Supporting Information

A practical metal-free homolytic aromatic alkylation protocol for the synthesis of 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylic acid

Navanita T. Thirumoorthi and Vikrant A. Adsool*

*Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology, and Research (A*STAR), 8 Biomedical Grove, Neuros Building, #07-01/02/03, Singapore 138665 (Singapore) Fax: (65) 6464 2102; Tel: (65) 6799 8503*

**E-mail: vikrant_adsool@ices.a-star.edu.sg*

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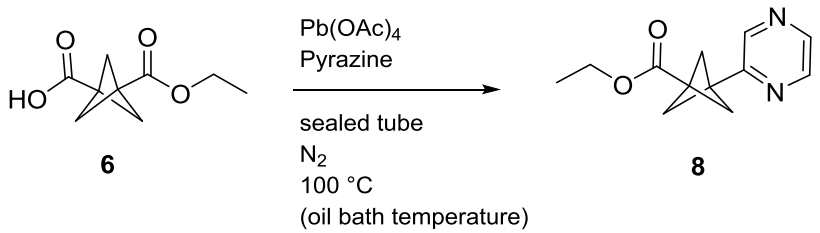
1 General Information

Materials. All chemicals were purchased from Sigma-Aldrich, Alfa Aesar, Merck, TCI and Apollo Scientific and were used as received. Reaction progress was monitored using Merck 60 F254, 0.25 µm silica gel plates (TLC plates) and spots were visualized by UV and/or ceric ammonium molybdate stain. Flash column chromatography was carried out using Merck 60 F254, 0.040-0.063 µm silica gel. Preparative thin layer chromatography was carried out using Merck 60 F254, 0.25 µm silica gel plates. Names of structures were generated using ChemBioDraw Ultra 14.0.0.117.

Instrumentation. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker 400 MHz with CryoProbe. Chemical shifts for protons were reported in parts per million (ppm) that are referenced to residual protium in the NMR solvent ($CDCl_3$: 7.26 ppm; CD_3OD: 3.31 ppm; $(CD_3)_2CO$: 2.05ppm). Chemical shifts for carbon were reported in ppm referenced to the carbon resonances of the NMR solvent ($CDCl_3$: 77.16 ppm; CD_3OD: 49.0 ppm). NMR spectra were processed using MestReNova 10.0.1-14719. ^{1}H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, br t = broad triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration. High resolution electrospray ionization (HRMS ESI) mass spectra were recorded using Agilent 6210 Time-of-Flight LC/MS. Infrared (IR) spectra were measured on a PerkinElmer Spectrum100 FT-IR spectrophotometer. Microwave reactions were carried out on the Anton Paar Monowave 300 Microwave Synthesis Reactor with G10 (maximum 6 mL filling volume) and G30 (maximum 20 mL filling volume) microwave vials (according to Anton Paar nomenclature). UV reactions were carried out using a Rayonet Reactor, RMR-600, at 254 nm. Yield refers to isolated yield of analytically pure material unless otherwise noted.

2 Synthetic Protocols

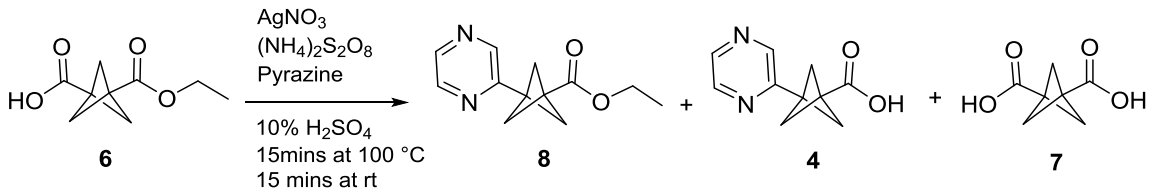
2.1 Lead tetraacetate reaction



3-(ethoxycarbonyl)bicyclo[1.1.1]pentane-1-carboxylic acid (**6**) (50 mg, 0.27 mmol), pyrazine (1 g) and lead tetraacetate (144 mg, 0.326 mmol) were weighed into a microwave vial and the vial was sealed. The sealed vial was subjected to vacuum and nitrogen replacement thrice. Then, the reaction mixture was heated at 100 °C (oil bath temperature) for 6 h. The reaction mixture was cooled to room temperature, washed with water and extracted with ethyl acetate thrice. The organics were washed with saturated sodium bicarbonate, dried over sodium sulphate and concentrated *in vacuo*. The crude reaction mixture was purified by preparative TLC with 50% ethyl acetate: hexanes solvent system to afford the desired product, ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate as a yellow oil (**8**) (16.1 mg, 27.2%). (The reaction did not go to completion and 22.8 mg of unreacted starting material, 3-(ethoxycarbonyl)bicyclo[1.1.1]pentane-1-carboxylic acid was recovered during work-up.)

2.2 Minisci reactions

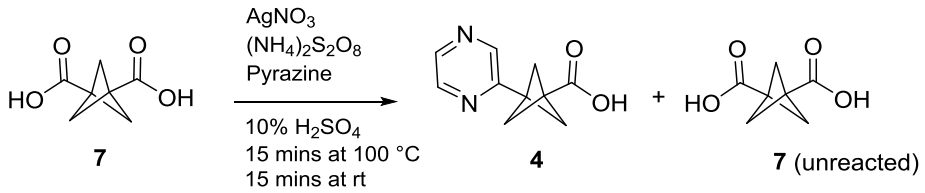
2.2.1 Minisci reaction with 6 as starting material



To 3-(ethoxycarbonyl)bicyclo[1.1.1]pentane-1-carboxylic acid (**6**) (50 mg, 0.27 mmol), pyrazine (217 mg, 2.71 mmol), silver nitrate (27.7 mg, 0.16 mmol) was added 10% H_2SO_4 (2.71 mL) and acetonitrile (0.5 mL). The reaction mixture was heated at 100 °C (bath temperature) for 15

mins. Then, ammonium persulfate (186 mg, 0.81 mmol) in water (1.88 mL) was added dropwise to the heated reaction mixture. The reaction mixture was heated for a further 15 mins at 100 °C (bath temperature) and then stirred at room temperature for 15 mins. The reaction mixture was extracted with EA thrice, combined organics dried over sodium sulphate and concentrated *in vacuo* to obtain white solids (41.3 mg). ^{1}H NMR analysis (taken in CD_3OD solvent) showed that there was presence of bicyclo[1.1.1]pentane-1,3-dicarboxylic acid (major) and 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylic acid (minor, approximately 5% by ^{1}H NMR).

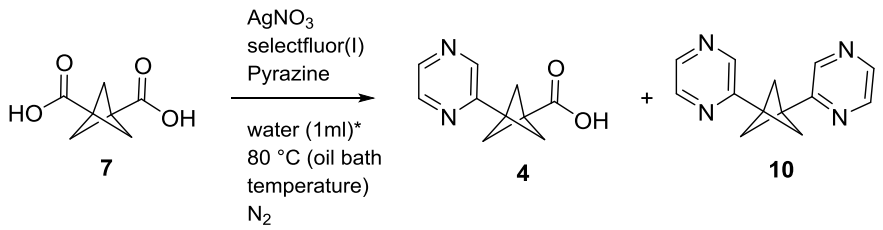
2.2.2 Minisci reaction with 7 as starting material



To bicyclo[1.1.1]pentane-1,3-dicarboxylic acid (**7**) (50 mg, 0.32 mmol), pyrazine (256 mg, 3.20 mmol), silver nitrate (32.6 mg, 0.19 mmol) was added 10% H_2SO_4 (3.20 mL) and acetonitrile (1 mL). The reaction mixture was heated at 100 °C (bath temperature) for 15mins. Then, ammonium persulfate (219.2 mg, 0.96 mmol) in water (2.24 mL) was added dropwise to the heated reaction mixture. The reaction mixture was heated for a further 15mins at 100 °C (bath temperature) and then stirred at room temperature for 15mins. The reaction mixture was extracted with ethyl acetate thrice, combined organics dried over sodium sulphate and concentrated *in vacuo* to obtain white solids (48.7 mg). ^{1}H NMR analysis (taken in CD_3OD solvent) showed that there was presence of the unreacted **7** (major) and **4** (minor, approximately 9% by ^{1}H NMR).

2.3 Homolytic aromatic substitution studies with Li's protocol

2.3.1 Li's protocol employing selectfluor(I)



A mixture of bicyclo[1.1.1]pentane-1,3-dicarboxylic acid (**7**) (50 mg, 0.32 mmol, 1 eq), pyrazine, silver nitrate and selectfluor(I) was subjected to three cycles of vacuum and nitrogen replacement. Degassed DI water[1] was then added and the reaction mixture was heated at 80 °C (oil bath temperature) under nitrogen atmosphere for the respective duration (refer to Table 1). The reaction mixture was cooled to room temperature, washed with water and extracted thrice with ethyl acetate. The combined organics were dried over sodium sulphate and concentrated *in vacuo* to obtain the crude reaction mixture. DMF (24.8 µL, 0.32 mmol, 1 eq) was added to the crude reaction mixture and it was dissolved in acetone-d6 for ^{1}H NMR analysis.

Table 1. Homolytic aromatic substitution studies with Li's protocol

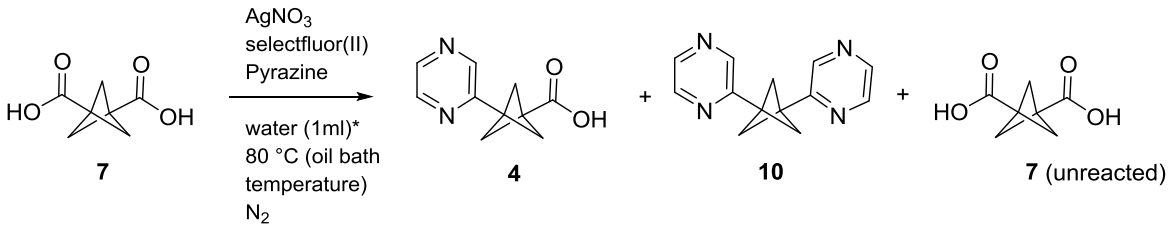
Conditions	Pyrazine (eq)	$AgNO_3$ (eq)	Selectfluor(I) (eq)	% yield[2]
3.5h	40	0.2	3	30
Addition of 0.1 eq $AgNO_3$ and 1 eq selectfluor(I) in 3 parts over 3h 50mins at t = 0h, 3/4h, 1.5h	10	0.3	3	31
1.5h	10	0.2	4	32
3h	10	0.2	6	24
mixed 2 pots separately, then added together, pot 1 – pyrazine, selectfluor (3 eq), DI water (1 mL) pot 2 – starting material, $AgNO_3$, DI	10	0.2	6	24

[1] 1 mL degassed DI water used, unless otherwise stated
[2] ^{1}H NMR yields were calculated employing DMF as an internal standard

water (1 mL), 3h				
mixed 2 pots separately, then added together, pot 1 – pyrazine, selectfluor (3 eq), DI water (0.7 mL) pot 2 – starting material, $AgNO_3$, DI water (0.8 mL), 3h	10	0.2	4	27
1.5h	2	0.2	4	12.5
1.5h	4	0.2	4	20.2
1.5h	6	0.2	4	25

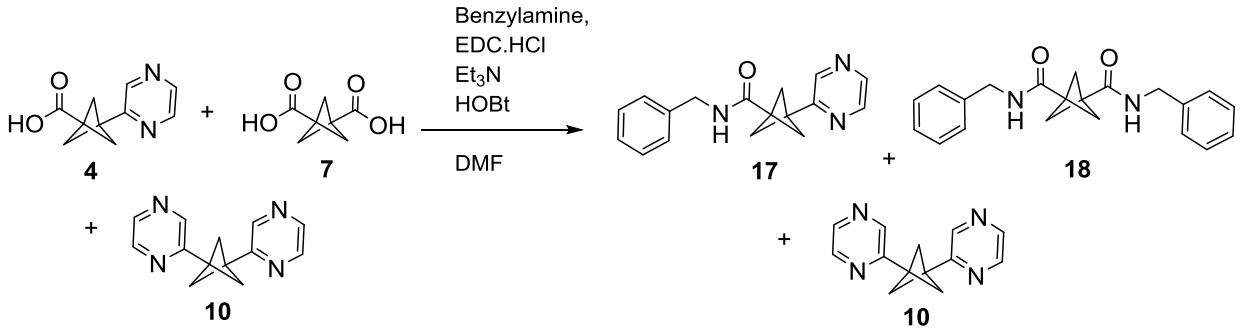
2.3.2 Li's protocol employing selectfluor (II)

<u>Step 1: Homolytic aromatic substitution</u>



To bicyclo[1.1.1]pentane-1,3-dicarboxylic acid (**7**) (50 mg, 0.32 mmol, 1 eq), pyrazine (10 eq), silver nitrate and selectfluor(II) was added degassed DI water (1 mL). The reaction mixture was heated at 80 °C (oil bath temperature) under nitrogen atmosphere for the respective duration (refer to table II). The reaction mixture was cooled to room temperature, washed with water and extracted thrice with ethyl acetate. The combined organics were dried over sodium sulphate and concentrated *in vacuo* to obtain the crude reaction mixture that was carried forward to step 2.

Step 2: Amide coupling



To a solution of the crude reaction mixture from step 1 in anhydrous DMF (1 mL), was added benzylamine (69.9 µL, 0.64 mmol), EDC.HCl (92 mg, 0.48 mmol), HOBt (64.9 mg, 0.48 mmol) and triethylamine (44.4 µL, 0.32 mmol). The reaction mixture was stirred under nitrogen atmosphere for 1.5 to 2h. The reaction mixture was washed with water, extracted thrice with ethyl acetate, the combined organics were dried over sodium sulphate and concentrated in vacuo to obtain the crude reaction mixture. The crude reaction mixture was reconstituted with minimal ethyl acetate for loading on to preparative TLC. However, the by-product **18** (11.6 mg) was not soluble in ethyl acetate, was filtered and isolated as off-white solids. The organics that were soluble in ethyl acetate were purified by preparative TLC with 2% methanol: ethyl acetate solvent system to afford N-benzyl-3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxamide, **17** as white solids (22 mg, 25%)[3], TLC R_f 0.58. 1,3-di(pyrazin-2-yl)bicyclo[1.1.1]pentane, **10** was also isolated as white solids (10.5 mg), TLC R_f 0.51.

Table II. Homolytic aromatic substitution studies with Li's protocol, followed by amide coupling

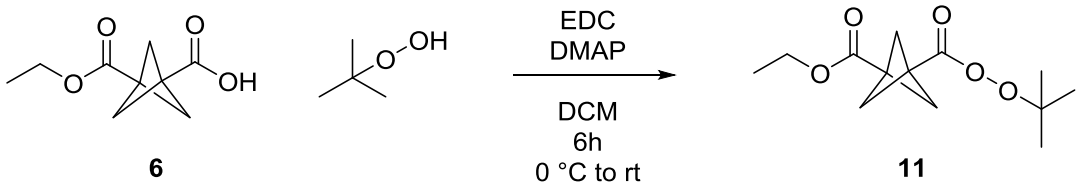
Conditions for Step 1	**Pyrazine (eq)**	**$AgNO_3$ (eq)**	**Selectfluor(II) (eq)**	**Isolated yield of compound 17 after step 2 (%)**
18h	10	0.2	1	16
18h	10	0.2	2	16
6h	10	0.2	3	25
2h	10	1	3	24
2h 45mins	10	1	3	24
3.5h, bubbled with air	10	1	3	12

[3] Best isolated yield for the amide coupling reaction. For isolated yield of other experiments, refer to Table II (above).

2.5h, Addition of $AgNO_3$ in parts, 0.2 eq + 0.1 eq + 0.1 eq	10	0.4	3	25

2.4 Synthesis of compound 11

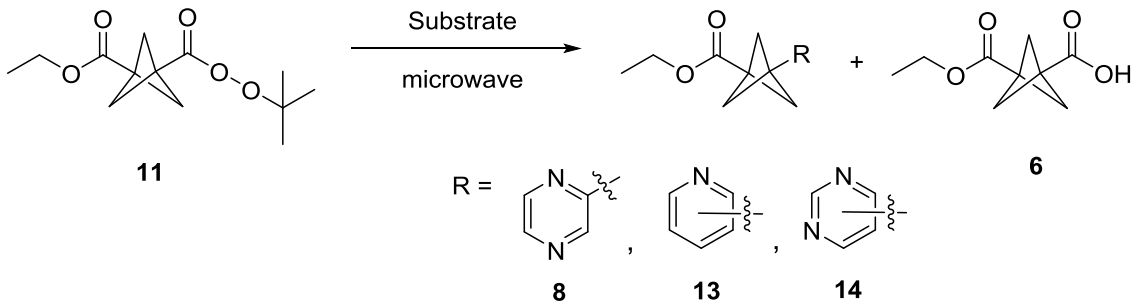
ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (11)[4]



A solution of 3-(ethoxycarbonyl)bicyclo[1.1.1]pentane-1-carboxylic acid (3 g, 16.3 mmol) in anhydrous dichloromethane (81 mL) was cooled to 0 °C in an ice bath and stirred under nitrogen atmosphere for 5 minutes. EDC.HCl (3.43 g, 17.9 mmol), DMAP (159 mg, 1.30 mmol) and 70% w/w solution of *tert*-butylhydroperoxide in water (2.31 mL, 17.9 mmol) were added successively to the reaction mixture and stirred at 0 °C under nitrogen atmosphere for 10 mins. The ice bath was then removed and the reaction mixture was stirred under nitrogen atmosphere for 6 h. The reaction mixture was washed with water and extracted with dichloromethane thrice. The combined organics were washed with saturated sodium thiosulphate solution, followed by saturated sodium bicarbonate solution, dried over anhydrous sodium sulphate and concentrated *in vacuo* to obtain a light yellow oil. The crude product was purified by silica gel flash column chromatography, with gradient elution of 100:0 to 20:80 ethyl acetate: hexanes to afford ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (**11**) as a colourless oil (3.92 g, 93.9%).

