Discovery of a potent thiadiazole class of histamine H_3 receptor antagonist for the treatment of diabetes

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Experimental Procedures

Analytical methods: All reactions were carried out under nitrogen atmosphere in anhydrous conditions, unless otherwise noted. Solvents were purchased from Aldrich and Acros without further purification. Reagents and chemicals were purchased from commercial sources with purity ≥ 95% without further purification. Flash chromatography was carried out with EM Science silica gel 60 (neutral, 230-400 mesh). ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Avance 500 NMR Spectrometer. LCMS analyses were performed on Applied Biosystem API-150. HRMS analyses were recorded on a Micromass QT of Ultima API US Mass Spectrometer by FAB.

All of the biologic assays and experiments with animal models performed in Merck & Co., Inc. were in accordance with all national or local guidelines and regulations.

Determination of binding affinities in a [^{3}H]-*N*-*α*-methylhistamine human and mouse recombinant assay: For binding assays, membranes (P2 pellet) from rHu H_3-HEK cells (3 μg protein) were incubated in 200 μL 50 mM Tris·HCl, pH 7.4, with 1 nM [^{3}H]-*N*-*α*-methylhistamine (82 Ci/mmol) and compounds at concentrations equivalent to half orders of magnitude over a five-order of magnitude range. Nonspecific binding was determined in the presence of 10^{-5} M thioperamide. After 30 min at 30 °C, assay mixtures were filtered through 0.3% polyethylenimine-soaked GF/B glass fiber filters, which were rinsed thrice with buffer, dried, impregnated with Meltilex wax scintillant, and counted. K_i values were determined from curves fit to the data using GraphPad Prism nonlinear, least-squares, curve-fitting program.

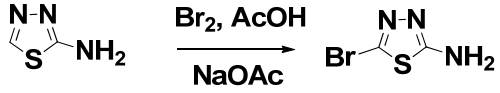
Determination of ex vivo receptor occupancy: ICR, Imprinting Control Region mice (from Taconics, nomenclature IcrTacICR). Individual mouse cortexes were dissected and homogenized in ice cold assay buffer, 50 mM Na_2HPO_4–KH_2PO_4 buffer (pH 6.8). Samples were then frozen at –80 °C for at least 12 hours. Protein concentration was determined by BCA Protein Assay. A 30 min room temperature incubation was performed, containing 140 μg/assay homogenized cortex sample, 0.1% BSA, 1 nM [^{3}H]-*N*-α-Methylhistamine and 10 μM thioperamide for non-specific binding or assay buffer for total binding. Incubations were performed in quadruplet and stopped by rapid

filtration on a Brandel Harvester using Unifilter-96, GF/B plates presoaked in 0.3% PEI for 30 min. The remaining radioactivity was measured on a Packard TopCount-NTX. Specific binding was calculated by subtracting the non-specific binding from the total.

Determination of effects on non-fasting glucose in mouse: Five week old male ICR mice (Taconic, Tac:Icr:Ha(ICR)fBR) were fed high fat/cholesterol diet (45% Kcal fat and 0.12% cholesterol, Research Diets D04012801) for 3 weeks, and were given streptozotocin (STZ) intraperitoneally at 80 mg/kg to induce type 2 diabetes. T2D mice were chosen for the study two weeks after STZ injection (n=12 per group, with non-fasting glucose between 250 and 500 mg/dl) and were balanced into groups. Compounds were administered by PO dosing with 0.4% MC daily before onset of dark. Non-fasting glucose was monitored daily at 4 hours after the lights were on. Individual body weight and food intake were monitored daily.

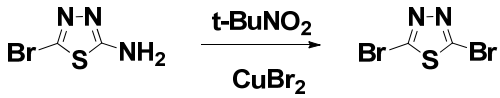
Determination of 12 day change in HbA_{1c} assay: HbA_{1c} was monitored before and after the treatment by A1CNow kit from Bayer Health Care.

General procedure for the preparation of analogs was illustrated by the synthesis of compound 5c



1 2

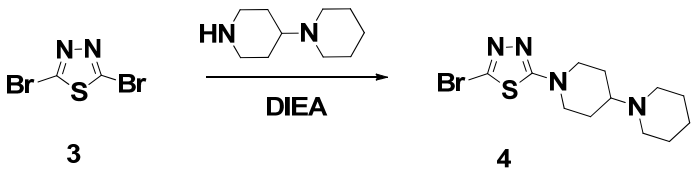
To a heterogenous mixture of 2-amino-1,3,4-thiadiazole (**1**) (100 g, 988.8 mmol) and sodium acetate (324.5 g, 3955.2 mmol, 4.0 eq.) in acetic acid (1.4 L) was added bromine (2 M in AcOH, 524 mL, 1048 mmol, 1.06 eq.) dropwise over 2 h under cooling with ice. The reaction mixture was allowed to stir at room temperature overnight. The reaction mixture gradually solidified upon stirring overnight. Added 500 mL of AcOH and the reaction was stirred longer upon which the excess AcOH was concentrated and H_2O (1 L) was added. The reaction mixture was agitated and the solids were filtered, washed with water and dried over P_2O_5 under vacuum to yield **2** (162.4 g, 91%) as a brown solid.



2 3

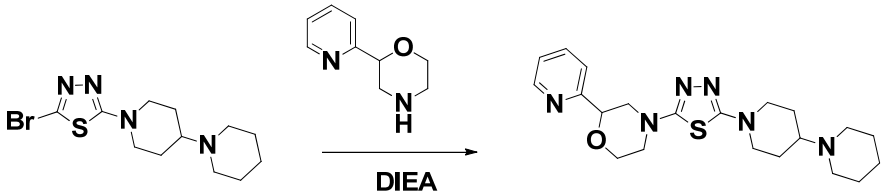
To a heterogeneous solution of $CuBr_2$ (37.25 g, 166.6 mmol, 1.2 eq.) and *t*-$BuNO_2$ (24.8 mL, 208.31 mmol, 1.5 eq.) in CH_3CN (600 mL) was added 2-amino-5-bromo-1,3,4-thiadiazole **2** (25.0 g, 138.87 mmol) slowly and stirred at room temperature under nitrogen overnight. The reaction mixture was quenched with 700 mL of saturated NH_4Cl (aq.) and extracted with diethyl ether (3 x 500 mL) and the organic layer was dried over $MgSO_4$, filtered and concentrated in *vacuo* to provide the crude product which

was triturated in 200 mL MeOH. The solid product was collected by filtration to provide 23.7 g of compound **3** (Yield: 70%).



3 **4**

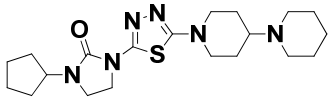
To a solution of 2,5-dibromo-1,3,4-thiadiazole **3** (10 g, 41 mmol) and 4-piperidinopiperidine (8.3 g, 49.2 mmol, 1.2 eq.) in 1,4-dioxane (150 mL) was added *N,N*-diisopropylethylamine (8.6 mL, 49.2 mmol, 1.2 eq.). The resulting reaction was heated to 120 ^{o}C under nitrogen and allowed to stir at this temperature for 2 h, then cooled to room temperature. The cooled solution was filtered and the collected solid was dried over vacuum to provide 8.6 g of compound **4** (Yield: 63%).



4 **5r**

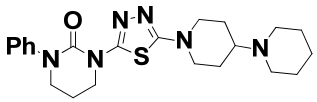
To a solution of compound **4** (100 mg, 0.30 mmol) in a mixture of $PhCF_3$ (2.0 mL) and 1,4-dioxane (1.0 mL) was added 2-(pyridin-2-yl)morpholine (59 mg, 0.36 mmol, 1.2 eq.) and *N,N*-diisopropylethylamine (132 μL, 0.75 mmol, 2.5 eq.). The resulting reaction mixture was heated in a microwave at 220 ^{o}C for 3 h, then cooled to room temperature and concentrated in *vacuo*. The residue was purified by reverse phase column chromatography to provide 75 mg of compound **5r** (Yield: 60%).

