Supplementary Information (SI) for RSC Advances.
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Supporting information

Continuous-flow synthesis of 1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-c]pyridines towards key intermediates of clinical candidates JNJ-54175446 and zanvipixant (JNJ-55308942) with antidepressant activity

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Contents

General

Solvents and chemicals were purchased from commercial vendors and were used without any further purification. Cyclohexane (≥99.8%), ethyl-acetate (≥99.8%), toluene (≥99.7%) and methanol (≥99.8%) were obtained from Honeywell.

^{1}H and ^{13}C-DEPTQ NMR measurements were performed on a Bruker Avance III HDX 500 MHz NMR spectrometer equipped with a ^{1}H {^{13}C/^{15}N} 5 mm TCI CryoProbe, or a Bruker Avance III HDX 800 MHz NMR spectrometer equipped with a ^{1}H-^{19}F{^{13}C/^{15}N} 5 mm TCI CryoProbe (Bruker Corporation, Billerica, MA, USA). ^{1}H and ^{13}C chemical shifts are given on the delta scale as parts per million (ppm) with tetramethylsilane (TMS) as reference. d_6-Dimethyl sulfoxide (DMSO-d_6) was used as solvent. ^{1}H–^{1}H scalar spin-spin connectivity, direct ^{1}H–^{13}C connectivity, and long-range ^{1}H–^{13}C connectivity were established from two-dimensional (2D) COSY, HSQC and HMBC experiments, respectively. ^{1}H–^{1}H spatial proximities and relative configurations were determined by using the 2D NOESY or ROESY experiments. The enantiomeric counterparts of **(*R*)-5** and **(*R*)-6** were confirmed based on the equivalence of their ^{1}H, ^{13}C-DEPTQ and HSQC NMR spectra. NMR spectra were processed with Bruker TopSpin 3.5 pl 6 (Bruker Corporation, Billerica, MA, USA) and ACD/Spectrus Processor version 2017.1.3 (Advanced Chemistry Development, Inc., Toronto, ON, Canada).

Supercritical fluid chromatography: SFC–MS analysis was performed using a Shimadzu SFC–MS system (CBM-20A; CO_2 pump: LC30ADSF; solvent delivery pump: LC20ADXR; make-up pump: LC20AD; autosampler: SIL30AC; oven: CTO-20AC; LC–MS: LCMS-2020; software: LabSolutions 6.114; FCV column selection valve). Chromatographic separations were carried out on a CHIRALART Cellulose-SC column (250 × 4.6 mm, 5 μm; YMC America) using a mobile phase consisting of CO_2 (solvent A) and methanol containing 0.025% ammonia (solvent B). The gradient elution program was as follows: 0 min – 5% B; 8 min – 55% B; 13 min – 55% B; 13.1 min – 5% B; 16 min – 5% B. The flow rate was set to 3.0 mL/min, the column temperature was maintained at 40 °C, and the injection volume was 10 μL. The sample concentration was 1 mg/mL, dissolved in dichloromethane:methanol (1:1, v/v). UV detection was carried out at a wavelength of 230 nm. The mass spectrometer was operated in APCI mode. Drying gas nitrogen was used with a flow rate of 12 L/min, and the nebulizer gas flow was set to 1.5 L/min.

Chromatographic separation was performed on Nexera UC Prep, from Shimadzu Corporation (Kyoto, Japan). Nexera UC Prep was composed of a system controller (CBM40), a CO_2 pump (LC-40Psf), a co-solvent/modifier pump (LC-20AP), a column oven (CTO-40C), a PDA (SPD-M40) and LCMS-2050 detector were used in parallel mode. An autosampler (SIL-40C) and a BPR (SFC-40P) were used. For fraction

collection, a make up pump (LC-20AR), two switching valves (FCV-20AH2) and a fraction collector (FRC-40) were also used. All modules were provided by Shimadzu Corporation, Kyoto, Japan. All chromatograms were recorded on LabSolutions version 5.128 (Shimadzu Corporation, Kyoto, Japan). The stationary phase YMC ChiralArt Cellulose-SC 250 × 10 mm, 5 μm was used. The separation was performed in isocratic mode with 25/30% (according to the different impurity profiles of samples) modifier (0,025% NH_4OH in MeOH) with a flow rate of 15 mL/min, an oven temperature of 40 °C, and a back-pressure of 16 MPa.

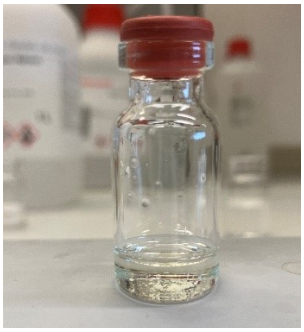
Safety note

Organic azides are potentially explosive compounds and should be handled with care even in small quantities, avoiding mechanical impacts and heating of pure compounds. Thermal stability evaluation of the azide (**4**) has been evaluated in the Janssen article (ref. 1). Suitable personal protective equipment and blast shields are always required. Organic and inorganic azide containing waste streams and discarded aqueous phases were treated by 10% aqueous solution of sodium nitrite to decompose the remaining azide, prior to their disposal. Except for micro-scale batch experiments (0.1 mmol), all experiments were performed exclusively in the flow system, up to 1.25 mmol (200-300 mg) scale. No incidents of any kind occurred.

Solubility studies for the cycloaddition side-product (9)

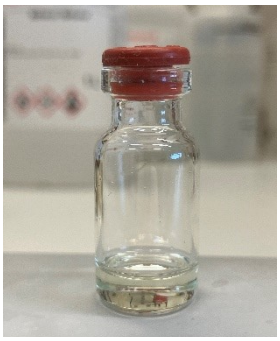
Solubility tests were conducted at room temperature utilizing 10 mg of the side-product (**9**) and 500 µl of solvent. The visual appearance of the Toluene-MeOH mixtures is shown on **Figure S1**.

Toluene



Toluene-MeOH 5:1

Toluene-MeOH 3:1



Toluene-MeOH 1:1

Figure S1. Solubility studies for the cycloaddition side-product (9)

General procedure for the continuous-flow cycloaddition step to form (S)-7 and (S)-8

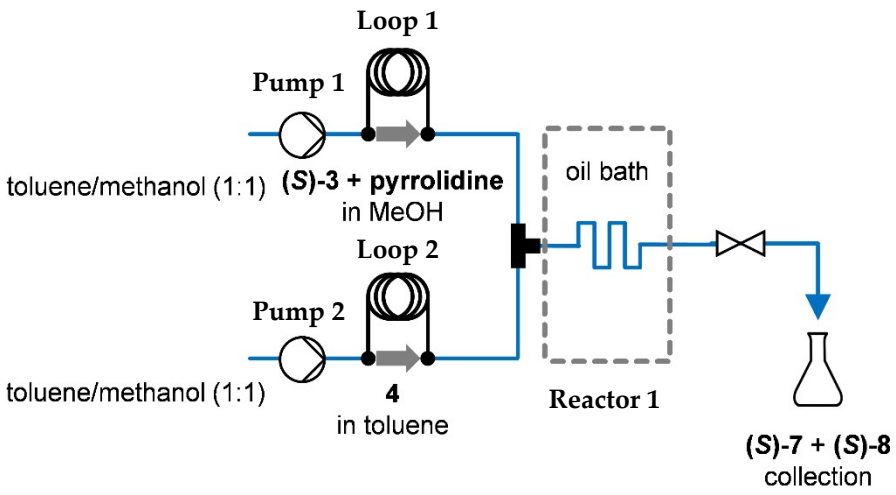


Figure S2. Schematic representation of the continuous-flow cycloaddition

A system was constructed (**Figure S2**) consisting of an Asia Syringe Pump (Syrris, Royston, United Kingdom, Pump 1) having two separate flow channels, two of which were connected to two Asia Reagent Injectors (Syrris, Royston, United Kingdom, 1 mL (loop 1), 1 mL (loop 2) respectively). The two channels of the injectors were connected to a T-mixer (PEEK T-mixer, 0.5 mm i.d, IDEX Health & Science LLC), which was followed by a reactor (PTFE tube 0.8 mm i.d., 1.5 mL net volume, Reactor 1). The output of Reactor 1 was connected to a back pressure regulator (250 psi), after which the reaction mixture was collected. The system was washed with methanol, followed by the 1:1 mixture of toluene:methanol. The reactor was heated using a thermostated oil bath. At lower than ambient temperatures, Reactor 1 was replaced with a Microreactor chip (Syrris, Royston, United Kingdom, 1 mL) and the temperature was set using an Asia Chip Climate Controller (Syrris, Royston, United Kingdom). Washing with toluene-methanol solvent mixture was upheld until steady temperature was reached. Other parts were kept at ambient temperature.

The 1:1 solvent mixture of toluene and methanol was transferred on both channels of Pump 1 with a flow rate of 150 μl/min. Loop 1 was filled with the solution of *tert*-butyl(2*S*)-2-methyl-4-oxopiperidine-1-carboxylate (**(*S*)-3**, 53 mg, 0.25 mmol of which 0.05 mmol was injected) and pyrrolidine (36 mg, 0.5 mmol) in methanol (1 mL), while loop 2 was filled with the solution of **4** (35 mg, 0.25 mmol) in toluene (1 mL). The reaction was controlled using the Asia Manager computer program, which was responsible for switching the injector valves at appropriate times. The dead volume was discarded to the waste, until the reaction mixture appeared at the output. Then, the reaction mixture was collected in a vial for analysis. After collection, the program was shut-down and the system was washed with methanol. Optimization data for the cycloaddition reaction of **(*S*)-3** are shown in **Table S1** and **Table S2.**

Calibration measurements using 2-methoxynaphthalene as internal standard

An internal standard was required to track the consumption of **(*S*)-3** and the formation of ***(S)-7*** and ***(S)-8***, as **(*S*)-3** is detectable by GC-MS, and ***(S)-7*** and ***(S)-8*** by LC-MS, with neither method being suitable for detecting the others.

Calibration for the consumption of ***(S)-3***

For the calibration measurements 10 mL 0.01 mol/L stock solutions of **(*S*)-3** (21.3 mg 0.1 mmol) and 2-methoxynaphthalene (15.82 mg, 0.1 mmol) were prepared in two volumetric flasks. As the purpose of the calibration was to determine the consumption of **(*S*)-3**, the range of the calibration was between 1% and 10% ratio of **(*S*)-3** compared to 2-methoxynapthalene (**Figure S3**).

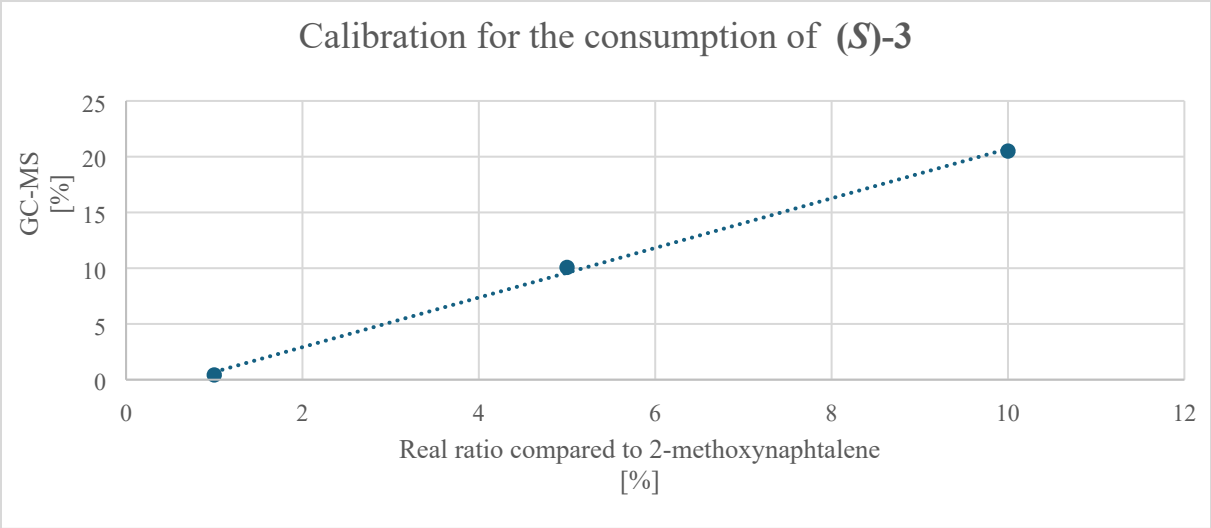


Figure S3. Calibration measurements for the consumption of (*S*)-3

Calibration for the formation of ***(S)-7*** *and* ***(S)-8***

For the calibration measurements 10 mL 0.01 mol/L stock solutions of **(*S*)-7** (40.5 mg 0.1 mmol) and 2-methoxynaphthalene (15.82 mg, 0.1 mmol) were prepared in two volumetric flasks. As the purpose of the calibration was to determine the formation of **(*S*)-7**, the range of the calibration was between 20% and 70% ratio of **(*S*)-7** compared to 2-methoxynapthalene (**Figure S4**).

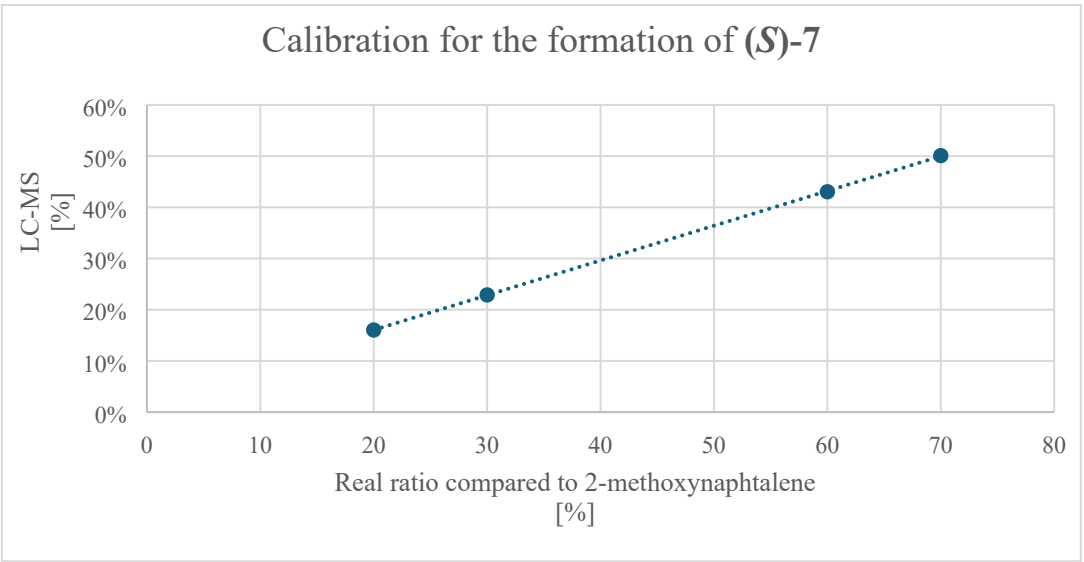


Figure S4. Calibration measurements for the formation of (*S*)-7

Table S1. Optimization of reaction parameters for the cycloaddition reaction from (*S*)-3

Entry	Net flow rate [µl/min]	Residence time [min]	Pyrrolidine equiv.	2-azido-5-fluoropyrimidine equiv.	Temperature [°C]	Ratio of (*S*)-7[a] [%]	Ratio of (*S*)-8[a] [%]
1	300	5	2	1	-15	38	62
2	300	5	2	1	0	39	61
3	300	5	2	1	20	39	61
4	300	5	2	1	45	40	60
5	**300**	**5**	**2**	**1**	**60**	**37**	**63**
6	300	5	2	1	70	41	59
7	300	5	2	1	80	44	56
8	300	5	2	1	90	46	54
9	300	5	2	1	100	51	49
10	300	5	2	1	110	59	41
11	300	5	2	1	120	76	24
12	**300**	**5**	**2**	**1**	**130**	**83**	**17**
13	300	5	2	1	140	82	18
14	300	5	2	1	150	72	28

[a] Determined by LC-MS assay (area%) @240 nm

Table S2. Effects of the molar ratio of 2-azido-5-fluoropyrimidine (4) on the consumption of (*S*)-3 and the formation of (*S*)-7 and (*S*)-8

Entry[a]	Molar ratio of 4	Conversion of (*S*)-3[b] [%]	Crude assay of (*S*)-7 + (*S*)-8[c] [%]	Relative HPLC area of (*S*)-7 : (*S*)-8 [%]	Observations
1	1 eq.	87	49	62:38	clear solution
2	2 eq.	97	68	61:39	clear solution
3	3 eq.	98	72	61:39	precipitation
4	4 eq.	92	53	61:39	precipitation

[a] Experimental conditions: 0.25 mol/L substrate concentration, 60°C, 5 min, in batch.[b] Determined by GCMS, based on TIC (Total Ion Chromatogram), after calibration. [c] Determined by LC-MS assay (area%) at 240 nm, after calibration.

Continuous-flow synthesis of ***(S)-7***

The title compound was prepared according to the general procedure, after replacement of both Loop 1 and Loop 2 with 5 mL loops and performing the reaction on a 1.25 mmol scale. The first loop was filled with the solution of **(*S*)-3** (267 mg, 1.25 mmol) in methanol (5 mL) and the second loop was filled with the solution of **4** (348 mg, 2.5 mmol) in toluene (5 mL). The temperature of Reactor 1 was set to 130°C. The reaction mixture was collected in a flask, and upon completion, the reaction mixture

was diluted with water, the phases were separated and the aqueous phase was extracted by EtOAc (3x15 mL), and the combined organic phase was washed with brine. The aqueous phase and azide containing waste streams were treated by 10% aqueous solution of sodium nitrite to decompose the remaining azide, prior to their disposal. The organic phase was dried on Na_2SO_4, which was filtered, and the solvent was evaporated using a water bath thermostated to 40°C. The product was isolated and purified by column chromatography on silica gel (cyclohexane-ethyl-acetate 1:1) to give the title compound (**(*S*)-7**) as a yellow oil in 46% yield.

Continuous-flow synthesis of ***(S)-8***

The title compound was prepared following the procedure described for the synthesis of **(*S*)-7** with the exception that the temperature of Reactor 1 was set to 60°C. The work-up was carried out as previously described. The product was isolated and purified by column chromatography on silica gel (cyclohexane-ethyl-acetate 1:1) to give the title compound (**(*S*)-8**) as a yellow oil in 50% yield.

General procedure for the continuous-flow oxidation step to form (S)-5 and (S)-6

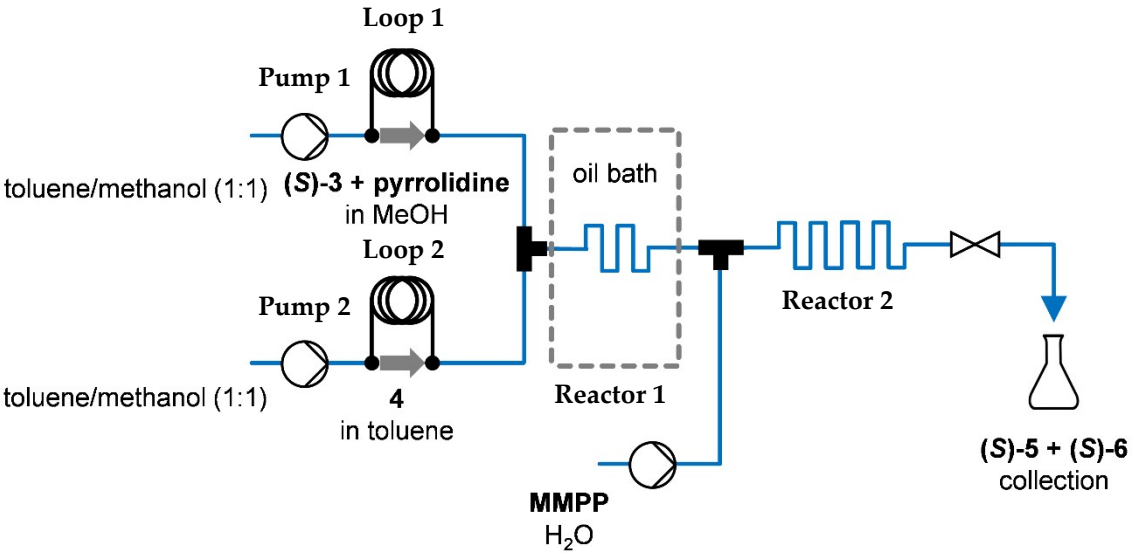


Figure S5. Calibration measurements for the consumption of (*S*)-3

The output of the reactor from the cycloaddition step and an Asia Syringe Pump (Syrris, Royston, United Kingdom, Pump 2) were connected to a mixer, followed by a reactor (PTFE tubing, 0.8 mm i.d., 1.6 mm o.d., Reactor 2). The output of Reactor 2 was connected to a back pressure regulator (250 psi), after which the reaction mixture was collected (**Figure S5**). The reaction parameters of the first cycloaddition step were not modified, as the previously described parameters were applied throughout.

The solution of MMPP and $NaHCO_3$ (both 0.12 mol/L in water) was streamed on Pump 2 with the flow rates and size of Reactor 2 were investigated. The reaction was

controlled using the Asia Manager computer program, which was responsible for switching the injector valves at appropriate times. The dead volume was discarded to the waste, until the reaction mixture appeared at the output. Then, the reaction mixture was collected in a vial for analysis. After collection, the program was shut-down and the system was washed with methanol. Optimization data for the oxidation are shown in **Table S3**.

