Supporting Information

Development of fluorinated peptoid-based histone deacetylase (HDAC) inhibitors for therapy-resistant acute leukemia

Nina Reßing,[‡1,2] Julian Schliehe-Diecks,[‡3] Paris R. Watson[4], Melf Sönnichsen[3], Abigail D. Cragin[4], Andrea Schöler,[2] Jing Yang,[3,5] Linda Schäker-Hübner,[1] Arndt Borkhardt,[3] David W. Christianson[4], Sanil Bhatia,[3] and Finn K. Hansen*[1]*

[1]Pharmaceutical Institute, Pharmaceutical and Cell Biological Chemistry, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany.
[2]Institute for Drug Discovery, Medical Faculty, Leipzig University, Brüderstraße 34, 04103 Leipzig, Germany.
[3]Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich Heine University Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany.
[4]Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, Pennsylvania 19104-6323, United States
[5]Department of Medicine, Yangzhou Polytechnic College, West Wenchang Road 458, Yangzhou, 225009, P.R. China

[‡] These authors contributed equally to this work.

Corresponding authors:
Sanil Bhatia, Tel. +49-211-81-04896, E-Mail: sanil.bhatia@uni-duesseldorf.de
Finn K. Hansen, Tel. +49-228-73-5213, E-Mail: finn.hansen@uni-bonn.de

Contents

1. Supplementary Tables

Table S1. Effect on cellular vaibility (IC50 values in nM) upon **10h**, **10p**, CI994 or ricolinostat exposure aganist selected leukemia cells and two healthy fibroblast control cells.

Cell line	10h	10p	CI994	Ricolinostat
REH	203.2 ± 17.3	>25000	3170.7 ± 158.9	1073.9 ± 57.6
HAL01	1206.3 ± 333.3	>25000	2140 ± 669.8	601.8 ± 12
697	227.7 ± 3.6	10995 ± 1887.1	2450.7 ± 238.5	1563 ± 38.8
PEER	175.3 ± 22	>25000	2605.3 ± 186.7	1036.4 ± 94.4
SUPB15	291.9 ± 50.6	13000.3 ± 1237.6	3382.3 ± 1046.6	1637 ± 232.6
KASUMI	175.3 ± 8.4	>25000	2553 ± 87.7	1779 ± 142.4
DND41	565 ± 23.6	>25000	7970 ± 563.2	3370.7 ± 6.1
Healthy Fibroblast 1	5979 ± 339.7	>25000	>25000	>25000
Healthy Fibroblast 2	3134.3 ± 135.9	>25000	>25000	16524.7 ± 1812.6
HL60	228.6 ± 37.8	>25000	1076.4 ± 302.2	1008.8 ± 248.6
MV4-11	258.8 ± 14.4	>25000	1366.5 ± 1055.7	2450.3 ± 166.2
K562	820.2 ± 791	24342 ± 930.6	14985.3 ± 4805.4	19299.3 ± 662.5
JURKAT	357.6 ± 91.2	>25000	4928.3 ± 125	3344 ± 233.2
MOLM13	94.7 ± 30.9	>25000	1690.3 ± 241.6	2413.7 ± 444

Table S2. Data collection and refinement statistics.

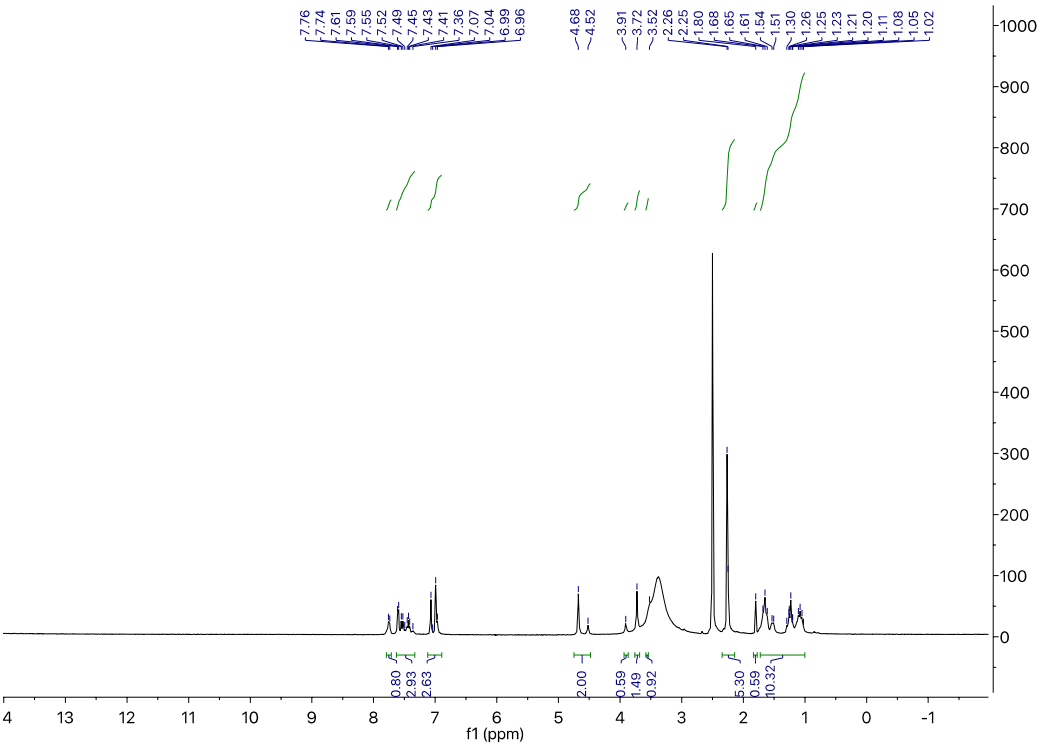
	HDAC6-**10h** complex[a]
Space group	$P2_1$
a,b,c (Å)	78.34, 95.13, 98.14
α, β, γ (°)	90.0, 98.73, 90.0
R_{merge}[b]	0.141 (0.416)
R_{pim}[c]	0.104 (0.298)
$CC_{1/2}$[d]	0.980 (0.720)
Redundancy	1.7 (1.7)
Completeness (%)	97.9 (97.7)
I/σ	5.4 (2.0)
Refinement	
Resolution (Å)	48.5 - 1.85 (1.916 - 1.85)
No. reflections	205228 (19929)
R_{work}/R_{free}[e]	0.172/ 0.215 (0.218/ 0.278)
Number of Atoms[f]	
Protein	11158
Ligand	228
Solvent	1047
Average B factors (Å^2)	
Protein	12
Ligand	15
Solvent	18
RMS Deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.3
Ramachandran Plot[g]	
Favored	97.00
Allowed	3.00
Outliers	0.00

[a]Values in parentheses refer to the highest-resolution shell indicated. [b]$R_{merge} = \sum_{hkl}\sum_i|I_{i,hkl} - \langle I\rangle_{hkl}|/\sum_{hkl}\sum_i I_{i,hkl}$, where $\langle I\rangle_{hkl}$ is the average intensity calculated for reflection *hkl* from replicate measurements. [c]$R_{p.i.m.} = (\sum_{hkl}(1/(N-1))^{1/2}\sum_i|I_{i,hkl} - \langle I\rangle_{hkl}|)/\sum_{hkl}\sum_i I_{i,hkl}$, where $\langle I\rangle_{hkl}$ is the average intensity calculated for reflection *hkl* from replicate measurements and N is the number of reflections. [d]Pearson correlation coefficient between random half-datasets. [e]$R_{work} = \sum||F_o| - |F_c||/\sum|F_o|$ for reflections contained in the working set. $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively. R_{free} is calculated using the same expression for reflections contained in the test set held aside during refinement. [f]Per asymmetric unit. [g]Calculated with MolProbity.

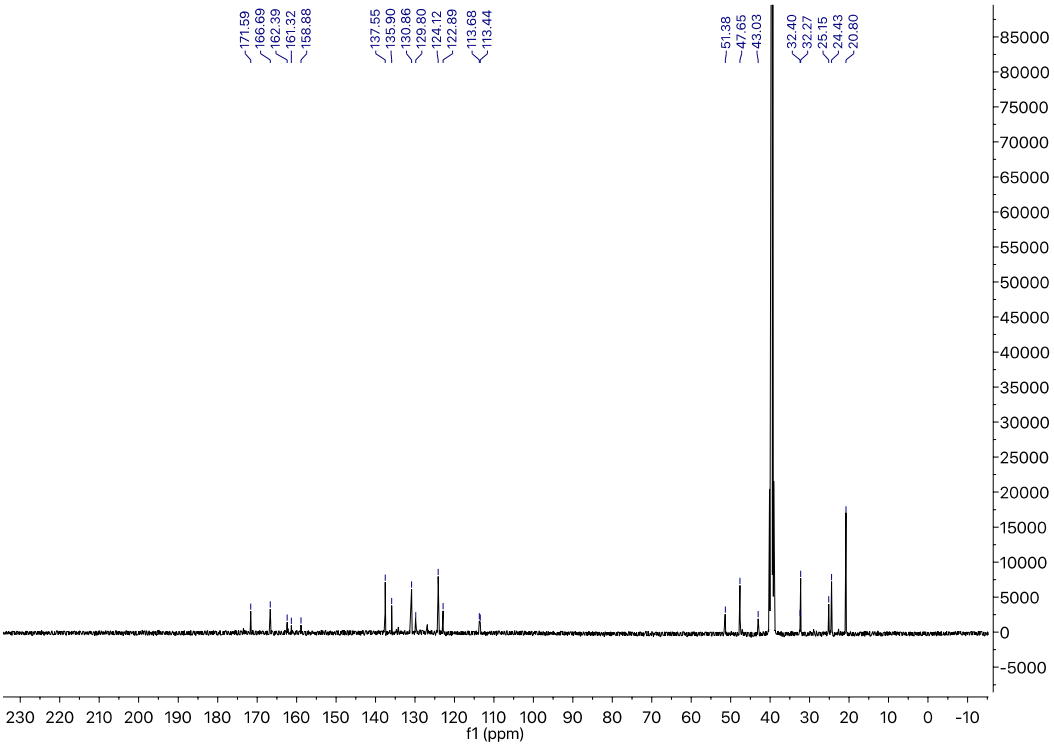
2. ^{1}H and ^{13}C NMR spectra

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(3,5-dimethylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10a**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

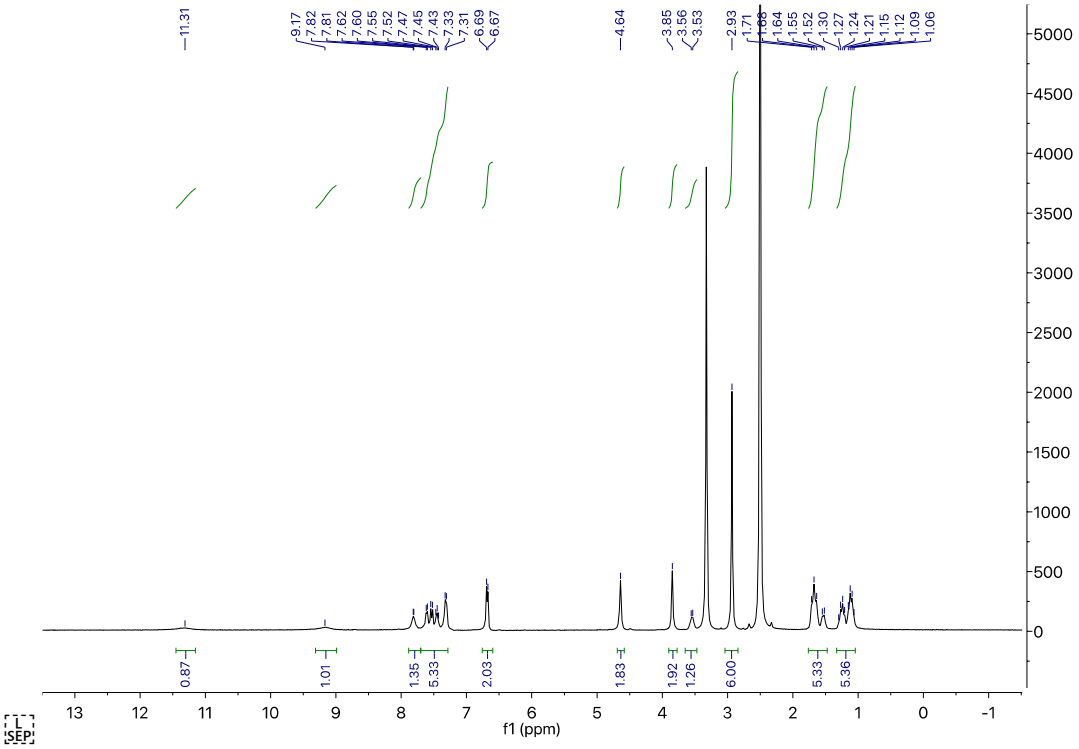


^{13}C NMR (101 MHz, DMSO-d_6)

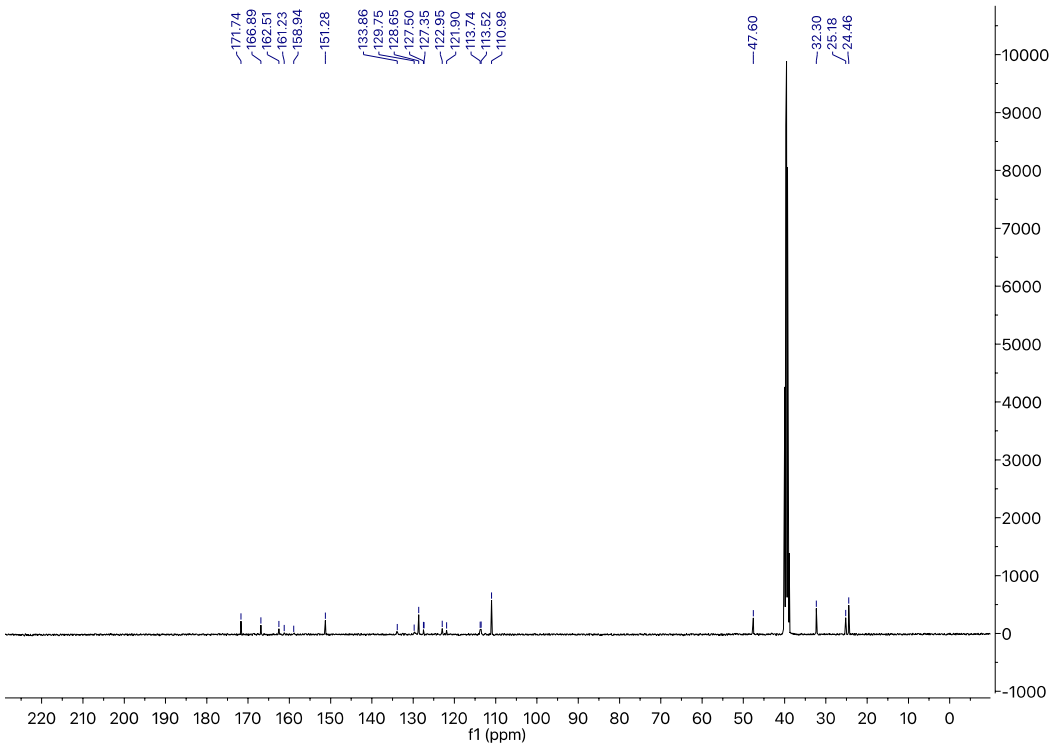


4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-[4-(dimethylamino)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10b**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

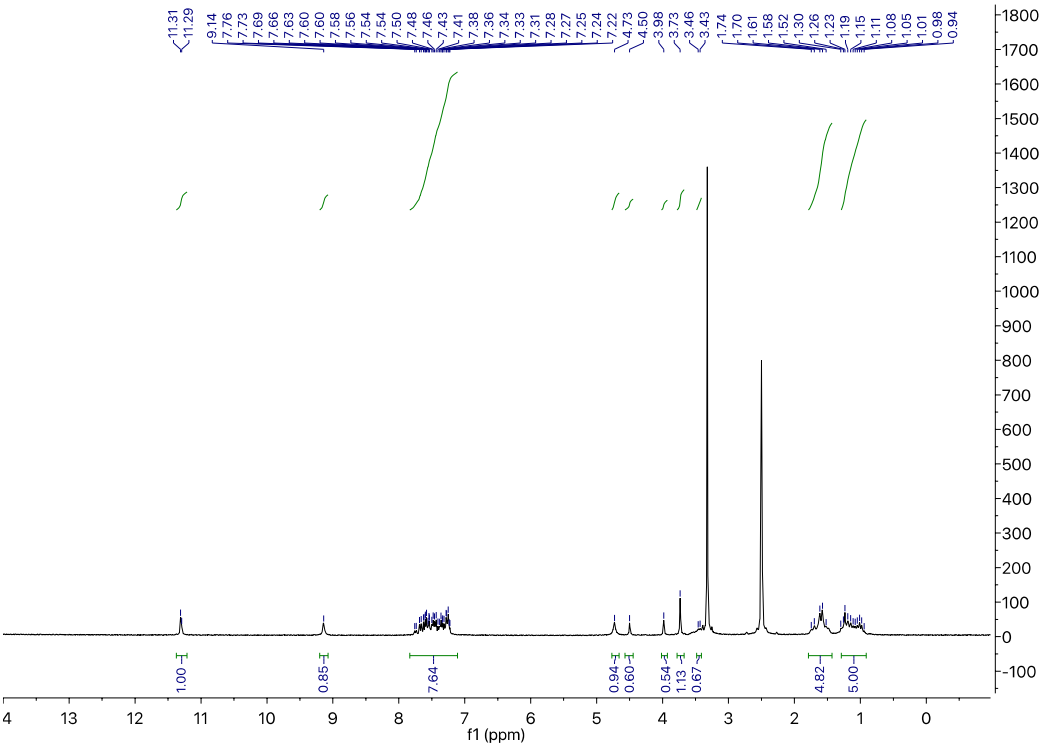


^{13}C NMR (101 MHz, DMSO-d_6)

