ChemMedChem

Supporting Information

Exploring the Translational Gap of a Novel Class of *Escherichia coli* IspE Inhibitors

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Table of content

1. Structure-Based Virtual Screening

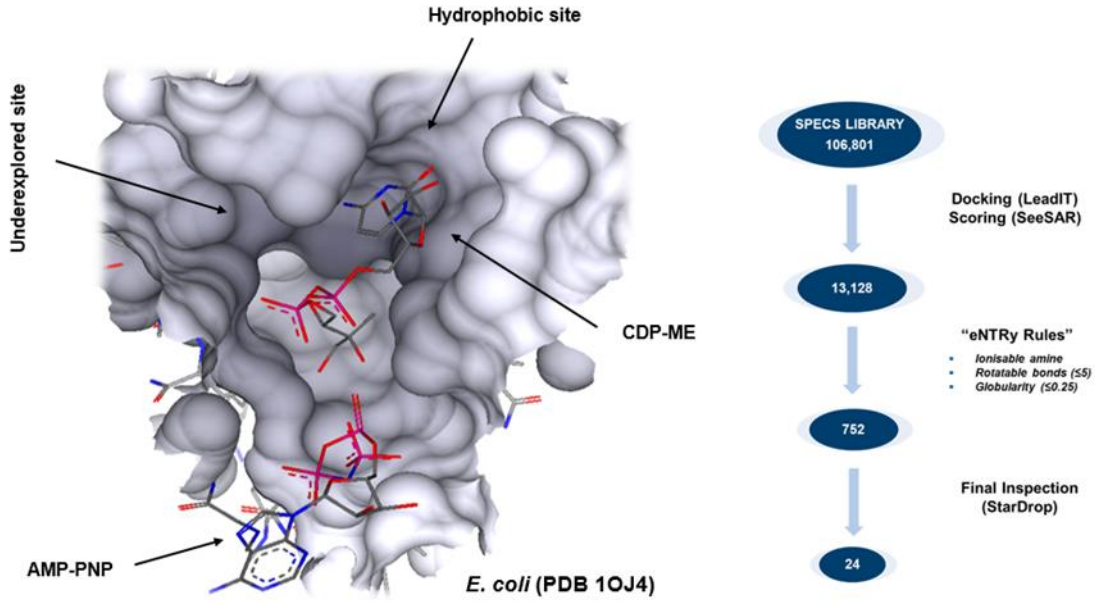


Fig. S1: The used binding pocket for the virtual screening with *Ec*IspE (PDB 1OJ4) and summary of the workflow. The figure was created with SeeSAR 8.2.[14d]

General Workflow

The VS campaign was completed using BioSolveIT software, where LeadIT was used for docking and SeeSAR for scoring. [14c] After scoring, the compounds were filtered and only compounds with estimated HYDE-affinity (<1 mM), torsional angles (green or orange) and a total number of poses (≥2) were selected further. In total, 13,128 compounds passed through these filters. After the final inspection of the poses and clustering compound classes in StarDrop, we purchased 24 compounds and tested them against *Ec*IspE, *E. coli* wild-type K12 and mutant strain Δ*tolC*. The selection included a mixture of compounds with different degrees of ionisation of the ionisable amine. In addition, we also selected a few compounds simply with the highest estimated HYDE-binding affinities and a few compounds based on a novel antibacterial scoring profile developed by StarDrop.

1.1 Prediction of Druggable Pockets

DoGSiteScorer was used to identify druggable pockets for *E. coli* IspE (PDB 1OJ4).[13] Overall, only P_0 with the druggability score of 0.81 was above the ideal druggability score (>0.80). Thus, pocket P_0 was selected as the binding pocket, occupying the main catalytic region in monomer A (Fig. S1).

Descriptors of the P_0 pocket from DogSiteScorer. [14b]

Size and shape descriptors

volume = 809.93 Å³
surface = 1210.48 Å²
depth = 18.80 [Å]
ellipsoid main axis ratio c/a = 0.17
ellipsoid main axis ratio b/a = 0.43
enclosure = 0.16

Element descriptors

pocket atoms =163
carbons = 108
nitrogens = 27
oxygens = 27
sulfurs = 1
other elements = 0

Functional group descriptors

hydrogen bond donors = 23
hydrogen bond acceptors = 55
metals = 0
hydrophobic interactions = 65
hydrophobicity ratio = 0.45

Amino acid composition

apolar amino acid ratio = 0.39
polar amino acid ratio = 0.41
positive amino acid ratio = 0.12
negative amino acid ratio = 0.07

Amino acid descriptors

ALA = 3
ARG = 0
ASN = 1
ASP = 2
CYS = 1
GLN = 1
GLU = 1
GLY = 9
HIS = 2
ILE = 1
LEU = 5
LYS = 3
MET = 0
PHE = 2
PRO = 2
SER = 1
THR = 3
TRP = 0
TYR = 1
VAL = 3

Virtual Screening of Compounds

The compound library was obtained from SPECS containing in total 106, 801 compounds. The library consisted of compounds with MW 250–500 Da fulfilling the Lipinski's rule of five[29] and any promiscuous compounds were filtering out by applying PAINS[30] and Eli Lilly rules[31]. Additionally, only compounds that were available (>2 mg) at the time of the library creation were included.

Protein Preparation

The crystal structure of *E. coli* IspE (PDB 1OJ4) in the absence of the co-crystallised ligands, ATP and CDP-ME, was used for the VS. The binding pocket was defined by selecting the following amino acids manually, as obtained in the druggability assessment: LYS10, ASN12, LEU15, GLN20, GLY24A, TYR25A, HIS26A, LEU28, THR30, PHE32, PRO99, GLY101, GLY102, GLY103, LEU104, GLY105, LEU136, GLY139, ALA140, ASP141, VAL144, ALA153, VAL156, GLY157, GLU158, LEU160, HIS174, VAL177, SER178, ILE179, THR181, PRO182, PHE185, LYS186, GLY239, THR240, GLY241, ALA242, CYS243, LYS76 (chain B) and ASP80 (chain B).

Virtual Screening

The KNIME Analytics Platform was used to run the VS workflow using the following nodes (Generate 3D Coordinates, LeadIT, SeeSAR) from BioSolveIT. First, the 3D-coordinates were generated and then compounds were docked by using LeadIT (version 2.3.2).[14d] The default settings were used for docking and for each molecule, ten poses were calculated. The resulting poses were then scored using the HYDE function in SeeSAR (version 8.1). The compounds were filtered based on binding affinities, torsional angles and number of poses. Compounds with red-flagged torsional angles and compounds having a low binding affinity (>1 mM) were filtered out. For the remaining set of compounds, compounds only with a single pose fulfilling the stricter criteria were removed.

Filtering based on the eNTRy rules and StarDrop Antibacterial Scoring Profile

Globularity and amphiphilic moment of the compounds was calculated using MOE (Molecular Operating Environment, version 2018.1). The compound filtering was completed using StarDrop (version 6.5.1) with the additional functions to calculate the number of rotatable bonds and filters to identify ionisable amines were performed in StarDrop. Compounds with a high number of rotatable bonds (>5) and high globularity (>0.25) were filtered out. All 3D parameters were calculated for the docked poses. The Antibacterial Scoring Profile was calculated in StarDrop and compounds with a score of 0.4–0.6 were considered ideal.

Visual Inspection

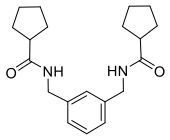
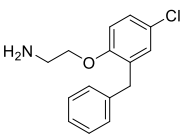
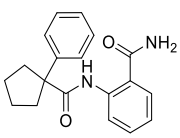
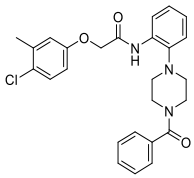
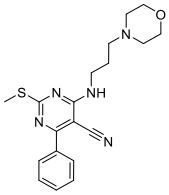
Using the SeeSAR evaluation node in StarDrop, the poses were analysed visually for ideal interactions and consistency of the binding modes. Additionally, compounds were screened for PAINS and compounds with high similarity of CHEMBL (>0.9) and PDB (>0.7) were filtered out. The best compounds were clustered and a final selection of total 24 compounds was made. The compounds were purchased from SPECS and used for biological testing without further purification in DMSO stock solutions (Table S 1). The purchased compounds were of >90% purity.

Table S1: The purchased compounds in their categories.

Category	Number of Compounds
Ionisable primary amine/globularity/rotatable bonds *(1^{ry} amine)*	3
Ionisable secondary amine/globularity/rotatable bonds *(2^{ry} amine)*	2
Ionisable tertiary amine/globularity/rotatable bonds *(3^{ry} amine)*	3
Non-ionisable primary amine/globularity/rotatable bonds *(-sNH_2)*	7
Scoring Profile (0.4–06) *(Scoring)*	4
High HYDE Affinity (≤500 nM) *(HYDE)*	6

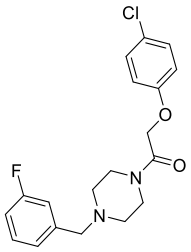
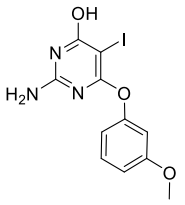
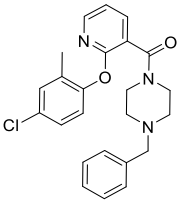
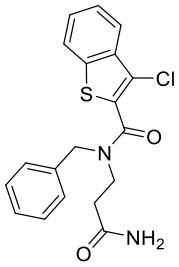
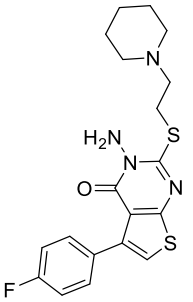
1.2 Summary of Purchased Compounds

Table S2: Virtual screening hits – Section 1.

	 **1**	 **2**	 **3**	 **4**	 **5**
SPECS ID	AK-968/ 41926654	AO-080/ 43442029	AP-970/ 43482379	AP-970/ 42444960	AS-871/ 43477312
Filter category	HYDE	1^{ry} amine	HYDE	HYDE	HYDE
HYDE estimated affinity lower boundary (nM)[a]	58.3	88.7	104.3	120.3	198.9
HYDE estimated affinity upper boundary (nM)[a]	5794	8809	1.036e^{+04}	1.196e^{+04}	1.976e^{+04}
Torsion Quality[a]	yellow	yellow	yellow	yellow	yellow
Docking E total[a]	–20.31	–13.53	–22.7	–25.45	–24.8
Globularity[b]	0.1321	0.1085	0.1478	0.0800	0.0544
Rotatable bonds[c]	6	5	4	6	7
Ionisable amine[c]	N/A	1^{ry} amine	N/A	N/A	3^{ry} amine
Amphiphilic Moment[b]	0.8374	4.7429	4.6133	2.8772	3.4566
Gram-negative antibacterial scoring profile_Score[c]	0.0001381	1.073e^{-08}	0.01162	0.01887	0.1664
Enzyme Activity					
*Ec*IspE IC_{50} (µM)	>500	>500	>500	144 ± 7	>500
PK/LDH IC_{50} (µM)	n.d.	n.d.	n.d.	39 ± 19	n.d.
T_m (°C) (ΔT_m (°C))[d]	n.d.	50.42 ± 0.09 (–1.1)	n.d.	51.31 ± 0.37 (–0.2)	n.d.
Antibacterial Activity (Minimum Inhibitory Concentration or Percentage inhibition at 100 µM)					
E. coli K12	>50 µM	99 ± 2 µM	>100 µM	>50 µM	>50 µM
E. coli Δ*tolC*	>50 µM	101 ± 6 µM	>100 µM	>50 µM	>50 µM
E. coli D22	n.d.	105 ± 7 µM	n.d.	n.d.	n.d.
B. subtilis	>50 µM	>100 µM	>100 µM	>50 µM	>50 µM
S. aureus	n.d.	n.i.	n.d.	n.d.	n.d.
P. aeruginosa	n.d.	53 ± 9%	n.d.	n.d.	n.d.
A. baumannii	n.d.	100 ± 0 µM	n.d.	n.d.	n.d.
Cytotoxicity Inhibitory Concentration (IC_{50}) or Percentage inhibition at 100 µM					
HepG2	n.d.	92 ± 0%	n.d.	n.d.	n.d.
HEK293	n.d.	96 ± 2%	n.d.	n.d.	n.d.
A549	n.d.	90 ± 3%	n.d.	n.d.	n.d.

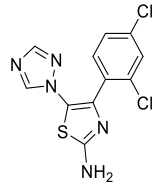
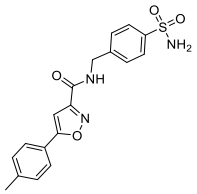
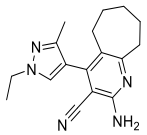
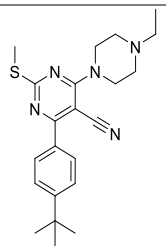
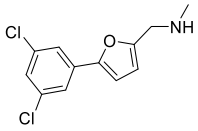
[a] BioSolveIT (LeadIT 2.3.2 and SeeSAR 8.1) [b] MOE 2018.01 [c] StarDrop v. 6.5.1 [d] See reference Table S for the blank protein.n.d.: not determined, n.i.: no inhibition, if inhibition <10%, N/A: not applicable.

Table S3: Virtual screening hits – Section 2.

					
	6	**7**	**8**	**9**	**10**
SPECS ID	AT-057/ 43313800	AG-670/ 11098007	AO-990/ 15068150	AN-329/ 43465228	AO-476/ 43417219
Filter category	3^{ry} amine	Scoring (0.4–0.6)	HYDE	HYDE	3^{ry} amine
HYDE estimated affinity lower boundary (nM)[a]	416.8	741.2	7.529	11.24	516.3
HYDE estimated affinity upper boundary (nM)[a]	4.141e^{+04}	7.364e^{+04}	748	1117	5.13e^{+04}
Torsion Quality[a]	green	green	yellow	yellow	yellow
Docking E total[a]	–19.26	–26.96	–21.8	–19.66	–27.74
Globularity[b]	0.03966	0.2713	0.08249	0.09595	0.07712
Rotatable bonds[c]	5	3	5	6	5
Ionisable amine[c]	3^{ry} amine	N/A	3^{ry} amine	N/A	3^{ry} amine
Amphiphilic Moment[b]	3.1070	5.1538	2.7088	1.5968	3.7062
Gram-negative antibacterial scoring profile_Score[c]	1.224e^{-09}	0.5045	1.609e^{-09}	0.00199	0.005626
Enzyme Activity					
*Ec*IspE IC_{50} (µM)	>500	>500	>500	>500	253 ± 24
PK/LDH IC_{50} (µM)	n.d.	n.d.	n.d.	n.d.	>500
T_m(°C) (ΔT_m (°C))[d]	n.d.	n.d.	n.d.	n.d.	50.75 ± 0.08 (–0.8)
Antibacterial Activity (Minimum Inhibitory Concentration or Percentage Inhibition at 100 µM)					
E. coli K12	>50 µM	>100 µM	>50 µM	>50 µM	10 ± 3%
E. coli ΔtolC	>50 µM	>100 µM	>50 µM	>50 µM	49 ± 6%
B. subtilis	>50 µM	>100 µM	>50 µM	>50 µM	>100 µM

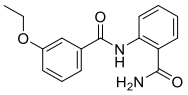
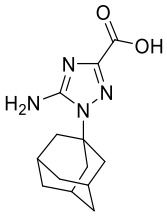
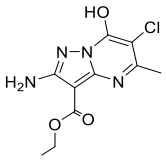
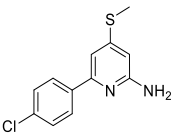
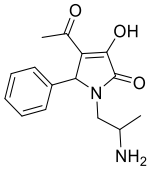
[a] BioSolveIT (LeadIT 2.3.2 and SeeSAR 8.1) [b] MOE 2018.01 [c] StarDrop v. 6.5.1 [d] See reference Table S for the blank protein. n.d.: not determined, N/A: not applicable.

Table S4: Virtual screening hits – Section 3.

					
	11	**12**	**13**	**14**	**15**
SPECS ID	AG-205/ 40649878	AS-871/ 43475260	AK-968/ 41017405	AS-871/ 43477283	AN-465/ 43411641
Filter category	-sNH_2	-sNH_2	-sNH_2	3^{ry} amine	2^{ry} amine
HYDE estimated affinity lower boundary (nM)[a]	790.8	808.7	1301	1608	1938
HYDE estimated affinity upper boundary (nM)[a]	7.857e^{+04}	8.035e^{+04}	1.292e^{+05}	1.597e^{+05}	1.925e^{+05}
Torsion Quality[a]	green	yellow	green	green	green
Docking E total[a]	–21.06	–31.89	–22.87	–21.27	–15.52
Globularity[b]	0.0849	0.02412	0.1226	0.07662	0.05732
Rotatable bonds[c]	2	5	2	5	3
Ionisable amine[c]	N/A	N/A	N/A	3^{ry} amine	2^{ry} amine
Amphiphilic Moment[b]	2.5254	7.1005	2.9562	2.6410	4.5464
Gram-negative antibacterial scoring profile_Score[c]	0.02025	0.1208	0.05725	7.622e^{-09}	4.249e^{-09}
Enzyme Activity					
*Ec*IspE IC_{50} (µM)	>500	29 ± 5	>500	>500	253 ± 24
PK/LDH IC_{50} (µM)	n.d.	21 ± 6	n.d.	n.d.	500
T_m(°C) (ΔT_m (°C)) [d]	n.d.	51.79 ± 0.12 (+0.3)	n.d.	51.23 ± 0.09 (–0.3)	50.81 ± 0.09 (–0.7)
Antibacterial Activity (Minimum Inhibitory Concentration or Percentage inhibition at 100 µM)					
E. coli K12	>100 µM	>50 µM	>100 µM	>50 µM	71 ± 6%
E. coli Δ*tolC*	>100 µM	>50 µM	>100 µM	33 ± 16 µM	74 ± 4%
B. subtilis	>100 µM	>50 µM	>100 µM	91 ± 4%	>100 µM
S. aureus	n.d.	n.d.	n.d.	>50 µM	13 ± 16%
P. aeruginosa	n.d.	n.d.	n.d.	>100 µM	25 ± 11%
A. baumannii	n.d.	n.d.	n.d.	>50 µM	41 ± 28%
Cytotoxicity (Inhibitory Concentration (IC_{50}) or Percentage inhibition at 50 µM)					
HepG2	n.d.	n.d.	n.d.	85 ± 1%	25 ± 2 µM
HEK293	n.d.	n.d.	n.d.	81 ± 0%	76 ± 1%
A549	n.d.	n.d.	n.d.	22 ± 13%	42 ± 2 µM

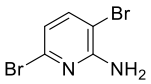
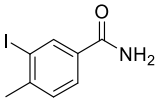
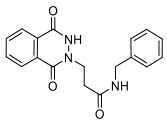
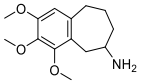
[a] BioSolveIT (LeadIT 2.3.2 and SeeSAR 8.1) [b] MOE 2018.01 [c] StarDrop v. 6.5.1 [d] See reference Table S for blank protein. n.d.: not determined, N/A: not applicable.

Table S5: Virtual screening hits – Section 4.

					
	16	**17**	**18**	**19**	**20**
SPECS ID	AP-970/ 41518174	AK-968/ 40064644	AE-641/ 11517590	AT-417/ 43484814	AQ-149/ 43285071
Filter category	-sNH$_2$	Scoring (0.4–0.6)	Scoring (0.4–0.6)	-sNH$_2$	1^{ry} amine
HYDE estimated affinity lower boundary (nM)[a]	2936	3447	5527	6472	9195
HYDE estimated affinity upper boundary (nM)[a]	2.917e^{+05}	3.425e^{+05}	5.492e^{+05}	6.43e^{+05}	9.136e^{+05}
Torsion Quality[a]	green	green	green	yellow	green
Docking E total[a]	–27.46	–15.34	–23.68	–18.23	–15.51
Globularity[b]	0.01951	0.1926	0.05019	0.102	0.0847
Rotatable bonds[c]	5	2	3	2	4
Ionisable amine[c]	N/A	N/A	N/A	N/A	1^{ry} amine
Amphiphilic Moment[b]	4.1186	5.6843	3.4603	5.2405	4.6577
Gram-negative antibacterial scoring profile_Score[c]	0.02997	0.4238	0.4706	6.881e^{-09}	0.08908
Enzyme Activity					
*Ec*IspE IC_{50} (µM)	>500	>500	>500	322 ± 57	>500
PK/LDH IC_{50} (µM)	n.d.	n.d.	n.d.	>500	n.d.
T_m (°C) (ΔT_m (°C))[d]	n.d.	n.d.	n.d.	51.23 ± 0.13 (–0.3)	n.d.
Antibacterial Activity (Minimum Inhibitory Concentration or Percentage inhibition at 100 µM)					
E. coli K12	>100 µM	>100 µM	>100 µM	>50 µM	>50 µM
E. coli Δ*tolC*	>100 µM	>100 µM	>100 µM	>50 µM	>50 µM
B. subtilis	>100 µM	>100 µM	>100 µM	>50 µM	>50 µM

[a] BioSolveIT (LeadIT 2.3.2 and SeeSAR 8.1) [b] MOE 2018.01 [c] StarDrop v. 6.5.1 [d] See reference Table S for blank protein. n.d.: not determined, N/A: not applicable.

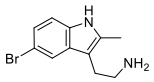
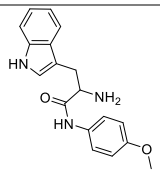
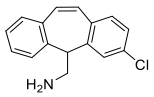
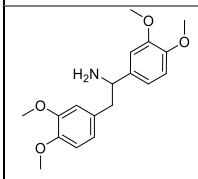
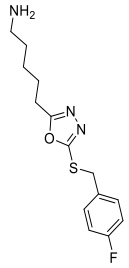
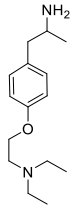
Table S6: Virtual screening hits – Section 5.

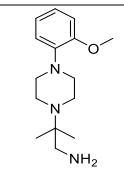
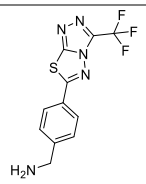
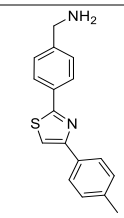
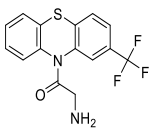
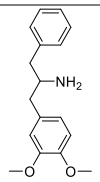
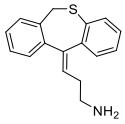
				
	21	**22**	**23**	**24**
SPECS ID	AC907/ 34104030	AN-329/ 41437602	AJ-292/ 41083380	AE-848/ 34162059
Filter category	-sNH$_2$	-sNH$_2$	Scoring (0.4–0.6)	1^{ry} amine
HYDE estimated affinity lower boundary (nM)[a]	1.108e^{+04}	1.795e^{+04}	1.291e^{+04}	1.487e^{+04}
HYDE estimated affinity upper boundary (nM)[a]	1.101e^{+06}	1.784e^{+06}	1.283e^{+06}	1.477e^{+06}
Torsion Quality[a]	not rotatable	green	green	green
Docking E total[a]	–12.67	–17.13	–23.13	–14.23
Globularity[b]	0.01404	0.02625	0.05944	0.0717
Rotatable bonds[c]	0	1	5	3
Ionisable amine[c]	N/A	N/A	N/A	1^{ry} amine
Amphiphilic Moment[b]	4.3979	5.8192	1.1035	4.2183
Gram-negative antibacterial scoring profile_Score[c]	3.216e^{-08}	2.54e^{-08}	0.5087	3.398e^{-08}
Enzyme Activity				
*Ec*IspE IC_{50} (µM)	>500	>500	>500	>500
PK/LDH IC_{50} (µM)	n.d.	n.d.	n.d.	n.d.
Antibacterial Activity (Minimum Inhibitory Concentration)				
E. coli K12	>100 µM	>100 µM	>100 µM	>100 µM
E. coli ΔtolC	>100 µM	>100 µM	>100 µM	>100 µM
B. subtilis	>100 µM	>100 µM	>100 µM	>100 µM

[a] BioSolveIT (LeadIT 2.3.2 and SeeSAR 8.1) [b] MOE 2018.01 [c] StarDrop v. 6.5.1. n.d.: not determined, N/A: not applicable.

2. Summary of Biological Results

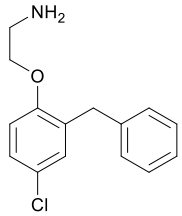
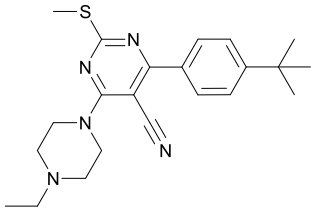
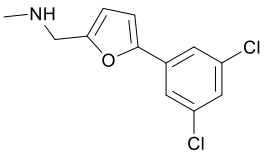
Table S7: Primary amines from the SPECS library (**25**—**36**).

		IC_{50} (μM)[a]		Percentage Inhibition at 100 μM or Minimum Inhibitory Concentration (MIC)			
Structure	**SPECS ID**	***Ec*IspE**	**PK/LDH**	***E. coli* K12**	***E. coli* Δ*tolC***	***P. aeruginosa***	***A. baumannii***
 **25**	AG-205/14785177	>500	n.d.	21 ± 10%	28 ± 3%	12 ± 4%	14 ± 1%
 **26**	AG-690/15435945	>500	n.d.	n.i.	40 ± 6%	n.d.	n.i.
 **27**	AE-641/06348040	>500	n.d.	50 ± 8%	81 ± 0%	23 ± 9%	34 ± 4%
 **28**	AE-848/30721050	>500	n.d.	n.i.	n.i.	n.d.	n.d.
 **29**	AS-871/43475867	>500	n.d.	n.i.	23 ± 5%	n.d.	n.d.
 **30**	AE-641/00601040	>500	n.d.	n.i.	14 ± 4%	n.d.	n.d.

 **31**	AS-871/ 4338735 0	>500	n.d.	n.i.	n.i.	n.d.	n.d.
 **32**	AP-970/ 4349217 6	>500	n.d.	n.i.	14 ± 1%	n.d.	n.d.
 **33**[b]	AS-871/ 4347521 0	>500	n.d.	76 ± 16%	98 ± 11 µM	16 ± 4%	9 ± 4% (at 50 µM)
 **34**	AG-690/ 1370253 8	>500	n.d.	n.i.	66 ± 6%	n.i.	n.i.
 **35**	AE-641/ 3011500 7	>500	n.d.	n.i.	13 ± 3%	n.d.	n.d.
 **36**	AE-641/ 3015305 5	>500	n.d.	57 ± 4%	79 ± 1%	24 ± 11%	24 ± 3%

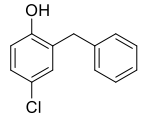
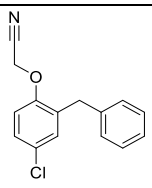
[a] Where *Ec*IspE activity was measured as IC_{50} >500 µM, no replicate or pyruvate kinase and lactate dehydrogenase (PK/LDH) inhibition was determined. [b] **33** HepG2 IC_{50} = 19 ± 3 µM and *S. aureus* MIC = 26 ± 1 µM. n.d.: not determined. n.i.: no inhibition, if inhibition <10%.

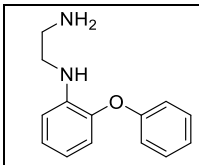
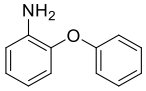
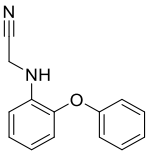
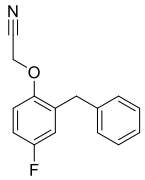
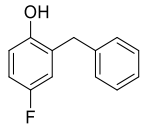
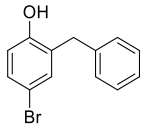
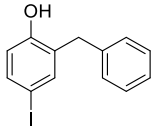
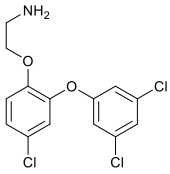
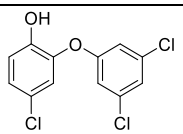
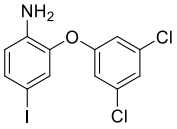
Table S8: Top-three hits of the virtual screening based on the cellular activities.

	 **2**	 **14**	 **15**
Enzyme activity			
*Ec*IspE IC_{50} (µM)	>500	>500	>500
T_m(°C) (ΔT_m (°C))	50.42 ± 0.09 (–1.1)	51.23 ± 0.09 (–0.3)	50.81 ± 0.09 (–0.7)
K_D	~ 700 µM	n.d.	n.d.
Minimum Inhibitory Concentration (MIC) or Percentage Inhibition at 100 µM			
E. coli K12	99 ± 2 µM	11 ± 9%[a]	71 ± 6%
E. coli Δ*tolC*	97 ± 4 µM	33 ± 16 µM	74 ± 4%
P. aeruginosa	50 ± 7%	n.i.	25 ± 11%
A. baumannii	100 ± 0 µM	n.i.[a]	41 ± 28%
S. aureus	47 ± 8%	48 ± 30%[a]	13 ± 15%
B. subtilis	n.i.	91 ± 4%[a]	>100 µM
Cytotoxicity Inhibitory Concentration (IC_{50}) or Percentage Inhibition at 50 µM			
HepG2	21 ± 1 µM	85 ± 1%	25 ± 2 µM
HEK293	96 ± 2%	81 ± 0%	76 ± 1%
A549	90 ± 3%	22 ± 13%	42 ± 2 µM
Calculated properties			
*c*logD (pH 7.4)[b]	1.6	2.9	2.5
Most basic pK_a[b]	9.1	9.3	8.4
Amphiphilic moment[c]	5.0	2.5	4.4

[a] Experiment performed at 50 µM. n.d.: not determined. n.i.: no inhibition, if inhibition <10%. [b] Calculated with StarDrop 7.0.1. [c] Calculated with MOE 2020.09 for the docked pose

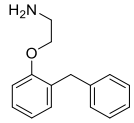
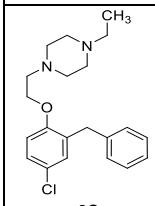
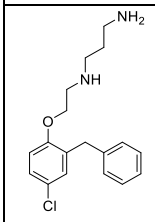
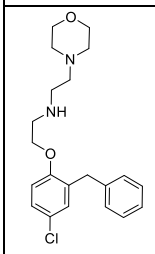
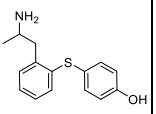
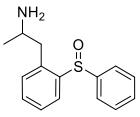
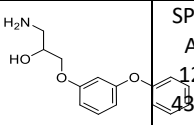
Table S9: Summary of biological data for **37**, **38**, **58a**, **58b**, **59b**, **59**—**64**, **68** and **77a**.

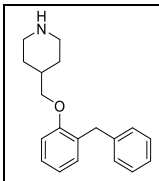
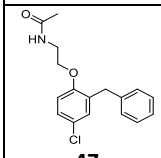
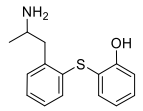
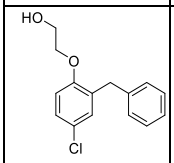
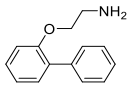
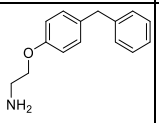
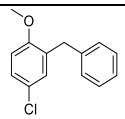
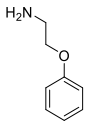
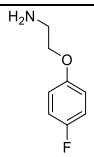
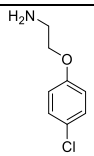
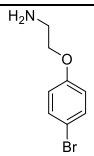
	IC_{50} (µM) [a]		**Percentage Inhibition at 100 µM or Minimum Inhibitory Concentration (MIC)**							
Structure and code	***Ec*IspE**	**PK/LDH**	***E. coli* K12**	***E. coli ΔtolC***	***E. coli ΔacrB***	***E. coli* D22**	***A. baumannii***	***P. aeruginosa***	***S. aureus***	***HepG2***
 **37**	>500	n.d.	50 ± 10%	19 ± 1 µM	33 ± 13 µM	95 ± 0 µM	27 ± 4%	46 ± 3%	95 ± 7 µM	91 ± 6%
 **38**	>500	n.d.	13 ± 2%	38 ± 1 µM	54 ± 5%	35 ± 5	2%*[b]	9 ± 5%	18 ± 24%	80 ± 3%

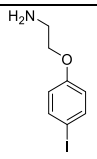
 **58**	n.d.	n.d.	29 ± 2%	24 ± 7%	29 ± 20%	n.i.	n.d.	n.d.	n.i.	48 ± 7%
 **58a**	>500	n.d.	12 ± 6%	13 ± 4%	n.i.	11 ± 1%	n.d.	n.d.	n.i.	45 ± 4%
 **58b**	n.d.	n.d.	10 ± 1%	44 ± 15%	n.i.	14 ± 2%	n.d.	n.d.	n.i.	n.d.
 **59b**	n.d.	n.d.	n.i.[b]	56 ± 27%[b]	n.d.	n.d.	n.d.	n.i.[b]	n.i.	n.d.
 **62**	n.d.	n.d.	30 ± 3%	80 ± 14 µM	n.d.	n.d.	n.d.	n.d.	82 ± 8%	n.d.
 **63**	n.d.	n.d.	18 ± 3%	14 ± 0 µM	n.d.	n.d.	n.d.	n.d.	85 ± 7 µM	n.d.
 **64**	n.d.	n.d.	23 ± 6 µM[b]	12 ± 0 µM	n.d.	n.d.	n.d.	n.d.	56 ± 33 µM	n.d.
 **67**	159 ± 4	>500	85 ± 6 µM	41 ± 2 µM	44 ± 1 µM	86 ± 4 µM	64 ± 2 µM	63 ± 8%	99 ± 6 µM	15 ± 3 µM
 **68**	40 ± 6	46 ± 1	58 ± 6[b]	3 ± 0 µM	n.d.	n.d.	n.d.	n.d.	5 ± 0 µM	92 ± 3% (IC_{50} = 33 ± 2 µM)
 **77a**	>500	n.d.	n.i.[b]	45 ± 7[b]	n.d.	n.d.	n.d.	n.d.	n.d.	IC_{50} = 15 ± 8 µM

[a] Only where *Ec*IspE activity was measured as IC_{50} >500 µM, no replicate pyruvate kinase and lactate dehydrogenase (PK/LDH) inhibition were determined. [b] Experiment performed at 50 µM. n.d.: not determined. n.i.: no inhibition, if inhibition <10%. *Value of a single measurement.

Table S10: Summary of biological data for the commercially available derivatives (**39**—**56**).

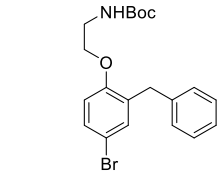
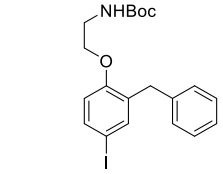
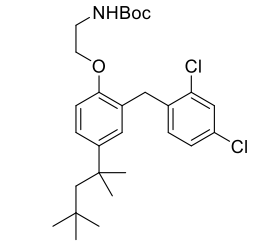
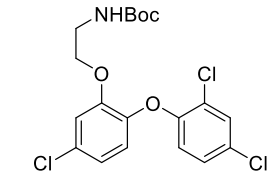
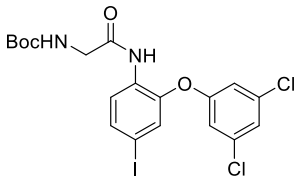
		IC_{50} (μM) [a]		Percentage Inhibition at 100 μM or Minimum Inhibitory Concentration (MIC)								
Structure and code	**External code**	***Ec*IspE IC_{50} (μM)** [a]	**PK/LDH IC_{50} (μM)**	***E. coli* K12**	***E. coli* ΔtolC**	***E. coli* ΔacrB**	***E. coli* D22**	***E. coli* Omp8**	***A. baumannii***	***P. aeruginosa***	***S. aureus***	***HepG2***
 **39**	Ambinter 68700 79	>500	n.d.	31 ± 5%	41 ± 0%	42 ± 1%	n.i.	n.i.	22 ± 1%	n.i.	11 ± 7%	88 ± 5%
 **40**	Ambinter 86046 46	>500	n.d.	17 ± 8%	48 ± 4 μM	95 μM*	40*	n.d.	25 ± 5%	n.i.	35 ± 16%	94 ± 4%
 **41**	Ambinter 86129 87	>500	n.d.	83 ± 4%	80 ± 14 μM	80 ± 0 μM	93 ± 4 μM	n.d.	100 ± 0 μM	95 ± 0 μM	103 ± 4 μM	93 ± 5%
 **42**	Ambinter 86128 33	>500	n.d.	16 ± 7%	80 ± 7 μM	95 μM*	35%*	n.d.	14 ± 4%	15 ± 5%	33 ± 13%	93 ± 4%
 **43**	SPECS AE-641/ 30177 026	447*	361 ± 80	33 ± 4%	47 ± 20%	n.d.	n.d.	n.d.	18 ± 2%	n.i.	15 ± 8%	IC_{50} = 45 ± 6 μM
 **44**	SPECS AE-641/ 30177 033	>500	n.d.	12 ± 1%	n.i.	n.d.	n.d.	n.d.	n.i.	n.i.	n.i.	n.i.
 **45**	SPECS AP-124/ 43382 853	>500	n.d.	28 ± 5%	40 ± 4%	n.d.	n.d.	n.d.	12 ± 0%	10 ± 8%	n.i.	85 ± 5%

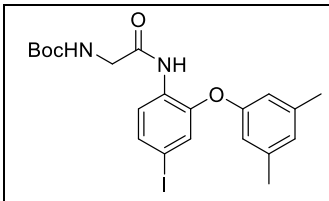
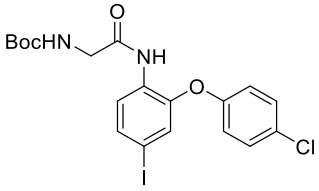
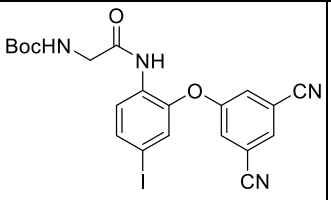
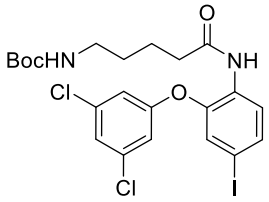
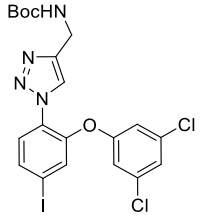
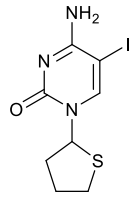
 **46**	SPECS AN-329/ 43448394	>500	n.d.	13 ± 11%	85 ± 8%	108 ± 4 µM	67 ± 4%	58 ± 5%	14 ± 6%	43 ± 9%	20 ± 10%	IC_{50} = 21 ± 4 µM
 **47**	SPECS AO-080/ 43441925	>500	n.d.	8 ± 8%	88 ± 11 µM	30 ± 12%[b]	34 ± 6[%b]	n.i.[b]	n.i.[b]	n.i.[b]	22 ± 6%	48 ± 4%
 **48**	SPECS AE-641/ 30177024	338 ± 45	>500	48 ± 3%	57 ± 7 µM	52 ± 3%	n.i.	n.d.	19 ± 1%	n.i.	14 ± 16%	86 ± 8%
 **49**	Enamine Z1182353799	>500	n.d.	27 ± 3%	50 ± 0 µM	103 ± 3 µM	22 ± 4%	104 ± 2 µM	n.i.[b]	n.i.	32 ± 21%	78 ± 13%
 **50**	Enamine Z57921595	>500	n.d.	42 ± 6%	28 ± 11%	n.d.	n.d.	n.d.	22 ± 4%	n.i.	n.i.	38 ± 2%
 **51**	Enamine Z2901973293	>500	n.d.	41 ± 9%	45 ± 15%	n.d.	n.d.	n.d.	13 ± 4%	12 ± 1%	n.i.	51 ± 8%
 **52**	Enamine Z54182174	>500	n.d.	n.i.[b]	49 ± 1%	n.d.	n.d.	n.d.	n.i.[b]	n.i.	36 ± 27%	n.i.
 **53**	CAS 17959-64-7	n.d.	n.d.	n.i.	n.i.	n.d.	n.d.	n.d.	n.d.	n.d.	n.i.	n.d.
 **54**	CAS 263409-81-0	n.d.	n.d.	n.i.	n.i.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
 **55**	CAS 106038-00-0	n.d.	n.d.	n.i.	n.i.	11 ± 3%	n.d.	n.d.	n.d.	n.d.	n.d.	n.i.
 **56**	CAS 663941-79-5	n.d.	n.d.	n.i.	n.i.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

 **57**	CAS 15197 8-97-1	n.d.	n.d.	n.i.	10 ± 5%	18 ± 2%	n.d.	n.d.	n.d.	n.d.	n.i.	n.i.

[a] Only where *Ec*IspE activity was measured as IC_{50} >500 µM, no replicate pyruvate kinase and lactate dehydrogenase (PK/LDH) inhibition were determined. [b] Experiment performed at 50 µM. n.d.: not determined. n.i.: no inhibition, if inhibition <10%. *Value of a single measurement.

