Supporting Information for

7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine based inhibitors of calcium dependent protein kinase 1 have distinct inhibitory and oral pharmacokinetic characteristics compared with 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine based inhibitors.

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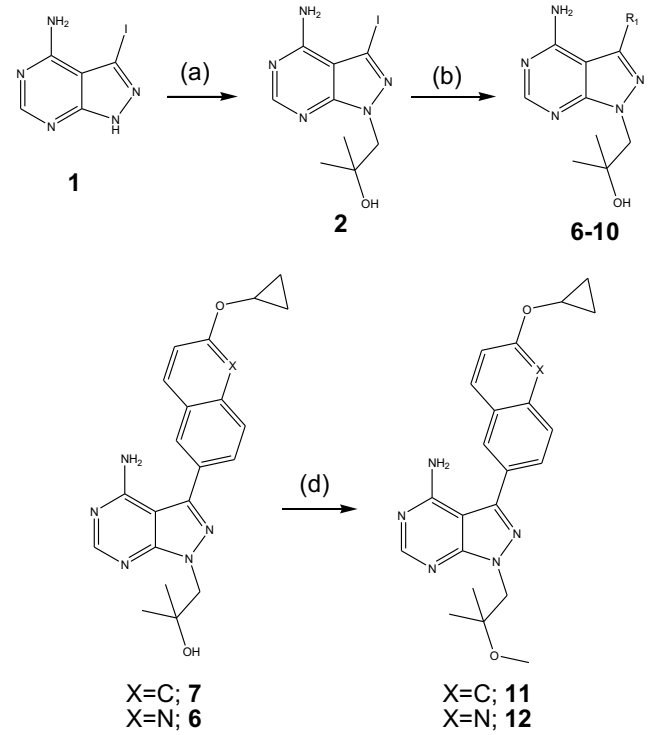
Contents:

General Synthetic Procedures.

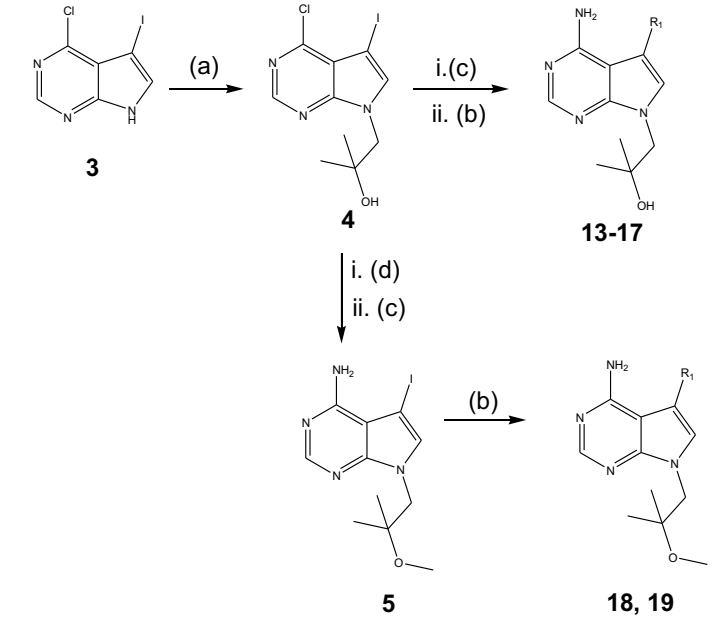
All chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. Reactions were monitored with thin-layer chromatography using silica gel 60 F254 coated glass plates (EM Sciences). Compound purification was performed with an IntelliFlash 280 automated flash chromatography system using pre-packed RedisepRF 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 (*method 1*) or H_2O/MeOH (*method 2*) gradient solvent systems (+0.05% TFA) run over 30 min. Products were detected by UV at λ=220 and 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 (**6-19**) are shown in Schemes 1-2. Synthesis and purification methods for compounds **6, 7, 8, 9** in Table 1 were described in previous publications. [13, 21] Synthesis and purification methods for compound **13** in Table 2 was described in a previous publication[3]**.** Compounds **10-12** in Table 1 and compounds **14-19** in table 2 are described below.

Scheme 1



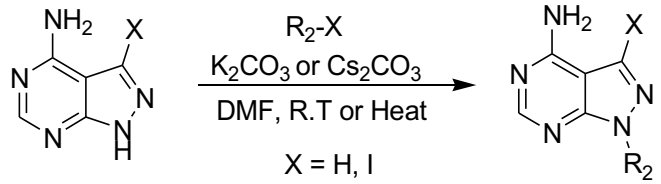
Scheme 2



Schemes 1 & 2. (a) epoxide, NaH_2PO_4:K_2CO_3 (1:1), DMF, 80 °C; (b) aryl boronic ester/acid, Na_2CO_3 or K_3PO_4, $PdCl_2(dppf).DCM$; or $Pd(pph_3)_4$, 1, 4-Dioxane:H_2O, 85 °C (microwave);(c)NH_4OH, 1,4-Dioxane, 60 °C (microwave); (d) CH_3I, NaH, DMF.

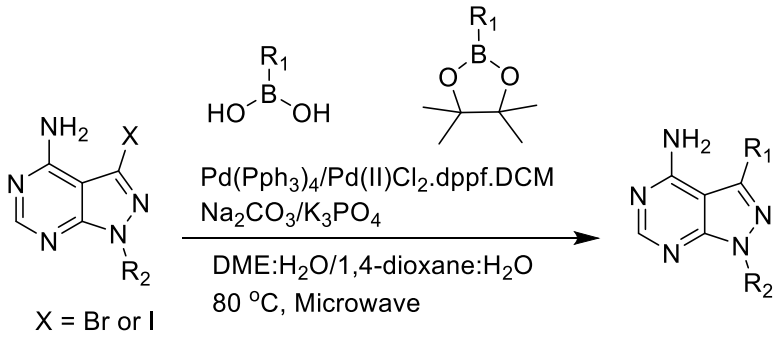
Syntheses and compound characterization data for all intermediates were described below. We reported many synthetic protocols in previous publications.[13, 21]

General R_2 alkylation procedure:



Pyrazolopyrimidine (1 equiv.), K_2CO_3 or Cs_2CO_3 or K_2CO_3:NaH_2PO_4 (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 completion, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. 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 Suzuki coupling procedure:

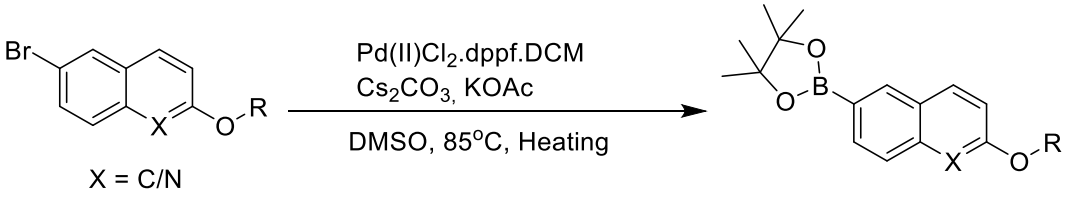


3-Iodopyrazolopyrimidines or 3-Bromopyrazolopyrimidines (1 equiv.), Na_2CO_3 or K_3PO_4 (2-4 equiv.), $Pd(PPh_3)_4$ or Pd(II)Cl_2dppf.DCM, (0.05 equiv.), and boronic acids or boronate pinacol esters (1-2 equiv.) were dissolved in a mixture of dimethoxyethane (1.5 mL) and water (0.5 mL) and then heated in a microwave at 80 °C for one hour. After cooling, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl

acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. 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.

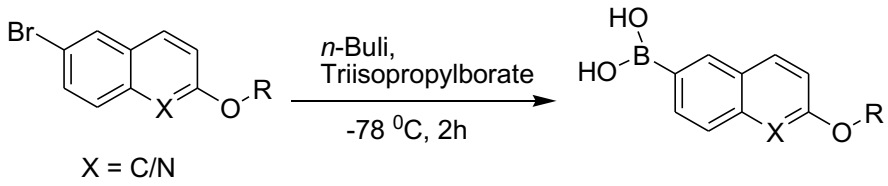
Synthesis and spectral data of various intermediates

General pinacol ester formation procedure:



Alkylated naphthols or quinolones (1 equiv.), Cs_2CO_3 (1.5-2 equiv.), pinacolatodiborane (2.0 equiv.), Pd(II)Cl_2(dppf).DCM (0.05 equiv.), and KOAc (1 equiv.) in dry DMSO were heated at 85 °C for 5-8 h. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified *via* flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient.

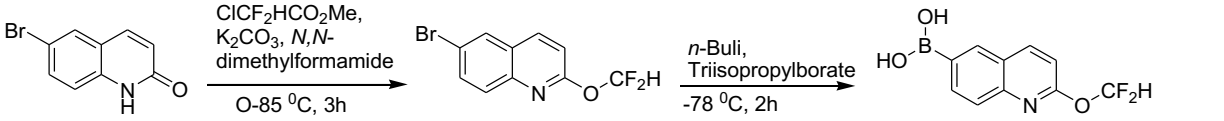
General procedure for boronylation using triisopropylborate:



Aryl halides (1 equiv.) and triisopropylborate (1.5 equiv.) were dissolved in tetrahydrofuran:toluene (2:8), cooled to -78 °C, and *n*-Buli (1.7 equiv.) was added dropwise over 30-40 min. After addition, the reaction was stirred at -78 °C for 1 h. After 1 h, the reaction was allowed to warm to 0 °C and stirred for 15-25 min followed by addition of 2N HCl slowly. The organic layer was separated and concentrated *in vacuo* to afford the desired crude product as a

white crystalline product or by collecting and washing with water the white crystalline solid that forms upon addition of 2N HCl.

2-(Difluoromethoxy)quinolin-6-ylboronic acid (20)

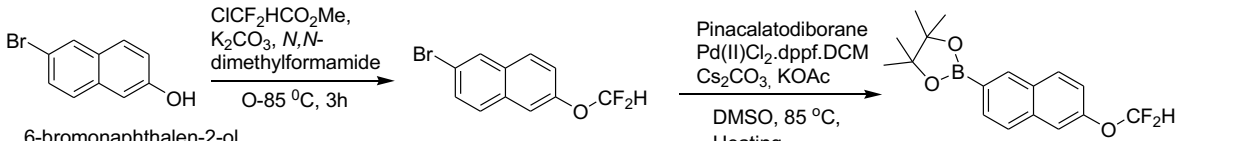


6-bromoquinolin-2(1*H*)-one — 6-bromo-2-(difluoromethoxy)quinoline — 2-(difluoromethoxy)quinolin-6-ylboronic acid

6-Bromo-2-(difluoromethoxy)quinoline: Mehylchlorodifluroacetate (2.90 g, 19.8 mmol) was added to the solution of 6-Bromo-quinolin-2(1*H*)-one (1.50 g, 6.6 mmol, 1 equiv.), K_2CO_3 (2.73 g, 19.8 mmol) in dry DMF (10 mL), all ingredients were heated at 100 °C for 3 h. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was further extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified *via* flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient to afford 1.2 g (35% yield) of pure product. ^{1}H NMR (301 MHz, $CDCl_3$) δ 8.10 (br t, J = 58.62 Hz 1H), 7.73-7.65 (m, 2H), 7.66-7.53 (m, 2H), 6.61 (d, J = 9.47 Hz, 1H); MS (ESI) 275.5 *m/z* [MH^+], $C_{10}H_7BrF_2NO$ requires 275.2.

2-(Difluoromethoxy)quinolin-6-ylboronic acid: *6-Bromo-2-(difluoromethoxy)quinoline* (1.01 g, 3.60 mmol, 1 equiv.) and triisopropylborate (0.829 mg, 4.41 mmol, 1.2 equiv.) were subjected to ***General procedure for boronylation using triisopropylborate*** to afford the desired pure product (0.52 g, 60% yield); ^{1}H NMR (300 MHz, DMSO) δ 8.67-8.57 (m, 2H), 8.45-8.26 (m, 2H), 7.98-7.87 (m, 1H), 7.27 (d, J = 8.70Hz, 1H); MS (ESI) 239.2 *m/z* [MH^+], $C_{10}H_9BF_2NO_3$ requires 239.2.

2-(6-(Difluoromethoxy)naphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (21)



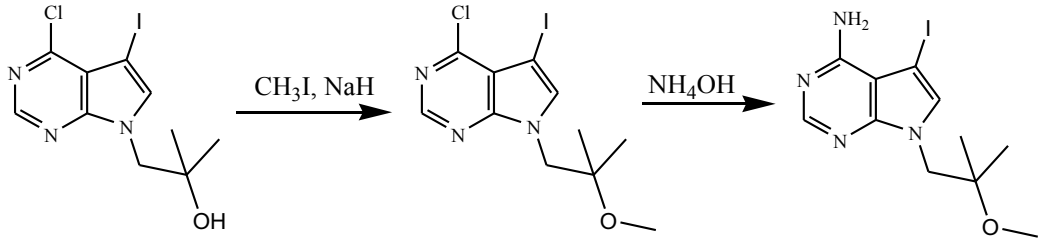
6-bromonaphthalen-2-ol — 2-bromo-6-(difluoromethoxy) naphthalene — 2-(6-(difluoromethoxy)naphthalen-2-yl) -4,4,5,5-tetramethyl-1,3,2-dioxaborolane

***2-Bromo-6-(difluoromethoxy)naphthalene*:** Mehylchlorodifluroacetate (2.00 g, 13.8 mmol) was added to the solution of 6-Bromonaphthalen-2-ol (1.0 g, 4.48 mmol, 1 equiv.), K_2CO_3 (1.90 g, 13.8 mmol) in dry DMF (10 mL), all ingredients were heated at 100 °C for 3 h. After completion, ethyl acetate and water were added and the organic phase was separated. The water phase was further extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was then purified *via* flash chromatography over silica, eluting with a hexanes/EtOAc solvent gradient to afford 0.364 g (30% yield) of pure product. ^{1}H NMR (301 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.74 (d, J = 8.81 Hz, 1H), 7.65 (d, J = 8.02 Hz, 1H), 7.56 (d, J = 8.80 Hz, 1H), 7.47 (s, 1H),7.28 (d, J = 8.80 Hz, 1H), 6.62 (br t, J = 74.46 Hz, 1H); MS (ESI) 274.2 *m/z* [MH^+], $C_{11}H_8BrF_2O$ requires 274.2.

2-(6-(Difluoromethoxy)naphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane: 2-Bromo-6-(difluoromethoxy)naphthalene was subjected to the ***General pinacol ester formation procedure*** to afford 0.147 g, (50% yield) of a white crystalline product. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.35 (s, 1H), 7.89 (m, 2H) 7.88 (d, J = 8.80 Hz, 1H), 7.47 (s, 1H), 7.26 (m, 1H), 6.65 (br t, J = 73.50 Hz, 1H); MS (ESI): 321.5 *m/z* [MH+], $C_{17}H_{20}BF_2O_3$ requires 321.2.

Synthetic intermediats:

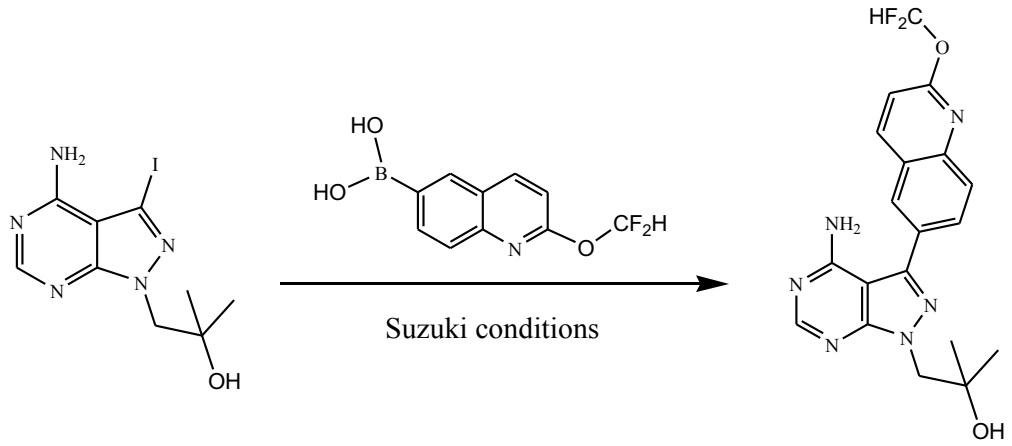
5-Iodo-7-(2-methoxy-2-methylpropyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (5)



Sodium hydride was added to the solution of 1-(4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-methylpropan-2-ol, followed by methyl iodide at O^{o}C, reaction was stirred for 3h at room temperature. Reaction was quenched with saturated solution of ammonium chloride, organic layer was extracted with ethyl acetate, dry over sodium sulfate. The crude product was taken to further steps without purification. 4-chloro-5-iodo-7-(2-methoxy-2-methylpropyl)-7*H*-

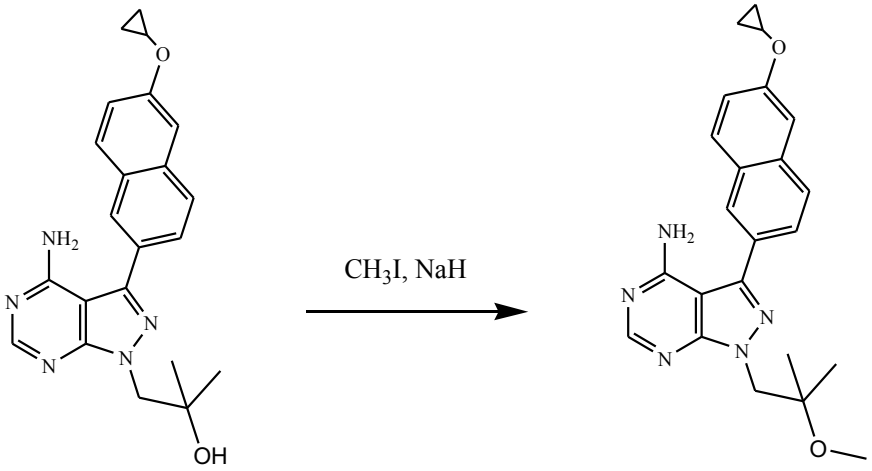
pyrrolo[2,3-*d*]pyrimidine purified was subjected ammonolysis at microwave, The crude product was purified by silica gel using ethylacetate/hexane gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H), 7.30 (s, 1H), 4.20 (s, 2H), 3.27 (s, 3H), 1.24 (s, 6H); MS (ESI) 347.4 *m/z* [MH+], $C_{11}H_{16}IN_4O$ requires 347.2.

1-(4-Amino-3-(2-(difluoromethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol: (10);



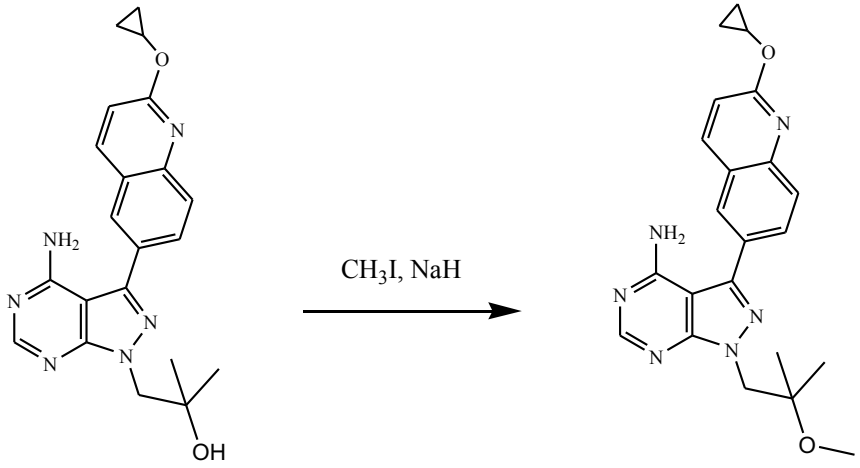
2-(difluoromethoxy)quinolin-6-ylboronic acid (**20**) and 1-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-2-methylpropan-2-ol[13] were subjected to the ***General Suzuki coupling procedure.*** The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.45 (d, J = 8.91 Hz, 1H), 8.28-8.21 (m, 2H), 8.08-8.04 (m, 2H), 7.83 (br t, J = 57.84 Hz, 1H), 7.17 (d, J = 8.70 Hz, 1H), 4.42 (s, 2H), 1.28 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.29, 158.20, 156.41, 155.42, 145.94, 143.88, 140.87, 130.38, 129.42, 127.72, 126.52, 116.02, 113.99, 113.41, 98.70, 71.48, 58.51, 27.33; HRMS Calc. for $C_{19}H_{19}F_2N_6O_2$ *m/z* 401.1532 [MH+] found 401.1521.