LC/MS conditions: Agilent 6140 Quadrupole LC/MS System; Column Zorbax SB-C18, 3.0 x 50.0 mm, 1.8 micron; Mobile phase A: H_2O/0.05% TFA/0.5% acetic acid; Mobile phase B: acetonitrile/0.5% acetic acid; Flow: 1.0 ml/min; Gradient: 0 min 10% B, 1.5 min 95% B, 2.7 min 95% B, 2.8 min 10% B, Stop 3.6 min; Column temperature: 50 ^{o}C.



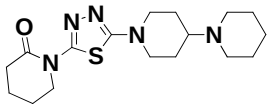
5a

1-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-3-cyclopentylimidazolidin-2-one: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.32 (p, 1 H, J = 8 Hz, J = 16 H), 4.04 (dd, 2 H, J = 8 Hz, J = 9.5 Hz), 3.98-3.95 (m, 2 H), 3.55 (dd, 2 H, J = 7 Hz, J = 8 Hz), 3.02 (dt, 2 H, J = 2.5 Hz, J = 12.5 Hz, J = 14.5 Hz), 2.65-2.48 (m, 5 H), 1.96-1.85 (m, 5 H), 1.74-1.56 (m, 11 H), 1.51-1.42 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 405.2 (98% LC/MS purity).



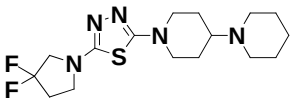
5b

1-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-3-phenyltetrahydropyrimidin-2(1H)-one: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42-7.39 (m, 1 H), 7.31-7.29 (m, 1 H), 7.27-7.24 (m, 3 H), 4.23 (t, 2H, J = 6.5 Hz), 4.05-4.00 (m, 2 H), 3.81 (t, 2 H, J = 6 Hz), 3.04 (t, 2 H, J = 14 Hz), 2.90-2.6 (m, 5 H), 2.32-2.28 (m, 2 H), 2.21-2.08 (m, 2 H), 1.86-1.53 (m, 8 H) ppm; ES-LC/MS $(M + H)^+$ = 427.2 (99% LC/MS purity).



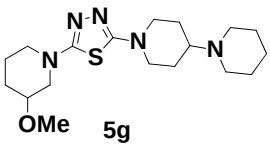
5c

1-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)piperidin-2-one: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.10 (t, 2 H, *J* = 6 Hz), 4.02 (dd, 2 H, *J* = 2.5 Hz, *J* = 11 Hz), 3.03 (dt, 2 H, *J* = 2.5 Hz, *J* = 12.5 Hz, *J* = 15 Hz), 2.63 (t, 2 H, *J* = 7 Hz), 2.50 (t, 4 H, *J* = 5 Hz), 2.01-1.96 (m, 2 H), 1.94-1.87 (m, 4 H), 1.70-1.56 (m, 7 H), 1.45-1.42 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 350.2 (99% LC/MS purity).



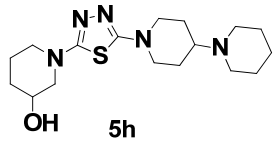
5d

2-([1,4'-bipiperidin]-1'-yl)-5-(3,3-difluoropyrrolidin-1-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 4.03-3.98 (m, 4 H), 3.86 (t, 2 H, *J* = 7.5 Hz), 3.57-3.53 (m, 3 H), 3.06 (t, 2 H, *J* = 12 Hz), 2.73-2.65 (m, 2 H), 2.29-2.27 (m, 2 H), 2.02-1.85 (m, 8 H), 1.59-1.53 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 358.2 (99% LC/MS purity).



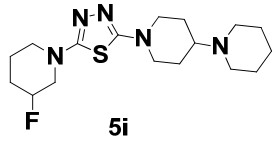
5g

2-([1,4'-bipiperidin]-1'-yl)-5-(3-methoxypiperidin-1-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.95 (d, 2 H, *J* = 13 Hz), 3.71-3.48 (m, 8 H), 3.38 (s, 3 H), 3.32-3.25 (m, 2 H), 3.04 (t, 2 H, *J* = 12.5 Hz), 2.25 (d, 2 H, *J* = 12 Hz), 2.02-1.81 (m, 8 H), 1.7-1.51 (m, 4 H) ppm; ES-LC/MS $(M + H)^+$ = 366.2 (99% LC/MS purity).



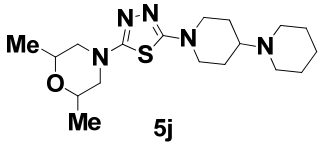
5h

1-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)piperidin-3-ol: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.95 (d, 2 H, *J* = 11 Hz), 3.72 (dd, 1 H, *J* = 2.5 Hz, *J* = 13.5 Hz), 3.63-3.44 (m, 4 H), 3.32-3.28 (m, 3 H), 3.07 (t, 2 H, *J* = 10.5 Hz), 2.27 (d, 2 H, *J* = 11.5 Hz), 2.06-1.86 (m, 7 H), 1.77-1.68 (m, 3 H), 1.56-1.39 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 352.2 (94% LC/MS purity).



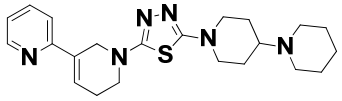
5i

2-([1,4'-bipiperidin]-1'-yl)-5-(3-fluoropiperidin-1-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.96 (dd, 2 H, *J* = 2 Hz, *J* = 11.5 Hz), 3.92-3.85 (m, 1 H), 3.77-3.67 (m, 2 H), 3.56-3.46 (m, 5 H), 3.09-3.04 (m, 3 H), 2.25 (d, 2 H, *J* = 12 Hz), 2.09-1.76 (m, 12 H), 1.59-1.51 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 354.2 (93% LC/MS purity).



5j

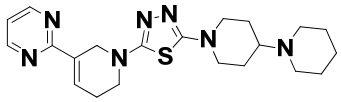
4-**(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-2,6-dimethylmorpholine**: ^{1}H NMR (CD_3OD, 500 MHz) δ 3.96 (dd, 2 H, *J* = 2.0 Hz, *J* = 11 Hz), 3.82-3.77 (m, 2 H), 3.62 (dd, 2 H, *J* = 2 Hz, *J* = 12.5 Hz), 3.56-3.50 (m, 3 H), 3.36-3.30 (m, 2 H), 3.09-2.99 (m, 4 H), 2.27 (d, 2 H, *J* = 12.5 Hz), 2.02-1.85 (m, 7 H), 1.57-1.53 (m, 1 H), 1.24 (d, 6 H, *J* = 6.5 Hz) ppm; ES-LC/MS $(M + H)^+$ = 366.2 (99% LC/MS purity).



5k

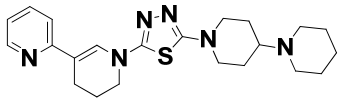
2-([1,4'-bipiperidin]-1'-yl)-5-(5',6'-dihydro-[2,3'-bipyridin]-1'(2'H)-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.52 (d, *J* = 5.0 Hz, 1H), 8.05 (ddd, *J* = 1.5, 7.5, 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.50 (dd, *J* = 5.5, 7.5 Hz, 1H), 6.82 (m, 1H), 4.41 (dd, *J* = 2.0, 4.0 Hz, 2H), 3.88 (dt, *J* = 2.5, 13.5 Hz, 2H), 3.59 (t, *J* = 6.0 Hz, 2H), 3.44 (d, *J* = 11.5 Hz, 2H), 3.37 (m, 1H), 3.12 (t, *J* = 11.0 Hz, 2H), 2.94 (t, *J* = 12.5 Hz,

2H), 2.53 (m, 2H), 2.11 (d, *J* = 12.0 Hz, 2H), 1.91-1.65 (m, 7H), 1.44 (m, 1H) ppm; ES-LC/MS $(M + H)^+$ = 411.2 (96% LC/MS purity).



