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Supplementary Information

Synthetic studies of cystobactamids as antibiotics and bacterial imaging carriers lead to compounds with high in vivo efficacy

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1 EXPERIMENTAL PART

1.1 BIOLOGY

1.1.1 Minimal inhibitory concentrations (MIC) determination

All microorganisms were handled according to standard procedures and were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) or the American Type Culture Collection (ATCC). Tsx-deficient *Escherichia* coli and corresponding parental strains were purchased from the Coli Genetic Stock Center (CGSC). Fluoroquinolone-resistant strains *Escherichia coli* WT-3 and *E. coli* wild-type were kindly provided by Prof. Dr. P. Heisig (University of Hamburg, Germany). *E. coli* ΔTolC has an *E. coli* K12 background. *Pseudomonas aeruginosa* PA14 and PA14Δ*mexAB* were kindly provided by Prof. Dr. S. Häußler (Twincore, Hannover). Cystobactamids were prepared as DMSO stocks (5 mg mL^{-1}). Minimum inhibitory concentrations were determined according to standard procedures as described elsewhere.[1] Single colonies of the bacterial strains were suspended in cation-adjusted Müller-Hinton broth to achieve a final inoculum of approximately 10^6 $CFUmL^{-1}$. Serial dilutions of cystobactamids (0.03 to 64 µg mL^{-1}) were prepared in sterile 96-well plates and the bacterial suspension was added. Growth inhibition was assessed after overnight incubation (16-18 h) at 30-37°C. MIC values are defined as the lowest concentration of antibiotic that inhibited visible growth.

Bacterial strains used in the primary panel for assessing antibiotic activity:

- *Escherichia coli* DSM-1116 (WT)
- *Escherichia coli* TolC-deficient (derived from *E. coli* K12)
- *Pseudomonas aeruginosa* PA14
- *Pseudomonas aeruginosa* PA14Δ*mexAB*
- *Staphylococcus aureus* ATCC29213

Larger panel of bacterial strains used for the characterization of selected analogs:

- *Enterococcus faecalis* ATCC-29212
- *Staphylococcus epidermidis* DSM-28765
- *Acinetobacter baumannii* DSM-30008
- *Escherichia coli* WT-3 (clinical isolate from P. Heisig, Hamburg)
- *Escherichia coli* WT-3 [*gyrA*(S83L,D87G)]
- *Enterobacter aerogenes* DSM-30053
- *Enterobacter cloacae* DSM-30054
- *Pseudomonas aeruginosa* DSM-24600 (ESBL 1)

- *Pseudomonas aeruginosa* DSM-46316 (ESBL 2)
- *Klebsiella pneumoniae* DSM-30104
- *Citrobacter freundii* DSM-30039
- *Serratia marcescens* DSM-30121
- *Proteus vulgaris* DSM-2140
- *Proteus mirabilis* DSM-4479

1.1.2 **DNA supercoiling assay**

The half-inhibitory concentrations (IC_{50}) against gyrase were determined by two different methods:

1.1.2.1 Method a)

Commercial *E. coli* gyrase supercoiling assay kits (Inspiralis, Norwich, UK) were used to determine half-inhibitory concentrations (IC_{50}). Cystobactamids were prepared as DMSO stocks [assay concentration range: 0.05 to 25 µM, DMSO concentration: 0.1 % (*v/v*)] and the reference drug ciprofloxacin was prepared as acidified (HCl) aqueous solution. Assays were performed according to the manufacturer's protocol. In brief, *E. coli* gyrase (1 U) was mixed with the inhibitors and 0.5 µg relaxed plasmid was added after 10 min equilibration at room temperature. Reactions were quenched after 30 min at 37 °C by the addition of DNA gel loading buffer containing 10 % (*w/v*) SDS. Relaxed (REL) and supercoiled (SC) plasmids were separated on 0.8 % (w/v) agarose gels and visualized by ethidium bromide staining. Each gel was additionally loaded with two control reaction samples (1: without enzyme; 2: solvent control). Image analysis (intensity of REL and SC plasmid bands) was performed in ImageJ[2]. The respective values were normalized to the controls and the ratio SC/REL was used to determine IC_{50} values by sigmoidal curve fitting in Origin (OriginLab, Northampton, MA).
Using method a), IC_{50}'s of 0.11 µM for ciprofloxacin and 0.08 µM for **22** have been determined. Method a) was applied for compounds **2**, **4**, **22**, **57** and **28**.

1.1.2.2 Method b)

Relaxed plasmid DNA was prepared by mixing 25 µg of circular pUC19 with 6.5 U of Topoisomerase I [Thermo] in a total volume of 50 µl Topoisomerase buffer (250 mM Tris, [pH 7.5], 250 mM KCl, 50 mM $MgCl_2$, 2.5 mM DTT, 0.5 mM EDTA, 150 µg/ml BSA). The reaction was run for 90 min at 37°C and eventually purified via DNA spin-columns according to the vendor's manual. DNA-concentration was adjusted to 25 ng/µl according to OD_{260nm}.

Supercoiling assays were performed in 0.2 ml reaction tubes. For a single reaction, 7.8 µl of H_2O were mixed with 3 µl of 5× gyrase buffer [NEB] and 0.2 µl of DNA-gyrase [NEB]. Then 1 µl of pre-diluted compound of interest or 1 µl of solvent (negative control) was added followed by 3 µl of relaxed plasmid DNA (= 75 ng). The reaction was carried out for 30 min at 37°C and subsequently stopped by setting temperature to 60°C for 10 min. The concentrations of compounds tested were either 50 µM, 25 µM or 10 µM with three-fold serial dilutions down to 0.07 µM, 0.03 µM or 0.01 µM, respectively.

All reactions were separated on an Ethidium bromide-free agarose gel. After electrophoresis, gels were stained for 5 min in Ethidium bromide solution (10 mg/ml) and UV-fluorescence was recorded.

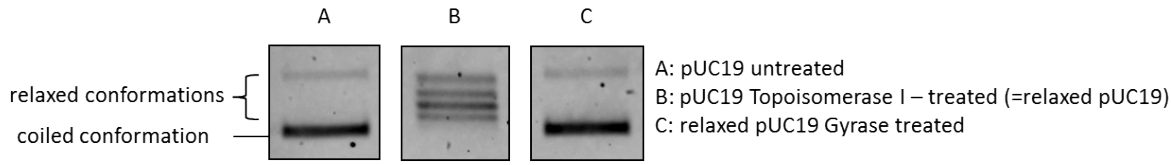


Figure S 1. DNA supercoiling assay result.

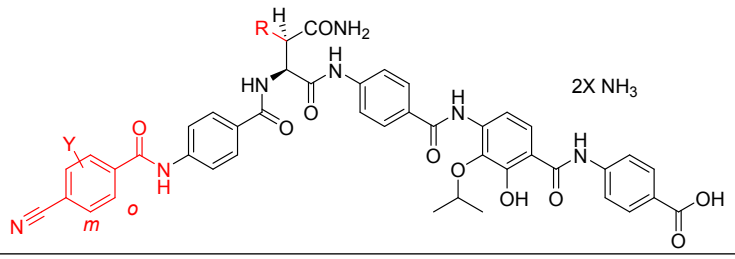
For evaluation, result images were loaded to the software Image Lab 5.0 [BioRad]. Digitally, each gel was divided into lanes and bands corresponding to coiled pUC19 were detected in each lane by densitometric analysis. Hereafter, intensities of each band were expressed relatively to the intensity of the untreated control. These values were then plotted on an x/y graph and IC_{50} was calculated by non-linear regression using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Using method b), IC_{50}'s of 0.17 µM for ciprofloxacin and 0.12 µM for **22** have been determined.

Method b) was applied for all compounds except of **2**, **4**, **57** and **28**.

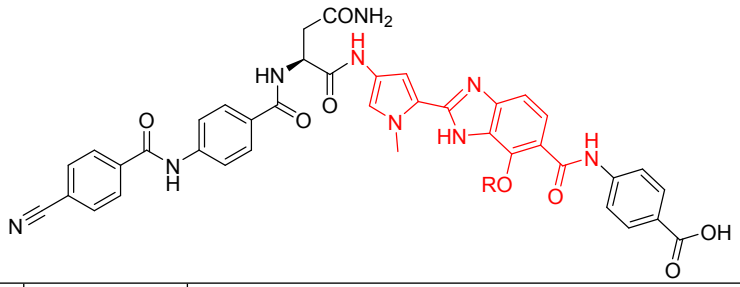
1.1.3 **Supplementary antibacterial activity data**

Table S 1. Biological activities of analogs bearing a second substituent on the *p*-cyanobenzoic acid unit.



			MIC [µg/mL]					EC gyrase IC_{50} [µM]
Compound	R	Y	*E. coli* ΔtolC	*E. coli* WT	*P. ae.* ΔmexAB	*P. ae.* WT	*S. aureus*	
Reference ***22***	H	/	0.06	0.5	0.25	2	0.25	0.08
88	H	CH_3 (o)	0.03	0.5	2	8*	64	1.7
89	H	CH_3 (m)	0.03	0.5	0.5	4	2	1.6
90	H	F (m)	≤ 0.03	0.25	0.5	8	2	0.4
91	H	Cl (m)	<0.03	<0.03	0.25	>64	<0.03	0.1
92	H	OCH_3 (m)	<0.03	<0.03	0.25	>64	>64	1.8
93	OMe	/	<0.03	<0.03	<0.03	0.5	<0.03	0.4

Table S 2. Biological activities of analogs incorporating a DNA minor groove recognition element.



		MIC [µg/mL]					EC gyrase IC_{50} [µM]
Compound	**R**	*E. coli* ΔtolC	*E. coli* WT	*P. ae.* ΔmexAB	*P. ae.* WT	*S. aureus*	
Reference ***22***		0.06	0.5	0.25	2	0.25	0.08
57	Me	>64	>64	>64	>64	>64	10.9
56	H	>64	>64	>64	>64	>64	nd

Table S 3. Biological characterization of the triazole-containing cystobactamid **61**.

	MIC [µg/ml]					EC gyrase IC_{50} [µM]
compound	*E. coli* ΔtolC	*E. coli* WT	*P. ae.* ΔmexAB	*P. ae.* WT	*S. aureus*	
1	0.06	0.1	0.1	0.25	0.4	0.11
22	0.06	0.5	0.25	2	0.25	0.08
61	<0.03	0.06	2	8	<0.03	1.5

1.1.4 **Cytotoxicity assay**

HEpG2-cells were seeded in a 96well-plate (Nunc, Roskilde, Denmark) and grown in DMEM supplemented with 10 % FCS to 75 % confluency at 37°C and 5 % CO_2. The respective cystobactamid was added in final concentrations up to 10 µg/ml. Untreated cells, pure medium and completely damaged cells served as controls. To damage cells, they were treated with 0.5 % Triton X-100 1 h prior to addition of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma). After 24 h, MTT diluted in PBS (stock solution 5 mg/ml) was added to the wells at a final concentration of 1 mg/ml. The cells were incubated for 4 h at 37°C and 5 % CO_2. Medium was removed, and 0.04 M HCl in 2-propanol was added. The cells were incubated at room temperature for 15 min. Then the supernatant was transferred to a 96well-plate. The samples were measured at 560 nm and at 670 nm as a reference wavelength on a Tecan Sunrise ELISAReader using Magellan software. Data was normalized using the following formula: (A-B)/(C-B) with 'A' as the respective data point, 'B' as the value of the uninfected Triton X-100-treated control and 'C' as the untreated and uninfected control. The experiment was repeated at least three times. For the cystobactamids **2** and **22** no signs of impaired viability were visible within the tested concentration range of 0.1 - 10 µM. This is in line with previously published cytotoxicity data on cystobactamid analogs.[1,3]

1.1.5 **Expression and purification of AlbD**

The coding sequence for the *Pantoea dispersa* albicidin detoxification enzyme (AlbD, U96453.1) was synthetized by Genscript as TRX-AlbD fusion construct flanked by NdeI and XhoI restriction nuclease sites. The synthetic DNA fragment was cloned into pCOOFY 38 [4] using the NdeI and XhoI restriction sites of the vector. The resulting construct pCOOFY38-TRX-AlbD-H6 creates an in frame fusion to the C-terminal His6 Tag derived from the vector backbone. Positive clones were verified by restriction digest with NdeI and XhoI, generating a 1049 bp insert and additional DNA sequence analysis.

The expression vector was isolated and transformed into *E. coli* Rosetta 2 (DE3). A 250 ml preculture was grown in TB-kan (50µg/ml) at 37 °C at 140 rpm in a baffled flask to a density of 4.8. The culture was transferred to a new flask and 250 ml of cold TB-kan medium (4°C) was added to reduce the temperature quickly to 18 °C. The induction of the expression of the AlbD fusion protein was done by adding IPTG at a concentration of 250 µM. The expression was continued over night for 18 hours at 18°C and 130 rpm. The cells were harvested by centrifugation at 6000 rpm for 15 minutes. The cell pellet was washed using 30 ml PBS. The biomass was shock frozen using liquid nitrogen and stored at -80°C.

The cell pellet (8 g wet-weight) was thawed on ice and resuspended in 50 ml lysis buffer (50mM Tris-HCl, pH7.4, 300 mM NaCl, 5% glycerol containing a Roche EDTA-free mini complete protease inhibitor tablet) and lysed in two rounds using an Avestin Emulsiflex C3 homogenizer according to the recommendations of the supplier.

The extract was clarified by centrifugation for 30 minutes at 16.000 g. The TRX-AlbD-H6 fusion protein was captured using a HisTrap column (GE) using an ÄKTA start chromatography system. Fractions were collected after washing the column with loading buffer (50mM Tris-HCl, pH7.4, 300 mM NaCl, 5% glycerol) containing different concentrations of Imidazole. Washing and elution were done according to the instruction leaflet of GE. Collected fractions were analyzed by SDS-PAGE and pooled. Soluble fractions of TRX-AlbD-H6 were dialyzed against loading buffer and subsequently separated on a Superdex 75 16/60 column equilibrated with loading buffer. The collected fractions containing the fusion protein were concentrated to 1 mg/ml using Vivaspin 20 /MWCO 10K and shock frozen in aliquots for further enzymatic analysis. The procedure for AlbD expression and purification was similar to a recently published literature protocol.[5]

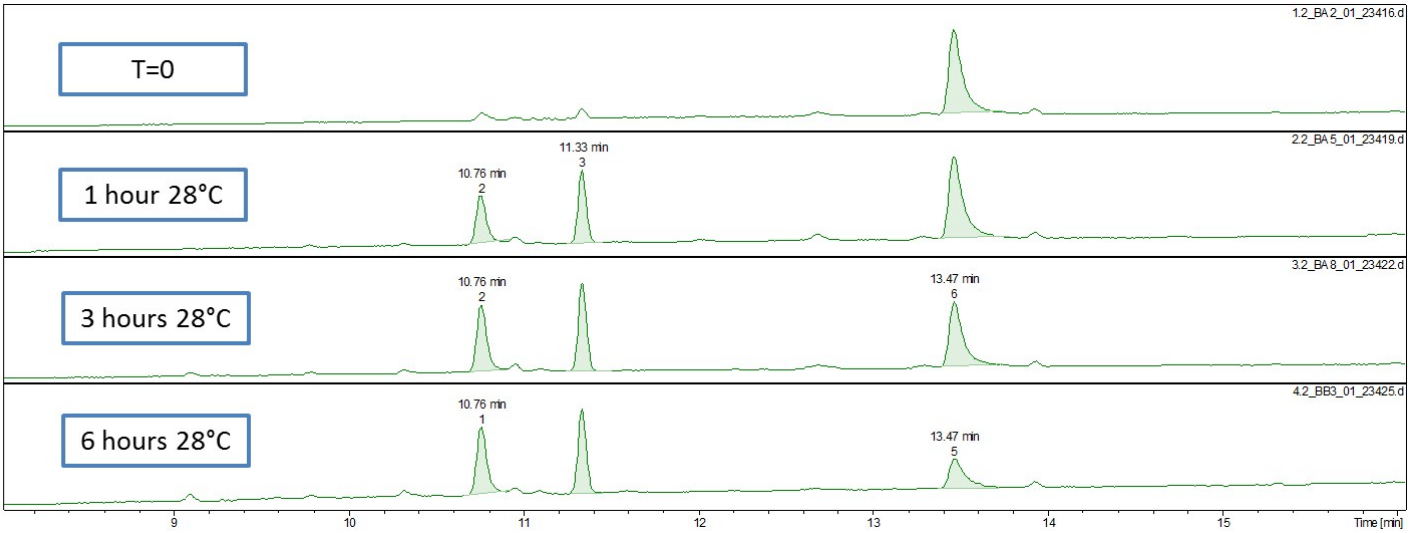
1.1.6 **LC/UV/MS-based monitoring of AlbD-mediated antibiotic degradation**

The assay mixture had a total volume of 100 µL in 0.2 M sodium phosphate buffer (pH 7.0), containing purified AlbD (final concentration: 10 µM) and albicidin or cystobactamid derivatives (final concentration: 120 µM). An AlbD-free assay mixture of 100 µL containing only the corresponding compound and the buffer solution was used as a negative control.

The assay mixtures were incubated for the indicated time (0, 1, 3 or 6h) at 28°C. The enzymatic reaction was then stopped through enzyme precipitation by adding 350 µL of methanol. Following centrifugation for 20 min at 20000 g, 250 µL of each supernatant were sampled and evaporated using a speed vac (2h at 30°C). Dried samples were dissolved in 100 µL methanol and analyzed with an HPLC (Thermo Scientific Dionex Ultimate 3000, column Phenomenex Kinetex 1,7 µm, C18, 100A, 150*2,1mm) coupled to an HRMS spectrometer (Bruker Maxis HD), with a 20 min gradient from 1 to 100% acetonitrile in water (0,1% HCOOH) with 0,3 ml/min flow rate. UV spectra were recorded using a photo diode array detector in the range of λ= 190-400 nm. HPLC Chromatograms were processed with the software Data Analysis. The procedure for the enzymatic assay was similar to a recently published literature protocol. [5]

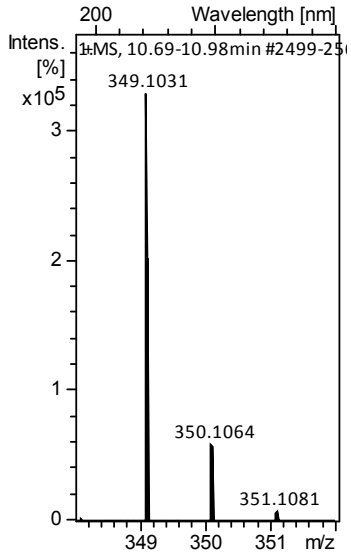
Results obtained for Albicidin:

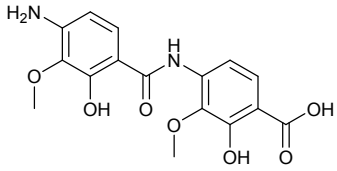
A)



B)

10.76 min : Fragment 1

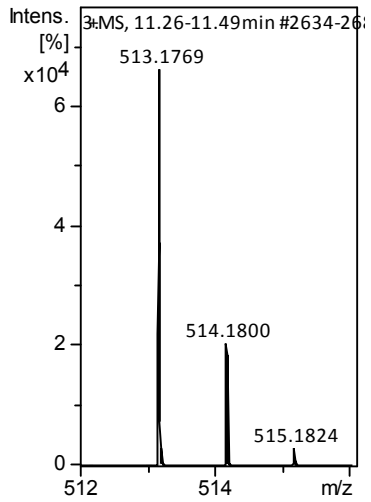


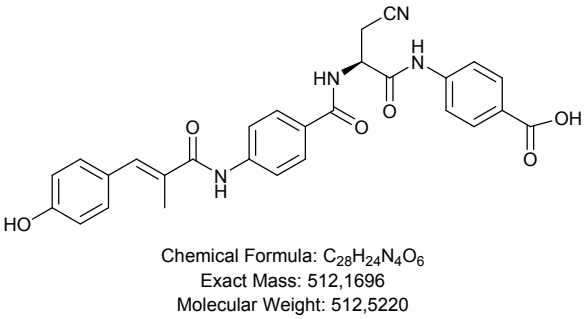


Chemical Formula: $C_{16}H_{16}N_2O_7$
Exact Mass: 348,0958
Molecular Weight: 348,3110

C)

11.33 min: Fragment 2

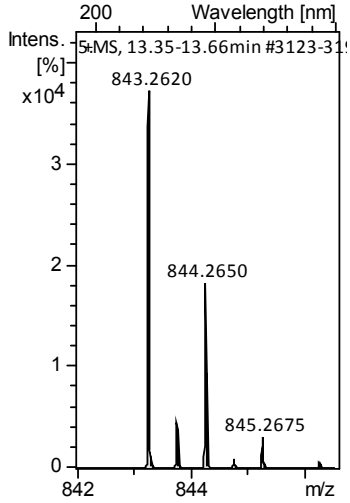


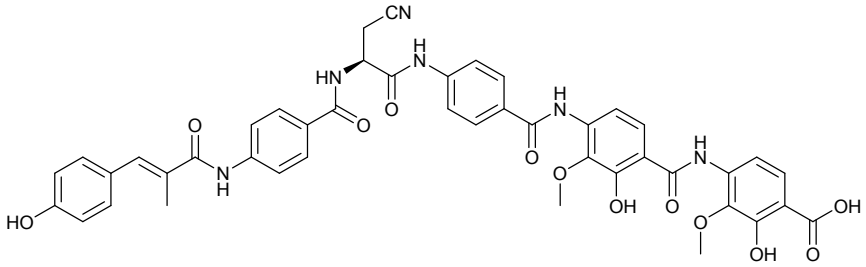


Chemical Formula: $C_{28}H_{24}N_4O_6$
Exact Mass: 512,1696
Molecular Weight: 512,5220

D)

13.47 min: Albicidin



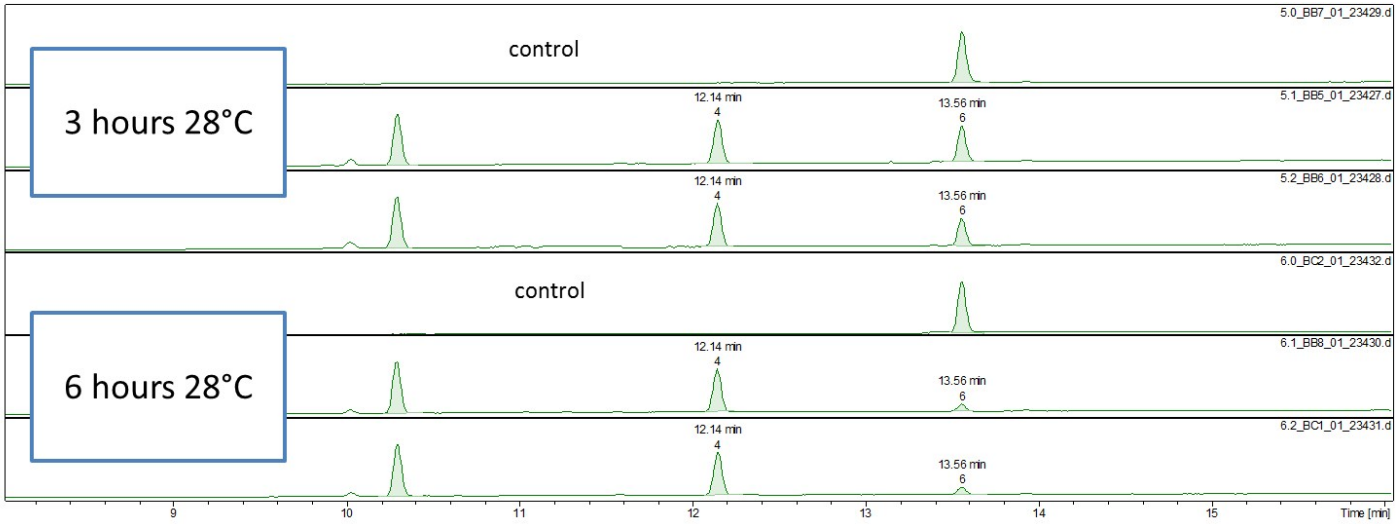


Chemical Formula: $C_{44}H_{38}N_6O_{12}$
Exact Mass: 842,2548
Molecular Weight: 842,8180

Figure S 2. Stability of albicidin upon exposure to AlbD. A) HPLC chromatograms after 0, 1, 3 and 6 hours of incubation of albicidin with AlbD at 28°C. B) High resolution mass spectrum (left; zoom into molecular ion) and structure (right) of fragment 1 of albicidin C) High resolution mass spectrum (left; zoom into molecular ion) and structure of fragment 2 of albicidin. D) High resolution mass spectrum (left; zoom into molecular ion) and structure (right) of albicidin.

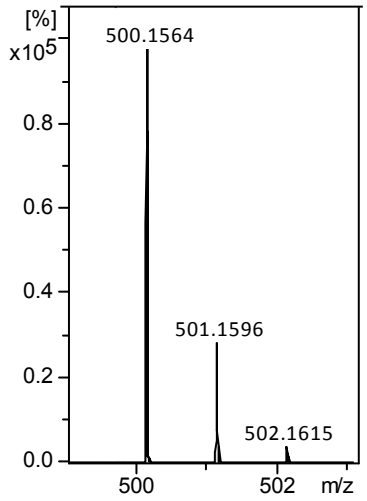
Results obtained for 22:

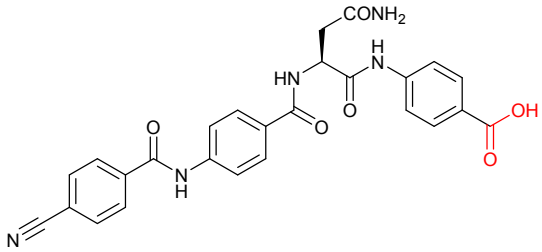
A)



B)

10.29 min: Fragment 1

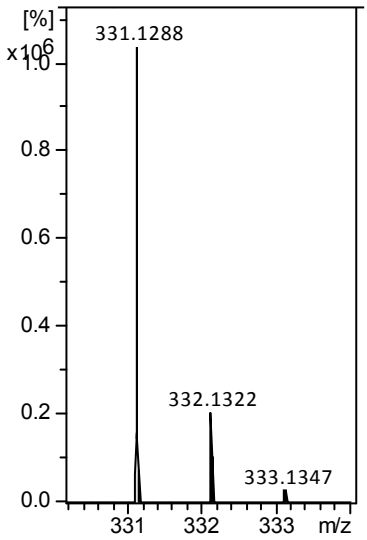


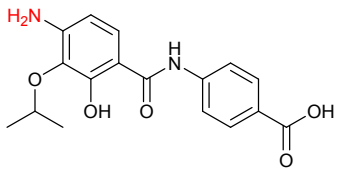


Chemical Formula: $C_{26}H_{21}N_5O_6$
Exact Mass: 499,1492
Molecular Weight: 499,4830

C)

12.14 min: Fragment 2

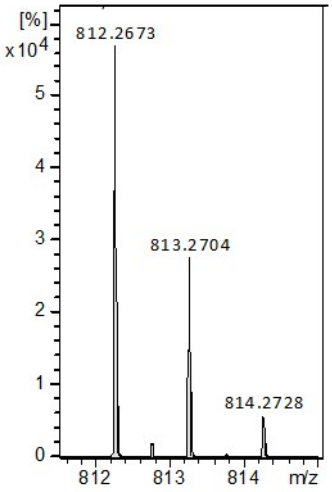


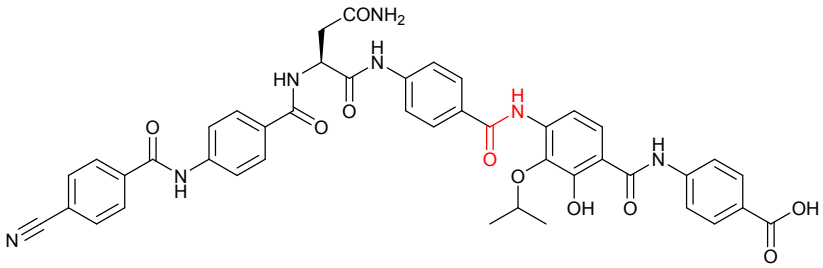


Chemical Formula: $C_{17}H_{18}N_2O_5$
Exact Mass: 330,1216
Molecular Weight: 330,3400

D)

13.56 min: **22**



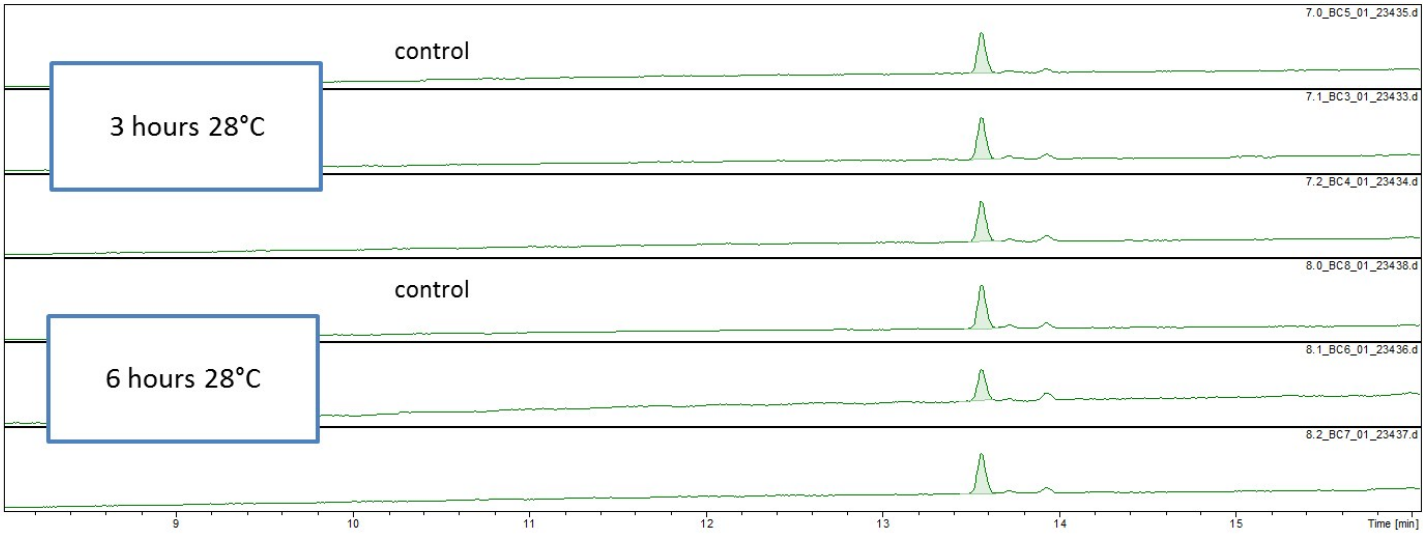


Chemical Formula: $C_{43}H_{37}N_7O_{10}$
Exact Mass: 811,2602
Molecular Weight: 811,8080

Figure S 3. Stability of **22** upon exposure to AlbD. A) HPLC chromatograms after 3 and 6 hours of incubation of **22** with AlbD at 28°. The compound incubated the same time without the enzyme was used as a control. B) High resolution mass spectrum (left; zoom into molecular ion) and structure (right) of fragment 1, generated by AlbD-mediated cleavage of **22**. C) High resolution mass spectrum (left; zoom into molecular ion) and structure of fragment 2, generated by AlbD-mediated cleavage of **22**. D) High resolution mass spectrum (left; zoom into molecular ion) and structure (right) of **22**.

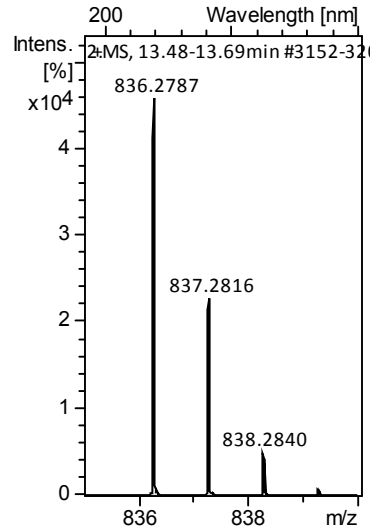
Results obtained for triazole 61:

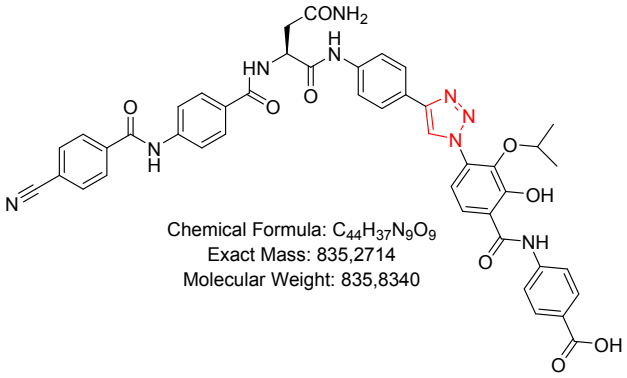
A)



B)

13.56 min: **61**





Chemical Formula: $C_{44}H_{37}N_9O_9$
Exact Mass: 835,2714
Molecular Weight: 835,8340

Figure S 4. Stability of **61** upon exposure to AlbD. A) HPLC chromatograms after 3 and 6 hours of incubation of Triazole with AlbD at 28°C. The compound incubated the same time without the enzyme was used as a control. B) High resolution mass spectrum (left; zoom into molecular ion) and structure (right) of **61**.

1.1.7 Cystobactamid-based bacterial imaging

Materials and methods

Visualizing and monitoring uptake with FAP-system

FAP6.2 was recombinantly expressed and purified as described by Ferreira *et al*.[6] For measuring intensity of fluorescence emission, **86** and **87** were incubated with recombinant FAP at a molar ratio of 1:2 in PBS in 96 well black µclear plates (Greiner Bio-One, Kremsmünster, Austria) and fluorescence emission was quantified at λ_{ex} 610nm and λ_{em} 655nm using a Tecan Infinite 200 pro reader (Tecan, Switzerland) in bottom read mode. Measurements were done in triplicate. For comparability of compounds with different fluorescent intensities, a correction factor c_f was calculated. c_f corresponds to the ratio of fluorescence intensity of free malachite green (MG, **87**) divided by the fluorescence intensity of the MG-conjugate **86**. Monitoring uptake by MG-fluorescence in FAP6.2 expressing *E. coli* Origami™ B (DE3) pLysS (Novagen/Merck, Darmstadt, Germany, *E. coli*_FAP6.2) and correction of fluorescence values with c_f was performed as described by Ferreira *et al*.[6]

Confocal microscopy - FAP-system

Confocal microscopy of *E. coli*_FAP6.2 was performed upon 16h incubation with **86** and **87** at a final concentration of 10 µM. Bacterial cells were placed into chamber slides (Ibidi GmbH, Martinsried, Germany) and covered with a pad consisting of 1% agarose. Imaging was performed using the confocal microscope ECLIPSE Ti (Nikon) equipped with UltraVIEWVoX spinning disc (Perkin Elmer, Waltham, US), ORCA-R2 camera (Hamamatsu Photonics, Japan) and Volocity software 6.1.1 (Perkin Elmer, Waltham, US).

Absorbance and fluorescence emission spectra of bodipy-conjugates

Compounds were diluted in DMSO to a final concentration of 1 µM and 100 µl were filled per well into 96 well black µclear plates (Greiner Bio-One, Kremsmünster, Austria). Absorbance spectra were recorded using a Tecan Infinite 200 pro reader (Tecan, Switzerland) with 5 nm wavelength step size. The fluorescence spectra were measured from the same samples with a λ_{ex} of 480 nm using the Tecan Infinite 200 pro reader (Tecan, Switzerland) in bottom read mode with 5 nm emission wavelength step size. Each sample was measured in duplicate.

Bacterial growth inhibition assay

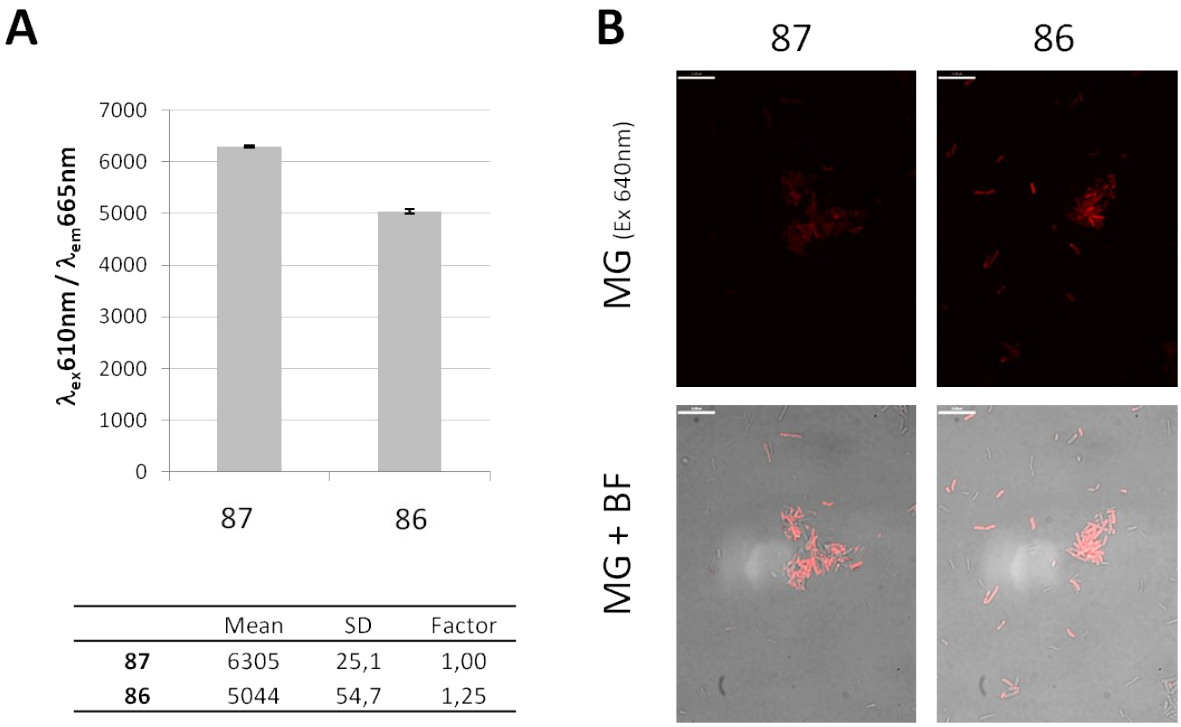
To evaluate the effect of **85** and **84** on growth of *E. coli* WT2 an antimicrobial activity assay was performed. Bacteria were cultured at 37°C in a shaker at 150 rpm. Overnight cultures contained 5 ml of LB medium. Overnight cultures were diluted 1:100 with 5 ml LB- medium and grown to an OD_{600} of 0.5-0.8. Cultures were diluted to a final OD_{600} of 0.01 and 100 µl of diluted culture were transferred each well of 96 well clear area plates. Test compounds were dissolved in DMSO, were added to a maximum final concentration of 1% of DMSO and were serially diluted over the plate. The plate was wrapped with parafilm and incubated in a shaker at 150 rpm and 37°C for 24h. Bacterial growth was determined by measuring OD_{600} using a microplate spectrophotometer power wave XS2 (Bio Tek, Winooski, US). Each concentration was measured in triplicate in two biological replicates. IC_{50} values were calculated using Sigma Plot (Systat Software GmbH, Erkrath, Germany) and four parameter logistics curve regression. Mean values of IC_{50} obtained from two biological replicates are reported.

Flow cytometry of bacteria treated with bodipy-conjugates

For analysing uptake by flow cytometry *E. coli* WT2 was incubated for 1 or 5 h in PBS with **85** or **84** at a final concentration of 10 µM. Cells were washed twice with PBS and the OD_{600} was adjusted to 0.025 with sterile-filtered PBS. At least 10000 cells per sample were analyzed with the 488 nm laser in combination with 525/50 nm band pass filter (bl525-A) of a LSRFortessaTM with FACSDivaTM software (BD Biosciences, Heidelberg, Germany). Dot plots depict Bodipy-fluorescence (bl525-A) vs SSC-A (side scatter), and histograms depict Bodipy-fluorescence vs cell count. Data was analyzed using FlowJo (FLOWJO LLC, Oregon, US) Version 10.0.8. For quantifiying the amount of dead cells, propidium iodide (PI) was added for 10 min at the end of incubation at a concentration of 2 µg/ml. PI fluorescence was monitored with 488 nm laser in combination with 685/35 nm band pass filter (bl685-A). Dot plots depict PI-fluorescence (bl685-A) vs SSC-A (side scatter) and histograms depict PI-fluorescence vs cell count.

Confocal microscopy - bodipy-conjugates

Labeling of *A. baumannii, K. pneumoniae, E. coli* (all grown in LB-medium) and *S. aureus* (grown in TSY-medium) with **85** or **84** was monitored by confocal microscopy. Bacteria were imaged after incubation with the compounds at a final concentration of 10 µM for 4 h in PBS. Bacterial cells were washed twice with PBS, placed into chamber slides (Ibidi GmbH, Martinsried, Germany) and covered with a pad consisting of 1% agarose in PBS. Imaging was performed using the confocal microscope ECLIPSE Ti (Nikon) equipped with UltraVIEWVoX spinning disc (Perkin Elmer, Waltham, US), ORCA-R2 camera (Hamamatsu Photonics, Japan) and Volocity software 6.1.1 (Perkin Elmer, Waltham, US).



	Mean	SD	Factor
87	6305	25,1	1,00
86	5044	54,7	1,25

Figure S 5. Monitoring uptake with the FAP system. A. Recombinant FAP was incubated with MG-conjugate or free MG at a molar ratio of 1:2 and fluorescence emission was quantified at indicated wavelengths. Measurements were done in triplicate. The factor corresponds to the ratio of fluorescence intensity of free MG divided by the fluorescence intensity of the MG-conjugate and was applied for correcting for the difference in fluorescence intensity. B. Confocal microscopy images of E. coli_FAP6.2 upon incubation with 10 µM of **87** or **86** for 16h. BF=bright field. Scale bars: 11 mm.

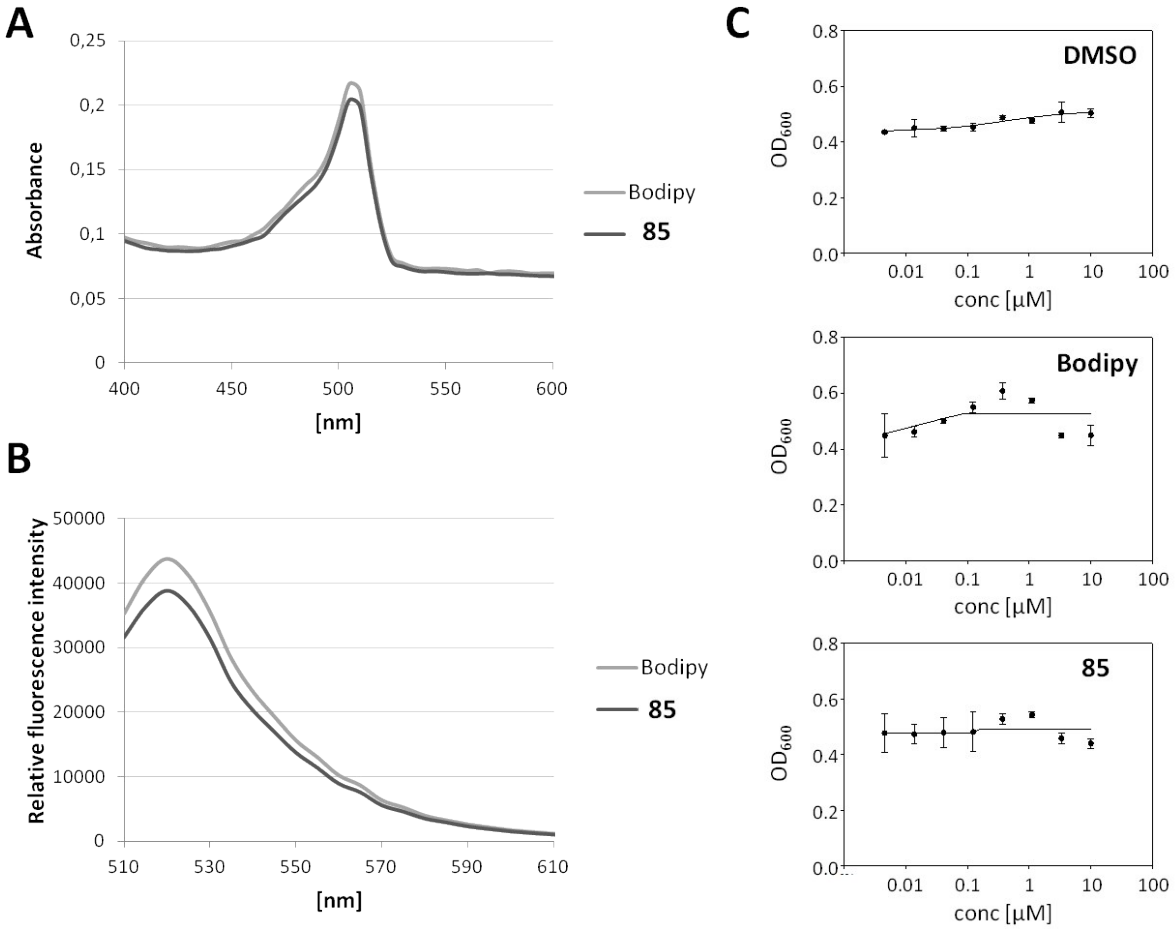


Figure S 6. Absorbance and emission spectra of bodipy-conjugates and effect on the growth of *E. coli*. A. Absorbance spectrum of **85** and bodipy FL azide. B. Fluorescence spectrum of **85** and bodipy FL azide at excitation of 480 nm. C. The effect of **85** and bodipy FL azide on the growth of *E. coli* BW25113 (wt) was assessed after 24 h incubation by quantifying OD_{600}.

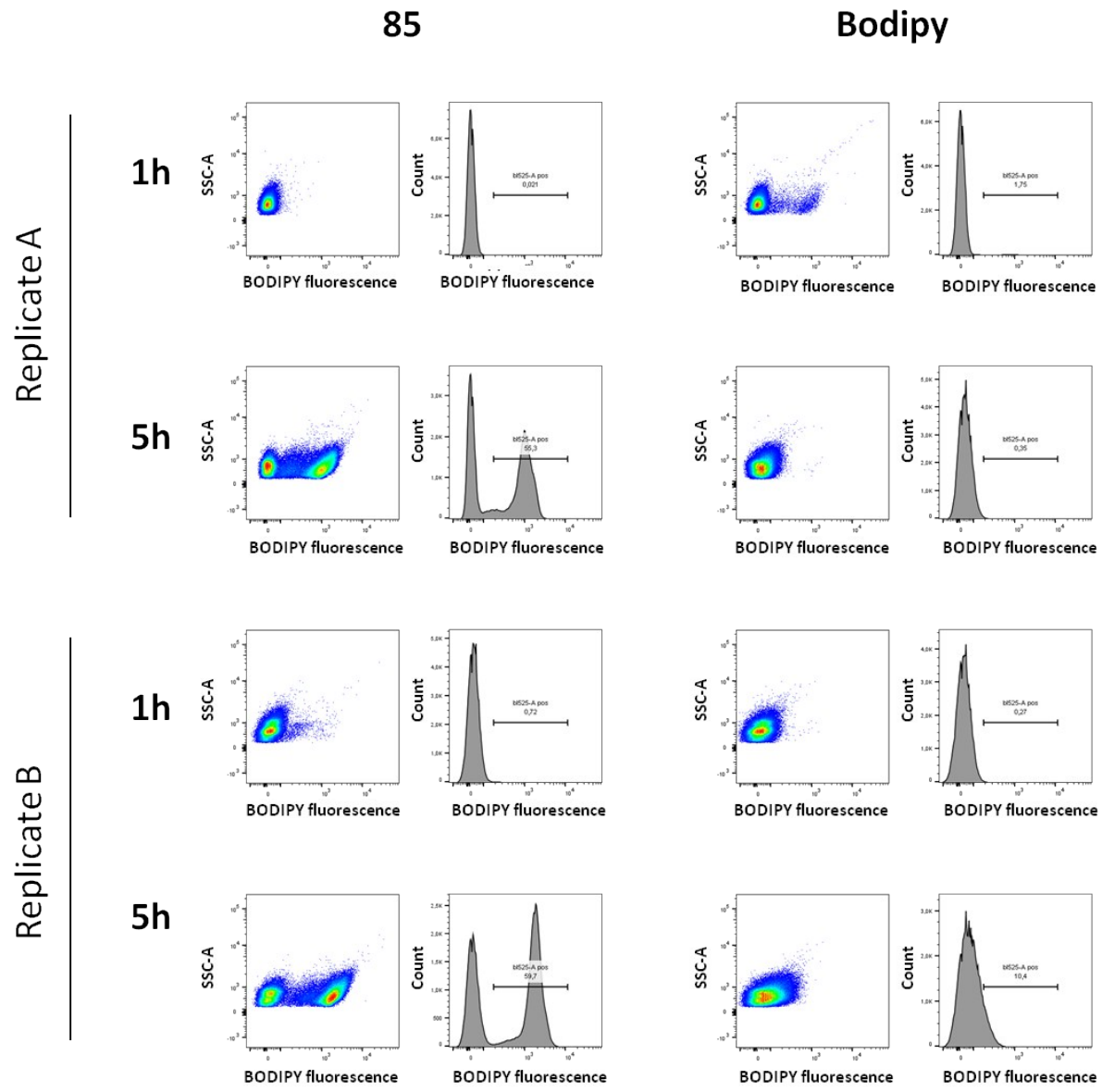


Figure S 7. Quantification of uptake by flow cytometry. *E. coli* wt was incubated for 1 or 5h in PBS with **85** or bodipy FL azide at a final concentration of 10 µM. Fluorescence of bodipy FL azide or **85** was quantified by flow cytometry. Dot plots depict bodipy-fluorescence vs SSC-A (side scatter), and histograms depict Bodipy-fluorescence vs cell count. Results of two replicate experiments are shown.

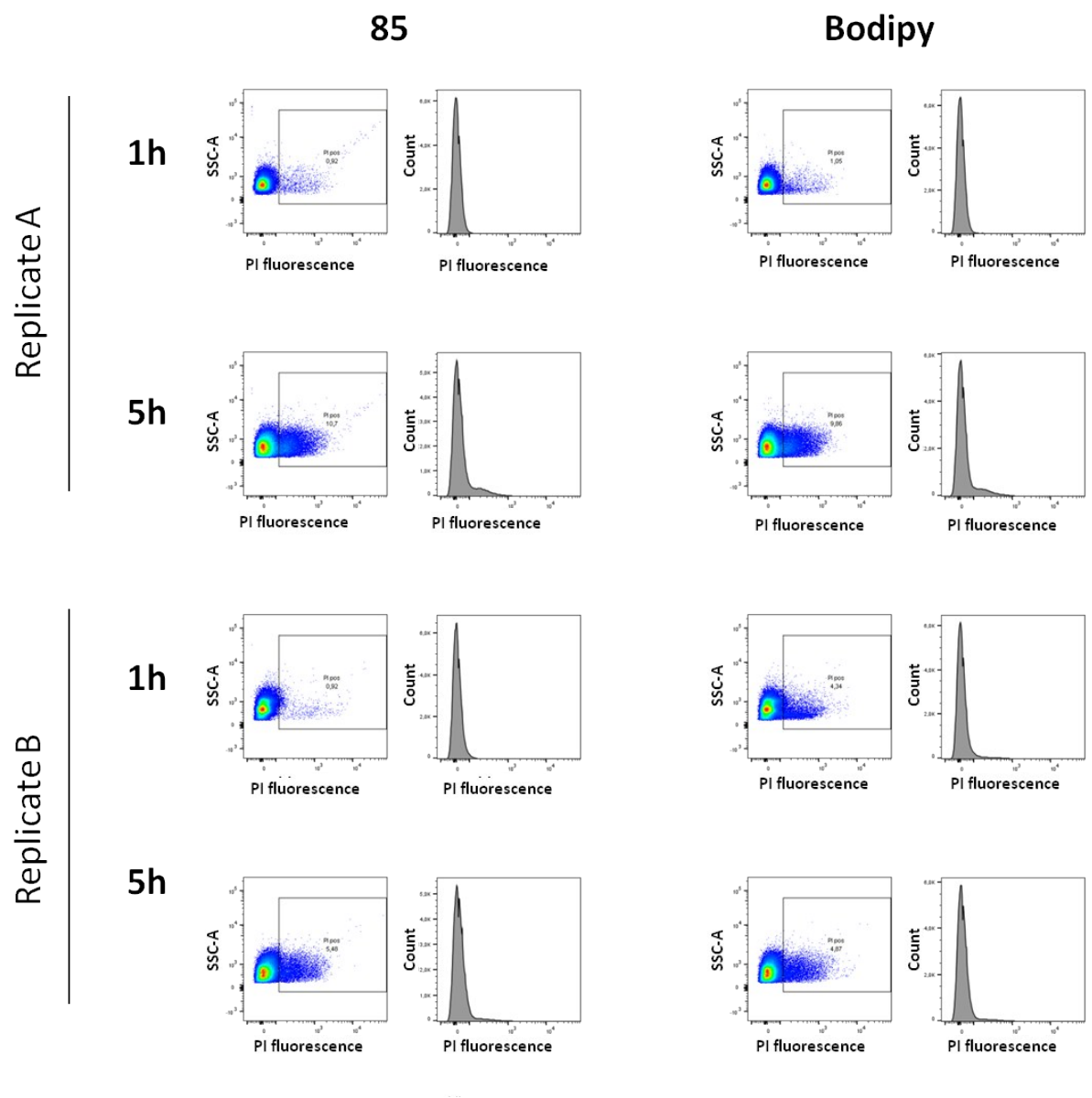


Figure S 8. Quantification of dead cells by monitoring propidium iodide (PI) staining signal by flow cytometry. *E. coli* wt was incubated for 1 or 5h in PBS with **85** or bodipy FL azide at a final concentration of 10 µM. Fluorescence of PI was quantified by flow cytometry. Dot plots depict PI-fluorescence vs. SSC-A (side scatter) and histograms depict PI-fluorescence vs cell count. Results of two replicate experiments are shown.

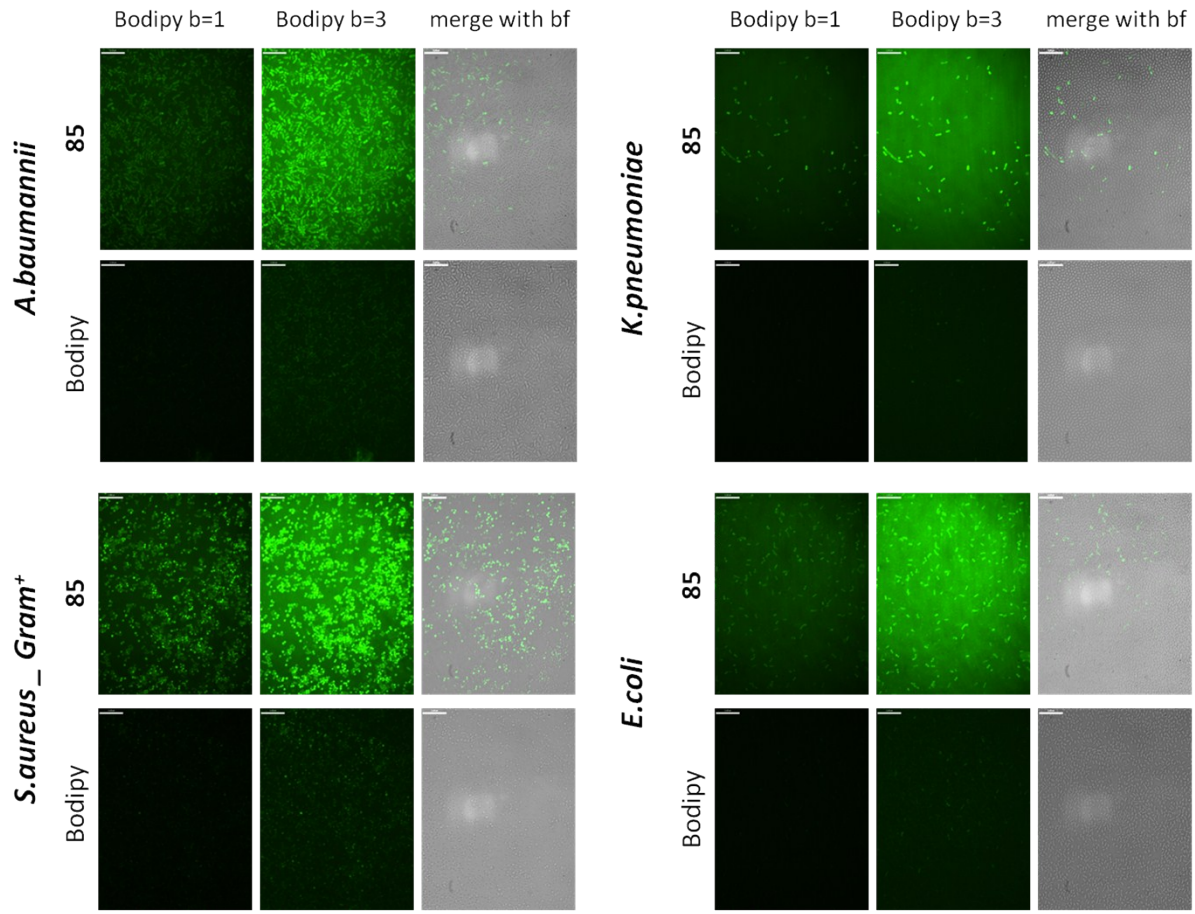


Figure S 9. Labeling of bacterial pathogens with **85** or **84** as monitored by confocal microscopy. Bacteria were incubated with **85** or **84** at a final concentration of 10 μM for 4 h in PBS and imaged by confocal microscopy. b = brightness. Scale bars: 11 mm.

1.1.8 **Resistance experiments**

1.1.8.1 Frequency of resistance

The screen was performed as previously described[7] with a few minor modifications. Bacterial culture was resuspended in saline solution followed by plating on CASO agar plates. CASO agar plates contained antibacterial agent at 4X MIC and were plated with 10^6, 10^7, 10^8, 10^9 and 10^{10} CFU. Plates were incubated for 24 hours at 37°C. Frequency of resistance was determined as ratio between number of bacteria growing divided by number of bacteria in the inoculum.

Table S 4: Frequency of resistance determined on *E. coli* ATCC-25922ΔtolC, *E. coli* MG1655/K12, *P. aeruginosa* Pa14ΔmexAB and *A. baumannii* DSM-30008 at 4X MIC for **22** and ciprofloxacin (CIP).

	FoR (4xMIC)	
Strain	**22**	CIP
E. coli MG1655/K12	3.8×10^{-8}	1.5×10^{-10}
E. coli ATCC-25922ΔtolC	3.1×10^{-8}	2.8×10^{-10}
Pa14ΔmexAB	1.1×10^{-8}	2.2×10^{-8}
A. baumannii DSM-30008	1.6×10^{-9}	6.9×10^{-10}

1.1.8.2 Fitness cost

Optical density (OD600nm) of a bacterial overnight culture was measured and adjusted to 0.001 in fresh MHBII medium. OD600 was measured at selected time points and plotted against time.

Table S 5: Determination of fitness loss of *E. coli* ATCC-25922ΔtolC, *E. coli* MG1655/K12, *P. aeruginosa* Pa14ΔmexAB and *A. baumannii* DSM-30008 resistant to **22** compared to their respective wild type.

strain	**Fitness loss**
E. coli MG1655/K12	no
E. coli ATCC-25922ΔtolC	
Pa14ΔmexAB	
A. baumannii DSM-30008	yes

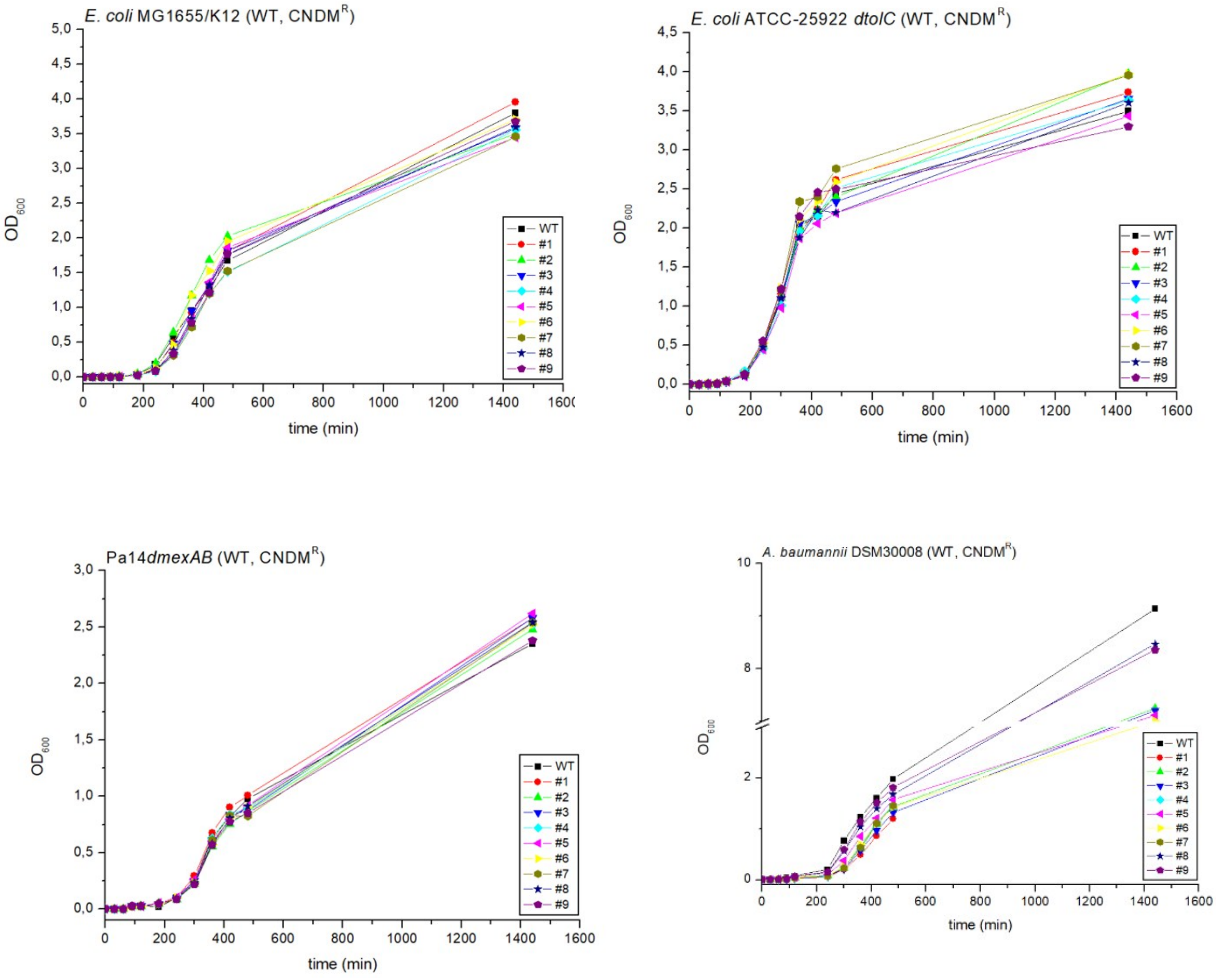


Figure S 10. Growth curves of wild type and resistant clones of *E. coli* MG1655/K12 (upper left panel), *E. coli* ATCC-25922ΔtolC (upper right panel), Pa14ΔmexAB (lower left panel) and *A. baumannii* DSM-30008 (lower right panel).

1.1.8.3 Serial passaging experiments

MIC values were first determined and recorded. After incubation, serial passaging was initiated by harvesting bacterial cells growing at the highest concentration of the antimicrobial (below the MIC) and inoculating into fresh MH2 medium. This inoculum was subjected to another MIC assay. After an 18– 24 h incubation period, cells growing in the highest concentration of the antimicrobial from the previous passage were once again harvested and assayed for the MIC. The process was repeated for 10 passages or when a 256 MIC-fold was reached. Antimicrobial concentrations were adjusted during the process to compensate for rising MIC values.

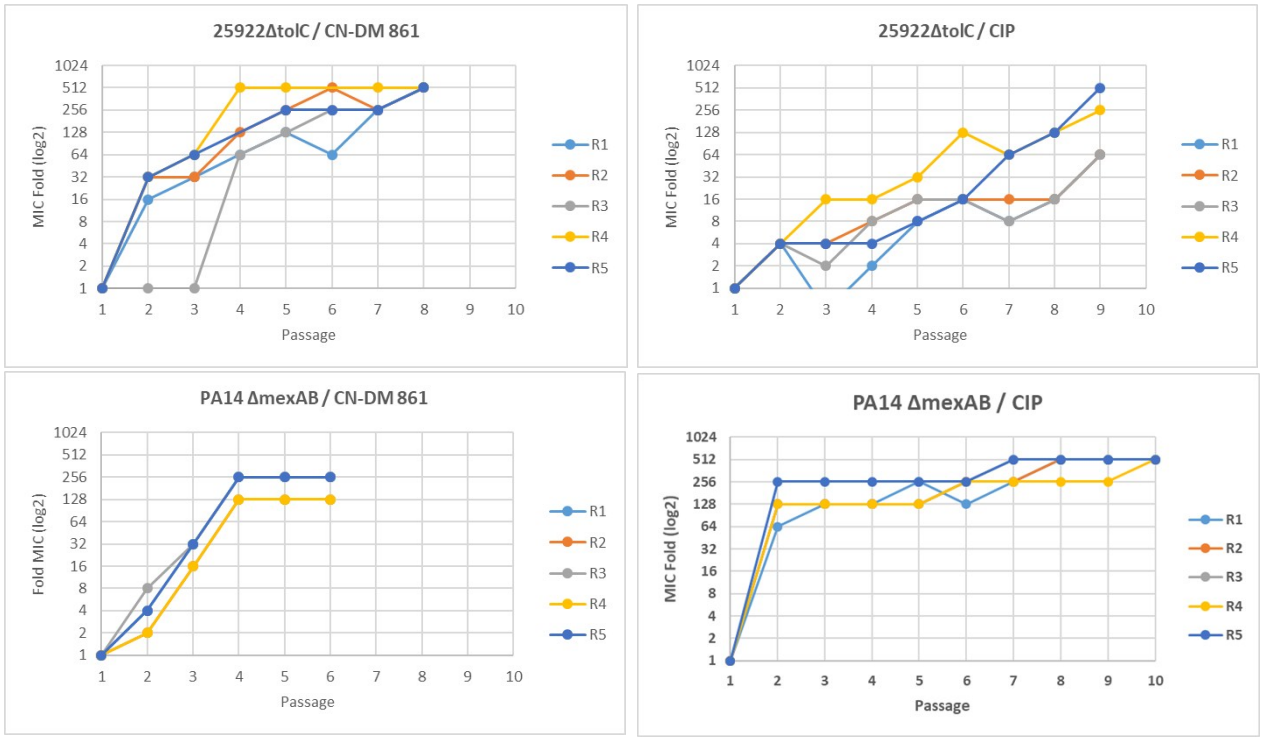


Figure S 11. Resistance development through serial passaging. Shifts in MIC for *E. coli* ATCC25922Δ*tolC* (upper panel) and *P. aeruginosa* PA14Δ*mexAB* (lower panel) after the number of serial passages indicated are depicted on a log2 scale. Curves for **22** (=CN-DM-861) are given in the left column, and curves for ciprofloxacin (=CIP) are given in the right column. Some curves are not visible due to overlaps: PA14: R2+R4, R1+R5 are overlaid for CN-DM-861; R3+R4 are overlaid for CIP.

1.1.9 **In vivo experiments (pharmacokinetics, neutropenic thigh infection model, s.c. and i.v. administration, bioanalysis**

1.1.9.1 Mice

For pharmacokinetic experiments, outbred male CD-1 mice (Charles River, Netherlands), 4 weeks old, were used. For pharmacodynamic experimens, outbred male CD-1 mice (Charles River, Netherlands), 6 weeks old, were used. The animal studies were conducted in accordance with the recommendations of the European Community (Directive 86/609/EEC, 24 November 1986). All animal procedures were performed in strict accordance with the German regulations of the Society for Laboratory Animal Science (GV- SOLAS) and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA). Animals were excluded from further analysis if sacrifice was necessary according to the human endpoints established by the ethical board. The experiments were approved by the ethical board of the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Oldenburg, Germany (LAVES; permit No. and 33.19-42502-04-15/1857).

1.1.9.2 Pharmacokinetic (PK) studies

Compound **22** was dissolved in 20 % DMSO, 10 % ethanol, 20 % Tween 80, 15 % PEG400 and 35 % water. For single dosing PK studies mice (n=3 for each route of administration) were administered **22** intravenously (i.v.) or subcutaneously (s.c.) at 5 mg/kg. About 20 µl of whole blood was collected serially from the lateral tail vein at time points 0.25, 0.5, 1, 2, 4 and 8 h post administration. After 24 h mice were sacrificed, and blood was collected from the heart. Whole blood was collected into Eppendorf tubes coated with 0.5 m EDTA and immediately spun down at 13.000 rpm for 10 min at 4°C. The plasma was transferred into a new Eppendorf tube and then stored at -80°C until analysis. For the multiple dosing PK study mice (n=3) were administered 10 mg/kg i.v. at t= 0 h and 10 mg/kg s.c. at t= 2, 6 and 10 h. Plasma was obtained analogously to the single dose PK studies.

1.1.9.3 Neutropenic thigh infection model in E. coli, s.c. and i.v. administration

Male CD-1 mice were rendered neutropenic by administration of 150 mg/kg and 100 mg/kg cyclophosphamide i.p. on day -4 and -1, respectively.
The *E. coli* strain ATCC25922 was used, that was sensitive against **22** (MIC = 0.02 µg/mL) and levofloxacin (LVX) (MIC = 0.04 µg/mL). On the day of infection (day 0), mice received

30 µl of the *E. coli* strain ATCC 25922 into each thigh (inoculum $3x10^6$ cfu/ml). The inoculum of the *E. coli* strain ATCC 25922 was prepared as follows: on day -1 the *E. coli* strain was streaked out onto a blood agar plate and incubated at 37°C. Then one single colony was inoculated into LB medium (diluted 1:15 in water) and incubated at 120 rpm and 37°C. On day 0 bacteria were centrifuged and washed twice in PBS. Then they were adjusted to an OD of 1 and diluted 1:100 in PBS. The inoculum was plated onto LB agar plates in serial dilutions and incubated at 37°C. After infection mice were treated as follows: vehicle treated animals received the formulation of **22** only (20 % DMSO, 10 % ethanol, 20 % Tween 80, 15 % PEG400 and 35 % water) at t=2 h post infection i.v. and at t= 4, 8 and 12 h post infection s.c. Levofloxacin treated animals received 8.2 mg/kg i.p. at t= 2,6 and 10 h post infection. The high dosing group received 7.5 mg/kg i.v. at t=2 h and 20 mg/kg s.c. at t=4, 8 and 12 h post infection whereas the low dosing group received 7.5 mg/kg i.v. at t=2 h and 10 mg/kg s.c. at t: 4,8 and 12 h post infection. 24 h after infection mice were sacrificed, kidneys, lung and muscles were aseptically removed. Organs were homogenized in PBS, plated onto LB agar plates in serial dilutions and incubated at 37°C for 18-20 h.

1.1.9.4 PK and PD sample preparation and analysis

All PK and PD plasma samples were analyzed via HPLC-MS/MS using an Agilent Infinity II HPLC system equipped with a diode array UV detector and coupled to an AB Sciex QTrap 6500plus mass spectrometer. First, a calibration curve was prepared by spiking different concentrations of **22** into mouse plasma, mouse urine, homogenized muscle, homogenized lung or homogenized kidney from CD-1 mice. The lower limits of quantification are indicated in Table S2. Glipizide was used as an internal standard. In addition, quality control samples (QCs) were prepared for **22** in plasma, urine, homogenized kidney, lung and muscle. The following extraction procedures were used: (1) 7.5 µl of a plasma sample (calibration samples, QCs or PK samples) was extracted with 22.5 µl of a methanol/THF mixture (1125 µl THF and 750 µl methanol), (2) 15 µl of a urine sample (calibration samples, QCs or PK samples) was extracted with 22.5 µl of a methanol/THF mixture (1125 µl THF and 750 µl methanol) and (3) 15 µl of a muscle/kidney or lung sample (calibration samples, QCs or PK and PD samples) was extracted with 22.5 µl of a methanol/THF mixture (1125 µl THF and 750 µl methanol) and 15 µl of water containing 1 % formic acid for 5 min at 2000 rpm on an Eppendorf MixMate® vortex mixer. Then samples were spun down at 13.000 rpm for 5 min. 20 µl of supernatant (from plasma, kidney, lung or muscle) or 30 µl of supernatant (from urine), respectively, were transferred to standard HPLC-glass vials containing 10 µl of DMSO with 12.5 ng/ml glipizide as internal standard. HPLC conditions were as follows: column: Agilent Zorbax Eclipse Plus C18, 50x2.1 mm, 1.8 µm; temperature: 30°C; injection volume: 1 µl for plasma and 10 µl for other matrices; flow rate: 700 µl/min; solvent A: water + 0.1 % formic acid; solvent B:

acetonitrile + 0.1 % formic acid; gradient: 99 % A at 0 min, 99 % - 0% A from 0.1 min to 4.00 min, 1 % A until 4.50 min, 0 % - 99 % A from 4.00 min to 4.50 min, 99 % A until 6.00 min. Mass spectrometric conditions were as follows: Scan type: MRM, negative mode; Q1 and Q3 masses for glipizide, **22** can be found in Table S3; peak areas of each sample and of the corresponding internal standard were analyzed using MultiQuant 3.0 software (AB Sciex). Peak areas of the respective sample of **22** were normalized to the internal standard peak area. For **22**, *m/z* 810.1 -> 432.0 was used for quantification and *m/z* 810.1 -> 264.0 was used for qualification. For glipizide *m/z* 444.1 -> 318.900 was used for quantification and *m/z* 444.1 -> 169.900 was used for qualification. Peaks of PK/PD samples were quantified using the calibration curve. The accuracy of the calibration curve was determined using QCs independently prepared on different days (Table S4). PK parameters were determined using a non-compartmental analysis with PKSolver[8].

Table S 6. Limits of quantification, lower limit of qualification and accuracy for **22** in different matrices.

ID	limits of quantification [ng/ml]	lower limit of qualification [ng/ml]	accuracy [%]
plasma	2.5-8000	1	101.11-113.23
urine	500-8000	250	102.09-109.11
kidney	2.5-4000	50	85.18-114.48
muscle	100-8000	5	85.62-111.83
lung	10-8000	5	91.59-111.49

Table S 7. Instrument parameters for the quantification of **22** by tandem mass spectrometry.

ID	Q1 Mass [Da]	Q3 Mass [Da]	time [msec]	CE [volts]	CXP [volts]
22	810.054	432.000	50.0	-58	-53
22	810.054	264.000	50.0	-56	-3
glipizide	444.084	318.900	50.0	-30	-25
glipizide	444.084	169.900	50.0	-42	-17

Table S 8. Pharmacokinetic data for **22** after i.v. and s.c. administration of 5 mg/kg.

PK parameter	**22**	
	i.v., 5 mg/kg	s.c., 5 mg/kg
C_0 [ng/ml]	2938.8 ± 65.0	
$t_{1/2}$ [h]	0.98 ± 0.1	1.65 ± 0.4
V/F [l/kg]	3.54 ± 1.2	22.82 ± 3.1
CL/F [ml/kg/min]	41.5 ± 11.4	162.9 ± 20.3
AUC [ng/ml*h]	2087.0 ± 573.1	517.0 ± 64.3
MRT [h]	1.08 ± 0.1	3.13 ± 0.5
C_{max} [ng/ml]		128.53 ± 27.5
T_{max} [h]		1.67 ± 0.6

Table S 9. Levels of **22** in muscle, kidney and lung in PD study samples 24 h after infection.

Compound (*dose*)	muscle	kidney	lung
22 *67.5 mg/kg/day*	102.11 ± 3.6	10.3 ± 9.3	18.61 ± 7.1
22 *37.5 mg/kg/day*	100.43 ± 3.2	2.43 ± 0.6	11.90 ± 4.7

1.1.10 **In vivo experiments (neutropenic thigh infection model with i.v. administration and tolerance study)**

1.1.10.1 *Preparation of pathogens and test articles*

For the infection, *E. coli* ATCC25922 was prepared from frozen stock and then was washed twice in PBS and adjusted to a concentration of 1 x 10^7 cfu/ml by optical density measurement. The *E. coli* strain ATCC25922 was sensitive to **22** (MIC = 0.02 µg/mL) and to ciprofloxacin (MIC = 0.01 µg/ml). Inoculum concentrations were confirmed by plating a sample onto drug-free agar. Test article was and solubilized in hydroxypropyl-beta cyclodextrin/Tris buffer 1% at pH8 (20%/80%; w/v) for final concentration required for a 5 ml/kg dosing formulation.

1.1.10.2 Animals and housing

This study was performed in performed in strict accordance under the UK Home office License and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA). The study was approved by the Agenda Resources (Alderley Park) Animal Welfare and Review Board (Project Licence No. PA67E0BAA E5). All animal experiments were performed by experienced technicians that had completed the UK Home Office Personal License course and held current personal licenses. Specific pathogen-free male ICR mice (4 weeks old, 11-15 g, Charles River Margate, UK) were housed on sterile aspen chip bedding with free access to food and water in individual ventilated cages, exposed to a 12-hour light/dark cycle, HEPA-filtered air at 22°C, and a relative humidity of 60%. Mice were allowed to acclimatize for at least seven days prior to the start of studies. During infection, mice had additional access to wet food.

1.1.10.3 Neutropenic murine thigh infection model

Mice were immunosuppressed using cyclophosphamide at 150 mg/kg on day minus 4 and at 100 mg/kg on day minus 1. Prior to infection on day 1, mice were anaesthetized with 2.5% isofluorane/97.5% oxygen. Once anesthesia was confirmed by absence of pedal reflex, both thighs were infected by intramuscular injection with 50 µl of bacterial suspension containing ~5 x 10^5 colony forming units (CFU). Mice were returned to cages in a warming cabinet with frequent observations until full recovery from anesthesia. Treatment was initiated 1 h post-infection by IV injection of 5 ml/kg test article via the lateral caudal vein. Treatment was administered every 6h over the 24h period. Animals of pre-treatment groups were euthanized 1 h post infection, and animals of treatment groups 25 h post infection by a pentobarbitone overdose. Immediately following confirmation of death thighs were removed and weighed. Thighs were placed individually in bead-beating tubes containing 3 mL of phosphate buffered saline (PBS) + 10% glycerol and subjected to mechanical disruption. Organ homogenates were diluted in PBS and cultured quantitatively on drug-free agar, resulting in two data points per animal. Colony forming units per gram of tissue (CFU/g) from each treatment group were converted to the $\log_{10}$ of the group geometric mean ($\log_{10}$ CFU/g). The terminal bioburden resulting from a specific regimen was expressed as the $\log_{10}$ CFU/g difference between pre-treatment and treatment groups ($\Delta\log_{10}$ CFU/g).

#Group	Treatment	Dose (mg/kg/dose)	Total dose (mg/kg/day)	Treatment time (post-infection)	End of study	No. Mice
1	Pre-treatment	-	-	-	1	5
2	Vehicle	-	-	1, 7, 13, 19	25	5
3	CN-DM-861	5	20	1, 7, 13, 19	25	5
4	CN-DM-861	10	40	1, 7, 13, 19	25	5
5	CN-DM-861	12.5	50	1, 7, 13, 19	25	5
6	Ciprofloxacin	10	20	1, 13	25	4

Table S 10. Study design of thigh infection model, i.v. administration.

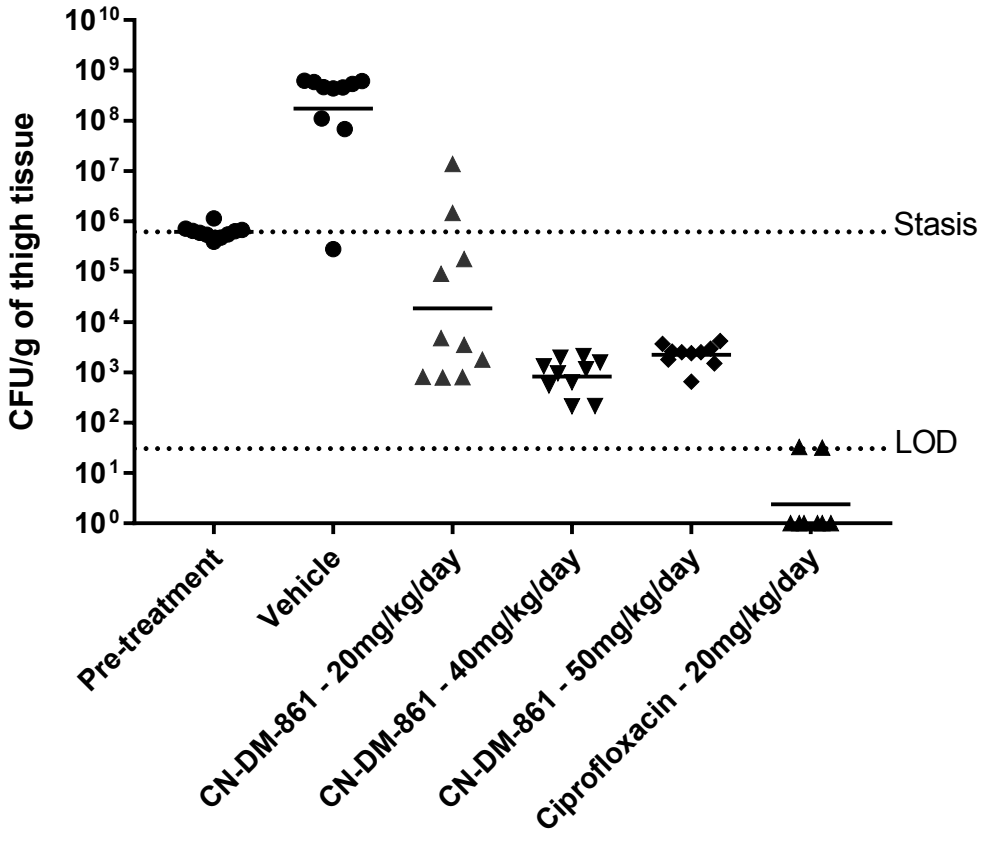


Figure S 12. Neutropenic murine thigh infection model of **22** (CN-DM-861). **22** and the positive control ciprofloxacin were administered as described under 1.1.10.3. and Table S 10. **22** reduced bacterial burden by 3.97, 5.32 and 4.89 $\log_{10}$ CFU/g compared to vehicle treated group at doses levels of 20, 40 and 50mg/kg/day (5, 10 and 12.5mg/kg/dose) respectively. This reduction corresponds to 1.52, 2.87 and 2.44 $\log_{10}$ CFU/g below the stasis.

Statistics

Kruskal-Wallis: all pairwise comparisons (Conover-Iman)		
Critical t (52 df) = 2.006647		
Pre-treatment and Vehicle		significant
(9.8 > 6.316097)		P = 0.003
Vehicle and CN-DM-861 - 20mg/kg/day		significant
(22.2 > 6.316097)		P < 0.0001
Vehicle and CN-DM-861 - 40mg/kg/day		significant
(35.7 > 6.316097)		P < 0.0001
Vehicle and CN-DM-861 - 50mg/kg/day		significant
(26.3 > 6.316097)		P < 0.0001
Vehicle and Ciprofloxacin - 20mg/kg/day		significant
(47.8 > 6.699232)		P < 0.0001
CN-DM-861 - 20mg/kg/day and Ciprofloxacin - 20mg/kg/day		significant
(25.6 > 6.699232)		P < 0.0001
CN-DM-861 - 40mg/kg/day and Ciprofloxacin - 20mg/kg/day		significant
(12.1 > 6.699232)		P = 0.0007
CN-DM-861 - 50mg/kg/day and Ciprofloxacin - 20mg/kg/day		significant
(21.5 > 6.699232)		P < 0.0001

Table S 11. Study results of thigh infection model, i.v. administration.

	Group Geometric mean (CFU/g)	**Log_{10} Geometric mean (CFU/g)**	**Log_{10} Standard Deviation (CFU/g)**	**Log_{10} variation from vehicle control (CFU/g)**	**Log_{10} variation from stasis (CFU/g)**
Pre-treatment	6.15×10^5	5.79	0.12	-2.45	0.00
Vehicle	1.73×10^8	8.24	0.98	0.00	2.45
CN-DM-861 20mg/kg/day	1.87×10^4	4.27	1.44	-3.97	-1.52
CN-DM-861 40mg/kg/day	8.27×10^2	2.92	0.34	-5.32	-2.87
CN-DM-861 50mg/kg/day	2.24×10^3	3.35	0.22	-4.89	-2.44
Ciprofloxacin 20mg/kg/day	2.39×10^0	0.38	0.65	-7.86	-5.41

1.1.10.4 In vivo tolerance study

A preliminary in vivo tolerance study was conducted with male CD-1 mice that were housed in cages of two. As vehicle, a formulation of HP-β-CD/Tris 1% at pH 9 (20%/80% w/v) was applied. Because the pH of the final formulations was >9.0, unsuitable for dosing IV, all dosing solutions were acidified to ~8.0 – 8.5 by addition of 1M HCl. The solutions were slightly diluted, resulting in the animals being treated with slightly lower doses (19.6, 9.8 and 4.9 mg/kg instead of 20, 10 and 5mg/kg). The study design is outlined in Table S 12.

Results: No adverse effect was observed at 5 and 10mg/kg doses. One animal in the group treated with **22** at 20mg/kg showed a moderate loss of activity, appeared ungroomed and pale between the 3rd and 4th dose. These signs remained the same until 24h. The necropsy of this animal showed blood in the caecum and colon as well as an abnormal aspect of the liver. As a result, **22** was administered at 5, 10 and 12.5 mg/kg in subsequent studies to avoid any toxicity issue.

Table S 12. Design of *in vivo* tolerance study.

Number of animals	Treatment	Dose (mg/kg/dose)	Frequency	Number of doses	Total dose (mg/kg/day)	Route	End of study
1	Vehicle	-	Q6h	4	-	IV	24h
2	22	5	Q6h	4	20	IV	24h
2	22	10	Q6h	4	40	IV	24h
2	22	20	Q6h	4	80	IV	24h

1.2 CHEMISTRY

1.2.1 **Materials and methods**

Commercially available reagents and solvents were used as supplied. All reactions were performed in oven-dried glassware under an atmosphere of nitrogen gas unless otherwise stated.

NMR spectra were recorded using a Bruker Advance-III HD 500 MHz or Bruker Advance-III HD 700 MHz spectrometer. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, septet (hept), m = multiplet, br = broad signal. Signals showing unexpected multiplicities are indicated by the prefix “pseudo”. Chemical shift values of ^{1}H and ^{13}C NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard.

High resolution mass spectra were recorded on a Bruker maXis HD spectrometer using positive or negative electrospray ionization (ESI).

LCMS measurements were performed using a Agilent technologies 1200 series (LC) coupled to Bruker amaZon SL (ion trap MS) using a Gemini-NX 3u C18 110A 50x2.0 mm column (for Marfey’s method), or on Agilent technologies 1260 Infinity II (LC) coupled to Agilent technologies 6130 a (quadrupol MS) using an Agilent poroshell 120 SB-C18 2.7 µm 2.1x30 mm column (for reaction monitoring).

Analytical thin-layer chromatography was performed using pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt), and the spots were visualized with UV light at 254 nm or alternatively by staining with potassium permanganate or cerium sulfate.

Chromatographic separations were performed by automated flash chromatography using Grace Reveleris® X2 flash chromatography system or via flash chromatography using silica gel 60M MACHEREY-NAGEL (0.040-0.063 mm; 230–400 mesh).

Preparative reversed phase high performance liquid chromatography was carried out with a Thermo Scientific Dionex (UltiMate 3000 HPLC system) with a Phenomenex 006-4252-P0 Luna C18 (250 mm × 21.2 mm, 5 µm) column.

For microwave assisted reaction, Biotage® Initiator+ was used.

Freezedrying was done using LYO Christ alpha 1-4 coupled to high vacuum oil pump. UV spectra were recorded using a microplate spectrophotometer PowerWave™ XS/XS2.

1.2.1.1 Marfey assay

The sample (5 µmol) is treated with HCl 6N at 110 °C for 6 hours. The resulting mixture/solution is dried via Freeze-drying, the residue thus obtained is treated with $NaHCO_3$ saturated solution (100 µL) and a solution 1% of Marfey reagent (FDAA) in acetone (200 µL). Reaction stirred at

40 °C for 1 h and quenched with HCl 1 N (100 µL). Sample analyzed by LCMS. (column, Gemini-NX 3u C18 110 A 50.0x2.0 mm)
Results are given in % of the two diasteroisomers formed upon derivatization. The method itself, presumably during the hydrolysis step, entails partial racemization, which was quantified in around 5%.

1.2.1.2 Preparative RP-HPLC purifications

Purifications via preparative RP-HPLC were carried out using two possible conditions:

- Condition A: 10-95% CH_3CN + 0.1% HCOOH in water + 0.1% HCOOH in 40 min.
  The sample was dissolved in DMSO and loaded on the HPLC system
- Condition B: gradient 10-70% CH_3CN in water 10 mM NH_4HCO_3 in 40 min.
  The sample was dissolved in THF (1 mL) and cooled to 0 °C. Then a few drops of DMSO and 1M aqueous NH_4HCO_3 solution (1 mL) were added. The solution pH was adjusted to 9 ca. by dropwise addition of 1M NaOH. The mixture was filtered through a syringe filter (CHROMAFIL® PET-45/15, 45 µm pore size, 15 mm diameter) and directly injected into the HPLC system.

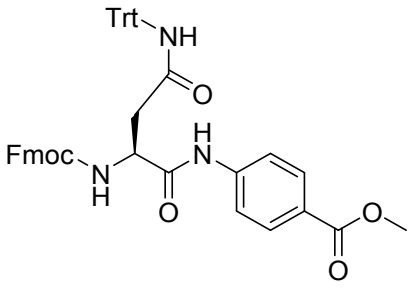
The collected fractions were lyophilized after their identity and purity was verified by LCMS.

1.2.2 **Experimental procedures**

A citation in the name of the molecule indicates that the compound has already been synthesized in the cited document or that the experimental procedure used for the reaction has been adapted from the cited document.

1.2.2.1 Synthesis of cystobactamid des-methoxy 861-2

Methyl (S)-4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoate (10)[9]



Chemical Formula: $C_{46}H_{39}N_3O_6$
Exact Mass: 729,28

$POCl_3$ (2.22 mL; 23.84 mmol) was added at 0 °C under N_2 atmosphere to a stirred solution of Fmoc-Asn(Trt)OH (14.22 g; 23.84 mmol), TEA (5.51 mL; 39.74 mmol) and methyl 4-aminobenzoate (3.00 g; 19.87 mmol) in DCM (330 mL). Reaction stirred at 0 °C for 2 hours, quenched with HCl 1N and ice. Organic phase washed with HCL 1 N (300 mL), brine (330 mL) and dried over sodium sulphate. The solvent was removed under reduced pressure, the residue thus obtained was chromatographed on silica gel with a gradient 0-10% EtOAc in DCM to give 13.97 g of a white solid(19.16 mmol; y= 96%).

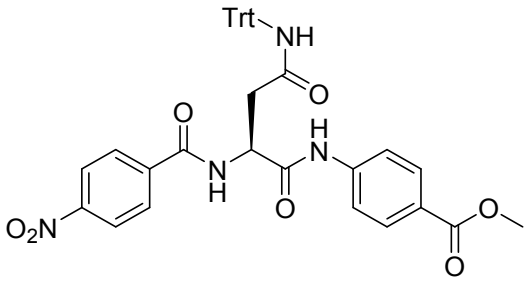
^{1}H NMR (700 MHz, DMSO) δ 10.44 (s, 1H), 8.62 (s, 1H), 7.91 (dd, *J* = 15.9, 8.2 Hz, 4H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.78 – 7.72 (m, 4H), 7.41 (q, *J* = 7.6 Hz, 2H), 7.30 (dt, *J* = 22.8, 7.3 Hz, 2H), 7.23 – 7.15 (m, 15H), 4.48 – 4.43 (m, 1H), 4.36 (dd, *J* = 10.6, 7.1 Hz, 1H), 4.30 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 2.75 (dd, *J* = 14.6, 9.8 Hz, 1H), 2.62 (dd, *J* = 14.6, 5.0 Hz, 1H).

^{13}C NMR (176 MHz, DMSO) δ 170.9, 168.4, 165.8, 155.8, 144.7, 143.8, 143.8, 143.4, 140.7, 130.2, 128.6, 127.6, 127.4, 127.1, 126.3, 125.3, 125.2, 124.0, 120.1, 118.7, 69.4, 65.8, 52.9, 51.9, 46.7, 38.3, 20.8.

HRMS (ESI) calculated for $C_{46}H_{40}N_3O_6$ ($M+H^+$) 730.2912, found 730.2925.

Marfey: 96.5% S enantiomer, 3.5% R enantiomer

Methyl (S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoate (94)



Chemical Formula: $C_{38}H_{32}N_4O_7$
Exact Mass: 656,23

Methyl (S)-4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoate (12.26 g; 16.82 mmol) was dissolved in a 20% solution of diethylamine in acetonitrile (195 mL), solution stirred for 30 min. The solvent was removed under vacuum, the residue was dissolved in CH_3CN and evaporated twice. The pale yellow gum and 4-Nitrobenzoic acid (3.09 g; 18.5 mmol) were suspended in CH_3CN (140 mL), HBTU (7.02 g; 18.5 mmol) followed by DiPEA (6.754 mL, 38.85 mmol) were added at 0 °C. The reaction mixture was stirred for 3 hours and quenched with $NaHCO_3$ saturated solution. The solvent was partially evaporated under reduced pressure, the residue was dissolved in EtOAc (300 mL). Organic phase washed with $NaHCO_3$ saturated solution (300 mL), HCl 1 N (300 mL), brine (300 mL) dried over sodium sulphate and evaporated under vacuum. The residue thus obtained was triturated with Pet. Et. and chromatographed on silica gel with a gradient 0-10% EtOAc in DCM to give 8.64 g of a yellow solid (13.17 mmol; y= 78%).

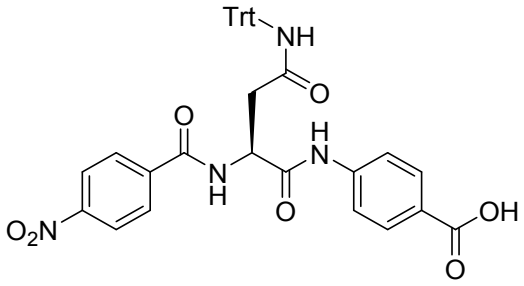
^{1}H NMR (500 MHz, DMSO) δ 10.57 (s, 1H), 9.20 (d, *J* = 7.6 Hz, 1H), 8.68 (s, 1H), 8.41 – 8.35 (m, 2H), 8.18 – 8.13 (m, 2H), 7.95 – 7.90 (m, 2H), 7.80 – 7.75 (m, 2H), 7.23 – 7.13 (m, 15H), 4.93 (m, 1H), 3.82 (s, 3H), 2.98 (dd, *J* = 14.9, 10.5 Hz, 1H), 2.75 (dd, *J* = 14.8, 4.6 Hz, 1H).

^{13}C NMR (126 MHz, DMSO) δ 170.5, 168.3, 165.8, 164.6, 149.2, 144.7, 143.3, 139.3, 130.2, 129.0, 128.5, 127.4, 126.4, 124.1, 123.6, 118.8, 69.4, 52.1, 51.9, 37.9.

HRMS (ESI) calculated for $C_{38}H_{33}N_4O_7$ ($M+H^+$) 657.2344, found 657.2348.

Marfey: 94.2% S enantiomer, 5.8% R enantiomer

(S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoic acid (6)[10]



Chemical Formula: $C_{37}H_{30}N_4O_7$
Exact Mass: 642,21

Methyl (S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoate (5.7 g; 8.69 mmol) and lithium iodide (9.32 g; 69.52 mmol) were mixed in EtOAc (80 mL) and heated to 90 °C for 5 days. After cooling, mixture diluted with EtOAc (200 mL) and HCl 1 N (200 mL), organic phase washed with water (2x 200 mL), brine (200 mL), dried over sodium sulphate and reduced under vacuum. The residue was chromatographed on silica gel with a gradient 0-20% MeOH in DCM to give 5.39 g of yellow solid (7.44 mmol; y= 86%).

^{1}H NMR (700 MHz, DMSO) δ 12.72 (s, 1H), 10.52 (s, 1H), 9.19 (d, *J* = 7.6 Hz, 1H), 8.67 (s, 1H), 8.40 – 8.36 (m, 2H), 8.17 – 8.13 (m, 2H), 7.91 – 7.88 (m, 2H), 7.76 – 7.72 (m, 2H), 7.22 – 7.14 (m, 15H), 4.93 (m, 1H), 2.98 (dd, *J* = 14.9, 10.6 Hz, 1H), 2.75 (dd, *J* = 14.9, 4.5 Hz, 1H).

^{13}C NMR (176 MHz, DMSO) δ 170.4, 168.3, 166.9, 164.6, 149.2, 144.7, 142.9, 139.3, 130.3, 129.0, 128.5, 127.4, 126.4, 123.6, 118.6, 69.4, 66.3, 52.0, 38.0.

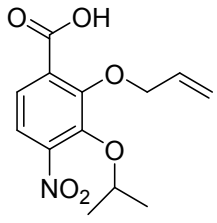
^{13}C NMR (176 MHz, DMSO) δ 170.4, 168.3, 166.9, 164.6, 149.2, 144.7, 142.9, 139.3, 130.3, 129.0, 128.5, 127.4, 126.4, 123.6, 118.6, 69.4, 52.0, 38.0.

HRMS (ESI) calculated for $C_{37}H_{29}N_4O_7$ ($M-H^+$) 641.2041, found 641.2045.

Marfey: 94.0 % S enantiomer, 6.0% R enantiomer

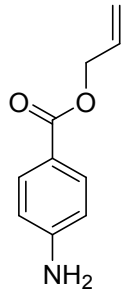
2-(Allyloxy)-3-isopropoxy-4-nitrobenzoic acid (95)

Bulding block PABA-D was synhesized according to our previously described experimental method.[3]



Chemical Formula: $C_{13}H_{15}NO_6$
Exact Mass: 281,0899

Allyl 4-aminobenzoate (96)



Chemical Formula: $C_{10}H_{11}NO_2$
Exact Mass: 177,0790

K_2CO_3 (2.5 g, 17.95 mmol) was added to a stirred mixture of 4-nitrobenzoic acid (5.0 g; 30.0 mmol) and Allyl bromide (2.9 mL; 33.0 mmol) in DMF (50 mL). Reaction was stirred for 24 hours. Et_2O (500 mL) and water (500 mL) were added, the organic phase washed then with $NaHCO_3$ satured solution twice (400 mL) and once with brine (400 mL), dried over sodium sulphate, evaporated under reduced pressure to obtain 6.1 g (30.0 mmol; y= q.) of a yellow oil. Product was used in the next step without further purification.

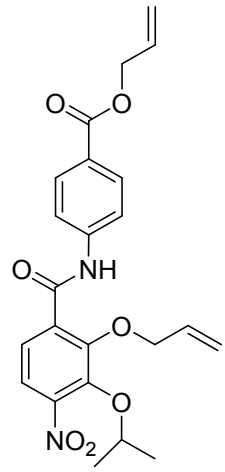
Zinc dust (27 g; 541 mmol) was added over 30 min to a solution of allyl 4-nitrobenzoate(5.6 g; 27 mmol) in acetic acid (100 mL). Reaction stirred overnight at r.t.. It was quenched with $NaHCO_3$, watery phase extracted twice with EtOAc, washed again with $NaHCO_3$ and brine, dried over sodium sulphate and reduced under vacuum to give around 4.5 g of a crude residue, which was chromatographed on silica gel with a gradient 5-30% EtOAc in Pet. Et. to afford 3.42 g (19 mmol; y= 71%) of a white solid.

^{1}H NMR (500 MHz, DMSO) δ 7.74 – 7.55 (m, 2H), 6.62 – 6.51 (m, 2H), 6.00 (m, 3H), 5.34 (pseudo dq, *J* = 17.2, 1.7 Hz, 1H), 5.22 (pseudo dq, *J* = 10.5, 1.4 Hz, 1H), 4.68 (dt, *J* = 5.3, 1.5 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 165.5, 153.6, 133.3, 131.1, 117.2, 115.6, 112.6, 64.0.

HRMS (ESI) calculated for $C_{10}H_{12}NO_2$ ($M+H^+$) 178.0863, found 178.0867.

Allyl 4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (97)



Chemical Formula: $C_{23}H_{24}N_2O_7$
Exact Mass: 440,1584

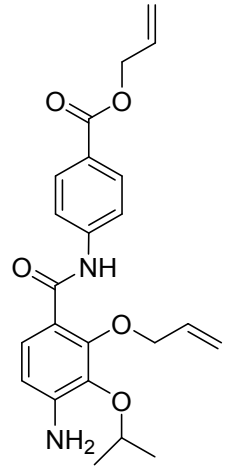
$POCl_3$ (0.68 mL; 7.32 mmol) was added at 0 °C to a stirred solution of 2-(allyloxy)-3-isopropoxy-4-nitrobenzoic acid (7.32 mmol, crude), TEA (1.7 mL; 12.20 mmol) and allyl 4-aminobenzoate (1.08 g; 6.10 mmol) in DCM (100 mL) under nitrogen. Reaction stirred for 3 h, then quenched with $NaHCO_3$ saturated solution, solvent partially reduced under vacuum, then diluted with EtOAc (150 mL) and water (150 mL), aqueous phase extracted again twice with EA (2x 100 mL), organic phases reun ited washed with HCl 1 N (300 mL) and brine (300 mL), dried over sodium sulphate and reduced under vacuum to give a crude material, which was chromatographed on silica gel with a gradient 5-30% to give 2.22 g (5.04 mmol; y= 83%) of a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.17 (s, 1H), 8.08 (d, *J* = 8.7 Hz, 3H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 1H), 6.24 – 5.87 (m, 2H), 5.55 – 5.22 (m, 4H), 4.80 (dd, *J* = 20.6, 5.9 Hz, 4H), 4.64 (hept, *J* = 12.4, 6.3 Hz, 1H), 1.34 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, $CDCl_3$) δ 165.7, 161.1, 151.5, 148.2, 144.7, 141.9, 132.3, 131.5, 131.0, 130.5, 126.2, 126.1, 121.1, 120.0, 119.4, 118.3, 78.7, 75.7, 65.5, 22.4.

HRMS (ESI) calculated for $C_{23}H_{25}N_2O_7$ ($M+H^+$) 441.1656, found 441.1654

Allyl 4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)benzoate (7)



Chemical Formula: $C_{23}H_{26}N_2O_5$
Exact Mass: 410,1842

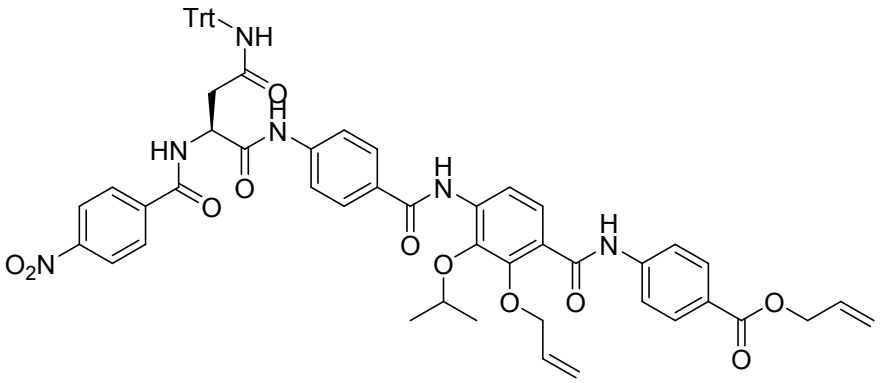
Tin(II) chloride dehydrate (4.3 g; 19.09 mmol), allyl 4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (1.4 g; 3.18 mmol) were dissolved in EtOH (32 mL), the solution was stirred at r.t. overnight. The solvent was removed under vacuum, $NaHCO_3$ saturated solution (300 mL) and EtOAc (300 mL) were added to the residue. The aqueous phase extracted again with EtOAc (200 mL). The organic phases reunited were washed with brine (400 mL), dried over sodium sulphate and reduced under vacuum. The crude residue thus obtained was chromatographed on silica gel with a gradient EtOAc 10-40% in Pet. Et. to give 1.22 g (2.98 mmol; 95%)of a yellow oil.

^{1}H NMR (500 MHz, DMSO) δ 10.26 (s, 1H), 8.01 – 7.86 (m, 2H), 7.86 – 7.74 (m, 2H), 7.39 (d, *J* = 8.6 Hz, 1H), 6.56 (d, *J* = 8.6 Hz, 1H), 6.26 – 5.93 (m, 2H), 5.59 (s, 2H), 5.48- 5.35 (m, 2H), 5.30- 5.20 (m, 2H) 4.78 (dt, *J* = 5.4, 1.5 Hz, 2H), 4.61 (dt, *J* = 5.6, 1.4 Hz, 2H), 4.46 (hept, *J* = 6.1 Hz, 1H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 165.0, 163.9, 150.6, 147.8, 143.7, 135.3, 133.6, 132.8, 130.3, 126.1, 123.6, 118.8, 118.1, 117.7, 114.6, 109.9, 74.4, 73.7, 64.8, 40.1, 40.0, 39.9, 39.8, 39.8, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 22.2.

HRMS (ESI) calculated for $C_{23}H_{27}N_2O_5$ ($M+H^+$) 411.1914, found 411.1900

Allyl (S)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)benzoate (11)



Chemical Formula: $C_{60}H_{54}N_6O_{11}$
Exact Mass: 1034,3851

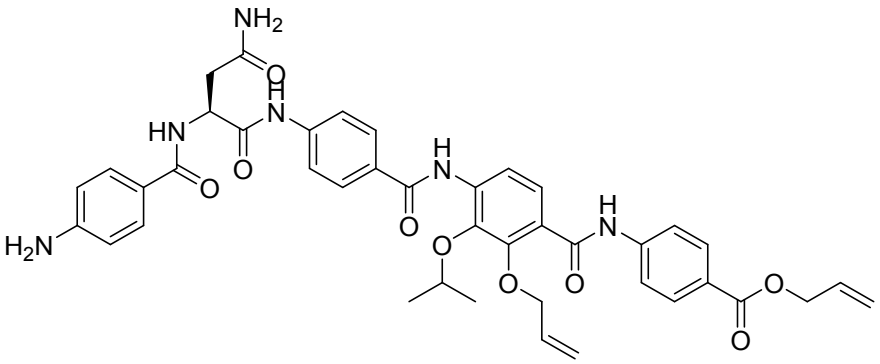
$POCl_3$ (3.20 mmol) as a solution in DCM (1:9) was added dropwise to a solution of allyl 4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)benzoate (0.53 g; 1.28 mmol) and (S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoic acid (2.05 g, 3.20 mmol) in THF (13 mL) at 0 °C, followed by DiPEA (1.78 mL; 10.24 mmol) as a solution in DCM (1:1). Reaction stirred at r.t. for 6 h, quenched with HCl 1 N and ice, solvent partially reduced under vacuum and residue diluted with EtOAc (250 mL) and HCl 1N (250 mL), organic phase washed with brine (250 mL) and dried over sodium sulphate. Solvent removed under vacuum, the crude residue was chromatographed on silica gel with a gradient EtOAc 20-75% in Pet. Et to give 940 mg of a orange residue (0.95 mmol; 54%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.20 (s, 1H), 9.63 (s, 1H), 8.74 (s, 1H), 8.50 (d, *J* = 8.9 Hz, 1H), 8.47 (d, *J* = 6.2 Hz, 1H), 8.29 (d, *J* = 8.8 Hz, 2H), 8.07 (dd, *J* = 8.8, 4.9 Hz, 3H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 8.7 Hz, 2H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.33 – 7.24 (m, 15H), 7.15 (s, 1H), 6.15 (m, 1H), 6.05 (m, 1H), 5.50 (dd, *J* = 17.1, 1.2 Hz, 1H), 5.43 (m, 2H), 5.30 (dd, *J* = 10.4, 1.1 Hz, 1H), 5.07 (m, 1H), 4.82 (d, *J* = 5.6 Hz, 2H), 4.76 (dt, *J* = 12.3, 6.2 Hz, 1H), 4.71 (d, *J* = 5.9 Hz, 2H), 3.31 (dd, *J* = 15.6, 2.7 Hz, 1H), 2.76 (dd, *J* = 15.6, 7.3 Hz, 1H), 1.39 (dd, *J* = 6.1, 3.9 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 168.3, 166.9, 165.0, 164.6, 164.3, 149.5, 149.2, 144.7, 143.5, 142.6, 142.2, 139.3, 135.6, 133.7, 132.8, 130.6, 130.3, 129.0, 128.5, 128.4, 128.0, 127.4, 127.2, 126.4, 124.2, 123.6, 119.0, 118.9, 118.7, 117.8, 117.8, 76.3, 74.3, 69.4, 64.8, 52.1, 38.0, 22.3.

HRMS (ESI) calculated for $C_{60}H_{55}N_6O_{11}$ ($M+H^+$) 1035.3923, found 1035.3943.

Allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (12)



Chemical Formula: $C_{41}H_{42}N_6O_9$
Exact Mass: 762,3013

Zn dust (0.95 g; 15.5 mmol) was added portionwise over few minutes to a stirred solution of allyl (S)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)-butanamido)benzamido)benzamido)benzoate (1.30 g; 1.26 mmol) in THF (6.0 mL), EtOH (4.8 mL) and AcOH (1.2 mL). Reaction stirred for 5 h, the mixture was filtered through celite, the solvent was reduced under vacuum. The crude was used in the next step without further purification.

The residue was dissolved in DCM (18.7 mL), Tips (0.775 mL; 3.78 mmol) followed by TFA (6.3 mL) were added at 0 °C. Reaction stirred 2 h at r.t. then solved removed under vacuum, residue take up and evaporated twice with DCM (15 mL) then triturated 3x with ice cold Pet. Et.. The crude thus obtained was purified on silica gel with a gradient 0-10% MeOH in DCM to give 1.02 g of a yellow solid (1.18 mmol; y= 94%).

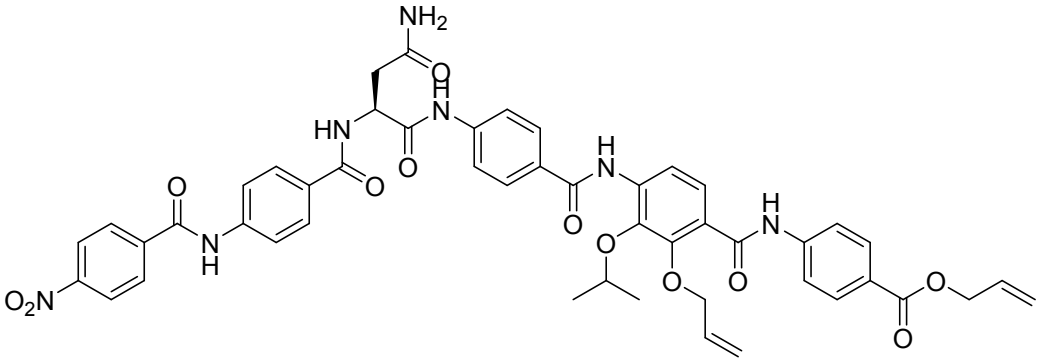
^{1}H NMR (500 MHz, DMSO) δ 10.59 (s, 1H), 10.38 (s, 1H), 9.52 (s, 1H), 8.23 (d, *J* = 7.3 Hz, 1H), 7.98 (t, *J* = 9.4 Hz, 4H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.80 (dd, *J* = 11.0, 8.7 Hz, 3H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 6.97 (s, 1H), 6.56 (d, *J* = 8.7 Hz, 2H), 6.11 – 5.97 (m, 2H), 5.68 (s, 2H), 5.44 – 5.35 (m, 2H), 5.31 - 5-18 (m, 2H), 4.85 (dd, *J* = 14.1, 7.1 Hz, 1H), 4.80 (d, *J* = 5.3 Hz, 2H), 4.61 (d, *J* = 5.5 Hz, 2H), 4.53 – 4.44 (m, 1H), 2.65 (d, *J* = 7.0 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 171.5, 171.1, 166.4, 165.0, 164.7, 164.3, 151.9, 149.5, 143.5, 142.6, 142.5, 135.7, 133.7, 132.8, 130.4, 129.1, 128.4, 128.2, 127.1, 124.2, 123.6, 120.4, 119.0, 118.8, 117.8, 117.8, 116.0, 112.5, 76.3, 74.3, 64.9, 51.5, 40.1, 40.0, 39.9, 39.8, 39.8, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 36.9, 22.3, 20.8.

HRMS (ESI) calculated for $C_{41}H_{43}N_6O_9$ ($M+H^+$) 763.3086, found 763.3085.

Marfey: 94.0% *S* enantiomer, 6.0% *R* enantiomer

Allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (13)[11]



Chemical Formula: $C_{48}H_{45}N_7O_{12}$
Exact Mass: 911,3126

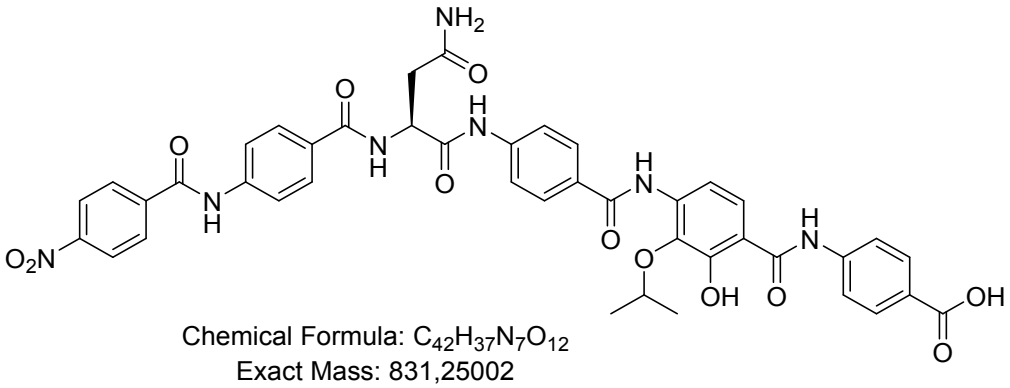
Collidine (0.166 mL; 1.26 mmol) was added dropwise at 0 °C to a solution of 4-nitro benzoic acid (92 mg; 0.551 mmol) and bis(trichloromethyl) carbonate (49 mg; 0.165 mmol) in THF (10 mL). Reaction stirred at r.t. for 20 min then added to a solution of allyl (S)-2-(allyloxy)-4-(2-(allyloxy)-4-(4-(2-(4-aminobenzamido)-3-cyanopropanamido)benzamido)-3-methoxybenzamido)-3-methoxybenzoate (120 mg; 0.157 mmol) and DiPEA (0.273 mL; 1.57 mmol) in THF (10 mL). Reaction stirred for 4 h then quenched with HCl 1 N and ice. Solvent partially reduced under vacuum, EtOAc (40 mL) and HCl 1N (40
mL) were added, organic phase washed with $NaHCO_3$ saturated solution (30 mL), brine (30 mL) and dried over sodium sulphate. The solvent was removed under reduced pressure, the residue was purified on silica gel with a gradient 0-10% MeOH in DCM to give 114 mg of a yellow residue (0.125 mmol; y= 80%).

^{1}H NMR (700 MHz, DMSO) δ 10.79 (s, 1H), 10.58 (s, 1H), 10.45 (s, 1H), 9.52 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.41 – 8.36 (m, 2H), 8.23 – 8.19 (m, 2H), 8.00 – 7.95 (m, 4H), 7.94 (m, 2H), 7.91 (m, 2H), 7.87 (d, *J* = 8.6 Hz, 2H), 7.81 (m, 3H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.99 (s, 1H), 6.04 (m, 2H), 5.39 (m, 2H), 5.28 (ddd, *J* = 10.5, 2.9, 1.4 Hz, 1H), 5.20 (ddd, *J* = 10.5, 2.9, 1.3 Hz, 1H), 4.92 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.79 (d, *J* = 5.3 Hz, 2H), 4.61 (d, *J* = 5.5 Hz, 2H), 4.49 (tt, *J* = 12.2, 6.1 Hz, 1H), 2.69 (d, *J* = 7.9 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 165.8, 164.9, 164.7, 164.3, 164.2, 149.5, 149.3, 143.5, 142.6, 142.4, 141.5, 140.3, 135.7, 133.7, 132.8, 130.3, 129.3, 129.2, 128.4, 128.3, 128.3, 127.1, 124.2, 123.6, 119.5, 119.0, 119.0, 118.8, 117.8, 117.8, 76.2, 74.3, 64.8, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for $C_{48}H_{46}N_7O_{12}$ ($M+H^+$) 912.3199, found 912.3196.

(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (4)



Chemical Formula: $C_{42}H_{37}N_7O_{12}$
Exact Mass: 831,25002

Phenyl silane (0.012 mL; 0.099 mmol) followed by palladium-tetrakis(triphenylphosphine (6.3 mg; 0.0055 mmol) was added to a solution of allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (20 mg; 0.022 mmol) in THF (2.75 mL). Reaction stirred overnight and purified by preparative RP-HPLC to afford to obtain 3.8 mg of a white material (0.0046 mmol; y= 21%).

According to the purification method used, the desired structure could be obtained either in its protonated form or as ammonium salt.

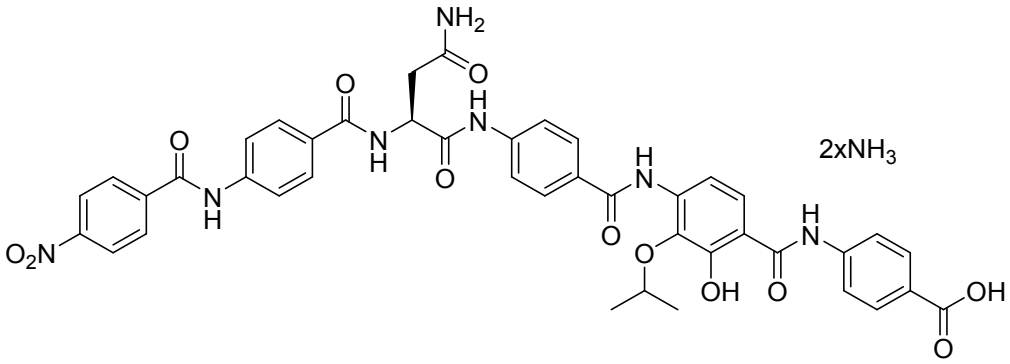
Condition A:

protonated form

^{1}H NMR (700 MHz, DMSO) δ 12.82 (s, 1H), 12.29 (s, 1H), 10.79 (s, 1H), 10.60 (s, 1H), 10.46 (s, 1H), 9.40 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.41 – 8.35 (m, 2H), 8.24 – 8.18 (m, 2H), 7.99 – 7.92 (m, 6H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.85 (m, 3H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.57 – 4.51 (m, 1H), 2.69 (d, *J* = 7.6 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

Condition B:

ammonium salts (retention time: 20.9 min)



Chemical Formula: $C_{42}H_{37}N_7O_{12}$
Exact Mass: 831,25002

^{1}H NMR (700 MHz, DMSO) δ 15.41 (br, 1H), 10.80 (s, 1H), 10.46 (s, 1H), 8.87 (s, 1H), 8.72 (d, *J* = 7.3 Hz, 1H), 8.41 – 8.35 (m, 2H), 8.23 – 8.18 (m, 2H), 7.96 – 7.92 (m, 2H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.84 (d, *J* = 8.9 Hz, 2H), 7.81 (d, *J* = 8.9 Hz, 2H), 7.76 (d, *J* = 7.8 Hz, 2H), 7.59 (br, 2H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.42 (s, 1H), 7.09 (d, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 5.06 – 4.99 (m, 1H), 4.93 (q, *J* = 7.1 Hz, 1H), 2.69 (d, *J* = 7.4 Hz, 2H), 1.19 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.6, 166.9, 165.7, 165.3, 164.2, 163.1, 149.3, 142.0, 141.5, 140.3, 137.5, 129.5, 129.3, 129.3, 128.3, 127.6, 124.2, 123.7, 123.6, 119.5, 119.0, 117.7, 70.3, 51.6, 36.8, 22.7.

HRMS (ESI) calculated for $C_{42}H_{38}N_7O_{12}$ ($M+H^+$) 832.2573, found 832.2580.

Marfey: 91.2% *S* enantiomer, 8.8% *R* enantiomer

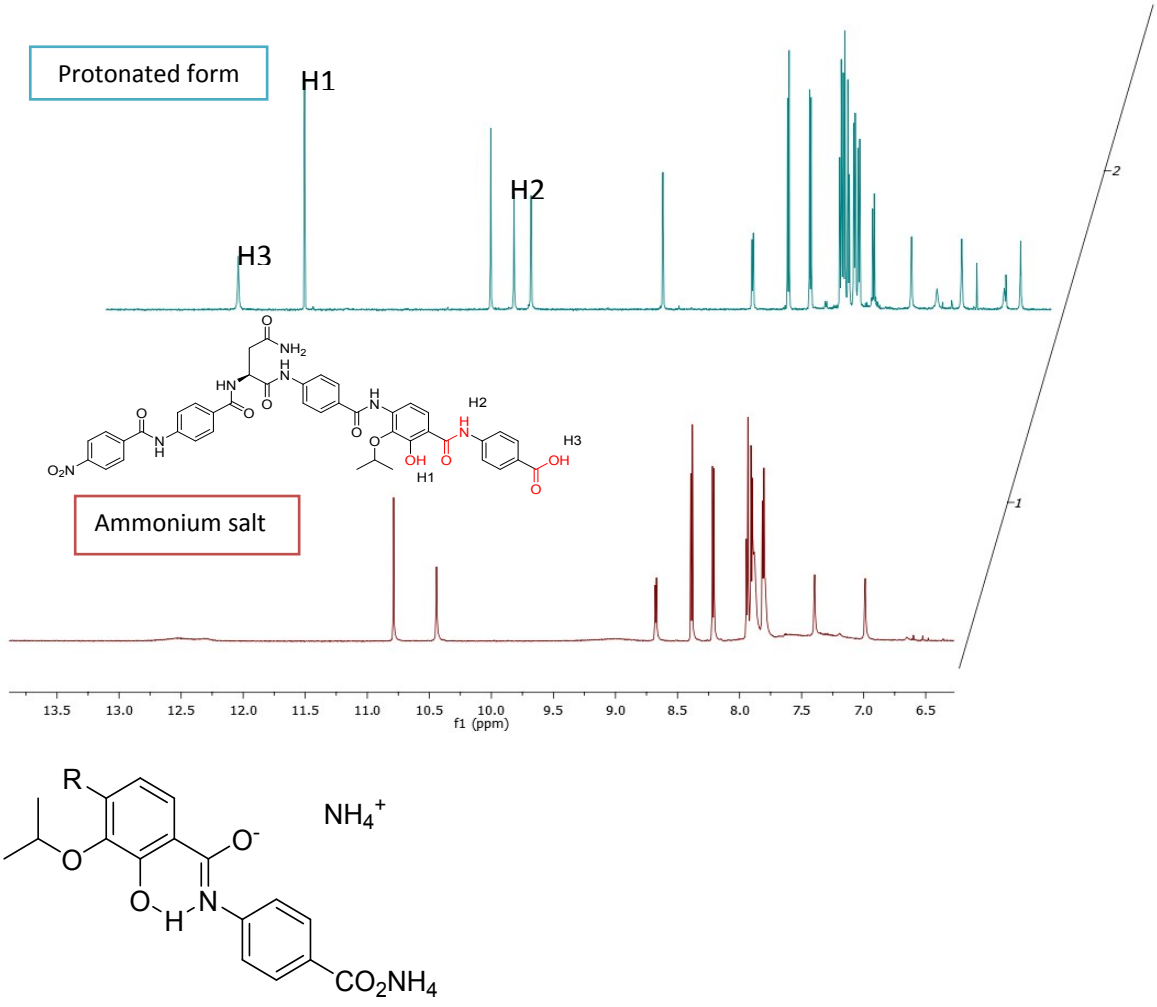
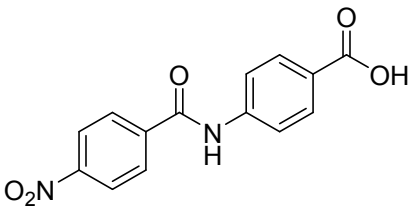


Figure S 103. NMR comparison of **4** in protonated and salt forms. The spectra demonstrate the acidity of the amide conneting rings D and E. The structure depicts the putative intramolecular hydrogen bond that is supposed to account for the unusual acidity of the amide.

1.2.2.2 N-terminal optimization

1.2.2.2.1 Synthesis of variants of building block A

4-(4-Nitrobenzamido)benzoic acid (98)



Chemical Formula: $C_{14}H_{10}N_2O_5$
Exact Mass: 286,0590

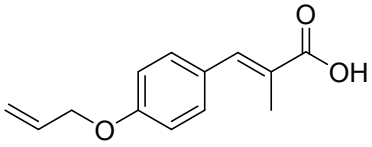
4-Aminobenzoic acid (800 mg; 5.84 mmol) was dissolved in THF (4.0 mL) and $NaHCO_3$ saturated solution (4.0 mL), to it 4-nitrobenzoyl chloride (1.08 g; 5.84 mmol) was added at 0 °C. Reaction stirred for 2 hours, precipitate collected by filtration, washed with water and THF, dried under high vacuum to give 1.30 g of a solid (4.55 mmol; y= 77%).

^{1}H NMR (500 MHz, DMSO) δ 12.80 (br, 1H), 10.83 (s, 1H), 8.43 – 8.35 (m, 2H), 8.25 – 8.14 (m, 2H), 7.99 – 7.94 (m, 2H), 7.94 – 7.90 (m, 2H).

^{13}C NMR (126 MHz, DMSO) δ 166.9, 164.3, 149.3, 142.8, 140.3, 130.3, 129.4, 126.0, 123.6, 119.7.

HRMS (ESI) calculated for $C_{14}H_9N_2O_5$ ($M\text{-}H^+$) 285.0517, found 285.0536.

(E)-3-(4-(Allyloxy)phenyl)-2-methylacrylic acid (99)[11]



Chemical Formula: $C_{13}H_{14}O_3$
Exact Mass: 218,0943

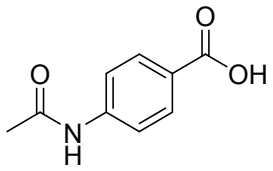
Synthesized according to exp. procedure reported.

^{1}H NMR (700 MHz, DMSO) δ 12.35 (br, 1H), 7.54 (d, *J* = 1.1 Hz, 1H), 7.46 – 7.42 (m, 2H), 7.03 – 6.99 (m, 2H), 6.05 (ddt, *J* = 17.3, 10.5, 5.2 Hz, 1H), 5.40 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.27 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.61 (dt, *J* = 5.3, 1.5 Hz, 2H), 2.03 (d, *J* = 1.5 Hz, 3H).

^{13}C NMR (176 MHz, DMSO) δ 169.6, 158.3, 137.4, 133.5, 131.4, 128.1, 126.2, 117.6, 114.7, 68.2, 13.9.

HRMS (ESI) calculated for $C_{13}H_{13}O_3$ ($M\text{-}H^+$) 217.0870, found 217.0873.

4-Acetamidobenzoic acid (100)[12]



Chemical Formula: $C_9H_9NO_3$
Exact Mass: 179,0582

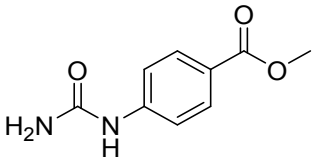
Synthesized according to exp. procedure reported.

^{1}H NMR (500 MHz, DMSO) δ 12.67 (br, 1H), 10.23 (s, 1H), 7.90 – 7.84 (m, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 2.08 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 168.8, 166.9, 143.3, 130.4, 124.9, 118.1, 24.1.

HRMS (ESI) calculated for $C_9H_8NO_3$ ($M-H^+$) 178.0510, found 178.0510.

Methyl 4-ureidobenzoate (101)



Chemical Formula: $C_9H_{10}N_2O_3$
Exact Mass: 194,0691

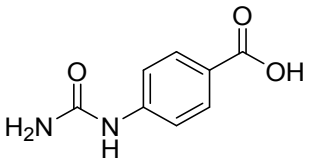
A solution of methyl 4-aminobenzoate (500 mg; 3.31 mmol) and DiPEA (0.86 mL; 4.97 mmol) in DCM (10 mL) was added dropwise at 0 °C to a solution of BTC (328 mg; 1.10 mmol) in DCM (20 mL). Reaction stirred at 0 °C for 2 h. Solvent reduced under vacuum, residue dissolved in 5 mL of DCM and added to a cooled solution of NH_4OH conc.. The mixture was stirred for 2 hours, the solid was collected by filtration, washed with water and twice with Et_2O, dried under high vacuum to give 410 mg of a white powder (2.11 mmol; y= 64%).

^{1}H NMR (500 MHz, DMSO) δ 8.98 (s, 1H), 7.87 – 7.79 (m, 2H), 7.57 – 7.48 (m, 2H), 6.05 (s, 2H), 3.79 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 166.0, 155.6, 145.3, 130.3, 121.6, 116.8, 51.7.

HRMS (ESI) calculated for $C_9H_{11}N_2O_3$ ($M+H^+$) 195.0764, found 195.0766.

4-Ureidobenzoic acid (102)



Chemical Formula: $C_8H_8N_2O_3$
Exact Mass: 180,0535

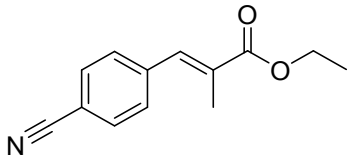
Methyl 4-ureidobenzoate (150 mg; 0.77 mmol) was suspended in THF (4 mL) and water (2 mL). A solution of LiOH (185 mg; 7.7 mmol) in water (2 mL) was added to the suspension at 0 °C. Reaction stirred overnight, pH adjusted to 1, the precipitate was collected by filtration washed with water and three times with Et_2O, dried at high vacuum to give 80 mg of a white powder (0.44 mmol; y= 58%).

^{1}H NMR (500 MHz, DMSO) δ 12.50 (br, 1H), 8.90 (s, 1H), 7.84 – 7.77 (m, 2H), 7.54 – 7.45 (m, 2H), 6.02 (s, 1H).

^{13}C NMR (126 MHz, DMSO) δ 167.1, 155.6, 144.9, 130.4, 122.9, 116.7.

HRMS (ESI) calculated for $C_8H_7N_2O_3$ ($M\text{-}H^+$) 179.0462, found 179.0470.

Ethyl (E)-3-(4-cyanophenyl)-2-methylacrylate (103)



Chemical Formula: $C_{13}H_{13}NO_2$
Exact Mass: 215,0946

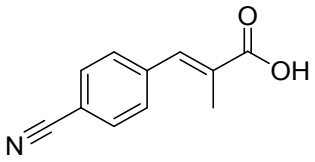
4-Hydroxybenzaldehyd (500 mg; 3.82 mmol) was dissolved in DCM (3.0 mL) at rt, ethyl-2-(triphenylphosphoranylidene)propionate (1.06 g; 2.94 mmol) was added. The mixture was stirred overnight at 37°C then concentrated under reduced pressure. The crude was purified on silica gel with a gradient 1-50% EtOAc in Pet. Et. to give 570 mg of white solid (2.65 mmol; y= 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 – 7.66 (m, 2H), 7.64 (s, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.10 (d, *J* = 1.5 Hz, 3H), 1.35 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9, 140.6, 136.4, 132.1, 131.8, 130.0, 118.6, 111.7, 61.3, 14.3, 14.2.

HRMS (ESI) calculated for $C_{13}H_{14}NO_2$ ($M\text{+}H^+$) 216.1019, found 216.1014.

(E)-3-(4-Cyanophenyl)-2-methylacrylic acid (104)



Chemical Formula: $C_{11}H_9NO_2$
Exact Mass: 187,0633

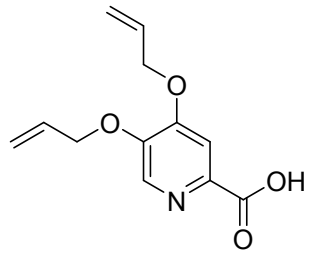
Ethyl (E)-3-(4-cyanophenyl)-2-methylacrylate (150 mg; 0.67 mmol) was dissolved in THF (3.35 mL) and water (1.68 mL), solution cooled to 0 °C and a mixture of LiOH (167 mg; 6.98 mmol) in water (1.68 mL) added to it. Reaction stirred at 0 C for 10 min. then to r.t. overnight. Reaction quenched adjusting pH to 1, then diluted with EtOAc (20 mL) and HCl 1 N (20 mL), organic solvent washed with brine and dried over sodium sulphate. Solvent reduce under vacuum to afford 125 mg of a white solid (0.67 mmol; y= q.).

^{1}H NMR (500 MHz, DMSO) δ 7.91 – 7.87 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.61 (s, 1H), 2.02 (d, *J* = 1.5 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 168.9, 140.4, 135.8, 132.3, 130.3, 127.6, 118.7, 110.6, 14.0.

HRMS (ESI) calculated for $C_{11}H_{10}NO_2$ ($M+H^+$) 188.0706, found 188.0709.

4,5-Bis(allyloxy)picolinic acid (105)[13]



Chemical Formula: $C_{12}H_{13}NO_4$
Exact Mass: 235,0845

Synthesized according experimental procedures reported using allyl bromide instead of benzyl bromide.

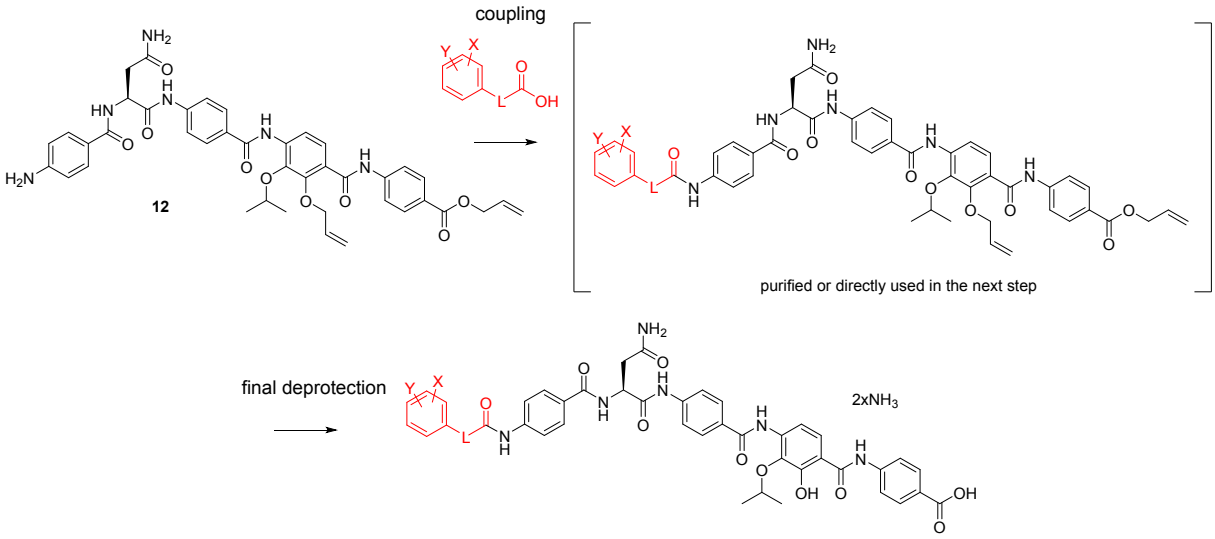
^{1}H NMR (500 MHz, MeOD) δ 8.16 (s, 1H), 7.82 (s, 1H), 6.11 (m, 2H), 5.54 – 5.44 (m, 2H), 5.36 (m, 2H), 4.86 (dt, *J* = 5.3, 1.5 Hz, 2H), 4.77 (dt, *J* = 5.3, 1.5 Hz, 2H).

^{13}C NMR (126 MHz, MeOD) δ 165.4, 159.9, 149.2, 144.4, 133.6, 132.9, 131.0, 119.5, 119.2, 110.5, 72.0, 71.5.

HRMS (ESI) calculated for $C_{12}H_{14}NO_4$ ($M+H^+$) 236.0917, found 236.0922.

1.2.2.2.2 General scheme

Synthetic scheme used for the synthesis of analogs bearing modifications of ring A. In all cases, amine **12** was coupled to a carboxylic acid and then deprotection of allyl groups followed.



Scheme S 1. General synthetic scheme adopted for the synthesis of ring A analogs.

1.2.2.2.3 General procedures

Coupling:

The last aromatic ring was installed activating the carboxylic acid to acyl chloride or activated ester by means of:

- A. BTC collidine
- B. oxalyl chloride
- C. EDC, HOAt

A. Collidine (8 eq.) was added dropwise at 0 °C to a solution of desired carboxylic acid (3.5 eq.) and bis(trichloromethyl) carbonate (1.05 eq.) in THF (M= 0.02). Reaction stirred at r.t. for 20 min then added to a solution of amine **12** (1 eq.) and DiPEA (10 eq.) in THF (M= 0.02). Reaction stirred for few hours then quenched with HCl 1 N and ice. Solvent partially reduced under vacuum, EtOAc and HCl 1N were added, organic phase washed with $NaHCO_3$ saturated solution, brine and dried over sodium sulphate. The solvent was removed under reduced pressure, the residue was purified on silica gel or directly used in the next step without further purification.

B. The desired carboxylic acid (1.0 eq.) is suspended in DCM (M= 0.5), oxalyl chloride (1.5 eq.) followed by catalytic DMF were added at 0 °C. Reaction stirred until all the carboxylic acid

has reacted, reaction monitored by TLC. Solvent reduced under vacuum, residue dissolved again in DCM and evaporated twice.
The desired acyl chloride (1.5 eq.) as a solution in THF (M= 0.08) was added at 0 °C to a stirred solution of amine **12** (1.0 eq.) and DiPEA (5 eq.) in THF (M= 0.08), reaction stirred at 0 °C for 10 min then warmed to r.t., stirring prolonged until completion of the reaction (monitored by TLC and/or LCMS). Reaction quenched with HCl 1N/ice, solvent partially reduced under vacuum, mixture diluted with EtOAc and HCl 1 N, organic phase washed with brine and dried over sodium sulphate. The residue thus obtained could be purified on silica gel or directly used in the next step without further purification.

C. Carboxylic acid (3.0 eq.), EDC (3.0 eq), HOAt (3.5 eq.) were mixed together in DMF (M= 0.2), to this solution collidine was added (8.0 eq.), reaction stirred for 20 min then added to a solution of amine **12** (1.0 eq.) in DMF (M= 0.2). Reaction heated to 40 °C for several hours until reaction completed. Reaction diluted with EtOAc and HCl 1 N, organic phase washed with brine and dried over sodium sulphate, solvent reduced under vacuum, the residue was used in the next step without further purification.

Final deprotection

Phenyl silane (2 eq. x n of allyl group) followed by tetrakis(triphenylphosphine)palladium^0 (0.25 eq.) were added to a solution of allyl protected cystobactamid derivative (1.0 eq.) in THF (M= 0.01). Reaction stirred overnight and purified by preparative RP-HPLC using condition A or B (described in section 1.2.1.2).

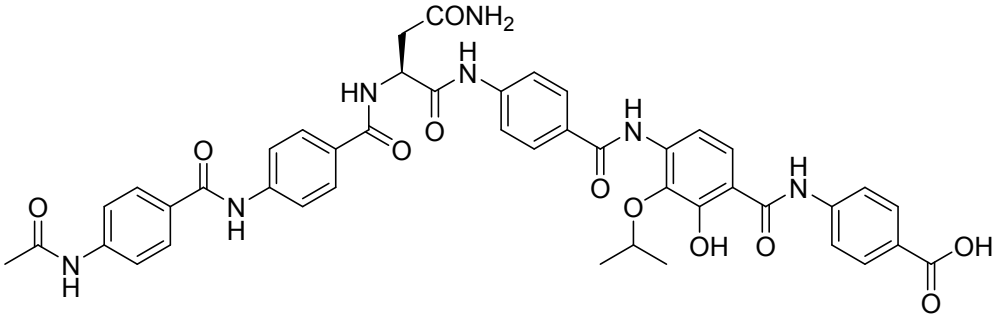
1.2.2.2.4 Analogs synthesized with this general method

For coupling step, final deprotection and purification, the general proceduers described above were used.

The carbocylic acids employed in the coupling step with amine **12** were purchased or synthesized according to procedures described herein.

All compounds purified according to condition B were obtained as ammonium salts. The number of counteranions varies and depends on the functional groups present on the molecule. The given numbers with the structures are estimations according to H-NMR data.

(S)-4-(4-(4-(2-(4-(4-Acetamidobenzamido)benzamido)-4-amino-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (14)



Chemical Formula: $C_{44}H_{41}N_7O_{11}$
Exact Mass: 843,2864

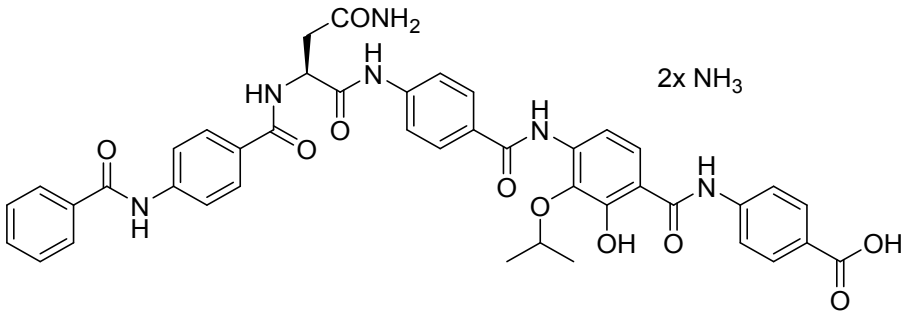
Amine **12** (15 mg, 0.016 mmol) was coupled with carboxylic acid **100** using coupling conditions C followed by final deprotection.

Desired compound purified by preparative RP-HPLC condition A (retention time: 29.4 min) , to obtain 1.1 mg of desired product as a white solid (0.0013 mmol, y= 8%), ^{1}H NMR (700 MHz, DMSO) δ 12.78 (br, 1H), 12.29 (s, 1H), 10.60 (s, 1H), 10.45 (s, 1H), 10.33 (s, 1H), 10.23 (s, 1H), 9.40 (s, 1H), 8.63 (d, *J* = 7.3 Hz, 1H), 7.95 (m, 6H), 7.92 – 7.87 (m, 3H), 7.85 (m, 3H), 7.82 (d, *J* = 8.7 Hz, 2H), 7.71 (m, 3H), 7.39 (s, 1H), 7.35 (dd, *J* = 8.0, 1.4 Hz, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.54 (dt, *J* = 12.3, 6.1 Hz, 1H), 2.69 (d, *J* = 8.1 Hz, 2H), 2.09 (s, 3H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.8, 168.8, 168.5, 166.9, 165.8, 165.2, 164.2, 154.1, 142.5, 142.5, 142.2, 142.0, 137.0, 136.3, 133.9, 133.7, 130.2, 129.7, 128.7, 128.7, 128.5, 128.3, 128.2, 127.3, 126.3, 122.8, 120.7, 119.3, 119.0, 118.1, 112.4, 112.2, 74.9, 51.6, 36.8, 24.1, 22.3.

HRMS (ESI) calculated for $C_{44}H_{42}N_7O_{11}$ ($M-H^+$) 844.2937, found 844.2930.

(S)-4-(4-(4-(4-Amino-2-(4-benzamidobenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (15)



Chemical Formula: $C_{42}H_{38}N_6O_{10}$
Exact Mass: 786,2649

Amine **12** (40 mg, 0.052 mmol) was coupled with benzoic acid using coupling conditions A followed by final deprotection.

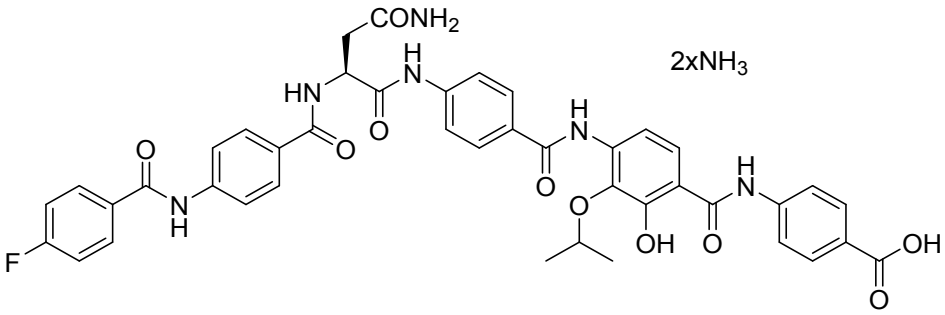
Desired compound purified by preparative RP-HPLC condition B (retention time: 20.86 min) , to obtain 13.8 mg of desired product as a white solid (0.018 mmol, y= 34%).

^{1}H NMR (700 MHz, DMSO) δ 12.74 (br, 1H), 12.30 (br, 1H), 10.48 (s, 1H), 10.45 (s, 1H), 9.33 (br, 1H), 8.65 (d, *J* = 7.3 Hz, 1H), 7.99 – 7.96 (m, 2H), 7.94 (dd, *J* = 15.6, 6.5 Hz, 4H), 7.92 – 7.89 (m, 4H), 7.83 (dd, *J* = 17.1, 8.7 Hz, 4H), 7.79 (s, 1H), 7.61 (dd, *J* = 11.6, 4.2 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 2H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.61 (s, 1H), 2.69 (d, *J* = 7.9 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.3, 168.3, 166.9, 165.8, 164.0, 142.4, 142.1, 136.5, 134.7, 131.8, 130.2, 128.7, 128.4, 128.3, 128.2, 127.7, 127.4, 123.0, 120.4, 119.3, 118.9, 51.6, 36.8, 22.4.

HRMS (ESI) calculated for $C_{42}H_{37}N_6O_{10}$ ($M\text{-}H^+$) 785.2577, found 785.2577.

(S)-4-(4-(4-(4-Amino-2-(4-(4-fluorobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (16)



Chemical Formula: $C_{42}H_{37}FN_6O_{10}$
Exact Mass: 804,2555

Amine **12** (40 mg, 0.052 mmol) was coupled with 4-fluorobenzoic acid using coupling conditions A followed by final deprotection.

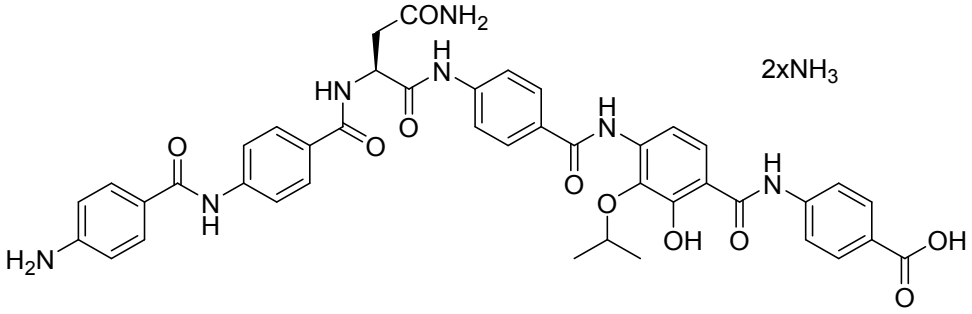
Desired compound purified by preparative RP-HPLC condition B (retention time: 18.2 min), to obtain 3.2 mg of desired product as a white solid (0.004 mmol, y= 8%).

^{1}H NMR (500 MHz, DMSO) δ 12.80 (br, 1H), 12.29 (br, 1H), 10.62 (br, 1H), 10.49 (s, 1H), 10.46 (s, 1H), 9.39 (s, 1H), 8.65 (d, *J* = 7.3 Hz, 1H), 8.09 – 8.03 (m, 2H), 7.96 (t, *J* = 8.4 Hz, 4H), 7.90 (q, *J* = 9.0 Hz, 4H), 7.84 (dd, *J* = 17.4, 8.8 Hz, 5H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.43 – 7.35 (m, 3H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.1 Hz, 1H), 4.59 – 4.51 (m, 1H), 2.69 (d, *J* = 7.2 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 171.3, 170.8, 168.4, 166.9, 165.8, 164.7, 164.2, 163.2, 142.5, 142.0, 136.4, 131.1, 130.6, 130.5, 130.2, 128.8, 128.3, 122.8, 120.6, 119.4, 118.9, 115.5, 115.3, 112.5, 74.8, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for $C_{42}H_{36}FN_6O_{10}$ ($M\text{-}H^+$) 803.2482, found 803.2498.

(S)-4-(4-(4-(4-Amino-2-(4-(4-aminobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (17)



Chemical Formula: $C_{42}H_{39}N_7O_{10}$
Exact Mass: 801,2758

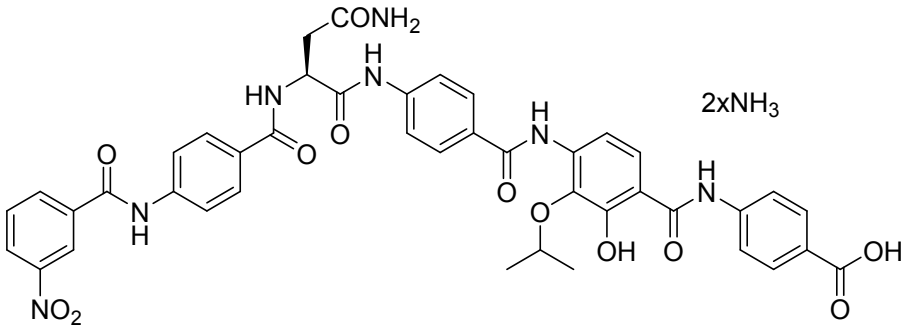
Compound **17** was isolated as a by-product of the synthesis of **4,** using preparative RP-HPLC condition B (retention time: 18.7 min) obtained 0.7 mg.

^{1}H NMR (700 MHz, DMSO) δ 15.31 (s, 1H), 10.46 (s, 1H), 9.98 (s, 1H), 8.87 (s, 1H), 8.68 (d, *J* = 7.0 Hz, 1H), 7.86 (d, *J* = 9.9 Hz, 4H), 7.82 (dd, *J* = 19.7, 8.8 Hz, 4H), 7.75 (dd, *J* = 11.3, 8.6 Hz, 4H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.44 (d, *J* = 8.7 Hz, 2H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 6.60 (d, *J* = 8.6 Hz, 2H), 5.80 (s, 2H), 5.02 (dt, *J* = 12.4, 6.1 Hz, 1H), 4.91 (dd, *J* = 14.2, 7.2 Hz, 1H), 2.69 (d, *J* = 7.0 Hz, 2H), 1.19 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 169.8, 166.8, 165.9, 165.5, 165.3, 163.1, 152.4, 142.7, 142.0, 141.5, 137.5, 134.0, 129.6, 129.5, 128.2, 127.9, 127.5, 123.7, 120.7, 119.0, 119.0, 117.5, 116.1, 112.5, 100.6, 70.3, 51.7, 36.9, 22.7.

HRMS (ESI) calculated for $C_{42}H_{38}N_7O_{10}$ ($M-H^+$) 800.2686, found 800.2733.

(S)-4-(4-(4-(4-Amino-2-(4-(3-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (18)

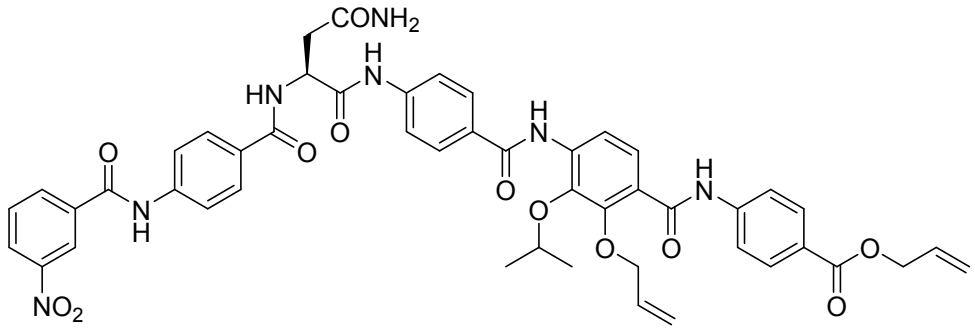


Chemical Formula: $C_{42}H_{37}N_7O_{12}$
Exact Mass: 831,2500

Amine **12** (40 mg, 0.052 mmol) was coupled with 3-nitrobenzoic acid using coupling conditions A. Allyl protected derivative was purified on silica gel with a gradient 0-10% MeOH in DCM to give 45 mg of white solid (0.049 mmol, y= 95%).

The final deprotection was done on 20 mg (0.022 mmol) of intermediate. The desired compound was purified by preparative RP-HPLC using condition B (retention time: 15.1 min), to obtain 6.8 mg of desired product (0.008 mmol, y= 31%) and 2.2 mg of compound **19** (retention time: 14.8 min), which was formed as a by-product of the reaction.

Allyl protected intermediate:



Chemical Formula: $C_{48}H_{45}N_7O_{12}$
Exact Mass: 911,3126

^{1}H NMR (700 MHz, DMSO) δ 10.80 (s, 1H), 10.58 (s, 1H), 10.45 (s, 1H), 9.52 (s, 1H), 8.82 (t, *J* = 2.0 Hz, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.46 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 8.44 – 8.41 (m, 1H), 8.00 – 7.96 (m, 4H), 7.96 – 7.93 (m, 2H), 7.93 – 7.89 (m, 2H), 7.86 (t, *J* = 8.0 Hz, 3H), 7.83 – 7.79 (m, 3H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.99 (s, 1H), 6.08 – 5.99 (m, 2H), 5.39 (m, 2H), 5.24 (m, 2H), 4.93 (dd, *J* = 14.0, 7.3 Hz, 1H), 4.79 (dd, *J* = 3.9, 1.4 Hz, 2H), 4.61 (d, *J* = 5.5 Hz, 2H), 4.52 – 4.46 (m, 1H), 2.71 – 2.68 (m, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 165.8, 164.9, 164.7, 164.3, 163.6, 149.5, 147.8, 143.5, 142.6, 142.4, 141.5, 136.0, 135.7, 134.3, 133.7, 132.8, 130.3, 130.3, 129.2, 128.4, 128.3, 127.1, 126.4, 124.2, 123.6, 122.5, 119.6, 119.0, 118.8, 117.8, 117.8, 76.2, 74.3, 64.8, 51.6, 36.8, 22.3.

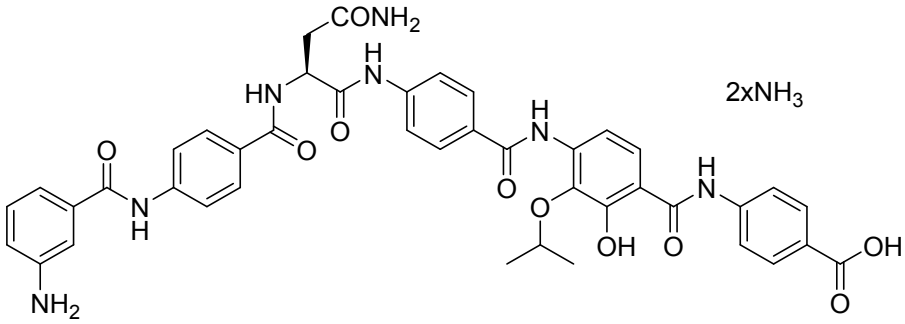
Final compound 18:

^{1}H NMR (700 MHz, DMSO) δ 12.79 (br, 1H), 12.29 (br, 1H), 10.80 (s, 1H), 10.62 (br, 1H), 10.46 (s, 1H), 9.36 (s, 1H), 8.82 (t, *J* = 1.8 Hz, 1H), 8.68 (d, *J* = 7.2 Hz, 1H), 8.46 (dd, *J* = 8.2, 1.5 Hz, 1H), 8.43 (d, *J* = 7.9 Hz, 1H), 7.98 – 7.89 (m, 8H), 7.86 (dd, *J* = 15.4, 7.9 Hz, 3H), 7.82 (d, *J* = 8.7 Hz, 3H), 7.66 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.93 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.58 (s, 1H), 2.70 (d, *J* = 7.5 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.4, 166.9, 165.8, 164.1, 163.6, 147.8, 142.5, 141.5, 136.4, 136.0, 134.3, 130.3, 130.2, 129.2, 128.3, 128.3, 126.4, 122.9, 122.5, 120.6, 119.6, 118.9, 74.5, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for $C_{42}H_{38}N_7O_{12}$ ($M+H^+$) 832.2573, found 832.2565.

(S)-4-(4-(4-(4-Amino-2-(4-(3-aminobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (19)



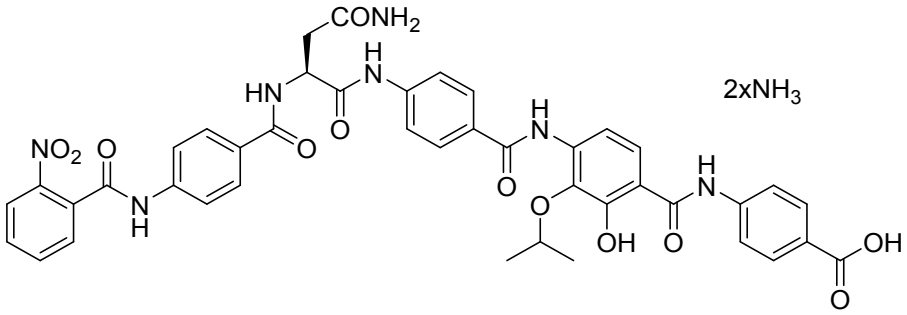
Chemical Formula: $C_{42}H_{39}N_7O_{10}$
Exact Mass: 801,2758

^{1}H NMR (700 MHz, DMSO) δ 15.86 (br, 1H), 12.45 (br, 1H), 10.43 (s, 1H), 10.30 (s, 1H), 8.94 (br, 1H), 8.62 (d, *J* = 7.3 Hz, 1H), 7.91 – 7.83 (m, 9H), 7.79 (dd, *J* = 20.6, 8.7 Hz, 4H), 7.49 (br, 1H), 7.40 (s, 1H), 7.16 (t, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 1.8 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 6.98 (s, 1H), 6.76 (dd, *J* = 7.9, 1.5 Hz, 1H), 5.33 (s, 2H), 4.95 (br, 1H), 4.92 (dd, *J* = 14.0, 7.3 Hz, 1H), 2.71 – 2.66 (m, 2H), 1.20 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 167.5, 167.2, 166.6, 165.9, 163.3, 148.8, 142.3, 142.1, 135.6, 130.4, 128.8, 128.5, 128.2, 127.7, 123.6, 119.2, 119.0, 117.0, 114.8, 113.0, 51.6, 36.8, 22.6.

HRMS (ESI) calculated for $C_{42}H_{38}N_7O_{10}$ ($M-H^+$) 800.2686, found 800.2691.

(S)-4-(4-(4-(4-Amino-2-(4-(2-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (20)



Chemical Formula: $C_{42}H_{37}N_7O_{12}$
Exact Mass: 831,2500

Amine **12** (20 mg, 0.026 mmol) was coupled with 2-nitrobenzoic acid using coupling conditions B followed by final deprotection.

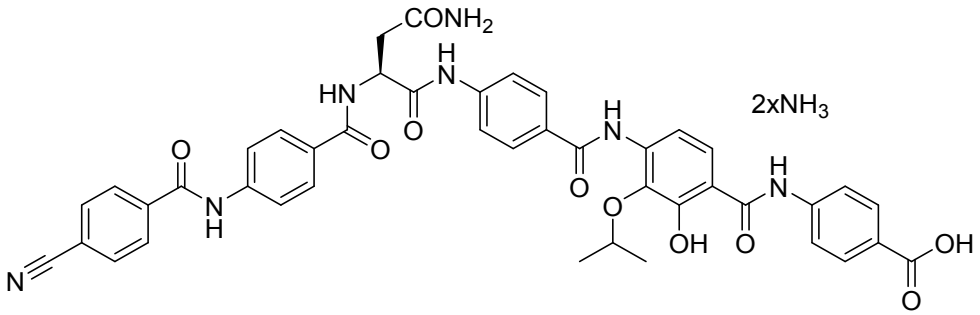
Desired compound was purified by preparative RP-HPLC condition B (retention time: 22.95 min), to obtain 2.4 mg of desired product as a white solid (0.003 mmol, y= 11%).

^{1}H NMR (700 MHz, DMSO) δ 12.72 (br, 1H), 12.30 (br, 1H), 10.91 (s, 1H), 10.45 (s, 1H), 9.29 (br, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.17 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.93 (t, *J* = 8.8 Hz, 6H), 7.89 (td, *J* = 7.5, 0.9 Hz, 1H), 7.85 – 7.80 (m, 6H), 7.80 – 7.75 (m, 4H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 13.9, 7.3 Hz, 1H), 4.64 (br, 1H), 2.69 (dd, *J* = 6.8, 3.4 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.2, 166.9, 165.7, 164.4, 164.0, 146.4, 142.4, 141.6, 136.6, 134.2, 132.4, 131.1, 130.3, 129.3, 129.0, 128.5, 128.1, 124.3, 123.0, 118.9, 118.7, 51.6, 36.8, 22.4.

HRMS (ESI) calculated for $C_{42}H_{36}N_7O_{12}$ ($M-H^+$) 830.2427, found 830.2439.

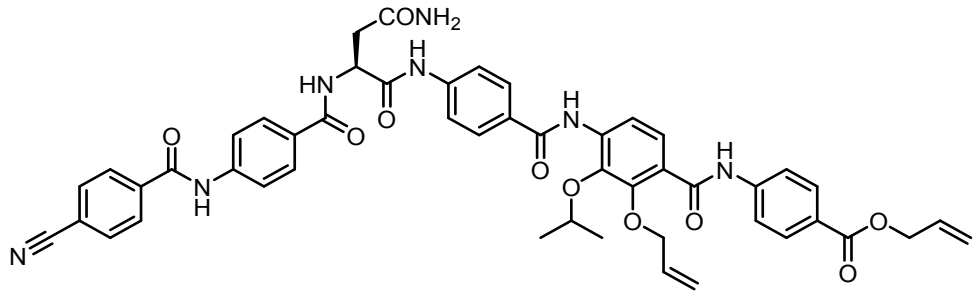
(S)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (22)



Chemical Formula: $C_{43}H_{37}N_7O_{10}$
Exact Mass: 811,2602

Amine **12** (25 mg, 0.033 mmol) was coupled with 4-cyanobenzoic acid using coupling conditions A. Allyl protected intermediate was purified on silica gel with a gradient 0-10% MeOH in DCM to give 18 mg (0.02 mmol, y= 61%) of allyl protected cystobactamid derivative. Final deprotection afforded the desired compound, which was purified by preparative RP-HPLC using condition A (retention time: 27.41 min) to obtain 3.3 mg of desired product (0.004 mmol, y= 20%).