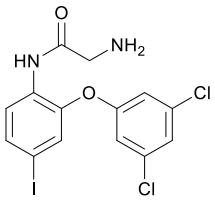
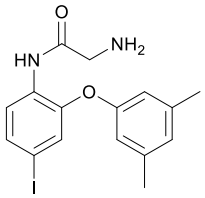
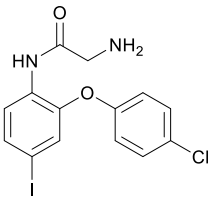
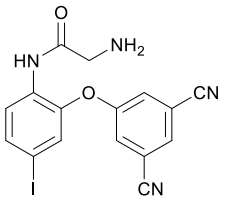
Table S11: Biological data of NHBoc derivatives (**65**, **66, 75, 76**, **86**–**91**) and compound **93.**

	IC_{50} (µM) [a]		**Percentage Inhibition at 100 µM or Minimum Inhibitory Concentration (MIC)**		
Structure and code	***Ec*IspE**	**PK/LDH**	***E. coli* K12**	***E. coli* Δ*tolC***	***HepG2***
 **65**	n.d.	n.d.	n.i.	27 ± 4%	n.d.
 **66**	n.d.	n.d.	n.i.	15 ± 0%[b]	n.d.
 **75**	47 ± 2 µM	15 ± 7%	n.i.	n.i.	50 ± 7%
 **76**	158 ± 31 µM	n.d.	n.i.	n.i.	84 ± 2%; IC_{50}= 31 ± 2 µM
 **86**	> 500	> 500	n.i.	16 ± 3%	72 ± 7%; IC_{50}= 20 ± 2 µM

 **87**	269 ± 122	137 ± 132	10 ± 4	18 ± 12%	81 ± 2%; IC_{50} = 29 ± 7 µM
 **88**	371 ± 52 µM	38 ± 25	48 ± 3%[b]	16 ± 3%	93 ± 3%; IC_{50} = 48 ± 17 µM
 **89**	395 ± 83 µM	n.d.	n.i.	31 ± 6%	61 ± 4%; IC_{50} = 64 ± 3 µM
 **90**	416 ± 217 µM	22 ± 8	n.i.	n.i.	44 ± 14%[c]
 **91**	>500	n.d.	n.i.	n.i.	n.i.
 **93**	>500 µM	n.d.	n.d.	13 ± 4 % inh. at 100 µM	n.d.

[a] Only where *Ec*IspE activity was measured as IC_{50} >500 µM, no replicate pyruvate kinase and lactate dehydrogenase (PK/LDH) inhibition were determined. [b] Experiment performed at 25 µM; [c] experiment performed at 50 µM. n.d.: not determined. n.i.: no inhibition, if inhibition <10%.

Table S12. Summary of the biological results for aniline derivatives

	 **77**	 **78**	 **79**	 **80**
Enzyme activity				
*Ec*IspE IC_{50} (μM)	63 ± 15	>500	302 ± 30	>500
PK/LDH IC_{50} (μM)	>500	n.d.	n.d.	n.d.
T_m(°C) (ΔT_m (°C))	48.38 ± 0.29 (–3.1)	n.d.	n.d.	n.d.
K_D	~20 μM	n.d.	n.d.	n.d.
Minimum Inhibitory Concentration (MIC) or Percentage Inhibition at 100 μM				
E. coli K12	47 ± 2 μM	92 ± 2 μM	46 ± 1 μM	11 ± 1%
E. coli D22	31 ± 12 μM	n.d.	n.d.	n.d.
E. coli Δ*tolC*[a]	46 ± 1 μM	23 ± 0 μM	38 ± 2 μM	63 ± 18%
E. coli Δ*acrB*[a]	23.3 ± 0.4 μM	n.d.	45 ± 2 μM	49 ± 7%
P. aeruginosa	64 ± 7%	n.d.	n.d.	n.d.
A. baumannii	43 ± 4 μM	n.d.	n.d.	n.d.
S. aureus	87 ± 7%	n.d.	n.d.	n.d.
B. subtilis	30 ± 10 μM	n.d.	n.d.	n.d.
Cytotoxicity Inhibitory Concentration (IC_{50})				
HepG2	9 ± 1 μM	12 ± 1 μM	28 ± 6 μM	63 ± 1 μM
HEK293	8.5 ± 0.1 μM	n.d.	n.d.	n.d.
A549	10.5 ± 4.4 μM	n.d.	n.d.	n.d.
Calculated properties				
*c*logD[b]	2.8	1.3	1.5	0.8
Most basic pK_a[c]	7.8	7.9	7.9	8.0
Amphiphilic moment[d]	5.3	5.0	5.9	4.1

[a] Efflux pump mutant. [b] Calculated with StarDrop 7.0.1 at pH 7.4. [c] Calculated with StarDrop 7.0.1. [d] Calculated with MOE 2020.09 for the energy-minimized molecule. n.d.: not determined. N/A: not applicable.

3. Biophysical Assays

3.1 Thermal Shift Assay

The thermal shift assay (TSA) was performed in triplicates on a 96-well plate. Each well contained DMSO-ligand (200 μM), *Ec*IspE (2.5 μM), 10% (V/V) x50 Protein Thermal Shift dye (LOT 1707029) and 75% (V/V) TBS-buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl). The ligands were pipetted from a 4 mM DMSO stock solution. The protein stock in a concentration of 25 μM was centrifuged at 4 °C, 14,000 rpm for 5 min. The blank (protein-only) and the positive control (natural substrate CDP-ME) contained DMSO with the same volume as the ligand instead of the ligand (5% (V/V)). The positive control additionally contained 500 μM CDP-ME (8 mM stock solution). The total sample volume in each well was 20 μL. The well plate was covered with a PCR-membrane, centrifuged at 4 °C, 1400 rpm for 1 min and placed into a StepOnePlus Real-Time PCR System (Serial no. 272003367, Applied Biosystems). "Detect melting point" method was used with a temperature ramp over 20–90 °C proceeding in steps of 0.3 °C (1 min per step). Protein Thermal Shift Software Version 1.3 was used to determine melting points (T_m) at least from two independent replicates (Table S13).

Table S13: Summary of the thermal shift assay results.

Compound	T_m (°C)	ΔT_m (°C)[a]
Protein only	51.52 ± 0.14	–
CDP-ME	52.28 ± 0.09	+0.8
2	50.42 ± 0.09	–1.1
4	51.31 ± 0.37	–0.2
10	50.75 ± 0.08	–0.8
12	51.79 ± 0.12	+0.3
14	51.23 ± 0.09	–0.3
15	50.81 ± 0.09	–0.7
19	51.23 ± 0.13	–0.3
43	50.82 ± 0.12	–0.7
44	51.52 ± 0.10	0.0
48	51.10 ± 0.08	–0.4
69	51.31 ± 0.08	–0.2
67	49.60 ± 0.20	–1.9
60	49.99 ± 0.09	–1.5
77	48.38 ± 0.29	–3.1

[a] T_m (*Ec*IspE with compound) – T_m (*Ec*IspE without compound).

3.2 Microscale Thermophoresis

The microscale thermophoresis (MST) (Serial no. 201709-BR-N024, Monolith NT.115 Micro Scale Thermophoresis, NanoTemper Technologies GmbH) was performed according to the standard protocol from the manufacturer NanoTemper Technologies GmbH using the Monolith His-Tag Labeling Kit RED-tris-NTA 2nd Generation kit (LOT #20L018-010). For **2**, 1st Generation kit was used. The buffer used was HEPES (50 mM), pH 7.6, $MgCl_2$ (5 mM) and Tween (0.05%). The protein concentration of 50 nM was used and the ligand was tested at the highest soluble concentration, which was 0.5 mM for most of the compounds under the assay conditions. A 1:1 dilution of the ligand over 16 samples was performed using a stock of 10% DMSO ligand stock in HEPES buffer. Non-hydrophobic capillary tubes (LOT #20K022_003) were used. A pretest to check for the labelling and compound fluorescence was performed before every sample, followed by a binding affinity (K_D) determination. Each sample was measured after 15 min and 60 min incubation time at rt and analysed in MO Control version 1.6 (Table S14).

Table S14: Summary of the microscale thermophoresis results.

	Time	K_D 1 [µM]	K_D 2 [µM]	K_D 3 [µM]	Average K_D [µM]
CDP-ME	15 min	1.3	0.2	0.4	0.3 ± 0.2
	1 h	(0.4)	0.1	0.1	0.1 ± 0.0
2	15 min	664.0[a]	733.0[a,b]	n.d.	699 ± 49
	1 h	403.0[a]	215.0[a,b]	n.d.	309 ± 133
67	15 min	79.4	57.4	57.4	65 ± 13
	1 h	60.6	60.0	60.0	60 ± 0
77	15 min	21.9	4.4	33.8	20 ± 15
	1 h	(281)	12.0	17.3	15 ± 4

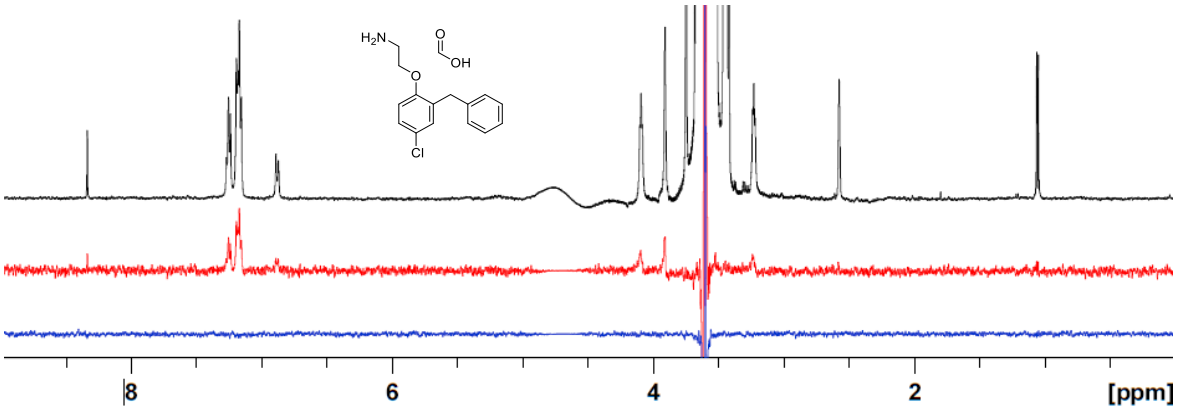
[a] 1st Generation His-Tag Kit [b] Measured in Tris-HCl buffer. *Value of a single measurement. n.d.: not determined

3.3 Saturation Transfer Difference (STD)-NMR spectroscopy

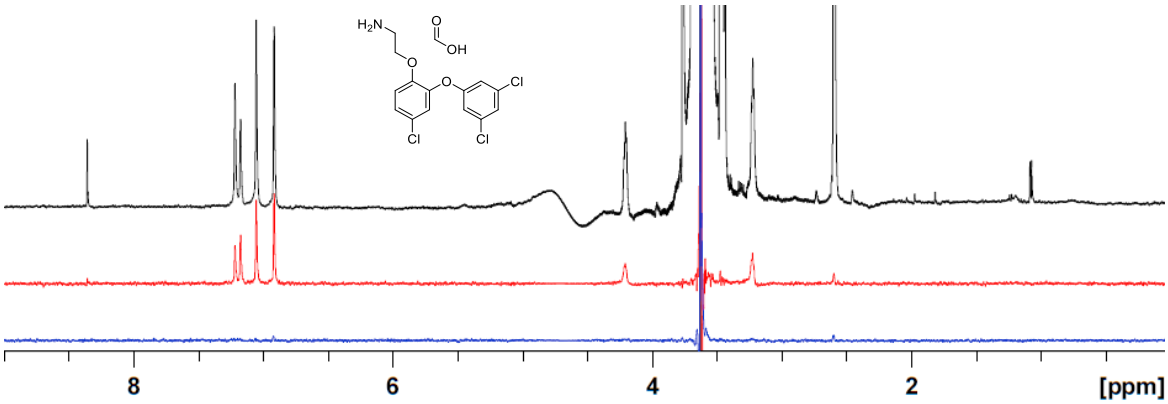
The STD experiments were recorded at 298 K on a Bruker Fourier spectrometer (500 MHz). The samples contained a 100- to 200-fold excess of compound (500 µM) relative to *Ec*IspE (2.5 µM or 5.0 µM – see Table S15) in D_2O buffer with Tris-HCl (50 mM) and $MgCl_2$ (5 mM) at pD = 7.6. The compounds were dissolved in DMSO-d_6 and added to the buffer to reach a final concentration of 2.4% DMSO-d_6.
All experiments were performed using the stddiffesgp.3 pulse program by Bruker. Blank spectra (compound in buffer without protein) were recorded to establish the parameters at which no residual compound signals were visible (Table S15). The screening experiments were all recorded with a carrier set at –2 or –3 ppm for the on-resonance and –40 ppm for the off-resonance irradiation. Selective protein saturation was carried out at 0.5 s or 1.0 s (d20 parameter in TopSpin) by using a train of 50 ms Gauss-shaped pulses, each separated by a 1 or 2 s delay (d1 parameter in TopSpin). In all cases, 256 scans were recorded. Binding was confirmed when a visible difference in peak intensity between off-resonance and STD spectrum could be observed.

Table S15: Measurement and experimental parameters that differed from general procedure.

Compound	d1 (s)	d20 (s)	On-resonance frequency (ppm)	*Ec*IspE (µM)
2	2.0	0.5	–2	5.0
67	1.0	0.5	–3	2.5



Spectrum S1: Blank (blue), STD (red) and off-resonance (black) spectrum of **2**. Binding for all compound signals visible; differences in intensities confirm specific binding. Only weak binding of formic acid (8.3 ppm). Epitope mapping not possible because peaks cannot be assigned unambiguously.



Spectrum S2: Blank (blue), STD (red) and off-resonance (black) spectrum of compound **67**. Binding for all compound signals visible; differences in intensities confirm specific binding. Only weak binding of formic acid (8.3 ppm). Epitope mapping not possible because peaks cannot be assigned unambiguously.

4. Biological Assays

4.1 IspE Enzymatic Assay

E. coli IspE for IspE assays was expressed, isolated and purified as previously described.[17] All other enzymes and chemicals used in the assays were purchased from Sigma-Aldrich (Taufkirchen, Germany).

For testing of compounds in the IspE assay, CDP-ME (0.2 mM) in 100 mM Tris-HCl, pH 7.6, 0.02% NaN_3 (30 µL) was added to a well of the 384-well microplate, preloaded either with DMSO or with test compound dissolved in DMSO (3 µL). The reaction was started by addition of 100 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 60 mM KCl, 10 mM dithiothreitol, 0.02% NaN_3, 1 mM NADH, 2 mM phosphoenolpyruvate, 2 mM ATP, pyruvate kinase (1 U mL^{-1}), lactate dehydrogenase (1 U mL^{-1}) and *E. coli* IspE (0.05 U mL^{-1}) (27 µL per microplate well).

IC_{50} values were measured at CDP-ME concentration 100 µM.

In order to find out if tested compounds are active *vs* auxiliary enzymes from the IspE assay, they were additionally tested in the pyruvate kinase assay. For this purpose, 1 mM ADP in 100 mM Tris-HCl, pH 7.6 (30 µL) was added to a well of the 384-well microplate which had been preloaded with DMSO or with test compound solved in DMSO (3 µL). The reaction was started by addition of 100 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 60 µM KCl, 10 mM dithiothreitol, 0.02% NaN_3, 1 mM NADH, 2 mM phosphoenolpyruvate, pyruvate kinase (0.05 U mL^{-1}) and lactate dehydrogenase (0.05 U mL^{-1}) (27 µL per microplate well).

For both kind of assays the OD values in the microplate wells were monitored photometrically at 340 nm (room temperature) for 30 to 90 min in a plate reader (SpectraMax5, Molecular Dynamics, USA). Initial rate values were evaluated with a nonlinear regression method using the program Dynafit.[33]

4.2 Antibacterial Assay

Assays regarding the determination of the minimum inhibitory concentration (MIC) were performed as described previously. [34] The experiments were based on a variety of *E. coli* strains/mutants (K12, D22, Δ*tolC*, Δ*acrB* and BL21(DE3)omp8) as well as *B. subtilis* (*ssp. subtilis*), *S. aureus* (Newman strain), *P. aeruginosa* (PA14, ΔoprF, Δ*omph*,, Δ*mexB* and Δ*mexA*) and *A. baumannii* (DSM30007). In the case that no MIC value could be determined due to activity reasons, the percentage (%) of inhibition at 100 µM (or lower, depending on the solubility of the compounds) was determined. All samples were run in duplicate for each condition, and experiments were performed independently at least twice.

4.3 Cytotoxicity

Cytotoxicity assays based on the human hepatocellular carcinoma (HepG2), human embryonic kidney (HEK293) and human lung adenocarcinoma (A549) cell lines were performed as described previously. [35] All samples were run in duplicate for each condition, and experiments were performed independently at least twice.

4.4 Comparative Phenotype Profiling

To validate interference with the IspE pathway in live bacteria, we compared the effect of compound **15** to that of IspE or Dxr depletion in *Bacillus subtilis* via comparative phenotype profiling. To this end, *B. subtilis* 168 *trpC2* wildtype and the mutant strains *kd-ispE* (BEC00460) and *kd-dxr* (BEC16550) [36] were grown in lysogeny broth (LB) at 37 °C to an optical density at 600 nm (OD_{600}) of 0.2. Then, the *kd-ispE* and *kd-dxr* mutants were supplemented with 1% xylose to repress either IspE or Dxr expression, respectively. Similarly, *B. subtilis* wildtype was grown at 37 °C to an OD_{600} of 0.2 and compound **15** was added. Bacterial cultures were further grown for 3.5 h until sampling. For microscopy, cells were mounted on microscopy slides coated with 1% agarose in water to immobilize cells. Images were acquired using the Nikon Eclipse Ti equipped with Perfect Focus system (Nikon Instruments Europe BV, Netherlands), an Orca Flash 4.0 camera (Hamamatsu, Photonics, Japan) and CFI Plan-Apo DM× 100/1.45 Oil Ph3 objective (Nikon). Images were processed using the NIS elements AR software package (Nikon). Our results show that compound **15** induced a characteristic bulging phenotype that was followed by cell lysis events. The same phenotype was observed for mutants with depleted IspE or Dxr.

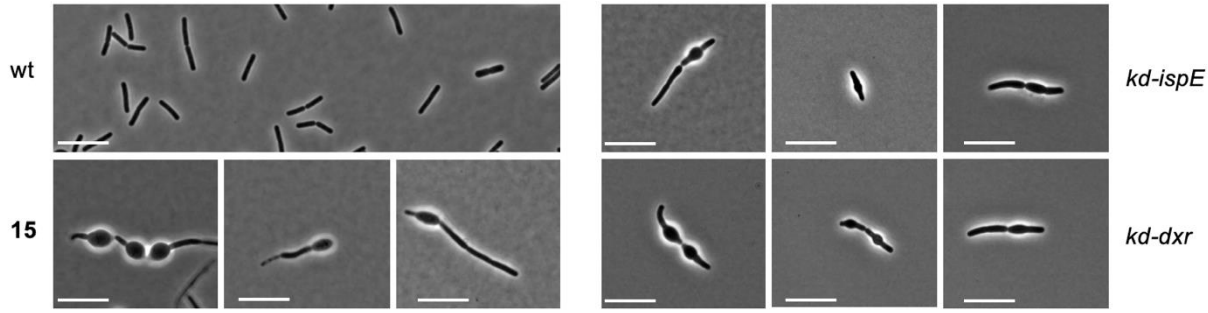
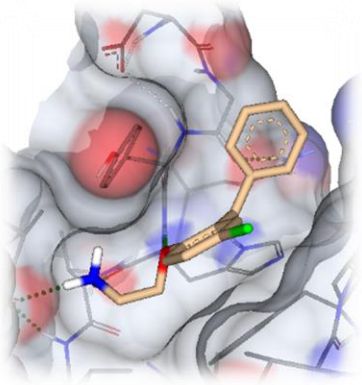
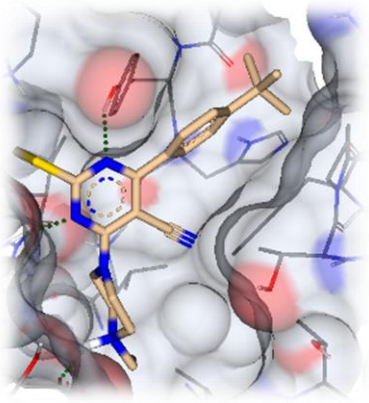
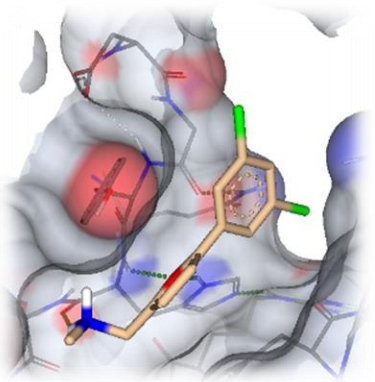
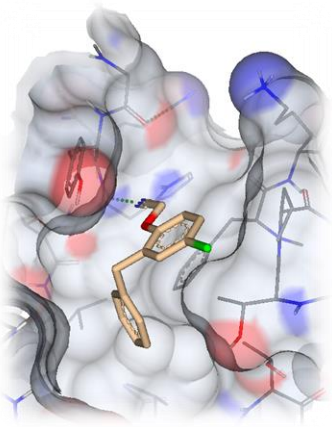


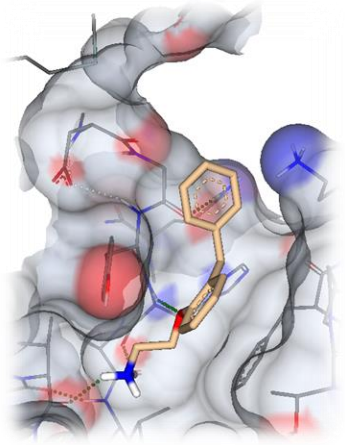
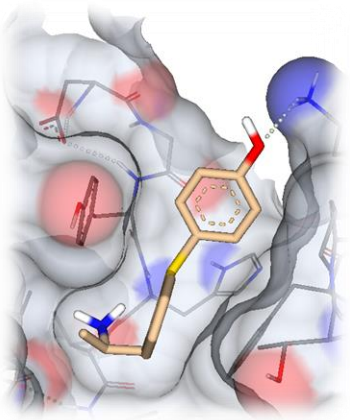
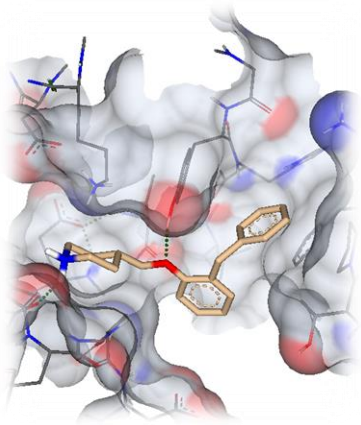
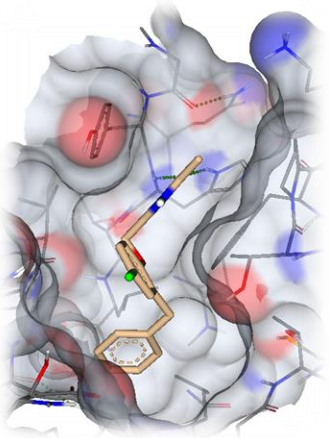
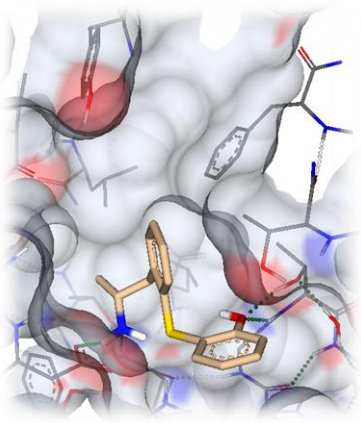
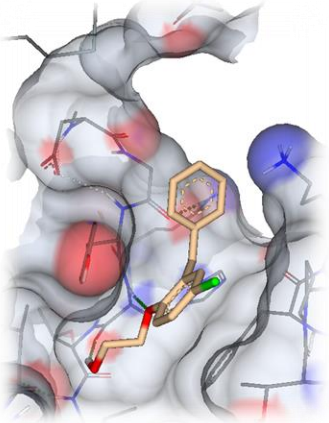
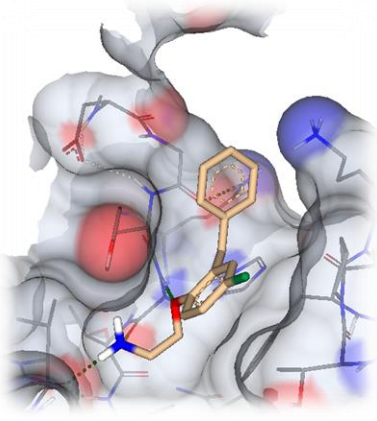
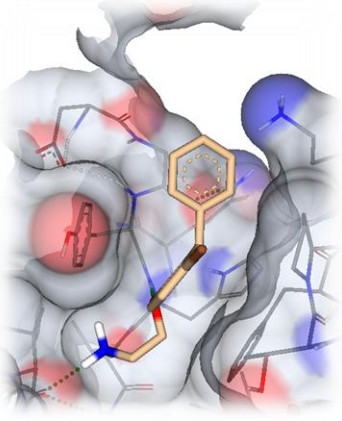
Figure S2: Comparative phenotype profiling. Treatment with compound **15** results in a similar bulging phenotype than a repression of IspE or Dxr expression. Scale bars, 10 µm. Images are representative of at least two biological replicate cultures.

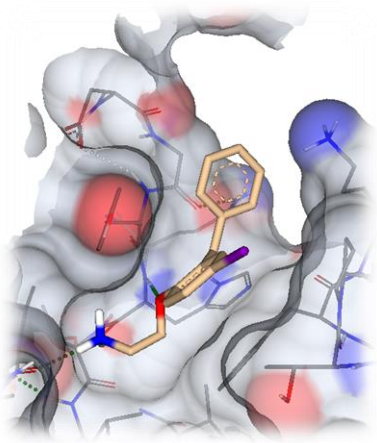
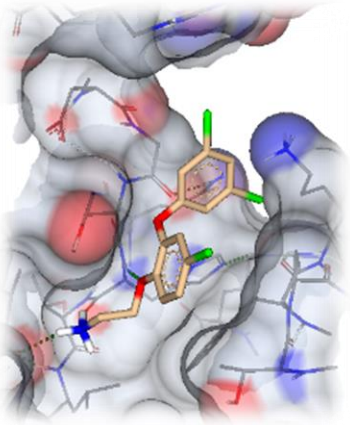
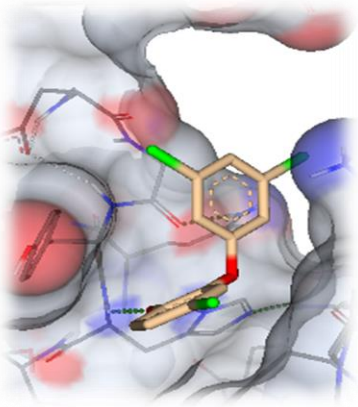
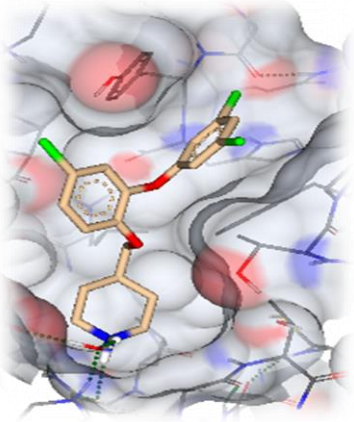
5. Docking

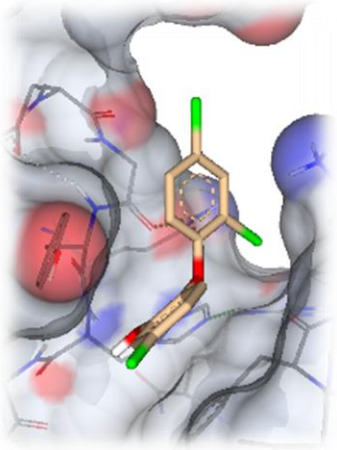
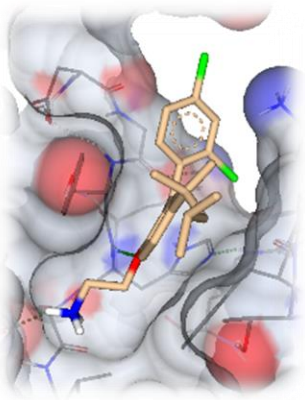
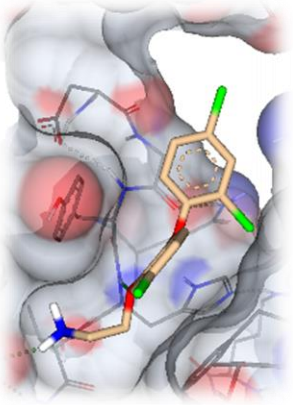
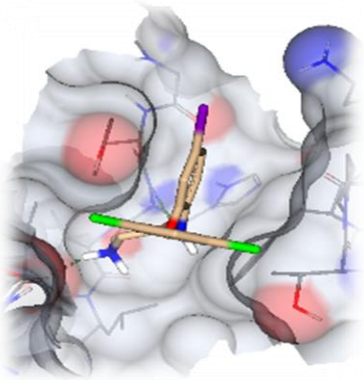
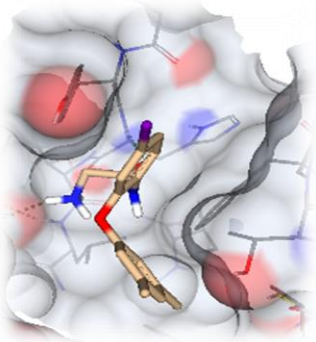
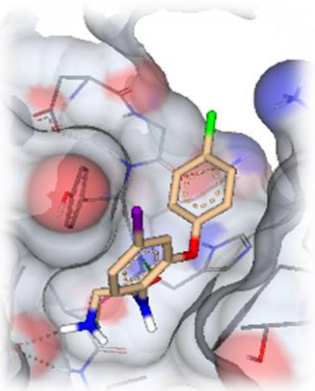
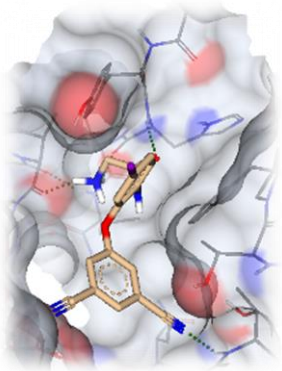
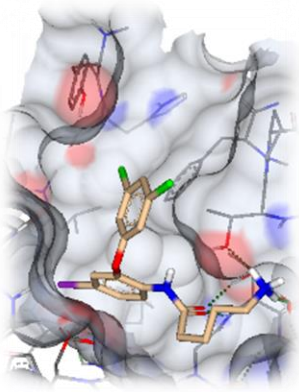
All compounds that were tested against *Ec*IspE were docked with SeeSAR 12.1 into the binding pocket of *Ec*IspE (PDB 1OJ4) and the figures were created in StarDrop 7.0.1.Ten poses were generated per compound and the HYDE score was calculated. The pose with the highest score was visually analyzed for its binding mode.

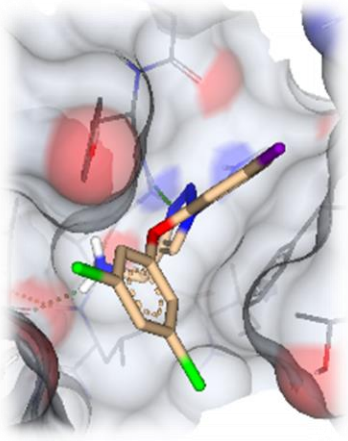
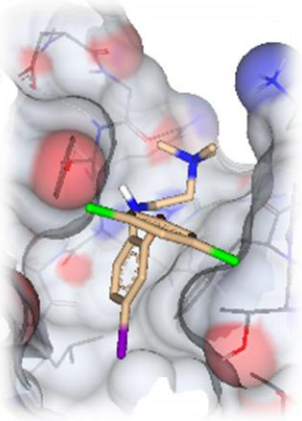
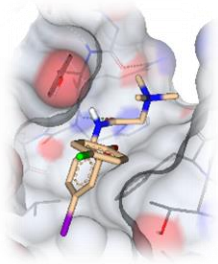
Table S16: Docking poses of some compounds described in the text

	Docking pose in *Ec*IspE (1OJ4)[a]		**Docking pose in *Ec*IspE (1OJ4)[a]**
2		**14**	
15		**38**	

39		**43**	
46		**47**	
48		**49**	
59		**60**	

61		**67**	
68		**69**	n.p. no poses can be generated
70		**71**	n.p. no poses can be generated

72		**73** [b]	
74		**77**	
78		**79**	
80		**81**	

82		**83**	
84			

[a] The poses were generated in SeeSAR 12.1 and the figures were created in StarDrop 7.0.1. [b] Docking of compound **73** only possible if one methyl group is removed.

6. Synthesis

6.1 Chemicals, Materials and Methods

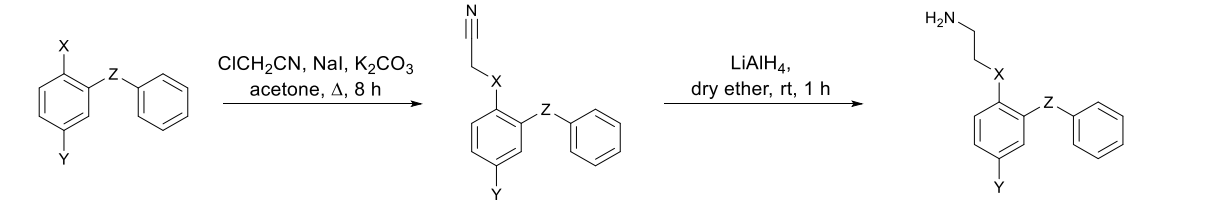
NMR experiments were run on a Bruker Ultrashield plus 500 (500 MHz) spectrometer. Spectra were acquired at 300 K, using deuterated dimethylsulfoxide (DMSO-d_6) as solvent. Chemical shifts for ^{1}H and ^{13}C spectra were recorded in parts per million (ppm) using the residual non-deuterated solvent as the internal standard (for DMSO- d_6: 2.50 ppm, ^{1}H; 39.52 ppm, ^{13}C; for $CDCl_3$: 7.27 ppm, ^{1}H; 77.00, ^{13}C; for methanol-d_4: 4.87 ppm, ^{1}H; 49.15 ppm, ^{13}C; for acetone-d_6, 2.05 ppm, ^{1}H; 29.32 ppm, ^{13}C). Coupling constants (*J*) are given in Hertz (Hz). Data are reported as follows: chemical shift, multiplicity (s = singlet, d =doublet, t = triplet, m = multiplet, br = broad and combinations of these) coupling constants and integration. Flash chromatography was performed using the automated flash chromatography system CombiFlash Rf+ (Teledyne Isco, Lincoln, NE, USA) equipped with RediSepRf silica columns (Axel Semrau, Sprockhövel Germany). TLC was performed with aluminium-backed silica TLC plates (Macherey-Nagel MN ALUGRAM Sheets SIL G/UV 254 20 x 20cm 818133) with a suitable solvent system and was visualized using UV fluorescence (254 & 366 nm). All reactions were carried out in oven-dried glassware under an atmosphere of argon. Anhydrous DMF was purchased from Aldrich and used directly.

Mass spectrometry was performed on a SpectraSystems-MSQ LCMS system (Thermo Fisher, Dreieich, Germany) using a Hypersil Gold column, 150 x 3 mm, 5 µm. At a flow rate of 700 µL/min, the gradient of H_2O (0.1% FA) and ACN (0.1% FA) starting from 30% ACN and then increased to 95% over 12 min. The mass spectrum was measured in positive and negative mode in a range from 100–600 m/z. The UV spectrum was recorded at 254 nm. High-resolution mass spectra (HR-MS) were recorded with a ThermoScientific system where a Dionex Ultimate 3000 RSLC was coupled to a Q Exactive Focus mass spectrometer with an electrospray ion (ESI) source. An Acquity UPLC® BEH C8, 150 x 2.1 mm, 1.7 µm column equipped with a VanGuard Pre-Column BEH C8, 5 x 2.1 mm, 1.7 µm (Waters, Germany) was used for separation. At a flow rate of 250 µL/min, the gradient of (A) H2O + 0.1% FA and (B) ACN + 0.1% FA was held at 10% B for 1 min and then increased to 95% B over 4 min. It was held there for 1.2 min before the gradient was decreased to 10% B over 0.3 min where it was held for 1 min. The mass spectrum was measured in positive mode in a range from 120–1000 m/z. UV spectrum was recorded at 254 nm. The compounds which did not give a good ionization at the Q Exactive, have been measured on a Dionex Ultimate 3000 RSLC system using a BEH C18, 100 x 2.1 mm, 1.7 µm dp column (Waters, Germany). Separation of 1 µl sample was achieved by a linear gradient from (A) H_2O + 0.1% FA to (B) ACN + 0.1% FA at a flow rate of 600 µL/min and 45 °C. The gradient was initiated by a 0.5 min isocratic step at 5% B, followed by an increase to 95% B in 18 min to end up with a 2 min step at 95% B before re-equilibration under the initial conditions. UV spectra were recorded by a DAD in the range from 200 to 600 nm. The LC flow was split to 75 µL/min before entering the maXis 4G hr-ToF mass spectrometer (Bruker Daltonics, Germany) using the Apollo ESI source. Mass spectra were acquired in centroid mode ranging from 150–2500 m/z at a 2 Hz scan rate.
Compounds were purified by prep. HPLC eluting with an alternating gradient of 5–100% ACN with 0.05% FA in H_2O with 0.05% FA. Compounds that were not clean enough for testing after FCC were purified by prep. HPLC on a small scale. All the compounds have a purity > 95% according to LC-MS

6.2 Abbreviations

Acetonitrile (ACN), dichloromethane (DCM), *meta*-chloroperoxybenzoic acid (CPBA), *N*,*N*-diisopropylethylamine (DIPEA), dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), ethyl acetate (EtOAc), formic acid (FA), flash column chromatography (FCC), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LCMS), *tetra*-*n*-butylammonium iodide (TBAI), trifluoroacetic acid (TFA), tetrahydrofuran (THF), thin-layer chromatography (TLC), tetramethylsilane (TMS). Other abbreviations used are: aqueous (aq.), calculated (calcd.), hours (h), minutes (min), room temperature (rt), overnight (on), saturated (sat.).