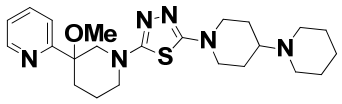
5l

2-([1,4'-bipiperidin]-1'-yl)-5-(3-(pyrimidin-2-yl)-5,6-dihydropyridin-1(2H)-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.65 (d, *J* = 4.5 Hz, 1H), 7.40 (m, 1H), 7.22 (dd, *J* = 4.5, 5.0 Hz, 1H), 4.44 (br, 2H), 3.88 (d, *J* = 11.0 Hz, 2H), 3.62 (t, *J* = 5.5 Hz, 2H), 3.44 (d, *J* = 12.0 Hz, 2H), 3.38 (m, 1H), 3.14 (t, *J* = 14.5 Hz, 2H), 2.95 (t, *J* = 14.5 Hz, 2H), 2.54 (m, 2H), 2.12 (d, *J* = 13.0 Hz, 2H), 1.92-1.67 (m, 7H), 1.45 (m, 1H) ppm; ES-LC/MS $(M + H)^+$ = 412.2 (98% LC/MS purity).



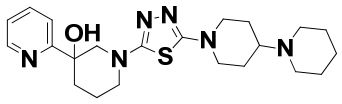
5m

2-([1,4'-bipiperidin]-1'-yl)-5-(5',6'-dihydro-[2,3'-bipyridin]-1'(4'H)-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.34 (d, *J* = 5.5 Hz, 1H), 8.22 (t, *J* = 9.0 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.92 (s, 1H), 7.51 (t, *J* = 7.0 Hz, 1H), 3.92 (m, 2H), 3.78 (dd, *J* = 5.5, 6.0 Hz, 2H), 3.43 (d, *J* = 6.5 Hz, 2H), 3.38 (m, 1H), 3.14 (t, *J* = 13.5 Hz, 2H), 2.94 (t, *J* = 11.5 Hz, 2H), 2.54 (t, *J* = 6.0 Hz, 2H), 2.14-2.08 (m, 4H), 1.90-1.68 (m, 7H), 1.43 (m, 1H) ppm; ES-LC/MS $(M + H)^+$ = 411.2 (96% LC/MS purity).



5n

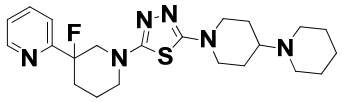
2-([1,4'-bipiperidin]-1'-yl)-5-(3-methoxy-3-(pyridin-2-yl)piperidin-1-yl)-1,3,4-thiadiazole: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.58 (d, *J* = 5.0 Hz, 1H), 7.72 (t, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.21 (dd, *J* = 5.0, 7.5 Hz, 1H), 3.91-3.73 (m, 4H), 3.64 (d, *J* = 13.0 Hz, 1H), 3.11 (s, 3H), 2.96 (t, *J* = 10.5 Hz, 2H), 2.52-2.37 (m, 4H), 2.22-2.00 (m, 3H), 1.85 (m, 2H), 1.69-1.53 (m, 9H), 1.43 (m, 2H) ppm; ES-LC/MS $(M + H)^+$ = 443.2 (95% LC/MS purity).



5o

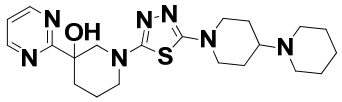
1-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-3-(pyridin-2-yl)piperidin-3-ol: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.55 (d, *J* = 1.0, 5.0 Hz, 1H), 8.26 (ddd, *J* = 1.5, 7.5, 8.5 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.68 (ddd, *J* = 1.0, 5.5, 7.5 Hz, 1H), 3.76-3.68 (m, 4H), 3.57 (d, *J* = 14.0 Hz, 1H), 3.34-3.25 (m, 4H), 3.08 (t, *J* = 13.5 Hz, 2H), 2.84 (t, *J* = 12.5

Hz, 2H), 2.17 (ddd, *J* = 4.0, 13.0, 13.0 Hz, 1H), 2.07-1.99 (m, 3H), 1.86-1.57 (m, 10H), 1.34 (m, 1H) ppm; ES-LC/MS $(M + H)^+$ = 429.2 (99% LC/MS purity).



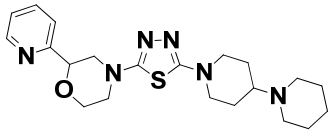
5p

2-([1,4'-bipiperidin]-1'-yl)-5-(3-fluoro-3-(pyridin-2-yl)piperidin-1-yl)-1,3,4-thiadiazole: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.48 (d, *J* = 5.0 Hz, 1H), 7.81 (ddd, *J* = 2.0, 7.5, 7.5 Hz, 1H), 7.56 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.30 (ddd, *J* = 1.5, 5.0, 8.0 Hz, 1H), 3.98-3.79 (m, 5H), 3.41-3.32 (m, 4H), 3.13 (t, *J* = 12.5 Hz, 2H), 2.91 (t, *J* = 14.0 Hz, 2H), 2.25 (m, 1H), 2.10-2.00 (m, 3H), 1.88-1.64 (m, 9H), 1.40 (m, 1H) ppm; ES-LC/MS $(M + H)^+$ = 431.2 (95% LC/MS purity).



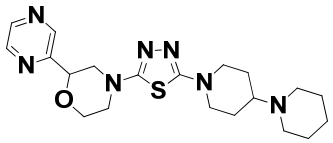
5q

1-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-3-(pyrimidin-2-yl)piperidin-3-ol: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.72 (d, *J* = 5.0 Hz, 2H), 7.31 (dd, *J* = 5.0, 5.0 Hz, 1H), 4.04 (d, *J* = 13.5 Hz, 1H), 3.83 (m, 2H), 3.73 (m, 1H), 3.66 (m, 1H), 3.46-3.32 (m, 4H), 3.12 (m, 2H), 2.92 (m, 2H), 2.21 (ddd, *J* = 1.5, 4.0, 11.5 Hz, 1H), 2.10-2.03 (m, 3H), 1.94-1.63 (m, 9H), 1.42 (m, 1H) ppm; ES-LC/MS $(M + H)^+$ = 430.2 (97% LC/MS purity).



5r

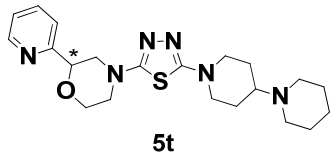
4-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-2-(pyridin-2-yl)morpholine: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.62 (d, 1 H, *J* = 4.5 Hz), 7.78 (dt, 1 H, *J* = 1.5 Hz, *J* = 7.5 Hz, *J* = 9.0 Hz), 7.54 (d, 1 H, *J* = 7.5 Hz), 7.28 (dd, 1 H, *J* = 4.5 Hz, *J* = 7.0 Hz), 4.81 (dd, 1 H, *J* = 2.5 Hz, *J* = 10.5 Hz), 4.20 (dd, 1 H, *J* = 3.5 Hz, *J* = 12.0 Hz), 4.03-3.84 (m, 4 H), 3.31 (dt, 1 H, *J* = 3.5 Hz, *J* = 12.5 Hz, *J* = 15.5 Hz), 3.20 (t, 1 H, *J* = 10.5 Hz), 3.06 (t, 2 H, *J* = 12.5 Hz), 2.61-2.47 (m, 4 H), 1.91 (d, 2 H, *J* = 12 Hz), 1.72-1.64 (m, 7 H), 1.50 (d, 2 H, *J* = 1.5 Hz) ppm; ES-LC/MS $(M + H)^+$ = 415.2 (99% LC/MS purity).