Allyl protected intermediate:



Chemical Formula: $C_{49}H_{45}N_7O_{10}$
Exact Mass: 891,3228

^{1}H NMR (700 MHz, DMSO) δ 10.70 (s, 1H), 10.57 (s, 1H), 10.45 (s, 1H), 9.52 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), 7.98 (dd, *J* = 8.7, 6.9 Hz, 4H), 7.93 (d, *J* = 8.8 Hz, 2H), 7.88 (dd, *J* = 15.2, 8.7 Hz, 4H), 7.81 (dd, *J* = 8.6, 5.6 Hz, 3H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.99 (s, 1H), 6.09 – 5.98 (m, 2H), 5.43 – 5.34 (m, 2H), 5.30 – 5.18 (m, 2H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.79 (d, *J* = 5.3 Hz, 2H), 4.61 (d, *J* = 5.4 Hz, 2H), 4.49 (dt, *J* = 12.3, 6.1 Hz, 1H), 2.69 (d, *J* = 7.5 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 165.8, 165.0, 164.7, 164.5, 164.3, 149.5, 143.5, 142.6, 142.4, 141.6, 138.7, 135.7, 133.7, 132.8, 132.5, 130.3, 129.1, 128.6, 128.4, 128.3, 127.1, 124.2, 123.6, 119.5, 119.0, 118.8, 117.8, 117.8, 114.0, 76.3, 74.3, 64.8, 51.6, 36.8, 22.3.

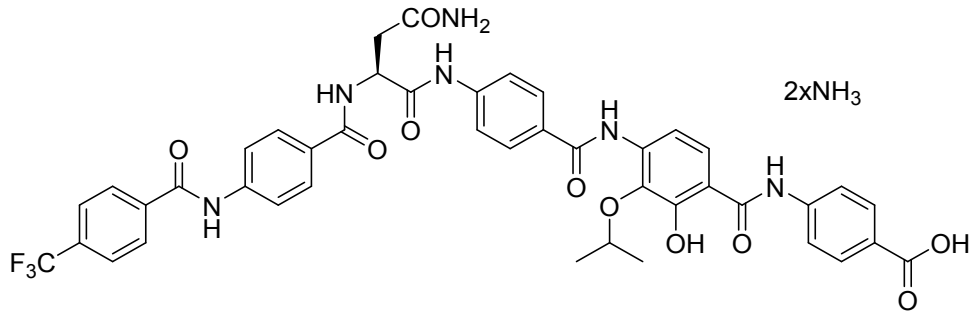
Final compound 22:

^{1}H NMR (500 MHz, DMSO) δ 12.81 (br, 1H), 12.29 (br, 1H), 10.70 (s, 1H), 10.61 (br, 1H), 10.46 (s, 1H), 9.37 (br, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.15 – 8.10 (m, 2H), 8.07 – 8.03 (m, 2H), 7.98 - 7.93 (m, 4H), 7.93 – 7.87 (m, 4H), 7.83 (dd, *J* = 16.0, 8.8 Hz, 5H), 7.67 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (q, *J* = 7.1 Hz, 1H), 4.56 (br, 1H), 2.69 (d, *J* = 7.2 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.4, 166.9, 165.8, 164.5, 164.1, 142.5, 141.6, 138.7, 136.4, 132.5, 130.2, 129.1, 128.6, 128.3, 128.3, 122.9, 120.6, 119.5, 118.9, 118.3, 114.0, 74.8, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for $C_{43}H_{36}N_7O_{10}$ ($M\text{-}H^+$) 810.2529, found 810.2538.

(S)-4-(4-(4-(4-Amino-4-oxo-2-(4-(4-(trifluoromethyl)benzamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (23)



Chemical Formula: $C_{43}H_{37}F_3N_6O_{10}$
Exact Mass: 854,2523

Amine **12** (15 mg, 0.020 mmol) was coupled with 4-trifluoromethyl benzoic acid using coupling conditions A followed by final deprotection.

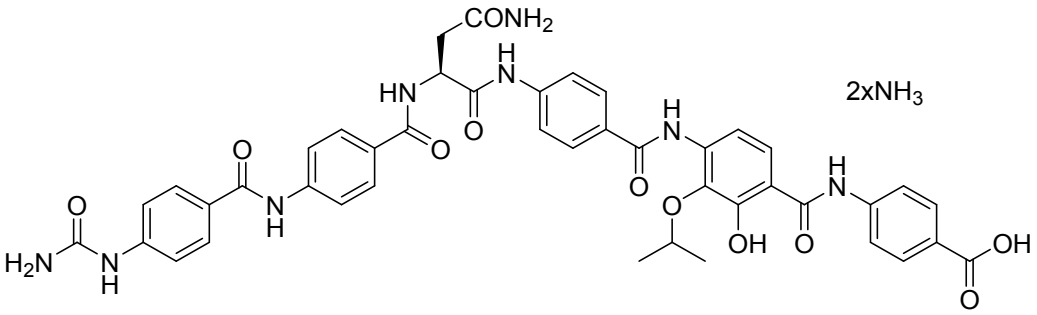
Desired compound purified by preparative RP-HPLC using condition B (retention time: 19.91 min), to obtain 5.3 mg of desired product as a white solid (0.0062 mmol, y= 31%).

^{1}H NMR (700 MHz, DMSO) δ 12.80 (br, 1H), 12.29 (br, 1H), 10.69 (s, 1H), 10.62 (br, 1H), 10.46 (s, 1H), 9.37 (br, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 2H), 7.98 – 7.92 (m, 8H), 7.90 (d, *J* = 8.9 Hz, 2H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.82 (d, *J* = 8.8 Hz, 3H), 7.67 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (q, *J* = 7.2 Hz, 1H), 4.57 (br, 1H), 2.69 (d, *J* = 7.6 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.4, 166.9, 165.8, 164.7, 164.1, 142.5, 141.7, 138.5, 136.9, 136.4, 131.6, 131.4, 130.2, 129.1, 128.7, 128.3, 128.3, 125.4, 125.4, 124.7, 123.1, 122.9, 120.6, 119.5, 118.9, 74.7, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for $C_{43}H_{36}F_3N_6O_{10}$ ($M\text{-}H^+$) 853.2450, found 853.2438.

(S)-4-(4-(4-(4-Amino-4-oxo-2-(4-(4-ureidobenzamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (21)



Chemical Formula: $C_{43}H_{40}N_8O_{11}$
Exact Mass: 844,2817

Amine **12** (20 mg, 0.026 mmol) was coupled with carboxylic acid **102** using coupling conditions C followed by final deprotection.

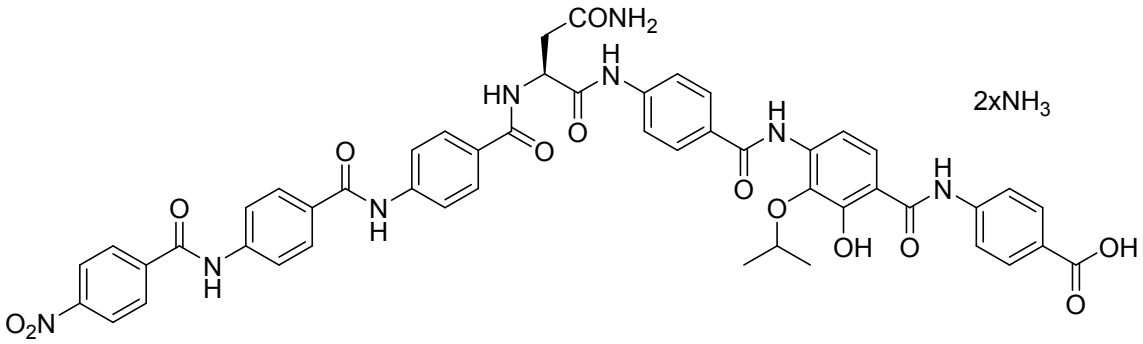
Desired compound purified by preparative RP-HPLC condition B (retention time: 19.94 min) to obtain 6.2 mg of desired product as a white solid (0.0073 mmol, y= 28%).

^{1}H NMR (700 MHz, DMSO) δ 12.55 (br, 1H), 10.44 (s, 1H), 10.26 (s, 1H), 9.15 (br, 1H), 8.89 (s, 1H), 8.63 (d, *J* = 7.3 Hz, 1H), 7.93 – 7.87 (m, 10H), 7.81 (d, *J* = 8.8 Hz, 4H), 7.66 (br, 1H), 7.56 – 7.52 (m, 2H), 7.39 (s, 1H), 6.99 (s, 1H), 6.01 (s, 2H), 4.92 (dd, *J* = 14.0, 7.3 Hz, 1H), 4.77 (br, 1H), 2.69 (d, *J* = 8.2 Hz, 2H), 1.23 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 167.9, 167.0, 165.9, 165.3, 163.8, 155.7, 144.0, 142.3, 142.3, 130.3, 128.8, 128.4, 128.2, 128.0, 126.6, 123.2, 119.2, 119.0, 116.6, 51.6, 36.8, 22.5.

HRMS (ESI) calculated for $C_{43}H_{39}N_8O_{11}$ (M-H$^+$) 843.2744, found 843.2759.

(S)-4-(4-(4-(4-Amino-2-(4-(4-(4-nitrobenzamido)benzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (24)



Chemical Formula: $C_{49}H_{42}N_8O_{13}$
Exact Mass: 950,2871

Amine **12** (15 mg, 0.020 mmol) coupled with carboxylic acid **98** using coupling conditions C followed by final deprotection.

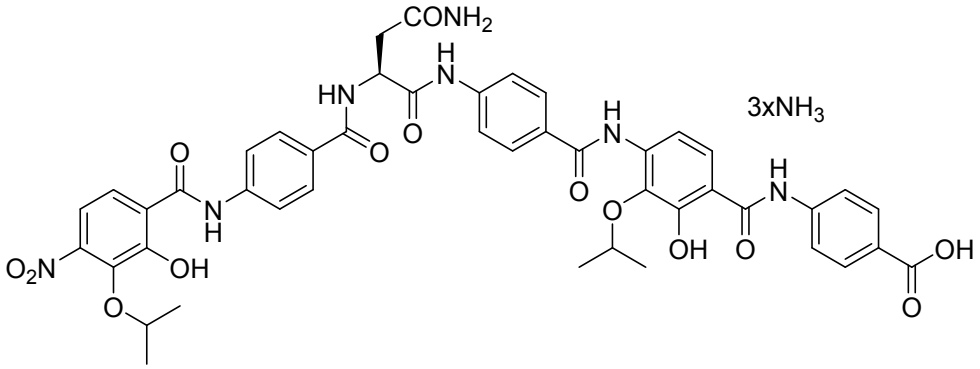
Desired compound was purified by preparative RP-HPLC condition B (retention time: 13.74 min), to obtain 3.9 mg of desired product as a white solid (0.0041 mmol, y= 20%).

^{1}H NMR (700 MHz, DMSO) δ 12.61 (br, 1H), 12.32 (br, 1H), 10.84 (s, 1H), 10.44 (s, 1H), 10.41 (s, 1H), 9.16 (br, 1H), 8.65 (d, *J* = 7.2 Hz, 1H), 8.42 – 8.37 (m, 2H), 8.25 – 8.19 (m, 2H), 8.04 (d, *J* = 8.7 Hz, 2H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.94 – 7.86 (m, 9H), 7.82 (d, *J* = 8.7 Hz, 4H), 7.67 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.93 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.76 (br, 1H), 2.69 (d, *J* = 7.7 Hz, 2H), 1.23 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 167.0, 165.8, 165.1, 164.3, 163.7, 149.3, 142.3, 142.1, 141.9, 140.3, 130.3, 129.8, 129.3, 128.7, 128.6, 128.3, 128.0, 123.6, 123.2, 119.6, 119.3, 119.0, 51.6, 36.8, 22.5.

HRMS (ESI) calculated for $C_{49}H_{41}N_8O_{13}$ (M-H$^+$) 949.2799, found 949.2848.

(S)-4-(4-(4-(4-Amino-2-(4-(2-hydroxy-3-isopropoxy-4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (25)

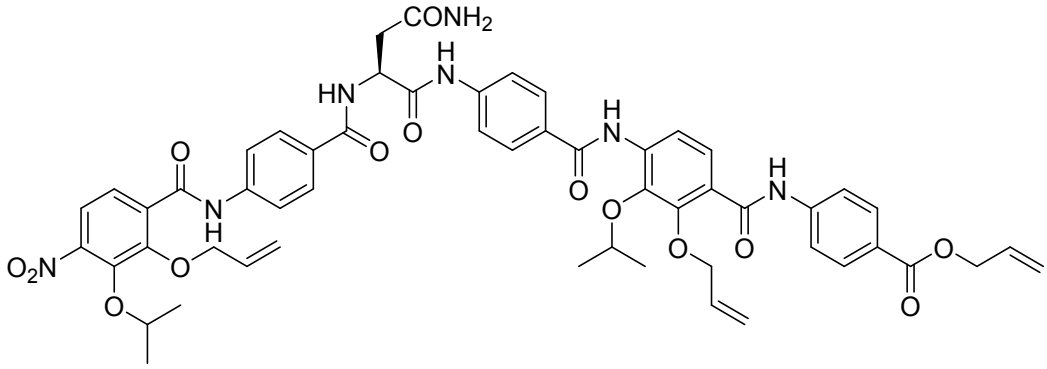


Chemical Formula: $C_{45}H_{43}N_7O_{14}$
Exact Mass: 905,2868

Amine **12** (40 mg, 0.052 mmol) was coupled with carboxylic acid **95** using coupling conditions A, the intermediate was purified on silica gel with a gradient 0-10% MeOH in DCM to give 40 mg (0.095 mmol, y= 87%) of allyl protected cystobactamid derivative.

Followed final deprotection on 15 mg of intermediate (0.015 mmol), the desired compound was purified by preparative RP-HPLC using condition B to obtain 2.5 mg of desired product (0.0028 mmol, y= 18%) and 1.2 mg of compound **26**, which was formed as a by-product of the reaction.

Allyl protected intermediate:



Chemical Formula: $C_{54}H_{55}N_7O_{14}$
Exact Mass: 1025,3807

^{1}H NMR (700 MHz, DMSO) δ 10.66 (s, 1H), 10.58 (s, 1H), 10.44 (s, 1H), 9.52 (s, 1H), 8.66 (d, *J* = 7.3 Hz, 1H), 7.98 (dd, *J* = 8.7, 6.6 Hz, 4H), 7.92 (d, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 8.6 Hz, 2H), 7.83 – 7.76 (m, 5H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.99 (s, 1H), 6.09 – 5.93 (m, 3H), 5.43 - 5.36 (m, 2H), 5.36 - 5.26 (m, 2H), 5.22 – 5.16 (m, 2H), 4.92 (dd, *J* = 13.9, 7.4 Hz, 1H), 4.81 – 4.78 (m, 2H), 4.70 – 4.65 (m, 1H), 4.62 – 4.59 (m, 4H), 4.52 – 4.46 (m, 1H), 2.71 – 2.67 (m, 1H), 1.26 (d, *J* = 6.1 Hz, 6H), 1.24 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 165.7, 164.9, 164.7, 164.3, 163.7, 149.9, 149.5, 146.8, 143.5, 143.4, 142.6, 142.4, 141.4, 136.1, 135.7, 133.7, 133.1, 132.8, 130.3, 129.1,

128.5, 128.4, 128.3, 127.1, 124.2, 123.6, 123.2, 119.2, 119.0, 118.8, 118.8, 118.3, 117.8, 117.8, 77.2, 76.2, 74.6, 74.3, 64.8, 51.6, 36.8, 22.3, 22.1.

Final compound 25:

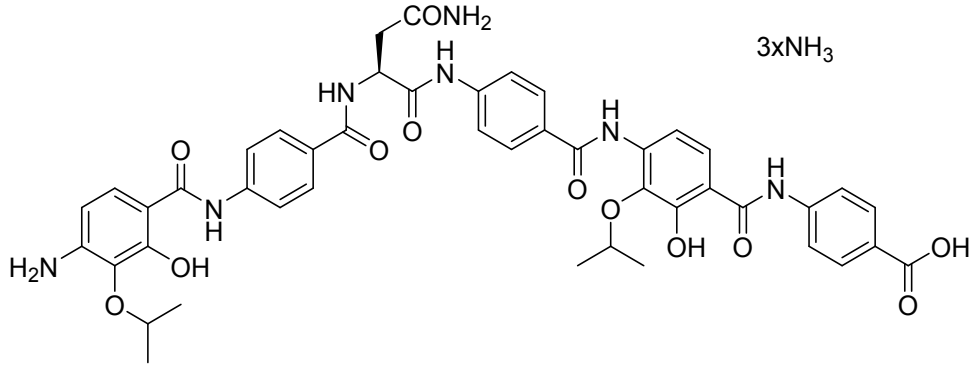
^{1}H NMR (700 MHz, DMSO) δ 12.82 (br, 1H), 12.29 (br, 1H), 10.61 (br, 1H), 10.43 (s, 1H), 9.40 (s, 1H), 8.60 (d, *J* = 7.2 Hz, 1H), 7.96 (dd, *J* = 14.7, 8.7 Hz, 4H), 7.91-7.75 (m, 9H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.40 (s, 1H), 6.98 (s, 1H), 6.48 (br, 1H), 5.10 (br, 1H), 4.91 (dd, *J* = 13.9, 7.2 Hz, 1H), 4.54 (dt, *J* = 11.9, 5.9 Hz, 1H), 2.69 (dd, *J* = 6.7, 3.2 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H), 1.14 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.8, 168.5, 166.3, 166.0, 164.2, 154.1, 147.2, 142.5, 137.0, 136.4, 130.2, 128.5, 128.3, 123.4, 122.8, 120.9, 120.7, 118.9, 118.8, 112.5, 112.2, 74.8, 51.6, 36.8, 22.3, 22.2.

HRMS (ESI) calculated for $C_{45}H_{44}N_7O_{14}$ ($M+H^+$) 906.2941, found 906.2940.

Side product 26:

(S)-4-(4-(4-(4-Amino-2-(4-(4-amino-2-hydroxy-3-isopropoxybenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (26)



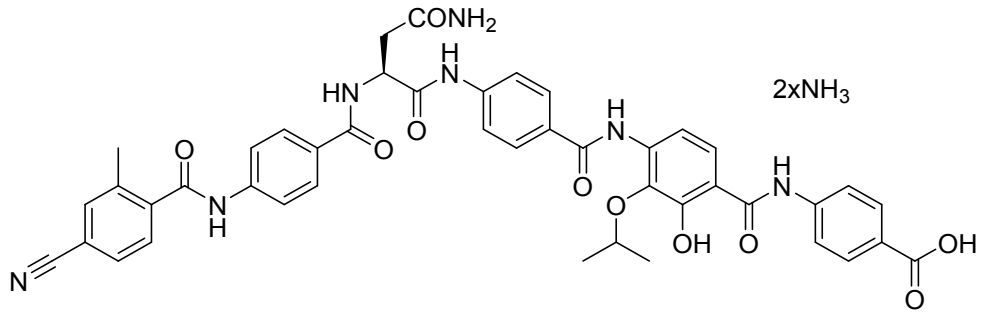
Chemical Formula: $C_{45}H_{45}N_7O_{12}$
Exact Mass: 875,3126

^{1}H NMR (700 MHz, DMSO) δ 12.82 (br, 1H), 12.63 (s, 1H), 12.28 (br, 1H), 10.61 (br, 1H), 10.45 (s, 1H), 10.11 (s, 1H), 9.39 (s, 1H), 8.65 (d, *J* = 7.3 Hz, 1H), 7.95 (dd, *J* = 12.0, 8.7 Hz, 4H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.83 (dd, *J* = 24.7, 8.7 Hz, 4H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.68 (br, 2H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 6.26 (d, *J* = 8.8 Hz, 1H), 5.63 (s, 2H), 4.92 (dd, *J* = 14.0, 7.3 Hz, 1H), 4.55 (br, 1H), 4.46 (dt, *J* = 12.3, 6.1 Hz, 1H), 2.70 – 2.67 (m, 2H), 1.26 (d, *J* = 6.1 Hz, 6H), 1.22 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 169.4, 168.4, 166.9, 165.9, 164.2, 155.7, 148.1, 142.5, 142.0, 141.3, 137.0, 136.4, 130.2, 129.6, 128.8, 128.3, 128.3, 128.2, 123.6, 122.9, 120.6, 120.3, 118.9, 105.3, 103.4, 72.8, 51.6, 36.8, 22.3, 22.2.

HRMS (ESI) calculated for $C_{45}H_{46}N_7O_{12}$ ($M+H^+$) 876.3199, found 876.3200.

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-2-methylbenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (88)



Chemical Formula: $C_{44}H_{39}N_7O_{10}$
Exact Mass: 825,2758

Amine **12** (20 mg, 0.026 mmol) was coupled with 4-Cyano-2-methylbenzoic acid using coupling conditions A followed by final deprotection.

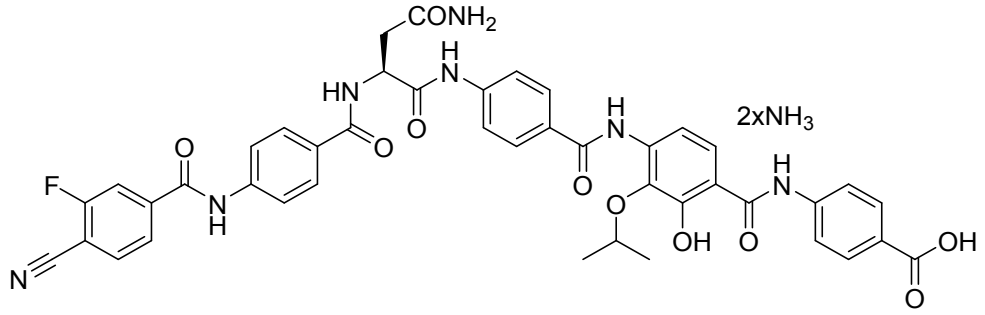
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 18.40 min) to obtain 4.5 mg of desired product as a white solid (0.005 mmol, y= 21%).

^{1}H NMR (700 MHz, DMSO) δ 12.75 (br, 1H), 12.29 (br, 1H), 10.73 (s, 1H), 10.46 (s, 1H), 9.31 (br, 1H), 8.66 (d, *J* = 7.3 Hz, 1H), 7.93 (m, 6H), 7.82 (m, 7H), 7.78 (br, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.65 – 7.55 (br, 2H), 7.39 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.63 (br, 1H), 2.69 (d, *J* = 8.1 Hz, 2H), 2.42 (s, 3H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.3, 166.9, 166.6, 165.7, 164.0, 142.4, 141.6, 141.1, 136.8, 136.5, 134.0, 130.2, 129.7, 129.0, 128.4, 128.2, 123.0, 120.3, 118.9, 118.9, 118.4, 112.3, 51.6, 36.8, 22.4, 18.8.

HRMS (ESI) calculated for $C_{44}H_{38}N_7O_{10}$ ($M\text{-}H^+$) 824.2686, found 824.2705.

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-3-fluorobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (90)



Chemical Formula: $C_{43}H_{36}FN_7O_{10}$
Exact Mass: 829,2508

Amine **12** (20 mg, 0.026 mmol) was coupled with 4-Cyano-3-fluorobenzoic acid using coupling conditions A followed by final deprotection.

Desired compound was purified by preparative RP-HPLC condition B (retention time: 17.4 min), to obtain 5.7 mg of desired product as a white solid (0.007 mmol, y= 27%).

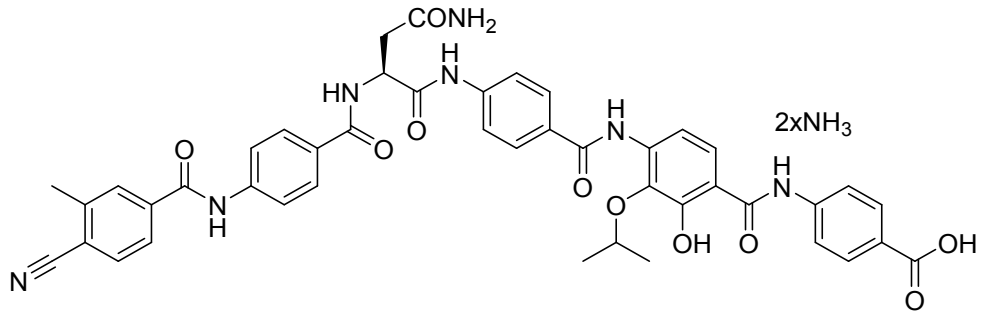
^{1}H NMR (500 MHz, DMSO) δ 12.78 (br, 1H), 12.29 (br, 1H), 10.74 (s, 1H), 10.46 (s, 1H), 9.35 (br, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.15 (dd, *J* = 8.0, 6.7 Hz, 1H), 8.08 (dd, *J* = 10.1, 1.5 Hz, 1H), 7.97 (m, 2H), 7.94 (d, *J* = 8.7 Hz, 5H), 7.88 (d, *J* = 8.9 Hz, 2H), 7.83 (dd, *J* = 15.2, 8.8 Hz, 5H), 7.64 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.1 Hz, 1H), 4.59 (br, 1H), 2.69 (d, *J* = 7.2 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.7, 168.4, 166.9, 165.7, 164.1, 161.5, 142.5, 141.5, 141.5, 141.3, 136.4, 134.3, 129.3, 128.4, 128.2, 124.7, 124.7, 122.9, 120.5, 119.6, 118.9, 115.8, 115.7, 113.6, 102.9, 102.8, 51.6, 36.8, 22.3.

^{19}F NMR (471 MHz, DMSO) δ -107.68 (dd, *J* = 9.8, 6.8 Hz).

HRMS (ESI) calculated for $C_{43}H_{35}FN_7O_{10}$ (M-H$^+$) 828.2435, found 28.2447.

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-3-methylbenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (89)



Chemical Formula: $C_{44}H_{39}N_7O_{10}$
Exact Mass: 825,2758

Amine **12** (20 mg, 0.026 mmol) was coupled with 4-Cyano-3-methylbenzoic acid using coupling conditions A followed by final deprotection.

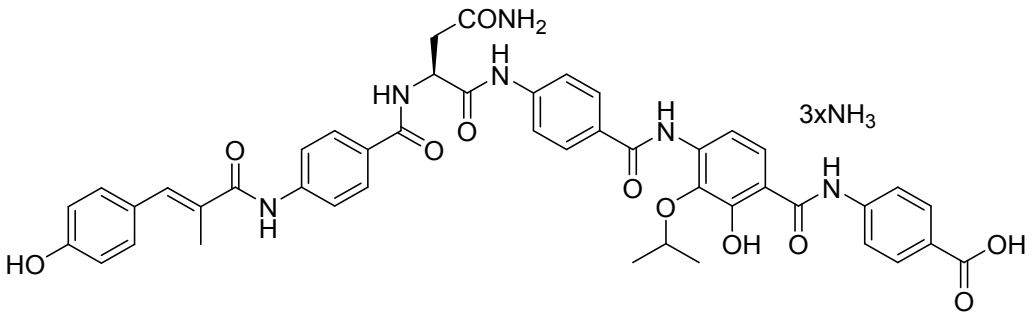
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 18.40 min) to obtain 5.2 mg of desired product as a white solid (0.006 mmol, y= 24%).

^{1}H NMR (700 MHz, DMSO) δ 12.77 (br, 1H), 12.29 (br, 1H), 10.66 (s, 1H), 10.46 (s, 1H), 9.34 (br, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.02 (s, 1H), 7.98 – 7.90 (m, 8H), 7.90 – 7.87 (m, 2H), 7.87 – 7.76 (m, 5H), 7.67 – 7.58 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.60 (br, 1H), 2.69 (d, *J* = 7.5 Hz, 2H), 2.59 (s, 3H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.3, 166.9, 165.8, 164.6, 164.1, 142.4, 141.9, 141.6, 138.6, 136.5, 132.8, 132.6, 130.2, 129.4, 129.2, 129.1, 128.3, 128.2, 125.8, 125.4, 122.9, 120.4, 119.5, 118.9, 117.5, 114.4, 51.6, 36.8, 22.3, 20.0.

HRMS (ESI) calculated for $C_{44}H_{38}N_7O_{10}$ (M-H$^+$) 824.2686, found 824.2694.

(S,E)-4-(4-(4-(4-Amino-2-(4-(3-(4-hydroxyphenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (45)

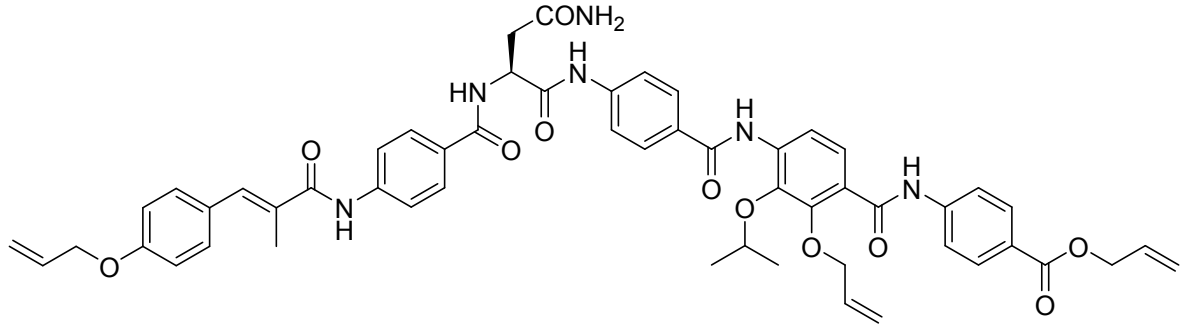


Chemical Formula: $C_{45}H_{42}N_6O_{11}$
Exact Mass: 842,2912

Amine **12** (40 mg, 0.052 mmol) was coupled with carboxylic acid **99** coupling conditions A, the intermediate was purified on silica gel with a gradient 0-10% MeOH in DCM to give 50 mg (0.052 mmol, y= q.) of allyl protected cystobactamid derivative.

Followed final deprotection on 15 mg of intermediate (0.016 mmol), the desired compound was purified by preparative RP-HPLC using condition B (retention time: 14.9 min) to obtain 1.0 mg of desired product (0.0012 mmol, y= 7%).

Allyl protected intermediate:



Chemical Formula: $C_{54}H_{54}N_6O_{11}$
Exact Mass: 962,3851

^{1}H NMR (700 MHz, DMSO) δ 10.58 (s, 1H), 10.44 (s, 1H), 10.12 (s, 1H), 9.52 (s, 1H), 8.62 (d, *J* = 7.3 Hz, 1H), 7.98 (dd, *J* = 8.8, 7.0 Hz, 4H), 7.90 – 7.85 (m, 4H), 7.85 – 7.79 (m, 5H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.30 (s, 1H), 7.06 – 7.01 (m, 2H), 6.99 (s, 1H), 6.09 - 5.98 (m, 3H), 5.44 – 5.35 (m, 3H), 5.28 (dq, *J* = 10.5, 1.4 Hz, 2H), 5.20 (ddd, *J* = 10.5, 2.9, 1.3 Hz, 1H), 4.91 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.79 (dd, *J* = 4.0, 1.4 Hz, 2H), 4.63 - 4.60 (m, 4H), 4.49 (dt, *J* = 12.3, 6.2 Hz, 1H), 3.85 – 3.73 (m, 1H), 2.69 (d, *J* = 7.8 Hz, 2H), 2.12 (d, *J* = 1.3 Hz, 3H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.8, 168.7, 165.9, 165.0, 164.7, 164.3, 158.0, 149.5, 143.5, 142.6, 142.4, 142.3, 135.7, 133.7, 133.6, 133.3, 13 2.8, 131.1, 130.7, 130.3, 128.4, 128.3, 128.2, 127.1, 124.2, 123.6, 119.1, 119.0, 118.8, 117.8, 117.8, 117.6, 114.7, 107.0, 97.2, 76.3, 74.3, 68.2, 64.8, 51.6, 36.8, 22.3, 14.5.

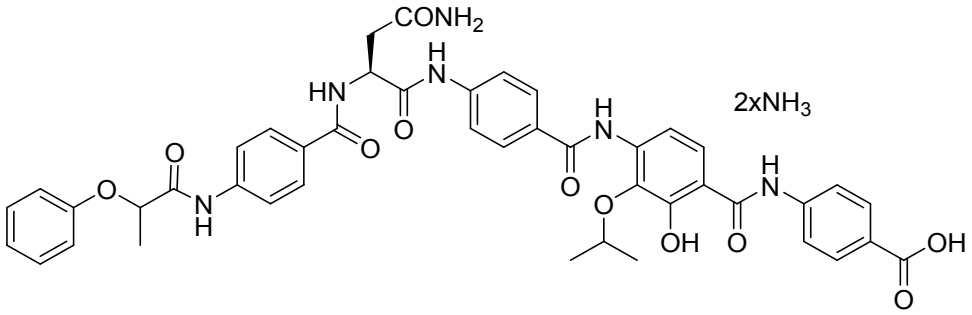
Final compound 45:

^{1}H NMR (700 MHz, DMSO) δ 12.79 (br, 1H), 12.29 (br, 1H), 10.60 (br, 1H), 10.45 (s, 1H), 10.08 (s, 1H), 9.76 (s, 1H), 9.38 (br, 1H), 8.61 (d, *J* = 7.2 Hz, 1H), 7.98 – 7.90 (m, 4H), 7.90 – 7.76 (m, 10H), 7.39 (s, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.26 (s, 1H), 6.99 (s, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.91 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.56 (br, 1H), 2.68 (d, *J* = 7.5 Hz, 2H), 2.11 (d, *J* = 1.2 Hz, 3H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.8, 168.8, 166.9, 165.9, 164.1, 162.3, 162.2, 157.5, 142.4, 133.8, 131.3, 130.2, 129.5, 128.2, 127.3, 126.6, 122.8, 120.6, 119.0, 118.9, 115.4, 51.6, 36.8, 22.3, 14.5.

HRMS (ESI) calculated for $C_{45}H_{41}N_6O_{11}$ ($M\text{-}H^+$) 841,2839, found 841,2844.

4-(4-(4-((2S)-4-Amino-4-oxo-2-(4-(2-phenoxypropanamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (51)

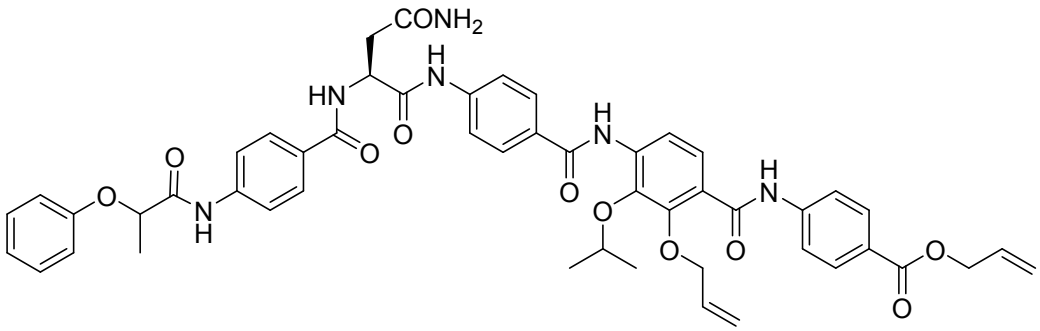


Chemical Formula: $C_{44}H_{42}N_6O_{11}$
Exact Mass: 830,2912

Amine **12** (40 mg, 0.052 mmol) was coupled with 2-phenoxypropanoic acid using coupling conditions A, the intermediate was purified on silica gel with a gradient 0-10% MeOH in DCM to give 30 mg (0.033 mmol, y= 63%) of allyl protected cystobactamid derivative.

Followed final deprotection on 15 mg of intermediate (0.016 mmol), the desired compound was purified by preparative RP-HPLC using condition B (retention time: 22.27 min) to obtain 7.5 mg of desired product (0.009 mmol, y= 56%).

Allyl protected intermediate:



Chemical Formula: $C_{50}H_{50}N_6O_{11}$
Exact Mass: 910,3538

^{1}H NMR (500 MHz, Acetone) δ 10.25 (s, 1H), 10.02 (s, 1H), 9.52 (s, 1H), 8.99 (s, 1H), 8.40 (dd, *J* = 8.8, 2.3 Hz, 1H), 8.37 (d, *J* = 7.5 Hz, 1H), 8.08 – 8.03 (m, 2H), 8.00 – 7.96 (m, 2H), 7.94 – 7.88 (m, 4H), 7.88 – 7.82 (m, 5H), 7.34 – 7.29 (m, 2H), 7.15 (s, 1H), 7.05 – 7.01 (m, 2H), 6.99 (tt, *J* = 7.4, 1.0 Hz, 1H), 6.54 (s, 1H), 6.27 - 6.17 (m, 1H), 6.14 – 6.04 (m, 1H), 5.50 (m, 2H), 5.32 (m, 2H), 5.10 – 5.05 (m, 1H), 4.89 (q, *J* = 6.7 Hz, 1H), 4.83 – 4.76 (m, 5H), 2.93 (qd, *J* = 15.9, 6.0 Hz, 2H), 1.61 (d, *J* = 6.7 Hz, 3H), 1.39 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, Acetone) δ 173.7, 171.5, 171.0, 167.2, 166.0, 165.0, 163.9, 158.3, 150.7, 144.4, 143.6, 142.6, 140.8, 138.6, 138.3, 134.1, 133.9, 131.5, 130.6, 130.4, 130.2, 130.1, 129.2, 126.9, 126.2, 123.8, 122.7, 122.0, 120.2, 120.1, 120.0, 119.6, 118.1, 116.7, 116.6, 116.6, 115.9, 77.5, 75.9, 75.6, 65.9, 52.5, 37.1, 22.9, 18.8.

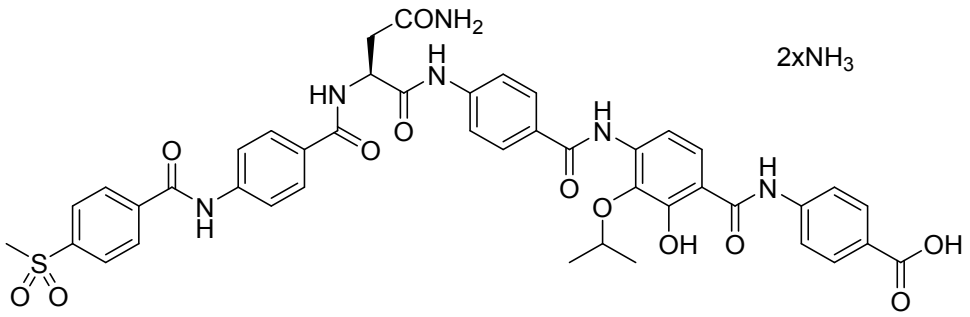
Final compound 51:

^{1}H NMR (700 MHz, DMSO) δ 12.76 (br, 1H), 12.29 (br, 1H), 10.71 (br, 1H), 10.44 (s, 1H), 10.36 (s, 1H), 9.33 (br, 1H), 8.62 (d, *J* = 7.3 Hz, 1H), 7.94 (dd, *J* = 15.5, 8.6 Hz, 4H), 7.85 (dd, *J* = 16.2, 8.7 Hz, 4H), 7.80 (d, *J* = 8.7 Hz, 3H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.62 (br, 1H), 7.38 (s, 1H), 7.30 (dd, *J* = 8.6, 7.4 Hz, 2H), 6.96 (dd, *J* = 13.2, 7.6 Hz, 4H), 4.93 – 4.87 (m, 2H), 4.60 (br, 1H), 2.69 – 2.65 (m, 2H), 1.56 (d, *J* = 6.6 Hz, 3H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 170.9, 170.3, 170.1, 168.0, 166.6, 165.3, 163.7, 156.8, 142.1, 140.9, 136.1, 129.9, 129.2, 128.4, 128.0, 127.8, 122.6, 120.9, 120.0, 118.5, 118.4, 114.7, 73.3, 51.2, 36.4, 22.0, 18.2.

HRMS (ESI) calculated for $C_{44}H_{41}N_6O_{11}$ ($M\text{-}H^+$) 829.2839, found 829.2830.

(S)-4-(4-(4-(4-Amino-2-(4-(4-(methylsulfonyl)benzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (27)



Chemical Formula: $C_{43}H_{40}N_6O_{12}S$
Exact Mass: 864,2425

Amine **12** (25 mg, 0.032 mmol) was coupled with 4-(methylsulfonyl)benzoic acid using coupling conditions A followed by final deprotection.

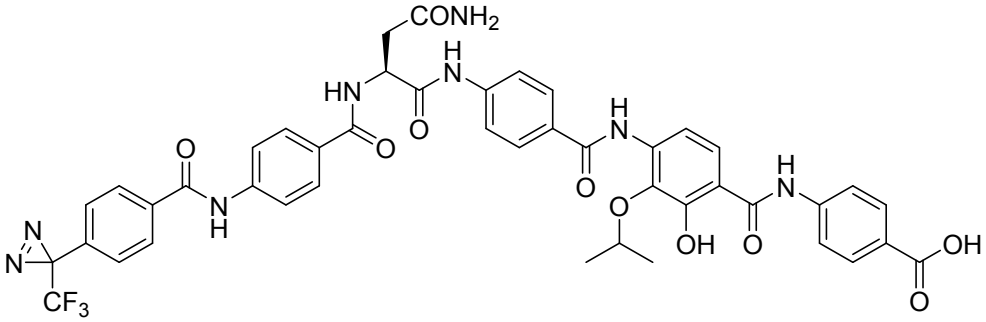
Desired compound was purified by preparative RP-HPLC using condition B (retention time: 20.10 min) to obtain 8.6 mg of desired product as a white solid (0.010 mmol, y= 31%).

^{1}H NMR (700 MHz, DMSO) δ 15.35 (s, 1H), 10.76 (s, 1H), 10.57 (s, 1H), 8.90 (s, 1H), 8.87 (s, 1H), 8.21 (d, *J* = 8.1 Hz, 2H), 8.09 (d, *J* = 8.1 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 8.4 Hz, 2H), 7.83 (s, 4H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.51 (s, 1H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 5.02 (dt, *J* = 12.0, 5.9 Hz, 1H), 4.92 (dd, *J* = 13.4, 7.2 Hz, 1H), 3.30 (s, 3H), 2.73 (dd, *J* = 14.8, 8.7 Hz, 1H), 2.68 (dd, *J* = 14.8, 5.1 Hz, 1H), 1.20 (d, *J* = 5.7 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 166.9, 165.7, 165.3, 164.6, 163.1, 143.3, 142.0, 141.8, 141.6, 139.2, 137.5, 134.0, 133.4, 129.7, 129.5, 129.2, 128.8, 128.4, 128.3, 127.6, 127.1, 127.0, 126.8, 123.7, 119.5, 119.0, 117.6, 116.0, 100.6, 70.3, 51.9, 43.3, 36.9, 22.7.

HRMS (ESI) calculated for C43H39N6O12S ($M-H^+$) 863.2352, found 863.2364.

(S)-4-(4-(4-(4-amino-4-oxo-2-(4-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (28)

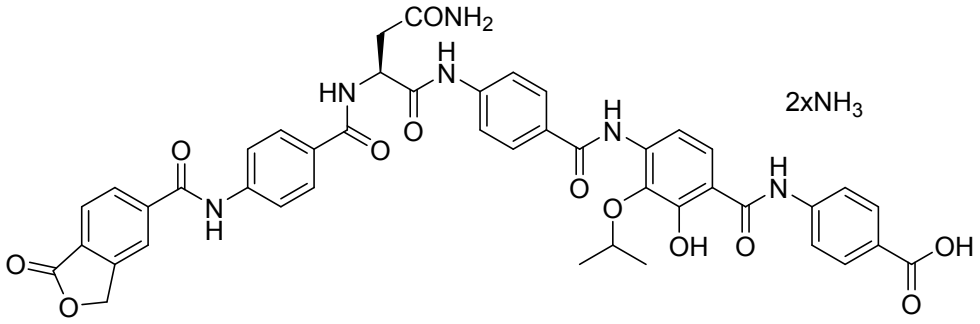


Chemical Formula: $C_{44}H_{37}F_3N_8O_{10}$
Exact Mass: 894,2585

Amine **12** (25 mg, 0.032 mmol) was coupled with 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoic acid using coupling conditions A followed by final deprotection.
Desired compound was purified by preparative RP-HPLC using condition B (retention time: 16.70 min) to obtain 4.6 mg of desired product as a white solid (0.005 mmol, y= 16%).

^{1}H NMR (700 MHz, DMSO-d_6) δ 12.81 (br, 1H), 12.29 (s, 1H), 10.61 (s, 2H), 10.46 (s, 1H), 9.39 (s, 1H), 8.66 (d, *J* = 7.3 Hz, 1H), 8.16 – 8.05 (m, 2H), 8.00 – 7.90 (m, 6H), 7.90 – 7.79 (m, 7H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.55 – 7.34 (m, 2H), 6.99 (s, 1H), 4.92 (q, *J* = 7.2 Hz, 1H), 4.54 (p, *J* = 6.1 Hz, 1H), 2.71 – 2.66 (m, 2H), 1.26 (d, *J* = 6.2 Hz, 6H).
^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.5, 166.9, 165.8, 164.8, 164.2, 142.5, 142.0, 141.7, 137.0, 136.3, 136.3, 130.7, 130.2, 129.6, 129.0, 128.7, 128.3, 126.5, 126.3, 122.8, 122.5, 121.0, 120.7, 119.4, 118.9, 112.4, 74.8, 51.6, 36.8, 22.3.
HRMS (ESI) calculated for $C_{44}H_{36}F_3N_8O_{10}$ ($M\text{-}H^+$) 893.2512, found 893.2506.

(S)-4-(4-(4-(4-Amino-4-oxo-2-(4-(1-oxo-1,3-dihydroisobenzofuran-5-carboxamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (29)



Chemical Formula: $C_{44}H_{38}N_6O_{12}$
Exact Mass: 842,2548

Amine **12** (25 mg, 0.032 mmol) was coupled with 1-oxo-1,3-dihydroisobenzofuran-5-carboxylic acid using coupling conditions A followed by final deprotection.

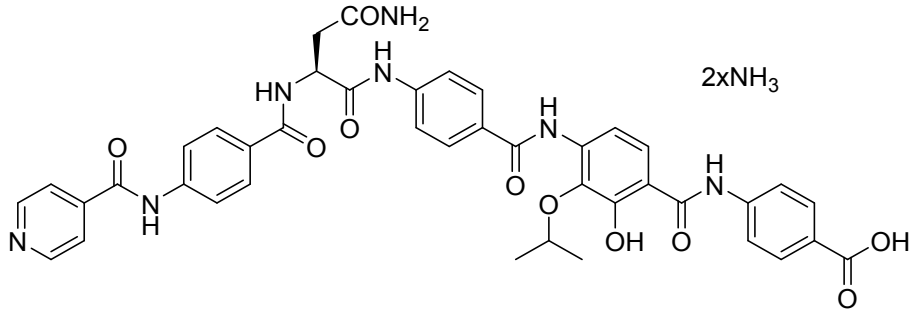
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 20.14 min) to obtain 4.9 mg of desired product as a white solid (0.0058 mmol, y= 18%).

^{1}H NMR (700 MHz, DMSO) δ 15.33 (s, 1H), 10.81 (s, 1H), 10.56 (s, 1H), 8.87 (s, 2H), 8.23 (s, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.7 Hz, 2H), 7.83 (q, *J* = 9.1 Hz, 4H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.51 (s, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 5.51 (s, 2H), 5.05 – 5.00 (m, 1H), 4.92 (dd, *J* = 13.9, 7.6 Hz, 1H), 2.73 (dd, *J* = 15.1, 8.6 Hz, 1H), 2.68 (dd, *J* = 15.1, 5.5 Hz, 1H), 1.19 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 170.0, 166.8, 165.7, 165.3, 165.0, 163.1, 147.4, 142.0, 141.6, 140.0, 137.5, 134.0, 133.4, 131.2, 129.7, 129.5, 129.1, 128.5, 128.3, 128.1, 127.5, 127.4, 127.1, 125.0, 124.2, 123.7, 122.5, 120.0, 119.5, 119.0, 117.5, 116.0, 100.6, 70.3, 70.1, 51.9, 36.9, 22.7.

HRMS (ESI) calculated for C44H37N6O12 ($M-H^+$) 841.2475, found 841.2478.

(S)-4-(4-(4-(4-Amino-2-(4-(isonicotinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (106)



Chemical Formula: $C_{41}H_{37}N_7O_{10}$
Exact Mass: 787,2602

Amine **12** (25 mg, 0.032 mmol) was coupled with isonicotinic acid using coupling conditions A followed by final deprotection.

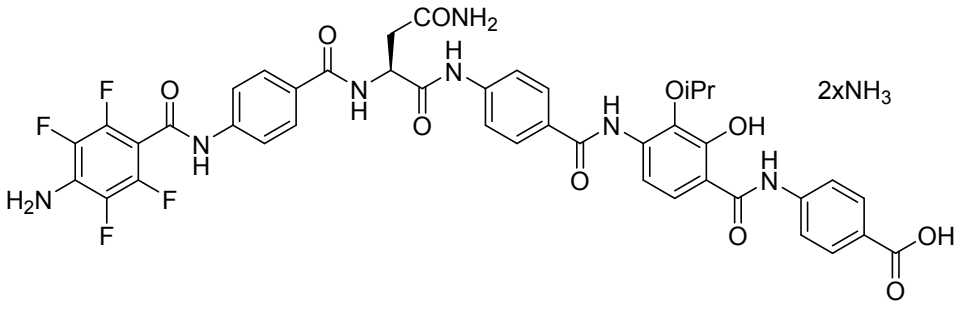
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 17.7 min) to obtain 5.5 mg of desired product as a white solid (0.007 mmol, y= 22%).

^{1}H NMR (700 MHz, DMSO) δ 15.70 (br, 1H), 12.48 (br, 1H), 10.73 (s, 1H), 10.46 (s, 1H), 8.88 (s, 1H), 8.80 (d, *J* = 5.8 Hz, 2H), 8.73 (d, *J* = 7.0 Hz, 1H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.91 – 7.86 (m, 4H), 7.86 – 7.77 (m, 6H), 7.70 (br, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.42 (s, 1H), 7.10 (d, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 5.01 (dt, *J* = 12.2, 6.0 Hz, 1H), 4.92 (q, *J* = 7.1 Hz, 1H), 2.69 (d, *J* = 7.0 Hz, 2H), 1.20 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 167.1, 165.7, 165.3, 164.3, 163.2, 150.3, 142.0, 141.7, 141.4, 137.5, 134.2, 130.0, 129.5, 129.3, 128.3, 127.6, 124.2, 123.7, 121.6, 119.5, 119.0, 117.9, 115.7, 100.9, 70.4, 51.7, 36.8, 22.7.

HRMS (ESI) calculated for C41H38N7O10 ($M-H^+$) 788.2675, found 788.2659.

(S)-4-(4-(4-(4-Amino-2-(4-(4-amino-2,3,5,6-tetrafluorobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (107)



Chemical Formula: $C_{42}H_{35}F_4N_7O_{10}$
Exact Mass: 873,2382

Amine **12** (25 mg, 0.033 mmol) was coupled with 4-azido-2,3,5,6-tetrafluorobenzoic acid using coupling conditions A followed by final deprotection.

Purification by preparative RP-HPLC following condition B (retention time: 21.01 min) was done to obtain 4.3 mg of amine 54 as a degradation byproduct of the desired azide (0.0069 mmol, y= 15%).

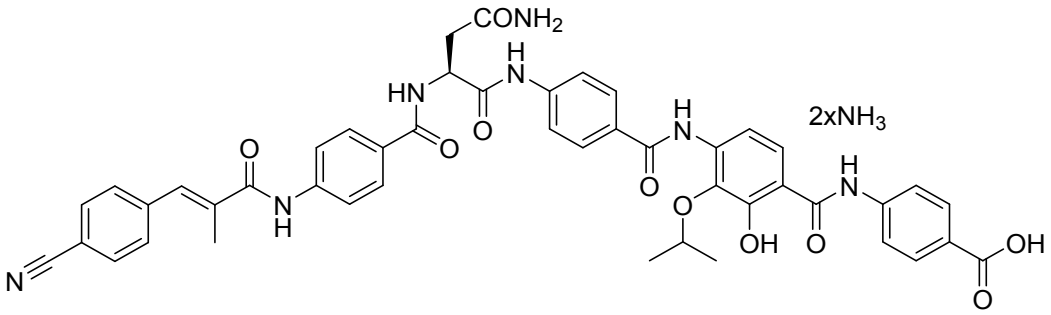
^{1}H NMR (500 MHz, DMSO) δ 12.64 (br, 2H), 10.83 (s, 1H), 10.45 (s, 1H), 9.23 (br, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 7.96 – 7.88 (m, 6H), 7.82 (dd, *J* = 8.7, 7.1 Hz, 4H), 7.76 (d, *J* = 8.7 Hz, 2H), 7.72 (br, 1H), 7.51 (br, 1H), 7.39 (s, 1H), 6.99 (s, 1H), 6.49 (s, 2H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.70 (br, 1H), 2.70 – 2.66 (m, 2H), 1.24 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.1, 167.0, 165.7, 163.9, 157.2, 144.4, 143.0, 142.3, 141.2, 136.7, 135.8, 134.3, 130.3, 129.2, 128.6, 128.1, 123.1, 119.9, 118.9, 118.6, 100.95 (t, *J* = 19.5 Hz, C), 51.6, 36.8, 22.4.

^{19}F NMR (471 MHz, DMSO) δ -145.12 (d, *J* = 16.0 Hz), -161.56 (d, *J* = 16.1 Hz).

HRMS (ESI) calculated for C42H36F4N7O10 ($M+H^+$) 874.2454, found 874.2458.

(S,E)-4-(4-(4-(4-Amino-2-(4-(3-(4-cyanophenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (46)



Chemical Formula: $C_{46}H_{41}N_7O_{10}$
Exact Mass: 851,2915

Amine **12** (25 mg, 0.032 mmol) was coupled with carboxylic acid **104** using coupling conditions A followed by final deprotection.

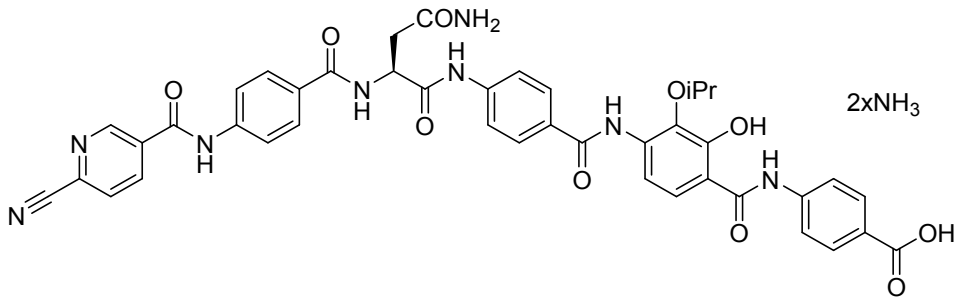
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 22.88 min) to obtain 6.4 mg of desired product as a white solid (0.0075 mmol, y= 24%).

^{1}H NMR (700 MHz, DMSO) δ 12.80 (br, 1H), 12.29 (br, 1H), 10.65 (br, 1H), 10.47 (s, 1H), 10.26 (s, 1H), 9.37 (s, 1H), 8.64 (d, *J* = 7.2 Hz, 1H), 7.95 (dd, *J* = 11.5, 8.8 Hz, 4H), 7.91 (dd, *J* = 10.4, 8.6 Hz, 4H), 7.88 – 7.80 (m, 7H), 7.67 (d, *J* = 8.3 Hz, 3H), 7.40 (s, 1H), 7.37 (s, 1H), 6.99 (s, 1H), 4.92 (q, *J* = 7.1 Hz, 1H), 4.59 – 4.53 (m, 1H), 2.69 (d, *J* = 7.0 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.8, 168.4, 168.1, 166.9, 165.8, 164.1, 142.5, 142.1, 142.0, 140.6, 136.9, 136.4, 135.8, 132.4, 132.0, 131.7, 130.2, 130.1, 128.6, 128.3, 126.9, 126.1, 122.9, 120.6, 119.2, 118.9, 118.7, 112.5, 110.3, 74.7, 51.6, 36.8, 22.3, 14.6.

HRMS (ESI) calculated for C46H41N7NaO10 ($M+Na^+$) 874.2807, found 874.2814.

(S)-4-(4-(4-(4-Amino-2-(4-(6-cyanonicotinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (30)



Chemical Formula: $C_{42}H_{36}N_8O_{10}$
Exact Mass: 812,2554

Amine **12** (25 mg, 0.033 mmol) was coupled with 6-cyanonicotinic acid using coupling conditions A followed by final deprotection.

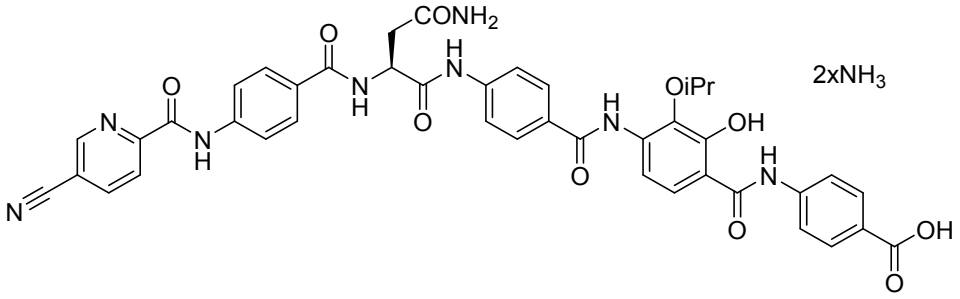
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 20.11 min) to obtain 4.6 mg of desired product (0.0057 mmol, y= 17%).

^{1}H NMR (500 MHz, DMSO) δ 12.76 (br, 1H), 12.31 (br, 1H), 10.87 (s, 1H), 10.47 (s, 1H), 9.34 (br, 1H), 9.26 – 9.22 (m, 1H), 8.69 (d, *J* = 7.2 Hz, 1H), 8.54 (dd, *J* = 8.1, 2.2 Hz, 1H), 8.26 (d, *J* = 8.1 Hz, 1H), 7.95 (dt, *J* = 6.7, 3.2 Hz, 6H), 7.91 – 7.74 (m, 7H), 7.63 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.1 Hz, 1H), 4.60 (s, 1H), 2.69 (d, *J* = 7.0 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.7, 168.3, 166.9, 165.7, 164.1, 163.0, 150.2, 142.4, 141.3, 137.3, 136.5, 134.5, 133.4, 130.2, 129.4, 128.8, 128.4, 128.2, 122.9, 120.4, 119.5, 118.9, 117.1, 74.4, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for C42H35N8O10 ($M-H^+$) 811.2482, found 811.2480.

(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanopicolinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (31)



Chemical Formula: $C_{42}H_{36}N_8O_{10}$
Exact Mass: 812,2554

Amine **12** (25 mg, 0.033 mmol) was coupled with 5-cyanopicolinic acid using coupling conditions A followed by final deprotection.

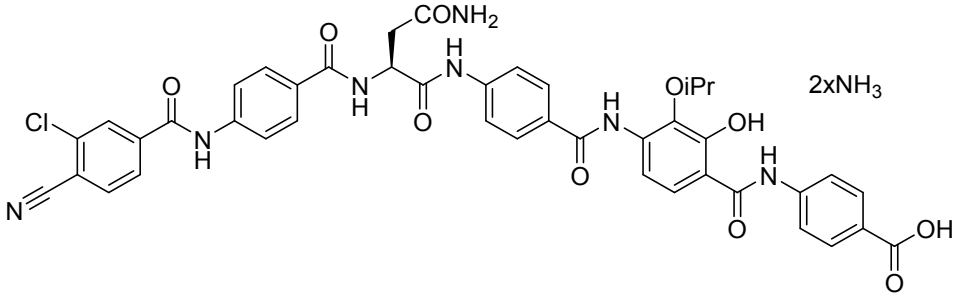
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 20.71 min) to obtain 2.5 mg of desired product (0.0031 mmol, y= 9%).

^{1}H NMR (500 MHz, DMSO) δ 12.76 (br, 1H), 12.30 (br, 1H), 11.03 (s, 1H), 10.46 (s, 1H), 9.33 (br, 1H), 9.22 (d, *J* = 1.3 Hz, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.60 (dd, *J* = 8.2, 2.0 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.00 – 7.89 (m, 6H), 7.83 (dd, *J* = 13.2, 8.8 Hz, 5H), 7.62 (s, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.60 (br, 1H), 2.69 (d, *J* = 7.3 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.3, 166.9, 165.8, 164.0, 161.6, 152.3, 151.5, 142.4, 142.3, 140.9, 136.5, 130.2, 129.4, 128.3, 128.2, 122.9, 122.5, 120.3, 119.7, 118.9, 116.6, 111.7, 51.6, 36.8, 22.4.

HRMS (ESI) calculated for C42H35N8O10 (M-H$^+$) 811.2482, found 811.2461.

(S)-4-(4-(4-(4-Amino-2-(4-(3-chloro-4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (91)



Chemical Formula: $C_{43}H_{36}ClN_7O_{10}$
Exact Mass: 845,2212

Amine **12** (25 mg, 0.033 mmol) was coupled with 3-chloro-4-cyanobenzoic acid using coupling conditions A followed by final deprotection.

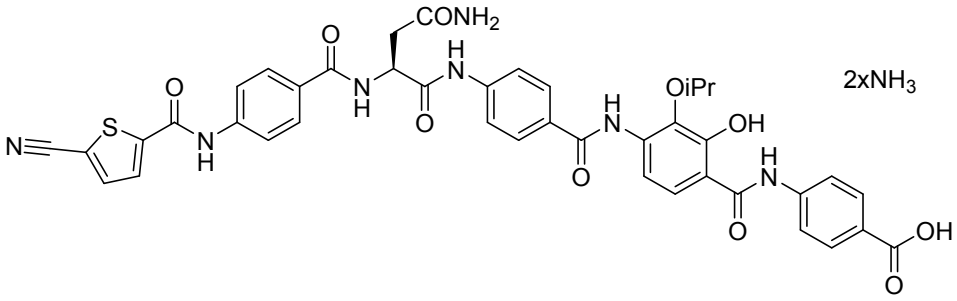
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 22.85 min) to obtain 4.5 mg of desired product (0.0053 mmol, y= 16%).

^{1}H NMR (500 MHz, DMSO) δ 12.78 (br, 1H), 12.30 (br, 1H), 10.76 (s, 1H), 10.46 (s, 1H), 9.36 (br, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.29 (d, *J* = 1.6 Hz, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 8.07 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.98 – 7.91 (m, 5H), 7.91 – 7.78 (m, 6H), 7.65 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (q, *J* = 7.1 Hz, 1H), 4.57 (br, 1H), 2.69 (d, *J* = 7.1 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.7, 168.4, 166.9, 165.7, 164.1, 163.1, 142.5, 141.3, 140.3, 136.4, 135.6, 134.9, 130.2, 129.3, 128.9, 128.4, 128.2, 127.3, 122.9, 120.5, 119.5, 118.9, 115.6, 114.5, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for C43H37ClN7O10 (M+H^{+}) 846.2285, found 846.2297.

(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanothiophene-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (32)



Chemical Formula: $C_{41}H_{35}N_7O_{10}S$
Exact Mass: 817,2166

Amine **12** (25 mg, 0.033 mmol) was coupled with 5-cyanothiophene-2-carboxylic acid using coupling conditions A followed by final deprotection.

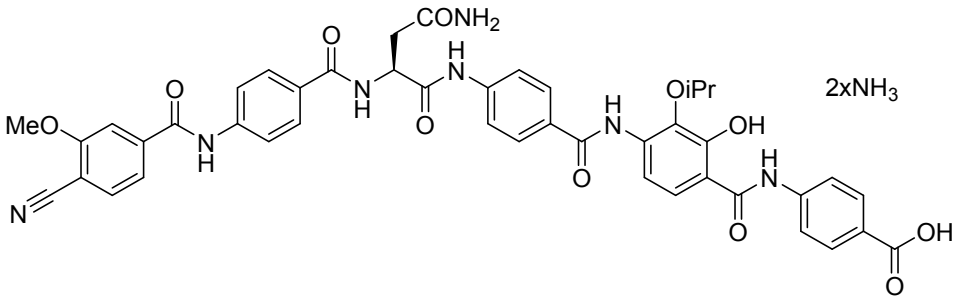
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 21.17 min) to obtain 6.6 mg of desired product (0.0053 mmol, y= 24%).

^{1}H NMR (500 MHz, DMSO) δ 12.71 (br, 1H), 12.35 (br, 1H), 10.79 (s, 1H), 10.46 (s, 1H), 9.34 (br, 1H), 8.70 (d, *J* = 7.3 Hz, 1H), 8.15 (d, *J* = 4.1 Hz, 1H), 8.08 (d, *J* = 4.1 Hz, 1H), 7.98 - 7.92 (m, 6H), 7.87 - 7.77 (m, 7H), 7.64 (br, 1H), 7.40 (s, 1H), 7.00 (s, 1H), 4.93 (dd, *J* = 14.1, 7.1 Hz, 1H), 4.61 (br, 1H), 2.70 (d, *J* = 7.3 Hz, 2H), 1.26 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.7, 168.3, 166.9, 165.7, 164.1, 158.5, 146.8, 142.4, 140.8, 139.7, 136.7, 136.5, 130.2, 129.5, 129.2, 128.4, 128.2, 125.8, 122.9, 120.4, 119.7, 118.9, 113.8, 112.8, 112.5, 74.3, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for C41H36N7O10S (M+H^{+}) 818.2239, found 818.2237.

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-3-methoxybenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (92)



Chemical Formula: $C_{44}H_{39}N_7O_{11}$
Exact Mass: 841,2708

Amine **12** (25 mg, 0.033 mmol) was coupled with 4-cyano-3-methoxybenzoic acid using coupling conditions A followed by final deprotection.

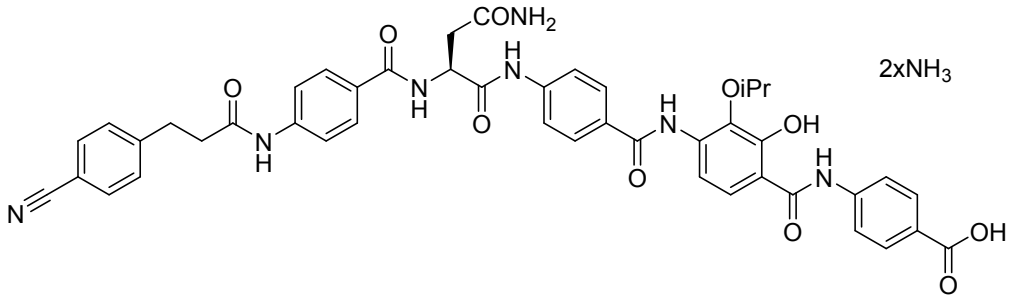
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 22.02 min) to obtain 4.8 mg of desired product (0.0057 mmol, y= 17%).

^{1}H NMR (500 MHz, DMSO) δ 12.80 (br, 1H), 12.29 (br, 1H), 10.66 (s, 1H), 10.47 (s, 1H), 9.38 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 7.98 – 7.92 (m, 7H), 7.91 – 7.79 (m, 7H), 7.72 (d, *J* = 1.3 Hz, 1H), 7.69 – 7.63 (m, 2H), 7.40 (s, 1H), 7.00 (s, 1H), 4.92 (q, *J* = 7.1 Hz, 1H), 4.61 – 4.53 (m, 1H), 4.03 (s, 3H), 2.69 (d, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 8.4 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.7, 168.4, 166.9, 165.7, 164.4, 164.1, 160.8, 142.5, 142.1, 141.5, 140.7, 136.9, 136.4, 133.9, 130.2, 129.2, 128.3, 128.3, 126.1, 122.9, 120.6, 120.1, 119.6, 118.9, 115.9, 112.6, 111.4, 103.0, 74.6, 56.6, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for C44H40N7O11 ($M+H^+$) 842.2780, found 842.2784.

(S)-4-(4-(4-(4-Amino-2-(4-(3-(4-cyanophenyl)propanamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (48)



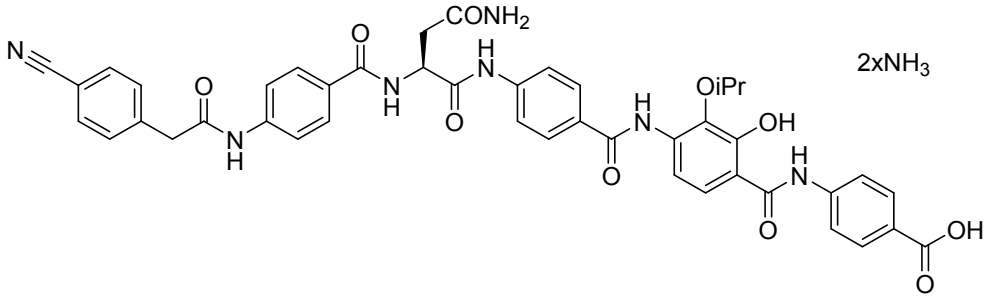
Chemical Formula: $C_{45}H_{41}N_7O_{10}$
Exact Mass: 839,2915

Amine **12** (25 mg, 0.033 mmol) was coupled with 3-(4-cyanophenyl)propanoic acid using coupling conditions A followed by final deprotection.

The desired compound was purified by preparative RP-HPLC condition B (retention time: 21.17 min) to obtain 4.7 mg of desired product (0.0057 mmol, y= 17%).

^{1}H NMR (500 MHz, DMSO) δ 12.80 (br, 1H), 12.29 (br, 1H), 10.63 (br, 1H), 10.44 (s, 1H), 10.19 (s, 1H), 9.39 (s, 1H), 8.60 (d, *J* = 7.3 Hz, 1H), 8.00 – 7.91 (m, 4H), 7.89 – 7.78 (m, 7H), 7.78 – 7.74 (m, 2H), 7.72 – 7.64 (m, 3H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.38 (s, 1H), 6.98 (s, 1H), 4.90 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.55 (dt, *J* = 12.1, 6.0 Hz, 1H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.71 (t, *J* = 7.6 Hz, 2H), 2.67 (d, *J* = 7.6 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).
^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 170.4, 168.4, 166.9, 165.8, 164.2, 147.3, 142.5, 142.0, 141.9, 137.0, 136.3, 132.2, 130.2, 129.4, 128.4, 128.3, 128.2, 126.2, 122.8, 120.6, 119.0, 118.9, 118.1, 112.5, 112.2, 108.9, 74.8, 51.6, 37.1, 36.8, 30.6, 22.3.
HRMS (ESI) calculated for C45H42N7O10 (M+H^{+}) 840.2988, found 840.2971.

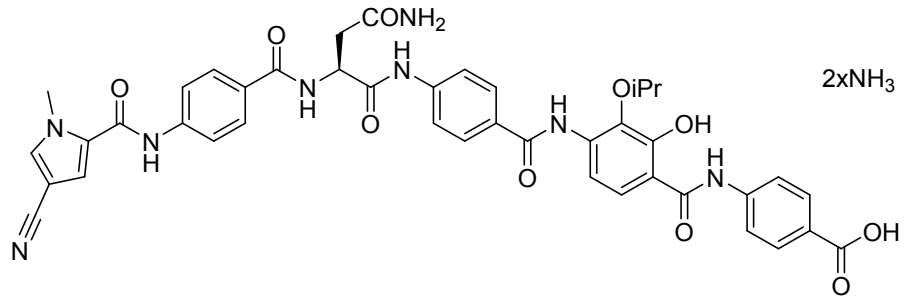
(S)-4-(4-(4-(4-Amino-2-(4-(2-(4-cyanophenyl)acetamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (47)



Chemical Formula: $C_{44}H_{39}N_7O_{10}$
Exact Mass: 825,2758

Amine **12** (25 mg, 0.033 mmol) was coupled with 2-(4-cyanophenyl)acetic acid using coupling conditions A followed by final deprotection.
The desired compound was purified by preparative RP-HPLC condition B (retention time: 19.98 min) to obtain 6.4 mg of desired product (0.0078 mmol, y= 24%).
^{1}H NMR (500 MHz, DMSO) δ 12.74 (br, 1H), 12.30 (br, 1H), 10.49 (s, 1H), 10.44 (s, 1H), 9.35 (br, 1H), 8.62 (d, *J* = 7.3 Hz, 1H), 7.94 (dd, *J* = 11.1, 8.9 Hz, 4H), 7.90 – 7.74 (m, 9H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.65 (br, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.39 (s, 1H), 6.98 (s, 1H), 4.90 (q, *J* = 7.1 Hz, 1H), 4.58 (s, 1H), 3.82 (s, 2H), 2.67 (d, *J* = 7.0 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).
^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.5, 168.4, 166.9, 165.7, 164.1, 142.4, 141.8, 141.5, 136.4, 132.2, 132.1, 131.9, 130.5, 130.2, 128.4, 128.2, 122.9, 122.5, 120.5, 118.9, 118.2, 109.5, 74.4, 51.6, 43.0, 36.8, 22.3.
HRMS (ESI) calculated for C44H38N7O10 (M-H^{+}) 824.2686, found 824.2689.

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-1-methyl-1H-pyrrole-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (34)



Chemical Formula: $C_{42}H_{38}N_8O_{10}$
Exact Mass: 814,2711

Amine **12** (20 mg, 0.033 mmol) was coupled with 4-cyano-1-methyl-1H-pyrrole-2-carboxylic acid using coupling conditions C. Allyl protected intermediate was purified on silica gel with a gradient 0-10% MeOH in DCM.

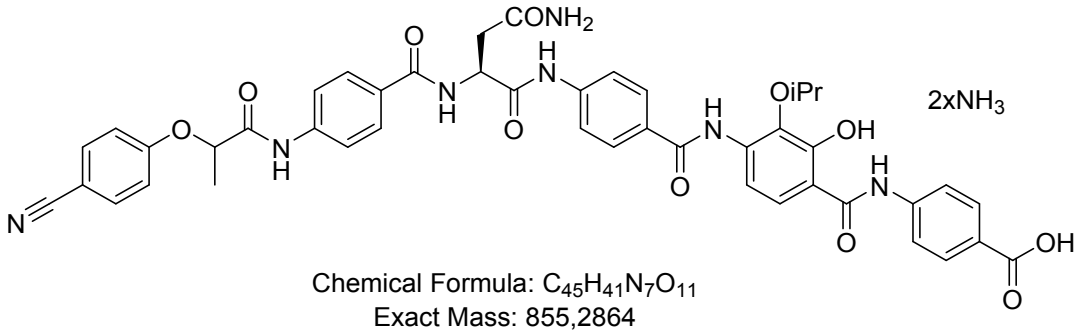
Final deprotection afforded the desired compound, which was purified by preparative RP- RP-HPLC using condition B (retention time: 19.77 min) to obtain 1.4 mg of desired product (0.002 mmol, y= 8%).

^{1}H NMR (500 MHz, DMSO) δ 12.51 (br, 1H), 10.43 (s, 1H), 10.24 (s, 1H), 8.99 (br, 1H), 8.64 (d, *J* = 7.3 Hz, 1H), 7.92 – 7.88 (m, 2H), 7.88 – 7.83 (m, 5H), 7.83 – 7.75 (m, 6H), 7.53 (br, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.39 (s, 1H), 7.23 (br, 1H), 6.98 (s, 1H), 4.92 (dd, *J* = 14.1, 7.1 Hz, 2H), 3.92 (s, 3H), 2.68 (d, *J* = 7.2 Hz, 2H), 1.21 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 167.6, 167.1, 165.7, 163.4, 158.6, 142.1, 141.6, 135.0, 130.4, 129.2, 128.7, 128.3, 127.8, 126.9, 123.5, 119.1, 119.0, 116.4, 115.9, 90.0, 51.6, 37.1, 36.8, 22.6.

HRMS (ESI) calculated for C42H39N8O10 ($M+H^+$) 815.2784, found 815.2777.

4-(4-(4-((2S)-4-Amino-2-(4-(2-(4-cyanophenoxy)propanamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (49)



Chemical Formula: $C_{45}H_{41}N_7O_{11}$
Exact Mass: 855,2864

Amine **12** (25 mg, 0.033 mmol) was coupled with 2-(4-cyanophenoxy)propanoic acid using coupling conditions A followed by final deprotection.

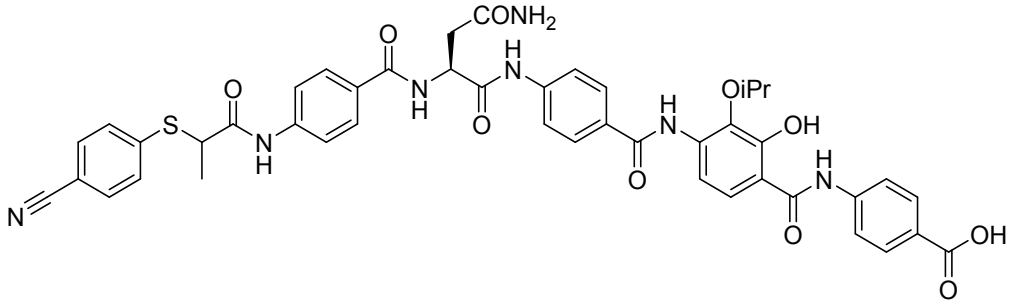
The desired compound was purified by preparative RP-HPLC condition B (retention time: 20.88 min) to obtain 6.9 mg of desired product (0.0081 mmol, y= 24%).

^{1}H NMR (500 MHz, DMSO) δ 12.61 (br, 2H), 10.45 (s, 2H), 9.27 (br, 1H), 8.64 (d, *J* = 7.3 Hz, 1H), 7.97 – 7.85 (m, 6H), 7.85 – 7.77 (m, 6H), 7.75 (br, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.55 (br, 1H), 7.39 (s, 1H), 7.15 – 7.08 (m, 2H), 6.98 (s, 1H), 5.07 (q, *J* = 6.6 Hz, 1H), 4.90 (q, *J* = 7.1 Hz, 1H), 4.66 (br, 1H), 2.70 – 2.65 (m, 2H), 1.59 (d, *J* = 6.6 Hz, 3H), 1.24 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 171.0, 169.4, 168.2, 167.0, 165.7, 163.9, 160.7, 142.4, 141.1, 136.6, 134.3, 130.3, 128.9, 128.6, 128.4, 128.1, 123.0, 120.1, 119.0, 118.9, 118.9, 116.0, 103.5, 73.7, 51.6, 36.8, 22.4, 18.3.

HRMS (ESI) calculated for C45H42N7O11 ($M+H^+$) 856.2937, found 856.2938.

4-(4-(4-((2S)-4-Amino-2-(4-(2-((4-cyanophenyl)thio)propanamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (50)



Chemical Formula: $C_{45}H_{41}N_7O_{10}S$
Exact Mass: 871,2636

Amine **12** (25 mg, 0.033 mmol) was coupled with 2-((4-cyanophenyl)thio)propanoic acid using coupling conditions A followed by final deprotection.

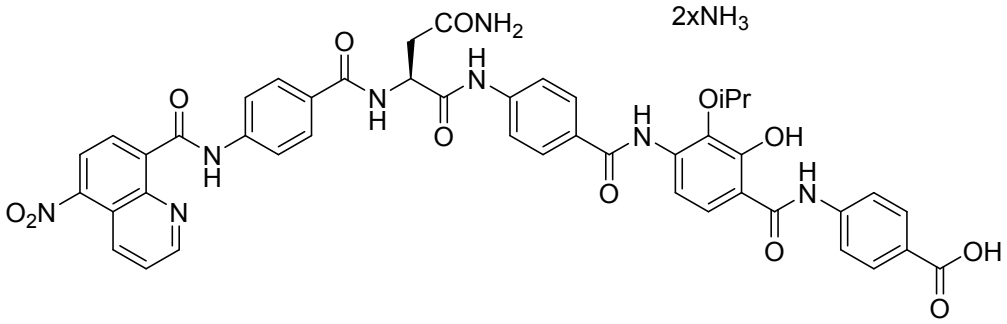
The desired compound was purified by preparative RP-HPLC using condition B (retention time: 22.37 min) to obtain 6.0 mg of desired product (0.0069 mmol, y= 21%).

^{1}H NMR (500 MHz, DMSO) δ 12.78 (br, 1H), 12.30 (br, 1H), 10.66 (br, 1H), 10.50 (s, 1H), 10.45 (s, 1H), 9.35 (br, 1H), 8.63 (d, *J* = 7.3 Hz, 1H), 7.95 (t, *J* = 9.5 Hz, 5H), 7.91 – 7.83 (m, 5H), 7.80 (ddd, *J* = 8.7, 4.6, 2.6 Hz, 6H), 7.66 (d, *J* = 8.8 Hz, 4H), 7.59 – 7.49 (m, 3H), 7.39 (s, 1H), 6.98 (s, 1H), 4.90 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.58 (s, 1H), 4.34 (q, *J* = 6.9 Hz, 1H), 2.69 – 2.66 (m, 2H), 1.53 (d, *J* = 6.9 Hz, 3H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 171.3, 170.7, 169.6, 168.4, 166.9, 165.7, 164.1, 142.5, 142.0, 141.4, 136.5, 132.7, 130.2, 128.9, 128.8, 128.5, 128.2, 122.9, 120.5, 118.9, 118.6, 118.5, 108.5, 51.6, 45.1, 36.8, 22.3, 17.8.

HRMS (ESI) calculated for C45H42N7O10S ($M+H^+$) 872.2708, found 872.2715.

(S)-4-(4-(4-(4-Amino-2-(4-(5-nitroquinoline-8-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (36)



Chemical Formula: $C_{45}H_{38}N_8O_{12}$
Exact Mass: 882,2609

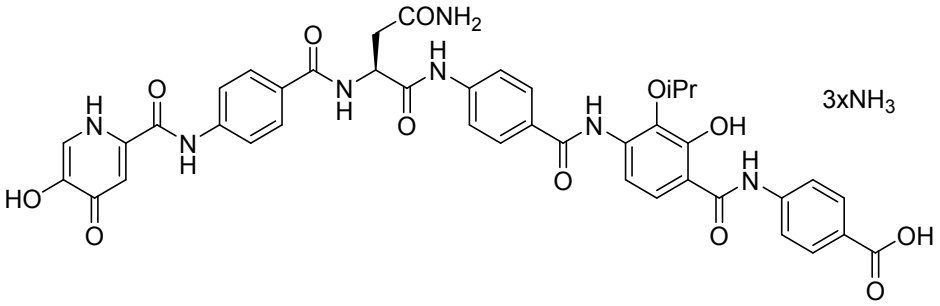
Amine **12** (25 mg, 0.033 mmol) was coupled with 5-nitroquinoline-8-carboxylic acid using coupling conditions A followed by final deprotection.

The desired compound was purified by preparative RP-HPLC using condition B (retention time: 25.29 min) to obtain 2.1 mg of desired product (0.0024 mmol, y= 7%).

^{1}H NMR (500 MHz, DMSO) δ 12.83 (br, 1H), 12.29 (br, 1H), 12.08 (s, 1H), 10.62 (br, 1H), 10.48 (s, 1H), 9.40 (s, 1H), 9.25 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.93 (dd, *J* = 8.9, 1.6 Hz, 1H), 8.71 (d, *J* = 7.3 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.02 – 7.90 (m, 8H), 7.84 (dd, *J* = 13.5, 8.7 Hz, 5H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.41 (s, 1H), 7.01 (s, 1H), 4.94 (dd, *J* = 14.1, 7.0 Hz, 1H), 4.59 – 4.51 (m, 1H), 2.74 – 2.67 (m, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

HRMS (ESI) calculated for C45H39N8O12 ($M+H^+$) 883.2682, found 883.2682.

(S)-4-(4-(4-(4-Amino-2-(4-(5-hydroxy-4-oxo-1,4-dihydropyridine-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (35)



Chemical Formula: $C_{41}H_{37}N_7O_{12}$
Exact Mass: 819,2500

Amine **12** (25 mg, 0.033 mmol) was coupled with 4,5-bis(allyloxy)picolinic acid (**105**) using coupling conditions A followed by final deprotection.

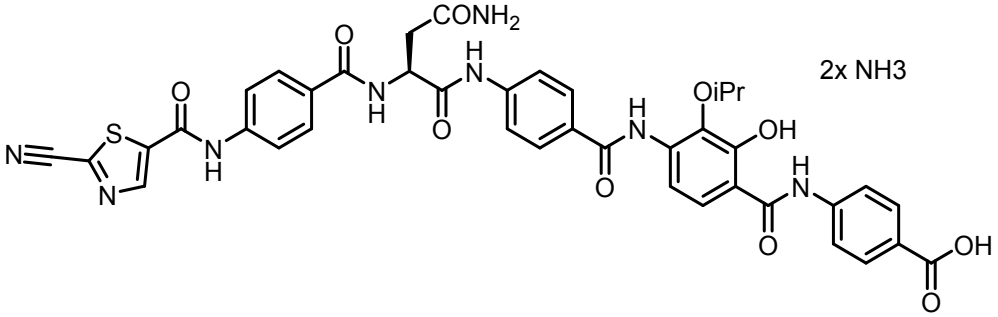
Purification by preparative RP-HPLC following condition B (retention time: 12.99 min) was done to obtain 11.5 mg of desired compound (0.0136 mmol, y= 41%).

^{1}H NMR (700 MHz, DMSO) δ 15.83 (br, 1H), 10.51 (s, 1H), 10.41 (s, 1H), 8.88 (s, 1H), 8.62 (d, *J* = 7.4 Hz, 1H), 8.49 (s, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.83 (t, *J* = 8.0 Hz, 5H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.39 (s, 1H), 7.32 (br, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 6.97 (s, 1H), 5.03 – 4.98 (m, 1H), 4.91 (dd, *J* = 14.1, 7.3 Hz, 1H), 2.68 (d, *J* = 8.1 Hz, 2H), 1.19 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.4, 170.7, 170.3, 166.9, 165.9, 165.3, 164.6, 163.1, 142.0, 141.7, 137.5, 134.0, 131.1, 129.7, 129.5, 128.3, 128.1, 127.6, 127.5, 127.0, 124.2, 123.7, 119.1, 118.0, 117.6, 116.0, 107.7, 100.7, 70.4, 51.7, 36.9, 22.7.

HRMS (ESI) calculated for C41H38N7O12 ($M+H^+$) 820.2573, found 820.2571.

(S)-4-(4-(4-(4-amino-2-(4-(5-cyanothiophene-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (33)



Chemical Formula: $C_{40}H_{34}N_8O_{10}S$
Exact Mass: 818,2119

Amine **12** (25 mg, 0.033 mmol) was coupled with 2-cyanothiazole-5-carboxylic acid using coupling conditions A followed by final deprotection.

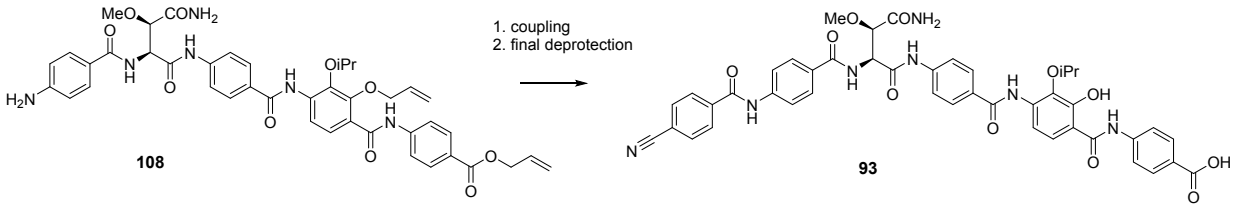
Purification by preparative RP-HPLC following condition B (retention time: 20.07 min) was done to obtain 7 mg of desired compound (0.0086 mmol, y= 26%).

^{1}H NMR (700 MHz, DMSO) δ 12.30 (br, 1H), 10.97 (s, 1H), 10.46 (s, 1H), 9.34 (br, 1H), 8.91 (s, 1H), 8.70 (d, *J* = 7.3 Hz, 1H), 7.94 (t, *J* = 9.2 Hz, 6H), 7.87 – 7.76 (m, 7H), 7.63 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.60 (br, 1H), 2.69 (d, *J* = 7.8 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.7, 168.3, 166.9, 165.7, 164.1, 157.3, 145.2, 142.4, 141.9, 140.6, 138.9, 136.5, 130.2, 129.7, 128.5, 128.2, 122.9, 120.3, 119.7, 118.9, 112.9, 51.6, 36.7, 22.3.

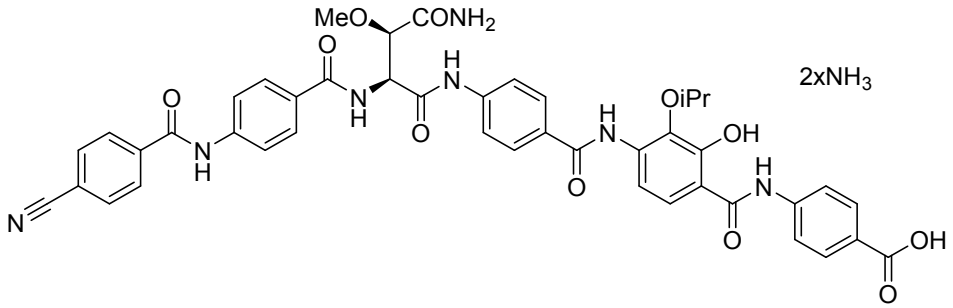
HRMS (ESI) calculated for C40H35N8O10S (M+H) 819.2191, found 819.2191.

For the synthesis of compound **93**, amine **108**, synthesied as previously published, [14] was used for the last two steps**.**



Scheme S 2 Last two steps of the synthesis of compound bearing the methoxy asparagine as for natural cystobactamid 861-2.

4-(4-(4-((2S,3R)-4-Amino-2-(4-(4-cyanobenzamido)benzamido)-3-methoxy-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (93)



Chemical Formula: $C_{44}H_{39}N_7O_{11}$
Exact Mass: 841,27076

Amine **108** (12 mg, 0.015 mmol) was coupled to 4-cyanobenzoic acid using coupling conditions A followed by final deprotection.

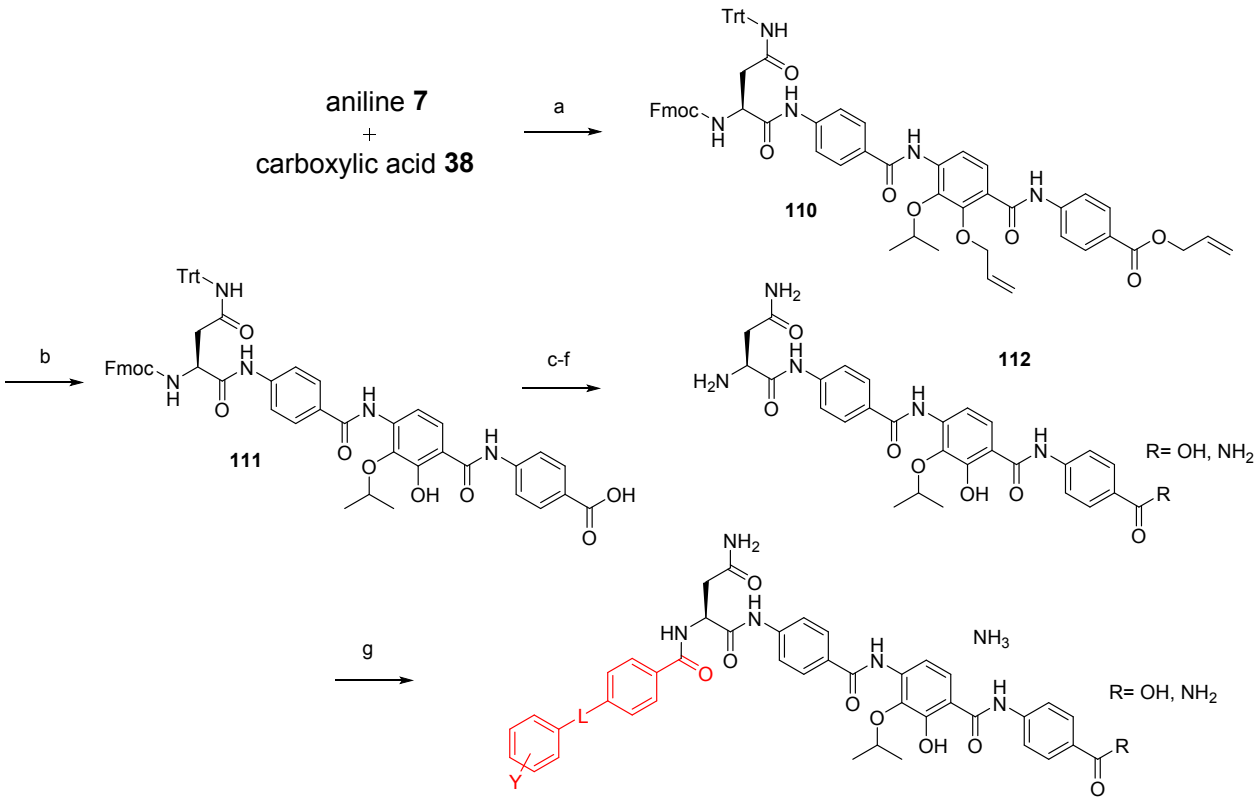
Purification by preparative RP-HPLC using condition B (retention time: 21.59 min) afforded 5.2 mg of desired compound (0.0062 mmol, y= 41%).

^{1}H NMR (700 MHz, DMSO) δ 12.70 (br, 1H), 12.28 (br, 1H), 10.72 (s, 1H), 10.57 (s, 1H), 9.34 (br, 1H), 8.46 (d, *J* = 8.1 Hz, 1H), 8.14 – 8.11 (m, 2H), 8.04 (d, *J* = 8.4 Hz, 2H), 7.95 (dd, *J* = 8.2, 5.4 Hz, 4H), 7.92 – 7.86 (m, 4H), 7.84 (d, *J* = 8.6 Hz, 4H), 7.80 (br, 1H), 7.62 (br, 1H), 7.54 (s, 1H), 7.47 (s, 1H), 4.92 (t, *J* = 8.1 Hz, 1H), 4.60 (br, 1H), 4.09 (d, *J* = 8.1 Hz, 1H), 3.31 (s, 3H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 170.9, 168.7, 168.3, 166.9, 165.5, 164.5, 164.1, 142.2, 141.8, 138.7, 136.5, 132.5, 130.2, 128.9, 128.6, 128.3, 122.9, 120.4, 119.6, 119.0, 118.3, 114.0, 107.0, 80.0, 57.7, 55.8, 45.8, 22.4.

HRMS (ESI) calculated for C44H40N7O11 ($M+H^+$) 842.2780, found 842.2771.

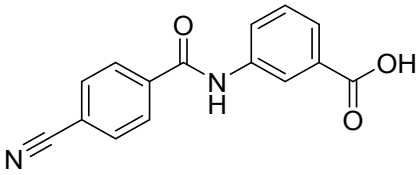
1.2.2.2.5 Second synthetic access, modification rings A-B



Scheme S 3. Reagent and conditions: a) $POCl_3$, DiPEA, THF/DCM 1:1, rt, 6 h (76%); b) $Pd(PPh_3)_4$; $PhSiH_3$, THF, rt, 3h (58%); c) DAST, DCM, 0°C, 20 min, d) NH_3 in MeOH, 0 °C, 15 min, e) TFA, Tips, DCM, rt, 2h, f) 20% Et_2NH in CH_3CN, rt, 30 min, g) coupling with activated building block A1 (y=5-32% o3-5s). In the case of the compounds bearing R=OH, steps c) and d) were skipped.

1.2.2.2.5.1 Fragment synthesis

3-(4-Cyanobenzamido)benzoic acid (113)

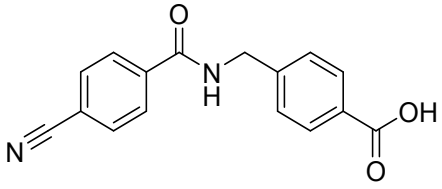


Chemical Formula: $C_{15}H_{10}N_2O_3$
Exact Mass: 266,0691

3-Aminobenzoic acid (230 mg; 1.68 mmol) was dissolved in THF (1.2 mL) and $NaHCO_3$ saturated solution (1.2 mL), to it 4-cyanobenzoyl chloride (277 mg; 1.68 mmol) was added at 0 °C. The reaction was stirred for 2 hours, pH adjusted to 1, the precipitate was collected by filtration, washed with HCl 1 N, triturated with Et_2O and dried at high vacuum to give 280 mg of a white solid (1.05 mmol; y= 63%).

^{1}H NMR (500 MHz, DMSO) δ 13.02 (br, 1H), 10.67 (s, 1H), 8.42 (t, *J* = 1.8 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 8.4 Hz, 3H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 1H).
^{13}C NMR (126 MHz, DMSO) δ 167.1, 164.3, 139.0, 138.6, 132.5, 131.3, 129.0, 128.6, 124.8, 124.5, 121.2, 118.3, 114.0.
HRMS (ESI) calculated for C15H9N2O3 (M-H^{+}) 265.0619, found 265.0606.

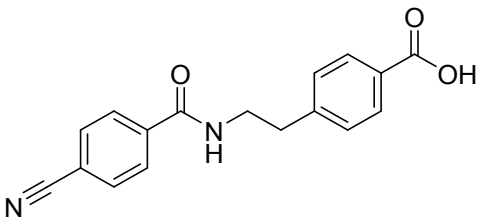
4-((4-Cyanobenzamido)methyl)benzoic acid (114)



Chemical Formula: $C_{16}H_{12}N_2O_3$
Exact Mass: 280,0848

4-(Aminomethyl)benzoic acid (287 mg; 1.9 mmol) was dissolved in THF (2.0 mL) and NaOH 1 N (5.7 mL), to it 4-cyanobenzoyl chloride (314 mg; 1.9 mmol) was added at 0 °C. Reaction stirred for 2 hours, pH adjusted to 1, compound extracted with EtOAc (50 mL), organic phase washed with brine (50 mL) dried over sodium sulphate and reduced under vacuum. The crude was chromatographed on silica gel with a gradient 0-10% MeOH in DCM to give 100 mg of a white solid (0.36 mmol; y= 19%).
^{1}H NMR (500 MHz, DMSO) δ 12.87 (br, 1H), 9.37 (t, *J* = 5.9 Hz, 1H), 8.08 – 8.02 (m, 2H), 8.01 – 7.96 (m, 2H), 7.94 – 7.88 (m, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 4.56 (d, *J* = 5.9 Hz, 2H).
^{13}C NMR (126 MHz, DMSO) δ 167.2, 165.0, 144.3, 138.1, 132.5, 129.4, 128.1, 127.2, 118.3, 113.7, 42.6.
HRMS (ESI) calculated for C16H11N2O3 (M-H^{+}) 279.0775, found 279.0794.

4-(2-(4-Cyanobenzamido)ethyl)benzoic acid (115)



Chemical Formula: $C_{17}H_{14}N_2O_3$
Exact Mass: 294,1004

4-(2-Aminoethyl)benzoic acid (165 mg; 0.82 mmol) was dissolved in THF (3.0 mL) and NaOH 1 N (3.5 mL), to it 4-cyanobenzoyl chloride (108 mg; 0.66 mmol) was added at 0 °C. Reaction stirred for 1.5 hours, pH adjusted to 1, compound extracted with EtOAc (30 mL), organic phase washed with brine (30 mL) dried over sodium sulphate and reduced under vacuum. The crude

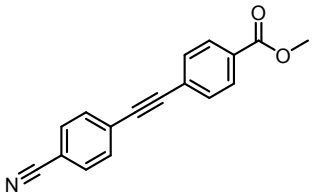
was chromatographed on silica gel with a gradient 0-10% MeOH in DCM to give 45 mg of a white solid (0.14 mmol; y= 21%).

^{1}H NMR (500 MHz, DMSO) δ 12.82 (br, 1H), 8.83 (t, *J* = 5.6 Hz, 1H), 7.98 – 7.92 (m, 4H), 7.88 – 7.84 (m, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 3.53 (dd, *J* = 13.0, 7.1 Hz, 2H), 2.93 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 167.2, 164.8, 144.7, 138.5, 132.4, 129.4, 128.9, 128.0, 118.3, 113.5, 40.5, 34.8.

HRMS (ESI) calculated for C17H13N2O3 (M-H^{+}) 293.0932, found 293.0951.

Methyl 4-((4-cyanophenyl)ethynyl)benzoate (116)



Chemical Formula: $C_{17}H_{11}NO_2$
Exact Mass: 261,0790

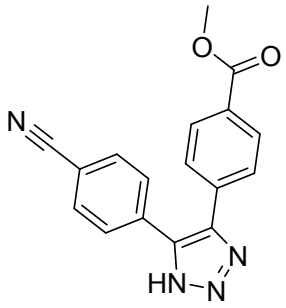
4-Iodobenzonitrile (100 mg, 0.437 mmol), methyl 4-ethynylbenzoate (77 mg, 0.481 mmol), Bis(triphenylphosphine)palladium(II) dichloride (15 mg, 0.022 mmol), copper iodide (4.2 mg, 0.022 mmol) and triphenylphosphine (5.7 mg, 0.022 mmol) were mixed together under a N_2 atmosphere in THF (1.7 mL), subsequently TEA (1.7 mL) was added. The reaction was stirred for 5 hours and then diluted with EtOAc (20 mL) and water (20 mL). The watery phase was extracted with EtOAc (2x5 mL). The combined organic phases were washed with HCl 1 N, $NaHCO_3$ saturated solution and brine, dried over sodium sulphate and reduced under vacuum. The crude residue was purified on silica gel with a gradient 2-20% EtOAc in Pet.Et. to give 440 mg of desired product (0.440 mmol, y=q.).

^{1}H NMR (500 MHz, DMSO) δ 8.04 – 7.99 (m, 2H), 7.95 – 7.91 (m, 2H), 7.81 – 7.77 (m, 2H), 7.77 – 7.72 (m, 2H), 3.88 (s, 3H).

^{13}C NMR (126 MHz, DMSO) δ 165.5, 132.7, 132.3, 132.0, 129.9, 129.5, 126.5, 126.1, 118.4, 111.5, 92.1, 90.6, 52.4.

HRMS (ESI) calculated for C17H12NO2 (M+H) 262.0863, found 262.0861.

Methyl 4-(5-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)benzoate (117)



Chemical Formula: $C_{17}H_{12}N_4O_2$
Exact Mass: 304,0960

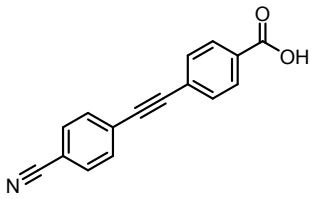
Alkyne **116** (31 mg, 0.119 mmol) and NaN_3 were mixed in DMF (1.2 mL), the reaction mixture was heated to 100 °C for 1 hour using a MW reactor. The reaction was diluted with EtOAc (12 mL) and brine (12 mL) and the layers were separated , the watery phase was extracted twice with EtOAc (5 mL). The combined organic phases were dried over sodium sulphate and reduced under vacuum. Te residue thus obtained was chromatographed on silica gel with a gradient 5-40% EtOAc in Pet.Et to give 21 mg of desired product (0.069 mmol, y= 58%).

^{1}H NMR (500 MHz, MeOD/THF-d8 3:1) δ 8.11 – 8.04 (m, 2H), 7.82 – 7.76 (m, 2H), 7.76 – 7.70 (m, 2H), 7.68 – 7.62 (m, 2H), 3.93 (s, 3H).

^{13}C NMR (126 MHz, MeOD/THF-d8 3:1) δ 167.7, 133.9, 131.7, 131.2, 130.1, 129.6, 119.5, 113.4, 52.9.

HRMS (ESI) calculated for C17H13N4O2 (M+H) 305.1033, found 305.1033.

4-((4-Cyanophenyl)ethynyl)benzoic acid (118)



Chemical Formula: $C_{17}H_{11}NO_2$
Exact Mass: 261,0790

Alkyne **116** (150 mg, 0.574 mmol) was dissolved in THF (2.9 mL) and water (1.4 mL), the solution was cooled to 0 °C and a solution of LiOH (137 mg, 5.74 mmol) in water (1.5 mL) was added. The reaction was stirred overnight at r.t., the pH was adjusted to 2, the precipitate thus formed was extracted with EtOAc (3x 20mL). The organic phases reunited were dried over sodium sulphate and reduced under vacuum to give 143 mg of desired product (0.578 mmol, y= q.).

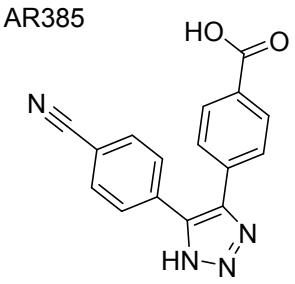
^{1}H NMR (700 MHz, DMSO) δ 13.21 (s, 1H), 8.04 – 7.95 (m, 2H), 7.95 – 7.89 (m, 2H), 7.84 – 7.75 (m, 2H), 7.75 – 7.69 (m, 2H).

^{13}C NMR (176 MHz, DMSO) δ 166.6, 132.7, 132.3, 131.8, 131.2, 129.6, 126.6, 125.7, 118.4, 111.4, 92.3, 90.3.

HRMS (ESI) calculated for C16H10NO2 (M+H) 248.0706, found 248.0707.

4-(5-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)benzoic acid (119)

AR385



Chemical Formula: $C_{17}H_{12}N_4O_2$
Exact Mass: 304,0960

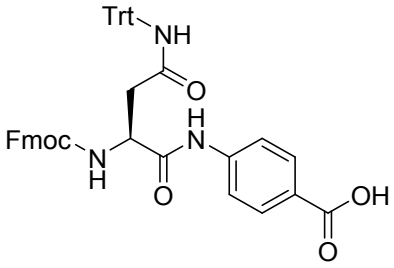
Triazole **117** (163 mg, 0.536 mmol) was dissolved in THF (2.7 mL) and water (1.2 mL), the solution was cooled to 0 °C and a solution of LiOH (128 mg, 5.36 mmol) in water (1.5 mL) was added. The reaction was stirred overnight at r.t., the pH was adjusted to 2, the precipitate thus formed was extracted with EtOAc (3x 20mL). The organic phases reunited were dried over sodium sulphate and reduced under vacuum to give 143 mg of desired product (0.493 mmol, y= 92%).

^{1}H NMR (500 MHz, DMSO) δ 15.65 (br, 1H), 13.10 (br, 1H), 8.00 (d, *J* = 8.3 Hz, 2H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.72 – 7.65 (m, 2H), 7.66 – 7.55 (m, 2H).

^{13}C NMR (126 MHz, DMSO) δ 166.9, 142.6, 132.8, 130.8, 129.9, 128.5, 128.2, 124.9, 118.6, 111.0.

HRMS (ESI) calculated for C16H11N4O2 (M+H) 291.0877, found 291.0879.

(S)-4-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoic acid (38)



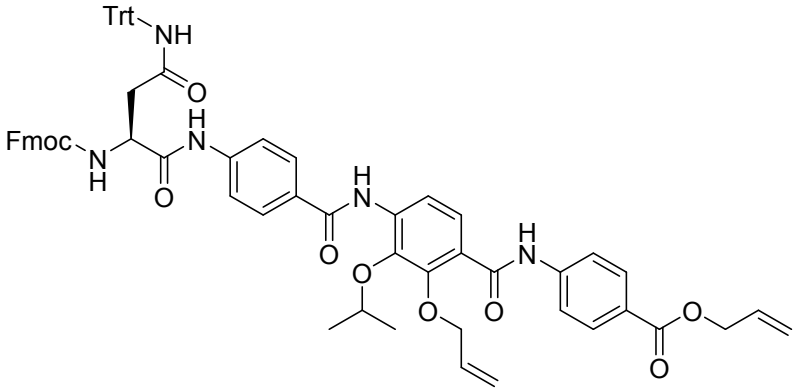
Chemical Formula: $C_{45}H_{37}N_3O_6$
Exact Mass: 715,26824

Methyl (S)-4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoate (1.0 g; 1.37 mmol), lithium iodide (1.8 g; 13.72 mmol) were mixed in EtOAc (14 mL) and heated at 110 °C for 12 h in a microwave synthesizer. Mixture was diluted with

EtOAc (100 mL) and HCL 1N (100 mL), organic phase washed with brine (100 mL), dried over sodium sulphate and reduced under vacuum. The residue was chromatographed on silica gel with a gradient 0-5% MeOH in DCM to give 0.74 g of a white solid (1.03 mmol; y= 75%).
^{1}H NMR (500 MHz, DMSO) δ 12.70 (s, 1H), 10.41 (s, 1H), 8.62 (s, 1H), 7.90 (dd, *J* = 8.1, 3.9 Hz, 4H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.77 – 7.70 (m, 4H), 7.42 (dd, *J* = 13.7, 7.0 Hz, 2H), 7.34 – 7.26 (m, 2H), 7.25 – 7.14 (m, 15H), 4.49 – 4.41 (m, 1H), 4.36 (dd, *J* = 10.4, 7.0 Hz, 1H), 4.30 (dd, *J* = 10.4, 7.0 Hz, 1H), 4.23 (t, *J* = 6.9 Hz, 1H), 2.75 (dd, *J* = 14.6, 9.8 Hz, 1H), 2.66 – 2.58 (dd, *J* = 14.0, 4.4 Hz, 1H).
^{13}C NMR (126 MHz, DMSO) δ 170.8, 168.4, 166.9, 155.8, 144.7, 143.8, 143.0, 140.7, 130.3, 128.6, 127.6, 127.4, 127.1, 126.3, 125.3, 125.2, 120.1, 118.6, 69.4, 65.8, 52.8, 46.7, 38.4.
HRMS (ESI) calculated for C45H38N3O6 ($M+H^+$) 716.2755, found 716.2745.
Marfey: 93.9% S enantiomer, 6.1% R enantiomer

1.2.2.2.5.2 Final assembly

Allyl (S)-4-(4-(4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-(allyloxy)-3-isopropoxybenzamido)benzoate (110)



Chemical Formula: $C_{68}H_{61}N_5O_{10}$
Exact Mass: 1107,44184

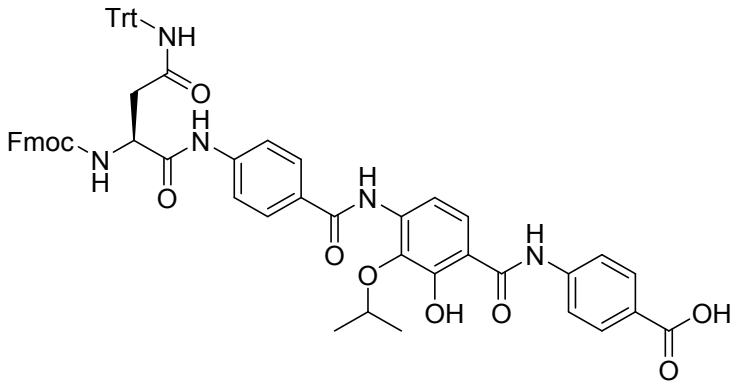
$POCl_3$ (1.92 mmol) as a solution in DCM (1:9) was added dropwise to a solution of allyl 4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)benzoate (0.315 g; 0.77 mmol) and (S)-4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoic acid (1.37 g, 1.92 mmol) in THF (8 mL) and DCM (4 mL) at 0 °C, followed by DiPEA (1.78 mL; 10.24 mmol) as a solution in DCM (1:1). Reaction stirred at r.t. for 6 h, quenched with HCL 1 N and ice, solvent partially reduced under vacuum and residue diluted with EtOAc (200 mL) and HCl 1N (200 mL), organic phase washed with brine (200 mL) and dried over sodium sulphate. Solvent removed under vacuum, the crude residue was chromatographed on silica gel with a gradient EtOAc 20-75% in Pet. Et to give 750 mg of a orange residue (0.68 mmol; 76%).

^{1}H NMR (700 MHz, DMSO) δ 10.58 (s, 1H), 10.42 (s, 1H), 9.53 (s, 1H), 8.64 (s, 1H), 7.99 (d, *J* = 8.6 Hz, 2H), 7.93 – 7.85 (m, 4H), 7.81 (m, 4H), 7.78 – 7.72 (m, 3H), 7.42 (m, 3H), 7.35 – 7.26 (m, 3H), 7.26 – 7.14 (m, 15H), 6.04 (m, 2H), 5.39 (m, 2H), 5.24 (m, 2H), 4.80 (d, *J* = 5.3 Hz, 2H), 4.62 (d, *J* = 5.5 Hz, 2H), 4.52 – 4.44 (m, 2H), 4.37 (m, 1H), 4.31 (m, 1H), 4.24 (dd, *J* = 14.3, 7.1 Hz, 1H), 2.77 (td, *J* = 14.1, 9.9 Hz, 1H), 2.64 (td, *J* = 15.1, 5.0 Hz, 1H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 170.8, 168.5, 166.9, 165.0, 164.7, 164.3, 155.8, 149.5, 144.7, 143.8, 143.5, 143.0, 142.6, 142.3, 140.7, 135.7, 133.7, 132.8, 130.3, 128.6, 127.4, 127.1, 126.3, 125.3, 125.2, 124.2, 123.6, 120.1, 119.0, 118.8, 118.6, 117.8, 117.8, 76.3, 74.3, 69.4, 65.8, 64.8, 52.9, 46.7, 30.4, 22.3.

HRMS (ESI) calculated for C68H62N5O10 ($M+H^+$) 1108.4491, found 1108.4514.

(S)-4-(4-(4-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (111)



Chemical Formula: $C_{62}H_{53}N_5O_{10}$
Exact Mass: 1027,37924

Phenyl silane (0.25 mL; 2.02 mmol) followed by palladium-tetrakis(triphenylphosphine (138 mg; 0.12 mmol) was added to a solution of allyl (S)-4-(4-(4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-(allyloxy)-3-isopropoxybenzamido)benzoate (530 mg; 0.48 mmol) in THF (19.0 mL). Reaction stirred for 3 hours, quenched by addition of few drops of acetic acid and filtered through celite. Solvent removed under reduced pressure, the crude residue was purified on silica gel with a gradient 0-10% MeOH in DCM +1% acetic acid to give 285 mg of a yellow solid (0.28 mmol; y= 58%).

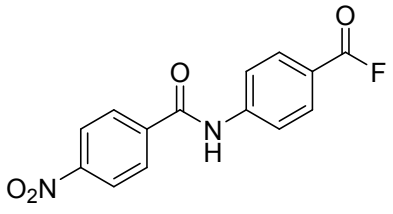
^{1}H NMR (500 MHz, DMSO) δ 12.36 (br, 1H), 12.30 (s, 1H), 10.61 (s, 1H), 10.43 (s, 1H), 9.41 (s, 1H), 8.64 (s, 1H), 8.00 – 7.93 (m, 3H), 7.90 (d, *J* = 7.6 Hz, 2H), 7.86 (m, 3H), 7.81 (dd, *J* = 8.0, 4.8 Hz, 3H), 7.75 (dd, *J* = 7.4, 3.8 Hz, 2H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.42 (dd, *J* = 12.6, 7.4 Hz, 2H), 7.35 – 7.27 (m, 3H), 7.27 – 7.15 (m, 15H), 4.58 – 4.51 (m, 1H), 4.48 (dd, *J* = 14.2, 8.4 Hz, 1H), 4.38 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.31 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.24 (t, *J* = 6.8 Hz, 1H), 2.78 (dd, *J* = 14.5, 9.7 Hz, 1H), 2.64 (dd, *J* = 14.2, 5.2 Hz, 1H), 1.27 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 172.0, 170.8, 168.5, 168.5, 166.9, 164.2, 155.8, 154.1, 144.7, 143.8, 142.4, 142.0, 140.7, 137.0, 136.3, 130.2, 128.6, 128.3, 127.7, 127.4, 127.1, 126.3, 126.3, 125.3, 125.2, 124.9, 122.8, 120.7, 120.1, 118.9, 112.4, 112.2, 74.9, 69.4, 65.8, 52.9, 46.7, 30.4, 22.3.

HRMS (ESI) calculated for C62H52N5O10 (M-H$^+$) 1026.3720, found 1026.3716.

Marfey: 96.2% S enantiomer, 3.8% R enantiomer

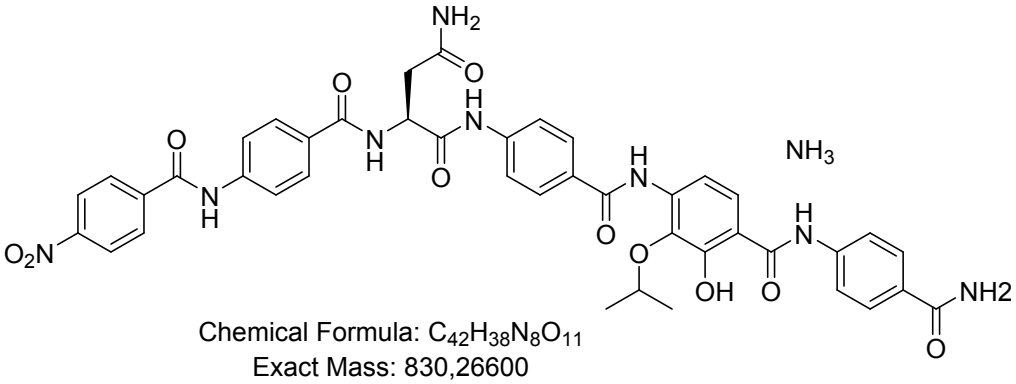
4-(4-Nitrobenzamido)benzoyl fluoride (120)



Chemical Formula: $C_{14}H_9FN_2O_4$
Exact Mass: 288,0546

Diethylaminosulfur trifluoride (13 µL; 0.095 mmol) was added was added at 0 °C to a mixture of 4-(4-nitrobenzamido)benzoic acid (**98**) (50 mg; 0.17 mmol) in DCM (1.5 mL). The reaction was stirred for 20 minutes, then diluted with DCM and washed with ice water, organic phase dried over sodium sulphate and reduced under vacuum to give 4-(4-nitrobenzamido)benzoyl fluoride.

(S)-N1-(4-((4-((4-Carbamoylphenyl)carbamoyl)-3-hydroxy-2-isopropoxyphenyl)carbamoyl)phenyl)-2-(4-(4-nitrobenzamido)benzamido)succinamide (68)



Chemical Formula: $C_{42}H_{38}N_8O_{11}$
Exact Mass: 830,26600

Diethylaminosulfur trifluoride (13 µL; 0.095 mmol) was added at 0 °C to a mixture of carboxylic acid **111** (65 mg; 0.063 mmol) in DCM (0.7 mL). The reaction was stirred for 20 minutes, then diluted with DCM (20 mL) and washed with ice water (20 mL), organic phase dried over sodium sulphate and reduced under vacuum.

The residue was dissolved in THF (0.5 mL), the solution added to a NH_3 in MeOH 7N at 0 °C. Reaction stirred 15 min then solvent removed under vacuum. Compound dissolved in DCM (2

mL), Tips (0.1 mL) followed by TFA (0.5 mL) were added at 0 °C. The reaction was stirred for 2 hours, the solvent was reduced under vacuum, the residue dissolved again in DCM and evaporated twice. The crude thus obtained was dissolved in a 20% solution of diethylamine in acetonitrile (2 mL), the solution was stirred for 30 min. The solvent was removed under reduced pressure, the residue was dissolved in CH_3CN and evaporated twice. Residue triturated three times with Pet. Et. and used in the coupling step.

A solution of 4-(4-nitrobenzamido)benzoyl fluoride (0.069 mmol) in THF (1 mL) was added to a solution of the crude from the previous step (0.048 mmol) and DiPEA (42 µL; 0.24 mmol) in DCM/THF 1:1 (1 mL). The reaction was stirred for 3 hours and then purified by RP-HPLC using method B (retention time: 26.20 min) to obtain 3.2 mg of desired compound (0.0039 mmol; y= 6%).

^{1}H NMR (700 MHz, DMSO) δ 12.40 (br, 1H), 10.79 (s, 1H), 10.58 (br, 1H), 10.46 (s, 1H), 9.35 (br, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.41 – 8.37 (m, 2H), 8.23 – 8.19 (m, 2H),7.94 (dd, *J* = 8.9, 2.2 Hz, 4H), 7.90 (dd, *J* = 8.6, 5.7 Hz, 5H), 7.82 (d, *J* = 8.8 Hz, 3H), 7.78 (d, *J* = 8.7 Hz, 2H), 7.66 (br, 1H), 7.40 (s, 1H), 7.28 (br, 1H), 6.99 (s, 1H), 4.93 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.58 (br, 1H), 2.69 (d, *J* = 7.7 Hz, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.4, 167.3, 165.8, 164.2, 164.1, 149.3, 142.5, 141.5, 140.3, 136.4, 129.3, 129.2, 128.6, 128.2, 123.6, 122.8, 120.5, 119.6, 118.9, 51.6, 36.8, 22.3.

HRMS (ESI) calculated for $C42H37N8O11$ ($M-H^+$) 829.2587, found 829.2588.

1.2.2.2.5.3 Derivatives synthesized

General procedures:

Trityl deprotection

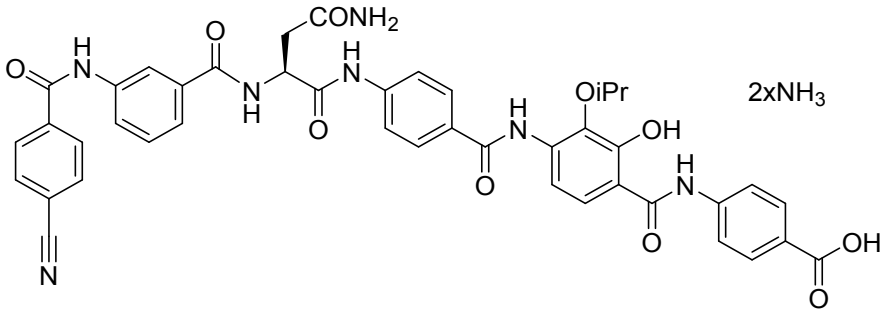
The compound was dissolved in DCM (M= 0.1), Tips (3 eq.) followed by TFA (20%) were added at 0 °C. The reaction was stirred for 2 hours at r.t. then the solvent was removed under vacuum, the residue was suspended and evaporated twice with DCM, finally it was triturated 3x with ice cold Pet. Et..

Fmoc deprotection

The compound was dissolved in a 20% solution of diethylamine in acetonitrile (M= 0.05- 0.1) and the reaction was stirred until completion as monitored by TLC or LCMS (in general reaction time 30 min-1 hour). The solvent was removed under reduced pressure and the residue was dissolved in CH_3CN and evaporated twice. Finally it was left at high vacuum overnight.

Starting from intermediate **111**, after Trityl and Fmoc deprotection (according to general procedures described above), the crude amine **112** was coupled to a carboxylic acid (diaryl fragments) to obtain the following compounds:

(S)-4-(4-(4-(4-Amino-2-(3-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (41)



Chemical Formula: $C_{43}H_{37}N_7O_{10}$
Exact Mass: 811,2602

Carboxylic acid **113** was activated to the corresponding pentafluorophenyl ester following reported procedure [15].

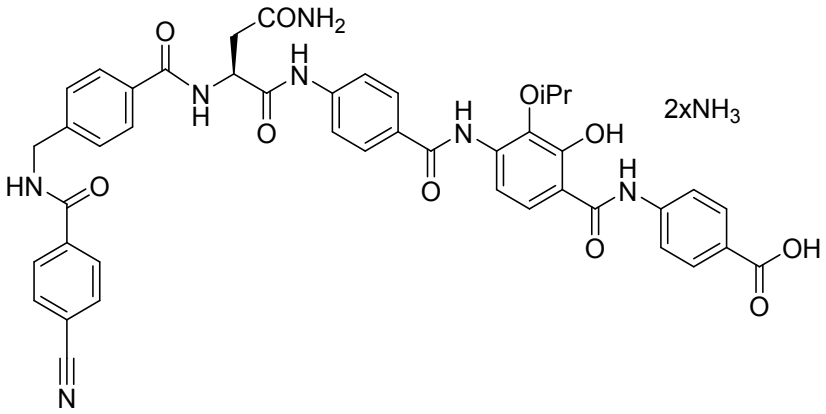
Activated ester (17.3 mg; 0.04 mmol) was dissolved in DMF (0.1 mL) and added at 0°C to a stirred solution of amine **112** (0.02 mmol) and DiPEA (18 µL; 0.10 mmol). The reaction was stirred for three hours at r.t. then diluted with EtOAc and a solution of ice cold HCl, organic phase washed with brine and evaporated under vacuum. The residue was purified by preparative RP-HPLC using condition B (retention time: 20.61 min) to give 0.9 mg of desired product (0.0011 mmol; y= 5%).

^{1}H NMR (700 MHz, DMSO) δ 15.36 (br, 1H), 10.70 (s, 1H), 10.48 (s, 1H), 8.87 (s, 1H), 8.83 (d, *J* = 6.6 Hz, 1H), 8.54 (s, 1H), 8.25 (s, 1H), 8.15 (d, *J* = 8.3 Hz, 2H), 8.04 (d, *J* = 8.3 Hz, 2H), 8.01 (d, *J* = 7.9 Hz, 1H), 7.82 (dd, *J* = 22.5, 8.7 Hz, 4H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 2H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 2H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.97 (s, 1H), 5.02 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.93 (dd, *J* = 13.9, 7.3 Hz, 1H), 2.72 – 2.68 (m, 2H), 1.19 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.5, 166.2, 165.4, 165.2, 164.2, 163.1, 142.0, 138.8, 137.5, 134.6, 134.0, 132.5, 129.7, 129.5, 128.6, 127.6, 123.7, 123.3, 122.9, 120.1, 119.0, 118.3, 117.6, 116.0, 113.9, 100.7, 70.3, 51.7, 36.8, 22.7.

HRMS (ESI) calculated for C43H38N7O10 ($M+H^+$) 812.2675, found 812.2671.

(S)-4-(4-(4-(4-Amino-2-(4-((4-cyanobenzamido)methyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (39)



Chemical Formula: $C_{44}H_{39}N_7O_{10}$
Exact Mass: 825,2758

Carboxylic acid **114** was activated to the corresponding pentafluorophenyl ester following reported procedure [15].

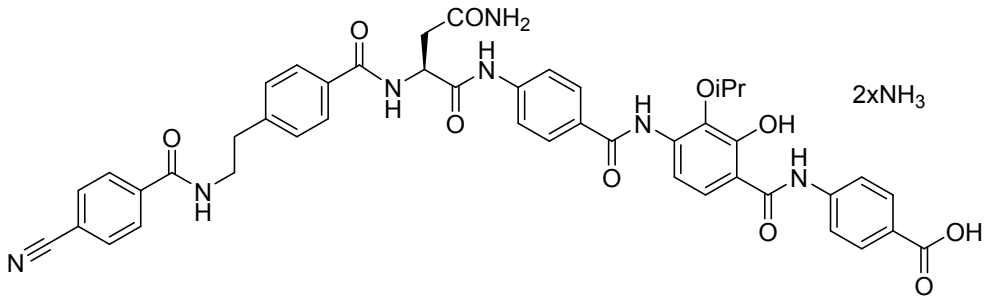
Activated ester (18.0 mg; 0.04 mmol) was dissolved in DMF (0.1 mL) and added at 0°C to a stirred solution of amine **112** (0.02 mmol) and DiPEA (18 µL; 0.10 mmol). The reaction was stirred for three hours at r.t. then diluted with EtOAc and a solution of ice cold HCl, organic phase washed with brine and evaporated under vacuum. The residue was purified by preparative RP-HPLC using condition B (retention time: 16.87 min) to give 3.2 mg of desired product (0.0039 mmol; y= 19%).

^{1}H NMR (700 MHz, DMSO) δ 12.49 (br, 1H), 10.44 (s, 1H), 9.36 (t, *J* = 5.9 Hz, 1H), 9.26 (s, 1H), 8.69 (d, *J* = 7.3 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 2H), 7.98 (d, *J* = 8.4 Hz, 2H), 7.93 (dd, *J* = 13.9, 8.6 Hz, 4H), 7.88 – 7.78 (m, 6H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.55 (br, 1H), 7.43 (d, *J* = 8.2 Hz, 2H), 7.38 (s, 1H), 6.98 (s, 1H), 4.91 (dd, *J* = 13.9, 7.3 Hz, 1H), 4.65 (br, 1H), 4.56 (d, *J* = 5.9 Hz, 2H), 2.68 (dd, *J* = 6.6, 4.4 Hz, 2H), 1.24 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.6, 168.2, 167.0, 166.1, 165.0, 163.9, 142.8, 142.4, 138.2, 136.6, 132.5, 130.3, 128.6, 128.1, 127.6, 127.0, 123.0, 120.1, 118.9, 118.3, 113.7, 51.6, 42.6, 36.8, 22.4.

HRMS (ESI) calculated for C44H40N7O10 ($M+H^+$) 826.2831, found 826.2822.

(S)-4-(4-(4-(4-Amino-2-(4-(2-(4-cyanobenzamido)ethyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (40)



Chemical Formula: $C_{45}H_{41}N_7O_{10}$
Exact Mass: 839,2915

The carboxylic **115** was activated to the corresponding pentafluorophenyl ester following reported procedure [15].

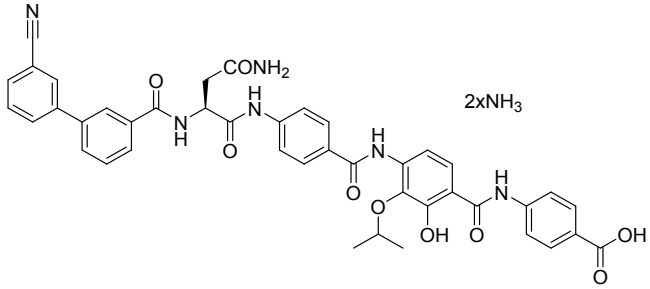
Activated ester (18.4 mg; 0.04 mmol) was dissolved in DMF (0.1 mL) and added at 0°C to a stirred solution of amine **112** (0.02 mmol) and DiPEA (18 µL; 0.10 mmol). The reaction was stirred for three hours at r.t. then diluted with EtOAc and a solution of ice cold HCl, organic phase washed with brine and evaporated under vacuum. The residue was purified by preparative RP-HPLC using condition B (retention time: 18.63 min) to give 5.3 mg of desired product (0.0063 mmol; y= 32%).

^{1}H NMR (700 MHz, DMSO) δ 15.34 (s, 1H), 10.53 (s, 1H), 8.85 (m, 3H), 7.95 (s, 4H), 7.82 (ddd, *J* = 25.7, 15.6, 8.3 Hz, 8H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.47 (s, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.96 (s, 1H), 5.02 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.90 (dd, *J* = 13.8, 7.6 Hz, 1 H), 3.54 (dd, *J* = 13.2, 6.9 Hz, 2H), 2.92 (t, *J* = 7.1 Hz, 2H), 2.68 (ddd, *J* = 20.6, 15.1, 7.1 Hz, 2H), 1.19 (d, *J* = 6.0 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 166.9, 166.1, 165.3, 164.8, 163.1, 143.1, 142.0, 141.7, 138.5, 137.5, 134.0, 132.4, 131.9, 129.7, 129.5, 128.6, 128.0, 127.6, 127.5, 124.2, 123.7, 119.0, 118.3, 117.6, 116.0, 113.5, 100.6, 70.3, 51.8, 40.7, 36.9, 34.7, 22.7.

HRMS (ESI) calculated for C45H42N7O10 ($M+H^+$) 840.2988, found 840.2994.

(S)-4-(4-(4-(4-amino-2-(3'-cyano-[1,1'-biphenyl]-3-carboxamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (42)



Chemical Formula: $C_{42}H_{36}N_6O_9$
Exact Mass: 768,2544

3'-cyano-[1,1'-biphenyl]-3-carboxylic acid was activated to the corresponding pentafluorophenyl ester following reported procedure.[15]

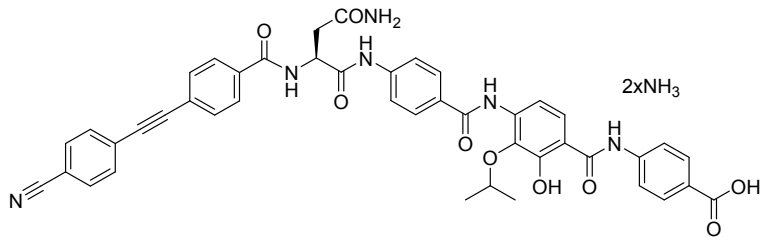
The activated ester was dissolved in DMF (0.1 mL) and added at 0°C to a stirred solution of crude amine **112** (0.02 mmol) and DiPEA (18 µL; 0.10 mmol). Reaction stirred three hours at r.t. then diluted with EtOAc and a solution of ice cold HCl, organic phase washed with brine and evaporated under vacuum. Residue purified by preparative RP-HPLC using condition B (retention time: 15.62 min) to give 0.9 mg of desired product (0.0011 mmol; y= 5%).

^{1}H NMR (700 MHz, DMSO) δ 12.55 (br, 1H), 10.47 (s, 2H), 9.08 (br, 1H), 8.91 (d, *J* = 7.4 Hz, 1H), 8.30 – 8.21 (m, 2H), 8.15 – 8.08 (m, 1H), 7.95 (dd, *J* = 7.7, 1.7 Hz, 2H), 7.92 – 7.85 (m, 5H), 7.81 (dd, *J* = 14.1, 8.7 Hz, 4H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.60 (br, 1H), 7.41 (s, 1H), 7.33 (br, 1H), 6.99 (s, 1H), 4.99 (dd, *J* = 13.8, 7.6 Hz, 1H), 4.83 (br, 2H), 2.72 (ddd, *J* = 23.7, 15.4, 7.1 Hz, 2H), 1.22 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.5, 167.8, 167.1, 166.0, 163.6, 142.2, 140.7, 138.0, 137.1, 134.7, 131.7, 131.4, 130.4, 130.3, 130.3, 129.9, 129.2, 127.9, 127.7, 125.9, 123.4, 119.0, 118.8, 112.2, 51.6, 36.9, 22.5.

HRMS (ESI) calculated for C42H37N6O9 ($M+H^+$) 769.2617, found 769.2610.

(S)-4-(4-(4-(4-amino-2-(4-((4-cyanophenyl)ethynyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (43)



Chemical Formula: $C_{44}H_{36}N_6O_9$
Exact Mass: 792,2544

4-((4-Cyanophenyl)ethynyl)benzoic acid (**118**) was activated to the corresponding pentafluorophenyl ester following reported procedure.[15]

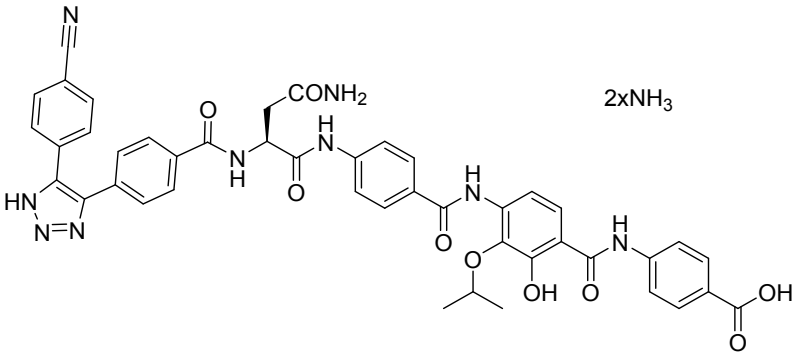
Activated ester (18.0 mg; 0.04 mmol) was dissolved in DMF (0.1 mL) and added at 0°C to a stirred solution of crude amine **112** (0.02 mmol) and DiPEA (18 µL; 0.10 mmol). Reaction stirred three hours at r.t. then diluted with EA and a solution of ice cold HCl, organic phase washed with brine and evaporated under vacuum. Residue purified by preparative RP-HPLC using condition B to give 3.2 mg of desired product (0.0039 mmol; y= 19%).

^{1}H NMR (700 MHz, DMSO) δ 10.46 (s, 1H), 9.18 (br, 1H), 8.88 (d, *J* = 7.2 Hz, 1H), 7.98 (d, *J* = 8.3 Hz, 2H), 7.92 (dd, *J* = 15.0, 7.6 Hz, 6H), 7.85 – 7.76 (m, 6H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.68 (br, 1H), 7.45 (br, 1H), 7.39 (s, 1H), 6.99 (s, 1H), 4.94 (dd, *J* = 14.0, 7.3 Hz, 1H), 4.73 (br, 1H), 2.73 – 2.67 (m, 2H), 1.23 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.2, 170.5, 168.0, 167.0, 165.5, 163.8, 142.2, 134.3, 132.7, 132.3, 131.6, 130.3, 128.0, 126.8, 124.3, 123.2, 119.0, 118.4, 111.3, 92.6, 89.8, 51.7, 36.7, 22.5.

HRMS (ESI) calculated for C44H37N6O9 ($M+H^+$) 793.2617, found 793.2608.

(S)-4-(4-(4-(4-amino-2-(4-(5-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (44)



Chemical Formula: $C_{44}H_{37}N_9O_9$
Exact Mass: 835,2714

The carboxylic acid **119** was activated to the corresponding pentafluorophenyl ester following reported procedure.[15]

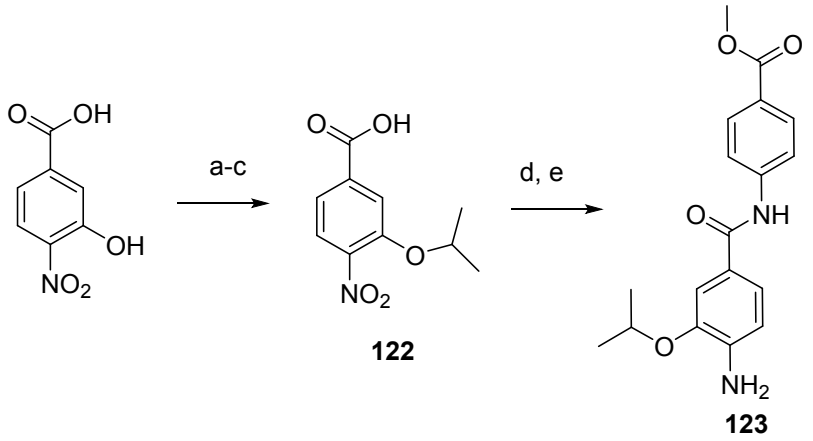
Activated ester (18.4 mg; 0.04 mmol) was dissolved in DMF (0.1 mL) and added at 0°C to a stirred solution of crude amine **112** (0.02 mmol) and DiPEA (18 µL; 0.10 mmol). Reaction stirred three hours at r.t. then diluted with EA and a solution of ice cold HCl, organic phase washed with brine and evaporated under vacuum. Residue purified by preparative RP-HPLC using condition B (retention time: 17.09 min) to give 5.3 mg of desired product (0.0063 mmol; y= 32%).

^{1}H NMR (700 MHz, DMSO) δ 15.34 (s, 1H), 10.45 (s, 1H), 8.87 (s, 1H), 8.70 (d, *J* = 7.1 Hz, 1H), 7.86 – 7.80 (m, 6H), 7.77 (dd, *J* = 16.7, 8.2 Hz, 3H), 7.66 (dd, *J* = 23.4, 8.3 Hz, 4H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.53 - 7.50 (m, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.42 (s, 1H), 7.08 (d, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 5.01 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.93 (dd, *J* = 14.1, 7.1 Hz, 1H), 2.69 (d, *J* = 7.0 Hz, 2H), 1.20 (d, *J* = 6.1 Hz, 6H).

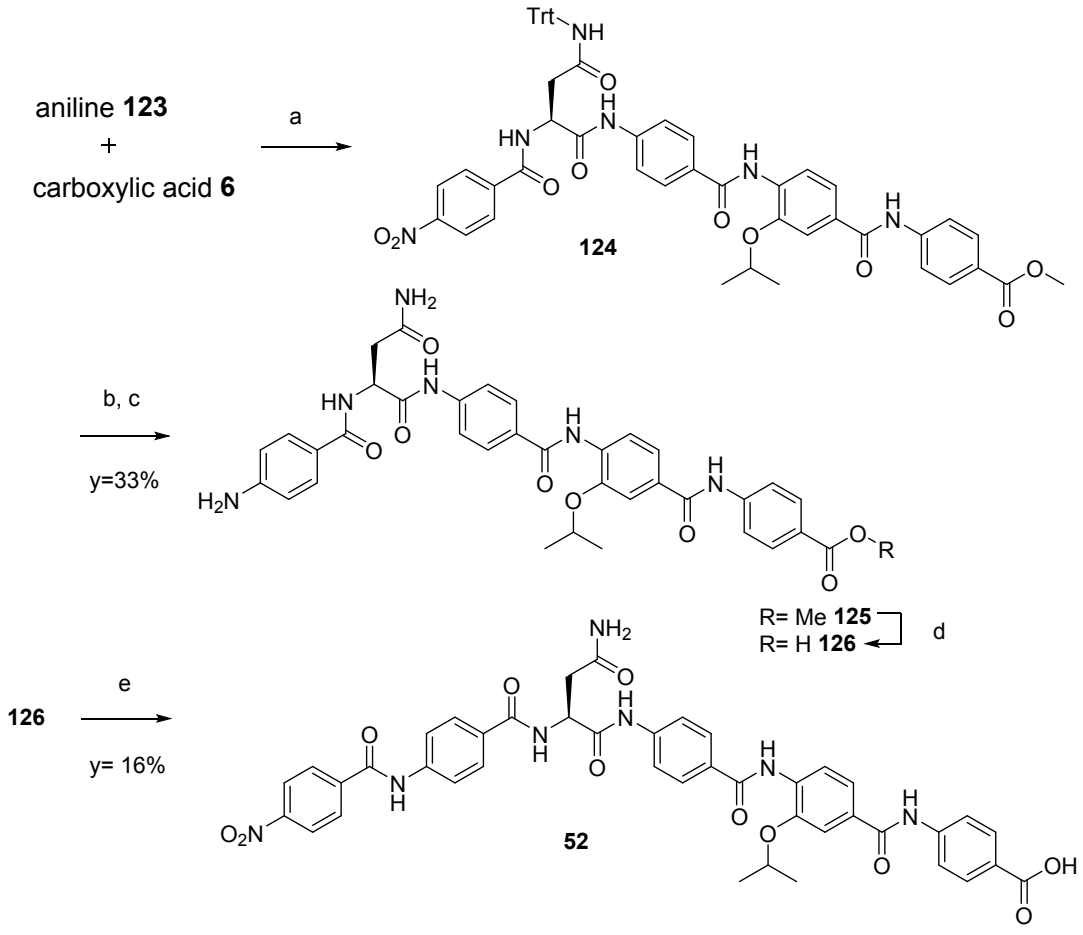
^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 170.3, 167.9, 166.9, 166.3, 165.3, 163.1, 142.0, 141.7, 141.3, 141.0, 140.1, 139.6, 138.9, 137.6, 134.0, 132.0, 130.8, 130.6, 129.7, 129.5, 127.6, 127.5, 127.3, 127.0, 126.0, 126.9, 126.4, 126.0, 124.2, 123.7, 119.5, 119.1, 117.6, 116.0, 106.8, 100.7, 70.4, 51.7, 36.9, 22.7.

HRMS (ESI) calculated for C44H38N9O9 ($M+H^+$) 836.2787, found 836.2807.

1.2.2.3 C-terminal OPTIMIZATION



Scheme S 4. Reagent and conditions: a) $SOCl_2$, MeOH, 1 h, b) *i*-Pr-Br, K_2CO_3, DMF, 70°C, overnight, c) LiOH, THF/water 1:1, rt, 3 h (96% o3s); d) EDC, HOAt, DMF, rt, overnight (60%); e) Pd/C, MeOH, rt, overnight (93%).

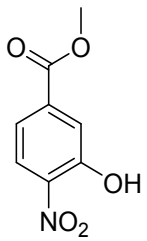


Scheme S 5. Reagent and conditions: a) EDC, HOAt, DMF, rt, 6 d (46%); b) TFA, Tips, DCM, rt, 2h, c) $SnCl_2$, EtOH, rt, overnight (33%); d) LiOH, THF/water 1:1, rt, 2.5 d (25%); e) 4-Nitrobenzoyl chloride, THF/$NaHCO_3$ sat. sol., rt, 2.5 h (16%).

Starting from 3-hydroxy-4-nitrobenzoic acid, functional group interconversions led to arene **122**. This was coupled to the methyl ester of PABA by means of EDCI and finally

the nitro group was reduced to amino under hydrogenolytic conditions to yield the desired diaryl unit **123**. Final assembly of the molecule followed the same order previously described. Due to the instability of the full length molecule under basic conditions, the methyl ester **125** was hydrolyzed to the corresponding acid **126** before the installation of the last PNBA unit.

Methyl 3-hydroxy-4-nitrobenzoate (121)



Chemical Formula: $C_8H_7NO_5$
Exact Mass: 197,0324

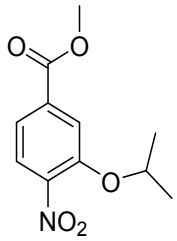
3-Hydroxy-4-nitrobenzoic acid (2.5 g; 13.66 mmol) was dissolved in a MeOH (35.0 mL), thionyl chloride (2.5 mL; 34.15mmol) was added dropwise at 0 °C, the solution was warmed to r.t. and then heated to 70 °C for 1 hour. The solvent was evaporated under reduced pressure, the residue was diluted with water (90 mL) and EtOAc (90 mL), water again extracted twice with EtOAc (2x90 mL). Combined organic phases washed with brine (150 mL), dried over sodium sulphate and evaporated under reduced pressure to give 2.64 g of a yellow solid (13.40 mmol; y= 98%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 10.50 (s, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 7.83 (d, *J* = 1.8 Hz, 1H), 7.62 (dd, *J* = 8.8, 1.8 Hz, 1H), 3.97 (s, 3H).

^{13}C NMR (176 MHz, $CDCl_3$) δ 164.9, 154.7, 138.0, 135.8, 125.3, 121.7, 120.6, 53.0.

HRMS (ESI) calculated for C8H6NO5 ($M-H^+$) 196.0251, found 196.0249.

Methyl 3-isopropoxy-4-nitrobenzoate (127)



Chemical Formula: $C_{11}H_{13}NO_5$
Exact Mass: 239,0794

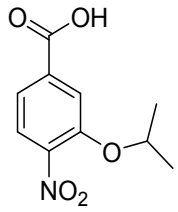
Methyl 3-hydroxy-4-nitrobenzoate (1.94 g; 9.85 mmol) and K_2CO_3 (1.72 g; 11.82 mmol) were mixed in DMF (32 mL). 2-Bromopropane (1.55 mL; 15.76 mmol) was added and the mixture heated to 70 °C overnight. Reaction diluted with Et_2O (320 mL) and water (320 mL). Organic

phase washed with brine (300 mL), dried over sodium sulphate and reduce under vacuum to give 2.25 g of a yellow oil (9.41 mmol; y= 96%).

^{1}H NMR (700 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.3 Hz, 1H), 7.74 (d, *J* = 1.6 Hz, 1H), 7.64 (dd, *J* = 8.3, 1.6 Hz, 1H), 4.77 (hept, *J* = 6.1 Hz, 1H), 3.96 (s, 3H), 1.41 (dd, *J* = 6.0, 2.5 Hz, 6H).

^{13}C NMR (176 MHz, $CDCl_3$) δ 165.4, 150.8, 143.8, 134.5, 125.1, 121.1, 117.0, 73.0, 52.8, 21.8.

3-Isopropoxy-4-nitrobenzoic acid (122)



Chemical Formula: $C_{10}H_{11}NO_5$
Exact Mass: 225,0637

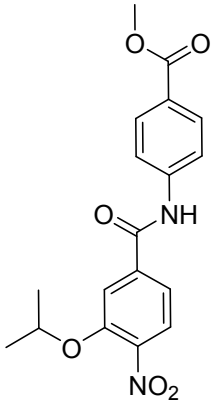
Compound **127** (1.45 g; 6.07 mmol) was dissolved in THF (33 mL) and water (15 mL). To this mixture, a solution of LiOH (1.45 g; 60.70 mmol) in water (18 mL) was added. Reaction stirred at r.t. for 3 h., pH adjusted to 1 and solvent partially reduced under vacuum. The precipitate thus formed was collected by filtration and washed with Pet. Et. twice to give 1.36 g of a pale yellow solid (6.04 mmol; y= q.).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.77 (m, 2H), 7.73 (dd, *J* = 8.3, 1.5 Hz, 1H), 4.79 (hept, *J* = 6.1 Hz, 1H), 1.43 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.4, 150.8, 133.3, 125.2, 121.8, 117.4, 73.2, 21.8.

HRMS (ESI) calculated for C10H10NO5 ($M-H^+$) 224.0564, found 224.0565.

Methyl 4-(3-isopropoxy-4-nitrobenzamido)benzoate (128)



Chemical Formula: $C_{18}H_{18}N_2O_6$
Exact Mass: 358,1165

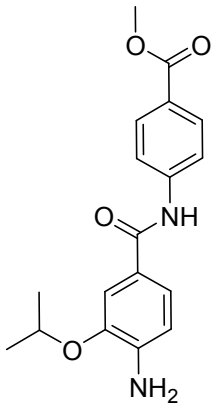
Methyl 4-aminobenzoate (79 mg; 0.52 mmol), 3-isopropoxy-4-nitrobenzoic acid (100 mg; 0.44) and HOAt (91; 0.67 mmol) were mixed together in DMF (1.2 mL). To this yellow solution, EDC (102 mg; 0.53 mmol) followed by lutidine (0.258 mL; 2.22 mmol) were added. Reaction stirred overnight, diluted with EtOAc (20 mL) and HCl 1 N (20 mL), watery phase extracted again with EtOAc (20 mL). Organic phases reunited dried over sodium sulphate and reduced under vacuum, the residue thus obtained chromatographed on silica gel with a gradient 3-40% EtOAc in Pet. Et. to give 112 mg (0.31 mmol; y= 60%) of a pale yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 8.06 (m, 2H), 7.98 (s, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.76 – 7.72 (m, 2H), 7.67 (d, *J* = 1.6 Hz, 1H), 7.36 (dd, *J* = 8.3, 1.7 Hz, 1H), 4.85 – 4.74 (m, 1H), 3.92 (s, 3H), 1.42 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 163.9, 151.7, 143.1, 141.4, 139.2, 131.0, 126.6, 125.7, 119.5, 117.2, 115.8, 73.3, 52.2, 21.8.

HRMS (ESI) calculated for C18H19N2O6 ($M+H^+$) 359.1238, found 359.1239.

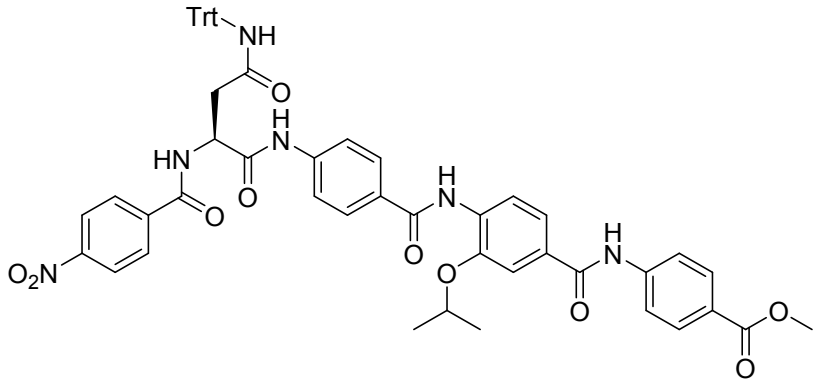
Methyl 4-(4-amino-3-isopropoxybenzamido)benzoate (123)



Chemical Formula: $C_{18}H_{20}N_2O_4$
Exact Mass: 328,1423

Methyl 4-(3-isopropoxy-4-nitrobenzamido)benzoate (800 mg; 2.23 mmol) was dissolved in MeOH (24 mL). The solution was degassed with Argon for 10 min. Pd (80 mg) was added, reaction stirred under an H_2 atmosphere overnight. Pd filtered out over a pad of celite and solvent evaporated under vacuum. The oil thus obtained was chromatographed on silica gel with a gradient 10-40% EtOAc in Pet. Et. to give 790 mg (2.40 mmol; y= 93%) of a white solid.
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 – 8.03 (m, 1H), 8.03 – 7.98 (m, 2H), 7.78 – 7.71 (m, 2H), 7.46 (d, *J* = 1.8 Hz, 1H), 7.28 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 1H), 4.71 – 4.62 (m, 1H), 3.91 (s, 3H), 1.37 (d, *J* = 6.0 Hz, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 165.5, 145.7, 142.7, 130.9, 125.4, 119.7, 119.0, 114.6, 112.9, 71.2, 52.1, 22.2.
HRMS (ESI) calculated for C18H21N2O4 ($M+H^+$) 329.1496, found 329.1495.

Methyl (S)-4-(3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)benzoate (124)

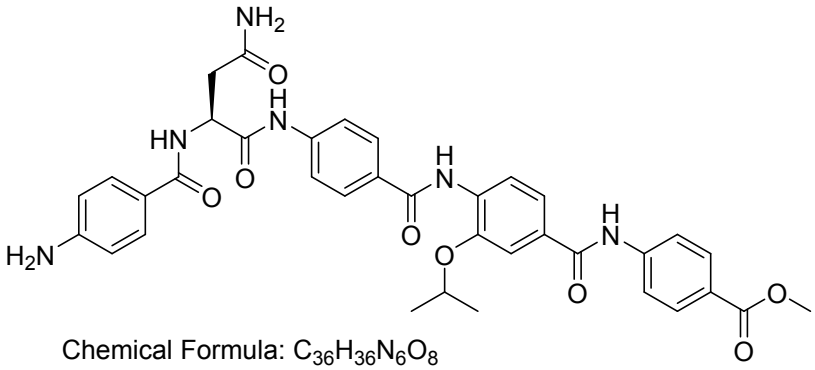


Chemical Formula: $C_{55}H_{48}N_6O_{10}$
Exact Mass: 952,34319

(S)-4-(2-(4-Nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoic acid (325 mg; 0.506 mmol), methyl 4-(4-amino-3-isopropoxybenzamido)benzoate (194 mg; 0.592 mmol), HOAt (103 mg; 0.759 mmol) were mixed in DMF (1.25 mL). To this solution, EDC (116 mg; 0.607 mmol) followed by lutidine (0.295 mL; 2.530 mmol) were added. Reaction stirred at r.t. for 6 days, reaction diluted with EtOAc (75 mL) and HCl 1 N (75 mL), the organic phase washed with $NaHCO_3$ saturated solution (50 mL) and brine (50 mL), dried over sodium sulphate and reduced under vacuum to give an orange material, which was chromatographed on silica gel with a gradient 0-3% MeOH in DCM to give 220 mg of desired product (0.23 mmol; y= 46%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.80 (s, 1H), 8.69 (d, *J* = 8.4 Hz, 1H), 8.48 (d, *J* = 6.3 Hz, 1H), 8.31 – 8.26 (m, 2H), 8.09 – 8.05 (m, 2H), 7.99 (s, 1H), 7.98 – 7.94 (m, 2H), 7.88 – 7.84 (m, 2H), 7.78 – 7.74 (m, 2H), 7.61 (d, *J* = 1.9 Hz, 1H), 7.60 – 7.55 (m, 2H), 7.41 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.32 – 7.24 (m, 15H), 7.14 (s, 1H), 5.09 – 5.04 (m, 1H), 4.88 – 4.76 (m, 1H), 3.92 (s, 3H), 3.31 (dd, *J* = 15.7, 2.7 Hz, 1H), 2.75 (dd, *J* = 15.6, 7.3 Hz, 1H), 1.47 (dd, *J* = 6.0, 2.9 Hz, 6H).

^{13}C NMR (176 MHz, $CDCl_3$) δ 171.2, 168.8, 166.6, 165.7, 165.1, 164.5, 150.1, 146.6, 143.9, 142.2, 141.0, 138.3, 132.4, 131.0, 130.4, 129.4, 128.6, 128.6, 128.2, 128.2, 127.4, 125.8, 124.0, 119.8, 119.2, 118.9, 118.8, 112.1, 72.0, 71.4, 52.1, 51.2, 37.5, 22.3.
HRMS (ESI) calculated for C55H49N6O10 ($M+H^+$) 953.3505, found 953.3483.

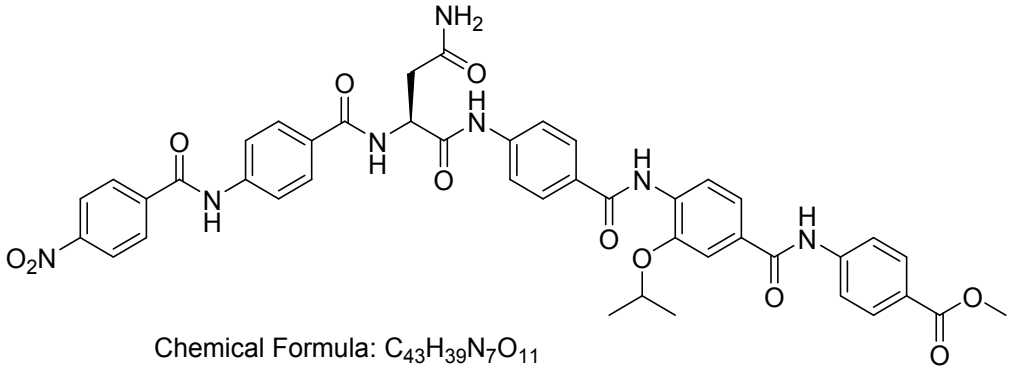
Methyl (S)-4-(4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (125)



Chemical Formula: $C_{36}H_{36}N_6O_8$
Exact Mass: 680,25946

Methyl (S)-4-(3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)-benzamido)benzamido)benzoate (238 mg; 0.25 mmol) was dissolved in DCM (7.5 mL), Tips (0.154 mL; 0.75 mmol) followed by TFA (2.5 mL) were added at 0 °C. Reaction stirred 2 h at r.t. then solved removed under vacuum, residue take up and evaporated twice with DCM (10 mL) then triturated 3x with ice cold Pet. Et..
Tin(II) chloride dehydrate (338 mg; 1.50 mmol) was mixed with the crude residue coming from the former step (0.25 mmol) in EtOH (5 mL), the solution was stirred at r.t. overnight.
Solvent was evaporated under vacuum, the residue dissolved in EtOAc (50 mL), washed with $NaHCO_3$ (50 mL) saturated solution, which was extracted twice again with EtOAc (2x30 mL). Organic phases reunited washed with brine (100 mL), dried over sodium sulphate and reduced under vacuum. The residue was chromatographed on silica gel with a gradient 0-10% MeOH in DCM to give 50 mg of desired product (0.082 mmol; y= 33%).
^{1}H NMR (700 MHz, DMSO) δ 10.47 (s, 1H), 10.38 (s, 1H), 9.25 (s, 1H), 8.22 (d, *J* = 7.4 Hz, 1H), 8.20 (d, *J* = 8.9 Hz, 1H), 7.99 – 7.90 (m, 6H), 7.82 – 7.78 (m, 2H), 7.64 (dd, *J* = 6.9, 1.8 Hz, 2H), 7.62 – 7.59 (m, 2H), 7.37 (s, 1H), 6.97 (s, 1H), 6.57 – 6.54 (m, 2H), 5.67 (s, 2H), 4.85 (q, *J* = 7.1 Hz, 1H), 4.79 (hept, *J* = 6.0 Hz, 1H), 3.84 (s, 3H), 2.64 (d, *J* = 7.1 Hz, 2H), 1.37 (d, *J* = 6.0 Hz, 6H).
^{13}C NMR (176 MHz, DMSO) δ 171.4, 171.1, 166.4, 165.8, 165.3, 164.2, 151.9, 147.8, 143.7, 142.5, 131.7, 130.4, 130.1, 129.1, 128.4, 128.3, 124.2, 121.3, 120.5, 120.4, 119.7, 118.8, 113.1, 112.4, 71.5, 51.9, 51.5, 36.9, 21.8.
HRMS (ESI) calculated for C36H37N6O8 ($M+H^+$) 681.2667, found 681.2673.

Methyl (S)-4-(4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (129)

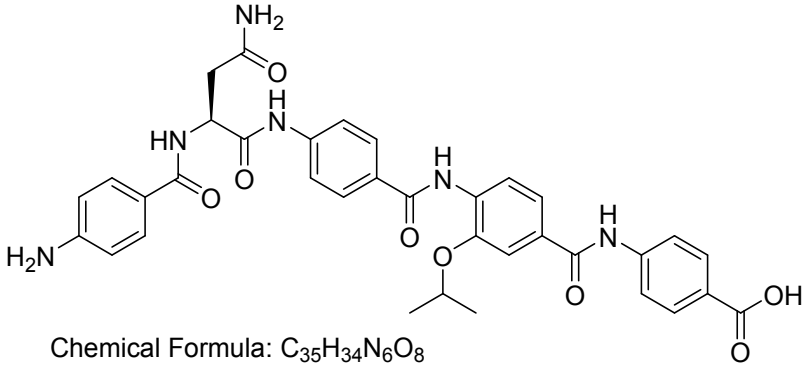


Chemical Formula: $C_{43}H_{39}N_7O_{11}$
Exact Mass: 829,2708

To a solution of Methyl (S)-4-(4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (7 mg; 0.0103 mmol) in THF (0.2 mL) and $NaHCO_3$ saturated solution (0.2 mL), 4-nitrobenzoyl chloride (3.8 mg; 0.0206 mmol) was added at 0 °C. Reaction stirred for 2.5 hours then quenched with HCl 1 N, solvent partially removed under vacuumn, residue dissolved in DMSO, salts filtered out, and purified by preparative RP-HPLC using condition A (retention time: 20.70 min) to obtain 2.1 mg of desired compound (0.0025 mmol; y= 25%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 10.47 (s, 1H), 10.46 (s, 1H), 9.26 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.43 – 8.34 (m, 2H), 8.26 – 8.13 (m, 3H), 8.00 – 7.87 (m, 10H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.64 (dd, *J* = 6.5, 1.8 Hz, 2H), 7.40 (s, 1H), 7.20 (s, 1H), 7.00 (s, 1H), 6.65 (s, 1H), 4.93 (dd, *J* = 14.2, 7.1 Hz, 1H), 4.79 (dt, *J* = 12.2, 6.1 Hz, 1H), 3.84 (s, 3H), 2.69 (d, *J* = 7.1 Hz, 2H), 1.37 (d, *J* = 6.0 Hz, 9H).

