Supporting Information

Sensitive Quantification of Short-Chain Fatty Acids Combined with Global Metabolomics in Microbiome Cultures

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1. General

All non-aqueous reactions were performed using flame- or oven dried glassware under an atmosphere of dry nitrogen. All reagents and solvents were purchased from Sigma-Aldrich or Fischer Scientific and were used without further purification. Mass spectrometry grade solvents were used for UHPLC-ESI-MS analysis. Solutions were concentrated in vacuo on a Heidolph or a IKA rotary evaporator. Thin Layer Chromatography (TLC) was performed on silica gel 60 F-254 plates. Chromatographic purification of products was accomplished using flash column chromatography on Merck silica gel 60 (40−63 μm. All synthesized compounds were ≥95% pure as determined by NMR. NMR spectra were recorded on a Bruker 600 MHz spectrometer (^{1}H NMR: 600.18 MHz, ^{13}C NMR: 150.92 MHz) or Agilent 400 MHz spectrometer (^{1}H NMR: 399.97 MHz, ^{13}C NMR: 100.58 MHz). Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Glass vials used for handling magnetic beads were microwave vials from Biotage (0.2-0.5 mL or 0.5-2.0 mL).

2. Description of procedures

2.1 UPLC-MS analysis.

The UPLC-MS analysis was performed in a Maxis II ETD Q-TOF mass spectrometer using an electrospray ionization (ESI) source with an Elite UHPLC system and equipped with a Waters ACQUITY UPLCVR HSS T3 column (1.8 mm, 100 2.1 mm). Milli-Q water with 0.1% formic acid was used as mobile phase A and LCMS grade methanol with 0.1% formic acid was used as mobile phase B. The column temperature was kept at 40 °C, and the autosampler was kept at 4 °C. The flow rate was set to 0.22 mL/min. The gradient used was as follows: 0–2 min, 0% B; 2–15 min, 0–100% B; 15–16 min, 100% B; 16–17 min, 100-0% B; 17–23 min, 0% B. The system was controlled using the Compass HyStar software package from Bruker. High-resolution mass spectra were acquired in positive mode at a mass range of m/z 50–1200. The samples were introduced into the q-TOF using positive electrospray ionization. A solution of sodium formate (0.5 mM in 2-propanol: water, 90:10, v/v) was used to calibrate the instrument. Data acquisition was performed in MSE mode. The samples were injected to the UPLC-MS system in a randomized order with QC samples injected in the beginning and end of the sample list in both ionization modes, as well as after every eight samples (7 QCs in each ionization mode in total).

2.2 Preparation of magnetic bead-bound, SCFAs quantification probe 1.

MagnaBind Amine Derivatized Beads slurry (50 µL, Thermo Scientific™) was transferred into a 1.5 mL Eppendorf tube. Original solution from supplier was taken out by magnetic separation. The beads were washed with THF (2×150 µL) followed by phosphate buffer (2×150 µL, 25 nM, pH 7.5). DMF (150 µL) was added to the Eppendorf followed by 5 µL DIPEA and then vortexed for at least 30 s to yield the unprotonated amine. The beads were washed with DMF (150 µL) followed by DCM (150 µL). An amide coupling solution (4.5 mM PyBop, 3.3 mM HOBT, 1% DIPEA v/v in DCM) and probe solution (3 mM in DMF) were freshly prepared in separate. The probe solution (100 µL) and amide coupling solution (100 µL) were combined into the Eppendorf tube containing magnetic beads. The mixture was shaken and incubated using a Thermomixer (1,500 rpm, 25 ℃, overnight.). The solution was removed and the beads consecutively washed with 2×150 µL THF and 2×150 µL DCM. After removal of all the solution, DCM (190 µL) and TFA (10 µL) were added in sequence to the Eppendorf for Boc deprotection. The mixture was shaken and incubated with a Thermomixer (1,500 rpm, 25 ℃, 5 h). The reaction mixture was removed and followed by washing with THF (2×150 µL). DCM (150 µL) and DIPEA (10 µL) were added in sequence to the Eppendorf for amine deprotonation and TFA neutralization. The beads were washed with DMF (2×150 µL). The beads were suspended in the DMF (100 µL), ready to be used for sample treatment.

2.3 Bacteria cultures.

Anaerobic Cultivated Human Intestinal Microflora system (ACHIM) culture was originally obtained from the fresh feces of a healthy Scandinavian donor on an ordinary Western diet. The culture has been re-cultivated every week in anaerobic conditions as described previously[1] (Patent Number: WO 2013/053836A). This ACHIM culture is referred to as "healthy gut microbiota" in this paper and the 1:10 dilution of the ACHIM culture is represented here as "weak gut microbiota". *Salmonella typhimurium* SL1344 at 107 or 106 colony forming units (CFU) were co-cultured with the "Healthy" and "Weak" gut microbiota in vitro, respectively. Further antibiotic treatment with 50 µg/mL of Spectinomycin was added to both conditions to evaluate the influence of the antibiotic treatment on the interaction between the gut microbiota and *Salmonella*. "Healthy" and "Weak" gut microbiota without *Salmonella* nor antibiotic were used as negative control for the analysis. *Salmonella* culture without gut microbiota culture is considered the positive control for the CFU comparison.

All tubes were grown anaerobically in a 9 mL culture system. On day 1 and day 5 after co-culture, *Salmonella* and gut microbial numbers were counted by series dilution and plated on CHROMagar *Salmonella* agar plates (CHROMagar, Paris France) and modified YCFA plates (recommended from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) supplemented with 2 g/L maltose and 2 g/L cellobiose, all reagents from Sigma-Aldrich, Germany), and grown aerobically and anaerobically, respectively as published before.[2]

In addition, on day 1 and day 5, 2 mL supernatant without bacteria from all the conditions was collected after filtering the culture mix through the 0.2 μm PES-membrane filter (Whatman Uniflo, Cytva UK). Afterwards, the supernatant was mixed with 8 ml ice-cold methanol (Sigma-Aldrich, Germany) and kept at -80°C until further *quant*-SCHEMA analysis.

2.4 Preparation of bacterial metabolite extracts.

10 μL bacterial extract (2 μL bacterial sample + 8 μL MeOH) was direct used in the bead treatment.

2.5 Treatment of bacterial metabolite extracts.

A suspension of activated beads **1** in DMF (100 μL) was used to treat the bacterial extract. A capture solution (4.8 mM HBTU, 3.6 mM HOBT, 1% DIPEA v/v in DCM) was freshly prepared in separate and 100 μL of this solution was added into Eppendorf for SCFAs capture before it was shaken for 16 h at 1,500 rpm and 25 ℃. The remaining bacterial extract solution was removed from the beads and the beads were washed with THF (2 x 200 μL) before being resuspended in THF (300 μL).

2.6 Cleavage of the bead-bound chemical probe 13.

The suspension of beads was transferred to a glass vial. Triphenylphosphine (97.0 μL, 12.9 mM in THF, 1.25 μmol) and dimethylbarbituric acid (90.0 μL, 30.7 mM in THF, 2.76 μmol) solutions were added to the vial, followed by palladium (II) acetate solution (84.0 μL, 6.53 mM in THF, 549 nmol). Then ^{13}**C$_6$-3** mixture (depend on SCFAs concentration in the samples) was spiked into the beads solution as internal standard. The vial was quickly sealed and a stream of nitrogen was passed through until approximately half the volume of the suspension remained. The vial was agitated at intervals on a vortexer and the reaction was allowed to continue 16 h at 25 ℃. In parallel, a sample of unmodified beads was treated with the same cleavage conditions as the activated beads treated with human sample extract and used as control sample.

The supernatant was removed from the beads using magnetic separation and the solvent removed using a vacuum centrifuge. The residues were redissolved in MeOH (30 µL each) and triphenylphosphine and triphenylphosphine oxide were precipitated through the addition of water (120 µL each). The suspension was centrifuged (benchtop centrifuge, 12,000 g, 5 min), the supernatant removed, and the solvent was again removed with the vacuum centrifuge. The residues were redissolved in water/acetonitrile solution (95:5 v/v) and submitted for LC-MS analysis.

2.7 LOQ measurement.

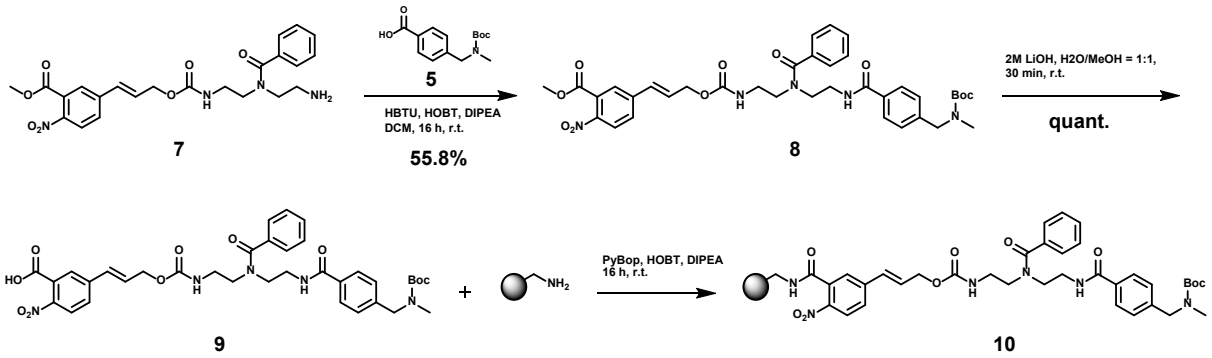
Synthetic conjugated SCFAs **3a-f** was prepared in a solution of water and acetonitrile (95:5 v/v) at a range of concentrations (1 µM, 100 nM, 50 nM, 10 nM, 1 nM, 0.5 nM, 0.1 nM) before being submitted for UPLC-MS analysis. The measurement was based on the conventional concepts that signal to noise ratios at least higher than 10 corresponding to Limit of Quantification (LOQ), respectively. The signal to noise ratios were calculated according to European Pharmacopoeia guidelines by using DataAnalysis 5.0 (Bruker).

2.8 Global metabolomics analysis.

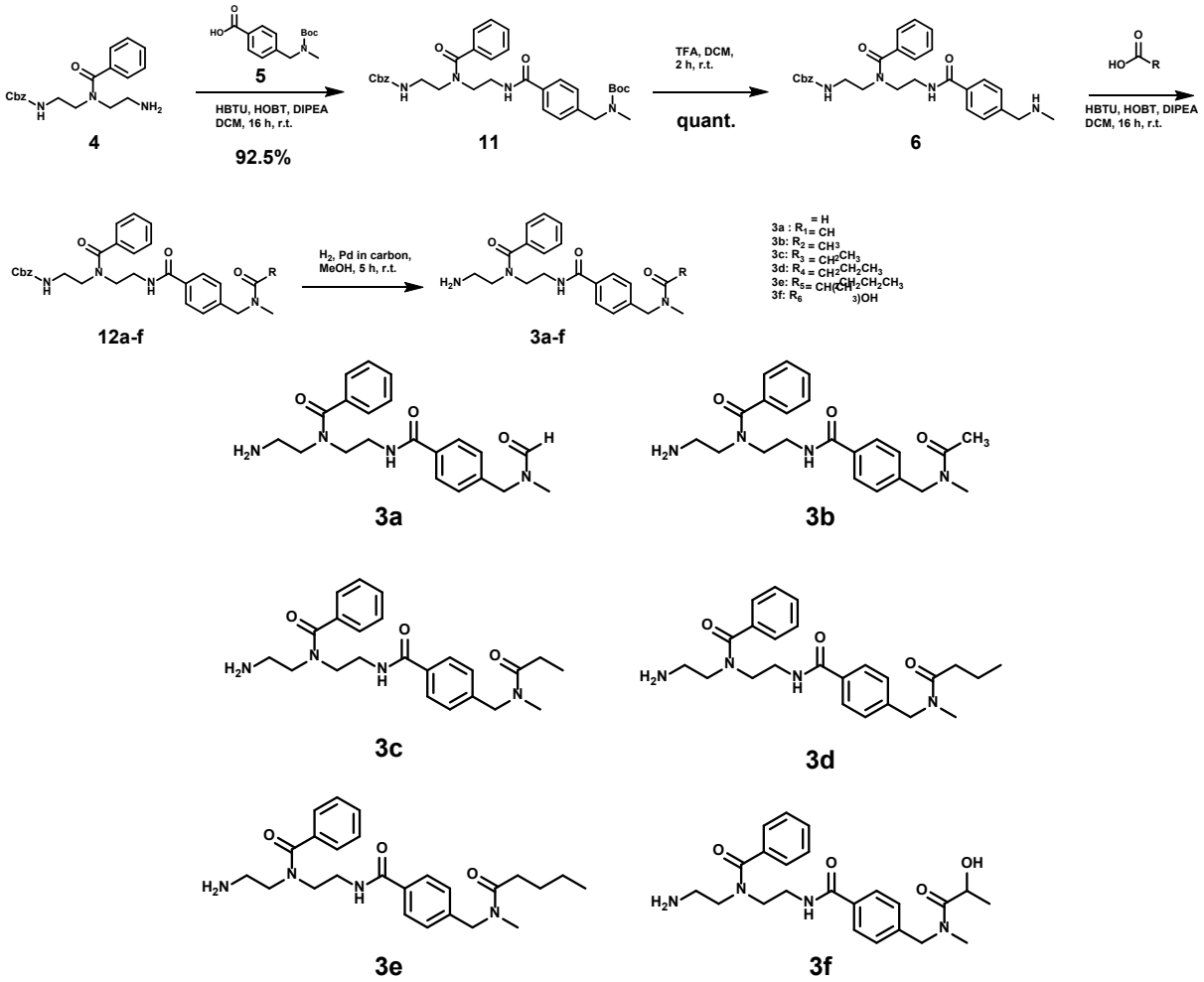
490 µL bacterial extract (98 µL bacterial sample + 392 µL MeOH) was transferred into an empty Eppendorf tube. As an internal standard (I.S.), a mixture of C-13 isotopically labelled tyrosine (5 mg/mL), phenylalanine (10 mg/mL) and valine (30 mg/mL) was used. The mixture was dried under vacuum on a Speedvac and re-suspended with 50 µL water:acetonitrile (95:5) prior to UPLC-MS/MS analysis. Quality control (QC) samples were prepared by 3 µL aliquots from all samples.

The chromatograms and mass spectra were processed using the XCMS R package (version 1.4.414) for peak alignment and retention time correction in both positive and negative ionization mode. From the corresponding feature lists obtained from the software, features with intensities > 30,000 ion count, rt > 1.5 min and %CV of the QCs < 30 were selected for further statistical analysis as considerably higher than noise. An overview of the data was provided by principal component analysis (PCA) and heatmap, prior to which the data was autoscaled using the metabolomics platform Metaboanalyst. The normality of the test statistics and p values were evaluated using the same platform, and the data were distributed normally.

3. Supporting Schemes

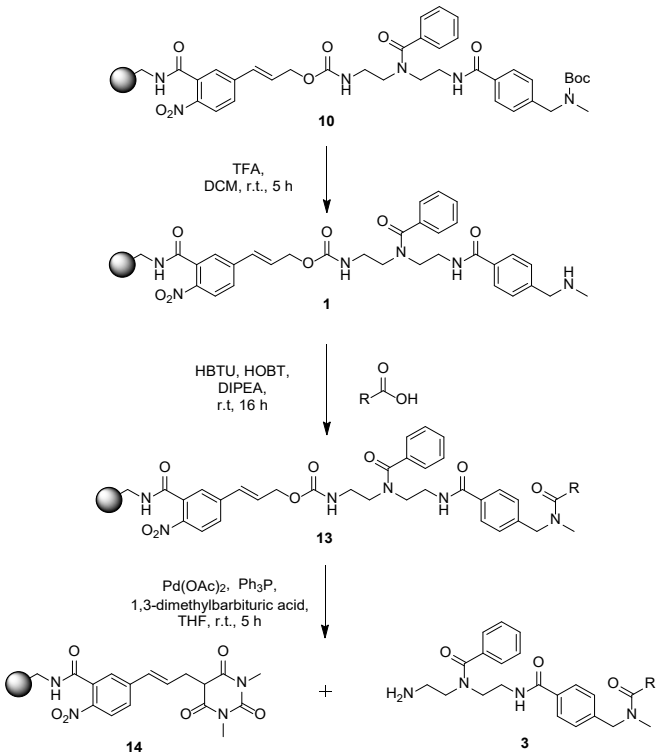


Scheme S1. Synthesis of the SCFAs full-length probe **10**



Scheme S2. Synthesis of the SCFAs conjugates **3a-f**.

Note: Phenyl-$^{13}C_6$ labelled compounds have been synthesized following the same synthetic route and marked as **3a***, **3b***, **3c***, **3d***, **3e*, and 3f***.



Scheme S3. Chemistry for SCFAs capture with the chemoselective probe immobilized to magnetic beads.

4. Supporting Figures

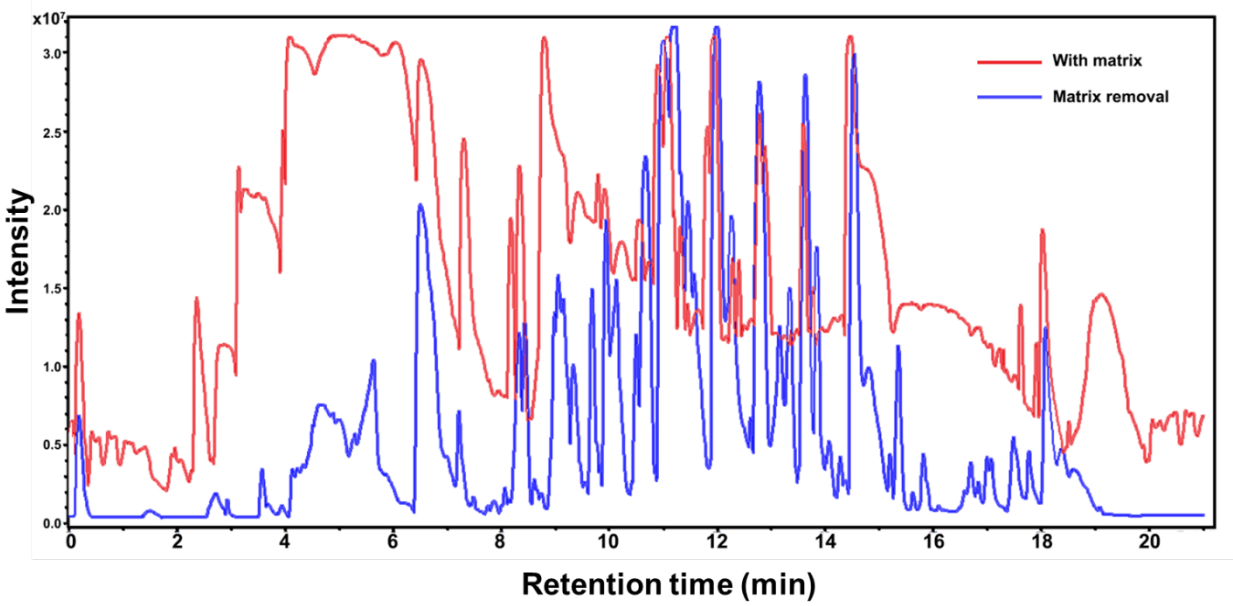


Figure S1. Comparison of TIC within sample matrix and without sample matrix.

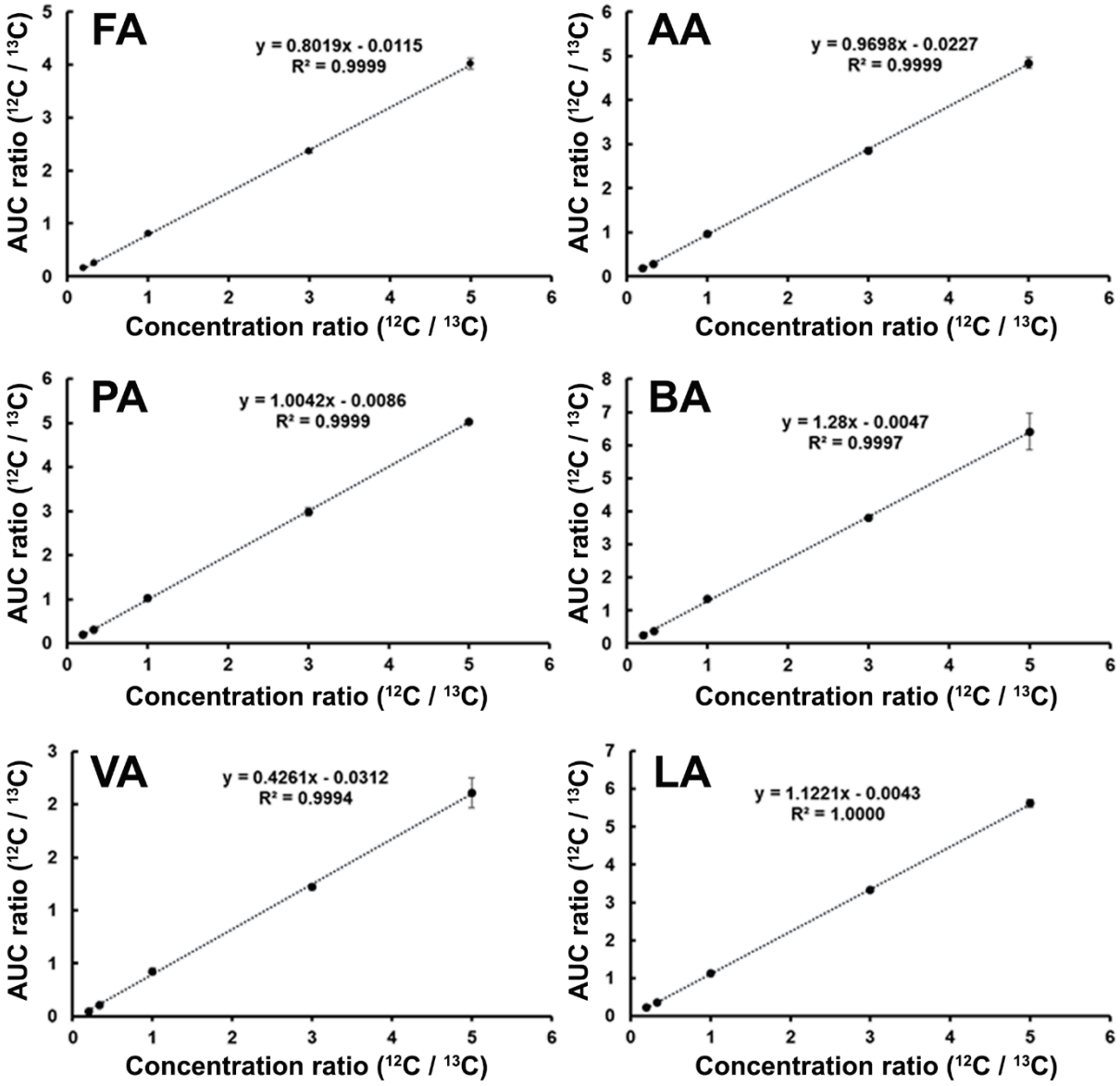


Figure S2. Calibration curve for formic acid (FA), acetic acid (AA), propanoic acid (PA), butyric acid (BA), valeric acid (VA), and lactic acid (LA) conjugates. Data are presented as mean ± SD from experimental replicates (N = 3).

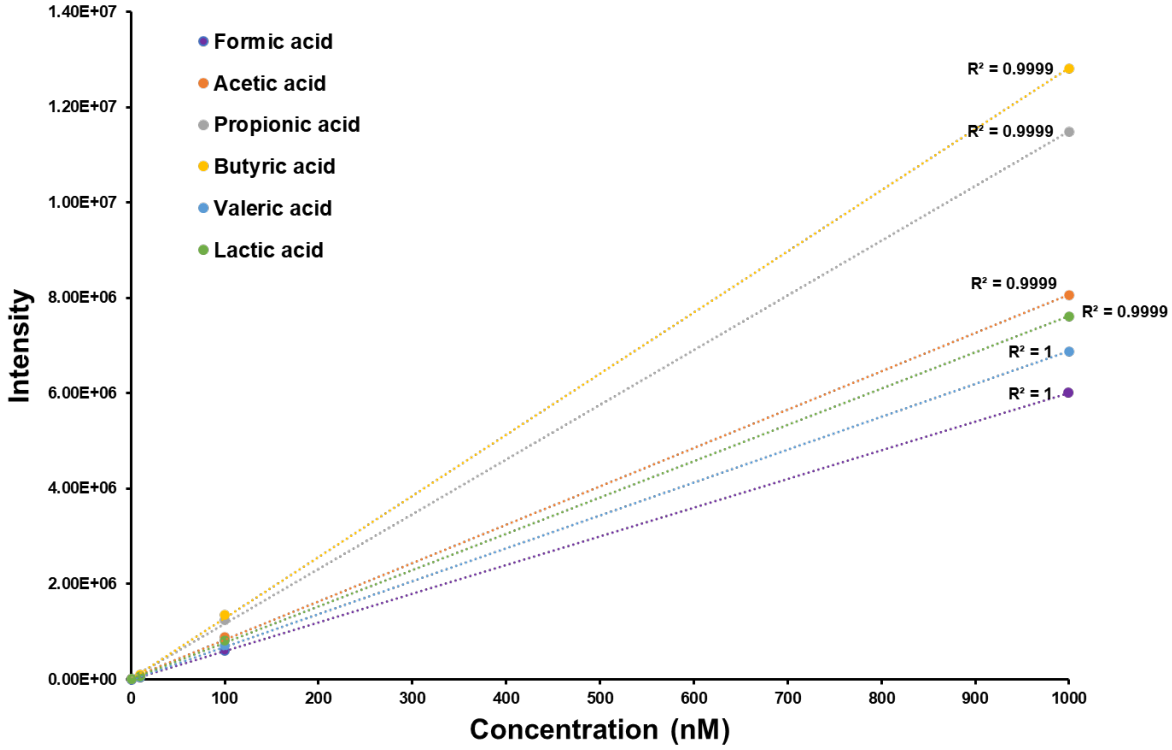


Figure S3. The linear concentration ranges from 1 nM to 1000 nM.

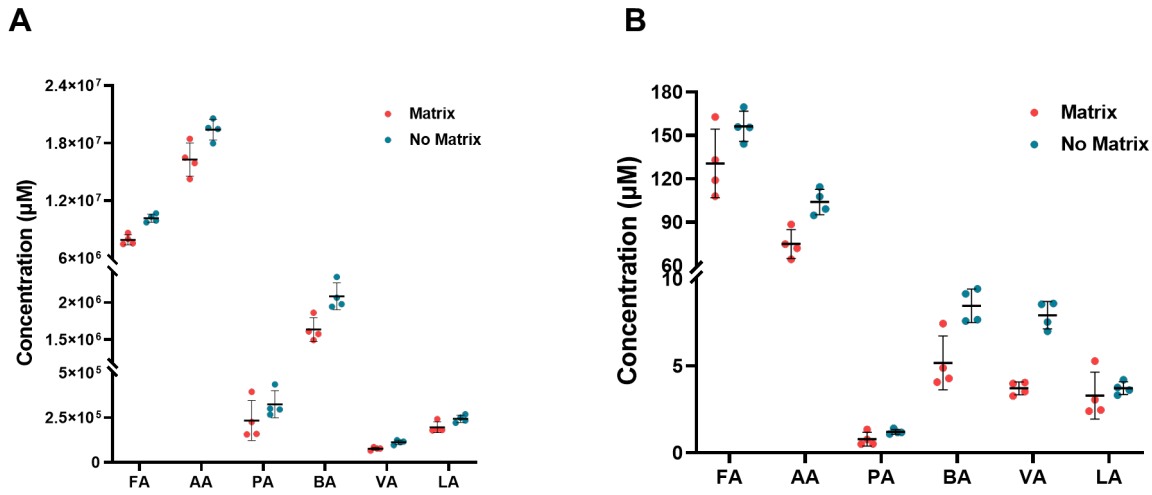


Figure S4. Comparison results of the impact of sample matrix. A) Area under curve, B) quantification results (N=4).

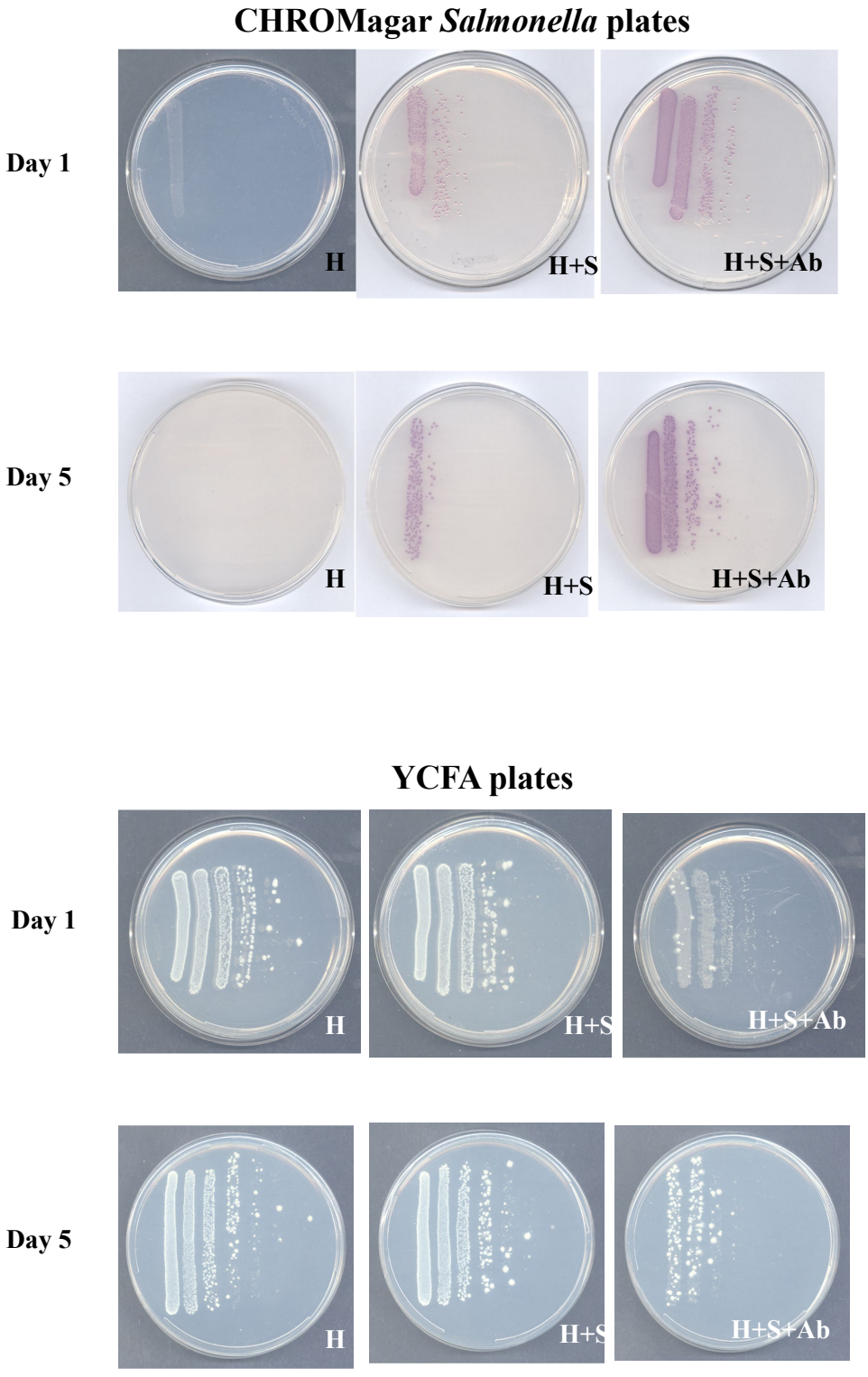


Figure S5. *Salmonella* colony forming units (CFU).