5s

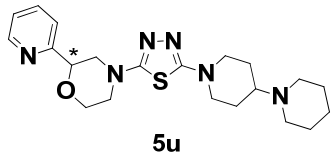
4-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-2-(pyrazin-2-yl)morpholine: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.85 (s, 1 H), 8.59 (dd, 2 H, *J* = 2.5 Hz, *J* = 7.5 Hz), 4.88 (dd, 1 H, *J* = 2.5 Hz, *J* = 10.0 Hz), 4.22 (dd, 1 H, *J* = 4.0 Hz, *J* = 12 Hz), 4.02 (dd, 2 H, *J* = 2.5

Hz, *J* = 11.5 Hz), 3.91 (d, 2 H, *J* = 13 Hz), 3.81 (dd, 1 H, *J* = 1.5 Hz, *J* = 13 Hz), 3.34 (dt, 1 H, *J* = 3.5 Hz, *J* = 12 Hz, *J* = 15.5 Hz), 3.23 (t, 1 H *J* = 10.5 Hz), 3.06 (t, 2 H, *J* = 13 Hz), 2.57-2.55 (m, 3 H), 2.53-2.47 (m, 1 H), 1.92 (d, 2 H, *J* = 12 Hz), 1.75-1.58 (m, 7 H), 1.50-1.48 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 416.2 (100% LC/MS purity).



5t

4-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-2-(pyridin-2-yl)morpholine: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.62 (d, 1 H, *J* = 4.5 Hz), 7.78 (dt, 1 H, *J* = 2 Hz, *J* = 8 Hz, *J* = 9.5 Hz), 7.54 (d, 1 H, *J* = 7.5 Hz), 7.28 (dd, 1 H, *J* = 5.0 Hz, *J* = 7.5 Hz), 4.81 (dd, 1 H, *J* = 2.5 Hz, *J* = 10.5 Hz), 4.20 (dd, 1 H, *J* = 3.0 Hz, *J* = 11.5 Hz), 4.03-3.85 (m, 4 H), 3.31 (dt, 1 H, *J* = 3.5 Hz, *J* = 12.5 Hz, *J* = 16.0 Hz), 3.20 (t, 1 H, *J* = 10.5 Hz), 3.05 (t, 2 H, *J* = 12.5 Hz), 2.60-2.46 (m, 4 H), 1.91 (d, 2 H, *J* = 12 Hz), 1.74-1.59 (m, 7 H), 1.51-1.47 (m, 2 H) ppm; ES-LC/MS $(M + H)^+$ = 415.2 (98% LC/MS purity).



5u

4-(5-([1,4'-bipiperidin]-1'-yl)-1,3,4-thiadiazol-2-yl)-2-(pyridin-2-yl)morpholine: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.62 (d, 1 H, *J* = 4.5 Hz), 7.78 (t, 1 H, *J* = 7.5 Hz), 7.54 (d, 1 H, *J* = 7.5 Hz), 7.28 (dd, 1 H, *J* = 5.0 Hz, *J* = 7.5 Hz), 4.81 (dd, 1 H, *J* = 2.5 Hz, *J* = 10.5 Hz), 4.20 (dd, 1 H, *J* = 3.0 Hz, *J* = 11.5 Hz), 4.03-3.85 (m, 4 H), 3.31 (dt, 1 H, *J* = 3.5 Hz, *J* = 12.5 Hz, *J* = 16.0 Hz), 3.20 (t, 1 H, *J* = 10.5 Hz), 3.05 (t, 2 H, *J* = 12.5 Hz), 2.61-2.46 (m, 4 H), 1.94-1.91 (m, 2 H), 1.75-1.60 (m, 7 H), 1.51-1.47 (m, 2 H) ppm; ^{13}C NMR ($CDCl_3$) δ 165.4, 165.0, 158.1, 149.0, 136.7, 122.8, 120.6, 77.5, 66.0, 62.2, 55.5, 50.1, 50.0, 48.4, 27.1, 26.2, 24.6. ES-LC/MS $(M + H)^+$ = 415.2 (98% LC/MS purity).

MERCK

Date: October 19, 2011

To: Ashwin Rao

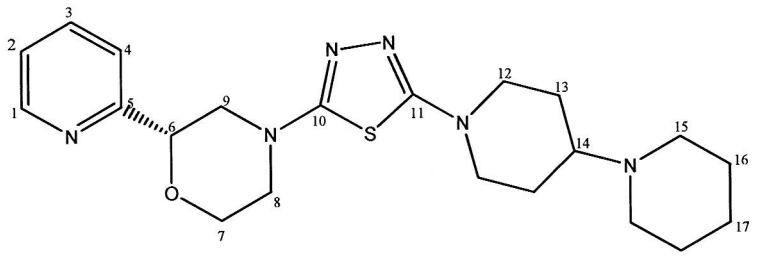
Copy: Tze-Ming Chan

From: Mary Senior

Reference: L-004871319

11nhf0806-0810

NMR data acquired at 500 MHz in chloroform-d for sample L-004871319 are consistent with the structure shown:



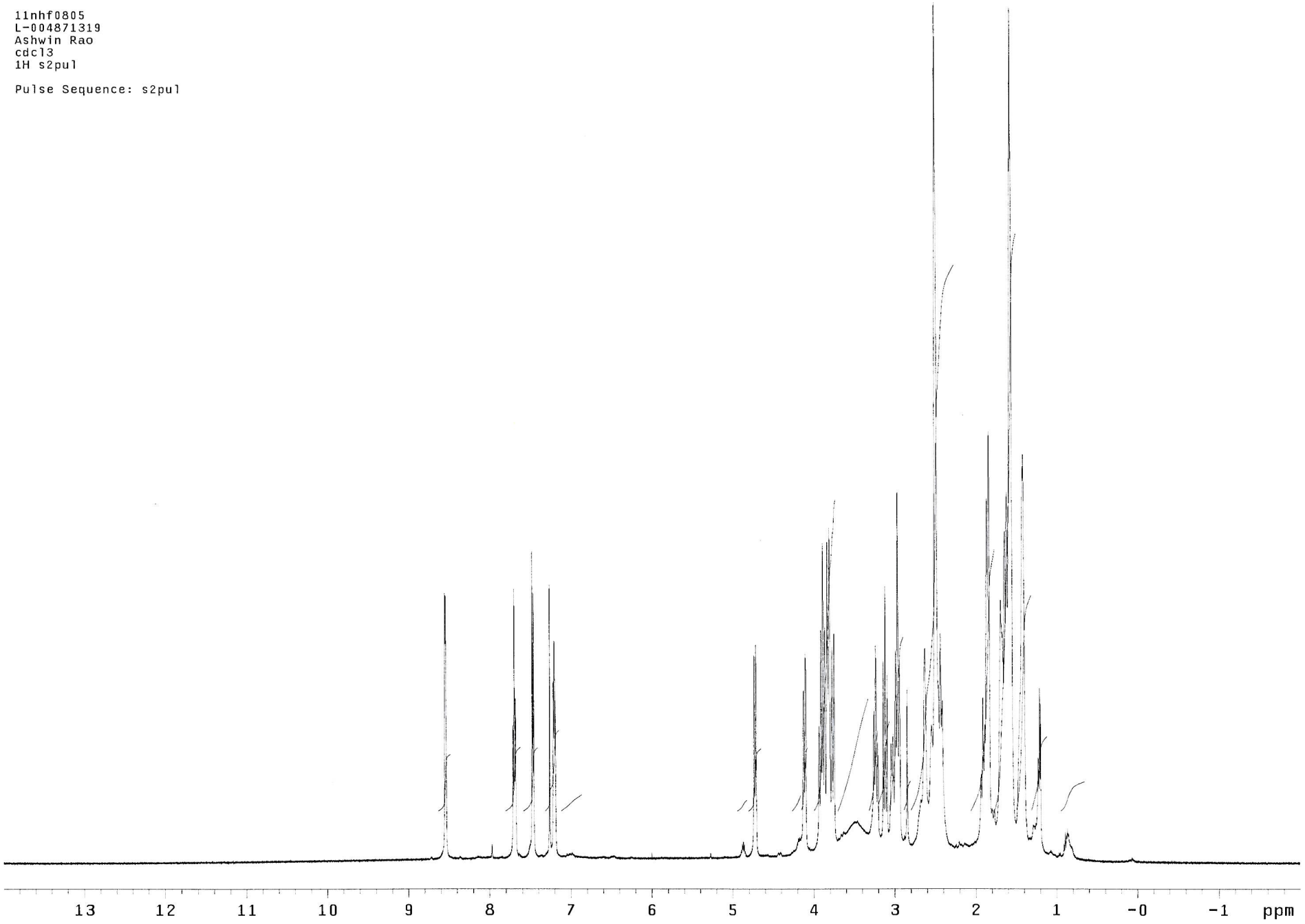
MW = 414.6

The expected number of protons and carbons were detected for this structure. The structural skeleton was confirmed with 2D HSQC, COSY, HSQC-TOCSY, and HMBC data sets.