(S)-4-(4-(4-(4-Amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoic acid (126)



Chemical Formula: $C_{35}H_{34}N_6O_8$
Exact Mass: 666,2438

Methyl (S)-4-(4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate (20 mg, 0.029 mmol) was mixed into THF (1.4 mL) and water (1.4 mL). To this mixture, a solution 0.1 M of LiOH (0.59 mL; 0.059 mmol) was added at 0 °C. Reaction stirred for 2.5 days. Reaction quenched with acetic acid, solvent reduced under

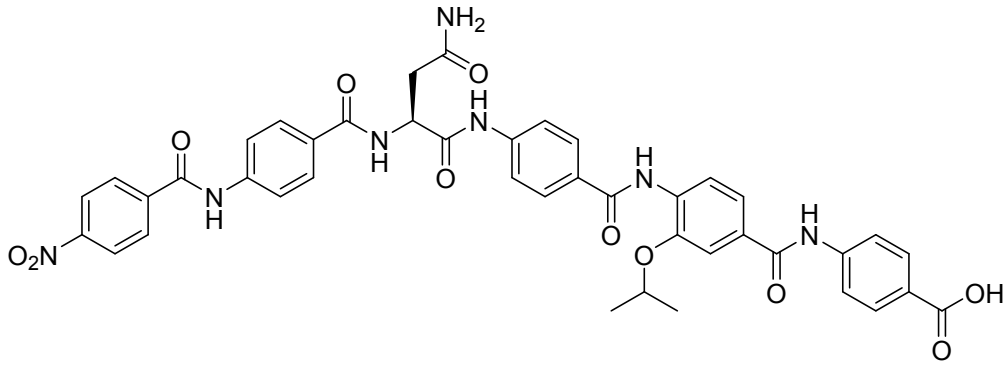
vacuum and residue purified by preparative HPLC with a gradient 20-90% CH_3CN in water both phases +0.1 % TFA to obtain 4.8 mg of desired product (0.0073 mmol; y= 25%).

^{1}H NMR (500 MHz, DMSO) δ 12.78 (br, 1H), 10.45 (s, 1H), 10.40 (s, 1H), 9.26 (s, 1H), 8.24 (d, *J* = 7.2 Hz, 1H), 8.19 (d, *J* = 8.9 Hz, 1H), 7.92 (m, 6H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.66 – 7.59 (m, 4H), 7.38 (s, 1H), 6.98 (s, 1H), 6.57 (d, *J* = 8.6 Hz, 2H), 5.72 (br, 1H), 4.85 (q, *J* = 7.0 Hz, 1H), 4.79 (dt, *J* = 12.1, 6.0 Hz, 1H), 2.67 – 2.60 (m, 2H), 1.37 (d, *J* = 6.0 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.4, 171.0, 166.9, 166.4, 165.2, 164.2, 147.8, 143.3, 142.5, 131.7, 130.4, 130.2, 129.1, 128.5, 128.3, 125.4, 121.3, 120.5, 119.6, 118.8, 113.1, 112.6, 71.6, 51.5, 36.9, 21.8.

HRMS (ESI) calculated for C35H35N6O8 ($M+H^+$) 667.2511, found 667.2512.

(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoic acid (52)



Chemical Formula: $C_{42}H_{37}N_7O_{11}$
Exact Mass: 815,2551

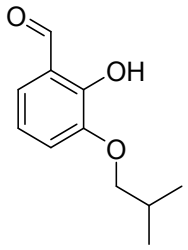
To a suspension of (S)-4-(4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoic acid (4.8 mg; 0.0072 mmol) in THF (0.25 mL) and $NaHCO_3$ saturated solution (0.25 mL), 4-nitrobenzoyl chloride (2.6 mg; 0.014 mmol) was added at 0 °C. Reaction stirred for 2.5 hours then quenched with AcOH, solvent partially removed under vacuumn, residue dissolved in DMSO, salts filtered out, and purified by preparative RP-HPLC using condition A (retention time: 16.83 min)to obtain 1 mg of desired compound (0.0012 mmol; y= 16%).

^{1}H NMR (700 MHz, DMSO) δ 12.75 (br, 1H), 10.79 (s, 1H), 10.46 (s, 1H), 10.44 (s, 1H), 9.26 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.40 – 8.37 (m, 2H), 8.21 (dt, *J* = 17.0, 6.2 Hz, 3H), 7.96 – 7.89 (m, 10H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.65 – 7.63 (m, 2H), 7.40 (s, 1H), 6.99 (s, 1H), 4.93 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.79 (hept, *J* = 6.1 Hz, 1H), 2.69 (d, *J* = 7.8 Hz, 2H), 1.37 (d, *J* = 6.0 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 166.9, 165.8, 165.2, 164.2, 149.3, 147.8, 143.3, 141.5, 140.3, 131.7, 130.5, 130.2, 129.3, 129.2, 128.6, 128.3, 128.3, 125.4, 123.6, 121.4, 120.5, 119.6, 118.9, 113.1, 71.6, 51.6, 36.8, 21.8.

HRMS (ESI) calculated for C42H38N7O11 ($M+H^+$) 816.2624, found 816.2633.

2-Hydroxy-3-isobutoxybenzaldehyde (130)



Chemical Formula: $C_{11}H_{14}O_3$
Exact Mass: 194,0943

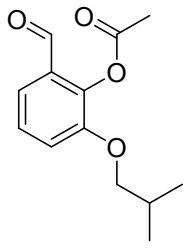
A solution of 2,3-dihydroxybenzaldehyde (15 g; 109 mmol) in DMSO (75 mL) was added drop wise, keeping low the temperature with an ice bath, to a previously prepared suspension of NaH (8.72 g; 218 mmol) in DMSO (180 mL). The mixture stirred at r.t. for two hours, then isobutyl bromide (11.9 mL; 109 mmol) was added slowly keeping the temperature low. The reaction mixture was stirred for 42 hours, quenched with HCl followed by NH_4Cl until pH 5 was reached. Work up done in several portions as follows: 300 mL of the mixture were further diluted with H_2O (1200 mL) and extracted with Et_2O (3x 200 mL). Organic phases dried over Na_2SO_4 and reduced under pressure to give 30 g of dark oil. The crude residue was chromatographed on silica gel using isocratic condition (Pet. Et. DCM 7:3) to give 8.04 g of a yellow oil (41 mmol, y= 38%).

^1H NMR (500 MHz, $CDCl_3$) δ 10.98 (s, 1H), 9.92 (s, 1H), 7.17 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.11 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.93 (t, *J* = 7.9 Hz, 1H), 3.81 (d, *J* = 6.7 Hz, 2H), 2.17 (dp, *J* = 13.3, 6.7 Hz, 1H), 1.05 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 196.5, 152.1, 147.9, 124.5, 121.0, 119.7, 119.5, 75.9, 28.2, 19.2.

HRMS (ESI) calculated for C11H15O3 ($M+H^+$) 195.1016, found 195.1014.

2-Formyl-6-isobutoxyphenyl acetate (131)



Chemical Formula: $C_{13}H_{16}O_4$
Exact Mass: 236,1049

Acetyl chloride (5.93 mL; 55.2 mmol) was added dropwise to a stirred solution of 2-hydroxy-3-isobutoxybenzaldehyde (**109**) (7.15 g; 36.8 mmol) and pyridine (8.90 mL; 110.0 mmol) in DCM (340 mL) at 0 °C. The reaction was stirred for 5 min. at 0 °C then temperature raised to r.t.,

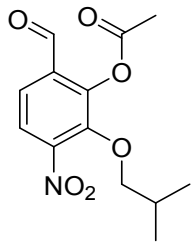
stirring was prolonged for 1 h. The reaction was quenched with HCl 1 N, organic phase partially reduced under vacuum, washed with HCl 1 N (200 mL), brine (200 mL), dried over sodium sulphate and reduced under vacuum. The crude was chromatographed on silica gel with a gradient 2-10% EtOAc in Pet. Et to give 8.46 g of a pale yellow oil (35.85 mmol; y= 97%).

^{1}H NMR (700 MHz, DMSO) δ 10.12 (s, 1H), 7.46 (dd, *J* = 7.2, 2.5 Hz, 1H), 7.42 – 7.37 (m, 2H), 3.82 (d, *J* = 6.3 Hz, 2H), 2.34 (s, 3H), 2.00 (dp, *J* = 13.2, 6.6 Hz, 1H), 0.96 (d, *J* = 6.8 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 190.0, 168.5, 150.9, 141.1, 128.9, 127.0, 120.4, 119.4, 74.6, 27.7, 20.2, 18.8.

HRMS (ESI) calculated for C13H16NaO4 ($M+Na^{+}$) 259.0941, found 259.0943.

6-Formyl-2-isobutoxy-3-nitrophenyl acetate (132)



Chemical Formula: $C_{13}H_{15}NO_6$
Exact Mass: 281,0899

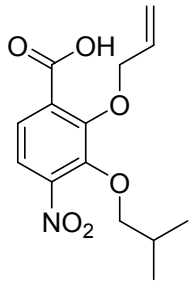
Fuming nitric acid (5.0 mL, 118.4 mmol) was cooled to −40 °C under a nitrogen atmosphere. A solution of 2-formyl-6-isobutoxyphenyl acetate (**110**) (3.50 g, 14.8 mmol) in 8.0 mL of dry DCM was added dropwise while the mixture was vigorously stirred and kept at −40 °C. The solution was stirred for an additional 1.5 hours before being poured into 100 mL of ice water. The mixture was then extracted with DCM (4 × 50 mL) and the combined organic extracts were dried over sodium sulphate. The solvent was removed under vacuum, the crude thus obtained was chromatographed on silica gel with a gradient 5-40% EtOAc in Pet. Et. to afford 2.45 g of the desired compound (8.73 mmol, y= 59%).

^{1}H NMR (500 MHz, DMSO) δ 10.13 – 10.09 (s 1H), 8.03 – 8.00 (m, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 3.84 (d, *J* = 6.2 Hz, 2H), 2.44 (s, 3H), 2.01 – 1.93 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 189.4, 168.5, 147.5, 145.2, 144.7, 132.0, 125.5, 122.3, 81.5, 28.5, 20.4, 18.5.

HRMS (ESI) calculated for C13H15NNaO6 ($M+Na^{+}$) 304.0792, found 304.0792.

2-(Allyloxy)-3-isobutoxy-4-nitrobenzoic acid (133)



Chemical Formula: $C_{14}H_{17}NO_6$
Exact Mass: 295,1056

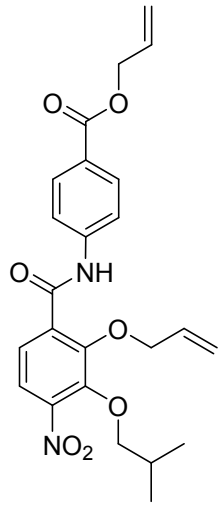
6-Formyl-2-isobutoxy-3-nitrophenyl acetate (**111**) (4.10 g; 14.6 mmol) was dissolved in THF (70 mL) and water (35 mL), then LiOH (3.50 g; 146.0 mmol) dissolved in water (35 mL) was added at 0 °C, the reaction was stirred overnight. In the morning, pH adjusted to 1, solvent partially reduced under vacuum and watery phase extracted with $CHCl_3$ (150 mL) three times, combined organic phases dried over sodium sulphate and reduced under vacuum to give a yellow oil, which was used in the next step without further purification. Residue was dissolved in DMF (30 mL), K_2CO_3 (4.03 g; 29.2 mmol) followed by allyl bromide (1.89 mL; 21.9 mmol) were added, the reaction was stirred for 24 h at r.t.. Reaction mixture diluted with water (200 mL) and EtOAc (200 mL), aqueous phase extracted with EtOAc (150 mL). Combined organic phases washed with brine (300 mL), dried over sodium sulphate and reduced under vacuum to give a crude material, which was dissolved with 2-Methyl-2-butene (15.5 mL; 146 mmol) in *t*-BuOH (100 mL). Then a solution of $NaClO_2$ 80% (1.98 g; 17.52 mmol) in monosodium phosphate monohydrate solution 1 N (16.2 mL) was added dropwise to the solution. Reaction stirred for 1 h, t hen quenched by adding a solution of Na_2SO_3 (34.0 mmol in 10 mL). Mixture partially reduced under vacuum, diluted with EtOAc (200 mL) and HCl 1 N (200 mL), aqueous phase extracted again with EtOAc (100 mL), organic phases reunited washed with brine (250 mL) and dried over sodium sulphate. Solvent reduced under vacuum, crude chromatographed on silica gel with a gradient 0-10% MeOH in DCM to afford 3.01 g of the desired compound (10.22 mmol, y= 70%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.7 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 1H), 6.08 (m, 1H), 5.44 (dq, *J* = 17.1, 1.3 Hz, 1H), 5.39 (ddd, *J* = 10.3, 1.9, 0.9 Hz, 1H), 4.81 – 4.77 (m, 2H), 3.93 (d, *J* = 6.5 Hz, 2H), 2.12 (dp, *J* = 13.3, 6.7 Hz, 1H), 1.03 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.0, 152.7, 148.2, 146.4, 131.1, 127.5, 126.8, 121.7, 119.8, 82.0, 76.5, 29.0, 19.0.

HRMS (ESI) calculated for C14H16NO6 ($M\text{-}H^+$) 294.0983, found 294.0995.

Allyl 4-(2-(allyloxy)-3-isobutoxy-4-nitrobenzamido)benzoate (134)



Chemical Formula: $C_{24}H_{26}N_2O_7$
Exact Mass: 454,1740

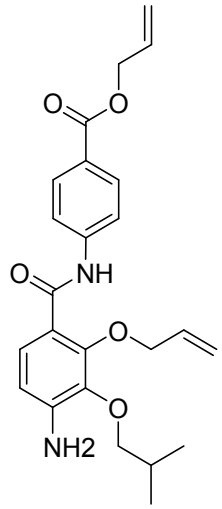
To a stirred solution of 2-(allyloxy)-3-isobutoxy-4-nitrobenzoic acid (500 mg; 1.69 mmol), allyl 4-aminobenzoate (250 mg; 1.41 mmol) and TEA (0.38 mL; 2.82 mmol) in dry DCM (28 mL), $POCl_3$ (0.16 mL; 1.69 mmol) was added drop wise at 0 °C. Reaction stirred for 4 hours then quenched by addition of $NaHCO_3$ saturated solution, solvent partially reduced under vacuum and residue dissolved in EtOAc (50 mL). The organic phase was washed with $NaHCO_3$ saturated solution (50 mL), HCl 1 N (50 mL), brine (50 mL), dried over sodium sulphate and reduced under vacuum. The crude thus obtained was purified on silica gel with a gradient 2-20% EtOAc in Pet. Et. to give 400 mg of desired product (0.88 mmol; y= 62%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.09 (m, 3H), 7.79 – 7.73 (m, 2H), 7.64 (d, *J* = 8.8 Hz, 1H), 6.08 (m, 2H), 5.48 (dq, *J* = 17.1, 1.3 Hz, 1H), 5.45 – 5.39 (m, 2H), 5.30 (dq, *J* = 10.5, 1.3 Hz, 1H), 4.83 (dt, *J* = 5.7, 1.4 Hz, 2H), 4.76 (dt, *J* = 6.1, 1.0 Hz, 2H), 3.97 (d, *J* = 6.5 Hz, 2H), 2.20 – 2.11 (m, 1H), 1.06 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.6, 161.1, 151.3, 147.3, 146.5, 141.8, 132.3, 131.5, 131.0, 130.8, 126.3, 126.1, 121.2, 120.1, 119.4, 118.3, 82.0, 76.2, 65.5, 29.1, 19.0.

HRMS (ESI) calculated for C24H27N2O7 ($M+H^+$) 455.1813, found 455.1806.

Allyl 4-(2-(allyloxy)-4-amino-3-isobutoxybenzamido)benzoate (135)



Chemical Formula:
$C_{24}H_{28}N_2O_5$
Exact Mass: 424,1998

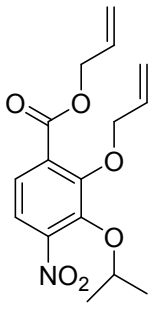
Allyl 4-(2-(allyloxy)-3-isobutoxy-4-nitrobenzamido)benzoate (350 mg; 0.77 mmol) was dissolved in EtOH (13.5 mL) and AcOH (1.5 mL), the solution was cooled to 0 °C and to it Zn dust (500 mg; 7.70 mmol) was added portion wise over few minutes. Reaction stirred at r.t. for 4 hours then mixture filtered over a pad of celite and solvent reduced under vacuum to afford 295 mg of desired compound (0.70 mmol; y= 90%).

^{1}H NMR (500 MHz, DMSO) δ 10.24 (s, 1H), 7.98 – 7.90 (m, 2H), 7.84 – 7.78 (m, 2H), 7.41 – 7.36 (m, 1H), 6.58 (d, *J* = 8.6 Hz, 1H), 6.05 (m, 2H), 5.54 (s, 2H), 5.41 (ddq, *J* = 17.1, 15.3, 1.6 Hz, 2H), 5.27 (m, 2H), 4.78 (dt, *J* = 5.4, 1.5 Hz, 2H), 4.61 (dt, *J* = 5.6, 1.3 Hz, 2H), 3.67 (d, *J* = 6.5 Hz, 2H), 2.11 (tt, *J* = 13.6, 6.8 Hz, 1H), 1.01 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 165.0, 163.9, 150.4, 146.6, 143.6, 137.2, 133.5, 132.8, 130.3, 126.2, 123.7, 118.8, 118.3, 117.8, 114.8, 110.2, 78.5, 74.4, 64.8, 28.5, 19.2.

HRMS (ESI) calculated for C24H27N2O5 ($M-H^+$) 423.1925, found 423.1933.

Allyl 2-(allyloxy)-3-isopropoxy-4-nitrobenzoate (136)



Chemical Formula: $C_{16}H_{19}NO_6$
Exact Mass: 321,1212

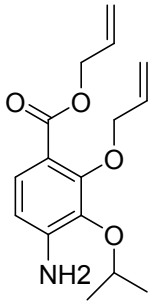
6-Formyl-2-isopropoxy-3-nitrophenyl acetate (0.9 g; 3.37 mmol) was dissolved in THF (17 mL) and water (8.5 mL), then LiOH (0.81 g; 33.70 mmol) dissolved in water (8.5 mL) was added at 0 °C, reaction stirred overnight. In the morning, pH adjusted to 1, solvent partially reduced under vacuum and watery phase extracted with $CHCl_3$ (100 mL) three times, combined organic phases dried over sodium sulphate and reduced under vacuum to give a yellow oil. This was dissolved with 2-Methyl-2-butene (3.75 mL; 35.39 mmol) in *t*-BuOH (25 mL), then a solution of $NaClO_2$ 80% (0.46 g; 4.04 mmol) in monosodium phosphate monohydrate solution 1 N (3.75 mL) was added dropwise to the solution. The reaction was stirred for 1 h, it was then quenched by adding a solution of Na_2SO_3 (8.0 mmol in 5 mL). Mixture partially reduced under vacuum, diluted with EtOAc (100 mL) and HCl 1 N (100 mL), aqueous phase extracted again with EtOAc (50 mL), organic phases reunited washed with brine (150 mL) and dried over sodium sulphate. Solvent removed under vacuum, the residue was dissolved in DMF (9.0 mL), K_2CO_3 (1.40 g; 10.11 mmol) followed by allyl bromide (0.73 mL; 8.43 mmol) were added, reaction stirred 24 h at r.t.. The reaction was diluted with water (100 mL) and EtOAc (100 mL), aqueous phase extracted with EtOAc (50 mL). Combined organic phases washed with brine (100 mL), dried over sodium sulphate and reduced under vacuum, the crude material was chromatographed on silica gel with a gradient 0-10% EtOAc in Pet. Et. to give 0.84 g a yellow oil (2.63 mmol; y= 79%).

^{1}H NMR (500 MHz, DMSO) δ 7.70 (d, *J* = 8.5 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 6.08 – 5.97 (m, 2H), 5.40 (m, 2H), 5.28 (m, 2H), 4.81 (dt, *J* = 5.6, 1.4 Hz, 2H), 4.64 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.56 (dt, *J* = 5.8, 1.4 Hz, 2H), 1.20 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 164.1, 151.7, 147.8, 143.8, 133.2, 132.1, 130.4, 124.8, 119.1, 118.7, 118.5, 77.2, 74.8, 66.0, 22.0.

HRMS (ESI) calculated for C16H19NNaO6 ($M+Na^+$) 344.1105, found 344.1105.

Allyl 2-(allyloxy)-4-amino-3-isopropoxybenzoate (137)



Chemical Formula: $C_{16}H_{21}NO_4$
Exact Mass: 291,1471

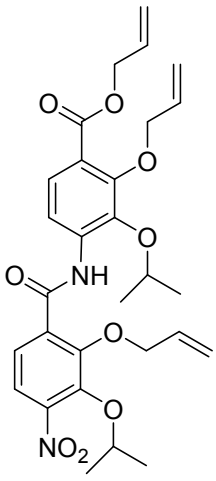
Allyl 2-(allyloxy)-3-isopropoxy-4-nitrobenzoate 700 mg; 2.17 mmol) was dissolved in EtOH (19.8 mL) and AcOH (2.2 mL), the solution was cooled to 0 °C and to it Zn dust (1.42 g; 21.7 mmol) was added portion wise. The reaction was stirred at r.t. for 4 hours then mixture filtered over a pad of celite and solvent reduced under vacuum, the residue was chromatographed on silica gel with a gradient 1-20% EtOAc in Pet. Et. to afford 550 mg of desired compound (1.89 mmol; y= 87%).

^{1}H NMR (500 MHz, DMSO) δ 7.33 (d, *J* = 8.6 Hz, 1H), 6.46 (d, *J* = 8.7 Hz, 1H), 6.10 – 5.94 (m, 2H), 5.62 (s, 2H), 5.34 (m, 2H), 5.20 (m, 2H), 4.66 (dt, *J* = 5.4, 1.5 Hz, 2H), 4.45 – 4.38 (m, 3H), 1.21 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 164.6, 153.1, 148.5, 136.2, 134.6, 133.2, 127.2, 117.5, 116.8, 111.0, 109.2, 74.1, 73.6, 64.2, 40.0, 39.9, 39.8, 39.8, 39.7, 39.5, 39.3, 39.2, 39.0, 22.1.

HRMS (ESI) calculated for C16H22NO4 ($M+H^+$) 292.1543, found 292.1541.

Allyl 2-(allyloxy)-4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)-3-isopropoxybenzoate (138)



Chemical Formula: $C_{29}H_{34}N_2O_9$
Exact Mass: 554,2264

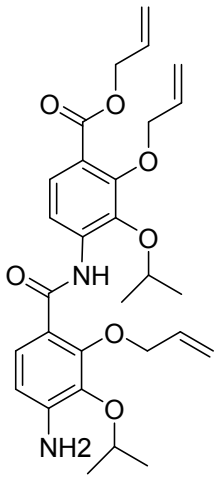
Collidine (1.44 mL; 11.00 mmol) was added dropwise at 0 °C to a solution of 2-(allyloxy)-3-isopropoxy-4-nitrobenzoic acid (579 mg; 2.06 mmol) and bis(trichloromethyl) carbonate (204 mg; 0.69 mmol) in THF (13 mL). The reaction was stirred at r.t. for 20 min then added to a cooled solution of allyl 2-(allyloxy)-4-amino-3-isopropoxybenzoate (400 mg; 1.37 mmol) and DiPEA (2.38 mL; 13.7 mmol) in THF (13 mL). The reaction was stirred for 20 hours at r.t., then quenched with water and solvent partially reduced under vacuum, the mixture was diluted with Et_2O (50 mL) and HCl 1N (50 mL), watery phase extracted again with Et_2O (50 mL). The combined organic phases were washed with brine (100 mL), dried over sodium sulphate and reduced under vacuum. The crude was chromatographed on silica gel with a gradient 1-20% EtOAc in Pet. Et. to give 535 mg of desired product (0.97 mmol; y= 70%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.73 (s, 1H), 8.42 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 6.18 – 6.00 (m, 3H), 5.45 – 5.35 (m, 2H), 5.34 – 5.27 (m, 2H), 5.26 (m, 2H), 4.83 – 4.76 (m, 5H), 4.65 (dt, *J* = 12.3, 6.2 Hz, 1H), 4.58 (dt, *J* = 5.9, 1.3 Hz, 2H), 1.37 (d, *J* = 6.2 Hz, 6H), 1.28 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.0, 161.3, 151.8, 151.2, 147.9, 145.0, 140.4, 137.4, 133.8, 132.2, 131.7, 131.6, 127.0, 125.9, 121.8, 121.1, 119.9, 118.5, 118.0, 115.1, 78.7, 76.3, 76.2, 74.9, 65.6, 22.5, 22.4.

HRMS (ESI) calculated for $C_{29}H_{35}N_2O_9$ ($M+H^+$) 555.2337, found 555.2335.

Allyl 2-(allyloxy)-4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)-3-isopropoxybenzoate (139)



Chemical Formula: $C_{29}H_{36}N_2O_7$
Exact Mass: 524,2523

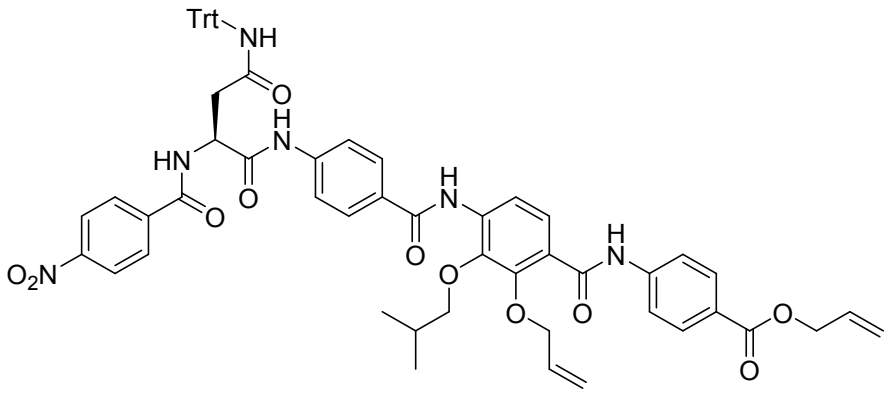
Allyl 2-(allyloxy)-4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)-3-isopropoxybenzoate (250 mg; 0.45 mmol) was dissolved in EtOH (9.0 mL) and AcOH (1.0 mL), the solution was cooled to 0 °C and to it Zn dust (295 mg; 4.50 mmol) was added portion wise. The reaction was stirred at r.t. for 5 hours then the mixture was filtered over a pad of celite and solvent reduced under vacuum, residue dissolved in DCM (30 mL), organic phase washed with $NaHCO_3$ saturated solution and dried over sodium sulphate. The solvent was removed under reduced pressure to give 220 mg of desired product (0.42 mmol; y= 93%).

^{1}H NMR (500 MHz, DMSO) δ 10.75 (s, 1H), 8.34 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 6.58 (d, *J* = 8.7 Hz, 1H), 6.12 – 5.98 (m, 3H), 5.70 (s, 2H), 5.43 – 5.36 (m, 2H), 5.31 (m, 2H), 5.27 – 5.19 (m, 2H), 4.75 (dt, *J* = 5.5, 1.5 Hz, 2H), 4.67 (d, *J* = 6.5 Hz, 2H), 4.61 – 4.55 (m, 1H), 4.53 (dt, *J* = 5.7, 1.4 Hz, 2H), 4.45 – 4.39 (m, 1H), 1.29 (d, *J* = 6.2 Hz, 6H), 1.23 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 164.5, 162.9, 151.2, 150.6, 148.3, 139.5, 138.5, 135.1, 134.0, 133.0, 132.7, 126.7, 126.2, 119.8, 119.3, 118.1, 117.5, 114.2, 113.1, 110.3, 75.7, 74.8, 74.2, 74.1, 65.0, 22.2, 22.0.

HRMS (ESI) calculated for C29H37N2O7 ($M+H^+$) 525.2595, found 525.2591.

Allyl (S)-4-(2-(allyloxy)-3-isobutoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)benzoate (140)



Chemical Formula: $C_{61}H_{56}N_6O_{11}$
Exact Mass: 1048,4007

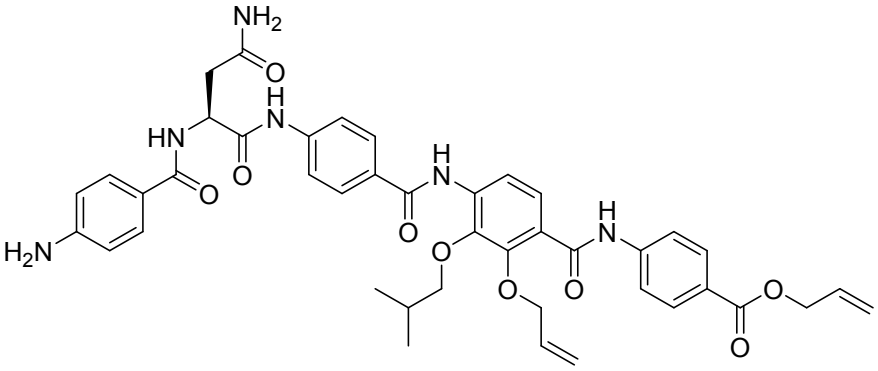
$POCl_3$ (2.3 mmol) as a solution in DCM (1:9) was added dropwise to a solution allyl 4-(2-(allyloxy)-4-amino-3-isobutoxybenzamido)benzoate (0.390 g; 0.92 mmol) and (S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoic acid (1.48g, 2.3 mmol) in THF (9.2 mL) and DCM (7.0 mL) at 0 °C, followed by DiPEA (1.28 mL; 7.36 mmol) as a solution in DCM (1:1). The reaction was stirred at r.t. for 4 hours, then it was quenched with HCL 1 N and ice, the solvent was partially reduced under vacuum and residue diluted with EtOAc (200 mL) and HCl 1N (200 mL), organic phase washed with brine (200 mL) and dried over sodium sulphate. The solvent was removed under vacuum, the crude residue was chromatographed on silica gel with a gradient EtOAc 20-90% in Pet. Et to give 626 mg of an orange residue (0.60 mmol; 65%).

^{1}H NMR (500 MHz, DMSO) δ 10.58 (s, 1H), 10.52 (s, 1H), 9.58 (s, 1H), 9.21 (d, *J* = 7.6 Hz, 1H), 8.70 (s, 1H), 8.41 – 8.37 (m, 2H), 8.19 – 8.15 (m, 2H), 7.99 (dd, *J* = 8.8, 2.0 Hz, 4H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.23 – 7.14 (m, 15H), 6.10 – 5.96 (m, 2H), 5.44 – 5.38 (m, 1H), 5.36 (ddd, *J* = 17.2, 3.1, 1.6 Hz, 1H), 5.28 (ddd, *J* = 10.5, 2.6, 1.3 Hz, 1H), 5.19 (dd, *J* = 10.5, 1.3 Hz, 1H), 4.97 – 4.91 (m, 1H), 4.79 (d, *J* = 5.3 Hz, 2H), 4.60 (d, *J* = 5.5 Hz, 2H), 3.82 (d, *J* = 6.2 Hz, 2H), 3.00 (dd, *J* = 14.8, 10.3 Hz, 1H), 2.77 (dd, *J* = 14.8, 4.5 Hz, 1H), 1.99 (tt, *J* = 13.4, 6.7 Hz, 1H), 0.94 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 168.3, 164.9, 164.6, 164.5, 149.4, 149.2, 144.8, 144.7, 143.5, 142.2, 139.3, 134.8, 133.6, 132.8, 130.3, 129.0, 128.5, 128.5, 127.6, 127.4, 126.4, 124.2, 123.6, 119.9, 119.0, 118.7, 117.8, 79.4, 74.6, 69.4, 64.8, 52.1, 38.0, 28.6, 19.0.

HRMS (ESI) calculated for C61H57N6O11 ($M+H^+$) 1049.4080, found 1049.4096.

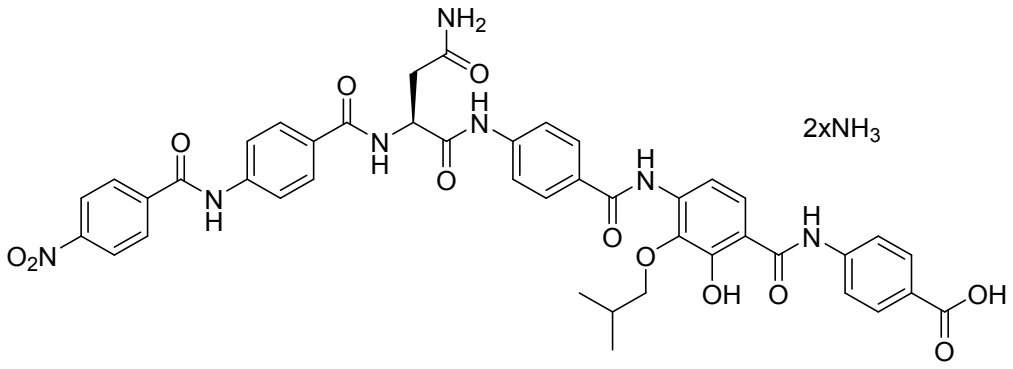
Allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isobutoxybenzamido)benzoate (141)



Chemical Formula: $C_{42}H_{44}N_6O_9$
Exact Mass: 776,3170

Zn dust (0.314 g; 4.8 mmol) was added portion wise over few minutes to a stirred solution of intermediate **140** (0.25 g; 0.24 mmol) in THF (1.5 mL), EtOH (1.4 mL) and AcOH (0.15 mL). The reaction was stirred for 5 hours, the mixture was filtered through celite and the solvent was reduced under vacuum. The crude was used in the next step without further purification.
The residue was dissolved in DCM (9.0 mL), Tips (0.148 mL; 0.72 mmol) followed by TFA (3.0 mL) were added at 0 °C. The reaction was stirred for 2 hours at r.t. then the solvent was removed under vacuum, the residue take up and evaporated twice with DCM (5 mL) then triturated 3x with ice cold Pet. Et.. The crude thus obtained was purified on silica gel with a gradient 0-10% MeOH in DCM to give 140 mg of a yellow solid (0.18 mmol; y= 76%).
^{1}H NMR (500 MHz, DMSO) δ 10.58 (s, 1H), 10.36 (s, 1H), 9.56 (s, 1H), 8.22 (d, *J* = 7.4 Hz, 1H), 7.98 (dd, *J* = 11.9, 5.0 Hz, 4H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.9 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.63 – 7.59 (m, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 6.58 – 6.54 (m, 2H), 6.10 – 5.96 (m, 2H), 5.70 (br, 2H), 5.41 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.36 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.28 (ddd, *J* = 10.5, 3.0, 1.4 Hz, 1H), 5.19 (ddd, *J* = 10.5, 3.0, 1.3 Hz, 1H), 4.84 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.79 (dt, *J* = 5.3, 1.4 Hz, 2H), 4.60 (d, *J* = 5.5 Hz, 2H), 3.81 (d, *J* = 6.2 Hz, 2H), 2.66 – 2.62 (m, 2H), 1.99 (dp, *J* = 13.2, 6.6 Hz, 1H), 0.94 (d, *J* = 6.7 Hz, 6H).
^{13}C NMR (126 MHz, DMSO) δ 171.5, 171.0, 166.4, 165.0, 164.6, 164.5, 151.8, 149.4, 144.7, 143.5, 142.4, 134.8, 133.6, 132.8, 130.4, 129.1, 128.4, 128.3, 127.6, 124.2, 123.5, 120.4, 119.9, 119.0, 118.6, 117.8, 112.5, 79.4, 74.6, 64.9, 51.5, 36.8, 28.6, 19.0.
HRMS (ESI) calculated for C42H45N6O9 ($M+H^+$) 777.3243, found 777.3225.

(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isobutoxybenzamido)benzoic acid (53)



Chemical Formula: $C_{43}H_{39}N_7O_{12}$
Exact Mass: 845,2657

Collidine (0.048 mL; 0.36 mmol) was added dropwise at 0 °C to a solution of 4-nitro benzoic acid (26 mg; 0.16 mmol) and bis(trichloromethyl) carbonate (14.0 mg; 0.047 mmol) in THF (2.3 mL). Reaction stirred at r.t. for 20 min then added to a solution of amine **141** (35 mg; 0.045 mmol) and DiPEA (0.078 mL; 0.45 mmol) in THF (2.3 mL). The reaction was stirred for 3 hours then quenched with HCl 1 N and ice. The solvent was partially reduced under vacuum, EtOAc (25 mL) and HCl 1N (25 mL) were added, organic phase washed with $NaHCO_3$ saturated solution (20 mL), brine (20 mL) and dried over sodium sulphate. The solvent was removed under reduced pressure, the residue thus obtained was used in the next step without further purification.

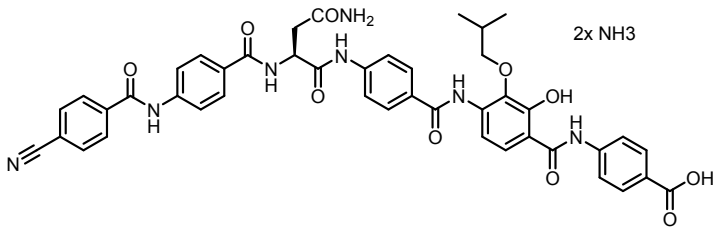
Phenyl silane (0.022 mL; 0.18 mmol) followed by palladium-tetrakis(triphenylphosphine (13.0 mg; 0.011 mmol) was added to a solution of the crude residue (0.045 mmol) in THF (4.5 mL). Reaction stirred for 3 hours and purified by preparative RP-HPLC using condition B (retention time: 22.69 min) to afford 3.0 mg of desired product (0.0036 mmol; y= 8%).

^{1}H NMR (700 MHz, DMSO) δ 12.78 (br, 1H), 12.27 (br, 1H), 10.79 (s, 1H), 10.61 (br, 1H), 10.44 (s, 1H), 9.36 (br, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.41 – 8.36 (m, 2H), 8.24 – 8.19 (m, 2H), 7.99 – 7.92 (m, 6H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.79 (d, *J* = 8.8 Hz, 3H), 7.53 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 13.9, 7.3 Hz, 1H), 3.83 (d, *J* = 6.4 Hz, 2H), 2.69 (dd, *J* = 6.9, 2.9 Hz, 2H), 2.01 (dp, *J* = 13.3, 6.6 Hz, 1H), 0.95 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.3, 166.9, 165.7, 164.3, 164.2, 149.3, 142.4, 141.5, 140.3, 130.2, 129.3, 129.2, 128.3, 128.3, 123.6, 122.9, 120.4, 119.5, 118.7, 78.5, 51.6, 36.8, 28.6, 19.1.

HRMS (ESI) calculated for C43H38N7O12 ($M-H^+$) 844.2584, found 844.2593.

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isobutoxybenzamido)benzoic acid (54)



Chemical Formula: $C_{44}H_{39}N_7O_{10}$
Exact Mass: 825,2758

Compound **54** was synthesized starting from amine **141** (30 mg; 0.038 mmol) and 4-Cyanobenzoic acid (18 mg; 0.12 mmol) using the same experimental procedure employed for the synthesis of compound **53**.

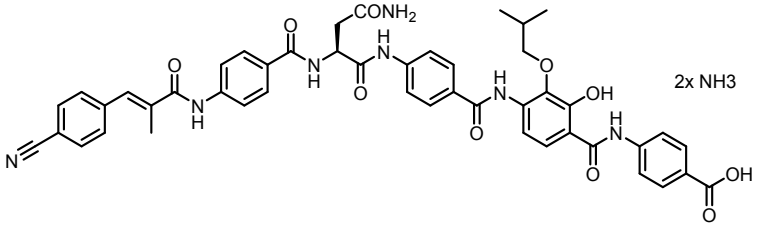
The final compound was purified by preparative RP-HPLC using condition B (retention time: 22.02 min) to afford 8.5 mg of desired product (0.010 mmol; y=27%).

^{1}H NMR (700 MHz, DMSO) δ 12.27 (br, 1H), 10.70 (s, 1H), 10.44 (s, 1H), 9.34 (br, 1H), 8.67 (d, *J* = 7.0 Hz, 1H), 8.17 – 8.10 (m, 2H), 8.08 – 8.01 (m, 2H), 7.99 – 7.87 (m, 8H), 7.84 (d, *J* = 7.5 Hz, 2H), 7.82 – 7.75 (m, 3H), 7.51 (br, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.91 (dd, *J* = 12.7, 6.1 Hz, 1H), 3.83 (d, *J* = 5.9 Hz, 2H), 2.69 (d, *J* = 6.5 Hz, 2H), 2.00 (m, 1H), 0.95 (dd, *J* = 6.6, 2.0 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.2, 166.9, 165.8, 164.5, 164.3, 141.6, 138.7, 132.5, 130.2, 129.1, 128.6, 128.3, 123.0, 120.3, 119.5, 118.7, 118.3, 114.0, 51.6, 36.8, 28.6, 19.1.

HRMS (ESI) calculated for C44H40N7O10 (M+H) 826.2831, found 826.2828.

(S,E)-4-(4-(4-(4-amino-2-(4-(3-(4-cyanophenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isobutoxybenzamido)benzoic acid (55)



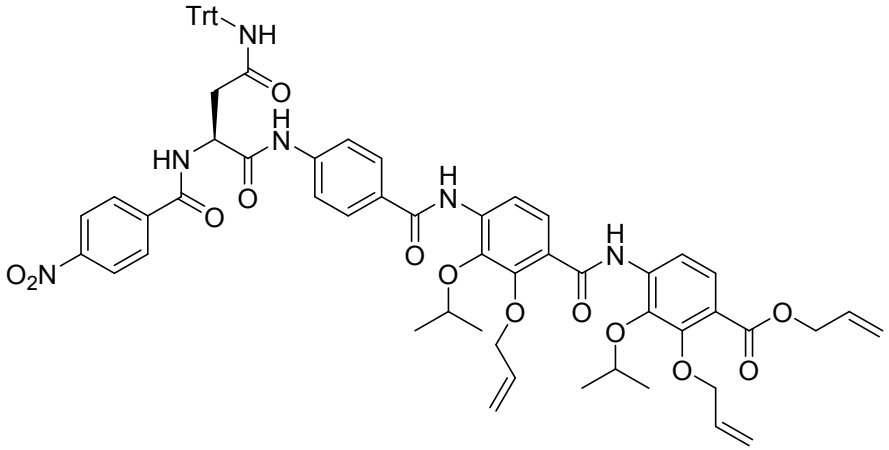
Chemical Formula: $C_{47}H_{43}N_7O_{10}$
Exact Mass: 865,3071

Compound **55** was synthesized starting from amine **141** (30 mg; 0.038 mmol) and carboxylic acid **104** (21 mg; 0.12 mmol) using the same experimental procedure employed for the synthesis of compound **53**.

The final compound was purified by preparative RP-HPLC using condition B (retention time: 24.21 min) to afford 5.5 mg of desired product (0.0064 mmol; y=18%).

^{1}H NMR (700 MHz, DMSO) δ 12.73 (br, 1H), 12.27 (br, 1H), 10.63 (br, 1H), 10.44 (s, 1H), 10.26 (s, 1H), 9.40 (s, 1H), 8.63 (d, *J* = 7.3 Hz, 1H), 7.96 (dd, *J* = 8.7, 3.2 Hz, 4H), 7.91 (dd, *J* = 13.1, 8.6 Hz, 4H), 7.84 (dd, *J* = 18.2, 8.8 Hz, 5H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.39 (s, 1H), 7.37 (s, 1H), 6.99 (s, 1H), 4.91 (dd, *J* = 14.0, 7.2 Hz, 1H), 3.82 (d, *J* = 6.4 Hz, 2H), 2.73 – 2.65 (m, 2H), 2.13 (d, *J* = 1.3 Hz, 3H), 2.01 (m, 1H) 0.95 (d, *J* = 6.7 Hz, 6H).
^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 168.4, 168.1, 166.9, 165.8, 164.4, 142.4, 142.0, 140.6, 138.5, 136.2, 135.8, 132.4, 131.7, 130.2, 130.1, 128.6, 128.3, 128.3, 126.2, 122.9, 120.6, 119.2, 118.7, 112.8, 110.3, 78.6, 51.6, 36.7, 28.6, 19.1, 14.6.
HRMS (ESI) calculated for C47H44N7O10 (M+H) 866.3144, found 866.3141.

Allyl (S)-2-(allyloxy)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)-3-isopropoxybenzoate (142)



Chemical Formula: $C_{66}H_{64}N_6O_{13}$
Exact Mass: 1148,4531

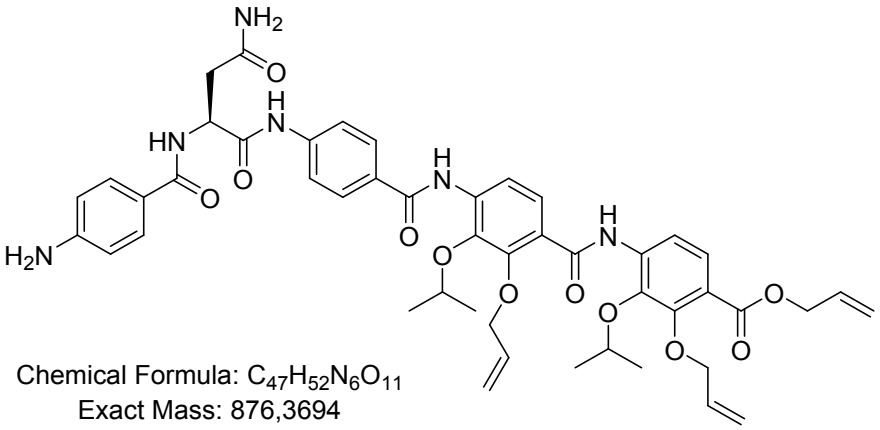
$POCl_3$ (1.42 mmol) as a solution in DCM (1:9) was added dropwise to a solution of allyl 2-(allyloxy)-4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)-3-isopropoxybenzoate (0.300 g; 0.57 mmol) and (S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoic acid (0.914 g, 1.42 mmol) in THF (7.2 mL) and DCM (5.0 mL) at 0 °C, followed by DiPEA (0.79 mL; 4.56 mmol) as a solution in DCM (1:1). Reaction stirred at r.t. for 5 h, quenched with HCl 1 N and ice, solvent partially reduced under vacuum and residue diluted with EtOAc (200 mL) and HCl 1N (200 mL), organic phase washed with brine (200 mL) and dried over sodium sulphate. Solvent removed under vacuum, the crude residue was chromatographed on silica gel with a gradient EtOAc 20-90% in Pet. Et to give 407 mg of an orange residue (0.35 mmol; 62%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 10.73 (s, 1H), 9.63 (s, 1H), 8.74 (s, 1H), 8.47 (d, *J* = 2.3 Hz, 1H), 8.45 (d, *J* = 2.4 Hz, 1H), 8.44 (s, 1H), 8.27 – 8.24 (m, 2H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.97 – 7.93 (m, 3H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.60 – 7.57 (m, 2H), 7.30 – 7.22 (m, 15H), 6.20 – 6.00 (m, 3H), 5.40 (tq, *J* = 17.2, 1.5 Hz, 2H), 5.33 – 5.25 (m, 2H), 5.25 – 5.21 (m,

2H), 5.12 – 5.06 (m, 1H), 4.80 (dt, *J* = 5.7, 1.3 Hz, 2H), 4.79 – 4.72 (m, 2H), 4.70 (d, *J* = 6.7 Hz, 2H), 4.59 (dt, *J* = 5.9, 1.3 Hz, 2H), 3.34 – 3.22 (m, 1H), 2.78 (dd, *J* = 15.6, 7.2 Hz, 1H), 1.41 (dd, *J* = 6.1, 3.3 Hz, 6H), 1.29 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 168.8, 165.6, 165.1, 164.1, 162.8, 151.9, 149.9, 149.0, 143.9, 141.1, 140.2, 139.2, 138.3, 138.2, 137.2, 133.9, 132.9, 132.3, 128.6, 128.1, 128.0, 127.3, 127.2, 127.0, 123.8, 122.6, 120.7, 120.3, 119.8, 119.4, 119.2, 118.4, 117.8, 115.5, 115.0, 76.6, 76.0, 75.7, 74.8, 71.2, 65.5, 51.2, 37.6, 22.8, 22.5.

HRMS (ESI) calculated for C66H65N6O13 ($M+H^+$) 1149.4604, found 1149.4593.

Allyl (S)-2-(allyloxy)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)-3-isopropoxybenzoate (143)



Chemical Formula: $C_{47}H_{52}N_6O_{11}$
Exact Mass: 876,3694

Zn dust (0.56 g; 8.6 mmol) was added portionwise over few minutes to a stirred solution of allyl (S)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)-butanamido)benzamido)benzamido)benzoate (0.49 g; 0.43 mmol) in THF (2.7 mL), EtOH (2.4 mL) and AcOH (0.27 mL). Reaction stirred for 5 hours, the mixture was filtered through celite, the solvent was reduced under vacuum. The crude was used in the next step without further purification.

The residue was dissolved in DCM (16.5 mL), Tips (0.264 mL; 1.29 mmol) followed by TFA (5.5 mL) were added at 0 C. Reaction stirred 2 h at r.t. then solved removed under vacuum, residue take up and evaporated twice with DCM (10 mL) then triturated 3x with ice cold Pet. Et.. The crude thus obtained was purified on silica gel with a gradient 0-10% MeOH in DCM to give 263 mg of a yellow solid (0.30 mmol; y= 70%).

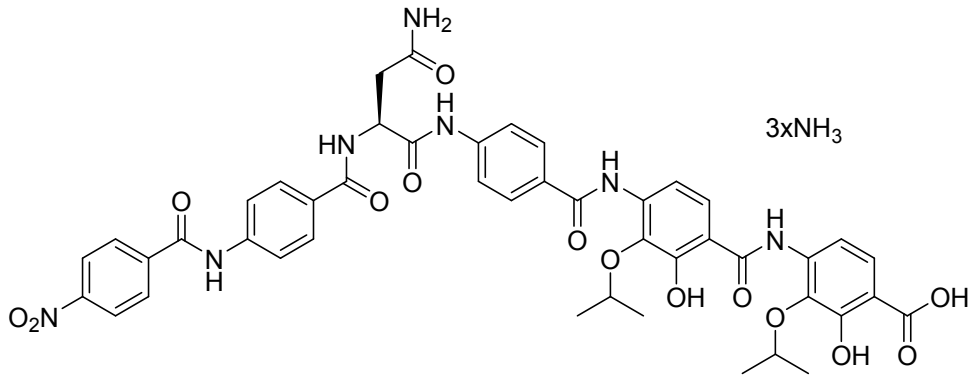
^{1}H NMR (500 MHz, DMSO) δ 10.89 – 10.54 (m, 1H), 10.39 (t, *J* = 7.9 Hz, 1H), 9.58 – 9.53 (m, 1H), 8.35 (d, *J* = 8.8 Hz, 1H), 8.22 (d, *J* = 7.4 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.84 – 7.77 (m, 3H), 7.63 – 7.59 (m, 2H), 7.56 (dd, *J* = 11.9, 8.7 Hz, 1H), 7.38 (s, 1H), 6.97 (s, 1H), 6.58 – 6.53 (m, 2H), 6.13 – 5.86 (m, 3H), 5.67 (br, 2H), 5.46 – 5.22 (m, 5H), 5.10 – 5.01 (m, 1H), 4.86 (m, 2H), 4.81 – 4.71 (m, 2H), 4.68 (d, *J* = 5.4 Hz, 1H), 4.62 (dt, *J* = 12.3,

6.1 Hz, 1H), 4.54 (d, *J* = 5.7 Hz, 1H), 4.46 (m, 2H), 2.64 (d, *J* = 7.1 Hz, 2H), 1.29 (dd, *J* = 12.4, 7.4 Hz, 3H), 1.24 (m, 6H), 1.19 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 171.5, 171.1, 168.7, 166.4, 164.5, 164.4, 152.9, 151.9, 149.8, 142.4, 141.3, 139.9, 137.7, 137.4, 136.6, 136.3, 134.0, 132.7, 132.2, 129.1, 128.5, 126.2, 125.5, 123.5, 120.4, 118.8, 118.5, 118.1, 117.7, 116.5, 114.6, 112.5, 76.6, 75.4, 74.2, 65.7, 65.1, 51.5, 36.9, 22.3, 22.1.

HRMS (ESI) calculated for C47H53N6O11 ($M+H^+$) 877.3767, found 877.3780.

(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (78)



Chemical Formula: $C_{45}H_{43}N_7O_{14}$
Exact Mass: 905,2868

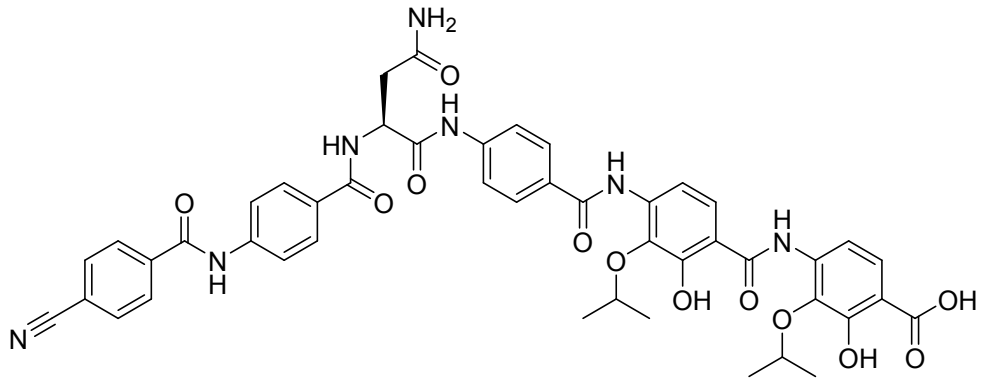
Collidine (0.036 mL; 0.272 mmol) was added dropwise at 0 °C to a solution of 4-nitro benzoic acid (20 mg; 0.12 mmol) and bis(trichloromethyl) carbonate (11.6 mg; 0.039 mmol) in THF (1.75 mL). Reaction stirred at r.t. for 20 min then added to a solution of amine **143** (30 mg; 0.034 mmol) and DiPEA (0.060 mL; 0.34 mmol) in THF (1.75 mL). Reaction stirred for 3 h then quenched with HCl 1 N and ice. Solvent partially reduced under vacuum, EtOAc (25 mL) and HCl 1N (25 mL) were added, organic phase washed with $NaHCO_3$ saturated solution (20 mL), brine (20 mL) and dried over sodium sulphate. The solvent was removed under reduced pressure, the residue thus obtained was used in the next step without further purification.

Phenyl silane (0.026 mL; 0.21 mmol) followed by palladium-tetrakis(triphenylphosphine (9.8 mg; 0.0085 mmol) was added to a solution of the crude residue (0.034 mmol) in THF (3.5 mL). The reaction was stirred overnight and purified by preparative RP-HPLC using condition B (retention time: 22.03 min) to afford 1.7 mg of desired product (0.0046 mmol; y= 6%).

^{1}H NMR (700 MHz, DMSO) δ 11.26 (s, 1H), 10.90 (s, 1H), 10.79 (s, 1H), 10.45 (s, 1H), 9.60 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.42 – 8.35 (m, 2H), 8.24 – 8.18 (m, 2H), 7.99 – 7.88 (m, 8H), 7.80 (dd, *J* = 8.7, 5.3 Hz, 4H), 7.54 – 7.48 (m, 2H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.69 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.31 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.71 – 2.65 (m, 2H), 1.31 – 1.23 (m, 12H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 165.8, 164.3, 164.2, 163.6, 162.3, 150.4, 149.3, 142.4, 141.5, 140.3, 138.4, 136.3, 134.0, 129.3, 129.2, 128.4, 128.3, 125.0, 124.8, 123.6, 119.5, 118.8, 116.4, 115.3, 112.5, 109.9, 75.7, 74.0, 51.6, 36.8, 22.0, 21.9.
HRMS (ESI) calculated for C45H42N7O14 (M-H$^+$) 904.2795, found 904.2786.

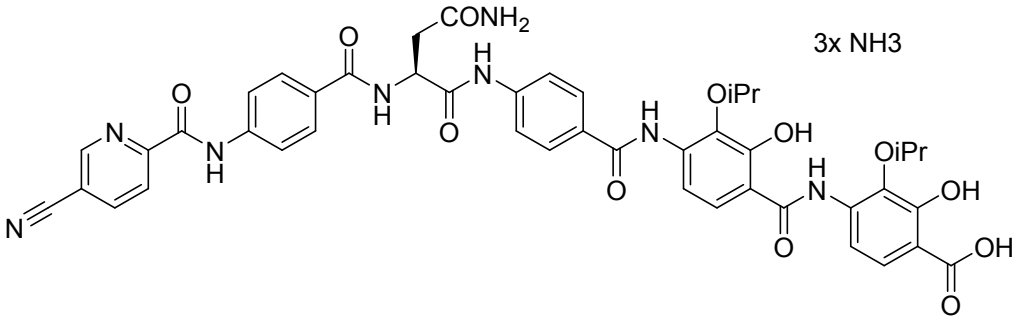
(S)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (79)



Chemical Formula: $C_{46}H_{43}N_7O_{12}$
Exact Mass: 885,2970

Compound **79** was synthesized starting from amine **143** (30 mg; 0.034 mmol) and 4-Cyanobenzoic acid (18 mg; 0.12 mmol) using the same experimental procedure employed for the synthesis of compound **78**.
The compound was purified by preparative RP-HPLC using condition B (retention time: 20.90 min) to afford 8.7 mg of desired product (0.010 mmol; y=29%).
^{1}H NMR (700 MHz, DMSO) δ 11.25 (s, 1H), 10.93 (s, 1H), 10.70 (s, 1H), 10.44 (s, 1H), 9.60 (s, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), 7.96 (t, *J* = 8.9 Hz, 3H), 7.91 (dd, *J* = 26.2, 8.8 Hz, 4H), 7.80 (m, 3H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.0, 7.3 Hz, 1H), 4.69 (dp, *J* = 12.3, 6.1 Hz, 1H), 4.30 (td, *J* = 12.1, 6.0 Hz, 1H), 2.71 – 2.67 (m, 2H), 1.27 (dd, *J* = 7.5, 6.3 Hz, 12H).
^{13}C NMR (176 MHz, DMSO) δ 172.0, 171.3, 170.7, 165.8, 164.5, 164.3, 163.6, 150.4, 142.4, 141.6, 138.7, 138.4, 136.3, 133.9, 132.5, 129.1, 128.6, 128.4, 128.3, 125.0, 124.9, 119.5, 118.8, 118.3, 116.4, 115.4, 114.0, 110.1, 75.7, 74.1, 51.6, 40.0, 39.9, 39.8, 39.6, 39.5, 39.4, 39.3, 39.2, 36.8, 22.0, 21.9.
HRMS (ESI) calculated for C46H42N7O12 (M-H$^+$) 884.2897, found 884.2917.

(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanopicolinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (80)



Chemical Formula: $C_{45}H_{42}N_8O_{12}$
Exact Mass: 886,2922

Compound **80** was synthesized starting from amine **143** (20 mg; 0.023 mmol) and 5-cyanopicolinic acid (12 mg; 0.08 mmol) using the same experimental procedure employed for the synthesis of compound **78**.

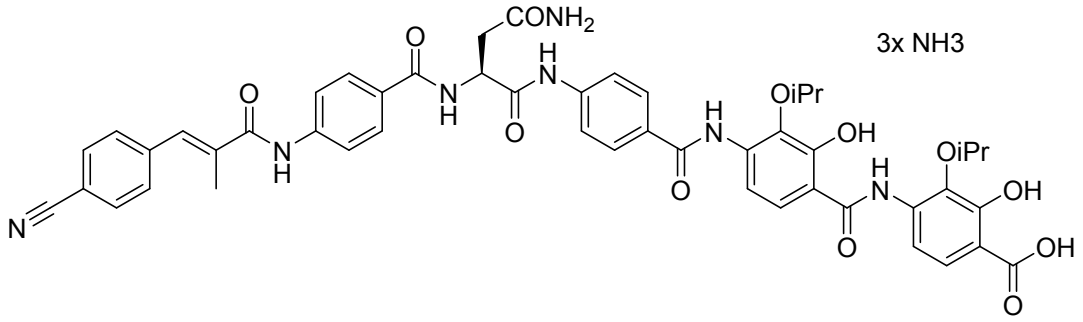
Preparative RP-HPLC using condition B (retention time: 23.53 min) afforded 5.6 mg of desired product (0.0063 mmol; y=27%).

^{1}H NMR (700 MHz, DMSO) δ 11.26 (s, 1H), 11.03 (s, 1H), 10.89 (s, 1H), 10.43 (s, 1H), 9.59 (s, 1H), 9.22 (dd, *J* = 1.9, 0.7 Hz, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.60 (dd, *J* = 8.2, 2.0 Hz, 1H), 8.35 – 8.27 (m, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.93 (d, *J* = 8.7 Hz, 3H), 7.80 (dd, *J* = 8.7, 4.8 Hz, 3H), 7.51 (t, *J* = 9.5 Hz, 2H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 13.9, 7.4 Hz, 1H), 4.72 – 4.66 (m, 1H), 4.31 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.73 – 2.65 (m, 2H), 1.27 (dd, *J* = 5.8, 5.1 Hz, 12H).

^{13}C NMR (176 MHz, DMSO) δ 171.9, 171.3, 170.7, 165.8, 164.3, 163.6, 161.6, 152.3, 151.5, 150.4, 142.4, 142.3, 140.9, 138.4, 136.3, 134.0, 129.4, 128.4, 128.3, 124.9, 124.8, 122.5, 119.7, 118.8, 116.6, 116.4, 115.3, 111.7, 109.9, 75.7, 74.0, 51.6, 36.8, 22.0, 21.9.

HRMS (ESI) calculated for C45H43N8O12 ($M+H^+$) 887.2995, found 887.2992.

(S,E)-4-(4-(4-(4-Amino-2-(4-(3-(4-cyanophenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (82)



Chemical Formula: $C_{49}H_{47}N_7O_{12}$
Exact Mass: 925,3283

Compound **82** was synthesized starting from amine **143** (25 mg, 0.033 mmol) and carboxylic acid **104** (12 mg; 0.08 mmol) using the same experimental procedure employed for the synthesis of compound **78**.

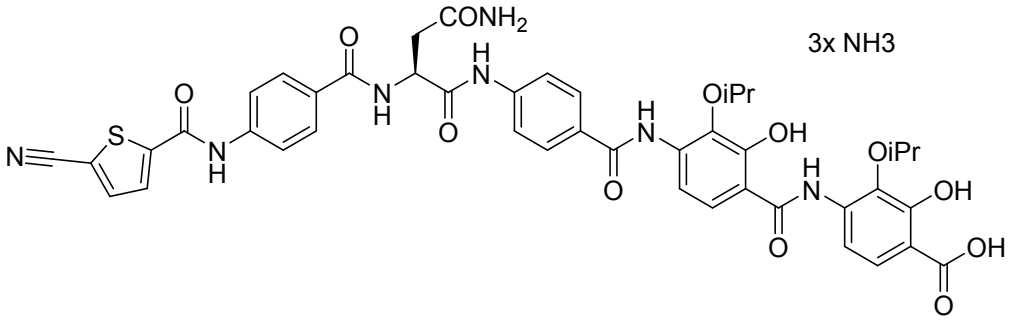
Preparative RP-HPLC using condition B (retention time: 26.18 min) afforded 6.6 mg of desired product (0.0071 mmol, y= 22%).

^{1}H NMR (700 MHz, DMSO) δ 11.26 (s, 1H), 10.91 (s, 1H), 10.44 (s, 1H), 10.26 (s, 1H), 9.60 (s, 1H), 8.64 (d, *J* = 7.2 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 6.3 Hz, 1H), 7.91 (dd, *J* = 11.4, 8.6 Hz, 4H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.80 (dd, *J* = 8.8, 1.9 Hz, 3H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.40 (s, 1H), 7.37 (s, 1H), 6.99 (s, 1H), 4.92 (dd, *J* = 14.0, 7.1 Hz, 1H), 4.69 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.31 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.69 (d, *J* = 7.5 Hz, 2H), 2.13 (d, *J* = 0.8 Hz, 3H), 1.27 (t, *J* = 6.2 Hz, 12H).

^{13}C NMR (176 MHz, DMSO) δ 172.0, 171.3, 170.7, 168.1, 165.8, 164.3, 163.6, 162.3, 155.0, 150.4, 142.4, 142.0, 140.6, 138.4, 136.3, 135.8, 134.0, 132.4, 131.7, 130.1, 128.6, 128.4, 128.3, 125.0, 124.8, 119.2, 118.8, 116.4, 115.4, 110.3, 110.1, 75.7, 74.1, 51.6, 36.8, 22.0, 21.9, 14.6.

HRMS (ESI) calculated for C49H48N7O12 ($M+H^+$) 926.3355, found 926.3337.

(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanothiophene-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid (81)



Chemical Formula: $C_{44}H_{41}N_7O_{12}S$
Exact Mass: 891,2534

Compound **81** was synthesized starting from amine **143** (20 mg; 0.023 mmol) and 5-cyanothiophene-2-carboxylic acid (12 mg; 0.08 mmol) using the same experimental procedure employed for the synthesis of compound **78**.

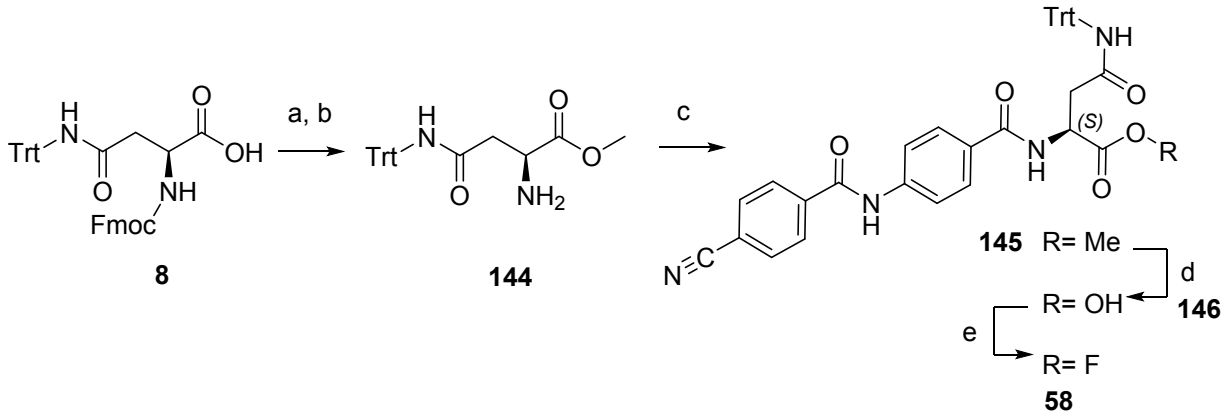
Preparative RP-HPLC using condition B (retention time: 24.41 min) afforded 4.7 mg of desired product (0.0053 mmol; y=23%).

^{1}H NMR (700 MHz, DMSO) δ 11.27 (br, 1H), 10.88 (br, 1H), 10.78 (s, 1H), 10.44 (s, 1H), 9.59 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.14 (d, *J* = 4.1 Hz, 1H), 8.08 (d, *J* = 4.0 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.92 (br, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.80 (m, 3H), 7.51 (t, *J* = 7.8 Hz, 2H), 7.39 (s, 1H), 6.98 (s, 1H), 4.92 (dd, *J* = 14.0, 7.2 Hz, 1H), 4.69 (dt, *J* = 12.3, 6.1 Hz, 1H), 4.31 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.73 – 2.65 (m, 2H), 1.27 (m, 12H).

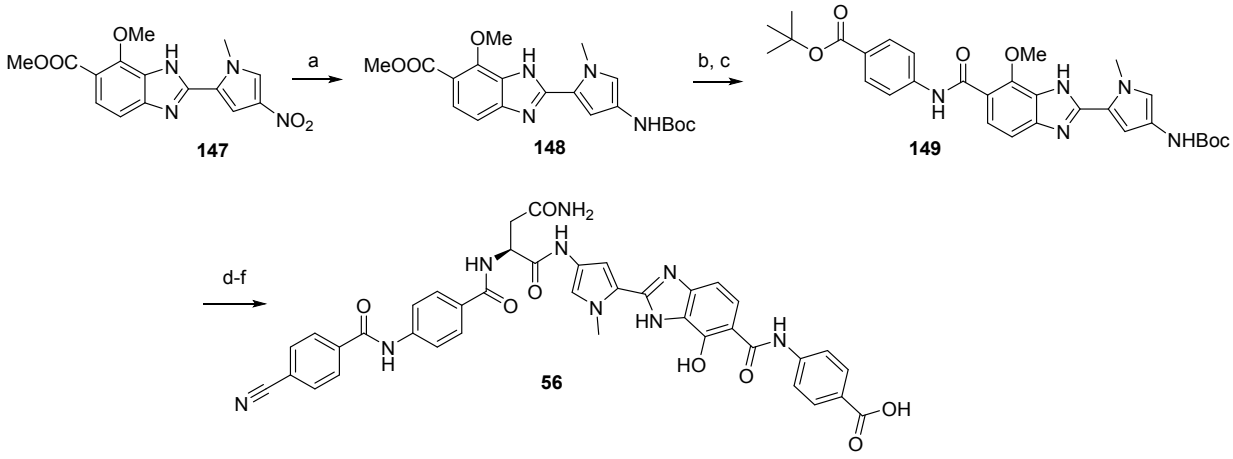
^{13}C NMR (176 MHz, DMSO) δ 171.9, 171.3, 170.7, 165.7, 164.3, 163.6, 158.5, 150.4, 146.8, 142.4, 140.8, 139.7, 138.4, 136.2, 134.0, 129.5, 129.2, 128.4, 124.9, 124.7, 119.7, 118.8, 116.4, 115.3, 113.8, 112.5, 109.9, 75.7, 73.9, 51.6, 36.8, 22.0, 21.9.

HRMS (ESI) calculated for C44H42N7O12S ($M+H^+$) 892.2607, found 892.2615.

1.2.2.3.1 rescaffolding - introduction of minor groove binder moiety.

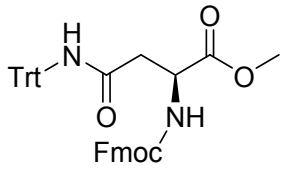


Scheme S 6. Reagents and conditions: a) MeI, K_2CO_3, DMF, rt, 3 h (98%); b) piperidine, DMF, rt, 3 h (88%); c) **64**, HBTU, DiPEA, DMF, rt, 21 h (92%); d) LiI, EtOAc, MW, 110 °C, 1.5 h (98%); e) DAST, DCM, rt, 1 h (84%).



Scheme S 7. Reagents and conditions: a) Pd/C, DMF, rt, 5 h, then Boc_2O, DiPEA, rt, overnight (73%); b) NaOH, THF/water, 50 °C, 2 d (57%); c) *t*-But *p*-aminobenzoate, EDC, HOAt, collidine, DMF, rt, 3 d (99%) d) TFA, DCM, rt, 2 h, then **58**, DiPEA, THF, rt, 42 h (91%), e) LiI, EtOAc, 110 °C, 1.5 h, f) TFA, Tips, DCM, rt, 1.5 h (37% o2s).

Fmoc-Asn(Trt)-OMe (150)



$C_{39}H_{34}N_2O_5$
610.71 g/mol

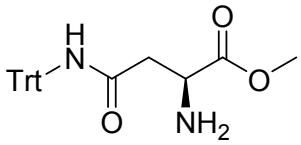
To a stirring solution of Fmoc-Asn(Trt)-OH (**10**) (3.00 g, 5.03 mmol, 1.0 eq.) and K_2CO_3 (0.83 g, 6.03 mmol, 1.2 eq.) in dry DMF (50 mL) was added dropwise MeI (0.47 mL, 7.54 mmol, 1.5 eq.) at 0 °C. After being stirred for 30 minutes at 0 °C, the mixture was allowed to warm to room temperature and stirred for an additional 3 hours. Then the solution was cooled to 0 °C and a solution of $Na_2S_2O_3$ (7.5 g) in water (50 mL) was added. The mixture was extracted with EtOAc (3 × 75 mL) and the combined organic extracts were washed with brine (5 × 100 mL) and dried over Na_2SO_4. The solvent was removed by distillation at reduced pressure and the residue was dried at high vacuum affording methyl ester **150** as a white solid (3.01 g, 4.93 mmol, 98%).

^{1}H NMR (500 MHz, DMSO-*d*$_6$, 300 K): δ (ppm) = 8.67 (s, 1H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.75 – 7.69 (m, 2H), 7.45 – 7.38 (m, 2H), 7.35 – 7.12 (m, 18H+CON*H*), 4.41 – 4.33 (m, 2H), 4.30 (dd, *J* = 10.5, 6.9 Hz, 1H), 4.24 (t, *J* = 6.9 Hz, 1H), 3.61 (s, 3), 2.72 – 2.65 (m, 2H).

^{13}C NMR (125 MHz, DMSO-*d*$_6$, 300 K): δ (ppm) = 172.1, 168.5, 155.8, 144.6, 143.7, 140.7, 128.5, 127.6, 127.4, 127.1, 126.4, 125.2, 125.1, 120.1, 69.4, 65.7, 52.1, 50.9, 46.6, 38.0.

HRMS (ESI+): *m/z* for $C_{394}N_2O_5$ $[M+H]^+$: calculated: 611.2540, found: 611.2546.

Asn(Trt)-OMe (144)



$C_{24}H_{24}N_2O_3$
388.47 g/mol

To a stirring solution of methyl ester **150** (2.50 g, 4.10 mmol, 1.0 eq.) in dry DMF (45 mL), piperidine (5.0 mL) was added dropwise. After 3 hours, the mixture was cooled to 0 °C and 100 mL of saturated aqueous NH_4Cl solution were added. The mixture was extracted with EtOAc (3 × 75 mL) and the combined organic extracts were washed with water (2 × 100 mL) and brine (2 × 100 mL) and dried over Na_2SO_4. The solvent was removed by distillation at reduced pressure and the residue was suspended in petroleum ether (100 mL). The suspension was stirred for 3 hours, before the solid was separated by filtration, washed with

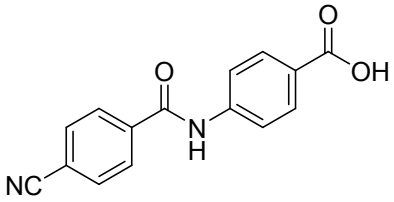
petroleum ether and dried at high vacuum to afford amine **144** as a white powder (1.40 g, 3.61 mmol, 88%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 9.16 (s, 1H), 7.30 – 7.24 (m, 6H), 7.22 – 7.14 (m, 9H), 3.64 (dd, J = 7.0, 5.5 Hz, 1H), 3.60 (s, 3), 2.57 – 2.51 (m, 2H), 2.03 (br, 2H).

^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 175.1, 169.4, 144.9, 128.5, 127.5, 126.4, 69.3, 51.6, 51.2, 40.4.

HRMS (ESI+): *m/z* for $C_{24}H_4N_2O_3$ $[M+H]^+$: calculated: 389.1860, found: 389.1864.

4-(4-cyanobenzamido)benzoic acid (64)



$C_{15}H_{10}N_2O_3$
266.26 g/mol

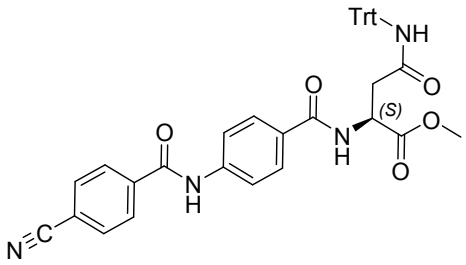
4-aminobenzoic acid (750 mg, 4.53 mmol, 1.0 eq.) was dissolved in a mixture of THF (6.0 mL) and saturated aqueous $NaHCO_3$ solution (6.0 mL). The solution was cooled to 0 °C and 4-cyanobenzoyl chloride (621 mg, 4.53 mmol, 1.0 eq.) was added in small portions. The mixture was allowed to warm to room temperature and stirred for 18 hours. The solution was acidified with 1M HCl until a white precipitate was formed. The precipitate was collected by filtration and washed first with 1M HCl (20 mL), then with Et_2O (2 × 20 mL) and dried at high vacuum. Amide **64** was afforded as a white solid (1.08 g, 4.06 mmol, 90%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.79 (s, 1H), 8.13 – 8.03 (m, 4H), 7.97 – 7.89 (m, 4H).

^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.9, 164.6, 142.8, 138.7, 132.5, 130.3, 128.7, 125.9, 119.6, 118.2, 114.1.

HRMS (ESI+): *m/z* for $C_{15}H_{10}N_2O_3$ $[M+H]^+$: calculated: 267.0764, found: 267.0764.

Methyl N^2-(4-(4-cyanobenzamido)benzoyl)-N^4-trityl-L-asparaginate (145)



$C_{39}H_{32}N_4O_5$
636.71 g/mol

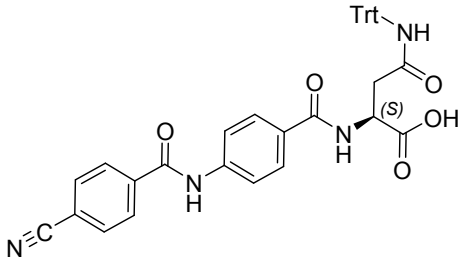
To a stirring solution of carboxylic acid **64** (0.76 g, 3.35 mmol, 1.0 eq.) and HBTU (1.27 g, 3.35 mmol, 1.0 eq.) in DMF (5.5 mL) under N_2 atmosphere was added DIPEA (1.7 mL, 10.1 mmol, 3.0 eq.) and the mixture was stirred for an additional 15 minutes. A solution of amine **144** (1.30 g, 3.35 mmol, 1.0 eq.) in DMF (5.5 mL) was added dropwise to the solution containing the pre-activated carboxylic acid. The mixture was stirred at room temperature for 21 hours, before H_2O (50 mL) and EtOAc (50 mL) were added and the pHses were separated. The aqueous pHse was extracted with EtOAC (3 × 50 mL) and the combined organic extracts were washed with saturated aqueous NH_4Cl solution (50 mL), saturated aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was removed by distillation at reduced pressure and the residue was dried at high vacuum overnight yielding compound **145** as a white powder (1.97 g, 3.09 mmol, 92%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.72 (s, 1H), 8.74 (d, *J* = 7.7 Hz, 1H), 8.71 (s, 1H), 8.15 – 8.04 (m, 4H), 7.94 – 7.86 (m, 4H), 7.23 – 7.14 (m, 15H), 4.76 (ddd, *J* = 9.3, 7.7, 5.3 Hz, 1H), 3.64 (s, 3), 2.89 (dd, *J* = 15.0, 9.1 Hz, 1H), 2.80 (dd, *J* = 15.0, 5.3 Hz, 1H).

^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 172.1, 168.6, 165.5, 164.5, 144.6, 141.7, 138.7, 132.5, 128.9, 128.6, 128.5, 128.2, 127.4, 126.4, 119.6, 118.3, 114.0, 69.4, 52.1, 50.0, 38.3.

HRMS (ESI+): *m/z* for $C_{392}N_4O_5$ $[M+H]^+$: calculated: 637.2445, found: 637.2444.

N^2-(4-(4-cyanobenzamido)benzoyl)-N^4-trityl-L-asparagine (146)



$C_{38}H_{30}N_4O_5$
622.68 g/mol

Methyl ester **145** (800 mg, 1.26 mmol, 1.0 eq.) was suspended in dry EtOAc (12.5 mL) and heated under microwave irradiation for 1.5 hours at 110 °C. The mixture was then diluted with

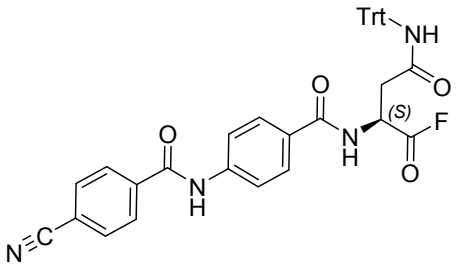
EtOAc (50 mL) and washed first with saturated aqueous $Na_2S_2O_3$ solution (50 mL), then with water (50 mL) and brine (50 mL). The aqueous pHses were combined, acidified to pH 3 by addition of 1M HCl and extracted with EtOAc (3 × 50 mL). The organic phases were combined and dried over Na_2SO_4. The solvent was removed by distillation at reduced pressure and the residue subjected to flash chromatography (DCM/ MeOH) and dried at high vacuum yielding carboxylic acid **146** as a beige solid (767 mg, 1.23 mmol, 98%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (br, 1H), 10.73 (s, 1H), 9.10 (br, 1H), 8.46 (d, *J* = 7.3 Hz, 1H), 8.15 – 8.03 (m, 4H), 7.93 – 7.85 (m, 4H), 7.23 – 7.15 (m, 15H), 4.59 (d, *J* = 13.2, 7.3 Hz, 1H), 2.83 – 2.70 (m, 2H).

^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 173.1, 169.1, 165.1, 164.4, 144.8, 141.5, 138.7, 132.5, 129.0, 128.6, 128.5, 128.3, 128.0, 126.3, 119.6, 118.3, 114.0, 69.3, 55.8, 38.3.

HRMS (ESI+): *m/z* for $C_{380}N_4O_5$ $[M+H]^+$: calculated: 623.2289, found: 623.2273.

N^2-(4-(4-cyanobenzamido)benzoyl)-N^4-trityl-L-asparaginoyl fluoride (58)



$C_{38}H_{29}FN_4O_4$
624.67 g/mol

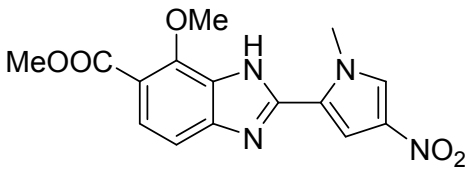
Carboxylic acid **146** (100 mg, 0.16 mmol, 1.0 eq.) was dissolved in 1.5 mL of dry DCM under a nitrogen atmosphere. The stirred solution was cooled to 0 °C and a solution of DAST (26 µL, 0.19 mmol, 1.2 eq.) in 100 µL of dry DCM was added slowly. After 30 minutes the mixture was allowed to warm to room temperature and stirred for an additional 30 minutes, before being diluted with 20 mL of DCM. The mixture was washed with ice water (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4 and the solvent was removed by distillation at reduced pressure to afford acyl fluoride **58** as a yellow solid (84 mg, 0.14 mmol, 84%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.85 (s, 1H), 8.88 (s, 1H), 8.18 – 8.15 (m, 2H), 8.08 – 8.02 (m, 4H), 7.98 – 7.95 (m, 2H), 7.14 – 7.04 (m, 16H), 4.77 (t, *J* = 4.4 Hz, 1H), 3.38 (dd, *J* = 15.8, 5.1 Hz, 1H), 2.78 (dd, *J* = 15.8, 3.9 Hz, 1H).

^{13}C NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 178.3, 168.1, 164.6, 160.8, 144.3, 143.8, 138.6, 132.5, 129.0, 128.7, 128.5, 128.4, 128.3, 126.4, 120.2, 118.3, 114.1, 69.5, 61.8, 37.0.

^{19}F-NMR (471 MHz DMSO-d_6, 300 K): δ (ppm) = 28.3.

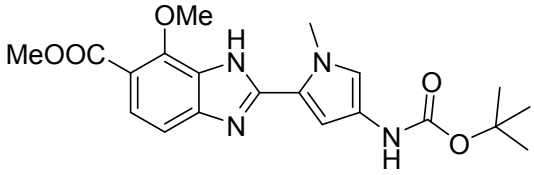
Methyl 4-methoxy-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)-3*H*-benzimidazole-5-carboxylate (147)



$C_{15}H_{14}N_4O_5$
330.30 g/mol

Compound **147** was synthesized according to a modified literature procedure.[16]
^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.16 (s, 1H), 8.27 (d, *J* = 2.0 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 4.37 (s, 3), 4.18 (s, 3), 3.80 (s, 3).
^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.6, 151.3, 143.9, 138.9, 134.9, 134.6, 128.0, 125.7, 123.7, 114.2, 106.5, 104.8, 61.0, 51.7, 38.0.
HRMS (ESI+): *m/z* for $C_{15}H_{14}N_4O_5$ $[M+H]^+$: calculated: 331.1037, found: 331.1035.

Methyl 2-(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrol-2-yl)-4-methoxy-3*H*-benzimidazole-5-carboxylate (148)

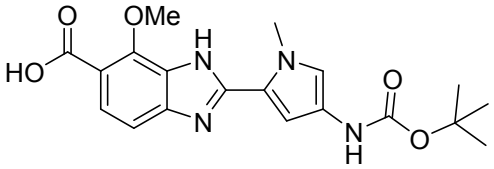


$C_{20}H_{24}N_4O_5$
400.44 g/mol

A stirring solution of compound **147** (450 mg, 1.36 mmol, 1.0 eq.) in dry DMF (12 mL) under N_2 atmosphere was purged with N_2 for 15 minutes. Palladium on activated carbon (170 mg, 10wt% loading) was added and the mixture set under H atmosphere. The mixture was stirred at room temperature for 5 hours until completion of the reaction was indicated by TLC. Then, Boc_2O (446 mg, 2.04 mmol, 1.5 eq.) and DIPEA (5.5 mL) were added. After stirring overnight, the reaction mixture was filtered through a pad of cellite. The residue was washed with EtOAc (50 mL) and water (50 mL) was added to the filtrate. The pHses were separated and the aqueous pHse was extracted with EtOAc (2 × 50 mL). The combined organic extracts where then washed with water (2 × 50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was removed by distillation at reduced pressure and the residue was purified by flash chromatography (petrol ether/ethyl acetate) affording compound **148** as a light brown solid (399 mg, 1.00 mmol, 73%).
^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.76 (s, 1H), 9.19 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 6.98 (s, 1H), 6.81 (s, 1H), 4.34 (s, 3), 4.03 (s, 3), 3.79 (s, 3), 1.47 (s, 9).

^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.7, 152.8, 150.8, 146.0, 139.1, 135.0, 124.7, 123.6, 119.7, 116.1, 113.9, 104.4, 102.8, 78.3, 60.8, 51.6, 36.4, 28.2.
HRMS (ESI+): *m/z* for $C_{20}H_4N_4O_5$ [M+H]$^+$: calculated: 401.1819, found: 401.1820.

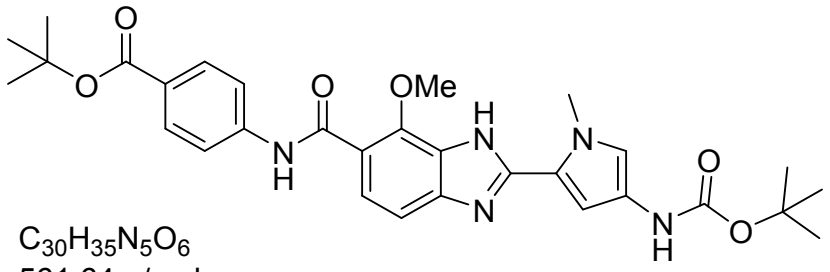
2-(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrol-2-yl)-4-methoxy-3*H*-benzimidazole-5-carboxylic acid (151)



$C_{19}H_{22}N_4O_5$
386.41 g/mol

Methyl 2-(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrol-2-yl)-4-methoxy-3*H*-benzimidazole-5-carboxylate (**148)** (262 mg, 0.65 mmol, 1.0 eq.) was dissolved in 13 mL of THF and 13 mL of 2M NaOH were added. The mixture was heated to 50 °C for two days. The mixture was then cooled to 0 °C, acidified to pH 3 by addition of 1M HCl and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed by distillation at reduced pressure. The crude product was purified by flash chromatography (DCM/ MeOH) yielding compound **151** as a pale yellow solid (143 mg, 0.37 mmol, 57%).
^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.73 (s, 1H), 12.25 (br, 1H), 9.19 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 1.9 Hz, 1H), 6.81 (s, 1H), 4.34 (s, 3), 4.03 (s, 3), 1.47 (s, 9).
^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.8, 152.8, 150.6, 145.9, 138.9, 135.0, 125.0, 123.6, 119.8, 116.1, 114.9, 104.4, 102.7, 78.3, 60.8, 36.4, 28.2.
HRMS (ESI+): *m/z* for $C_{20}H_4N_4O_5$ [M+H]$^+$: calculated: 387.1663, found: 387.1680.

***tert*-butyl 4-(2-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrol-2-yl)-7-methoxy-1*H*-benzo[*d*]imidazole-6-carboxamido)benzoate (149)**



$C_{30}H_{35}N_5O_6$
561.64 g/mol

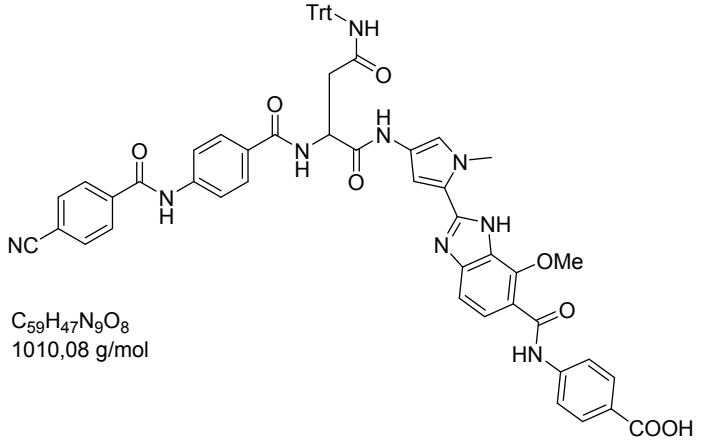
Carboxylic acid **151** (58 mg, 0.15 mmol, 1.0 eq.), *tert*-butyl 4-aminobenzoate (43 mg, 0.22 mmol, 1.5 eq.) and HOAt (30 mg, 0.22 mmol, 1.5 eq.) were dissolved in DMF (1.5 mL). EDC · HCl (34 mg, 0.18 mmol, 1.2 eq.) and collidine (99 µL, 0.74 mmol, 5.0 eq.) were added to the solution and the mixture was stirred for 3 days. Water (15 mL) was added and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with 1M HCl (15 mL), saturated aqueous $NaHCO_3$ solution (15 mL) and brine (15 mL) before the solvent was removed by distillation at reduced pressure. The residue was dried at high vacuum yielding compound **149** as a pale brown solid (83 mg, 0.15 mmol, 99%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.77 (s, 1H), 10.42 (s, 1H), 9.20 (s, 1H), 7.88 (s, 4H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 7.00 (s, 1H), 6.83 (s, 1H), 4.53 (s, 3) , 4.05 (s, 3), 1.55 (s, 9), 1.47 (s, 9).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.3, 164.6, 148.3, 146.0, 143.3, 138.6, 134.2, 130.9, 130.1, 125.7.1, 124.0, 118.8, 117.6, 116.1, 112.5, 104.7, 102.8, 80.3, 78.3, 61.1, 36.4, 28.2, 27.9.

HRMS (ESI+): *m/z* for $C_{305}N_5O_6$ $[M+H]^+$: calculated: 562.2660, found: 562.2661.

4-(2-(4-(2-(4-(4-cyanobenzamido)benzamido)-4-oxo-4-(tritylamino)butanamido)-1-methyl-1*H*-pyrrol-2-yl)-7-methoxy-1*H*-benzimidazole-6-carboxamido)benzoic acid (152)



$C_{59}H_{47}N_9O_8$
1010,08 g/mol

Compound **149** (68 mg, 0.12 mmol, 1.0 eq.) was dissolved in dry DCM (2.4 mL) under nitrogen atmosphere. The solution was cooled to 0 °C, protected from light and TFA (0.6 mL) was

added. The mixture was allowed to warm to room temperature and stirred for 2 hours. The solvent was evaporated, the residue suspended in DCM (5 mL) and evaporated again. Suspension in DCM (5 mL) and evaporation was repeated twice.

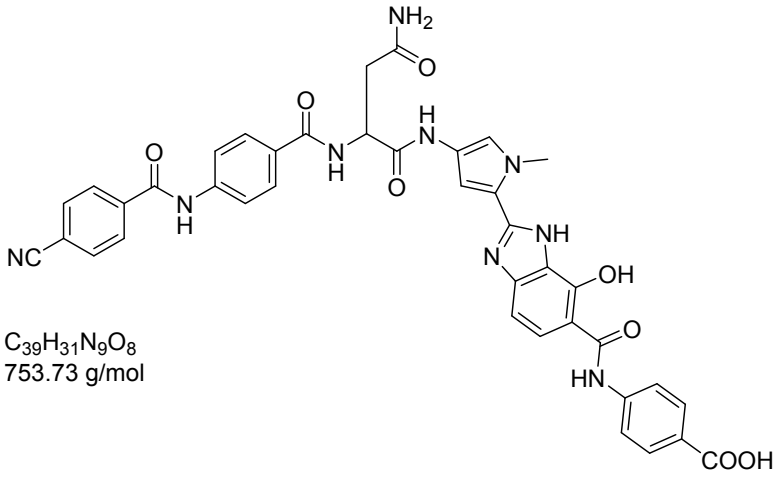
The crude product was then dissolved in dry THF (1 mL) and cooled to 0 °C again. Acyl fluoride **58** (75 mg, 0.12 mmol, 1.0 eq.) was suspended in dry THF (5 mL) and added to the previously prepared, cooled solution. DIPEA (0.11 mL, 0.60 mmol, 5.0 eq.) was added and the mixture was allowed to slowly warm to room temperature. The mixture was stirred under protection from light for 42 hours. Ethy acetate (100 mL) was added and the organic layer was washed with 0.5M HCl (2 × 100 mL) and brine (100 mL) and dried over Na_2SO_4. The solvent was then removed by distillation at reduced pressure and the product was dried at high vacuum yielding compound **152** (racemic) as a brownish solid containing some impurities (110 mg, 0.11 mmol, 91%).

^{1}H NMR (500 MHz, DMSO-*d*$_6$, 300 K): δ (ppm) = 10.72 (s, 1H), 10.44 (s, 1H), 10.17 (s, 1H), 8.71 (d, *J* = 8.0 Hz, 1H,), 8.62 (s, 1H,), 8.16 – 8.13 (m, 2H), 8.06 – 8.04 (m, 2H), 7.99 – 7.97 (m, 2H), 7.94 – 7.88 (m, 6H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.23 – 7.16 (m, 15H), 7.13 – 7.10 (m, 2H), 6.98 (s, 1H), 4.89 (ddd, *J* = 11.5, 7.8, 4.1 Hz, 1H), 4.48 (s, 3), 4.08 (s, 3), 3.00 (dd, *J* = 14.5, 11.0 Hz, 1H), 2.64 (dd, *J* = 14.4, 4.0 Hz, 1H).

^{13}C NMR (126 MHz, DMSO-*d*$_6$, 300 K): δ (ppm) = 168.7, 168.5, 166.9, 165.6, 165.3, 164.5, 158.3, 158.1, 144.7, 143.8, 143.3, 141.5, 138.7, 132.5 , 130.4 , 129.0, 128.6, 128.6, 128.3, 127.4, 126.3, 126.2 (), 125.1, 123.0, 119.5, 118.9, 118.3, 116.3, 114.7, 114.0, 69.4, 61.2, 51.4, 38.7, 36.5.

HRMS (ESI+): *m/z* for $C_{59}H_{47}N_9O_8$ $[M+H]^+$: calculated: 1010.3620, found: 1010.3551.

4-(2-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)-1-methyl-1*H*-pyrrol-2-yl)-7-hydroxy-1*H*-benzimidazole-6-carboxamido)benzoic acid (56)



$C_{39}H_{31}N_9O_8$
753.73 g/mol

Compound **152** (10 mg, 9.90 µmol, 1.0 eq.) was suspended in dry ethyl acetate (2 mL). Lithium iodide (13.3 mg, 99.0 µmol, 10.0 eq.) was added and the mixture was heated by microwave irradiation for 1.5 hours at 110 °C. The solution was then diluted with ethyl acetate (100 mL),

washed with saturated aqueous $Na_2S_2O_3$ solution (25 mL), water (25 mL) and brine (25 mL) and dried over Na_2SO_4. The solvent was evaporated and the product was dried at high vacuum.

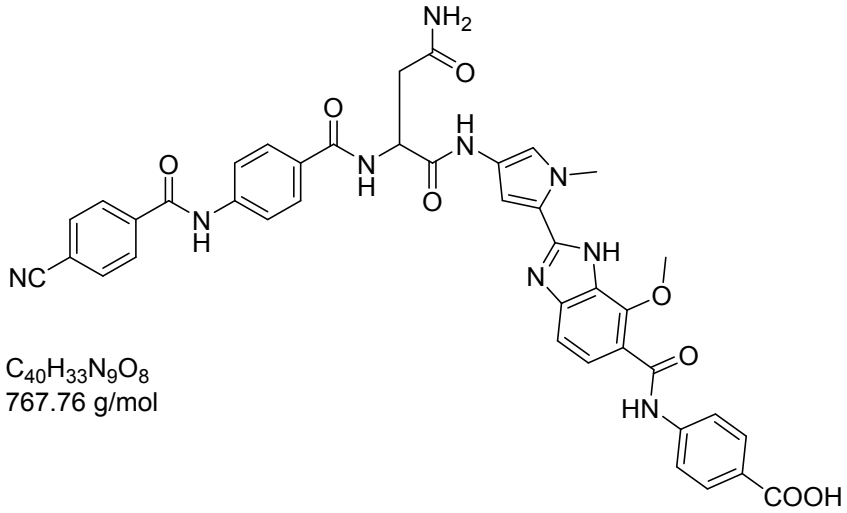
The crude product was then suspended in DCM (0.75 mL) and TIPS (6.1 µL, 29.7 µmol, 3.0 eq.) was added. The solution was cooled to 0 °C before TFA (0.25 mL) was added. The mixture was stirred for 1.5 hours and the solvent was evaporated. The residue was suspended in DCM (3 mL) and evaporated again. Suspension in DCM (3 mL) and evaporation was repeated twice. The crude product was purified by preparative RP-HPLC using condition B (retention time: 17.65 min) to give compound **56** (racemic) as a white solid (2.8 mg, 3.71 µmol, 37%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.00 (s, 1H), 12.77 (s, 1H), 10.70 (s, 1H), 10.56 (s, 1H), 10.09 (s, 1H), 8.60 (d, *J* = 7.6 Hz, 2H), 8.16 – 8.10 (m, 2H), 8.08 – 8.03 (m, 2H), 7.98 – 7.86 (m, 9H), 7.36 (s, 1H), 7.26 (d, *J* = 1.8 Hz, 1H), 7.02 (d, *J* = 8.6 Hz, 1H), 6.96 (s, 1H), 6.92 (d, *J* = 1.8 Hz, 1H), 4.90 (q, *J* = 7.4, 7.0 Hz, 1H), 4.08 (s, 3), 2.66 (d, *J* = 7.0 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.5, 169.2, 168.4, 166.9, 165.6, 164.4, 146.0, 142.5, 141.5, 138.7, 133.2, 132.5, 130.2, 130.2, 129.4, 128.6, 128.3, 123.0, 122.6, 120.7, 120.4, 120.0, 119.5, 118.3, 117.3, 114.0, 107.8, 103.3, 102.1, 50.9, 37.2, 36.4.

HRMS (ESI+): *m/z* for $C_{391}N_9O_8$ $[M+H]^+$: calculated: 754.2368, found: 754.2368.

4-(2-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)-1-methyl-1*H*-pyrrol-2-yl)-7-methoxy-1*H*-benzimidazole-6-carboxamido)benzoic acid (57)



$C_{40}H_{33}N_9O_8$
767.76 g/mol

Compound **152** (10 mg, 9.90 µmol, 1.0 eq.) was suspended in DCM (0.75 mL) and TIPS (6.1 µL, 29.7 µmol, 3.0 eq.) was added. The solution was cooled to 0 °C before TFA (0.25 mL) was added. The mixture was stirred for 1.5 hours and the solvent was evaporated. The residue was suspended in DCM (3 mL) and evaporated again. Suspension in DCM (3 mL) and evaporation was repeated twice. The crude product was purified by preparative RP-HPLC using

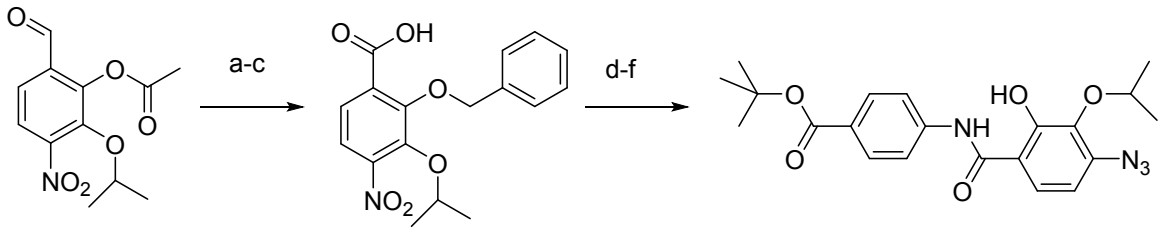
condition B (retention time: 22.57 min) to give compound **57** (racemic) as a white solid (2.2 mg, 2.87 µmol, 29%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (s, 1H), 12.71 (br, 1H), 10.70 (s, 1H), 10.43 (s, 1H), 10.10 (s, 1H), 8.60 (d, *J* = 7.6 Hz, 1H), 8.16 – 8.11 (m, 2H), 8.08 – 8.03 (m, 2H), 7.96 – 7.86 (m, 8H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.36 (s, 1H), 7.28 (d, *J* = 1.8 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 6.96 (m, 2H), 4.90 (q, *J* = 7.2 Hz, 1H), 4.54 (s, 3), 4.08 (s, 3), 2.66 (d, *J* = 7.1 Hz, 2H).

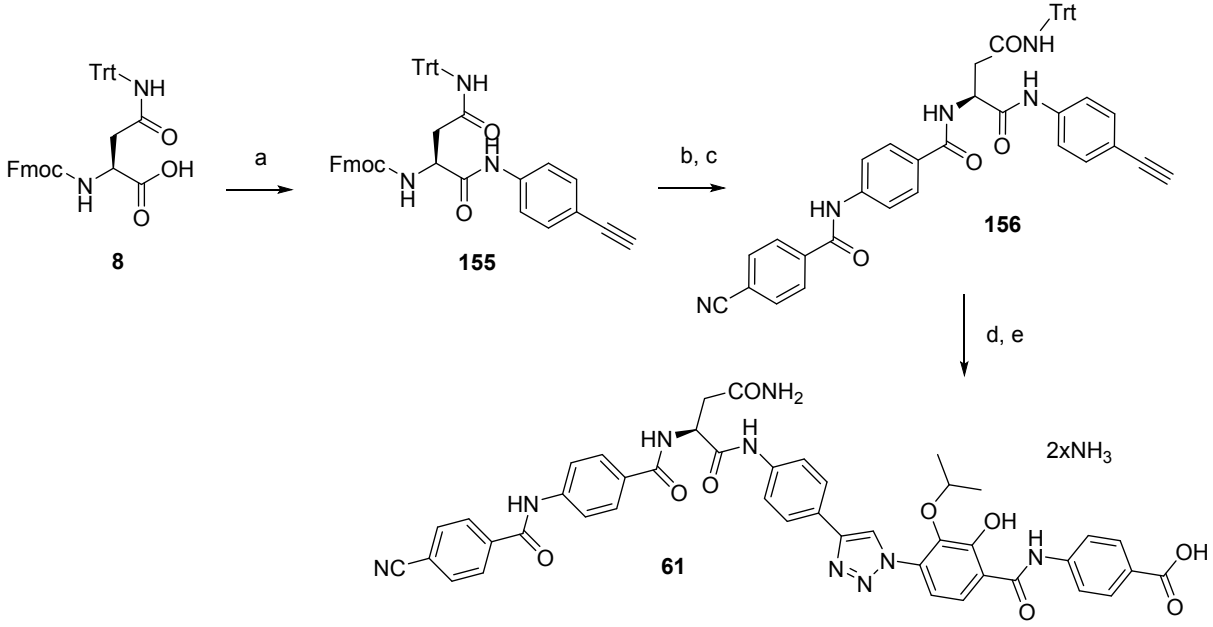
^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.5, 168.4, 167.0, 165.6, 165.3, 164.4, 148.4, 145.9, 143.3, 141.5, 138.7, 138.6, 134.2, 132.5, 130.4, 129.4, 128.6, 128.3, 125.1, 124.1, 123.0, 119.7, 119.5, 118.9, 118.3, 117.7, 117.6, 114.0, 104.7, 103.4, 61.1, 50.9, 37.2, 36.5.

HRMS (ESI+): *m/z* for $C_{403}N_9O_8$ $[M+H]^+$: calculated: 768.2525, found: 768.2529.

1.2.2.3.2 AlbD-stable cystobactamid

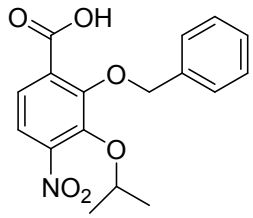


Scheme S 8. Reagent and conditions: a) LiOH, THF/water 1:1, rt, 3 h, b) Bn-Br, K_2CO_3, DMF, rt, 24 h, c) 2-Methyl-2-butene, $NaClO_2$, *t*-BuOH/NaH_2PO_4 1 N solution, rt, 1 h (69% o3s); d) $POCl_3$, TEA, DCM, rt, 2.5 h (55%), e) Pd/C, MeOH, rt, 2 h (53% o2s); f) *t*-BuONO, TMS-N_3, CH_3CN, rt, 2 h (70%).



Scheme S 9. Reagent and conditions: a) 4-ethynylaniline, $POCl_3$, TEA, DCM, rt, 2 h (87%); b) diethylamine, CH_3CN, rt, 1 h; c) **64**, HBTU, DiPEA, DMF, rt, 3 h (69%); d) **154**, sodium ascorbate, TBTA, $CuSO_4$, DMSO/THF/water, rt, 2 h; e) TFA, Tips, DCM, rt, 3 h (55% o2s).

2-(Benzyloxy)-3-isopropoxy-4-nitrobenzoic acid (153)



Chemical Formula:
$C_{17}H_{17}NO_6$
Exact Mass: 331,1056

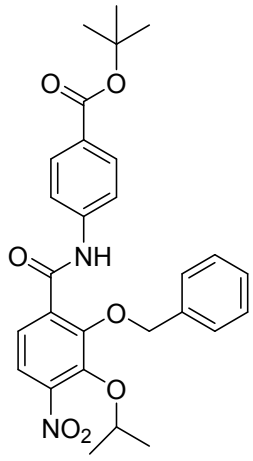
6-Formyl-2-isopropoxy-3-nitrophenyl acetate[14] (**66**) (2.0 g; 7.49 mmol) was dissolved in THF (38 mL) and water (19 mL), then LiOH (1.42 g; 74.9 mmol) dissolved in water (19 mL) was added at 0 C, reaction stirred overnight. In the morning, the pH was adjusted to 1, solvent partially reduced under vacuum and aqueous phase extracted with $CHCl_3$ (150 mL) three times, combined organic phases dried over sodium sulphate and reduced under vacuum to give a yellow oil, which was used in the next step without further purification. Residue was dissolved in DMF (18 mL), K_2CO_3 (2.07 g; 14.98 mmol) followed by benzyl bromide (1.34 mL; 11.24 mmol) were added, reaction stirred 24 h at r.t.. Reaction diluted with water (200 mL) and EtOAc (200 mL), aqueous phase extracted with EtOAc (150 mL). Combined organic phases washed with brine (300 mL), dried over sodium sulphate and reduced under vacuum to give a crude material, which was dissolved with 2-Methyl-2-butene (8.35 mL; 78.65 mmol) in *t*-BuOH (45 mL). Then a solution of $NaClO_2$ 80% (1.02 g; 8.99 mmol) in Monosodium phosphate monohydrate solution 1 N (8.4 mL) was added dropwise to the solution. Reaction stirred for 1 h, then quenched by adding a solution of Na_2SO_3. Mixture partially reduced under vacuum, diluted with EtOAc (200 mL) and HCl 1 N (200 mL), aqueous phase extracted again with EtOAc (100 mL), organic phases reunited washed with brine (250 mL) and dried over sodium sulphate. Solvent reduced under vacuum, crude chromatographed on silica gel with a gradient 0-10% MeOH in DCM to afford 1.7 g of the desired compound (5.14 mmol, y= 69%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.7 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 1H), 7.46 – 7.37 (m, 5H), 5.31 (s, 2H), 4.74 – 4.67 (m, 1H), 1.34 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7, 153.0, 149.0, 144.8, 134.4, 129.4, 129.1, 128.9, 127.6, 126.6, 119.7, 78.7, 77.3, 22.3.

HRMS (ESI) calculated for $C_{17}H_{17}NNaO_6$ ($M+Na^+$) 354.0948, found 354.0950.

Tert-butyl 4-(2-(benzyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (157)



Chemical Formula:
$C_{28}H_{30}N_2O_7$
Exact Mass: 506,2053

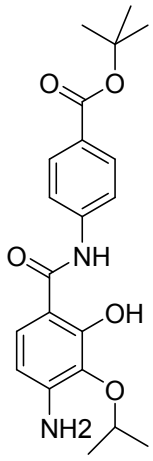
$POCl_3$ (0.031 mL; 0.33 mmol) was added at 0 °C to a stirred solution of tert-butyl 4-(2-(benzyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (**153**) (110 mg; 0.33 mmol), TEA (0.077mL; 0.55 mmol) and tert-butyl 4-aminobenzoate (53 mg; 0.27 mmol) in DCM (4.5 mL) under nitrogen. Reaction stirred 2.5 h, then quenched with $NaHCO_3$ saturated solution, solvent partially reduced under vacuum , then diluted with EtOAc (20 mL) and $NaHCO_3$ saturated solution (20 mL), organic phase then washed with HCl 1 N and brine, dried over sodium sulphate and reduced under vacuum to give around 200 mg of crude material which was chromatographed on silica gel with a gradient 5-30% EtOAc in PetEt to give 75 mg of a yellow oil (0.15 mmol; y= 55%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.94 (s, 1H), 8.09 (d, *J* = 8.8 Hz, 1H), 7.89 – 7.85 (m, 2H), 7.66 (d, *J* = 8.8 Hz, 1H), 7.47 – 7.36 (m, 5H), 7.28 – 7.23 (m, 2H), 5.31 (s, 2H), 4.73 (hept, *J* = 6.2 Hz, 1H), 1.60 (s, 9H), 1.41 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 160.9, 151.8, 148.1, 144.5, 141.1, 134.5, 130.6, 130.4, 129.6, 129.2, 129.2, 127.8, 126.3, 119.9, 119.1, 81.0, 78.7, 77.7, 28.2, 22.4.

HRMS (ESI) calculated for $C_{28}H_{31}N_2O_7$ ($M+H^+$) 507.2126, found 507.2120.

Tert-butyl 4-(4-amino-2-hydroxy-3-isopropoxybenzamido)benzoate (158)



Chemical Formula:
$C_{21}H_{26}N_2O_5$
Exact Mass: 386,1842

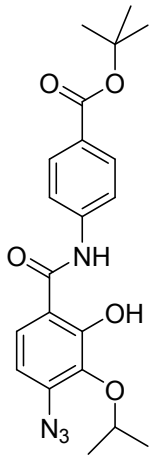
Tert-butyl 4-(2-(benzyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (1.22 g; 2.41 mmol) was dissolved in MeOH (35 mL). The solution was purged with N_2, then Pd/C (240 mg) was added and solution purged with H_2. The reaction was stirred under an H_2 atmosphere for 2 h, afterwards the mixture was filtered over a pad of celite and solvent removed under reduced pressure. The crude thus obtained was chromatographed on silica gel with gradient 0-20% MeOH in DCM to give 906 mg of desired product (2.37 mmol; y= 97%).

^{1}H NMR (500 MHz, DMSO) δ 12.57 (s, 1H), 10.15 (s, 1H), 7.92 – 7.84 (m, 2H), 7.83 – 7.78 (m, 2H), 7.59 (d, *J* = 8.9 Hz, 1H), 6.26 (d, *J* = 8.8 Hz, 1H), 5.66 (s, 2H), 4.46 (dt, *J* = 12.3, 6.1 Hz, 1H), 1.55 (s, 9H), 1.22 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 169.9, 165.1, 156.2, 148.7, 143.1, 130.3, 130.0, 126.6, 124.2, 120.7, 105.8, 103.9, 80.8, 73.2, 28.3, 22.7.

HRMS (ESI) calculated for $C_{21}H_{27}N_2O_5$ ($M+H^+$) 387.1914, found 387.1902.

Tert-butyl 4-(4-azido-2-hydroxy-3-isopropoxybenzamido)benzoate (154)



Chemical Formula:
$C_{21}H_{24}N_4O_5$
Exact Mass: 412,1747

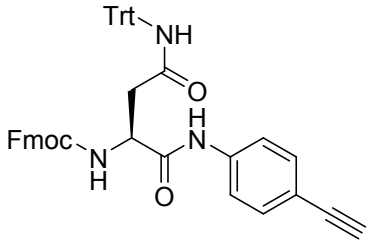
Tert-butyl 4-(4-amino-2-hydroxy-3-isopropoxybenzamido)benzoate (40 mg, 0.10 mmol) was dissolved in acetonitrile (2 mL) and the reaction mixture was cooled to 0 °C, then terbutylnitrite (18.5 µL, 0.15 mmol) and trimethylsilylazide (20.4 µL, 0.15 mmol) were subsequently added dropwise and the reaction mixture was allowed to stir at room temperature for 2 hours. After evaporation of the volatiles, the crude mixture was subjected to purification by flash column chromatography using petroleum ether / ethyl acetate 8:2 as an eluent to obtain 30 mg of a pale yellow orange solid (0.072 mmol, y= 70 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.49 (s, 1H), 8.56 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 1H), 6.65 (d, *J* = 8.8 Hz, 1H), 4.76 (hept, 6.1 Hz, 1H), 1.61 (s, 9H), 1.37 (d, *J* = 6.2 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 165.2, 154.0, 140.9, 138.0, 137.9, 130.7, 128.2, 122.6, 119.6, 113.1, 110.9, 81.0, 76.0, 28.2, 22.2

HRMS (ESI) calculated for $C_{21}H_{23}N_4O_5$ (M-H$^+$) 411.1674, found 411.1662.

(9H-Fluoren-9-yl)methyl (1-((4-ethynylphenyl)amino)-1,4-dioxo-4-(tritylamino)butan-2-yl)carbamate (155)



Chemical Formula: $C_{46}H_{37}N_3O_4$
Exact Mass: 695,2784

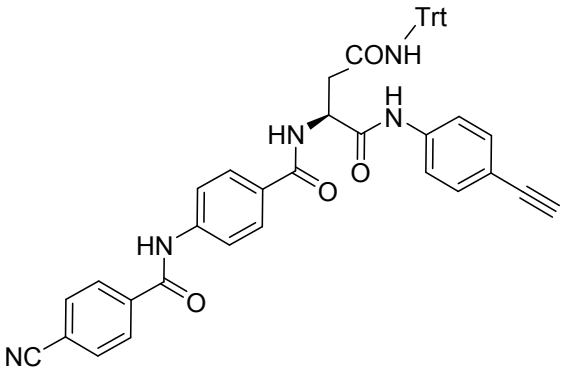
$POCl_3$ (40 µL, 0.43 mmol) was added at 0 °C to a stirred solution of Fmoc-Asn(Trt)-OH (254 mg, 0.43 mmol), triethylamine (60 µL, 0.43 mmol) and 4-ethynylaniline (25 mg, 0.215 mmol) in DCM (4 mL) under nitrogen. The reaction was stirred at °0 C for 2 hours. $NaHCO_3$ (5 mL) saturated solution and EtOAc (20 mL) were added, the organic phase washed again with $NaHCO_3$ (5 mL) and brine (20 mL), dried over sodium sulphate and reduced under vacuum to give a yellow oil which was chromatographed on silica gel with a solution Hexane/EtOAc 7:3 to give 131 mg of a white solid (0.19 mmol; y= 87 %).

^{1}H NMR (500 MHz, DMSO) δ 10.27 (s, 1H), 8.61 (s, 1H), 7.90 (d, J = 7.5 Hz, 2H), 7.78 (d, J = 8.0 Hz, 1H), 7.74 (dd, J = 7.3, 4.3 Hz, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.46 – 7.38 (m, 3H), 7.37 – 7.25 (m, 2H), 7.25 – 7.12 (m, 15H), 4.44 (td, J = 9.0, 5.3 Hz, 1H), 4.36 (dd, J = 10.4, 7.0 Hz, 1H), 4.29 (dd, J = 10.4, 7.0 Hz, 1H), 4.23 (t, J = 6.9 Hz, 1H), 4.09 (s, 1H), 2.75 (dd, J = 14.5, 9.8 Hz, 1H), 2.61 (dd, J = 14.5, 5.0 Hz, 1H).

^{13}C NMR (126 MHz, DMSO) δ 170.5, 168.5, 155.8, 144.7, 143.8, 140.7, 139.5, 132.3, 128.6, 127.7, 127.4, 127.1, 126.3, 125.3, 125.2, 120.1, 119.1, 116.2, 83.6, 79.9, 69.4, 65.8, 52.8, 46.7, 38.4.

HRMS (ESI) calculated for $C_{46}H_{36}N_3O_4$ (M-H$^+$) 694.2711, found 694.2690.

(S)-2-(4-(4-Cyanobenzamido)benzamido)-N1-(4-ethynylphenyl)-N4-tritylsuccinamide (156)



Chemical Formula: $C_{46}H_{35}N_5O_4$
Exact Mass: 721,2689

To a solution of 4-(4-cyanobenzamido) benzoic acid (15.5 mg, 0.058 mmol) and HBTU (27 mg, 0.77 mmol) in dry DMF (0.4 mL) DIPEA (30 µL, 0.174 mmol) was added. The mixture was stirred for 15 minutes before being added to a solution of 2-amino-*N*1-(4-ethynylphenyl)-*N*4-tritylsuccinamide (34 mg, 0.07 mmol) in dry DMF (0.6 mL), which was obtained cleaving the Fmoc protecting group from (9H-fluoren-9-yl)methyl (1-((4-ethynylphenyl)amino)-1,4-dioxo-4-(tritylamino)butan-2-yl)carbamate using standard conditions as already described herein (a 20% solution of diethylamine in CH_3CN).

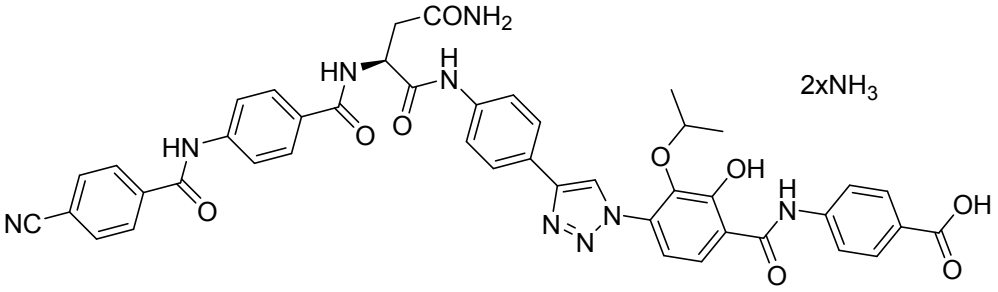
The solution was stirred for 3 hours, diluted with brine (5 mL) and water (5 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were washed with 1N HCl (5 mL), saturated aqueous $NaHCO_3$ solution (5 mL) and brine (5 mL). After drying over anhydrous Na_2SO_4, the solvent was removed by distillation at reduced pressure and the residue was subjected to flash chromatography with a gradient MeOH 0-5% in DCM to afford 29 mg of desired product as a pale yellow solid (0.04 mmol, y= 69%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.75 (s, 1H), 8.07 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 8.1 Hz, 4H), 7.59 (d, *J* = 7.9 Hz, 2H), 7.42 (s, 1H), 7.37 (s, 4H), 7.25 – 7.13 (m, 15H), 5.05 – 4.94 (m, 1H), 3.19 (d, *J* = 12.3 Hz, 1H), 3.05 (s, 1H), 2.70 (dd, *J* = 15.3, 6.7 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9, 169.1, 167.0, 164.3, 143.9, 141.1, 138.2, 138.0, 132.8, 132.4, 128.7, 128.6, 128.3, 128.0, 127.2, 120.0, 119.6, 117.9, 117.8, 115.3, 83.3, 77.0, 71.0, 51.1, 37.9.

HRMS (ESI) calculated for $C_{46}H_{34}N_5O_4$ (M-H$^+$) 720.2616, found 720.2618.

4-(4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)phenyl)-1H-1,2,3-triazol-1-yl)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (61)



Chemical Formula: $C_{44}H_{37}N_9O_9$
Exact Mass: 835,2714

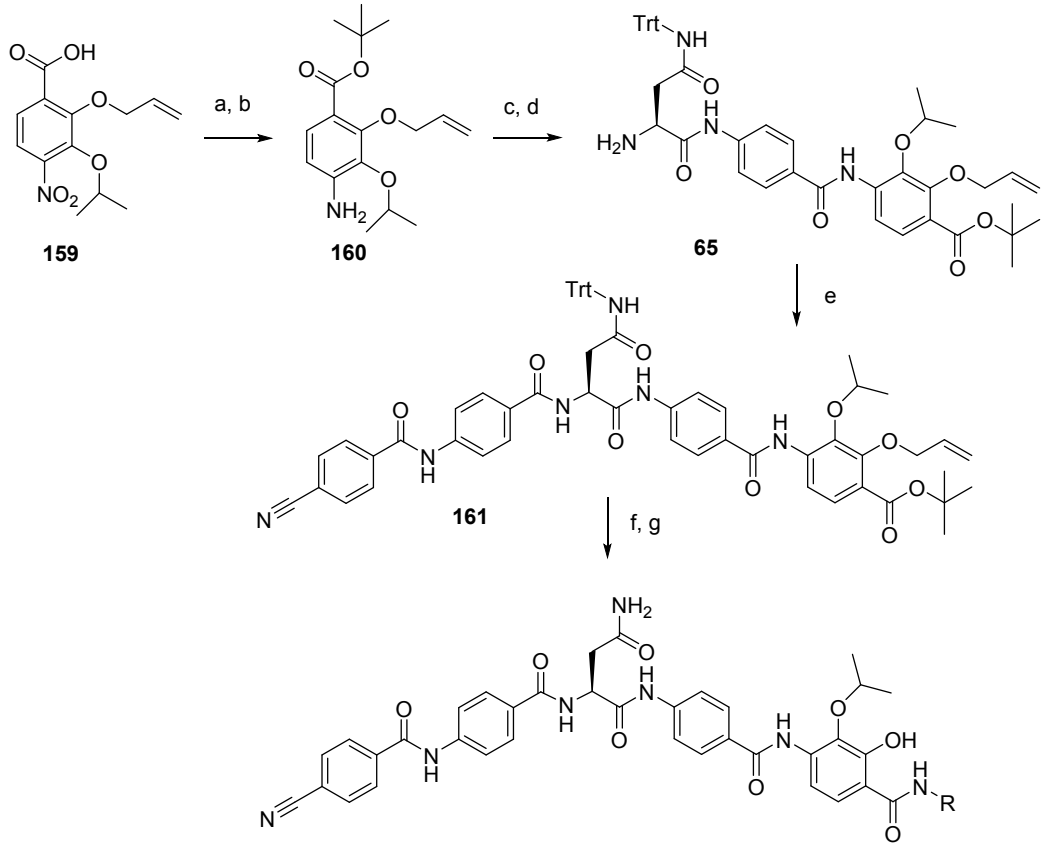
(S)-2-(4-(4-Cyanobenzamido)benzamido)-N1-(4-ethynylphenyl)-N4-tritylsuccinamide (9 mg, 0.012 mmol) and tert-butyl 4-(4-azido-2-hydroxy-3-isopropoxybenzamido)benzoate (5 mg, 0.012 mmol) were dissolved in 300 µL DMSO/THF mixture (2:1) and then sodium ascorbate (1.4 mg, 0.0072 mmol) previously dissolved in 10 µL of water was added followed by TBTA (2.5 mg, 0.0048 mmol) previously dissolved in DMSO (10 µL). Finally; copper sulfate (0.2 mg, 0.0012 mmol) was added as a solid and the reaction mixture was allowed to stir at room temperature for 2 hours. After extraction with ethyl acetate (3x1 mL), the organic layer was washed with NH_4Cl saturated solution, water and brine, dried over sodium sulfate and evaporated under reduced pressure to obtain 14 mg (0.012 mmol, y= q.) of a yellow oil. The residue was used in the next step without further purification. Part of the residue (10 mg, 0.0088 mmol) was dissolved in DCM (250 µL), then TFA (50µL) and Tips (10 µL) were subsequently added and the reaction mixture was allowed to stir at room temperature for 3 hours. After evaporation of the volatiles, the crude residue was purified by preparative RP-HPLC using condition B (retention time: 17.45 min) to afford the pure compound as a white solid (4 mg, 0.0048 mmol, 55%).

^{1}H NMR (700 MHz, DMSO) δ 15.18 (s, 1H), 10.74 (s, 1H), 10.31 (s, 1H), 8.80 (d, *J* = 5.7 Hz, 1H), 8.69 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 8.3 Hz, 2H), 7.95 (d, *J* = 8.7 Hz, 2H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.87 (d, *J* = 8.6 Hz, 2H), 7.79 (d, *J* = 8.1 Hz, 2H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.47 (s, 1H), 6.97 (s, 1H), 6.34 (d, *J* = 8.5 Hz, 1H), 4.92 (dd, *J* = 13.8, 7.7 Hz, 1H), 4.83 (hept, *J* = 6.1 Hz, 1H), 2.74 – 2.65 (m, 2H), 0.93 (d, *J* = 6.0 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.4, 170.2, 169.8, 166.7, 166.3, 165.7, 164.4, 145.1, 141.5, 141.1, 138.7, 132.5, 129.7, 129.2, 128.6, 128.3, 126.4, 125.9, 125.6, 123.9, 122.5, 119.7, 119.5, 119.4, 118.3, 117.7, 103.1, 70.8, 51.7, 40.0, 37.0, 22.2.

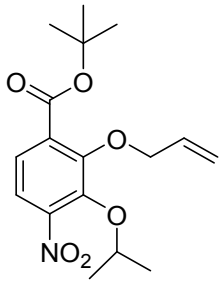
HRMS (ESI) calculated for $C_{44}H_{36}N_9O_9$ ($M-H^+$) 834.2641, found 834.2661.

1.2.2.3.3 Ring E modifications



Scheme S 10. Reagent and conditions: a) DCC, *t*-BuOH, DMAP, DCM, rt, 5 h (71%); b) zinc dust, THF/EtOH, rt, 30 min. (89%); c) **109**, $POCl_3$, DiPEA, DCM/THF, rt, 6.5 h, d) diethylamine, CH_3CN, rt, 20 min. (66% o2s); **64**, HBTU, DIPEA, DMF, rt, 6 h (67%); f); TFA, Tips, DCM, rt, 1.5 h, g) amine, HBTU, DiPEA, DMF, rt.

tert-butyl 2-(allyloxy)-3-isopropoxy-4-nitrobenzoate (162)



2-Alloxy-3-isopropoxy-4-nitrobenzoic acid[14] (2.18 g; 7.75 mmol), DMAP (95 mg; 0.78 mmol) and *tert*-butyl alcohol (7.3 mL; 77.5 mmol) were dissolved in 17 mL of dry DCM. The stirred solution was cooled to 0 °C and DCC (1.76 g; 7.75 mmol) was added in small portions. Stirring continued at 0 °C for 10 minutes, before the mixture was allowed to warm to room temperature and stirred for an additional 5 hours. The precipitate was removed by filtration. The clear solution was diluted with ethyl acetate (100 mL), washed with 0.5M HCl (2 × 100 mL) and saturated aqueous $NaHCO_3$ solution (100 mL) and dried over anhydrous Na_2SO_4. The solvent was removed by distillation at reduced pressure and the crude product was purified by flash

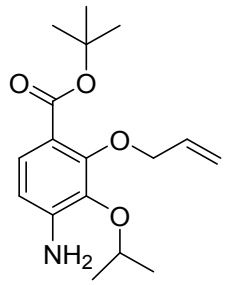
chromatography (petroleum ether/ ethyl acetate) affording *tert*-butyl 2-allyloxy-3-isopropoxy-4-nitrobenzoate as a pale yellow oil (1.85 g; 5.48 mmol; 71%).

^{1}H-NMR (500 MHz, DMSO): δ (ppm) = 7.67 (d, *J* = 8.5 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 6.06 (ddt, *J* = 17.2, 10.7, 5.4 Hz, 1H), 5.40 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.28 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.62 (hept, *J* = 6.2 Hz, 1H), 4.57 (dt, *J* = 5.4, 1.5 Hz, 2H), 1.54 (s, 9H), 1.19 (d, *J* = 6.2 Hz, 6H).

^{13}C-NMR (126 MHz, DMSO-d_{6H}): δ (ppm) = 164.0, 150.8, 147.3, 143.8, 133.1, 132.6, 124.2, 119.2, 117.8, 82.5, 77.2, 74.2, 27.6, 22.0.

HRMS (ESI+): *m/z* for $C_{17}H_3NO_6$ [M+Na]$^+$: calculated: 360.1418, found: 360.1419.

tert-butyl 2-(allyloxy)-4-amino-3-isopropoxybenzoate (160)



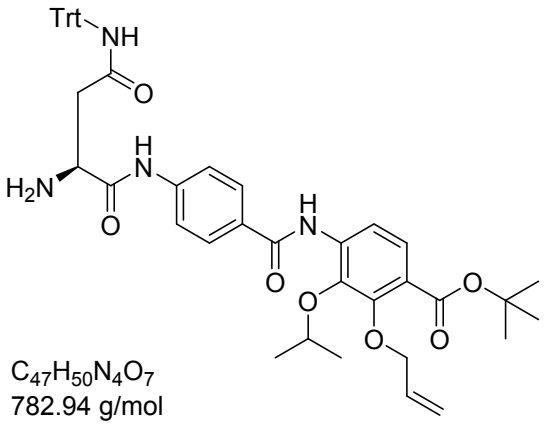
To a stirred solution of tert-Butyl 2-allyloxy-3-isopropoxy-4-nitrobenzoate (1.71 g; 5.07 mmol) in THF (9.0 mL) and EtOH (7.2 mL) was added glacial acidic acid (1.8 mL). The solution was cooled to 0 °C and zinc dust (3.31 g; 50.7 mmol) was added in small portions. The mixture was stirred at room temperature for 30 minutes, before the solid was filtered off. The filtrate was diluted with saturated aqueous $NaHCO_3$ solution (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed by distillation at reduced pressure. The crude material was purified by flash chromatography (petrol ether/ ethyl acetate) and dried at high vacuum yielding *tert*-butyl 2-allyloxy-4-amino-3-isopropoxybenzoate as a white solid (1.39 g; 4.53 mmol; 89%).

^{1}H-NMR (500 MHz, DMSO): δ (ppm) = 7.20 (d, *J* = 8.5 Hz, 1H), 6.43 (d, *J* = 8.5 Hz, 1H), 6.06 (ddt, *J* = 17.3, 10.5, 5.2 Hz, 1H), 5.48 (s, 2H), 5.35 (dd, *J* = 17.3, 1.8 Hz, 1H), 5.20 (dd, *J* = 10.5, 1.8 Hz, 1H), 4.45 – 4.38 (m, 3H), 1.48 (s, 9H), 1.20 (d, *J* = 6.1 Hz, 6H).

^{13}C-NMR (126 MHz, DMSO): δ (ppm) = 164.9, 152.4, 147.7, 136.1, 134.8, 126.9, 116.2, 113.6, 109.1, 79.2, 74.0, 73.3, 28.0, 22.2.

HRMS (ESI+): *m/z* for $C_{17}H_5NO_4$ [M+H]$^+$: calculated: 308.1852, found: 308.1856.

***tert*-butyl (*S*)-2-allyloxy-4-(4-(2-amino-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzoate (65)**



$C_{47}H_{50}N_4O_7$
782.94 g/mol

A stirred solution of (S)-4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoic acid (**38**) (1.29 g, 1.81 mmol, 1.7 eq.) and *tert*-butyl 2-Allyloxy-4-amino-3-isopropoxybenzoate (**160**) (327 mg, 1.06 mmol, 1.0 eq.) in 10.5 mL of dry DCM and 10.5 mL of dry THF under N_2 atmosphere was cooled to 0 °C. $POCl_3$ (0.17 mL, 1.81 mmol, 1.7 eq.) and DIPEA (0.93 mL, 5.31 mmol, 5.0 eq.) were added dropwise and the reaction mixture was allowed to warm to room temperature after stirring for 10 minutes. The solution was stirred for 6.5 hours before the reaction was quenched by addition of water (20 mL). The mixture was diluted with brine (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined, washed with 1M HCl (50 mL), saturated aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed by distillation at reduced pressure.

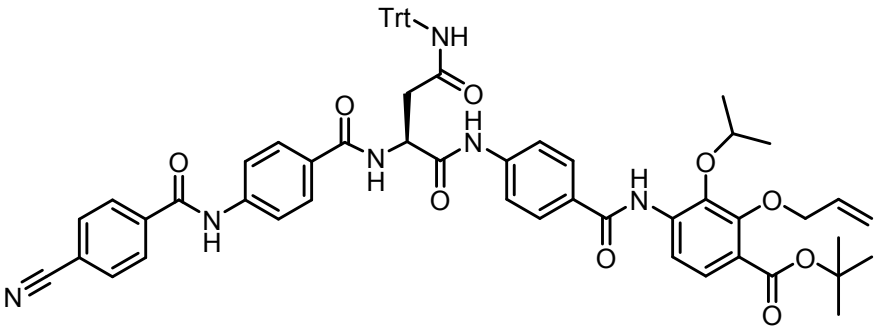
The crude reaction product was then dissolved in MeCN (40 mL) and diethylamine (10 mL) was added. The solution was stirred at room temperature for 20 minutes before the solvent was removed by distillation at reduced pressure. Purification by flash chromatography (DCM/ MeOH) afforded **65** as an off-white solid (545 mg, 0.70 mmol, 66%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 9.45 (s, 1H), 9.23 (s, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.83 (pseudo t, *J* = 8.4 Hz, 3H), 7.29 – 7.16 (m, 16H), 6.10 (ddt, *J* = 17.2, 10.5, 5.3 Hz, 1H), 5.40 (dq, *J* = 17.2, 1.8 Hz, 1H), 5.26 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.54 (dt, *J* = 5.4, 1.5 Hz, 2H), 4.47 (hept, *J* = 6.1 Hz, 1H), 3.73 (dd, *J* = 8.2, 5.1 Hz, 1H), 2.64 – 2.53 (m, 2H), 1.53 (s, 1), 1.23 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 173.8, 169.9, 164.8, 164.2, 150.1, 144.9, 142.4, 136.5, 134.1, 128.5, 128.4, 128.2, 127.5, 126.3, 124.9, 123.9, 118.7, 118.0, 116.9, 81.0, 76.1, 73.8, 69.3, 52.9, 41.0, 27.8, 22.3.

HRMS (ESI+): *m/z* for $C_{47}H_{50}N_4O_7$ $[M+H]^+$: calculated: 783.3752, found: 783.3757.

tert-butyl (S)-2-(allyloxy)-4-(4-(2-(4-(4-cyanobenzamido)benzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzoate (161)



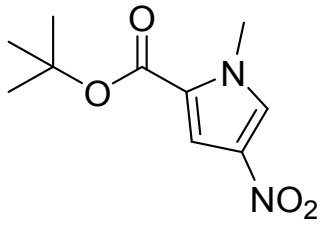
To a solution of 4-(4-cyanobenzamido)benzoic acid (173 mg; 0.77 mmol) and HBTU (292 mg; 0.77 mmol) in dry DMF (1.5 mL) was added DIPEA (0.4 mL; 2.30 mmol). The mixture was stirred for 15 minutes before being added to a solution of tert-butyl (S)-2-(allyloxy)-4-(4-(2-amino-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzoate (500 mg; 0.64 mmol) in dry DMF (3.5 mL). The solution was stirred for 6 hours, diluted with brine (50 mL) and water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with 1N HCl (50 mL), saturated aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL). After drying over anhydrous Na_2SO_4, the solvent was removed by distillation at reduced pressure and the residue was subjected to flash chromatography (DCM/ MeOH) to afford the desired product as an off-white solid (440 mg; 0.43 mmol; 67%).

^{1}H-NMR (500 MHz, DMSO): δ (ppm) = 10.73 (s, 1H), 10.51 (s, 1H), 9.45 (s, 1H), 8.78 (d, *J* = 7.5 Hz, 1H), 8.68 (s, 1H), 8.17 – 8.04 (m, 4H), 8.00 – 7.90 (m, 6H), 7.85 – 7.77 (m, 3H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.25 – 7.15 (m, 15H), 6.10 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 1H), 5.40 (dq, *J* = 17.3, 1.8 Hz, 1H), 5.26 (dq, *J* = 10.5, 1.6 Hz, 1H), 4.95 – 4.88 (m, 1H), 4.54 (d, *J* = 5.7 Hz, 1H), 4.46 (hept, *J* = 6.1 Hz, 1H), 3.06 – 2.96 (m, 1H), 2.74 – 2.69 (m, 1H), 1.53 (s, 9H), 1.22 (d, *J* = 6.2 Hz, 6H).

^{13}C-NMR (125 MHz, DMSO): δ (ppm) = 170.9, 168.4, 165.7, 164.8, 164.5, 164.2, 150.7, 144.7, 142.5, 142.4, 141.6, 138.7, 136.5, 134.1, 132.5, 129.1, 128.6, 128.6, 128.4, 128.3, 127.4, 126.4, 124.9, 123.9, 119.5, 118.8, 118.3, 118.1, 116.9, 114.0, 81.0, 76.1, 73.8, 69.5, 52.0, 38.2, 27.8, 22.3.

HRMS (ESI+): *m/z* for $C_{62}H_{58}N_6O_9$ $[M+H]^+$: calculated: 1031.4338, found: 1031.4334.

***tert*-butyl 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylate (163)**



$C_{10}H_{14}N_2O_4$
226.23 g/mol

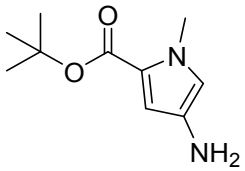
1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (500 mg, 2.94 mmol, 1.0 eq.) and DMAP (36 mg, 0.29 mmol, 0.1 eq.) were suspended in a mixture of dry DCM (6.0 mL) and *t*BuOH (2.8 mL, 29.4 mmol, 10.0 eq.) under a nitrogen atmosphere. The mixture was cooled to 0 °C and DCC (667 mg, 3.23 mmol, 1.1 eq.) was added in small portions. The mixture was stirred for 5 minutes at 0 °C before being allowed to warm to room temperature. Stirring continued overnight and the solid was filtered off. The solvent was removed by distillation at reduced pressure and the crude product was purified by flash chromatography (petroleum ether/ ethyl acetate) yielding *tert*-butyl 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylate as a white solid (550 mg, 2.43 mmol, 83%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 8.22 (dd, J = 2.1, 0.6 Hz, 1H), 7.22 (d, J = 2.1 Hz, 1H), 3.90 (d, J = 0.6 Hz, 3), 1.25 (s, 9).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 158.7, 133.9, 129.2, 124.0, 111.4, 81.7, 37.6, 27.8.

HRMS (ESI+): *m/z* for $C_{10}H_{14}N_2O_4$ $[M+H]^+$: calculated: 227.1026, found: 227.1033.

***tert*-butyl 4-amino-1-methyl-1*H*-pyrrole-2-carboxylate (164)**



$C_{10}H_{16}N_2O_2$
196.25 g/mol

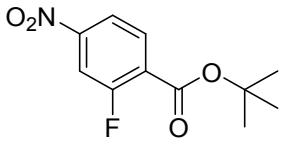
Compound **163** (200 mg, 0.88 mmol, 1.0 eq.) was dissolved in dry MeOH (30 mL) under a nitrogen atmosphere. The solution was purged with nitrogen for 10 minutes, before Pd/C (10%wt, 100 mg) was added. The mixture was then purged with hydrogen for 5 minutes and set under a hydrogen atmosphere. After stirring overnight, the mixture was filtered through a pad of celite and the solvent was removed by distillation at reduced pressure. The product was dried at high vacuum affording **164** as yellow oil (160 mg, 0.81 mmol, 92%). The product was stored under nitrogen at −20°C to prevent decomposition.

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 6.33 (d, *J* = 2.2 Hz, 1H), 6.14 (d, *J* = 2.2 Hz, 1H), 3.88 (br, 2H), 3.66 (s, 3), 1.46 (s, 9).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 160.0, 132.2, 120.1, 116.1, 106.8, 79.0, 35.8, 28.1.

HRMS (ESI+): *m/z* for $C_{10}H_{16}N_2O_2$ $[M+H]^+$: calculated: 197.1285, found: 197.1289.

tert-butyl 2-fluoro-4-nitrobenzoate (165)



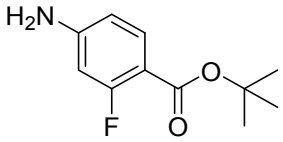
To a stirred solution of 2-fluoro-4-nitrobenzoic acid (0.5 g; 2.7 mmol) in DCM (30.0 mL), DMAP (0.16 g; 1.35 mmol) and triethylamine (0.82 g; 8.1 mmol) were added. Boc anhydride (0.88 g; 4.03 mmol) was added slowly at 0°C then the mixture was stirred at room temperature overnight. Excess solvent was removed under reduced pressure. The residue was then dissolved in ethyl acetate and then washed with 1N HCl (2 x 30 mL). The organic layer was then washed with saturated aqueous $NaHCO_3$ (30 mL). The organic extracts were dried over $MgSO_4$ and the solvent was removed by distillation at reduced pressure. The crude material was purified by flash chromatography (petrol ether/ ethyl acetate) to yield *tert*-butyl 2-fluoro-4-nitrobenzoate (0.26 g; 1.07 mmol; 40%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.10 – 8.01 (m, 2H), 8.00 – 7.95 (m, 1H), 1.61 (s, 9H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) = 163.08, 161.97, 161.92, 159.58, 150.72, 150.61, 133.07, 133.05, 126.65, 126.51, 118.84, 118.78, 113.11, 112.74, 83.75, 28.25.

HRMS (ESI+): *m/z* for $C_{11}H_{13}FNO_4$ $[M+H]^+$: calculated: 242.0823, found: 242.0833.

tert-butyl 4-amino-2-fluorobenzoate (166)

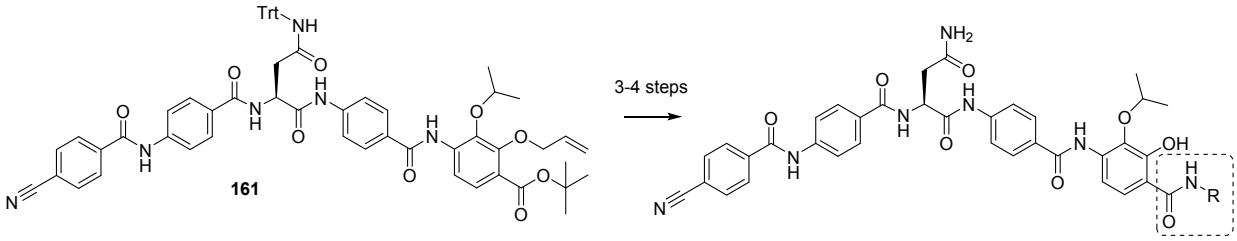


Compound **(165)** (0.23 g; 0.95 mmol), Fe (0.26 g; 4.75 mmol) and NH_4Cl (0.025 g; 0.47 mmol) in a mixture of EtOH/H_2O (20mL/10mL) were heated at 100°C for 2 hours. The solution was filtered over celite then excess solvent was removed under reduced pressure. The residue was diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The organic extracts were dried over $MgSO_4$ and the solvent was removed by distillation at reduced pressure to yield *tert*-butyl 4-amino-2-fluorobenzoate (0.18 g; 0.85 mmol; 90%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.68 (m, 1H), 6.41 – 6.35 (m, 1H), 6.34 – 6.27 (m, 1H), 4.09 (s, 2H), 1.56 (s, 9H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) = 165.70, 163.84, 163.78, 162.28, 152.18, 152.02, 133.79, 133.75, 109.94, 109.91, 109.81, 109.78, 102.12, 101.78, 80.88, 28.46.
HRMS (ESI+): *m/z* for $C_{11}H_{15}FNO_2$ $[M+H]^+$: calculated: 212.1081, found: 212.1070.

General procedure for synthesis of derivatives starting from compound 161



Deprotection

Compound **161** (20.0 mg – 30.0 mg, 19.4 µmol – 29.1 µmol, 1.0 eq.) was dissolved in 800 µL of dry DCM. TIPS (12 µL – 18 µL, 38.8 µmol – 58.2 µmol, 2.0 eq.) was added and the solution was cooled to 0 °C. 200 µL of TFA were added to the mixture and the solution was allowed to warm to room temperature and stirred for 1.5 hours. The solvent was removed by distillation at reduced pressure. The residue was taken up in 1 mL of DCM and the solvent was evaporated again. This procedure was repeated twice.

Coupling

HBTU (6.2 mg – 11.1 mg, 19.4 µmol – 29.1 µmol, 1.0 eq.) and DMF (300 µL) and DIPEA (17 µL – 25 µL, 97.0 µmol – 146 µmol, 5.0 eq.) were then added to the residue. The solution was stirred at room temperature for 30 minutes before a solution of the amine (5.0 eq., 3.0 eq. in case of the presence of a free acid moiety) in DMF (300 µL) was added. The mixture was then stirred until completion of the reaction monitoring the reaction progress by LCMS. The reaction mixture was quenched by addition of 25 mL of 1M HCl (or 25 mL of water in the presence of a pyridine or amine moiety in the product), diluted with brine (25 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with water (25 mL) and brine (25 mL) and dried over Na_2SO_4. The solvent was removed by distillation at reduced pressure.

Allyl deprotection

The residue was then dissolved in dry THF (1.0 mL) under a nitrogen atmosphere. $PhSiH_3$ (4.8 µL – 6.3 µL, 38.3 µmol – 58.µmol, 2.0 eq.) and a freshly prepared solution of $Pd(PP)_4$ (5.6 mg – 8.4 mg, 4.9 – 7.3 µmol, 0.25 eq.) in 0.9 mL of dry THF were added and the reaction mixture was stirred for 3 hours. The solvent was then removed by distillation at reduced pressure.

tert-Butyl or Boc deprotection

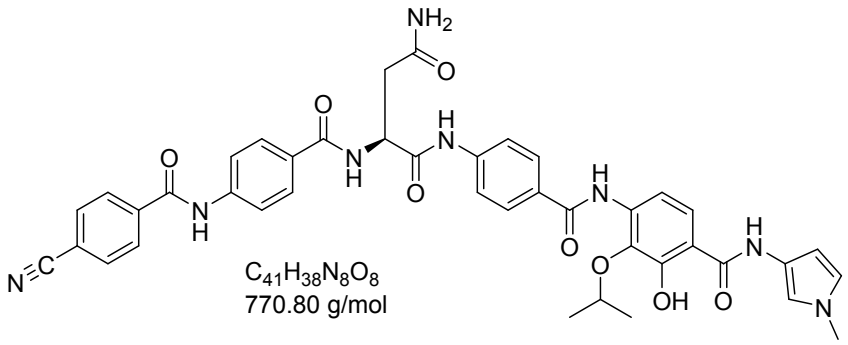
In case of the presence of a *tert*-butyl ester or Boc protecting group on the coupled amine, the residue was dissolved in dry DCM (800 µL) and cooled to 0 °C. TFA (200 µL) was added and

the reaction mixture was stirred until full conversion was observed by LCMS. The solvent was removed by distillation at reduced pressure. The residue was taken up in 1 mL of DCM and the solvent was evaporated again. This procedure was repeated twice.

Purification

The crude product was then purified using preparative reversed-phase HPLC according to condition A or B of section 1.2.1.2.

(*S*)-2-(4-(4-Cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-((1-methyl-1*H*-pyrrol-3-yl)carbamoyl)phenyl)carbamoyl)phenyl)succinamide (167)



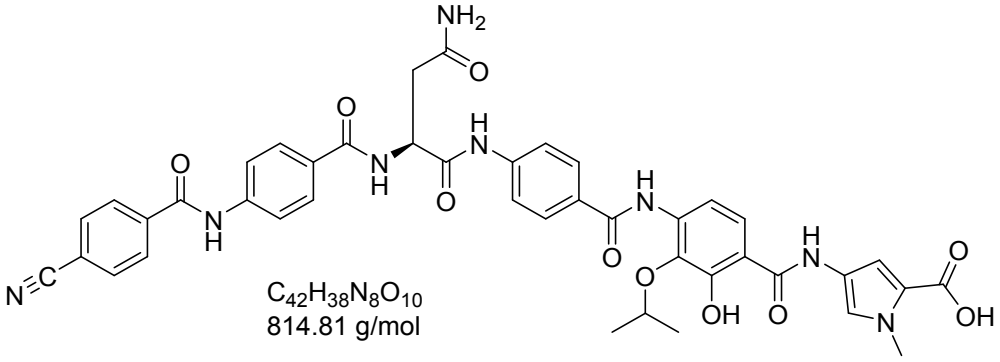
Compound **167** was synthesized according to the general procedure using intermediate **161** (20.0 mg, 19.4 µmol, 1.0 eq.) and amine **164** (19.0 mg, 97.0 µmol, 5.0 eq.) yielding a white solid (9.00 mg, 11.7 µmol, 60%, retention time: 20.43 min).

^{1}H NMR (700 MHz, DMSO-*d*$_6$, 300 K): δ (ppm) = 13.33 (s, 1H), 10.70 (s, 1H), 10.46 (s, 1H), 10.40 (s, 1H), 9.31 (s, 1H), 8.67 (d, *J* = 7.4 Hz, 1H), 8.15 – 8.02 (m, 4H), 7.97 – 7.87 (m, 6H), 7.84 – 7.77 (m, 3H), 7.65 (d, *J* = 8.9 Hz, 1H), 7.40 (s, 1H), 7.17 (t, *J* = 2.1 Hz, 1H), 6.99 (s, 1H), 6.62 (t, *J* = 2.5 Hz, 1H), 6.17 (dd, *J* = 2.8, 1.7 Hz, 1H), 4.92 (q, *J* = 7.1 Hz, 1H), 4.56 (hept, *J* = 6.1 Hz, 1H), 3.61 (s, 3), 2.69 (d, *J* = 7.1 Hz, 2H), 1.25 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO-*d*$_6$, 300 K): δ (ppm) = 171.3, 170.7, 166.4, 165.8, 164.5, 164.1, 154.6, 142.5, 141.6, 138.7, 136.2, 136.1, 133.0, 132.5, 132.4, 129.1, 128.3, 128.2, 121.9, 121.6, 119.8, 119.5, 118.9, 118.3, 114.0, 113.1, 111.6, 111.5, 101.2, 74.6, 51.6, 36.8, 36.0, 22.4.

HRMS (ESI+): *m/z* for $C_{41}H_{38}N_8O_8$ $[M+H]^+$: calculated: 771.2885, found: 771.2890.

(*S*)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid (74)



1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (170 mg, 1.00 mmol, 1.0 eq.) was dissolved in dry DMF (5 mL) under a nitrogen atmosphere. The solution was purged with nitrogen for 15 minutes before Pd/C (80 mg, 10%wt) was added. The mixture was then purged with hydrogen for 5 minutes, set under a hydrogen atmosphere and stirred overnight. The mixture

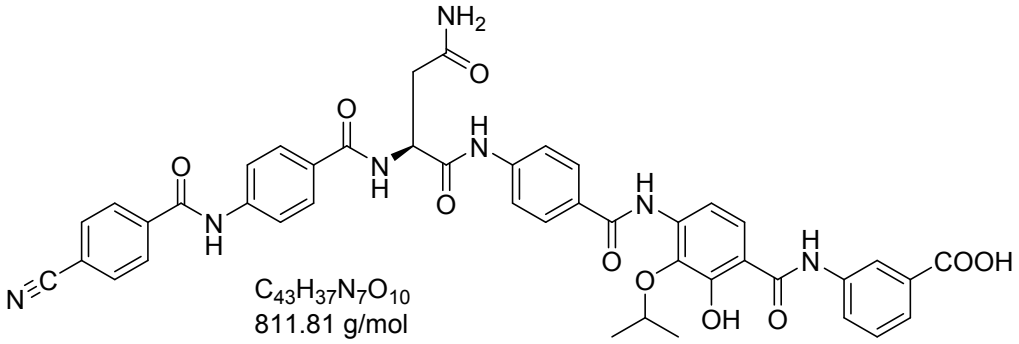
was then passed through a syringe filter (CHROMAFIL® PET-45/15, 45 µm pore size, 15 mm diameter). Compound **74** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and the previously prepared solution of crude amine (730 µL, 146 µmol, 5.0 eq.) yielding a white solid containing some residual triphenylphosphine oxide (6.16 mg, 7.7 µmol, 26%, retention time: 20.68 min).

^{1}H NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.71 (s, 1H), 10.47 (s, 1H), 9.26 (s, 1H), 8.69 (d, *J* = 7.3 Hz, 1H), 8.15 – 8.02 (m, 4H), 7.95 – 7.88 (m, 6H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.72 (br, 1H), 7.68 – 7.50 (m, 2H), 7.45 (d, *J* = 2.0 Hz, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 6.89 (pseudo br, 1H), 4.92 (pseudo q, *J* = 7.1 Hz, 1H), 4.63 (pseudo br, 1H), 3.86 (s, 3), 2.69 (d, *J* = 7.0 Hz, 2H), 1.24 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3, 170.7, 166.7, 165.7, 164.4, 163.9, 162.0, 142.4, 141.6, 138.7, 136.3, 132.5, 129.1, 128.6, 128.5, 128.3, 128.1, 120.8, 119.5, 118.9, 118.3, 114.0, 108.7, 51.6, 36.8, 36.2, 22.4.

HRMS (ESI+): *m/z* for $C_{42}H_{38}N_8O_{10}$ [M+H]$^+$: calculated: 815.2784, found: 815.2793.

(*S*)-3-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (72)



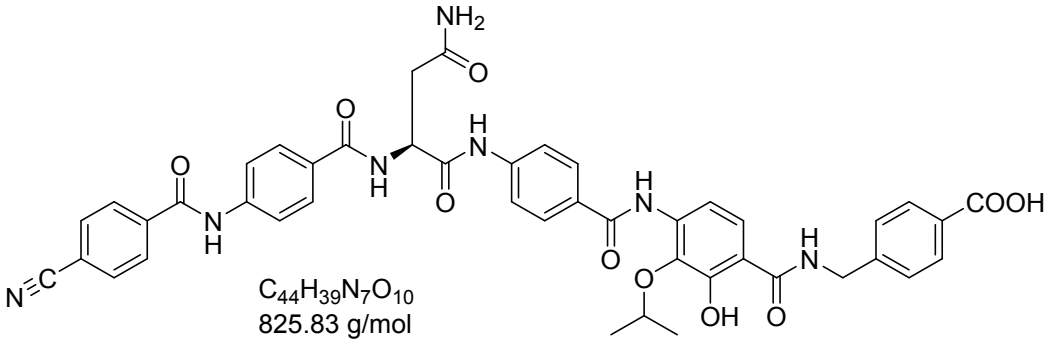
Compound **72** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and *m*-aminobenzoic acid (12.0 mg, 87.3 µmol, 3.0 eq.) yielding a white solid (3.90 mg, 4.8 µmol, 17%, retention time: 20.70 min).

^{1}H NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.04 (br, 1H), 12.51 (br, 1H), 10.70 (s, 1H), 10.46 (s, 1H), 9.36 (s, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.33 (t, *J* = 2.0 Hz, 1H), 8.14 – 8.03 (m, 4H), 7.99 – 7.80 (m, 11H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.68 (pseudo br, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (pseudo q, *J* = 7.1 Hz, 1H), 4.58 (pseudo br, 1H), 2.71 – 2.68 (m, 2H), 1.26 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3, 170.7, 168.5, 167.1, 165.8, 164.4, 164.1, 142.5, 141.6, 138.7, 136.3, 132.5, 131.3, 129.1, 129.0, 128.6, 128.3, 128.2, 122.6, 122.2, 119.5, 118.9, 118.3, 114.0, 54.9, 51.6, 36.8, 22.4.

HRMS (ESI+): *m/z* for $C_{43}H_{37}N_7O_{10}$ [M+H]$^+$: calculated: 812.2675, found: 812.2683.

(*S*)-3-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (71)



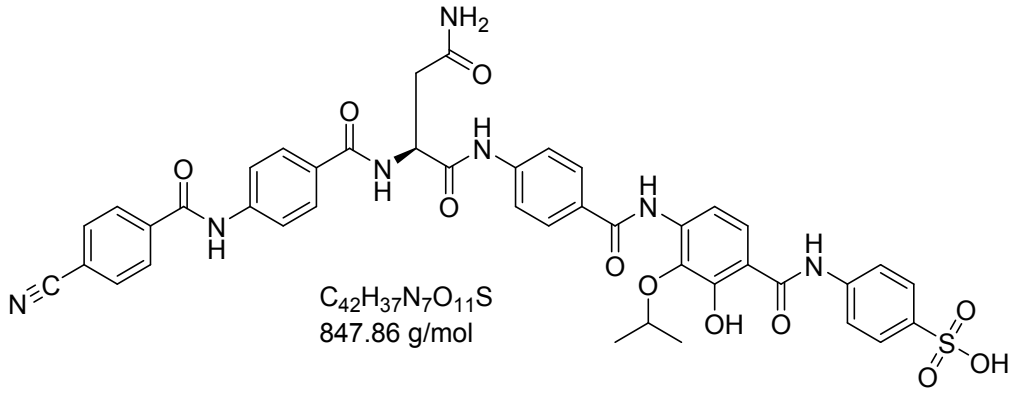
Compound **71** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and 4-(aminomethyl)benzoic acid (13.2 mg, 87.3 µmol, 3.0 eq.) yielding a white solid (3.42 mg, 4.2 µmol, 14%, retention time: 21.96 min).

^{1}H-NMR (700 MHz, DMSO): δ (ppm) = 13.20 (br s, 1H), 10.70 (s, 1H), 10.47 (s, 1H), 9.53 (br s, 1H), 9.30 (s, 1H), 8.69 (d, *J* = 7.3 Hz, 1H), 8.15 – 8.02 (m, 4H), 7.96 – 7.87 (m, 8H), 7.81 (d, *J* = 8.6 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.40 (d, J = 2.5 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 4.92 (pseudo q, J = 7.1 Hz, 1H), 4.60 – 4.52 (m, 3H), 2.69 (d, *J* = 7.1 Hz, 2H), 1.24 (d, J = 6.1 Hz, 6H).

^{13}C-NMR (176 MHz, DMSO): δ (ppm) = 171.3, 170.7, 169.8, 167.2, 165.7, 164.4, 164.1, 142.5, 141.6, 138.7, 136.5, 136.1, 132.5, 129.5, 129.1, 128.6, 128.4, 128.3, 128.2, 127.2, 121.7, 119.5, 118.9, 118.3, 114.0, 74.5, 51.6, 42.2, 36.8, 22.4.

HRMS (ESI+): *m/z* for $C_{44}H_{39}N_7O_{10}$ $[M+H]^+$: calculated: 826.2831, found: 826.2852.

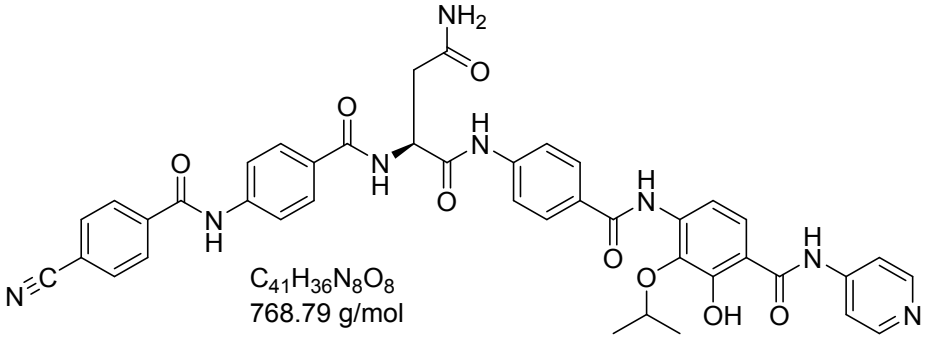
(*S*)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzenesulfonic acid (75)



Compound **75** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and sulfanilic acid (15.1 mg, 87.3 µmol, 3.0 eq.) yielding a white solid (3.53 mg, 4.2 µmol, 14%, retention time: 18.51 min).

^{1}H NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.70 (s, 1H), 10.44 (s, 1H), 9.10 (br, 1H, SO_3H), 8.67 (d, J = 7.3 Hz, 1H), 8.15 – 8.03 (m, 4H), 7.95 – 7.80 (m, 9H), 7.67 – 7.59 (m, 3H), 7.53 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 2.4 Hz, 1H), 6.98 (d, J = 2.4 Hz, 1H), 4.93 (q, J = 7.2 Hz, 1H), 4.80 (pseudo br, 1H), 2.72 – 2.66 (m, 2H), 1.23 (d, J = 6.1 Hz, 6H).
^{13}C NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3, 170.7, 165.7, 164.4, 142.2, 141.6, 138.7, 132.5, 129.2, 128.6, 128.3, 127.9, 126.1, 119.5, 119.0, 118.3, 114.0, 51.6, 36.8, 22.5.
HRMS (ESI+): *m/z* for $C_{427}N_7O_{11}S$ $[M+H]^+$: calculated: 848.2345, found: 848.2363.

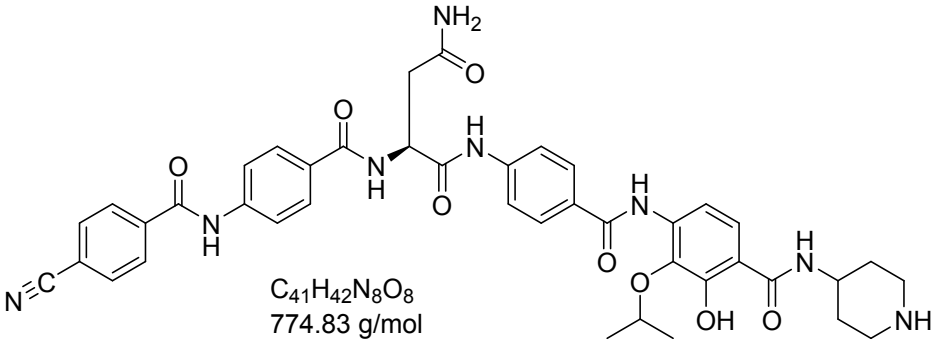
(*S*)-2-(4-(4-Cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-(pyridin-4-ylcarbamoyl)phenyl)carbamoyl)phenyl)succinamide (77)



$C_{41}H_{36}N_8O_8$
768.79 g/mol

Compound **77** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and 4-aminopyridine (13.7 mg, 146 µmol, 5.0 eq.) yielding a white solid (10.39 mg, 13.5 µmol, 46%, retention time: 22.99 min).
^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.98 (s, 1H), 10.71 (s, 1H), 10.45 (s, 1H), 9.09 (s, 1H), 8.67 (d, J = 7.3 Hz, 1H), 8.42 – 8.38 (m, 2H), 8.15 – 8.01 (m, 6H), 7.95 – 7.86 (m, 6H), 7.81 (d, J = 8.9 Hz, 2H), 7.58 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 2.4 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 4.92 (pseudo q, J = 7.2 Hz, 1H), 4.81 (hept, J = 6.2 Hz, 1H), 2.69 (d, J = 7.1 Hz, 2H), 1.22 (d, J = 6.2 Hz, 6H).
^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3, 170.7, 168.3, 165.7, 164.4, 163.6, 156.9, 150.1, 146.5, 144.9, 142.2, 141.6, 138.7, 137.2, 135.6, 132.5, 129.1, 128.9, 128.6, 128.3, 127.9, 123.5, 119.5, 119.0, 118.3, 114.2, 114.0, 108.8, 72.2, 51.6, 36.8, 22.5.
HRMS (ESI+): *m/z* for $C_{416}N_8O_8$ $[M+H]^+$: calculated: 769.2729, found: 769.2722.

