Supporting Information

For

Stereospecific amination of mesylated cyclobutanol in continuous flow

Matthieu Tissot*, Jérôme Jacq, Patrick Pasau

UCB Biopharma SPRL, avenue de l' industrie, 1420 Braine l' Alleud, Belgium
E-mail : matthieu.tissot@ucb.com

Table of Contents

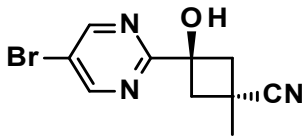
1. General experimental details

Commercially available starting materials were used as received without further purification. All capillary tubing and microfluidic fittings were purchased from IDEX Health & Science. Flow reactions were performed on either R2+ and R4 system from Vapourtec or on Uniqsis FlowSyn using PTFE tubing. Yields refer to isolated yields of compounds estimated to be >95% pure as determined by ^{1}H-NMR spectroscopy. ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker ADVANCE 400 MHz spectrometer at ambient temperature (298K) using the residual solvent peak in CDCl3 (δH = 7.26 ppm, δC = 77.16 ppm) and using TMS as internal standard. The multiplicity of ^{1}H NMR signals are indicated as s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, or combinations of thereof. Coupling constants J are quoted in Hz and are reported to the nearest 0.1 Hz. Where appropriate, averages of the signals that display multiplicity were used to calculate the value of the coupling constant. High resolution mass spectra (HRMS) were recorded on SYNAPT G2-SI Waters Q-TOF instrument equipped with an ESI source and a Waters Acquity H-class UPLC with diode array detector. Melting point were performed on Büchi Melting point B-545

2. Synthetic procedures and characterization of compounds

1.1. Alcohol 3 and 3' characterization

3-(5-bromopyrimidin-2-yl)-3-hydroxy-1-methyl-cyclobutanecarbonitrile (3-*trans*)



Signals of the mixture of products are given.

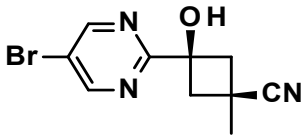
1H NMR (400 MHz, Chloroform-d) δ 8.83 (s, 2H), 4.79 (s, 1H), 3.33 (d, J = 13.5 Hz, 2H), 2.47 (d, J = 13.6 Hz, 2H), 1.78 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 169.01, 157.72, 125.03, 118.89, 72.61, 47.64, 26.48, 24.01.

HRMS (ESI) $[M+H]^+$ calcd. for $C_{10}H_{11}BrN_3O$=268.0085; found 268.0078

Melting point = 168°C

3-(5-bromopyrimidin-2-yl)-3-hydroxy-1-methyl-cyclobutanecarbonitrile (3'-*cis*)



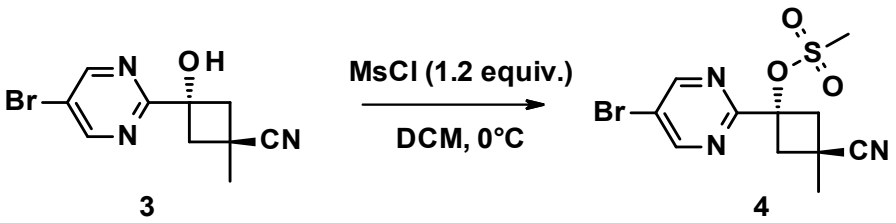
Signals of the mixture of products are given.

1H NMR (400 MHz, Chloroform-d)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.82 (s, 2H), 4.78 (s, 1H), 2.98 (d, *J* = 13.7 Hz, 2H), 2.85 (d, *J* = 13.7 Hz, 2H), 1.71 (s, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.14, 157.65, 125.61, 118.87, 72.53, 47.76, 24.80, 23.87.

HRMS (ESI) $[M+H]^+$ calcd. for $C_{10}H_{11}BrN_3O$=268.0085; found 268.0084
Melting point = 129°C

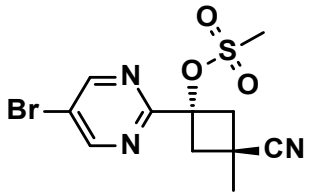
1.2. Typical Procedure for mesylation



Scheme S1 : Mesylation

A solution of 3-(5-bromopyrimidin-2-yl)-3-hydroxy-1-methyl-cyclobutanecarbonitrile **3** (368 g, 1.37 mol) in dichloromethane (3 L) was cooled to 0 °C, and triethylamine (290 ml, 2.058 mol) was added.
A solution of methanesulfonyl chloride (127.9 ml, 1.647 mmol) in dichloromethane (570 mL) was added to the mixture over 35 min with a peristaltic pump (20 ml/min), maintaining the internal temperature to 0°C. The mixture was stirred for 30 min at 0° C, followed by successive addition of a solution of aqueous KHSO4 10% (2 L, w/w) and water (1 L), the resulting mixture was then vigorously stirred at 20-25 °C. The phases were separated, and the aqueous phases were extracted with dichloromethane (1 L). The organic phases were combined and washed with water (1.5 L). Finally, the organic phases were dried with $MgSO_4$ and evaporated to give the crude mesylated alcohol **4**. To the crude mixture was added TBDME (1.5 L), and the resulting slurry was stirred for 30 min. The slurry was filtered, and the cake was washed with two portion of TBDME (2x500 mL). The solids were dried in a vacuum oven at 22 °C for 12 h to afford [1-(5-bromopyrimidin-2-yl)-3-cyano-3-methyl-cyclobutyl] methanesulfonate **4** as a white solid (439 g, 92% yield, NMR purity>99%).

[1-(5-bromopyrimidin-2-yl)-3-cyano-3-methyl-cyclobutyl] methanesulfonate (4-*trans*)



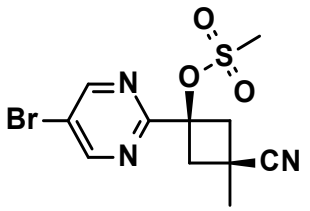
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.88 (s, 2H), 3.58 (d, *J* = 14.2 Hz, 2H), 3.16 (s, 3H), 2.94 (d, *J* = 14.2 Hz, 2H), 1.80 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 165.40, 158.18, 123.65, 119.85, 83.10, 45.20, 40.64, 26.29, 24.92.

HRMS (ESI) $[M+H]^+$ calcd. for $C_{11}H_{13}BrN_3O_3S$=345.9861; found 345.9856

Melting point = 175°C

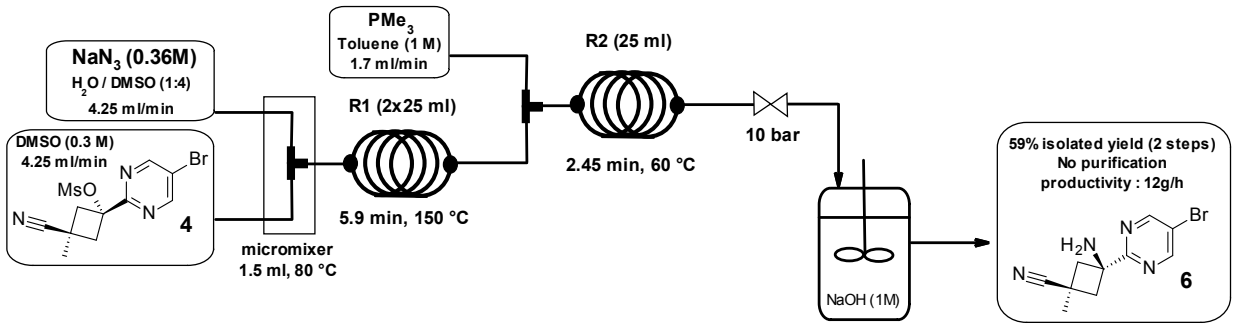
[1-(5-bromopyrimidin-2-yl)-3-cyano-3-methyl-cyclobutyl] methanesulfonate (4'-*cis*)



^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.84 (s, 2H), 3.36 (d, J = 14.8 Hz, 2H), 3.20 (s, 3H), 3.10 (d, J = 14.8 Hz, 2H), 1.59 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 165.69, 158.08, 124.35, 119.71, 84.02, 44.87, 41.09, 25.11, 24.72.

1.3. Procedure for Flow-Scale-up Azidation / Staudinger reduction



Scheme S2 : Flow set-up azidation / Staudinger reduction

Using a Uniqsis Flowsyn[1], a solution of [1-(5-bromopyrimidin-2-yl)-3-cyano-3-methyl-cyclobutyl] methanesulfonate **4** (20.77 g, 60 mmol, 0.3 M in DMSO) was pumped at a rate of 4.25 ml/min and mixed in a micromixer (1.6 ml, 80 °C) with a solution of sodium azide (4.68 g, 72 mmol, 0.36 M in a mixture DMSO/Water (4:1, v/v)) pumped at a rate of 4.25 ml/min (1.2 equiv.)(both solutions were engaged simultaneously to the flow set up). The mixture was then passed through a 2x25 mL PTFE reactor at 150 °C (total flow rate of 8.5 ml/min for a residence time of 5.9 min). The resulting solution of intermediate azide **5** was then mixed in a T-piece with a solution of trimethylphosphine (1 M in toluene), pumped at a rate of 1.7 mL/min (1.1 equiv). This was directed to a 25 mL reactor at 60 °C (total flow rate of 10.2 ml/min for a residence time of 2.45 min). The resulting mixture of amine **6** was passed through a static back pressure regulator (10 bars). Finally, the output stream was collected under an atmosphere of nitrogen in a batch reactor containing aqueous 1 M NaOH (600 ml) (scheme 1).

