The design of experiments (DoE) in optimization of an aerobic flow Pd-catalyzed oxidation of alcohol towards an important aldehyde precursor in the synthesis of phosphatidylinositide 3-kinase inhibitor (CPL302415)

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1. Materials and Methods

Solvents and chemicals were obtained from commercial suppliers and were used without any further purification unless otherwise noted. The {5-[2-(difluoromethyl)-2,3-dihydro-1,3-benzodiazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-2-yl}methanol (**1**) was synthesized according to the procedure published by us. [1,2] Oxygen was purchased from Air Product. Experiments were performed using a combined two Vapourtec easy-Medchem with four standard PFA tubular reactors (10mL each, id=1mm). All tubes and mixers were bought from Vapourtec.
Pressure to the system was delivered to the system using a second Vapourtec SF-10 pump and back pressure regulator (BPR) Vapourtec SF-10 pump was used as a mass flow controller.

Main components utilized within a continuous flow reactor	
Pump	Vapourtec V-3 Peristaltic pump
Mass flow meter	Vapourtec SF-10
Mixer	Vapourtec Y-type mixer Material: PTFE i.d.= 1 mm <0.1mL
Reactor	Vapourtec PFA 10 mL coil reactor i.d=1mm Temperature control: Vapourtec easy-Medchem
Back pressure regulator	Vapourtec SF-10

	Adjustable pressure
Miscellaneous fittings	Vapourtec PFA tubes i.d.=1mm

1.1 Design of Experiments (DoE)

The DoE study and statistical analysis were performed by using the design of experiment tools of STATISTICA software (v.13.3). The experimental data were fitted by using multiple linear regression. The main and interaction effects were generated based on multivariate ANOVA. The statistical significance level was set up to 0.05. The goodness of fit of the models was expressed in regression coefficient R^2.

1.2 High Field NMR

^1H and ^{13}C NMR spectra were performed on JOEL JNMR-ECZR 600 MHz spectrometers with ^1H being observed at 600 MHz and ^{13}C at 151 MHz. Chemical shifts for ^1H and ^{13}C were reported in *d* (ppm) using the residual proton in a deuterated solvent. Mass spectra (Atmospheric Pressure Ionization Electrospray, API-ES) were obtained on Agilent 6130 LC/MSD spectrometer or Agilent 1290 UHPLC coupled with Agilent QTOF 6545 mass spectrometer.

1.3 UHPLC analytical method

Reaction in-process monitoring was conducted by the RP UHPLC method using the parameters listed in Table 1.3.1:

Table 1.3.1. Chromatographic conditions

Parameters	**Range**	
Instrumentation	Ultra-high performance liquid chromatograph equipped with an UV/DAD detector, autosampler, and column heater	
Column	Acquity UPLC CSH C18; 2.1 mm x 100 mm, 1.7 µm	
Mobile phases	Phase A: 0.1% ortho-phosphoric acid in water Phase B: 0.1% ortho-phosphoric acid in ACN	
Diluent	Methanol	
Flow	0.5 mL/min	
Run time	11 min	
Column temperature	30°C	
Autosampler temperature	10°C	
Injection volume	1 µL	
Detection Wavelength	254 nm	
Typical Retention Time	Alcohol **1** about 4.1 min, Aldehyde **3** about 5.5 min	
Rinsing the column	After analysis rinse the column for 10 min using ACN : water (10:90, v/v) solution then during 10 min using ACN; store the column with ACN	
Gradient program		
Time, min	Mobile phase A, %	Mobile phase B, %
0.0	90.0	10.0
6.0	40.0	60.0
7.5	10.0	90.0
9.0	10.0	90.0
9.10	90.0	10.0
11.0	90.0	10.0

Reporting of results and calculations

System suitability:

- The resolution between the peaks of alcohol **1** and its nearest impurity must not be less than 1.5;

- Symmetry factor for alcohol **1** peak: within the range from 0.8 to 1.5;

Evaluation of chromatograms

- Disregard peaks from the blank matrix and diluents;
- Disregard peaks less than 0.05%;

Calculations

The progress of the reaction (product aldehyde yield) was controlled based on the normalization procedure according to the following formula:

$$X = \frac{A_X \cdot CF \cdot 100\%}{\Sigma A_{Xi}},$$

where:

X – aldehyde product **3** percentage of in the chromatogram of the sample solution,

A_X – aldehyde **3** peak area in the chromatogram of the sample solution,

CF – correction factor of aldehyde **3** versus alcohol **1**

SA_{Xi} – the sum of the areas of all integrated peaks in the chromatogram of the sample solution.

1.4 Note of Caution

The mixtue of O_2 with organic solvents vapours is extremely flammable! Pressurized equipment should be operated with care! Before conducting any experiments, an individual, careful safety assessment including reaction kinetics and explosive hasards should be carried out!

2. Experimental Details

General procedure

Alcohol **1** was dissolved in an appropriate solvent or mixture (200 mg/20 mL) (Reagent A).
Palladium acetate was dissolved in toluene, then pyridine was added using an automatic pipette (Reagent B). The solvent bottle was filled with toluene for the reaction, 2 mL of each solution was used. Both reagents and oxygen were pumped at an appropriate flow rate. Reagent A was mixed with oxygen using the Y-shaped mixer, run through a 28 cm long tube to saturate it with gas, and later combined with reagent B in the Y-shape mixer. The reaction was performed at different temperatures at first within two heated PFA tubular reactors. Next, in order to extend the reaction time, the reaction mixture feed was supplemented with oxygen and transferred into next two heated reactors at the same temperature. FlowWizard™ software, which calculated reaction time, controlled the easy-Medchem system and the collection/waste valve. The reaction mixture (4 mL) was collected offline into the flask and analyzed by UHPLC.

Synthesis of compound 3 in flow – 1g test

Alcohol **1** (1.05 g; 2.61 mmol) was dissolved in 105 mL mixture of toluene: ethyl acetate 1:1 and kept in an oil bath at 80°C (Reagent A). Palladium acetate (0.12 g; 0.524 mmol; 20%mol) was dissolved in 105 mL toluene, then pyridine (60 uL; 0.745mmol; 26%mol) was added using automatic pipette (Reagent B). Upon addition of pyridine to palladium acetate solution (orange) brightened to yellow. The solvent bottle was filled with toluene (for both reagents). For the reaction, 100 mL of each solution was used (1.00 g of substrate; 2.49 mmol). Both reagents were pumped at flow rate = 1mL/min, and oxygen was pumped with flow rate of 0.1 mL/min. Reagent A was mixed with oxygen using the Y-shaped mixer, run through a 28 cm long tube to saturate it with gas, and later combined with reagent B in the Y-shape mixer. The reaction was performed at 120°C at first within two heated PFA tubular reactors. Next, in order to extend the reaction time, the reaction mixture feed was supplemented with oxygen and transferred into next two heated reactors. FlowWizard™ software which calculated reaction time, controlled the easy-Medchem system and the collection/waste valve. The fraction obtained during "steady-state" operation, which corresponded to an operating time of 93 min (186 mL; 0.93 g of substrate) collected into the round bottom flask, vaporized, and purified via column chromatography. 0.671 g of yellow solid was obtained (yield =72.6%). 1H-NMR (400 MHz, CDCl3) δ 10.21 (s, 1H), 7.93 (m, 1H), 7.70 (m, 1H), 7.49-7.42 (m, 2H), 7.44-7,16 (t, 1H), 7.11 (s, 1H), 6.53 (s, 1H), 4.05-3.92 (m, 8H)

Synthesis of compound 3 in batch autoclave using $Pd(OAc)_2$

The reaction was prepared under un inert argon atmosphere. Alcohol **1** (100 mg; 0,25 mmol) was placed in 35 mL vial under argon and solubilised with dry and degassed toluene/EtOAc 1:1 mixture (25mL). The palladium acetate (11.4 mg; 0.05 mmol; 20%mol) was also placed in a 35mL vial under argon and solubilised with toluene/EtOAc 1:1 mixture (15mL) and then mixed with pyridine (5.2 uL; 0.0649mmol; 26%mol). Next, the content of the both vials was transferred under argon to a 300 mL Büchi miniclave containing a magnetic stirrer bar. After three purges with O_2 the reactor was then pressurised at 5 bar O_2 at ambient temperature then heated at 120°C during 2h. After the reaction, the autoclave was cooled at ambient temperature. The vented and the crude mixture was filtered throught silica gel pad and analysed by using UHPLC. Resulted with 45% yield of **3**.

Synthesis of compound 3 in batch using MnO_2[1]

According to the described procedure described by us in Ref [1]
The 2L batch reactor was loaded with 27 g of compound **1** (0.067mol), 58,4 g activated manganese oxide (0.667 mol), 350 mL and toluene, and 350 mL butyl acetate. The reaction mixture was heated and mixed (200 rpm). The temperature was set to 120°C. After reaching reflux, the timer was set to 1.5h. The reaction was controlled using TLC. After reaction completion, the mixture was cooled to 25°C. Reaction mixture was filtered on Schott funnel G-4 using 100 g Celite® 545 and washed with 200 mL DCM. Filtrate was evaporated resulting in 18.2 g creamy solid (yield=68.1%) 1H-NMR (600 MHz, CDCl3) δ 10.21 (s, 1H), 7.94-7.92 (m, 1H), 7.71-7.69 (m, 1H), 7.48-7.42 (m, 2H), 7.39-7.21 (t, 1H), 7.11-7.10 (m, 1H), 6.53 (s, 1H), 4.04-3.95 (m, 8H)

Synthesis of compound 3 in batch using Dess-Martin periodinane[1]

According to the described procedure described by us in Ref [1]

Table S1. Additional conditions screened for flow $Pd(OAc)_2$/Pyridine catalyzed aerobic oxidation of **1** in Toluene.