6.3 Synthesis Schemes

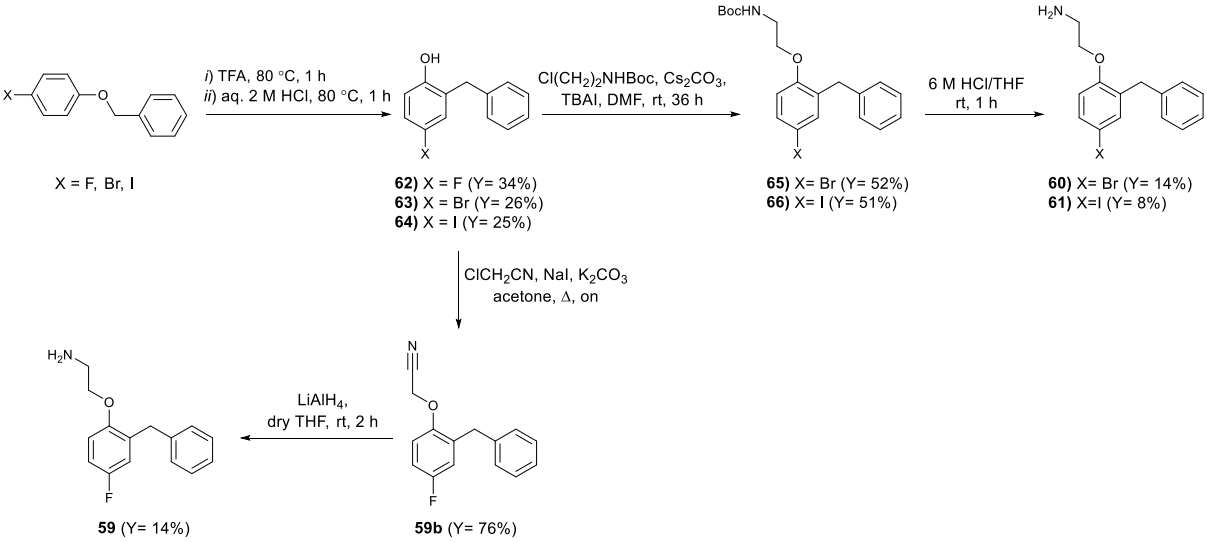


37) X = OH; Y = Cl; Z = CH_2
58a) NH_2; Y = H; Z = O

38) X = O; Y = Cl; Z = CH_2 (Y= 80%)
58b) X = NH; Y = H; Z = O (Y= 33%)

2) X = O; Y = Cl; Z = CH_2 (Y= 17%)
58) X = NH; Y = H; Z = O (Y= 2%)

Scheme S1: Synthesis of **2** and **58**.



X = F, Br, I

62) X = F (Y= 34%)
63) X = Br (Y= 26%)
64) X = I (Y= 25%)

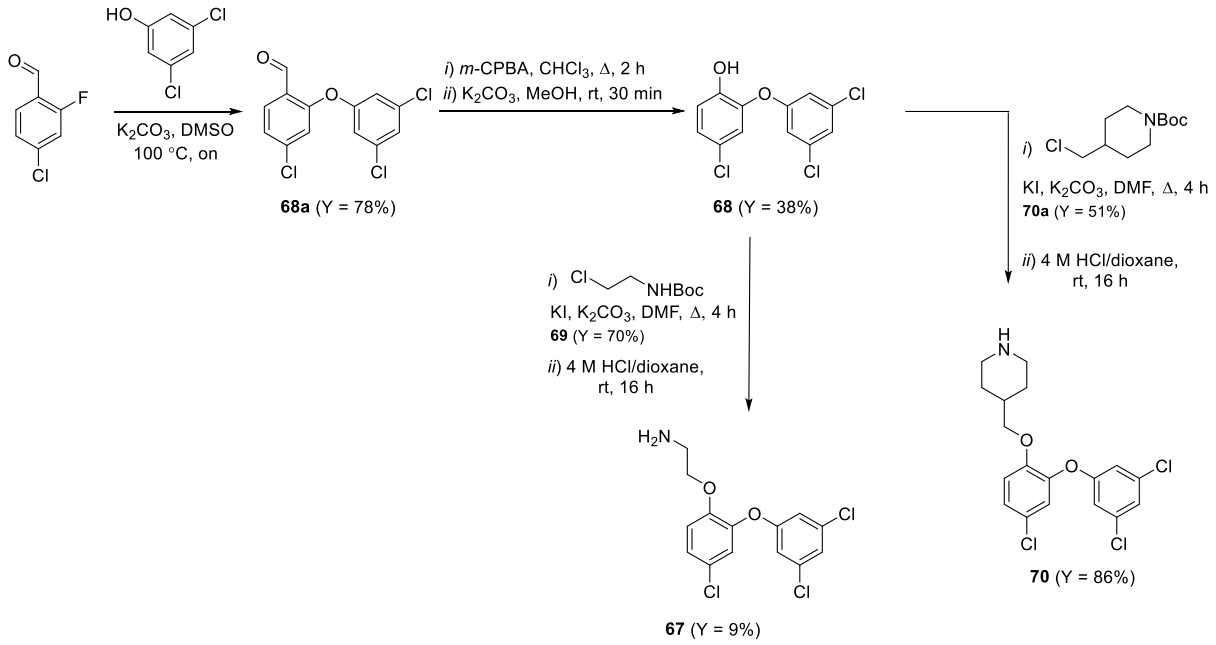
65) X= Br (Y= 52%)
66) X= I (Y= 51%)

60) X= Br (Y= 14%)
61) X=I (Y= 8%)

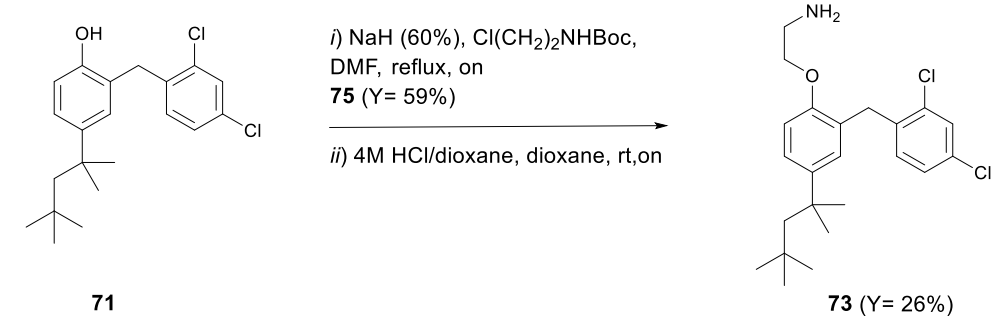
59 (Y= 14%)

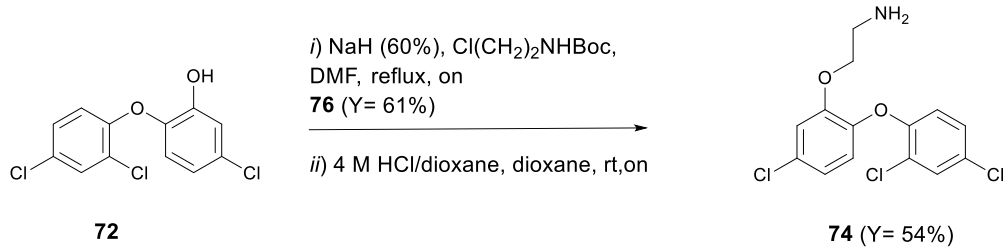
59b (Y= 76%)

Scheme S2: Synthesis of **59**–**66.**

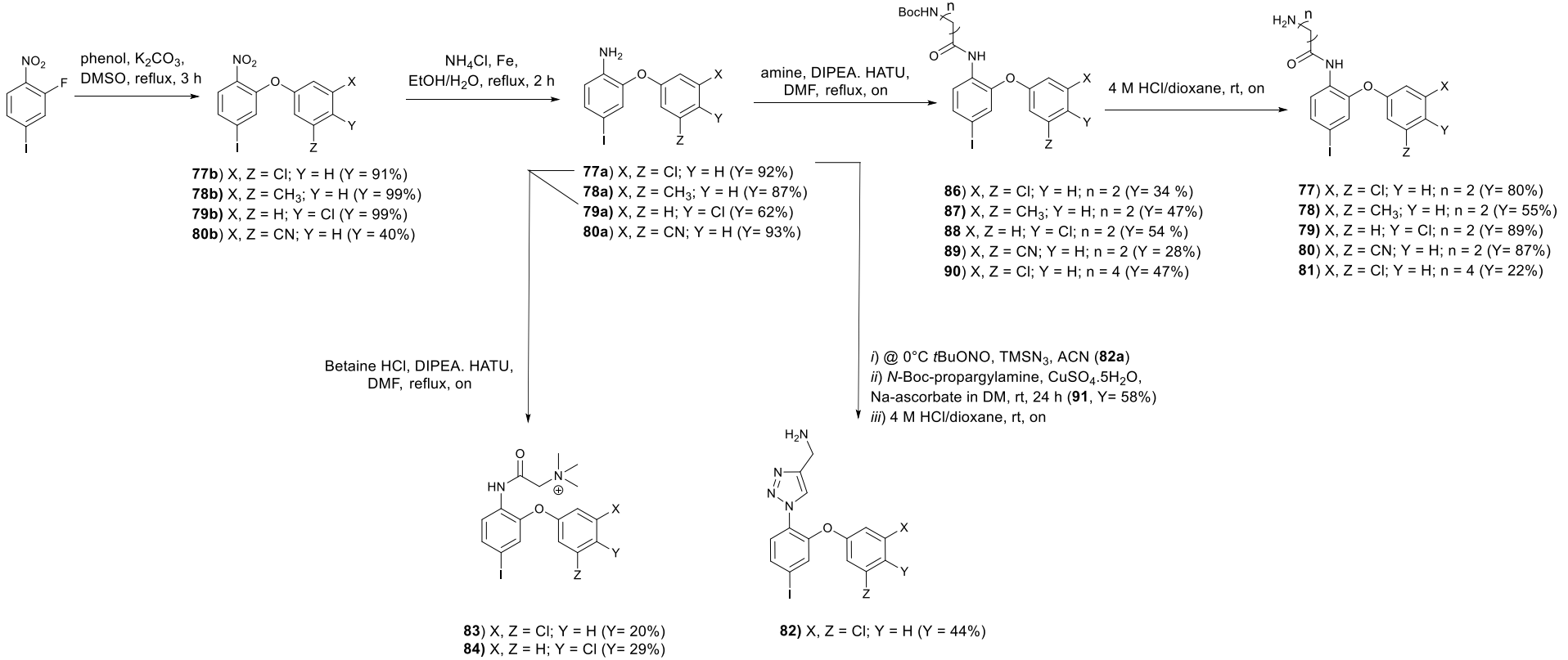


Scheme S3: Synthesis of **67**–**70**.





Scheme S4: Synthesis of **73**–**76**.



77b) X, Z = Cl; Y = H (Y = 91%)
78b) X, Z = CH_3; Y = H (Y = 99%)
79b) X, Z = H; Y = Cl (Y = 99%)
80b) X, Z = CN; Y = H (Y = 40%)

77a) X, Z = Cl; Y = H (Y= 92%)
78a) X, Z = CH_3; Y = H (Y= 87%)
79a) X, Z = H; Y = Cl (Y= 62%)
80a) X, Z = CN; Y = H (Y= 93%)

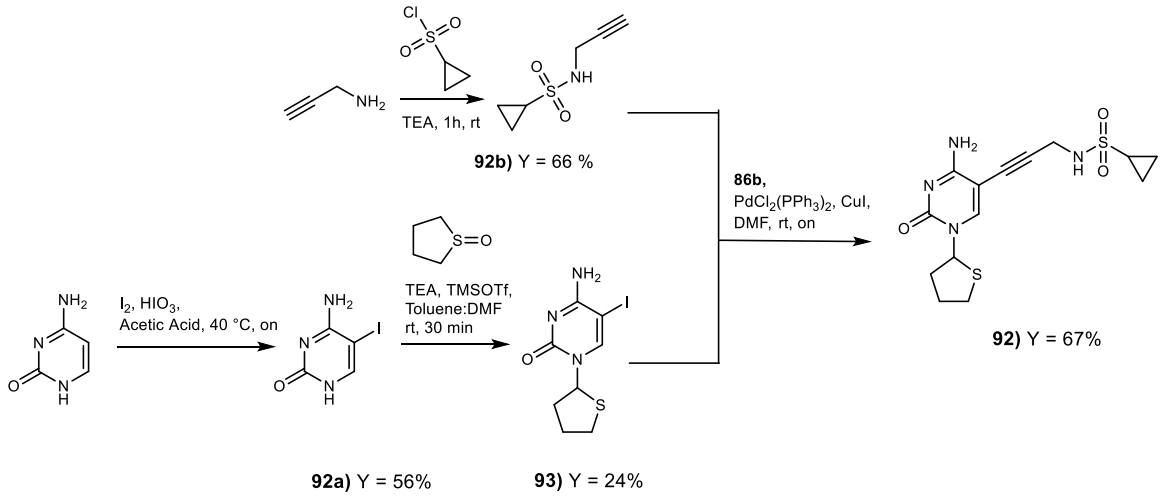
86) X, Z = Cl; Y = H; n = 2 (Y= 34 %)
87) X, Z = CH_3; Y = H; n = 2 (Y= 47%)
88 X, Z = H; Y = Cl; n = 2 (Y= 54 %)
89) X, Z = CN; Y = H; n = 2 (Y = 28%)
90) X, Z = Cl; Y = H; n = 4 (Y= 47%)

77) X, Z = Cl; Y = H; n = 2 (Y= 80%)
78) X, Z = CH_3; Y = H; n = 2 (Y= 55%)
79) X, Z = H; Y = Cl; n = 2 (Y= 89%)
80) X, Z = CN; Y = H; n = 2 (Y= 87%)
81) X, Z = Cl; Y = H; n = 4 (Y= 22%)

83) X, Z = Cl; Y = H (Y= 20%)
84) X, Z = H; Y = Cl (Y= 29%)

82) X, Z = Cl; Y = H (Y = 44%)

Scheme S5: Synthesis of **77**–**84**.



SchemeS6: Synthesis of **92** and **93.**

6.4 Synthesis of Compounds

6.4.1 General Procedures

General Procedures A (GPA): Activity handle *via* S_N2

The respective derivative of 2-benzyl-4-(halogen)phenol or 2-phenoxyaniline (1.0 eq.) was dissolved in dry acetone under N_2 flow. Anhydrous K_2CO_3 (1.0 eq.) and NaI (0.2 eq.) were added followed by a dropwise addition of chloroacetonitrile (1.0 eq.). The mixture was refluxed for the given time, then cooled down to rt and diluted with acetone. The mixture was filtered through celite and the remaining filtrate was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–10% EtOAc in petroleum benzene (40–60 °C) or cyclohexane to afford the title compounds.

General Procedure A-1 (GPA-1): Activity handle *via* S_N2

To a stirred solution of the respective derivative of 2-benzyl-4-(halogen)phenol derivative (1.0 eq.) in dry DMF under N_2 flow, Cs_2CO_3 (3.0 eq.) and TBAI (0.1 eq.) were added. The mixture was stirred at rt for 1 h followed by the addition of *tert*-butyl (2-chloroethyl)carbamate (2.5 eq.). The reaction was stirred overnight, and where not complete, followed by another addition of *tert*-butyl (2-chloroethyl)carbamate (2.5 eq.). The mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–100% EtOAc in petroleum benzene (40–60 °C) to afford the respective title compounds.

General Procedure B (GPB): Adapted Fries/Duff Rearrangement [37]

In a pressure sealed vial, the specific halogen derivative of 1-(benzyloxy)-4-(halogen)benzene (1.0 eq.) was flushed with N_2. TFA (4 mL) was added under N_2 flow, and the pressure vial was sealed. The mixture was stirred at 80 °C for 1 h. After 1 h, aq. 2 M HCl (4 mL) was added dropwise and stirred at 80 °C for 1 h. The reaction mixture was cooled down to rt and carefully extracted with DCM (2 x 10 mL). The organic layers were then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–10% EtOAc in petroleum benzene (40–60 °C) leaving TFA traces. The crude was dissolved in (aq. 2 M HCl), basified with aq. sat. $NaHCO_3$ and finally extracted with DCM. The organic layer was then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–10% EtOAc in petroleum benzene (40–60 °C) or cyclohexane, affording the respective title compounds.

General Procedure C (GPC): Boc-deprotection

To a stirred solution of the respective Boc-derivative (1.0 eq.) in THF or dioxane (0.1 M, as stated), was added dropwise at 0 °C the deprotecting agent (aq. 6 M HCl or 4 M HCl/dioxane, 4.0 to 20 eq. as stated). The mixture was stirred at rt until full conversion. The reaction mixture was basified with sat. aq. $NaHCO_3$ and extracted with organic solvent (DCM or diethyl ether). The organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford the desired compounds as crudes that were purified as described in detail to afford the respective title compounds.

General Procedure D (GPD): Amidic coupling

To a solution of the corresponding handle as carboxylic acid (1.0 eq.) in dry DMF (0.2 M), DIPEA (1.1 eq.) was added. After 10 min, HATU (1.2 eq.) was added to the solution, and the reaction mixture was stirred for 10 min. Then, aniline (1.1 eq.) was added, and the mixture was heated to reflux. The reaction mixture was cooled down to rt, quenched with NH_4Cl and extracted with EtOAc (3 x). The organic layers were washed with water (5 x), sat. aq. $NaHCO_3$ (1 x) and sat. aq. NaCl solution (2 x). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford the desired compounds as crude products that were further purified as described in detail for each compound.

General Procedure E (GPE): Nitro reduction

To a stirring solution of nitro aryl derivatives (1.0 eq.) in EtOH/H_2O (2:1, 0.1 M), Fe (5.0 eq.) and NH_4Cl (10 eq.) were added at rt, and the mixture was stirred at reflux until the starting material was consumed (usually 2 h, otherwise stated) as indicated by TLC and UPLC-MS analyses. Once cooled, the reaction solution was passed through a bed of Celite® and washed with copious amounts of EtOH. The solvent was then removed under reduced pressure. The residue was taken up in EtOAc washed with $NaHCO_3$ (3 x 10 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford the desired compounds as crude products.

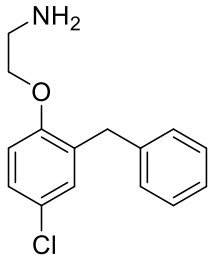
General Procedure F (GPF): Nucleophilic aromatic substitution

Phenol (1.0 eq.) and K_2CO_3 (1.2 eq.) were suspended in DMSO (0.5 M) and stirred at rt for 20 min. Then, aryl fluoride (1.0 eq.) was added, and the reaction mixture was stirred at 90 °C for 3 h. The suspension was cooled to rt, washed with water and extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified by FCC.

6.4.2 Synthesis and Characterization of Compounds 2, 38, 58–70, 73–91.

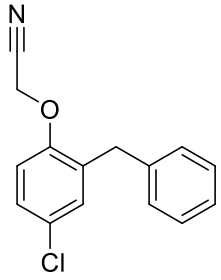
General observations: In the proton spectra of compounds **58**, **59**, **60**, **61** and **67** there is one NH-proton missing.

2-(2-Benzyl-4-chlorophenoxy)ethan-1-amine (2)



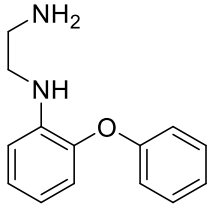
38 (0.20 g, 0.77 mmol, 1.0 eq.) was dissolved in dry diethyl ether (1.0 mL) and flushed with N_2. $LiAlH_4$ (0.06 g, 1.55 mmol, 2.0 eq.) was carefully dissolved in another flask in dry diethyl ether (2.5 mL) under N_2 flow. The solution of **38** was then added dropwise to the $LiAlH_4$ mixture and left to stir at rt for 2 h. The reaction mixture was quenched with ice and left to cool down on an ice bath forming a white precipitate. The white precipitate was filtered off, dissolved in EtOAc and concentrated *in vacuo*. The crude was purified with prep. HPLC to afford **2** as a white powder (0.039 g as formate salt, 17%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.34 (s, 2H), 7.26–7.29 (m, 2H), 7.23–7.25 (m, 2H), 7.21–7.23 (m, 1H), 7.16–7.20 (m, 1H), 7.14–7.16 (m, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 4.02 (t, *J* = 5.3 Hz, 2H), 3.94–3.95 (m, 2H), 3.02 (t, *J* = 5.3 Hz, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 154.7, 140.2, 132.0, 129.5, 128.9, 128.4, 127.0, 126.0, 124.2, 113.3, 67.8, 39.5, 34.9. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{15}H_{17}ClNO$ [*M* + H]$^+$: 262.09932, found: 262.09867.

2-(2-Benzyl-4-chlorophenoxy)acetonitrile (38)



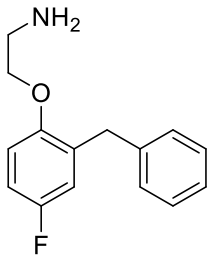
Using GPA, 2-benzyl-4-chlorophenol (0.50 g, 2.28 mmol, 1.0 eq.) was refluxed for 8 h with anhydrous K_2CO_3 (0.32 g, 2.28 mmol, 1.0 eq.), NaI (0.07 g, 0.46 mmol, 20 mol%) and chloroacetonitrile (140 µL, 2.28 mmol, 1.0 eq.) in dry acetone (5 mL) to afford FCC purified compound **38** as a clear oil (0.47 g, 80%). Further prep. HPLC purification (0.04 g) afforded **38** as a beige powder, (0.03 g). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.34 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.26–7.29 (m, 2H), 7.25–7.28 (m, 1H), 7.21–7.23 (m, 2H), 7.17–7.23 (m, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 5.21 (s, 2H), 3.91 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 153.4, 140.2, 132.8, 130.6, 129.1, 128.9, 127.7, 126.6, 126.5, 116.9, 114.6, 54.4, 35.2. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{15}H_{11}ClNO$ [*M* – H]$^-$: 256.05346, found: 256.05315.

***N*^{1}-(2-Phenoxyphenyl)ethane-1,2-diamine (58)**



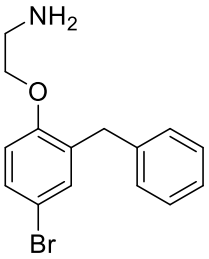
2-((2-Phenoxyphenyl)amino)acetonitrile **58b** (0.15 g, 0.69 mmol, 1.0 eq.) was dissolved in dry diethyl ether (1.0 mL) and flushed with N_2. $LiAlH_4$ (0.053 g, 1.39 mmol, 2.0 eq.) was carefully dissolved in another flask in dry diethyl ether (1.8 mL) under N_2 flow. The solution of **58b** was then added dropwise to the $LiAlH_4$ mixture and left to stir at rt for 2 h. The mixture was quenched with aq. 2 M NaOH (2 mL) under cooling. The aqueous layer was extracted with diethyl ether (2 x 10 mL). The organic phase was concentrated *in vacuo* and purified *via* prep. HPLC to afford **58** as an off-white powder, (0.005 g formate salt, 2%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.41 (br s, 1H), 7.29–7.38 (m, 2H), 6.98–7.09 (m, 1H,) 6.98–7.04 (m, 1H), 6.92 (br d, *J* = 8.4 Hz, 2H), 6.74–6.78 (m, 1H), 6.73–6.80 (m, 1H), 6.55–6.61 (m, 1H), 5.39 (br s, 1H), 3.21–3.27 (m, 2H), 2.80–2.91 (m, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 157.4, 142.6, 140.3, 129.8, 125.0, 122.6, 119.3, 117.3, 116.1, 111.2, 42.3, 38.7. **HRMS (ESI$^+$)** *m/z* calcd. For $C_{14}H_{17}N_2O$ [*M* + H]$^+$: 229.13354, found: 229.13301.

2-(2-Benzyl-4-fluorophenoxy)ethan-1-amine (59)



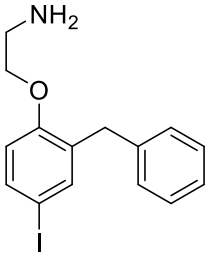
2-(2-Benzyl-4-fluorophenoxy)acetonitrile **59b** (0.025 g, 1.11 mmol, 1.0 eq.) was dissolved in dry THF (2 mL) under N_2 flow. $LiAlH_4$ (2.4 M in THF) (100 µL, 0.207 mmol, 2.0 eq.) was added dropwise to the mixture. The mixture was stirred at rt for 2 h and was quenched with aq. 2 M NaOH (2 mL) under cooling. The mixture was extracted with THF (2 x 5 mL). The organic layers were combined and concentrated *in vacuo*. The crude was purified with prep. HPLC to afford **59** as a crystalline, colourless powder (0.005 g as formate salt, 14%). **^{1}H NMR (Methanol-*d*$_4$, 500 MHz)** δ 8.43 (br s, 1H), 7.19–7.27 (m, 2H), 7.13–7.17 (m, 2H), 7.13–7.18 (m, 1H), 6.92–6.95 (m, 1H), 6.87–6.91 (m, 1H), 6.76 (dd, *J* = 9.2, 2.9 Hz, 1H), 4.09–4.12 (m, 2H), 3.98–4.00 (m, 2H), 3.22–3.25 (m, 2H). **^{13}C NMR (Methanol-*d*$_4$, 126 MHz)** δ 156.6, 151.5, 140.3, 132.2, 128.4, 128.1, 125.8, 116.6, 113.0, 112.9, 64.8, 38.8, 35.1. **^{19}F NMR (Methanol-*d*$_4$, 470 MHz)** δ ppm –124.49 (s). **HRMS (ESI$^+$)** *m/z* calcd. For $C_{15}H_{17}FNO$ [*M* + H]$^+$: 246.12887, found: 246.12816.

2-(2-Benzyl-4-bromophenoxy)ethan-1-amine (60)



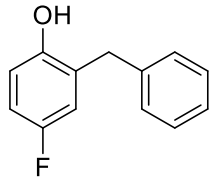
Using GPC, to a stirred solution of *tert*-butyl (2-(2-benzyl-4-bromophenoxy)ethyl)carbamate **65** (0.020 g, 0.049 mmol) in THF (1 mL), aq. 6 M HCl (1 mL) was added. The mixture was stirred at rt for 1 h to afford prep. HPLC purified **60** as a white powder, (0.002 g formate salt, 14%). **^{1}H NMR (Methanol-*d*$_4$, 500 MHz)** δ 8.50 (br s, 1H), 7.26–7.30 (m, 1H), 7.20–7.25 (m, 2H), 7.16–7.18 (m, 1H), 7.12–7.15 (m, 3H), 6.82–6.85 (m, 1H), 3.98–4.03 (m, 2H), 3.93 (s, 2H), 3.02–3.08 (m, 2H). **^{13}C NMR (Methanol-*d*$_4$, 126 MHz)** δ 156.9, 141.9, 134.4, 133.8, 131.5, 129.9, 129.7, 127.4, 114.6, 114.4, 68.5, 41.1, 36.9. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{15}H_{17}BrNO$ [*M* + H]$^+$: 306.04881, found: 306.04807.

2-(2-Benzyl-4-iodophenoxy)ethan-1-amine (61)



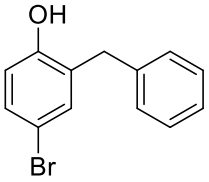
Using GPC, to a stirred solution of *tert*-butyl (2-(2-benzyl-4-iodophenoxy)ethyl)carbamate **66** (0.03 g, 0.06 mmol) in THF (1.1 mL), aq. 6 M HCl (1.1 mL) was added. The mixture was stirred at rt for 1 h to afford **61** as a white powder after prep. HPLC purification (0.002 g formate salt, 7%). **^{1}H NMR (Methanol-*d*$_4$, 500 MHz)** δ 8.49 (br s, 1H), 7.46 (br d, *J* = 8.7 Hz, 1H), 7.33–7.37 (m, 1H), 7.20–7.25 (m, 2H), 7.10–7.15 (m, 3H), 6.72 (d, *J* = 8.5 Hz, 1H), 4.01 (t, *J* = 4.5 Hz, 2H), 3.91 (s, 2H), 3.06 (br t, *J* = 4.5 Hz, 2H). **^{13}C NMR (Methanol-*d*$_4$, 126 MHz)** δ 170.4, 157.6, 141.9, 140.4, 137.8, 134.2, 129.9, 129.7, 127.4, 115.2, 68.0, 41.0, 36.8. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{15}H_{17}INO$ [*M* + H]$^+$: 354.03494, found: 354.03393.

2-Benzyl-4-fluorophenol (62)



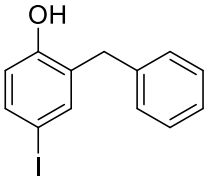
Using GPB, 1-(benzyloxy)-4-fluorobenzene (0.21 g, 1.03 mmol) was reacted to afford compound **62** as a yellow oil after FCC (0.064 g, 34%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.42 (br s, 1H), 7.24–7.29 (m, 2H), 7.20–7.24 (m, 2H), 7.15–7.19 (m, 1H), 6.83–6.86 (m, 1H), 6.80–6.86 (m, 1H), 6.75–6.80 (m, 1H), 3.84 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 155.8, 151.7, 141.0, 129.6, 129.1, 128.7, 126.3, 116.8, 116.1, 113.6, 35.6. **^{19}F NMR (DMSO-*d*$_6$, 470 MHz,)** δ ppm –125.96 (s). **HRMS (ESI$^+$)** *m/z* calcd. for $C_{13}H_{10}FO$ [*M* – H]$^-$: 201.07211, found: 201.07115.

2-Benzyl-4-bromophenol (63)



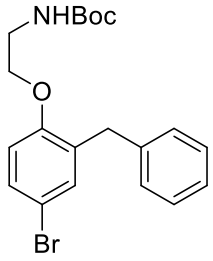
Using GPB, 1-(benzyloxy)-4-bromobenzene (0.21 g, 0.79 mmol) was reacted to afford FCC-purified compound **63** as a colourless oil, (0.054 g, 26%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.80 (br s, 1H), 7.24–7.29 (m, 2H), 7.21–7.23 (m, 2H), 7.15–7.19 (m, 3H), 6.73–6.78 (m, 1H), 3.84 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 154.4, 140.5, 132.4, 130.4, 129.7, 128.7, 128.3, 125.9, 117.1, 109.9, 34.8. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{13}H_{10}BrO$ [*M* – H]$^-$: 260.99205, found: 260.99173.

2-Benzyl-4-iodophenol (64)



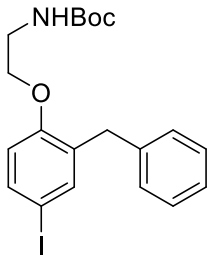
Using GPB, 1-(benzyloxy)-4-iodobenzene (0.21 g, 0.67 mmol) was reacted to afford FCC-purified compound **64** as a colourless oil, (0.053 g, 25%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.77 (br s, 1H), 7.30–7.33 (m, 1H), 7.29–7.34 (m, 1H), 7.23–7.29 (m, 2H), 7.19–7.23 (m, 2H), 7.14–7.19 (m, 1H), 6.64 (br d, *J* = 9.2 Hz, 1H), 3.81 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 155.6, 141.1, 138.7, 136.1, 131.3, 129.1, 128.8, 126.3, 118.2, 81.3, 35.2. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{13}H_{10}IO$ [*M* – H]$^-$: 308.97818, found: 308.97791.

***Tert*-butyl (2-(2-benzyl-4-bromophenoxy)ethyl)carbamate (65)**



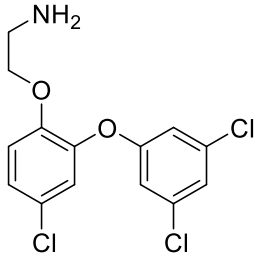
Using GPA-1, into a solution of **63** (0.10 g, 0.38 mmol, 1.0 eq.) in anhydrous DMF (4 mL), Cs_2CO_3 (0.375 g, 1.14 mmol, 3.0 eq.) and TBAI (0.014 g, 0.038 mmol, 10 mol%) were added. The mixture was stirred at rt for 1 h, followed by the addition of *tert*-butyl (2-chloroethyl)carbamate (0.17 g, 0.95 mmol, 2.5 eq.). The reaction was stirred overnight followed by another addition of *tert*-butyl (2-chloroethyl)carbamate (0.171 g, 0.95 mmol, 1.0 eq.) with a total reaction time of 36 h to afford FCC-purified compound **65** as a yellow oil (0.08 g, 52%). Further prep. HPLC purification (0.040 g) afforded **65** as a white powder, (0.013 g). **^{1}H NMR (Methanol-*d*$_4$, 500 MHz)** δ 7.22–7.24 (m, 1H), 7.19–7.22 (m, 2H), 7.14–7.17 (m, 2H), 7.09–7.14 (m, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 4.60 (br s, 1H), 3.91 (t, *J* = 5.5 Hz, 2H), 3.88 (s, 2H), 3.36 (t, *J* = 5.5 Hz, 2H), 1.38 (s, 9H). **^{13}C NMR (Methanol-*d*$_4$, 126 MHz)** δ 158.6, 157.2, 141.9, 134.0, 131.3, 130.1, 129.6, 128. 3, 127.3, 114.5, 113.9, 80.4, 68.5, 41.1, 36.8, 28.9. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{20}H_{25}BrNO_3$ $[M + H]^+$: 406.10124, found: 306.04829 without Boc-group.

***Tert*-butyl (2-(2-benzyl-4-iodophenoxy)ethyl)carbamate (66)**



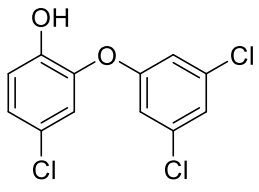
Using GPA-1, 2-benzyl-4-iodophenol **64** (0.12 g, 0.38 mmol, 1.0 eq.) was reacted with Cs_2CO_3 (0.38 g, 1.16 mmol, 3.1 eq.) and TBAI (0.04 g, 0.014 mmol, 4 mol%) in dry DMF (4 mL). The mixture was stirred at rt for 1 h followed by the addition of *tert*-butyl (2-chloroethyl)carbamate (0.17 g, 0.97 mmol, 2.6 eq.). The reaction was stirred overnight followed by another addition of *tert*-butyl (2-chloroethyl)carbamate (0.17 g, 0.97 mmol, 2.6 eq.) with a total reaction time of 36 h to afford FCC purified compound **66** as a yellow oil, (0.09 g, 51%). Further prep. HPLC purification (0.045 g) afforded compound **66** as a white powder (0.015 g). **^{1}H NMR (Methanol-*d*$_4$, 500 MHz)** δ 7.41 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.31 (d, *J* = 2.3 Hz, 1H), 7.18–7.23 (m, 2H), 7.13–7.18 (m, 2H), 7.09–7.13 (m, 1H), 6.68 (br d, *J* = 8.5 Hz, 1H), 4.60 (br s, 1H), 3.91 (br t, *J* = 5.4 Hz, 2H), 3.85 (s, 2H), 3.35 (br t, *J* = 5.4 Hz, 2H), 1.34 - 1.41 (m, 9H). **^{13}C NMR (Methanol-*d*$_4$, 126 MHz)** δ 157.9, 152.6, 142.0, 140.0, 137.6, 134.4, 130.1, 129.6, 127.2, 115.0, 83.7, 80.4, 68.3, 41.0, 36.7, 28.9. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{20}H_{25}INO_3$ $[M + H]^+$: 454.08737, found: 354.03433 without Boc-group.

2-(4-Chloro-2-(3,5-dichlorophenoxy)phenoxy)ethan-1-amine (67)



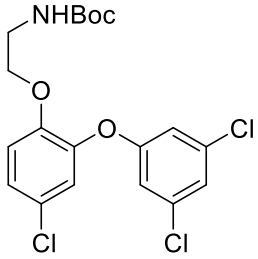
Using GPC, *tert*-butyl (2-(4-chloro-2-(3,5-dichlorophenoxy)phenoxy)ethyl)carbamate **69** (0.10 g, 0.23 mmol, 1.0 eq.) and 4 M HCl/dioxane (0.58 mL, 2.31 mmol, 10 eq.) in dioxane (0.5 mL) were stirred at rt overnight to afford **67** as a white powder (0.008 g as formate salt, 9%) after prep. HPLC. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.28 (s, 1H), 7.33 (d, *J* = 2.4 Hz, 1H), 7.31 (t, *J* = 1.6 Hz, 1H), 7.22 (d, *J* = 8.5 Hz, 1H), 7.09 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.95 (d, *J* = 1.6 Hz, 2H), 4.05 (t, *J* = 5.5 Hz, 2H), 2.81 (t, *J* = 5.5 Hz, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 164.3, 159.0, 150.9, 141.7, 134.7, 130.3, 123.5, 122.3, 121.5, 120.2, 115.4, 115.2, 69.5 (one peak under DMSO-***d*$_6$**). **HRMS (ESI$^+$)** *m/z* calcd. for $C_{14}H_{13}Cl_3NO_2$ $[M + H]^+$: 332.00064, found: 331.99998.

4-Chloro-2-(3,5-dichlorophenoxy)phenol (68)



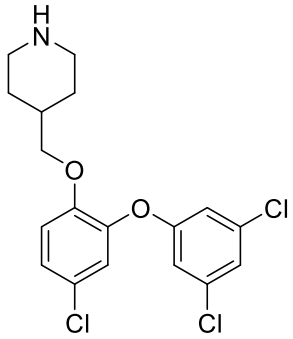
To a stirred solution of 4-chloro-2-(3,5-dichlorophenoxy)benzaldehyde **68a** (0.594 g, 1.97 mmol, 1.0 eq.) in chloroform (7 mL), *m*-CPBA (purity ~75%) (2.26 g, 9.85 mmol, 5.0 eq.) was added. The mixture was refluxed for 2 h. The mixture was then cooled down, quenched with aq. 10% $Na_2S_2O_3$ (10 mL) and extracted with DCM (3 x 15 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was dissolved in MeOH (15 mL) and K_2CO_3 (0.72 g, 5.18 mmol, 3.0 eq.) was added. The mixture was stirred at rt for 30 min followed by filtration. The filtrate was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–10% acetone in cyclohexane to afford **68** as a light-yellow sticky solid, (0.215 g, 38%). **^{1}H NMR ($CDCl_3$, 500 MHz)** δ 7.17 (t, *J* = 1.9 Hz, 1H), 7.10 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.01 (d, *J* = 8.6 Hz, 1H), 6.92–6.93 (m, 2H), 5.35 (s, 1H). **^{13}C NMR ($CDCl_3$, 126 MHz)** δ 157.5, 146.2, 142.6, 136.01, 135. 7, 126.1, 125.4, 124.5, 119.8, 117.7, 116.7, 116.1. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{12}H_6Cl_3O_2$ $[M - H]^-$: 286.94388, found: 286.94381.

