Supporting Information

Optimization of covalent MKK7 inhibitors via crude nanomole-scale libraries

Paul Gehrtz[1,2,#], Shir Marom[1,#], Mike Bührmann[3,4,#], Julia Hardick[3,4,#], Silke Kleinbölting[3,4], Amit Shraga[1,5], Christian Dubiella[1,6], Ronen Gabizon[1], Jan N. Wiese[3,4], Matthias P. Müller[3,4], Galit Cohen[7], Ilana Babaev[7], Khriesto Shurrush[7], Liat Avram[8], Efrat Resnick[1], Haim Barr[7], Daniel Rauh[3,4,*], Nir London[1,*]

[1] Department of Chemical and Structural Biology, Weizmann Institute of Science, 7610001 Rehovot, Israel

[3] Department of Chemistry and Chemical Biology, TU Dortmund University, Otto-Hahn-Strasse 4a, 44227, Dortmund, Germany.

[4] Drug Discovery Hub Dortmund (DDHD) am Zentrum für integrierte Wirkstoffforschung (ZIW), 44227 Dortmund, Germany

[7] The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, 7610001 Rehovot, Israel.

[8] Department of Chemical Research Support, Weizmann Institute of Science, 7610001, Rehovot, Israel

[#] These authors contributed equally.

[*] Corresponding:

N.L.: nir.london@weizmann.ac.il

D.R.: daniel.rauh@tu-dortmund.de

Keywords: CuAAC, Click-Chemistry, Covalent Inhibitors, MKK7, High-Throughput Chemistry

Contents

Supplementary Figures

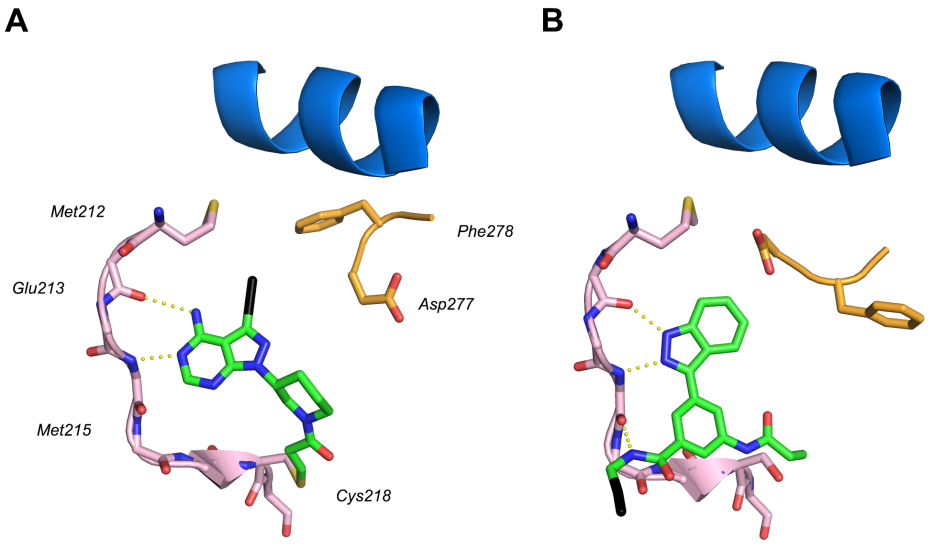


Figure S1. The binding modes of alkynes **1** and **2** in the ATP binding site of MKK7. (A) **1** co-crystalized in complex with MKK7 (PDB: 6IB0), where the alkyne (black) is protruding into the back pocket. (B) **2** co-crystalized in complex with MKK7 (PDB: 7CBX), where the alkyne (black) is pointing outside the binding pocket towards the solvent-exposed region.

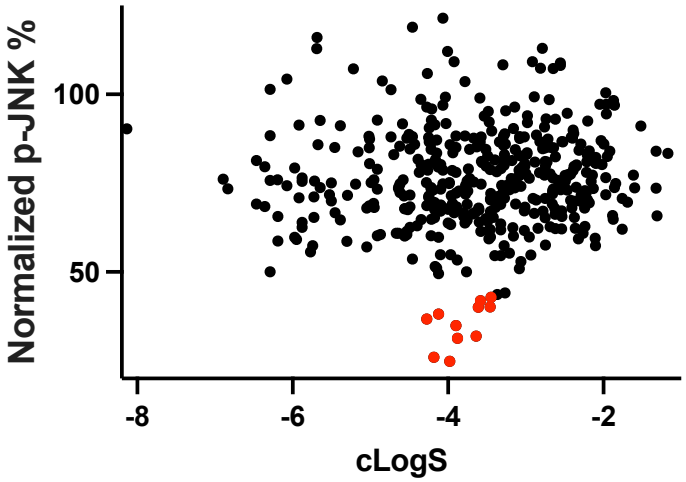


Figure S2. Plot of cLogS of series **1** members and their associated p-JNK levels determined via an ICW assay (13.8 µM, 2 h, U2OS cells). Top ten compounds from the series are highlighted in red.

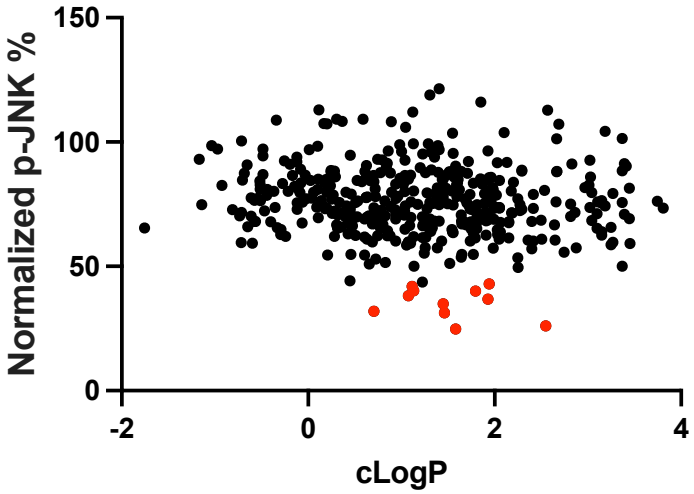


Figure S3. Plot of cLogP of series **1** members and their associated p-JNK levels determined via an ICW assay (13.8 µM, 2 h, U2OS cells). Top ten compounds from the series are highlighted in red.

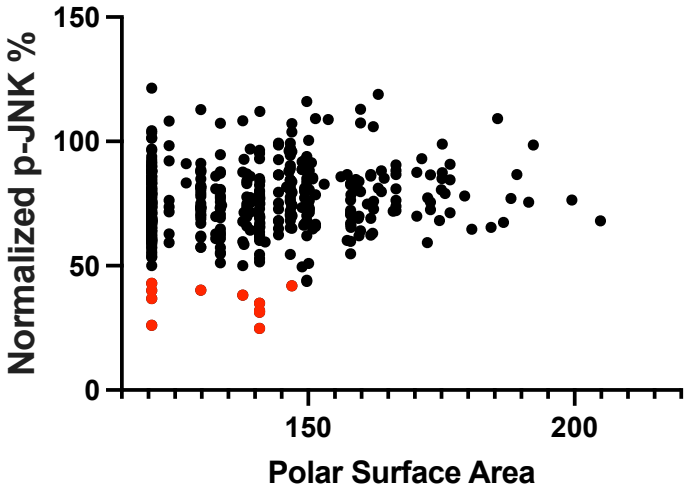


Figure S4. Plot of tPSA of series **1** members and their associated p-JNK levels determined via an ICW assay (13.8 µM, 2 h, U2OS cells). Top ten compounds from the series are highlighted in red.

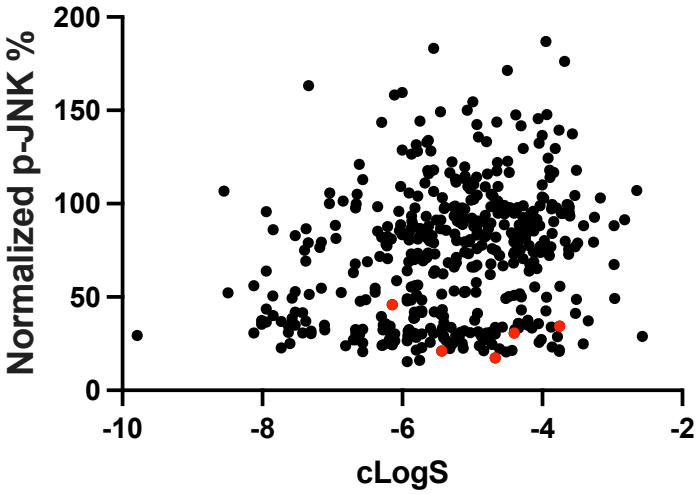


Figure S5. Plot of cLogS of series **2** members and their associated p-JNK levels determined via an ICW assay (10 µM, 2 h, U2OS cells). Selected five compounds from the series are highlighted in red.

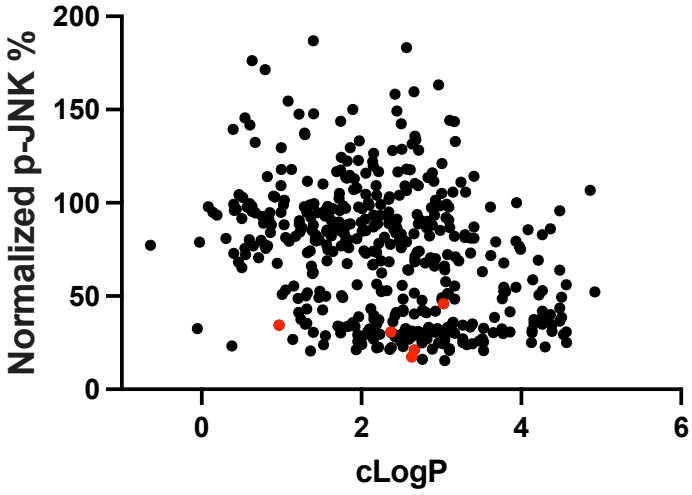


Figure S6. Plot of cLogP of series **2** members and their associated p-JNK levels determined via an ICW assay (10 µM, 2 h, U2OS cells). Selected five compounds from the series are highlighted in red.

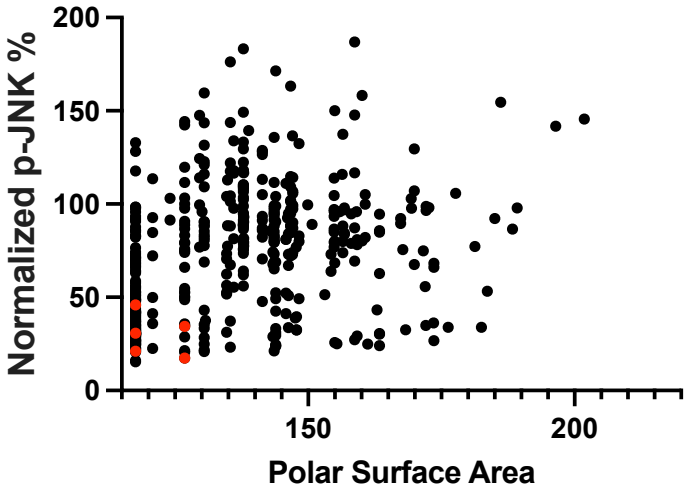


Figure S7. Plot of tPSA of series **2** members and their associated p-JNK levels determined via an ICW assay (13.8 µM, 2 h, U2OS cells). Selected five compounds from the series are highlighted in red.

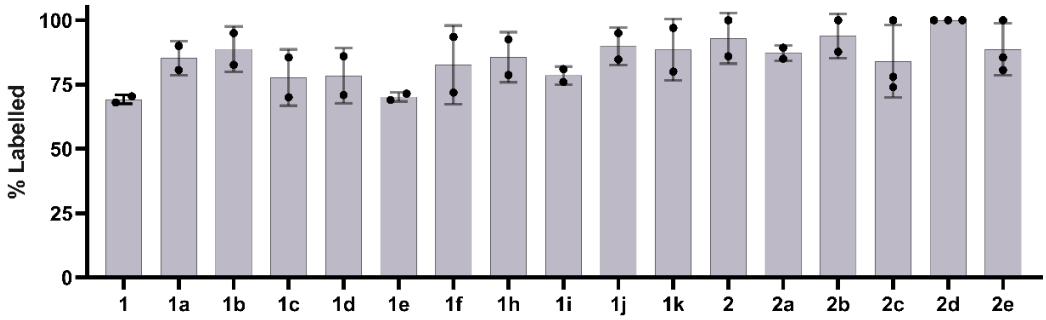


Figure S8. Intact protein LC/MS labelling experiment of re-synthesized compounds derived from top crude screening hits. Reaction conditions: 2 µM compound, 2 µM protein, 10 min, 4 °C, quenched with formic acid to a final concentration of 0.4% (v/v). In series **1**, all except compounds **1e**, showed improved binding abilities compared to precursor **1** with over 75% labelling. In series **2**, all compounds showed high binding abilities of over 75%.

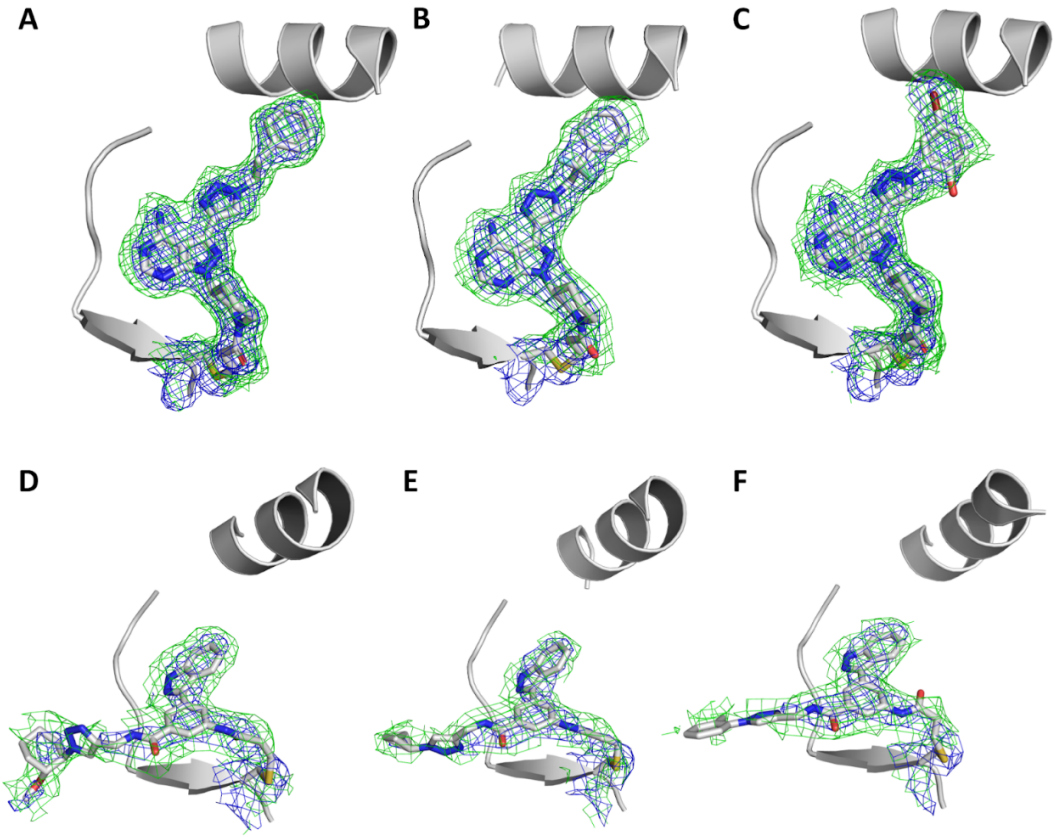
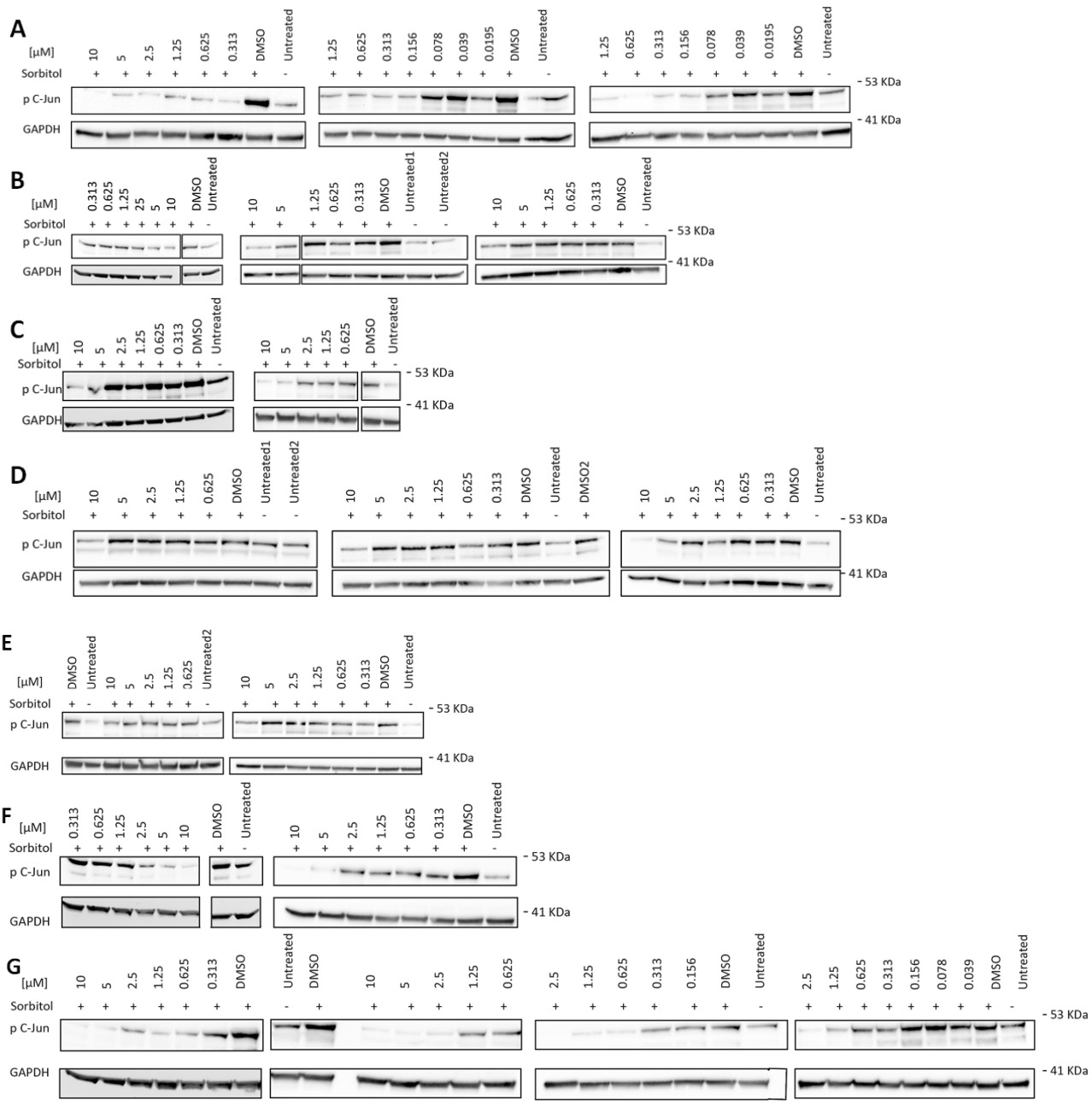


Figure S9. Omit maps of the co-crystallized MKK7 inhibitors. Performing a simulated annealing refinement, mFo-DFc omit maps (green, contoured at 2.5σ) were calculated for (A) **1h** (B) **1k**, (C) **1a**, (D) **2b**, (E) **2d**, and (F) **2c** as well as for the targeted Cys218. 2Fo-Fc maps for the ligands and Cys218 were contoured at 1.0σ (blue). Maps indicate partial occupancy for the compounds of series **2** in the area of the solvent-exposed triazolyl moiety.


A
[µM]
Sorbitol
p C-Jun
GAPDH
53 KDa
41 KDa
B
C
D
E
F
G

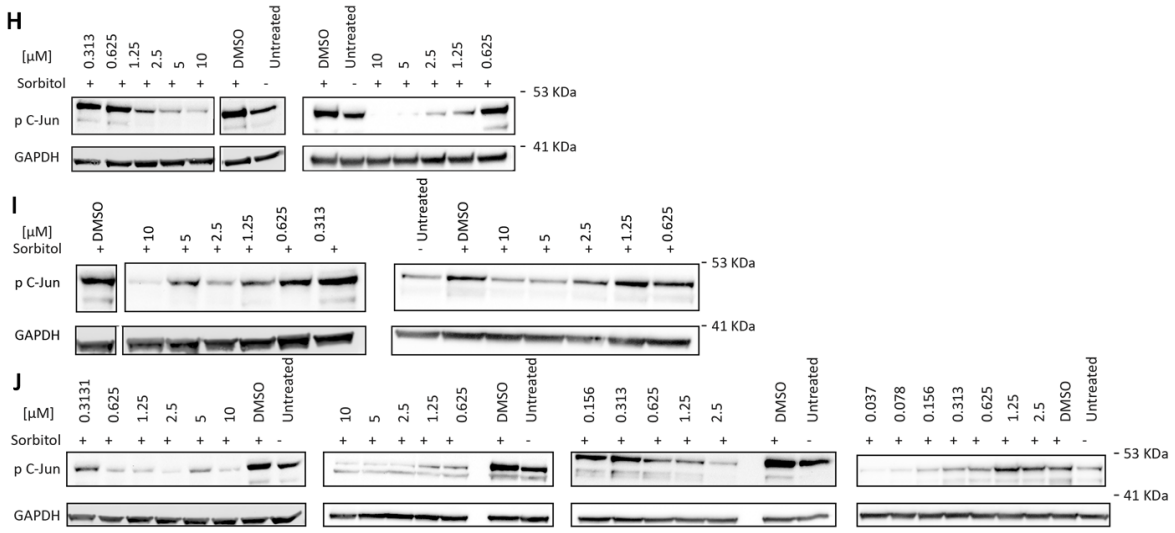


Figure S10. Dose response western blot with p-c-Jun as the readout and GAPDH as the housekeeping gene for normalization. (A) **2**, (B) **2a**, (C) **2b**, (D) **2c**, (E) **1**, (F) **1a**, (G) **1b**, (H) **1d**, (I) **1e**, (J) **1k**.

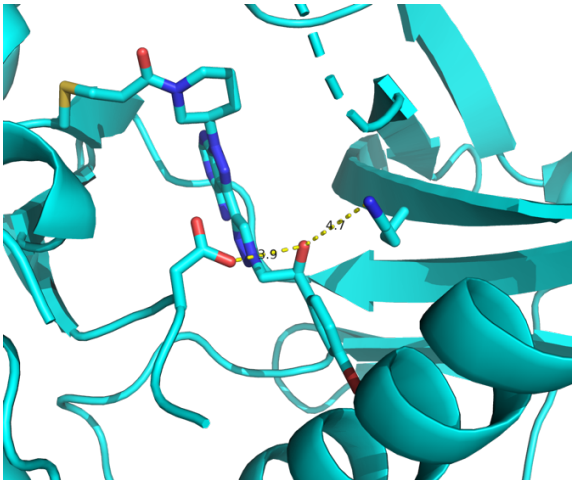


Figure S11. A model of compound **1e** based on the co-crystal structure of MKK7 in complex with **1k** shows the hydroxy side-chain may form a hydrogen bond with either Asp277 (similar to **1a**; see Fig. 4), or with Lys155. Note that the side-chains were not remodeled and may in fact adopt a rotamer that places closer to the hydroxy moiety.