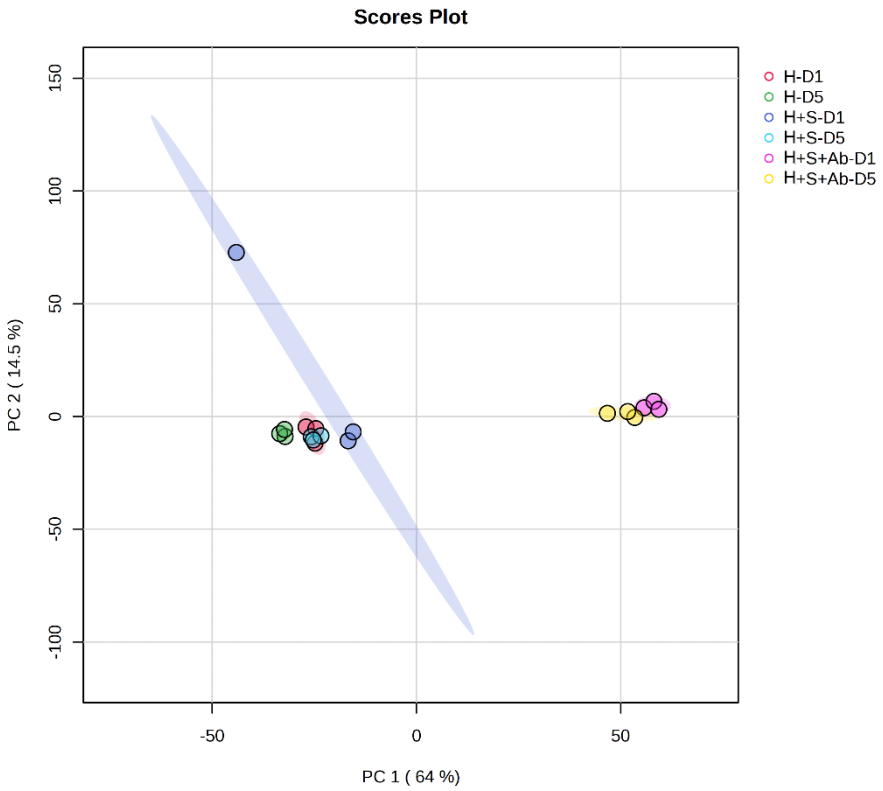
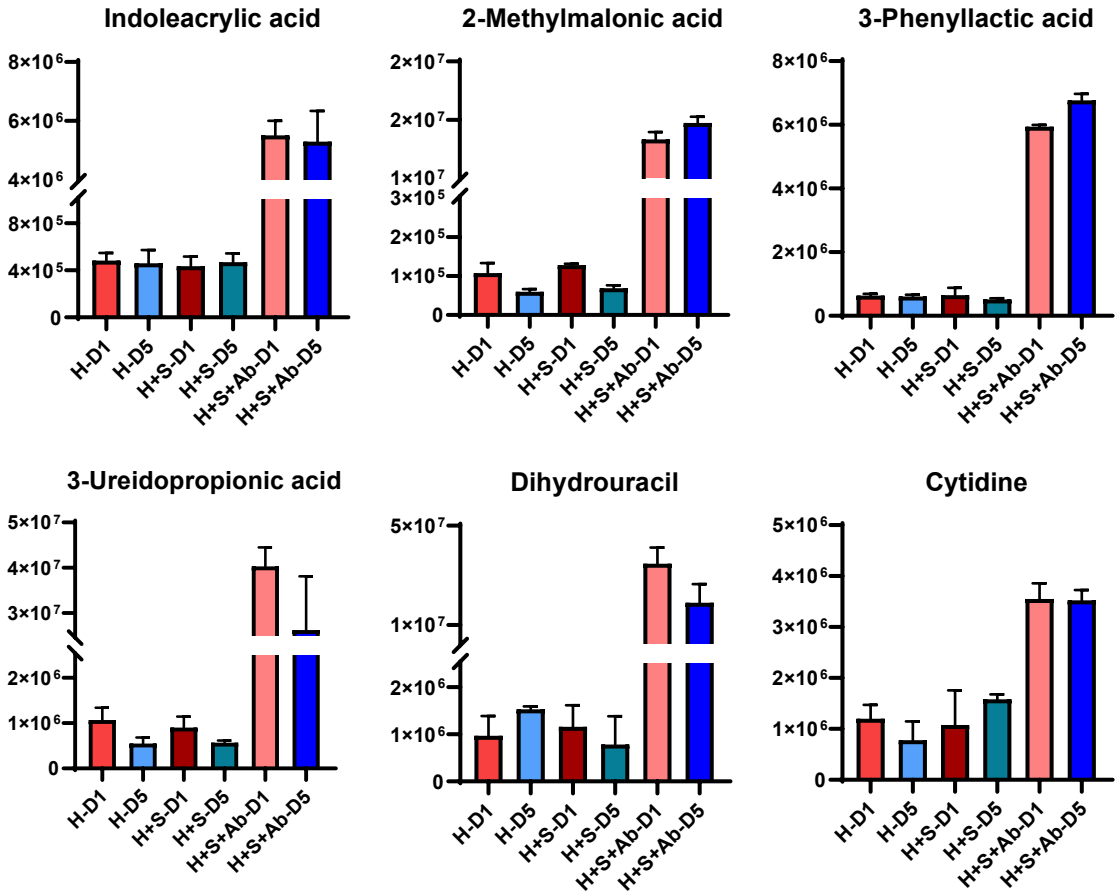


Figure S6. Multivariate analysis (PCA).



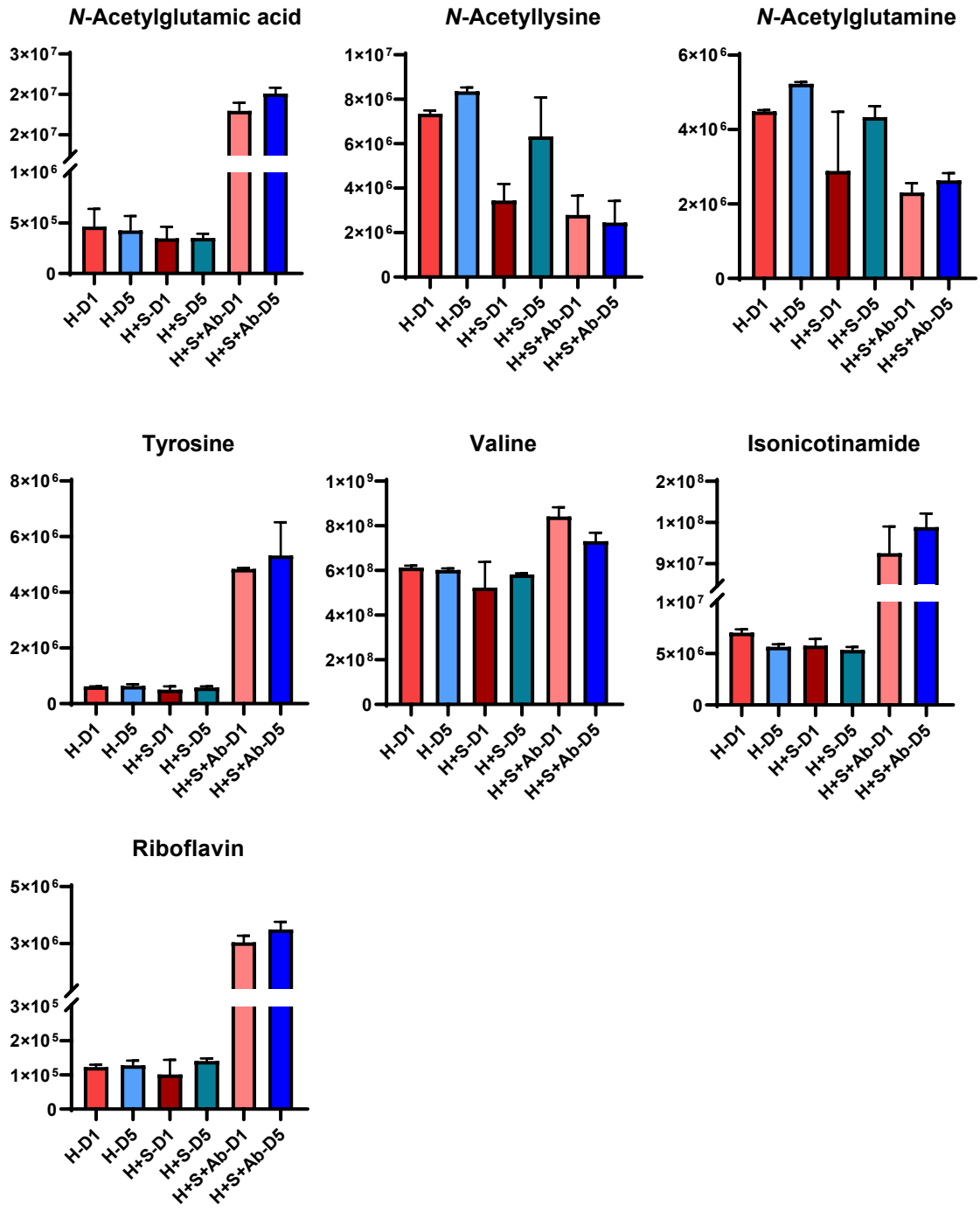


Figure S7. Boxplots of 13 validated metabolites. N = 3, error bars are standard deviation.

5. Supporting Tables

Table S1. LOD/LOQ experiment results in 1 μM, 100 nM, 10 nM and 1 nM. S/N = signal to noise ratio.

S/N	FA	AA	PA	BA	VA	LA
1 μM	1260.8	3497	7006.5	7269.4	3263.9	1963.6
100 nM	297.2	487.8	1840.4	1575.6	2082.4	155.7
10 nM	16	63	259.5	124.8	250	30
1 nM	ND	5.7	19.2	10.1	27.5	5.8
0.5 nM	ND	ND	ND	ND	ND	ND

Note: Formic acid (FA), acetic acid (AA), propanoic acid (PA), butyric acid (BA), valeric acid (VA), and lactic acid (LA)

Table S2. LOD/LOQ experiment results from calibration curve and linearity.

SCFAs	Linear Range(nM)	R^2	LOD(nM)	LOQ(nM)
Formic acid	**1-1000**	**1**	**3.36**	**10.19**
Acetic acid	**1-1000**	**0.9999**	**13.87**	**42.04**
Propionic acid	**1-1000**	**0.9999**	**12.59**	**38.17**
Butyric acid	**1-1000**	**0.9999**	**9.00**	**27.26**
Valeric acid	**1-1000**	**1**	**6.32**	**19.17**
Lactic acid	**1-1000**	**0.9999**	**9.53**	**28.88**

Table S3 LOD/LOQ Comparison with other studies.[3, 4]

SCFAs	This study		Other study from Borchers Lab		Other study from Molloy Lab	
	LOD (nM)	LOQ (nM)	LOD (nM)	LOQ (nM)	LOD (nM)	LOQ (nM)
Formic acid	3.36	10.19	-	-	-	-
Acetic acid	13.87	42.04	5.00	19.50	0.85	2.74
Propionic acid	12.59	38.17	1.20	4.90	0.36	1.30
Butyric acid	9.00	27.26	0.08	0.31	0.28	0.91
Valeric acid	6.32	19.17	0.02	0.06	0.31	1.11
Lactic acid	9.53	28.88	-	-	-	-

Table S4. Comparison of matrix effect in detection using area under curve (AUC).

Matrix	FA	AA	PA	BA	VA	LA
Exp.1	8639695	18451720	395641	1862294	78089	242071
Exp.2	8003012	16492046	226079	1577423	66085	180811
Exp.3	7573860	15920651	157094	1611376	78229	184810
Exp.4	7480087	14270476	159437	1493546	85568	179342
Mean	7924164	16283723	234563	1636160	76993	196759
SD	457744	1494038	97035	137422	6986	26238
RSD	5.8%	9.2%	41.4%	8.4%	9.1%	13.3%
Average RSD	**14.5%**					

No Matrix	FA	AA	PA	BA	VA	LA
Exp.1	9746659	20581568	267394	2342578	96999	269245
Exp.2	10327008	19592296	301084	2064538	113020	250381
Exp.3	10687108	19462392	436994	1978676	124480	234328
Exp.4	9933801	17973286	295962	1943592	116728	221785
Mean	10173644	19402386	325359	2082346	112807	243935
SD	362976	931727	65719	156555	10020	17784
RSD	3.6%	4.8%	20.2%	7.5%	8.9%	7.3%
Average RSD	**8.7%**					
Matrix effect	77.9%	83.9%	72.1%	78.6%	68.3%	80.7%
Average	76.9%					

Table S5. Comparison of matrix effect in quantification.

Matrix	FA	AA	PA	BA	VA	LA	
Exp.1	162.71	88.44	1.35	7.44	3.53	5.28	**μM**
Exp.2	133.01	74.83	0.77	4.89	3.28	2.47	**μM**
Exp.3	107.89	71.93	0.50	4.29	4.05	3.06	**μM**
Exp.4	118.91	64.35	0.52	4.07	3.99	2.40	**μM**
Mean	130.63	74.89	0.78	5.17	3.71	3.30	**μM**
SD	20.55	8.71	0.35	1.34	0.32	1.17	
RSD	15.7%	11.6%	44.0%	26.0%	8.6%	35.4%	
Average RSD	**23.6%**						

No Matrix	FA	AA	PA	BA	VA	LA	
Exp.1	169.55	114.37	1.17	9.15	7.53	4.20	**μM**
Exp.2	155.81	107.60	1.18	9.44	8.60	3.76	**μM**
Exp.3	155.49	99.00	1.41	7.59	8.55	3.61	**μM**
Exp.4	144.11	94.72	1.08	7.67	7.00	3.32	**μM**
Mean	156.24	103.92	1.21	8.46	7.92	3.72	**μM**
SD	9.01	7.61	0.12	0.84	0.68	0.32	
RSD	5.8%	7.3%	10.1%	9.9%	8.6%	8.6%	
Average RSD	**8.4%**						
Matrix effect	83.6%	72.1%	64.5%	61.1%	46.8%	88.7%	
Average	69.5%						

Table S6. LC-MS output for quality control by measuring internal standards.

Ion count	Val	Phe	Tyr
QC-1	10698395	11655347	5980158
QC-2	12114594	17797059	7452672
QC-3	13564925	18100738	7540969
QC-4	13481020	17435237	8136903
QC-5	13313388	18726162	7545794
QC-6	14114205	18242556	8349312
QC-7	12486019	17898733	7632020
Mean	12824649	17122262	7519690
SD	1070411	2262374	702813.4
RSD	8.35%	13.21%	9.35%

Table S7. Top100 significant features from ANOVA analysis and their annotation.

Features	P.value	FDR	Annotation	*m/z*	RT(min)
M101T298	7.78E-17	9.73E-14	Succinic anhydride	101.0229	4.96
M119T298	1.64E-16	1.02E-13	4-Hydroxy-2-oxobutanoic acid	119.0336	4.97
M222T343	3.2E-15	6.66E-13	9-(Methylaminomethyl)anthracene	222.1281	5.72
M361T479	3.46E-15	6.66E-13		361.1723	7.98
M173T749	2.32E-14	3.62E-12	6-oxononanoic acid	173.1174	12.48
M436T406	3.95E-14	5.46E-12		436.1751	6.76
M141T166_1	6.28E-14	6.83E-12	5-Ethyluracil	141.0657	2.77
M123T167	5.69E-14	6.83E-12	Isonicotinamide	123.055	2.78
M101T132	5.78E-14	6.83E-12		101.1169	2.21
M231T245	6.07E-14	6.83E-12		230.9775	4.08
M361T412	8.5E-14	8.85E-12		361.1729	6.87
M291T299	1.59E-13	1.59E-11		290.98	4.98
M140T204	1.66E-13	1.6E-11	3-Nitrophenol	140.034	3.40
M471T593_1	2.23E-13	1.91E-11		471.256	9.88
M197T302	2.27E-13	1.91E-11		196.9201	5.03
M279T444	2.37E-13	1.91E-11		279.1553	7.40
M141T193	3.31E-13	2.44E-11	5-Ethyluracil	141.0657	3.22
M145T396_2	9.15E-13	6.53E-11	Triacetic acid	145.0494	6.60
M127T262	1.33E-12	8.73E-11		127.0364	4.37
M197T158	1.47E-12	9.39E-11		197.0535	2.64
M170T743	1.73E-12	1.05E-10	1-Naphthyl isocyanate	170.0602	12.38
M279T416	1.88E-12	1.12E-10		279.1557	6.93
M141T298	2.77E-12	1.61E-10		141.0156	4.96
M304T649	3.33E-12	1.85E-10	3-hydroxyoctanoyl carnitine	304.212	10.82
M134T196	3.33E-12	1.85E-10		134.0439	3.27
M148T156	4.03E-12	2.19E-10	D-Glutamic acid	148.0603	2.61
M213T757	5.28E-12	2.75E-10	2',3'-Dideoxyuridine	213.0873	12.61
M401T745	6.26E-12	2.91E-10	Sinapic acid glucuronide	401.1093	12.41
M230T252	6.29E-12	2.91E-10		229.9748	4.21
M437T406	7.17E-12	3.26E-10		437.1784	6.77
M456T369	7.43E-12	3.31E-10		456.2094	6.15
M490T380	7.61E-12	3.33E-10		490.1906	6.33
M537T622	8.37E-12	3.57E-10		537.1439	10.36
M197T278_1	8.79E-12	3.66E-10		196.9201	4.64
M279T412	1E-11	4.04E-10	Phenylalanylleucine	279.1693	6.86
M189T622	1.05E-11	4.16E-10	2-(Sec-butyldisulfanyl)-1h-imidazole	189.0523	10.37
M237T352	1.28E-11	4.77E-10		237.1345	5.87
M186T222	1.46E-11	5.34E-10		185.985	3.70
M189T763	1.47E-11	5.34E-10		189.0786	12.72
M490T562	1.62E-11	5.79E-10		490.2748	9.37
M157T123	1.65E-11	5.82E-10	1,5-Dimethylbarbituric acid	157.061	2.05
M143T358	1.85E-11	6.26E-10	Piracetam	143.0814	5.96
M158T159	1.88E-11	6.26E-10	Succinimidyl acetate	158.045	2.65

M234T265	2.03E-11	6.59E-10	Hydroxypropionylcarnitine	234.1338	4.41
M416T203	2.01E-11	6.59E-10		416.0392	3.38
M314T247	2.07E-11	6.64E-10		314.0151	4.12
M420T288	2.82E-11	8.7E-10		420.023	4.81
M252T397	3.06E-11	9.2E-10		252.1532	6.62
M140T221	3.47E-11	1.02E-09	3-Nitrophenol	140.034	3.69
M112T205	3.81E-11	1.1E-09	2,4-Dihydroxypyridine	112.0392	3.41
M238T396_1	3.79E-11	1.1E-09		238.08	6.60
M207T279	3.9E-11	1.1E-09		206.9157	4.64
M379T297	4.84E-11	1.31E-09	6-Methylthiopurine ribonucleoside	379.0456	4.96
M129T189	5.13E-11	1.36E-09	Dihydrothymine	129.0656	3.14
M190T297	5.36E-11	1.41E-09	Glutarylglycine	190.0712	4.95
M190T622	5.4E-11	1.41E-09	Thiotepa	190.0554	10.36
M401T746	5.95E-11	1.53E-09		401.1468	12.44
M393T487	6.05E-11	1.54E-09		393.2232	8.12
M205T327	7.57E-11	1.91E-09		204.9795	5.45
M235T124_2	8.05E-11	1.99E-09		235.1288	2.07
M418T746	8.45E-11	2.07E-09		418.1165	12.44
M148T260	8.82E-11	2.14E-09	Daunosamine	148.0968	4.34
M130T120_1	9.23E-11	2.21E-09	4-Oxoproline	130.0497	2.00
M144T794	9.38E-11	2.21E-09	Quinaldine	144.0806	13.24
M228T746	9.44E-11	2.21E-09		228.042	12.43
M87T259	9.5E-11	2.21E-09		87.04358	4.32
M429T394_2	9.53E-11	2.21E-09		428.6937	6.56
M166T436	9.87E-11	2.26E-09	Phenyl-Alanine	166.0864	7.26
M534T649_1	1.01E-10	2.3E-09		534.3109	10.82
M235T351	1.06E-10	2.39E-09		235.1415	5.85
M277T530	1.12E-10	2.5E-09		277.1543	8.84
M295T370_1	1.18E-10	2.62E-09		294.6531	6.17
M503T324	1.21E-10	2.64E-09		503.2101	5.39
M127T326	1.25E-10	2.72E-09		127.0365	5.44
M364T388	1.36E-10	2.91E-09	Pyroglutamyl-histidyl-proline	364.1627	6.47
M182T328	1.48E-10	3.13E-09	D-Tyrosine	182.0813	5.47
M329T159	1.51E-10	3.18E-09		329.157	2.65
M251T398_2	1.53E-10	3.18E-09		251.1504	6.63
M250T398	1.62E-10	3.35E-09		250.1656	6.64
M488T502	1.65E-10	3.37E-09	Ac-Ser-Asp-Lys-Pro-OH	488.2356	8.36
M229T746_1	1.66E-10	3.37E-09	Aminoethylcysteine ketimine	229.0453	12.43
M490T580	1.69E-10	3.39E-09		490.2754	9.67
M188T745	1.73E-10	3.41E-09	Indoleacrylic acid	188.0708	12.41
M95T167	1.78E-10	3.48E-09	2-Aminopyridine	95.06002	2.79
M177T199	1.83E-10	3.55E-09	Alanylserine	177.0872	3.32
M377T608	1.84E-10	3.55E-09	Riboflavin	377.146	10.13
M235T124_1	2.03E-10	3.84E-09	Glutamylserine	235.0927	2.06
M275T746	2.14E-10	3.97E-09		275.0246	12.43
M359T344	2.14E-10	3.97E-09		359.1764	5.73

M530T590	2.18E-10	4E-09	Haloperidol decanoate	530.2823	9.83
M351T473	2.43E-10	4.43E-09		351.1766	7.88
M133T162	2.63E-10	4.58E-09	DL-Asparagine	133.0607	2.69
M208T671	2.64E-10	4.58E-09	Phenyl-Leucine	208.1332	11.18
M167T622	2.59E-10	4.58E-09	Tropate	167.0703	10.37
M670T520	2.58E-10	4.58E-09		670.3047	8.66
M213T746	2.64E-10	4.58E-09		213.0717	12.43
M315T302	2.78E-10	4.79E-09	7-Methoxy tropisetron	315.1702	5.03
M212T763	2.86E-10	4.9E-09		212.0684	12.71
M645T410	3.03E-10	5.05E-09		645.2481	6.83

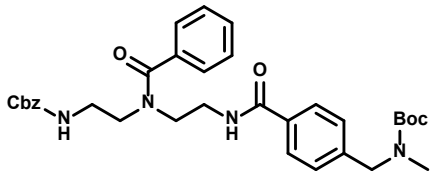
Table S8. Quantification results of an identical bacteria sample six times.

Conc.	**FA**	**AA**	**PA**	**BA**	**VA**	**LA**	
Rep.1	61.82	169.84	5.10	76.60	5.74	3.21	**μM**
Rep.2	74.65	205.62	6.16	55.44	4.72	3.12	**μM**
Rep.3	79.35	270.71	5.17	61.10	4.15	3.17	**μM**
Rep.4	63.29	211.88	5.30	46.69	4.54	3.30	**μM**
Rep.5	79.13	202.87	5.26	48.37	5.14	3.86	**μM**
Rep.6	90.45	200.91	5.05	67.13	5.37	2.62	**μM**
Mean:	74.78	210.31	5.34	59.22	4.94	3.21	**μM**
SD	9.8755	30.1503	0.3795	10.4675	0.5327	0.3630	
RSD	13.21%	14.34%	7.11%	17.68%	10.78%	11.30%	

6. Synthesis

Compounds **5**, **7** were synthesized according to the procedures from the literature.[5, 6]

6.1 Synthesis of tert-butyl (4-((2-(*N*-(2-(((benzyloxy)carbonyl)amino)ethyl)benzamido) ethyl)carbamoyl)benzyl)(methyl)carbamate (11)

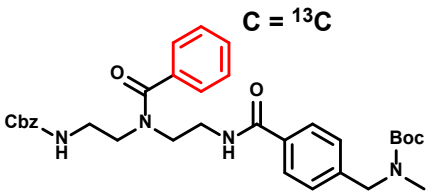


11

N_1-Cbz, N_4-benzyl diethylenetriamine **4** (41 mg, 120.1 μmol, 1 eq.), 4-(((tert-butoxycarbonyl)(methyl)amino) methyl)benzoic acid **5** (38.2 mg, 144.1 μmol, 1.2 eq.), HBTU (59.2 mg, 156.1 μmol, 1.3 eq.), HOBT (19.5 mg, 144.1 μmol, 1.2 eq.), DIPEA (63.0 μL, 360.3 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **11** (65.4 mg, 92.5 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (s, 2H), 7.37 – 7.07 (m, 12H), 5.59 (s, 1H), 5.15 – 4.98 (m, 2H), 4.43 (s, 2H), 3.93 – 3.14 (m, 8H), 2.79 (s, 3H), 1.45 (s, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.3, 167.8, 156.8, 156.4, 142.0, 136.5, 136.0, 132.9, 129.7, 128.7, 128.6, 128.2, 128.1, 127.5, 126.4, 80.0, 66.8, 52.5, 49.8, 45.0, 39.8, 39.1, 34.2, 28.5.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{33}H_{41}O_6N_4^+$: 589.3021; found 589.3019.

6.2 Synthesis of tert-butyl (4-((2-(*N*-(2-(((benzyloxy)carbonyl)amino)ethyl)benzamido) ethyl)carbamoyl)benzyl)(methyl)carbamate (phenyl-$^{13}C_6$) (11*)

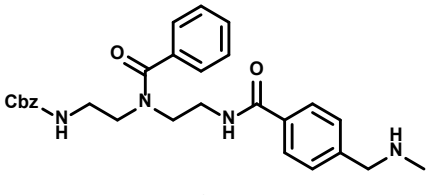


11*

N_1-Cbz, N_4-benzyl diethylenetriamine (phenyl-$^{13}C_6$) **4*** (43.9 mg, 126.3 µmol, 1 eq.), 4-(((tert-butoxycarbonyl)(methyl) amino)methyl)benzoic acid **5** (40.2 mg, 151.5 µmol, 1.2 eq.), HBTU (71.8 mg, 189.4 µmol, 1.5 eq.), HOBT (20.5 mg, 151.5 µmol, 1.2 eq.), DIPEA (63.0 µL, 360.3 µmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **11*** (68.3 mg, 91.0 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 2H), 7.66 – 6.90 (m, 12H), 5.02 (s, 2H), 4.42 (s, 2H), 3.95 – 3.05 (m, 8H), 2.79 (t, J = 10.9 Hz, 3H), 1.45 (s, 9H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.6, 167.8, 157.1, 156.4, 142.0, 136.6-125.8, 80.0, 66.8, 52.5, 49.8, 45.0, 39.6, 39.0, 34.2, 28.5.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{27}{}^{13}C_6H_{41}O_6N_4^+$: 595.3222; found 595.3217.

6.3 Synthesis of (2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl) carbamate (6)

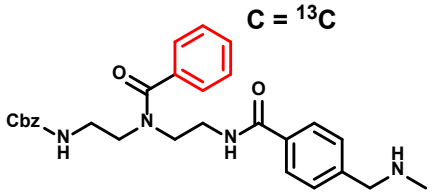


6

Tert-butyl(4-((2-(N-(2-(((benzyloxy)carbonyl)amino)ethyl)benzamido)ethyl)carbamoyl) benzyl)(methyl)carbamate **11** (65.4 mg, 111.1 µmol) was dissolve in DCM (1 mL), and trifluoroacetic acid (250 µL) was then added into the reaction mixture. The reaction was stirred at room temperature for 2 h and monitored by TLC. Upon full consumption of the starting material, the solvent is removed under reduced pressure with several additions of DCM in order

to remove trifluoroacetic acid. The product **6** (54.3 mg, quant.) was obtained as TFA salt form and directly used in the next step.

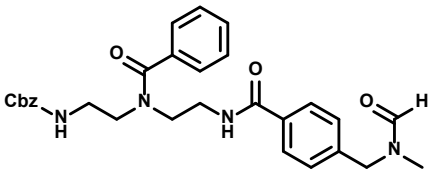
6.4 Synthesis of (2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido) ethyl)carbamate (phenyl-$^{13}C_6$) (6*)



6*

Tert-butyl(4-((2-(N-(2-(((benzyloxy)carbonyl)amino)ethyl)benzamido)ethyl)carbamoyl) benzyl)(methyl) carbamate (phenyl-$^{13}C_6$) **$^{13}C_6$-11** (68.3 mg, 114.9 µmol) was dissolve in DCM (1 mL), and trifluoroacetic acid (250 µL) was then added into the reaction mixture. The reaction was stirred at room temperature for 2 h and monitored by TLC. Upon full consumption of the starting material, the solvent is removed under reduced pressure with several additions of DCM in order to remove trifluoroacetic acid. The product **$^{13}C_6$-6** (56.9 mg, quant.) was obtained as TFA salt form and directly used in the next step.

6.5 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylformamido)methyl)benzamido) ethyl)benzamido) ethyl)carbamate (12a)



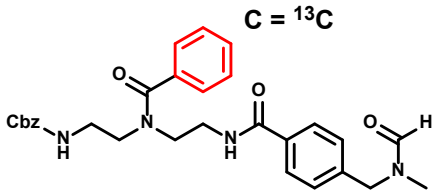
12a

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate **6** (9 mg, 18.4 µmol, 1 eq.), formic acid (3 µL, 55.3 µmol, 3 eq.), HBTU (10.5 mg, 27.6 µmol, 1.5 eq.), HOBT (3.0 mg, 22.1 µmol, 1.2 eq.), DIPEA (10.0 µL, 55.3 µmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography

on silica gel (1:19 MeOH/DCM) to afford the compound **12a** (5.1 mg, 53.6 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 44.7 Hz, 1H), 7.88 – 7.75 (m, 2H), 7.41 – 7.17 (m, 12H), 5.05 (s, 2H), 4.48 (d, *J* = 52.2 Hz, 2H), 3.94 – 3.24 (m, 8H), 2.80 (d, *J* = 28.4 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.9, 162.8, 136.5, 135.9, 133.5, 129.8, 128.8, 128.7, 128.5, 128.4, 128.2, 128.0, 127.7, 126.5, 67.0, 53.3, 47.7, 34.3, 29.8, 18.79, 17.6.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{29}H_{33}O_5N_4^+$ $[M+H]^+$: 517.2445; found 517.2450.

6.6 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylformamido)methyl)benzamido)ethyl) benzamido) ethyl)carbamate (phenyl-$^{13}C_6$) (12a*)

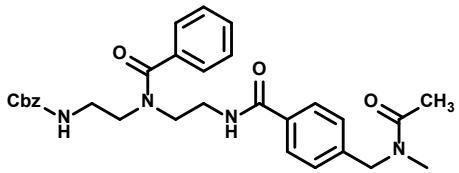


12a*

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) **6*** (9 mg, 18.2 μmol, 1 eq.), formic acid (3 μL, 54.6 μmol, 3 eq.), HBTU (10.4 mg, 27.3 μmol, 1.5 eq.), HOBT (3.0 mg, 21.8 μmol, 1.2 eq.), DIPEA (10.0 μL, 54.6 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12a*** (9.5 mg, 99.9 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 46.9 Hz, 1H), 7.82 (dd, *J* = 13.6, 7.7 Hz, 2H), 7.54 – 6.97 (m, 12H), 5.05 (s, 2H), 4.48 (d, *J* = 50.4 Hz, 2H), 3.96 – 3.21 (m, 8H), 2.80 (d, *J* = 29.6 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.0, 162.9, 136.4-125.9, 67.0, 53.4, 47.7, 34.4, 29.8.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{23}{}^{13}C_6H_{33}O_5N_4^+$: 577.2600; found 577.2599.

6.7 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylacetamido)methyl)benzamido)ethyl) benzamido) ethyl)carbamate (12b)

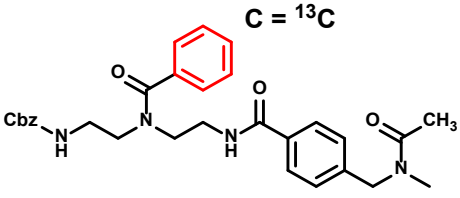


12b

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate **6** (6 mg, 12.3 µmol, 1 eq.), acetic acid (2 µL, 36.8 µmol, 3 eq.), HBTU (7.0 mg, 18.4 µmol, 1.5 eq.), HOBT (2.0 mg, 14.7 µmol, 1.2 eq.), DIPEA (6.0 µL, 36.8 µmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12b** (4.3 mg, 66.0 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (s, 2H), 7.39 – 7.14 (m, 12H), 5.05 (s, 2H), 4.59 (m, 2H), 3.96 – 3.17 (m, 8H), 2.93 (s, 3H), 2.20 (s, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.5, 135.9, 129.8, 128.8, 128.7, 128.3, 128.2, 127.7, 126.5, 67.0, 50.8, 50.0, 39.5, 36.2, 29.9, 28.6.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{30}H_{35}O_5N_4^+$: 531.2602; found 531.2598.

6.8 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylacetamido)methyl)benzamido) ethyl)benzamido) ethyl)carbamate (phenyl-$^{13}C_6$) (12b*)



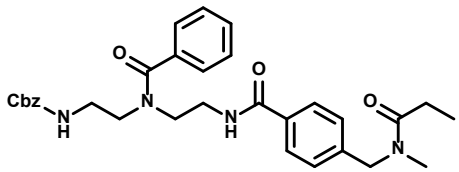
12b*

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) **6*** (9 mg, 18.2 µmol, 1 eq.), acetic acid (3 µL, 54.6 µmol, 3 eq.), HBTU (10.4 mg, 27.3 µmol,

1.5 eq.), HOBT (3.0 mg, 21.8 μmol, 1.2 eq.), DIPEA (10.0 μL, 54.6 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12b*** (9.9 mg, quant.) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 – 7.70 (m, 2H), 7.64 – 6.87 (m, 12H), 5.05 (s, 2H), 4.58 (d, J = 27.5 Hz, 2H), 4.03 – 3.14 (m, 8H), 2.92 (d, J = 6.4 Hz, 3H), 2.15 (d, J = 14.2 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.6-125.8, 67.0, 50.6, 49.9, 35.9, 34.1, 29.9, 21.8.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{24}{}^{13}C_6H_{35}O_5N_4^+$: 537.2803; found 537.2810.

