Discovery of a Potent and Selective Inhibitor of Cdk4/6 (PD 0332991)

SUPPORTING INFORMATION

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CONTENTS

Synthesis of:

4-(6-Nitro-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester. 5-Bromo-2-nitropyridine (203 g, 1.365 mol), tetra-n-butyl ammonium iodide (25.2 g, 0.068 mol), piperazine (152.8 g, 1.774 mol) and potassium carbonate (207.44 g, 1.50 mol) were mixed in DMSO (2.6 L). The reaction mixture was warmed to 80 °C and exothermed to 100 °C. The mixture was allowed to cool back to 80 °C and was maintained at this temperature overnight. After cooling to room temperature the reaction mixture was poured into water (7 L) and the resulting solid was collected by filtration. This solid was

triturated twice with dichloromethane (1 L each time). The aqueous mother liquor was extracted with chloroform (4 x 2 L) and the combined organic layers were washed with water (2 L) then brine (2 L). Re-extraction of the mother liquor with chloroform (3 x 2 L) was followed by a brine wash (1 5 L). The combined organic extracts were concentrated to provide an orange solid (490.46 g), which was used without further purification. This solid was dissolved in THF (2 L) and water (500 mL) and sodium bicarbonate (119.22 g, 1.419 mol) were added, followed by di-*tert*-butyl dicarbonate (262 g, 1.2 mol) portion-wise over 2.5 h such that the temperature did not rise above 26 °C. After 3 h the volatile materials were removed under reduced pressure and the residue was diluted with water (1 L) and extracted with dichloromethane (3 x 1 L). The organic layers were combined and washed with water (1 L). This water was then back-extracted with more dichloromethane (300 mL). The organic extracts were combined and dried with magnesium sulfate, filtered, and concentrated to afford a brown solid. This material was warmed in 2.0 L of ethyl acetate to 60 °C. While at 60 °C, the solids were removed by filtration to afford the product 4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester as an orange solid (190.93 g, 62%). MS (APCI) *m/z* 309.1 (M+1). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.16 (d, *J* = 9 Hz, 1H), 8.11 (d, *J* = 3 Hz, 1H), 7.19 (dd, *J* = 9, 3, Hz, 1H), 3.64-3.61 (m, 4H), 3.45-3.42 (m, 4H), 1.47 (s, 9H).

4-(6-Amino-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester (63). 4-(6-Nitro-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester (83 g, 0.269 mol) in methanol (1.3 L) plus Raney Nickel (15 g, 50% slurry in water) were placed in a Parr shaker and hydrogenated at 50 psi of hydrogen for 5 h. The reaction mixture was filtered

through a pad of celite and concentrated to a brown solid. This material was triturated with diethyl ether (120 mL) for 4 h. Heptane was added and the mixture was cooled to 0 °C for 45 min. The solid was collected by filtration and dried to afford **63** as a tan solid (62.46 g, 83%). mp 130-132 °C. MS (ESI) *m/z* 279 (M+1). Anal. ($C_{14}H_{22}N_4O_3$) C; H, N.

2,2-Dimethyl-4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester. 5-Bromo-2-nitropyridine (10.67 g, 52.6 mmol), tetra-*n*-butyl ammonium iodide (0.97 g, 02.63 mmol), 2,2-dimethyl-piperazine (6.60 g, 57.8 mmol) and potassium carbonate (8.00 g, 57.8 mmol) were mixed in DMSO (50 mL). The reaction mixture was warmed to 95 °C for 5 h. The reaction mixture was poured onto ice chips (approximately 200 mL) then extracted with dichloromethane (6 x 75 mL). The combined organics were dried over $MgSO_4$, the inorganic salts were removed by filtration and the remaining solvents were concentrated to provide an orange solid. This solid was dissolved in dichloromethane (100 mL) to which triethylamine (10.65 g, 14.7 mL, 105 mmol) and di-*tert*-butyl dicarbonate (13.8 g, 63.12 mmol) were added. After 16 h, more di-*tert*-butyl dicarbonate (3.8 g, 17.41 mmol) was added and the mixture was brought to reflux for 3 h. The reaction mixture was then cooled to room temperature and diluted with dichloromethane (100 mL) and washed with water (1 x 100 mL). The organic layer was then dried over $MgSO_4$, filtered, and the solvent evaporated to yield 2,2-dimethyl-4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester as an orange solid (14.91 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 9.3 Hz, 1H), 7.97 (d, J = 2.9 Hz,

1H), 7.01 (dd, *J* = 2.9, 9.0 Hz, 1H), 3.91 (m, 2H), 3.54 (m, 4H), 1.48 (s, 9H), 1.43 (s, 6H).

4-(6-Amino-pyridin-3-yl)-2,2-dimethyl-piperazine-1-carboxylic acid *tert*-butyl ester. 2,2-Dimethyl-4-(6-nitro-pyridin-3-yl)-piperazine-1-carboxylic acid *tert*-butyl ester (14.63 g, 43.5 mmol) was dissolved in THF (400 mL) to which Raney Nickel (6.8 g) was added. The reaction mixture was shaken under a hydrogen atmosphere (50 psi) for 4 h. The catalyst was removed by filtration and the solvent evaporated to give 4-(6-amino-pyridin-3-yl)-2,2-dimethyl-piperazine-1-carboxylic acid *tert*-butyl ester as a purple solid (11.26 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 2.4 Hz, 1H), 7.06 (dd, *J* = 2.9, 8.8 Hz, 1H), 6.51 (d, *J* = 8.8 Hz, 1H), 3.68 (m, 2H), 3.16 (m, 2H), 2.98 (s, 2H), 1.48 (s, 9H), 1.43 (s, 6H).