Important HMBC couplings detected include H_4, H_7, and H_9 to C_6; H_1, H_3, H_6, and H_9 to C_5; H_8 and H_9 to C_{10}; H_{12} to C_{11}; and H_{14} and H_{16} to C_{15}.

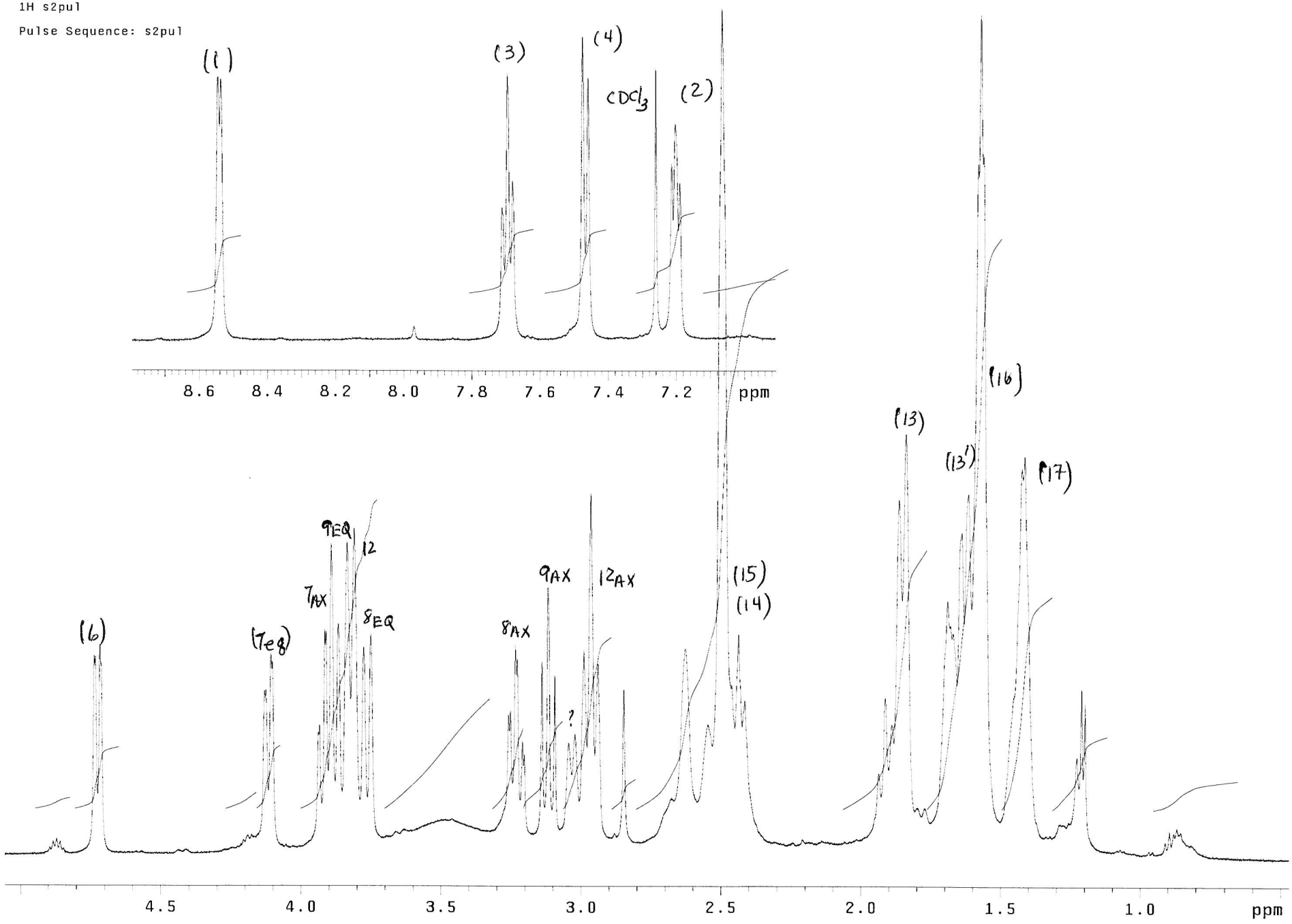
The stereochemistry shown, where H_6 is axial and the pyridine ring is equatorial, was determined from 1D coupling constants and 2D NOESY data. One large and one small coupling measured between H_6 and H_{9ax} and H_6 and H_9 equatorial (10 Hz & 2 Hz) agrees with that determined from modeling (11Hz & 3 Hz) . NOEs detected that support this stereochemistry include those between H_4 and H_{9ax} and between H_6 and H_{7ax}.

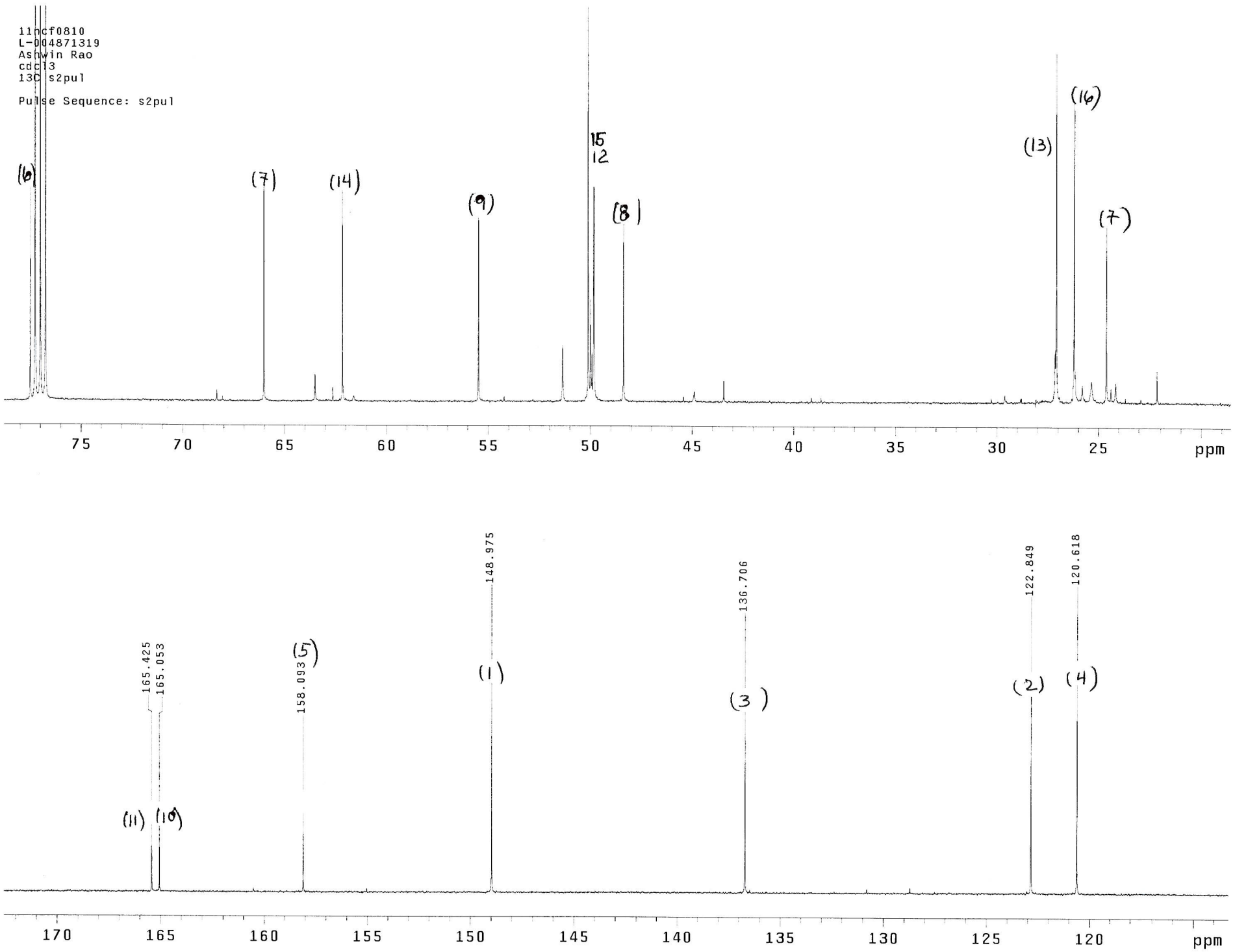
The molecular ion detected agrees with the molecular weight of this structure.


