Discovery of A New Human A_{2A} Adenosine Receptor Agonist, Truncated 2-Hexynyl-4'-thioadenosine

Xiyan Hou,[1] Hea Ok Kim,[1] Varughese Alexander,[1] Kyunglim Kim,[1] Sun Choi,[1] Seul-gi Park,[1] Jin Hee Lee,[1] Lena S. Yoo,[2] Zhan-Guo Gao,[2] Kenneth A. Jacobson,[2] and Lak Shin Jeong*[,1,3]

[1] Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea, [2]Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, and Digestive and Kidney Disease, National Institutes of Health, Bethesda, Maryland 20892, USA, and [3]Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Korea

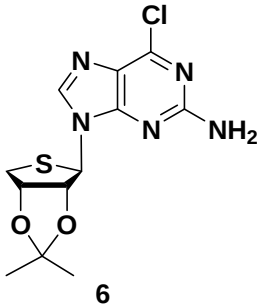
lakjeong@ewha.ac.kr

Supporting Information

General Methods. ^{1}H NMR spectra ($CDCl_3$, CD_3OD, or DMSO-d_6) were recorded on Varian

Unity Inova 400 MHz. Chemical shifts are reported in parts per million (δ) units relative to the solvent peak. The ^{1}H NMR data are reported as peak multiplicities: s for singlet; d for doublet; dd for doublet of doublets; t for triplet; pseudo t for pseudo triplet; brs for broad singlet; and m for multiplet. Coupling constants were reported in Hertz. ^{13}C NMR spectra ($CDCl_3$, CD_3OD, or DMSO-d_6) were recorded on Varian Unity Inova 100 MHz. NOE experiments were recorded on Varian Unity Inova 400 MHz, using $CDCl_3$ and DMSO-d_6 as solvent and a mixing time of 1 second. The chemical shifts are reported as ppm (δ) relative to the solvent peak. Mass spectra were recorded on Agilent Technologies 6220 Accurate-Mass TOF LC/MS spectrometer. Melting points were measured on IA9100 made by Barnstead. These are uncorrected. UV spectra were recorded on U-3000 made by Histachi in methylene chloride or methanol. Optical rotations were determined on Jasco III in methylene chloride or methanol. Infrared spectra were recorded on FT-IR (FTS-135) made by Bio-Rad. Reactions were checked with TLC (Merck precoated 60F254 plates). Spots were detected by viewing under a UV light, colorizing with charring after dipping in anisaldehyde solution with acetic acid and sulfuric acid and methanol. Column chromatography were performed on silica gel 60 (230-400 mesh, Merck). Reagents were purchased from Aldrich Chemical Company. Solvents were obtained from local suppliers. All the anhydrous solvents were distilled over CaH_2, P_2O_5, or Na/benzophenone prior to the reaction.

6-chloro-9-((3a*S*,4*R*,6a*R*)- tetrahydro-2,2-dimethylthieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-2-amine (6)

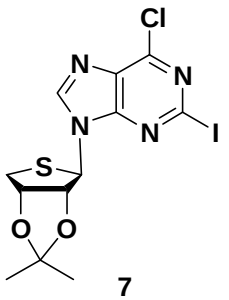


6

A solution of 2-amino-6-chloropurine (0.91 g, 5.35 mmol), ammonium sulfate (106 mg, 0.80 mmol) and HMDS (10 mL) were refluxed overnight, under dry and inert conditions. The solution was then carefully evaporated under high vacuum. The solid residue thus obtained, was dissolved in 1,2-dichloroethane (5 mL) and cooled to 0 ^{o}C. The solution of **5** (0.58 g, 2.68 mmol) in 1,2-dichloroethane (5 mL) was added dropwise to the above mixture. Then, TMSOTf (0.97 mL, 5.35 mmol) was dropwise added and the mixture was stirred for 30

minutes at 0 °C, for 1 h at rt, and finally heated at 90 °C for 2 h. The reaction mixture was cooled, diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. The extracted organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. The crude syrup was subjected to flash silica gel column chromatography (Hexane : EtOAc = 1:1) to give **6** (0.27 g, 30%) as a solid: mp 199.1-199.2 °C; UV (CH_2Cl_2) λ_{max} 305.0 nm; ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1 H), 5.73 (s, 1 H), 5.29 (brs, 2 H, NH_2), 5.16-5.21 (m, 2H), 3.64 (dd, 1 H, *J* = 4.4, 13.2 Hz), 3.20 (d, 1 H, *J* = 13.2 Hz), 1.58 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 159.14, 153.21, 151.91, 141.14, 126.24, 111.96, 89.50, 84.23, 69.48, 40.78, 26.54, 24.83; $[\alpha]^{25}_D$ -42.23 (*c* 0.16, CH_2Cl_2); (ESI+) ($M+Na^+$) m/z 350.0443; Anal. Calcd for $C_{12}H_{14}ClN_5O_2S$: C, 43.97; H, 4.30; Cl, 10.82; N, 21.37; O, 9.76; S, 9.78. Found: C, 43.98; H, 4.28; N, 20.99; S, 10.00.

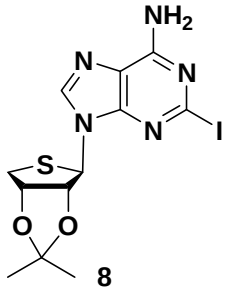
6-chloro-9-(3a*S*,4*R*,6a*R*)- tetrahydro-2,2-dimethylthieno[3,4-*d*][1,3]dioxol-4-yl)-2-iodo-9*H*-purine (7).



7

Isoamylnitrite (0.31mL, 2.27mmol) was added to a mixture of **6** (248 mg, 0.76mmol), I_2 (192 mg, 0.76 mmol), CH_2I_2 (0.95 mL, 7.60 mmol) and CuI (159 mg, 0.83mmol) in 10 mL THF at rt. The mixture was then allowed to reflux for 45 min and finally cooled to room temperature. Insoluble materials were removed by filtration and the filtrate thus obtained was concentrated to dryness. The crude residue was purified by means of silica gel column chromatography, which was washed with hexane until the color of iodine disappeared and then eluted with Hexane : EtOAc = 7 : 1, to give **7** (233mg 72%) as a syrup: UV (CH_2Cl_2) λ_{max} 282.0 nm; ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1 H), 5.84 (s, 1 H), 5.33 (pseudo t, 1 H, *J* = 5.2 Hz), 5.21 (d, 1 H, *J* = 5.6 Hz), 3.80 (dd, 1 H, *J* = 4.4, 12.8 Hz), 3.26 (d, 1 H, *J* = 12.8 Hz), 1.59 (s, 3 H), 1.37 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 151.91, 151.21, 144.39, 132.65, 116.74, 112.13, 89.97, 84.85, 70.73, 41.48, 26.60, 24.78; $[\alpha]^{25}_D$ -66.33 (*c* 0.10, CH_2Cl_2); (ESI+) ($M+H^+$) m/z 438.9492; Anal. Calcd. for $C_{12}H_{12}ClIN_4O_2S$: C, 32.86; H, 2.76; Cl, 8.08; I, 28.93; N, 12.77; O, 7.29; S, 7.31. Found: C, 32.99; H, 2.48; N, 12.99; S, 7.01.