6.9 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylpropionamido)methyl)benzamido)ethyl) benzamido)ethyl)carbamate (12c)

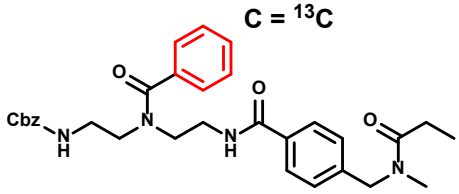


12c

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate **6** (4.8 mg, 9.9 μmol, 1 eq.), propionic acid (2 μL, 29.7 μmol, 3 eq.), HBTU (5.6 mg, 14.8 μmol, 1.5 eq.), HOBT (1.6 mg, 11.9 μmol, 1.2 eq.), DIPEA (5.0 μL, 29.7 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12c** (4.2 mg, 78.0 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 18.4, 7.7 Hz, 2H), 7.51 – 6.96 (m, 12H), 5.05 (s, 2H), 4.58 (d, *J* = 29.9 Hz, 2H), 3.96 – 3.15 (m, 8H), 2.92 (d, *J* = 16.9 Hz, 3H), 2.37 (dd, *J* = 19.9, 7.3 Hz, 2H), 1.17 (dt, *J* = 18.3, 7.3 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.5, 136.2, 129.7, 128.8, 128.7, 128.3, 128.2, 127.6, 126.5, 77.5, 77.2, 76.8, 66.9, 59.7, 50.7, 39.9, 30.0, 29.6.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{31}H_{37}O_5N_4^+$: 545.2758; found 545.2756

6.10 Synthesis of benzyl (2-(N-(2-(4-((N-methylpropionamido)methyl)benzamido)ethyl) benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) (12c*)

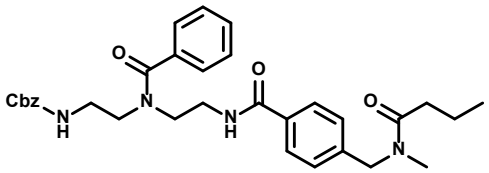


12c*

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) **6*** (9 mg, 18.2 µmol, 1 eq.), propionic acid (3 µL, 54.6 µmol, 3 eq.), HBTU (10.4 mg, 27.3 µmol, 1.5 eq.), HOBT (3.0 mg, 21.8 µmol, 1.2 eq.), DIPEA (10.0 µL, 54.6 µmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12c*** (9.9 mg, 98.8 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (s, 2H), 7.42 – 7.05 (m, 12H), 5.05 (s, 2H), 4.59 (d, *J* = 28.4 Hz, 2H), 4.00 – 3.17 (m, 8H), 2.92 (d, *J* = 14.5 Hz, 3H), 2.47 – 2.32 (m, 2H), 1.19 (t, *J* = 7.6 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.6-125.9, 67.0, 50.8, 49.9, 35.6, 34.9, 29.9, 26.8.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{25}{}^{13}C_6H_{37}O_5N_4^+$:551.2959; found 551.2963.

6.11 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylbutyramido)methyl)benzamido)ethyl) benzamido) ethyl)carbamate (12d)

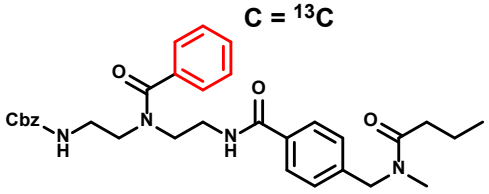


12d

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate **6** (4.8 mg, 9.9 µmol, 1 eq.), butyric acid (2 µL, 29.7 µmol, 3 eq.), HBTU (5.6 mg, 14.8 µmol, 1.5 eq.), HOBT (1.6 mg, 11.9 µmol, 1.2 eq.), DIPEA (5.0 µL, 29.7 µmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12d** (5.3 mg, 96.0 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.67 (m, 2H), 7.44 – 7.05 (m, 12H), 5.05 (s, 2H), 4.58 (d, J = 27.0 Hz, 2H), 3.98 – 3.18 (m, 8H), 2.91 (d, J = 9.8 Hz, 3H), 2.33 (dt, J = 19.9, 7.5 Hz, 2H), 1.69 (tq, J = 15.3, 7.8 Hz, 3H), 0.96 (dt, J = 26.5, 7.3 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.5, 136.0, 129.7, 128.8, 128.7, 128.3, 128.2, 127.6, 126.5, 67.0, 50.7, 49.9, 35.5, 35.1, 18.7, 14.1.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{32}H_{39}O_5N_4^+$: 559.2915; found 559.2911

6.12 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylbutyramido)methyl)benzamido)ethyl) benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) (12d*)



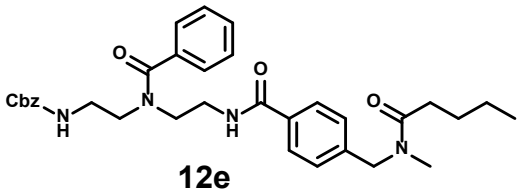
12d*

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) **6*** (4.5 mg, 9.1 µmol, 1 eq.), butyric acid (2 µL, 27.3 µmol, 3 eq.), HBTU (5.2 mg, 13.6 µmol,

1.5 eq.), HOBT (1.5 mg, 10.9 μmol, 1.2 eq.), DIPEA (5.0 μL, 27.3 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12d*** (3.8 mg, 74.0 %) as a light yellow liquid.

^1H NMR (400 MHz, $CDCl_3$): δ 7.80 (dd, *J* = 19.7, 7.8 Hz, 2H), 7.58 – 6.91 (m, 12H), 5.05 (s, 2H), 4.59 (d, *J* = 26.5 Hz, 2H), 3.96 – 3.22 (m, 8H), 2.91 (d, *J* = 9.5 Hz, 3H), 2.33 (dt, *J* = 20.1, 7.5 Hz, 2H), 1.71 (dp, *J* = 14.4, 7.3 Hz, 2H), 0.96 (dt, *J* = 26.4, 7.4 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.6-125.8, 67.0, 50.8, 50.0, 35.5, 35.2, 18.8, 14.1. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{26}{}^{13}C_6H_{39}O_5N_4^+$: 565.3116; found 565.3121.

6.13 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylpentanamido)methyl)benzamido)ethyl) benzamido)ethyl)carbamate (12e)

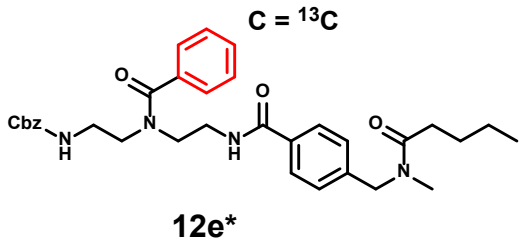


12e

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate **6** (7.2 mg, 14.8 μmol, 1 eq.), valeric acid (3 μL, 44.5 μmol, 3 eq.), HBTU (8.4 mg, 22.2 μmol, 1.5 eq.), HOBT (2.4 mg, 17.8 μmol, 1.2 eq.), DIPEA (8.0 μL, 44.5 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12e** (2.6 mg, 30.6 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 2H), 7.48 – 7.02 (m, 12H), 5.05 (s, 2H), 4.61 (s, 2H), 4.04 – 3.07 (m, 8H), 2.92 (s, 3H), 2.47 (s, 4H), 2.39 (s, 3H), 1.66 (p, *J* = 6.9, 6.5 Hz, 3H), 0.92 (d, *J* = 7.5 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.5, 136.0, 129.8, 129.7, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.2, 127.7, 126.8, 126.5, 67.0, 50.8, 49.9, 45.1, 35.2, 33.3, 28.5, 27.5, 22.7, 14.0.; HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{33}H_{41}O_5N_4^+$: 573.3071; found 573.3075

6.14 Synthesis of benzyl (2-(*N*-(2-(4-((*N*-methylpentanamido)methyl)benzamido)ethyl) benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) (12e*)

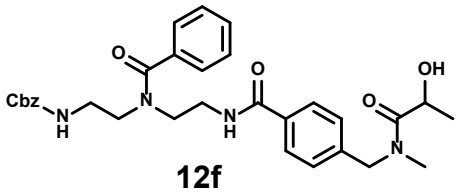


12e*

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) **6*** (4.5 mg, 9.1 μmol, 1 eq.), valeric acid (2 μL, 27.3 μmol, 3 eq.), HBTU (5.2 mg, 13.6 μmol, 1.5 eq.), HOBT (1.5 mg, 10.9 μmol, 1.2 eq.), DIPEA (5.0 μL, 27.3 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12e*** (4.7 mg, 89.3 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (dd, *J* = 19.4, 7.8 Hz, 2H), 7.43 – 6.98 (m, 12H), 5.05 (s, 2H), 4.58 (d, *J* = 25.9 Hz, 2H), 4.00 – 3.19 (m, 8H), 2.91 (d, *J* = 8.2 Hz, 3H), 2.35 (dt, *J* = 19.9, 7.5 Hz, 2H), 1.66 (dq, *J* = 14.8, 7.2 Hz, 5H), 1.36 (ddt, *J* = 29.4, 15.0, 7.5 Hz, 2H), 0.91 (dt, *J* = 24.5, 7.4 Hz, 3H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.6-125.9, 67.0, 50.7, 49.9, 43.4, 34.6, 33.3, 27.4, 22.7, 14.0. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{27}{}^{13}C_6H_{41}O_5N_4^+$: 579.3272; found 579.3269.

6.15 Synthesis of benzyl (2-(*N*-(2-(4-((2-hydroxy-*N*-methylpropanamido)methyl) benzamido)ethyl)benzamido)ethyl)carbamate (12f)

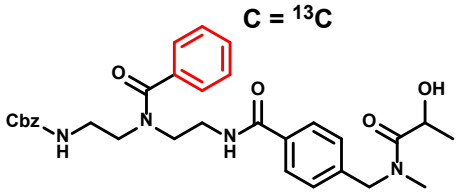


12f

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate **6** (7.2 mg, 14.8 µmol, 1 eq.), sodium lactate (5 mg, 44.5 µmol, 3 eq.), HBTU (8.4 mg, 22.2 µmol, 1.5 eq.), HOBT (2.4 mg, 17.8 µmol, 1.2 eq.), DIPEA (8.0 µL, 44.5 µmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12f** (6.9 mg, 83.1 %) as a light yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.73 (m, 2H), 7.46 – 7.09 (m, 12H), 5.01 (d, J = 35.3 Hz, 2H), 4.75 – 4.54 (m, 2H), 4.55 – 4.37 (m, 1H), 3.98 – 3.18 (m, 8H), 2.90 (d, J = 18.4 Hz, 3H), 1.45 (d, J = 4.7 Hz, 1H), 1.35 (dd, J = 13.1, 6.7 Hz, 2H).; ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.7, 136.5, 135.9, 133.5, 129.8, 128.7, 128.4, 128.2, 127.8, 126.9, 126.5, 67.0, 64.6, 64.5, 52.2, 51.4, 50.0, 45.1, 40.1, 39.6, 34.3, 34.2, 29.8, 28.5, 22.0, 21.1. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{31}H_{37}O_6N_4^+$: 561.2708; found 561.2710.

6.16 Synthesis of benzyl (2-(*N*-(2-(4-((2-hydroxy-*N*-methylpropanamido)methyl) benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) (12f*)

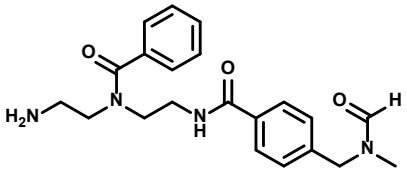


12f*

(2-(*N*-(2-(4-((methylamino)methyl)benzamido)ethyl)benzamido)ethyl)carbamate (phenyl-$^{13}C_6$) **6*** (4.5 mg, 9.1 μmol, 1 eq.), sodium lactate (3.1 mg, 27.3 μmol, 3 eq.), HBTU (5.2 mg, 13.6 μmol, 1.5 eq.), HOBT (1.5 mg, 10.9 μmol, 1.2 eq.), DIPEA (5.0 μL, 27.3 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **12f*** (1.2 mg, 23.3 %) as a light yellow liquid.

^{1}H NMR (601 MHz, $CDCl_3$) δ 7.89 – 7.74 (m, 2H), 7.54 – 6.95 (m, 12H), 5.05 (s, 2H), 4.76 – 4.55 (m, 2H), 4.51 (q, *J* = 6.5 Hz, 1H), 3.97 – 3.15 (m, 8H), 2.90 (d, *J* = 29.9 Hz, 3H), 1.35 (dd, *J* = 19.7, 6.6 Hz, 3H).; ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.7, 136.4,-125.9, 67.0, 64.6, 64.5, 52.2, 51.4, 49.9, 45.1, 40.2, 39.7, 34.2, 29.9, 22.0, 21.2. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{25}{}^{13}C_6H_{37}O_6N_4^+$: 567.2909; found 567.2910.

6.17 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylformamido)methyl) benzamido)ethyl)benzamide (3a)

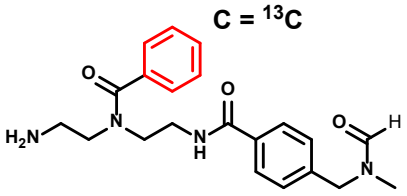


3a

Benzyl (2-(*N*-(2-(4-((*N*-methylformamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate **12a** (5.1 mg, 9.9 μmol) and palladium on carbon (10%, 5.1 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 μL HCl (1 M solution) and 100 μL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3a** (3.5 mg, 92.7%) as a viscous liquid.

^{1}H NMR (400 MHz, MeOD): δ 8.25 (d, *J* = 53.9 Hz, 1H), 7.81 (dt, *J* = 36.6, 10.5 Hz, 2H), 7.54 – 7.19 (m, 7H), 4.58 (d, *J* = 10.4 Hz, 2H), 3.89 – 3.33 (m, 8H), 2.84 (d, *J* = 68.7 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 175.0, 165.3, 165.2, 141.5, 137.7, 130.7, 130.4, 130.0, 129.7, 129.2, 129.0, 128.8, 127.8, 127.5, 54.0, 53.1, 50.2, 48.3, 46.1, 40.9, 40.2, 39.1, 39.0, 34.8, 30.8, 29.8. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{21}H_{27}O_3N_4^+$: 383.2078; found 383.2077.

6.18 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylformamido)methyl)benzamido) ethyl) benzamide (phenyl-$^{13}C_6$) (3a*)

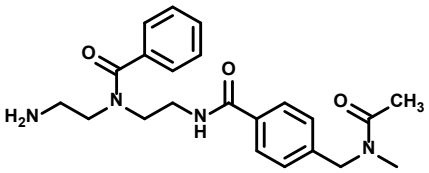


3a*

Benzyl (2-(*N*-(2-(4-((*N*-methylformamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate (phenyl-$^{13}C_6$) **12a*** (9.5 mg, 18.2 µmol) and palladium on carbon (10%, 9.5 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3a*** (6.2 mg, 87.8 %) as a viscous liquid.

^{1}H NMR (601 MHz, MeOD) δ 8.25 (d, *J* = 82.0 Hz, 1H), 7.80 (ddd, *J* = 56.6, 20.9, 9.0 Hz, 2H), 7.58 – 7.15 (m, 7H), 4.58 (dd, *J* = 15.6, 5.7 Hz, 2H), 3.88 – 3.35 (m, 8H), 3.04 – 2.69 (m, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 174.8, 165.3, 165.2, 141.5, 138.1-127.0, 54.0, 53.0, 50.3, 48.3, 46.1, 40.8, 40.2, 39.1, 39.0, 34.8, 30.8. 29.8.HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{15}{}^{13}C_6H_{27}O_3N_4^+$: 389.2279; found 389.2278.

6.19 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylacetamido)methyl)benzamido) ethyl) benzamide (3b)

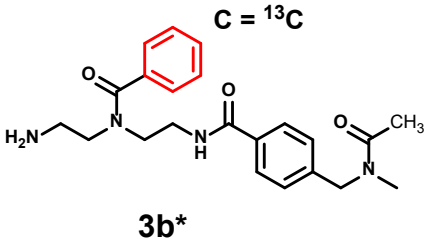


3b

Benzyl (2-(N-(2-(4-((N-methylacetamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate **12b** (4.3 mg, 8.1 µmol) and palladium on carbon (10%, 8.1 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3b** (3.2 mg, quant.) as a viscous liquid.

^{1}H NMR (400 MHz, MeOD): δ 7.77 (d, J = 28.9 Hz, 2H), 7.59 – 7.17 (m, 7H), 4.67 (d, J = 19.5 Hz, 2H), 3.84 – 3.37 (m, 6H), 3.11 (s, 1H), 2.98 (d, J = 34.4 Hz, 3H), 2.78 (s, 1H), 2.16 (d, J = 14.9 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 173.7, 130.8, 129.7, 129.1, 128.8, 128.7, 127.9, 127.8, 127.5, 54.9, 52.5, 51.4, 50.5, 47.7, 46.1, 40.6, 40.0, 39.1, 39.0, 36.4, 34.3, 21.6, 21.3. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{22}H_{29}O_3N_4^+$: 397.2234; found 397.2233

6.20 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylacetamido)methyl)benzamido) ethyl)benzamide (phenyl-$^{13}C_6$) (3b*)



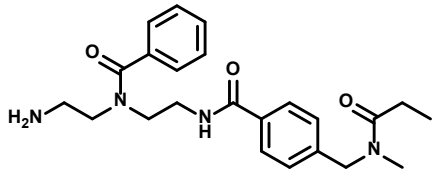
3b*

Benzyl (2-(*N*-(2-(4-((*N*-methylformamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate (phenyl-$^{13}C_6$) **12b*** (9.9 mg, 18.2 µmol) and palladium on carbon (10%, 9.9 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction

mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3b*** (3.9 mg, 52.5 %) as a viscous liquid.

^{1}H NMR (601 MHz, MeOD): δ 7.93 – 7.70 (m, 2H), 7.60 – 7.12 (m, 7H), 4.67 (d, *J* = 32.4 Hz, 2H), 3.86 – 3.36 (m, 6H), 3.06 (s, 1H), 3.04 – 2.90 (m, 3H), 2.76 (s, 1H), 2.16 (d, *J* = 22.8 Hz, 3H).;^{13}C NMR (151 MHz, MeOD): δ 173.7, 138.1-127.0, 54.9, 52.8, 51.4, 50.4, 49.4, 46.1, 40.7, 40.0, 39.1, 39.0, 36.4, 34.3, 21.6, 21.3. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{16}{}^{13}C_6H_{29}O_3N_4^+$: 403.2435; found 403.2436.

6.21 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylpropionamido)methyl) benzamido)ethyl)benzamide (3c)

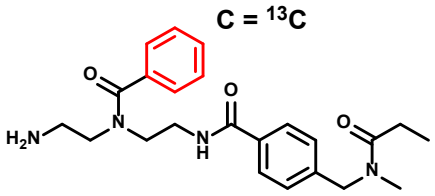


3c

Benzyl (2-(*N*-(2-(4-((*N*-methylpropionamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate **12c** (4.2 mg, 7.7 μmol) and palladium on carbon (10%, 4.2 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 μL HCl (1 M solution) and 100 μL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3c** (3.1 mg, quant.) as a viscous liquid.

^{1}H NMR (400 MHz, MeOD): δ 7.76 (d, *J* = 26.4 Hz, 2H), 7.47 – 7.26 (m, 7H), 4.66 (d, *J* = 18.1 Hz, 2H), 3.87 – 3.32 (m, 6H), 3.00 (s, 3H), 2.94 (s, 1H), 2.74 (s, 1H), 2.46 (dq, *J* = 15.0, 7.6 Hz, 2H), 1.12 (dt, *J* = 20.9, 7.4 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.7, 175.1, 130.7, 129.7, 129.0, 128.7, 127.8, 127.7, 127.5, 54.0, 53.0 51.6, 50.3, 46.1, 40.8, 40.1, 39.1, 35.6, 34.6, 27.5, 27.2, 9.9, 9.7. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{23}H_{31}O_3N_4^+$: 411.2391; found 411.2389.

6.22 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylpropionamido)methyl)benzamido) ethyl)benzamide (phenyl-$^{13}C_6$) (3c*)

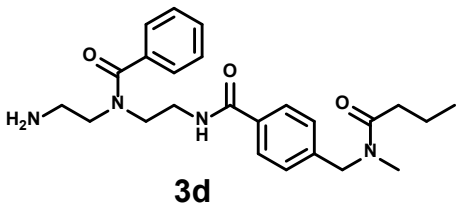


3c*

Benzyl (2-(*N*-(2-(4-((*N*-methylpropionamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate (phenyl-$^{13}C_6$) **12c*** (14.9 mg, 27.1 µmol) and palladium on carbon (10%, 14.9 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3c*** (3.0 mg, 26.6 %) as a viscous liquid.

^{1}H NMR (601 MHz, MeOD) δ 7.80 (ddd, *J* = 53.5, 27.5, 6.9 Hz, 2H), 7.61 – 7.13 (m, 7H), 4.67 (d, *J* = 24.9 Hz, 2H), 3.85 – 3.33 (m, 6H), 3.01 (d, *J* = 8.6 Hz, 3H), 2.95 (d, *J* = 6.3 Hz, 1H), 2.76 (d, *J* = 7.4 Hz, 1H), 2.54 – 2.37 (m, 2H), 1.13 (dt, *J* = 32.8, 7.3 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.7, 174.8, 138.1-127.0, 54.0, 53.0, 51.6, 50.3, 46.1, 40.8, 40.2, 39.0, 35.6, 34.6, 30.7, 27.5, 27.2, 9.9, 9.7. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{17}{}^{13}C_6H_{31}O_3N_4^+$: 417.2592; found 417.2593

6.23 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((N-methylbutyramido)methyl)benzamido) ethyl)benzamide (3d)



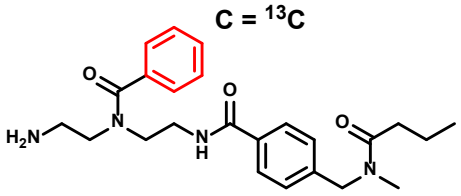
3d

Benzyl (2-(*N*-(2-(4-((*N*-methylbutyramido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate **12d** (6.1 mg, 10.9 µmol) and palladium on carbon (10%, 6.1 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen,

then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3d** (3.2 mg, 69.0%) as a viscous liquid.

^{1}H NMR (400 MHz, MeOD): δ 7.76 (d, *J* = 26.4 Hz, 2H), 7.47 – 7.26 (m, 7H), 4.66 (d, *J* = 18.1 Hz, 2H), 3.87 – 3.32 (m, 6H), 3.00 (s, 3H), 2.94 (s, 1H), 2.74 (s, 1H), 2.46 (dq, *J* = 15.0, 7.6 Hz, 2H), 1.12 (dt, *J* = 20.9, 7.4 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 175.9, 175.1, 130.7, 129.7, 129.0, 128.8, 128.7, 127.9, 127.7, 127.5, 54.1, 52.9, 51.6, 50.3, 46.1, 40.8, 40.1, 39.1, 39.0, 36.2, 35.9, 34.6, 19.9, 19.7, 14.2. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{24}H_{33}O_3N_4^+$: 425.2547; found 425.4544

6.24 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((N-methylbutyramido)methyl)benzamido) ethyl)benzamide (phenyl-$^{13}C_6$) (3d*)

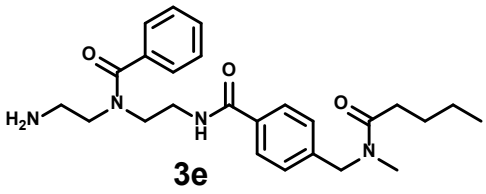


3d*

Benzyl (2-(*N*-(2-(4-((*N*-methylbutyramido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate (phenyl-$^{13}C_6$) **12d*** (3.8 mg, 6.7 μmol) and palladium on carbon (10%, 3.8 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 μL HCl (1 M solution) and 100 μL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3d*** (3.0 mg, quant.) as a viscous liquid.

^{1}H NMR (601 MHz, MeOD): δ 7.90 – 7.67 (m, 2H), 7.57 – 7.10 (m, 7H), 4.68 (d, *J* = 34.8 Hz, 2H), 3.84 – 3.39 (m, 6H), 3.18 – 2.97 (m, 3H), 2.95 (s, 1H), 2.79 (s, 1H), 2.42 (d, *J* = 37.8 Hz, 2H), 1.77 – 1.57 (m, 2H), 1.03 – 0.87 (m, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.1, 175.9, 138.1-127.0, 54.1, 51.6, 50.5, 47.5, 46.1, 40.5, 39.9, 39.2, 36.2, 35.9, 34.6, 26.7, 19.9, 19.7, 14.2. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{18}{}^{13}C_6H_{33}O_3N_4^+$: 431.2748; found 431.2749

6.25 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylpentanamido)methyl)benzamido) ethyl)benzamide (3e)

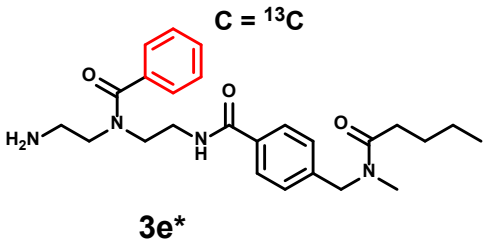


3e

Benzyl (2-(*N*-(2-(4-((*N*-methylpentanamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate **12e** (9.6 mg, 16.8 µmol) and palladium on carbon (10%, 9.6 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3e** (7.4 mg, quant.) as a viscous liquid.

NMR (601 MHz, MeOD): δ 8.03 – 7.63 (m, 2H), 7.54 – 7.17 (m, 7H), 4.61 (d, J = 51.2 Hz, 2H), 4.07 – 3.31 (m, 8H), 2.99 (d, J = 47.6 Hz, 3H), 2.45 (dt, J = 38.5, 7.5 Hz, 2H), 1.61 (dt, J = 31.7, 7.7 Hz, 2H), 1.36 – 1.26 (m, 2H), 1.06 – 0.85 (m, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.1, 175.8, 169.8, 143.0, 142.7, 136.7, 135.8, 134.0, 131.1, 130.2, 129.7 129.1, 128.8, 128.4, 128.1, 127.7, 54.2, 51.6, 51.0, 45.5, 39.3, 36.8, 35.9, 34.7, 34.0, 33.8, 30.7, 29.2, 28.8, 28.7, 28.5, 23.5, 23.4, 21.4, 14.2. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{25}H_{35}O_3N_4^+$: 439.2704; found 439.2704

6.26 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((*N*-methylpentanamido)methyl)benzamido) ethyl)benzamide (phenyl-$^{13}C_6$) (3e*)



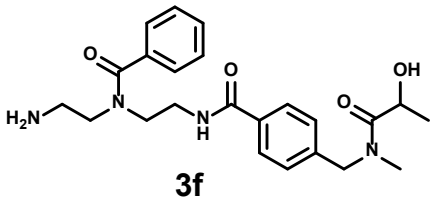
3e*

Benzyl (2-(*N*-(2-(4-((*N*-methylpentanamido)methyl)benzamido)ethyl)benzamido)ethyl) carbamate (phenyl-$^{13}C_6$) **12e*** (4.7 mg, 8.1 µmol) and palladium on carbon (10%, 4.7 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl

(1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3e*** (2.8 mg, quant.) as a viscous liquid.

^{1}H NMR (601 MHz, MeOD): δ 7.80 (ddd, *J* = 54.3, 29.9, 7.7 Hz, 2H), 7.61 – 7.06 (m, 7H), 4.76 – 4.58 (m, 2H), 3.89 – 3.31 (m, 6H), 3.02 (d, *J* = 10.2 Hz, 3H), 2.95 (d, *J* = 9.7 Hz, 1H), 2.75 (t, *J* = 7.3 Hz, 1H), 2.44 (dt, *J* = 39.7, 7.6 Hz, 2H), 1.68 – 1.52 (m, 2H), 1.46 – 1.26 (m, 2H), 0.93 (dt, *J* = 46.0, 7.4 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.3, 176.0, 142.9, 138.1-127.0, 54.2, 53.0, 51.6, 50.3, 46.1, 40.8, 40.2, 39.1, 39.0, 35.9, 34.6, 34.0, 33.8, 30.7, 28.7, 28.5, 23.5, 23.4, 14.2. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{19}{}^{13}C_6H_{35}O_3N_4^+$: 445.2905; found 445.2902

6.27 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((2-hydroxy-*N*-methylpropanamido)methyl) benzamido)ethyl)benzamide (3f)



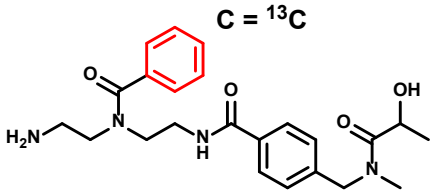
3f

Benzyl (2-(*N*-(2-(4-((2-hydroxy-*N*-methylpropanamido)methyl)benzamido)ethyl)benzamido) ethyl)carbamate **12f** (6.9 mg, 12.3 µmol) and palladium on carbon (10%, 6.9 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3e** (4.5 mg, 85.7 %) as a viscous liquid.

^{1}H NMR (400 MHz, MeOD) δ 7.78 (d, *J* = 26.1 Hz, 2H), 7.48 – 7.26 (m, 7H), 4.66 (s, 2H), 3.86 – 3.38 (m, 7H), 3.10 (d, *J* = 46.6 Hz, 3H), 2.88 (d, *J* = 13.3 Hz, 1H), 2.81 (s, 1H), 1.36 (d, *J* = 6.6 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.6, 175.4, 142.6, 130.8, 129.7, 129.0, 128.9, 128.8, 128.1, 127.9, 127.5, 66.3, 65.9, 53.3, 52.0, 50.6, 47.2, 46.1, 40.4, 39.9, 39.2, 35.1,

34.4, 28.7, 21.0, 20.5. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{23}H_{31}O_4N_4^+$: 427.2340; found 427.2341

6.28 Synthesis of *N*-(2-aminoethyl)-*N*-(2-(4-((2-hydroxy-*N*-methylpropanamido)methyl) benzamido)ethyl)benzamide (phenyl-$^{13}C_6$) (3f*)

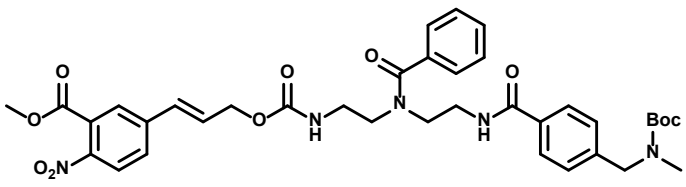


3f*

Benzyl (2-(*N*-(2-(4-((2-hydroxy-*N*-methylpropanamido)methyl)benzamido)ethyl)benzamido) ethyl)carbamate (phenyl-$^{13}C_6$) **12f*** (1.2 mg, 2.1 µmol) and palladium on carbon (10%, 1.2 mg) were dissolved in MeOH (2 mL), making sure that the catalyst is completely submerged. 2 µL HCl (1 M solution) and 100 µL H_2O were added into reaction mixture. Followed by flushing flask with nitrogen, then hydrogen, a balloon with hydrogen was attached and the reaction mixture was stirred for 5 h. Upon full consumption of the starting material by TLC monitoring, the reaction mixture was filtered through celite and washed through with additional MeOH. Solvent was removed and the residual **3f*** (1.0 mg, quant.) as a viscous liquid.

^{1}H NMR (601 MHz, MeOD): δ 7.92 – 7.64 (m, 2H), 7.61 – 7.12 (m, 7H), 4.61 (d, *J* = 51.0 Hz, 2H), 3.91 – 3.33 (m, 6H), 3.12 – 2.98 (m, 3H), 2.91 (s, 1H), 2.77 (s, 1H), 1.35 (dd, *J* = 19.3, 6.5 Hz, 3H).; ^{13}C NMR (151 MHz, MeOD): δ 176.8, 176.6, 138.1-127.0, 66.3, 65.9, 53.3, 52.0, 50.4, 49.4, 49.3, 49.1, 49.0, 48.9, 48.7, 48.6, 47.9, 46.1, 40.7, 40.0, 39.1, 35.1, 34.4, 30.8, 21.0, 20.5. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{17}{}^{13}C_6H_{31}O_4N_4^+$: 433.2541; found 433.2543

6.29 Synthesis of methyl (*E*)-5-(5-benzoyl-1-(4-(((tert-butoxycarbonyl)(methyl)amino) methyl)phenyl)-1,9-dioxo-10-oxa-2,5,8-triazatridec-12-en-13-yl)-2-nitrobenzoate (8)



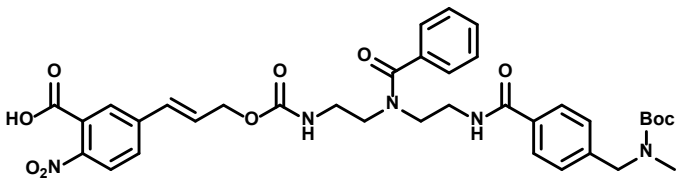
8

Methyl (*E*)-5-(3-(((2-(N-(2-aminoethyl)benzamido)ethyl)carbamoyl)oxy)prop-1-en-1-yl)-2-nitrobenzoate **7** (18.6 mg, 39.4 µmol, 1 eq.), 4-(((tert-butoxycarbonyl)(methyl)amino)

methyl)benzoic acid **5** (15.7 mg, 59.1 μmol, 1.5 eq.), HBTU (22.4 mg, 59.1, 1.5 eq.), HOBT (6.4 mg, 47.3 μmol, 1.2 eq.), DIPEA (21.0 μL, 118.3 μmol, 3 eq.) were dissolved in DCM (3 mL). The reaction was stirred at room temperature for 16 h and monitored by TLC. Upon full consumption of the starting material, the sat. $NaHCO_3$ (10 mL) was added to quench the reaction. Then the reaction mixture was extracted by DCM (3 X 20 mL). All the organic solvent were combined and washed by sat. $NaHCO_3$ (2 X 30 mL) and brine (2 X 30 mL). All the organic solvent were collected and dried over $MgSO_4$. The mixture was then filtered and concentrated in *vacuo* affording the yellow oil. The residual was purified by flash column chromatography on silica gel (1:19 MeOH/DCM) to afford the compound **11** (15.8 mg, 55.8 %) as a light yellow liquid.