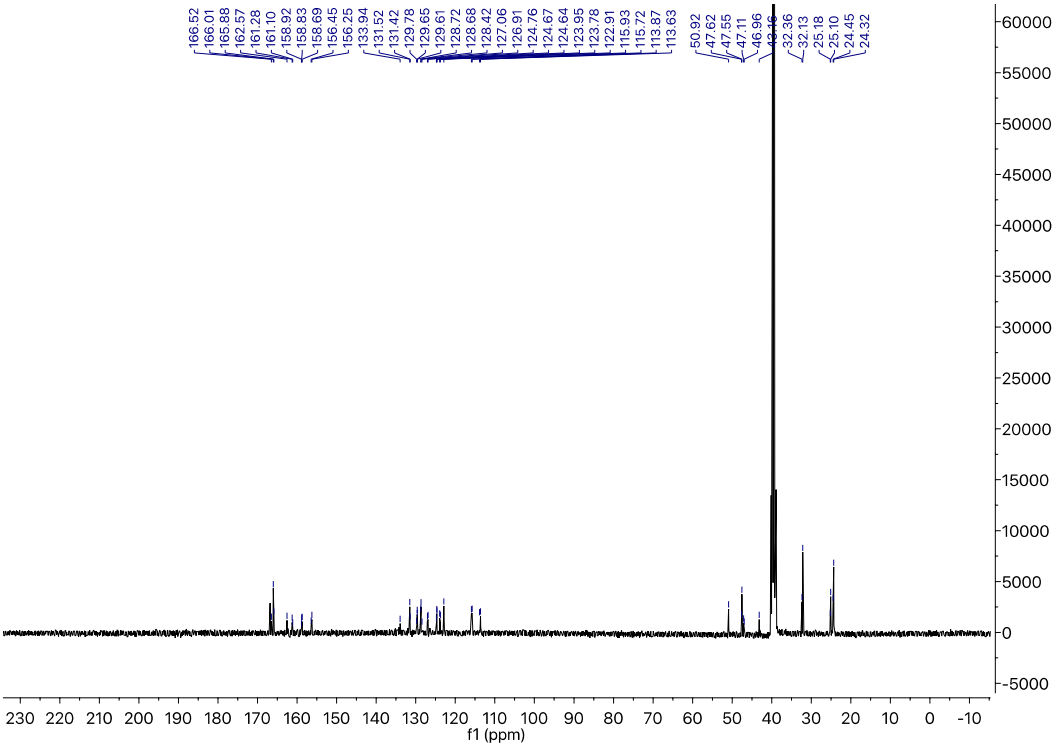


4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(2-fluorophenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10c**)

^{1}H NMR (300 MHz, DMSO-d_6, 20°C)

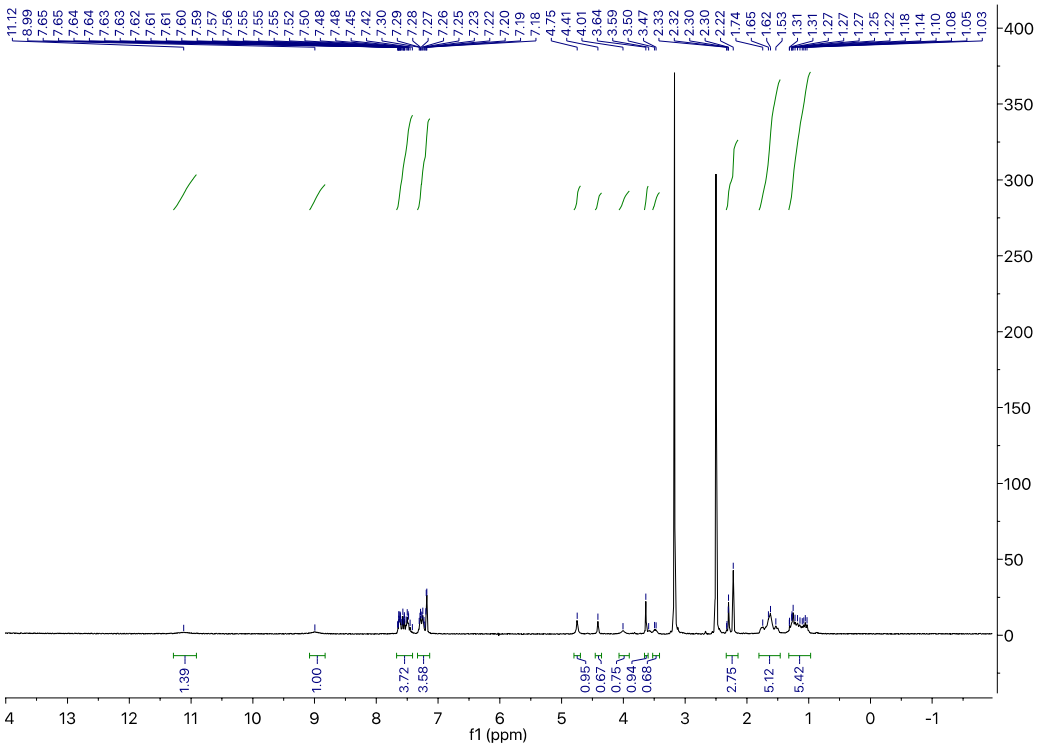


^{13}C NMR (101 MHz, DMSO-d_6)

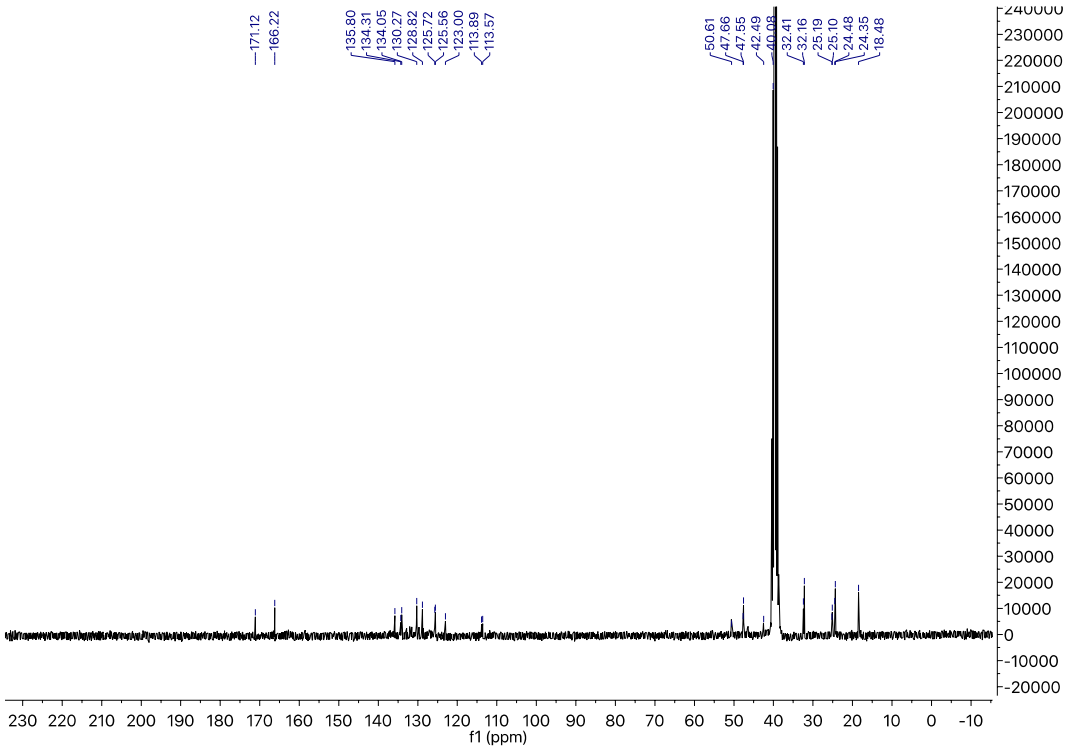


4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(2-methylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10d**)

^{1}H NMR (400 MHz, DMSO-d_6, 60 °C)

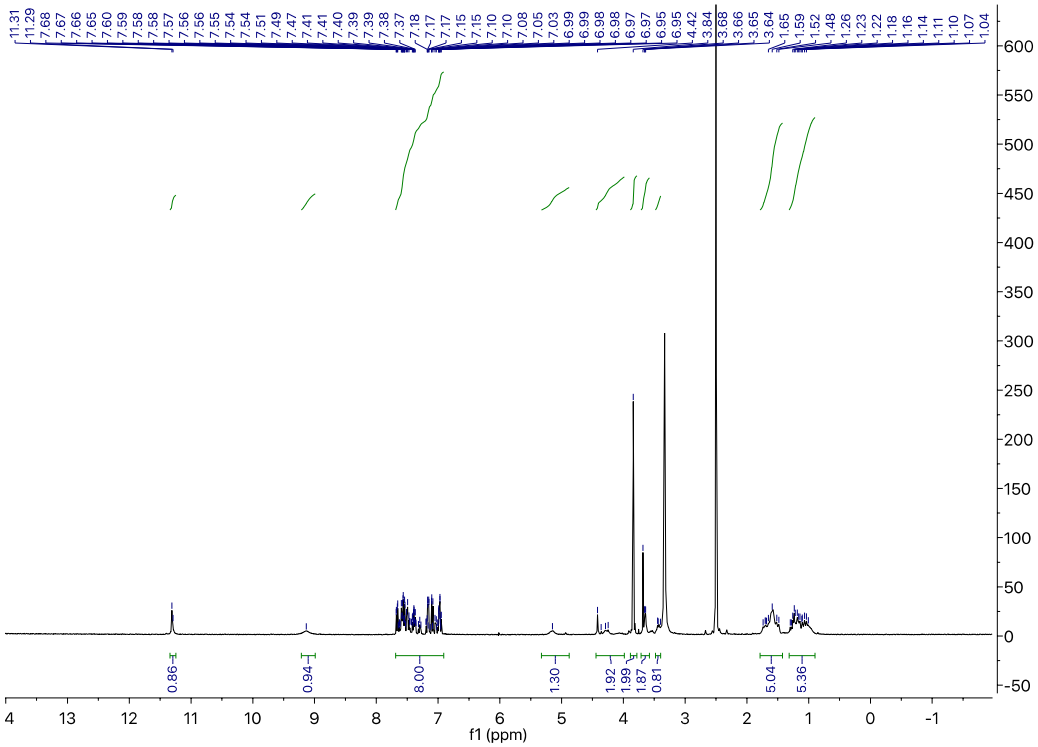


^{13}C NMR (75 MHz, DMSO-d_6)

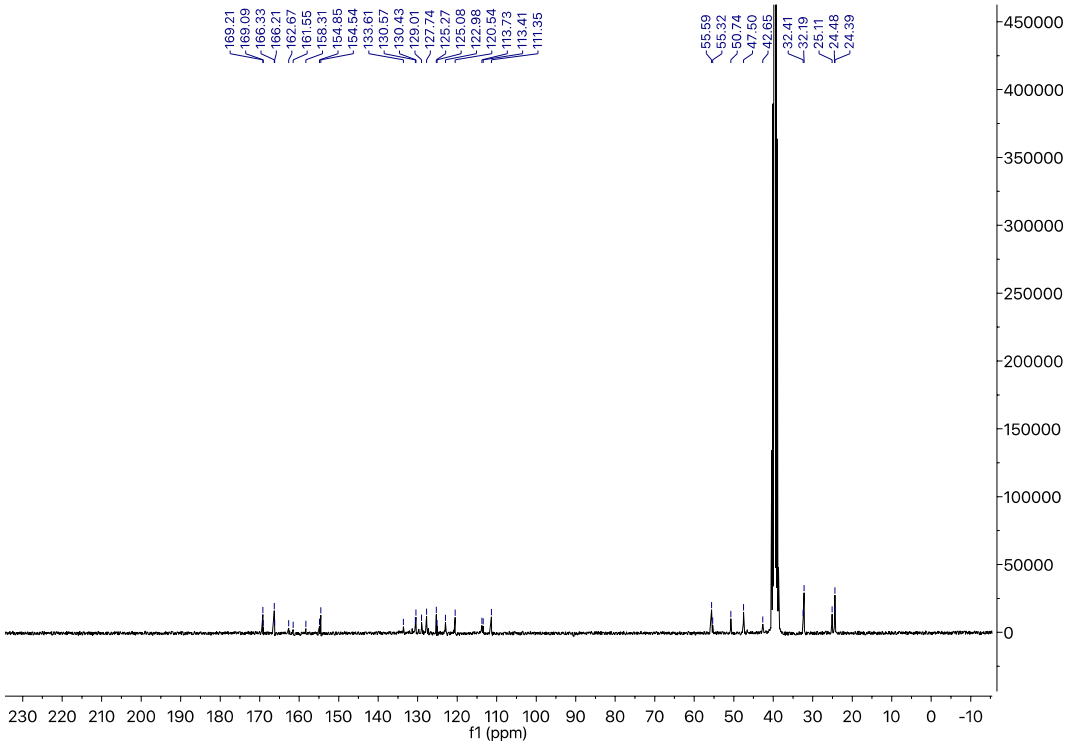


4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(2-methoxyphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10e**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

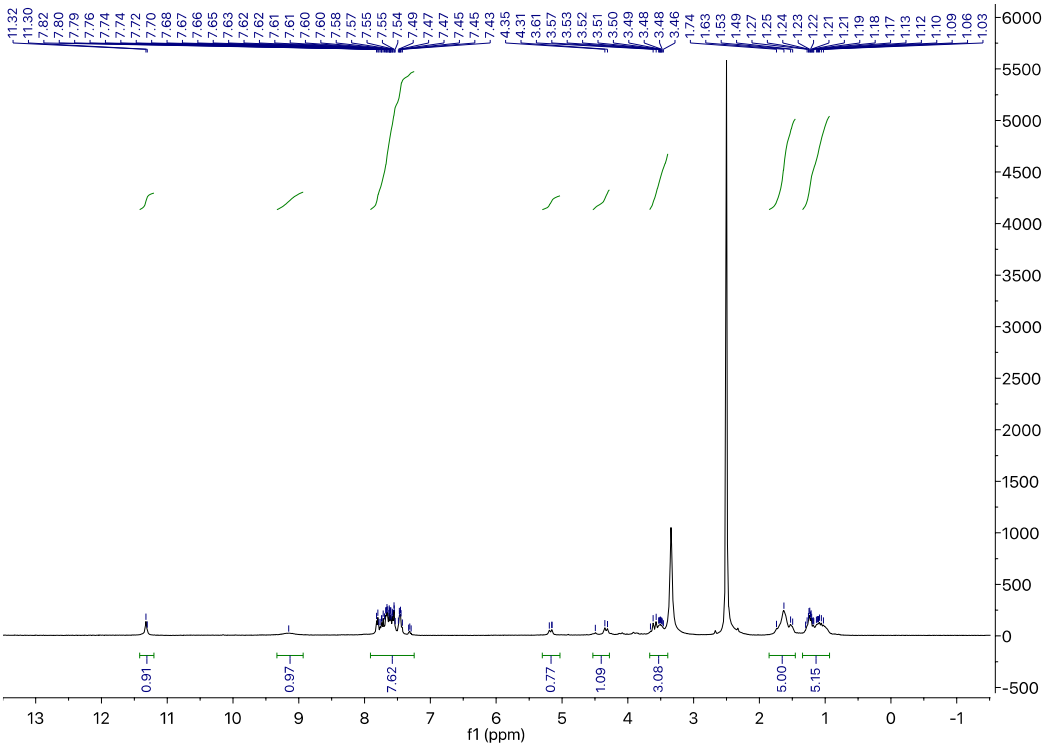


^{13}C NMR (75 MHz, DMSO-d_6)

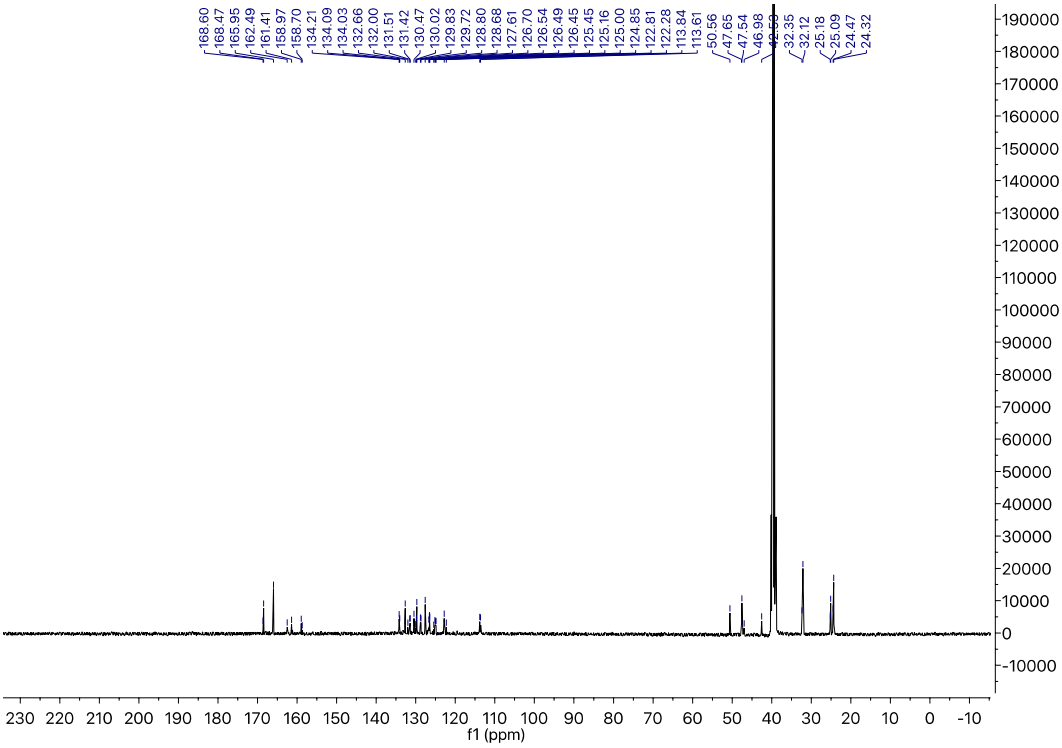


4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-[2-(trifluoromethyl)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10f**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