Table S3. Optimization of the oxidation reaction to form (*S*)-5 and (*S*)-*6*

Entry	Reactor size [mL]	MMPP equiv.	Type of mixer	Conversion [a] [%]
1	1	1	T	30
2	1	2	T	54
3	1	3	T	59
4	1	4	T	58
5	4	1	T	52
6	4	2	T	86
7	4	3	T	91
8	4	4	T	89
9	4	1	arrowhead	55
10	4	2	arrowhead	81
11	4	3	arrowhead	91
12	**4**	**4**	arrowhead	**100**
13	10	1	arrowhead	74
14	10	2	arrowhead	71
15	10	3	arrowhead	84
16	10	4	arrowhead	82

Reaction conditions: 300 μl/min flow rate from the first module, room temperature. [a]Determined by LC-MS, based on product area/(product area + **(*S*)-8** + **(*S*)-9**)

General procedure for the continuous-flow synthesis of 1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-c]pyridines

A system was constructed consisting of two Asia Syringe Pumps (Syrris, Royston, United Kingdom, Pump 1) both having two separate flow channels, two of which were connected to two Asia Reagent Injectors (Syrris, Royston, United Kingdom, 5 mL (loop 1), 5 mL (loop 2) respectively), the third channel (Pump 2) was used directly. The two channels of the injectors were connected to a T-mixer (PEEK T-mixer, 0.5 mm i.d, IDEX Health & Science LLC), which was followed by a reactor (PTFE tube 0.8 mm i.d., 1.5 mL net volume, Reactor 1). The output of the reactor and Pump 2 were connected to a arrowhead mixer (PEEK Static mixing Tee, 0.5 mm i.d, IDEX Health & Science LLC), followed by a second reactor (PTFE tubing, 0.8 mm i.d., 1.6 mm o.d., 4 mL, Reactor 2). The output of Reactor 2 was connected to a back pressure regulator (250 psi), after which the reaction mixture was collected. The system was washed with methanol,

followed by the 1:1 mixture of toluene:methanol. The reactor was heated to the required temperature (depending on the substrate) using a thermostated oil bath. Washing with toluene-methanol solvent mixture was upheld until steady temperature was reached. Other parts were kept at ambient temperature.

The 1:1 solvent mixture of toluene and methanol was transferred on both channels of Pump 1 with a flow rate of 150 µl/min. Loop 1 was filled with the solution of *tert*-butyl-2-methyl-4-oxopiperidine-1-carboxylate (267 mg, 1.25 mmol) and pyrrolidine (178 mg, 2.5 mmol) in methanol (5 mL), while loop 2 was filled with the solution of **4** (348 mg, 2.5 mmol) in toluene (5 mL). The flow rate of Pump 2 was set at 1.20 mL/min, and the solution of MMPP and $NaHCO_3$ (both 0.12 mol/L in water) was streamed on it. The reaction was controlled using the Asia Manager computer program, which was responsible for switching the injector valves at appropriate times. The dead volume was discarded to the waste, until the reaction mixture appeared at the output. Then, the reaction mixture was collected in a stirred flask. After collection, the program was shut-down and the system was washed with methanol. The reaction mixture was diluted with water, the phases were separated and the aqueous phase was extracted by EtOAc (3x15 mL), and the combined organic phase was washed with brine. The aqueous phase and azide containing waste streams were treated by 10% aqueous solution of sodium nitrite to decompose the remaining azide, prior to their disposal. The organic phase was dried on Na_2SO_4, which was filtered, and the solvent was evaporated using a water bath thermostated to 40°C. The crude product was purified by column chromatography on silica gel. Photograph of the flow system is shown in **Figure S6**, description of fluidic parts is detailed in **Table S4**.

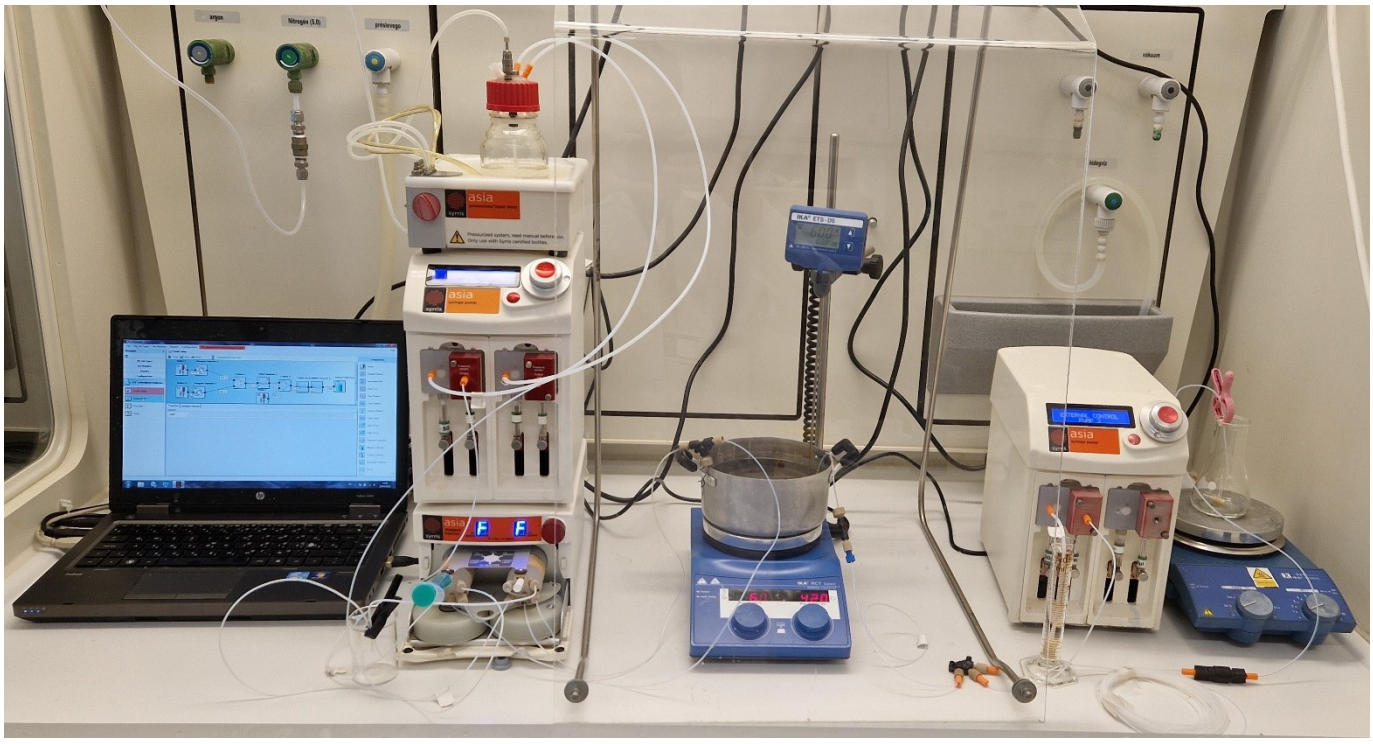
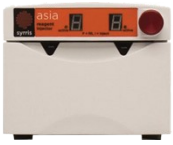


Figure S6. Photographic representation of the continuous-flow system

Table S4. Parts and consumables used for the construction of the flow system. Source of the images: refs. 2–4

Manufacturer:	Syrris Ltd. (Royston, UK)	
Description:	**Asia Syringe Pump** With Asia Pressurized Input Store	
Product No.	2200292	
Manufacturer:	Syrris Ltd. (Royston, UK)	
Description:	**Asia Reagent Injector** with 5mL and 2mL sample loops	
Product No.	2200520	
Source:	IDEX Health & Science LLC. (Rohnert Park, CA, USA)	
Description:	**PEEK Static mixing Tee,** 0.5 mm i.d.	
Part No.	U-466	
Source:	IDEX Health & Science LLC. (Rohnert Park, CA, USA)	
Description:	**PEEK T-mixer,** 0.5 mm i.d.	
Part No.	P-727	
Source:	Supelco Inc.	
Description:	**PTFE tubing**, 1/16 in. o.d. x 0.8 mm i.d.	
Product No.	58696-U	
Manufacturer:	Syrris Ltd.	
Description:	**Orange end fitting** with flangeless ferrules, for 1.6 mm o.d. tubing, 1/4″-28 UNF thread	
Product No.	2200618	
Source:	Supelco Inc.	
Description:	**PEEK one-piece fitting** for 1/16 in. o.d. tubing, 10-32 thread	
Product No.	55067-U	
Source:	Supelco Inc.	
Description:	**PEEK Fingertight Union** bore 0.020 in., 10-32 thread	
Product No.	57659	
Source:	IDEX Health & Science LLC.	
Description:	**PEEK Back Pressure Regulator** for 1/16 in o.d. tubing	
Product No.	P-788	

Supercritical fluid chromatography (SFC) purification of regioisomers

To purify the regioisomers SFC chromatography was employed. A method was developed to separate the regioisomers of a given enantiomer (**Figure S7**), as we used chiral starting materials (**(*S*)-3** and **(*R*)-3**). Furthermore, the method could also serve as confirmation that no racemization occurred during the process (since we used chiral stationary phase).

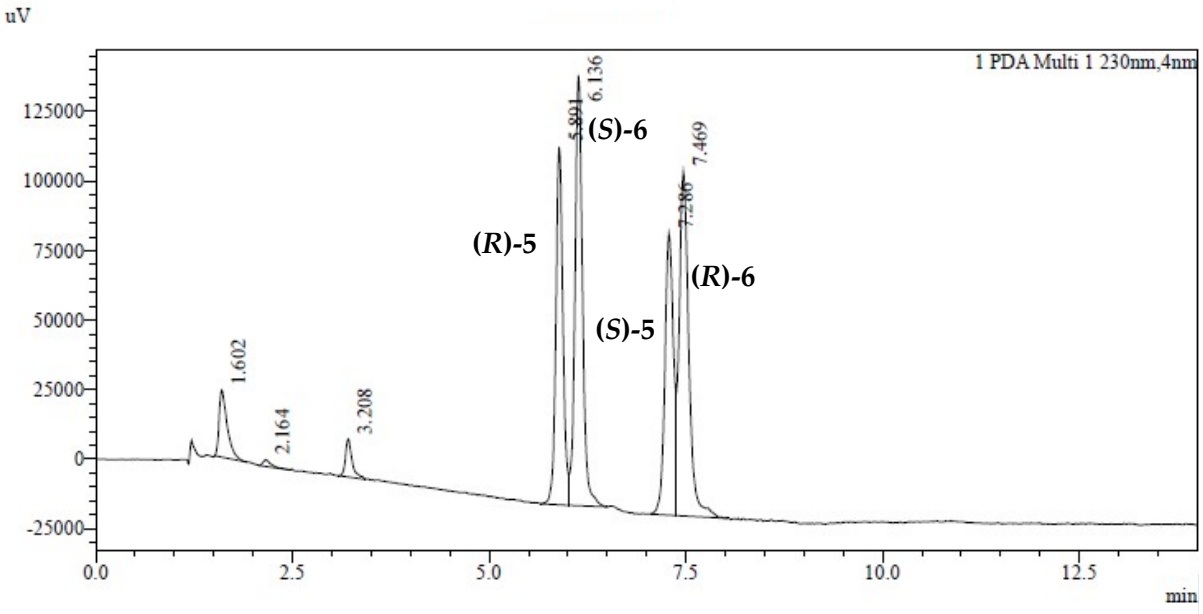


Figure S7. SFC chromatogram of the mixture of isomers (*R*)-5, (*S*)-5, (*R*)-6 and (*S*)-6

*Continuous-flow synthesis of **(S)-5***

The title compound was prepared according to the general procedure after filling Loop 1 with the solution of **(*S*)-3** and setting the temperature of Reactor 1 to 130°C. The product was isolated and purified by column chromatography on silica gel (cyclohexane-ethyl-acetate 1:1) to give the mixture of regioisomers as a white solid (45% yield, 91:9 **(*S*)-5:(*S*)-6** ratio of the regioisomers, **Figure S8**). An analytical sample was purified using preparative SFC chromatography (**Figure S9**) to obtain isomerically pure **(*S*)-5**. The major (**(*S*)-5**) and minor (**(*S*)-6**) regioisomers were subjected to NMR spectroscopy for analysis.

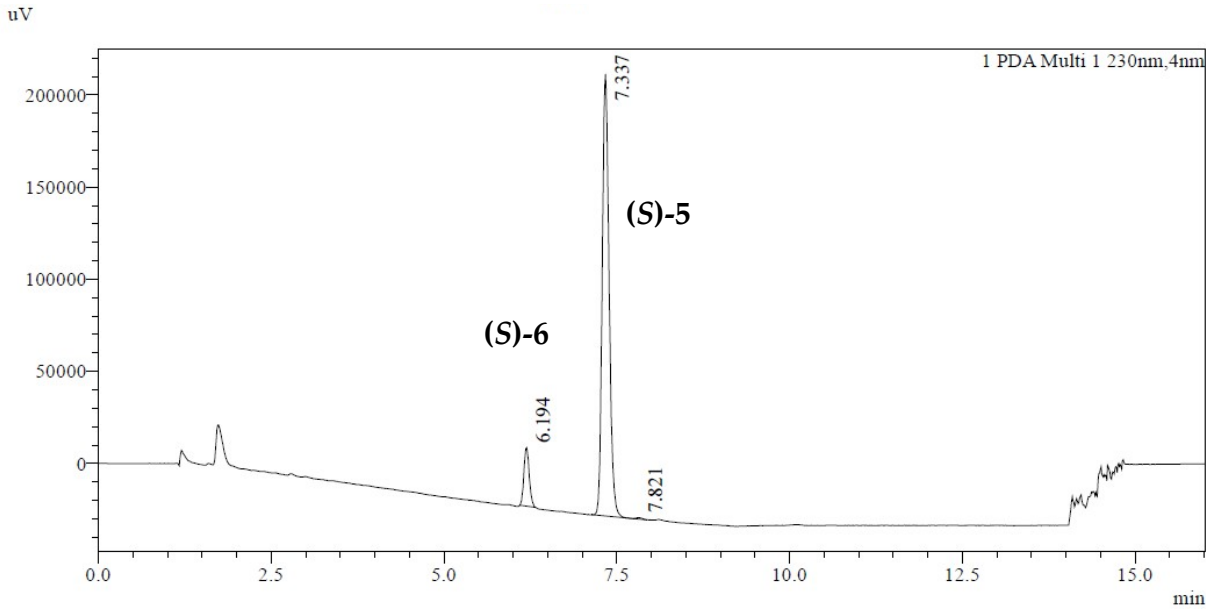


Figure S8. SFC chromatogram of the isolated mixture of regioisomers (*S*)-5 and (*S*)-6

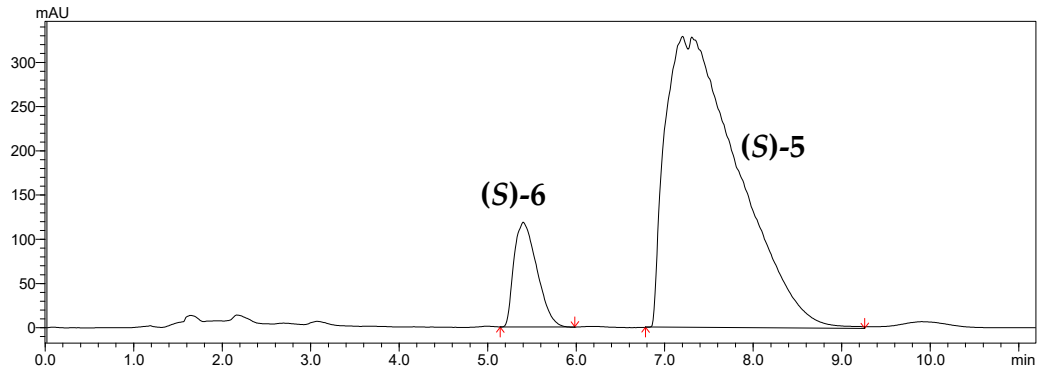


Figure S9. Preparative SFC separation of regioisomers (*S*)-5 and (*S*)-6 (UV detection at 254 nm)

Continuous-flow synthesis of ***(R)-6***

The title compound was prepared according to the general procedure after filling Loop 1 with the solution of **(*R*)-3** and setting the temperature of Reactor 1 to 60°C. The product was isolated and purified by column chromatography on silica gel (cyclohexane-ethyl-acetate 1:1) to give the mixture of regioisomers as a white solid ((48% yield, 58:42 **(*R*)-6: (*R*)-5** ratio of the regioisomers, **Figure S10**). An analytical sample was purified using preparative SFC chromatography (**Figure S11**) to obtain isomerically pure **(*R*)-6**. The major (**(*R*)-6**) and minor (**(*R*)-5**) regioisomers were subjected to NMR spectroscopy for analysis.

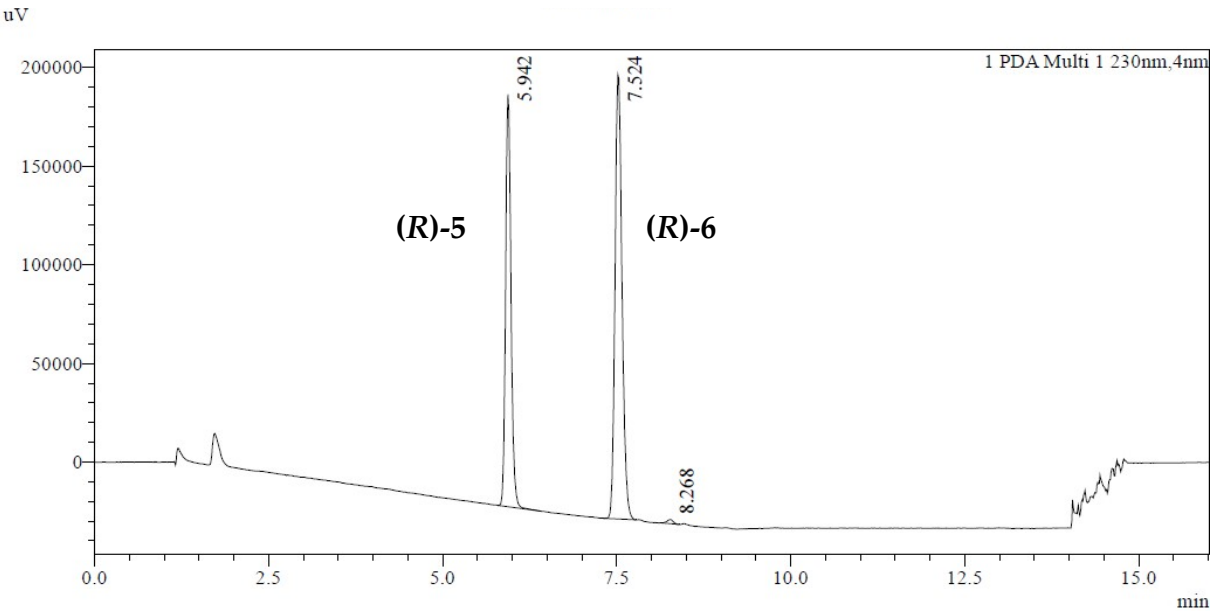


Figure S10. SFC separation of (*R*)-5 and (*R*)-6

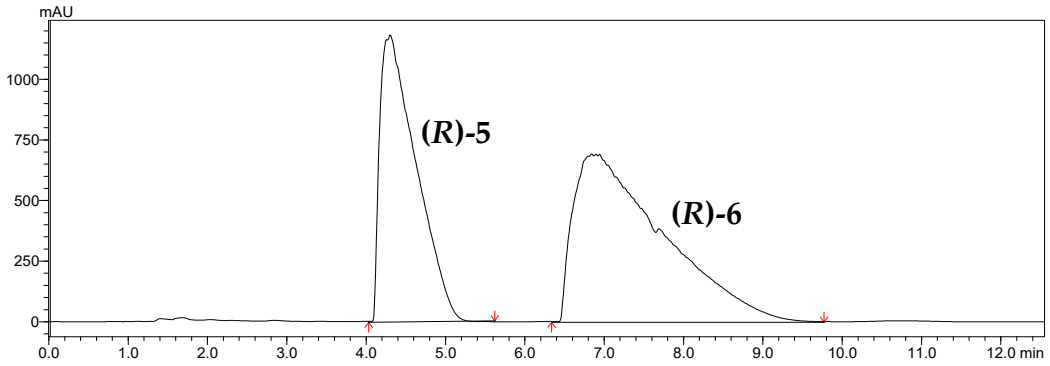
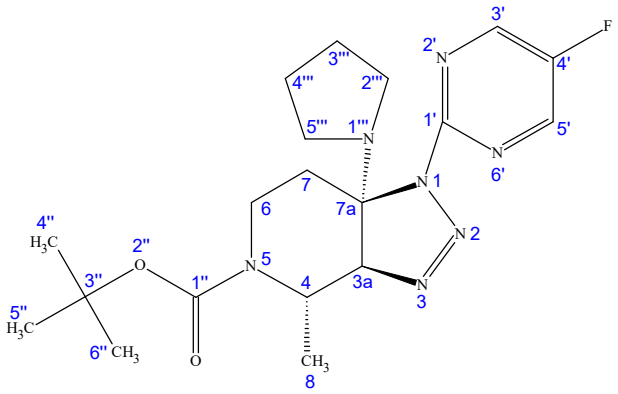


Figure S11. Preparative SFC separation of regioisomers (R)-5 and (*R*)-6 (UV detection at 254 nm)

Spectroscopic data

The HRMS and NMR spectra, including signal assignments and relevant interpretations, are provided below for all compounds. Signals corresponding to impurities are indicated by a red asterisk in the NMR spectra.