The reaction mixture was diluted in water (400 mL) and extracted three times with 400 mL of *tert*-butyl methyl ether . The organic phases were combined and washed with brine (400 mL). The organic phase was then evaporated. The crude product was next dissolved in 200 mL of EtOAc and extracted by addition of 1 M HCl (200 mL). The aqueous phase was carefully basified through slow addition of 12 M NaOH (10 mL) to pH 14, the solution was then extracted twice with EtOAc (2 x 150 mL). The organic phases were combined, evaporated and dried with $MgSO_4$ to generate the pure amine **6** as a white solid (9.4 g, 59% yield[2], NMR purity>99%).

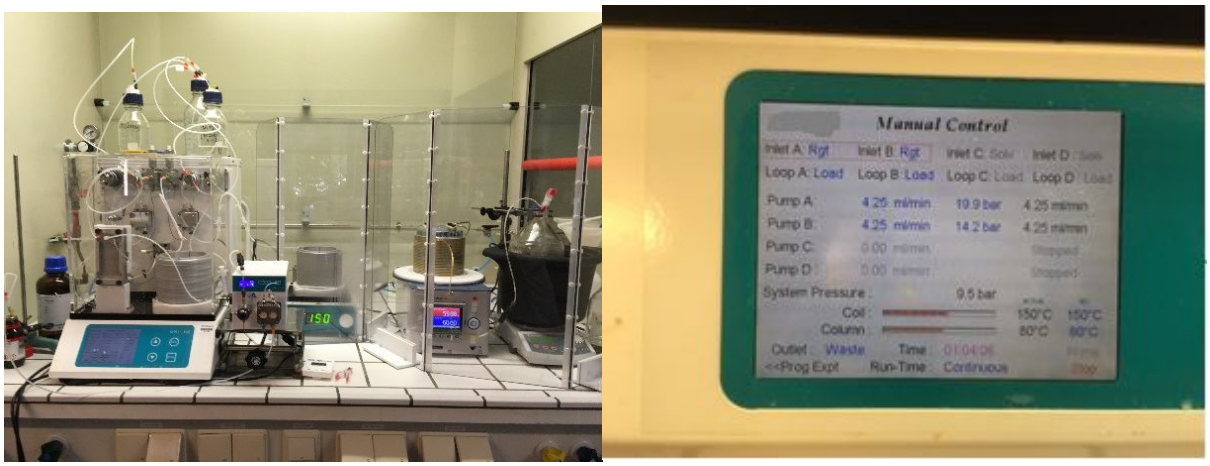
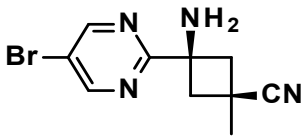


Figure S1 : Uniqsis flow set-up

[1] The whole system was first conditioned with DMSO for 20 min at 4.25 ml/min with R1=150 °C, R2=60 °C and mixer = 80 °C, same conditions were used to clean the system at the end of the process.
[2] Yield calculation was based on the total amount of reagents engaged in the process, 60 mmol of **4**.

3-amino-3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobutanecarbonitrile (6-*cis*)



^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.75 (s, 2H), 2.84 (m, 2H), 2.68 (m, 2H), 2.21 (s, 2H), 1.55 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 171.25, 157.74, 126.11, 118.35, 56.07, 46.71, 25.51, 24.62.

HRMS (ESI) $[M+H]^+$ calcd. for $C_{10}H_{12}BrN_4$=267.0245; found 267.0247

Melting point = 118°C

Molecular structure of the 6-*cis* compound

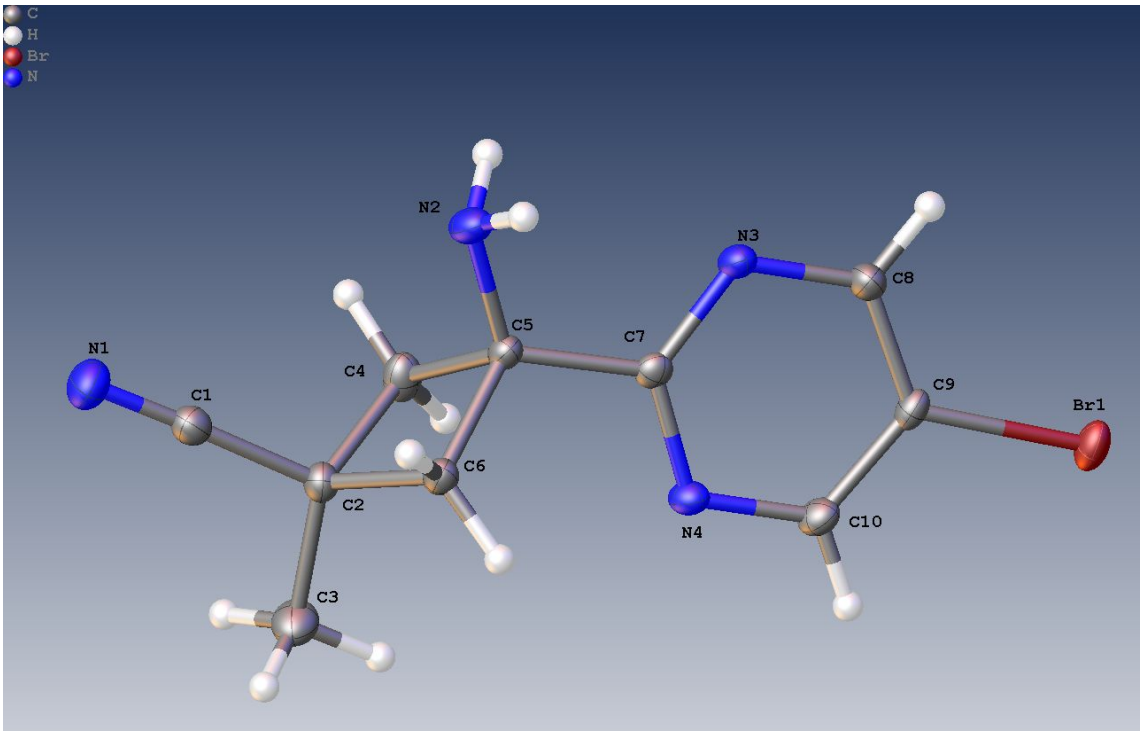


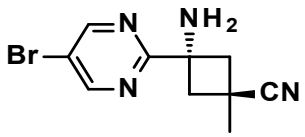
Figure S2. Asymmetric unit and labelling scheme of the of the **6**-*cis* compound.

Table S1. Crystal data and structure refinement for the 6-cis compound

Identification code	nt0663_UCB_DV016480-S-02_reMo
Empirical formula	$C_{10}H_{11}N_4Br$
Formula weight	267.14
Temperature/K	100(2)
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	6.0580(3)
b/Å	11.4750(4)
c/Å	15.7975(7)
α/°	90
β/°	98.286(4)
γ/°	90
Volume/Å^3	1086.72(8)
Z	4
ρ_{calc} /g·cm^{-3}	1.633
μ/mm^{-1}	3.755
F(000)	536.0
Crystal size/mm^3	0.68 × 0.37 × 0.29
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	4.404 to 65.492
Index ranges	$-8 \le h \le 9$, $-14 \le k \le 16$, $-14 \le l \le 23$
Reflections collected	7348
Independent reflections	3613 [R_{int} = 0.0259, R_{sigma} = 0.0430]
Data/restraints/parameters	3613/0/145
Goodness-of-fit on $\|F\|^2$	1.044
Final R indexes [I≥2σ (I)]	R_1 = 0.0313, wR_2 = 0.0649
Final R indexes [all data]	R_1 = 0.0450, wR_2 = 0.0695
Largest diff. peak/hole / e·Å^{-3}	0.69/-0.52

Crystal Data for C10H11N4Br (M =267.14 g/mol): monoclinic, space group P21/n (no. 14), a = 6.0580(3) Å, b = 11.4750(4) Å, c = 15.7975(7) Å, β = 98.286(4)°, V = 1086.72(8) Å3, Z = 4, T = 100(2) K, μ(MoKα) = 3.755 mm-1, Dcalc = 1.633 g/cm3, 7348 reflections measured (4.404° ≤ 2θ ≤ 65.492°), 3613 unique (Rint = 0.0259, Rsigma = 0.0430) which were used in all calculations. The final R1 was 0.0313 (I > 2σ(I)) and wR2 was 0.0695 (all data).

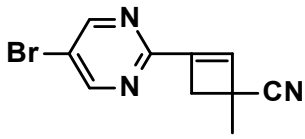
3-amino-3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobutanecarbonitrile (6'-*trans*)



^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.77 (s, 2H), 3.33 (d, J = 13.0 Hz, 2H), 2.26 (d, J = 13.0 Hz, 2H), 2.12 (s, 2H), 1.75 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 171.48, 157.86, 125.19, 118.44, 55.77, 45.98, 26.52, 24.07.