^{1}H NMR (400 MHz, CD_3Cl): δ 7.90 (s, 1H), 7.78 (s, 2H), 7.67 (s, 1H), 7.54 (d, *J* = 10.1 Hz, 1H), 7.29 (d, *J* = 37.5 Hz, 7H), 6.65 (d, *J* = 16.4 Hz, 1H), 6.42 (s, 1H), 5.41 (s, 1H), 4.72 (s, 2H), 4.44 (s, 2H), 3.92 (s, 3H), 3.83 (d, *J* = 26.5 Hz, 3H), 3.52 (d, *J* = 34.0 Hz, 4H), 3.32 (s, 1H), 2.81 (s, 3H), 1.45 (s, 9H).; ^{13}C NMR (101 MHz, CD_3Cl): δ 173.8, 167.9, 166.2, 156.1, 146.6, 141.8, 136.0, 133.0, 131.5, 130.0, 129.8, 129.2, 128.8, 127.5, 126.5, 124.8, 124.4, 80.1, 77.5, 77.4, 77.2, 76.8, 64.7, 61.4, 53.5, 50.0, 45.1, 39.7, 39.2, 34.3, 28.6. HRMS (ESI+) *m/z* $[M+H]^+$ calcd. for $C_{37}H_{44}O_{10}N_5^+$: 718.3083; found 718.3082

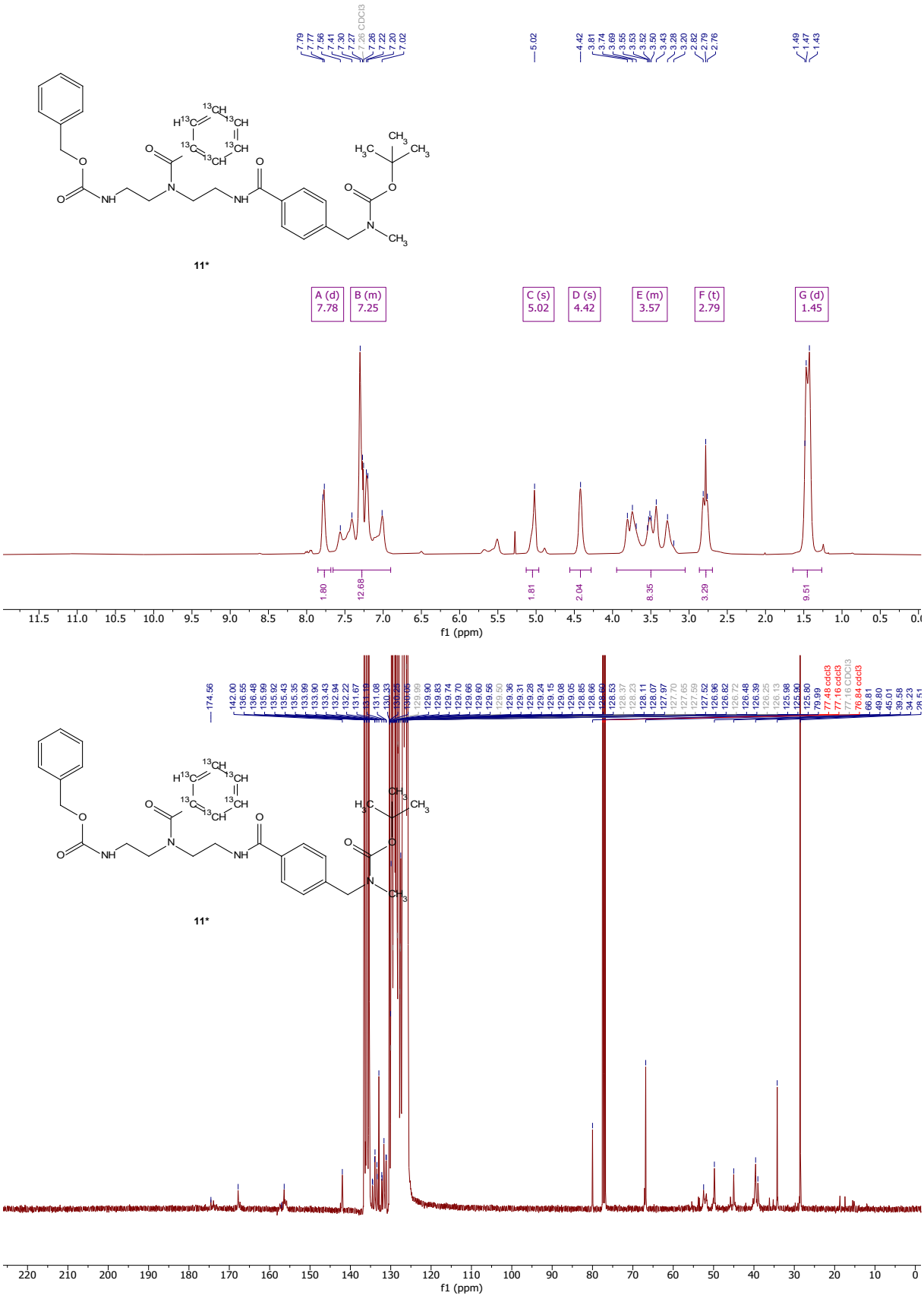
6.30 Synthesis of (*E*)-5-(5-benzoyl-1-(4-(((tert-butoxycarbonyl)(methyl)amino)methyl) phenyl)-1,9-dioxo-10-oxa-2,5,8-triazatridec-12-en-13-yl)-2-nitrobenzoic acid (9)



9

Methyl (E)-5-(5-benzoyl-1-(4-(((tert-butoxycarbonyl)(methyl)amino)methyl)phenyl)-1,9-dioxo-10-oxa-2,5,8-triazatridec-12-en-13-yl)-2-nitrobenzoate **8** was dissolved in MeOH (2 mL). 2M LiOH solution (200 μL) and H_2O (1 mL) were added into the reaction mixture. The reaction was stirred for 30 min and monitored by TLC. Upon full consumption of the starting material, 5 M HCl was used to neutralize the mixture until the solution has turn to clear from turbid yielding **9**. After solvent was removed under reduced pressure, the residual was redissolved in DMF and directly used for conjugation to magnetic beads.

7. NMR spectra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7.77
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7.41
7.30
7.27
7.26 CDCl3
7.22
7.20
7.02
5.02
4.42
3.81
3.74
3.69
3.55
3.53
3.52
3.50
3.43
3.28
3.20
2.82
2.79
2.76
1.49
1.47
1.43
11*
A (d) 7.78
B (m) 7.25
C (s) 5.02
D (s) 4.42
E (m) 3.57
F (t) 2.79
G (d) 1.45
1.80
12.68
1.81
2.04
8.35
3.29
9.51
f1 (ppm)
174.56
11*
f1 (ppm)


8.28
8.17
7.85
7.83
7.81
7.79
7.33
7.26 CDCl3
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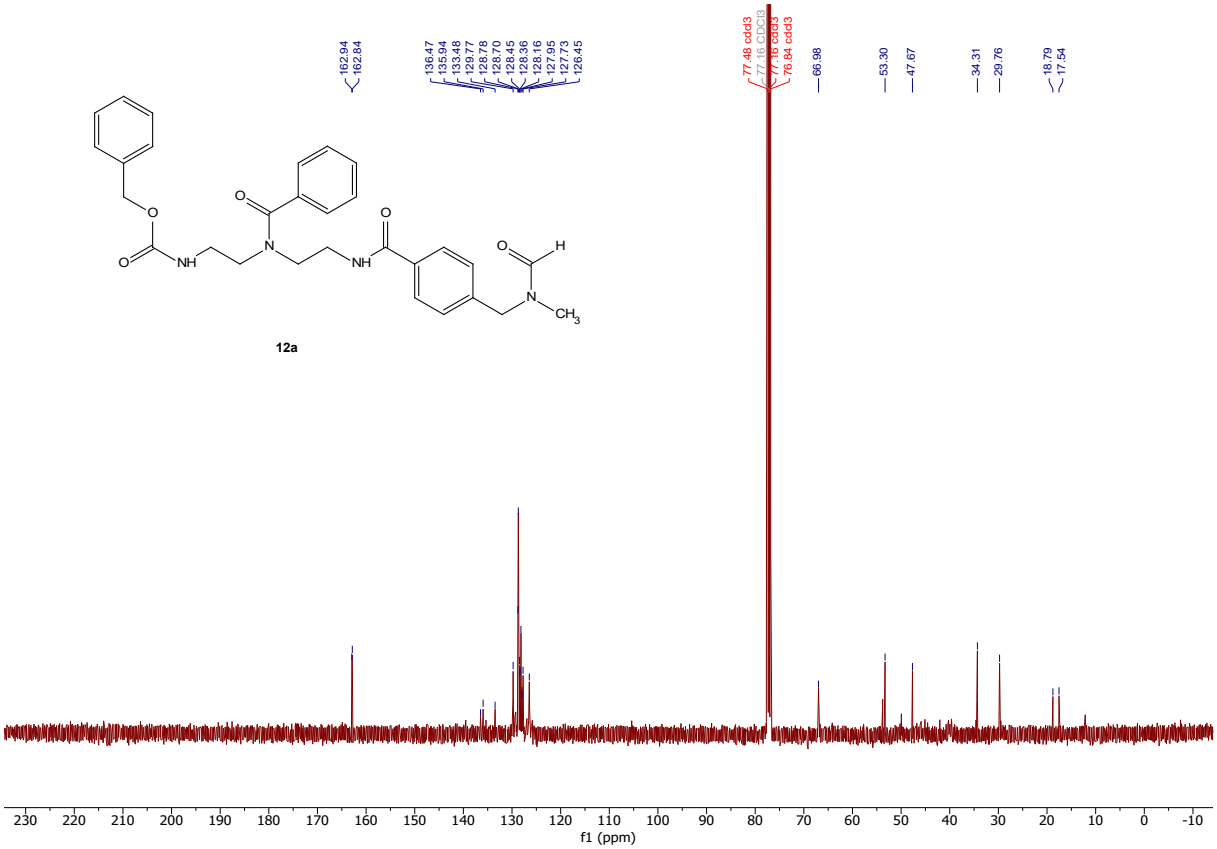
f1 (ppm)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128.78
128.70
128.45
128.36
128.16
127.95
127.73
126.45
77.48 cdd3
77.16 CDCl3
77.16 cdd3
76.84 cdd3
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47.67
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29.76
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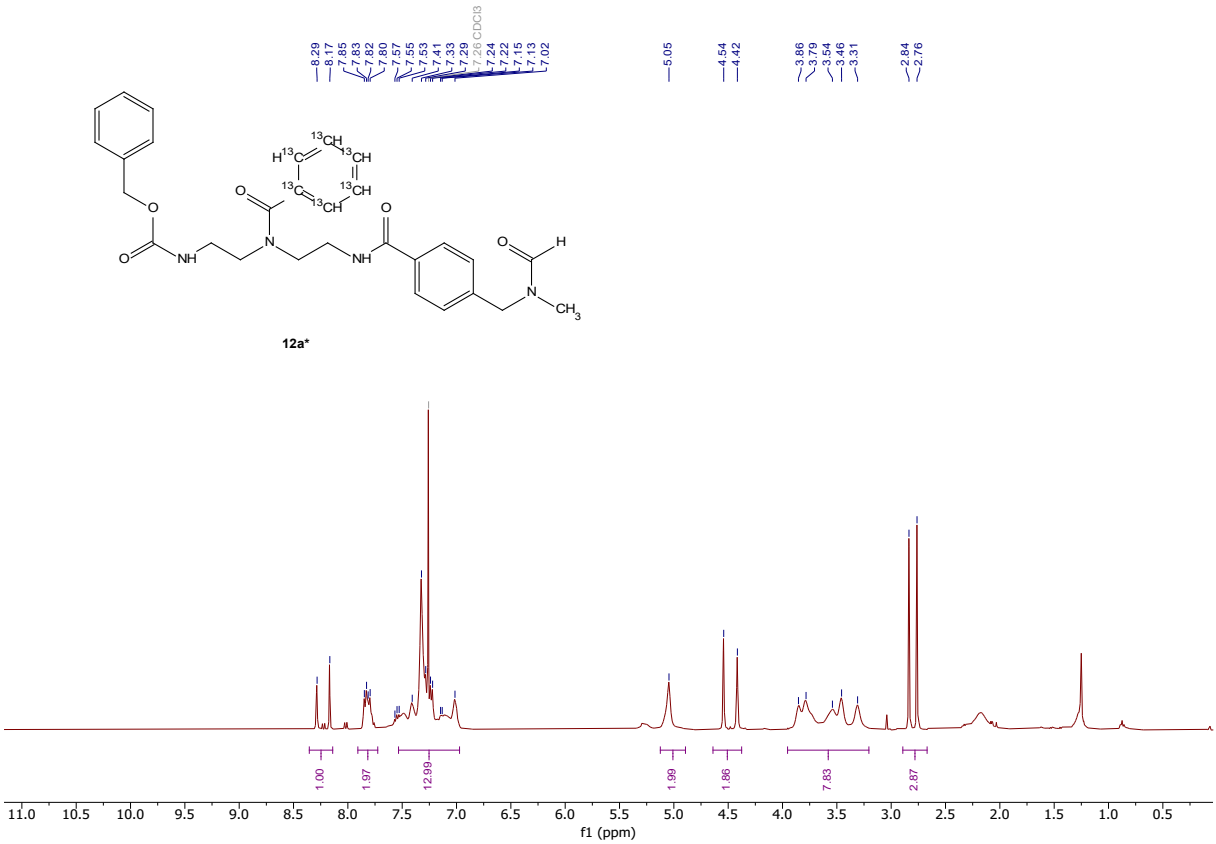
17.54
12a
f1 (ppm)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7.29
7.26 CDCl3
7.24
7.22
7.15
7.13
7.02
5.05
4.54
4.42
3.86
3.79
3.54
3.46
3.31
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2.76
12a*
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12.99
1.99
1.86
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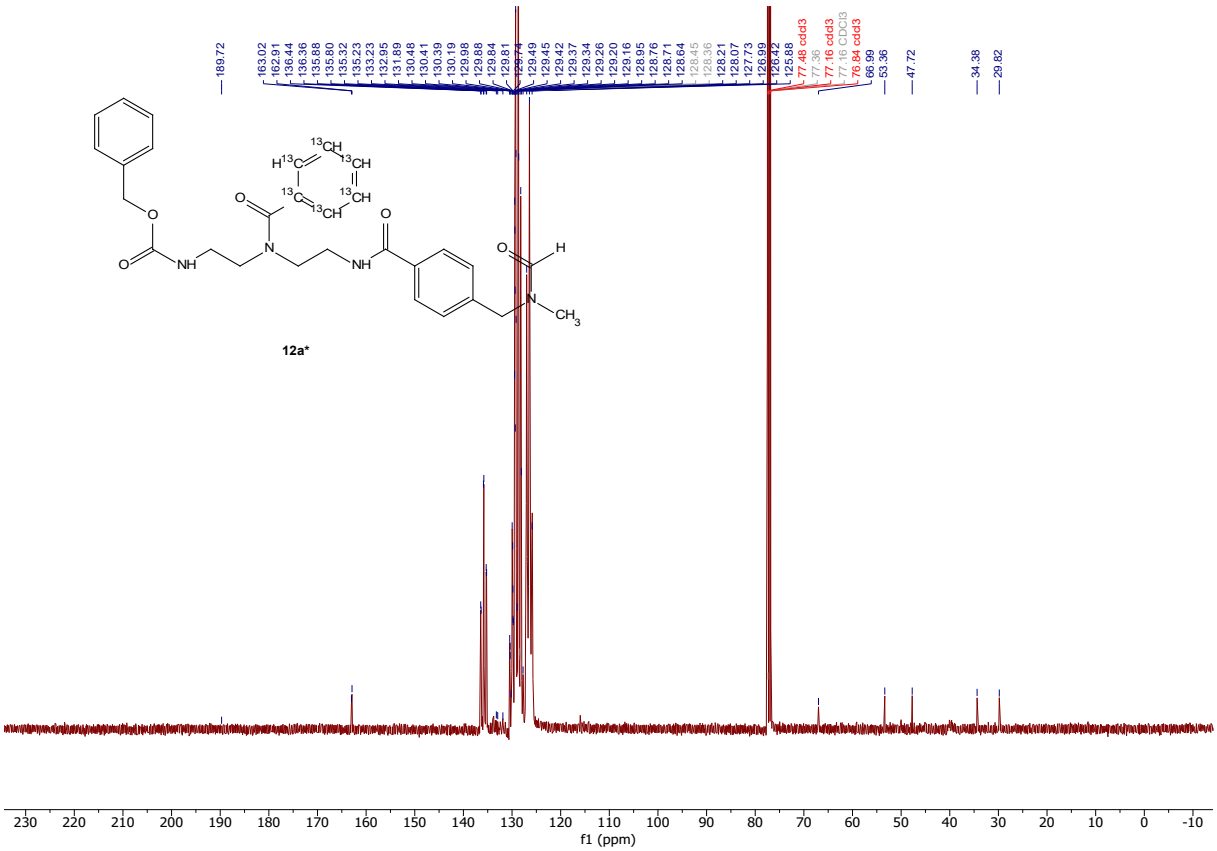
2.87
f1 (ppm)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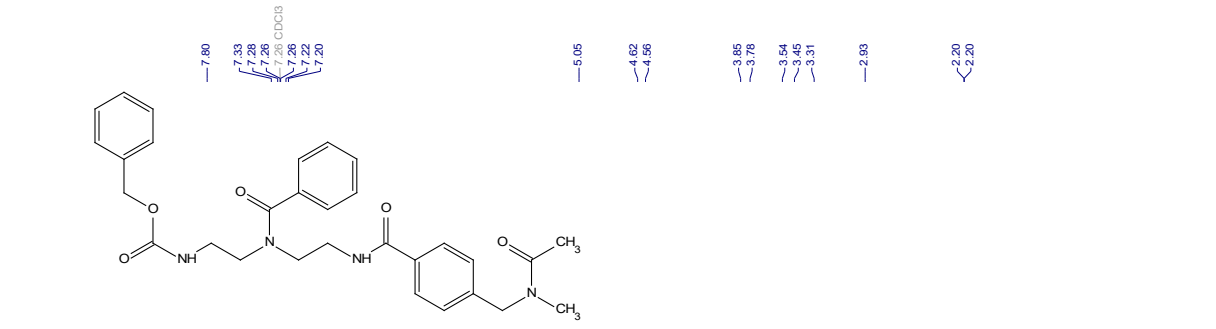
128.36
128.21
128.07
127.73
126.99
126.42
125.88
77.48 cdd3
77.36
77.16 cdd3
77.16 CDCl3
76.84 cdd3
66.99
53.36
47.72
34.38
29.82
12a*
f1 (ppm)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3.85
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3.31
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2.20
2.20
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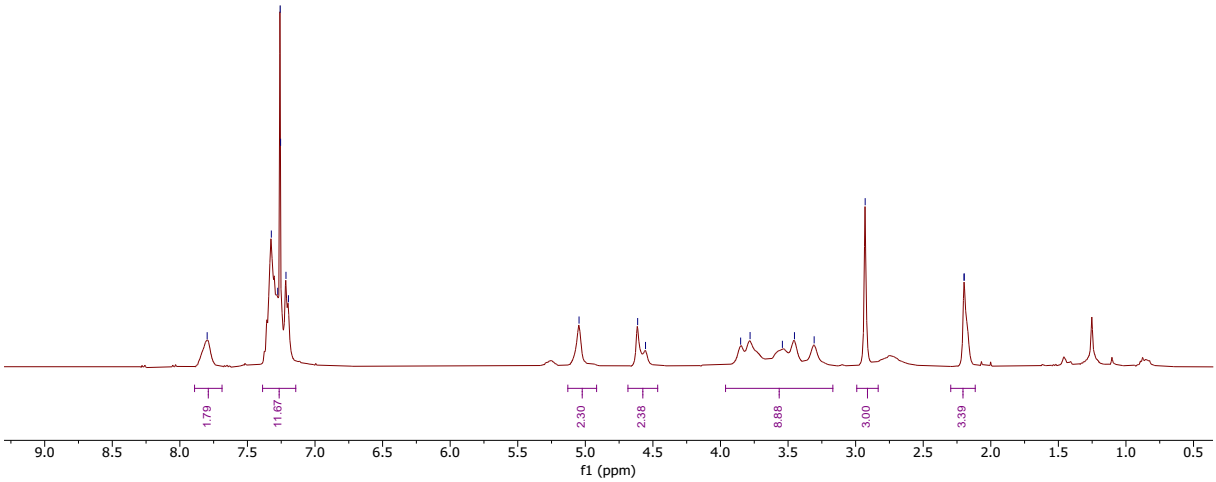
NH
N
O
CH3
N
CH3

12b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7.0
6.5
6.0
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4.0
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3.0
2.5
2.0
1.5
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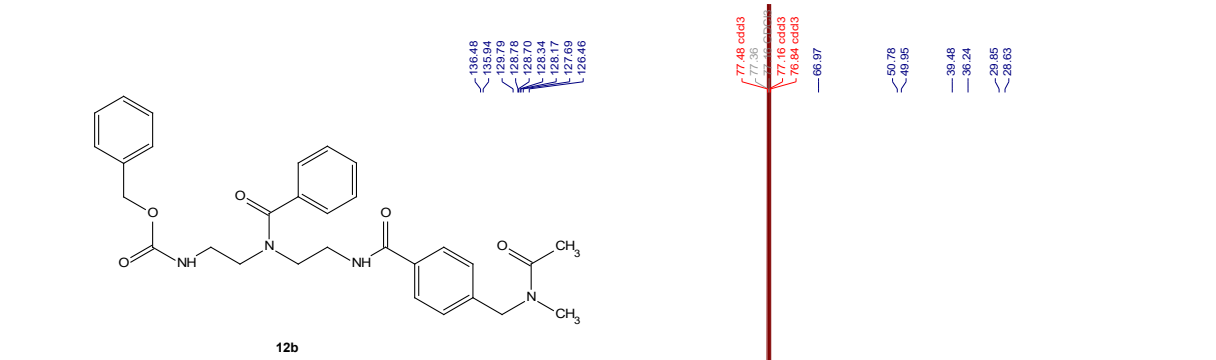
0.5
f1 (ppm)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77.48 cdd3
77.16 cdd3
76.84 cdd3
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39.48
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29.85
28.63
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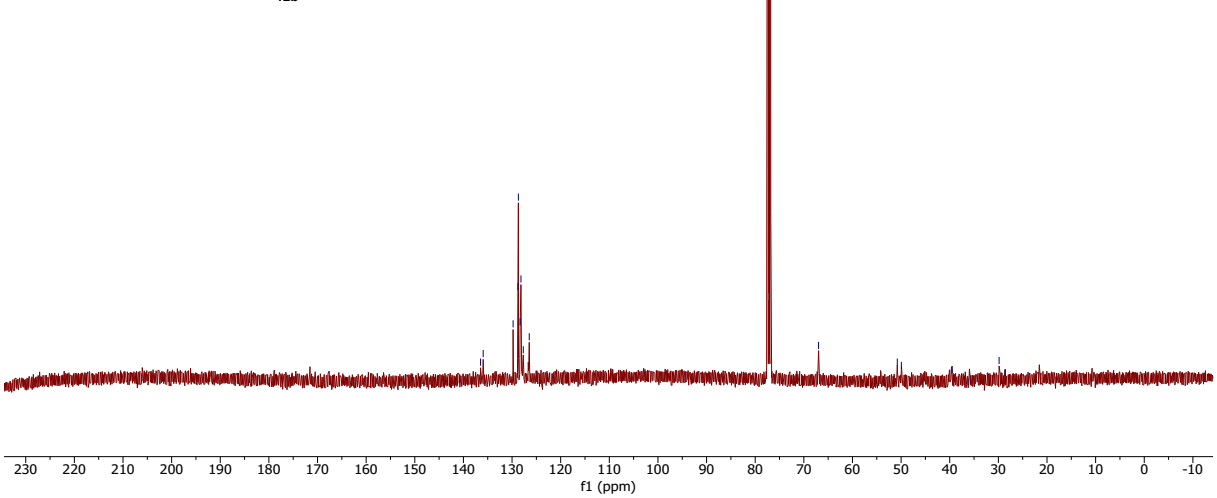
NH
N
O
CH3
N
CH3