Entry	Catalyst loading (%)	Pyridine eq. per catalyst	T (°C)	PO_2 (bar)	V of O_2 (mL/min)	V of reagents (mL/min)	Conv. of **1** (%)	Yield of **2** (%)	Yield of **3** (%)
1	20 mol%	4	100	4	0.1	0.1	68.87	0	63.16
2	20 mol%	4	100	5	0.222	0.222	72.3	0	72.3
3	20 mol%	4	100	5	2.02	2.02	10.82	0	10.82
4	20 mol%	1.3	130	5	1	0.15	0	0	0
5	20 mol%	1.3	130	5	1	0.1	0	0	0
6	20 mol%	1.3	130	5	0.5	0.25	54.32	0	54.32
7	20 mol%	1.3	130	5	1	0.15	13.65	0	13.65
8	20 mol%	1.3	130	5	1	0.25	100	0	0
9	20 mol%	1.3	120	5	0.4	0.4	94.16	0	89.14

[a] Standard reaction conditions: : substrate **1** = 20 mg (0.05 mmol) dissolved in 2 mL Toluene.

Table S2. Additional conditions screened for flow $Pd(OAc)_2$/Pyridine catalyzed aerobic oxidation of **1** in different solvents or solvent mixture.

Entry	Catalyst loading (%)	Pyridine eq. per catalyst	Solvent	T (°C)	PO_2 (bar)	V of O_2 (mL/min)	V of reagents (mL/min)	Conv. of **1** (%)	Yield of **2** (%)	Yield of **3** (%)	Yield of **4** (%)
1	20 mol%	0	Toluene/Caprolactone 1:1	130	5	1	0.15	18.53	0.17	16.98	0
2	20 mol%	1.3	Toluene/Caprolactone 1:1	130	5	0.5	0.15	83.3	1.16	77.67	0
3	20 mol%	1.3	Toluene/Caprolactone 1:1	130	5	1	0.15	77.78	0	77.78	0
4	5 mol%	1.3	Toluene/Caprolactone 1:1	120	5	0.1	0.55	10.05	0	4.25	0
5	5 mol%	1.3	Toluene/Caprolactone 1:1	130	5	0.1	1	11.19	0	5.18	0
6	20 mol%	4	Toluene/Caprolactone 1:1	100	3	0.1	0.1	39.52	0.6	37.90	0
7	20 mol%	4	Toluene/Caprolactone 1:1	100	3	0.22	0.1	49.0	0	48.96	0
8	20 mol%	4	Toluene/Caprolactone 1:1	100	3	0.22	0.33	53.62	0	53.62	0
9	20 mol%	4	Toluene/Caprolactone 1:1	100	5	0.4	0.4	52.86	0.27	52.58	0
10	20 mol%	4	Toluene/Caprolactone 1:1	120	5	0.4	0.4	78.99	0	78.99	0
11	20 mol%	4	Toluene/Caprolactone 1:1	100	5	0.1	0.1	41.2	0.67	39.4	0
12	10 mol%	4	DMA	100	2	0.11	0.11	2.0	0	0	0
13	10 mol%	4	DMA	130	5	0.11	0.11	25.01	0.69	7.4	0
14	20 mol%	4	DMA	150	8	0.25	0.25	60.1	0	16.3	9.5
15	20 mol%	1.3	EtOAc	130	5	1	0.1	66.12	0	66.12	0
16	20 mol%	1.3	EtOAc	130	5	1	0.15	45.32	0	44.06	0
17	20 mol%	1.3	EtOAc	120	5	0.4	0.4	26.64	0	24.23	0
				Air instead of O_2							
18	20 mol%	1.3	Toluene/Caprolactone 1:1	130	5	1	0.15	72.45	0	27.55	0
19	20 mol%	1.3	Toluene/Caprolactone 1:1	130	5	0.5	0.15	52.82	0	47.18	0

[a] Standard reaction conditions: substrate **1** = 20 mg (0.05 mmol) dissolved in 2 mL of solvent.

Table S3. Additional conditions screened for flow $Pd(OAc)_2$/Pyridine catalyzed aerobic oxidation of **1** in different solvents or solvent mixtures.

Entry	Catalyst loading (%)	Pyridine eq. per catalyst	Solvent	T (°C)	PO_2 (bar)	V of O_2 (mL/ min)	V of reagents (mL/min)	Conv. of **1** (%)	Yield of **2** (%)	Yield of **3** (%)	Yield of **4** (%)
1	5	1.3	Toluene/EtOAc 1:1	120	5	0.1	0.1	23.22	0	20.61	0
2	22.5	2.6	Toluene/ EtOAc 1:1	120	5	0.1	1	80.18	0	75.16	0
3	40	1.3	Toluene/ EtOAc 1:1	120	5	0.1	0.1	90.25	0	67.63	0
4	40	1.3	Toluene/ EtOAc 1:1	120	5	0.1	1	83.71	0	74.07	0
1	5	1.3	Toluene/EtOAc 1:1	120	5	0.1	0.1	23.22	0	20.61	0

[a] Standard reaction conditions: substrate **1** = 20 mg (0.05 mmol) dissolved in 2 mL of solvent.

Table S4. Additional conditions screened for flow $Pd(OAc)_2$/Pyridine catalyzed aerobic oxidation of **1** in Toluene/EtOAc - 1g synthesis.

Entry	Catalyst loading (%)	Pyridine eq. per catalyst	T (°C)	PO_2 (bar)	V of O_2 (mL/min)	V of reagents (mL/min)	Conv. of **1** (%)	Yield of **2** (%)	Yield of **3** (%)
1	20 mol%	1.3	120	5	0.1	1	100	16	84 (72.6%)[a]

[a] isolated yield.

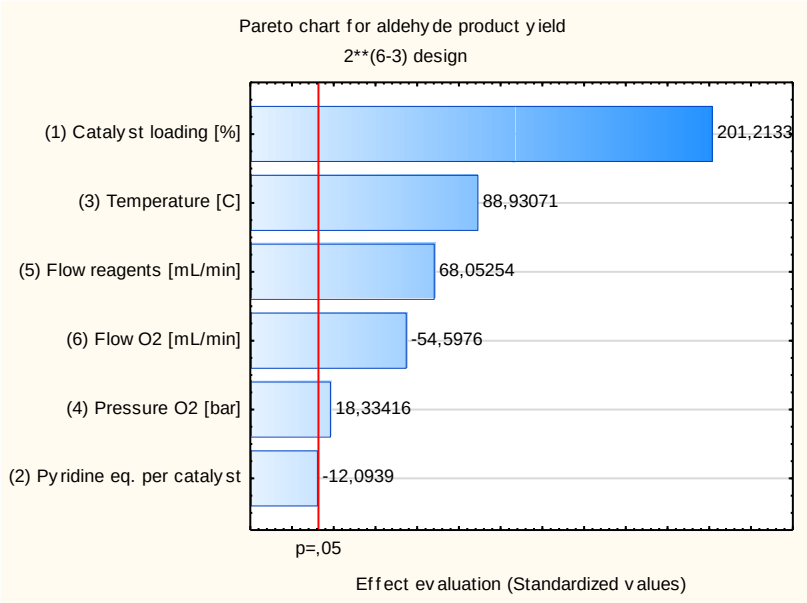
Remark 1: For the higher temperature (130°C) catalyst starts decomposing slowly in the reactor. It is easy to overlook, but after running a couple of experiments, palladium deposition on the reactor's walls becomes visible.

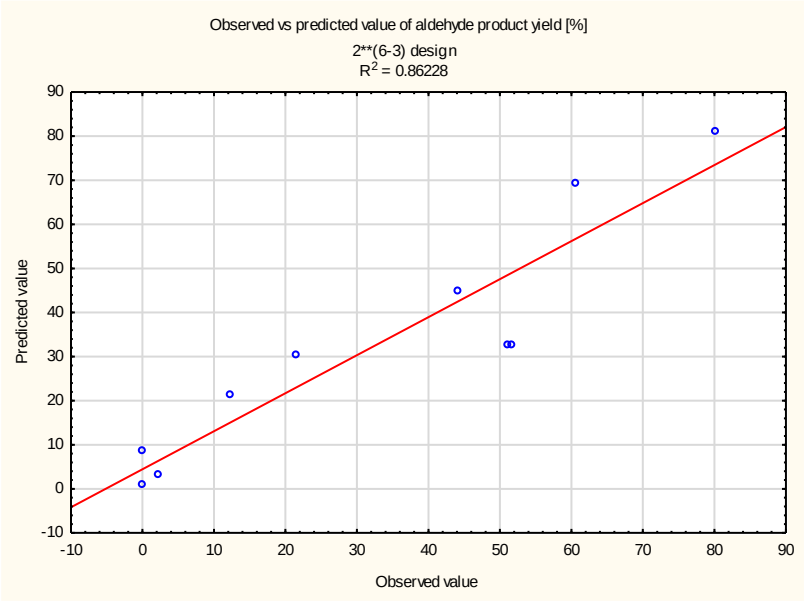
Remark 2: At a higher temperature (150°C) we observed the formation of the acidic product **4**.