3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobut-2-ene-1-carbonitrile (8)



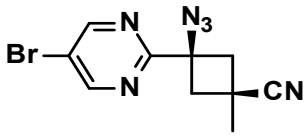
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.77 (s, 2H), 6.84 (s, 1H), 3.46 (d, J = 13.6 Hz, 1H), 2.93 (d, J = 13.7 Hz, 1H), 1.68 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ 157.84, 157.80, 145.64, 138.51, 121.87, 119.24, 41.92, 33.72, 22.96.

HRMS (ESI) $[M+H]^+$ calcd. for $C_{10}H_9BrN_3$=249.9980; found 249.9982

Melting point = 180°C

3-azido-3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobutanecarbonitrile (5)



^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.84 (s, 2H), 3.22 – 2.71 (m, 4H), 1.54 (s, 3H).

DSC analysis : performed on the pure compound

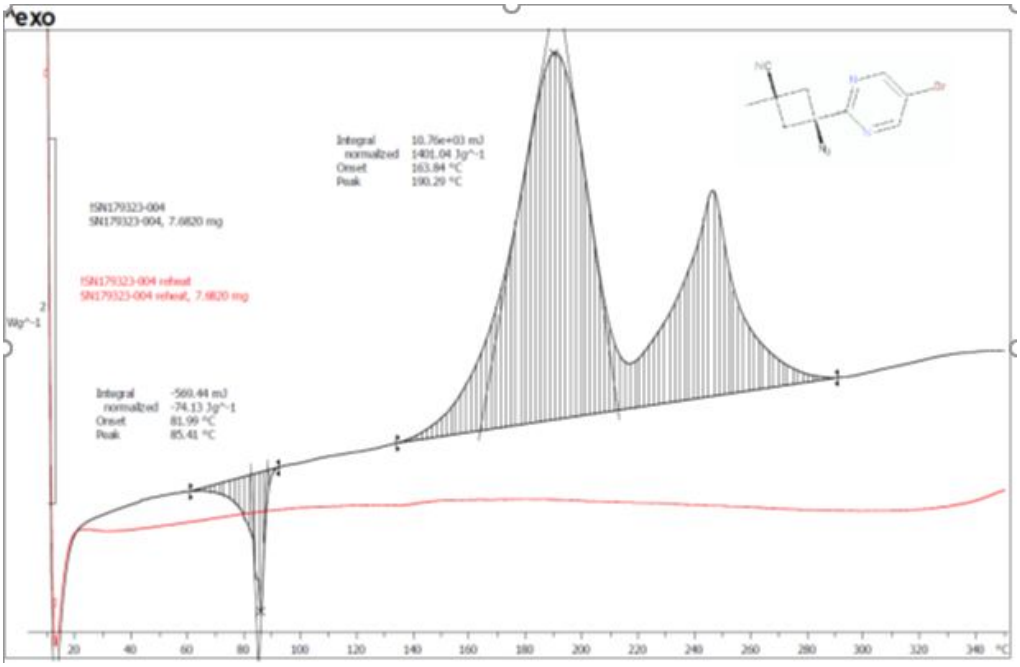


Figure S3 : DSC on pure compound **5**

DSC conditions : Sealed, high pressure, gold plated DSC crucible, 4°C/min from 10 to 350°C

-Endotherm 60 to 90°C, 74 J/g
-Major double exotherm 135 to 290°C, 1400 J/g

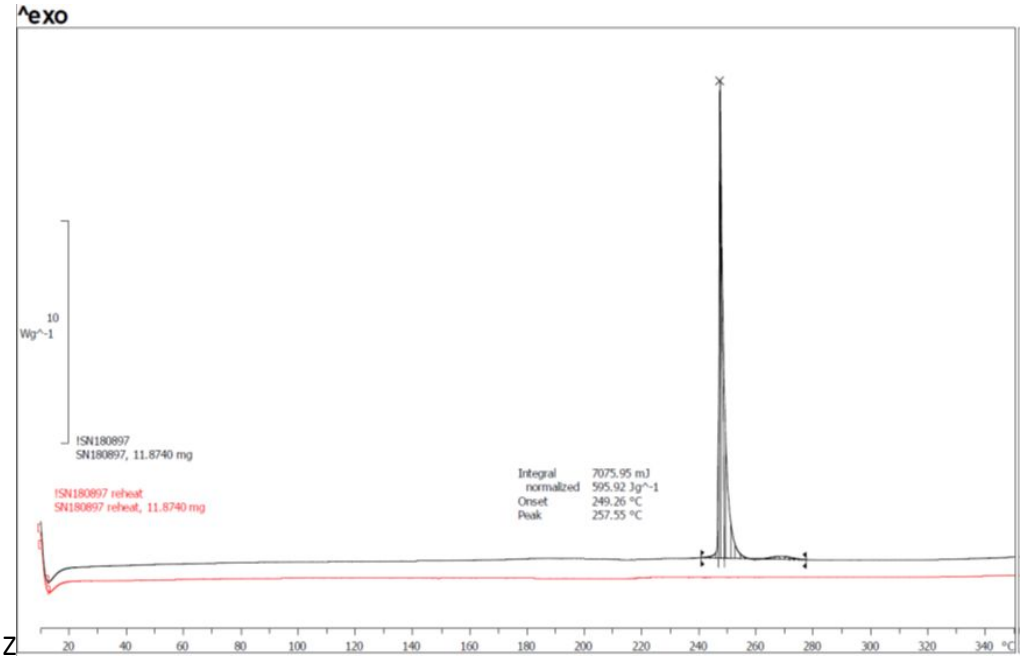
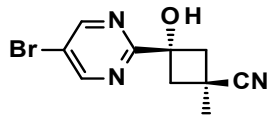


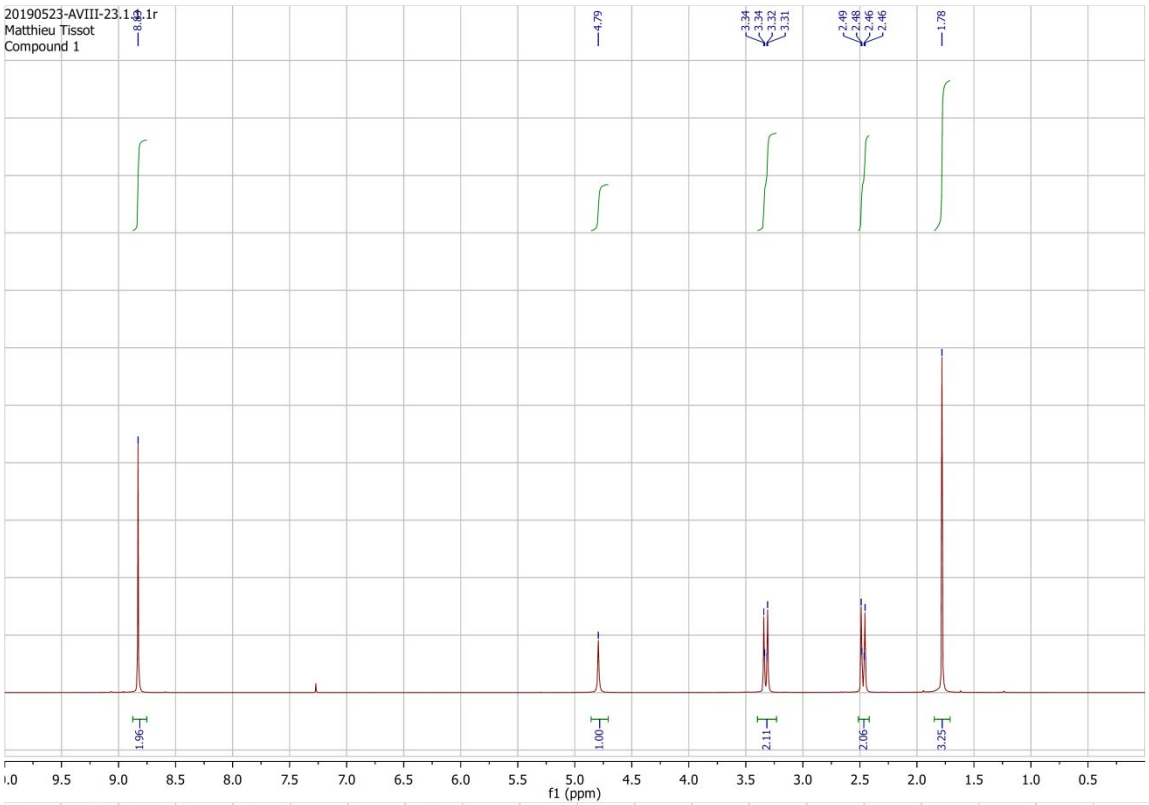
Figure S4 : DSC on the output mixture of entry 4, table 1

