Supporting Information

Development of Potent and Selective *Plasmodium falciparum* Calcium-Dependent Protein Kinase 4 (*Pf*CDPK4) Inhibitors that Block the Transmission of Malaria to Mosquitoes

Rama Subba Rao Vidadala[a], Kayode K. Ojo[b], Steven M. Johnson[a], Zhongsheng Zhang[c], Stephen E. Leonard[a], Arinjay Mitra[a], Ryan Choi[b], Molly C. Reid[b], Katelyn R. Keyloun[b], Anna M.W. Fox[b], Mark Kennedy[d], Tiffany Silver-Brace[d], Jen C. C. Hume[d], Stefan Kappe[d,e], Christophe L.M.J. Verlinde[c], Erkang Fan[c], Ethan A. Merritt[c], Wesley C. Van Voorhis[b,e,f], & Dustin J. Maly[a]

[a]Department of Chemistry, [b]Division of Allergy and Infectious Diseases, Department of Medicine, [c]Department of Biochemistry, University of Washington, Seattle, Washington, USA. [d]Seattle Biomedical Research Institute, Seattle, Washington, USA. Departments of Global Health[e] and Microbiology[f], University of Washington, Seattle, Washington, USA.

Correspondence should be addressed to: DJM (Tel: 206-543-1653. Fax 206-685 7002. E-mail: maly@chem.washington.edu) or WCVV (Tel: 206-543-2447. Fax: 206-616-4898. E-mail: wesley@uw.edu)

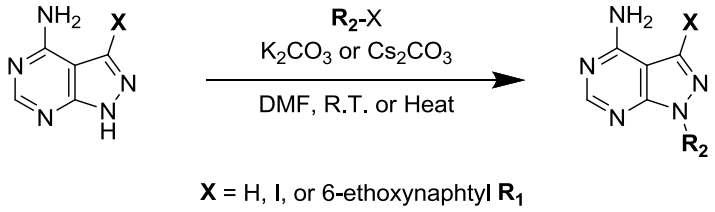
Table of Contents

General Synthetic Procedures.

all chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. Reactions were monitored with thin-layer chromatography using silica gel 60 F254 coated glass plates (EM Sciences). Compound purification was performed with an IntelliFlash 280 automated flash chromatography system using pre-packed Varian SuperFlash silica gel columns (hexanes/EtOAc or CH_2Cl_2/MeOH gradient solvent systems). A Varian Dynamax Microsorb 100-5 C_{18} column (250 mm x 21.4 mm), eluting with H_2O/CH_3CN or H_2O/ MeOH gradient solvent systems (+0.05% TFA) was used preparatory HPLC purification. The purity of all final compounds was determined by two analytical RP-HPLC methods, using an Agilent ZORBAX SB-C_{18} (2.1 mm x 150 mm) or Varian Microsorb-MV 100-5 C_{18} column (4.6 mm x 150 mm), and eluting with either H_2O/CH_3CN or H_2O/MeOH gradient solvent systems (+0.05% TFA) run over 30 min. Products were detected by UV at λ=254 nm, with all final compounds displaying >95% purity. NMR spectra were recorded on Bruker 300 or 500 MHz spectrometers at ambient temperature. Chemical shifts are reported in parts per million (δ) and coupling constants in Hz. ^{1}H-NMR spectra were referenced to the residual solvent peaks as internal standards (7.26 ppm for $CDCl_3$, 2.50 ppm for d_6-DMSO, and 3.34 ppm for CD_3OD). Mass spectra were recorded with a Bruker Esquire Liquid Chromatograph - Ion Trap Mass Spectrometer. The synthetic routes used to generate inhibitors are shown in Schemes 1-4.

Synthesis and purification methods for compounds **5b, 6b, 7a, 8a, 9a, 10a, 10b, 14a, 14b, 15a, 15b, 16a, 16b, 17a, 17b, 18a, 19a, 20a, 21a, 22b, 24a, 25a, 26a, 27a, 28a, 29a, 29b, and 35a** in Table 1 are described in previous publications [1,2]. Synthesis and purification methods for compounds **17, 15j, 16j, 17c, 17d, 17e, 17f, 17j, 17k, 17m ,17n, 17o, 19j, 20j, 21j, 24j, 25j, 29j, 30j,** and **36j** in Table 2 are described in previous publications [1,2]. Synthesis and purification methods for compounds **39b, 40a, 40b, 41b, 42b, 43a** and **43b** in Table 3 are described in previous publications [3]. Syntheses and compound characterization data for all other compounds are described below.

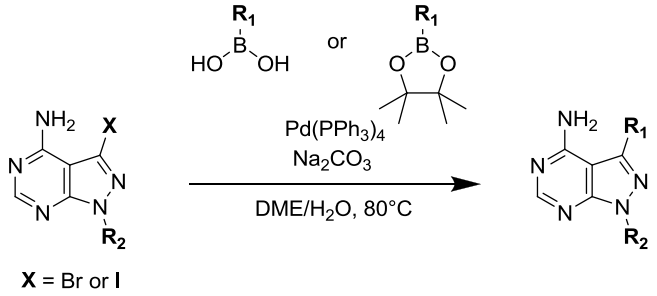
General R_2 alkylation procedure:



X = H, I, or 6-ethoxynaphtyl **R_1**

Pyrazolopyrimidine (1 equiv), K_2CO_3 or Cs_2CO_3 (1.5-2 equiv), and an alkylhalide (1.1 equiv) or alkylmesylate (1.1 equiv) were stirred in dry DMF at room temperature or 80 °C. The reaction was monitored by thin layer chromatography. After complete, the reaction was dissolved into EtOAc, washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified via flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. Product fractions were collected and concentrated to a solid.

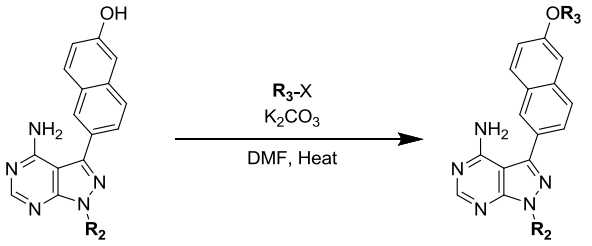
General Suzuki coupling procedure:



X = Br or I

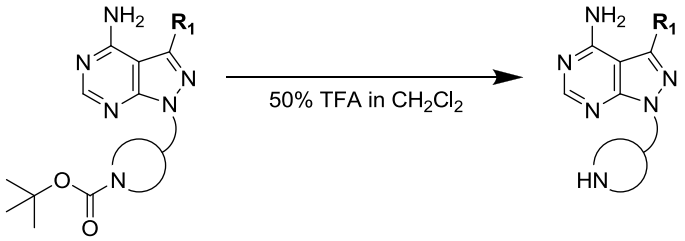
3-Iodopyrazolopyrimidines or 3-bromopyrazolopyrimidines (1 equiv), Na_2CO_3 (2-4 equiv), catalytic $Pd(PPh_3)_4$ (.05 equiv), and boronic acids or boronate pinacol esters (1-2 equiv) dissolved in a dimethoxyethane (1.5 ml) and water (0.5 ml) mixture were heated in a microwave at 80 °C for one hour. The reaction was then allowed to cooled to room temperature, diluted into ethyl acetate, washed with brine, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified *via* flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. If necessary, further purification was performed with preparatory RP-HPLC.

General naphthol alkylation procedure:



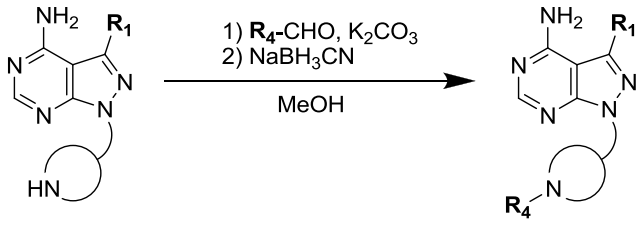
6-hydroxy-2-naphthalene pyrazolopyrimidines (1 equv), K_2CO_3 (1.5-2 equiv), and alkyl halides (1.1 equiv) were stirred in dry DMF at room temperature or 80°C and monitored by thin layer chromatography. After completion, the reaction was diluted with EtOAc, which was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was then purified *via* flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. If necessary, further purification was performed with preparatory RP-HPLC.

General boc-deprotection procedure:



Boc-amine-containing pyrazolopyrimidines and imidazopyrazines were stirred in a TFA/CH_2Cl_2 (1:1) mixture for ~3 h. The reaction was then concentrated and purified via preparatory RP-HPLC. After HPLC purification, the product was then re-concentrated from 1.25 M HCl in EtOH to afford the final, purified product as a bis-HCl salt.

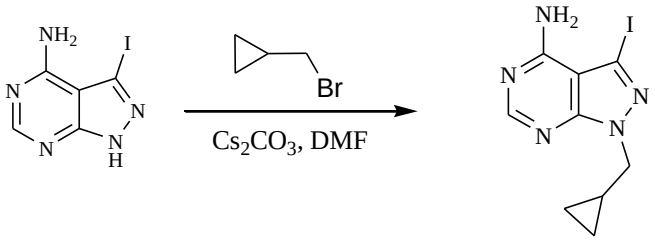
General reductive amination procedure:



Deprotected pyrazolopyrimidines (1 equiv) were dissolved in methanol and neutralized with sodium methoxide. A solution containing 2% acetic acid and an aldehyde or ketone (5-10 equiv) was stirred at room temperature for 10 min. Sodium cyanoborohydride (5 equiv) was then added and the reaction was stirred until reaching completion, as determined by thin layer chromatography (typically ~2 h). The reaction was then purified *via* preparatory RP-HPLC. After HPLC purification, the residue was dissolved in a small amount of 2 M HCl in methanol and, after concentration *in vacuo*, the final product was obtained as an HCl salt.

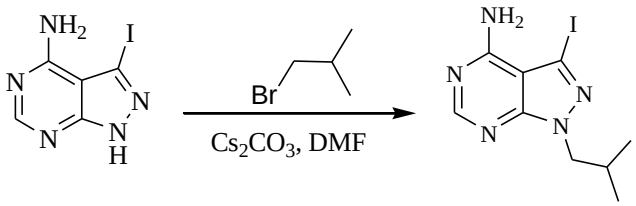
Synthesis and spectral data of various Intermediates

*1-(cyclopropylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**44**)*



44 was generating using the ***general R_2 alkylation procedure*** using 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine [1,2] (577.9 mg, 2.21 mmol) and (bromomethyl)cyclopropane (330.8 mg, 2.45 mmol), and Cs_2CO_3 (1.05 g, 3.25 mmol) in anhydrous DMF (10mL). The reaction was stirred at 70 °C for 18 h, affording **44** (211 mg, yield:30.1%) as a yellow solid after purification using a hexanes/EtOAc solvent gradient. ^{1}H-NMR (301 MHz, d_6-DMSO): δ ppm 8.20 (s, 1H), 4.14 (d, J=7.1 Hz, 2H), 1.26 (m, 1H), 0.47 (m, 2H), 0.38 (m, 2H); MS (ESI): 316.5 *m/z* [MH^+], $C_9H_{10}IN_5$ requires 316.1

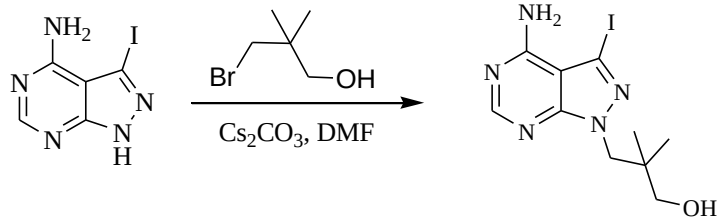
*3-iodo-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**45**)*



45 was generating using the ***general R_2 alkylation procedure*** using 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (500 mg, 1.92 mmol), 1-bromo-2-methylpropane (393 mg,

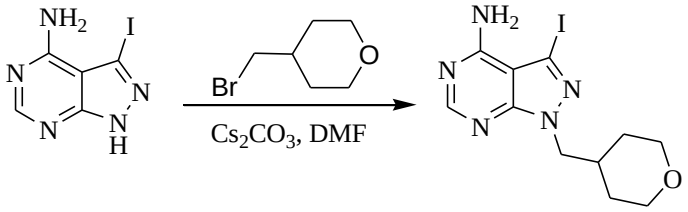
2.87 mmol), and Cs_2CO_3 (930 mg, 2.87 mmol) in anhydrous DMF (10mL). The reaction was stirred at 70 °C for 18 h, affording **45** (210 mg, yield:34%) as a yellow solid after purification using a hexanes/EtOAc solvent gradient. ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm 8.32 (s, 1H), 5.97 (br s, 2H), 4.18 (d, *J*=7.5 Hz, 2H), 2.36 (m, 1H), 0.92 (d, *J*=6.6 Hz, 6H); MS (ESI): 318.2 *m/z* [MH^+], $C_9H_{12}IN_5$ requires 318.1

*3-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol (**46**)*



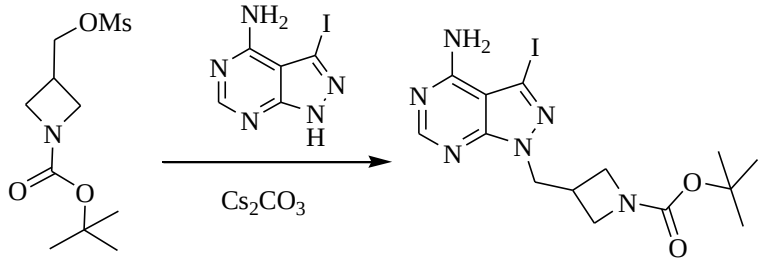
46 was generating using the ***general R_2 alkylation procedure*** using 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (500 mg, 1.92 mmol), 3-bromo-2,2-dimethylpropan-1-ol (642 mg, 3.84 mmol), and Cs_2CO_3 (1.24 g, 3.84 mmol) in anhydrous DMF (10mL). The reaction was stirred at 70 °C for 18 h, affording **46** (250.1 mg, yield:37.4%) as a yellow solid after purification using a hexanes/EtOAc solvent gradient. ^{1}H-NMR (300 MHz, CD_3OD): δ ppm 8.22 (s, 1H), 4.24 (s, 2H), 3.27 (s, 2H), 0.93 (s, 6H); MS (ESI): 348.5 *m/z* [MH^+], $C_{10}H_{14}IN_5O$ requires 348.1

*3-iodo-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**47**)*



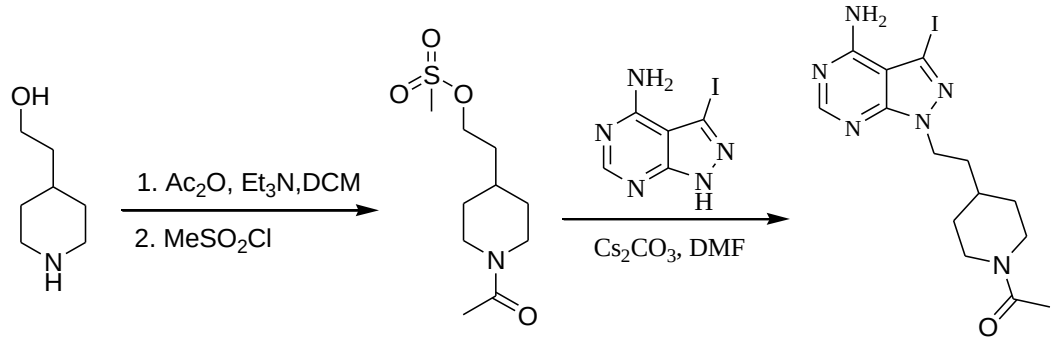
47 was generating using the ***general R_2 alkylation procedure*** using 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (504 mg, 1.93 mmol), 4-(bromomethyl)-tetrahydro-2H-pyran (379.9 mg, 2.12 mmol), and Cs_2CO_3 (941 mg, 2.89 mmol) in anhydrous DMF (10mL). The reaction was stirred at 70 °C for 18 h, affording **47** (425.8 mg, yield:61.5%) as a white solid after purification using a hexanes/EtOAc solvent gradient. ^{1}H-NMR (301 MHz, d_6-DMSO): δ ppm 8.20 (s, 1H), 4.18 (d, *J*=7.1 Hz, 2H), 3.81 (m, 2H), 3.23 (m, 2H), 2.13 (m, 1H), 1.37 (m, 2H), 1.24 (m, 2H); MS (ESI): 360.1 *m/z* [MH^+], $C_{11}H_{14}IN_5O$ requires 360.1

*tert-butyl-3-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl) azetidine-1-carboxylate (**48**)*



48 was generating using the ***general R_2 alkylation procedure*** using 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (245 mg, 0.93 mmol), *tert*-butyl 3-((methylsulfonyloxy)methyl)azetidine-1-carboxylate[5] (274 mg, 1.1 mmol), and Cs_2CO_3 (454 mg, 1.39 mmol) in 20 mL of DMF. The reaction was stirred at 70 °C for 18 h, affording **48** (241.8 mg, yield:60%) after purification using a methanol/dichloromethane solvent gradient. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.22 (s, 1H), 4.55 (d, *J*=7.0 Hz, 2H), 4.01 (t, *J*=8.5 Hz, 2H), 3.81 (q, *J*=8.3 Hz, 2H), 3.12 (m, 1H), 1.42 (s, 9H); MS (ESI): 431.2 *m/z* [MH+], $C_{14}H_{19}IN_6O_2$ requires 431.2

*1-(4-(2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone (**49**)*

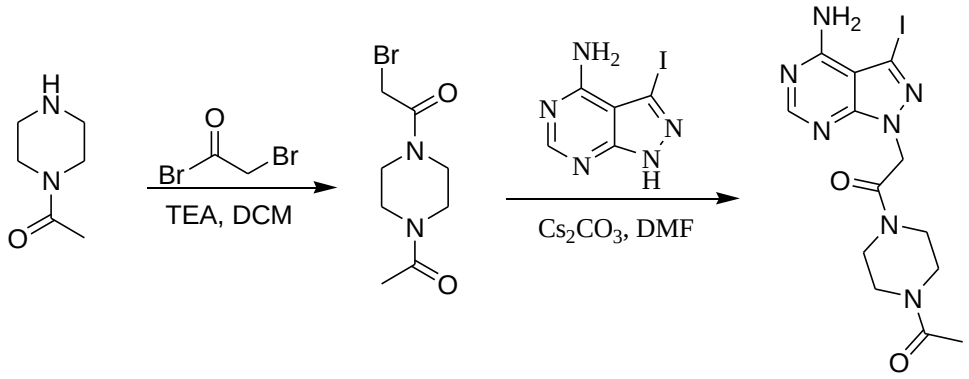


2-(1-acetylpiperidin-4-yl)ethyl methanesulfonate: 4-piperidineethanol (1220 mg, 9.44 mmol), acetic anhydride (0.94 mL, 9.96 mmol), and triethylamine (23.7 mmol) in (20 mL) dichloromethane were stirred at room temperature for 4 h. Methanesulfonyl chloride (1.10 mL, 14.2 mmol) was added slowly to the same vessel and stirred for 5 h at room temperature. The reaction was diluted with ethyl acetate, washed with dilute $NaHCO_3$, 1N HCl, brine (50ml), dried over sodium sulfate, filtered, and concentrated. 2-(1-Acetylpiperidin-4-yl)ethyl methanesulfonate (852 mg, yield:36.1%) was obtained after purification using a methanol/chloroform solvent

gradient. ^{1}H NMR (301 MHz, d_6-DMSO): δ ppm 4.34-3.78 (m, 4H), 3.19 (s, 3H), 2.98 (m, 1H), 1.98 (s, 3H), 1.67-1.57 (m, 4H), 1.16-0.86 (m, 4H); MS (ESI): 250.1 *m/z* [MH+], $C_{10}H_{19}NO_4S$ requires 249.3

(49): **49** was generated with 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine and 2-(1-acetylpiperidin-4-yl)ethyl methanesulfonate using the ***general R_2 alkylation procedure***. ^{1}H NMR (300 MHz, d_6-DMSO): δ ppm 8.20 (s, 1H), 4.32 (t, *J*=7.1 Hz, 2H), 3.75 (d, *J*=13.7 Hz, 2H), 2.90 (t, *J*=13.7 Hz, 1H), 2.40 (t, *J*=13.7 Hz, 1H), 1.96 (s, 3H), 1.72 (m, 4H), 1.36 (m, 1H), 1.17-0.88 (m, 2H); MS (ESI): 415.2 *m/z* [MH+], $C_{14}H_{19}IN_6O$ requires 415.2

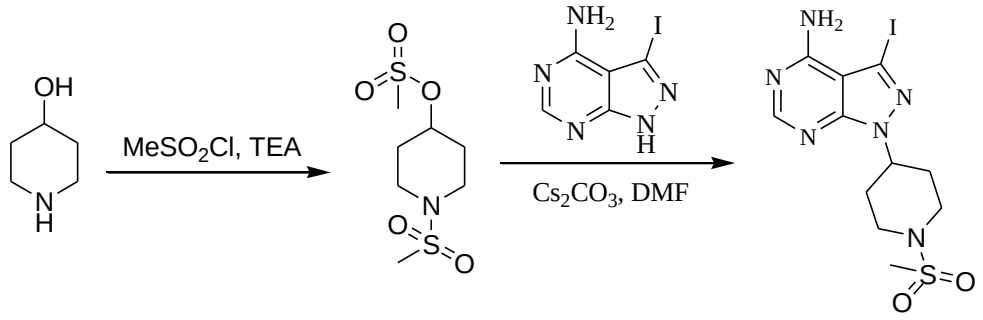
*1-(4-(2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrim, idin-1-yl)ethyl)piperazin-1-yl)ethanone (**50**)*



1-Acetyl-4-(bromoacetyl)piperazine: Bromoacetyl bromide (1.2 mL, 13.8 mmol) was added slowly to a stirring mixture of triethylamine (1.9 mL, 39.5 mmol) and N-acetylpiperidine (1470 mg, 11.5 mmol) in dichloromethane (12 mL). The reaction mixture was stirred at room temperature for 5 h. The reaction was then diluted with dichloromethane, washed with 1N HCl (2x20 mL), brine, dried over sodium sulfate, and concentrated *in vacuo* to afford 1-Acetyl-4-(bromoacetyl)piperazine (1525 mg, yield:53.9%) as a brown solid. ^{1}H NMR (301 MHz, d_6-DMSO): δ ppm 4.18 (s, 2H), 3.66-3.37 (m, 8H), 2.05 (s, 3H); MS (ESI): *m/z* 249.0 [MH+], $C_8H_{14}BrN_2O_2$ requires 249.0

50: **50** was generated with 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine and 1-acetyl-4-(bromoacetyl)piperazine using the ***general R_2 alkylation procedure***. ^{1}H NMR (301 MHz, d_6-DMSO): δ ppm 8.18 (s, 1H), 5.32 (d, *J*=4.9 Hz, 2H), 3.64-3.35 (m, 8H), 2.03 (s, 3H); MS (ESI): 430.2 *m/z* [MH+],$C_{13}H_{16}IN_7O_2$ requires 430.2.