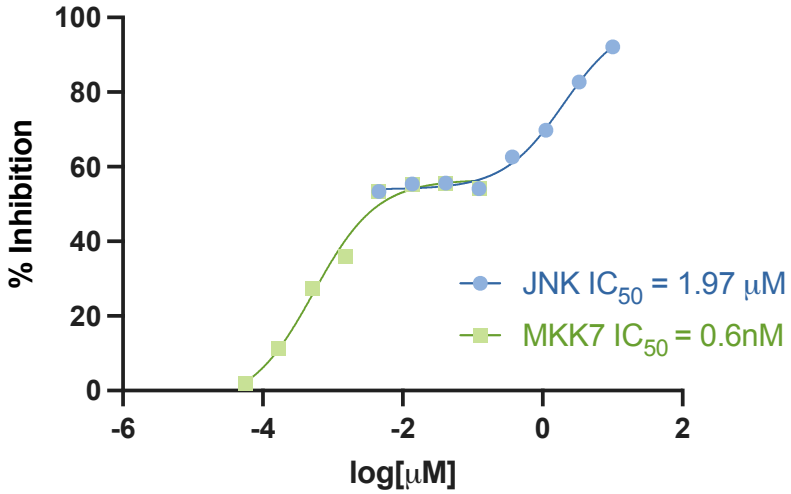


Figure S12. Biphasic in vitro kinase inhibition behavior by alkyne **2.** In a coupled JNK/ MKK7 in vitro kinase activity assay, alkyne **2** shows a biphasic inhibition curve. Each sigmoidal curve can be fitted separately with very potent inhibition of MKK7 at lower concentrations (IC_{50} = 0.6nM; R^2=0.986) and likely weak inhibition of JNK at higher concentrations (IC_{50}=1.975 μM; R^2=0.966).

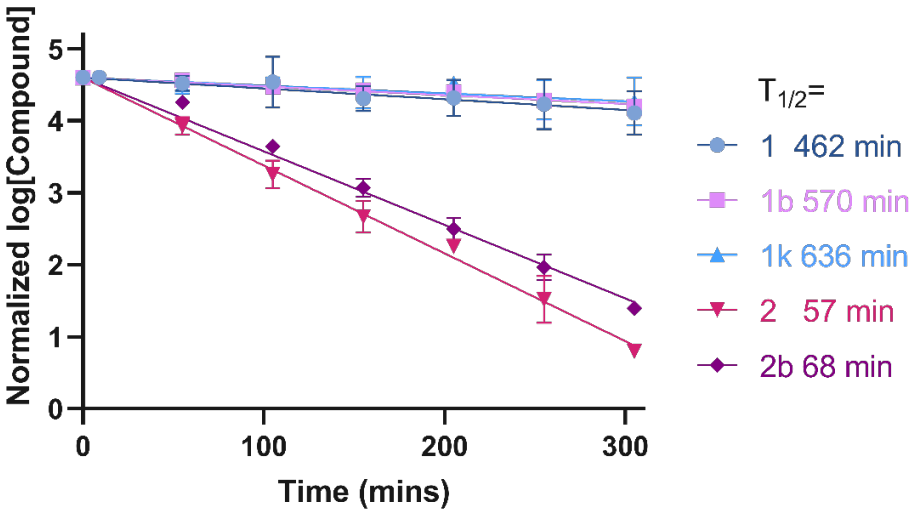


Figure S13. GSH consumption assay of selected compounds **1**, **1b**, **1k**, **2**, and **2b**. The assay was done at 37 °C using 100 μM reference compound 4-nitrobenzonitrile, 100 μM of tested compounds and 5 mM GSH. The buffer was 90% PBS and 10% DMF. Every 50 min, the vial was taken from the incubator, shaken well, and then 50 μL was taken and analyzed in the LC/MS. The series **1** compounds were significantly more stable and less reactive than **2** and **2b**. **2b** appeared slightly less reactive than the scaffold **2**.

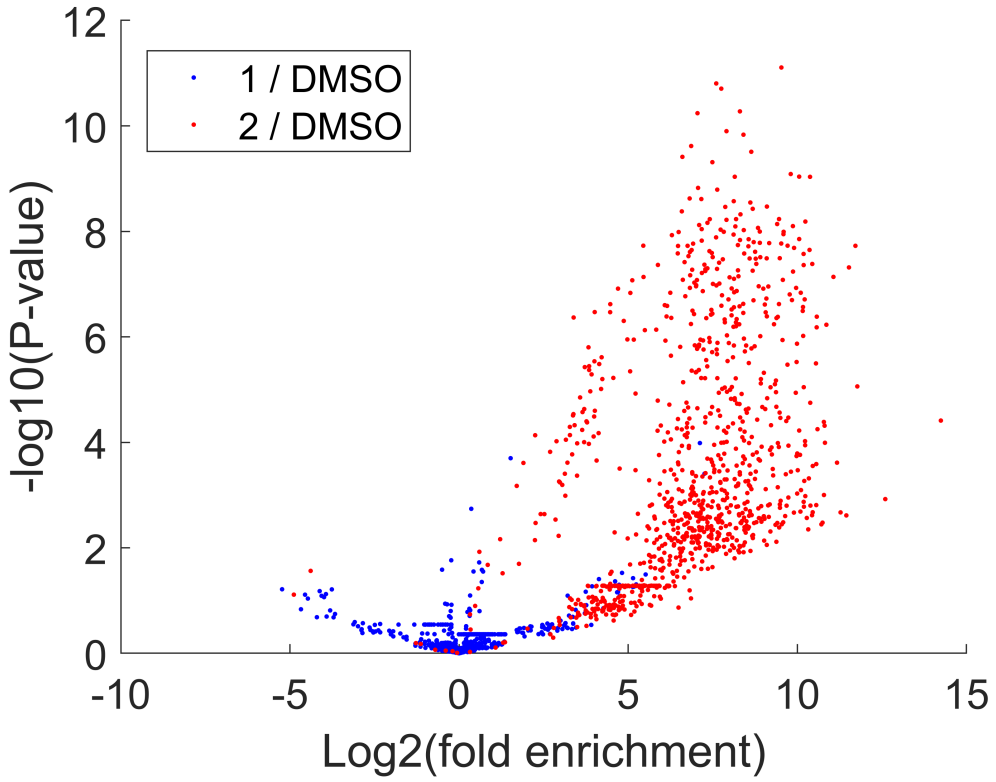


Figure S14. Pull down proteomics analysis of alkynes **1** and **2.** Volcano plot for proteins identified in pull-down proteomics experiments using molecules **1** and **2**, the x-axis shows Log2 fold enrichment of proteins detected in samples treated with either alkyne compared to DMSO. The y-axis shows the significance of the difference.

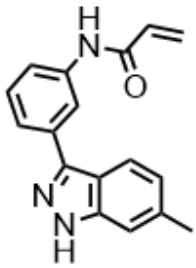


Figure S15. Chemical structure of compound **4**.

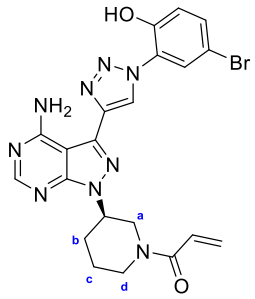
Supplementary Tables

	1a (PDB: 7OVK)	1h (PDB: 7OVI)	1k (PDB: 7OVJ)	2a (PDB: 7OVL)	2b (PDB: 7OVN)	2c (PDB: 7OVM)
Data collection						
Space group	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$
Cell dimensions						
a, b, c (Å)	61.72, 68.49, 83.99	61.05, 68.80, 83.22	61.08, 68.09, 85.08	60.23, 67.68, 84.73	60.91, 69.21, 84.29	60.65, 68.90, 84.08
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	50.0 – 2.05 (2.15 – 2.05)	50.0 – 1.95 (2.05 – 1.95)	50.0 – 2.35 (2.40 – 2.35)	50.0 – 2.90 (3.00 – 2.90)	50.0 – 2.90 (3.00 – 2.90)	50.0 – 2.90 (3.00 – 2.90)
R_{meas} (%)	6.9 (128.1)	5.2 (157.3)	7.1 (94.2)	11.2 (151.1)	18.2 (158.1)	11.0 (144.9)
I/σI	19.69 (2.23)	23.53 (1.55)	23.30 (3.21)	15.00 (1.57)	15.61 (2.55)	17.65 (2.18)
$CC_{1/2}$	99.9 (79.8)	100 (67.9)	99.9 (88.4)	99.9 (75.7)	99.8 (79.7)	99.9 (81.3)
Completeness (%)	100 (100)	99.5 (100)	100 (100)	100 (100)	99.7 (100)	100 (100)
Redundancy	13.37 (13.87)	13.00 (12.29)	13.22 (13.91)	12.99 (13.66)	13.13 (12.78)	13.14 (13.84)
Refinement						
Resolution (Å)	45.47 – 2.05	49.74 – 1.95	45.66 – 2.35	49.09 – 2.90	45.72 – 2.90	49.19 – 2.90
No. reflections	22883	26461	15130	8083	8276	8217
R_{work}/R_{free}	21.67/24.44	21.12/25.04	20.38/24.45	24.83/27.03	22.34/25.72	22.98/28.58
No. atoms						
Protein	2062	2125	2051	2163	2202	2177
Ligand/ion	33	35	39	37	36	34
Water	83	77	40	3	0	1
B-factors						
Protein	58.38	60.31	57.61	99.66	72.46	84.97
Ligand/ion	50.21	70.69	60.85	102.79	96.82	105.81
Water	56.71	56.74	61.97	75.15	-	74.77
rms deviations						
Bond lengths (Å)	0.009	0.002	0.001	0.002	0.006	0.002
Bond angles (°)	0.945	0.422	0.448	0.520	0.667	0.557
Wavelength (Å)	0.9999	0.9999	0.9197	0.9197	0.9197	0.9197
Temperature (K)	100	100	100	100	100	100
X-ray source	PXII at SLS, Villigen, CH	PXII at SLS, Villigen, CH	PXII at SLS, Villigen, CH	PXII at SLS, Villigen, CH	PXII at SLS, Villigen, CH	PXII at SLS, Villigen, CH
Detector	EIGER2 16M	EIGER2 16M	EIGER2 16M	EIGER2 16M	EIGER2 16M	EIGER2 16M
Ramachandran plot						
Residues in						
favored regions	99.22 %	97.76 %	97.60 %	97.42 %	98.55 %	98.18 %
allowed regions	0.78 %	2.24 %	2.40 %	2.21 %	1.09 %	1.82 %
outlier regions	0.00 %	0.00 %	0.00 %	0.37 %	0.36 %	0.00 %

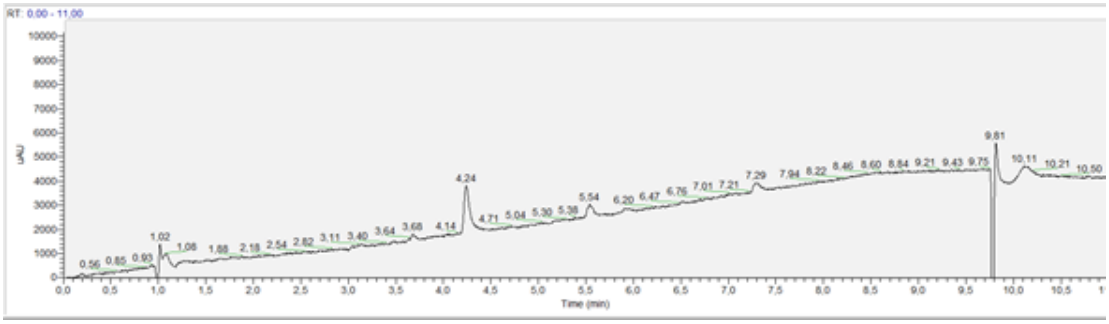
Table S1. Crystallographic statistics of MKK7 in complex with triazolyl inhibitor derivatives. Statistics for co-crystals with compounds **1a**, **1h**, **1k**, **2a**, **2b**, **2c** (PDB: 7OVI, 7OVJ, 7OVK, 7OVL, 7OVM, 7OVN). Values in parenthesis refer to the highest resolution shell.

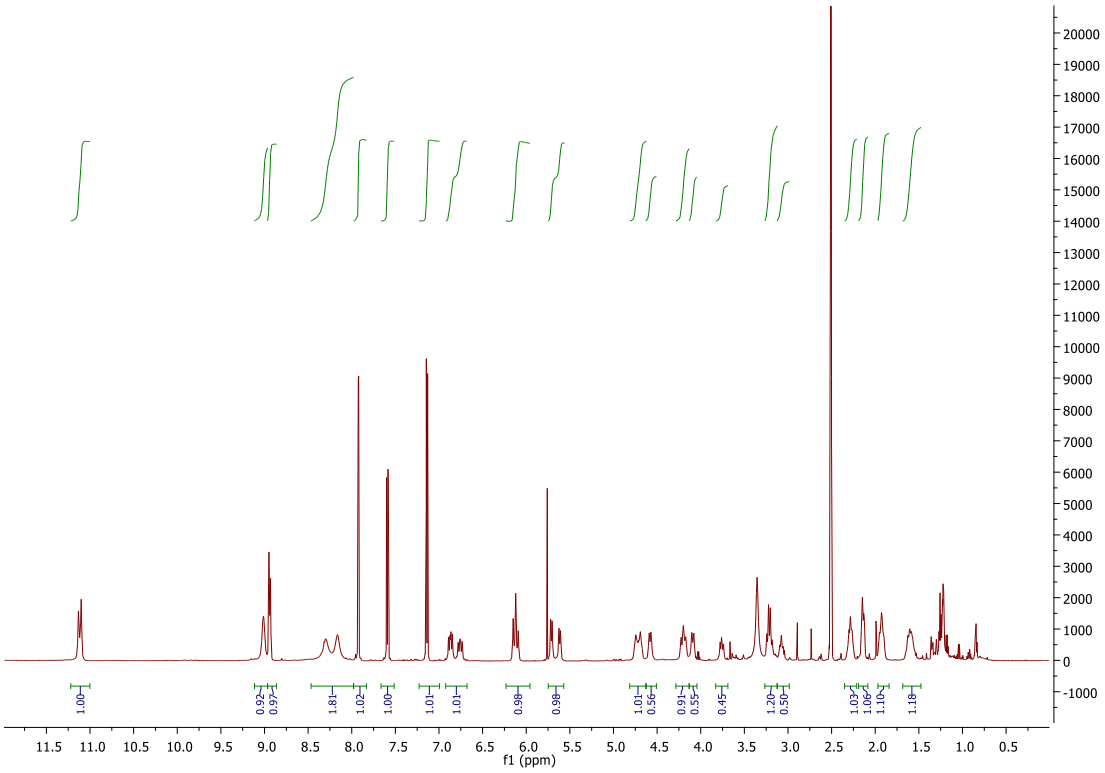
HPLC and NMR spectra of reported compounds

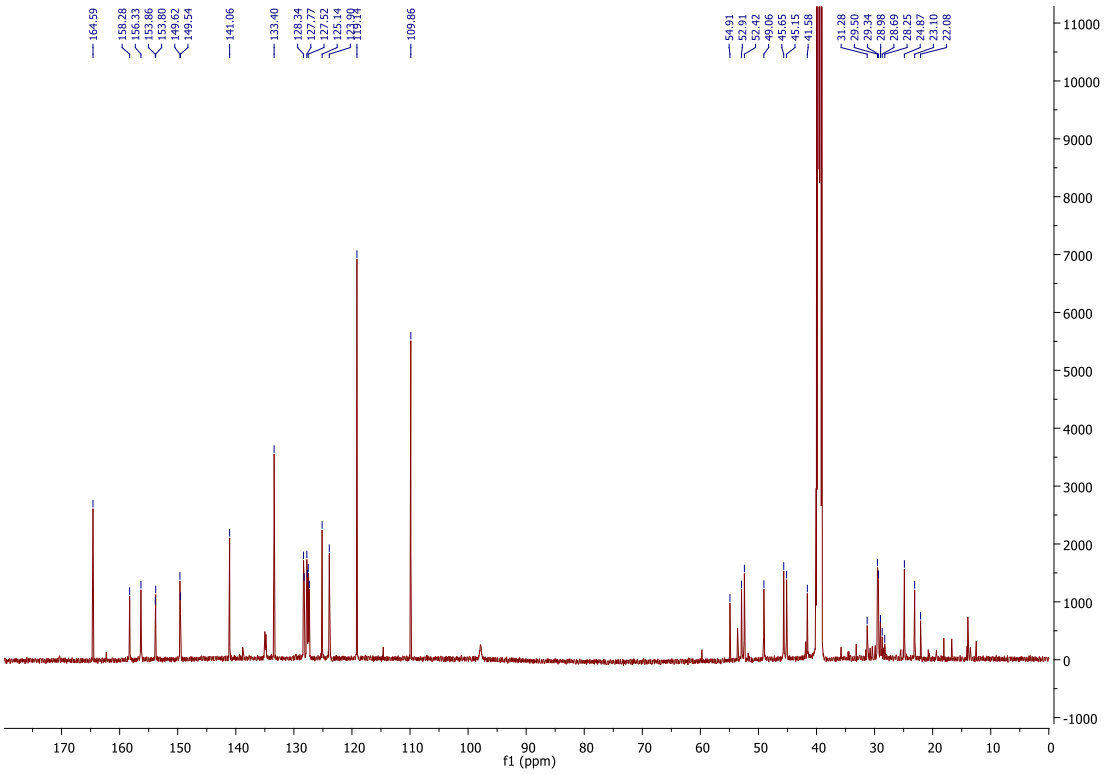
(*R*)-1-(3-(4-amino-3-(1-(4-bromo-2-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1a)



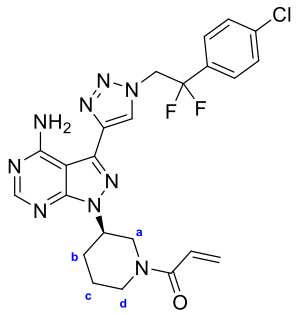
LC-MS chromatogram, detection at 280 nm



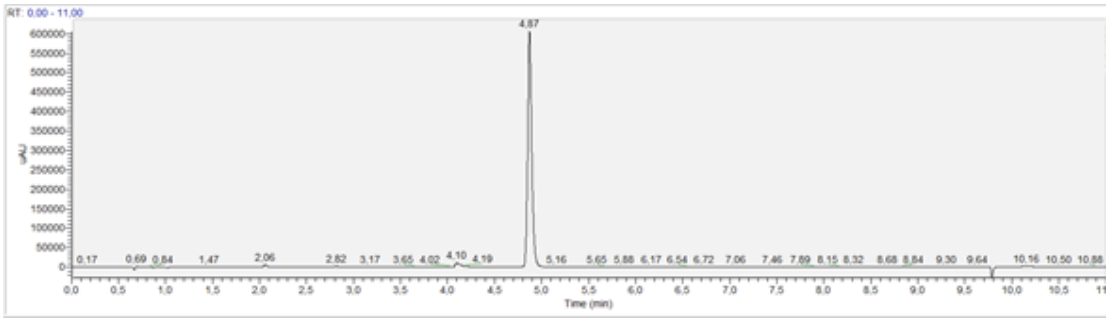

1.00
0.92
0.97
1.81
1.02
1.00
1.01
1.01
0.98
0.98
1.01
0.56
0.91
0.55
0.45
1.20
0.50
1.03
1.06
1.10
1.18
f1 (ppm)

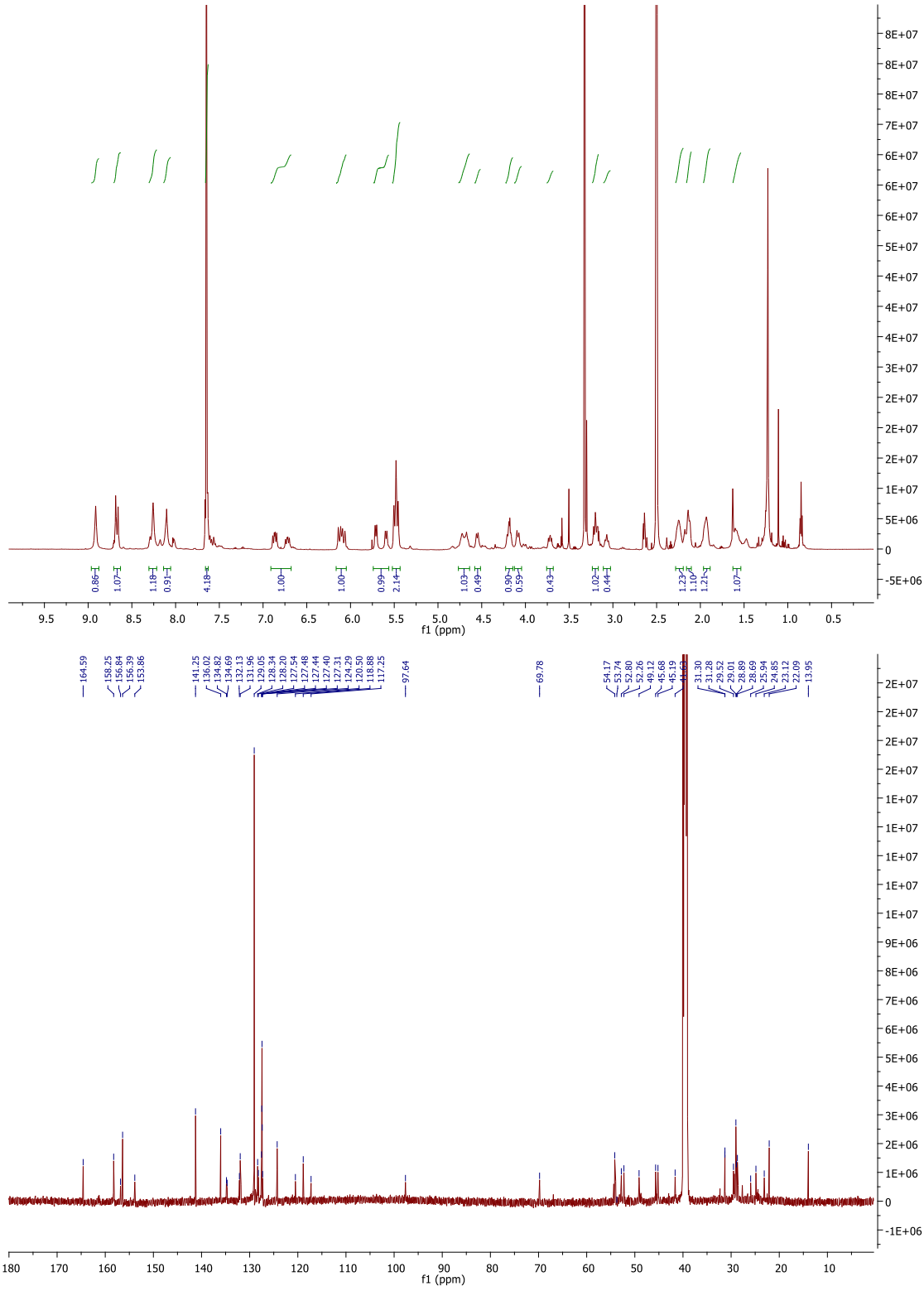

164.59
158.28
156.33
153.86
153.80
149.62
149.54
141.06
133.40
128.34
127.77
127.52
125.14
109.86
54.91
52.91
52.42
49.06
45.65
45.15
41.58
31.28
29.50
29.34
28.88
28.69
28.25
24.87
23.10
22.08
f1 (ppm)