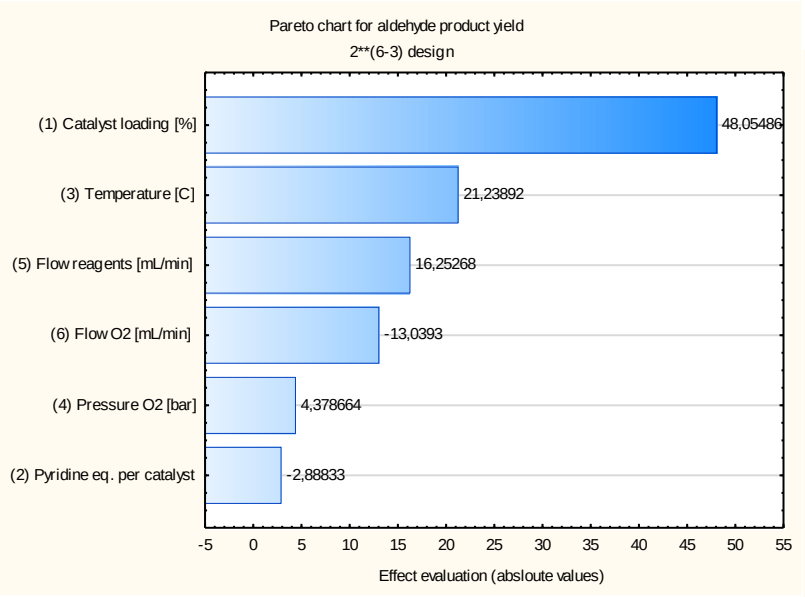
Remark 3: The additional microreactor **LTF-MS** (Volume 0,2 ml; id: 1mm) used as a mixer installed after the second reactor in order to increase the mixing of the additional O_2 portion did not increase the yield of the desired product.[2]

3. DoE Analysis

Figure S1. Summary of the model for screening DOE 2^(6-3) for product **3** yield.







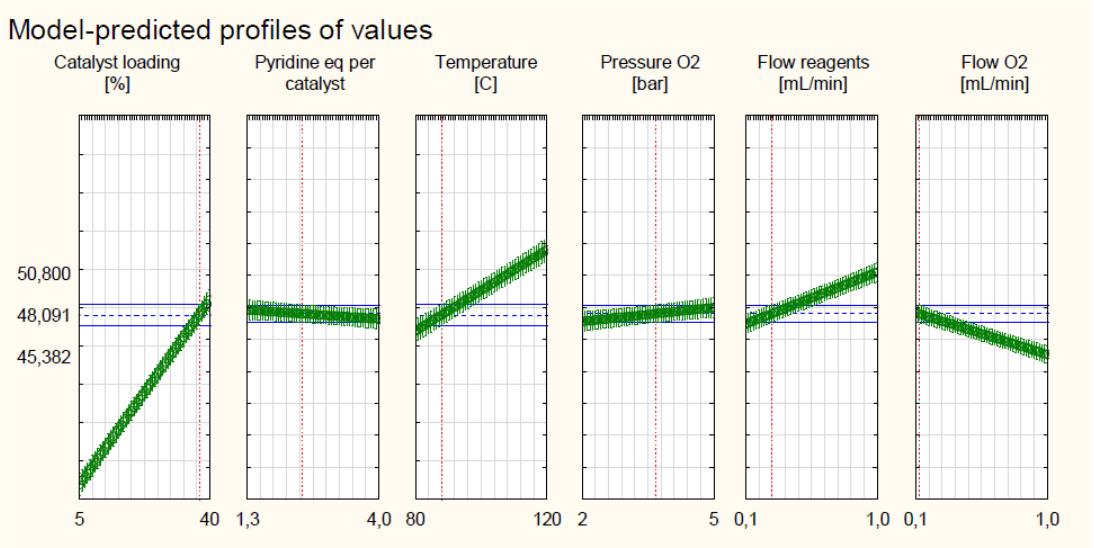
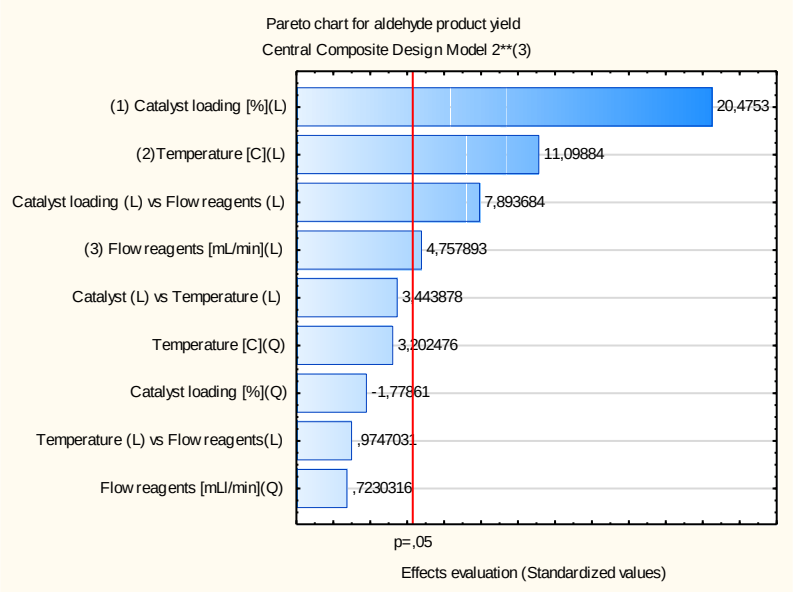
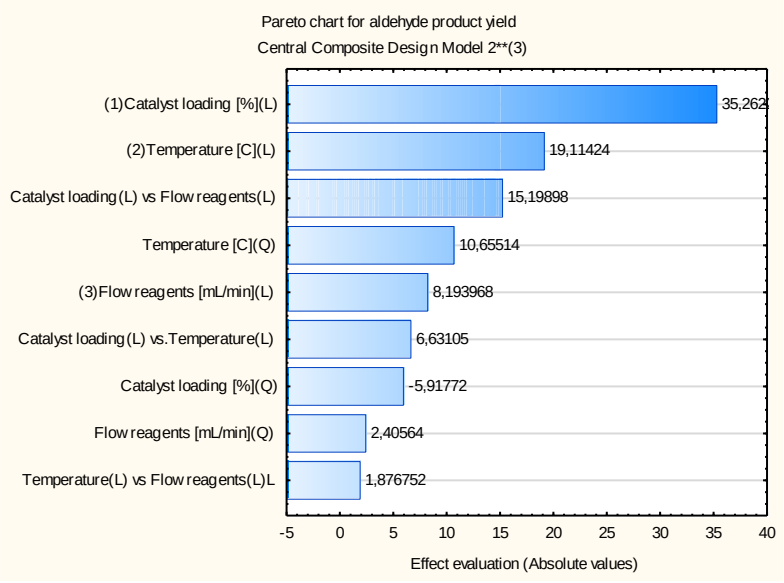
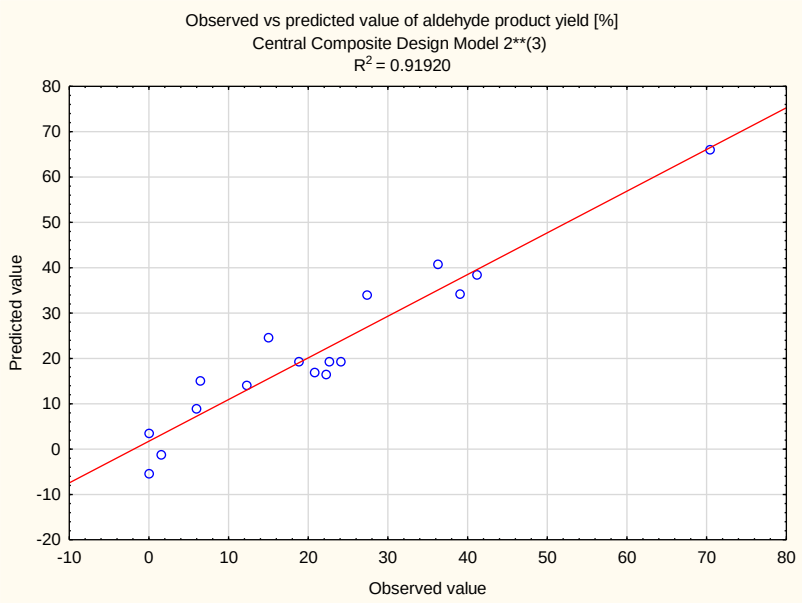
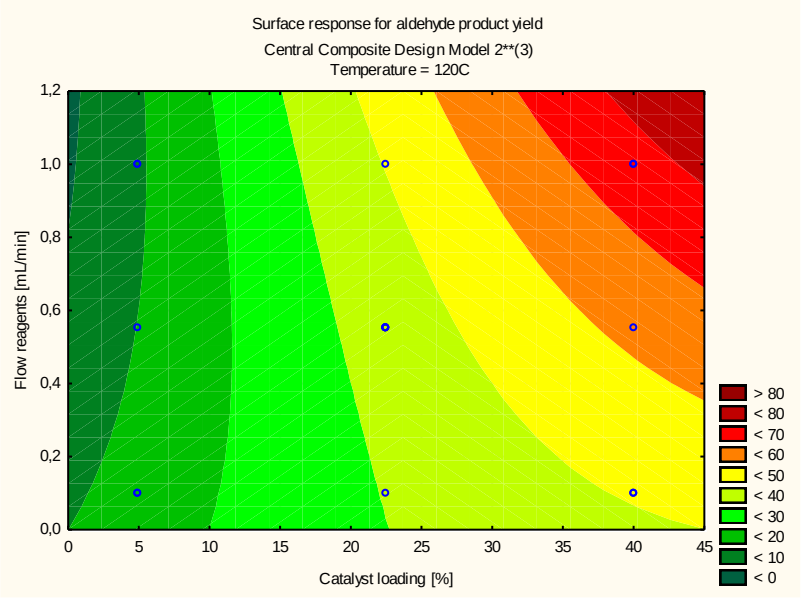