***Tert*-butyl (2-(4-chloro-2-(3,5-dichlorophenoxy)phenoxy)ethyl)carbamate (69)**



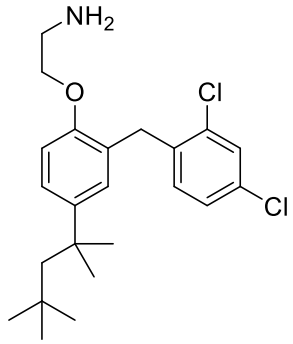
Into a solution of 4-chloro-2-(3,5-dichlorophenoxy)phenol **68** (0.16 g, 0.54 mmol, 1.0 eq.) in dry DMF (2 mL), K_2CO_3 (0.22 g, 1.63 mmol, 3.0 eq.) was added. The mixture was stirred at rt for 30 min followed by the addition of KI (0.18 g, 1.08 mmol, 2.0 eq.) and *tert*-butyl (2-chloroethyl)carbamate (0.12 g, 0.65 mmol, 1.2 eq.). The mixture was refluxed for 4 h. The reaction mixture was cooled down to rt, diluted with water (10 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (3 x 5 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–10% EtOAc in cyclohexane to afford **69** as a light-yellow oil, (0.16 g, 70 %). Further prep. HPLC purification (0.065 g) afforded compound **69** as a white powder, (0.031 g). **^{1}H NMR ($CDCl_3$, 500 MHz)** δ 7.02–7.06 (m, 2H), 6.99–7.01 (m, 2H), 6.79 (d, *J* = 1.6 Hz, 2H), 4.47 (br s, 1H), 3.99 (t, *J* = 5.0 Hz, 2H), 3.39 (q, *J* = 5.0 Hz, 2H), 1.44 (s, 9H). **^{13}C NMR ($CDCl_3$, 126 MHz)** δ 167.4, 152.1, 150.9, 149.1, 141.9, 135.5, 123.3, 122.8, 121.8, 115.0, 114.9, 79.8, 68.6, 53.7, 28.3. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{19}H_{21}Cl_3NO_4$ $[M + H]^+$: 432.05307, found: 332.00007 without Boc-group.

4-((4-Chloro-2-(3,5-dichlorophenoxy)phenoxy)methyl)piperidine (70)



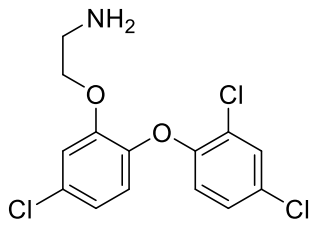
Using GPC, into a solution of **70a** (0.10 g, 0.21 mmol, 1.0 eq.) in dioxane (0.5 mL), 4 M HCl/dioxane (0.53 mL, 2.10 mmol, 10 eq.) was added and the reaction was stirred at rt for 7 h to afford a crude product as an oil, (0.070 g, 86%). Further prep. HPLC purification (0.060 g) afforded compound **70** as a beige powder (0.025 g as formate salt). **^{1}H NMR (DMSO-d_6, 500 MHz)** δ 8.38 (br s, 1H), 7.28–7.31 (m, 2H), 7.24 (d, *J* = 8.5 Hz, 1H), 7.07 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.91 (d, *J* = 1.8 Hz, 2H), 3.85 (d, *J* = 6.7 Hz, 2H), 3.03 (br d, *J* = 12.4 Hz, 2H), 2.58–2.65 (m, 2H), 1.74 (br s, 1H), 1.47 (br d, *J* = 12.4 Hz, 2H), 1.12 (br d, *J* = 10.1 Hz, 2H). **^{13}C NMR (DMSO-d_6, 126 MHz)** δ 165.9, 165.8, 159.3, 151.0, 141.2, 134.7, 130.5, 123.7, 122.1, 121.1, 115.0, 114.9, 72.5, 43.2, 33.8, 26.3. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{18}H_{19}Cl_3NO_2$ $[M + H]^+$: 386.04759, found: 386.04760.

2-(2-(2,4-Dichlorobenzyl)-4-(2,4,4-trimethylpentan-2-yl)phenoxy)ethan-1-amine (73)



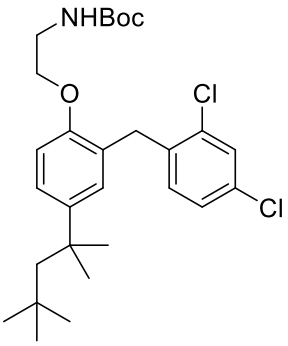
Using GPC, into a solution of *tert*-butyl (2-(2-(2,4-dichlorobenzyl)-4-(2,4,4-trimethylpentan-2-yl)phenoxy)ethyl)carbamate **75** (0.08 g, 0.16 mmol, 1.0 eq.) in dioxane (1.6 mL), 4 M HCl/dioxane (0.78 mL, 3.14 mmol, 20 eq.) was added and the reaction was stirred at rt overnight. Prep. HPLC purification afforded **73** as colorless solid (0.017 g as formate salt, 26%). **^{1}H NMR (DMSO-d_6, 500 MHz)** δ 8.32 (s, 1H), 8.23 (s, 0.2H, formic acid) 7.60 (d, *J* = 2.0 Hz, 1H), 7.34 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.03 (s, 1H), 6.86 (d, *J* = 8.6 Hz, 1H), 4.02 (s, 2H), 3.98 (t, *J* = 4.8 Hz, 2H), 2.98 (t, *J* = 4.8 Hz, 2H), 1.61 (s, 2H), 1.23 (s, 6H), 0.62 (s, 9H). **^{13}C NMR (DMSO-d_6, 126 MHz)** δ 164.6 (formic acid), 153.7, 141.3, 137.5, 134.2, 132.4, 131.4, 128.5, 128.2, 127.2, 125.8, 125.1, 110.8, 67.4, 56.2, 51.1, 48.6, 37.5, 32.8, 32.0, 31.6. **HR-MS** *m/z* calcd for $C_{23}H_{32}Cl_2NO$ $[M + H]^+$: 408.18555, found: 408.18533.

2-(5-Chloro-2-(2,4-dichlorophenoxy)phenoxy)ethan-1-amine (74)



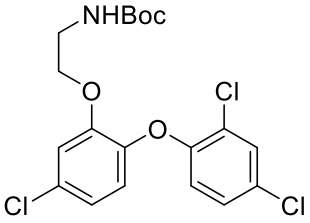
Using GPC, into a solution of *tert*-butyl (2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)ethyl)carbamate **76** (0.24 g, 0.56 mmol, 1.0 eq.) in dioxane (5.6 mL), 4 M HCl/dioxane (2.83 mL, 11.32 mmol, 20 eq.) was added and the reaction was stirred at rt overnight. Prep. HPLC purification afforded **74** as white solid (0.10 g as formate salt, 54%). **^{1}H NMR (DMSO-d_6, 500 MHz)** δ 8.29 (s, 1.5H, formic acid), 7.72 (d, *J* = 2.5 Hz, 1H), 7.34–7.28 (m, 2H), 7.10 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 1H), 4.01 (t, *J* = 5.6 Hz, 2H), 2.78 (t, *J* = 5.5 Hz, 2H). **^{13}C NMR (DMSO-d_6, 126 MHz)** δ 164.4 (formic acid), 151.9, 150.4, 142.7, 129.7, 129.6, 128.4, 127.0, 123.5, 122.2, 121.3, 118.7, 115.3, 69.9, 40.1. **HRMS** *m/z* calcd for $C_{14}H_{13}Cl_3NO_2$ $[M + H]^+$: 332.00064, found: 332.00037.

***Tert*-Butyl (2-(2-(2,4-dichlorobenzyl)-4-(2,4,4-trimethylpentan-2-yl)phenoxy)ethyl)carbamate (75)**



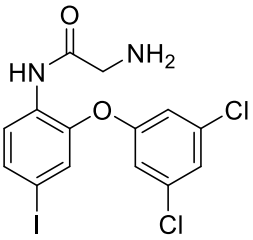
To a solution of **71** (0.10 g, 0.27 mmol, 1.0 eq.) in dry DMF (3 mL), NaH 60% (0.016 g, 0.41 mmol, 1.6 eq.) was added and the reaction mixture was stirred at rt for 30 min. Then, *tert*-butyl (2-chloroethyl)carbamate (0.097 g, 0.54 mmol, 2.0 eq.) was slowly added and the reaction was refluxed overnight. The mixture was then cooled down, quenched with HCl 2 M and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified *via* prep. HPLC to afford **75** as a colorless oil (0.08 g, 59%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.57 (d, *J* = 2.1 Hz, 1H), 7.32 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 7.16 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.05 (d, *J* = 2.2 Hz, 1H), 6.91 (t, *J* = 5.5 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 1H), 3.97 (s, 2H), 3.90 (t, *J* = 5.6 Hz, 2H), 3.28 (dd, *J* = 11.4, 5.7 Hz, 2H), 1.61 (s, 2H), 1.37 (s, 9H), 1.23 (s, 6H), 0.62 (s, 9H). **^{13}C NMR (126 MHz, DMSO)** δ 155.7, 153.9, 141.1, 137.4, 134.1, 132.6, 131.3, 128.4, 128.2, 127.1, 125.7, 125.0, 110.7, 77.7, 66.6, 56.2, 37.5, 35.8, 32.8, 31.9, 31.5, 31.5, 28.2. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{28}H_{40}Cl_2NO_3$ [*M* + H]$^+$: 508.23798, found: 408.18542, without Boc group (mass calculated without Boc group: 408.18555).

***Tert*-Butyl (2-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)ethyl)carbamate (76)**



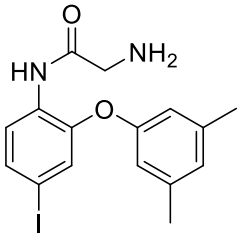
To a solution of **72** (0.30 g, 1.03 mmol, 1.0 eq.) in dry DMF (5.0 mL, 0.2 M), NaH 60 % (0.08 g, 2.07 mmol, 2.0 eq.) was added and the reaction mixture was stirred at rt for 30 min. Then, *tert*-butyl (2-chloroethyl)carbamate (0.37 g, 2.07 mmol, 2.0 eq.) was slowly added and the reaction was refluxed overnight. The mixture was then cooled down, quenched with 2 M HCl and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified *via* FCC (petroleum benzene/EtOAc 85:15) to afford **76** as a colorless oil (0.27 g, 61%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** 7.70 (d, *J* = 2.5 Hz, 1H), 7.31 (m, 2H), 7.04 (m, 2H), 6.81 (d, *J* = 8.9 Hz, 1H), 6.69 (t, *J* = 5.5 Hz, 1H), 4.00 (t, *J* = 6.0 Hz, 2H), 3.13 (q, *J* = 5.8 Hz, 2H), 1.35 (s, 9H). **^{13}C NMR (126 MHz, DMSO)** δ 155.5, 151.8, 150.5, 142.7, 129.7, 129.6, 128.4, 127.0, 123.5, 122.1, 121.2, 118.7, 115.2, 77.8, 67.4, 38.6, 28.2. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{19}H_{21}Cl_3NO_4$ [*M* + H]$^+$: 432.05307, found: 332.0003, without Boc group (mass calculated without Boc group: 332.00064).

2-Amino-*N*-(2-(3,5-dichlorophenoxy)-4-iodophenyl)acetamide (77)



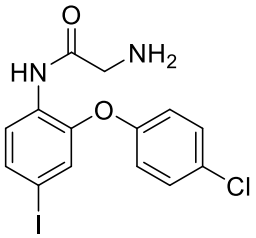
Using GPC, **85** (0.03 g, 0.05 mmol, 1.0 eq.) and 4 M HCl/dioxane (0.25 mL, 1.0 mmol, 20 eq.,) in dioxane (0.5 mL), afforded **77** as white solid (0.017 g as formate salt, 80%) after prep. HPLC purification. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.18 (s, 1H, formic acid) 8.06 (d, *J* = 8.6 Hz, 1H) 7.60 (dd, *J* = 8.6, 1.9 Hz, 1H) 7.43 (t, *J* = 1.7 Hz, 1H) 7.41 (d, *J* = 1.7 Hz, 1H) 7.10 (d, *J* = 1.9 Hz, 2H) 3.11 (s, 1H), 2.5 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 170.3, 163.0 (formic acid), 158.3, 145.9, 135.4, 134.6, 130.3, 128.4, 124.2, 123.7, 117.6, 87.9, 44.1. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{14}H_{12}Cl_2IN_2O_2$ [*M* + H]$^+$: 436.93151, found: 436.93052.

2-Amino-*N*-(2-(3,5-dichlorophenoxy)-4-iodophenyl)acetamide (78)



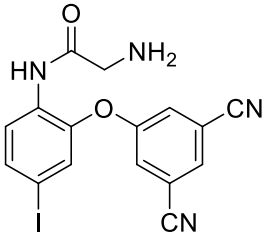
Using GPC, **86** (0.20 g, 0.43 mmol, 1.0 eq.) and 4 M HCl/dioxane (1mL, 4.3 mmol, 10 eq.) in dioxane (4 mL), afforded **78** as white powder (0.98 g as formate salt, 55%) after prep. HPLC purification. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.22 (s, 1H, formic acid), 8.14 (d, *J* = 8.6 Hz, 1H), 7.46 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.08 (d, *J* = 1.9 Hz, 1H), 6.85 (s, 1H), 6.68 (s, 2H), 3.35 (s, 2H), 2.27 (s, 6H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 170.7, 163.8 (formic acid), 155.7, 147.0, 139.7, 132.3, 129.3, 126.0, 125.9, 122.1, 116.5, 86.6, 44.3, 20.8. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{16}H_{18}IN_2O_2$ [*M* + H]$^+$: 397.04075, found: 397.04028.

2-Amino-*N*-(2-(4-chlorophenoxy)-4-iodophenyl)acetamide (79)



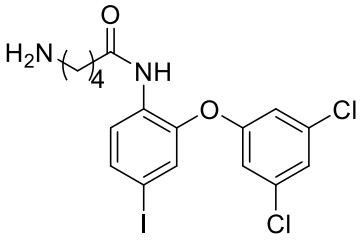
Using GPC, **87** (0.25 g, 0.5 mmol, 1.0 eq.) and 4 M HCl/dioxane (2.5 mL, 10 mmol, 20 eq.) in dioxane (5 mL), afforded **79** as white powder (0.20 g as formate salt, 89%) after prep. HPLC purification. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.23 (s, 1H, formic acid), 8.12 (d, *J* = 8.6 Hz, 1H), 7.52 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.47 (m, 2H), 7.21 (d, *J* = 1.9 Hz, 1H), 7.08 (m, 2H), 3.34 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 170.6, 163.9 (formic acid), 154.8, 146.3, 133.1, 130.1, 129.5, 128.0, 126.7, 122.5, 120.3, 86.8, 44.1. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{14}H_{13}ClIN_2O_2$ $[M + H]^+$: 402.97102, found: 402.97025.

2-Amino-*N*-(2-(3,5-dicyanophenoxy)-4-iodophenyl)acetamide (80)



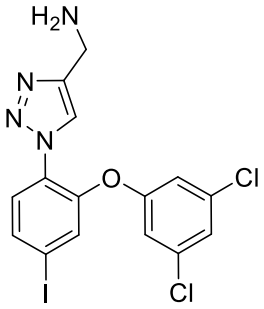
Using GPC, **88** (0.05 g, 0.09 mmol, 1.0 eq) and 4M HCl/dioxane (0.48 mL, 1.92 mmol, 21 eq.) in dioxane (1 mL), afforded **80** as white powder (0.035 g as formate salt, 87%) after prep. HPLC purification. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.27 (s, 1H), 8.20 (s, 1H, formic acid), 8.10 (d, *J* = 8.6 Hz, 1H), 7.94 (d, *J* = 1.2 Hz, 2H), 7.61 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 3.28 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 171.1, 163.6 (formic acid), 156.8, 145.0, 134.4, 131.5, 129.9, 127.9, 126.4, 123.0, 116.6, 114.3, 87.2, 44.4. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{16}H_{12}IN_4O_2$ $[M + H]^+$: 418.99995, found: 418.99829.

5-Amino-*N*-(2-(3,5-dichlorophenoxy)-4-iodophenyl)pentanamide (81)



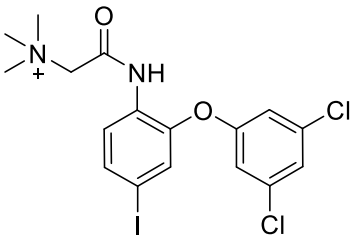
Using GPC, **89** (0.09 g, 0.16 mmol, 1.0 eq.) and 4 M HCl/dioxane (0.82 mL, 3.28 mmol, 20 eq.) in dioxane (3.3 mL, 0.05 M), afforded **81** as white powder (0.017 g, 22%) after prep. HPLC purification. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.60 (s, 1H), 8.42 (s, 1H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.57 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.41 (d, *J* = 1.8 Hz, 1H), 7.38 (t, *J* = 1.7 Hz, 1H), 7.00 (d, *J* = 1.8 Hz, 2H), 2.68 (t, *J* = 6.6 Hz, 2H), 2.28 (t, *J* = 6.8 Hz, 2H), 1.55–1.38 (m, *J* = 3.2 Hz, 4H). **^{13}C NMR (126 MHz, DMSO)** δ 171.5, 165.8, 158.1, 146.7, 134.7, 134.0, 130.4, 128.6, 126.3, 123.1, 116.8, 38.8, 35.3, 27.6, 22.1. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{17}H_{18}Cl_2IN_2O_2$ $[M + H]^+$: 478.97900, found: 478.97684.

(1-(2-(3,5-Dichlorophenoxy)-4-iodophenyl)-1H-1,2,3-triazol-4-yl)methanamine (82)



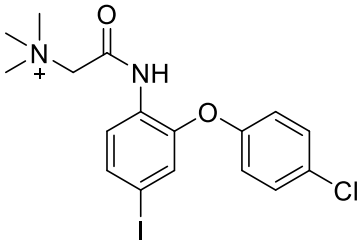
Using GPC, **90** (0.11 g, 0.20 mmol) and 4 M HCl/dioxane (0.98 mL, 3.93 mmol, 20eq.) in dioxane (4 mL, 0.05 M), afforded **82** as white powder (0.04 g as formate salt, 44%) after prep. HPLC purification. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.39 (s, 1H), 8.27 (s, 1H, formic acid), 7.80 (dd, *J* = 8.4, 1.8, 1H), 7.60 (d, *J* = 1.7, 1H), 7.57 (d, *J* = 8.4, 1H), 7.46 (t, *J* = 1.8, 1H), 7.24 (d, *J* = 1.8, 2H), 3.91 (s, 2H). **^{13}C NMR (126 MHz, DMSO)** δ 164.1 (formic acid), 156.7, 148.3, 147.3, 135.0, 134.4, 128.5, 128.2, 127.8, 124.5, 124.3, 118.2, 96.1, 35.9. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{15}H_{12}Cl_2IN_4O$ $[M + H]^+$: 460.94328, found: 460.94107.

2-((2-(3,5-Dichlorophenoxy)-4-iodophenyl)amino)-*N*,*N*,*N*-trimethyl-2-oxoethan-1-aminium (83)



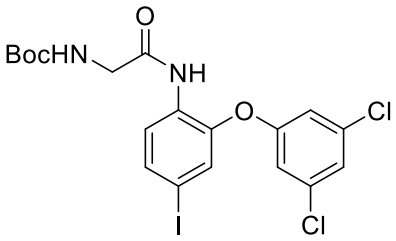
Using GPD, betaine HCl (0.03 g, 0.21 mmol, 0.9 eq.) DIPEA (0.04 mL, 0.23 mmol, 1.0 eq.), HATU (0.09 g, 0.25 mmol, 1.1 eq.) and **77a** (0.08 g, 0.23 mmol, 1.0 eq.) in dry DMF (1 mL), afforded **83** as white solid (0.020 g, 20%) after prep. HPLC. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 10.22 (s, 1H), 7.77 (d, *J* = 8.5, 1H), 7.63 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.45 (m, 2H), 7.06 (d, *J* = 1.5 Hz, 2H), 4.30 (s, 2H), 3.22 (s, 9H). **^{13}C NMR (126 MHz, DMSO)** δ 163.3, 158.3, 147.6, 135.4, 134.6, 129.2, 128.9, 126.7, 124.1, 117.6, 90.4, 64.5, 53.8. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{17}H_{19}Cl_2IN_2O_2^+$ $[M + H]^+$: 478.97845, found: 478.97836.

2-((2-(4-Chlorophenoxy)-4-iodophenyl)amino)-*N*,*N*,*N*-trimethyl-2-oxoethan-1-aminium (84)



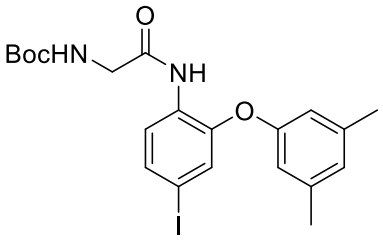
Using GPD with betaine HCl (0.11 g, 0.74 mmol, 1.0 eq.), DIPEA (0.14 mL, 0.81 mmol, 1.1 eq.), HATU (0.34 g, 0.88 mmol, 1.2 eq.) and **79a** (0.28 g, 0.74 mmol, 1.0 eq.) in dry DMF (3.7 mL) afforded **84** as white powder (0.096 g, 29%) after prep HPLC. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 10.26 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.55 (dd, *J* = 8.6 Hz, 1.7, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.22 (d, *J* = 1.6 Hz, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 4.32 (s, 2H), 3.22 (s, 9H). **^{13}C NMR (126 MHz, DMSO)** δ 162.8, 154.8, 148.5, 132.8, 130.0, 128.3, 128.0, 127.0, 126.1, 120.5, 89.6, 64.2, 53.3. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{17}H_{20}ClIN_2O_2^+$ [*M* + *H*]$^+$: 445.01742, found: 445.01715.

***Tert*-Butyl (2-((2-(3,5-dichlorophenoxy)-4-iodophenyl)amino)-2-oxoethyl)carbamate (86)**



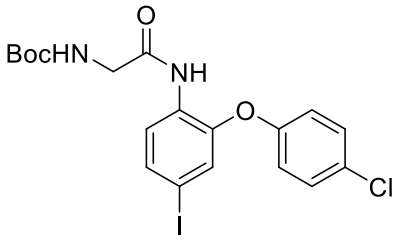
Using GPD, into a solution of *N*-Boc-glycine (0.03 g, 0.17 mmol, 1.0 eq.) in dry DMF (0.8 mL), DIPEA (0.03 mL, 0.18 mmol, 1.1 eq.), HATU (0.07 g, 0.20 mmol, 1.2 eq.) and **77a** (0.07 g, 0.18 mmol, 1.1 eq.) were added to afford **86** as a white solid, (0.03 g, 34%), after purification by FCC (hexane/EtOAc 6:4). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.44 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.39 (d, *J* = 12.4 Hz, 2H), 7.12 (t, *J* = 5.5 Hz, 1H), 7.05 (s, 2H), 3.69 (d, *J* = 5.8 Hz, 2H), 1.34 (s, 9H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 168.8, 157.9, 155.9, 146.0, 134.8, 133.9, 130.1, 127.9, 124.6, 123.5, 117.2, 87.6, 78.2, 43.9, 28.1. **HRMS (ESI$^-$)** *m/z* calcd. for $C_{19}H_{18}Cl_2IN_2O_4$ [*M* – H]$^-$: 534.9693, found: 534.9701.

***Tert*-Butyl (2-((2-(3,5-dimethylphenoxy)-4-iodophenyl)amino)-2-oxoethyl)carbamate (87)**



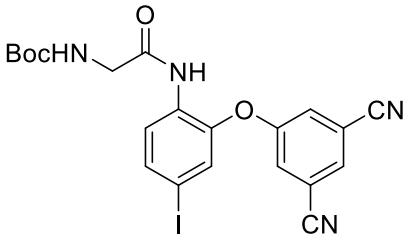
Using GPD, into a solution of *N*-Boc-glycine (0.14 g, 0.83 mmol, 1.0 eq.) in dry DMF (4.1 mL), DIPEA (0.16 mL, 0.91 mmol, 1.1 eq.), HATU (0.38 g, 0.99 mmol, 1.2 eq.) and **78a** (0.30 g, 0.91 mmol, 1.1 eq.) were added to afford **86** as a white solid after prep HPLC (0.21 g, 4 %). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.36 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.44 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.19 (t, *J* = 5.8 Hz, 1H), 7.04 (d, *J* = 1.8 Hz, 1H), 6.84 (s, 1H), 6.66 (s, 2H), 3.72 (d, *J* = 6.0 Hz, 2H), 2.26 (s, 6H), 1.34 (s, 9H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 168.8, 156.0, 155.7, 147.8, 139.6, 132.1, 129.3, 125.9, 125.9, 123.6, 116.7, 87.0, 78.4, 44.2, 28.1, 20.8. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{21}H_{24}IN_2O_4$ [*M* – H]$^-$: 495.07862, found: 495.07858.

***Tert*-Butyl (2-((2-(4-chlorophenoxy)-4-iodophenyl)amino)-2-oxoethyl)carbamate (88)**



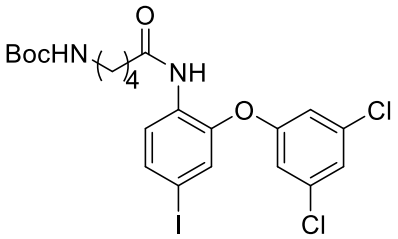
Using GPD, into a solution of *N*-Boc-glycine (0.18 g, 1.03 mmol, 1.0 eq.), DIPEA (0.19 mL, 1.14 mmol, 1.1 eq.), HATU (0.47 g, 1.24 mmol, 1.2 eq.) and **79a** (0.39 g, 1.14 mmol, 1.1 eq.) in dry DMF (5.7 mL) were added to afford **87** as a white solid (0.28 g, 54%) after prep. HPLC. **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.43 (s, 1H), 7.94 (d, *J* = 8.7 Hz, 1H), 7.49 (d, *J* = 10.2 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.16 (s, 2H), 7.06 (d, *J* = 8.6 Hz, 2H), 3.71 (d, *J* = 6.1 Hz, 2H), 1.34 (s, 9H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 168.7, 155.9, 154.9, 147.1, 132.8, 129.9, 129.6, 127.9, 126.6, 124.0, 120.5, 87.2, 78.2, 44.0, 28.10. **HRMS (ESI$^-$)** *m/z* calcd. for $C_{19}H_{19}ClIN_2O_4$ [*M* – H]$^-$: 501.00835, found: 501.00937.

***Tert*-Butyl (2-((2-(3,5-dicyanophenoxy)-4-iodophenyl)amino)-2-oxoethyl)carbamate (89)**



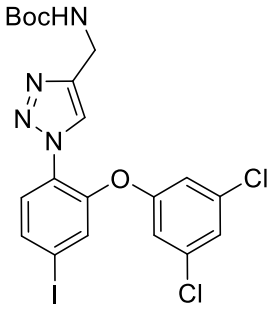
Using GPD, into a solution of *N*-Boc-glycine (0.13 g, 0.75 mmol, 1.0 eq.), DIPEA (0.14 mL, 0.83 mmol, 1.0 eq.), HATU (0.34 g, 0.90 mmol, 1.2 eq.) and **80a** (0.30 g, 0.83 mmol, 1.1 eq.) in dry DMF (3.7 mL) were added to afford **88** as a white solid, (0.11 g, 28%) after FCC (cyclohexane/EtOAc 6:4). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.49 (s, 1H), 8.23 (s, 1H), 7.85 (s, *J* = 11.9 Hz, 2H), 7.82 (s, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.44 (s, 1H), 7.09 (t, *J* = 5.5 Hz, 1H), 3.66 (d, *J* = 5.9 Hz, 2H), 1.34 (s, 9H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 169.1, 157.2, 156.5, 147.4, 134.3, 131.1, 130.1, 128.3, 126.3, 125.3, 116.6, 114.1, 88.1, 78.2, 30.7, 28.1. **HRMS (ESI$^-$)** *m/z* calcd. for $C_{21}H_{18}IN_4O_4$ [*M* – H]$^-$: 517.03782, found: 517.03833.

***Tert*-Butyl (5-((2-(3,5-dichlorophenoxy)-4-iodophenyl)amino)-5-oxopentyl)carbamate (90)**



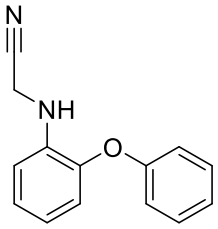
Using GPD, into a solution of Boc-5-Ava-OH (0.104 g, 0.48 mmol, 1.0 eq.), DIPEA (0.09 mL, 0.52 mmol, 1.1 eq.), HATU (0.22 g, 0.57 mmol, 1.2 eq.) and **77a** (0.20 g, 0.52 mmol, 1.0 eq.) in dry DMF (2.4 mL) were added to afford **89** as a white solid (0.13 g, 47%) after FCC (cyclohexane/EtOAc 7:3). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 9.48 (s, 1H), 7.73 (d, *J* = 8.6 Hz, 1H), 7.56 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.42 (d, *J* = 1.7 Hz, 1H), 7.37 (t, *J* = 1.7 Hz, 1H), 6.98 (d, *J* = 1.6 Hz, 2H), 6.74 (t, *J* = 5.5 Hz, 1H), 2.85 (dd, *J* = 12.9, 6.6 Hz, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.41 (m, 2H), 1.36 (s, 9H), 1.29 (m, 2H). **^{13}C NMR (126 MHz, DMSO)** δ 172.1, 158.6, 156.0, 147.1, 135.2, 134.5, 130.8, 129.1, 126.8, 123.6, 117.2, 88.6, 77.7, 40.5, 35.9, 29.4, 28.7, 22.8. **HRMS (ESI$^-$)** *m/z* calcd. for $C_{22}H_{24}Cl_2IN_2O_4$ [*M* – *H*]$^-$: 577.01633, found: 577.01721.

***Tert*-Butyl ((1-(2-(3,5-dichlorophenoxy)-4-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)carbamate (91)**



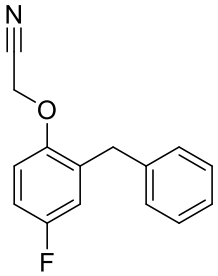
Into a solution of **77a** (0.19 g, 0.74 mmol, 1.0 eq.) and *N*-Boc-propargylamine (0.07 g, 0.47 mmol, 1.0 eq) in DMF (4.7 mL, 0.1 M), $CuSO_4.5H_2O$ (0.02 g, 0.095 mmol, 0.2 eq,) and Na-ascorbate (0.03 g, 0.14 mmol, 0.3 eq.,) were added. The reaction was stirred at rt for 24h. Then, EDTA salt and EtOAc were added and the mixture was stirred for 15 min. The mixture was washed with H_2O, aq. sat. NH_4Cl and brine and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified by FCC (cyclohexane/EtOAc 60:40) to afford **90** as a white powder (0.15 g, 58%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.25 (s, 1H), 7.79 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.57 (dd, *J* = 4.9, 3.3 Hz, 2H), 7.45 (s, 1H), 7.36 (t, *J* = 5.8 Hz, 1H), 7.25 (s, 2H), 4.20 (d, *J* = 5.7 Hz, 2H), 1.33 (s, 9H). **^{13}C NMR (126 MHz, DMSO)** δ 156.7, 155.6, 148.3, 146.1, 135.0, 134.3, 128.4, 128.2, 127.8, 124.5, 124.2, 118.2, 95.9, 77.9, 48.6, 35.4, 28.1. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{20}H_{20}Cl_2IN_4O_3$ [*M* + *H*]$^+$: 560.99571, found: 560.99536.

2-((2-Phenoxyphenyl)amino)acetonitrile (58b)



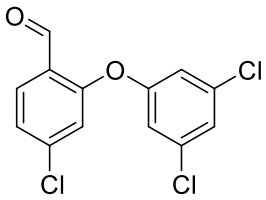
Using GPA with 2-phenoxyaniline (**58a**) (0.50 g, 2.69 mmol, 1.0 eq.) was refluxed for 16 h with anhydrous K_2CO_3 (0.373 g, 2.69 mmol, 1.0 eq.), NaI (0.081 g, 0.540 mmol, 0.2 eq.) and chloroacetonitrile (170 µL, 2.69 mmol, 1.0 eq.) in dry acetone (5 mL). After 16 h, the reaction was reloaded with K_2CO_3 (0.5 eq.), NaI (0.1 eq.) and chloroacetonitrile (1.0 eq.), and the mixture was refluxed for 60 h to afford FCC **58b** as a light-yellow oil, (0.20 g, 33%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.32–7.36 (m, 2H) 7.11 (td, *J* = 7.7, 1.4 Hz, 1H) 7.05–7.09 (m, 1H) 6.89 - 6.94 (m, 3H) 6.84 (dd, *J* = 7.7, 1.4 Hz, 1H) 6.73 (td, *J* = 7.7, 1.4 Hz, 1H) 5.97 (t, *J* = 6.9 Hz, 1H) 4.25 (d, *J* = 6.9 Hz, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 157.3, 143.0, 138.5, 129.8, 125.1, 122.7, 119.7, 118.5, 118.2, 117.1, 112.3, 31.5. **HRMS (ESI$^+$)** calcd. for $C_{14}H_{13}N_2O$ [*M* + H]$^+$: 225.10224, found: 225.10184.

2-(2-Benzyl-4-fluorophenoxy)acetonitrile (59b)



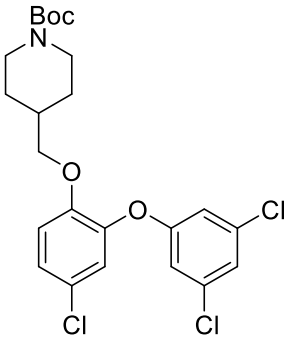
Using GPA, **62** (0.05 g, 0.25 mmol, 1.0 eq.), K_2CO_3 (0.034 g, 0.25 mmol, 1.0 eq.), NaI (0.007 g, 0.05 mmol, 20 mol%) and chloroacetonitrile (20 µL, 0.25 mmol, 1.0 eq.) in dry acetone (0.5 mL) were refluxed overnight to afford **59b** after FCC as a colourless oil, (0.045 g, 76%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.26–7.30 (m, 2H), 7.21–7.24 (m, 2H), 7.17–7.21 (m, 1H), 7.13–7.15 (m, 1H) 7.10–7.13 (m, 2H), 7.06–7.10 (m, 1H), 5.18 (s, 2H), 3.91 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 157.7, 150.8, 140.2, 132.8, 129.1, 128.9, 126.6, 117.7, 117.1, 114.5, 114.1, 54.8, 35.4. **^{19}F NMR (DMSO-*d*$_6$, 470 MHz,)** δ –121.37 (s). **HRMS (ESI$^+$)** *m/z* calcd. for $C_{15}H_{12}FNO$ [*M* – H]$^-$: 240.08301, found: 240.08250.

4-Chloro-2-(3,5-dichlorophenoxy)benzaldehyde (69a)



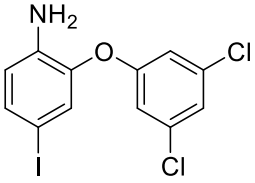
To a stirred solution of 4-chloro-2-fluorobenzaldehyde (0.50 g, 3.15 mmol, 1.0 eq.) and 3,5-dichlorophenol (0.58 g, 3.47 mmol, 1.1 eq.) in DMSO (6.5 mL), K_2CO_3 (0.52 g, 3.78 mmol, 1.2 eq.) was added. The mixture was stirred overnight at 100 °C. The reaction mixture was cooled down to rt and quenched with aq. 1 M HCl (15 mL) and extracted with diethyl ether (2 x 20 mL). The combined organic layers were washed with sat. aq. NaCl solution (20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–20% acetone in hexane to afford **69a** as a white sticky solid, (0.743 g, 78%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 10.23 (s, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.50 (t, *J* = 1.8 Hz, 1H), 7.44–7.48 (m, 1H), 7.32 (d, *J* = 1.7 Hz, 2H), 7.27 (d, *J* = 1.8 Hz, 1H). **^{13}C NMR (DMSO- *d*$_6$, 126 MHz)** δ 188.1, 158.0, 157.3, 140.5, 135.1, 130.6, 125.7, 125.3, 124.5, 119.8, 118.4. **LCMS** *m/z* (ESI$^+$) calcd. for $C_{13}H_8Cl_3O_2$ [*M* – H]$^-$: 300.5, found 300.9.

***Tert*-Butyl 4-((4-chloro-2-(3,5-dichlorophenoxy)phenoxy)methyl)piperidine-1-carboxylate (71a)**



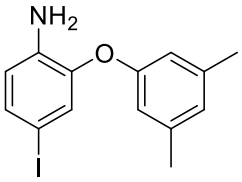
Into a solution of 4-chloro-2-(3,5-dichlorophenoxy)phenol **69** (0.11 g, 0.40 mmol, 1.0 eq.) in dry DMF (4 mL), K_2CO_3 (0.16 g, 1.2 mmol, 3.0 eq.), *tert*-butyl 4-(chloromethyl)piperidine-1-carboxylate (0.112 g, 0.480 mmol, 1.2 eq.) and KI (0.133 g, 0.80 mmol, 2.0 eq.) were added. The mixture was stirred at 80 °C for 4 h followed by an addition of *tert*-butyl 4-(chloromethyl)piperidine-1-carboxylate (0.094 g, 0.40 mmol, 1.0 eq.). The reaction was stirred overnight followed by another addition of *tert*-butyl 4-(chloromethyl)piperidine-1-carboxylate (0.019 g, 0.080 mmol, 0.2 eq.) with a total reaction time of 30 h. The crude was absorbed onto silica 0.063–0.200 mm and purified by FCC with an alternating gradient of 0–10% EtOAc in cyclohexane to afford **71a** as a colourless oil, (0.10 g, 51%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.24–7.29 (m, 3H), 7.07 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.89 (d, *J* = 1.7 Hz, 2H), 3.86 (br d, *J* = 6.3 Hz, 5H), 3.54 (d, *J* = 6.3 Hz, 1H), 1.63–1.73 (m, 2H), 1.36–1.38 (m, 9H), 1.09 (m, 1H), 0.89 (br dd, *J* = 12.3, 3.9 Hz, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 159.3, 153.8, 153.7, 151.1, 141.1, 134.7, 130.5, 123.7, 122.0, 121.0, 114.9, 114.8, 114.3, 78.6, 78.5, 72.5, 49.8, 37.8, 35.0, 28.4, 28.1. **LCMS** *m/z* (ESI$^+$) calcd. for $C_{23}H_{27}Cl_3NO_4$ [*M* + H]$^+$: 486.1, found 386.1 without Boc-group.

2-(3,5-Dichlorophenoxy)-4-iodoaniline (77a)



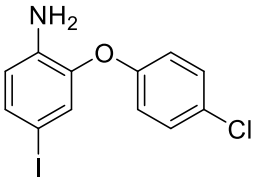
Using GPE, **77b** (0.70 g, 1.7 mmol, 1.0 eq.), Fe (0.47 g, 8.55 mmol, 5.0 eq.) and NH_4Cl (0.09 g, 1.71 mmol, 1.0 eq.) were mixed in EtOH/H_2O (18 mL) to afford **77a** as a crude brown solid (0.60 g, 92%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.27–7.30 (m, 2H), 7.20–7.22 (m, 1H), 6.87 (d, *J* = 1.8 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 1H), 5.30 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 158.8, 141.0, 140.6, 134.9, 134.6, 129.4, 122.0, 118.2, 115.3, 75.0. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{12}H_9Cl_2INO$ [*M* + H]$^+$: 379.91004, found: 379.90918.

2-(3,5-Dimethylphenoxy)-4-iodoaniline (78a)



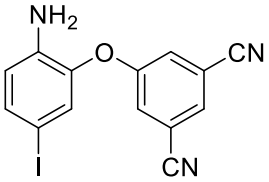
Using GPE, 2-(3,5-dimethylphenoxy)-4-iodo-1-nitrobenzene **78b** (1.5 g, 4.06 mmol, 1.0 eq.), Fe (1.13 g, 20.31 mmol, 5.0 eq.) and NH_4Cl (0.22 g, 4.06 mmol, 1.0 eq.) were mixed in EtOH/H_2O (27/13 mL) to afford **78a** as white solid (1.2 g, 87%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.17 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.94 (d, *J* = 1.9 Hz, 1H), 6.72 (s, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 6.53 (s, 2H), 5.11 (s, 2H), 2.23 (s, 6H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 156.9, 143.1, 140.5, 139.1, 133.1, 127.7, 124.5, 117.7, 114.8, 75.1, 20.9. **LCMS** MS (ESI+): *m/z* = 340 $[M - H]^+$.