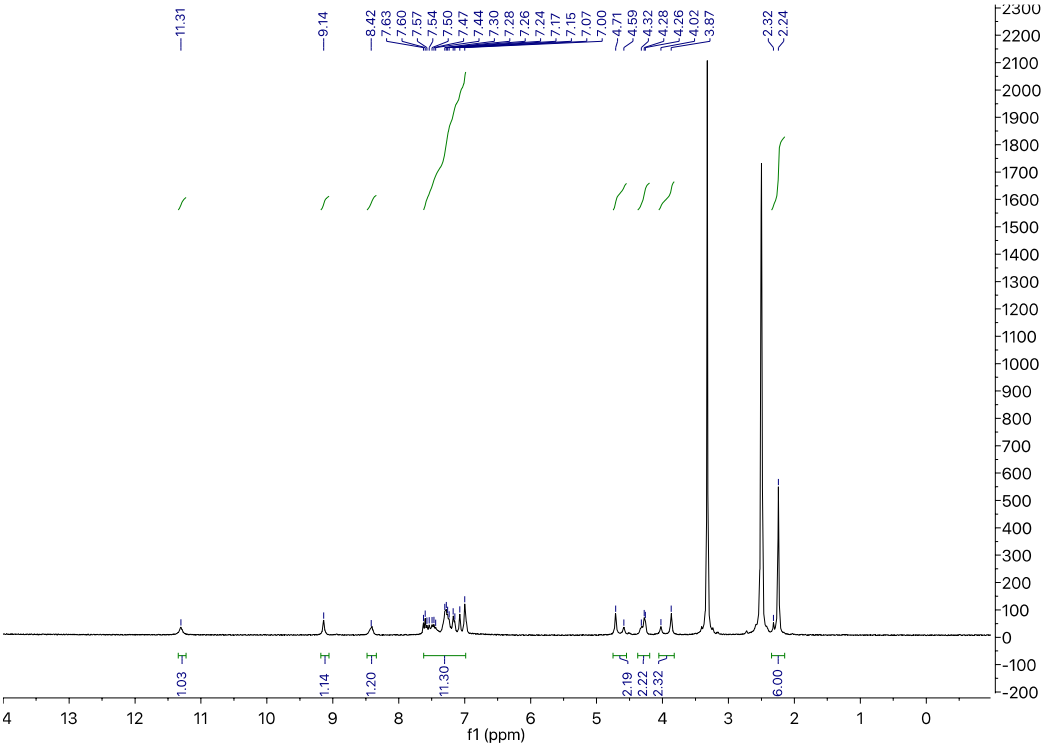


^{13}C NMR (101 MHz, DMSO-d_6)

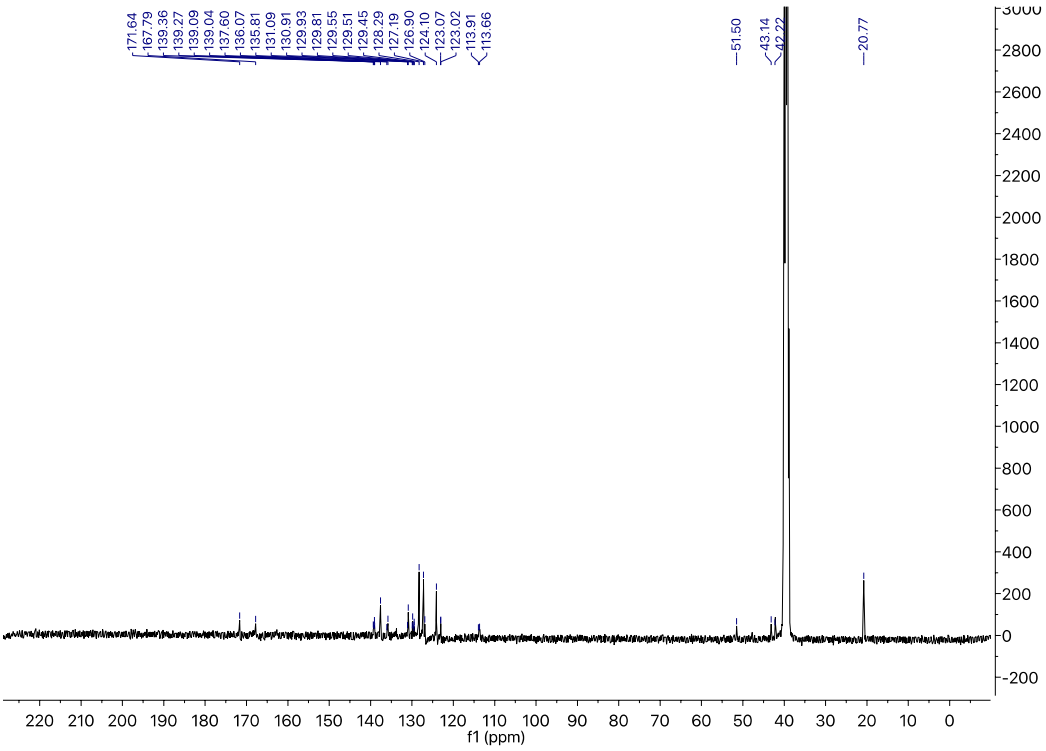


4-({*N*-[(benzylcarbamoyl)methyl]-1-(3,5-dimethylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10g**)

^{1}H NMR (300 MHz, DMSO-d_6, 20°C)

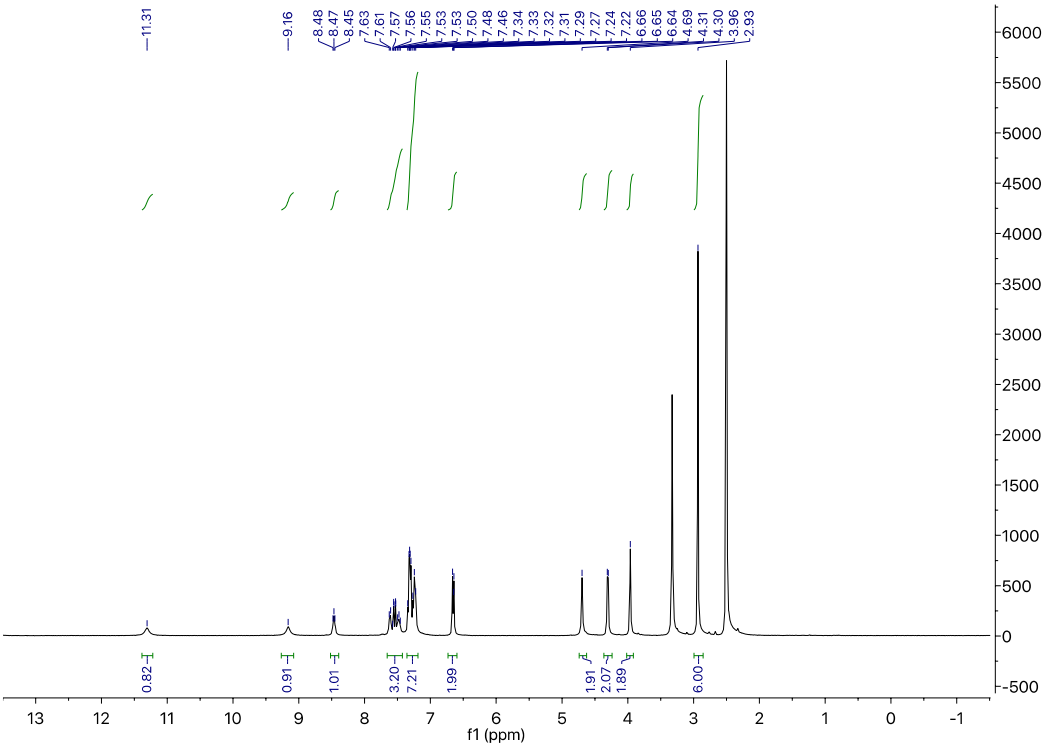


^{13}C NMR (101 MHz, DMSO-d_6)

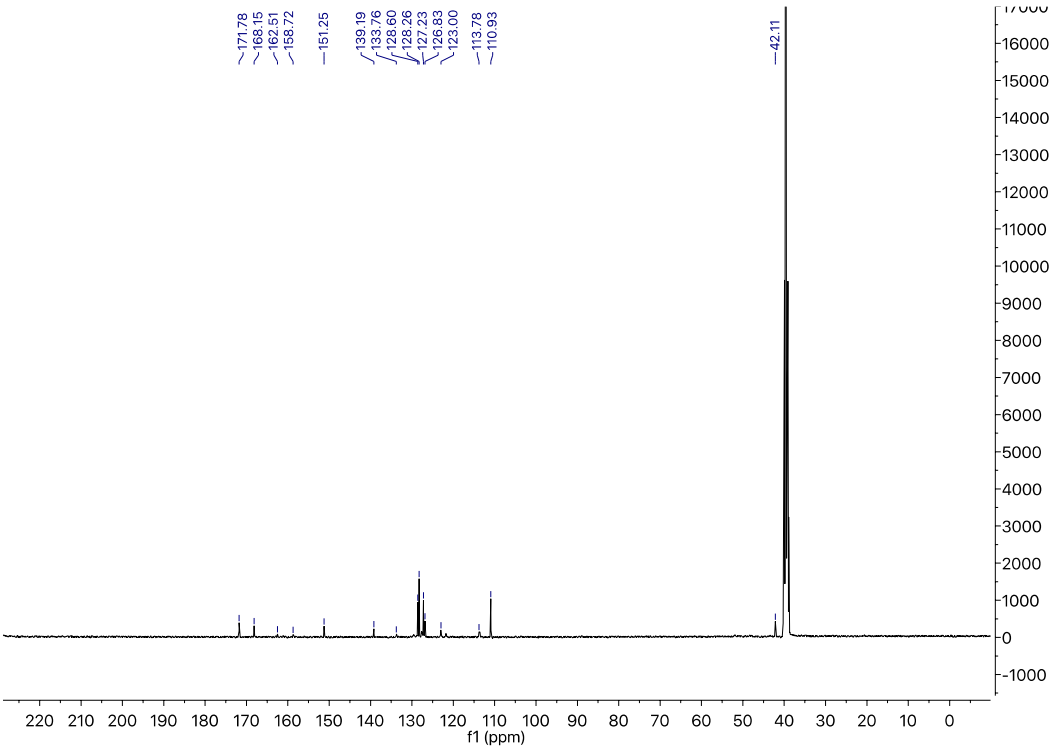


4-({*N*-[(benzylcarbamoyl)methyl]-1-[4-(dimethylamino)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10h**)

^{1}H NMR (400 MHz, DMSO-d_6, 20°C)

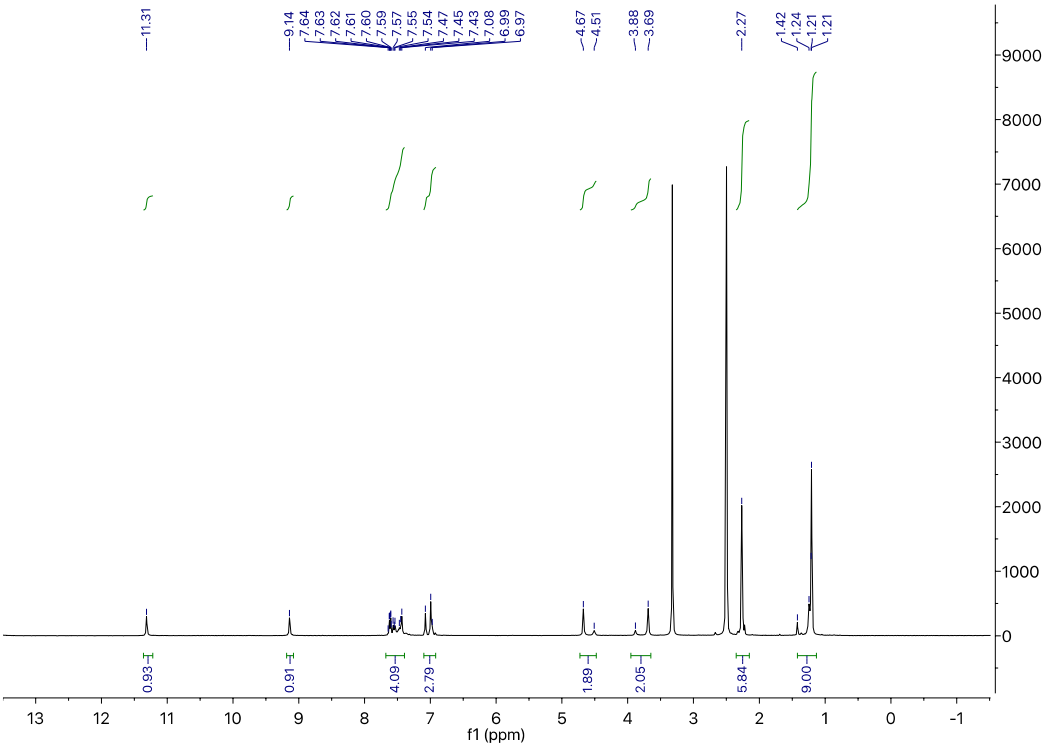


^{13}C NMR (101 MHz, DMSO-d_6)

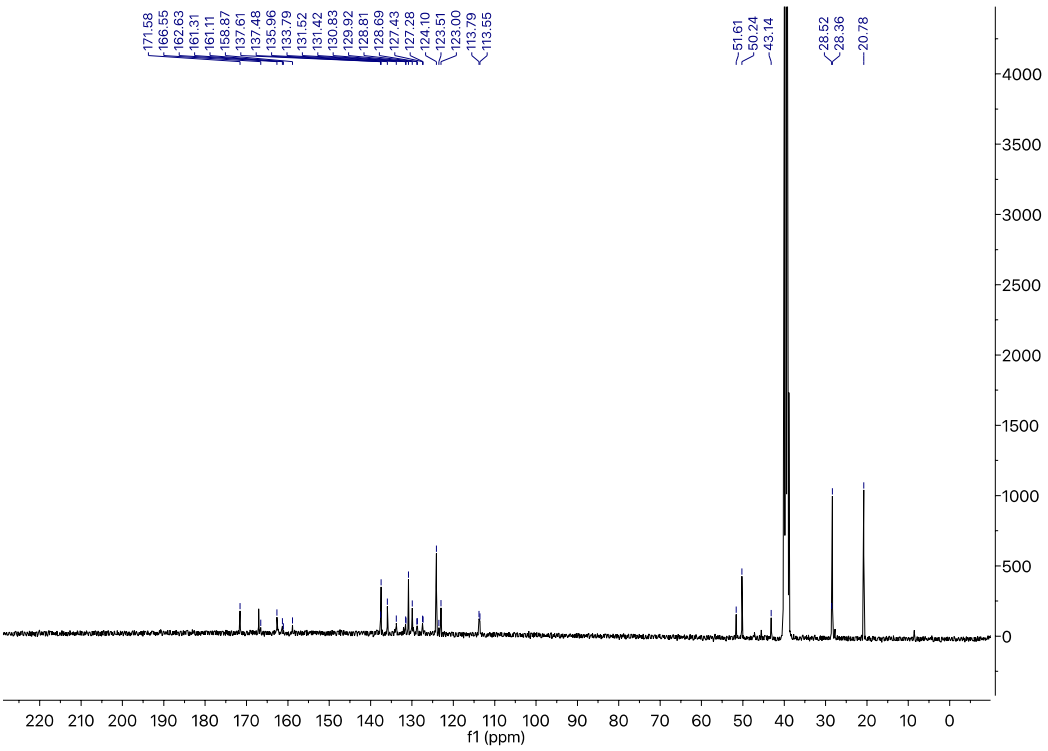


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(3,5-dimethylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10i**)

^{1}H NMR (400 MHz, DMSO-d_6, 20°C)

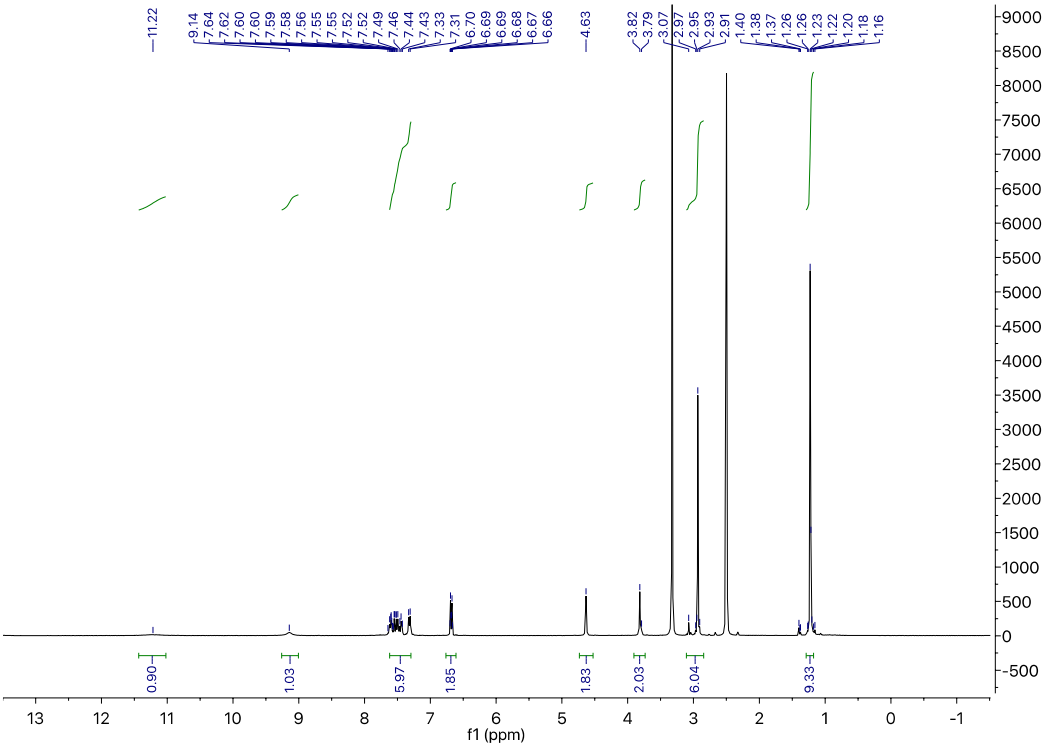


^{13}C NMR (101 MHz, DMSO-d_6)