*3-iodo-1-(1-(methylsulfonyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**51**)*

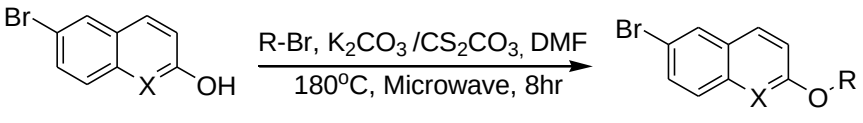


1-(methylsulfonyl)piperidin-4-yl methanesulfonate: Methanesulfonyl chloride (3.8 mL, 49.1 mmol) was added slowly to a mixture of piperidin-4-ol (1980 mg, 19.6 mmol), triethylamine (5.5 mL, 39.5 mmol) in dichloromethane (40 mL). The reaction mixture was stirred at room temperature for 4 h. The reaction was then diluted with ethyl acetate, washed with dilute $NaHCO_3$, 1 N HCl, brine, dried over sodium sulfate, and concentrated *in vacuo* to afford 1-(methylsulfonyl)piperidin-4-yl methanesulfonate (4670 mg, yield:93.2%) as a white powder. ^{1}H NMR (301 MHz, d_6-DMSO): δ ppm 4.84 (m, 1H), 3.28 (m, 2H), 3.23 (m, 2H), 3.14 (s, 3H), 2.91 (s, 3H), 2.09-1.93 (m, 2H), 1.91-1.74 (s, 2H); MS (ESI): 258.1 *m/z* [MH+], $C_7H_{16}NO_5S$ requires 258.1

51: **51** was generated with 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine and 1-(methylsulfonyl)piperidin-4-yl methanesulfonate using the ***general R_2 alkylation procedure***. ^{1}H NMR (301 MHz, d_6-DMSO): δ ppm 8.22 (s, 1H), 3.68 (m, 4H), 2.94 (s, 3H), 2.19-1.94 (m, 5H); MS (ESI): 423.2 *m/z* [MH+], $C_{11}H_{15}IN_6O_2S$ requires 423.2

Synthesis of various naphthalene and quinoline pinacol esters

General naphtol/quinolone alkylation procedure:

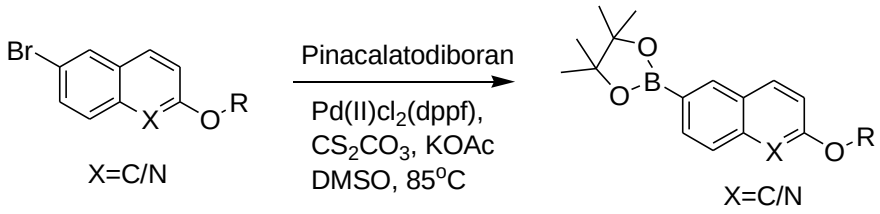


X= C/N X=C/N

naphthols or quinolones (1 equiv), K_2CO_3 or CS_2CO_3 (1.5-2 equiv), and alkyl halides (1.1 equiv) in dry DMF were heated at 180 °C in a microwave for 8 h. After the reaction was found to

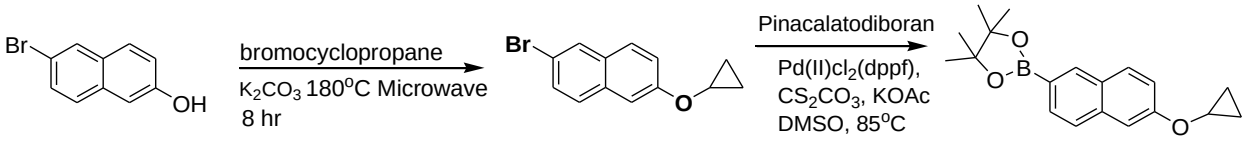
be complete by thin layer chromatography, it was diluted into EtOAc, washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired crude product. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient.

general pinacol ester formation procedure:



The alkylated naphthols or quinolones (1 equiv), CS_2CO_3 (1.5-2 equiv), and pinacalatodiboran (2.0 equiv), Pd(II)cl2(dppf) (0.05 equiv), KOAc (1 eqyuv) in dry DMSO were heated at 85 °C for 5-8 h. After the reaction was found to be complete by thin layer chromatography, it was diluted into EtOAc, washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired crude product. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient.

2-(6-cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane

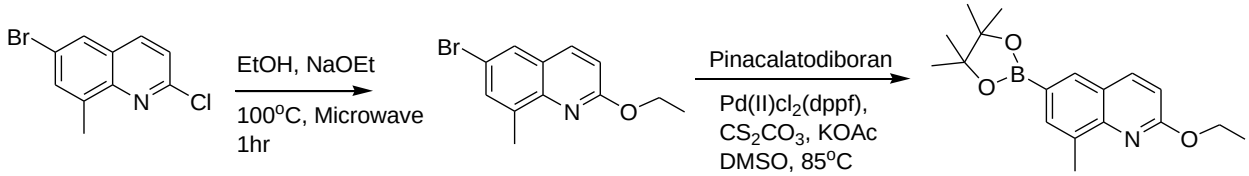


2-bromo-6-cyclopropoxynaphthalene: 6-bromonaphthalen-2-ol and bromocyclopropane were used in the ***general naphtol/quinolone alkylation procedure*** to afford 2-bromo-6-cyclopropoxynaphthalene. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.91 (m, 1H), 7.66-7.58 (dd, *J*=8.9, 4.6 Hz, 2H), 7.53-7.46 (dd, *J*=8.7, 1.9 Hz, 1H), 7.39 (d, *J*=2.1 Hz, 1H), 7.18-7.12 (dd, *J*=8.9, 2.3 Hz, 1H), 3.83 (m, 1H), 0.87-0.78 (m, 4H); MS (ESI): 264.2 *m/z* [MH+], $C_{13}H_{11}BrO$ requires 264.2

2-(6-cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane: 2-bromo-6-cyclopropoxynaphthalene was used in the **general pinacol ester formation procedure** to afford 2-(6-cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane. ^{1}H NMR (300

MHz, $CDCl_3$): δ ppm 8.27 (s, 1H), 7.77 (m, 3H) 7.45 (d, , *J*=2.3 Hz, 1H), 7.14 (dd, *J*=8.9, 2.48 Hz, 1H), 3.88 (m, 1H), 1.40 (s, 12H), 0.87 (m, 2H), 0.82 (m, 2H); MS (ESI): 311.5 *m/z* [MH+], $C_{19}H_{23}BO_3$ requires 311.2

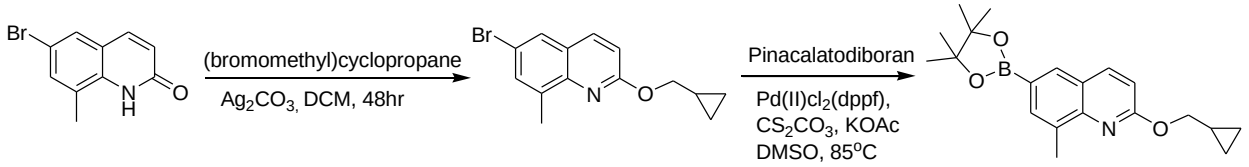
2-ethoxy-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline



6-bromo-2-ethoxy-8-methylquinoline: 6-bromo-2-chloro-8-methylquinoline (190 mg, 0.74 mmol) and NaOEt (201 mg, 2.96 mmol) in ethanol (2ml) were heated to 100 ^oC for 1 h in a microwave. The reaction was then extracted into ethyl acetate (3x), and the combined organic layers were washed with brine, dried over sodium sulfate, concentrated *in vacuo*. The crude product was flash purified using an EtOAc:hexanes solvent gradient to afford (180 mg, yield:91.8%) of 6-bromo-2-ethoxy-8-methylquinoline. ^1H NMR (300 MHz, $CDCl_3$): δ ppm 7.85 (d, *J*=8.9 Hz, 1H), 7.70 (s, 1H), 7.56 (s, 1H), 6.88 (d, *J*=8.1 Hz, 1H), 4.54 (q, *J*=6.8 Hz, 2H), 2.65 (s, 3H), 1.46 (t, *J*=6.8 Hz, 3H); MS (ESI): 267.2 *m/z* [MH+], $C_{12}H_{12}BrNO$ requires 267.1

2-ethoxy-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline: 6-bromo-2-ethoxy-8-methylquinoline was used in the **general pinacol ester formation procedure** to afford 2-ethoxy-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline. ^1H NMR (300 MHz, $CDCl_3$): δ ppm 8.07 (s, 1H), 7.97 (d, *J*=8.9 Hz, 1H), 7.86 (s, 1H), 6.86 (d, *J*=8.7 Hz, 1H), 4.57 (q, *J*=7.0 Hz, 2H), 2.67 (s, 3H), 1.46 (t, *J*=7.0 Hz, 3H), 1.39 (s, 12H); MS (ESI): 314.1 *m/z* [MH+], $C_{18}H_{24}BNO_3$ requires 314.2

2-(cyclopropylmethoxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline

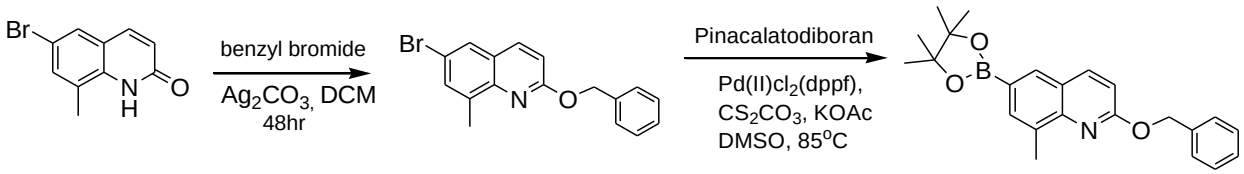


6-bromo-2-(cyclopropylmethoxy)-8-methylquinoline: 6-bromo-8-methylquinolin-2(1H)-one (200 mg, 0.89 mmol), Ag_2CO_3 (344 mg, 1.24 mmol), and bromomethyl cyclopropane (600 mg, 4.40 mmol) were dissolved in dichloromethane (5 ml) and stirred at room temperature for 48

h. The reaction was then filtered through a bed of celite-545 powder that was washed with ethyl acetate (10 mL). The crude reaction mixture was subjected to flash chromatography using an EtOAc/hexanes solvent gradient. ^{1}H NMR (301 MHz, $CDCl_3$): δ ppm 7.85 (d, *J*=8.9 Hz, 1H), 7.69 (s, 1H), 7.55 (s, 1H), 6.92 (d, *J*=8.7 Hz, 1H), 4.31 (d, *J*=7.3 Hz, 2H), 2.64 (s, 3H), 1.39 (m, 1H), 0.65 (m, 2H), 0.41 (m, 2H); MS (ESI): 293.4 *m/z* [MH+], $C_{14}H_{14}BrNO$ requires 293.1

2-(cyclopropylmethoxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline: 6-bromo-2-(cyclopropylmethoxy)-8-methylquinoline was used in the **general pinacol ester formation procedure** to afford 2-(cyclopropylmethoxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.07 (s, 1H), 7.97 (d, *J*=8.9 Hz, 1H), 7.86 (s, 1H), 6.90 (d, *J*=8.9 Hz, 1H,), 4.34 (d, *J*=7.0 Hz, 2H,), 2.67 (s, 3H), 1.38 (s, 13H), 0.64 (m, 2H), 0.42 (m, 2H); MS (ESI): 340.1 *m/z* [MH+], $C_{20}H_{26}BNO_3$ requires 340.1

2-(benzyloxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline

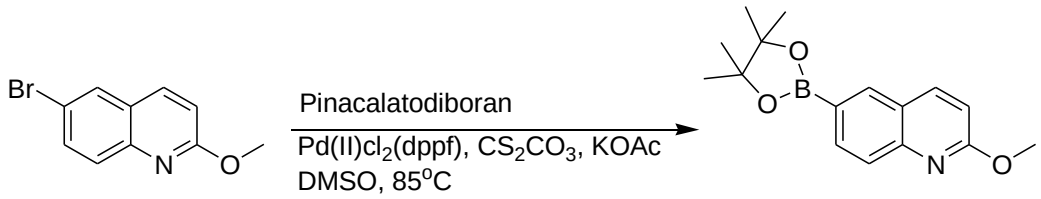


2-(benzyloxy)-6-bromo-8-methylquinoline: 6-bromo-8-methylquinolin-2(1H)-one (200 mg, 0.84 mmol), Ag_2CO_3 (323 mg, 1.17 mmol), and benzyl bromide (836 mg, 4.80 mmol) dissolved in dichloromethane (5ml) were stirred at room temperature for 48 h. The reaction was then filtered through a bed of celite-545 powder that was washed with ethyl acetate (10 mL). The crude reaction mixture was subjected to flash chromatography using an EtOAc/hexanes solvent gradient. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, *J*=8.7 Hz, 1H), 7.70 (d, *J*=2.1 Hz, 1H), 7.59-7.50 (m, 3H), 7.42-7.29 (m, 3H), 6.95 (d, *J*=8.9 Hz, 1H), 5.55 (s, 2H), 2.68 (s, 3H); MS (ESI): 329.3 *m/z* [MH+], $C_{17}H_{14}BrNO$ requires 329.2.

2-(benzyloxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline: 2-(benzyloxy)-6-bromo-8-methylquinoline was used in the **general pinacol ester formation procedure** to afford 2-(benzyloxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.09 (s, 1H), 7.98 (d, *J*=8.9 Hz, 1H), 7.89 (s,

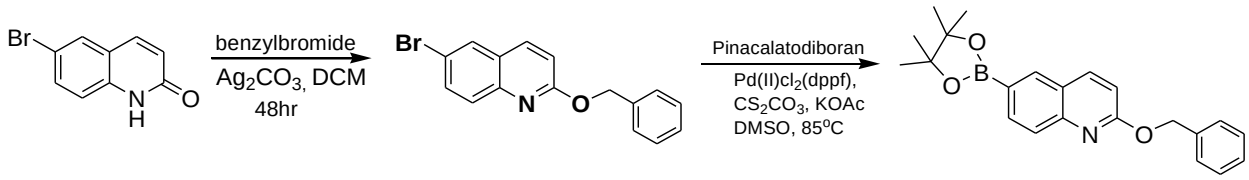
1H), 7.54 (d, *J*=7.4 Hz, 2H), 7.46-7.24 (m, 3H), 6.92 (d, *J*=8.7 Hz, 1H), 5.57 (s, 2H), 2.71 (s, 3H), 1.38 (s, 12H); MS (ESI): 376.1 *m/z* [MH+], $C_{23}H_{26}BNO_3$ requires 376.2

2-methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline



2-methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline: 6-bromo-2-methoxyquinoline [3] was used in the **general pinacol ester formation procedure** to afford 2-methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.22 (s, 1H), 8.00 (d, *J*=8.5 Hz, 2H), 7.83 (d, *J*=8.5 Hz, 1H), 6.88 (d, *J*=8.7 Hz, 1H), 4.08 (s, 3H), 1.38 (s, 12H); MS (ESI): 286.2 *m/z* [MH+], $C_{16}H_{20}BNO_3$ requires 286.1

2-(benzyloxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline

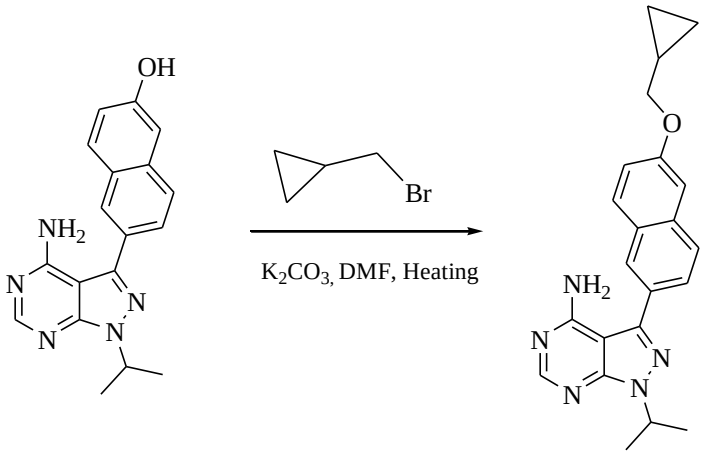


2-(benzyloxy)-6-bromoquinoline: 6-bromoquinolin-2(1H)-one (200 mg, 0.89 mmol), Ag_2CO_3 (344 mg, 1.24 mmol), and benzylbromide (885 mg, 5.17 mmol) were dissolved in DCM (5 mL) and stirred at room temperature for 48 h. The reaction was then filtered through celite-545 powder, which was washed with ethyl acetate (10 mL). The crude reaction mixture was taken to the next step without further purification.

*2-(benzyloxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline***:** 2-(benzyloxy)-6-bromoquinoline was used in the **general pinacol ester formation procedure** to afford 2-(benzyloxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.23 (s, 1H), 8.02 (d, *J*=8.7 Hz, 2H), 7.85 (d, *J*=8.2 Hz, 1H), 7.53 (d, *J*=7.0 Hz, 2H), 7.40-7.28 (m, 3H), 6.94 (d, *J*=8.7 Hz, 1H), 5.55 (s, 2H), 1.38 (s, 12H); MS (ESI): 362.1 *m/z* [MH+], $C_{22}H_{24}BNO_3$ requires 362.2

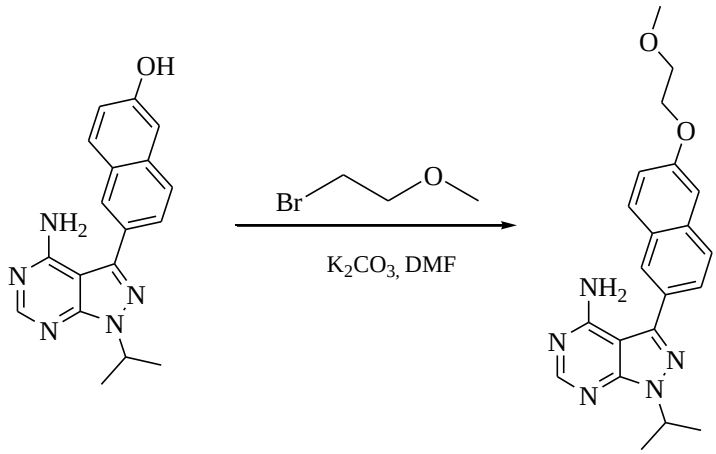
Final compounds from Table 1

3-(6-(cyclopropylmethoxy)naphthalen-2-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(22a)***



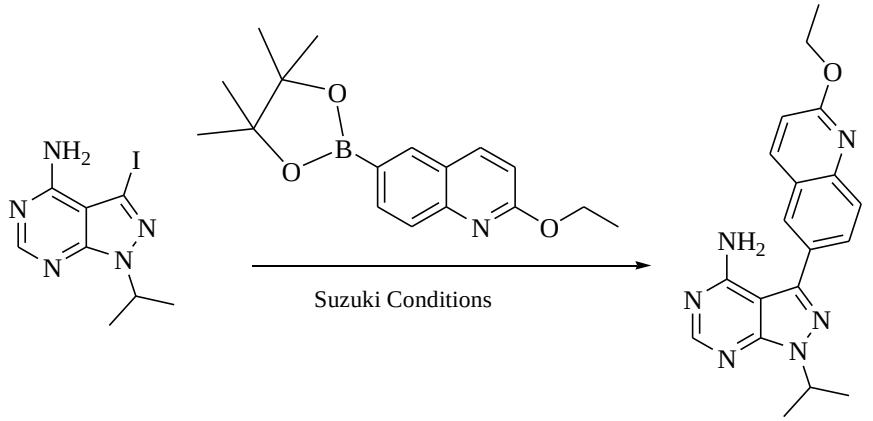
6-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-ol (**3**) [2] and bromomethyl cyclopropane were subjected to ***general naphthol alkylation procedure*** in order to afford 2**2a**.

1-isopropyl-3-(6-(2-methoxyethoxy)naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(23a)***



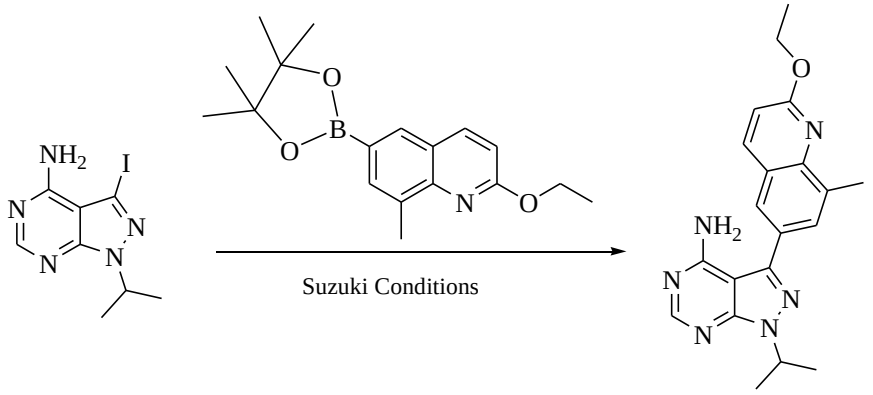
6-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)naphthalen-2-ol (**3**) [2] and 2-bromomethylethylether were subjected to ***general naphthol alkylation procedure*** in order to afford **23a**.