(*S*)-2-(4-(4-Cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-(piperidin-4-ylcarbamoyl)phenyl)carbamoyl)phenyl)succinamide (73)



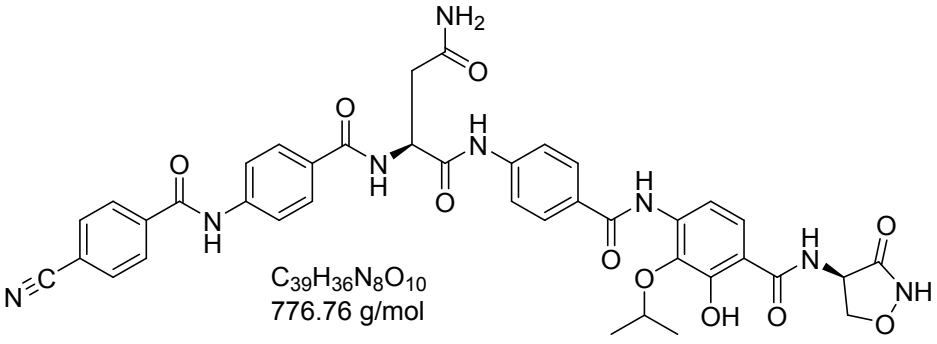
Compound **73** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and tert-butyl 4-aminopiperidine-1-carboxylate (29.1 mg, 146 µmol, 5.0 eq.) yielding a white solid (6.17 mg, 8.0 µmol, 27%, retention time: 20.59 min).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.71 (s, 1H), 10.47 (s, 1H), 9.24 (s, 1H), 8.69 (d, *J* = 7.2 Hz, 1H), 8.15 – 8.03 (m, 4H), 7.95 – 7.87 (m, 6H), 7.83 – 7.79 (m, 2H), 7.65 – 7.63 (m, 2H), 7.40 (d, *J* = 2.4 Hz, 1H), 6.99 (d, *J* = 2.4 Hz, 1H), 4.92 (pseudo q, *J* = 7.1 Hz, 1H), 4.57 (hept, *J* = 6.3 Hz, 1H), 4.05 – 3.96 (m, 1H), 3.20 – 3.15 (m, 2H), 2.81 (t, *J* = 11.9 Hz, 2H), 2.69 (d, *J* = 7.1 Hz, 2H), 1.92 – 1.86 (m, 2H), 1.68 – 1.57 (m, 2H), 1.22 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3, 170.7, 169.0, 165.8, 164.4, 164.0, 142.4, 141.6, 138.7, 136.2, 136.1, 133.1, 132.5, 132.3, 129.1, 128.6, 128.5, 128.3, 128.1, 122.0, 119.5, 118.9, 118.3, 114.0, 74.0, 51.6, 45.4, 43.5, 36.8, 30.1, 22.4.

HRMS (ESI+): *m/z* for $C_{41}H_{42}N_8O_8$ $[M+H]^+$: calculated: 775.3198, found: 775.3209.

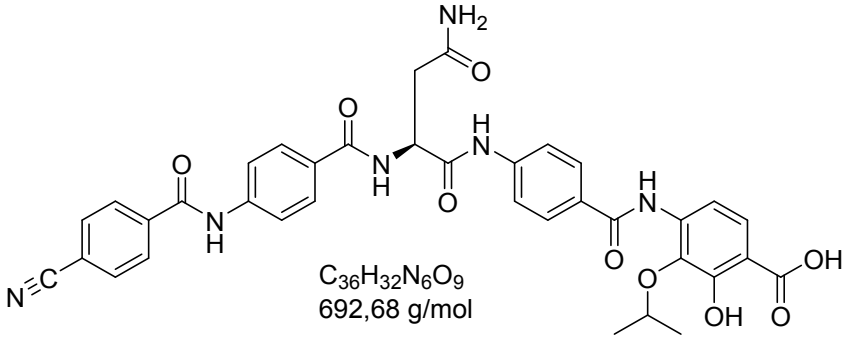
(*S*)-2-(4-(4-Cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-(((*R*)-3-oxoisoxazolidin-4-yl)carbamoyl)phenyl)carbamoyl)phenyl)succinamide (76)



Compound **76** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and (*R*)-(+)-cycloserine (14.9 mg, 146 µmol, 5.0 eq.) yielding a white solid still containing some allyl-protected compound (2.46 mg, 3.2 µmol, 11%, retention time: 20.11 min).

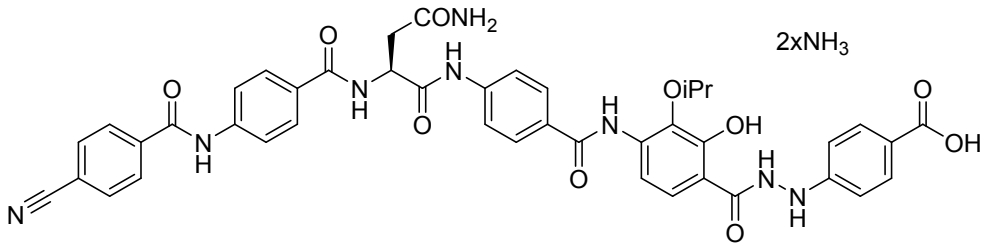
^{1}H NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.71 (s, 1H), 10.70 (s, 1H), 10.45 (s, 1H), 9.27 (s, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.14 – 8.03 (m, 4H), 7.95 – 7.87 (m, 6H), 7.82 – 7.80 (m, 2H), 7.63 – 7.58 (m, 2H), 7.39 (d, *J* = 2.4 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 4.92 (pseudo q, *J* = 7.1 Hz, 1H), 4.54 (hept, *J* = 6.2 Hz, 1H), 2.71 – 2.66 (m, 2H), 1.24 (d, *J* = 6.2 Hz, 6H).
^{13}C NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 172.6, 171.3, 170.7, 169.7, 165.8, 164.5, 164.1, 155.2, 142.5, 141.6, 138.7, 136.6, 135.9, 132.5, 131.6, 129.1, 128.6, 128.3, 128.2, 122.2, 119.5, 118.9, 118.3, 114.0, 111.3, 110.8, 74.5, 51.6, 36.8, 22.4.
HRMS (ESI+): *m/z* for $C_{39}H_{36}N_8O_{10}$ $[M+H]^+$: calculated: 777.2627, found: 777.2623.

(*S*)-4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzoic acid (67)



Compound **67** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and skipping the coupling step yielding a white solid (17.24 mg, 24.9 µmol, 86%, retention time: 20.70 min).
^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.71 (s, 1H), 10.46 (s, 1H), 9.28 (s, 1H), 8.68 (d, *J* = 7.3 Hz, 1H), 8.15 – 8.03 (m, 4H), 7.95 – 7.88 (m, 6H), 7.83 – 7.80 (m, 2H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.40 (s, 1H), 6.99 (s, 1H), 4.92 (pseudo q, *J* = 7.1 Hz, 1H), 4.56 (hept, *J* = 6.2 Hz, 1H), 2.69 (d, *J* = 7.1 Hz, 2H), 1.25 (d, *J* = 6.2 Hz, 6H).
^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 172.0, 171.3, 170.7, 165.7, 164.4, 164.1, 155.1, 142.5, 141.6, 138.7, 137.4, 135.4, 132.5, 129.1, 128.6, 128.4, 128.3, 128.2, 124.6, 119.5, 118.9, 118.3, 114.0, 111.4, 74.6, 51.6, 36.8, 22.4.
HRMS (ESI+): *m/z* for $C_{36}H_{32}N_6O_9$ $[M+H]^+$: calculated: 693.2304, found: 693.2288.

(S)-4-(2-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzoyl)hydrazineyl)benzoic acid (70)



Chemical Formula: $C_{43}H_{38}N_8O_{10}$
Exact Mass: 826,2711

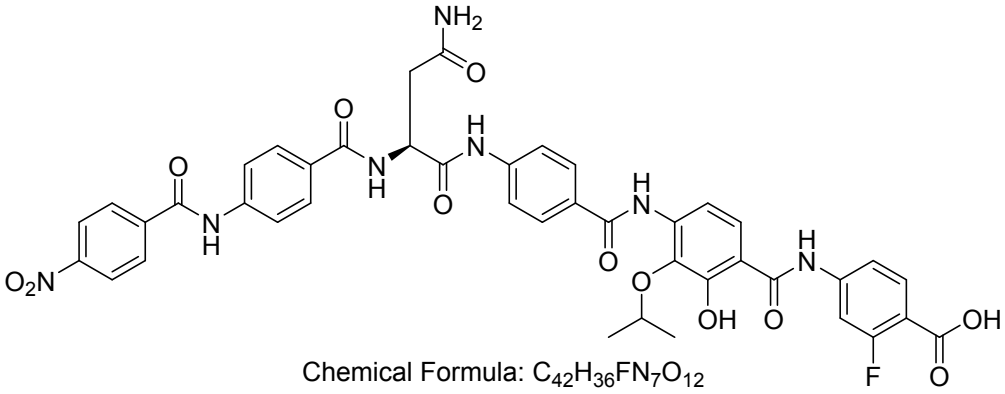
Compound **70** was synthesized according to the general procedure using intermediate **161** (30.0 mg, 29.1 µmol, 1.0 eq.) and 4-hydrazineylbenzoic acid (8.6 mg, 63 µmol, 2.2 eq.) yielding a white solid (1.2 mg, 1.5 µmol, 5%, retention time: 20.16 min).

^{1}H NMR (700 MHz, DMSO) δ 12.00 (br, 1H), 10.71 (s, 1H), 10.45 (s, 1H), 9.00 (s, *J* = 15.8 Hz, 1H), 8.97 (br, 1H), 8.70 (d, *J* = 7.1 Hz, 2H), 8.57 (br, 1H), 8.13 (d, *J* = 8.2 Hz, 2H), 8.04 (d, *J* = 8.3 Hz, 2H), 7.93 (d, *J* = 8.8 Hz, 2H), 7.91 – 7.84 (m, 4H), 7.82 – 7.78 (m, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.41 (s, 2H), 7.23 (br, 1H), 6.98 (s, 1H), 6.73 (d, *J* = 8.1 Hz, 2H), 4.92 (q, *J* = 7.1 Hz, 1H), 4.83 (br, 1H), 2.69 (d, *J* = 6.9 Hz, 2H), 1.21 (d, *J* = 6.0, 3.8 Hz, 6H).

^{13}C NMR (176 MHz, DMSO) δ 171.3, 170.7, 167.5, 165.7, 164.4, 163.4, 157.1, 142.1, 141.6, 138.7, 135.3, 133.9, 132.5, 130.9, 129.2, 128.6, 128.3, 127.8, 123.8, 119.5, 119.0, 118.3, 114.0, 110.3, 107.7, 72.9, 51.6, 36.8, 22.6, 22.5.

HRMS (ESI) calculated for C43H39N8O10 ($M+H^+$) 827.2784, found 827.2798.

(S)-4-(4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-fluorobenzoic acid (69)



Chemical Formula: $C_{42}H_{36}FN_7O_{12}$
Exact Mass: 849,24

Compound **69** was synthesized according to the general procedure using the nitro analogue of intermediate **161** (20.0 mg, 19 µmol, 1.0 eq.) and tert-butyl 4-amino-2-fluorobenzoate (8 mg,

38 µmol, 2 eq.), the crude was purified by preparative RP-HPLC using condition A (retention time: 24.5 min) to yield the desired product (6.5 mg, 7.6 µmol, 40%).

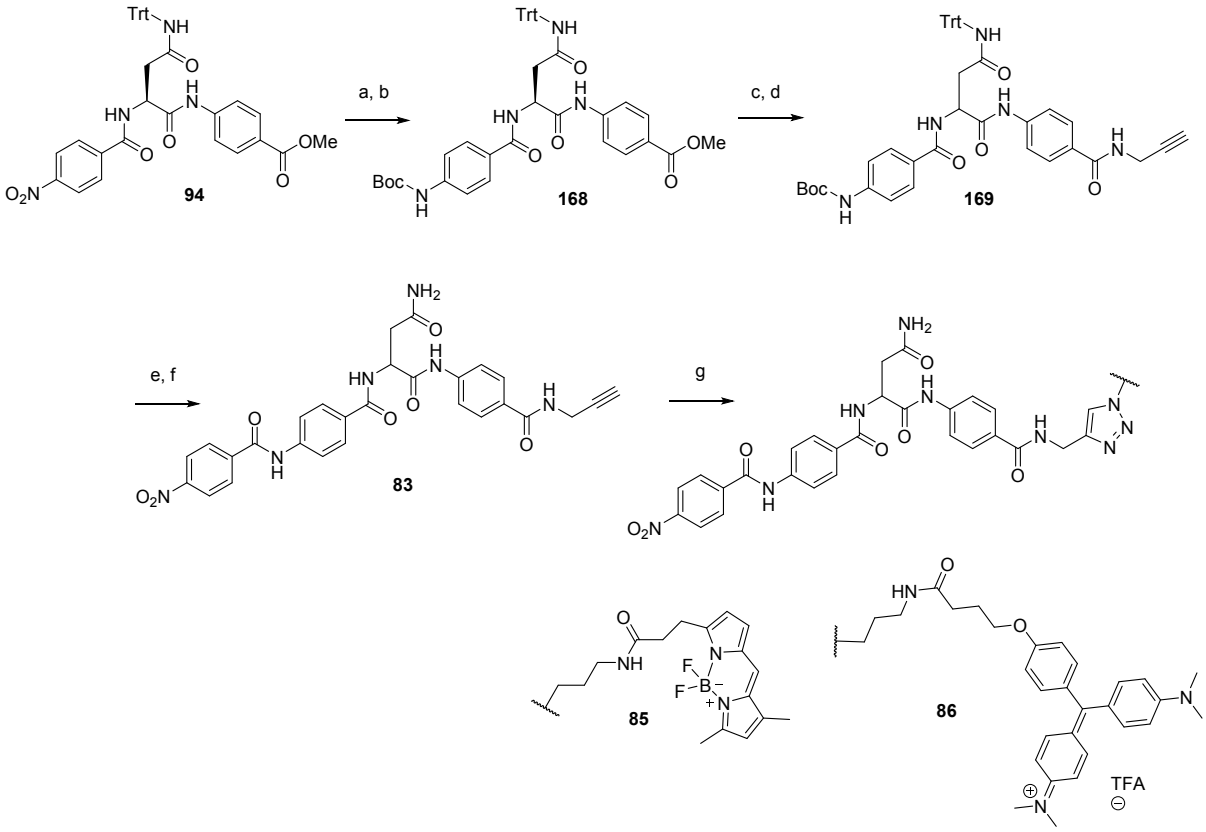
^{1}H NMR (500 MHz, DMSO) δ 13.00 (s, 1H), 12.03 (s, 1H), 10.79 (s, 2H), 10.47 (s, 1H), 9.40 (s, 1H), 8.68 (d, *J* = 7.0 Hz, 1H), 8.39 (d, *J* = 8.4 Hz, 2H), 8.21 (d, *J* = 8.3 Hz, 2H), 7.96 – 7.89 (m, 7H), 7.80 (dd, *J* = 19.5, 7.9 Hz, 4H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.61 (d, *J* = 8.5 Hz, 1H), 7.41 (s, 1H), 7.00 (s, 1H), 4.93 (dd, *J* = 13.6, 6.6 Hz, 1H), 4.58 – 4.51 (m, 1H), 2.70 (d, *J* = 6.5 Hz, 2H), 1.26 (d, *J* = 6.0 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 171.29, 170.77, 168.50, 165.79, 164.60, 164.57, 164.22, 164.21, 162.51, 160.48, 154.24, 149.29, 143.71, 143.62, 142.53, 141.57, 140.34, 137.10, 136.47, 132.63, 132.62, 129.35, 129.23, 128.38, 128.32, 123.61, 122.98, 119.57, 118.92, 116.21, 116.19, 114.15, 114.08, 112.72, 112.06, 108.50, 108.28, 74.80, 51.65, 36.79, 22.31.

HRMS (ESI) calculated for $C_{42}H_{37}FN_7O_{12}$ ($M+H^+$) 850.2479, found 850.2476.

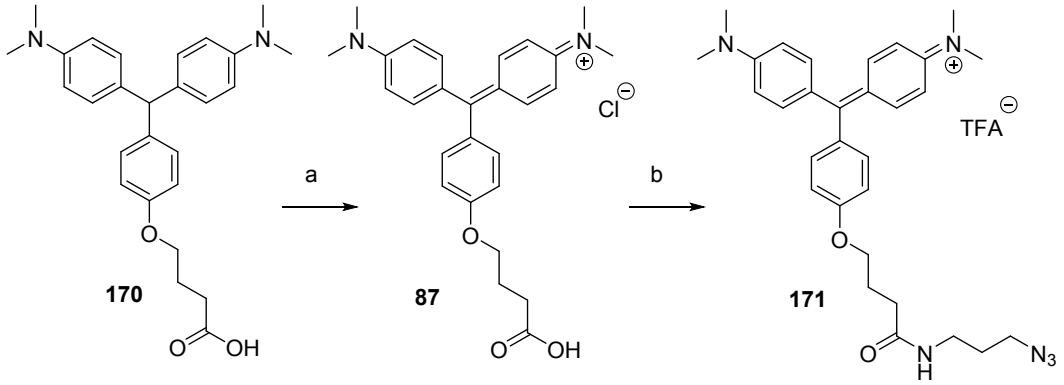
1.2.2.4 Cystobactamid-based bacterial imaging

Synthesis of the probes



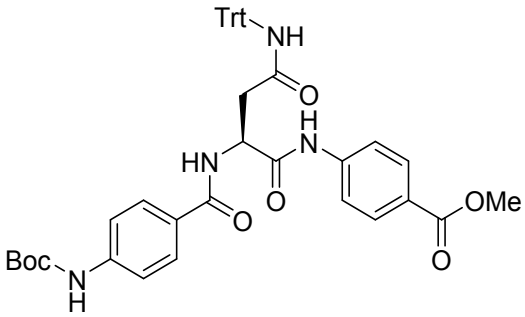
Scheme S 11. Reagents and conditions: a) Pd/C, MeOH, rt, 2 h, b) Boc_2O, THF/$NaHCO_3$ sat. sol., 3 d, rt (74% o2s); c) LiOH, THF/water, rt, overnight, d) propargyl amine, $POCl_3$, DiPEA, rt, 3 h (31% o2s); e) TFA, Tips, DCM, rt, 2 h, f) 4-nitrobenzoyl chloride, THF/$NaHCO_3$ sat. sol., rt, 30 min. (93% o2s); g) azide **84** or **171** sodium ascorbate, TBTA, $CuSO_4$, DMSO/*t*-BuOH/water, rt, 5h for **85**, overnight for **86** (77%, 35%)

Synthesis of clickable malachite green (MG)



Scheme S 12. Reagents and conditions: a) chloranil, CH_3CN, rt, overnight (65%); b) 3-azidopropan-1-amine, EDC, HOAt, DiPEA, DMF, rt, overnight (88%).

Methyl (S)-4-(2-(4-((tert-butoxycarbonyl)amino)benzamido)-4-oxo-4-(tritylamino)butanamido)benzoate (168)



Chemical Formula: $C_{43}H_{42}N_4O_7$
Exact Mass: 726,3053

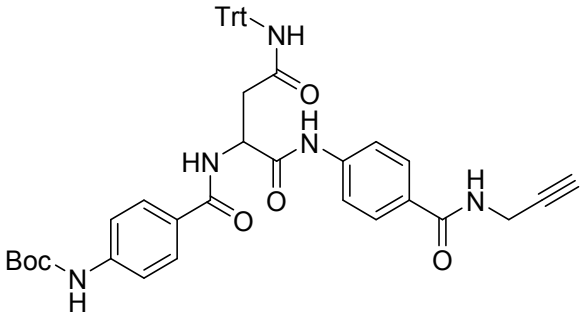
Nitro arene **94** (1 g; 1.52 mmol) was dissolved in MeOH (35 mL) and EtOAc (5 mL), Pd/C (100 mg) was added and the solution was purged with N_2 and successively with H_2. The reaction mixture was stirred for 2 hours under an atmosphere of H_2, then it was filtered over a pad of celite and the solvent was removed under vacuum to give 1 g of crude material which was emplozed directly in the step without further purification.

The crude material was dissolved in a mixture of THF / $NaHCO_3$ saturated solution 1:1 (15 mL), the solution was cooled to 0°C and di-*tert*-butyl dicarbonate (500 mg; 2.28 mmol) was added, the reaction was stirred at r.t. for 4 hours. TLC and LCMS check showed presence of mainly starting material, therefore further di-*tert*-butyl dicarbonate (500 mg; 2.28 mmol) was added. The reaction was stirred at r.t. for 3 days. The reaction mixture was diluted with EtOAc (100 mL) and water (100 mL), organic phase washed with brine, dried over sodium sulphate and evaporated under vacuum. The crude material was purified on silica gel with a gradient 20-70% EtOAc in PetEt to give 820 mg of desired product (0.82 g; y= 74%).

^{1}H NMR (500 MHz, DMSO) δ 10.51 (s, 1H), 9.66 (s, 1H), 8.65 (d, *J* = 7.6 Hz, 1H), 8.63 (s, 1H), 7.94 – 7.88 (m, 2H), 7.88 – 7.82 (m, 2H), 7.80 – 7.73 (m, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.24 – 7.12 (m, 15H), 4.86 (ddd, *J* = 10.8, 7.6, 4.5 Hz, 1H), 3.82 (s, 3H), 2.96 (dd, *J* = 14.5, 10.6 Hz, 1H), 2.67 (dd, *J* = 14.5, 4.4 Hz, 1H), 1.49 (s, 9H).

^{13}C NMR (126 MHz, DMSO) δ 171.0, 168.4, 165.8, 165.8, 152.6, 144.7, 143.4, 142.5, 130.2, 128.5, 128.4, 127.4, 127.0, 126.3, 124.0, 118.7, 117.0, 79.5, 69.4, 52.0, 51.9, 38.1, 28.1.

Tert-butyl (4-((1,4-dioxo-1-((4-(prop-2-yn-1-ylcarbamoyl)phenyl)amino)-4-(tritylamino)butan-2-yl)carbamoyl)phenyl)carbamate (169)



Chemical Formula: $C_{45}H_{43}N_5O_6$
Exact Mass: 749,3213

Methyl ester **168** (0.715 g; 0.98 mmol) was dissolved in THF (10 mL) and water (2 mL), then a solution of LiOH (0.118 g, 4.92 mmol) in water (8 mL) was added. The reaction was stirred overnight at r.t. and was quenched with HCl 1 N, the solvent was partially reduced under vacuum and then the mixture was diluted with EtOAc (100 mL) and water (100 mL), the organic phase was washed with brine, dried over sodium sulphate and reduced under vacuum. The crude residue was used in the next step without further purification.

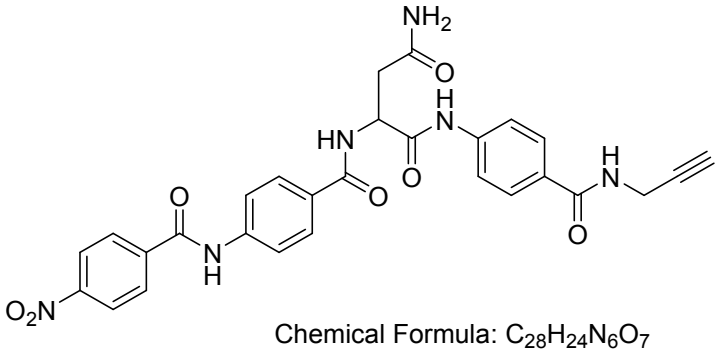
The crude carboxylic acid (0.98 mmol) was mixed with propargyl amine (52 µL; 0.82 mmol) and DiPEA (430 µL; 2.46 mmol), to this solution $POCl_3$ (91 µL; 0.98 mmol) was added dropwise at 0°C. The reaction was stirred for 3 hours at r.t. and then it was quenched with $NaHCO_3$ saturated solution, the solvent was partially reduced under vacuum, residue diluted with EtOAc (50 mL) and $NaHCO_3$ saturated solution (50 mL), the organic phase was washed with HCl 1 N (50 mL) and brine (50 mL), dried over sodium sulphate and reduced under vacuum. The residue was chromatographed on silica gel with a gradient 30-100% EtOAc in PetEt to give 230 mg of a colourless solid (0.31 mmol; y= 31%).

^{1}H NMR (500 MHz, DMSO) δ 10.38 (s, 1H), 9.66 (s, 1H), 8.80 (t, *J* = 5.6 Hz, 1H), 8.64 (d, *J* = 7.2 Hz, 2H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.82 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.23 – 7.13 (m, 15H), 4.91 – 4.79 (m, 1H), 4.03 (dd, *J* = 5.5, 2.5 Hz, 2H), 3.10 (td, *J* = 2.5, 1.0 Hz, 1H), 2.96 (dd, *J* = 14.4, 10.5 Hz, 1H), 2.67 (dd, *J* = 14.4, 4.3 Hz, 1H), 1.49 (s, 9H).

^{13}C NMR (126 MHz, DMSO) δ 170.8, 168.5, 165.8, 165.4, 152.6, 144.7, 142.5, 141.8, 128.6, 128.4, 128.3, 128.1, 127.4, 127.1, 126.3, 118.5, 117.0, 81.5, 79.5, 72.8, 69.4, 51.9, 28.4, 28.1.

HRMS (ESI) calculated for $C_{45}H_{44}N_5O_6$ ($M+H^+$) 750.3286, found 750.3281.

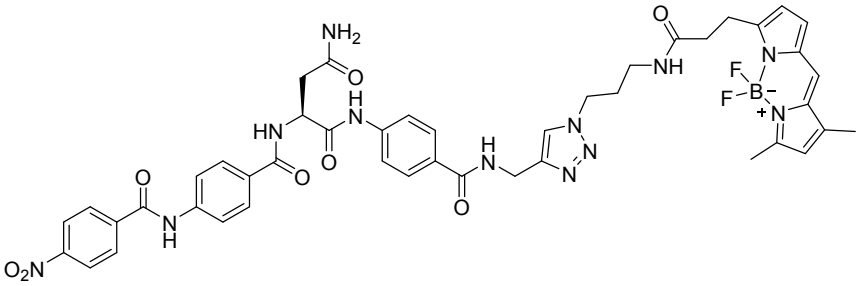
2-(4-(4-Nitrobenzamido)benzamido)-N1-(4-(prop-2-yn-1-ylcarbamoyl)phenyl)succinamide (83)



Chemical Formula: $C_{28}H_{24}N_6O_7$
Exact Mass: 556,17

TFA (6.0 mL) was added dropwise at 0°C to a stirred solution of Boc-protected aniline **169** (220 mg; 0.29 mmol) and Tips (0.50 mL) in DCM (14 mL). The reaction was stirred for 2 h, then the solvent was reduced under vacuum, the residue was taken up with DCM and evaporated again twice. The crude was triturated with cold PetEt (3x) and then it was dissolved in THF (2 mL) and NaHCO3 (3 mL). The mixture was cooled to 0°C and a solution of 4-nitrobenzoyl chloride (81 mg, 0.43 mmol) in THF (1 mL) was added dropwise. The reaction was stirred for 30 min, then it was diluted with water (100 mL) and EtOAc (100 mL), the watery phase were extracted again with EtOAc (2x 100 mL). The combined organic phases were washed with brine (200 mL), dried over sodium sulphate and reduced under vacuum to give 150 mg of product which was used in the next step without further purification.
^{1}H NMR (500 MHz, DMSO) δ 10.78 (s, 1H), 10.36 (s, 1H), 8.80 (t, *J* = 5.6 Hz, 1H), 8.66 (d, *J* = 7.3 Hz, 1H), 8.40 – 8.36 (m, 2H), 8.23 – 8.18 (m, 2H), 7.95 – 7.86 (m, 4H), 7.86 – 7.78 (m, 2H), 7.75 – 7.67 (m, 2H), 7.38 (s, 1H), 6.98 (s, 1H), 4.90 (dd, *J* = 14.0, 7.3 Hz, 1H), 4.03 (dd, *J* = 5.5, 2.5 Hz, 2H), 3.10 (t, *J* = 2.5 Hz, 1H), 2.67 (d, *J* = 7.8 Hz, 2H).
^{13}C NMR (126 MHz, DMSO) δ 171.3, 170.6, 165.7, 165.4, 164.2, 149.3, 141.9, 141.5, 140.3, 129.3, 129.2, 128.3, 128.1, 123.6, 119.5, 118.5, 81.5, 72.8, 51.6, 36.8, 28.4.
HRMS (ESI) calculated for $C_{28}H_{25}N_6O_7$ ($M+H^+$) 557.1779, found 557.1774.

(S)-N1-(4-(((1-(4-((2-(5,5-Difluoro-7,9-dimethyl-5H-5l4,6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)ethyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)phenyl)-2-(4-(4-nitrobenzamido)benzamido)succinamide (85)



Chemical Formula: $C_{45}H_{45}BF_2N_{12}O_8$
Exact Mass: 930,35

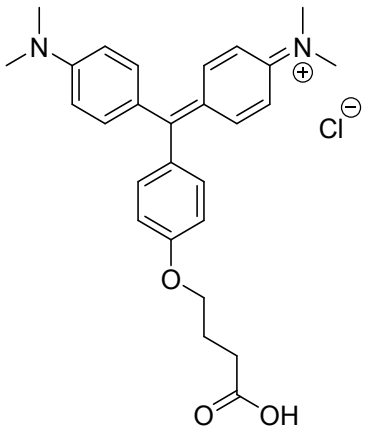
Alkyne **83** (3.3 mg; 0.0058 mmol), azide **84** (2 mg; 0.0053 mmol) and TBTA (0.28 mg; 0.00053 mmol) as a solution in DMSO (10 mg/mL) were dissolved in a mixture of DMSO/*t*-BuOH/water 2:1:1 (220 µL). $CuSO_4$ (0.042 mg; 0.0027 mmol) as a solution in water (2.5 mg/mL) and sodium ascorbate (0.63 mg; 0.0032 mmol) as a solution in water (15 mg/mL) were mixed together, the mixture turned quickly yellow and then it was added to the reaction solution. The reaction was stirred at r.t. for 5 hours and then it was purified by preparative RP-HPLC using condition A (retention time: 17.97 min.) to obtain 3.8 mg of desired product (0.0041 mmol; y= 77%).

^{1}H NMR (500 MHz, DMSO) δ 10.78 (s, 1H), 10.34 (s, 1H), 8.89 (t, *J* = 5.7 Hz, 1H), 8.65 (d, *J* = 7.3 Hz, 1H), 8.42 – 8.34 (m, 2H), 8.25 – 8.16 (m, 2H), 8.02 (t, *J* = 5.6 Hz, 1H), 7.98 (s, 1H), 7.95 – 7.87 (m, 4H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.9 Hz, 2H), 7.68 (s, 1H), 7.38 (s, 1H), 7.07 (d, *J* = 4.0 Hz, 1H), 6.98 (s, 1H), 6.34 (d, *J* = 4.0 Hz, 1H), 6.29 (s, 1H), 4.90 (dd, *J* = 14.1, 7.2 Hz, 1H), 4.49 (d, *J* = 5.6 Hz, 2H), 4.32 (t, *J* = 7.1 Hz, 2H), 3.11 – 3.03 (m, 4H), 2.67 (d, *J* = 7.6 Hz, 2H), 2.47 (s, 1H, CH_2 overlapping with DMSO-d6 peak), 2.46 (s, 3H), 2.25 (s, 3H), 1.93 (p, *J* = 6.9 Hz, 2H).

^{13}C NMR (126 MHz, DMSO) δ 171.3, 170.9, 170.5, 165.7, 165.5, 164.2, 159.2, 157.7, 149.3, 145.1, 144.1, 141.7, 141.5, 140.3, 134.4, 133.0, 129.3, 129.2, 128.9, 128.8, 128.7, 128.3, 128.1, 127.9, 125.3, 123.6, 123.0, 120.3, 119.5, 118.5, 116.6, 51.5, 47.1, 36.8, 35.8, 34.8, 33.8, 30.0, 24.0, 14.5, 11.0.

HRMS (ESI) calculated for $C_{45}H_{46}BF_2N_{12}O_8$ ($M+H^+$) 931.3617, found 931.3611.

N-(4-((4-(3-Carboxypropoxy)phenyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium (87)



Chemical Formula: $C_{27}H_{31}N_2O_3^+$
Exact Mass: 431,23

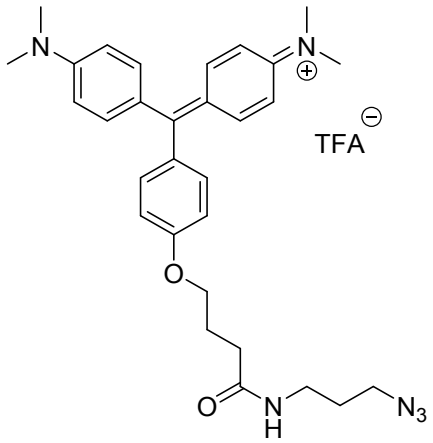
Carboxylic acid **170** [6] (9.6 mg; 0.023 mmol) was suspended in CH_3CN (1.2 mL), following chloranil (6.6 mg; 0.026 mmol) was added. The dark mixture was stirred at r.t. overnight, the solvent was partially removed under vacuum, then the mixture was dissolved in MeOH and loaded on silica gel. Column chromatography with a gradient 1-20% MeOH in DCM afforded 7 mg of desired product (0.015 mmol; y= 65%).

^{1}H NMR (500 MHz, MeOD) δ 7.46 – 7.41 (m, 4H), 7.40 – 7.33 (m, 2H), 7.22 – 7.14 (m, 2H), 7.05 (d, *J* = 9.4 Hz, 4H), 4.22 (t, *J* = 6.3 Hz, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.26 – 1.97 (m, 2H).

^{13}C NMR (126 MHz, MeOD) δ 179.9, 176.9, 165.9, 158.6, 142.0, 139.1, 133.4, 128.5, 116.2, 114.6, 69.0, 41.0, 31.4, 25.8.

HRMS (ESI) calculated for $C_{27}H_{31}N_2O_3$ (M^+) 431.2329, found 431.2325.

N-(4-((4-(4-((3-Azidopropyl)amino)-4-oxobutoxy)phenyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium trifluoroacetate (171)



Chemical Formula: $C_{30}H_{37}N_6O_2^+$
Exact Mass: 513,30

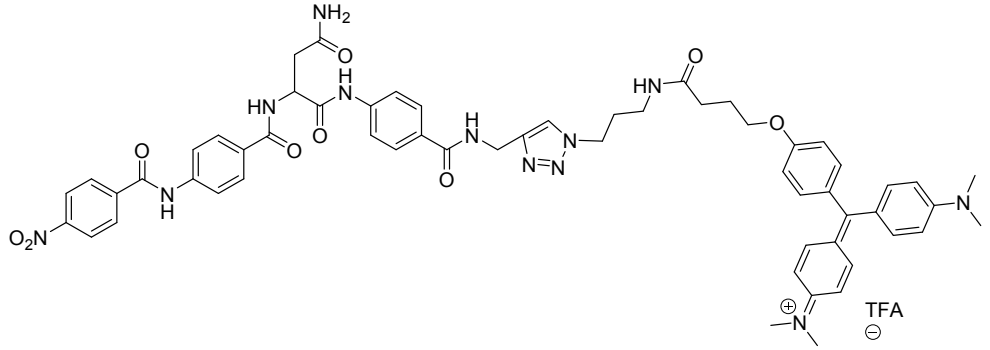
Carboxylic acid **87** (50 mg; 0.11 mmol), 3-azidopropan-1-amine (13 µL; 0.013 mmol) and HOAt (22 mg; 0.16 mmol) were mixed together in DMF (1.1 mL), to this dark solution EDC (23 mg; 0.13 mmol) followed by DiPEA (93 µL; 0.054 mmol) were added at r.t.. The reaction was stirred overnight. The purification was done by preparative RP-HPLC with a gradient 20-95% of acetonitrile in water, both phases buffered with 0.1% TFA, to obtain 58.6 mg of desired compound (0.094 mmol; y=88%).

^{1}H NMR (500 MHz, MeOD) δ 7.41 (d, *J* = 8.6 Hz, 4H), 7.35 (dd, *J* = 8.9, 1.0 Hz, 2H), 7.16 (dt, *J* = 4.5, 1.4 Hz, 2H), 7.03 (dd, *J* = 9.4, 1.0 Hz, 4H), 4.18 (t, *J* = 6.0 Hz, 2H), 3.33 (d, *J* = 6.8 Hz, 2H), 3.25 (t, *J* = 6.8 Hz, 2H), 2.41 (t, *J* = 7.3 Hz, 2H), 2.21 – 2.06 (m, 2H), 1.82 – 1.64 (m, 2H).

^{13}C NMR (126 MHz, MeOD) δ 178.4, 173.9, 164.3, 157.0, 140.5, 137.5, 131.9, 126.9, 114.6, 113.0, 67.6, 48.7, 39.4, 36.4, 31.9, 28.3, 24.9.

HRMS (ESI) calculated for $C_{30}H_{37}N_6O_2$ (M^+) 513.2973, found 513.2989.

N-(4-((4-(4-((3-(4-((4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)methyl)-1H-1,2,3-triazol-1-yl)propyl)amino)-4-oxobutoxy)phenyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium trifluoroacetate (86)



Alkyne **83** (4 mg; 0.0064 mmol) and azide **171** (3.6 mg; 0.005 mmol) were coupled together using the same experimental protocol described for the synthesis of conjugate **85**. The reaction was stirred at r.t. overnight and it was purified by using condition A (retention time: 19.13 min.) to obtain 2.2 mg of desired product (0.0021 mmol; y= 35%).

^{1}H NMR (700 MHz, MeOD) δ 8.39 – 8.35 (m, 2H), 8.17 – 8.11 (m, 2H), 7.92 – 7.88 (m, 3H), 7.88 – 7.84 (m, 2H), 7.80 – 7.77 (m, 2H), 7.71 – 7.66 (m, 2H), 7.37 (d, *J* = 8.9 Hz, 4H), 7.34 – 7.30 (m, 2H), 7.18 – 7.12 (m, 2H), 7.00 (d, *J* = 9.4 Hz, 4H), 5.04 (t, *J* = 6.8 Hz, 1H), 4.59 (s, 2H), 4.38 (t, *J* = 6.9 Hz, 2H), 4.17 (t, *J* = 6.2 Hz, 2H), 3.29 (s, 12H), 3.22 (t, *J* = 6.9 Hz, 2H), 2.93 (dd, *J* = 15.5, 6.5 Hz, 1H), 2.84 (dd, *J* = 15.5, 7.1 Hz, 1H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.13 (dt, *J* = 13.7, 6.8 Hz, 2H), 2.11 – 2.06 (m, 2H).

^{13}C NMR (176 MHz, MeOD) δ 179.8, 175.6, 175.1, 172.0, 169.6, 169.5, 166.9, 165.8, 158.5, 151.4, 146.7, 143.4, 143.2, 142.0, 139.0, 133.4, 130.9, 130.6, 130.3, 129.7, 129.4, 128.4, 124.9, 124.7, 121.4, 120.7, 116.2, 114.5, 69.0, 53.3, 41.0, 37.9, 37.6, 36.3, 33.4, 31.1, 26.3.

HRMS (ESI) calculated for $C_{58}H_{61}N_{12}O_9$ (M^+) 1069.4679, found 1069.4680.

2 PHYSICOCHEMICAL PROPERTY ANALYSIS

Table S 13. Selection of marketed antibiotics targeting Gram-negative bacteria, and their calculated physicochemical properties.

Gram-negative antibiotics	MW	LogP	LogD (7.4)	PSA (pH 7.4)	Sum of H bond donors and acceptors
Trimethoprim	290,32	1,28	1,1	105,5	14
*Rifampicin	822,9	3,62	2,87	220,2	31
Plazomycin	592,6	-6,1	-14,22	269,2	39
Streptomycin	581,5	-6,65	-12,13	331,43	47
Sulfamethoxazole	253,2	0,79	0	98,22	9
Chloramphenicol	323,1	0,88	0,86	112,7	20
Fosfomycin	138,1	-0,74	-3,18	79,9	10
Doxycycline	444,4	-0,66	-3,57	181,6	24
Eravacycline	558,5	0,23	-3,51	193,7	25
Tigecycline	585,6	0	-3,41	205,76	27
Ciprofloxacine	331,3	1,55	-0,85	72,88	11
Levofloxacin	361,3	1,56	-0,47	73,32	12
Nalidixic acid	232,2	1,24	-0,45	70,5	9
Gatifloxacin	375,4	1,81	-0,61	82,11	13
Benzylpenicillin	334,3	1,08	-2,29	86,7	12
Amoxicillin	365,4	-0,04	-2,67	132,9	18
Imipenem	299,3	-2,15	-3,8	113,72	16

*lack of activity against *P. aeruginosa, A. baumannii and* and *Enterobacteriaceae* species. MW= molecular weight, PSA= polar surface area.

Table S 14. Selection of marketed antibiotics targeting Gram-positive bacteria, and their calculated physicochemical properties.

Gram-positive antibiotics	MW	LogP	LogD (7.4)	PSA (pH 7.4)	Sum of H bond donors and acceptors
Vancomycin	1449,2	-1,18	-4,85	530,5	73
Dalfopristin	690,8	1,58	1,41	176,4	18
Mupirocin	500,6	2,45	-0,07	146,1	20
Fusidic acid	516,7	4,42	1,58	104,1	13
Novobiocin	612,6	3,26	1,36	196,1	24
Quinupristin	1022,2	2,95	2,07	231,2	27
Linezolid	337,3	0,64	0,64	71,1	9
Tedizolid	370,3	2,12	2,12	106,26	10
Lincomycin	406,5	-0,32	-0,99	122,49	20
Clindamycin	424,9	1,04	0,65	102,2	17
Erythromycin	733,9	2,6	1,57	193,9	30
Azithromycin	748,9	2,44	-1,23	180,1	29
Solithromycin	845	5,76	5,3	197,8	23

MW= molecular weight, PSA= polar surface area.

Table S 45. Calculated physicochemical properties for two selected cystobactamids.

cystobactamids	MW	LogP	LogD (7.4)	PSA (pH 7.4)	Sum of H bond donors and acceptors
CN-DM-861 (**22**)	811,8	4,18	1,05	279,14	30
Cys 861-2 (**2**)	861	4,19	1,05	307,7	36

MW= molecular weight, PSA= polar surface area.

For structure property prediction and calculation of antibiotics, the calculator plugins of ChemAxon were used with MarvinSketch. [17]

For the determination of globularity and rigidity, a so-called eNTRy analysis using an online tool provide under the web address www.entry-way.org (accessed on 15.07.2018) was used. According to the analysis, cystobactamid **22** had no amine, high flexibility due to 15 rotatable bonds (favorable for Gram-negative entry is a low flexibility with < 5 rotatable bonds) and a low globularity of 0.018 (favorable for Gram-negative entry is a low globularity of < 0.25).

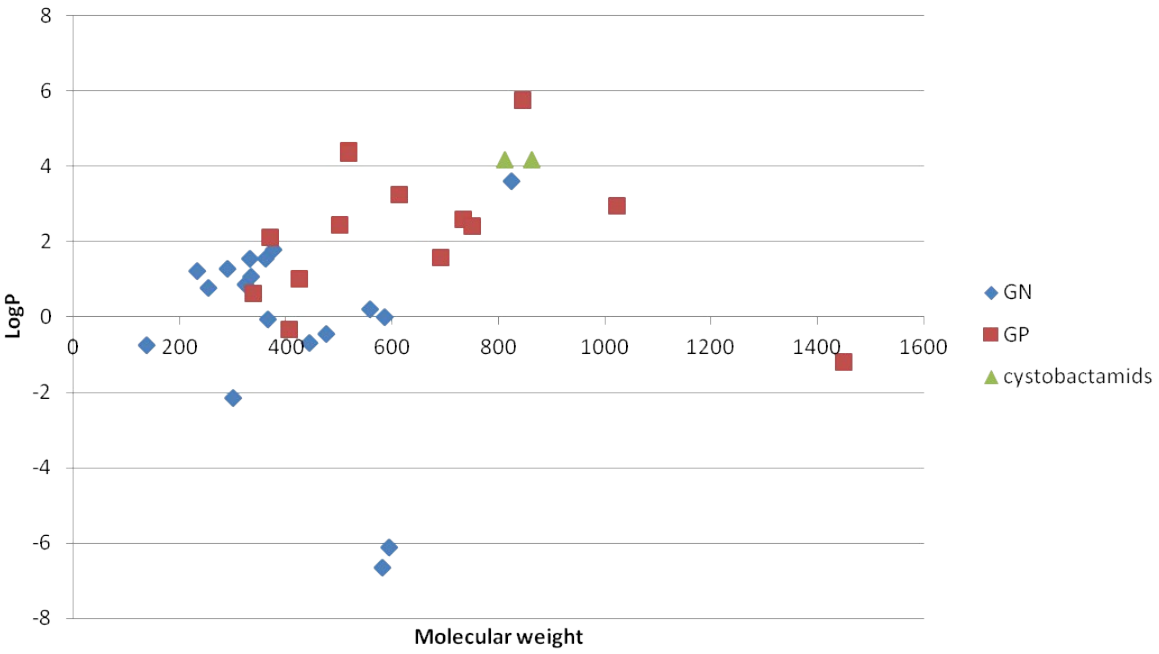
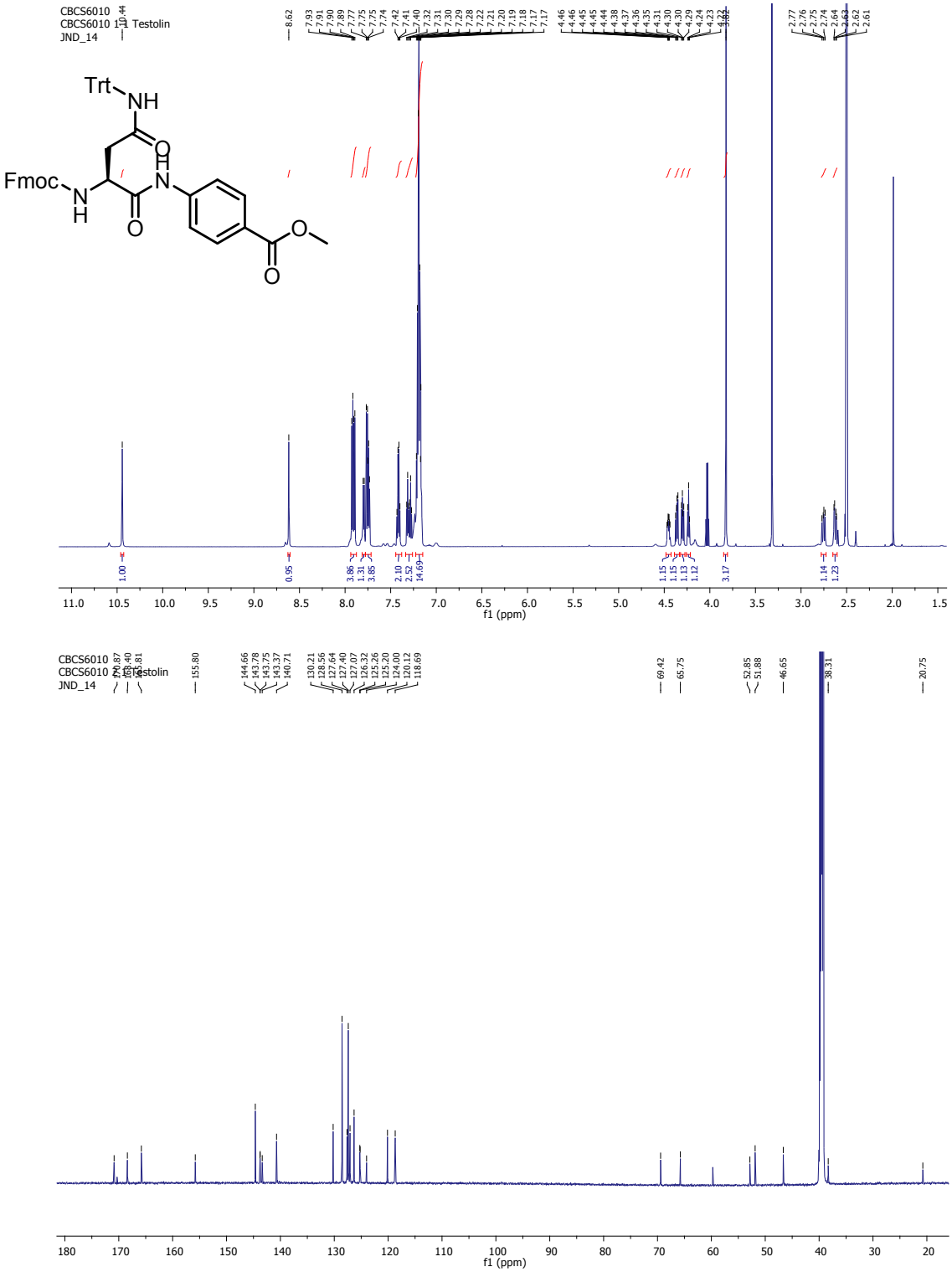


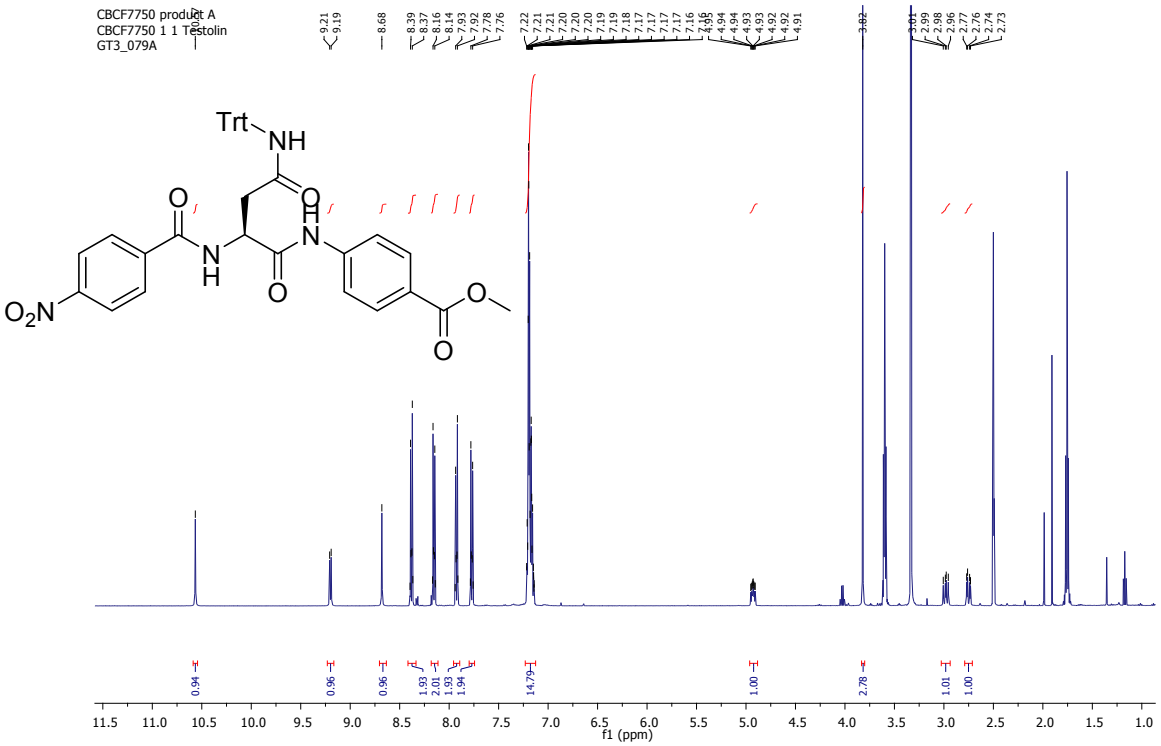
Figure S 114. Physicochemical properties of cystobactamids and selected marketed antibiotics. The molecular weight of antibiotics is plotted vs LogP. Cystobactamids are more similar to the GP antibiotics. GN = Gram-negative antibiotics, GP = Gram-positive only antibiotics. Cystobactamids **2** and **22** were used in the analysis (see Tables S8-S10).

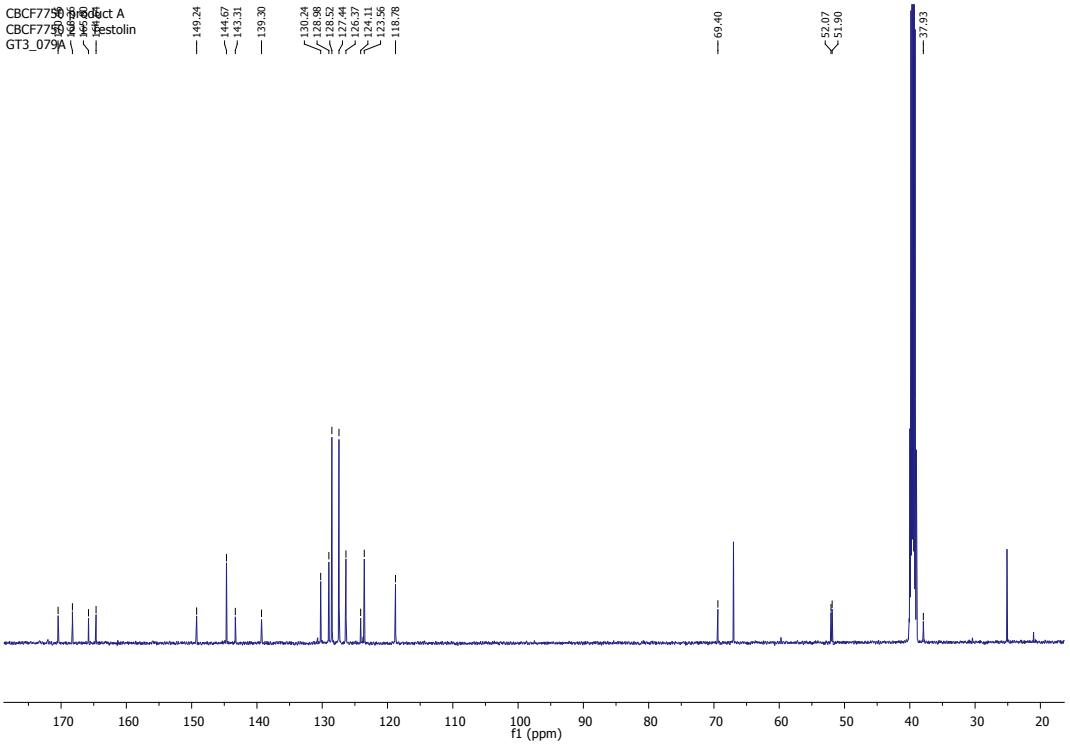
3 NMR SPECTRA

Methyl (S)-4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoate

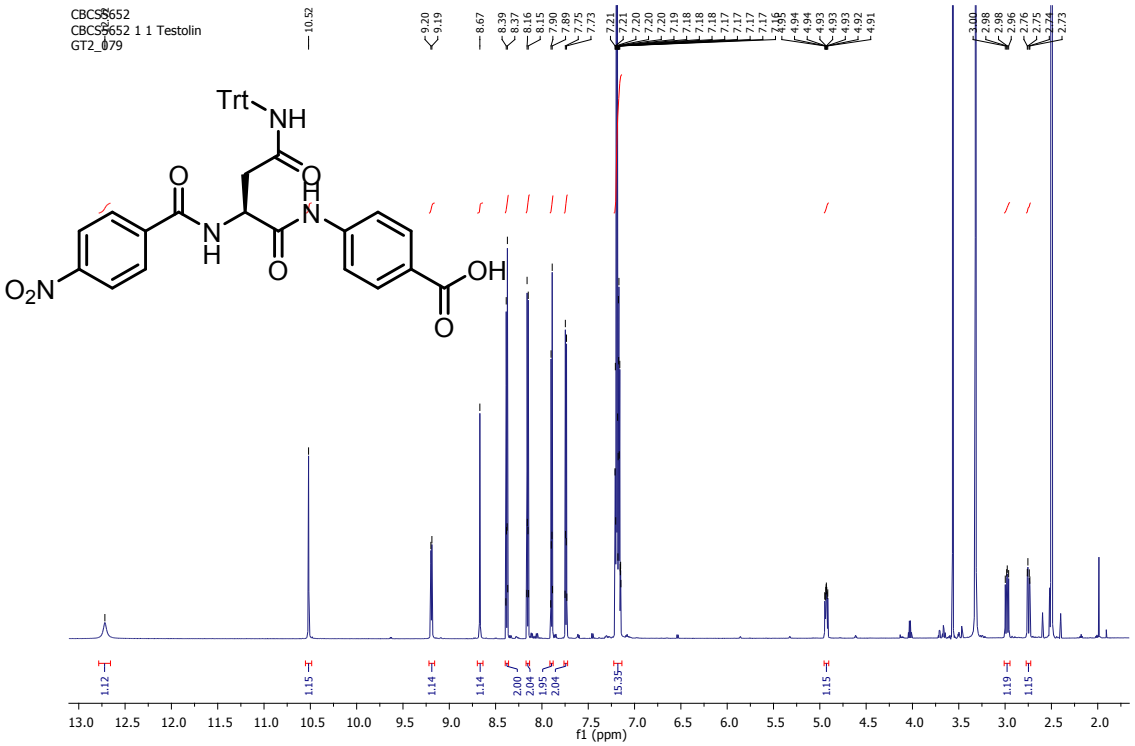


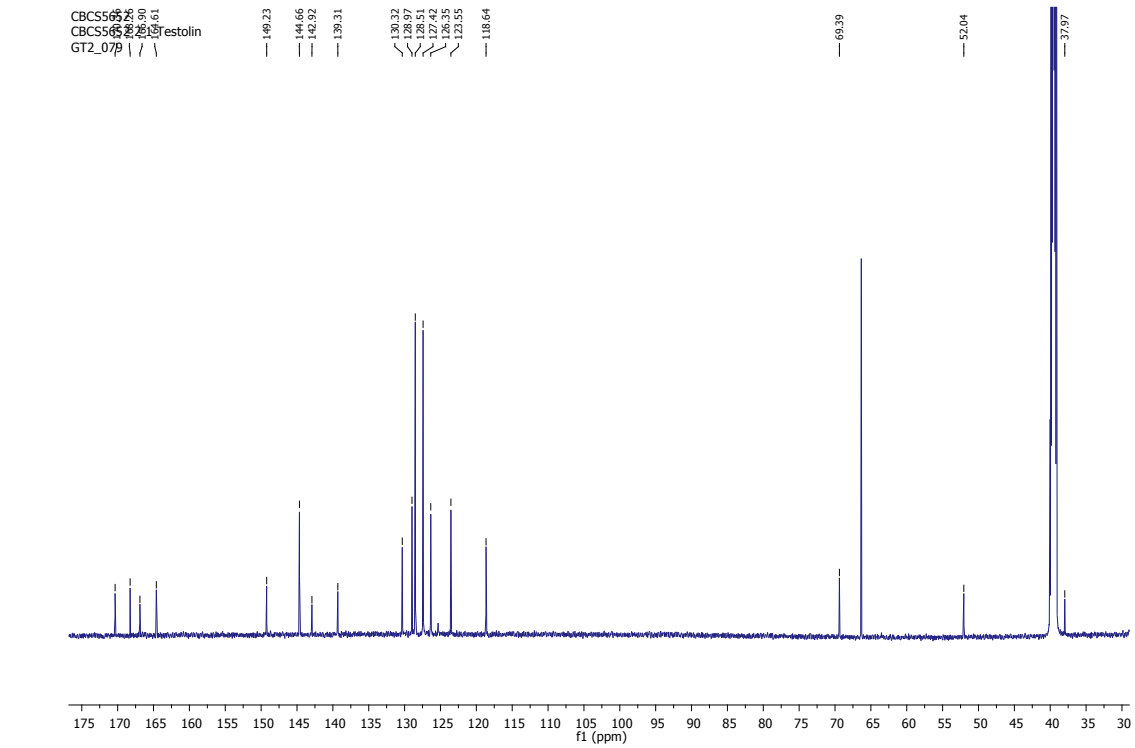
Methyl (S)-4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoate



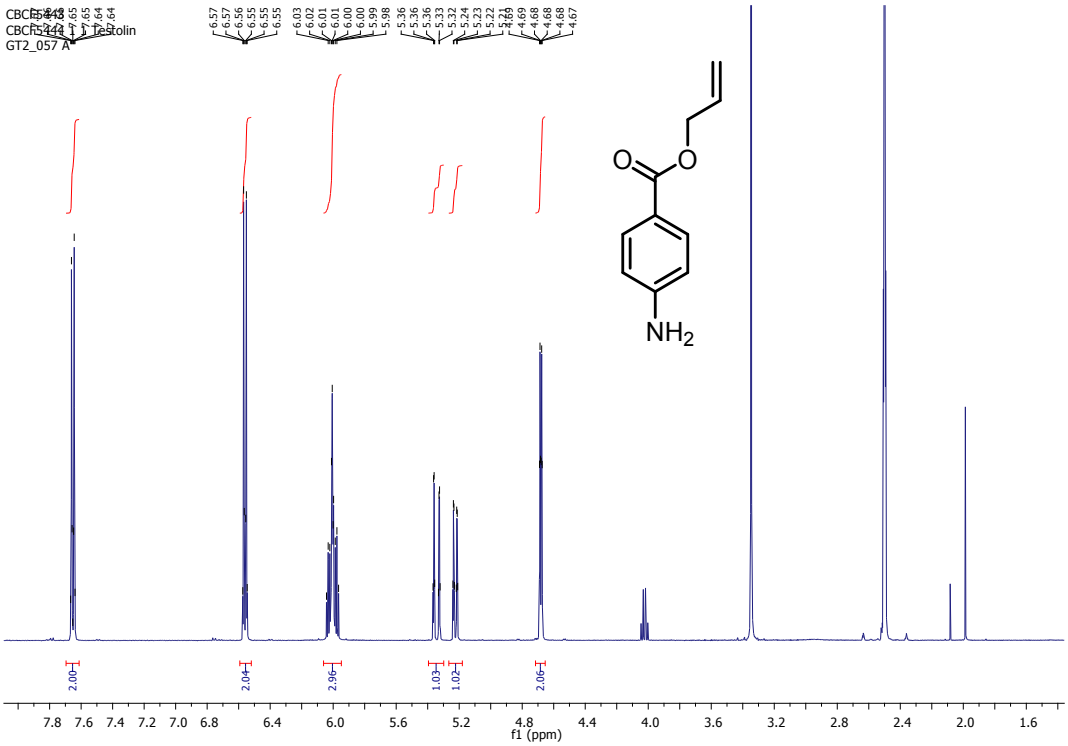


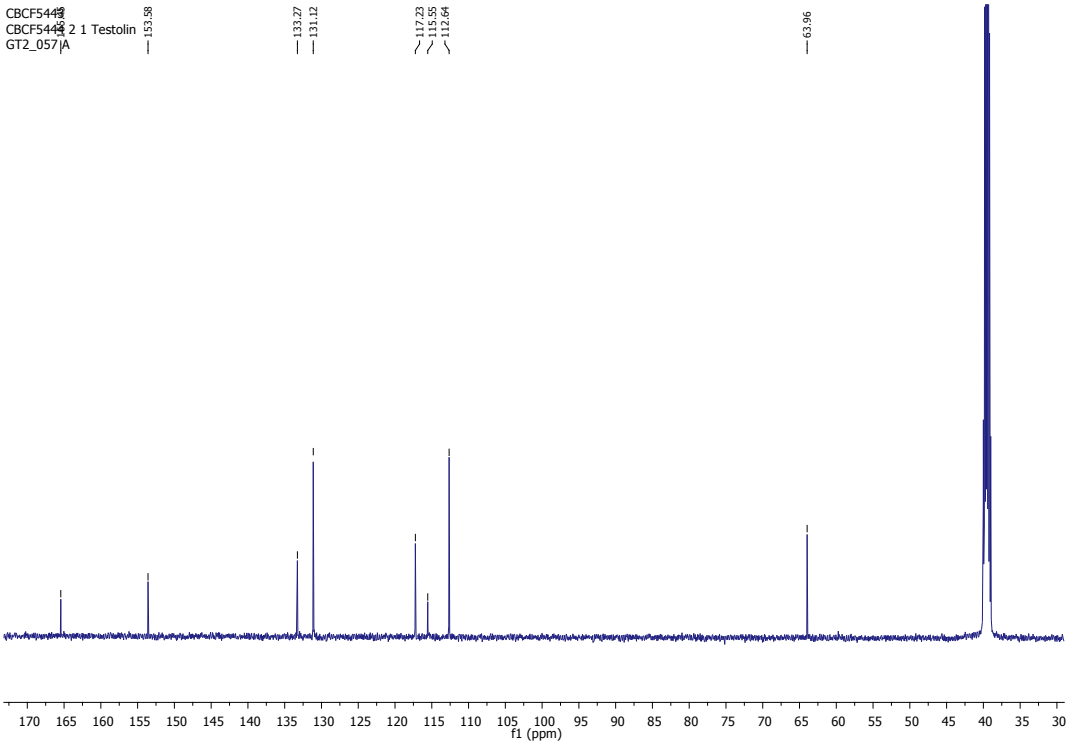
(S)-4-(2-(4-Nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzoic acid



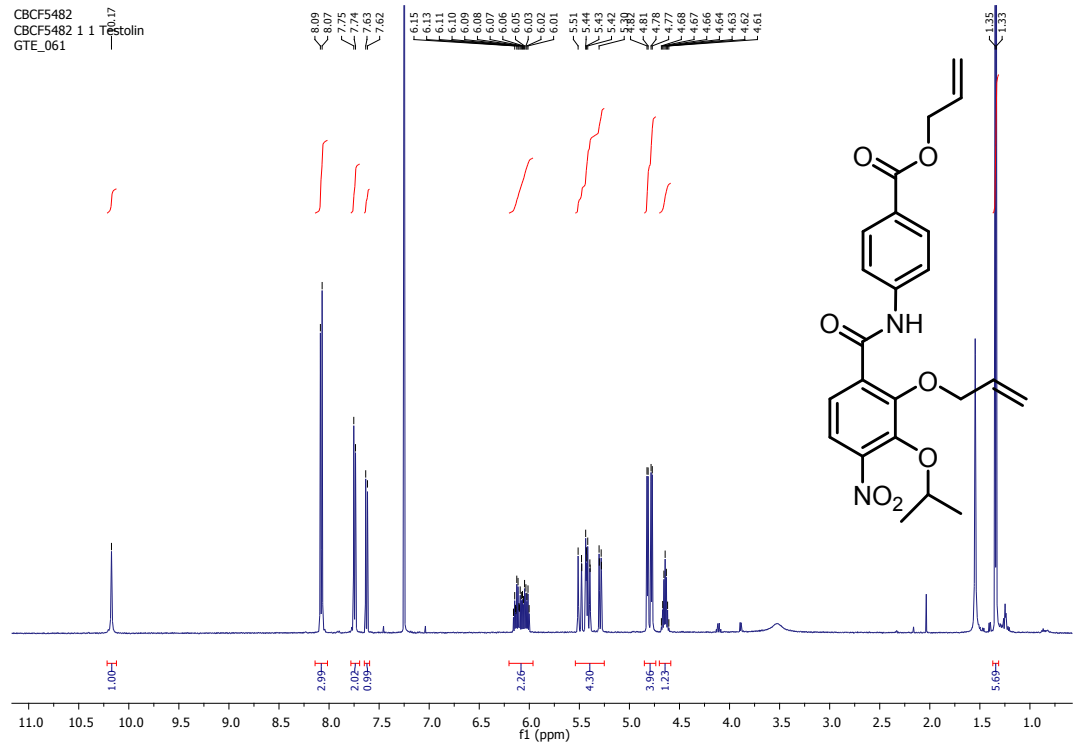


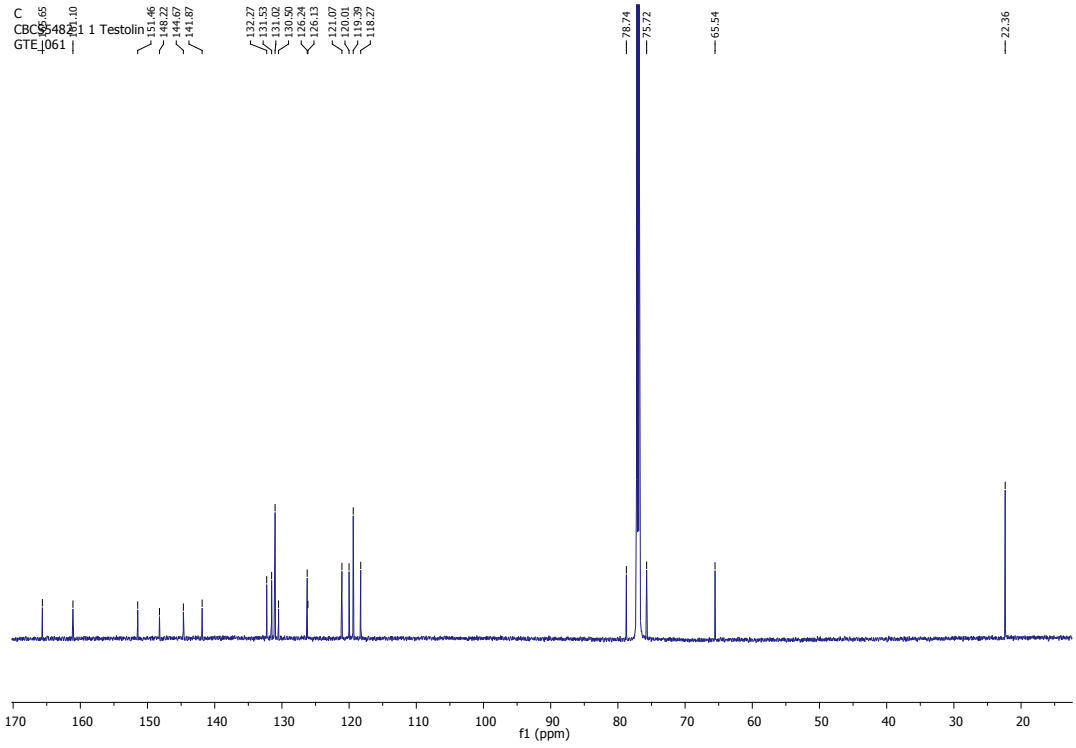
Allyl 4-aminobenzoate



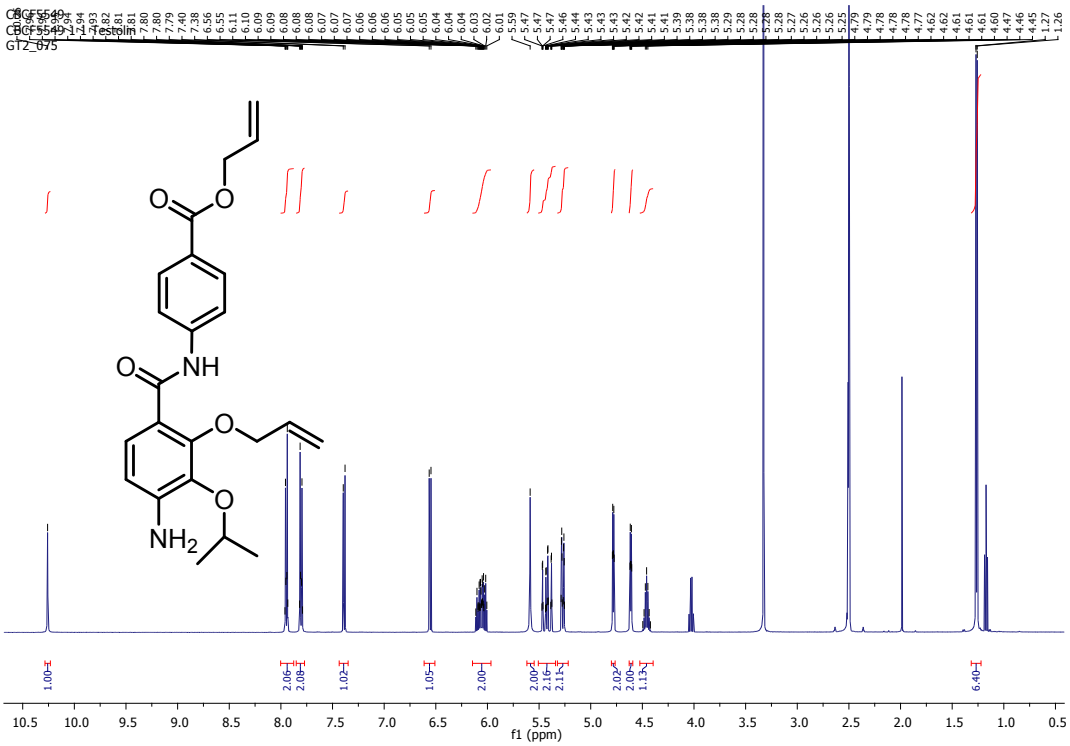


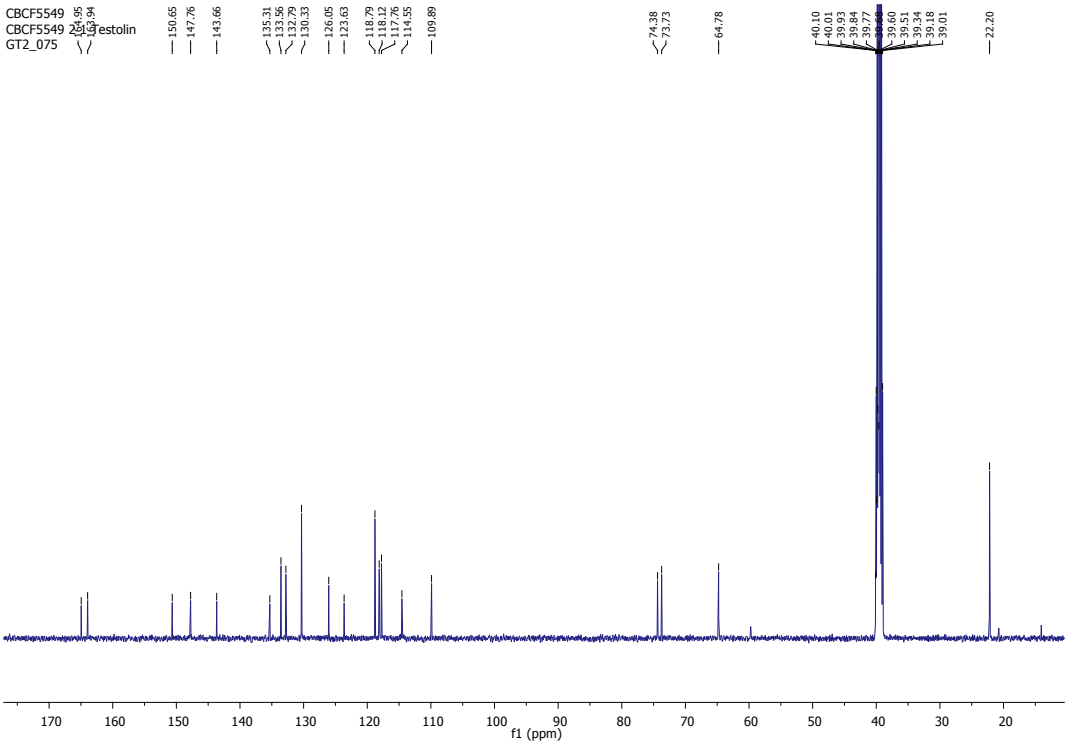
Allyl 4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate



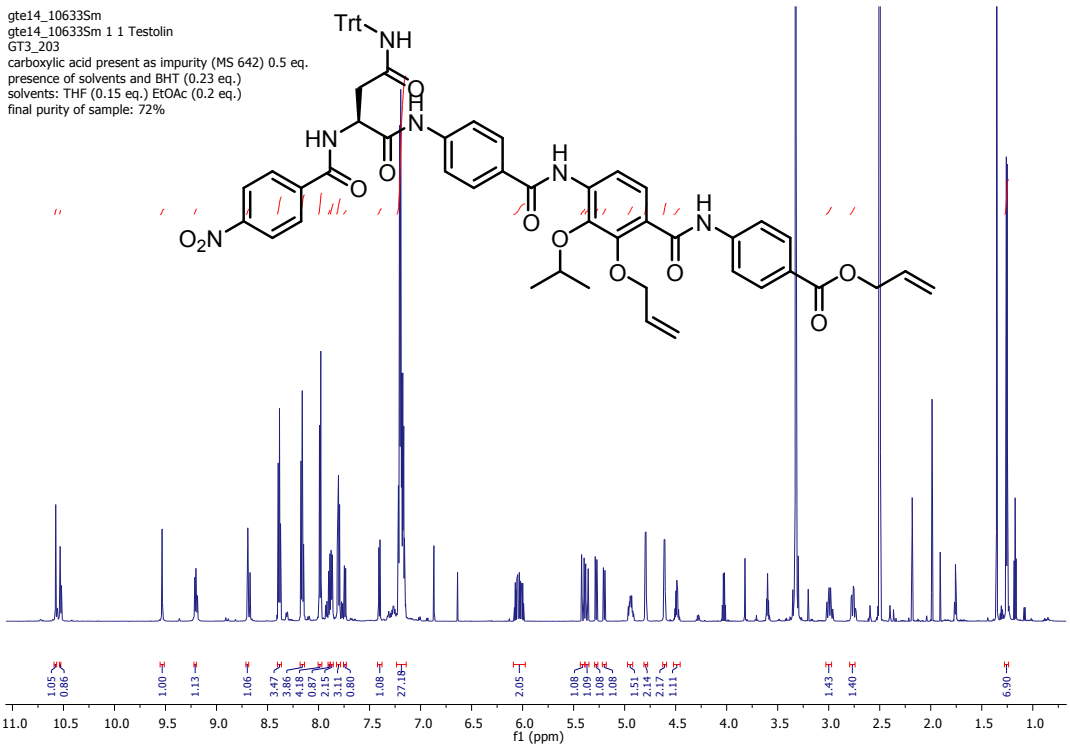


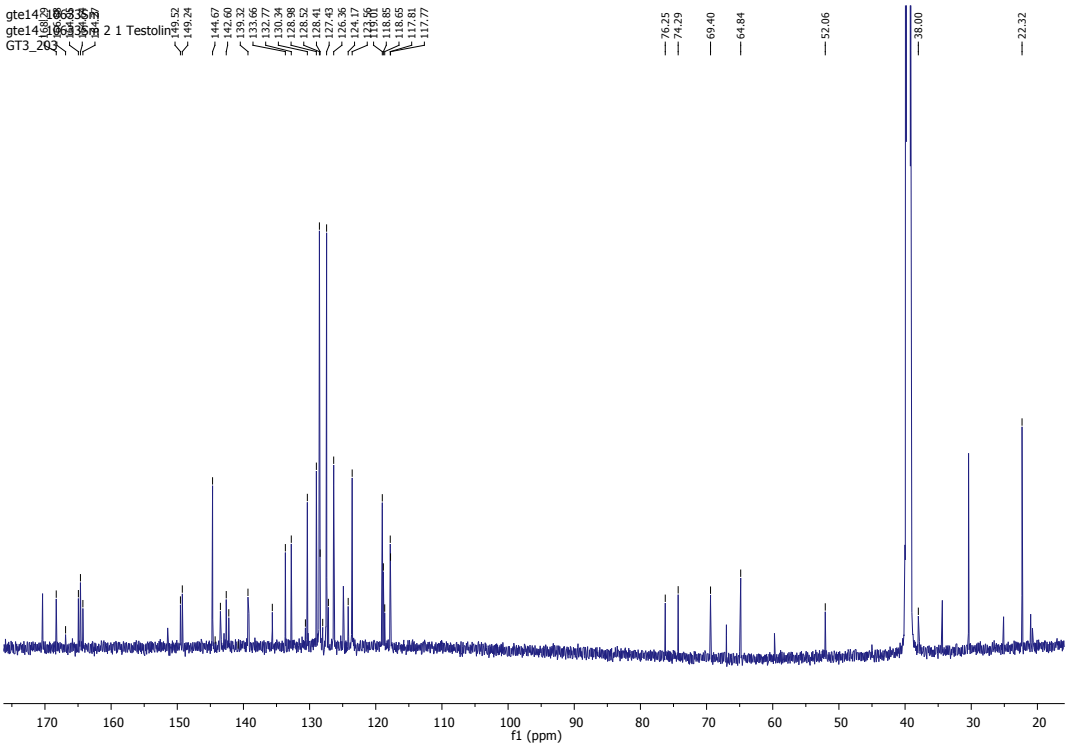
Allyl 4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)benzoate



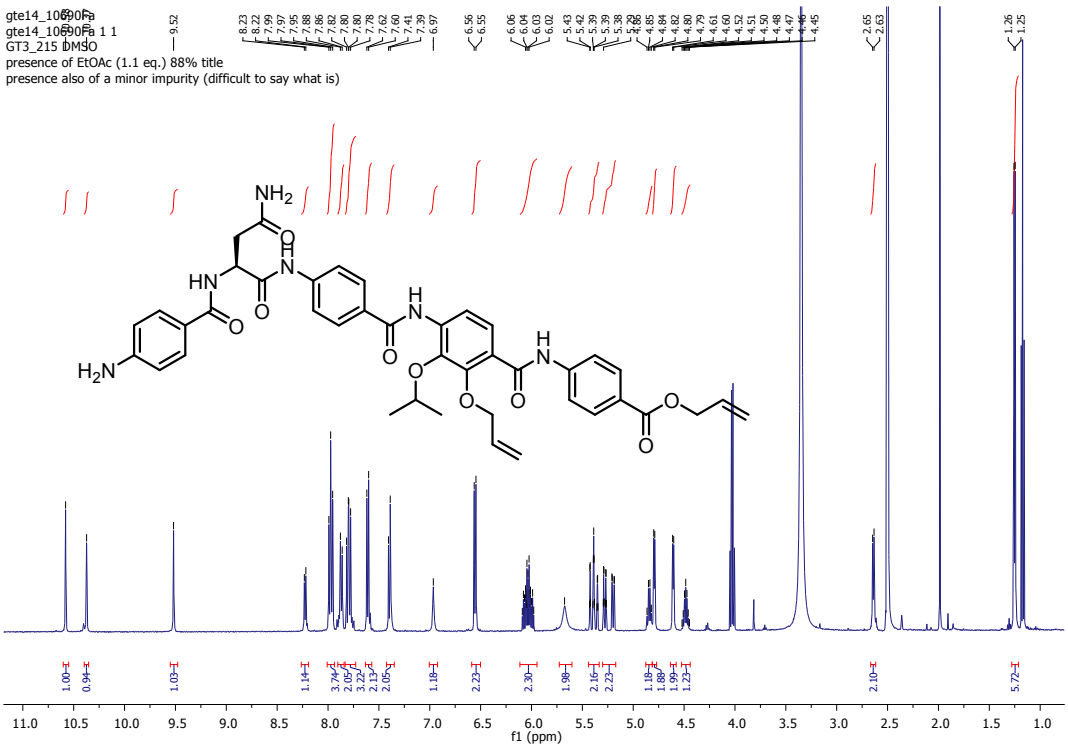


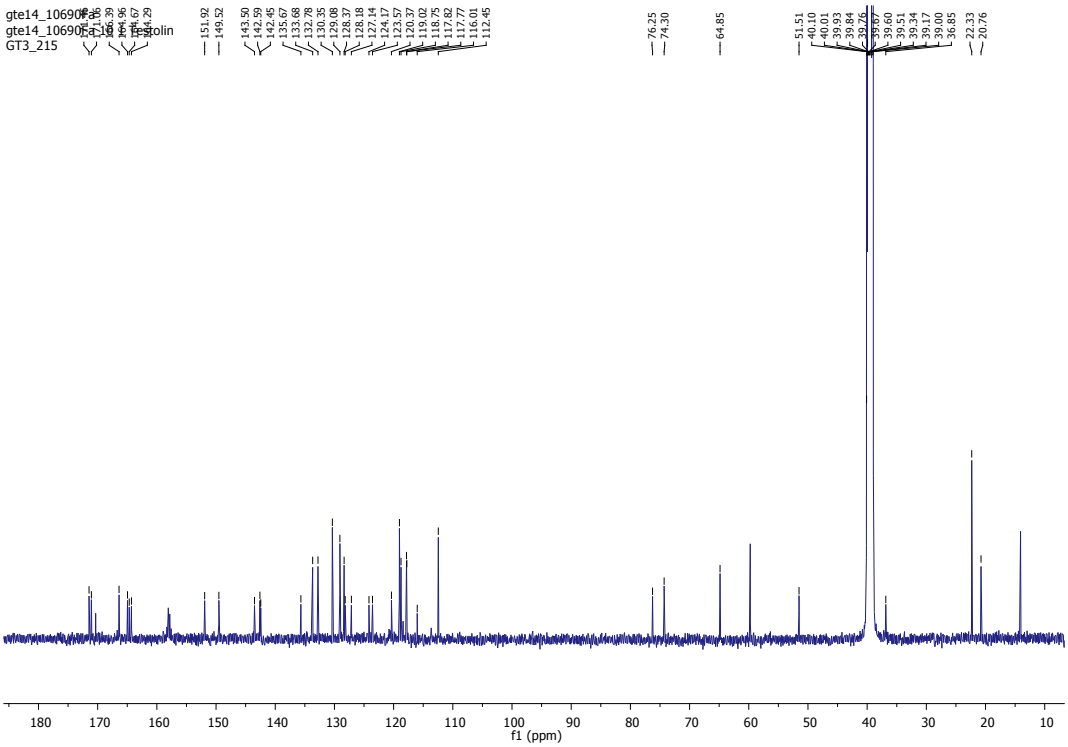
Allyl (S)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)benzoate



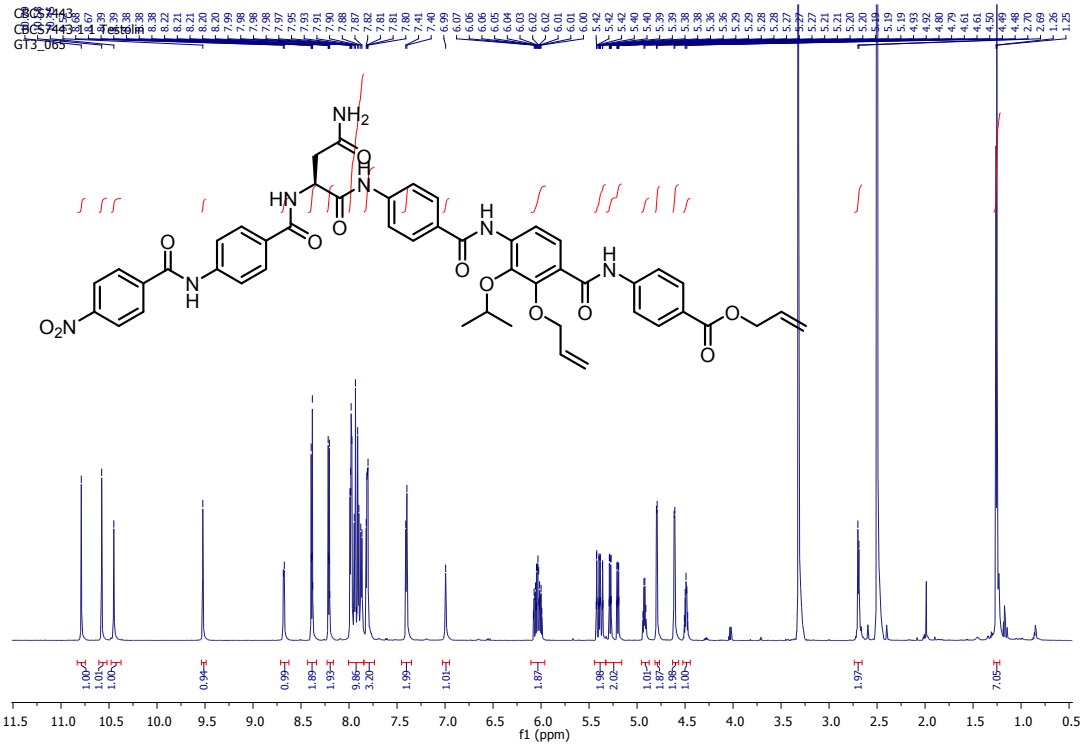


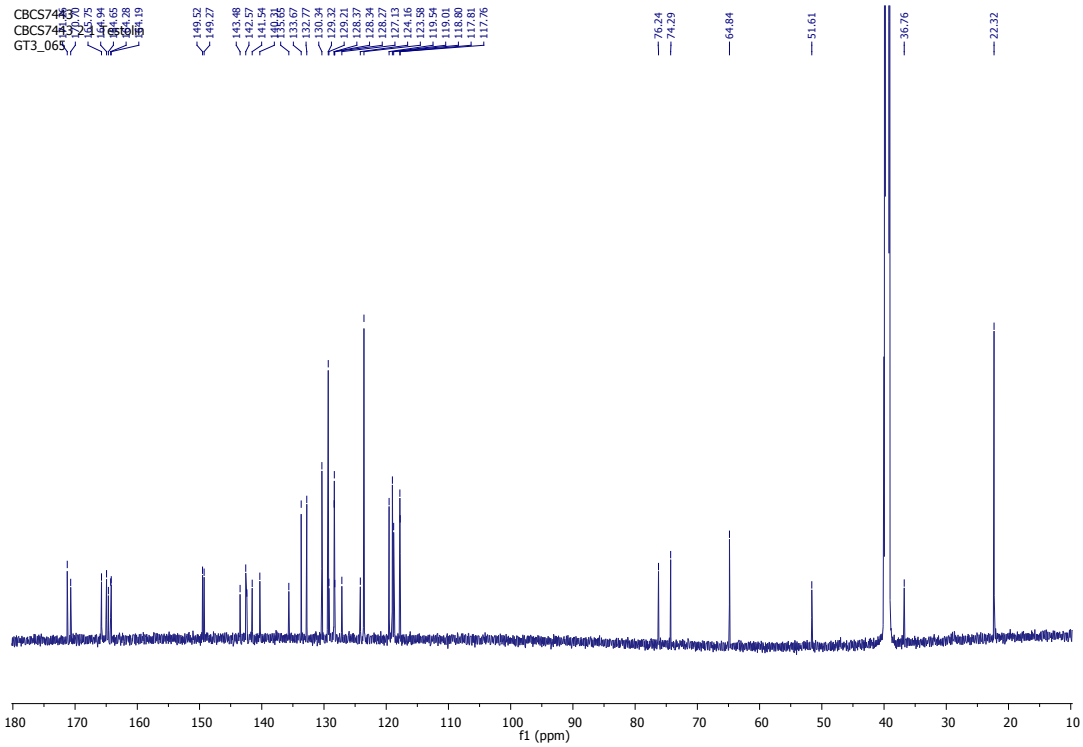
Allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate



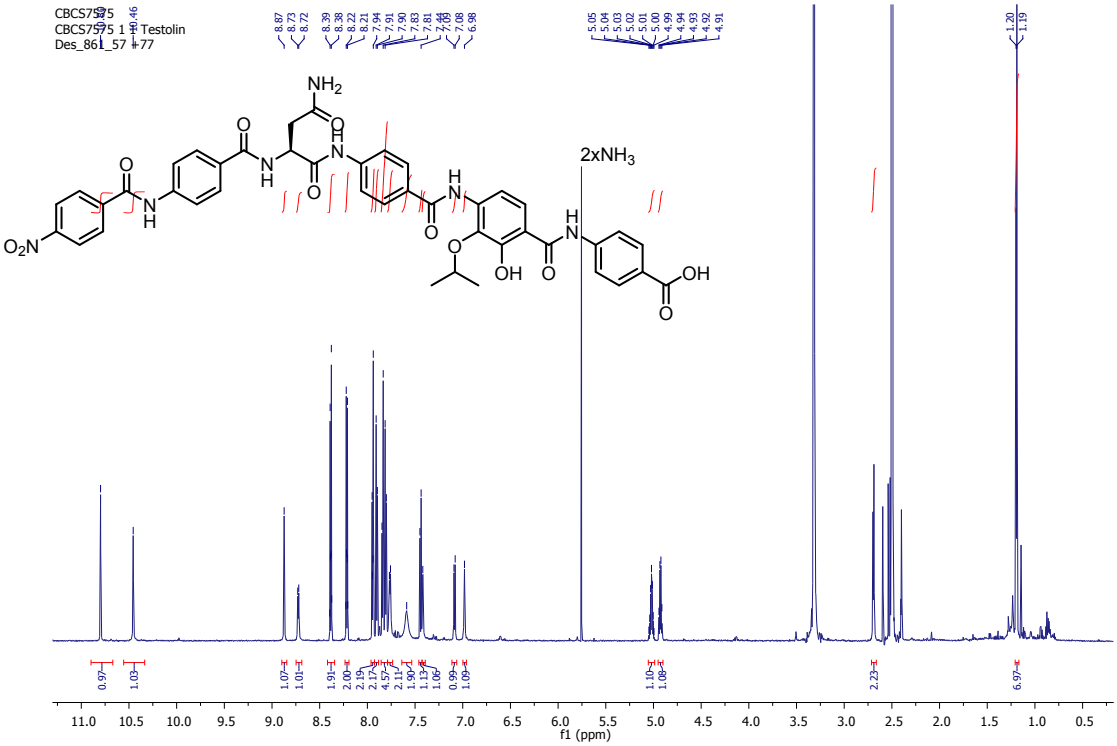


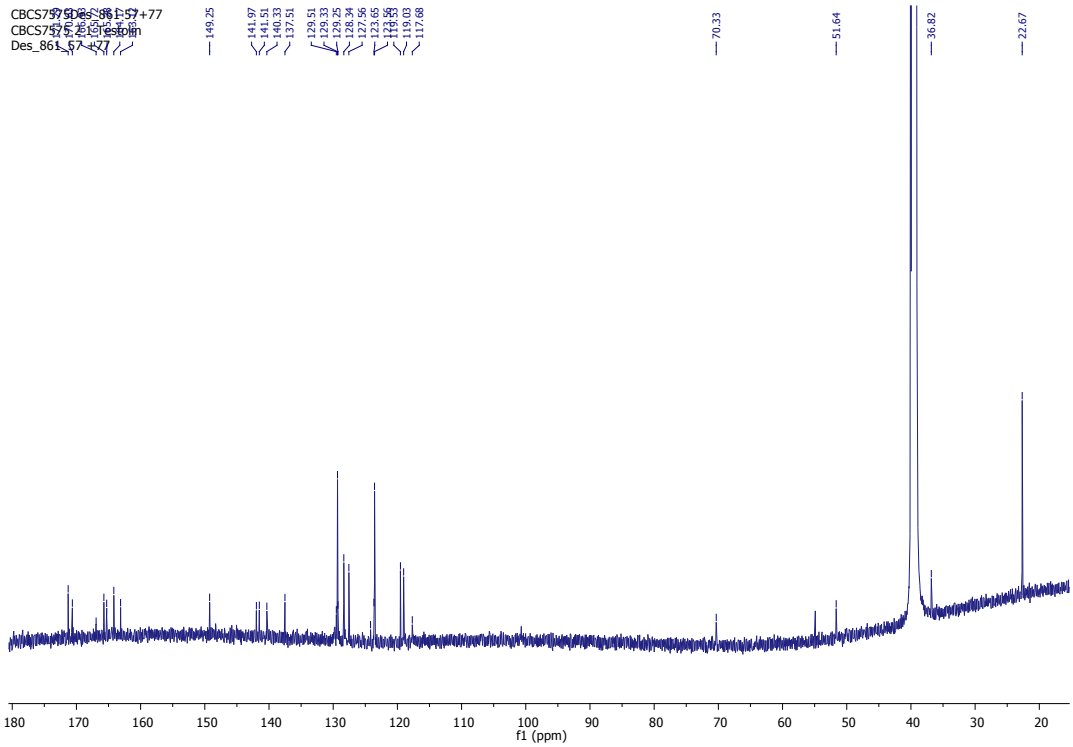
Allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate



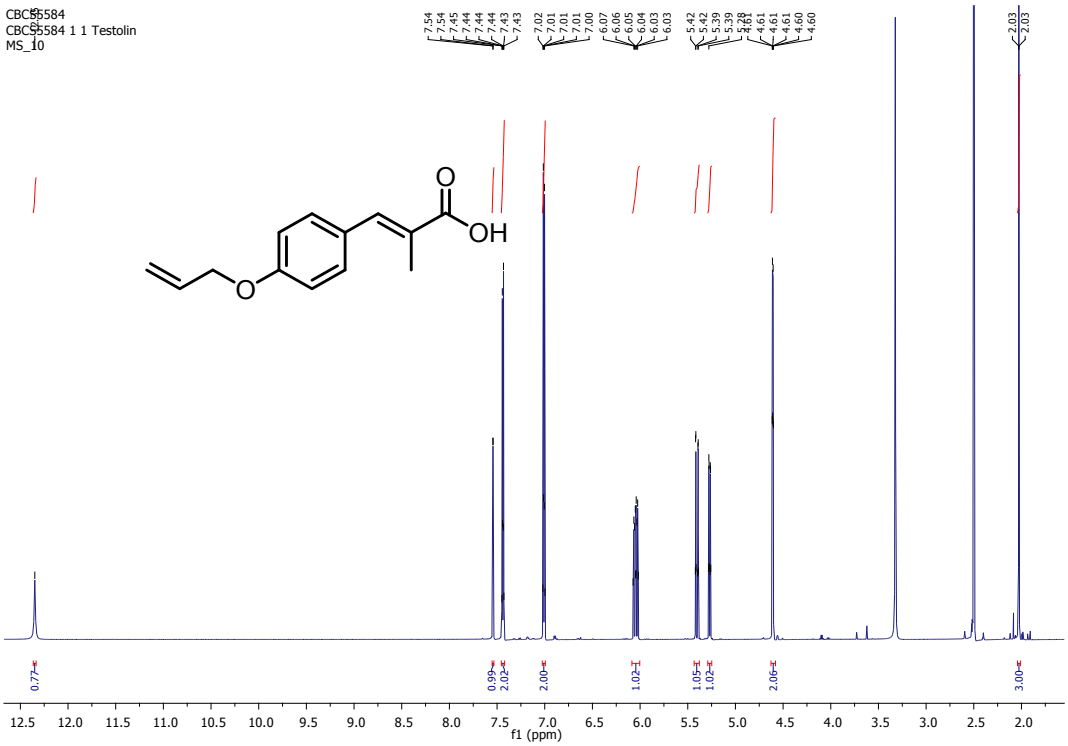


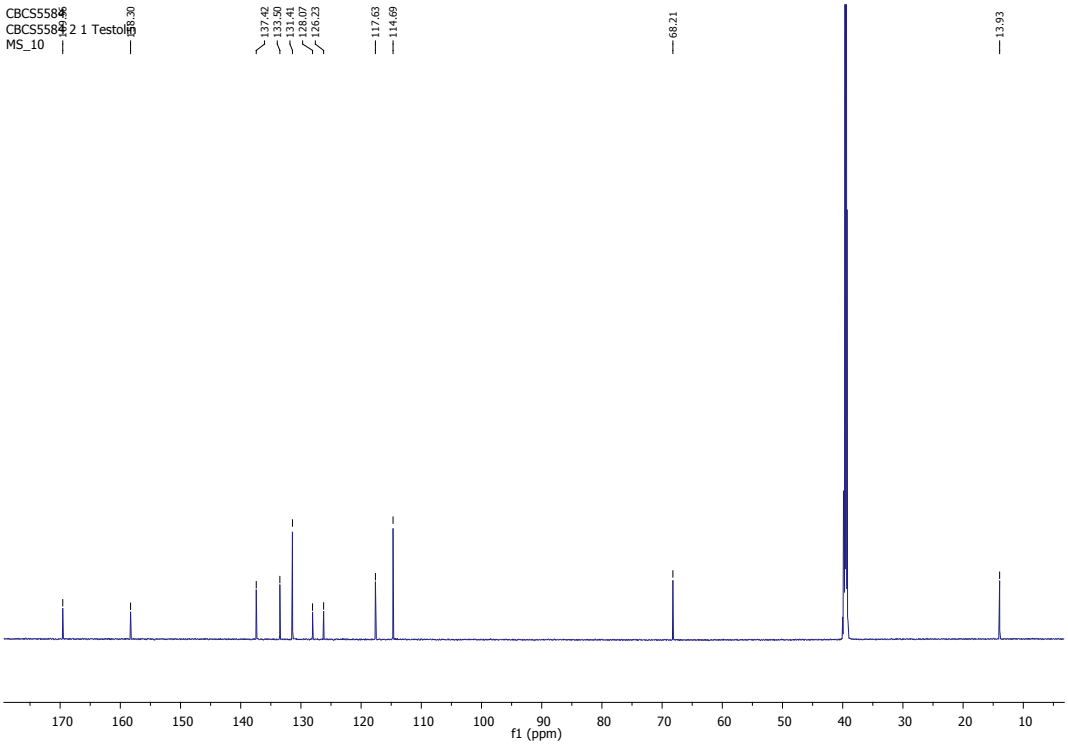
(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



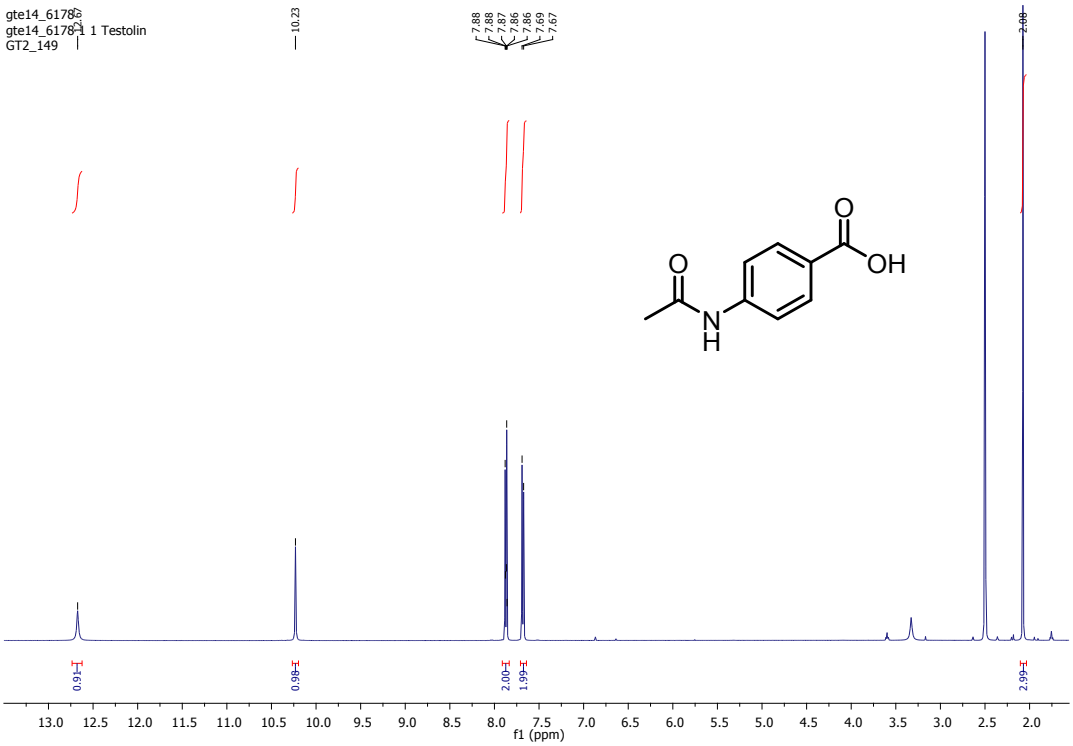


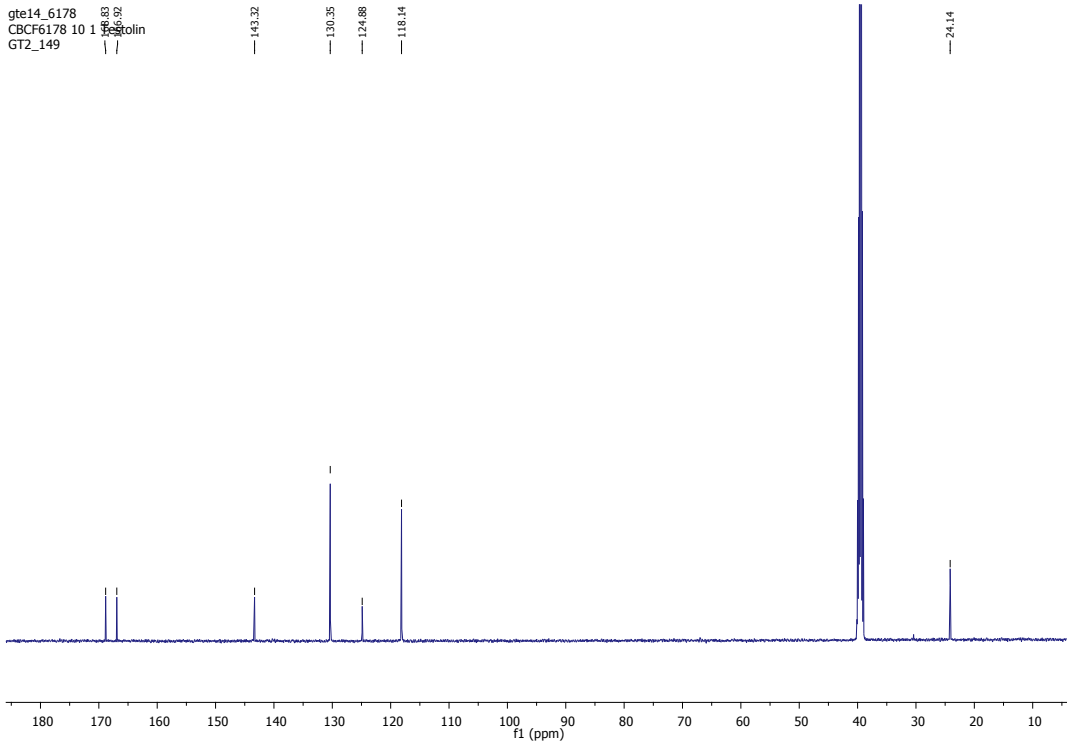
(E)-3-(4-(Allyloxy)phenyl)-2-methylacrylic acid



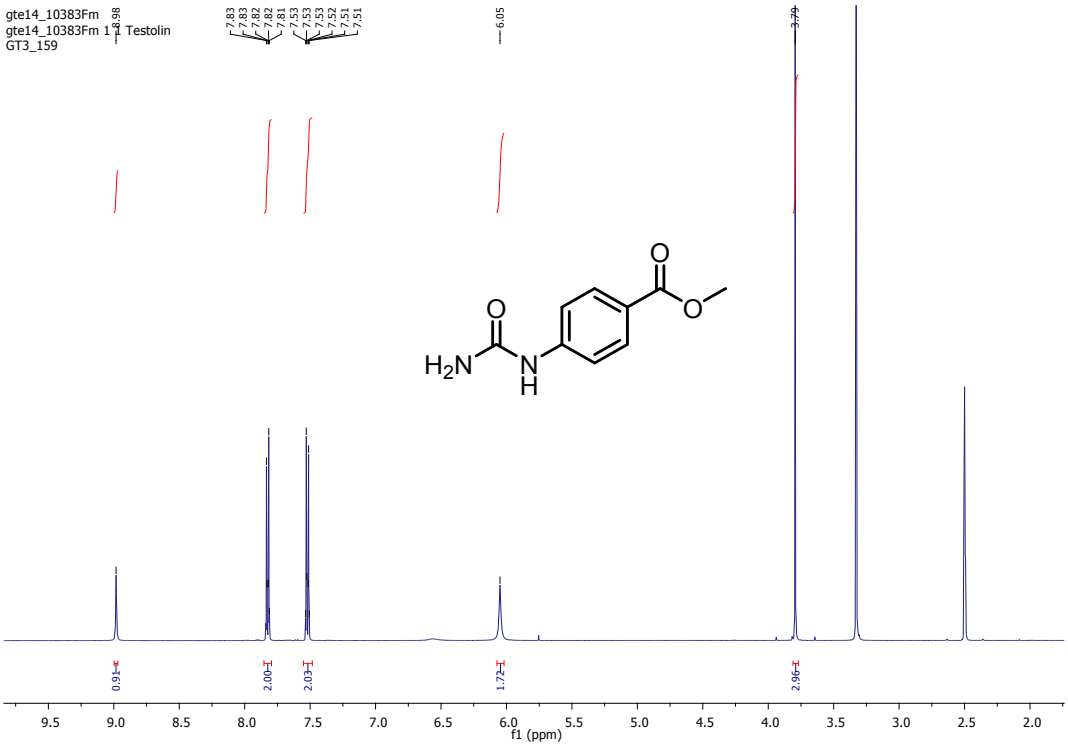


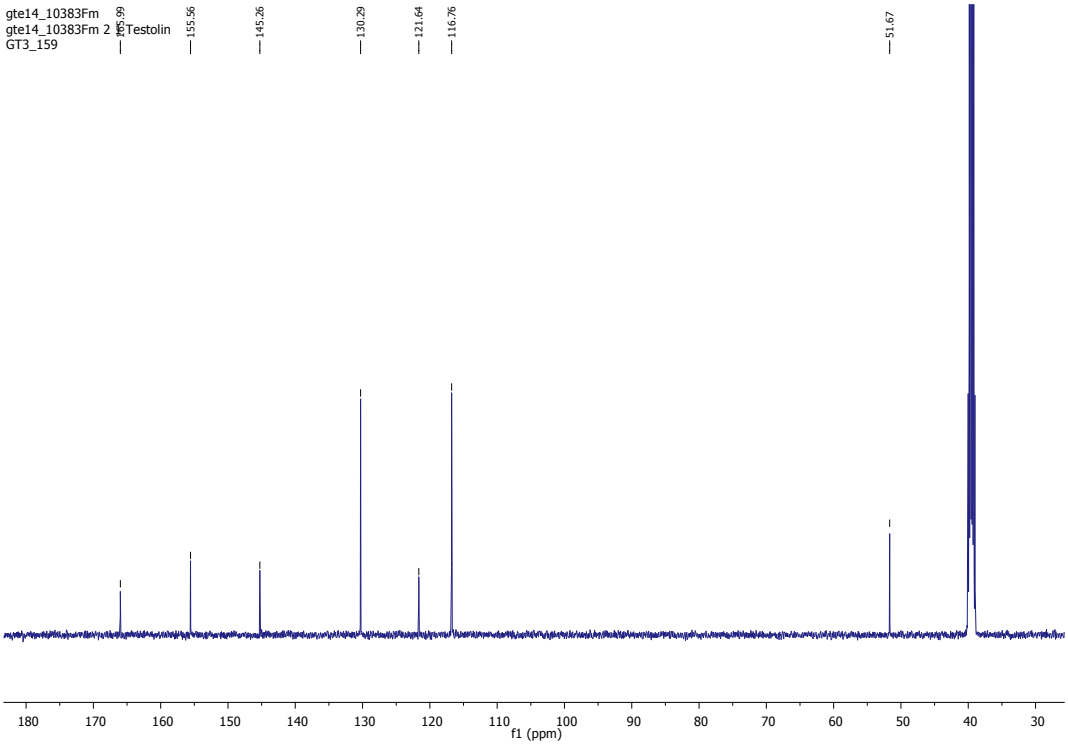
4-Acetamidobenzoic acid



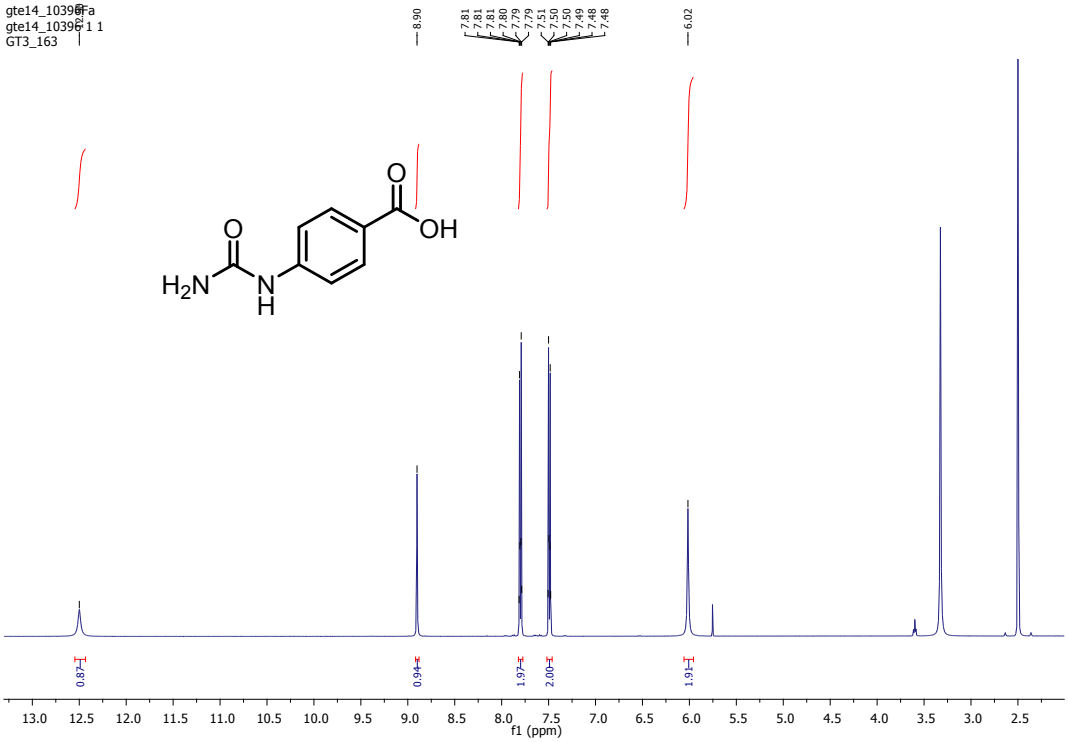


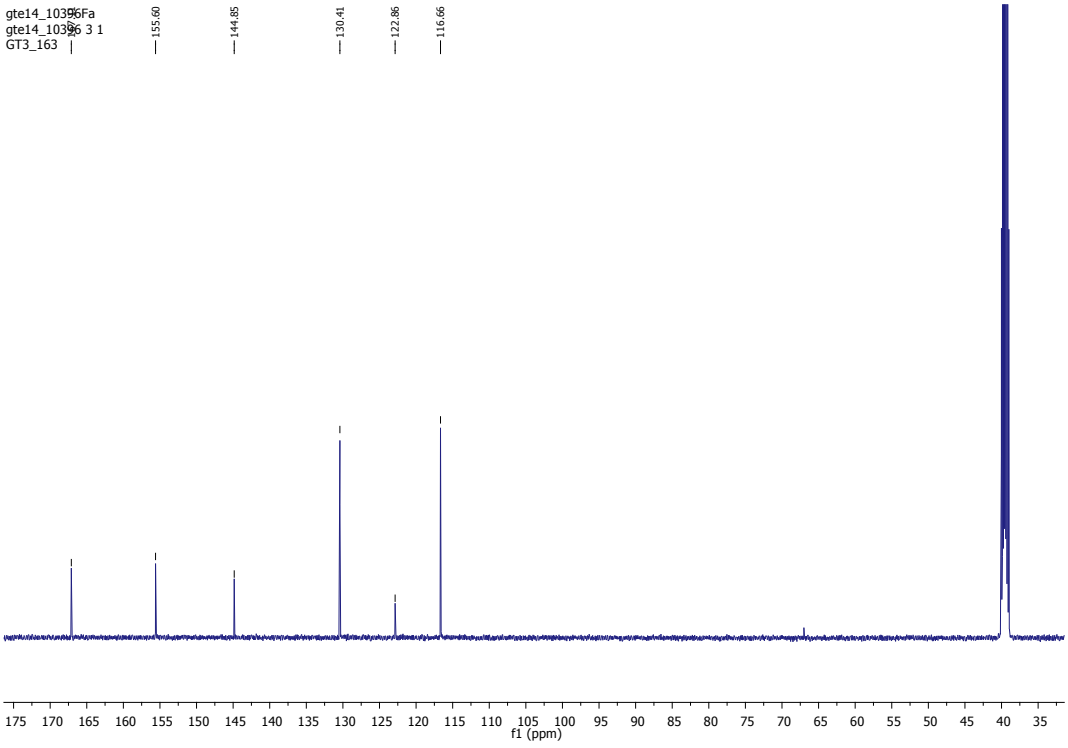
Methyl 4-ureidobenzoate



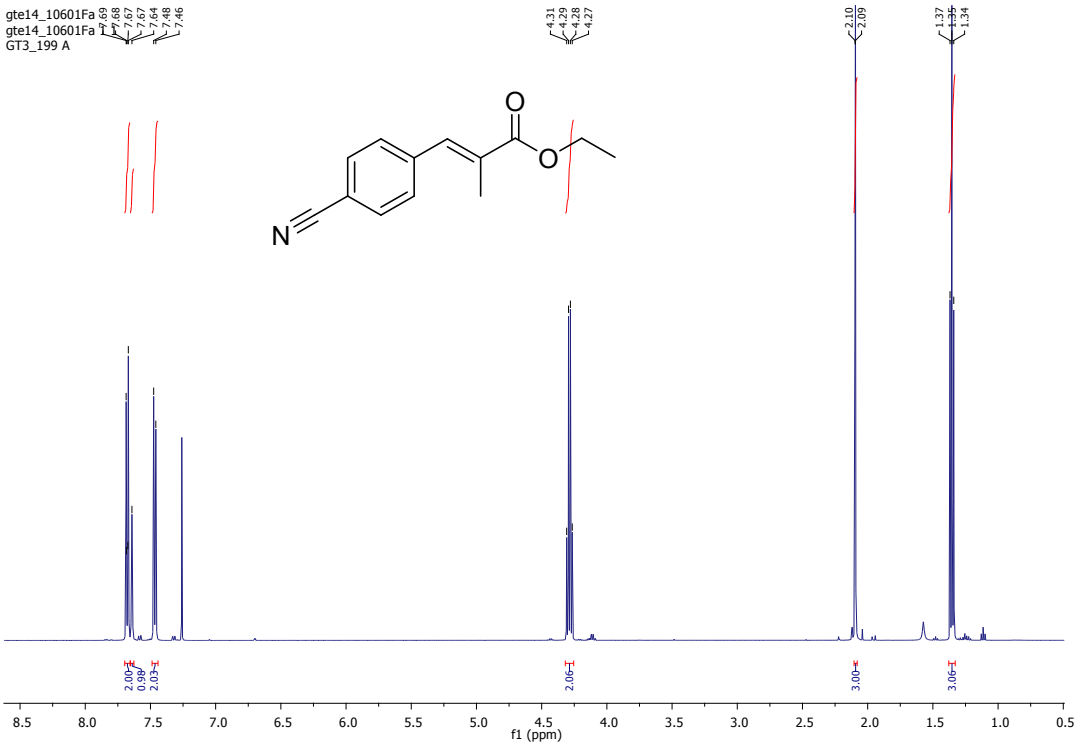


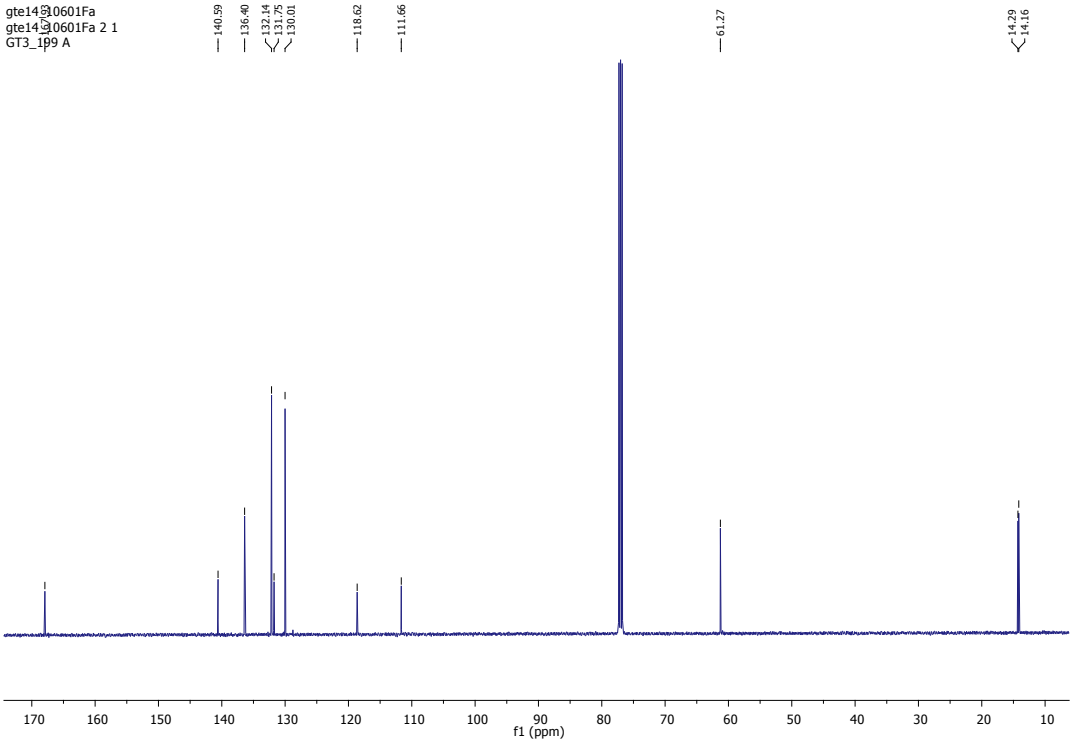
4-Ureidobenzoic acid



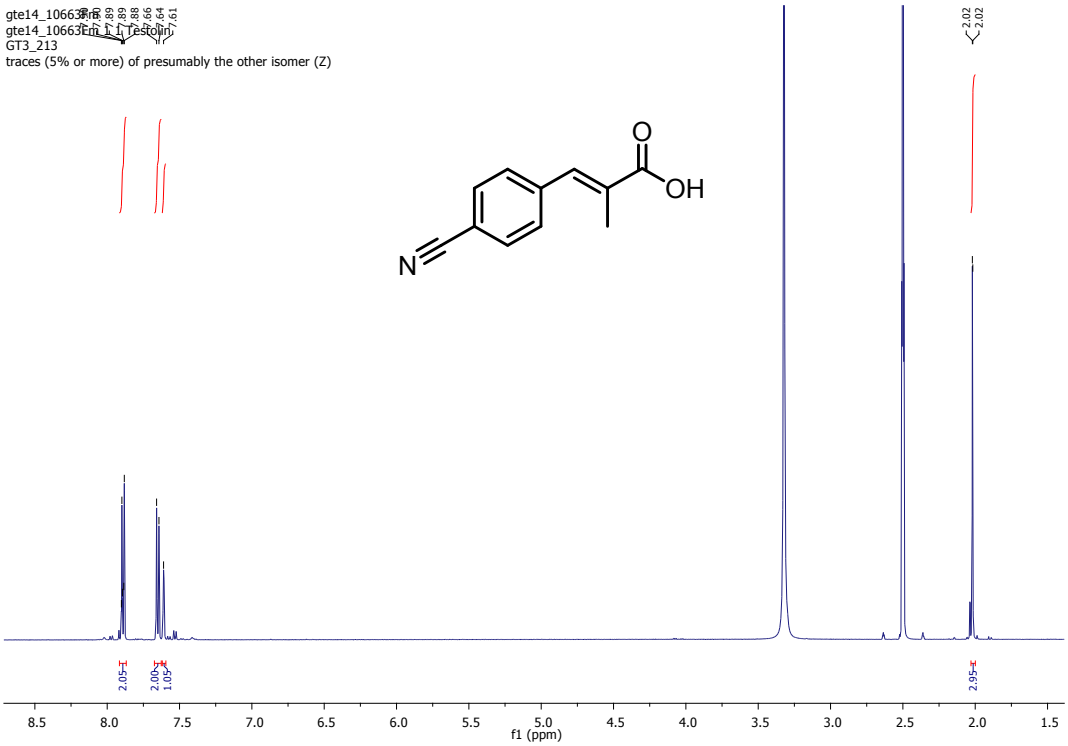


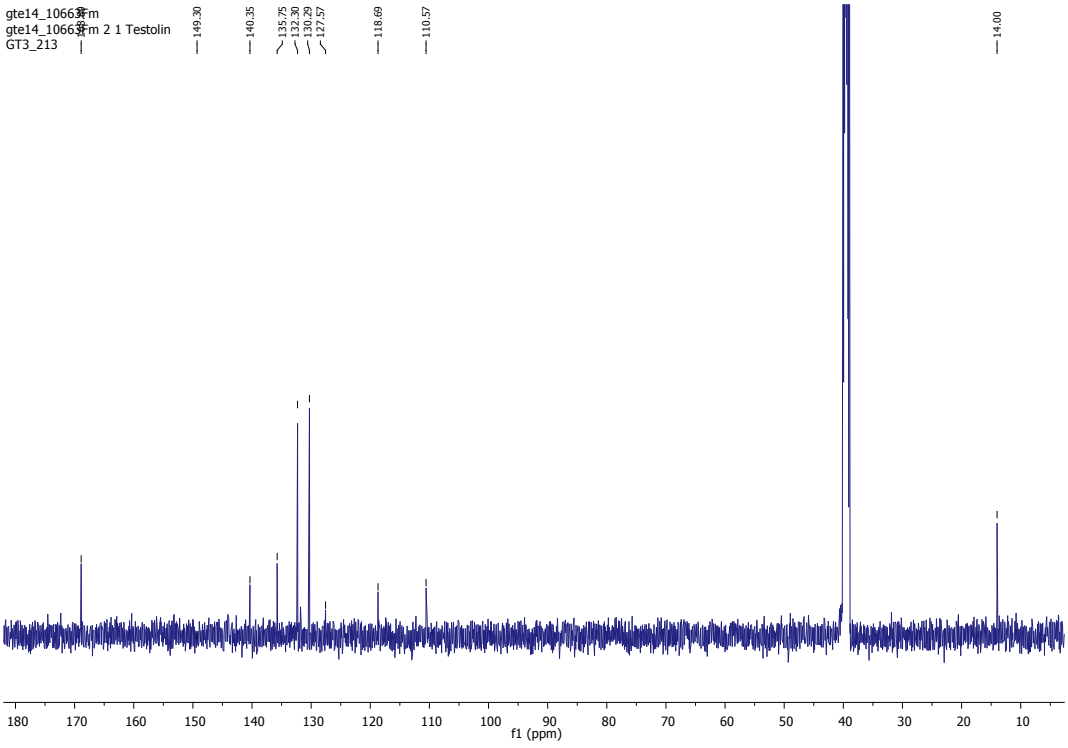
Ethyl (E)-3-(4-cyanophenyl)-2-methylacrylate



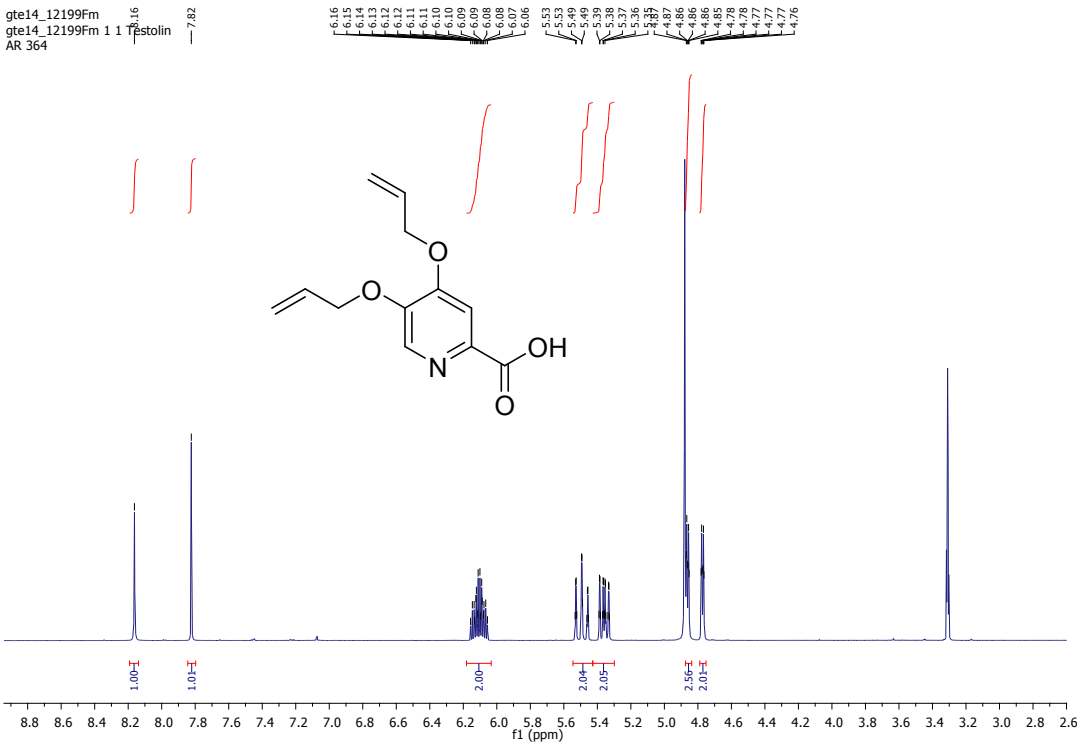


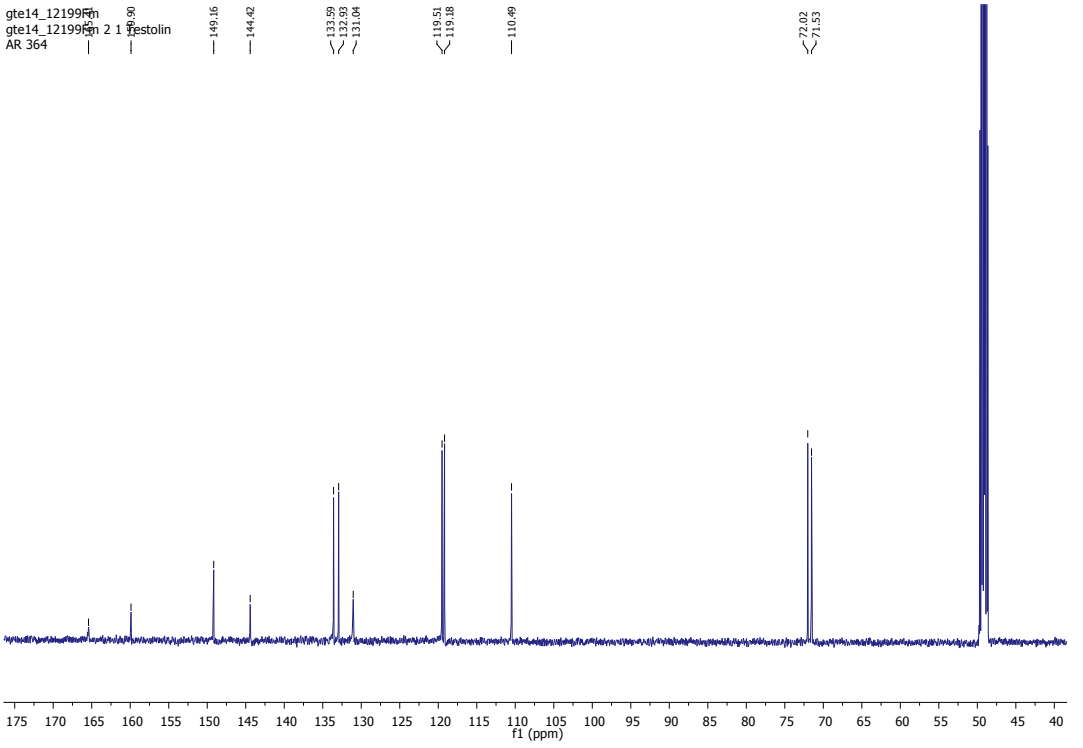
(E)-3-(4-Cyanophenyl)-2-methylacrylic acid



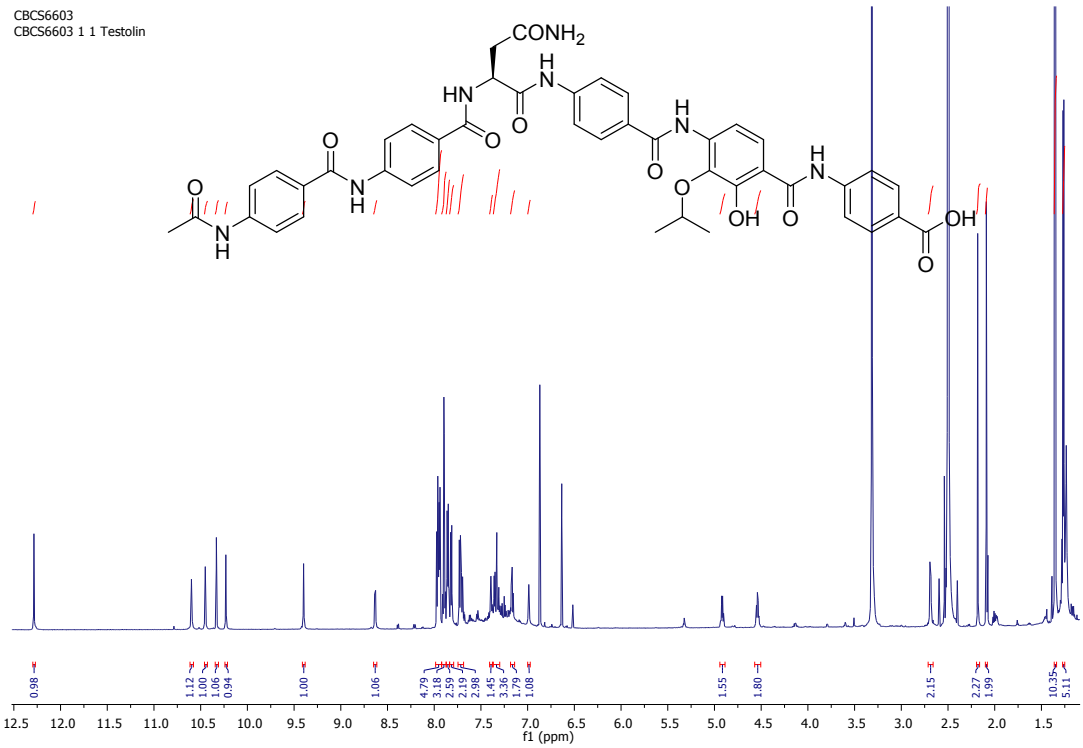


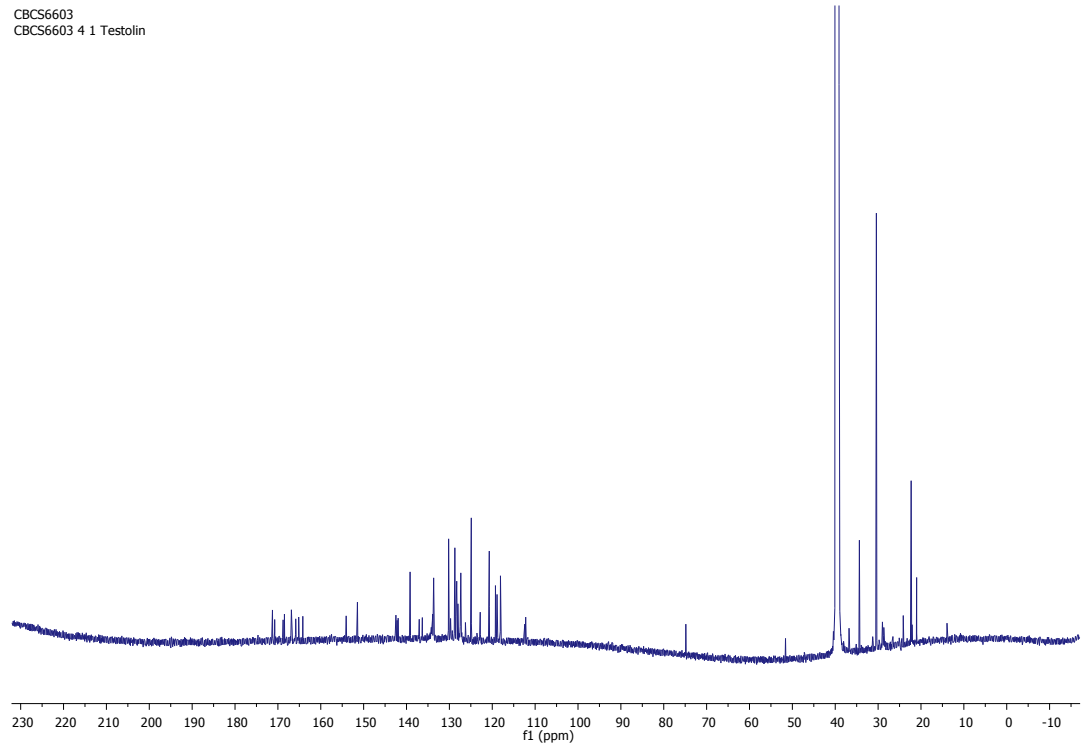
4,5-Bis(allyloxy)picolinic acid

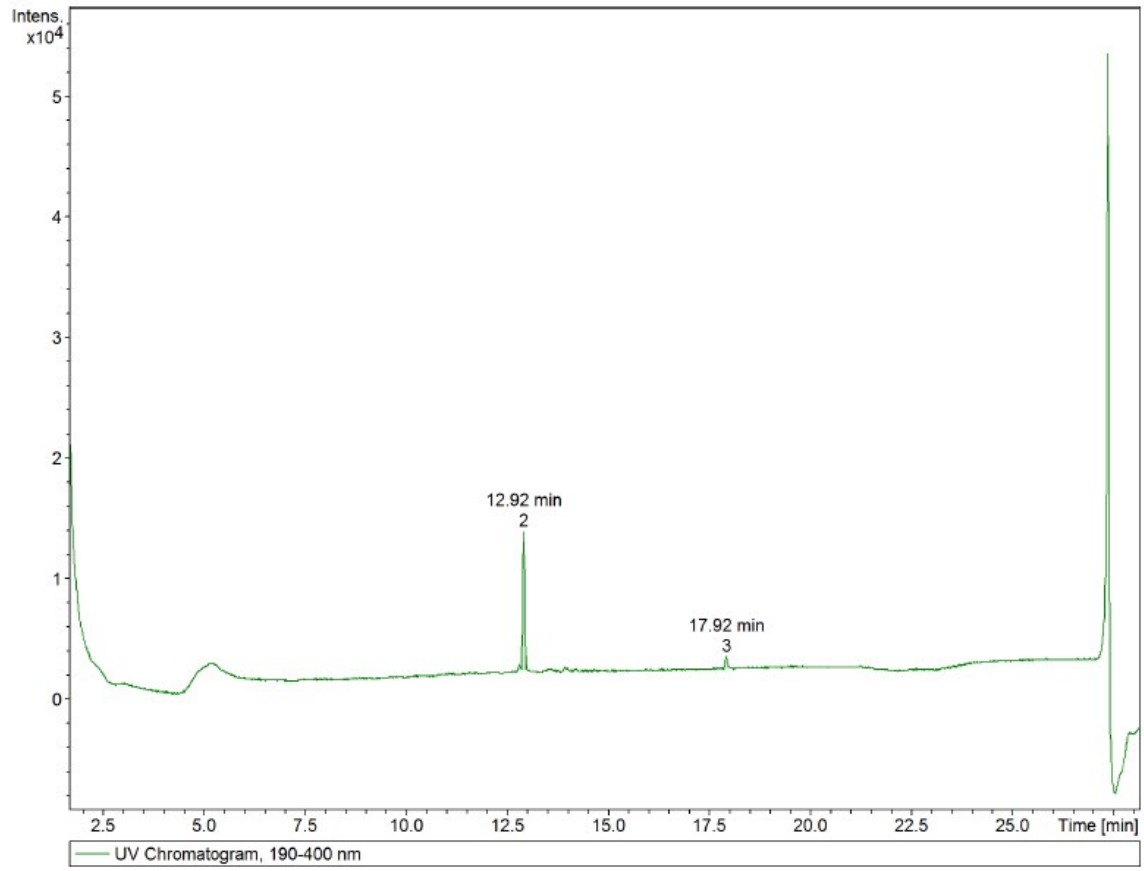




(S)-4-(4-(4-(2-(4-(4-Acetamidobenzamido)benzamido)-4-amino-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid







RT [min]	Area	Area Frac. %	Chromatogram
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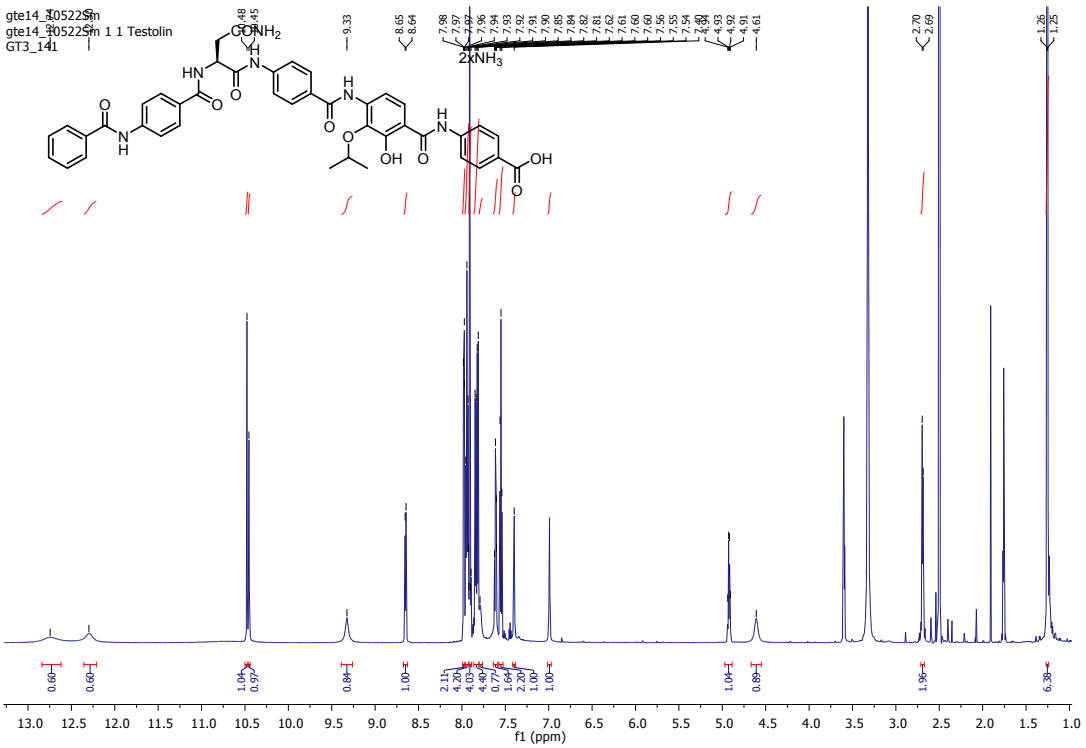
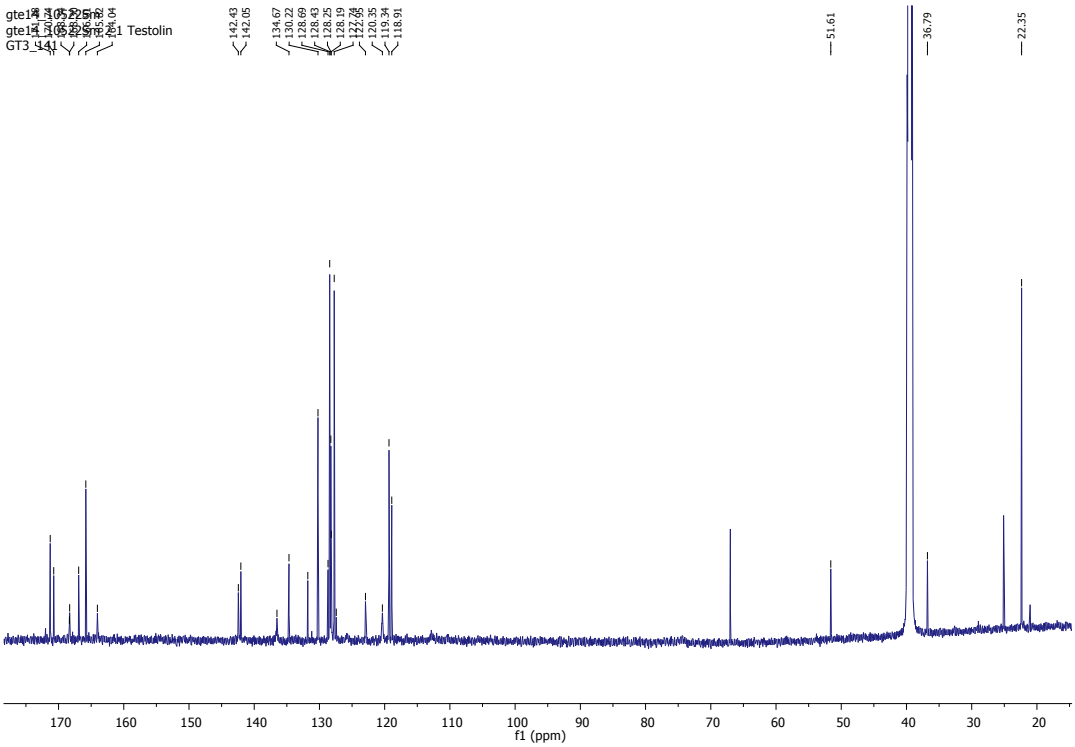
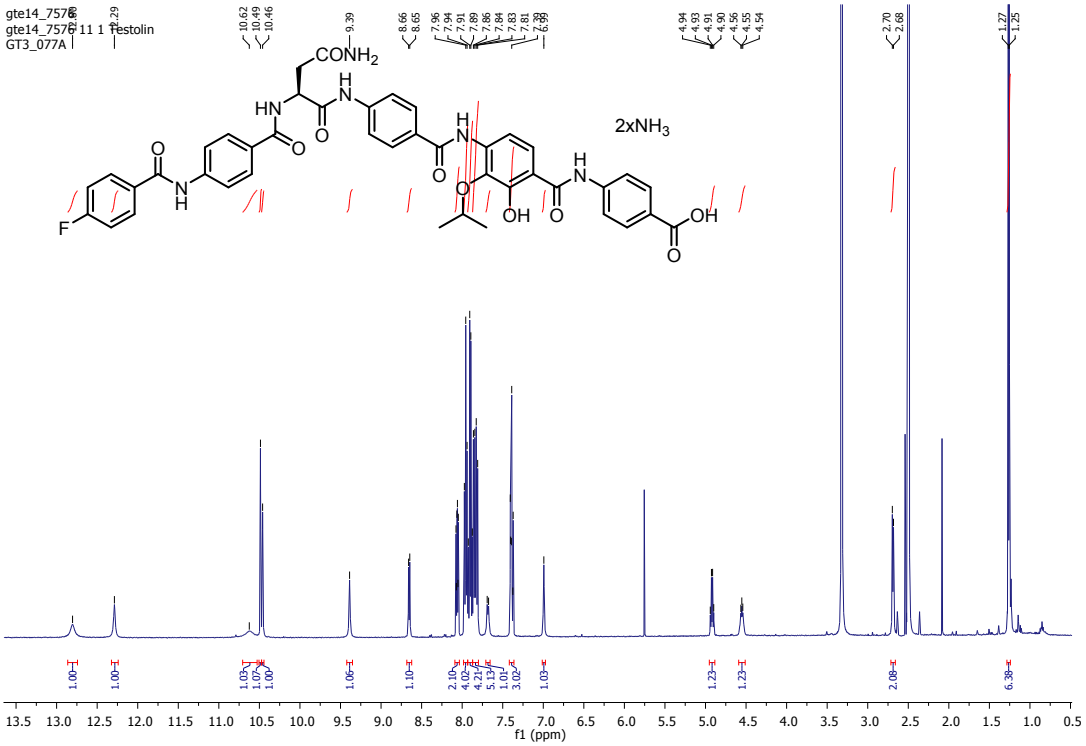
17.92	3097,1	7.90	UV Chromatogram, 190-400 nm

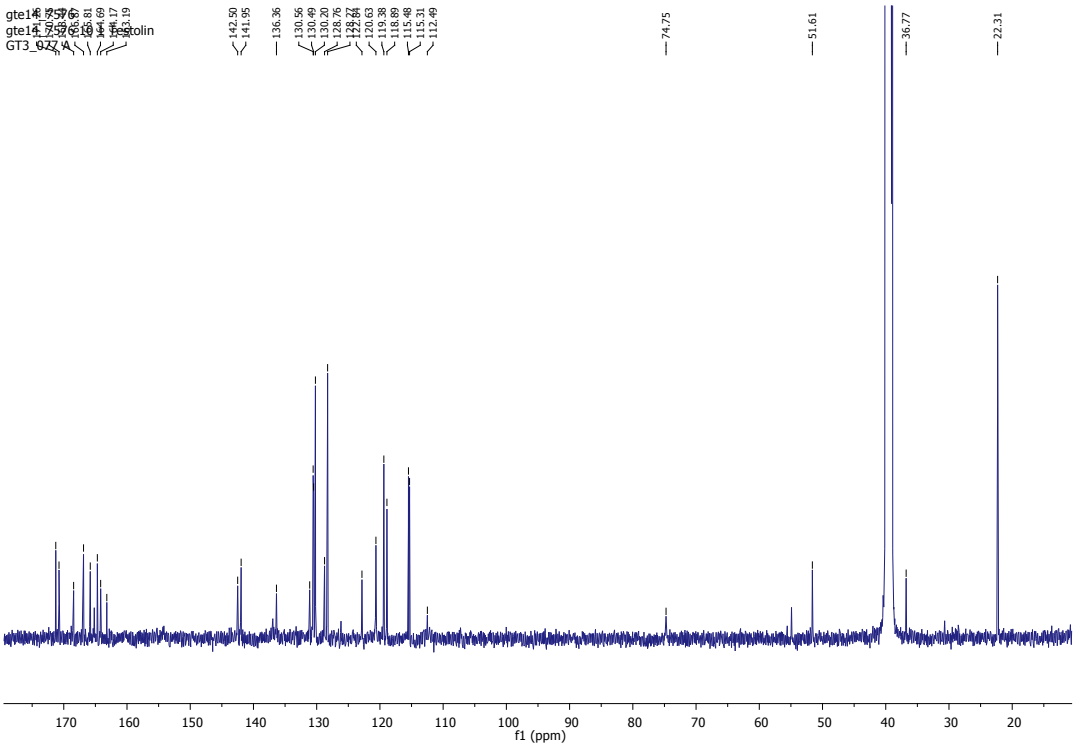
(S)-4-(4-(4-(4-Amino-2-(4-benzamidobenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



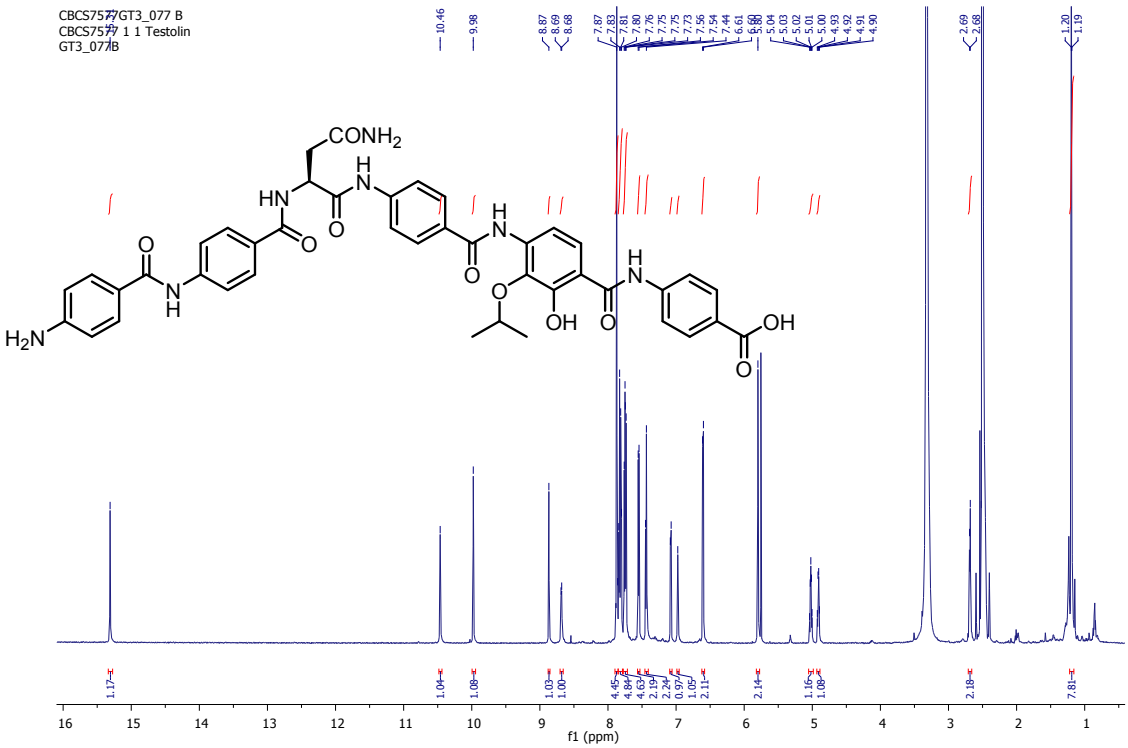


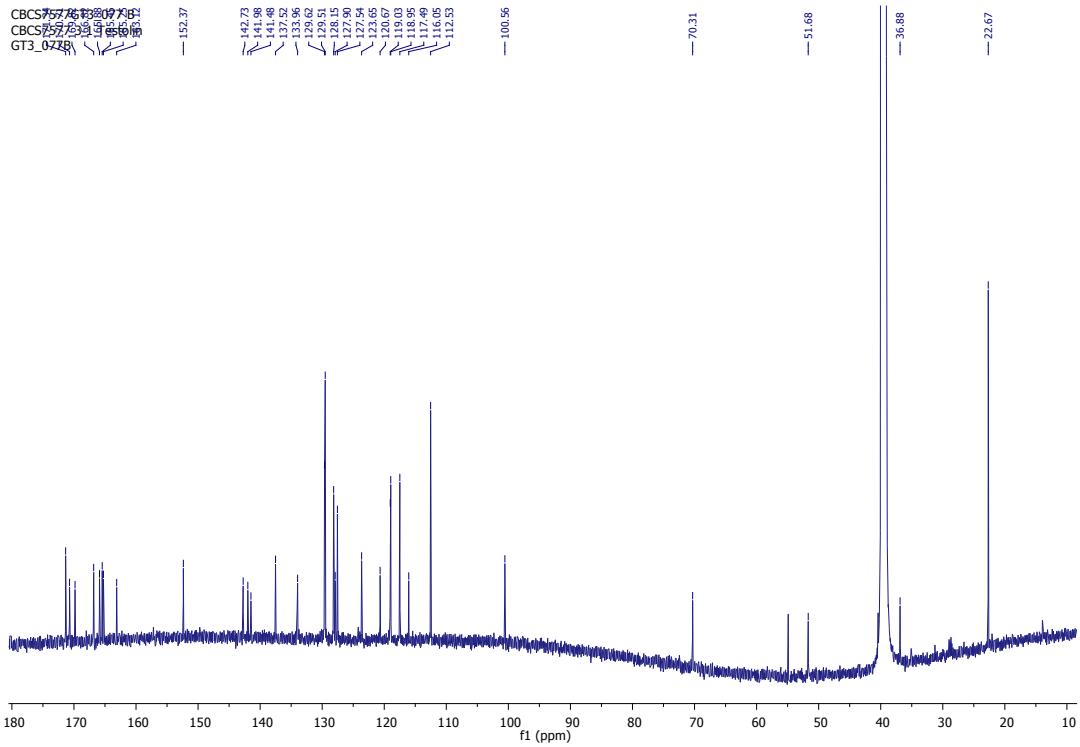
(S)-4-(4-(4-(4-Amino-2-(4-(4-fluorobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



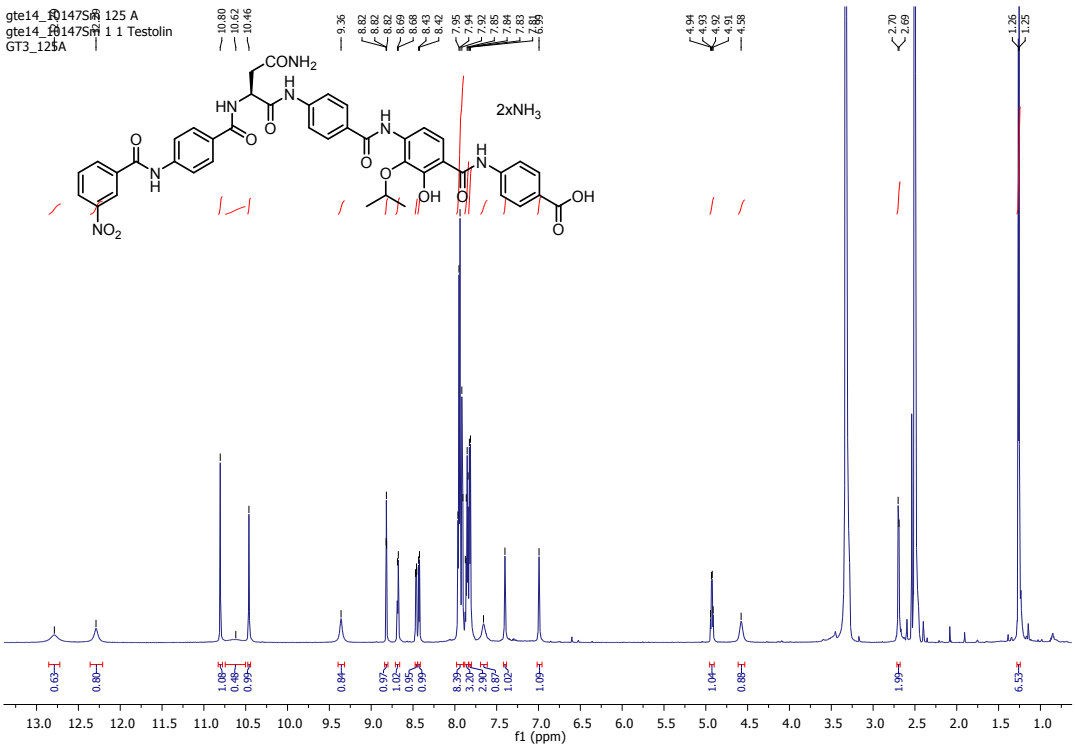


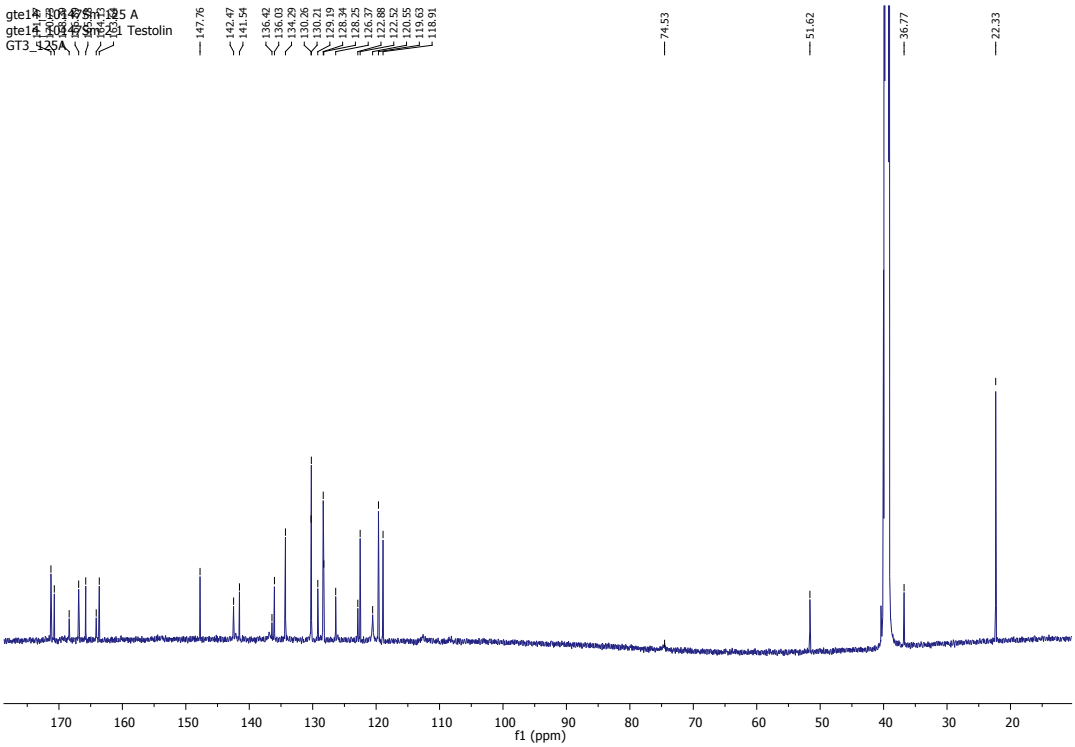
(S)-4-(4-(4-(4-Amino-2-(4-(4-aminobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