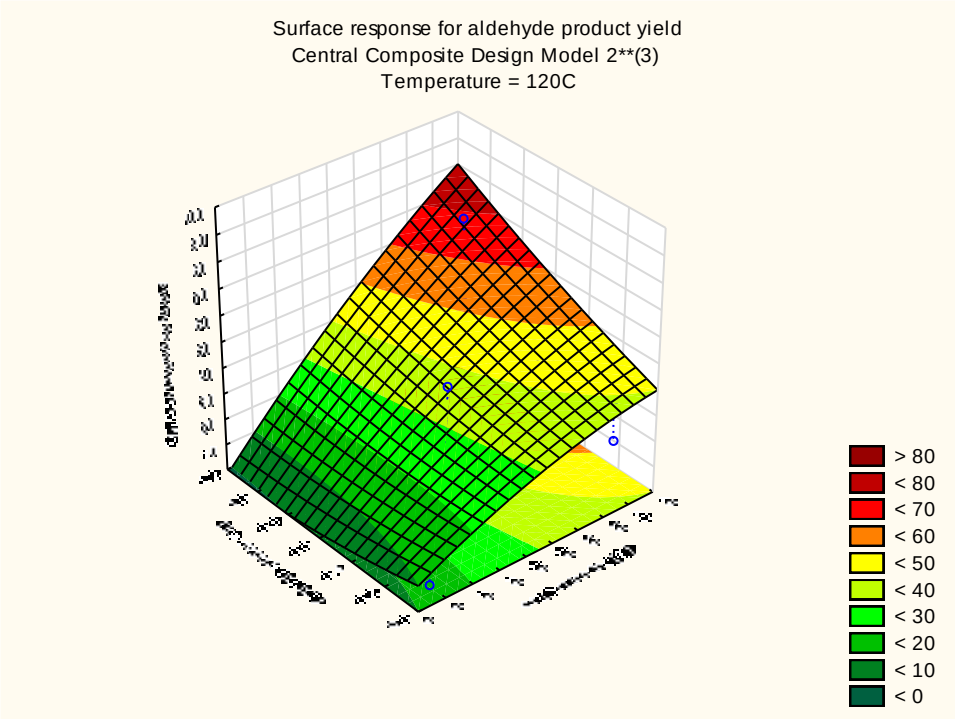
Figure S2. Summary of the Central Composite Design (CCD) model 2^(3) for product **3** yield.





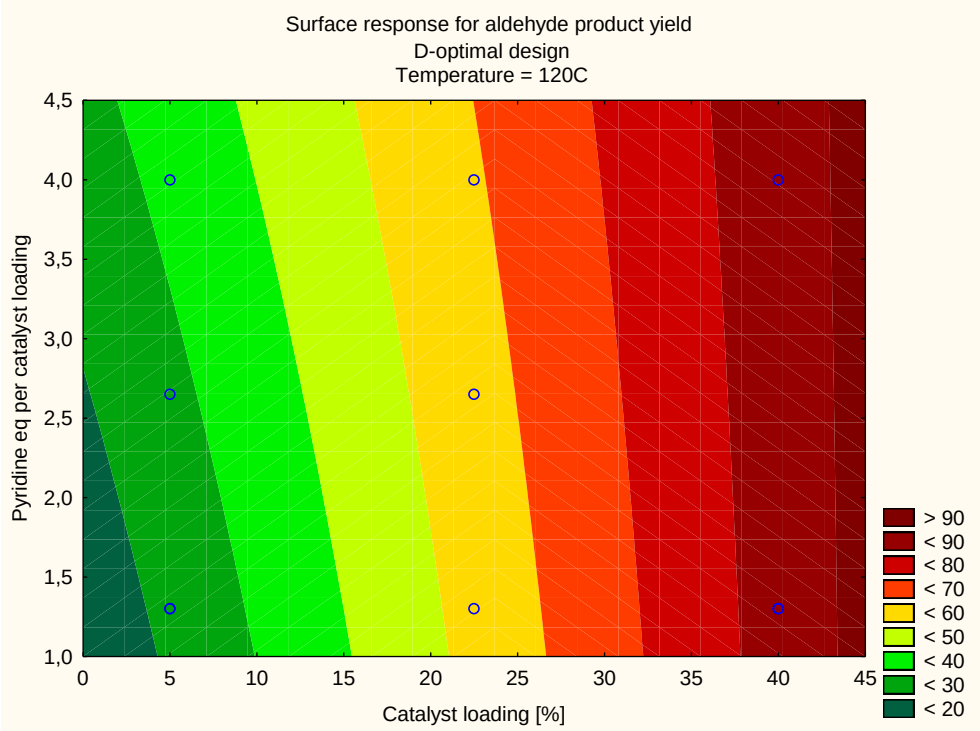






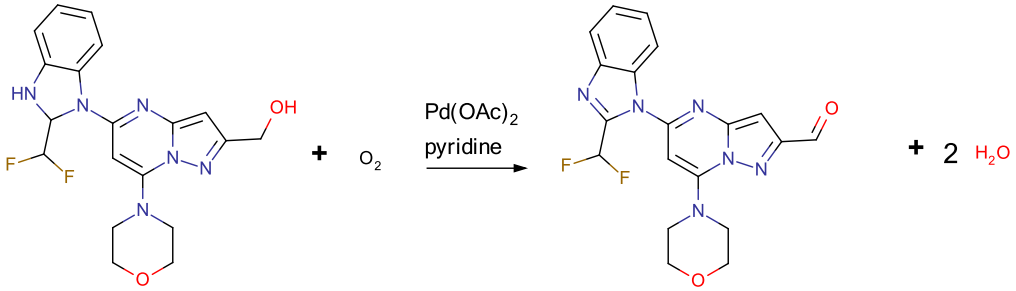
Surface response for aldehyde product yield, Central Composite Design Model 2**(3), Temperature = 120C

Figure S3. D-optimal design for product **3** yield.



4. Green metrics calculations [3-5]

4.1 $Pd(OAc)_2$/Pyridine - no workup



1 3

MW (g/mol)	
1	402.40
3	398.37
O_2	32.00

$$AE\ (\%) = \frac{Mol\ wt\ of\ product\ x100}{Sum\ of\ mol\ wts\ of\ reactants} = 91.71$$

Role	Chemical	Mass (g)	Volume (mL)	Density (g/mol)
Reaction				
Reactant	Alcohol (1)	0.3		
Reactant	Oxygen	0.133		
Catalyst	$Pd(OAc)_2$	0.106		
Reagent	Pyridine	0.049	0.054	0.982
Solvent	Toluene	120.95	150	0.867
Solvent	Ethyl acetate	41.94	50	0.902
	Reaction total	164.11		
Product				
Product	Aldehyde (3)	0.785		

$$RME\ (\%) = \frac{Mass\ of\ product\ x100}{Total\ mass\ of\ reactants} = 64.43$$

$$OE\ (\%) = \frac{RME\ x\ 100}{AE} = 70.26$$

$$PMI(reaction) = \frac{Total\ mass\ in\ reaction}{Mass\ of\ product} = 209.05$$

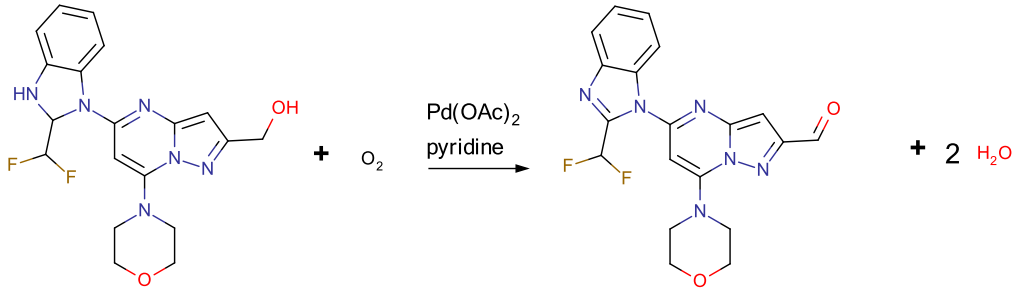
$$E = \frac{Total\ mass\ of\ waste}{Mass\ of\ product} = \frac{0.106 + 0.300}{0.785} = 0.52$$

$$WI = \frac{Total\ mass\ of\ waste}{Total\ mass\ input} = 0.00025$$

Mass of product (kg)	Time (h)	Reactor volume (m^3)
0.000785	1.55	0.00004

$$Spacetime\ yield = \frac{Mass\ of\ product}{Reaction\ time\ x\ volume\ of\ reactor\ used} = 12.66$$