*3-(2-ethoxyquinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**30a**)*



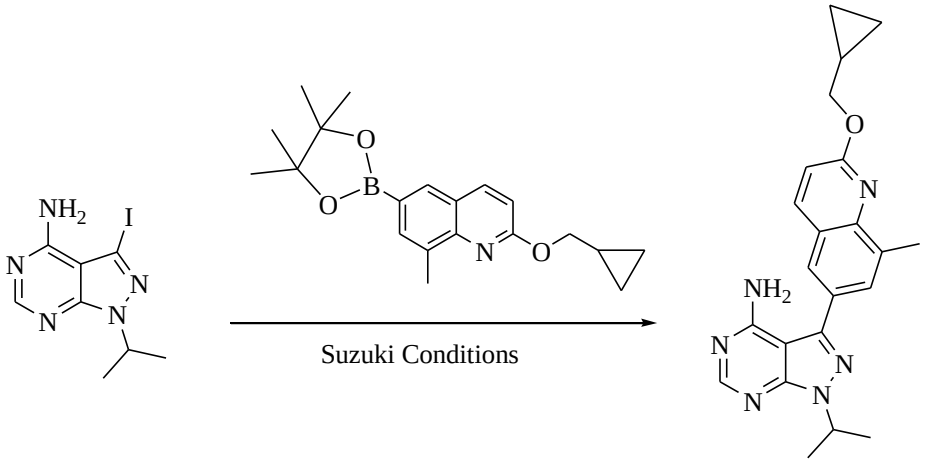
2-ethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinolinepinacol ester and 3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1**) were subjected to ***general Suzuki coupling procedure*** in order to afford **30a**.

*3-(2-ethoxy-8-methylquinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**31a**)*



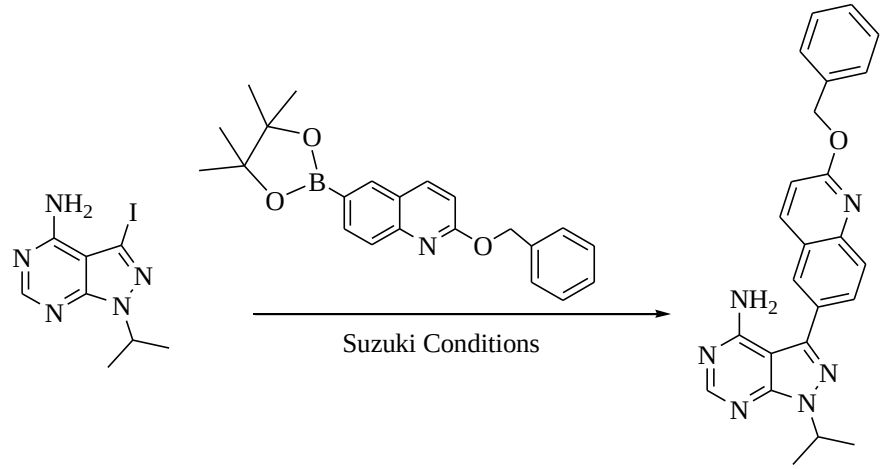
2-ethoxy-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and 3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1**) were used in the ***general Suzuki coupling procedure*** to afford **31a**.

*3-(2-(cyclopropylmethoxy)-8-methylquinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32a**)*



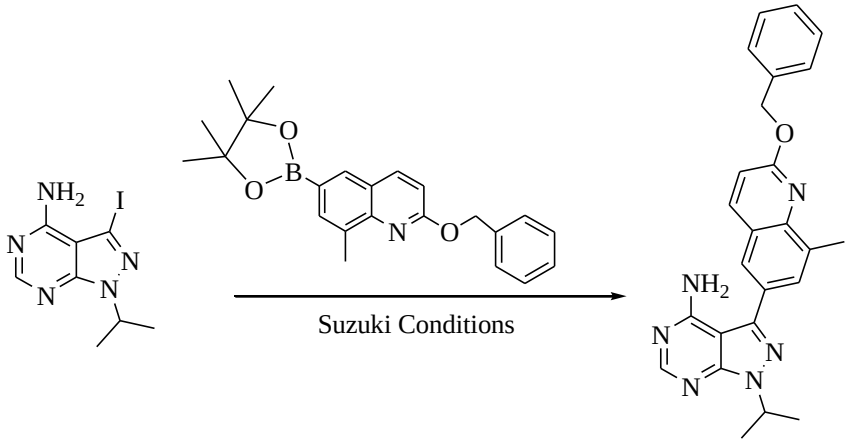
2-(cyclopropylmethoxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and 3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1**) were used in the ***general Suzuki coupling procedure*** to afford **32a**.

*3-(2-(benzyloxy)quinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**33a**)*



2-(benzyloxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and 3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1**) were used in the ***general Suzuki coupling procedure*** to afford 3**3a**.

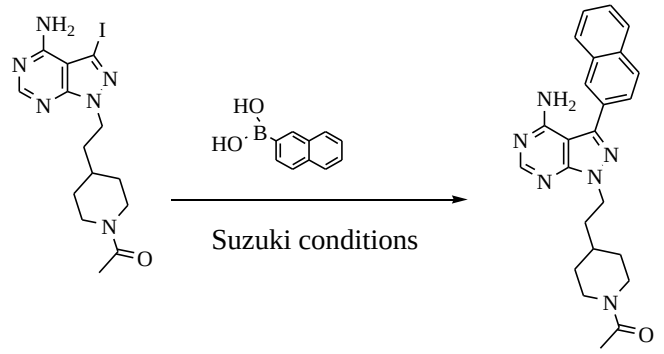
3-(2-(benzyloxy)-8-methylquinolin-6-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(34a)***



2-(benzyloxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and 3-iodo-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1**) were subjected to the ***general Suzuki coupling procedure*** to afford **34a**.

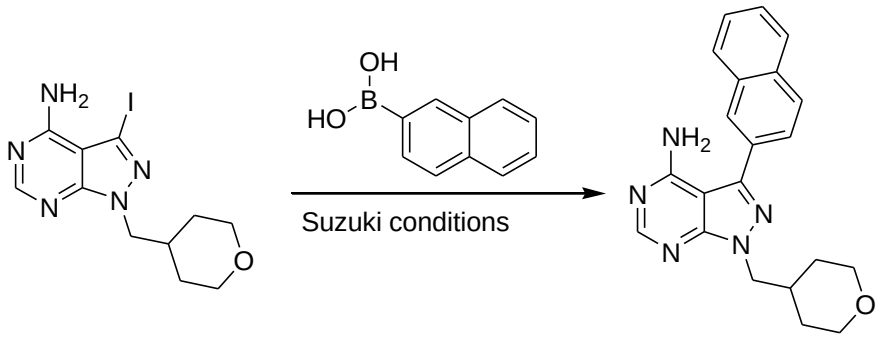
Table 2

1-(4-(2-(4-amino-3-(naphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone ***(15o)***



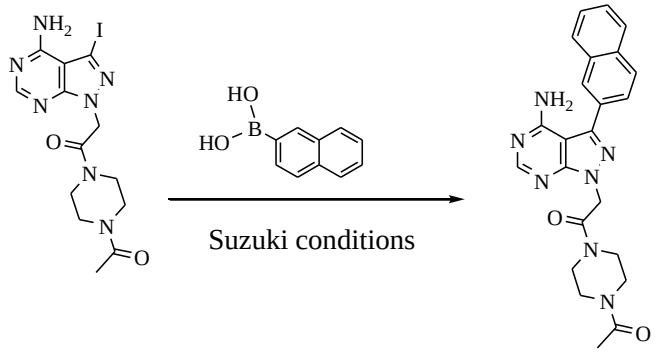
Naphthalen-2-ylboronic acid and 1-(4-(2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone (**49**) were subjected to the ***general Suzuki coupling procedure*** in order to afford **15o**.

*1-(naphthalen-2-yl)-3-((tetrahydro-2H-pyran-4-yl)methyl)imidazo[1,5-a]pyrazin-8-amine (**15p**)*



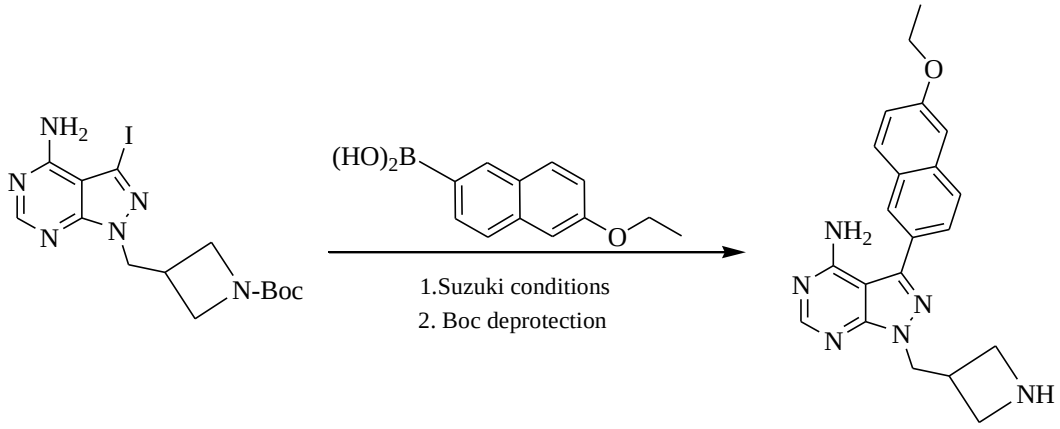
Naphthalen-2-ylboronic acid and 3-iodo-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**47**) were subjected to the ***general Suzuki coupling procedure*** in order to afford **15p**.

15t:



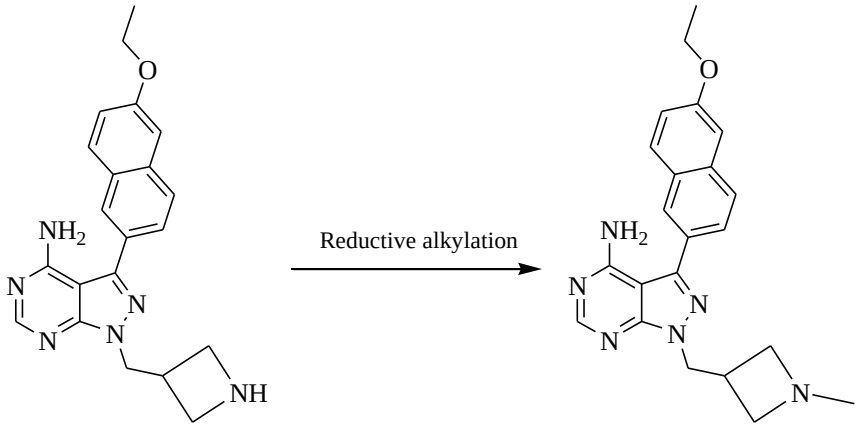
Naphthalen-2-ylboronic acid and 1-(4-(2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperazin-1-yl)ethanone (**50**) were subjected to ***general Suzuki coupling procedure*** in order to afford to **15t**.

1-(azetidin-3-ylmethyl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(17g)***



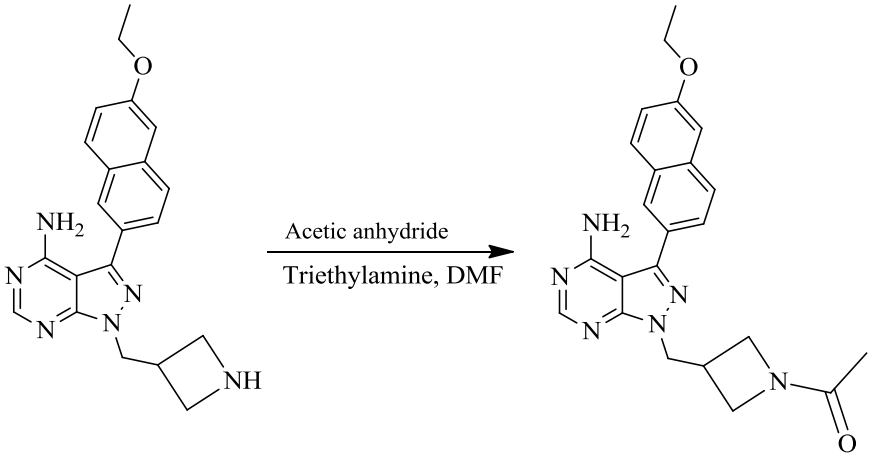
6-ethoxynaphthalen-2-ylboronic acid ester and *tert*-butyl 3-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidine-1-carboxylate (**48)** were subjected to the ***general Suzuki coupling procedure*** followed by the ***general boc-deprotection procedure*** in order to afford **17g**.

3-(6-ethoxynaphthalen-2-yl)-1-((1-methylazetidin-3-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(17h)***



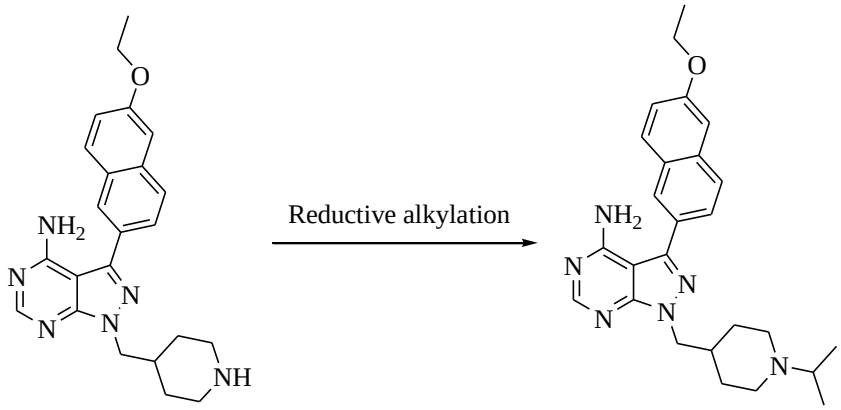
17g and formaldehyde were subjected to the ***general reductive amination procedure*** in order to afford **17h**.

*1-(3-((4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)azetidin-1-yl)ethanone (**17i**)*



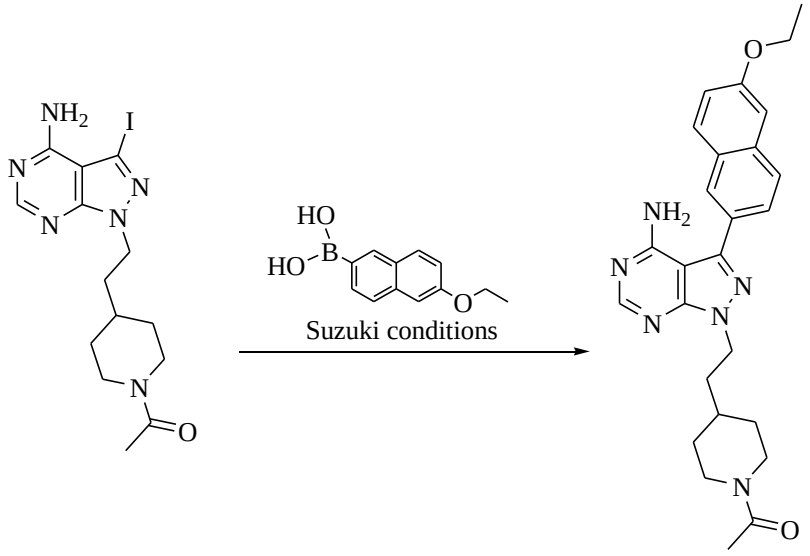
Triethylamine (5.9 mg, 0.058 mmol) was added to 1-(azetidin-3-ylmethyl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**17g**) (20 mg, 0.053 mmol) in DMF (5 mL). After 5min, acetic anhydride (6.80 mg, 0.058 mmol) was added. The reaction mixture was stirred for 5h. After completion, water (3ml) was added, and compound was extracted with ethyl acetate (3x). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. The crude reaction mixture was purified by flash chromatography using a CH_2Cl_2/MeOH gradient.

*3-(6-ethoxynaphthalen-2-yl)-1-((1-isopropylpiperidin-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**17l**)*



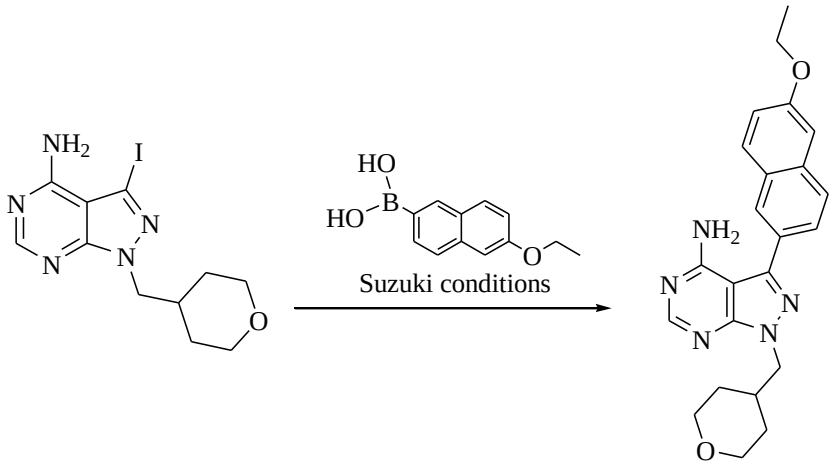
17j and acetone were subjected to the ***general reductive amination procedure*** in order to afford **17l**.

*1-(4-(2-(4-amino-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone (**17o**)*



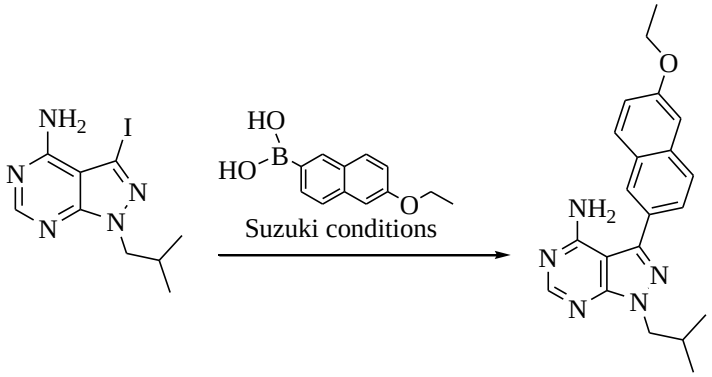
6-Ethoxynaphthalen-2-ylboronicacid and 1-(4-(2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone (**49**) were subjected to the ***general Suzuki coupling procedure*** to afford **17o**.

3-(6-ethoxynaphthalen-2-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(17p)***



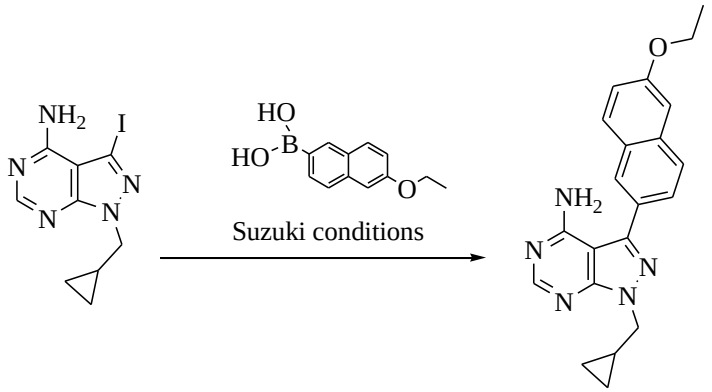
6-Ethoxynaphthalen-2-ylboronicacid and 3-iodo-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**47**) were subjected to the ***general Suzuki coupling procedure*** to afford **17p**.

3-(6-ethoxynaphthalen-2-yl)-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine ***(17q)***



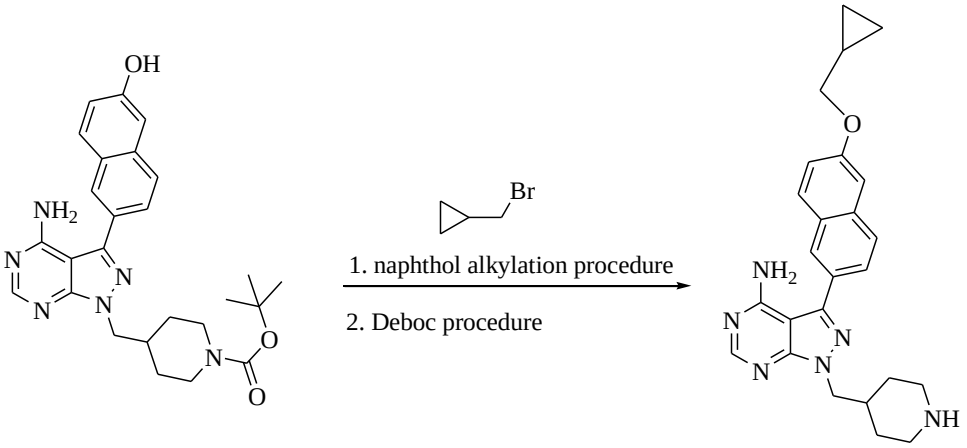
6-Ethoxynaphthalen-2-ylboronic acid and 3-iodo-1-isobutyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**45**) were subjected to the ***general Suzuki coupling procedure*** to afford **17q**.

1-(cyclopropylmethyl)-3-(6-ethoxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (***17r***)



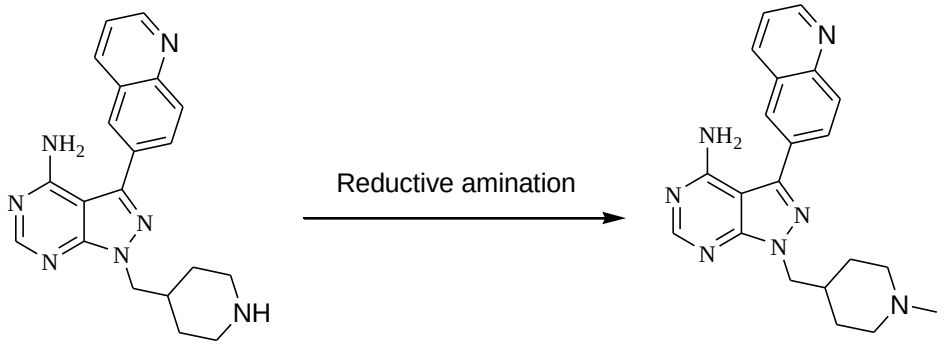
6-Ethoxynaphthalen-2-ylboronic acid and 1-(cyclopropylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**44**) were subjected to the ***general Suzuki coupling procedure*** to afford **17r**.