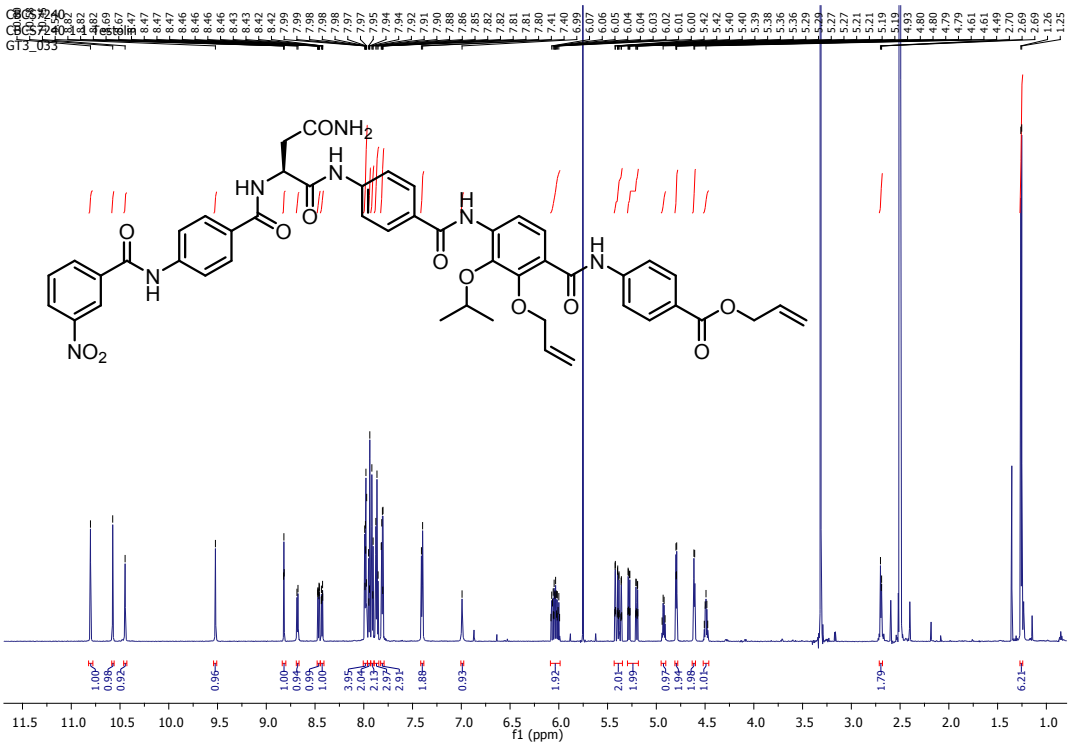


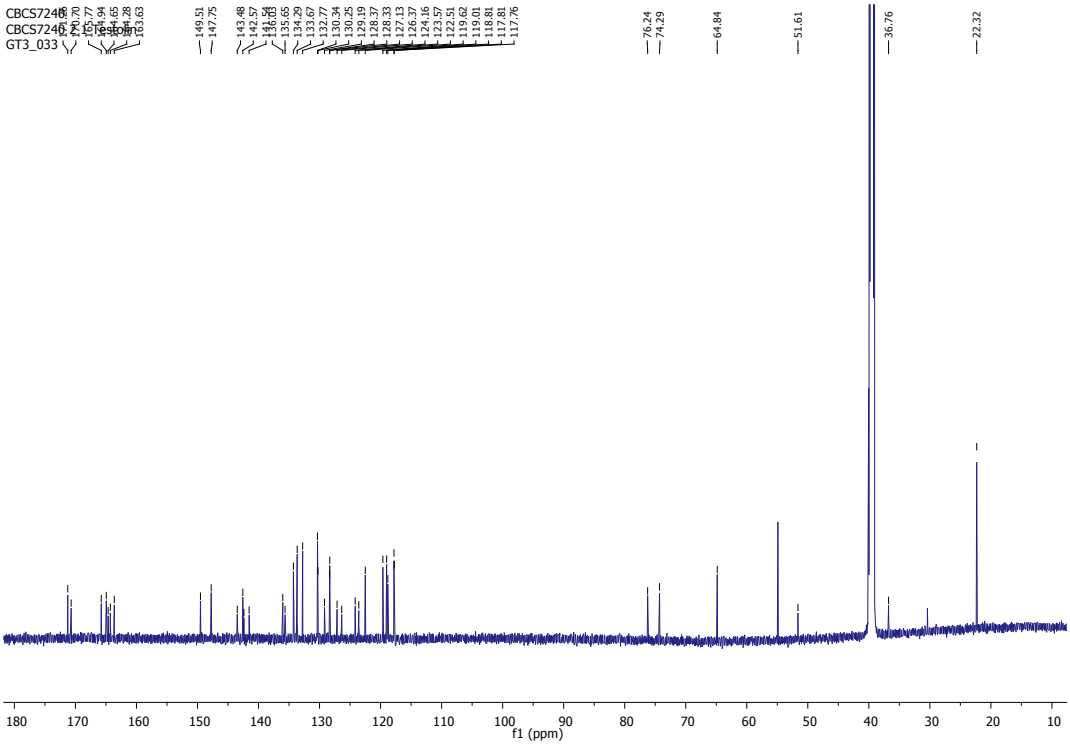
(S)-4-(4-(4-(4-Amino-2-(4-(3-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



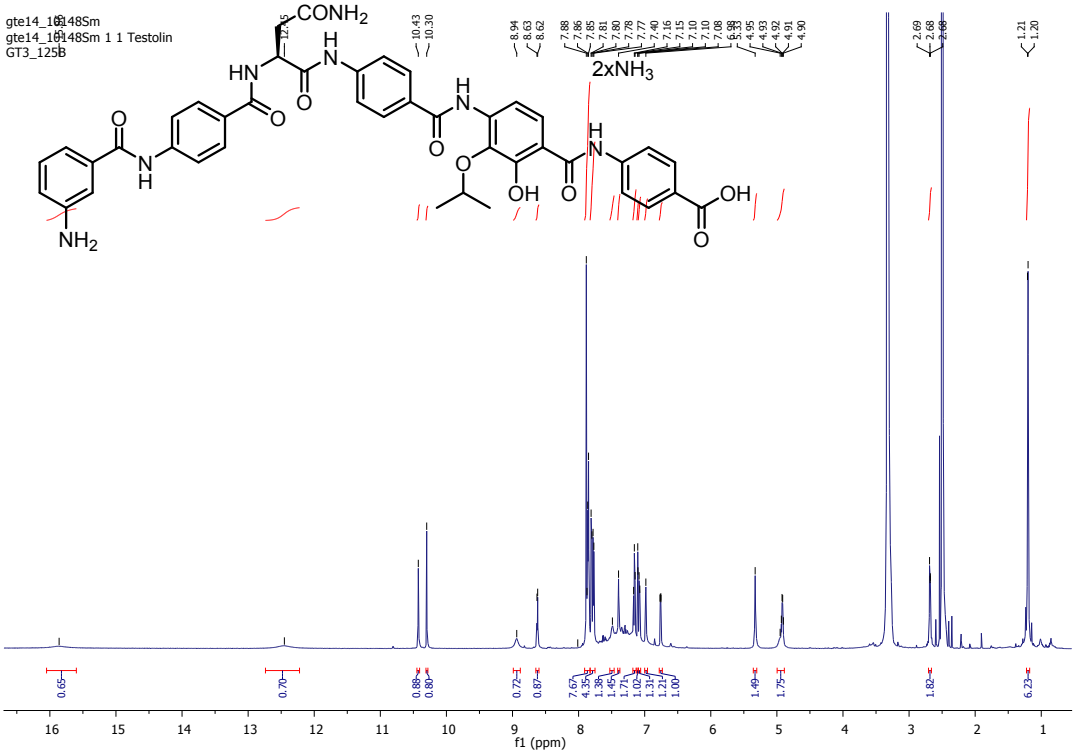


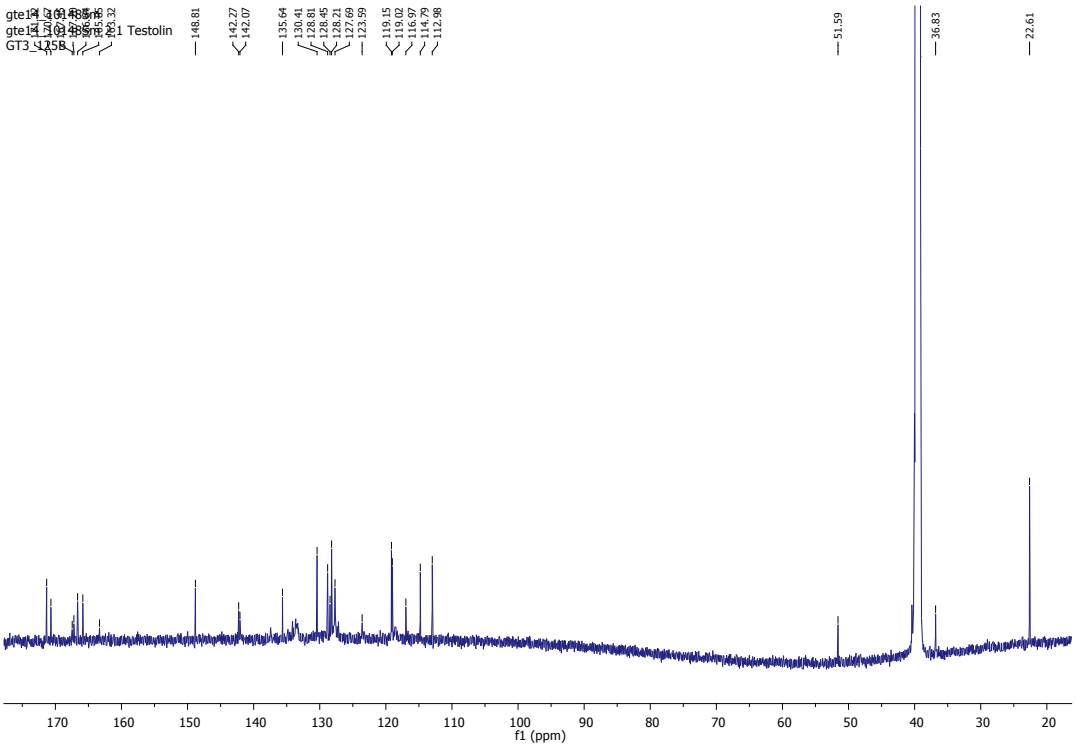
Allyl protected intermediate:



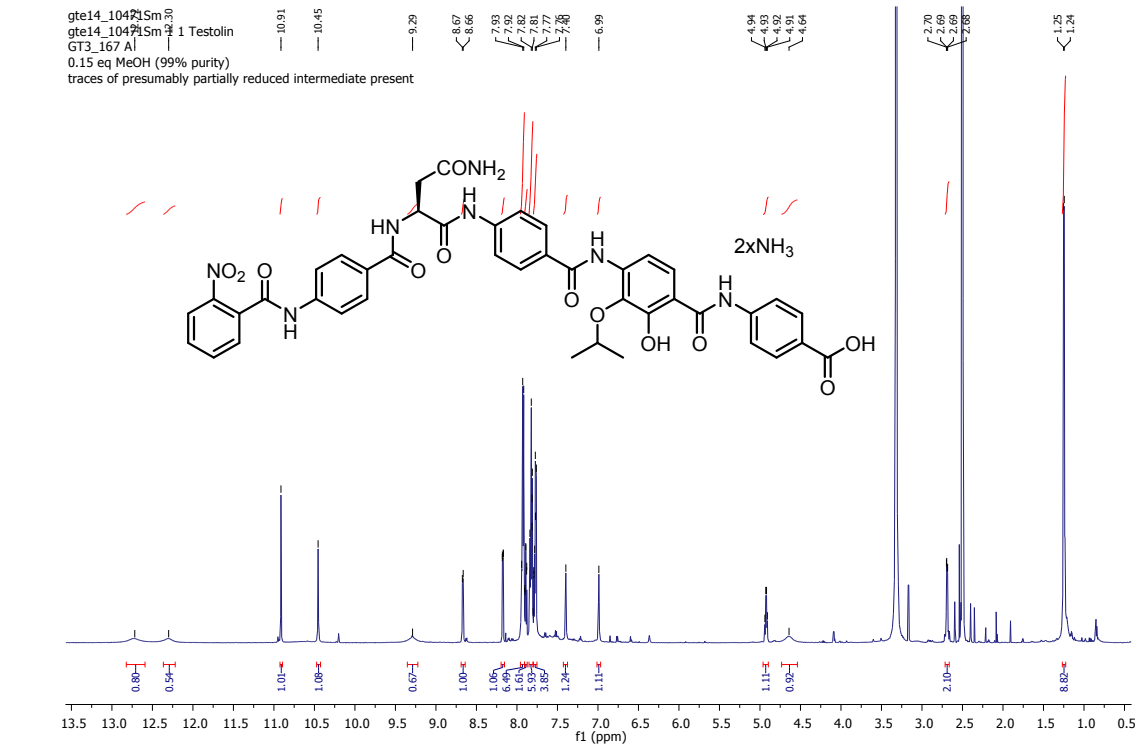


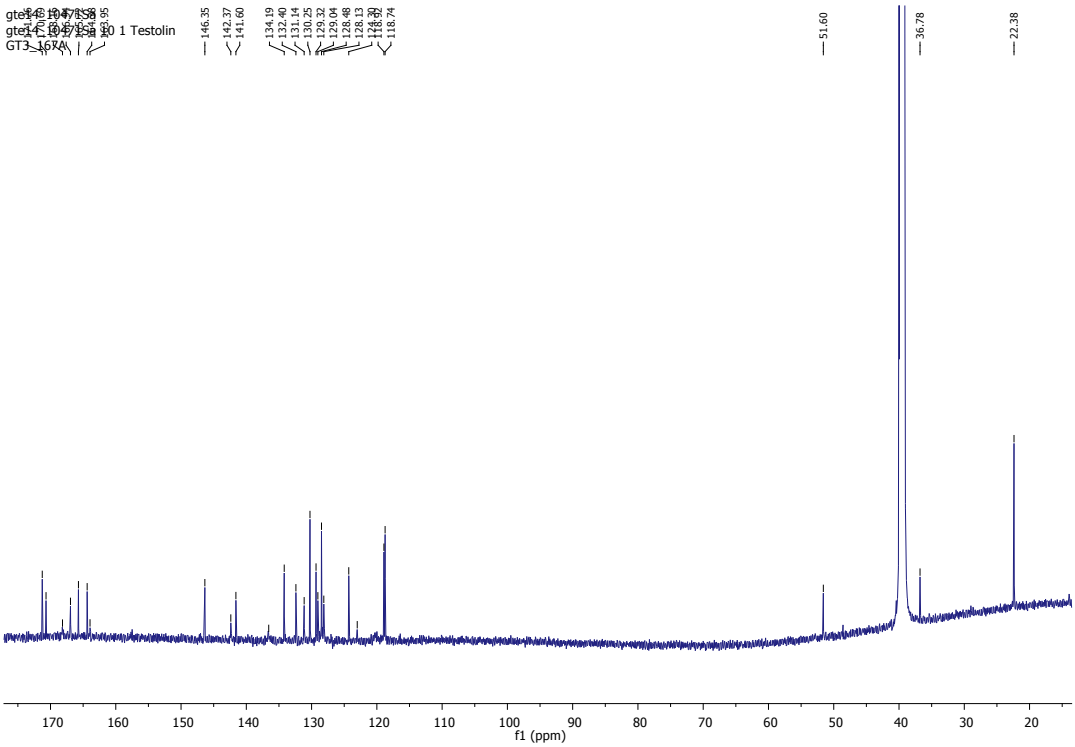
(S)-4-(4-(4-(4-Amino-2-(4-(3-aminobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

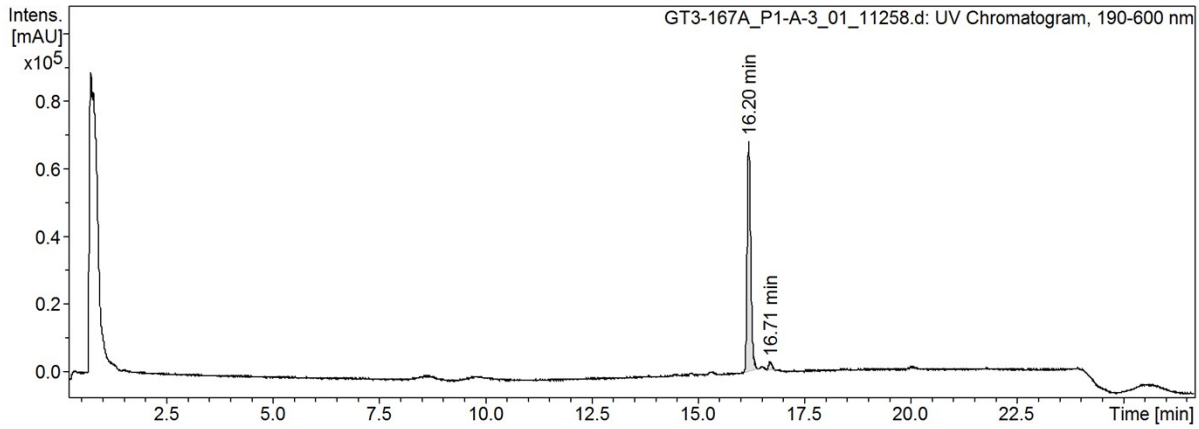




(S)-4-(4-(4-(4-Amino-2-(4-(2-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

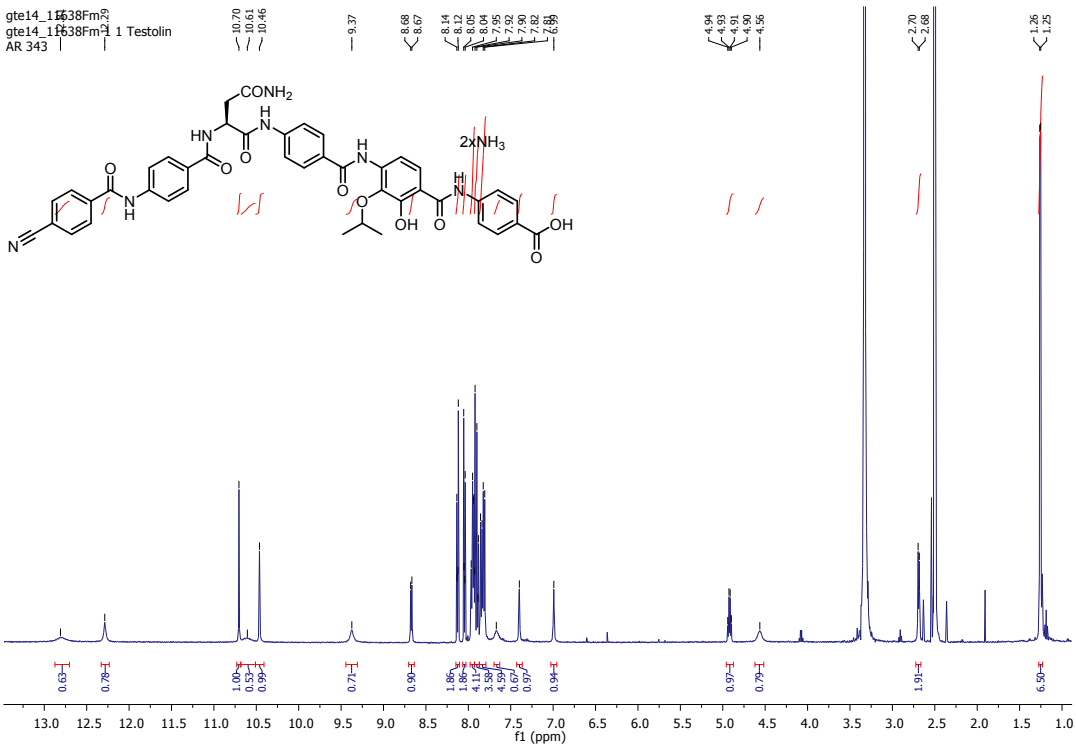


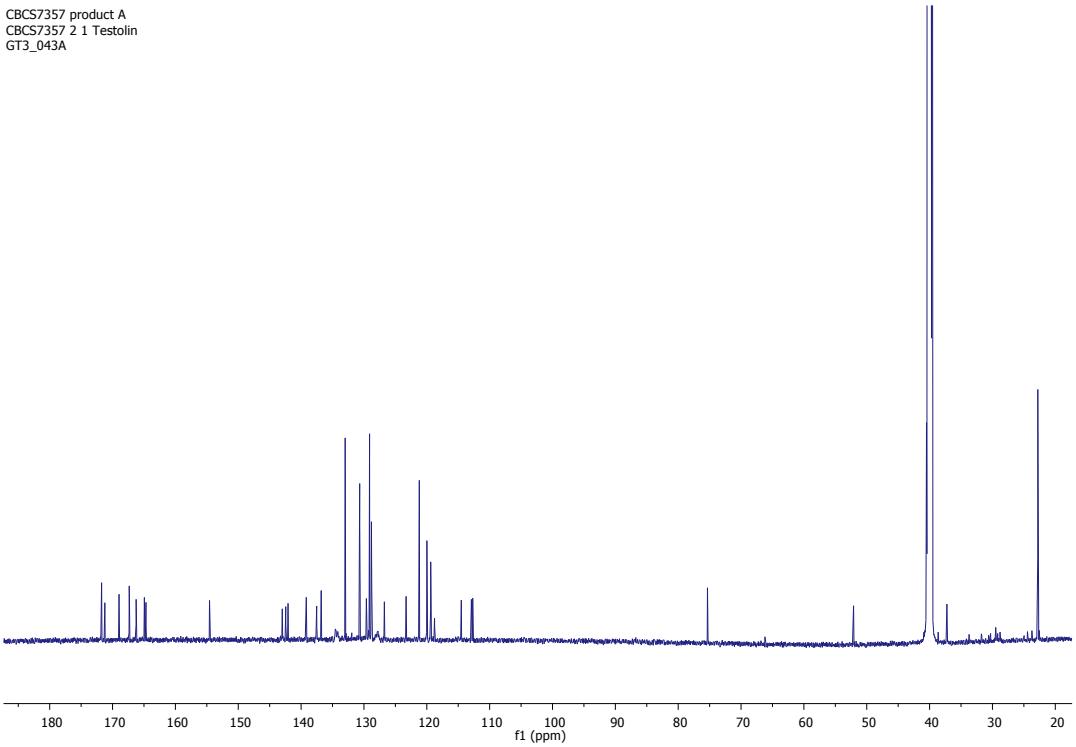




RT [min]	Area	Area Frac. %	Chromatogram
16.20	394461	96.83	UV Chromatogram, 190-600 nm
16.71	12912	3.17	UV Chromatogram, 190-600 nm

(S)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid





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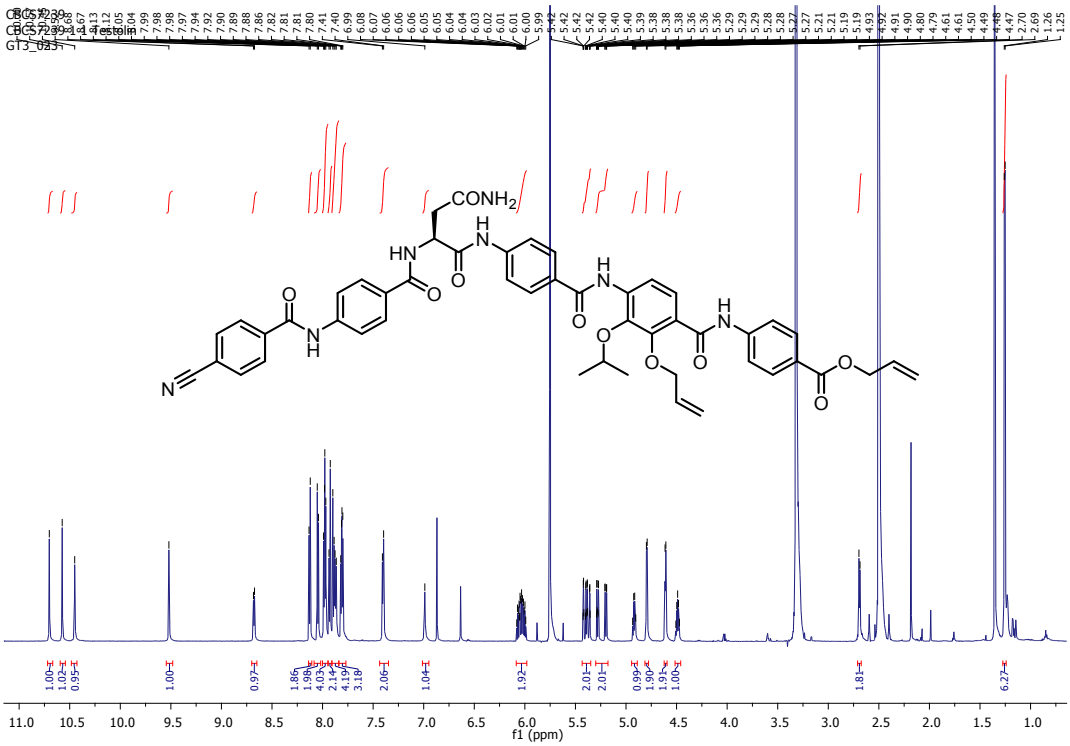
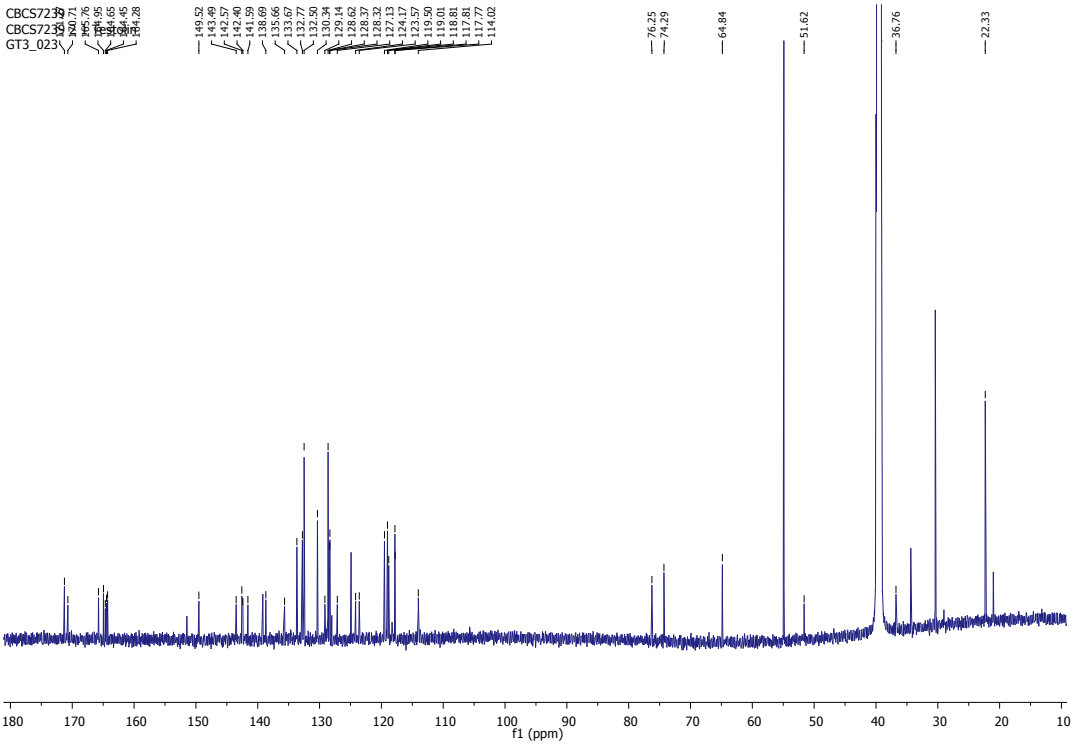
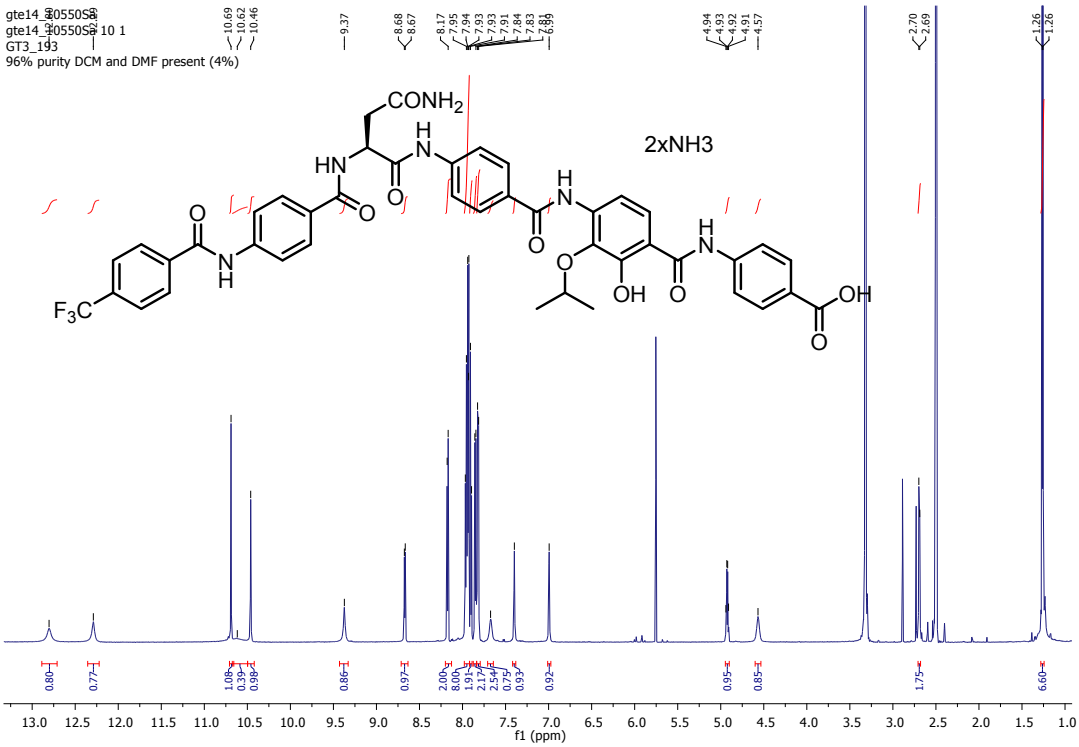
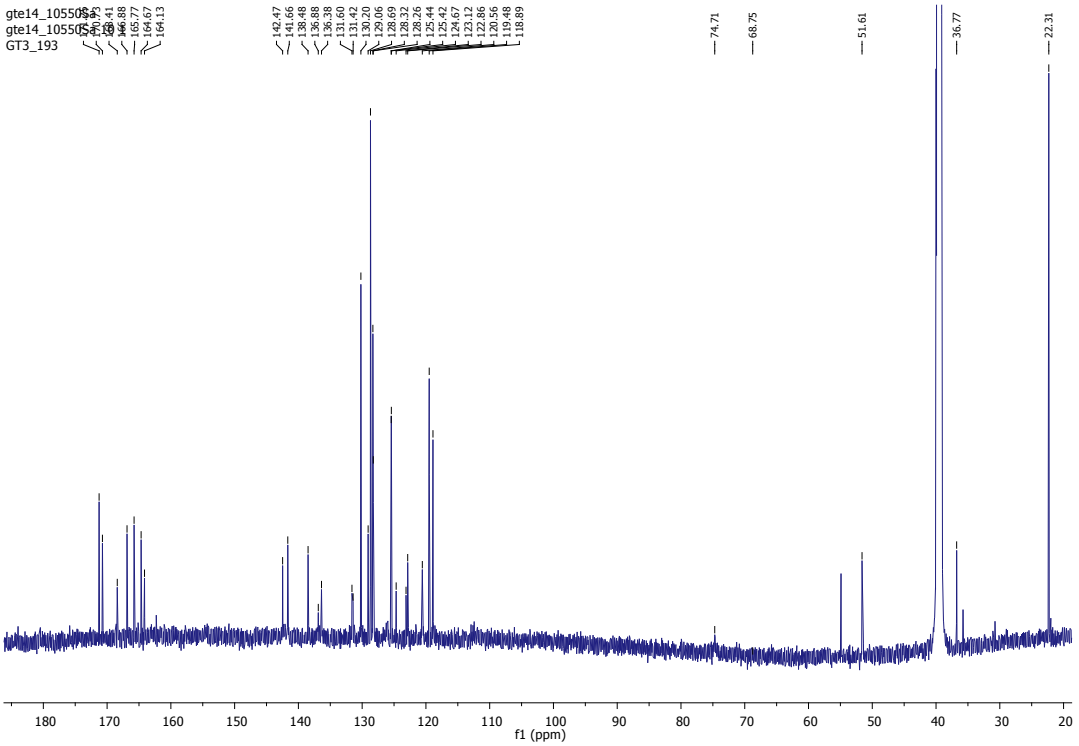




(S)-4-(4-(4-(4-Amino-4-oxo-2-(4-(4-(trifluoromethyl)benzamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid





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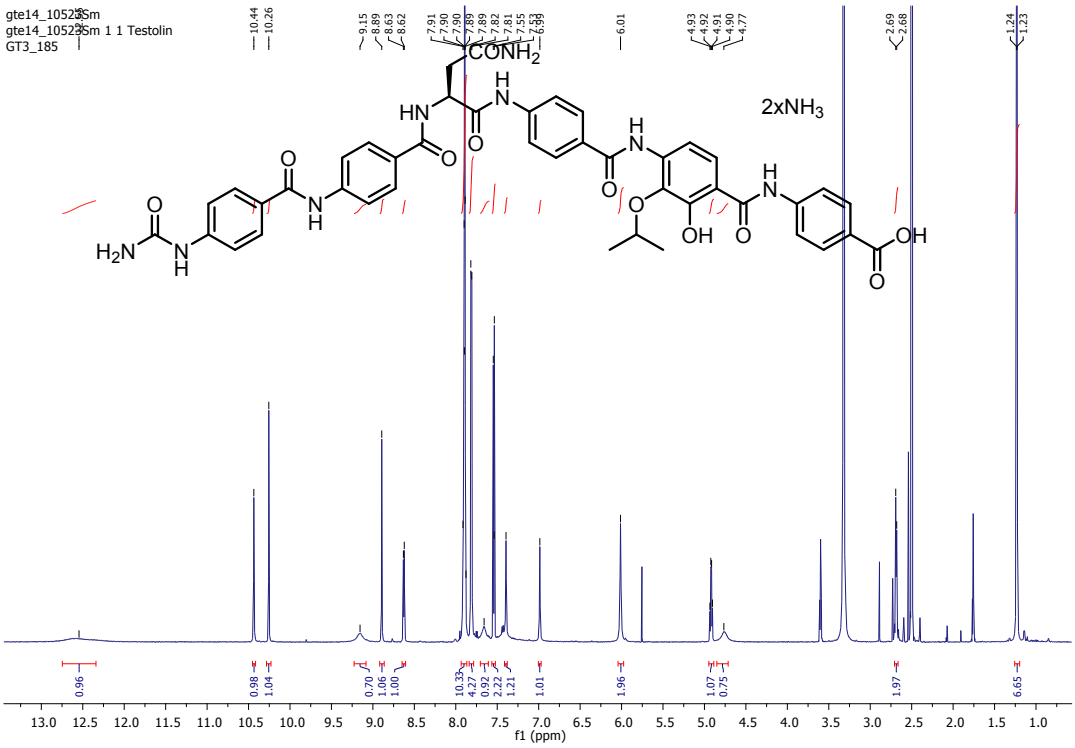
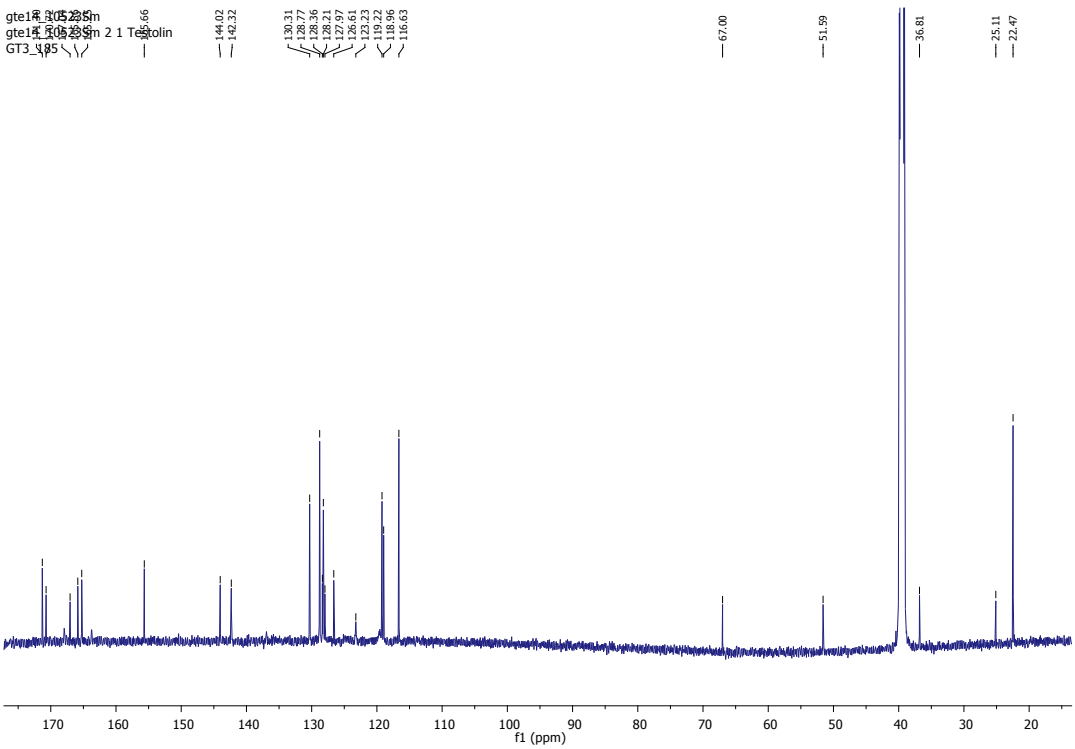
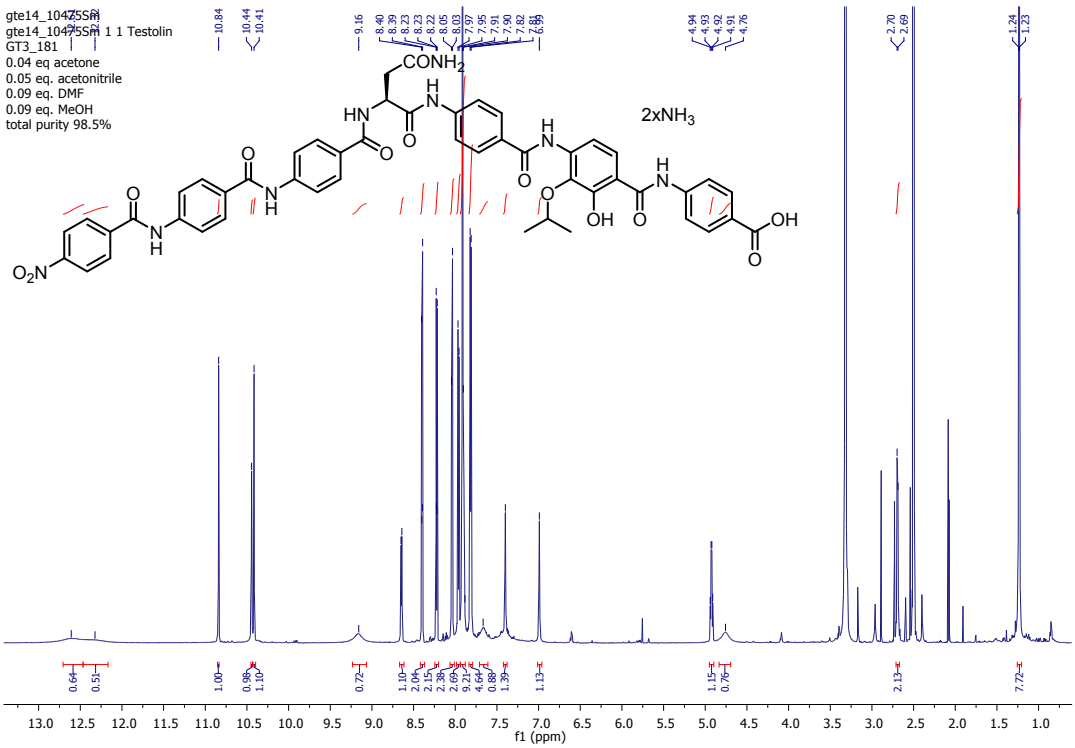
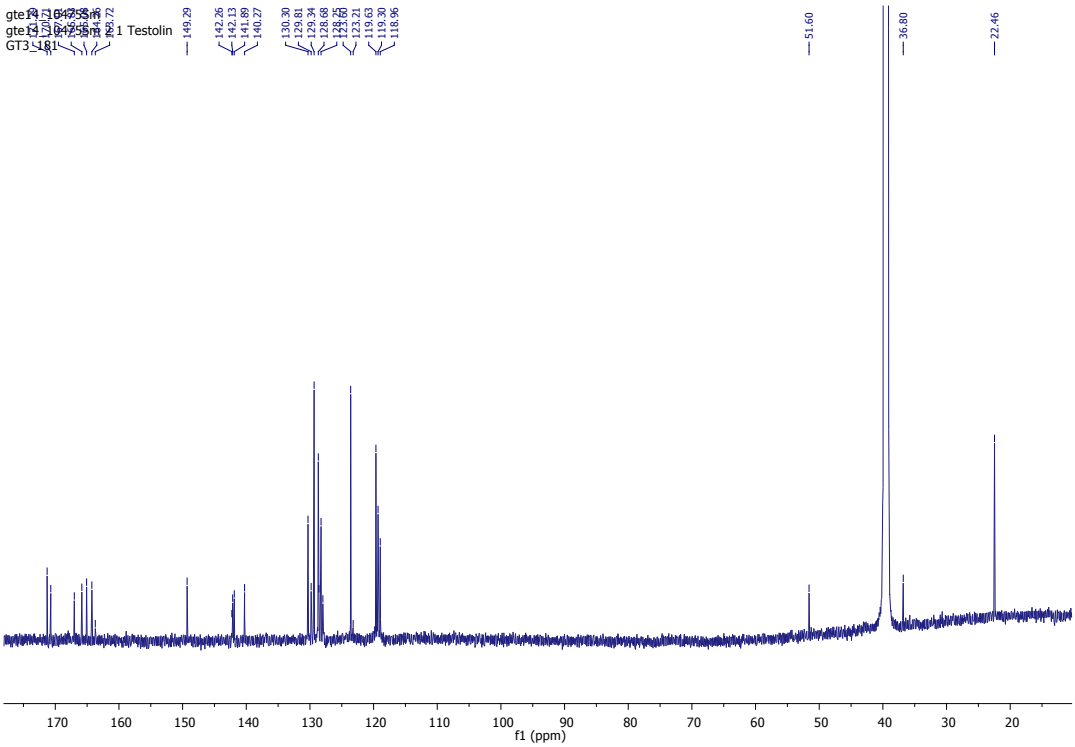


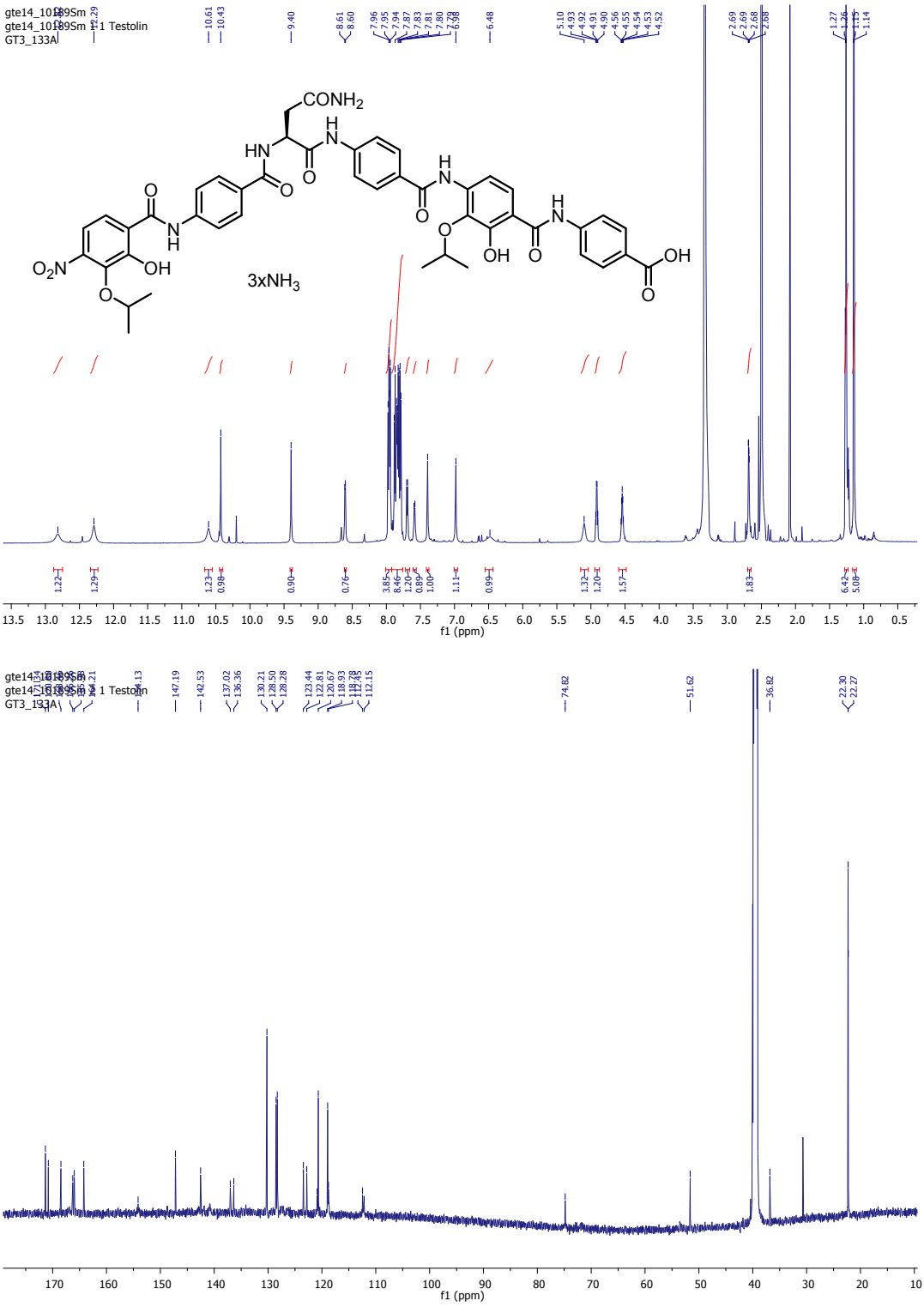


(S)-4-(4-(4-(4-Amino-2-(4-(4-(4-nitrobenzamido)benzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

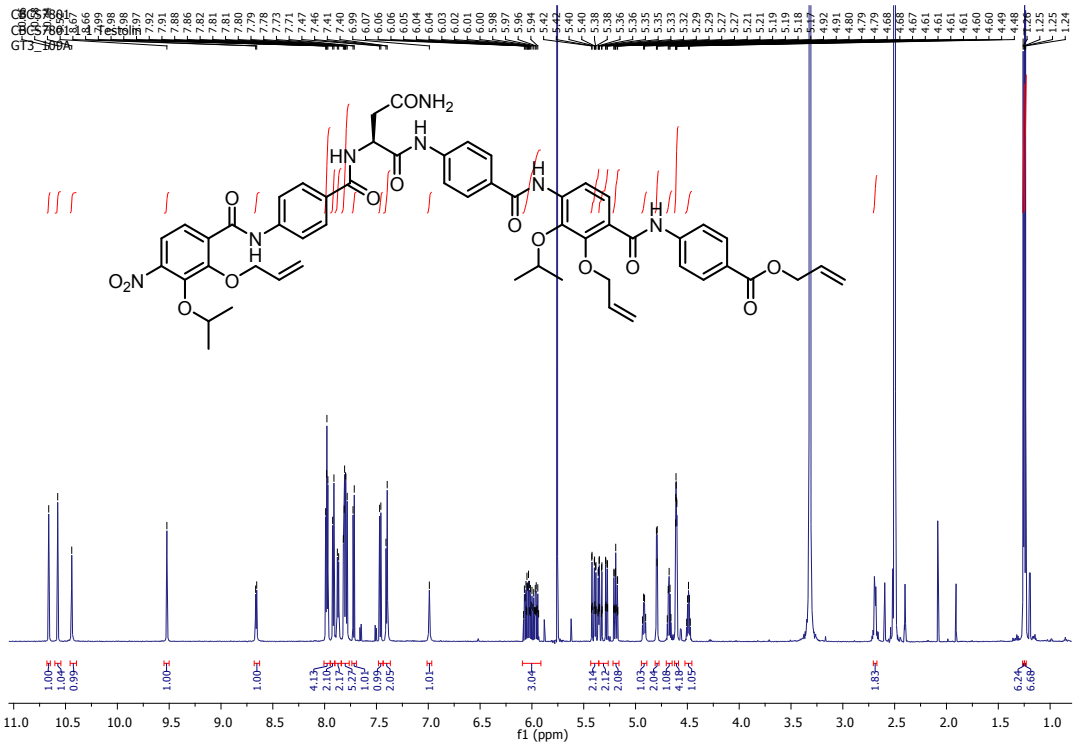


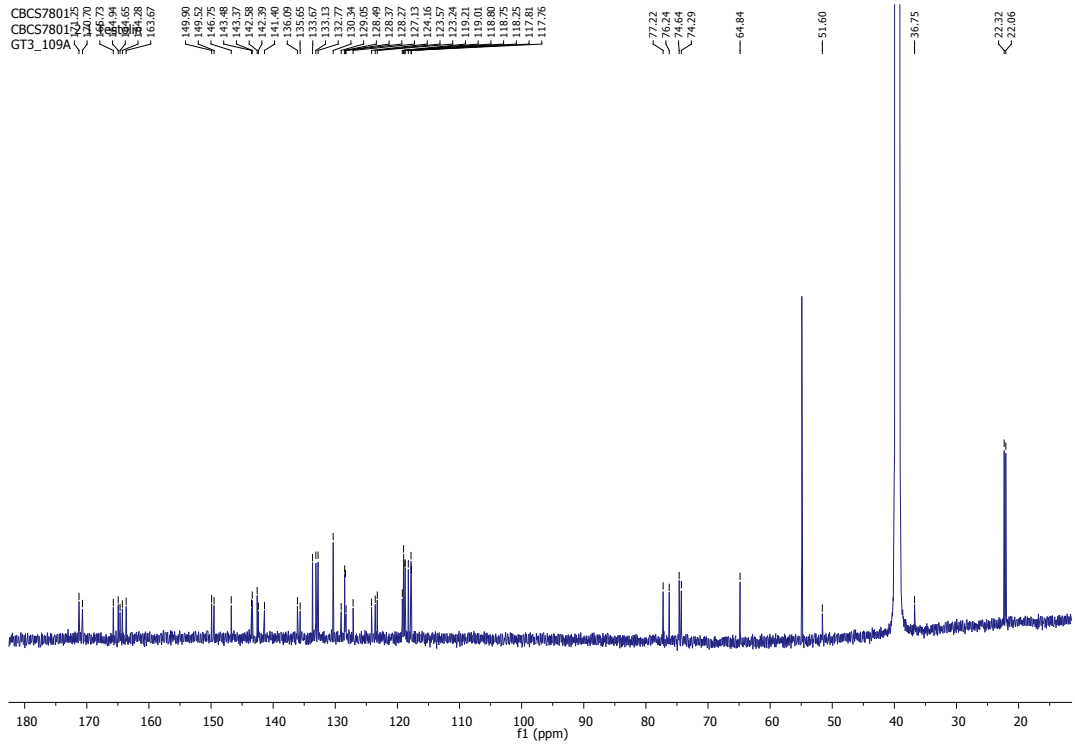


(S)-4-(4-(4-(4-Amino-2-(4-(2-hydroxy-3-isopropoxy-4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

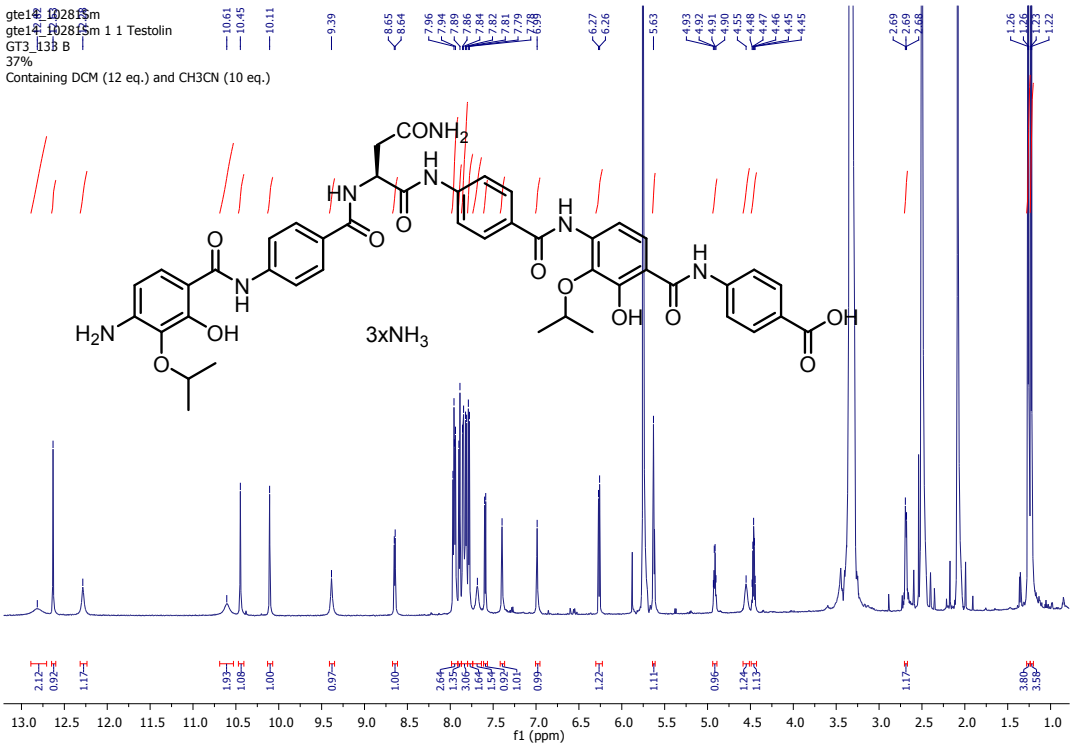


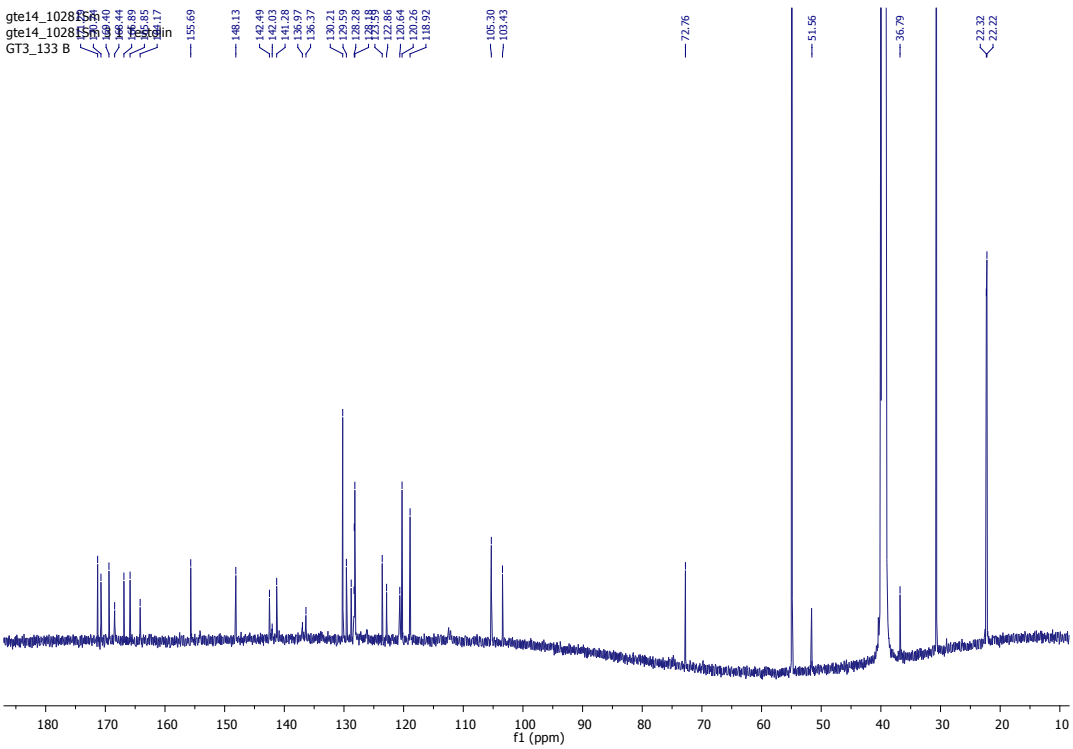
Allyl protected intermediate:



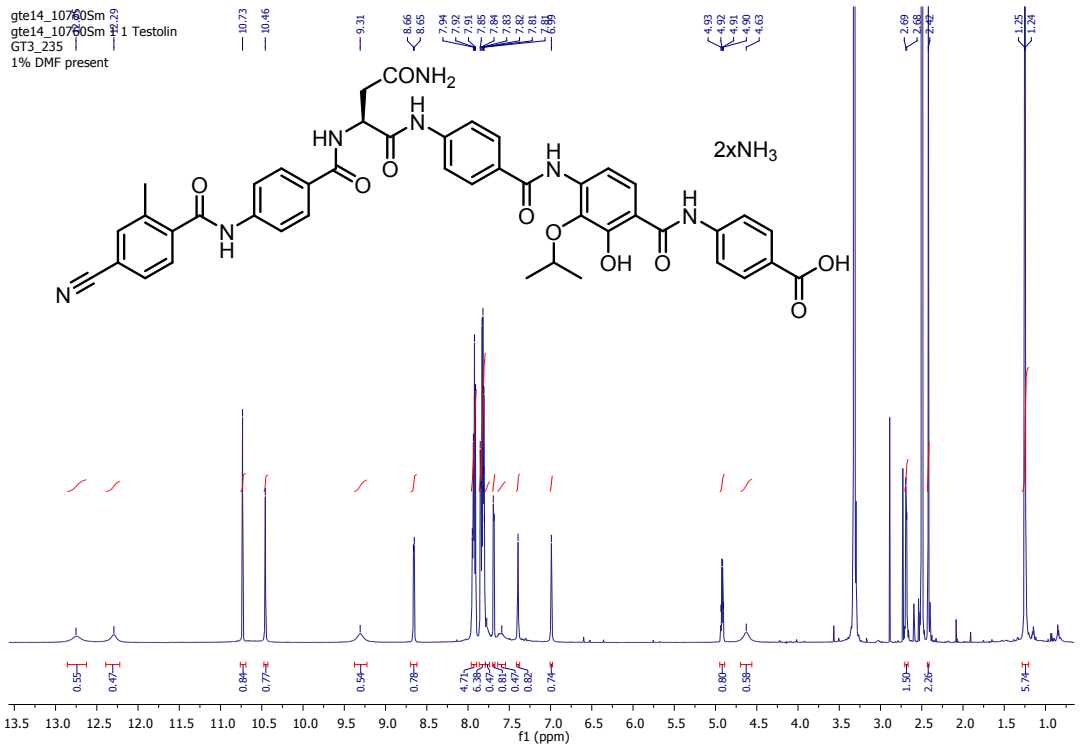


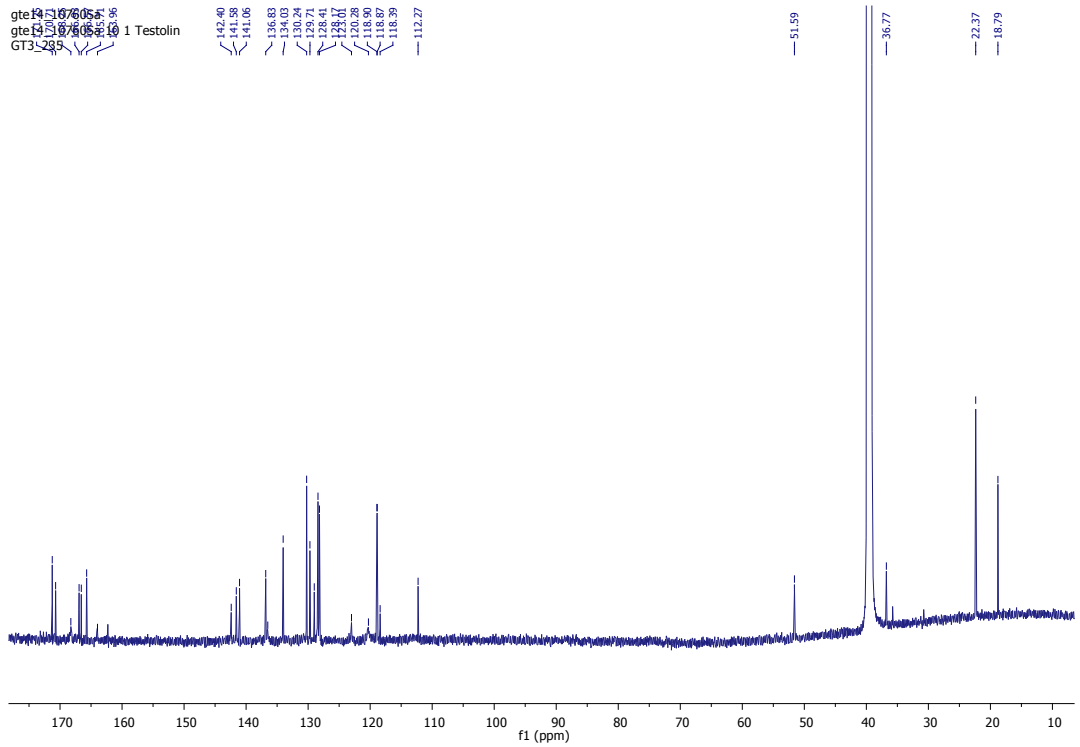
(S)-4-(4-(4-(4-Amino-2-(4-(4-amino-2-hydroxy-3-isopropoxybenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



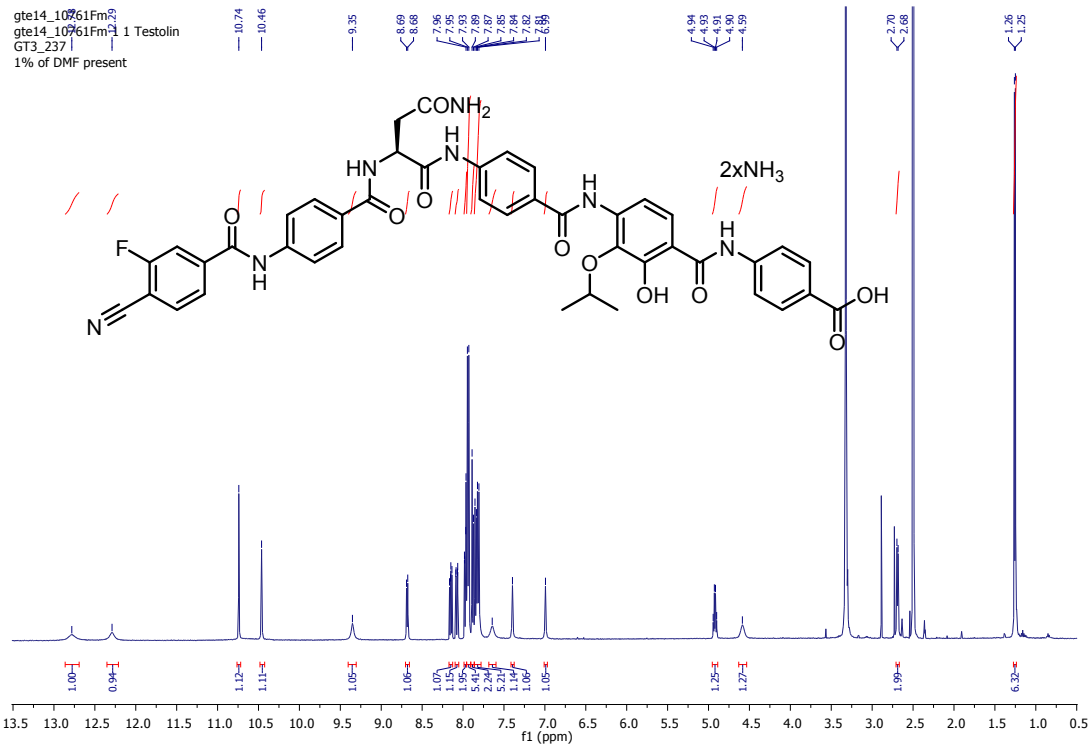


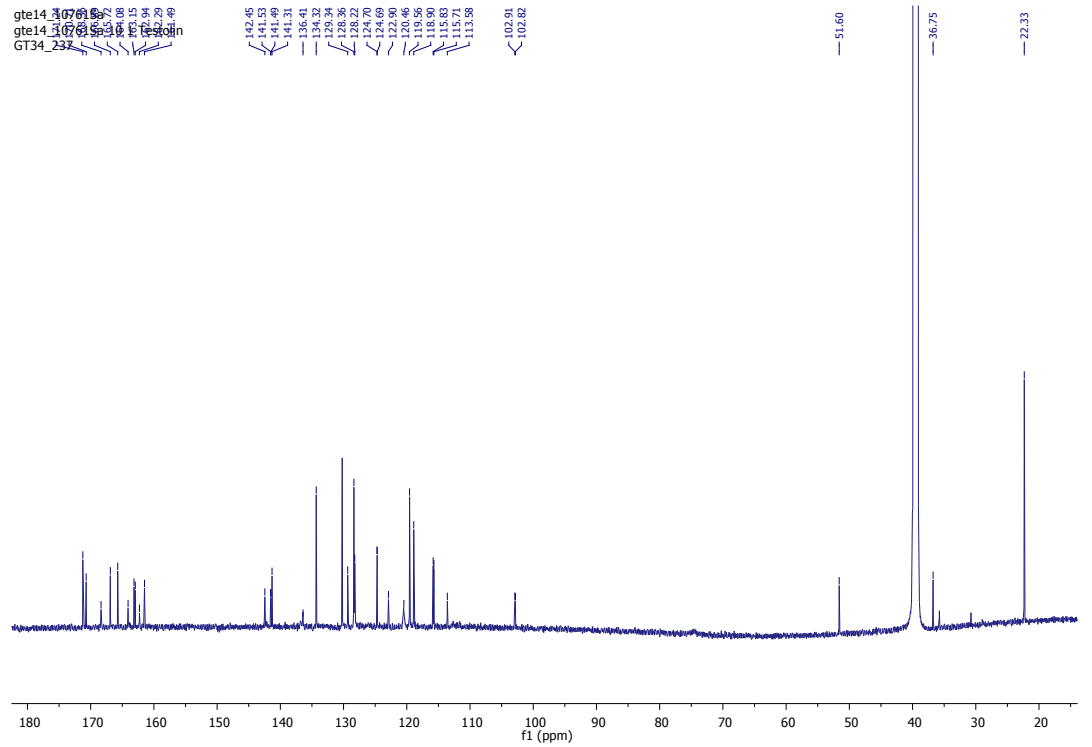
(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-2-methylbenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



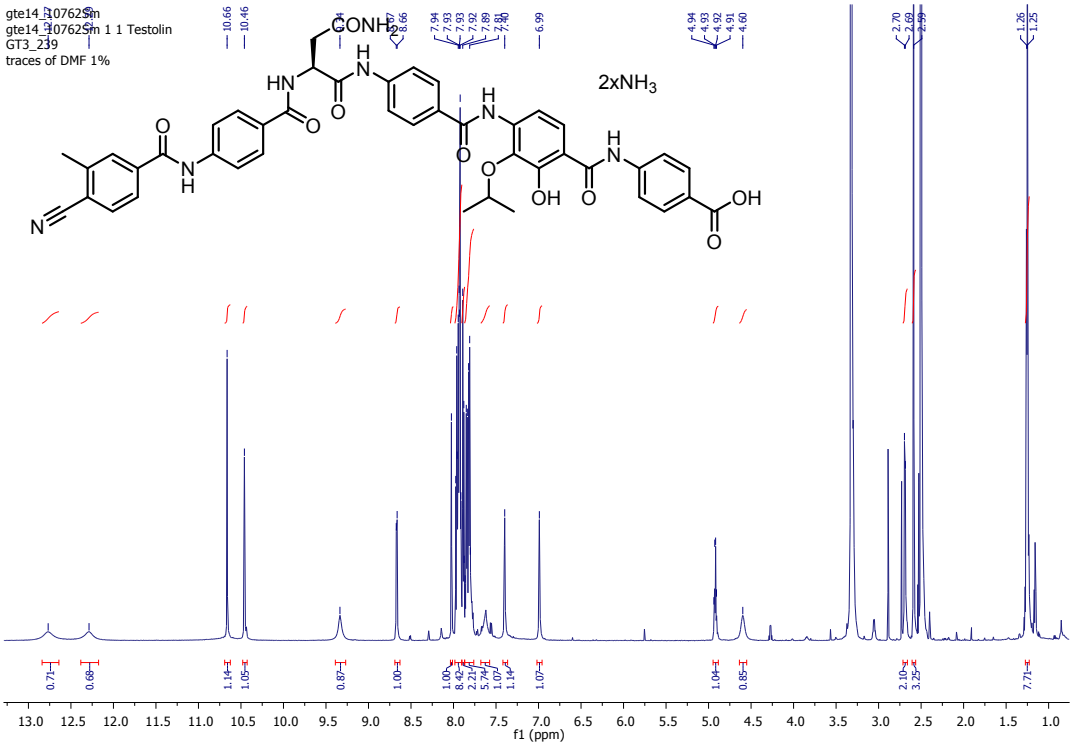


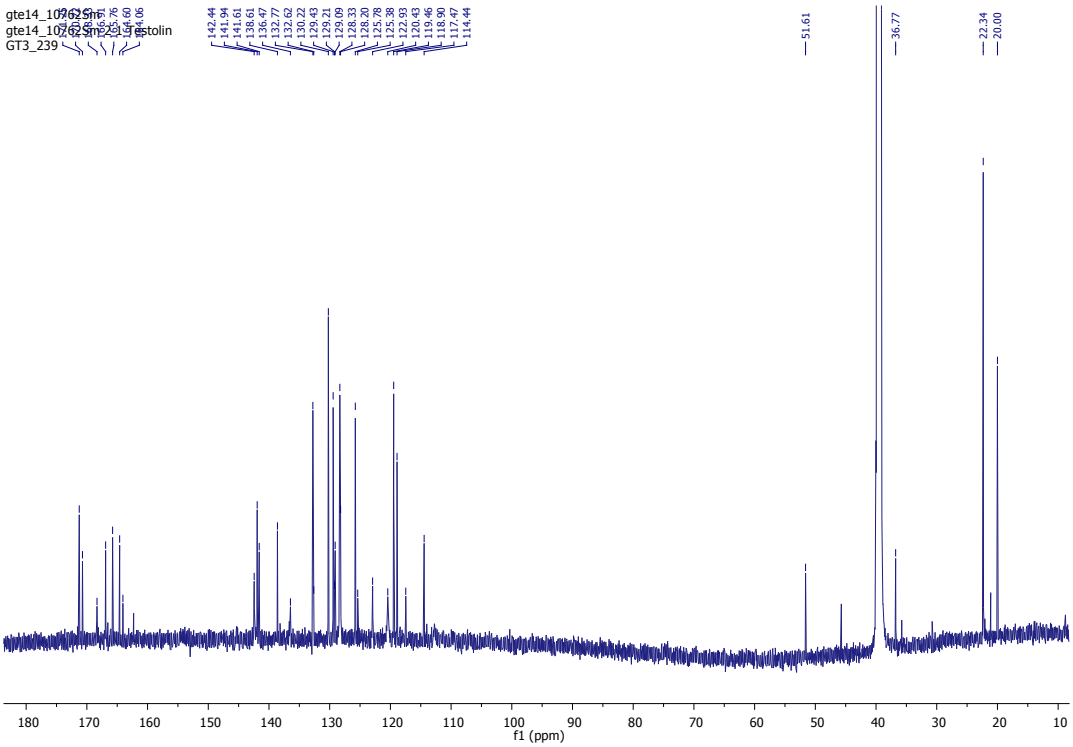
(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-3-fluorobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



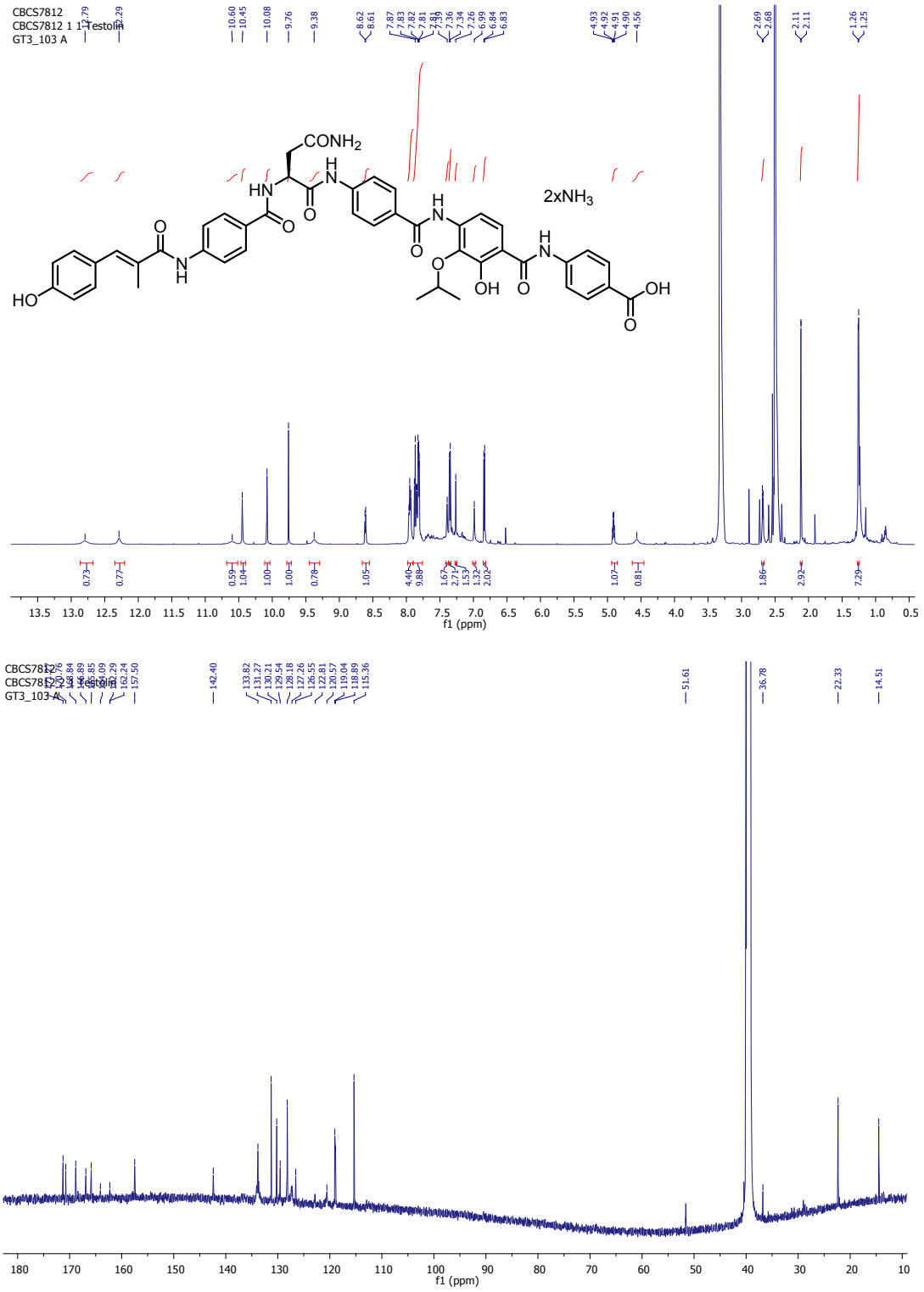


(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-3-methylbenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid





(S,E)-4-(4-(4-(4-Amino-2-(4-(3-(4-hydroxyphenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



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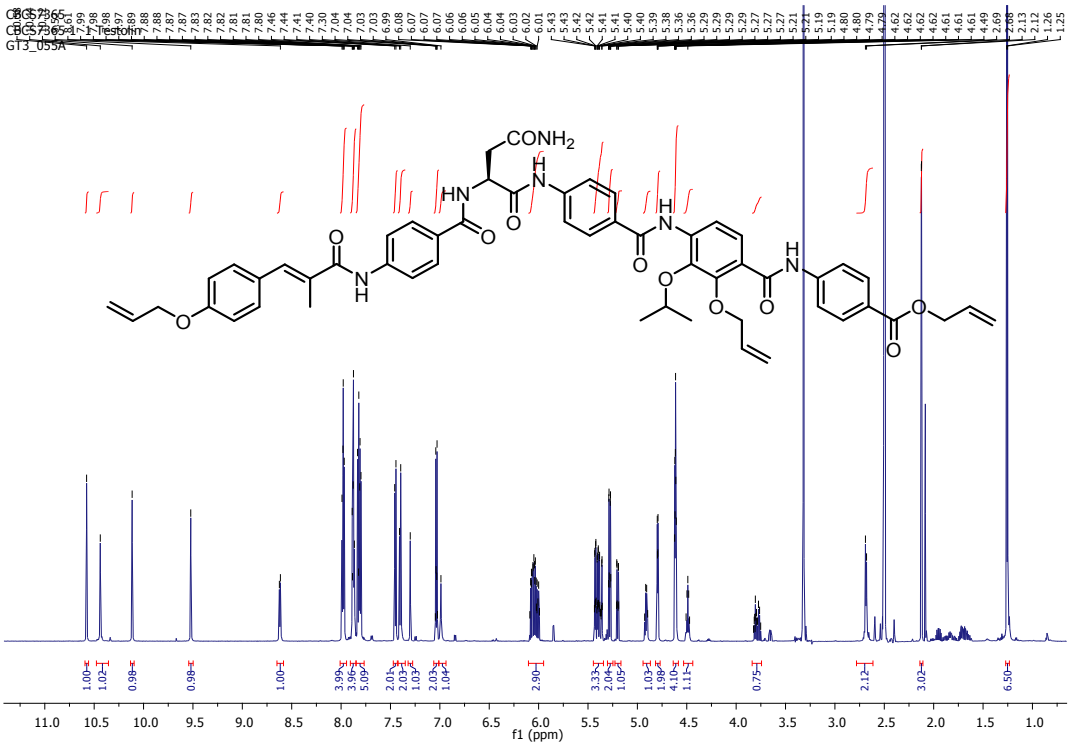
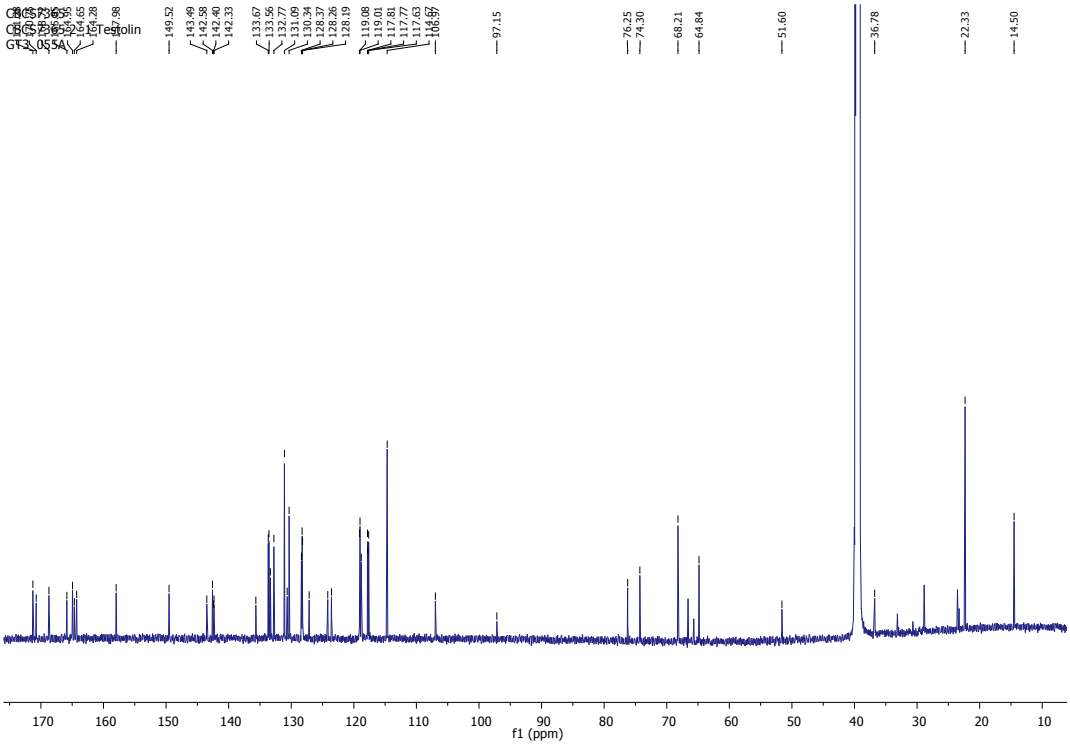
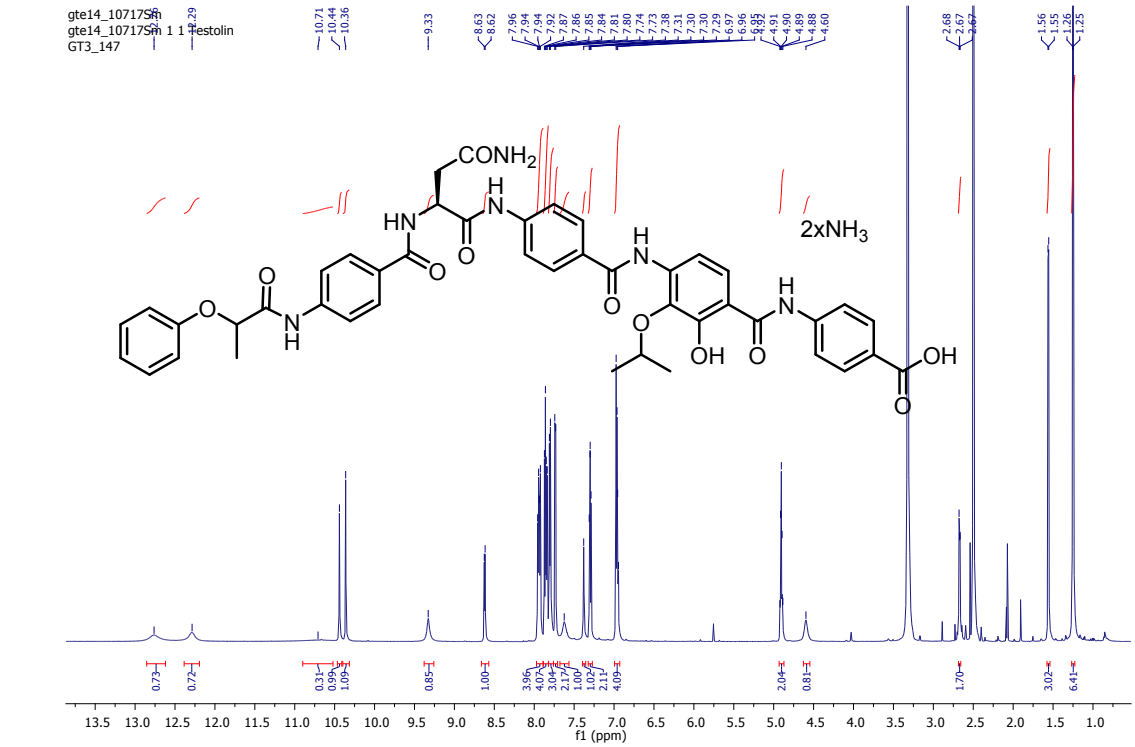
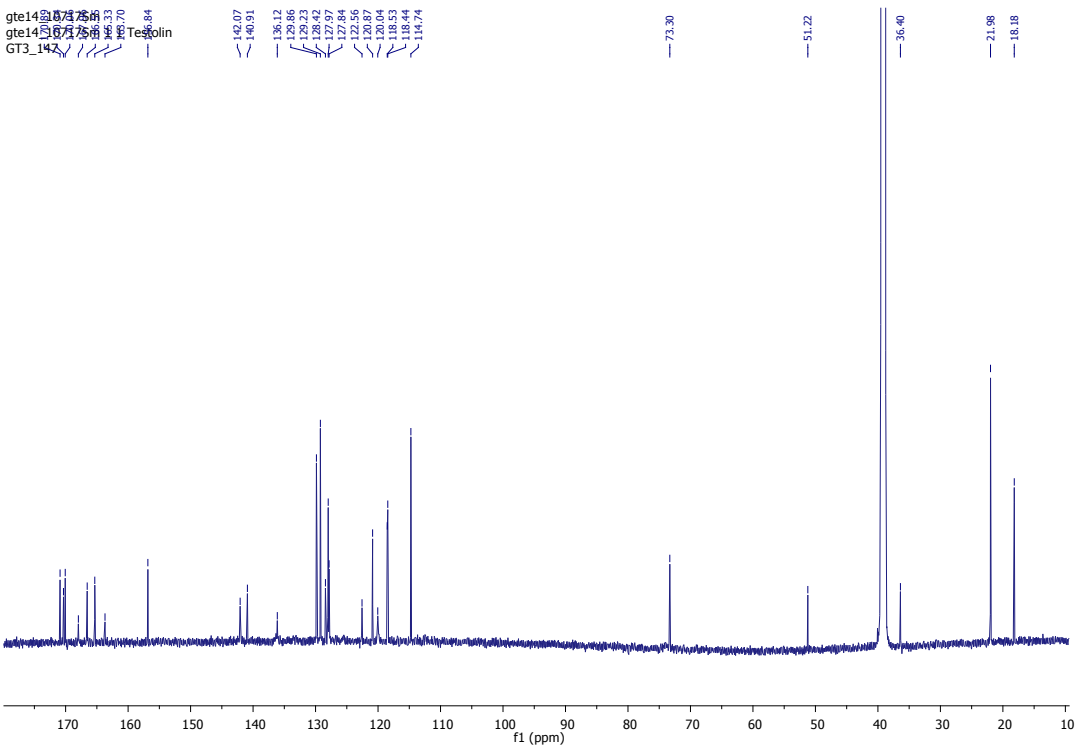




4-(4-(4-((2S)-4-Amino-4-oxo-2-(4-(2-phenoxypropanamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid





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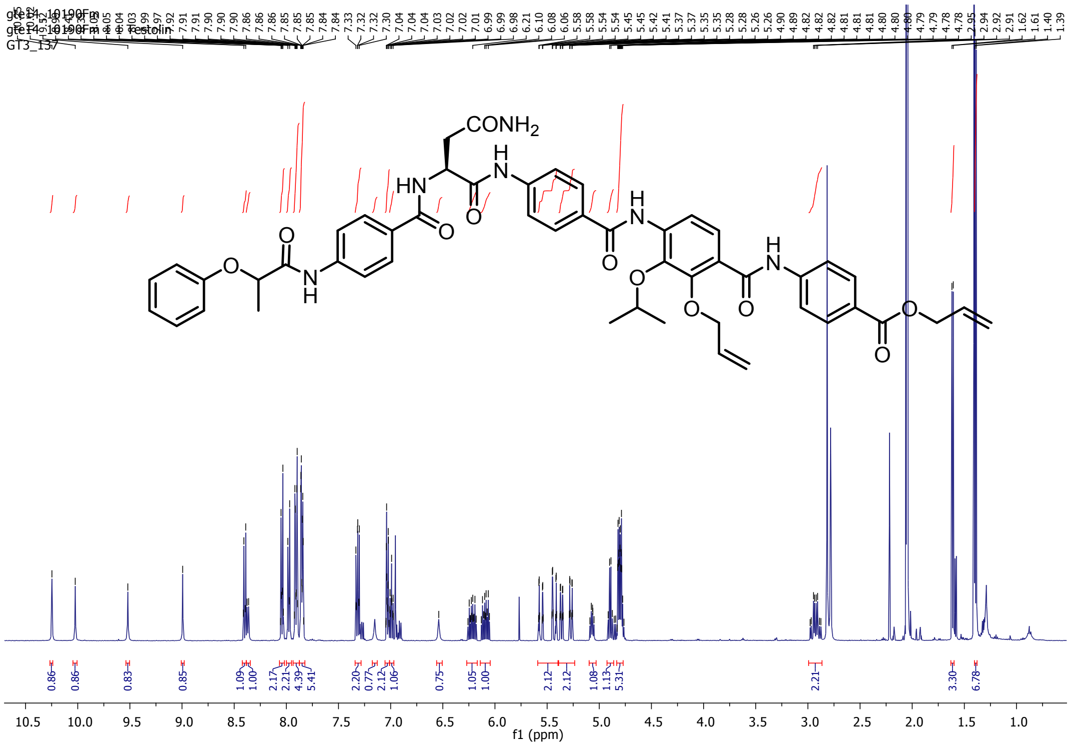
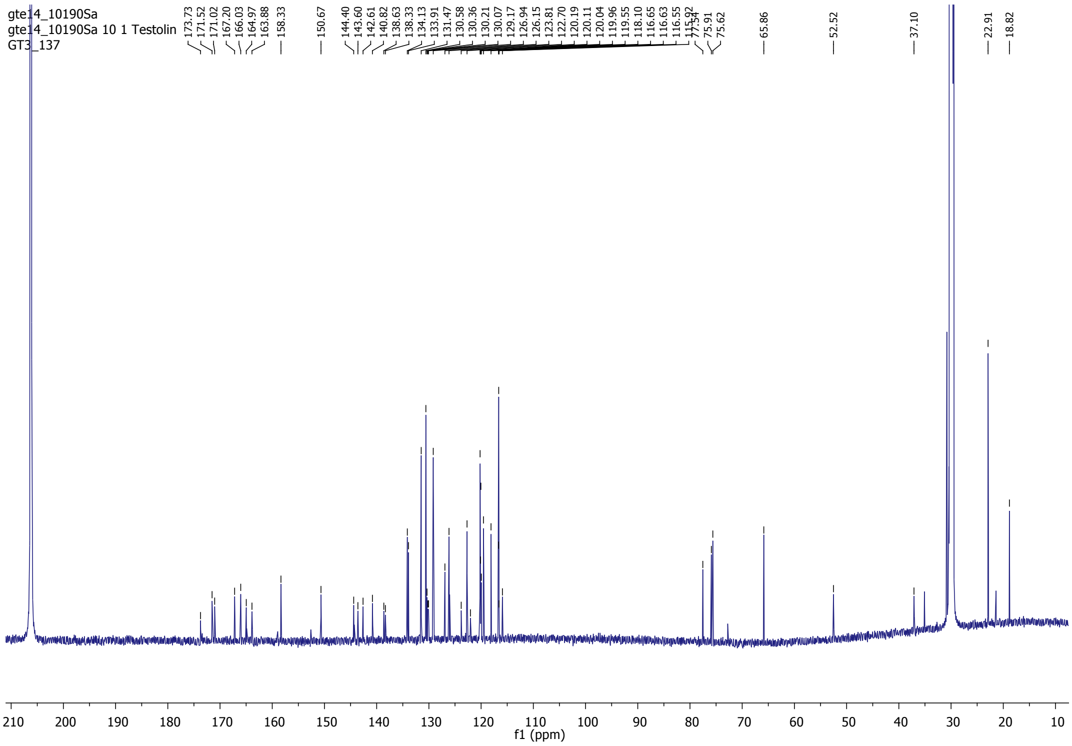
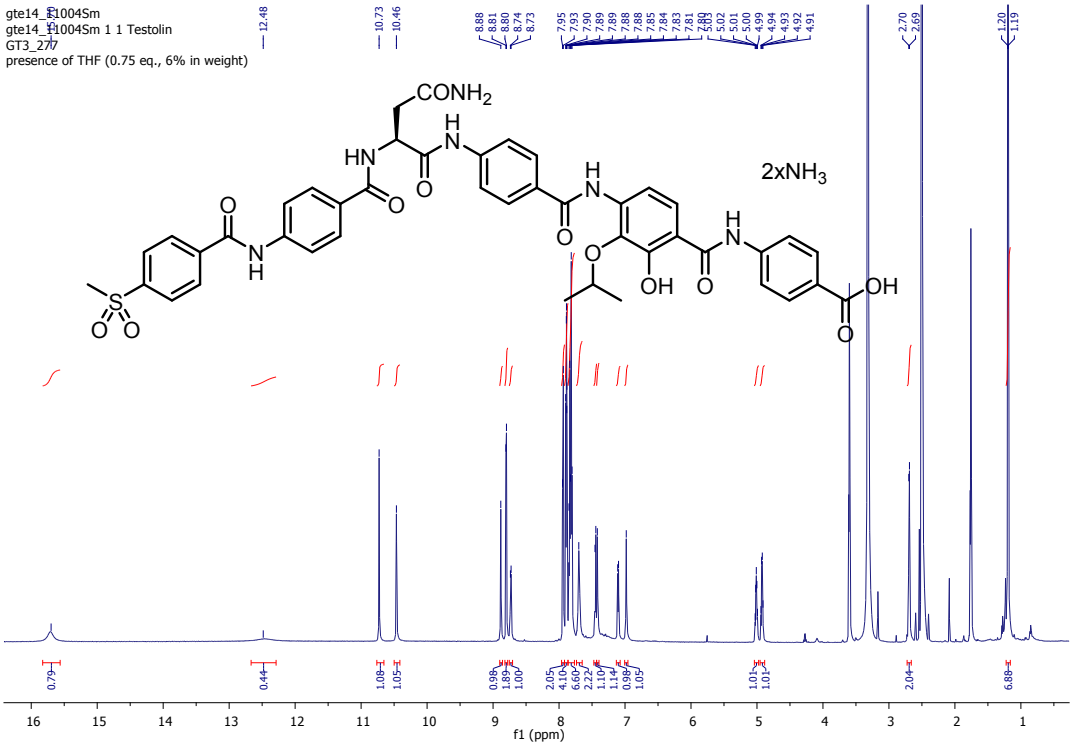
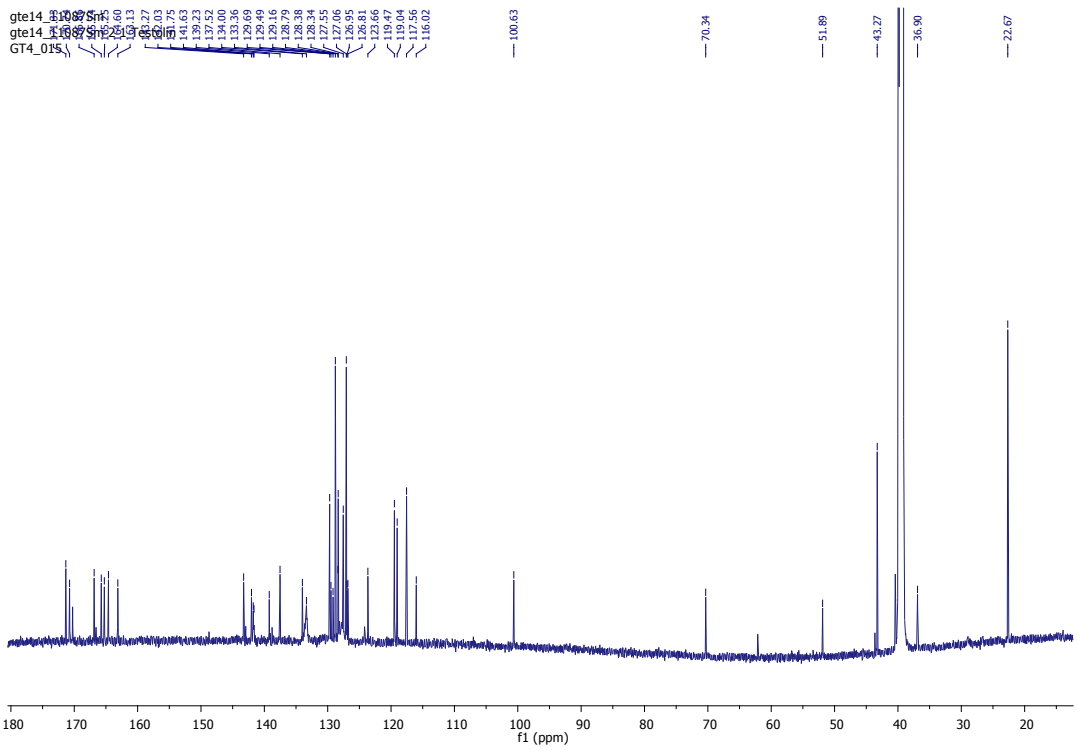


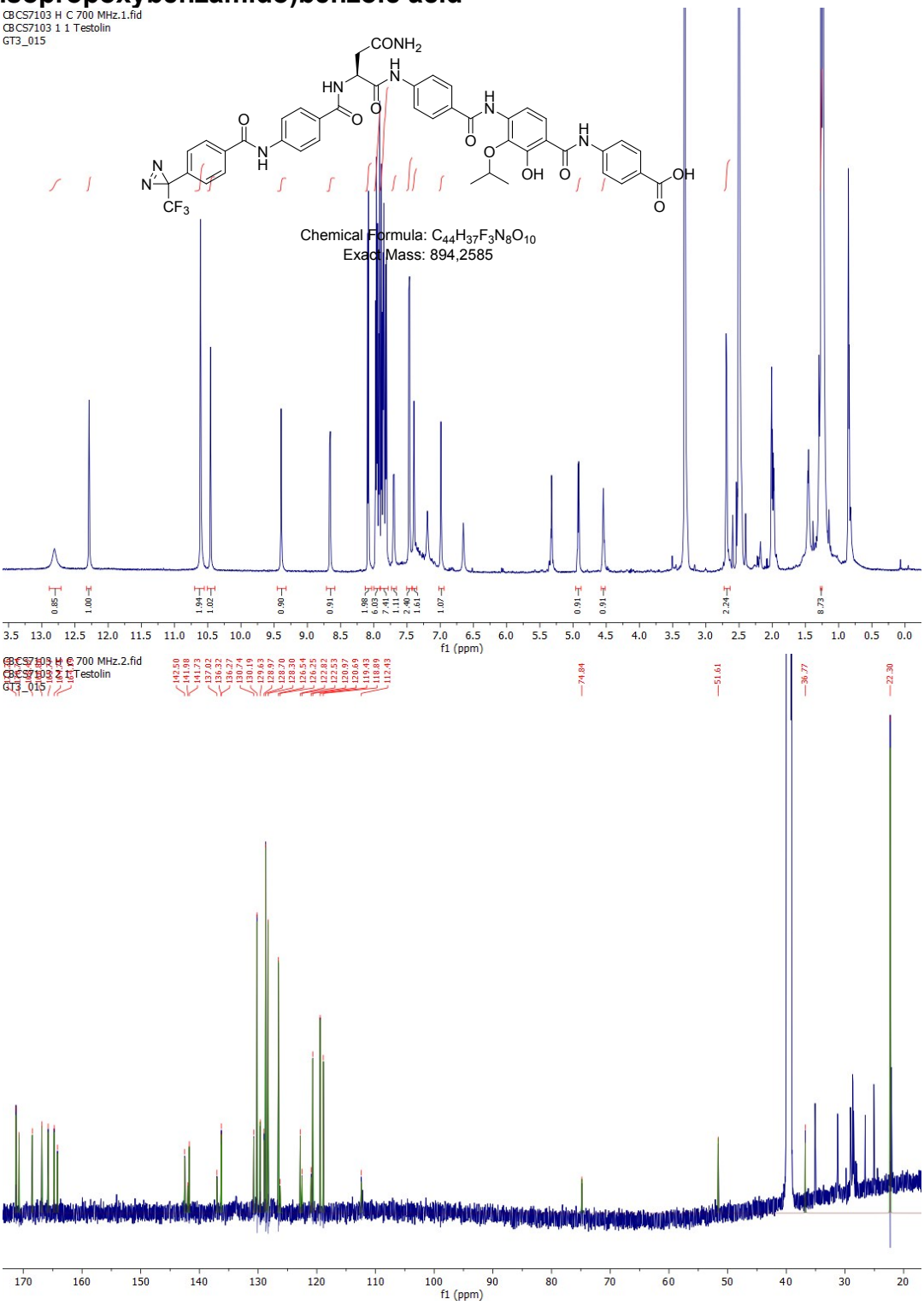


(S)-4-(4-(4-(4-Amino-2-(4-(4-(methylsulfonyl)benzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

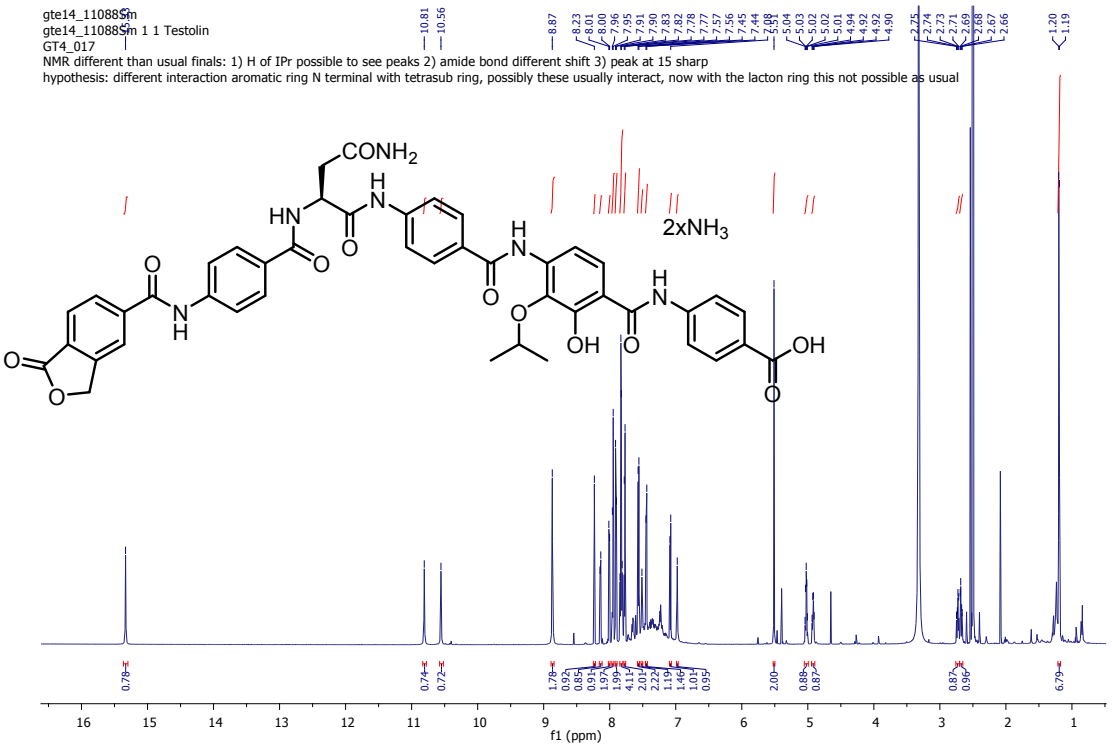


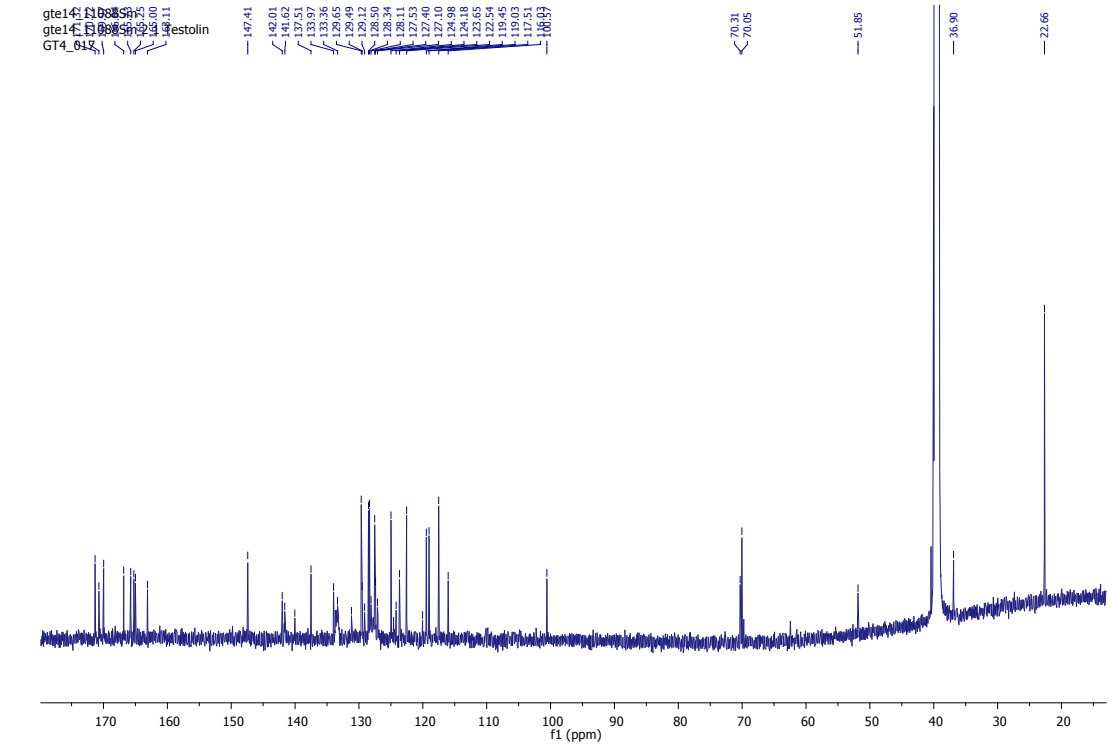


(S)-4-(4-(4-(4-amino-4-oxo-2-(4-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

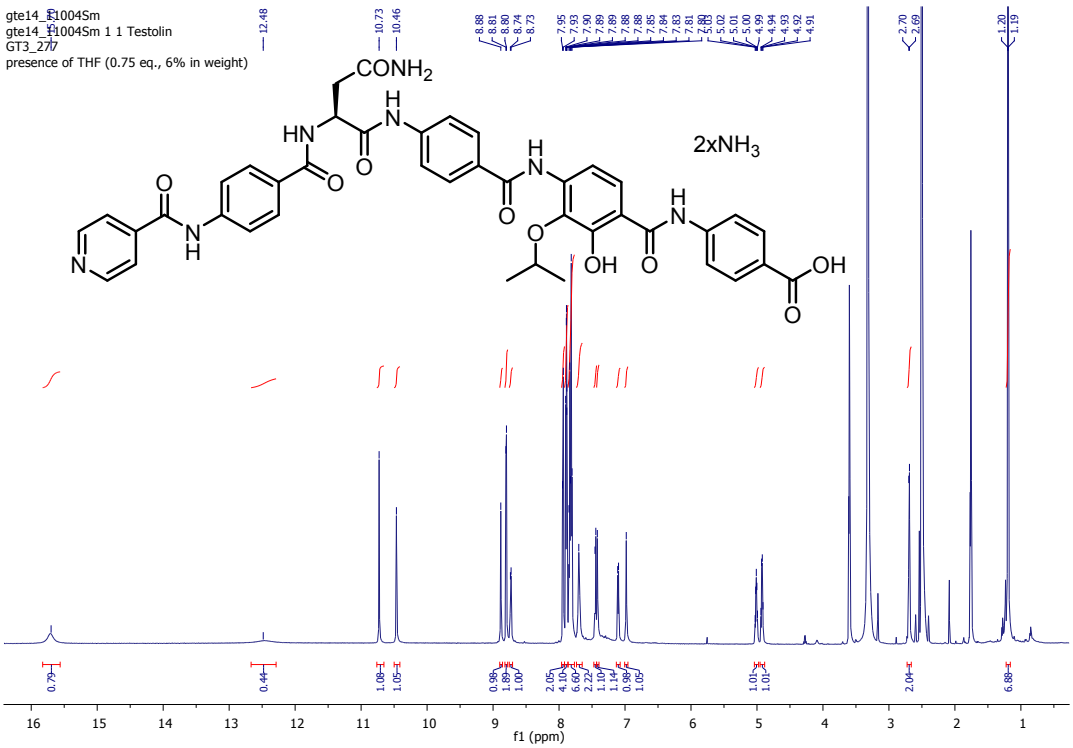


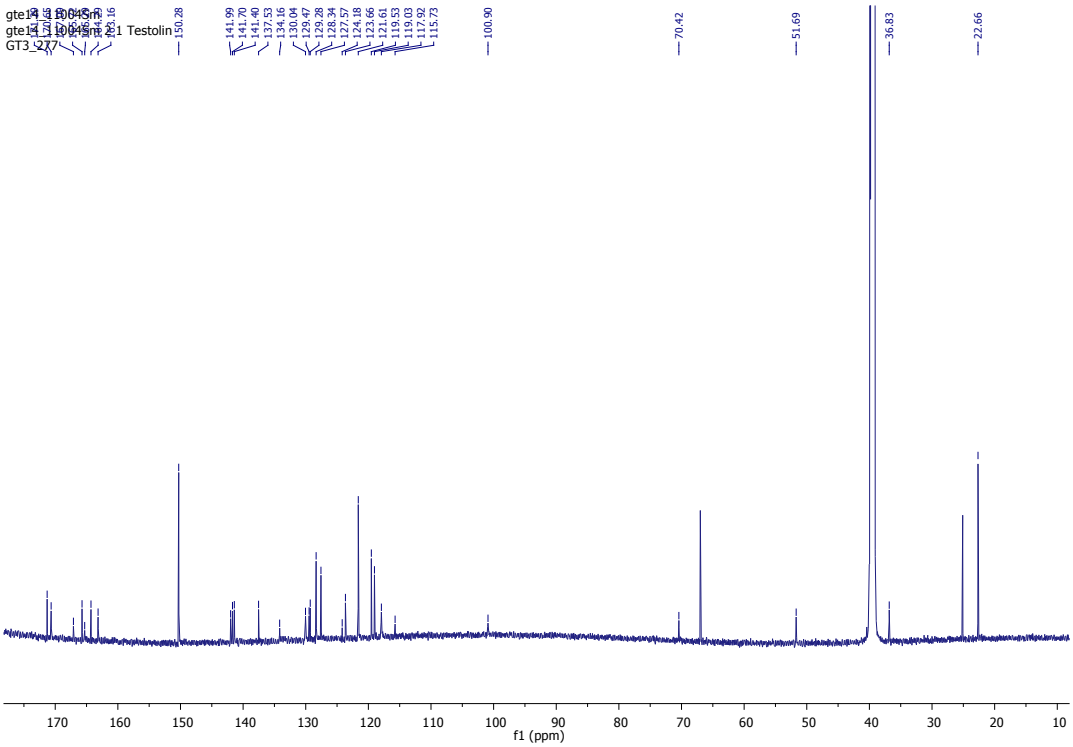
(S)-4-(4-(4-(4-Amino-4-oxo-2-(4-(1-oxo-1,3-dihydroisobenzofuran-5-carboxamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



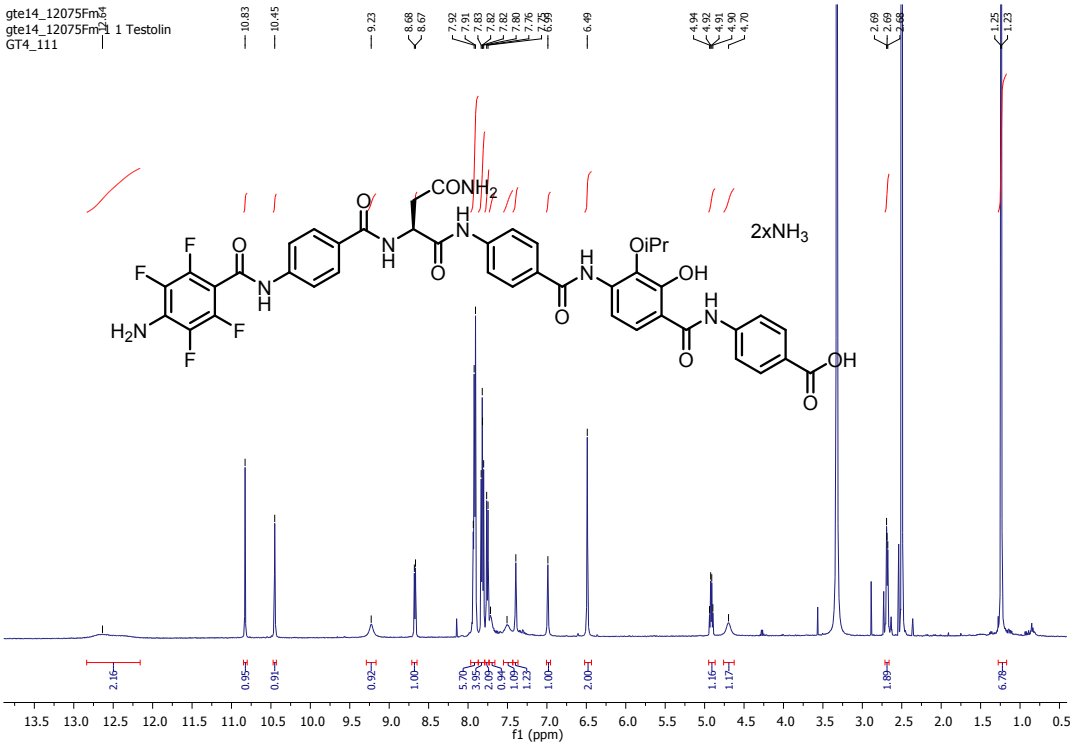


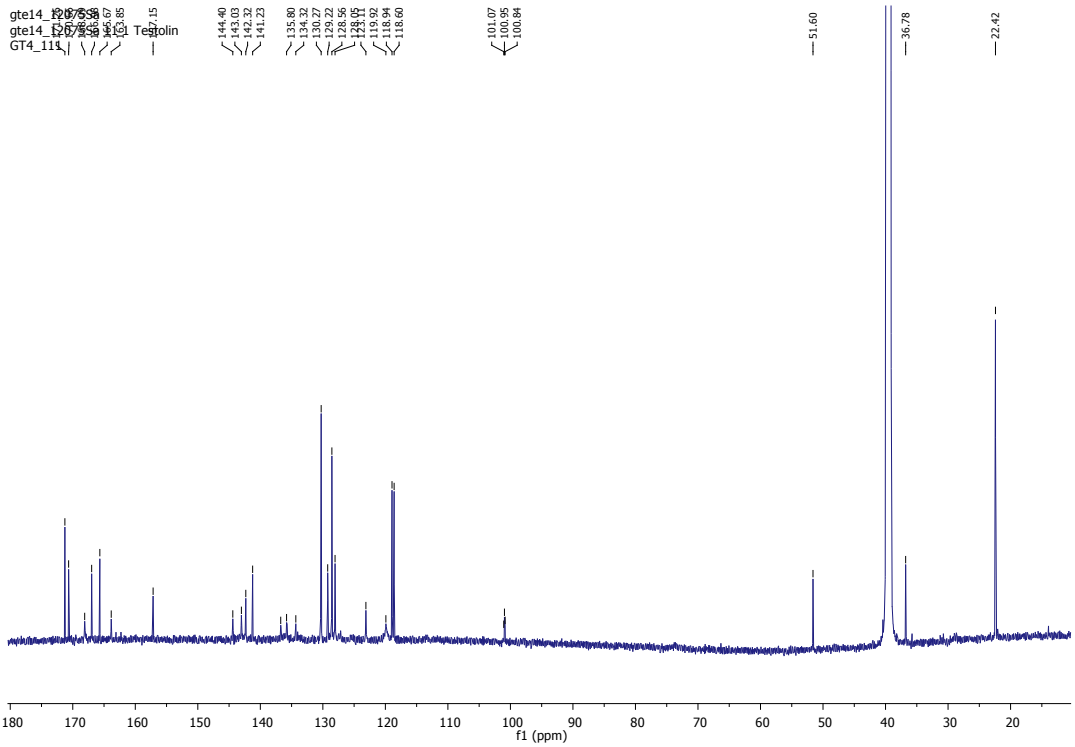
(S)-4-(4-(4-(4-Amino-2-(4-(isonicotinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



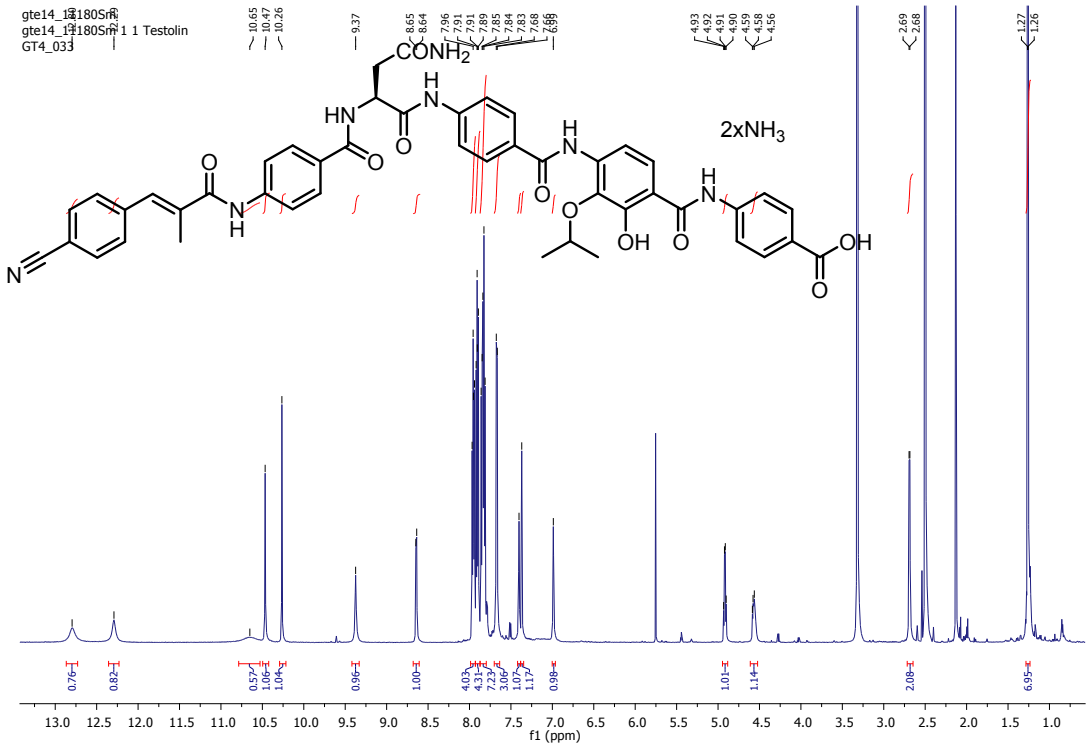


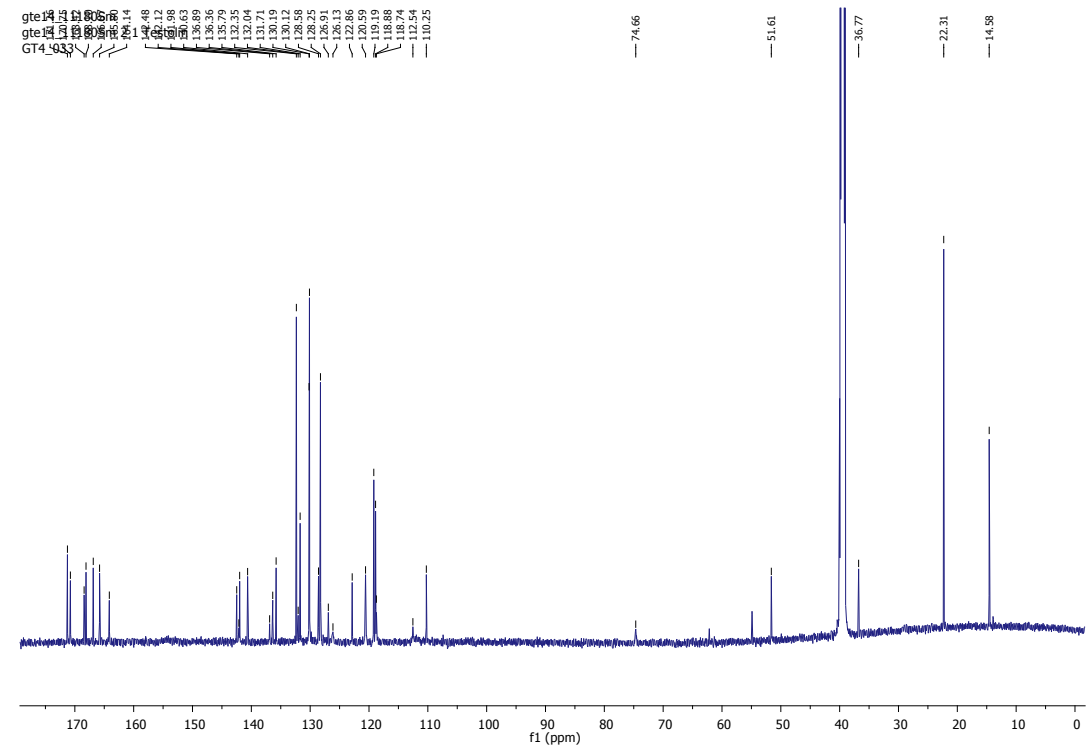
(S)-4-(4-(4-(4-Amino-2-(4-(4-amino-2,3,5,6-tetrafluorobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



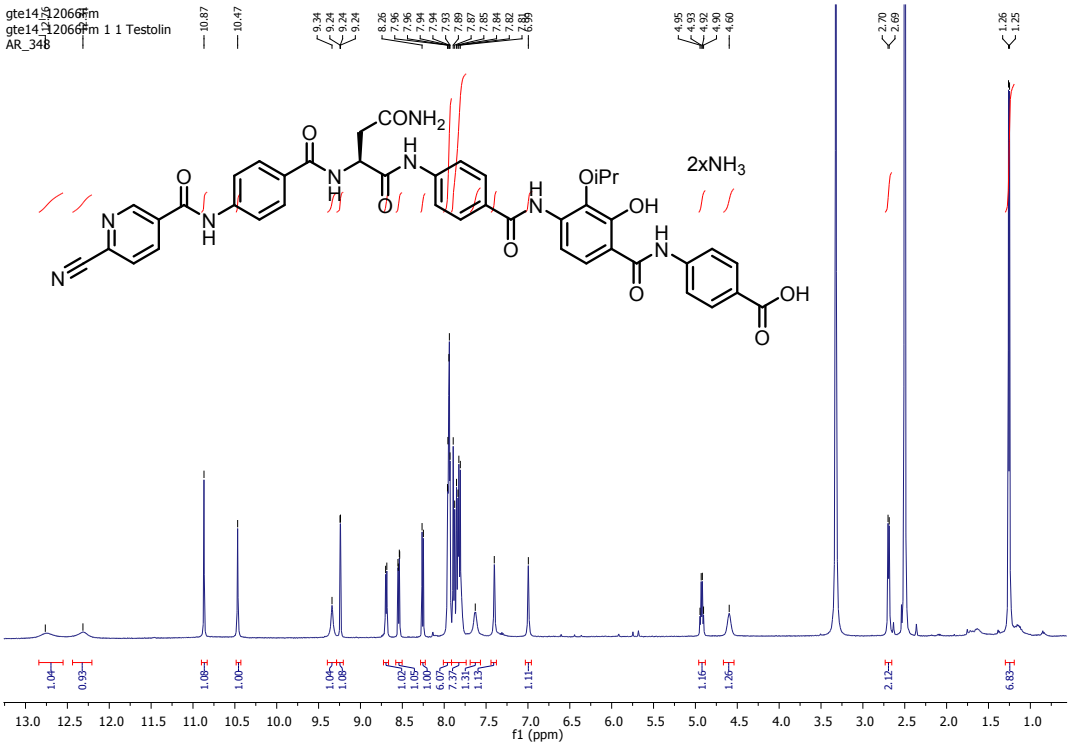


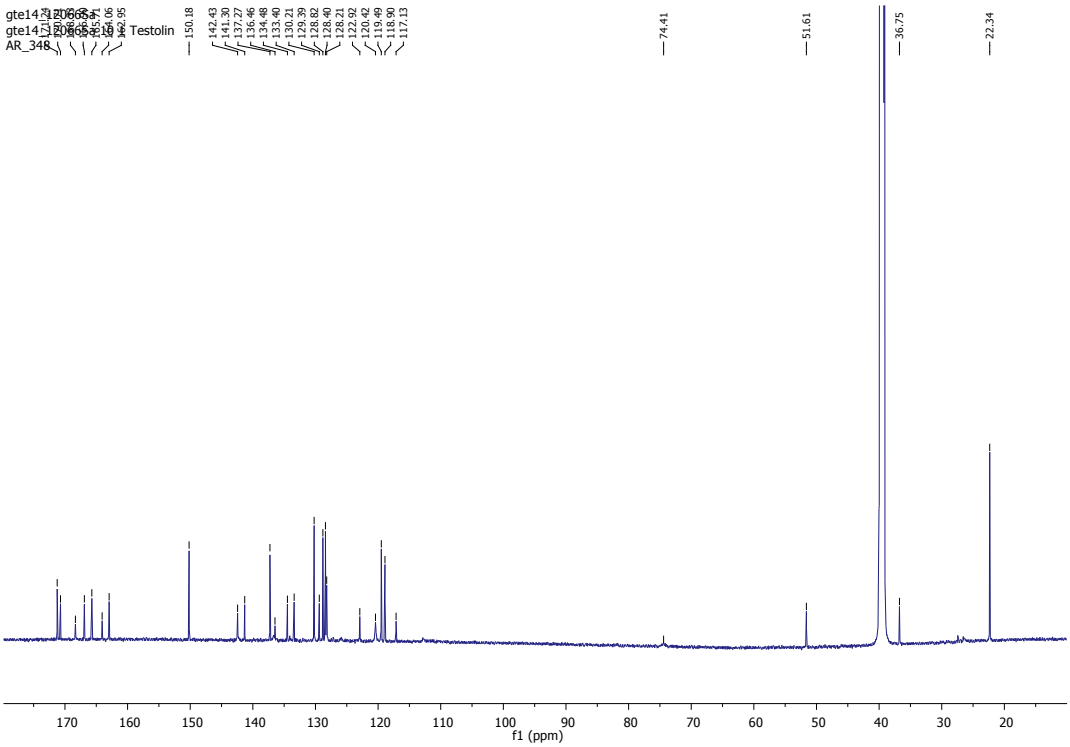
(S,E)-4-(4-(4-(4-Amino-2-(4-(3-(4-cyanophenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic aci



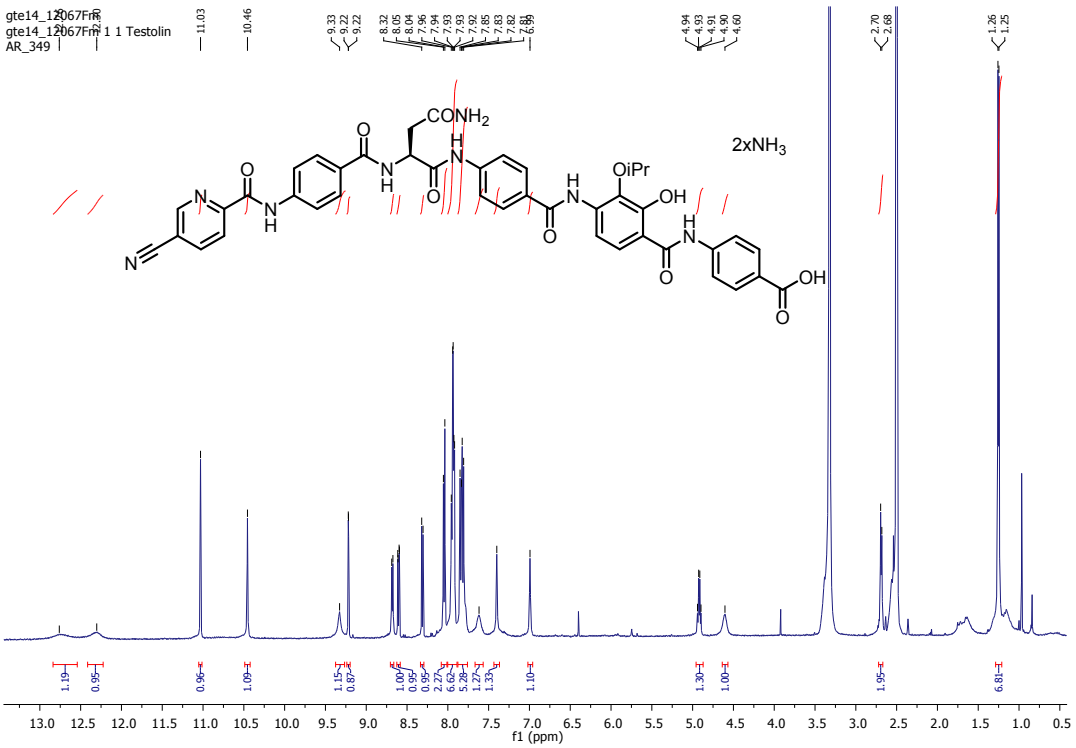


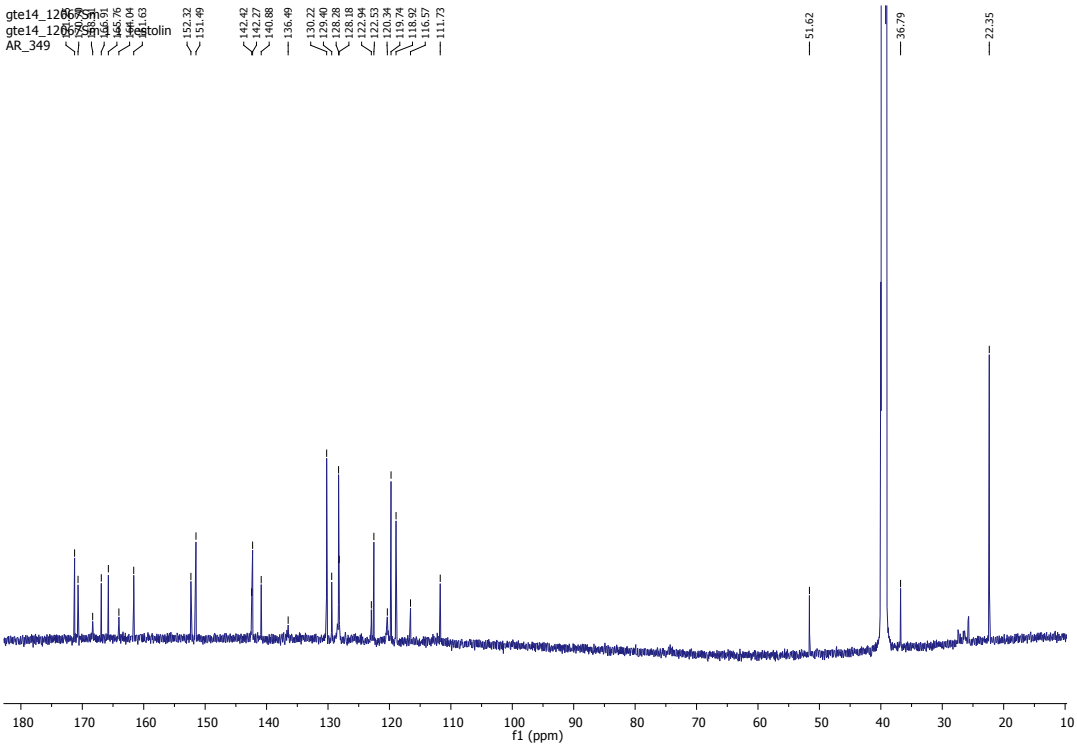
(S)-4-(4-(4-(4-Amino-2-(4-(6-cyanonicotinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



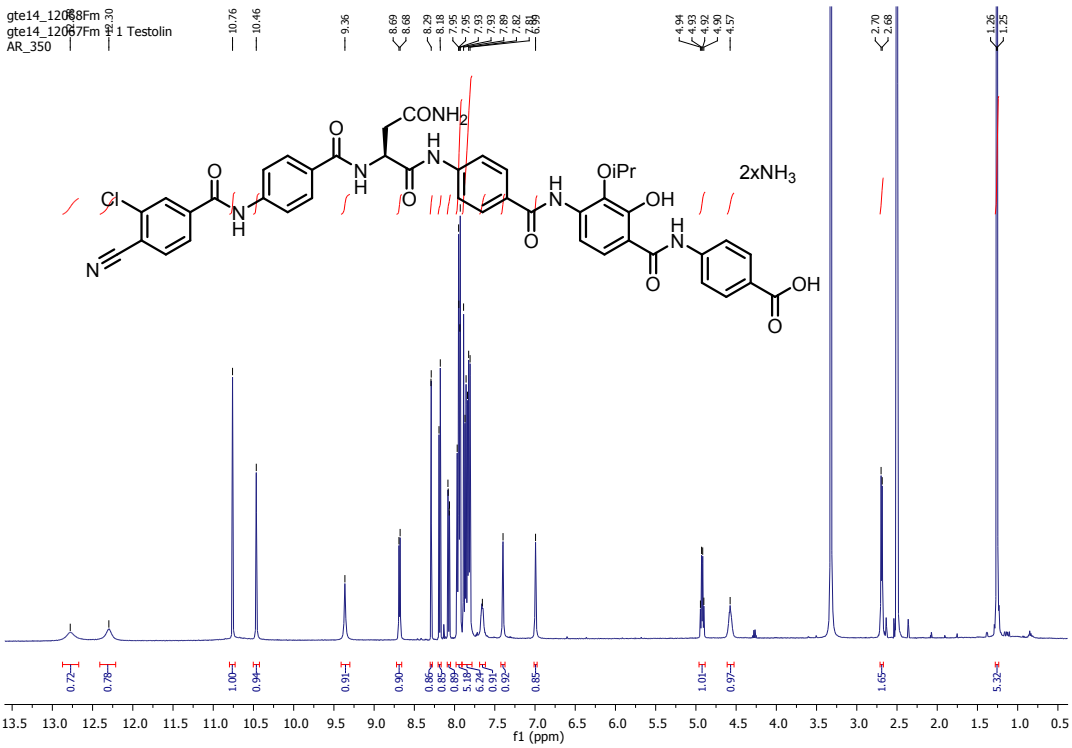


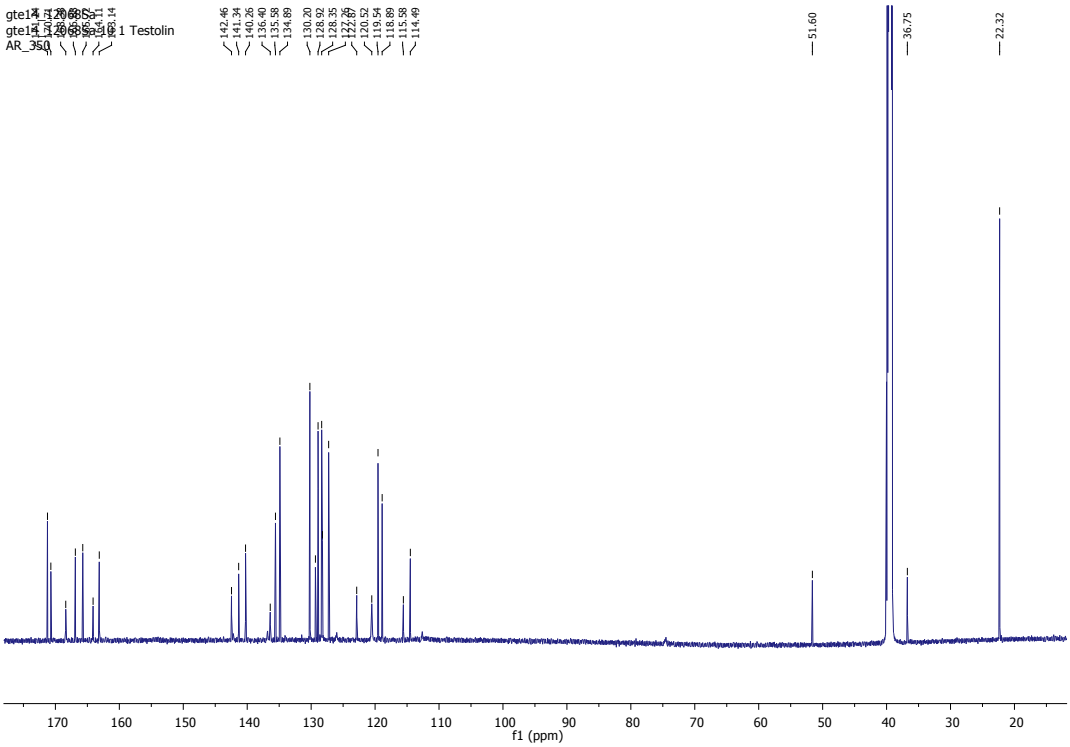
(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanopicolinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



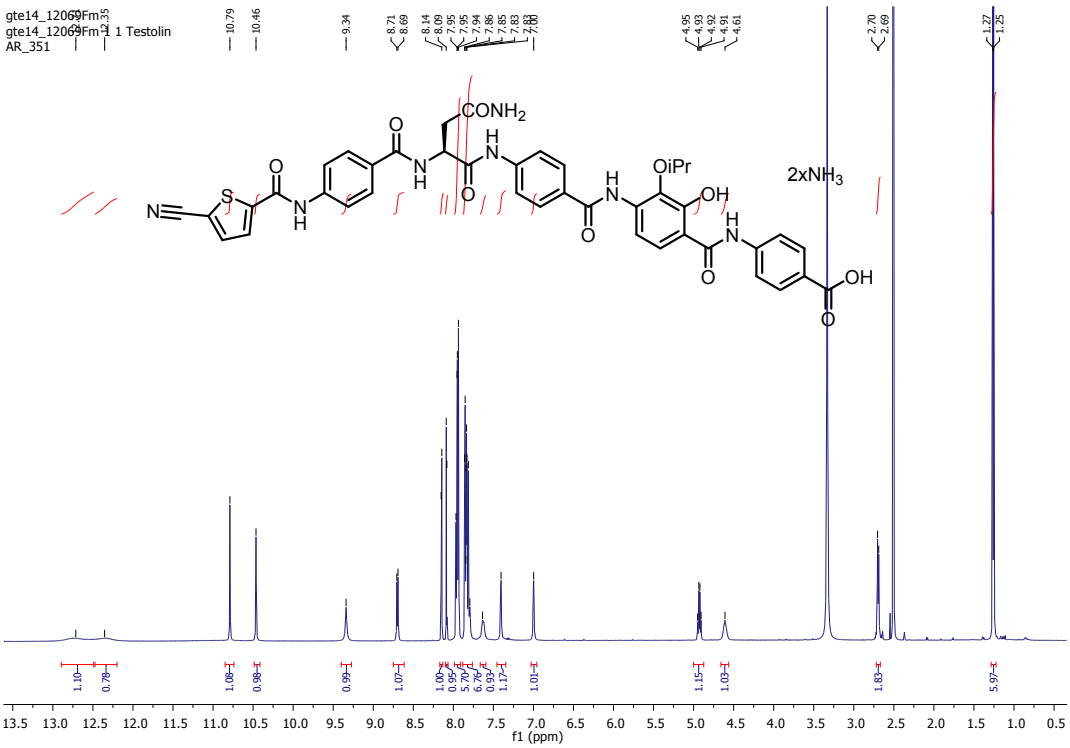


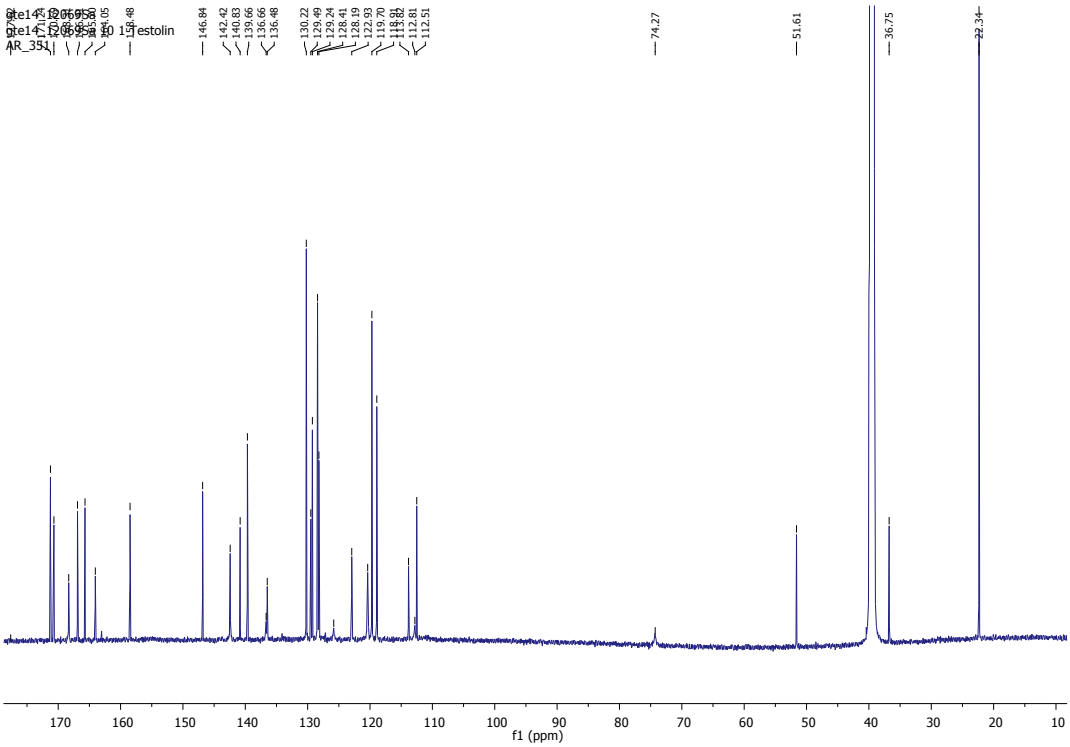
(S)-4-(4-(4-(4-Amino-2-(4-(3-chloro-4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



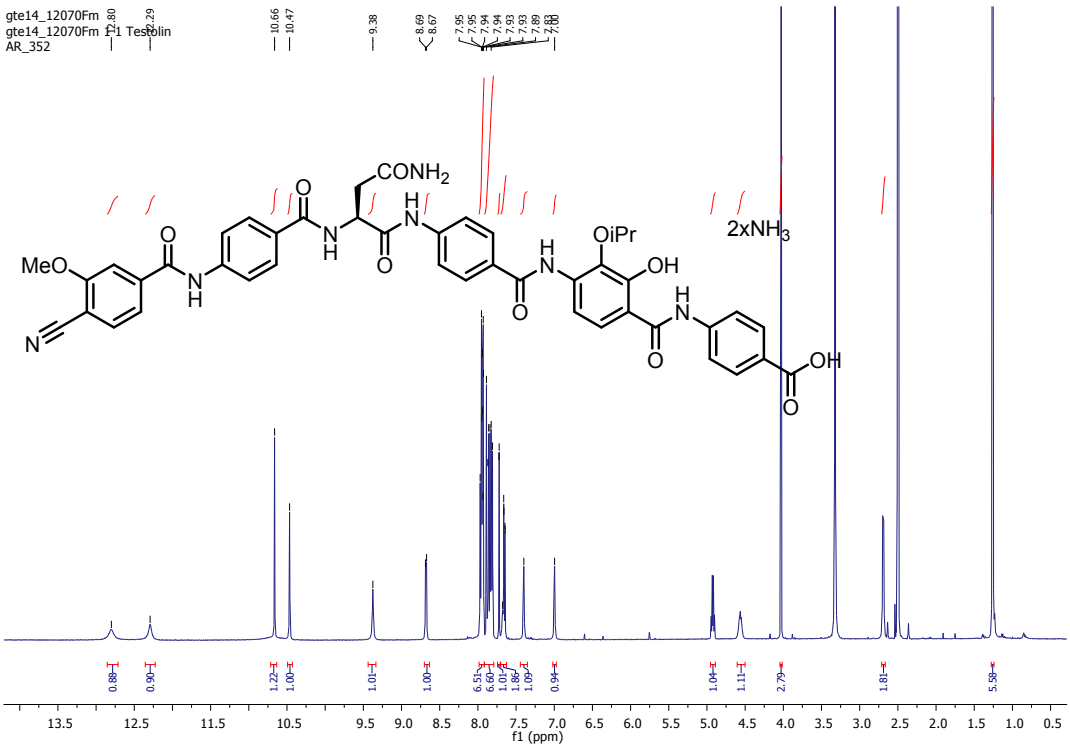


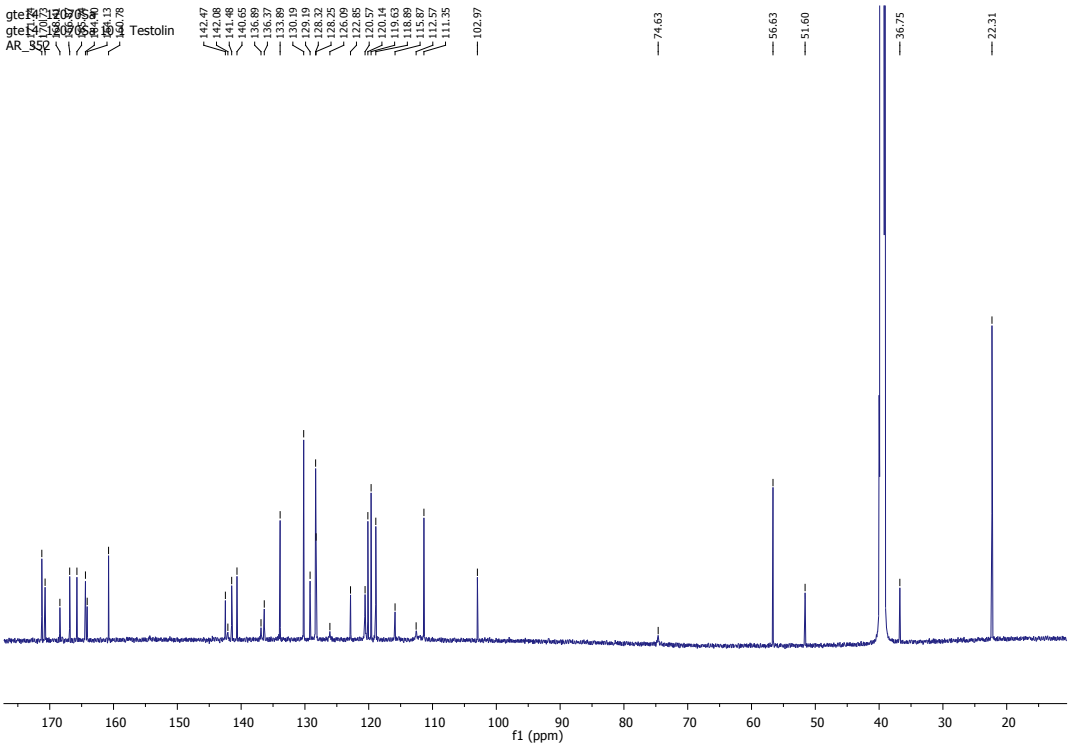
(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanothiophene-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



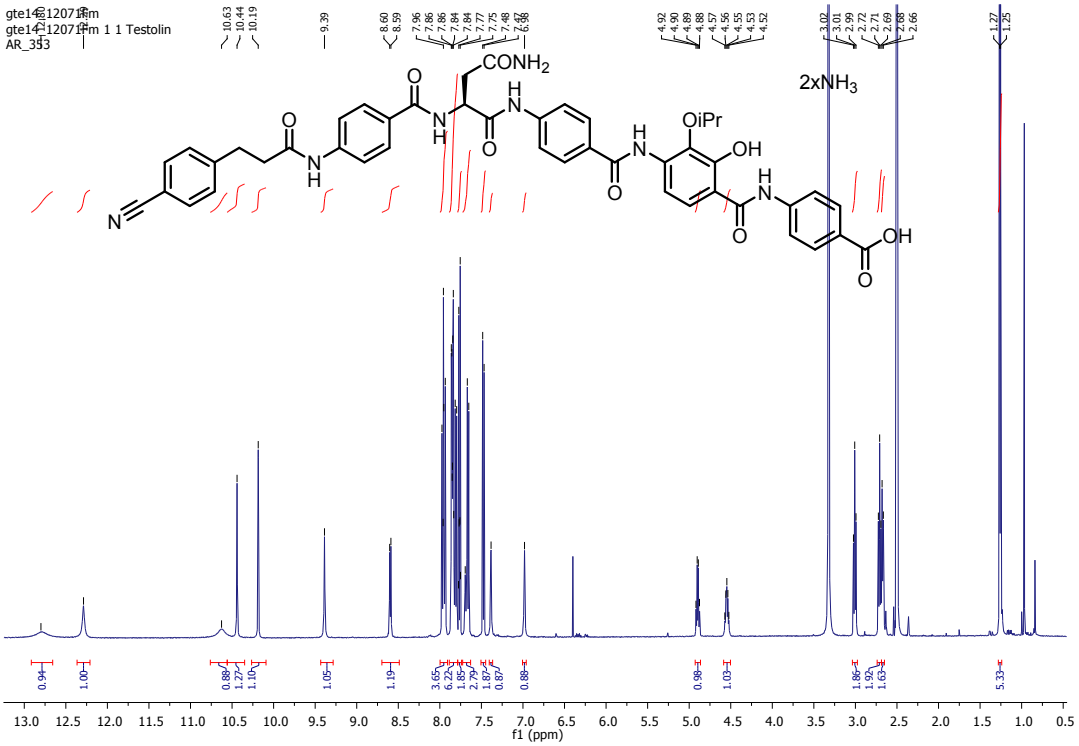


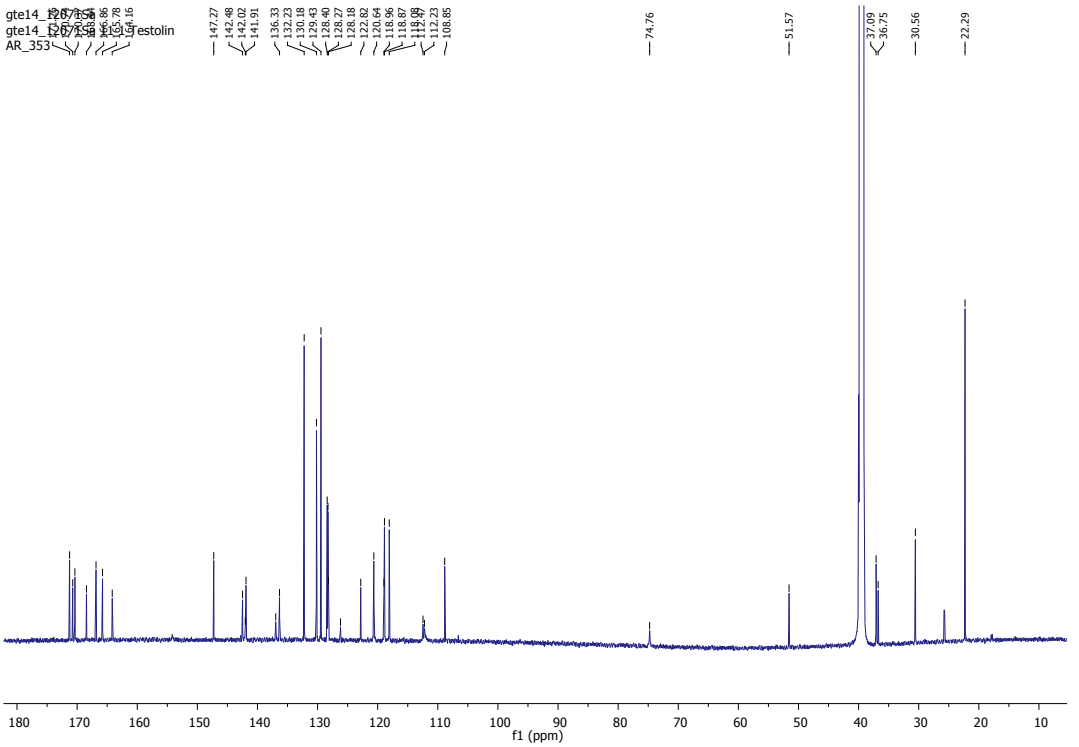
(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-3-methoxybenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



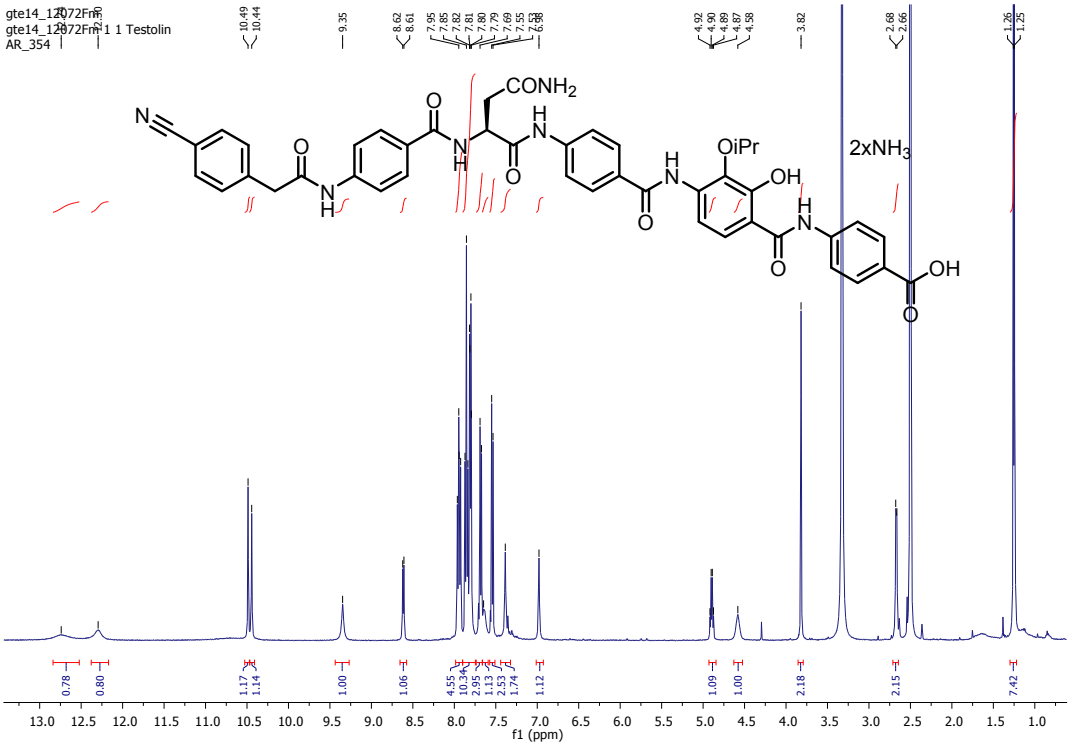


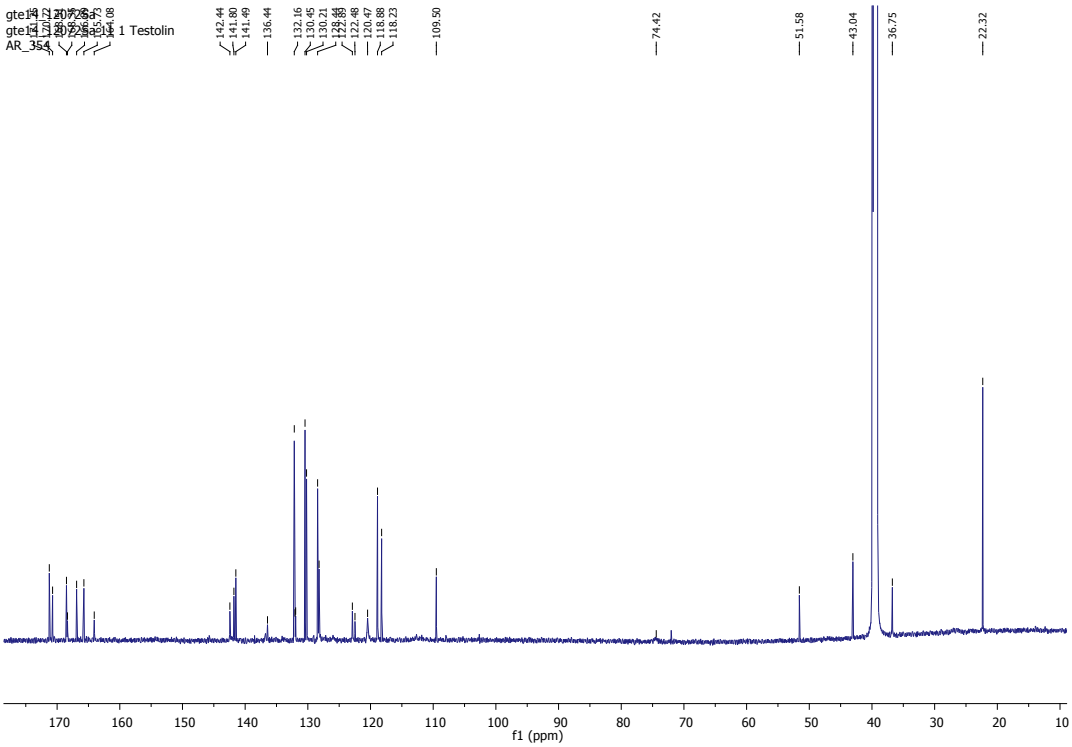
(S)-4-(4-(4-(4-Amino-2-(4-(3-(4-cyanophenyl)propanamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



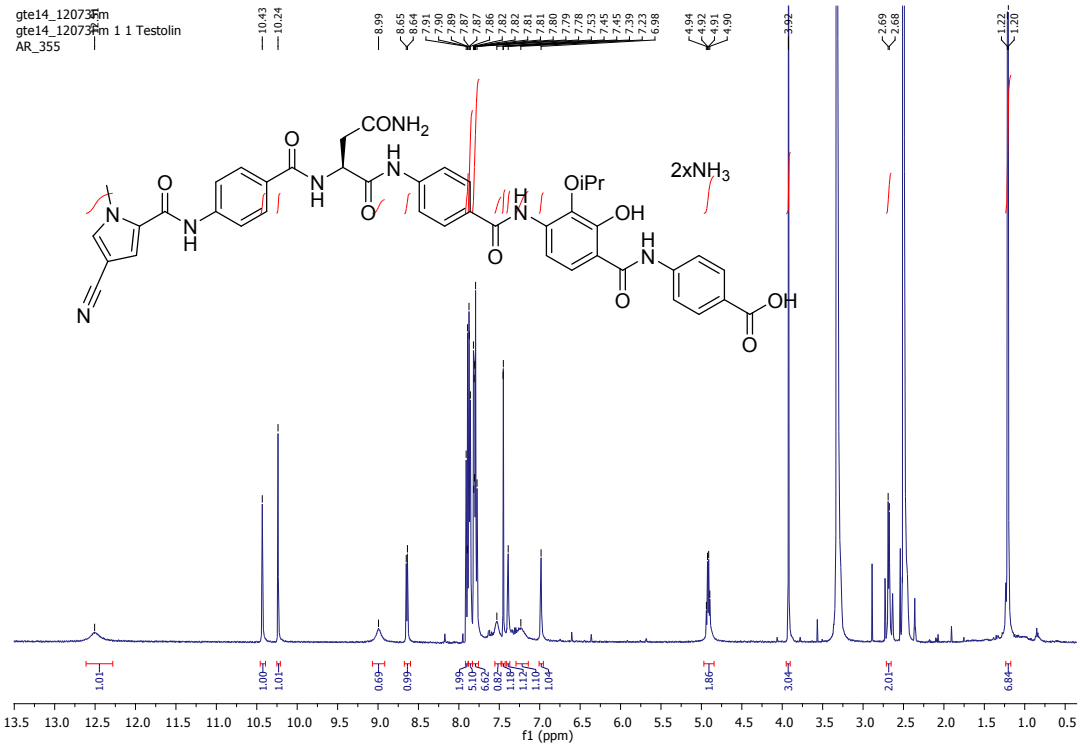


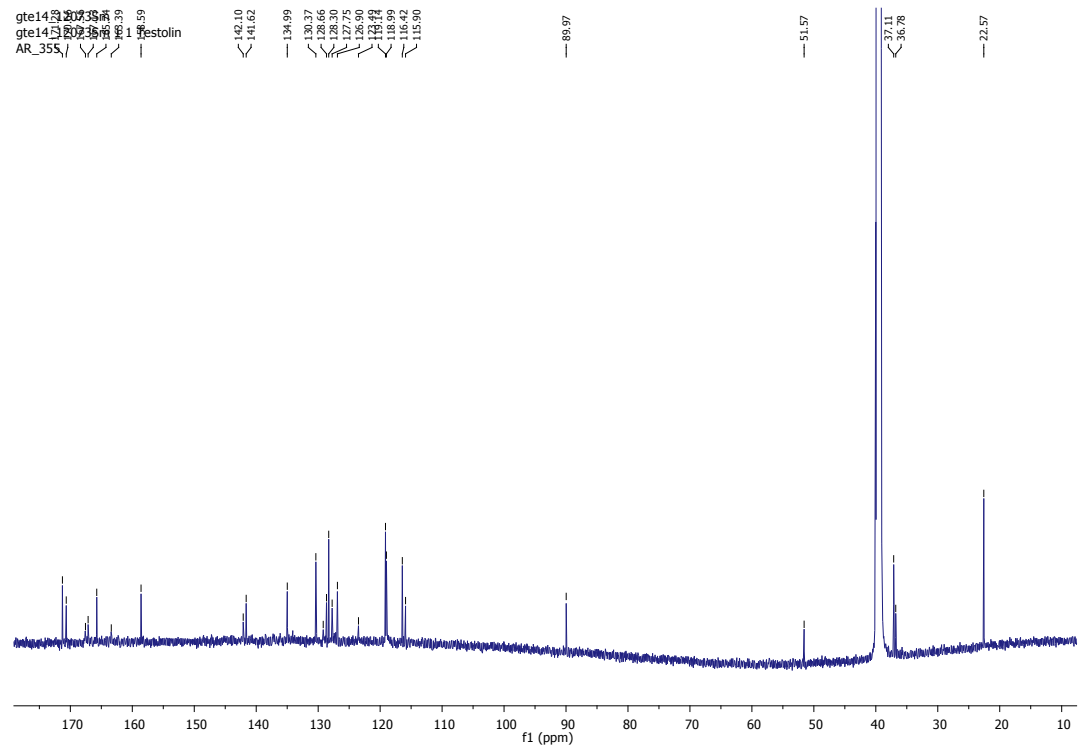
(S)-4-(4-(4-(4-Amino-2-(4-(2-(4-cyanophenyl)acetamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