11nhf0805
L-004871319
Ashwin Rao
cdcl3
1H s2pul
Pulse Sequence: s2pul
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm

11nhf0805
L-004871319
Ashwin Rao
cdcl3
1H s2pul

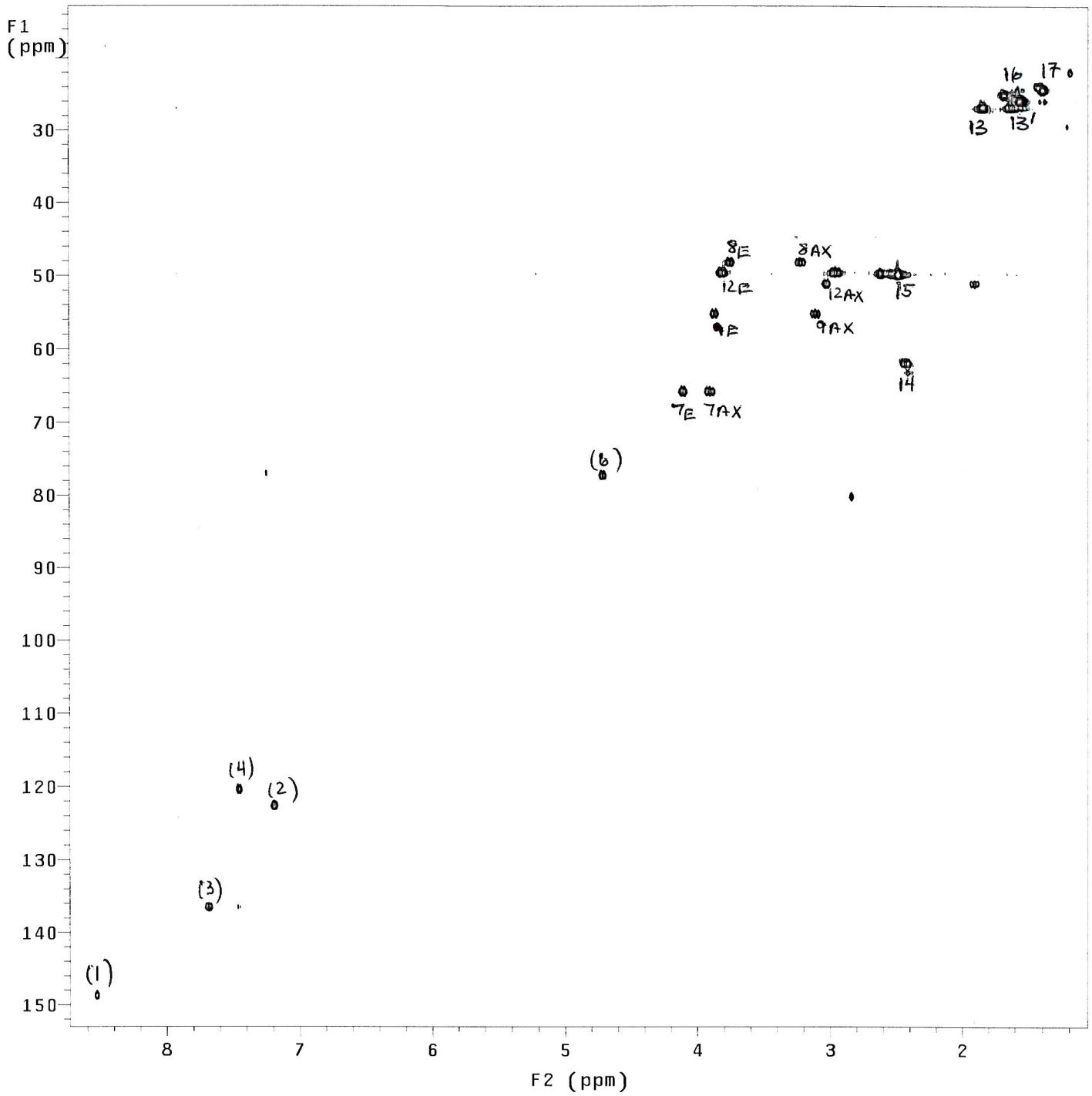
Pulse Sequence: s2pul




11ncf0810
L-004871319
Ashwin Rao
cdcl3
13C s2pul
Pulse Sequence: s2pul
(6)
(7)
(14)
(9)
15
12
(8)
(13)
(16)
(7)
75
70
65
60
55
50
45
40
35
30
25
ppm
165.425
165.053
158.093
148.975
136.706
122.849
120.618
(11)
(10)
(5)
(1)
(3)
(2)
(4)
170
165
160
155
150
145
140
135
130
125
120
ppm