2-(4-Chlorophenoxy)-4-iodoaniline (79a)



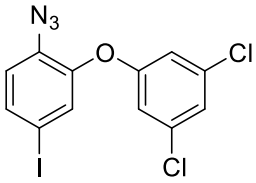
Using GPE, 2-(4-chlorophenoxy)-4-iodo-1-nitrobenzene **79b** (0.70 g, 1.86 mmol, 1.0 eq.), Fe (0.52 g, 9.3 mmol, 5.0 eq.) and NH_4Cl (0.10 g, 1.86 mmol, 1.0 eq.) were mixed in EtOH/H_2O (6/12 mL) to afford **79a** as a white solid (0.40 g, 62%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.39 (m, 2H), 7.22 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.04 (d, *J* = 1.9 Hz, 1H), 6.92 (m, 2H), 6.64 (d, *J* = 8.4 Hz, 1H), 5.21 (s, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 156.0, 142.3, 140.7, 133.8, 129.6, 128.3, 126.3, 118.5, 117.9, 74.9. **LCMS** MS (ESI^+): *m/z* = 346 $[M - H]^+$.

5-(2-Amino-5-iodophenoxy)isophthalonitrile (80a)



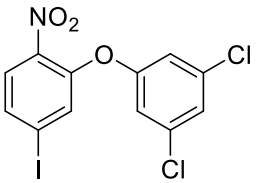
Using GPE, 5-(5-iodo-2-nitrophenoxy)isophthalonitrile **80b** (0.35 g, 0.89 mmol, 1.0 eq.), Fe (0.25 g, 4.45 mmol, 5.0 eq.) and NH_4Cl (0.05 g, 0.89 mmol, 1.0 eq.) in EtOH/H_2O (6/3 mL) were mixed to afford **80a** as a white powder (0.30 g, 93%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.15 (s, 1H), 7.65 (d, *J* = 1.0 Hz, 2H), 7.30 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.25 (d, *J* = 1.9 Hz, 1H), 6.67 (d, *J* = 8.5 Hz, 1H), 5.33 (s, 2H). **^{13}C NMR (126 MHz, DMSO)** δ 157.7, 140.8, 140.2, 135.1, 129.8, 129.3, 124.5, 118.3, 116.8, 113.8, 75.2. **LCMS** MS (ESI^+): *m/z* = 362 $[M - H]^+$.

1-Azido-2-(3,5-dichlorophenoxy)-4-iodobenzene (82a)



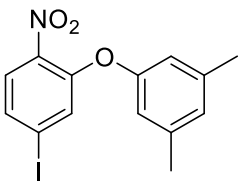
Into a solution of 2-(3,5-dichlorophenoxy)-4-iodoaniline **77a** (0,14 g, 0.47 mmol, 1.0 eq.) in ACN (4.7 mL, 0.1 M), *t*BuONO (0.100 g, 0.95 mmol, 2.0 eq.) and of $TMSN_3$ (0.100 g, 0.95 mmol, 2.0 eq.) were added at 0 °C. The reaction was stirred at rt for 5 h and after evaporation, the crude mixture was used directly in the next step.

2-(3,5-Dichlorophenoxy)-4-iodo-1-nitrobenzene (77b)



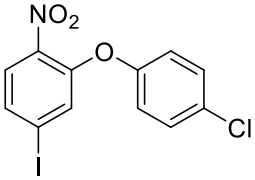
Using GPF, 3,5-dichlorophenol (0.30 g, 1.87 mmol, 1.0 eq.), K_2CO_3 (0.31 g, 2.24 mmol, 1.2 eq.) and 2-fluoro-4-iodo-1-nitrobenzene (0.50 g, 1.87 mmol, 1.0 eq.) in DMSO (9.35 mL) were used to afford **77b** as crude yellow powder, (0.70 g, 91%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.85–7.90 (m, 2H), 7.77 (d, *J* =1.4 Hz, 1H), 7.46 (t, *J* = 1.8 Hz, 1H), 7.22 (d, *J* = 1.8 Hz, 2H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 157.4, 147.8, 141.0, 135.1, 134.8, 131.0, 127.4, 124.1, 117.2, 103.1.

2-(3,5-Dimethylphenoxy)-4-iodo-1-nitrobenzene (78b)



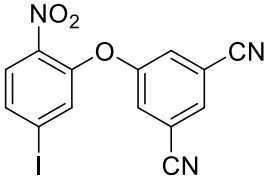
Using GPF, 3,5-dimethylphenol (0.50 g, 4.09 mmol, 1.0 eq.), K_2CO_3 (0.67 g, 4.91 mmol, 1.2 eq.) and 2-fluoro-4-iodo-1-nitrobenzene (1.09 g, 4.09 mmol, 1.0 eq.) in DMSO (8.2 mL) were used to afford **78b** as crude yellow powder (1.5 g, 99%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.82 (d, *J* = 8.5 Hz, 1H), 7.72 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.37 (d, *J* = 1.6 Hz, 1H), 6.88 (s, 1H), 6.72 (s, 2H), 2.27 (s, 6H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 155.0, 149.8, 140.5, 139.9, 132.7, 128.8, 127.0, 126.5, 116.4, 102.3.

2-(4-Chlorophenoxy)-4-iodo-1-nitrobenzene (77b)



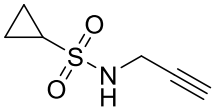
Using GPF, 4-chlorophenol (0.24 g, 1.87 mmol, 1.0 eq.), K_2CO_3 (0.31 g, 2.24 mmol, 1.2 eq.) and 2-fluoro-4-iodo-1-nitrobenzene (1.09 g, 4.09 mmol, 2.2 eq.) in DMSO (3.74 mL) were used to afford **77b** as crude light-yellow powder, (0.70 g, 99%). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.86 (d, *J* = 8.5 Hz, 1H), 7.79 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.54 (d, *J* = 1.4 Hz, 1H), 7.49 (d, *J* = 8.9 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 1H). **^{13}C NMR (DMSO-*d*$_6$, 126 MHz)** δ 154.4, 149.1, 140.7, 133.6, 130.3, 129.6, 128.6, 127.2, 120.5, 102.8.

5-(5-Iodo-2-nitrophenoxy)isophthalonitrile (80b)



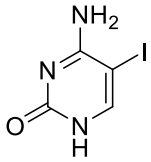
Using GPF, 5-hydroxyisophthalonitrile (0.33 g, 2.25 mmol, 1.0 eq.), K_2CO_3 (0.37 g, 2.7 mmol, 1.2 eq.) and 2-fluoro-4-iodo-1-nitrobenzene (0.60 g, 2.25 mmol, 1.0 eq.) in DMSO (4.5 mL) were used to afford **80b** as crude light-yellow powder, (0.35 g, 40%). **^{1}H NMR (500 MHz, DMSO-*d*$_6$)** δ 8.29 (t, *J* = 1.2 Hz, 1H), 8.05 (d, *J* = 1.2 Hz, 2H), 7.92 (s, *J* = 10.0 Hz, 2H), 7.84 (s, 1H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 156.8, 147.4, 141.1, 135.3, 131.6, 131.4, 127.5, 126.3, 116.5, 114.3, 103.5.

***N*-(Prop-2-yn-1-yl)cyclopropanesulfonamide (92b)**



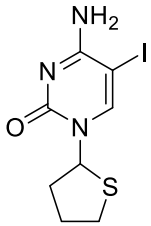
To a solution of propargylamine (0.25 mL, 3.9 mmol, 1.1 eq.) in dry DCM (3.5 mL, 1M) and TEA (0.54 mL, 3.9 mmol, 1.1 eq.), cyclopropanesulfonyl chloride (0.36 mL, 3.55 mmol, 1.0 eq.) was added dropwise at 0 °C and the reaction is stirred at rt for 1h. The reaction mixture was dried under vacuum and purified *via* FCC (product at 20% of ACN+0.1%FA) to afford **86b** as white solid (0.37 g, 66 %). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 7.58 (t, *J* = 5.4 Hz, 1H), 3.80 (dd, *J* = 5.7, 2.3 Hz, 2H), 3.26 (t, *J* = 2.4 Hz, 1H), 2.57 (tt, *J* = 7.7, 5.1 Hz, 1H), 0.99 – 0.90 (m, 4H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 80.7, 74.4, 31.8, 29.8, 5.0.

4-Amino-5-iodopyrimidin-2(1*H*)-one (92a)



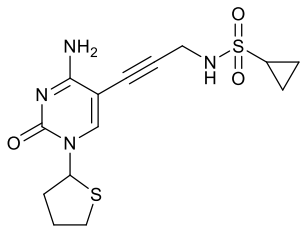
Cytosine (1.0 g, 9 mmol, 10 eq.), iodine (3.43 g, 13.5 mmol, 1.5 eq) and iodic acid (2.21 g, 12.6 mmol, 1.4 eq.) were stirred in acetic acid (30 mL, 0.3 M) at 40°C overnight. Then, the mixture was cooled and treated with $Na_2S_2O_3$ (20 mL) until a white suspension in obtained. The mixture was then neutralised with NaOH 5M. The resulting white solid was collected by filtration and dried under vacuum to give **86a** as white solid (2.0 g, 94 %). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 11.19 (br, 1H), 7.83 (s, 1H), 7.75 (br, 1H), 6.80 (br, 1H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 163.8, 154.6, 149.9, 55.2. **HRMS (ESI$^+$)** *m/z* calcd. for $C_4H_5IN_3O$ [*M* + H]$^+$: 237.94718, found: 237.94597.

4-Amino-5-iodo-1-(tetrahydrothiophen-2-yl)pyrimidin-2(1*H*)-one (93)



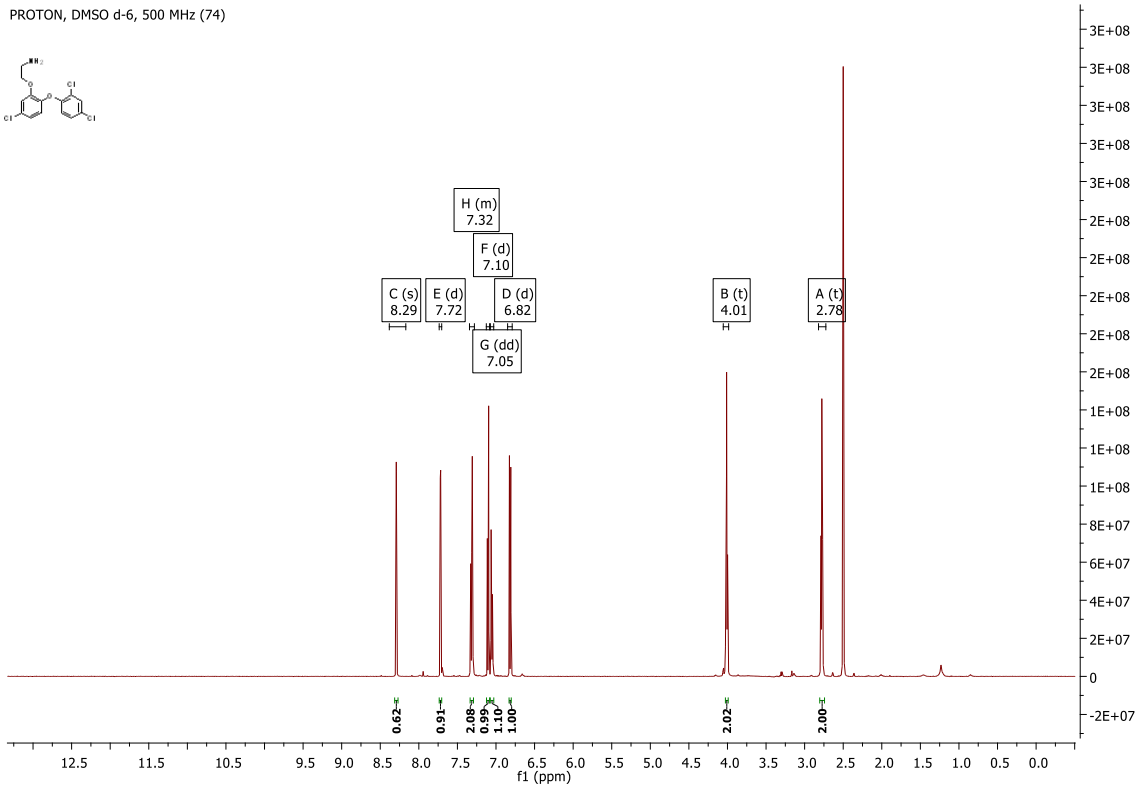
To a suspension of **86a** (0.30 g, 1.26 mmol, 1.0 eq) in a mixture toluene/DMF (2:1, 0.1 M, 12.6 mL), was added triethylamine (0.70 mL, 5.06 mmol, 4 eq) and TMSOTf (1.8 mL, 10.08 mmol, 8eq), and the mixture was stirred at rt for 15 min. Then, tetrahydrothiophene 1-oxide (0.11 mL, 1.26 mmol, 1 eq) was added dropwise followed by an additional amount of triethylamine (1.05 mL, 7.56 mmol, 6eq) to initiate the Pummerer reaction. After the mixture was stirred at rt for 30 min, the reaction was quenched by addition of ice and, it was partitioned between EtOAc and sat. aq. $NaHCO_3$ (3 x 10 mL). The crude product was purified by preparative HPLC (product at 30% ACN+0.1%FA) to afford **86** as light-brown solid (0.096 g, 24 %). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.15 (s, 1H), 7.84 (s, 1H), 6.64 (s, 1H), 6.12 (dd, *J* = 6.6, 5.2 Hz, 1H), 3.24 (dt, *J* = 10.0, 6.4 Hz, 1H), 2.85 (dt, *J* = 10.1, 6.3 Hz, 1H), 2.25 – 2.14 (m, 1H), 2.09 – 1.93 (m, 3H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 163.5, 154.1, 147.9, 64.0, 56.8, 36.7, 32.8, 29.1. **HRMS (ESI$^+$)** *m/z* calcd. for $C_8H_{11}IN_3OS$ [*M* + H]$^+$: 323.96620, found: 323.96448.

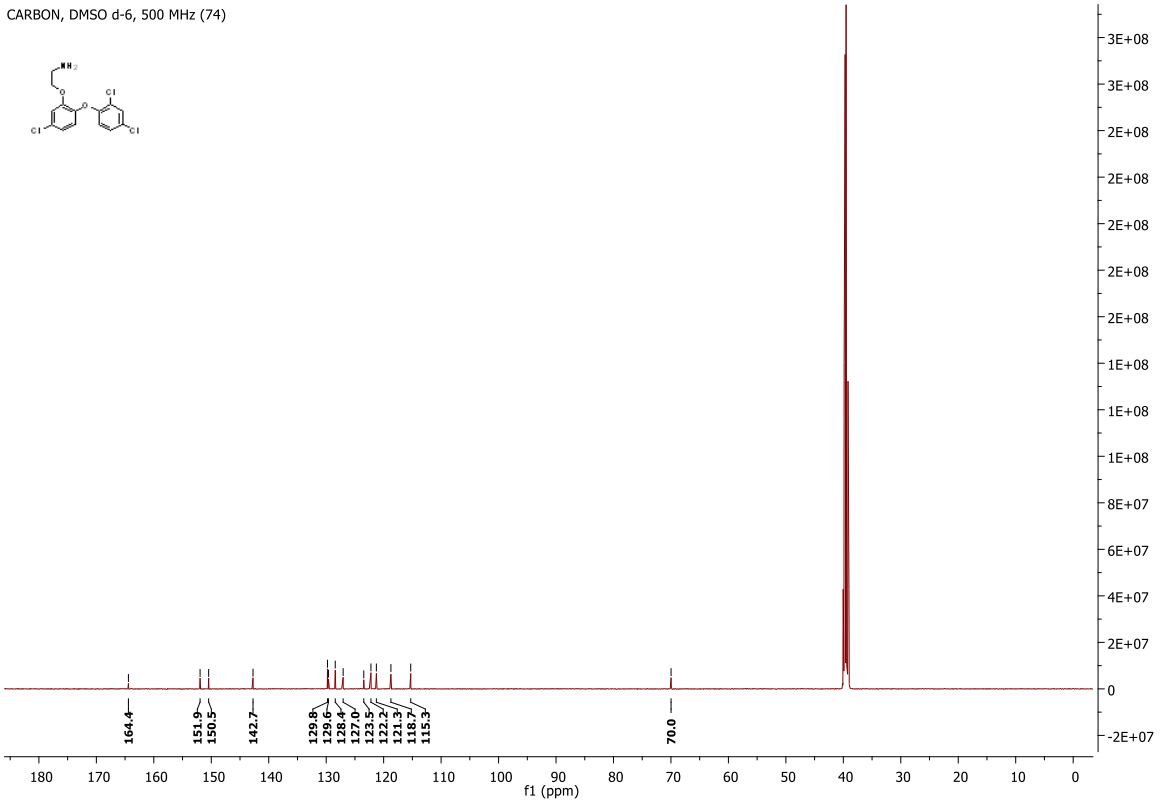
***N*-(3-(4-Amino-2-oxo-1-(tetrahydrothiophen-2-yl)-1,2-dihydropyrimidin-5-yl)prop-2-yn-1-yl)cyclopropanesulfonamide ± (92)**



To an air-degassed Schlenk flask, **93** (0.035 g, 0.12 mmol, 1.0 eq.), **92b** (0.039 g, 0.25 mmol, 2.0 eq.), TEA (0.05 mL, 0.36 mmol, 3.0 eq.), $PdCl_2(PPh_3)_2$ (0.008 g, 0.012 mmol, 0.1 eq.) and CuI (0.004 g, 0.024 mmol, 0.2 eq.) in dry DMF (1.2 mL, 0.1M) were stirred at rt on under N_2 atmosphere. Then, the mixture was filtered on a bed of celite and washed with EtOAc. The filtrate was evaporated to dryness to afford a dark brown residue that was purified by preparative HPLC (product at 35% ACN+0.1%FA) to afford **92** as white powder (0.028 g, 67 %). **^{1}H NMR (DMSO-*d*$_6$, 500 MHz)** δ 8.11 (s, 1H), 7.81 (br. s, 1H), 7.57 (t, *J* = 5.6 Hz, 1H), 6.85 (br. s, 1H), 6.14 (dd, J = 6.7, 4.8 Hz, 1H), 4.06 (d, *J* = 5.7 Hz, 2H), 3.23 (dt, *J* = 10.3, 6.3 Hz, 1H), 2.85 (dt, *J* = 10.0, 6.5 Hz, 1H), 2.69 – 2.62 (m, 1H), 2.19 (td, *J* = 12.9, 6.2 Hz, 1H), 2.07 – 1.91 (m, 3H), 0.98 – 0.93 (m, 4H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 164.1, 153.8, 145.2, 91.6, 89.4, 75.3, 64.3, 36.8, 33.0, 32.8, 29.5, 28.9, 5.0. **HRMS (ESI$^+$)** *m/z* calcd. for $C_{14}H_{19}N_4O_3S_2$ $[M + H]^+$: 355.08931, found: 355.08755.

6.4.3 ^{1}H Proton and ^{13}C spectra as well as LC-MS traces of some representative compounds

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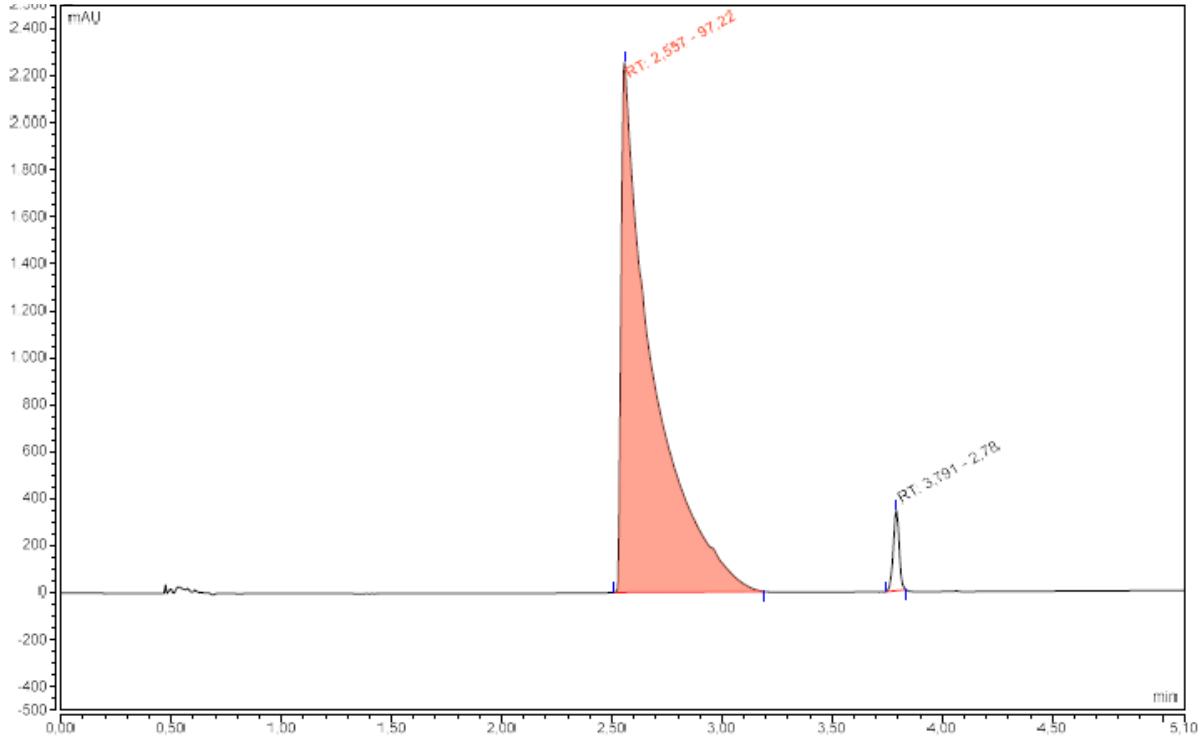
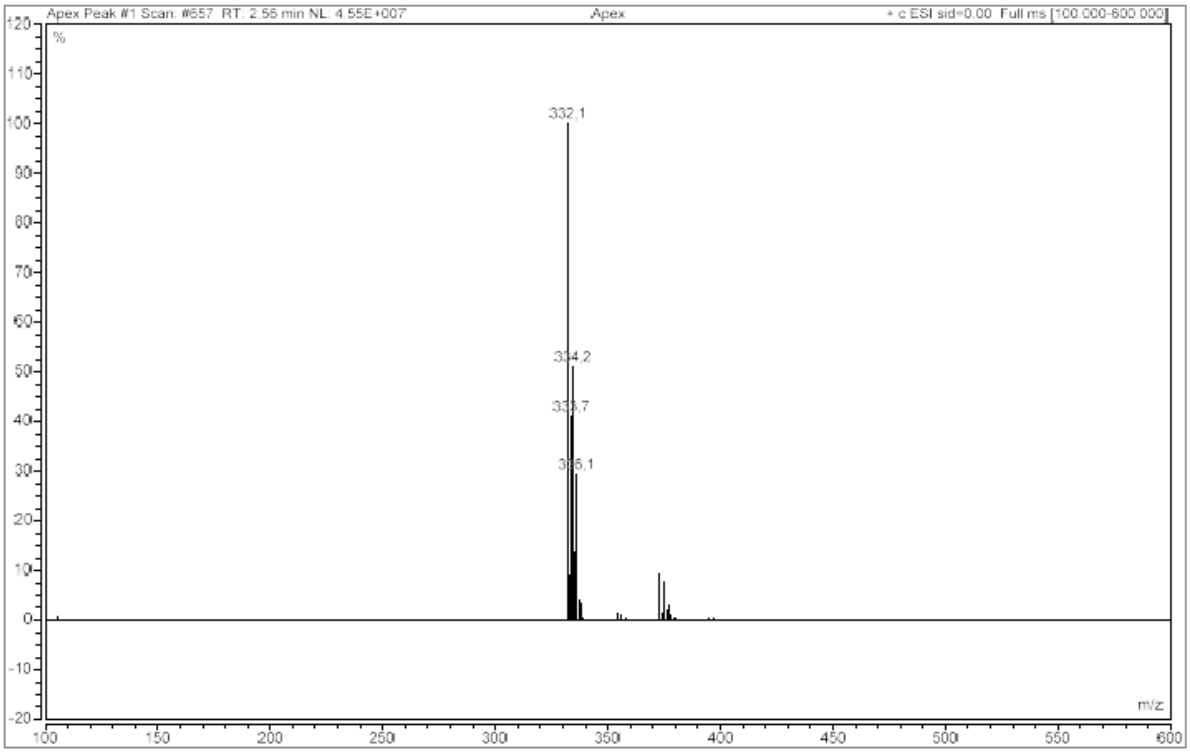
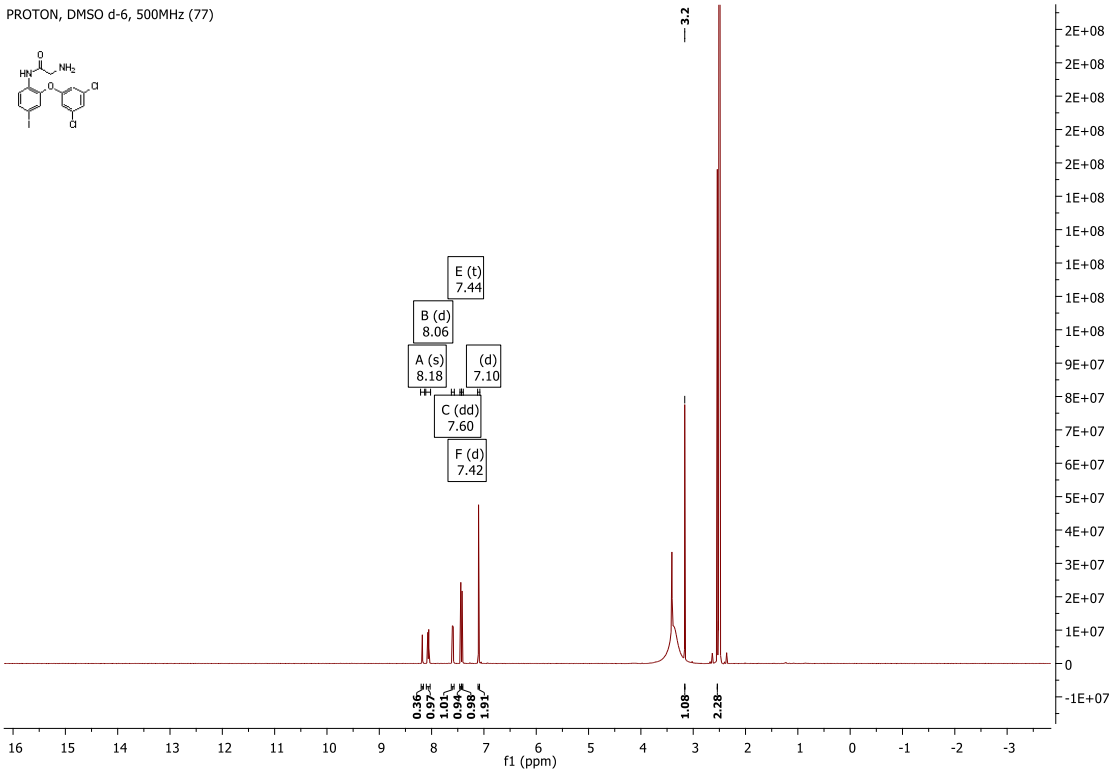
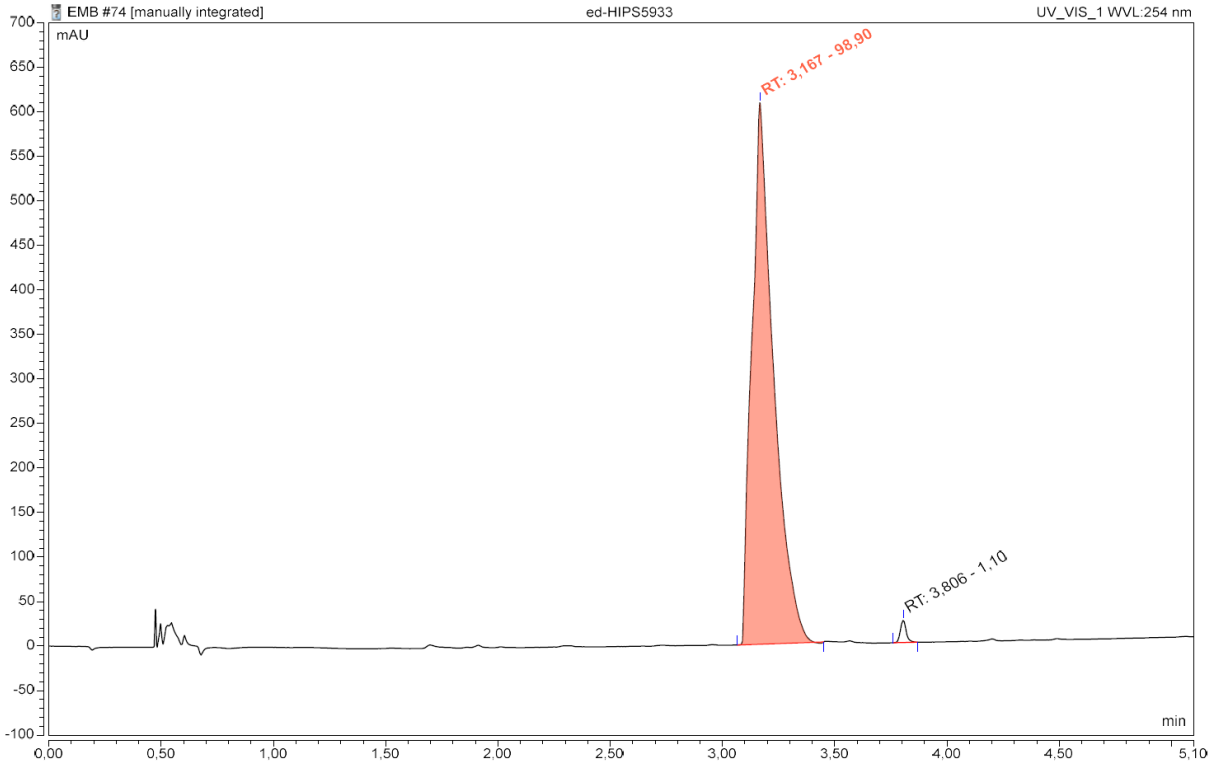
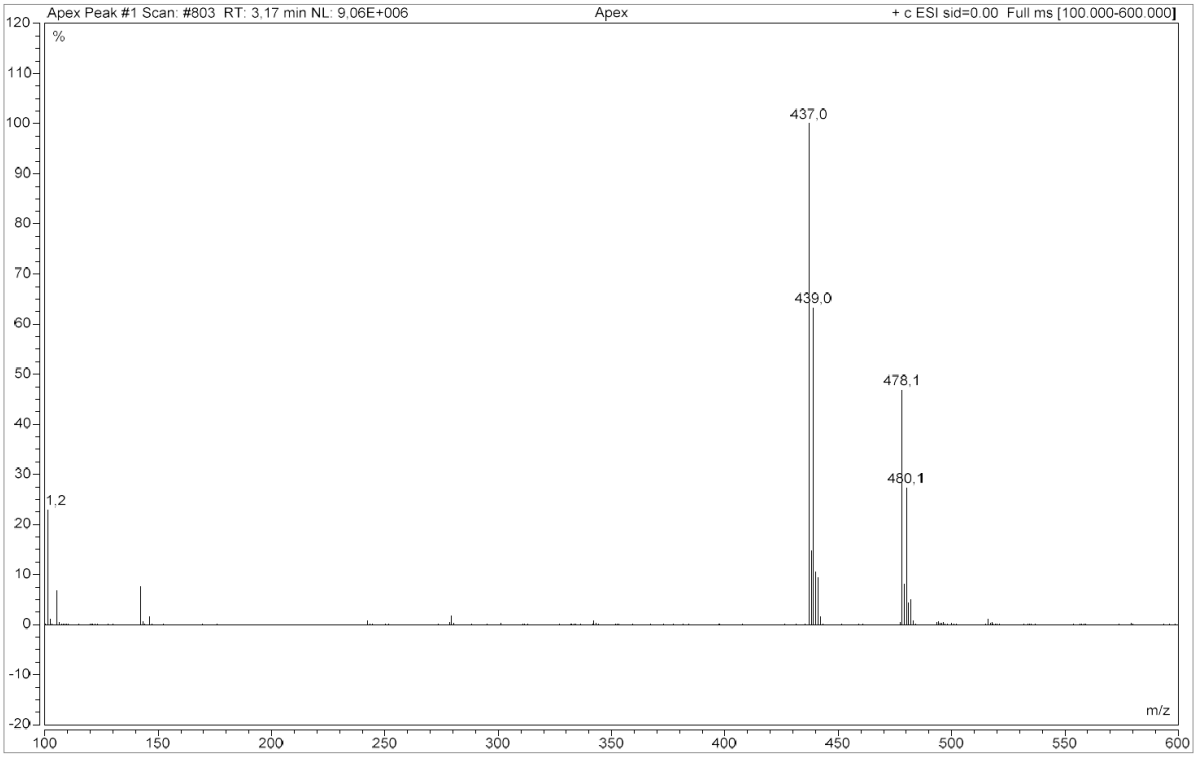
164.4
151.9
150.5
142.7
129.8
129.6
128.4
127.0
123.5
122.2
121.3
118.7
115.3
70.0
f1 (ppm)

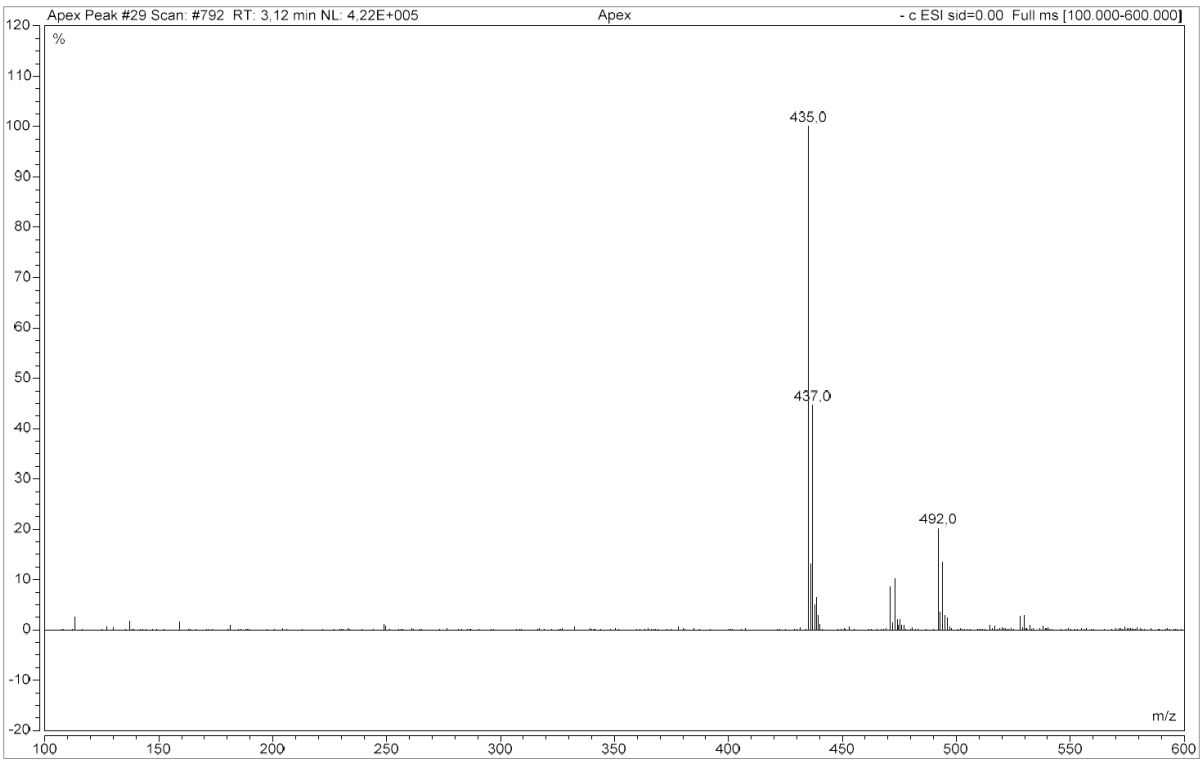

mAU
RT. 2.587 - 97.22
RT. 3.791 - 2.78
min

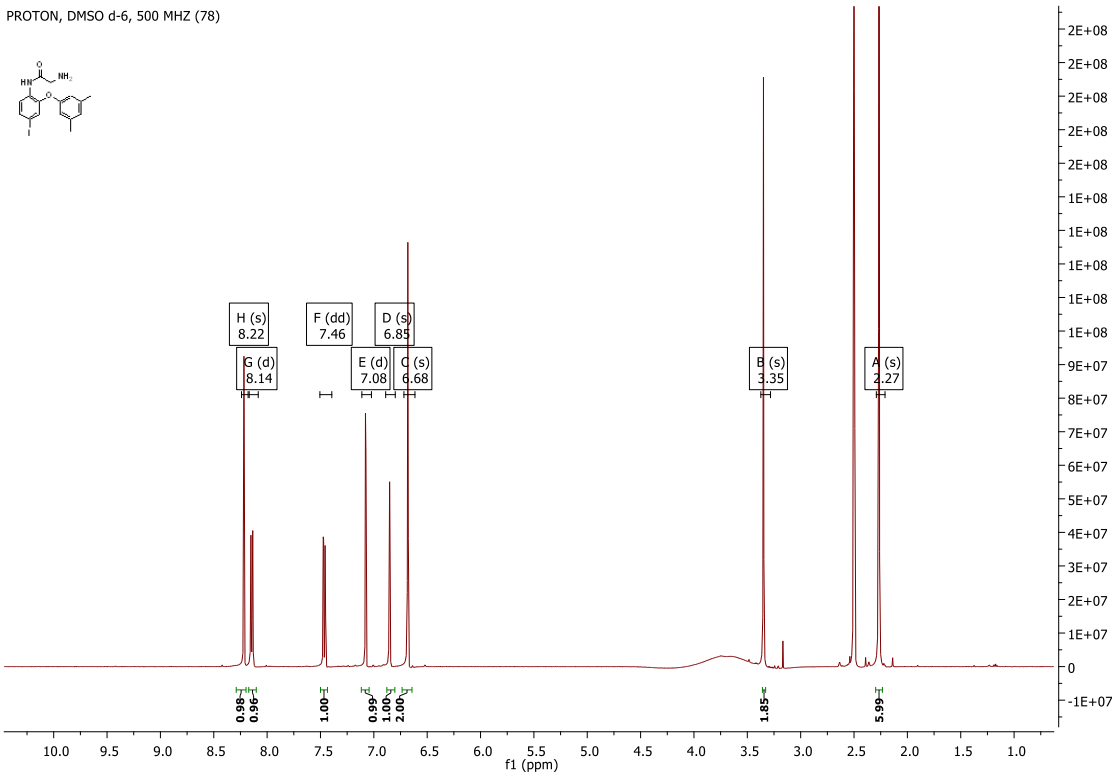

Apex Peak #1 Scan: #657 RT: 2.56 min NL: 4.55E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
332.1
334.2
333.7
335.1
m/z

