New ATP-competitive inhibitors of *E. coli* GyrB obtained from the mapping of hydrophobic floor at the binding site. Synthesis and biological evaluation.

Lucas Gutierrez,*[1] Peter Peršolja[2], Rodrigo Tosso[1], Nace Zidar*[2], Danijel Kikelj[2], Ricardo D. Enriz,[1]

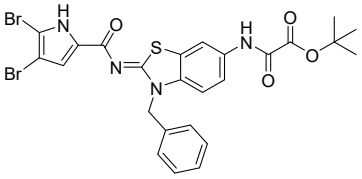
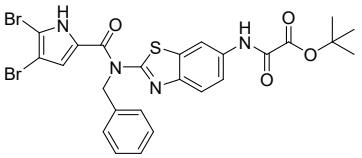
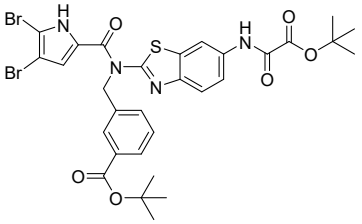
[1] Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis; Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-SL). Ejercito de los Andes 950, (5700) San Luis, Argentina.
[2]University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

Supplementary Information

Table 1. Different conformations obtained for complexes of compounds 3, 2, and 4.

Compound	*Conformation*	*ϕ (degrees)*	*ψ (degrees)*	*Relative energy (kcal/mol)*
3	A	90.79	-66.78	0.00
	B	90.79	-126.78	0.31
	C	90.79	-141.78	0.70
	D	90.79	38.22	5.53
	E	-74.21	-6.78	5.95
	F	-74.21	83.22	5.96
	G	-74.21	8.22	6.07
	H	-74.21	-111.78	9.14
2	A	119.94	48.83	0.00
	B	119.94	108.83	0.93
	C	134.94	-146.17	2.99
	D	134.94	33.83	3.07
	E	134.94	-86.17	4.35
	F	134.94	168.83	4.96
	G	179.94	63.83	7.61
	H	-135.06	-131.17	7.92
	I	-135.06	33.83	7.93
4	A	-128.85	9.68	0.00
	B	-128.85	99.68	0.13
	C	-83.85	114.68	1.37
	D	-98.85	-20.32	3.16
	E	111.15	144.68	3.63
	F	-38.85	24.68	3.63

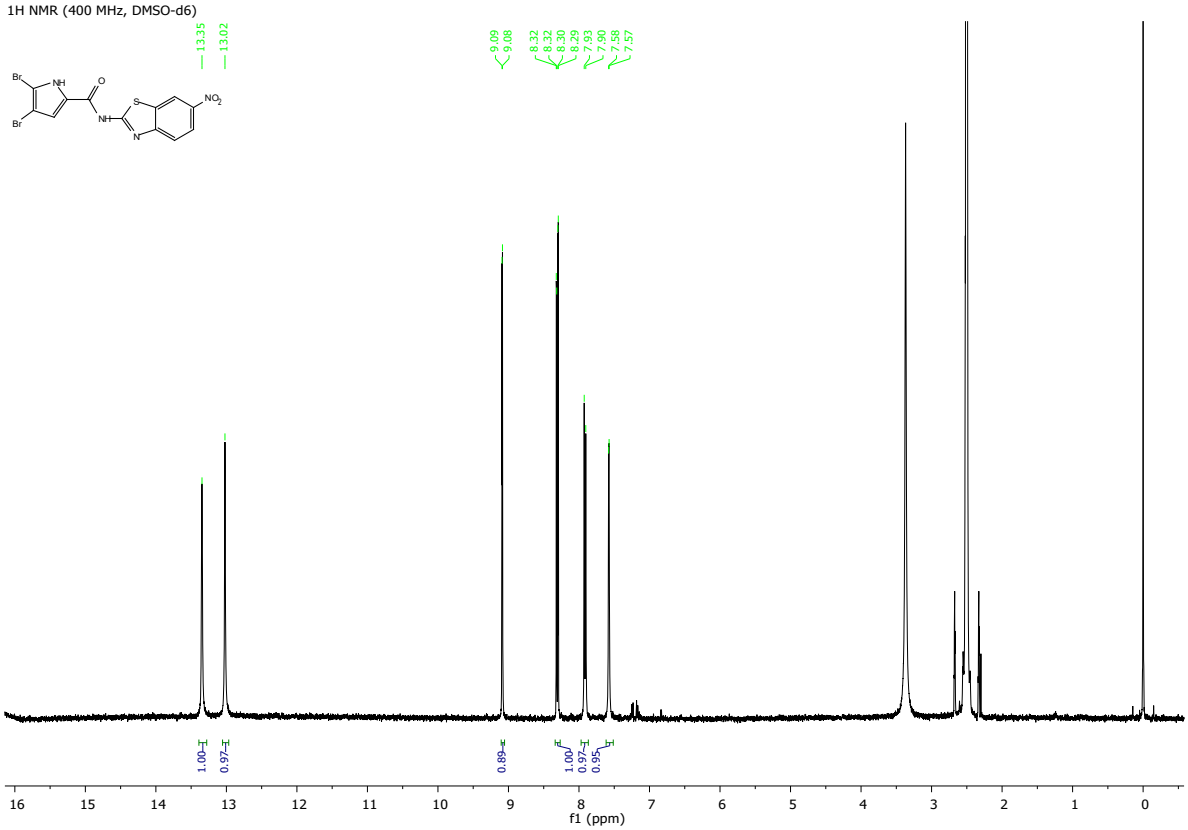
Table 2. Inhibitory activities of compounds 9 and 14 against DNA gyrase from *E. coli*.

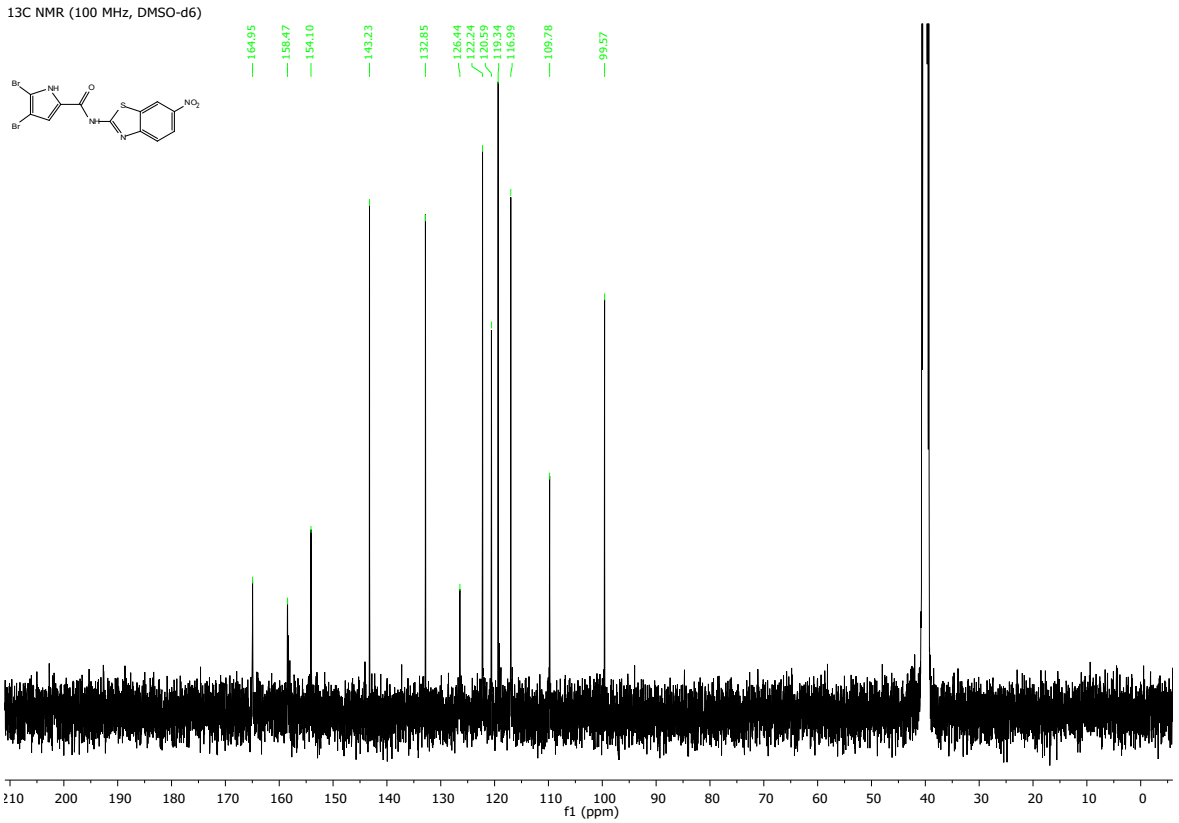
Compound	Structure	IC_{50} (nM)[a] *or RA (%)[b] E. coli* gyrase
9		2950 ± 470
14a		757 ± 146
14b		@10 μM = 77 ± 6%[b]

[a] Concentration of compound that inhibits the enzyme activity by 50%. [b] RA – Residual activity of the enzyme at the indicated concentration of the compound.
Mean values with standard deviations are reported. Biological assessments were performed in duplicate.

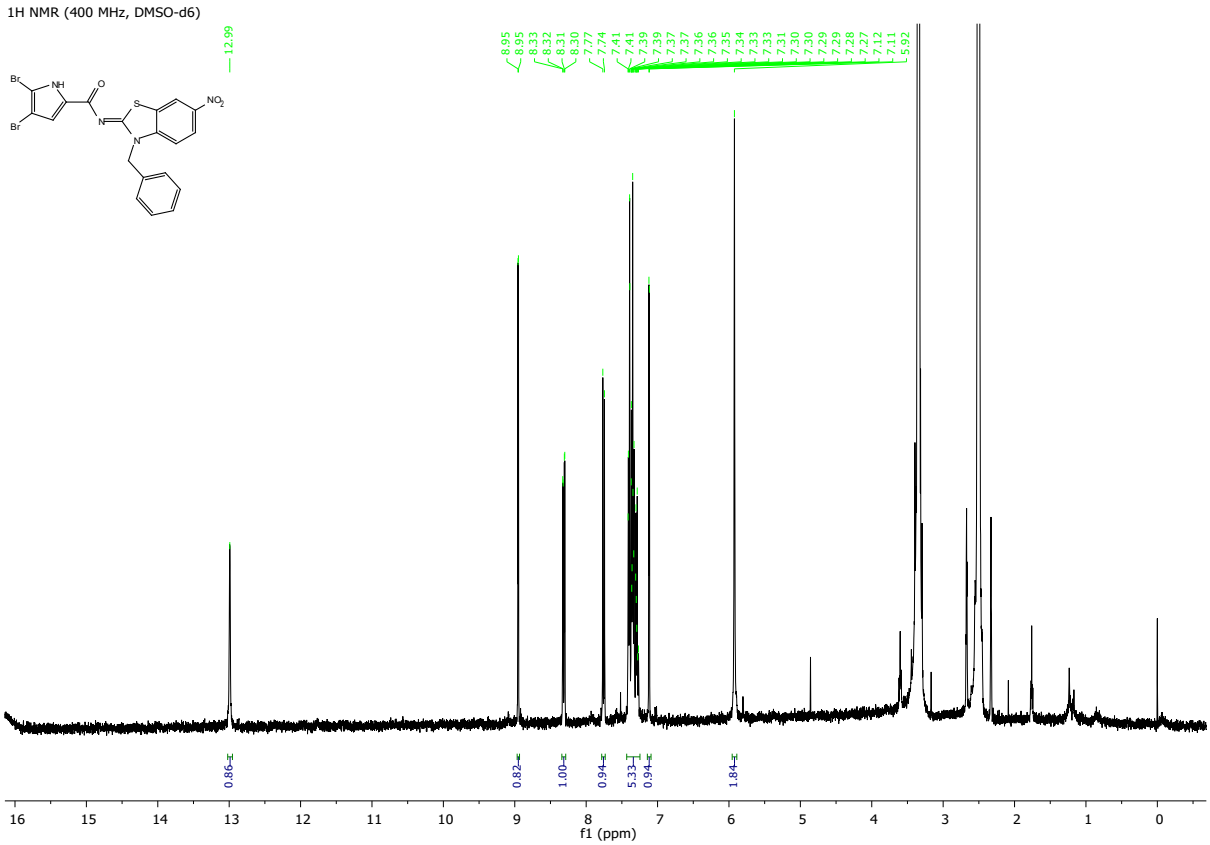
^{1}H and ^{13}C NMR spectra of the representative compounds

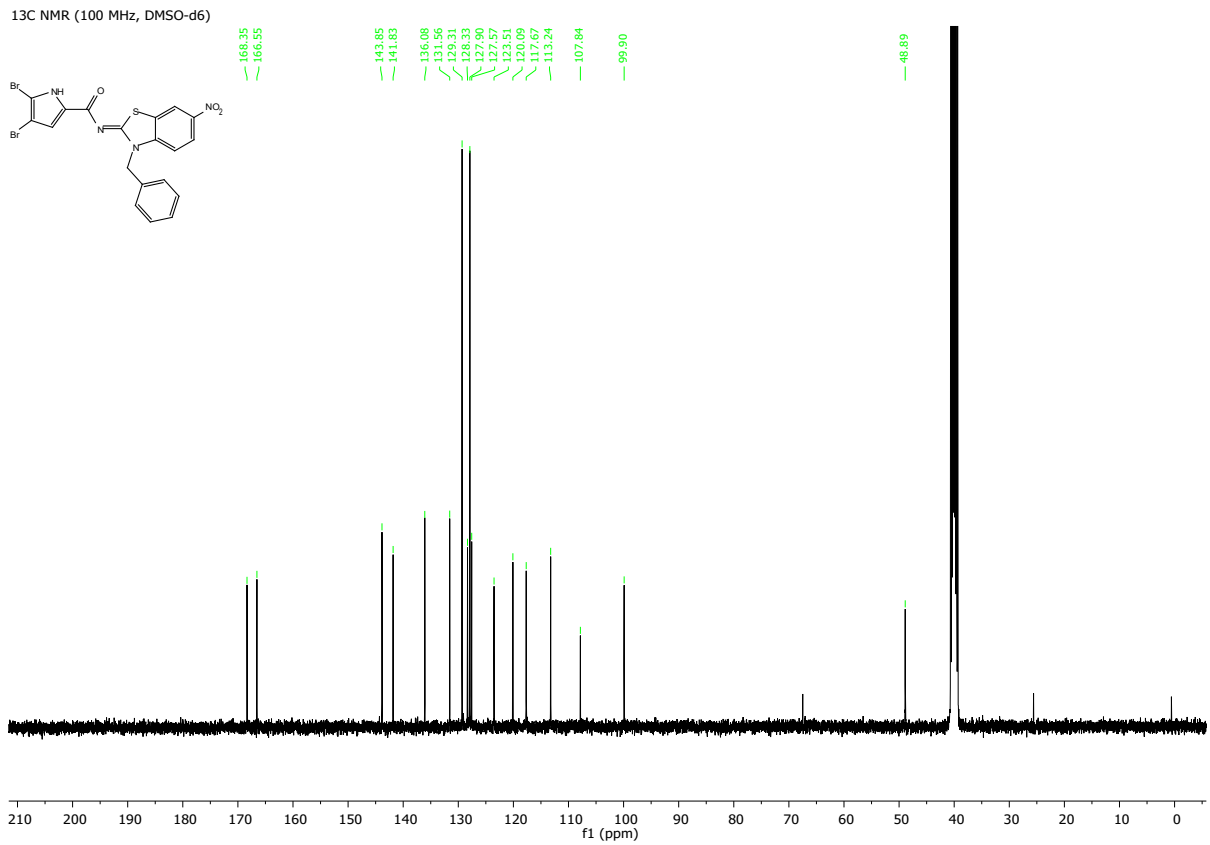
*4,5-Dibromo-N-(6-nitrobenzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamide (**6**)*



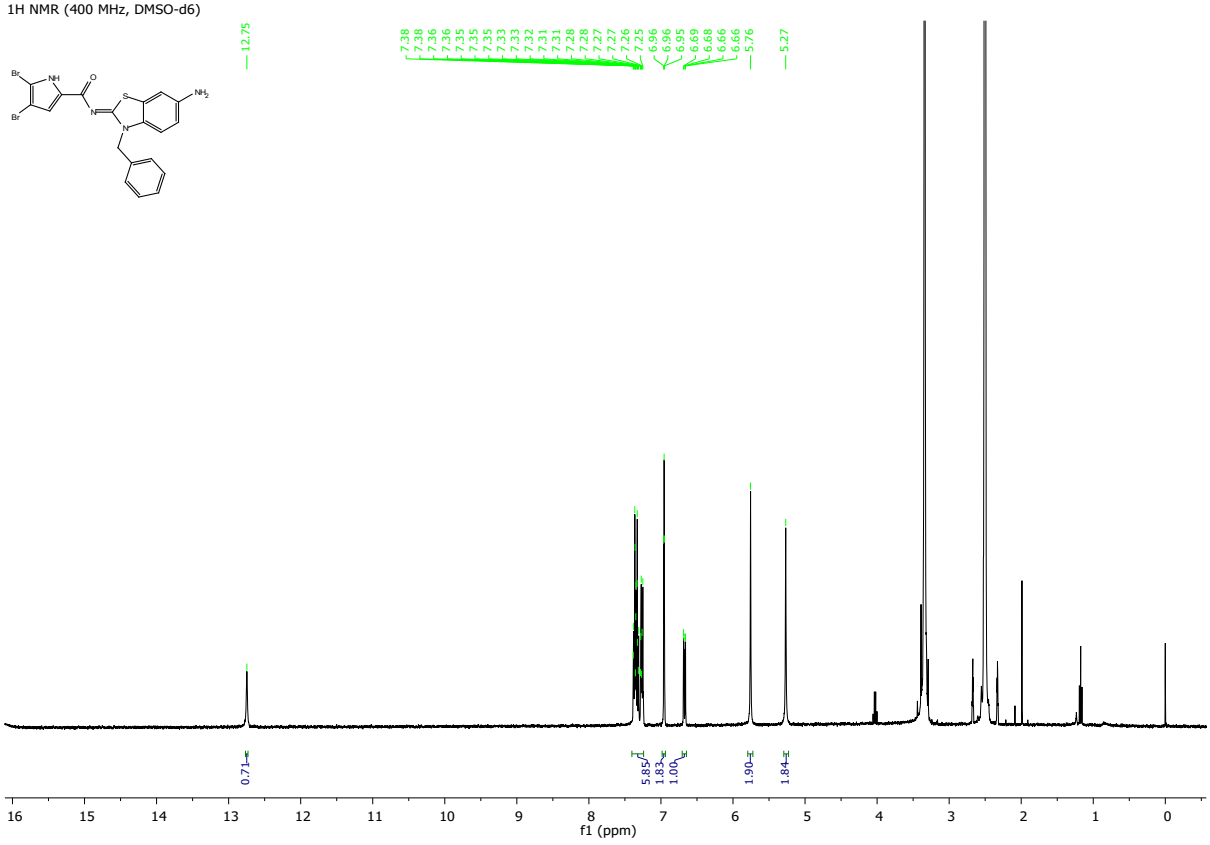

13C NMR (100 MHz, DMSO-d6)
164.95
158.47
154.10
143.23
132.85
126.44
122.24
120.59
119.34
116.99
109.78
99.57
Br
NH
O
Br
NH
S
N
NO2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