(*R*)-1-(3-(4-amino-3-(1-(2-(4-chlorophenyl)-2,2-difluoroethyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1b)

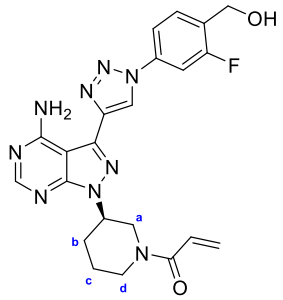


LC-MS chromatogram, detection at 280 nm

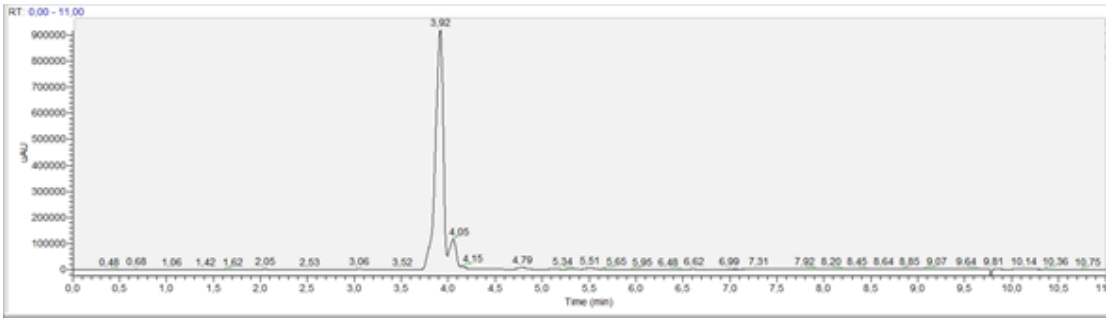


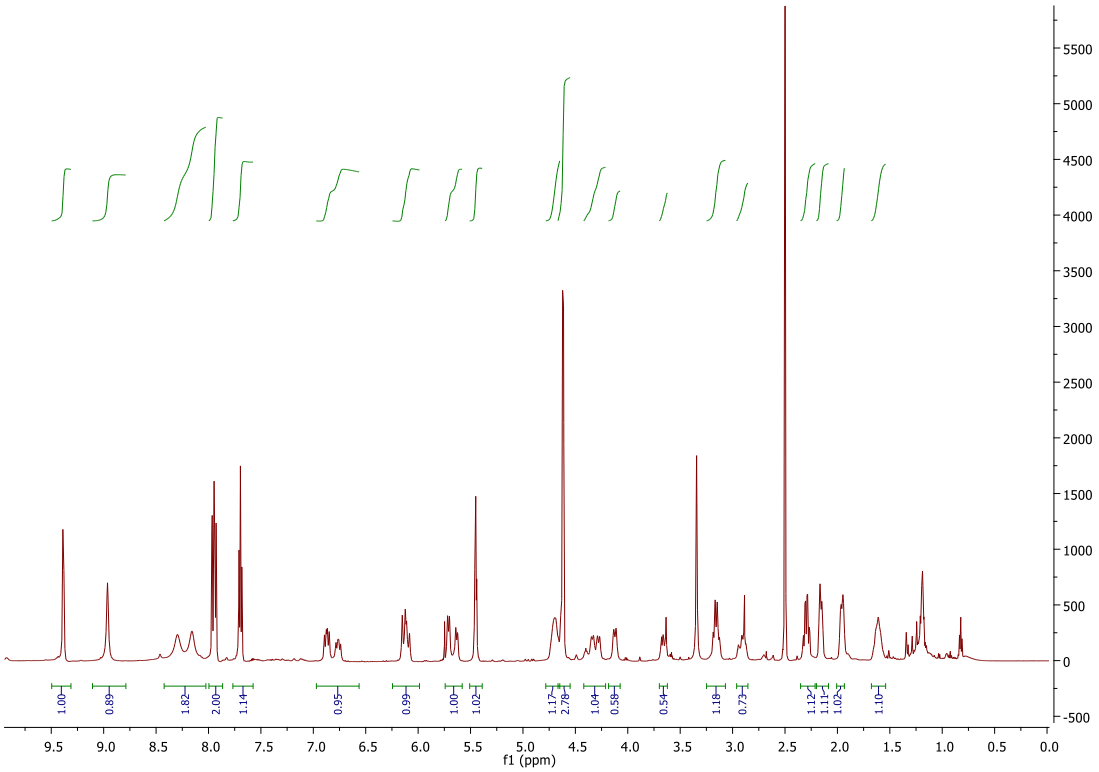


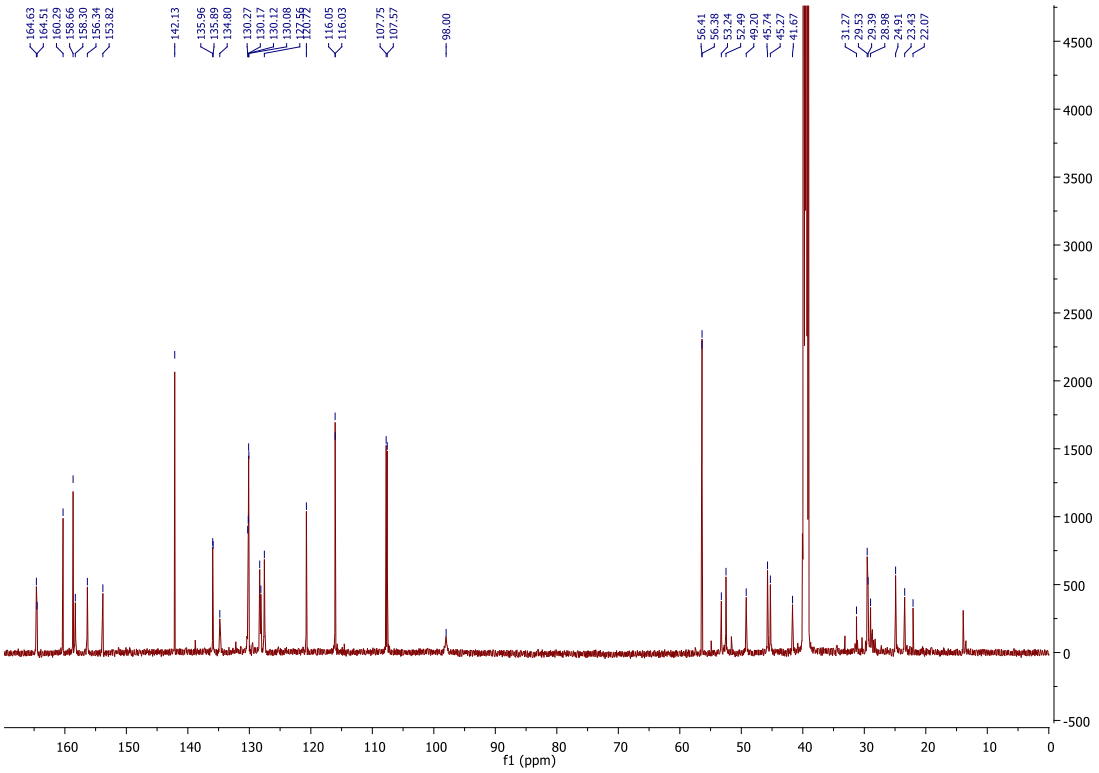
(*R*)-1-(3-(4-amino-3-(1-(3-fluoro-4-(hydroxymethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1c)



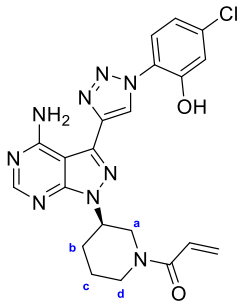
LC-MS chromatogram, detection at 280 nm



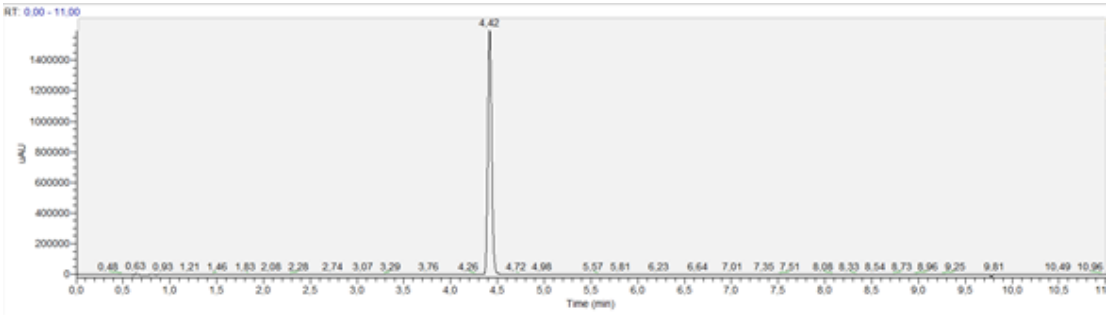

1.00
0.89
1.82
2.00
1.14
0.95
0.99
1.00
1.02
1.17
2.78
1.04
0.58
0.54
1.18
0.73
1.12
1.14
1.02
1.10
f1 (ppm)

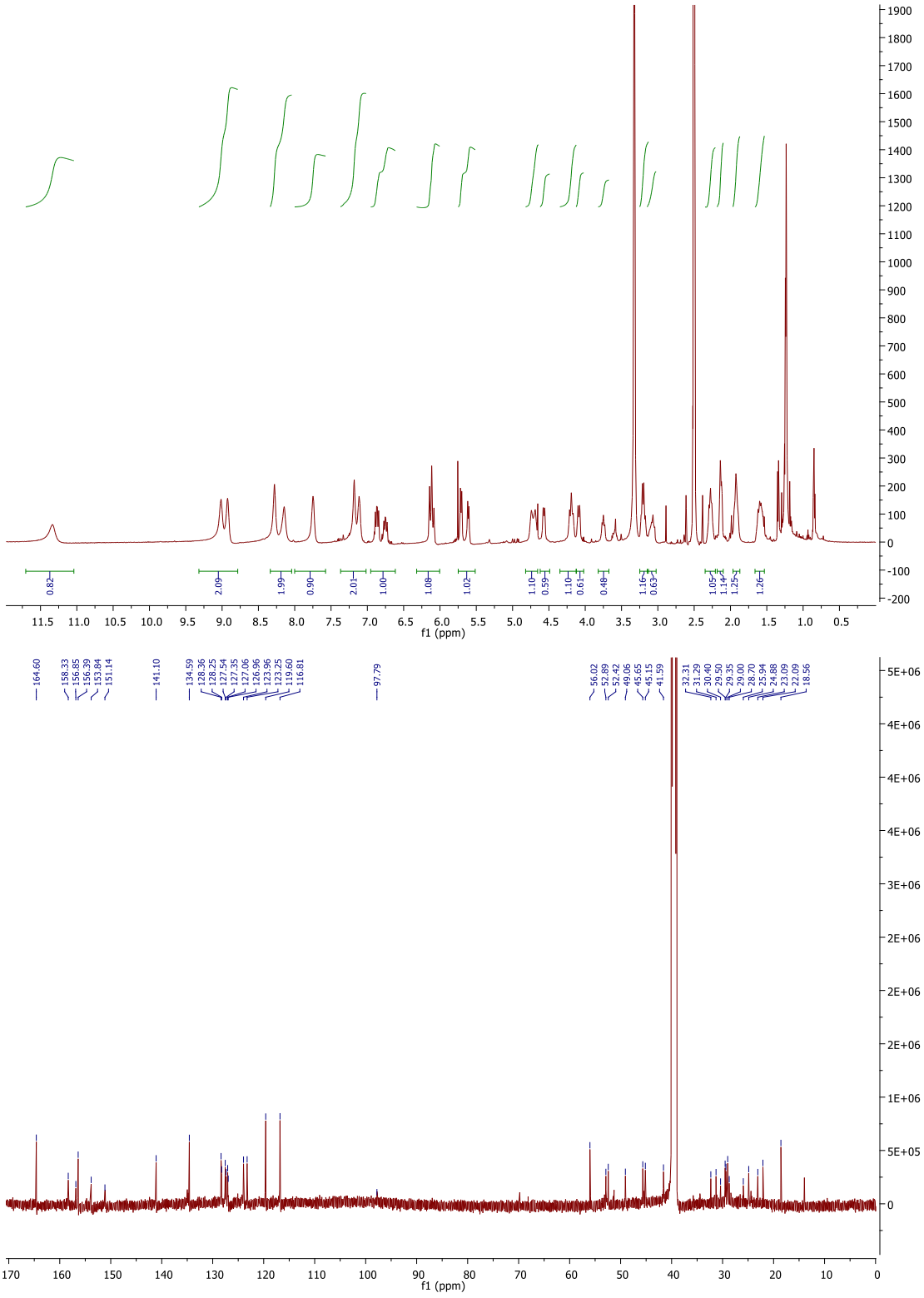

164.63
164.51
160.29
158.66
156.30
155.34
153.82
142.13
135.96
135.89
134.80
130.27
130.17
130.12
130.08
127.56
120.72
116.05
116.03
107.75
107.57
98.00
56.41
56.38
53.24
52.49
50.20
45.74
45.27
41.67
31.27
29.53
29.39
28.98
24.91
23.43
22.07
f1 (ppm)

(*R*)-1-(3-(4-amino-3-(1-(4-chloro-2-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1d)

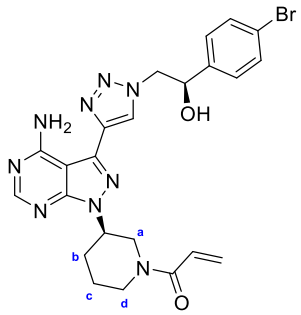


LC-MS chromatogram, detection at 280 nm

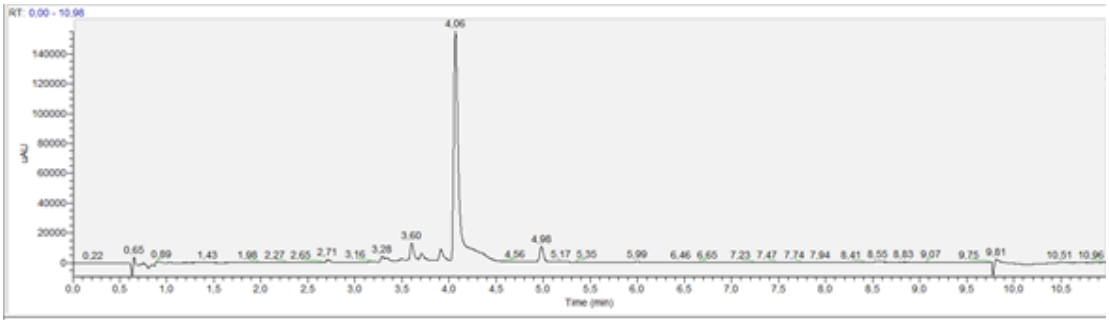


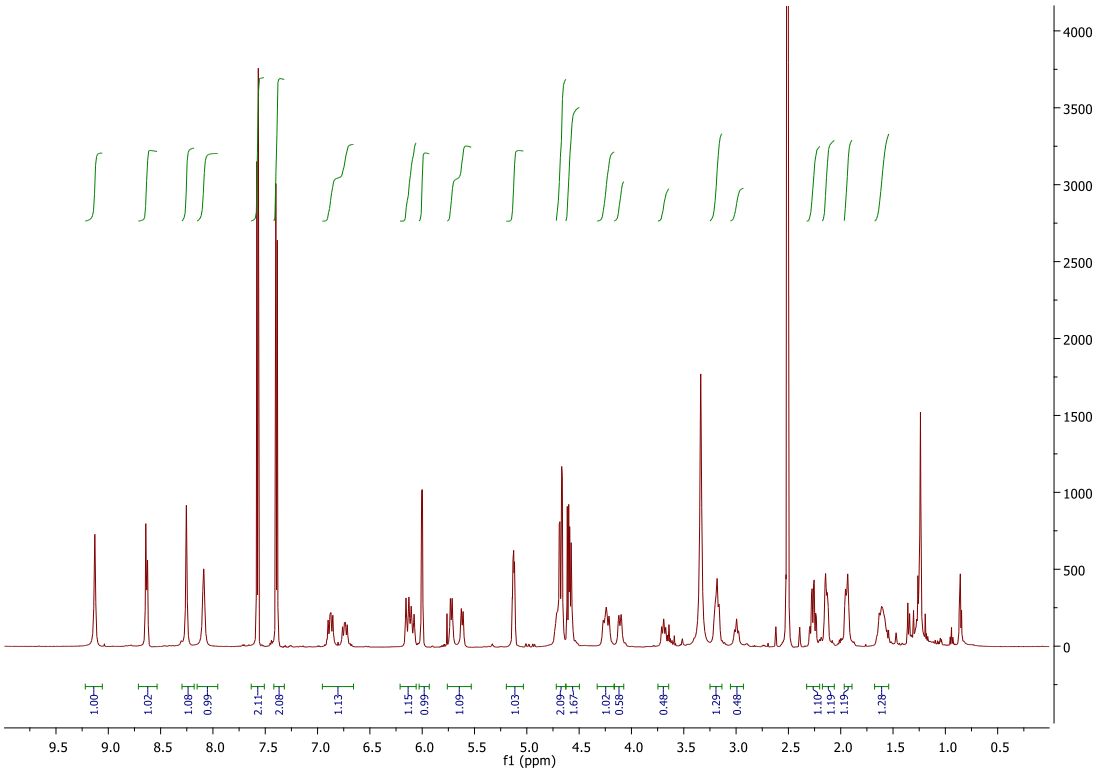

0.82
2.09
1.99
0.90
2.01
1.00
1.08
1.02
1.10
0.59
1.10
0.61
0.48
1.16
0.63
1.05
1.14
1.25
1.26
f1 (ppm)
164.60
158.33
156.85
156.39
153.84
151.14
141.10
134.59
128.36
128.25
127.54
127.35
127.06
126.96
123.96
123.25
119.60
116.81
97.79
56.02
52.89
52.42
49.06
45.65
45.15
41.59
32.31
31.29
30.40
29.50
29.35
29.00
28.70
25.94
24.88
23.09
22.09
18.56
f1 (ppm)

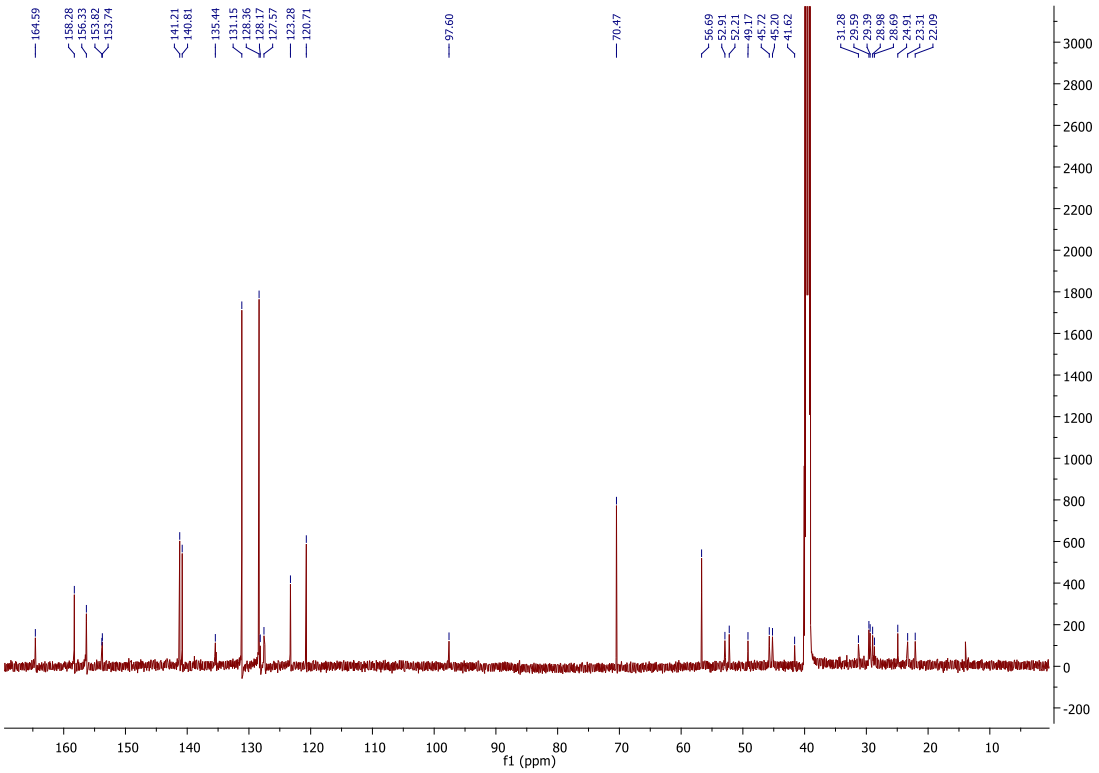
1-((*R*)-3-(4-amino-3-(1-((*R*)-2-(4-bromophenyl)-2-hydroxy-ethyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1e)



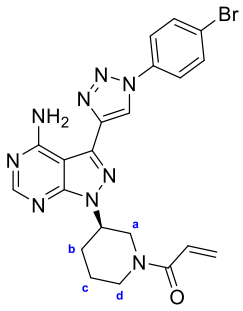
LC-MS chromatogram, detection at 280 nm



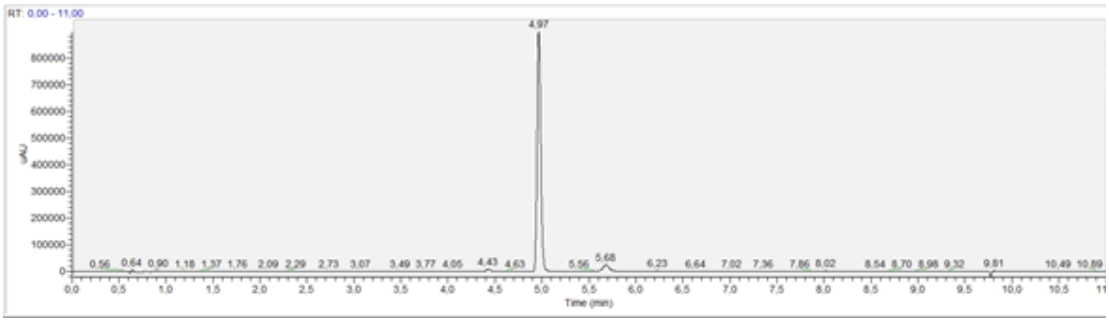

1.00
1.02
1.08
0.99
2.11
2.08
1.13
1.15
0.99
1.09
1.03
2.09
1.67
1.02
0.58
0.48
1.29
0.48
1.10
1.19
1.19
1.28
f1 (ppm)