*3-(6-(cyclopropylmethoxy)naphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**22j**)*



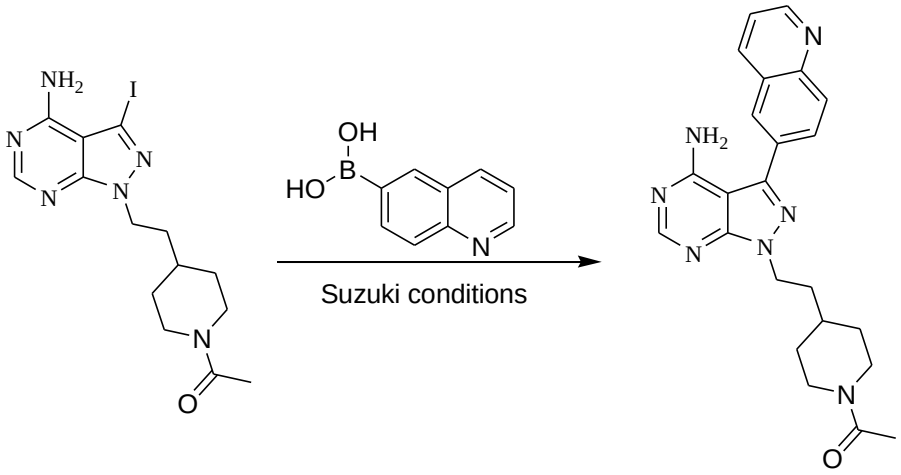
Tert-butyl4-((4-amino-3-(6-hydroxynaphthalen-2-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate [2] and bromomethylcylopropane were used in the ***general naphthol alkylation procedure,*** and then subjected to the ***general boc-deprotection procedure*** in order to afford **22j**.

*1-((1-methylpiperidin-4-yl)methyl)-3-(quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**29k**)*



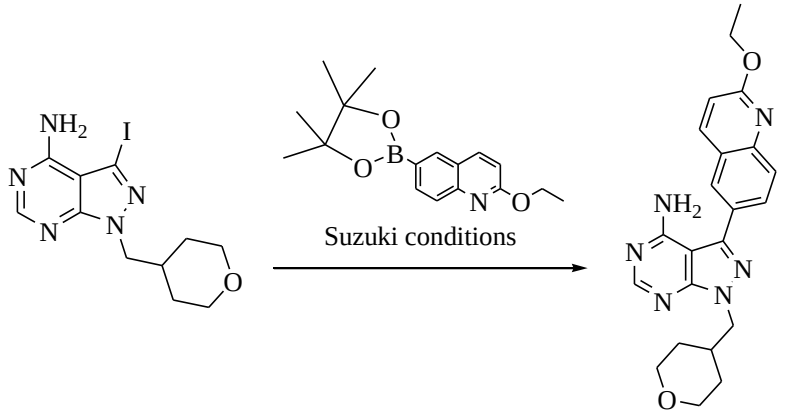
1-(piperidin-4-ylmethyl)-3-(quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**29j**) was subjected to the ***general reductive amination procedure*** in order to afford **29k**.

*1-(4-(2-(4-amino-3-(quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone (**29o**)*



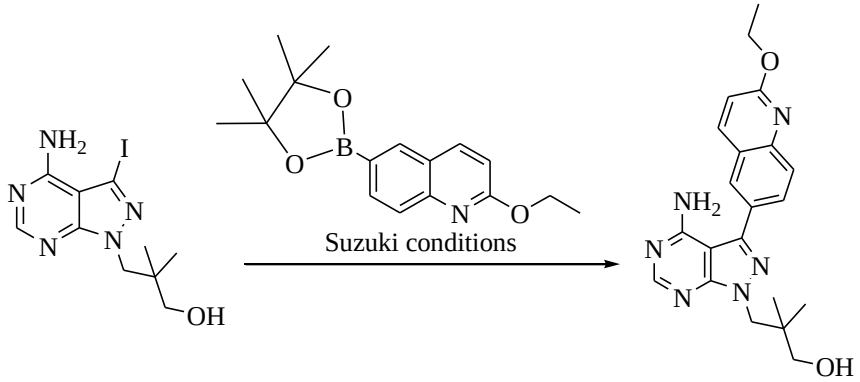
Quinolin-6-ylboronic acid and 1-(4-(2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)piperidin-1-yl)ethanone (**49**) were subjected to the ***general Suzuki coupling procedure*** to afford **29o**.

*3-(2-ethoxyquinolin-6-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**30p**)*



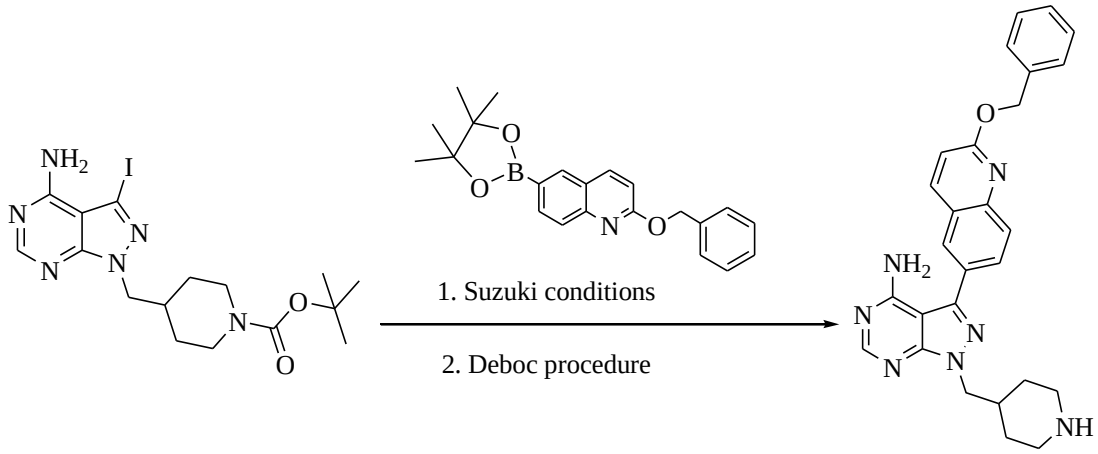
2-Ethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinolinepinacol ester and 3-iodo-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**47**) were subjected to the ***general Suzuki coupling procedure*** in order to afford **30p**.

*3-(4-amino-3-(2-ethoxyquinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol (**30u**)*



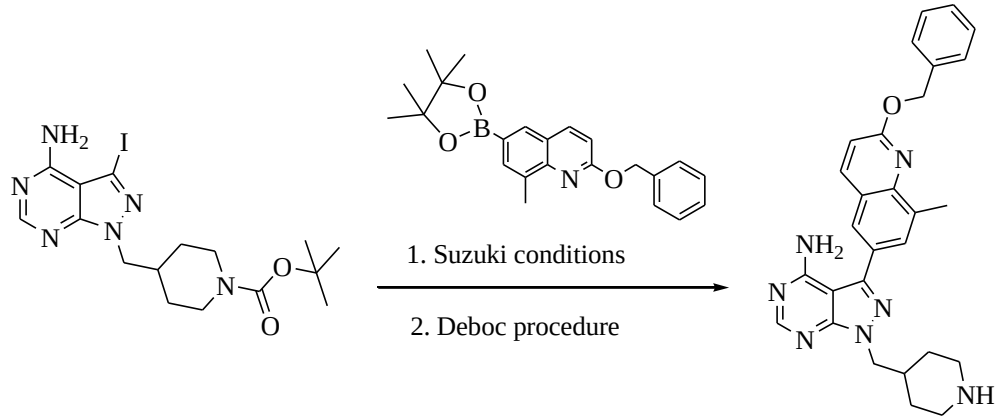
2-Ethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) quinolinepinacol ester and 3-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2,2-dimethylpropan-1-ol (**46**) were subjected to the ***general Suzuki coupling procedure*** in order to afford **30u**.

*3-(2-(benzyloxy)quinolin-6-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**33j**)*



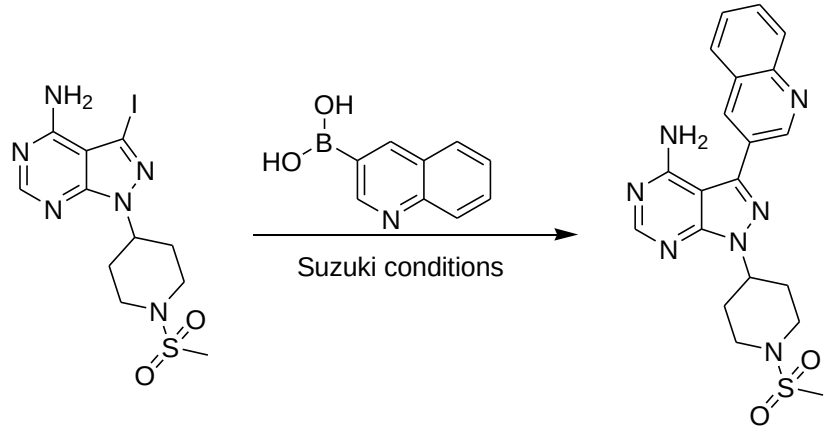
2-(benzyloxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and *tert*-butyl 4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate [2] were subjected to the ***general Suzuki coupling procedure*** followed by the ***general boc-deprotection procedure*** in order to afford **33j**.

*3-(2-(benzyloxy)-8-methylquinolin-6-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**34j**)*



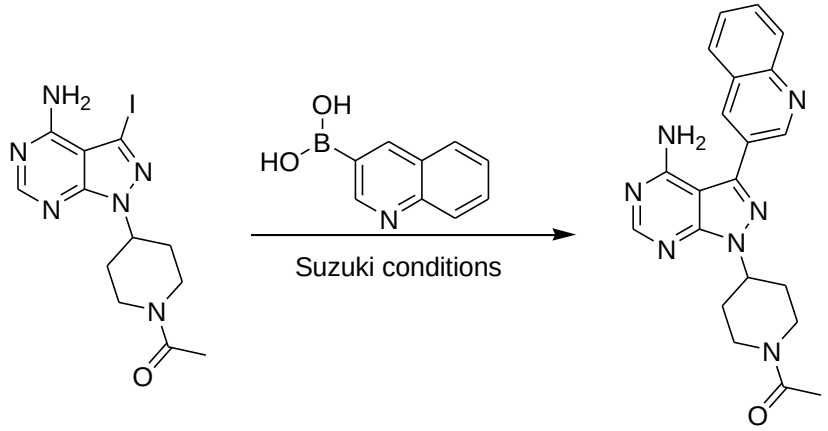
2-(benzyloxy)-8-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline and tert-butyl4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate [2] were subjected to the ***general Suzuki coupling procedure*** followed by the **g*eneral boc-deprotection procedure*** in order to afford **34j**.

*1-(1-(methylsulfonyl)piperidin-4-yl)-3-(quinolin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**35e**)*



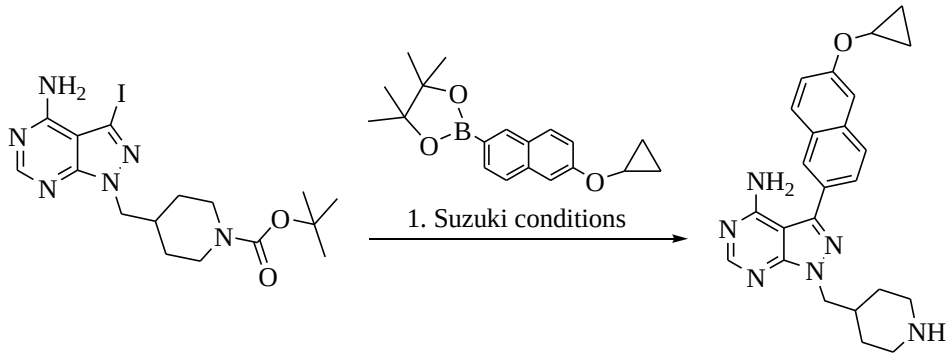
Quinolin-3-ylboronic acid and 3-iodo-1-(1-(methylsulfonyl)piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine as aryl halide (**51**) were subjected to the ***general Suzuki coupling procedure*** in order to afford **35e**.

*1-(4-(4-amino-3-(quinolin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethanone (**35f**)*



Quinolin-3-ylboronic acid and 1-(4-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethanone [2] were subjected to the ***general Suzuki coupling procedure*** in order to afford **35f**.

*3-(6-cyclopropoxynaphthalen-2-yl)-1-(piperidin-4-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**37j**)*

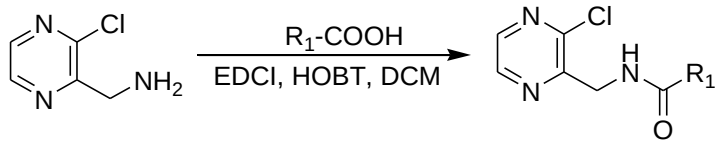


2-(6-cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and tert-butyl 4-((4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate as aryl halide, [2] were subjected to the ***general Suzuki coupling procedure*** followed by the **g*eneral boc-deprotection procedure*** in order to afford **37j**.

Imidazo Pyrazine scaffold

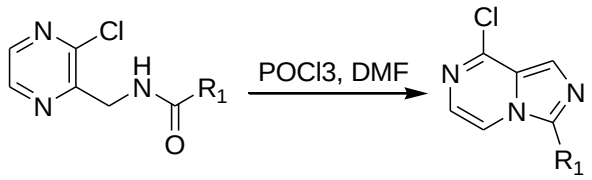
Synthetic procedure for various intermediates

General amide coupling reaction procedure:



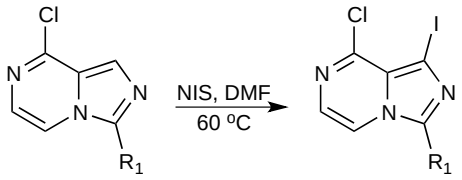
R_1.acid (1.0 eq), EDCI (1.5 eq), HOBt (1.0 eq) were taken in dry DCM, stirred at 0 ^oC for 10 min, followed by addition of (3-chloropyrazin-2-yl)methanamine (1 eq.), the reaction was stirred at room temperature for 3-6 h. Upon completion (monitored by thin layer chromatography), the reaction was extracted into DCM and washed with water, $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc gradient.

***General cyclizaiton reaction*:**



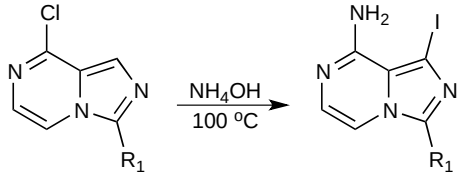
To the R1 substituted N-(3-chloropyrazin-2-yl)acetamide (1.0 eq) in acetonitrile, $POCl_3$ (5.0 eq), and a catalytic amount of DMF (0.01 eq) were added. The reaction mix was stirred at 55 ^oC for 3-6 h until completion (monitored by TLC (Thin layer chromatography)). The reaction mixture was concentrated *in vacuo* and triethylamine in methanol (1M, 5ml) was added at 0 ^oC. The reaction was extracted into ethyl acetate (3x10ml) and washed with brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc gradient.

General Iodonation procedure:



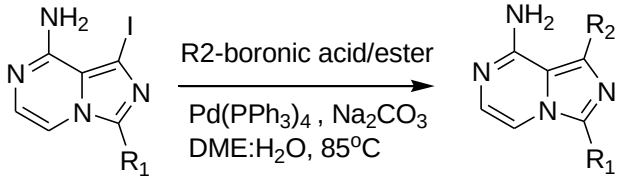
N-iodosuccinimide was added to an R_1-substituted 8-chloroimidazo[1,5-a]pyrazine in DMF. The reaction mixture was heated for 5-8 h until completion (monitored by TLC (Thin layer chromatography). The reaction was then diluted with EtOAc, washed with water, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified via flash chromatography over silica, eluting with a hexanes/EtOAc gradient.

General aminolysis:



Ammonium hydroxide in isopropanol (3 M in methanol) was added to an R_1-substituted 8-chloroimidazo[1,5-a]pyrazine. The reaction mixture was then heated to 100 ^oC for 1-3 h in a microwave. The crude reaction mixture was then concentrated *in vacuo* and taken to the next step without purification.

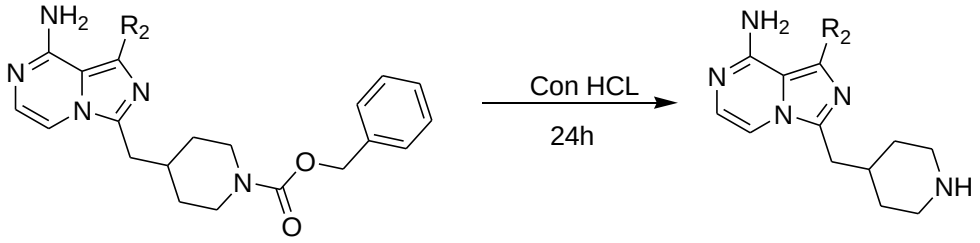
General Suzuki coupling reaction:



The respective iodo scaffold (1 eq.), Na_2CO_3 (2-4 eq.), $Pd(PPh_3)_4$ (0.05 eq.), and appropriate boronic acid or pinacol ester (1-2 eq.) were stirred in dimethoxyethane (DME, 1.5 ml) and water (0.5 ml) and heated in a microwave at 85 °C for 1 h. The reaction was then cooled

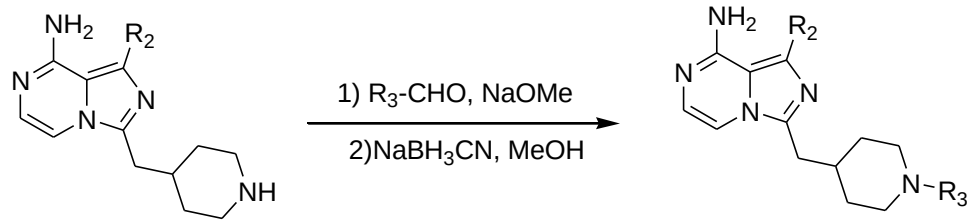
to room temperature, extracted into EtOAc, washed with brine, and the organic layer dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified via flash chromatography over silica, eluting with either a hexanes/EtOAc or CH_2Cl_2/MeOH gradient. Further purification, if necessary, was performed via preparatory RP-HPLC, eluting with either a H_2O/CH_3CN or H_2O/MeOH gradient (+0.05% TFA).

General CBZ-deprotection procedure:



The cbz-amine is stirred in conconcentrated HCl for ~24 h. The reaction was then dissolved in methanol, filtered, and subjected to preparatory RP-HPLC (eluting with either a H_2O/CH_3CN or H_2O/ MeOH gradient (+0.05% TFA)). After purification, the product was re-concentrated from 1.25 M HCl in EtOH to afford the final, purified product as a bis-HCl salt.

General reductive alkylation procedure:

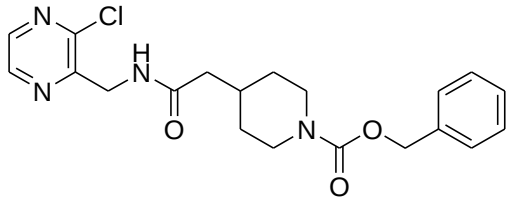


The TFA-amine salt (1 eq.) was dissolved in methanol and neutralized with sodium methoxide. A solution containing 2% acetic acid was added with the appropriate aldehyde (5-10 eq.) and stirred at room temperature for 10 min. Sodium cyanoborohydride (5 eq.) was then added and the reaction was left to stir until completion (typically ~2 h). The reaction was then filtered and purified via preparatory RP-HPLC, eluting with either a H_2O/CH_3CN or H_2O/MeOH gradient (+0.05% TFA). Product fractions were collected and concentrated. The residue was

dissolved in a small amount of 2 M HCl in ethanol and, after concentration *in vacuo*, the final product was obtained as an HCl salt.