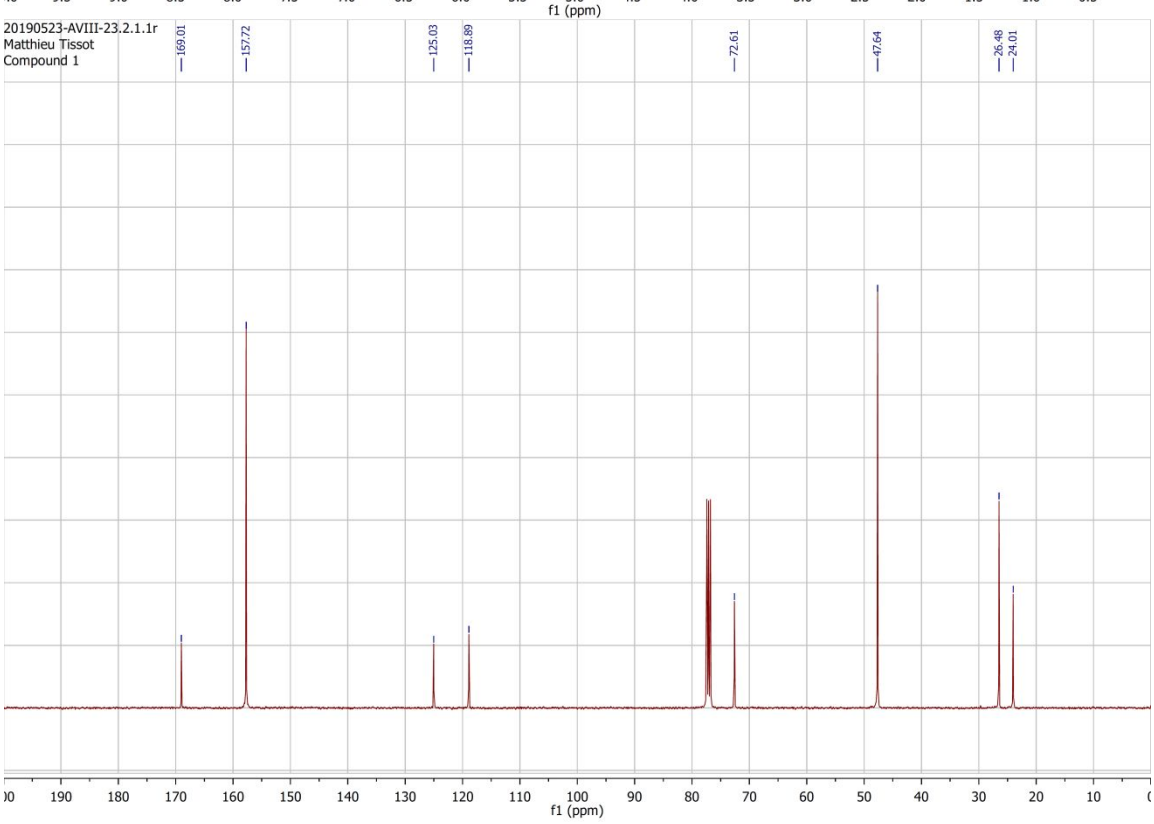
- exotherm 250°C, 595 J/g

3. NMR spectra

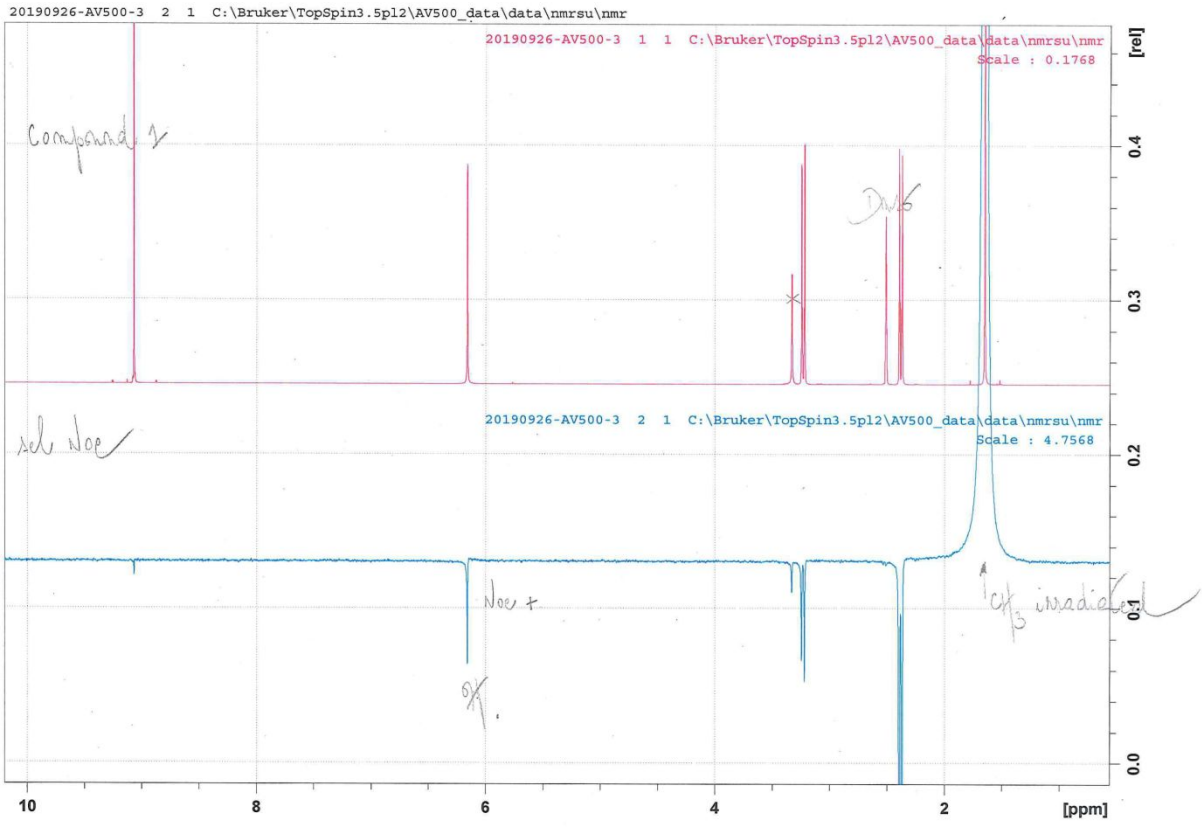
3-(5-bromopyrimidin-2-yl)-3-hydroxy-1-methyl-cyclobutanecarbonitrile (3-*trans*)