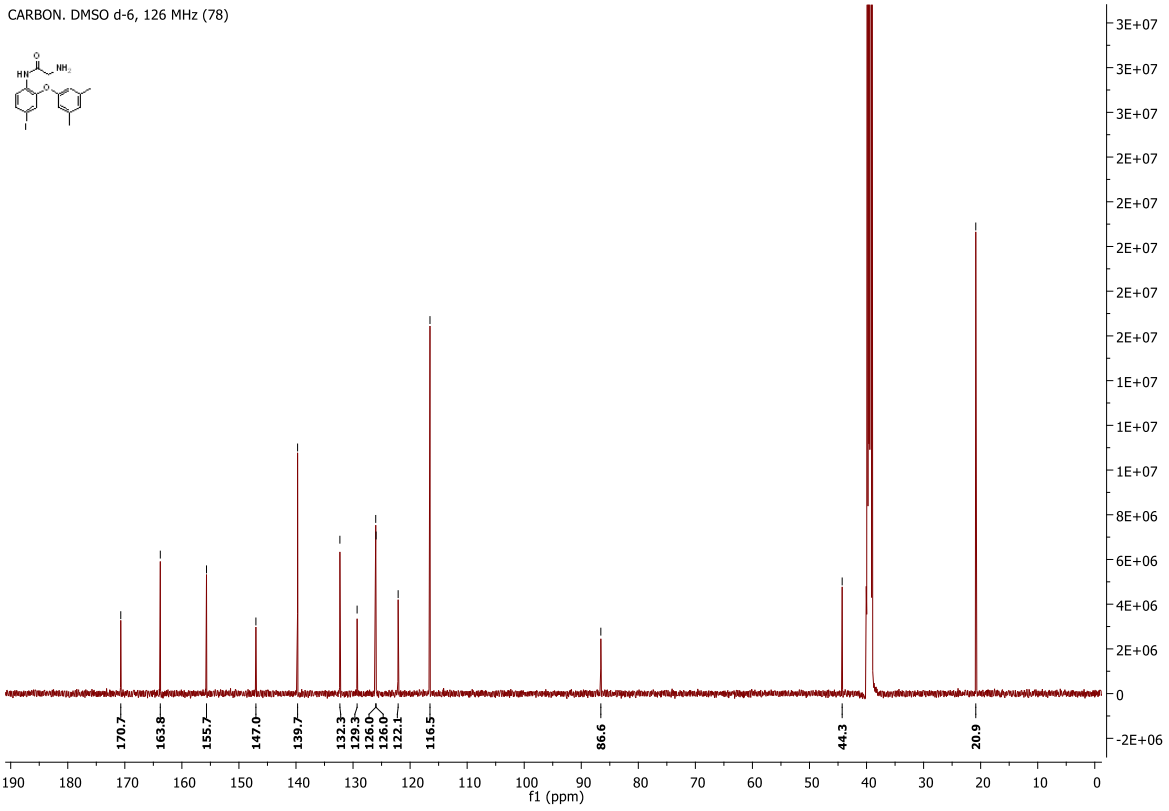

PROTON, DMSO d-6, 500MHz (77)
E (t) 7.44
B (d) 8.06
A (s) 8.18
(d) 7.10
C (dd) 7.60
F (d) 7.42
3.2
0.36
0.97
1.01
0.94
0.98
1.91
1.08
2.28
f1 (ppm)

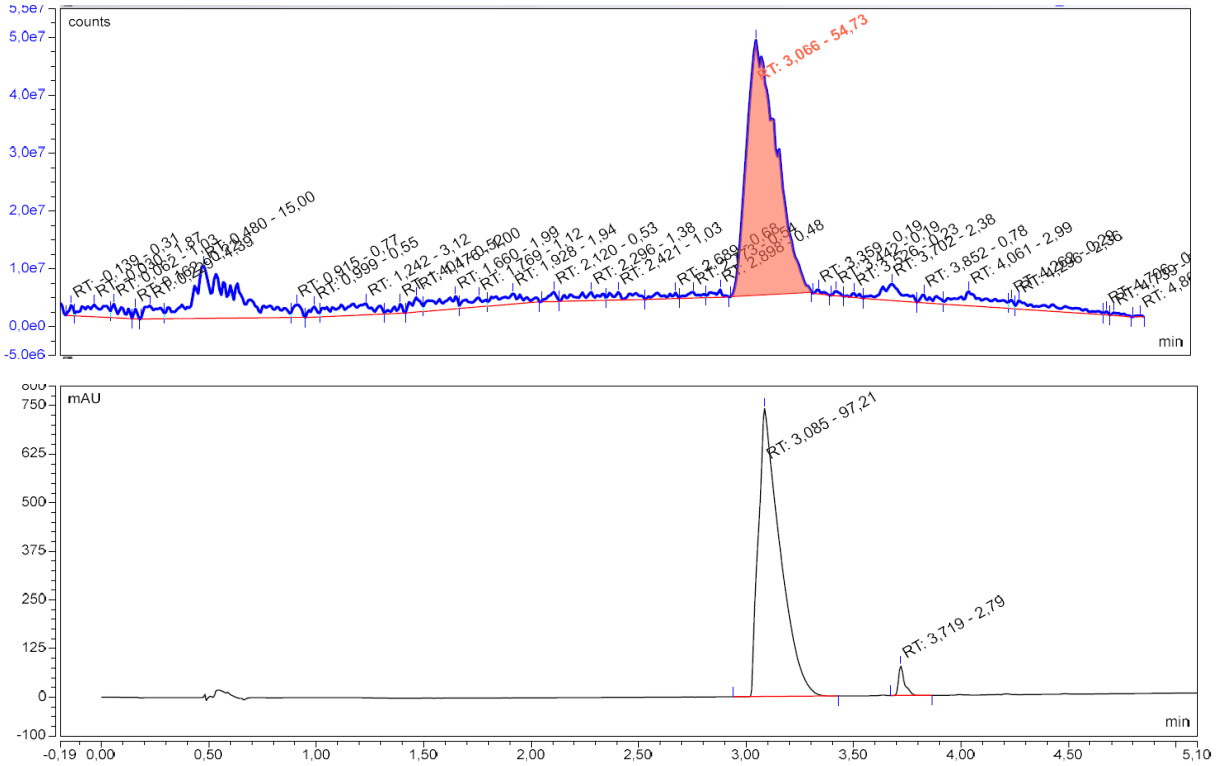

EMB #74 [manually integrated]
ed-HIPS5933
UV_VIS_1 WVL:254 nm
mAU
RT: 3,167 - 98,90
RT: 3,806 - 1,10
min

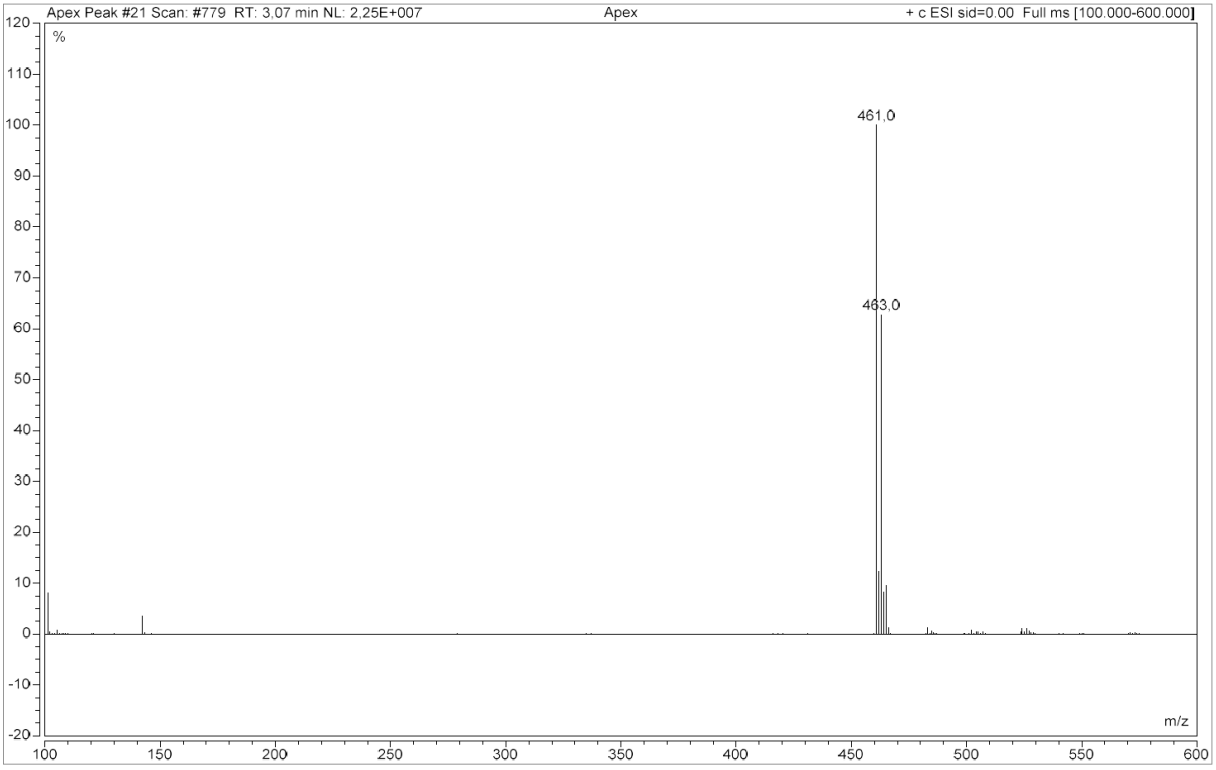

Apex Peak #1 Scan: #803 RT: 3,17 min NL: 9,06E+006
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
437,0
439,0
478,1
480,1
1,2
m/z

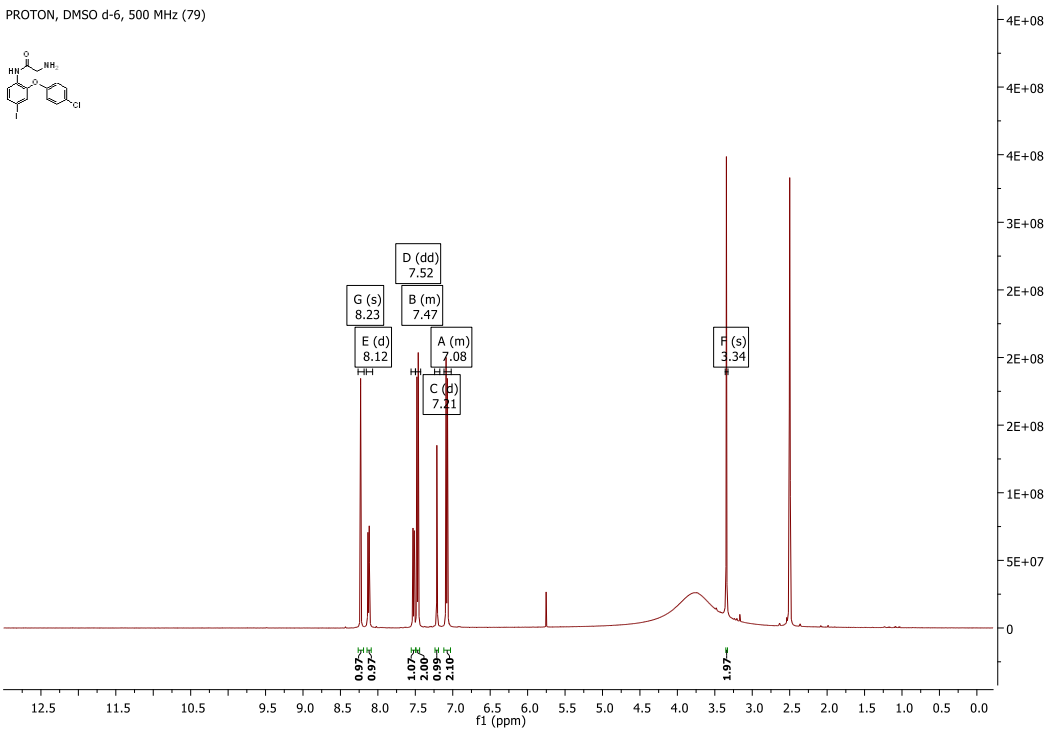

Apex Peak #29 Scan: #792 RT: 3,12 min NL: 4,22E+005
Apex
- c ESI sid=0.00 Full ms [100.000-600.000]
%
435.0
437.0
492.0
m/z
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
100
150
200
250
300
350
400
450
500
550
600

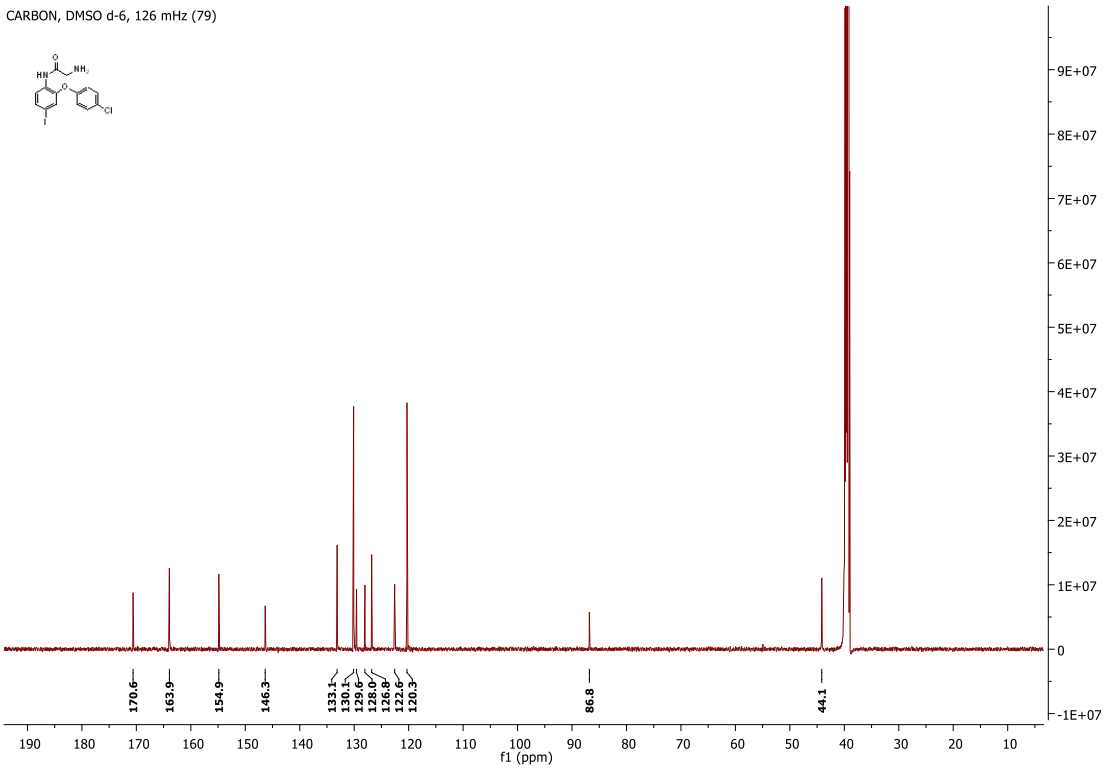

PROTON, DMSO d-6, 500 MHZ (78)
H (s) 8.22
G (d) 8.14
F (dd) 7.46
E (d) 7.08
D (s) 6.85
C (s) 6.68
B (s) 3.35
A (s) 2.27
0.98
0.96
1.00
0.99
1.00
2.00
1.85
5.99
f1 (ppm)

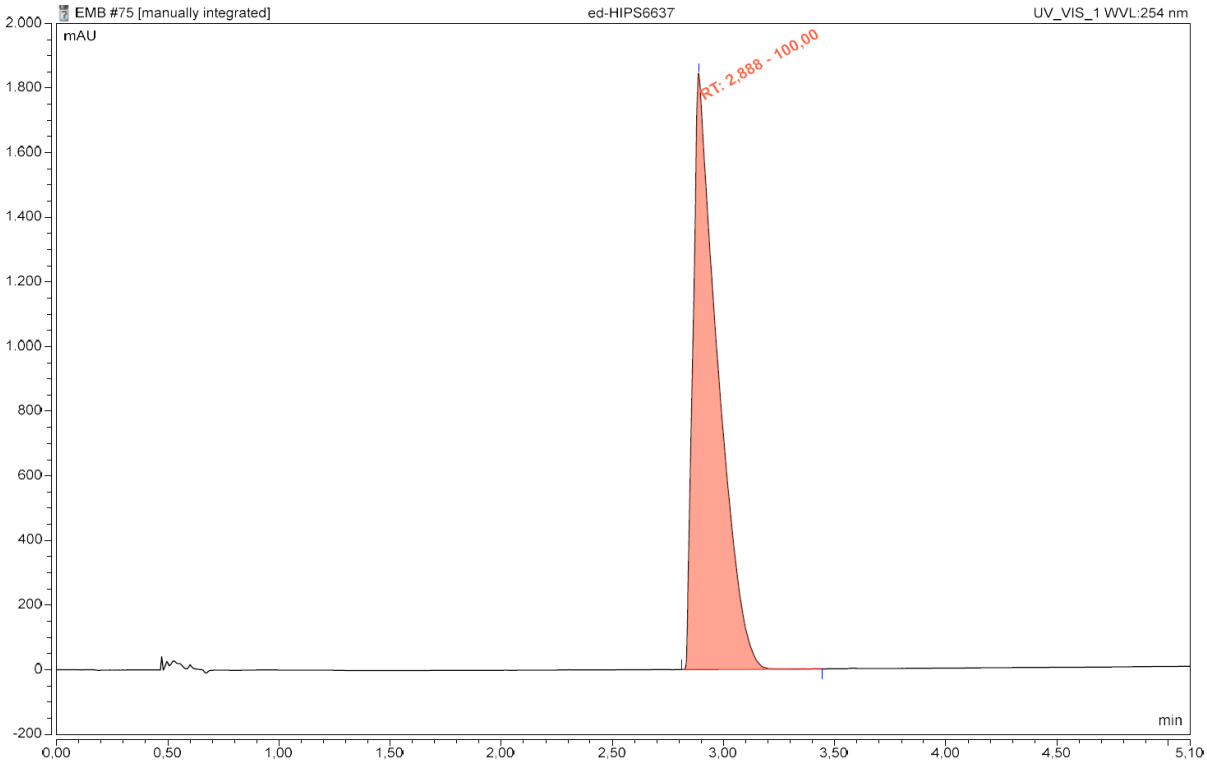

CARBON. DMSO d-6, 126 MHz (78)
170.7
163.8
155.7
147.0
139.7
132.3
129.3
126.0
126.0
122.1
116.5
86.6
44.3
20.9
f1 (ppm)

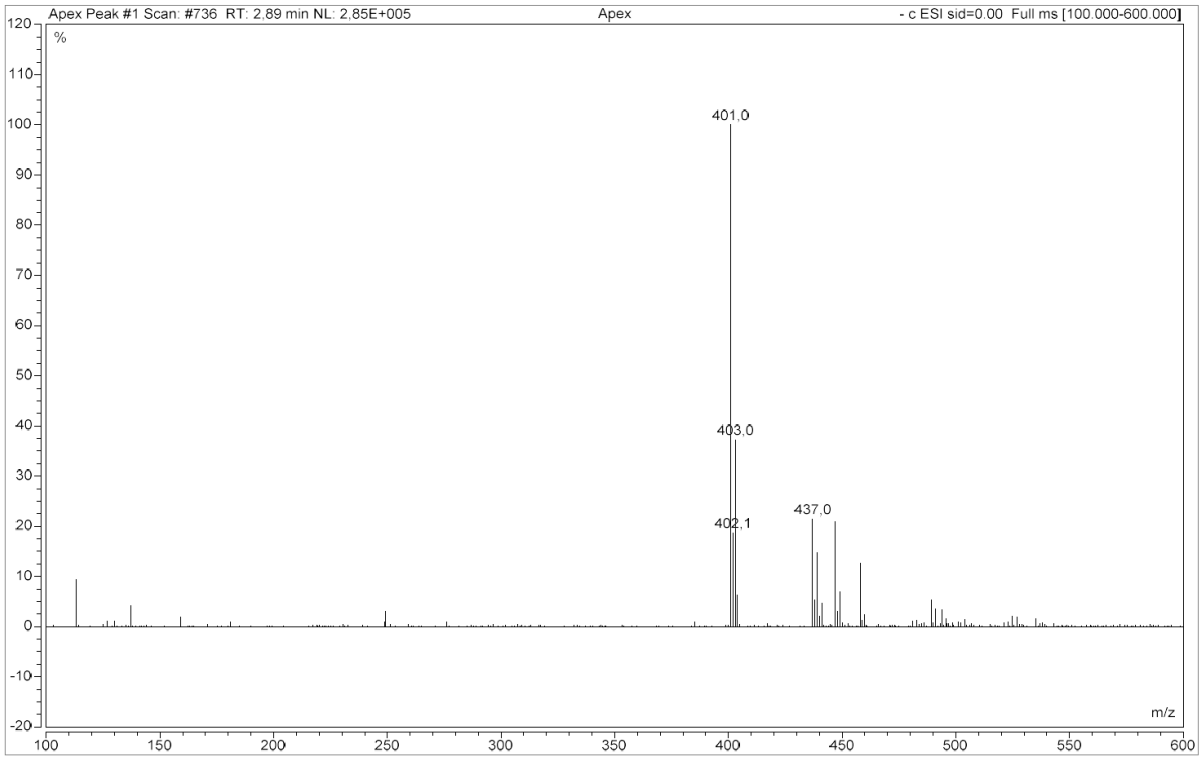

counts
RT: 3,066 - 54,73
RT: 0,480 - 15,00
RT: 0,915 - 0,77
RT: 1,242 - 3,12
RT: 1,660 - 1,99
RT: 1,769 - 1,12
RT: 1,928 - 1,94
RT: 2,120 - 0,53
RT: 2,296 - 1,38
RT: 2,421 - 1,03
RT: 3,359 - 0,19
RT: 3,526 - 0,19
RT: 3,702 - 2,38
RT: 3,852 - 0,78
RT: 4,061 - 2,99
min
mAU
RT: 3,085 - 97,21
RT: 3,719 - 2,79
min

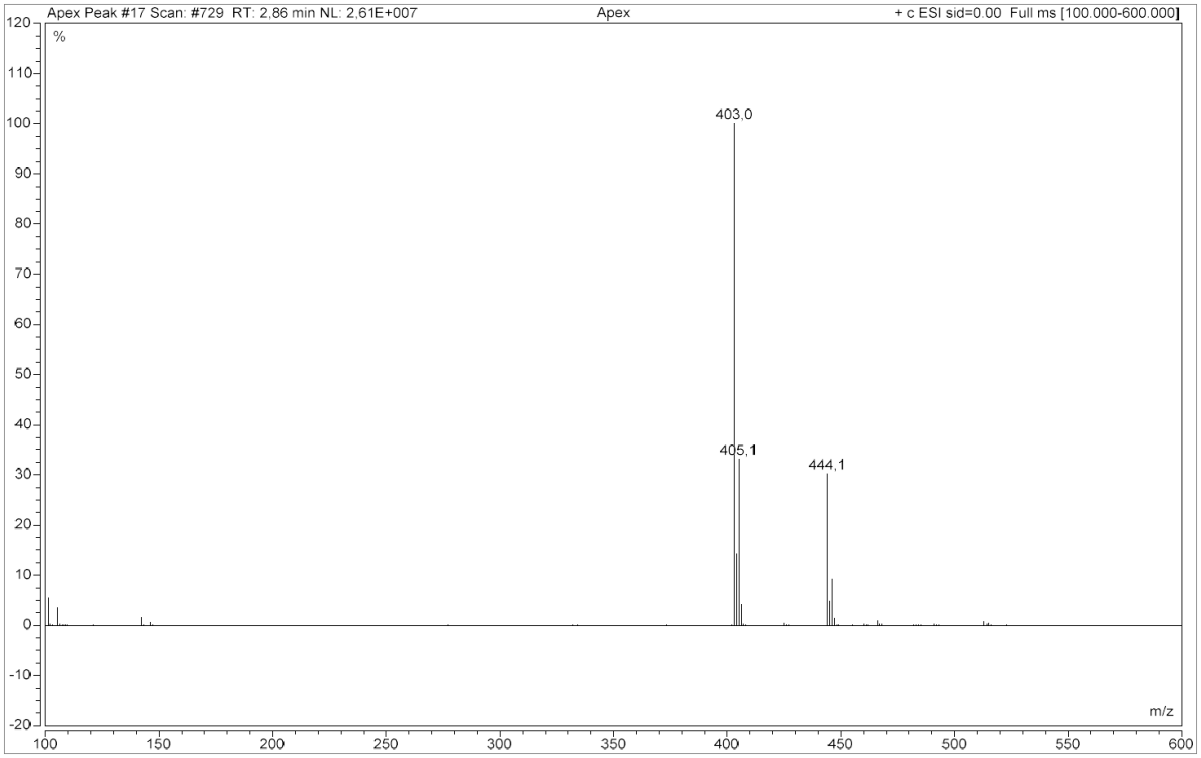

Apex Peak #21 Scan: #779 RT: 3,07 min NL: 2,25E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
461,0
463,0
m/z

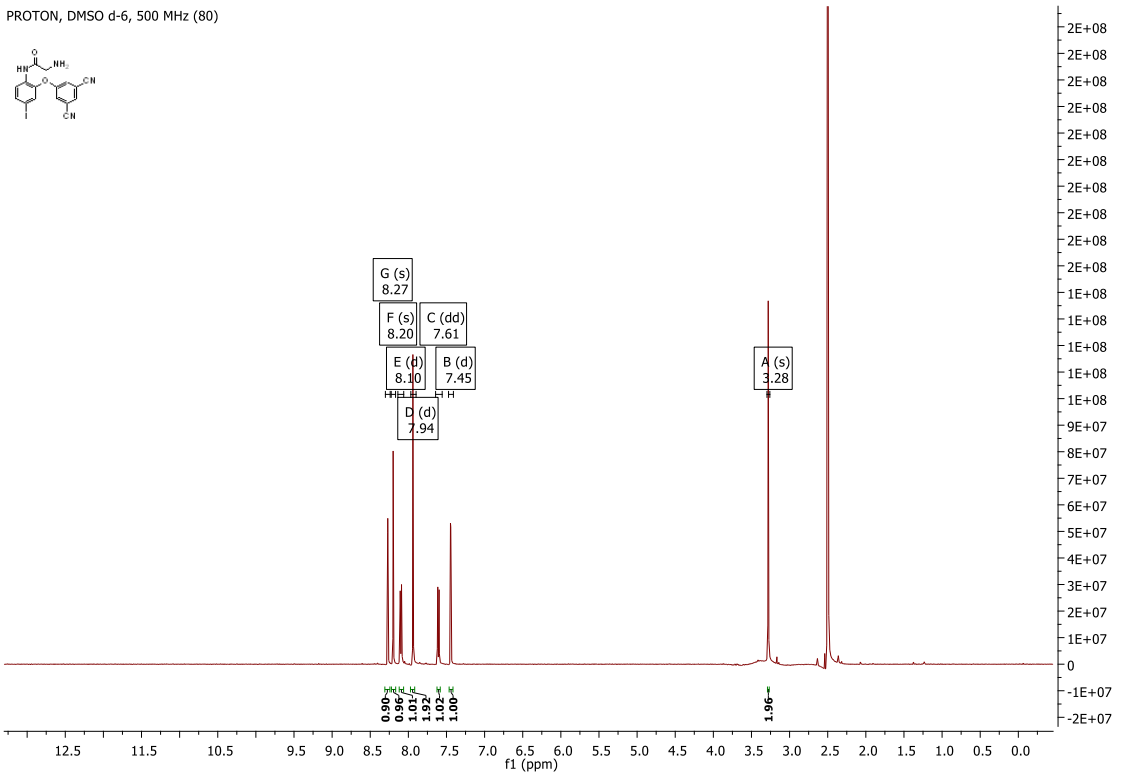

PROTON, DMSO d-6, 500 MHz (79)
G (s)
8.23
E (d)
8.12
D (dd)
7.52
B (m)
7.47
A (m)
7.08
C (d)
7.21
F (s)
3.34
0.97
0.97
1.07
2.00
0.99
2.10
1.97
f1 (ppm)

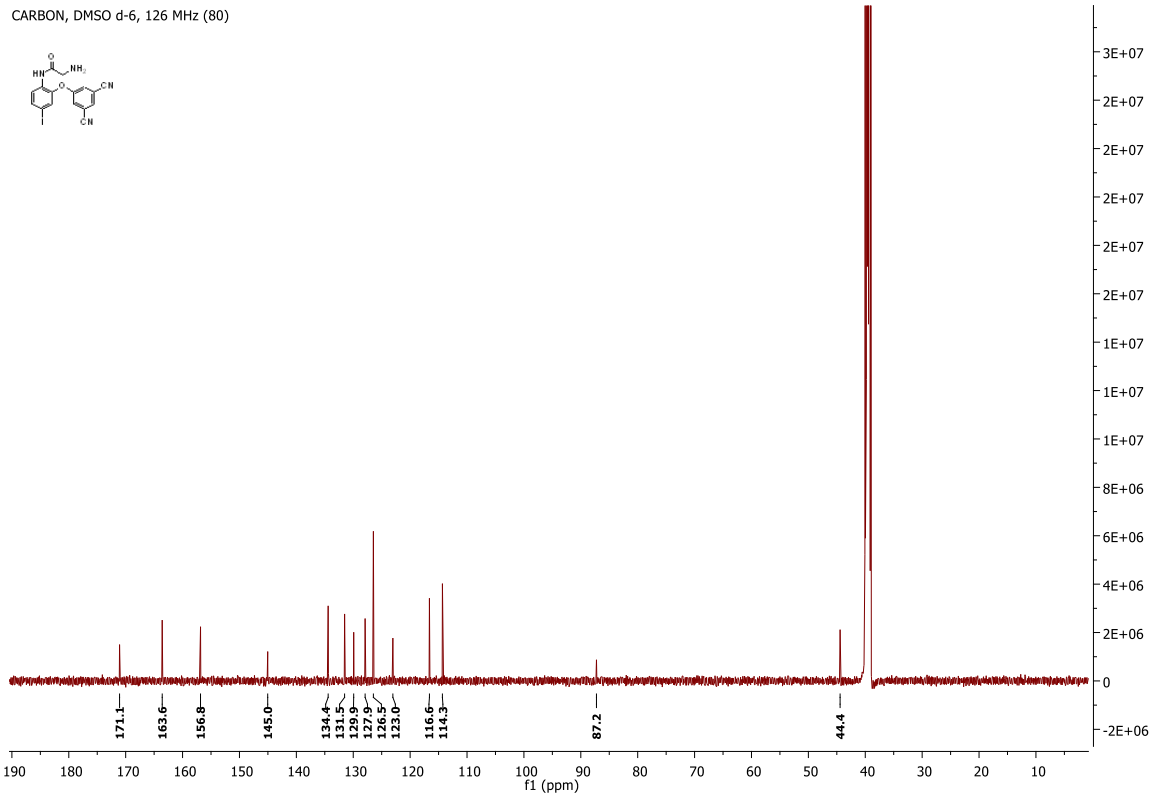

CARBON, DMSO d-6, 126 mHz (79)
170.6
163.9
154.9
146.3
133.1
130.1
129.6
128.0
126.8
122.6
120.3
86.8
44.1
f1 (ppm)

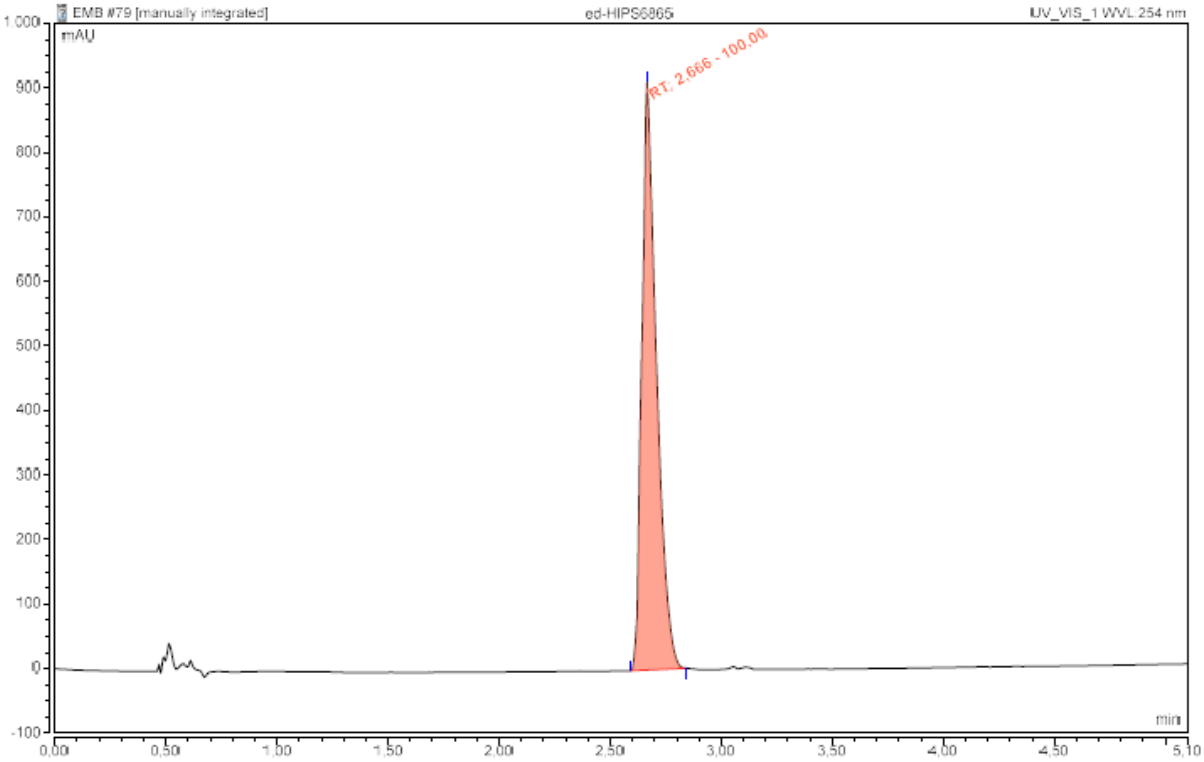

EMB #75 [manually integrated]
ed-HIPS6637
UV_VIS_1 WVL:254 nm
mAU
RT: 2,888 - 100,00
min

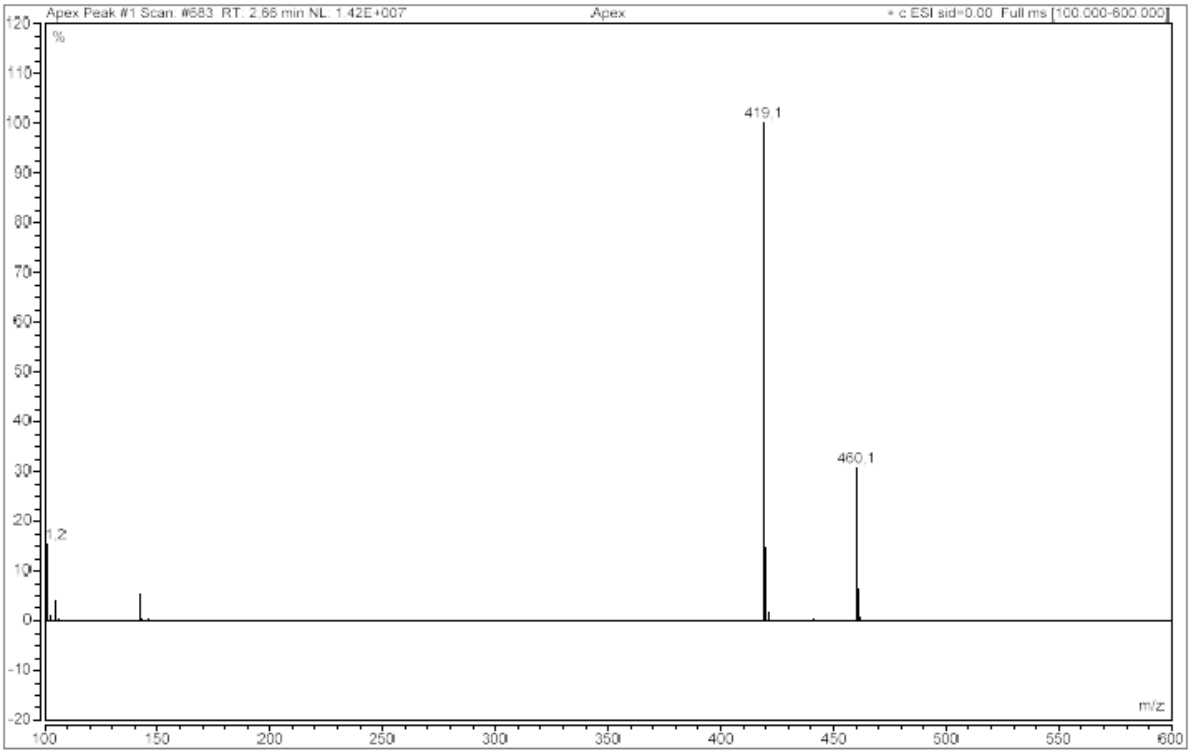

Apex Peak #1 Scan: #736 RT: 2,89 min NL: 2,85E+005
Apex
- c ESI sid=0.00 Full ms [100.000-600.000]
%
401,0
403,0
437,0
402,1
m/z

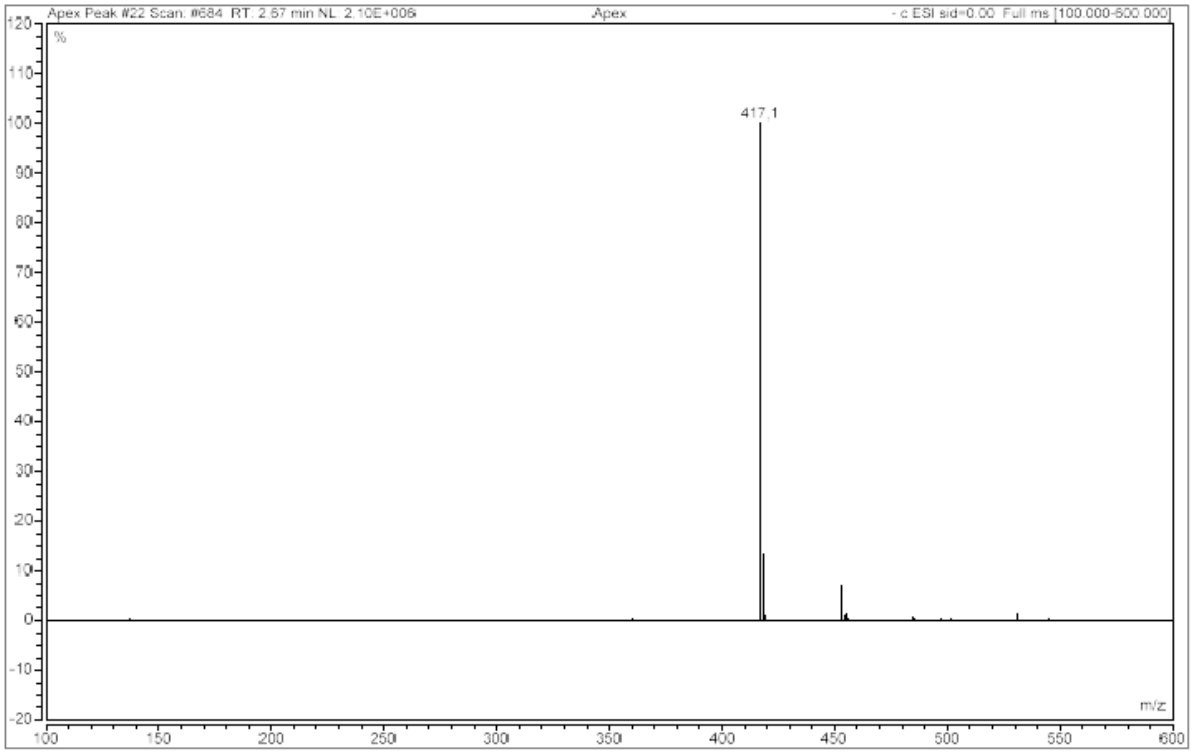

Apex Peak #17 Scan: #729 RT: 2,86 min NL: 2,61E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
403,0
405,1
444,1
m/z