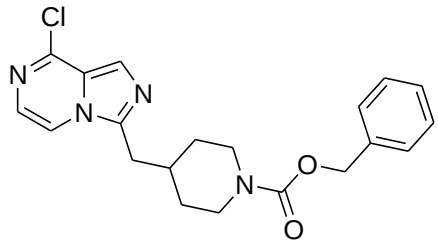
Synthesis and spectral data of various Intermediates

benzyl 4-(2-((3-chloropyrazin-2-yl)methylamino)-2-oxoethyl)piperidine-1-carboxylate



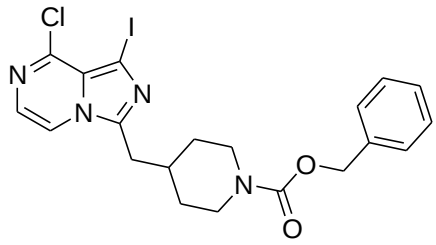
(3-chloropyrazin-2-yl)methanamine and 2-(1-(benzyloxycarbonyl)piperidin-4-yl)acetic acid were subjected to ***general amide coupling reaction procedure.*** ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.45 (s, 1H), 8.30 (s, 1H), 7.47-7.22 (m, 5H), 5.11 (s, 2H), 4.69 (d, *J*=4.5 Hz, 2H), 4.18 (br s, 2H), 2.81 (br s, 2H), 2.25 (d, *J*=7.0 Hz, 2H), 2.05 (m, 1H), 1.76 (m, 2H), 1.22 (m, 2H); MS (ESI): 403.2 *m/z* [MH+], $C_{20}H_{23}ClN_4O_3$ requires 403.2.

benzyl 4-((8-chloroimidazo[1,5-a]pyrazin-3-yl)methyl)piperidine-1-carboxylate



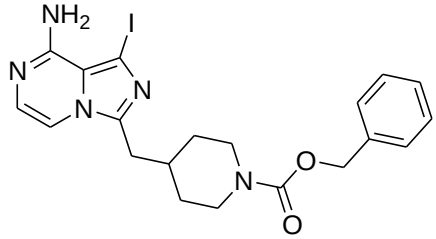
Benzyl 4-(2-((3-chloropyrazin-2-yl)methylamino)-2-oxoethyl)piperidine-1-carboxylate was subjected to the ***general cyclization procedure.*** ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.82 (s, 1H), 7.58 (d, *J*=4.9 Hz, 1H), 7.44-7.28 (m, 6H), 5.12 (s, 2H), 4.33-4.08 (br s, 2H), 2.93 (d, *J*=7.0 Hz, 2H), 2.88-2.66 (br s, 2H), 2.16 (m, 1H), 1.80-1.62 (d, *J*=11.8 Hz, 2H), 1.40-1.19 (m, 2H); MS (ESI): 385.3 *m/z* [MH^+], $C_{20}H_{21}ClN_4O_2$ requires 385.2.

benzyl 4-((8-chloro-1-iodoimidazo[1,5-a]pyrazin-3-yl)methyl)piperidine-1-carboxylate



Benzyl 4-((8-chloroimidazo[1,5-a]pyrazin-3-yl)methyl)piperidine-1-carboxylate was subjected to the ***general iodonation procedure.*** ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.61 (d, *J*=4.9 Hz, 1H), 7.43-7.26 (m, 5H), 7.08 (d, *J*=4.9 Hz, 1H), 5.12 (s, 2H), 4.29-4.12 (br s, 2H), 2.98-2.80 (m, 4H), 2.14 (m, 1H), 1.69 (d, *J*=11.1 Hz, 2H), 1.36-1.17 (m, 2H); MS (ESI): 511.1 *m/z* [MH^+], $C_{20}H_{20}ClIN_4O_2$ requires 511.2

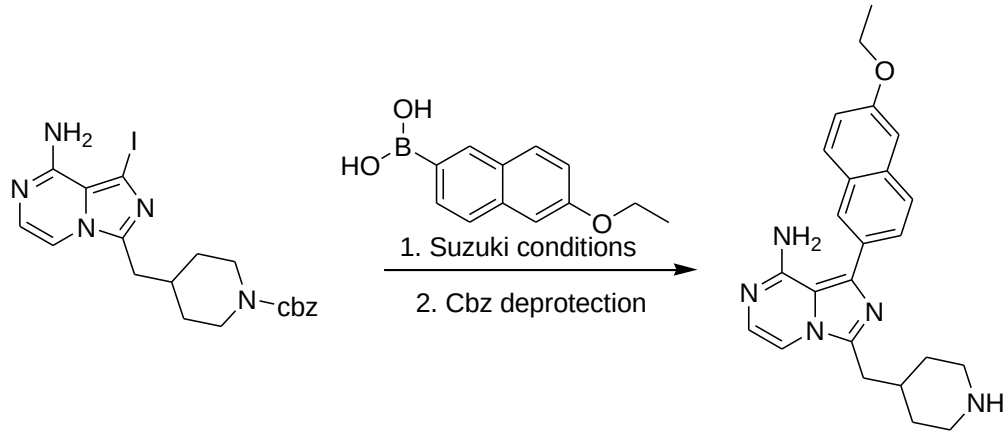
benzyl4-((8-amino-1-iodoimidazo[1,5-a]pyrazin-3-yl)methyl)piperidine-1-carboxylate



Benzyl 4-((8-chloro-1-iodoimidazo[1,5-a]pyrazin-3-yl)methyl)piperidine-1-carboxylate was subjected to ***general aminolysis procedure.*** ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.38-7.28 (m, 5H), 7.17 (d, *J*=4.9 Hz, 1H), 6.97 (d, *J*=4.9 Hz, 1H), 5.12 (s, 2H), 4.27-4.10 (br s, 2H), 2.81 (d, *J*=7.0 Hz, 2H), 2.79-2.56 (m, 2H), 2.07 (m, 1H), 1.68 (d, *J*=11.1 Hz, 2H), 1.35-1.16 (m, 2H); MS (ESI): 492.0 *m/z* [MH^+], $C_{20}H_{22}IN_5O_2$ requires 492.0.

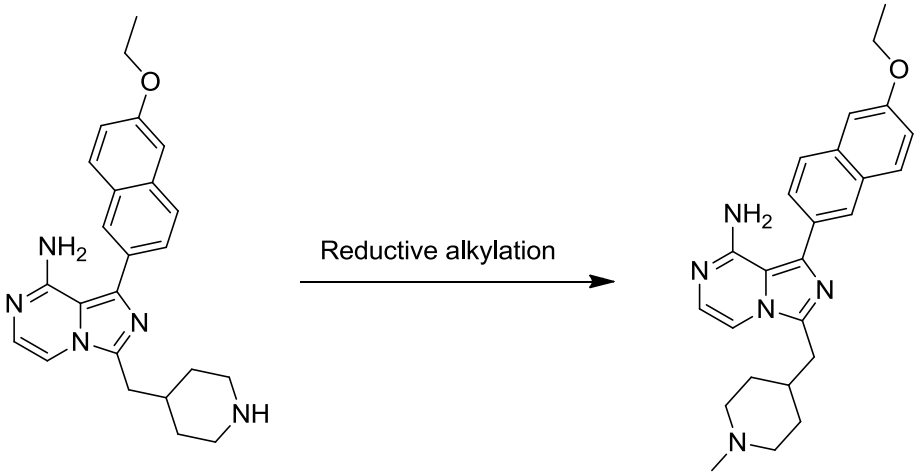
Synthesis and spectra data of final compounds (Table 3)

*1-(6-ethoxynaphthalen-2-yl)-3-(piperidin-4-ylmethyl)imidazo[1,5-a]pyrazin-8-amine **(43j)***



6-Ethoxynaphthalen-2-ylboronic acid and benzyl4-((8-amino-1-iodoimidazo[1,5-a]pyrazin-3-yl)methyl)piperidine-1-carboxylate were subjected to ***general Suzuki coupling procedure*** followed by ***general cbz-deprotection procedure*** in order to afford **43j**.

*1-(6-ethoxynaphthalen-2-yl)-3-((1-methylpiperidin-4-yl)methyl)imidazo[1,5-a]pyrazin-8-amine **(43k)***



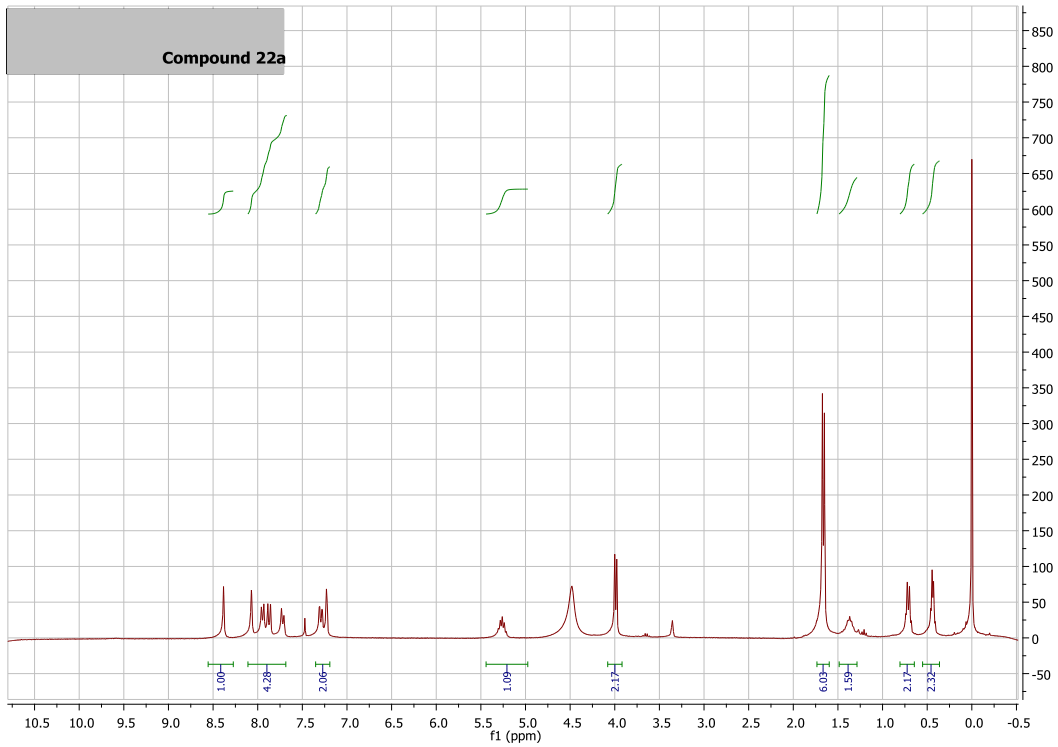
40j and formaldehyde were subjected to the ***general reductive alkylation procedure*** in order to afford **43k**.

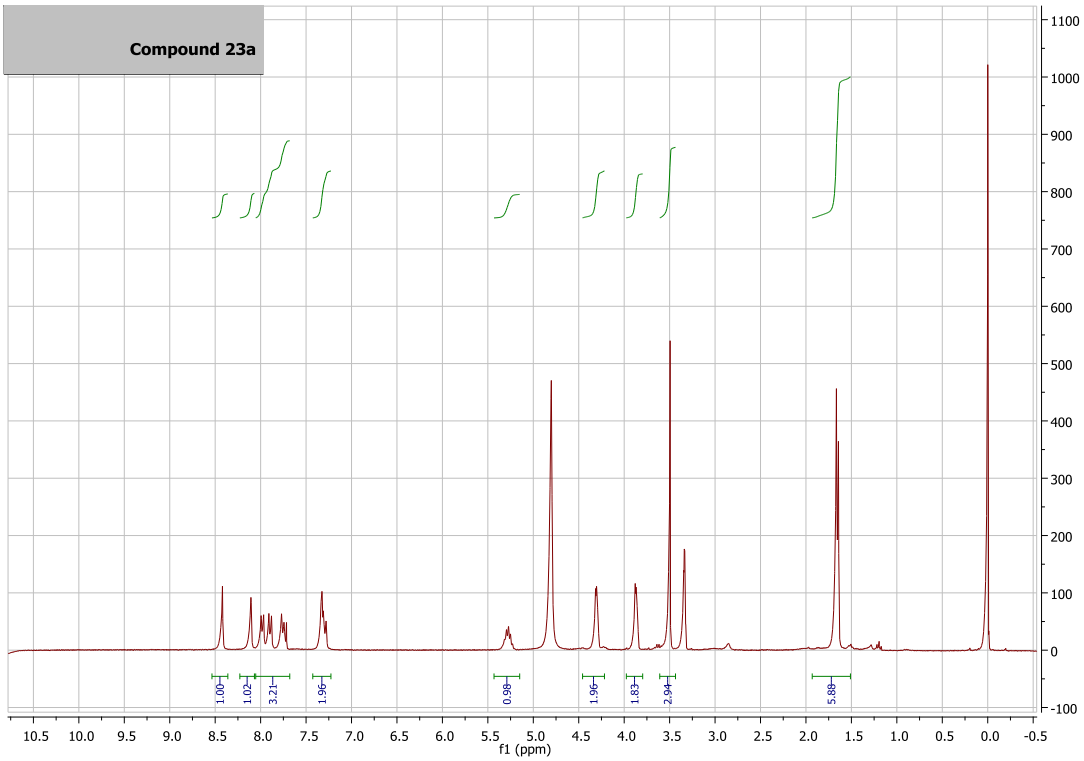
Supporting Information References:

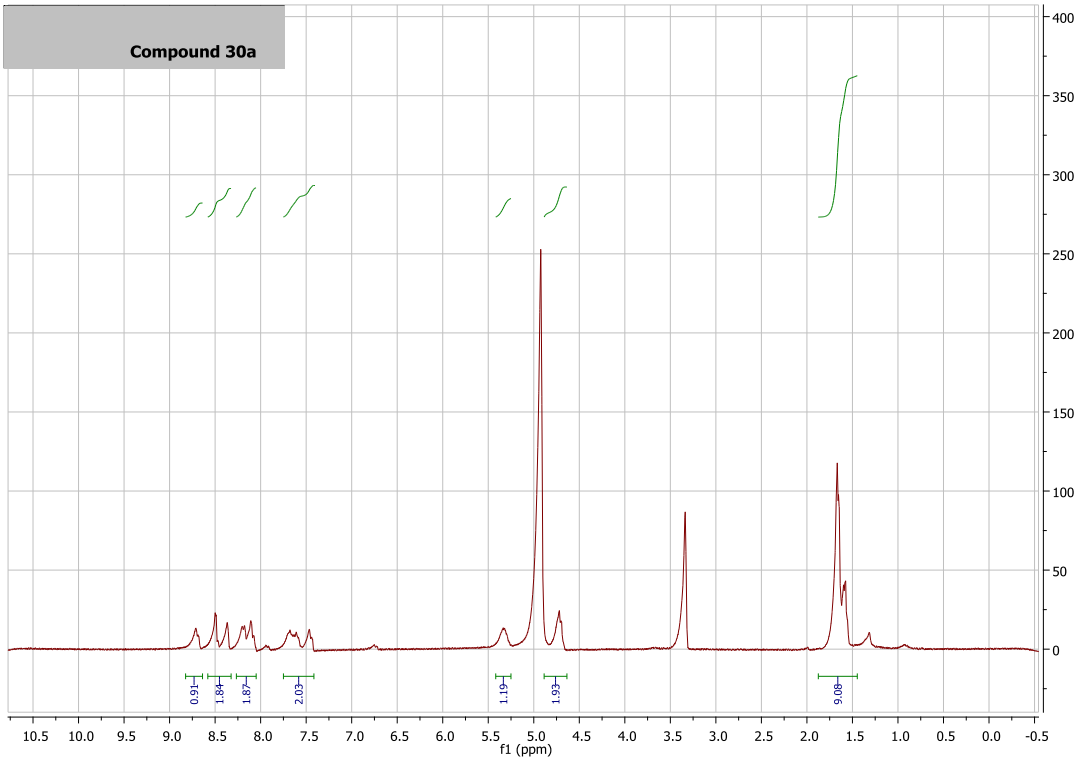
1. R.C. Murphy, K.K. Ojo, E.T. Larson, A. Castellanos-Gonzalez, B.G. Perera, K.R. Keyloun, J.E. Kim, J.G. Bhandari, N.R. Muller, C.L. Verlinde, A.C. White, Jr., E.A. Merritt, W.C. Van Voorhis, D.J. Maly, Discovery of Potent and Selective Inhibitors of Calcium-Dependent Protein Kinase 1 (CDPK1) from C. parvum and T. gondii, ACS Med. Chem. Lett., 1 (2010) 331-335.
2. S.M. Johnson, R.C. Murphy, J.A. Geiger, A.E. DeRocher, Z.S. Zhang, K.K. Ojo, E.T. Larson, B.G.K. Perera, E.J. Dale, P.Q. He, M.C. Reid, A.M.W. Fox, N.R. Mueller, E.A. Merritt, E.K. Fan, M. Parsons, W.C. Van Voorhis, D.J. Maly, Development of Toxoplasma gondii Calcium-Dependent Protein Kinase 1 (TgCDPK1) Inhibitors with Potent Anti-Toxoplasma Activity, J. Med. Chem., 55 (2012) 2416-2426.
3. W.C. Van Voorhis, G. J. H. Wilhelmus, E. T. Larson, D.J. Maly, E.A. Merritt, K.K. Ojo. U.S. Pat. Appl. Publ. (2013), US 20130018040 A1 20130117.
4. E. Baston, A. Palusczak, R. W. Hartmann, E. J. Med. Chem., 35 (2000) 931-940.
5. Pyranochromene derivatives as γ-secretase inhibitors and their preparation and use for the treatment of neurodegenerative diseases, W. Wen-Lian et al, PCT Int. Appl. (2012) 2012138678

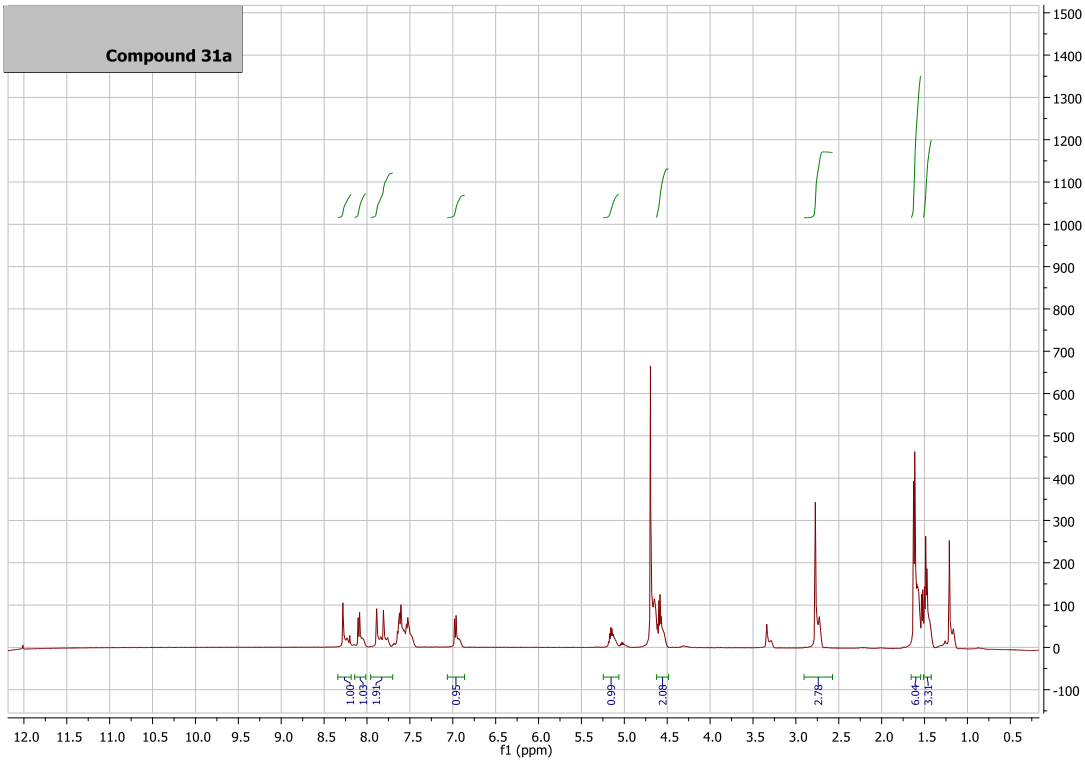
^{1}H-NMR spectra of New compounds

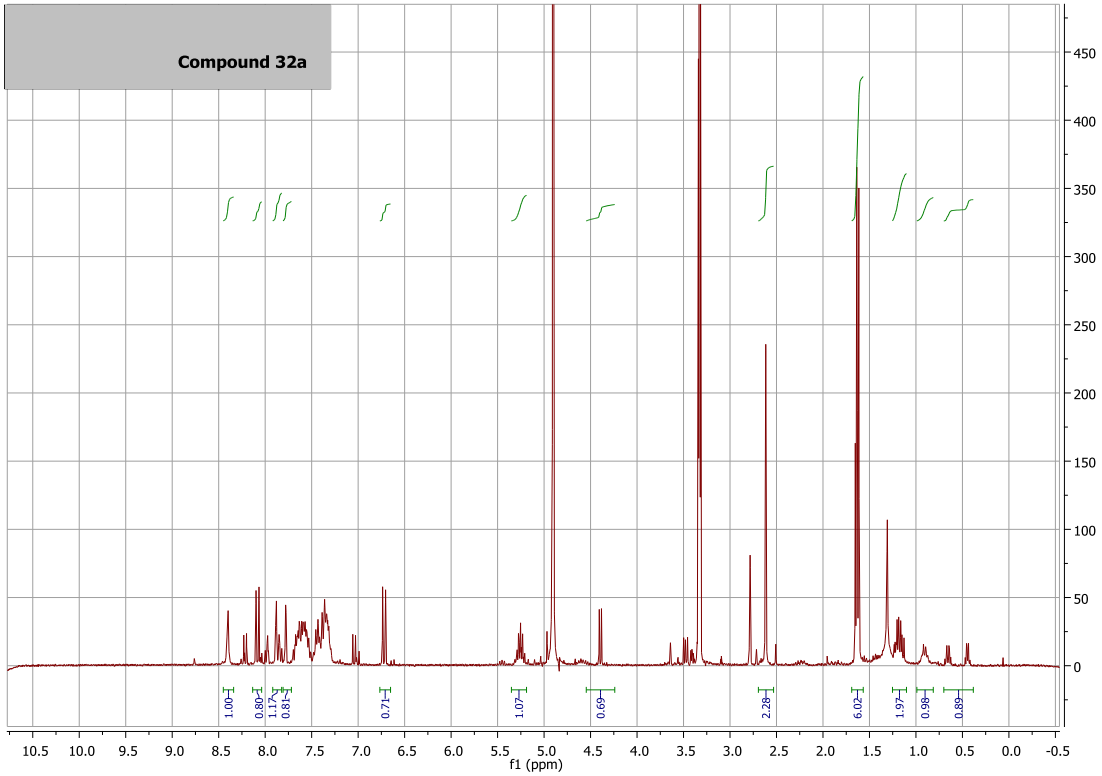
Table 1:

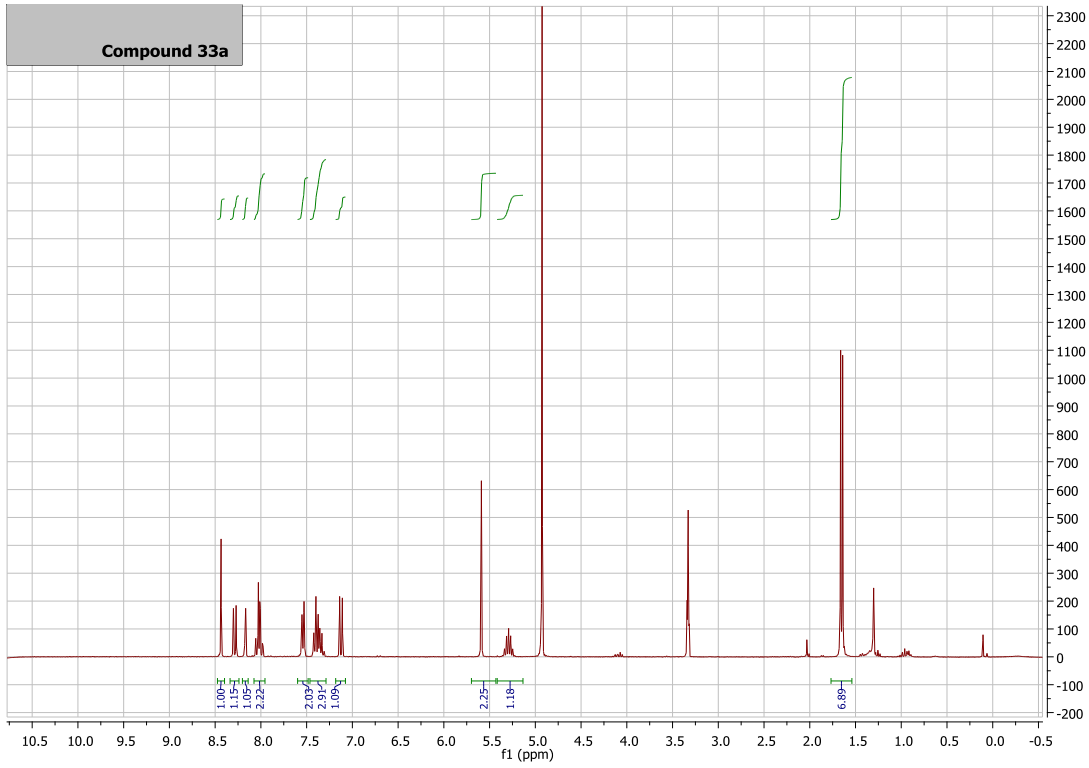





Compound 30a
0.91
1.84
1.87
2.03
1.19
1.93
9.08
f1 (ppm)