12b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40
30
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0
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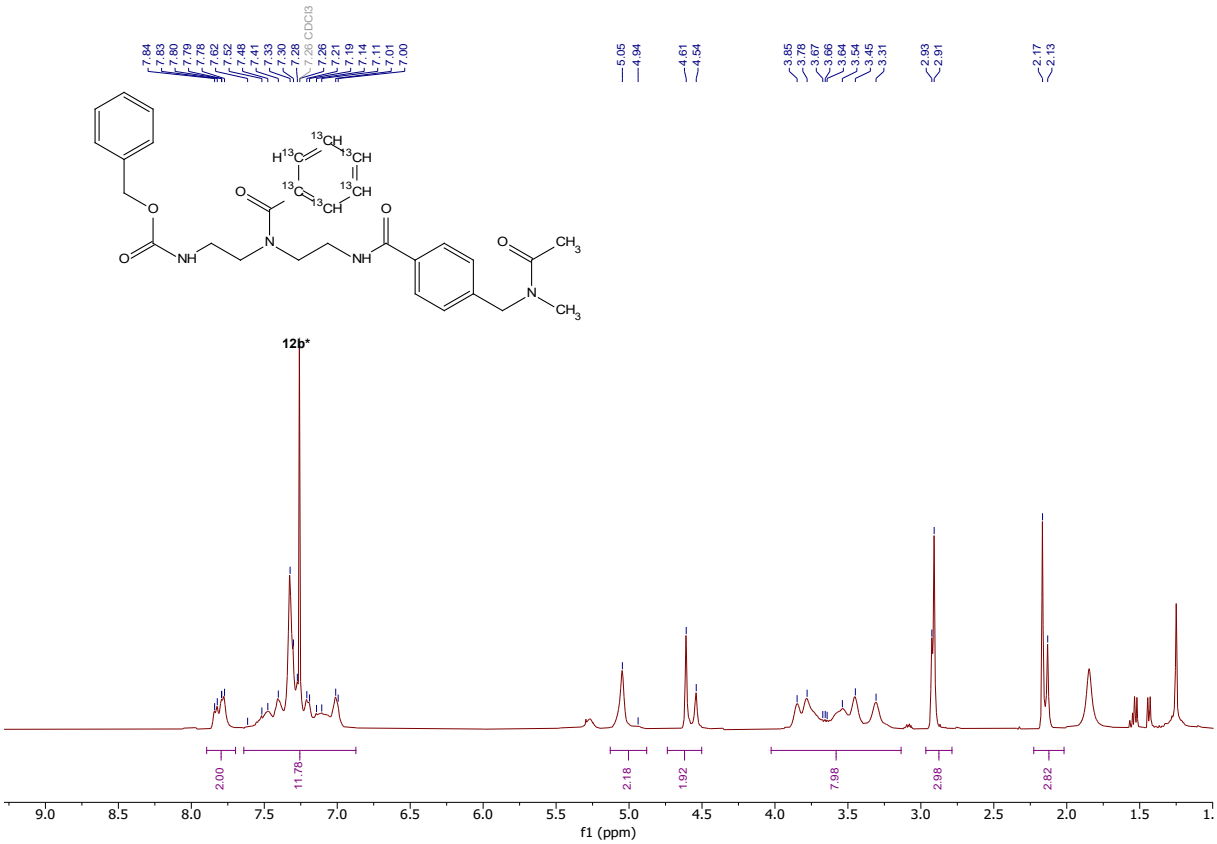
f1 (ppm)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7.80
7.79
7.78
7.62
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7.48
7.41
7.33
7.30
7.28
7.26 CDCl3
7.21
7.19
7.14
7.11
7.01
7.00
5.05
4.94
4.61
4.54
3.85
3.78
3.67
3.66
3.64
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3.31
2.93
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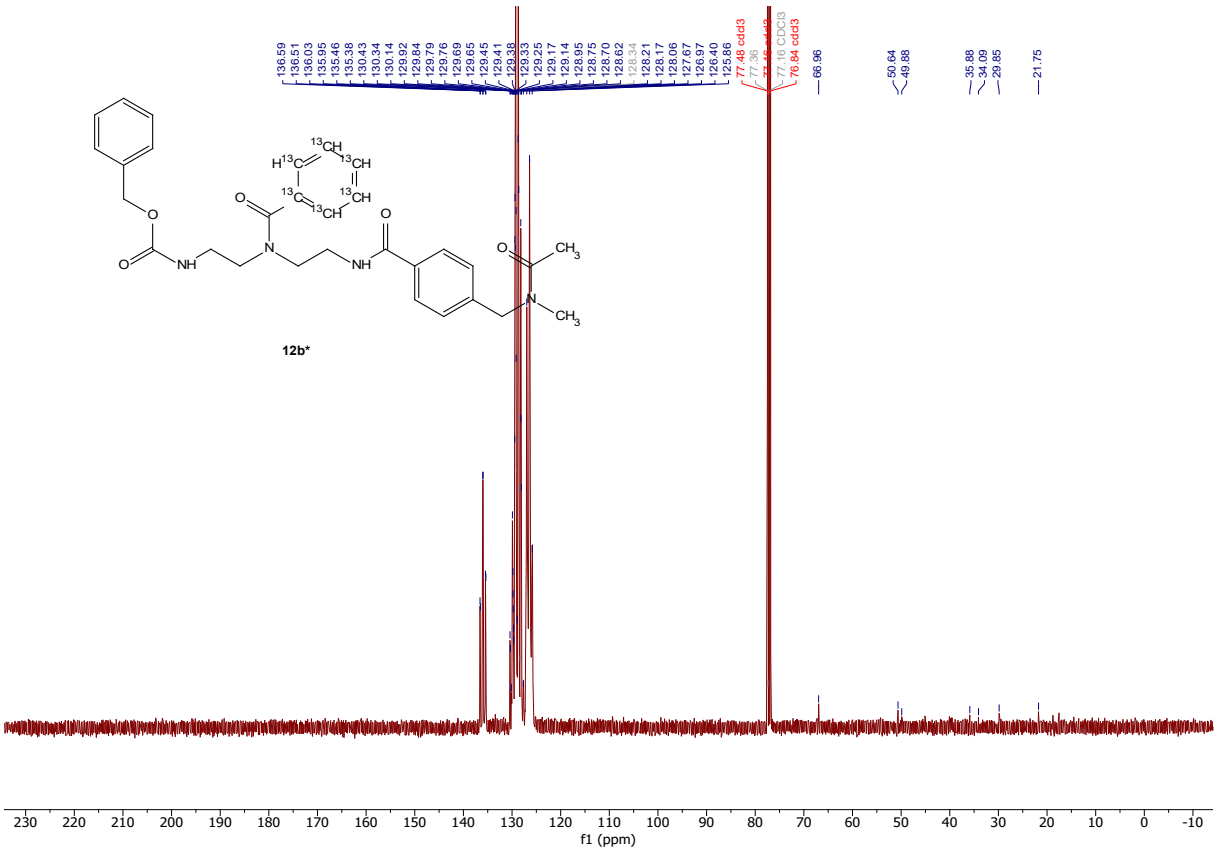
2.98
2.82
f1 (ppm)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129.65
129.45
129.41
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129.25
129.17
129.14
128.95
128.75
128.70
128.62
128.34
128.21
128.17
128.06
127.67
126.97
126.40
125.86
77.48 cdcl3
77.36
77.16 CDCl3
76.84 cdcl3
66.96
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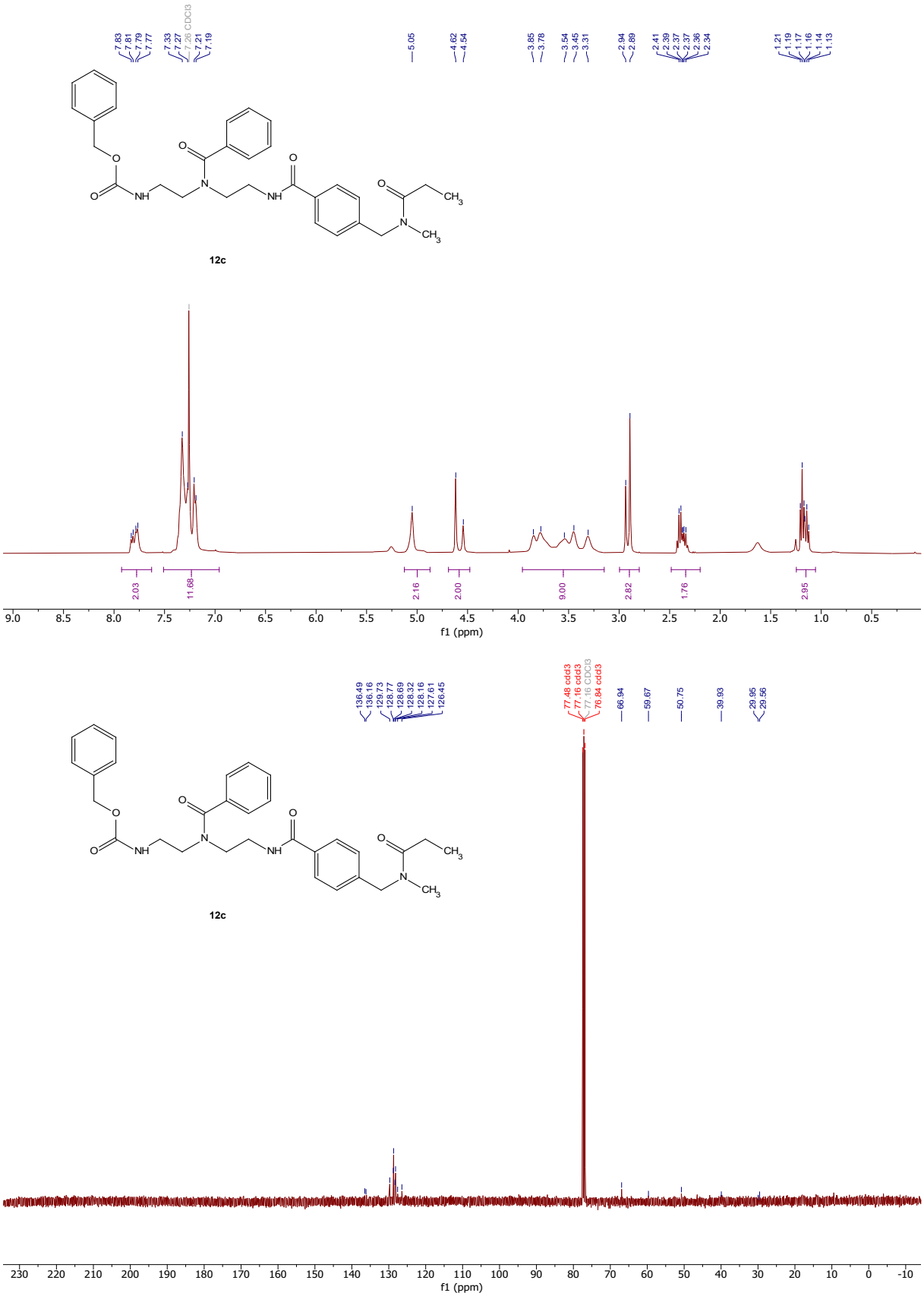
49.88
35.88
34.09
29.85
21.75
12b*
f1 (ppm)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7.27
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7.21
7.19
5.05
4.62
4.54
3.85
3.78
3.54
3.45
3.31
2.94
2.89
2.41
2.39
2.37
2.37
2.36
2.34
1.21
1.19
1.17
1.16
1.14
1.13
12c
2.03
11.68
2.16
2.00
9.00
2.82
1.76
2.95
f1 (ppm)
136.49
136.16
129.73
128.77
128.69
128.32
128.16
127.61
126.45
77.48 cdd3
77.16 cdd3
77.16 CDCl3
76.84 cdd3
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50.75
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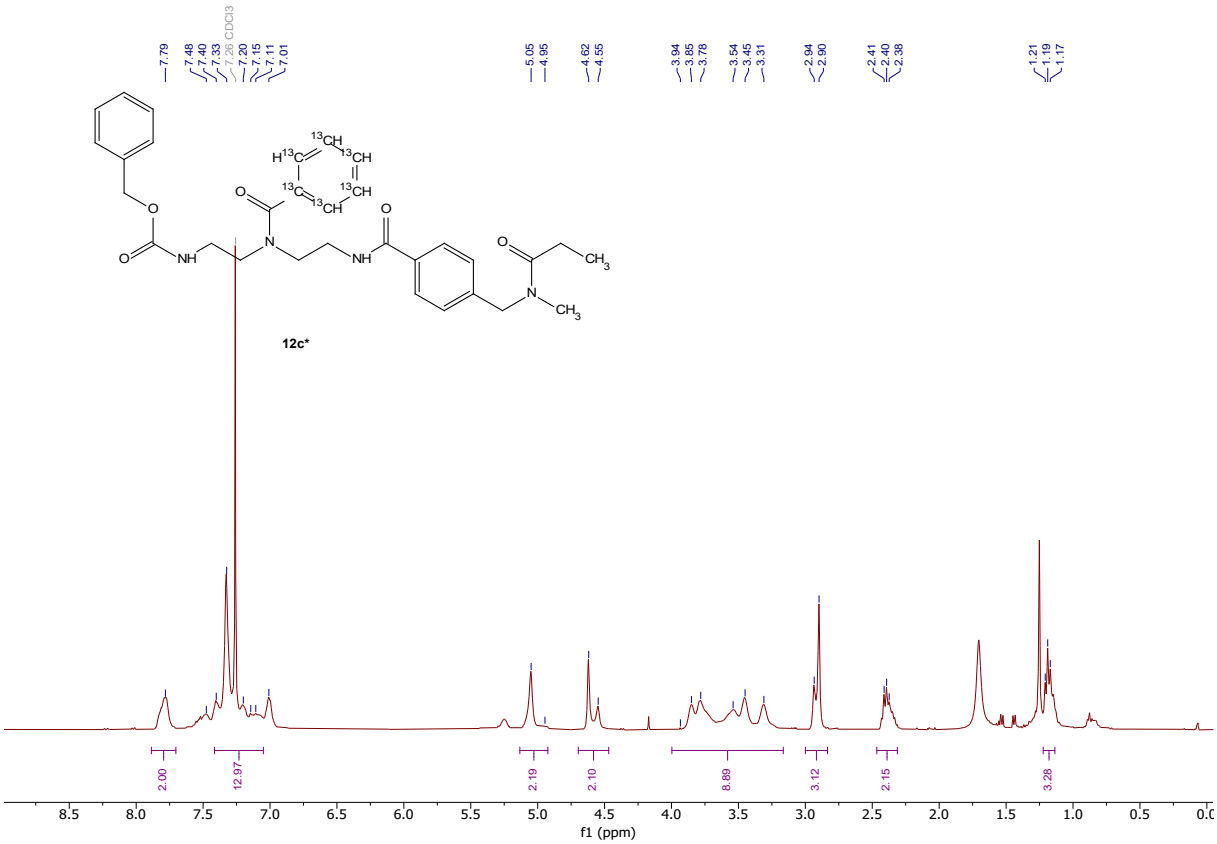
12c
f1 (ppm)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5.05
4.95
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4.55
3.94
3.85
3.78
3.54
3.45
3.31
2.94
2.90
2.41
2.40
2.38
1.21
1.19
1.17
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2.00
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2.19
2.10
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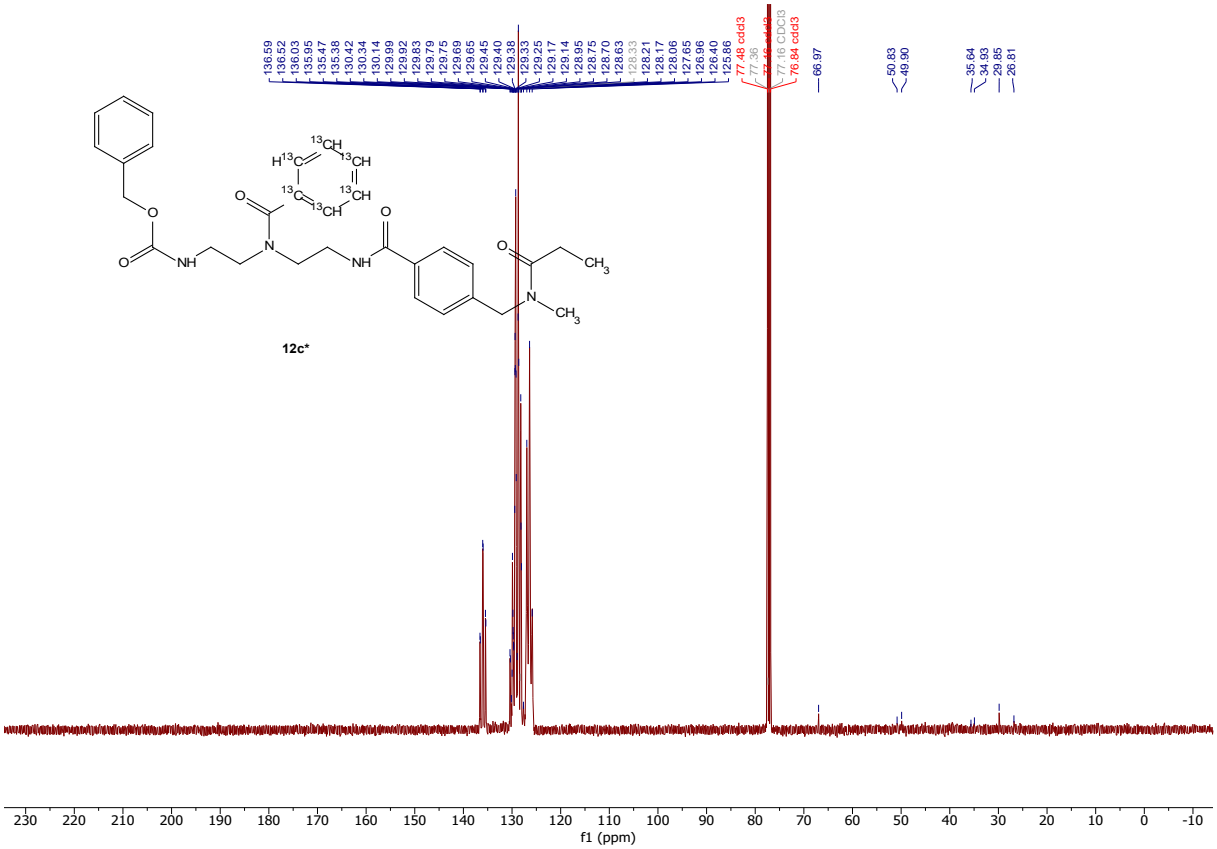
3.12
2.15
3.28
f1 (ppm)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129.33
129.25
129.17
129.14
128.95
128.75
128.70
128.63
128.33
128.21
128.17
128.06
127.65
126.96
126.40
125.86
77.48 cdd3
77.36
77.16 CDCl3
76.84 cdd3
66.97
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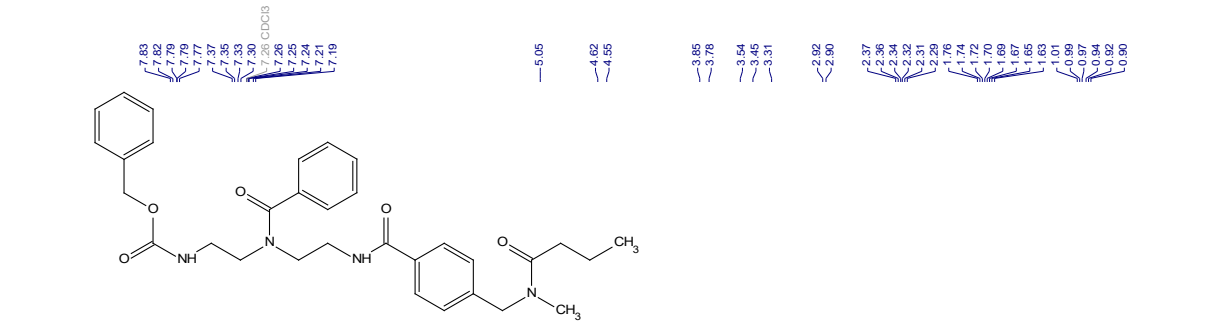
49.90
35.64
34.93
29.85
26.81
12c*
f1 (ppm)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7.26 CDCl3
7.26
7.25
7.24
7.21
7.19
5.05
4.62
4.55
3.85
3.78
3.54
3.45
3.31
2.92
2.90
2.37
2.36
2.34
2.32
2.31
2.29
1.76
1.74
1.72
1.70
1.69
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1.01
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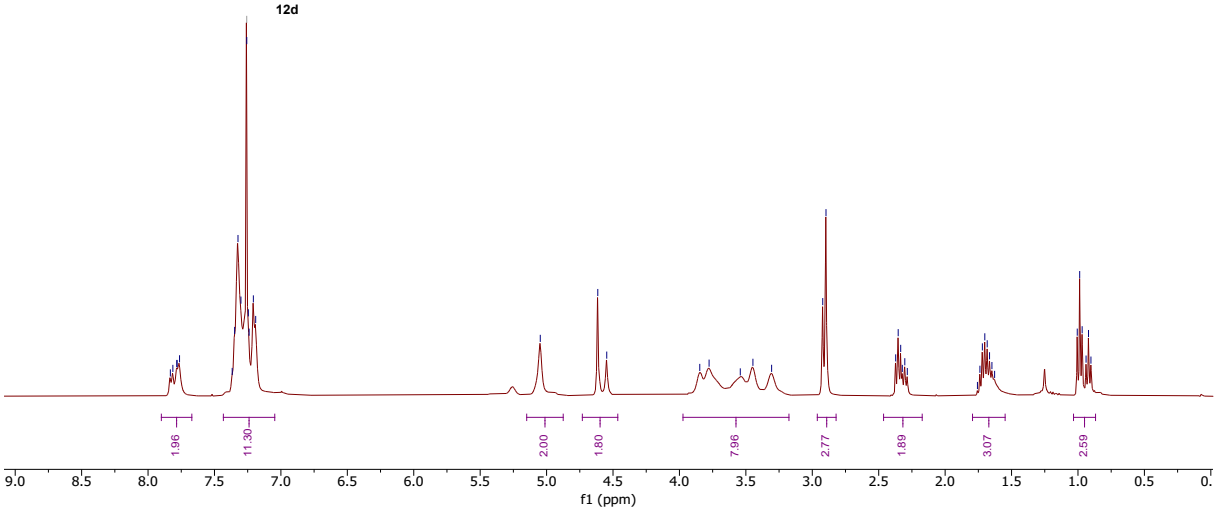
N
CH3
12d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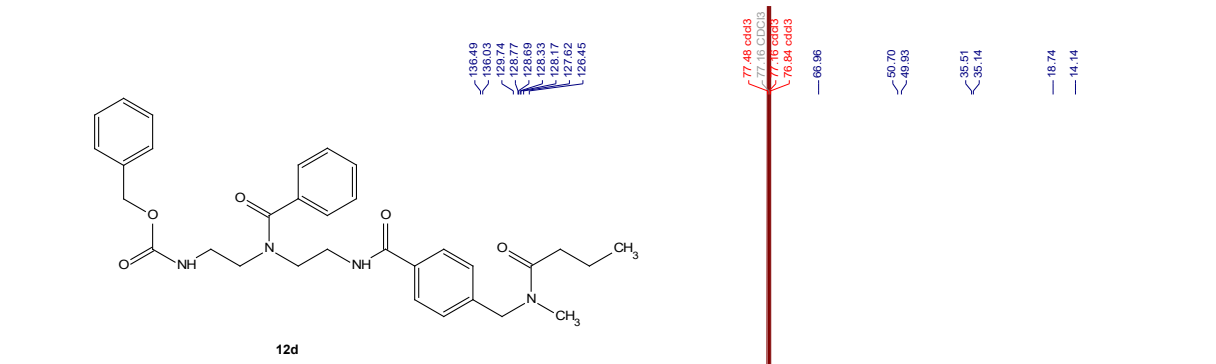
2.77
1.89
3.07
2.59
f1 (ppm)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126.45
77.48 cdd3
77.16 CDCl3
77.16 cdd3
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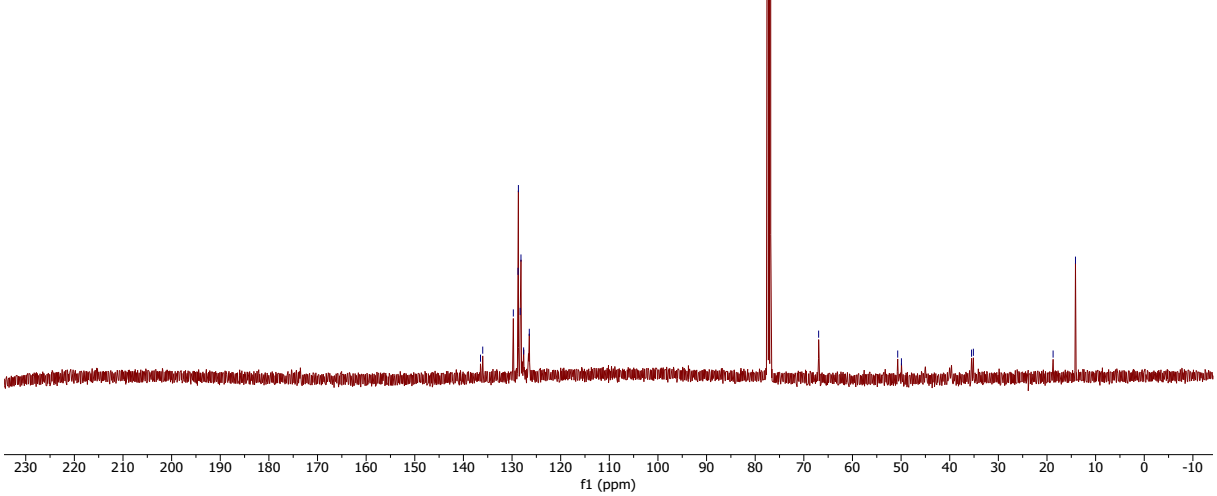
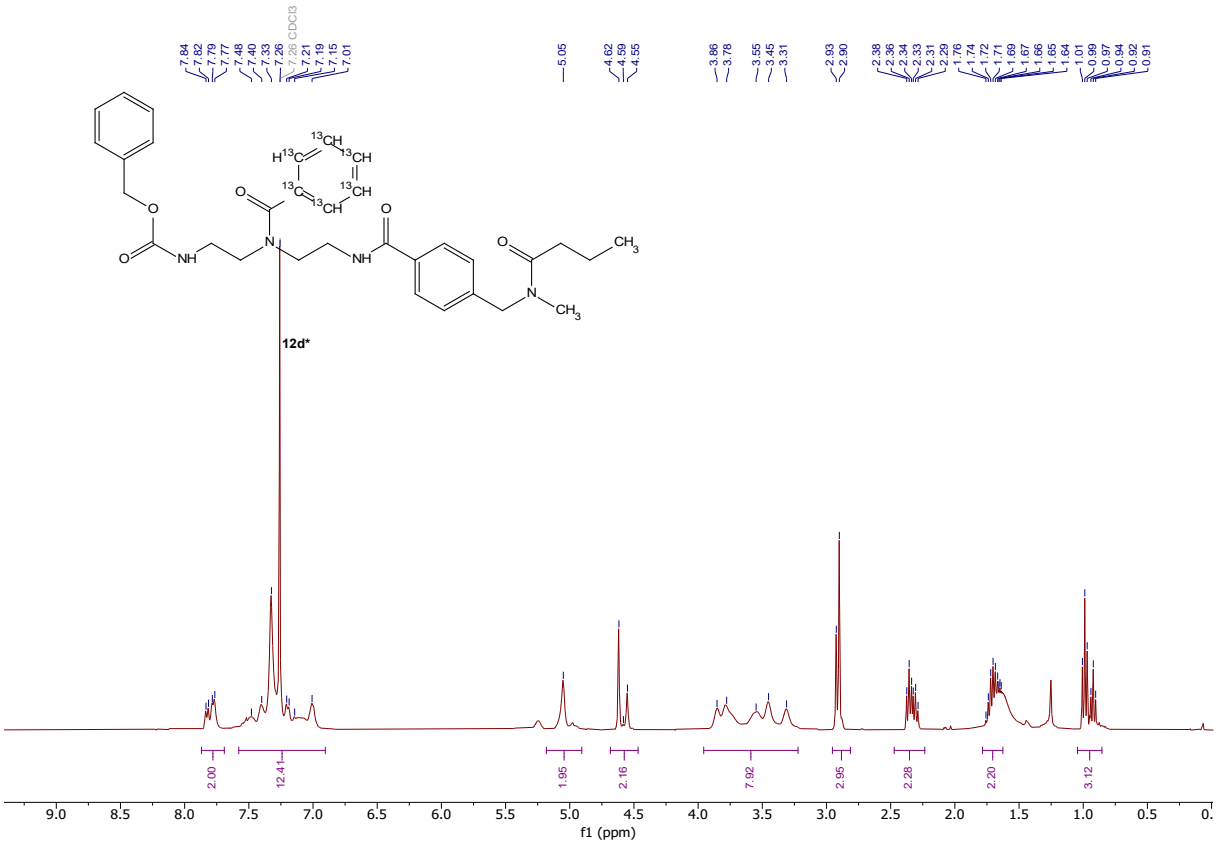
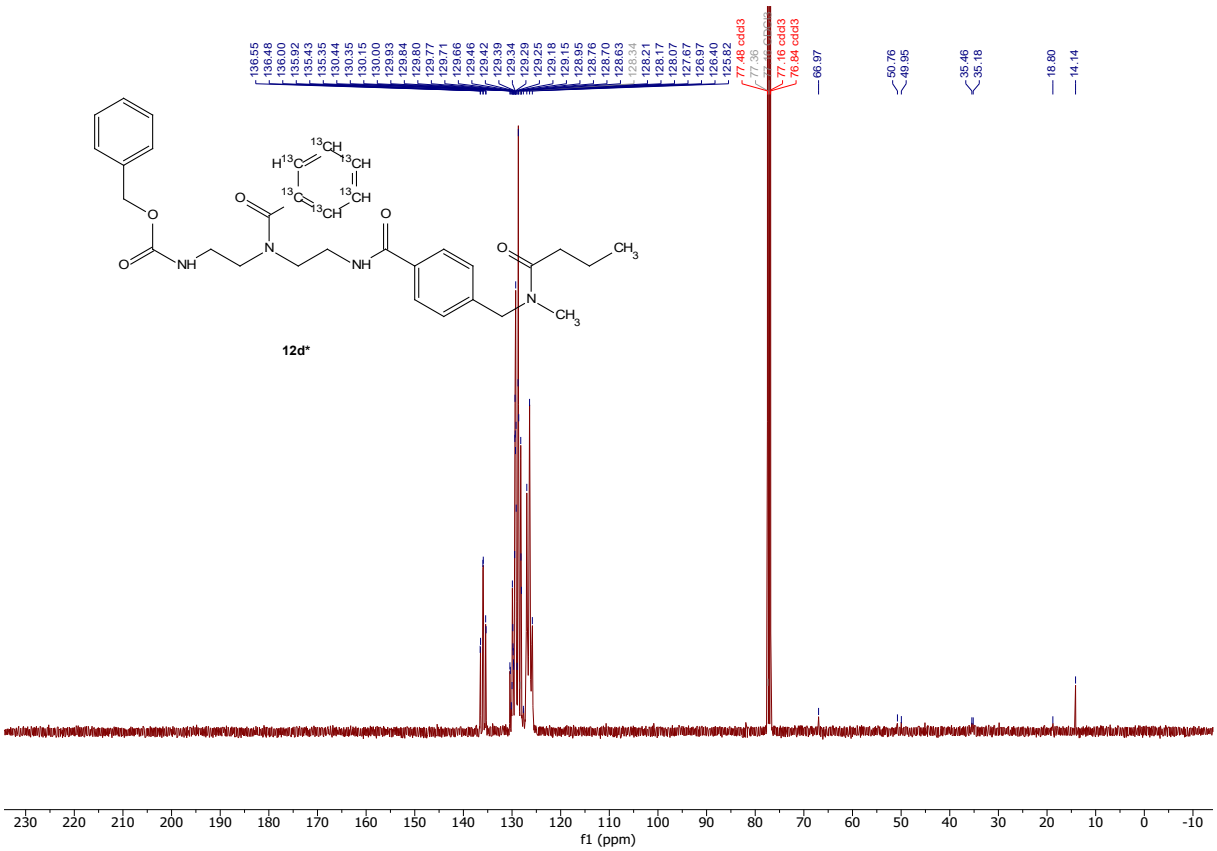
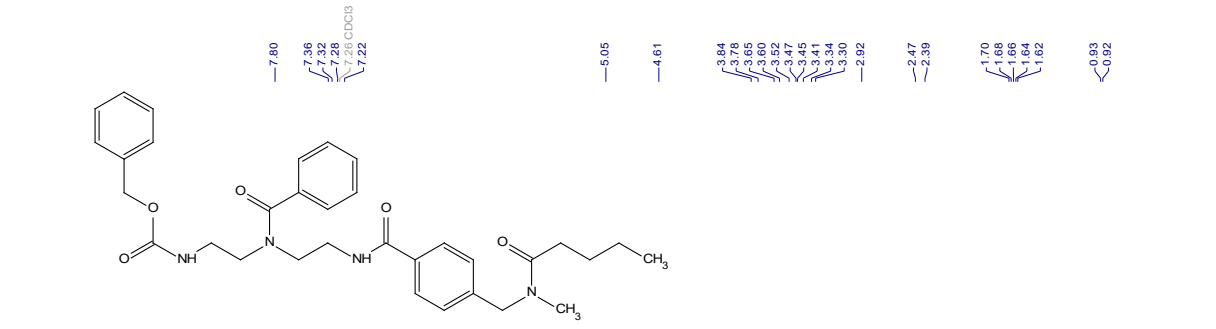
18.74
14.14
O
NH
N
CH3
12d


f1 (ppm)


12d*
f1 (ppm)


12d*
f1 (ppm)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3.78
3.65
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3.52
3.47
3.45
3.41
3.34
3.30
2.92
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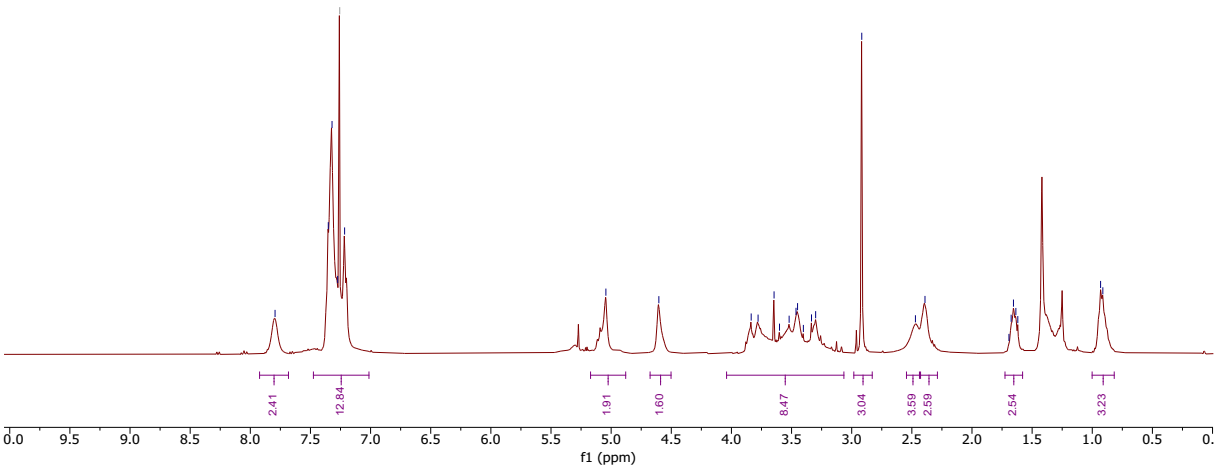
1.68
1.66
1.64
1.62
0.93
0.92
O
NH
N
CH3
12e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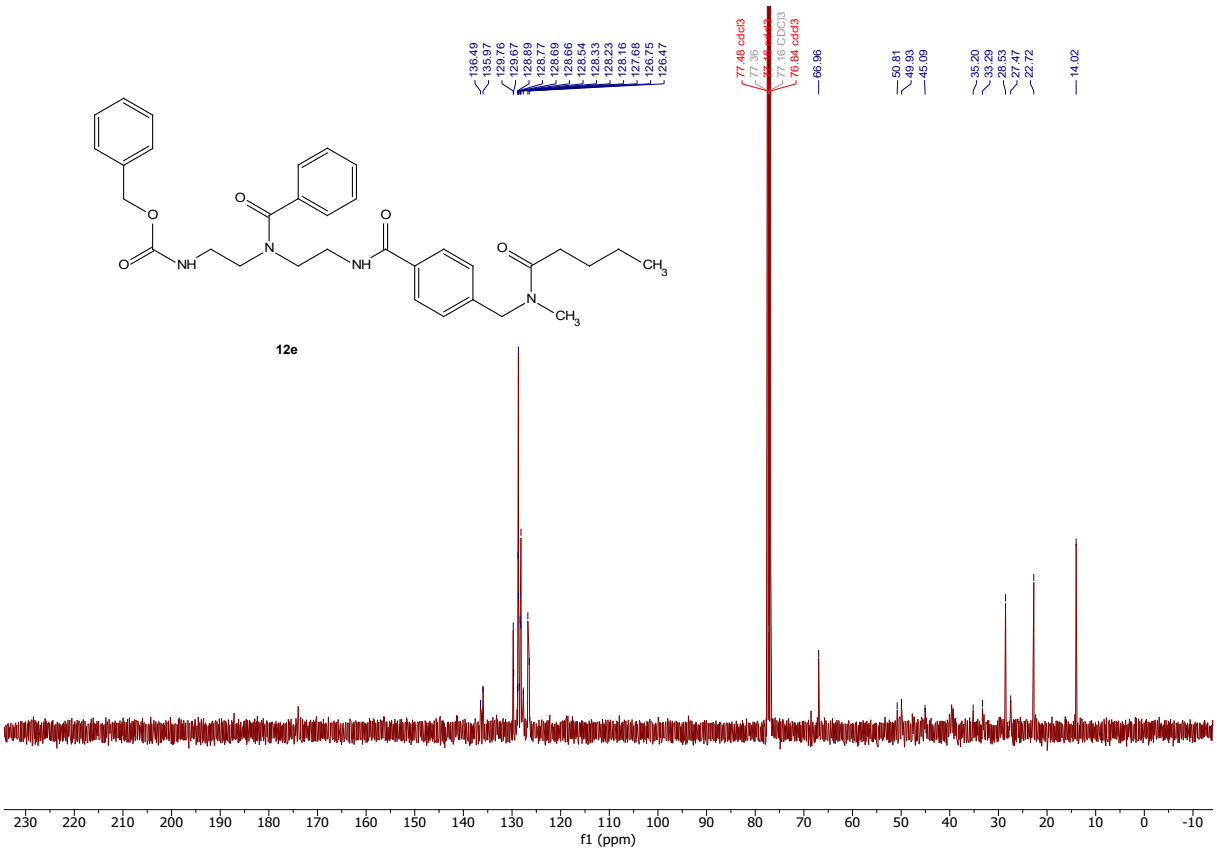
3.04
3.59
2.59
2.54
3.23
f1 (ppm)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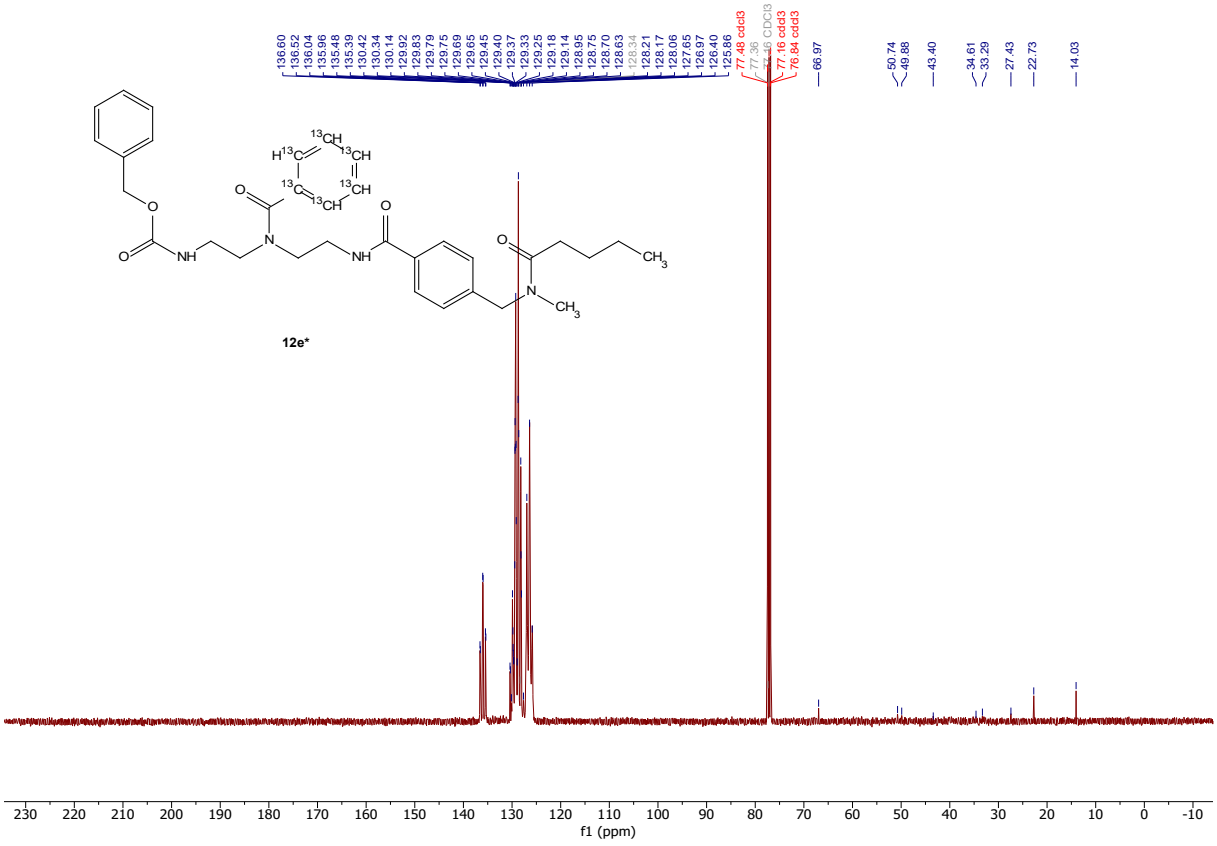
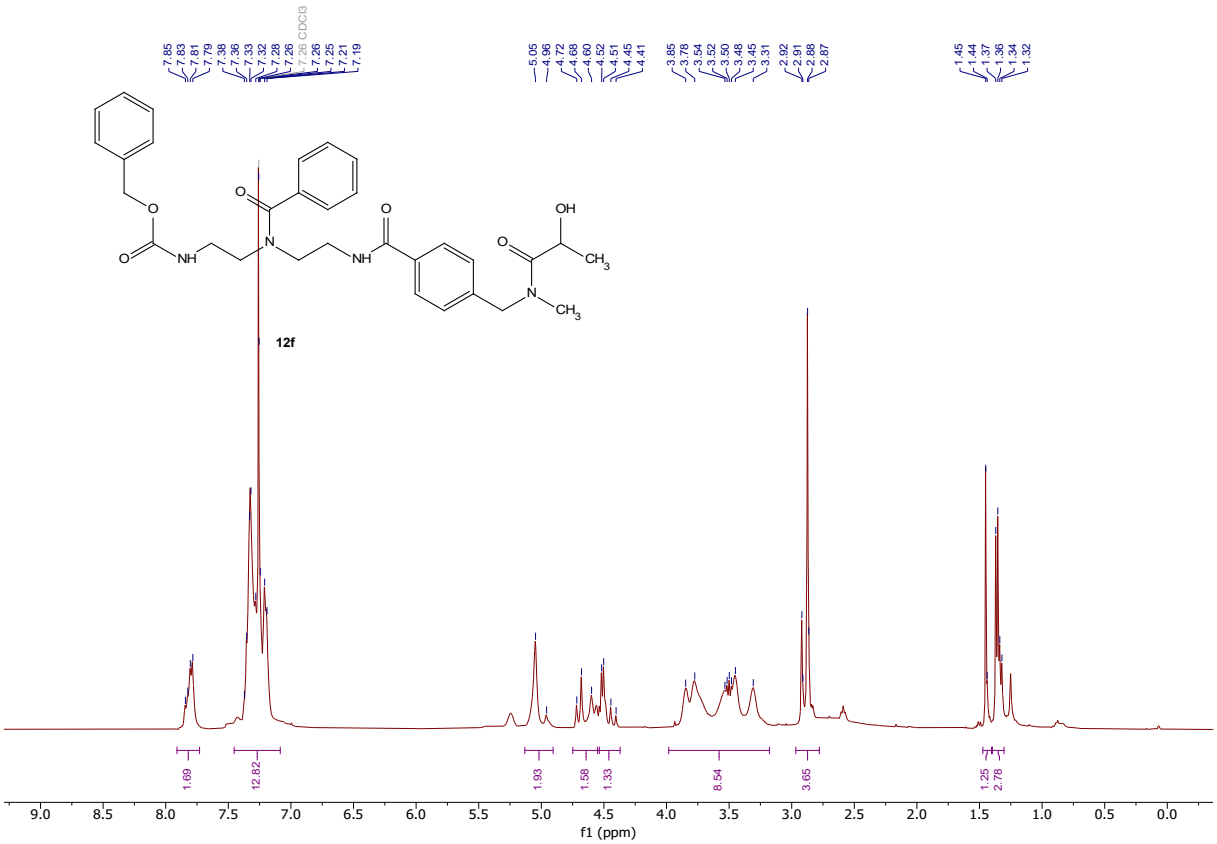
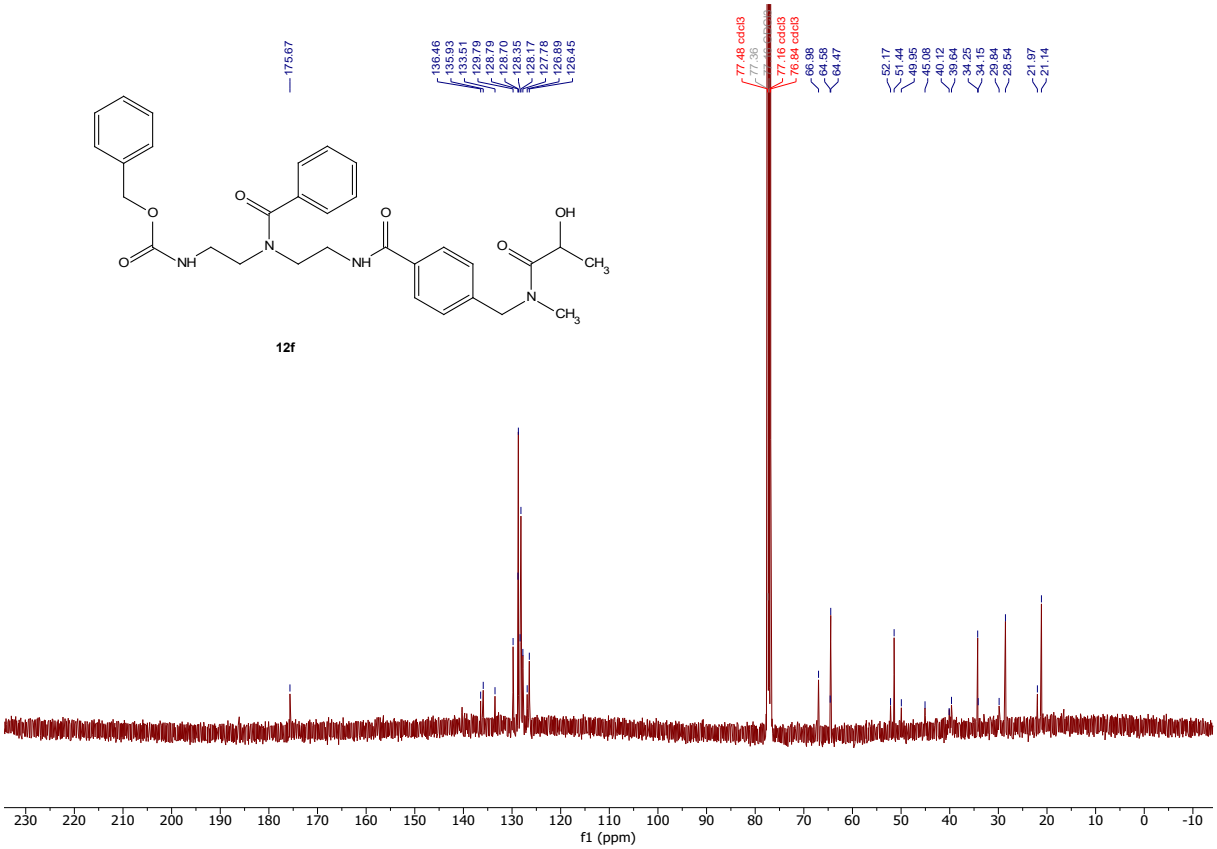
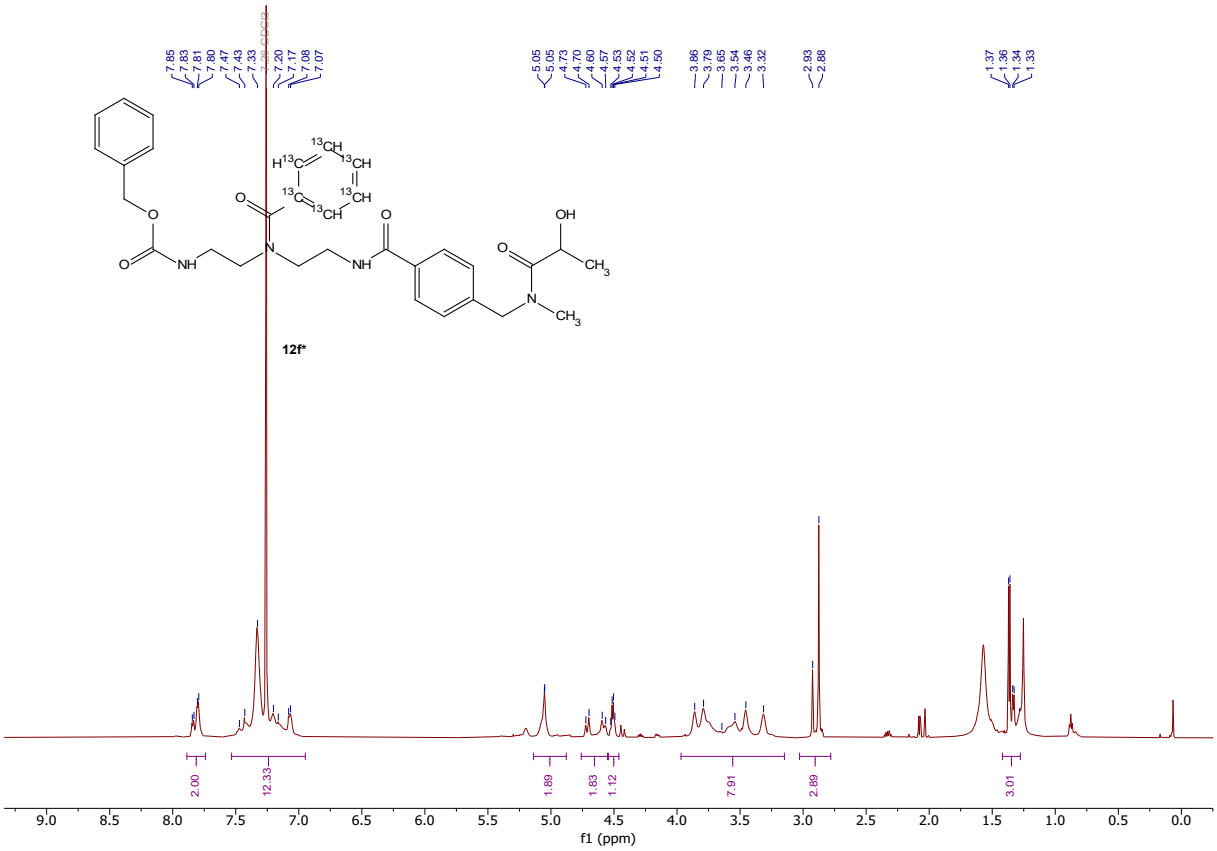
128.23
128.16
127.68
126.75
126.47
77.48 cdcl3
77.16 CDCl3
76.84 cdcl3
66.96
50.81
49.93
45.09
35.20
33.29
28.53
27.47
22.72
14.02
O
NH
N
CH3
12e
f1 (ppm)


12e*
A (dd) 7.80
B (m) 7.22
C (s) 5.05
D (d) 4.58
E (m) 3.59
F (d) 2.91
G (dt) 2.35
H (ddt) 1.36
I (dq) 1.66
J (dt) 0.91
f1 (ppm)


12e*
f1 (ppm)


12f
f1 (ppm)


12f
f1 (ppm)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7.08
7.07
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5.05
4.73
4.70
4.60
4.57
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4.51
4.50
3.86
3.79
3.65
3.54
3.46
3.32
2.93
2.88
1.37
1.36
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1.33
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1.83
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7.91
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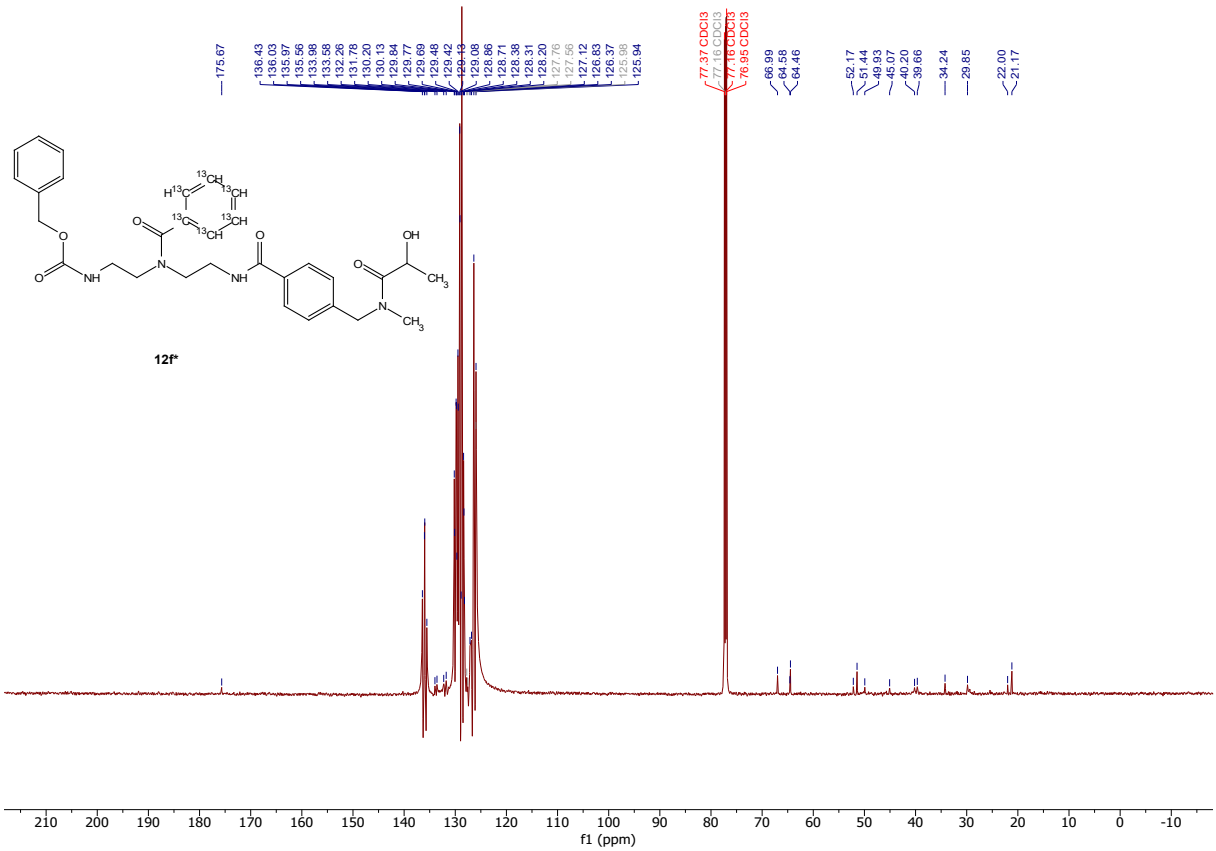
3.01
f1 (ppm)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129.69
129.48
129.42
129.08
128.86
128.71
128.38
128.31
128.20
127.12
126.83
126.37
77.37 CDCl3
77.16 CDCl3
76.95 CDCl3
68.99
64.58
64.46
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51.44
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45.07
40.20
39.66
34.24
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22.00
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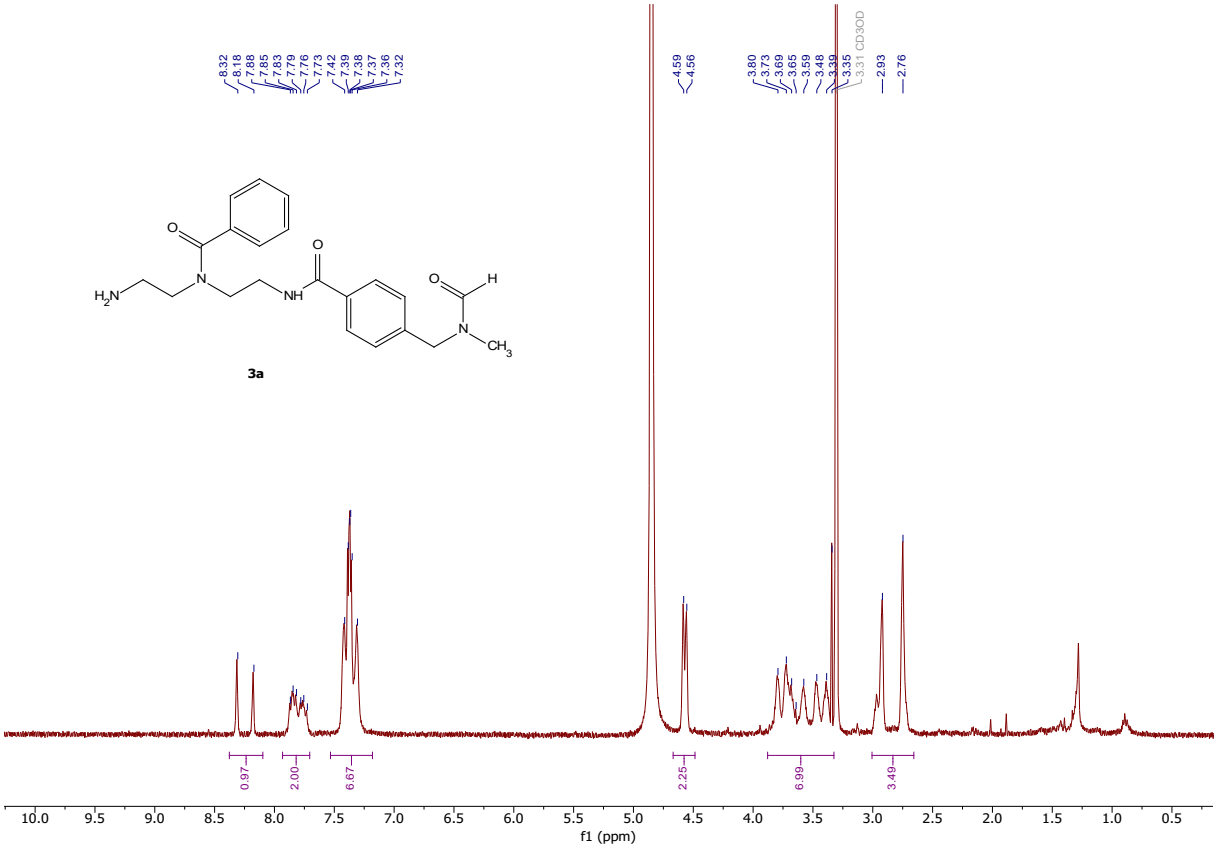
12f*
f1 (ppm)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7.76
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7.38
7.37
7.36
7.32
4.59
4.56
3.80
3.73
3.69
3.65
3.59
3.48
3.39
3.35
3.31 CD3OD
2.93
2.76
O
N
NH
O
O
H
H2N
N
CH3
3a
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2.00
6.67
2.25
6.99
3.49
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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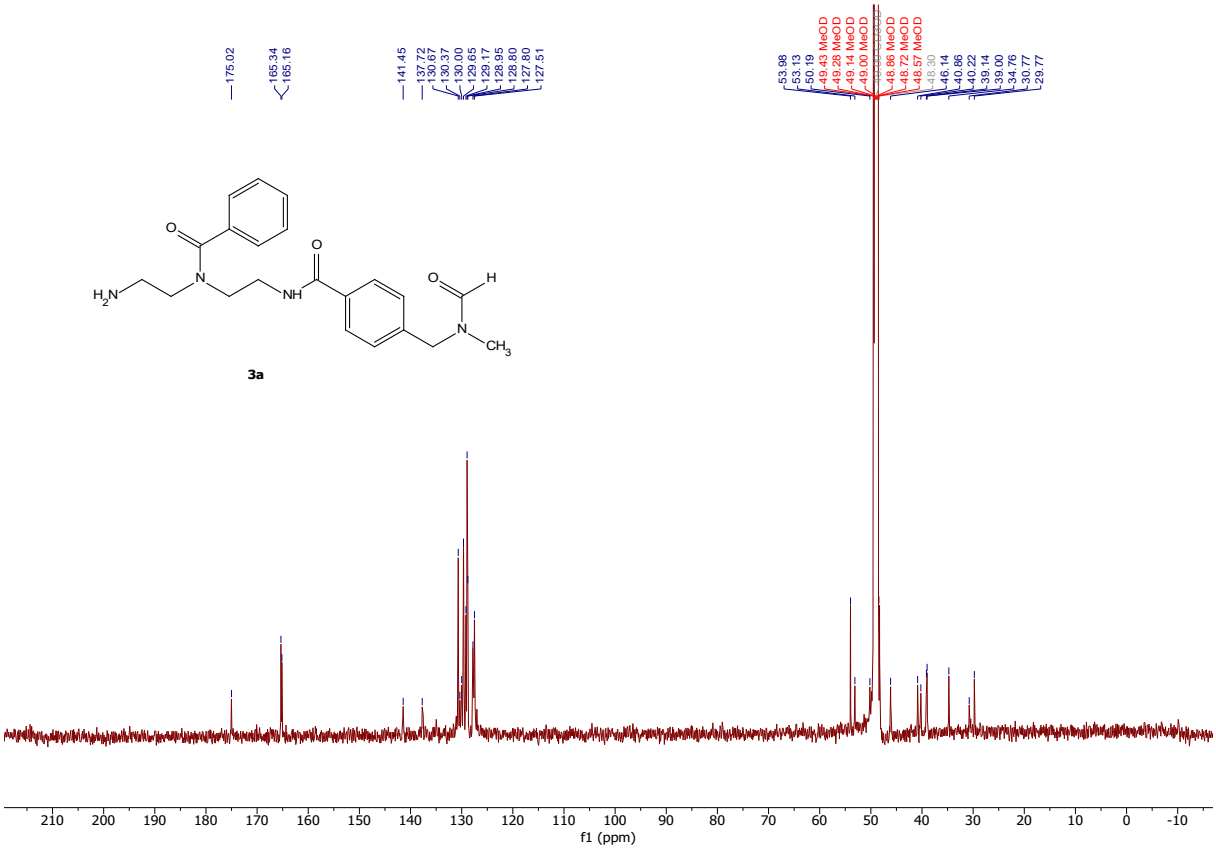
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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127.51
53.98
53.13
50.19
49.43 MeOD
49.29 MeOD
49.14 MeOD
49.00 MeOD
48.86 MeOD
48.72 MeOD
48.57 MeOD
48.30
46.14
40.86
40.22
39.14
39.00
34.76
30.77
29.77
O
N
NH
O
O
H
H2N
N
CH3
3a
210
200
190
180
170
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100
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80
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60
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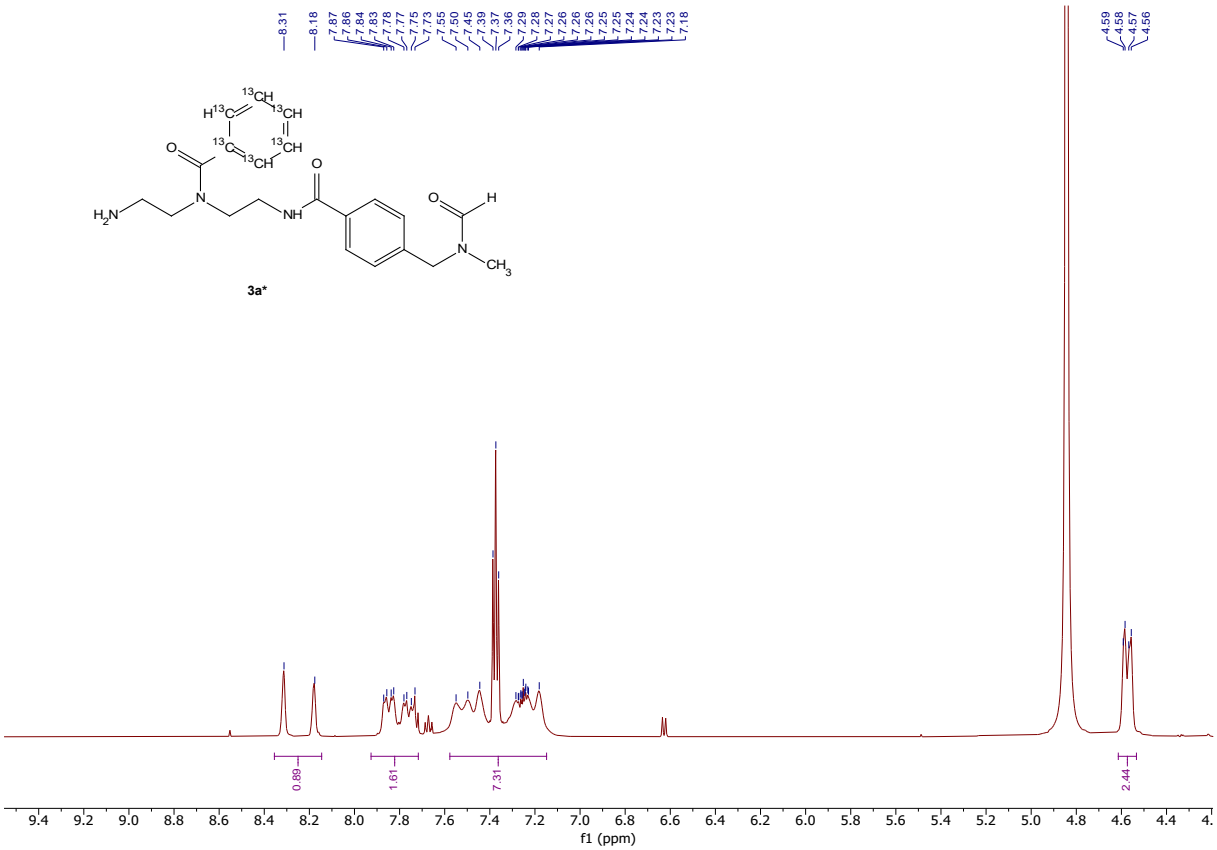
40
30
20
10
0
-10
f1 (ppm)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7.37
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7.18
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4.58
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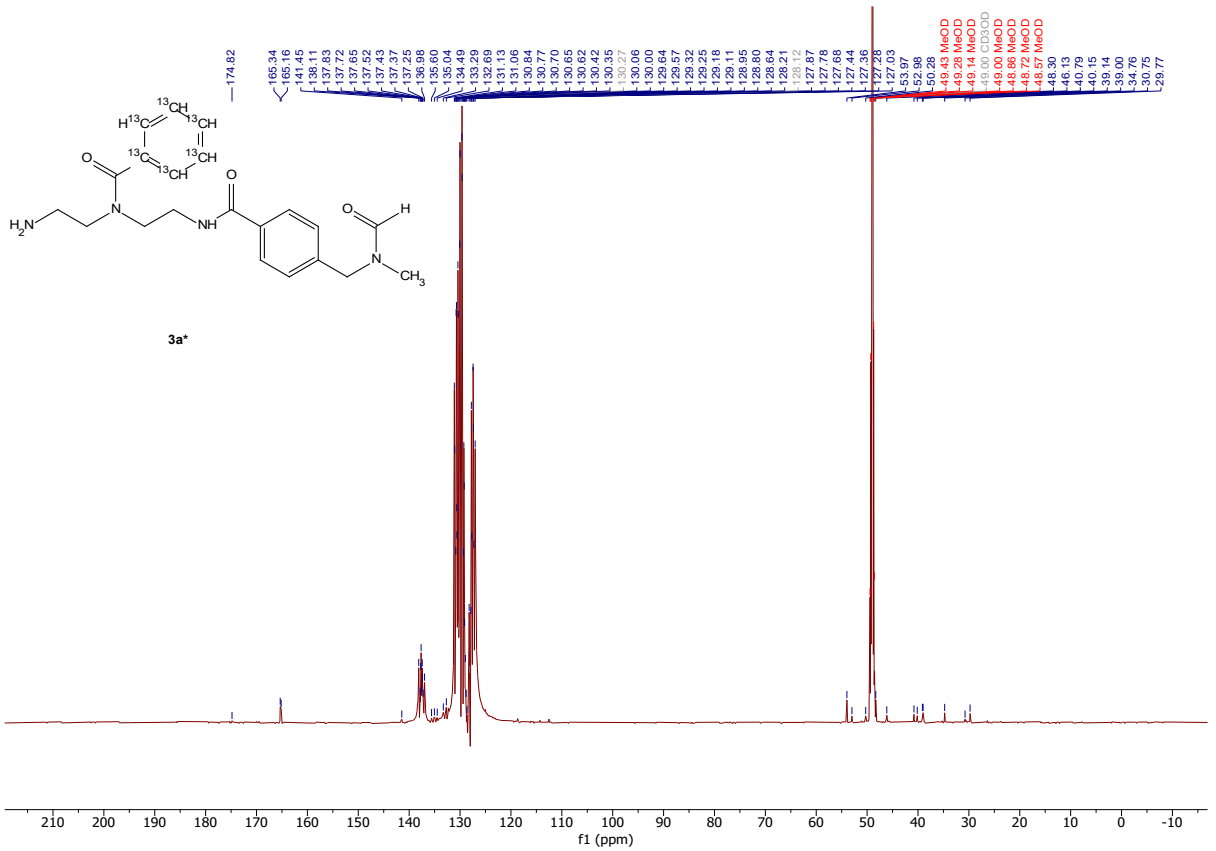
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48.57 MeOD
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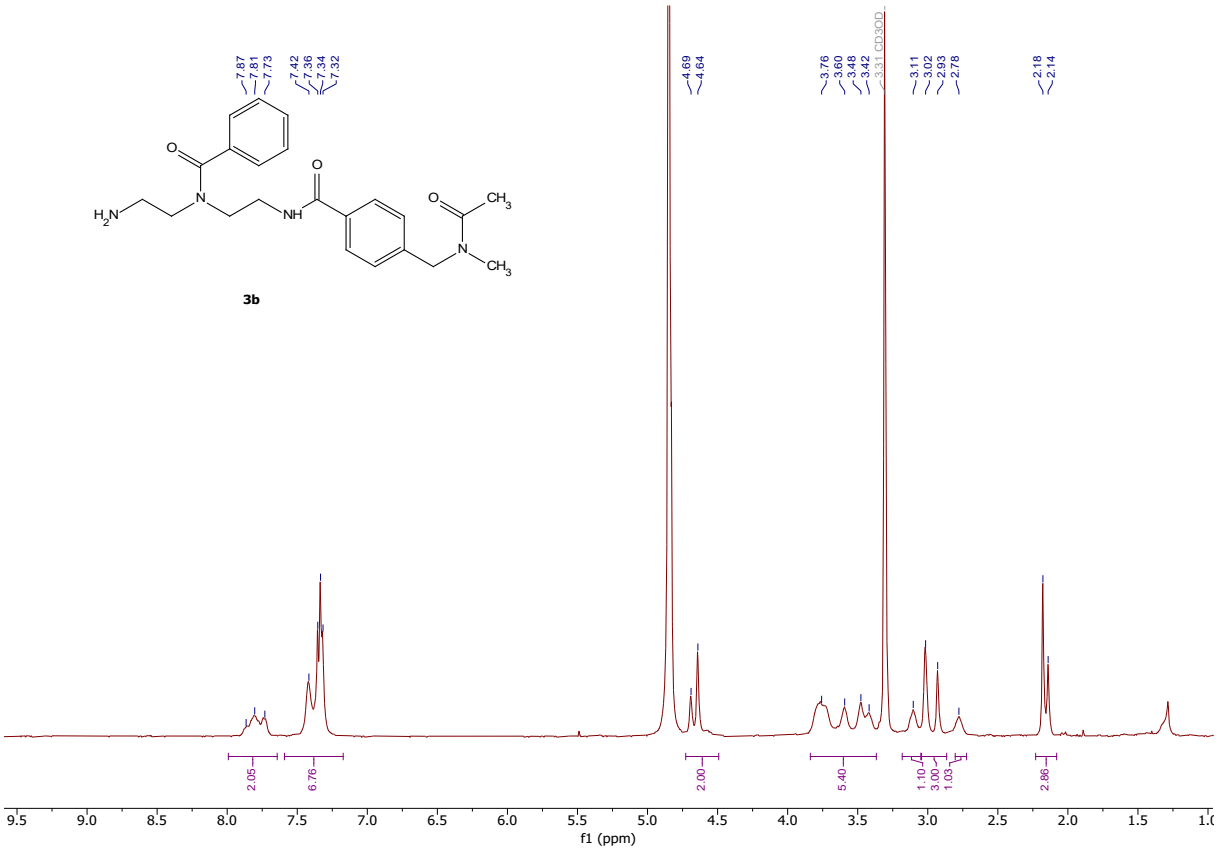
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3.42
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3.00
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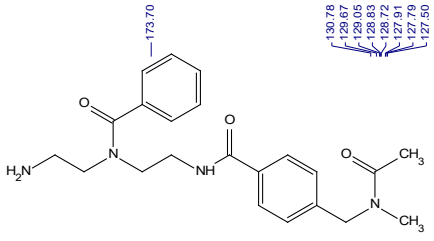
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49.00 MeOD
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48.72 MeOD
48.57 MeOD
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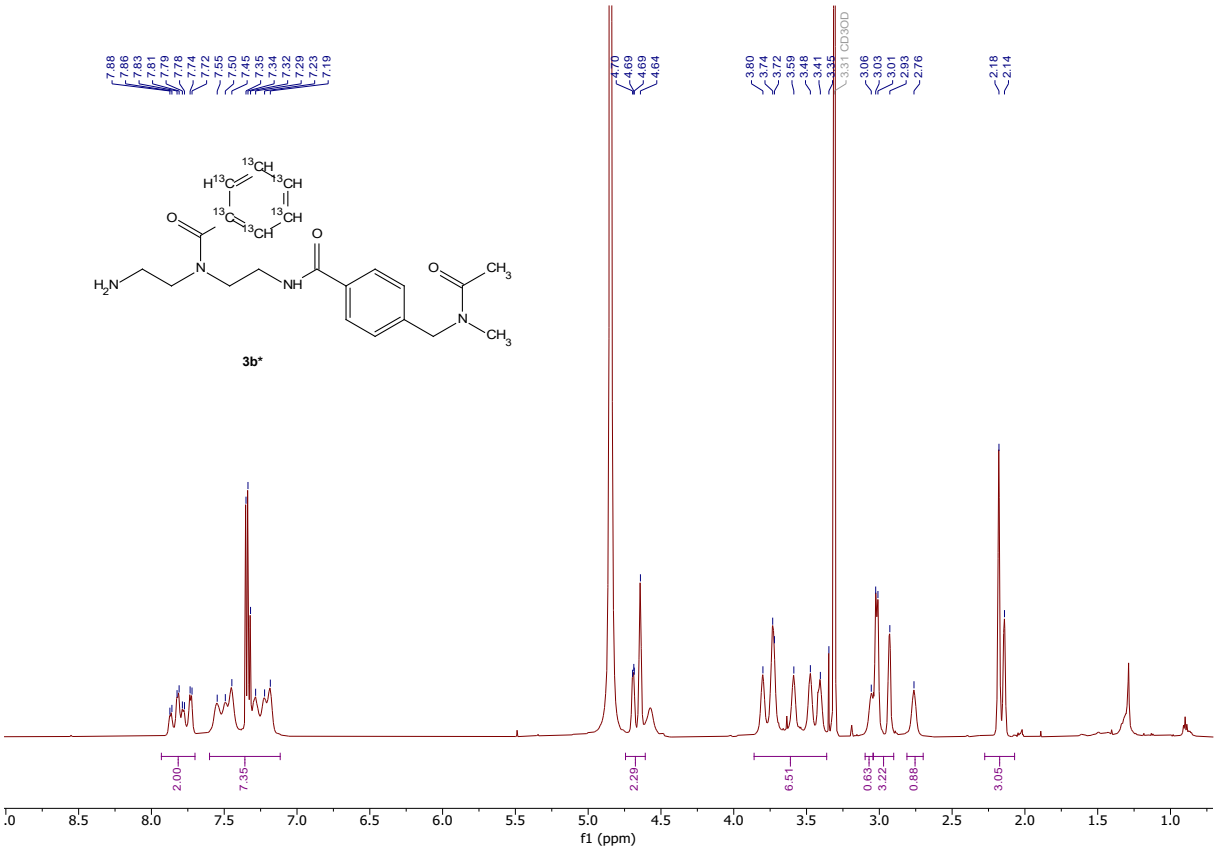
21.34
f1 (ppm)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7.29
7.23
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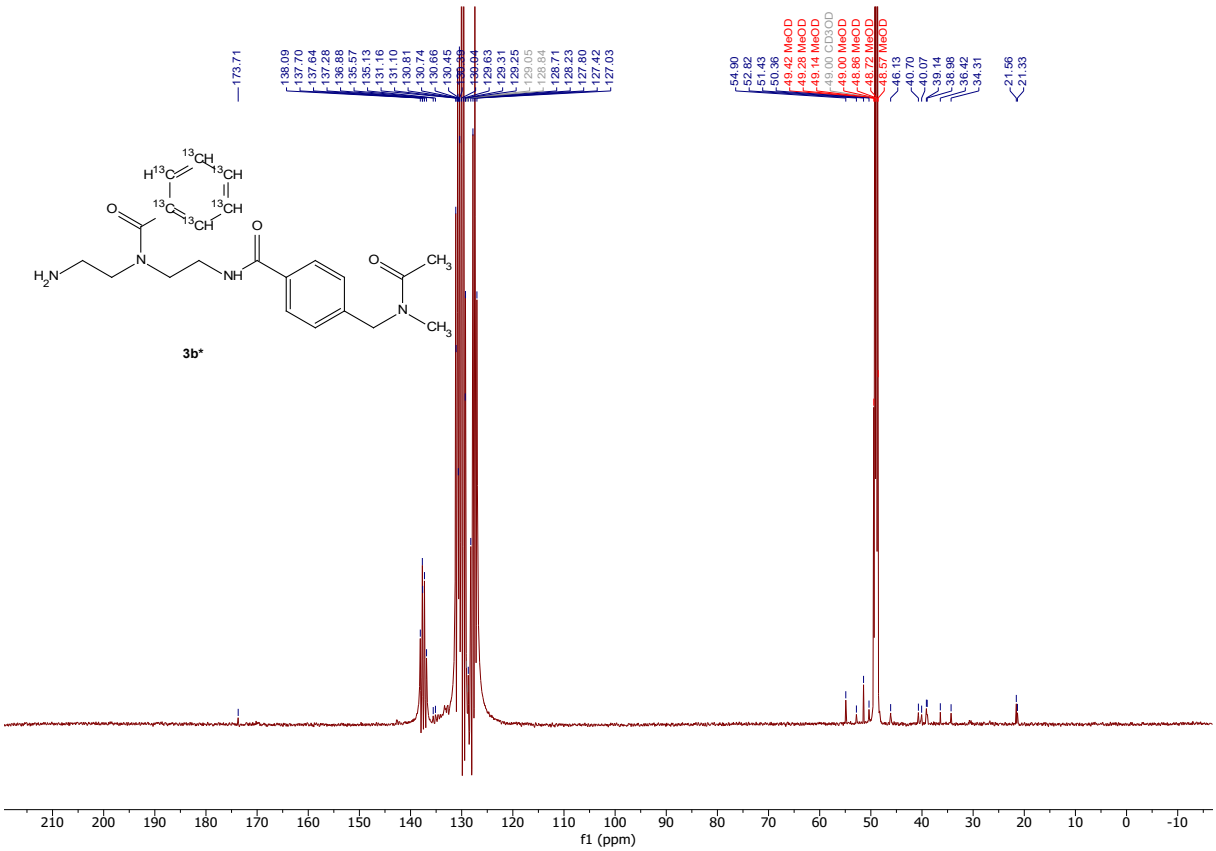
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48.57 MeOD
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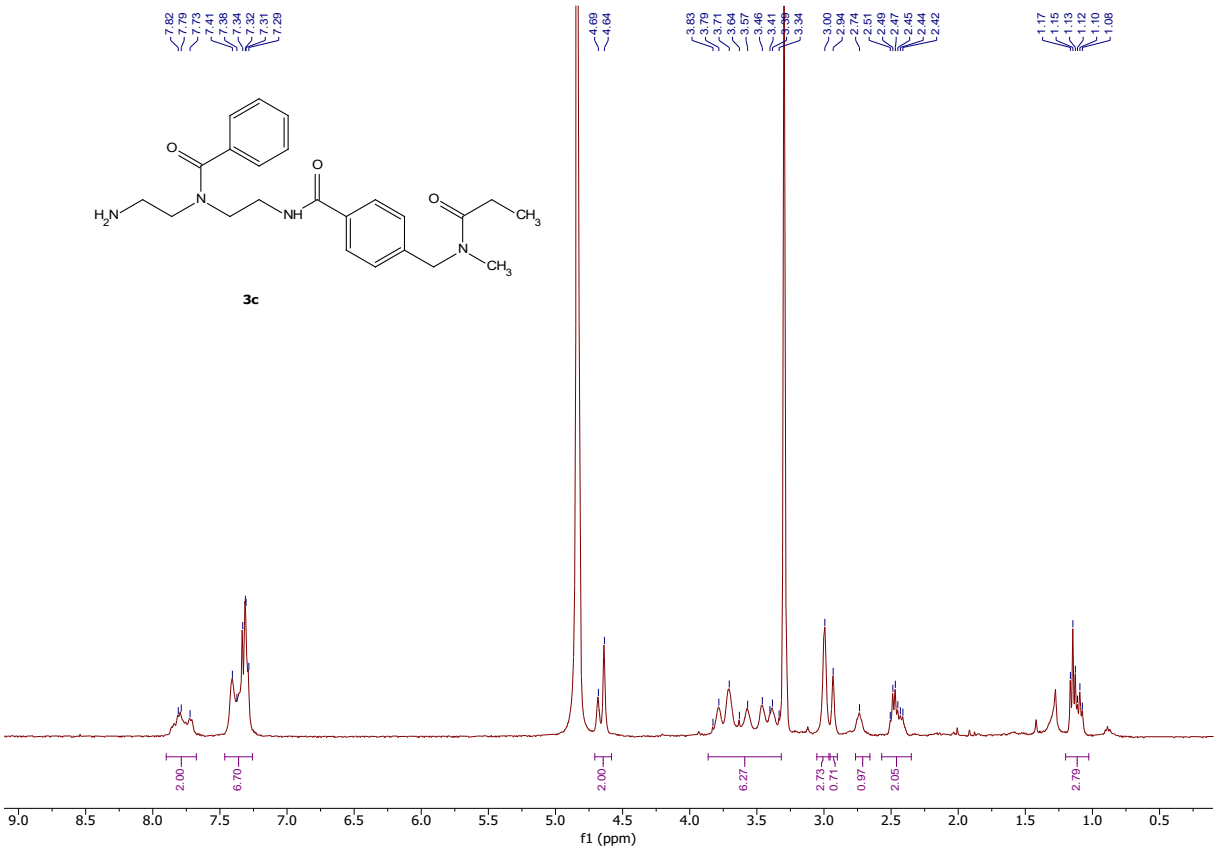
f1 (ppm)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1.17
1.15
1.13
1.12
1.10
1.08
2.00
6.70
2.00
6.27
2.73
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2.79
f1 (ppm)