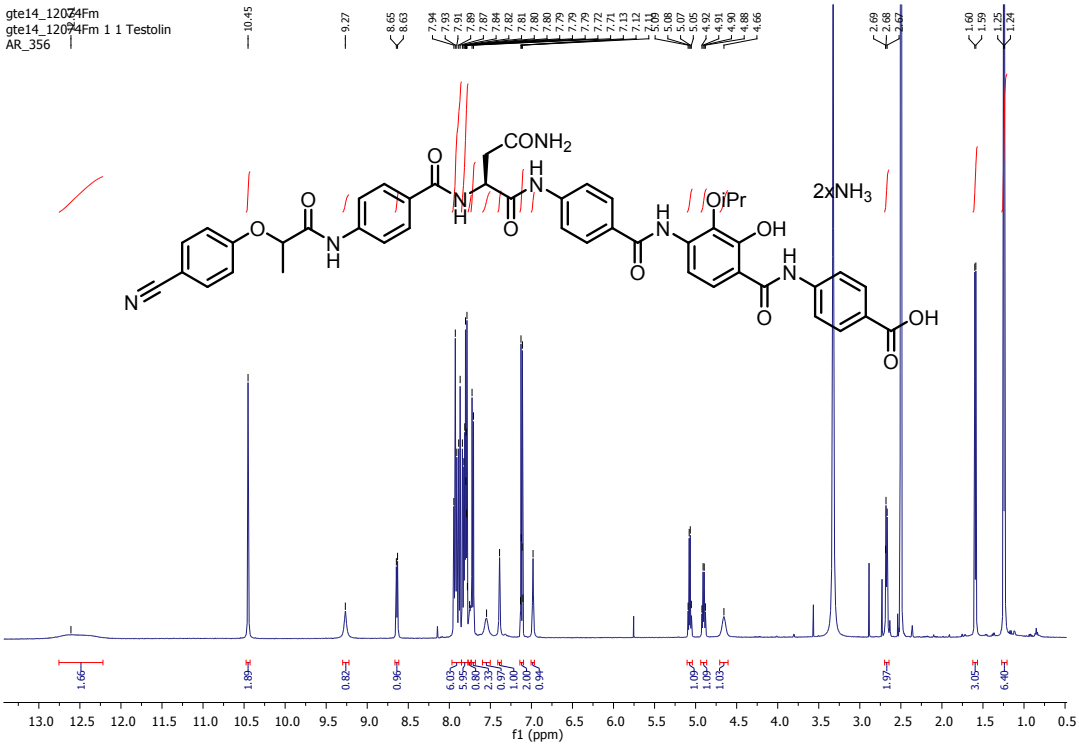


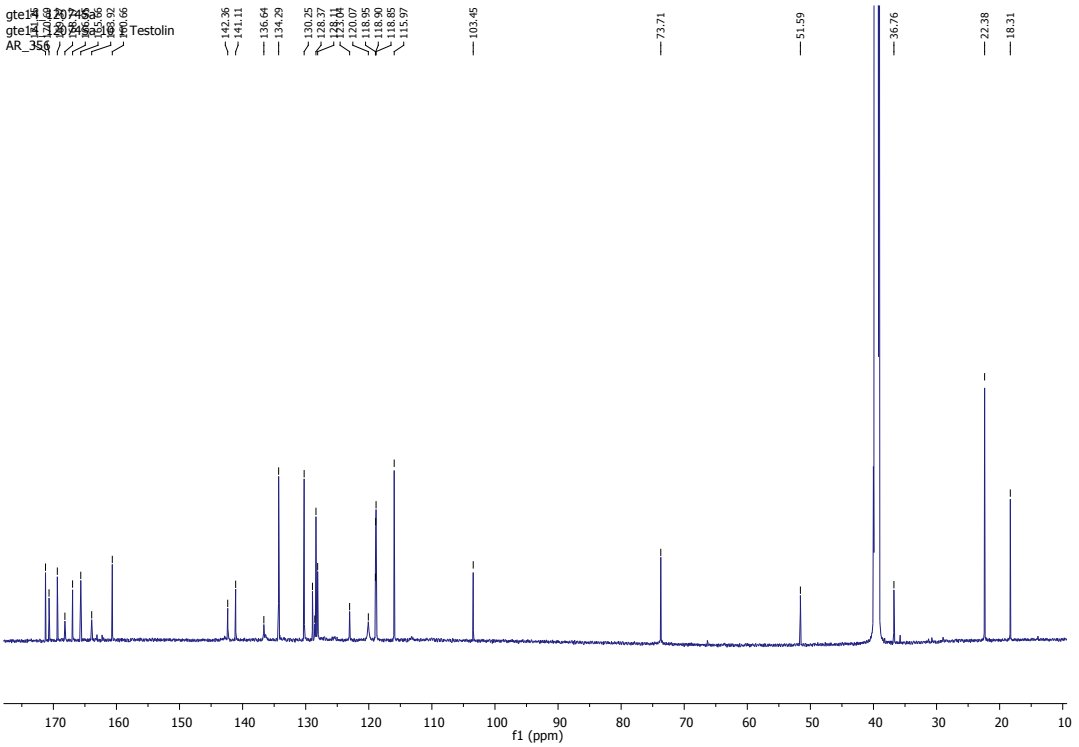
(S)-4-(4-(4-(4-Amino-2-(4-(4-cyano-1-methyl-1H-pyrrole-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



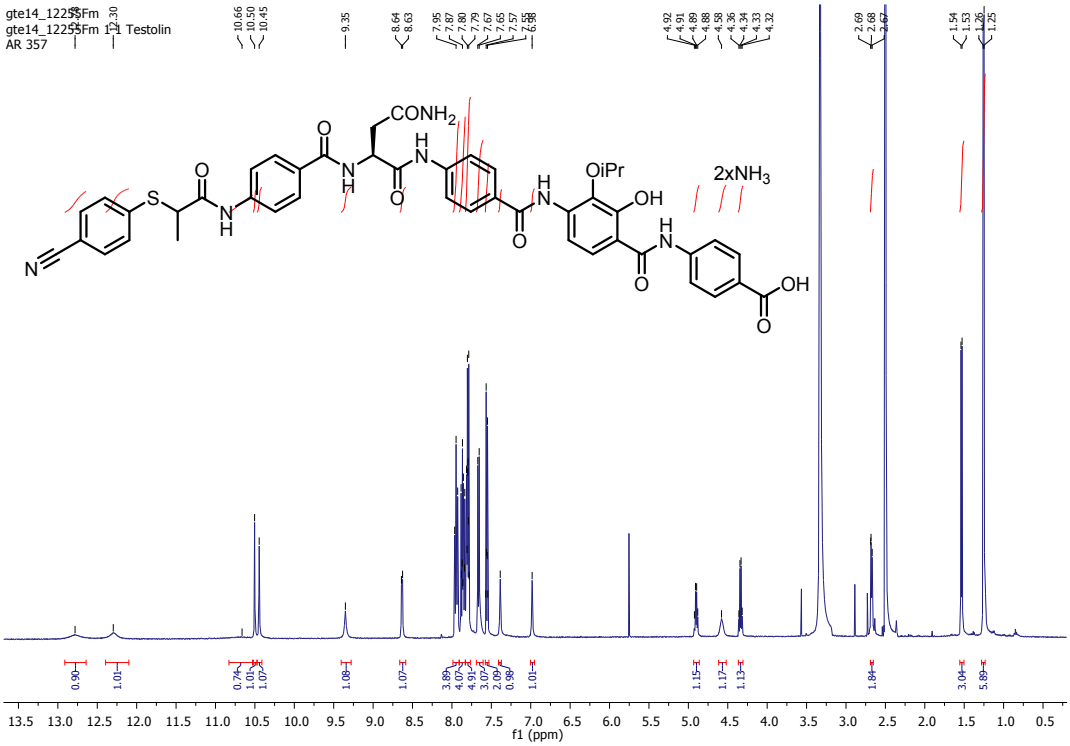


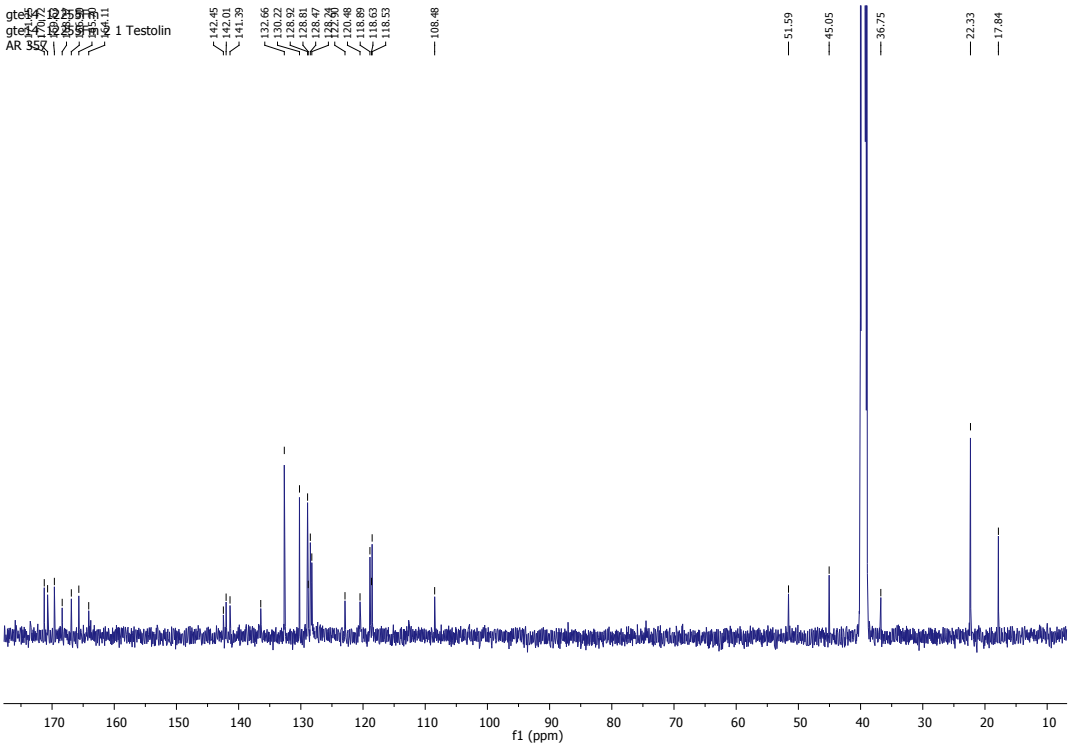
4-(4-(4-((2S)-4-Amino-2-(4-(2-(4-cyanophenoxy)propanamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



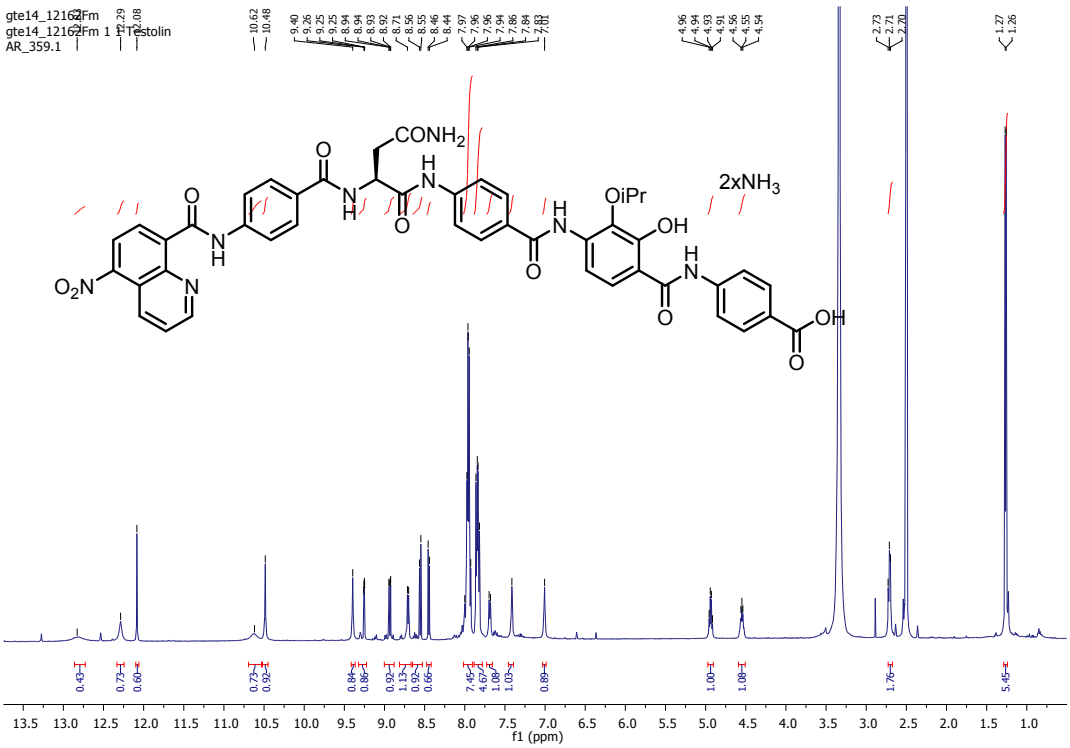


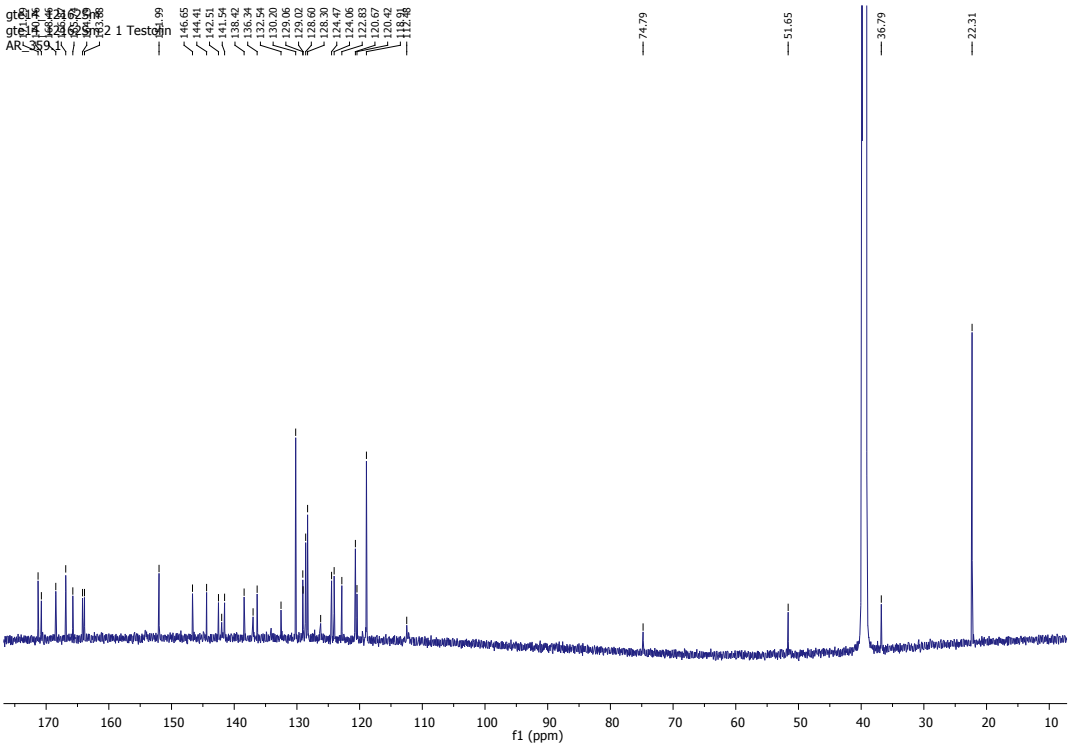
4-(4-(4-((2S)-4-Amino-2-(4-(2-((4-cyanophenyl)thio)propanamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



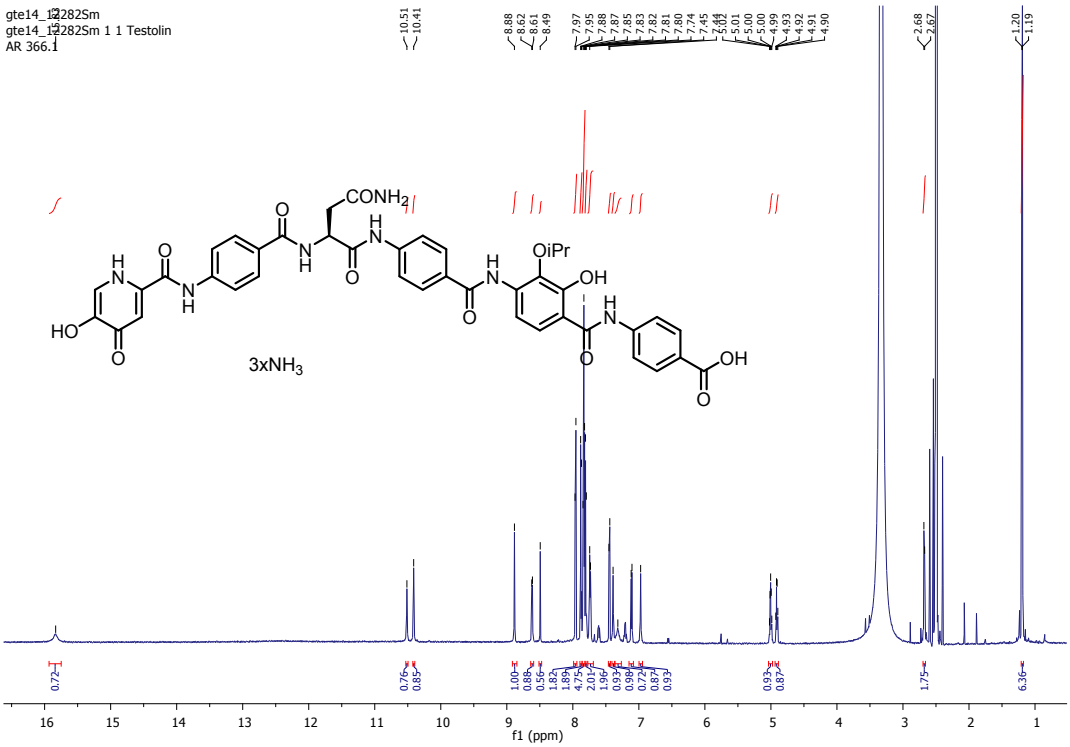


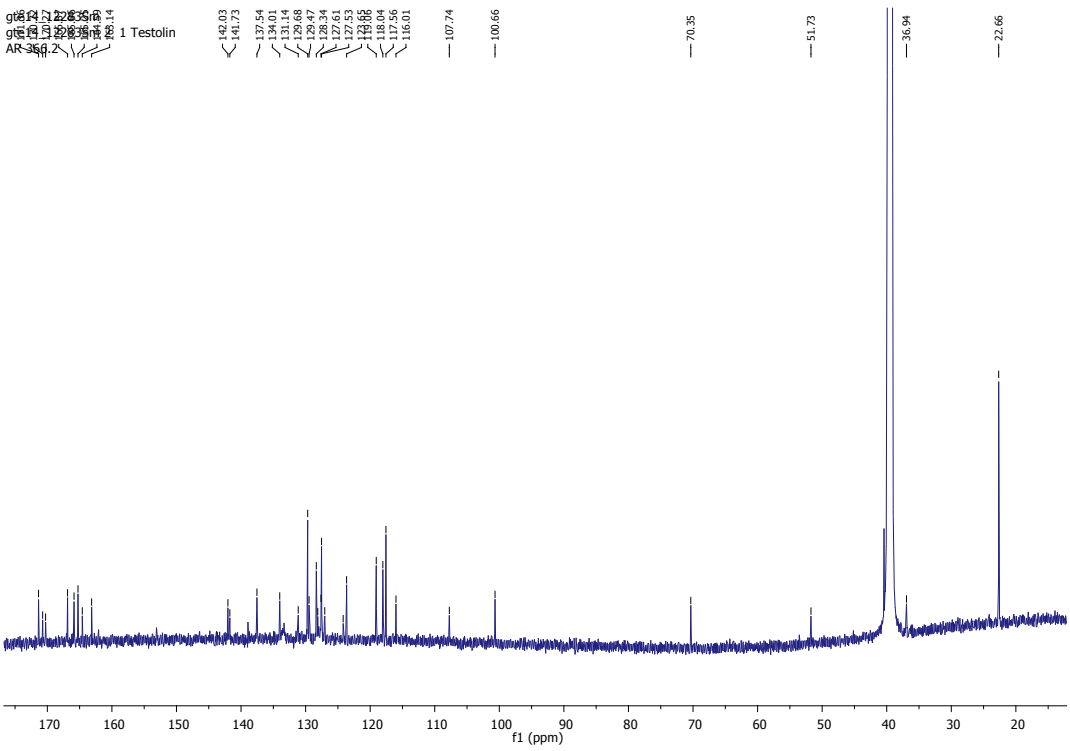
(S)-4-(4-(4-(4-Amino-2-(4-(5-nitroquinoline-8-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



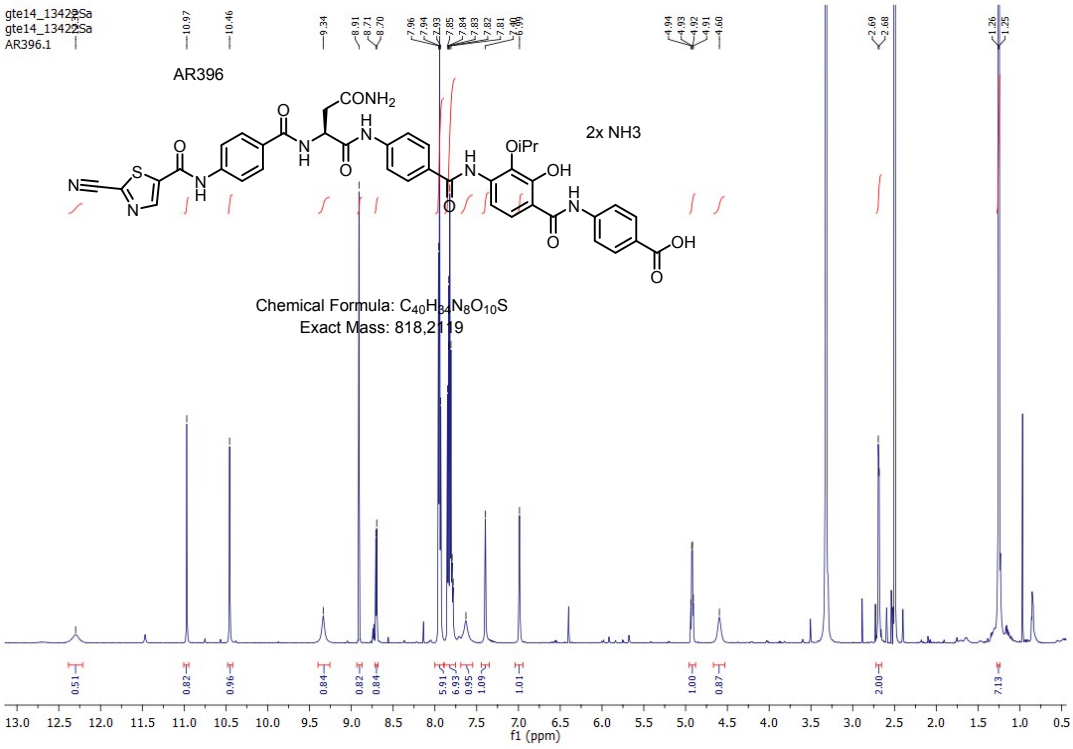


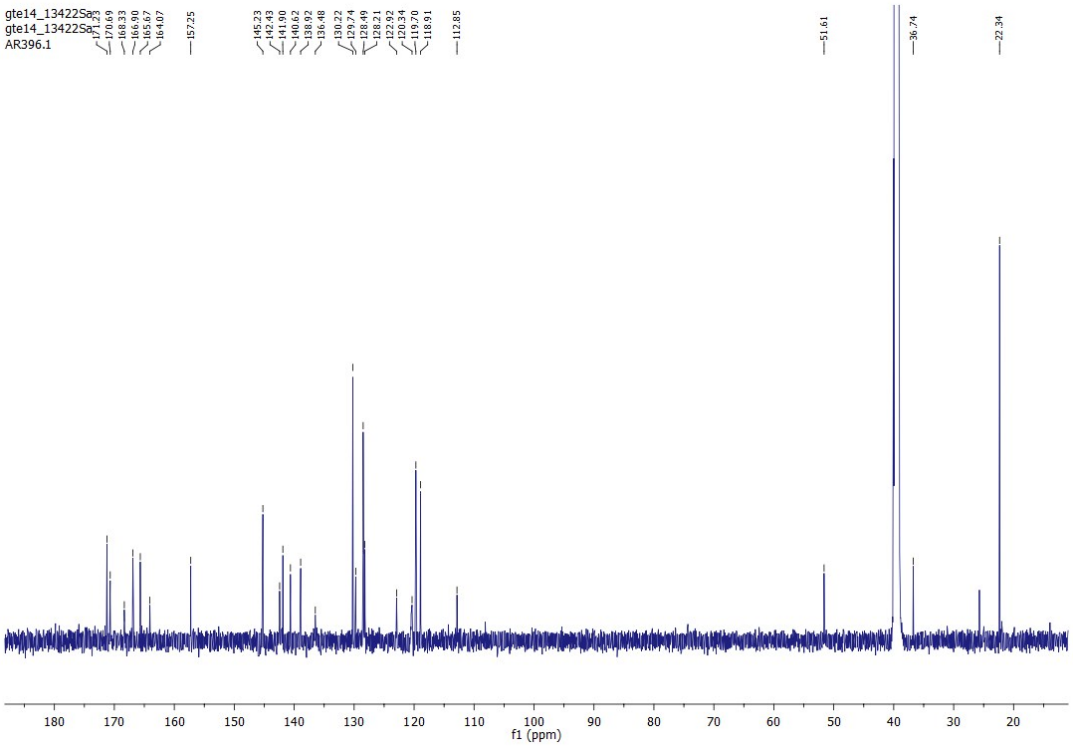
(S)-4-(4-(4-(4-Amino-2-(4-(5-hydroxy-4-oxo-1,4-dihydropyridine-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



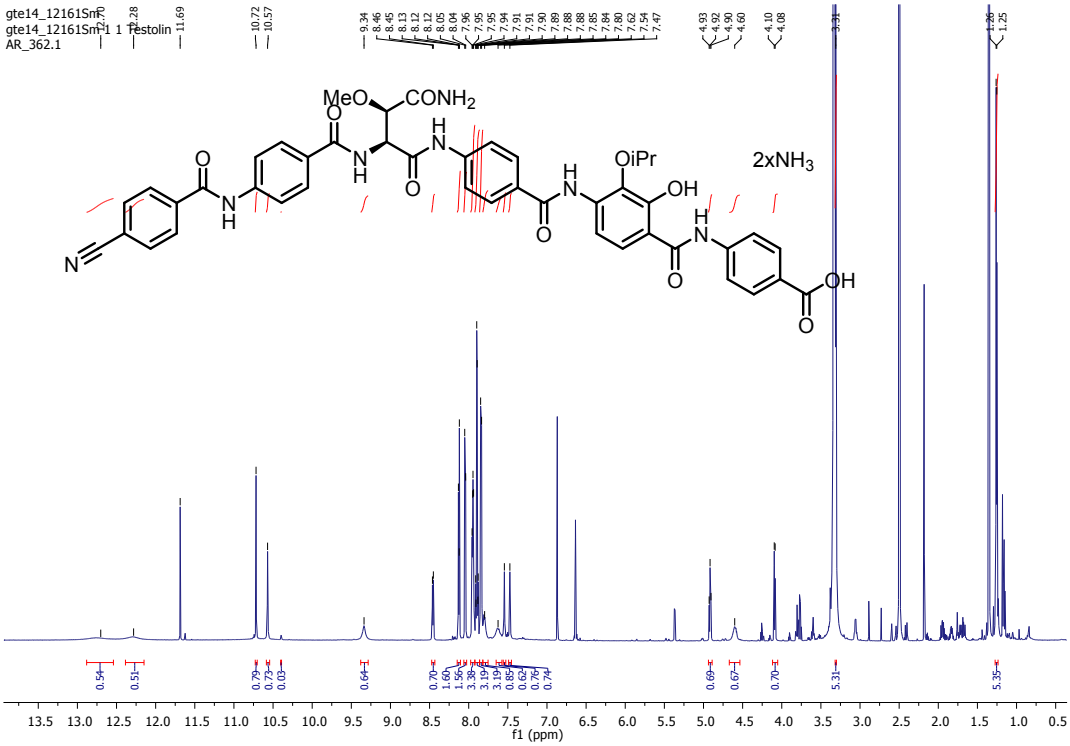


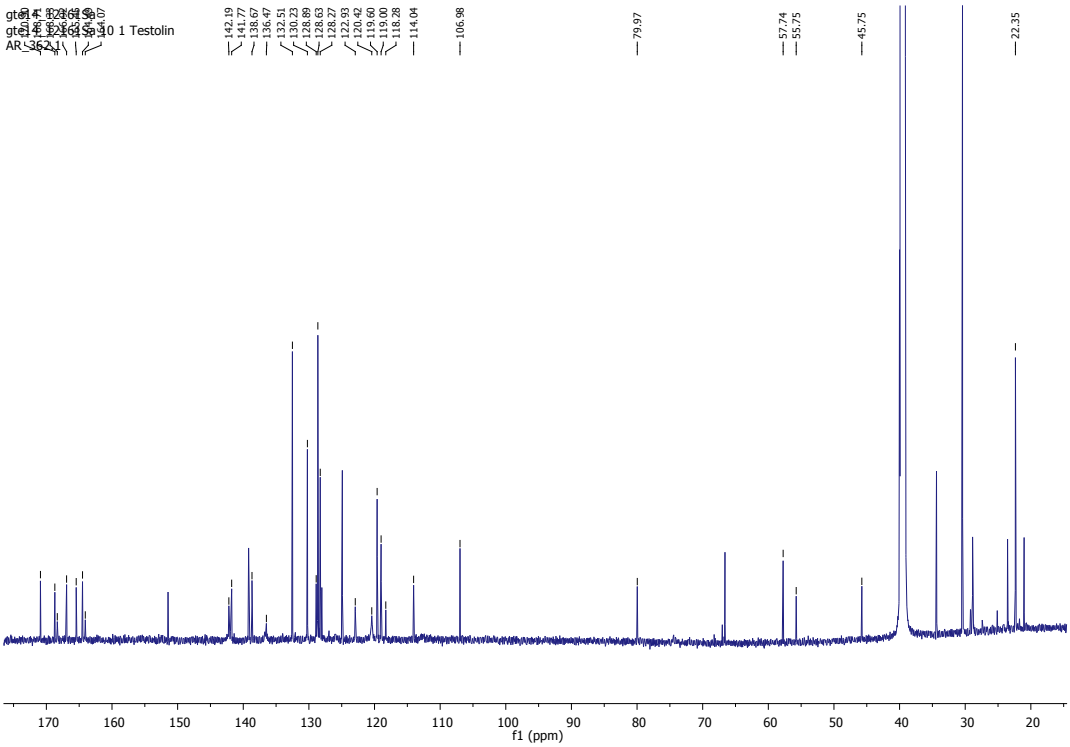
(S)-4-(4-(4-(4-amino-2-(4-(5-cyanothiophene-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid



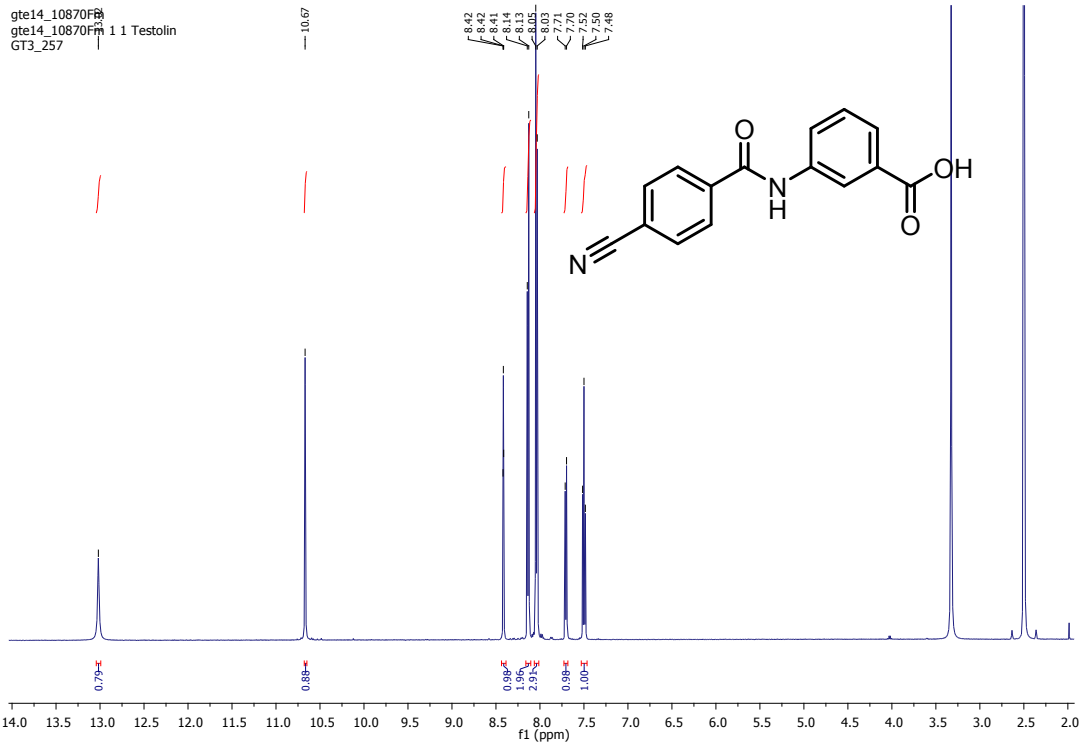


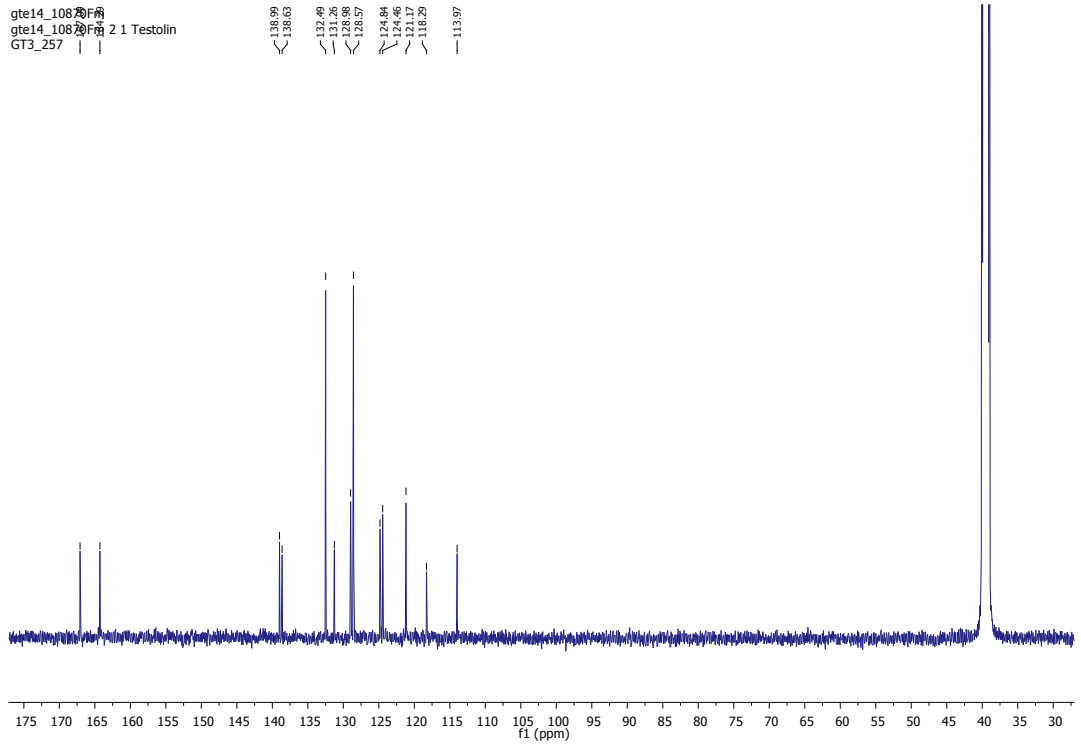
4-(4-(4-((2S,3R)-4-Amino-2-(4-(4-cyanobenzamido)benzamido)-3-methoxy-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



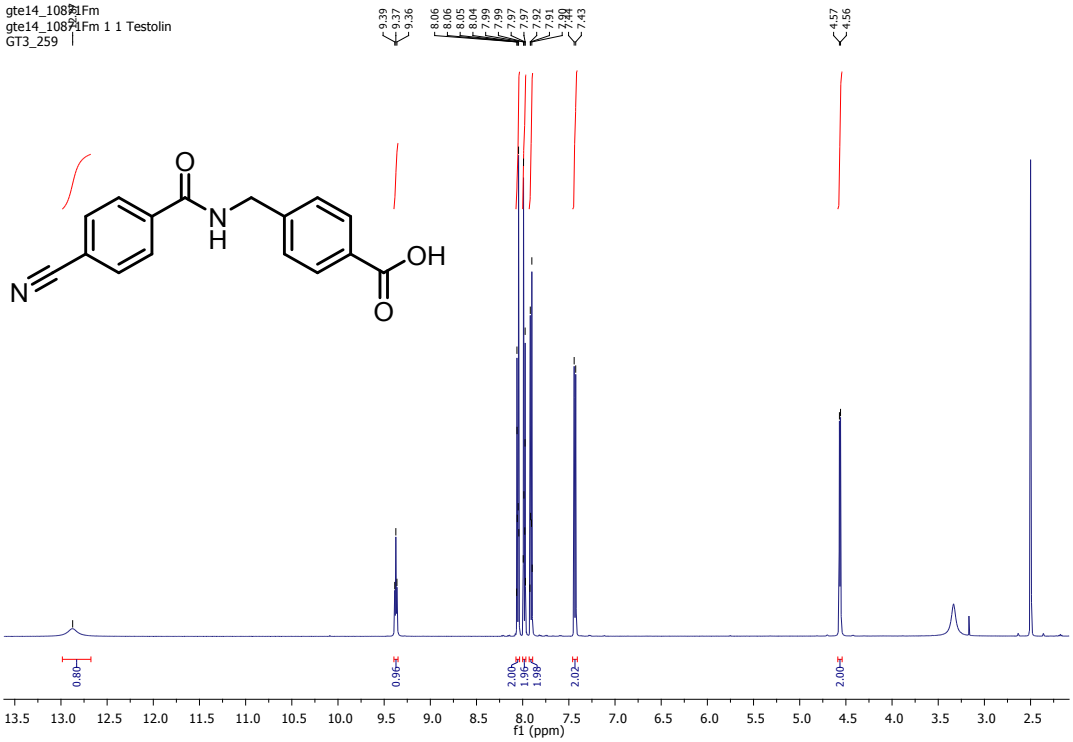


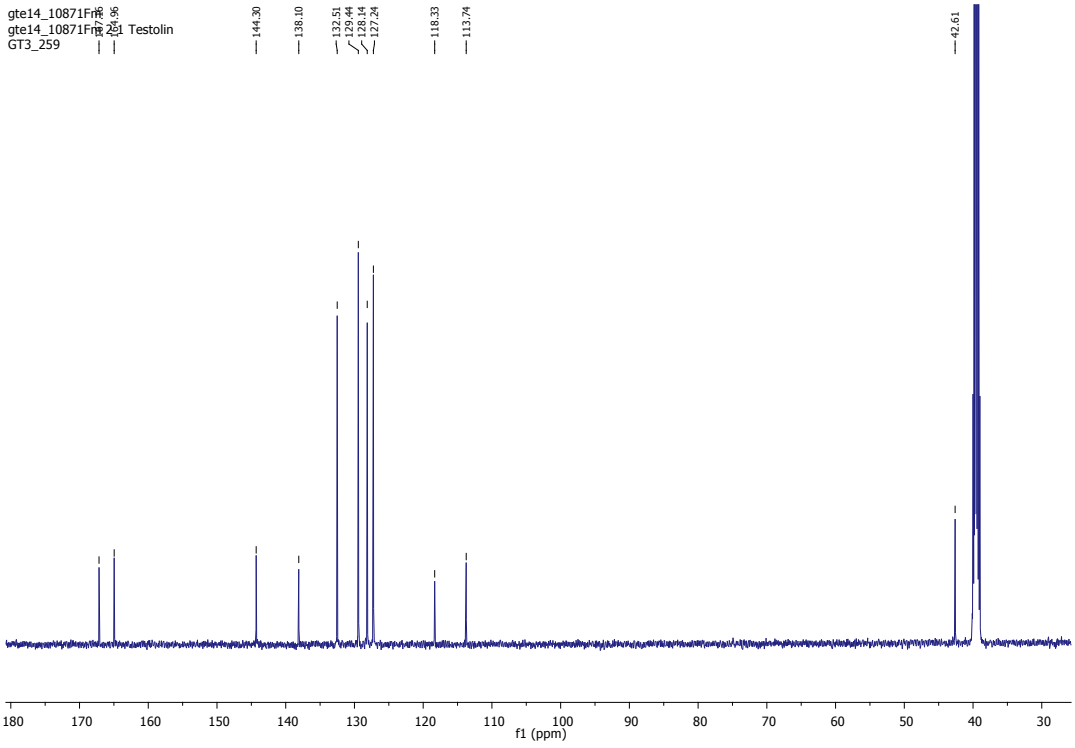
3-(4-Cyanobenzamido)benzoic acid



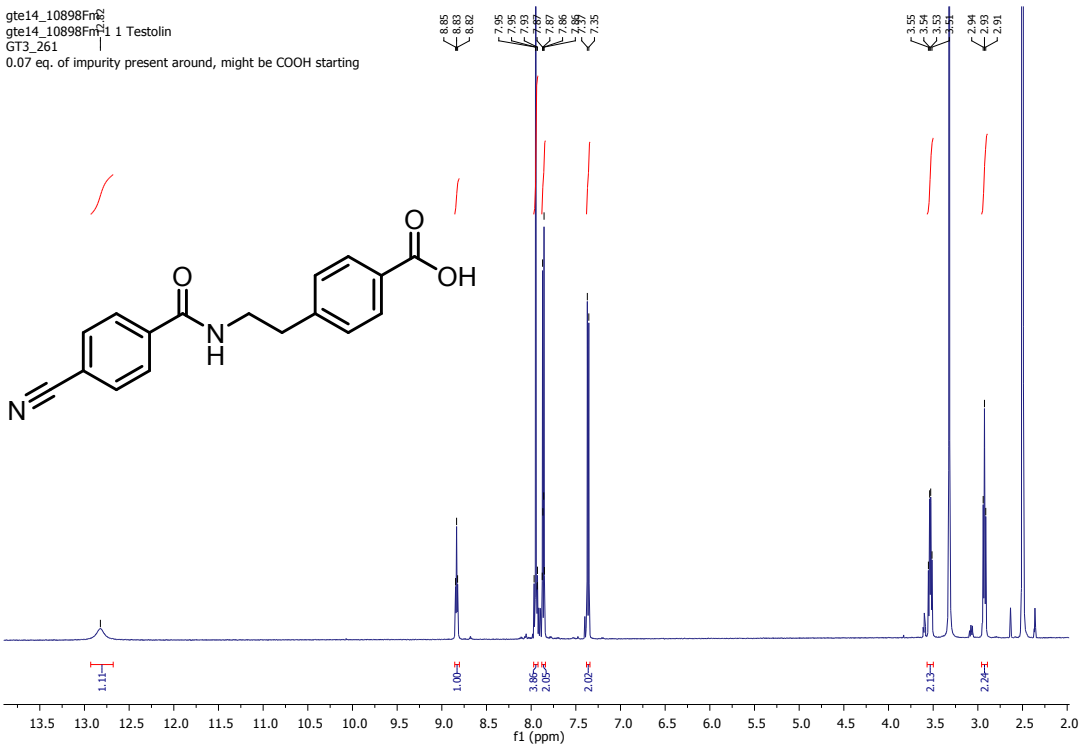


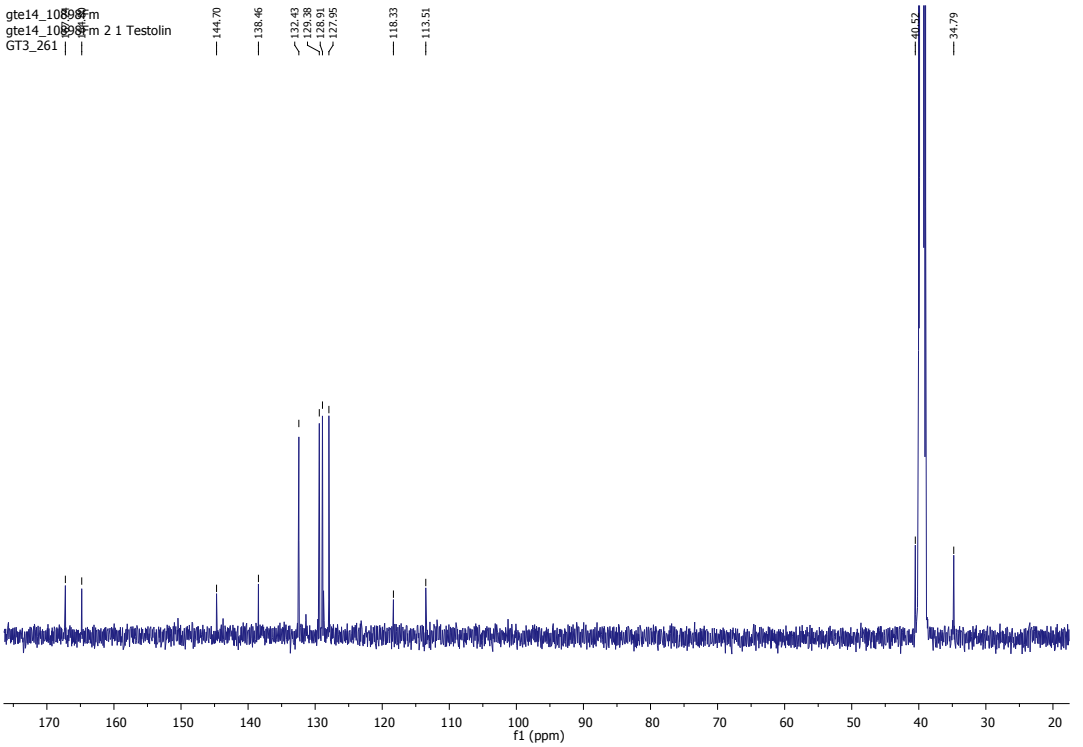
4-((4-Cyanobenzamido)methyl)benzoic acid



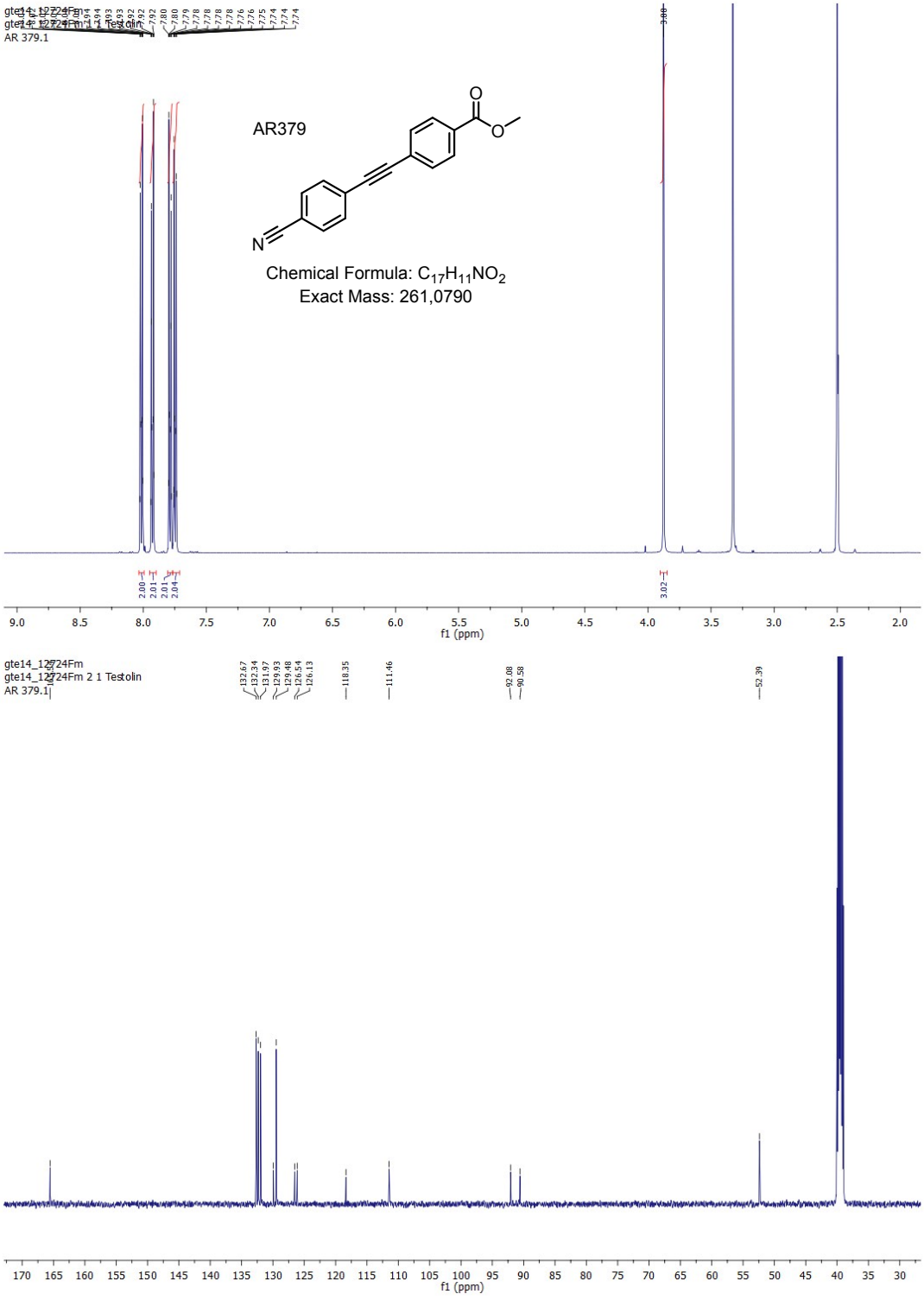


4-(2-(4-Cyanobenzamido)ethyl)benzoic acid

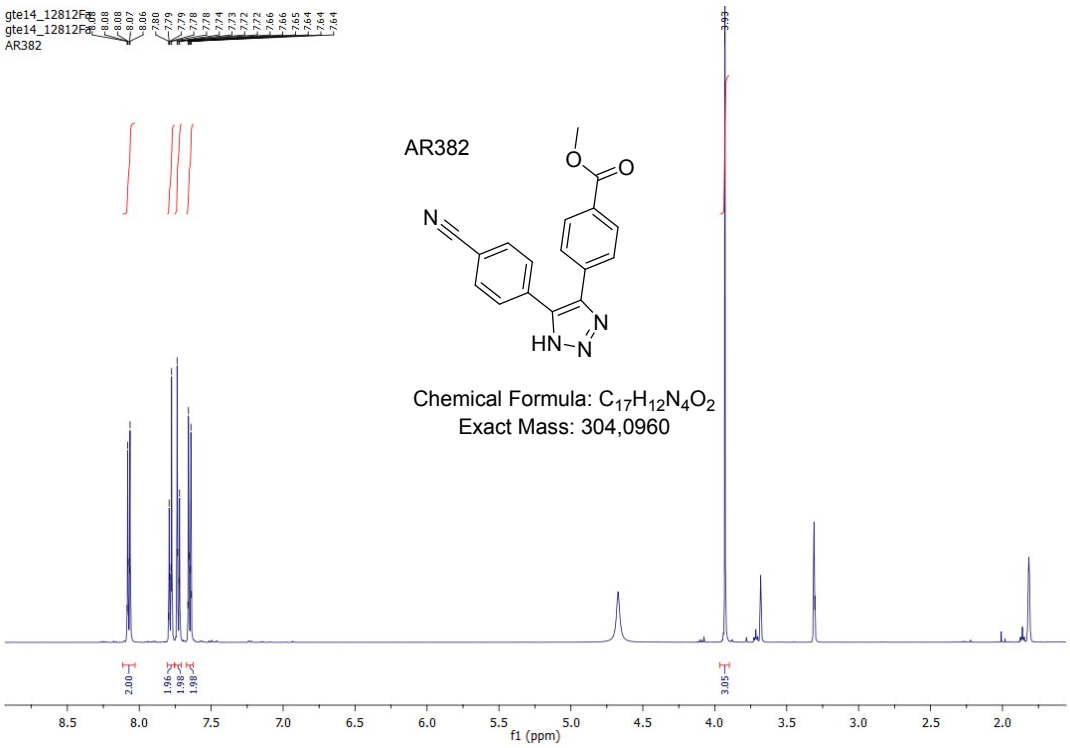


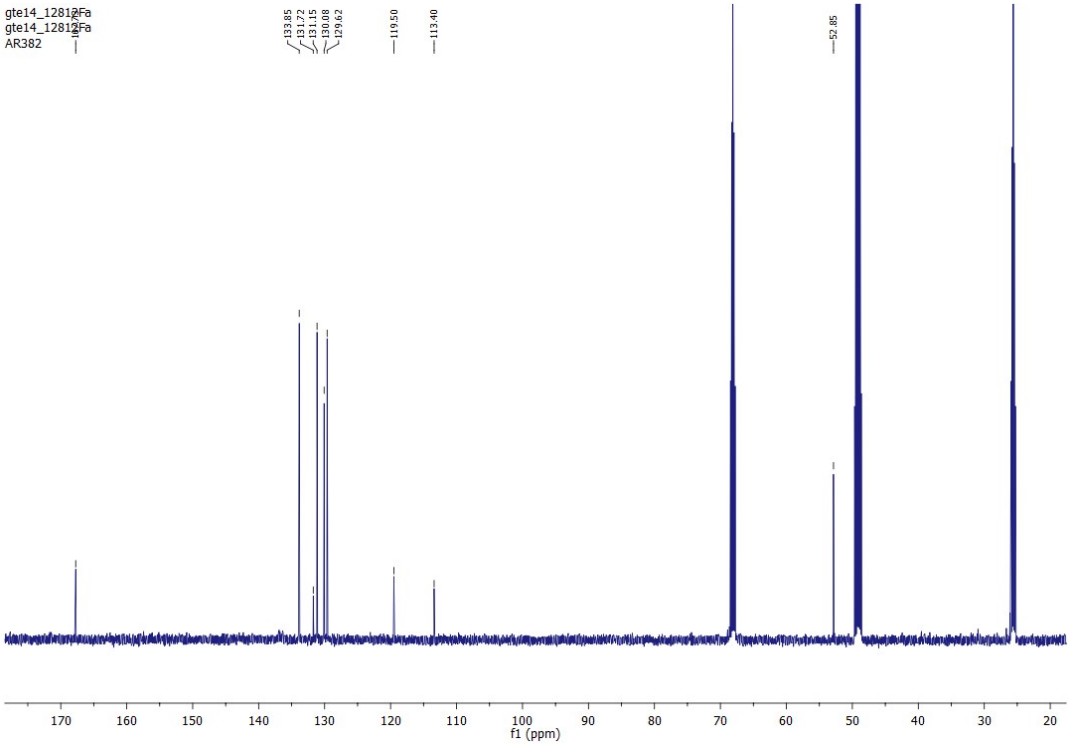


- **methyl 4-((4-cyanophenyl)ethynyl)benzoate**

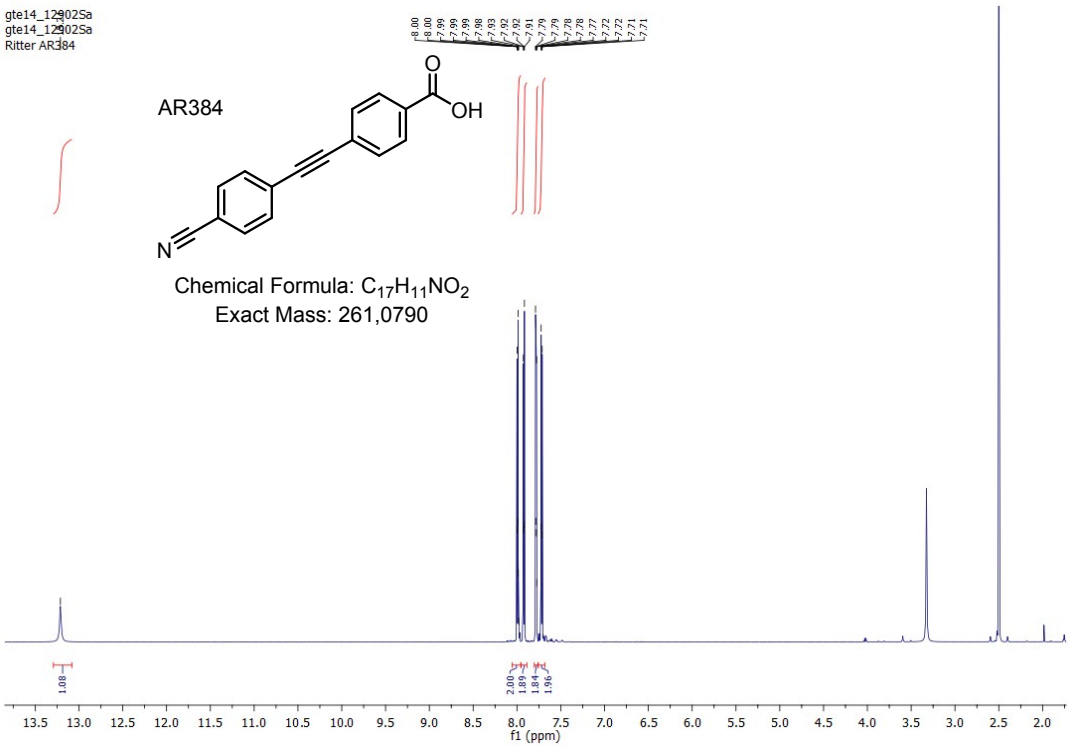


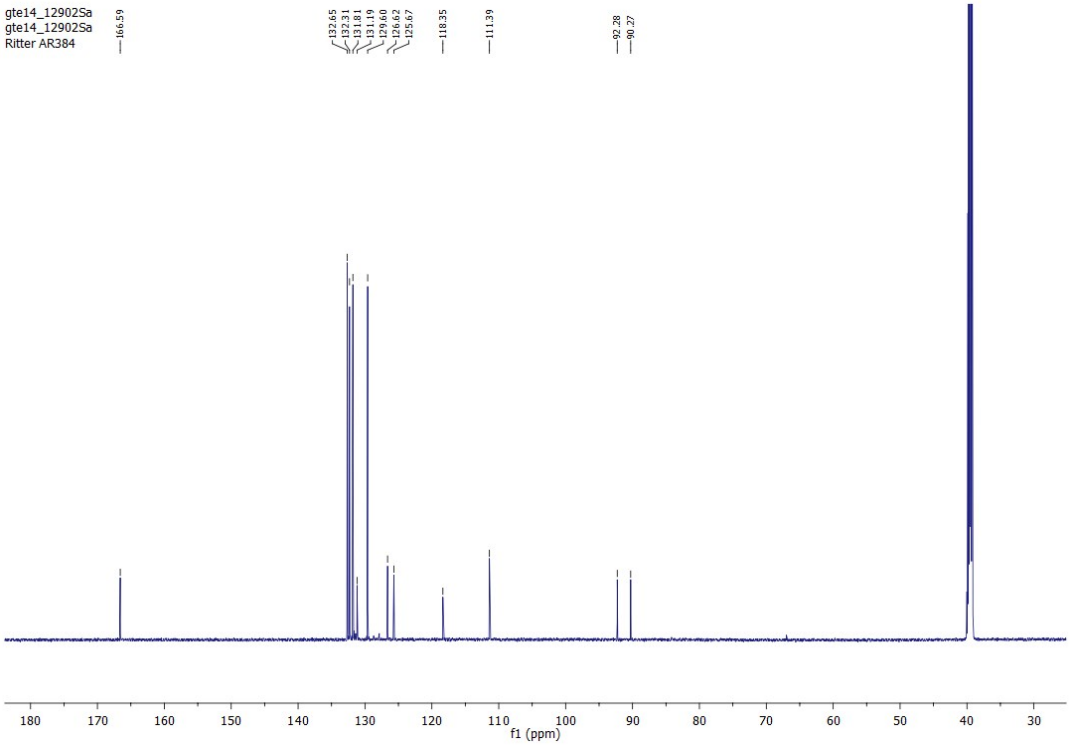
- **methyl 4-(5-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)benzoate**



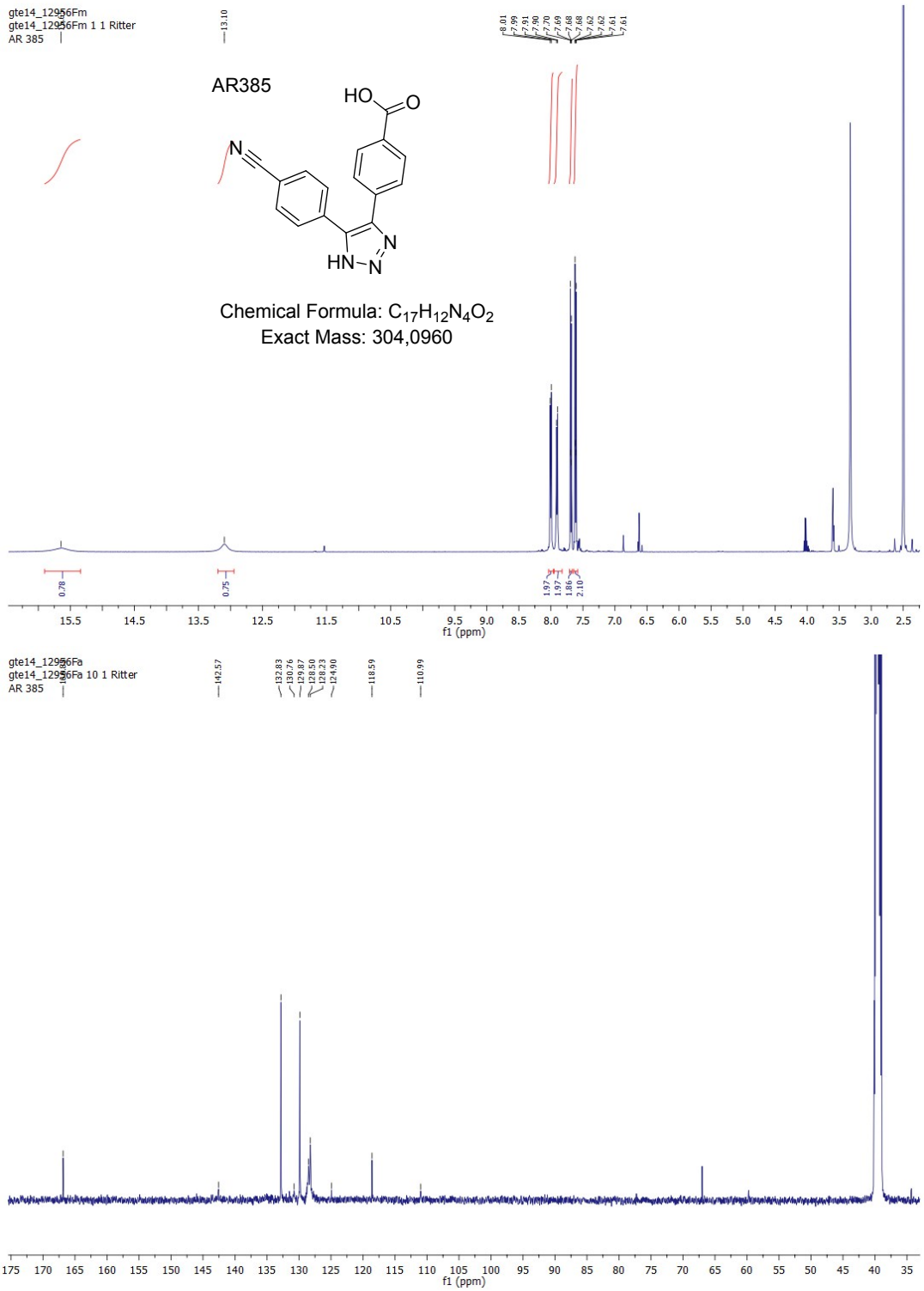


- **4-((4-cyanophenyl)ethynyl)benzoic acid**

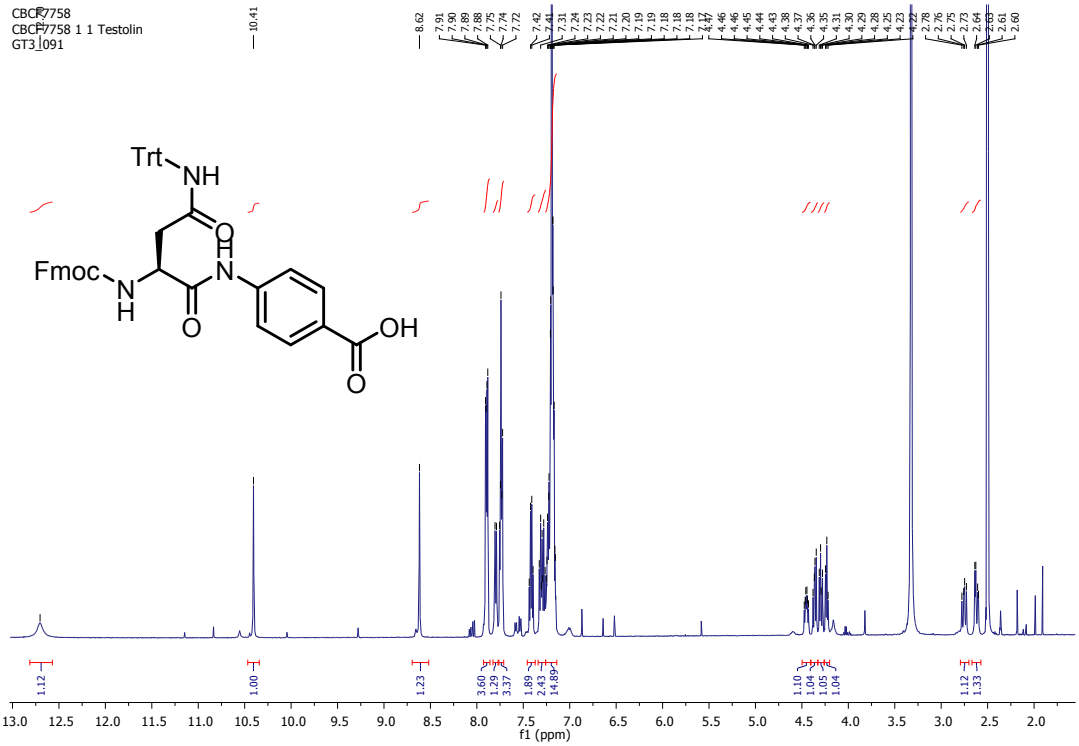


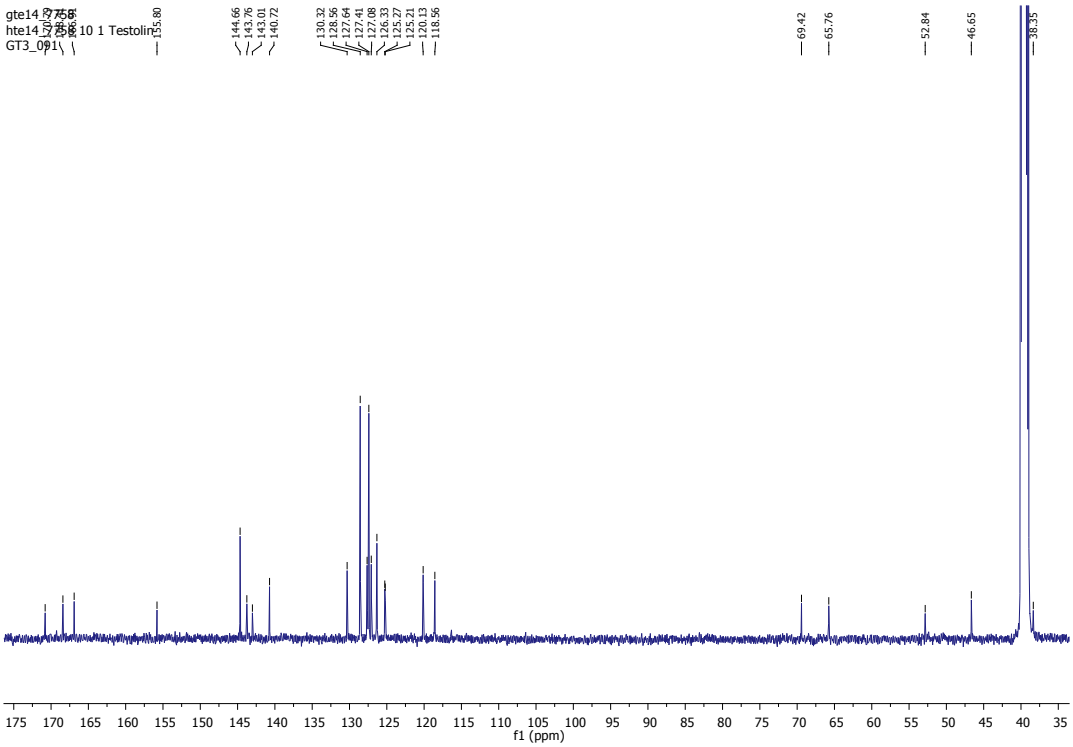


4-(5-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)benzoic acid

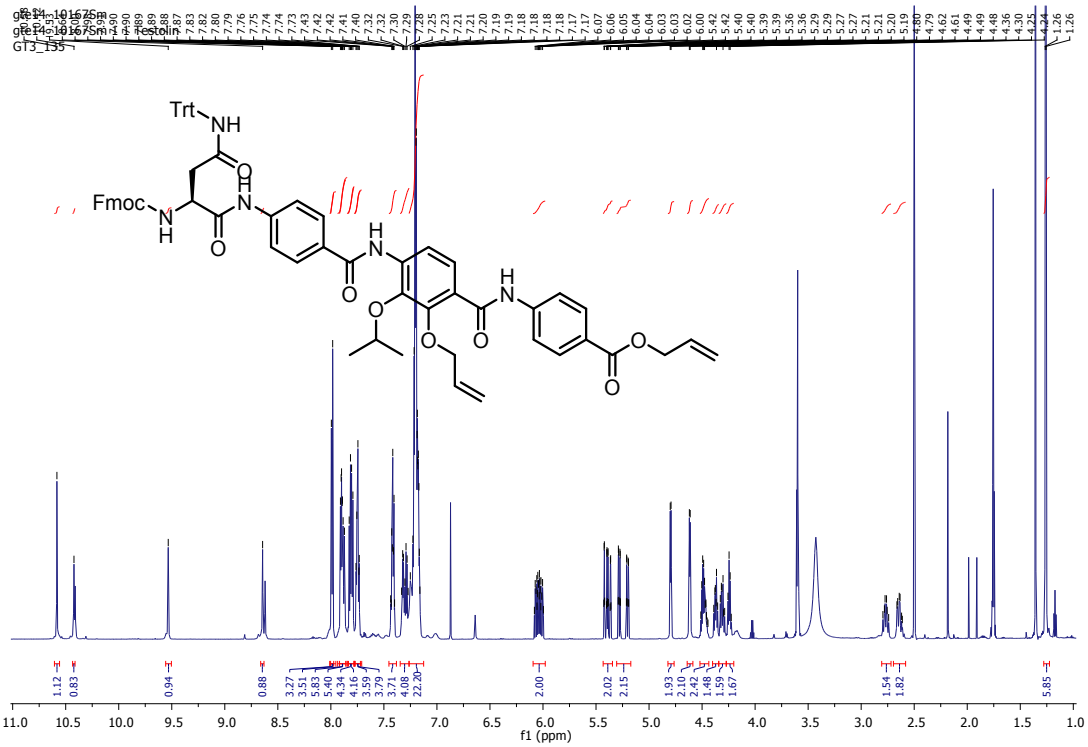


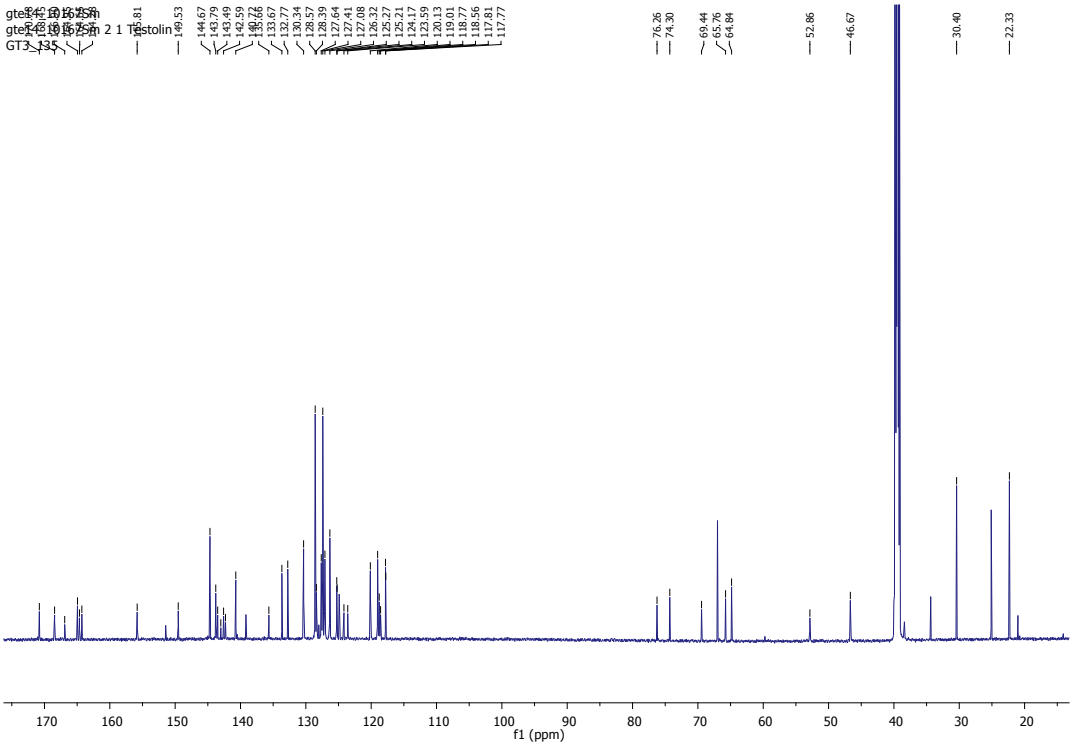
(S)-4-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzoic acid



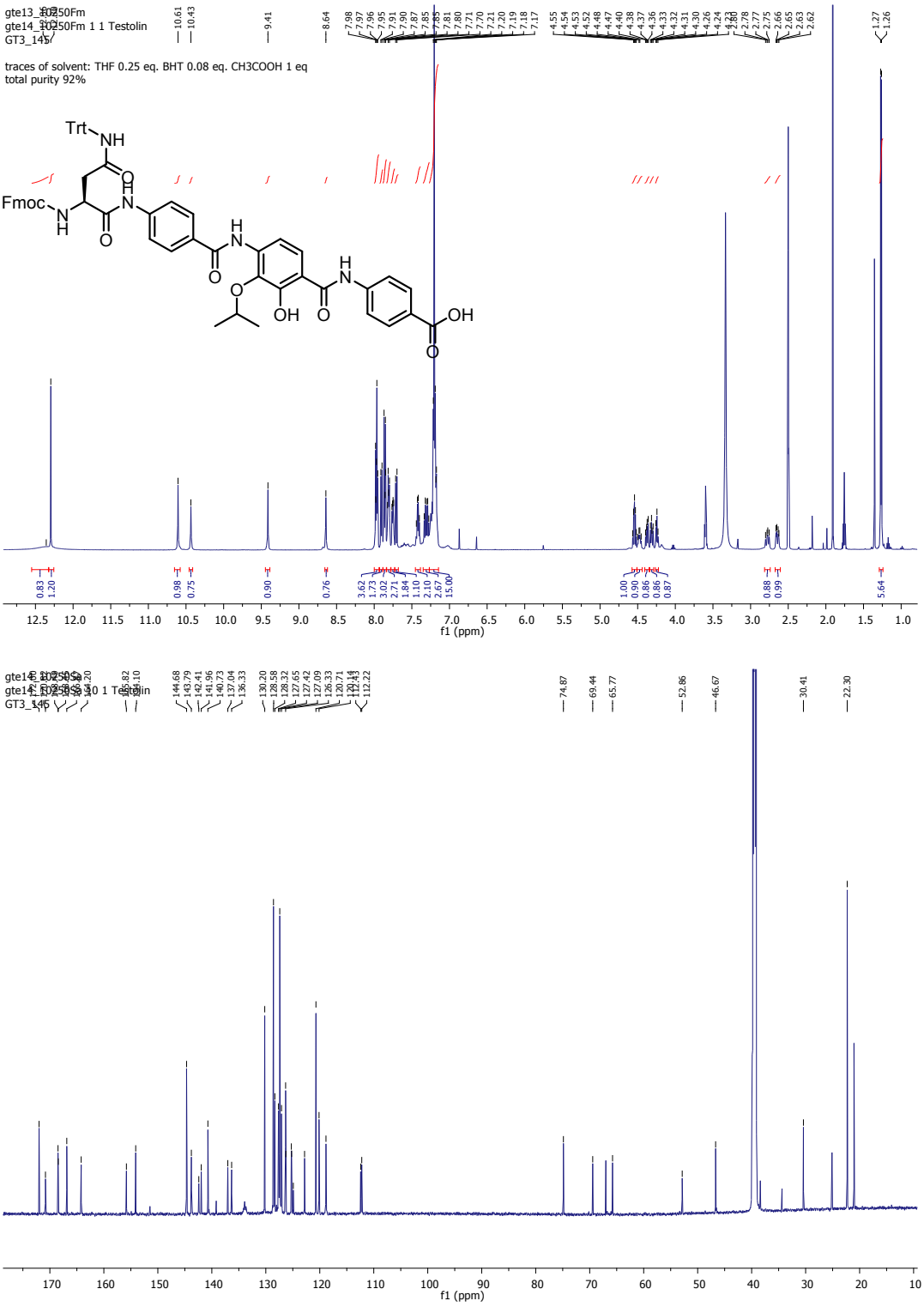


Allyl (S)-4-(4-(4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-(allyloxy)-3-isopropoxybenzamido)benzoate

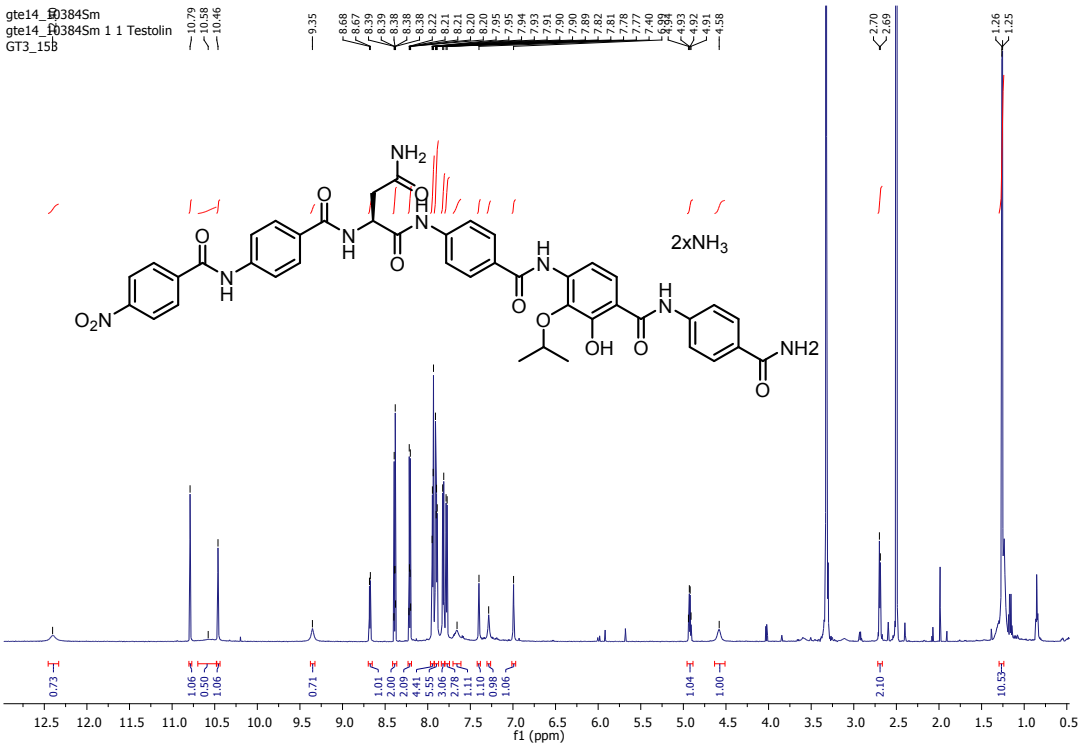


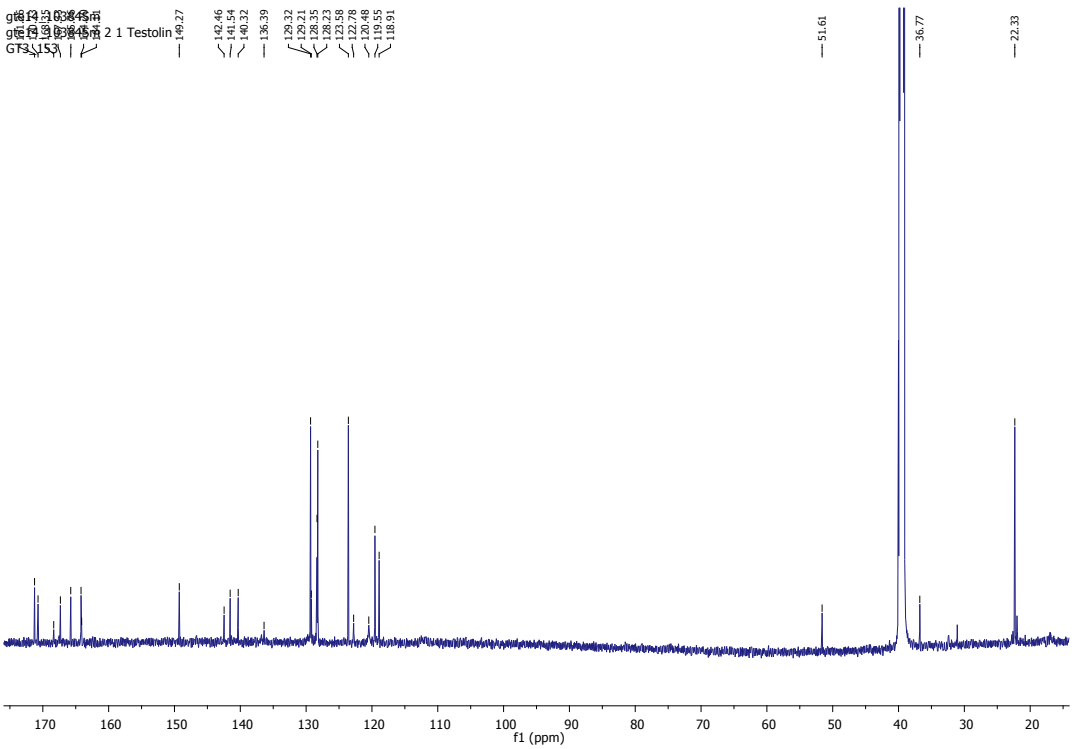


(S)-4-(4-(4-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

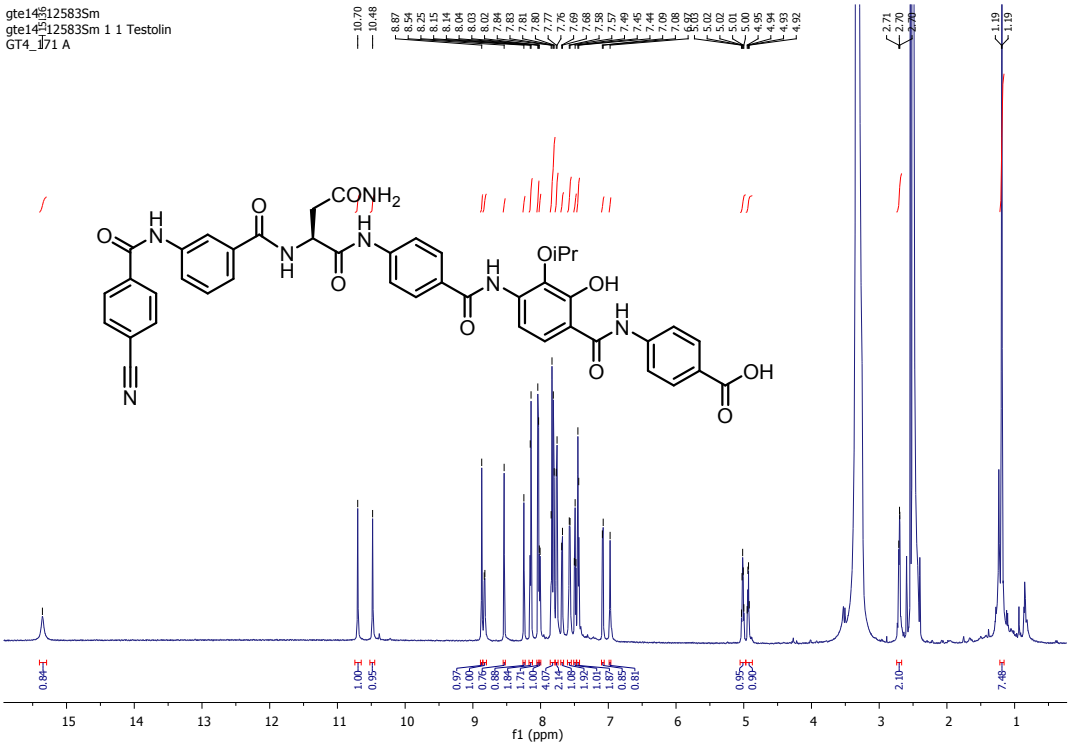


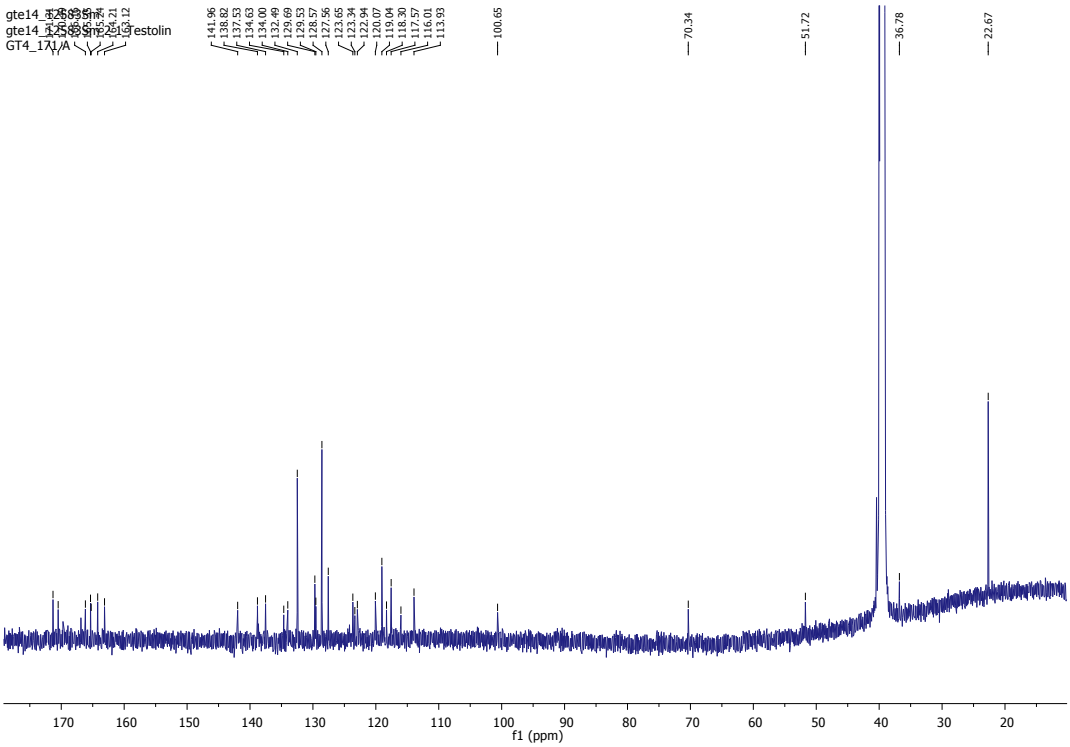
(S)-N1-(4-((4-((4-Carbamoylphenyl)carbamoyl)-3-hydroxy-2-isopropoxyphenyl)carbamoyl)phenyl)-2-(4-(4-nitrobenzamido)benzamido)succinamide



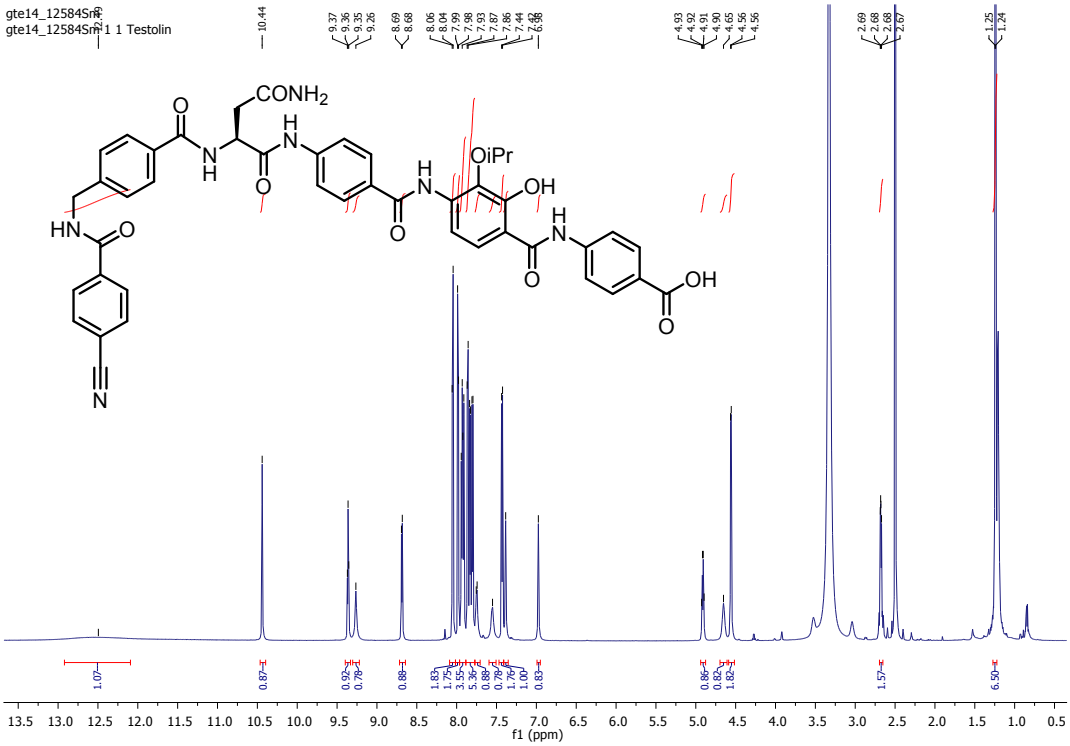


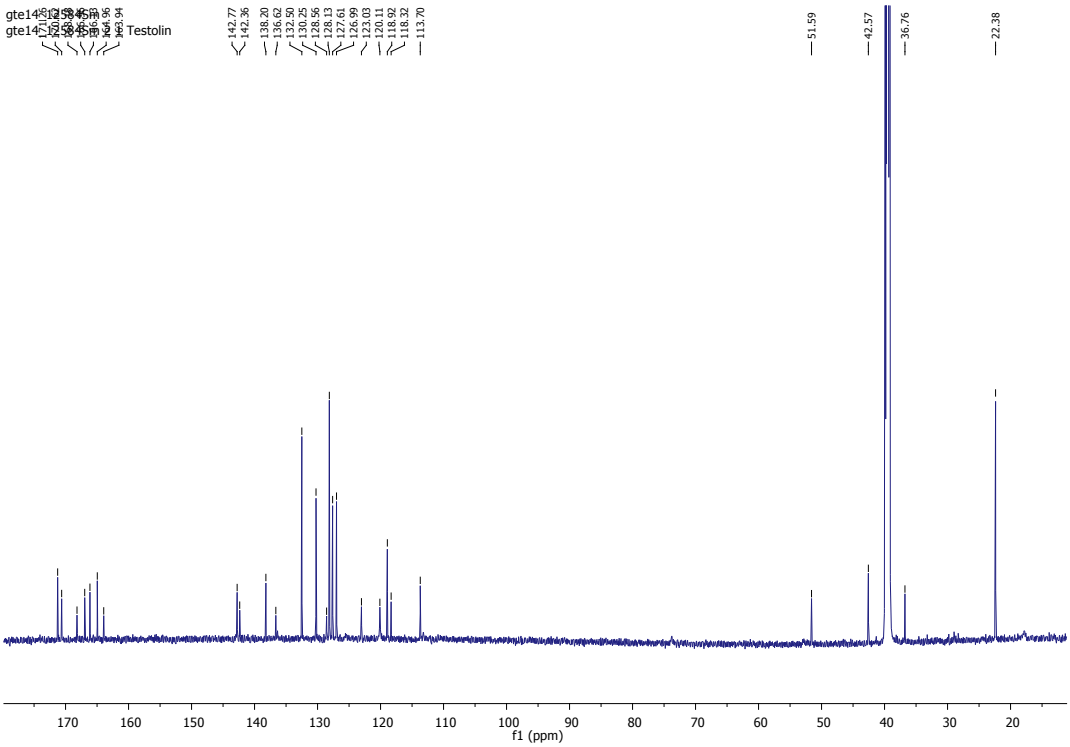
(S)-4-(4-(4-(4-Amino-2-(3-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



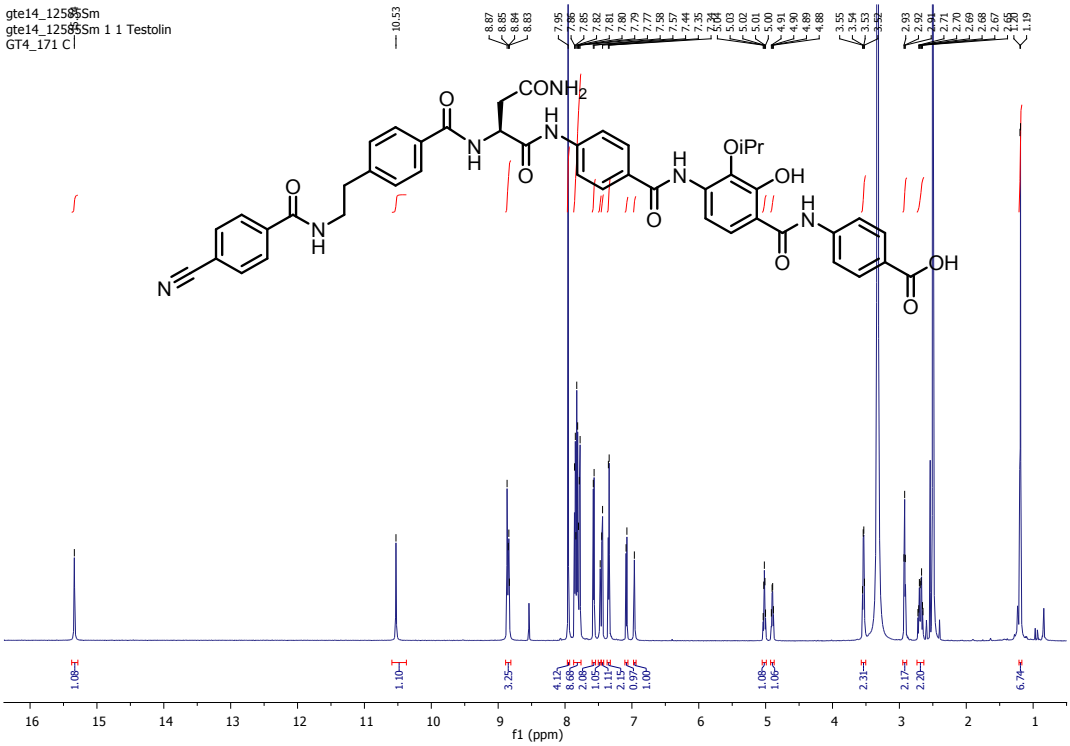


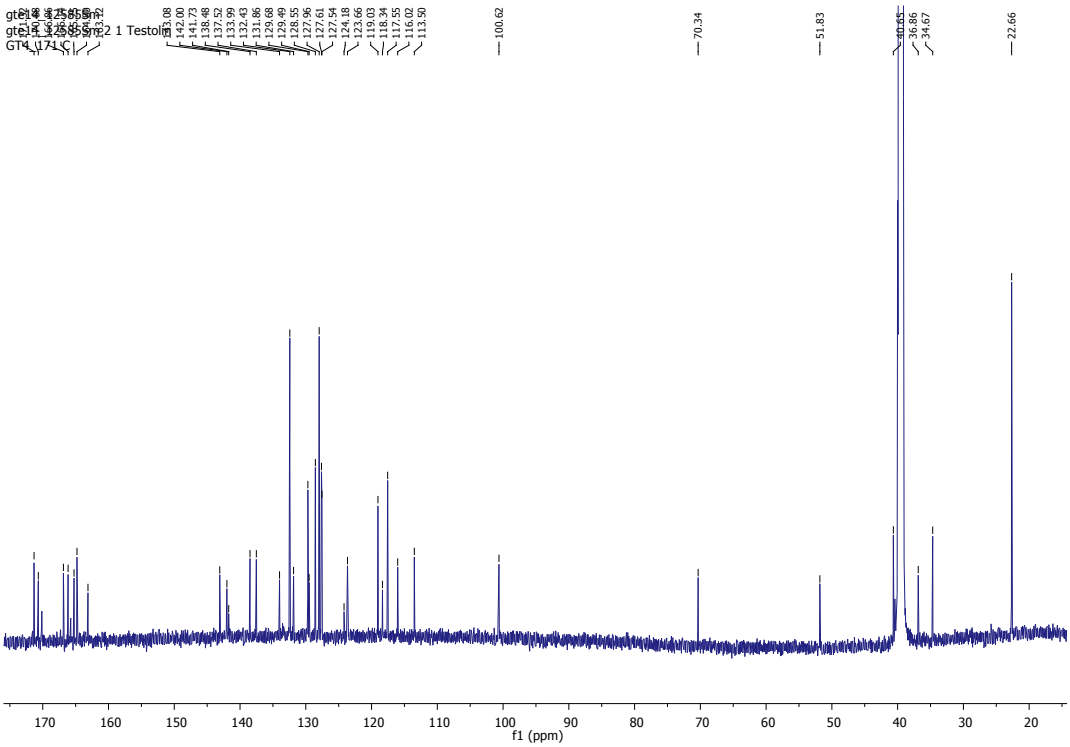
(S)-4-(4-(4-(4-Amino-2-(4-((4-cyanobenzamido)methyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



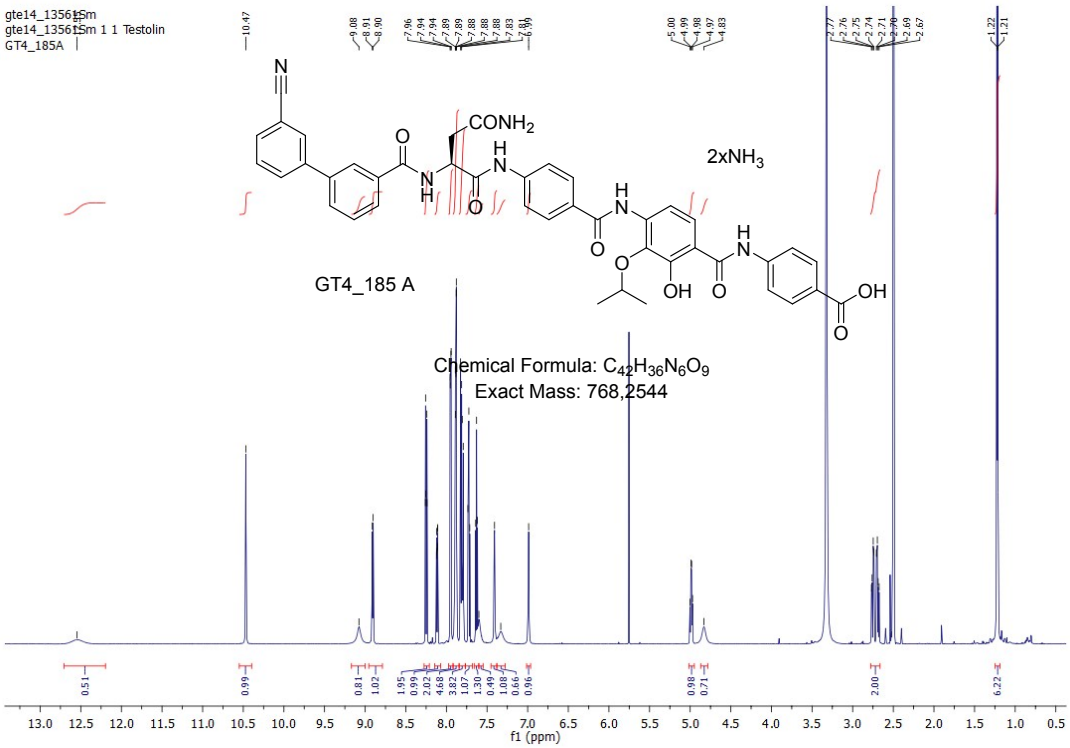


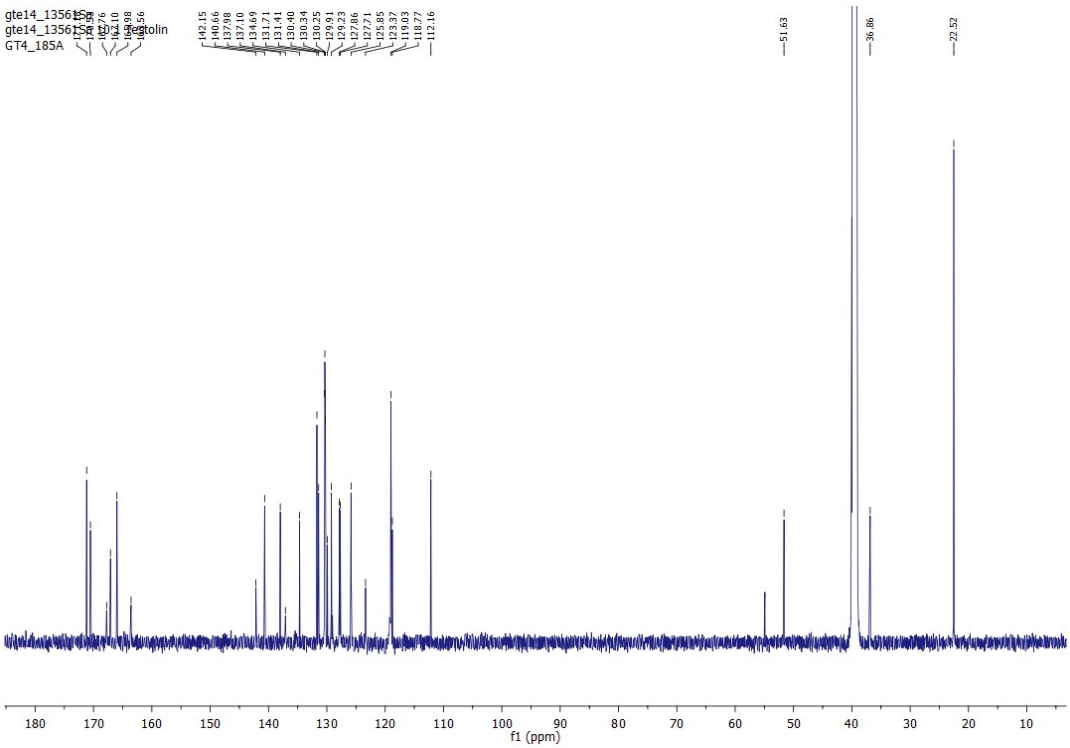
(S)-4-(4-(4-(4-Amino-2-(4-(2-(4-cyanobenzamido)ethyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



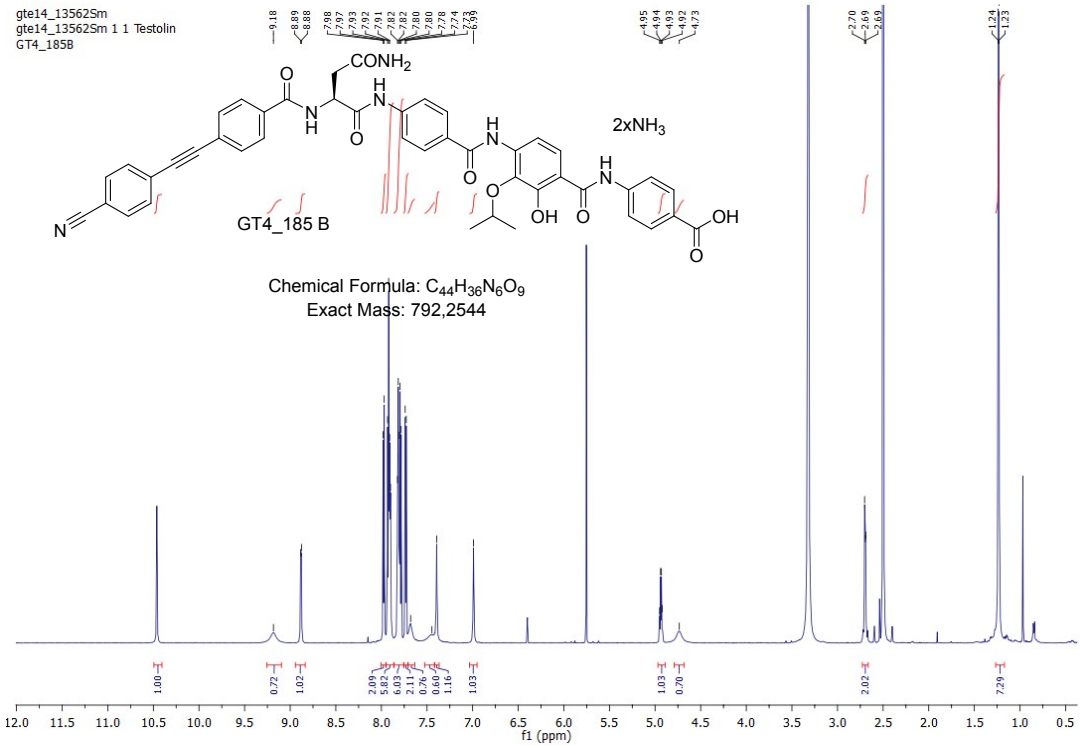


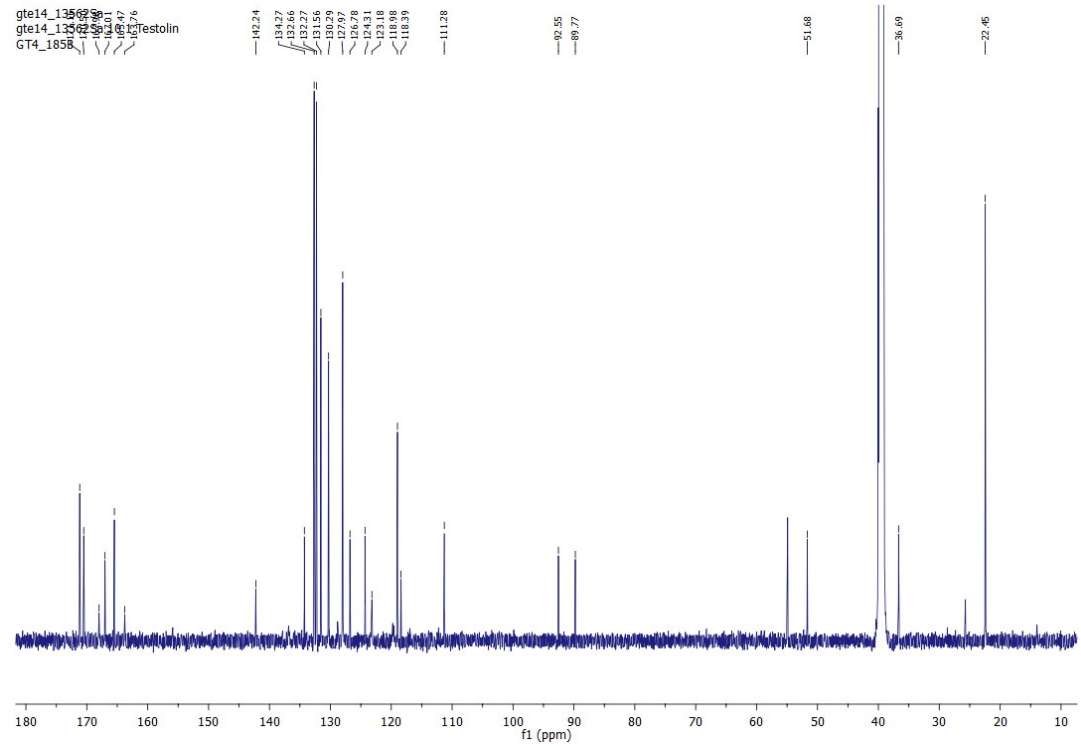
(S)-4-(4-(4-(4-amino-2-(3'-cyano-[1,1'-biphenyl]-3-carboxamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



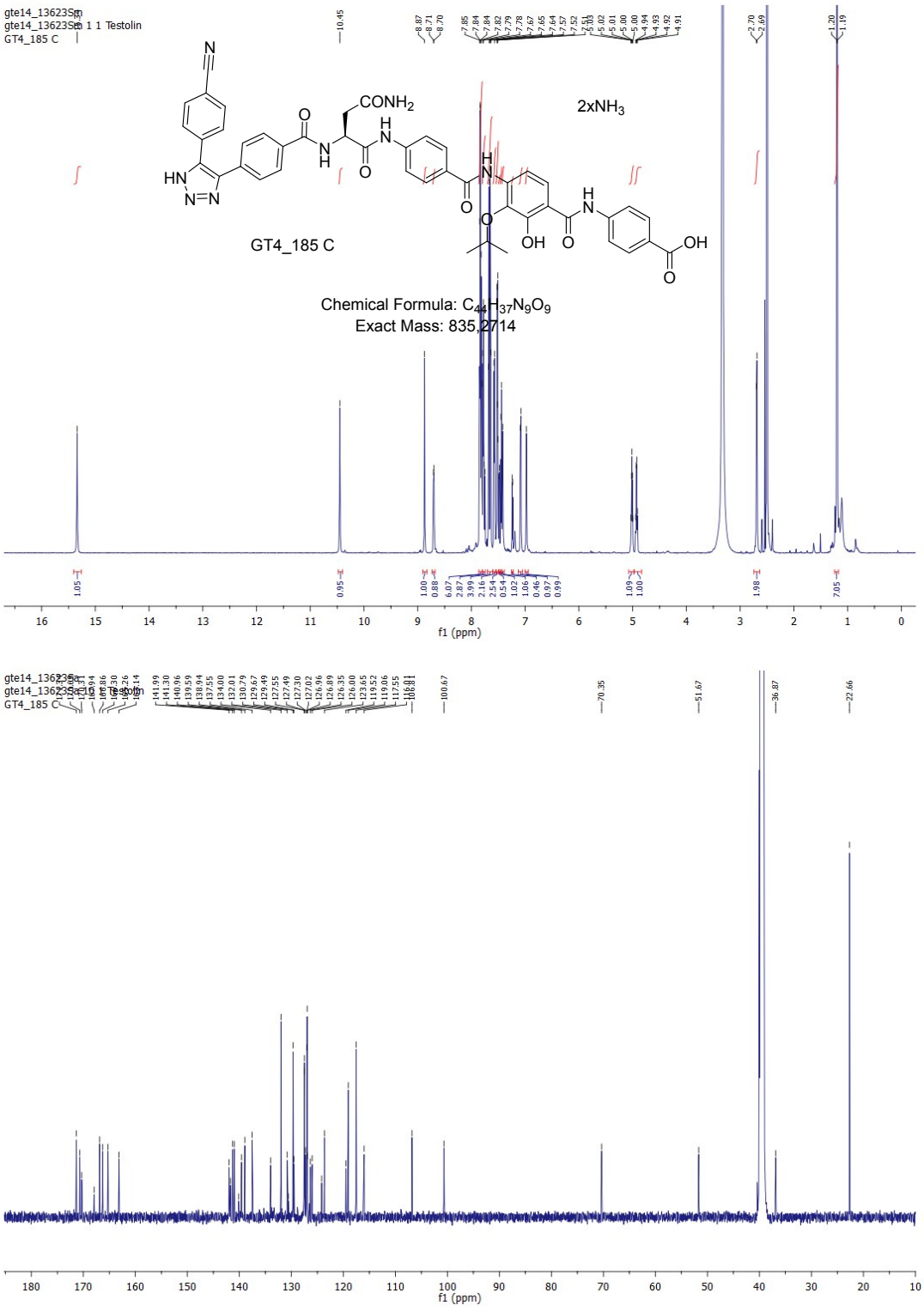


(S)-4-(4-(4-(4-amino-2-(4-((4-cyanophenyl)ethynyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

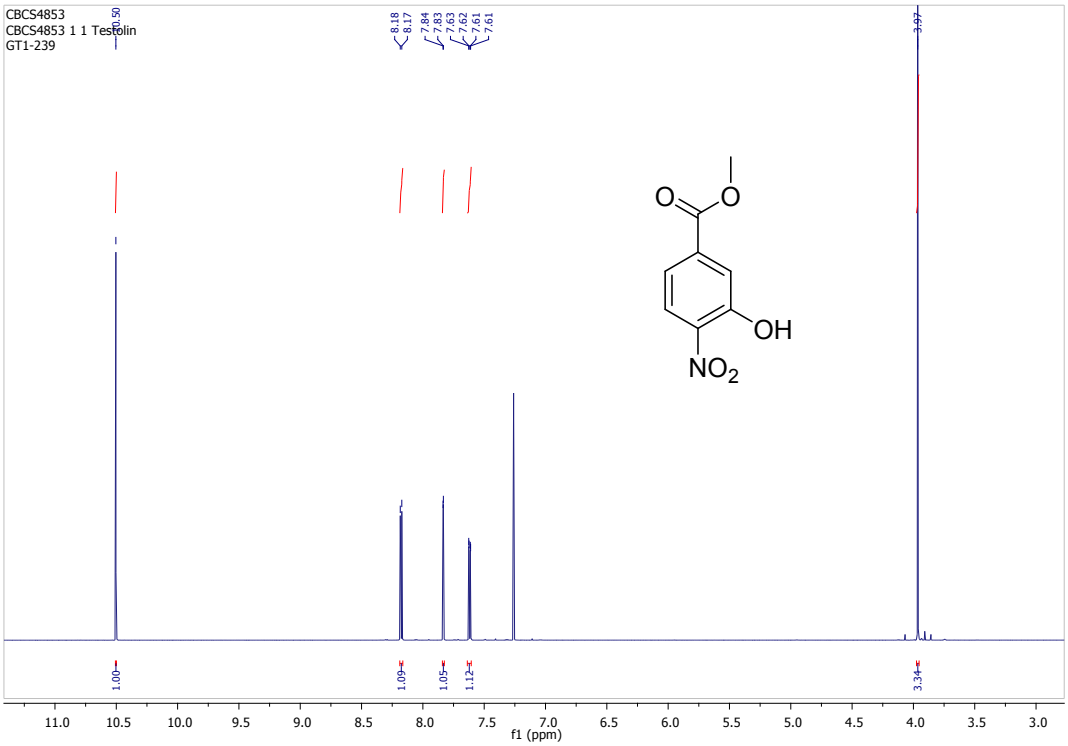


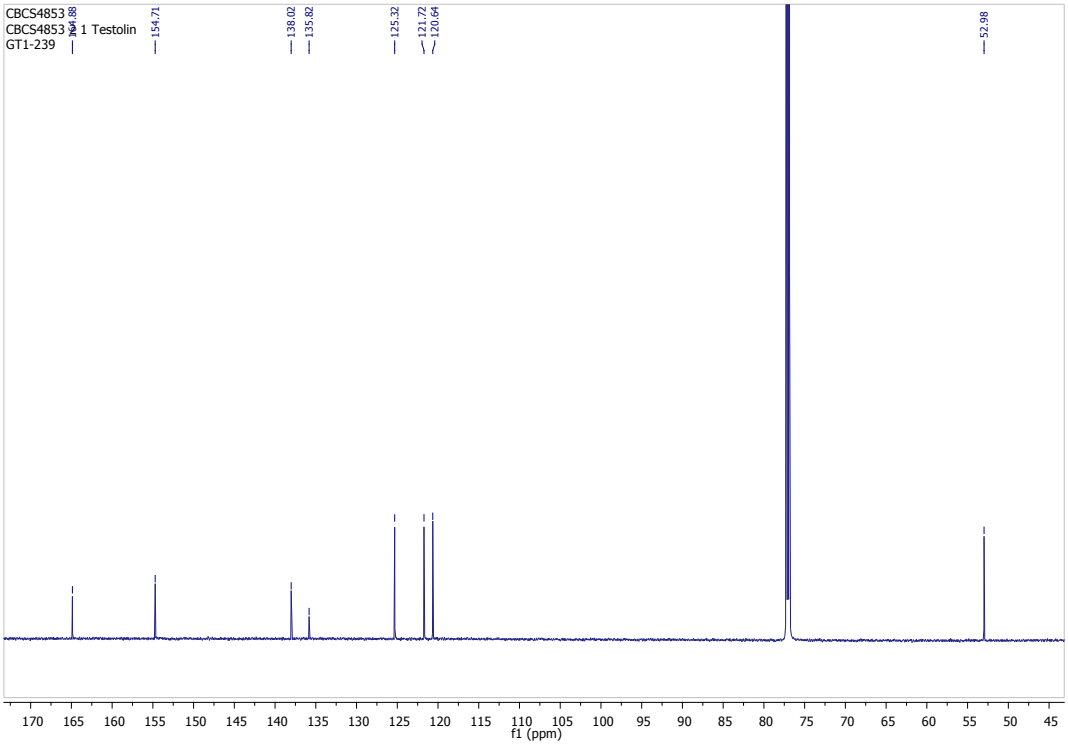


(S)-4-(4-(4-(4-amino-2-(4-(5-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid

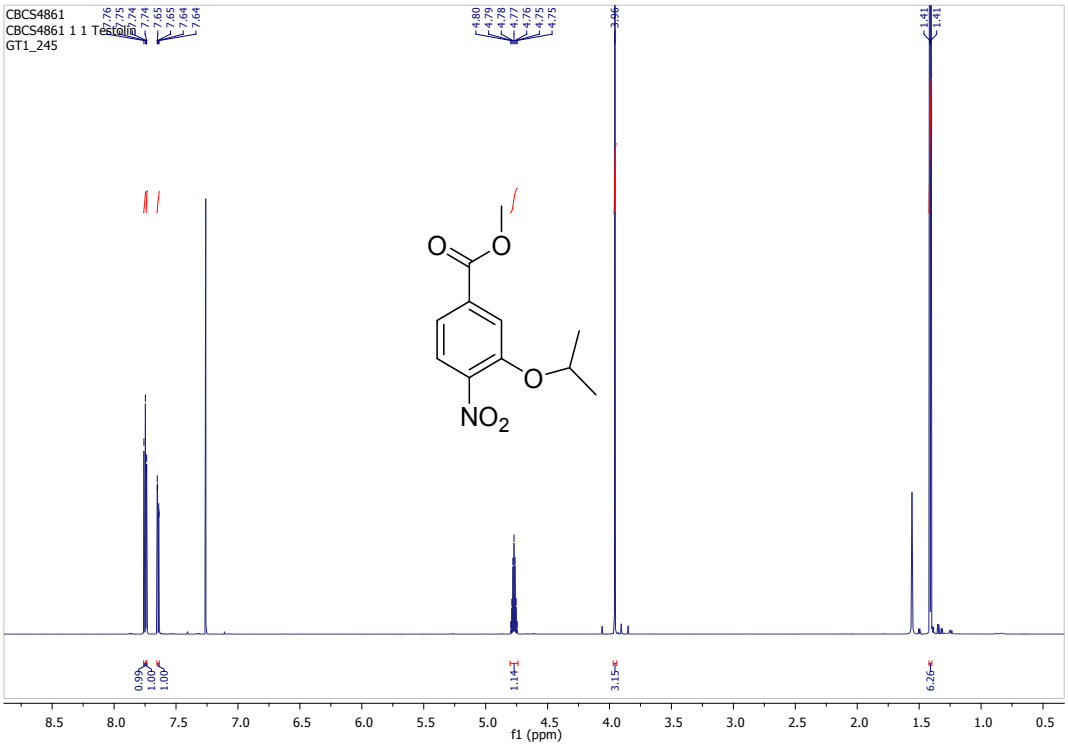


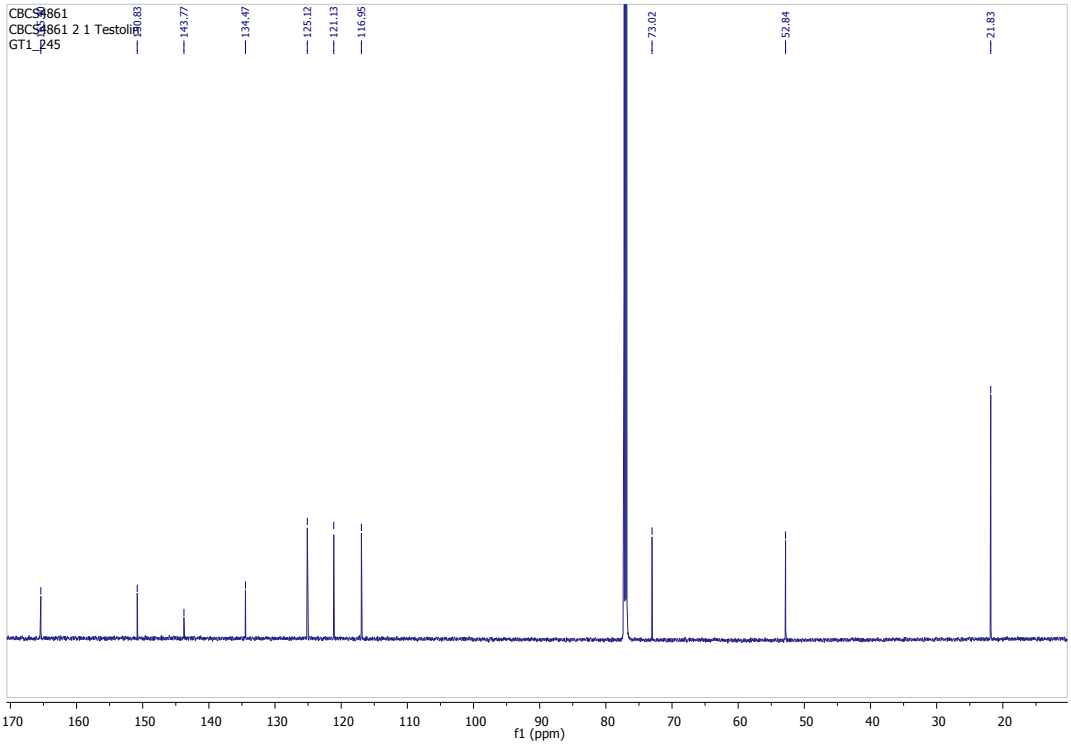
Methyl 3-hydroxy-4-nitrobenzoate



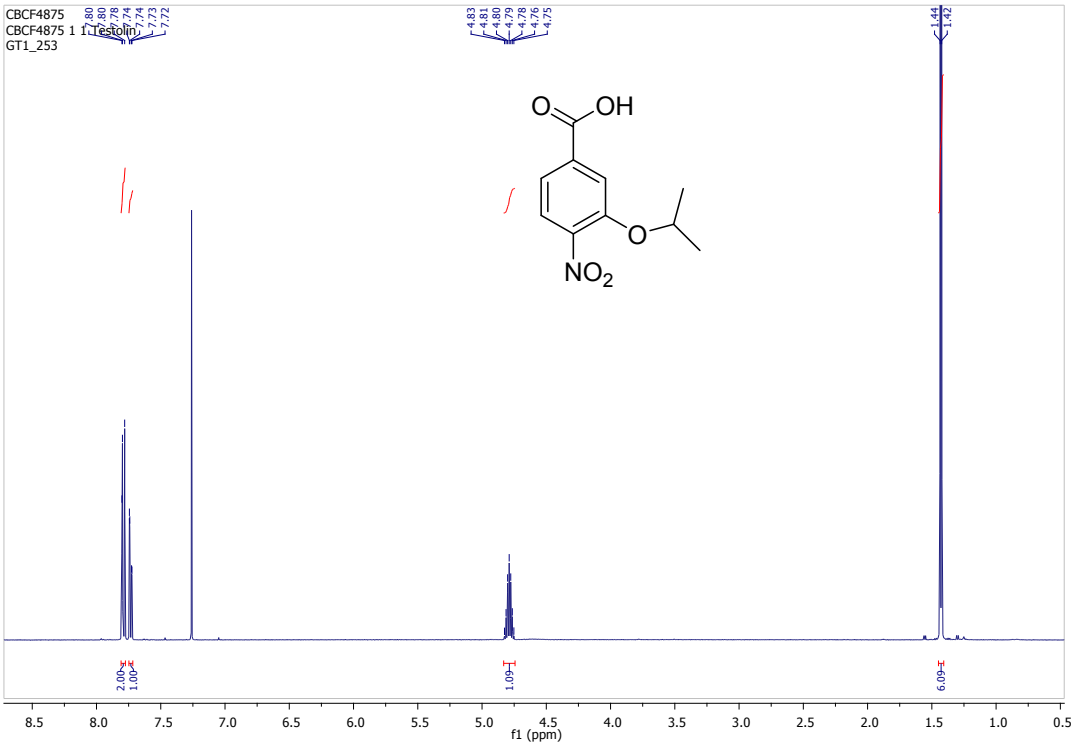


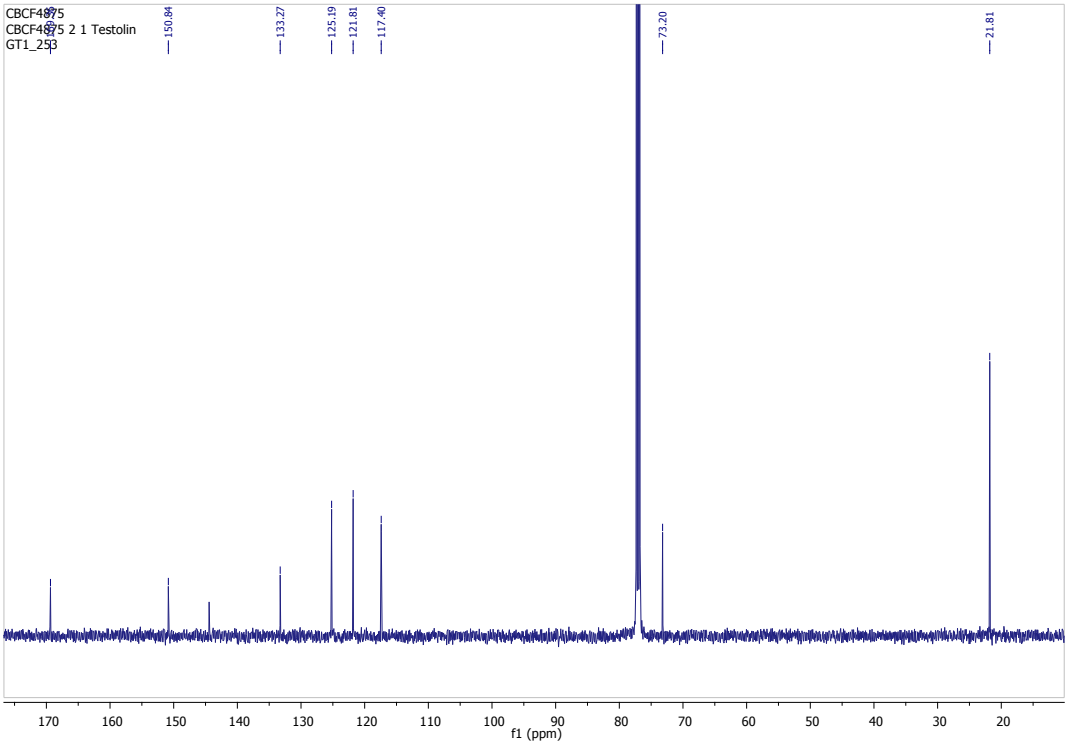
Methyl **3-isopropoxy-4-nitrobenzoate**



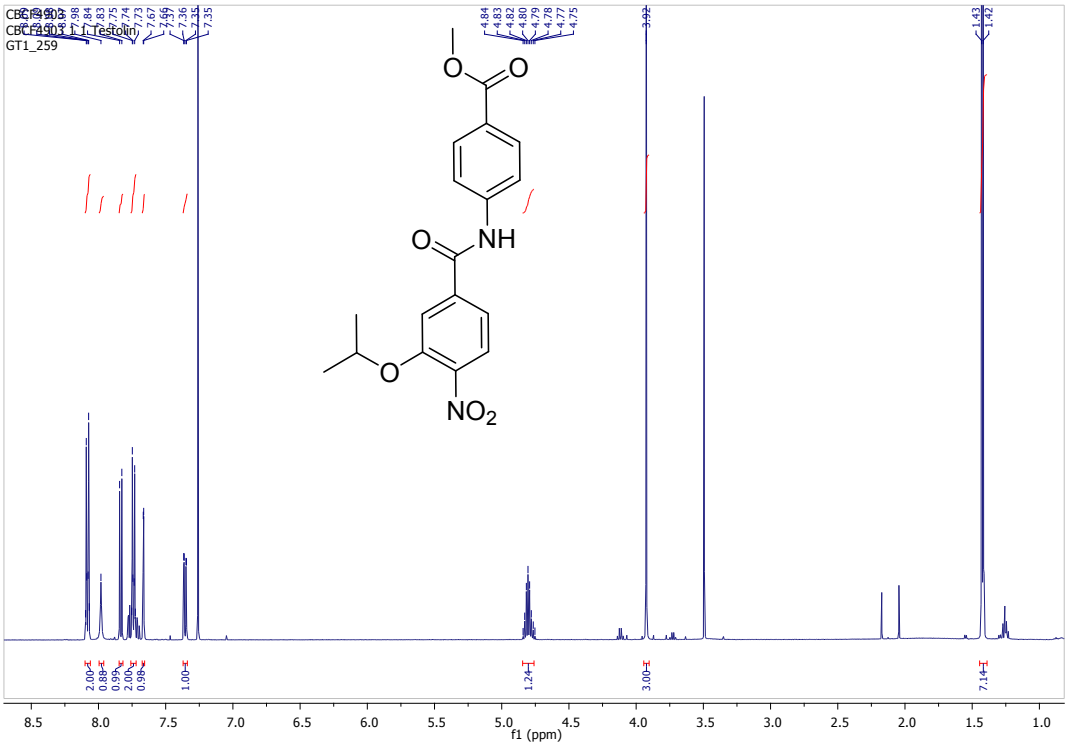


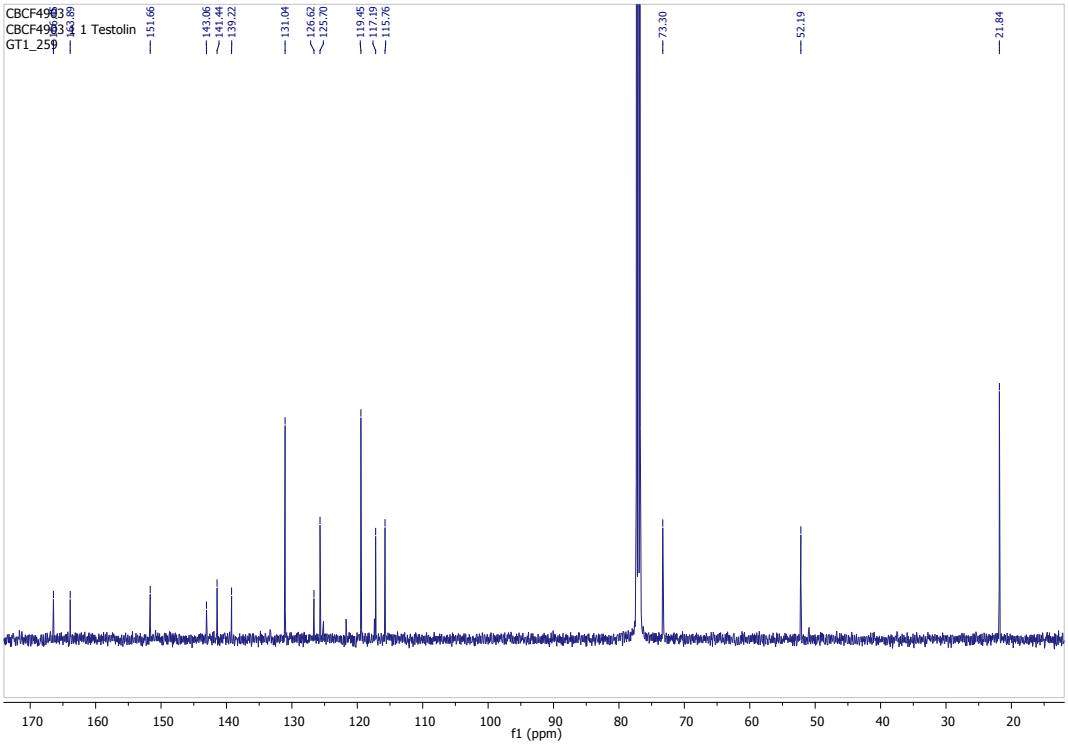
3-Isopropoxy-4-nitrobenzoic acid



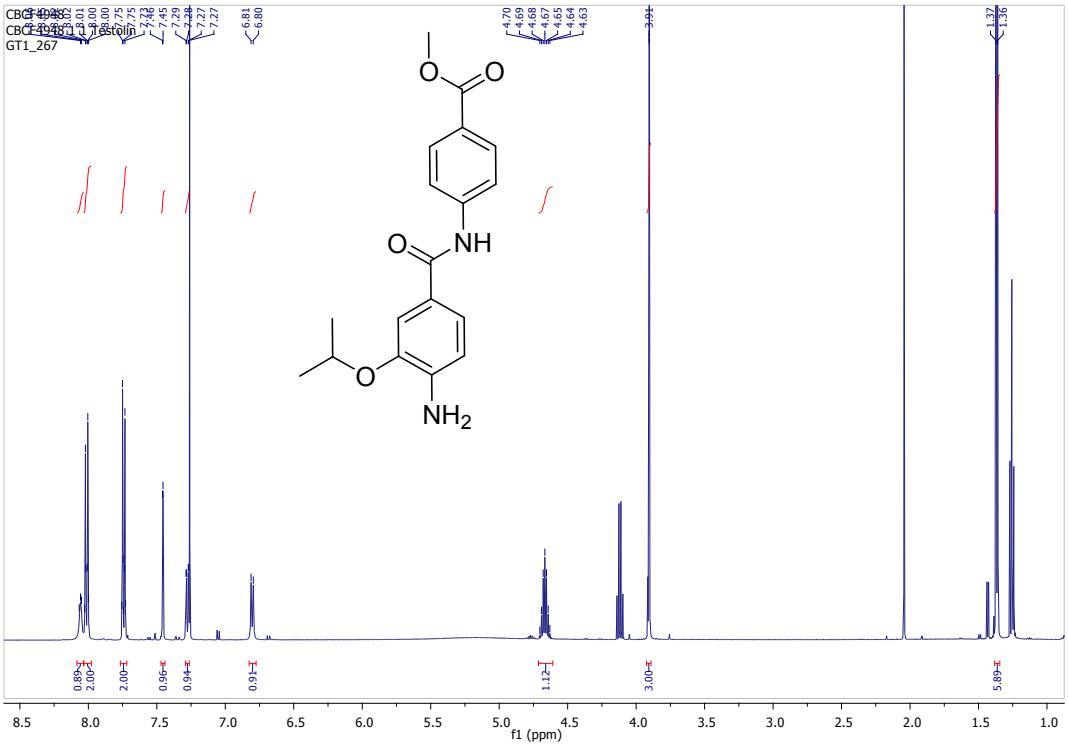


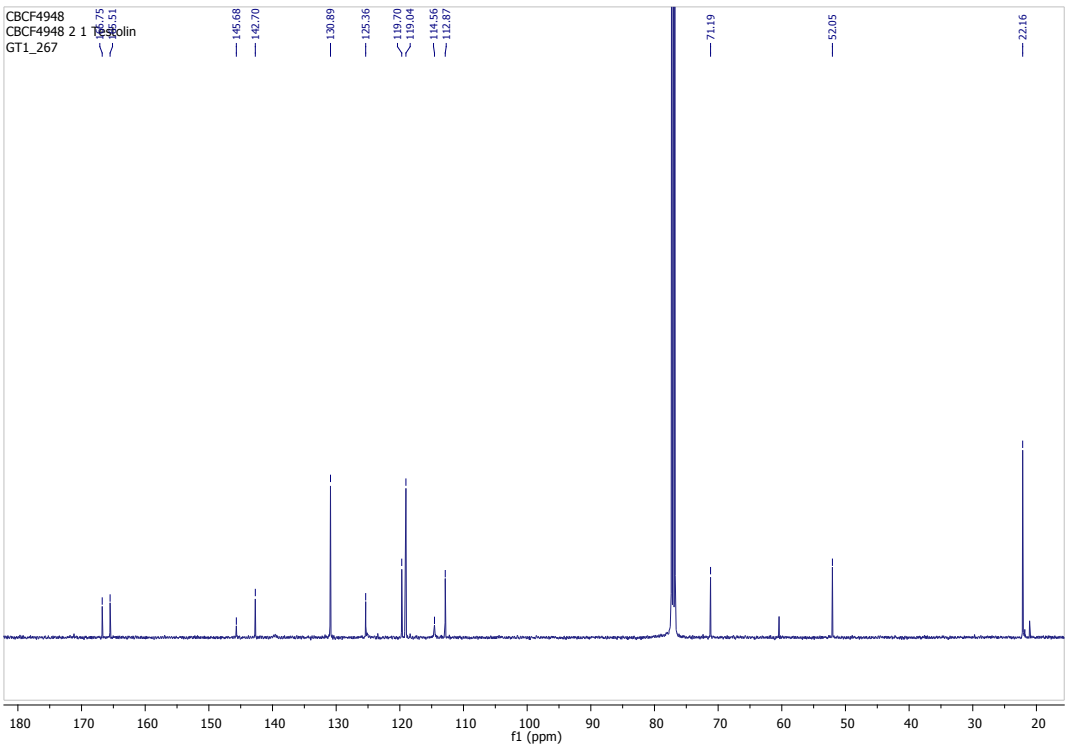
Methyl 4-(3-isopropoxy-4-nitrobenzamido)benzoate



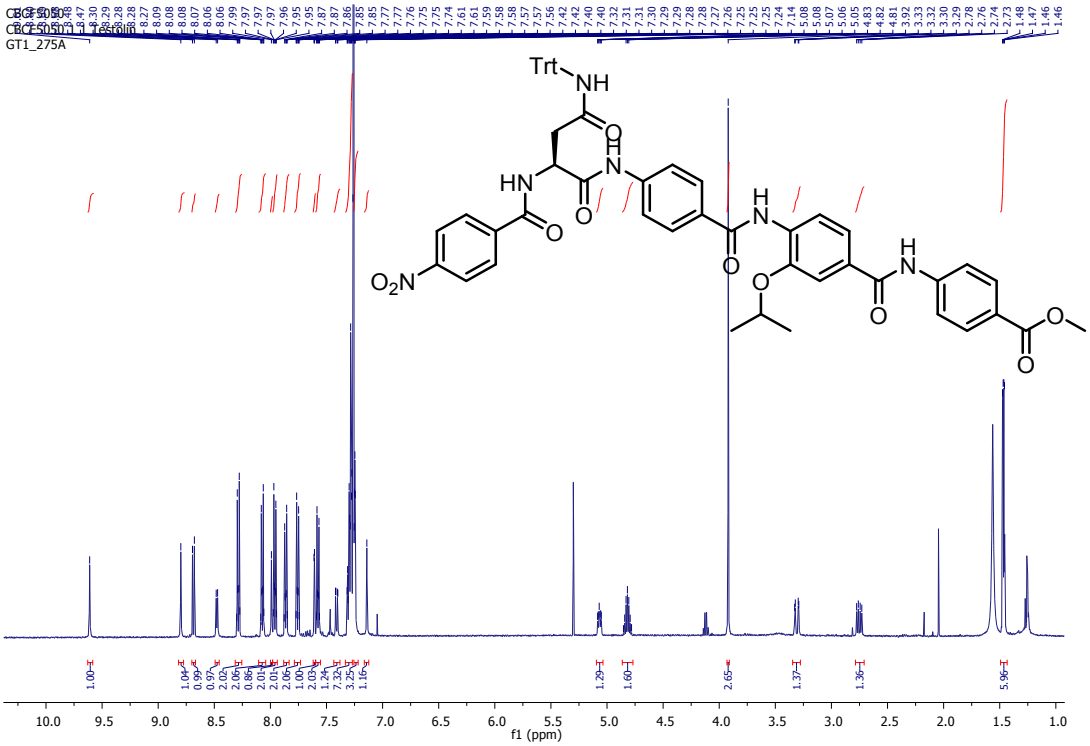


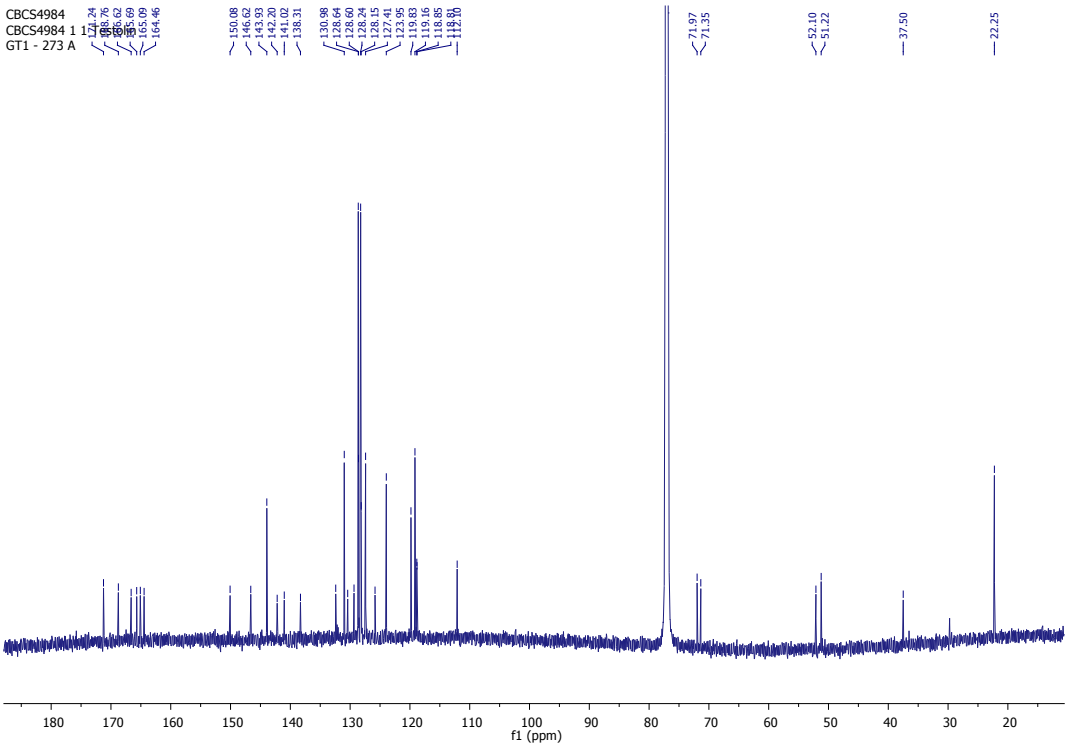
Methyl 4-(4-amino-3-isopropoxybenzamido)benzoate



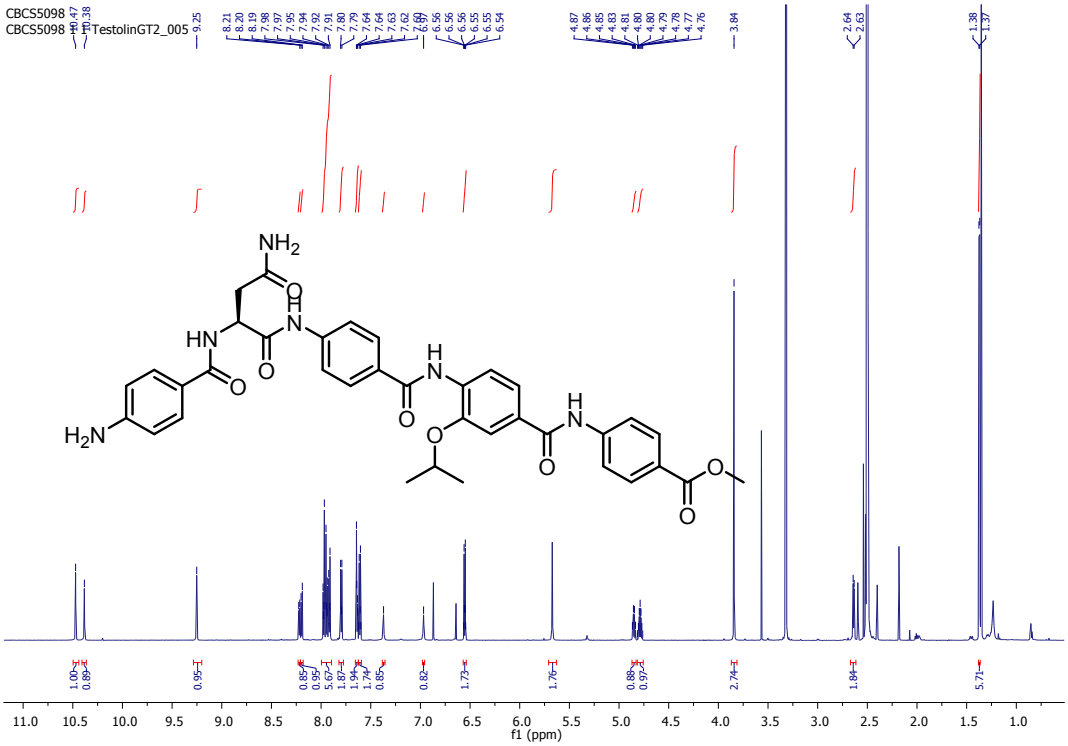


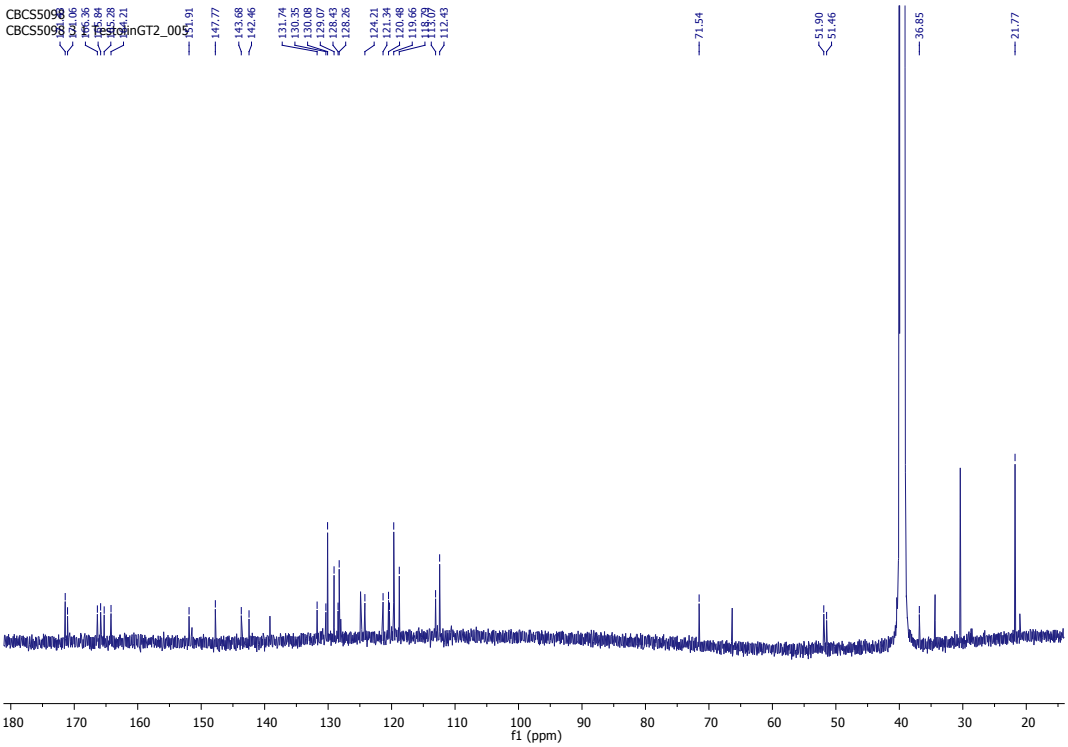
Methyl (S)-4-(3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)benzoate



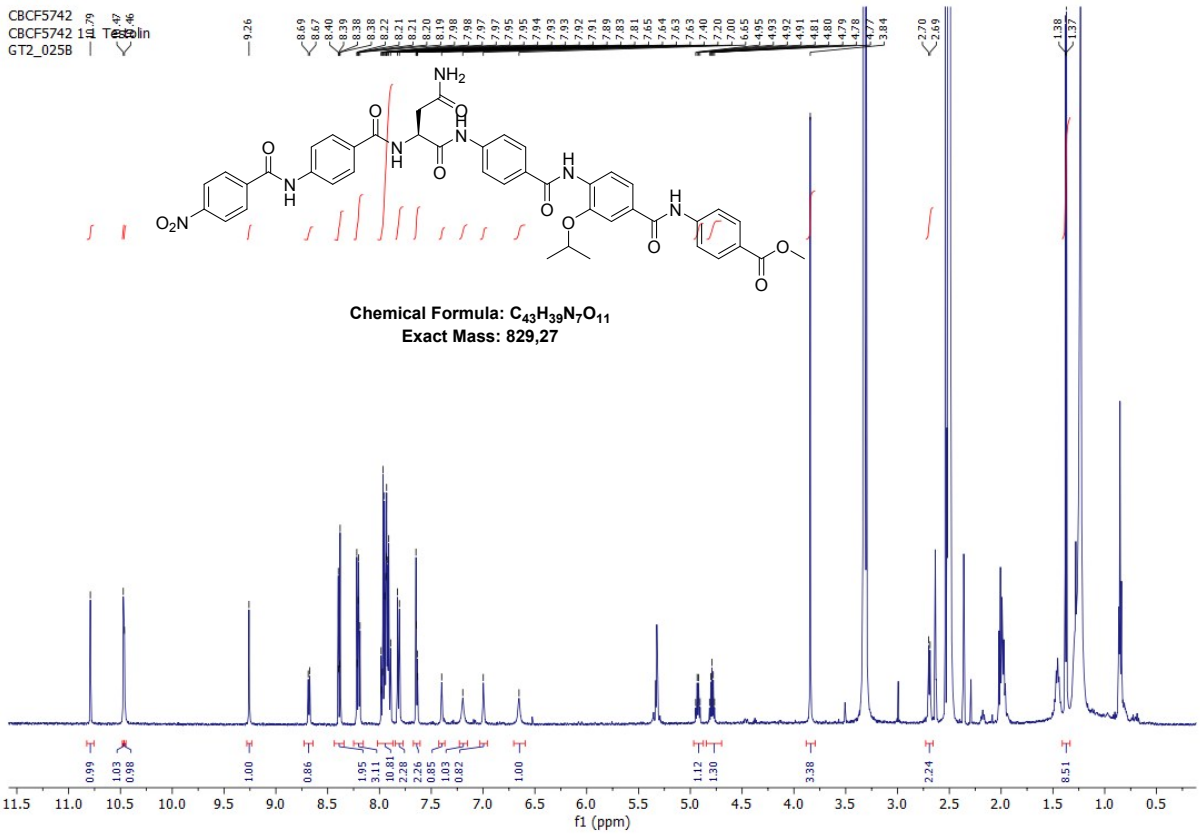


Methyl (S)-4-(4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate

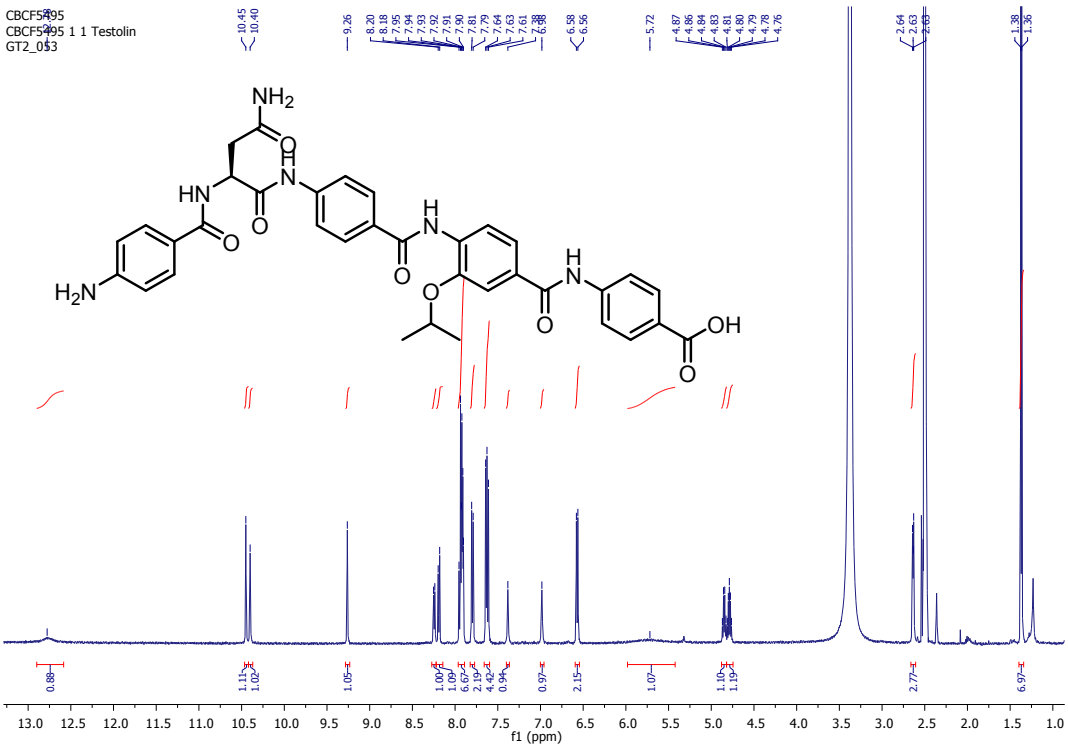


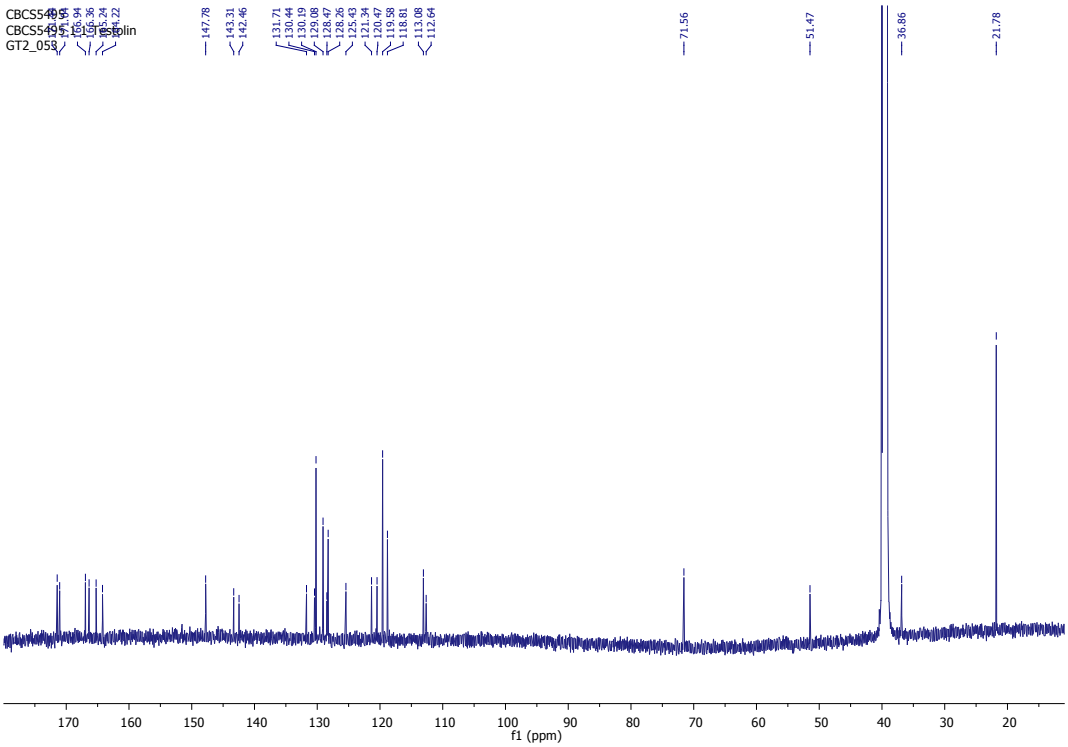


Methyl (S)-4-(4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate

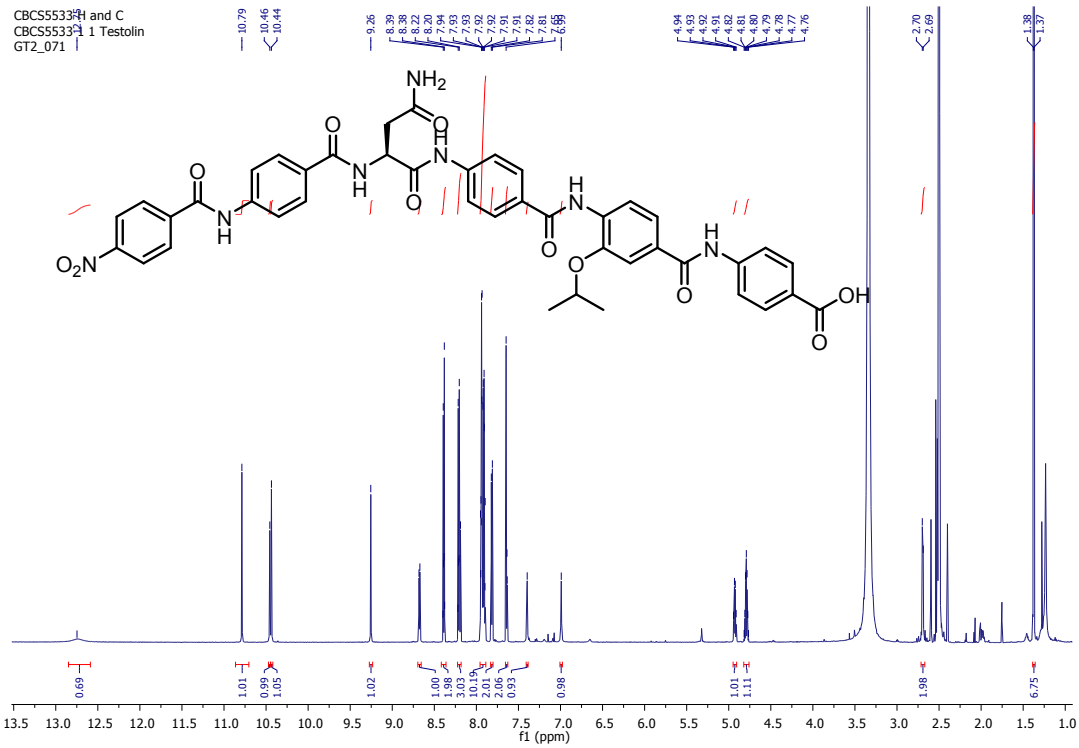


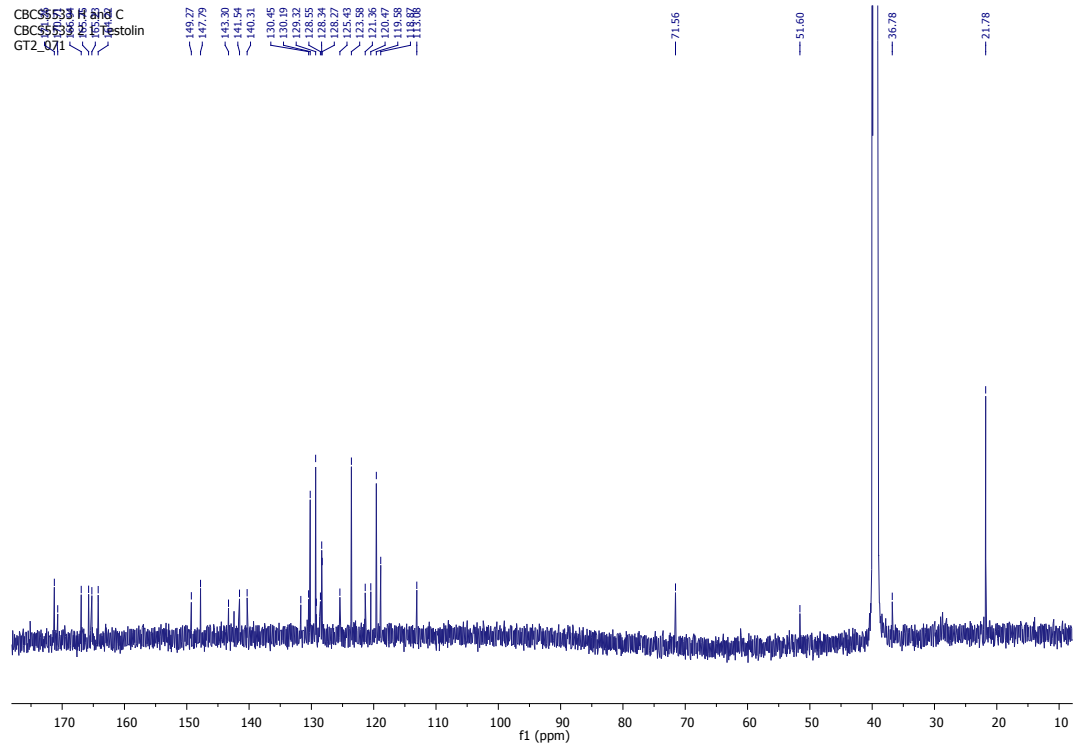
(S)-4-(4-(4-(4-Amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoic acid