Compound 31a
1.00
1.03
1.91
0.95
0.99
2.08
2.78
6.04
3.33
f1 (ppm)


Compound 32a
f1 (ppm)


Compound 33a
f1 (ppm)

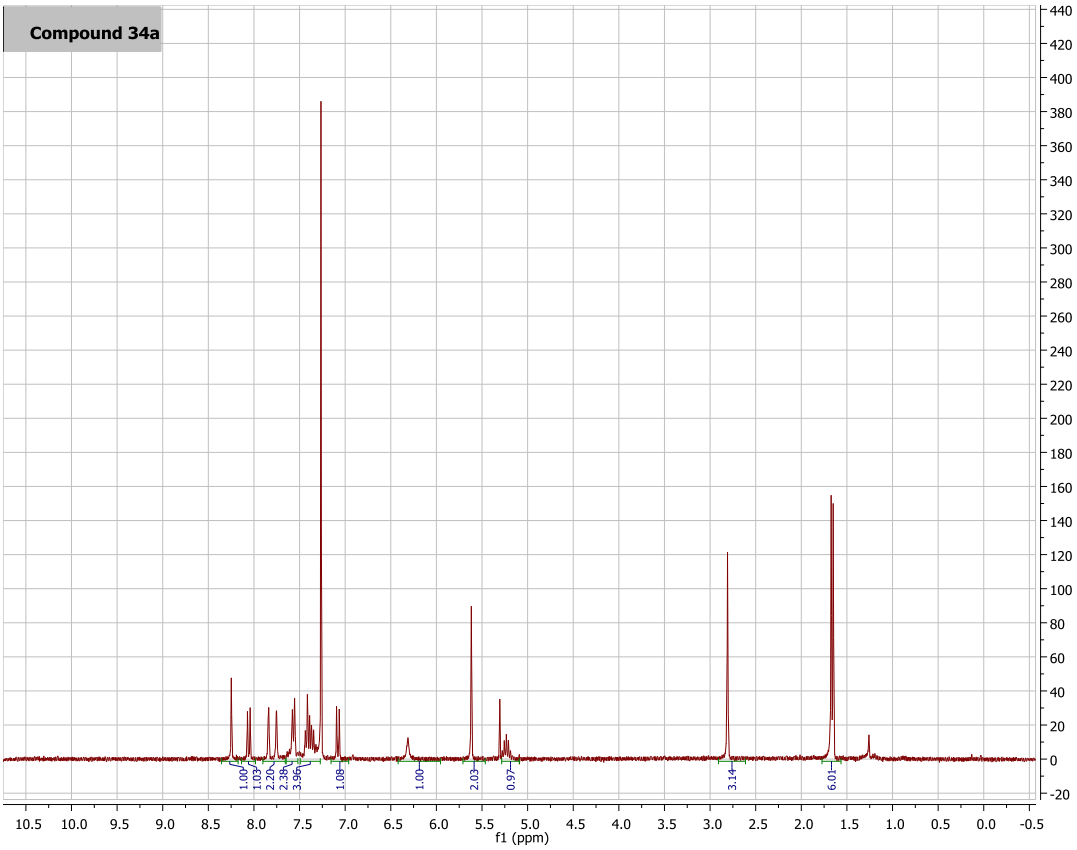
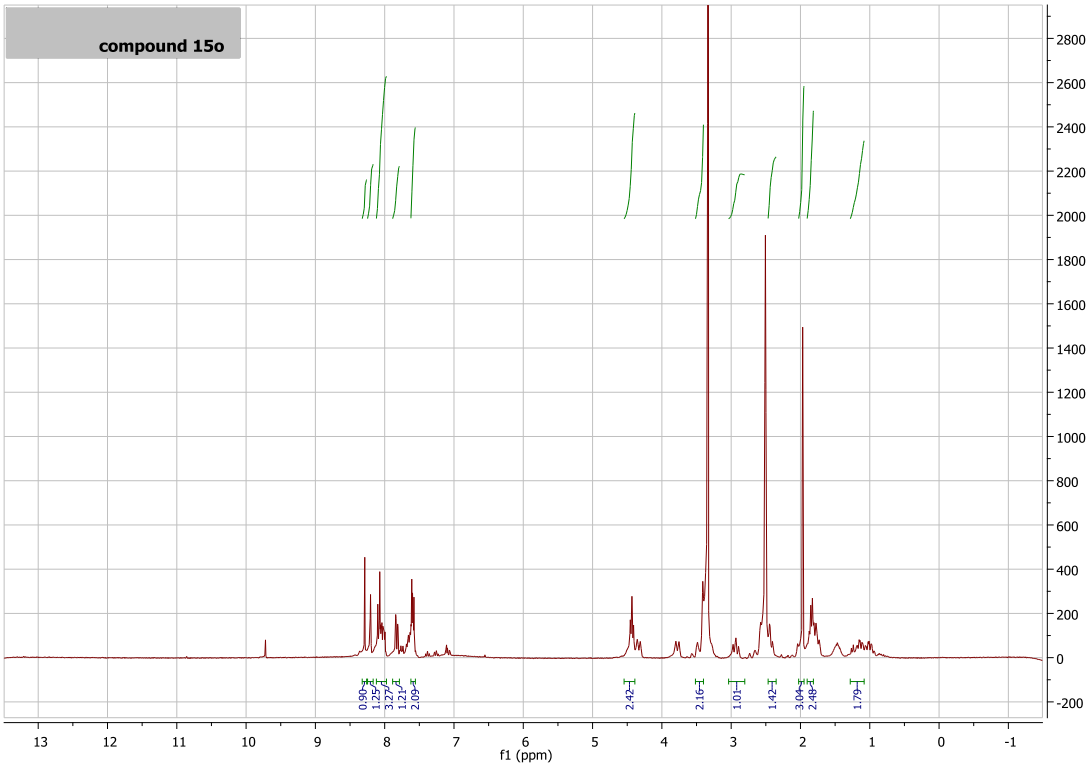
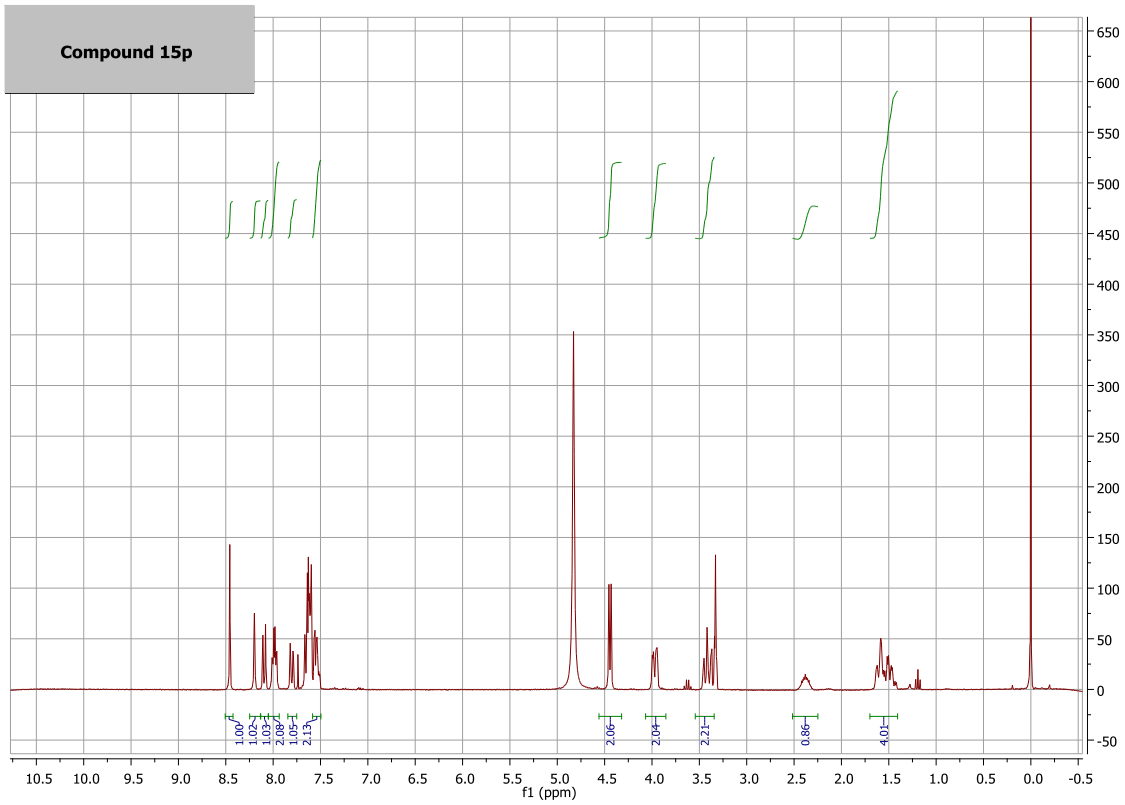
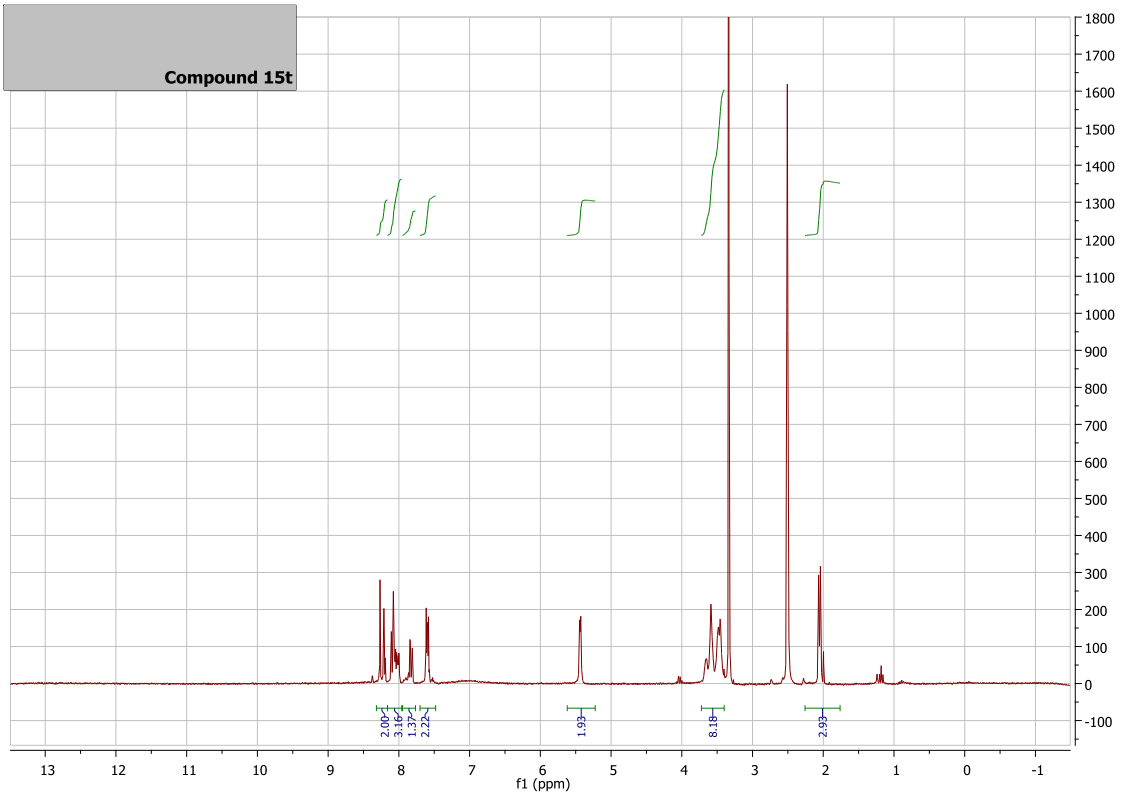
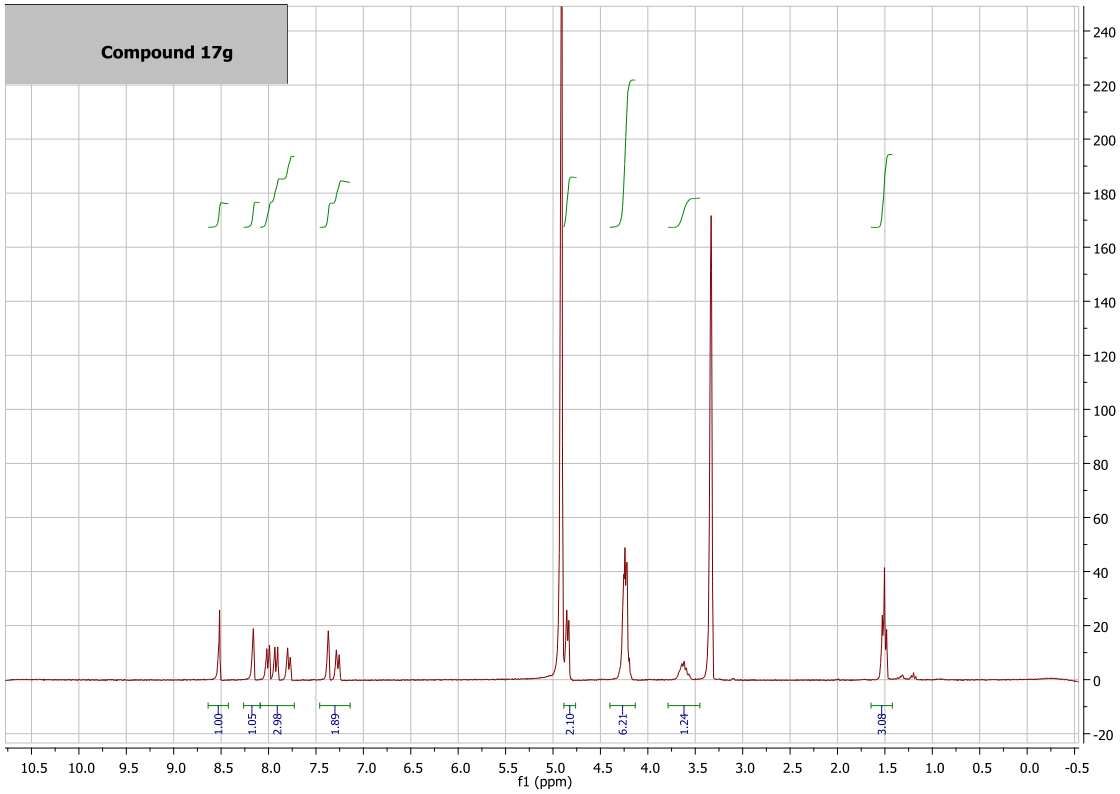


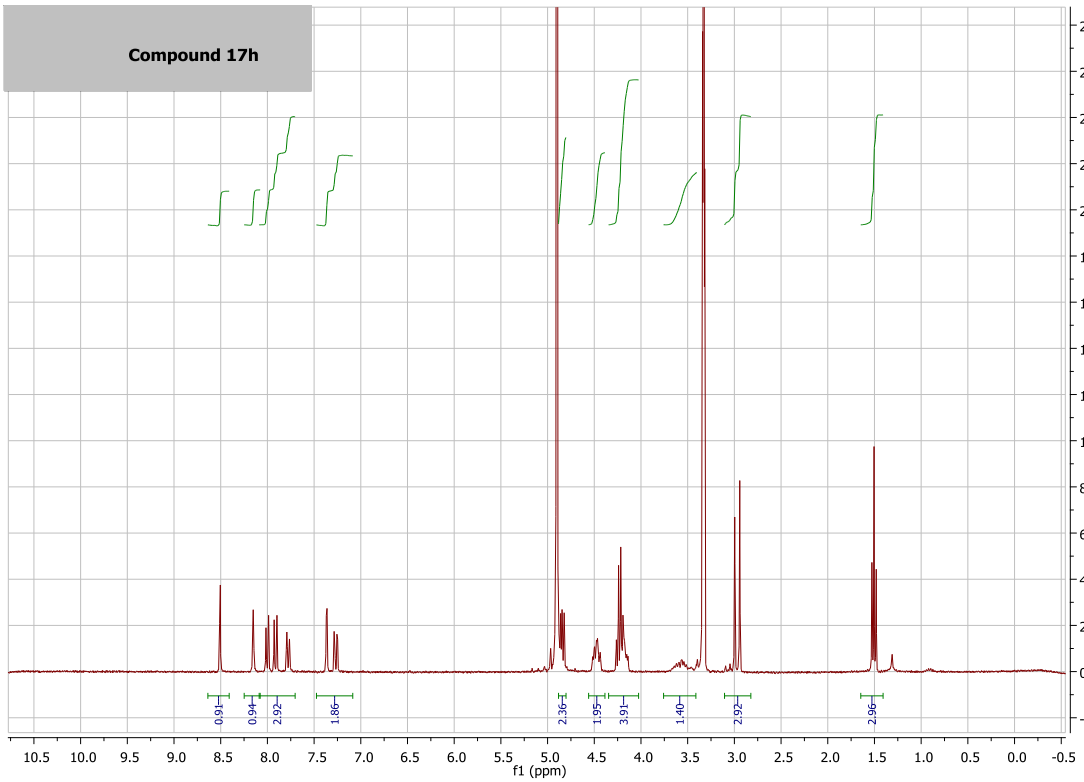
Table 2

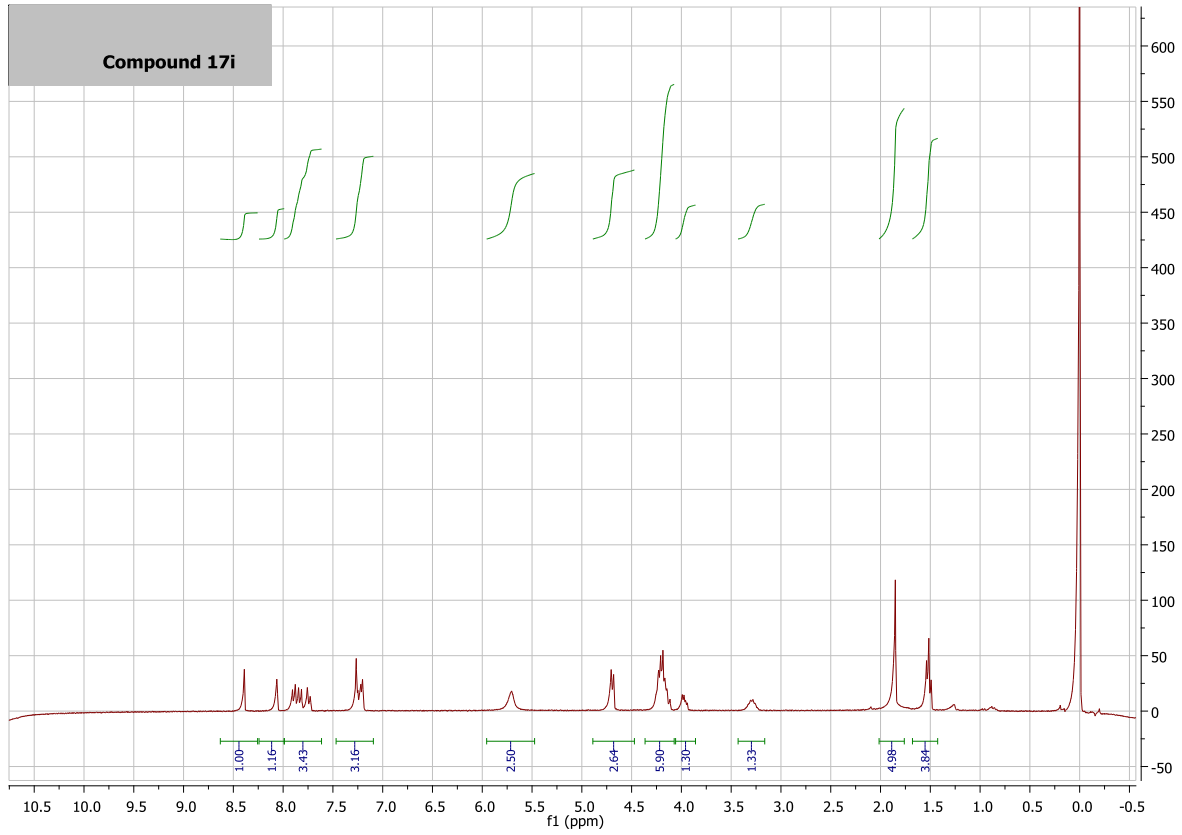


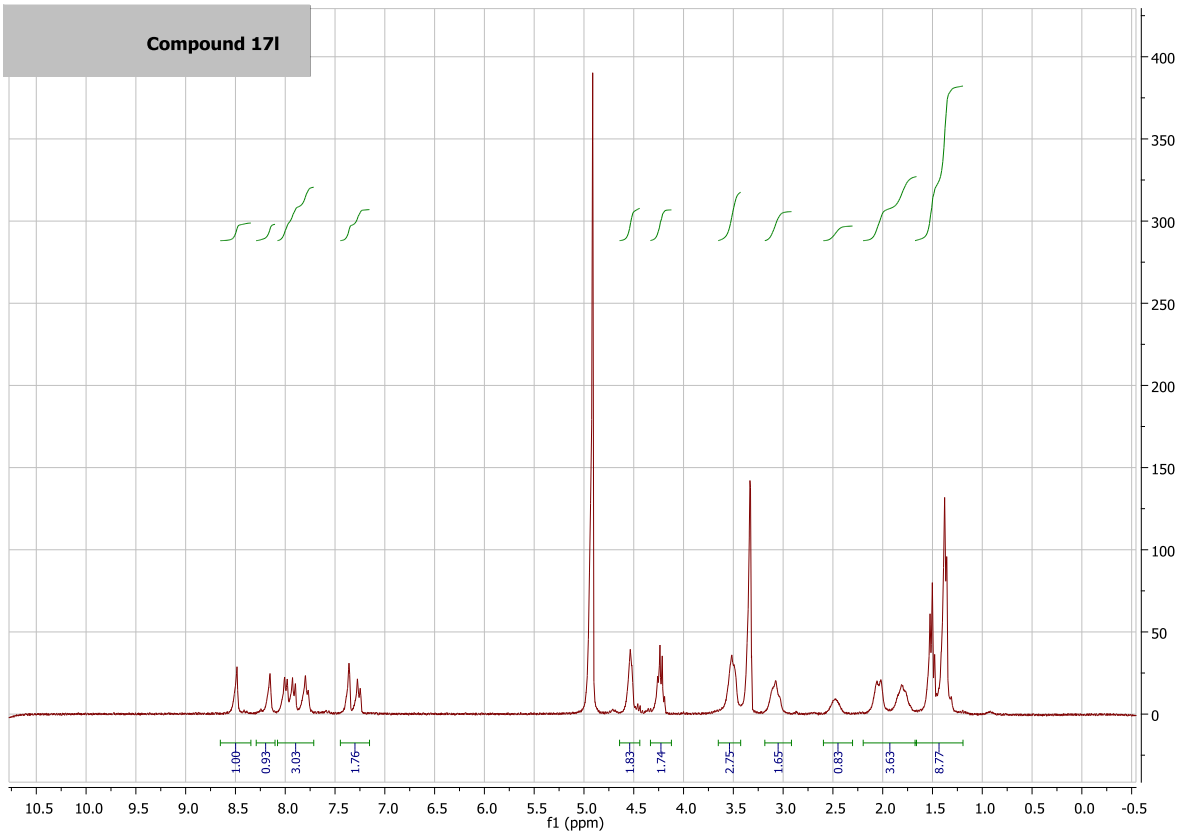

Compound 15p
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
1.00
1.02
1.03
2.08
1.05
2.13
2.06
2.04
2.21
0.86
4.01