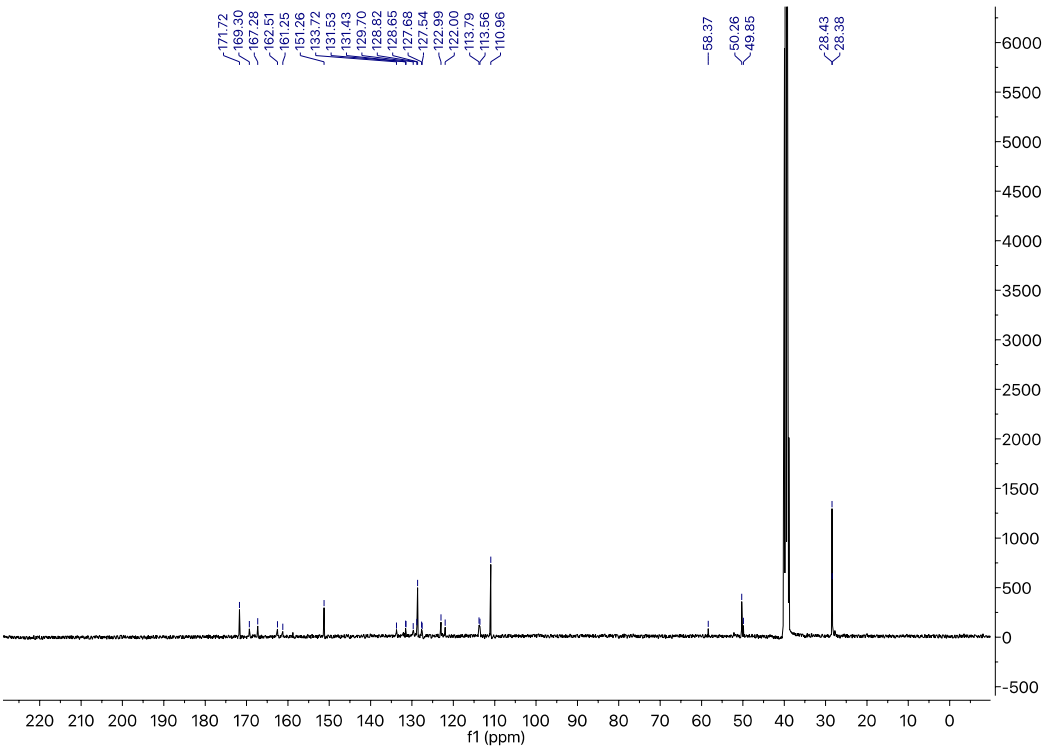


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-[4-(dimethylamino)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10j**)

^{1}H NMR (400 MHz, DMSO-d_6, 20°C)

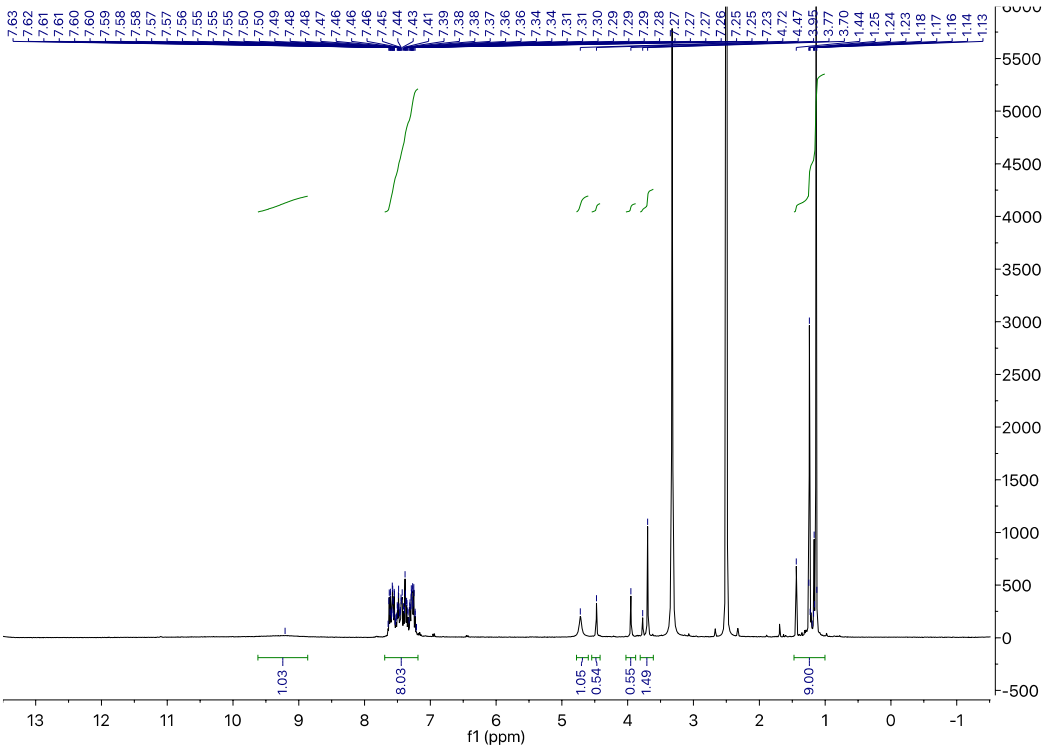


^{13}C NMR (101 MHz, DMSO-d_6)

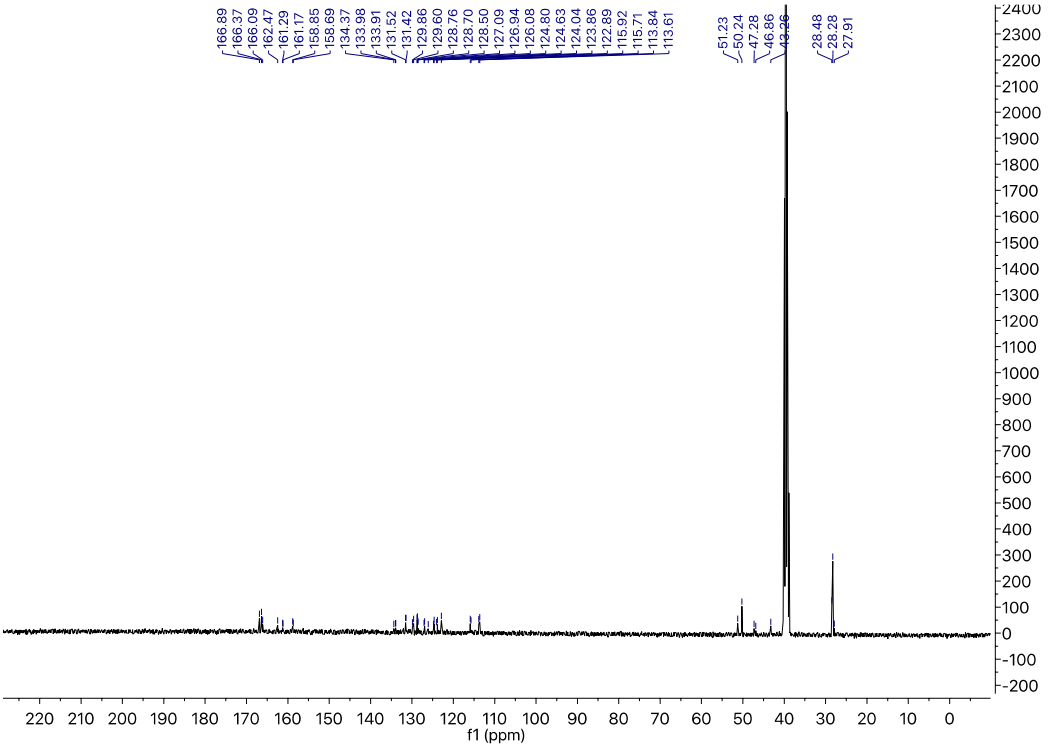


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-fluorophenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10k**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

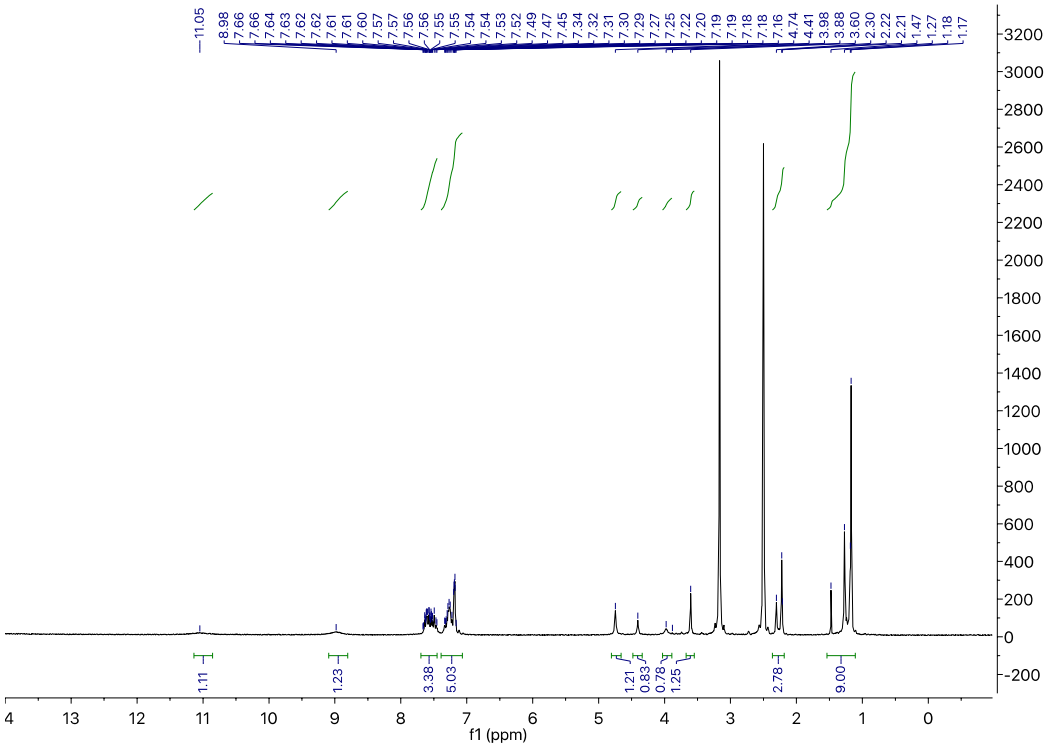


^{13}C NMR (101 MHz, DMSO-d_6)

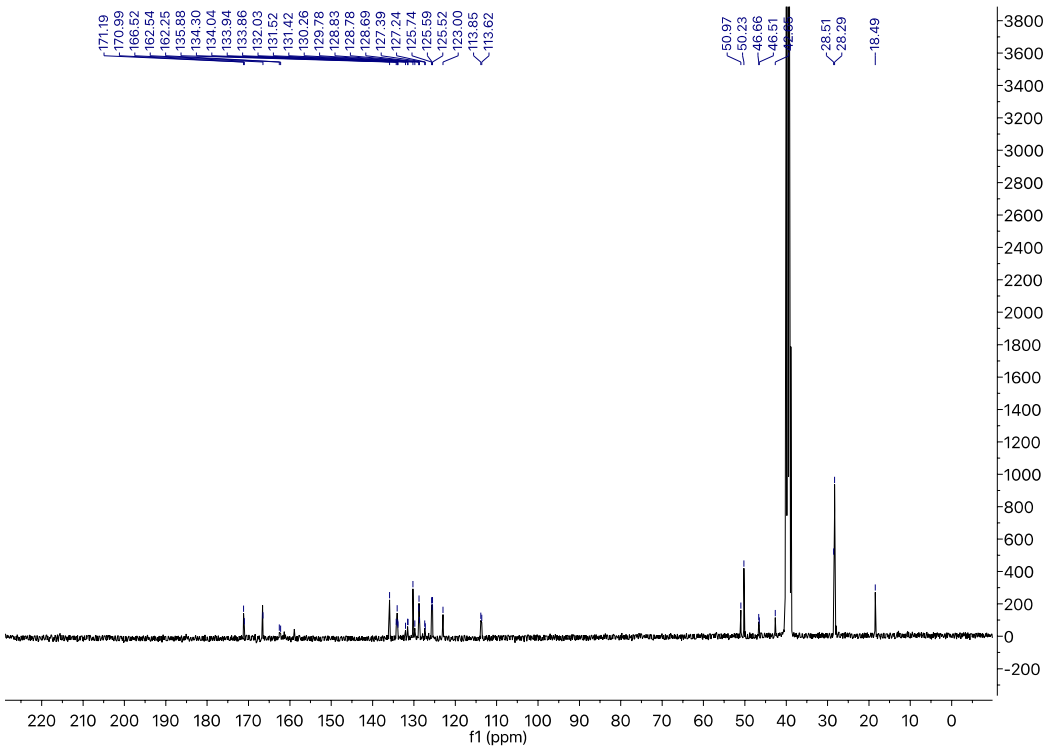


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-methylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10l**)

^{1}H NMR (300 MHz, DMSO-d_6, 60°C)

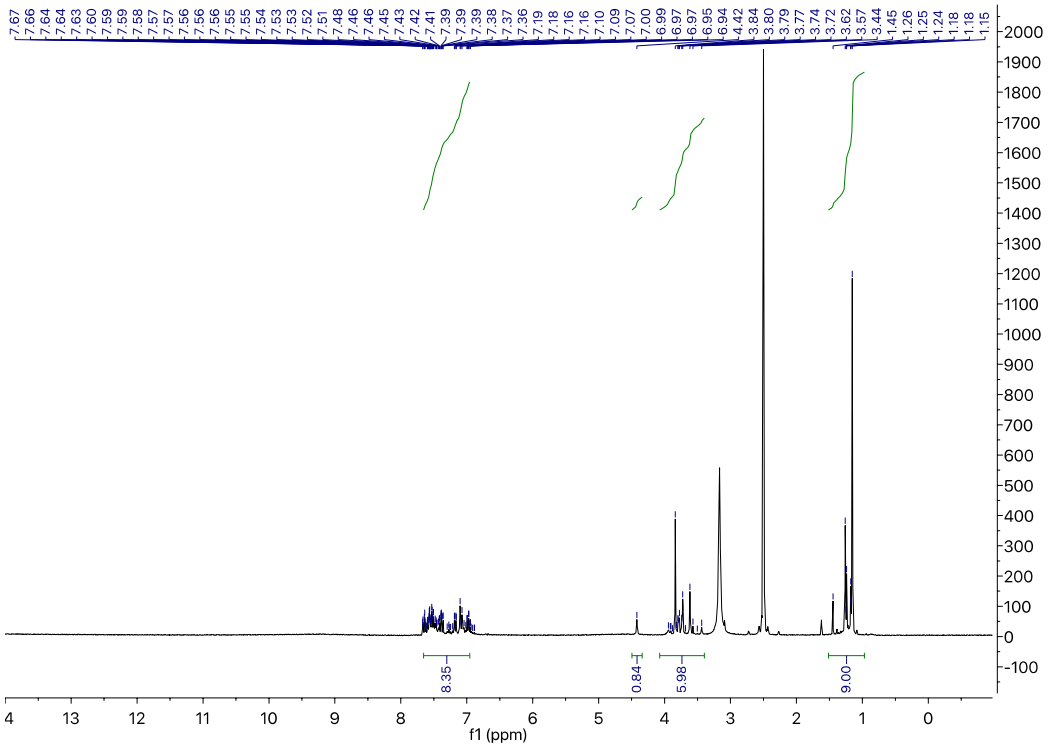


^{13}C NMR (101 MHz, DMSO-d_6)

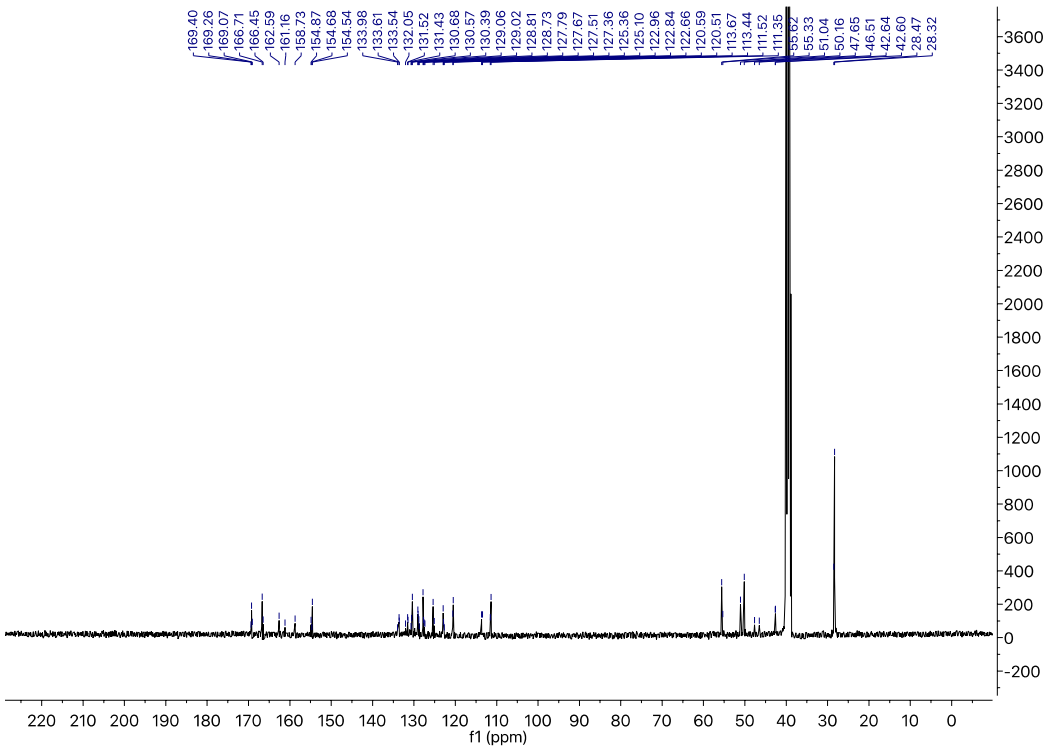


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-methoxyphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10m**)

^{1}H NMR (300 MHz, DMSO-d_6, 60 °C)