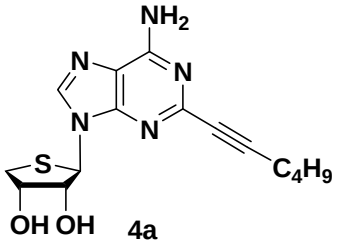
9-((3a*S*,4*R*,6a*R*)- tetrahydro-2,2-dimethylthieno[3,4-*d*][1,3]dioxol-4-yl)-2-iodo-9*H*-purin-6-amine (8)



8

A solution of **7** (535 mg, 1.22 mmol) in methanolic ammonia (5 mL) was stirred for 2 h at 80 °C. The reaction mixture was concentrated to dryness and the crude residue was subjected to flash silica gel column chromatography (Hexane : EtOAc = 1 : 1) to give **8** (439 mg, 85%) as a syrup: UV (CH_2Cl_2) λmax 267.0 nm; ^{1}H NMR (CD_3OD) δ 8.20 (s, 1 H), 5.97 (s, 1 H), 5.30 (pseudo t, 1 H, *J* = 5.2 Hz), 5.23 (d, 1 H, *J* = 5.6 Hz), 3.80 (dd, 1 H, *J* = 4.4, 12.8 Hz), 3.14 (d, 1 H, *J* = 12.8 Hz), 1.54 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR (CD_3OD) δ 156.11, 150.37, 141.97, 119.96, 117.99, 112.68, 91.19, 86.37, 71.30, 41.58, 26.84, 24.86. $[\alpha]^{25}_D$ -61.90 (*c* 0.08, CH_2Cl_2); (ESI+) ($M+H^+$) m/z 419.998; Anal. Calcd. for $C_{12}H_{14}IN_5O_2S$: C, 34.38; H, 3.37; I, 30.27; N, 16.70; O, 7.63; S, 7.65. Found: C, 33.56; H, 3.28; N, 16.99; S, 7.23.

(2*R*,3*S*,4*R*)-2-(6-amino-2-(hex-1-ynyl)-9*H*-purine-9-yl)-tetrahydrothiophene-3,4-diol (4a)

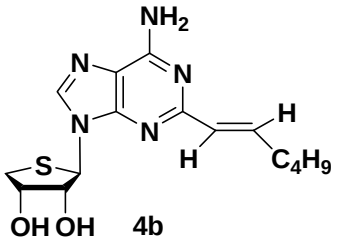


4a

Compound **8** (96 mg, 0.23 mmol) was dissolved in Et_3N (1.5 mL) and DMF (1 mL). After purging the solution with N_2, $(PPh_3)_2PdCl_2$ (16 mg, 0.023 mmol) and CuI (4.3 mg, 0.023 mmol) were added. 1-Hexyne (0.065 mL, 0.57 mmol) was subsequently added dropwise and the mixture was stirred at room temperature for 3 h. The solvents were removed under reduced pressure, to give the crude compound **9**. To a solution of **9** in THF (5 mL) was added 1 *N* hydrochloric acid (5 mL) and the mixture stirred at room temperature for 1 day. The crude residue was neutralized with 1 *N* NaOH solution, and then carefully evaporated under reduced pressure. The mixture was subjected to flash silica gel column chromatography

(CH_2Cl_2 : MeOH = 20 : 1) to give **4a** (50 mg , 65%) as a white solid: mp 234.2-234.8 ^oC; UV (MeOH) λ_{max} 289.5 nm; ^1H NMR (CD_3OD) δ 8.45 (s, 1 H), 5.98 (d, 1 H, *J* = 6.4 Hz), 4.56-4.59 (m, 1 H), 4.44 (dd, 1 H, *J* = 4.4, 8.0 Hz), 3.51 (dd, 1 H, *J* = 4.8, 10.8 Hz), 2.96 (dd, 1 H, *J* = 4.0, 10.8 Hz), 2.46 (t, 2 H, *J* = 6.8 Hz), 1.60-1.67 (m, 2 H), 1.48-1.57 (m, 2 H), 0.98 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (CD_3OD) δ 157.08, 151.32, 147.94, 142.27, 119.80, 88.50, 81.20, 80.90, 74.37, 63.94, 35.11, 31.55, 23.11, 19.46, 13.97. $[\alpha]^{25}_D$ -28.36 (*c* 0.20, MeOH); (ESI+) ($M+H^+$) m/z 334.1335; Anal. Calcd. for $C_{15}H_{19}N_5O_2S$: C, 54.04; H, 5.74; N, 21.01; O, 9.60; S, 9.62. Found: C, 54.44; H, 5.88; N, 20.98; S, 10.00.

(2*R*,3*S*, 4*R*)-2-(6-amino-2-(*E*)-hex-1-enyl)-9*H*-purine-9-yl)-tetrahydrothiophene-3,4-diol (4b)

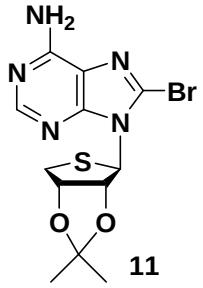


4b

A mixture of **8** (46 mg, 0.11 mmol), tetrakis(triphenylphosphine) palladium(0) (13mg, 0.011 mmol), sodium carbonate (35 mg, 0.33 mmol) and (*E*)-1-catecholboranylhexene *(Organic Syntheses, Coll. Vol. 8, p. 532* (**1993**)*; Vol. 68, p. 130* (**1990**)) (67 mg, 0.33 mmol) in DMF and H_2O (8 : 1, 5 mL) was stirred for overnight at 90 °C. The reaction mixture was filtered over a celite bed and the residue was concentrated to give the crude compound **10**. To a solution of **10** in THF (5 mL) was added 1 *N* hydrochloric acid (5 mL) and the mixture stirred at room temperature for 1 day. The mixture was neutralized with 1 *N* NaOH solution, and then carefully evaporated under reduced pressure. The crude residue was subjected to flash silica gel column chromatography (CH_2Cl_2 : MeOH = 20 : 1) to give **4b** (23 mg , 63%) as a white solid : mp 209.1-209.6 ^oC; UV (MeOH) λmax 274.5 nm; ^1H NMR (DMSO-d_6) δ 8.37 (s, 1 H), 7.11 (brs, 2 H, NH_2), 6.90 (tt, 1 H, *J* = 6.8, 15.6 Hz), 6.29 (tt, 1 H, *J* = 1.6, 15.2 Hz), 5.89 (d, 1 H, *J* = 7.2 Hz), 5.52 (d, 1 H, OH, *J* = 6.0 Hz), 5.36 (d, 1 H, OH, *J* = 4.0 Hz), 4.63-4.67 (m, 1 H), 4.37 (pseudo t, 1 H, *J* = 3.2 Hz), 3.42 (dd, 1 H, *J* = 4.4, 10.8 Hz), 2.80 (dd, 1 H, *J* = 2.8, 10.8 Hz); 2.20-2.26 (m, 2 H), 1.41-1.48 (m, 2 H), 1.29-1.38 (m, 2 H), 0.90 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 158.20, 155.49, 150.61, 139.60, 138.18, 130.44, 117.70, 78.51, 72.28, 61.02, 34.39, 31.40, 30.54, 21.73, 13.75. $[\alpha]^{25}_D$ -28.36 (*c* 0.20, MeOH);

(ESI+) ($M+H^+$) m/z 336.1494; $[\alpha]^{25}_D$ -44.80 (*c* 0.12, MeOH); Anal. Calcd for $C_{15}H_{21}N_5O_2S$: C, 53.71; H, 6.31; N, 20.88; O, 9.54; S, 9.56. Found: C, 53.98; H, 6.28; N, 20.96; S, 10.02.

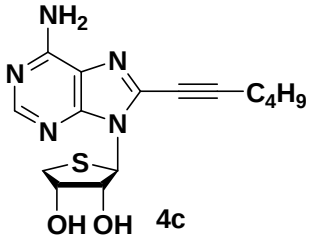
8-bromo-9-((3a*R*, 6R, 6a*S*)- tetrahydro-2,2-dimethylthieno[3,4-*d*][1,3]dioxol-6-yl)-9*H*-purin-2-amine (11).