3-(6-Cyclopropoxynaphthalen-2-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (11):



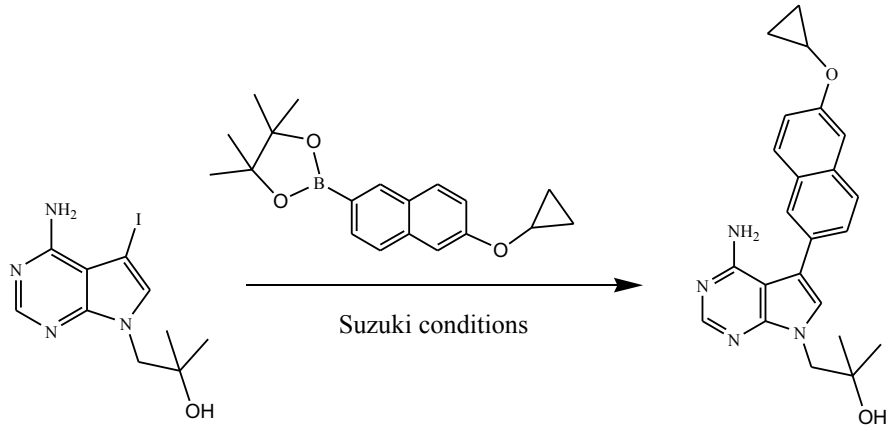
Sodium hydride was added to the solution of1-(4-Amino-3-(6-cyclopropoxynaphthalen-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-2-methylpropan-2-ol (**7**), followed by methyl iodide at O^{o}C, reaction was stirred for 3h at room temperature. Reaction was quenched with saturated solution of ammonium chloride, organic layer was extracted with ethyl acetate, dry over sodium sulfate. The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.37 (s, 1H), 8.12 (s, 1H), 7.99 (d, J = 8.70 Hz, 1H), 7.90 (d, J = 9.12 Hz, 1H), 7.77 (dd, J = 8.50, 1.80 Hz, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.29-7.22 (dd, J = 8.91, 2.40 Hz, 1H), 4.43 (s, 2H), 3.97 (m, 1H), 3.05 (s, 3H), 1.30 (s, 6H), 0.96-0.75 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.45, 156.94, 155.66, 146.23, 140.56, 136.36, 130.99, 130.68, 129.47, 129.25, 129.02, 127.57, 120.82, 120.51, 109.31, 72.32, 58.47, 52.18, 28.46, 27.83, 6.87; HRMS Calc. for $C_{23}H_{26}N_5O_2$ *m/z* 404.2081 [MH+] found 404.2087.

3-(2-Cyclopropoxyquinolin-6-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (12):



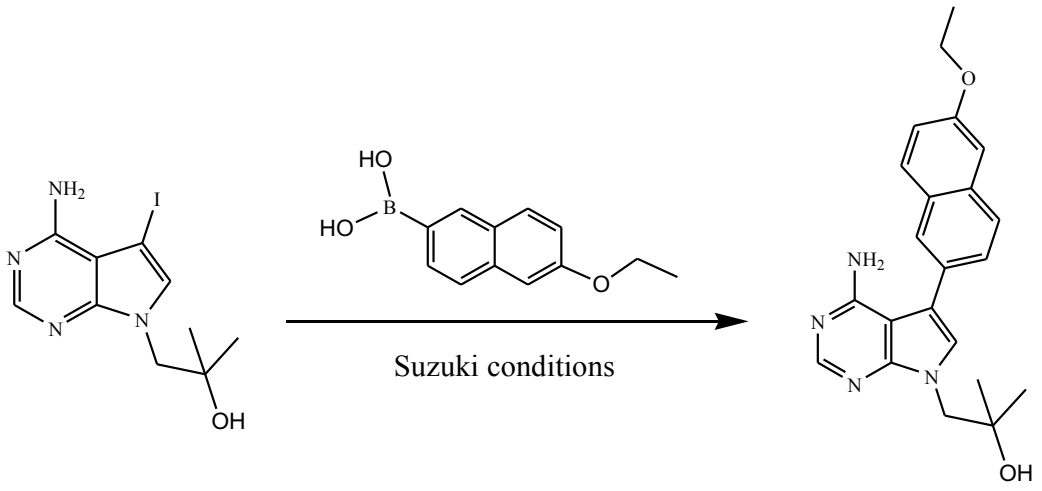
Sodium hydride was added to the solution of 1-(4-Amino-3-(2-cyclopropoxyquinolin-6-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-2-methylpropan-2-ol (**6**), followed by methyl iodide at 0^{o}C, reaction was stirred for 3h at room temperature. Reaction was quenched with saturated solution of ammonium chloride, organic layer was extracted with ethyl acetate, dry over sodium sulfate. The crude product was purified by silica gel using dichloromethane/methanol gradient. The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.37 (s, 1H), 8.29 (d, , J = 8.96 Hz, 1H), 8.14 (s, 1H), 8.06-7.98 (m, 2H), 7.08 (d, J = 8.96, Hz, 1H), 4.51 (m, 1H), 4.43 (s, 2H), 3.05 (s, 3H), 1.30 (s, 6H), 0.94-0.79 (m, 4H); HRMS Calc. for $C_{22}H_{25}N_6O_2$ *m/z* 405.2034 [MH+] found 405.2037.

1-(4-amino-5-(6-cyclopropoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol (14)



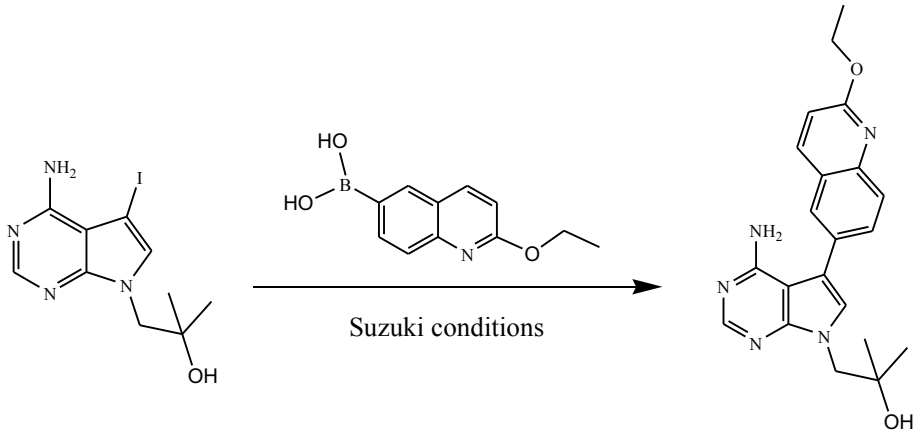
2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 1-(4-amino-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol[11] were subjected to the ***General Suzuki coupling procedure.*** The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.16 (s, 1H), 7.91 (m, 2H), 7.83 (d, J = 9.12 Hz, 1H), 7.67-7.55 (m, 2H), 7.34 (s, 1H), 7.23-7.17 (dd, J = 8.91, 2.20 Hz, 1H), 4.26 (s, 2H), 3.96 (m, 1H), 1.24 (s, 6H), 0.95-0.74 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.90, 158.71, 152.26, 151.98, 135.14, 131.22, 130.87, 130.47, 128.91, 128.74, 128.31, 126.68, 120.53, 117.77, 109.21, 101.93, 72.18, 55.88, 52.06, 27.48, 25.19, 6.86; HRMS Calc. for $C_{23}H_{25}N_4O_2$ *m/z* 389.1972 [MH+] found 389.1974.

1-(4-Amino-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol (15);



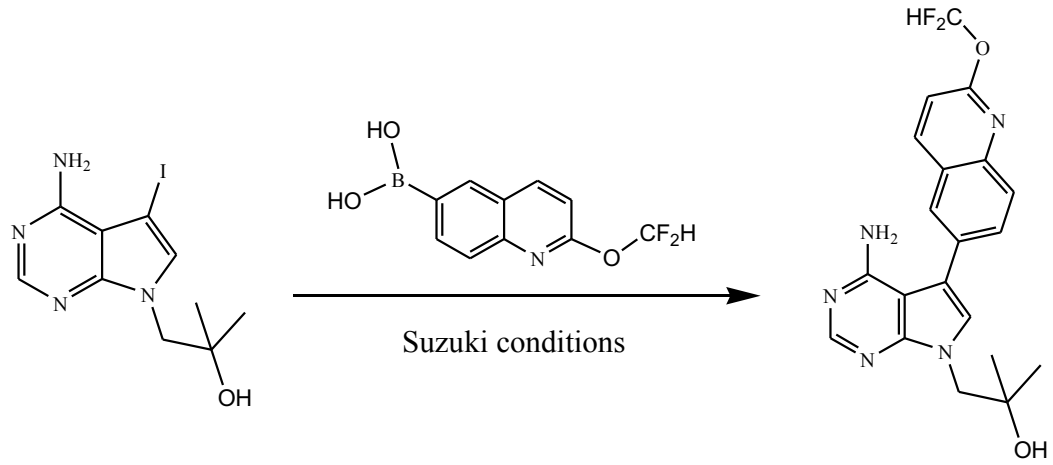
6-Ethoxynaphthalen-2-ylboronic acid and 1-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-methylpropan-2-ol[11] were subjected to the ***General Suzuki coupling procedure.*** The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.19 (s, 1H), 7.93-7.88 (m, 2H), 7.83 (d, J = 8.80 Hz, 1H), 7.62 (d, J = 8.43 Hz, 1H), 7.35 (s, 1H), 7.30 (s, 1H), 7.21 (dd, J = 8.80, 2.30 Hz, 1H), 4.27 (s, 2H), 4.24-4.18 (qt, J = 13.93, 6.96 Hz, 2H), 1.50 (t, J = 6.96 Hz, 3H), 1.25 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.01, 156.78, 150.24, 149.97, 133.34, 129.07, 128.66, 128.48, 126.82, 126.70, 126.32, 124.65, 118.90, 115.88, 105.69, 99.96, 70.22, 62.74, 53.88, 25.47, 13.29; HRMS Calc. for $C_{22}H_{25}N_4O_2$ *m/z* 377.1972 [MH+] found 377.1974.

1-(4-Amino-5-(2-ethoxyquinolin-6-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol (16):



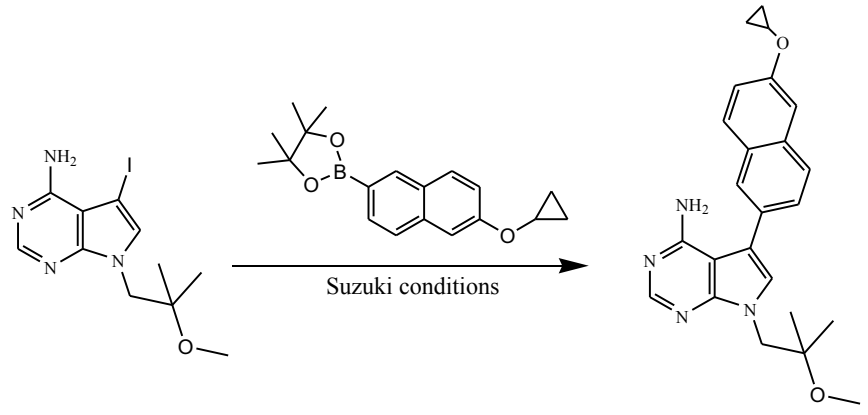
2-Ethoxyquinolin-6-ylboronic acid and 1-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-methylpropan-2-ol[11] were subjected to the ***General Suzuki coupling procedure.*** The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (500 MHz, CD_3OD) δ 8.22-8.16 (m, 2H), 7.95-7.90 (m, 2H), 7.82 (d, J = 8.80 Hz, 1H), 7.38 (s, 1H), 7.00 (d, J = 8.80 Hz, 1H), 4.56 (qt, J = 13.93, 6.96 Hz, 2H), 4.27 (s, 2H), 1.49 (t, J = 6.60 Hz, 3H), 1.25 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.10, 159.02, 152.32, 152.09, 147.23, 140.33, 131.90, 128.76, 128.32, 127.68, 126.95, 126.85, 117.22, 115.06, 101.91, 72.20, 63.04, 55.88, 27.47, 15.07; HRMS Calc. for $C_{21}H_{24}N_5O_2$ *m/z* 378.1925 [MH+] found 378.1927.

1-(4-amino-5-(2-(difluoromethoxy)quinolin-6-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol (17):



2-(Difluoromethoxy)quinolin-6-ylboronic acid (**20**) and 1-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-methylpropan-2-ol[11] were subjected to the ***General Suzuki coupling procedure.*** The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.40 (d, J = 8.96 Hz, 1H), 8.21 (s, 1H), 8.08-7.95 (m, 2H), 7.92 (m, 1H), 7.83 (br t, J = 73.70 Hz, 1H), 7.16 (dd, J = 8.70, 1.02 Hz, 1H), 7.40 (m, 1H), 4.26 (s, 2H), 1.24 (s, 6H); ^{13}C NMR (125 MHz, MeOD) δ 159.05, 152.45, 152.28, 146.12, 142.35, 133.80, 132.86, 129.50, 128.36, 128.12, 127.33, 116.83, 115.75, 113.77, 113.36, 101.86, 101.58, 72.28, 55.89, 27.52; HRMS Calc. for $C_{20}H_{20}F_2N_5O_2$ *m/z* 400.1580 [MH+] found 400.1583.

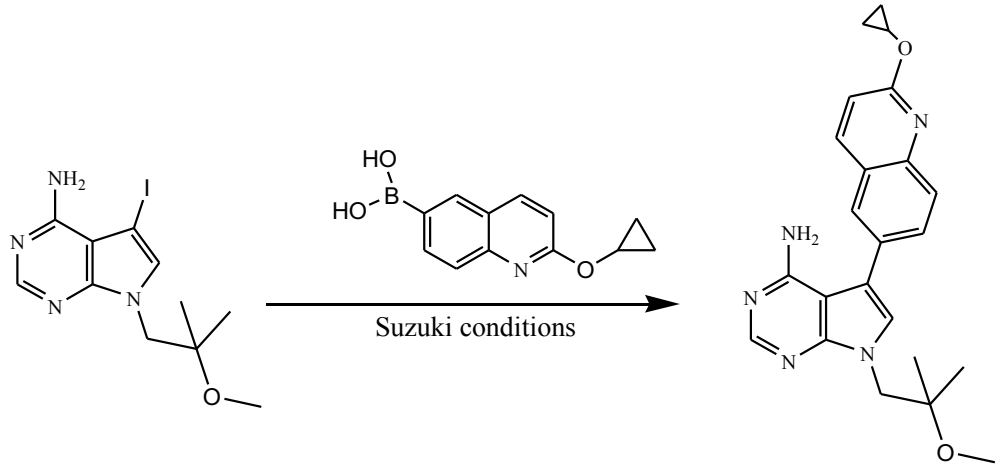
***5-(6-Cyclopropoxynaphthalen-2-yl)-7-(2-methoxy-2-methylpropyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (18*)*:**



2-(6-Cyclopropoxynaphthalen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and 5-iodo-7-(2-methoxy-2-methylpropyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**5**) were subjected to the

General Suzuki coupling procedure. The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.08 (s, 1H), 7.78 (m, 2H), 7.71 (d, J = 8.90 Hz, 1H), 7.52-7.41 (m, 2H), 7.17 (m, 1H), 7.11-7.01 (dd, J = 8.70, 1.70 Hz, 1H), 4.18 (s, 2H), 3.82 (m, 1H), 3.19 (s, 3H), 1.08 (s, 6H), 0.84-0.60 (m, 4H); ^{13}C NMR (125 MHz, MeOD) δ 158.69, 158.58, 152.19, 151.96, 134.99, 131.09, 130.70, 130.50, 128.98, 128.86, 128.47, 126.39, 120.67, 117.82, 109.30, 102.04, 76.91, 53.15, 52.21, 50.43, 23.22, 7.19; HRMS Calc. for $C_{24}H_{27}N_4O_2$ *m/z* 403.2129 [MH+] found 403.2129.

5-(2-Cyclopropoxyquinolin-6-yl)-7-(2-methoxy-2-methylpropyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (19):



2-Cyclopropoxyquinolin-6-ylboronic acid and 5-iodo-7-(2-methoxy-2-methylpropyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**5**) were subjected to the ***General Suzuki coupling procedure.*** The crude product was purified by silica gel using dichloromethane/methanol gradient. ^{1}H NMR (300 MHz, CD_3OD) δ 8.21 (s, 1H), 8.19 (s, 1H), 7.99 (d, J = 8.43 Hz, 1H), 7.92 (s, 2H), 7.83 (d, J = 8.80 Hz, 1H), 7.31 (s, 1H), 7.07 (d, J = 8.80, 1.70 Hz, 1H), 4.47 (m, 1H), 4.30 (s, 2H), 3.33 (s, 3H), 1.21 (s, 6H), 0.96-0.80 (m, 4H); ^{13}C NMR (125 MHz, MeOD) δ 163.23, 157.12, 151.92, 151.76, 146.08, 138.78, 131.02, 128.44, 127.11, 125.70, 125.24, 115.58, 113.75, 101.01, 100.92, 75.65, 52.28, 50.43, 49.77, 29.92, 22.52, 6.08; HRMS Calc. for $C_{23}H_{26}N_5O_2$ *m/z* 404.2081 [MH+] found 404.2088.

Biological Procedures

Enzymatic Inhibition Assay. A modified protocol from a previously reported study was used.[19] Inhibitors were evaluated in triplicate in eight-point dilutions (3-fold dilutions) during the enzymatic reactions. *Tg*CDPK1 and *Cp*CDPK1 enzymatic inhibition was determined with a coupled luciferase assay (Kinaseglo®). 2.1 nM *Tg*CDPK1 or 2.0 nM *Cp*CDPK1 and 20 μM BioSyntide-2 (Biotin-C6-PLARTLSVAGLPGKK (American Peptide Company, Inc. Sunnyvale, CA)) were incubated in 25 μL of buffer containing 1 mM EGTA (pH 7.2), 10 mM $MgCl_2$, 20 mM HEPES, pH 7.5 (KOH), 0.1% BSA, and 2 mM $CaCl_2$. The reaction was initiated with the addition of ATP at a 10 μM final concentration. After incubating at 30 °C for 90 min., changes in ATP concentration were determined by adding Kinaseglo® luciferase reagent (Promega, Madison, WI) and measuring luminescence with a MicroBeta2 multi-label plate reader (Perkin Elmer, Waltham, MA). Results were converted to percent inhibition, and IC_{50} values were calculated using nonlinear regression analysis in GraphPad Prism.

Src kinase enzymatic inhibition assay. A modified protocol from a previously reported study was used.[19] Inhibitors were evaluated in triplicate in eight-point dilutions (3-fold dilutions) during the enzymatic reactions. Src enzymatic inhibition was determined with a coupled luciferase assay (Kinaseglo®). 2 nM Src and 61 μM Src substrate peptide (sequence Ac-EIYGEFKKK, GenScript, Piscataway, NJ) were incubated in 25 μL of buffer containing 40 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM DTT, and 0.1% BSA. The reaction was initiated with the addition of ATP at a 10 μM final concentration. After incubating at 30 °C for 90 min., changes in ATP concentration were determined by adding Kinaseglo® luciferase reagent (Promega, Madison, WI) and measuring luminescence with a MicroBeta2 multi-label

plate reader (Perkin Elmer, Waltham, MA). Results were converted to percent inhibition, and IC_{50} values were calculated using nonlinear regression analysis in GraphPad Prism.