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O
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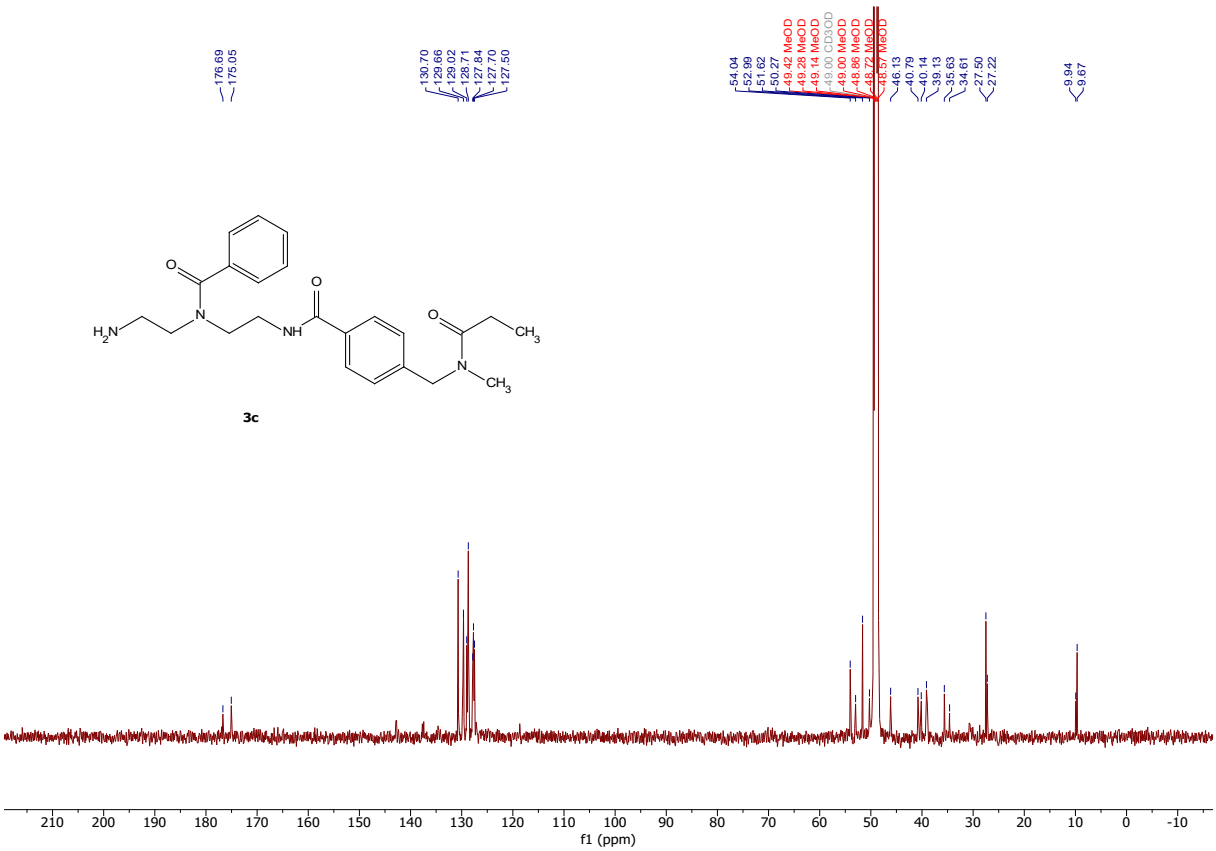
CH3
H2N
CH3

3c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54.04
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49.42 MeOD
49.28 MeOD
49.14 MeOD
49.00 CD3OD
48.86 MeOD
48.72 MeOD
48.57 MeOD
46.13
40.79
40.14
39.13
35.63
34.61
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9.67
f1 (ppm)

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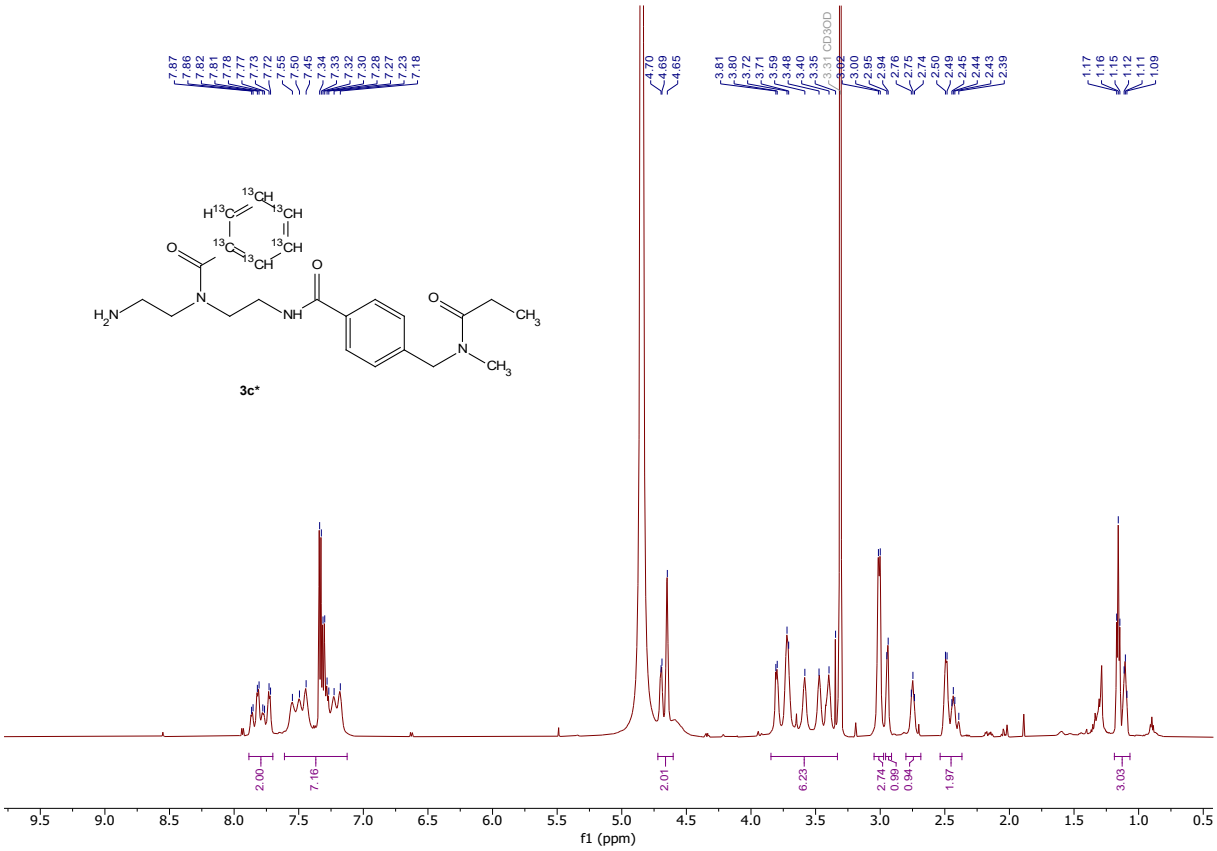
O
O
N
CH3
H2N
CH3

3c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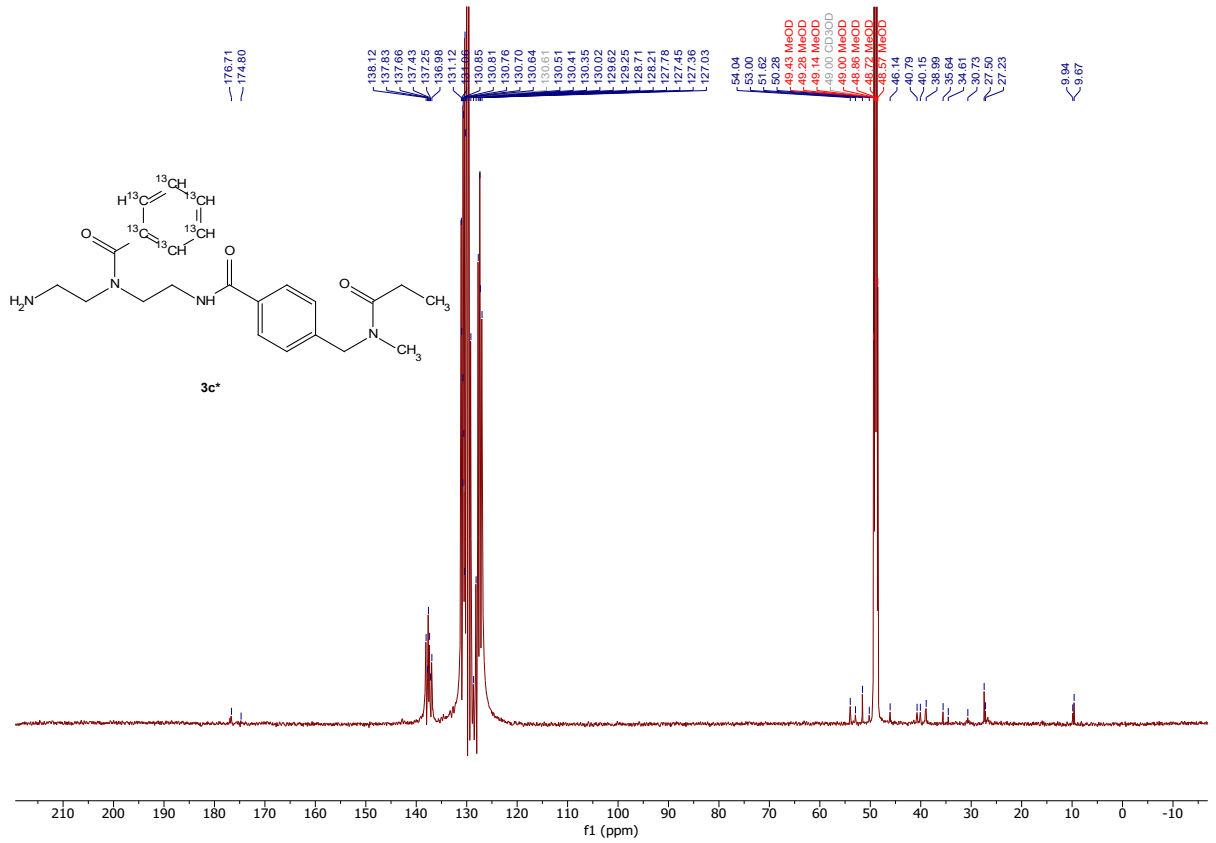
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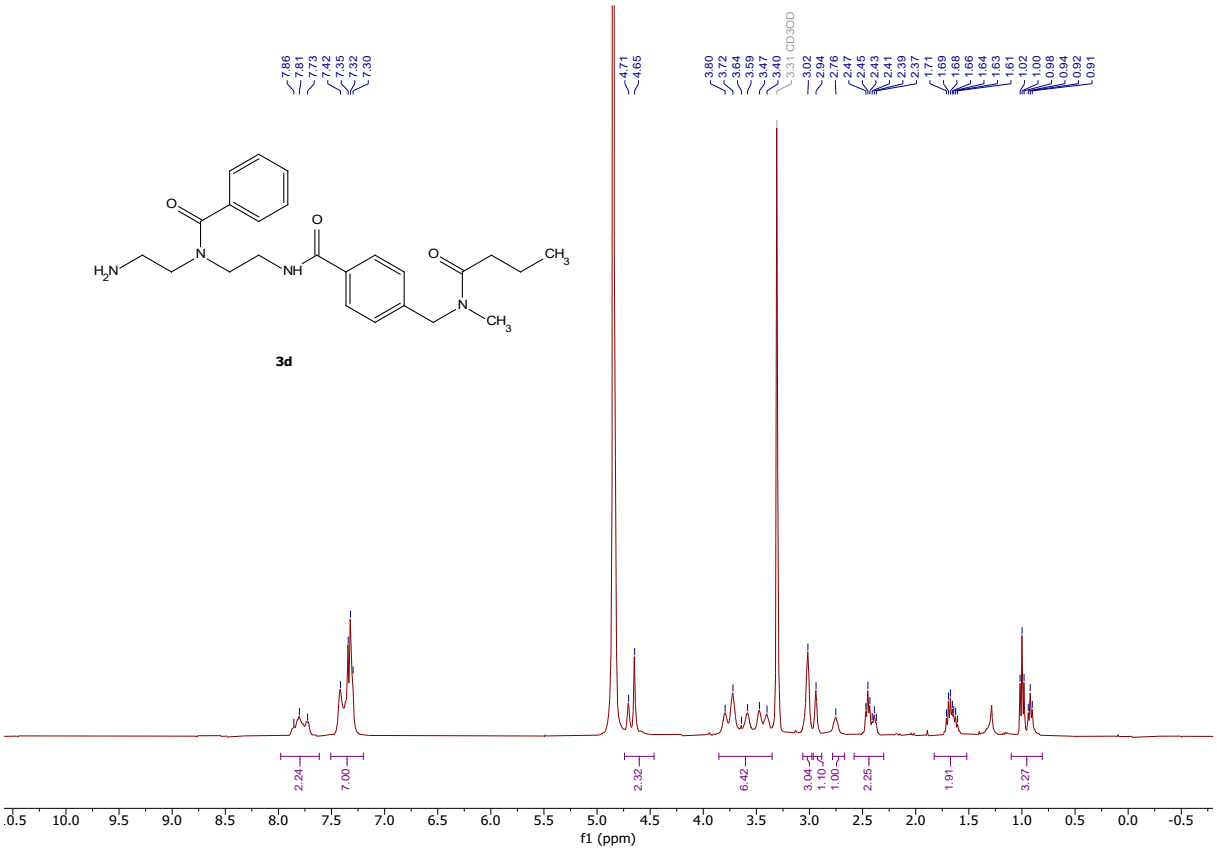
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3.47
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2.37
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1.69
1.68
1.66
1.64
1.63
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0.94
0.92
0.91
O
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NH
O
O
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CH3
CH3
H2N
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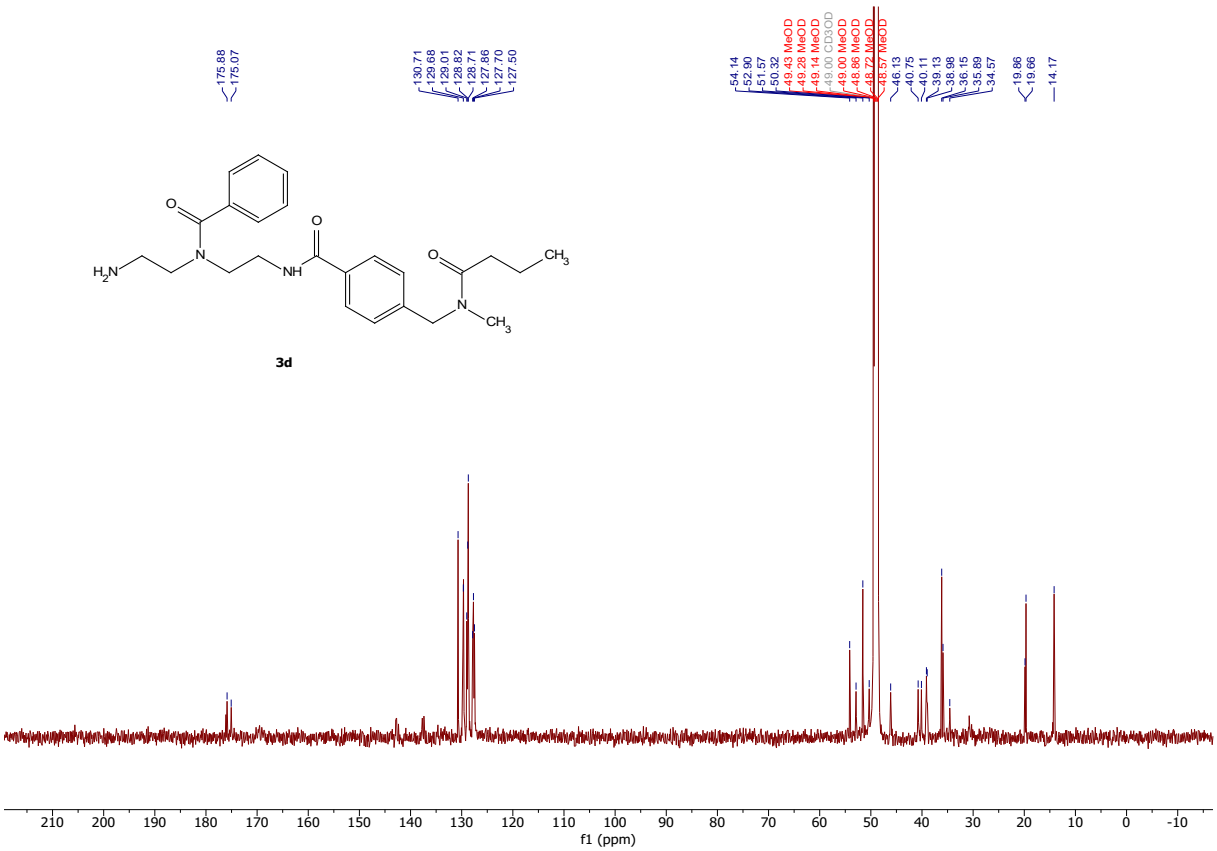
3.27
f1 (ppm)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50.32
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49.00 MeOD
48.86 MeOD
48.72 MeOD
48.57 MeOD
46.13
40.75
40.11
39.13
38.98
36.15
35.89
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O
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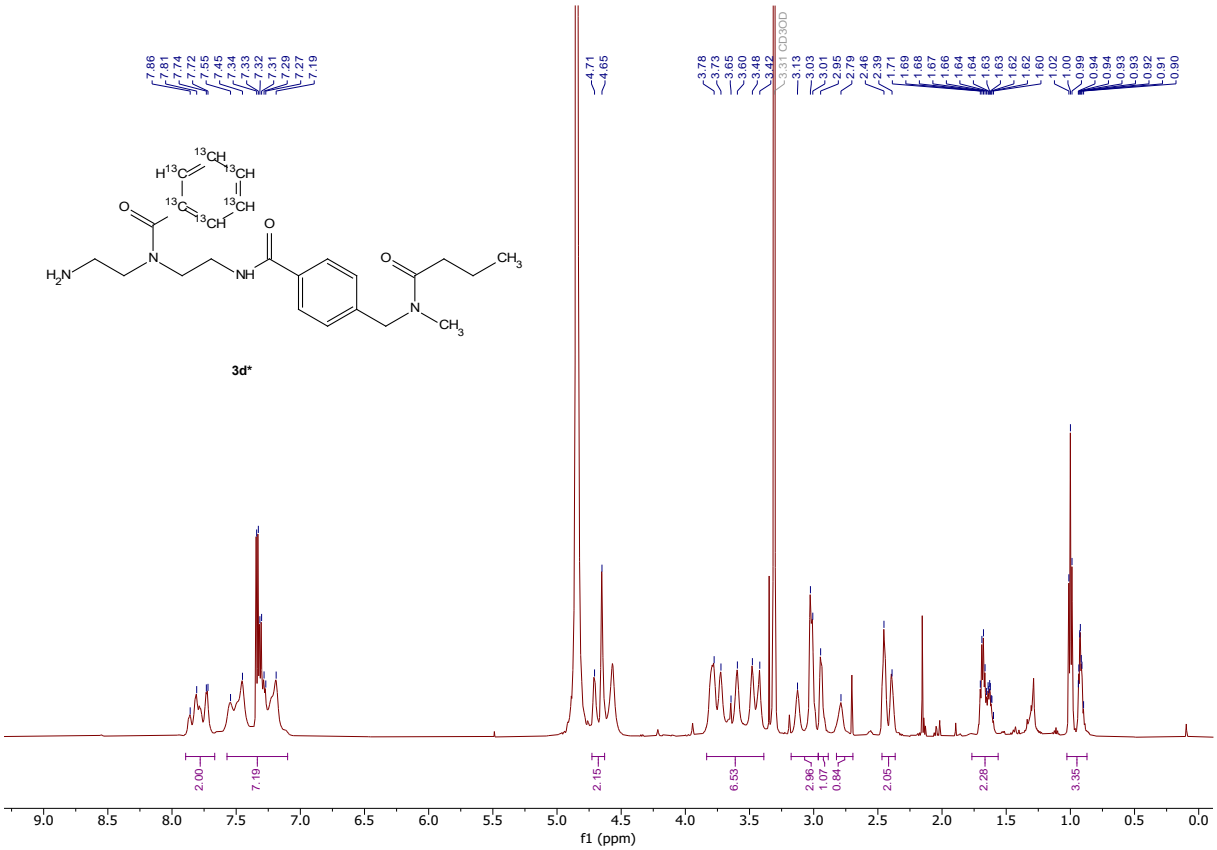
CH3
H2N
3d
f1 (ppm)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0.90
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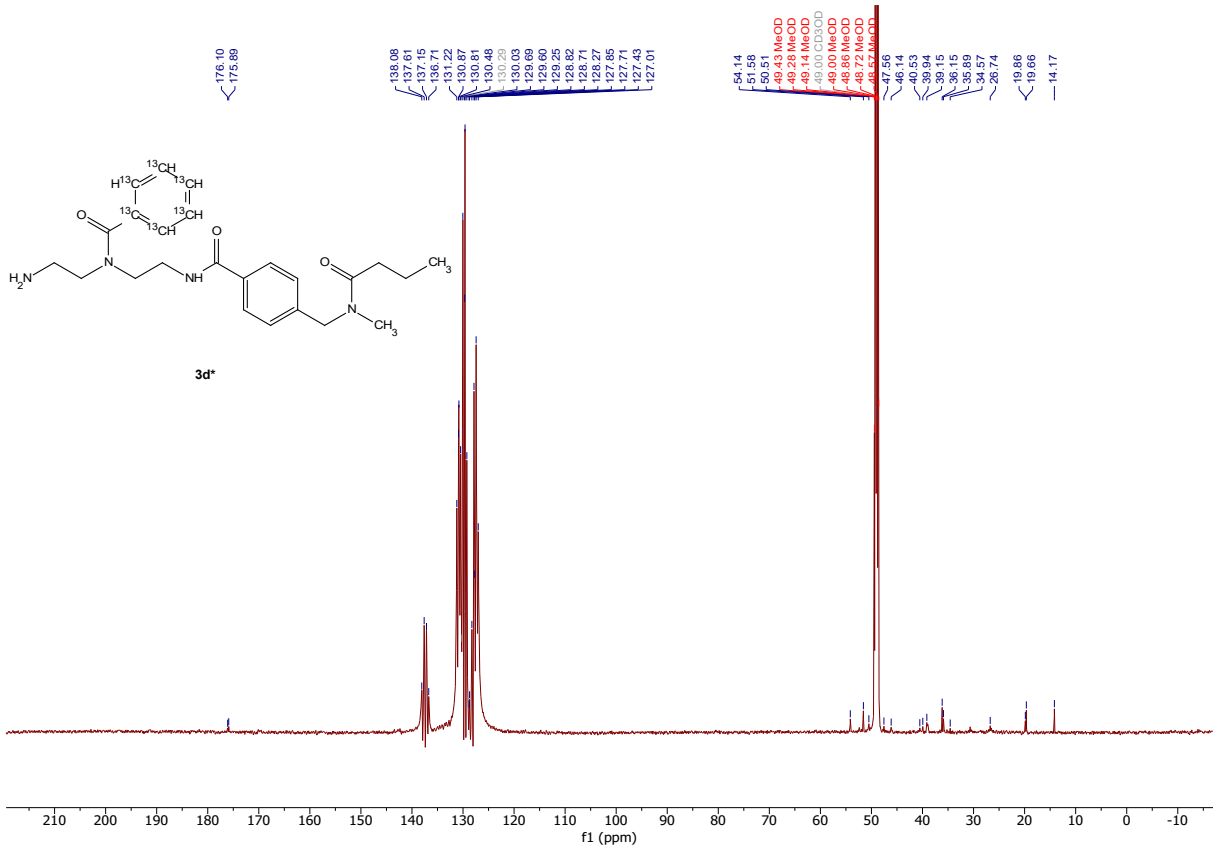
3.35
f1 (ppm)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127.85
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127.43
127.01
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51.58
50.51
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49.28 MeOD
49.14 MeOD
49.00 CD3OD
49.00 MeOD
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48.72 MeOD
48.57 MeOD
47.56
46.14
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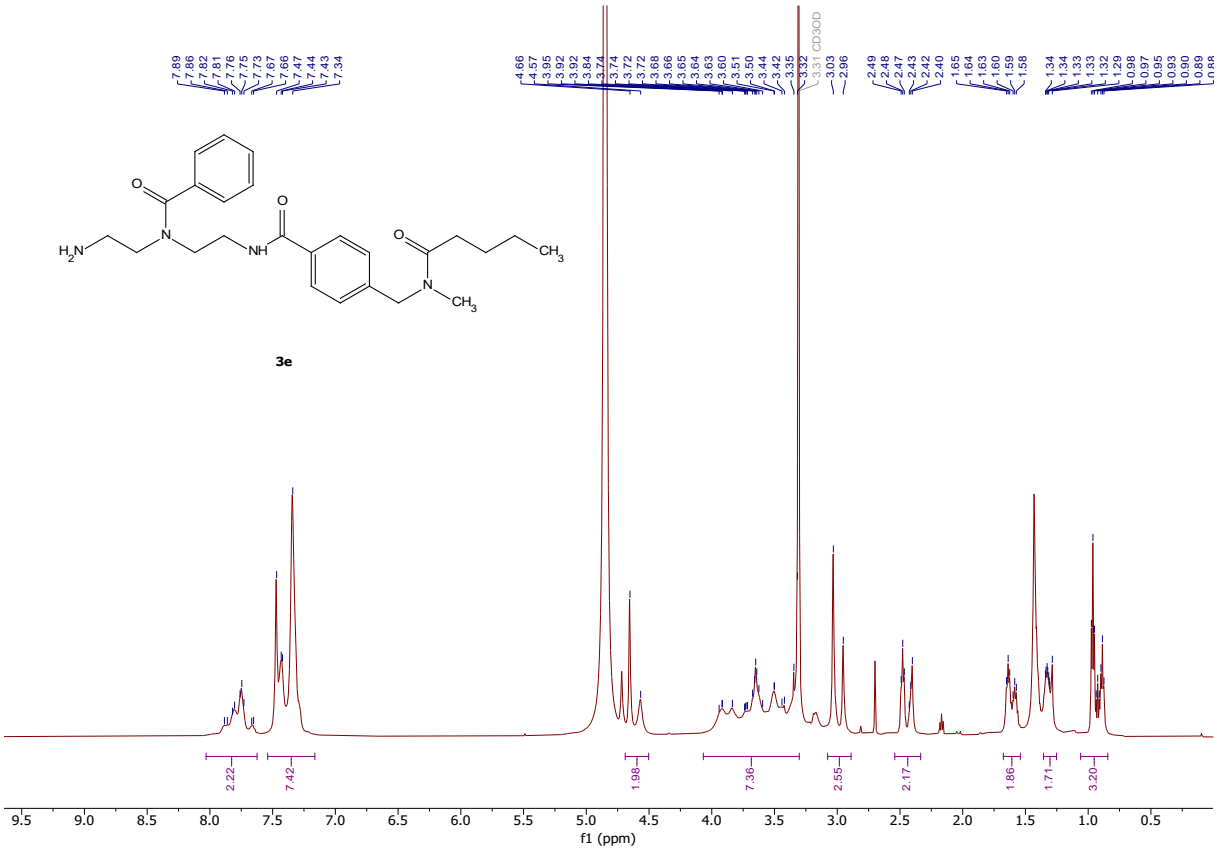
3d*
f1 (ppm)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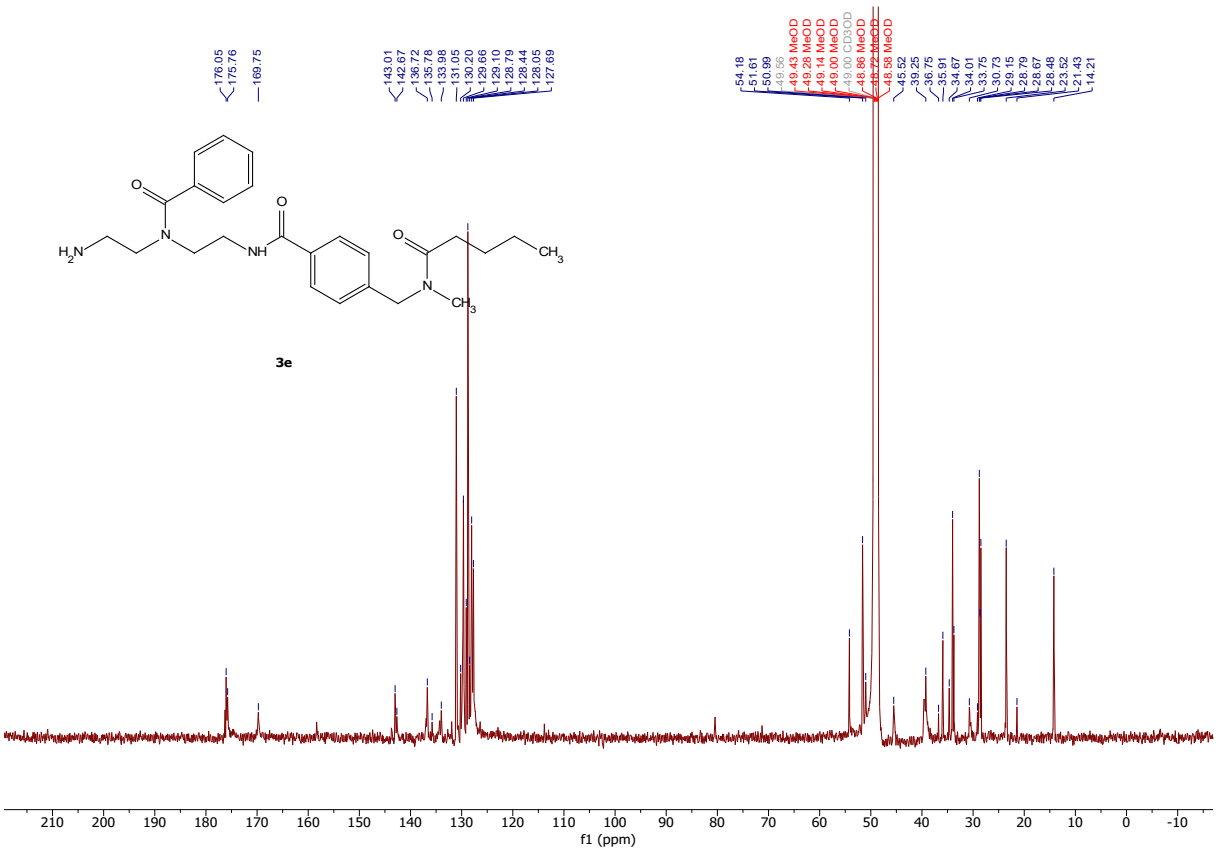
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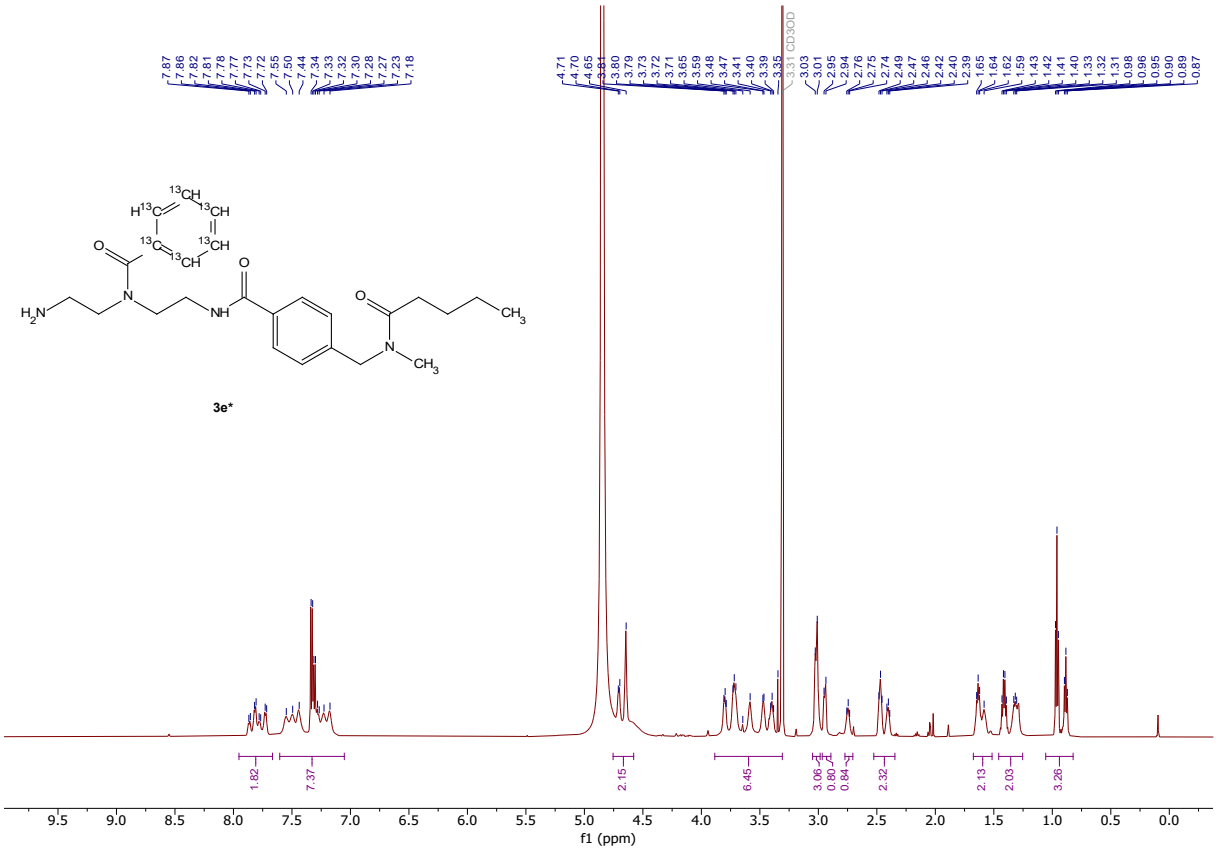
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3.79
3.73
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3.71
3.65
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0.95
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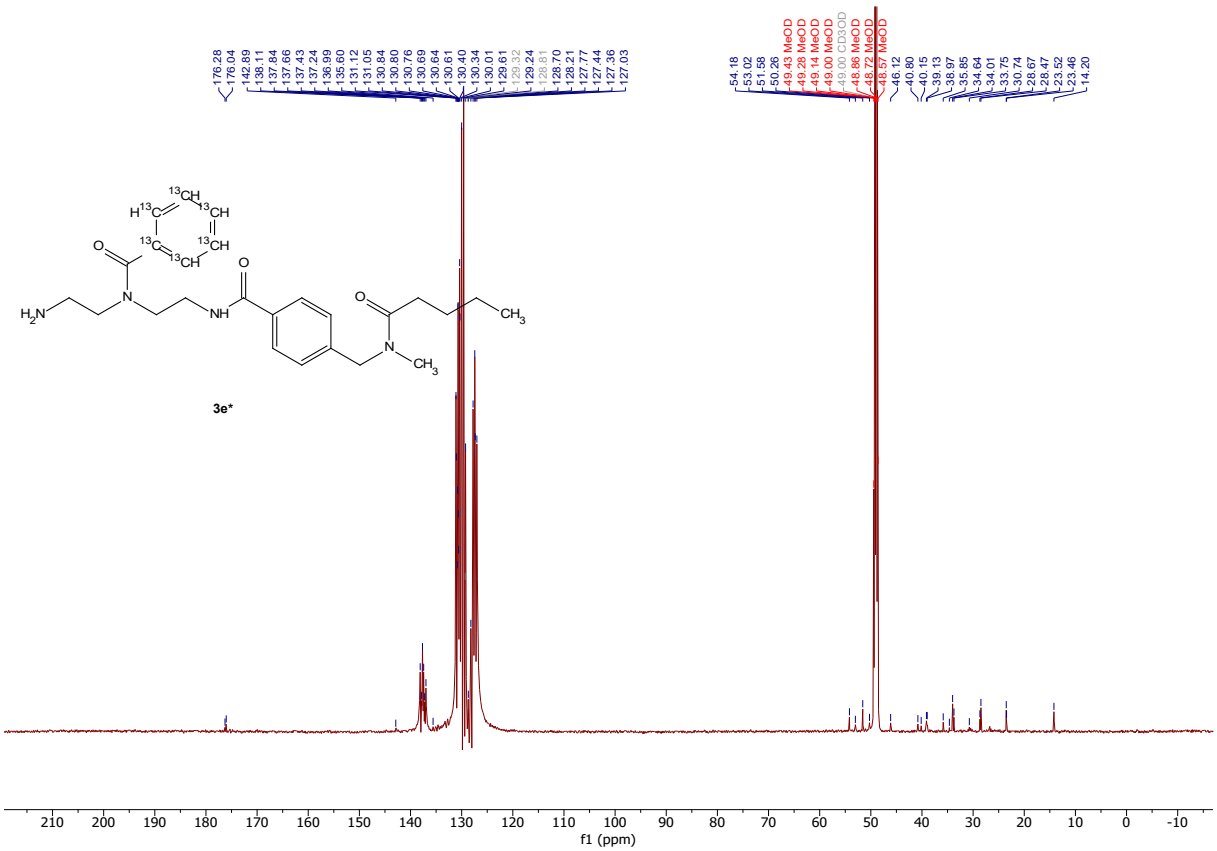
3e*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129.32
129.24
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128.21
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127.36
127.03
54.18
53.02
51.58
50.26
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49.28 MeOD
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49.00 CD3OD
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48.72 MeOD
48.57 MeOD
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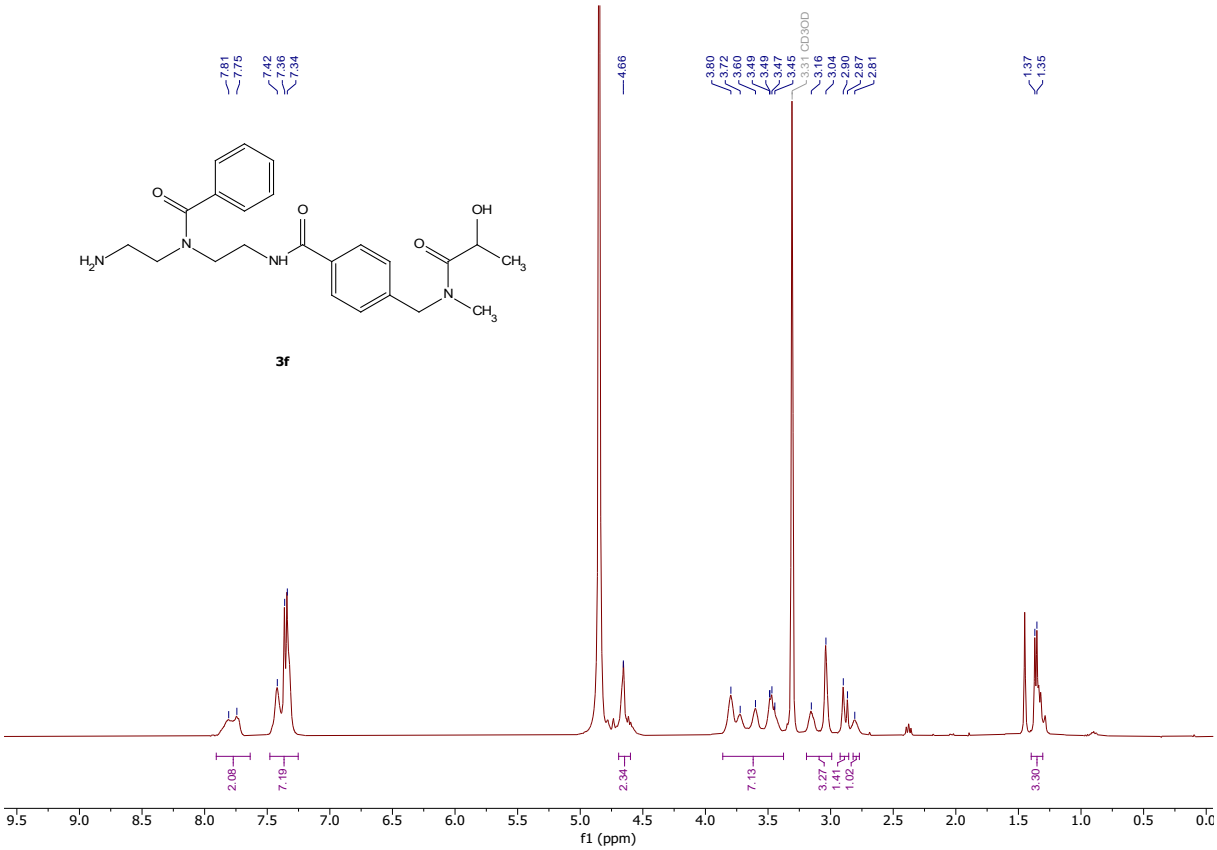
3e*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3.47
3.45
3.31 CD3OD
3.16
3.04
2.90
2.87
2.81
1.37
1.35
O
N
NH
O
OH
O
CH3
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CH3
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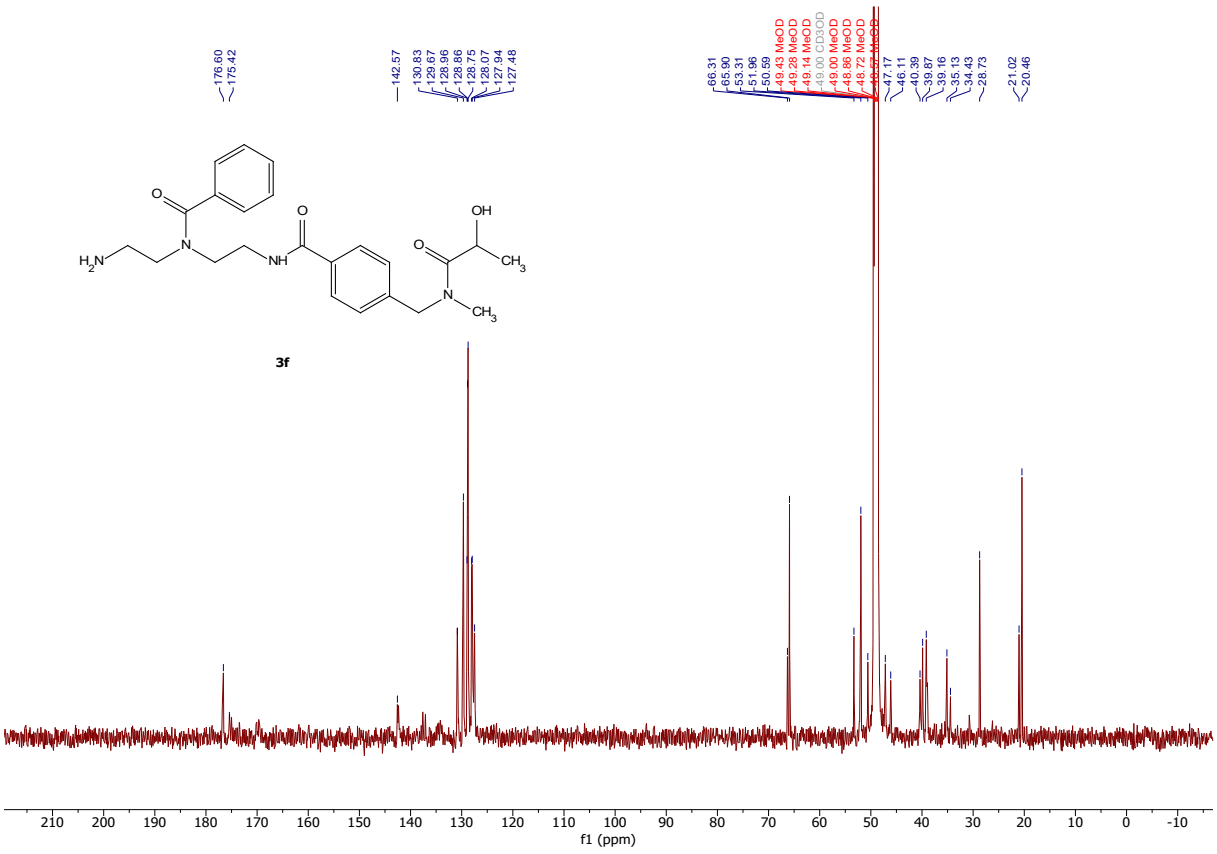
3f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53.31
51.96
50.59
49.43 MeOD
49.28 MeOD
49.14 MeOD
49.00 CD3OD
48.86 MeOD
48.72 MeOD
47.17
46.11
40.39
39.87
39.16
35.13
34.43
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O
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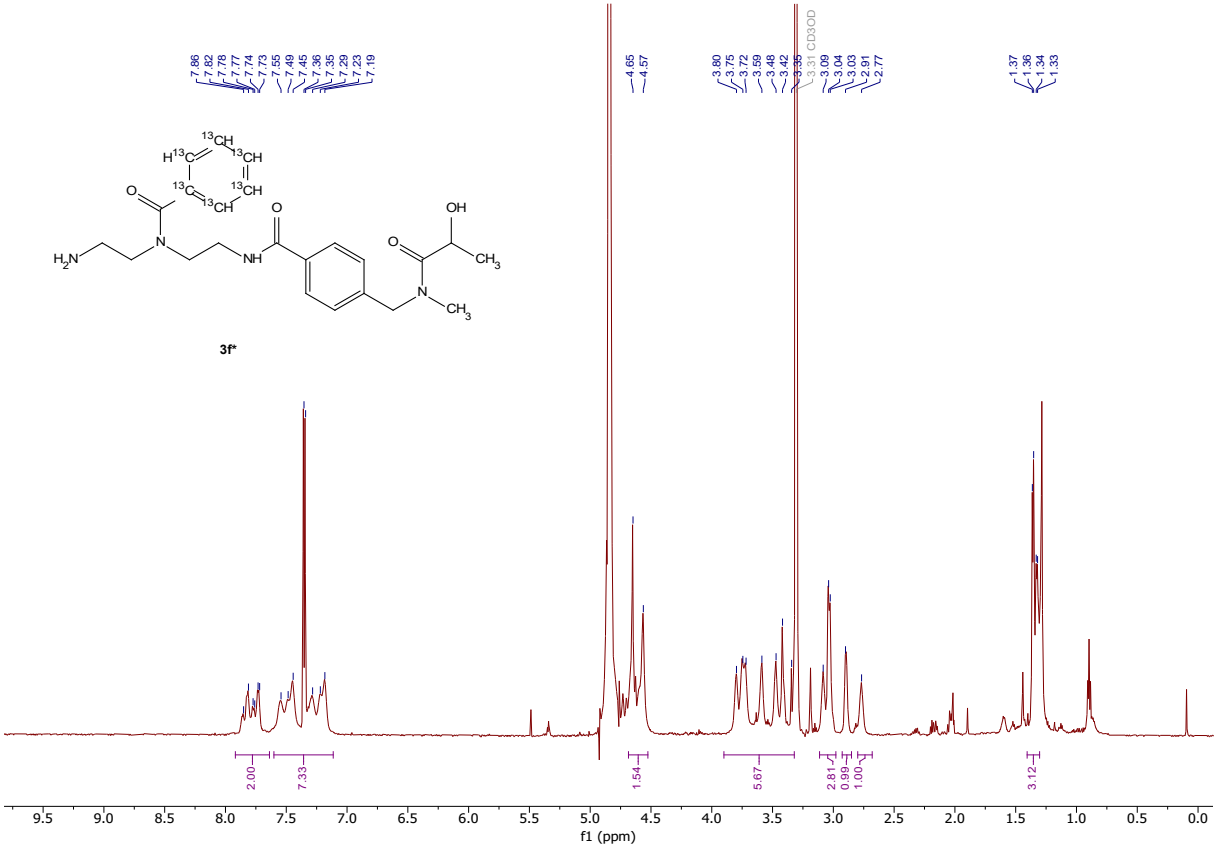
NH
O
OH
O
CH3
H2N
N
CH3
f1 (ppm)

3f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7.23
7.19
4.65
4.57
3.80
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3.72
3.59
3.48
3.42
3.35
3.31 CD3OD
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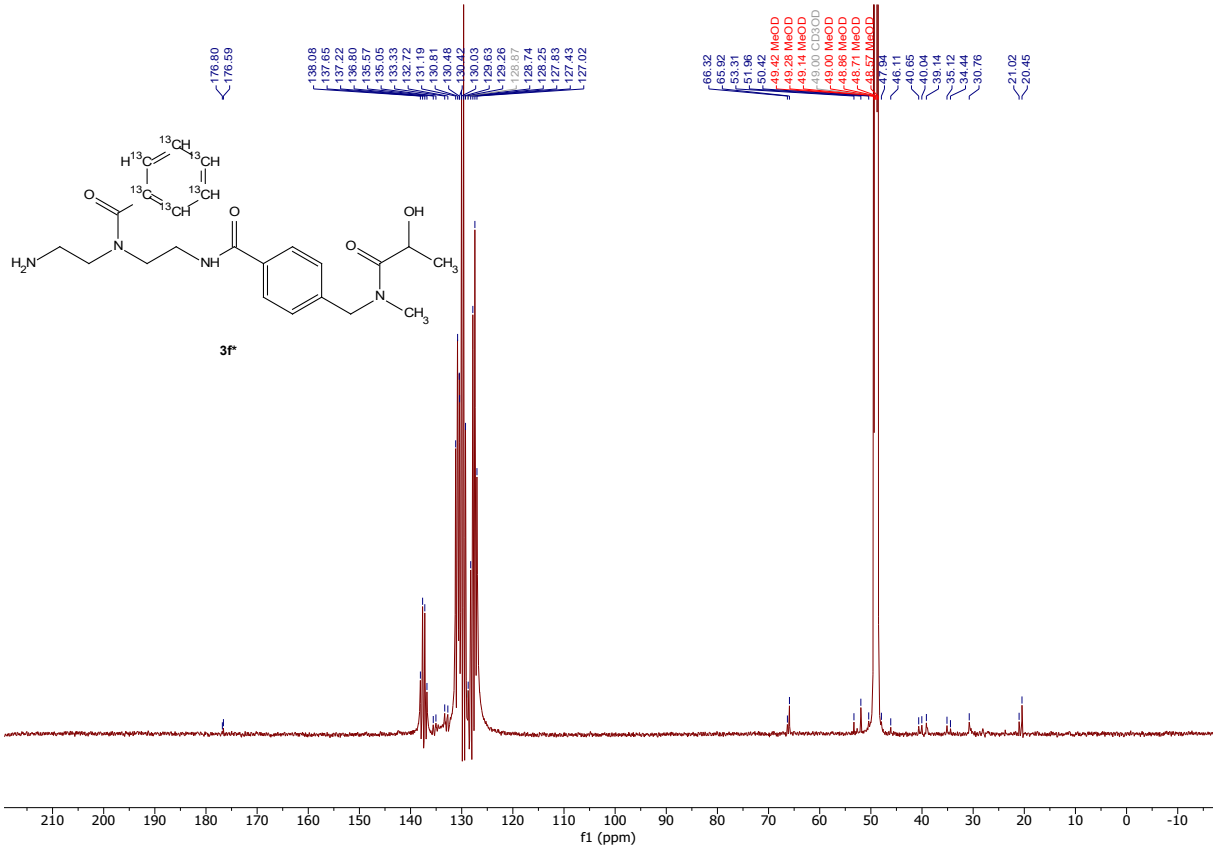
3f*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130.42
130.03
129.63
129.26
128.77
128.74
128.25
127.83
127.43
127.02
66.32
65.92
53.31
51.96
50.42
49.42 MeOD
49.28 MeOD
49.14 MeOD
49.00 CD3OD
49.00 MeOD
48.86 MeOD
48.71 MeOD
48.57 MeOD
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35.12
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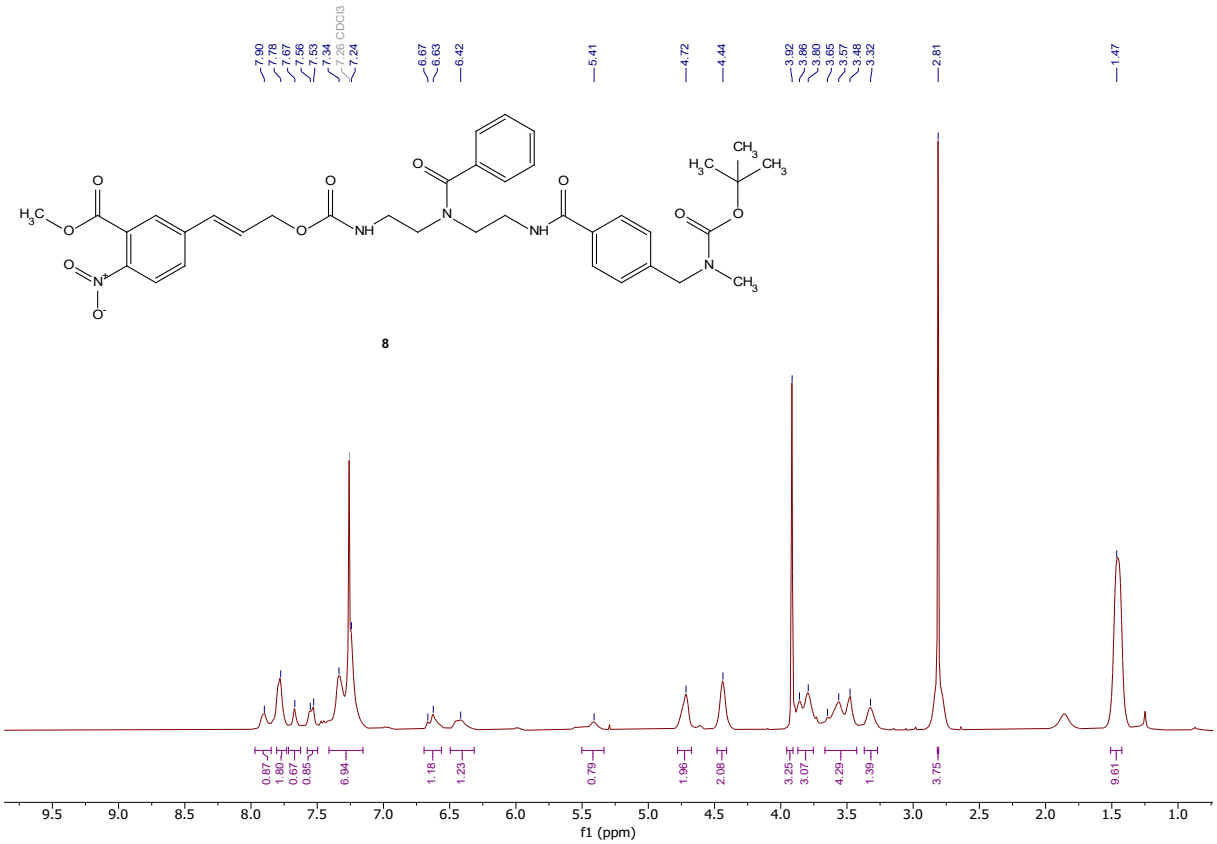
21.02
20.45
f1 (ppm)

3f*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4.44
3.92
3.86
3.80
3.65
3.57
3.48
3.32
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1.47
0.87
1.80
0.67
0.85
6.94
1.18
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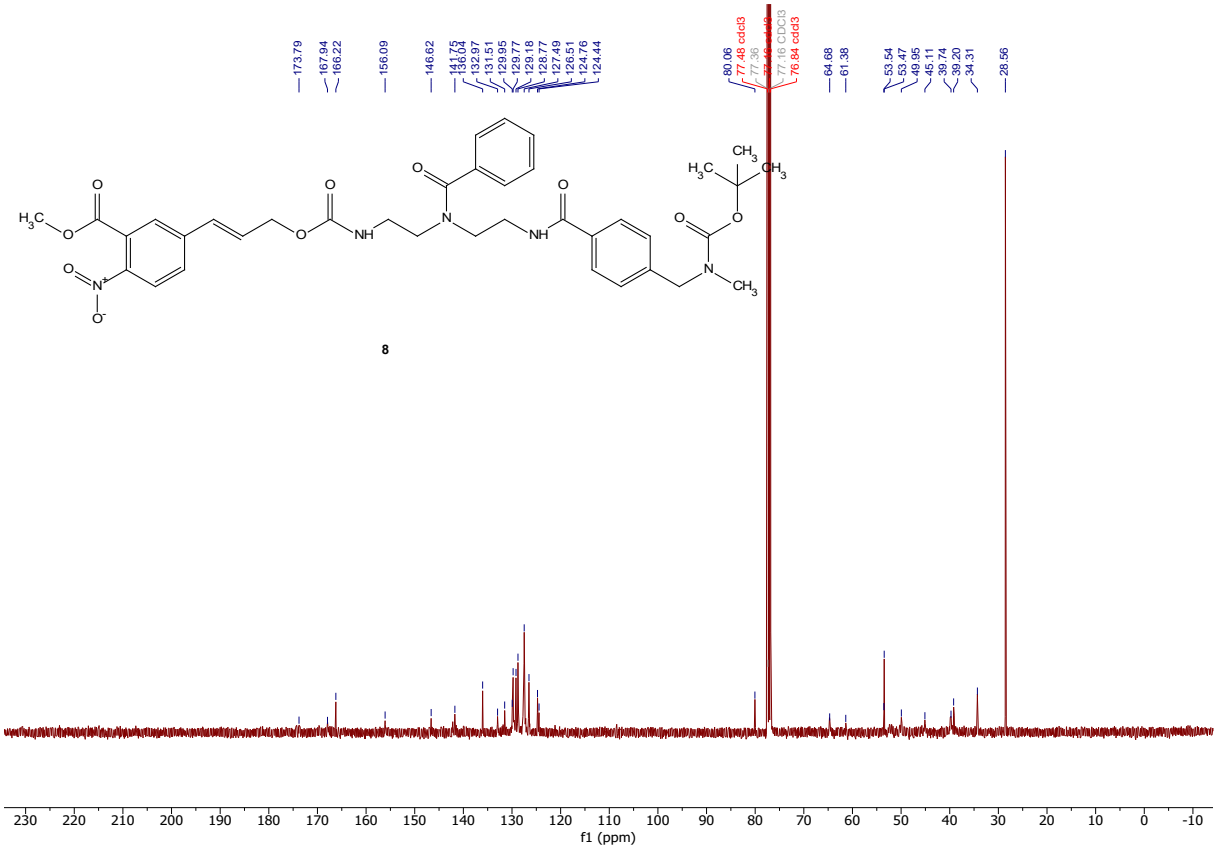
f1 (ppm)

8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128.77
127.49
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124.76
124.44
80.06
77.48 cdcl3
77.36
77.16 CDCl3
76.84 cdcl3
64.68
61.38
53.54
53.47
49.95
45.11
39.74
39.20
34.31
28.56
f1 (ppm)

8

8. References

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