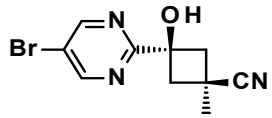


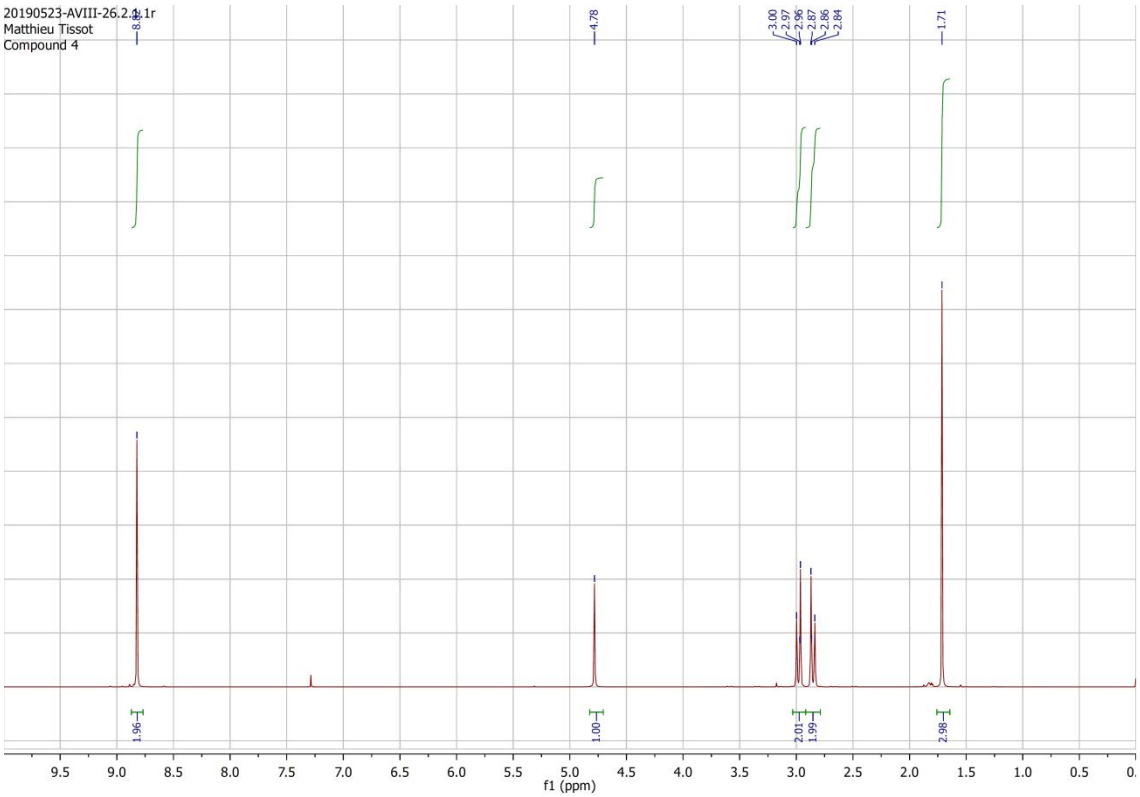


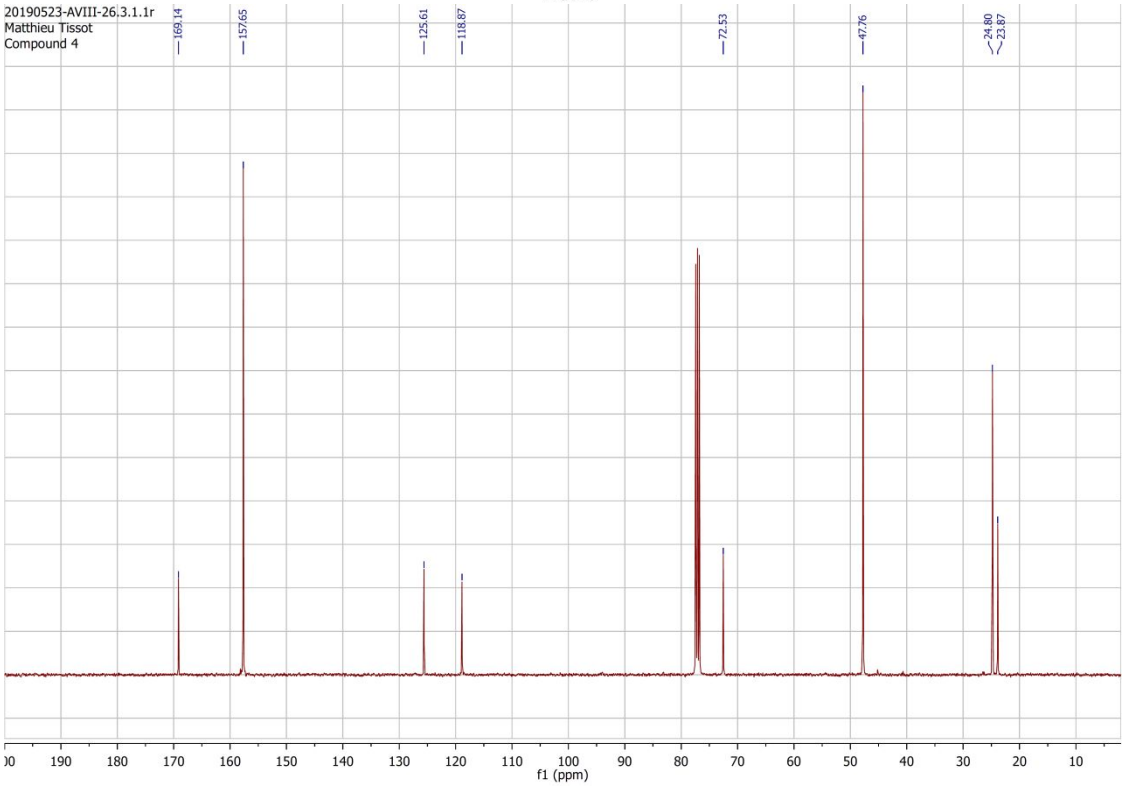
Selective 1D NOE experiment / CH3 irradiated in DMSO-d6



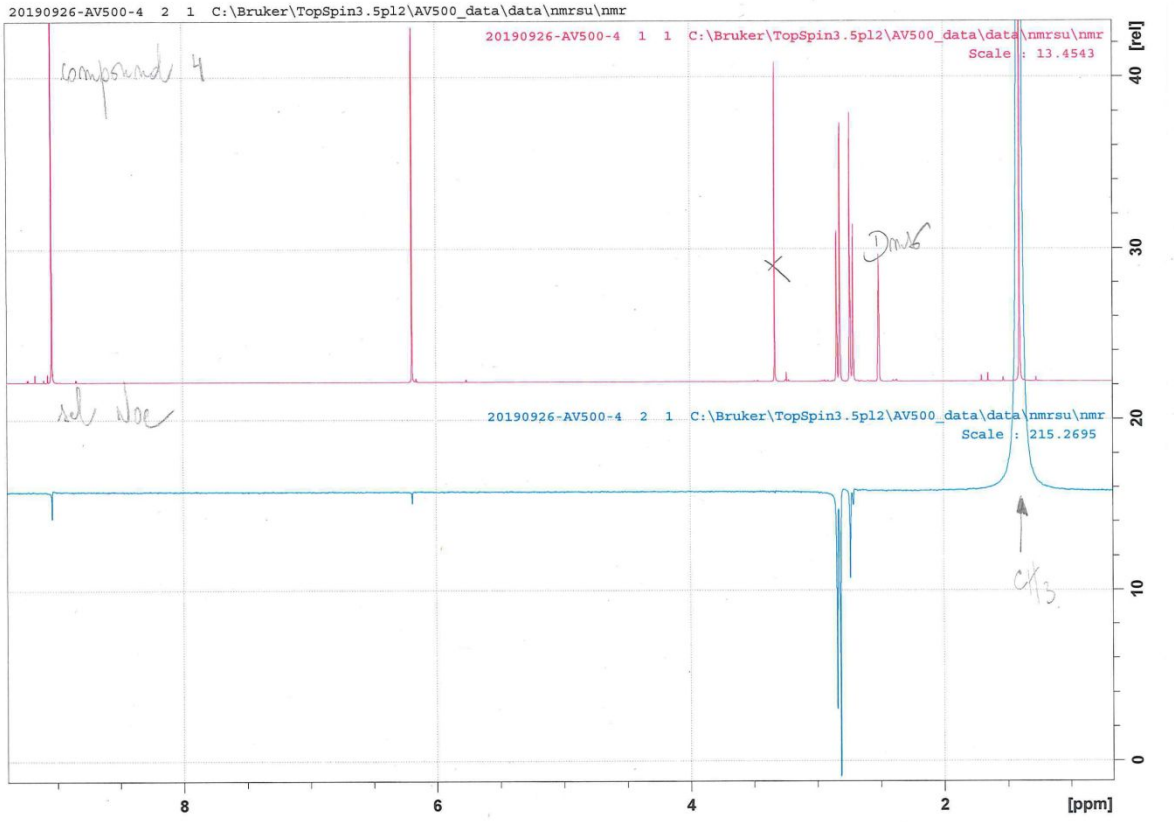
3-(5-bromopyrimidin-2-yl)-3-hydroxy-1-methyl-cyclobutanecarbonitrile (3-*cis*)