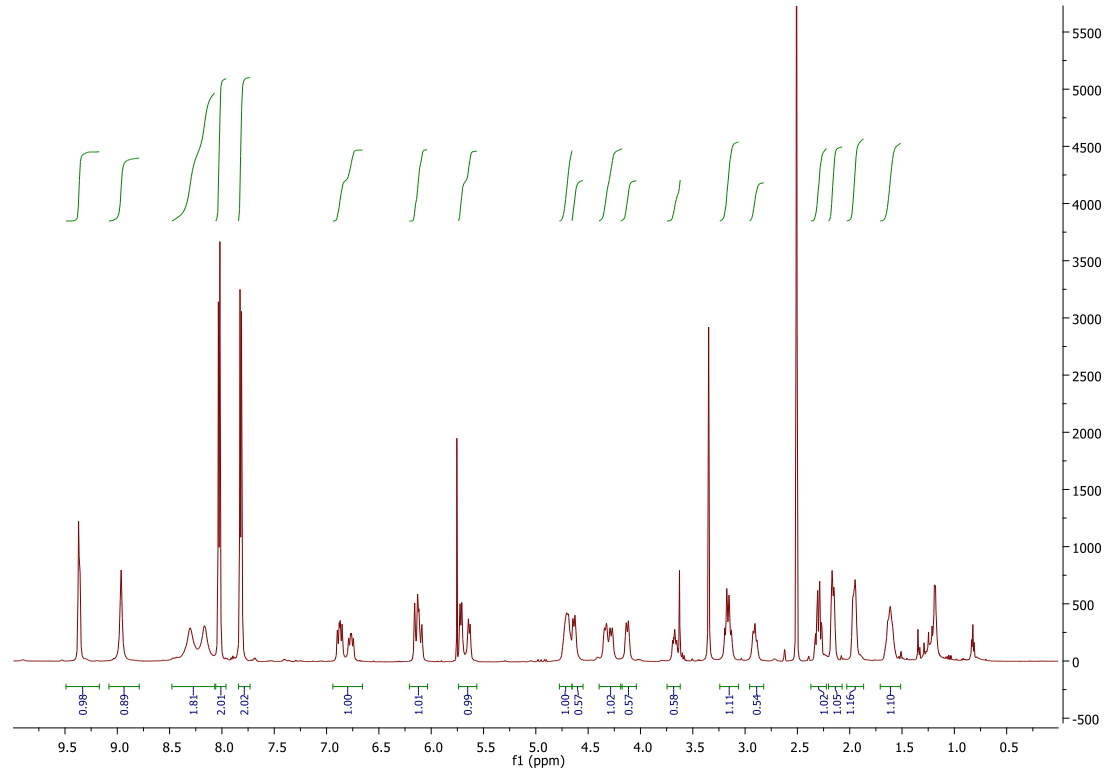

164.59
158.28
156.33
153.82
153.74
141.21
140.81
135.44
131.15
128.36
128.17
127.57
123.28
120.71
97.60
70.47
56.69
52.91
52.21
49.17
45.72
45.20
41.62
31.28
29.59
29.39
28.98
28.69
24.91
23.31
22.09
f1 (ppm)

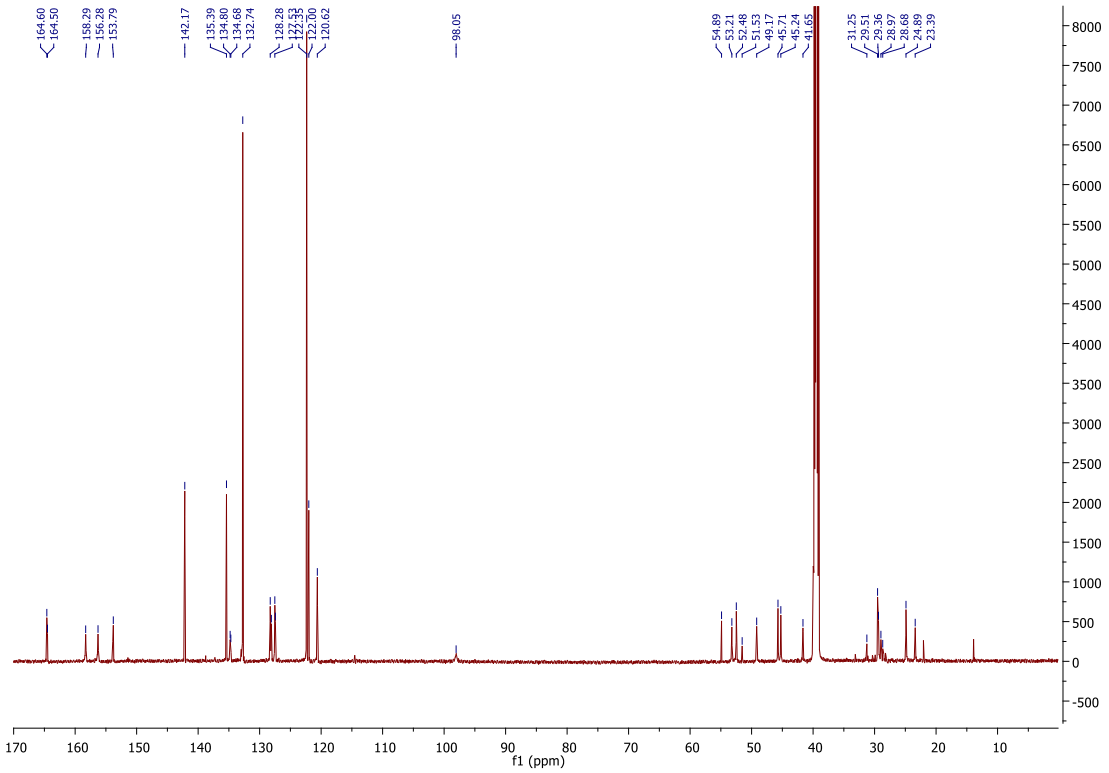
(*R*)-1-(3-(4-amino-3-(1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1f)



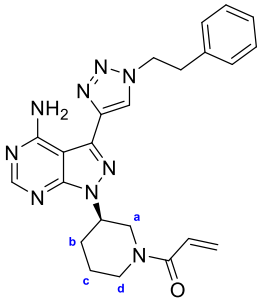
LC-MS chromatogram, detection at 280 nm



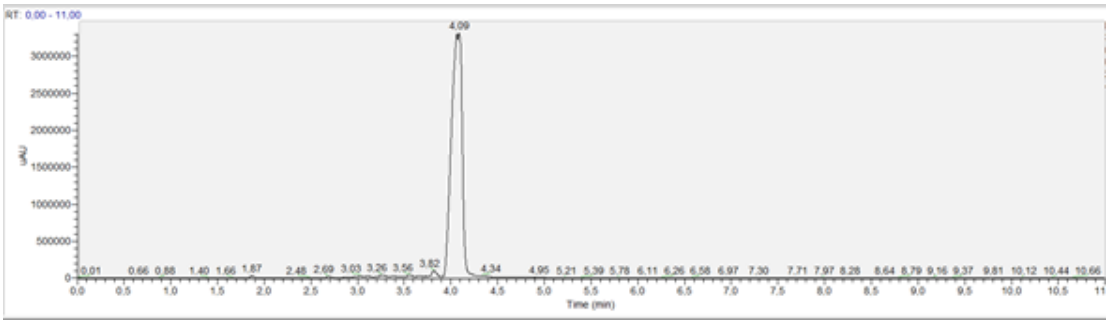

0.98
0.89
1.81
2.01
2.02
1.00
1.01
0.99
1.00
0.57
1.02
0.57
0.58
1.11
0.54
1.02
1.05
1.16
1.10
f1 (ppm)

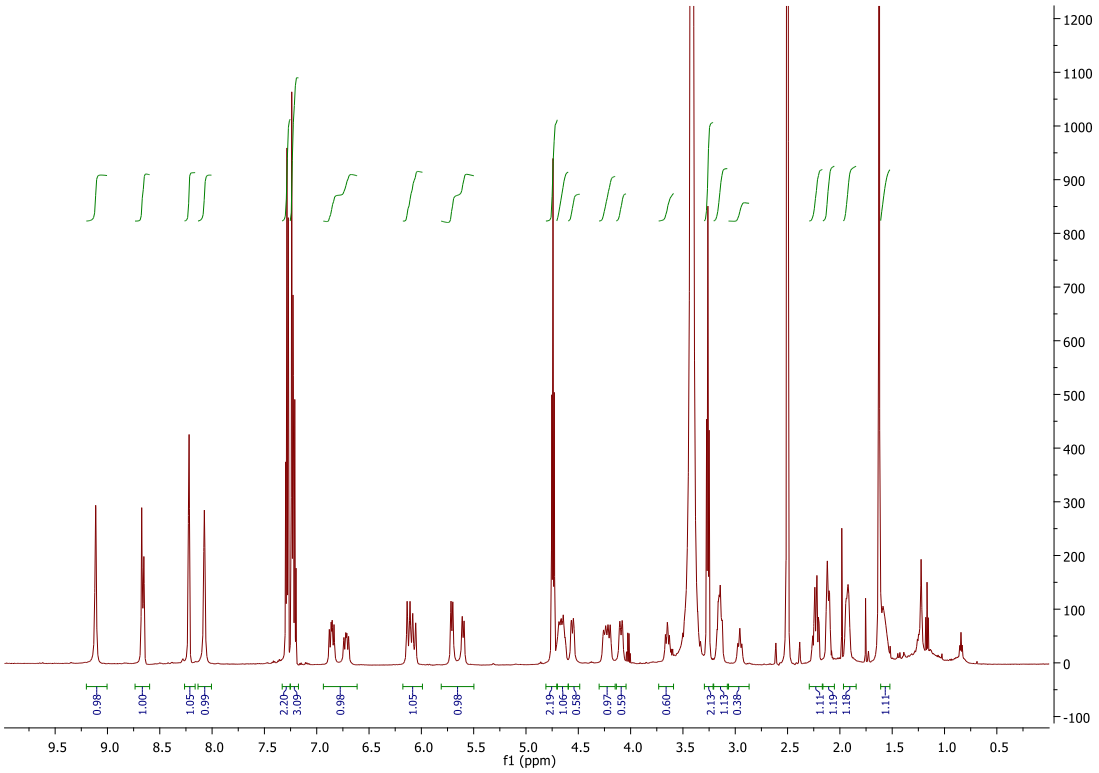

164.60
164.50
158.29
156.28
153.79
142.17
135.39
134.80
134.68
132.74
128.28
122.53
122.35
122.00
120.62
98.05
54.89
53.21
52.48
51.53
49.17
45.71
45.24
41.65
31.25
29.51
29.36
28.97
28.68
24.89
23.39
f1 (ppm)

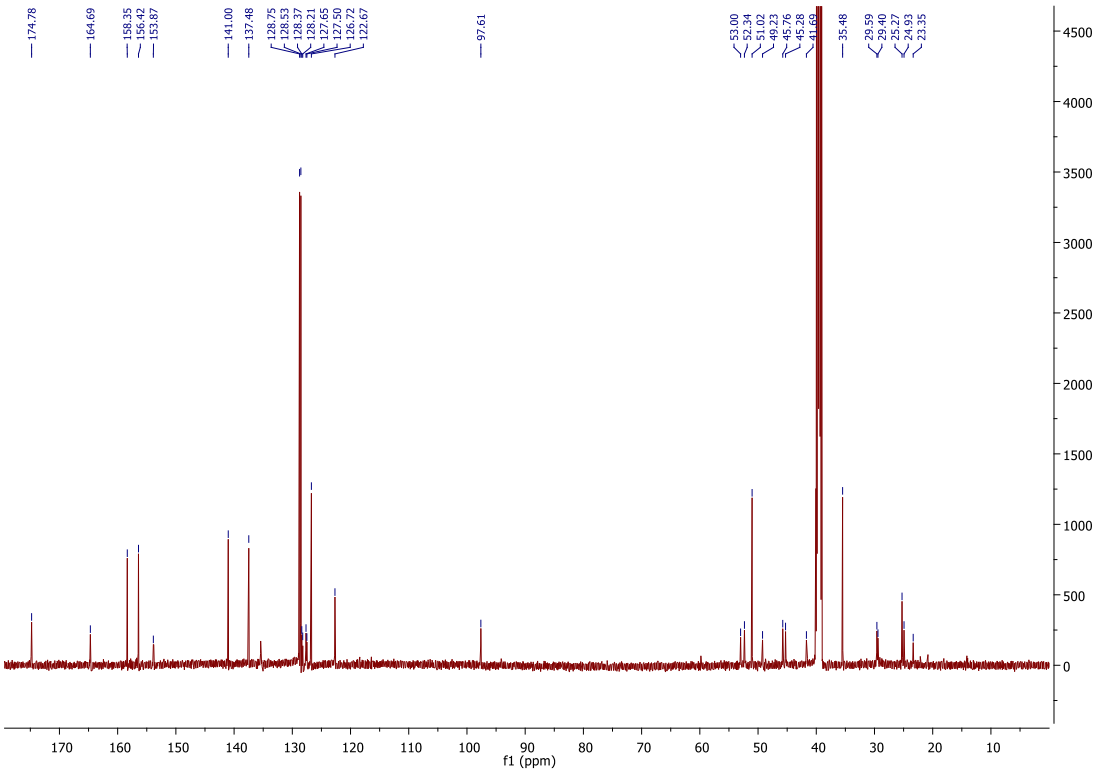
(*R*)-1-(3-(4-amino-3-(1-phenethyl-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1h)



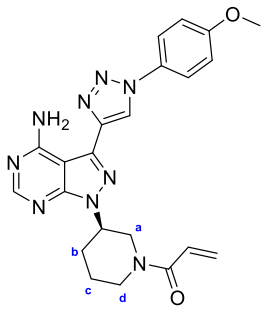
LC-MS chromatogram, detection at 280 nm



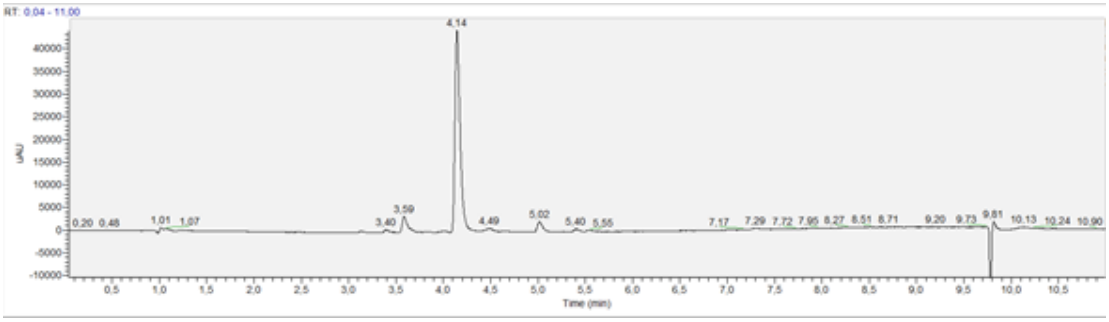

0.98
1.00
1.05
0.99
2.20
3.09
0.98
1.05
0.98
2.19
1.06
0.58
0.97
0.59
0.60
2.13
1.13
0.38
1.11
1.19
1.18
1.11
f1 (ppm)

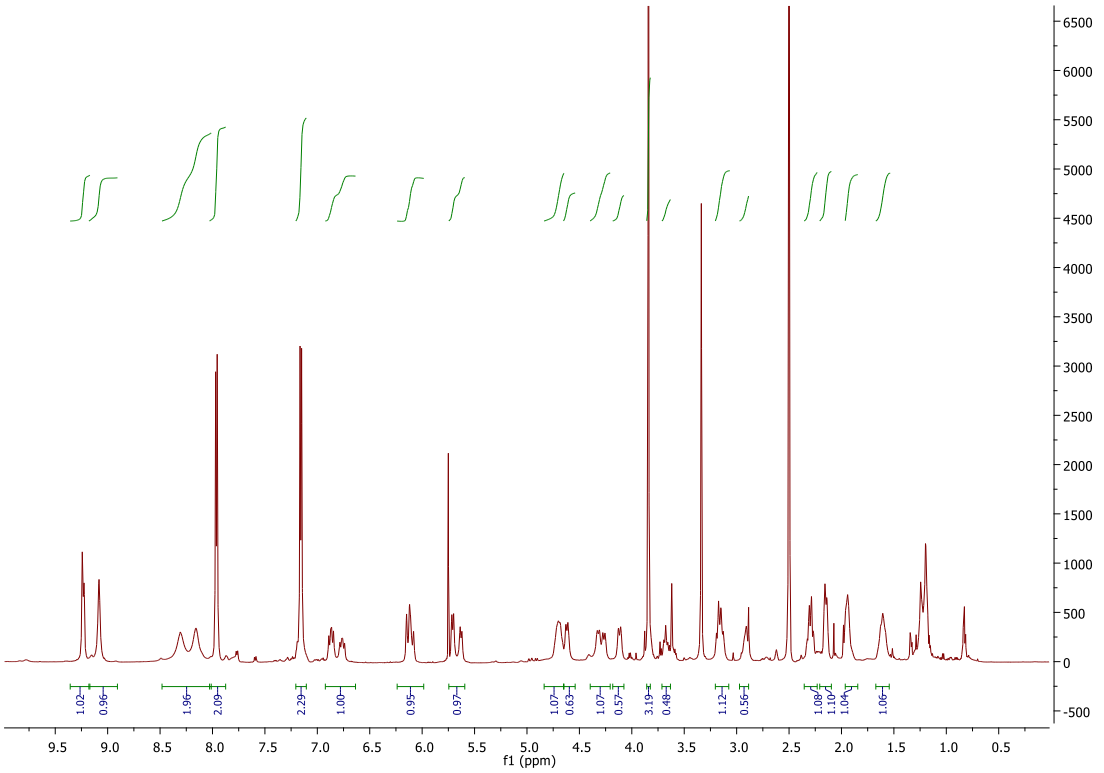

174.78
164.69
158.35
156.42
153.87
141.00
137.48
128.75
128.53
128.37
128.21
127.65
127.50
126.72
122.67
97.61
53.00
52.34
51.02
49.23
45.76
45.28
41.69
35.48
29.59
29.40
25.27
24.93
23.35
f1 (ppm)

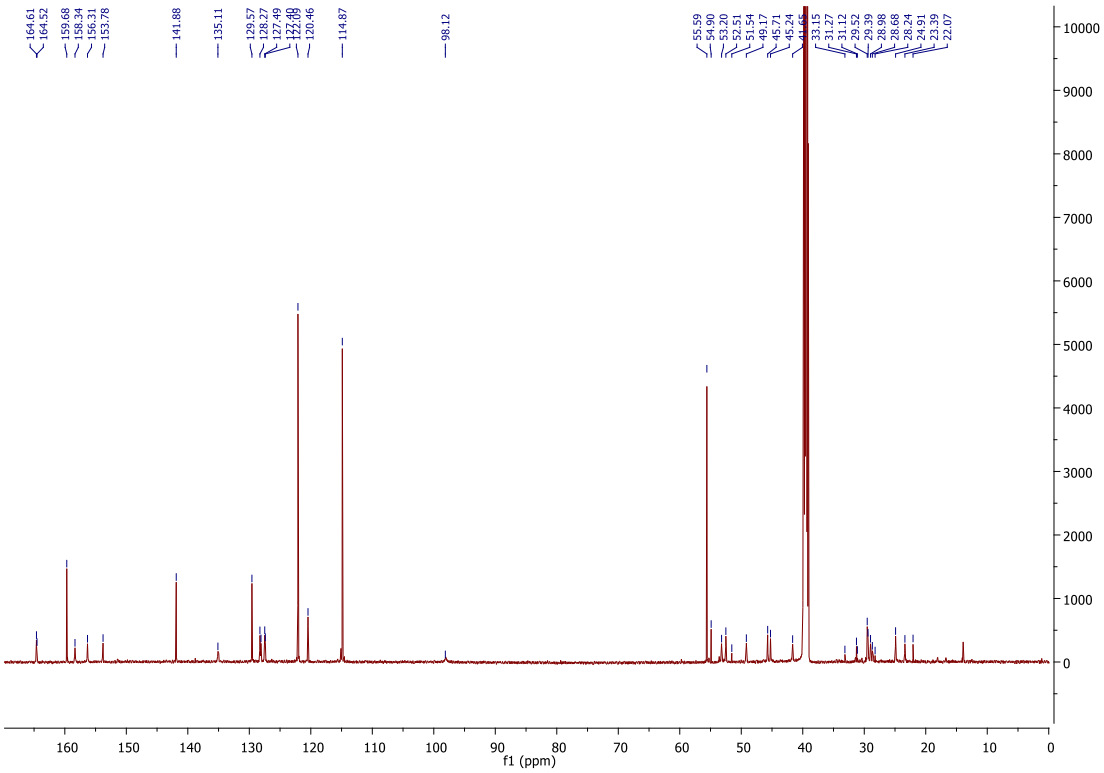
(*R*)-1-(3-(4-Amino-3-(1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1i)



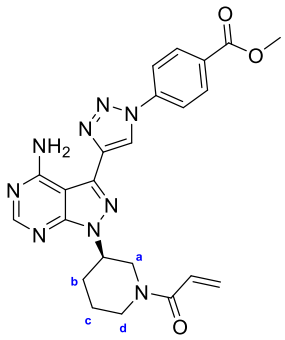
LC-MS chromatogram, detection at 280 nm



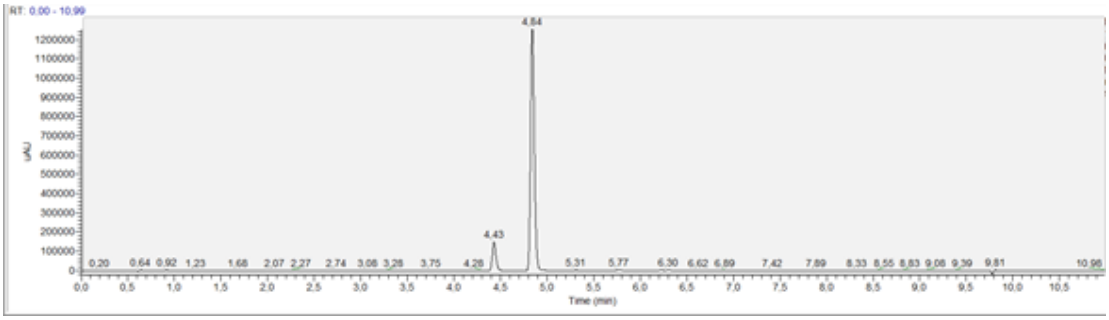

1.02
0.96
1.96
2.09
2.29
1.00
0.95
0.97
1.07
0.63
1.07
0.57
3.19
0.48
1.12
0.56
1.08
1.10
1.04
1.06
f1 (ppm)