11

8-Bromoadenine (*J. Org. Chem.* **2001**, *66*, 5463-5481) (0.40 g, 1.84 mmol), ammonium sulfate (37 mg, 0.27 mmol) and HMDS (10 mL) were refluxed for overnight, under dry and inert conditions. The solution was evaporated under high vacuum. The resulting solid was dissolved in 1,2-dichloroethane (5 mL) and cooled at 0 ^oC. The solution of **5** (0.20 g, 0.92 mmol) in 1,2-dichloroethane (5 mL) was dropwise added to the above cooled mixture. To this TMSOTf (0.33 mL, 1.84 mmol) was dropwise added and the mixture was stirred for 30 minutes at 0 ^oC, for 1 h at rt, and finally heated at 90 ^oC for 2 h. The mixture was cooled, diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. The extracted organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. The crude syrup was subjected to a flash silica gel column chromatography (Hexane : EtOAc = 1 : 1) to give **12** (69 mg, 20%) as a syrup: UV (MeOH) λmax 263.5 nm; ^1H NMR ($CDCl_3$) δ 8.25 (s, 1 H), 5.91 (s, 1 H), 5.81 (brs, 2 H, NH_2), 5.54 (d, 1 H, *J* = 5.2 Hz), 5.50 (pseudo t, 1 H, *J* = 5.2 Hz), 3.87 (dd, 1 H, *J* = 4.0, 12.4 Hz), 3.14 (d, 1 H, *J* = 12.4 Hz), 1.60 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 154.29, 153.03, 150.70, 127.76, 120.42, 111.41, 88.98, 85.99, 71.91, 41.35, 26.59, 24.65. $[\alpha]^{25}_D$ -77.07 (*c* 0.16, CH_2Cl_2); (ESI+) ($M+H^+$) m/z 373.0151; Anal. Calcd for $C_{12}H_{14}BrN_5O_2S$: C, 38.72; H, 3.79; Br, 21.47; N, 18.81; O, 8.60; S, 8.61. Found: C, 38.99; H, 3.77; N, 19.09; S, 8.26.

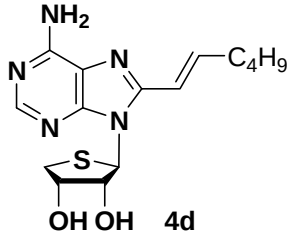
(2*R*,3*S*,4*R*)-2-(6-amino-8-(hex-1-y nyl)-9*H*-purine-9-yl)-tetrahydrothiophene-3,4-diol (4c)



4c

Compound **11** (69 mg, 0.19 mmol) was dissolved in Et_3N (1.5 mL) and DMF (1 mL). After purging the solution with N_2, $(PPh_3)_2PdCl_2$ (14 mg, 0.019 mmol) and CuI (3.7 mg, 0.019 mmol) were added. 1-hexyne (0.056 mL, 0.48 mmol) was subsequently added dropwise and the mixture was stirred at room temperature for 3 h. The solvents were removed under reduced pressure, to give the crude compound **12**. To a solution of **12** in THF (3 mL) was added 1 *N* hydrochloric acid (3 mL) and the mixture stirred at room temperature for 1 day. The mixture was neutralized with 1 *N* NaOH solution, and then carefully evaporated under reduced pressure. The crude residue was subjected to flash silica gel column chromatography (CH_2Cl_2 : MeOH = 20 : 1) to give **4c** (36 mg , 58%) as a white solid: mp 233.8-234.9 ^oC; UV (MeOH) λ_{max} 294.0 nm; ^1H NMR (DMSO-d_6) 8.16 (s, 1 H), 7.40 (brs, 2 H, NH_2), 6.03 (d, 1 H, J = 7.6 Hz), 5.44 (d, 1 H, -OH, J = 6.4 Hz), 5.33 (d, 1 H, OH, J = 4.0 Hz), 5.24-5.29 (m, 1 H), 4.40 (dd, 1 H, J = 1.6, 3.2 Hz), 3.42 (dd, 1 H, J = 3.6, 11.2 Hz), 2.80 (dd, 1 H, J = 2.0, 11.2 Hz), 2.59 (t, 2 H, J = 6.8 Hz), 1.55-1.62 (m, 2 H), 1.44-1.51 (m, 2 H), 0.93 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ155.71, 153.11, 149.10, 133.74, 118.91, 97.97, 76.51, 72.33, 70.72, 63.18, 35.52, 29.59, 21.38, 18.31, 13.41. $[\alpha]^{25}_D$ -112.2 (*c* 0.14, MeOH); (ESI+) ($M+H^+$) m/z 334.1339; Anal. Calcd for $C_{15}H_{19}N_5O_2S$: C, 54.04; H, 5.74; N, 21.01; O, 9.60; S, 9.62. Found: C, 54.00; H, 5.78; N, 20.99; S, 9.30.

(2*R*,3*S*,4*R*)-2-(6-amino-8-(hex-1-enyl)-9*H*-purine-9-yl)-tetrahydrothiophene-3,4-diol (4d)



4d

A mixture of **11** (42 mg, 0.12 mmol), tetrakis(triphenylphosphine) palladium(0) (14 mg, 0.012 mmol), sodium carbonate (37 mg, 0.35 mmol) and (*E*)-1-catecholboranylhexene (*Organic Syntheses, Coll. Vol. 8, p. 532* (**1993**); *Vol. 68, p. 130* (**1990**)) (72 mg, 0.35 mmol) in DMF and H_2O (8 : 1, 5 mL) was stirred overnight at 90 °C. The reaction mixture was filtered

by a bed of celite and the residue was concentrated to give the crude compound **13**. To a solution of **13** in THF (3 mL) was added 1 *N* hydrochloric acid (3 mL) and the mixture stirred at room temperature for 1 day. The mixture was neutralized with1 *N* NaOH solution, and then carefully evaporated under reduced pressure. The mixture was subjected to a flash silica gel column chromatography (CH_2Cl_2 : MeOH = 20 : 1) to give **4d** (25 mg , 65%) as a white solid: mp 248.6-248.8 ^oC; UV (MeOH) λ_{max} 297.5 nm; ^1H NMR (CD_3OD) δ 8.15 (s, 1 H), 6.97 (tt, 1 H, *J* = 6.8, 15.6 Hz), 6.75 (tt, 1 H, *J* = 1.6, 15.2 Hz), 6.15 (d, 1 H, *J* = 8.0 Hz), 5.27 (dd, 1 H, *J* = 3.6, 8.0 Hz), 4.48-4.50 (m, 1 H), 3.65 (dd, 1 H, *J* = 3.6, 11.6 Hz), 2.93 (dd, 1 H, *J* = 1.6, 11.6 Hz), 2.36-2.42 (m, 2 H), 1.53-1.58 (m, 2 H), 1.42-1.50 (m, 2 H), 0.98 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (CD_3OD) δ 156.54, 152.90, 151.82, 151.24, 143.93, 120.12, 117.42, 78.81, 74.39, 63.95, 36.27, 33.94, 32.02, 23.37, 14.28; $[\alpha]^{25}_D$ -71.56 (*c* 0.10, MeOH); (ESI+) ($M+H^+$) m/z 336.1494; Anal. Calcd for $C_{15}H_{21}N_5O_2S$: C, 53.71; H, 6.31; N, 20.88; O, 9.54; S, 9.56. Found: C, 53.99; H, 6.28; N, 20.98; S, 9.16.