Human Cell Growth Inhibition Assay. A modified protocol from a previously reported study was used.[13, 20] CRL-8155 human lymphocytic cells (ATCC, WIL2-NS) were cultured in RPMI-1640 growth medium supplemented with 10% heat inactivated fetal bovine serum (FBS), 10 mM HEPES, 1 mM sodium pyruvate, and 1 mM L-glutamine. HepG2 human hepatocyte (ATCC, HB-8065) were cultured in DMEM/F12 growth medium supplemented with 10% heat inactivated FBS. The Alamar Blue® assay (Invitrogen, Grand Island, NY), which measures general cellular metabolism, was used to quantify cell growth. Mid-log cells were seeded in 96-well flat-bottom plates (Corning, Corning, NY) at a density of $3x10^5$ cells/mL containing test compounds at six final concentrations (80 µL, 40 µL, 20 µL, 10 µL, 5 µL, 2.5 µL, and 1.25 µL) in triplicate and grown at 37°C for 48 hours in a 5% CO_2 humidified incubator. A 1/10th volume of Alamar Blue® developing reagent was added to each well and incubated for an additional 3 hours and fluorescence was measured at the respective excitation and emission wavelengths of 560 nm and 590 nm in a FLx800 microplate reader (Biotek, Winooski, VT). Percent growth inhibition by test compounds was calculated based on DMSO vehicle and positive controls (50 µL quinacrine), which corresponded to 0% and 100% growth inhibition, respectively.

Supplementary references

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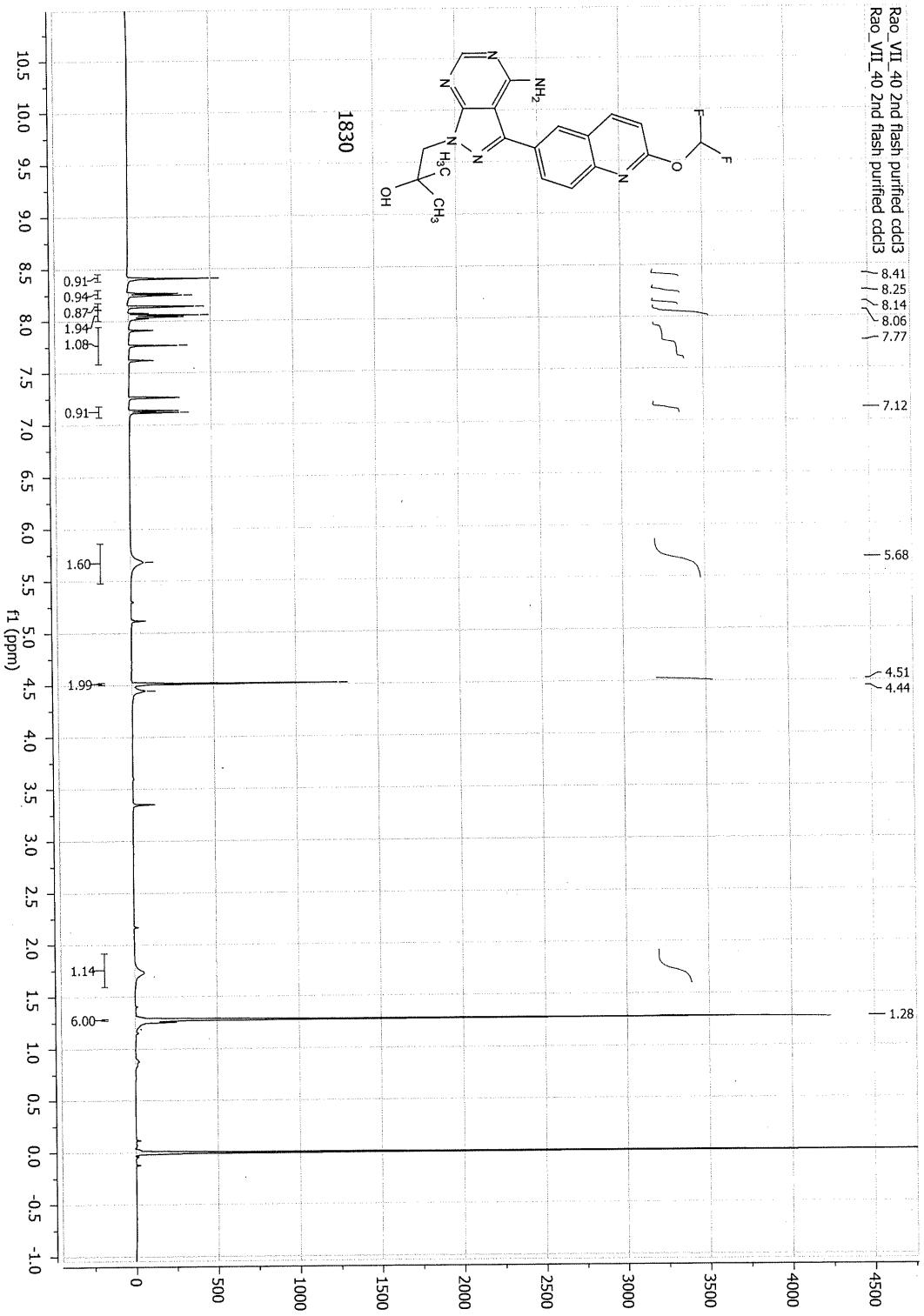
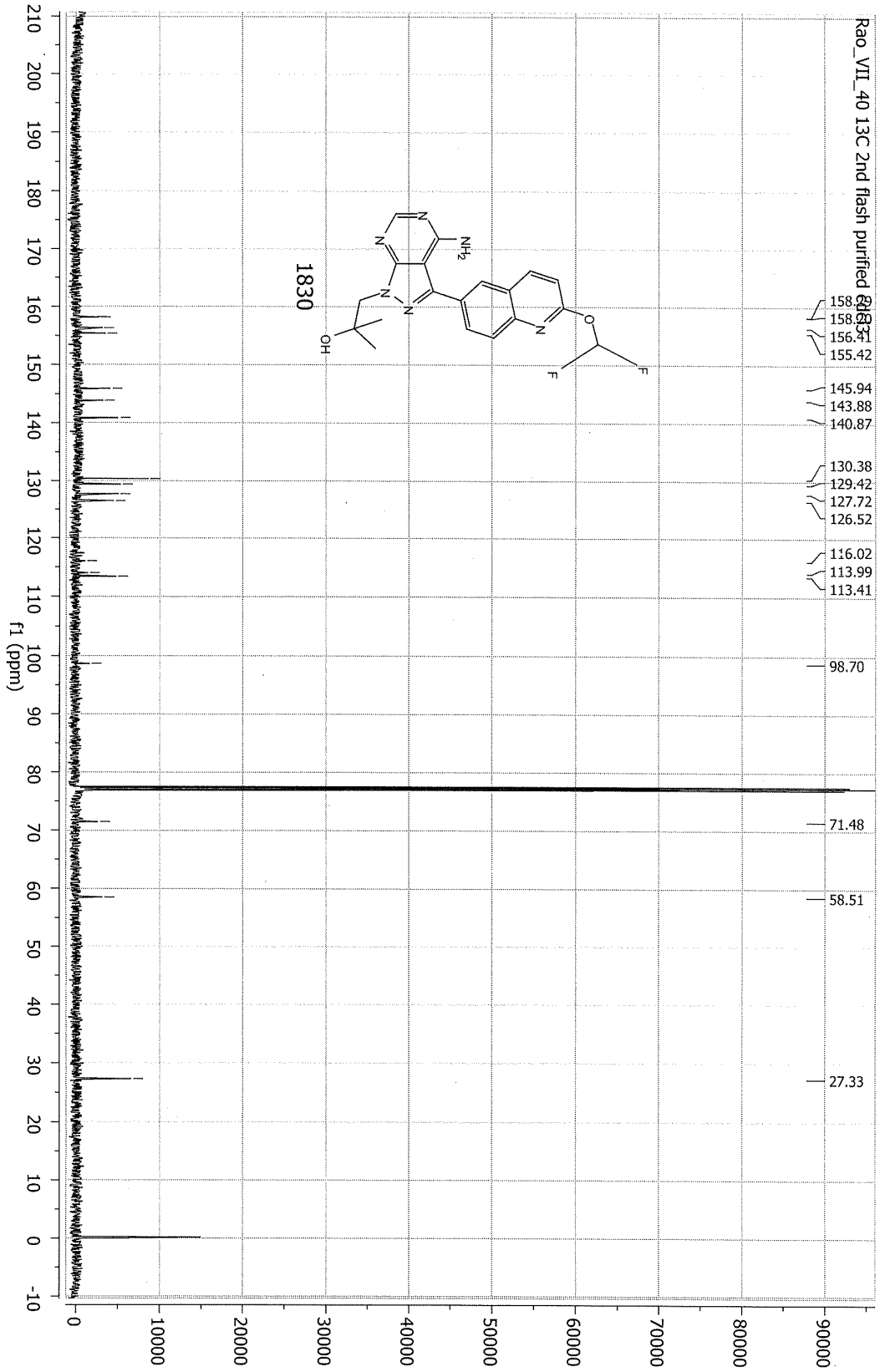
1-(4-Amino-3-(2-(difluoromethoxy)quinolin-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropan-2-ol

Compound 10

1H-NMR



13C-NMR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1830
158.93
158.41
156.41
155.42
145.94
143.88
140.87
130.38
129.42
127.72
126.52
116.02
113.99
113.41
98.70
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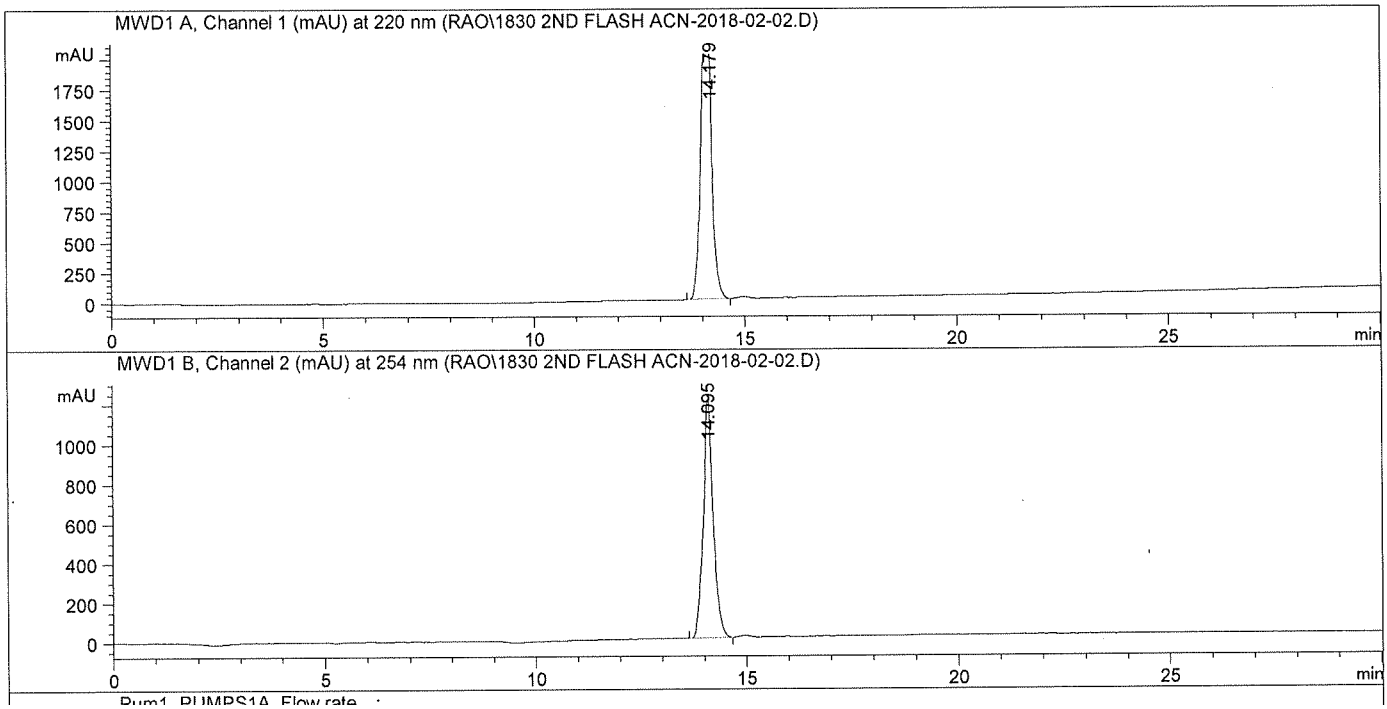
58.51
27.33
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



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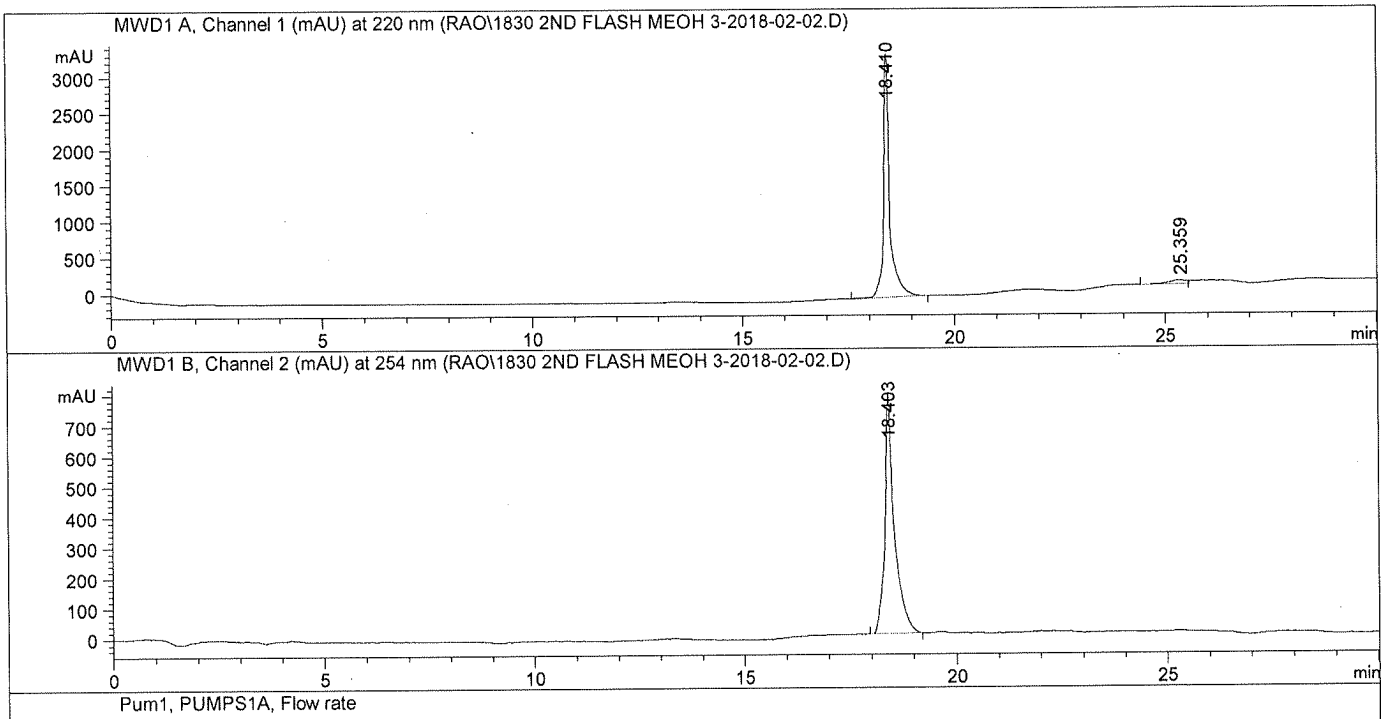
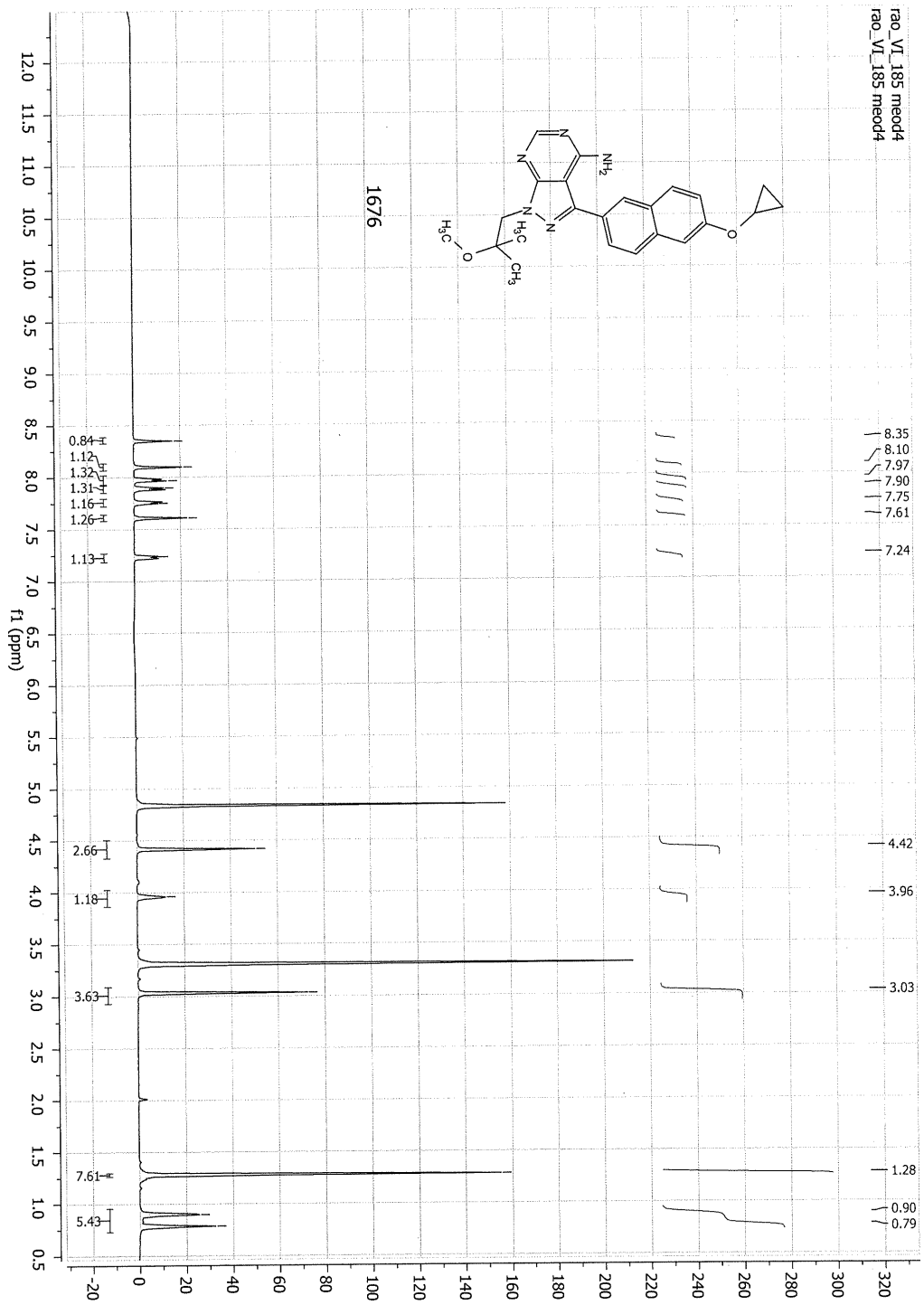


3-(6-Cyclopropoxynaphthalen-2-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

Compound 11 (1676)