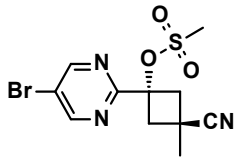


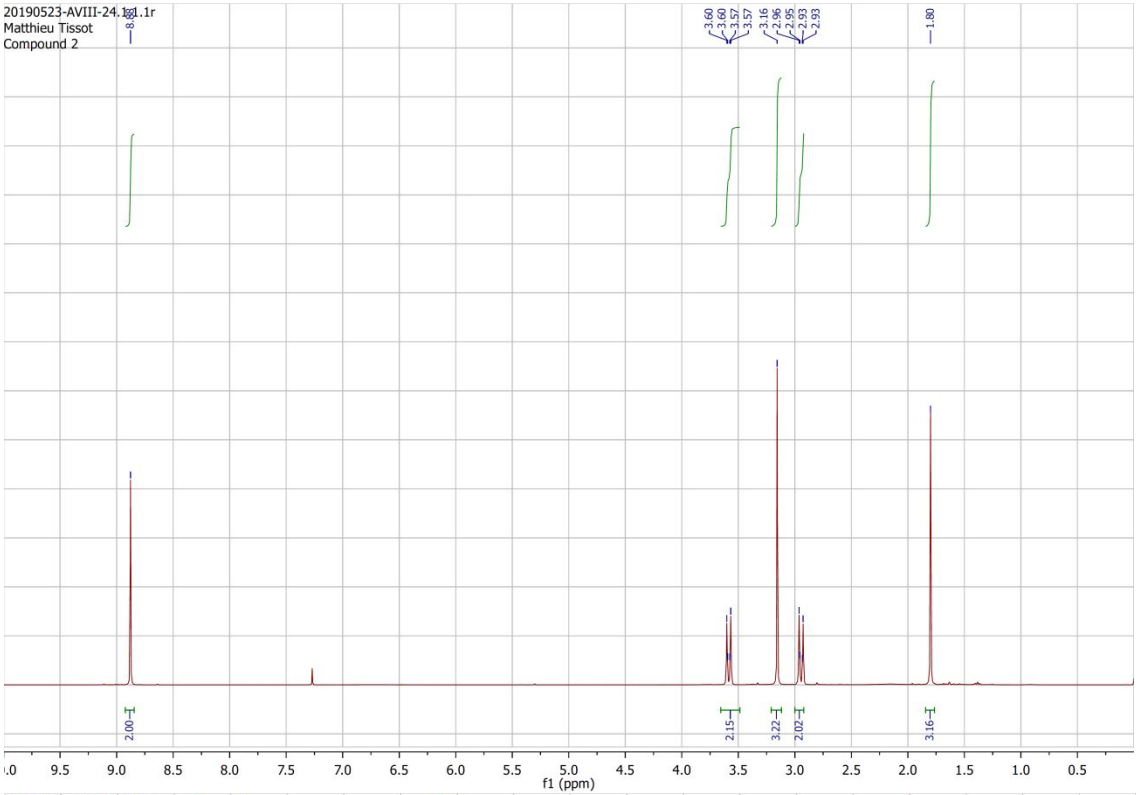


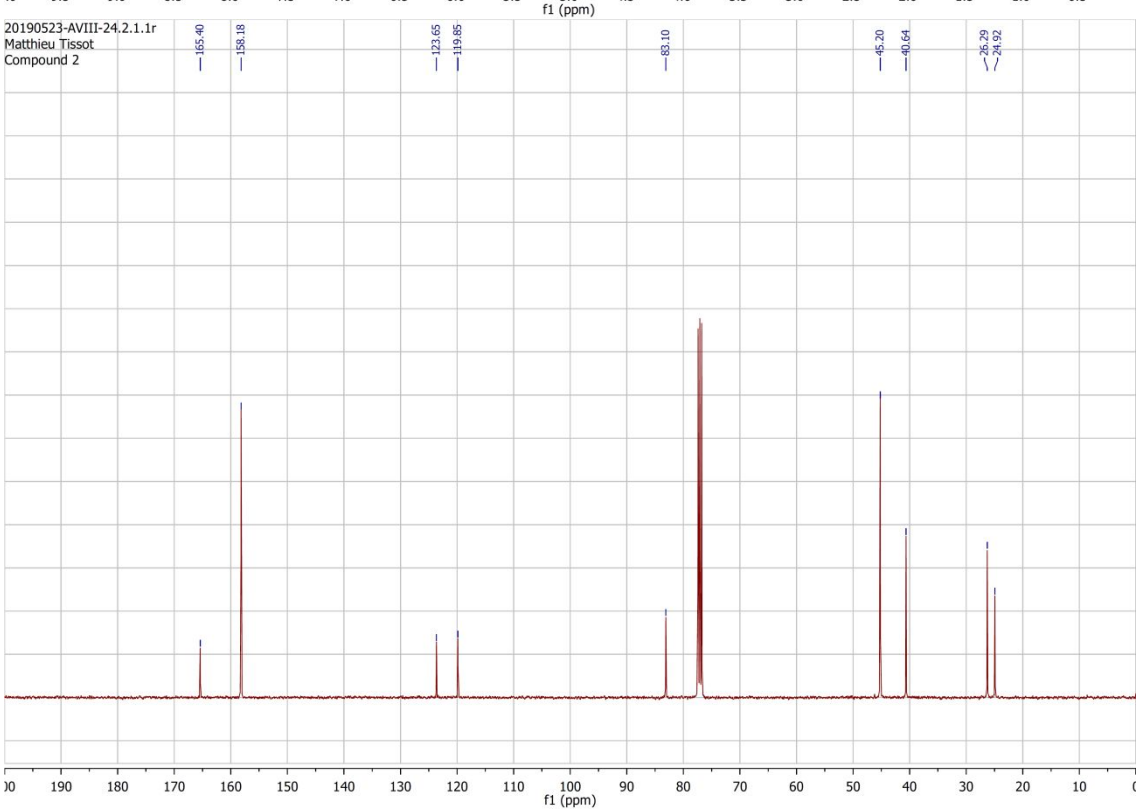
Selective 1D NOE experiment / CH3 irradiated in DMSO-d6 => Correlation OH and CH3 weak=>*cis* isomer



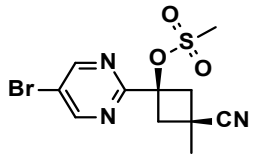
[1-(5-bromopyrimidin-2-yl)-3-cyano-3-methyl-cyclobutyl] methanesulfonate (4-*trans*)

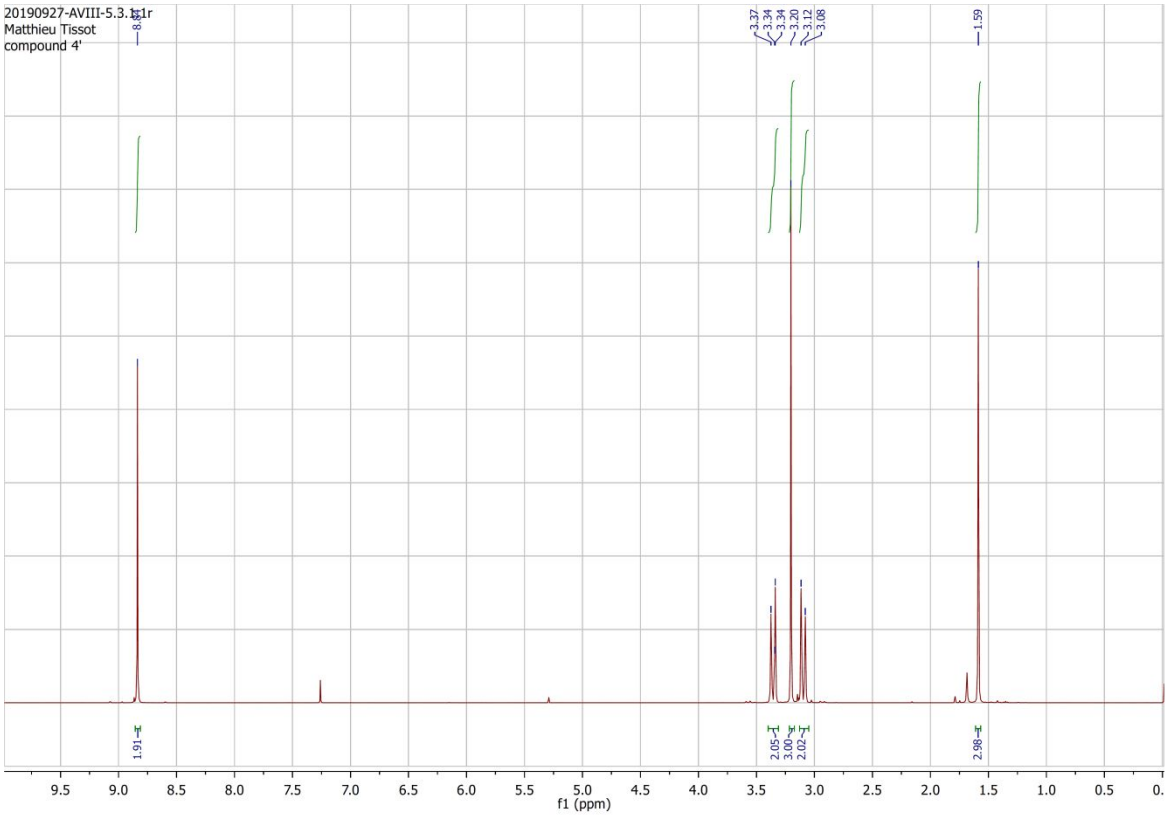


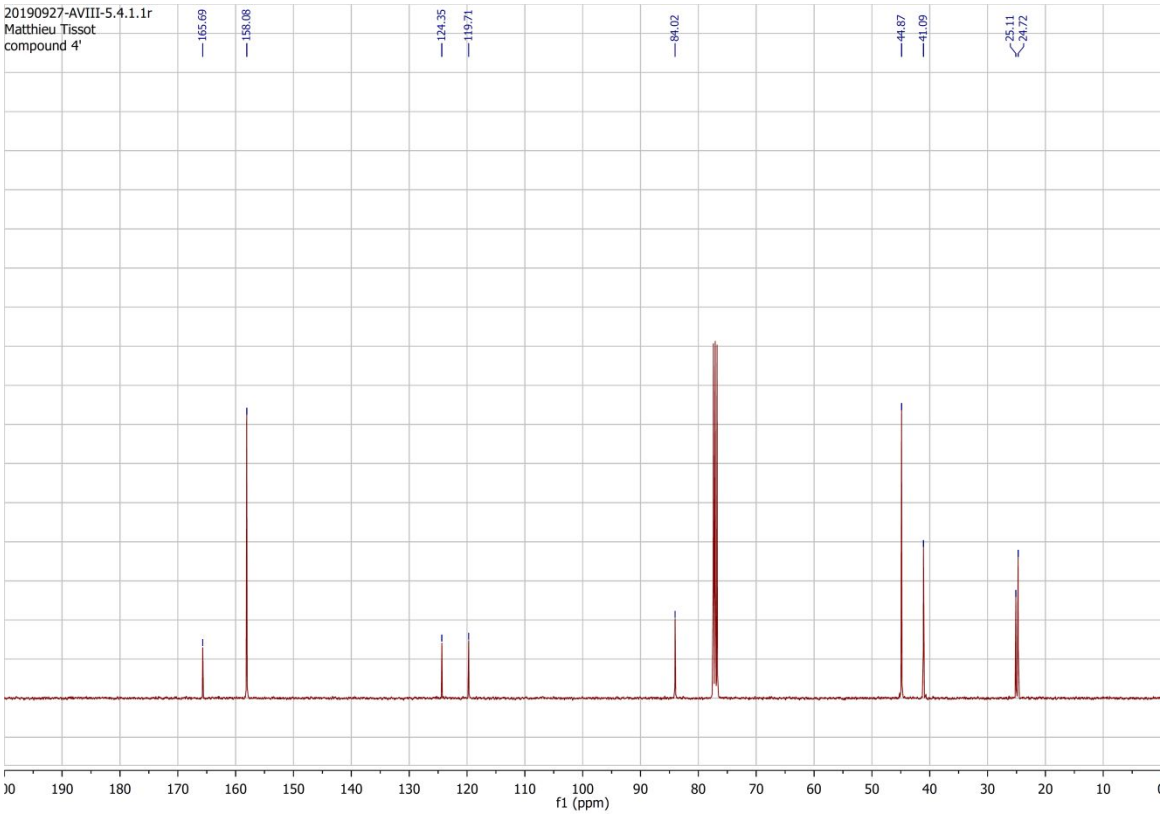




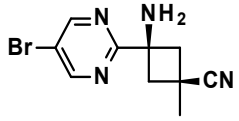
[1-(5-bromopyrimidin-2-yl)-3-cyano-3-methyl-cyclobutyl] methanesulfonate (4'-*cis*)

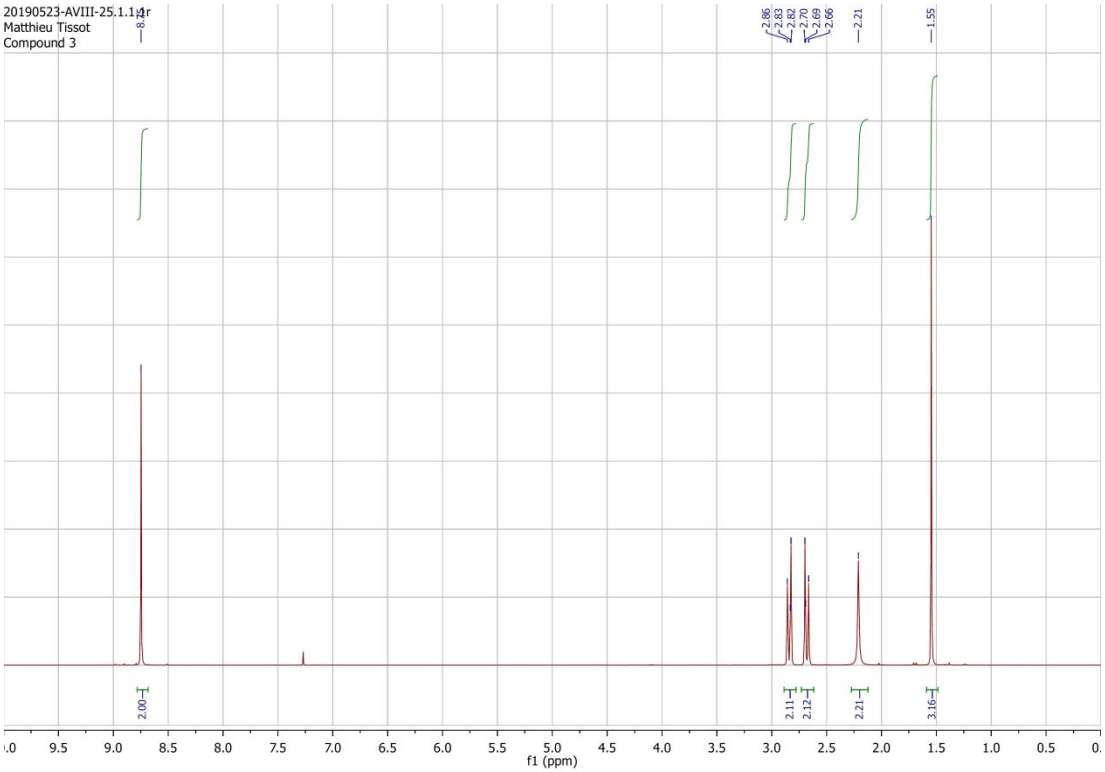


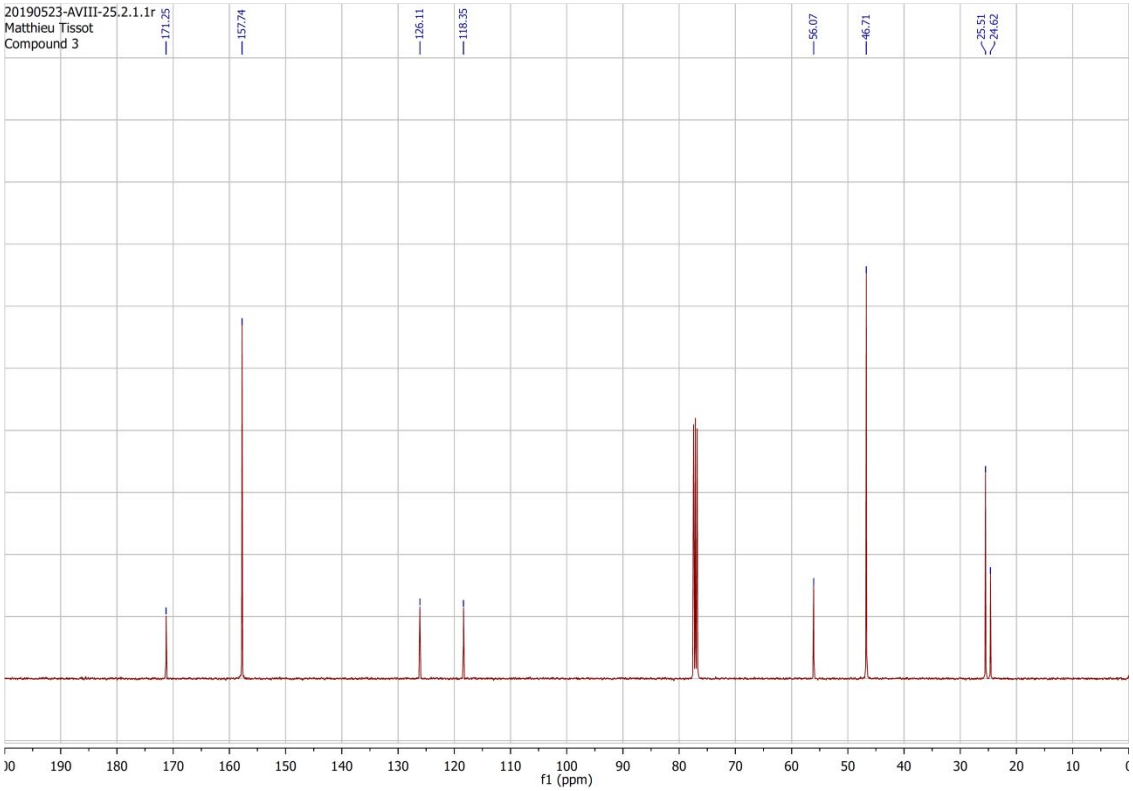




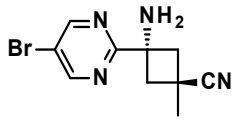
3-amino-3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobutanecarbonitrile (6-*cis*)