Molecular modeling

Ligand structures were generated with Concord and energy minimized using MMFF94s force field and MMFF94 charge until the rms of Powell gradient was 0.05 kcal $mol^{-1}A^{-1}$ in SYBYL 8.1.1 (Tripos International, St. Louis, MO, USA). The X-ray crystal structure of human A_{2A} adenosine receptor (PDB code: 3EML) was prepared using Biopolymer Structure Preparation Tool in SYBYL. The docking study was carried out by GOLD v.4.1.2 (Cambridge Crystallographic Data Centre, Cambridge, UK), which employs a genetic algorithm (GA) and allows for full ligand flexibility and partial protein flexibility. The binding site was defined as the region of 9 Å extent around the co-crystallized ligand (ZM-241385). The side chains of the eight residues (i.e. Thr88, Phe168, Glu169, Trp246, Leu249, Asn253, Ser277, and His278) in the binding site were set to be flexible with 'crystal mode'. The ligands were docked using the GoldScore scoring function with 30 GA runs and other parameters were set as suggested by the GOLD authors. The Fast Connolly surface of the A_{2A}AR and the Van der Waals surface of each ligand were generated by MOLCAD in SYBYL. All computation calculations were undertaken on Intel® Xeon™ Quad-core workstation with Linux Cent OS release 4.6.

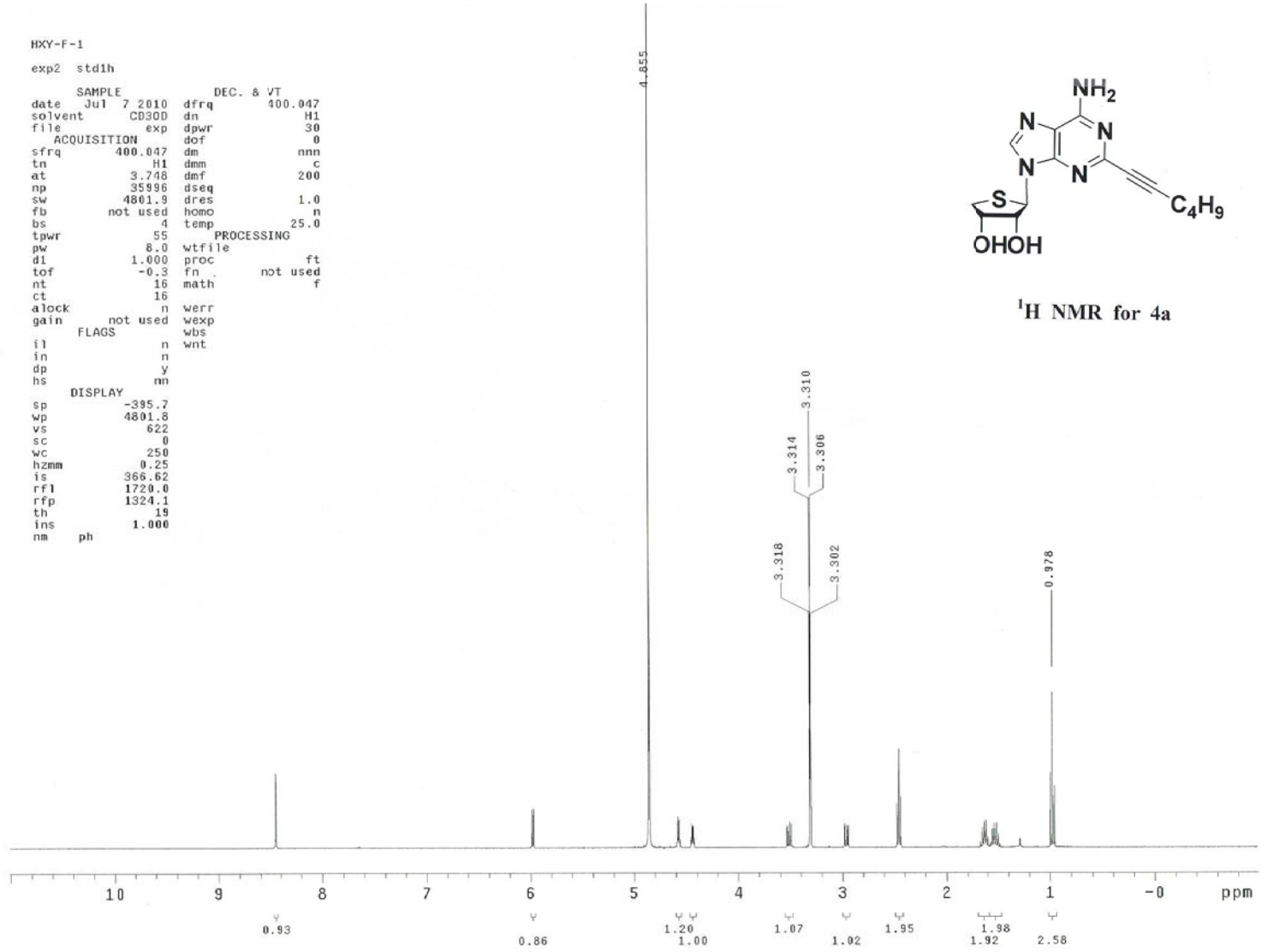
Binding assay

Human A_1 and A_{2A} Adenosine Receptors: For binding to human A_1 receptors, [^3H]PIA (1 nM) was incubated with membranes (40 μg/tube) from CHO cells stably expressing human A_1