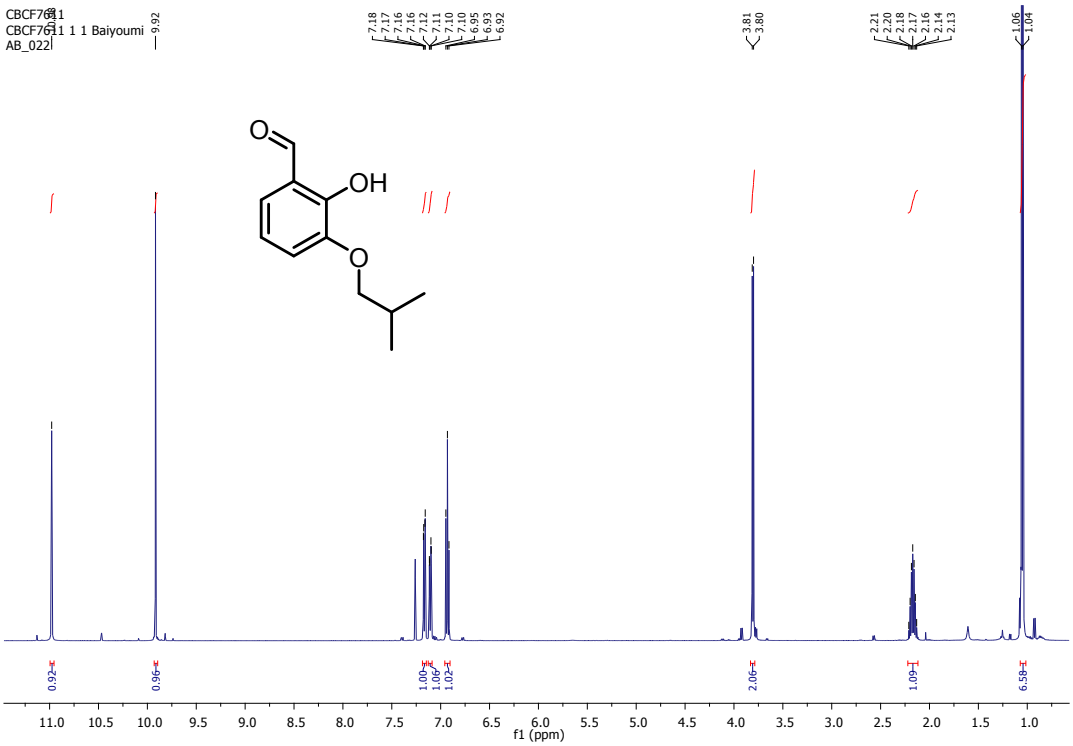


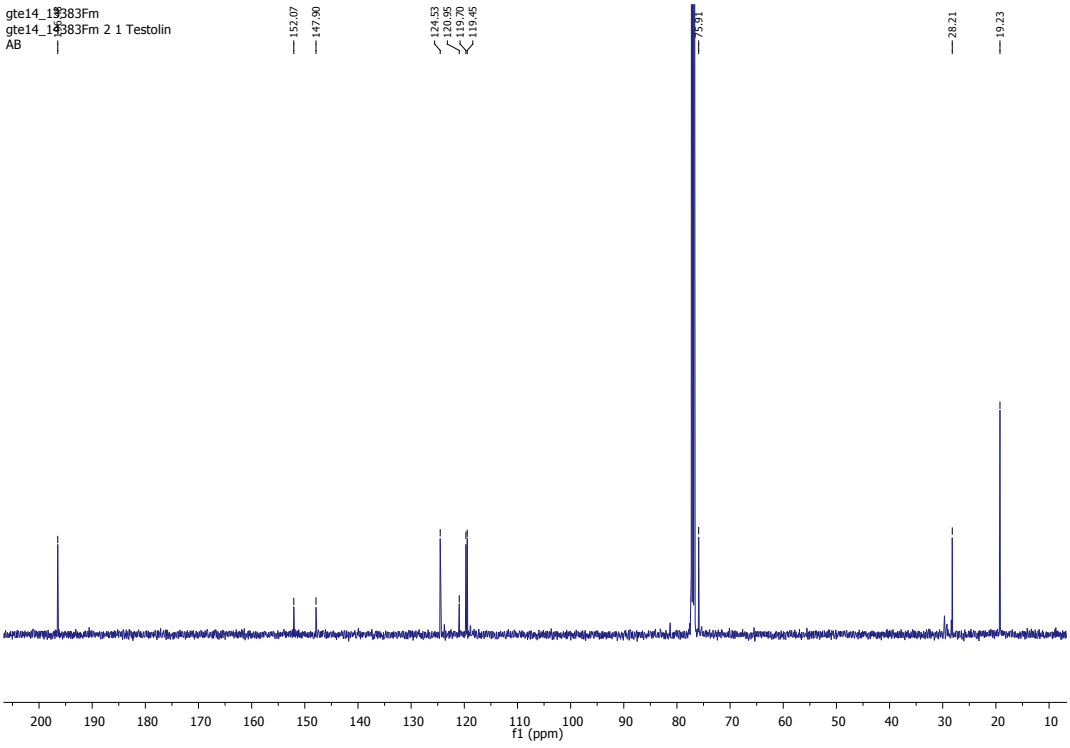
(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoic acid



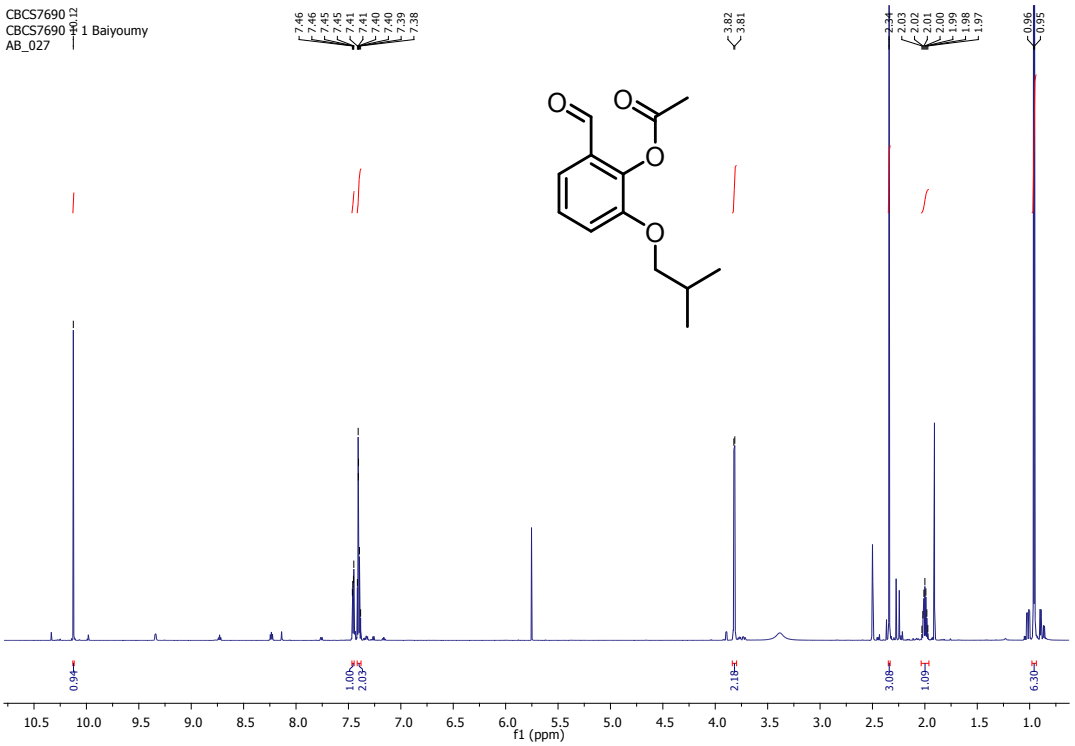


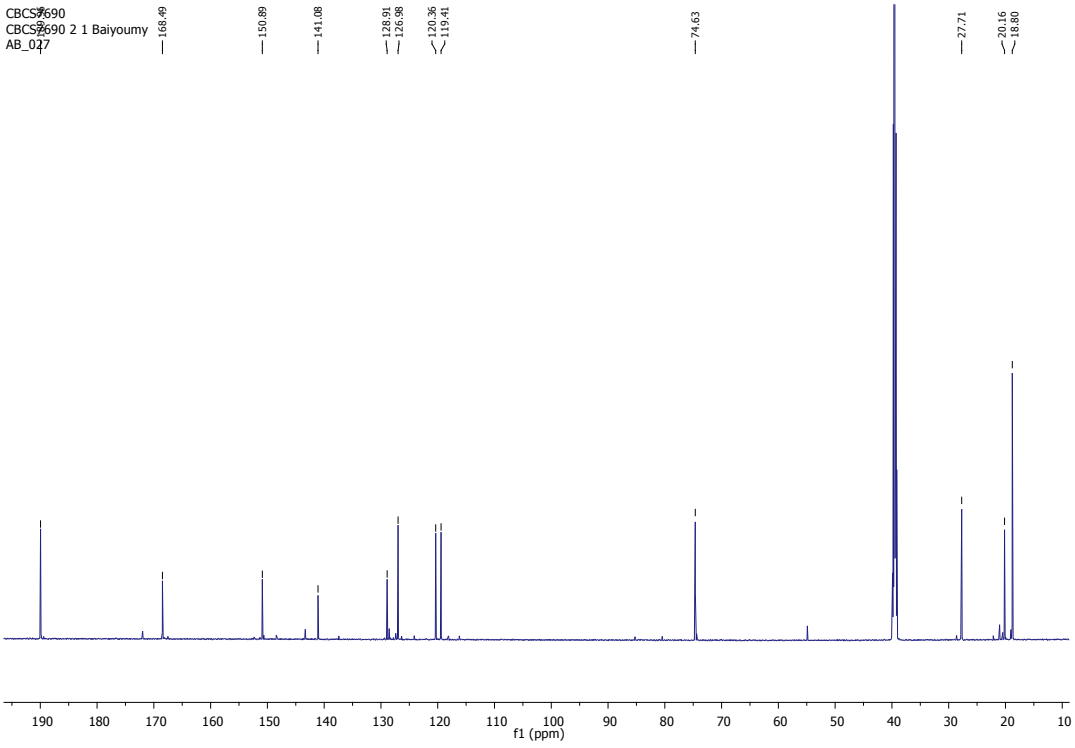
2-Hydroxy-3-isobutoxybenzaldehyde



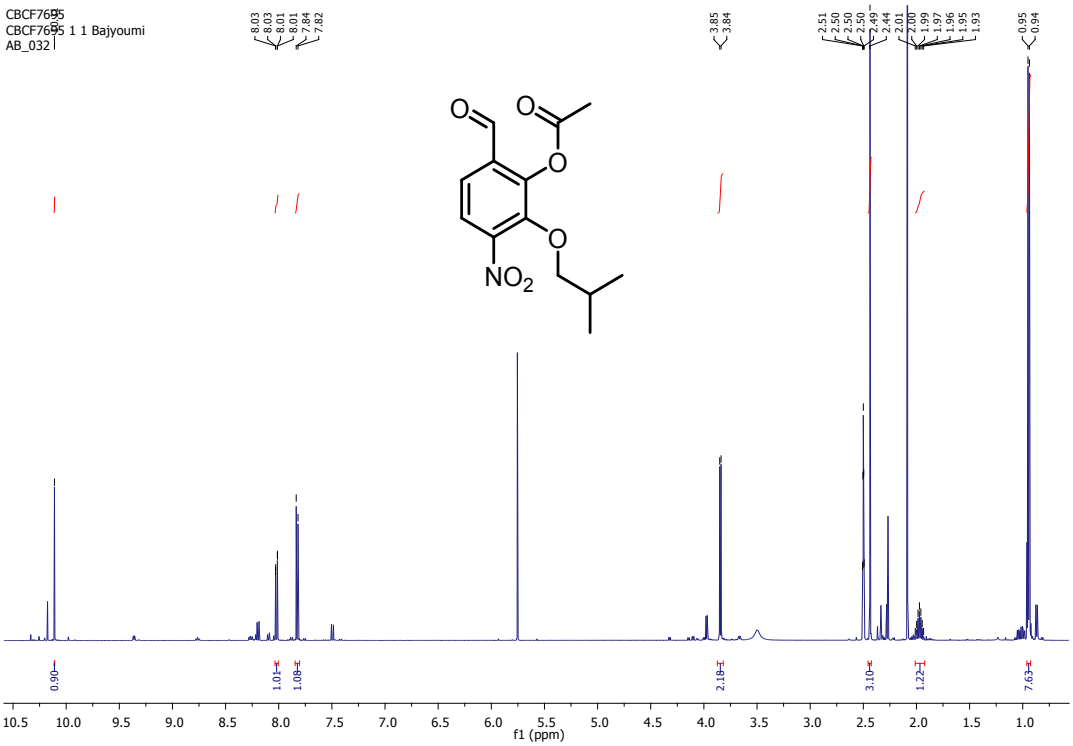


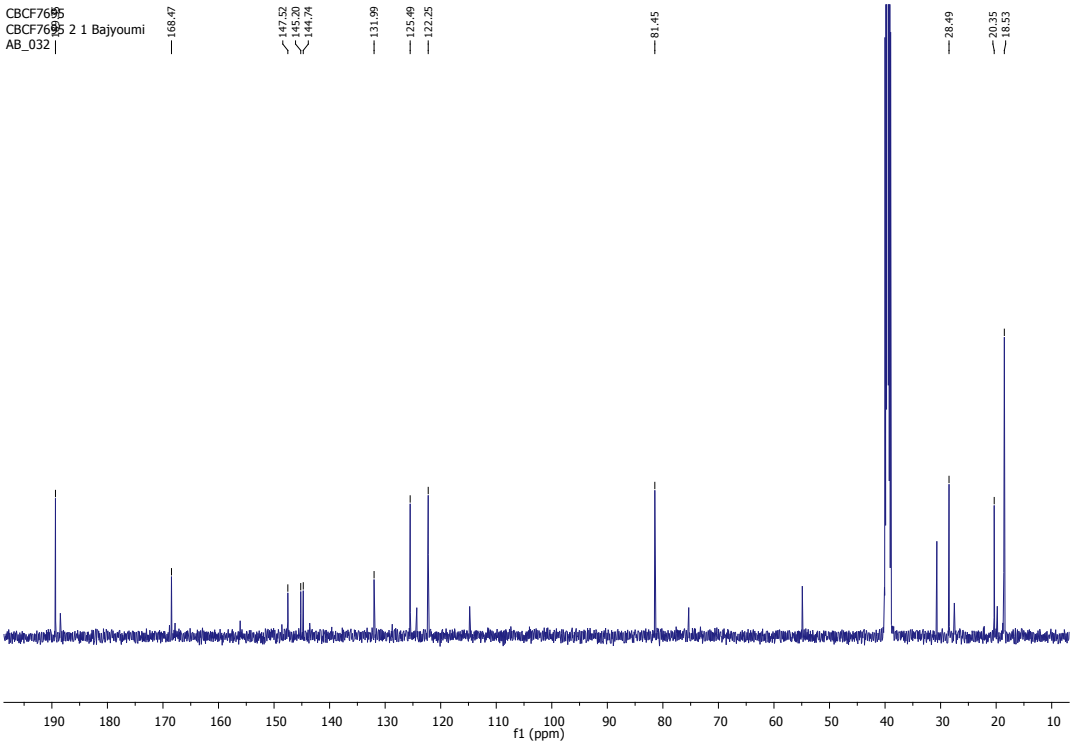
2-Formyl-6-isobutoxyphenyl acetate



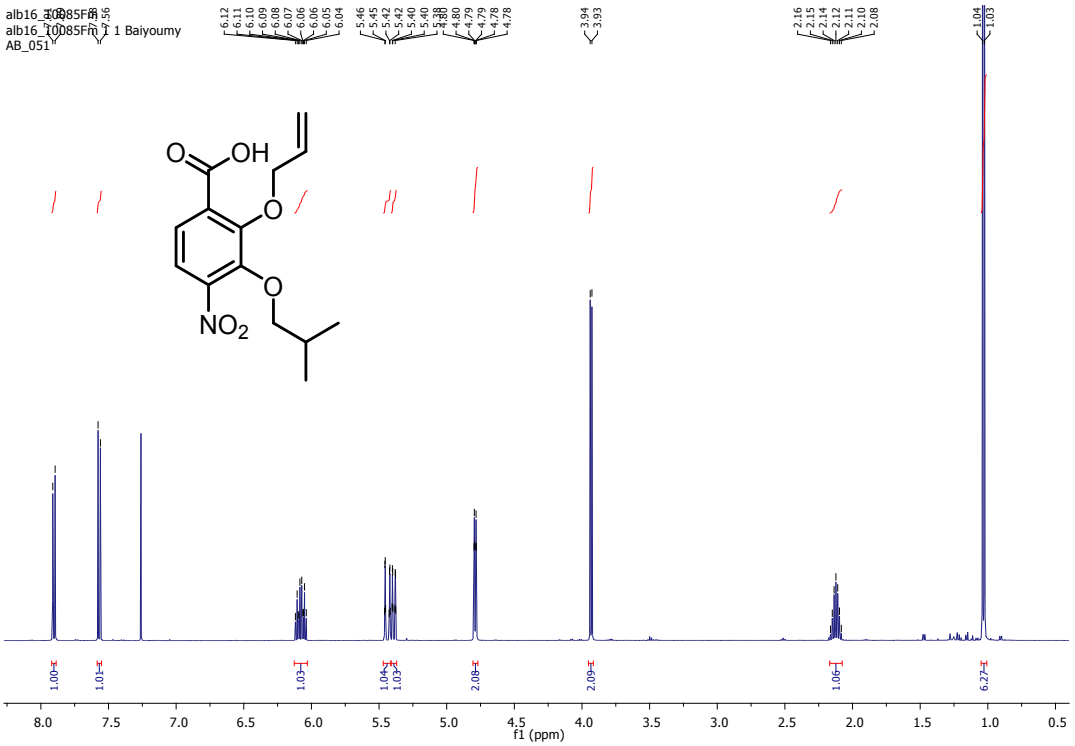


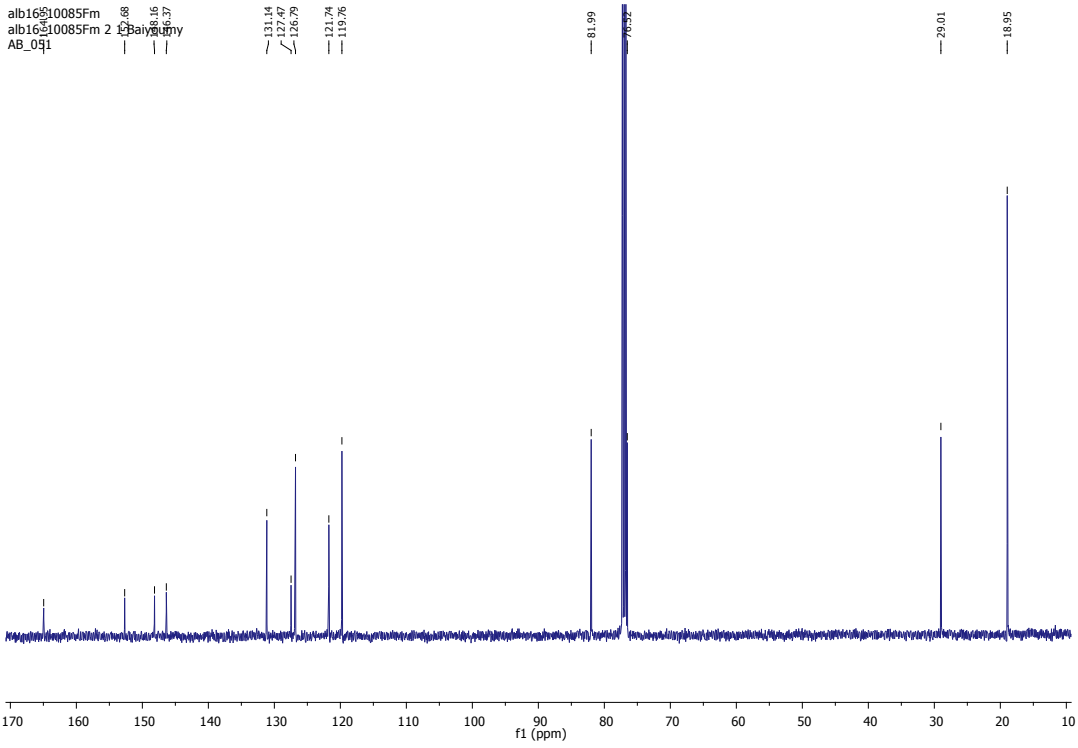
6-Formyl-2-isobutoxy-3-nitrophenyl acetate



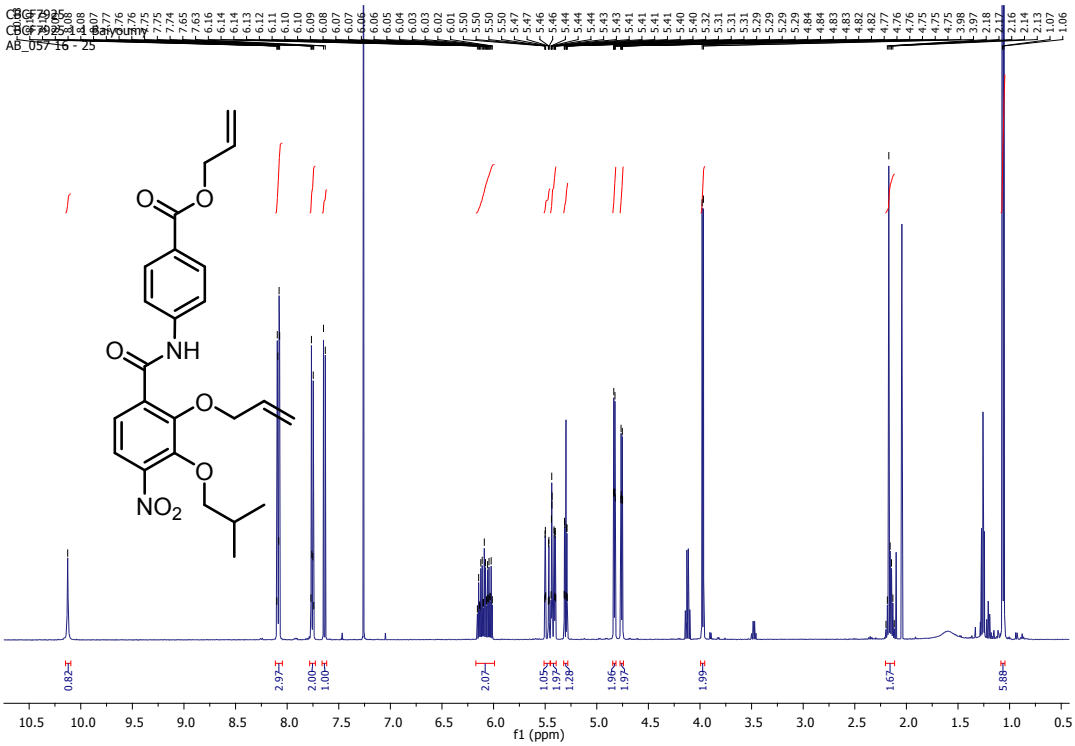


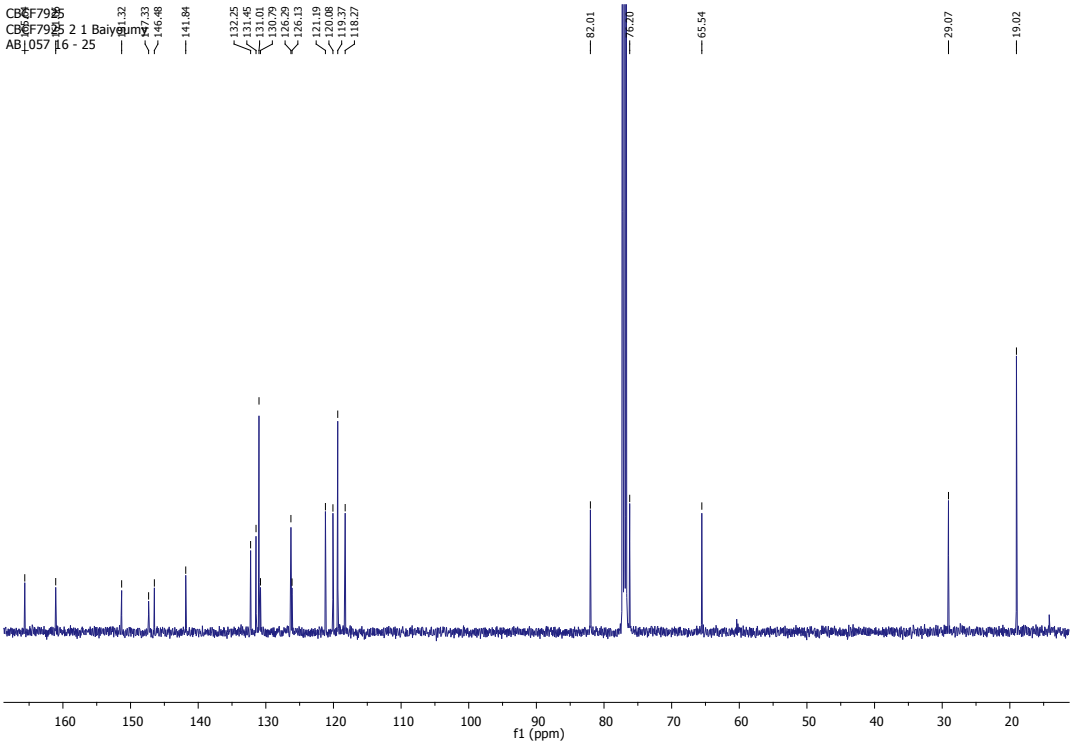
2-(Allyloxy)-3-isobutoxy-4-nitrobenzoic acid



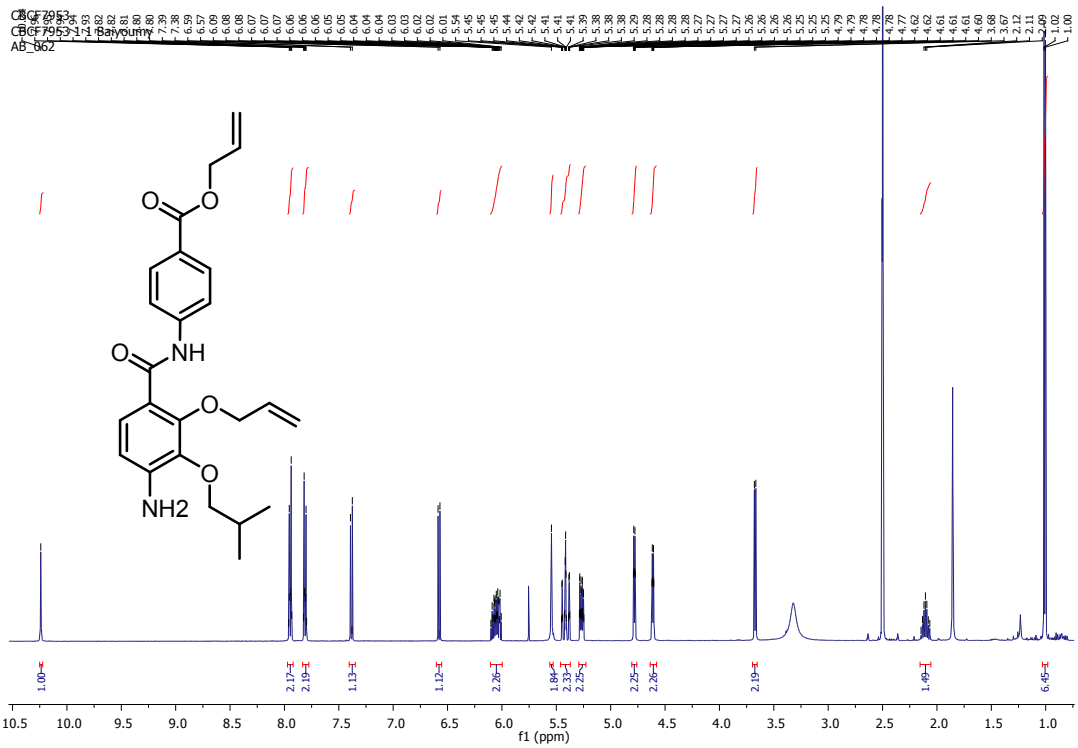


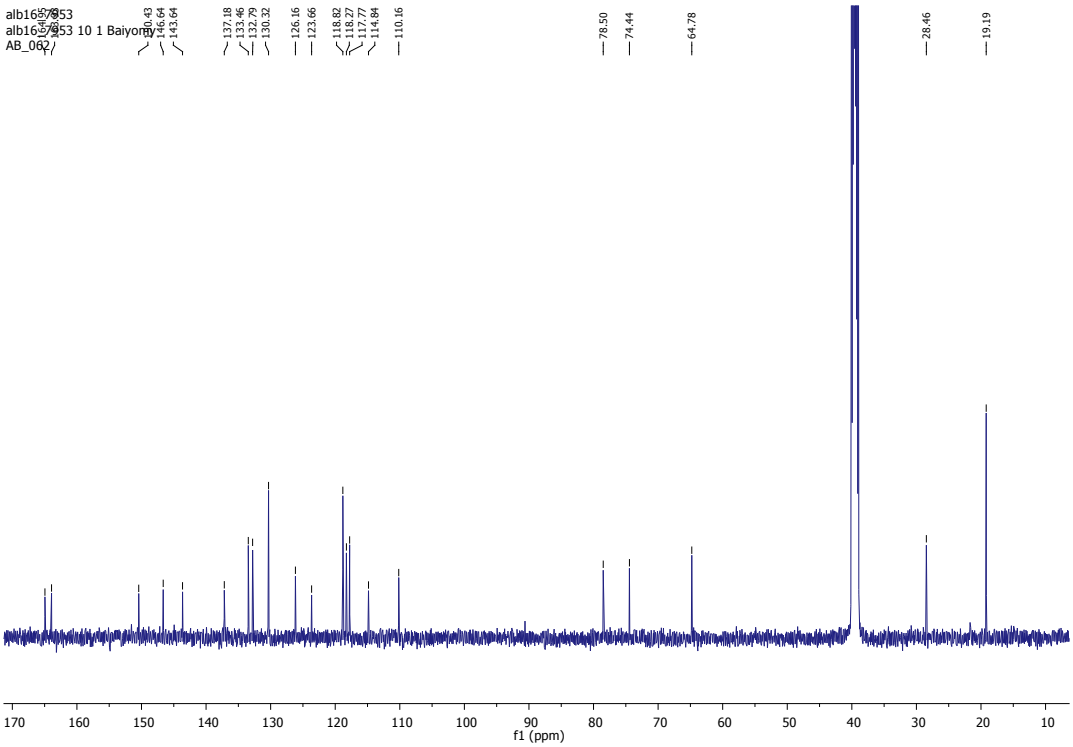
Allyl 4-(2-(allyloxy)-3-isobutoxy-4-nitrobenzamido)benzoate



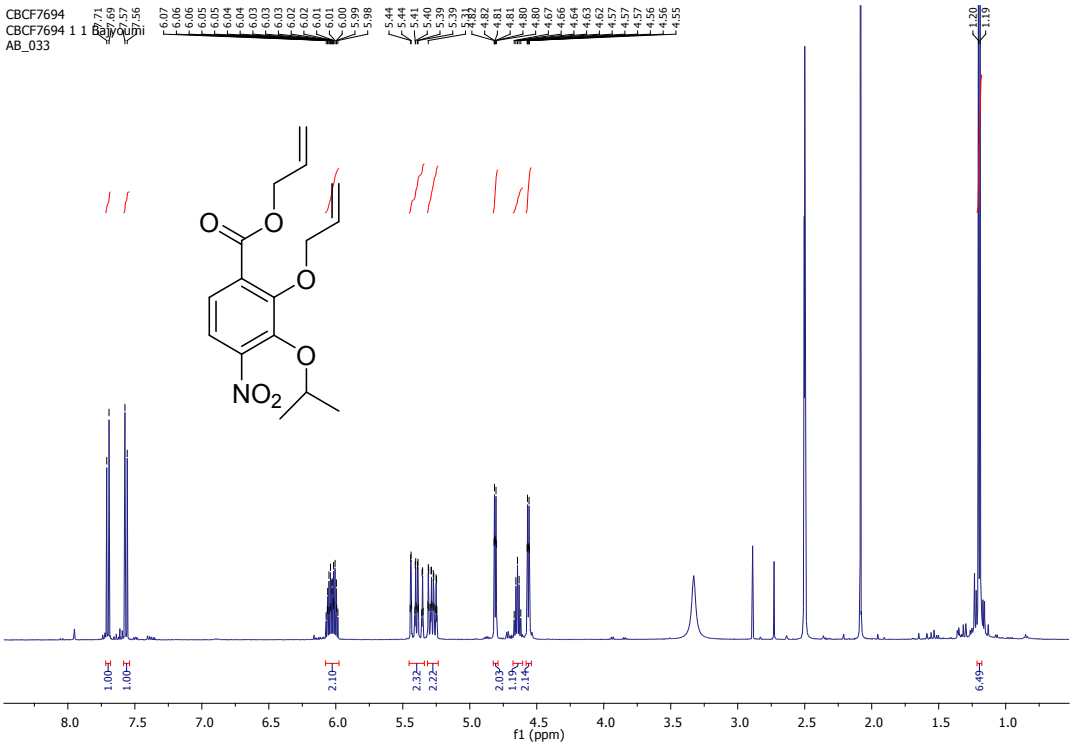


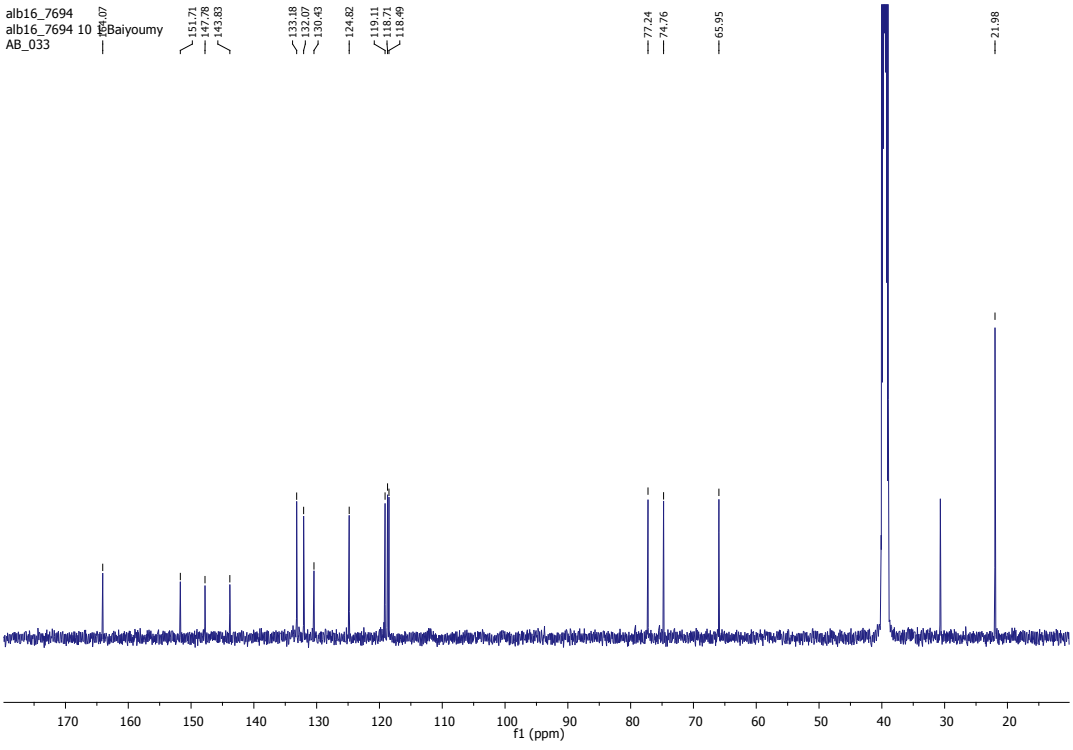
Allyl 4-(2-(allyloxy)-4-amino-3-isobutoxybenzamido)benzoate



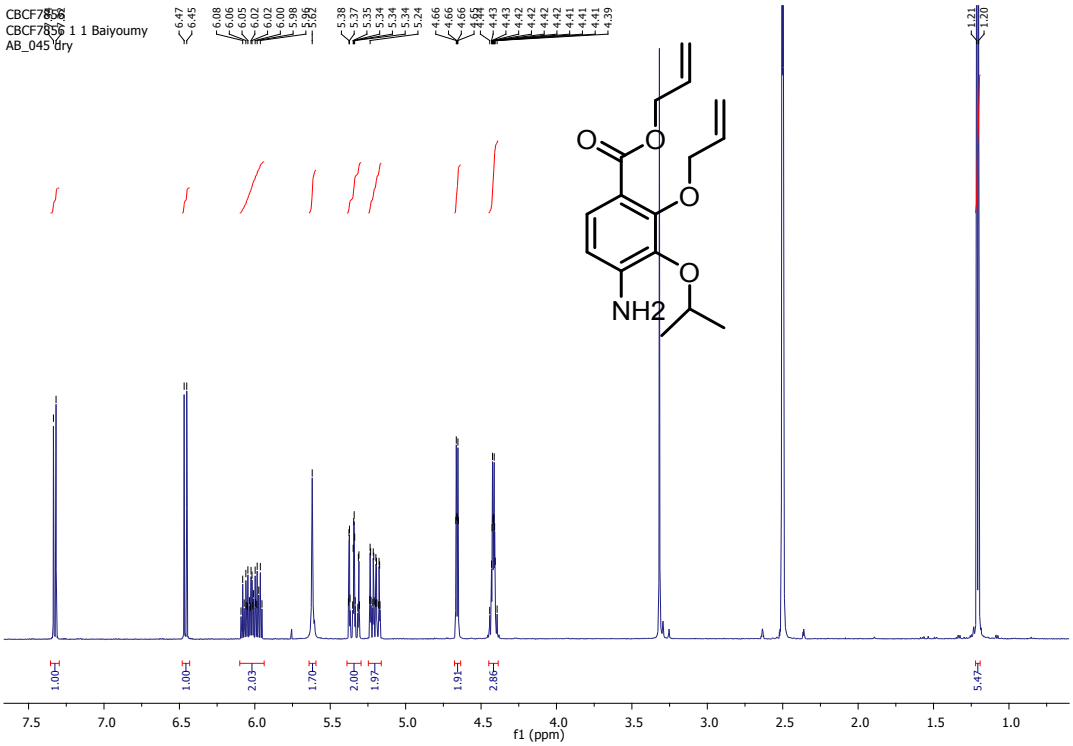


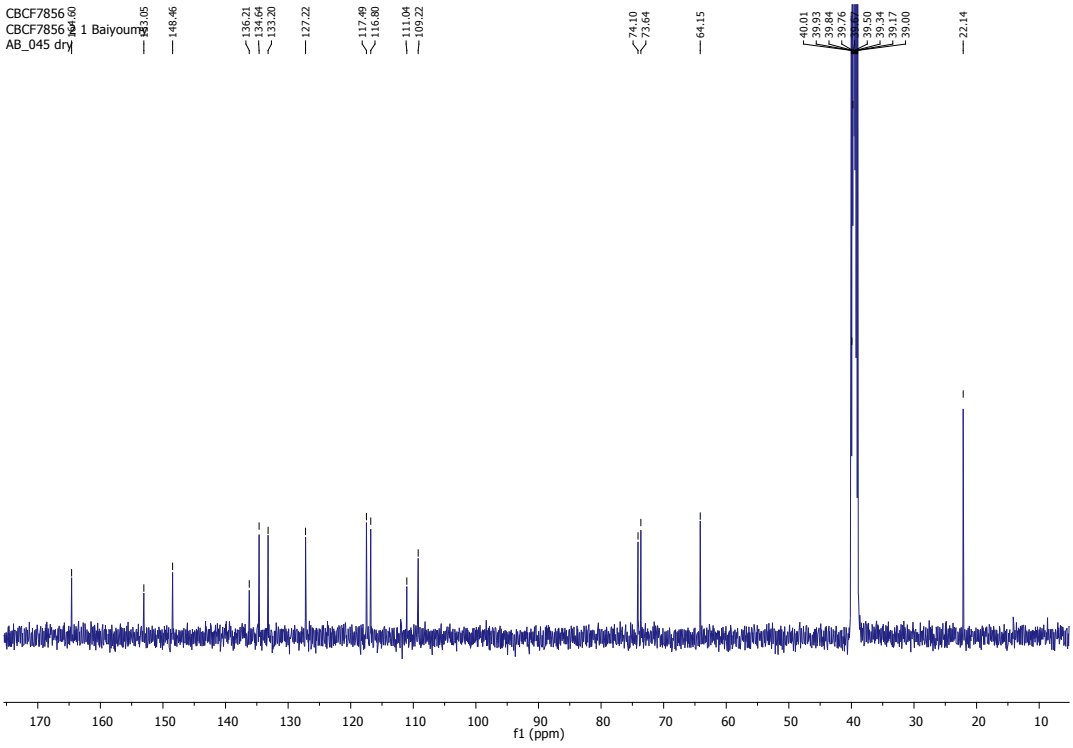
Allyl 2-(allyloxy)-3-isopropoxy-4-nitrobenzoate



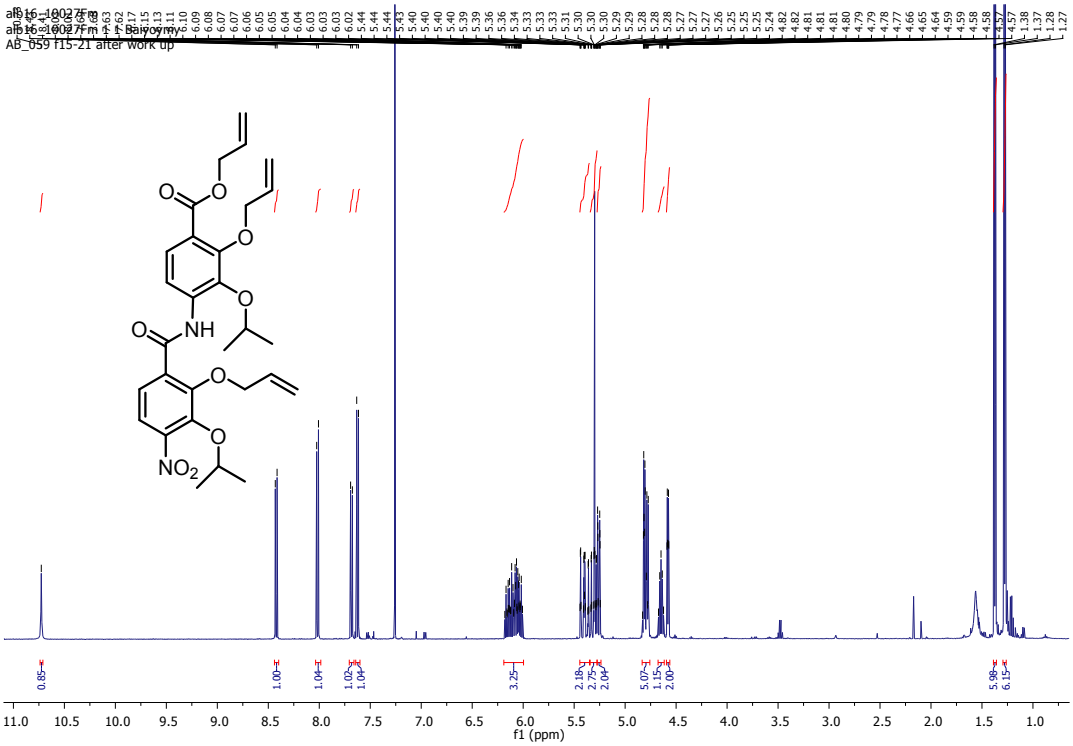


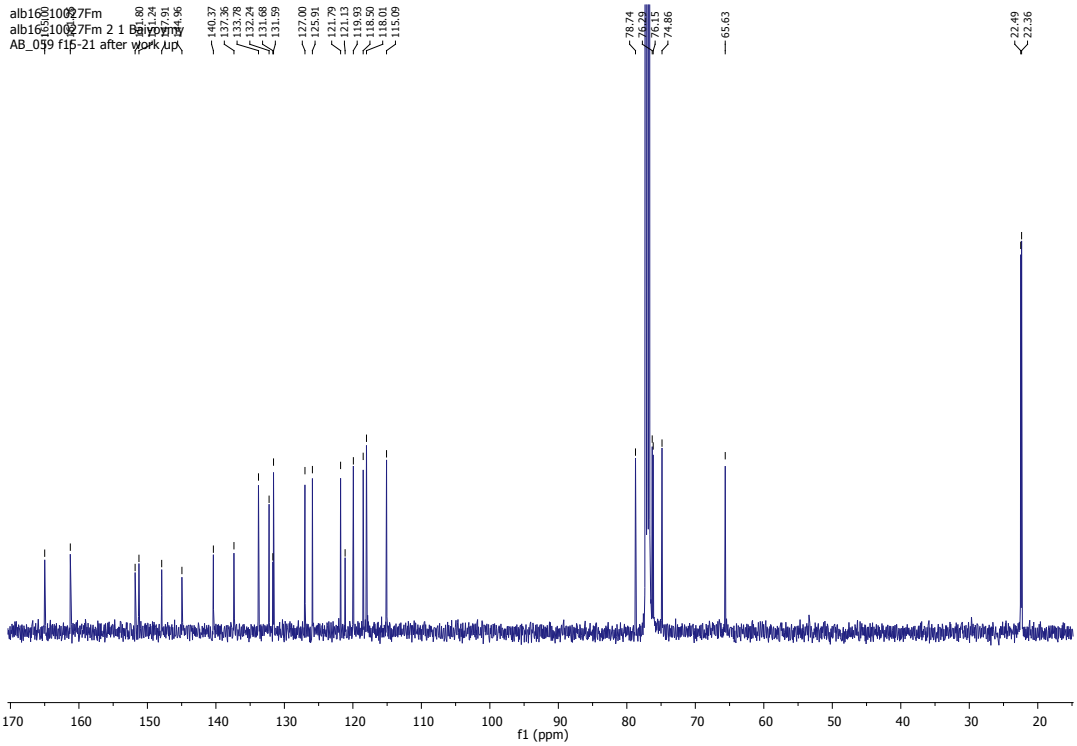
Allyl 2-(allyloxy)-4-amino-3-isopropoxybenzoate



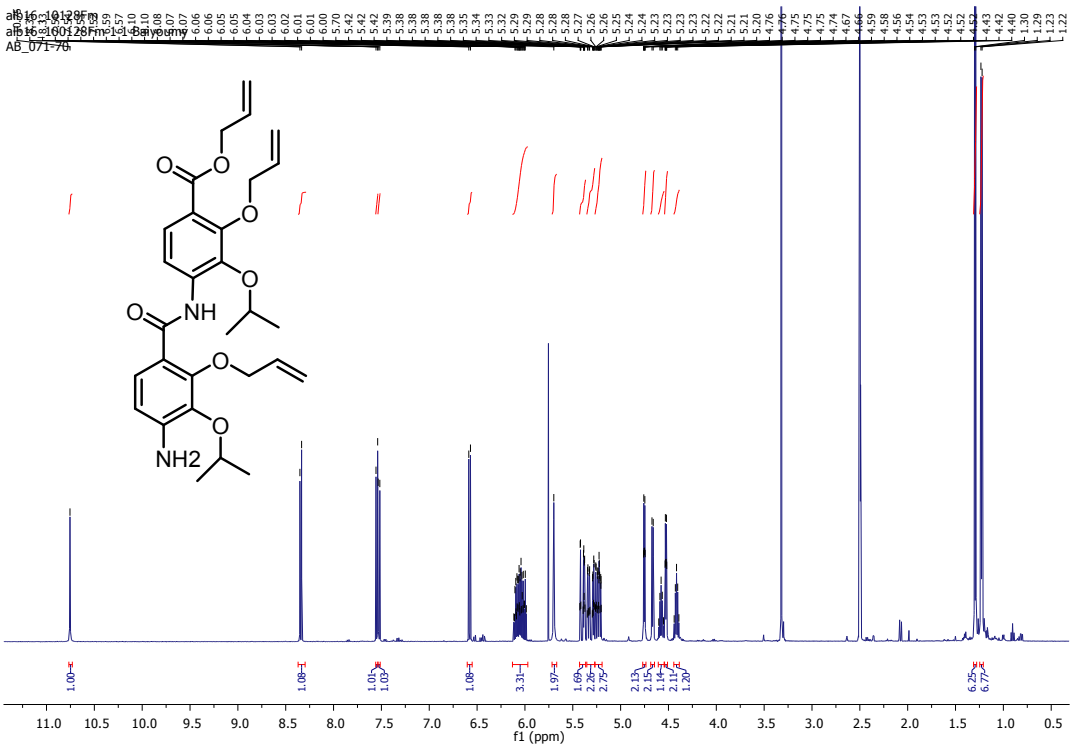


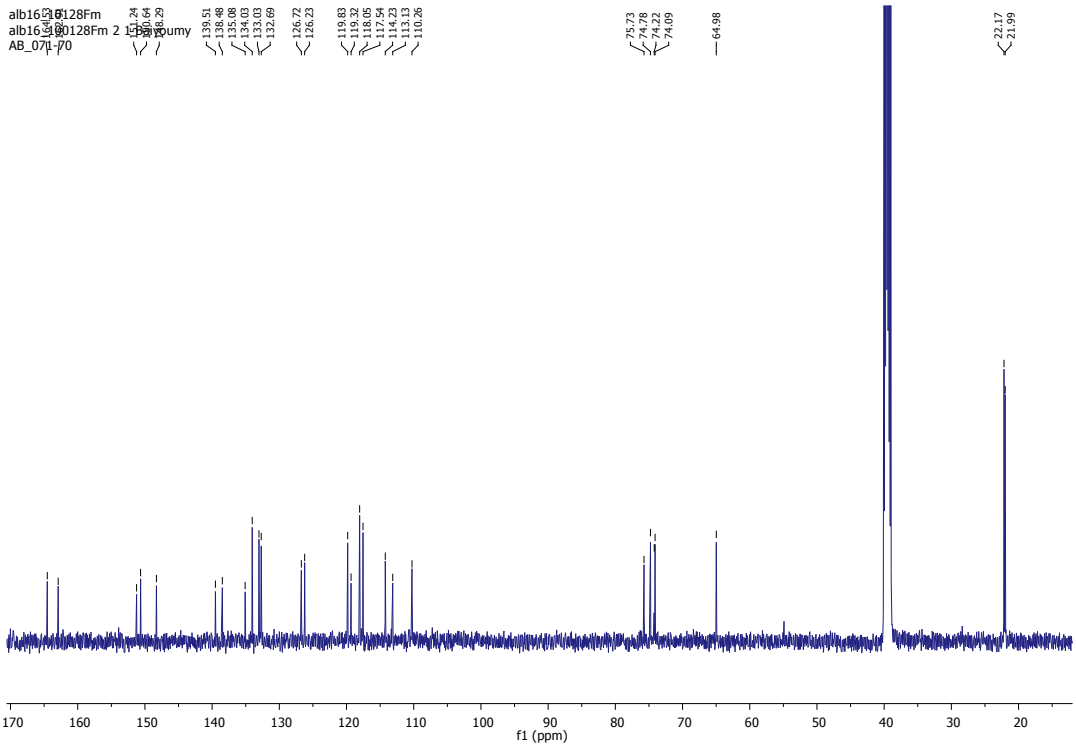
Allyl 2-(allyloxy)-4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)-3-isopropoxybenzoate



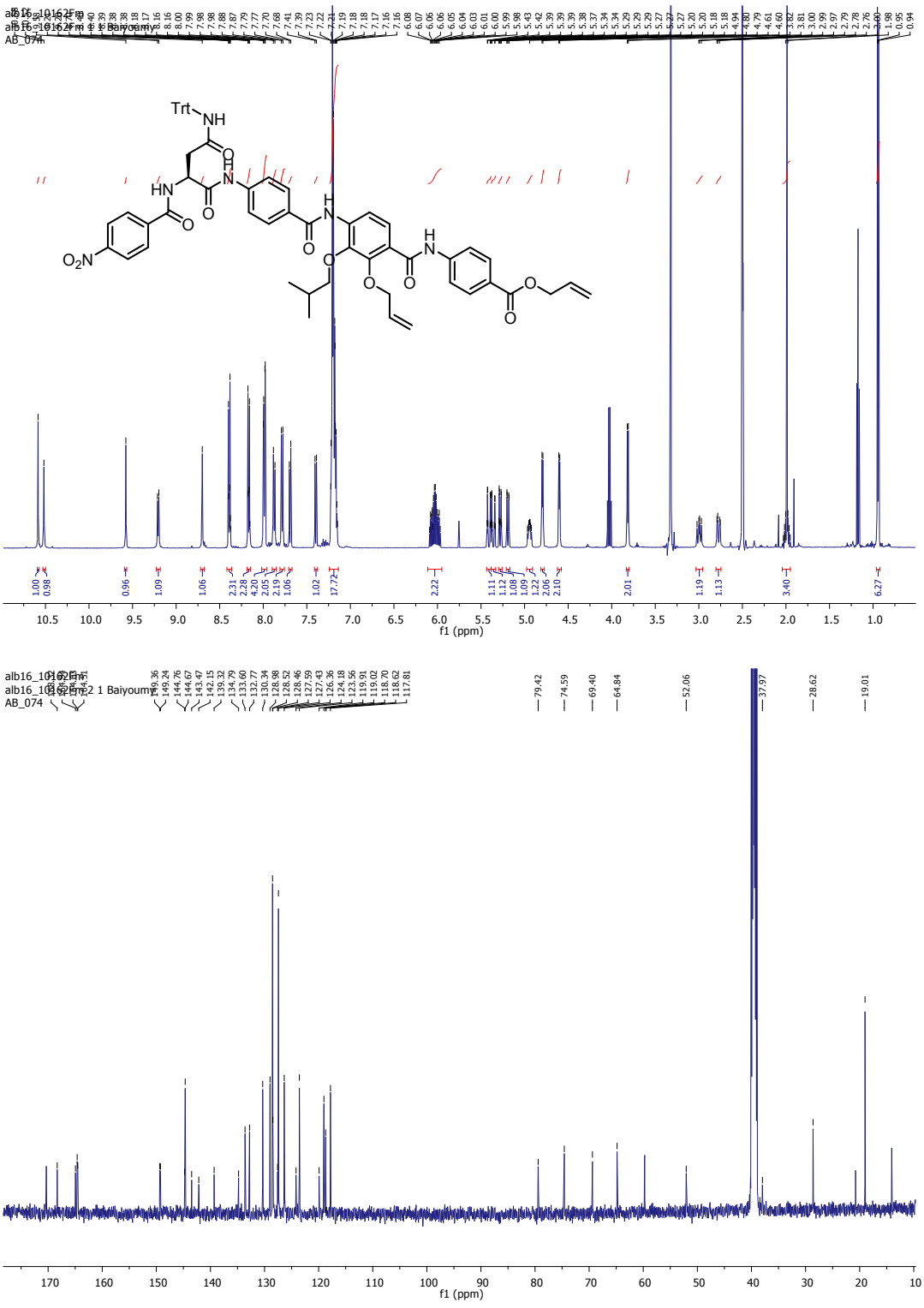


Allyl 2-(allyloxy)-4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)-3-isopropoxybenzoate

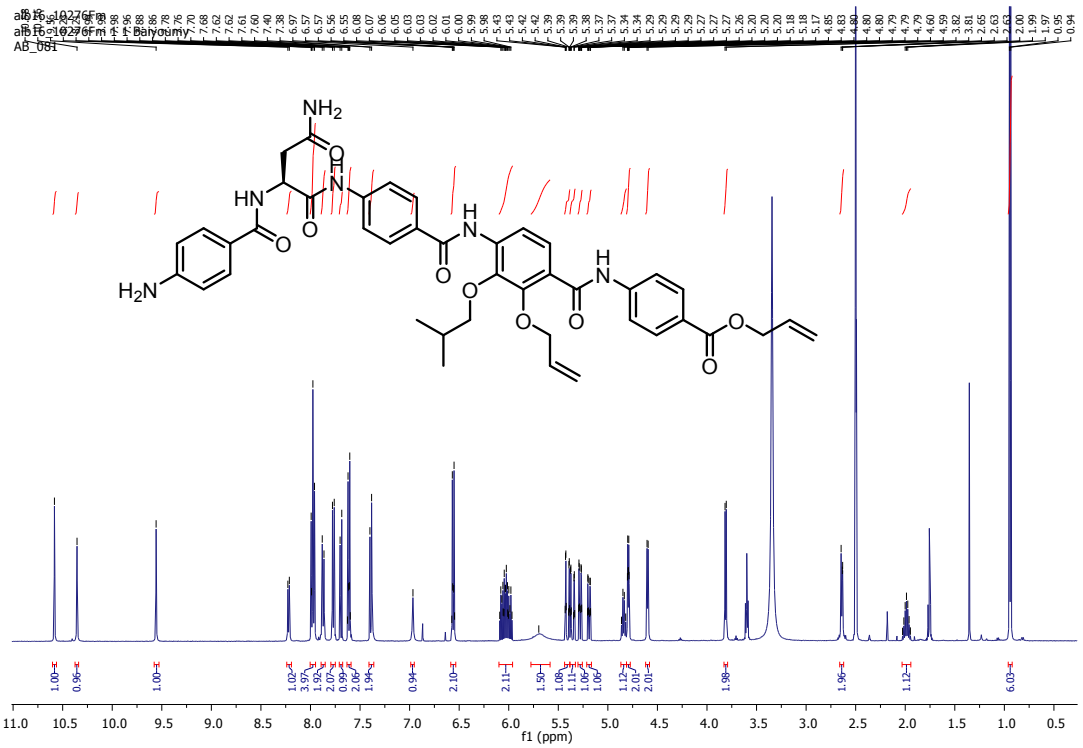


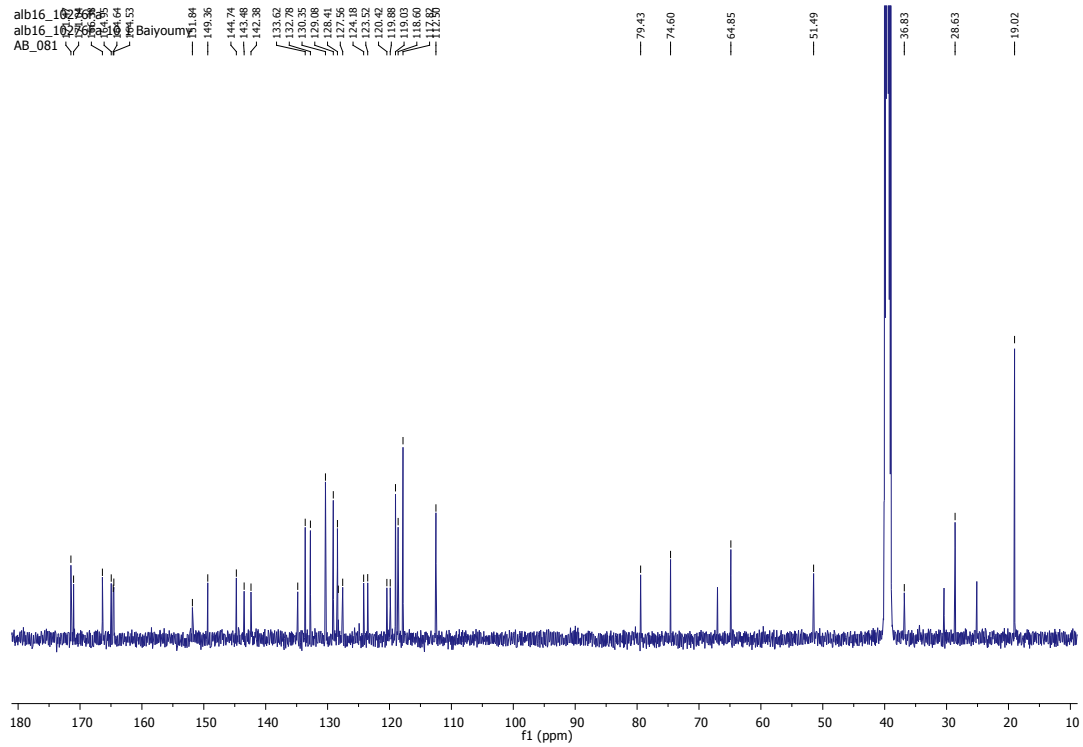


Allyl (S)-4-(2-(allyloxy)-3-isobutoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)benzoate

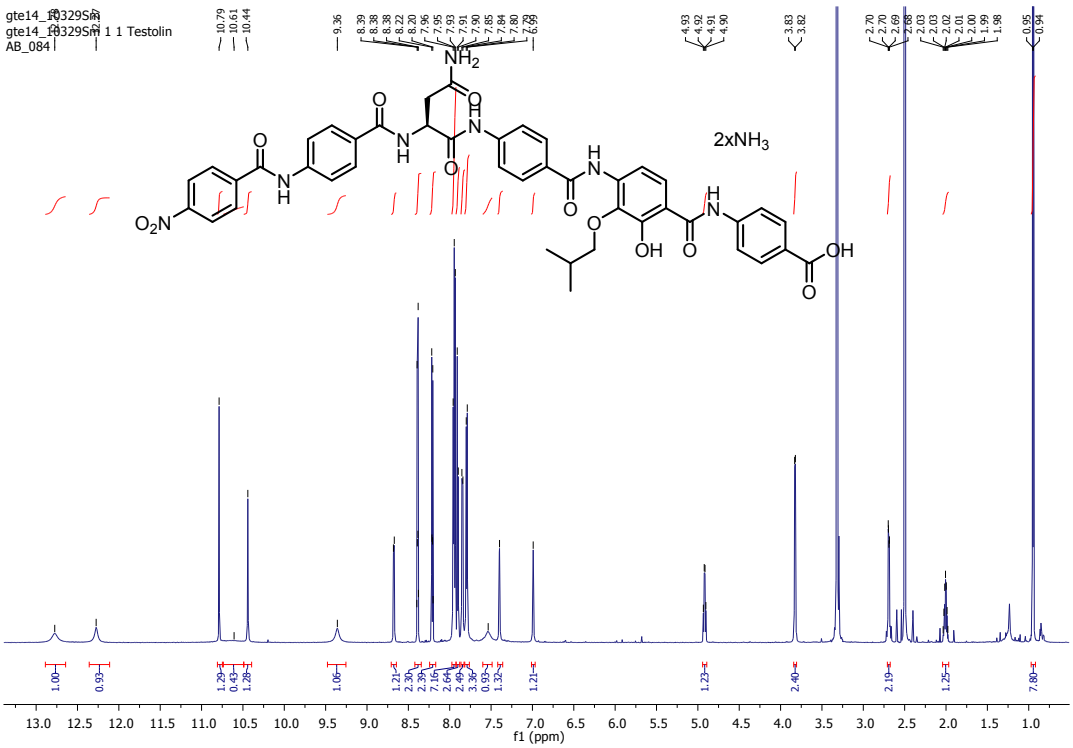


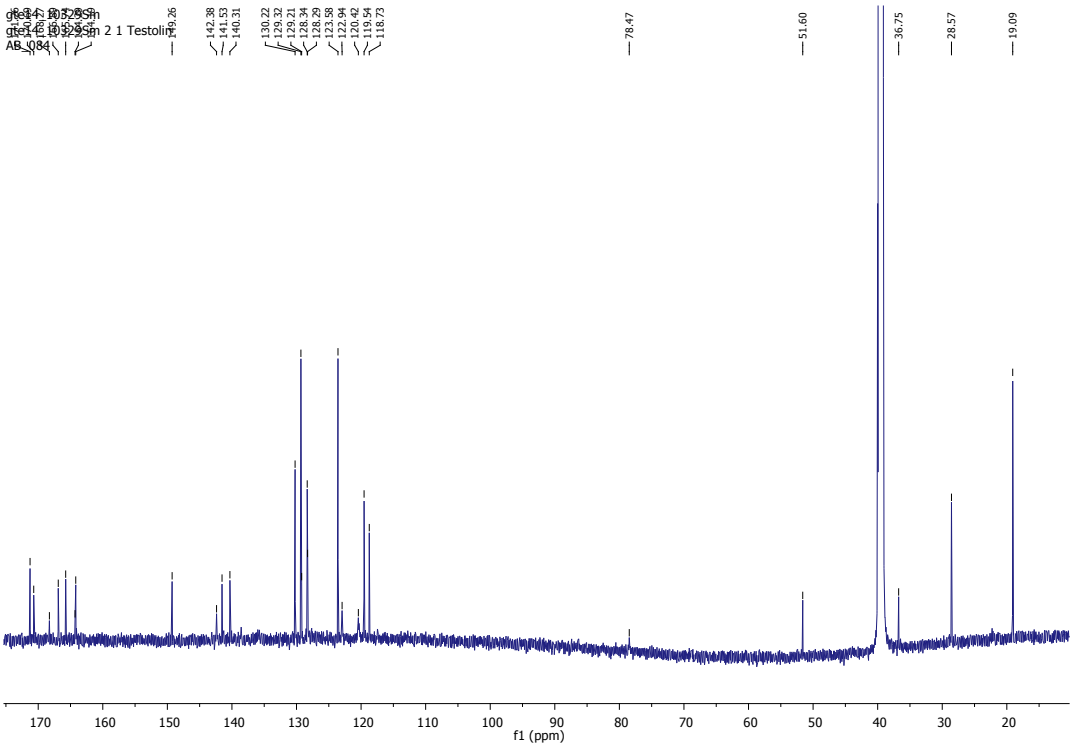
Allyl (S)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isobutoxybenzamido)benzoate



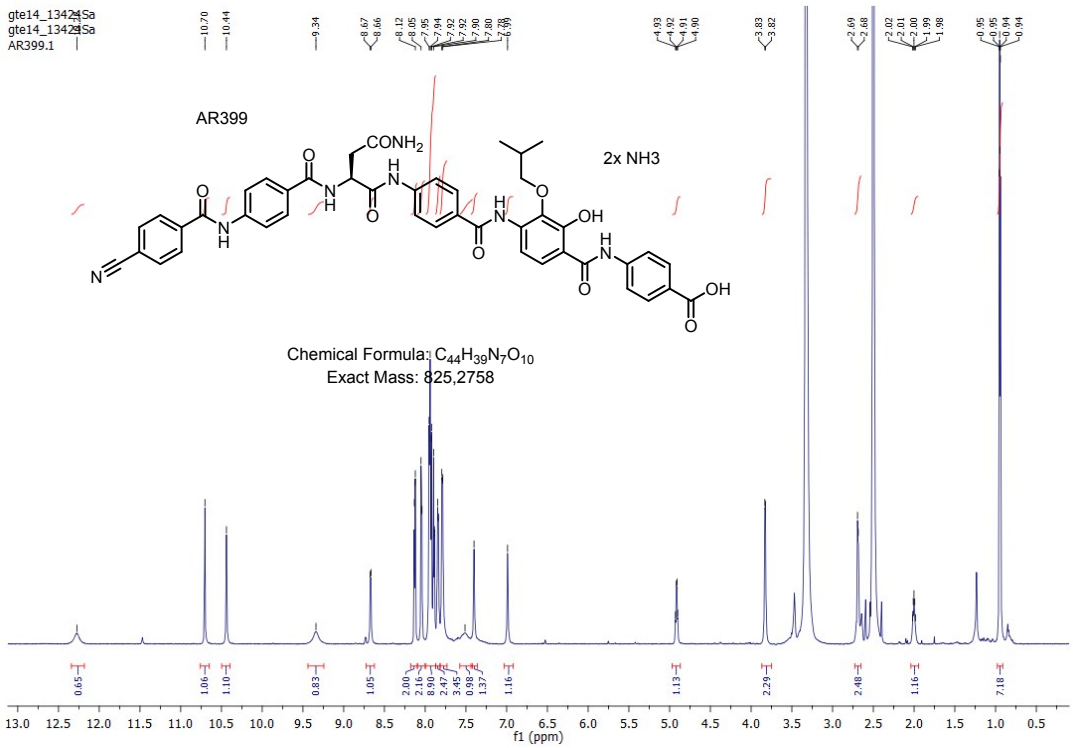


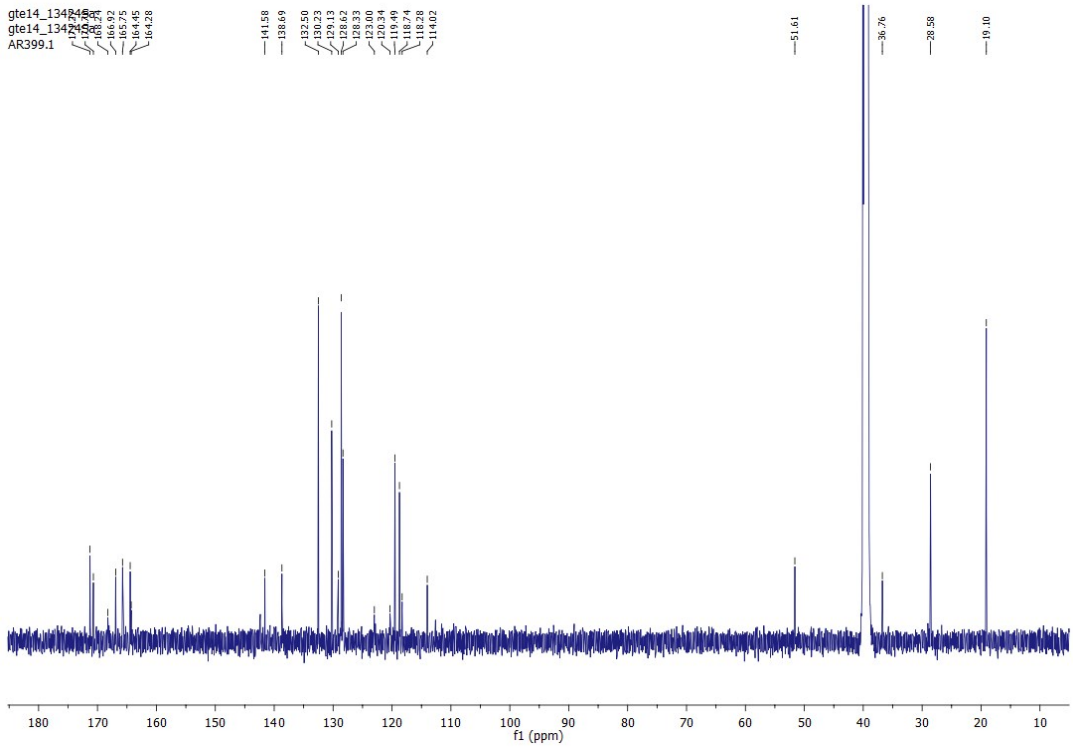
(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isobutoxybenzamido)benzoic acid



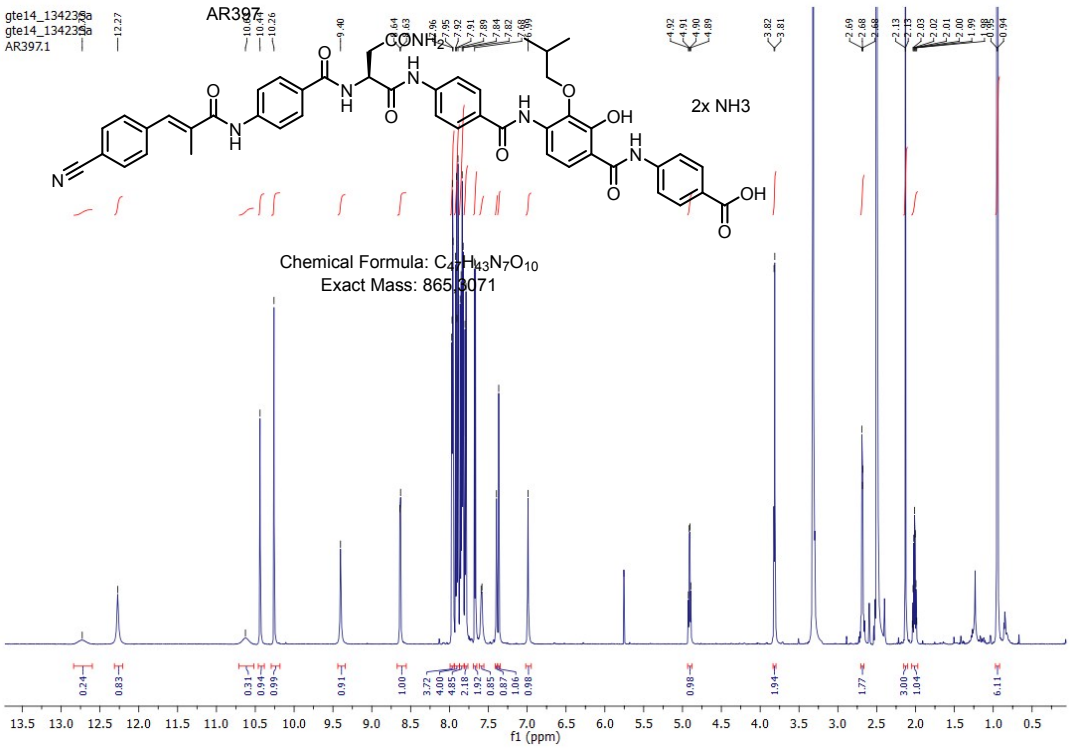


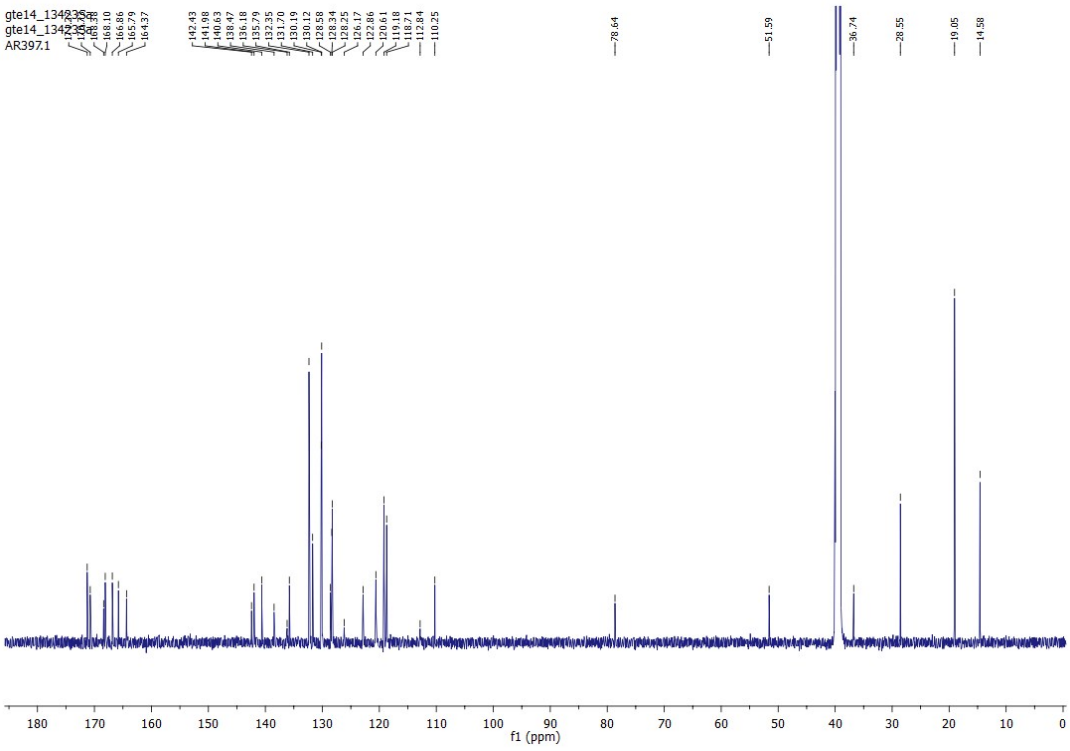
(S)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isobutoxybenzamido)benzoic acid



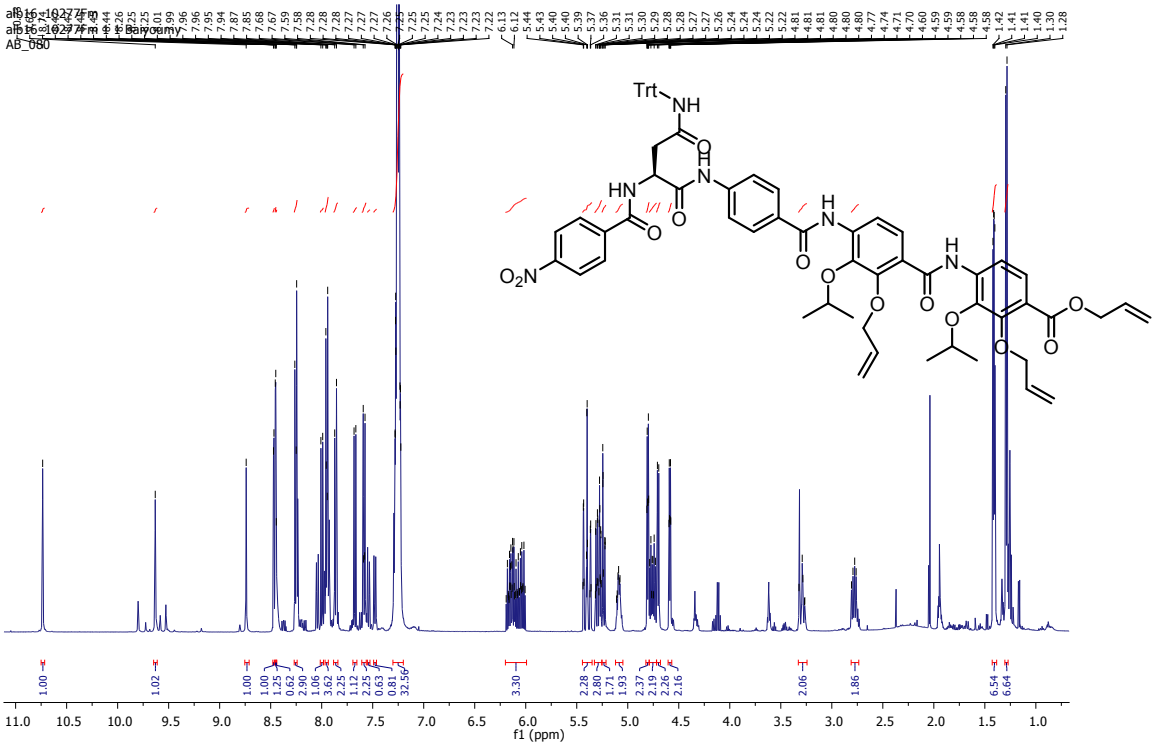


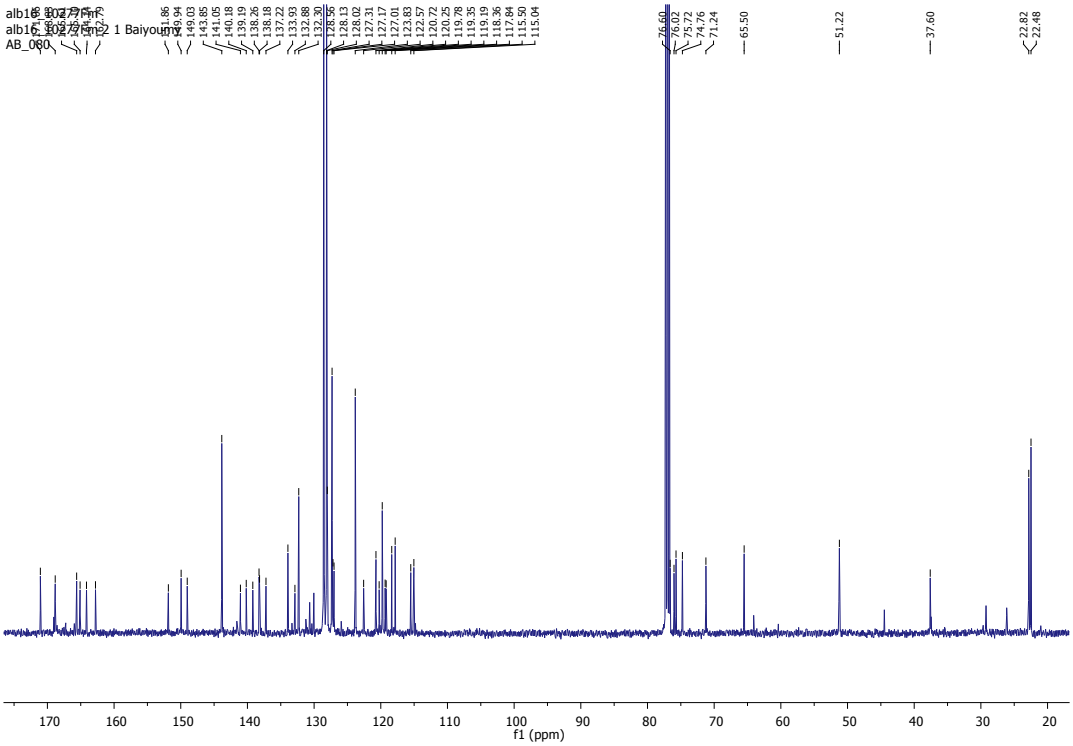
(S,E)-4-(4-(4-(4-amino-2-(4-(3-(4-cyanophenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isobutoxybenzamido)benzoic acid



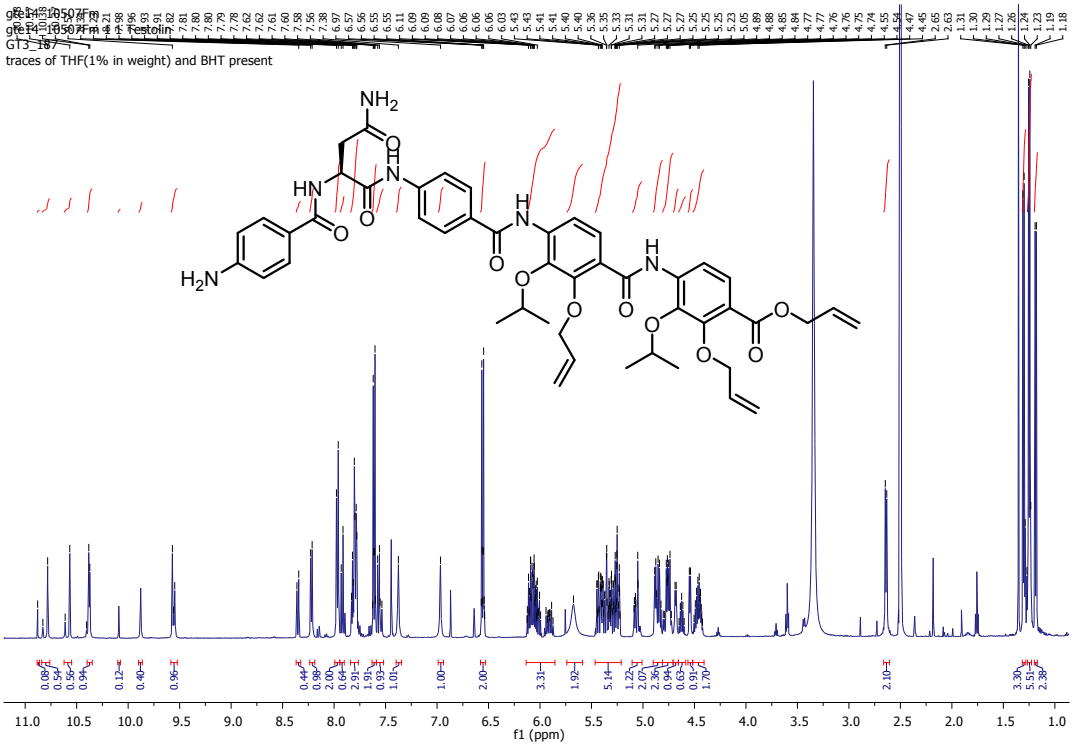


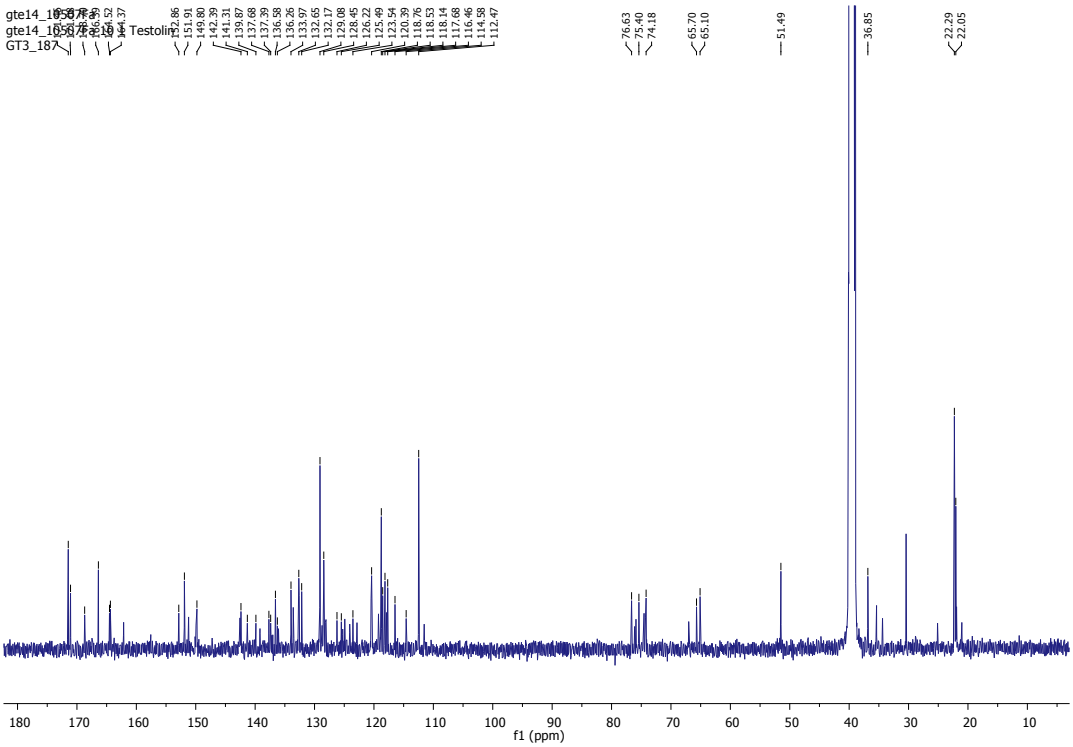
Allyl (S)-2-(allyloxy)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(2-(4-nitrobenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)benzamido)-3-isopropoxybenzoate



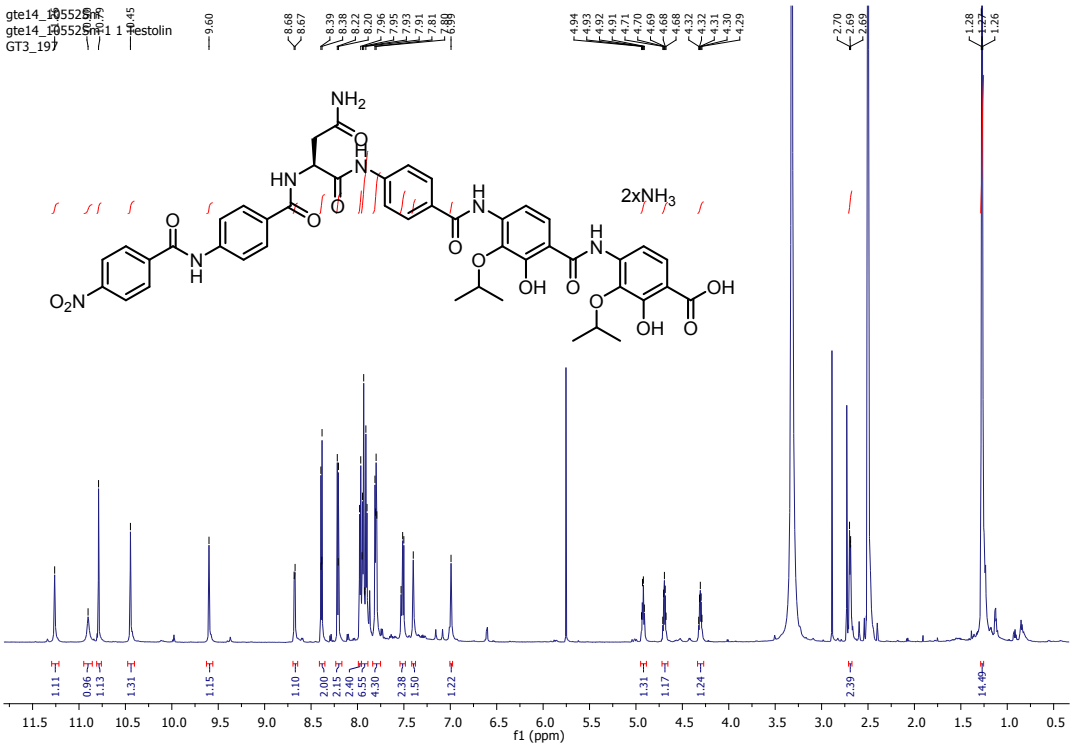


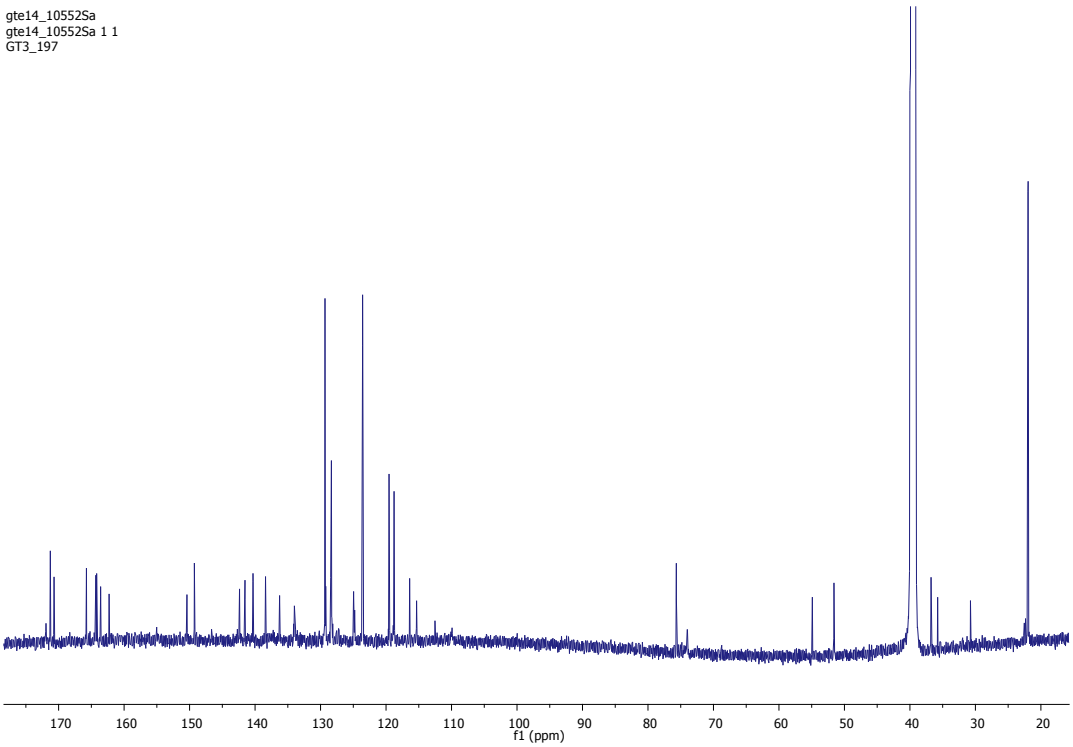
Allyl (S)-2-(allyloxy)-4-(2-(allyloxy)-4-(4-(4-amino-2-(4-aminobenzamido)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)-3-isopropoxybenzoate



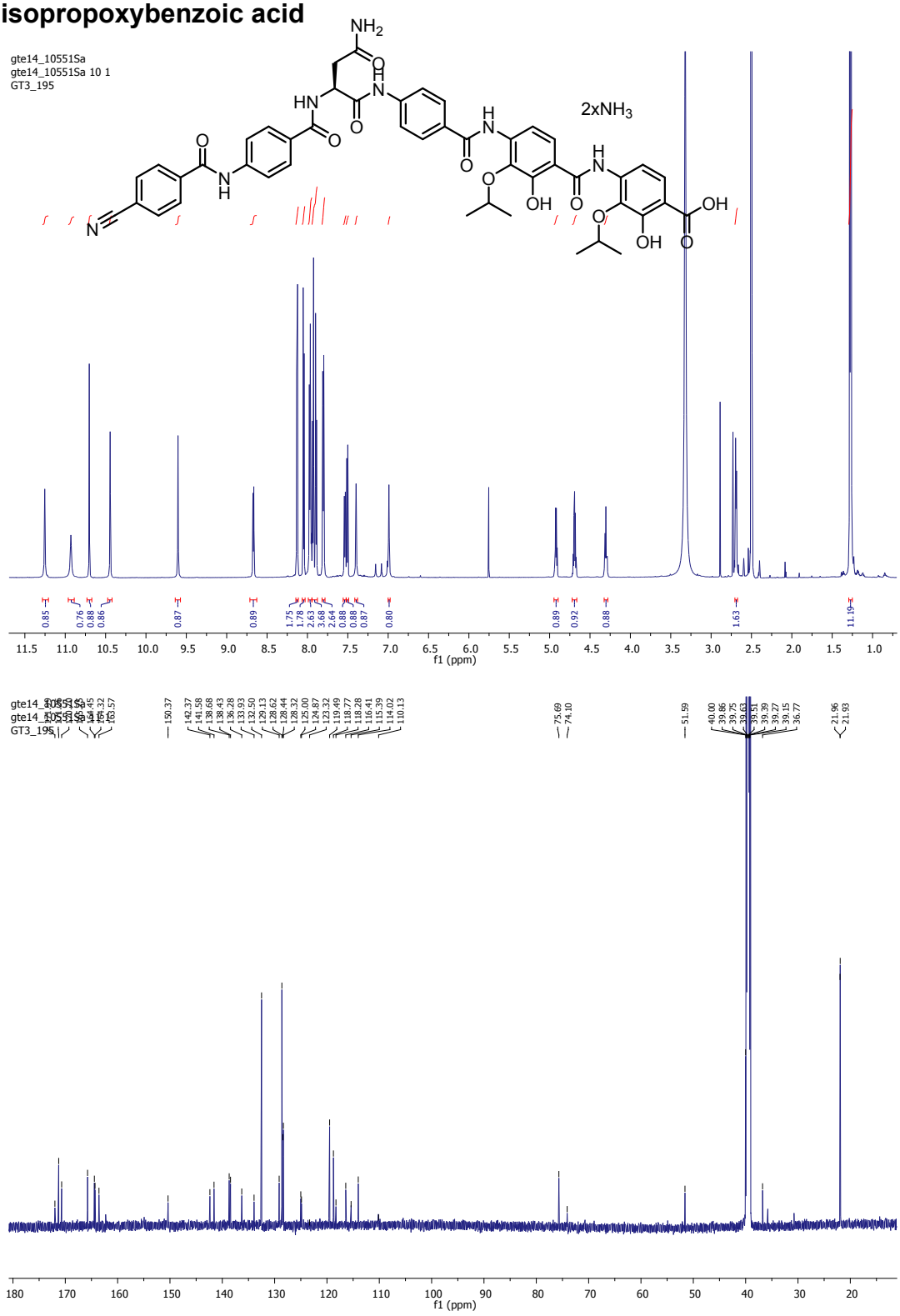


(S)-4-(4-(4-(4-Amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid

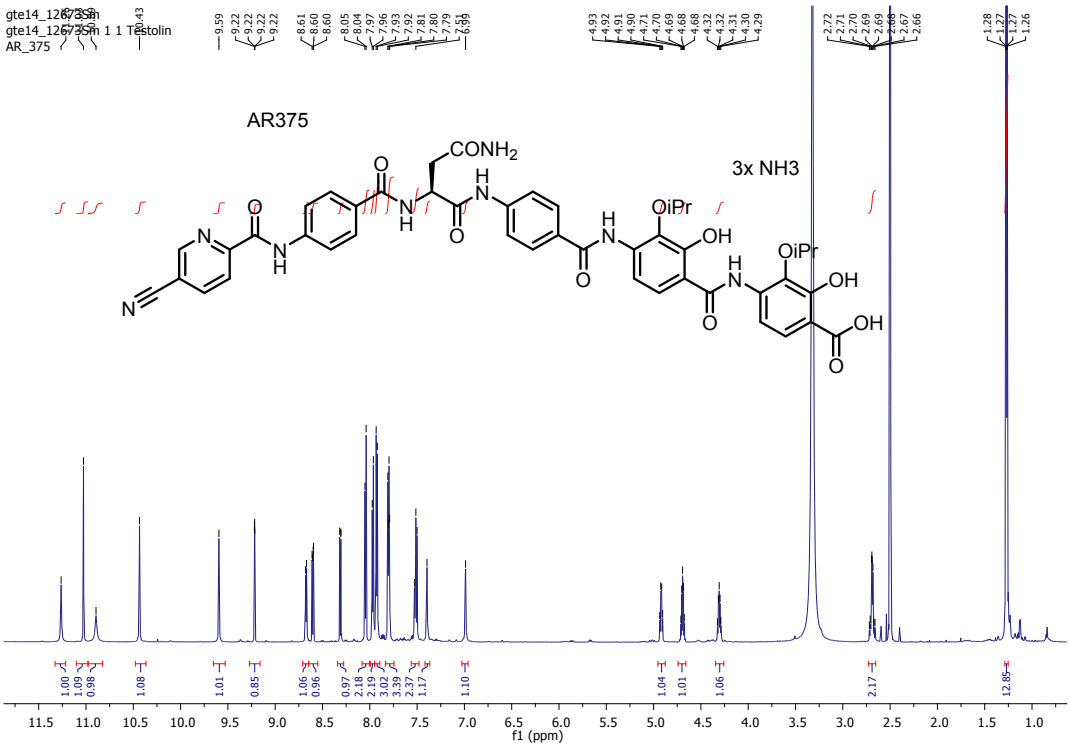


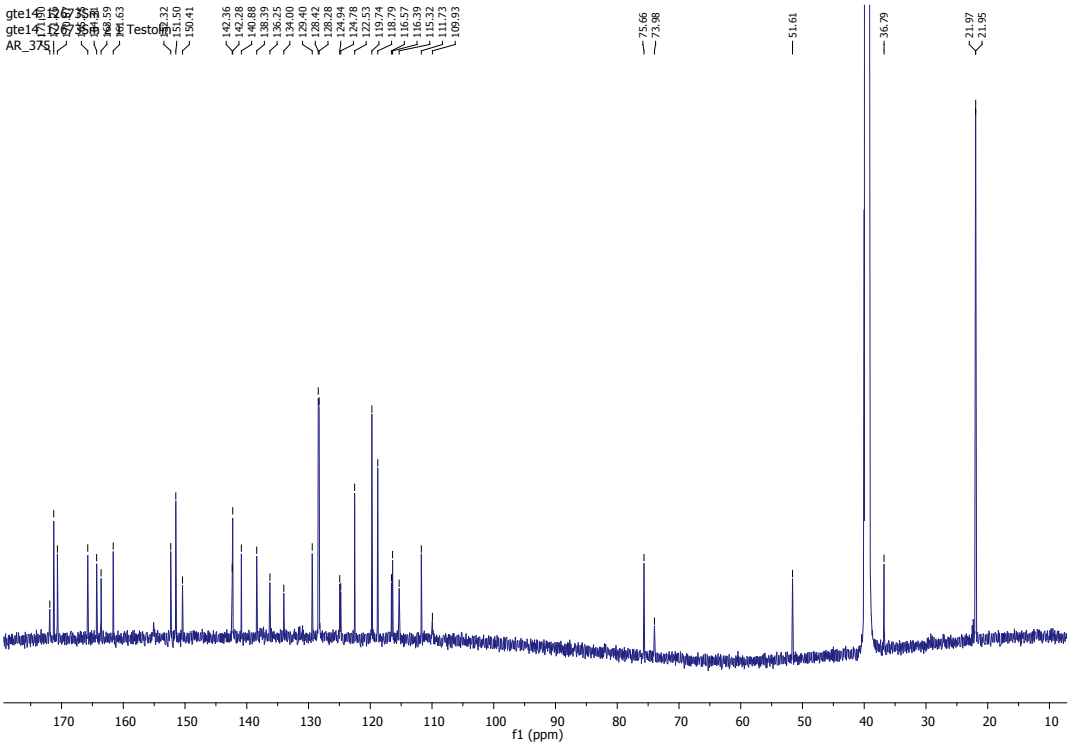


(S)-4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid

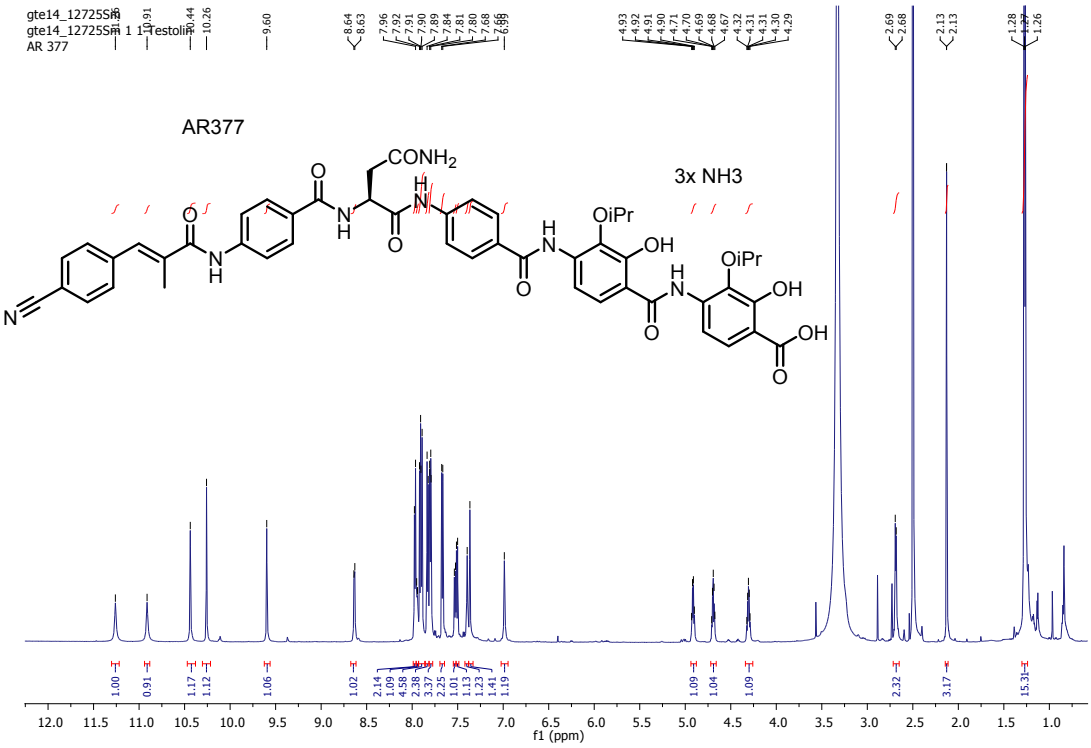


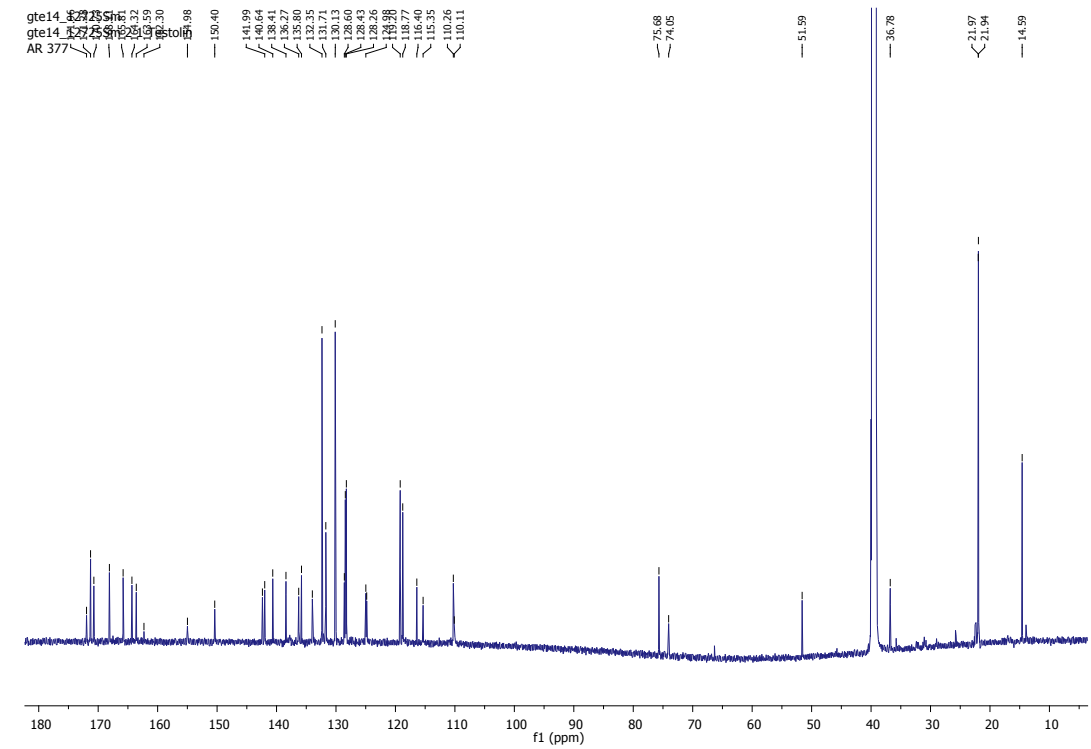
(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanopicolinamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid



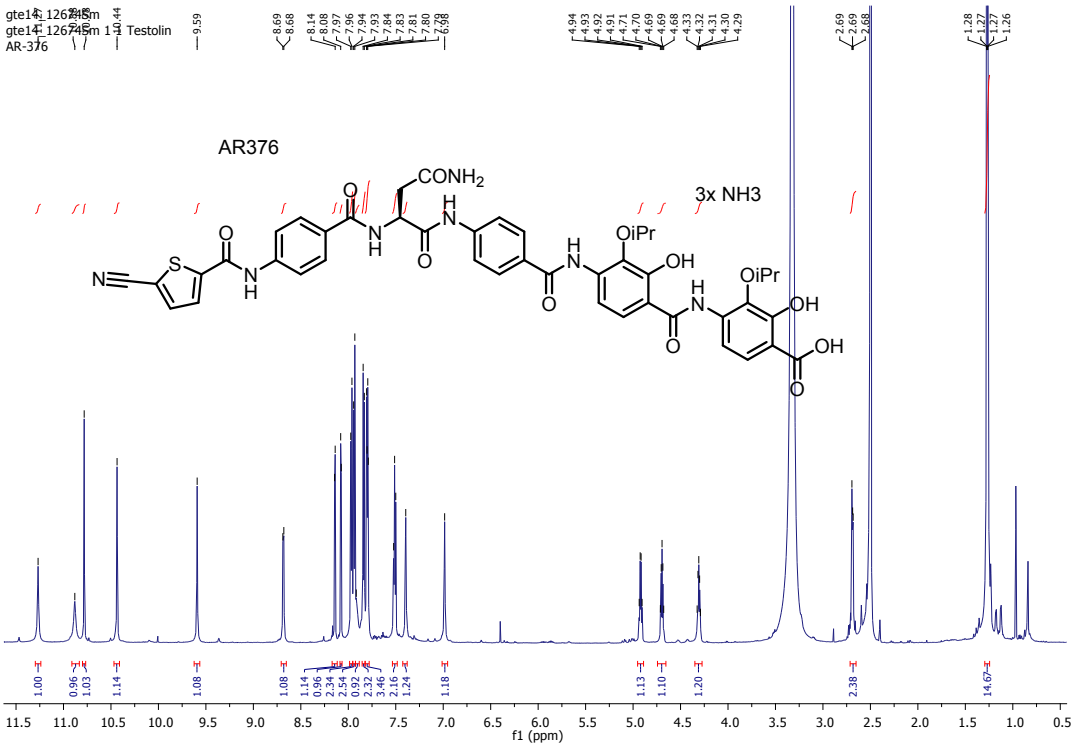


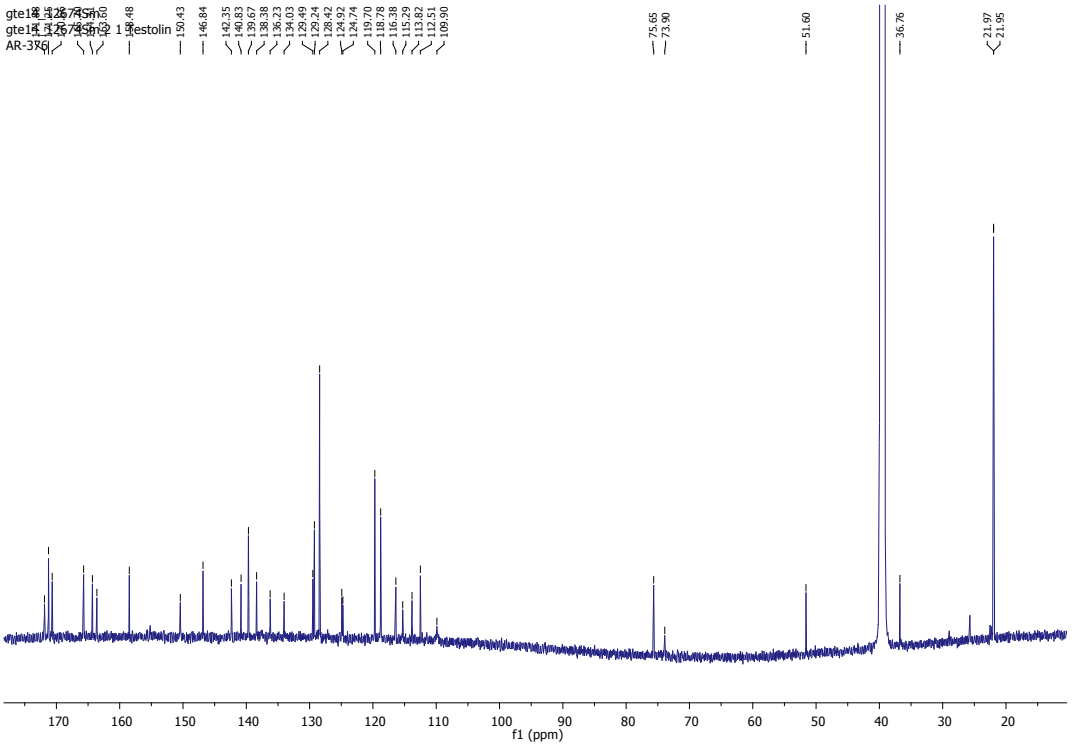
(S,E)-4-(4-(4-(4-Amino-2-(4-(3-(4-cyanophenyl)-2-methylacrylamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid



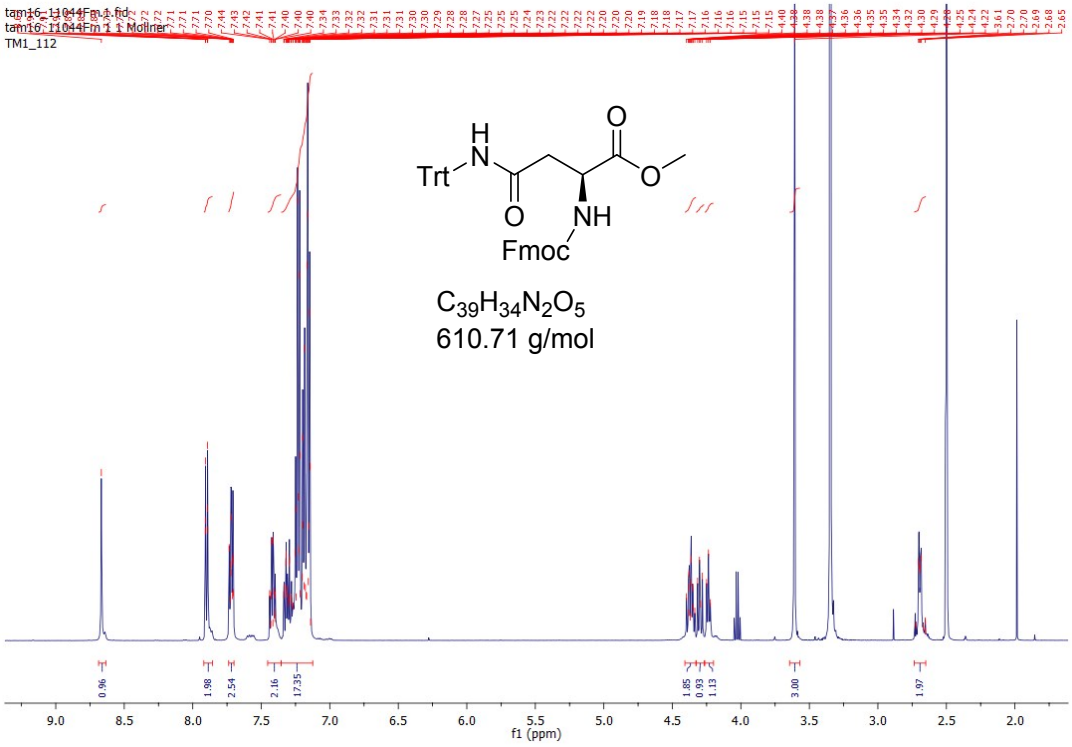


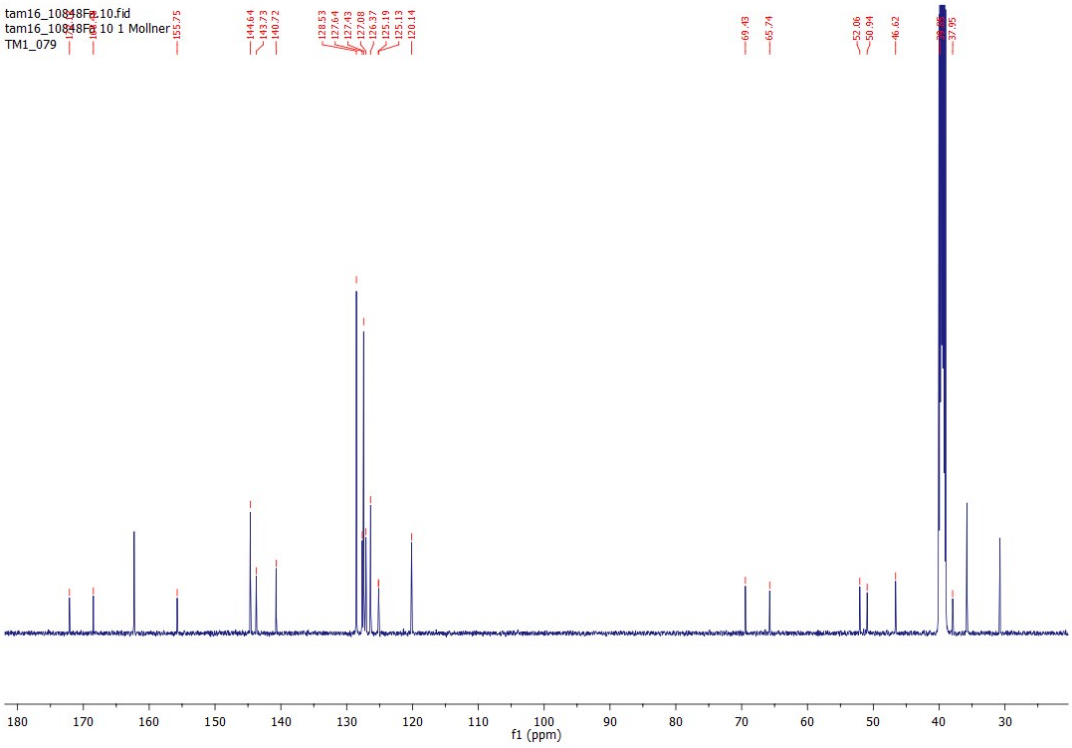
(S)-4-(4-(4-(4-Amino-2-(4-(5-cyanothiophene-2-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-hydroxy-3-isopropoxybenzoic acid



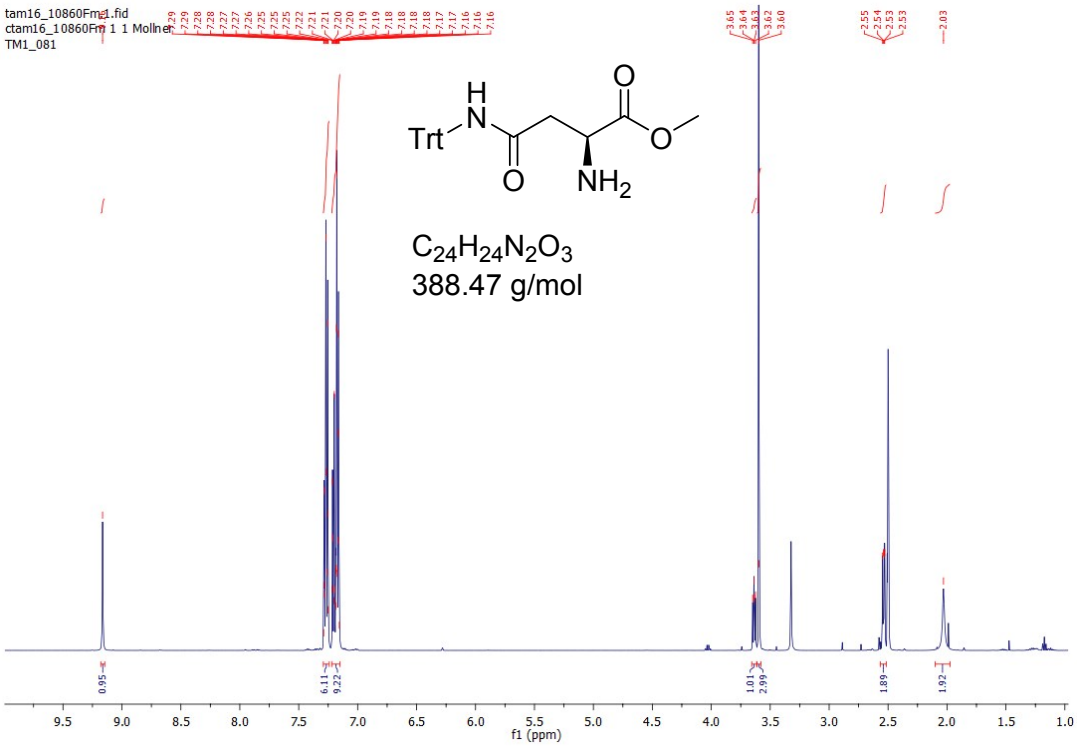


Fmoc-Asn(Trt)-OMe





Asn(Trt)-OMe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N
O
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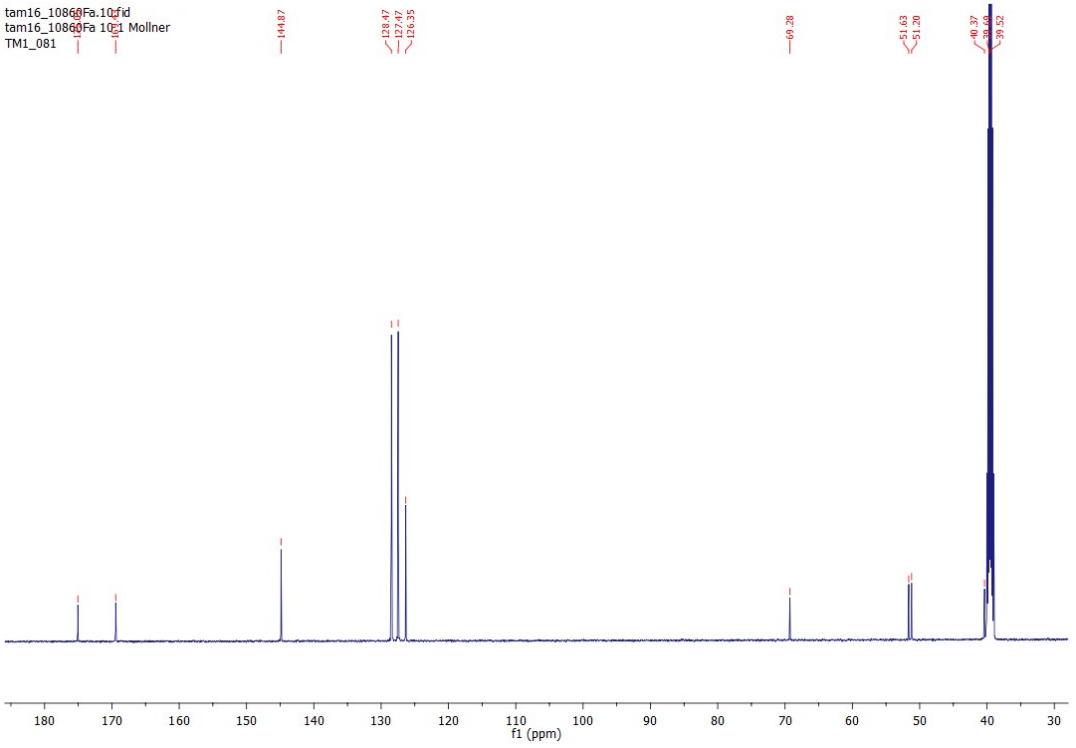
f1 (ppm)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40.37
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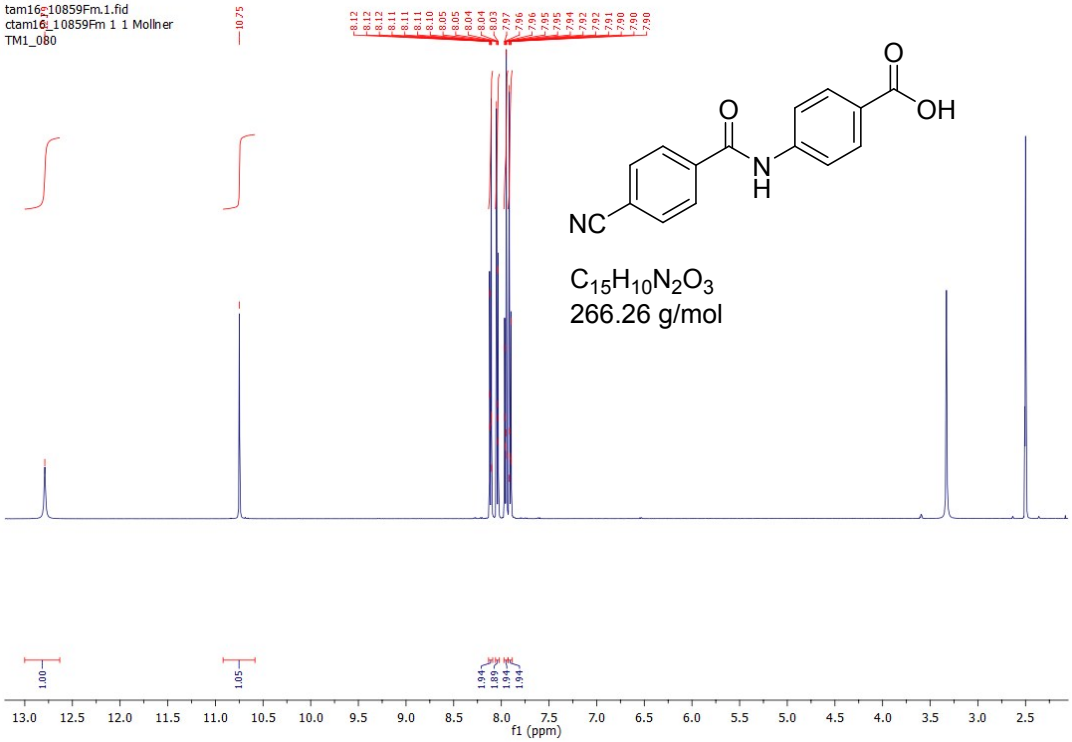
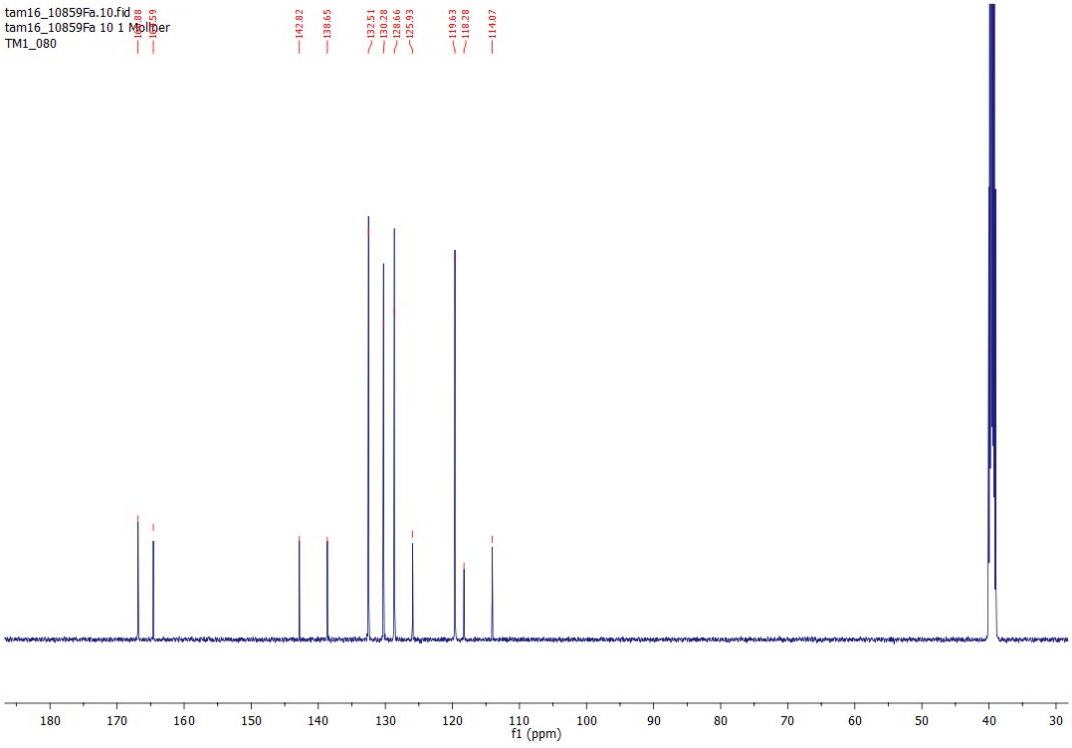
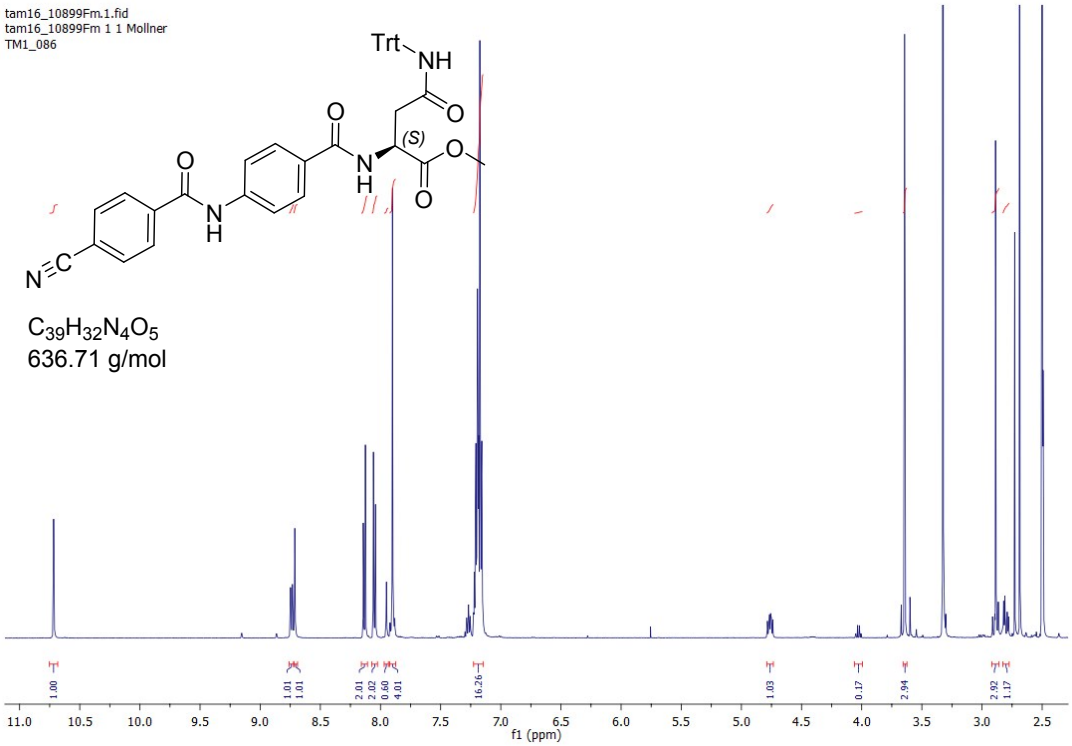
f1 (ppm)

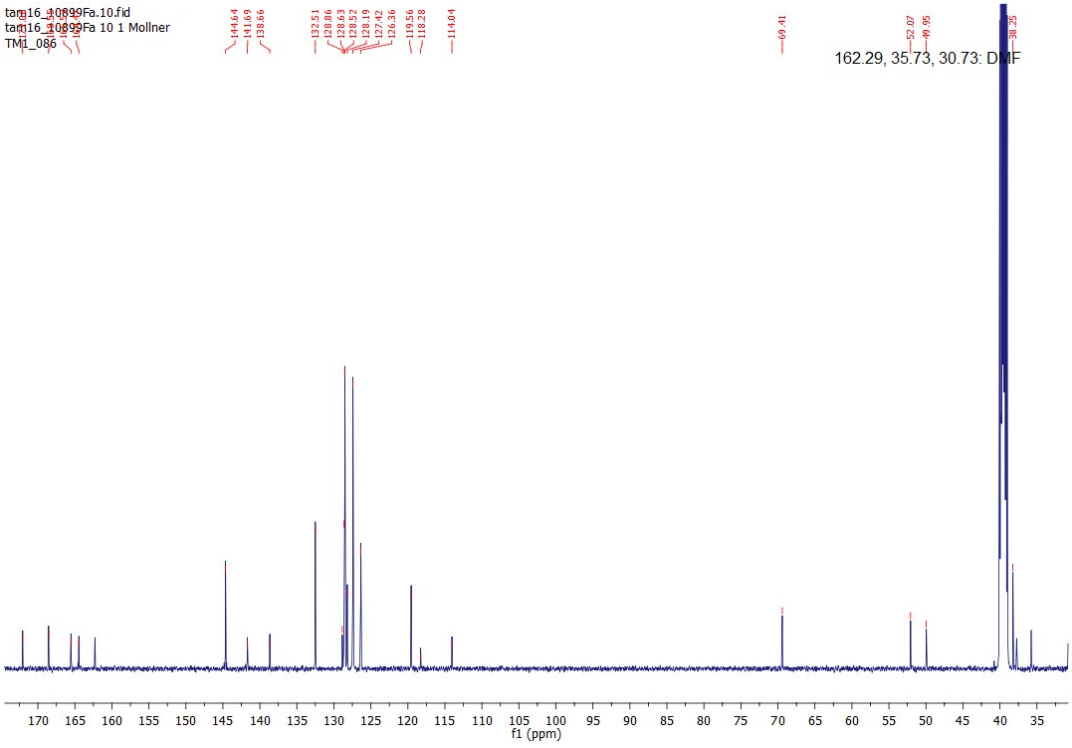
4-(4-cyanobenzamido)benzoic acid



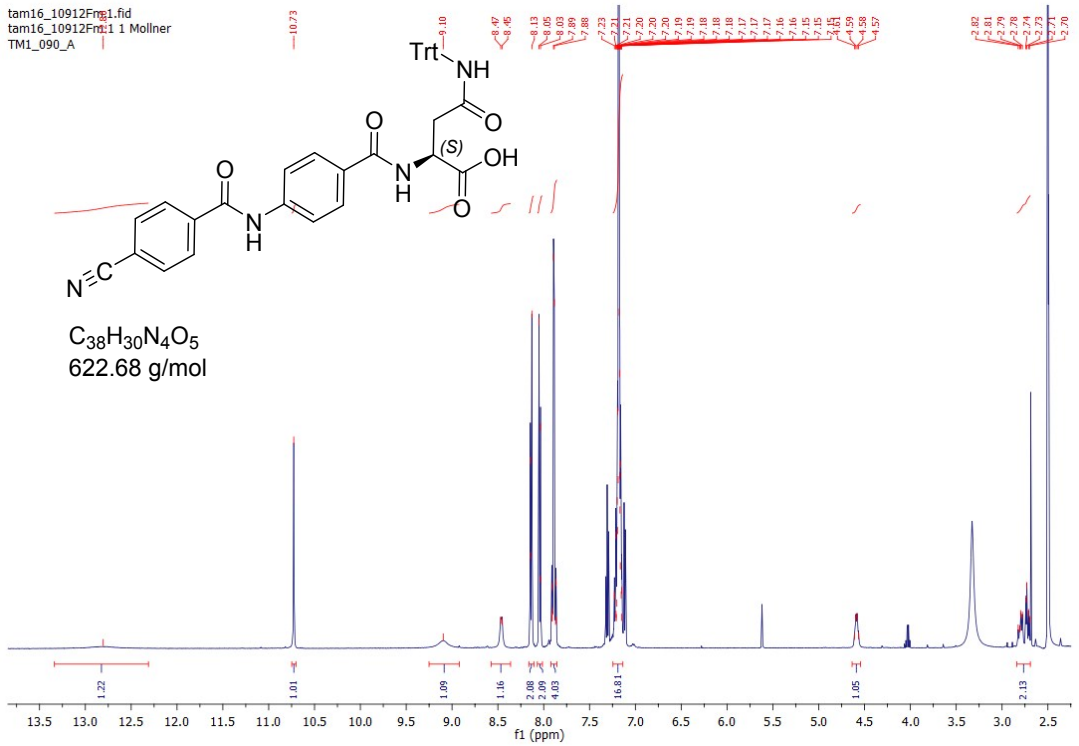


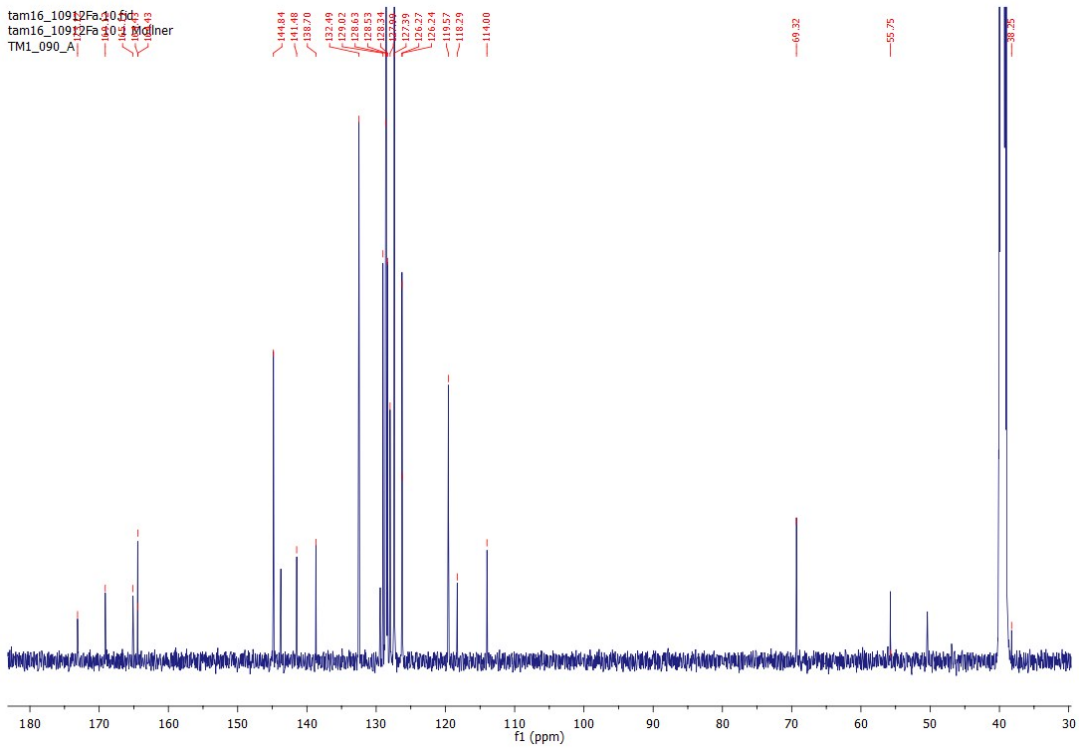
Methyl N^2-(4-(4-cyanobenzamido)benzoyl)-N^4-trityl-L-asparaginate



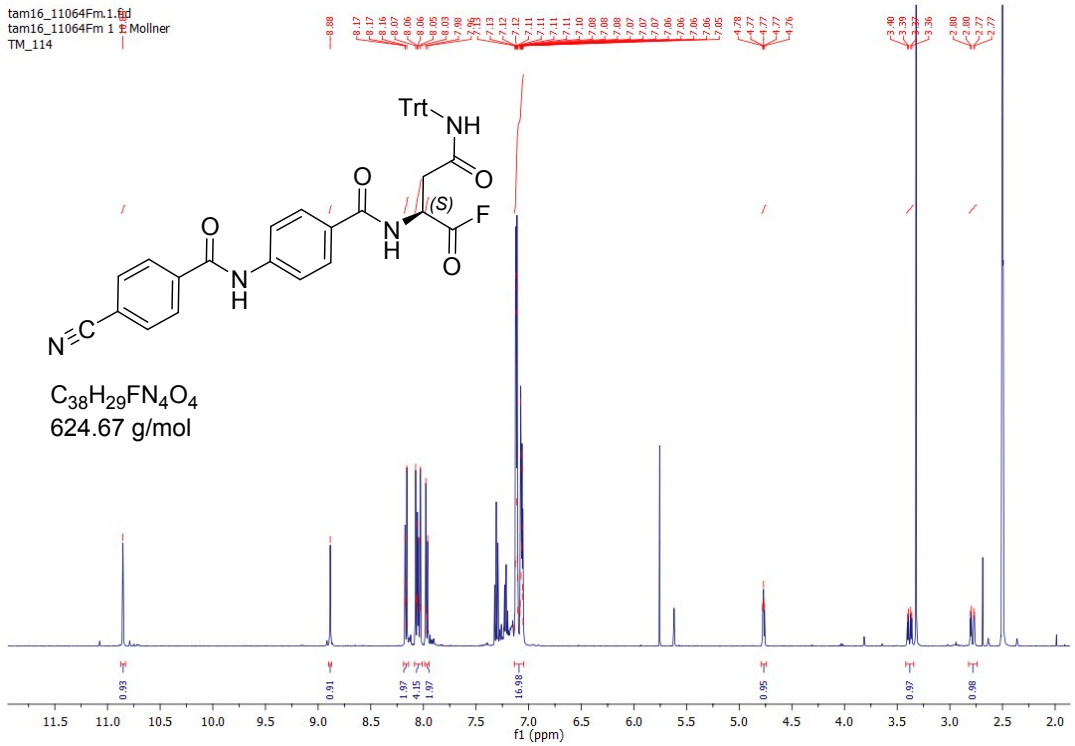


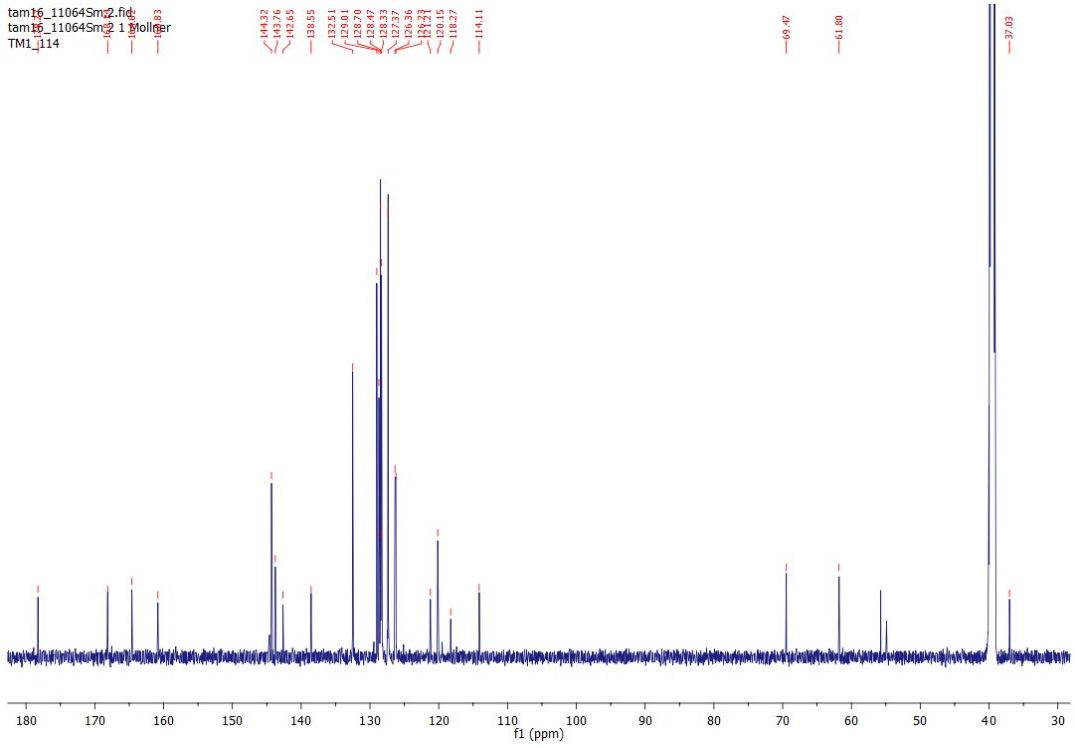
N^2-(4-(4-cyanobenzamido)benzoyl)-N^4-trityl-L-asparagine



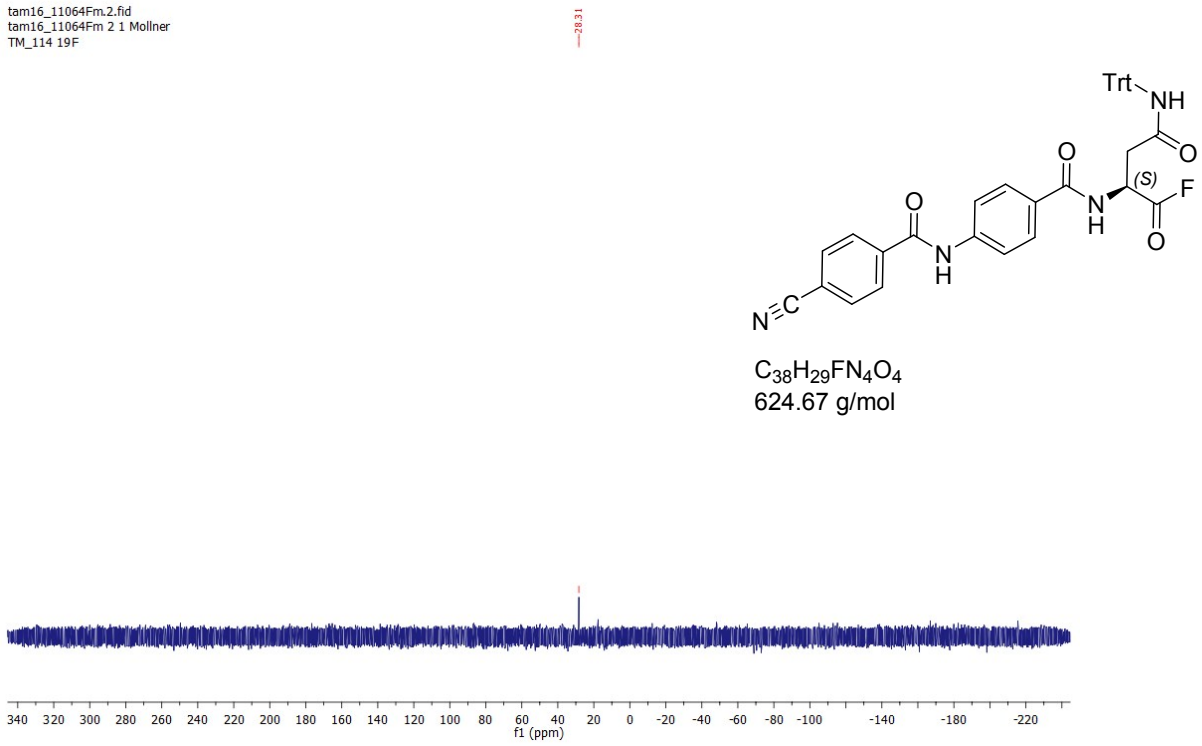


N^2-(4-(4-cyanobenzamido)benzoyl)-N^4-trityl-L-asparaginoyl fluoride



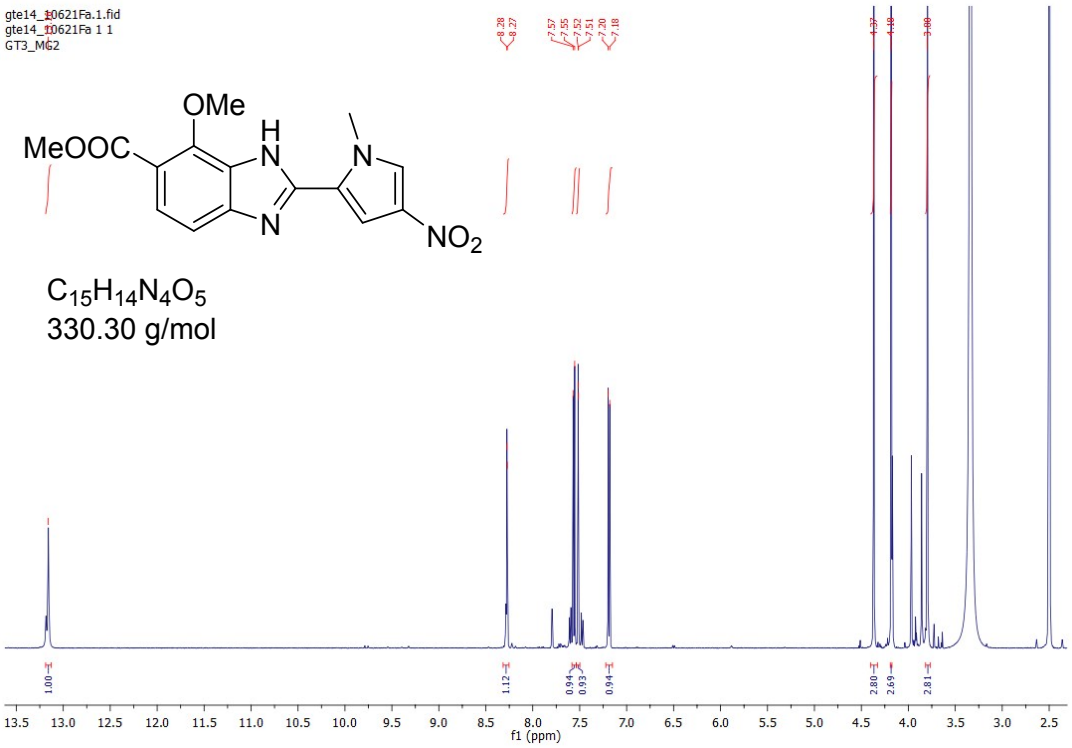


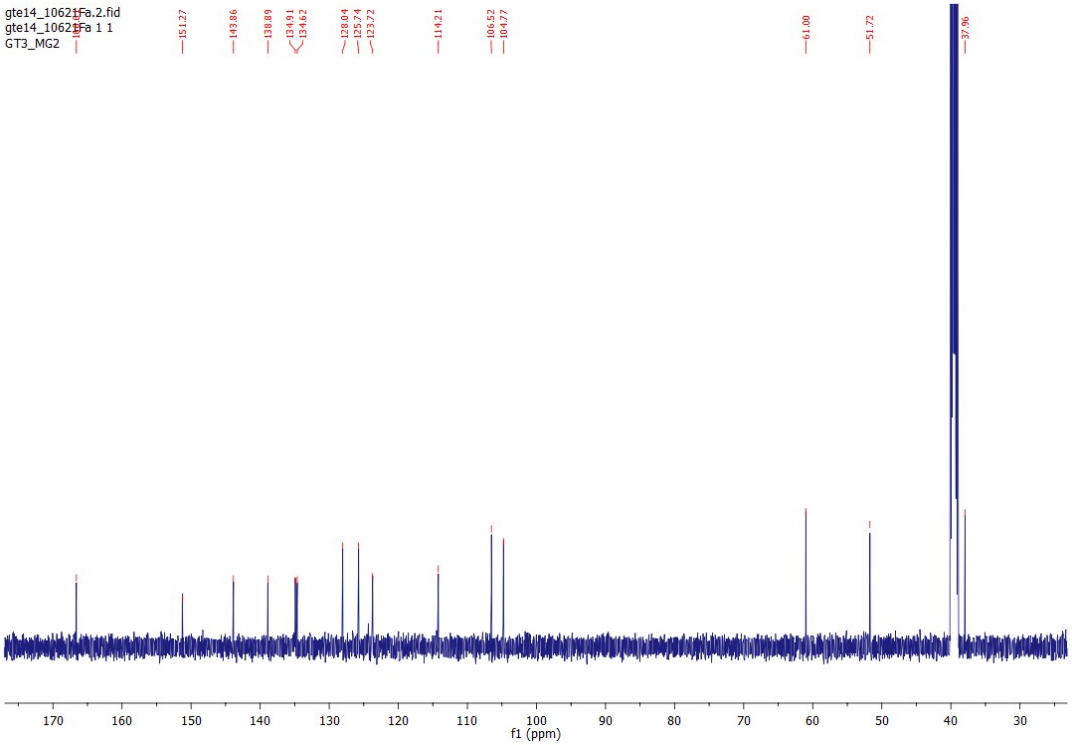
^{19}F spectrum



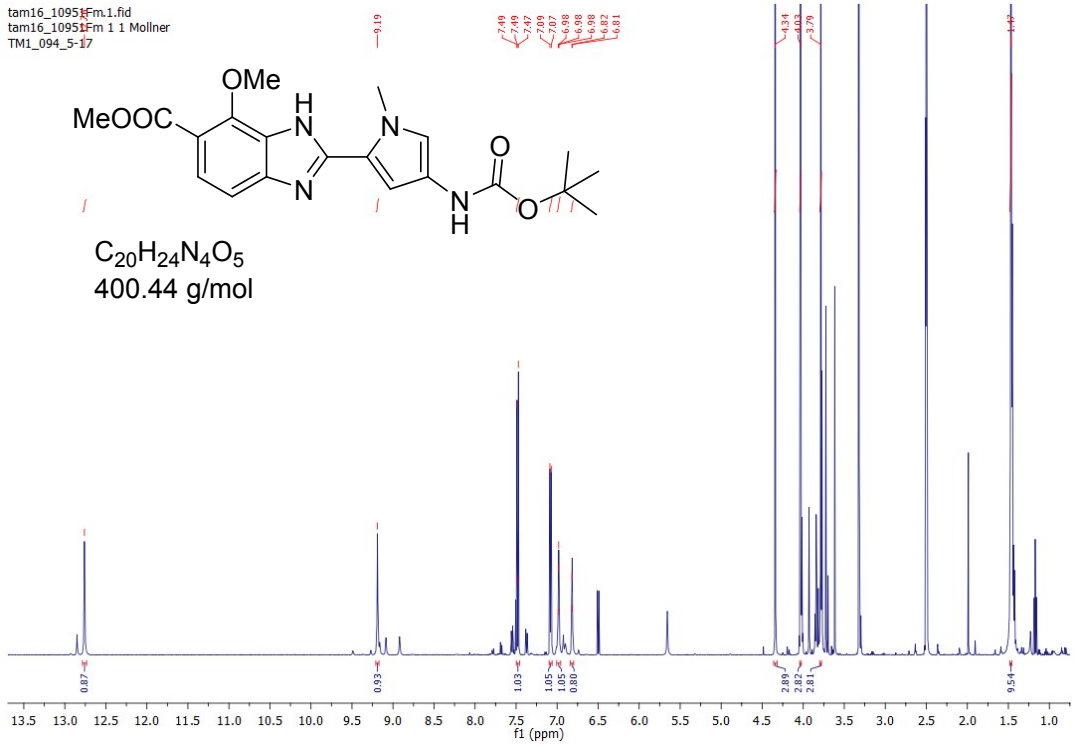
$C_{38}H_{29}FN_4O_4$
624.67 g/mol

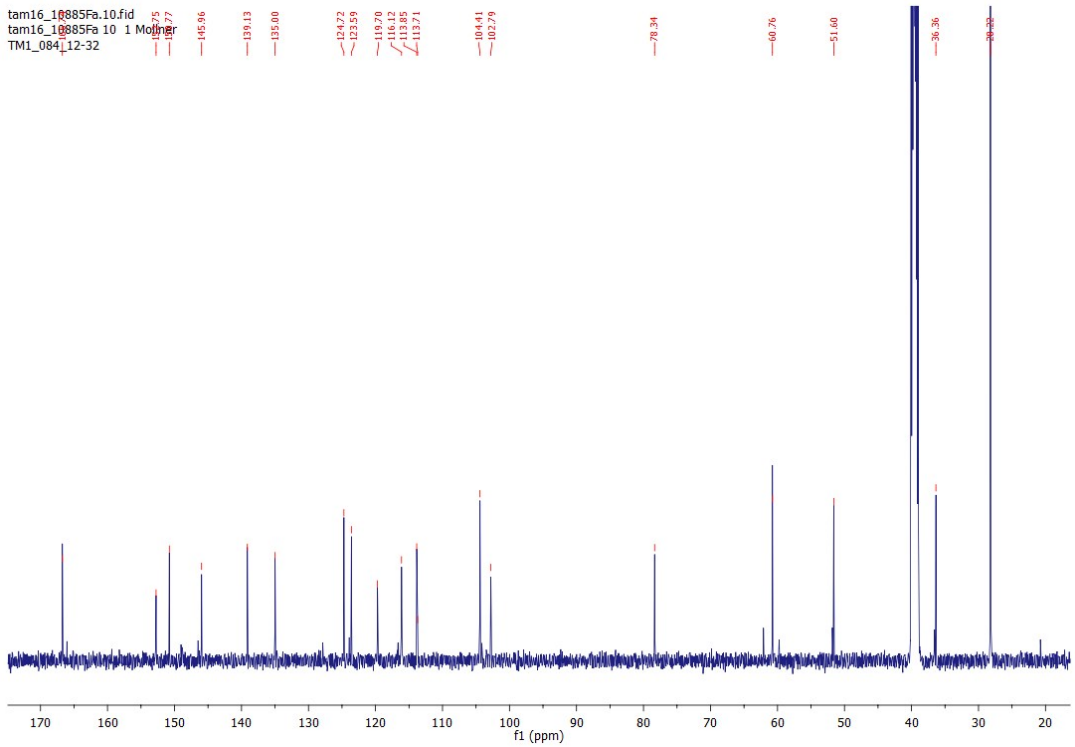
Methyl 4-methoxy-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)-3*H*-benzimidazole-5-carboxylate



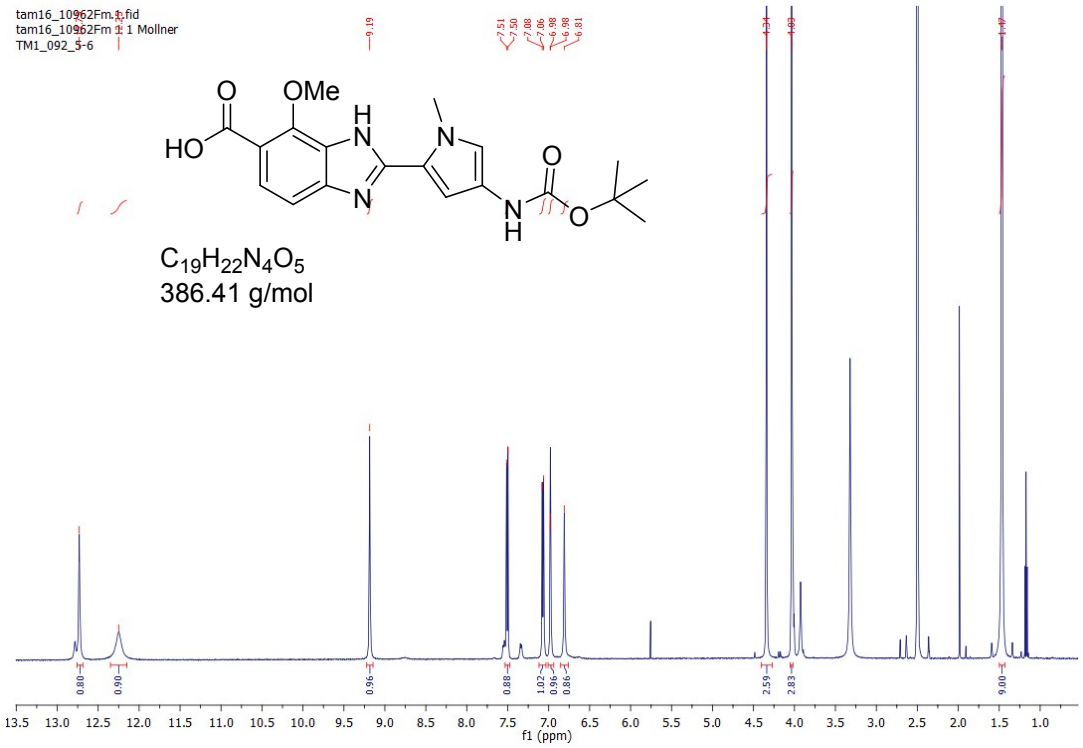


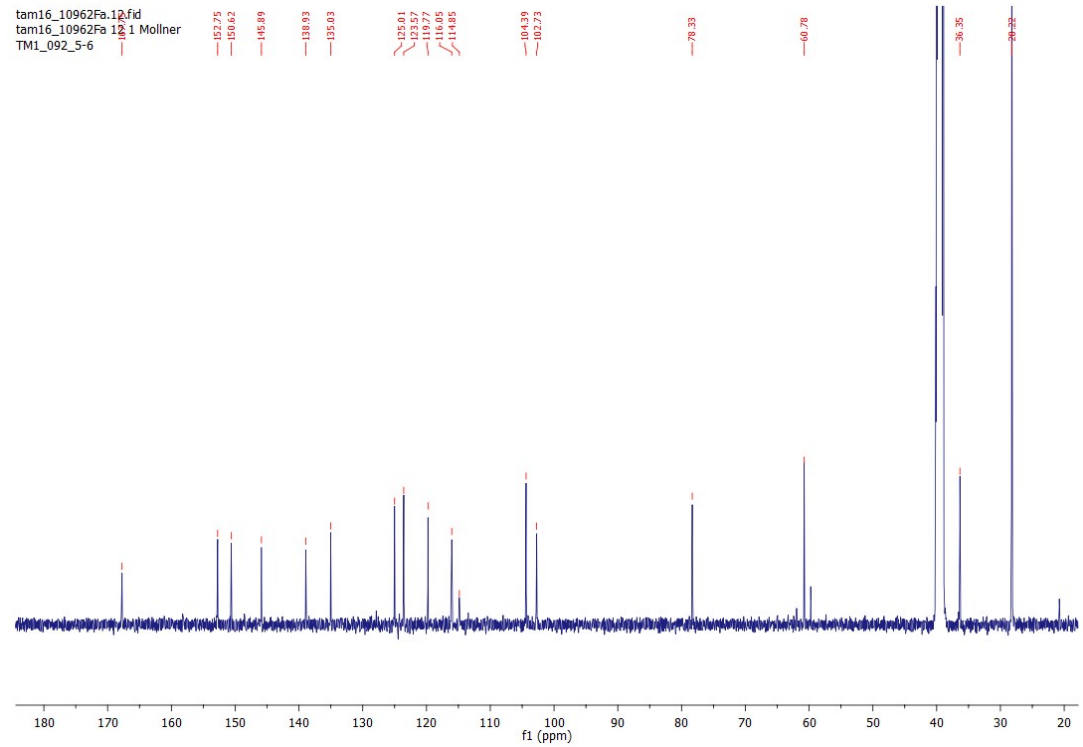
Methyl 2-(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrol-2-yl)-4-methoxy-3*H*-benzimidazole-5-carboxylate



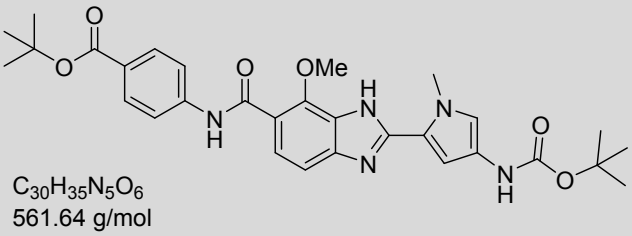


2-(4-*tert*-butoxycarbonylamino-1-methyl-1*H*-pyrrol-2-yl)-4-methoxy-3*H*-benzimidazole-5-carboxylic acid



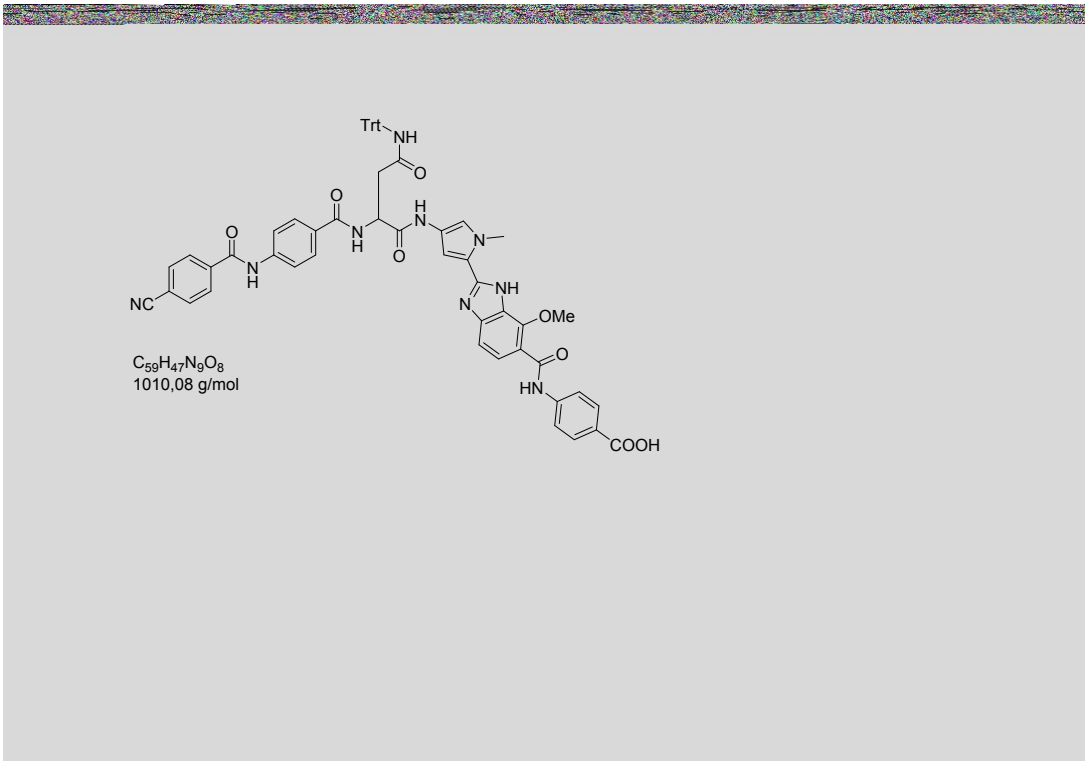


***tert*-butyl 4-(2-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-1*H*-pyrrol-2-yl)-7-methoxy-1*H*-benzo[*d*]imidazole-6-carboxamido)benzoate**