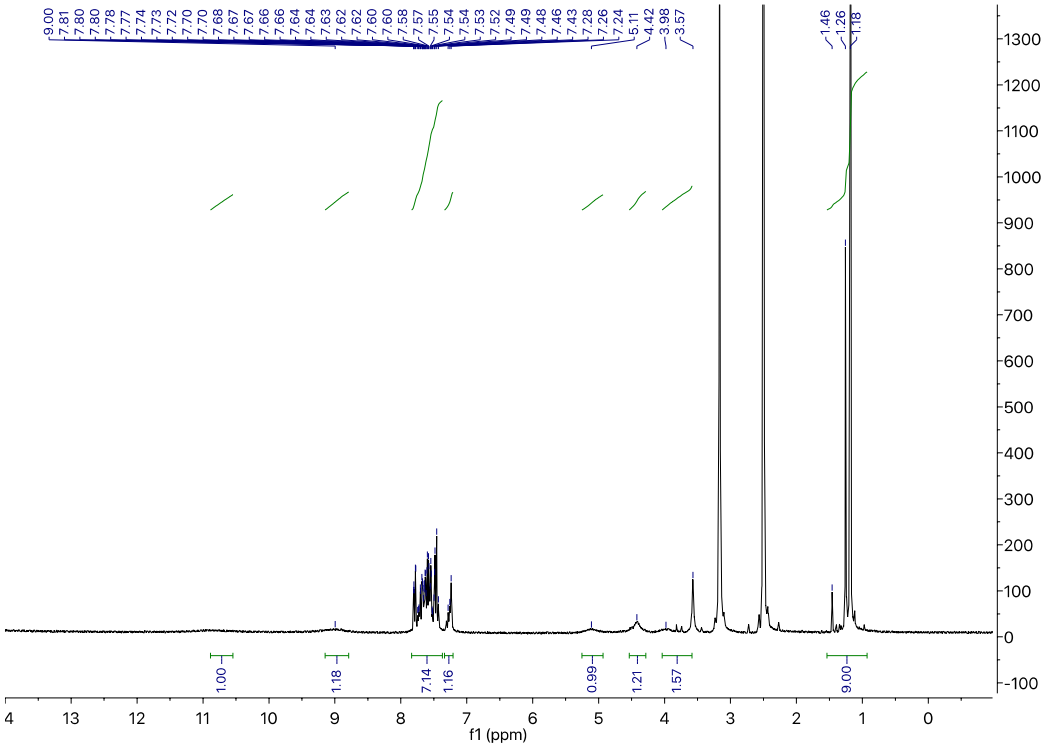


^{13}C NMR (101 MHz, DMSO-d_6)

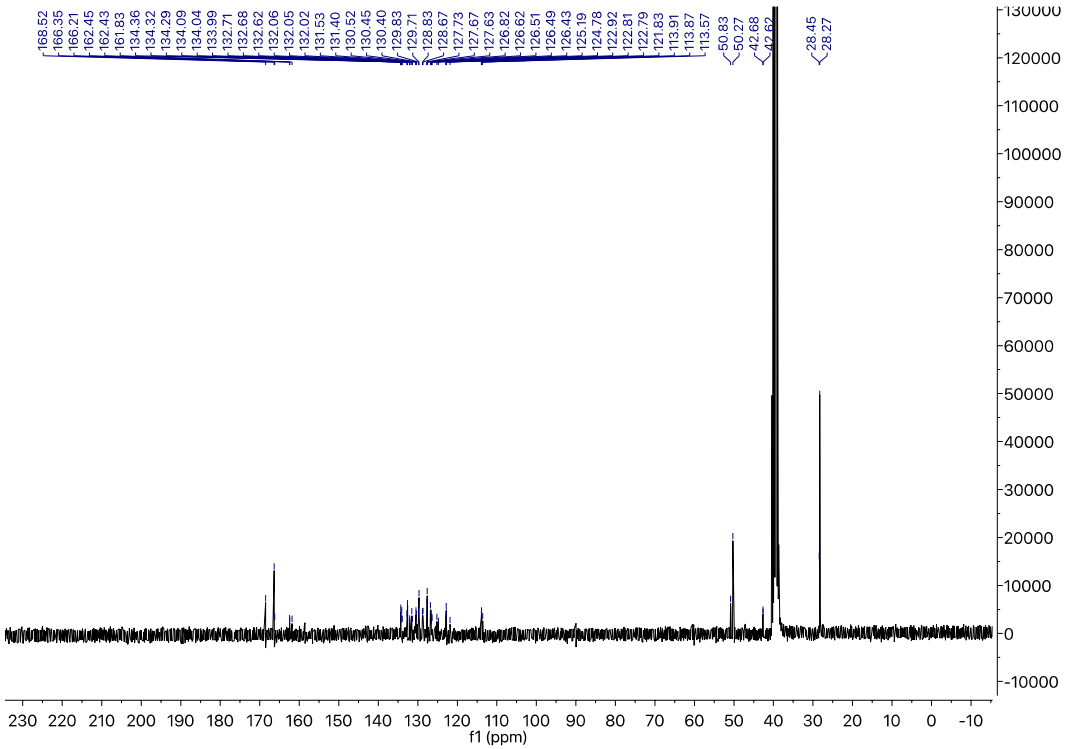


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-[2-(trifluoromethyl)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10n**)

^{1}H NMR (300 MHz, DMSO-d_6, 60 °C)

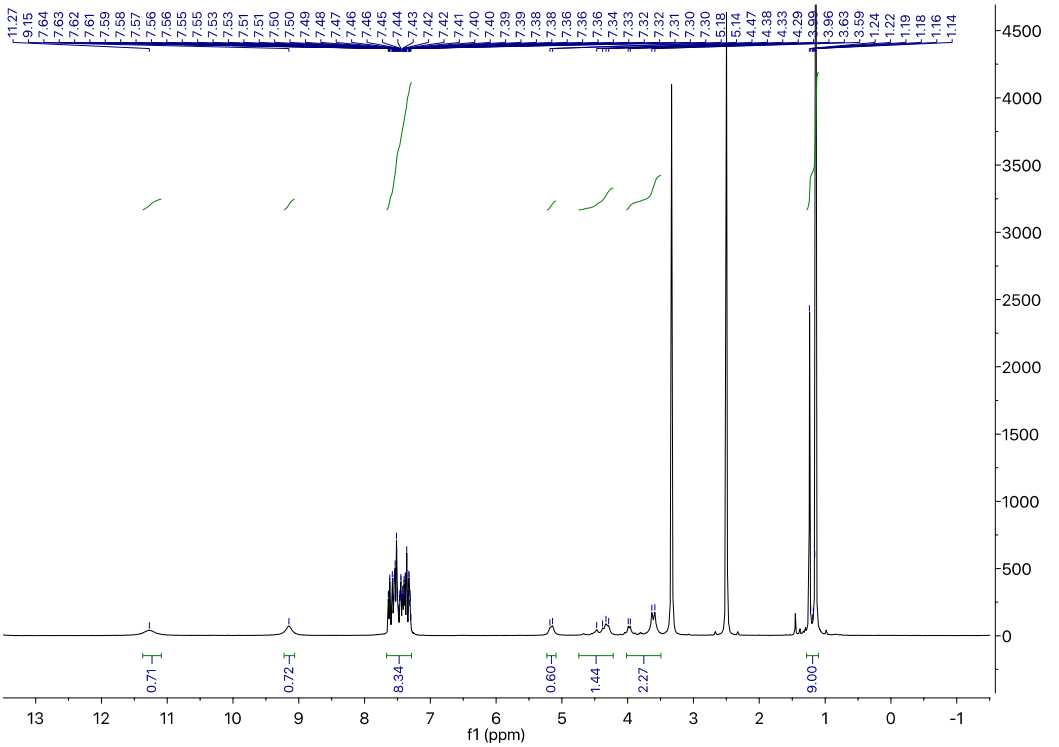


^{13}C NMR (75 MHz, DMSO-d_6)

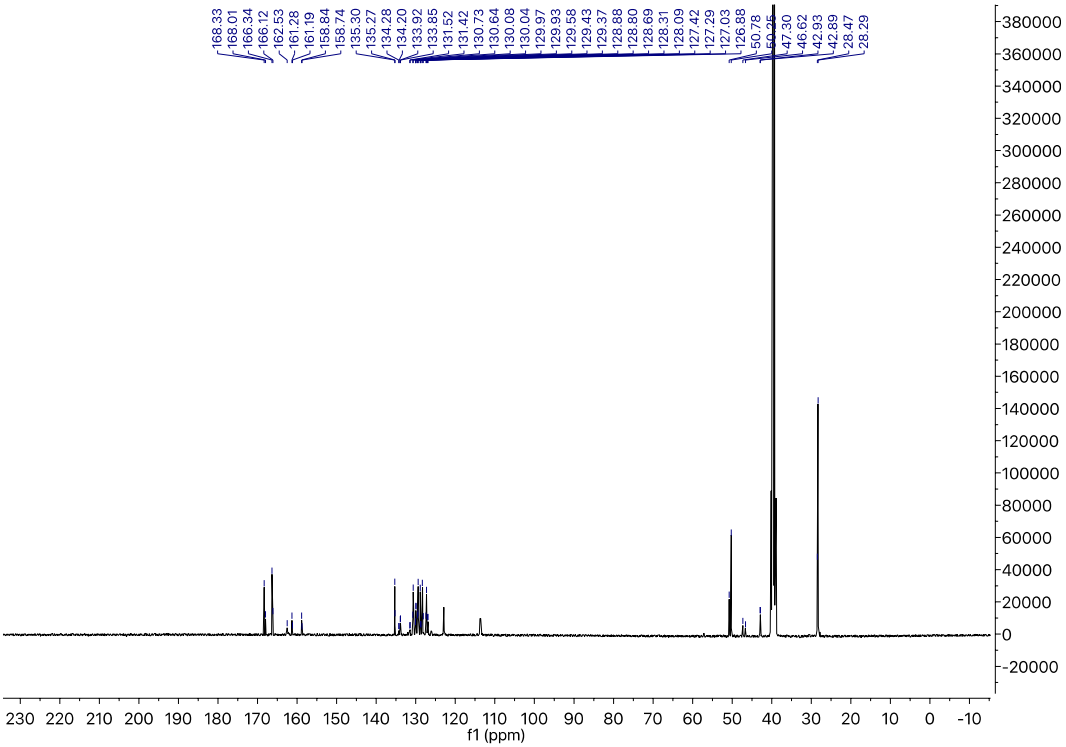


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-chlorophenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10o**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

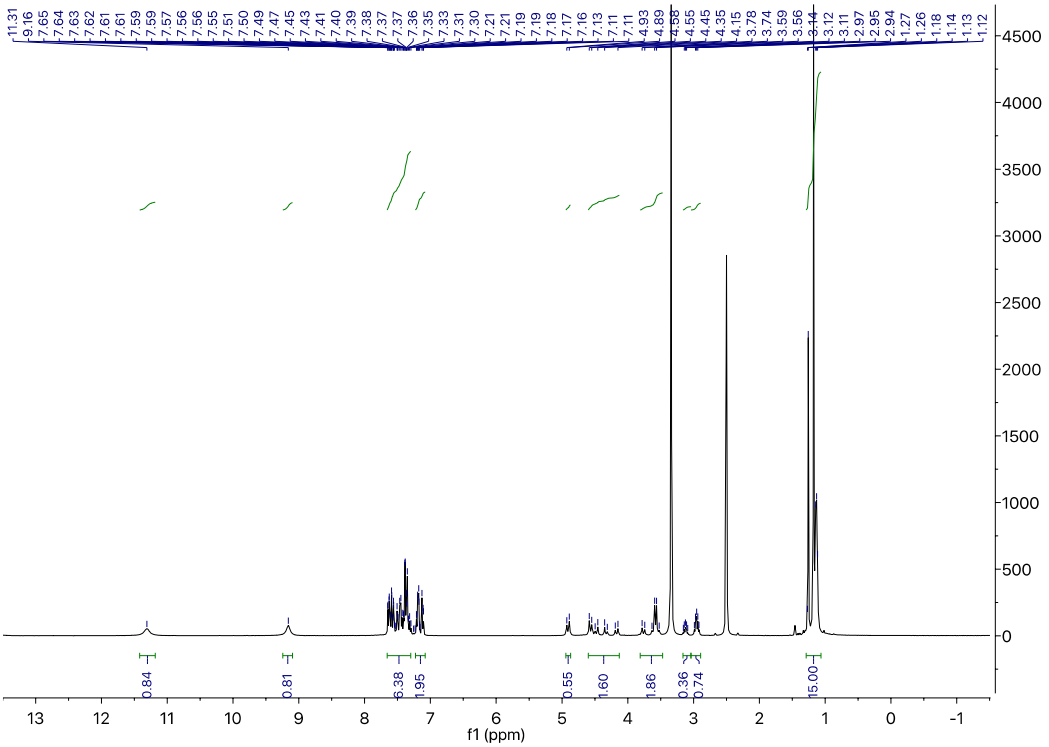


^{13}C NMR (101 MHz, DMSO-d_6)

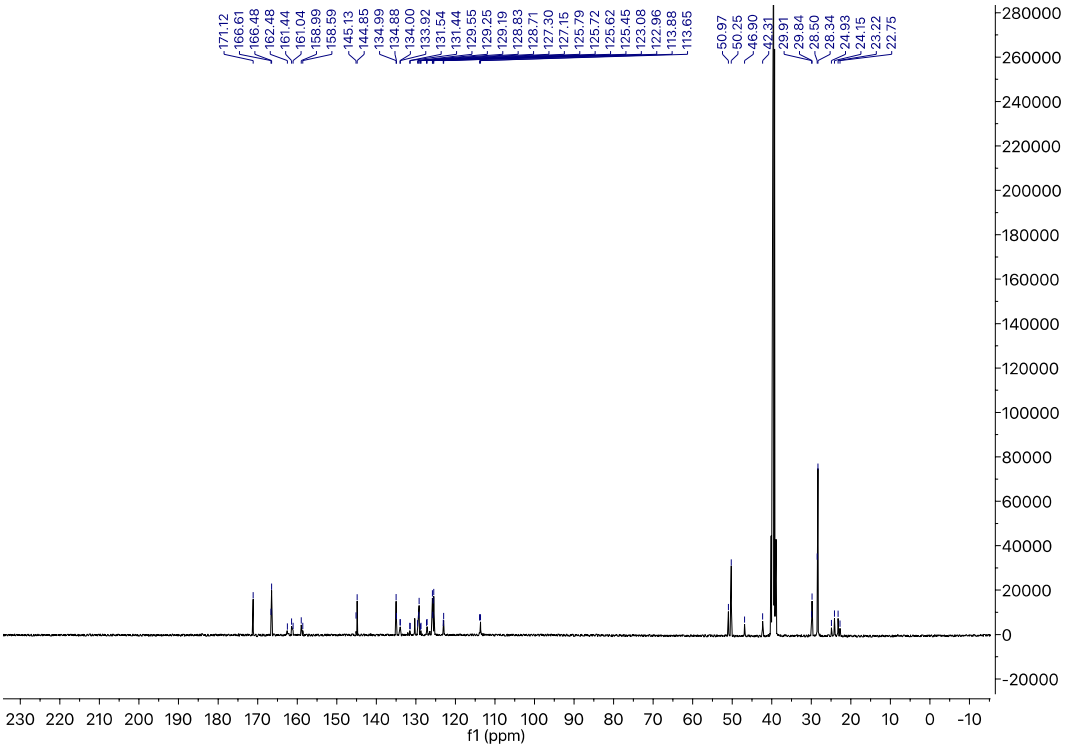


4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-[2-(propan-2-yl)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10p**)

^{1}H NMR (400 MHz, DMSO-d_6, 20 °C)

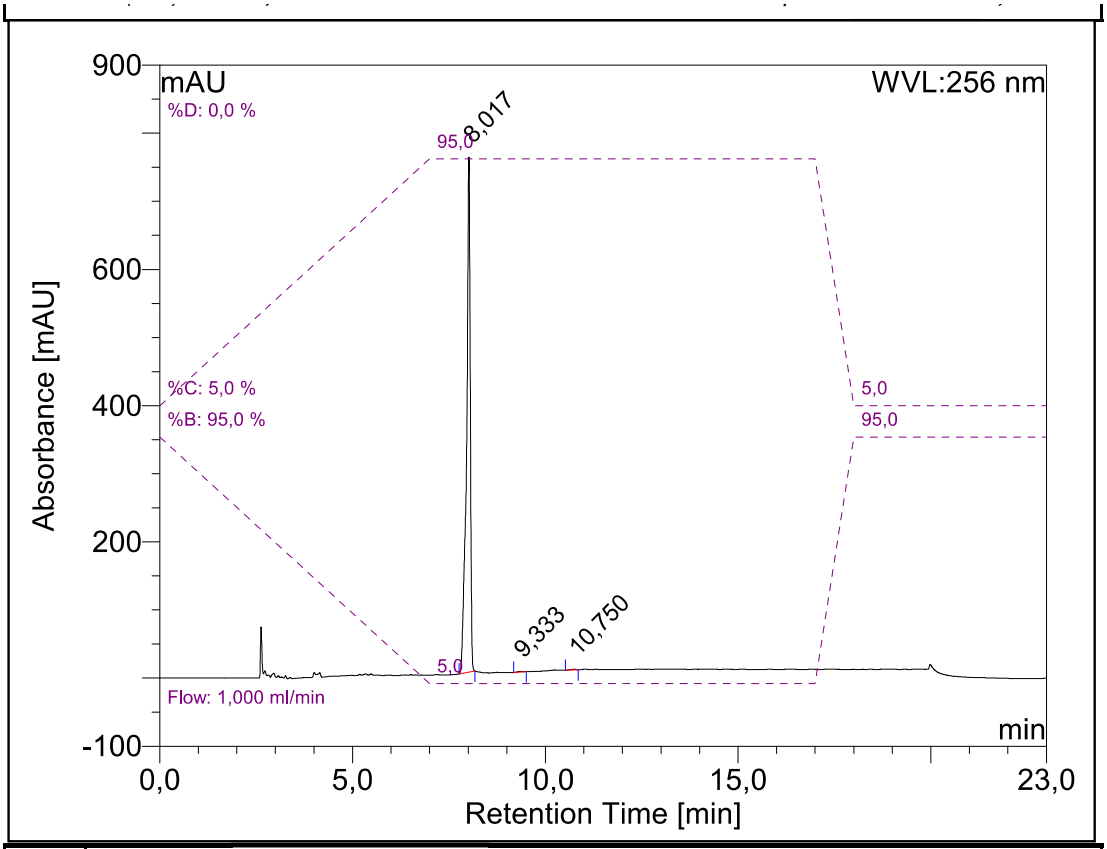


^{13}C NMR (101 MHz, DMSO-d_6)



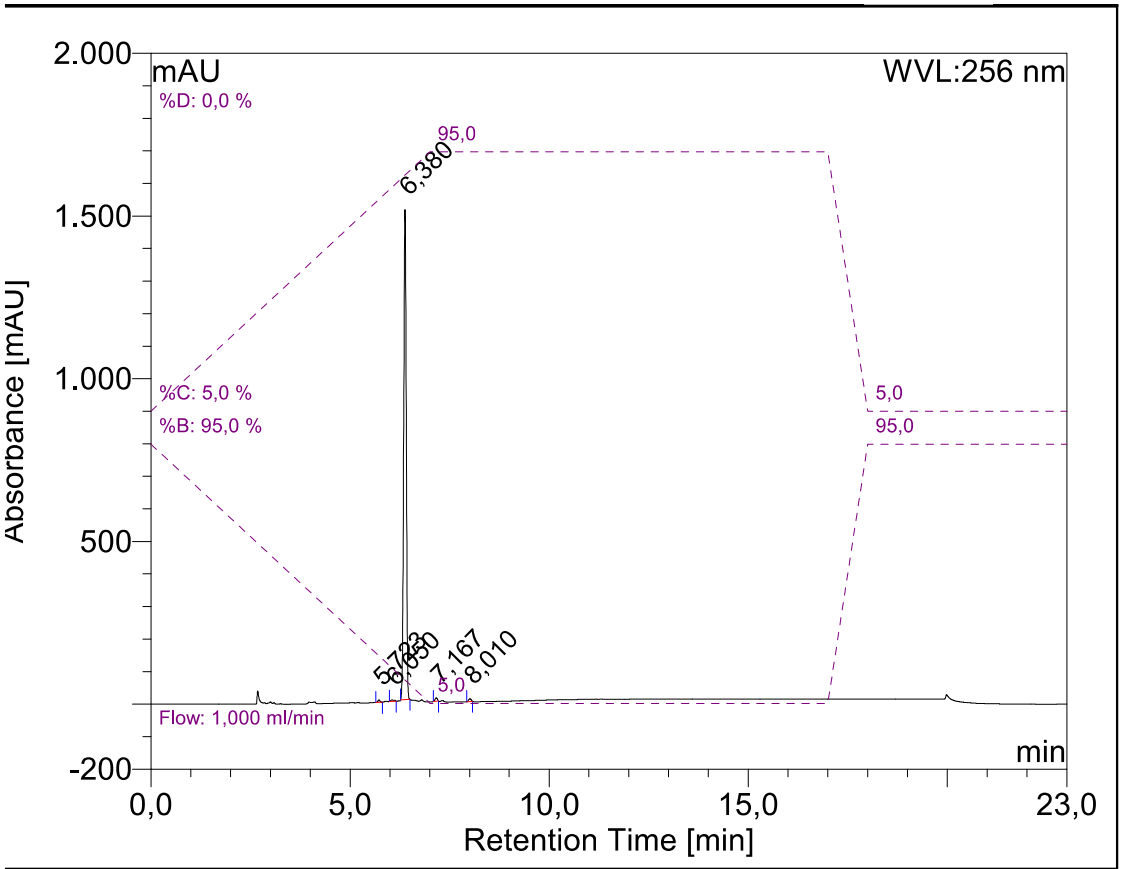
3. HPLC chromatograms

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(3,5-dimethylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10a**)



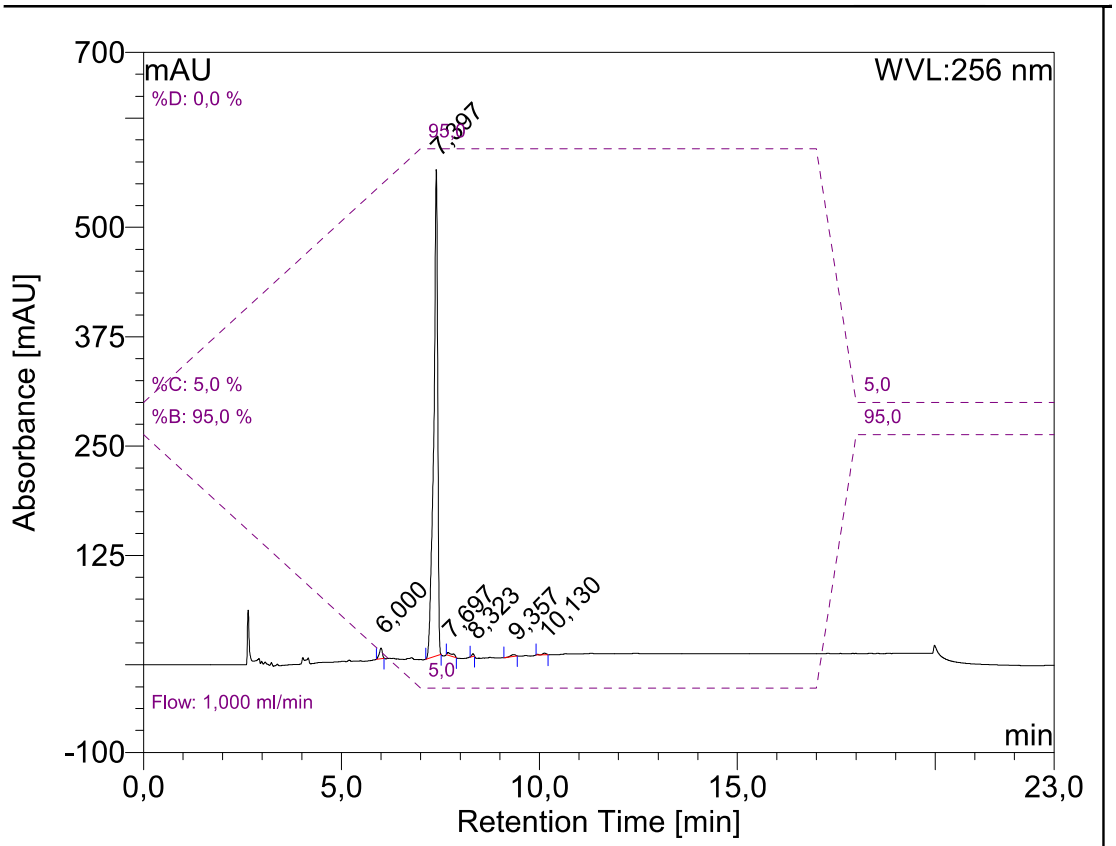
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8,02	n.a.	756,434	85,724	99,72	n.a.	BMB*
2	9,33	n.a.	0,876	0,111	0,13	n.a.	BMB*
3	10,75	n.a.	0,809	0,129	0,15	n.a.	BMB*
Total:			758,119	85,964	100,00	0,000	

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-[4-(dimethylamino)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10b**)



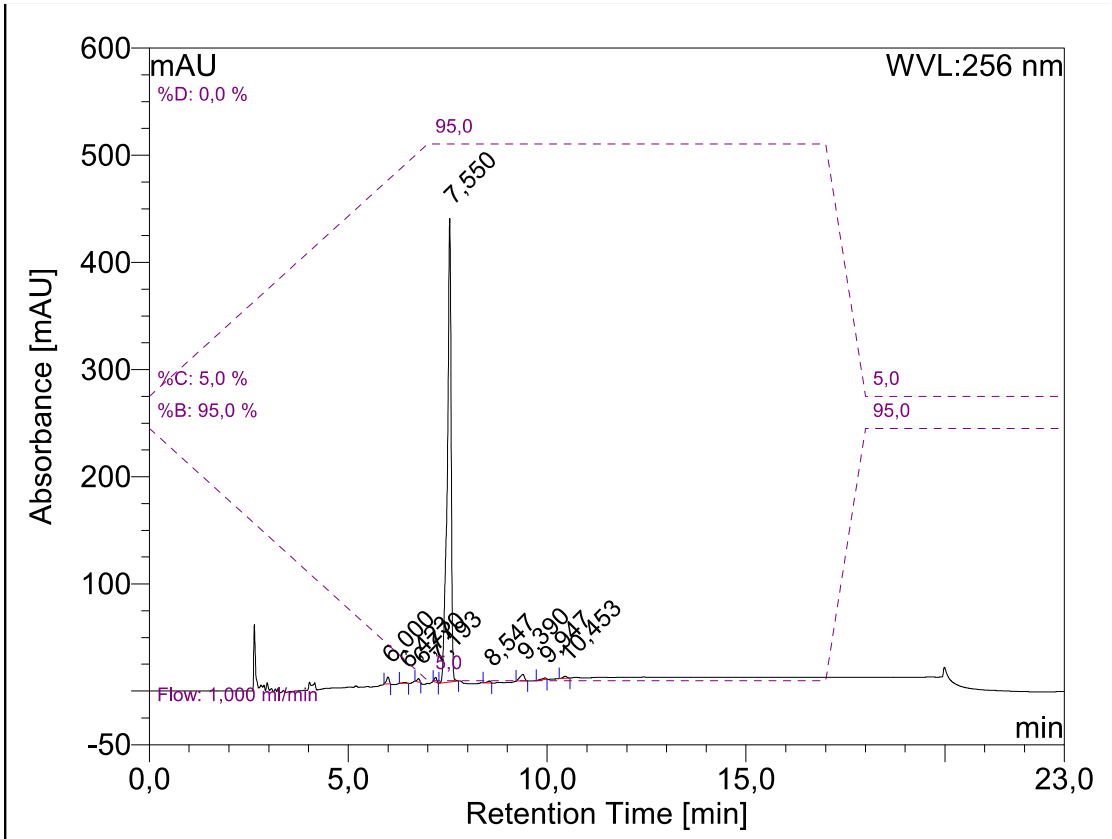
lo.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,72	n.a.	7,255	0,465	0,40	n.a.	BMB*
2	6,05	n.a.	3,878	0,429	0,37	n.a.	BMB*
3	6,38	n.a.	########	112,630	98,08	n.a.	BMB*
4	7,17	n.a.	10,328	0,715	0,62	n.a.	BMB*
5	8,01	n.a.	8,184	0,601	0,52	n.a.	BMB*
tal:			########	114,840	100,00	0,000	

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(2-fluorophenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10c**)



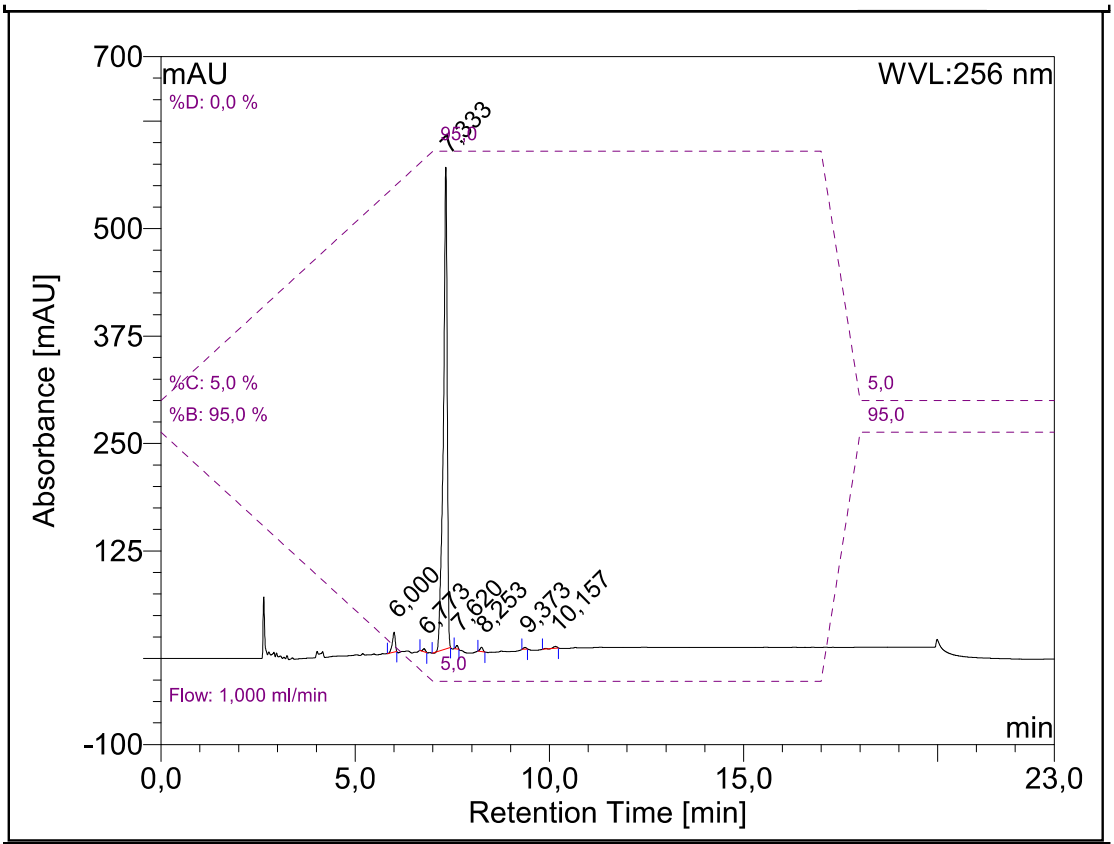
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,00	n.a.	12,112	1,061	1,60	n.a.	BMB*
2	7,40	n.a.	555,713	63,989	96,62	n.a.	BMB*
3	7,70	n.a.	2,917	0,549	0,83	n.a.	BMB*
4	8,32	n.a.	3,019	0,179	0,27	n.a.	BMB*
5	9,36	n.a.	1,951	0,274	0,41	n.a.	BMB*
6	10,13	n.a.	1,735	0,174	0,26	n.a.	BMB*
otal:			577,447	66,227	100,00	0,000	

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(2-methylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10d**)



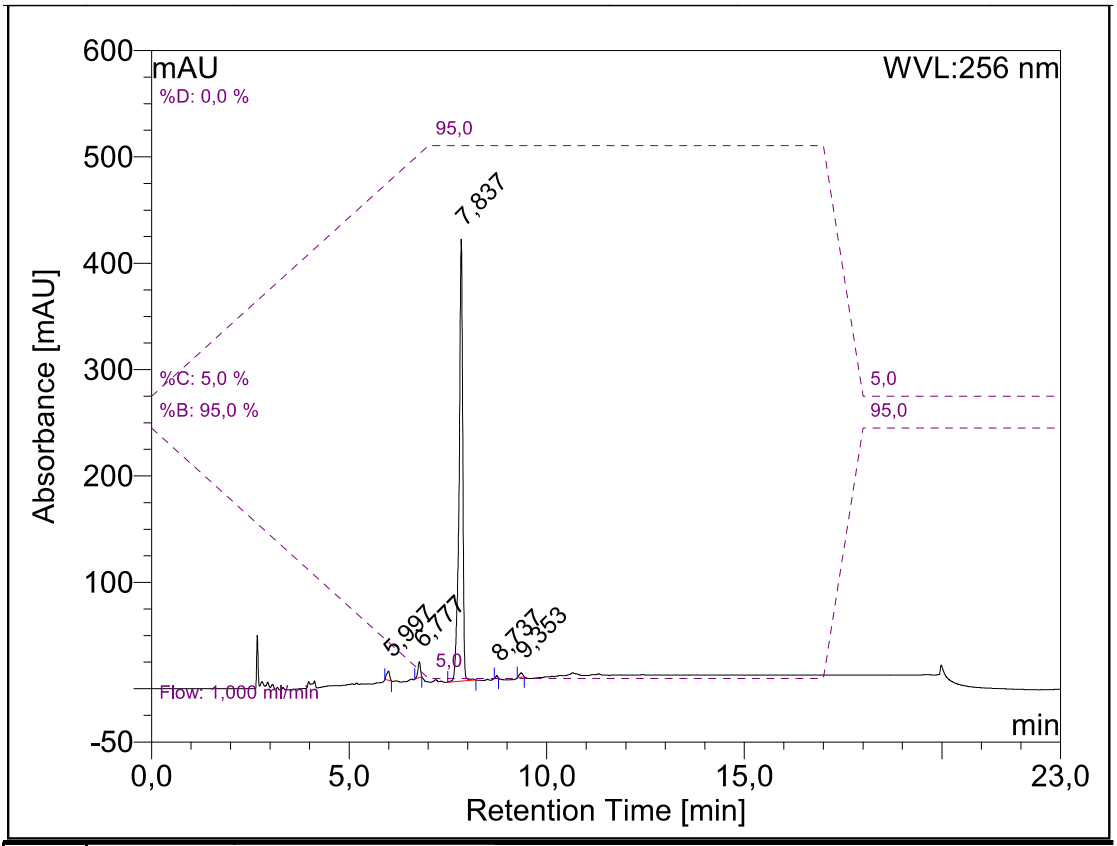
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,00	n.a.	6,453	0,499	0,97	n.a.	BMB*
2	6,42	n.a.	1,006	0,092	0,18	n.a.	BMB*
3	6,77	n.a.	3,072	0,219	0,43	n.a.	BMB*
4	7,19	n.a.	4,475	0,315	0,61	n.a.	BMB*
5	7,55	n.a.	432,672	48,955	95,07	n.a.	BMB*
6	8,55	n.a.	1,904	0,182	0,35	n.a.	BMB*
7	9,39	n.a.	6,247	0,833	1,62	n.a.	BMB*
8	9,95	n.a.	1,513	0,179	0,35	n.a.	BMB*
9	10,45	n.a.	1,954	0,221	0,43	n.a.	BMB*
Total:			459,297	51,496	100,00	0,000	

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-(2-methoxyphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10e**)