[4] Characterisation data was in accordance with literature values, reference: *Org. Biomol. Chem.*, 2015,13, 11597-11601

2.5 General reaction conditions for microwave reactions (Refer to table 1 from manuscript)



Substrate: Pyrazine, pyridine, pyrimidine

General methods

Isothermal heating at 115 °C

To ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (**11**) (50 mg, 0.195 mmol) in a G10 or G30 microwave vial, was added the respective substrate (1 g or 1 mL). The reaction mixture was incubated in the Anton Paar Monowave 300 Microwave Synthesis Reactor by employing the following method:

Step 1: Heat the reaction mixture from room temperature to 115 °C over 2 mins,

Step 2: Heat the reaction mixture at 115 °C for 2 h,

Step 3: Cool down the reaction mixture to 55 °C over 2 mins.

Gradient heating from 100 °C to 130 °C

To ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (50 mg, 0.195 mmol) in a G10 or G30 microwave vial, was added the respective substrate (1 g or 1 mL). The reaction mixture was incubated in the Anton Paar Monowave 300 Microwave Synthesis Reactor by employing the following method:

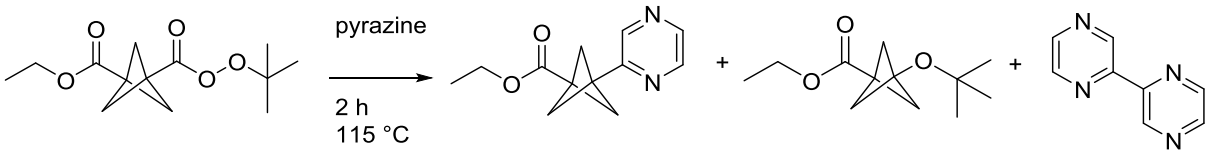
Step 1: heat the reaction mixture from room temperature to 100 °C over 2 mins,

Step 2: heat the reaction mixture at a gradient from 100 °C to 130 °C over 1 h,

Step 3: cool down the reaction mixture to 55 °C over 2 mins.

General work-up procedure

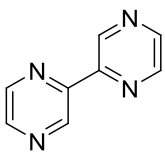
The reaction mixture was cooled to room temperature, washed with water and extracted thrice with ethyl acetate. The organics were washed with saturated sodium bicarbonate, dried over sodium sulphate and concentrated *in vacuo*. The crude reaction mixture was purified by preparative TLC.



11 **8** **12**

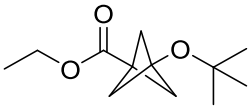
***ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate* (8)**

3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (**11**) (50 mg, 0.195 mmol) and pyrazine (1 g) in a G30 microwave vial were incubated in the Anton Paar Monowave 300 Microwave Synthesis Reactor at 115 °C for 2 h. The reaction mixture was washed with water and extracted thrice with ethyl acetate. The organics were washed with saturated sodium bicarbonate, dried over sodium sulphate and concentrated *in vacuo*. The crude reaction mixture was purified by preparative TLC with 75% ethyl acetate: hexanes solvent system (0.1 mL of ammonia was added to the solvent system during elution of the PTLC plate, and also during the subsequent extraction of the product from the silica gel). The desired product, ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate (**8**) was isolated as a colourless oil (24.4 mg, 57.3%).



2,2'-bipyrazine (12)

This compound was isolated as a by-product from the synthesis of ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate as yellow solids.



ethyl 3-(tert-butoxy)bicyclo[1.1.1]pentane-1-carboxylate (16)

This compound was isolated as a by-product from the synthesis of ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate as a colourless oil. Compound is not UV visible but stains well as a blue spot with CAM stain.

2.6 General microwave conditions for scale up reactions (200 mg starting material scale) (Refer to Table 2 from manuscript)

To ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (200 mg, 0.78 mmol) in a G30 microwave vial, was added the respective substrate (2 g or 2 mL). The microwave vial was sealed and the reaction mixture was subjected to three cycles of vacuum and gas (oxygen or nitrogen or air) replacement. The reaction mixture was then incubated in the Anton Paar Monowave 300 Microwave Synthesis Reactor by employing the following method:

Step 1: heat the reaction mixture from room temperature to 115 °C over 2 minutes,

Step 2: heat the reaction mixture at 115 °C for 2h,

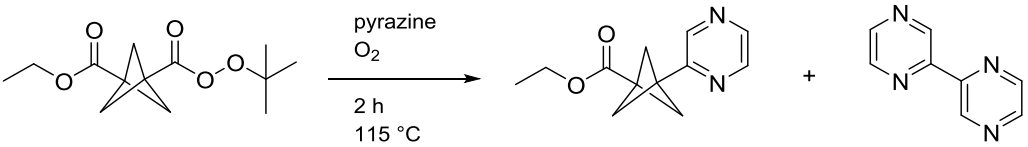
Step 3: cool down the reaction mixture to 55 °C over 2 mins.

Substrates employed for scale up: pyrazine, pyridine, pyrimidine

Work-up procedure

The reaction mixture was cooled to room temperature, washed with water and extracted thrice with ethyl acetate. The organics were washed with saturated sodium bicarbonate, dried over sodium sulphate and concentrated *in vacuo*. The crude reaction mixture was purified by silica gel column chromatography.

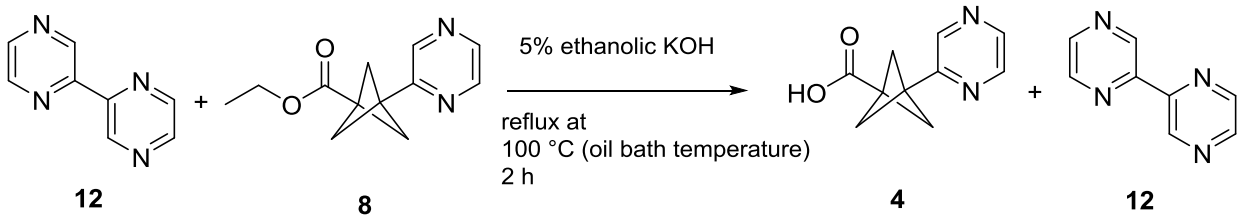
***ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate* (8)**



11 **8** **12**

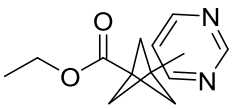
To ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (200 mg, 0.78 mmol) in a G30 microwave vial, was added pyrazine (2 g). The microwave vial was sealed and the reaction mixture was subjected to three cycles of vacuum and oxygen replacement. The reaction mixture was incubated in the Anton Paar Monowave 300 Microwave Synthesis Reactor at 115 °C for 2 h. The reaction mixture was cooled to room temperature, washed with water and extracted thrice with ethyl acetate. The organics were washed with saturated sodium bicarbonate, dried over sodium sulphate and concentrated *in vacuo*. The crude reaction mixture was purified by silica gel column chromatography with 25% ethyl acetate: hexanes solvent system to afford a mixture of the desired product, ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate (**8**, 93.7 mg, 55%) and by-product (**12**, 19.3 mg).[5]

3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylic acid (4)



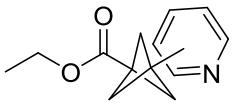
5% ethanolic KOH (6 mL) was added to the mixture of **8** and **12** (113 mg). The reaction mixture was refluxed at 100 °C under air atmosphere for 2 h. TLC showed complete consumption of ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate, (**8**), thus reflux was stopped and the reaction mixture was evaporated to dryness. The yellow precipitate obtained was dissolved in water and washed thrice with dichloromethane to afford the by-product, **12** (19.3 mg). The aqueous layer was acidified with 6M HCl and extracted thrice with dichloromethane. The combined organics were dried over sodium sulphate and concentrated *in vacuo* to afford 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylic acid (**4**) as cream coloured solids (68.5 mg, 83.9%).

[5]Purification attempt to isolate **8** gave an inseparable mixture of **8** and **12**. The given yield of **8** was calculated based on the amount of **12** recovered after the hydrolysis of **8** to **4**.



ethyl 3-(pyrimidin-n-yl)bicyclo[1.1.1]pentane-1-carboxylate (13) (where n = 2 or 4) Crude reaction mixture was purified by silica gel column chromatography with 25% ethyl acetate: petroleum ether solvent system to afford a mixture of isomers of the desired product as a yellow oil (92.6 mg, 54.4%) ^{1}H NMR ratio of 2: 4/6: 5 isomers of **14** was 0.38: 1: 0.17[6].

The mixture of isomers of the desired product of **13**[7] was purified by preparative TLC with 50% ethyl acetate: hexanes solvent system (developed twice) to afford a mixture of ethyl 3-(pyrimidin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate and ethyl 3-(pyrimidin-4-yl)bicyclo[1.1.1]pentane-1-carboxylate as a colourless oil (19 mg, 44.6%), TLC Rf 0.57. Ammonia was added in the solvent during extraction of product from silica gel. This was further purified by preparative TLC with 2% methanol: dichloromethane solvent system to afford the isomers of **13** in a relatively pure form[8,9].



ethyl 3-(pyridin-n-yl)bicyclo[1.1.1]pentane-1-carboxylate (14) (where *n*=2,3,4), Crude reaction mixture was purified by silica gel column chromatography via gradient elution with a solvent system of 0% to 40% ethyl acetate: petroleum ether to afford a mixture of isomers of the desired product as a yellow oil (73.3 mg, 43.2%) ^{1}H NMR ratio of 2/6: 3/5: 4 isomers of **14** was 1:0.46: 0.38[10].

The isomers of the desired product of **14**[11] were purified by preparative TLC with 50% ethyl acetate: hexanes and the isomers of the desired product were isolated from the preparative

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[6] ^{1}H NMR ratios were derived from the analysis of the crude reaction mixture.

[7] Reaction was performed on a 50 mg scale of starting material, microwave conditions: gradient heating from 100 to 130°C over 1h.

[8] Both isomers were contaminated with a trace of the other isomer.

[9] Only trace amounts of the third isomer observed from ^{1}H NMR analysis of the crude reaction mixture, and the third isomer was not identified.

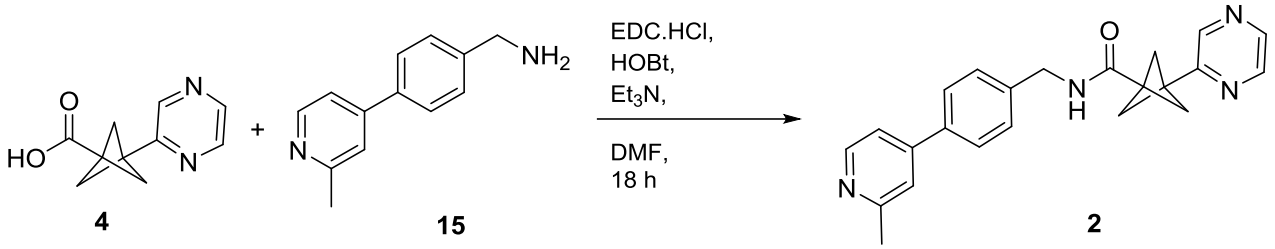
[10] ^{1}H NMR ratios were derived from analysis of the crude reaction mixture.

[11] Reaction was performed on a 50 mg scale of starting material, microwave conditions: gradient heating from 100 to 130°C over 1h.

TLC at TLC R_f 0.67, 0.62, 0.51 respectively. Ammonia was added in the solvent during extraction of product from silica gel. Overall yield of the isomers of **14** was 42.2%.

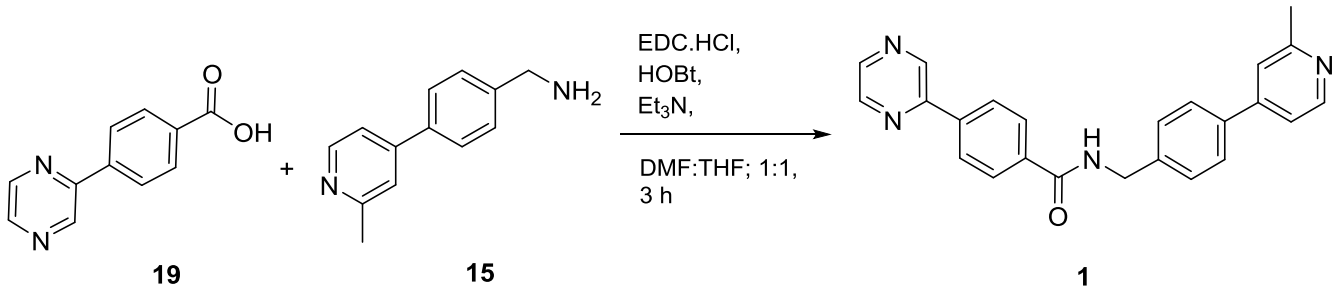
2.7 Synthesis of compounds for WNT assay

N-(4-(2-methylpyridin-4-yl)benzyl)-3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxamide (2)



To a solution of 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylic acid (**4**) (47.6 mg, 0.25 mmol) in anhydrous DMF (0.72 mL), was added EDC.HCl (65.4 mg, 0.34 mmol), HOBt (46.1 mg, 0.34 mmol), triethylamine (63.1 µL, 0.46 mmol) and (4-(2-methylpyridin-4-yl)phenyl)methanamine, **15**[12] (45.1 mg, 0.23 mmol). The reaction mixture was stirred for 18h under argon atmosphere at room temperature. The reaction mixture was washed with water and extracted thrice with ethyl acetate. The organics were dried over sodium sulphate and concentrated *in vacuo* to obtain the crude reaction mixture. The crude reaction mixture was purified by silica gel flash column chromatography with gradient elution of 0:100 to 9:91 methanol: ethyl acetate to afford the desired product (**2**) as a white solid (59.5 mg, 70.6%).

N-(4-(2-methylpyridin-4-yl)benzyl)-4-(pyrazin-2-yl)benzamide (1)



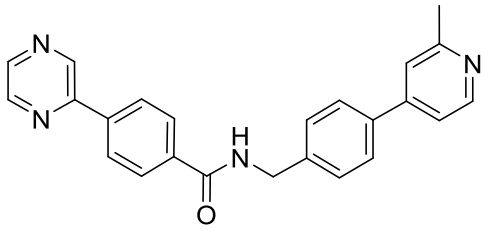
[12] **15** was synthesized by adapting known synthetic procedure from the patent: CUREGENIX INC.; AN, Songzhu; WO2013/185353; (2013); (A1).

To a solution of 4-(pyrazin-2-yl)benzoic acid, (**19**)[13] (100.7 mg, 0.50 mmol) in anhydrous DMF (2 mL) and anhydrous THF (2 mL)[14], was added EDC.HCl (51.6 mg, 0.27 mmol), HOBt (36.4 mg, 0.27 mmol), triethylamine (49.8 µL, 0.36 mmol) and (4-(2-methylpyridin-4-yl)phenyl)methanamine, (**15**) (35.6 mg, 0.18 mmol). The reaction mixture was stirred for 3h under argon atmosphere at room temperature. The reaction mixture was washed with water and extracted thrice with ethyl acetate. The organics were washed twice with saturated sodium bicarbonate, dried over sodium sulphate and concentrated *in vacuo* to obtain the crude reaction mixture. The crude reaction mixture was purified by silica gel flash column chromatography with gradient elution of 0: 100 to 6:94 methanol: chloroform to afford the desired product, **1** as a white solid (36.5 mg, 53.4%). **1** was further purified by trituration and washed with minimal amounts of diethyl ether followed by dichloromethane. The remaining solids were dissolved in methanol and concentrated to afford *N*-(4-(2-methylpyridin-4-yl)benzyl)-3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxamide, **1** as a white solid (32.7 mg, 47.8%).

[13] Purchased from Apollo Scientific.
[14] A mixture of solvents DMF: THF = 1:1 was used as **19** was not soluble in 100% DMF.

3 Characterisation Data

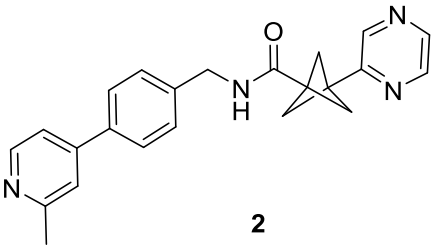
***N-(4-(2-methylpyridin-4-yl)benzyl)-4-(pyrazin-2-yl)benzamide* (*1*)**



1

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.08 (d, *J* = 1.6 Hz, 1H), 8.67 (dd, *J* = 2.5, 1.5 Hz, 1H), 8.59 – 8.53 (m, 2H), 8.13 (d, *J* = 8.6 Hz, 2H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.69 – 7.61 (m, 2H), 7.56 – 7.49 (m, 2H), 7.47 – 7.44 (m, 1H), 7.42 (d, *J* = 5.5 Hz, 1H), 6.61 (s, 1H), 4.76 (d, *J* = 5.8 Hz, 2H), 2.71 (s, 3H); **HRMS (ESI^{+})** Calcd for C24H20N4O + H, 381.1637, Found, 381.1708; **IR** (thin film, cm^{-1}) 3337, 1638, 1542, 1303, 1017, 842; **TLC** (4:96 Methanol/Chloroform): R_f = 0.38.

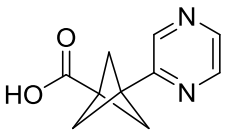
N-(4-(2-methylpyridin-4-yl)benzyl)-3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxamide (2)



2

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (d, *J* = 5.3 Hz, 1H), 8.53 – 8.49 (m, 2H), 8.46 (d, *J* = 2.4 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.46 – 7.39 (m, 3H), 7.37 (dd, *J* = 5.4, 1.8 Hz, 1H), 5.99 (s, 1H), 4.54 (d, *J* = 5.9 Hz, 2H), 2.68 (s, 3H), 2.47 (s, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 169.48, 158.04, 153.67, 149.64, 148.01, 144.03, 143.16, 142.66, 139.62, 137.13, 128.62, 127.51, 121.64, 119.17, 52.73, 43.04, 39.88, 39.44, 23.70; **HRMS (ESI^{+})** Calcd for C23H22N4O + H, 371.1794, Found, 371.1868; **IR** (thin film, cm^{-1}) 3279, 1642, 1608, 1543, 1229, 1018, 816; **TLC** (100% Ethyl acetate): R_f = 0.28.