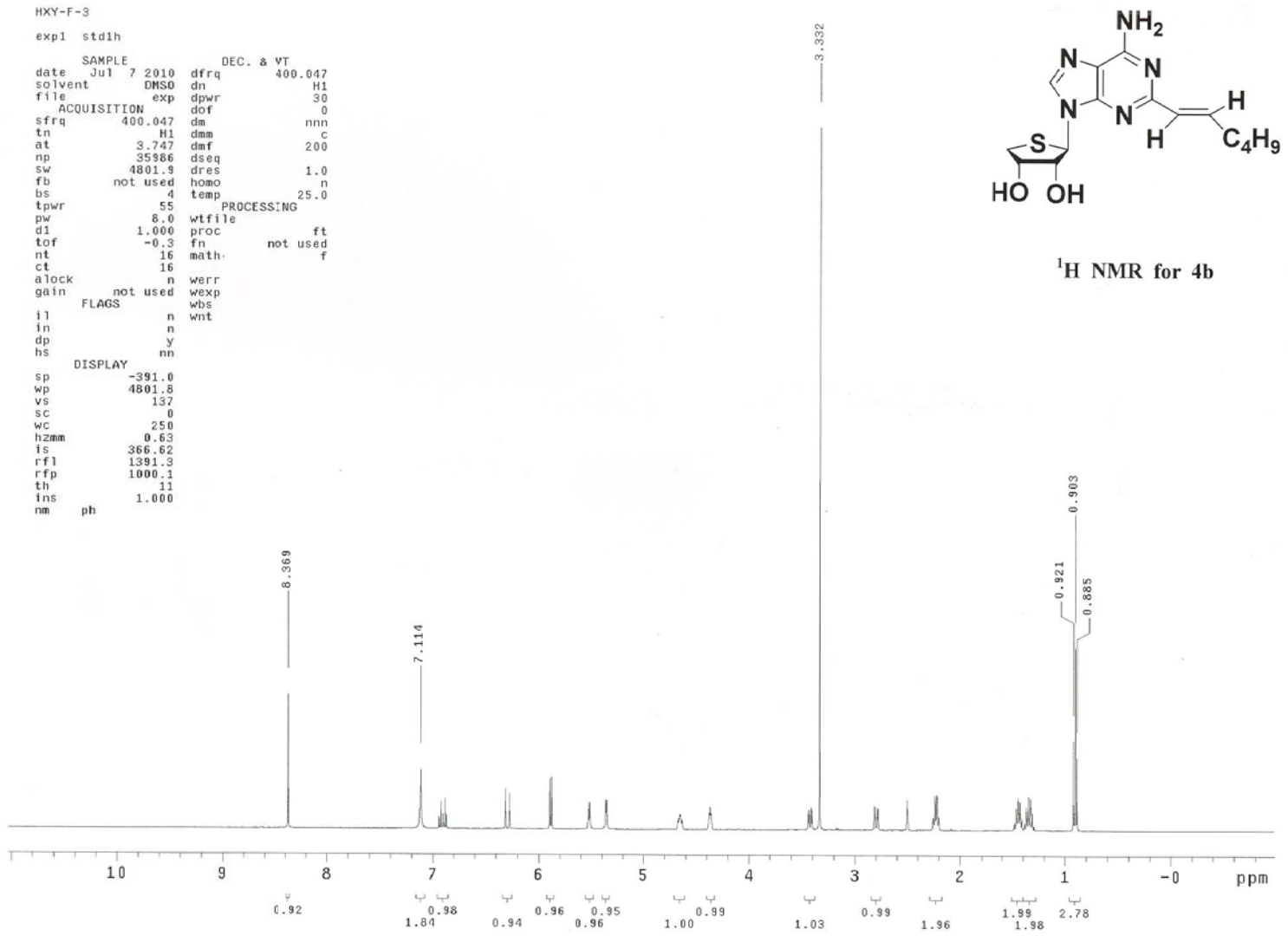
receptors at 25 °C for 60 min in 50 mM Tris·HCl buffer (pH 7.4; $MgCl_2$, 10 mM) in a total assay volume of 200 μL. Nonspecific binding was determined using 10 μM of CPA. For human A_{2A} receptor binding, membranes (20 μg/tube) from HEK-293 cells stably expressing human A_{2A}ARs were incubated with 15 nM [^{3}H]CGS21680 at 25 °C for 60 min in 200 μl 50 mM Tris·HCl, pH 7.4, containing 10 mM $MgCl_2$. Reaction was terminated by filtration with GF/B filters.

Human A_3 Adenosine Receptor: For competitive binding assay, each tube contained 100 μL suspension of membranes (20 μg protein) from CHO cells stably expressing the human A_3AR, 50 μL of [^{125}I]I-AB-MECA (0.5 nM), and 50 μL of increasing concentrations of the nucleoside derivative in Tris·HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA. Nonspecific binding was determined using 10 μM of Cl-IB-MECA in the buffer. The mixtures were incubated at 25 °C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburgh, MD, USA). Filters were washed three times with 9 mL ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter.

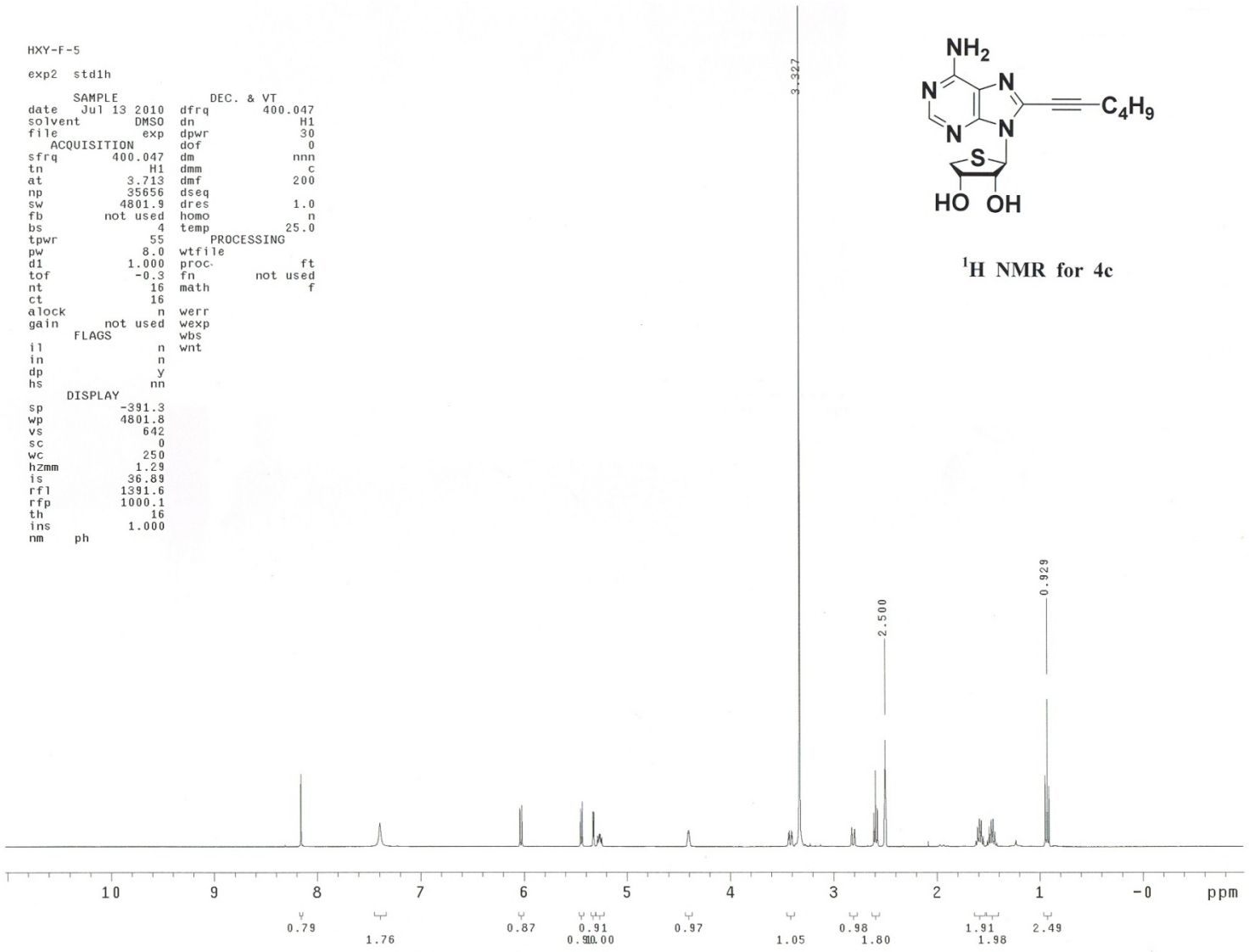
For binding at all three subtypes, K_i values are expressed as mean ± sem, n = 3-5 (outliers eliminated), and normalized against a non-specific binder, 5'-*N*-ethylcarboxamidoadenosine (NECA, 10 μM).[9] Alternately, for weak binding a percent inhibition of specific radioligand binding at 10 μM, relative to inhibition by 10 μM NECA assigned as 100%, is given.