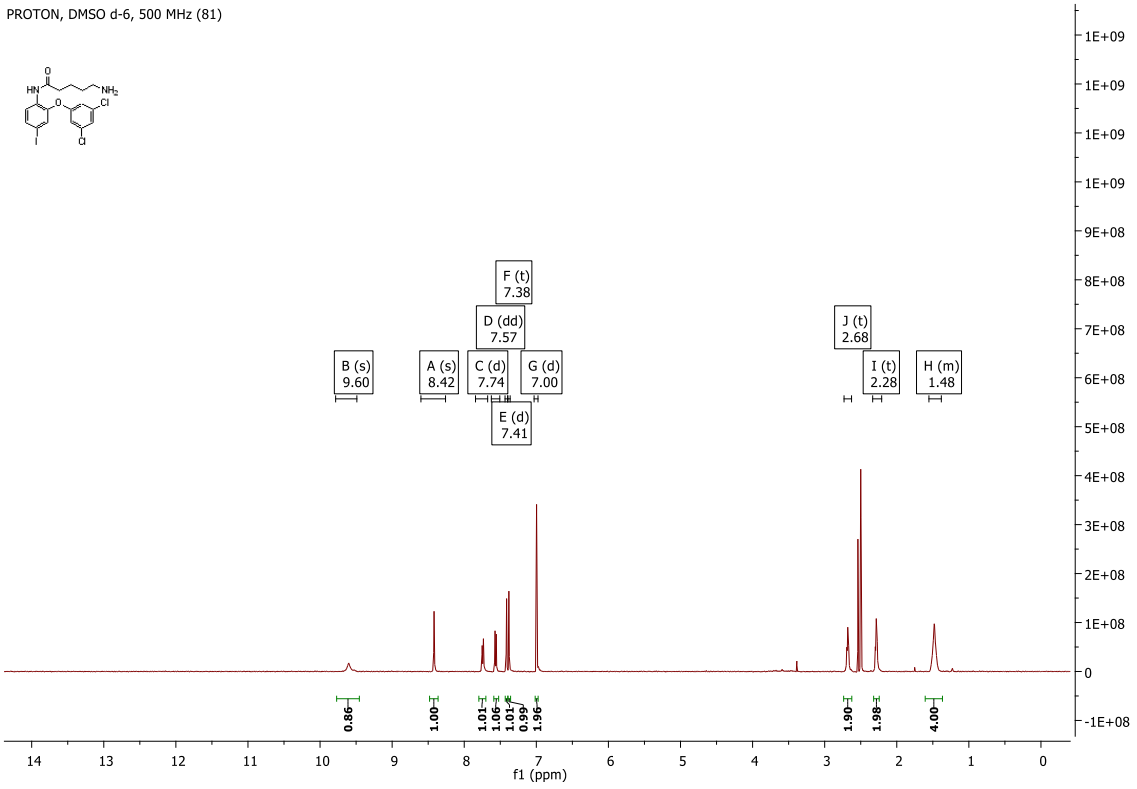

PROTON, DMSO d-6, 500 MHz (80)
G (s) 8.27
F (s) 8.20
C (dd) 7.61
E (d) 8.10
B (d) 7.45
D (d) 7.94
A (s) 3.28
0.90
0.96
1.01
1.92
1.02
1.00
1.96
f1 (ppm)

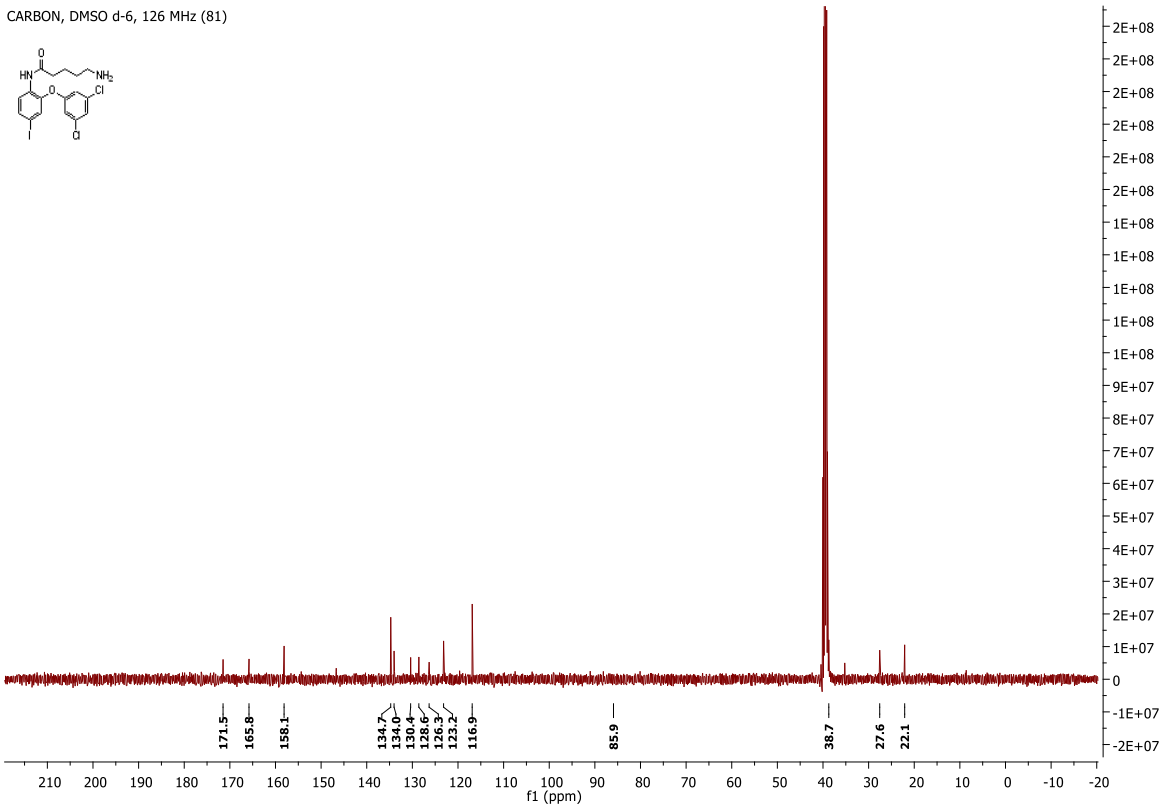

CARBON, DMSO d-6, 126 MHz (80)
171.1
163.6
156.8
145.0
134.4
131.5
129.9
127.9
126.5
123.0
116.6
114.3
87.2
44.4
f1 (ppm)

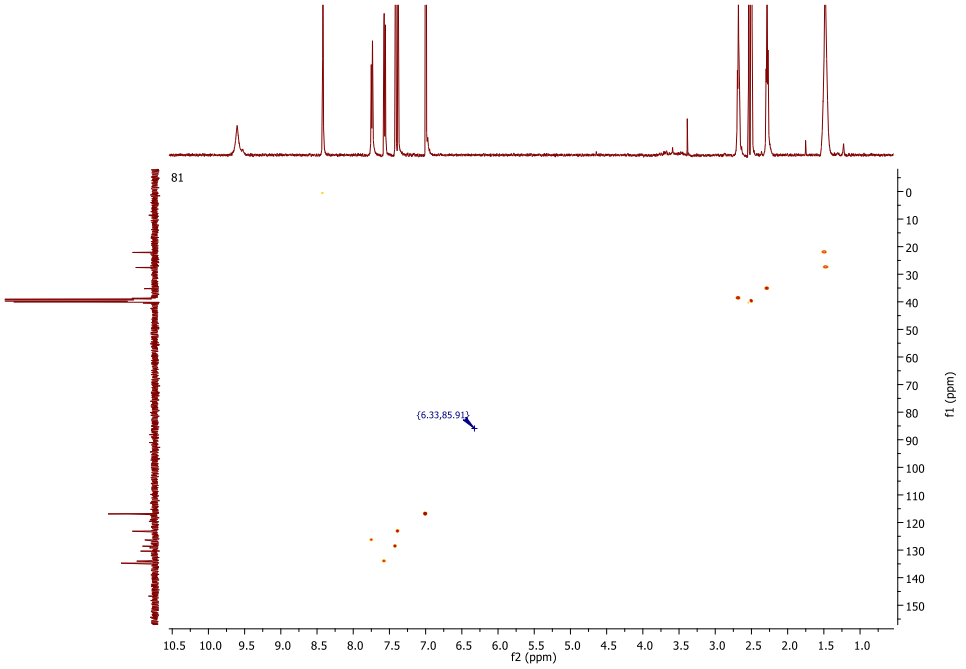

EMB #79 [manually integrated]
ed-HIPS6865
UV_VIS_1 WVL:254 nm
mAU
RT: 2.666 - 100.00
min

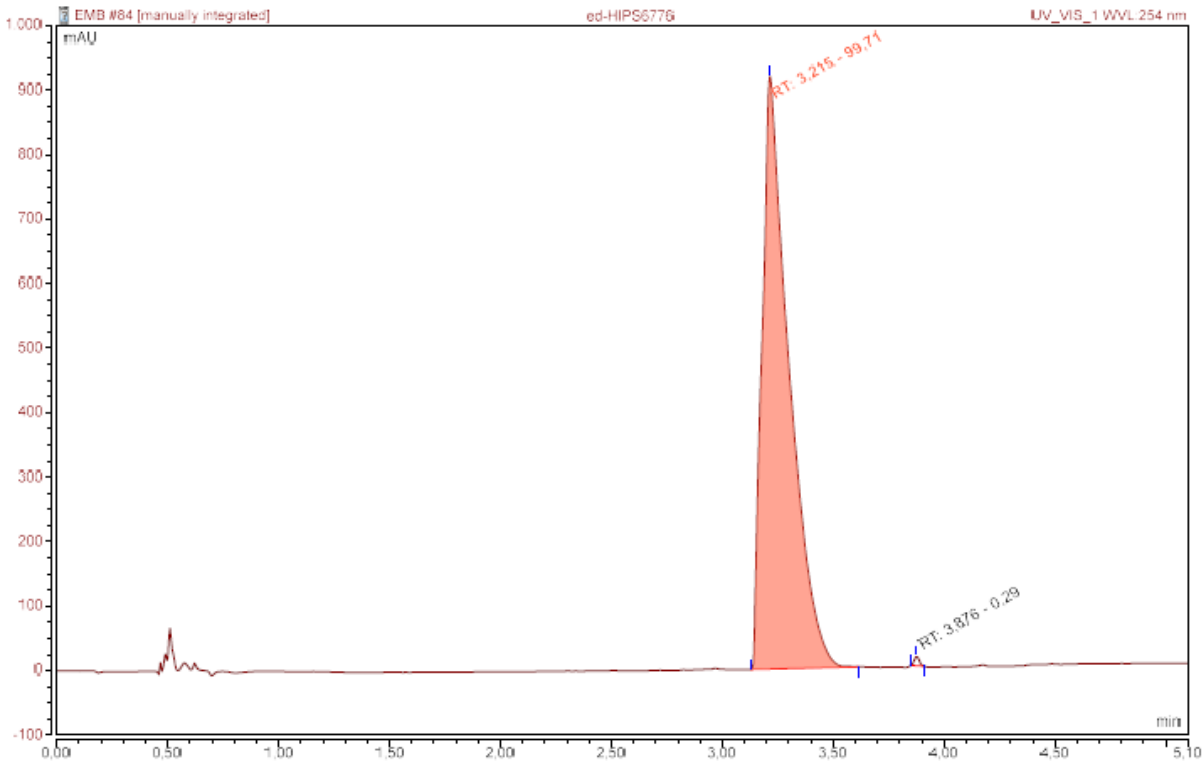

Apex Peak #1 Scan: #683 RT: 2.66 min NL: 1.42E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
419.1
460.1
1.2
m/z

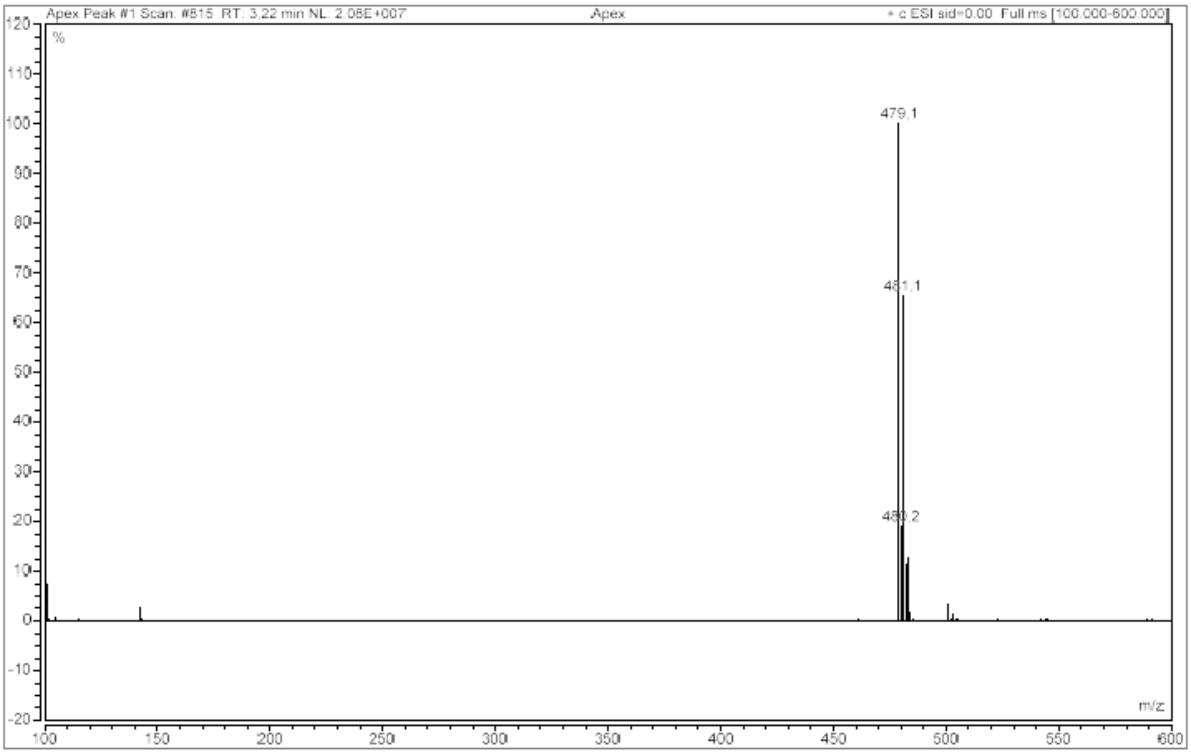

Apex Peak #22 Scan: #684 RT: 2.67 min NL: 2.10E+006
Apex
- c ESI sid=0.00 Full ms [100.000-600.000]
%
417.1
m/z
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
100
150
200
250
300
350
400
450
500
550
600

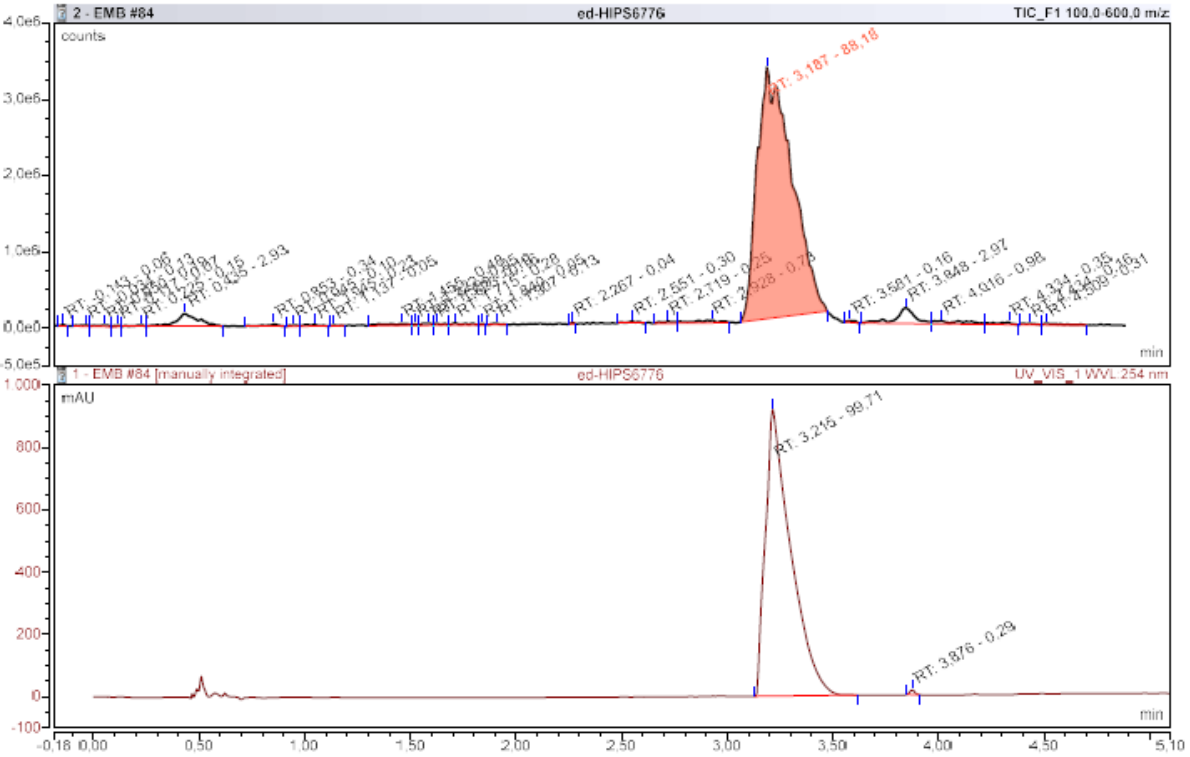

PROTON, DMSO d-6, 500 MHz (81)
B (s) 9.60
A (s) 8.42
C (d) 7.74
E (d) 7.41
D (dd) 7.57
F (t) 7.38
G (d) 7.00
J (t) 2.68
I (t) 2.28
H (m) 1.48
0.86
1.00
1.01
1.06
1.01
0.99
1.96
1.90
1.98
4.00
f1 (ppm)

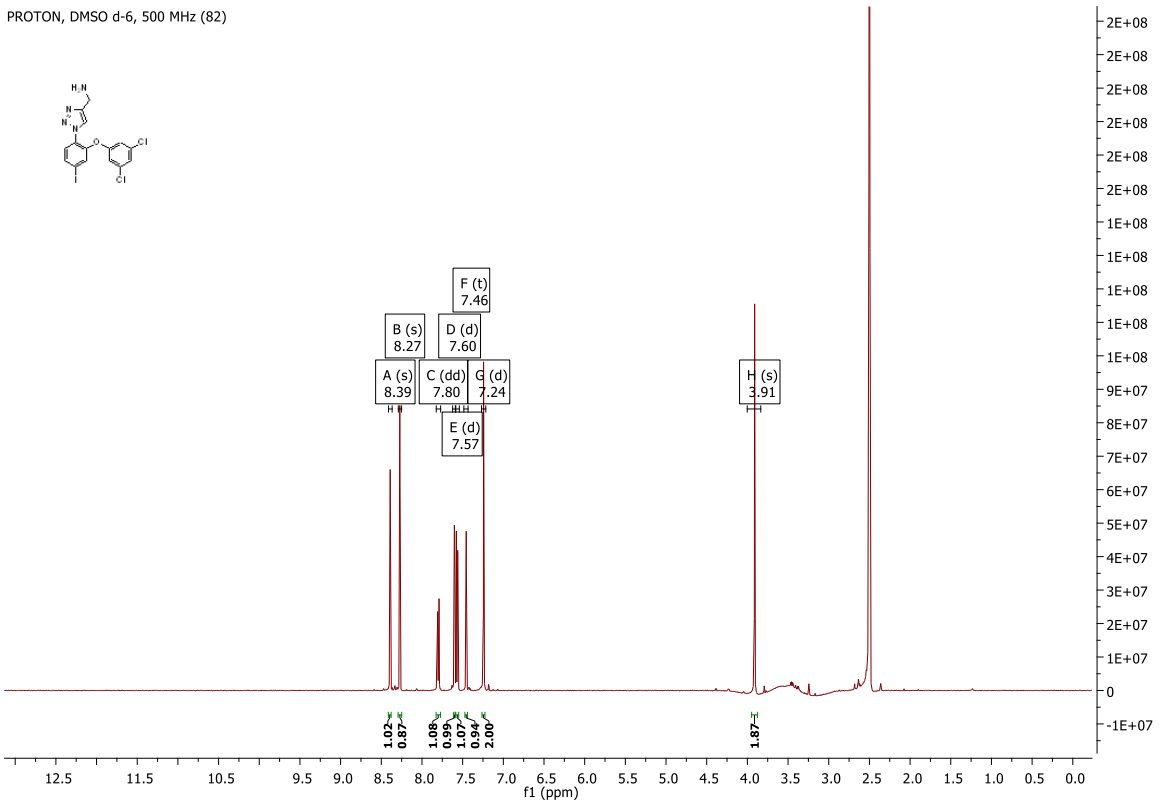

CARBON, DMSO d-6, 126 MHz (81)
171.5
165.8
158.1
134.7
134.0
130.4
128.6
126.3
123.2
116.9
85.9
38.7
27.6
22.1
f1 (ppm)

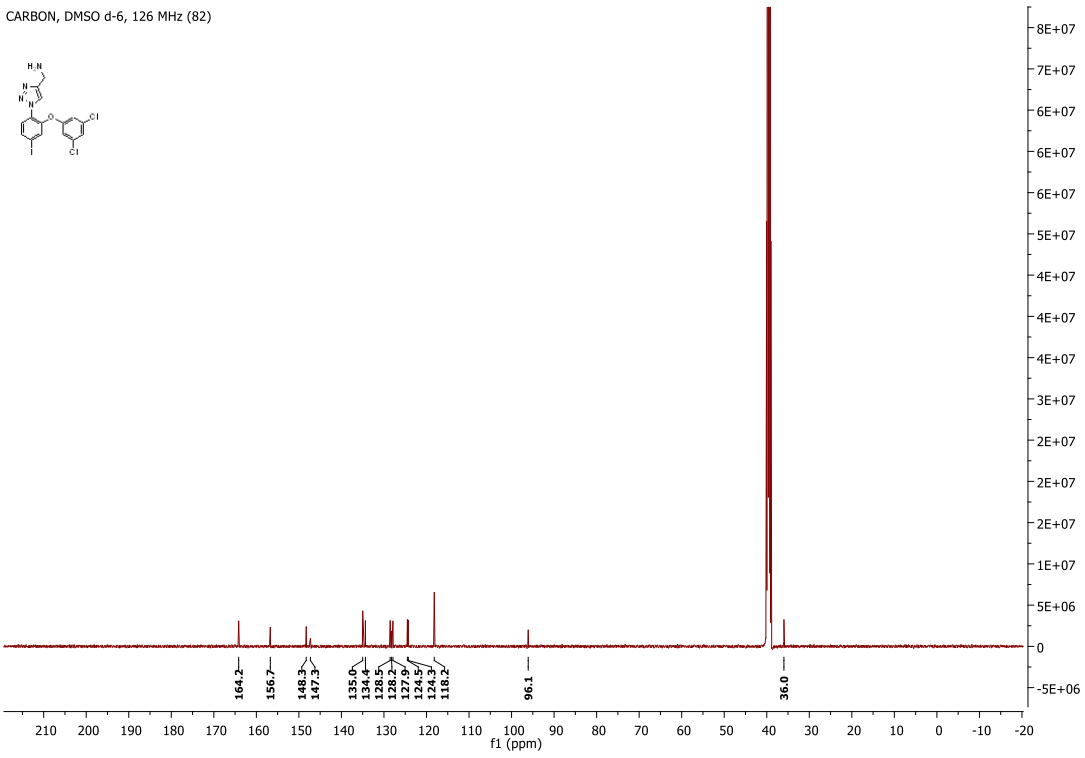

81
(6.33,85.91)
f1 (ppm)
f2 (ppm)

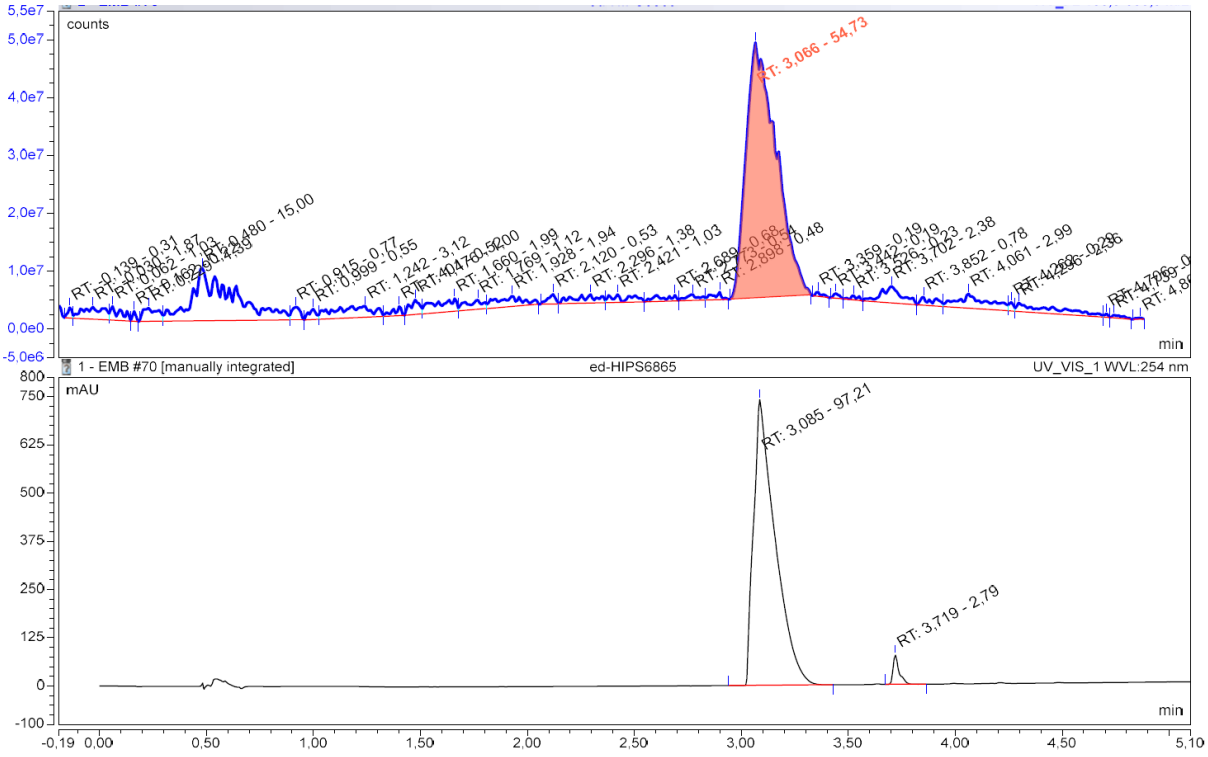

EMB #84 [manually integrated]
ed-HIPS6776
UV_VIS_1 WVL:254 nm
mAU
RT: 3.215 - 99.71
RT: 3.876 - 0.29
min

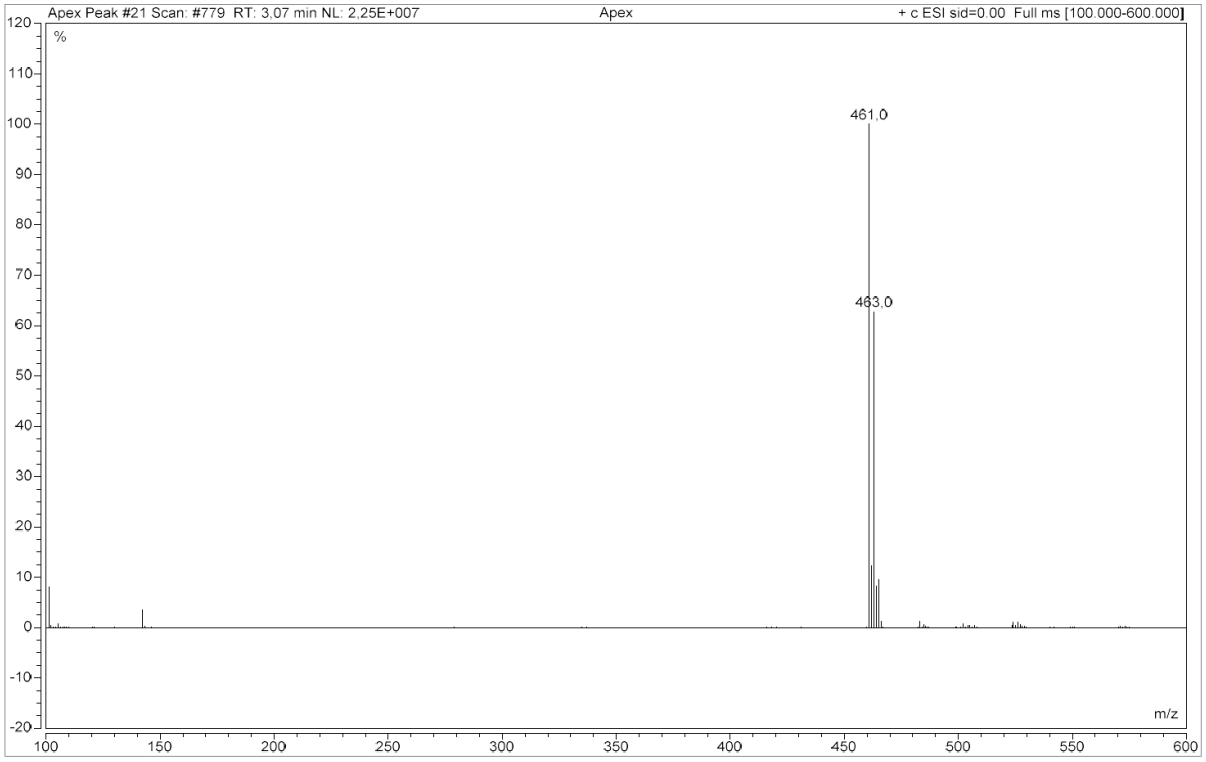

Apex Peak #1 Scan: #815 RT: 3.22 min NL: 2.08E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
479.1
481.1
483.2
m/z

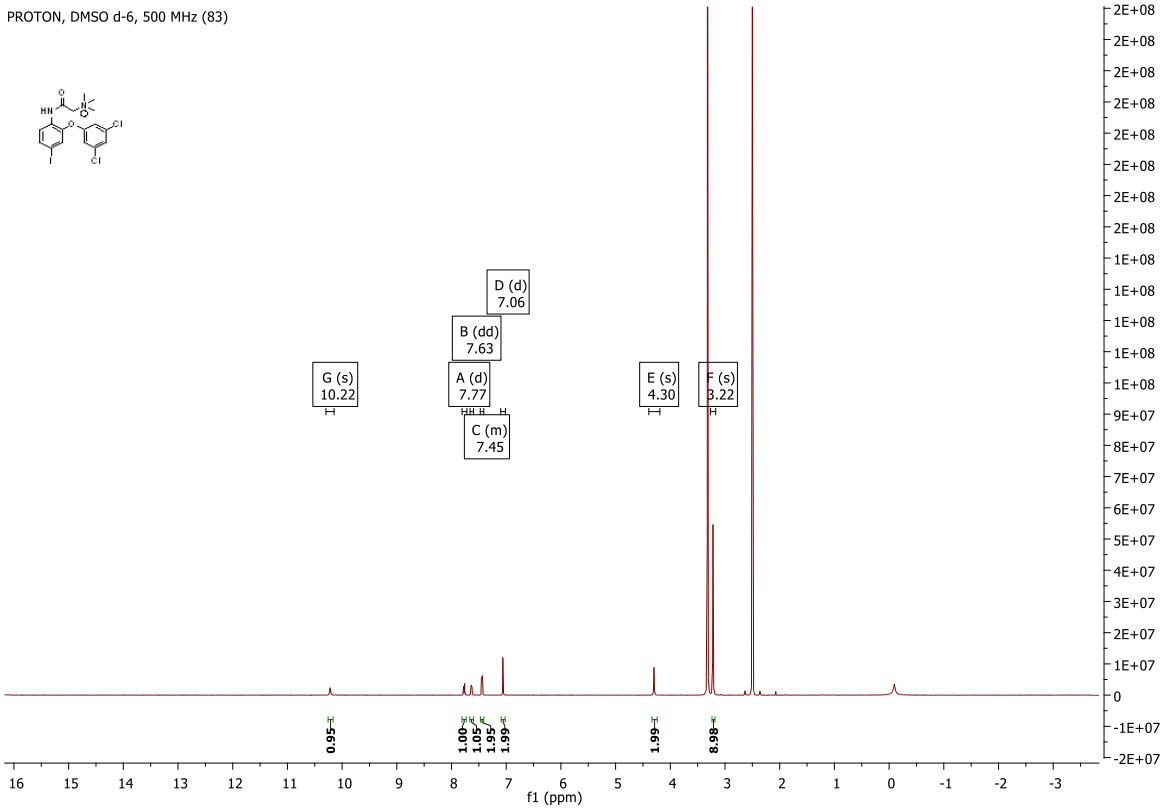

2 - EMB #84
ed-HIPS6776
TIC_F1 100.0-600.0 m/z
counts
RT. 3.187 - 88.18
min
1 - EMB #84 [manually integrated]
ed-HIPS6776
UV_VIS_1 WVL:254 nm
mAU
RT. 3.215 - 99.71
RT. 3.876 - 0.29
min

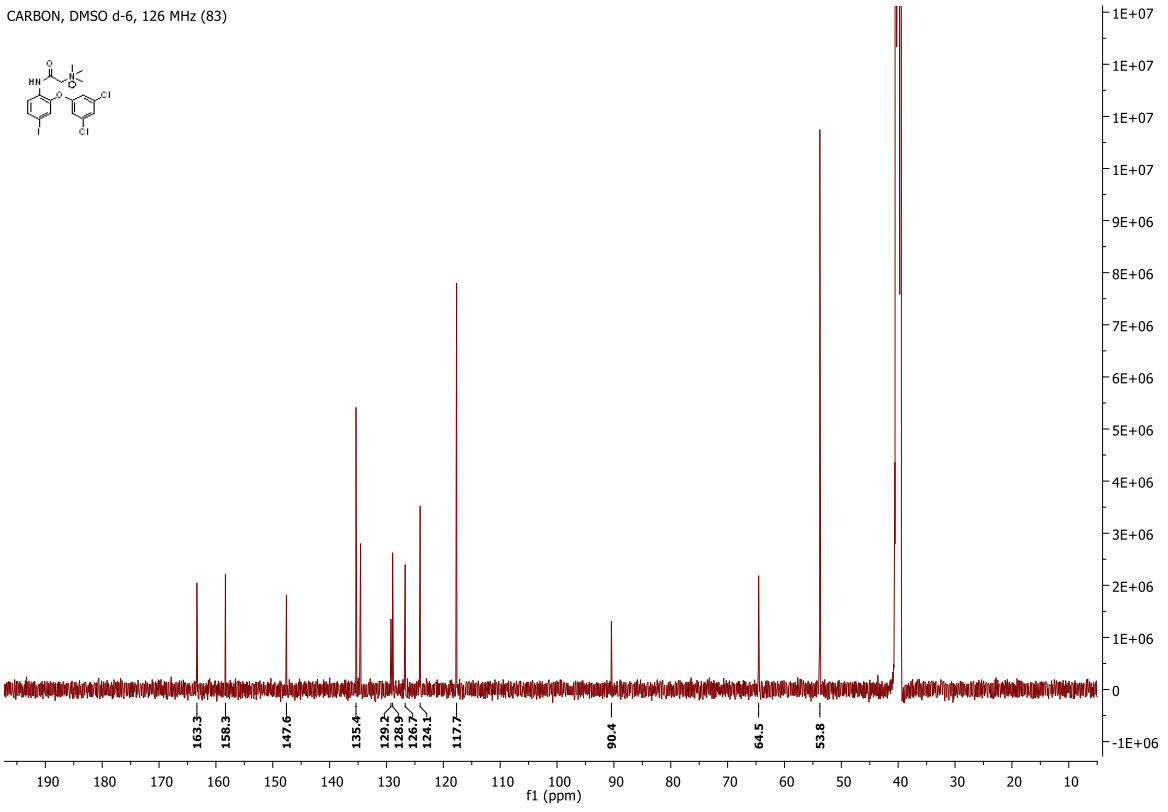

PROTON, DMSO d-6, 500 MHz (82)
F (t) 7.46
B (s) 8.27
D (d) 7.60
A (s) 8.39
C (dd) 7.80
G (d) 7.24
E (d) 7.57
H (s) 3.91
1.02
0.87
1.08
0.99
1.07
0.94
2.00
1.87
f1 (ppm)

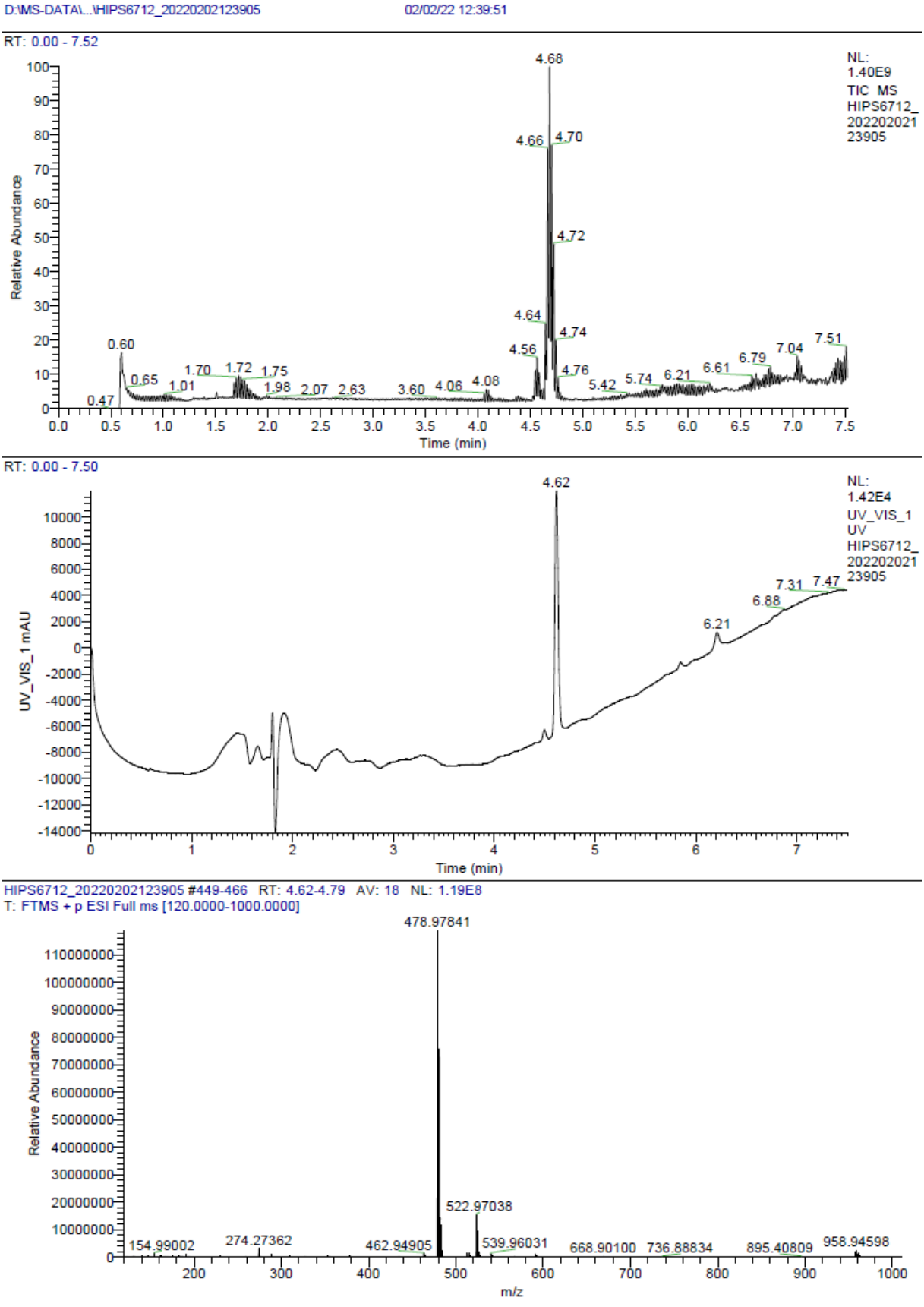

CARBON, DMSO d-6, 126 MHz (82)
164.2
156.7
148.3
147.3
135.0
134.4
128.5
128.2
127.9
124.5
124.3
118.2
96.1
36.0
f1 (ppm)