1H-NMR



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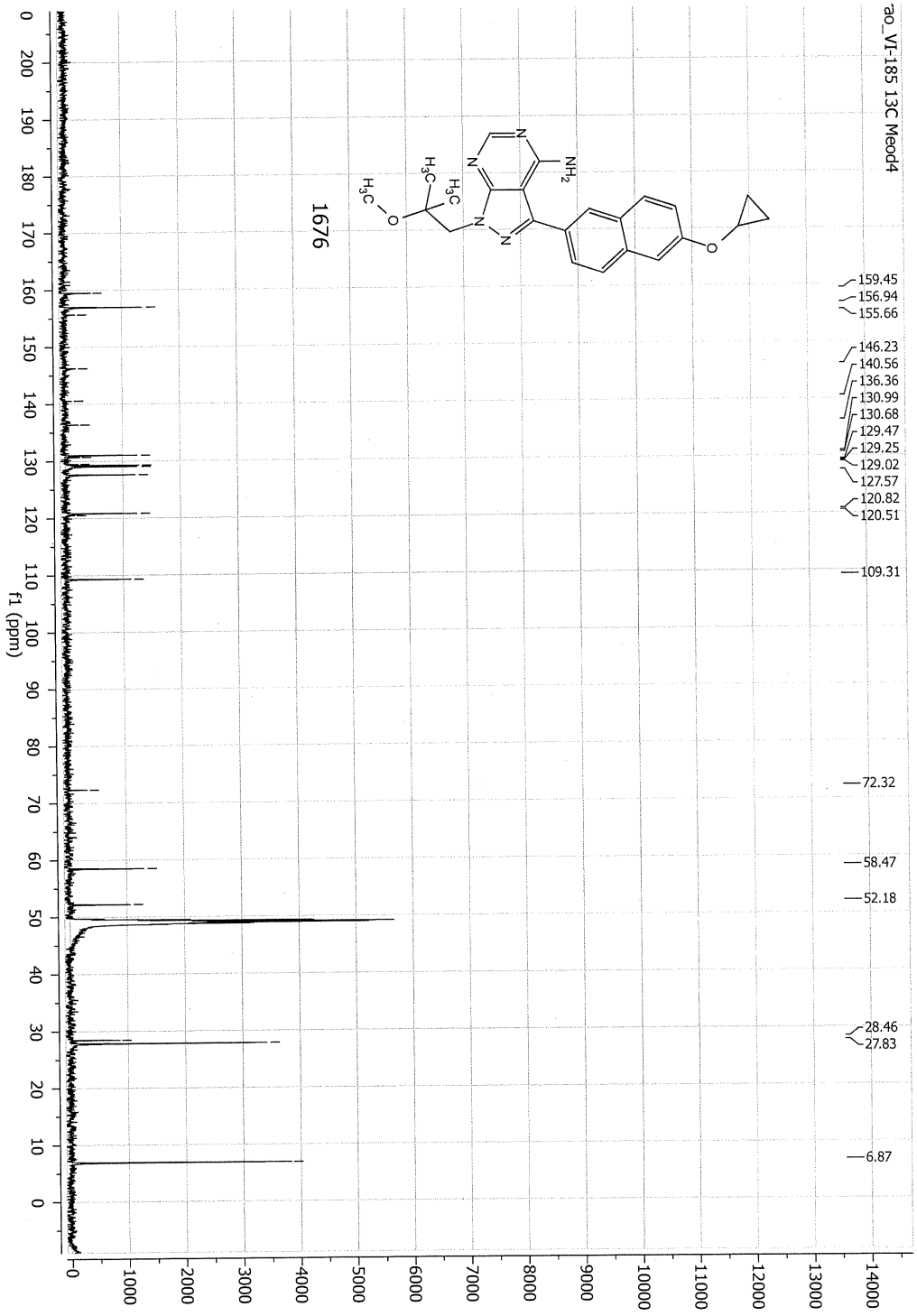
ao_VI-185 13C Meod4
1676
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146.23
140.56
136.36
130.99
130.68
129.47
129.25
129.02
127.57
120.82
120.51
109.31
72.32
58.47
52.18
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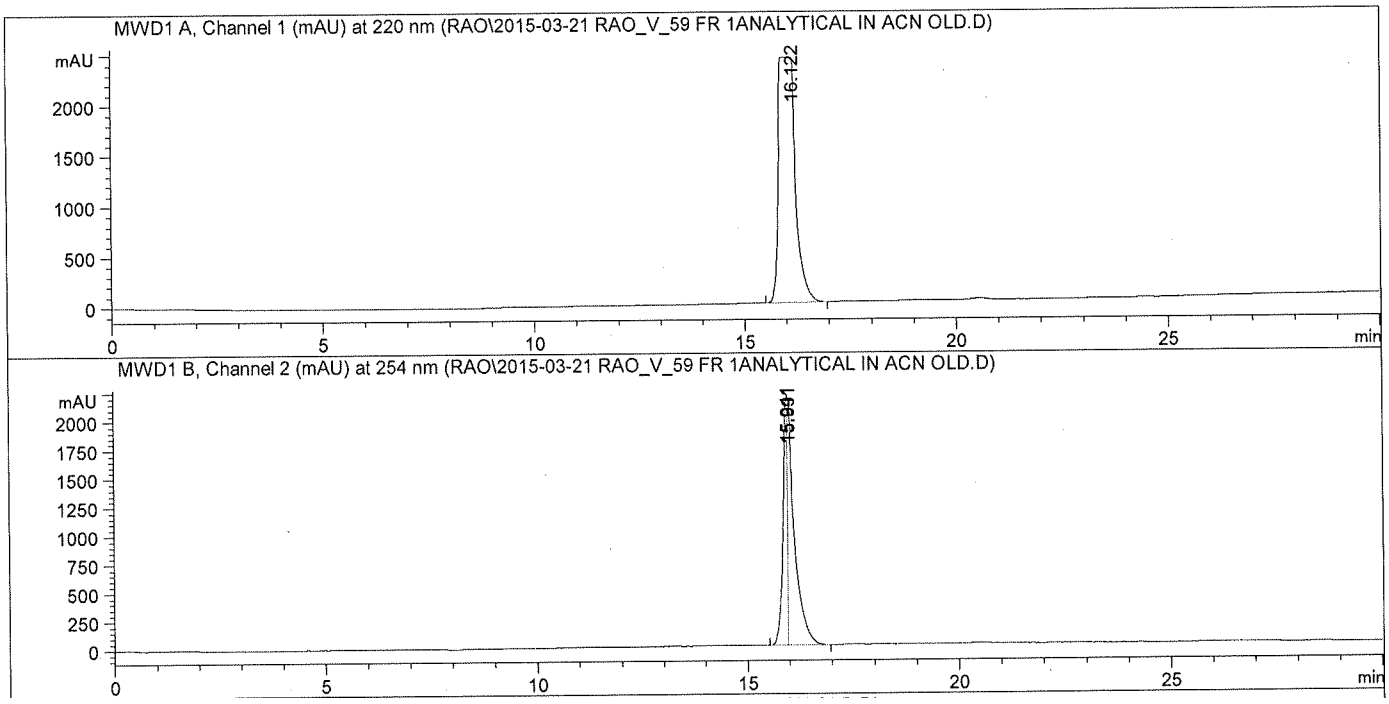
27.83
6.87
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



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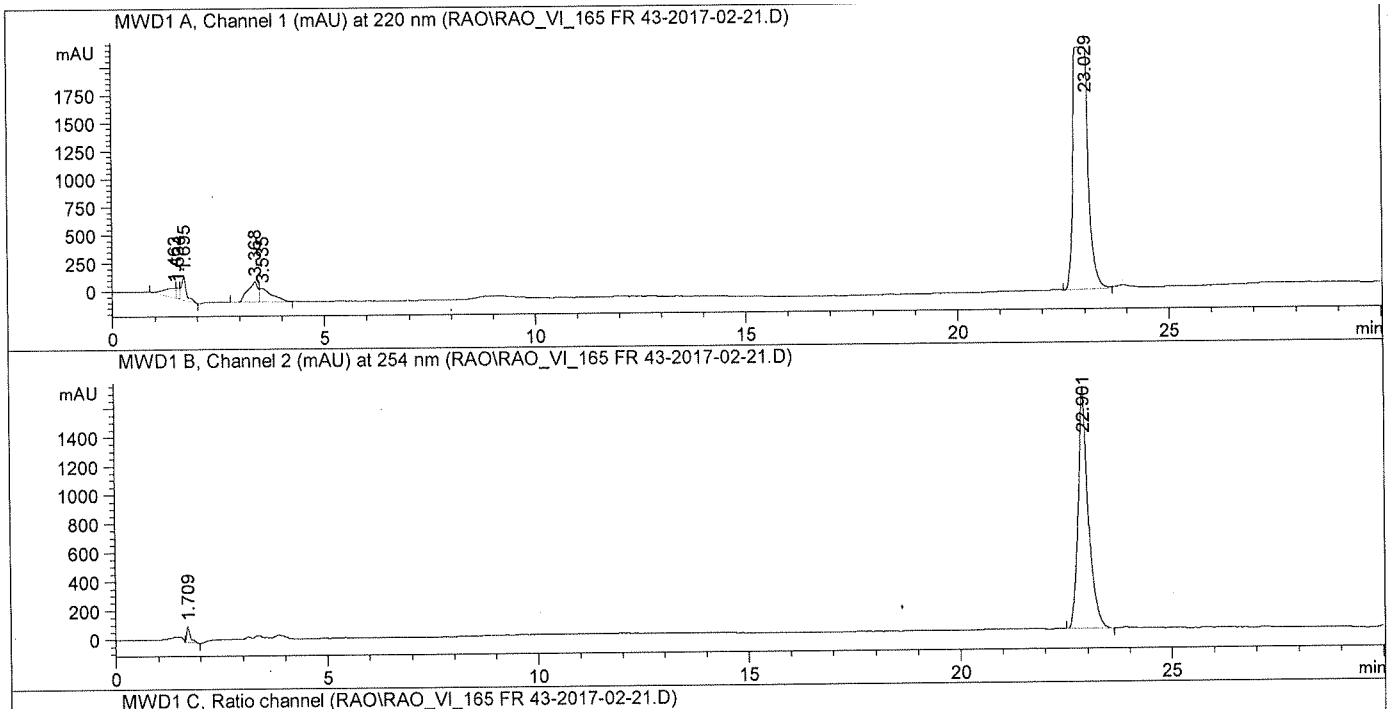
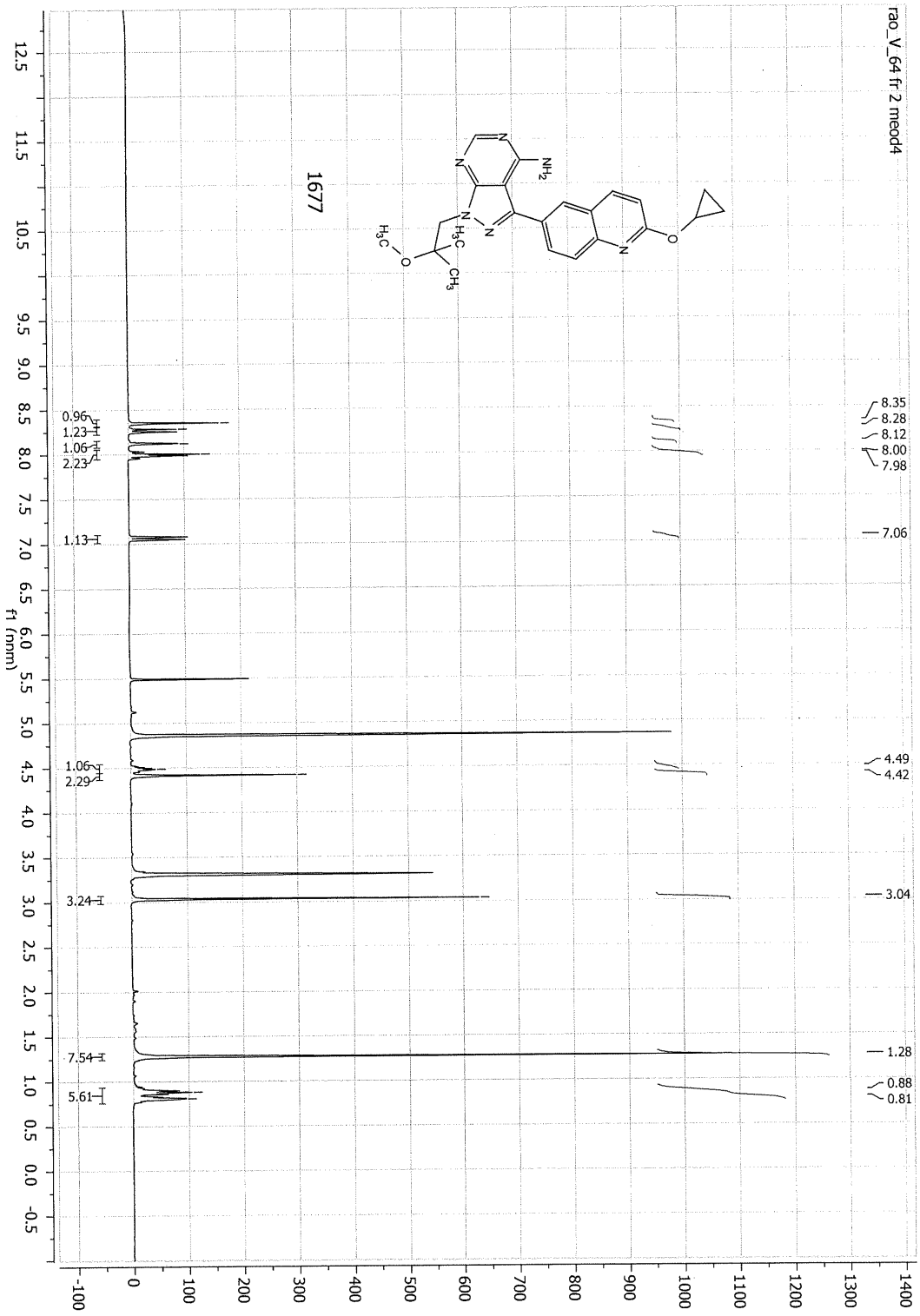


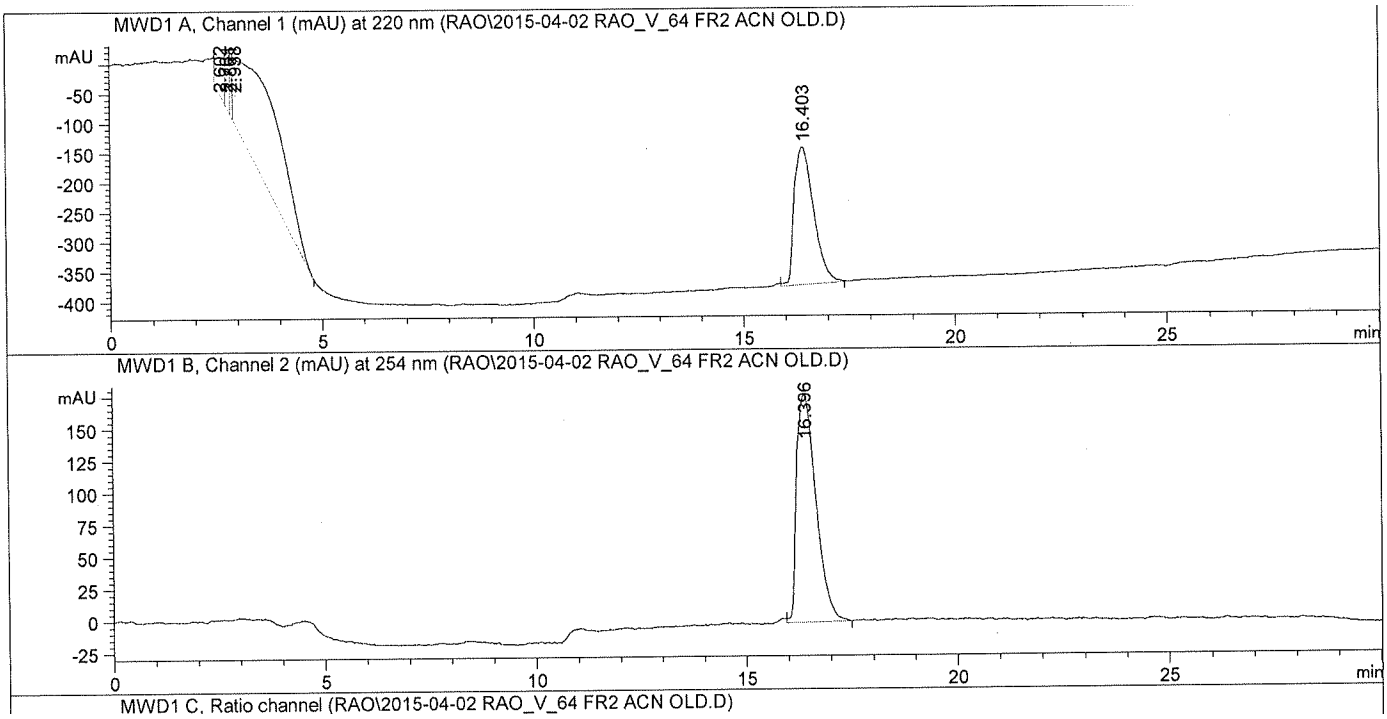
3-(2-Cyclopropoxyquinolin-6-yl)-1-(2-methoxy-2-methylpropyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

Compound 12 (1677)

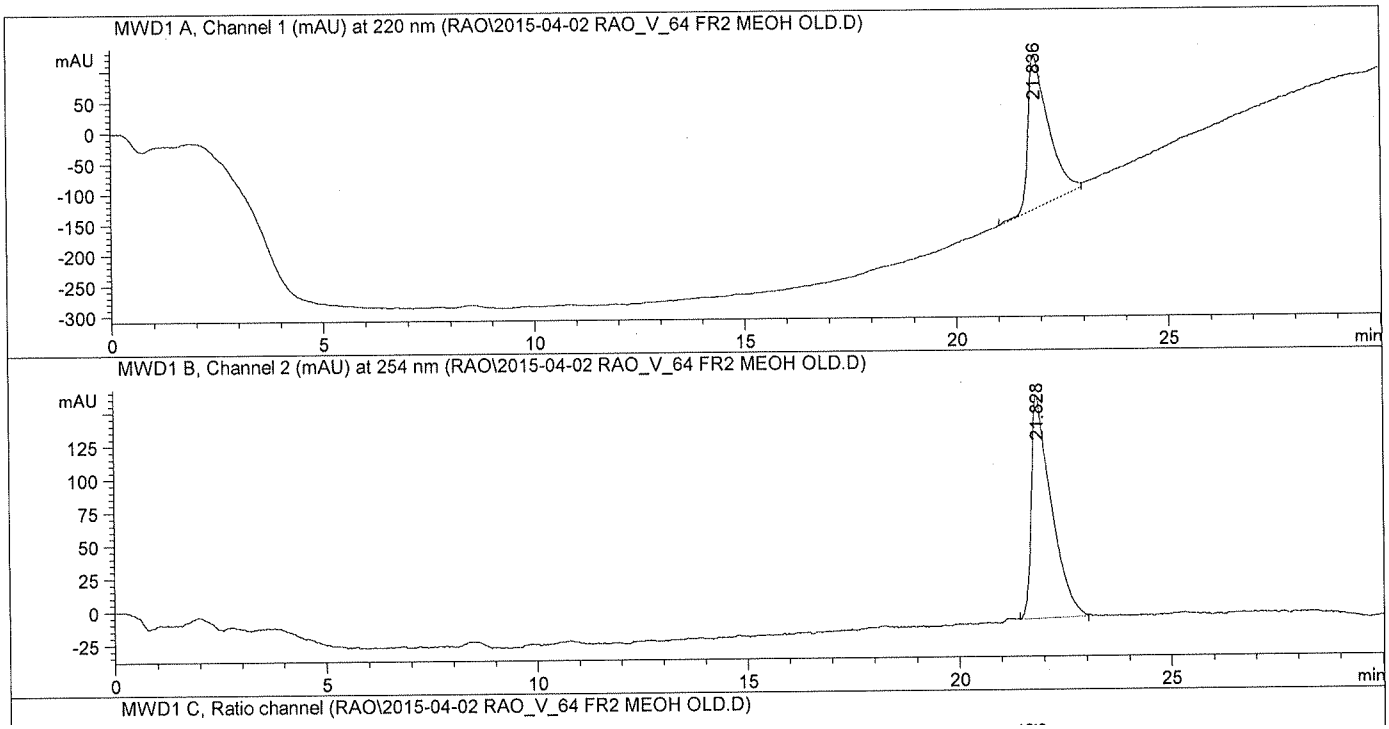
1H-NMR



Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



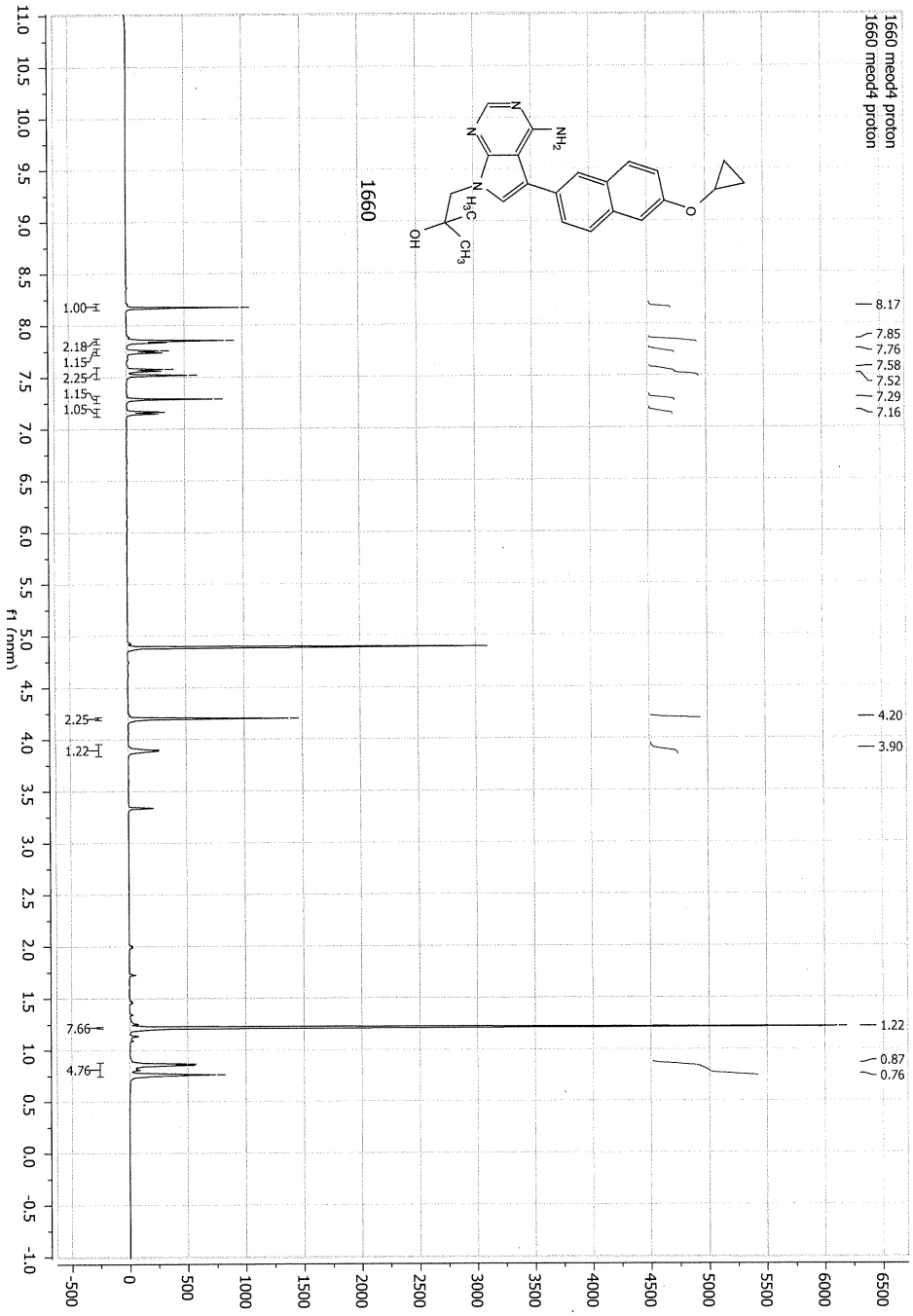
Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm



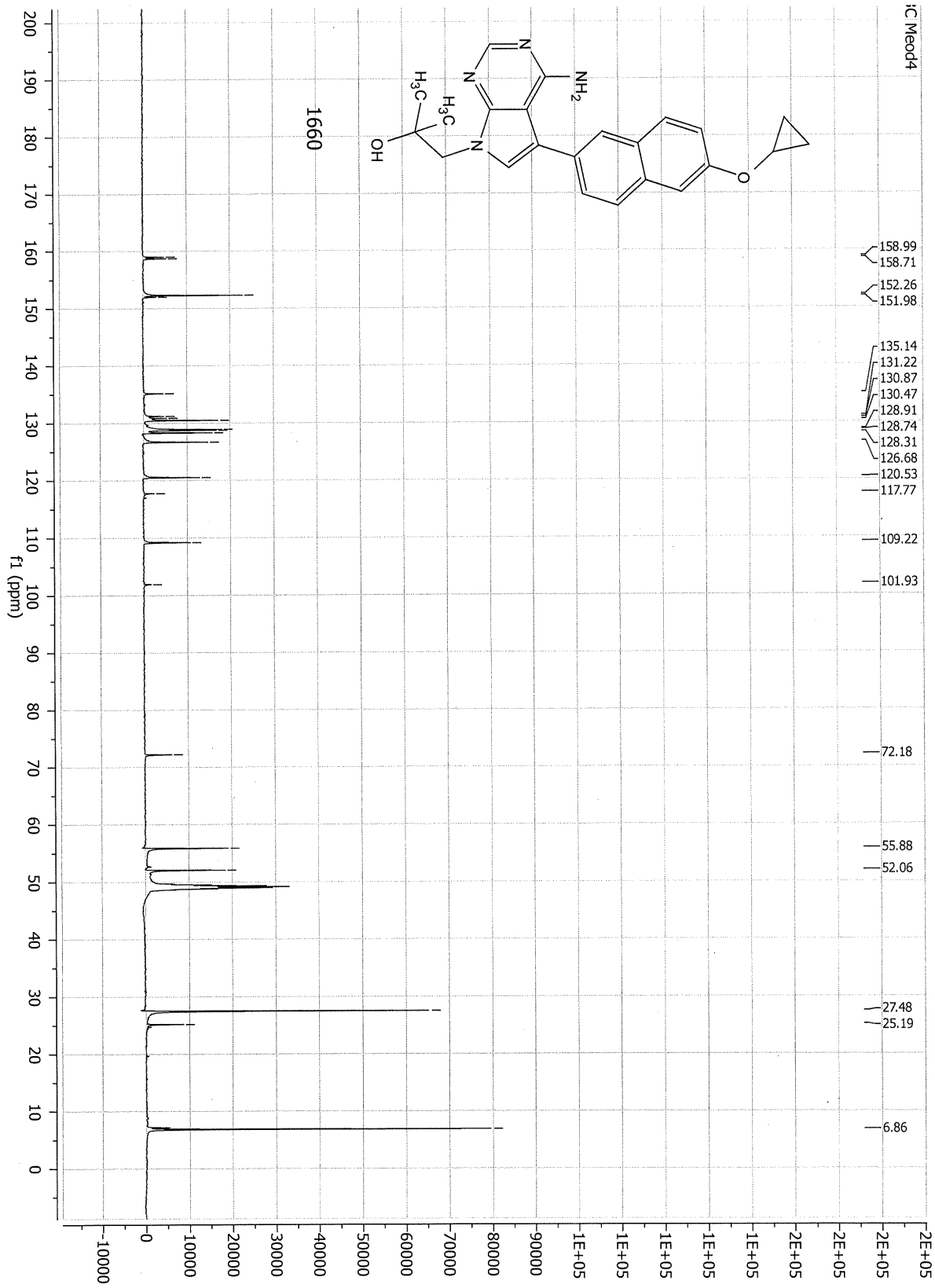
1-(4-amino-5-(6-cyclopropoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol

Compound 14

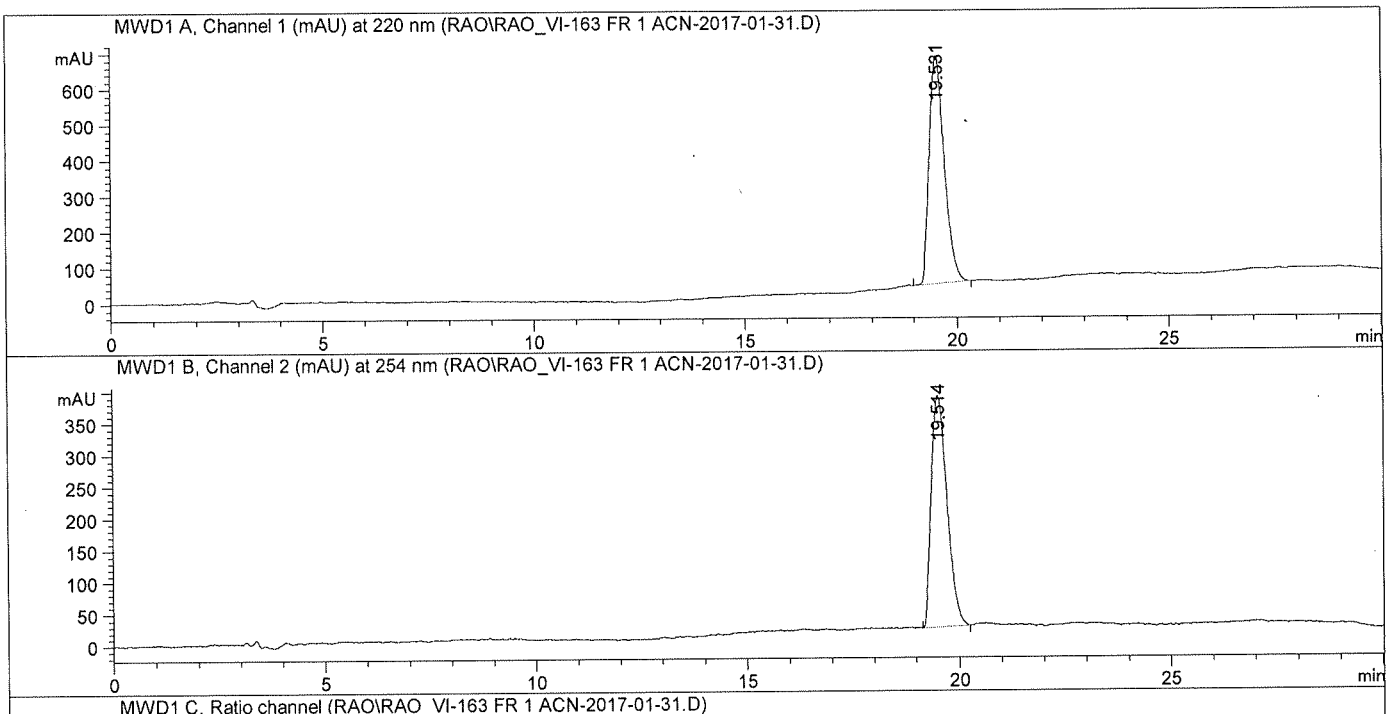
1H-NMR



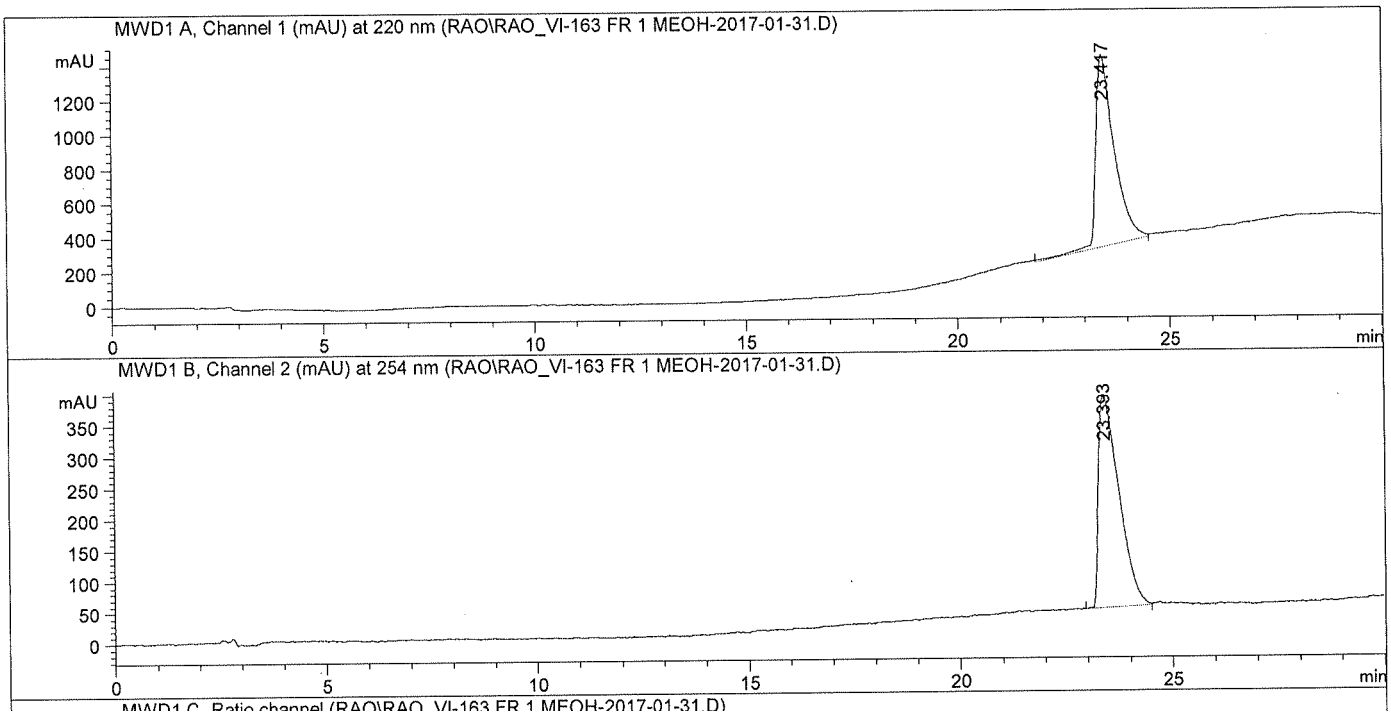
13C-NMR


1660
iC Meod4
158.99
158.71
152.26
151.98
135.14
131.22
130.87
130.47
128.91
128.74
128.31
126.68
120.53
117.77
109.22
101.93
72.18
55.88
52.06
27.48
25.19
6.86
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm

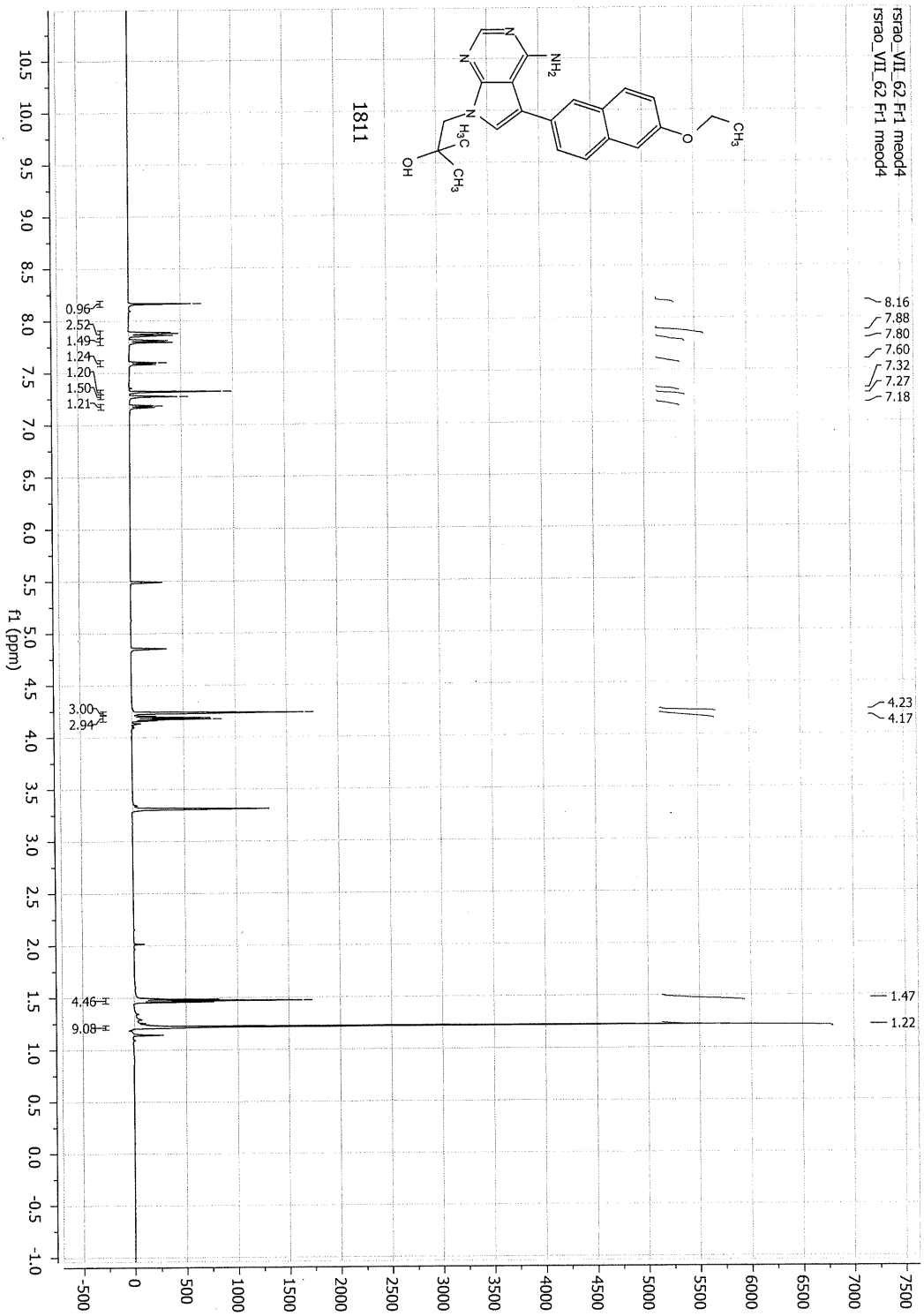


Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm

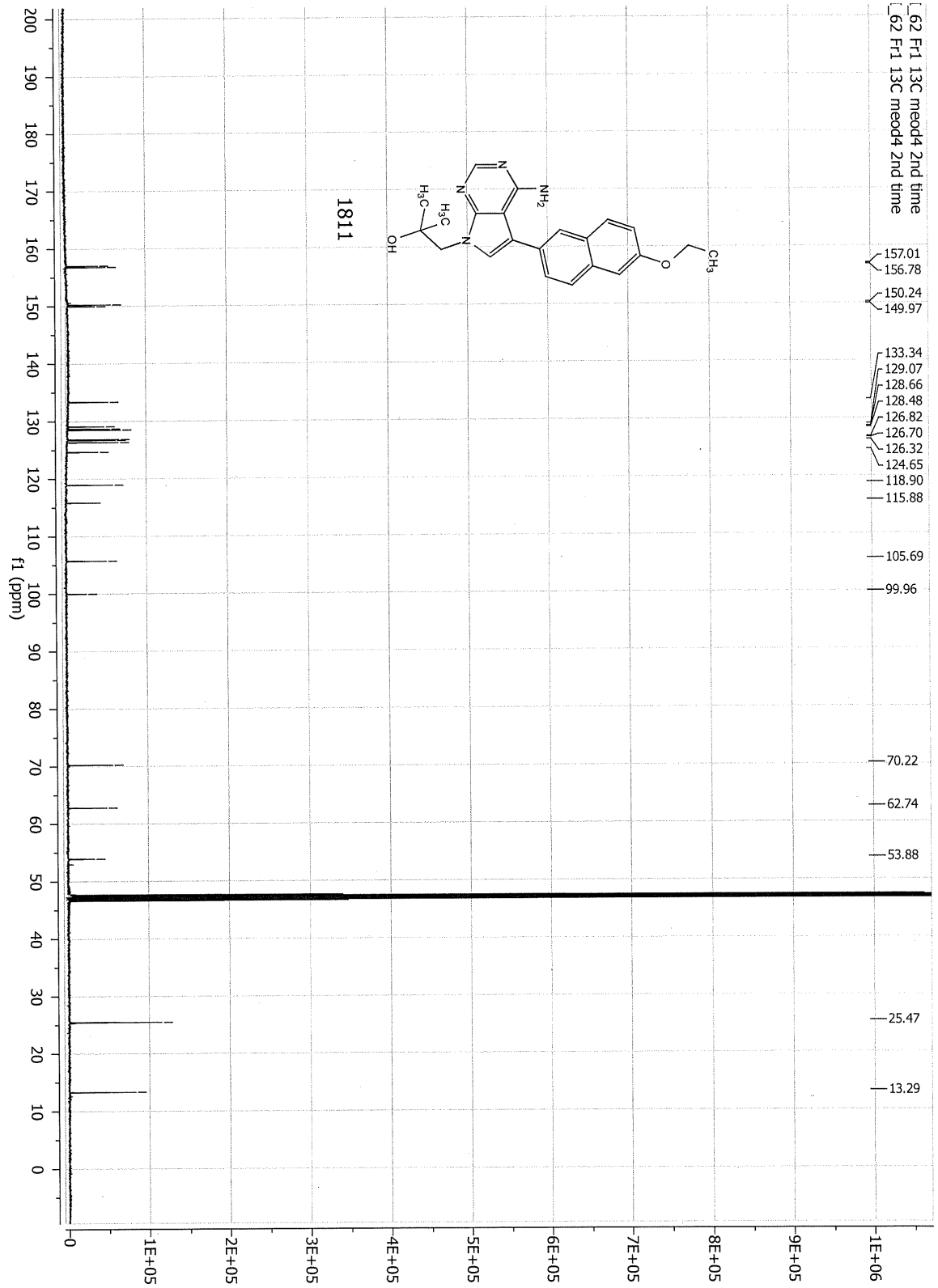


1-(4-Amino-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol
Compound 15 (1811)