HXY-F-1
exp2 std1h
SAMPLE
date Jul 7 2010
solvent CD3OD
file exp
ACQUISITION
sfrq 400.047
tn H1
at 3.748
np 35996
sw 4801.9
fb not used
bs 4
tpwr 55
pw 8.0
d1 1.000
tof -0.3
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -395.7
wp 4801.8
vs 622
sc 0
wc 250
hzmm 0.25
is 366.62
rfl 1720.0
rfp 1324.1
th 19
ins 1.000
nm ph
DEC. & VT
dfrq 400.047
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt
4.855
3.310
3.314
3.306
3.318
3.302
0.978
NH2
N
N
N
N
S
OHOH
C4H9
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.93
0.86
1.20
1.00
1.07
1.02
1.95
1.98
1.92
2.58

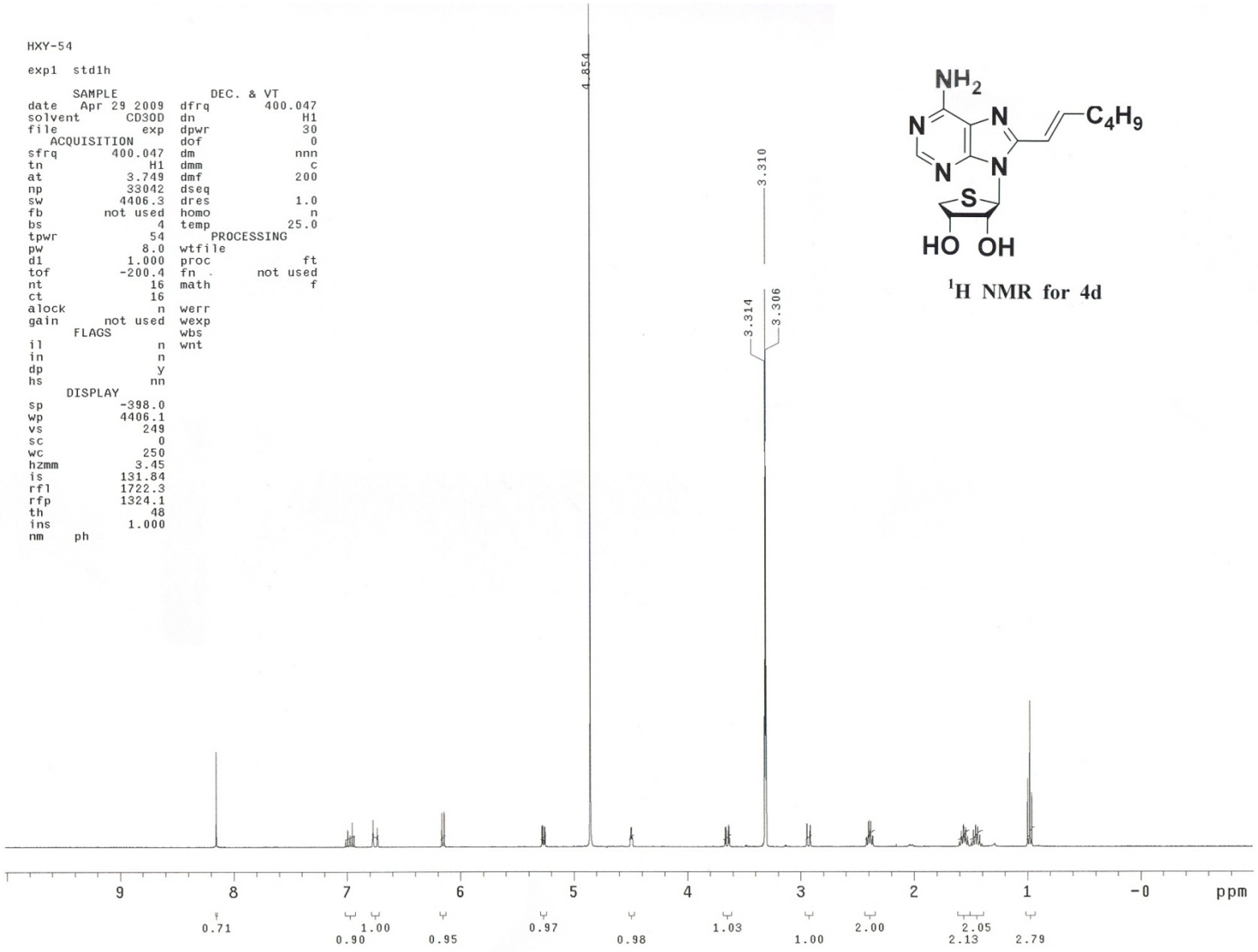
^{1}H NMR for 4a


HXY-F-3
expl stdlh
SAMPLE
date Jul 7 2010
solvent DMSO
file exp
ACQUISITION
sfrq 400.047
tn H1
at 3.747
np 35986
sw 4801.9
fb not used
bs 4
tpwr 55
pw 8.0
d1 1.000
tof -0.3
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -391.0
wp 4801.8
vs 137
sc 0
wc 250
hzmm 0.63
is 366.62
rfl 1391.3
rfp 1000.1
th 11
ins 1.000
nm ph
DEC. & VT
dfrq 400.047
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt
8.369
7.114
3.332
0.921
0.903
0.885
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.92
1.84
0.98
0.94
0.96
0.96
0.95
1.00
0.99
1.03
0.99
1.96
1.99
1.98
2.78