4.2 $Pd(OAc)_2$/Pyridine - with product isolation



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Reaction				
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Reactant	Oxygen	0.133		
Catalyst	$Pd(OAc)_2$	0.106		
Reagent	Pyridine	0.049	0.054	0.982
Solvent	Toluene	120.95	150	0.867
Solvent	Ethyl acetate	41.94	50	0.902
	Reaction total	164.64		
Work-up				
LC	Silica-gel column	47.5		
Solvent	Heptane	145.0	212	0.684
Solvent	Ethyl acetate	68.6	76	0.902
	Work-up total	261.1		
	Reaction and work-up total	425.7		
Product				
Product	Aldehyde (3)	0.671		

$$RME\ (\%) = \frac{Mass\ of\ product\ x100}{Total\ mass\ of\ reactants} = 55.09$$

$$OE\ (\%) = \frac{RME\ x\ 100}{AE} = 60.06$$

$$PMI(reaction) = \frac{Total\ mass\ in\ reaction}{Mass\ of\ product} = 244.57$$

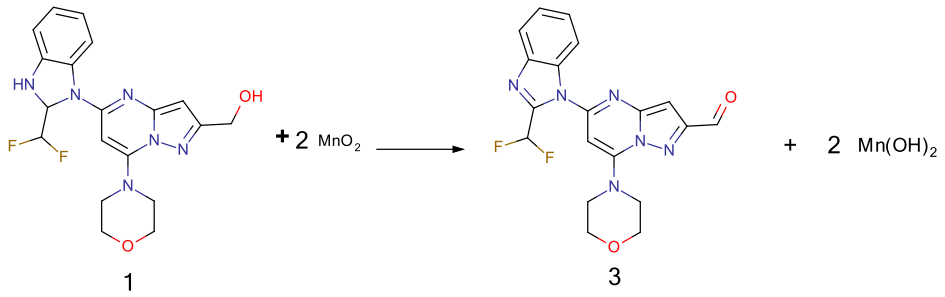
$$E = \frac{Total\ mass\ of\ waste}{Mass\ of\ product} = \frac{425.7 - 0.671}{0.671} = 632.69$$

$$WI = \frac{Total\ mass\ of\ waste}{Total\ mass\ input} = 0.998$$

Mass of product (kg)	Time (h)	Reactor volume (m^3)
0.000671	1.55	0.00004

$$Spacetime\ yield = \frac{Mass\ of\ product}{Reaction\ time\ x\ volume\ of\ reactor\ used} = 10.82$$

4.3 MnO_2



1 3

MW (g/mol)	
1	402.40
3	398.37
MnO_2	86.94

$$AE\ (\%) = \frac{Mol\ wt\ of\ product\ x100}{Sum\ of\ mol\ wts\ of\ reactants} = 69.13$$

Role	Chemical	Mass (g)	Volume (mL)	Density (g/mol)
Reaction				
Reactant	Alcohol (1)	27.0		
Reactant	MnO_2	58.4		
Solvent	Toluene	303.5	350	0.867
Solvent	Butyl acetate	308.7	350	0.882
	Reaction total	697.5		
Work-up				
Column	Celite®	100		
Solvent	DCM	265.0	200	1.325
	Work-up total	365.0		
	Reaction and work-up total	1062.5		
Product				
Product	Aldehyde (3)	18.2		

$$RME\ (\%) = \frac{Mass\ of\ product\ x100}{Total\ mass\ of\ reactants} = 31.62$$

$$OE\ (\%) = \frac{RME\ x\ 100}{AE} = 30.06$$

$$PMI(reaction) = \frac{Total\ mass\ in\ reaction}{Mass\ of\ product} = 38.32$$

$$PMI(workup) = \frac{Total\ mass\ used\ for\ workup}{Mass\ of\ product} = 20.05$$

$$PMI(total) = \frac{Total\ mass\ process}{Mass\ of\ product} = 58.37$$

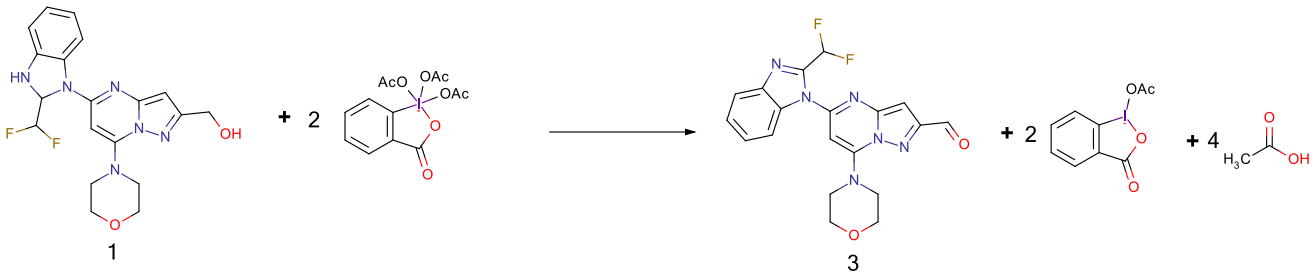
$$E = \frac{Total\ mass\ of\ waste}{Mass\ of\ product} = 57.37$$

$$WI = \frac{Total\ mass\ of\ waste}{Total\ mass\ input} = 0.983$$

Mass of product (kg)	Time (h)	Reactor volume (m^3)
0.0182	1.5	0.002

$$Spacetime\ yield = \frac{Mass\ of\ product}{Reaction\ time\ x\ volume\ of\ reactor\ used} = 6.07$$

4.4 Dess-Martin Periodinane



MW (g/mol)	
1	402.40
3	398.37
DMP	424.14

$$AE\ (\%) = \frac{Mol\ wt\ of\ product\ x100}{Sum\ of\ mol\ wts\ of\ reactants} = 31.85$$

Role	Chemical	Mass (g)	Volume (mL)	Density (g/mol)
Reaction				
Reactant	Alcohol (1)	0.9		
Reactant	DMP	1.31		
Solvent	DMF	24.54	26	0.944
	Reaction total	26.75		
Work-up				
Solvent	Ethyl acetate	13.53	15	0.902
LC	Silica-gel column	33.30		
Solvent	Heptane	103.97	152	0.684
Solvent	Ethyl acetate	51.41	57	0.902
	Work-up total	202.21		
	Reaction and work-up total	228.97		
Product				
Product	Aldehyde (3)	0.70		

$$RME\ (\%) = \frac{Mass\ of\ product\ x100}{Total\ mass\ of\ reactants} = 24.29$$

$$OE\ (\%) = \frac{RME\ x\ 100\%}{AE} = 76.26$$

$$PMI(reaction) = \frac{Total\ mass\ in\ reaction}{Mass\ of\ product} = 34.66$$

$$PMI(workup) = \frac{Total\ mass\ used\ for\ workup}{Mass\ of\ product} = 273.46$$

$$PMI(total) = \frac{Total\ mass\ process}{Mass\ of\ product} = 308.12$$

$$E = \frac{Total\ mass\ of\ waste}{Mass\ of\ product} = 307.12$$

$$WI = \frac{Total\ mass\ of\ waste}{Total\ mass\ input} = 0.997$$

Mass of product (kg)	Time (h)	Reactor volume (m^3)
0.00102	1	0.00005

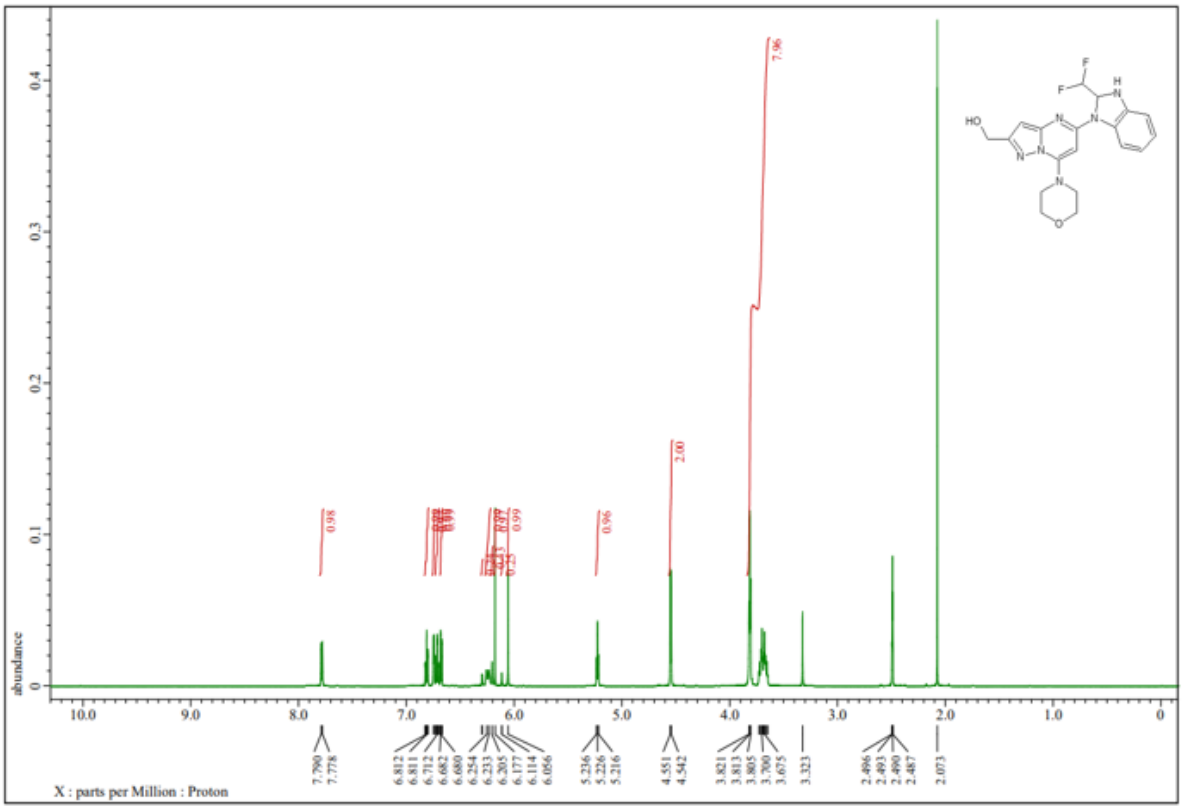
$$Spacetime\ yield = \frac{Mass\ of\ product}{Reaction\ time\ x\ volume\ of\ reactor\ used} = 20.4$$

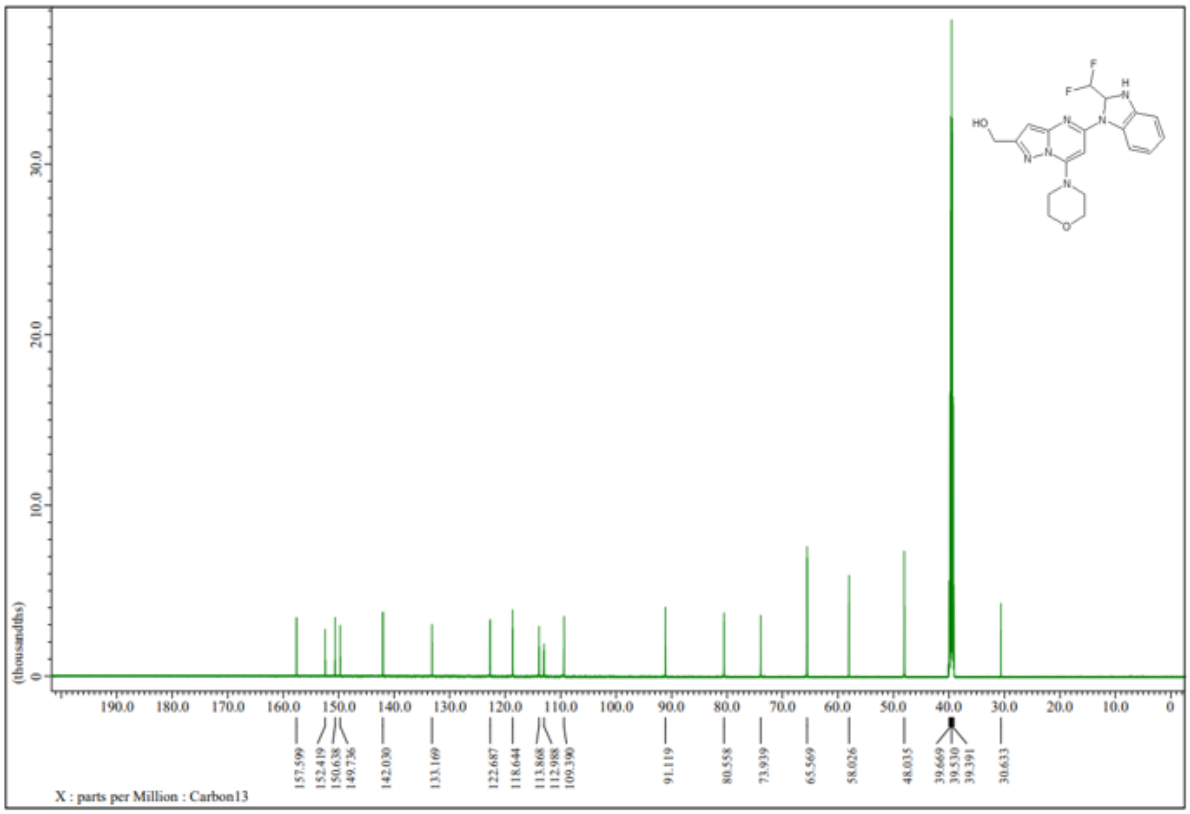
5. NMR spectrum

{5-[2-(difluoromethyl)-2,3-dihydro-*1H*-1,3-benzodiazol-1-yl]-7-(morpholin-4- yl)pyrazolo[1,5-a]pyrimidin-2-yl}methanol (**1**)

1H-NMR (600 MHz, DMSO-D6) δ 7.78 (d, 1H), 6.81 (td, 1.1 Hz, 1H), 6.75 (d,1H), 6.71 (td, J = 7.7, 1H), 6.67 (dd, 1H), 6.30-6.11(t, 1H, CF_2H), 6.24 (d, 1H), 6.18 (s, 1H), 6.06 (s, 1H), 5.23 (t, 1H), 4.55 (d, 2H), 3.82-3.65 (m, 8H); 13C-NMR (151 MHz, DMSO-D6) δ 157.6, 152.4, 150.6, 149.7, 142.0, 133.2, 122.7, 118.6, 113.9, 113.0, 109.4, 91.1, 80.6, 73.9, 65.6, 58.0, 48.0

HRMS (ESI/MS): *m/z* calculated for C19 H20 F2 N6 O2 $[M + H]^+$ 402.1616 found 402.1613.

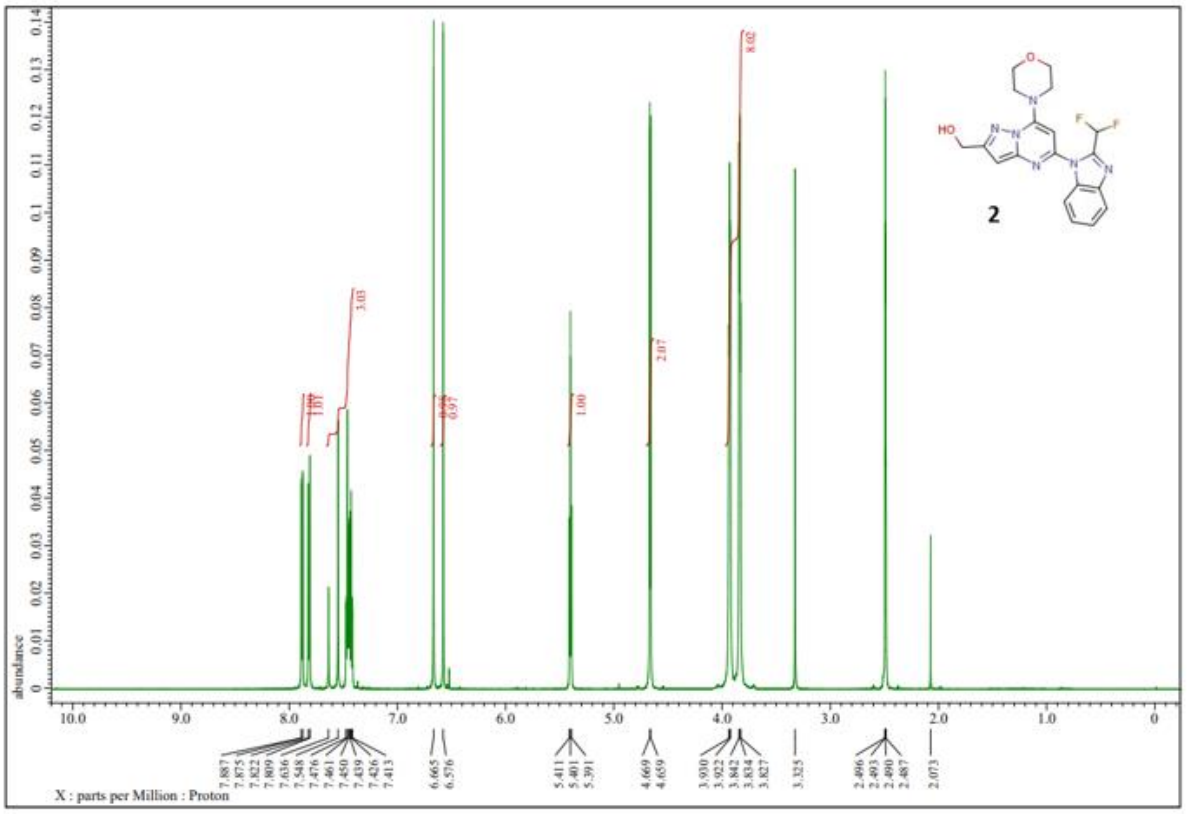


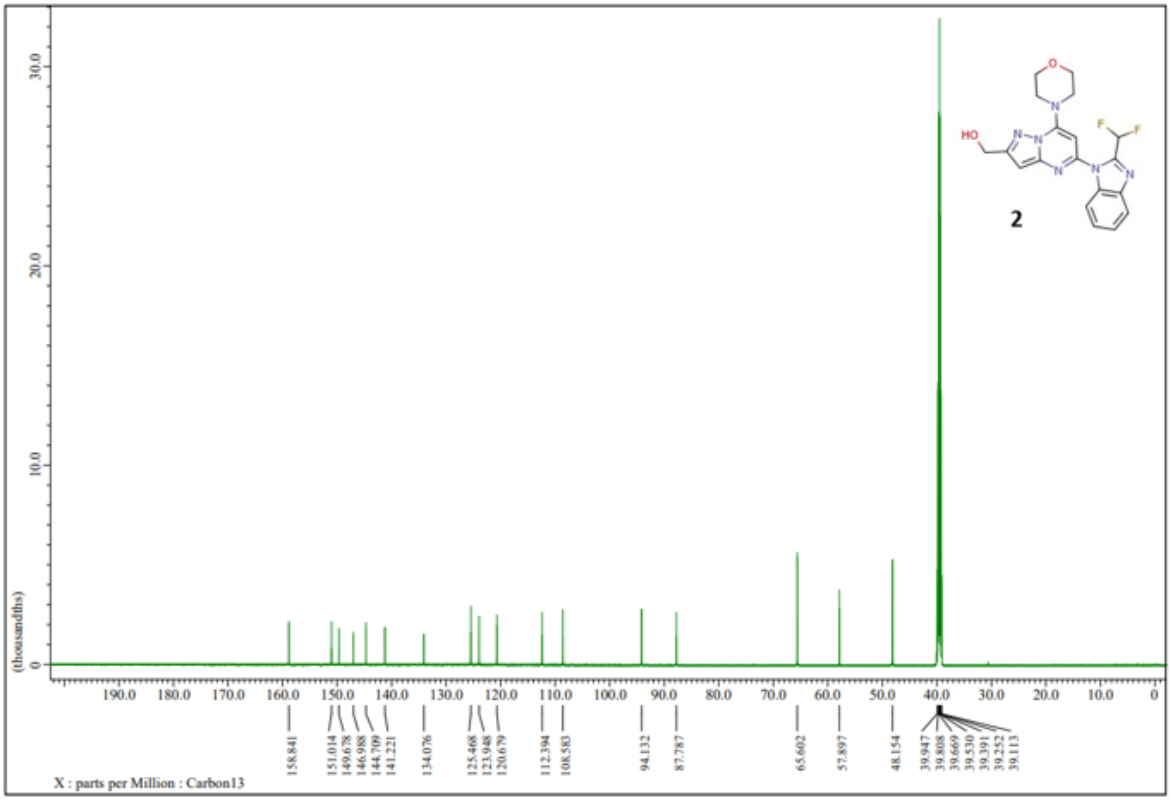


{5-[2-(difluoromethyl)-1H-1,3-benzodiazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-2-yl}methanol (**2**)

1H-NMR (600 MHz, DMSO-D6) δ 7.88 (d, 1H), 7.82 (d, 1H), 7.64-7.46 (t, 1H CF_2H), 7.48-7.41(m,2H) 6.67 (s, 1H), 6.58 (s, 1H), 5.40 (t, 1H), 4.66 (d, 2H), 3.94-3.83 (m, 8H); 13C-NMR (151 MHz, DMSO-D6) δ 158.8, 151.0, 149.7, 147.0, 144.7, 141.2, 134.1, 125.5, 123.9, 120.7, 112.4, 108.6, 94.1, 87.8, 65.6, 57.9, 48.2

HRMS (ESI/MS): *m/z* calculated for C19 H18 F2 N6 O2 $[M + H]^+$ 400.1459 found 400.1456.