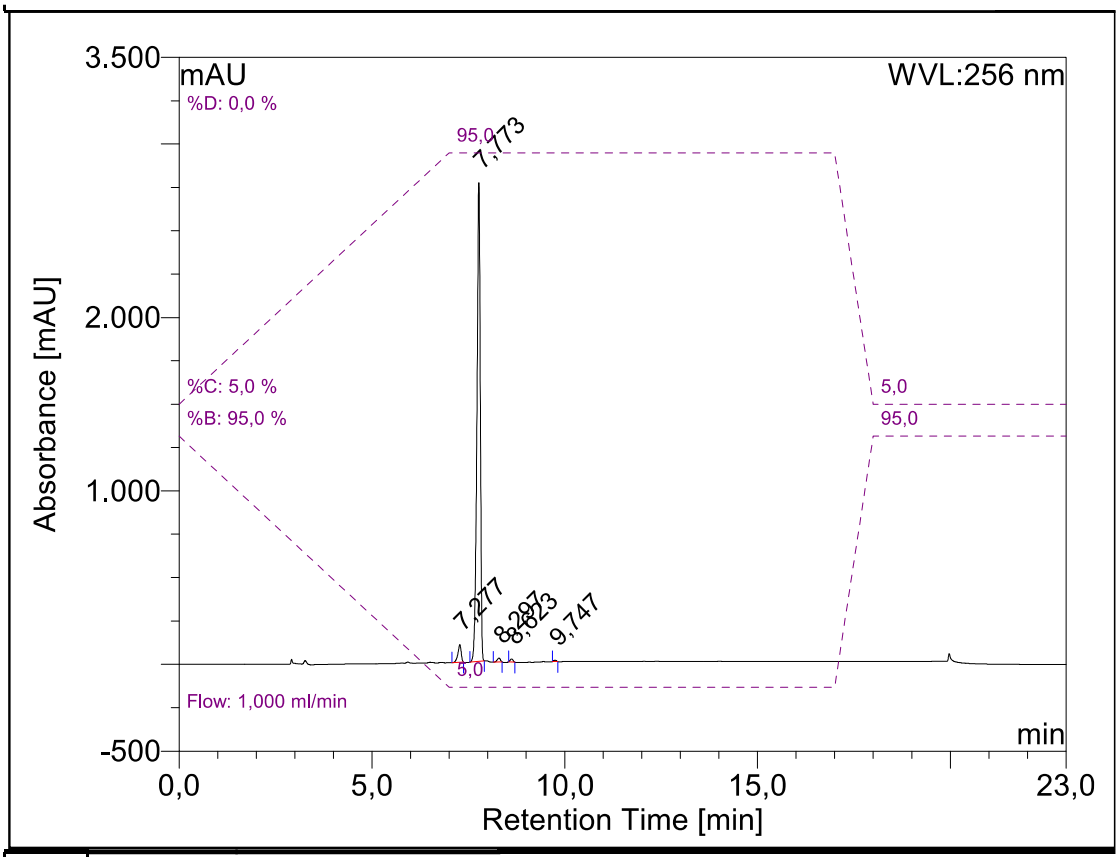
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,00	n.a.	23,227	1,979	2,92	n.a.	BMB*
2	6,77	n.a.	3,518	0,266	0,39	n.a.	BMB*
3	7,33	n.a.	560,359	64,409	95,06	n.a.	BMB*
4	7,62	n.a.	4,018	0,261	0,38	n.a.	BMB*
5	8,25	n.a.	4,892	0,399	0,59	n.a.	BMB*
6	9,37	n.a.	1,859	0,160	0,24	n.a.	BMB*
7	10,16	n.a.	2,135	0,283	0,42	n.a.	BMB*
Total:			600,009	67,756	100,00	0,000	

4-({*N*-[(cyclohexylcarbamoyl)methyl]-1-[2-(trifluoromethyl)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10f**)



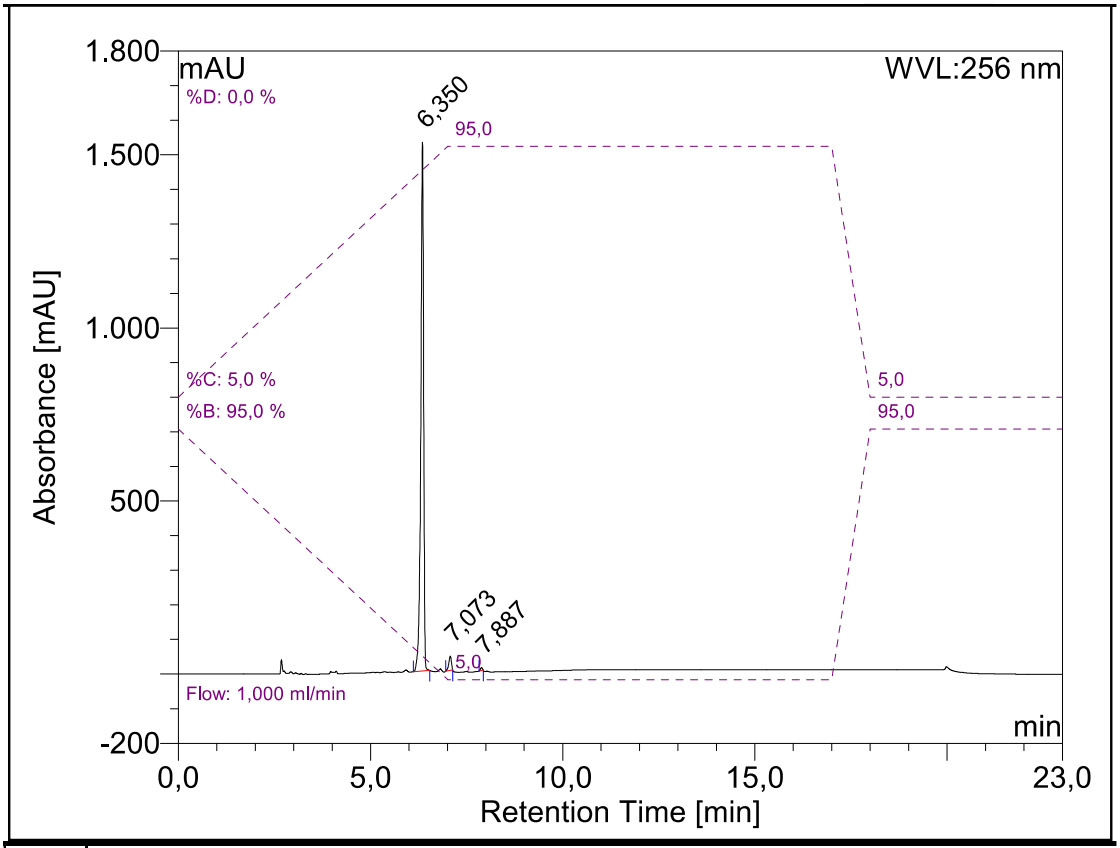
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,00	n.a.	8,523	0,769	1,62	n.a.	BMB*
2	6,78	n.a.	14,812	1,071	2,26	n.a.	BMB*
3	7,84	n.a.	415,314	45,086	95,02	n.a.	BMB*
4	8,74	n.a.	2,456	0,139	0,29	n.a.	BMB*
5	9,35	n.a.	3,914	0,386	0,81	n.a.	BMB*
Total:			445,018	47,450	100,00	0,000	

4-({*N*-[(benzylcarbamoyl)methyl]-1-(3,5-dimethylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10g**)



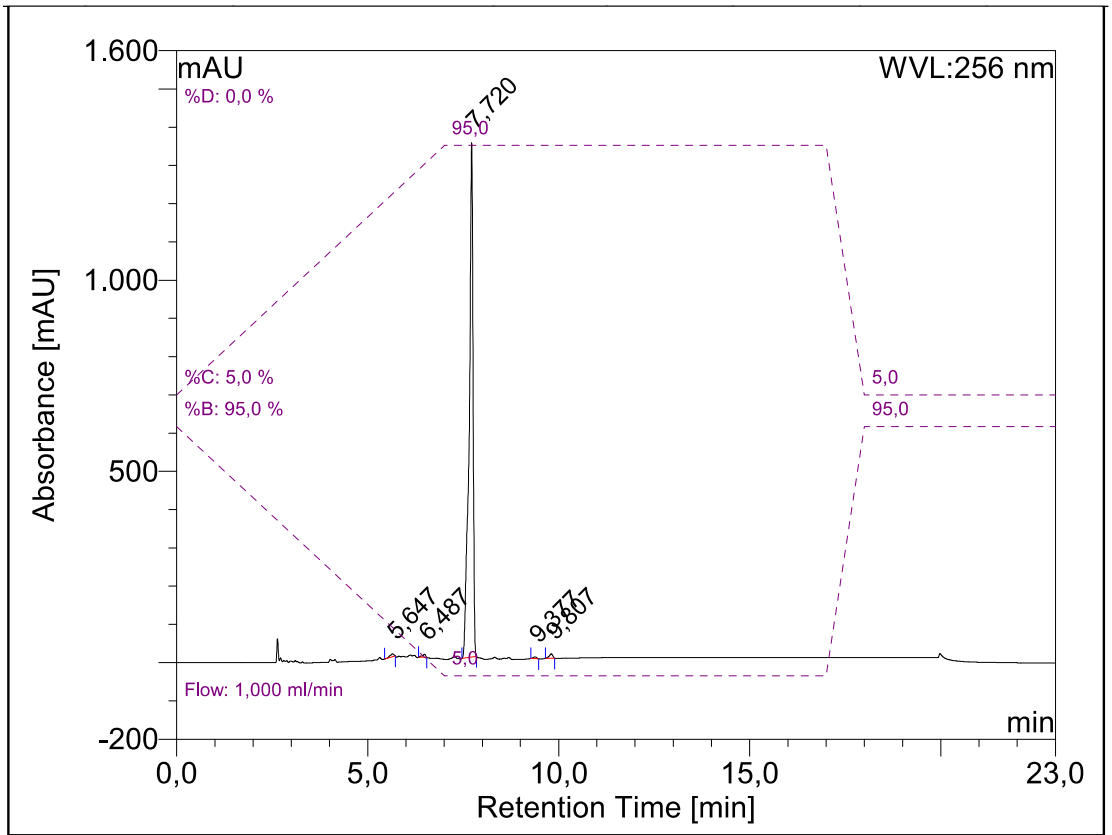
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7,28	n.a.	102,138	10,225	3,43	n.a.	BMB*
2	7,77	n.a.	########	283,953	95,18	n.a.	BMB*
3	8,30	n.a.	22,152	2,064	0,69	n.a.	BMB*
4	8,62	n.a.	17,619	1,463	0,49	n.a.	BMB*
5	9,75	n.a.	8,046	0,627	0,21	n.a.	BMB*
Total:			########	298,333	100,00	0,000	

4-({*N*-[(benzylcarbamoyl)methyl]-1-[4-(dimethylamino)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10h**)



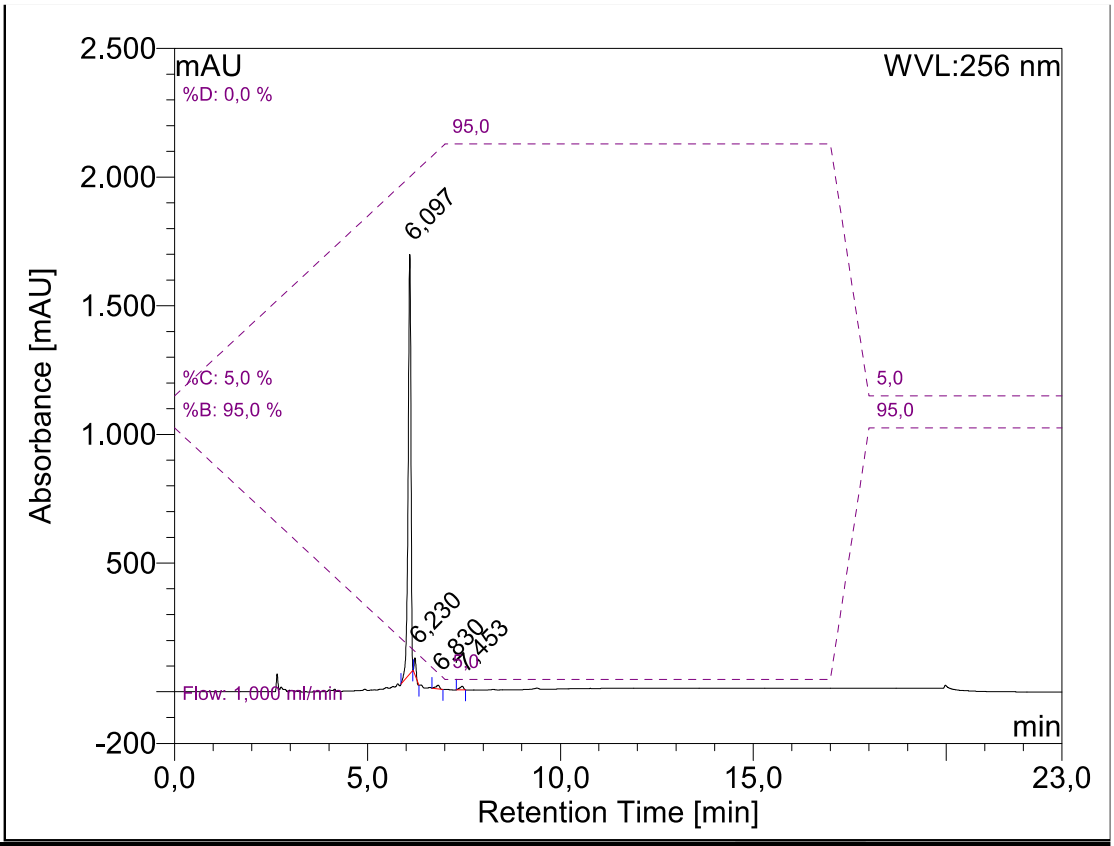
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,35	n.a.	########	130,046	97,24	n.a.	BMB*
2	7,07	n.a.	41,580	3,199	2,39	n.a.	BMB*
3	7,89	n.a.	8,265	0,498	0,37	n.a.	BMB*
Total:			########	133,743	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(3,5-dimethylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10i**)



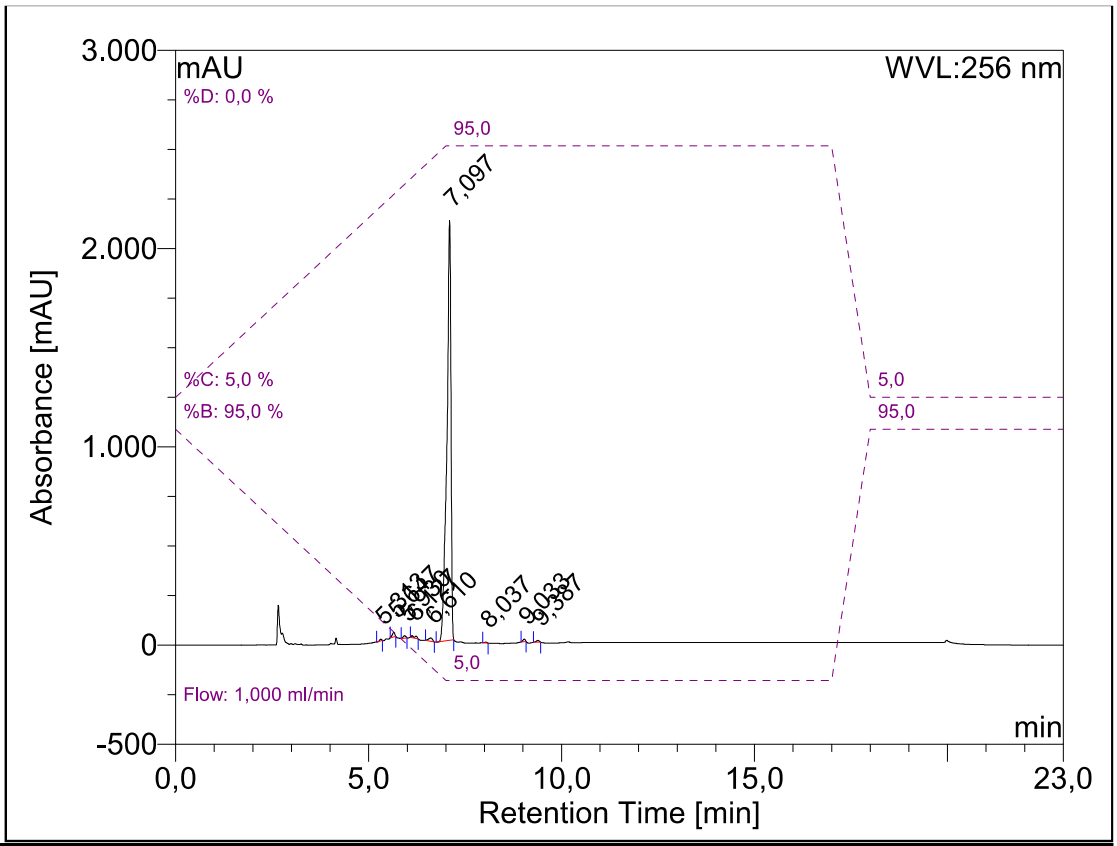
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,65	n.a.	7,964	0,909	0,56	n.a.	BMB*
2	6,49	n.a.	8,031	0,837	0,51	n.a.	BMB*
3	7,72	n.a.	#########	160,040	97,86	n.a.	BMB*
4	9,38	n.a.	4,857	0,512	0,31	n.a.	BMB*
5	9,81	n.a.	11,349	1,236	0,76	n.a.	BMB*
Total:			#########	163,534	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-[4-(dimethylamino)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10j**)