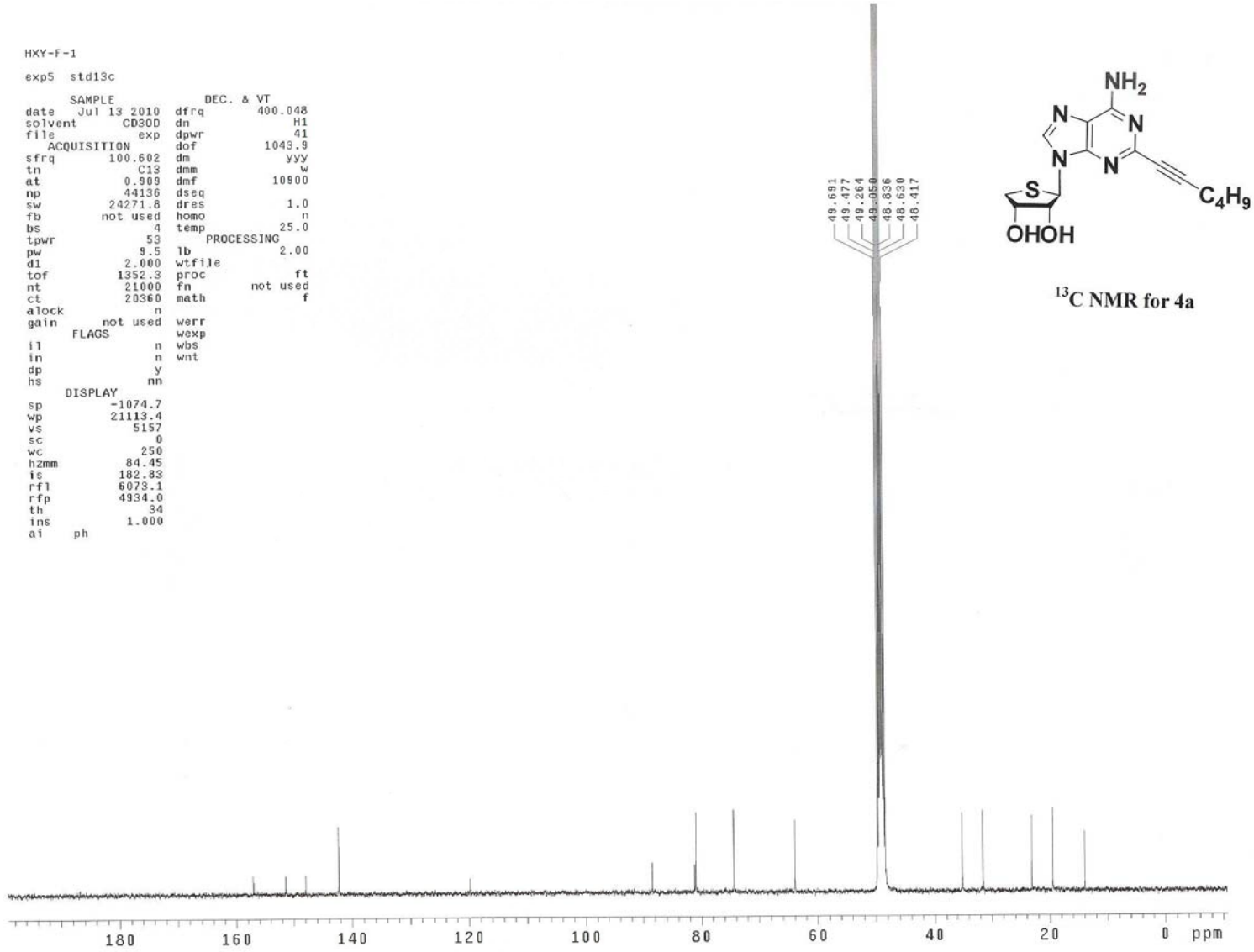
^{1}H NMR for 4b


HXY-F-5
exp2 std1h
SAMPLE
date Jul 13 2010
solvent DMSO
file exp
ACQUISITION
sfrq 400.047
tn H1
at 3.713
np 35656
sw 4801.9
fb not used
bs 4
tpwr 55
pw 8.0
d1 1.000
tof -0.3
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -391.3
wp 4801.8
vs 642
sc 0
wc 250
hzmm 1.29
is 36.89
rfl 1391.6
rfp 1000.1
th 16
ins 1.000
nm ph
DEC. & VT
dfrq 400.047
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt
3.327
2.500
0.929
NH2
N
N
N
N
S
HO
OH
C4H9
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.79
1.76
0.87
0.91
0.97
1.05
0.98
1.80
1.91
1.98
2.49

^{1}H NMR for 4c


HXY-54
exp1 std1h
SAMPLE
date Apr 29 2009
solvent CD3OD
file exp
ACQUISITION
sfrq 400.047
tn H1
at 3.749
np 33042
sw 4406.3
fb not used
bs 4
tpwr 54
pw 8.0
d1 1.000
tof -200.4
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -398.0
wp 4406.1
vs 249
sc 0
wc 250
hzmm 3.45
is 131.84
rfl 1722.3
rfp 1324.1
th 48
ins 1.000
nm ph
DEC. & VT
dfrq 400.047
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 25.0
PROCESSING
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt
4.854
3.310
3.314
3.306
NH2
N
N
N
N
S
C4H9
HO OH
9
8
7
6
5
4
3
2
1
-0
ppm
0.71
1.00
0.90
0.95
0.97
0.98
1.03
1.00
2.00
2.05
2.13
2.79