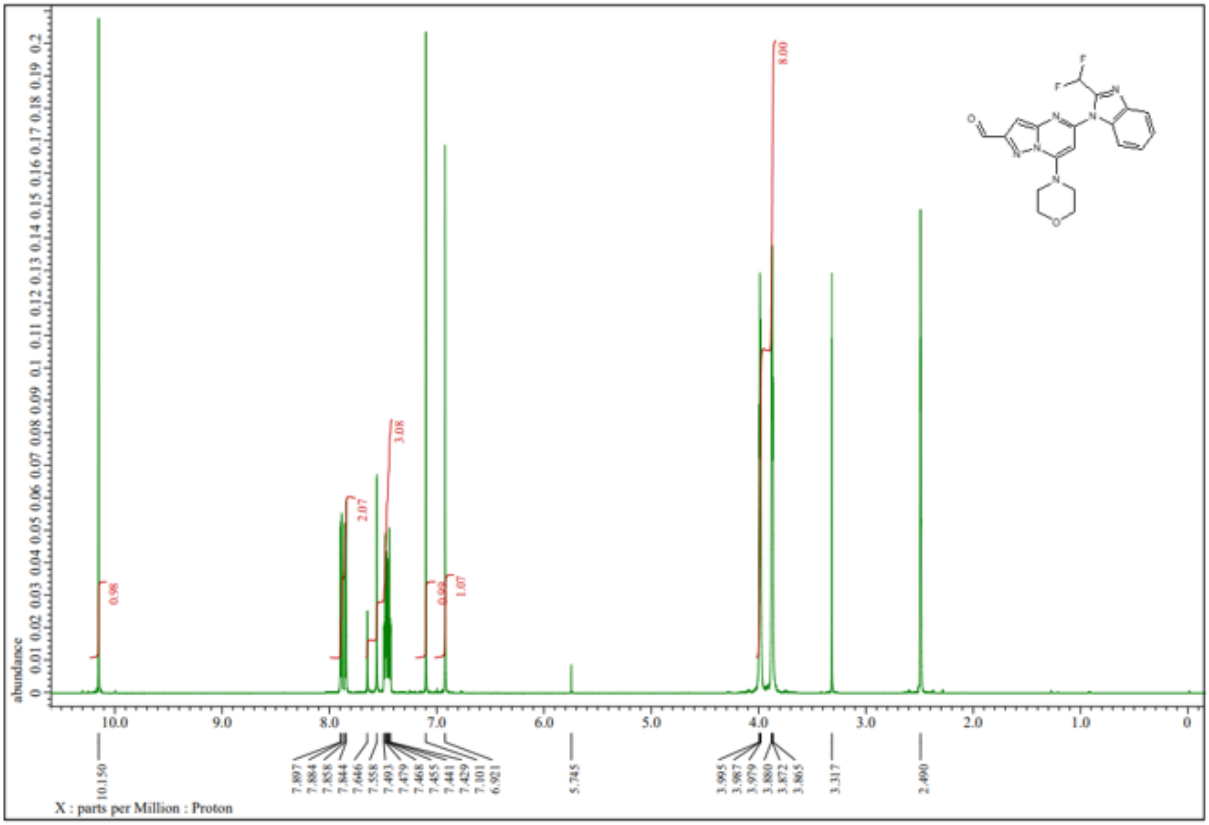


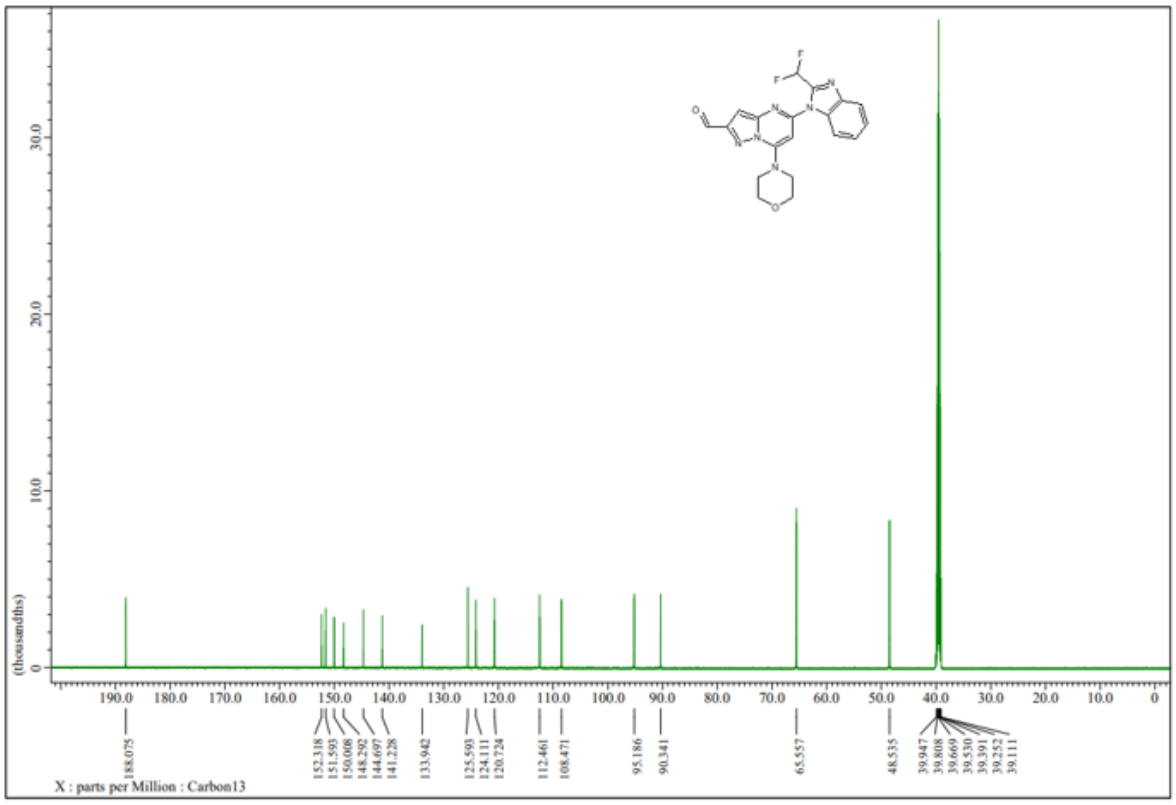
5-[2-(difluoromethyl)-*1H*-benzimidazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidine-2-carbaldehyde (3)

1H-NMR (600 MHz, DMSO-D6) δ 10.15 (s, 1H), 7.87 (dd, 2H), 7.65- 7,47 (t,1H; CF_2H) 7,49-7.43 (m, 2H), 7.10 (s, 1H), 6.92 (s, 1H), 3.99-3.86 (m, 8H); 13C-NMR (151 MHz, DMSO-D6) δ 188.1, 152.3, 151.6, 150.0, 148.3, 144.7, 141.2, 133.9, 125.6, 124.1,