Compound 15t
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)
2.00
3.16
1.37
2.22
1.99
8.18
2.93

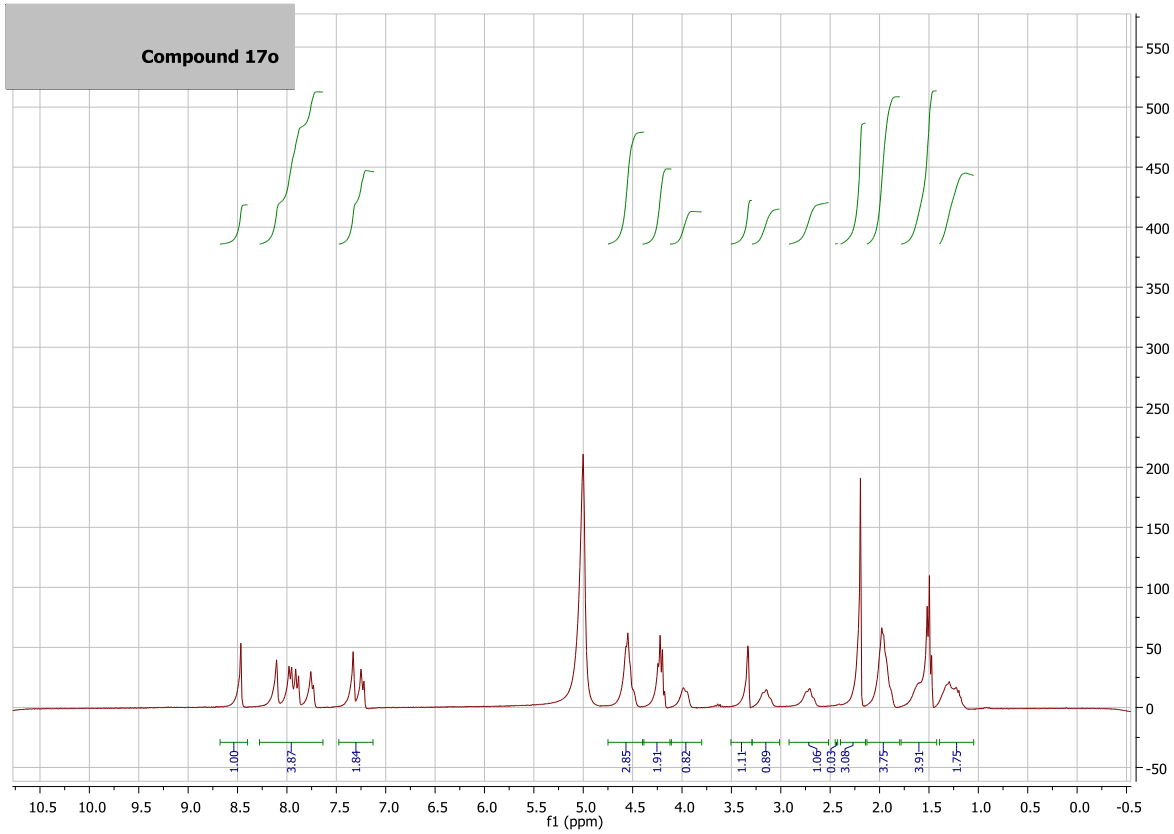

Compound 17g
1.00
1.05
2.98
1.89
2.10
6.21
1.24
3.08
f1 (ppm)


Compound 17h
0.91
0.94
2.92
1.86
2.36
1.95
3.91
1.40
2.92
2.96
f1 (ppm)

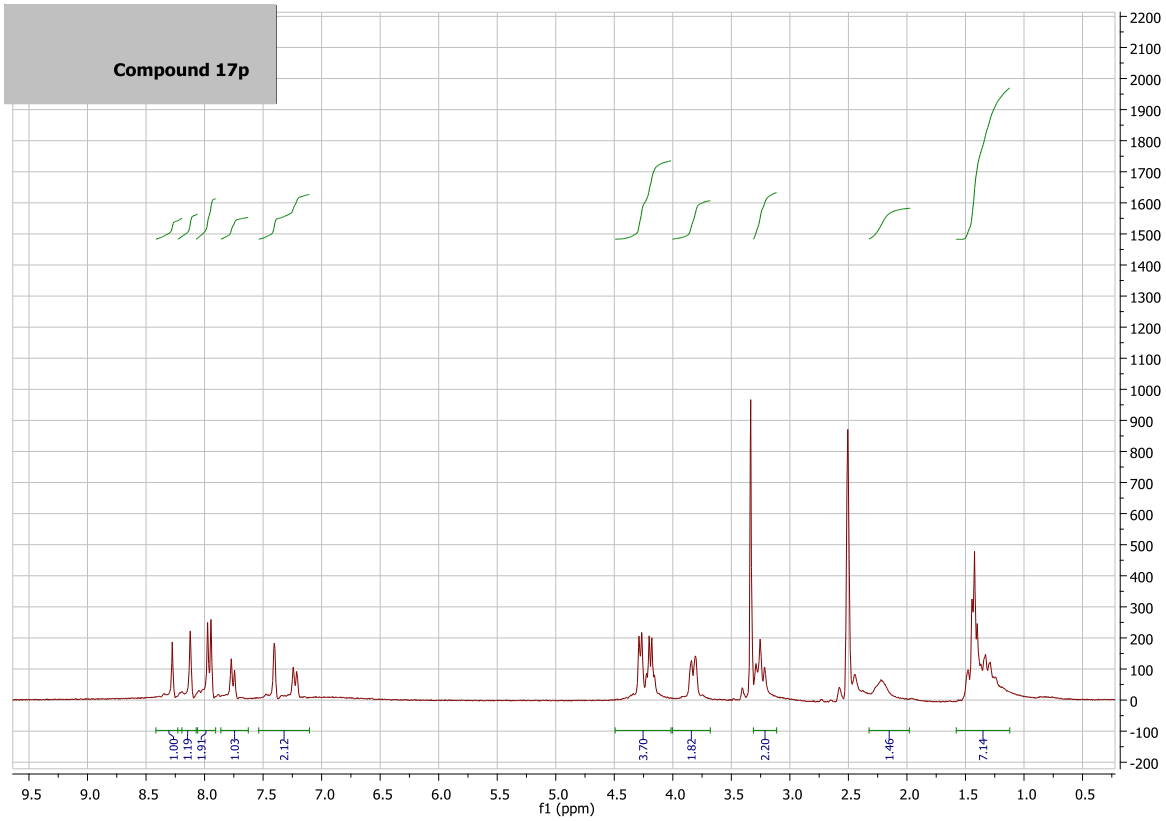

Compound 17i
1.00
1.16
3.43
3.16
2.50
2.64
5.90
1.30
1.33
4.98
3.84
f1 (ppm)


Compound 17l
1.00
0.93
3.03
1.76
1.83
1.74
2.75
1.65
0.83
3.63
8.77
f1 (ppm)