^{1}H NMR for 4d

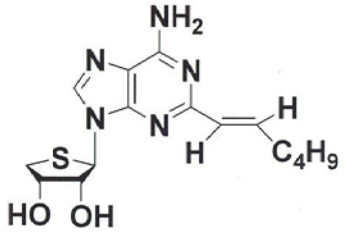


^{13}C NMR for 4a

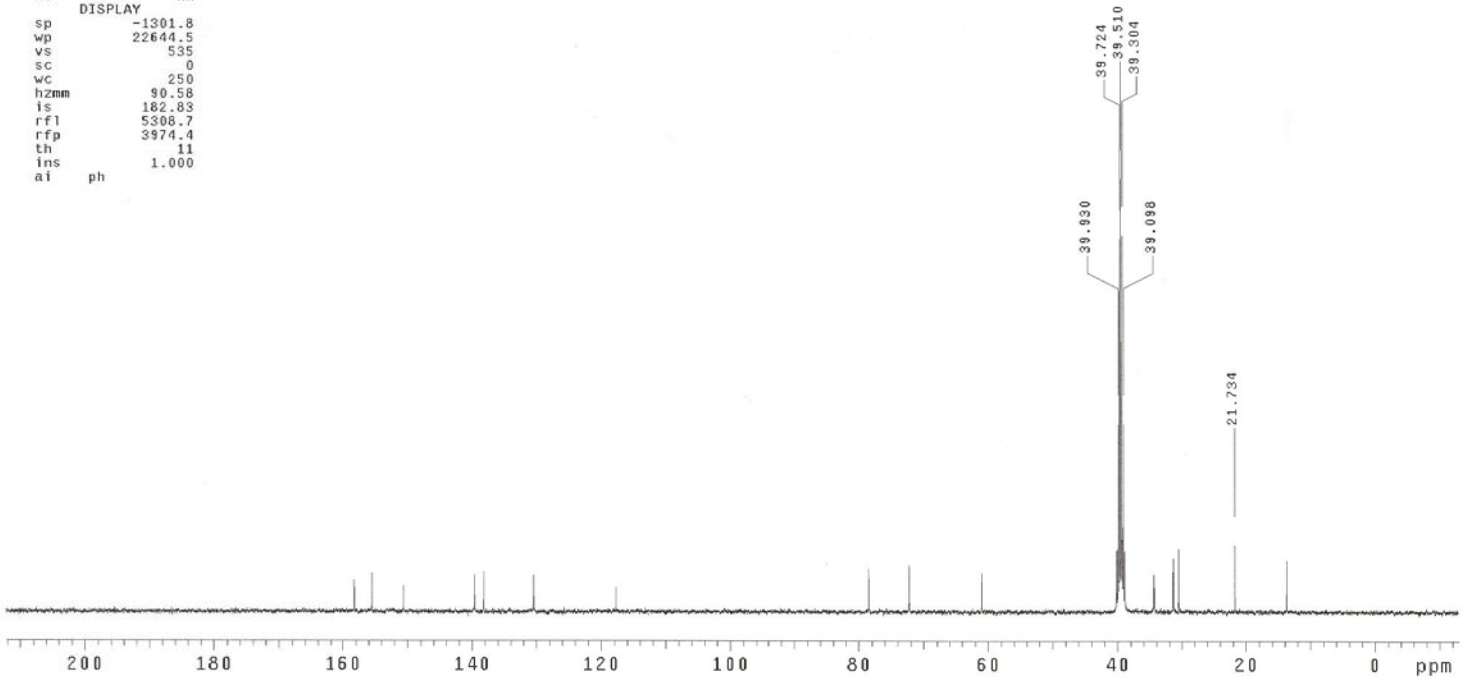
HXY-F-3

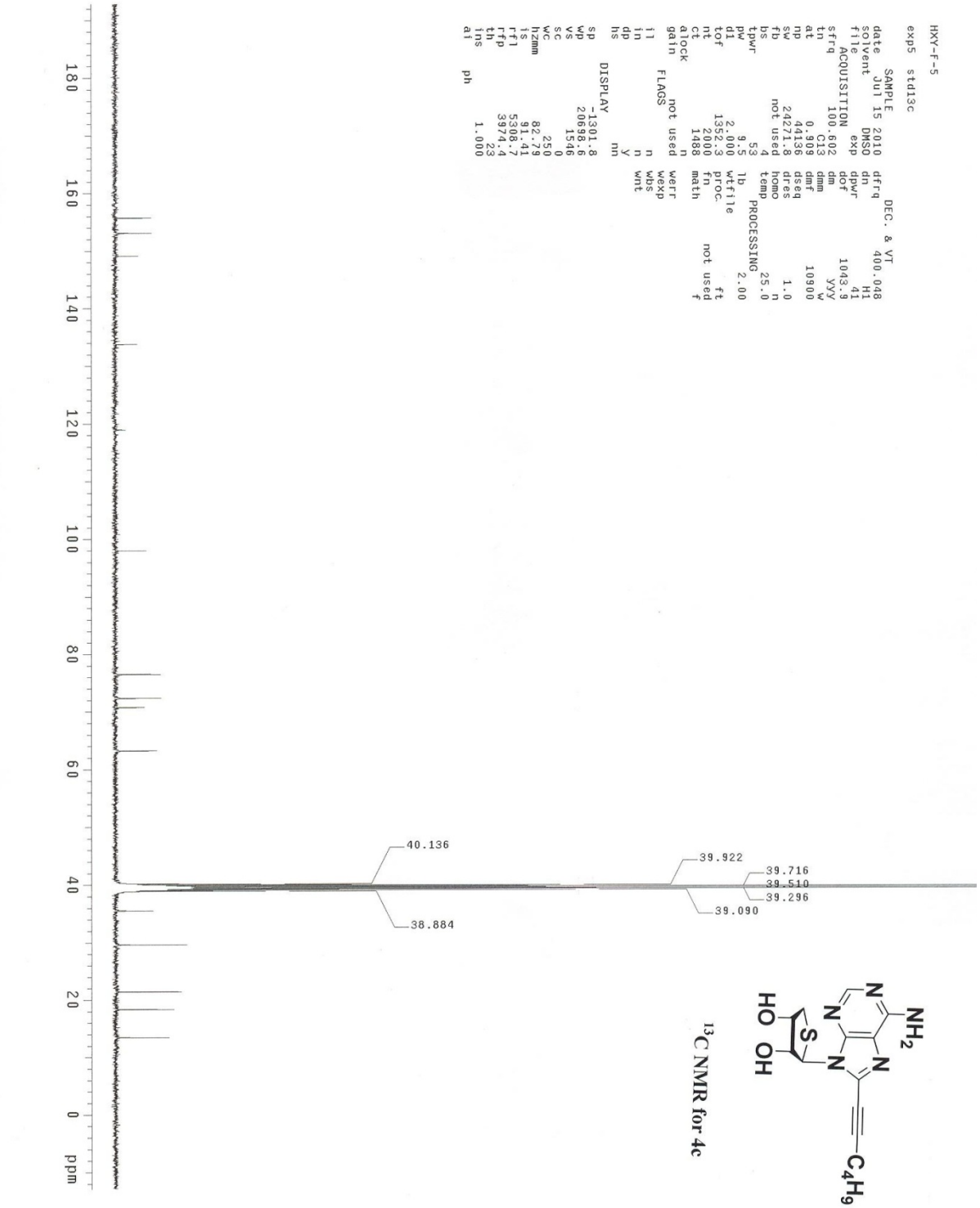
exp5 std13c

SAMPLE		DEC. & VT	
date	Jul 8 2010	dfrq	400.048
solvent	DMSO	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	1043.9
sfrq	100.602	dm	yyy
tn	C13	dmm	w
at	0.909	dmf	10900
np	44136	dseq	
sw	24271.8	dres	1.0
fb	not used	homo	n
bs	4	temp	25.0
tpwr	53	PROCESSING	
pw	9.5	lb	2.00
d1	2.000	wtfile	
tof	1352.3	proc	ft
nt	1500	fn	not used
ct	376	math	f
alock	n		
gain	not used	werr	
FLAGS		wexp	
il	n	wbs	
in	n	wnt	
dp	y		
hs	nn		
DISPLAY			
sp	-1301.8		
wp	22644.5		
vs	535		
sc	0		
wc	250		
hzmm	90.58		
is	182.83		
rfl	5308.7		
rfp	3974.4		
th	11		
ins	1.000		
ai	ph		

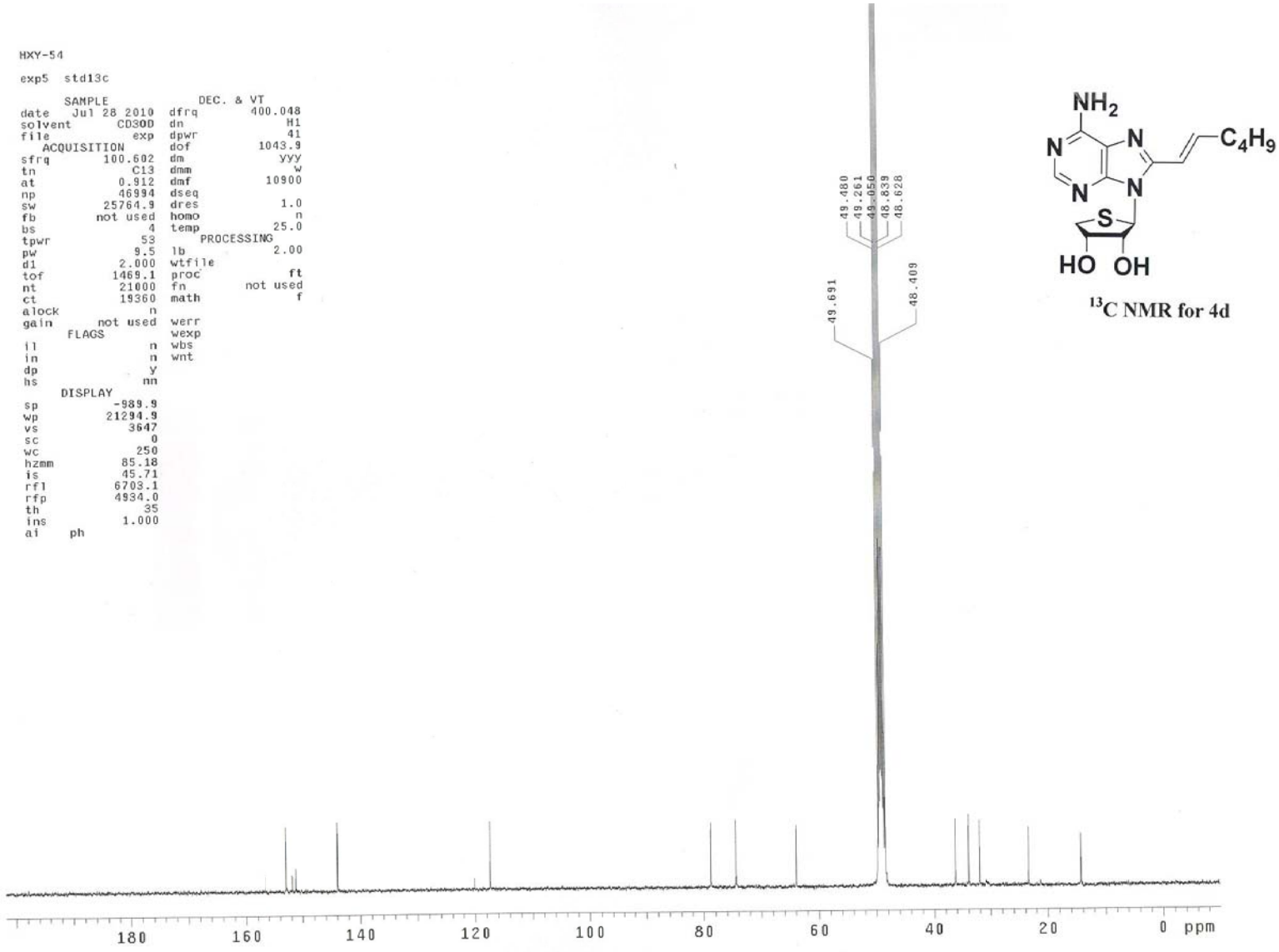


^{13}C NMR for 4b





^{13}C NMR for 4c



^{13}C NMR for 4d