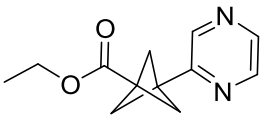
3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylic acid (4)



4

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (dd, *J* = 2.6, 1.5 Hz, 1H), 8.54 (d, *J* = 1.5 Hz, 1H), 8.49 (d, *J* = 2.5 Hz, 1H), 2.52 (s, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 173.98, 153.91, 144.18, 142.99, 142.54, 53.45, 40.72, 37.84; **HRMS (ESI^{+})** Calcd for C10H10N2O2 + H, 191.0742, Found, 191.0819; **IR** (thin film, cm^{-1}) 2924, 1708, 1359, 1299, 1212, 1159, 1030.

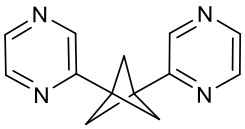
***ethyl 3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate* (8)**



8

^{1}H NMR (400 MHz, $CDCl_3$): δ8.51 (d, *J* = 2.5 Hz, 2H), 8.46 (d, *J* = 2.3 Hz, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 2.46 (s, 6H), 1.29 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ169.86, 154.08, 144.14, 143.10, 142.76, 60.87, 53.36, 40.68, 38.08, 14.34; **HRMS (ESI^{+})** Calcd for C12H14N2O2 + H, 219.1055 found, m/z 219.1136; **IR** (thin film, cm^{-1}) 2984, 1725, 1372, 1301, 1206, 1135, 1016, 772; **TLC** (75:25 Ethyl acetate/Hexane): R_f = 0.63.

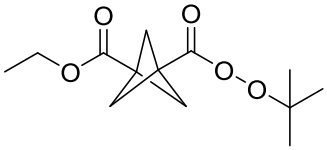
1,3-di(pyrazin-2-yl)bicyclo[1.1.1]pentane (10)



10

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 - 8.48 (m, 6H), 2.63 (s, 6H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ 154.43, 144.21, 143.16, 142.96, 53.86, 40.94; **HRMS (ESI^{+})** Calcd for C13H12N4 +H, 225.1062; Found, 225.1132 [M+H], **IR** (thin film, cm^{-1}) 569, 538, 529, 513, 501, 473; **TLC** (2:98 Methanol: Ethyl acetate): R_f = 0.51

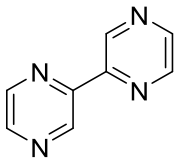
ethyl 3-((tert-butylperoxy)carbonyl)bicyclo[1.1.1]pentane-1-carboxylate (11)



11

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.13 (q, *J* = 7.1 Hz, 2H), 2.35 (s, 6H), 1.30 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 4H); **HRMS (ESI^{+})** Calcd for C13H20O5+Na, 279.1311; Found, 279.1217; **IR** (thin film, cm^{-1}) 2983, 1773, 1732, 1368, 1269, 1204, 1021; **TLC** (20:80 Ethyl acetate/Hexane): R_f = 0.58 (compound was visible under UV and stained well in $KMnO_4$)

2,2'-bipyrazine (12)

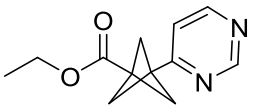


12

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.59 (d, 2H), 8.66 (s, 4H); **TLC** (75:25 Ethyl acetate/Hexane): R_f = 0.56.[15]

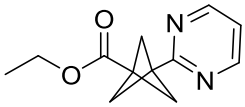
[15] Characterisation data was in accordance with literature values, references: *Tetrahedron*, *1994*, *50*, *11893-11902 and Beilstein J. Org. Chem.,* 2015, 11, 61–65.

Compound **13**



ethyl 3-(pyrimidin-4-yl)bicyclo[1.1.1]pentane-1-carboxylate (4/6-isomer, major isomer) colourless oil, 11.5 mg

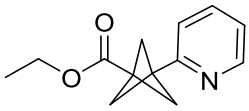
^{1}H NMR (400 MHz, $CDCl_3$): δ9.17 (d, *J* = 1.4 Hz, 1H), 8.67 (d, *J* = 5.1 Hz, 1H), 7.20 (dd, *J* = 5.2, 1.4 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 2.43 (s, 6H), 1.30 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.75, 166.86, 158.49, 156.83, 118.47, 60.92, 53.18, 41.71, 37.81, 14.32[16]; **HRMS (ESI^{+})** Calcd for C12H14N2O2 + H, 219.1055; Found, 219.1135 [M+H].



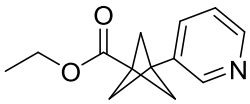
ethyl 3-(pyrimidin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate (2-isomer, minor isomer) colourless oil, 2 mg

^{1}H NMR (400 MHz, $CDCl_3$): δ8.71 (d, *J* = 4 Hz, 2H), 7.19 (t, *J* = 4.9Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 2.49 (s, 6H), 1.30 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.33, 167.15, 157.22, 119.43, 60.80, 53.46, 42.75, 37.67, 14.37; **HRMS (ESI^{+})** Calcd for C12H14N2O2 + H, 219.1055; Found, 219.1135 [M+H].

Compound **14**



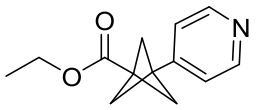
ethyl 3-(pyridin-2-yl)bicyclo[1.1.1]pentane-1-carboxylate (2/6 isomer), yellow oil (9.6 mg, 22.7%) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.60 – 8.56 (m, 1H), 7.71 (td, J = 7.7, 1.8 Hz, 1H), 7.26 – 7.21 (m, 2H), 4.16 (q, J = 7.1 Hz, 2H), 2.44 (s, 6H), 1.28 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.25, 158.07, 148.63, 137.48, 122.40, 121.34, 60.79, 53.34, 42.05, 37.55, 14.34; **HRMS (ESI^{+})** Calcd for C13H15NO2 + H, 218.1103, Found, 218.1168; **IR** (thin film, cm^{-1}) 2983, 1725, 1372, 1302, 1204, 1128, 1019; **TLC** (50:50 Ethyl acetate/Hexane): R_f = 0.67.



ethyl 3-(pyridin-3-yl)bicyclo[1.1.1]pentane-1-carboxylate (3/5 isomer), yellow oil (4.4 mg, 10.4%) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.60 – 8.45 (m, 2H), 7.72 – 7.60 (m, 1H), 7.37 (dd, J = 7.9, 4.9 Hz, 1H), 4.17 (qd, J = 7.1, 0.7 Hz, 2H), 2.38 (s, 6H), 1.29 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.74, 146.41, 146.21, 136.20, 135.86, 123.97, 60.91, 53.51, 39.65, 37.79, 14.34; **HRMS (ESI^{+})** Calcd for C13H15NO2 + H, 218.1103, Found,

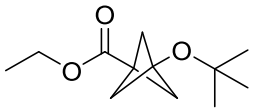
[16] Minor peaks observed in ^{13}C NMR spectra are from the other isomer of compound **13**.

218.1170; **IR** (thin film, cm^{-1}) 2982, 1726, 1373, 1299, 1208, 1194, 1123, 713; **TLC** (50:50 Ethyl acetate/Hexane): R_f = 0.62.



ethyl 3-(pyridin-4-yl)bicyclo[1.1.1]pentane-1-carboxylate (4-isomer), yellow oil (3.9 mg, 9.2%) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.59 (d, J = 5.4 Hz, 2H), 7.31 (d, J = 6.2 Hz, 2H), 4.18 (q, J = 7.1 Hz, 2H), 2.39 (s, 6H), 1.29 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.56, 151.65, 147.08, 122.47, 61.02, 53.42, 40.91, 37.64, 14.33; **HRMS (ESI$^+$)** Calcd for C13H15NO2 + H, 218.1103, Found, 218.1169; **IR** (thin film, cm^{-1}) 2984, 1727, 1373, 1299, 1207, 1121, 772; **TLC** (50:50 Ethyl acetate/Hexane): R_f = 0.51

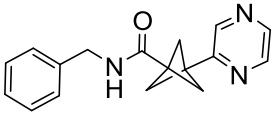
ethyl 3-(tert-butoxy)bicyclo[1.1.1]pentane-1-carboxylate (16)



16

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.13 (q, J = 7.1 Hz, 2H), 2.28 (s, 6H), 1.31 – 1.20 (m, 12H); **^{13}C NMR** (101 MHz, $CDCl_3$): δ170.3, 76.1, 63.8, 60.8, 56.9, 33.0, 29.4, 14.3; **HRMS (ESI$^+$)** Calcd for C12H20O3 + H, 213.1412, Found, 213.1492; **IR** (thin film, cm^{-1}) 2981, 1732, 1370, 1336, 1268, 1191, 1067; **TLC** (15:85 Ether/Petroleum ether): R_f = 0.74.

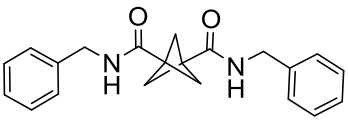
N-benzyl-3-(pyrazin-2-yl)bicyclo[1.1.1]pentane-1-carboxamide (17)



17

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 – 8.39 (m, 3H), 7.40 – 7.27 (m, 5H), 5.91 (bs, 1H), 4.47 (d, *J* = 5.7 Hz, 2H), 2.44 (s, 6H)ppm; **^{13}C NMR** (101 MHz, $CDCl_3$): δ 169.48, 153.93, 144.16, 143.24, 142.81, 138.13, 128.94, 128.03, 127.81, 52.84, 43.65, 39.97, 39.60; **HRMS (ESI$^+$)** Calcd for C17H17N3O +H, 280.1372; Found, 280.1443 [M+H], 302.1264 [M+Na]; **IR** (thin film, cm^{-1}); 3239, 2921, 1639, 1562, 1455, 1220; **TLC** (2:98 Methanol: Ethyl acetate): R_f = 0.58.

N^1,N^3-dibenzylbicyclo[1.1.1]pentane-1,3-dicarboxamide (18)



18

^{1}H NMR (400 MHz, CD_3OD): δ 7.35 – 7.19 (m, 10H), 4.36 (s, 4H), 2.25 (s, 6H); IR (thin film, cm^{-1}) 570, 558, 547, 499, 491, 483, 473; ; **^{13}C NMR** (101 MHz, CD_3OD): δ 172.19, 139.94, 129.50, 128.49, 128.19, 52.77, 43.90, 39.52; **HRMS (ESI$^+$)** Calcd for C21H22N2O2 +H, 335.1681; Found, 335.1750 [M+H]; **IR** (thin film, cm^{-1}) 2959, 2926, 2856, 1625, 1540, 1455, 1224.

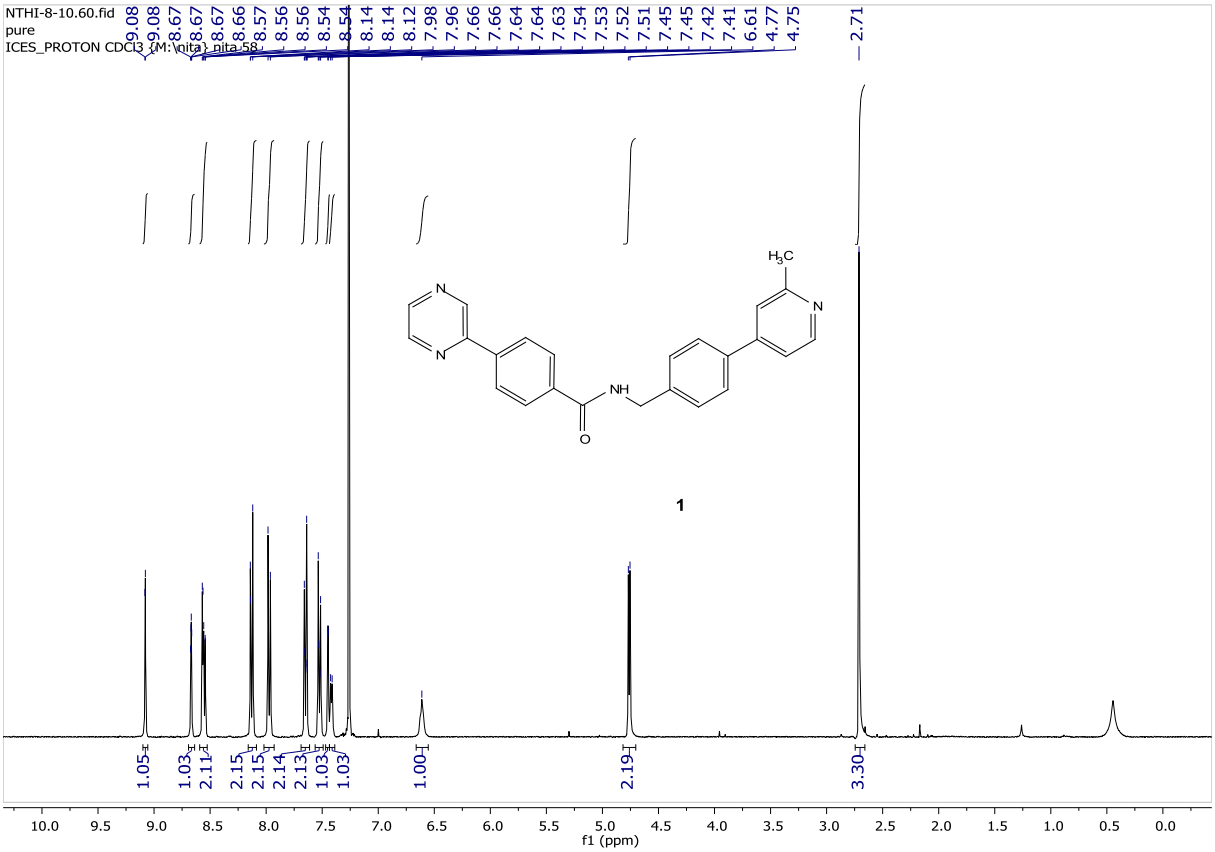
4 Cell-based Wnt reporter assay[17,18]

Preparation of STF3A cells and the assay conditions used to determine IC_{50} of the compounds were described in Coombs et al. (2010) with some modifications. $2x10^4$ STF3A cells were seeded in 75 µL culture medium in each well of a 96 well plate (Greiner 655098) and incubated overnight at 37 °C. Then 25 µL of serially diluted compounds were added to the cells to give final concentrations of 50 µM to 1.5 nM. One day after treatment, 100 µL of Steady-Glo Luciferase Assay reagent (Promega) was added to each well and incubated for 10 minutes at room temperature. Luminescence was measured using the Tecan Infinite M200 PRO microplate reader.

[17] Reference: Coombs, GS, Yu J, Canning CA, Veltri CA, Covey TM, Cheong JK, Utomo V, Banerjee N, Zhang ZH, Jadulco RC, Concepcion GP, Bugni TS, Harper MK, Mihalek I, Jones CM, Ireland CM, VIrshup DM. 2010 . WLS-dependent secretion of WNT3A r equires Ser209 acylation and vacuolar acidification. J Cell Sci. 123(Pt 19), 3357–3367.

[18] Assay performed by: Ke Zhiyuan and Lee May Ann from the Experimental Therapeutics Centre (ETC, A*STAR).

^{1}H & ^{13}C NMR SPECTRA


NTHI-8-10.60.fid
pure
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9.08
9.08
8.67
8.67
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8.56
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8.14
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7.41
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4.77
4.75
2.71
H3C
N
N
N
NH
O
1
1.05
1.03
2.11
2.15
2.15
2.14
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1.03
1.03
1.00
2.19
3.30
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
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2.5
2.0
1.5
1.0
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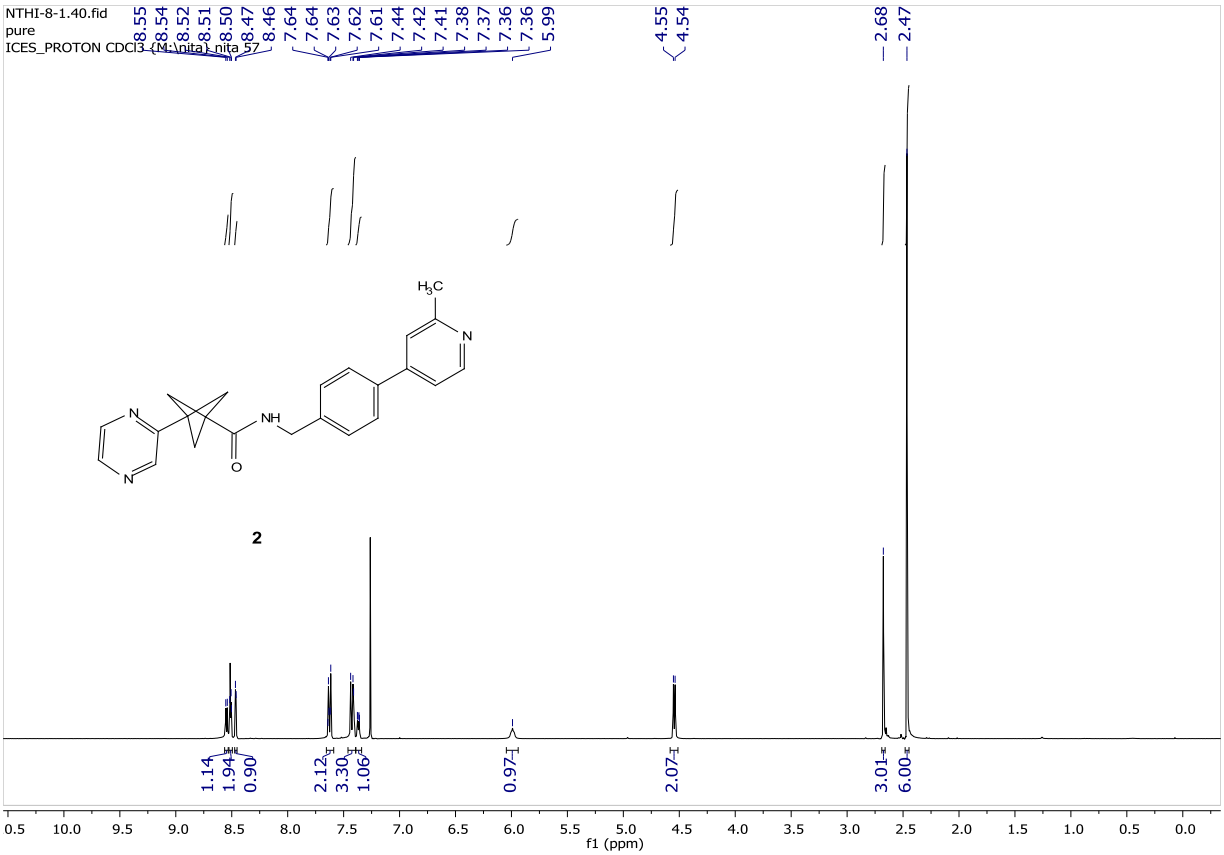
0.0
f1 (ppm)


NTHI-8-1.40.fid
pure
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8.52
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8.50
8.47
8.46
7.64
7.64
7.63
7.62
7.61
7.44
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7.41
7.38
7.37
7.36
7.36
5.99
4.55
4.54
2.68
2.47
H3C
N
NH
O
N
N
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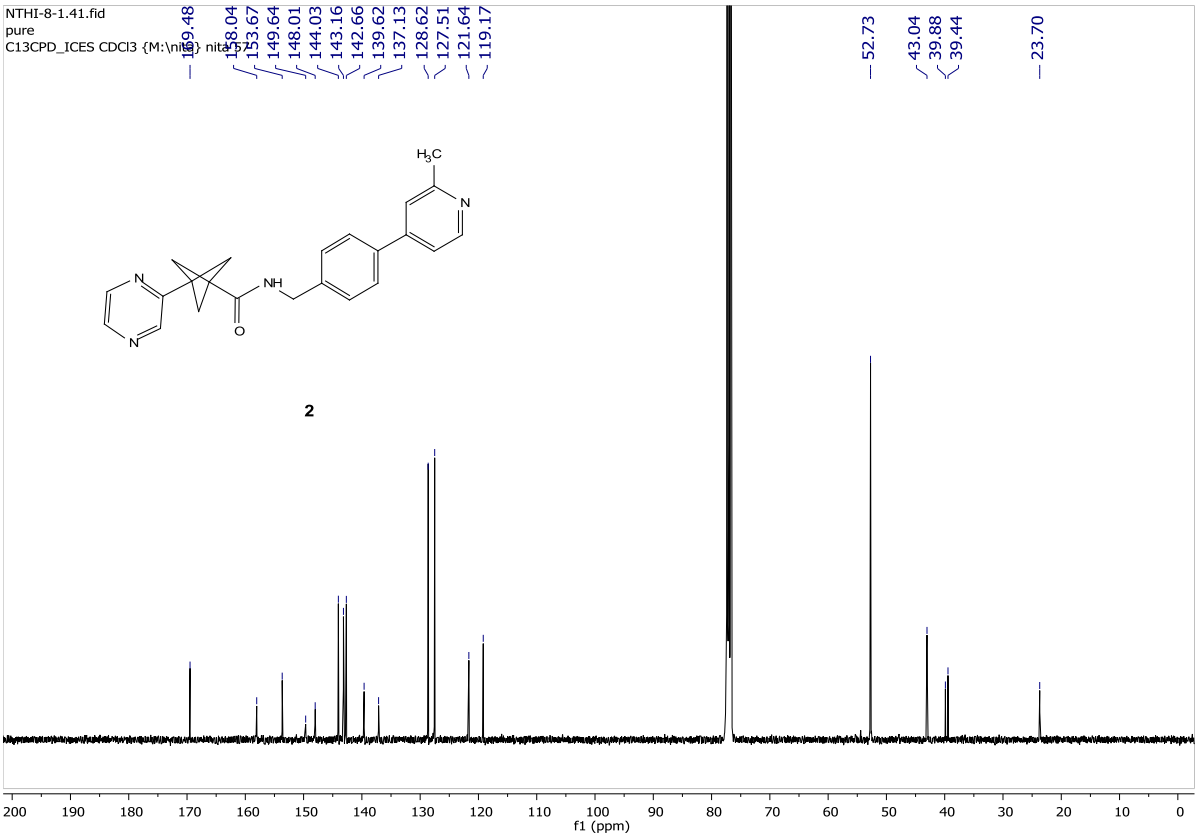
1.94
0.90
2.12
3.30
1.06
0.97
2.07
3.01
6.00
f1 (ppm)


NTHI-8-1.41.fid
pure
C13CPD_ICES CDCl3 {M:\nita} nita 57
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139.62
137.13
128.62
127.51
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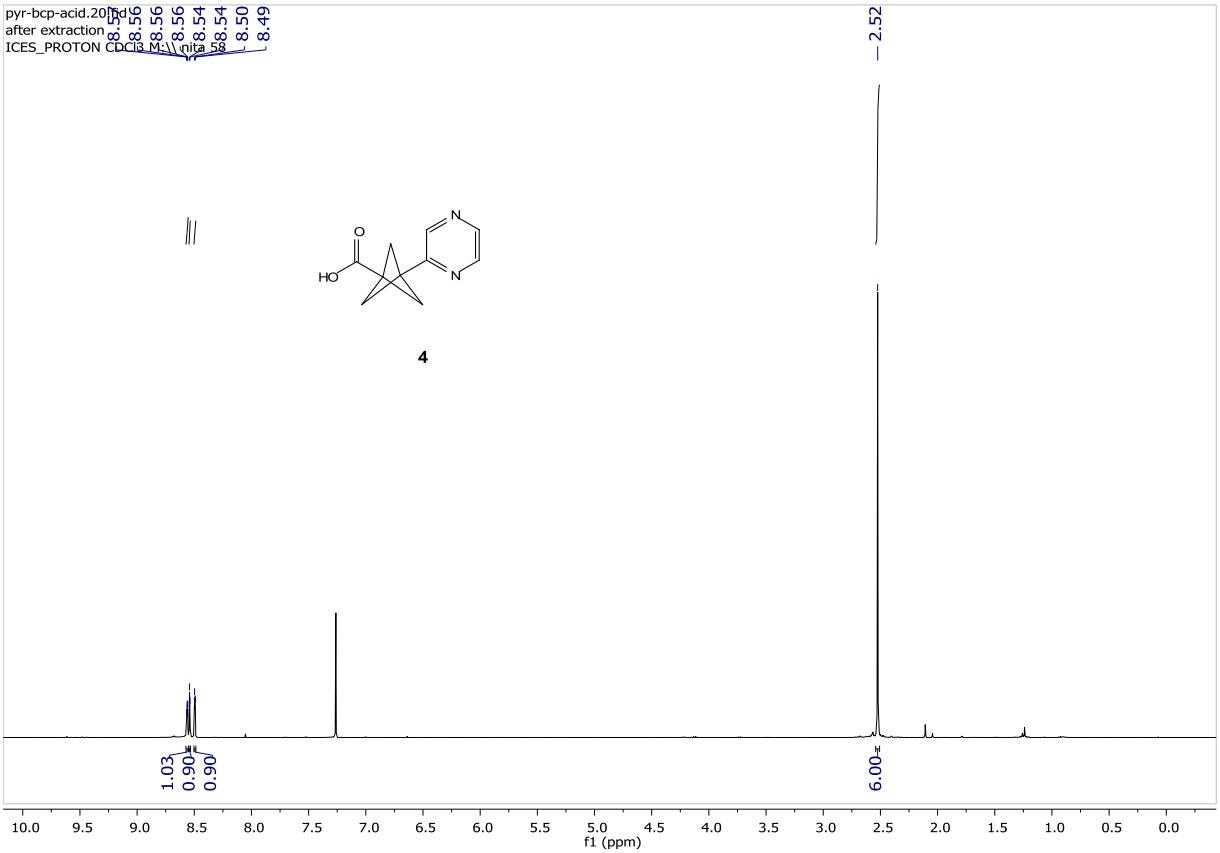
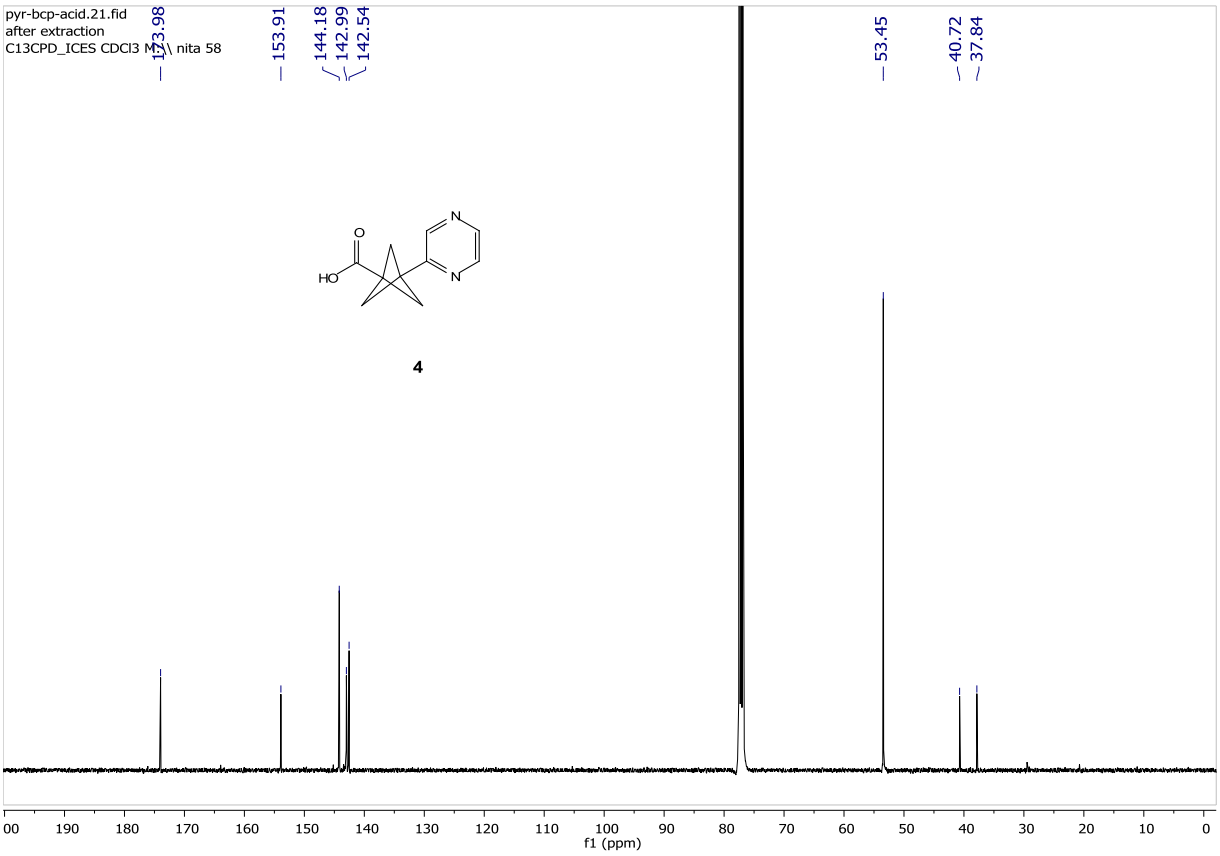
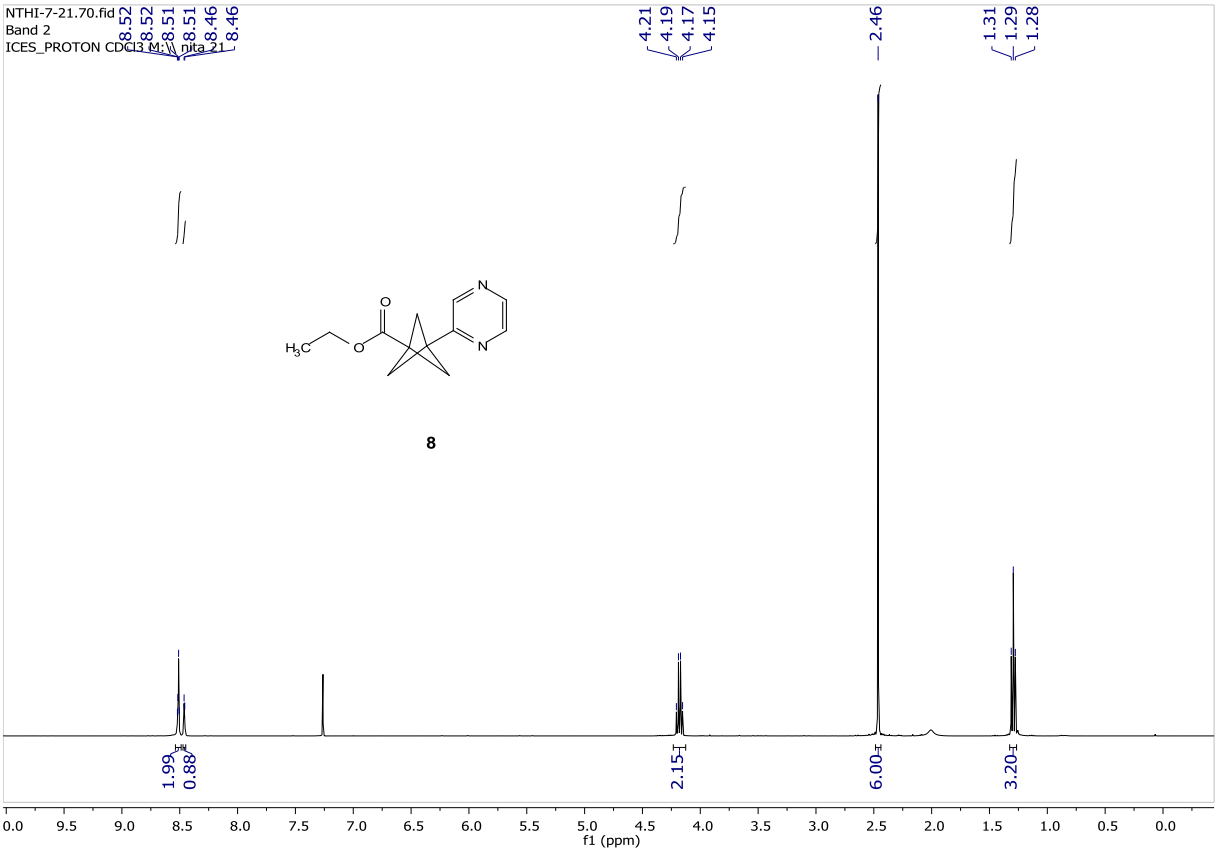
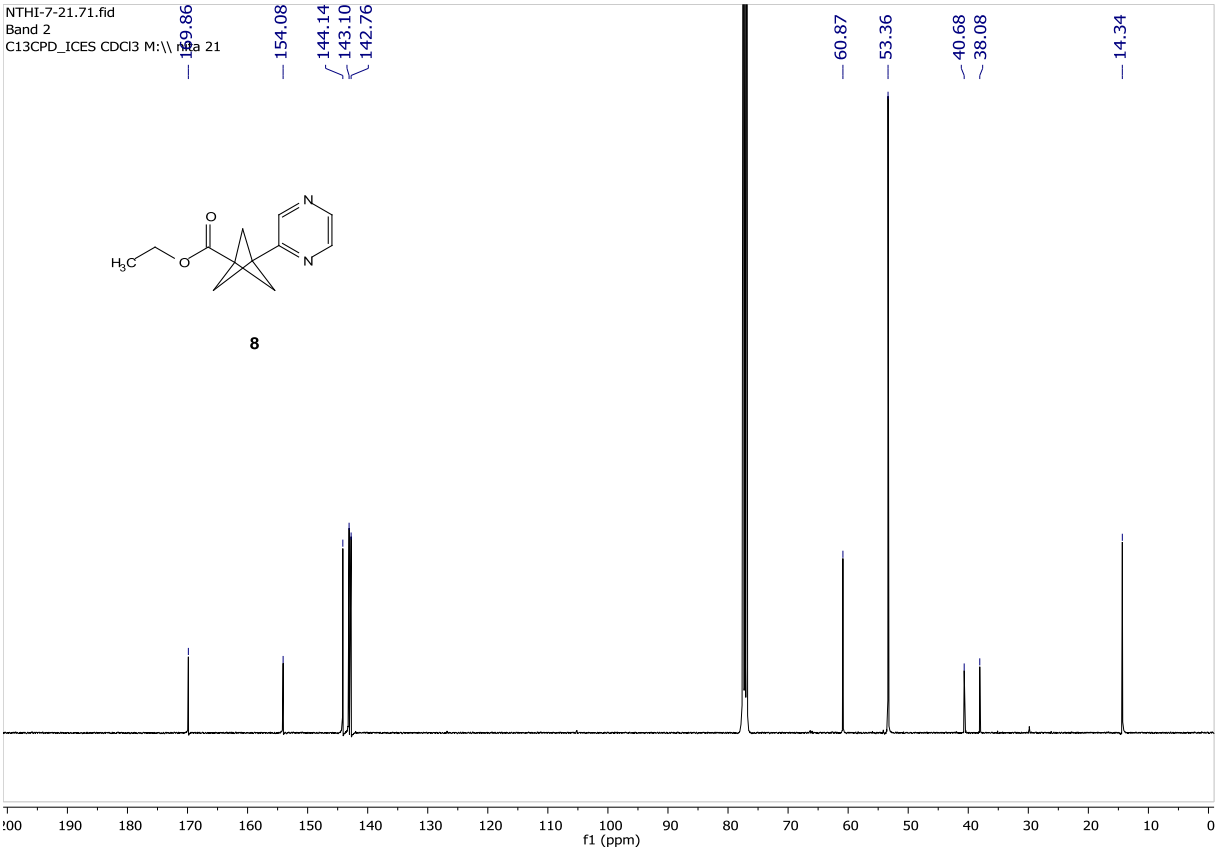
NH
O
N
N
2
f1 (ppm)


pyr-bcp-acid.20.fid
after extraction
ICES_PROTON CDCl3 M:\\ nita 58
8.57
8.56
8.56
8.56
8.54
8.54
8.50
8.49
2.52
1.03
0.90
0.90
6.00
4
f1 (ppm)

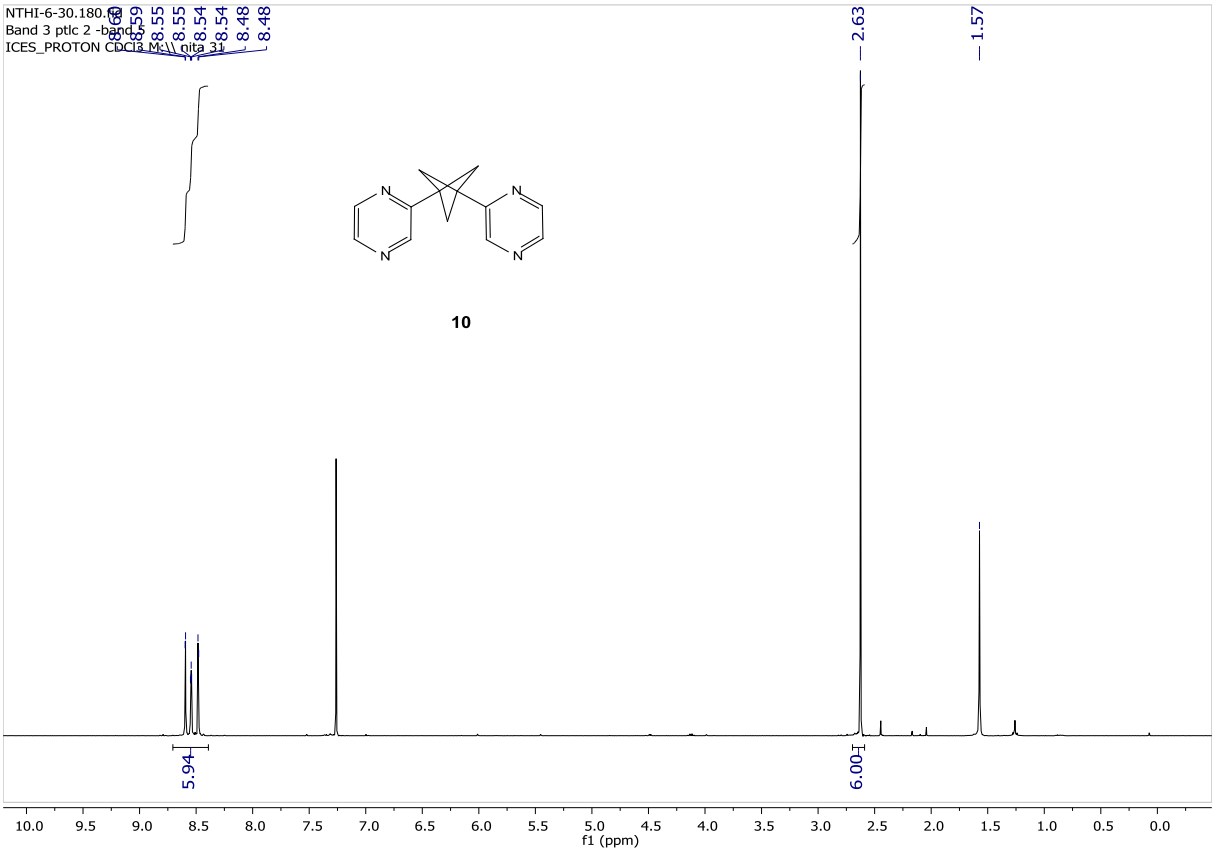

pyr-bcp-acid.21.fid
after extraction
C13CPD_ICES CDCl3 M:\\ nita 58
173.98
153.91
144.18
142.99
142.54
53.45
40.72
37.84
4
f1 (ppm)

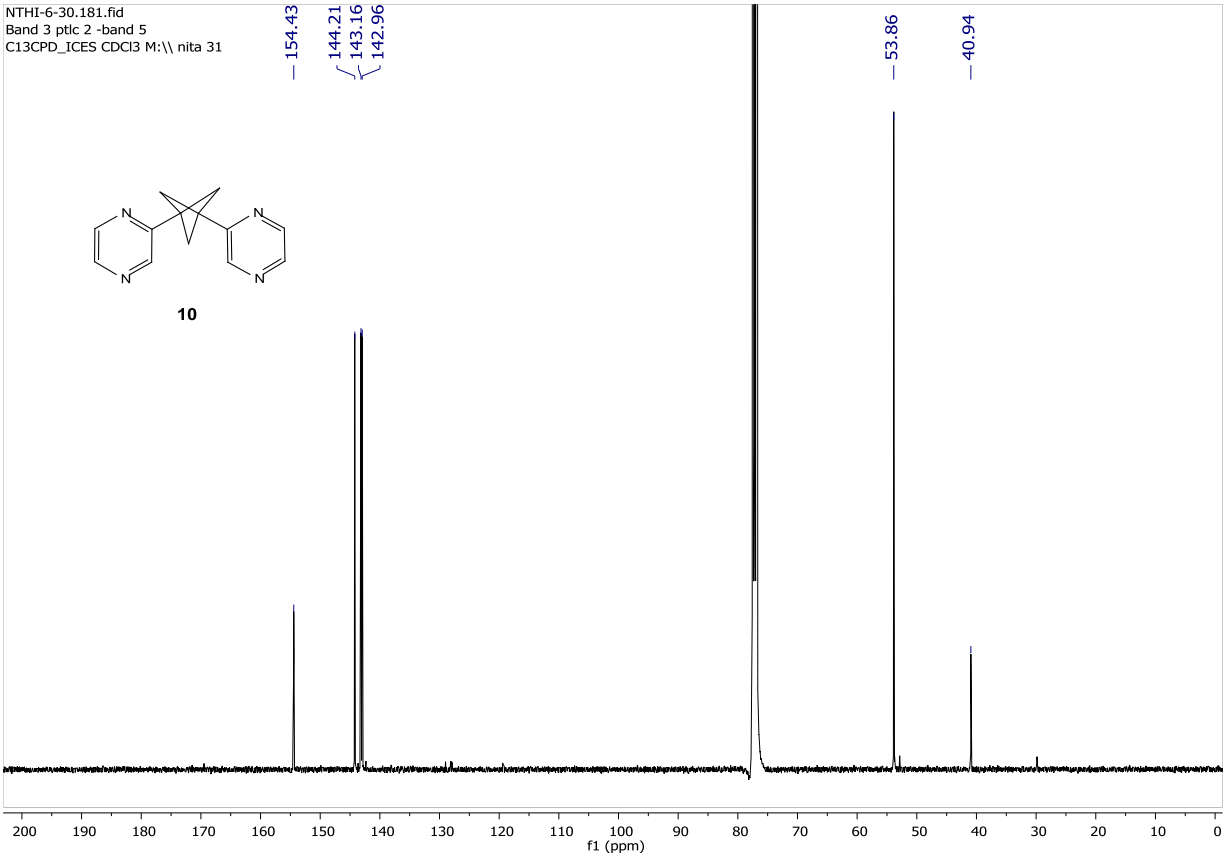

NTHI-7-21.70.fid
Band 2
ICES_PROTON CDCl3 M:\\ nita 21
8.52
8.52
8.51
8.51
8.46
8.46
4.21
4.19
4.17
4.15
2.46
1.31
1.29
1.28
1.99
0.88
2.15
6.00
3.20
f1 (ppm)

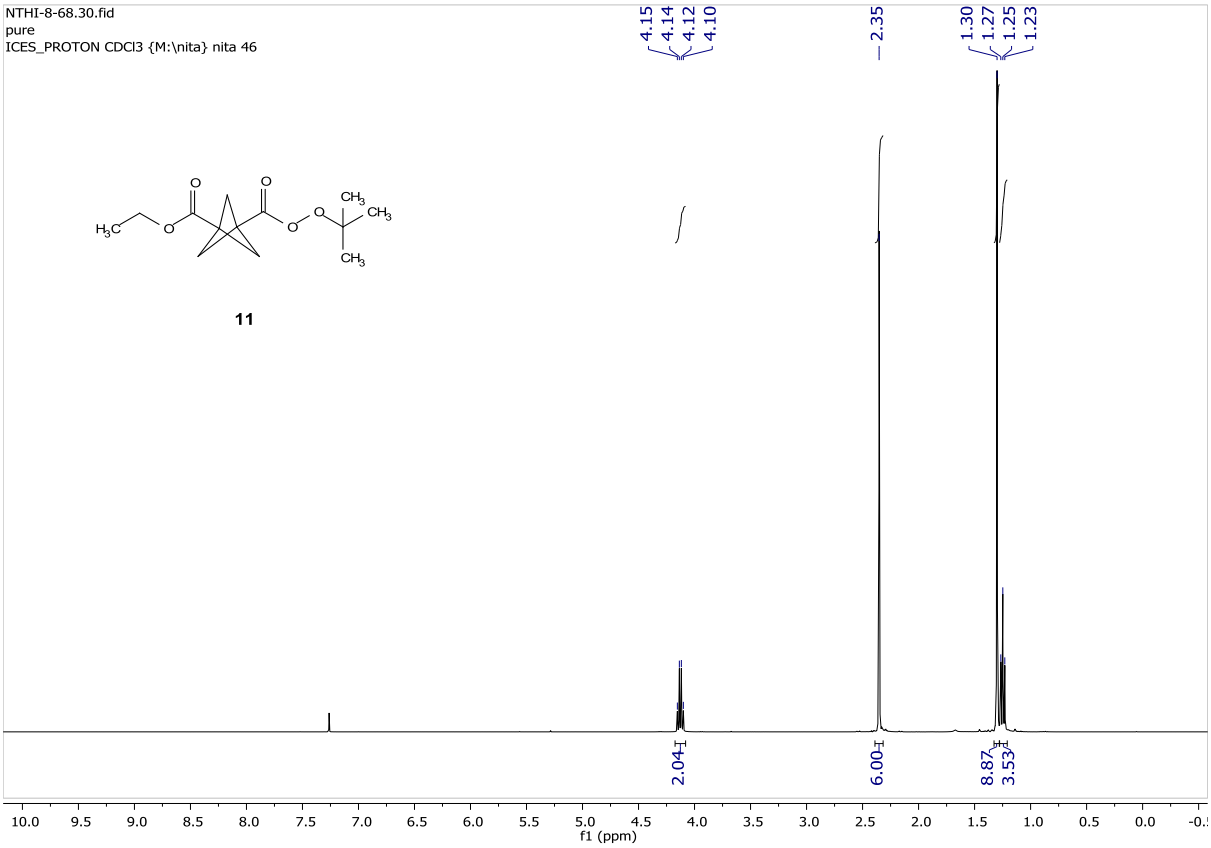
8


NTHI-7-21.71.fid
Band 2
C13CPD_ICES CDCl3 M:\\ nita 21
169.86
154.08
144.14
143.10
142.76
60.87
53.36
40.68
38.08
14.34
f1 (ppm)

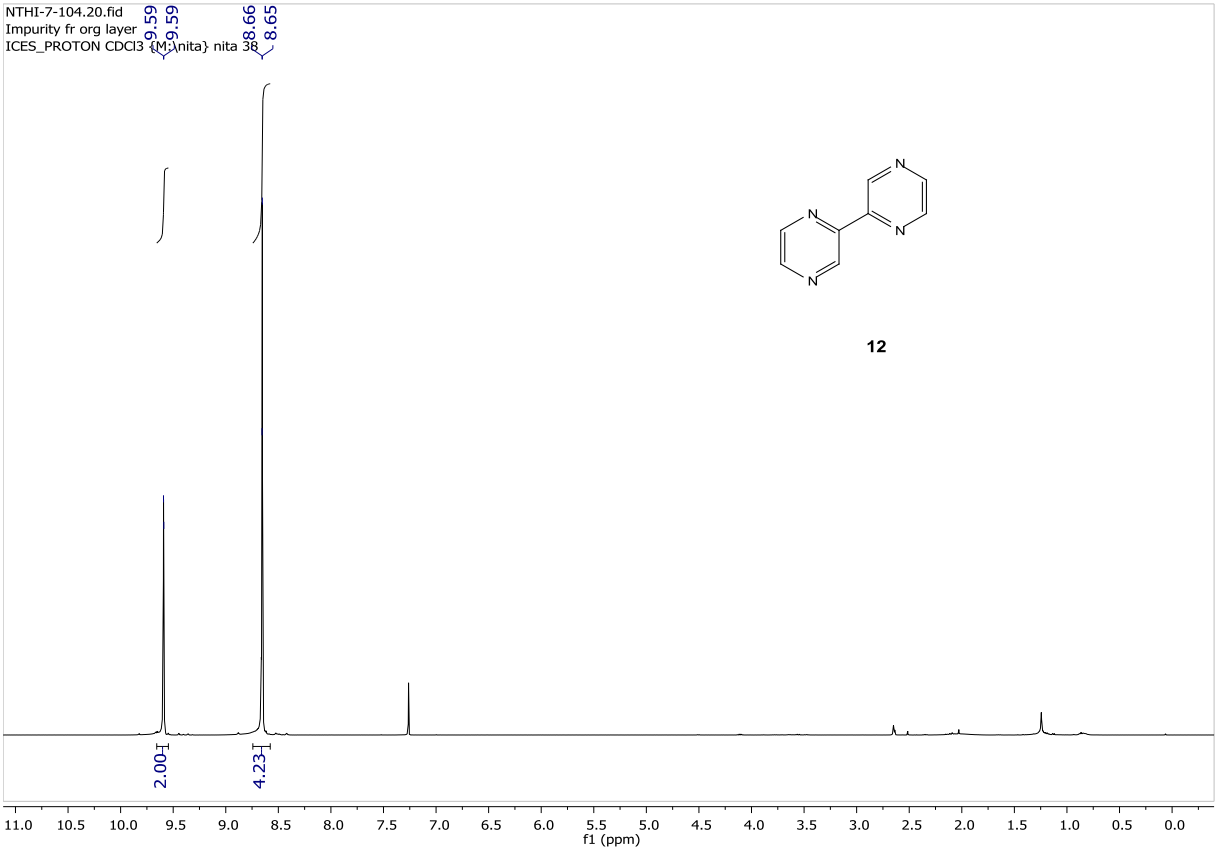
8


NTHI-6-30.180.fid
Band 3 ptlc 2 -band 5
ICES_PROTON CDCl3 M:\\ nita 31
8.60
8.59
8.55
8.54
8.54
8.48
8.48
2.63
1.57
10
5.94
6.00
f1 (ppm)

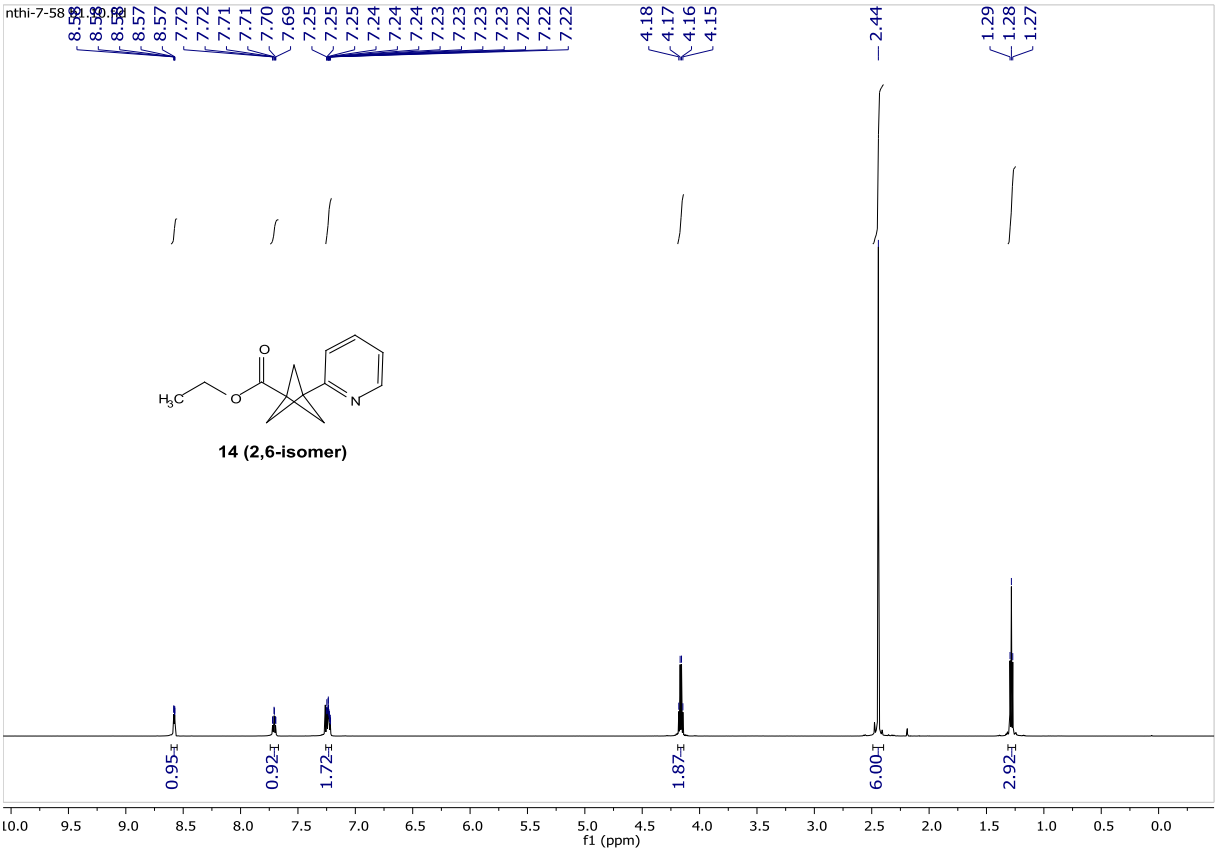

NTHI-6-30.181.fid
Band 3 ptlc 2 -band 5
C13CPD_ICES CDCl3 M:\\ nita 31
154.43
144.21
143.16
142.96
53.86
40.94
10
f1 (ppm)


NTHI-8-68.30.fid
pure
ICES_PROTON CDCl3 {M:\nita} nita 46
4.15
4.14
4.12
4.10
2.35
1.30
1.27
1.25
1.23
2.04
6.00
8.87
3.53
f1 (ppm)

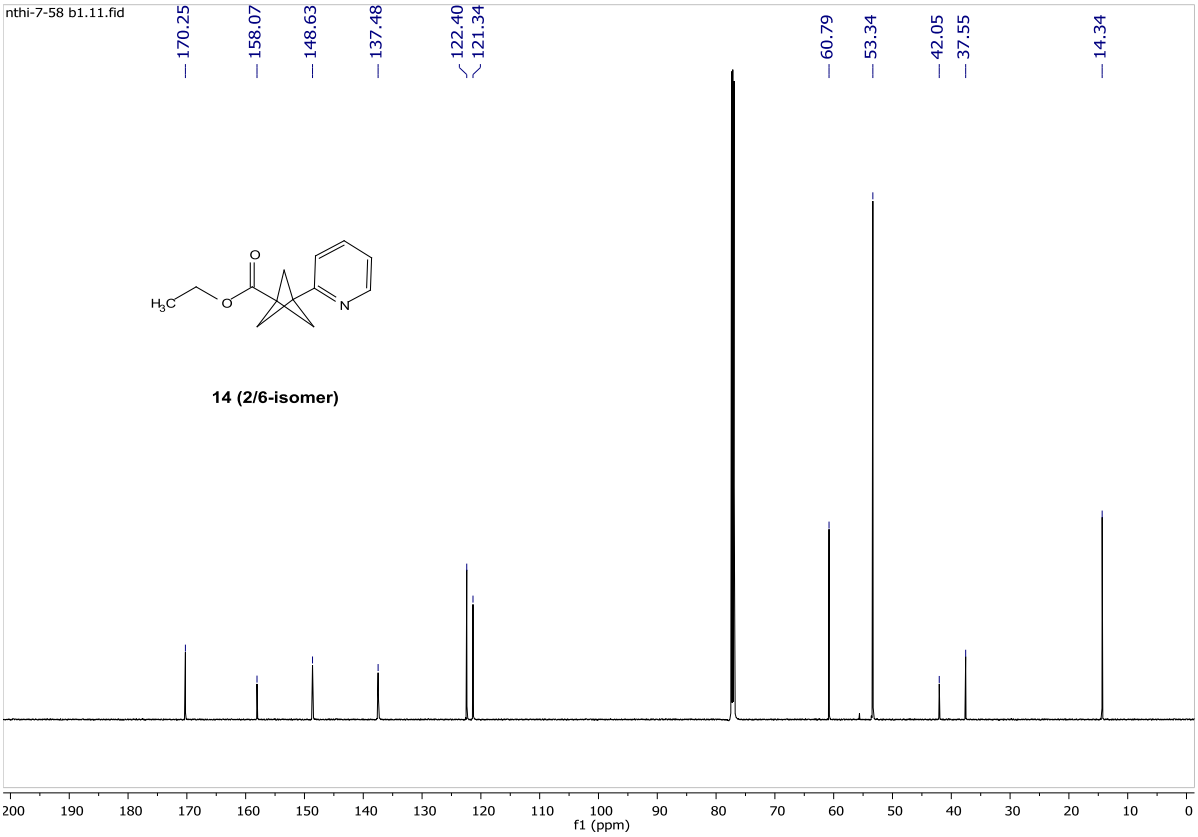
11


NTHI-7-104.20.fid
Impurity fr org layer
ICES_PROTON CDCl3 {M:\nita} nita 38
9.59
9.59
8.66
8.65
2.00
4.23
f1 (ppm)

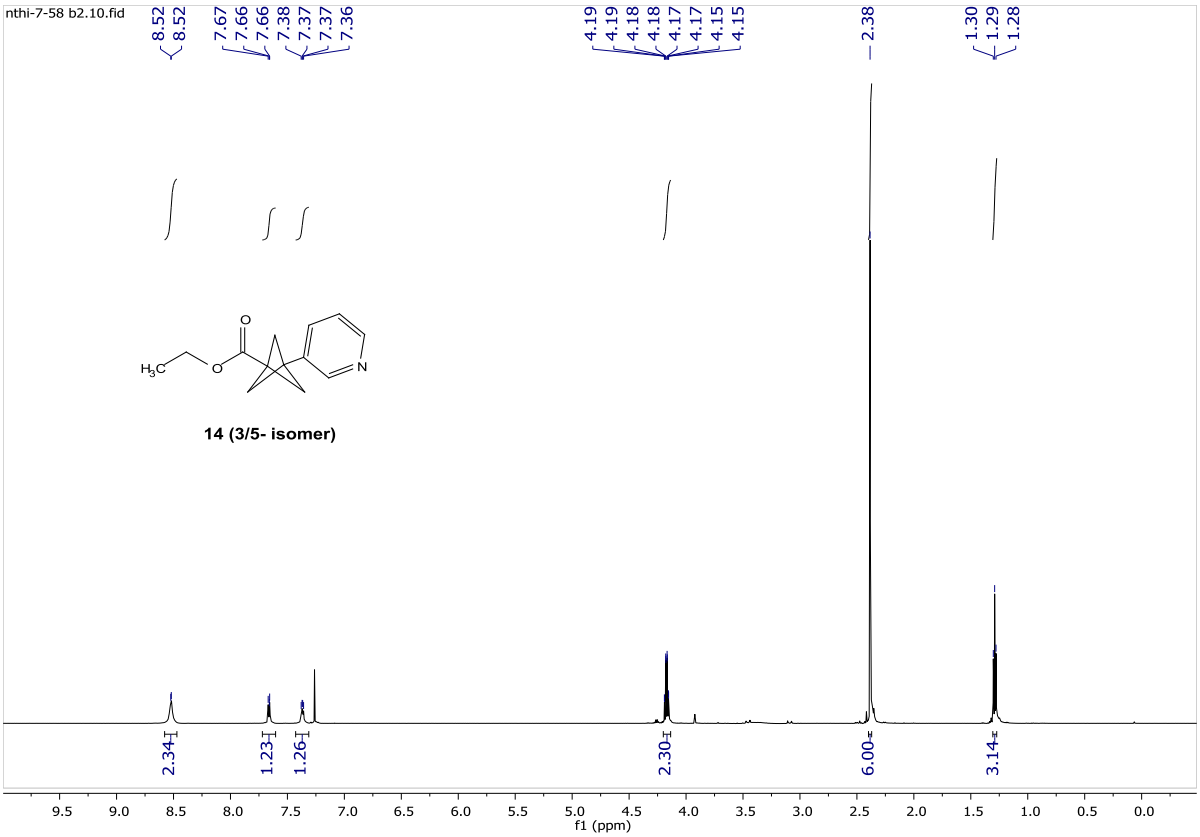
12



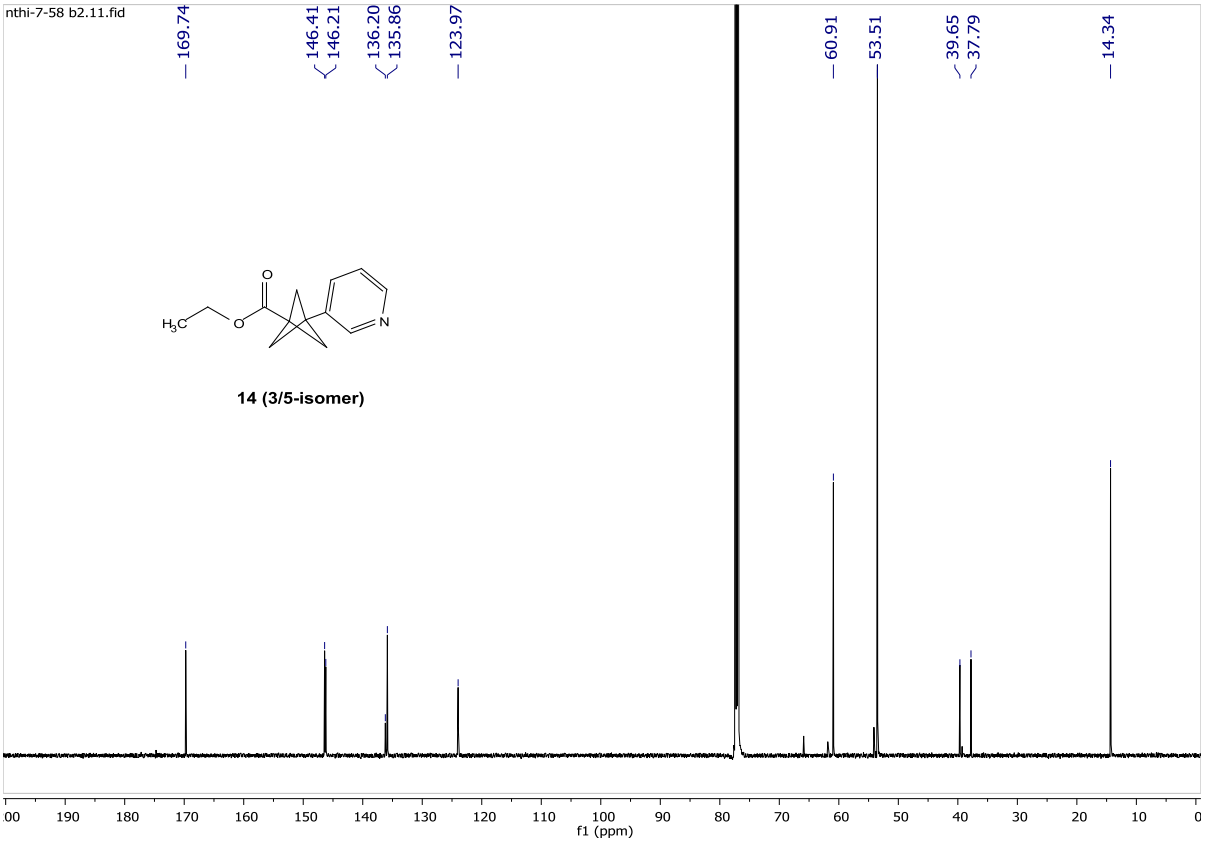
14 (2,6-isomer)



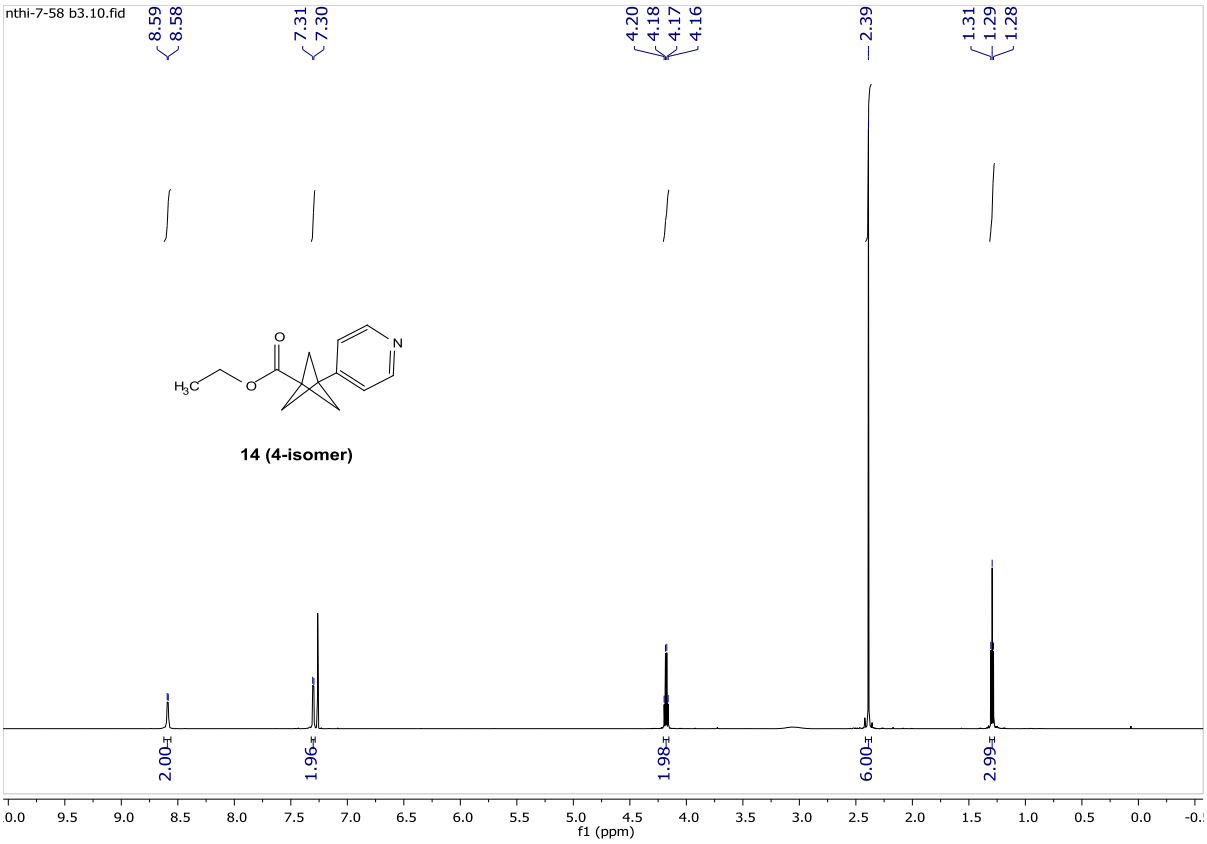
14 (2/6-isomer)

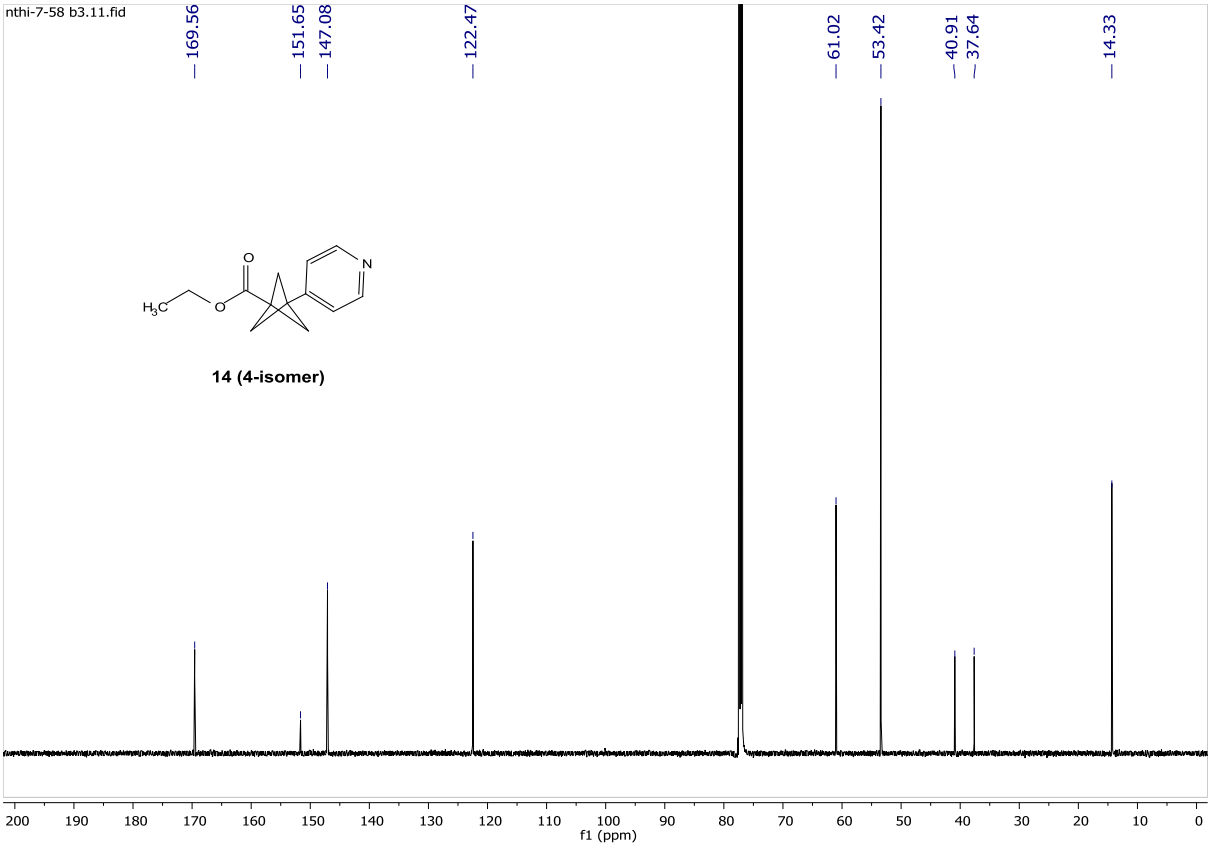


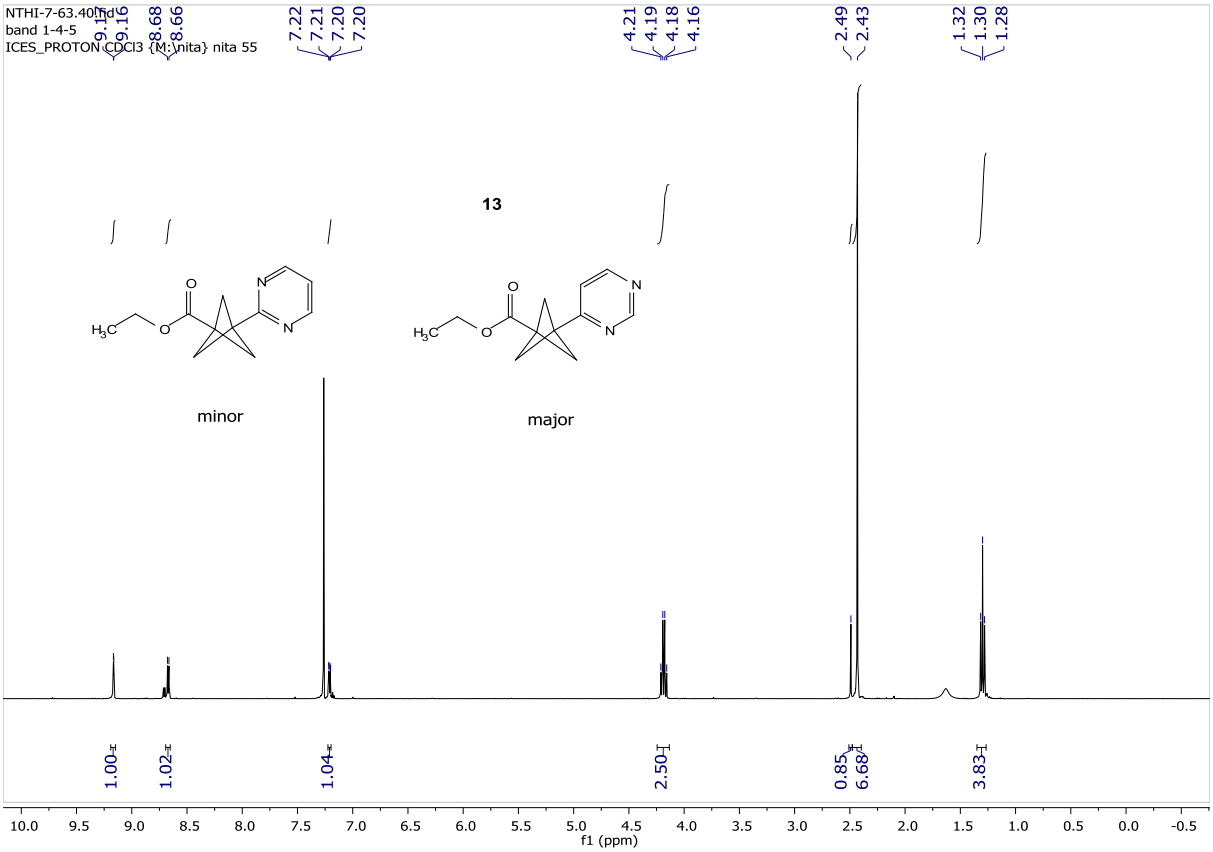
14 (3/5- isomer)

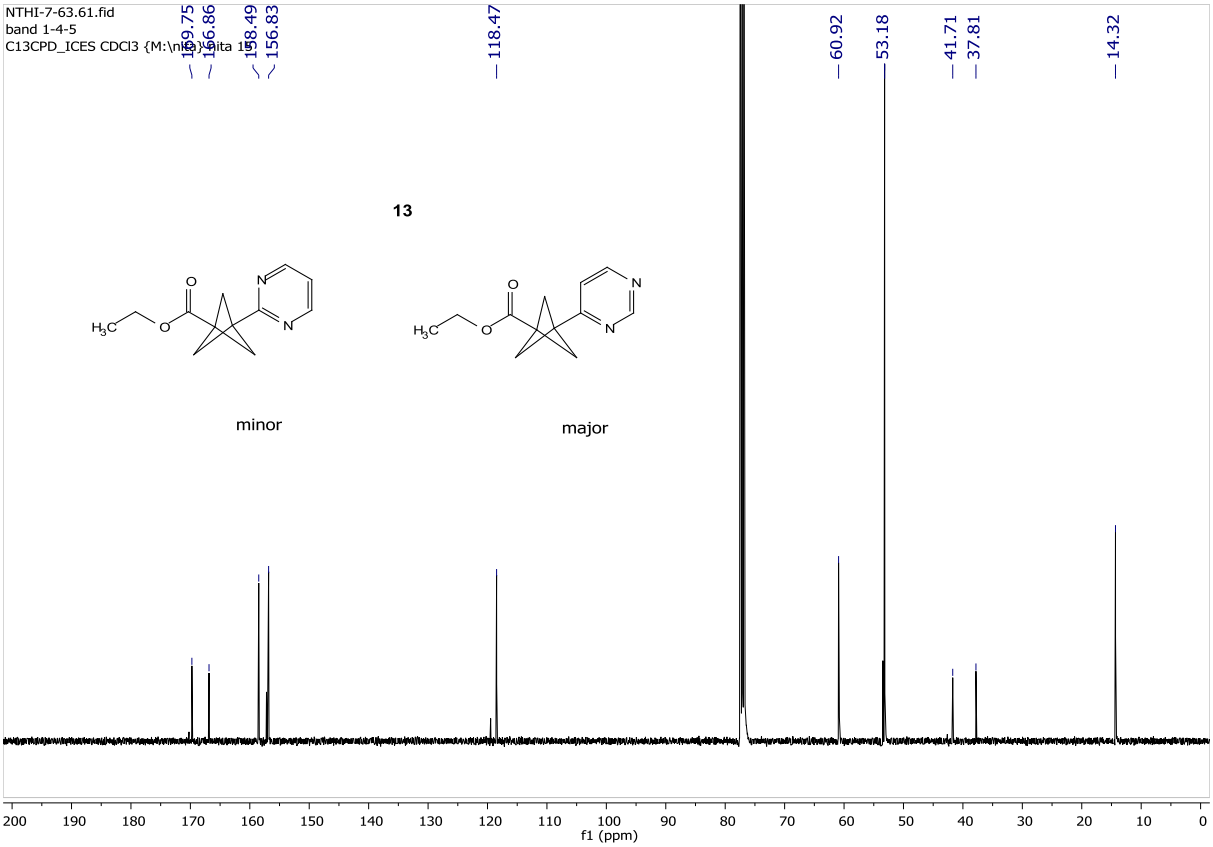


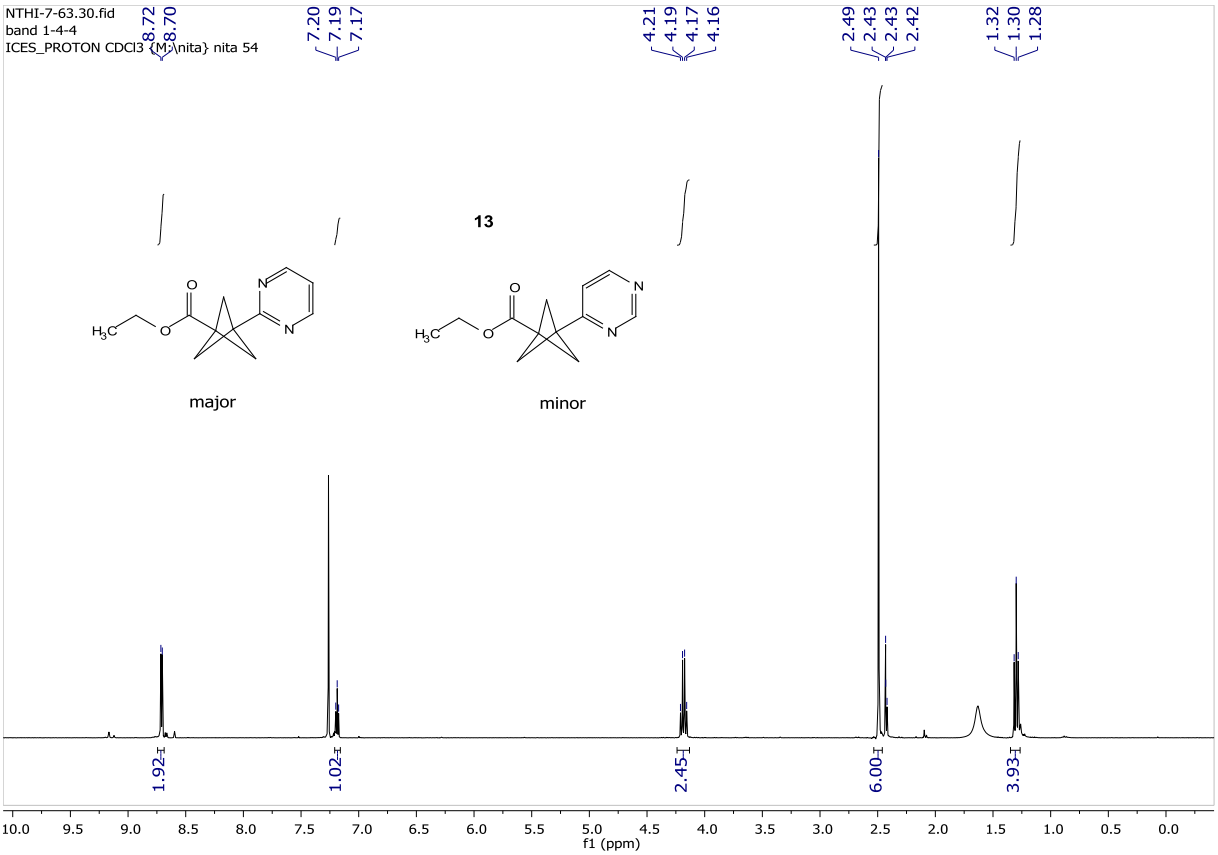
14 (3/5-isomer)

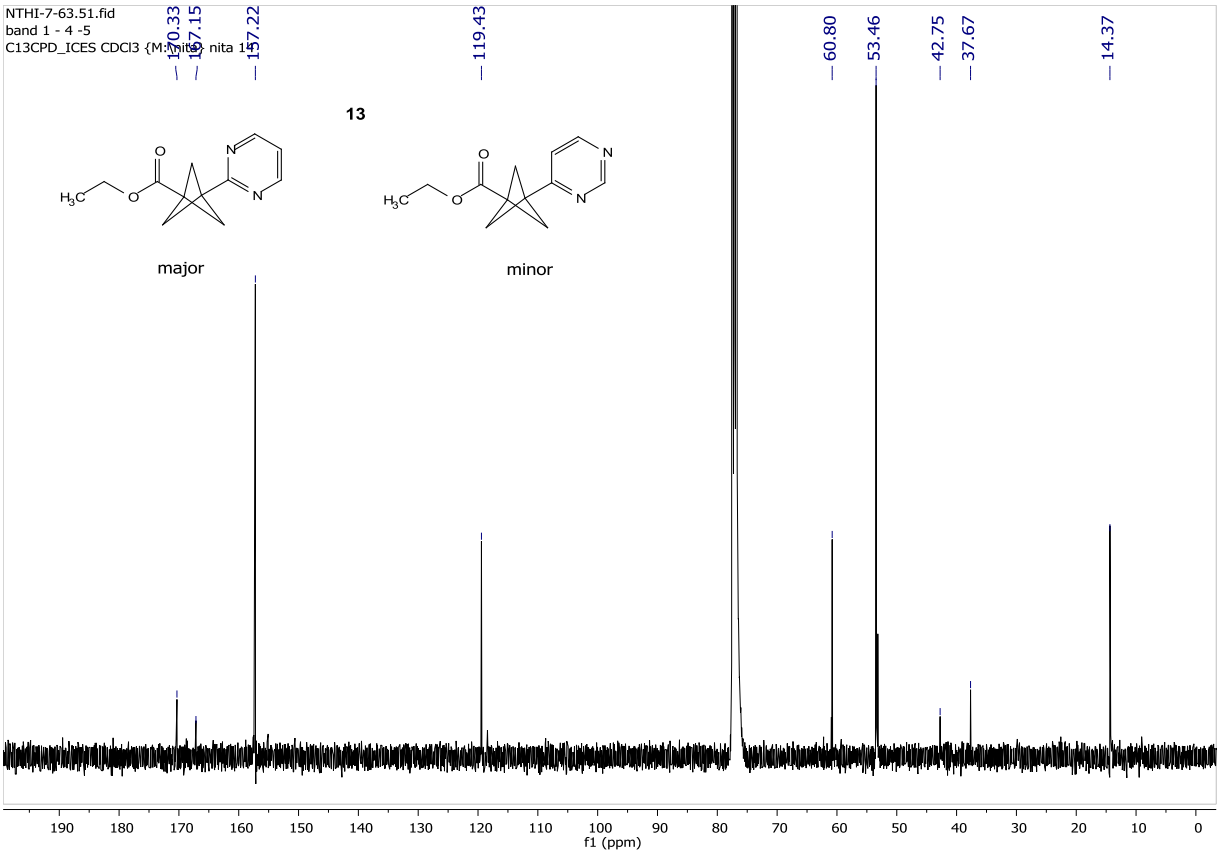

nthi-7-58 b3.10.fid
8.59
8.58
7.31
7.30
4.20
4.18
4.17
4.16
2.39
1.31
1.29
1.28
14 (4-isomer)
2.00
1.96
1.98
6.00
2.99
f1 (ppm)

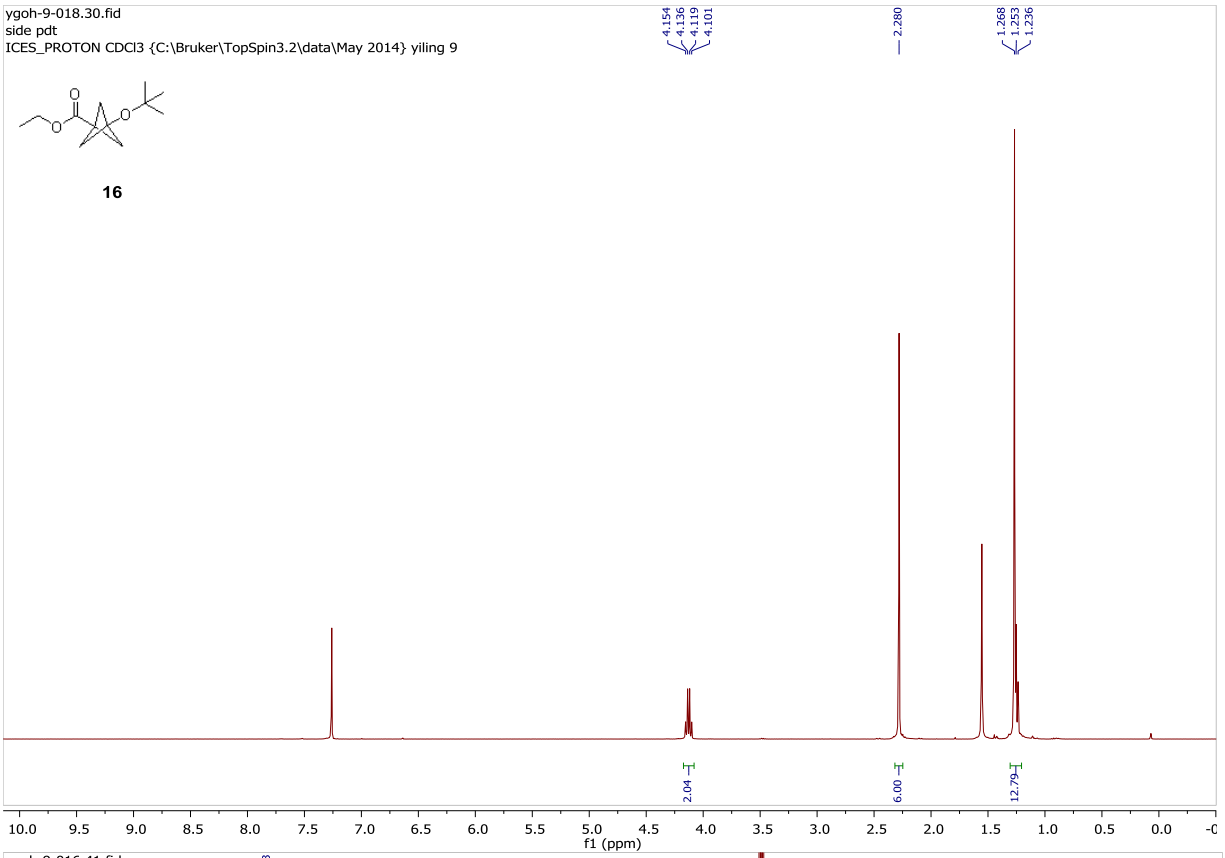

nthi-7-58 b3.11.fid
169.56
151.65
147.08
122.47
61.02
53.42
40.91
37.64
14.33
14 (4-isomer)
f1 (ppm)

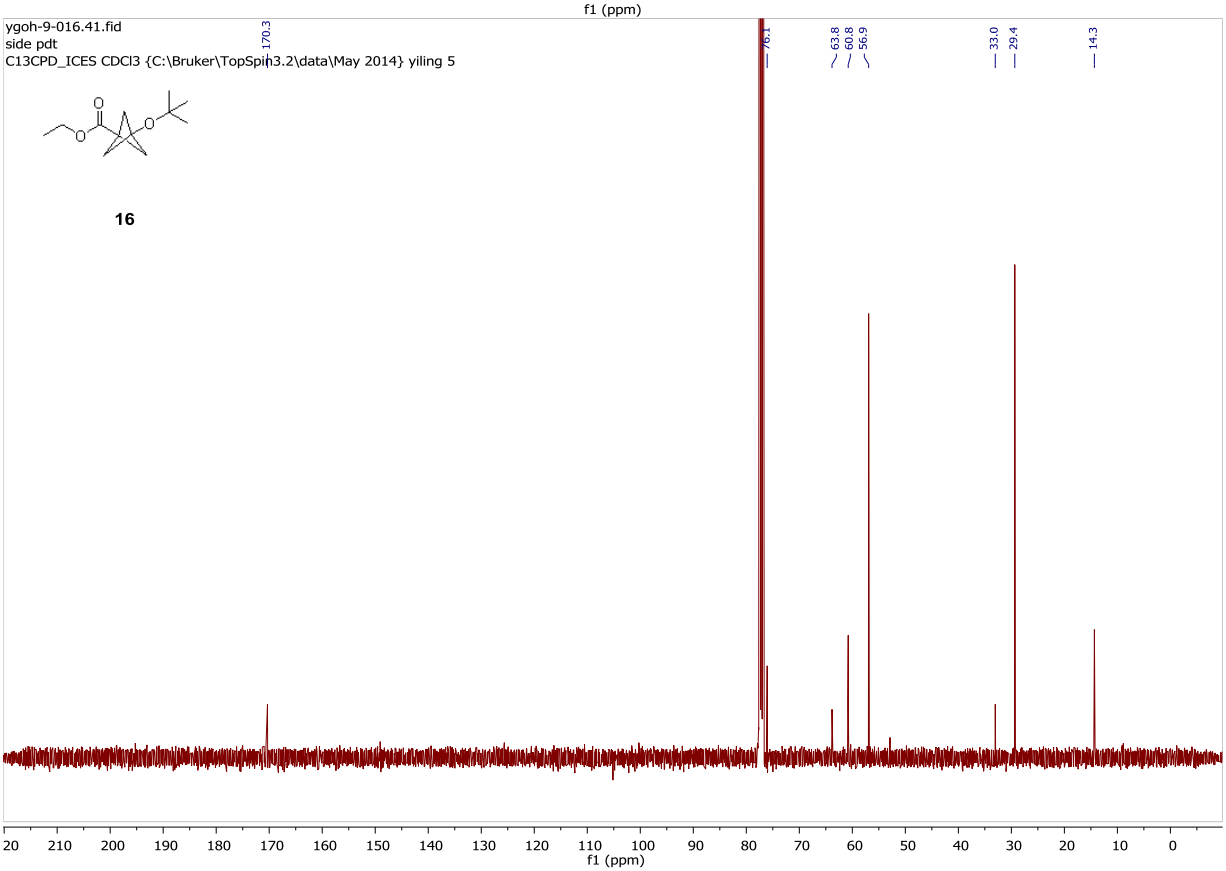

NTHI-7-63.40.fid
band 1-4-5
ICES_PROTON CDCl3 {M:\nita} nita 55
9.17
9.16
8.68
8.66
7.22
7.21
7.20
7.20
4.21
4.19
4.18
4.16
2.49
2.43
1.32
1.30
1.28
13
minor
major
1.00
1.02
1.04
2.50
0.85
6.68
3.83
f1 (ppm)

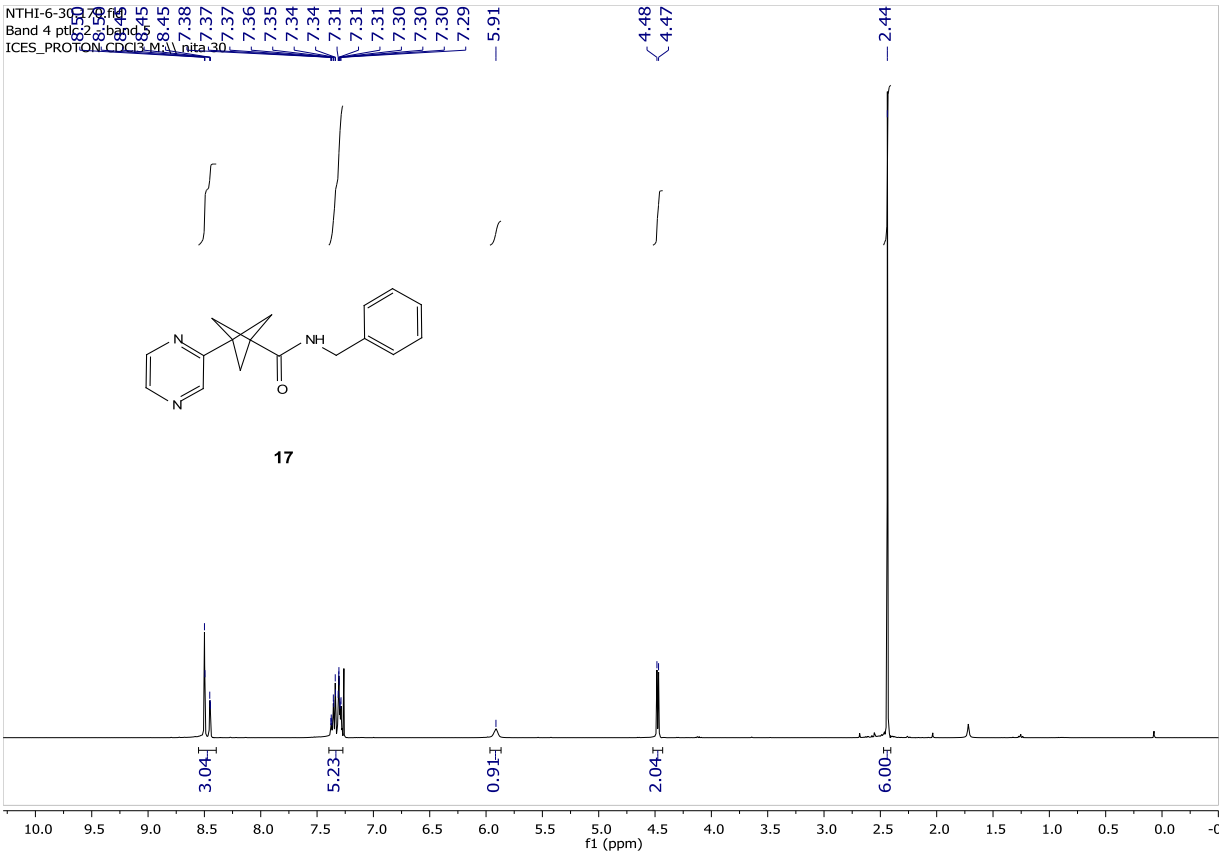

NTHI-7-63.61.fid
band 1-4-5
C13CPD_ICES CDCl3 {M:\nita} nita 15
169.75
166.86
158.49
156.83
118.47
60.92
53.18
41.71
37.81
14.32
13
minor
major
f1 (ppm)

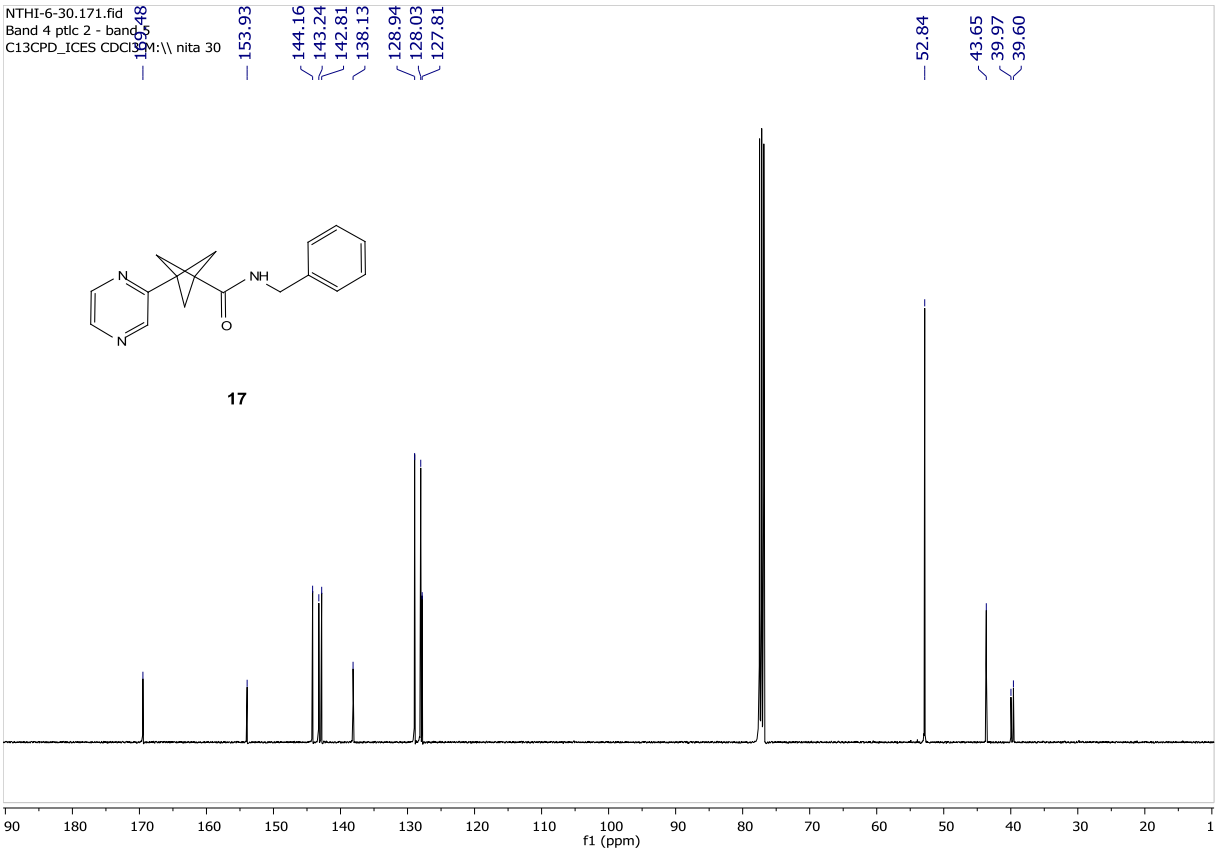

NTHI-7-63.30.fid
band 1-4-4
ICES_PROTON CDCl3 {M:\nita} nita 54
8.72
8.70
7.20
7.19
7.17
4.21
4.19
4.17
4.16
2.49
2.43
2.43
2.42
1.32
1.30
1.28
13
major
minor
1.92
1.02
2.45
6.00
3.93
f1 (ppm)

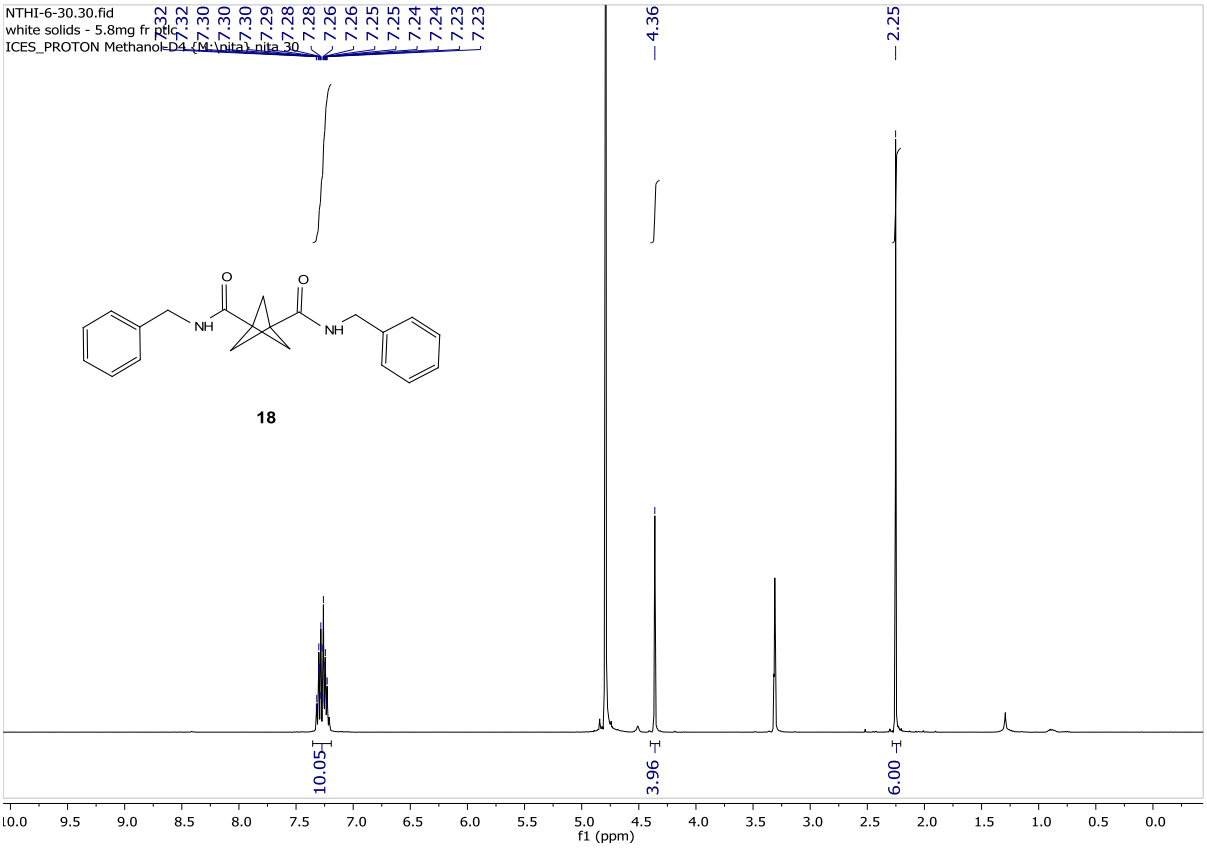

NTHI-7-63.51.fid
band 1 - 4 -5
C13CPD_ICES CDCl3 {M:\nita} nita 15
170.33
167.15
157.22
119.43
60.80
53.46
42.75
37.67
14.37
13
major
minor
f1 (ppm)

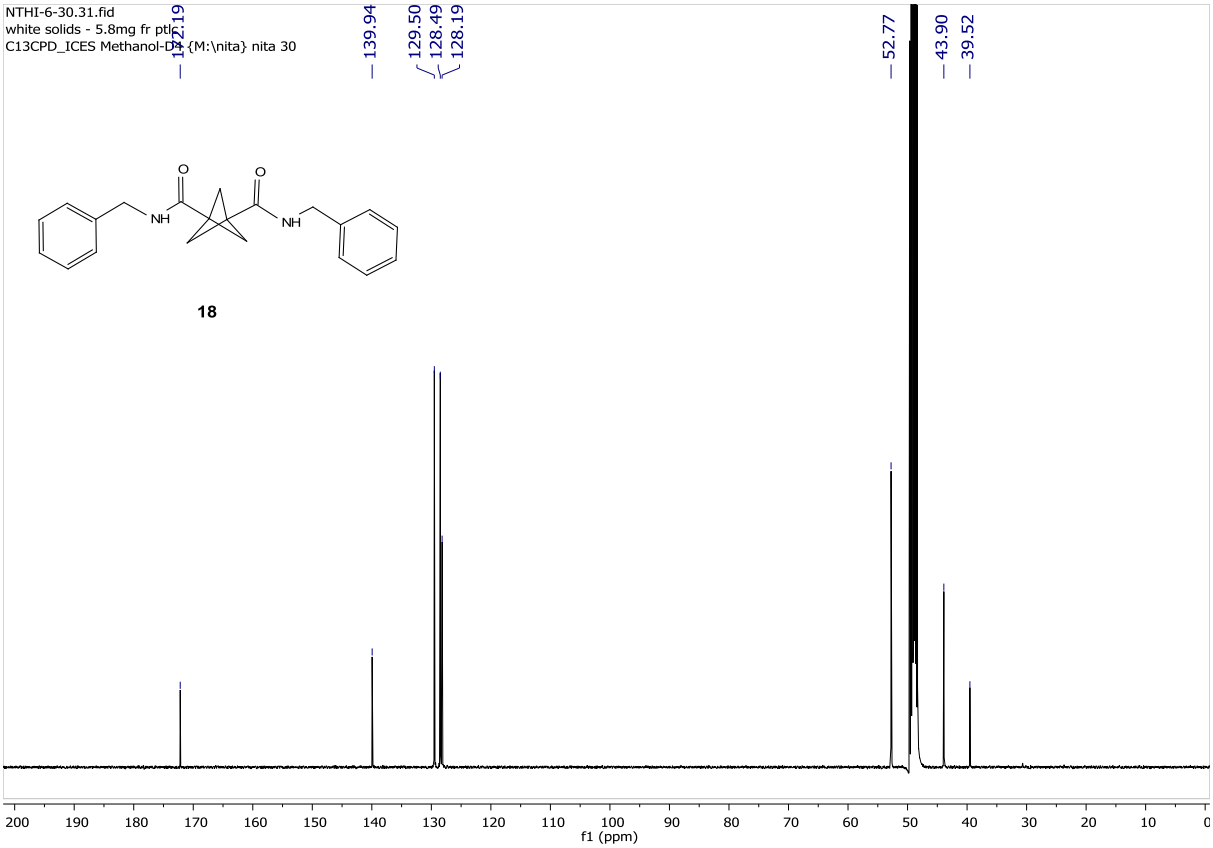

ygoh-9-018.30.fid
side pdt
ICES_PROTON CDCl3 {C:\Bruker\TopSpin3.2\data\May 2014} yiling 9
16
4.154
4.136
4.119
4.101
2.280
1.268
1.253
1.236
2.04
6.00
12.79
f1 (ppm)


ygoh-9-016.41.fid
side pdt
C13CPD_ICES CDCl3 {C:\Bruker\TopSpin3.2\data\May 2014} yiling 5
16
170.3
76.1
63.8
60.8
56.9
33.0
29.4
14.3
f1 (ppm)


NTHI-6-30.170.fid
Band 4 ptlc 2 - band 5
ICES_PROTON CDCl3 M:\\ nita 30
8.46
8.45
8.45
8.45
7.38
7.37
7.37
7.36
7.35
7.34
7.34
7.31
7.31
7.31
7.30
7.30
7.30
7.29
5.91
4.48
4.47
2.44
N
N
NH
O
17
3.04
5.23
0.91
2.04
6.00
f1 (ppm)


NTHI-6-30.171.fid
Band 4 ptlc 2 - band 5
C13CPD_ICES CDCl3 M:\\ nita 30
169.48
153.93
144.16
143.24
142.81
138.13
128.94
128.03
127.81
52.84
43.65
39.97
39.60
N
N
NH
O
17
f1 (ppm)


NTHI-6-30.30.fid
white solids - 5.8mg fr ptlc
ICES_PROTON Methanol-D4 {M:\nita} nita 30
7.32
7.32
7.30
7.30
7.30
7.29
7.28
7.28
7.26
7.26
7.25
7.25
7.24
7.24
7.23
7.23
4.36
2.25
10.05
3.96
6.00
NH
NH
O
O
18
f1 (ppm)


NTHI-6-30.31.fid
white solids - 5.8mg fr ptlc
C13CPD_ICES Methanol-D4 {M:\nita} nita 30
172.19
139.94
129.50
128.49
128.19
52.77
43.90
39.52
NH
NH
O
O
18
f1 (ppm)