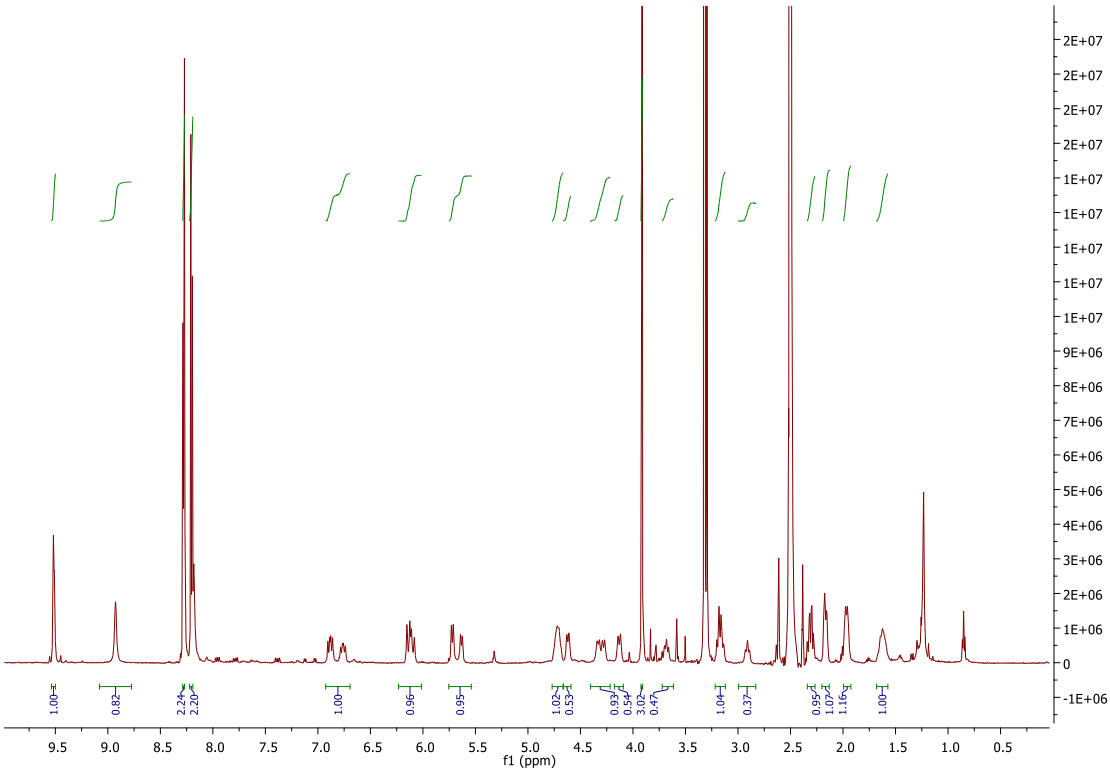

164.61
164.52
159.68
158.34
156.31
153.78
141.88
135.11
129.57
128.27
127.49
122.09
120.46
114.87
98.12
55.59
54.90
53.20
52.51
51.54
49.17
45.71
45.24
41.66
33.15
31.27
31.12
29.52
29.39
28.98
28.68
28.24
24.91
23.39
22.07
f1 (ppm)

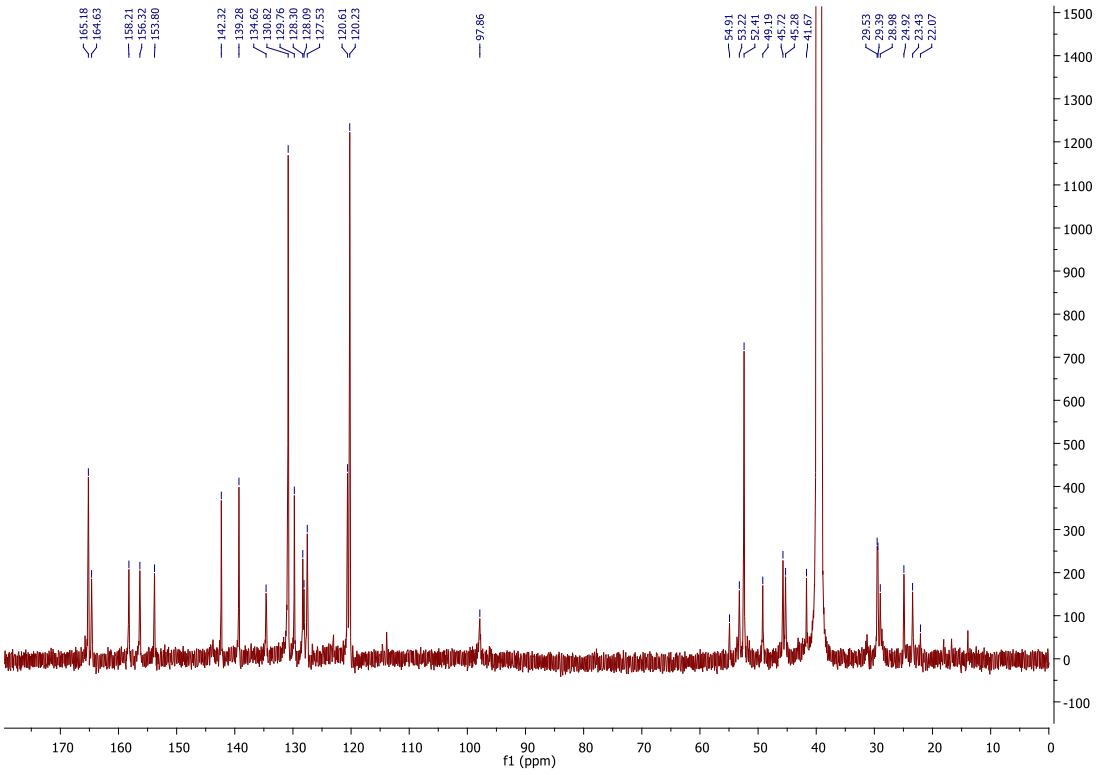
Methyl (*R*)-4-(4-(1-(1-acryloylpiperidin-3-yl)-4-amino-1*H*-pyrazolo [3,4-*d*]pyrimidin-3-yl)-1H-1,2,3-triazol-1-yl)benzoate (1j)



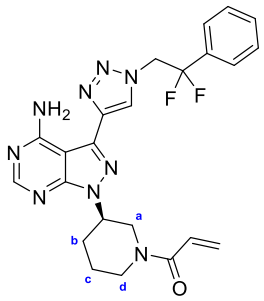
LC-MS chromatogram, detection at 280 nm



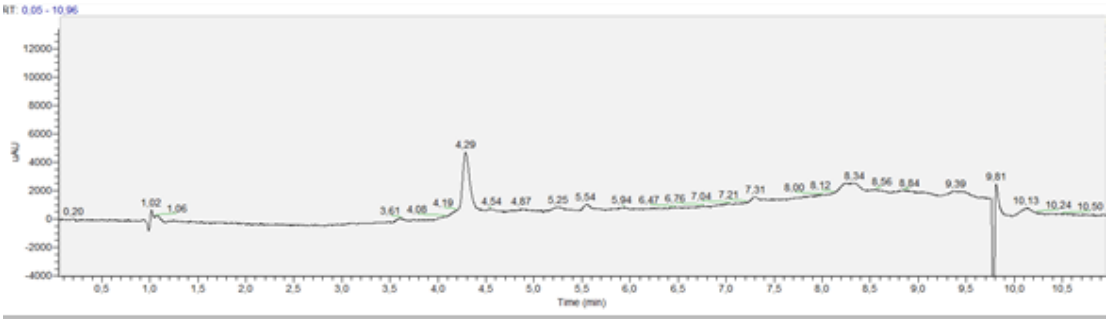

1.00
0.82
2.24
2.20
1.00
0.96
0.95
1.02
0.53
0.93
0.54
3.02
0.47
1.04
0.37
0.95
1.07
1.16
1.00
f1 (ppm)

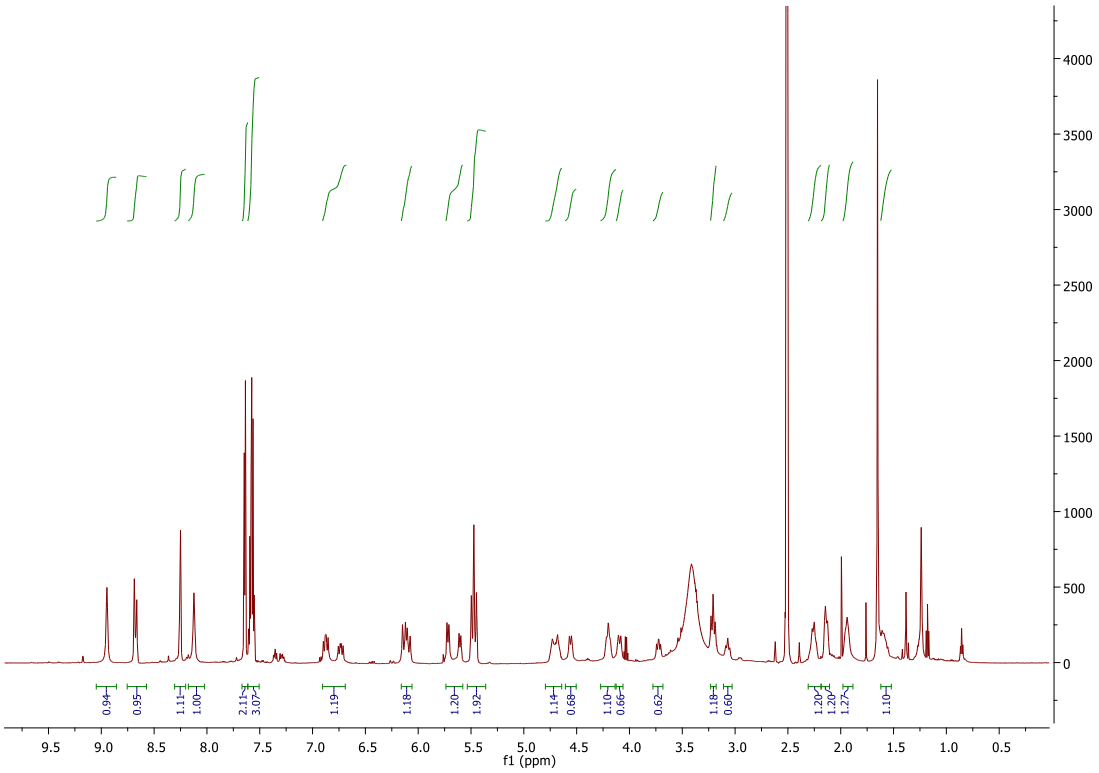

165.18
164.63
158.21
156.32
153.80
142.32
139.28
134.62
130.82
129.76
128.30
128.09
127.53
120.61
120.23
97.86
54.91
53.22
52.41
49.19
45.72
45.28
41.67
29.53
29.39
28.98
24.92
23.43
22.07
f1 (ppm)

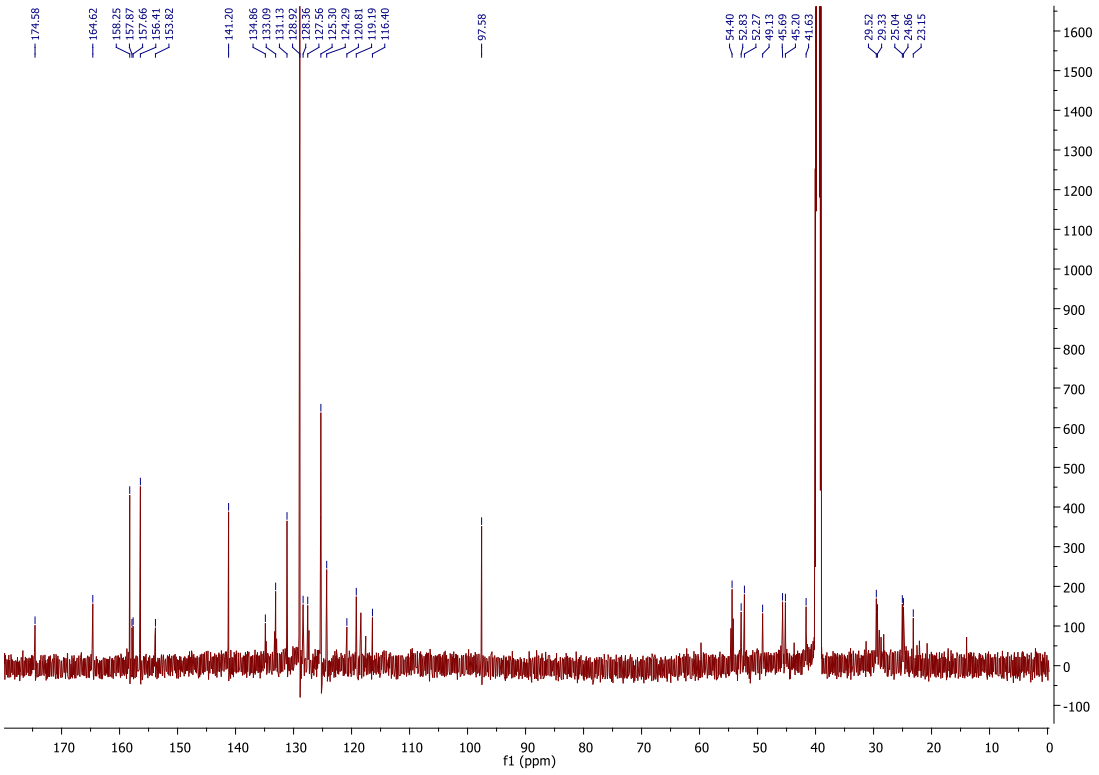
(*R*)-1-(3-(4-amino-3-(1-(2,2-difluoro-2-phenylethyl)-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (1k)



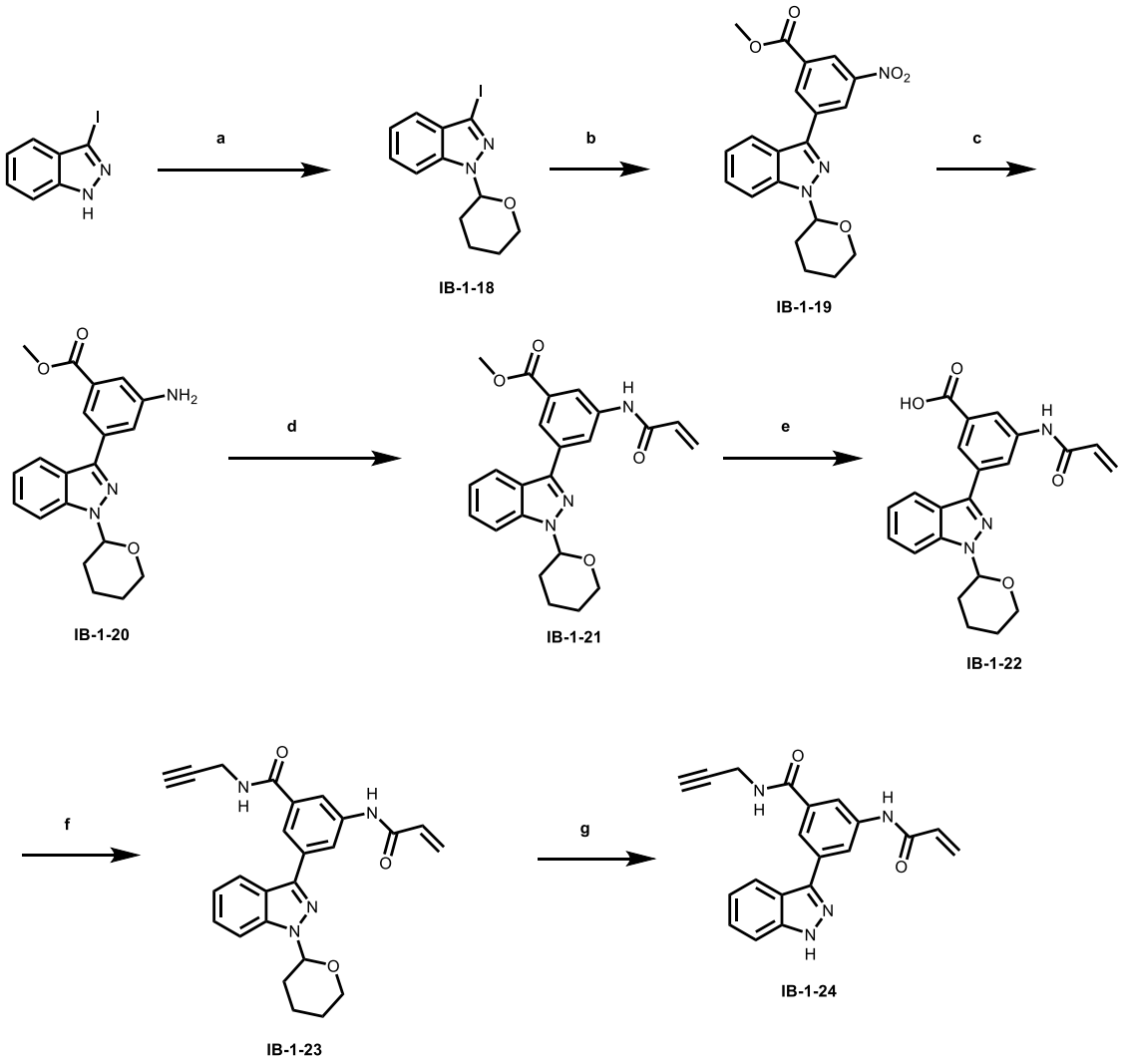
LC-MS chromatogram, detection at 280 nm



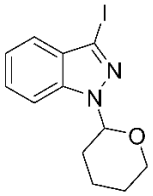

0.94
0.95
1.11
1.00
2.11
3.07
1.19
1.18
1.20
1.92
1.14
0.68
1.10
0.66
0.62
1.18
0.60
1.20
1.20
1.27
1.10
f1 (ppm)

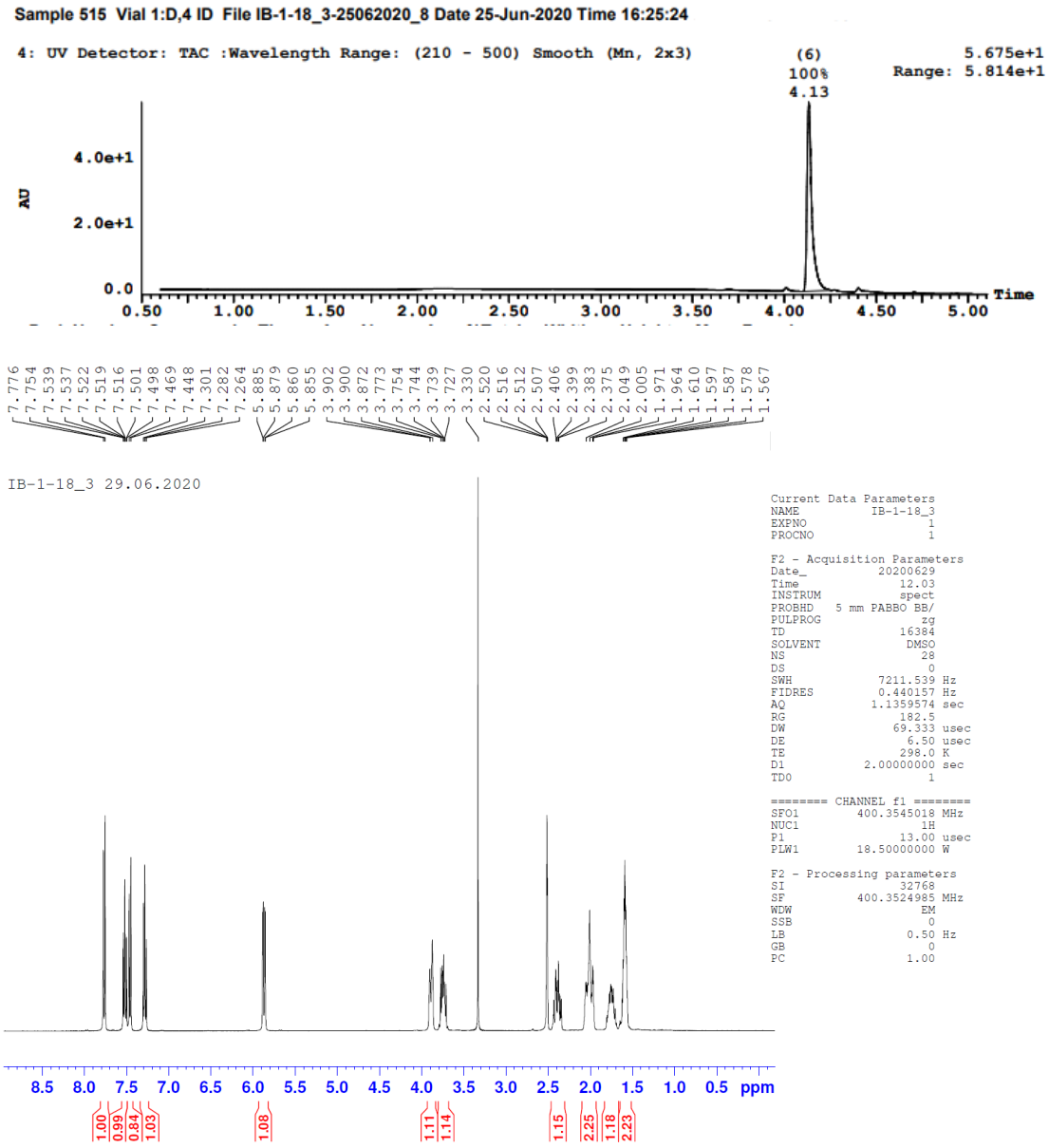

174.58
164.62
158.25
157.87
157.66
156.41
153.82
141.20
134.86
133.09
131.13
129.32
128.36
127.56
125.30
124.29
120.81
119.19
116.40
97.58
54.40
52.83
52.27
49.13
45.69
45.20
41.63
29.52
29.33
25.04
24.86
23.15
f1 (ppm)

Synthesis of IB-1-24*:*

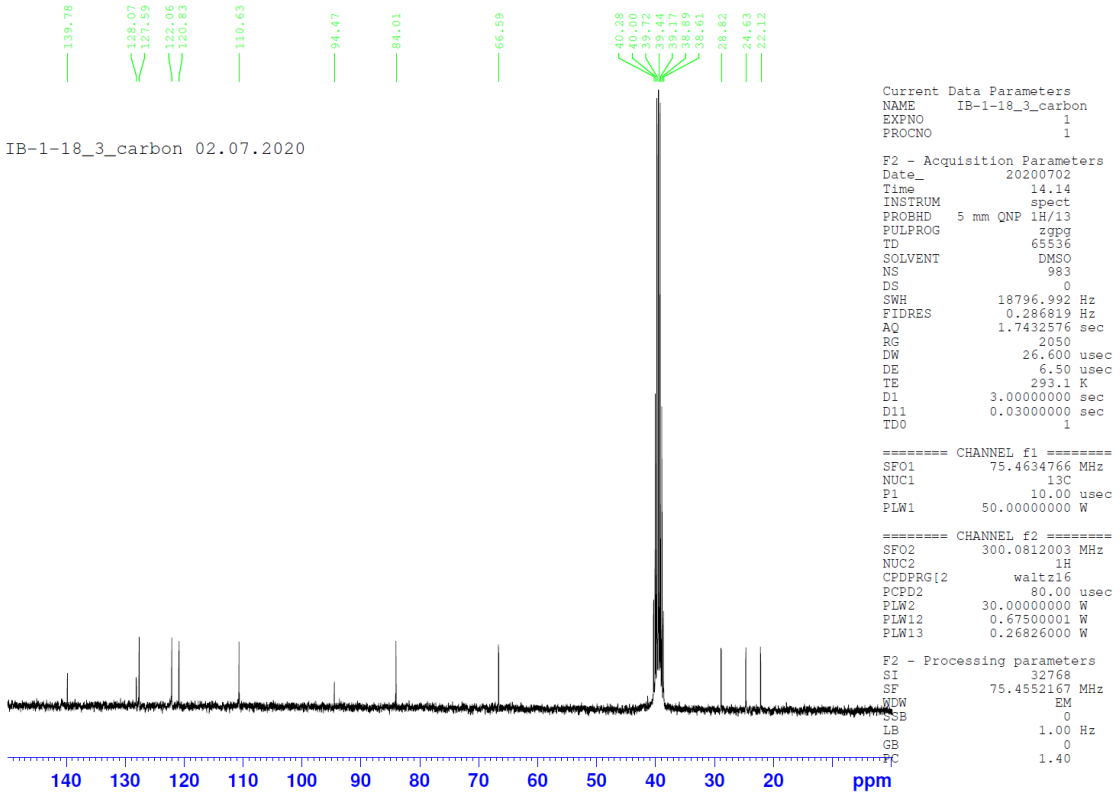


3-iodo-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole (IB-1-18)

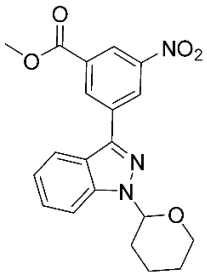


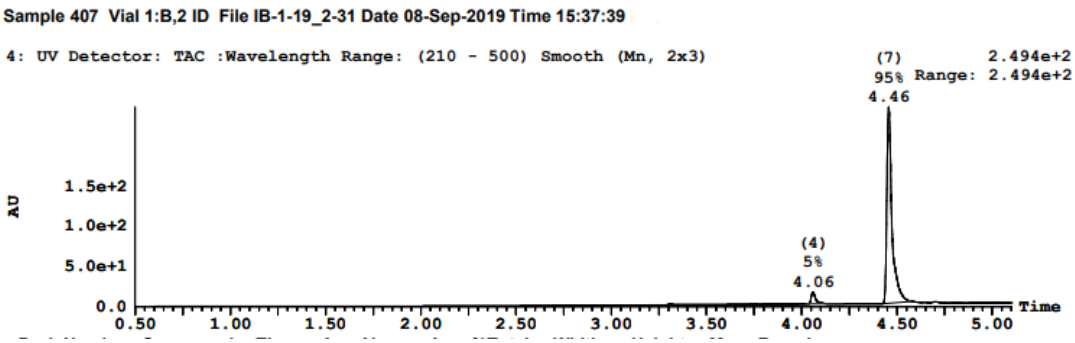


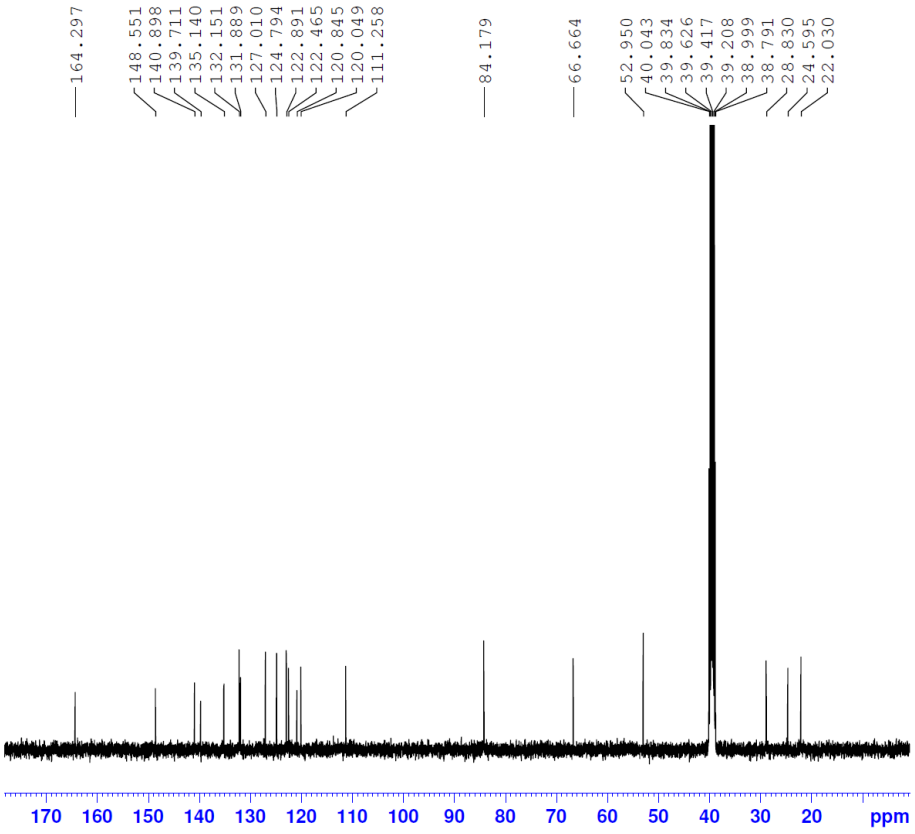

```
Current Data Parameters
NAME                  IB-1-18_3
EXPNO                         1
PROCNO                        1

F2 - Acquisition Parameters
Date_                  20200629
Time                      12.03
INSTRUM                   spect
PROBHD    5 mm PABBO BB/
PULPROG                      zg
TD                        16384
SOLVENT                    DMSO
NS                           28
DS                            0
SWH                  7211.539 Hz
FIDRES               0.440157 Hz
AQ                   1.1359574 sec
RG                        182.5
DW                     69.333 usec
DE                       6.50 usec
TE                      298.0 K
D1                   2.00000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1               400.3545018 MHz
NUC1                         1H
P1                       13.00 usec
PLW1               18.50000000 W

F2 - Processing parameters
SI                        32768
SF                 400.3524985 MHz
WDW                          EM
SSB                           0
LB                       0.50 Hz
GB                            0
PC                        1.00
```



Methyl 3-nitro-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)benzoate (IB-1-19).

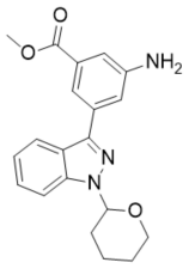




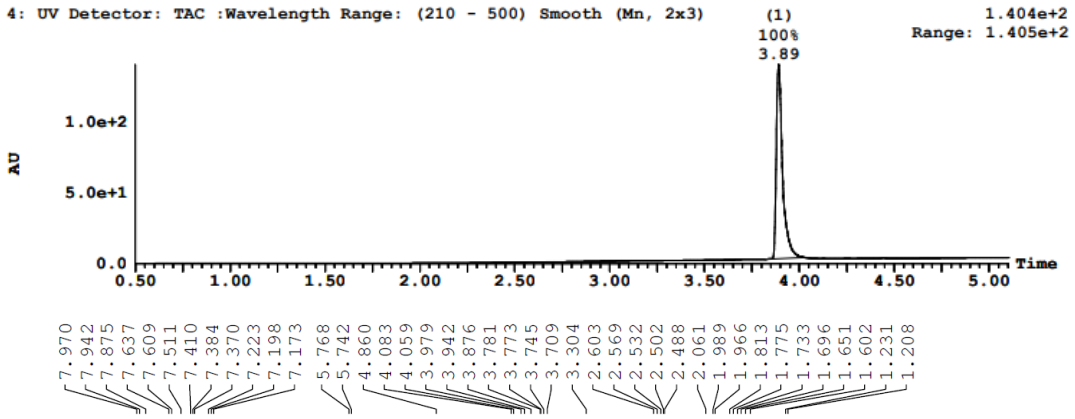


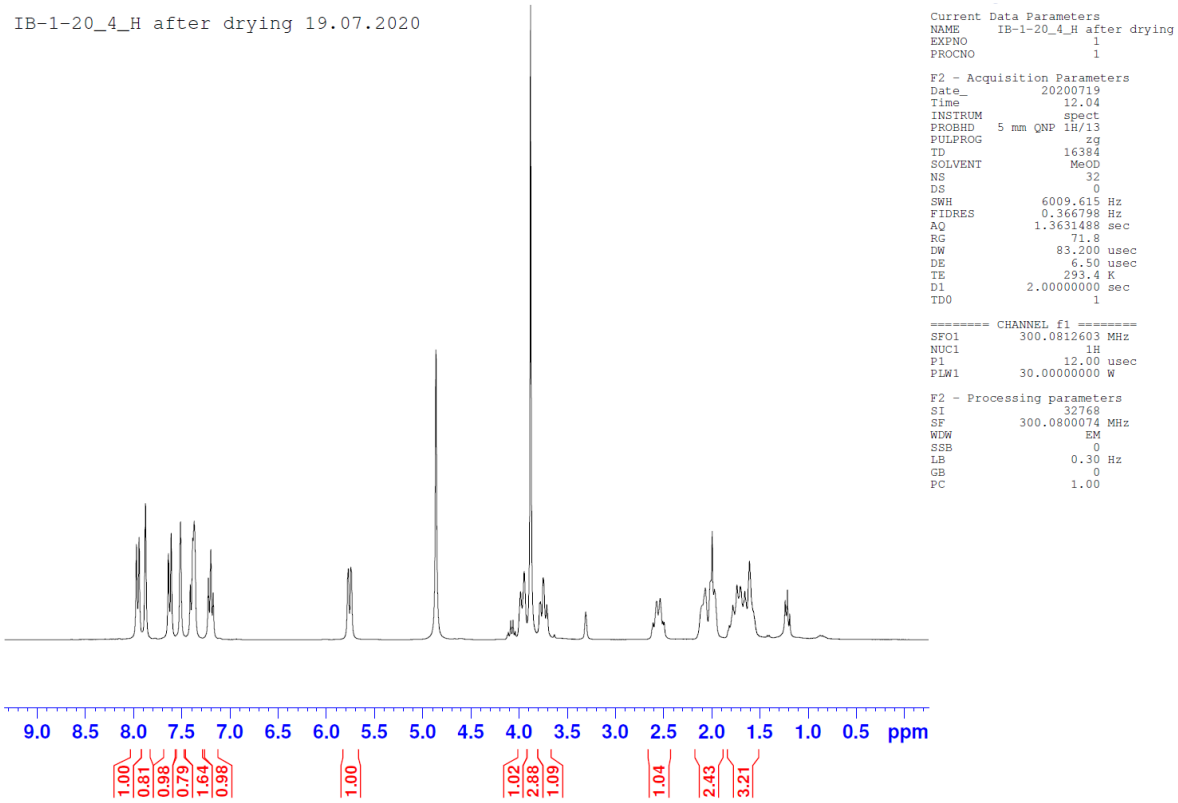

```
Current Data Parameters
NAME      KAS-2-409 LIKE IB-1-19_CARBON
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20200628
Time              12.49
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           zgpg
TD                65536
SOLVENT            DMSO
NS                  934
DS                    0
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976128 sec
RG                 1030
DW               19.800 usec
DE                6.50 usec
TE                298.2 K
D1            3.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6797896 MHz
NUC1                 13C
P1                  9.00 usec
PLW1         64.00000000 W

======== CHANNEL f2 ========
SFO2         400.3541014 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2         18.50000000 W
PLW12         0.41110000 W
PLW13         0.33291000 W

F2 - Processing parameters
SI                 131072
SF           100.6687754 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

Methyl 3-amino-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)benzoate (IB-1-20).

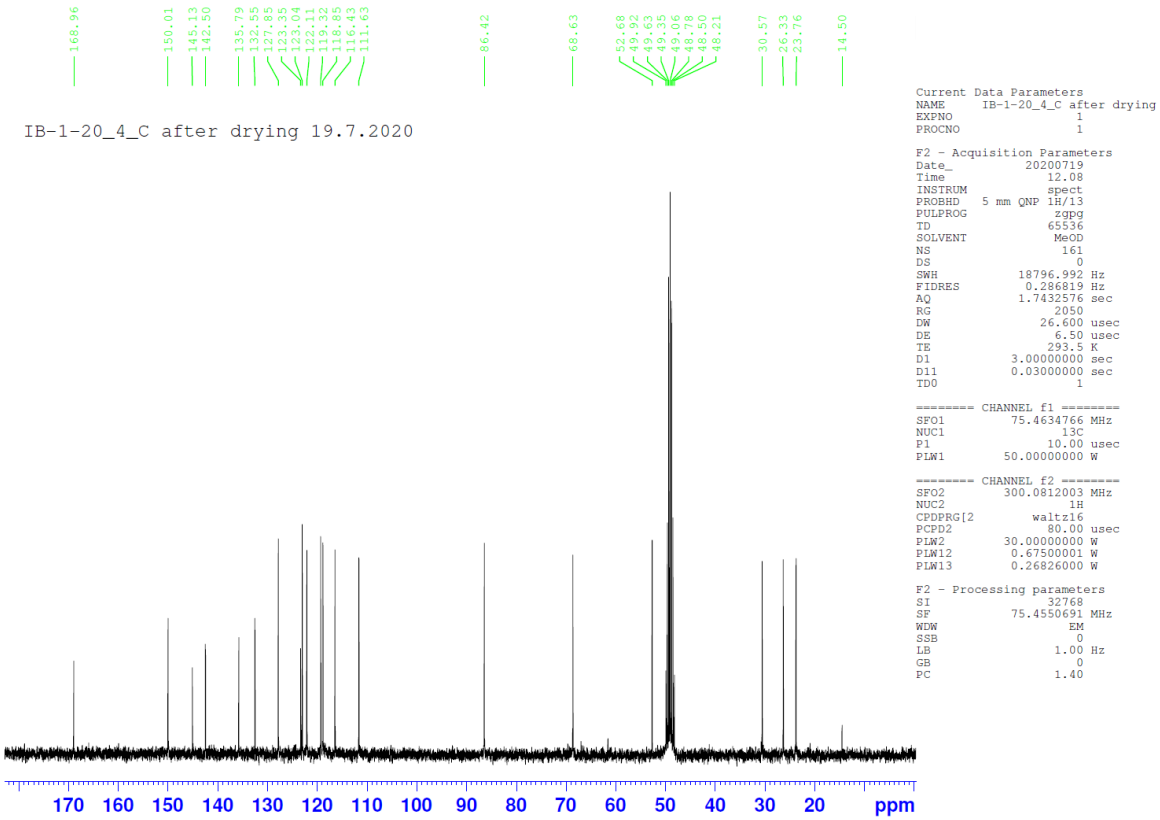


Sample 36 Vial 1:C,4 ID File IB-1-20_4-8 Date 05-Jul-2020 Time 17:13:52 Description combined f20-27 without f23

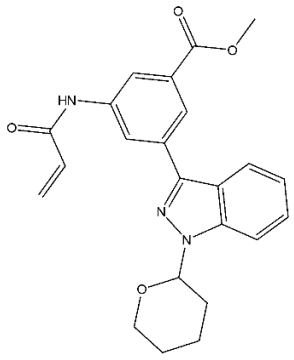


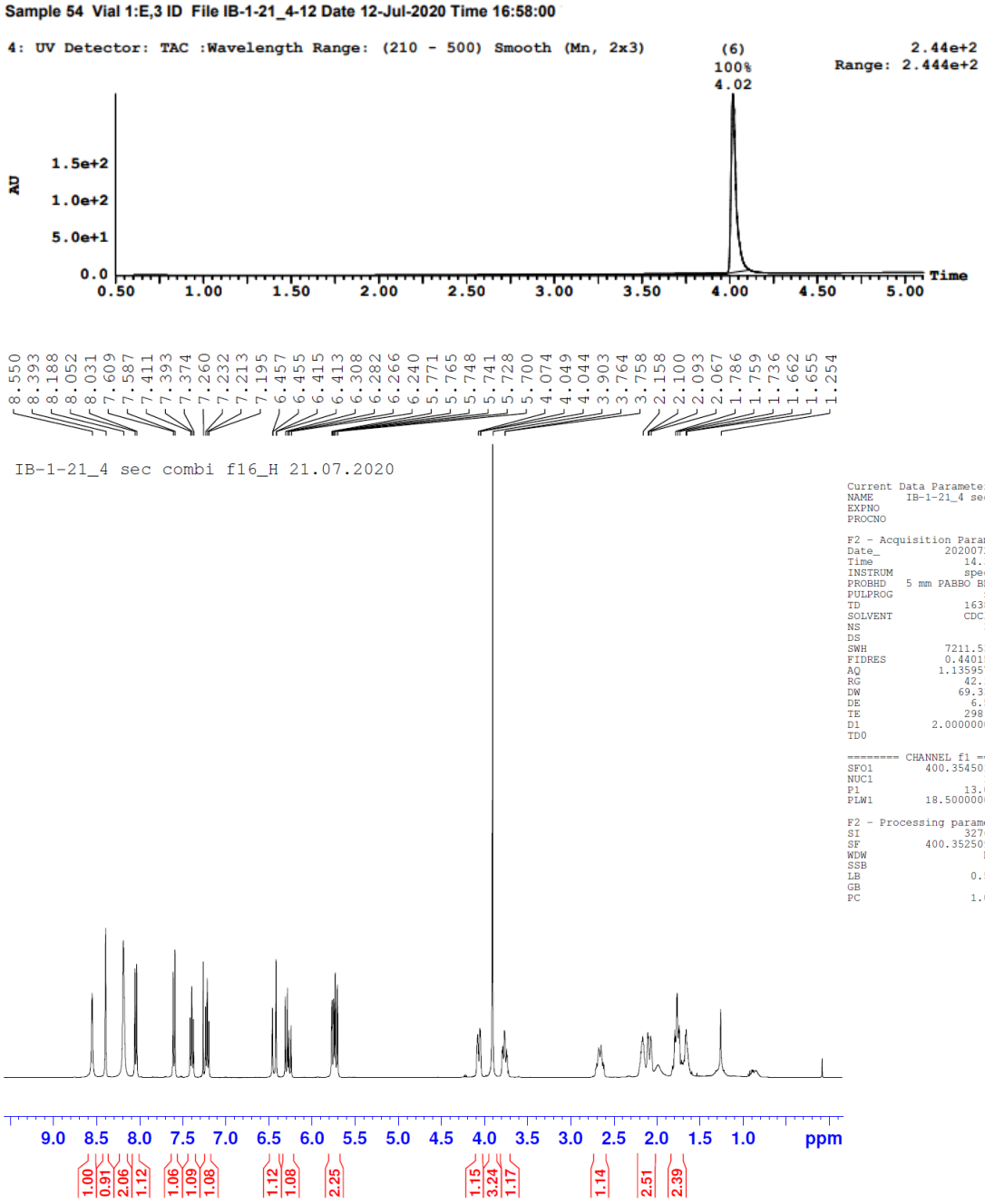


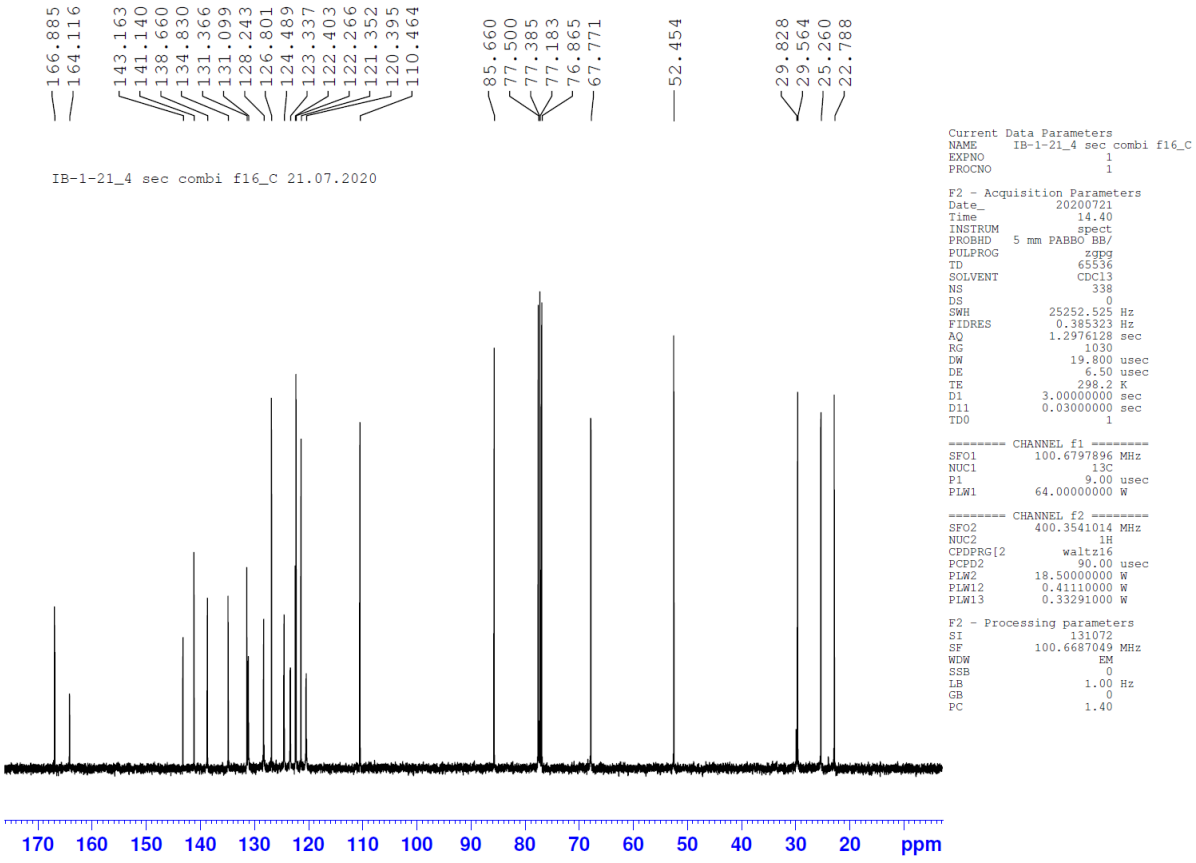

```
Current Data Parameters
NAME      IB-1-20_4_H after drying
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_           20200719
Time              12.04
INSTRUM           spect
PROBHD   5 mm QNP 1H/13
PULPROG             zg
TD                16384
SOLVENT            MeOD
NS                   32
DS                    0
SWH            6009.615 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                 71.8
DW                83.200 usec
DE                 6.50 usec
TE                293.4 K
D1             2.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         300.0812603 MHz
NUC1               1H
P1                12.00 usec
PLW1         30.00000000 W

F2 - Processing parameters
SI                32768
SF           300.0800074 MHz
WDW                EM
SSB                 0
LB              0.30 Hz
GB                  0
PC                1.00
```



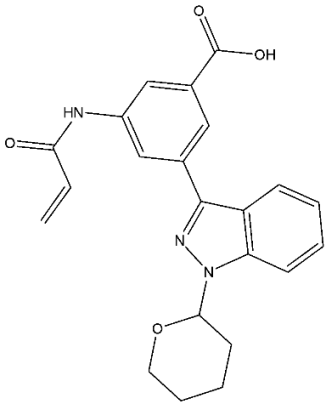
Methyl 3-acrylamido-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)benzoate (IB-1-21).



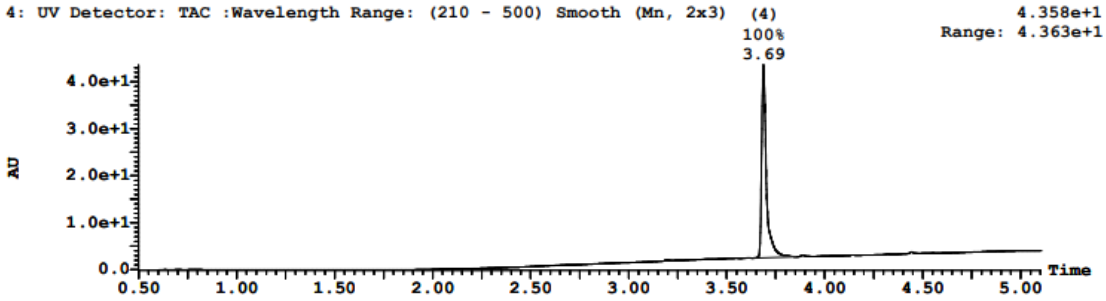

Sample 54 Vial 1:E,3 ID File IB-1-21_4-12 Date 12-Jul-2020 Time 16:58:00
4: UV Detector: TAC :Wavelength Range: (210 - 500) Smooth (Mn, 2x3)
(6)
100%
4.02
2.44e+2
Range: 2.444e+2
AU
1.5e+2
1.0e+2
5.0e+1
0.0
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Time
8.550
8.393
8.188
8.052
8.031
7.609
7.587
7.411
7.393
7.374
7.260
7.232
7.213
7.195
6.457
6.455
6.415
6.413
6.308
6.282
6.266
6.240
5.771
5.765
5.748
5.741
5.728
5.700
4.074
4.049
4.044
3.903
3.764
3.758
2.158
2.100
2.093
2.067
1.786
1.759
1.736
1.662
1.655
1.254
IB-1-21_4 sec combi f16_H 21.07.2020
Current Data Parameters
NAME IB-1-21_4 sec combi f16_H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200721
Time 14.32
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg
TD 16384
SOLVENT CDCl3
NS 34
DS 0
SWH 7211.539 Hz
FIDRES 0.440157 Hz
AQ 1.1359574 sec
RG 42.14
DW 69.333 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 400.3545018 MHz
NUC1 1H
P1 13.00 usec
PLW1 18.50000000 W
F2 - Processing parameters
SI 32768
SF 400.3525097 MHz
WDW EM
SSB 0
LB 0.50 Hz
GB 0
PC 1.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
0.91
2.06
1.12
1.06
1.09
1.08
1.12
1.08
2.25
1.15
3.24
1.17
1.14
2.51
2.39

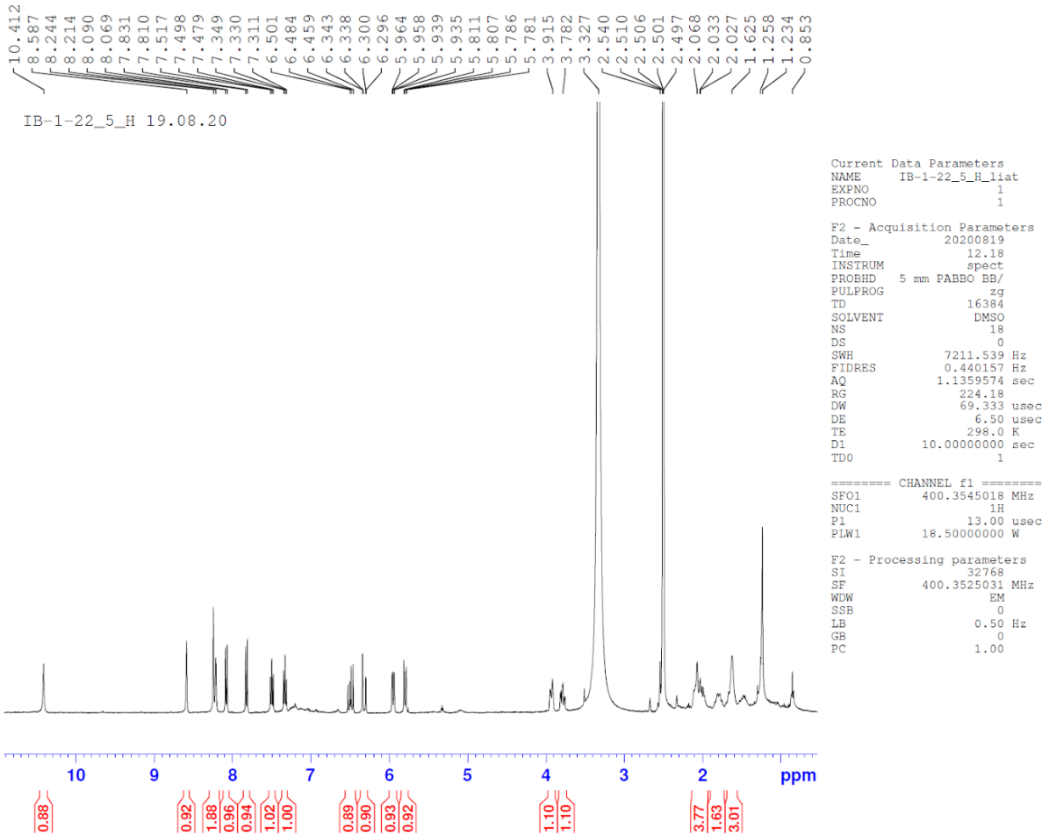


3-acrylamido-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)benzoic acid (IB-1-22).

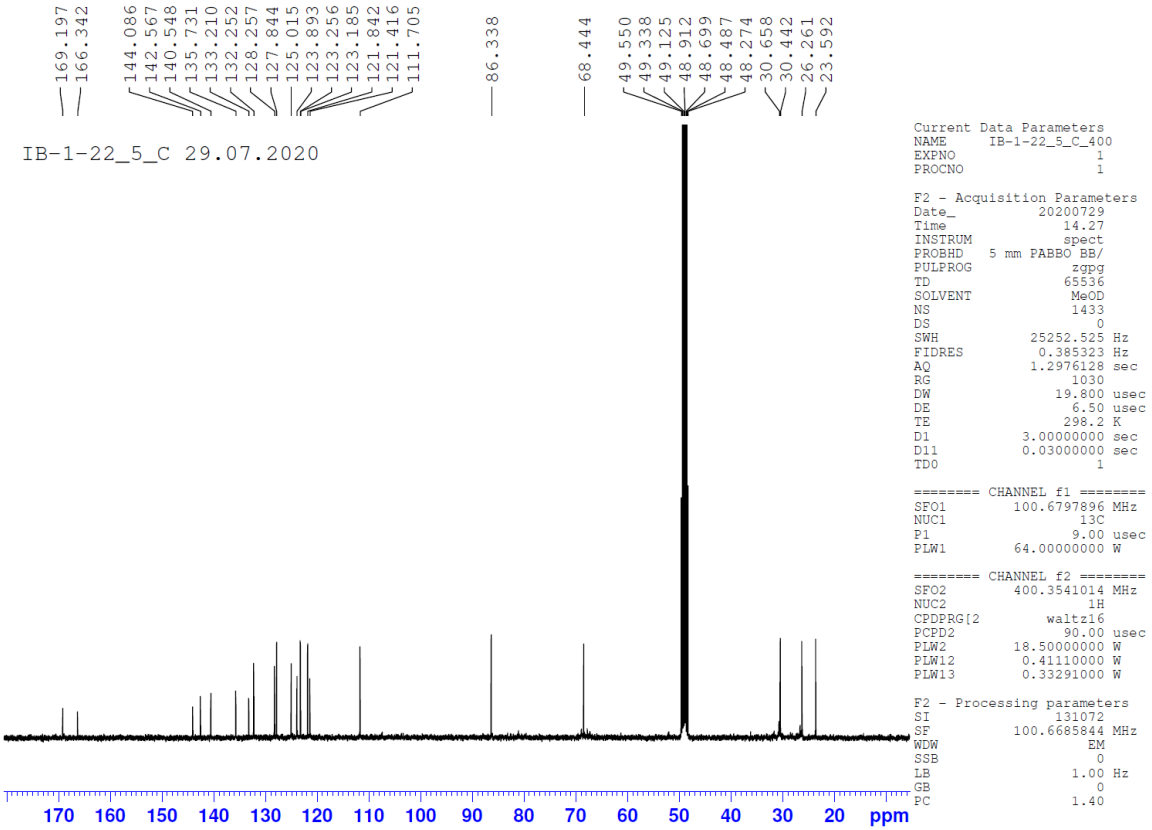


Sample 137 Vial 1:F,8 ID File IB-1-22_5-40 Date 05-Aug-2020 Time 15:46:17 Description after sec combi f20,22,23,24. after heating

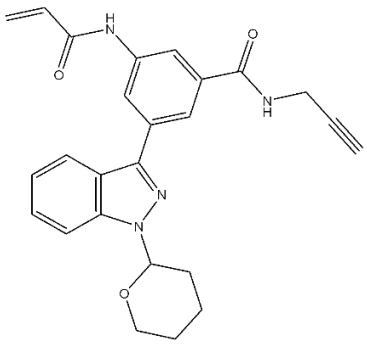


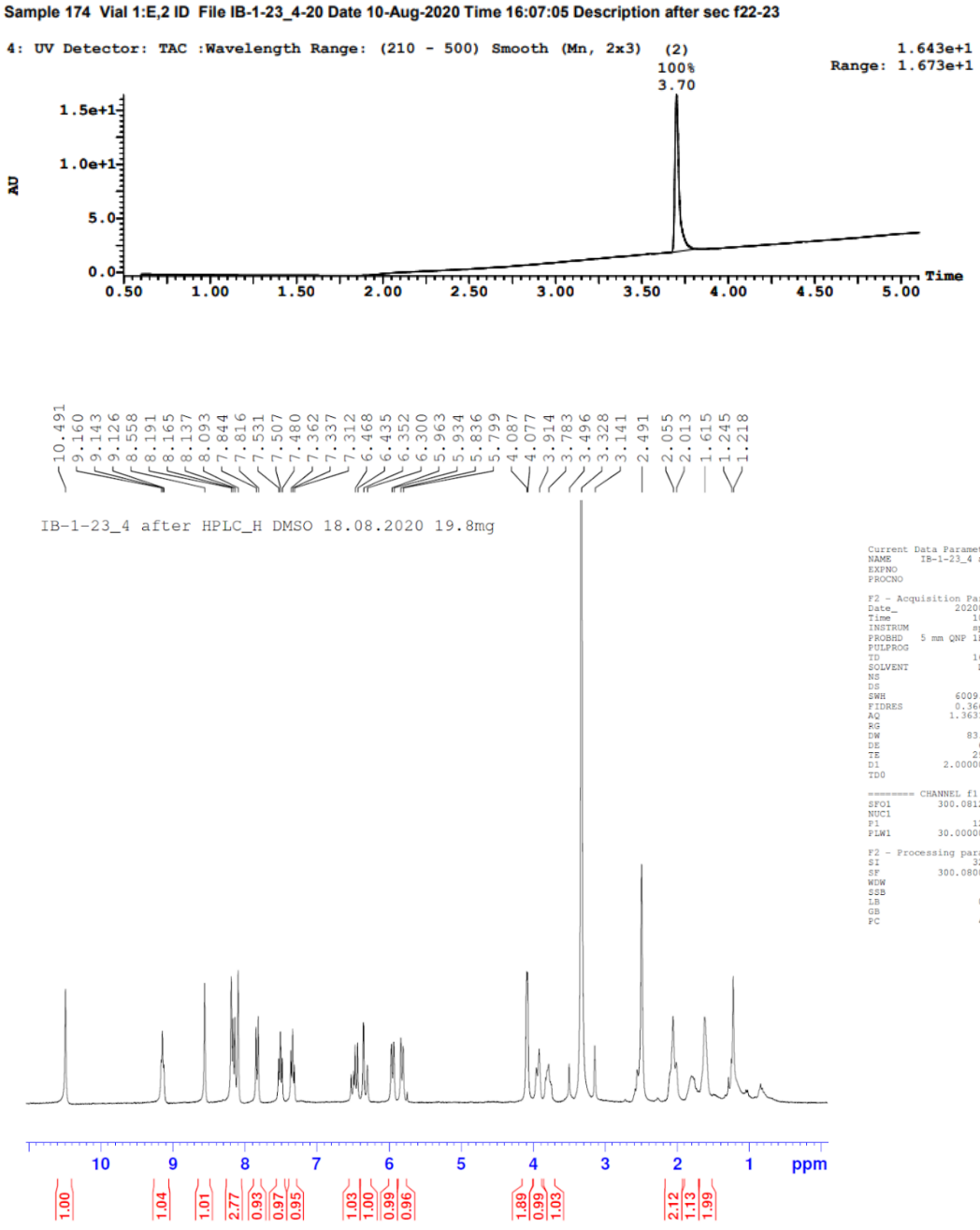


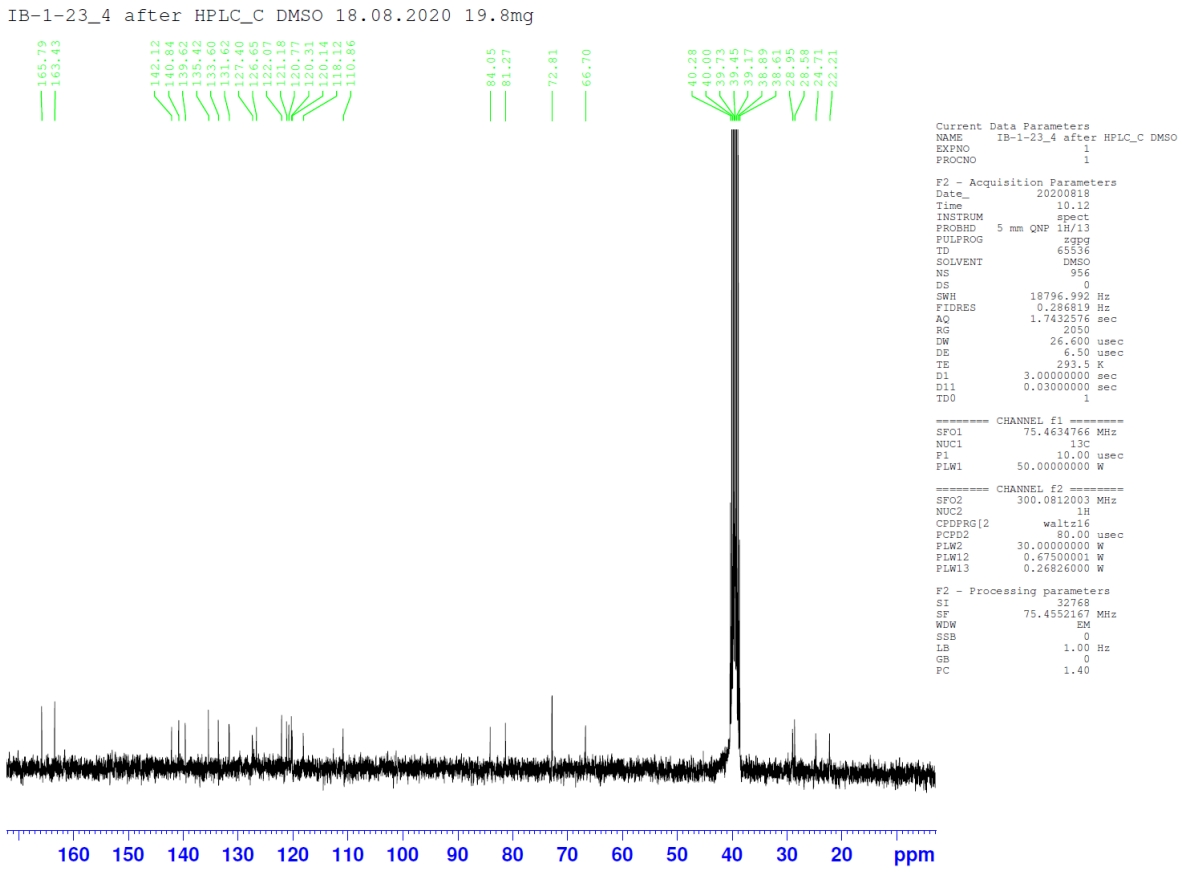

```
Current Data Parameters
NAME      IB-1-22_5_H_liat
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20200819
Time                 12.18
INSTRUM              spect
PROBHD    5 mm PABBO BB/
PULPROG                 zg
TD                   16384
SOLVENT               DMSO
NS                      18
DS                       0
SWH               7211.539 Hz
FIDRES            0.440157 Hz
AQ                1.1359574 sec
RG                   224.18
DW                  69.333 usec
DE                    6.50 usec
TE                  298.0 K
D1             10.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.3545018 MHz
NUC1                    1H
P1                   13.00 usec
PLW1          18.50000000 W

F2 - Processing parameters
SI                   32768
SF            400.3525031 MHz
WDW                     EM
SSB                      0
LB                    0.50 Hz
GB                       0
PC                    1.00
```



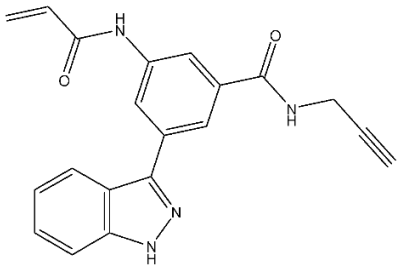
3-acrylamido-N-(prop-2-yn-1-yl)-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-3-yl)benzamide (IB-1-23).



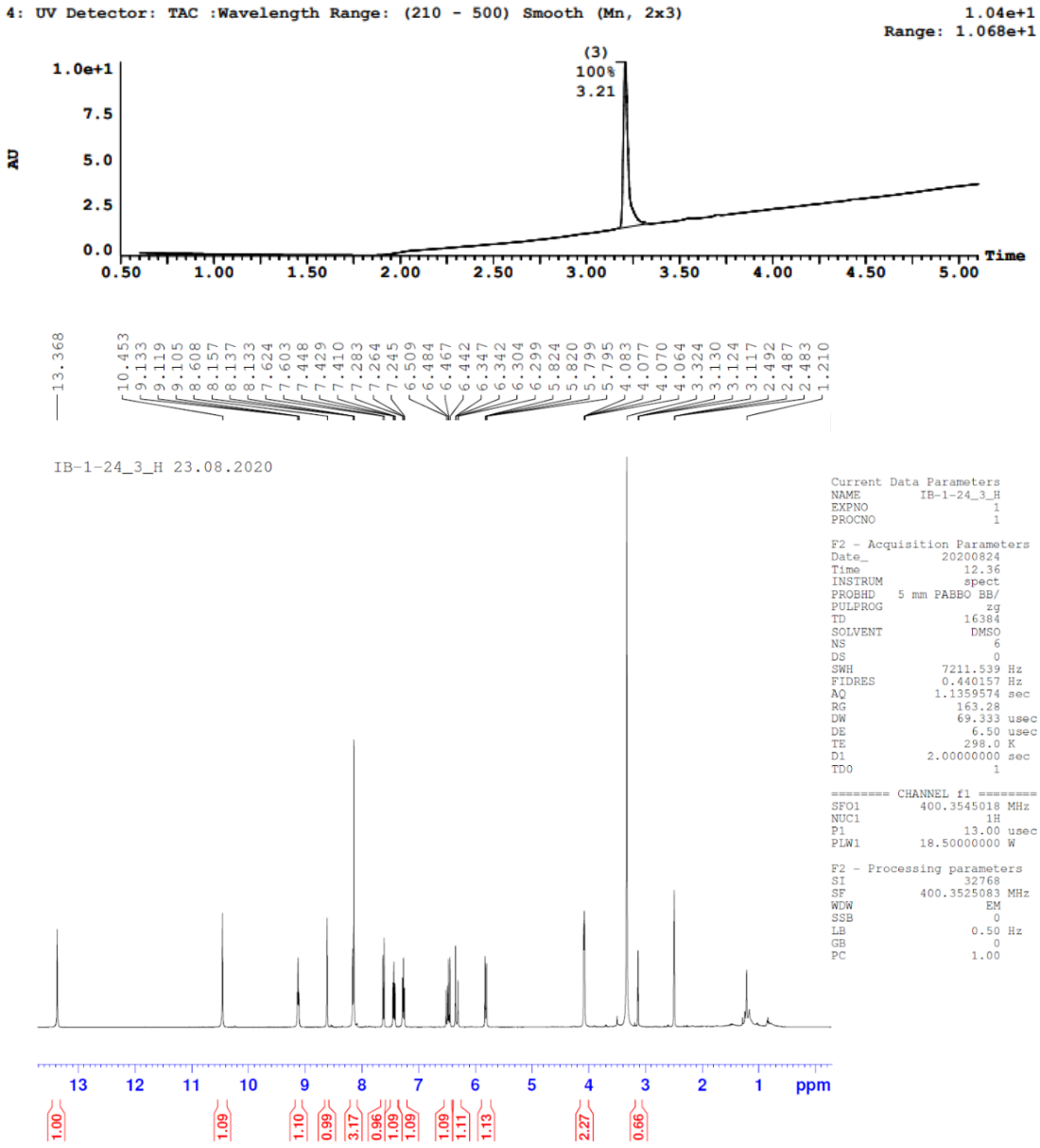

Sample 174 Vial 1:E,2 ID File IB-1-23_4-20 Date 10-Aug-2020 Time 16:07:05 Description after sec f22-23
4: UV Detector: TAC :Wavelength Range: (210 - 500) Smooth (Mn, 2x3) (2)
100%
3.70
1.643e+1
Range: 1.673e+1
AU
Time
10.491
9.160
9.143
9.126
8.558
8.191
8.165
8.137
8.093
7.844
7.816
7.531
7.507
7.480
7.362
7.337
7.312
6.468
6.435
6.352
6.300
5.963
5.934
5.836
5.799
4.087
4.077
3.914
3.783
3.496
3.328
3.141
2.491
2.055
2.013
1.615
1.245
1.218
IB-1-23_4 after HPLC_H DMSO 18.08.2020 19.8mg
Current Data Parameters
NAME IB-1-23_4 after HPLC_H DMSO
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200818
Time 10.07
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg
TD 16384
SOLVENT DMSO
NS 30
DS 0
SWH 6009.615 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 362
DW 83.200 usec
DE 6.50 usec
TE 293.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 300.0812603 MHz
NUC1 1H
P1 12.00 usec
PLW1 30.00000000 W
F2 - Processing parameters
SI 32768
SF 300.0800050 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 4.00
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.04
1.01
2.77
0.93
0.97
0.95
1.03
1.00
0.99
0.96
1.89
0.99
1.03
2.12
1.13
1.99

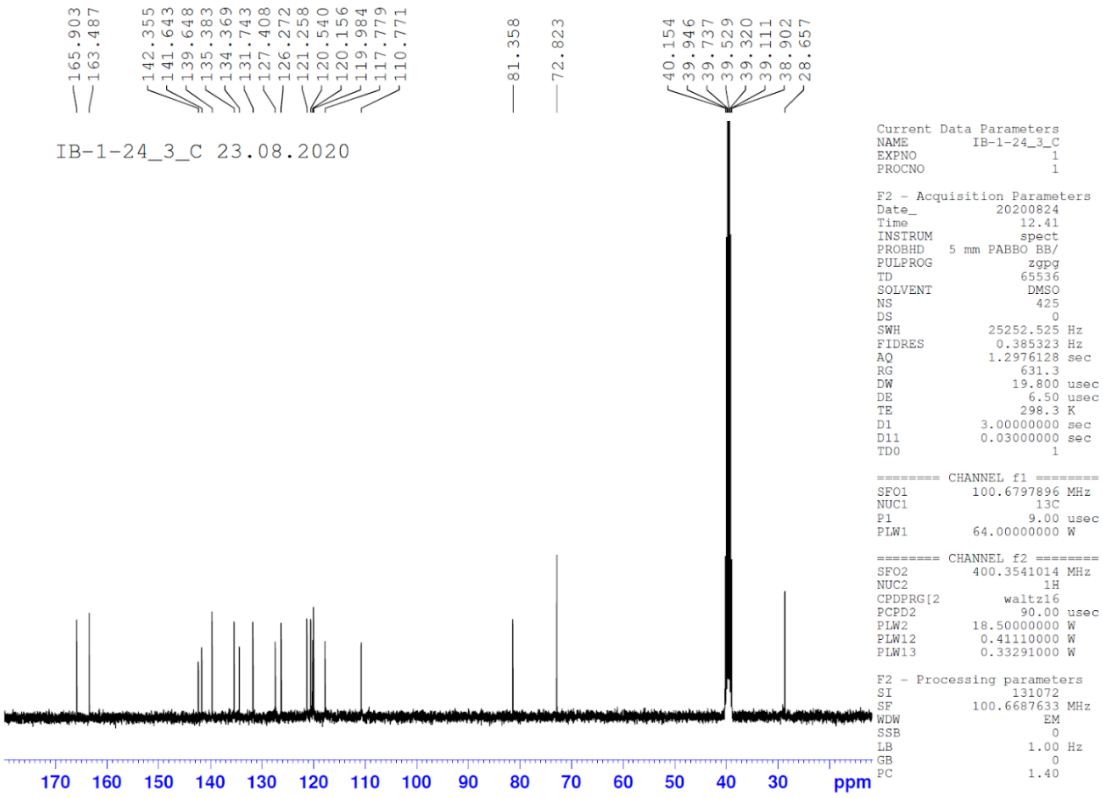


3-acrylamido-5-(1H-indazol-3-yl)-N-(prop-2-yn-1-yl)benzamide (IB-1-24).

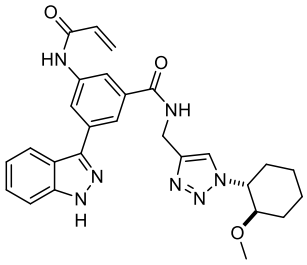


Sample 218 Vial 1:E,6 ID File IB-1-24_3-7 Date 17-Aug-2020 Time 17:04:14 Description after combi f11

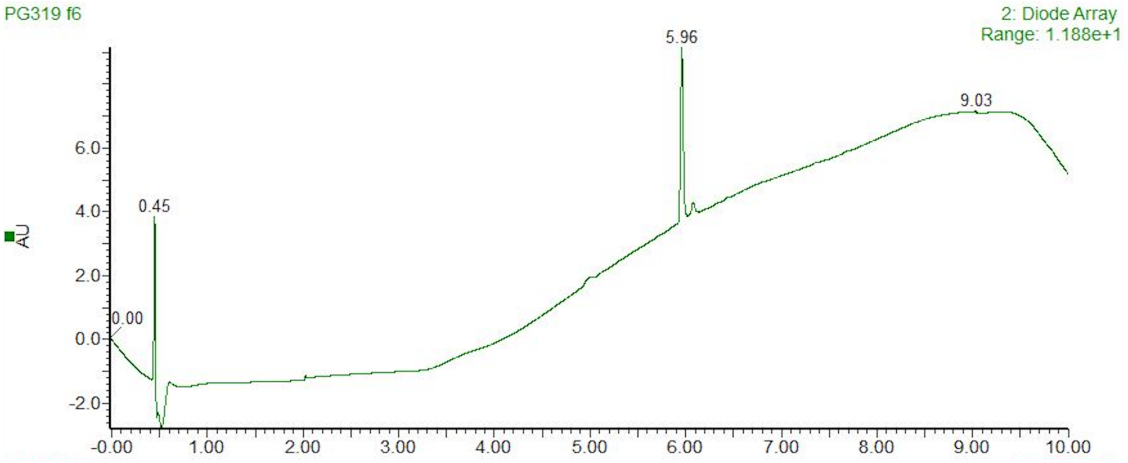


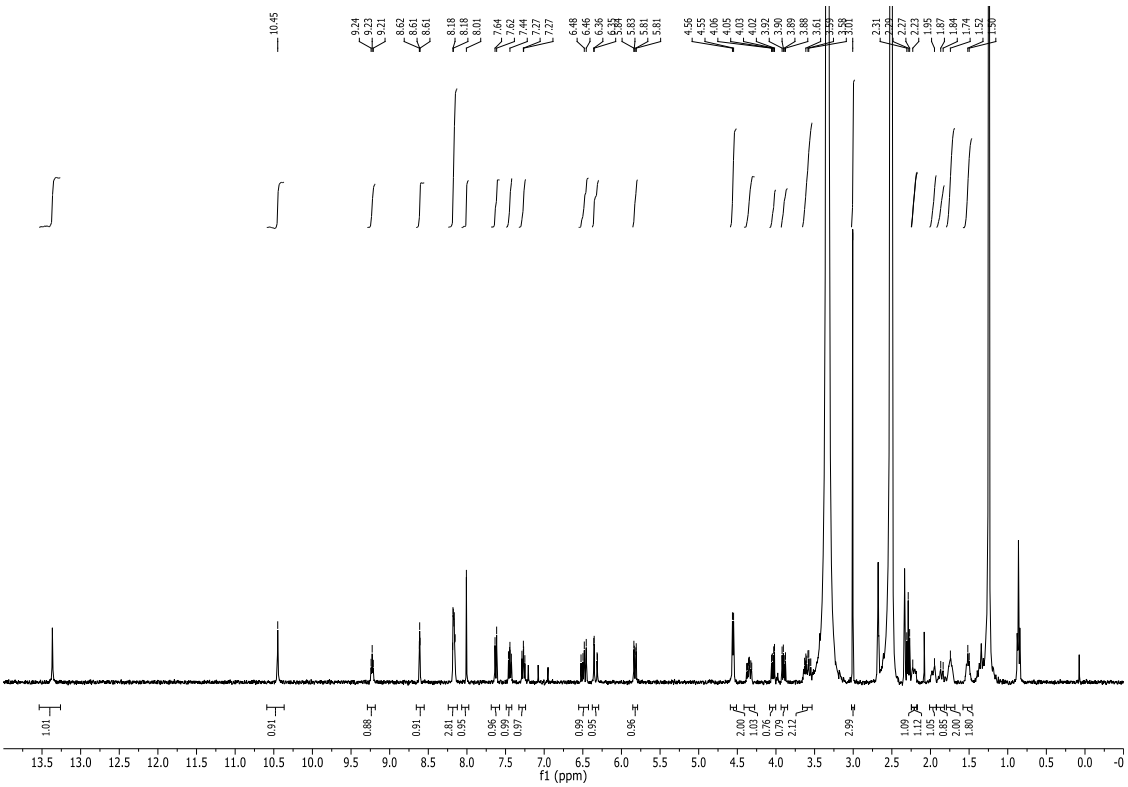


***Rac*-3-acrylamido-5-(1H-indazol-3-yl)-N-((1-((*trans*-)-2-methoxycyclohexyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (2a)**

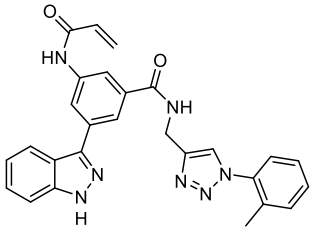


UPLC-MS chromatogram (averaged from 200 – 498 nm)

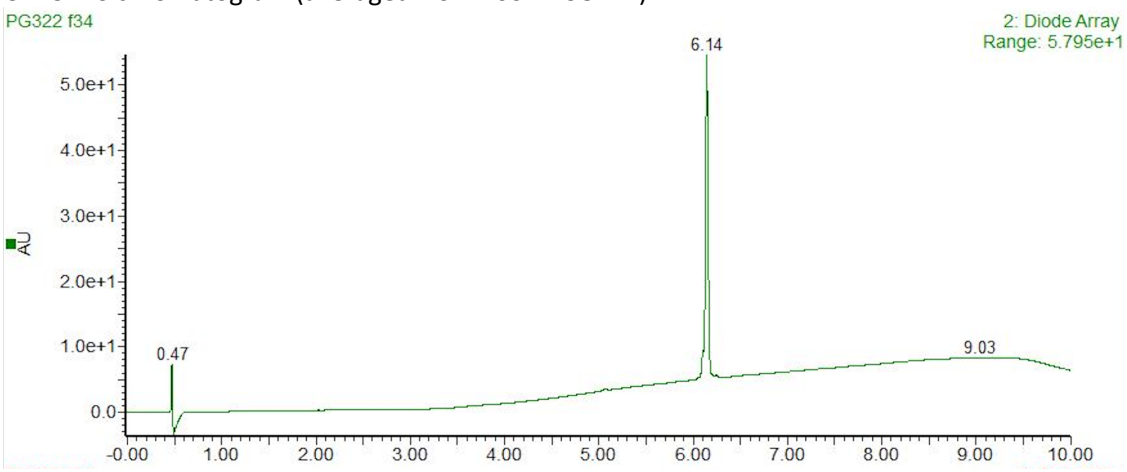


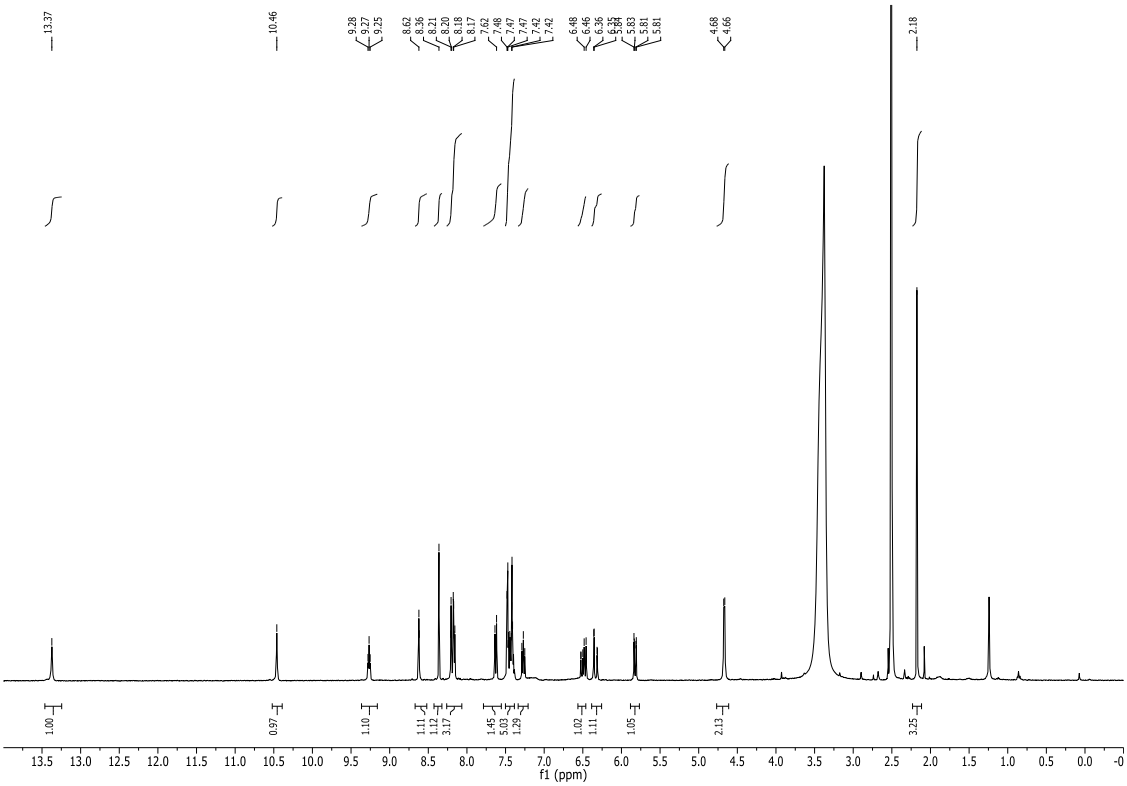


3-acrylamido-5-(1H-indazol-3-yl)-N-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (**2b**)

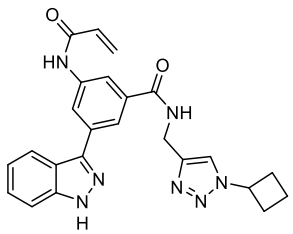


UPLC-MS chromatogram (averaged from 200 – 498 nm)

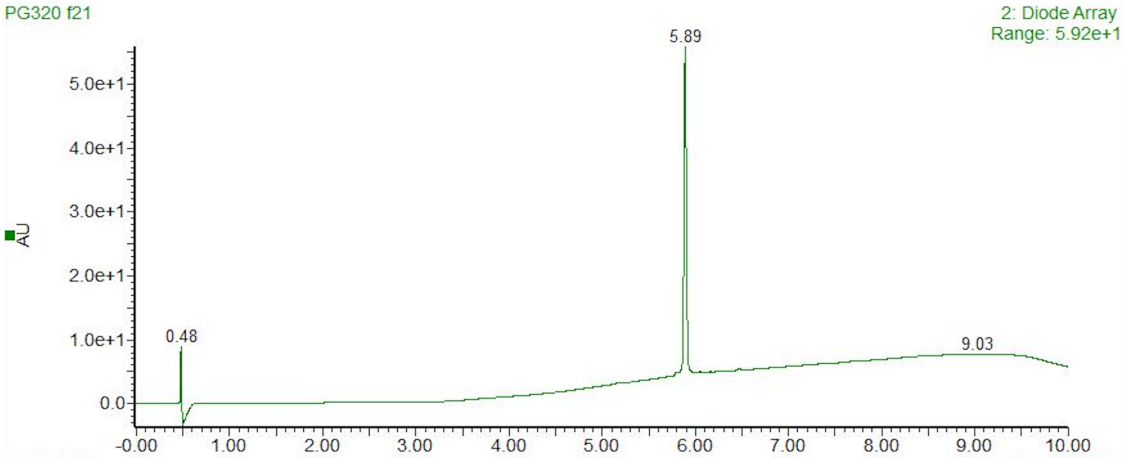


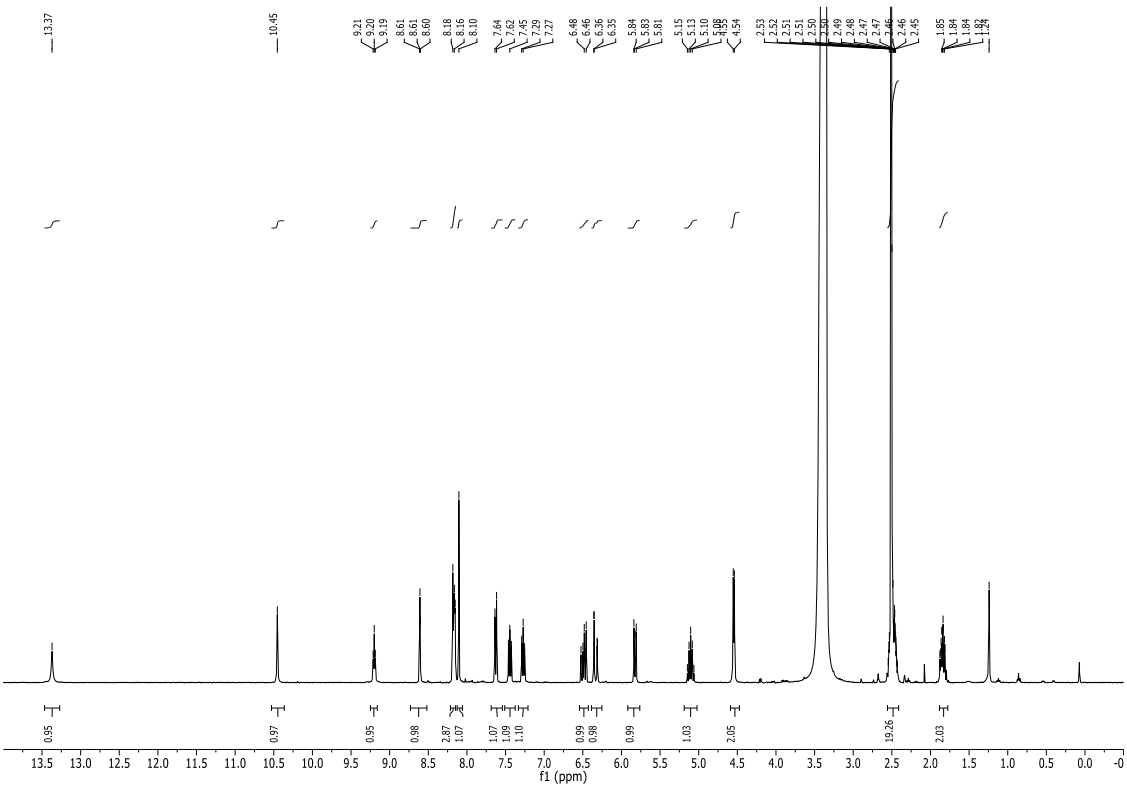


3-acrylamido-N-((1-cyclobutyl-1H-1,2,3-triazol-4-yl)methyl)-5-(1H-indazol-3-yl)benzamide (2c)

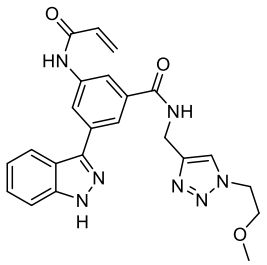


UPLC-MS chromatogram (averaged from 200 – 498 nm)

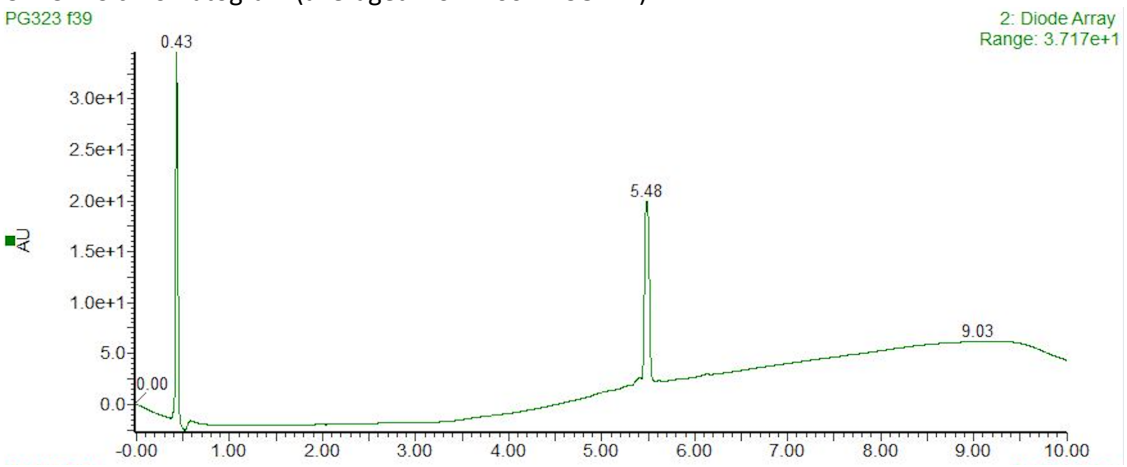


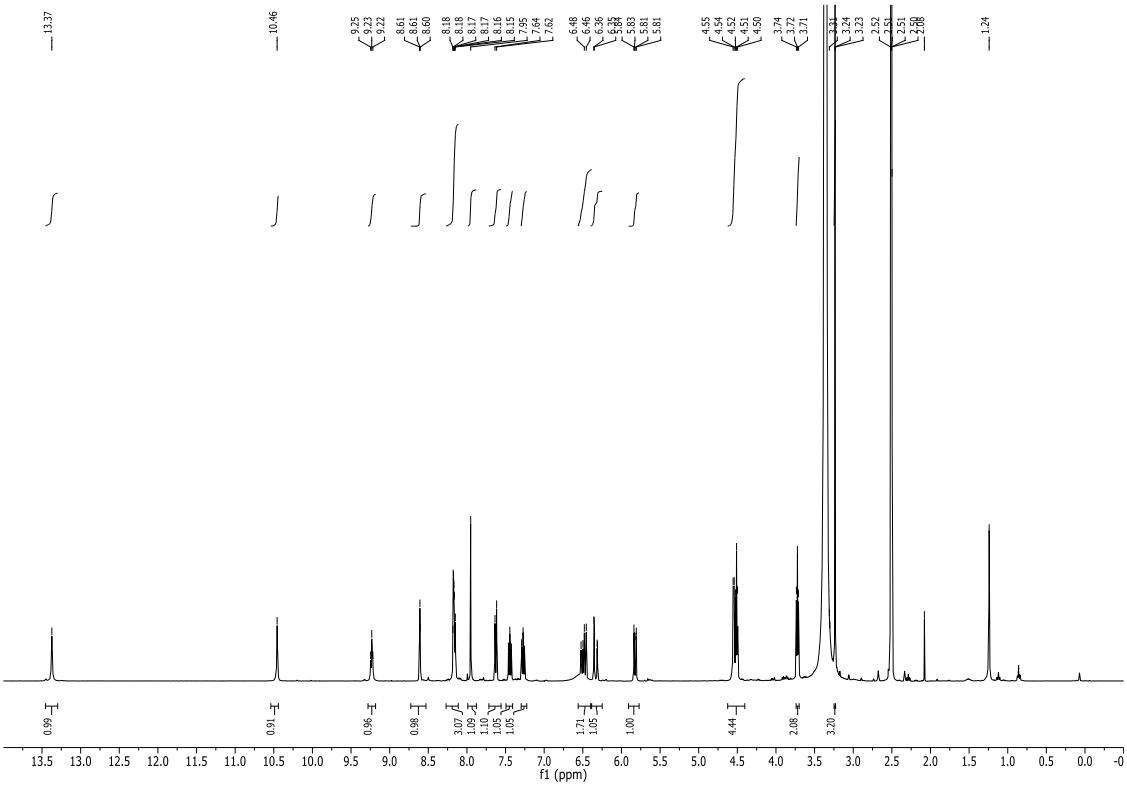


3-acrylamido-5-(1H-indazol-3-yl)-N-((1-(2-methoxyethyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (2d)

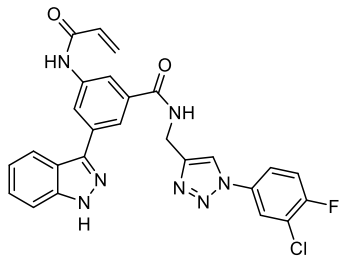


UPLC-MS chromatogram (averaged from 200 – 498 nm)





3-acrylamido-N-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1H-indazol-3-yl)benzamide (**2e**)



UPLC-MS chromatogram (averaged from 200 – 498 nm)