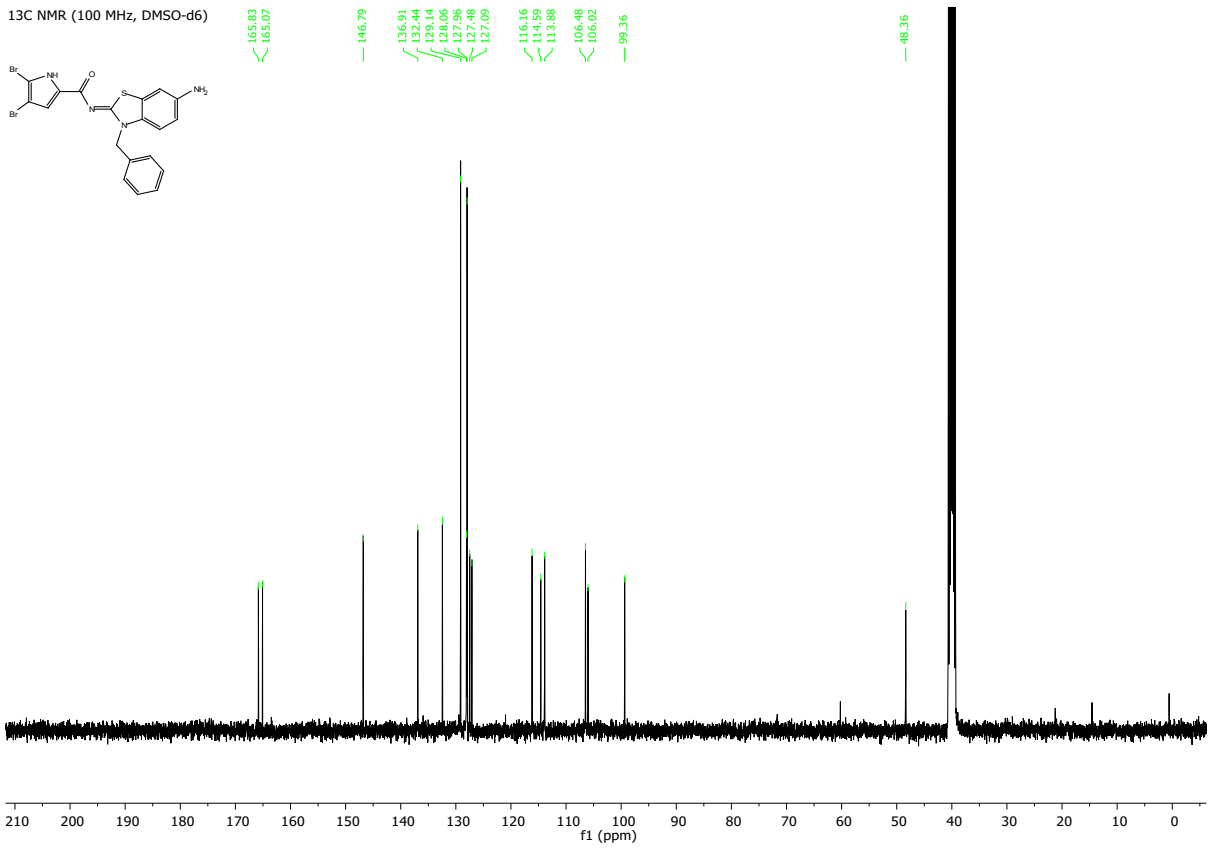
*(Z)-N-(3-Benzyl-6-nitrobenzo[d]thiazol-2(3H)-ylidene)-4,5-dibromo-1H-pyrrole-2-carboxamide (**7**)*



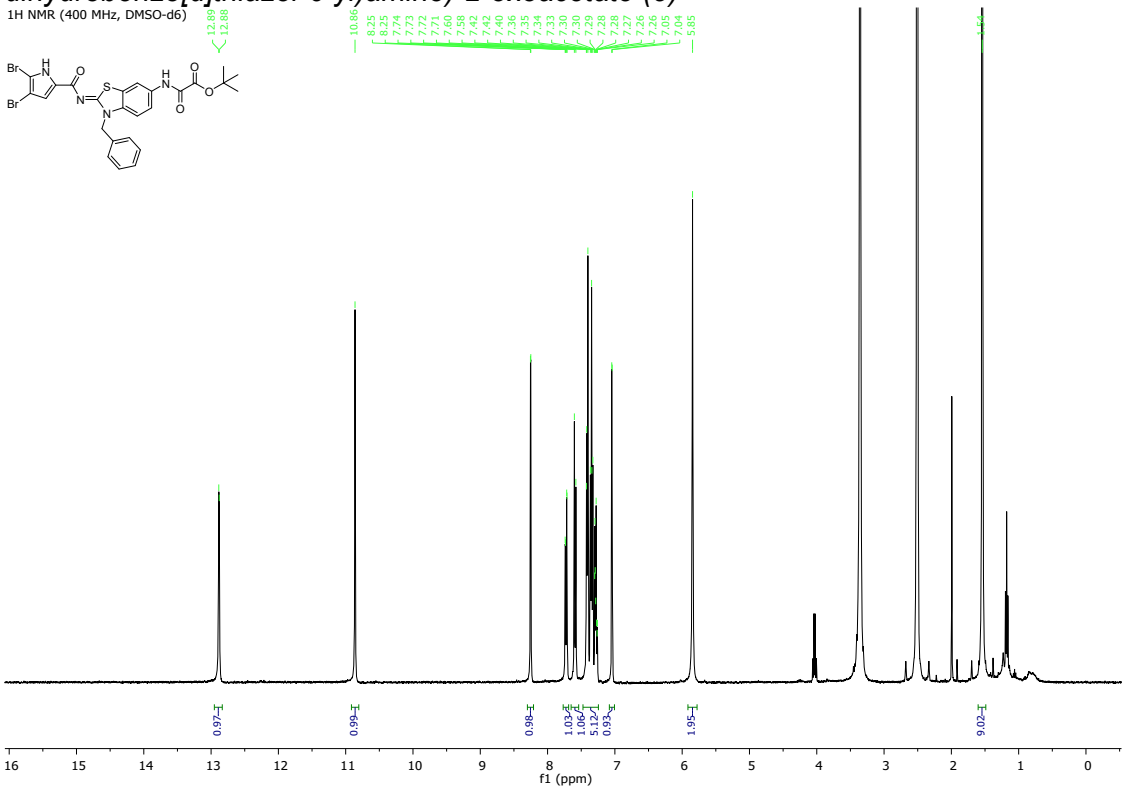


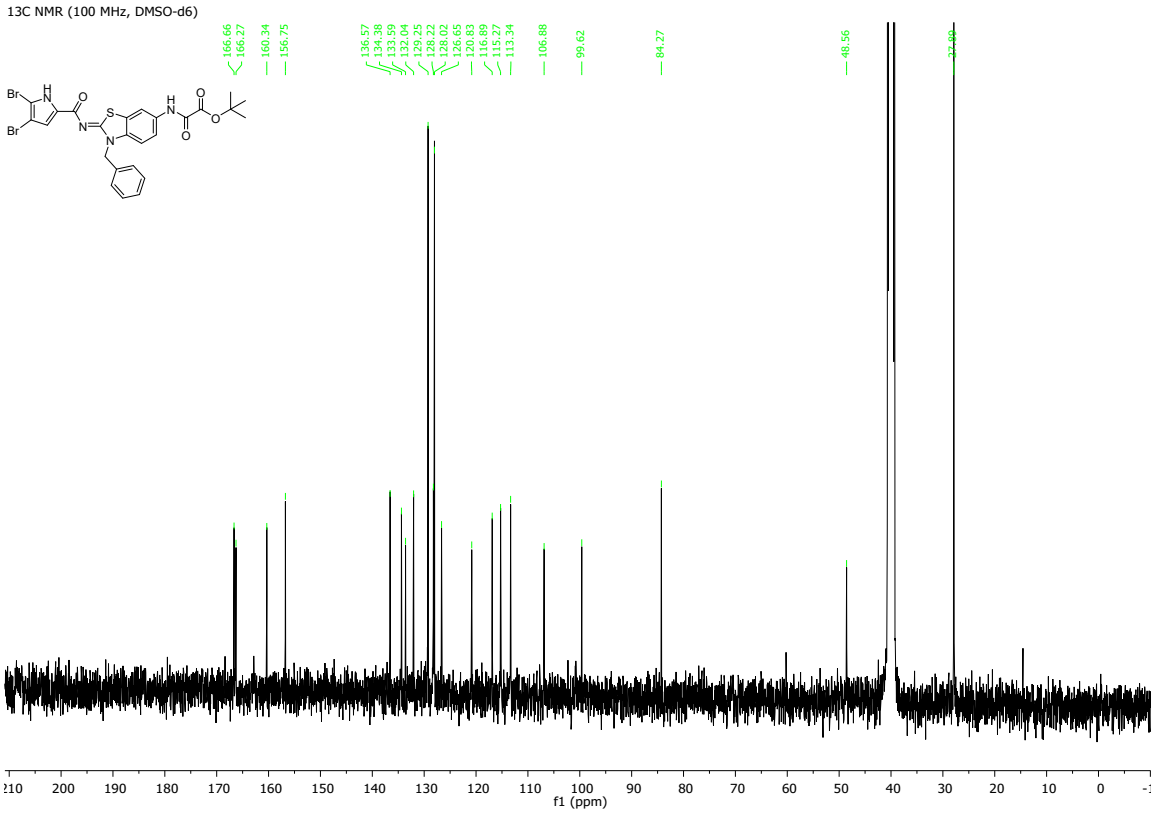
*(Z)-N-(6-Amino-3-benzylbenzo[d]thiazol-2(3H)-ylidene)-4,5-dibromo-1H-pyrrole-2-carboxamide (**8**)*



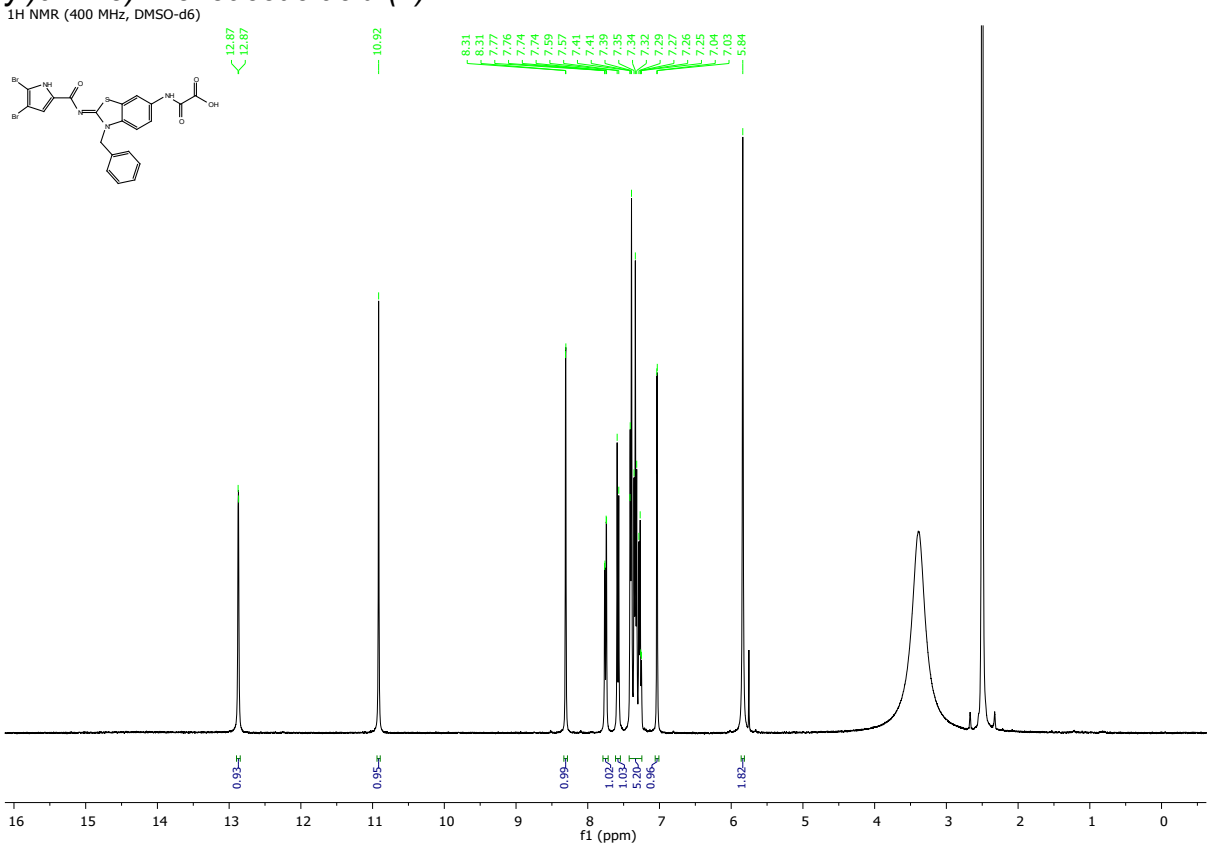


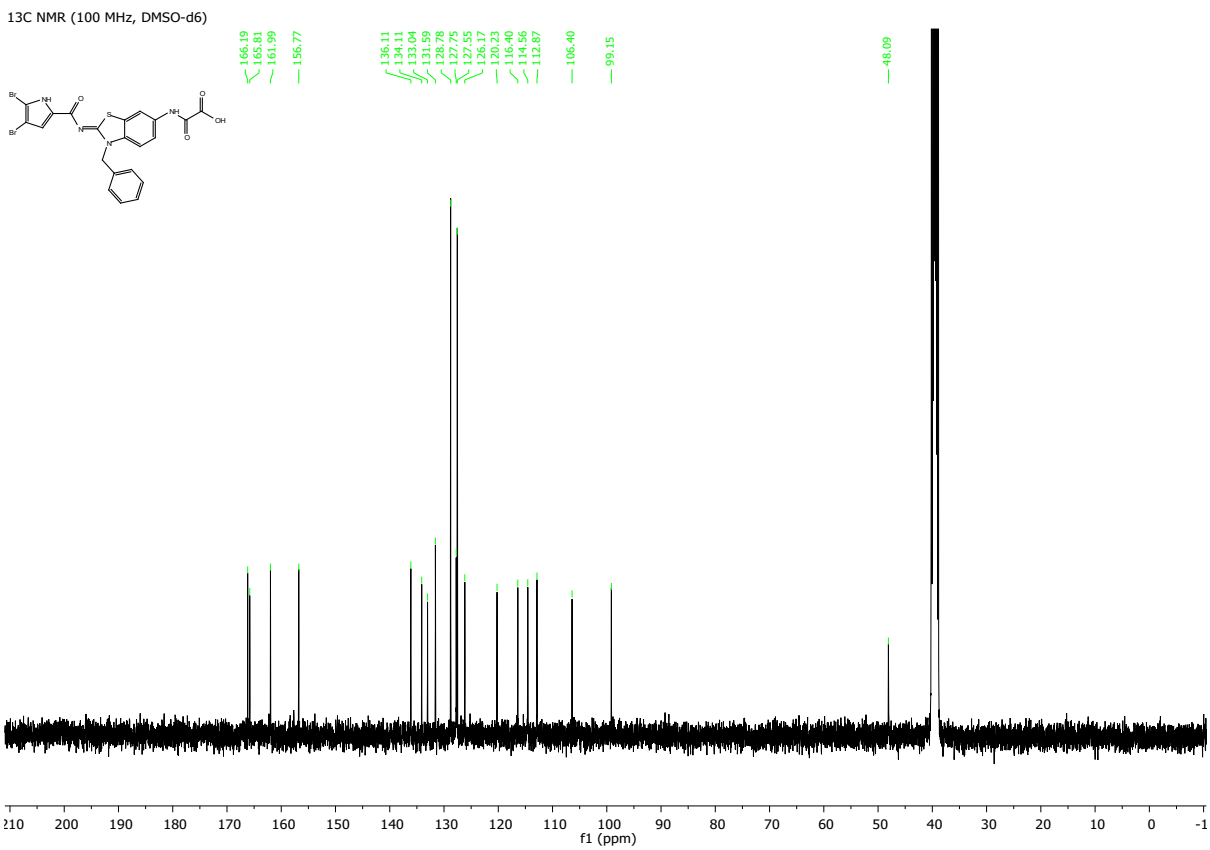
*tert-Butyl(Z)-2-((3-benzyl-2-((4,5-dibromo-1H-pyrrole-2-carbonyl)imino)-2,3-dihydrobenzo[d]thiazol-6-yl)amino)-2-oxoacetate (**9**)*





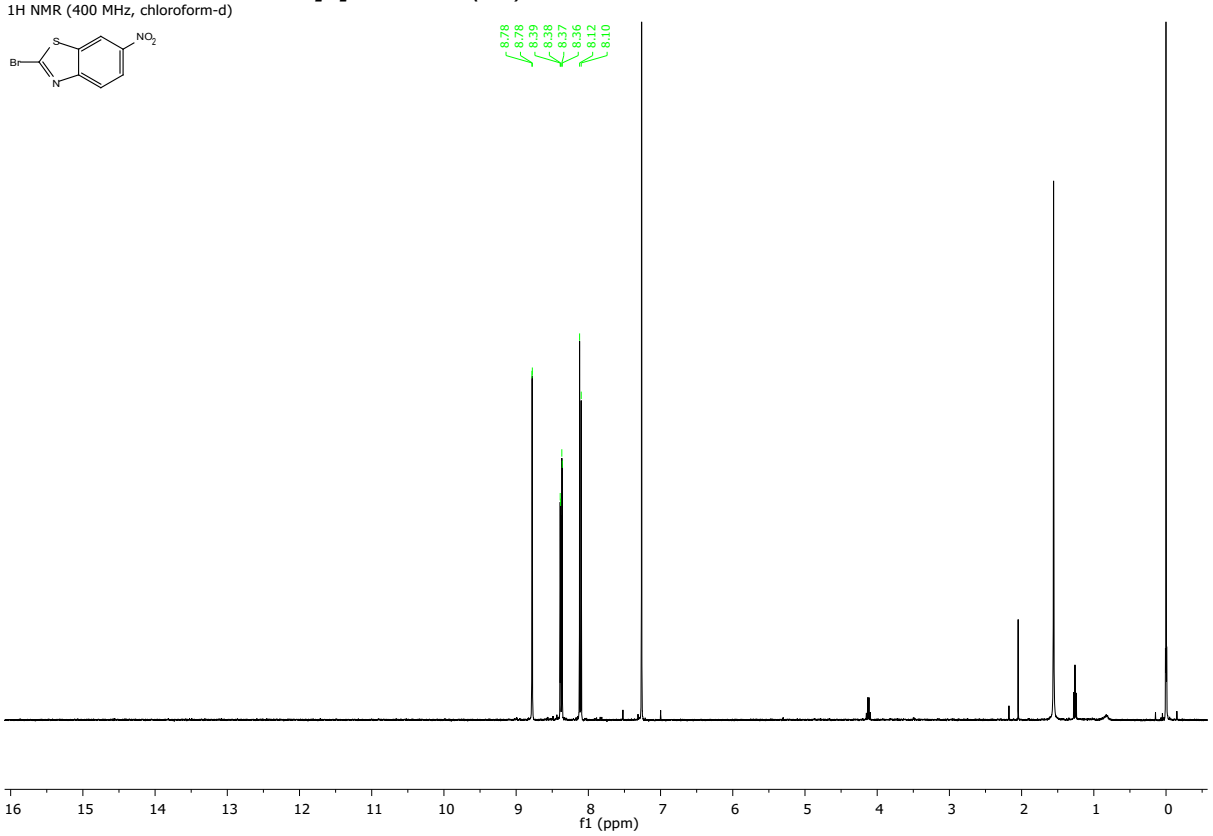
*(Z)-2-((3-Benzyl-2-((4,5-dibromo-1H-pyrrole-2-carbonyl)imino)-2,3-dihydrobenzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (**2**)*





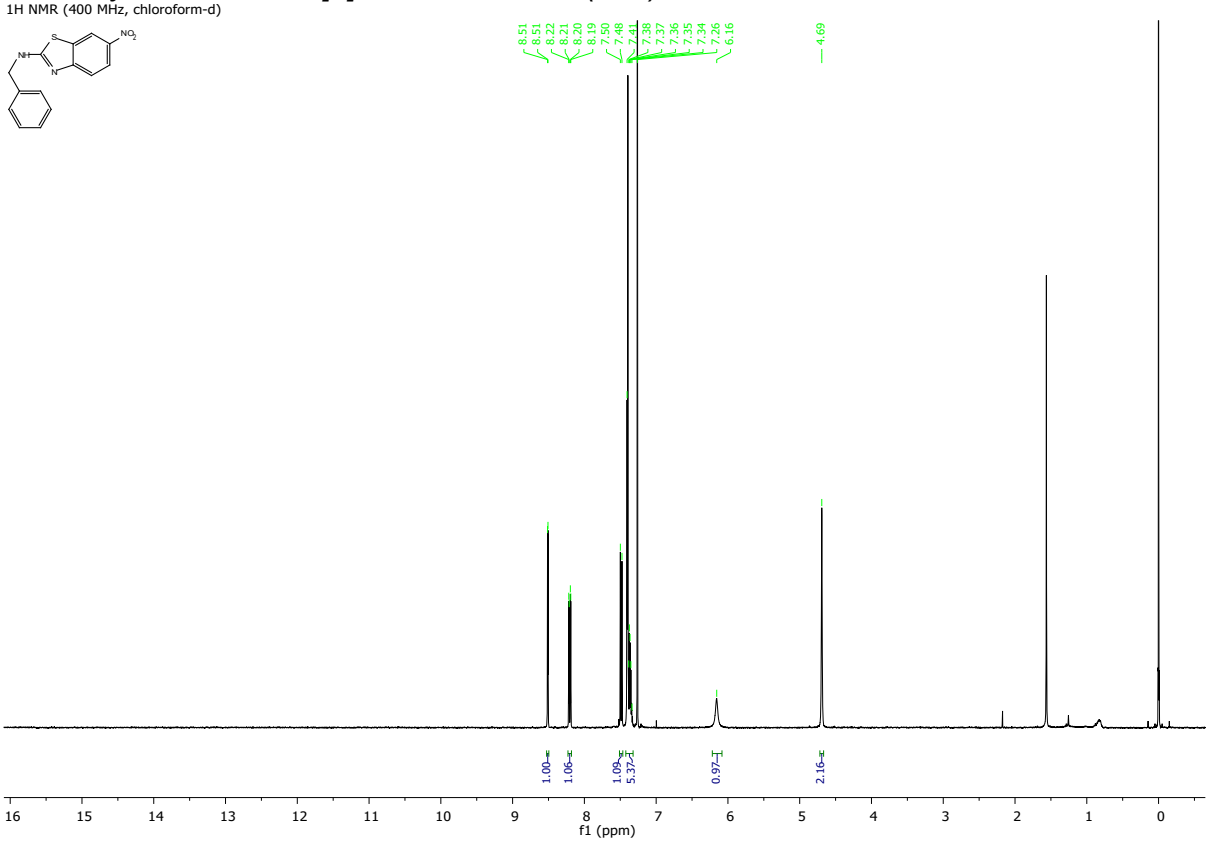
*2-Bromo-6-nitrobenzo[d]thiazole (**10**)*

1H NMR (400 MHz, chloroform-d)

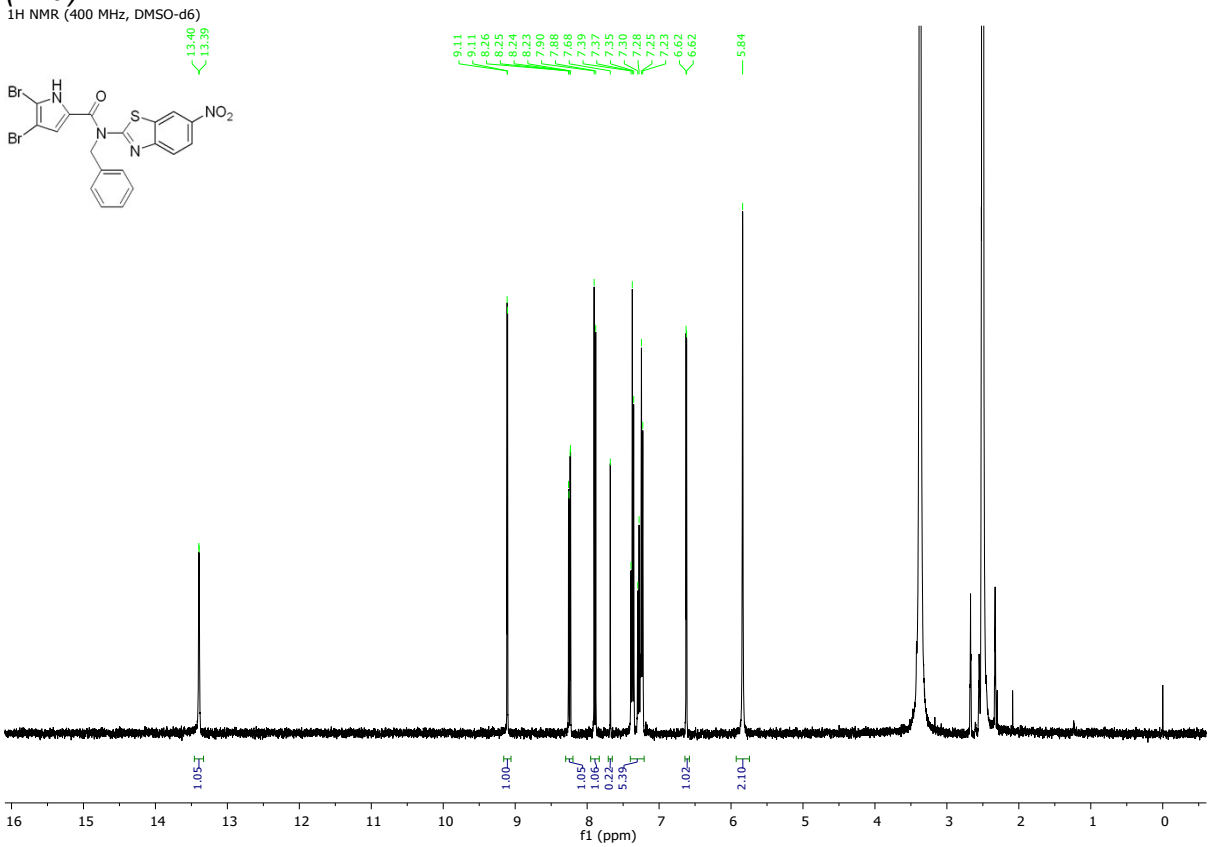


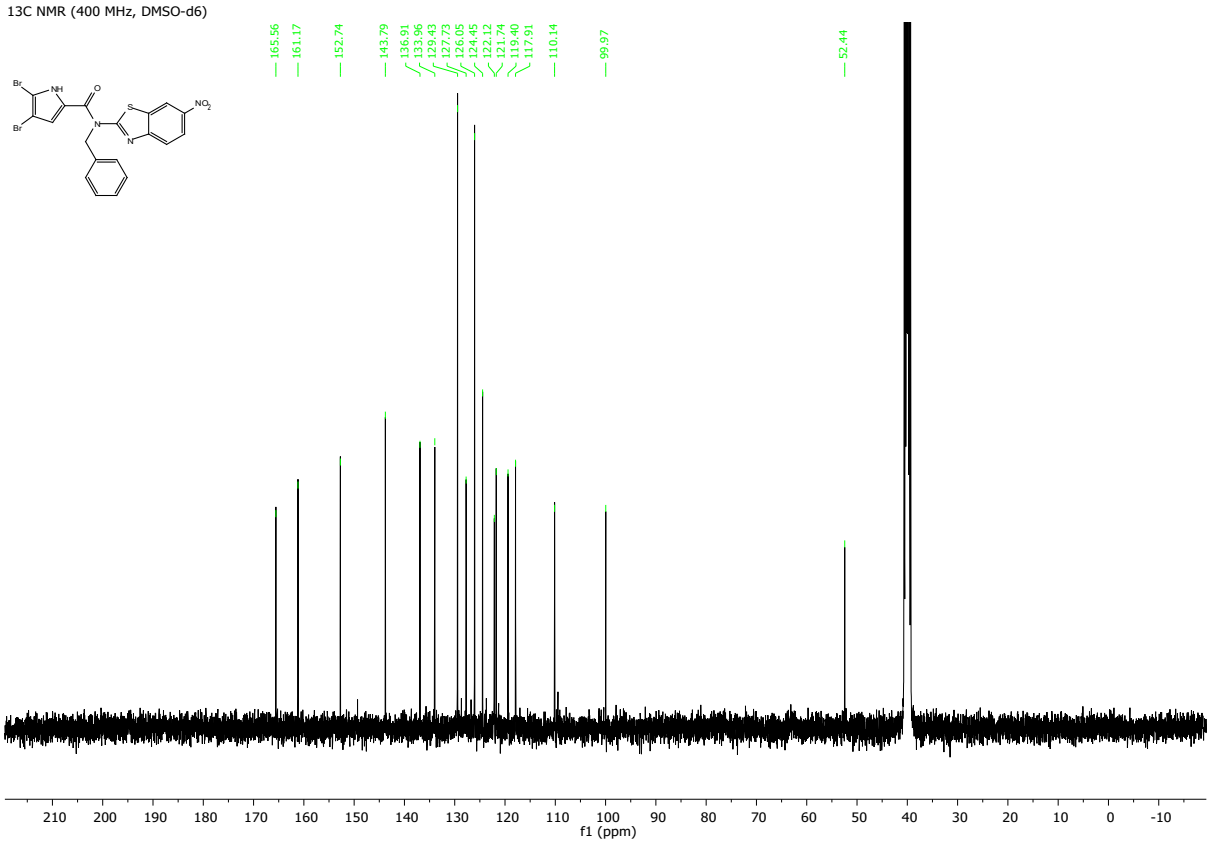
*N-Benzyl-6-nitrobenzo[d]thiazol-2-amine (**11a**)*

1H NMR (400 MHz, chloroform-d)



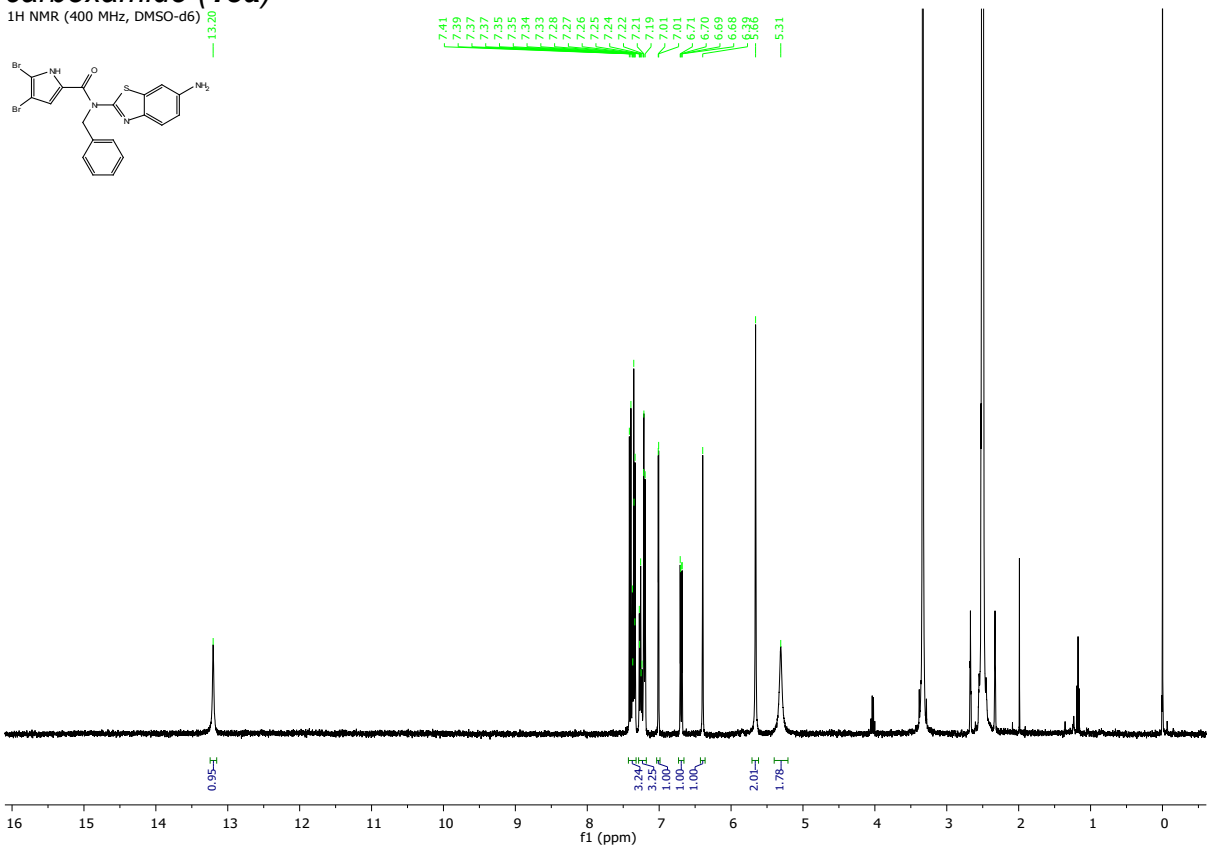
*N-Benzyl-4,5-dibromo-N-(6-nitrobenzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamide (**12a**)*



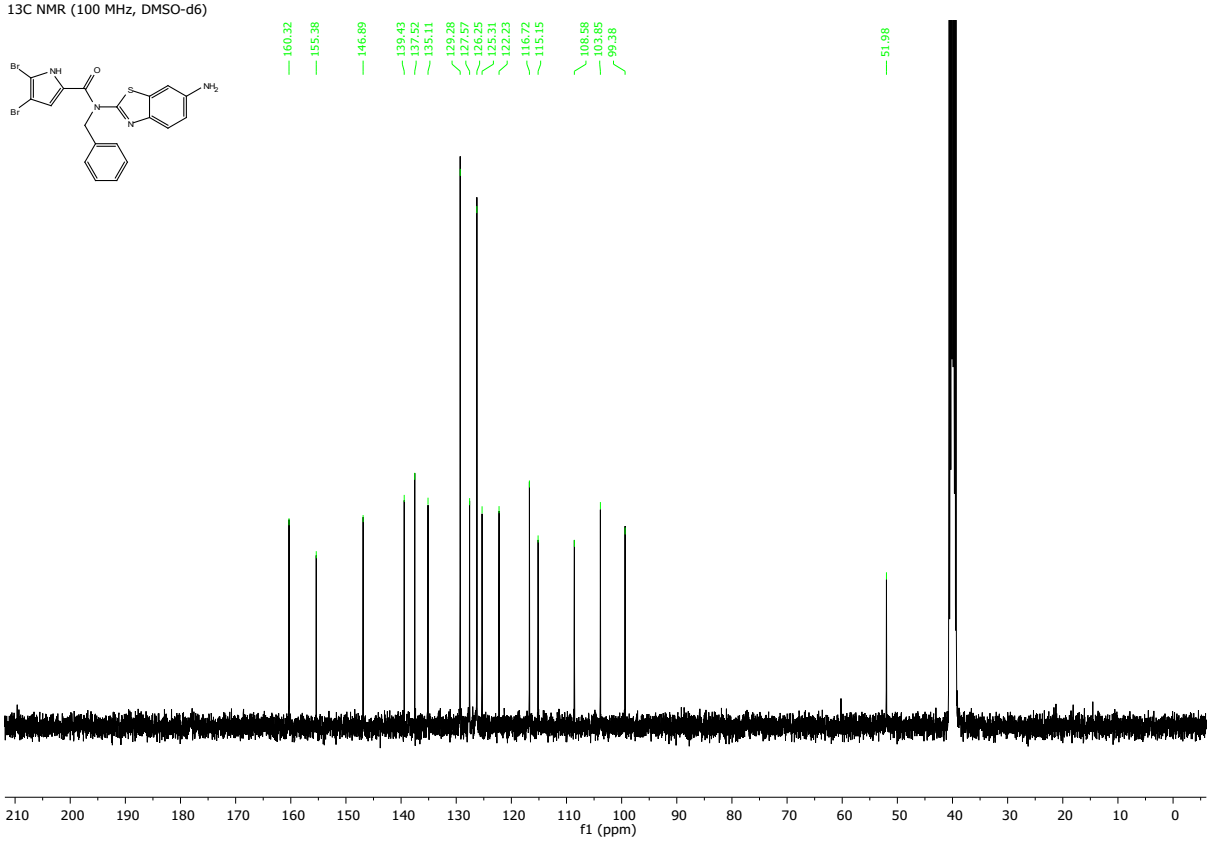


*N-(6-Aminobenzo[d]thiazol-2-yl)-N-benzyl-4,5-dibromo-1H-pyrrole-2-carboxamide (**13a**)*

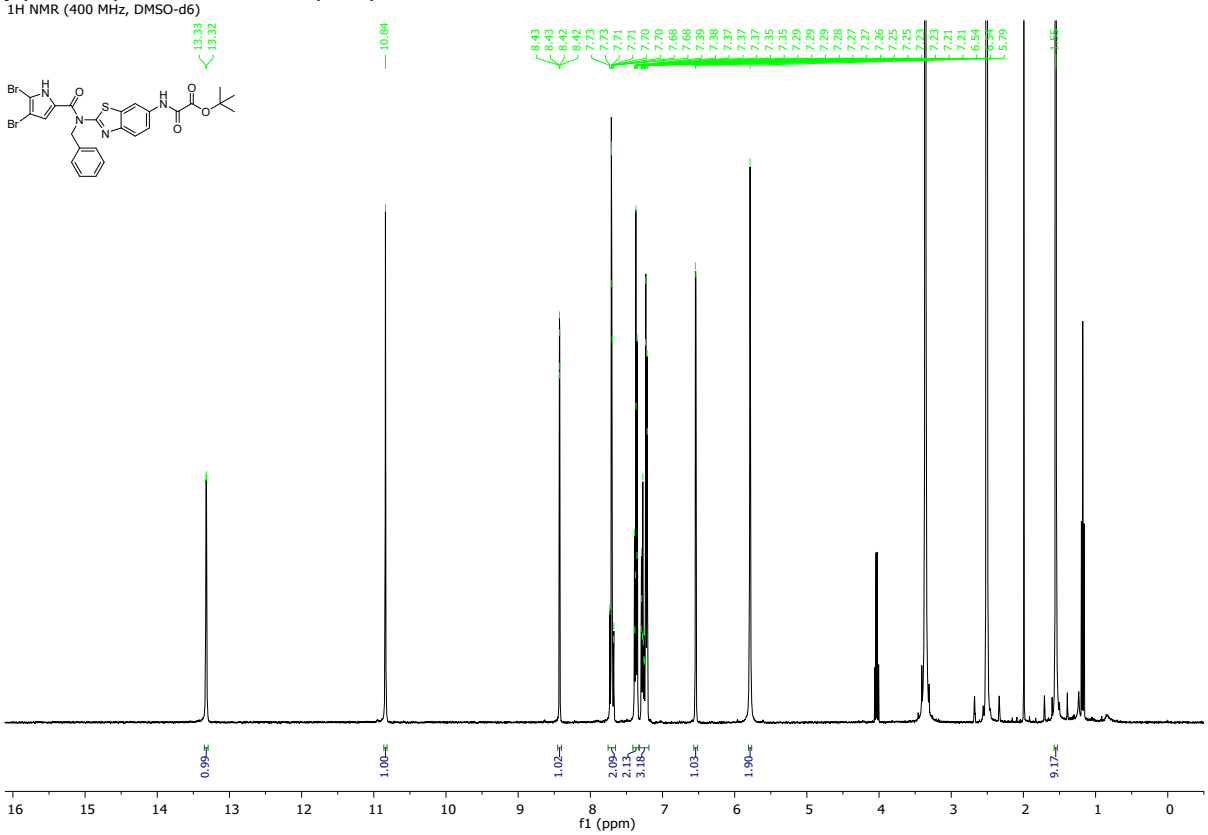
1H NMR (400 MHz, DMSO-d6)

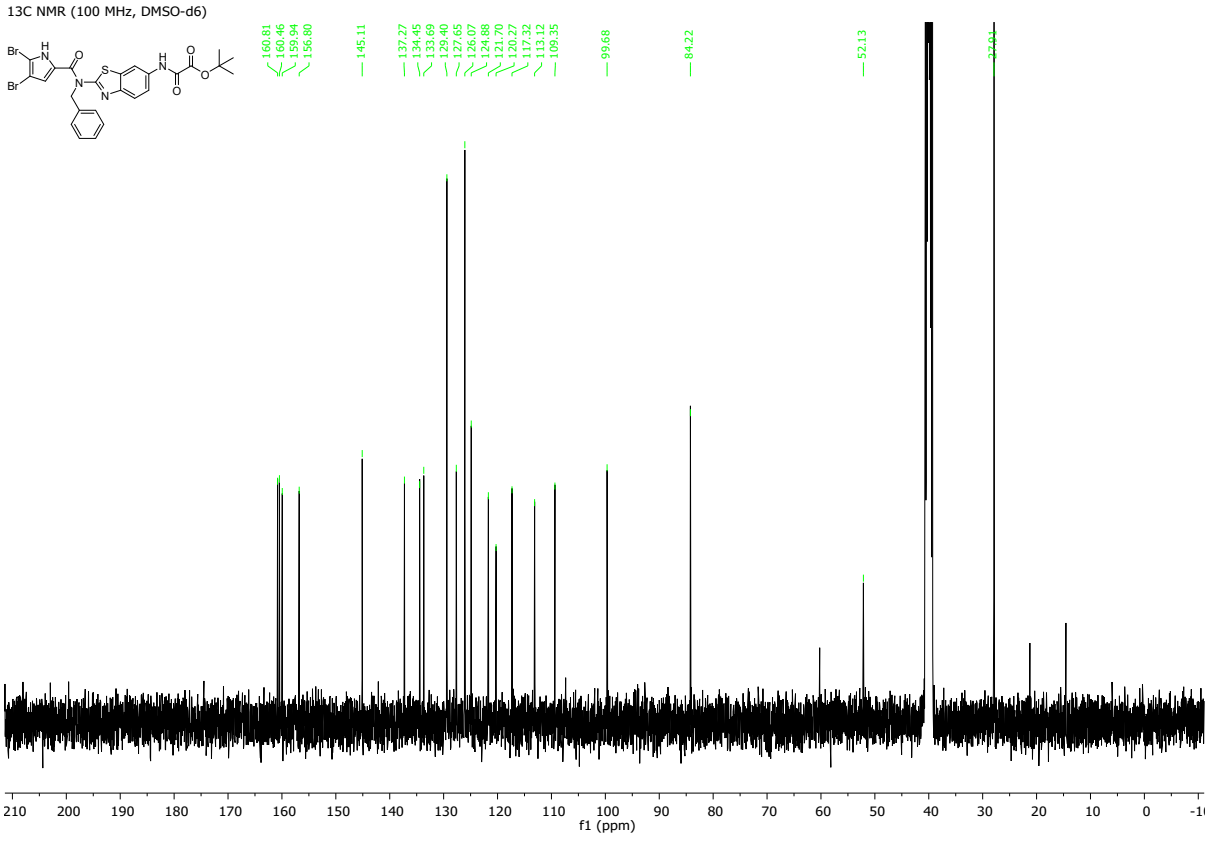


13C NMR (100 MHz, DMSO-d6)

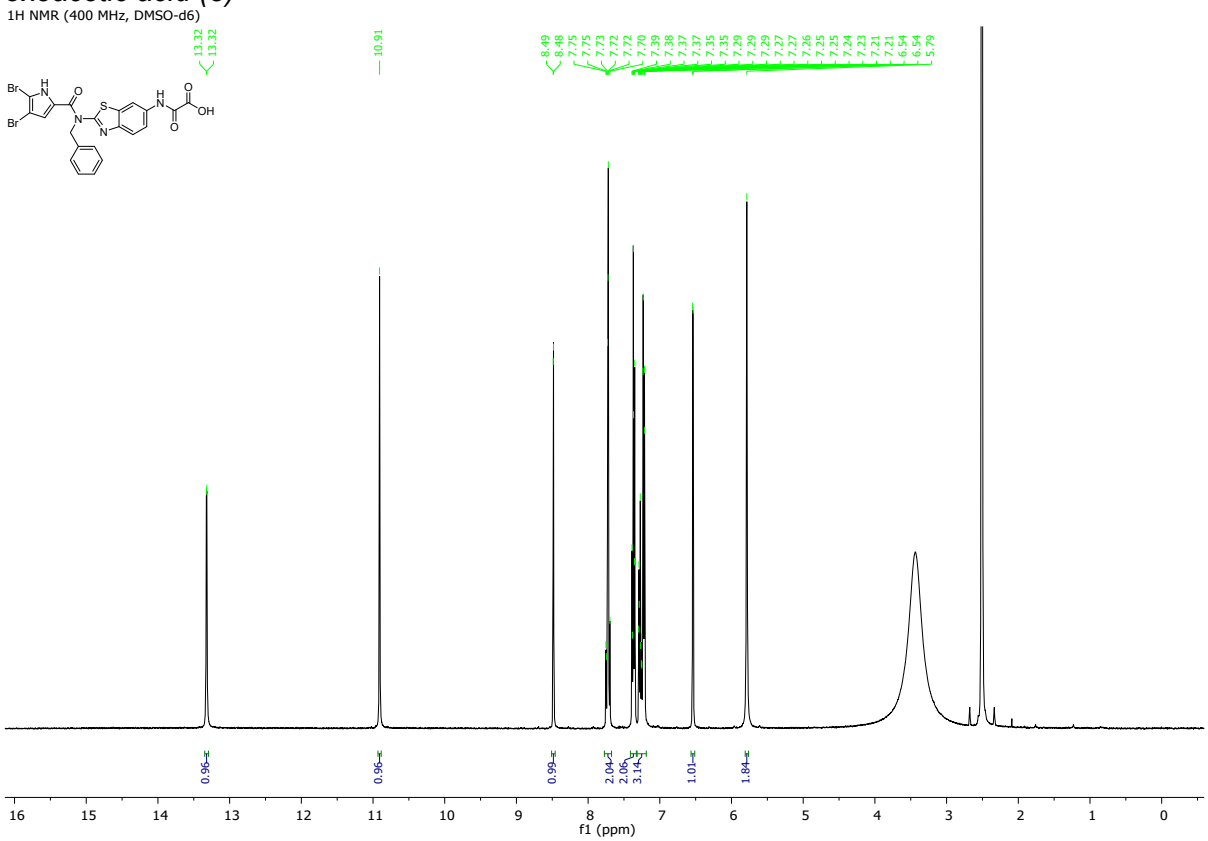


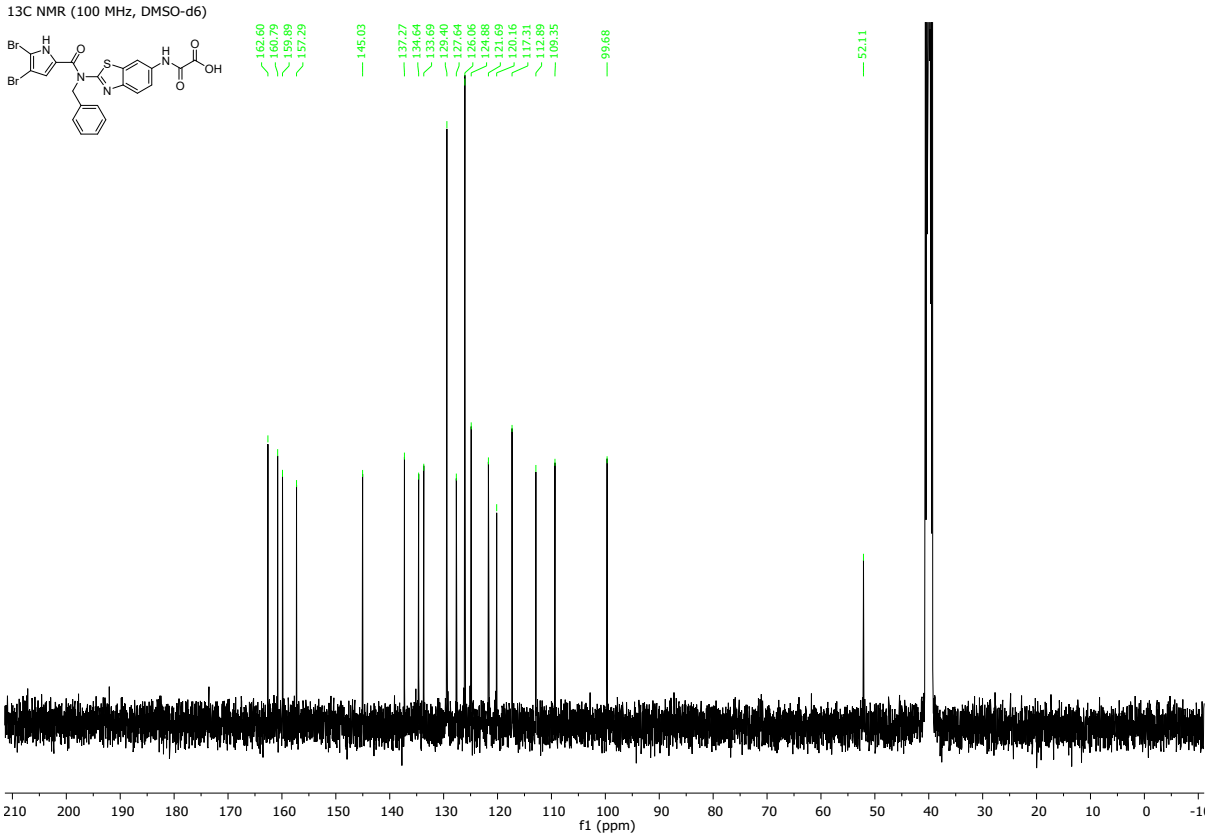
*tert-Butyl2-((2-(N-benzyl-4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (**14a**)*





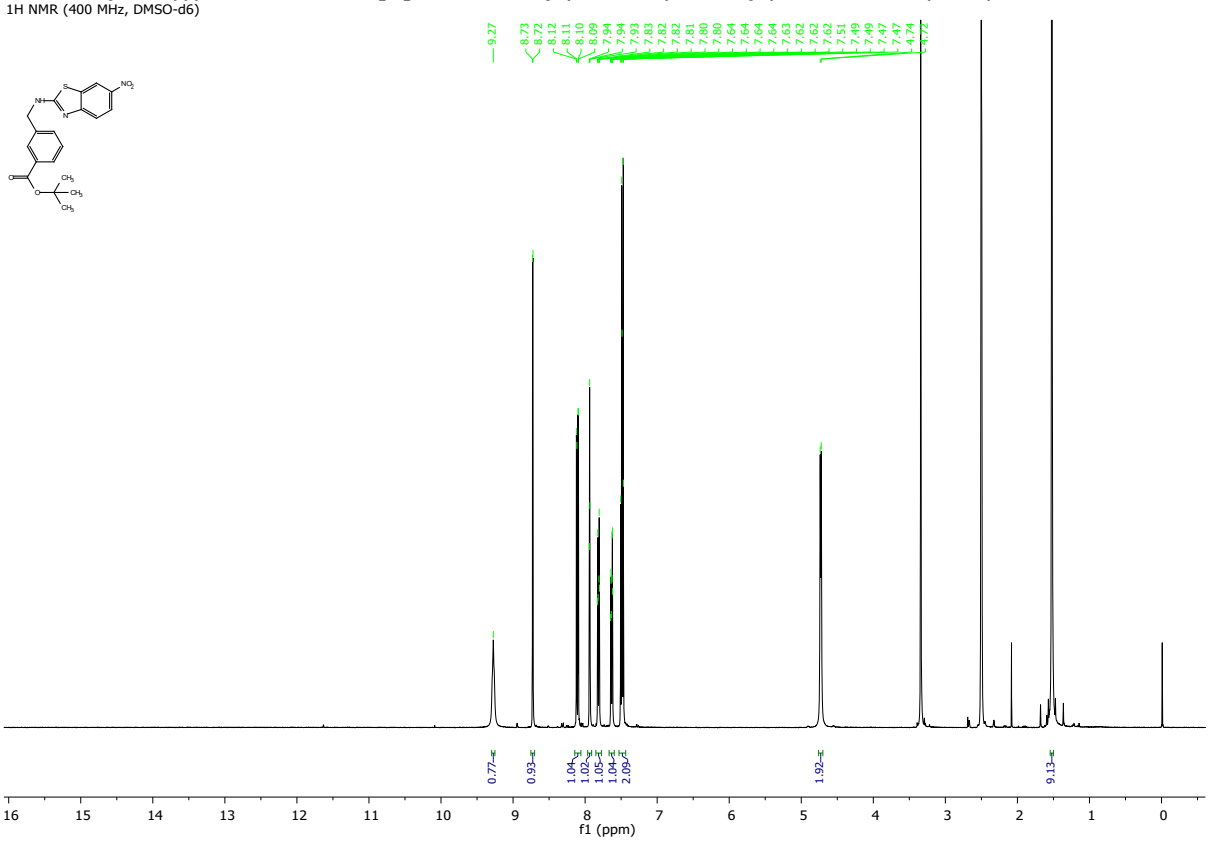
*2-((2-(N-Benzyl-4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (**3**)*



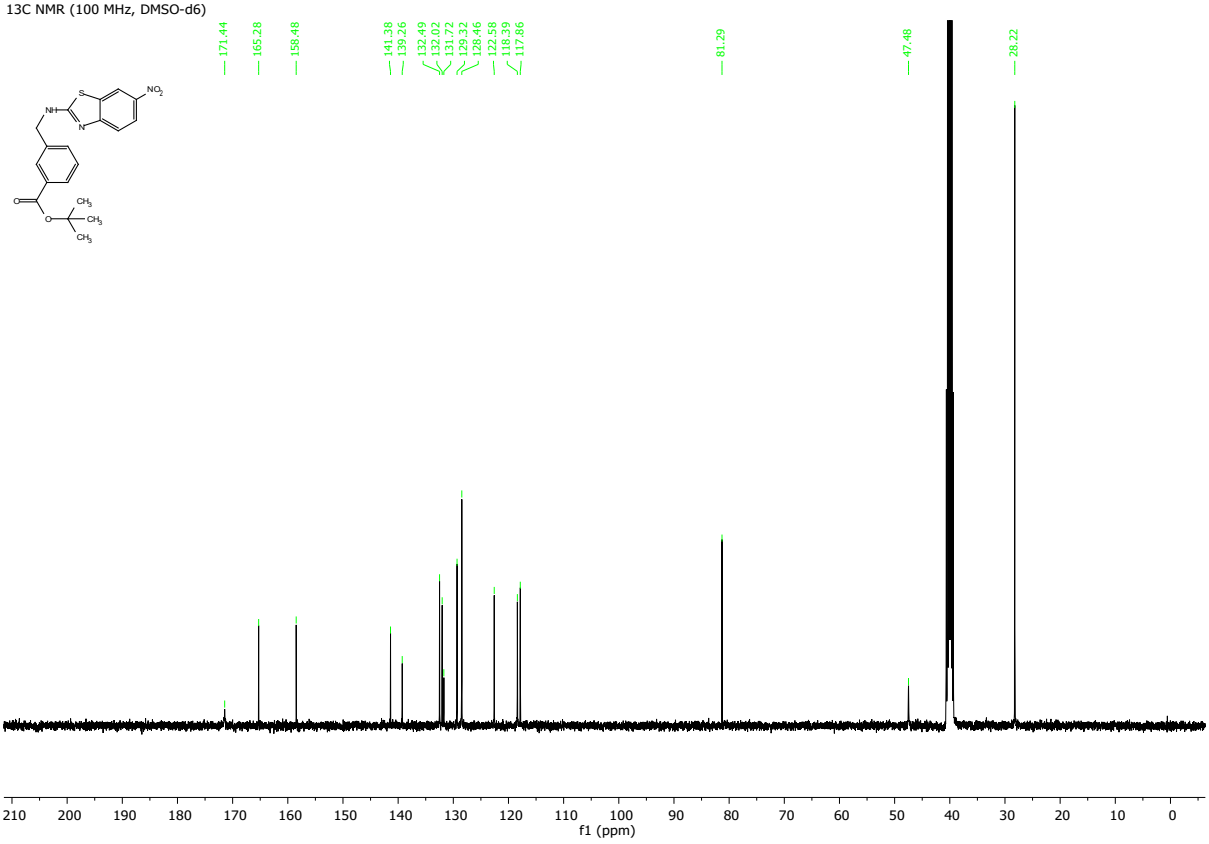


*tert-Butyl 3-(((6-nitrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (**11b**)*

1H NMR (400 MHz, DMSO-d6)

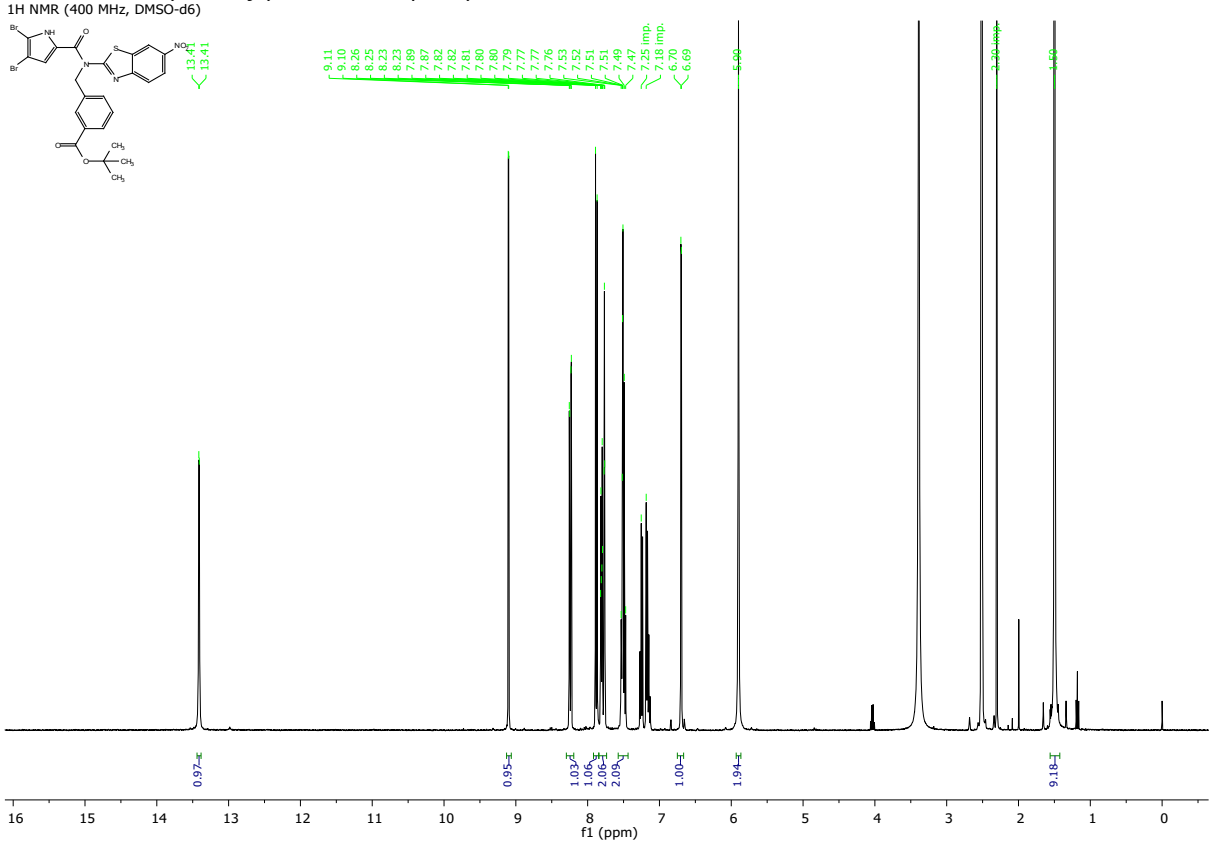


13C NMR (100 MHz, DMSO-d6)

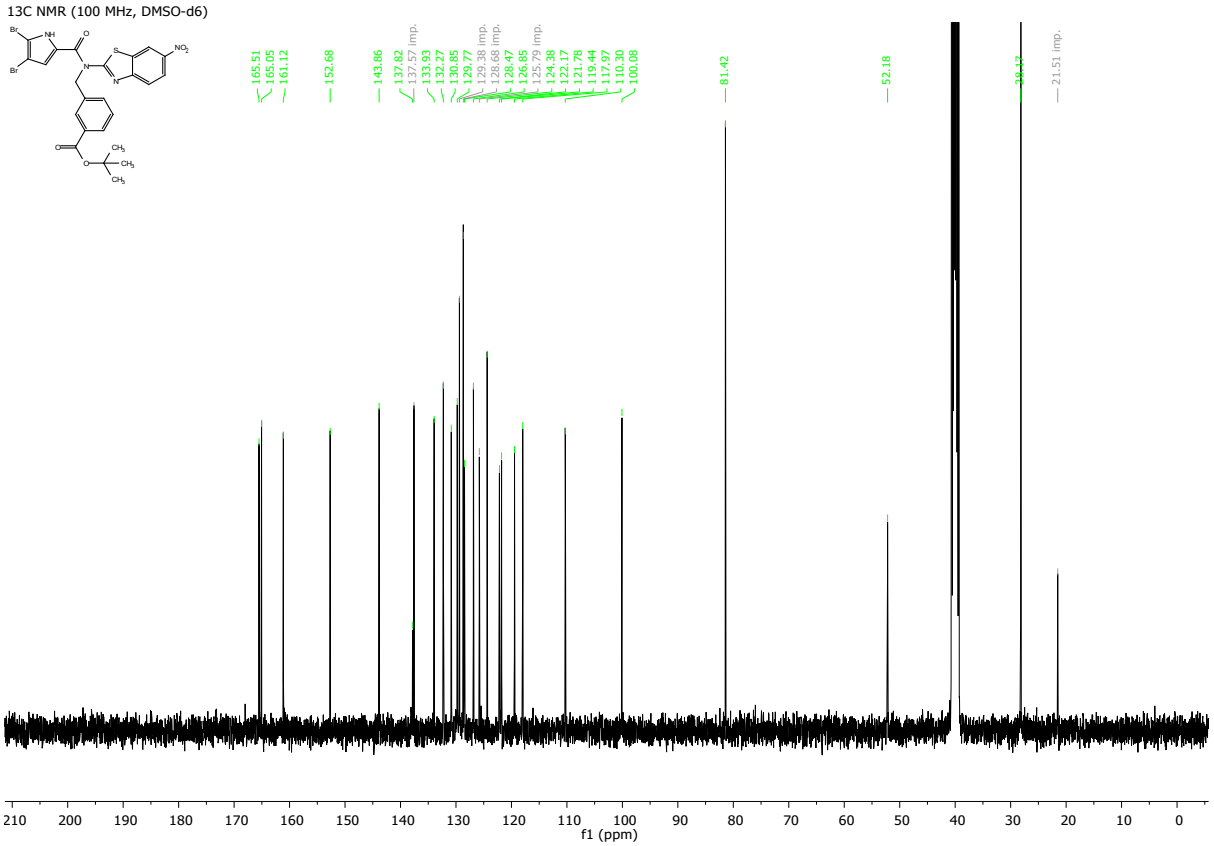


*tert-Butyl3-((4,5-dibromo-N-(6-nitrobenzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamido)methyl)benzoate (**12b**)*

1H NMR (400 MHz, DMSO-d6)

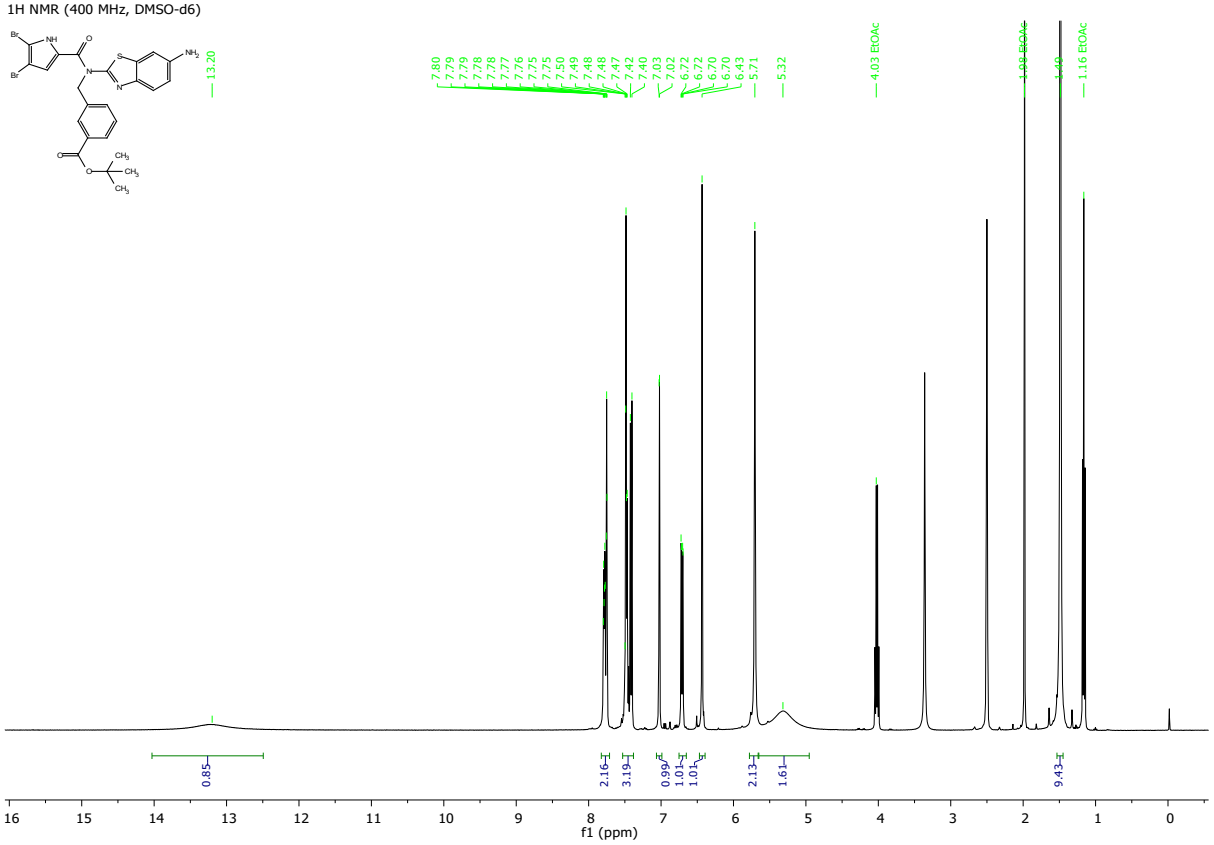


13C NMR (100 MHz, DMSO-d6)

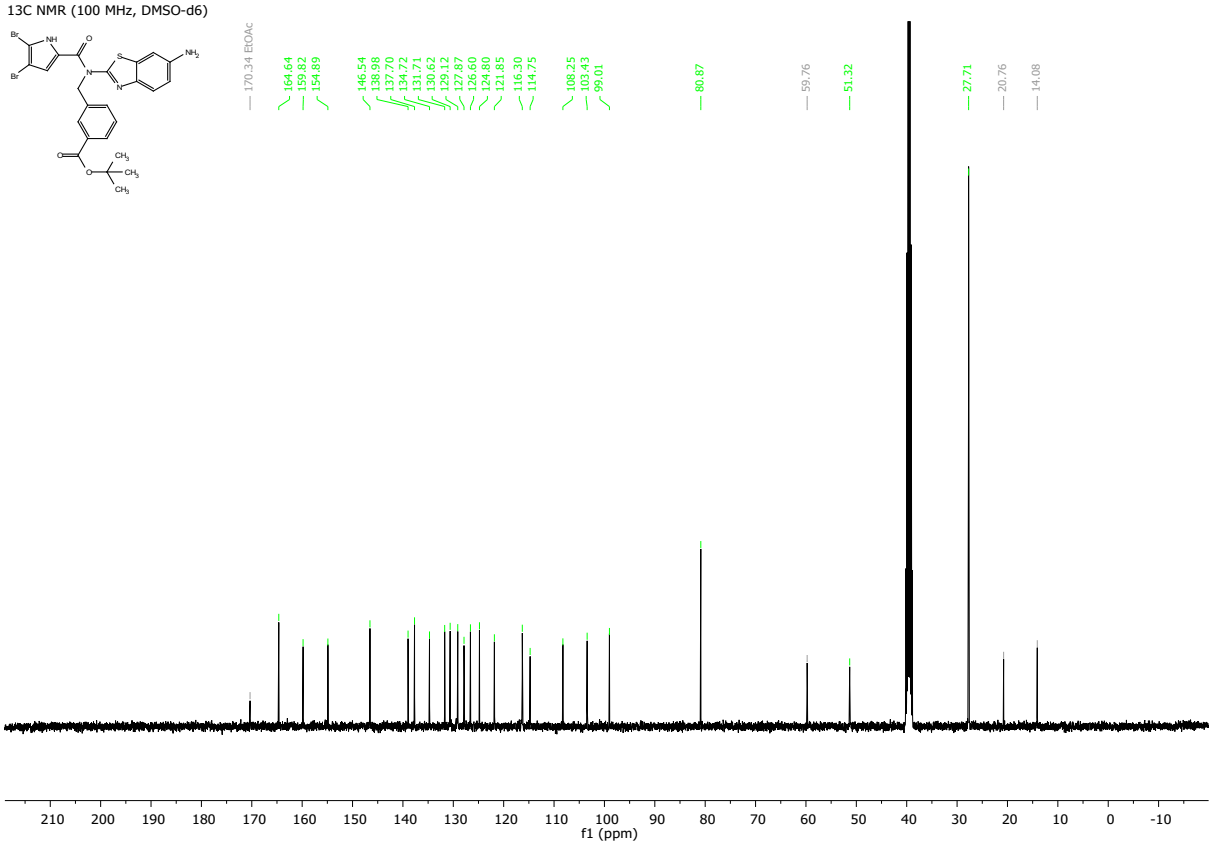


*tert-Butyl 3-((N-(6-aminobenzo[d]thiazol-2-yl)-4,5-dibromo-1H-pyrrole-2-carboxamido)methyl)benzoate (**13b**)*

1H NMR (400 MHz, DMSO-d6)

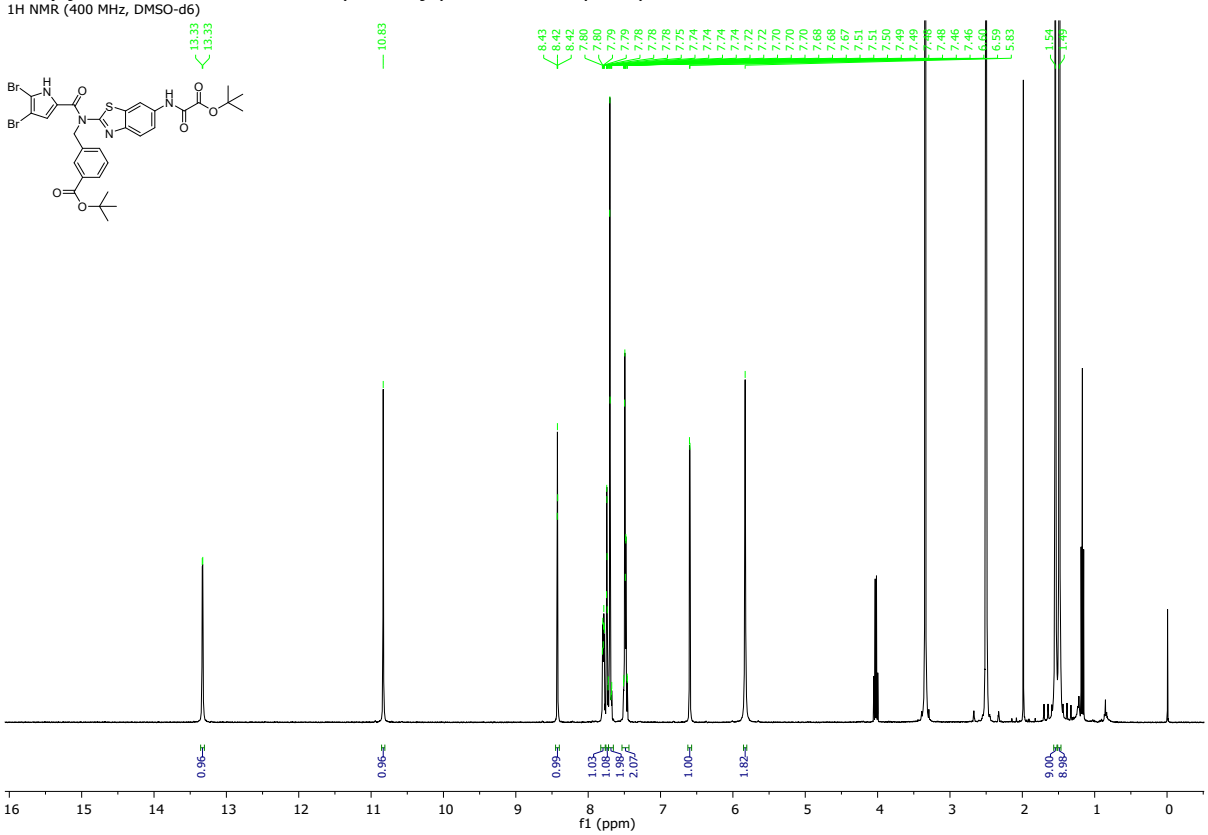


13C NMR (100 MHz, DMSO-d6)

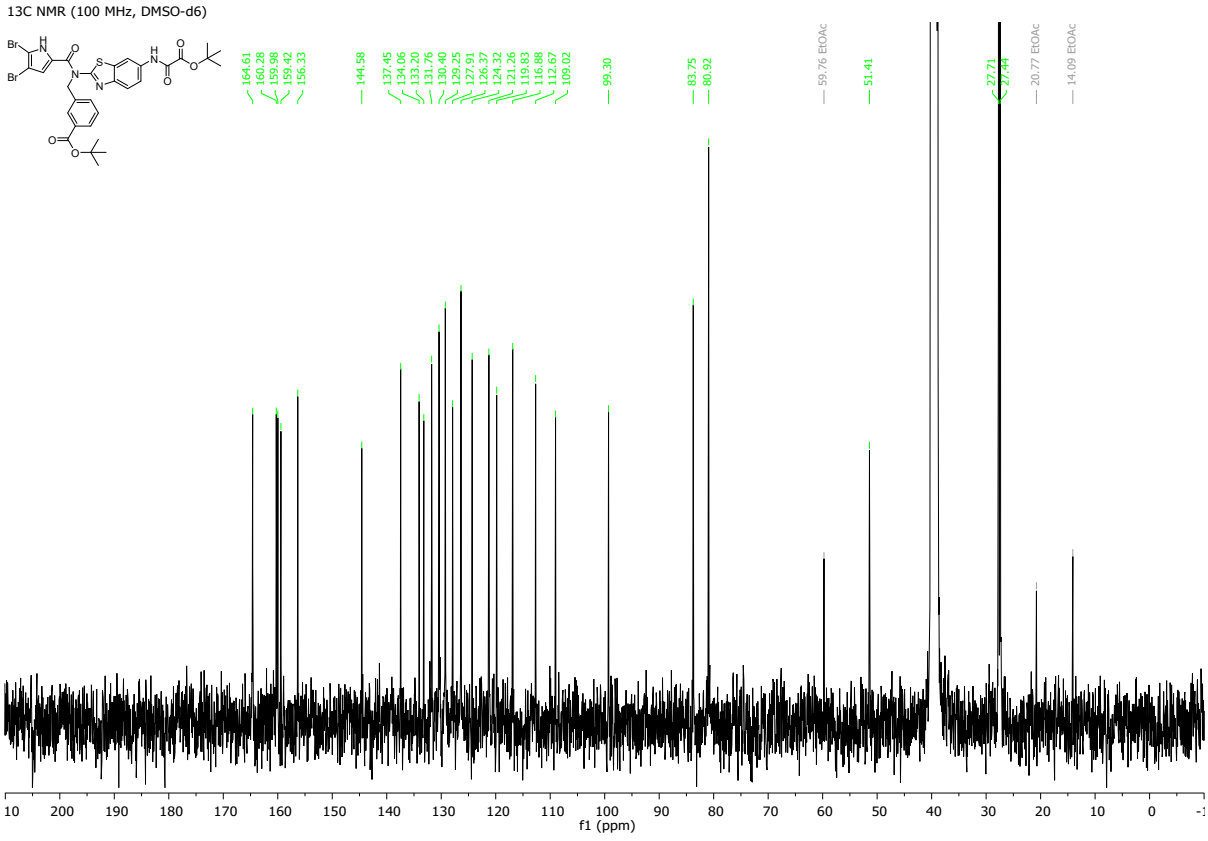


*tert-Butyl 3-((4,5-dibromo-N-(6-(2-(tert-butoxy)-2-oxoacetamido)benzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamido)methyl)benzoate (**14b**)*