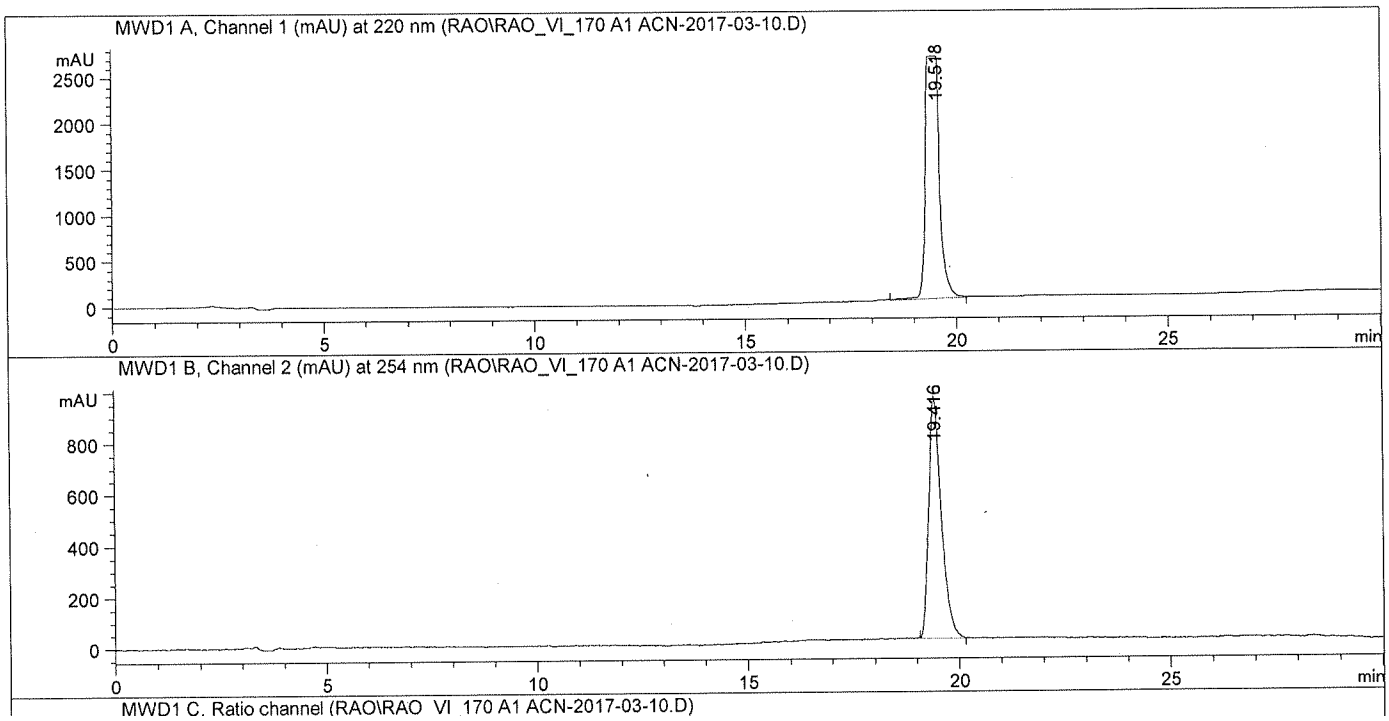
1H-NMR



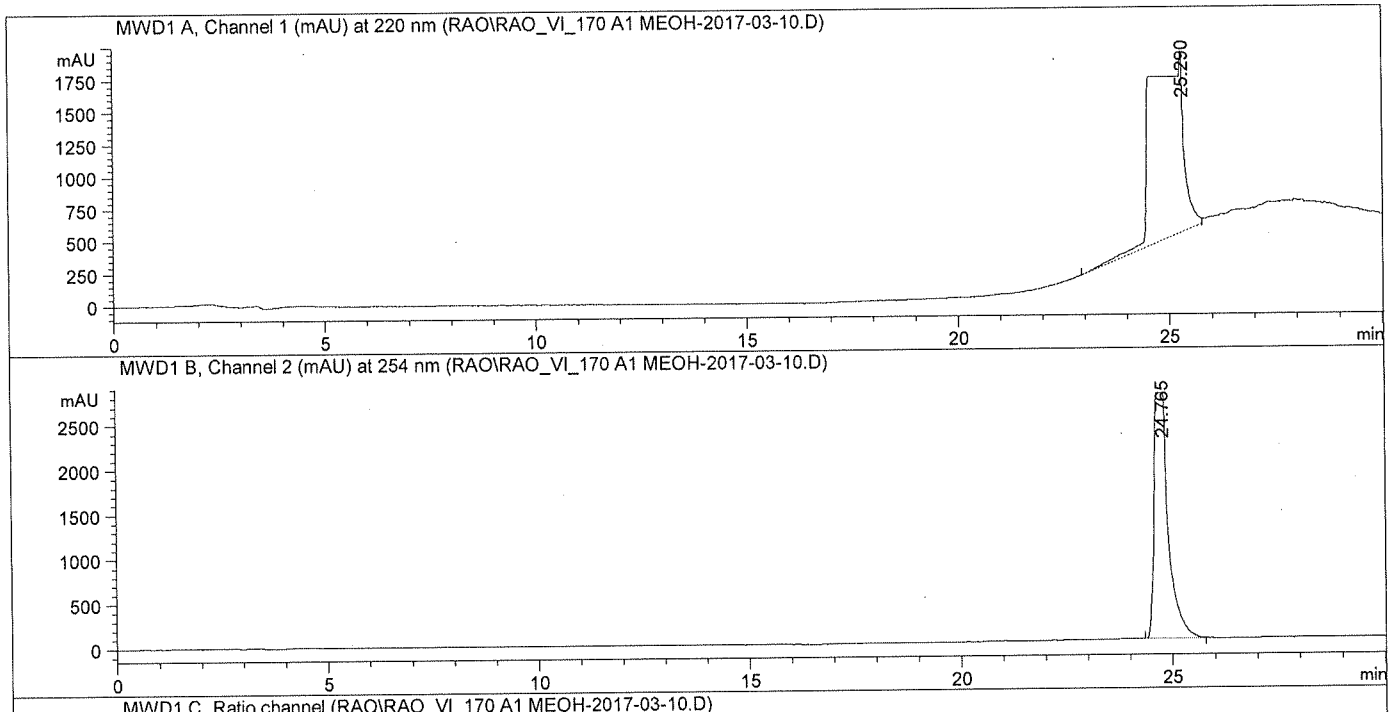
13C-NMR


_62 Fr1 13C meod4 2nd time
_62 Fr1 13C meod4 2nd time
1811
157.01
156.78
150.24
149.97
133.34
129.07
128.66
128.48
126.82
126.70
126.32
124.65
118.90
115.88
105.69
99.96
70.22
62.74
53.88
25.47
13.29
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm

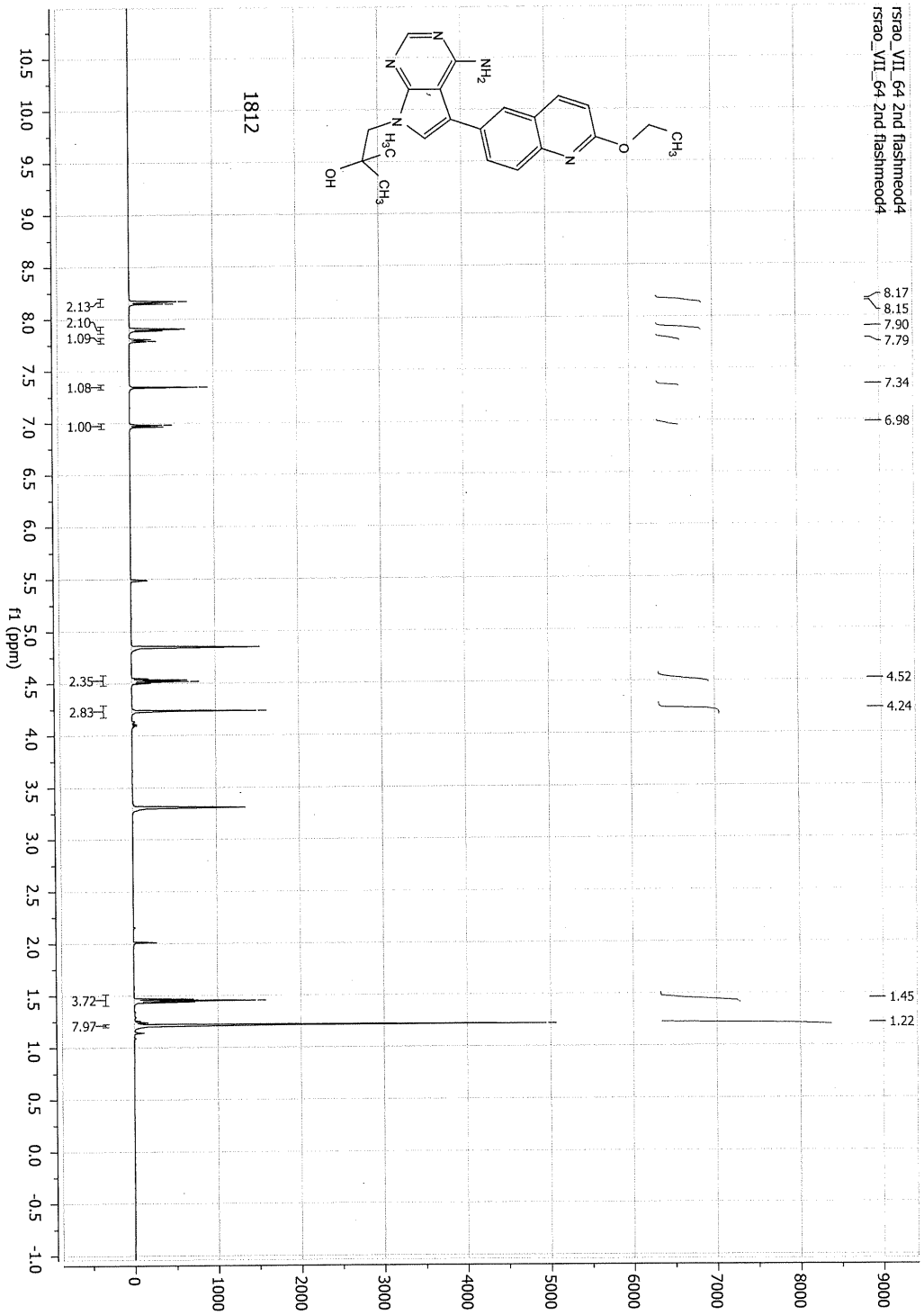


Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm

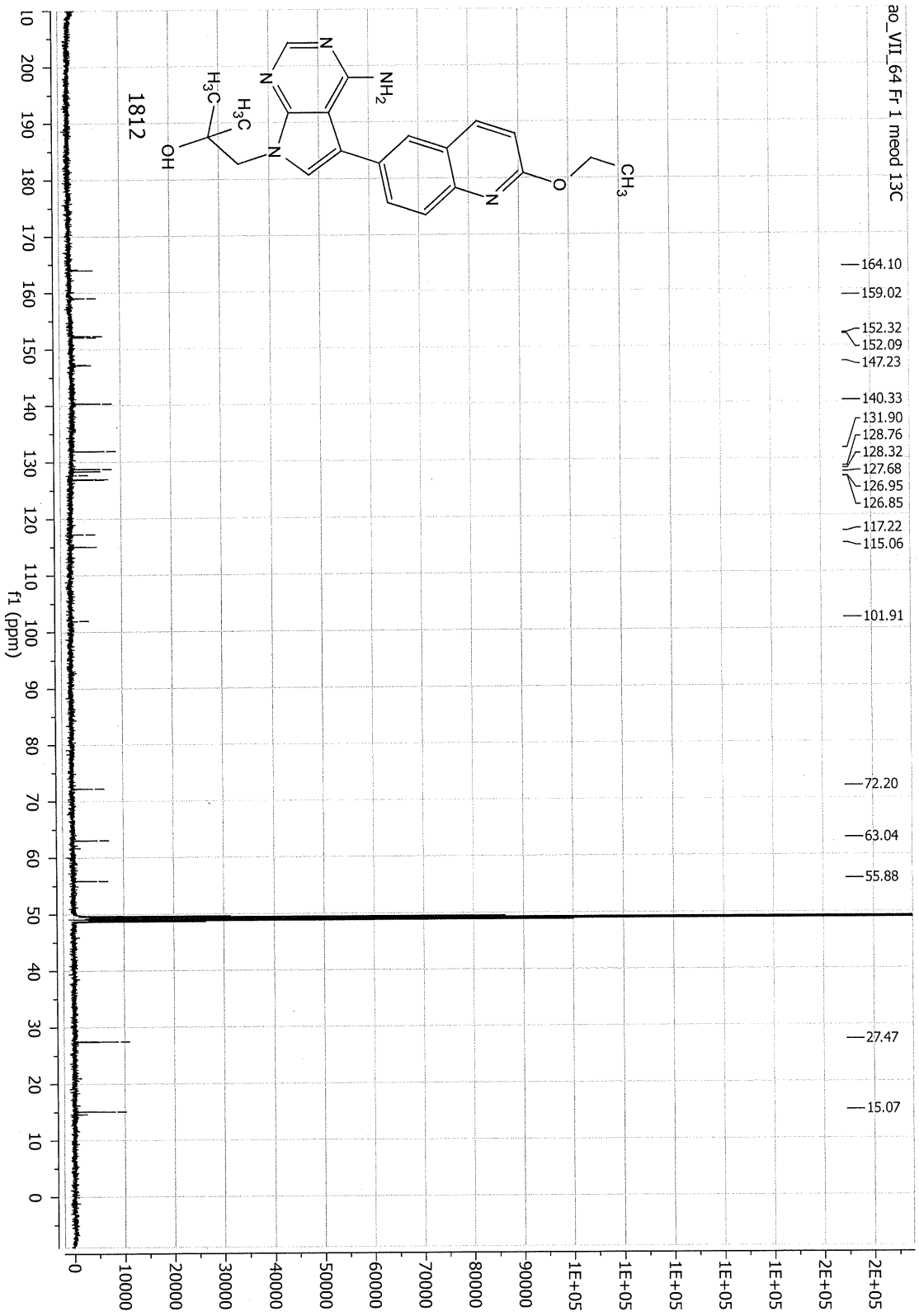


1-(4-Amino-5-(2-ethoxyquinolin-6-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol
Compound 16 (1812)

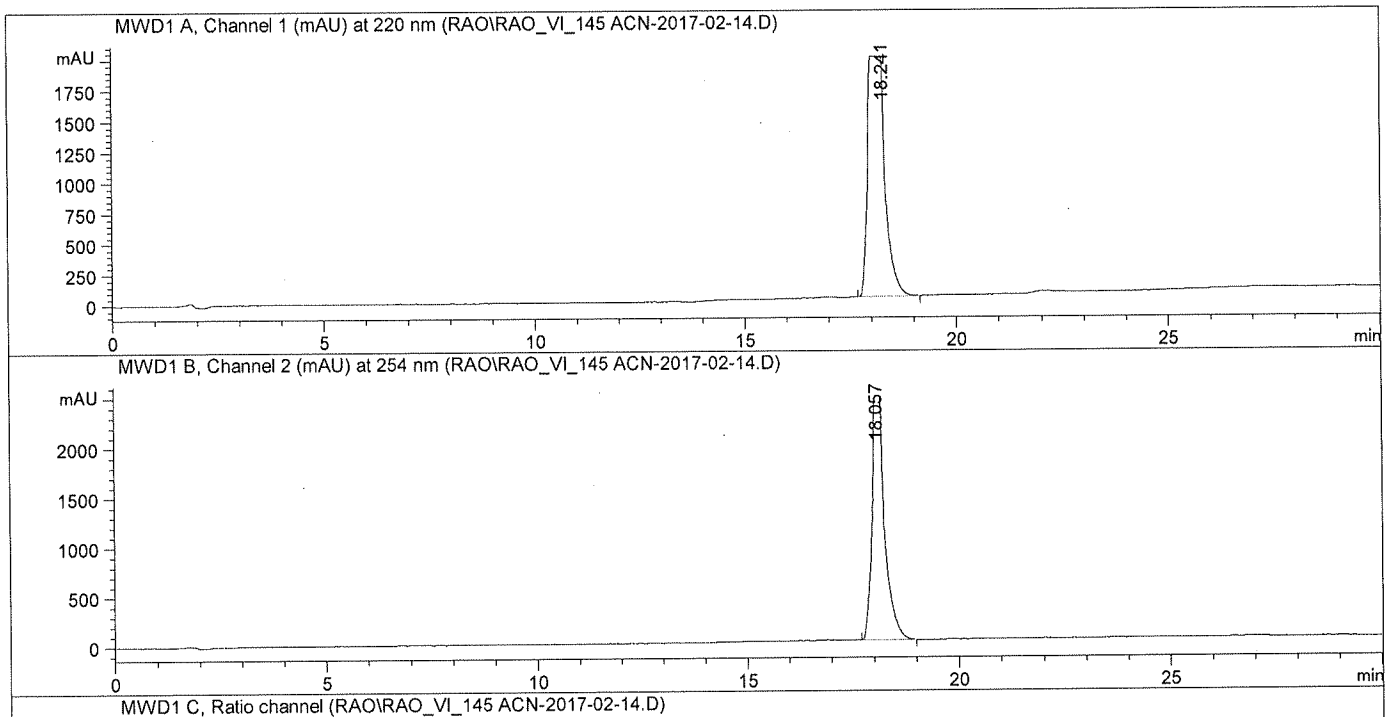
1H-NMR



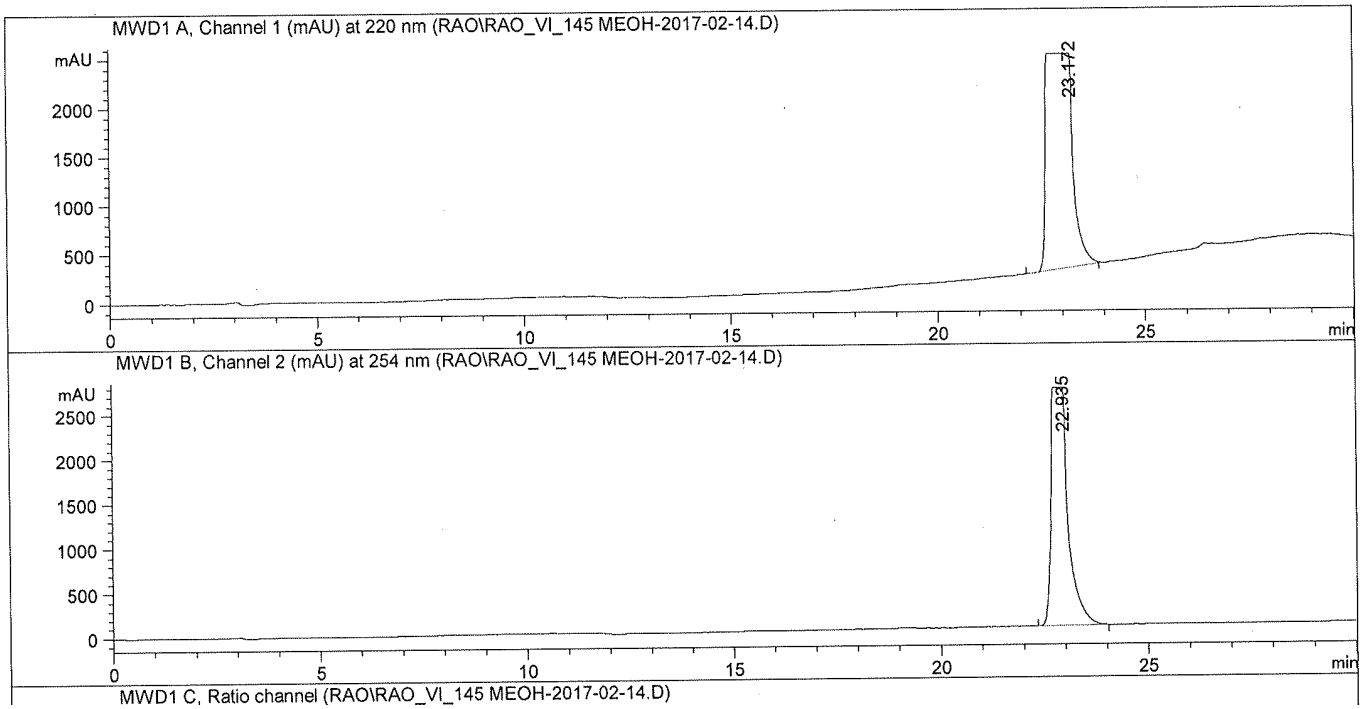
13C-NMR


ao_VII_64 Fr 1 meod 13C
1812
164.10
159.02
152.32
152.09
147.23
140.33
131.90
128.76
128.32
127.68
126.95
126.85
117.22
115.06
101.91
72.20
63.04
55.88
27.47
15.07
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