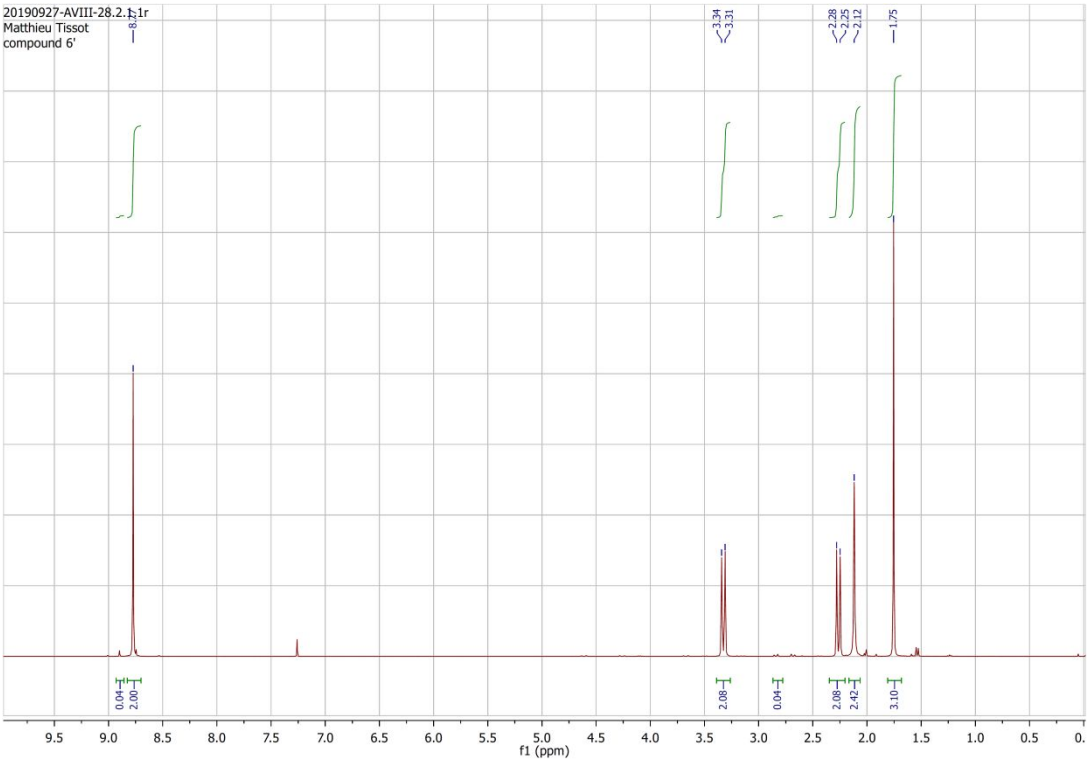


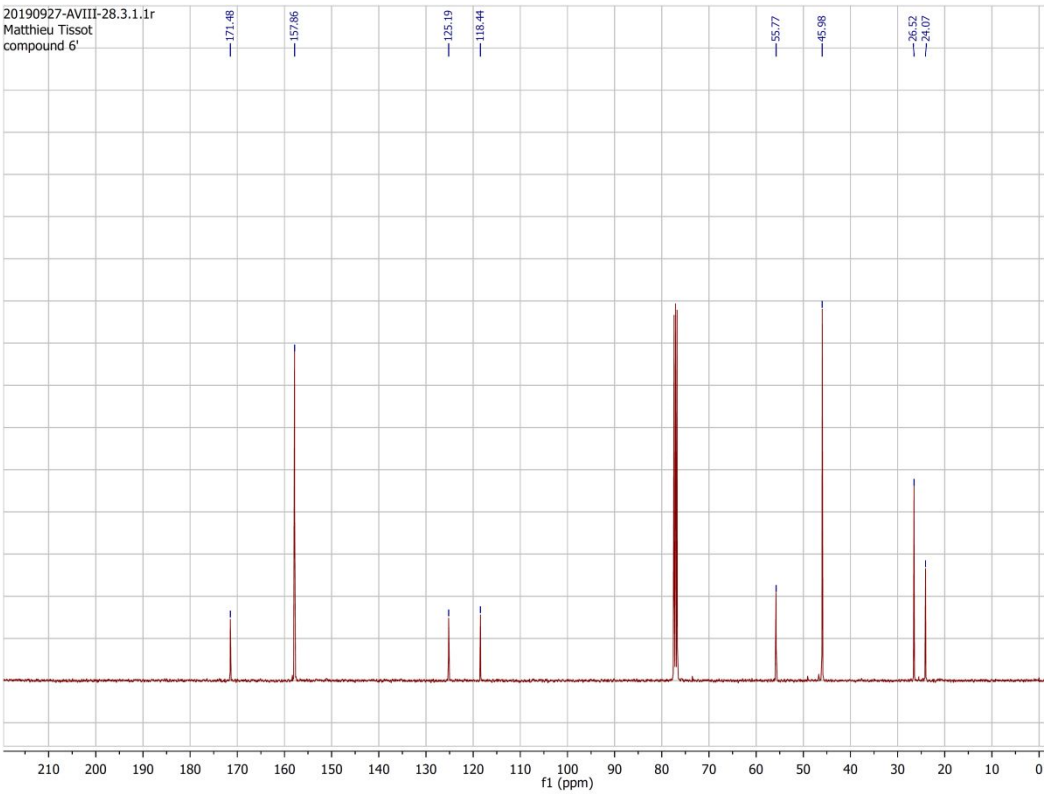




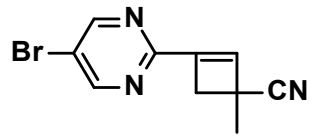
3-amino-3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobutanecarbonitrile (6'-*trans*)

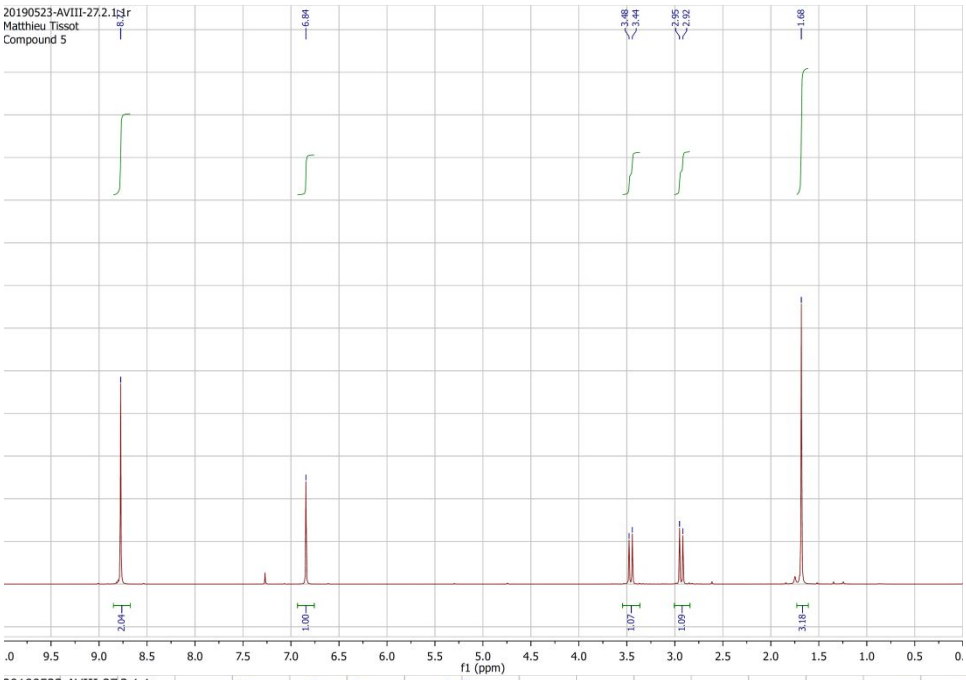


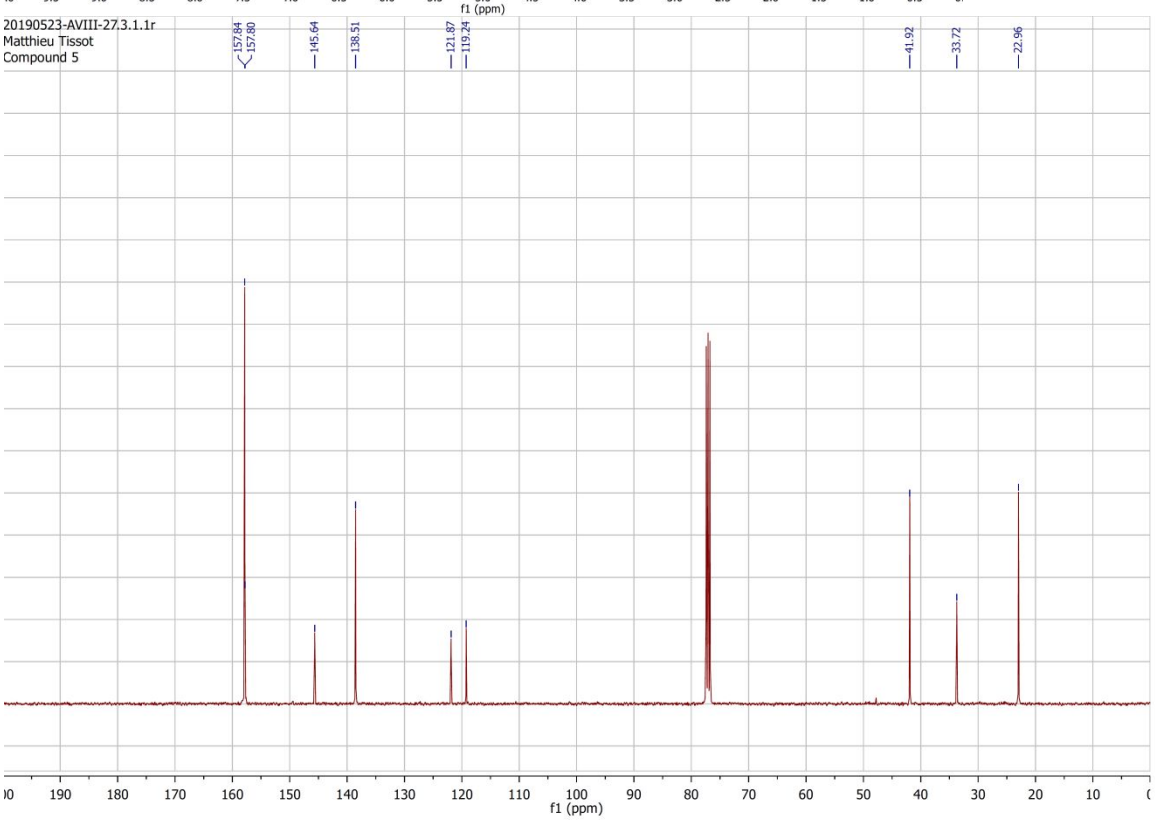




3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobut-2-ene-1-carbonitrile (8)







3-azido-3-(5-bromopyrimidin-2-yl)-1-methyl-cyclobutanecarbonitrile (5)