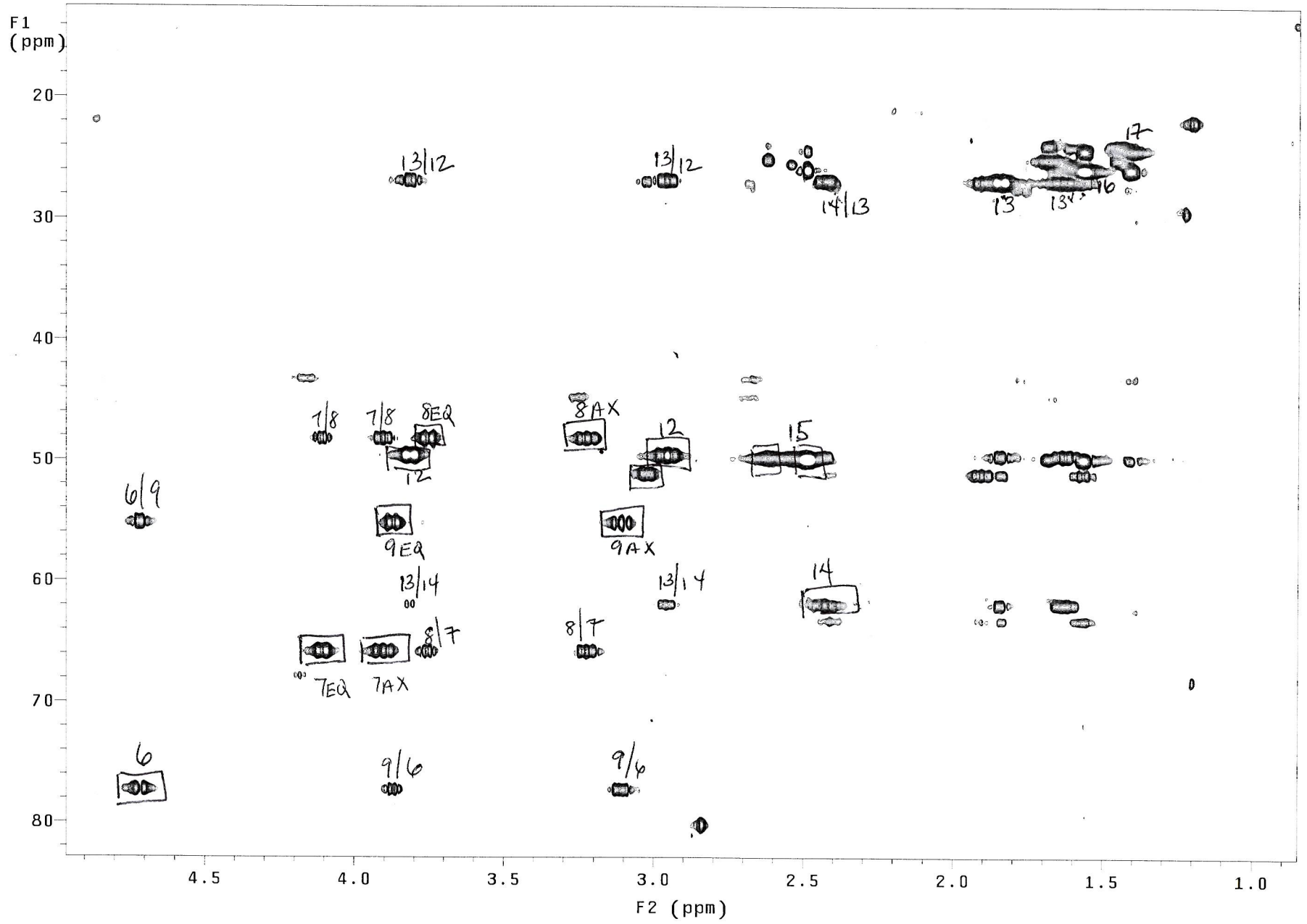
11nhf0806
L-004871319
Ashwin Rao
cdcl3
hsqc

Pulse Sequence: HSQC



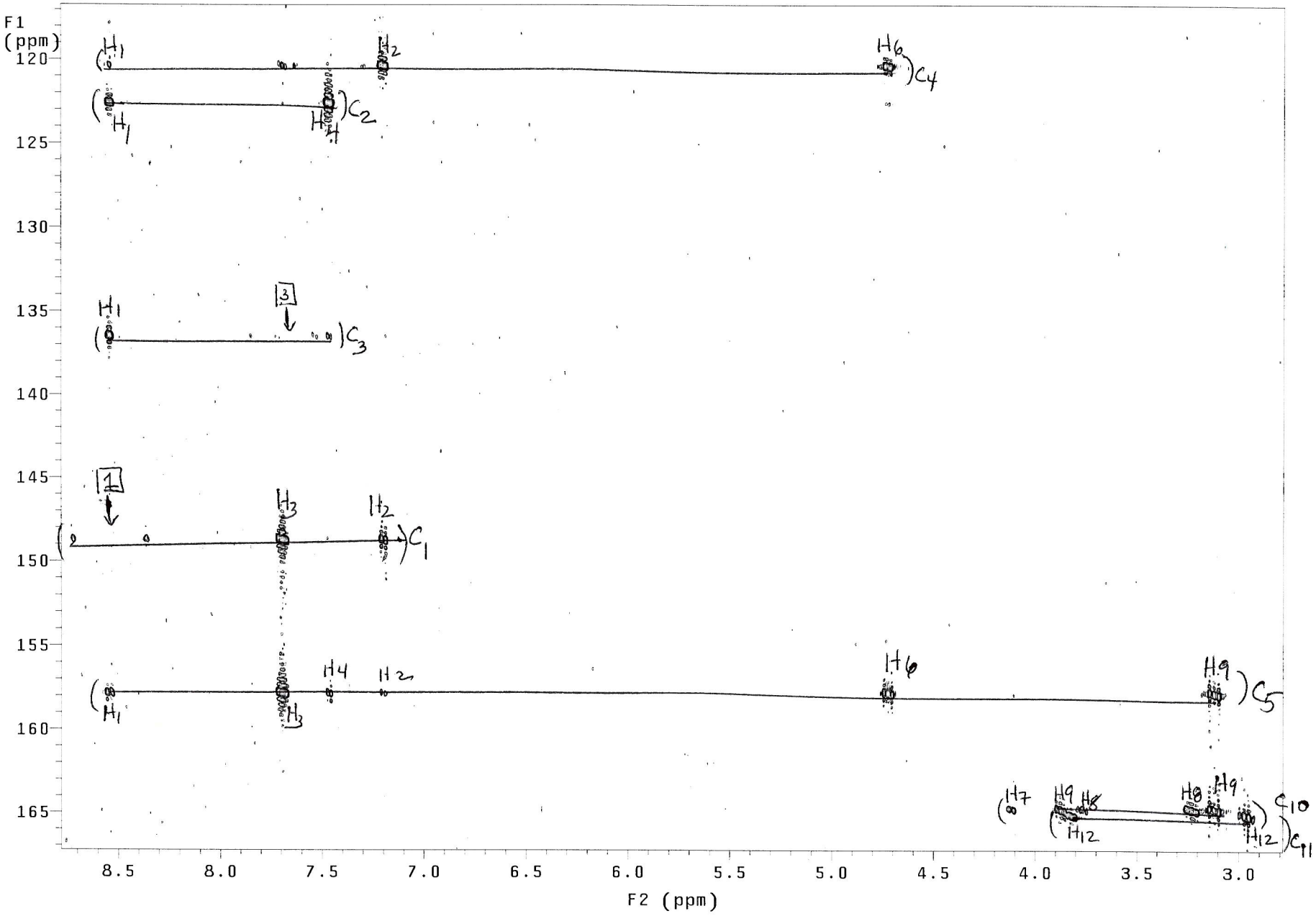
11nhf0808
L-004871319
Ashwin Rao
cdcl3
hsqctoxy