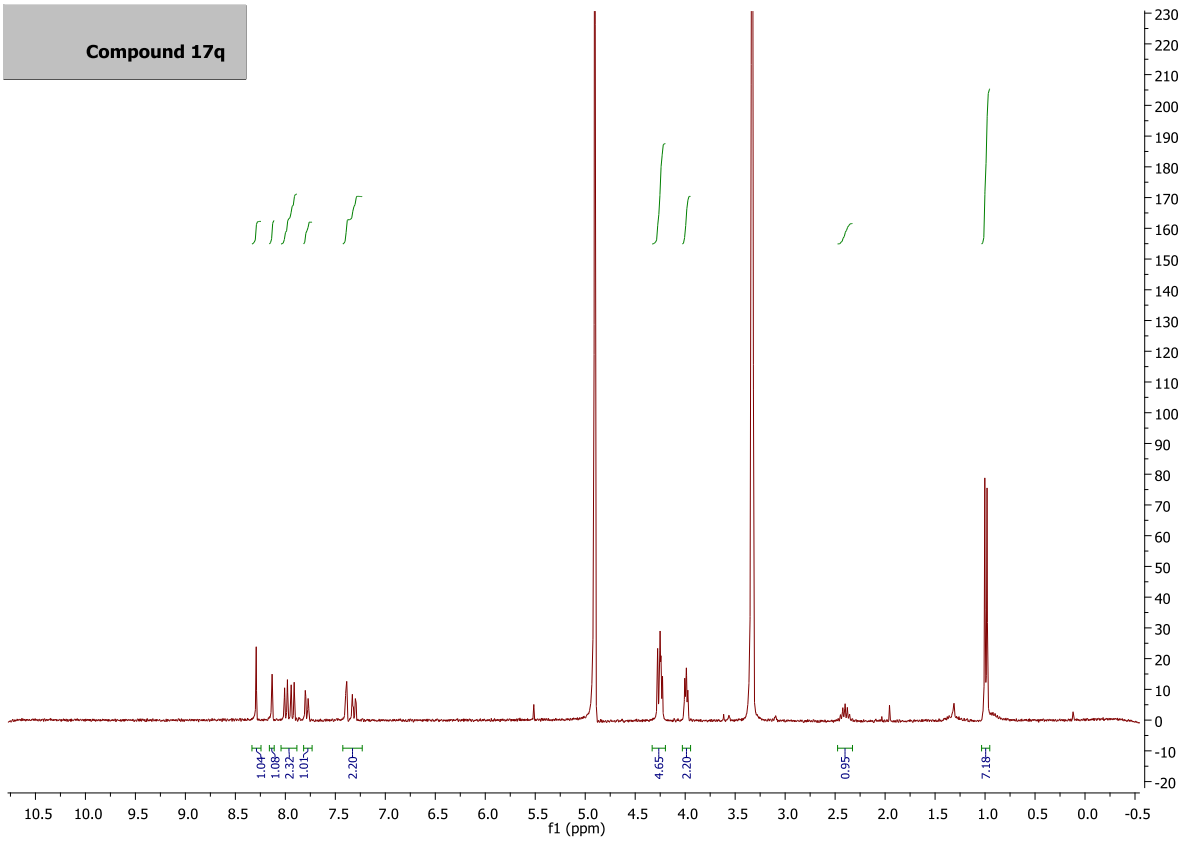
Compound 17o



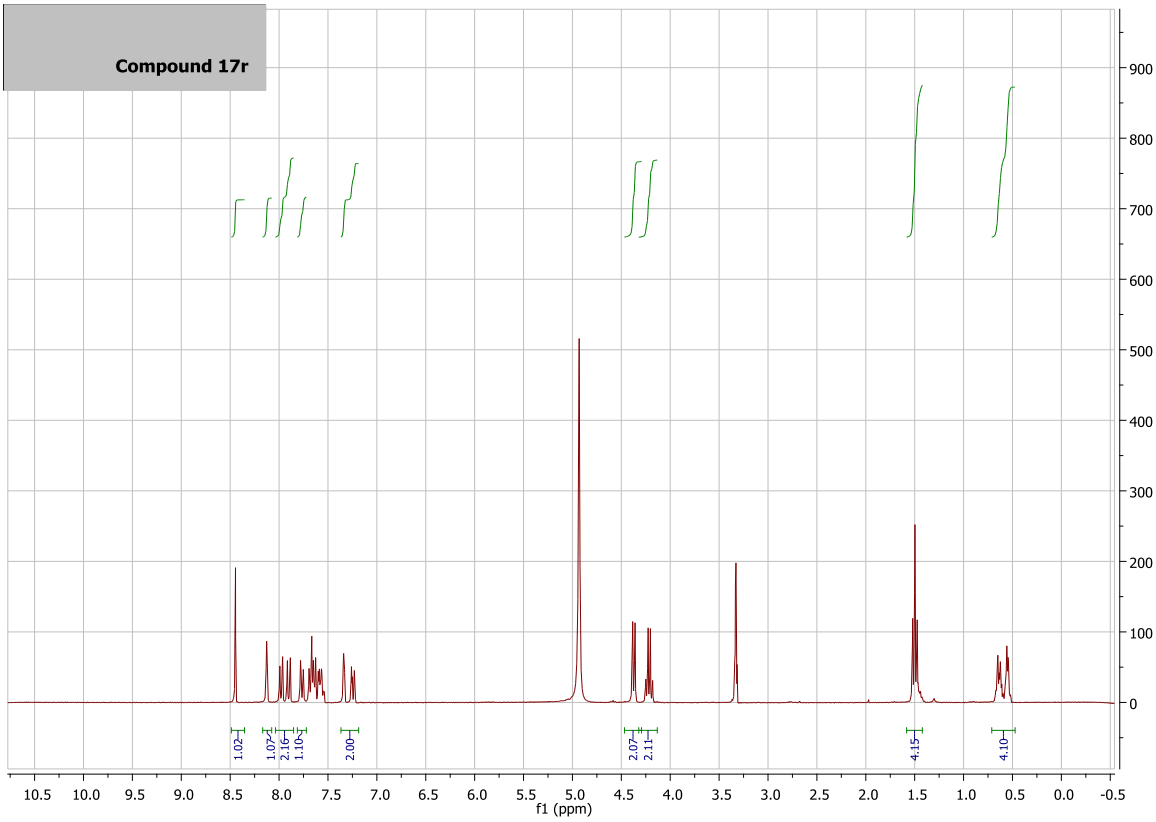
Compound 17p

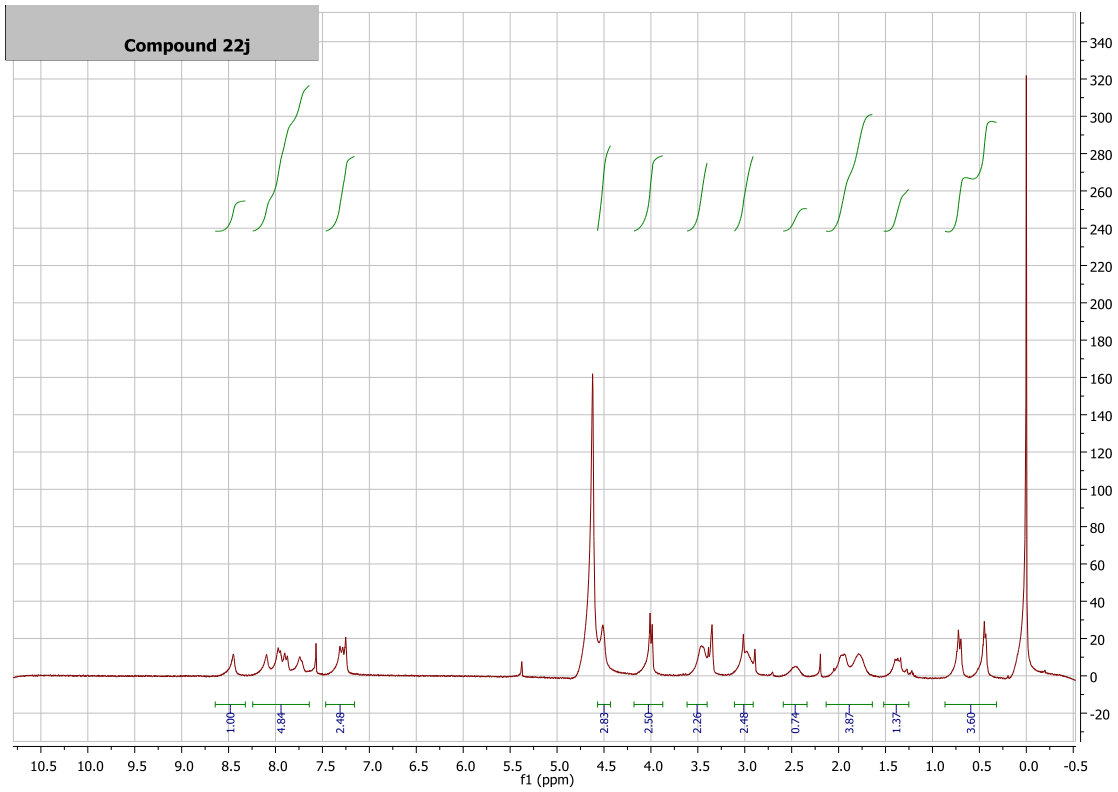


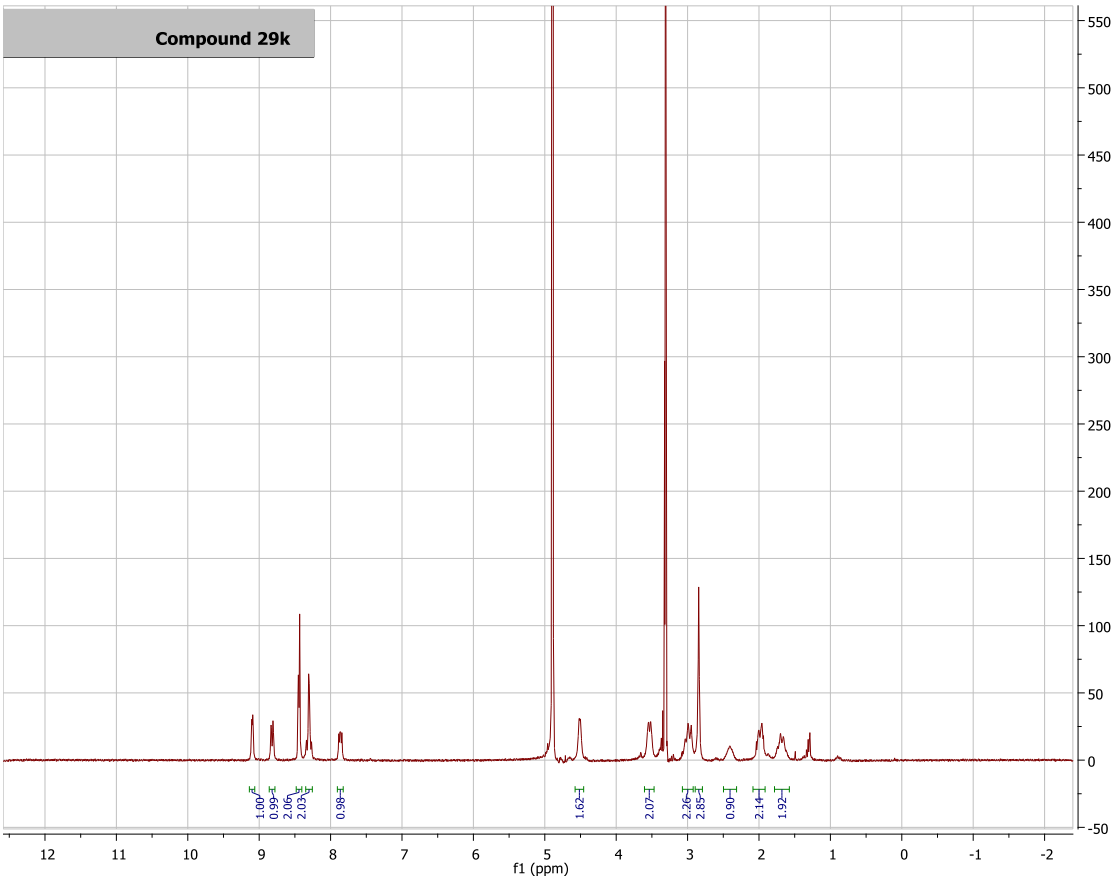
Compound 17q

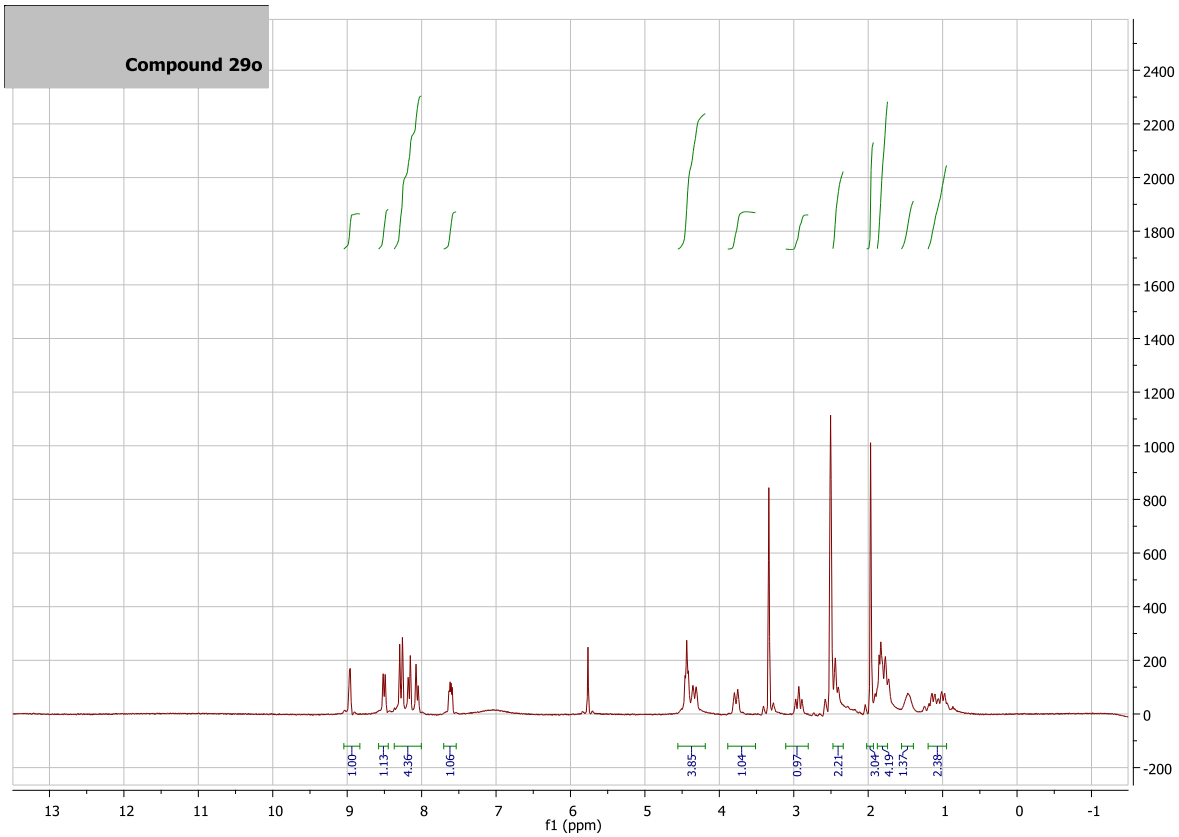


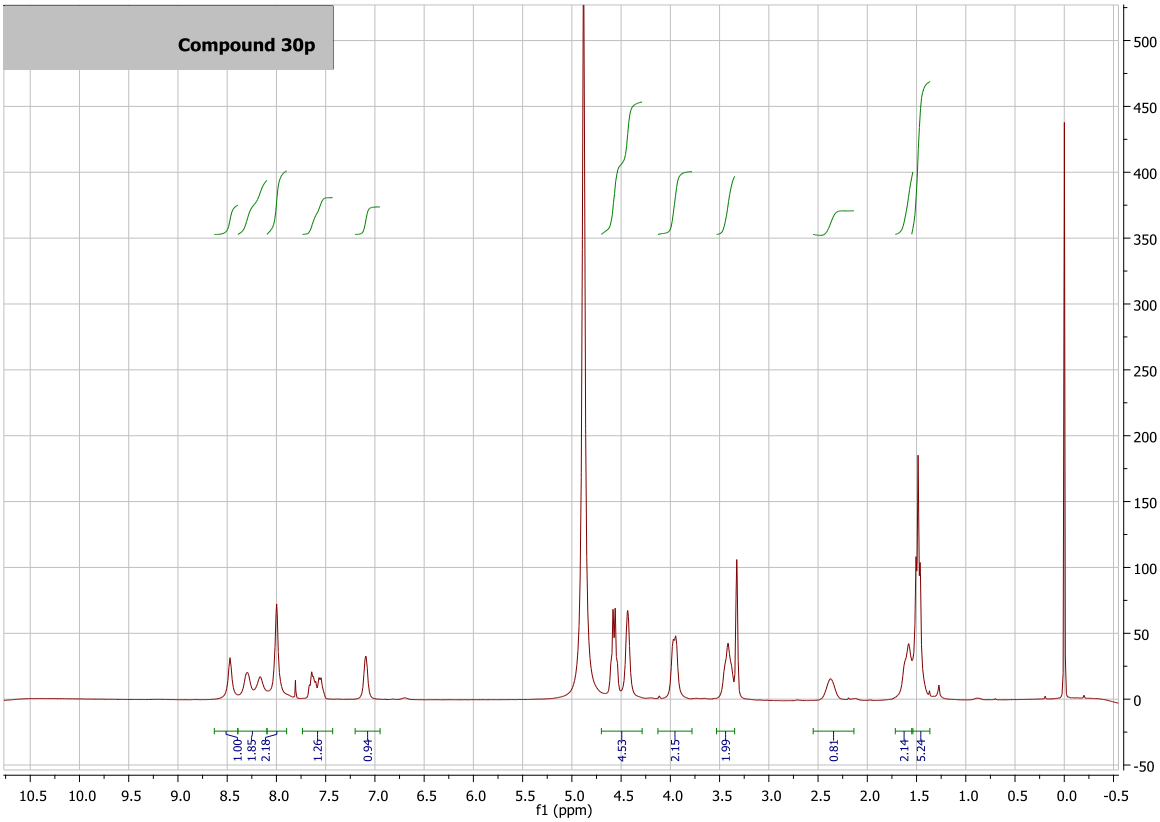
Compound 17r

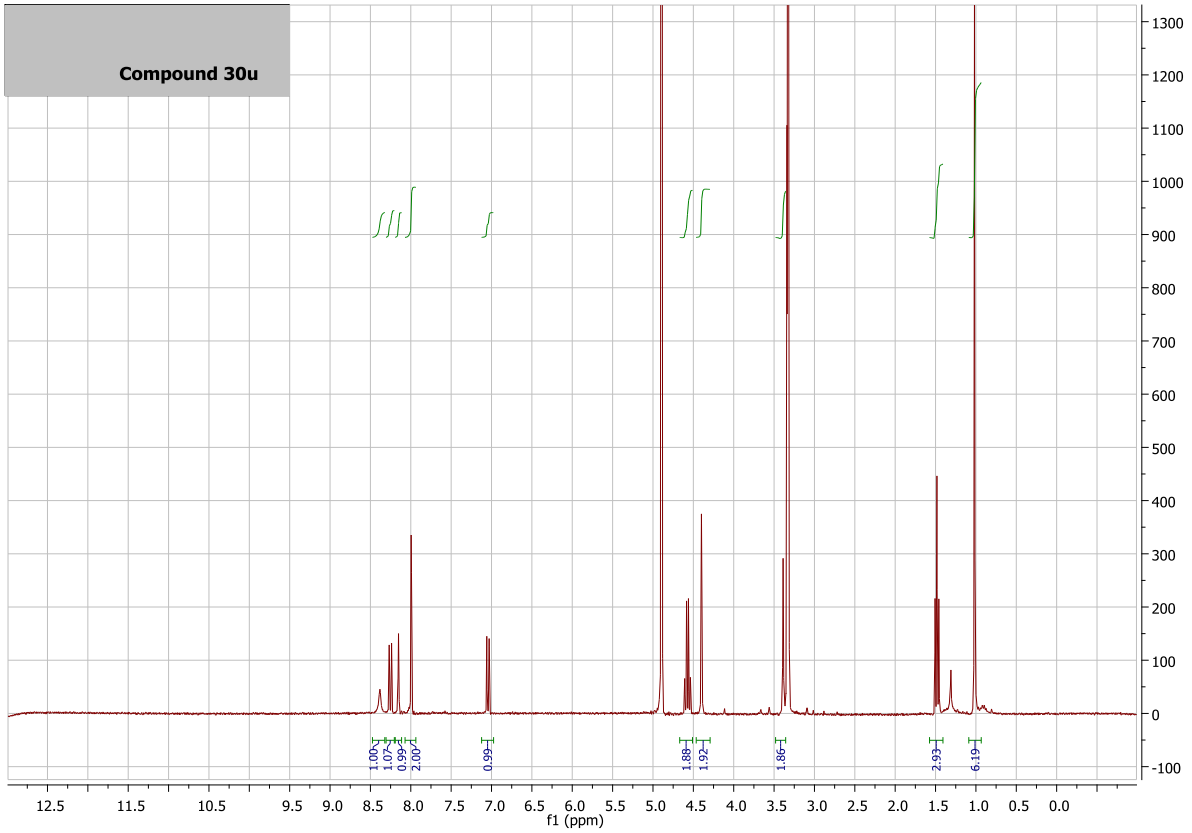


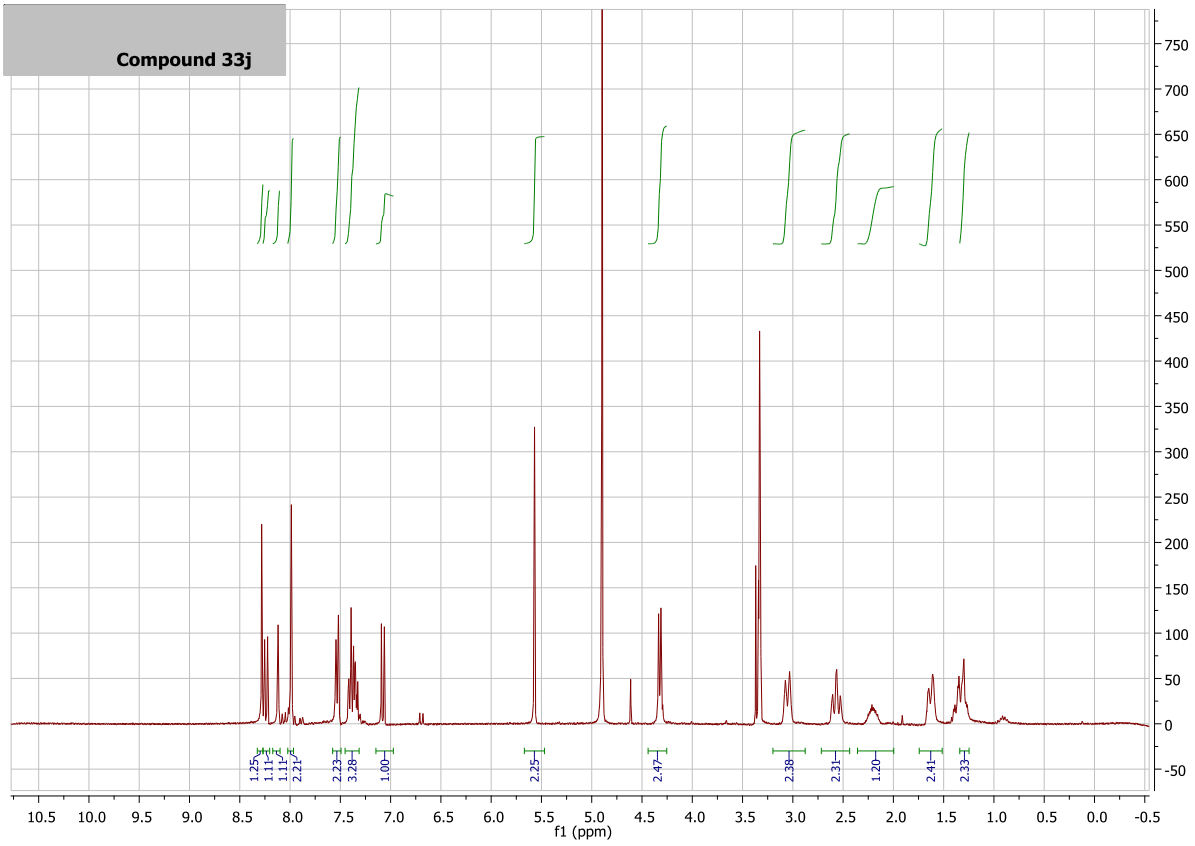

Compound 22j
f1 (ppm)

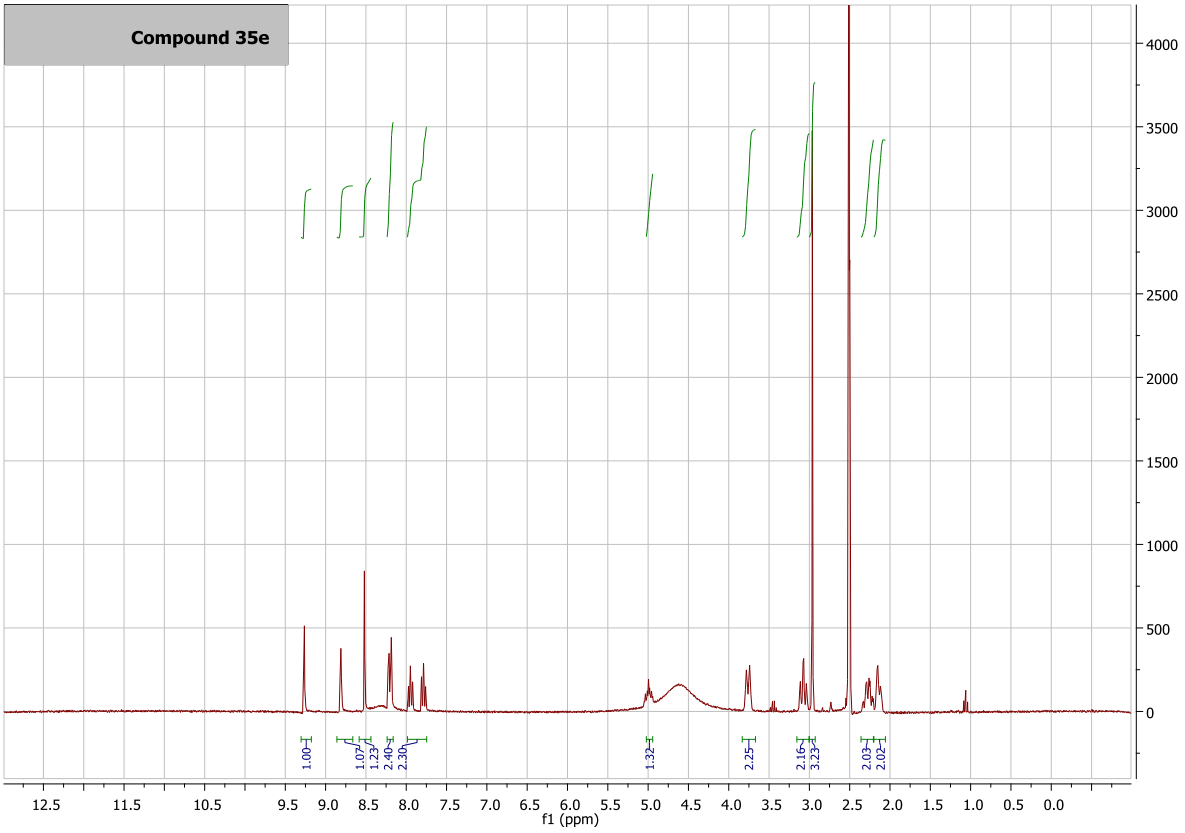

Compound 29k
f1 (ppm)

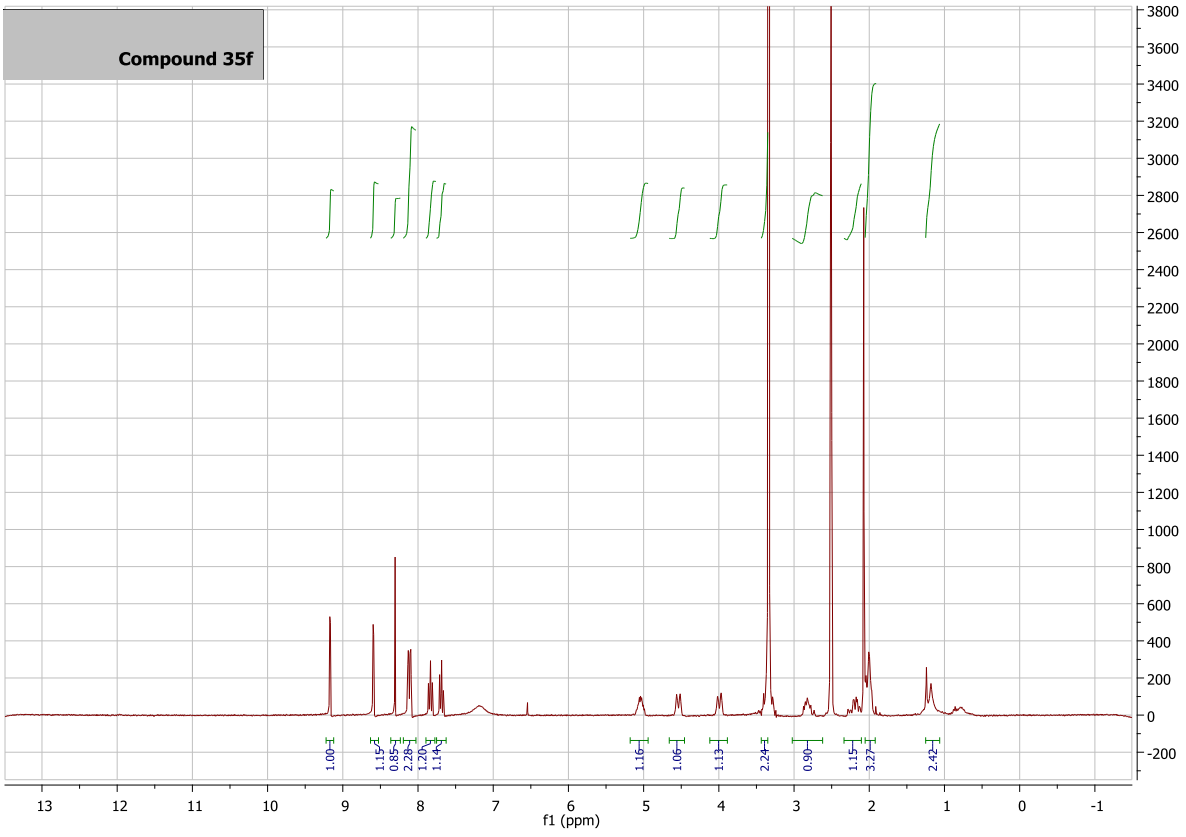

Compound 29o
f1 (ppm)


Compound 30p
f1 (ppm)


Compound 30u
1.00
1.07
0.99
2.00
0.99
1.88
1.92
1.86
2.93
6.19
f1 (ppm)


Compound 33j
1.25
1.11
1.11
2.21
2.23
3.28
1.00
2.25
2.47
2.38
2.31
1.20
2.41
2.33
f1 (ppm)


Compound 35e
4000
3500
3000
2500
2000
1500
1000
500
0
1.00
1.07
1.23
2.40
2.30
1.32
2.25
2.16
3.23
2.03
2.02
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Compound 35f
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
1.00
1.15
0.85
2.28
1.20
1.14
1.16
1.06
1.13
2.24
0.90
1.15
3.27
2.42
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

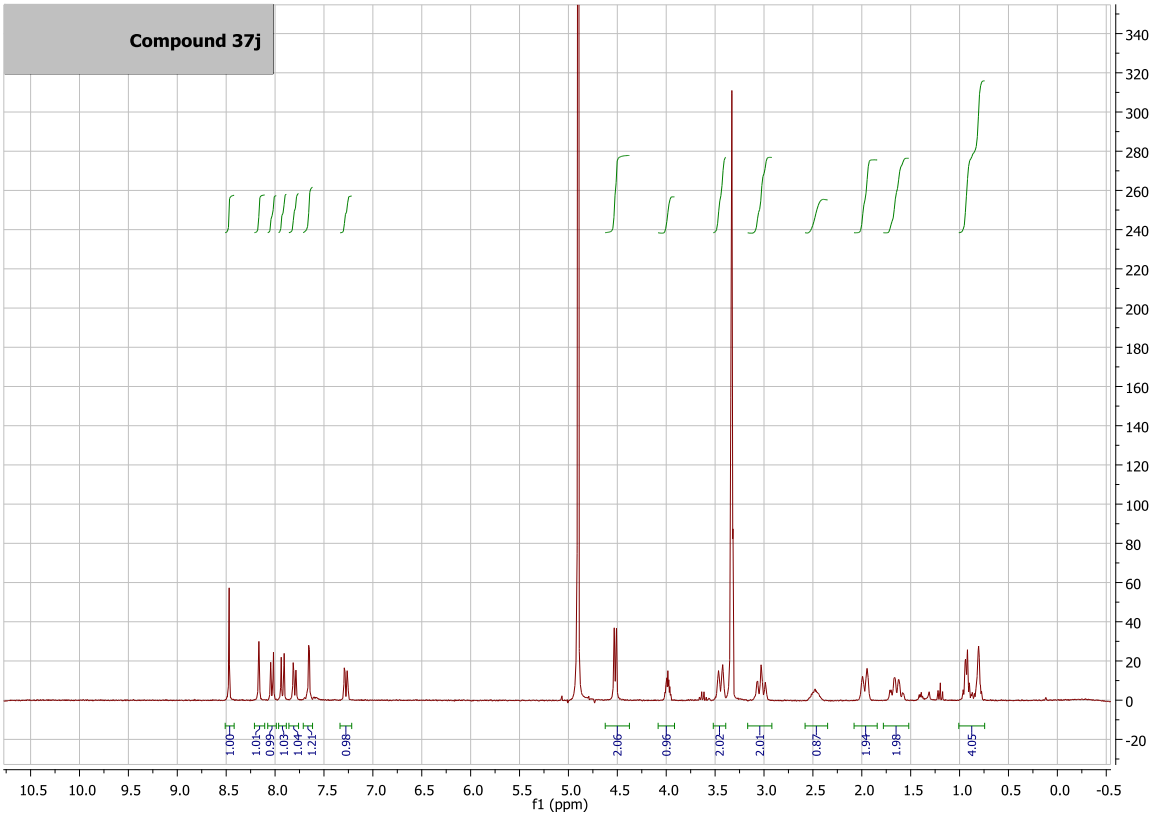

Compound 37j
f1 (ppm)

Table 3: