SUPPORTING INFORMATION

Discovery of a spirocyclic 3-bromo-4,5-dihydroisoxazole covalent inhibitor of hGAPDH with antiproliferative activity against pancreatic cancer cells

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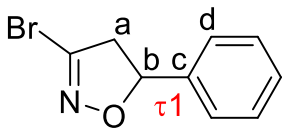
Overview, Summary

Table S1. cLogD, percentage of ionic forms, and calculated pKa values of compounds **1-12**.

Compd	Calculated pKa	Prevalent Ionic Form[a] (%)		cLogD	
		pH 7.4	**pH 7.6**	**pH 7.4**	**pH 7.6**
1	-	N (100)	N (100)	2.66	2.66
2	9.9 ± 0.4	N(100)	N(100)	2.22	2.22
3	4.1± 0.4	A(100)	A(100)	-0.08	-0.25
4	4.0± 0.4	A(100)	A(100)	-0.11	-0.27
5	3.9± 0.4	A(100)	A(100)	-0.26	-0.41
6	0.7± 0.4	A(100)	A(100)	-4.12	-4.12
7	10.1± 0.4	N(100)	N(100)	1.56	1.56
8	4.4± 0.4	A(100)	A(100)	-1.02	-1.13
9	-	N (100)	N (100)	2.60	2.60
10	3.6± 0.4	A(100)	A(100)	-0.70	-0.82
11	-	N (100)	N (100)	4.42	4.42
12	4.1± 0.4	A(100)	A(100)	0.96	0.79

[a] N = neutral; A = anionic. ACD/pKa GALAS algorithm of ACD/Percepta software.

Table S2. ΔE_{GM} values (kcal/mol) and torsional angle values (degrees) of the DFT conformer of (*R*)-**1**.



Enantiomer[a]	**ΔE_{GM} (kcal/mol)**	**Torsional Angle (°)**[b]
		τ1
R	0	-55.80

[a] The *S* enantiomer presents the same absolute value of τ1 with the opposite sign. [b]τ1= a,b,c,d.;

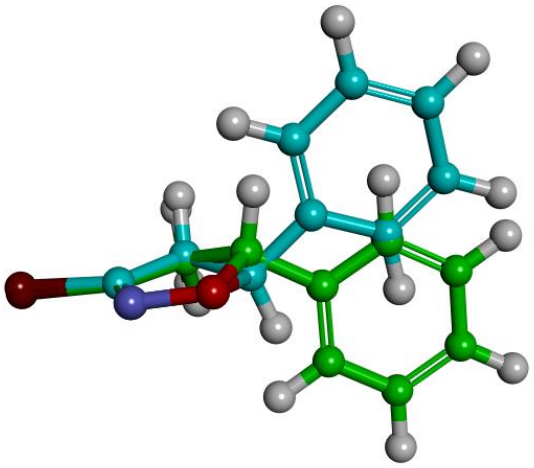
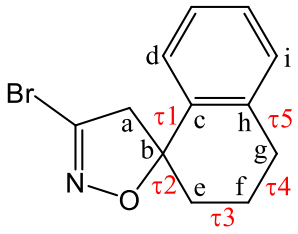


Figure S1. Superimposition by the 3-bromo-4,5-dihydroisoxazole ring atoms of the DFT conformer of the *R* (carbon atoms = green) and *S* enantiomer (carbon atoms = cyan) of **1**.

Table S3. ΔE_{GM} values (kcal/mol) and torsional angle values (degrees) of the DFT conformers of (*R*)-**11**.



Conf	Enantiomer[a]	ΔE_{GM} (kcal/mol)	Torsional Angles (°)[b]				
			τ1	**τ2**	**τ3**	**τ4**	**τ5**
1	*R*	0	-41.88	-173.85	62.12	-50.83	-158.20
2	*R*	0.02	-66.53	-82.55	-63.33	47.52	162.74

[a] The *S* enantiomer presents the same absolute values of torsional angles with the opposite sign. [b] τ1= a,b,c,d; τ2= a,b,e,f; τ3= b,e,f,g; τ4= e,f,g,h; τ5= f,g,h,i.

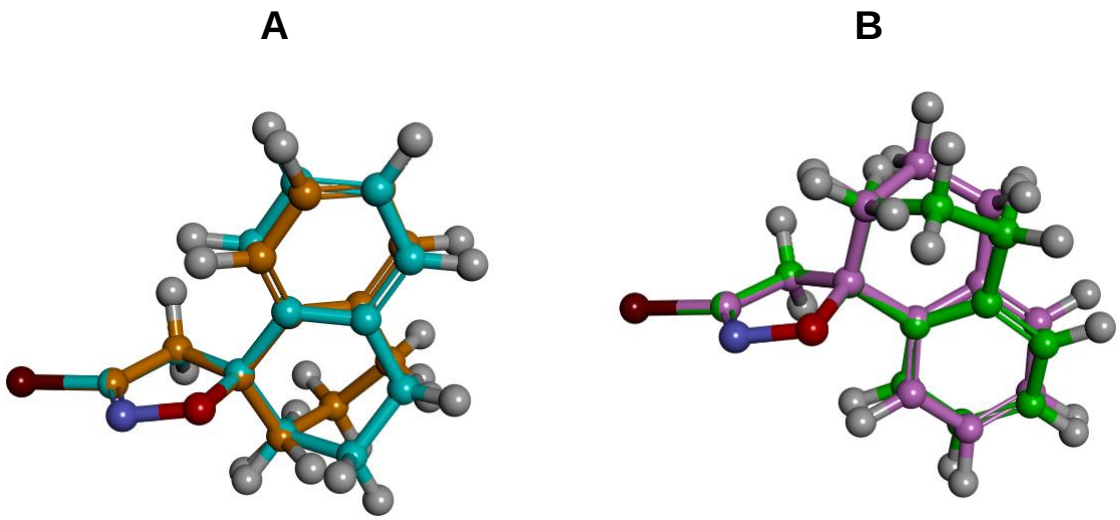


Figure S2. DFT conformers of the *S* and *R* enantiomers of **11**. Superimposition by the 3-bromo-4,5-dihydroisoxazole ring atoms of the calculated conformers of the *S* enantiomer (A) and the *R* enantiomer (B) of **11**. The carbon atoms of the GM conformer of the *S* enantiomer are colored in cyan (A), those of the R enantiomer are colored in green (B); note that the GM conformers presents the opposite flip of the bicylcic ring.

Table S4. X-ray structures of GAPDH used in the structural and bioinformatics analysis.

PDB ID	Organism	Structure
1ZNQ	Homo Sapiens	Liver holo-GAPDH
1U8F	Homo Sapiens	Placental holo-GAPDH
4WNC	Homo Sapiens	Holo-GAPDH
4WNI	Homo Sapiens	T229K mutant holo-GAPDH
5C7L	Homo Sapiens	Testis-specific apo GAPDH
5C7O	Homo Sapiens	Testis-specific holo GAPDH
3PFW	Homo Sapiens	Sperm-specific holo GAPDH
6ADE	Homo Sapiens	Mutant placental holo-GAPDH
6YND	Homo Sapiens	Apo GAPDH purified from the supernatant of HEK293F cells
6YNE	Homo Sapiens	Apo GAPDH purified from the supernatant of HEK293F cells
6YNF	Homo Sapiens	Apo GAPDH purified from the supernatant of HEK293F cells
6YNH	Homo Sapiens	Apo GAPDH purified from the supernatant of HEK293F cells
1DC4	Escherichia coli	Apo GAPDH in complex with the substrate Glyceraldehyde 3-phosphate
3KV3	Staphylococcus aureus subsp. aureus MRSA252	C151S mutant of GAPDH in complex with NAD^+ and 3-phosphoglyceric acid
5JYA	Streptococcus agalactiae	C152S mutant of GAPDH in complex with NAD^+ and the substrate Glyceraldehyde 3-phosphate
1NQA	Geobacillus stearothermophilus	C149A mutant of GAPDH in complex with NAD^+ and the substrate Glyceraldehyde 3-phosphate
1NQO	Geobacillus stearothermophilus	C149S mutant of GAPDH in complex with NAD^+ and the substrate Glyceraldehyde 3-phosphate
3CIF	Cryptosporidium parvum	C153S mutant of GAPDH in complex with NAD^+ and the substrate Glyceraldehyde 3-phosphate
3KSZ	Staphylococcus aureus subsp. aureus MRSA252	C151S and H178N mutant of GAPDH in complex with NAD^+ and 3-phosphoglyceric acid
3CMC	Bacillus stearothermophilus	GAPDH in complex with with NAD^+, the substrate Glyceraldehyde 3-phosphate and sulfate ions
1ML3	Trypanosoma cruzi	GAPDH in complex with $NAD^+$and the inhibitor 2-(2-phosphono-ethyl)-acrylic acid 4-nitro-phenyl ester
6QUQ	Arabidopsis thaliana	GAPDH in complex with glutathione, NAD^+ and sulfate ions
6IQ6	Homo Sapiens	GAPDH in complex with the inhibitor Monomethyl Fumarate
6M61	Homo Sapiens	GAPDH in complex with NAD^+ and the inhibitor heptelidic acid (koningic acid)
1I32	Leishmania mexicana	GAPDH in complex with the inhibitor N-naphthalen-1-ylmethyl-2'-[3,5-dimethoxybenzamido]-2'-deoxy-adenosine
1I33	Leishmania mexicana	GAPDH in complex with the inhibitor N-1,2,3,4-tetrahydronaphth-1-yl-2'-[3,5-dimethoxybenzamido]-2'-deoxy-adenosine
1GYQ	Leishmania mexicana	GAPDH in complex with the inhibitor N6-Benzyl-NAD
1IHY	Panulirus versicolor	GAPDH in complex with ADP-ribose and sulfate ions
1K3T	Trypanosoma cruzi	GAPDH in complex with the inhibitor Chalepin
3H9E	Homo Sapiens	Sperm-specific GAPDH in complex with NAD^+ and phosphate ions
3GDP	Homo Sapiens	Skeletal muscle GAPDH in complex with NAD^+ and sulfate ions
1S7C	Escherichia coli	GAPDH in complex with 2-(N-morpholino)-ethanesulfonic acid and sulfate ion
5TSO	Sus scrofa	GAPDH in complex with 1,10-Phenanthroline, NAD^+ and sulfate ions

7JWK	Mycoplasma genitalium G37	GAPDH in complex with NAD^+ and phosphate ions
6IO4	Escherichia coli K-12	GAPDH in complex with silver ion
7D1G	Clostridium beijerinckii NCIMB 8052	GAPDH in complex with magnesium ion and beta-mercaptoethanol
3IDS	Trypanosoma cruzi	Glycosomal GAPDH in complex with NAD^+ and the iodoacetamide inhibitor
6GFP	Thermosynechococcus elongatus BP-1	GAPDH in complex with NADP, malonate ion, formic acid and magnesium ion
6GFR	Thermosynechococcus elongatus BP-1	GAPDH in complex with NAD^+, acetate ion and magnesium ion
2I5P	Kluyveromyces marxianus	GAPDH in complex with beta-mercaptoethanol

Table S5. Summary of Procheck results obtained for the selected docked complexes of **1**/hGAPDH and **11**/hGAPDH.

Structure	Cmplx	Residues favored regions (%)	Residues allowed regions (%)	Residues generously allowed regions (%)	Residues disallowed regions (%)	Poor rotamer (%)
1*S*/hGAPDH	3	69.9	26.9	2.2	1.0	2.5
1R/hGAPDH	1	69.1	27.2	3.2	0.5	0.1
11*S*_Conf1/hGAPDH	4	68.5	28.2	2.3	1.0	2.1
11*R*_Conf2/hGAPDH	19	68.1	29.0	2.3	0.6	2.1

Table S6. Ligand-residue nonbonded interaction energies (kcal/mol) of the hGAPDH/(*S*)-**1** docked complex.

hGAPDH amino acids	**Substructures**	**Nonbonded interaction Energy (kcal/mol)**
I14	H1 helix	-0.086
T99	S6-H3 loop	-0.170
G100	S6-H3 loop	-0.138
P124	S7-S8 loop	-0.801
S151	S9-H4 loop	-0.388
C152	S9-H4 loop	-0.560
T153	S9-H4 loop	-0.545
T177	S10 strand	-0.140
H179	S10 strand	-1.219
A180	S10 strand	-0.141
T182	S-loop	-0.927
T184	S-loop	-0.176
A213	active-site segment	-0.115
R234	S11 strand	-1.906
N316	S16-H8 loop	-0.166
NAD^+	-	-4.899

Table S7. Ligand-residue nonbonded interaction energies (kcal/mol) of the hGAPDH/(*R*)-**1** docked complex.

hGAPDH amino acids	**Substructures**	**Nonbonded interaction Energy (kcal/mol)**
T99	S6-H3 loop	-0.132
A123	S7-S8 loop	-0.232
P124	S7-S8 loop	-1.273
S151	S9-H4 loop	-0.638
C152	S9-H4 loop	-0.491
T153	S9-H4 loop	-0.420
T177	S10 strand	-0.180
H179	S10 strand	-1.315
T182	S-loop	-0.743
A183	S-loop	-0.104
T184	S-loop	-0.497
A232	S11 strand	-0.103
R234	S11 strand	-1.712
N316	S16-H8 loop	-0.158
NAD+	-	-5.174

Table S8. Ligand-residue nonbonded interaction energies (kcal/mol) of the hGAPDH/(*S*)-**11** docked complex.

hGAPDH amino acids	**Substructures**	**Nonbonded interaction Energy (kcal/mol)**
S122	S7-S8 loop	-0.234
A123	S7-S8 loop	-0.336
P124	S7-S8 loop	-1.411
S125	S7-S8 loop	-0.242
S151	S9-H4 loop	-1.350
C152	S9-H4 loop	-1.354
T153	S9-H4 loop	-0.949
T154	H4 helix	-0.156
T177	S10 strand	-0.585
H179	S10 strand	-1.433
T184	S-loop	-0.357
A209	Active-site segment	-0.131
T211	Active-site segment	-0.754
A213	Active-site segment	-1.064
A214	Active-site segment	-0.552
A232	S11 strand	-0.432
R234	S11 strand	-1.499
Y314	S16 strand	-0.260
NAD^+	-	-1.510

Table S9. Ligand-residue nonbonded interaction energies (kcal/mol) of the hGAPDH/(*R*)-**11** docked complex**.**

hGAPDH amino acids	**Substructures**	**Nonbonded interaction Energy (kcal/mol)**
G12	H1 helix	-0.159
R13	H1 helix	-1.826
S151	S9-H4 loop	-0.311
C152	S9-H4 loop	-0.788
H179	S10 strand	-1.113
A180	S10 strand	-0.554
I181	S-loop	-1.524
T182	S-loop	-1.777
A183	S-loop	-0.301
R234	S11 strand	-0.637
T237	S11-S12 loop	-0.185
A238	S11-S12 loop	-0.556
N239	S11-S12 loop	-0.498
V240	S11-S12 loop	-0.092
N316	S16-H8 loop	-0.875
E317	S16-H8 loop	-1.179
V188 (subunit 2)	S-loop	-0.593
D189 (subunit2)	S-loop	-0.724
G190 (subunit 2)	S-loop	-0.229
P191 (subunit 2)	S-loop	-0.526
NAD^+	-	-3.536

Table S10. Ligand-protein nonbonded interaction energies (kcal/mol) of the docked complexes and NAD^+ displacement with respect to the original position (RMSD).

Complex	Number	Docking Procedure[a]	Nonbonded interaction energies (kcal/mol)	RMSD NAD^+ (Å)
1*S*/hGAPDH	3	II	-14.61	3.29
1R/hGAPDH	1	I	-15.70	2.51
11*S*_Conf1/hGAPDH	4	II	-17.50	3.08
11*R*_Conf2/hGAPDH	19	II	-20.16	4.25

[a] Docking procedure without constraints on the hydrogen bonds between NAD^+ and hGAPDH; II: docking procedure by using constraints on the hydrogen bonds between NAD^+ and hGAPDH.

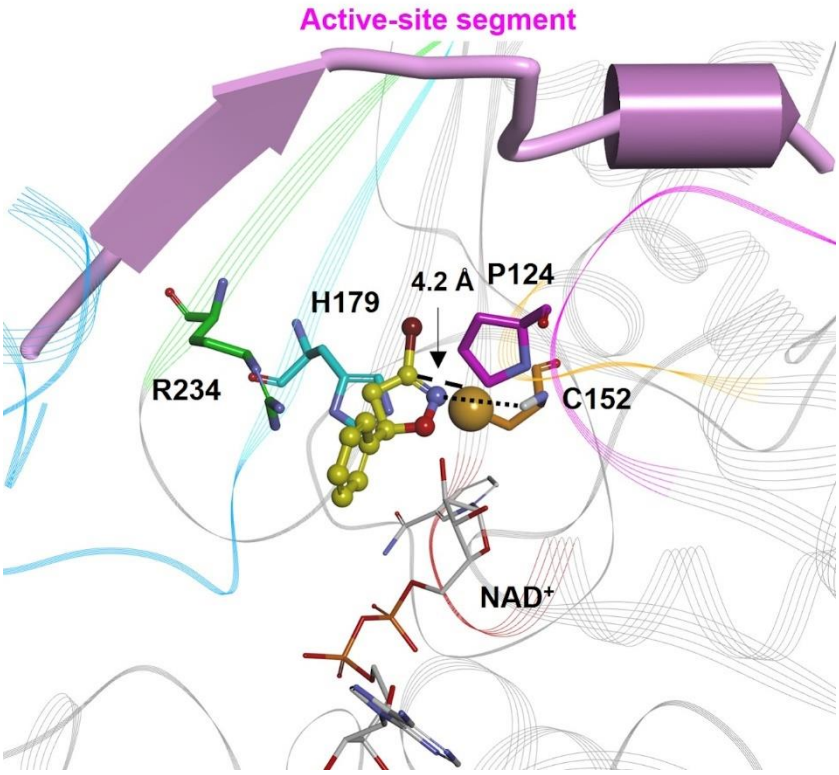


Figure S3. Docked complex of (*R*)-**1**/hGAPDH. hGAPDH is displayed as line ribbon and colored in grey. The S7-S8 loop (aa122-129) is colored in magenta, the S9-H14 loop (aa149-153) in orange, the S10 strand (aa171-180) in cyan, the S-loop (aa181-207) in light blue, the S11 strand (aa228-235) in green, and the S16-H8 loop (aa315-320) in brown. The active-site segment (aa208-220) is displayed in cartoon form and colored in pink. The ligand (carbon atoms colored in yellow) is displayed in ball&stick while the interacting residues and NAD^+ (carbon atoms colored in white) in stick. The sulfur atom of C152 is displayed in CPK (scaled by 50%). NAD^+, ligands and interacting residues are colored by atom type (N = blue, O = red, S = yellow, P = orange, Br = brown, H = white). The distance between the electrophilic carbon C3 of 3-bromo-4,5-dihydroisoxazole ring and the C152 sulfur atom is showed. Hydrogen atoms are omitted for clarity, except those involved in hydrogen bond interactions (black dashed lines).

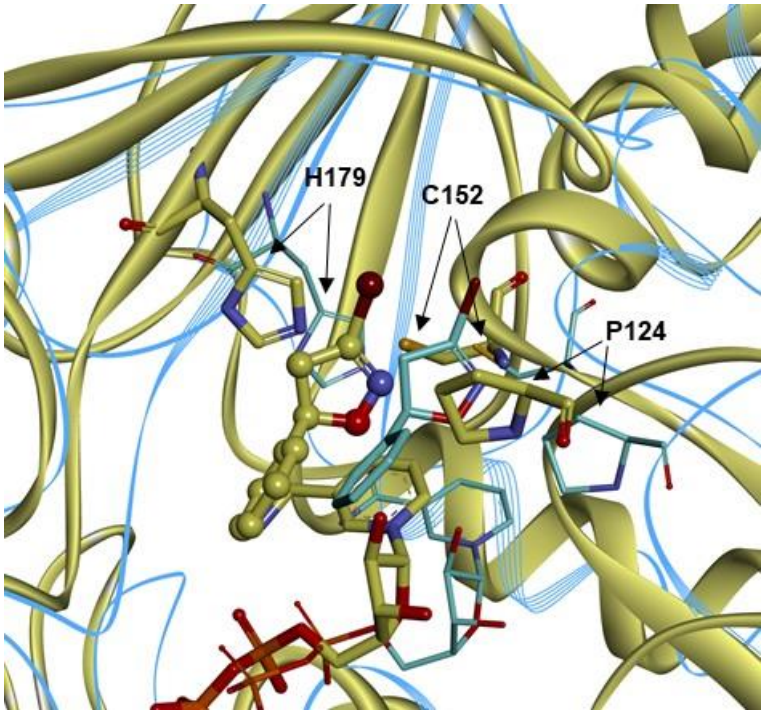


Figure S4. Superimposition of the best docked (*R*)-**1**/hGAPDH complex before (protein ribbons and ligand carbon atoms = cyan) and after (protein ribbons and ligand carbon atoms =light yellow; (*R*)-**1** in ball&stick) the unrestrained structural optimization (energy minimization). (*R*)-**1,** NAD^+, P124, C152, and H179 heteroatoms are colored by atom type (N: blue; O: red; P: orange; S: yellow).

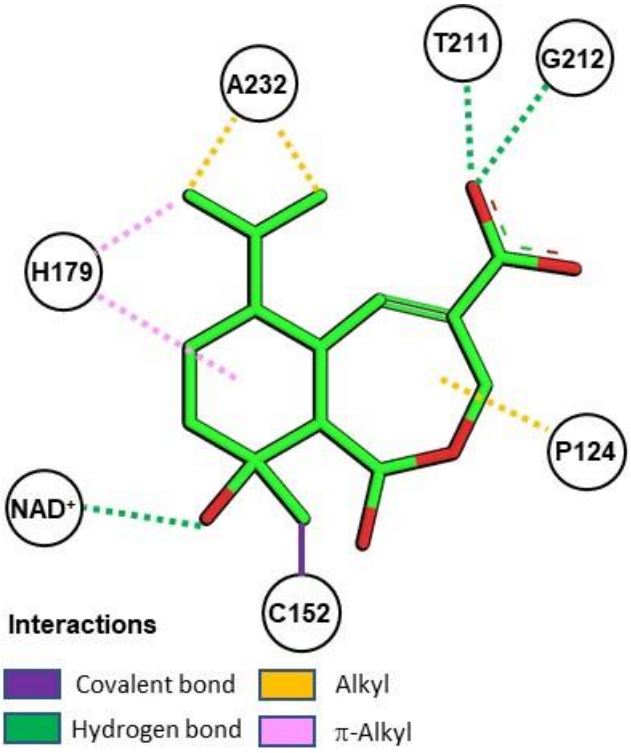


Figure S5. 2D representation of the interactions between koningic acid (KA) and hGAPDH (PDB ID: 6M61).

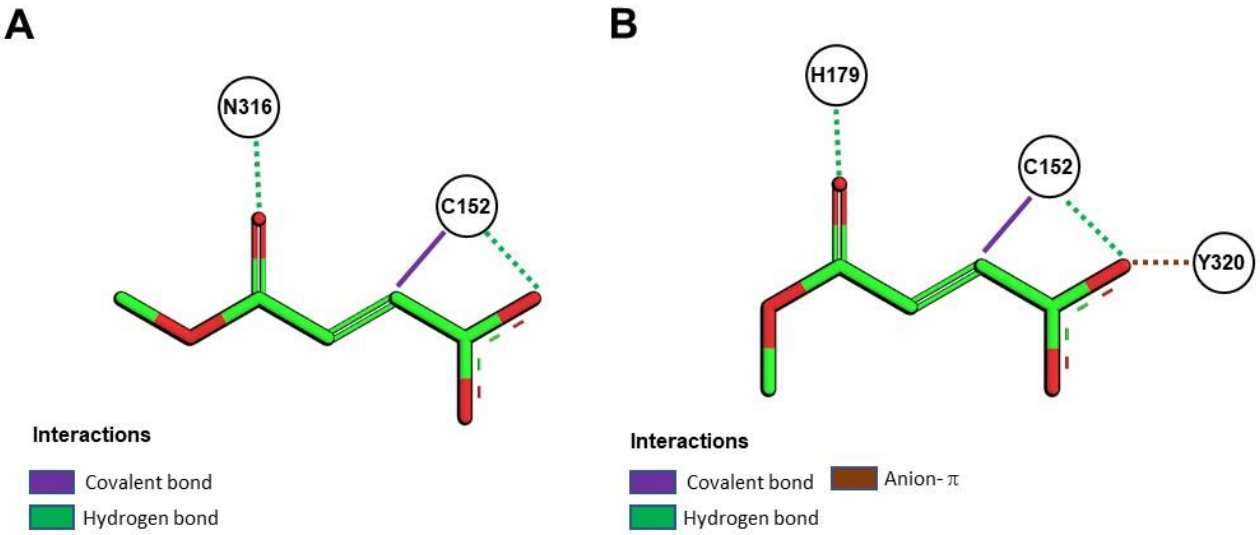
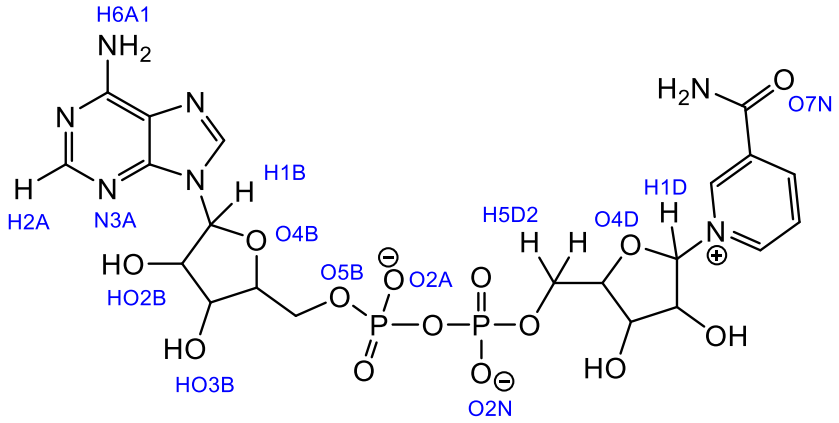


Figure S6. 2D representation of the interactions between monomethyl fumarate (MMF) and hGAPDH: binding mode 1 (A) and binding mode 2 (B) (PDB ID: 6IQ6).

Table S11. Hydrogen bonds between NAD^+ and substrate-free hGAPDH (PDB ID: 1ZNQ) restrained during the MC_II docking procedure. The involved atoms are labelled in blue on the NAD^+ structure. Unconventional hydrogen bonds are marked with an asterisk in the Table.



AD^+	GAPDH	
Atom	**Atom**	**Residue**
H2A*	OD1*	N9 ($CONH_2$)
N3A*	HA2*	G10 (Cα)
O5B*	HA1*	G12 (Cα)
O2N*	HA1*	G12 (Cα)
O2N	HN	R13 (NH_{bb})
O2A	HN	R13 (NH_{bb})
O2N	HN	I14 (NH_{bb})
H2A*	O*	N34 ($C=O_{bb}$)
HO2B	OD1	D35 (COO^-)
HO3B	OD2	D35 (COO^-)
H1B*	OD2*	D35 (COO^-)
H6A1	O	R80 ($C=O_{bb}$)
H5D2*	O*	S98 ($C=O_{bb}$)
O4B*	HA*	T99 (Cα)
H1D*	O*	S122 ($C=O_{bb}$)
O4D	HG	S122 (OH)
O7N	HD21	N316 ($CONH_2$)

Table S12. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **1**(*S*)/hGAPDH complexes obtained by MC_I simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.85	2.42	-63.10	129.04	131.97	80.35	92.40
2	3.05	3.82	-60.01	135.61	122.62	86.38	97.32
3	3.90	4.65	-59.48	131.91	128.04	83.55	97.08
4	3.96	4.45	-60.01	120.50	142.82	87.96	97.93
5	3.74	4.52	-64.11	117.77	140.47	89.62	100.97
6	2.23	5.25	-53.66	155.06	96.32	96.06	110.47
7	1.72	5.15	-55.83	144.85	103.07	78.11	92.65
8	2.16	5.89	-53.35	141.88	114.79	70.80	87.62
9	2.09	4.99	-52.48	152.77	97.74	76.95	93.40
10	2.08	4.89	-51.02	158.59	88.34	88.26	103.70
11	1.62	5.76	-56.33	100.43	134.20	74.47	88.94
12	1.89	5.21	-57.19	136.54	122.22	71.30	89.16
13	1.70	5.13	-56.80	127.87	132.90	71.81	87.19
14	1.68	5.31	-178.49	70.86	126.79	116.70	134.17
15	1.48	5.26	-179.12	70.00	128.22	115.42	132.76
16	1.00	4.90	-69.85	114.33	86.45	94.17	68.26
17	1.41	4.67	-68.63	137.66	83.87	101.77	74.21
18	0.96	5.20	-58.92	113.21	143.39	70.44	82.94
19	0.97	4.87	-176.81	69.63	132.27	109.01	125.08
20	1.02	4.81	-175.88	67.11	129.20	115.99	133.10
21	1.89	4.86	-52.79	137.70	103.83	129.65	136.58
22	1.69	4.75	-54.21	135.44	105.84	125.75	134.32
23	1.48	4.72	-55.27	137.66	113.80	106.51	113.57
24	1.80	4.37	-51.99	161.52	85.50	109.22	105.00
25	1.23	4.10	-58.38	143.24	98.82	75.20	98.83

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S13. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **1**(*S*)/hGAPDH complexes obtained by MC_II simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	2.00	2.46	-62.63	132.49	124.76	85.06	98.56
2	2.60	3.64	-62.63	122.14	140.69	70.15	81.66
3	2.44	3.04	-63.98	132.64	125.59	84.21	96.47
4	1.81	3.14	-66.77	140.63	95.62	112.41	89.49
5	1.87	3.14	-65.22	128.49	87.46	103.67	80.91
6	1.05	3.42	-62.88	76.68	97.57	96.78	72.84
7	1.46	3.78	-59.50	134.25	104.51	119.39	142.76
8	1.70	3.23	-69.88	127.38	103.33	140.80	117.66
9	1.67	3.23	-69.36	126.19	101.61	137.84	114.73
10	1.88	3.22	-63.93	137.69	94.89	121.57	147.11
11	0.94	3.39	-66.43	88.59	101.30	116.76	91.86
12	1.61	3.57	-55.72	151.32	79.90	139.55	149.42
13	1.77	3.25	-63.86	126.46	93.57	123.98	151.09
14	1.92	3.40	-69.70	121.89	113.08	145.88	124.89
15	1.87	3.60	-69.07	113.20	149.11	159.87	154.52
16	2.55	4.56	-67.45	125.98	111.05	144.09	124.94
17	2.36	4.37	-66.85	128.32	115.20	158.54	139.20
18	2.58	4.02	-65.79	133.70	124.51	171.92	154.08
19	2.98	4.55	-69.51	132.54	100.57	129.72	106.47
20	2.78	5.34	-70.14	114.39	98.30	103.05	79.86
21	1.35	2.82	-65.30	123.41	78.42	104.43	80.69
22	1.44	2.68	-63.78	121.39	79.10	103.60	82.66
23	1.59	2.86	-61.01	107.68	79.08	89.47	75.61
24	2.62	3.46	-68.03	149.42	98.07	131.84	114.86
25	2.48	3.84	-66.50	156.50	85.32	109.12	86.61

26	2.67	3.52	48.80	156.87	83.19	77.30	100.23
27	2.60	3.25	53.70	143.42	94.66	74.32	100.42
28	2.44	3.22	67.87	128.37	93.43	115.29	94.67
29	2.76	3.44	63.61	137.16	92.61	114.40	90.69
30	2.40	3.32	67.52	125.82	93.86	116.05	95.31

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S14. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **1**(*R*)/hGAPDH complexes obtained by MC_I simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.69	2.21	-63.37	131.27	122.11	82.40	93.84
2	3.58	4.05	-61.86	125.18	137.38	74.24	86.53
3	4.11	4.57	-60.52	133.91	122.72	84.02	97.51
4	3.42	4.07	-175.51	103.84	158.07	96.57	97.46
5	3.44	4.10	-175.86	104.34	157.45	96.00	96.55
6	2.32	6.37	-59.52	107.11	148.11	74.76	85.44
7	2.40	6.37	-55.62	110.98	155.87	81.86	83.60
8	3.30	5.04	-63.85	120.30	127.26	72.02	76.86
9	3.20	4.10	-67.35	136.27	122.69	83.71	96.42
10	2.58	3.98	-174.78	97.80	151.51	97.49	101.29
11	1.99	5.29	-55.84	108.15	147.59	92.46	103.57
12	1.62	5.19	-55.61	123.22	123.38	123.02	134.75
13	1.90	4.91	-54.46	152.65	88.89	108.51	105.35
14	2.18	4.76	-58.68	154.35	97.61	80.47	105.96
15	2.63	4.79	-67.12	144.17	111.77	120.32	105.93

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S15. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **1**(*R*)/hGAPDH complexes obtained by MC_II simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.96	2.72	-63.08	125.33	128.61	76.93	88.72
2	2.22	3.86	-176.06	107.07	122.94	95.65	104.55
3	1.96	3.63	-173.53	81.28	154.80	88.96	95.84
4	2.20	3.46	-176.57	85.05	160.75	89.48	95.34
5	2.45	3.58	-175.31	75.09	142.13	93.09	99.63
6	2.13	3.81	55.70	150.42	81.90	83.49	102.51
7	3.08	4.53	56.87	146.75	90.49	80.54	103.96
8	2.83	4.47	69.32	129.39	132.07	100.39	85.87
9	2.99	4.38	56.11	149.11	89.55	81.35	102.45
10	2.96	4.46	56.54	147.95	91.74	77.53	98.82

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S16. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*S*)_Conf1/hGAPDH complexes obtained by MC_I simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.93	2.64	-65.11	119.49	145.67	75.72	83.95
2	3.03	3.71	-61.88	131.04	129.89	81.07	97.52
3	3.57	4.06	-60.50	138.04	117.34	94.74	111.23
4	3.12	3.50	-59.93	131.79	123.65	92.79	110.04
5	2.95	4.18	-67.65	142.84	114.41	126.77	110.28
6	1.72	3.50	-64.19	104.64	157.45	114.33	108.01
7	1.87	3.52	-61.07	107.78	163.00	116.84	113.83
8	1.89	3.85	-63.29	113.71	152.19	108.08	103.66
9	1.71	3.88	-60.03	121.41	103.53	99.80	124.31
10	1.61	3.79	-62.90	123.39	137.48	136.80	142.76
11	1.48	3.79	-49.67	139.24	84.67	156.15	129.12
12	1.81	3.81	-64.83	120.28	146.53	137.85	138.40
13	1.47	3.76	-63.89	119.64	144.41	109.86	104.62
14	1.67	3.72	-63.05	112.09	157.91	114.80	113.15
15	2.16	3.78	62.23	102.11	85.32	105.79	130.99
16	1.70	3.07	-62.24	121.54	97.17	118.65	93.26
17	1.99	3.30	-175.79	102.40	157.99	102.61	106.56

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S17. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*S*)_Conf1/hGAPDH complexes obtained by MC_II simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.76	2.20	-63.95	118.35	140.36	78.07	83.27
2	2.86	3.24	-62.91	122.93	141.68	81.60	91.52
3	2.20	2.99	-62.54	115.07	150.38	73.93	83.83
4	2.34	2.91	-62.67	129.66	131.21	87.81	102.12
5	2.26	2.81	-62.90	124.50	139.52	78.70	89.51
6	1.55	3.86	-60.43	112.28	122.01	107.88	127.12
7	1.88	4.63	-170.50	61.63	124.32	148.77	167.07
8	2.73	4.66	-172.46	66.70	133.00	118.02	131.14
9	3.70	4.32	-174.13	78.25	106.05	135.93	158.91
10	4.43	4.64	-173.76	74.81	144.57	109.11	117.25
11	2.85	4.69	71.11	89.36	118.41	159.10	155.17
12	2.49	4.13	73.68	95.14	109.98	134.63	124.88
13	2.77	4.33	64.39	77.85	100.12	140.99	116.40
14	4.26	4.98	64.54	95.51	84.20	123.53	96.73
15	4.23	4.82	64.16	72.13	133.22	104.12	115.12
16	1.77	5.19	-65.58	95.41	117.01	113.16	133.06
17	2.08	5.14	-61.14	98.37	124.00	93.23	109.73
18	1.97	4.90	-172.85	63.37	126.72	140.19	157.92
19	2.31	5.02	-173.22	63.15	122.77	134.19	153.30
20	3.62	5.01	-174.81	70.67	112.36	143.17	155.37
21	2.48	4.49	179.40	73.97	143.09	96.63	104.61
22	2.81	4.53	178.37	76.45	147.15	97.22	102.73
23	2.48	4.30	176.95	69.64	125.89	122.03	138.50
24	2.30	3.99	175.92	69.63	120.61	127.18	146.17
25	2.69	3.65	173.41	73.33	131.37	123.52	138.72
26	2.51	4.20	177.85	67.08	129.47	119.52	131.33
27	2.04	4.53	177.79	66.67	128.30	112.71	124.75
28	2.09	4.38	178.27	66.95	126.29	113.69	126.68

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S18. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*S*)_Conf2/hGAPDH complexes obtained by MC_I simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.97	3.10	-62.58	132.53	127.73	74.98	89.69
2	2.08	3.45	-59.45	126.59	116.71	94.16	114.78
3	3.38	4.81	-68.07	135.10	123.55	120.32	105.91
4	3.40	4.43	-67.40	153.99	98.22	133.07	117.85
5	2.76	4.32	-66.45	133.24	127.67	130.49	126.52
6	0.98	3.90	-65.20	128.16	133.78	113.58	114.31
7	2.08	4.56	-65.79	139.33	107.80	144.29	137.43
8	2.49	4.70	-66.84	141.32	115.72	128.60	124.06
9	2.59	4.75	-66.56	140.29	117.36	128.76	126.02
10	2.31	4.69	-60.39	145.64	86.02	112.55	137.23
11	2.58	4.51	-66.74	132.72	128.21	152.12	148.26
12	3.84	5.27	-61.48	118.82	146.16	145.60	148.23
13	3.46	5.01	-61.61	116.10	148.12	143.63	144.69
14	3.49	4.99	-60.06	111.58	148.67	137.15	136.96
15	3.25	5.16	-59.31	116.88	98.19	132.05	140.69
16	3.04	4.82	-61.20	114.02	154.30	143.36	147.50
17	2.90	4.95	-63.36	117.65	149.08	148.11	154.68
18	3.17	4.97	-63.07	120.26	145.30	146.27	154.05
19	3.61	5.08	-62.72	119.48	144.65	142.76	147.18
20	3.44	5.02	-63.25	121.26	145.35	147.84	156.84
21	1.89	3.31	-164.89	66.03	123.50	141.38	145.73
22	1.52	3.19	-167.24	68.90	125.27	131.70	137.18
23	0.91	3.55	-166.76	64.50	120.74	144.13	150.68
24	1.06	3.68	-55.49	151.91	91.46	106.56	118.42
25	1.69	3.48	-64.18	110.82	159.82	127.61	122.12
26	2.58	3.36	-64.63	116.83	151.69	127.60	121.07
27	1.89	3.40	-63.77	108.46	158.74	124.17	117.15
28	2.25	3.69	-60.06	104.14	143.68	84.64	96.38
29	3.16	4.14	-63.01	68.90	125.27	131.70	137.18
30	2.38	3.96	-55.90	135.31	108.19	86.57	105.84
31	2.40	4.14	-64.18	110.82	159.82	127.61	122.12

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S19. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*S*)_Conf2/hGAPDH complexes obtained by MC_II simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.76	2.61	-62.44	138.23	116.10	79.15	93.44
2	2.08	1.91	-61.80	139.90	116.84	97.52	115.25
3	1.64	1.88	-178.75	86.61	139.03	109.09	102.72
4	1.93	2.16	-176.62	77.84	150.03	119.68	125.37
5	3.24	3.05	-178.62	107.59	93.80	89.41	115.27
6	2.98	4.06	-57.82	116.26	140.57	101.87	113.48
7	2.91	3.67	-55.93	121.28	144.05	102.34	113.44
8	2.83	2.89	-57.65	158.93	83.25	147.47	133.96
9	2.48	3.08	-57.55	146.82	88.33	145.17	146.04
10	2.59	2.81	-66.49	144.99	109.60	154.74	173.09
11	2.66	4.74	-57.95	105.79	167.66	81.21	85.42
12	2.91	4.89	-58.36	93.03	144.04	87.09	85.48
13	2.73	4.94	-57.59	90.33	109.32	91.73	84.76
14	2.50	5.20	-61.47	109.86	144.43	105.37	108.66
15	2.49	4.91	-54.49	90.10	109.64	96.74	86.49
16	2.30	4.90	-56.21	86.65	124.70	86.82	80.44
17	2.49	5.45	-57.98	93.53	121.10	87.00	82.52
18	2.68	4.82	-60.37	105.01	97.16	93.14	95.67
19	2.74	5.01	-62.66	117.48	114.30	101.49	102.62
20	2.75	4.41	-62.35	98.13	111.39	93.70	81.29

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S20. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*R*)_Conf1/hGAPDH complexes obtained by MC_I simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.89	2.24	-64.62	111.02	157.58	75.32	80.15
2	2.58	2.86	-62.80	120.63	143.24	79.95	89.13
3	2.24	2.58	-64.36	118.54	147.82	81.85	91.12
4	2.28	2.71	-63.31	112.39	152.00	76.81	83.12
5	2.12	2.65	-55.18	118.83	126.08	115.16	130.02

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S21. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*R*)_Conf1/hGAPDH complexes obtained by MC_II simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.83	1.98	-63.64	114.63	147.10	78.35	83.15
2	2.30	2.71	-62.90	118.03	146.86	77.88	87.36
3	2.18	2.15	-179.22	109.17	161.42	90.47	90.05
4	2.32	1.91	-178.14	95.69	169.36	101.30	104.98
5	2.45	2.09	-178.65	88.82	166.50	102.28	104.27
6	2.35	3.68	-57.62	89.65	134.70	104.25	108.84
7	1.80	4.81	-57.75	84.98	159.36	71.47	65.32
8	2.05	4.65	-53.23	81.74	153.39	76.00	68.65
9	2.01	5.23	-60.30	89.36	161.05	70.68	67.63
10	2.25	4.73	-59.01	84.93	157.04	72.54	66.73

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S22. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*R*)_Conf2/hGAPDH complexes obtained by MC_I simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.78	2.25	-63.80	113.81	154.47	77.13	82.81
2	3.17	3.59	-62.96	137.24	119.87	92.40	108.54
3	2.30	3.25	-62.54	124.59	114.70	104.67	89.70
4	1.97	3.21	-59.75	134.32	117.85	86.49	105.17
5	2.32	3.53	-63.92	144.62	107.78	88.45	107.19
6	1.92	3.34	-55.49	125.10	112.74	123.98	137.98
7	1.32	3.10	-52.13	155.52	91.26	116.35	115.51
8	1.16	3.63	-53.27	126.19	108.82	153.61	163.45
9	2.58	2.84	64.10	95.42	125.71	125.86	108.74
10	2.73	3.22	64.94	120.46	126.88	128.33	111.05
11	1.93	3.65	68.50	106.08	160.79	147.42	153.06
12	4.26	3.95	61.01	132.96	115.46	99.96	121.26
13	4.16	4.03	59.05	131.81	105.60	102.68	112.63
14	3.92	4.09	59.56	123.54	122.91	92.69	101.65
15	4.26	4.10	58.75	129.18	106.31	103.00	112.20
16	2.81	4.97	64.55	96.27	138.22	95.34	98.52
17	3.32	6.02	62.42	95.21	125.21	96.07	91.69
18	3.62	5.95	63.74	87.98	116.05	84.58	81.34
19	3.88	5.66	62.20	128.59	116.63	118.58	130.18
20	3.45	5.44	61.93	147.37	104.02	129.19	146.33
21	1.09	3.49	-49.76	147.54	84.93	141.51	123.18
22	1.44	3.18	-63.91	114.85	151.25	124.85	119.77
23	1.38	3.04	-62.86	112.36	152.88	127.81	123.87
24	1.43	3.08	-63.18	115.49	151.93	131.83	128.95
25	1.74	3.19	-63.34	106.27	168.07	140.56	142.18

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

Table S23. Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the **11**(*R*)_Conf2/hGAPDH complexes obtained by MC_II simulation.

Complex	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
				XDA[a]	DHA[a]	DAY[a]	HAY[a]
1	1.74	2.23	-64.32	116.23	143.12	74.01	81.37
2	1.70	2.66	-173.41	94.15	156.38	96.27	101.28
3	1.93	3.02	-173.45	94.63	146.17	96.11	103.98
4	1.66	2.91	-62.62	132.66	126.54	76.12	88.45
5	1.87	2.85	-61.73	140.88	114.80	86.33	101.93
6	1.58	3.12	-68.50	107.53	162.97	153.91	150.90
7	1.22	3.51	-57.56	134.84	103.19	116.80	133.57
8	1.14	3.74	-57.89	136.87	99.49	119.13	134.08
9	2.01	4.27	-55.24	136.55	99.15	133.30	141.18
10	1.82	4.09	-68.97	139.61	107.16	131.18	109.03
11	1.58	4.02	-56.20	118.56	115.28	124.94	143.48
12	1.33	4.01	-56.73	119.99	113.42	127.81	146.58
13	1.46	3.70	-68.74	122.35	106.93	139.94	116.63
14	1.39	3.35	-68.72	125.37	109.76	140.12	117.27
15	1.43	4.04	-70.28	116.81	118.00	139.31	118.81
16	1.90	4.09	-56.99	119.98	103.43	102.40	127.04
17	1.58	3.76	-53.96	134.97	86.84	111.25	137.78
18	1.57	3.02	-65.75	111.55	158.65	157.81	150.44
19	1.37	3.00	-62.14	119.31	147.40	157.62	146.66
20	1.82	3.34	-61.30	73.65	113.08	98.25	115.95

[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179.

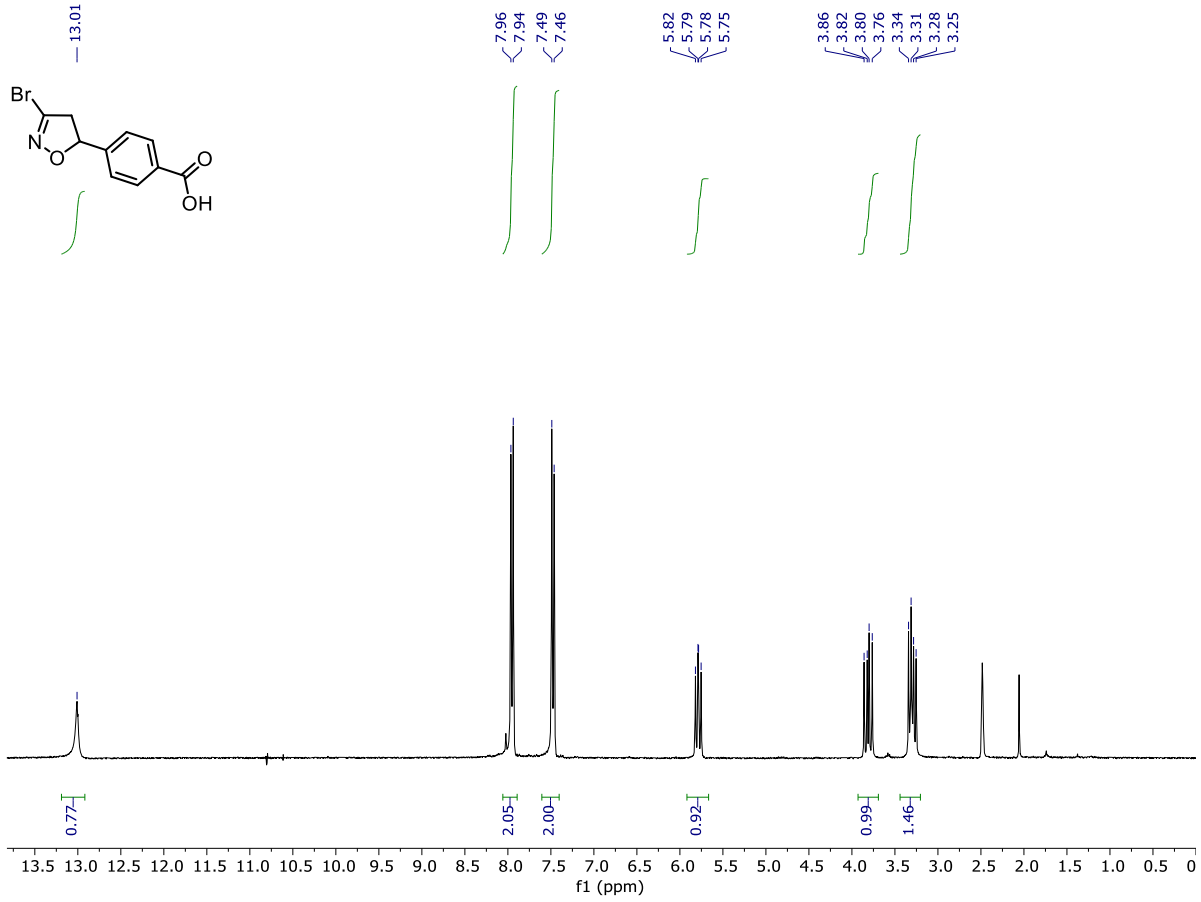
Table S24. Distance (Å) between the 3-bromo-4,5-dihydroisoxazole carbon atom C3 and the C152 sulfur atom, direction of the bromine atom towards the cofactor NAD+, the inside (inward) or outside (outward) of the catalytic site, Cα RMSD (Å) of C152 and H179 calculated with respect to the starting position, χ1 torsion angle of C152 (degrees) and angle values of the hydrogen bond between C152 and H179 (degrees) of the MC **1**/hGAPDH and **11**/hGAPDH complexes obtained after unrestrained structure optimization.

Lig	Cplx	Docking Proc.[c]	Dist. C3-S	Br Orientation	RMSD (C152)	RMSD (H179)	χ_1 (C152)	Angle values of the hydrogen bond between C152 and H179			
								XDA[a]	DHA[a]	DAY[a]	HAY[a]
1S	**1**	**I**	4.9	Outward	0.93	0.27	-74.62	155.46	95.92	115.62	127.73
1S	**1**	**II**	5.8	Outward	1.89	1.49	-75.23	143.14	110.44	101.38	112.58
1S	**3**[b]	**II**	3.7	Outward	2.26	2.22	-73.97	129.57	114.96	138.94	156.55
1R	**1**[b]	**I**	4.2	Outward	1.46	0.59	-72.13	145.55	109.91	106.27	120.58
11S_Conf1	**4**[b]	**II**	4.0	NAD^+	2.21	1.84	-66.44	135.44	126.48	121.90	107.14
11S_Conf2	**1**	**II**	4.0	Inward	1.31	0.64	-64.76	103.56	152.51	80.45	80.15
11S_Conf2	**2**	**II**	4.2	Inward	1.28	1.15	-68.09	138.62	117.54	120.28	134.58
11S_Conf2	**8**	**II**	6.2	Outward	1.94	1.13	-63.55	70.24	139.58	103.48	89.95
11S_Conf2	**10**	**II**	5.5	Outward	1.86	1.13	-68.97	110.49	113.49	95.17	81.70
11R_Conf1	**3**	**I**	4.7	NAD^+	0.75	0.94	-69.00	137.18	119.80	90.16	103.17
11R_Conf2	**5**	**II**	4.2	NAD^+	2.12	2.03	-82.81	149.87	106.69	118.71	94.98
11R_Conf2	**18**	**II**	5.2	Inward	2.19	2.91	-75.16	93.37	139.67	161.06	163.42
11R_Conf2	**19**[b]	**II**	3.6	Outward	2.37	3.05	-77.77	100.93	125.43	149.59	143.86

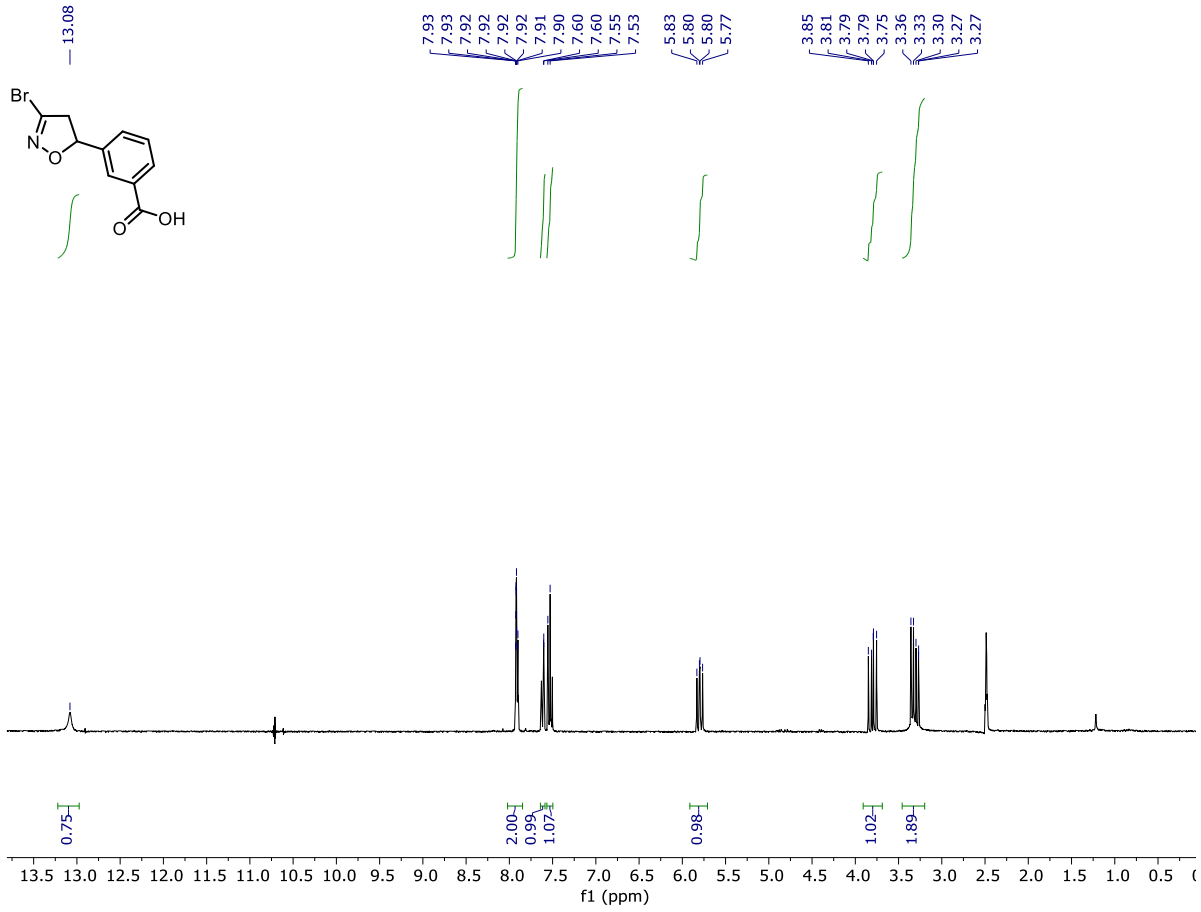
[a] X: C_β of C152; D: S_γ of C152; A: N_τ of H179; H: H_γ of C152 and Y: C_δ of H179. [b]Selected complex.[c] I: docking procedure without constraints on the hydrogen bonds between NAD^+ and hGAPDH; II: docking procedure with constraints on the hydrogen bonds between NAD^+ and hGAPDH.

^{1}H NMR Spectra

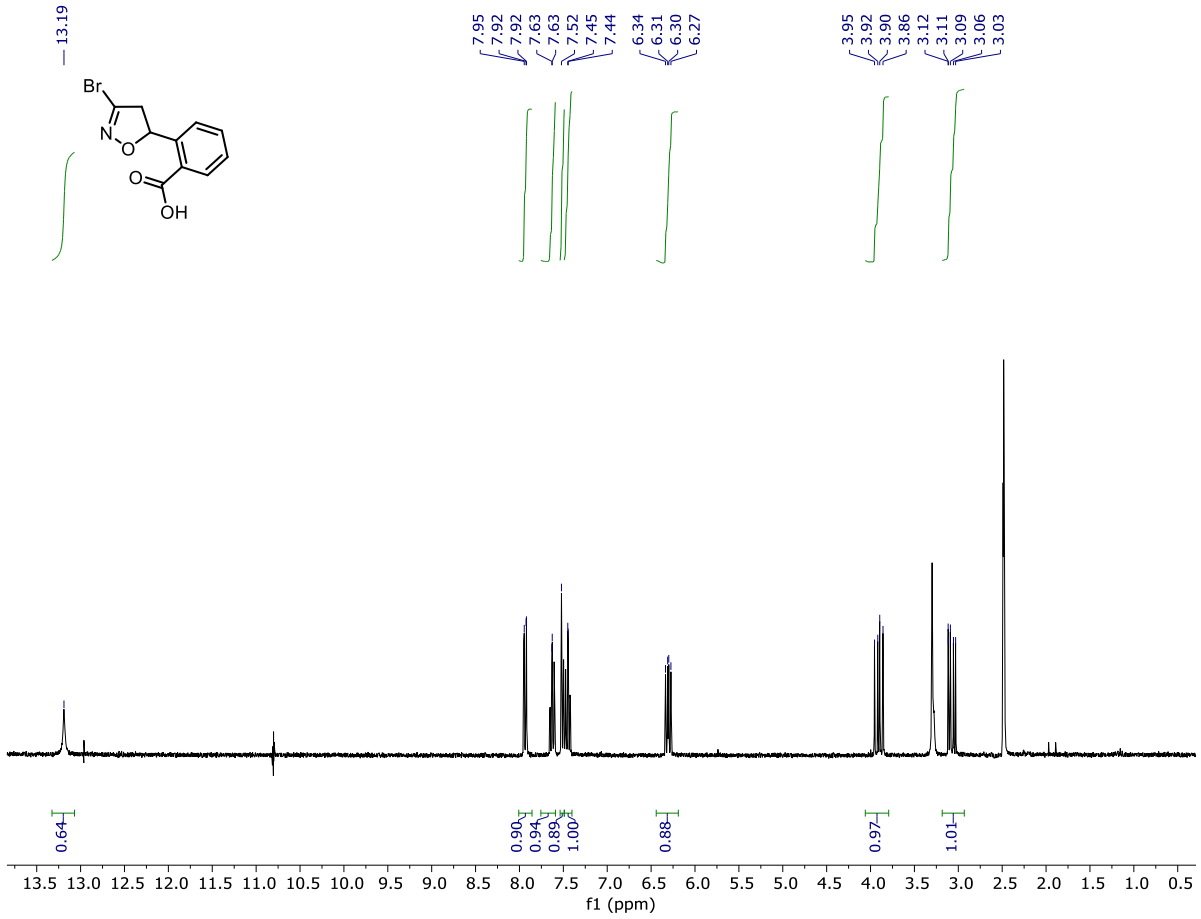
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**3**):



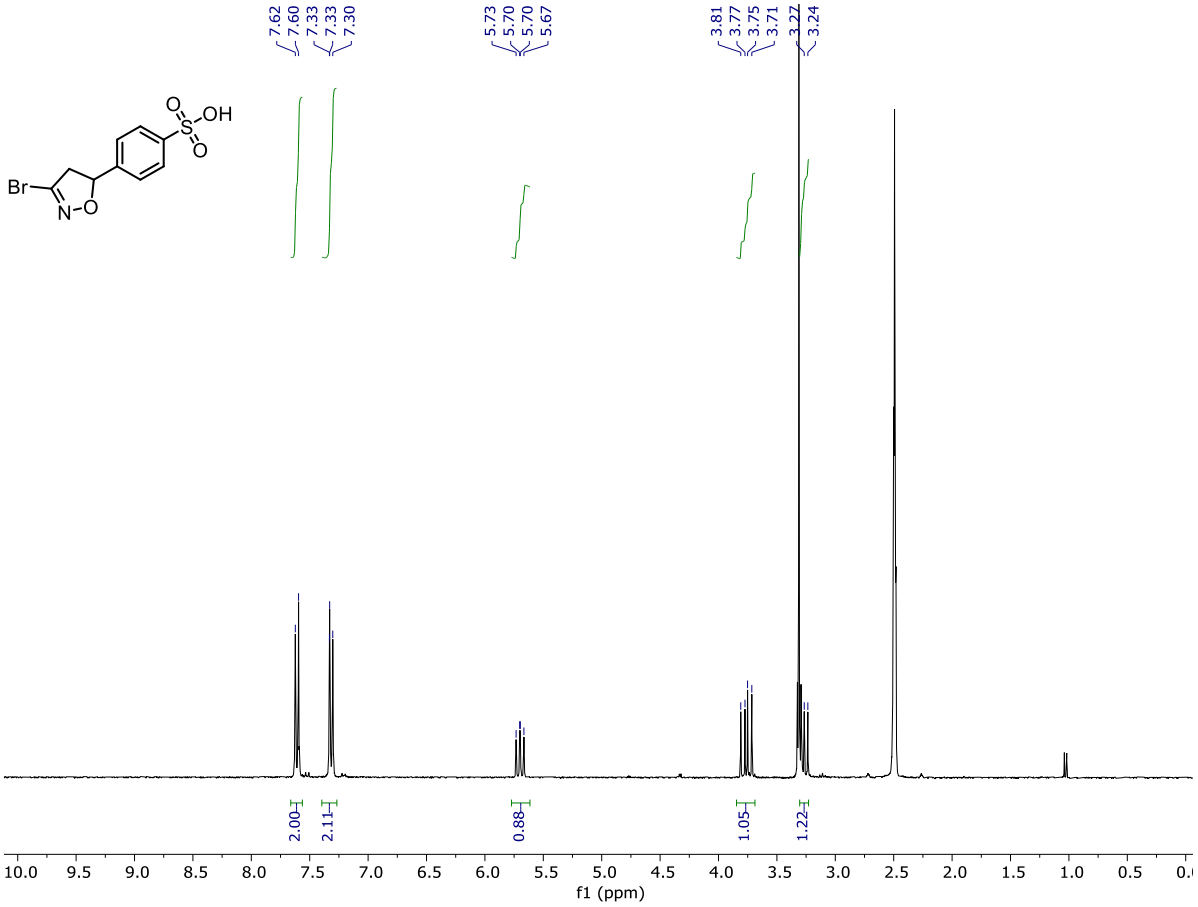
3-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**4**):



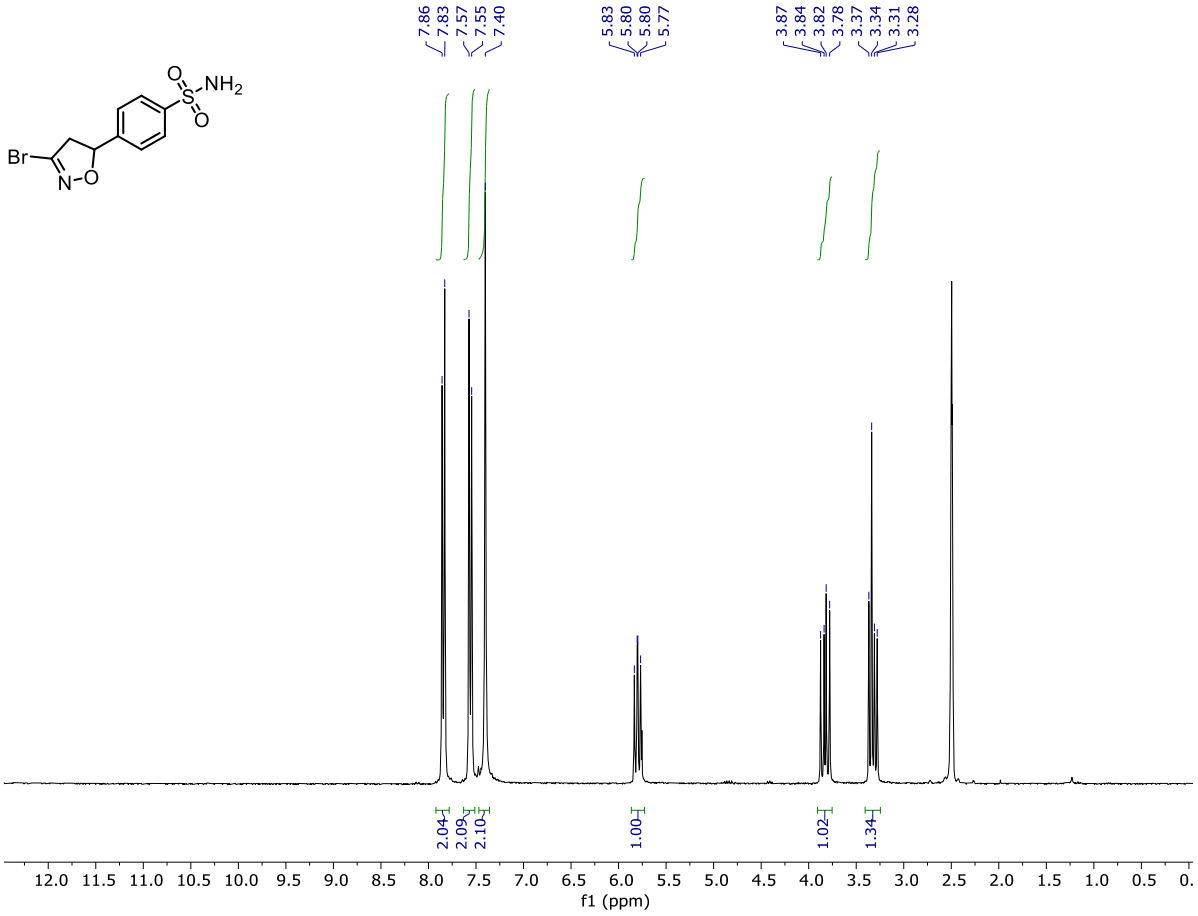
2-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**5**):



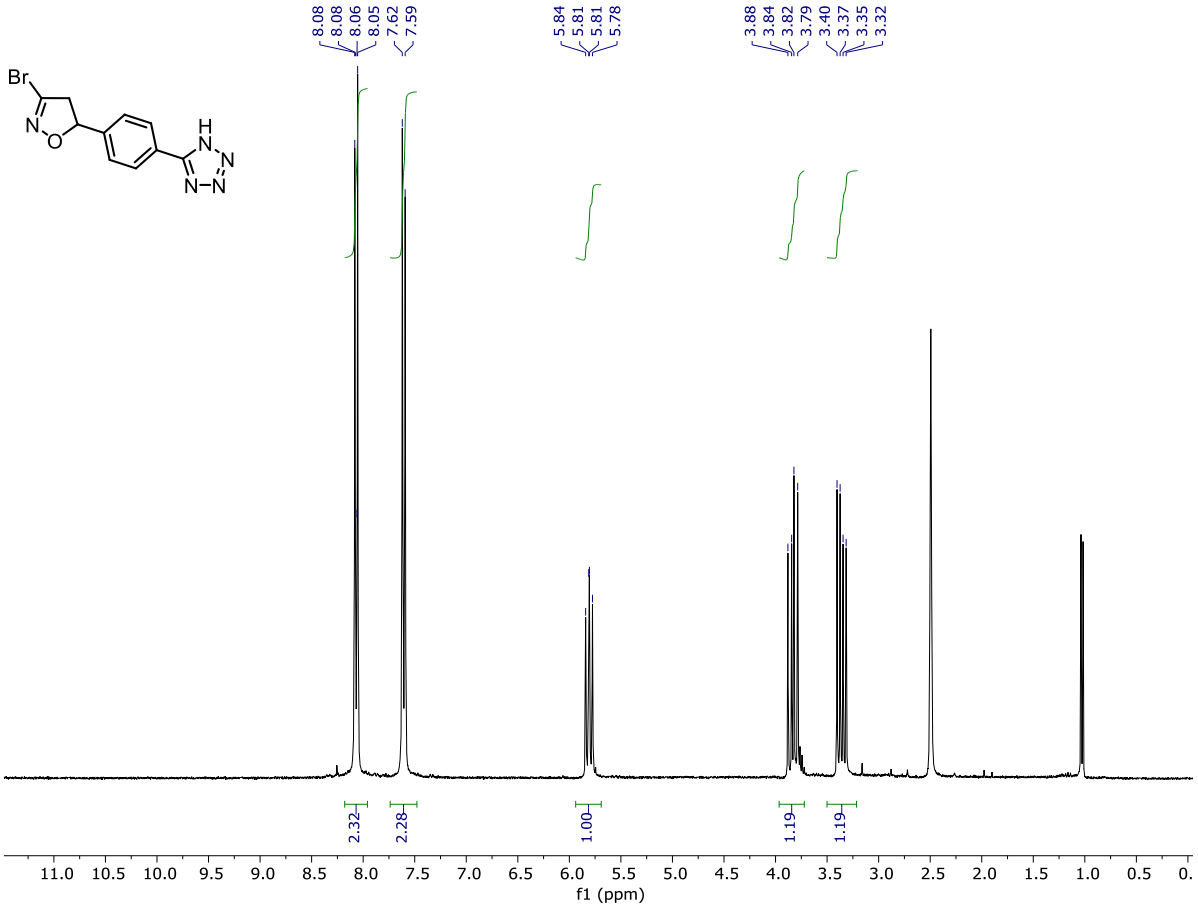
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonic acid (**6**):



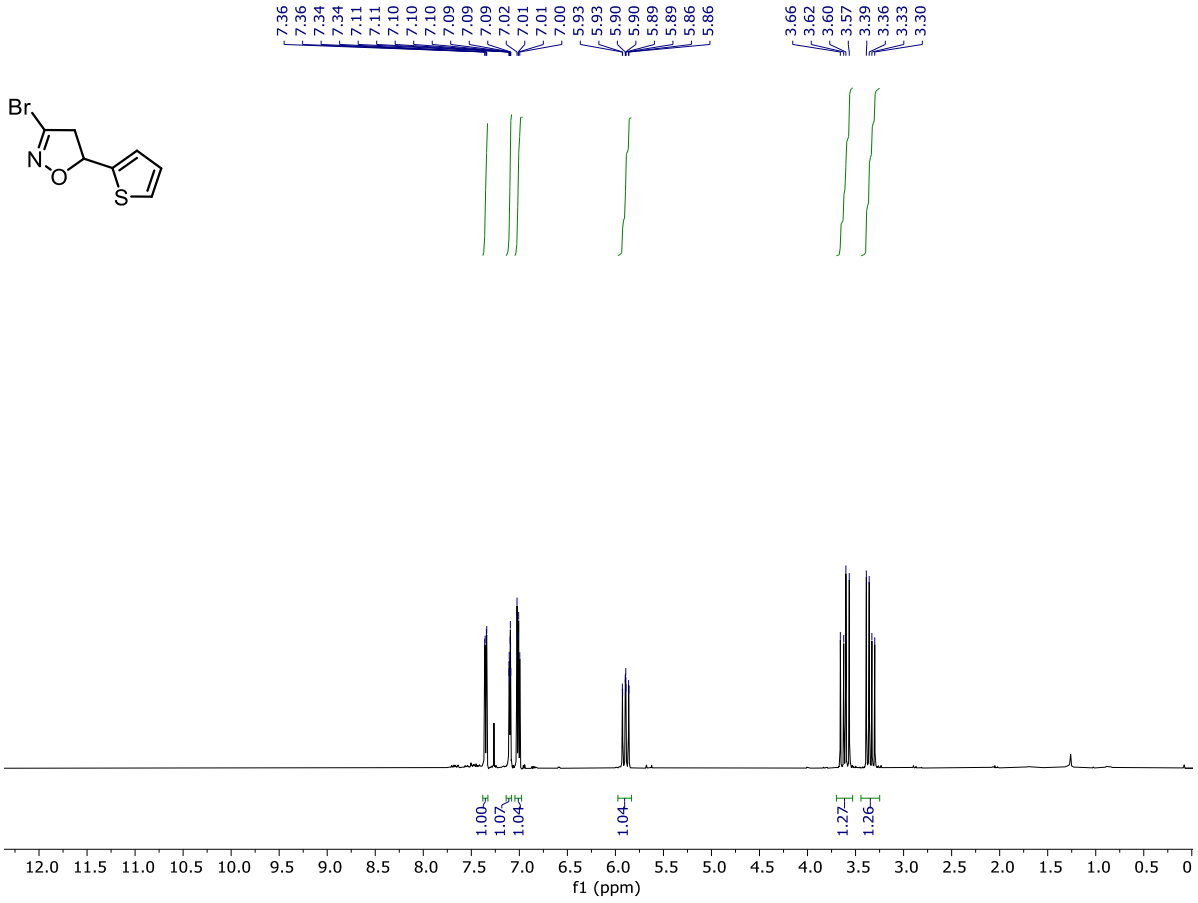
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonamide (**7**):



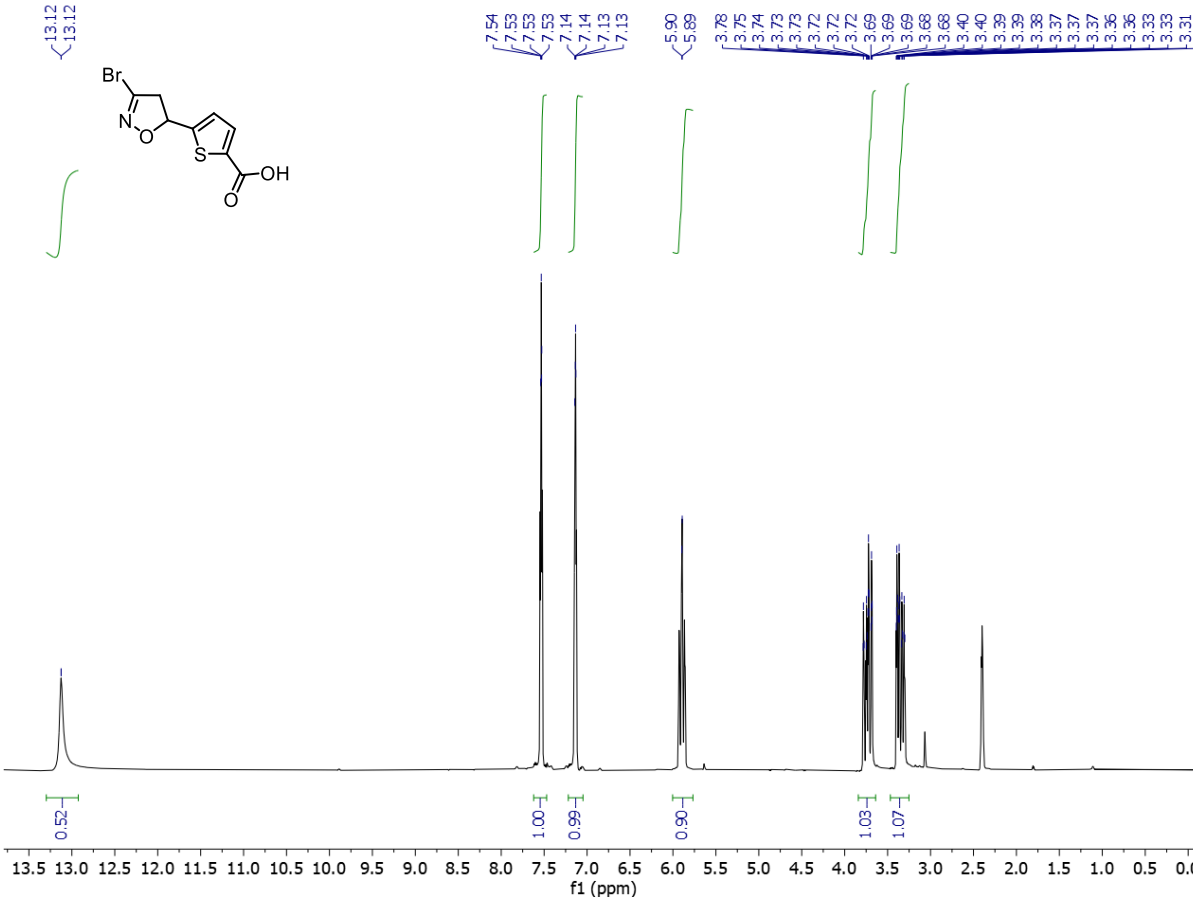
5-(4-(1*H*-Tetrazol-5-yl)phenyl)-3-bromo-4,5-dihydroisoxazole (**8**):



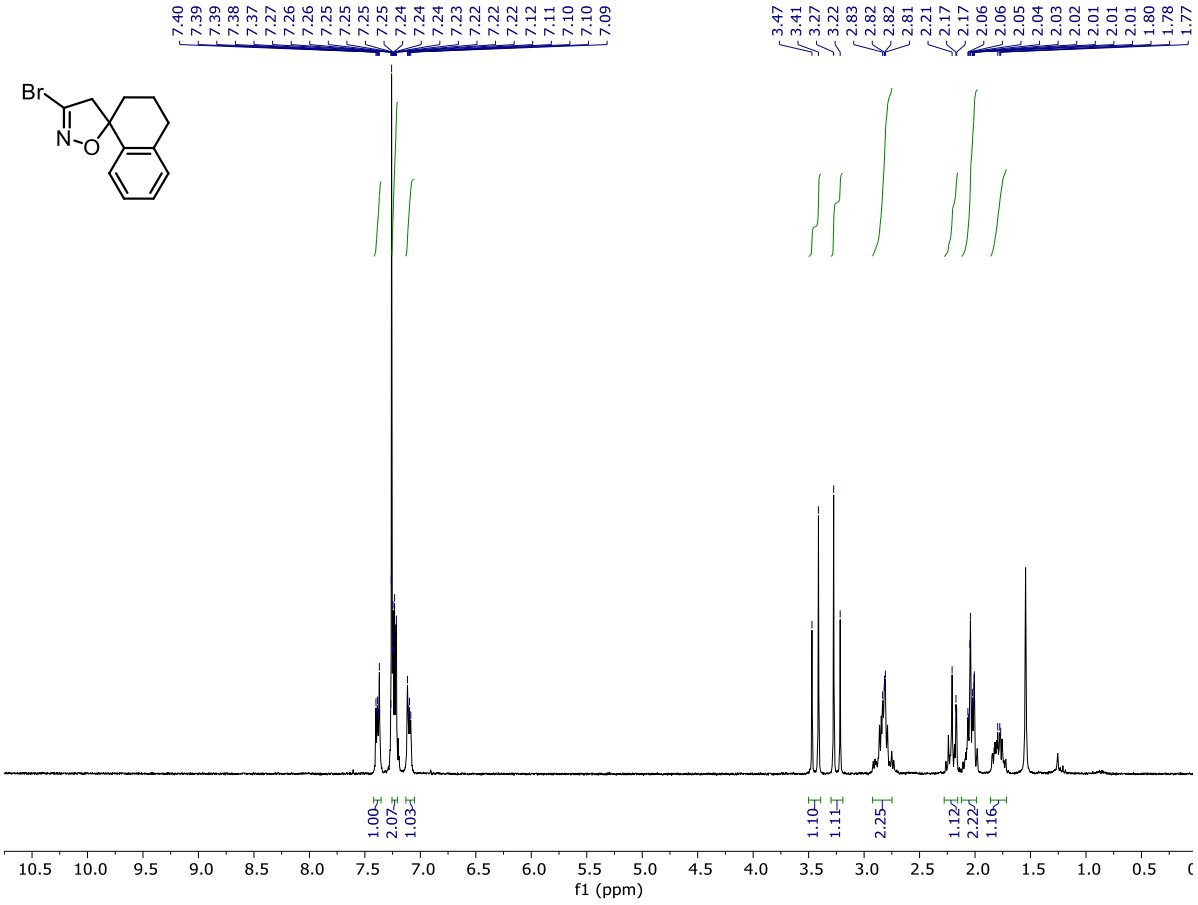
3-Bromo-5-(thiophen-2-yl)-4,5-dihydroisoxazole (**9**):



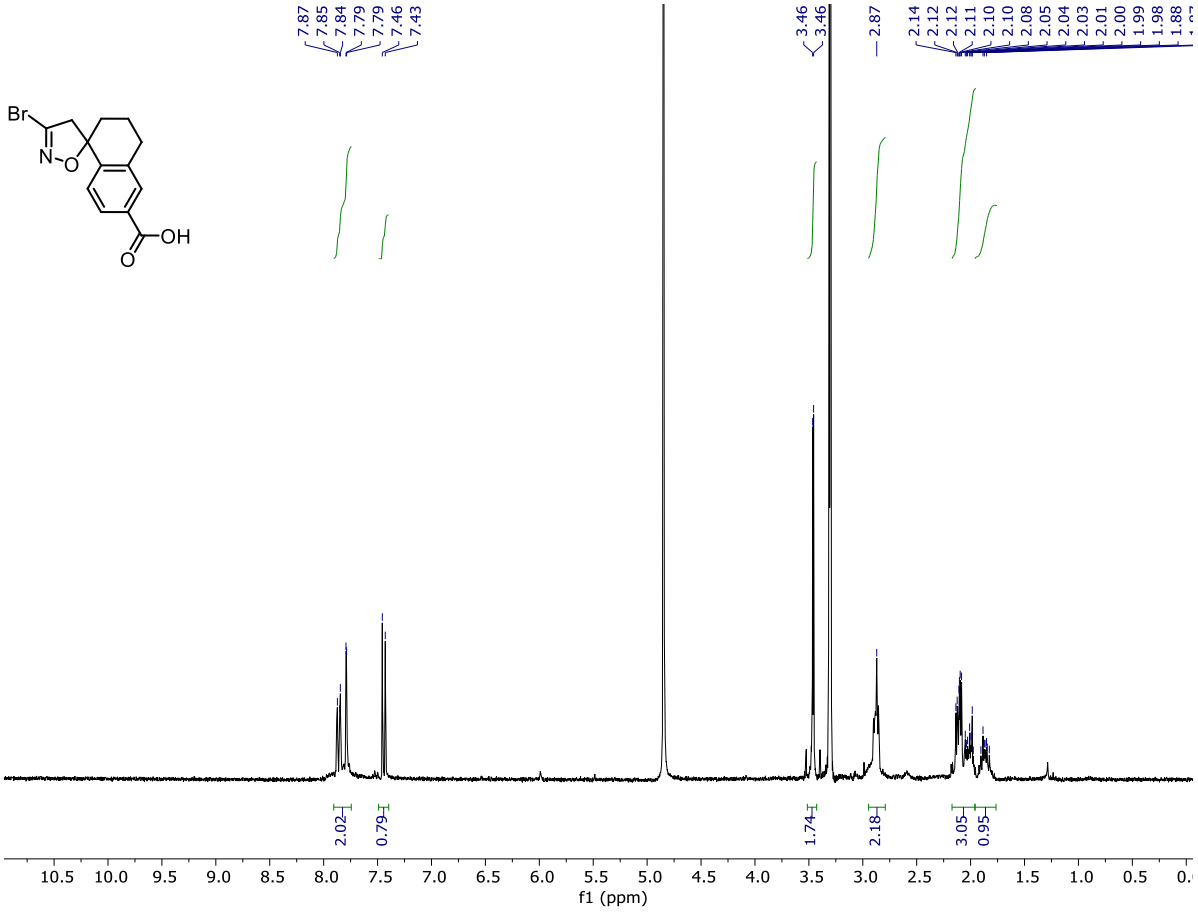
5-(3-Bromo-4,5-dihydroisoxazol-5-yl)thiophene-2-carboxylic acid (**10**):



3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene] (**11**):

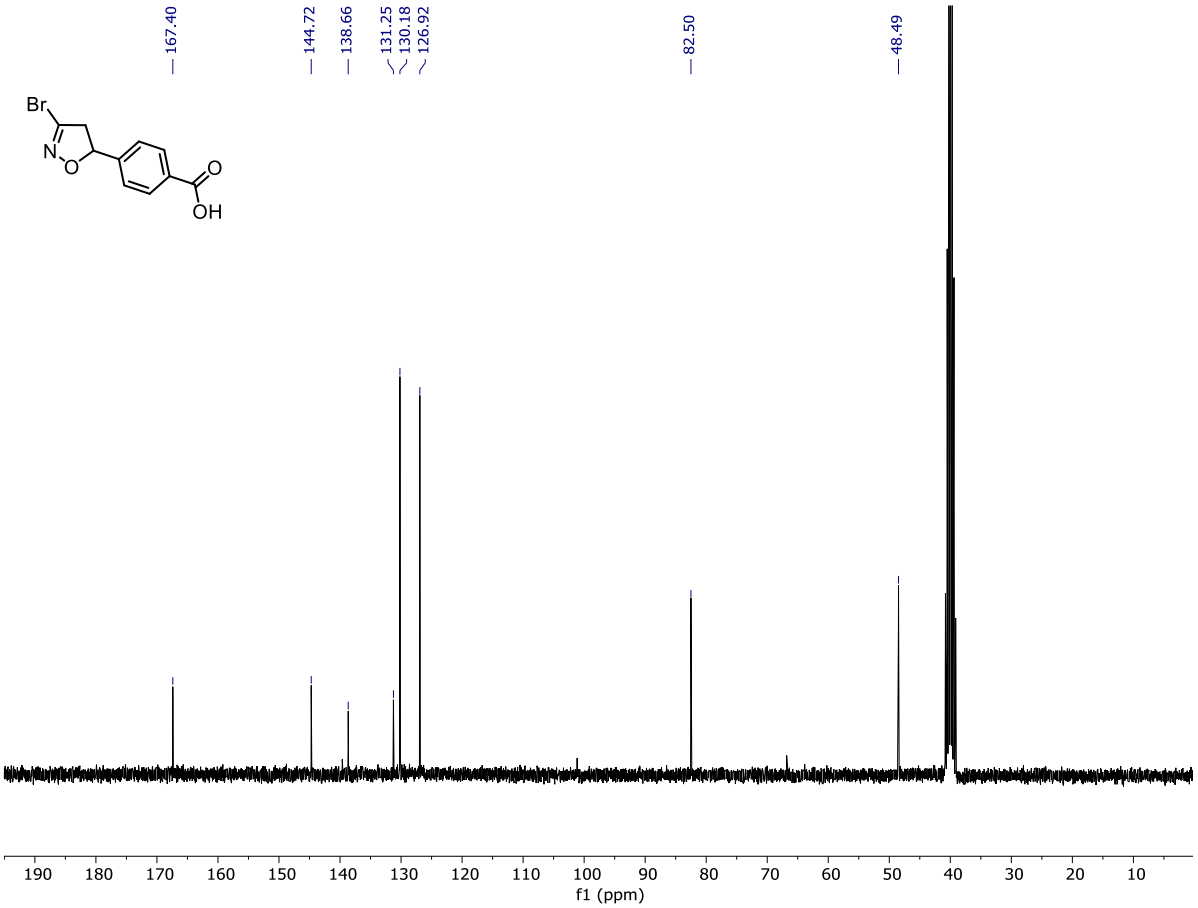


3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene]-6'-carboxylic acid (**12**):

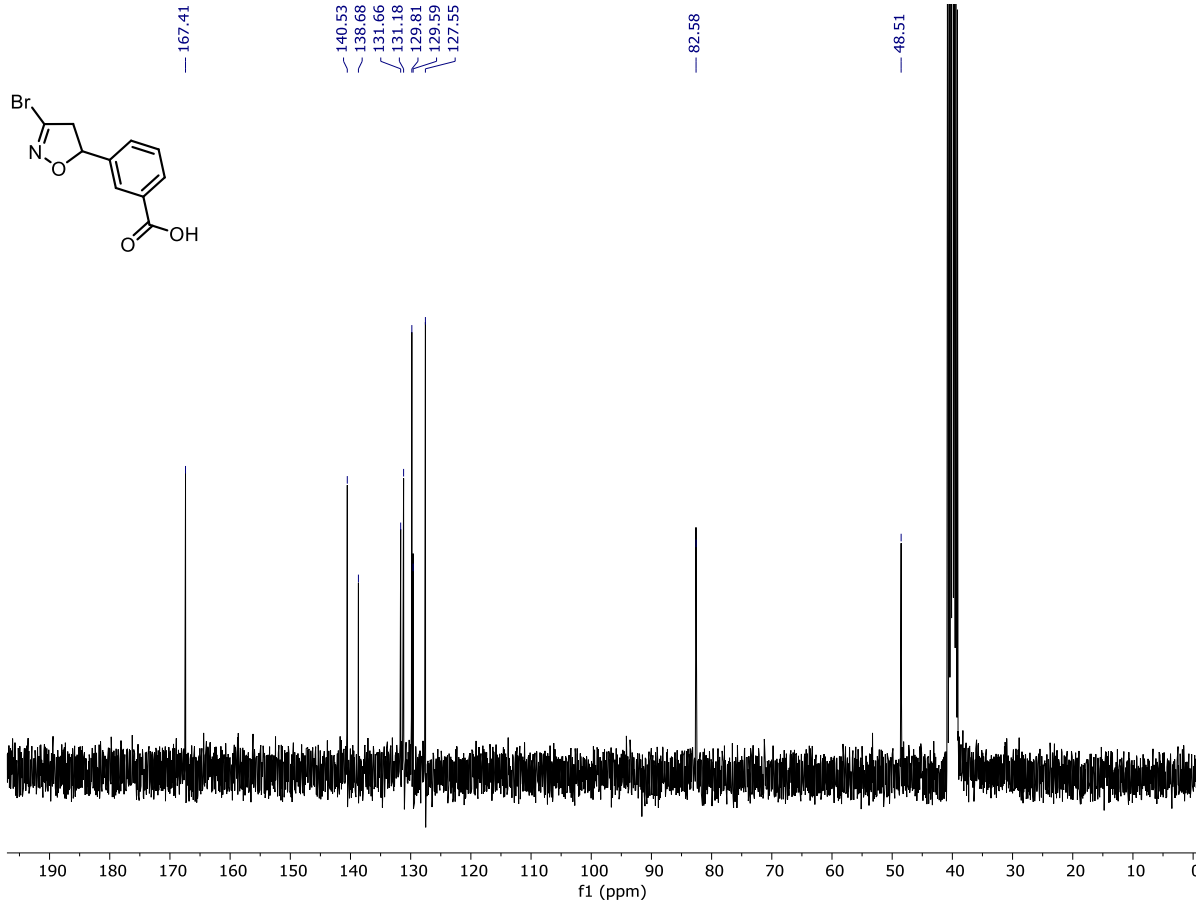


$^{13}C\{^{1}H\}$ NMR Spectra

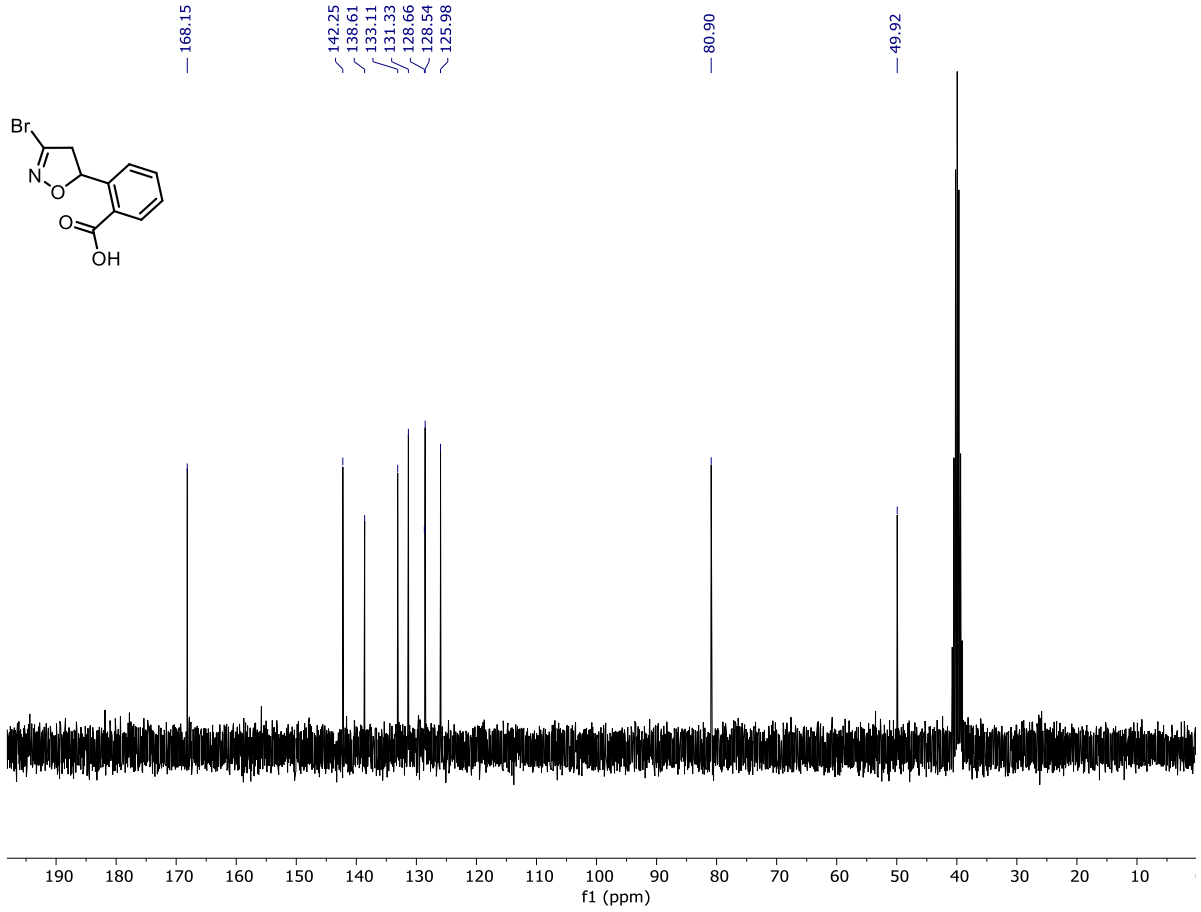
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**3**):



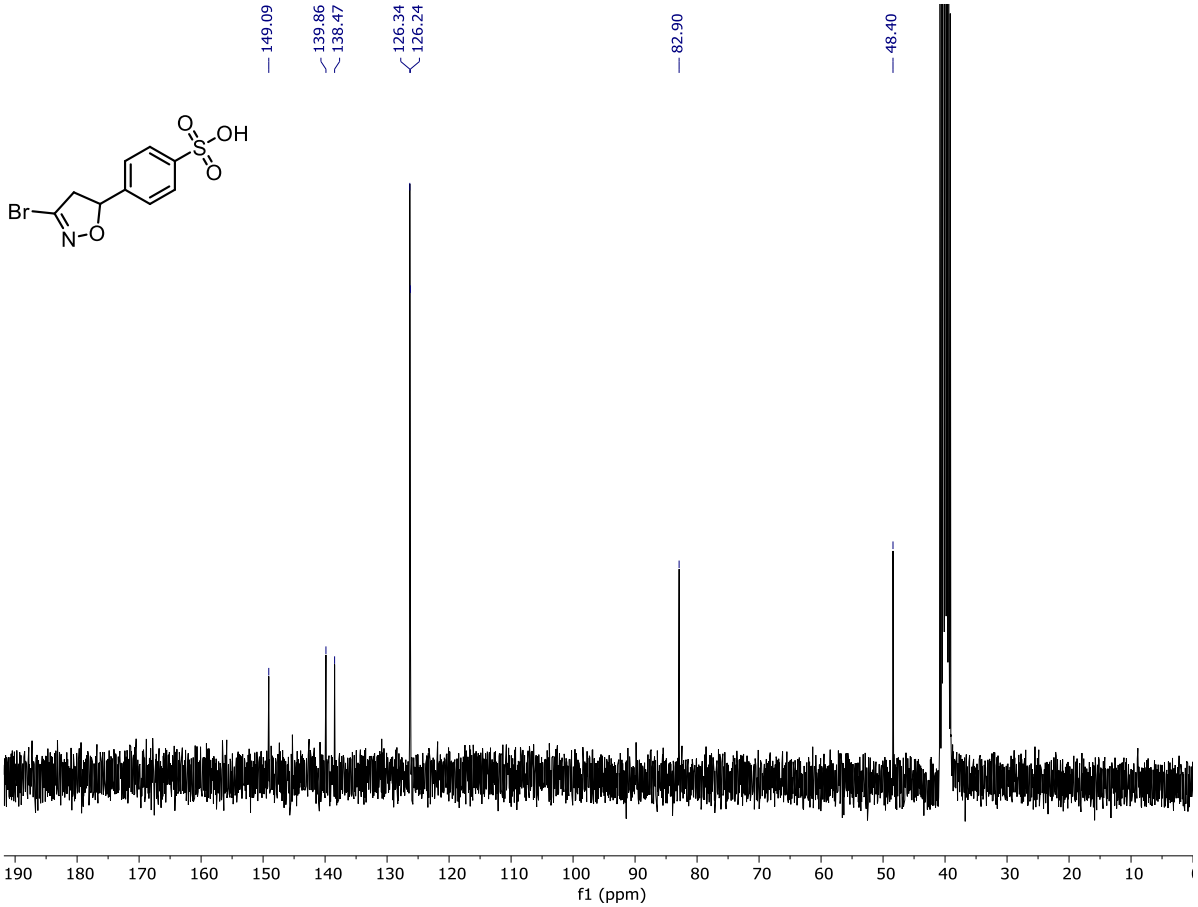
3-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**4**):



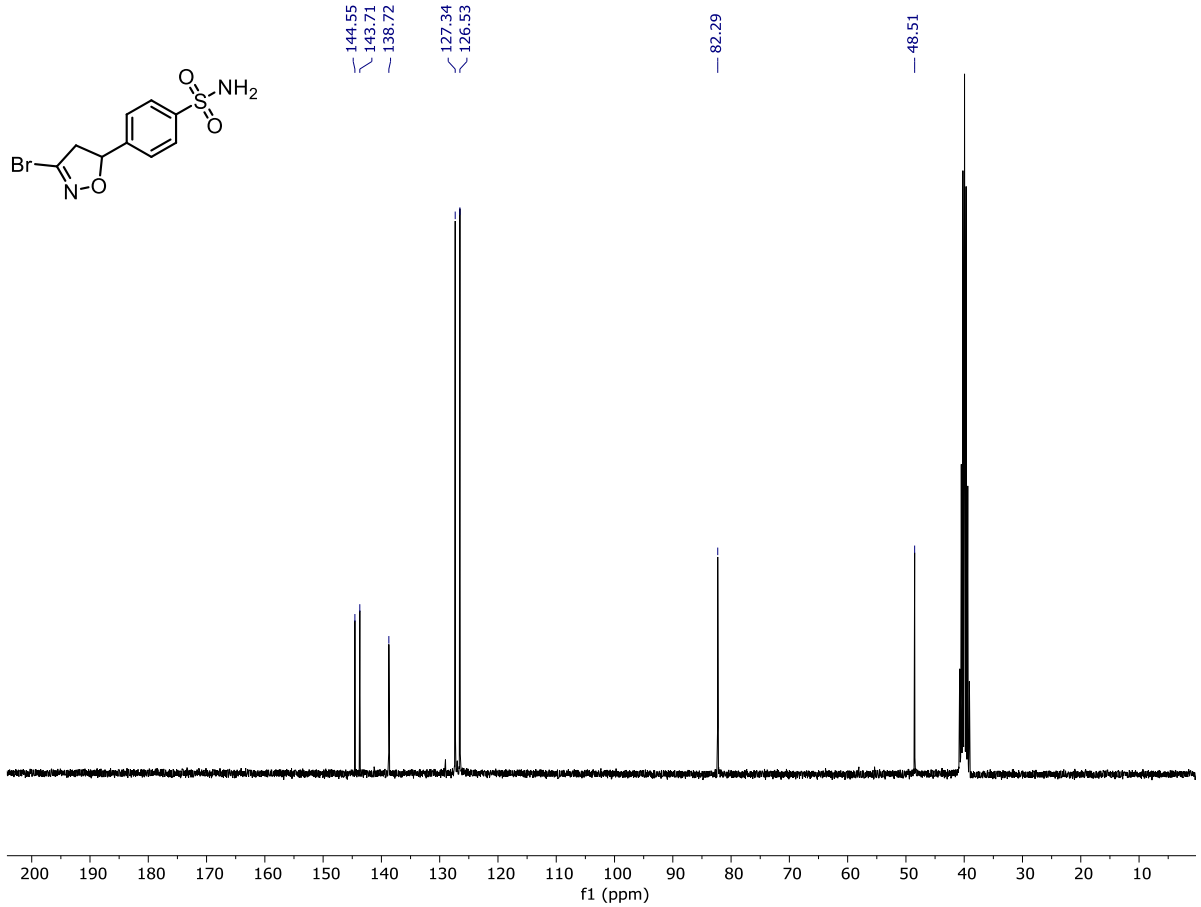
2-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**5**):



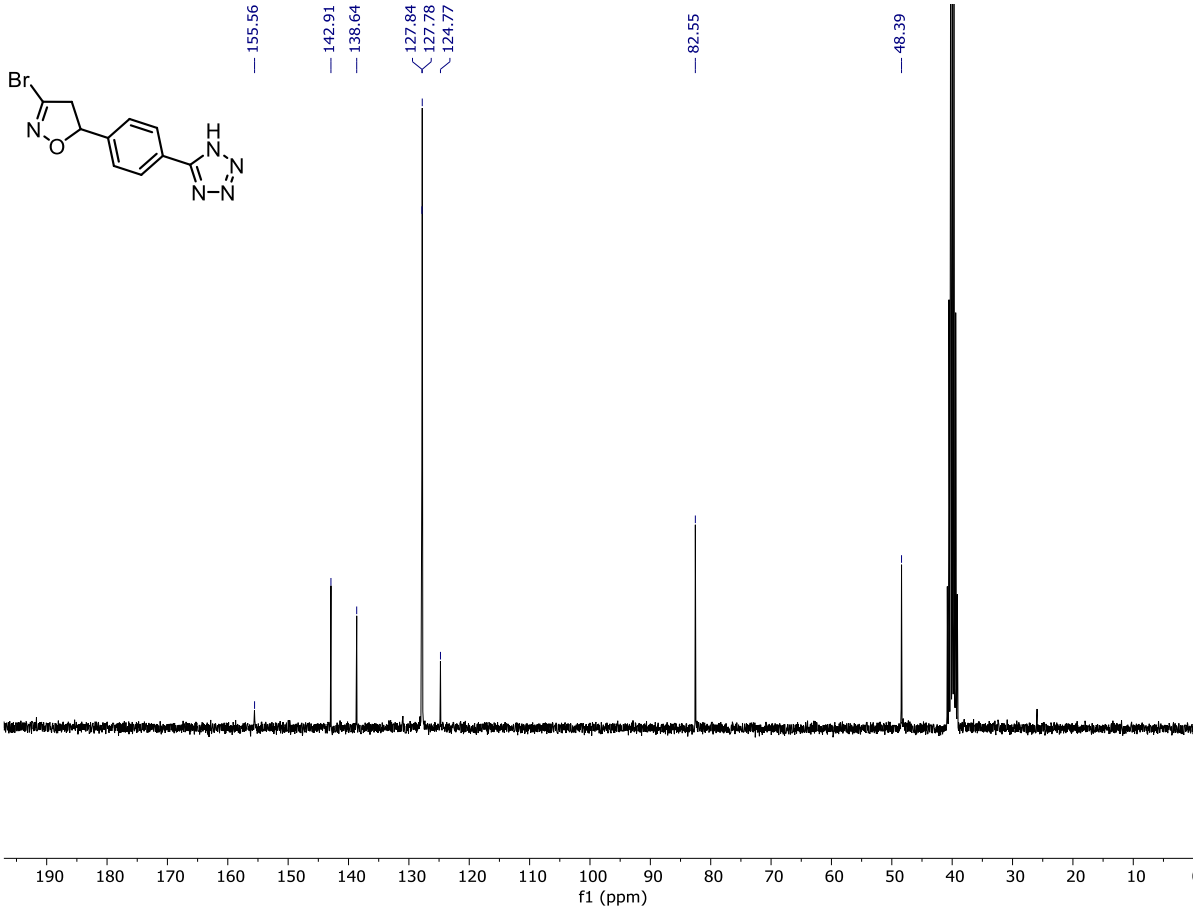
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonic acid (**6**):



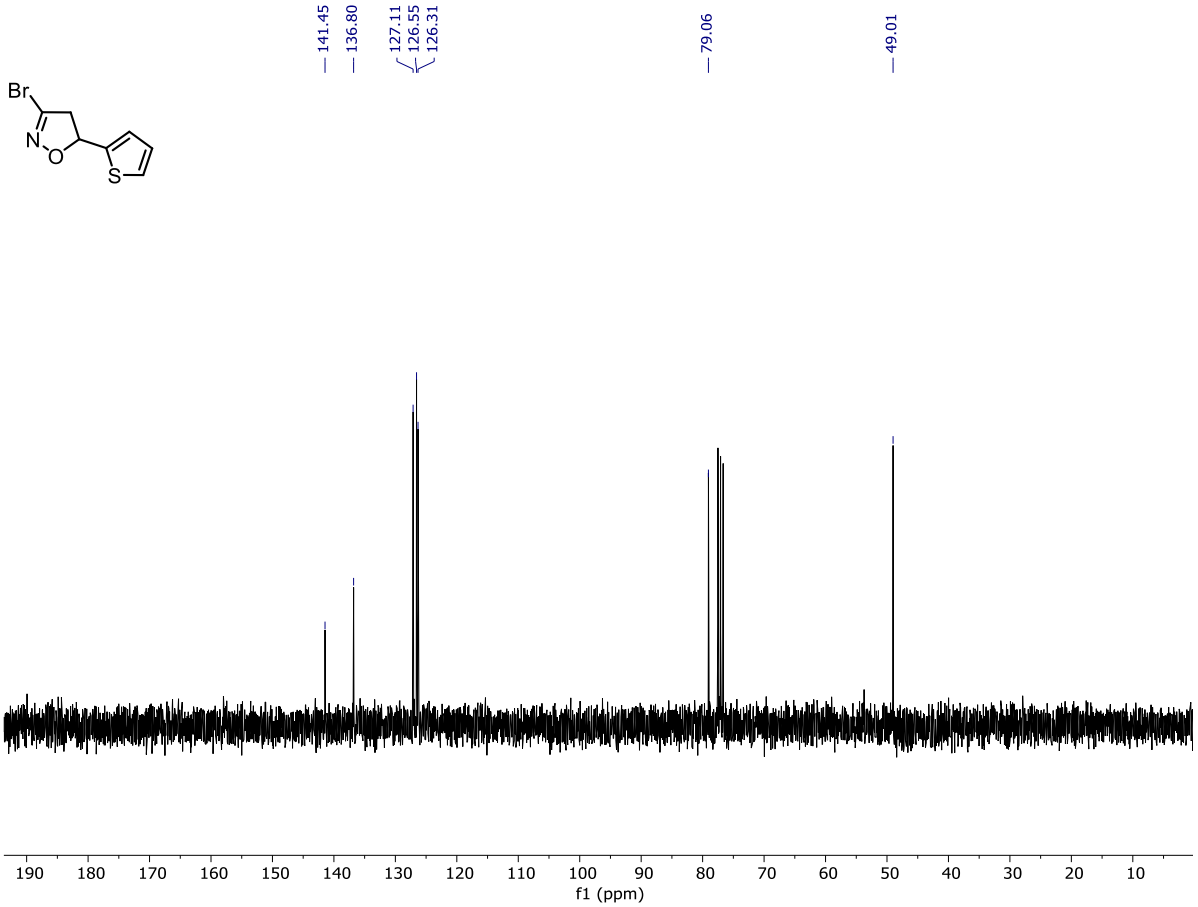
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonamide (**7**):



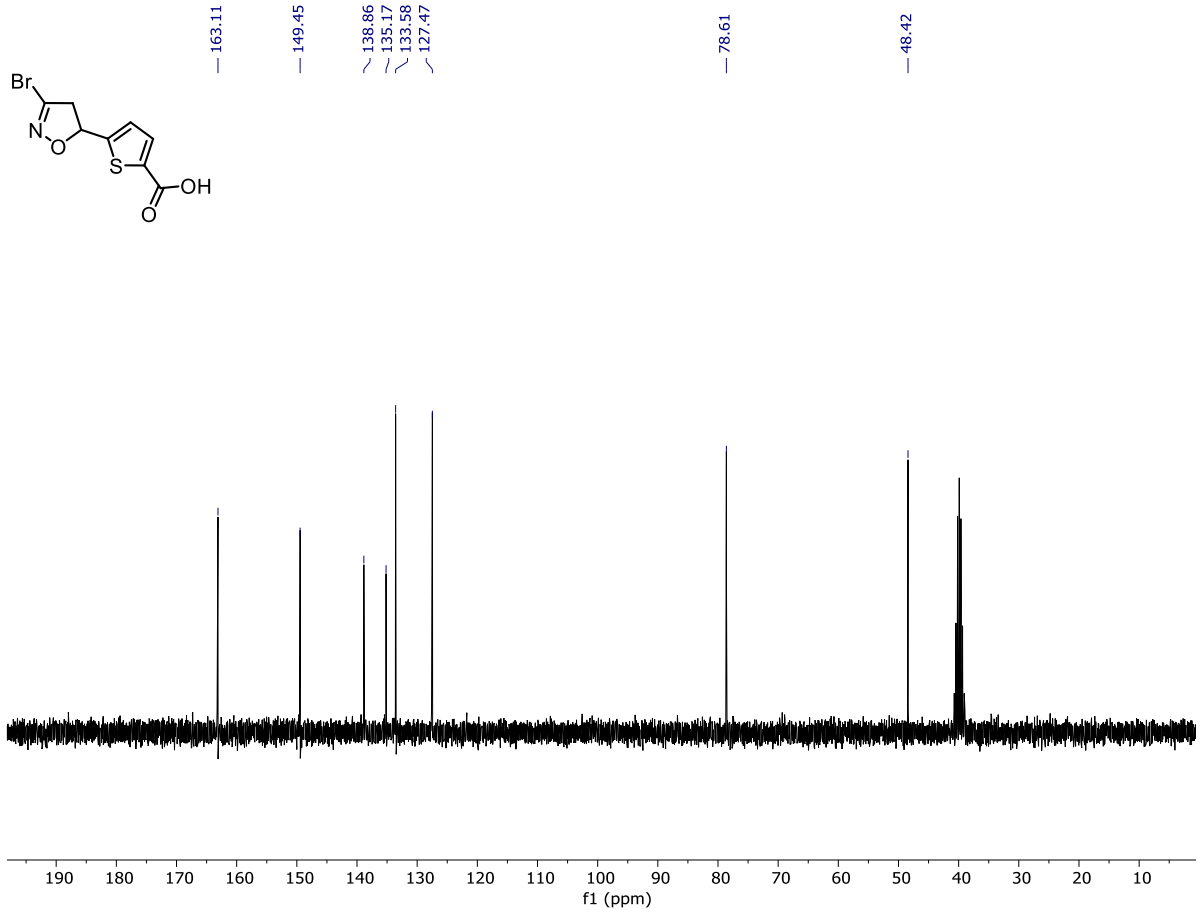
5-(4-(1*H*-Tetrazol-5-yl)phenyl)-3-bromo-4,5-dihydroisoxazole (**8**):



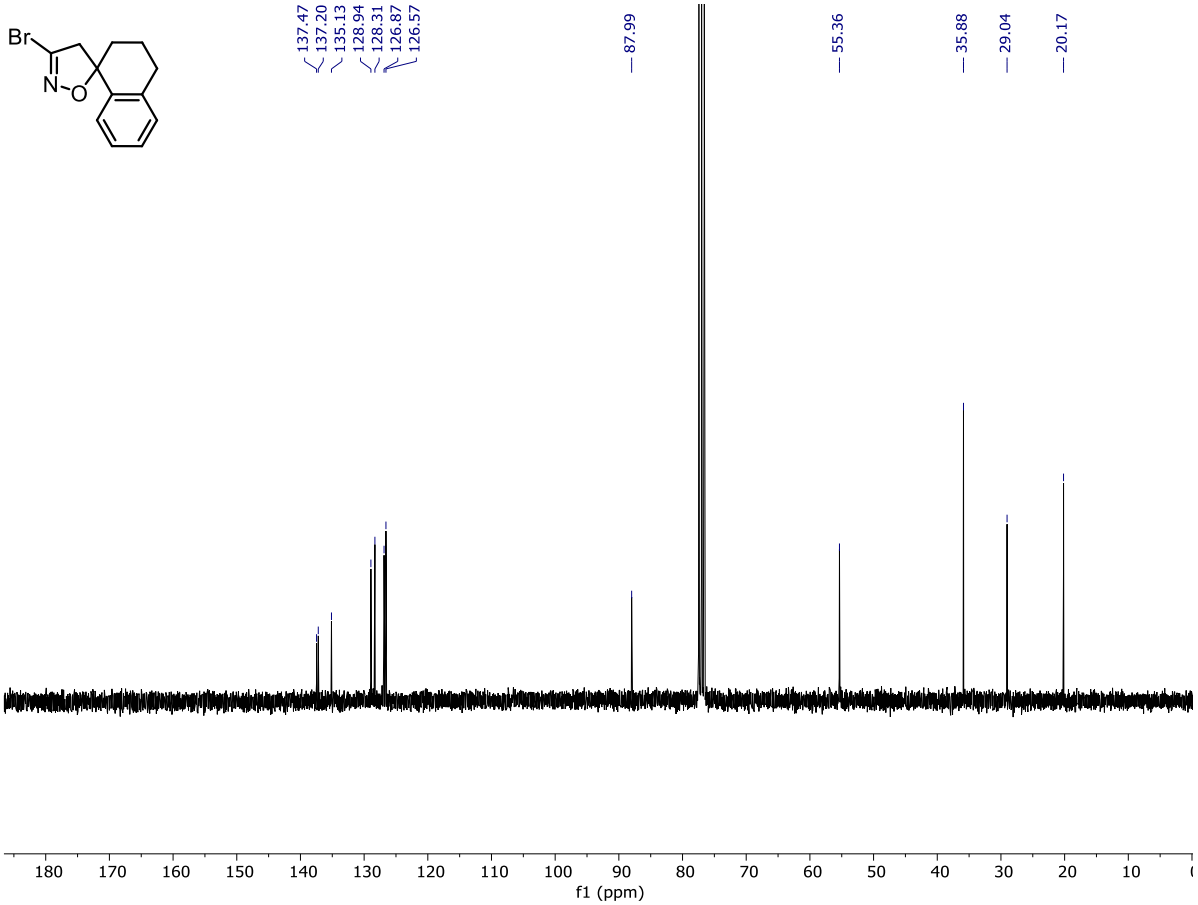
3-Bromo-5-(thiophen-2-yl)-4,5-dihydroisoxazole (**9**):



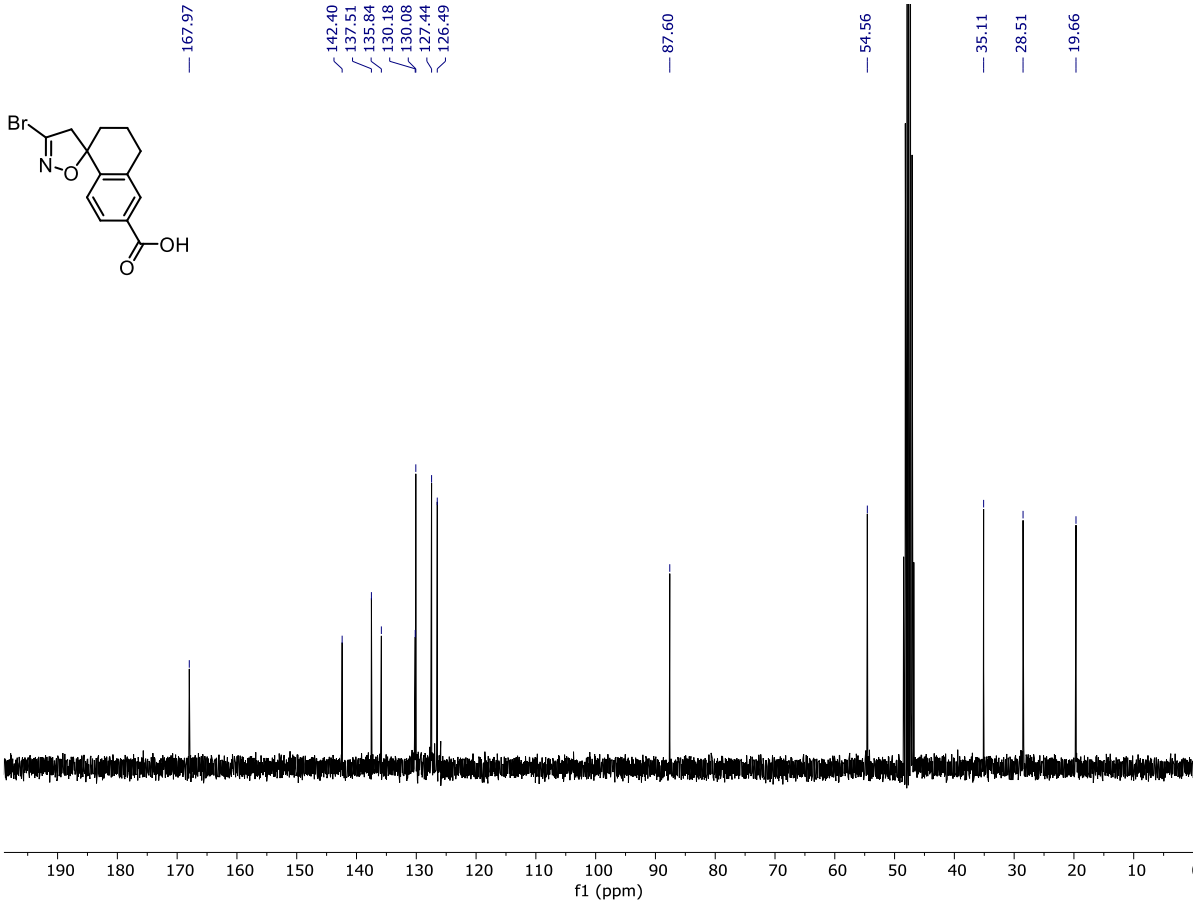
5-(3-Bromo-4,5-dihydroisoxazol-5-yl)thiophene-2-carboxylic acid (**10**):



3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene] (**11**):

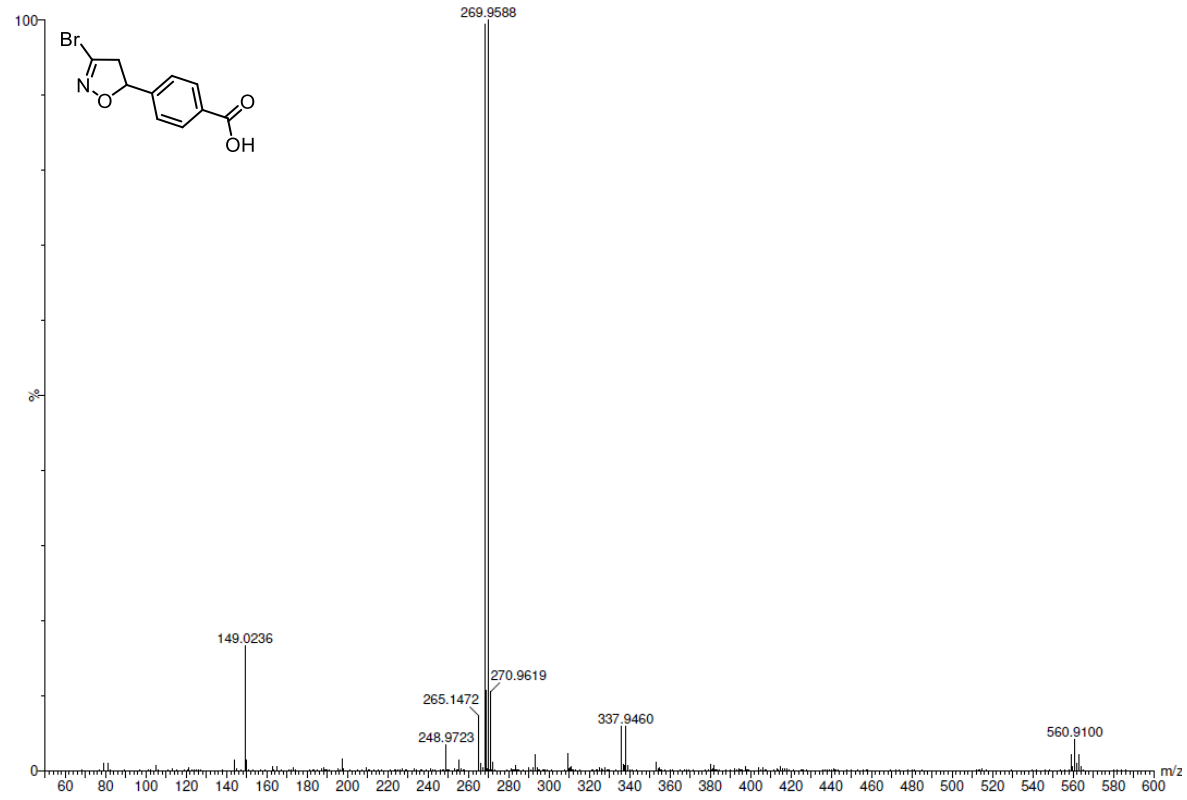


3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene]-6'-carboxylic acid (**12**):

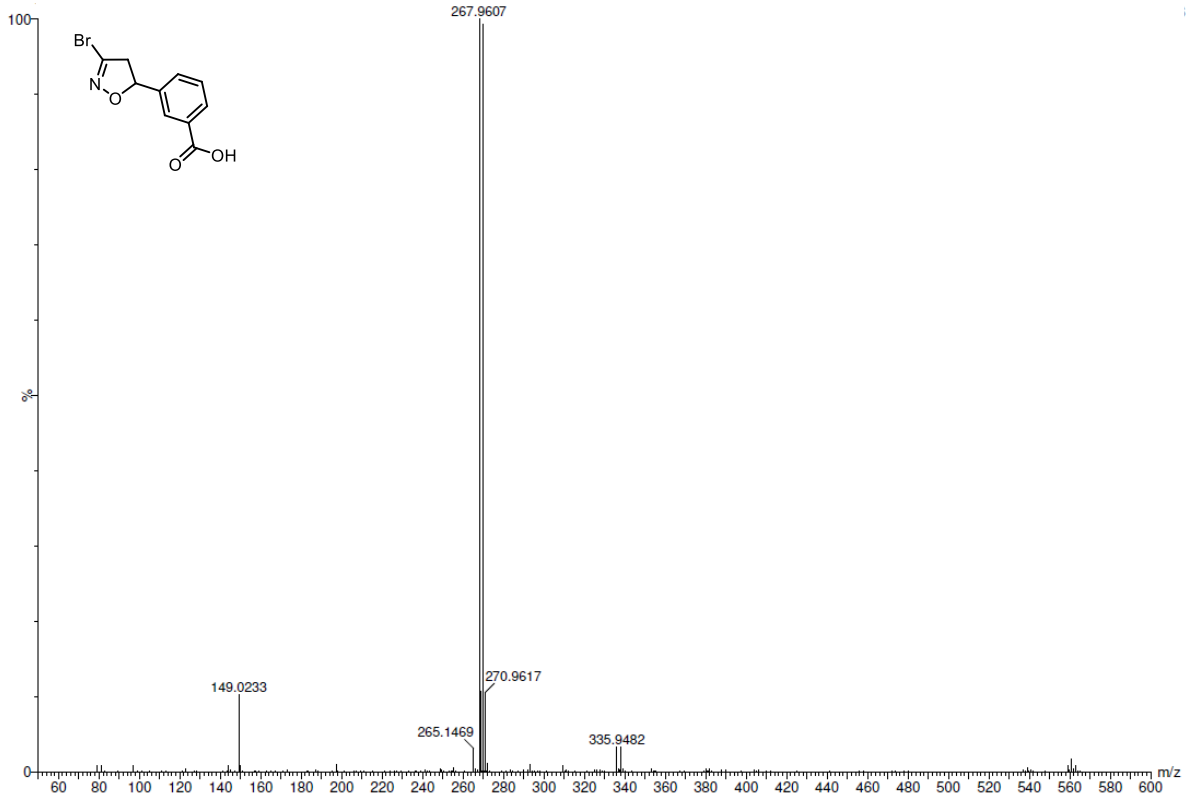


HRMS Spectra

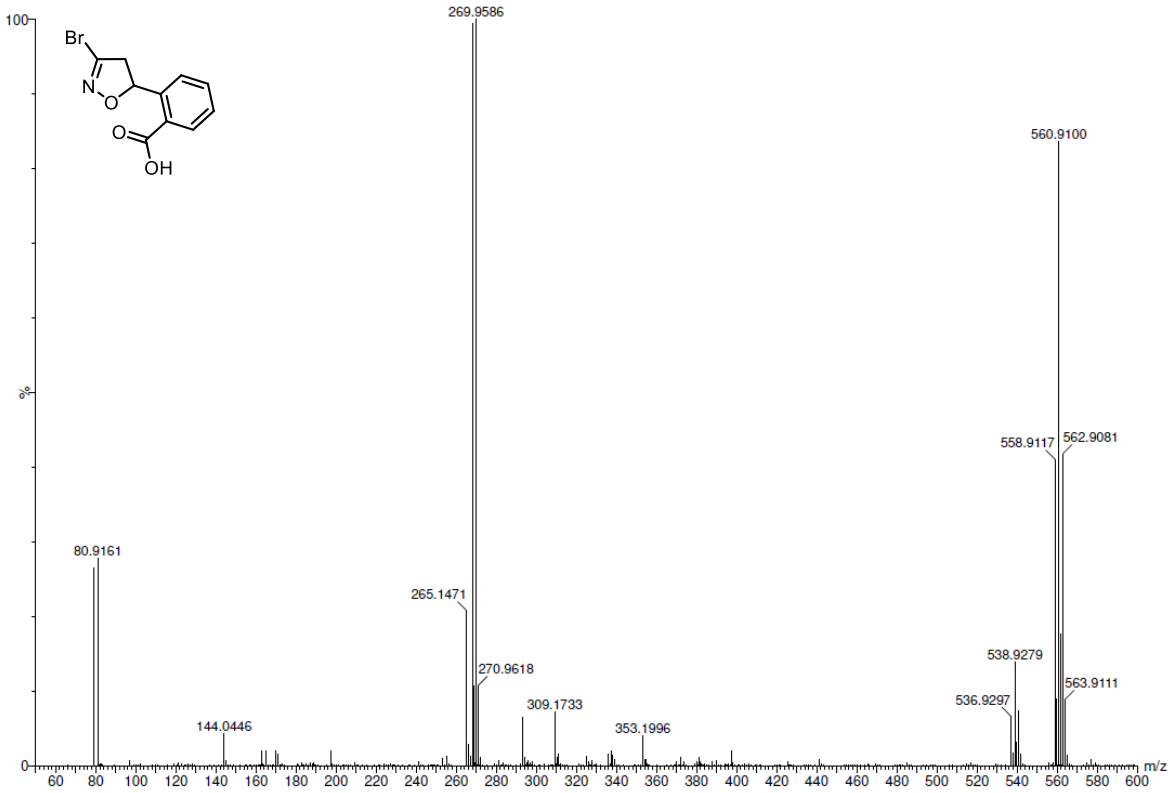
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**3**):



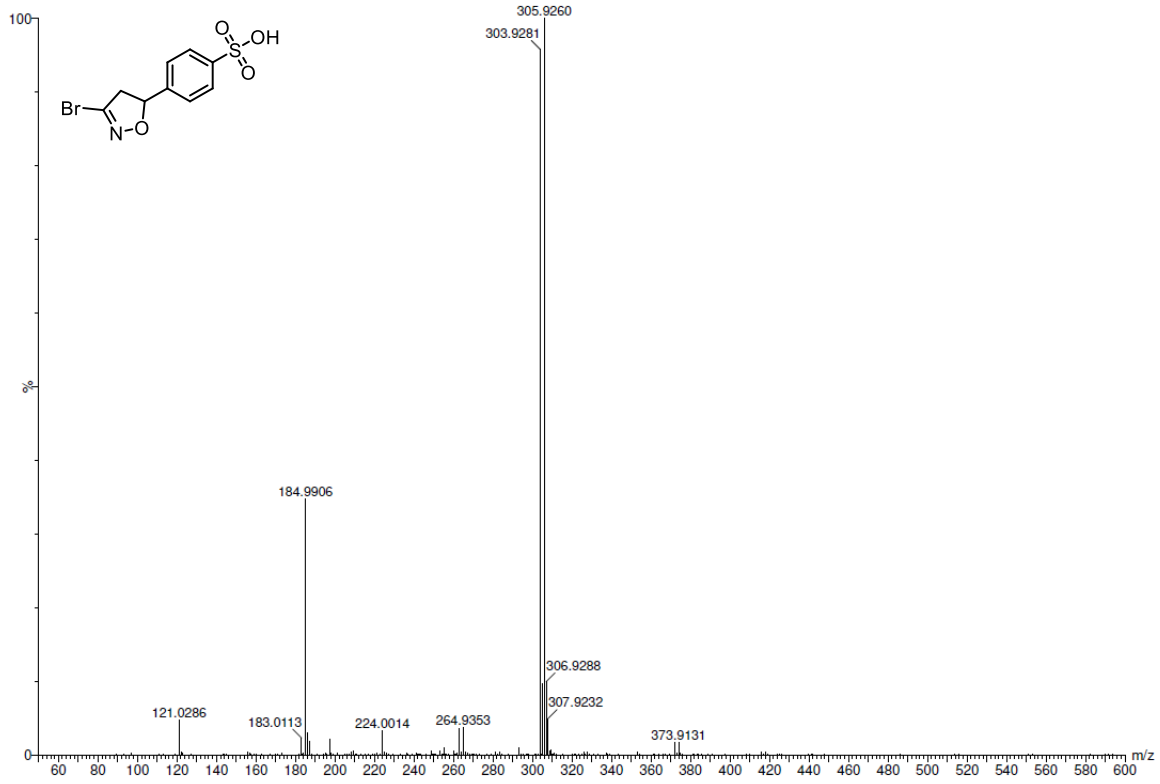
3-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**4**):



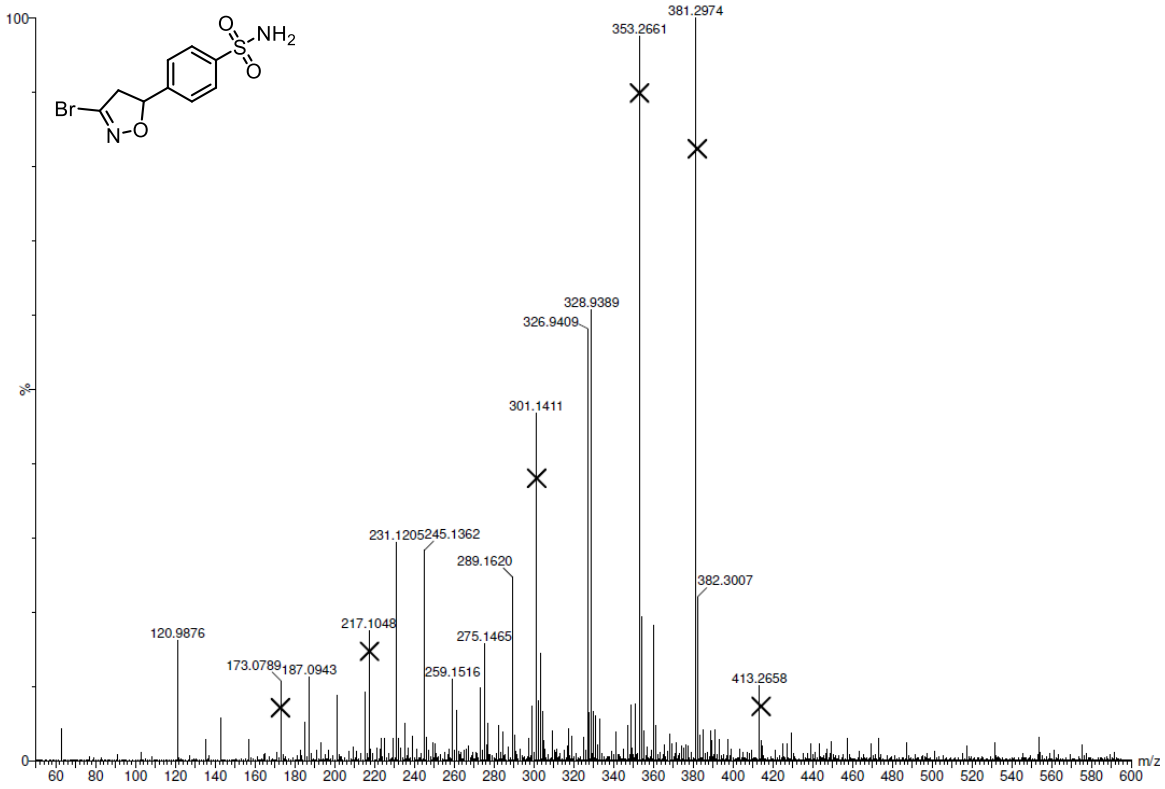
2-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**5**):



4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonic acid (**6**):

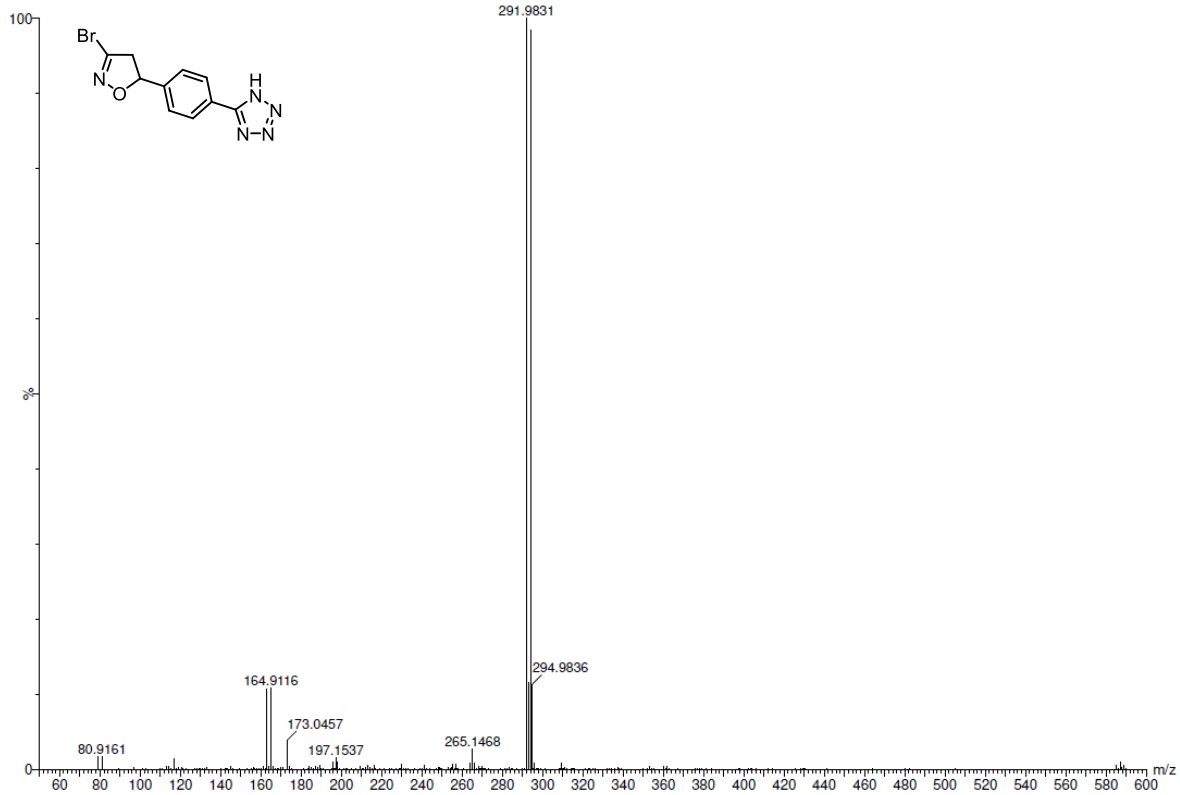


4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonamide (**7**):

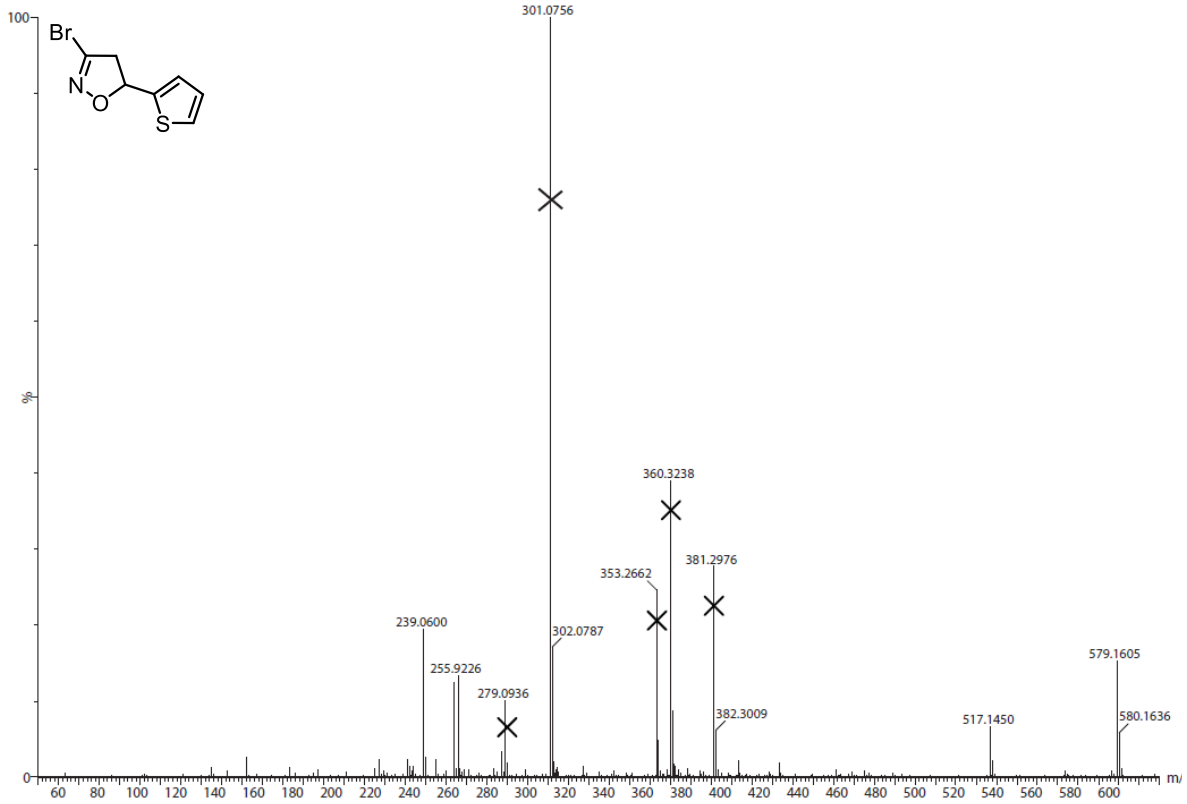


Crossed peaks are the interferences and contaminants observed in mass spectrometry, as described in Ref. [1].

5-(4-(1*H*-Tetrazol-5-yl)phenyl)-3-bromo-4,5-dihydroisoxazole (**8**):

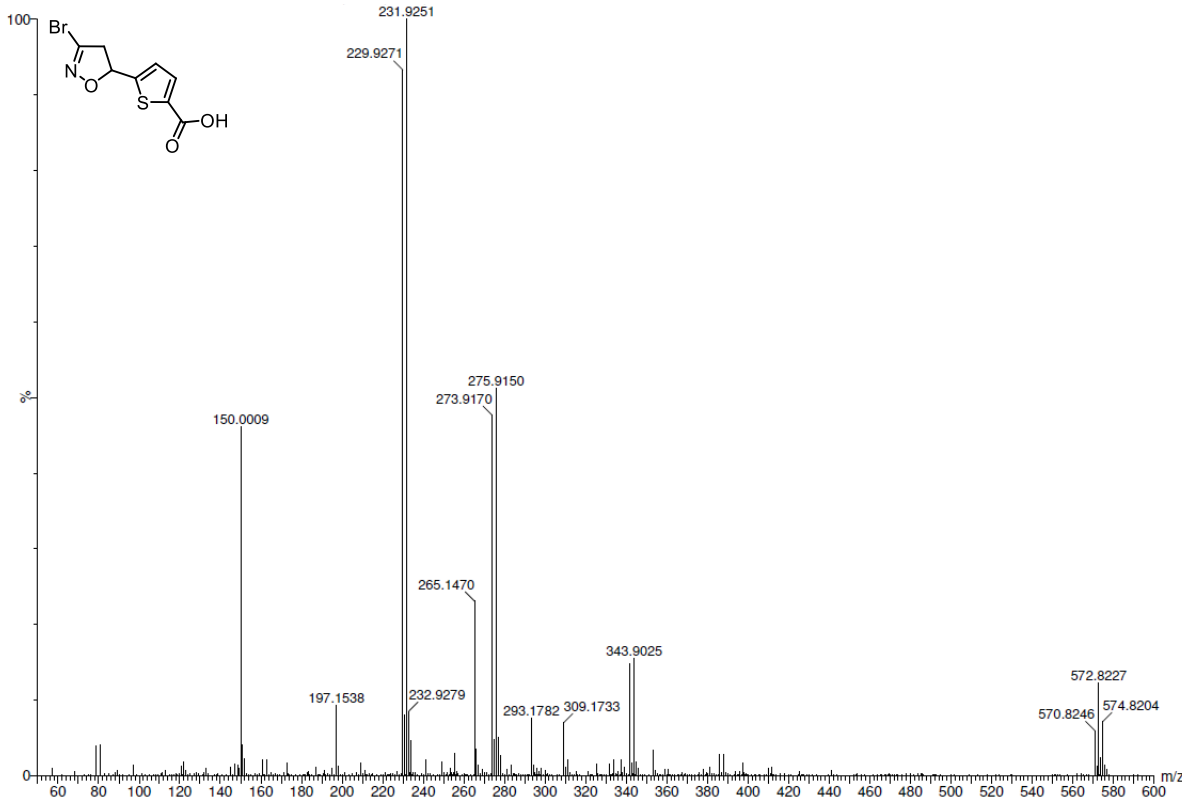


3-Bromo-5-(thiophen-2-yl)-4,5-dihydroisoxazole (**9**):

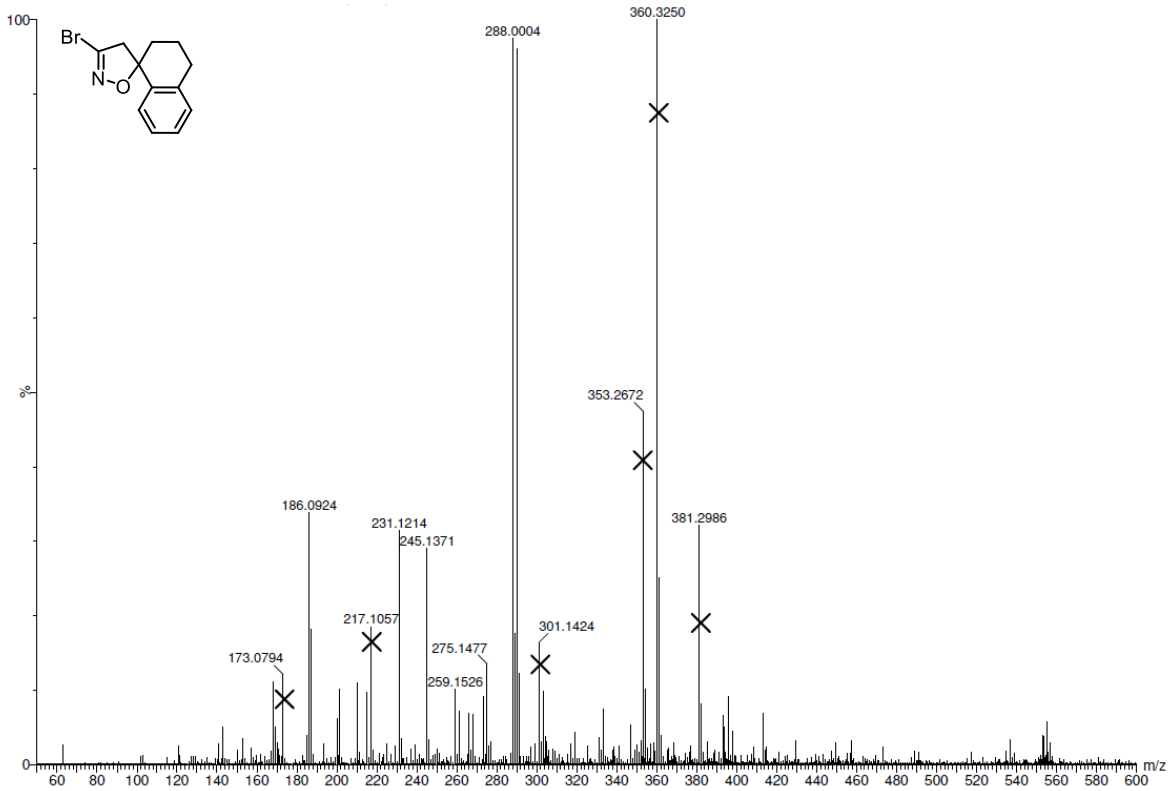


Crossed peaks are the interferences and contaminants observed in mass spectrometry, as described in Ref. [1].

5-(3-Bromo-4,5-dihydroisoxazol-5-yl)thiophene-2-carboxylic acid (**10**):

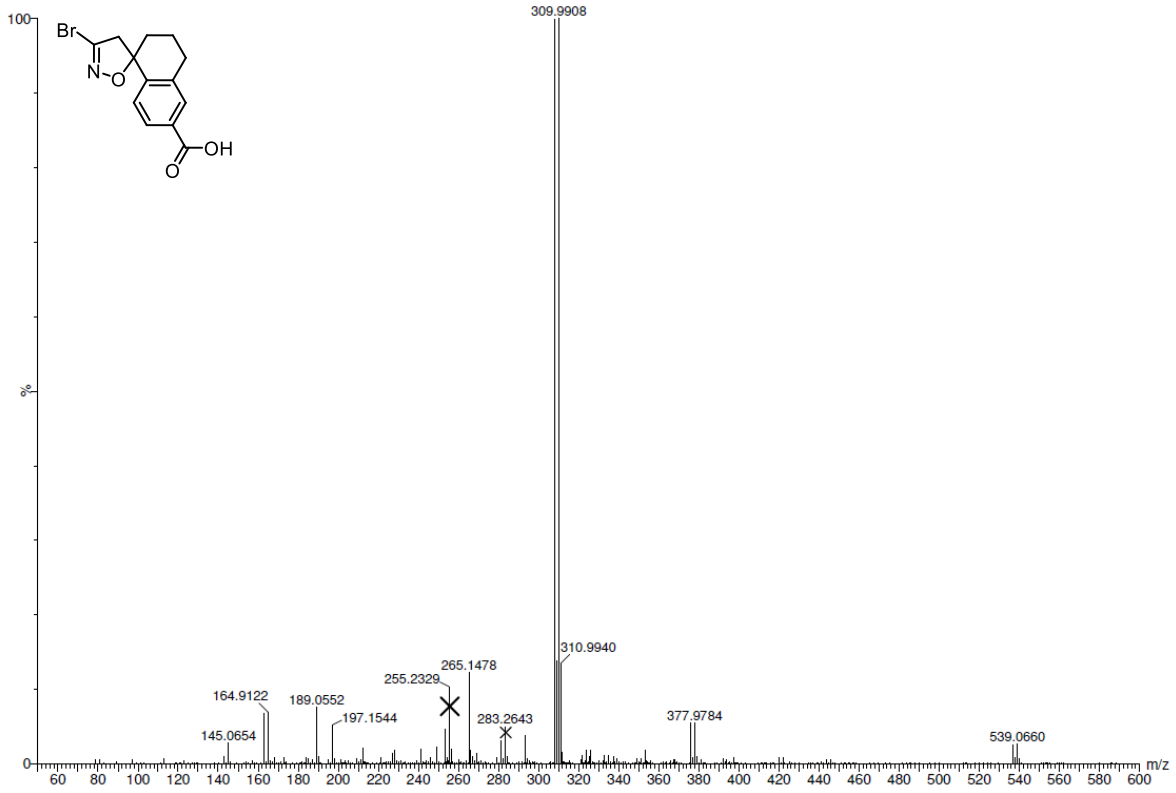


3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene] (**11**):



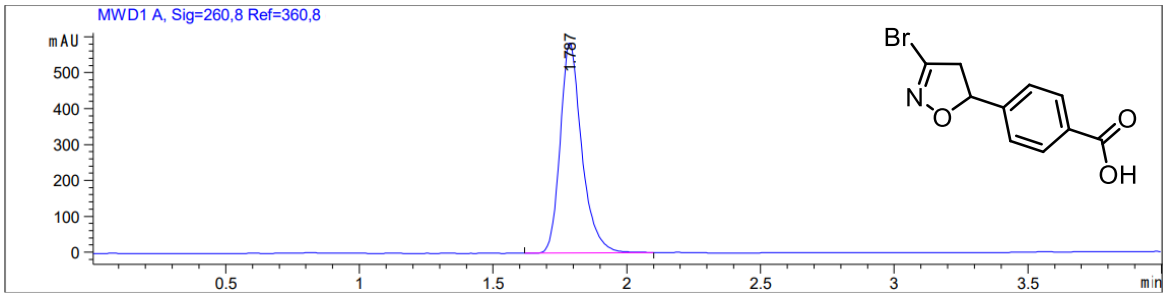
Crossed peaks are the interferences and contaminants observed in mass spectrometry, as described in Ref. [1].

3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene]-6'-carboxylic acid (**12**):

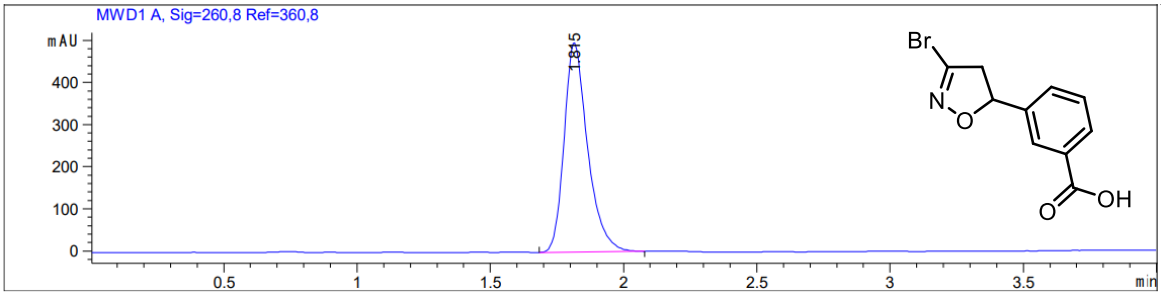


HPLC Chromatograms

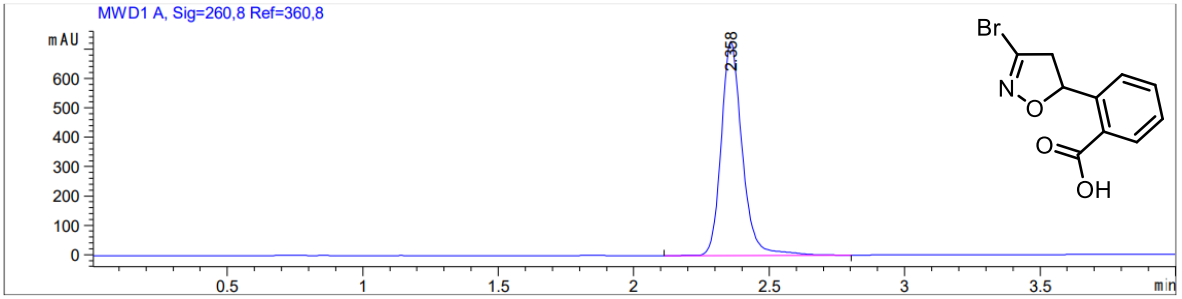
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**3**):



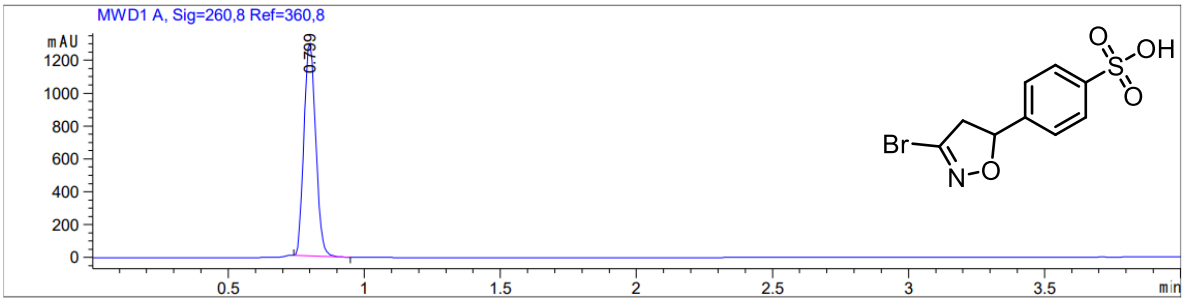
3-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**4**):



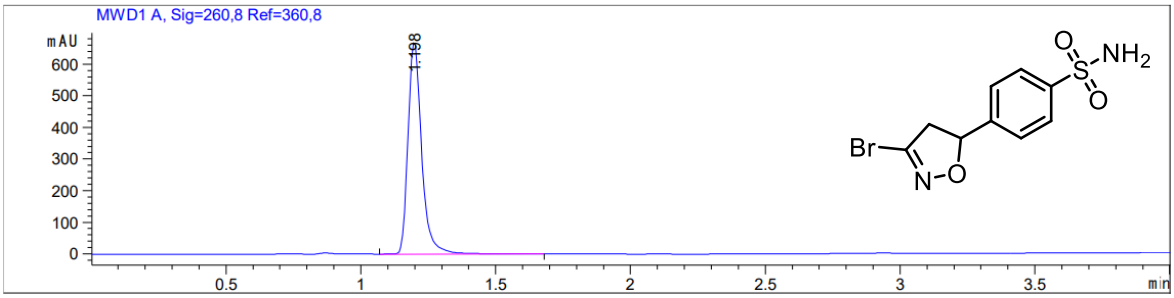
2-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzoic acid (**5**):



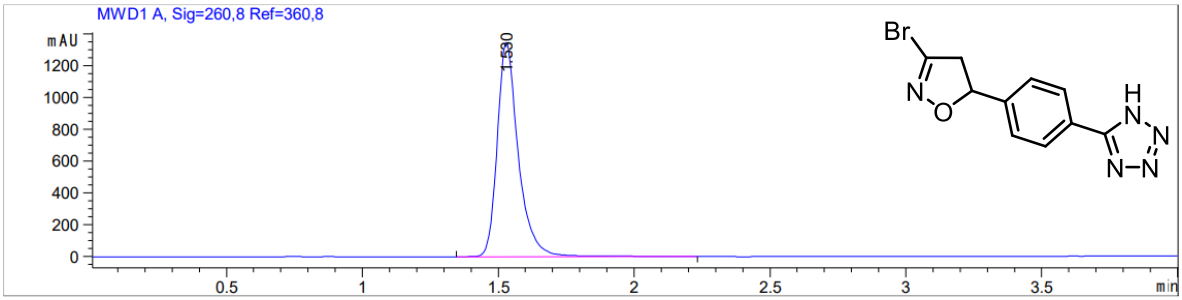
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonic acid (**6**):



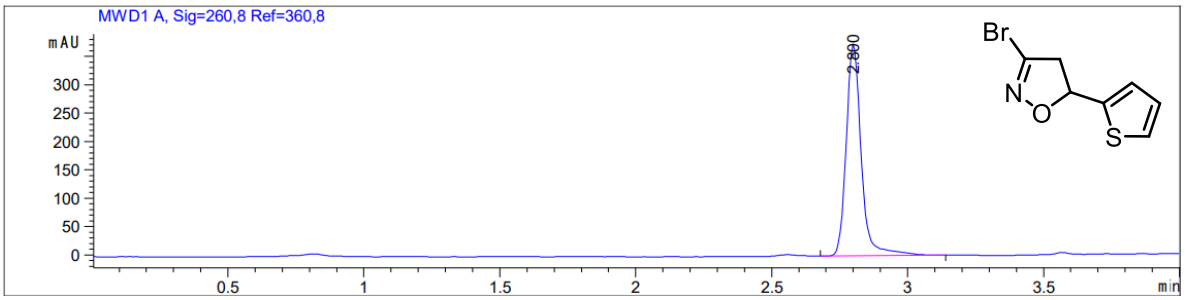
4-(3-Bromo-4,5-dihydroisoxazol-5-yl)benzenesulfonamide (**7**):



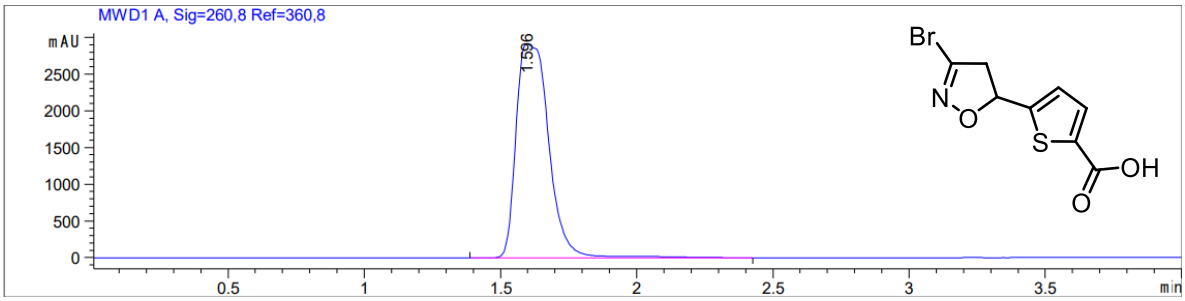
5-(4-(1*H*-Tetrazol-5-yl)phenyl)-3-bromo-4,5-dihydroisoxazole (**8**):



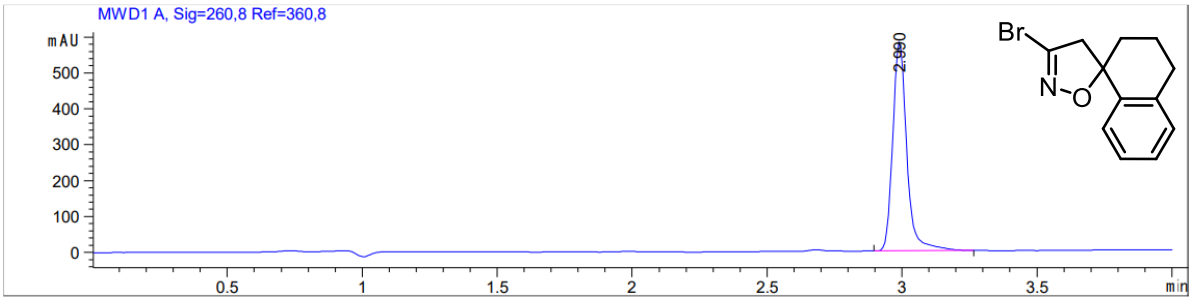
3-Bromo-5-(thiophen-2-yl)-4,5-dihydroisoxazole (**9**):



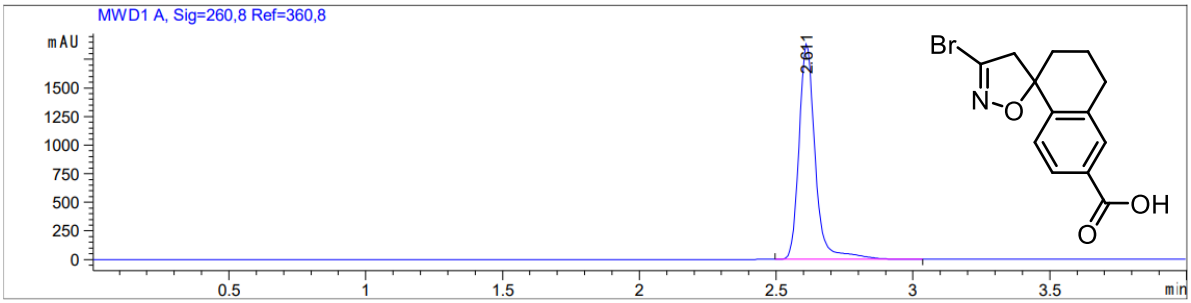
5-(3-Bromo-4,5-dihydroisoxazol-5-yl)thiophene-2-carboxylic acid (**10**):



3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene] (**11**):

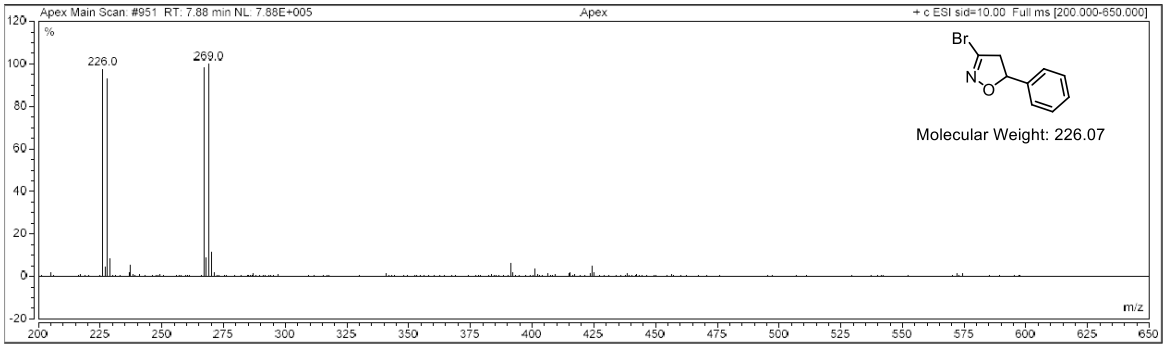


3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene]-6'-carboxylic acid (**12**):

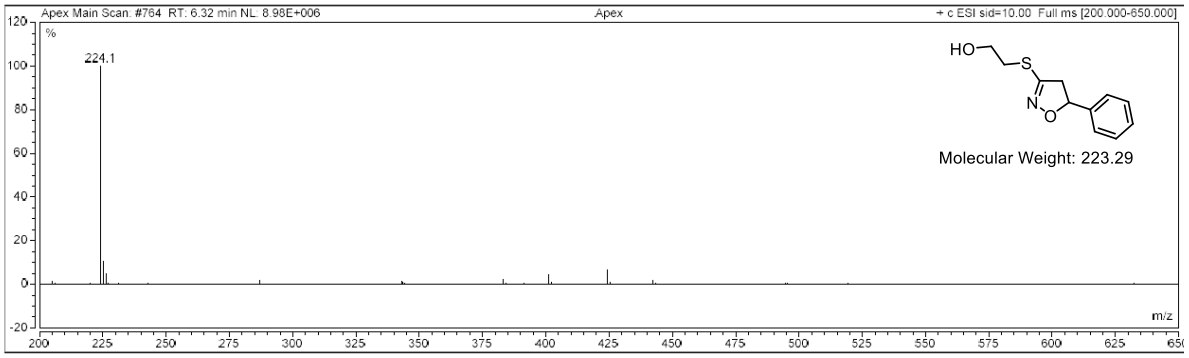


LC-MS Results: compounds and βME reaction

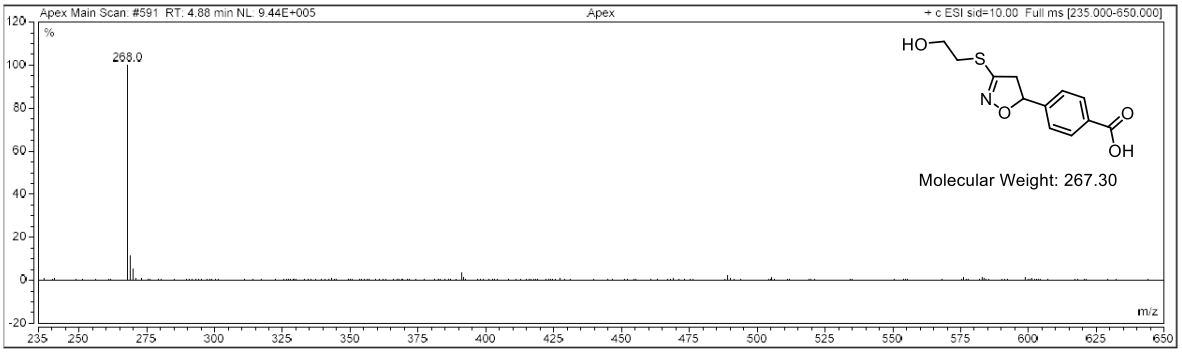
3-bromo-5-phenyl-4,5-dihydroisoxazole (**1**):



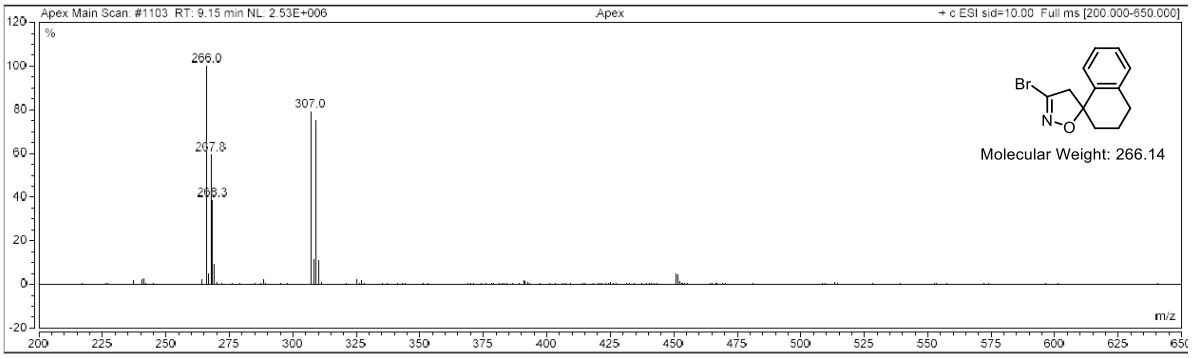
2-((5-phenyl-4,5-dihydroisoxazol-3-yl)thio)ethan-1-ol (**1-βME**):



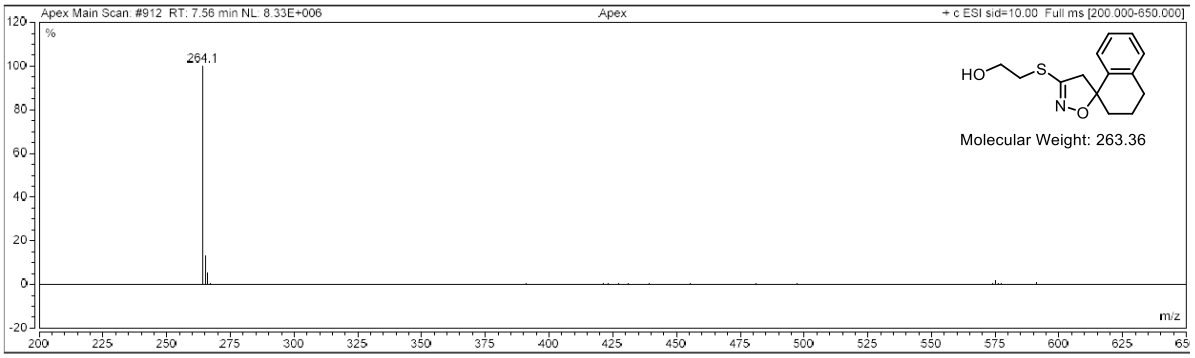
4-(3-((2-hydroxyethyl)thio)-4,5-dihydroisoxazol-5-yl)benzoic acid (**3-βME**)



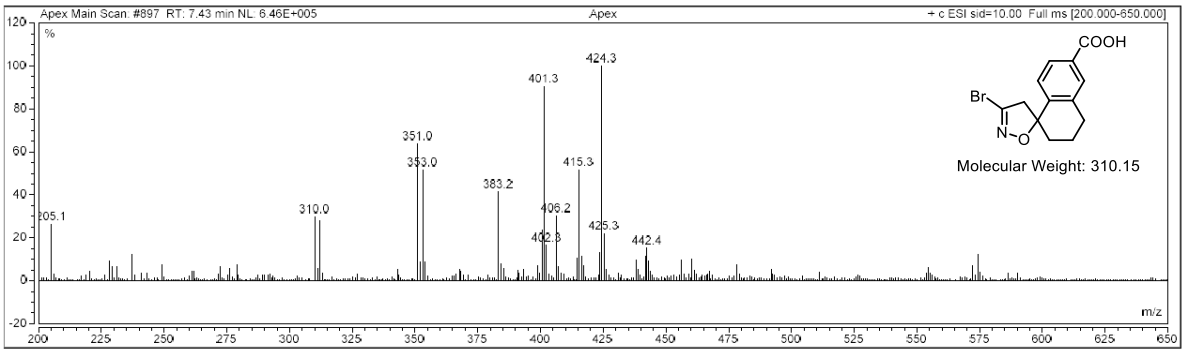
3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene] (**11**):



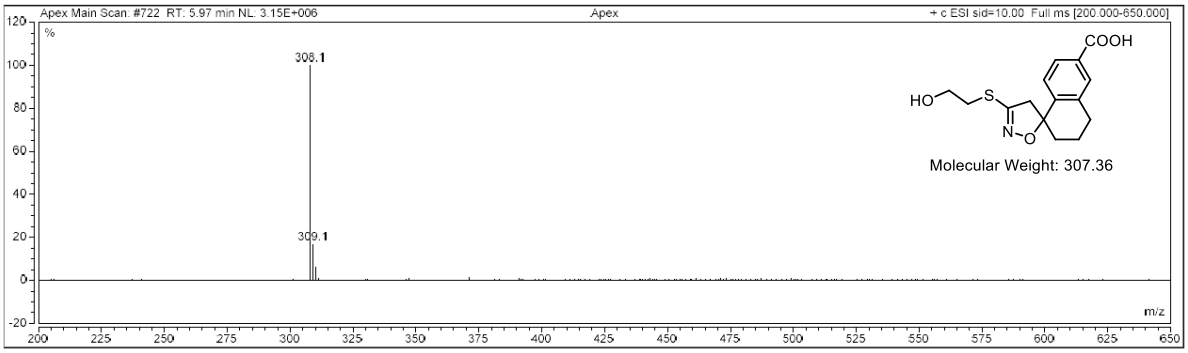
2-((3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalen]-3-yl)thio)ethan-1-ol (**11-βME**)



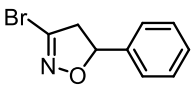
3-Bromo-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene]-6'-carboxylic acid (**12**):



3-((2-hydroxyethyl)thio)-3',4'-dihydro-2'*H*,4*H*-spiro[isoxazole-5,1'-naphthalene]-6'-carboxylic acid (**12-βME**)



Chemical Structures. Final Compounds.



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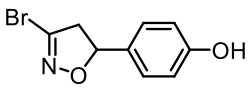

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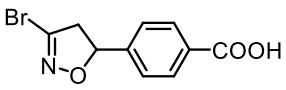

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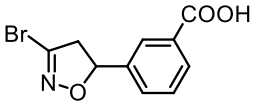

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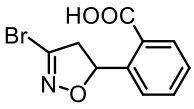

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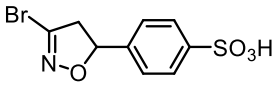

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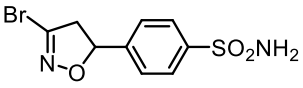

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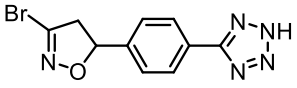

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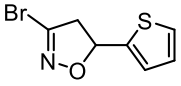

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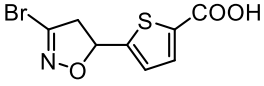

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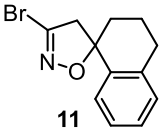

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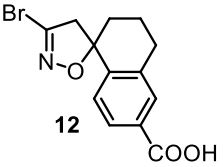
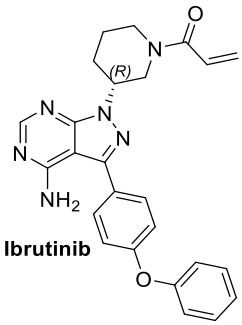
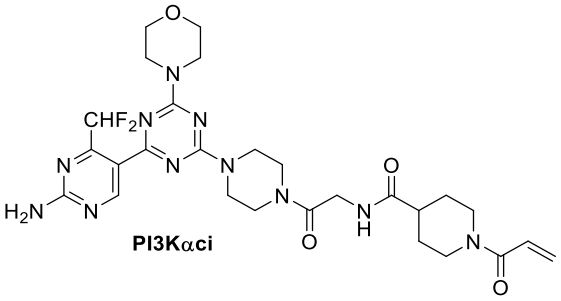


12

Chemical Structures. Reference compounds.



Ibrutinib



PI3Kαci

References

[1] B.O. Keller, J. Sui, A.B. Young, R.M. Whittal, Interferences and contaminants encountered in modern mass spectrometry, Anal Chim Acta, 627 (2008) 71-81.