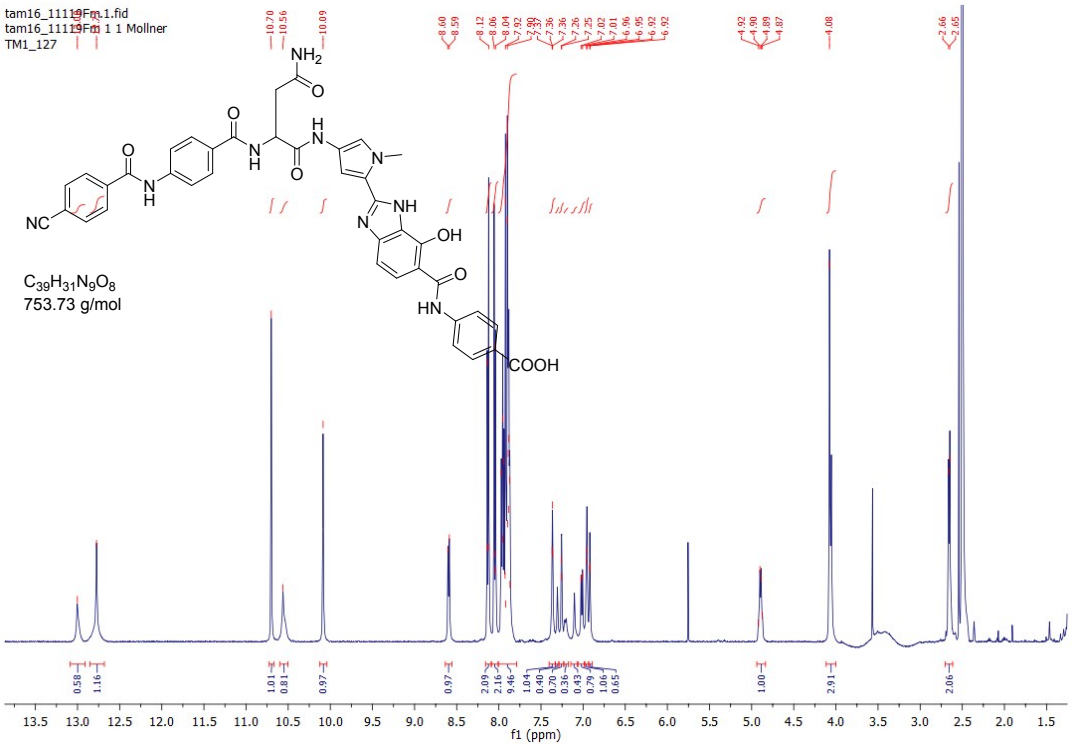
$C_{30}H_{35}N_5O_6$
561.64 g/mol

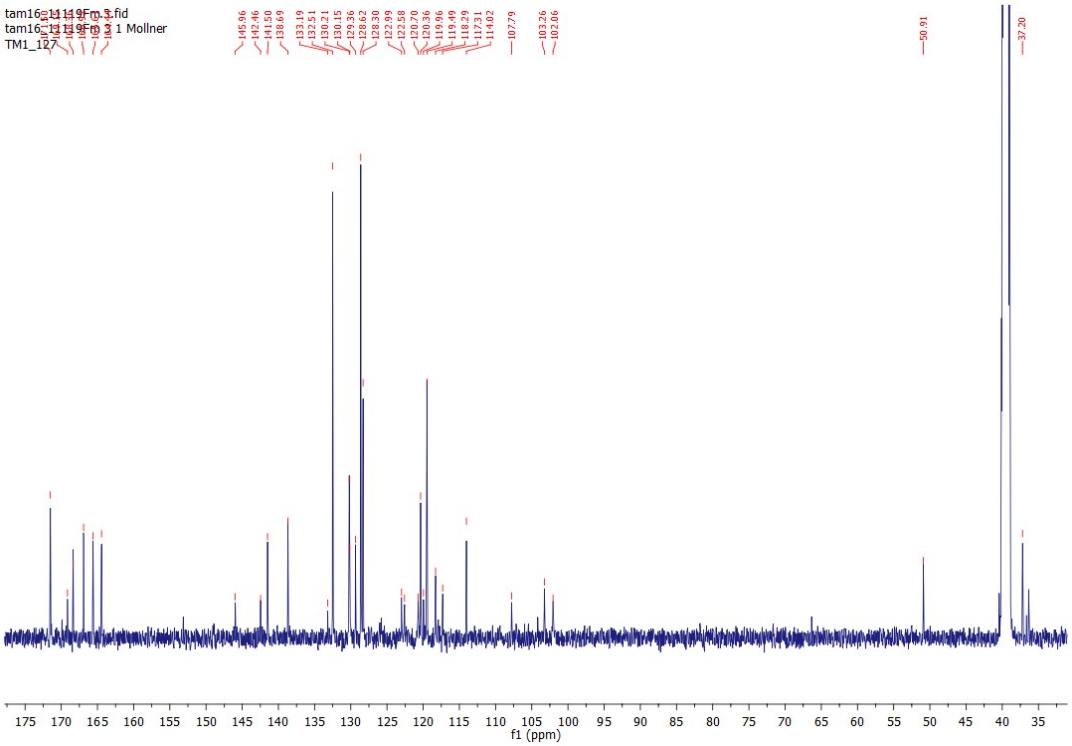
4-(2-(4-(2-(4-(4-cyanobenzamido)benzamido)-4-oxo-4-(tritylamino)butanamido)-1-methyl-1*H*-pyrrol-2-yl)-7-methoxy-1*H*-benzimidazole-6-carboxamido)benzoic acid



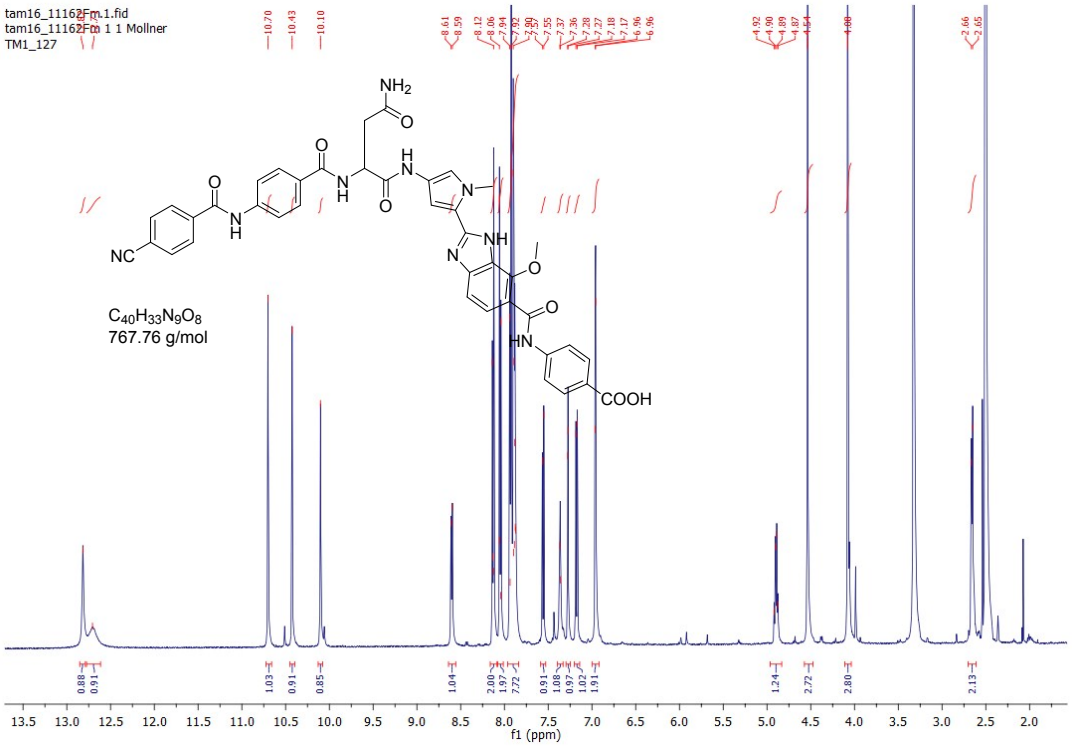
$C_{59}H_{47}N_9O_8$
1010,08 g/mol

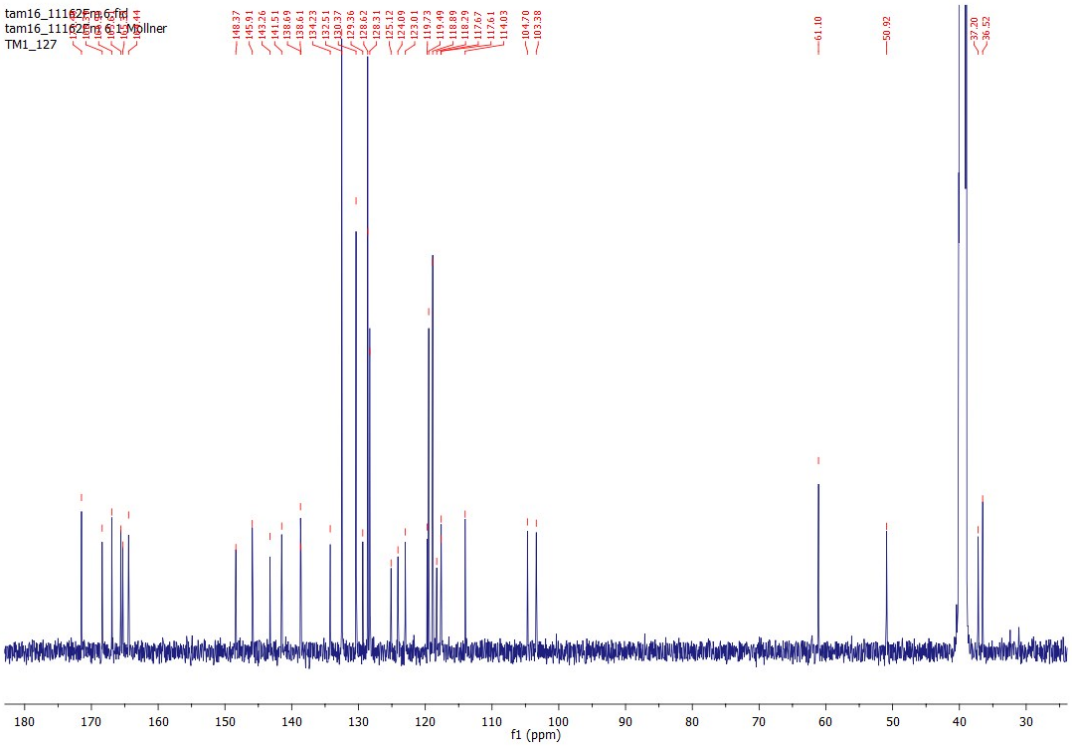
4-(2-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)-1-methyl-1*H*-pyrrol-2-yl)-7-hydroxy-1*H*-benzimidazole-6-carboxamido)benzoic acid



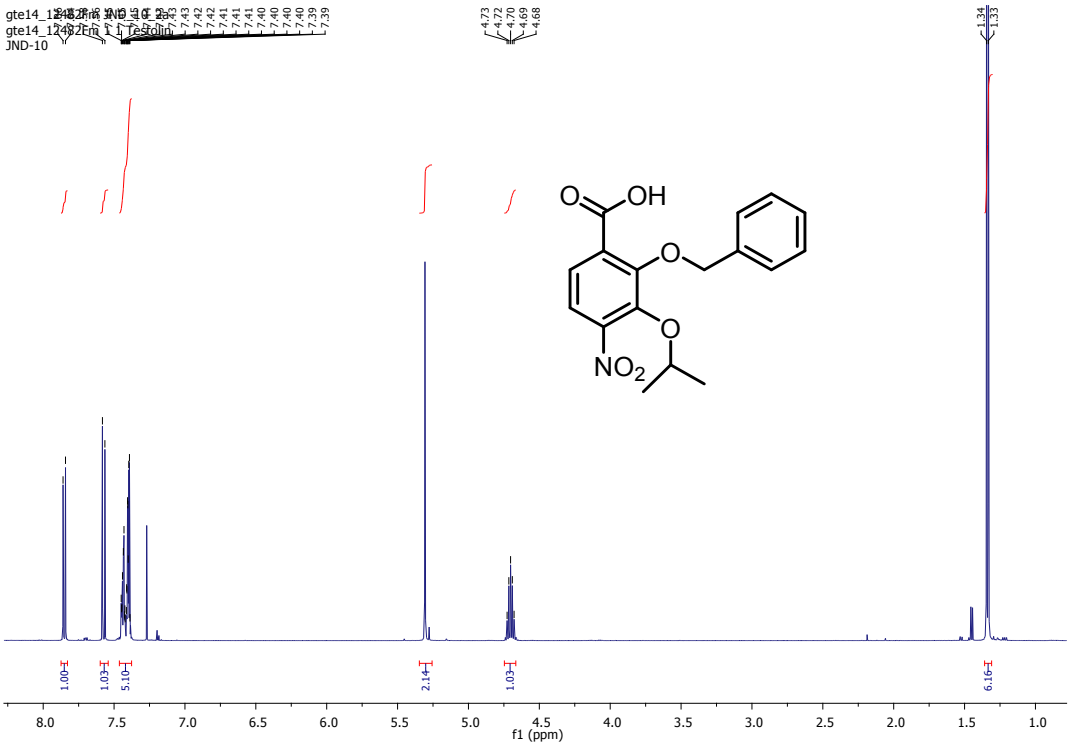


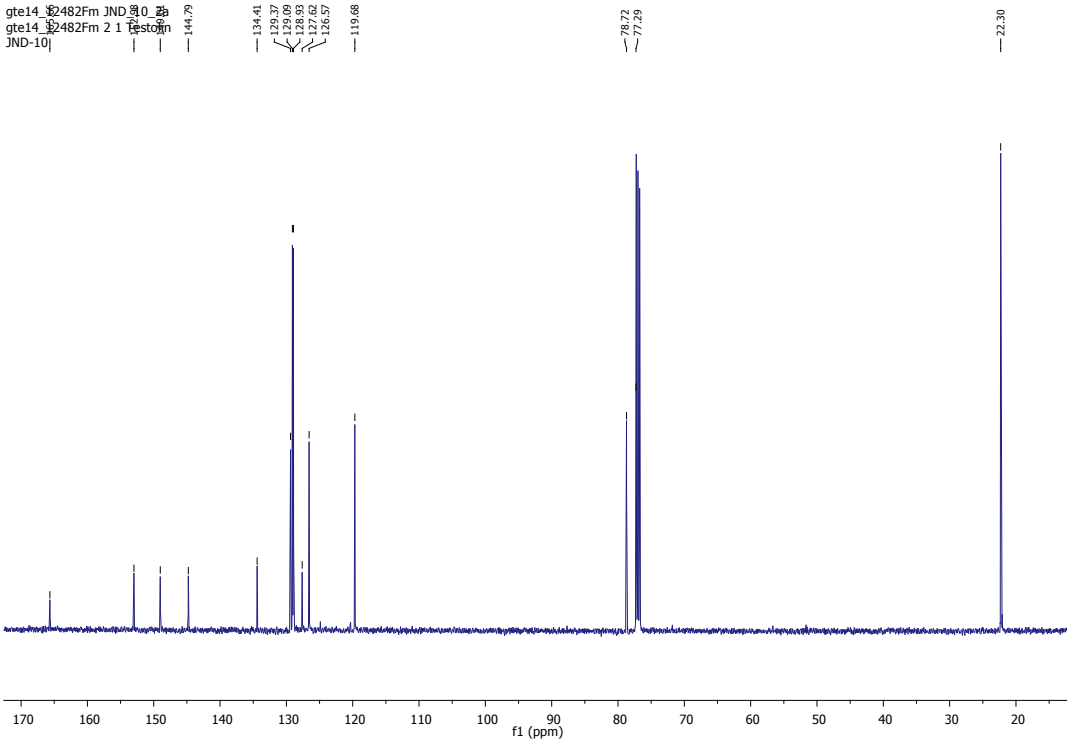
4-(2-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)-1-methyl-1*H*-pyrrol-2-yl)-7-methoxy-1*H*-benzimidazole-6-carboxamido)benzoic acid



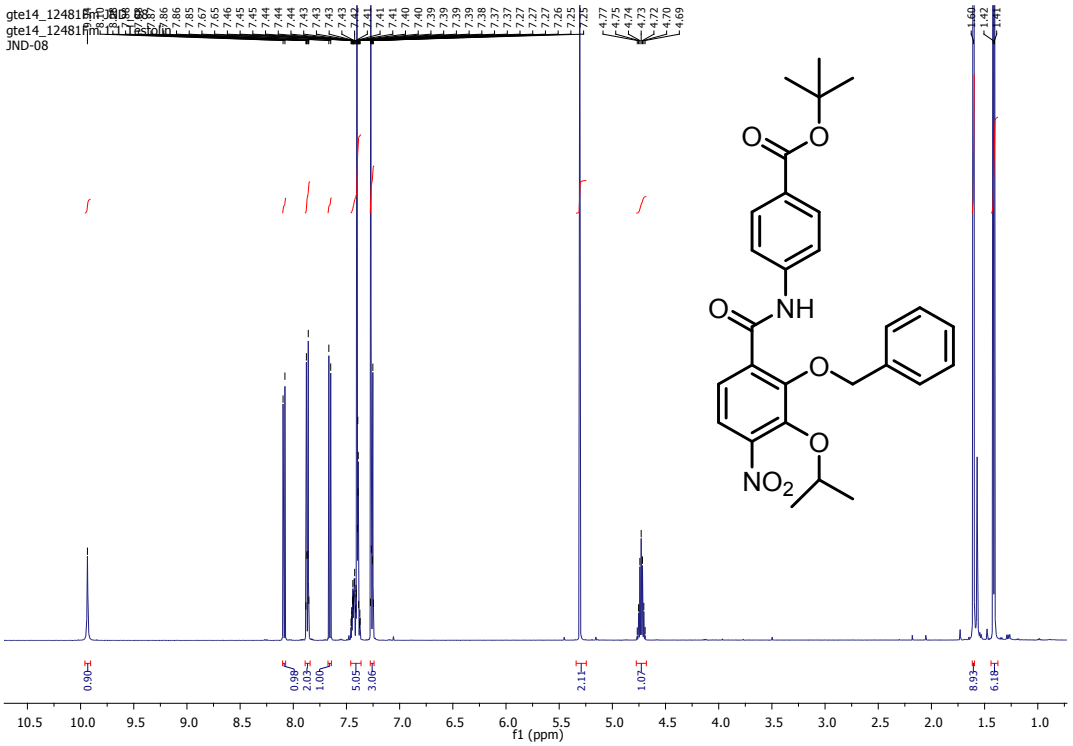


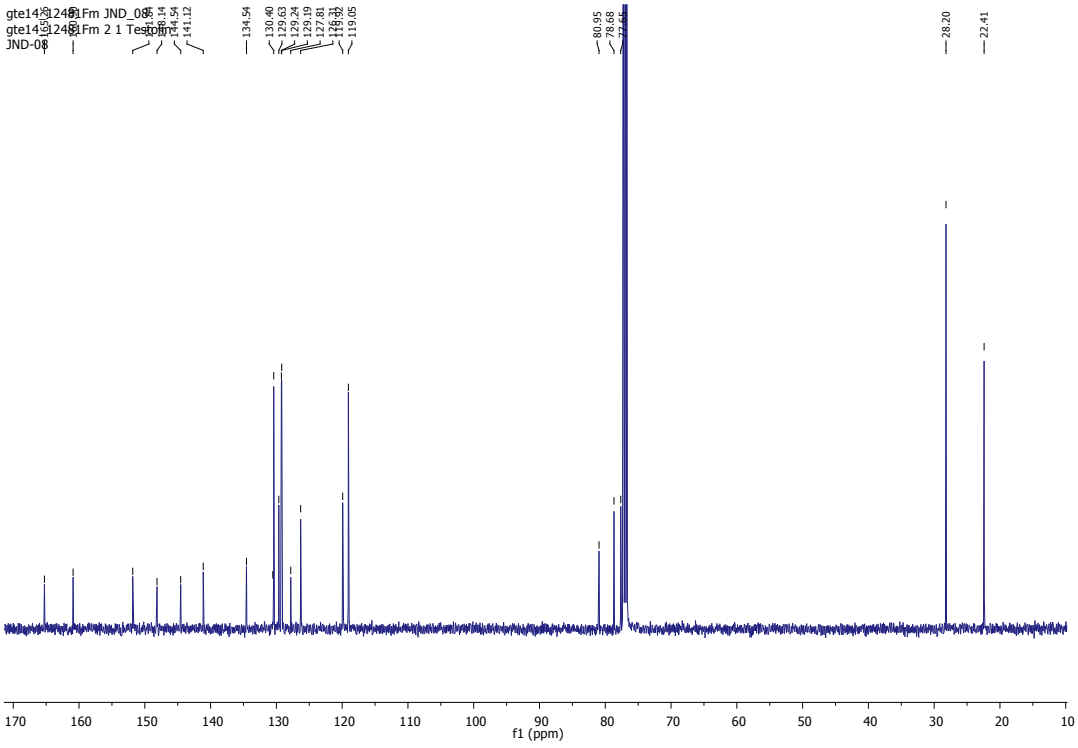
2-(Benzyloxy)-3-isopropoxy-4-nitrobenzoic acid



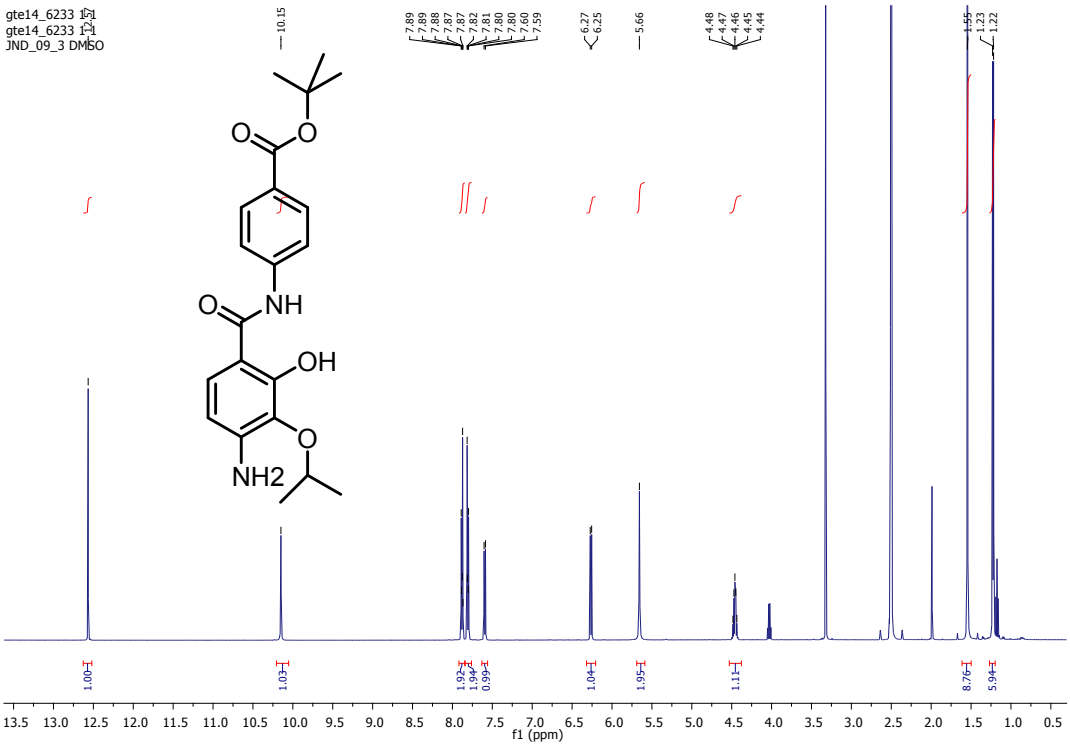


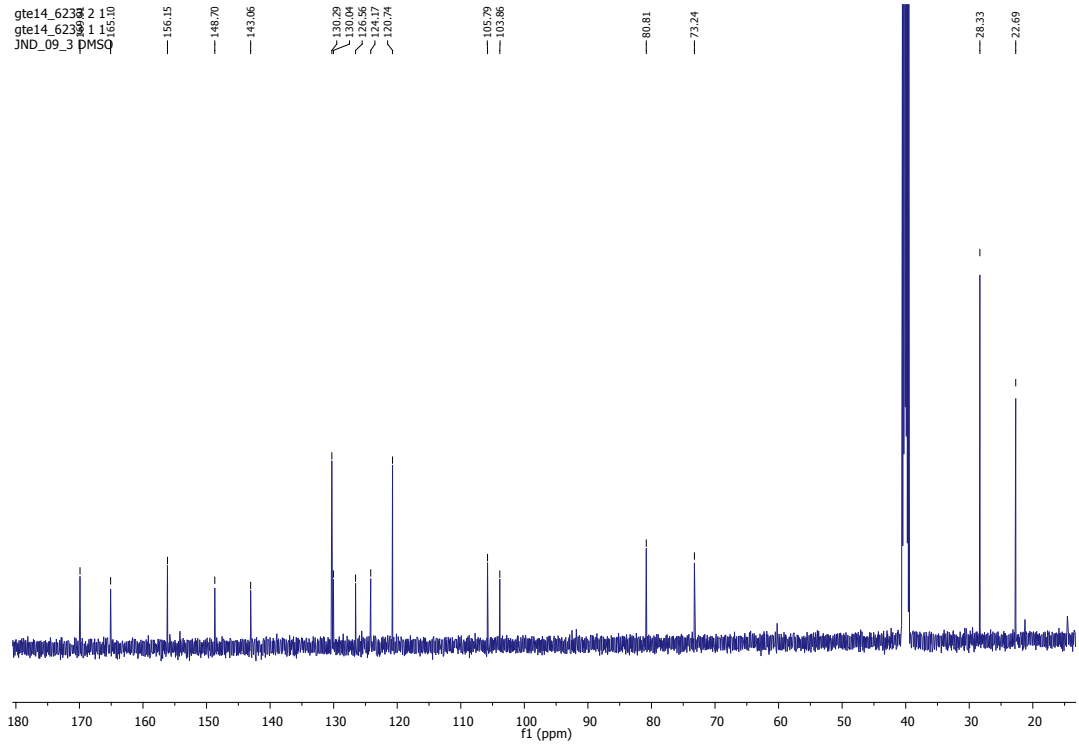
Tert-butyl 4-(2-(benzyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate



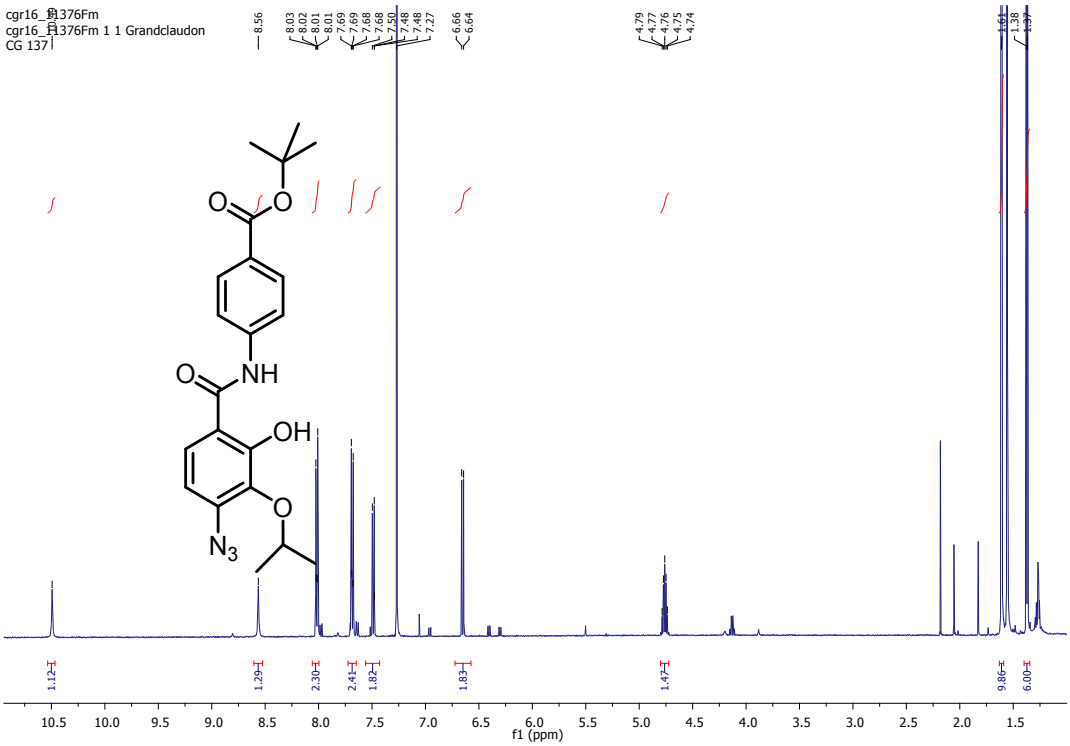


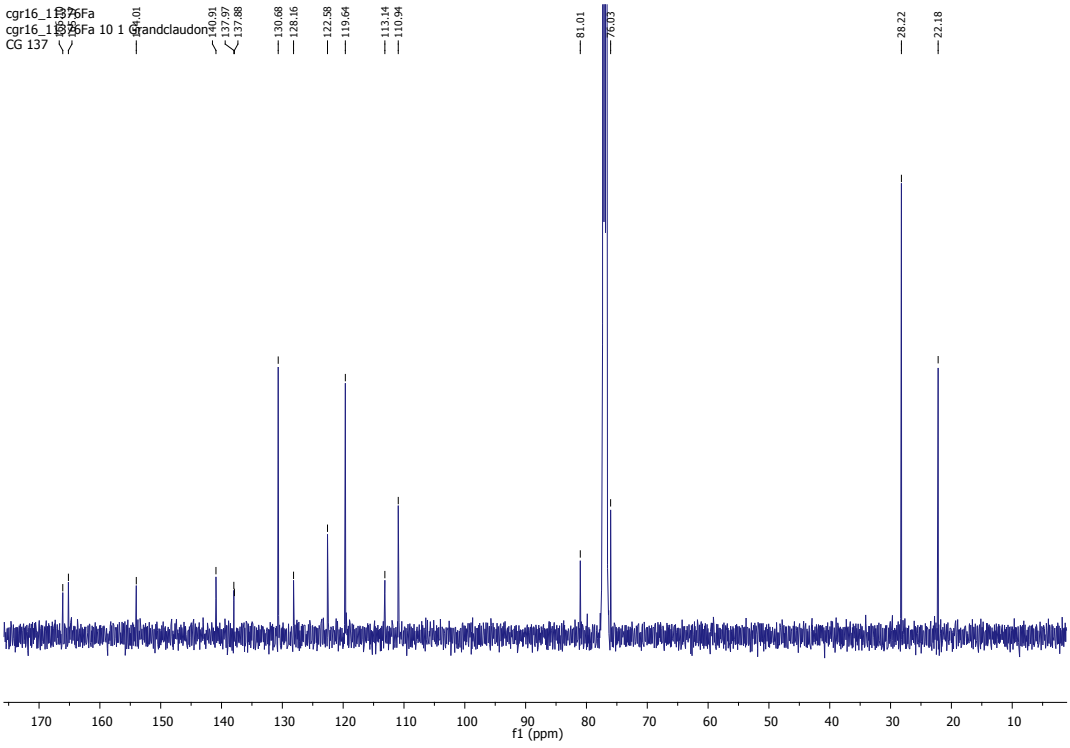
Tert-butyl 4-(4-amino-2-hydroxy-3-isopropoxybenzamido)benzoate



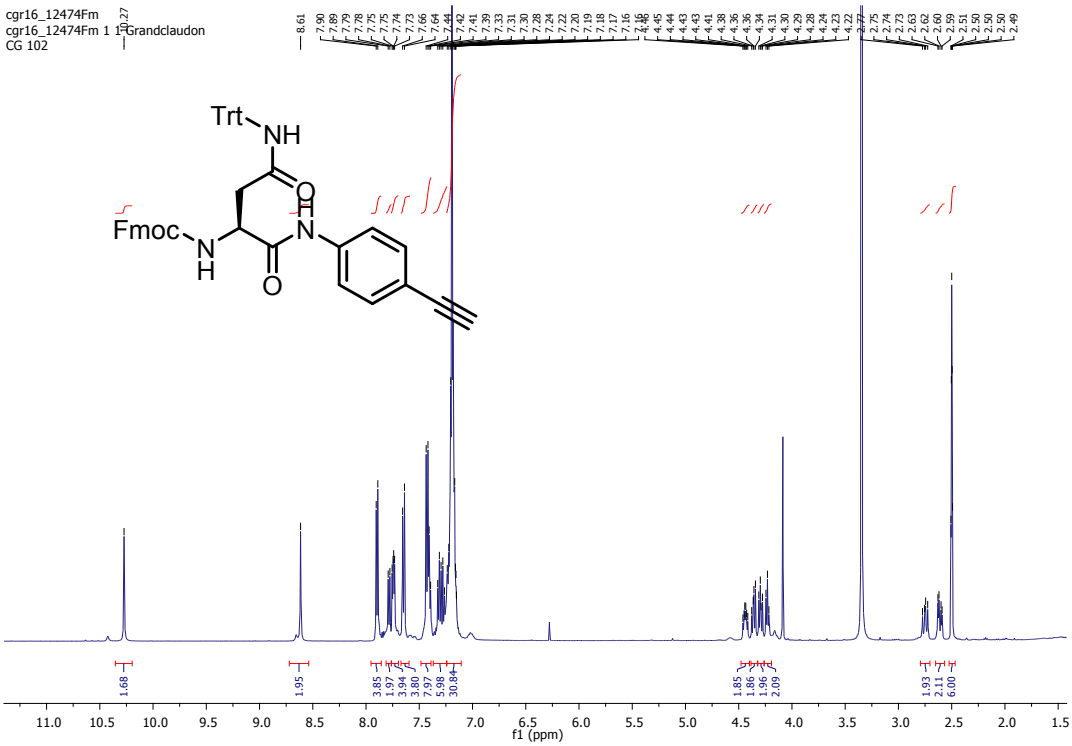


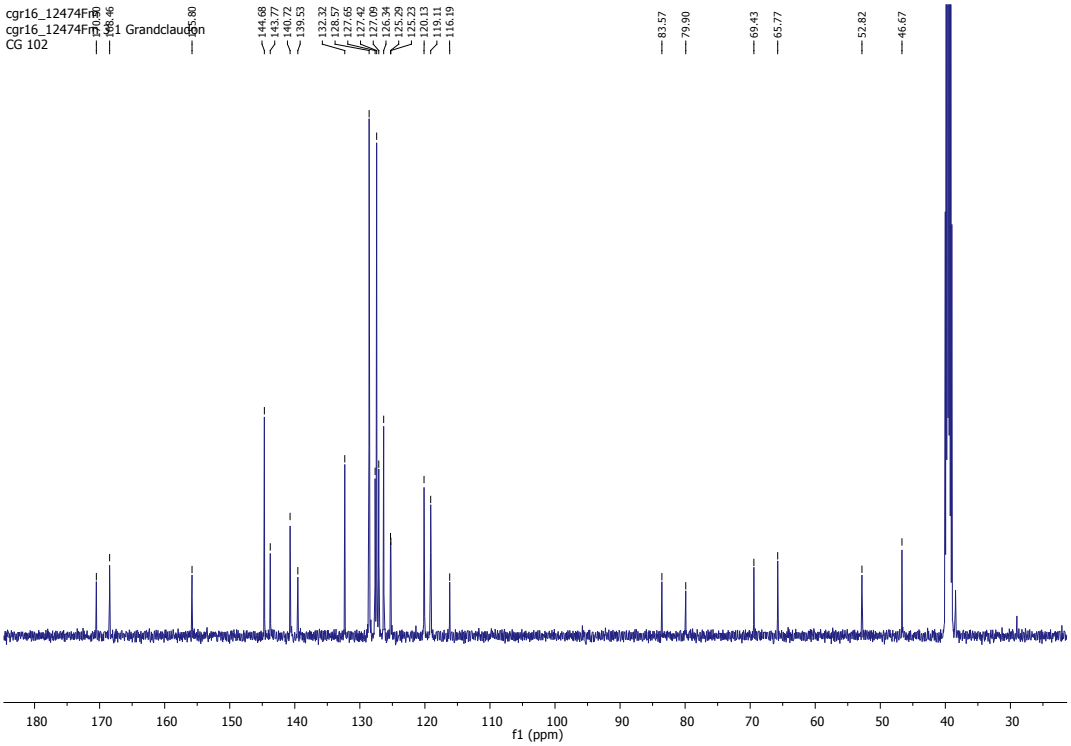
Tert-butyl 4-(4-azido-2-hydroxy-3-isopropoxybenzamido)benzoate



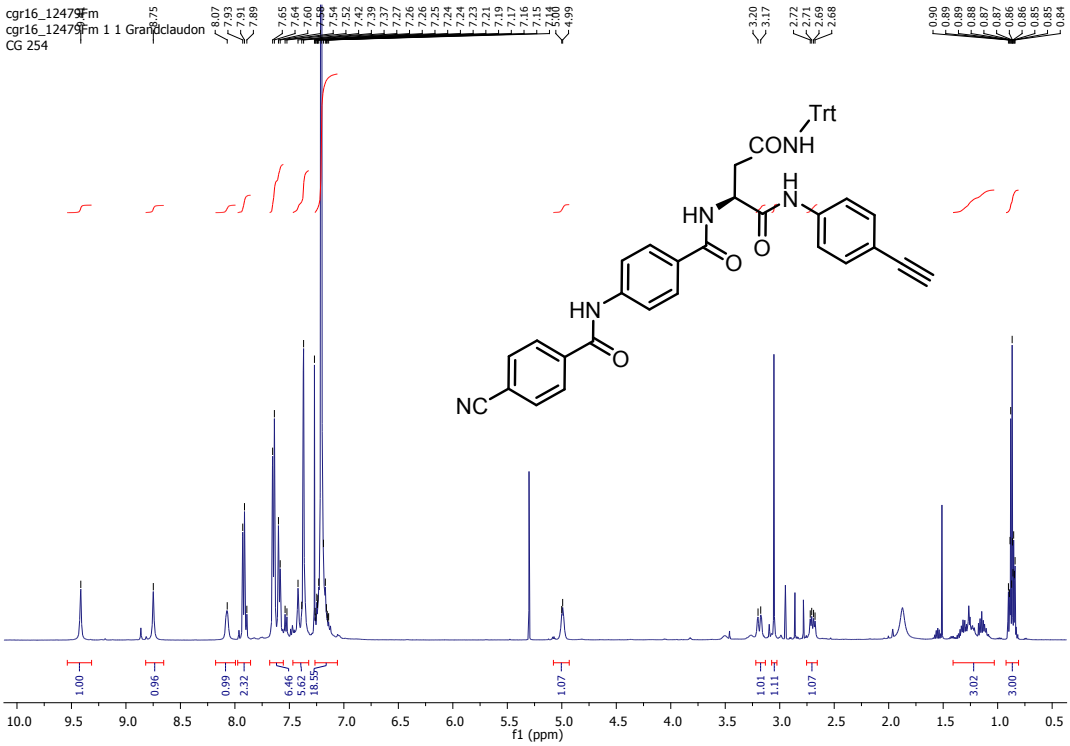


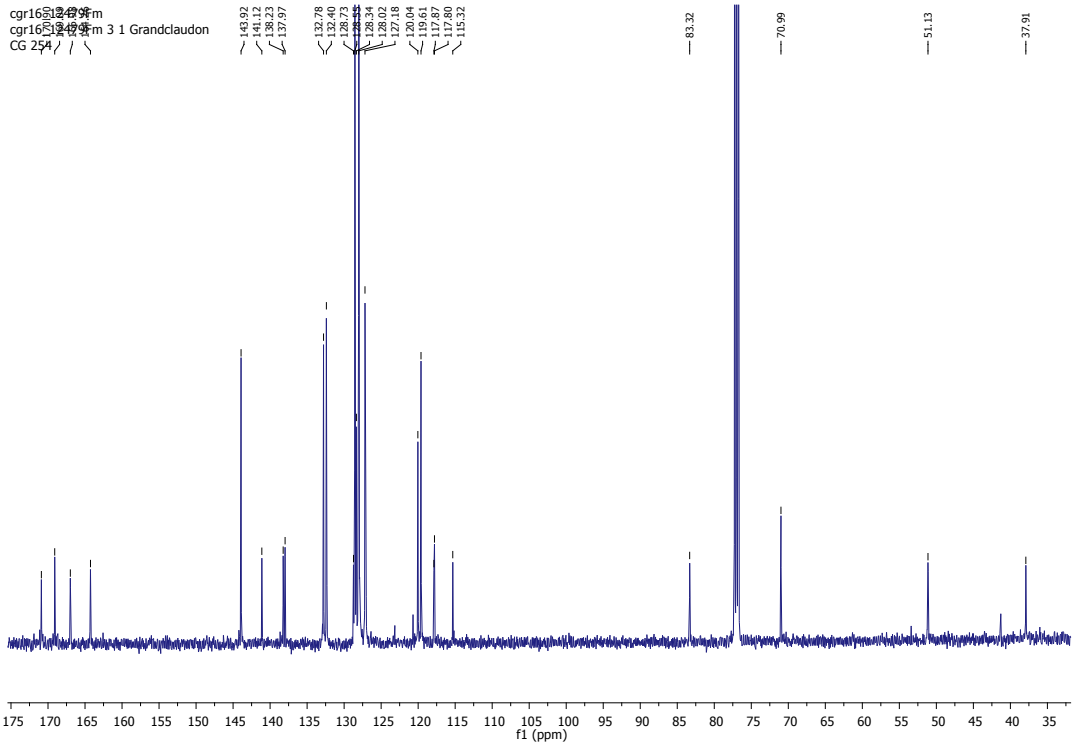
(9H-Fluoren-9-yl)methyl (1-((4-ethynylphenyl)amino)-1,4-dioxo-4-(tritylamino)butan-2-yl)carbamate



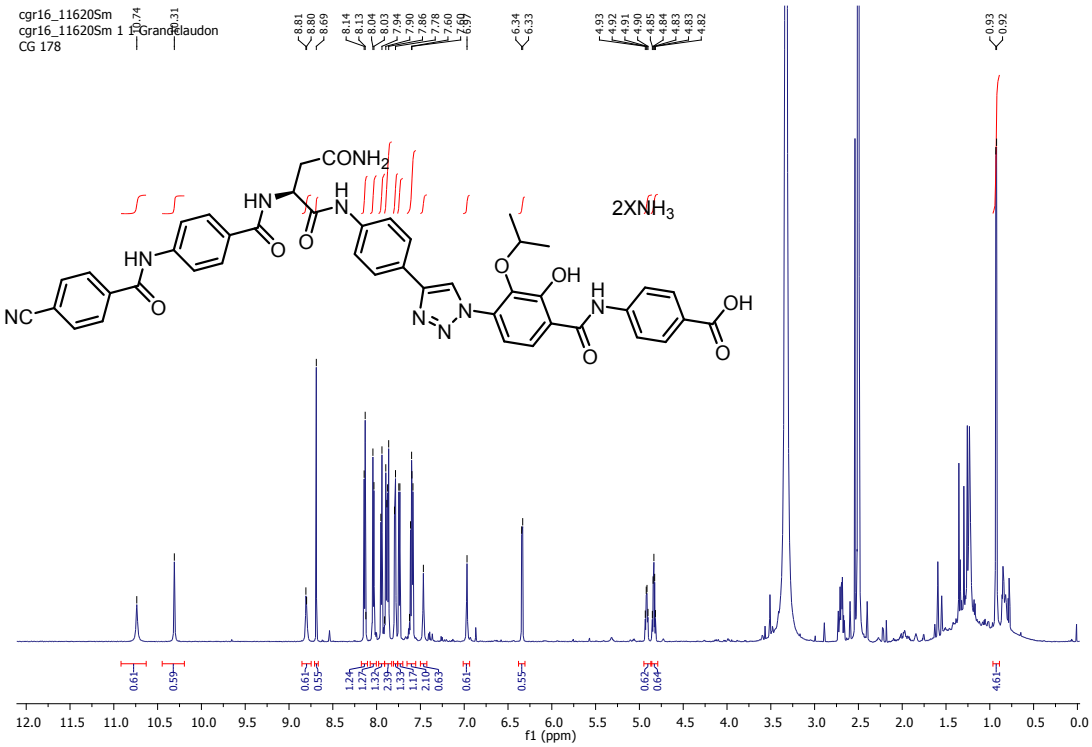


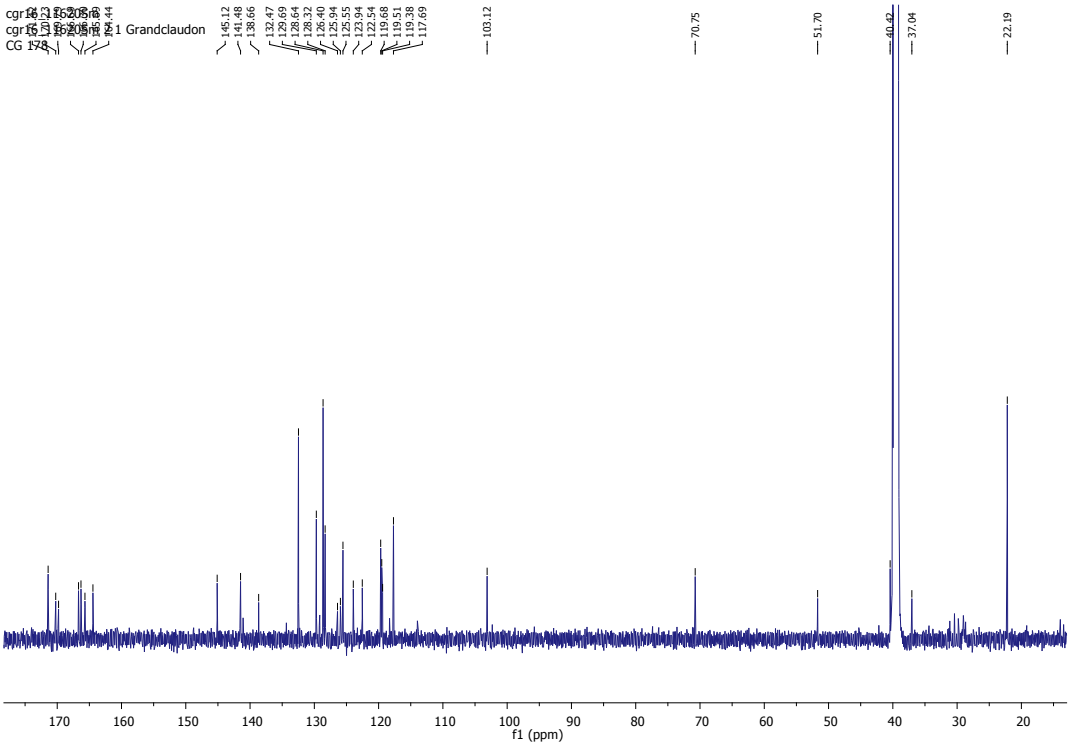
(S)-2-(4-(4-Cyanobenzamido)benzamido)-N1-(4-ethynylphenyl)-N4-tritylsuccinamide



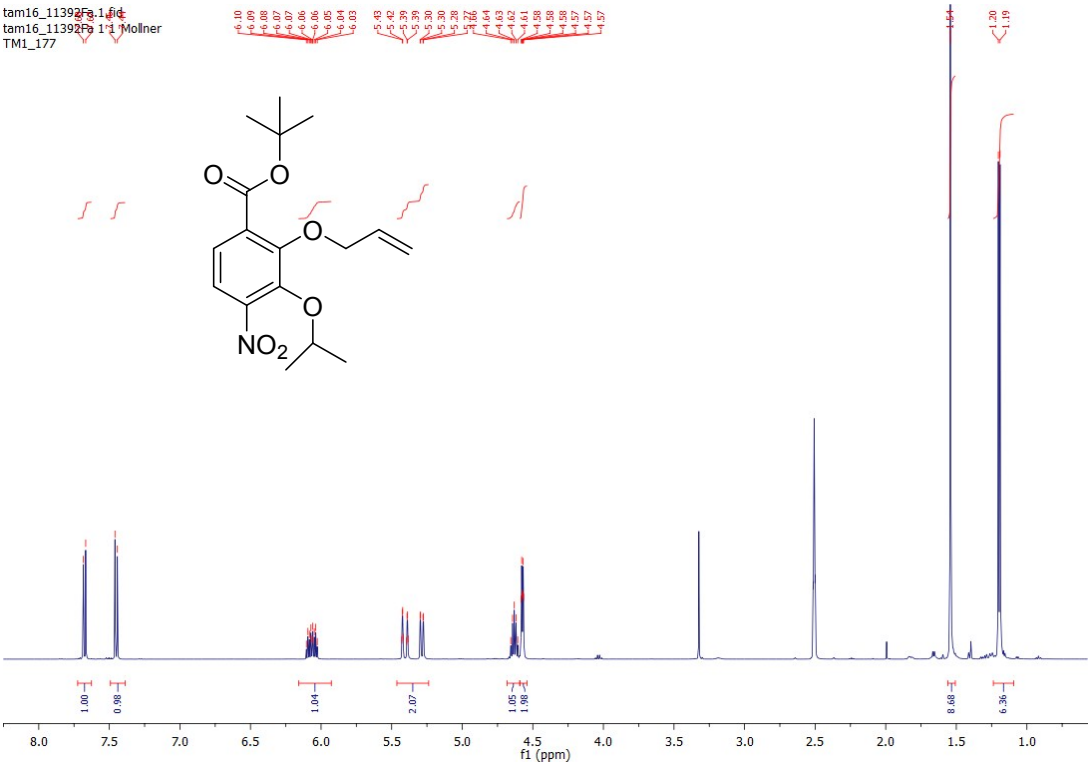


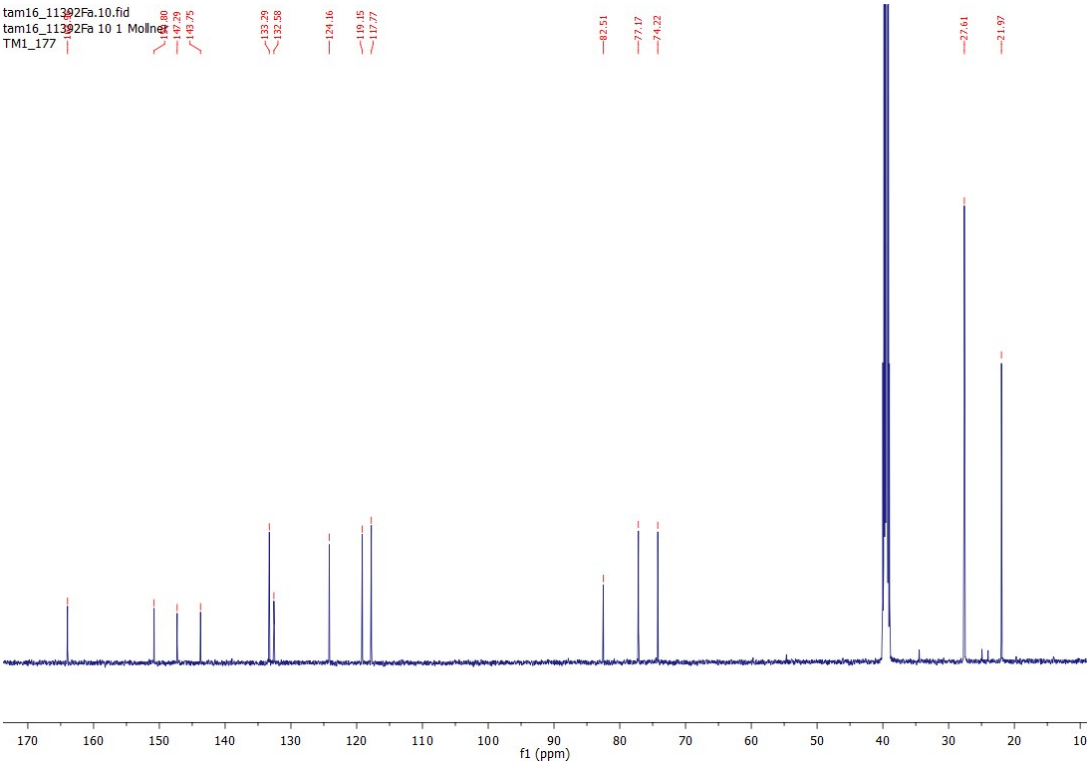
4-(4-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)phenyl)-1H-1,2,3-triazol-1-yl)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



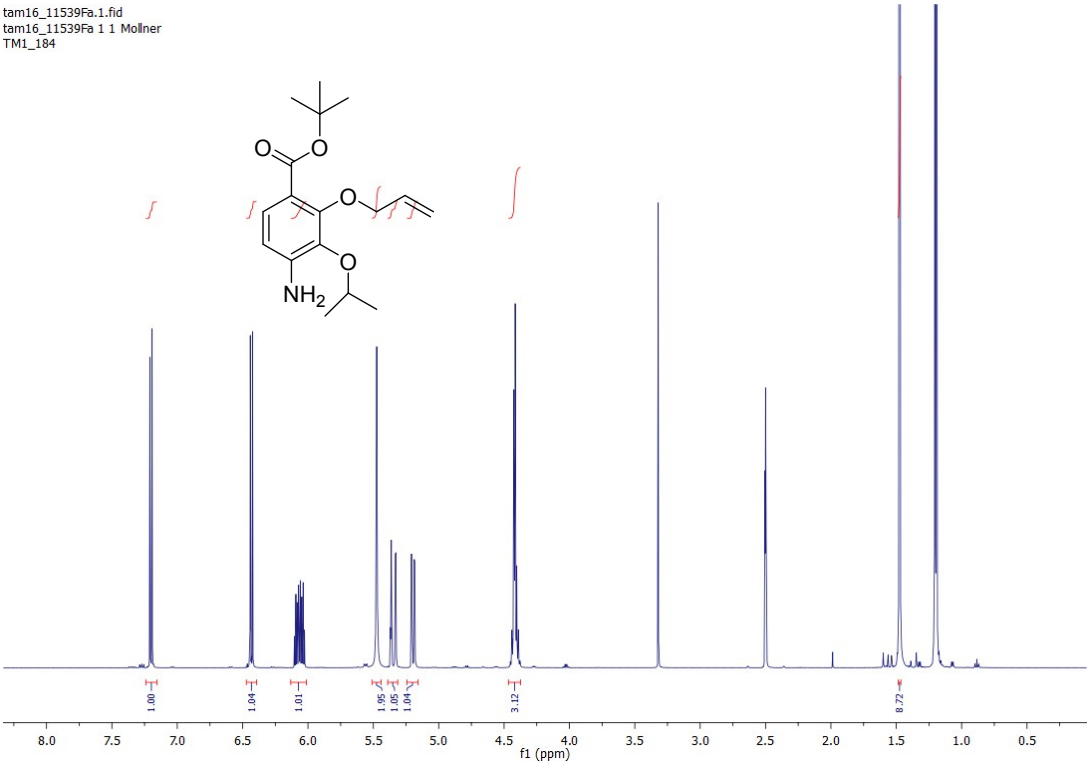


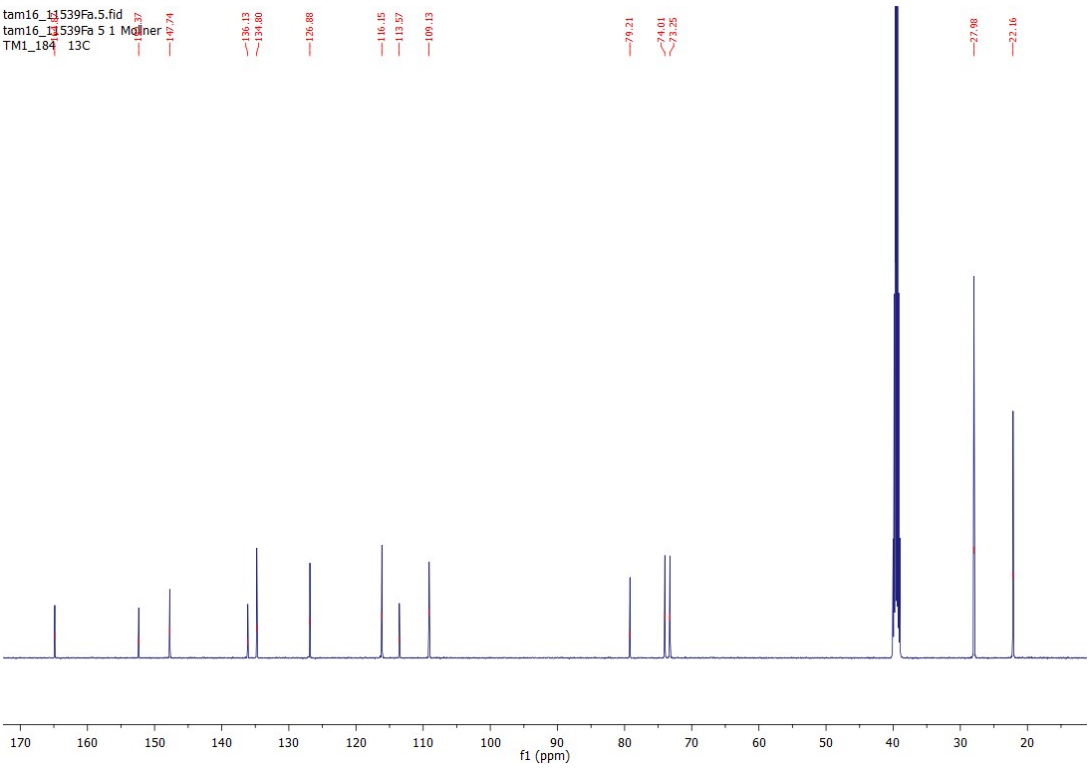
tert-butyl 2-(allyloxy)-3-isopropoxy-4-nitrobenzoate



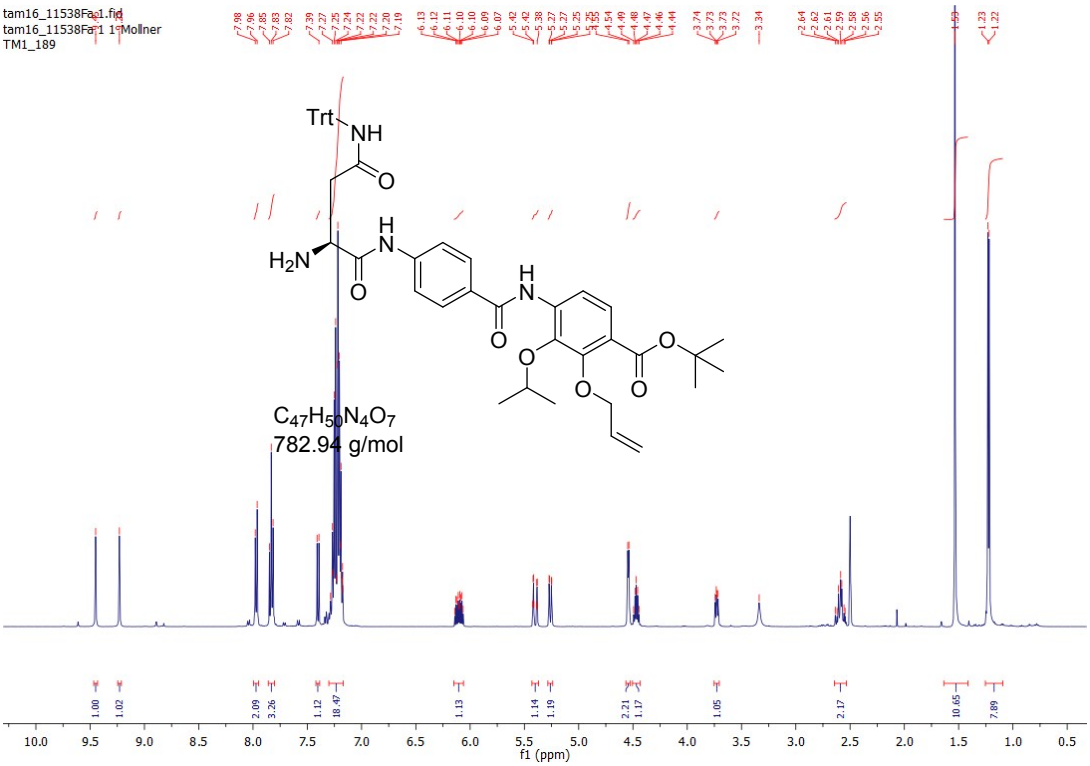


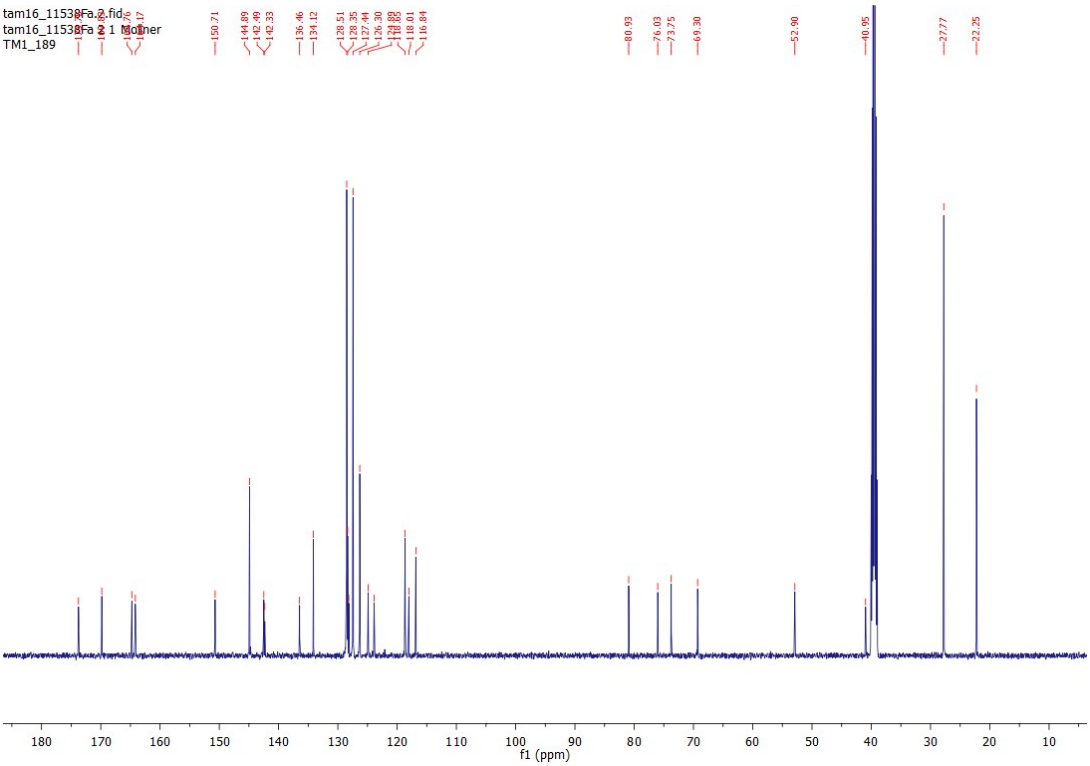
tert-butyl 2-(allyloxy)-4-amino-3-isopropoxybenzoate



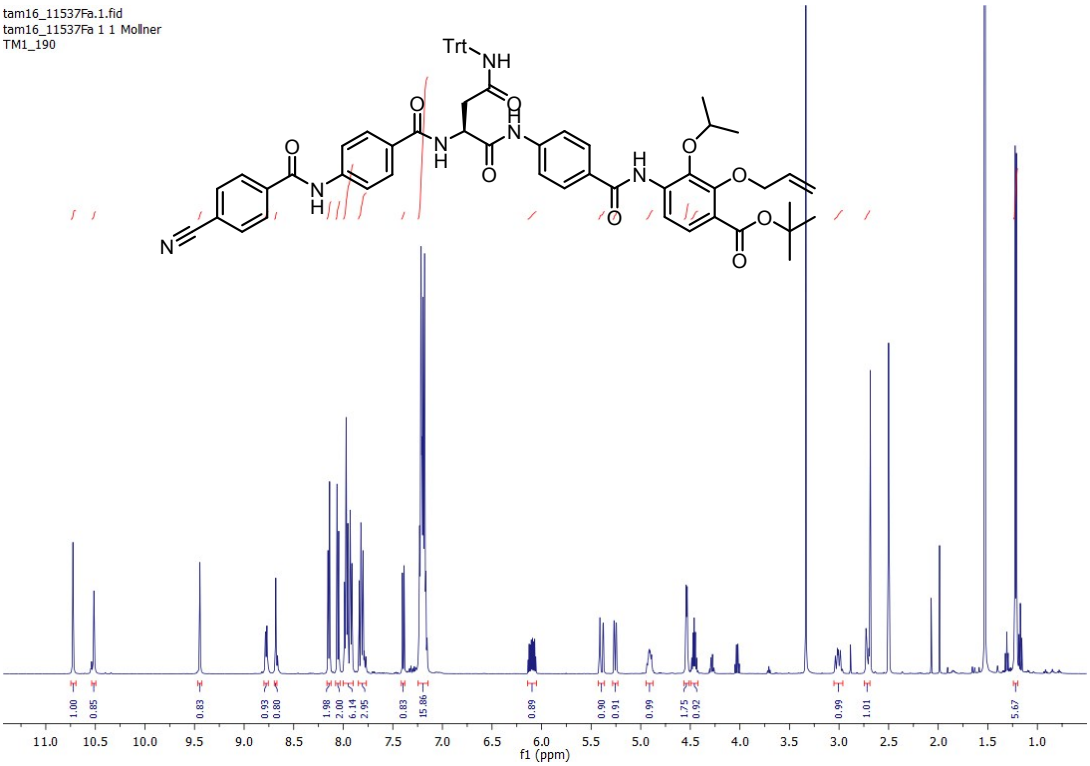


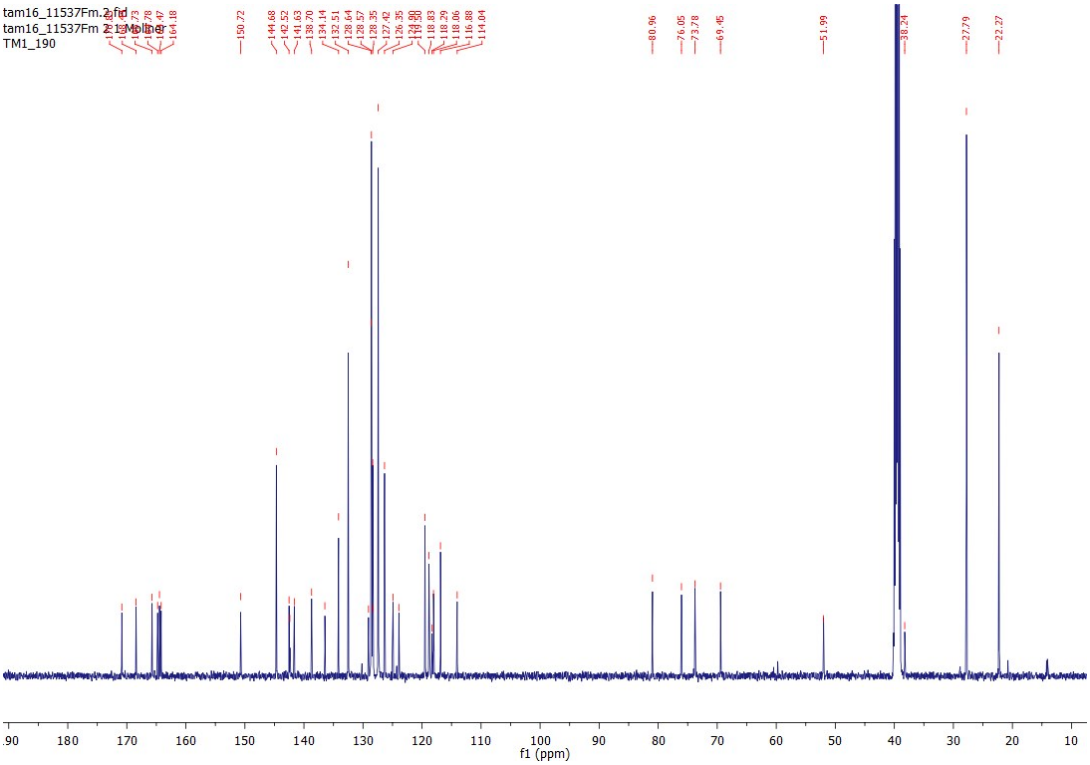
tert-butyl (*S*)-2-allyloxy-4-(4-(2-amino-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzoate



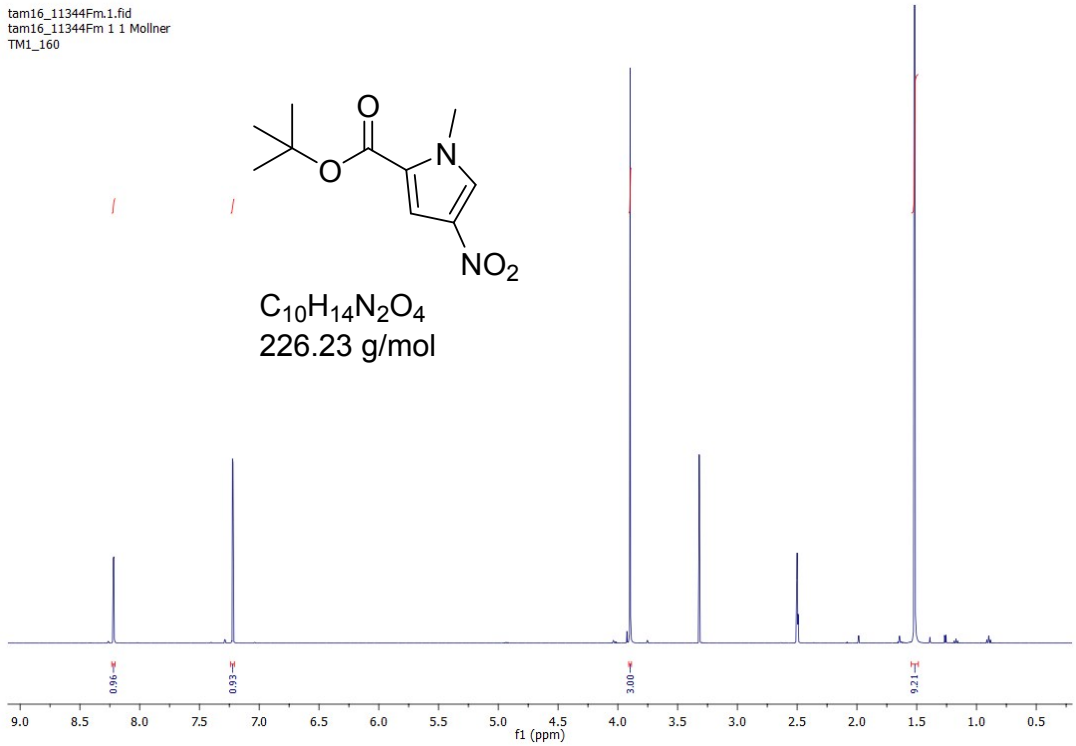


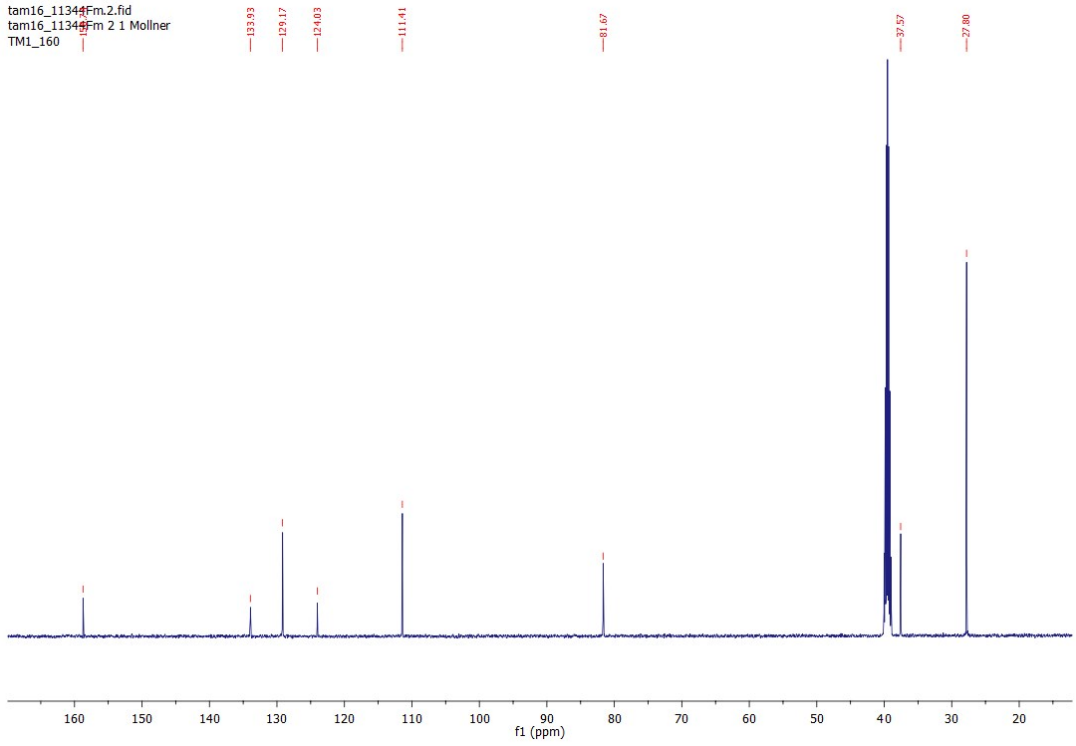
tert-butyl (S)-2-(allyloxy)-4-(4-(2-(4-(4-cyanobenzamido)benzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)-3-isopropoxybenzoate



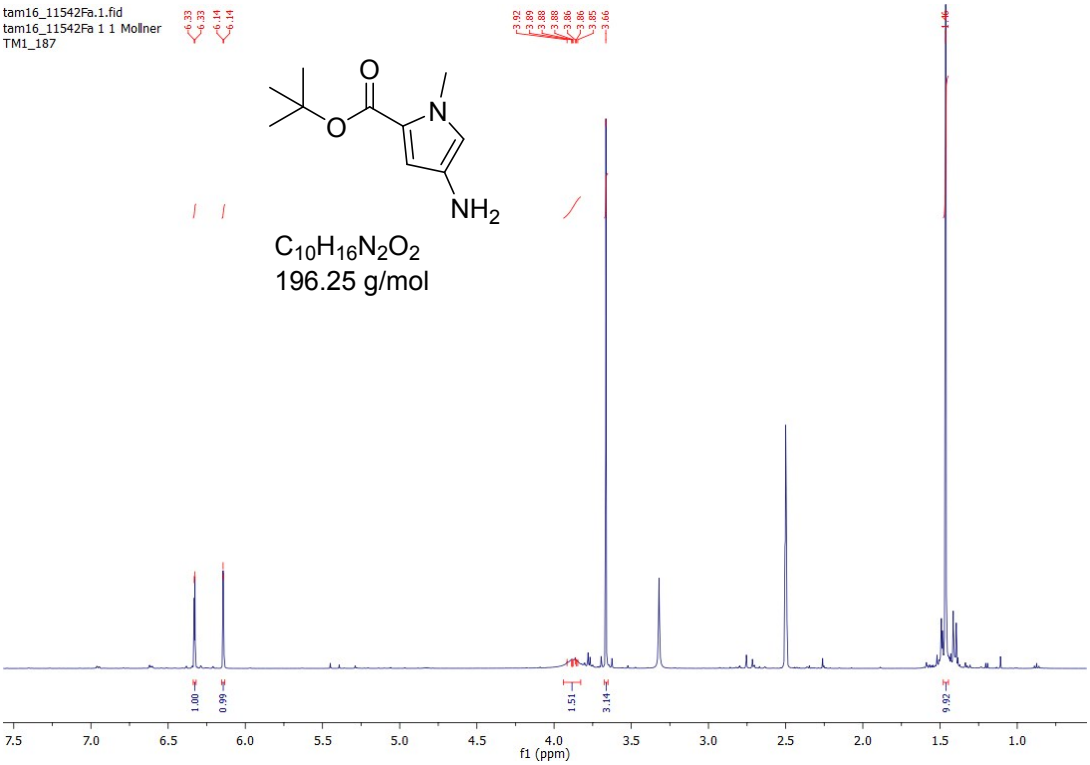


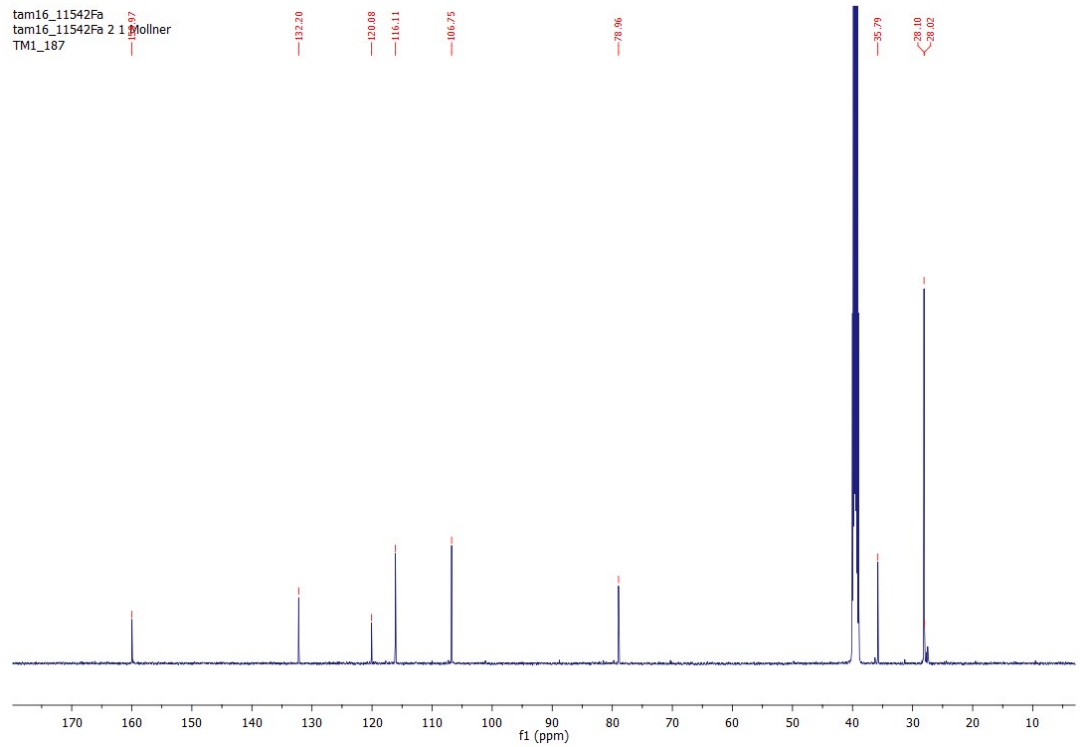
tert-butyl 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylate



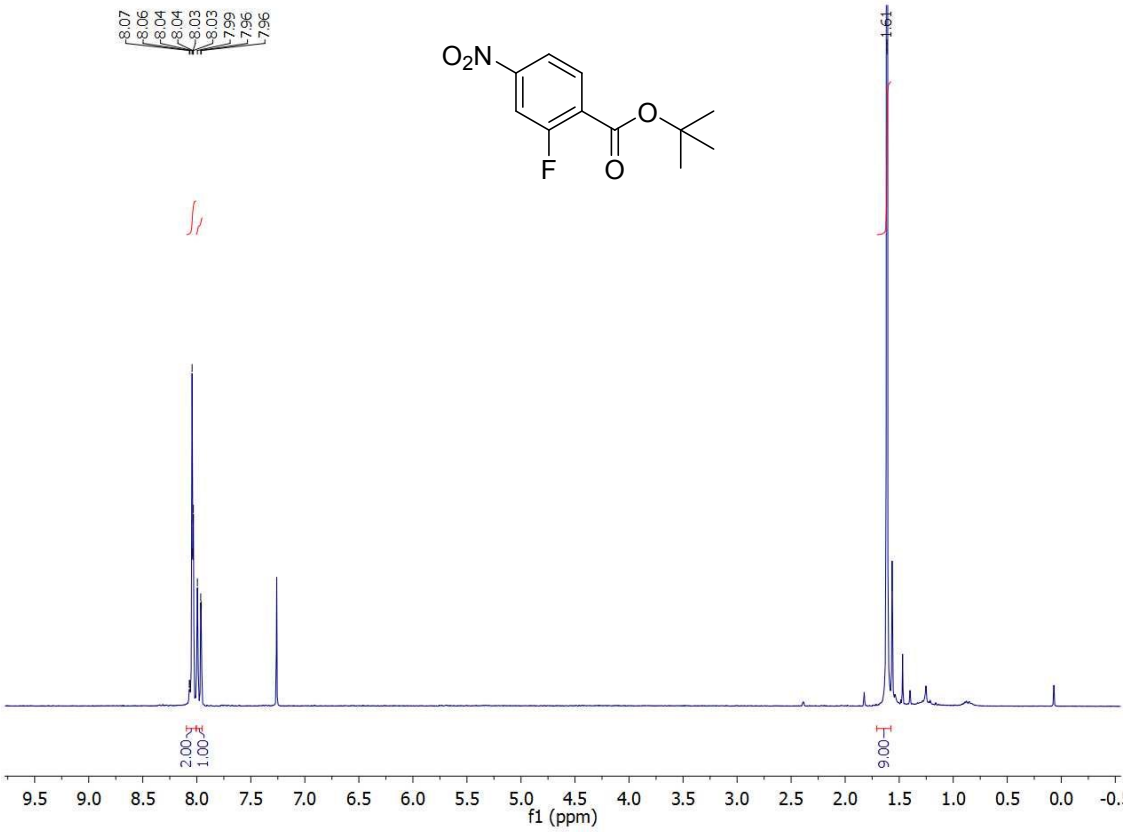


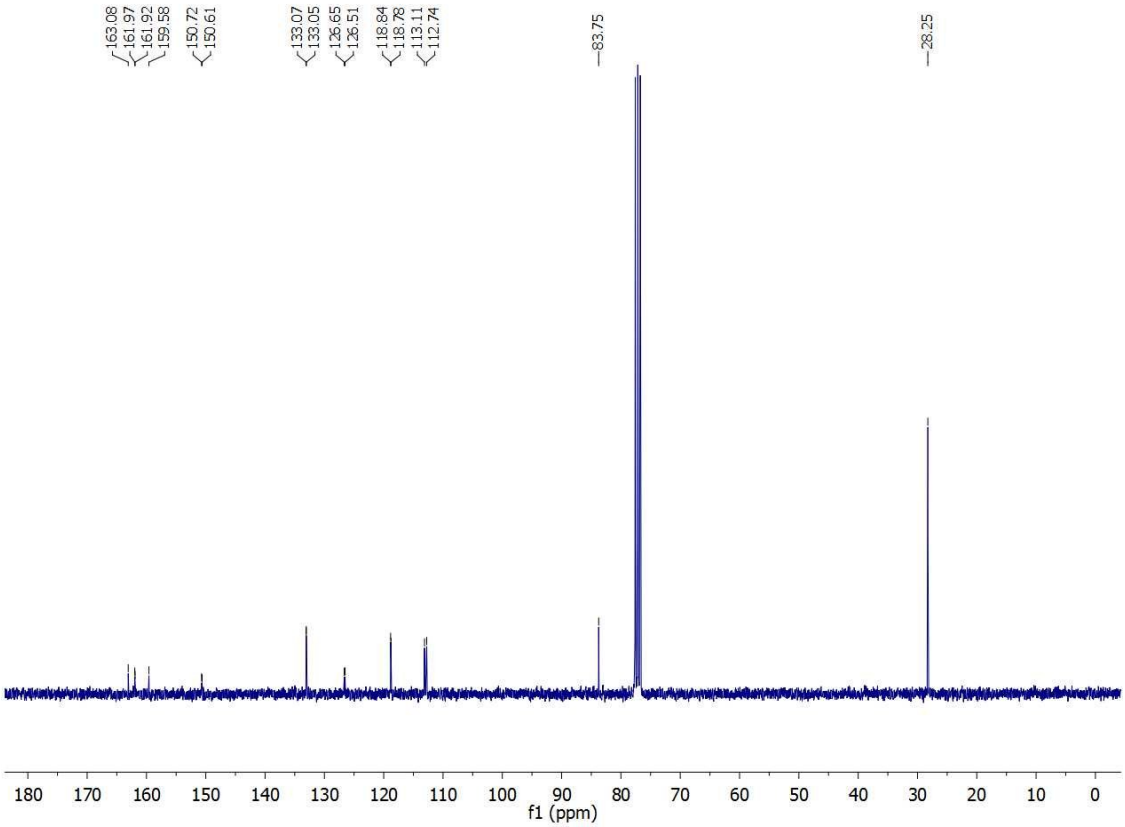
tert-butyl 4-amino-1-methyl-1*H*-pyrrole-2-carboxylate



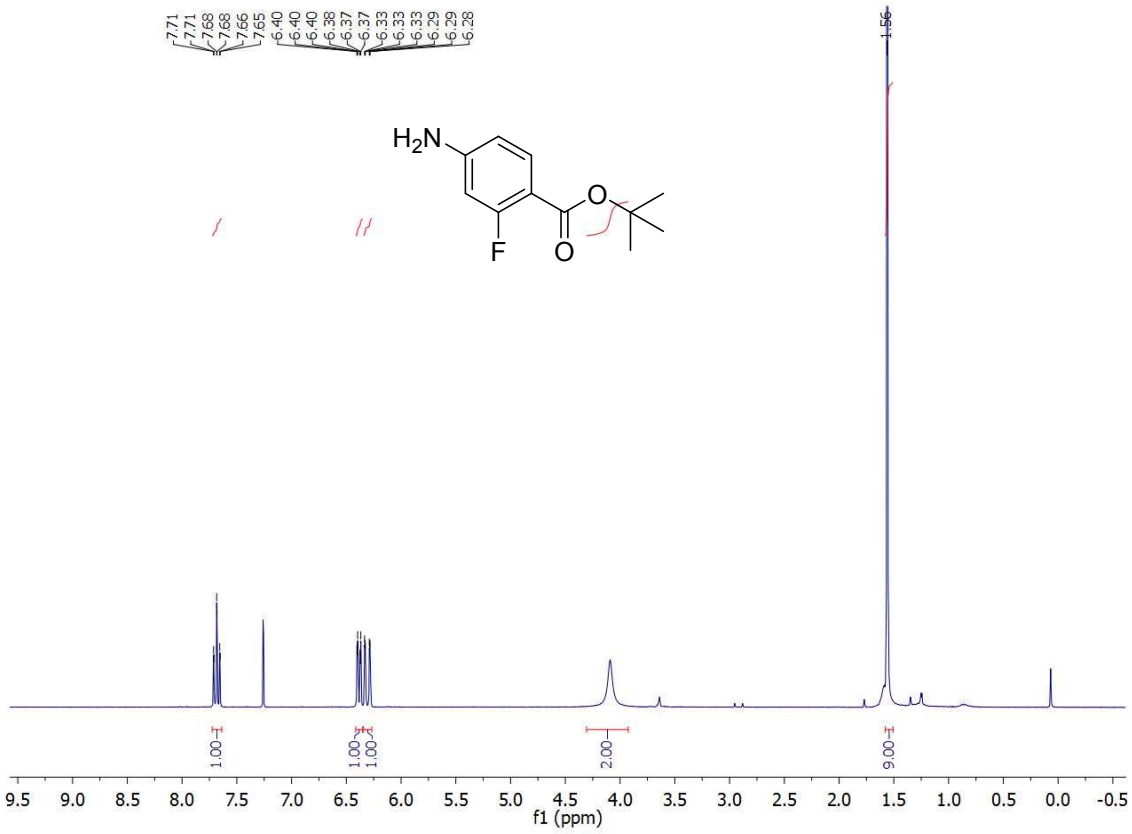


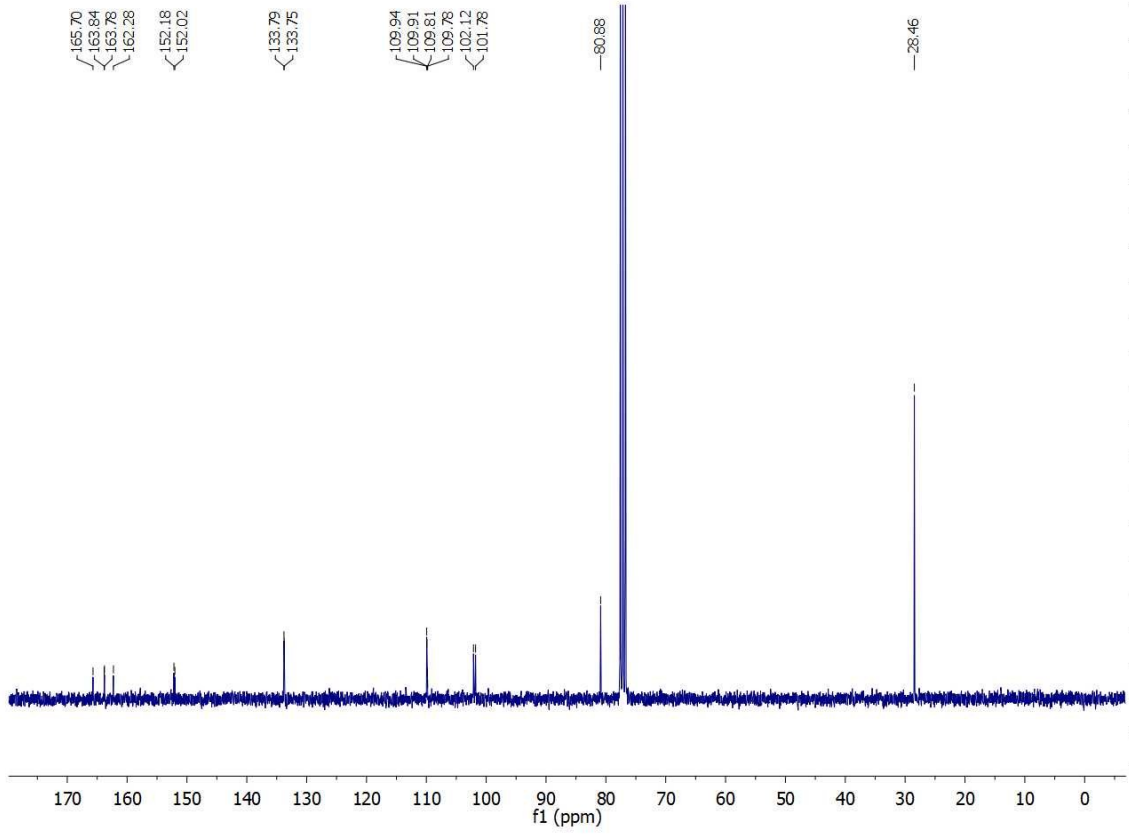
***tert*-butyl 2-fluoro-4-nitrobenzoate**



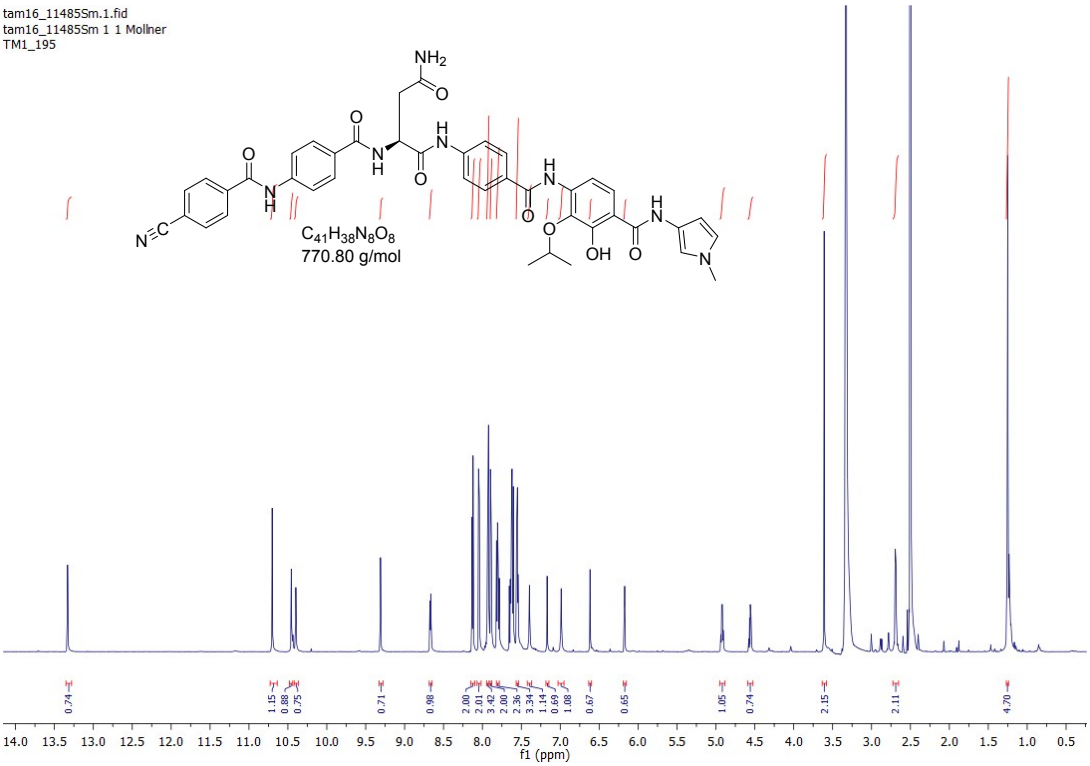


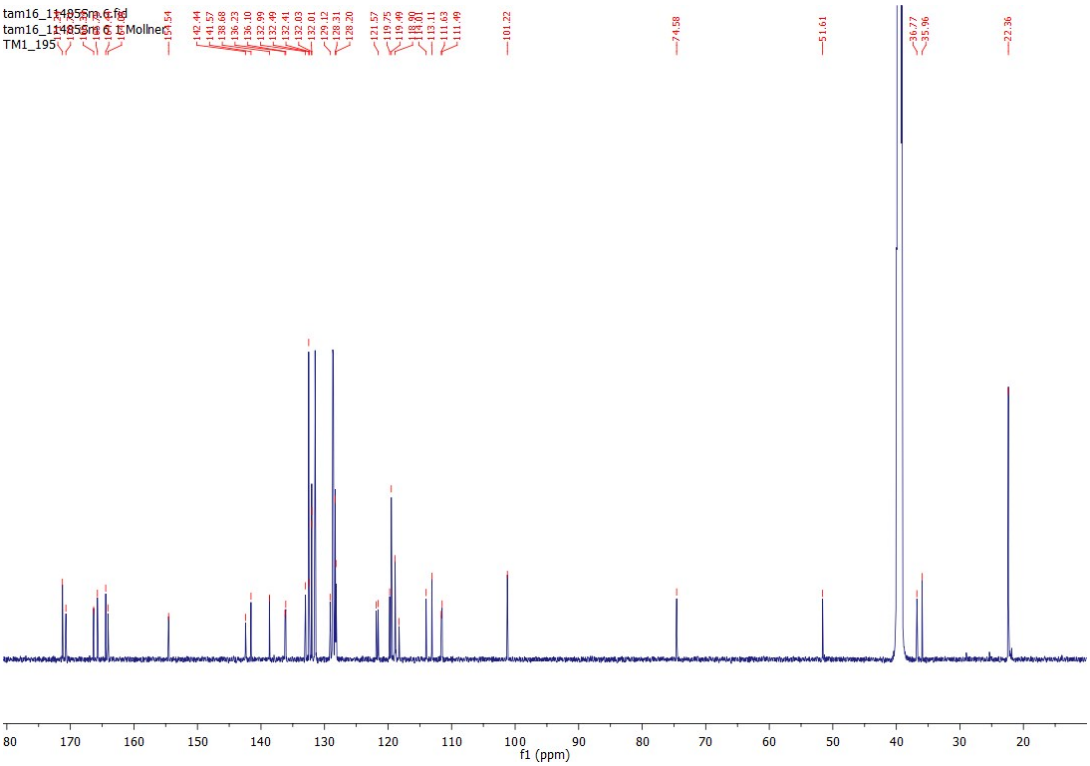
***tert*-butyl 4-amino-2-fluorobenzoate**



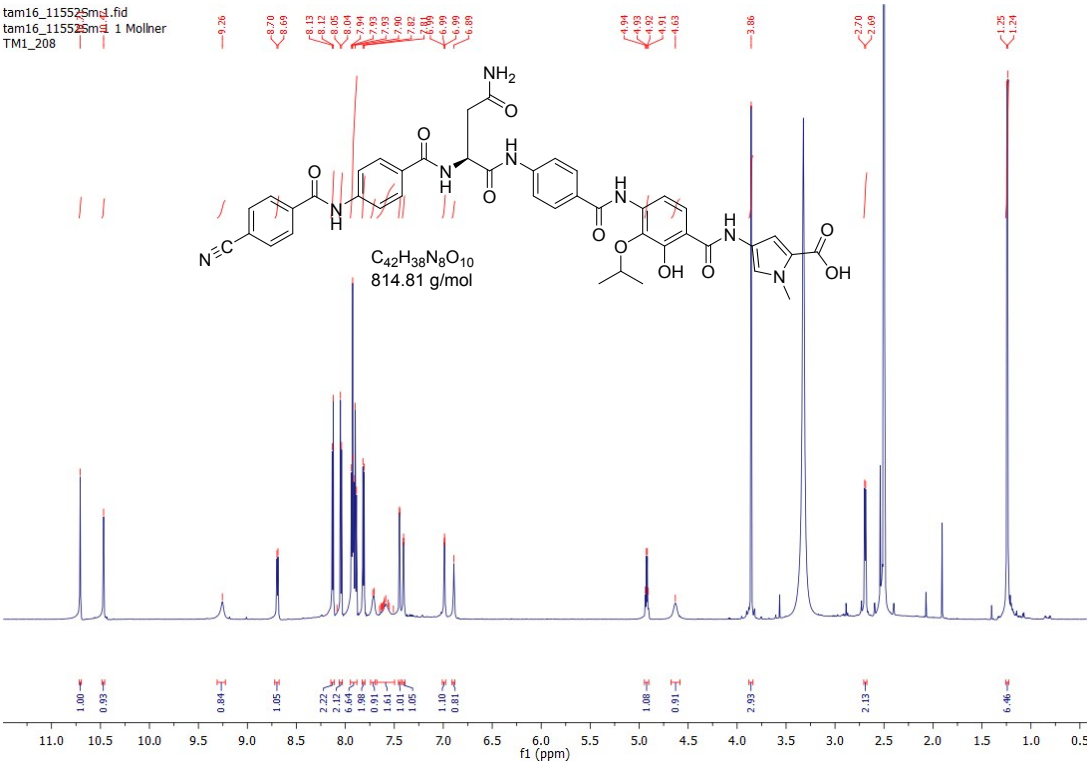


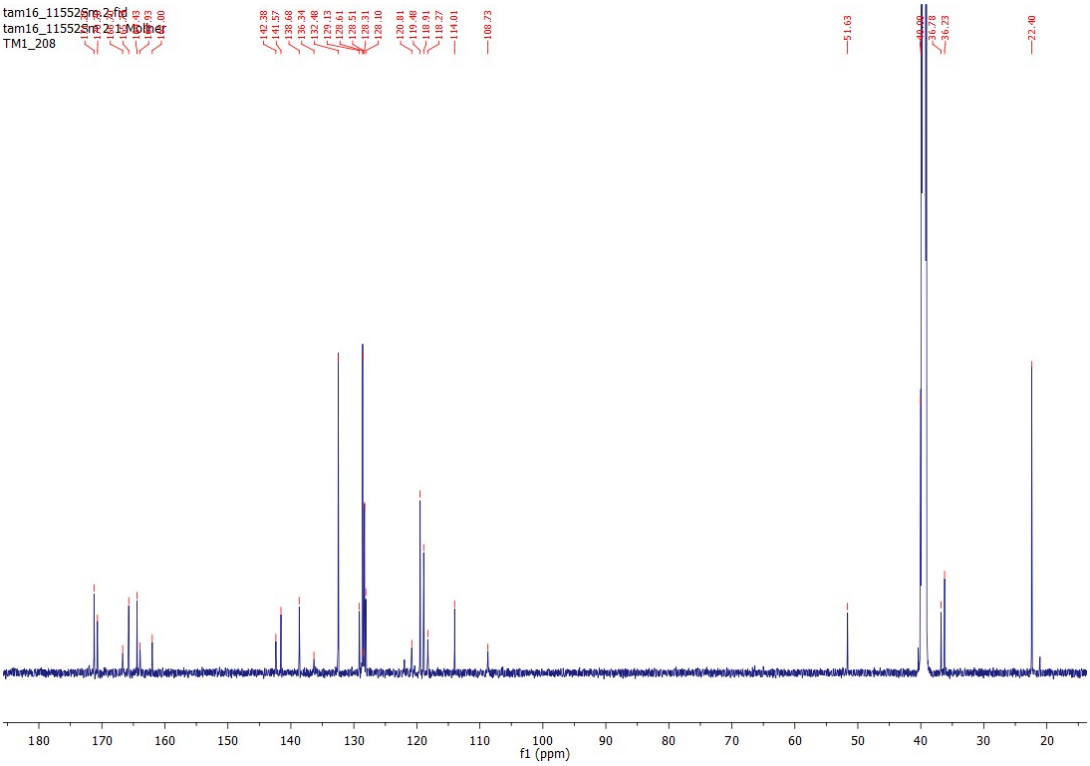
(*S*)-2-(4-(4-cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-((1-methyl-1*H*-pyrrol-3-yl)carbamoyl)phenyl)carbamoyl)phenyl)succinamide



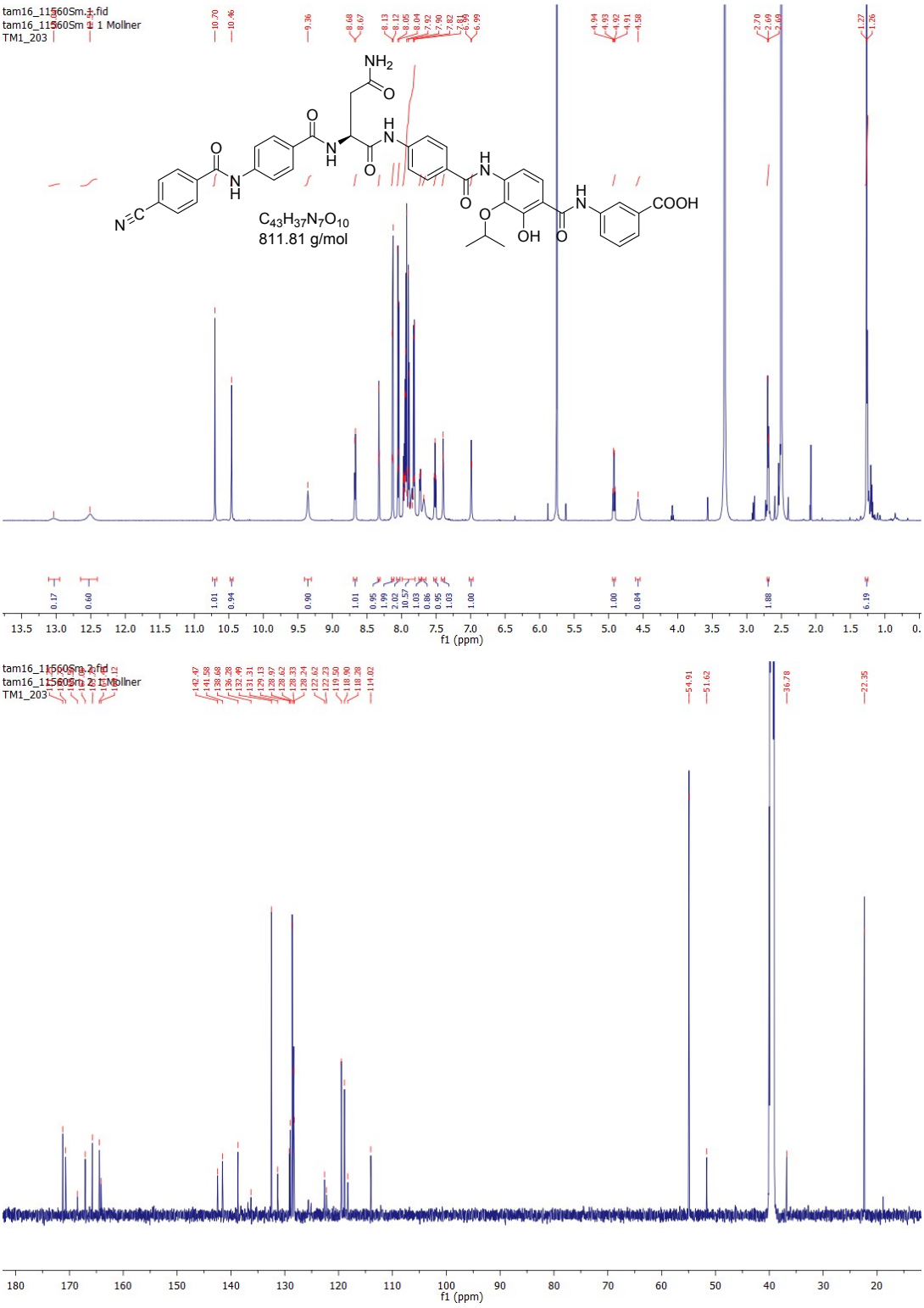


(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid

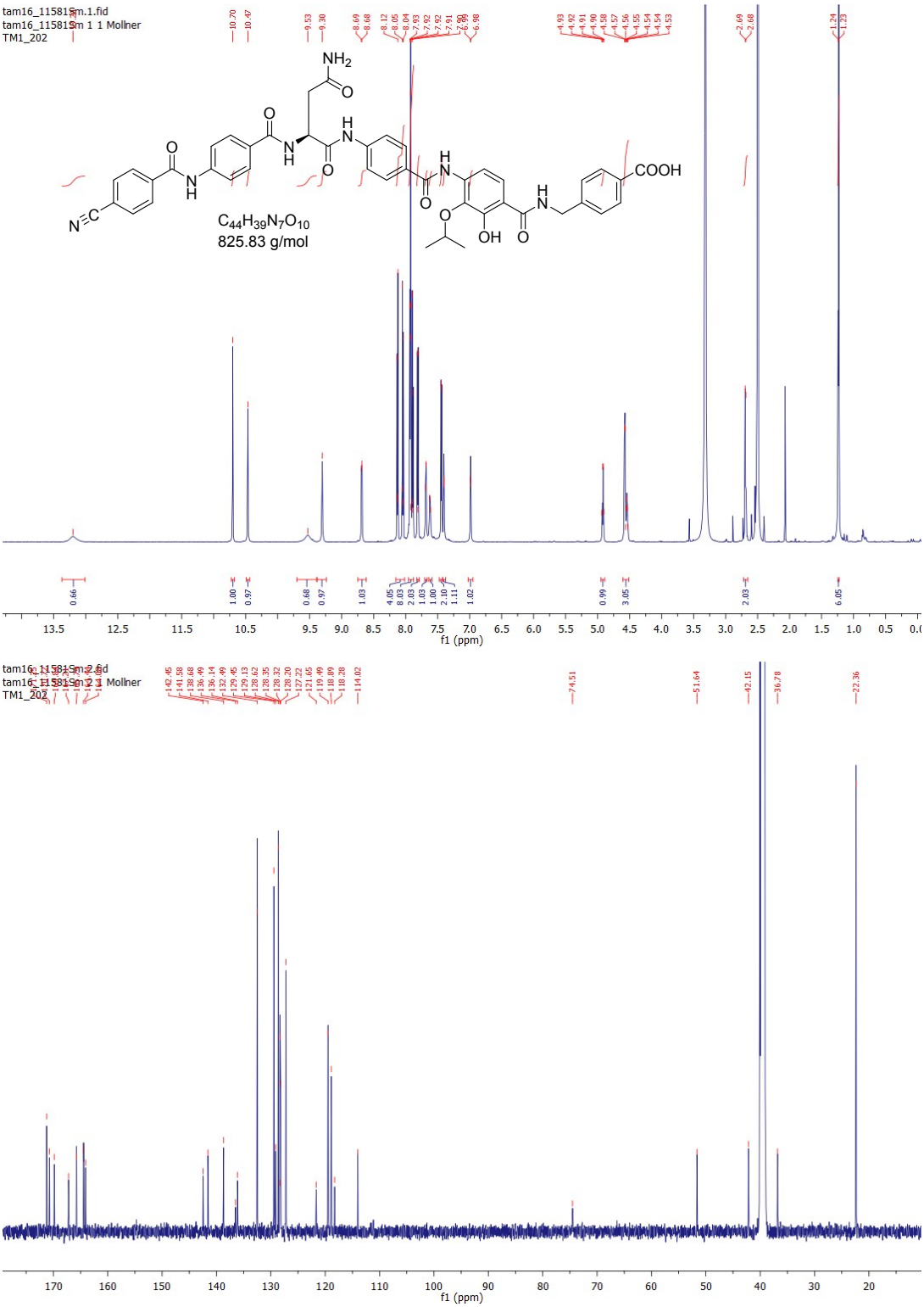




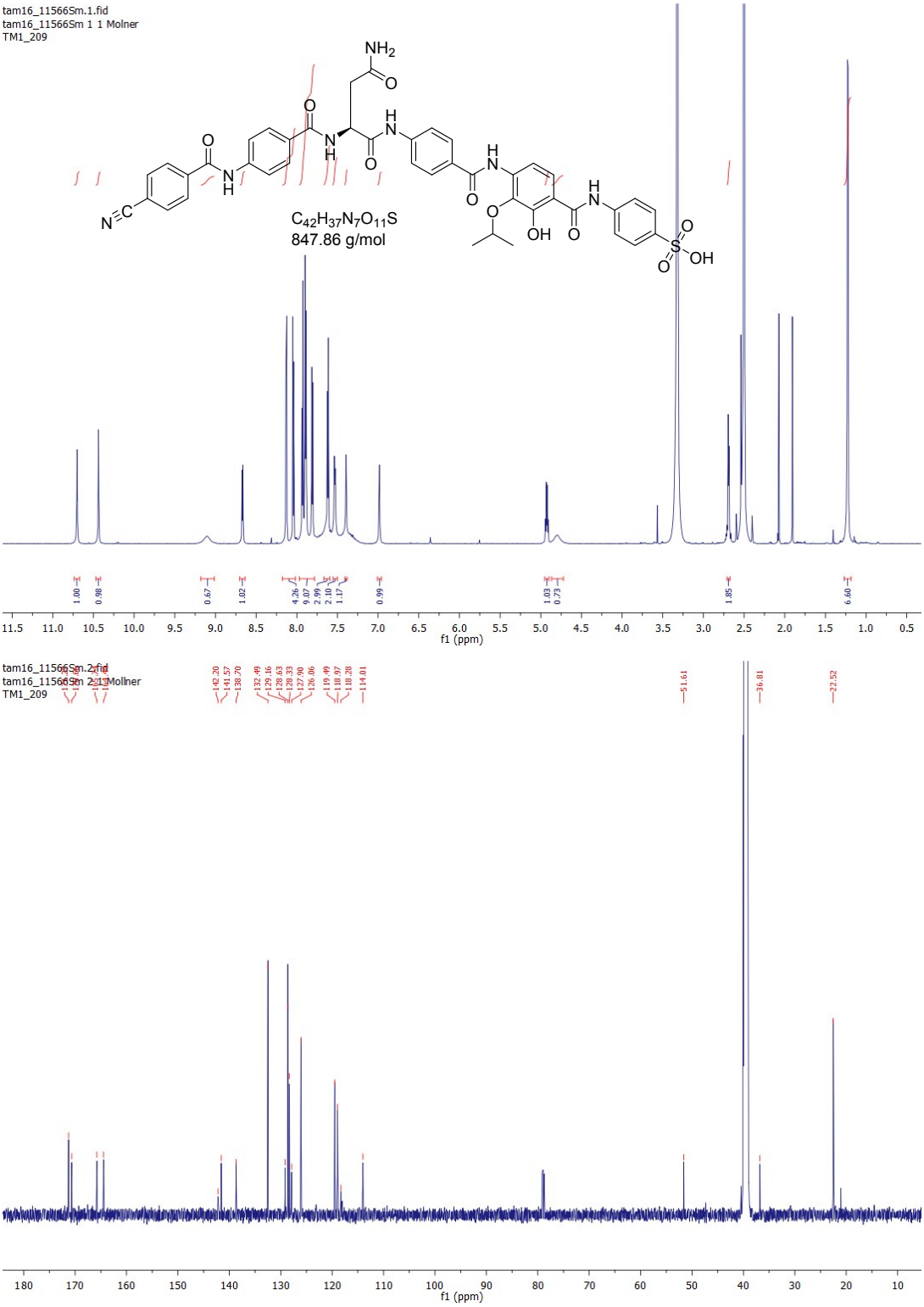
(*S*)-3-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



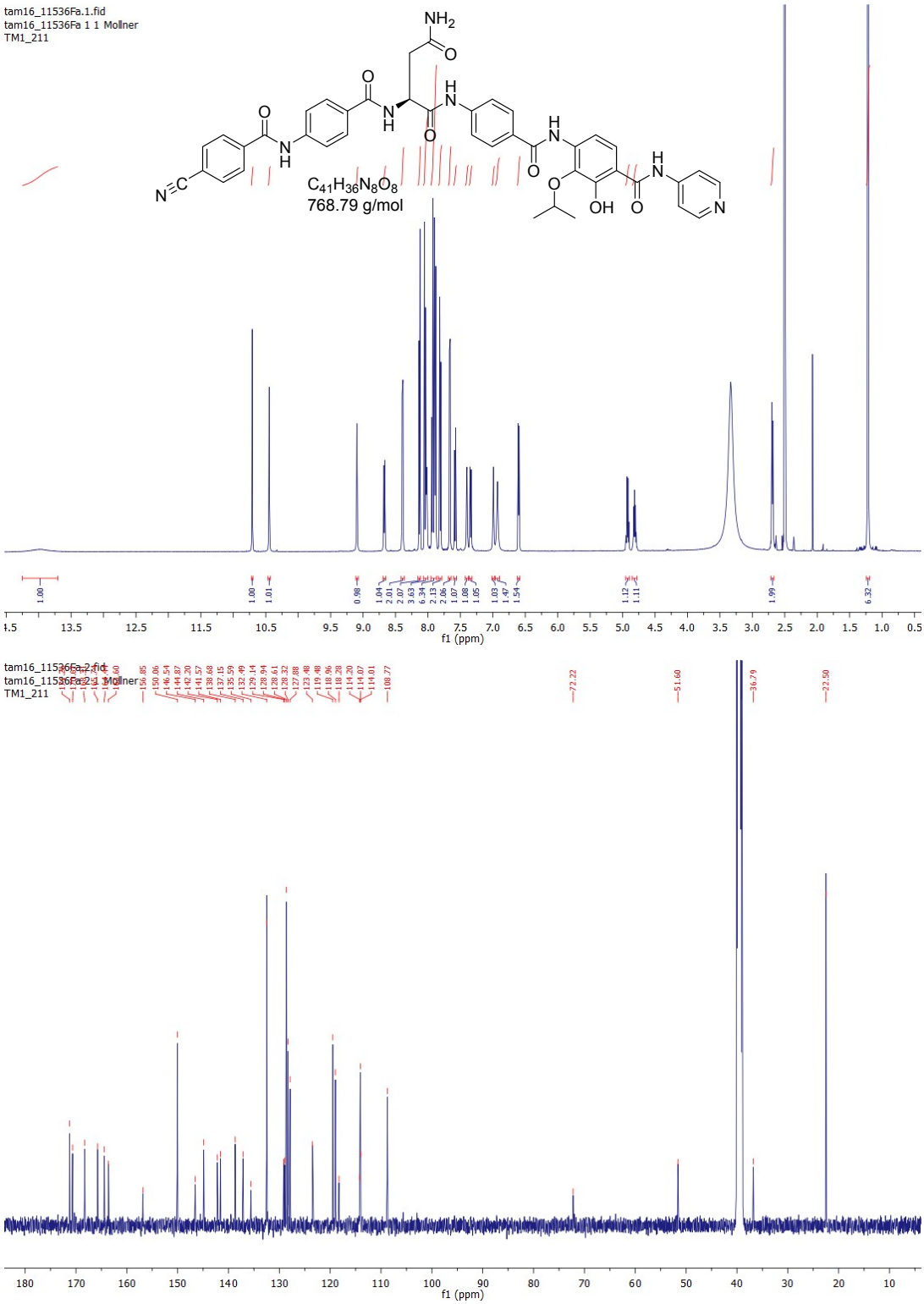
(*S*)-3-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid



(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzenesulfonic acid



(*S*)-2-(4-(4-cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-(pyridin-4-ylcarbamoyl)phenyl)carbamoyl)phenyl)succinamide



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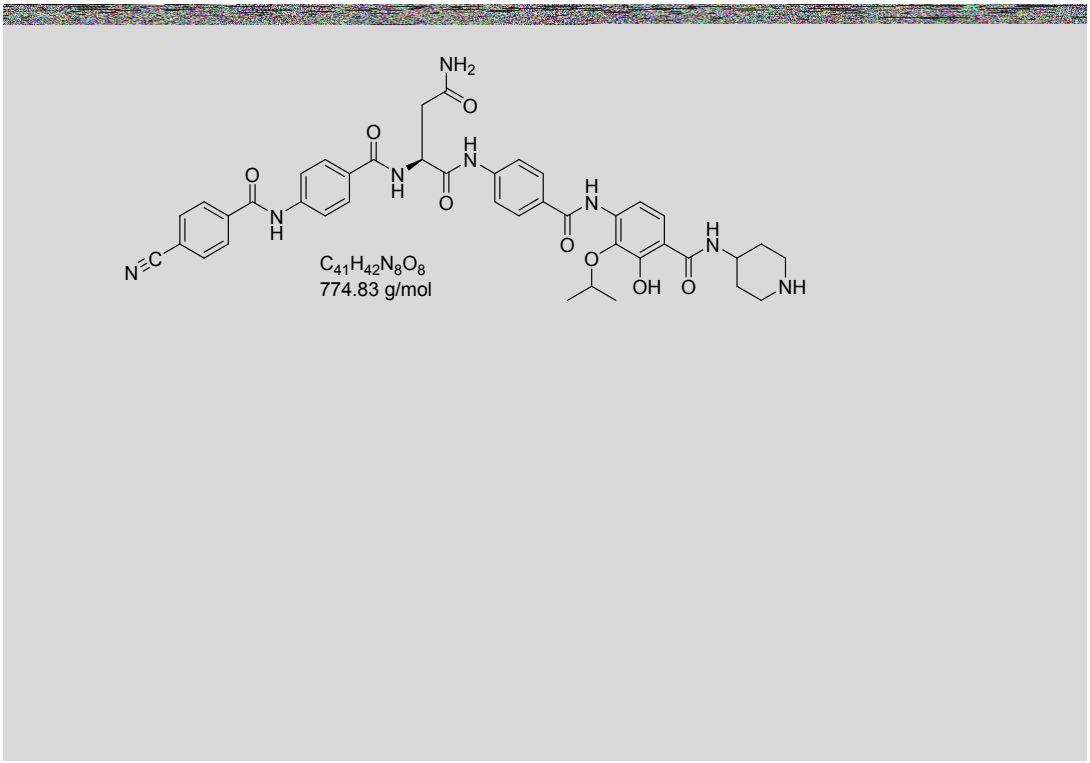
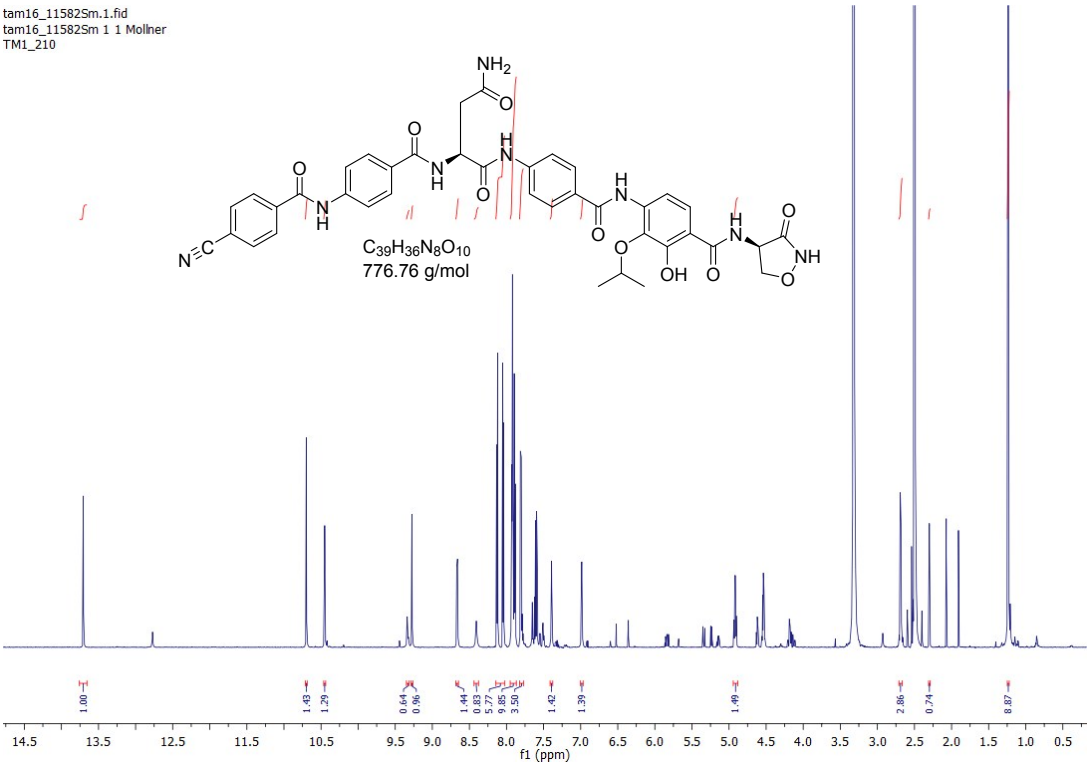
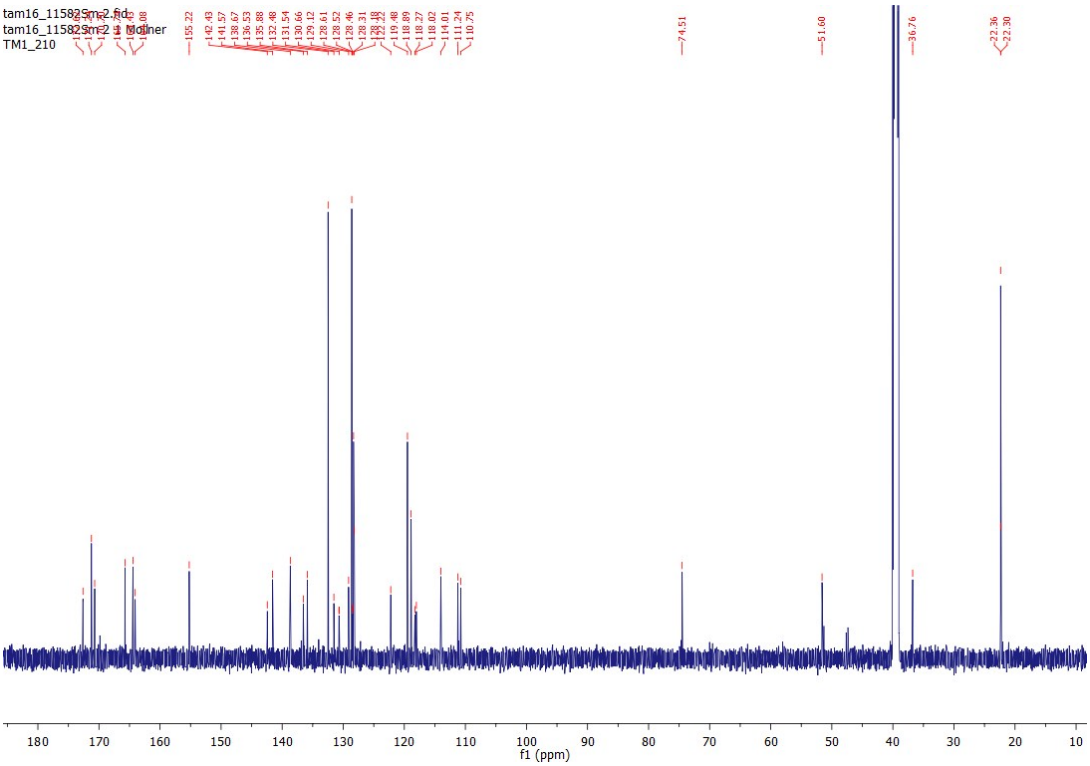
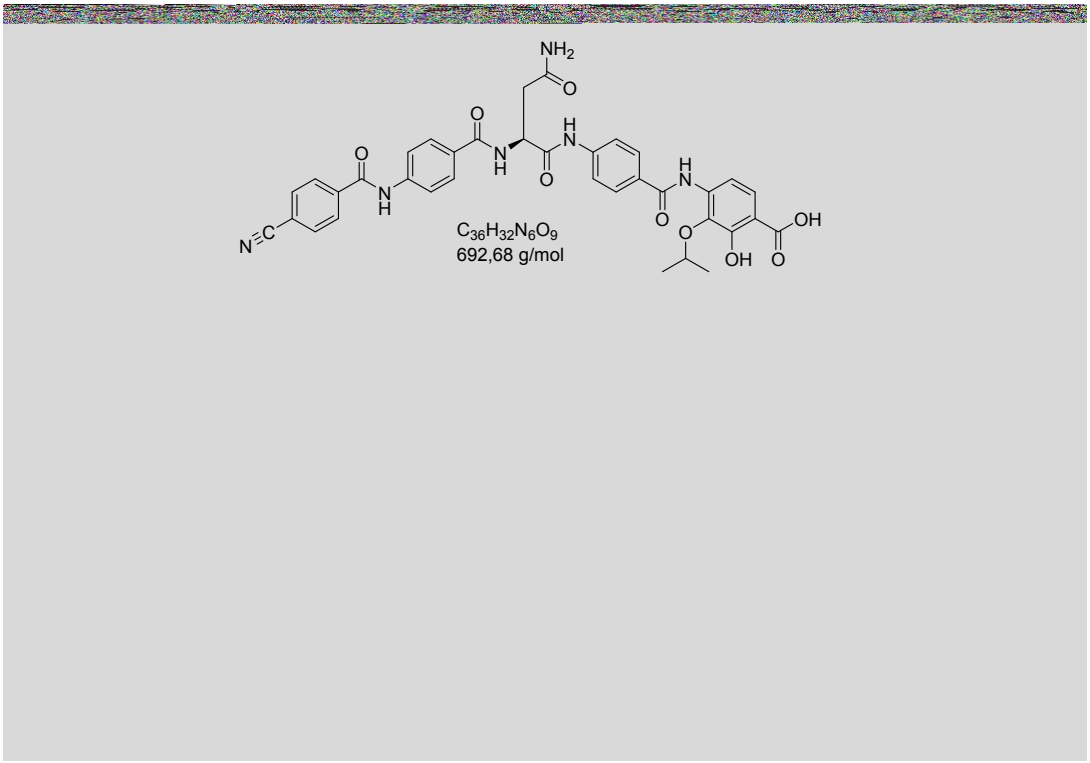


(*S*)-2-(4-(4-cyanobenzamido)benzamido)-N^1-(4-((3-hydroxy-2-isopropoxy-4-(((*R*)-3-oxoisoxazolidin-4-yl)carbamoyl)phenyl)carbamoyl)phenyl)succinamide

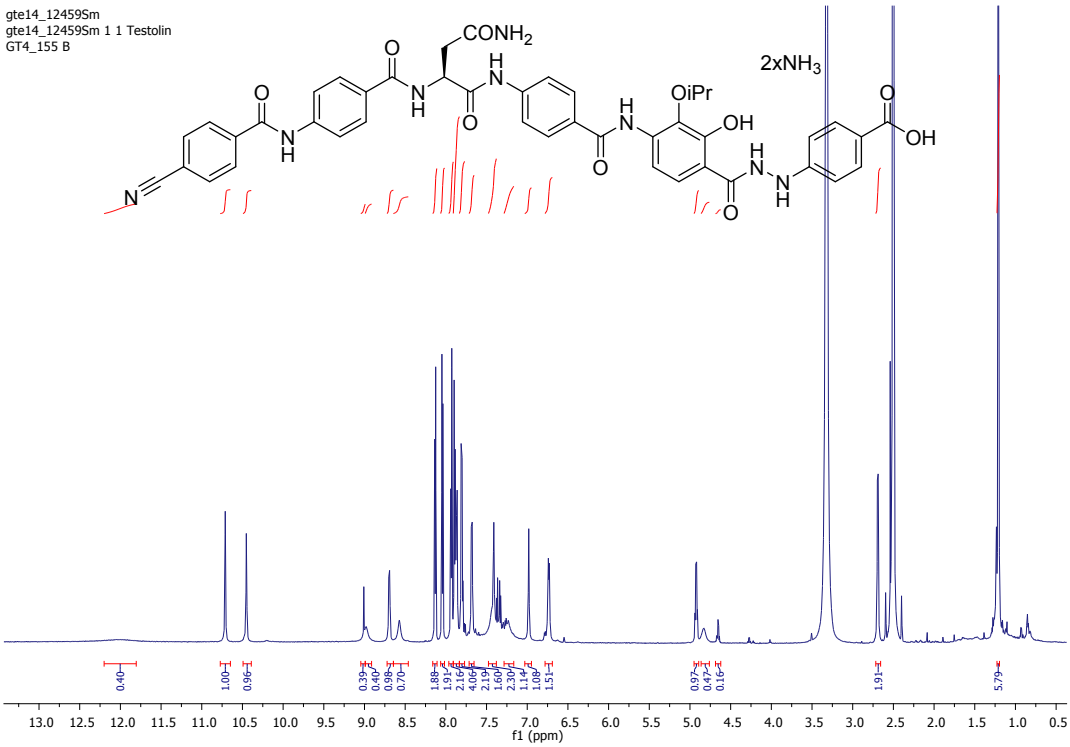


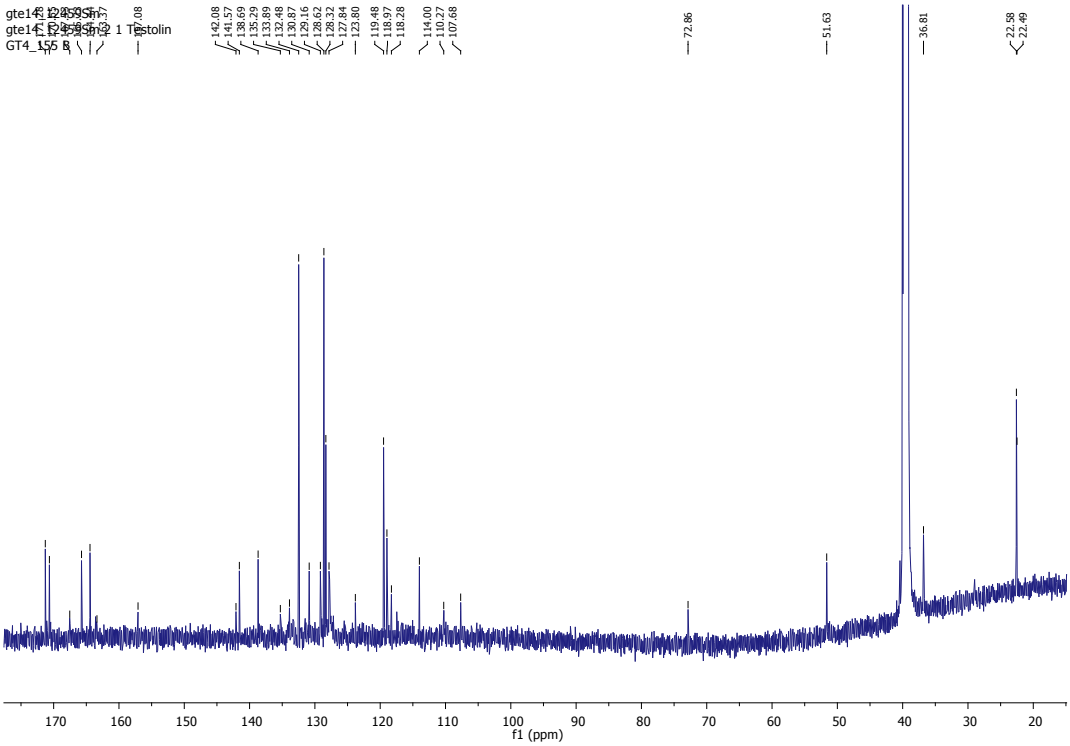


(*S*)-4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzoic acid

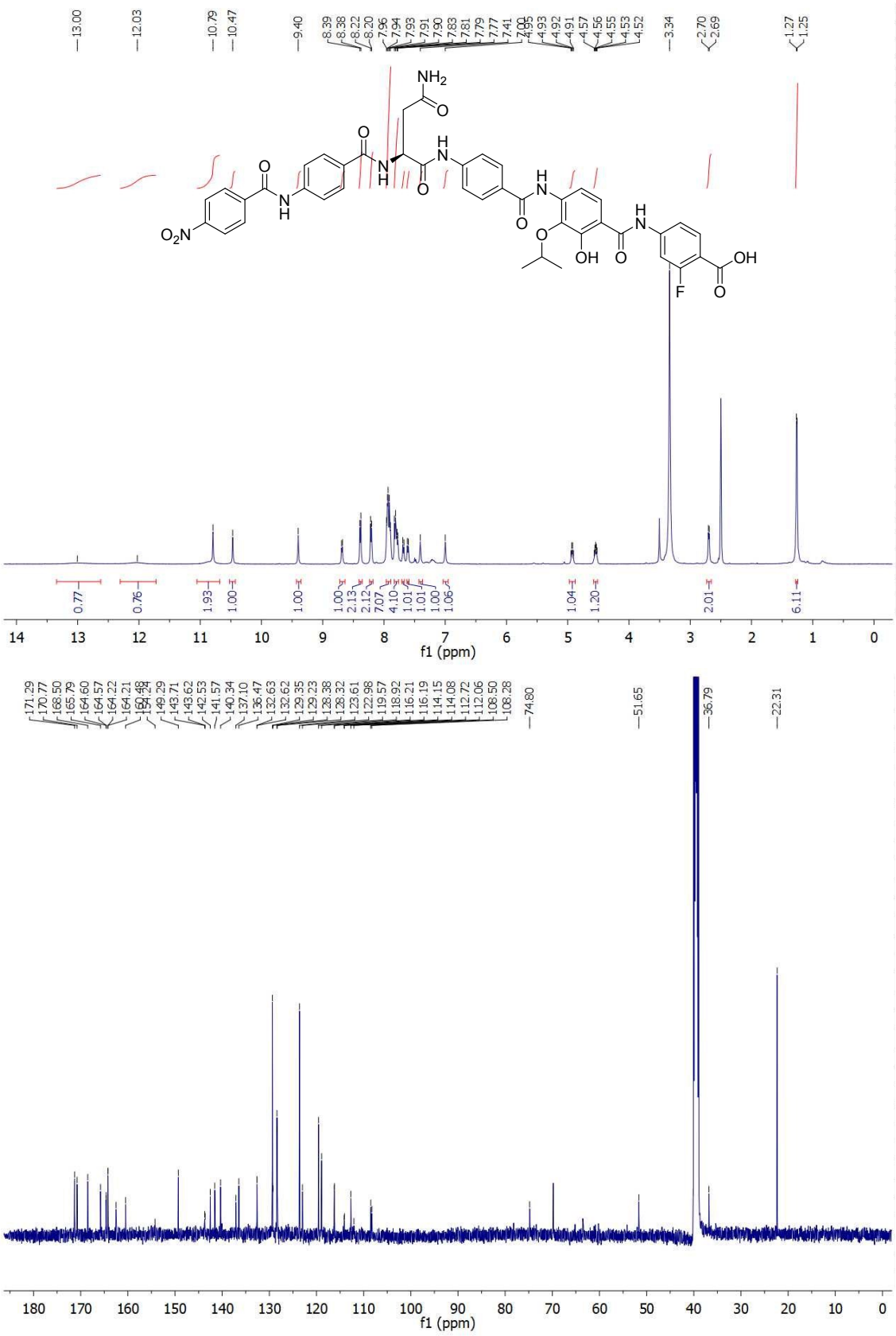


(S)-4-(2-(4-(4-(4-Amino-2-(4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzoyl)hydrazineyl)benzoic acid

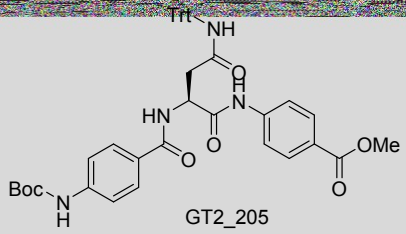




(S)-4-(4-(4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)-2-fluorobenzoic acid



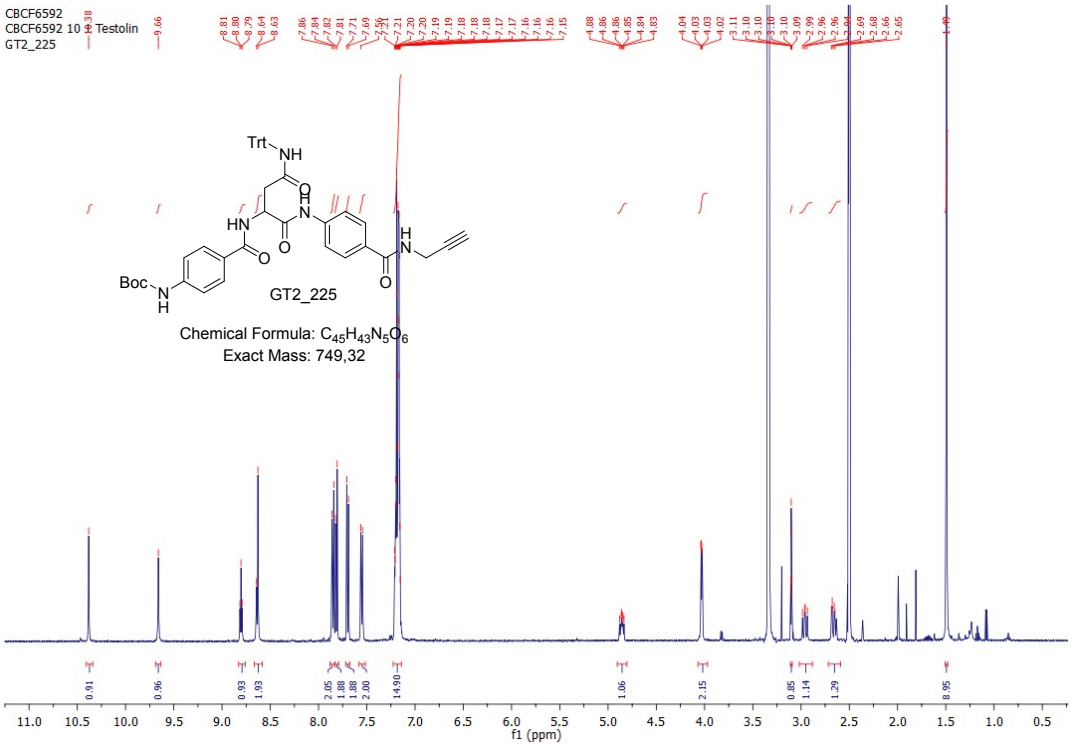
methyl (S)-4-(2-(4-((tert-butoxycarbonyl)amino)benzamido)-4-oxo-4-(tritylamino)butanamido)benzoate

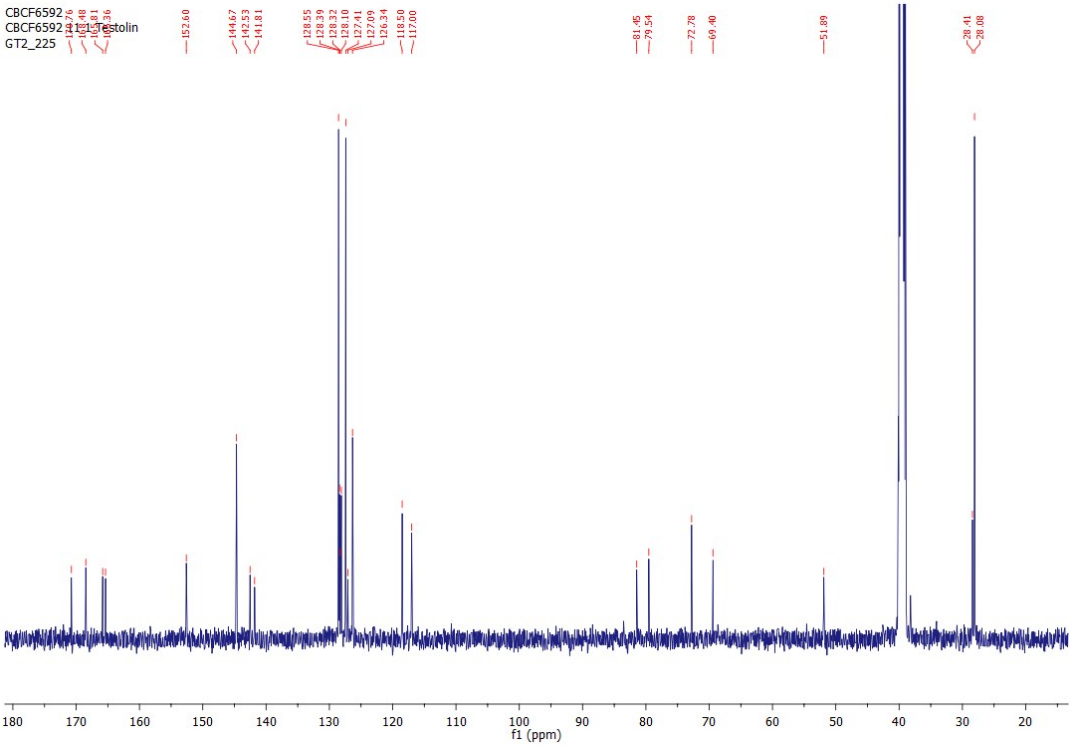


GT2_205

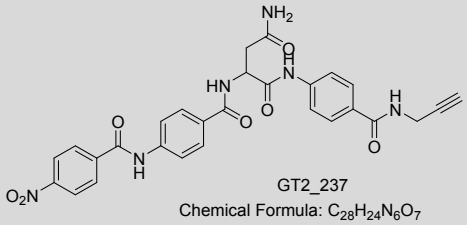
Chemical Formula: $C_{43}H_{42}N_4O_7$
Exact Mass: 726,31

tert-butyl (4-((1,4-dioxo-1-((4-(prop-2-yn-1-ylcarbamoyl)phenyl)amino)-4-(tritylamino)butan-2-yl)carbamoyl)phenyl)carbamate



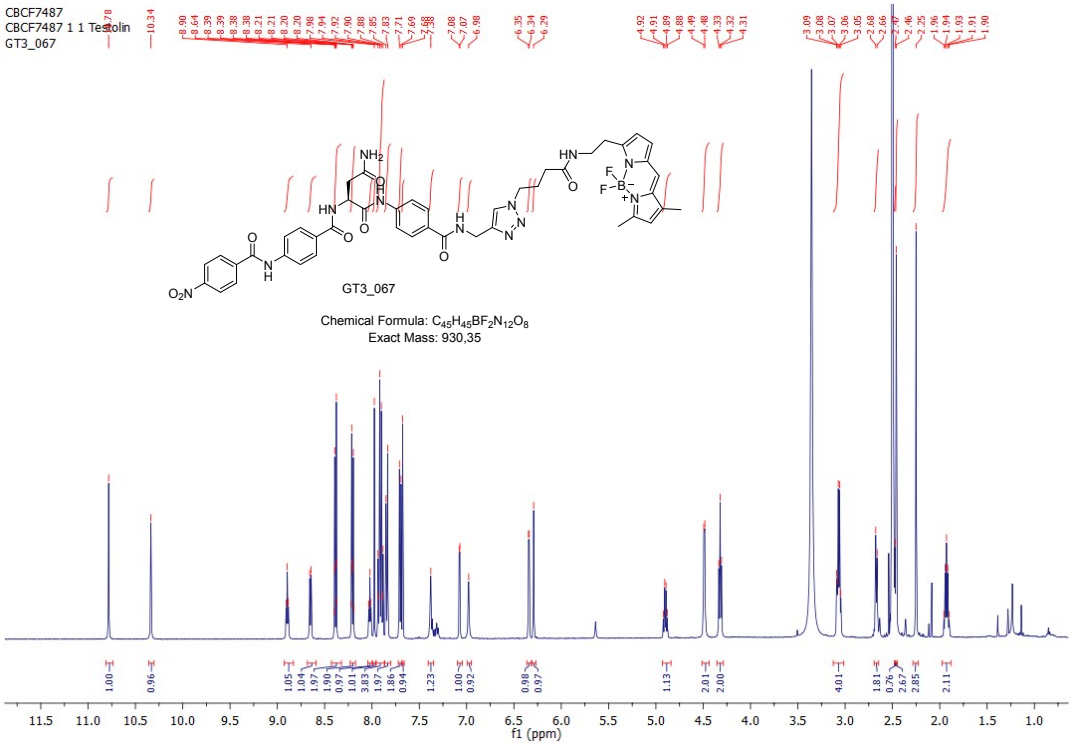


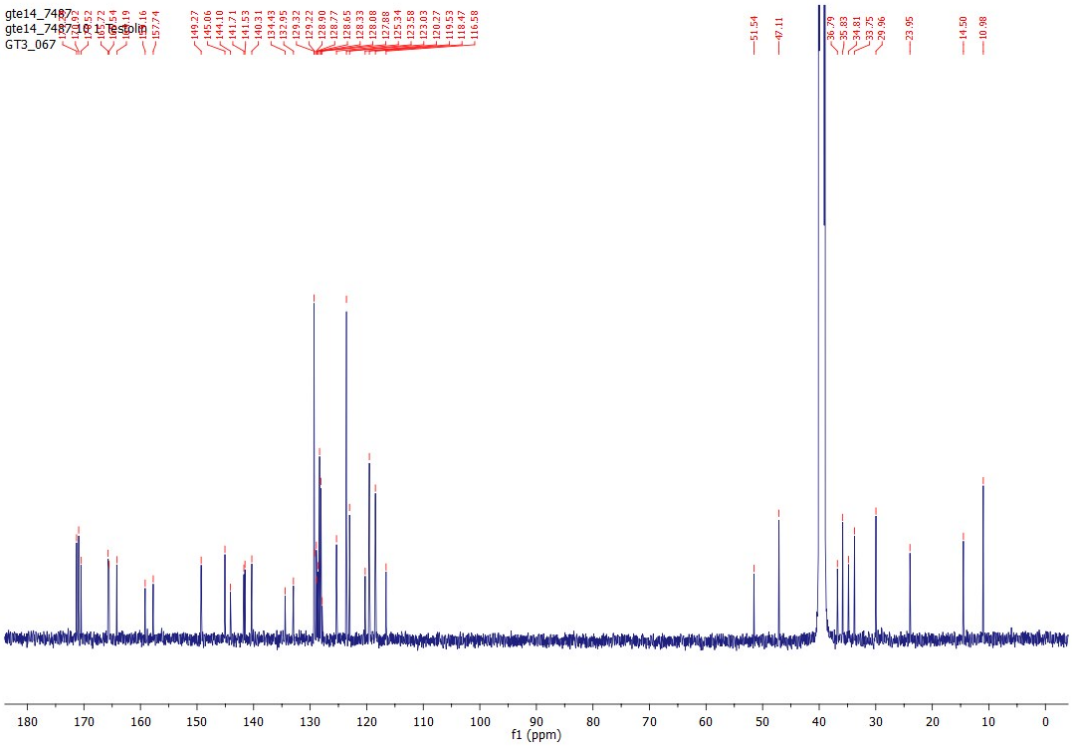
2-(4-(4-nitrobenzamido)benzamido)-N1-(4-(prop-2-yn-1-ylcarbamoyl)phenyl)succinamide



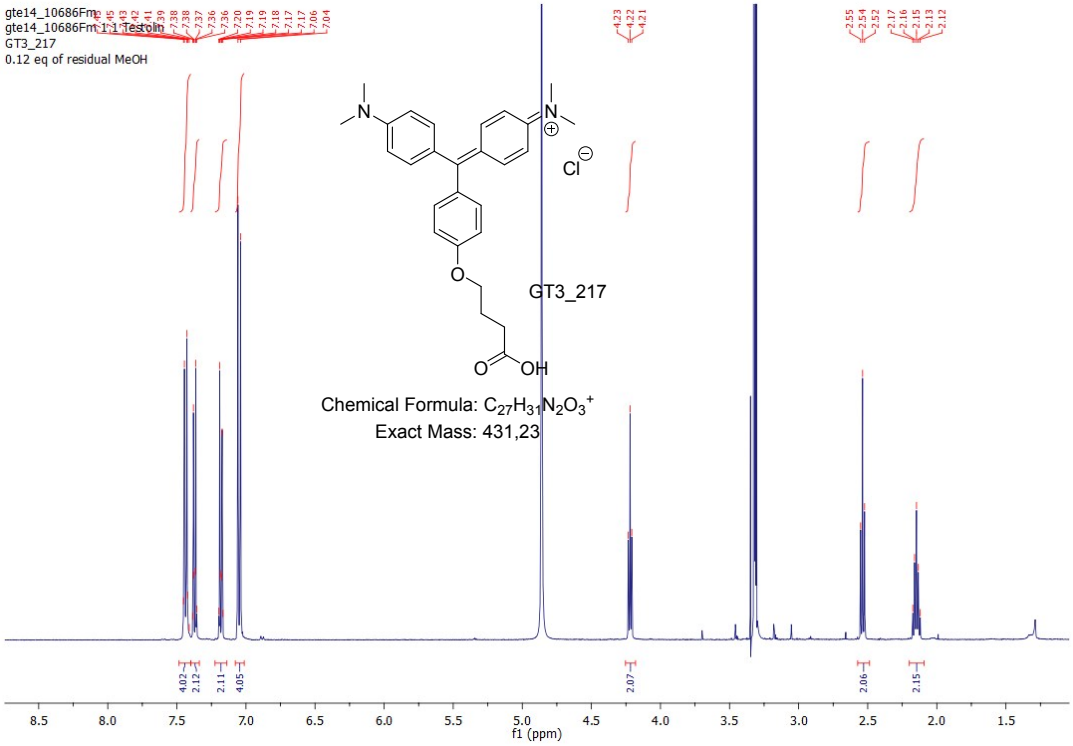
GT2_237
Chemical Formula: $C_{28}H_{24}N_6O_7$
Exact Mass: 556,17

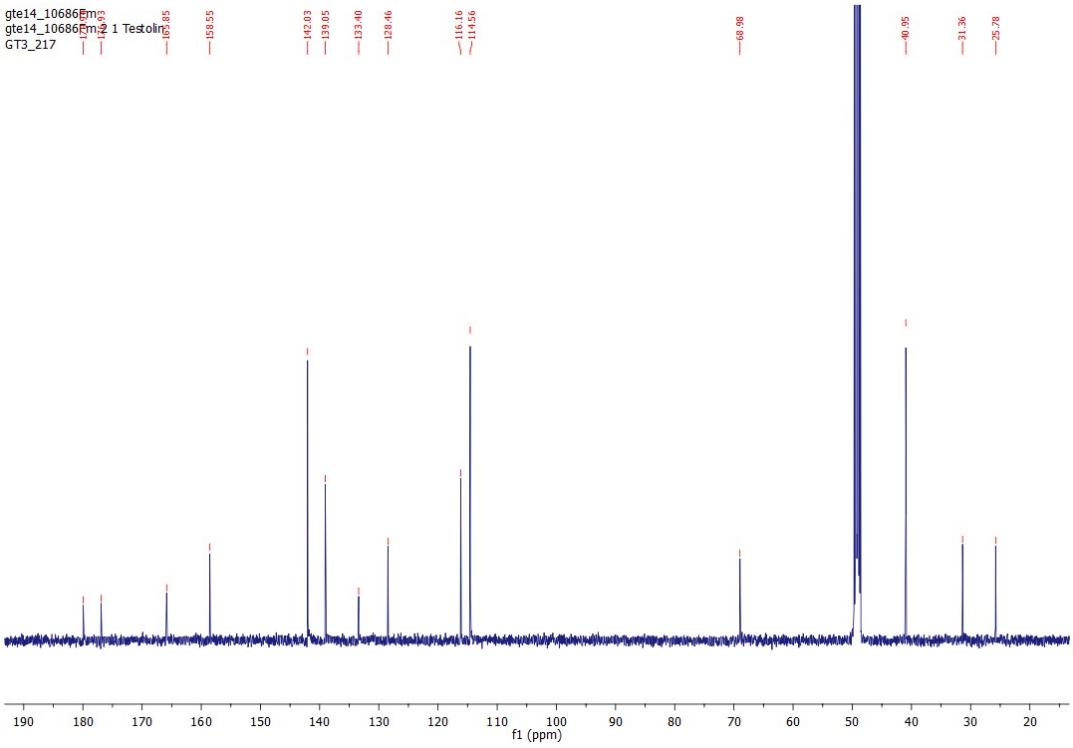
(S)-N1-(4-(((1-(4-((2-(5,5-difluoro-7,9-dimethyl-5H-5l4,6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)ethyl)amino)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)phenyl)-2-(4-(4-nitrobenzamido)benzamide



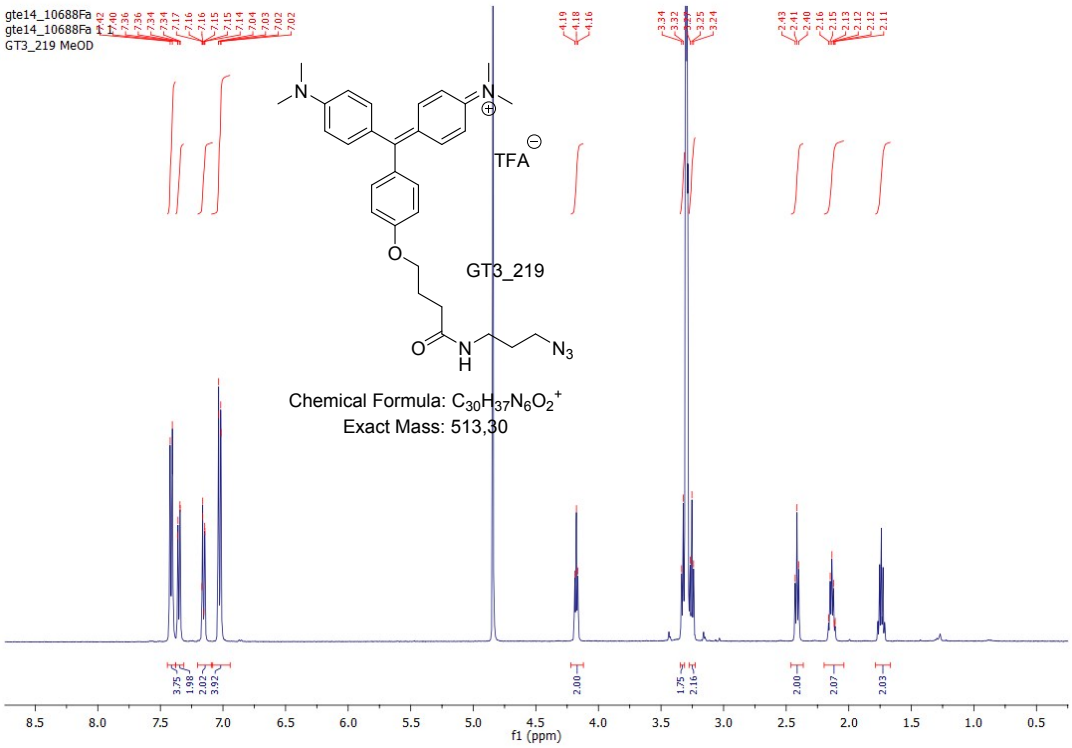


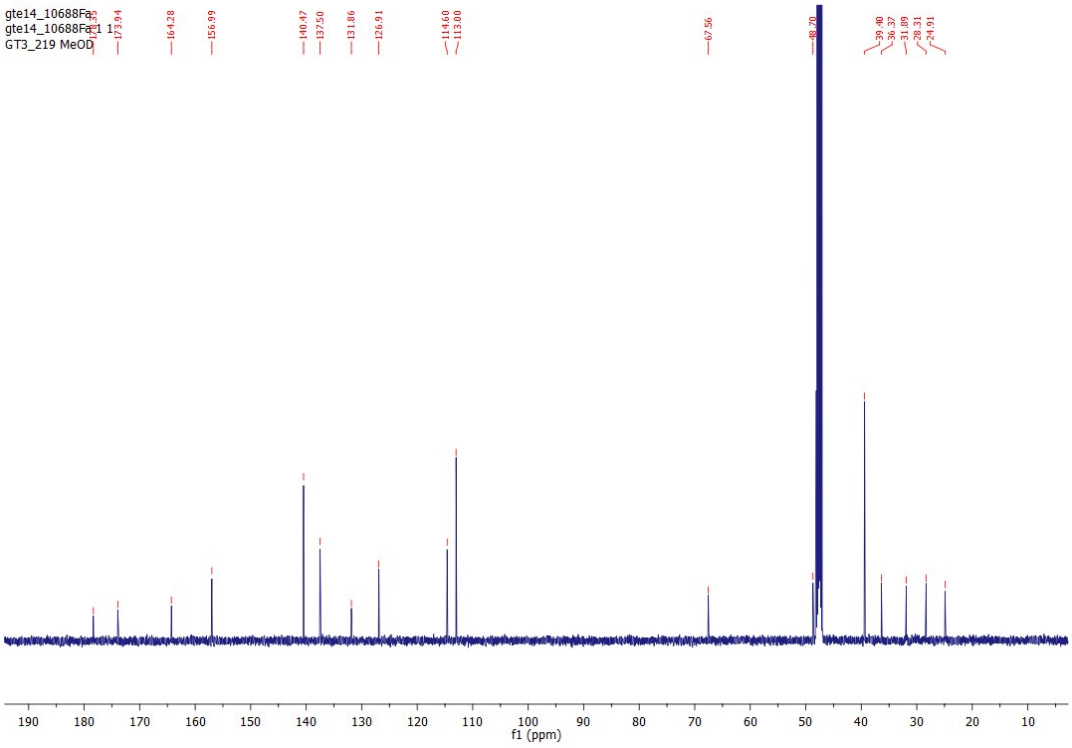
N-(4-((4-(3-carboxypropoxy)phenyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium



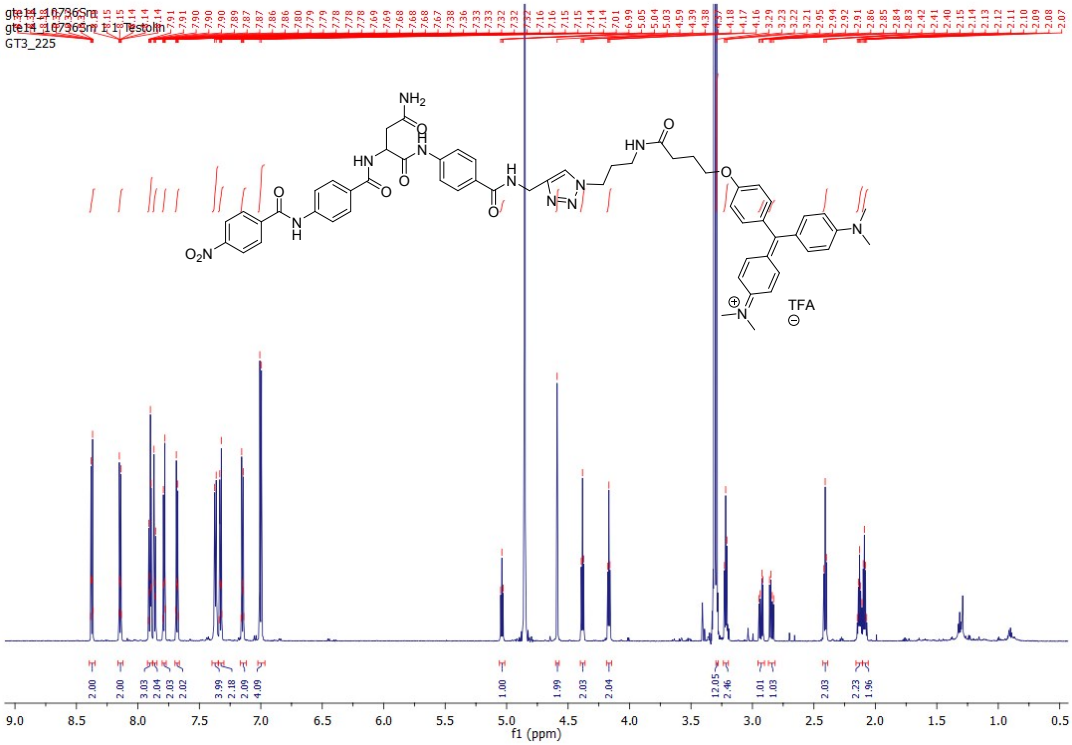


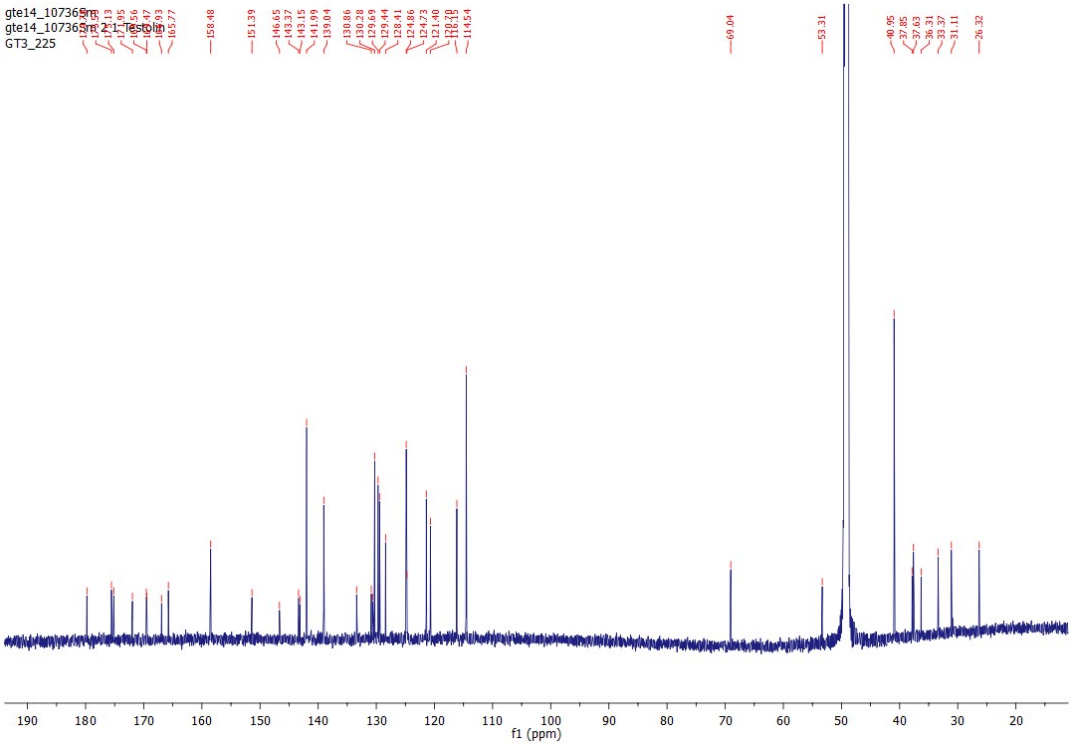
N-(4-((4-(4-((3-azidopropyl)amino)-4-oxobutoxy)phenyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium trifluoroacetate





N-(4-((4-(4-((3-(4-(((4-(4-amino-2-(4-(4-nitrobenzamido)benzamido)-4-oxobutanamido)benzamido)methyl)-1H-1,2,3-triazol-1-yl)propyl)amino)-4-oxobutoxy)phenyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)-N-methylmethanaminium trifluoroacetate





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