120.7, 112.5, 108.5, 95.2, 90.3, 65.6, 48.5

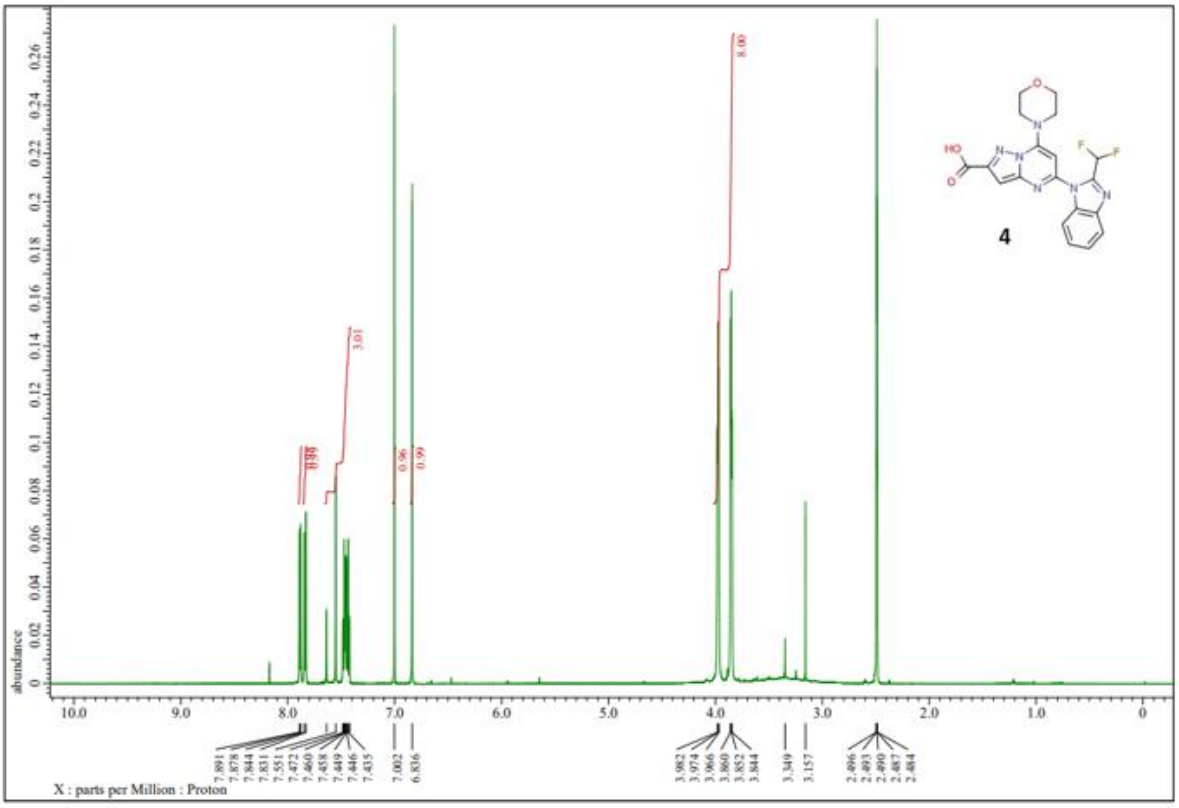
HRMS (ESI/MS): *m/z* calculated for C19 H16 F2 N6 O2 $[M + H]^+$ 398.1303 found 398.1303.

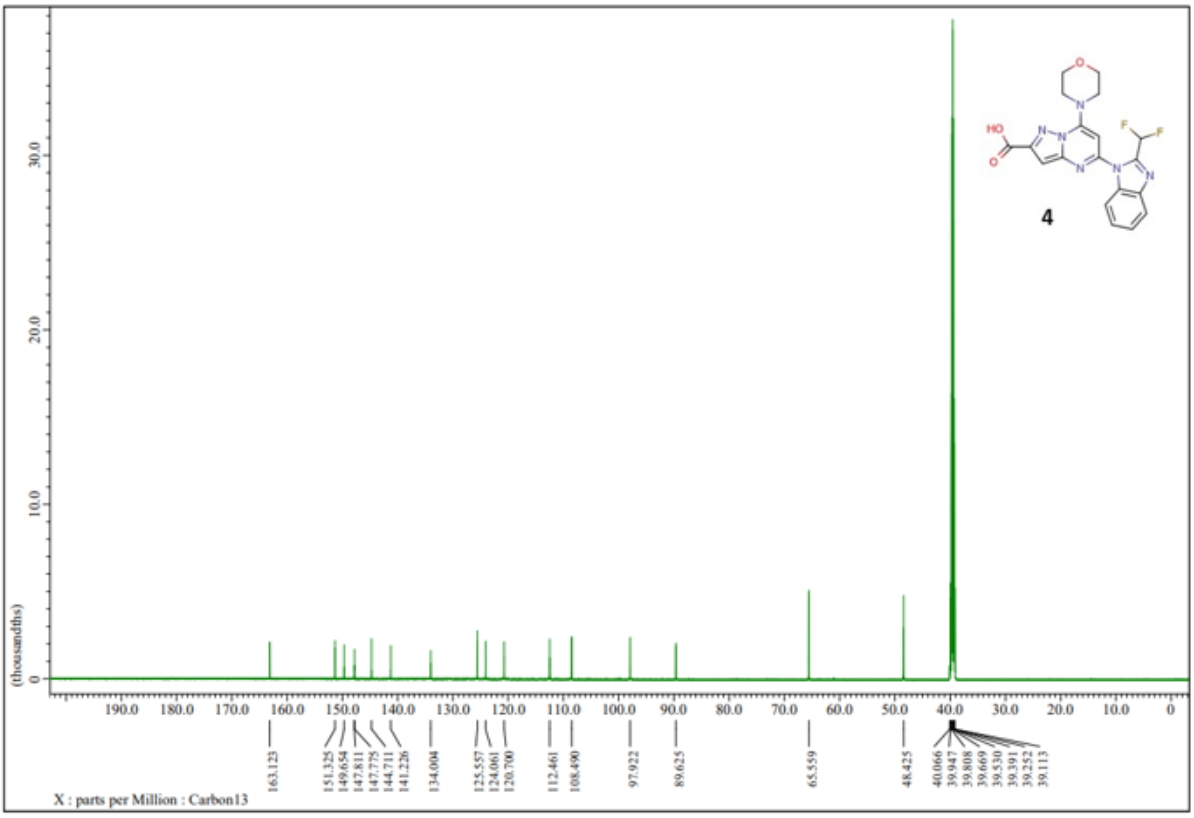




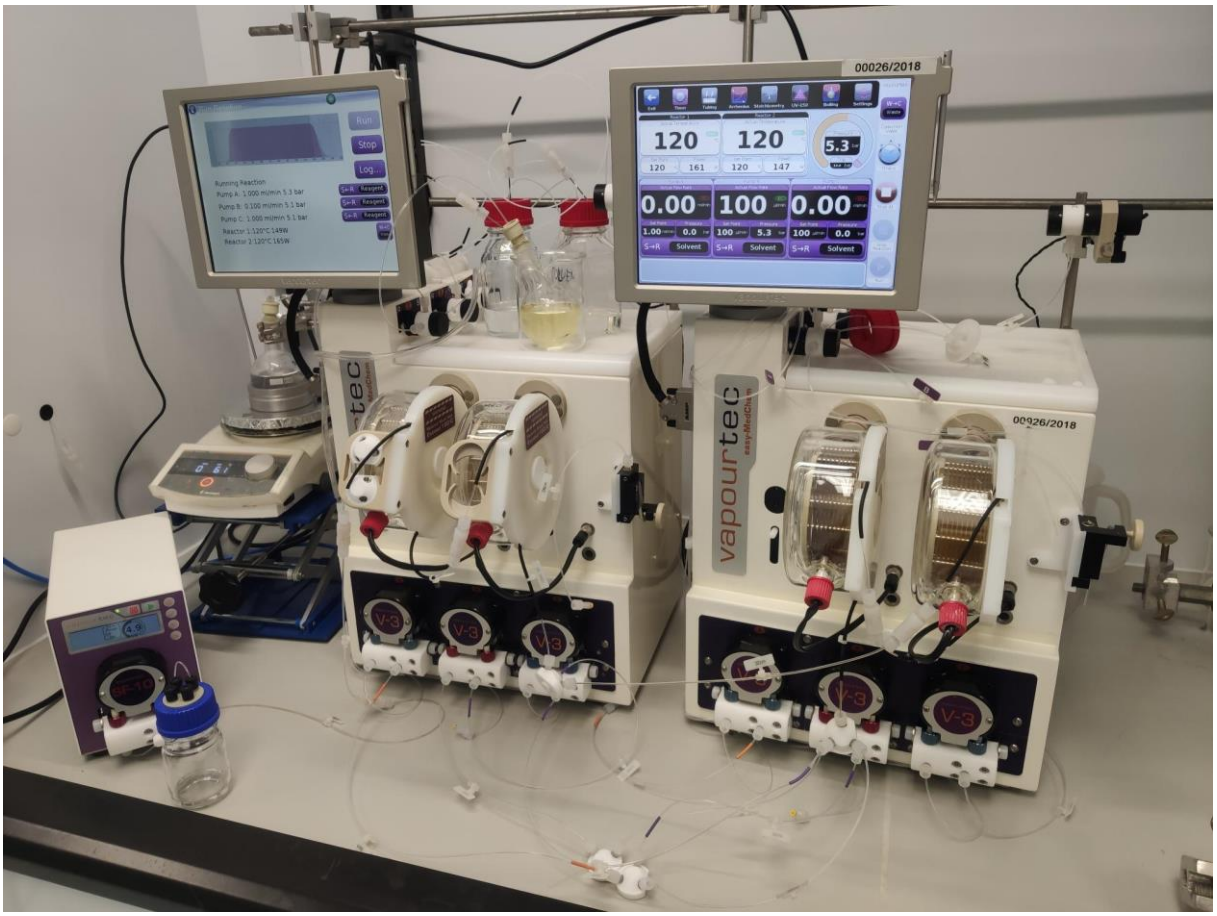
5-[2-(difluoromethyl)-1H-1,3-benzimidazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidine-2-carboxylic acid (4)
1H-NMR (600 MHz, DMSO-D6) δ 7.88 (d, 1H), 7.84 (d, 1H), 7.64-7.46 (t, 1H CF_2H), 7.49-7.42 (m, 2H), 7.00 (s, 1H), 6.84 (s, 1H), 3.98-3.84 (m, 8H) 13C-NMR (151 MHz, DMSO-D6) δ 163.1, 151.3, 149.7, 147.8, 147.8, 144.7, 141.2, 134.0, 125.6, 124.1, 120.7, 112.5, 108.5, 97.9, 89.6, 65.6, 48.4

HRMS (ESI/MS): *m/z* calculated for $C_{19}H_{16}F_2N_6O_3$ $[M + H]^+$ 414.12519 found 414.12546.





6. Real photo of the flow equipment



7.

8. References

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