Pulse Sequence: HSQCTOXY



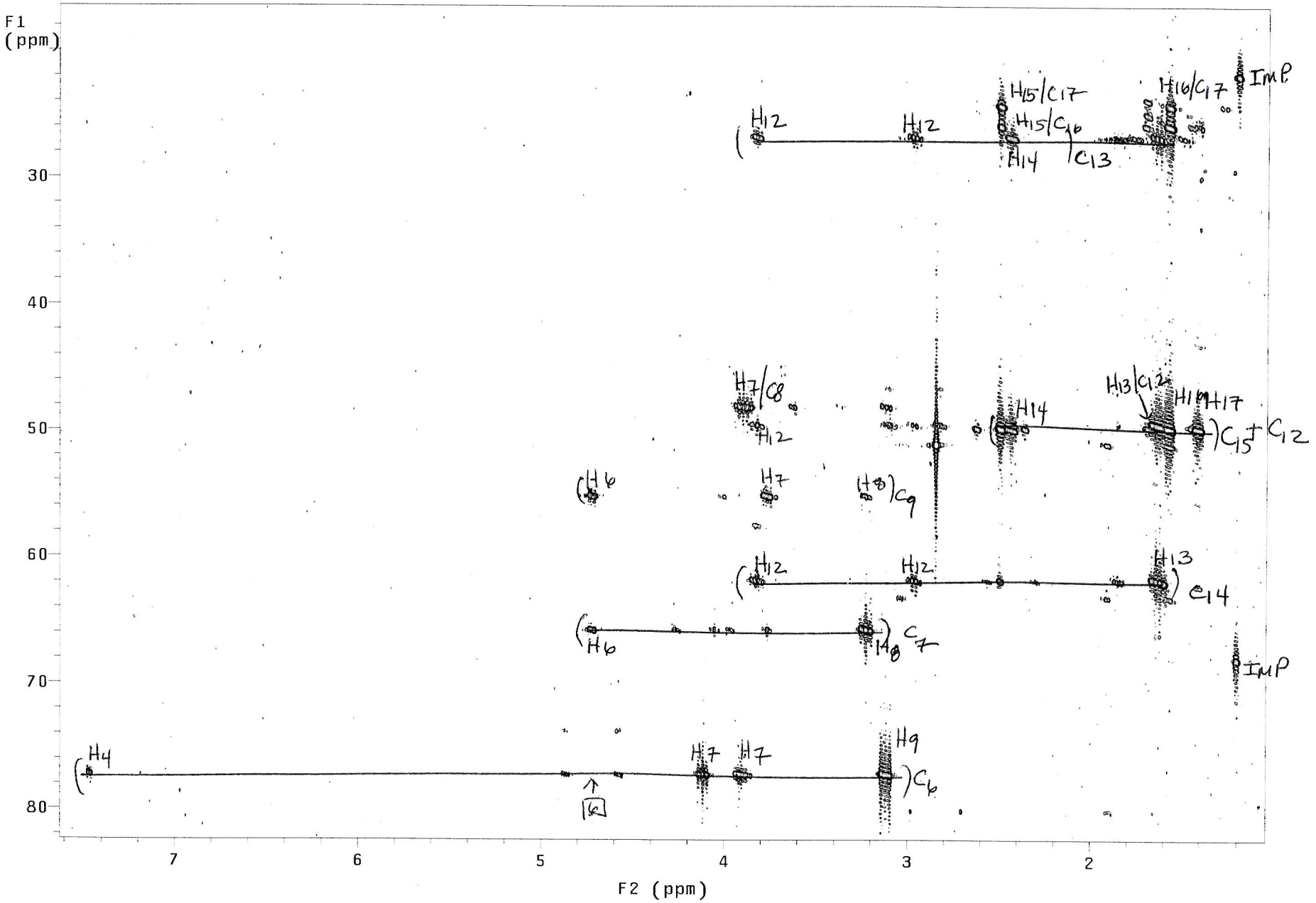
11nhf0809
L-004871319
Ashwin Rao
cdcl3
ghmbc

Pulse Sequence: gHMBC



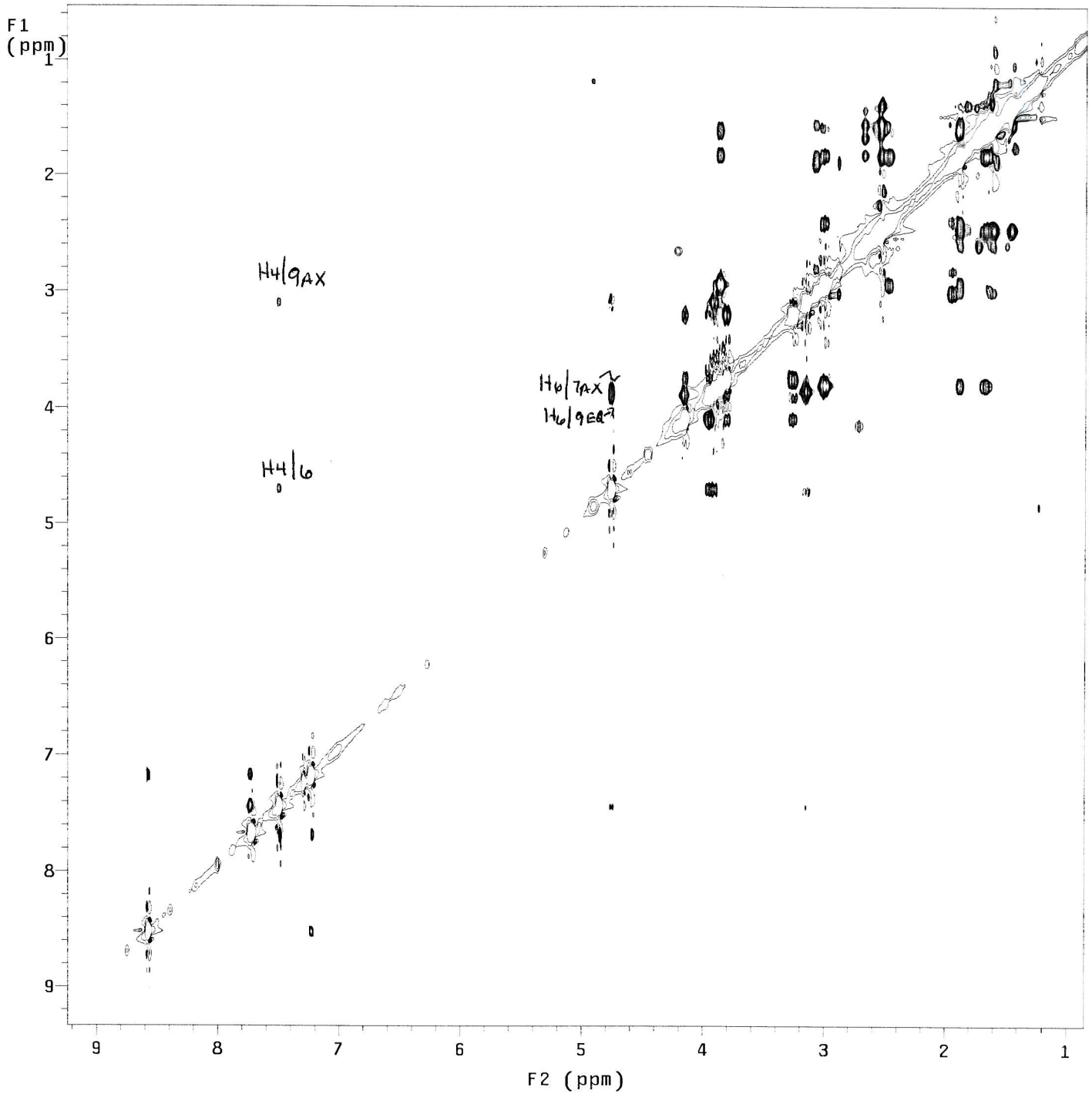
11nhf0809
L-004871319
Ashwin Rao
cdcl3
ghmbc

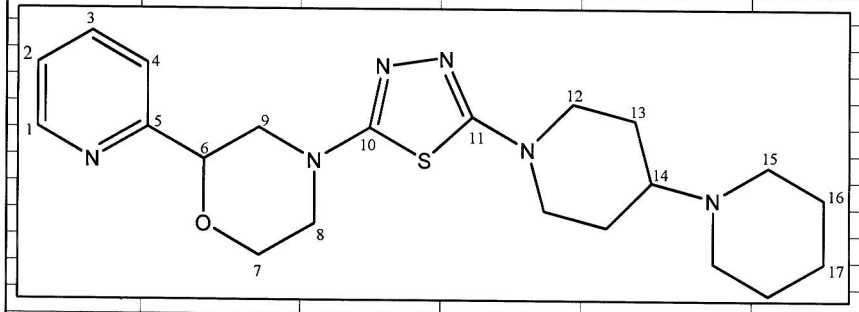
Pulse Sequence: gHMBC



11nhf0815
L-004871319
Ashwin Rao
cdcl3
noesy

Pulse Sequence: noesy





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3	7.70	136.7
4	7.47	120.6
5	XXXX	158.1
6	4.72	77.5
7	3.91, 4.11	66.0
8	3.75, 3.21	48.4
9	3.87, 3.10	55.5
10	XXXX	165.0
11	XXXX	165.4
12	3.81, 2.96	50.0
13	1.83, 1.62	27.1
14	2.43	62.2
15	2.62, 2.42	50.1
16	1.56	26.2
17	1.40	24.6