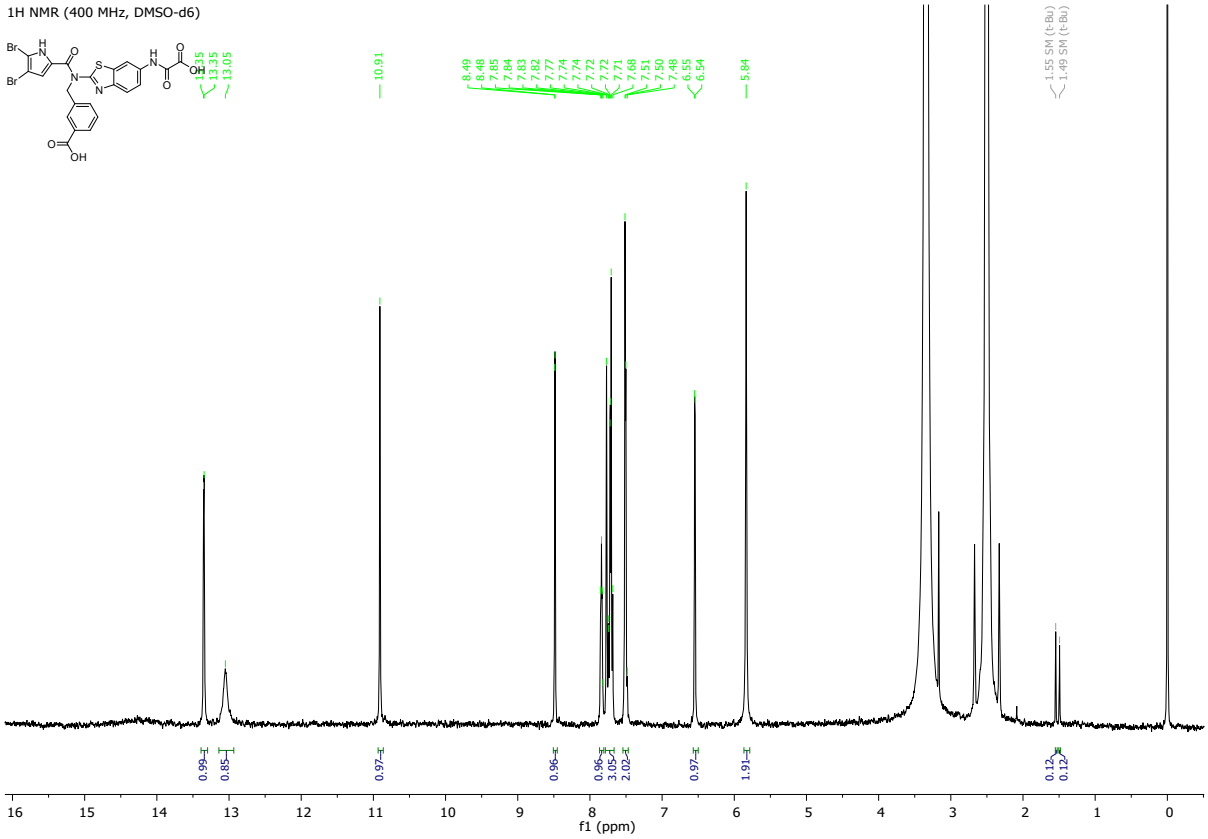
1H NMR (400 MHz, DMSO-d6)

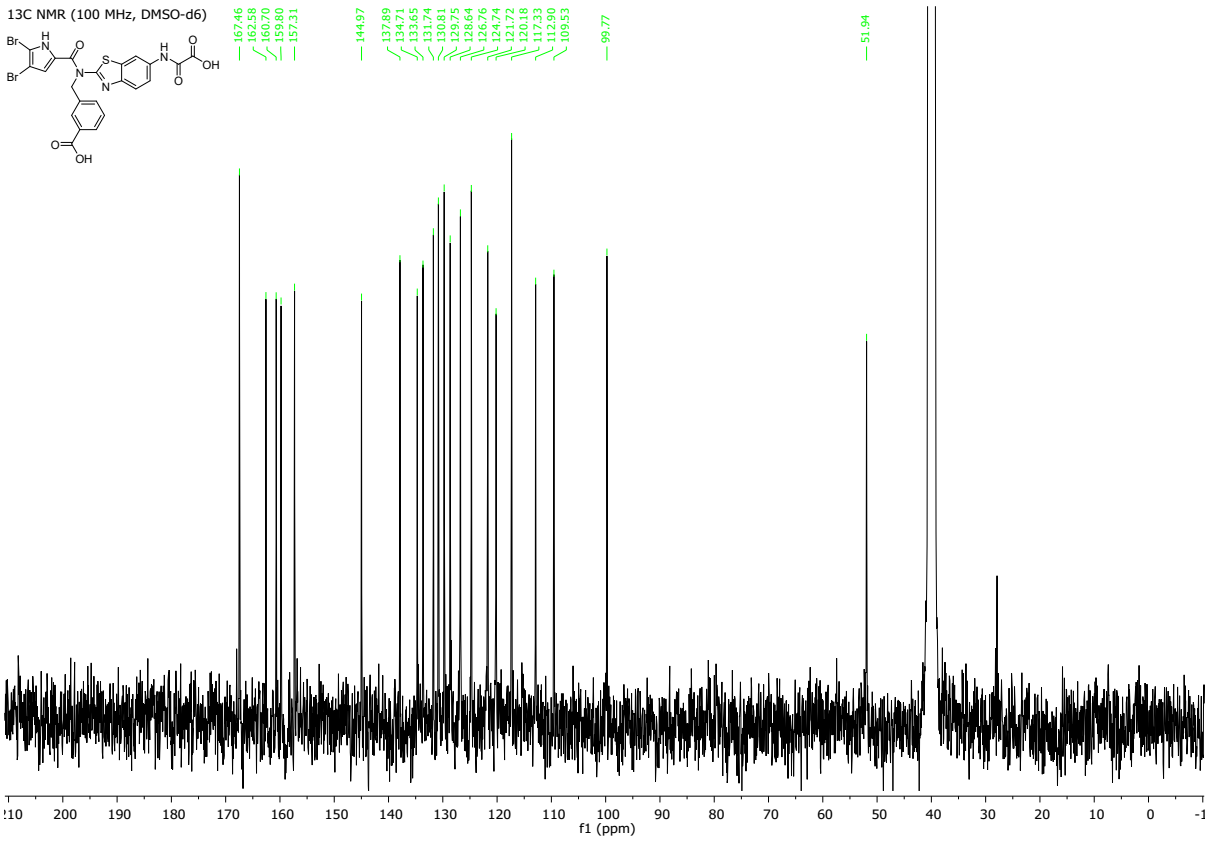


13C NMR (100 MHz, DMSO-d6)



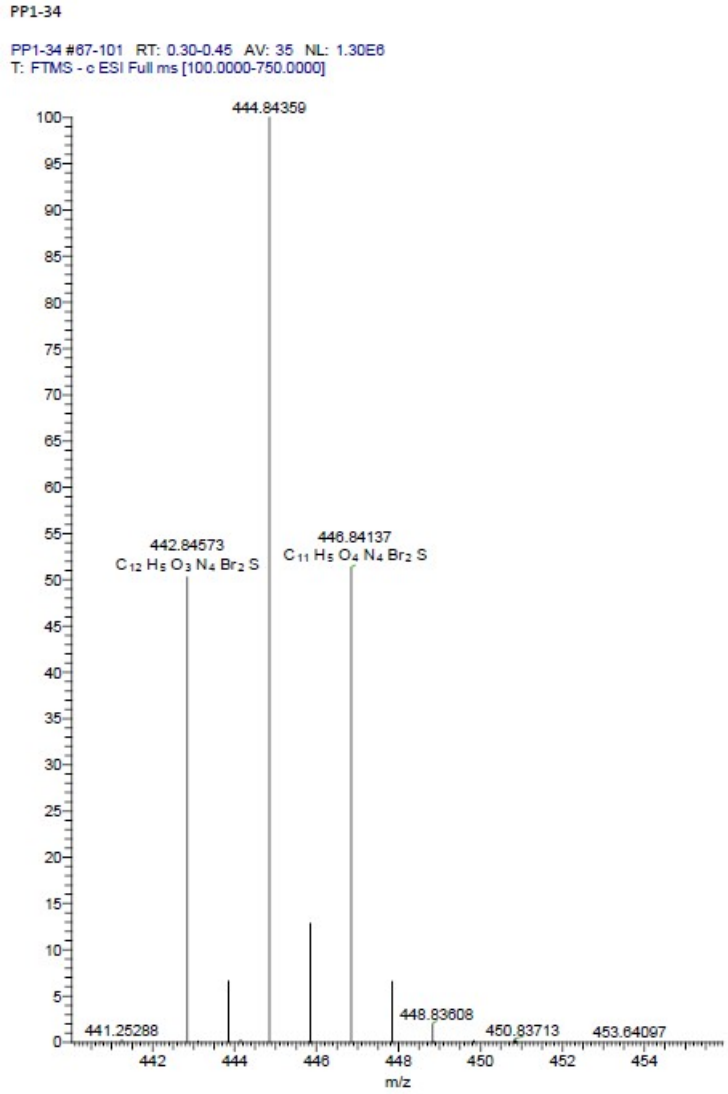
*3-((4,5-Dibromo-N-(6-(carboxyformamido)benzo[d]thiazol-2-yl)-1H-pyrrole-2 carboxamido)methyl)benzoic acid (**4**)*





HRMS spectra of the representative compounds

*4,5-Dibromo-N-(6-nitrobenzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamide (**6**)*



Elemental composition search on mass 442.84573

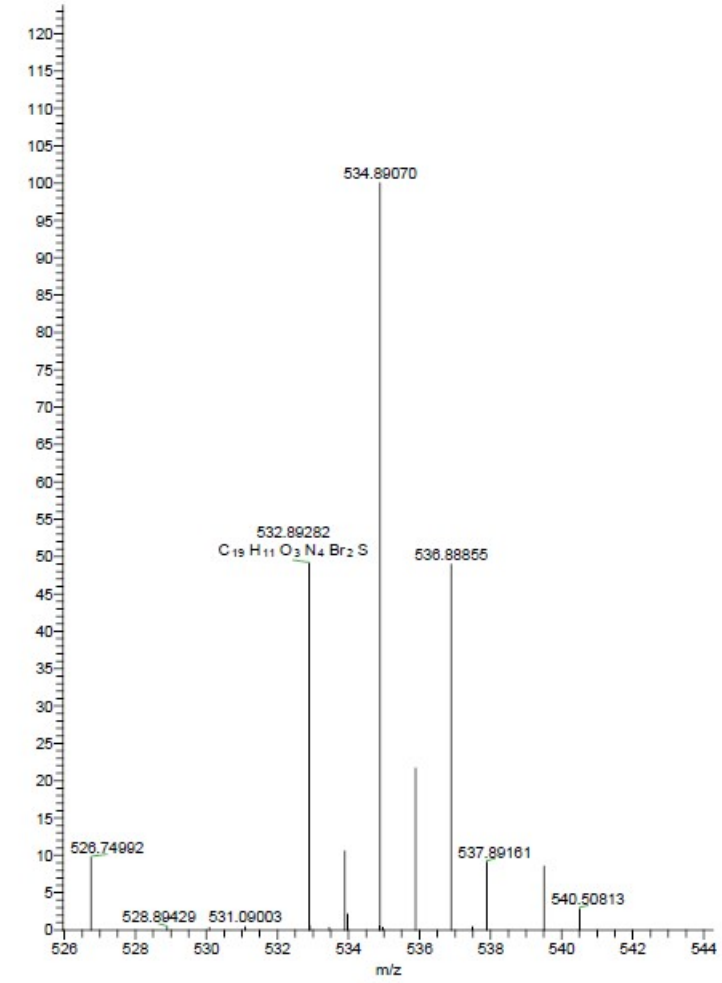
m/z= 437.84573-447.84573

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
442.84573	442.84546	0.61	11.5	$C_{12}H_5O_3N_4Br_2S$

*(Z)-N-(3-Benzyl-6-nitrobenzo[d]thiazol-2(3H)-ylidene)-4,5-dibromo-1H-pyrrole-2-carboxamide (**7**)*

PP1-41-F1

PP1-41-F1 #120-133 RT: 0.53-0.59 AV: 14 NL: 5.44E4
T: FTMS - c ESI Full ms [100.0000-750.0000]



Elemental composition search on mass 532.89282

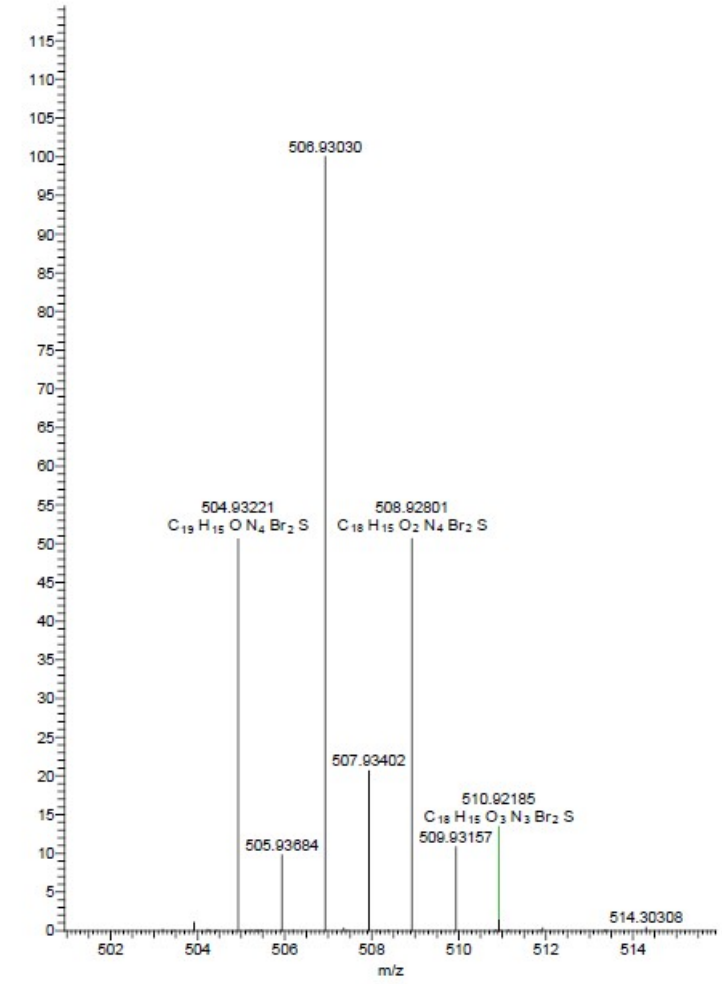
m/z= 527.89282-537.89282

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
532.89282	532.89241	0.77	15.5	C19 H11 O3 N4 Br2 S

*(Z)-N-(6-Amino-3-benzylbenzo[d]thiazol-2(3H)-ylidene)-4,5-dibromo-1H-pyrrole-2-carboxamide (**8**)*

PP1-47-F1

PP1-47-F1 #21-32 RT: 0.09-0.14 AV: 12 NL: 3.70E6
T: FTMS + c ESI Full ms [100.0000-750.0000]



Elemental composition search on mass 504.93221

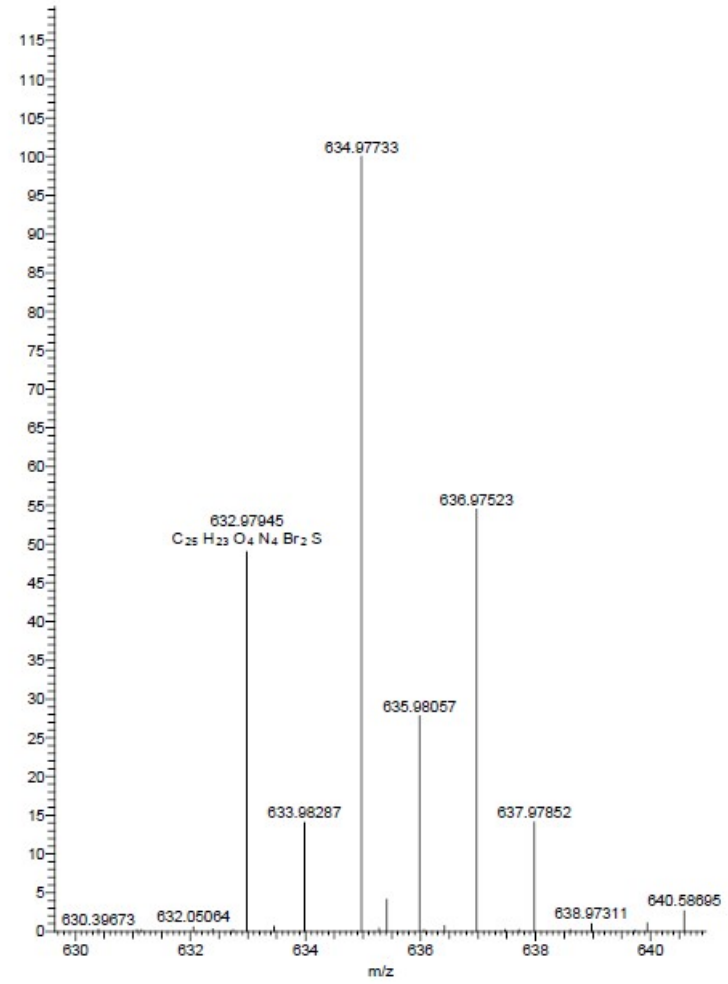
m/z= 499.93221-509.93221

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
504.93221	504.93278	-1.14	13.5	C19 H15 O N4 Br2 S

*tert-Butyl(Z)-2-((3-benzyl-2-((4,5-dibromo-1H-pyrrole-2-carbonyl)imino)-2,3-dihydrobenzo[d]thiazol-6-yl)amino)-2-oxoacetate (**9**)*

PP1-51-C3-F12

PP1-51_C3-F12 #34-46 RT: 0.15-0.20 AV: 13 NL: 4.61E5
T: FTMS + c ESI Full ms [100.0000-750.0000]