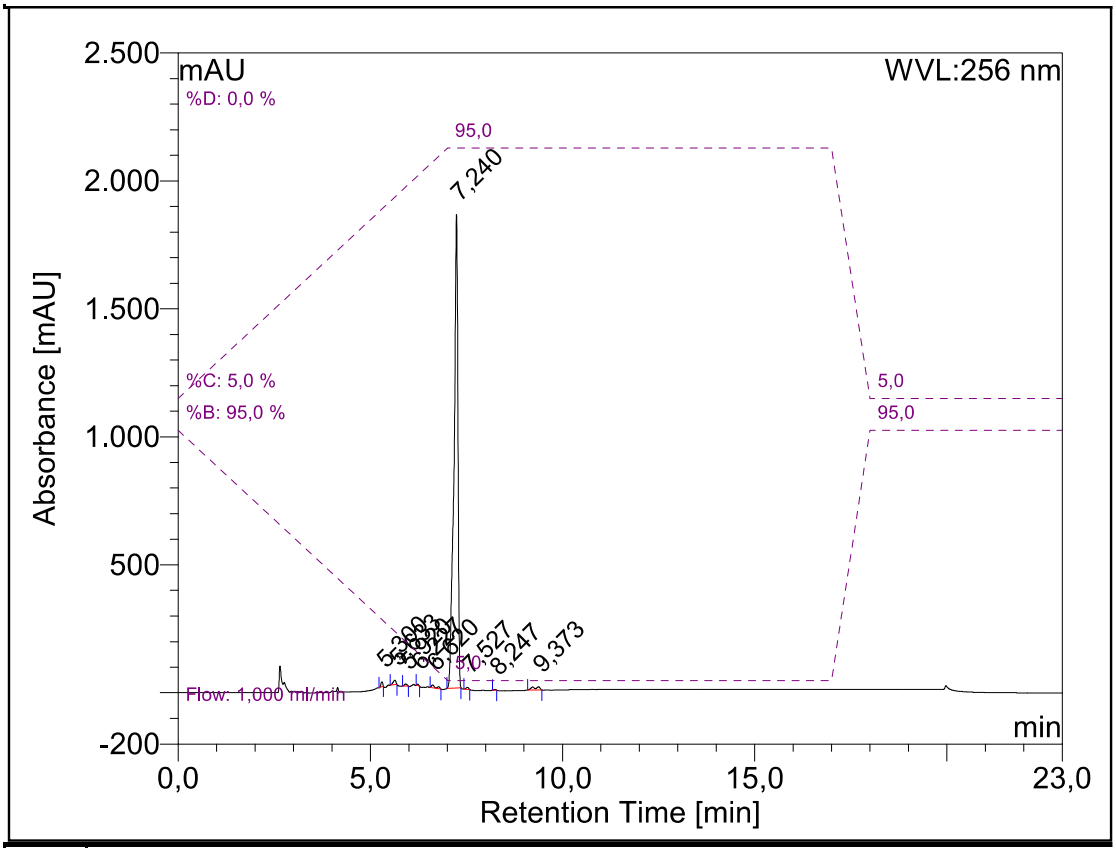
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,10	n.a.	########	139,570	95,46	n.a.	BMB*
2	6,23	n.a.	65,683	3,326	2,27	n.a.	BMB*
3	6,83	n.a.	15,276	1,673	1,14	n.a.	BMB*
4	7,45	n.a.	14,745	1,638	1,12	n.a.	BMB*
Total:			#########	146,206	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-fluorophenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10k**)



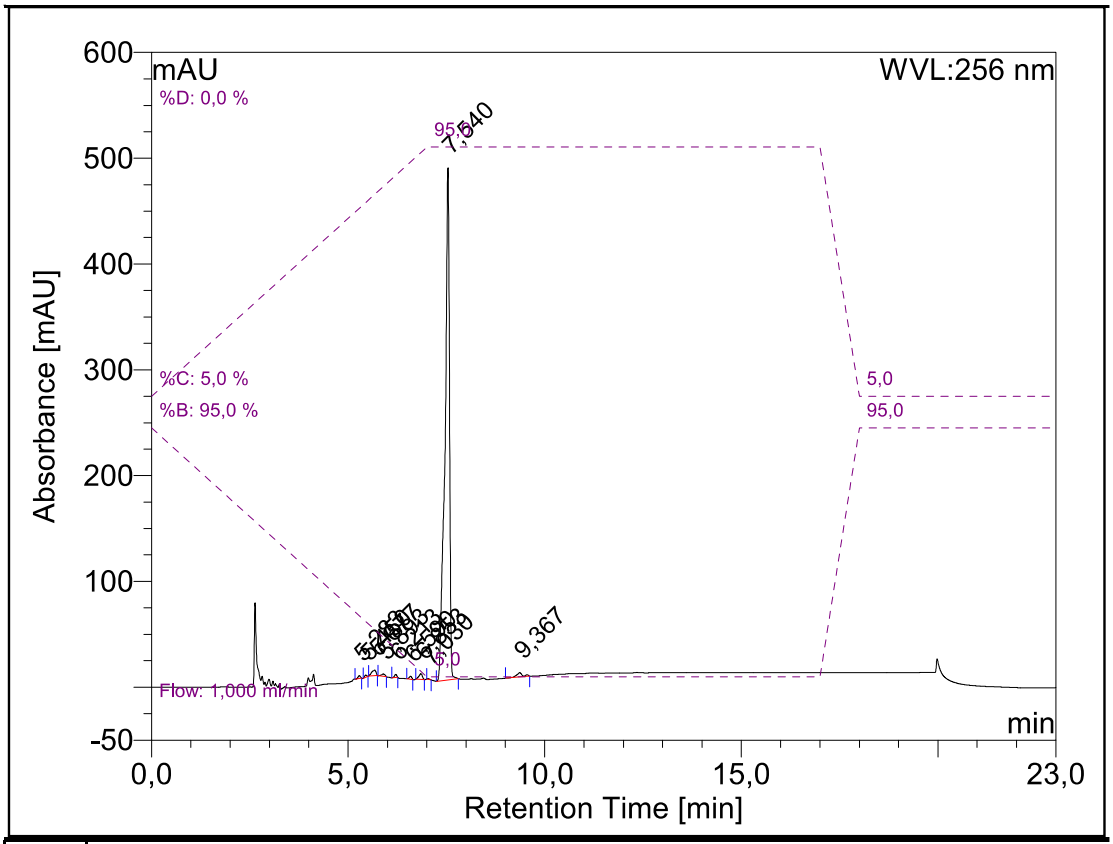
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,31	n.a.	9,767	0,547	0,20	n.a.	BMB*
2	5,65	n.a.	23,662	1,717	0,64	n.a.	BMB*
3	5,93	n.a.	11,607	0,910	0,34	n.a.	BMB*
4	6,13	n.a.	10,134	1,356	0,51	n.a.	BMB*
5	6,61	n.a.	16,290	1,993	0,75	n.a.	BMB*
6	7,10	n.a.	########	258,534	96,76	n.a.	BMB*
7	8,04	n.a.	4,209	0,290	0,11	n.a.	BMB*
8	9,03	n.a.	13,369	0,940	0,35	n.a.	BMB*
9	9,39	n.a.	9,563	0,916	0,34	n.a.	BMB*
Total:			########	267,203	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-methylphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10l**)



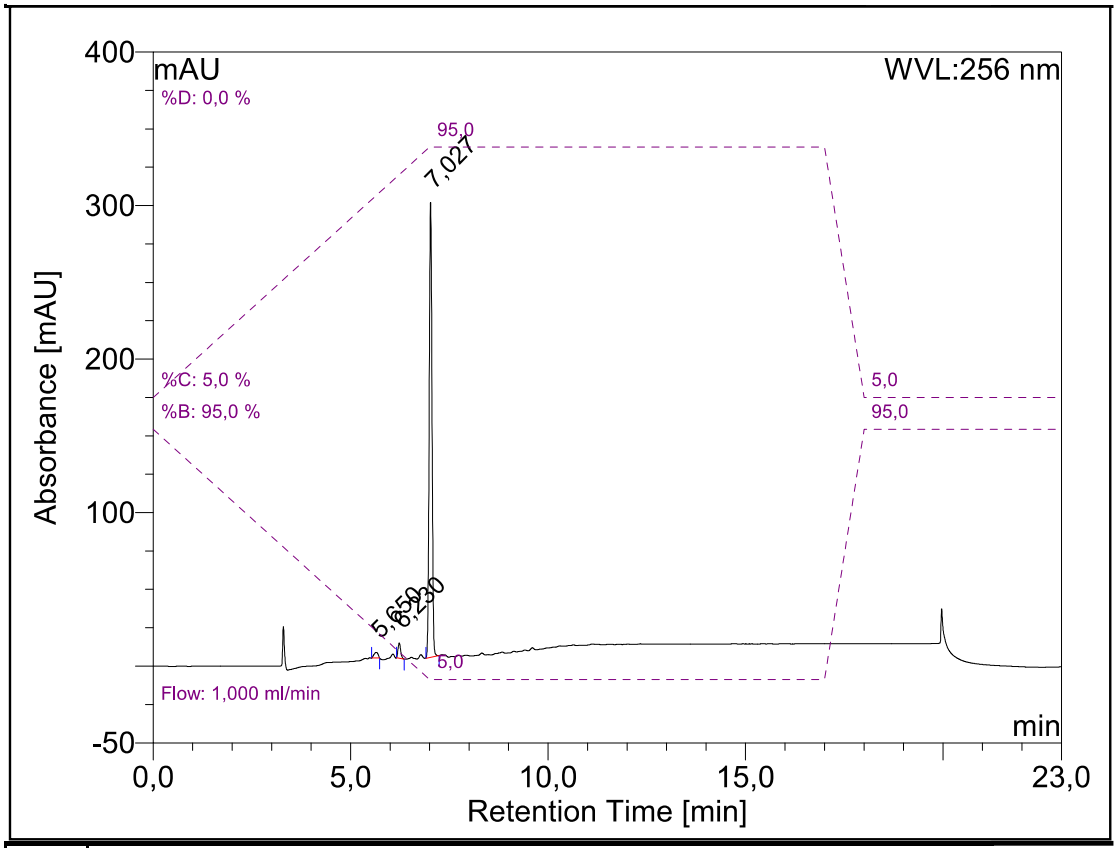
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,30	n.a.	18,691	1,042	0,46	n.a.	BMB*
2	5,63	n.a.	17,920	1,567	0,69	n.a.	BMB*
3	5,92	n.a.	8,234	0,620	0,27	n.a.	BMB*
4	6,23	n.a.	5,203	0,271	0,12	n.a.	BMB*
5	6,62	n.a.	10,859	1,451	0,64	n.a.	BMB*
6	7,24	n.a.	########	217,882	96,43	n.a.	BMB*
7	7,53	n.a.	7,259	0,554	0,25	n.a.	BMB*
8	8,25	n.a.	2,951	0,173	0,08	n.a.	BMB*
9	9,37	n.a.	11,601	2,400	1,06	n.a.	BMB*
Total:			########	225,960	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-methoxyphenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10m**)



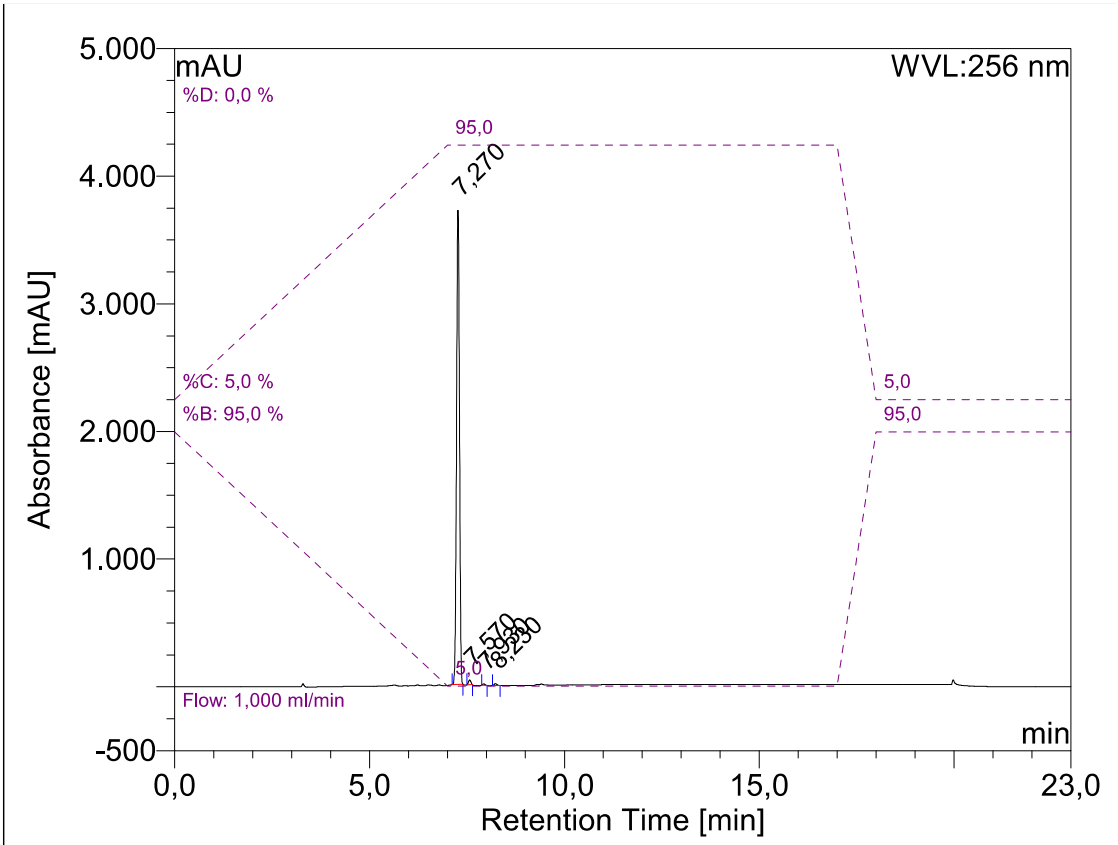
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,28	n.a.	2,730	0,183	0,27	n.a.	BMB*
2	5,45	n.a.	2,231	0,138	0,21	n.a.	BMB*
3	5,68	n.a.	5,251	0,652	0,98	n.a.	BMB*
4	5,89	n.a.	2,239	0,245	0,37	n.a.	BMB*
5	6,21	n.a.	2,849	0,188	0,28	n.a.	BMB*
6	6,59	n.a.	2,842	0,205	0,31	n.a.	BMB*
7	6,85	n.a.	5,421	0,543	0,81	n.a.	BMB*
8	7,03	n.a.	0,593	0,049	0,07	n.a.	BMB*
9	7,54	n.a.	484,106	63,662	95,59	n.a.	BMB*
10	9,37	n.a.	4,085	0,731	1,10	n.a.	BMB*
Total:			512,347	66,596	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-[2-(trifluoromethyl)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10n**)



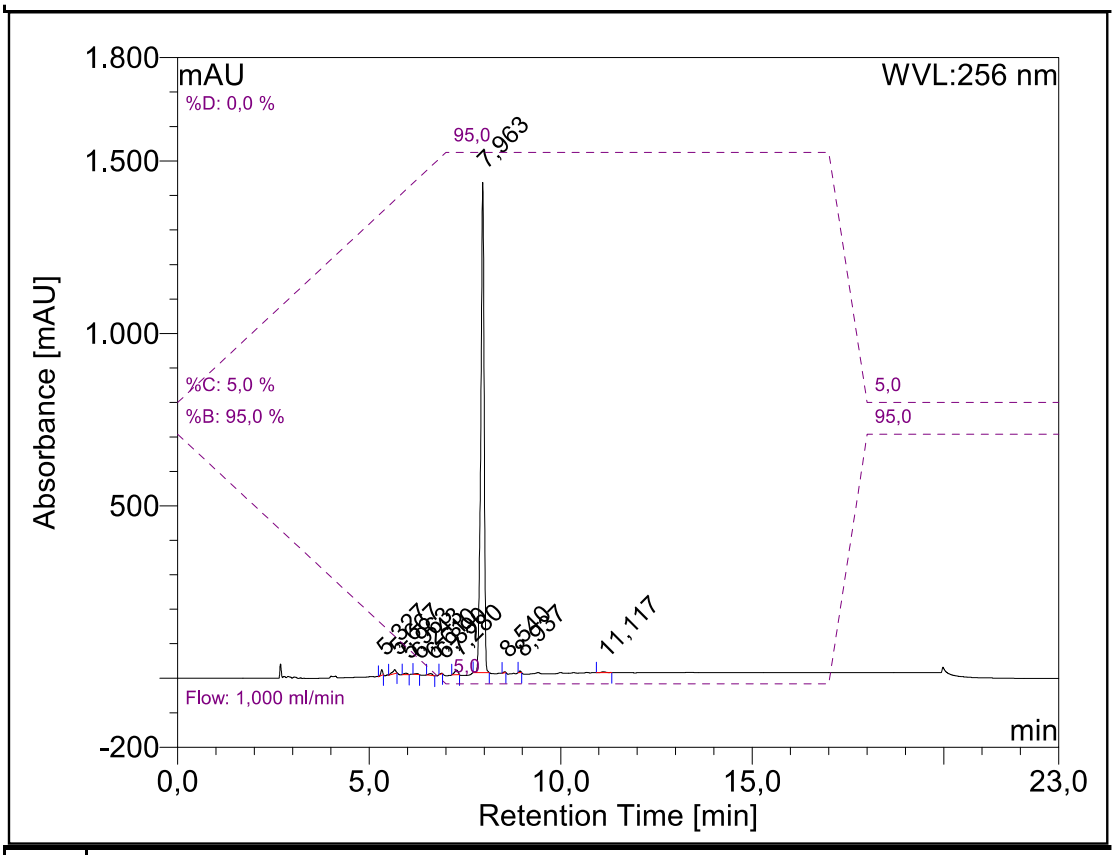
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,65	n.a.	3,712	0,450	1,76	n.a.	BMB*
2	6,23	n.a.	10,095	0,799	3,13	n.a.	BMB*
3	7,03	n.a.	296,196	24,261	95,10	n.a.	BMB*
Total:			310,003	25,510	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-(2-chlorophenyl)formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10o**)



No.	Ret.Time min		Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7,27	n.a.		########	322,378	98,56	n.a.	BMB*
2	7,57	n.a.		37,183	2,726	0,83	n.a.	BMB*
3	7,93	n.a.		10,692	0,753	0,23	n.a.	BMB*
4	8,23	n.a.		15,348	1,228	0,38	n.a.	BMB*
Total:				########	327,085	100,00	0,000	

4-({*N*-[(*tert*-butylcarbamoyl)methyl]-1-[2-(propan-2-yl)phenyl]formamido}methyl)-3-fluoro-*N*-hydroxybenzamide (**10p**)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5,33	n.a.	15,654	0,815	0,55	n.a.	BMB*
2	5,67	n.a.	11,328	1,118	0,76	n.a.	BMB*
3	5,96	n.a.	3,677	0,360	0,24	n.a.	BMB*
4	6,24	n.a.	2,570	0,219	0,15	n.a.	BMB*
5	6,61	n.a.	4,983	0,534	0,36	n.a.	BMB*
6	6,89	n.a.	1,790	0,104	0,07	n.a.	BMB*
7	7,28	n.a.	13,440	1,412	0,96	n.a.	BMB*
8	7,96	n.a.	########	141,929	96,35	n.a.	BMB*
9	8,54	n.a.	3,457	0,202	0,14	n.a.	BMB*
10	8,94	n.a.	4,819	0,272	0,18	n.a.	BMB*
11	11,12	n.a.	2,353	0,338	0,23	n.a.	BMB*
Total:			#########	147,303	100,00	0,000	