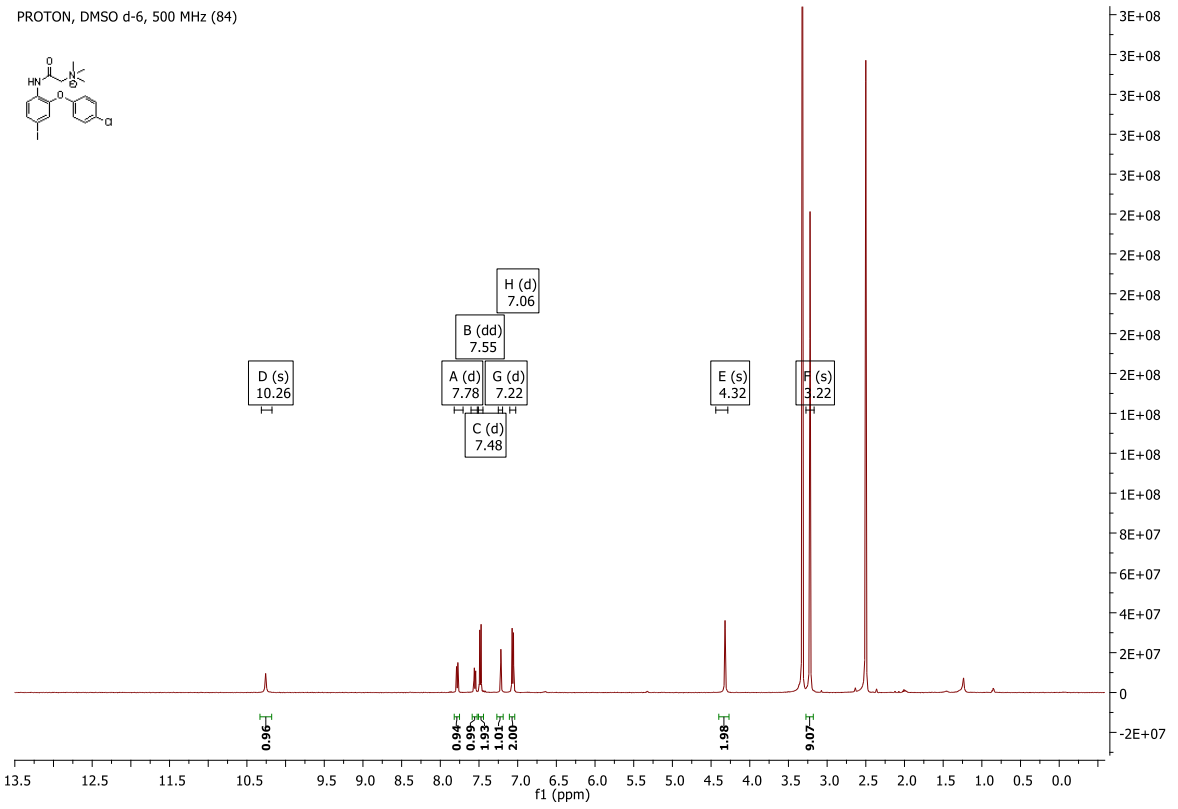

counts
RT: 3,066 - 54,73
RT: 0,480 - 15,00
RT: 0,915 - 0,77
RT: 1,242 - 3,12
RT: 1,660 - 1,99
RT: 1,769 - 1,12
RT: 1,928 - 1,94
RT: 2,120 - 0,53
RT: 2,296 - 1,38
RT: 2,421 - 1,03
RT: 3,359 - 0,19
RT: 3,526 - 0,19
RT: 3,702 - 2,38
RT: 3,852 - 0,78
RT: 4,061 - 2,99
min
1 - EMB #70 [manually integrated]
ed-HIPS6865
UV_VIS_1 WVL:254 nm
mAU
RT: 3,085 - 97,21
RT: 3,719 - 2,79
min

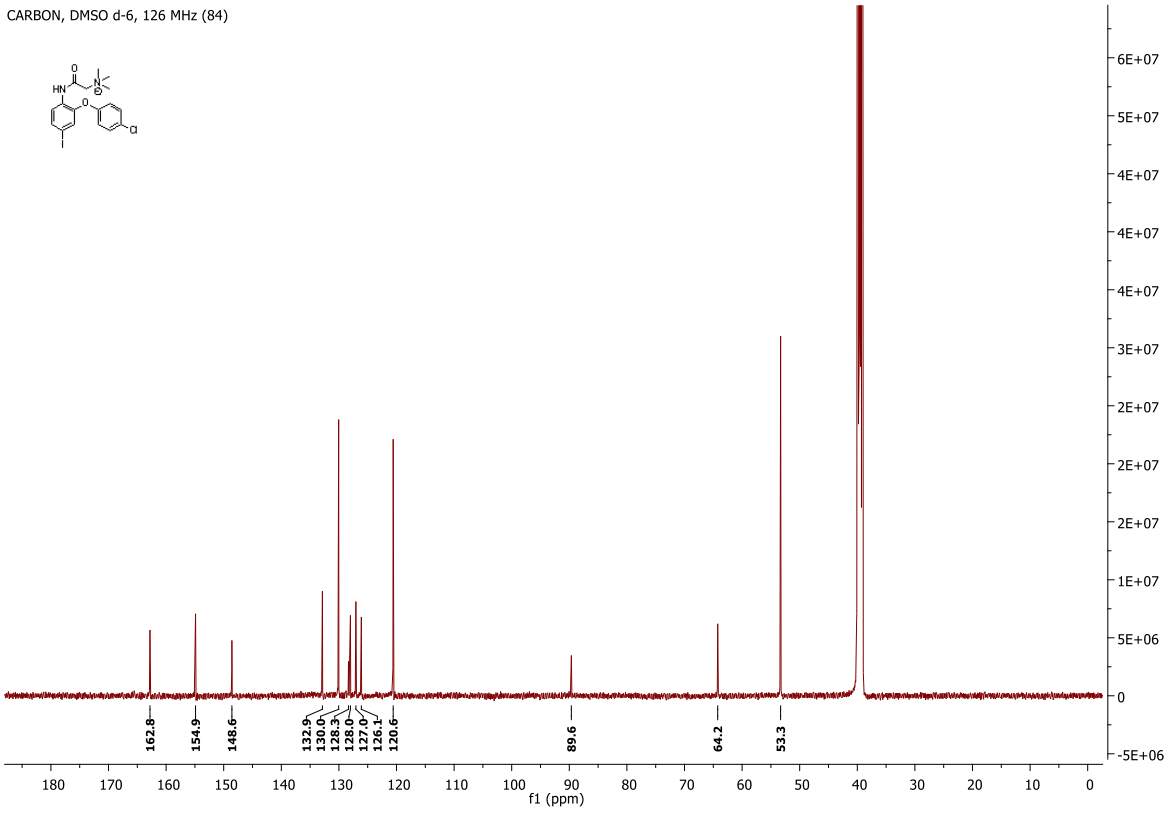

Apex Peak #21 Scan: #779 RT: 3,07 min NL: 2,25E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
461,0
463,0
m/z

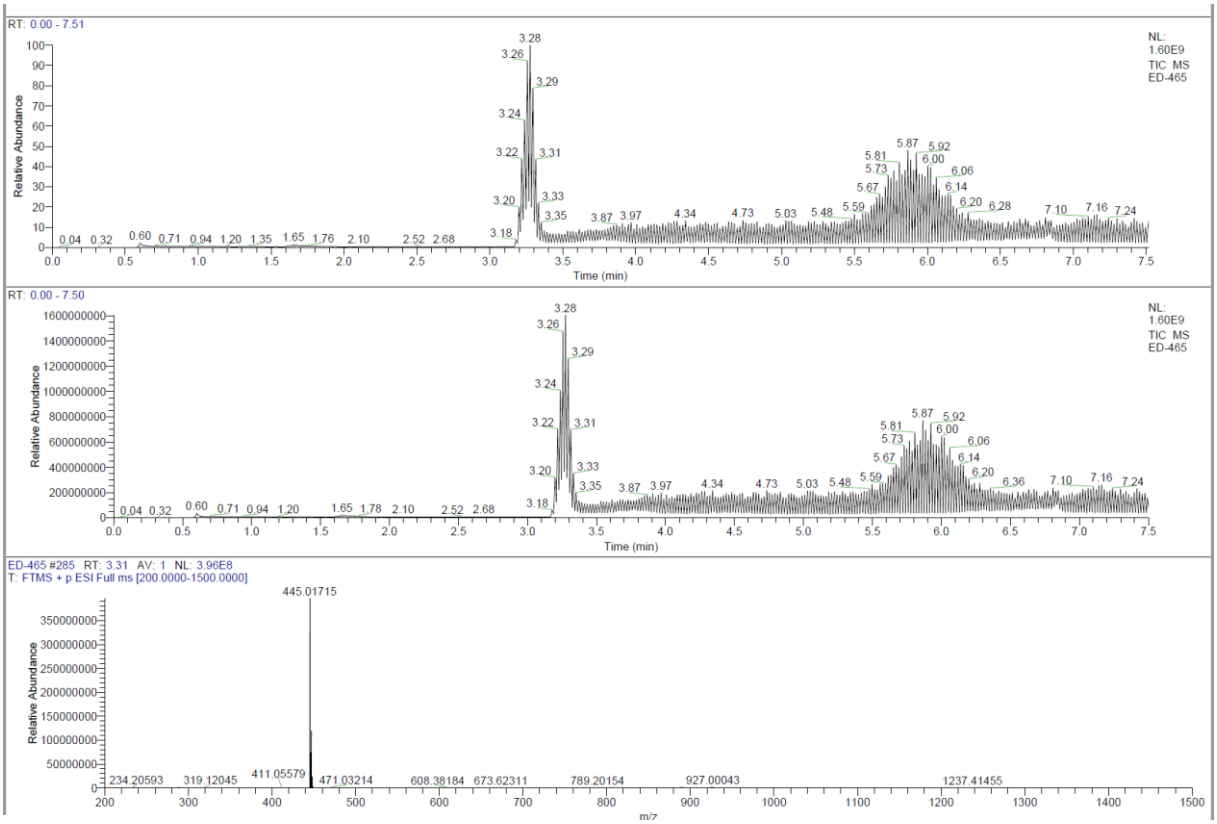

PROTON, DMSO d-6, 500 MHz (83)
G (s)
10.22
A (d)
7.77
B (dd)
7.63
C (m)
7.45
D (d)
7.06
E (s)
4.30
F (s)
3.22
0.95
1.00
1.05
1.95
1.99
1.99
8.98
f1 (ppm)


CARBON, DMSO d-6, 126 MHz (83)
163.3
158.3
147.6
135.4
129.2
128.9
126.7
124.1
117.7
90.4
64.5
53.8
f1 (ppm)

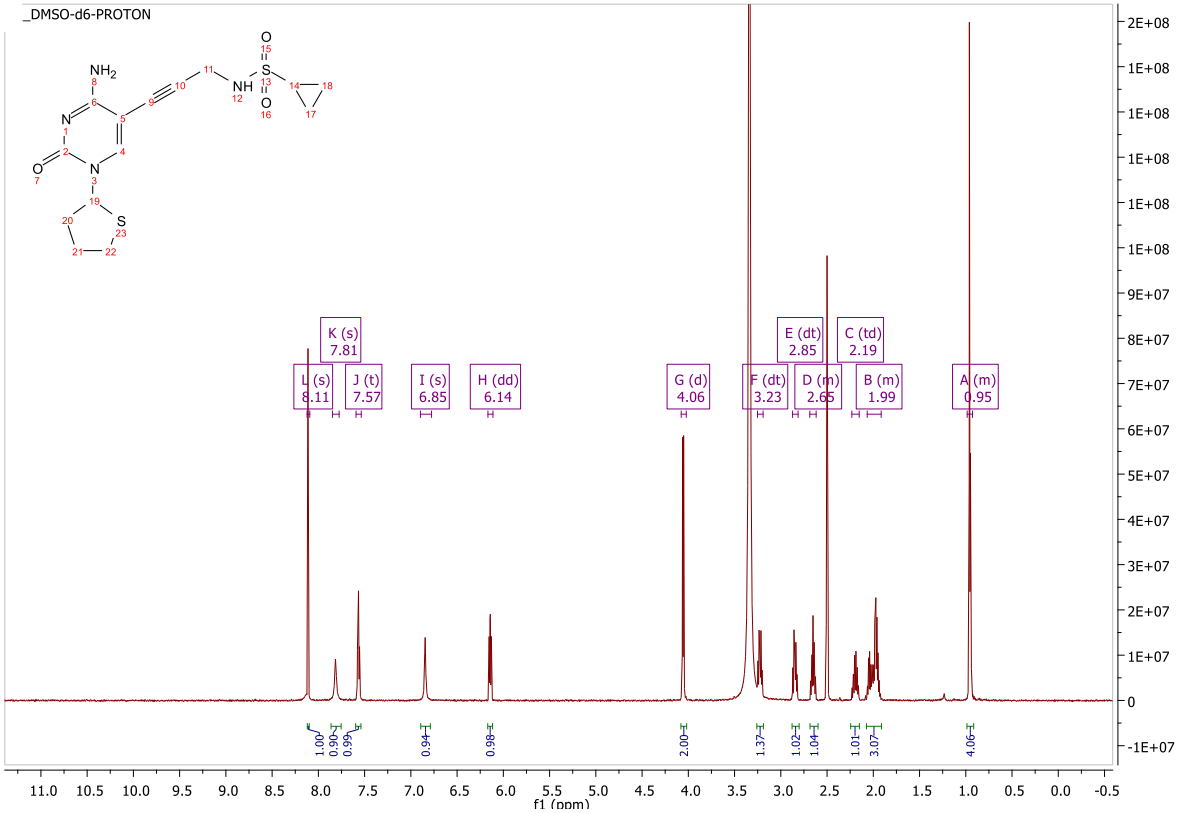

D:\MS-DATA\...\HIPS6712_20220202123905 02/02/22 12:39:51
RT: 0.00 - 7.52
NL: 1.40E9 TIC MS HIPS6712_202202021 23905
Relative Abundance
Time (min)
RT: 0.00 - 7.50
NL: 1.42E4 UV_VIS_1 UV HIPS6712_202202021 23905
UV_VIS_1 mAU
Time (min)
HIPS6712_20220202123905 #449-466 RT: 4.62-4.79 AV: 18 NL: 1.19E8
T: FTMS + p ESI Full ms [120.0000-1000.0000]
478.97841
522.97038
154.99002
274.27362
462.94905
539.96031
668.90100
736.88834
895.40809
958.94598
Relative Abundance
m/z

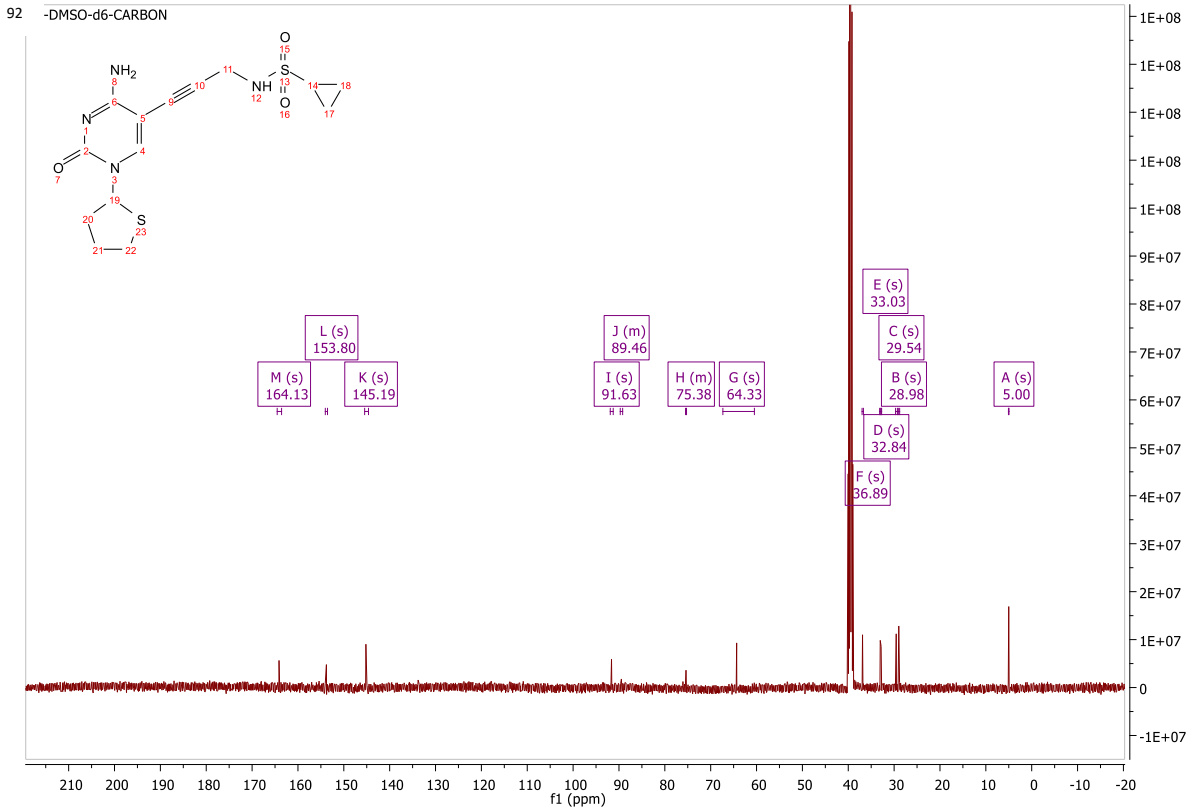

PROTON, DMSO d-6, 500 MHz (84)
D (s)
10.26
H (d)
7.06
B (dd)
7.55
A (d)
7.78
G (d)
7.22
C (d)
7.48
E (s)
4.32
F (s)
3.22
0.96
0.94
0.99
1.93
1.01
2.00
1.98
9.07
f1 (ppm)

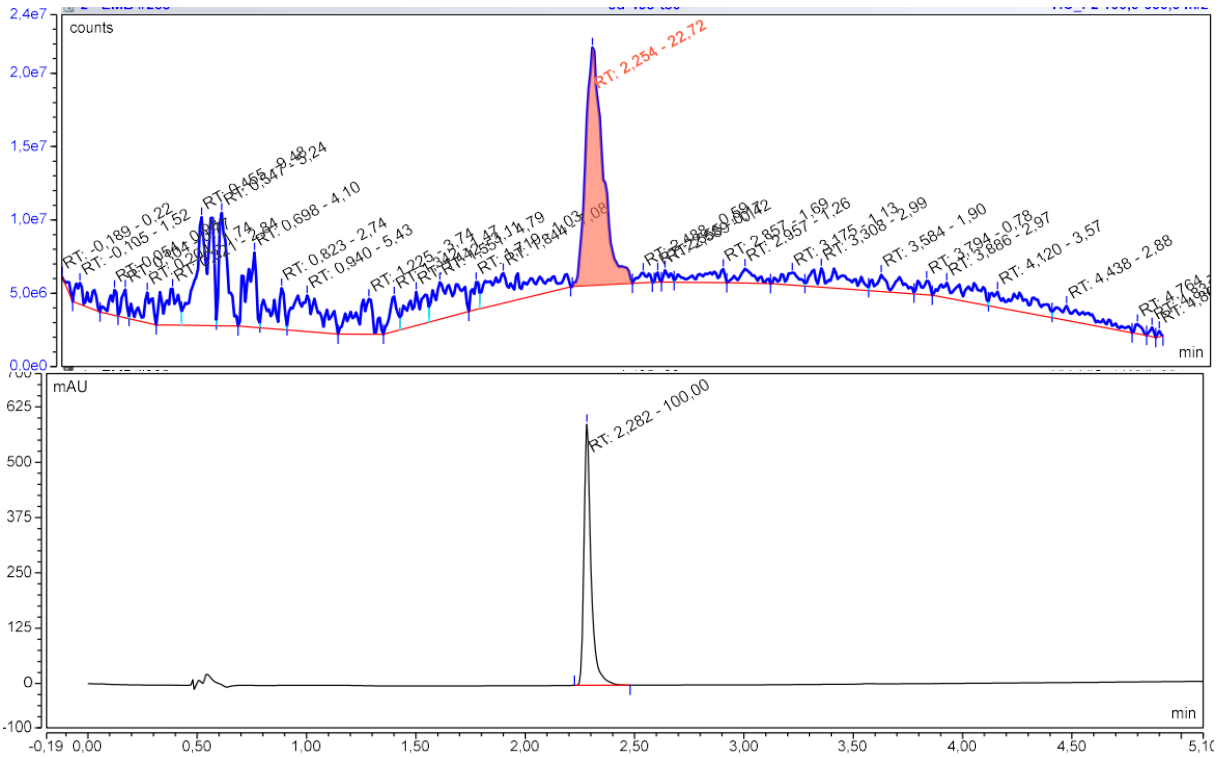

CARBON, DMSO d-6, 126 MHz (84)
162.8
154.9
148.6
132.9
130.0
128.3
128.0
127.0
126.1
120.6
89.6
64.2
53.3
f1 (ppm)

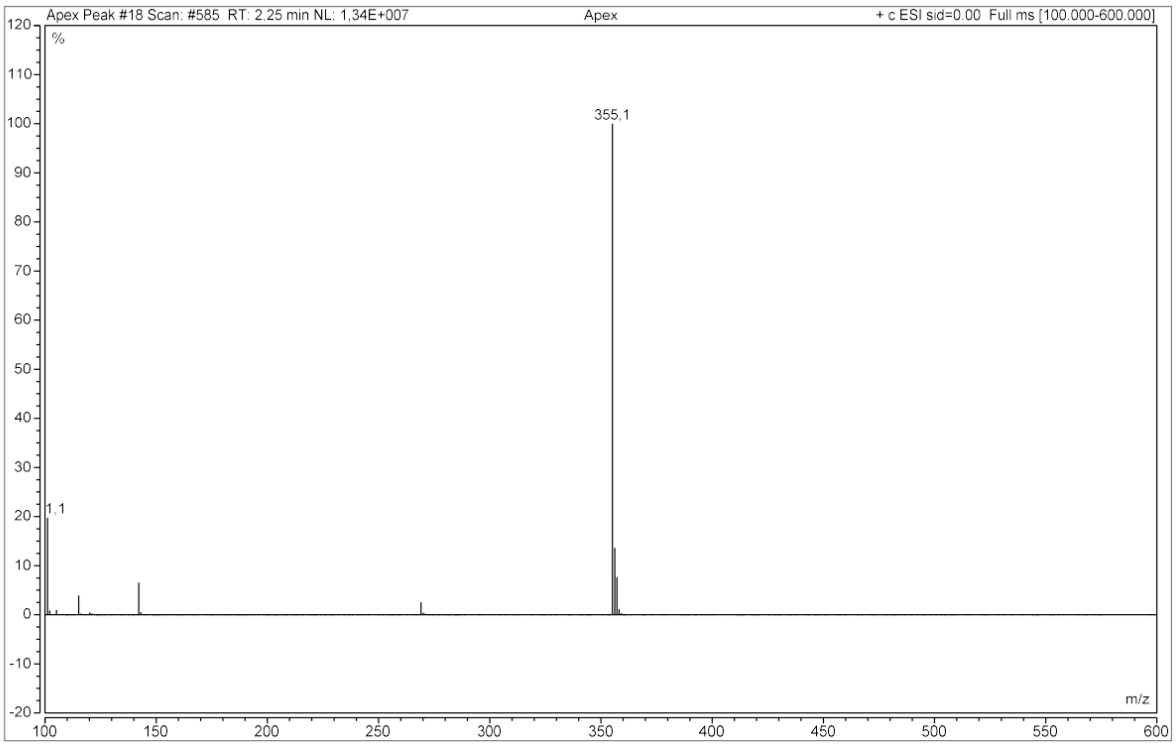

RT: 0.00 - 7.51
Relative Abundance
Time (min)
NL:
1.60E9
TIC MS
ED-465
RT: 0.00 - 7.50
Relative Abundance
Time (min)
NL:
1.60E9
TIC MS
ED-465
ED-465 #285 RT: 3.31 AV: 1 NL: 3.96E8
T: FTMS + p ESI Full ms [200.0000-1500.0000]
445.01715
Relative Abundance
m/z

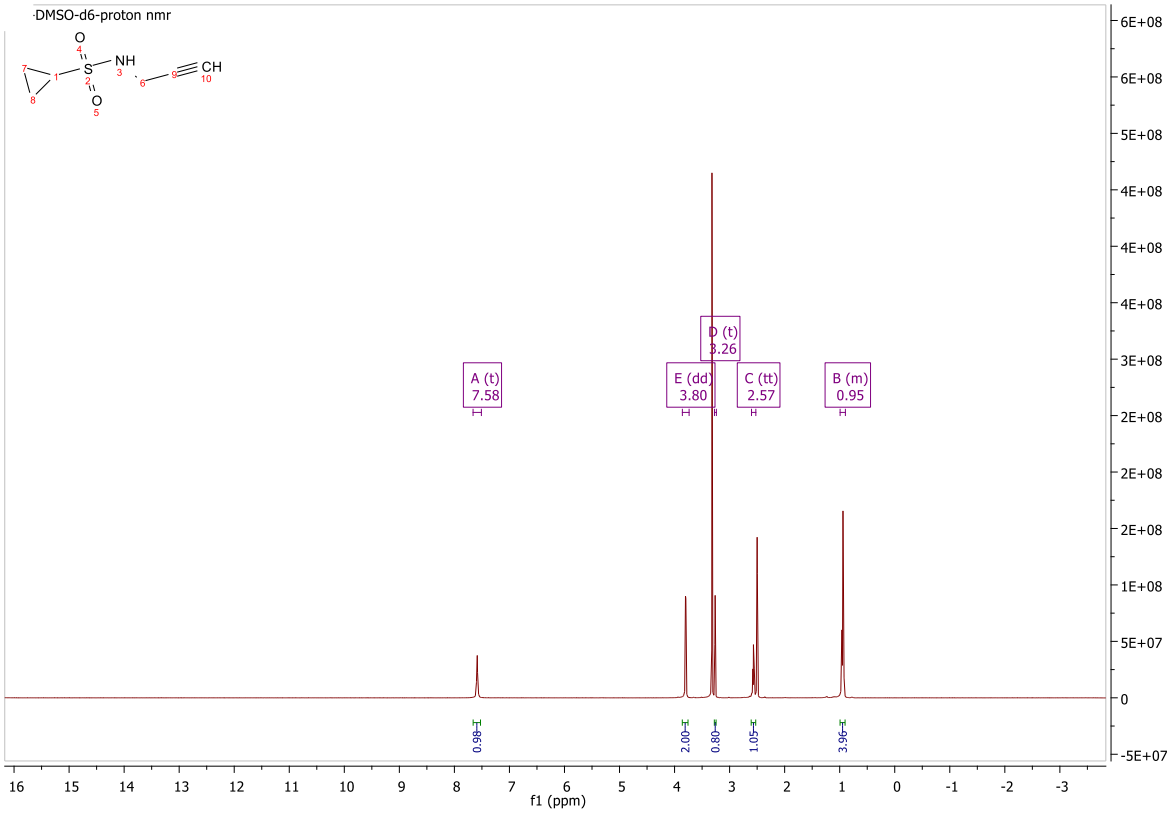
92

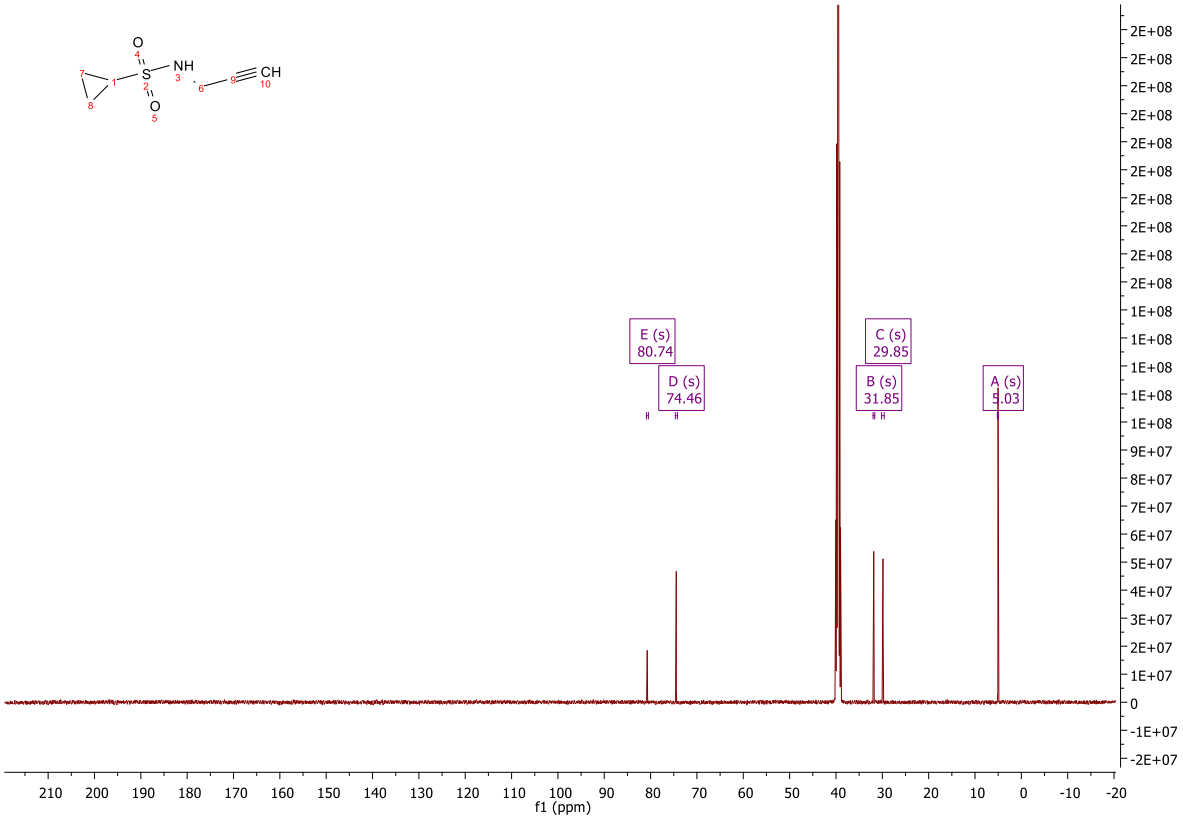

_DMSO-d6-PROTON
K (s) 7.81
L (s) 8.11
J (t) 7.57
I (s) 6.85
H (dd) 6.14
G (d) 4.06
F (dt) 3.23
E (dt) 2.85
D (m) 2.65
C (td) 2.19
B (m) 1.99
A (m) 0.95
f1 (ppm)

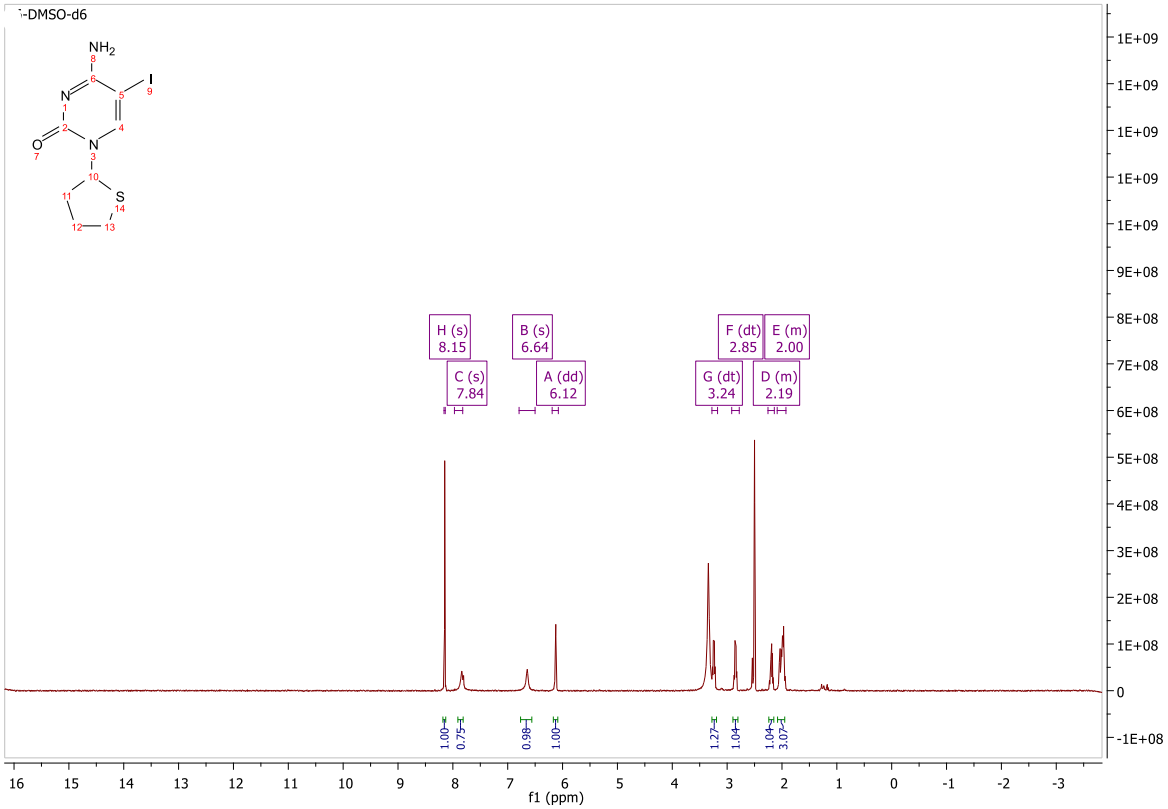

92
-DMSO-d6-CARBON
L (s) 153.80
M (s) 164.13
K (s) 145.19
J (m) 89.46
I (s) 91.63
H (m) 75.38
G (s) 64.33
E (s) 33.03
C (s) 29.54
B (s) 28.98
D (s) 32.84
F (s) 36.89
A (s) 5.00
f1 (ppm)

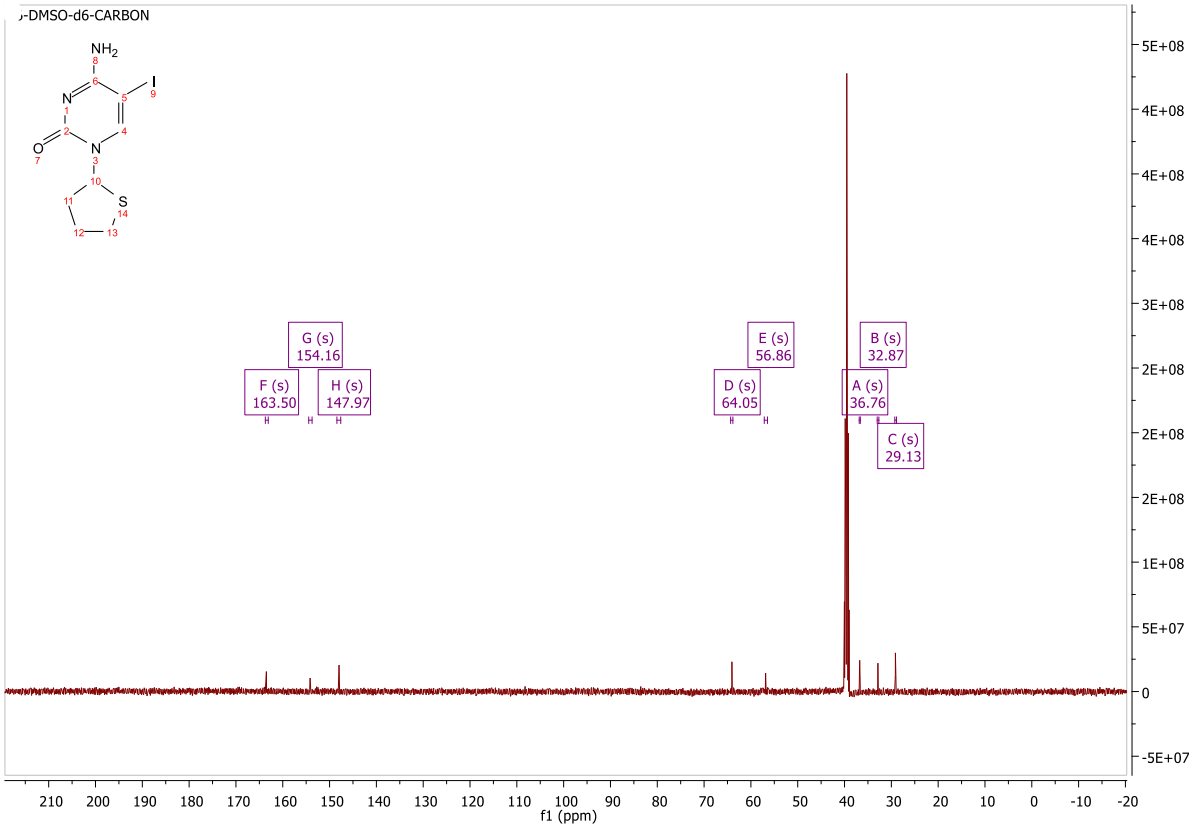

counts
RT: 2,254 - 22,72
min
mAU
RT: 2,282 - 100,00
min


Apex Peak #18 Scan: #585 RT: 2.25 min NL: 1,34E+007
Apex
+ c ESI sid=0.00 Full ms [100.000-600.000]
%
355,1
1,1
m/z


DMSO-d6-proton nmr
A (t)
7.58
H
E (dd)
3.80
H
D (t)
3.26
C (tt)
2.57
H
B (m)
0.95
H
0.98
2.00
0.80
1.05
3.96
f1 (ppm)


E (s)
80.74
D (s)
74.46
C (s)
29.85
B (s)
31.85
A (s)
5.03
f1 (ppm)


H (s)
8.15
C (s)
7.84
B (s)
6.64
A (dd)
6.12
F (dt)
2.85
E (m)
2.00
G (dt)
3.24
D (m)
2.19
f1 (ppm)



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