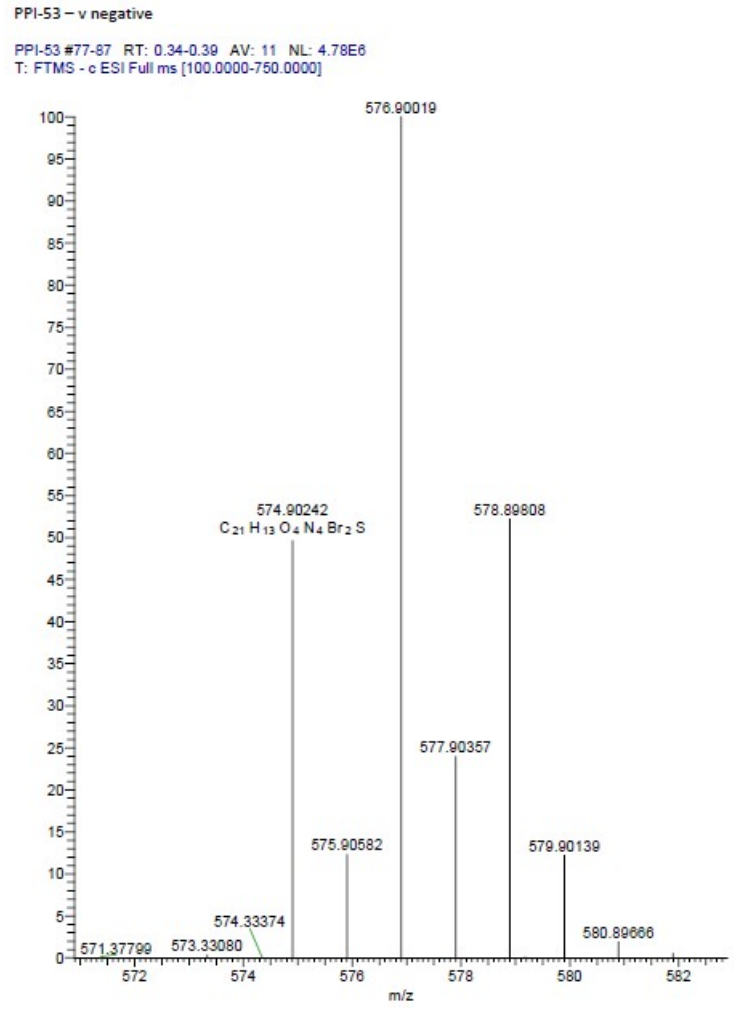


Elemental composition search on mass 632.97945

m/z= 627.97945-637.97945

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
632.97945	632.98013	-1.07	15.5	$C_{25}H_{23}O_4N_4Br_2S$

*(Z)-2-((3-Benzyl-2-((4,5-dibromo-1H-pyrrole-2-carbonyl)imino)-2,3-dihydrobenzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (**2**)*

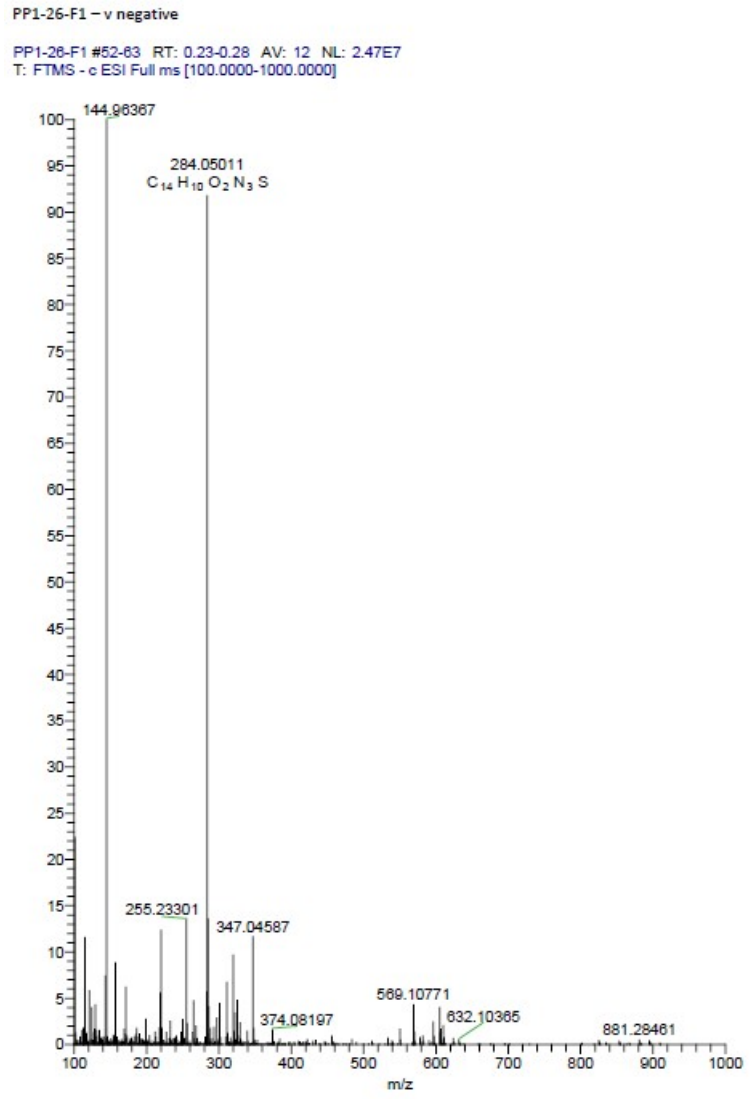


Elemental composition search on mass 574.90242

m/z= 569.90242-579.90242

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
574.90242	574.90297	-0.96	16.5	$C_{21}H_{13}O_4N_4Br_2S$
	574.90057	3.22	13.5	$C_{19}H_{14}O_4N_4Br_2NaS$

*N-Benzyl-6-nitrobenzo[d]thiazol-2-amine (**11a**)*

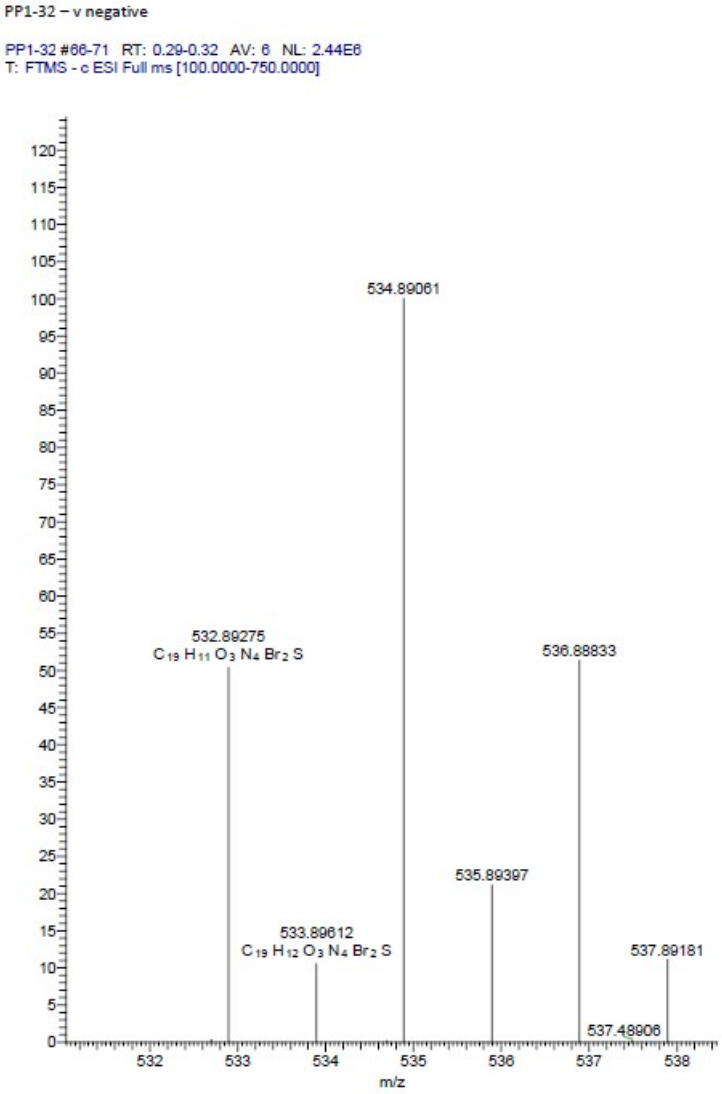


Elemental composition search on mass 284.05011

m/z= 279.05011-289.05011

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
284.05011	284.04992	0.67	11.5	$C_{14}H_{10}O_2N_3S$

*N-Benzyl-4,5-dibromo-N-(6-nitrobenzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamide (**12a**)*

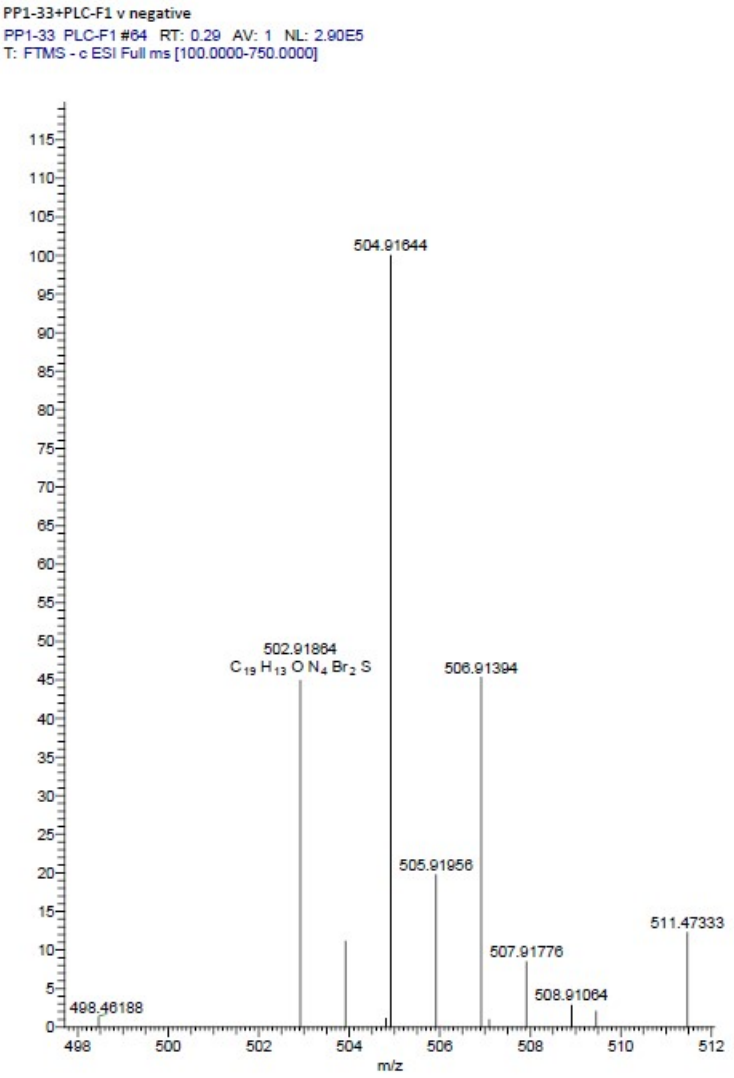


Elemental composition search on mass 532.89275

m/z= 527.89275-537.89275

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
532.89275	532.89241	0.64	15.5	$C_{19}H_{11}O_3N_4Br_2S$

*N-(6-Aminobenzo[d]thiazol-2-yl)-N-benzyl-4,5-dibromo-1H-pyrrole-2-carboxamide (**13a**)*

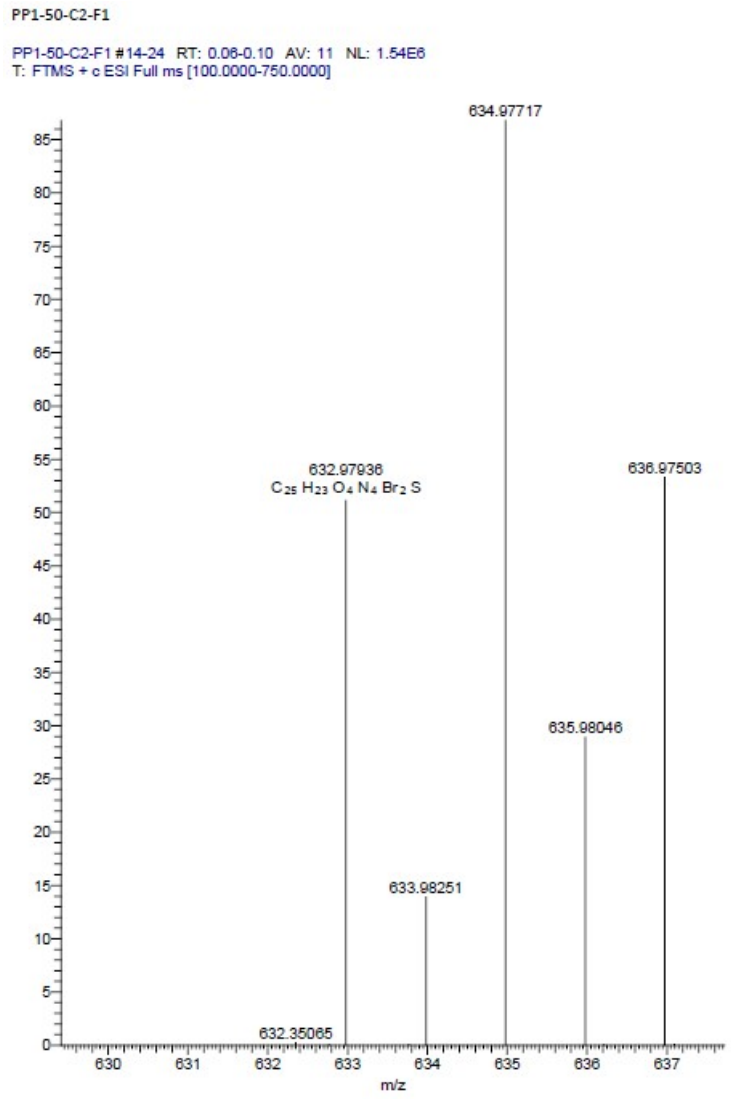


Elemental composition search on mass 502.91864

m/z= 497.91864-507.91864

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
502.91864	502.91823	0.81	14.5	C19 H13 O N4 Br2 S

*tert-Butyl2-((2-(N-benzyl-4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (**14a**)*

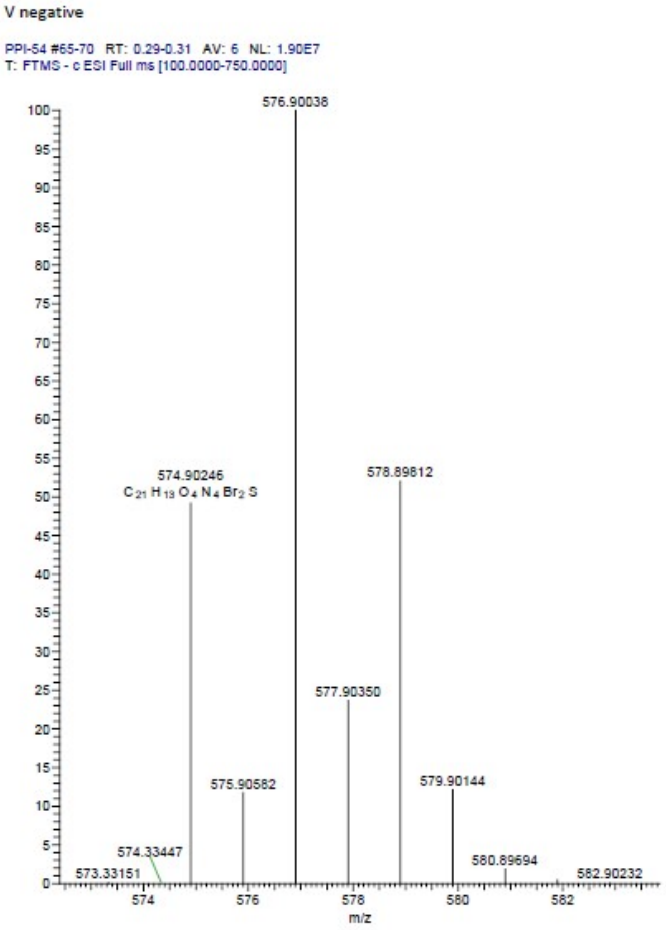


Elemental composition search on mass 632.97936

m/z= 627.97936-637.97936

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
632.97936	632.98013	-1.21	15.5	$C_{25}H_{23}O_4N_4Br_2S$

*2-((2-(N-Benzyl-4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (**3**)*

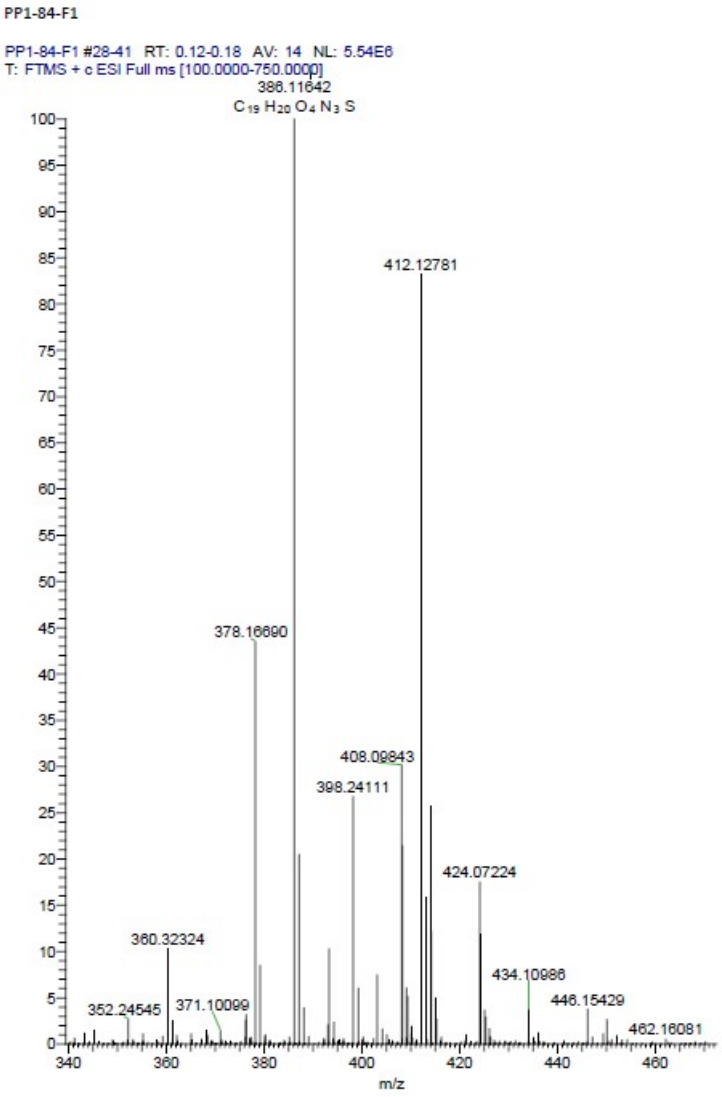


Elemental composition search on mass 574.90246

m/z= 569.90246-579.90246

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
574.90246	574.90297	-0.89	16.5	$C_{21}H_{13}O_4N_4Br_2S$
	574.90057	3.29	13.5	$C_{19}H_{14}O_4N_4Br_2NaS$