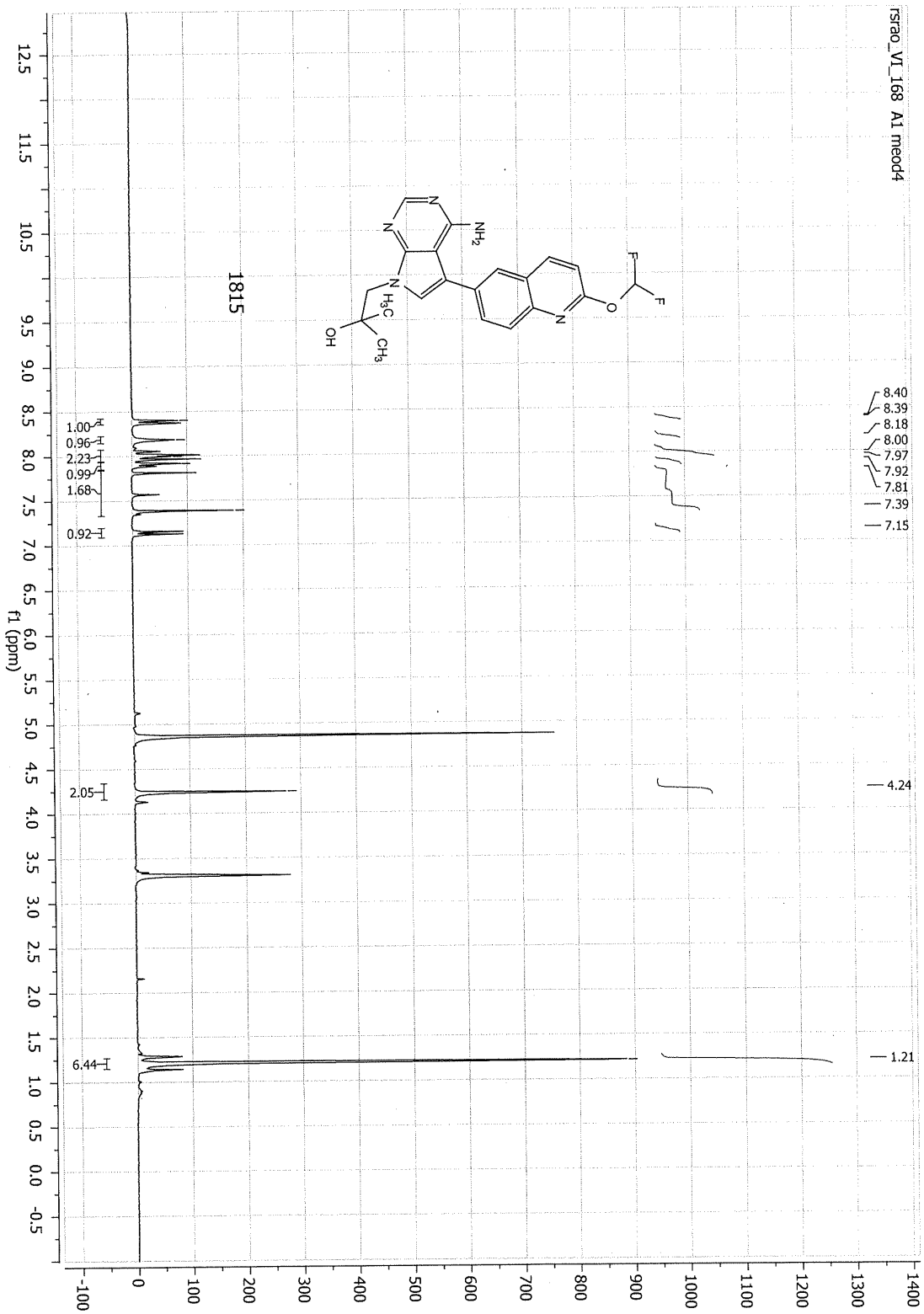
Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm



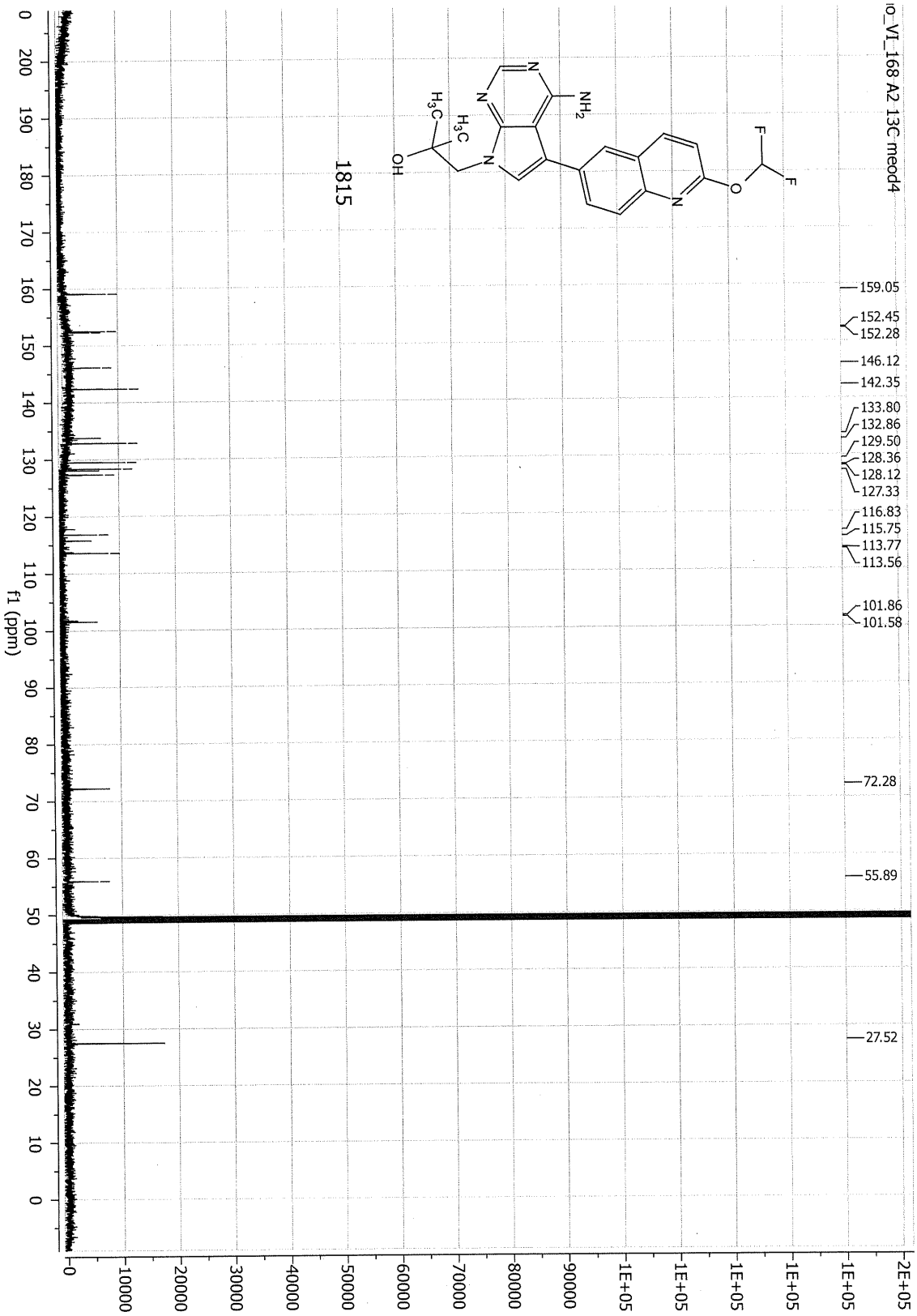
1-(4-amino-5-(2-(difluoromethoxy)quinolin-6-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-methylpropan-2-ol

Compound 17 (1815)

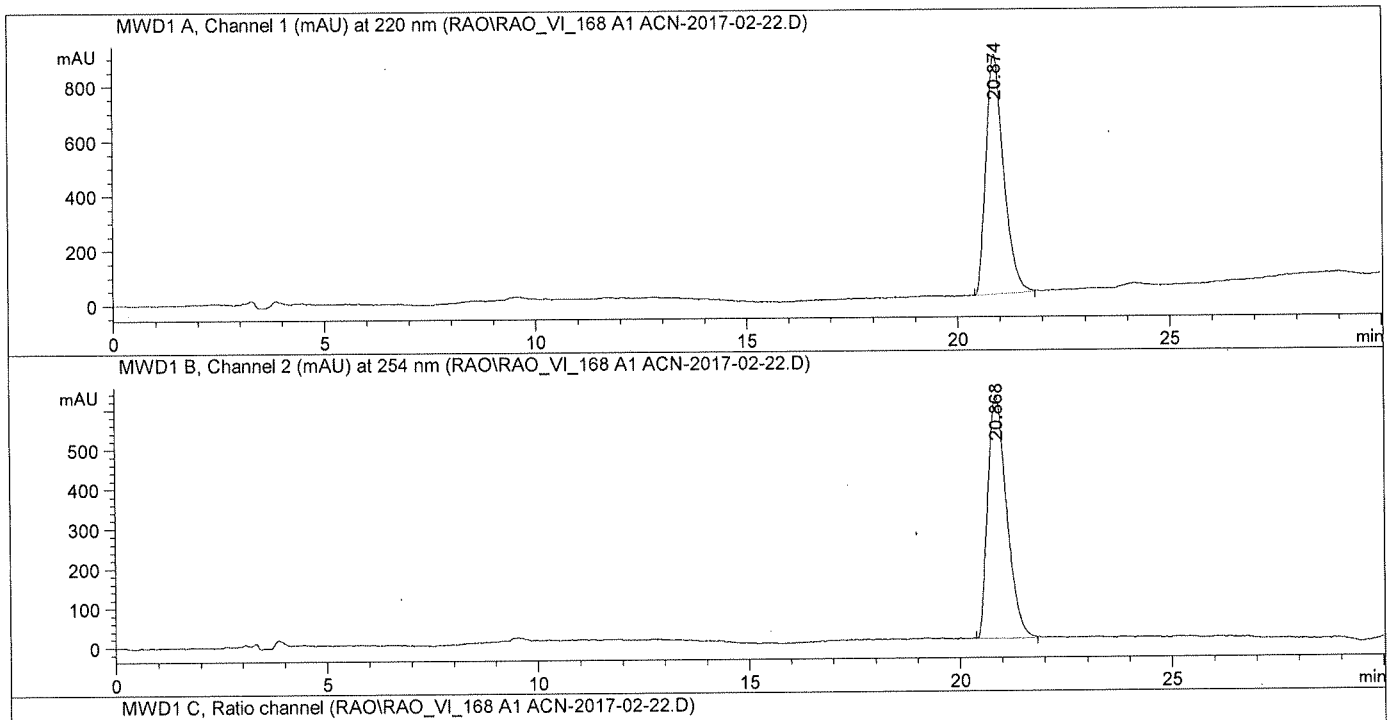
1H-NMR



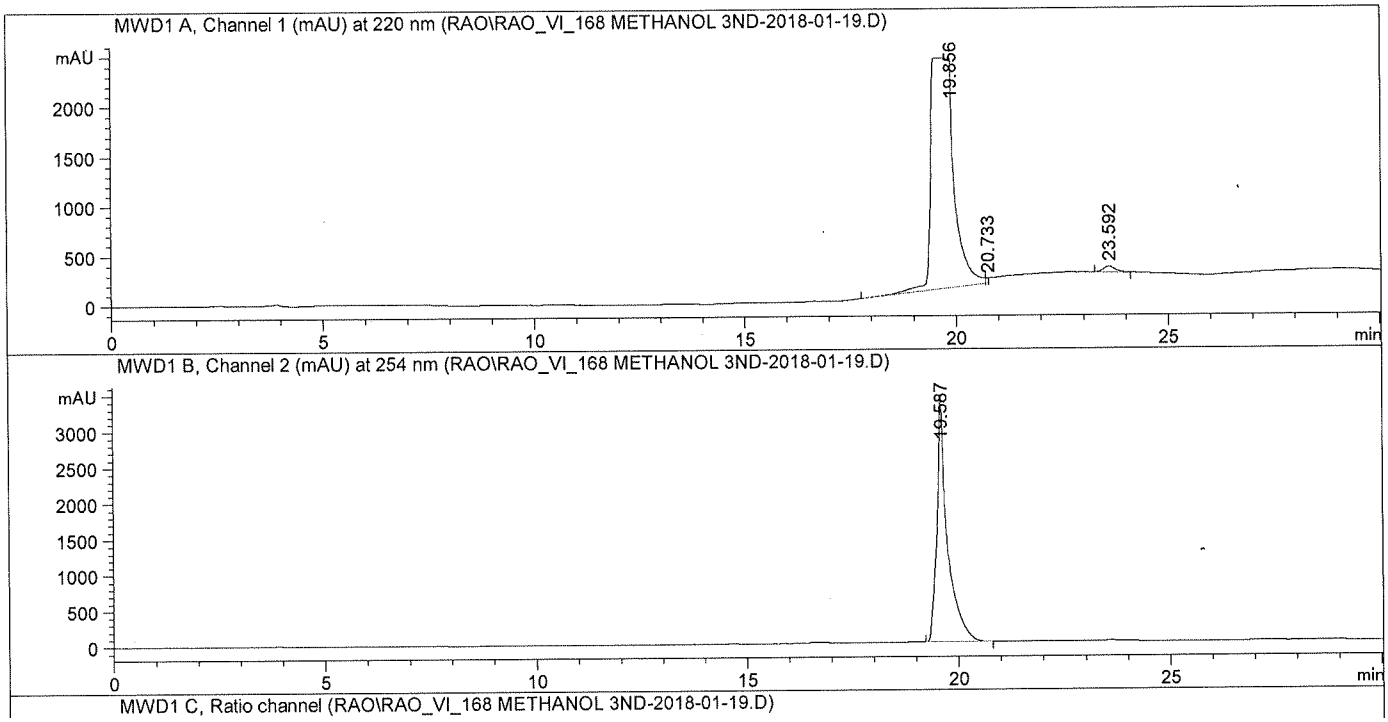
13C-NMR


iO_VI_168 A2 13C meod4
1815
159.05
152.45
152.28
146.12
142.35
133.80
132.86
129.50
128.36
128.12
127.33
116.83
115.75
113.77
113.56
101.86
101.58
72.28
55.89
27.52
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



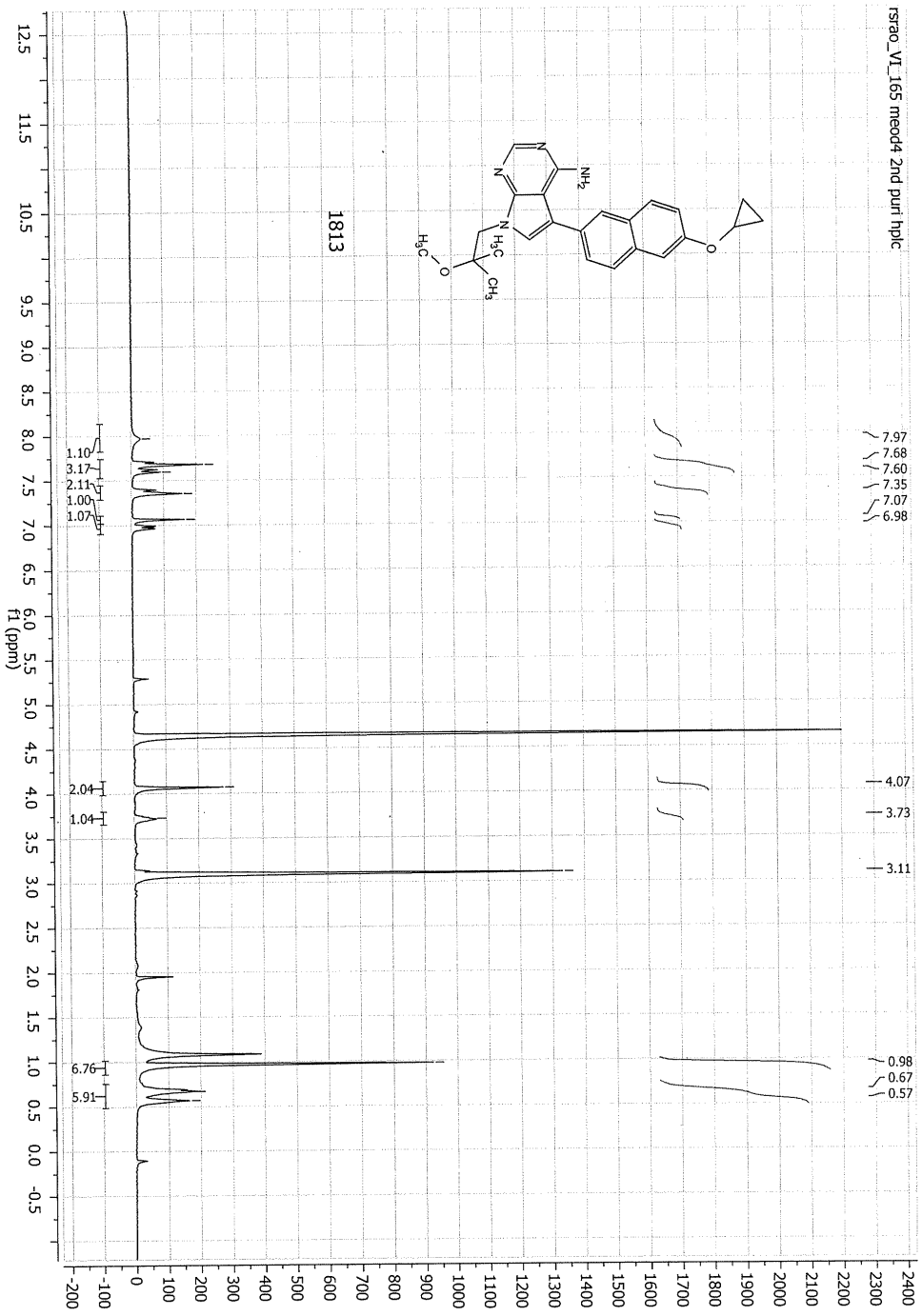
Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm



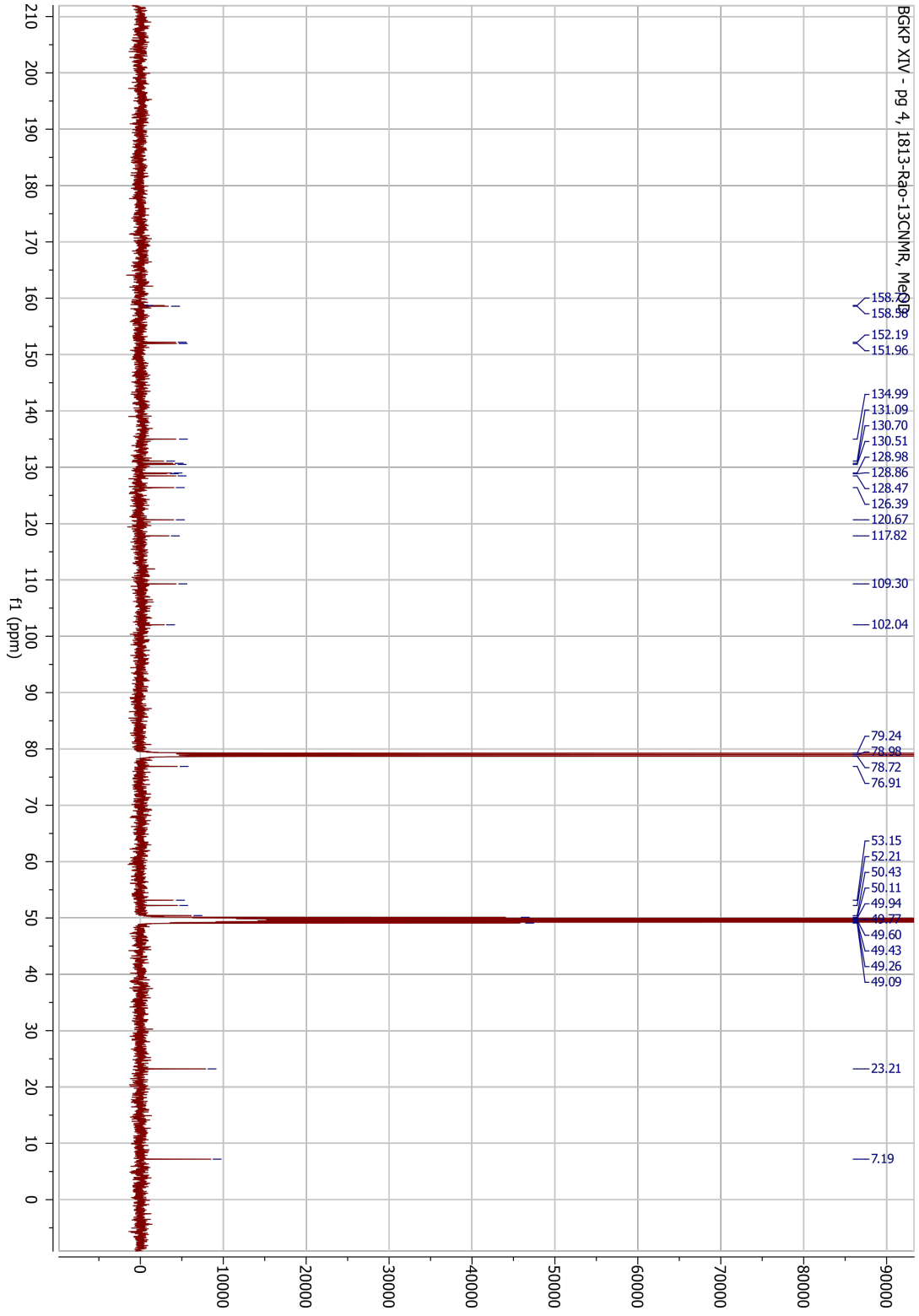
5-(6-Cyclopropoxynaphthalen-2-yl)-7-(2-methoxy-2-methylpropyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine

Compound 18 (1813)

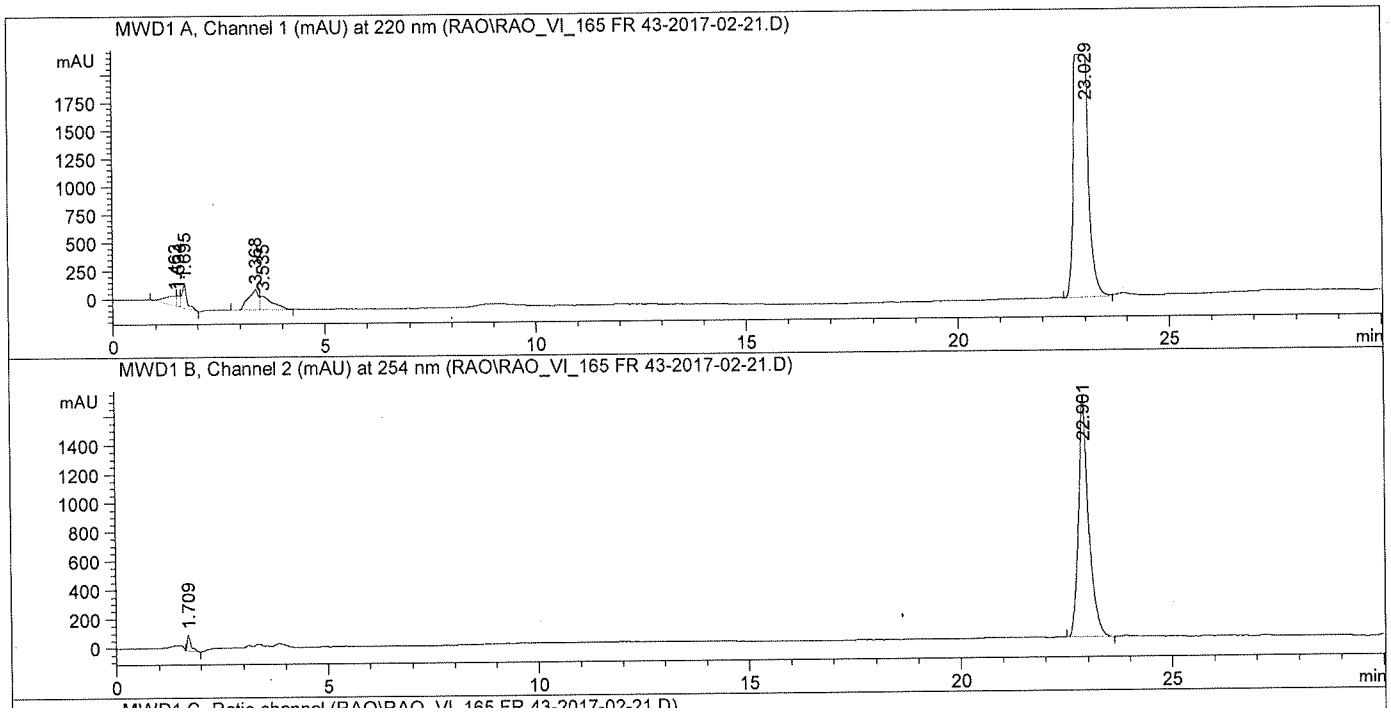
1H-NMR



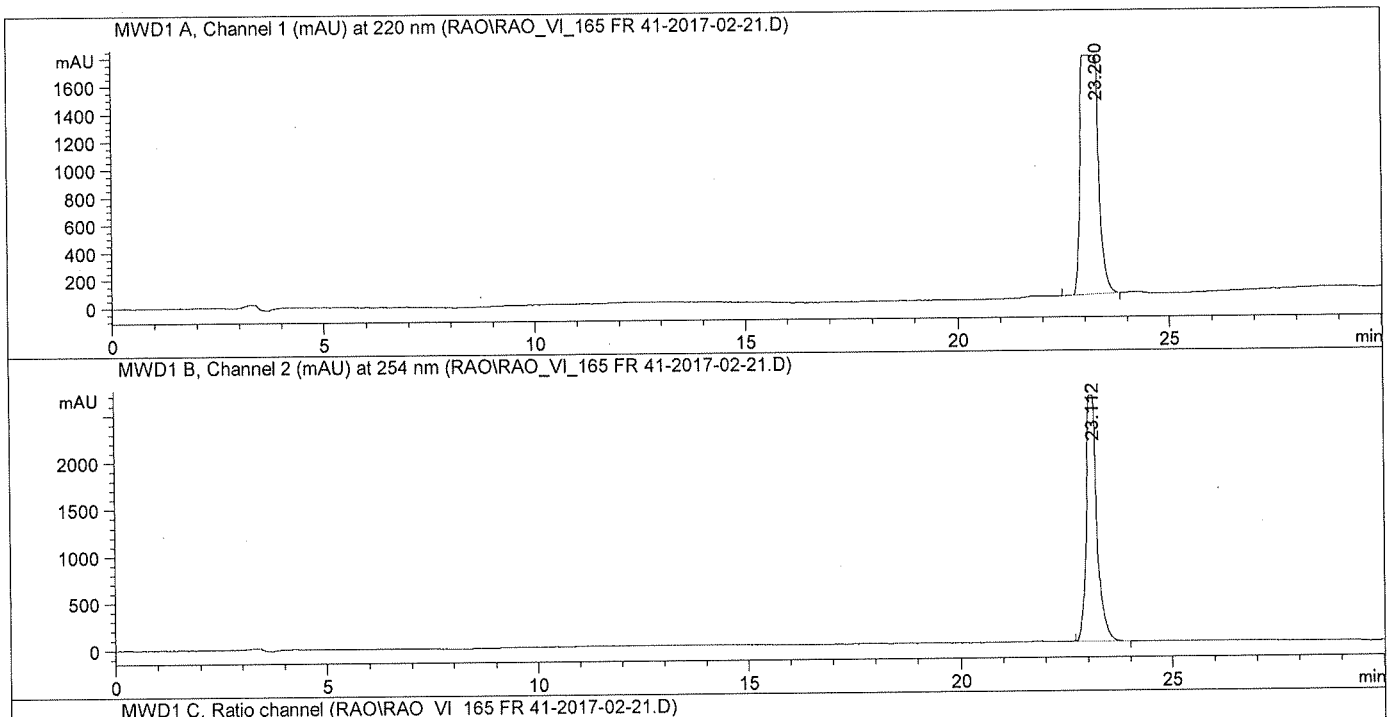
13C-NMR


BGKP XIV - pg 4, 1813-Rao-13CNMR, MeOD
158.74
158.58
152.19
151.96
134.99
131.09
130.70
130.51
128.98
128.86
128.47
126.39
120.67
117.82
109.30
102.04
79.24
78.98
78.72
76.91
53.15
52.21
50.43
50.11
49.94
49.77
49.60
49.43
49.26
49.09
23.21
7.19
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



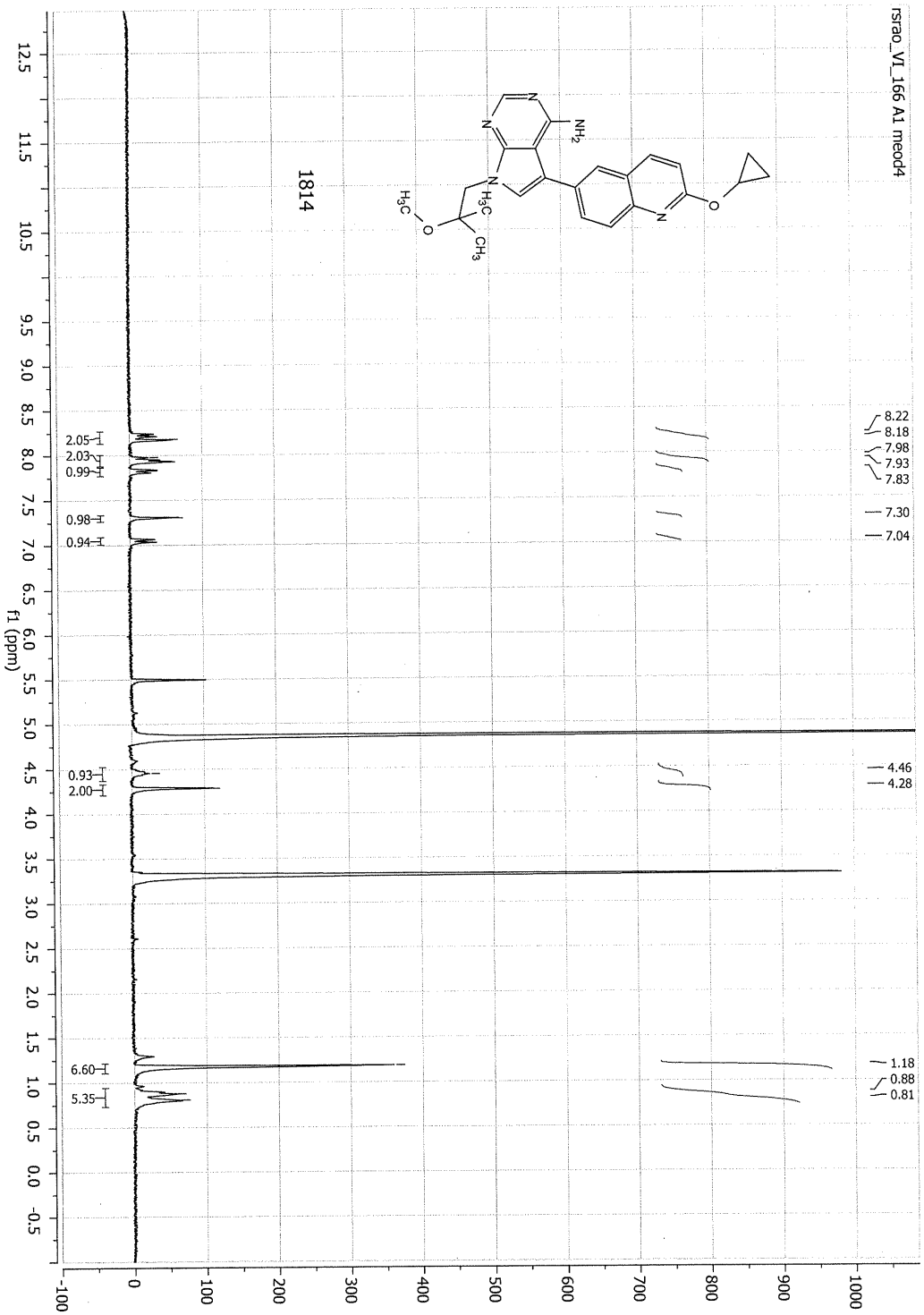
Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm



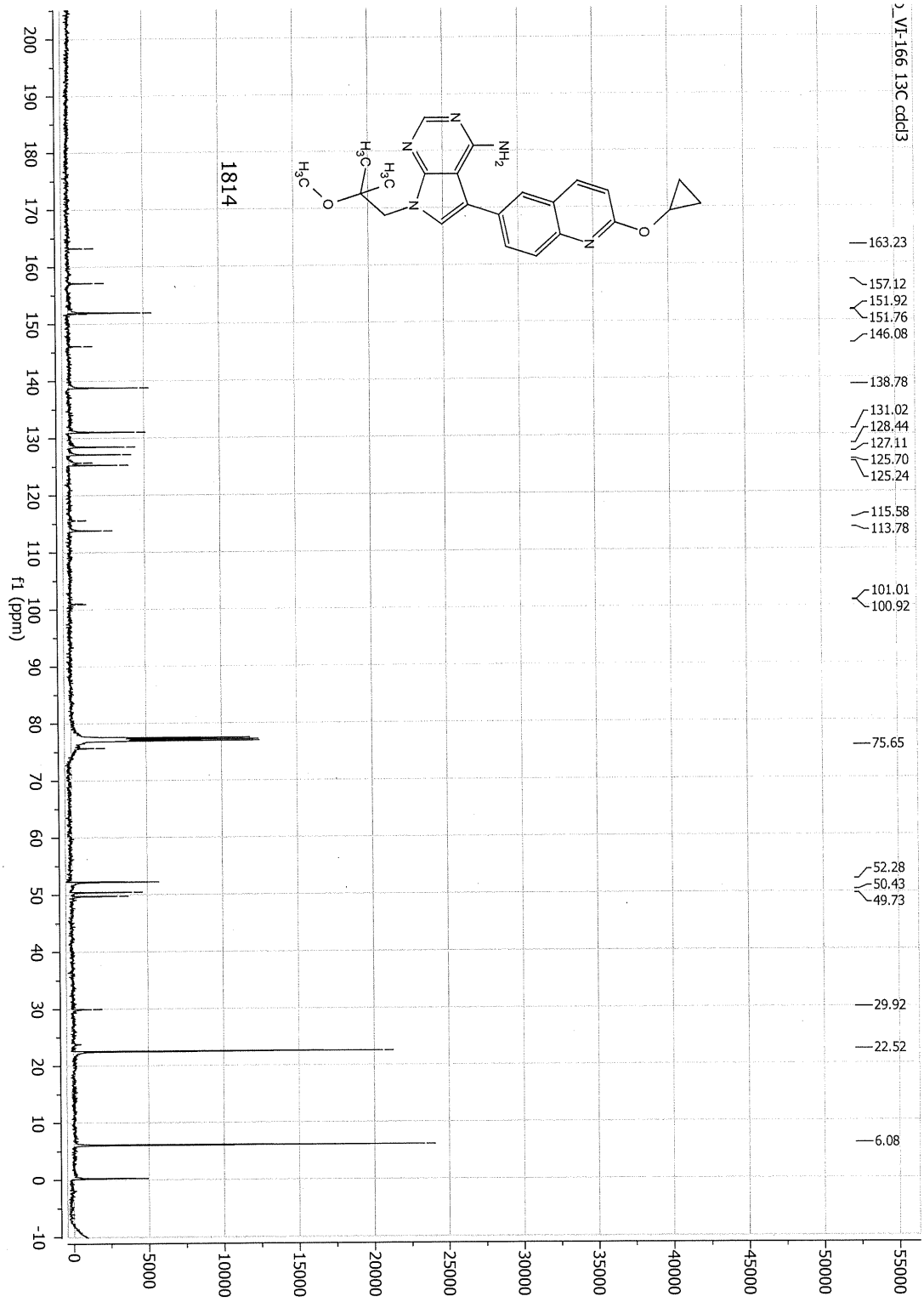
5-(2-Cyclopropoxyquinolin-6-yl)-7-(2-methoxy-2-methylpropyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine

Compound 19 (1814)

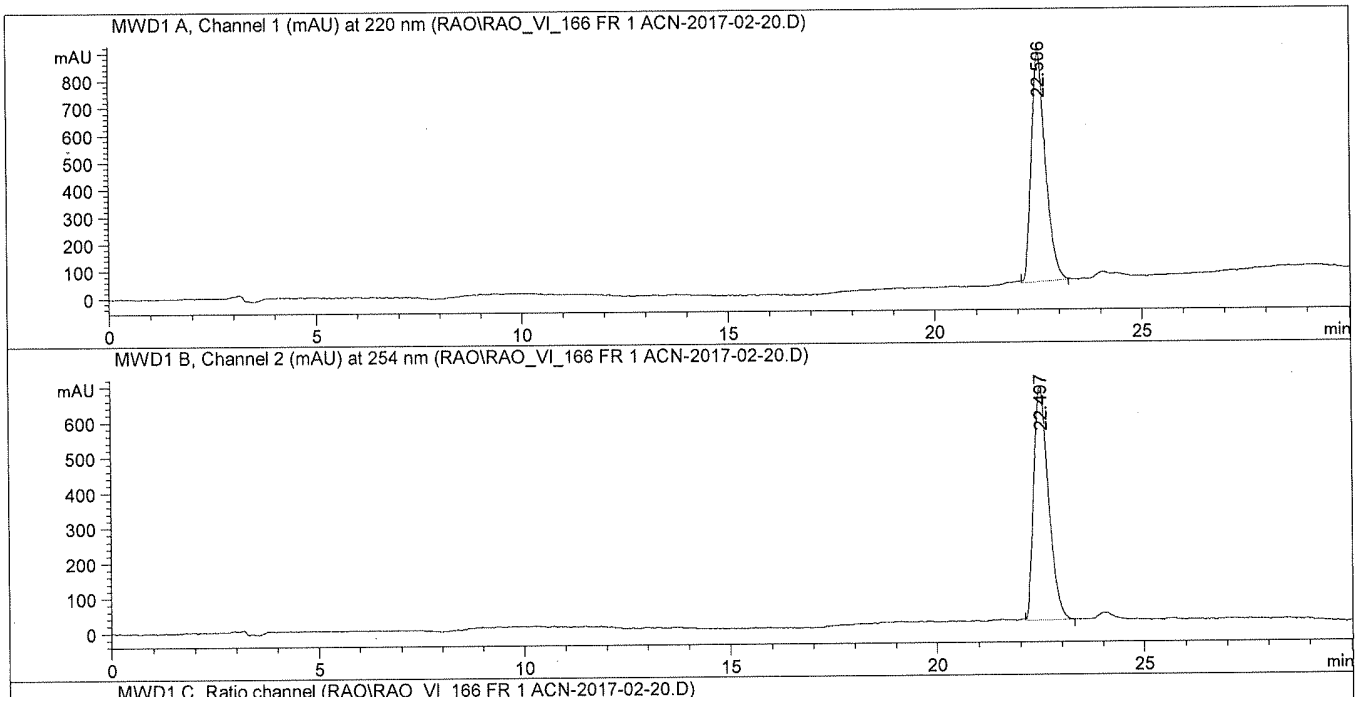
1H-NMR



13C-NMR


1814
163.23
157.12
151.92
151.76
146.08
138.78
131.02
128.44
127.11
125.70
125.24
115.58
113.78
101.01
100.92
75.65
52.28
50.43
49.73
29.92
22.52
6.08
f1 (ppm)

Analytical HPLC: H_2O/CH_3CN (method 1) Top λ=220 nm; bottom λ=254 nm



Analytical HPLC: H_2O/CH_3OH (method 2) Top λ=220 nm; bottom λ=254 nm