***Tert*-butyl (4*S*)-1-(5-fluoropyrimidin-2-yl)-4-methyl-7a-(pyrrolidin-1-yl)-1,3a,4,6,7,7a-hexahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate ((*S*)-8)**

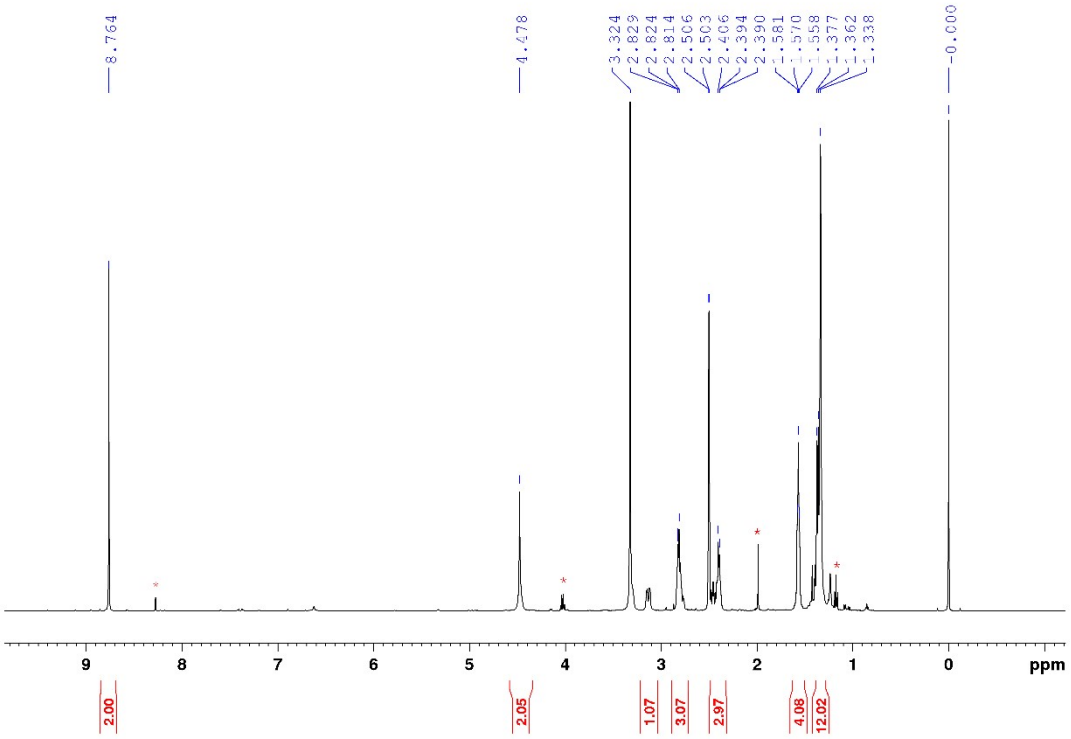


^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) 1.34 (9H; s; H_3-4'', H_3-5'', H_3-6''); 1.37 (3H; d; J=7.4 Hz; H_3-8); 1.52-1.62 (4H; m; H_2-3''', H_2-4'''); 2.35-2.49 (3H; m; H_{ax}-7, H_x-2''', H_x-5'''); 2.72-2.88 (3H; m; H_{ax}-6, H_y-2''', H_y-5'''); 3.14 (1H; ~dd; J=14.1, 2.6 Hz; H_{eq}-7); 3.26-3.35 (1H; m; H_{eq}-6); 4.42-4.53 (2H; m; H-3a, H-4); 8.76 (2H; s; H-3', H-5').

^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) 20.9 (C-8); 23.4 (C-3''', C-4'''); 24.7 (C-7); 27.9 (C-4'', C-5'', C-6''); 38.0 (br, C-6); 45.1 (C-2'', C-5''); 46.7 (C-4)*; 77.8 (C-7a); 78.8 (C-3''); 83.8 (C-3a) 146.3 (d; J=22.1 Hz; C-3', C-5'); 154.0 (C-1''); 154.3 (d; J=254.7 Hz; C-4'); 154.7 (d; J=2.5 Hz; C-1').

* Due to severe line broadening in the ^{13}C spectrum, this ^{13}C resonance was verified by the ^{1}H-^{13}C HSQC experiment

ESI-HRMS: calcd for $C_{19}H_{29}O_2N_7F$ $[M+H]^+$: 406.23613; found: 406.23574; delta= -0.95 ppm.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1.558
1.377
1.362
1.338
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9
8
7
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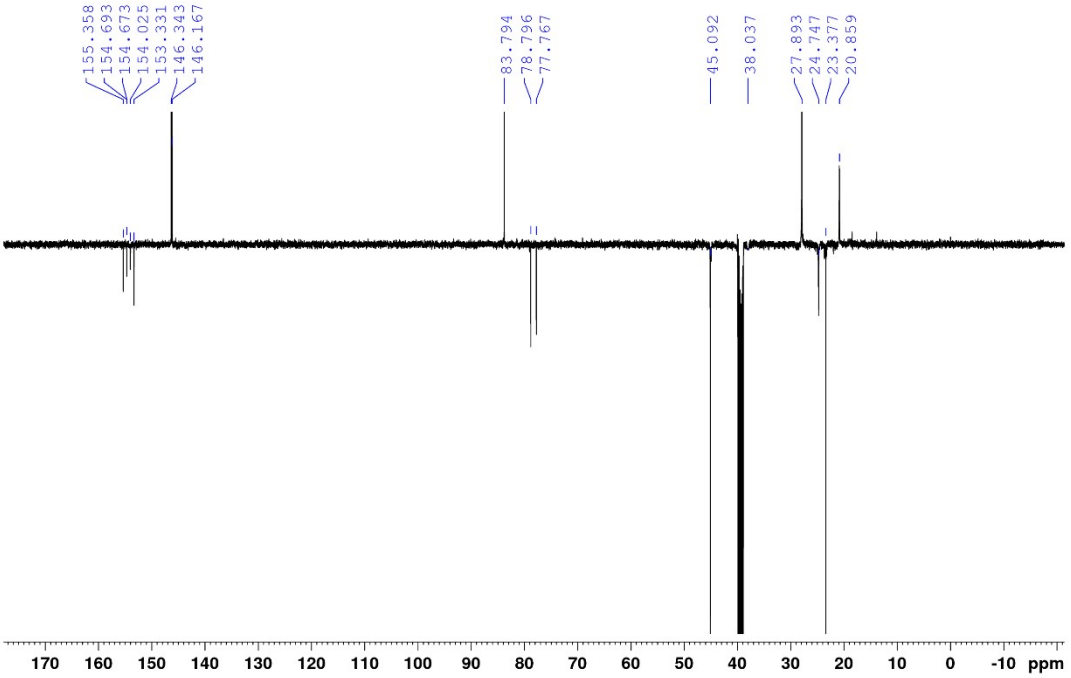
3.07
2.97
4.08
12.02

1H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153.331
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146.167
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77.767
45.092
38.037
27.893
24.747
23.377
20.859
170
160
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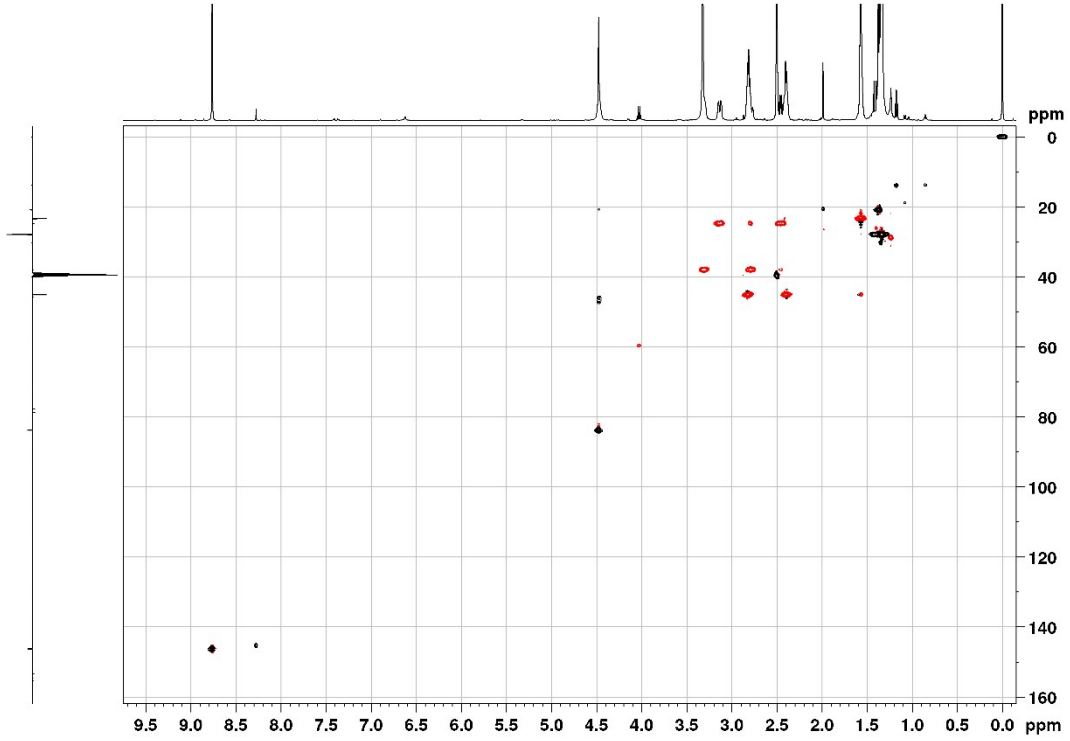
ppm

13C-DEPTQ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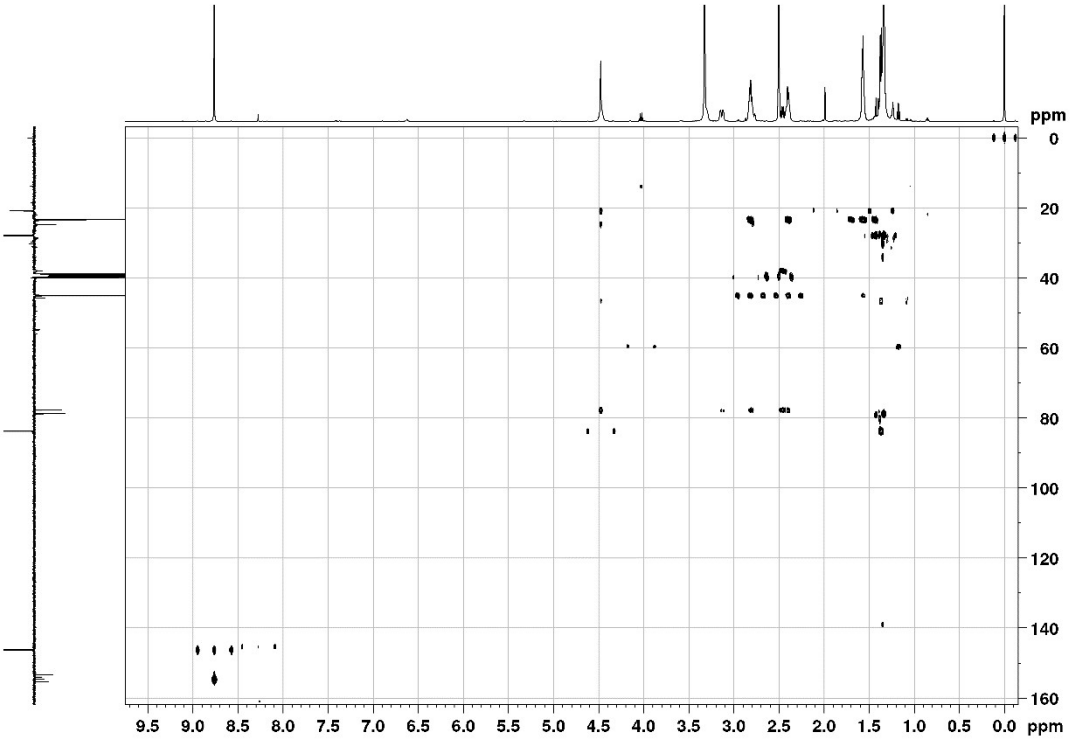
HSQC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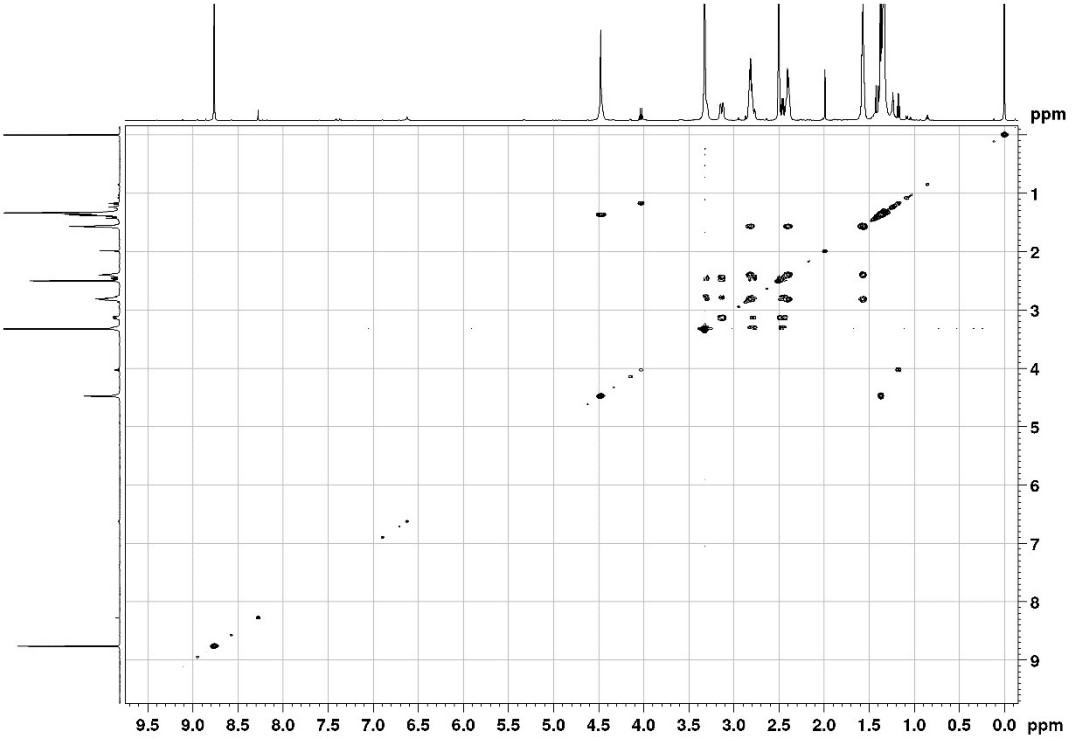
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

HMBC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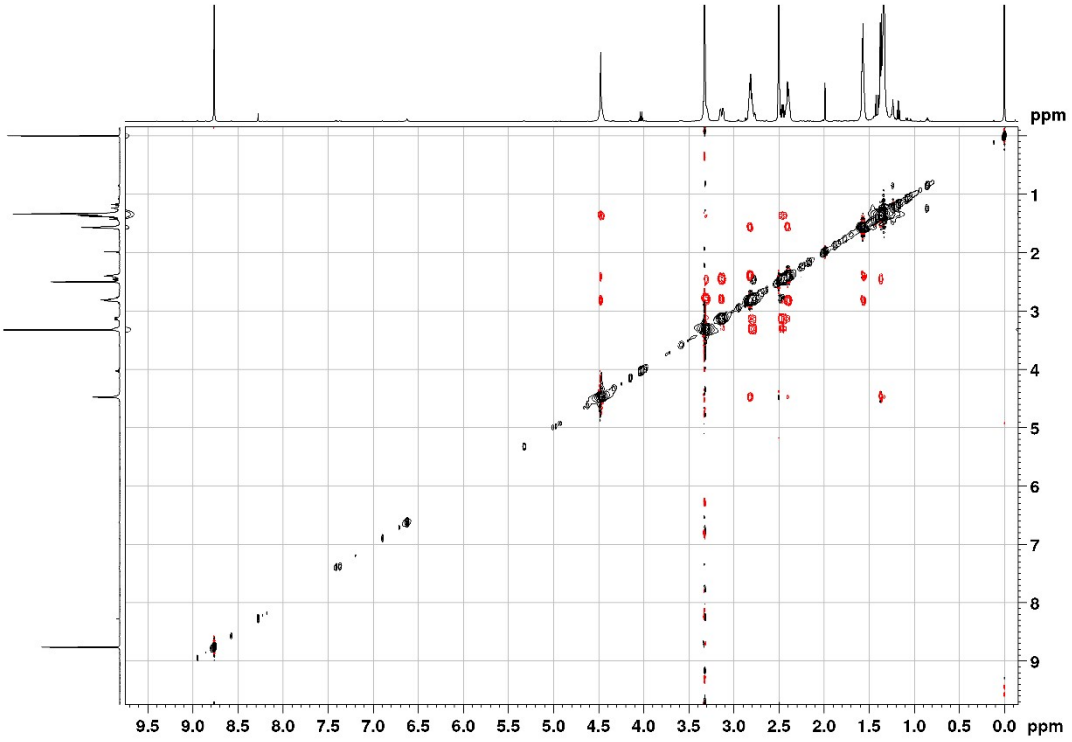
COSY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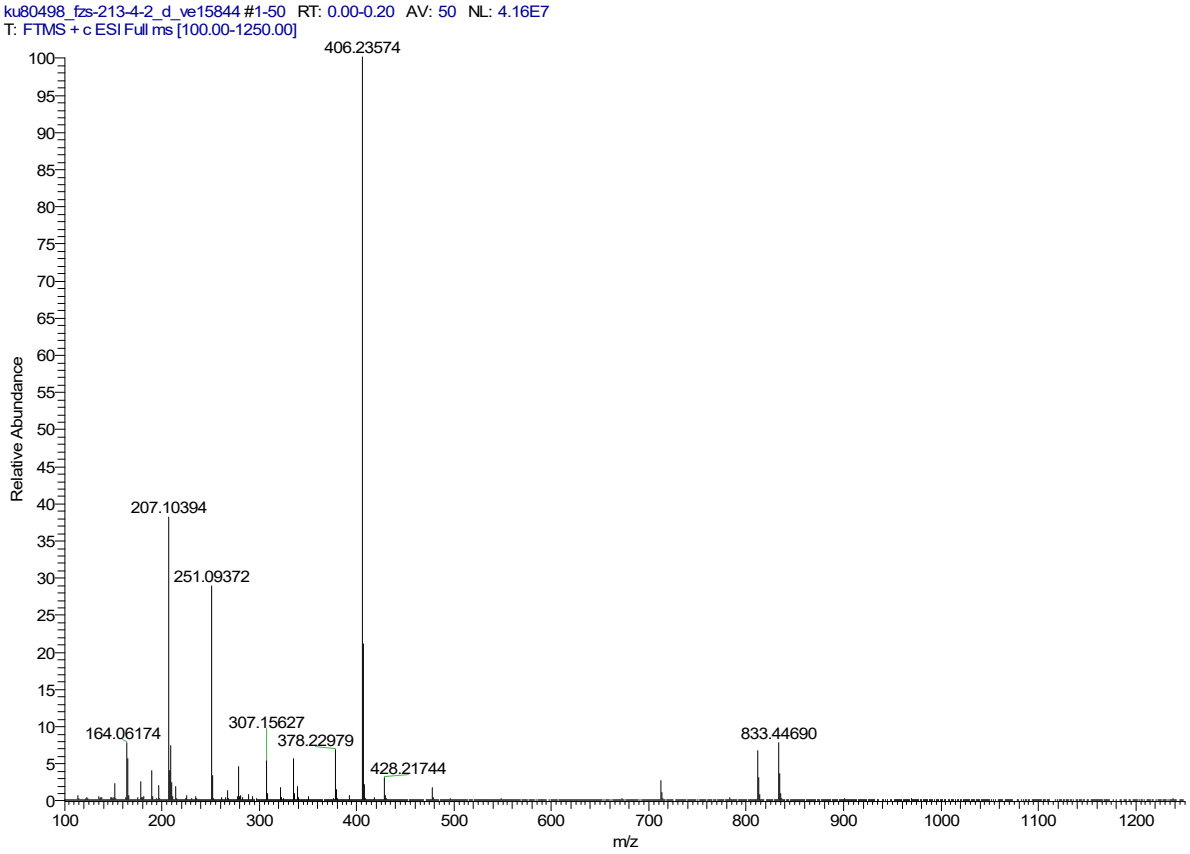
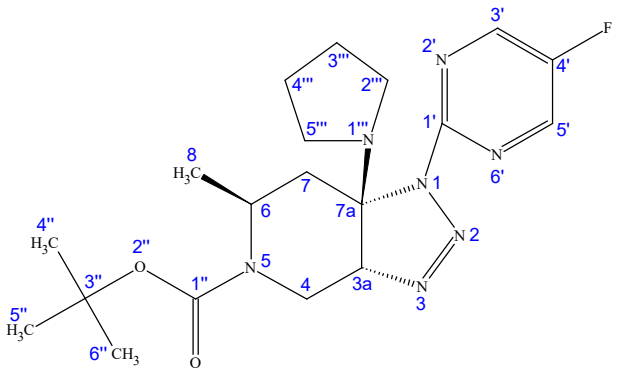
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

ROESY



ESI-HRMS spectrum

***Tert*-butyl (6*S*)-1-(5-fluoropyrimidin-2-yl)-6-methyl-7a-(pyrrolidin-1-yl)-1,3a,4,6,7,7a-hexahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate ((*S*)-7)**



Two rotamers observed approximately in a 1:1 ratio at 298 K in DMSO.

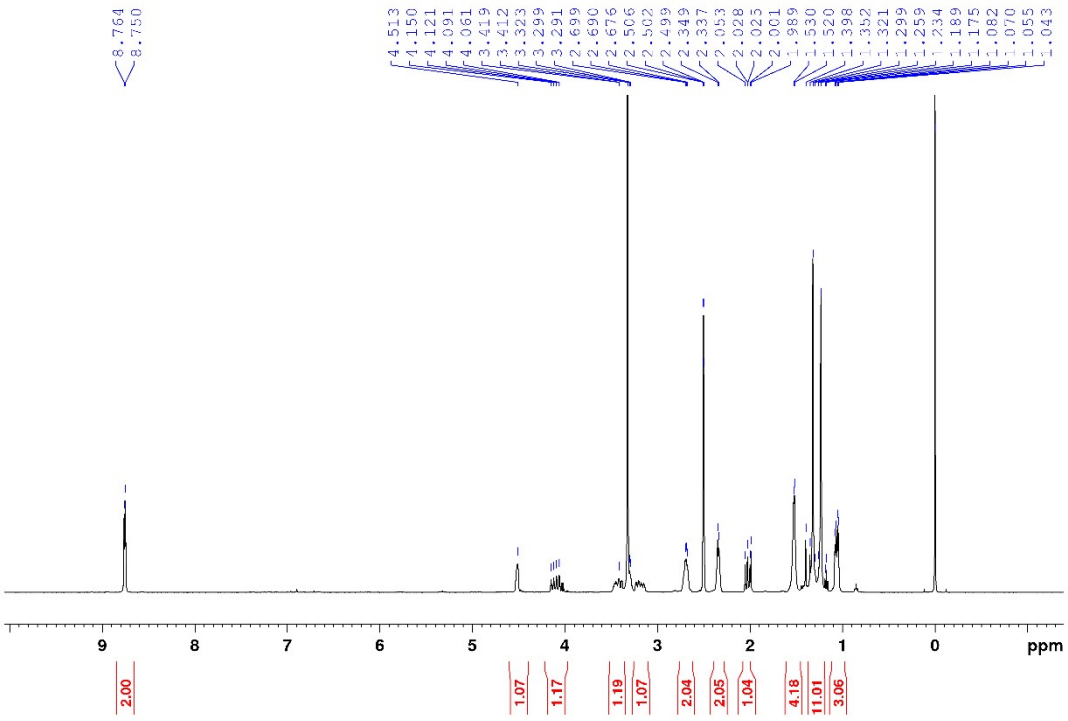
^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) (rotamer A) 1.05 (3H; d; J=6.0 Hz; H_3-8); 1.32 (9H; s; H_3-4'', H_3-5'', H_3-6''); 1.47-1.59 (4H; m; H_2-3''', H_2-4'''); 2.03 (1H; dd; J=13.9, 12.2 Hz; H_{ax}-7); 2.29-2.40 (2H; m; H_x-2''', H_x-5'''); 2.62-2.76 (2H; m; H_y-2''', H_y-5'''); 3.22 (1H; dd; J=14.1, 5.7 Hz; H_{eq}-7); 3.25-3.35 (1H; m; H_{eq}-4); 3.42-3.52 (1H; m; H_{ax}-6); 4.08 (1H; br d; J=14.7 Hz; H_{ax}-4); 4.48-4.55 (1H; m; H_{eq}-3a); 8.75 (2H; s; H-3', H-5').

^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) (rotamer A) 19.2 (C-8); 23.3 (C-3‴, C-4‴); 27.8 (C-4″, C-5″, C-6″); 32.9 (C-7); 38.6 (C-4); 45.0 (C-2″, C-5″); 45.3 (C-6); 77.7 (C-7a); 78.5 (C-3″); 81.3 (C-3a) 146.2 (d; *J*=22.0 Hz; C-3′, C-5′); 153.4 (C-1″); 154.3 (d; *J*=254.8 Hz; C-4′); 155.1 (C-1′).

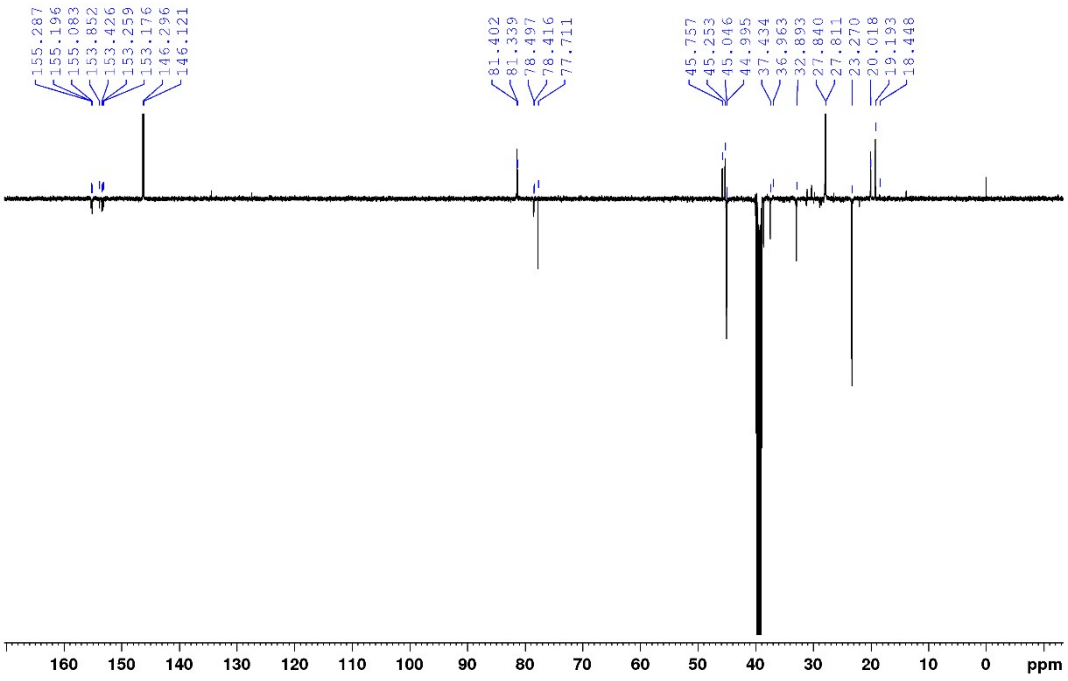
^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) (rotamer B) 1.08 (3H; d; *J*=5.9 Hz; H_3-8); 1.23 (9H; s; H_3-4″, H_3-5″, H_3-6″); 1.47-1.59 (4H; m; H_2-3‴, H_2-4‴); 2.03 (1H; dd; *J*=13.9, 12.2 Hz; H_{ax}-7); 2.29-2.40 (2H; m; H_x-2‴, H_x-5‴); 2.62-2.76 (2H; m; H_y-2‴, H_y-5‴); 3.16 (1H; dd; *J*=14.2, 5.8 Hz; H_{eq}-7); 3.25-3.35 (1H; m; H_{ax}-6); 3.40 (1H; dd; *J*=14.8, 3.6 Hz; H_{eq}-4); 4.14 (1H; br d; *J*=14.5 Hz; H_{ax}-4); 4.48-4.55 (1H; m; H_{eq}-3a); 8.76 (2H; s; H-3′, H-5′).

^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) (rotamer B) 20.0 (C-8); 23.3 (C-3‴, C-4‴); 27.8 (C-4″, C-5″, C-6″); 32.9 (C-7); 37.4 (C-4); 45.1 (C-2″, C-5″); 45.8 (C-6); 77.7 (C-7a); 78.4 (C-3″); 81.4 (C-3a) 146.2 (d; *J*=22.0 Hz; C-3′, C-5′); 153.9 (C-1″); 154.2 (d; *J*=253.9 Hz; C-4′); 155.1 (C-1′).

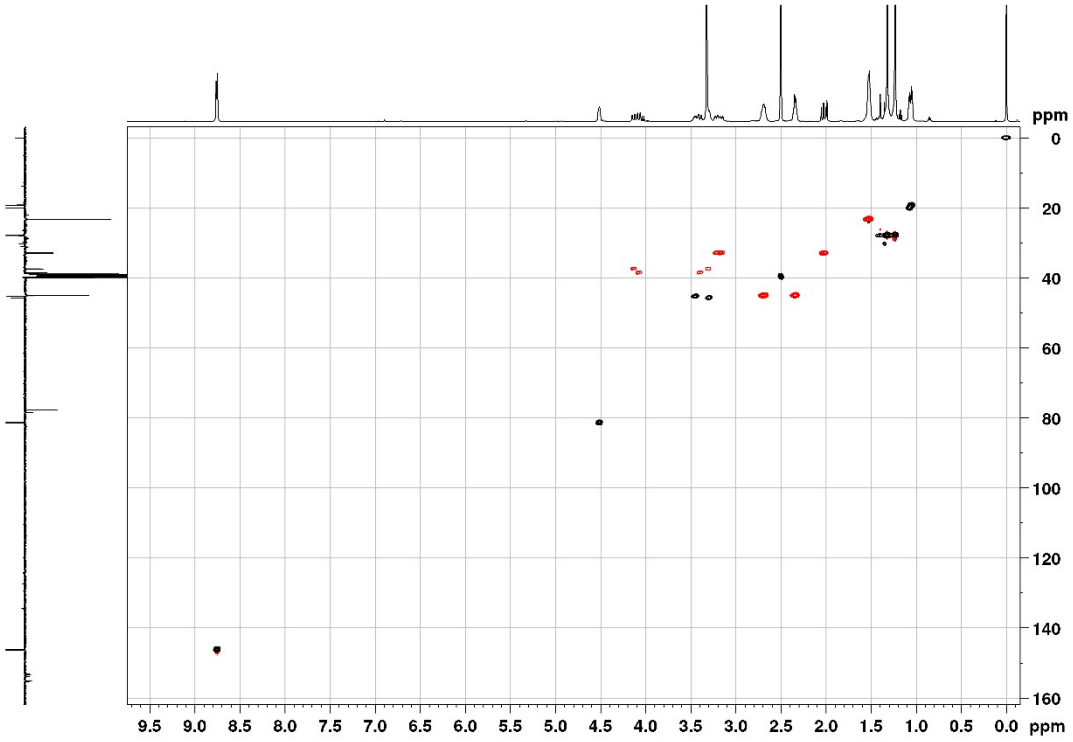
ESI-HRMS: calcd for $C_{19}H_{29}O_2N_7F$ $[M+H]^+$: 406.23613; found: 406.23591; delta= -0.54 ppm.



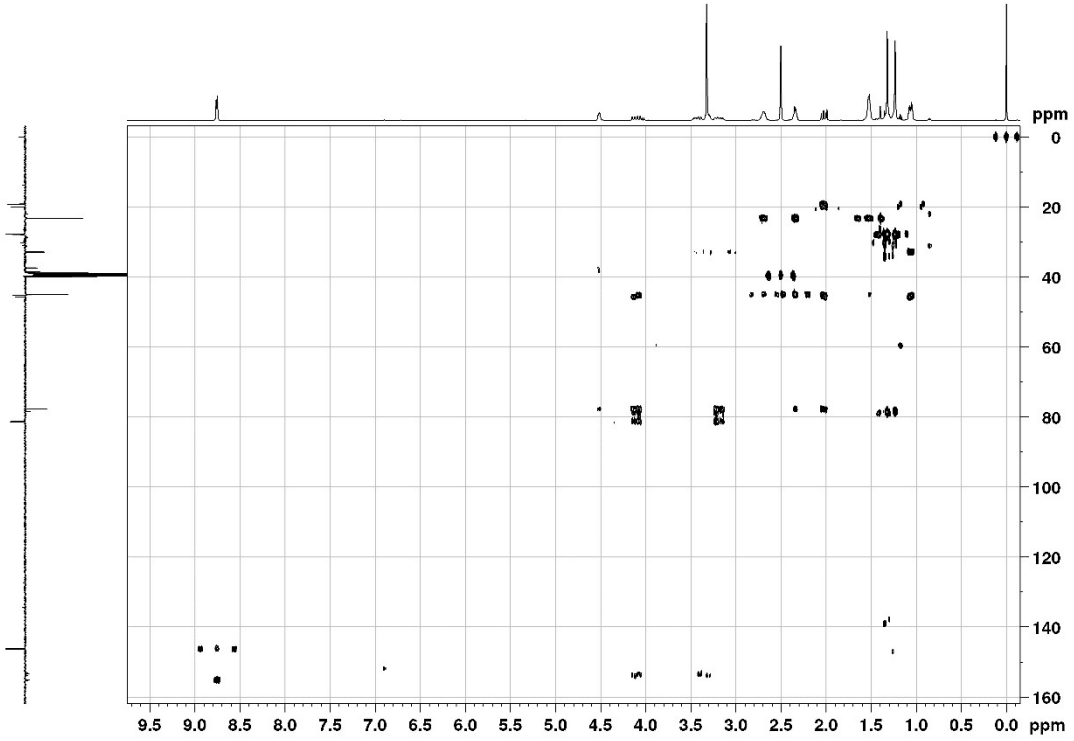
1H



13C-DEPTQ



HSQC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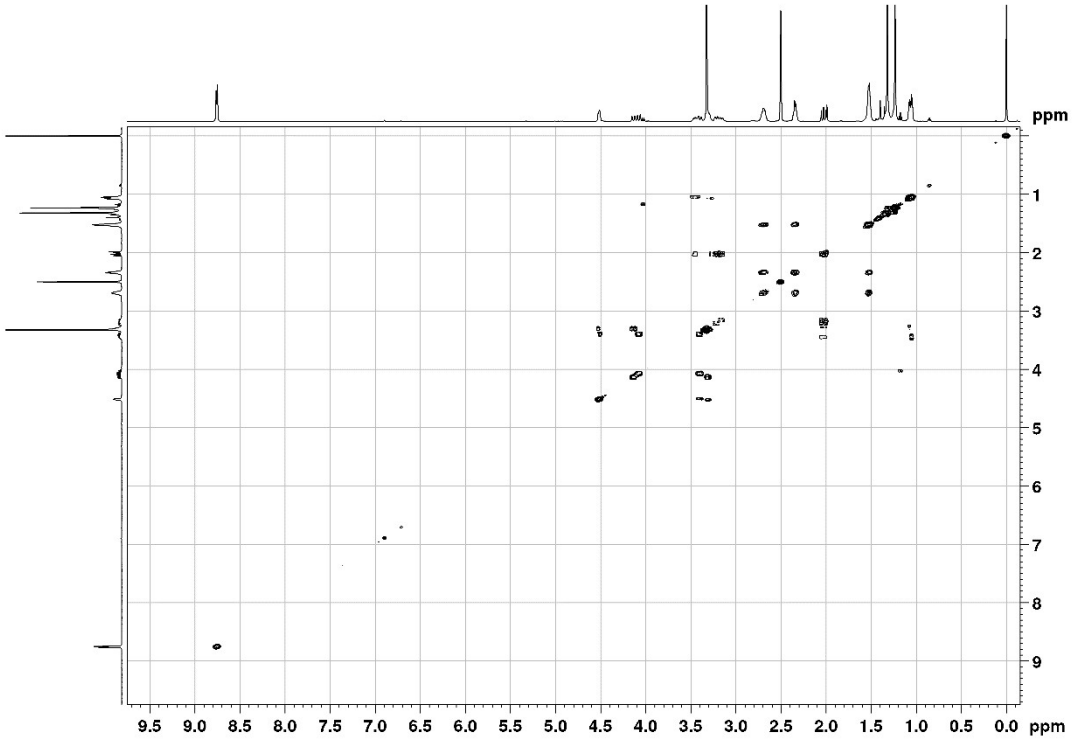
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

HMBC


ppm
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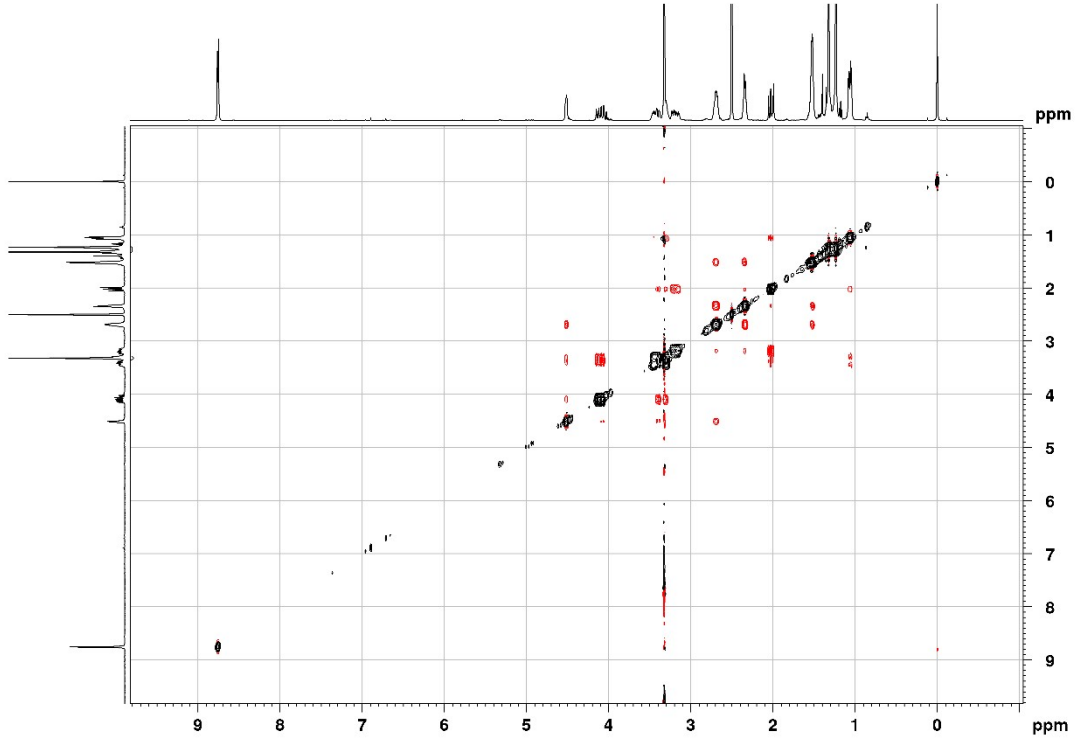
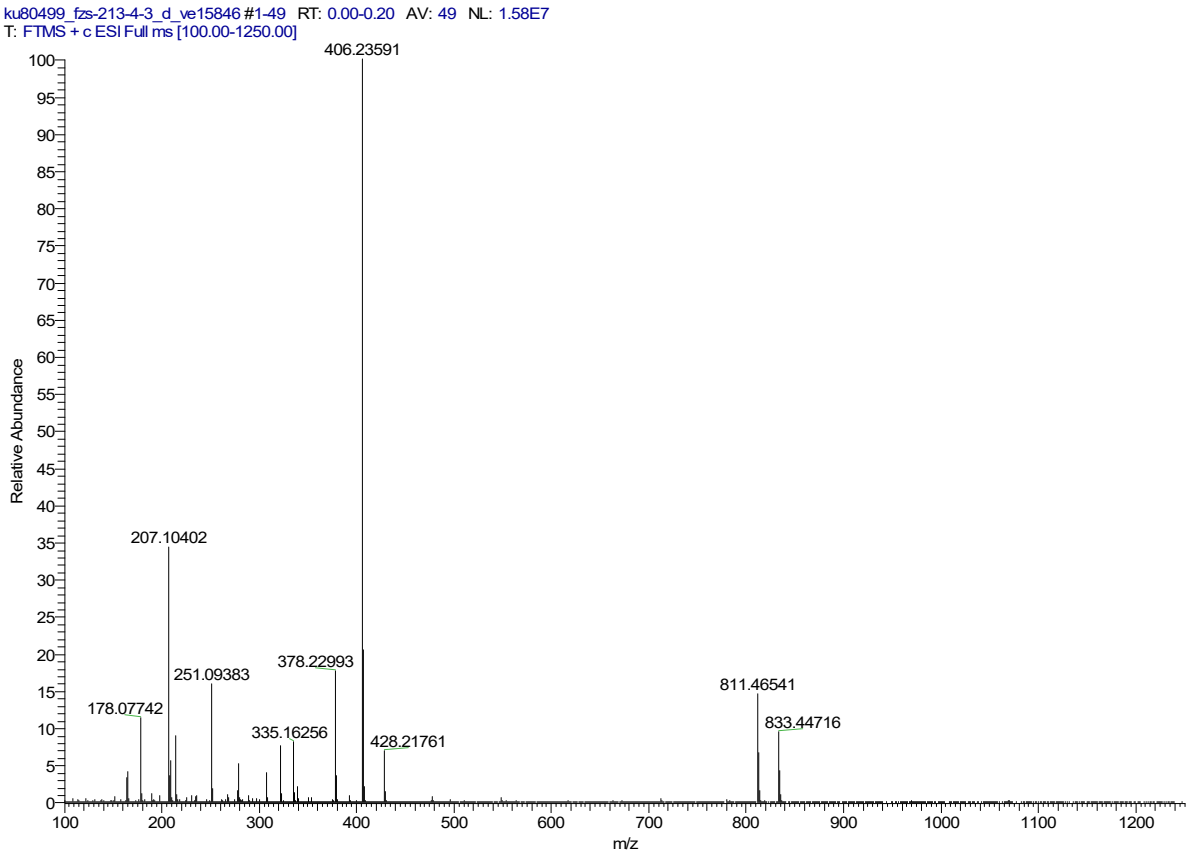
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

COSY

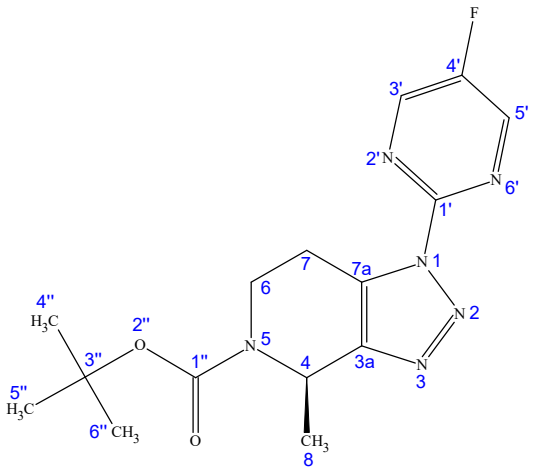


ROESY



ESI-HRMS spectrum

***Tert*-butyl (4*R*)-1-(5-fluoropyrimidin-2-yl)-4-methyl-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate ((*R*)-6)**

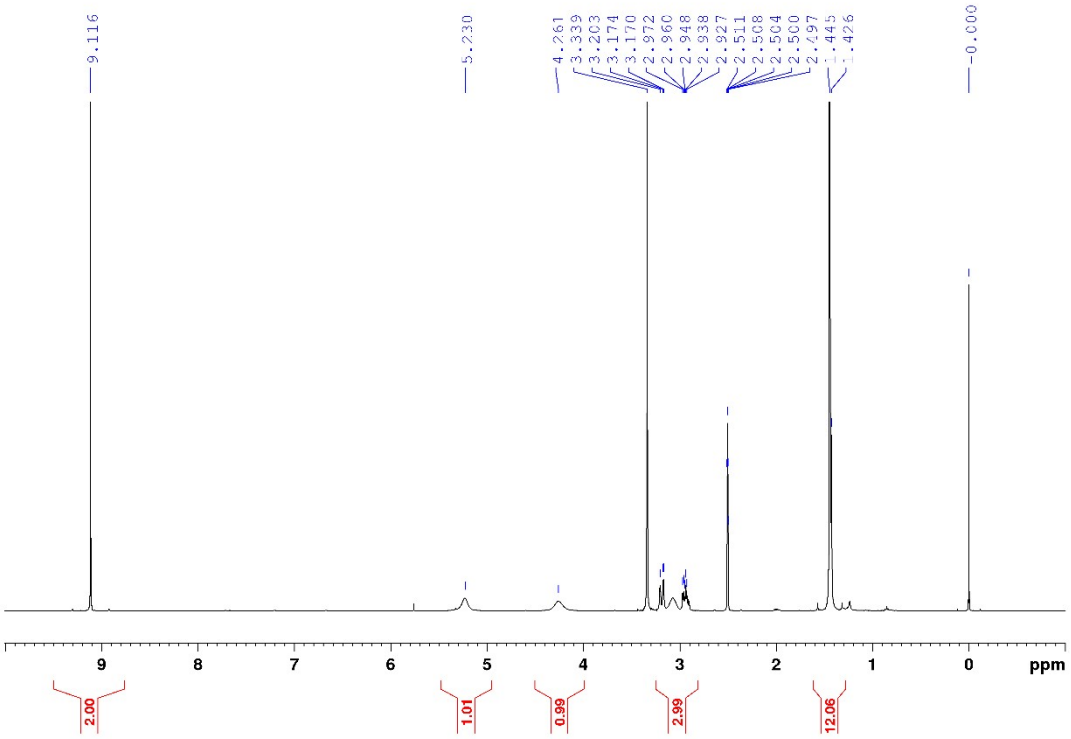


^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) 1.40-1.48 (12H; m; H_3-8, H_3-4'', H_3-5'', H_3-6''); 2.94 (1H; ~ddd; *J*=17.0, 11.8, 5.6 Hz; H_x-7); 3.07 (1H; br s; H_x-6); 3.19 (1H; ~br dd; *J*=16.6, 2.1 Hz; H_y-7); 4.26 (1H; br s; H_y-6); 5.23 (1H; br s; H-4); 9.12 (2H; s; H-3', H-5').

^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) 18.7 (C-8); 23.6 (br s; C-7) 27.9 (C-4'', C-5'', C-6''); 35.2 (C-6)*; 46.4 (C-4)*; 79.4 (C-3''); 131.9 (C-7a); 145.3 (C-3a); 147.4 (d; *J*=23.1 Hz, C-3', C-5'); 150.6 (d; *J*=3.3 Hz; C-1'); 153.5 (C-1''); 156.7 (d; *J*=261.2 Hz; C-4').

* Due to severe line broadening in the ^{13}C spectrum, these ^{13}C resonances were verified by the ^{1}H-^{13}C HSQC experiment

ESI-HRMS: calcd for $C_{15}H_{20}O_2N_6F$ $[M+H]^+$: 335.16263; found: 335.16215; delta= -1.43 ppm.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2.508
2.504
2.500
2.497
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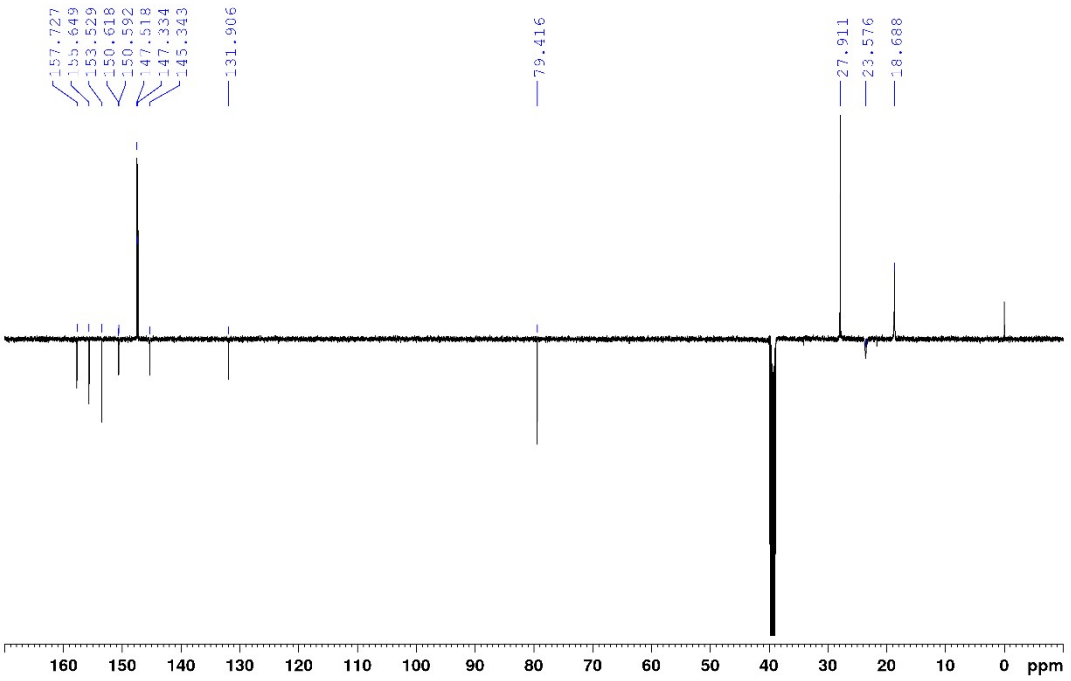
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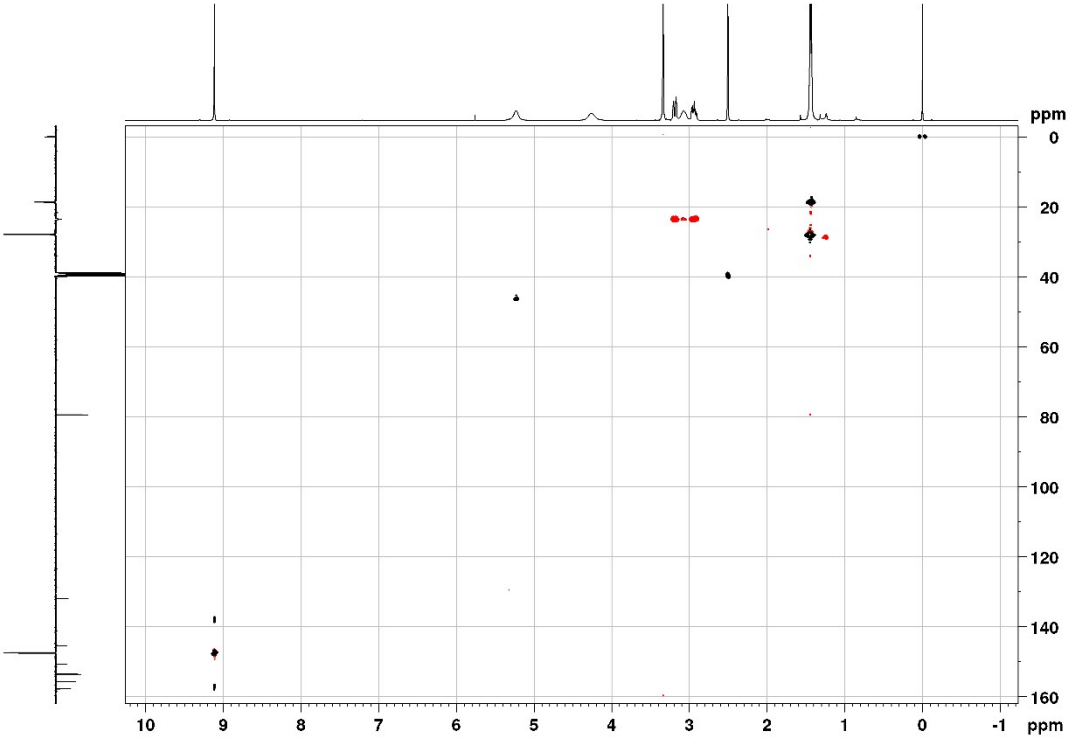
13C-DEPTQ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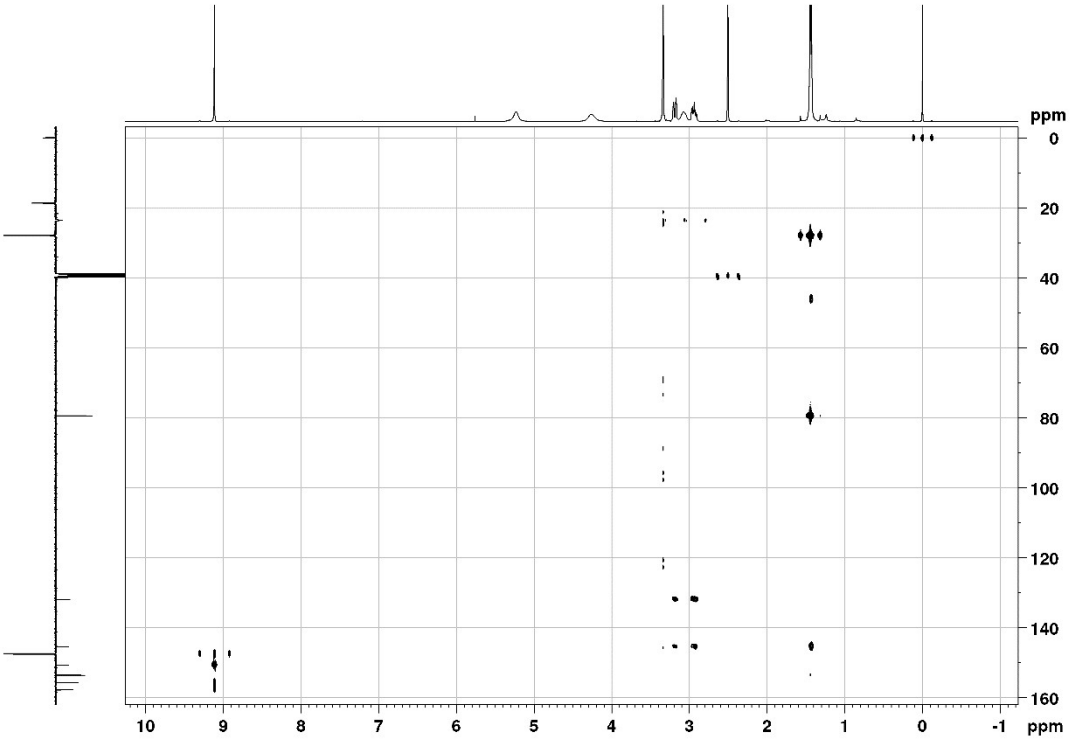
HSQC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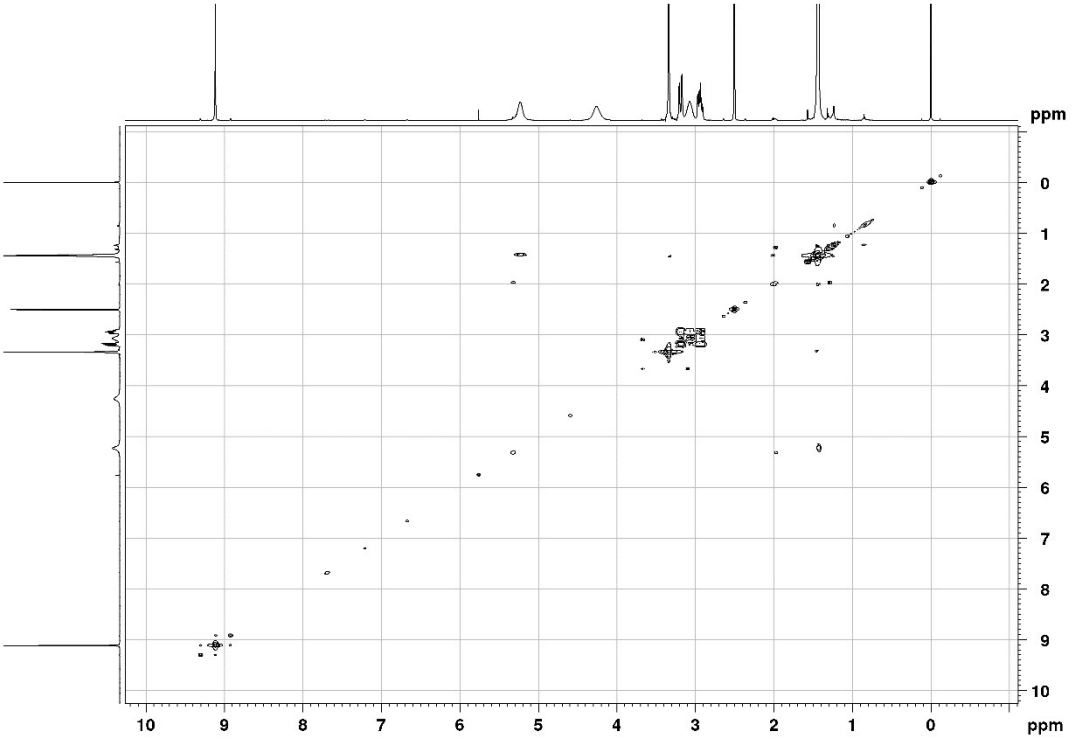
HMBC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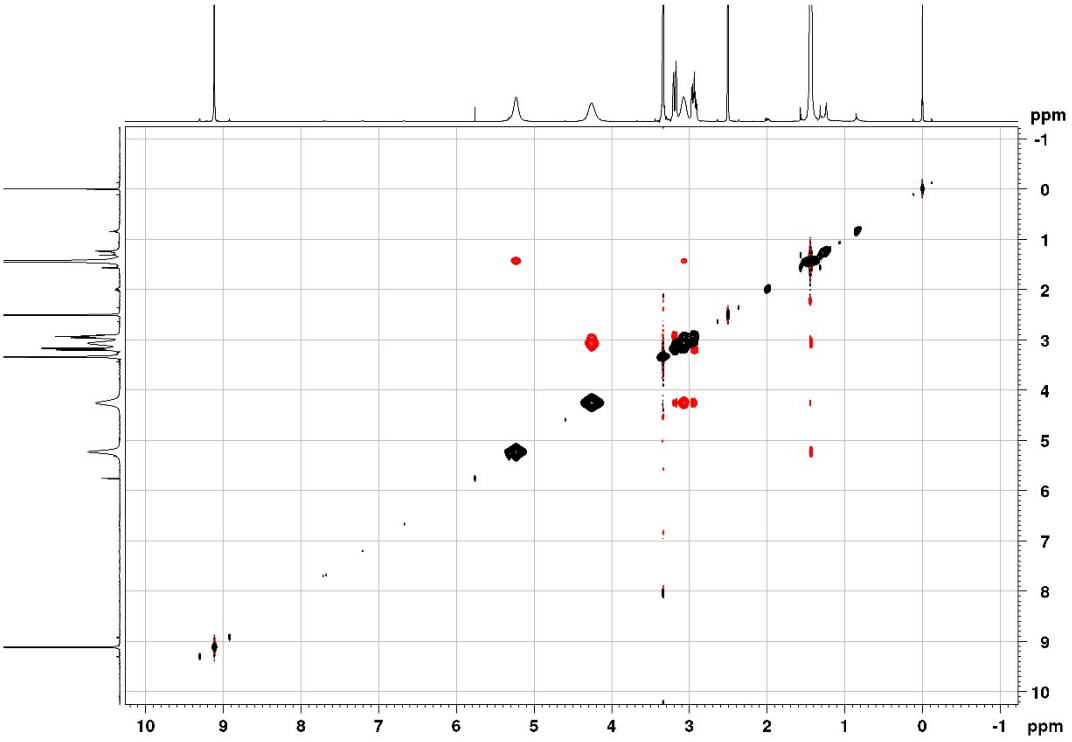
COSY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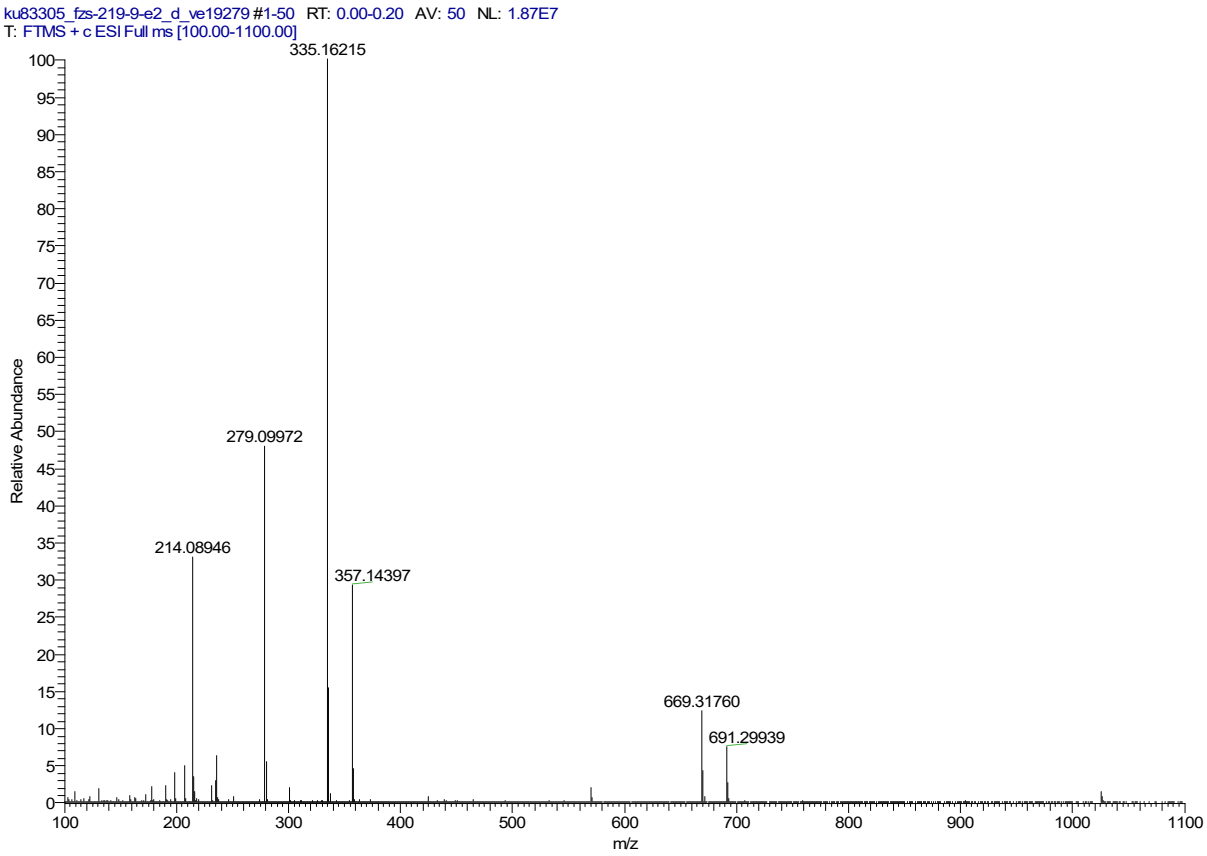
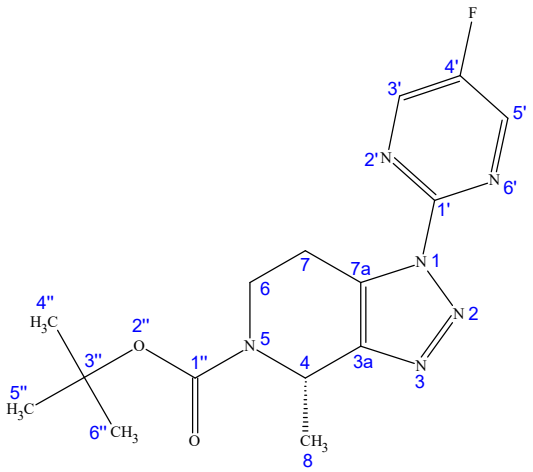
NOESY



ESI-HRMS spectrum

Tert-butyl (4*S*)-1-(5-fluoropyrimidin-2-yl)-4-methyl-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate ((*S*)-6)

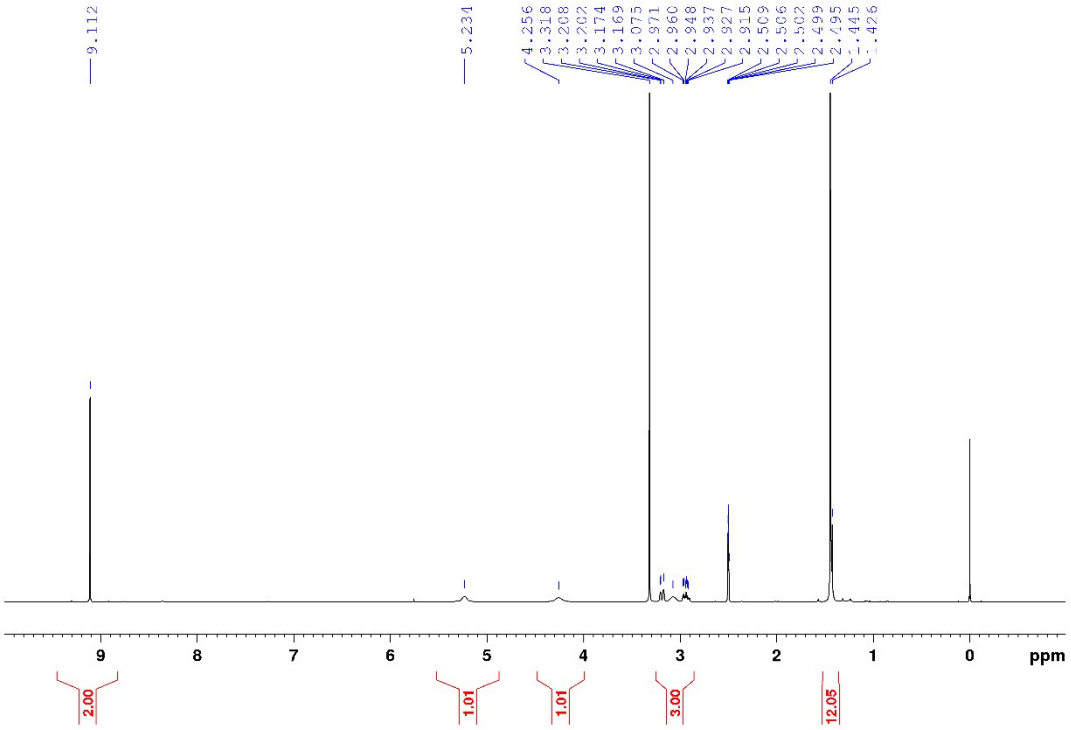


^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) 1.40-1.48 (12H; m; H_3-8, H_3-4'', H_3-5'', H_3-6''); 2.94 (1H; ~ddd; J=17.0, 11.8, 5.6 Hz; H_x-7); 3.07 (1H; br s; H_x-6); 3.19 (1H; ~br dd; J=16.6, 2.1 Hz; H_y-7); 4.26 (1H; br s; H_y-6); 5.23 (1H; br s; H-4); 9.12 (2H; s; H-3', H-5').

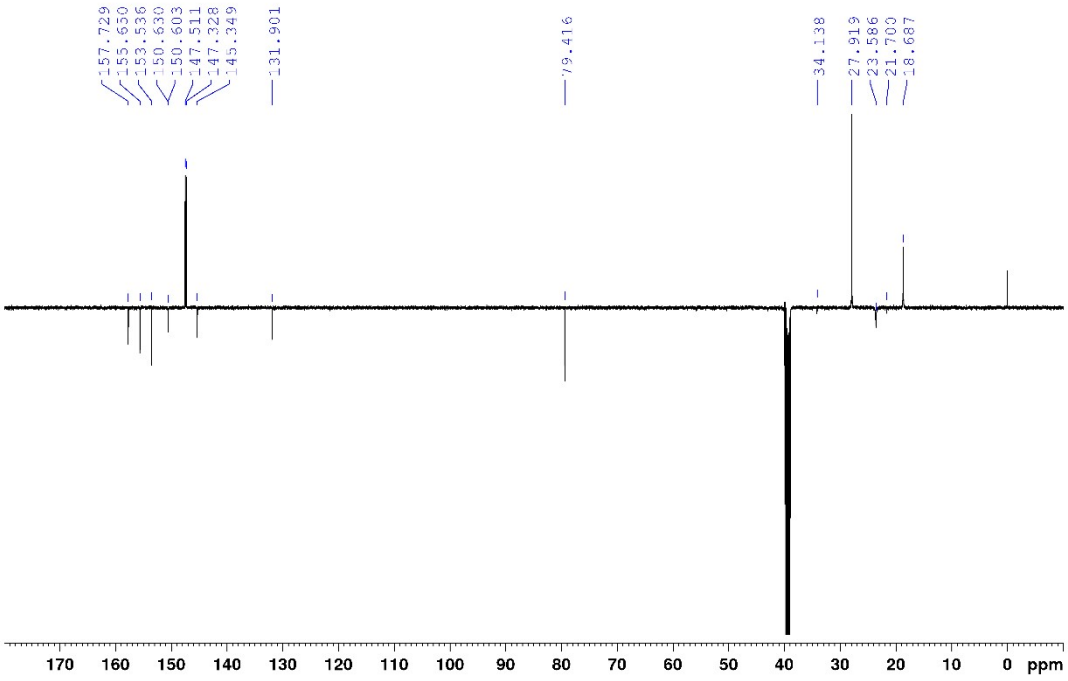
^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) 18.7 (C-8); 23.6 (br s; C-7) 27.9 (C-4'', C-5'', C-6''); 35.2 (C-6)*; 46.4 (C-4)*; 79.4 (C-3''); 131.9 (C-7a); 145.3 (C-3a); 147.4 (d; J=23.1 Hz, C-3', C-5'); 150.6 (d; J=3.3 Hz; C-1'); 153.5 (C-1''); 156.7 (d; J=261.2 Hz; C-4').

* Due to severe line broadening in the ^{13}C spectrum, these ^{13}C resonances were verified by the ^{1}H-^{13}C HSQC experiment

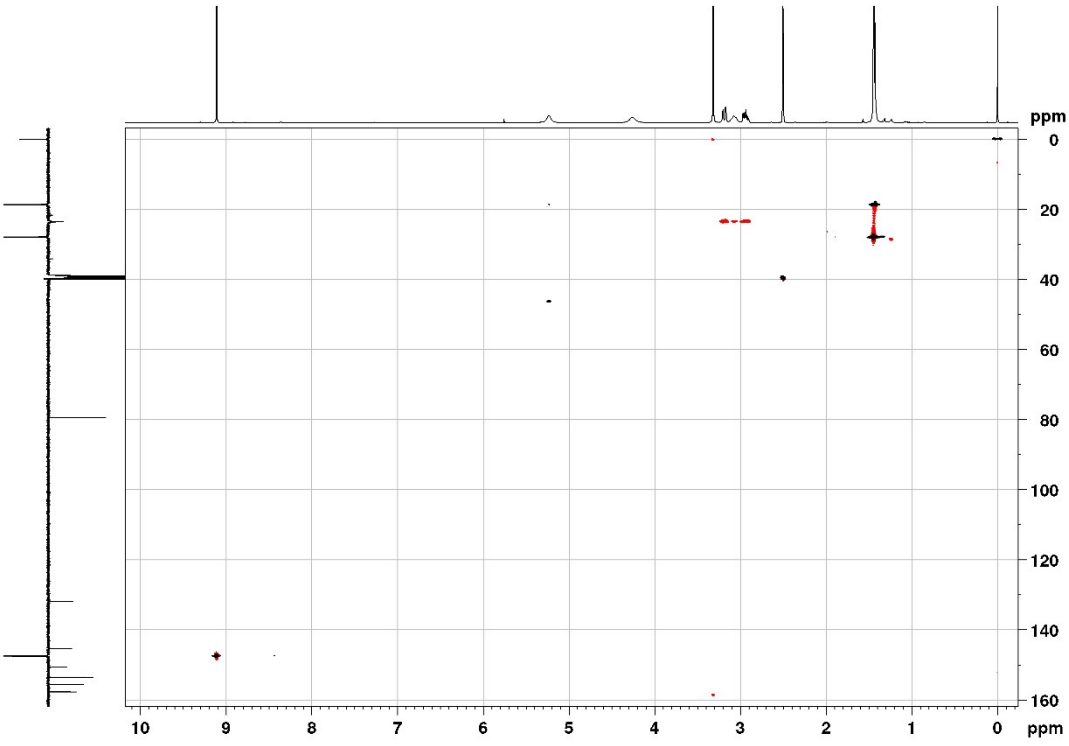
ESI-HRMS: calcd for $C_{15}H_{20}O_2N_6F$ $[M+H]^+$: 335.16263; found: 335.16219; delta= -1.31 ppm.



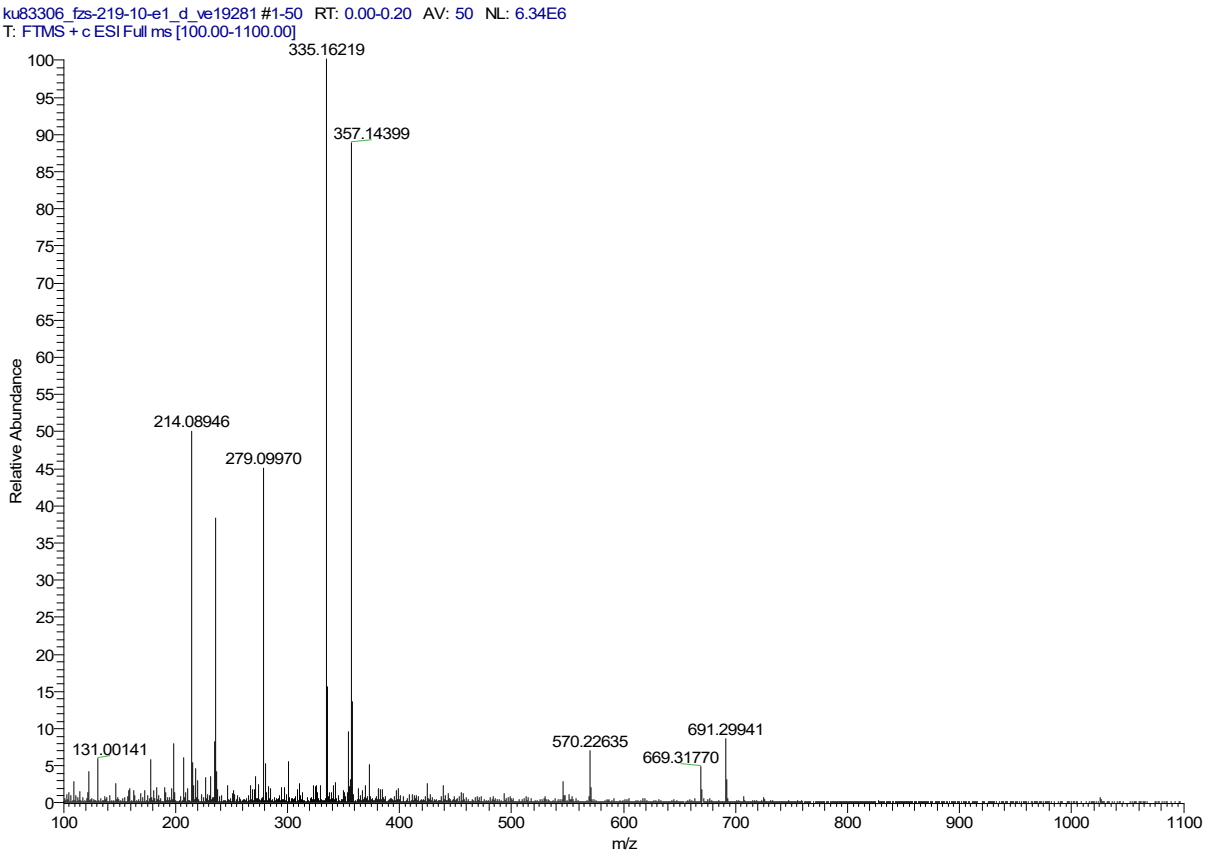
1H



13C-DEPTQ

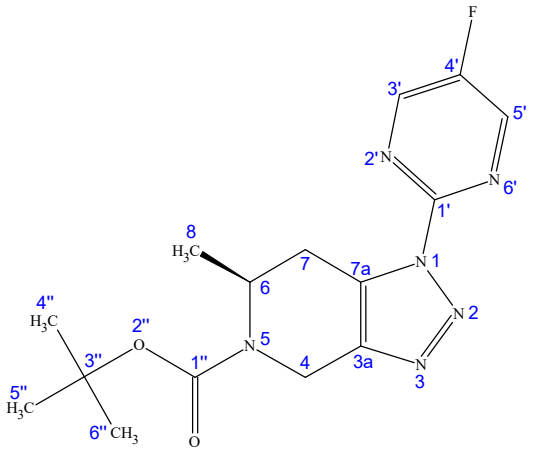


HSQC



ESI-HRMS spectrum

Tert-butyl (6_S_)-1-(5-fluoropyrimidin-2-yl)-6-methyl-1,4,6,7-tetrahydro-5_H_-[1,2,3]triazolo[4,5-_c_]pyridine-5-carboxylate ((_S_)-5)

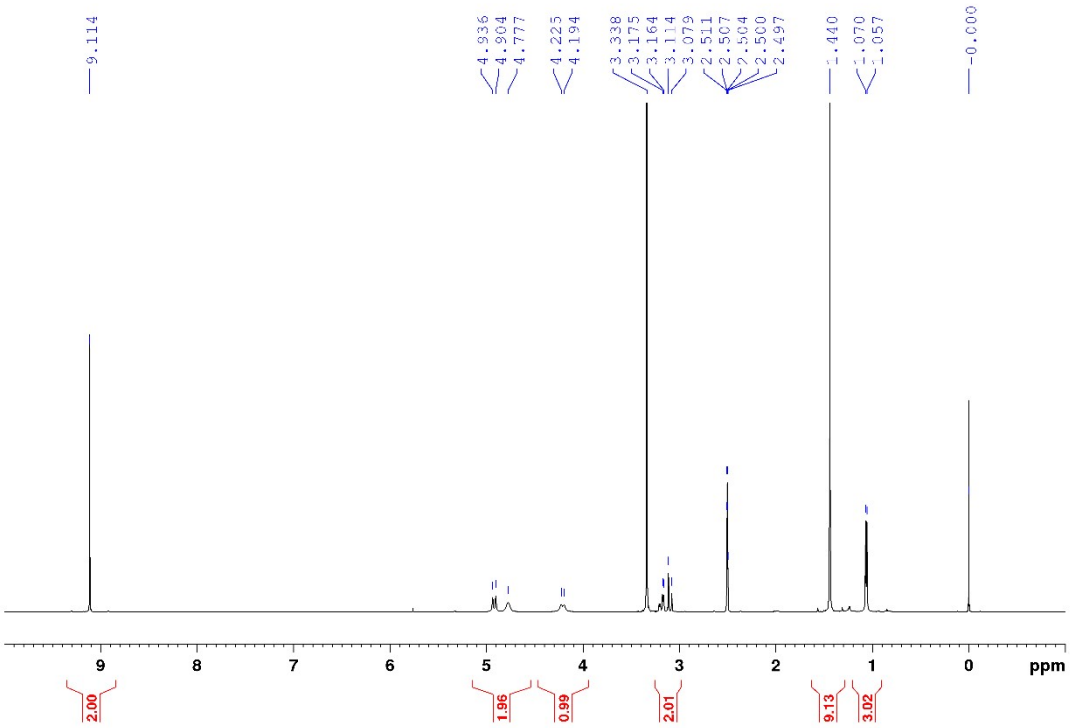


^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) 1.06 (3H; d; J=6.9 Hz; H_3-8); 1.44 (9H; s; H_3-4'', H_3-5'', H_3-6''); 3.10 (1H; ~br d; J=17.3 Hz; H_x-7); 3.19 (1H; ~br dd; J=17.4, 6.0 Hz; H_y-7); 4.21 (1H; br d; J=15.6 Hz; H_x-4); 4.78 (1H; br s; H-6); 4.92 (1H; d; J=16.2 Hz; H_y-4); 9.11 (2H; s; H-3', H-5').

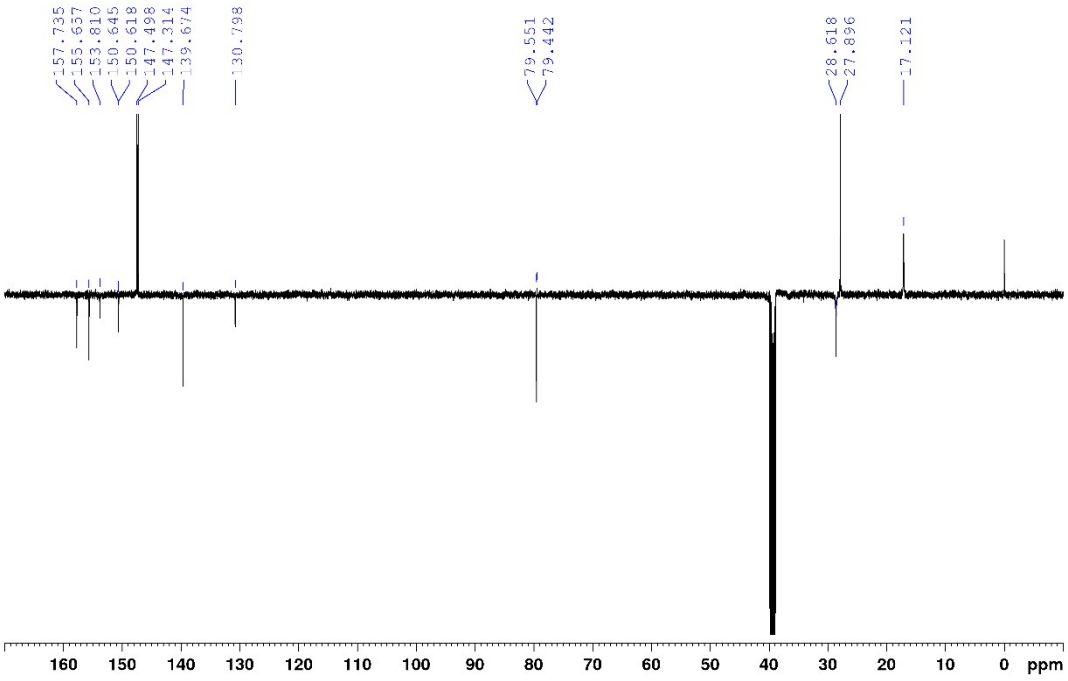
^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) 17.1 (C-8); 27.9 (C-4'', C-5'', C-6''); 28.6 (C-7); 36.5 (C-4)*; 43.6 (C-6)*; 79.6 (C-3''); 130.8 (C-7a); 139.7 (C-3a); 147.4 (d; J=23.0 Hz, C-3', C-5'); 150.6 (d; J=3.3 Hz; C-1'); 153.8 (C-1''); 156.7 (d; J=261.1 Hz; C-4').

* Due to severe line broadening in the ^{13}C spectrum, these ^{13}C resonances were verified by the ^{1}H-^{13}C HSQC experiment

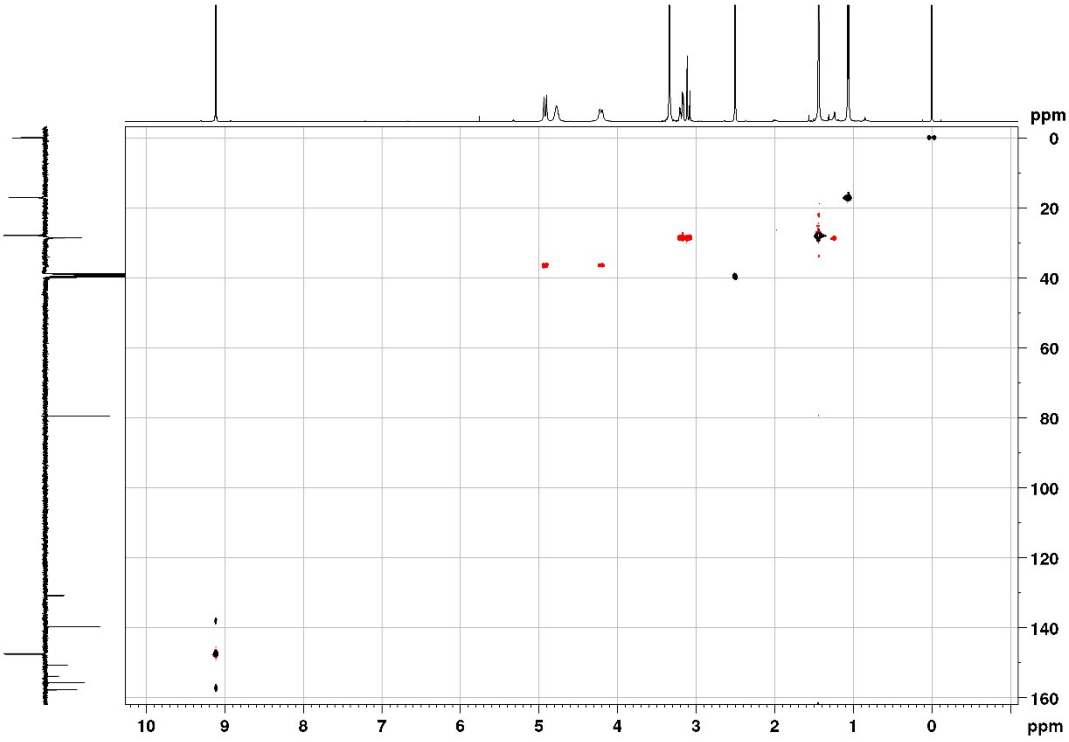
ESI-HRMS: calcd for $C_{15}H_{20}O_2N_6F$ $[M+H]^+$: 335.16263; found: 335.16195; delta= -2.02 ppm.



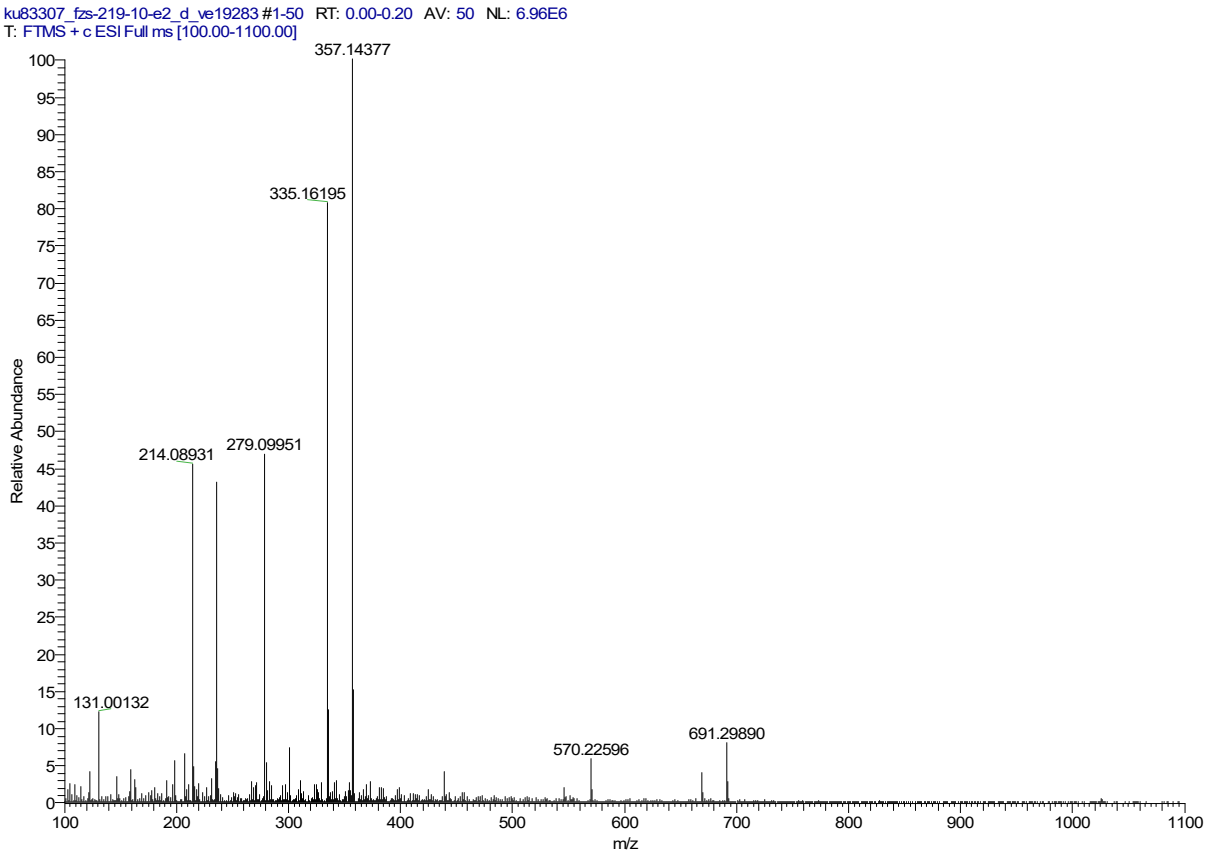
1H



13C-DEPTQ

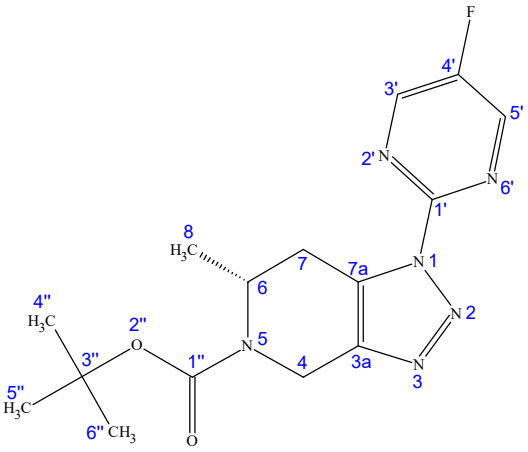


HSQC



ESI-HRMS spectrum

***Tert*-butyl (6*R*)-1-(5-fluoropyrimidin-2-yl)-6-methyl-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate ((*R*)-5)**

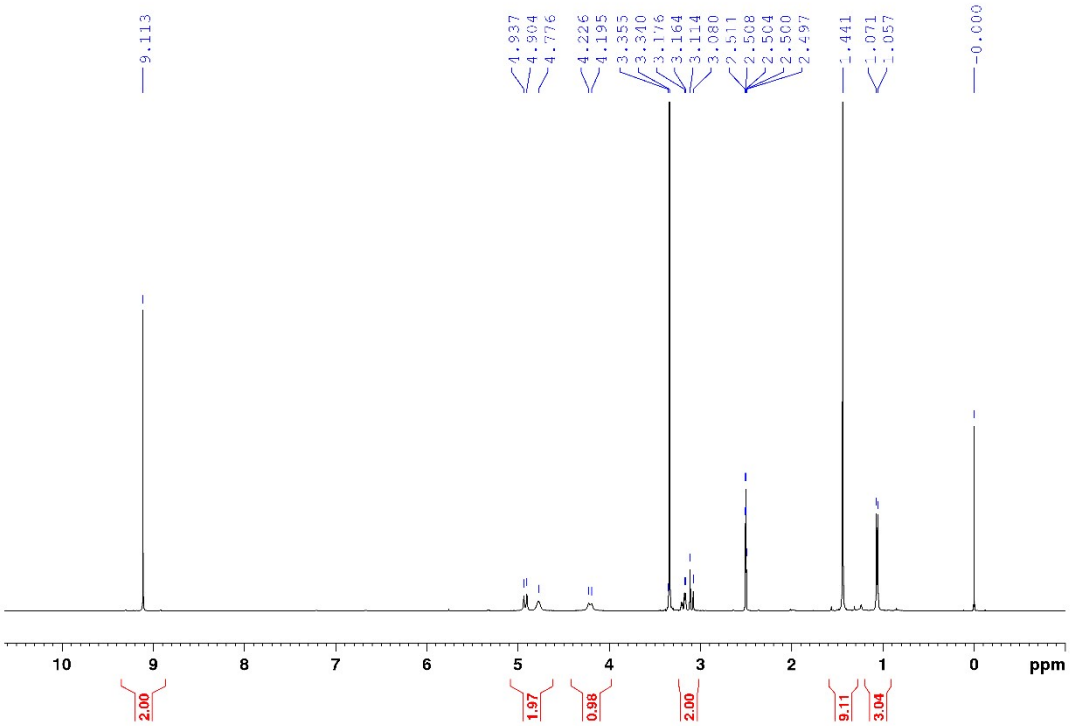


^{1}H NMR (499.9 MHz; DMSO-d_6): δ (ppm) 1.06 (3H; d; J=6.9 Hz; H_3-8); 1.44 (9H; s; H_3-4'', H_3-5'', H_3-6''); 3.10 (1H; ~br d; J=17.3 Hz; H_x-7); 3.19 (1H; ~br dd; J=17.4, 6.0 Hz; H_y-7); 4.21 (1H; br d; J=15.6 Hz; H_x-4); 4.78 (1H; br s; H-6); 4.92 (1H; d; J=16.2 Hz; H_y-4); 9.11 (2H; s; H-3', H-5').

^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) 17.1 (C-8); 27.9 (C-4'', C-5'', C-6''); 28.6 (C-7); 36.5 (C-4)*; 43.6 (C-6)*; 79.6 (C-3''); 130.8 (C-7a); 139.7 (C-3a); 147.4 (d; J=23.0 Hz, C-3', C-5'); 150.6 (d; J=3.3 Hz; C-1'); 153.8 (C-1''); 156.7 (d; J=261.1 Hz; C-4').

* Due to severe line broadening in the ^{13}C spectrum, this ^{13}C resonance was verified by the ^{1}H-^{13}C HSQC experiment

ESI-HRMS: calcd for $C_{15}H_{20}O_2N_6F$ $[M+H]^+$: 335.16263; found: 335.16216; delta= -1.40 ppm.



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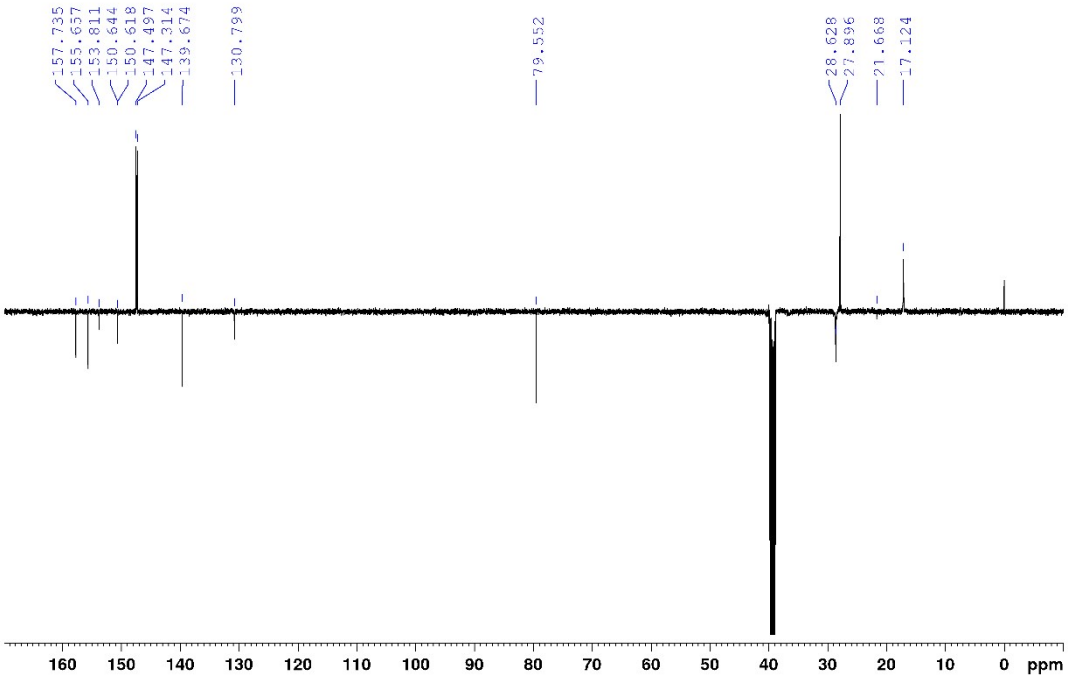
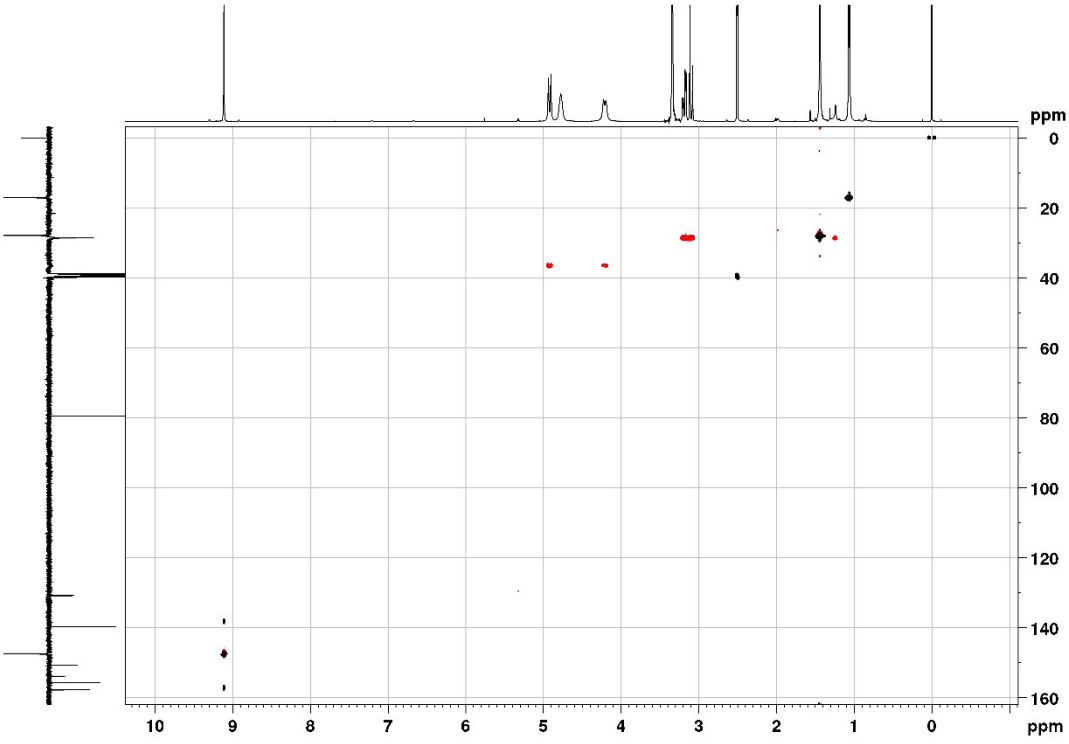
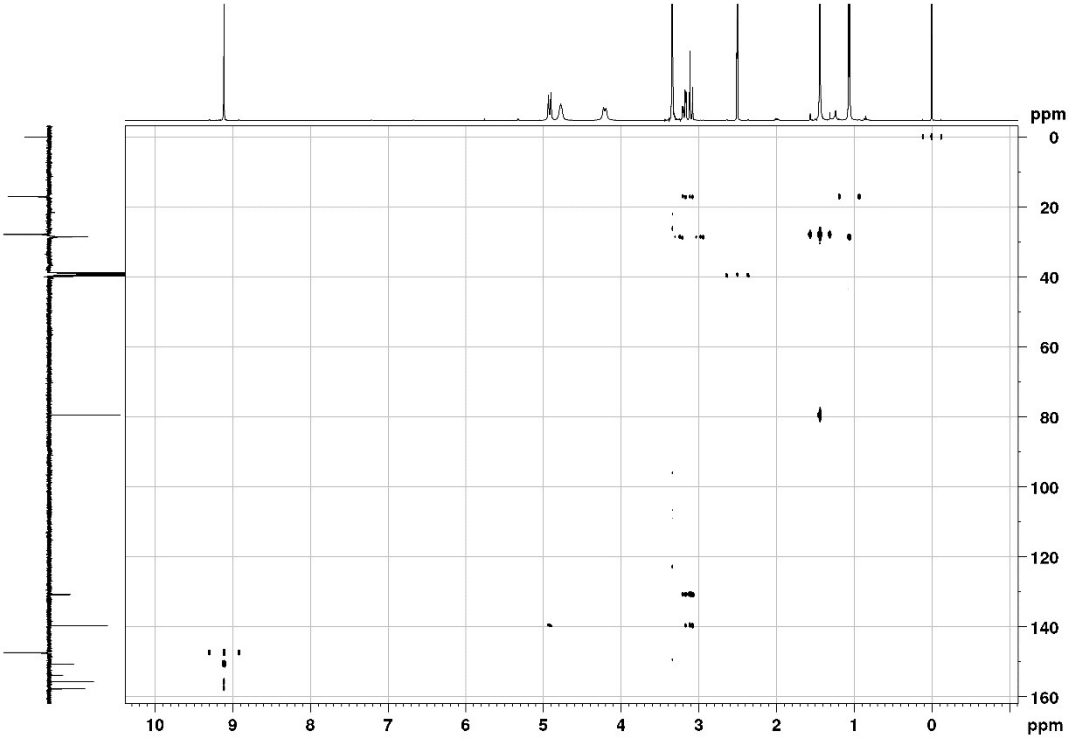


13C-DEPTQ



HSQC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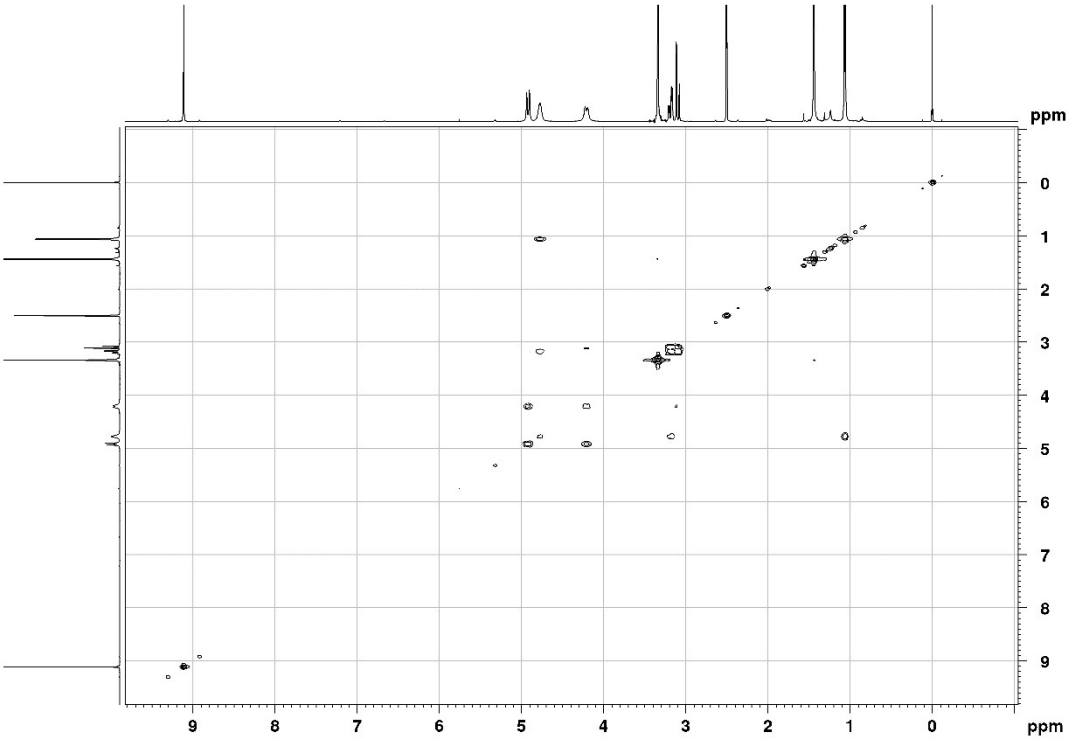
ppm

HMBC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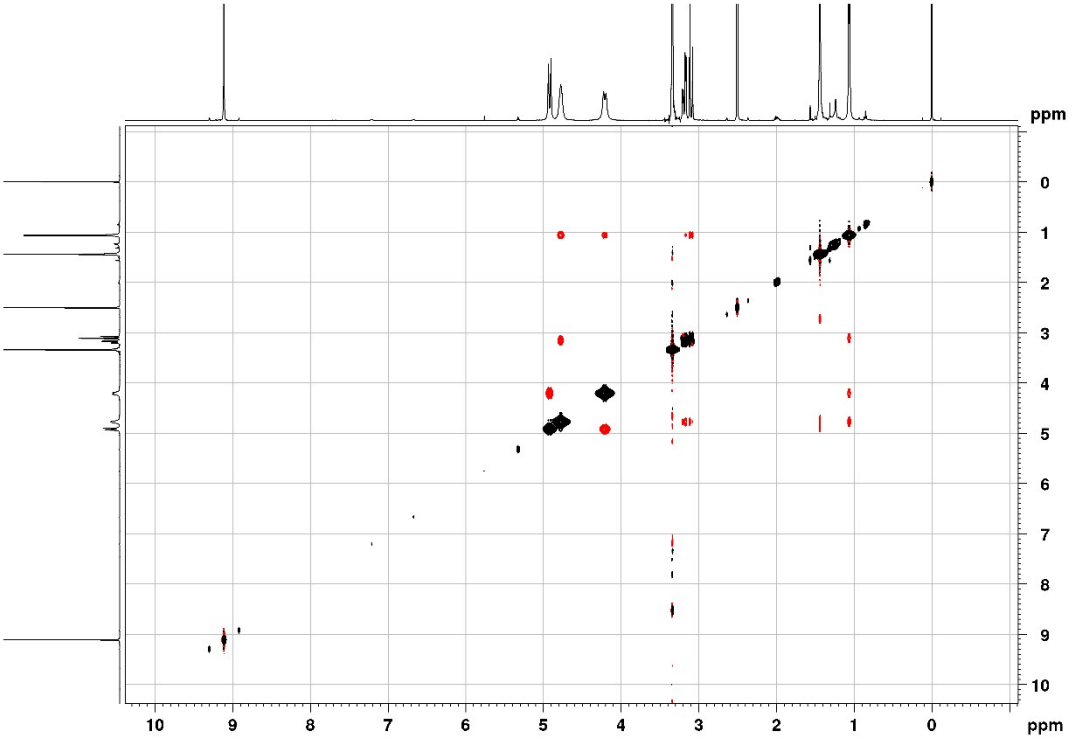
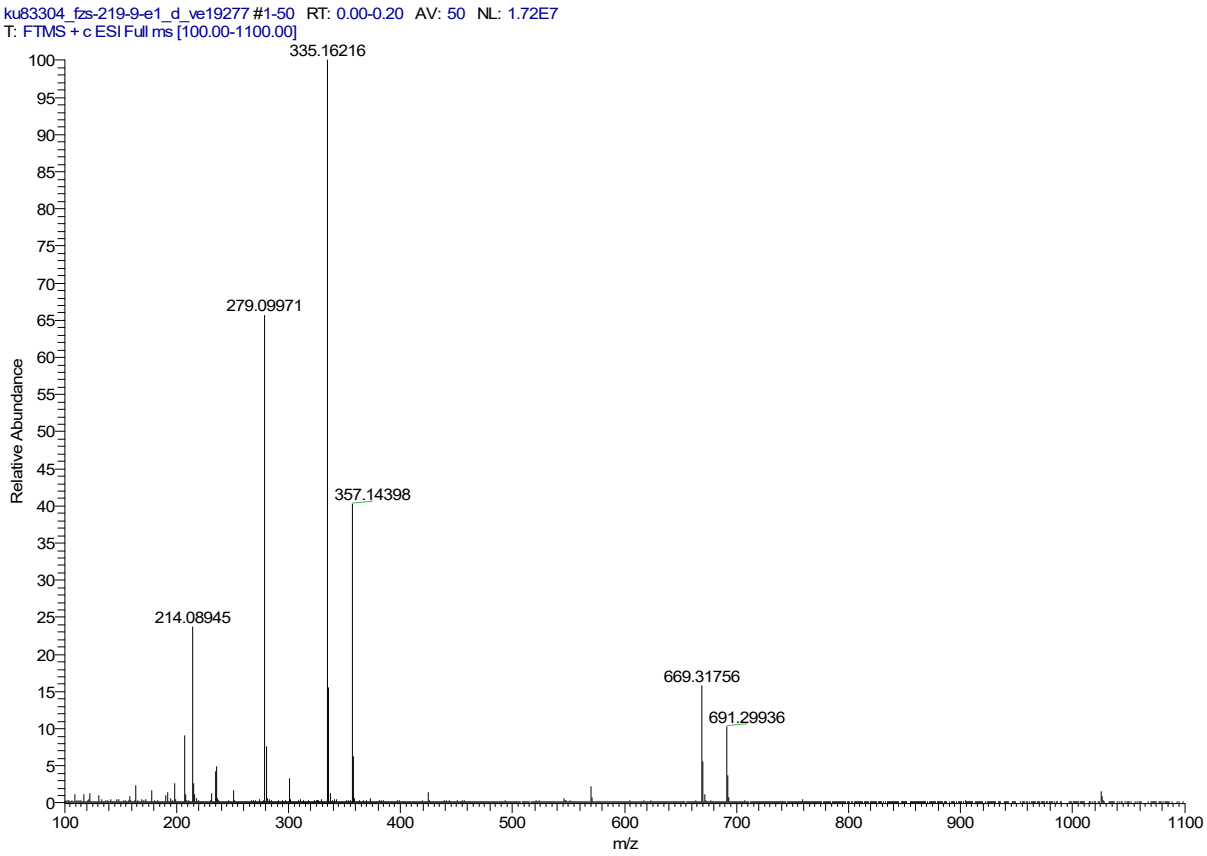
ppm

COSY

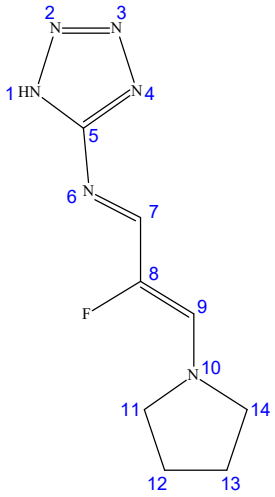


NOESY



ESI-HRMS spectrum

(1*E*,2*Z*)-2-fluoro-3-(pyrrolidin-1-yl)-*N*-(1*H*-tetrazol-5-yl)prop-2-en-1-imine (9)



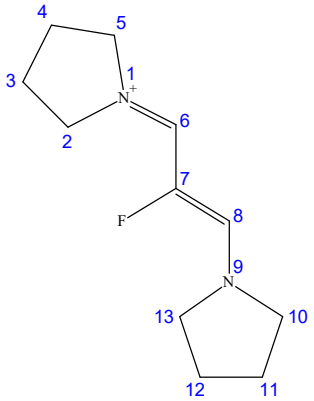
^{1}H NMR (799.7 MHz; DMSO-d_6): δ (ppm) 1.86-1.93 (4H; m; H_2-12, H_2-13); 3.59-3.66 (4H; m; H_2-11, H_2-14); 7.39 (1H; d; *J*=29.0 Hz; H-9); 8.23 (1H; d; *J*=24.0 Hz; H-7).

^{13}C NMR (200.1 MHz; DMSO-d_6): δ (ppm) 24.7 (C-12, C-13); 51.2 (C-11, C-14)*; 137.1 (d; *J*=231.2 Hz; C-8); 139.2 (C-9)*; 151.8 (C-7)*; 162.5 (C-5).

* Due to severe line broadening in the ^{13}C-DEPTQ spectrum, these ^{13}C resonances were verified by the ^{1}H-^{13}C HSQC experiment

ESI-HRMS: calcd for $C_8H_{12}N_6F$ $[M+H]^+$: 211.11020; found: 211.11087; delta= 3.18 ppm.

Note: due to the rapid decomposition of the compound in solution, the NMR spectra below contain impurity signals, including pyrrolidine (denoted with green asterisks) and a "dimeric" analogue (denoted with blue asterisks); the proposed structure and signal assignments of the latter are provided below.

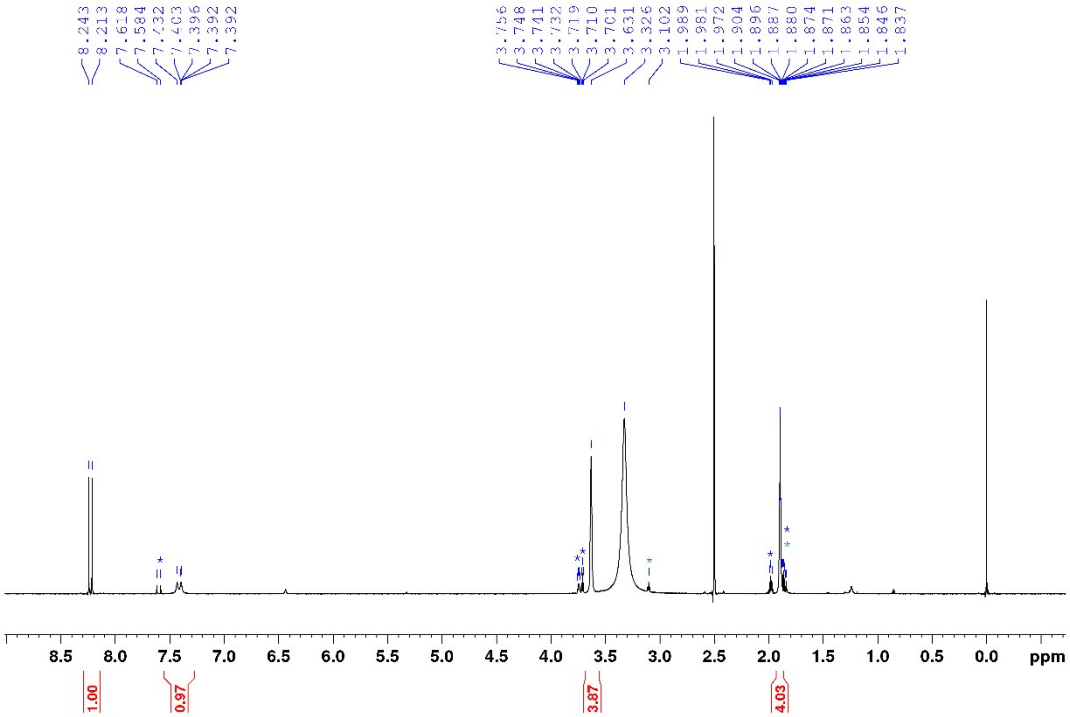


^{1}H NMR (799.7 MHz; DMSO-d_6): δ (ppm) 1.86 (4H; qui; J=6.8 Hz), 1.98 (4H; qui; J=7.1 Hz) (H_2-3, H_2-4, H_2-11, H_2-12); 3.71 (4H; t; J=6.9 Hz), 3.74 (br q; J=6.0 Hz) (H_2-2, H_2-5, H_2-10, H_2-13); 7.60 (2H; d; J=27.9 Hz; H-6, H-8).

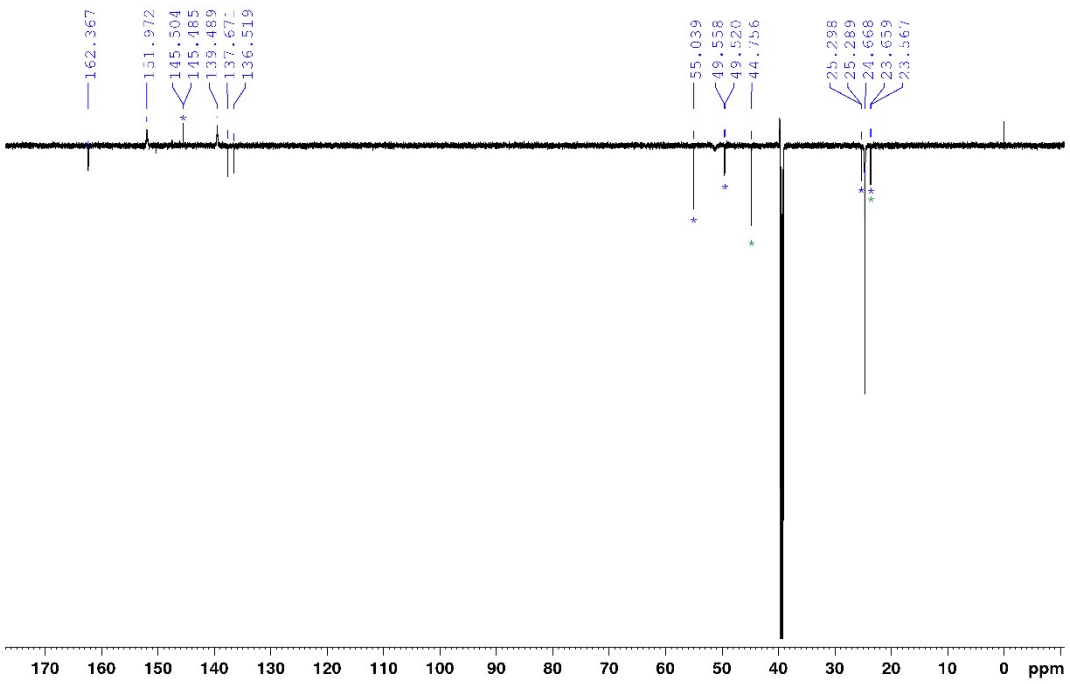
^{13}C NMR (200.1 MHz; DMSO-d_6): δ (ppm) 23.7, 25.3 (C-3, C-4, C-11, C-12); 49.5, 49.6, 55.0 (C-2, C-5, C-10, C-13); 132.9 (C-7)*; 145.9 (d; J=3.8 Hz; C-6, C-8).

* Due to insufficient signal-to-noise ratio in the ^{13}C-DEPTQ spectrum, this ^{13}C resonance was verified by the ^{1}H-^{13}C HMBC experiment.

ESI-HRMS: calcd for $C_{11}H_{18}N_2F$ $[M+H]^+$: 197.14485; found: 197.14548; delta= 3.18 ppm.



1H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137.67
136.519
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49.520
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25.289
24.668
23.659
23.567
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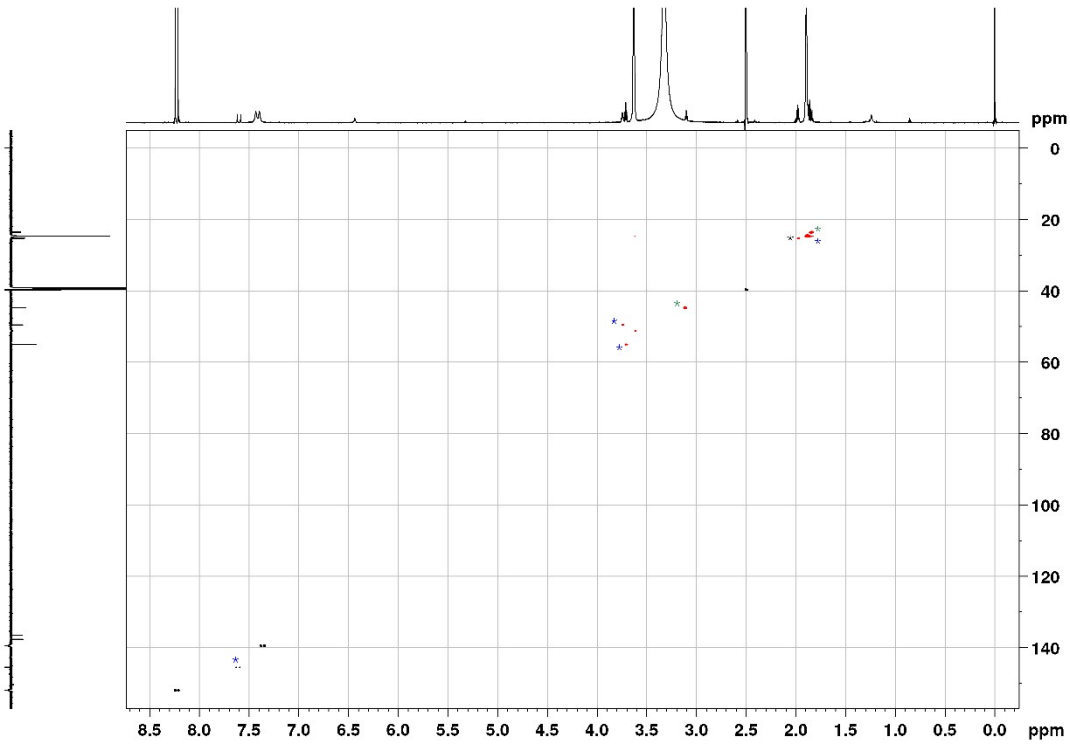
13C-DEPTQ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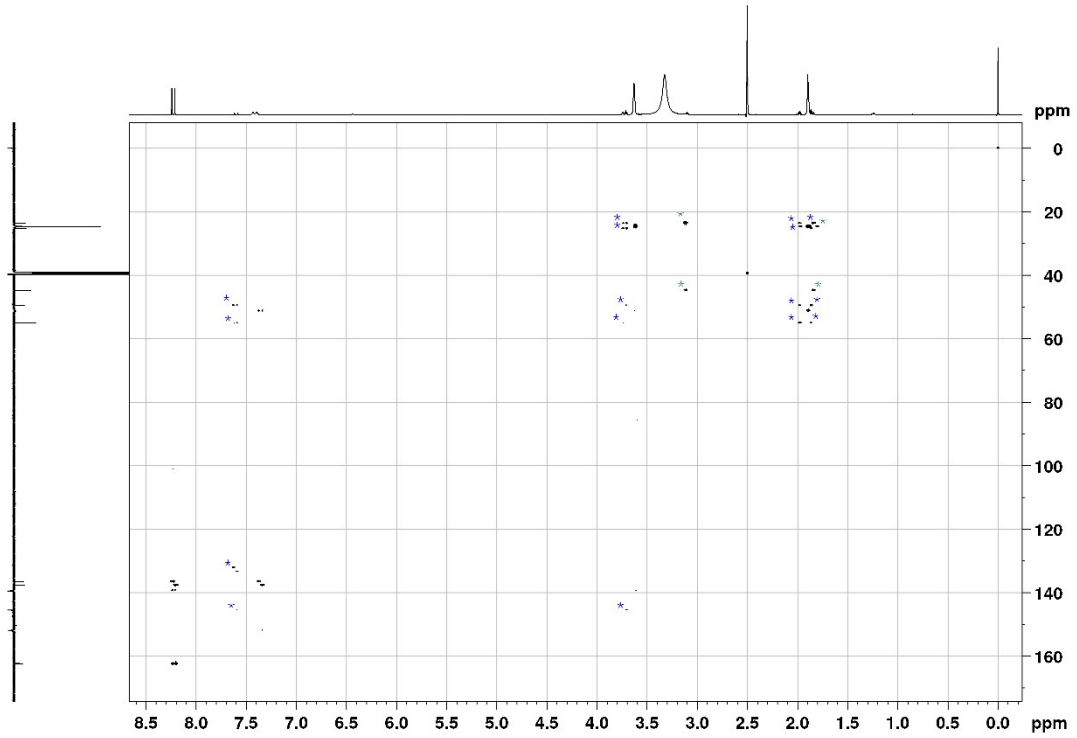
HSQC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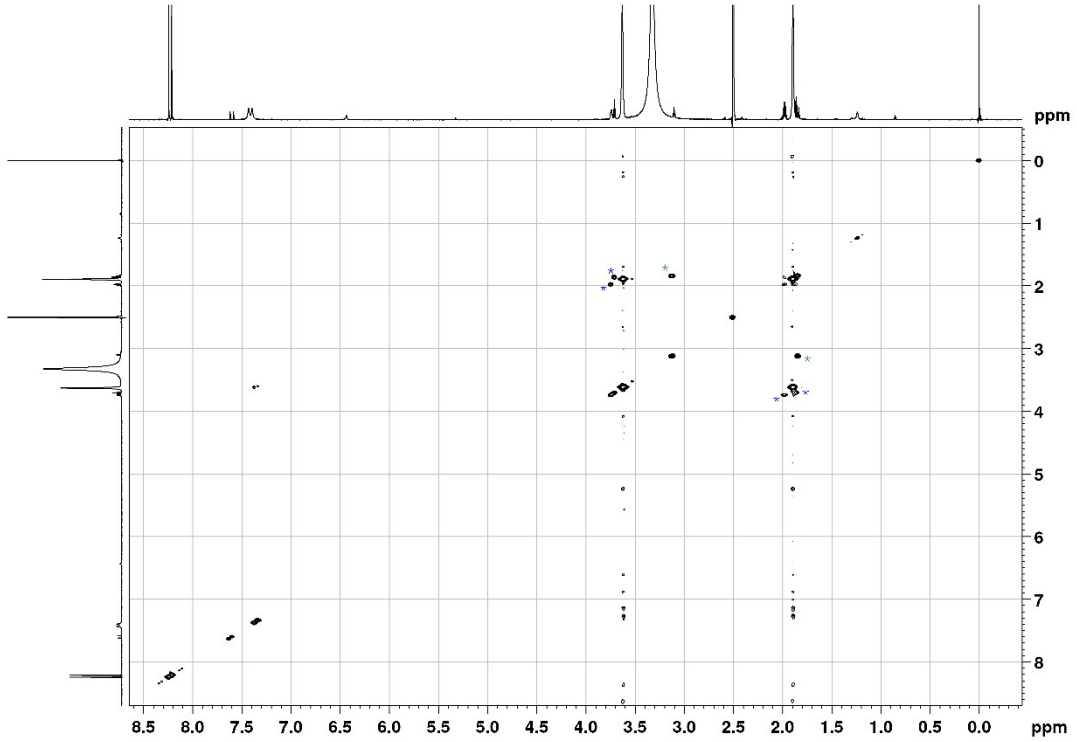
HMBC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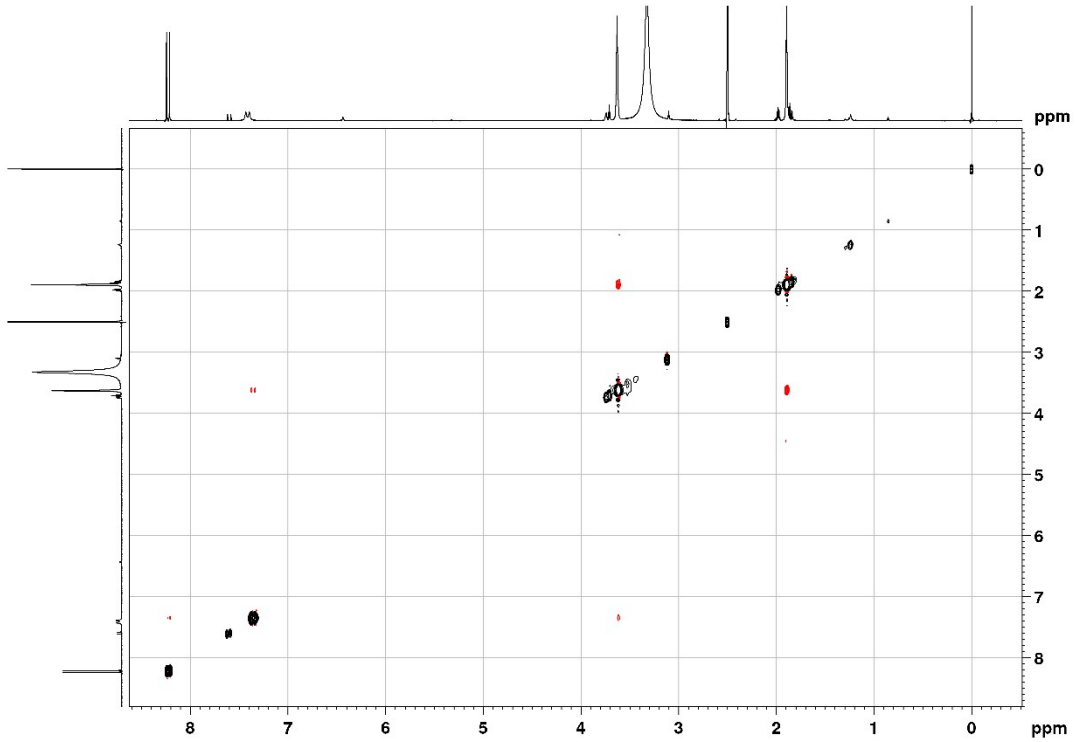
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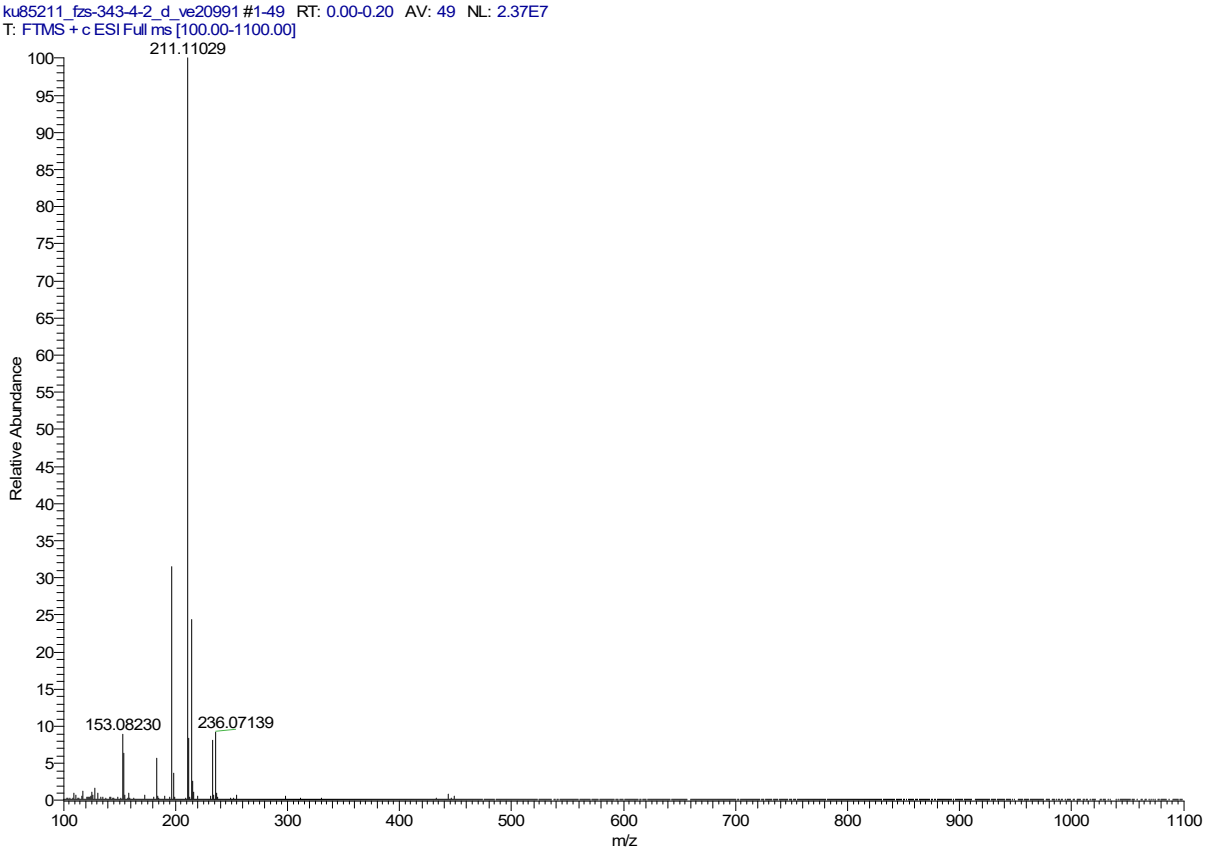
NOESY



ESI-HRMS

References

(1) C. C. Chrovian, A. Soyode-Johnson, A. A. Peterson, C. F. Gelin, X. Deng, C. A. Dvorak, N. I. Carruthers, B. Lord, I. Fraser, L. Aluisio, K. J. Coe, B. Scott, T. Koudriakova, F. Schoetens, K. Sepassi, D. J. Gallacher, A. Bhattacharya and M. A. Letavic, *J Med Chem*, 2018, **61**, 207–223.

(2) https://www.syrris.com.

(3) https://www.idex-hs.com.

(4) https://www.sigmaaldrich.com.