*tert-Butyl 3-(((6-nitrobenzo[d]thiazol-2-yl)amino)methyl)benzoate (**11b**)*

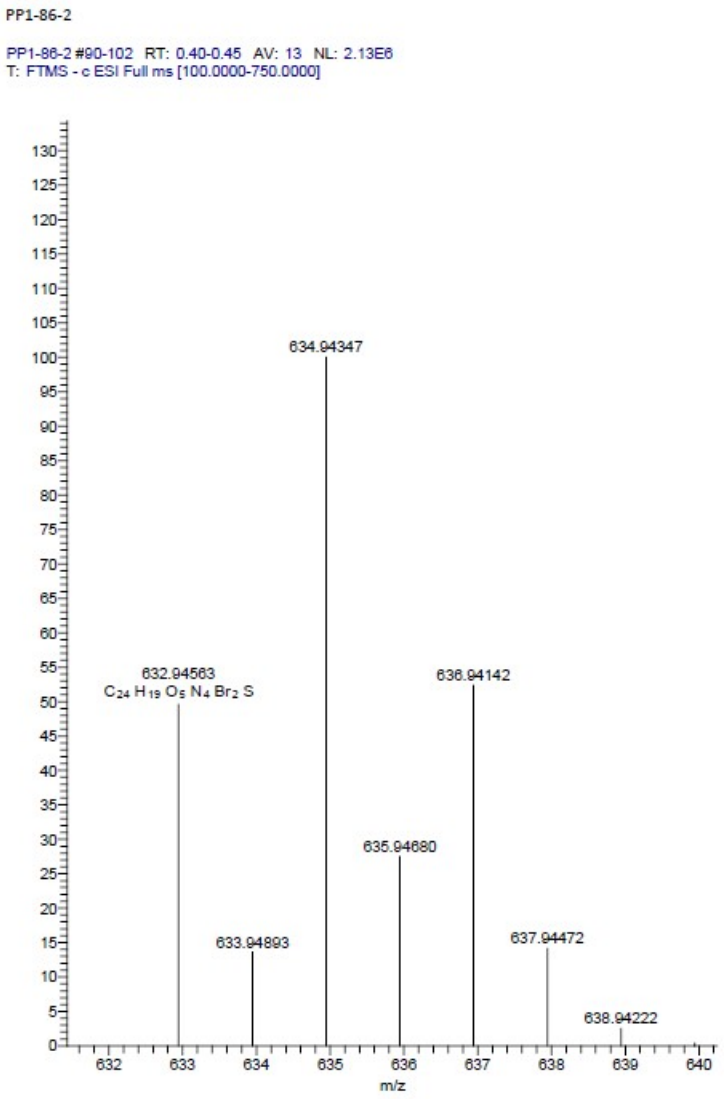


Elemental composition search on mass 386.11642

m/z= 381.11642-391.11642

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
386.11642	386.11690	-1.25	11.5	$C_{19}H_{20}O_4N_3S$

*tert-Butyl3-((4,5-dibromo-N-(6-nitrobenzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamido)methyl)benzoate (**12b**)*



Elemental composition search on mass 632.94563

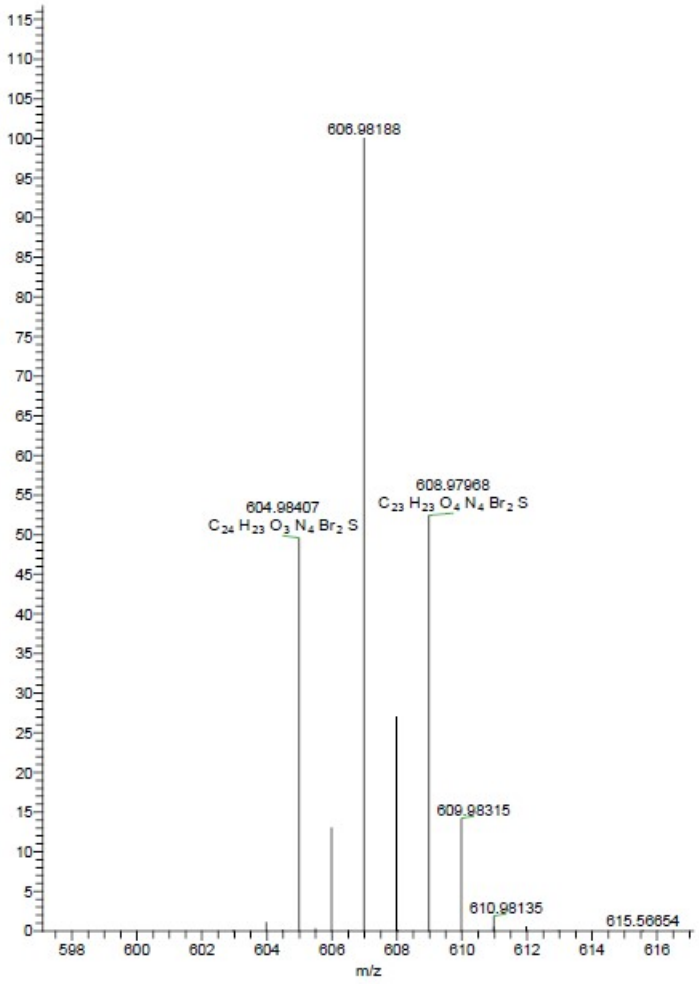
m/z= 627.94563-637.94563

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
632.94563	632.94484	1.25	16.5	$C_{24}H_{19}O_5N_4Br_2S$

*tert-Butyl 3-((N-(6-aminobenzo[d]thiazol-2-yl)-4,5-dibromo-1H-pyrrole-2-carboxamido)methyl)benzoate (**13b**)*

PP1-105-2

PP1-105-2 #17-29 RT: 0.07-0.13 AV: 13 NL: 1.00E7
T: FTMS + c ESI Full ms [100.0000-750.0000]



Elemental composition search on mass 604.98407

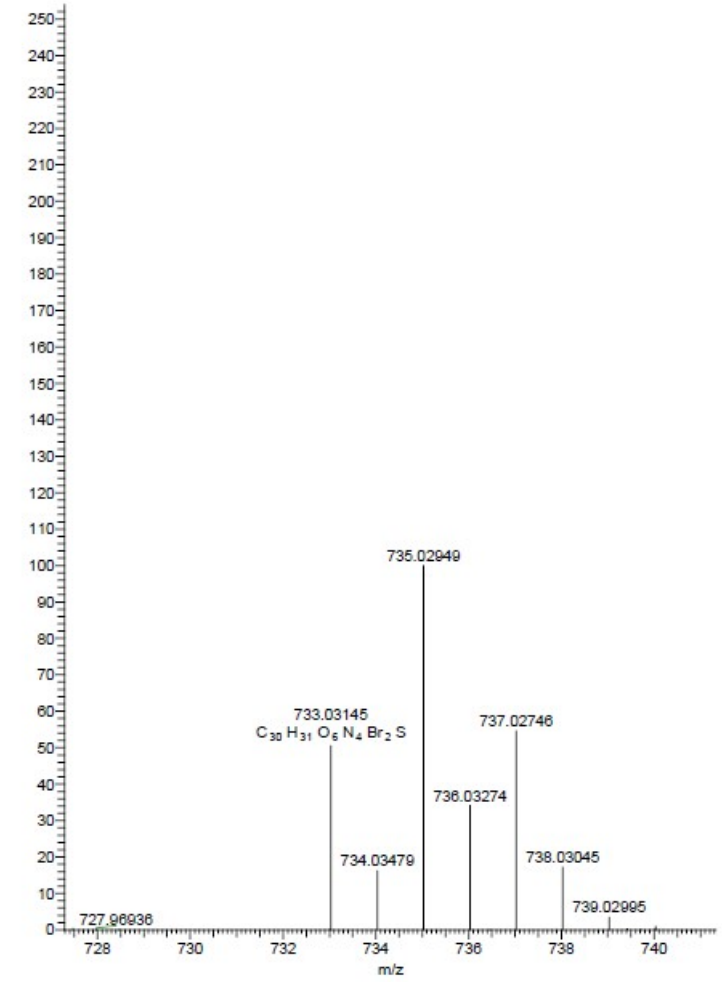
m/z= 599.98407-609.98407

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
604.98407	604.98521	-1.89	14.5	C24 H23 O3 N4 Br2 S

*tert-Butyl 3-((4,5-dibromo-N-(6-(2-(tert-butoxy)-2-oxoacetamido)benzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamido)methyl)benzoate (**14b**)*

PP1-107-F1

PP1-107-F1 #31-38 RT: 0.14-0.17 AV: 8 NL: 6.94E5
T: FTMS + c ESI Full ms [100.0000-750.0000]



Elemental composition search on mass 733.03145

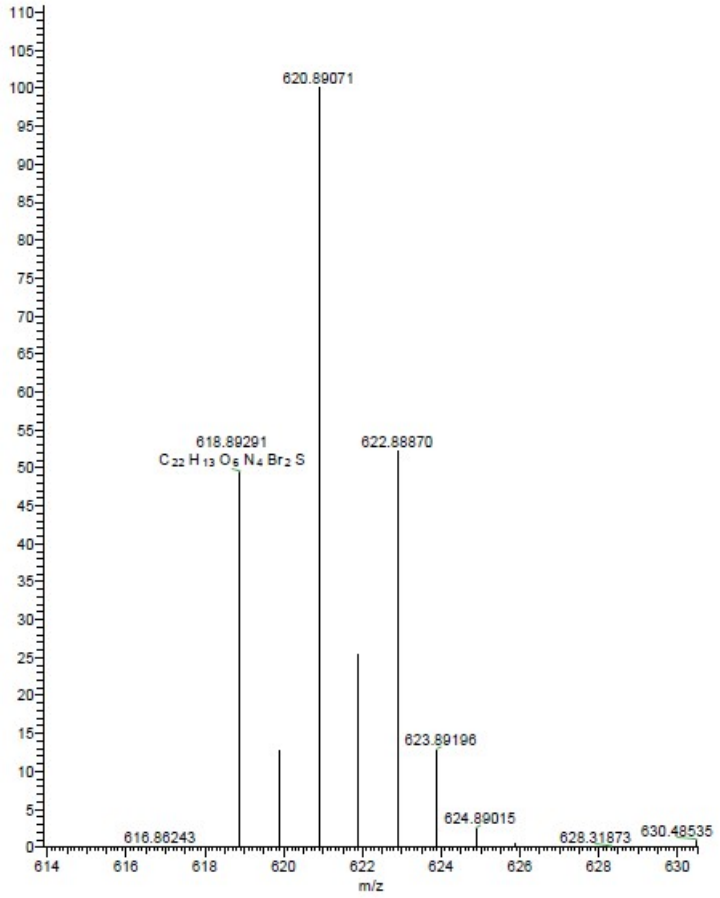
m/z= 728.03145-738.03145

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
733.03145	733.03256	-1.51	16.5	$C_{30}H_{31}O_6N_4Br_2S$

*3-((4,5-Dibromo-N-(6-(carboxyformamido)benzo[d]thiazol-2-yl)-1H-pyrrole-2 carboxamido)methyl)benzoic acid (**4**)*

PPI-109-3

PPI-109-3 #56-66 RT: 0.25-0.29 AV: 11 NL: 4.66E6
T: FTMS - c ESI Full ms [100.0000-1000.0000]



Elemental composition search on mass 618.89291

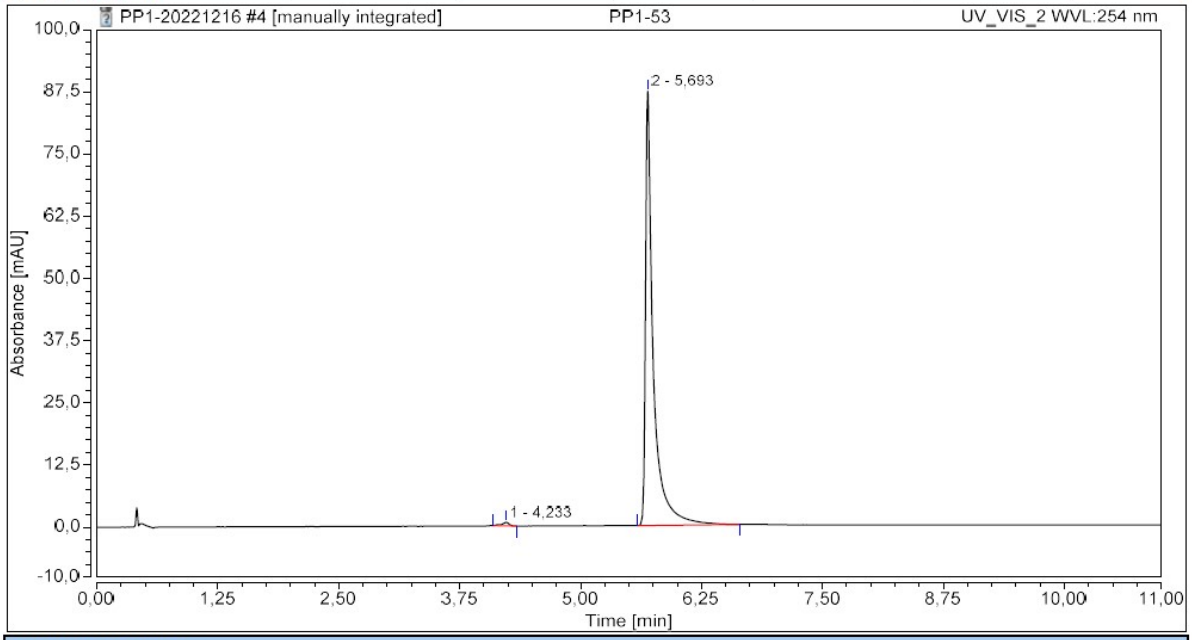
m/z= 613.89291-623.89291

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
618.89291	618.89280	0.17	17.5	$C_{22}H_{13}O_6N_4Br_2S$
	618.89040	4.06	14.5	$C_{20}H_{14}O_6N_4Br_2NaS$

HPLC chromatograms of the final compounds

*(Z)-2-((3-Benzyl-2-((4,5-dibromo-1H-pyrrole-2-carbonyl)imino)-2,3-dihydrobenzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (**2**)*

Chromatogram

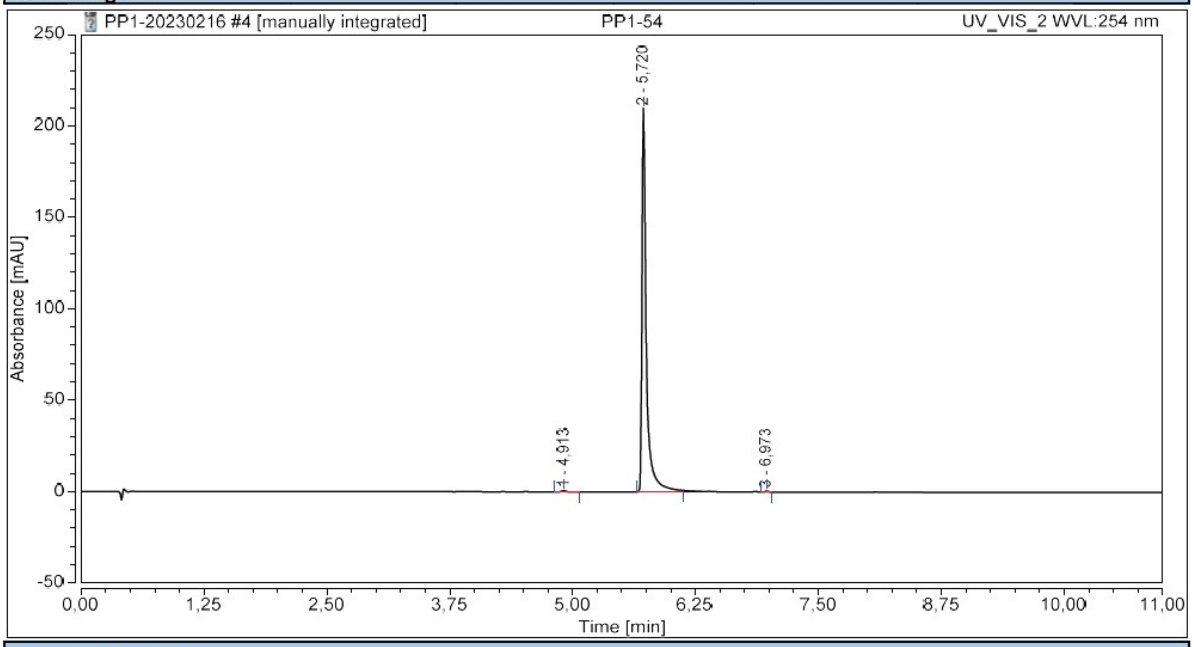


Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		4,233	0,061	0,728	0,74	0,83	n.a.
2		5,693	8,094	87,297	99,26	99,17	n.a.
Total:			**8,155**	**88,024**	**100,00**	**100,00**	

*2-((2-(N-Benzyl-4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetic acid (**3**)*

Chromatogram

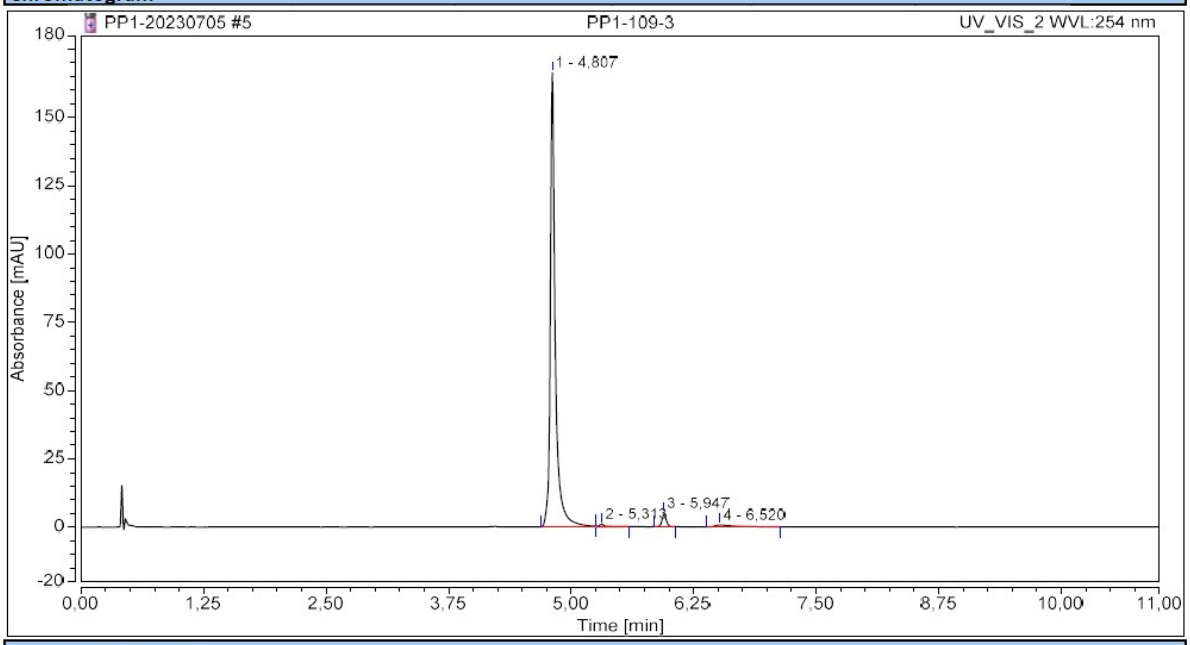


Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		4,913	0,036	0,705	0,33	0,33	n.a.
2		5,720	10,839	209,932	99,53	99,46	n.a.
3		6,973	0,015	0,428	0,14	0,20	n.a.
Total:			**10,891**	**211,065**	**100,00**	**100,00**	

*3-((4,5-Dibromo-N-(6-(carboxyformamido)benzo[d]thiazol-2-yl)-1H-pyrrole-2-carboxamido)methyl)benzoic acid (**4**)*

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		4,807	9,905	166,438	95,40	96,13	n.a.
2		5,313	0,072	1,002	0,69	0,58	n.a.
3		5,947	0,241	4,974	2,32	2,87	n.a.
4		6,520	0,165	0,732	1,59	0,42	n.a.
Total:			**10,382**	**173,146**	**100,00**	**100,00**	