4-(6-Amino-pyridin-3-yl)-2,6-dimethyl-piperazine-1-carboxylic acid *tert*-butyl ester. 5-Bromo-2-nitropyridine (10.81 g, 53.3 mmol), tetra-*n*-butyl ammonium iodide (0.98 g, 2.66 mmol), 2,6-dimethyl-piperazine (6.69 g, 58.6 mmol) and potassium carbonate (8.10 g, 58.6 mmol) were mixed in DMSO (50 mL). The reaction mixture was warmed to 80 °C for 4 h by which time the reaction was complete by TLC analysis. The reaction mixture was diluted with dichloromethane and washed with water (3 x 75 mL). The combined organics were dried over $MgSO_4$, the inorganic salts were removed by filtration and the remaining solvents were concentrated to provide an orange solid. This solid was dissolved in dichloromethane (150 mL) to which triethylamine (10.8 g, 14.8 mL, 108 mmol) and di-*tert*-butyl dicarbonate (13.95 g, 63.9 mmol) were added. The

reaction mixture was heated to reflux for 3 h then cooled to room temperature and diluted with dichloromethane (100 mL) and washed with water (1 x 100 mL). The organic layer was then dried over $MgSO_4$, filtered and the solvent evaporated to yield an orange solid. The orange solid was dissolved in THF (500 mL) to which Raney Nickel (9.23 g) was added. The reaction mixture was shaken under a hydrogen atmosphere (50 psi) for 4 h. The catalyst was removed by filtration, and the solvent evaporated to give a crude purple solid. This solid was purified by chromatography eluting with ethyl acetate to give 4-(6-amino-pyridin-3-yl)-2,6-dimethyl-piperazine-1-carboxylic acid *tert*-butyl ester as a purple solid (4.36 g, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 2.4 Hz, 1H), 7.18 (dd, J = 2.9, 8.8 Hz, 1H), 6.51 (d, J = 8.8 Hz, 1H), 4.35 (s, 2H), 4.21 (m, 2H), 3.08 (dd, J = 4.4, 11.7 Hz 2H), 1.48 (s, 9H), 1.35 (d, J = 6.8 Hz, 6H).

4-(6-Nitro-pyridin-3-yl)-morpholine. 5-Bromo-2-nitropyridine (5.14 g, 25.3 mmol), tetra-*n*-butyl ammonium iodide (0.467 g, 1.27 mmol), morpholine (2.43 g, 27.9 mmol) and potassium carbonate (3.85 g, 27.9 mmol) were mixed in DMSO (50 mL). The reaction mixture was warmed to 80 °C for 15 h. The reaction mixture was diluted with ethyl acetate and the solids removed by filtration. The organic filtrate was washed with water, then the solvent evaporated. The residue was then triturated with a dichloromethane/hexanes mixture to provide 4-(6-nitro-pyridin-3-yl)-morpholine as brown needles (2.90 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (m, 1H), 7.97 (d, J = 2.9 Hz, 1H), 7.15 (dd, J = 3.2, 9.3 Hz, 1H), 3.45 (m, 4H), 1.72 (m, 4H).

5-Morpholin-4-yl-pyridin-2-ylamine. 4-(6-Nitro-pyridin-3-yl)-morpholine (2.86 g, 13.7 mmol) was dissolved in THF (100 mL) to which Raney Nickel (1.03 g) was added. The reaction mixture was shaken under a hydrogen atmosphere (50 psi) for 4 h. The catalyst was removed by filtration and the solvent evaporated to give 5-morpholin-4-yl-pyridin-2-ylamine as a purple solid (1.91 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 2.0 Hz, 1H), 7.16 (dd, *J* = 2.7, 8.8 Hz, 1H), 6.50 (d, *J* = 8.8 Hz, 1H), 4.24 (s, 2H), 3.84 (m, 4H), 3.16 (m, 4H), 3.01 (m, 4H).

6'-Nitro-3,4,5,6-tetrahydro-2H-[1,3']bipyridinyl. 5-Bromo-2-nitropyridine (5.6 g, 27.6 mmol), tetra-*n*-butyl ammonium iodide (0.510 g, 1.38 mmol), piperidine (2.58 g, 30.3 mmol) and potassium carbonate (3.85 g, 30.3 mmol) were mixed in DMSO (50 mL). The reaction mixture was warmed to 80 °C for 4 h. The reaction mixture was diluted with ethyl acetate and filtered. The volume was reduced to remove ethyl acetate, the remaining solution was diluted with water (50 mL). A precipitate immediately formed and was collected by filtration and washed on the funnel with water to provide 6'-nitro-3,4,5,6-tetrahydro-2H-[1,3']bipyridinyl as an orange - brown solid (4.90 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.15 (d, *J* = 7.3 Hz, 1H), 6.49 (d, *J* = 8.5 Hz, 1H), 3.84 (m, 5H), 3.00 (m, 4H), 2.60 (s, 1H).

3,4,5,6-Tetrahydro-2H-[1,3']bipyridinyl-6'-ylamine. 6'-Nitro-3,4,5,6-tetrahydro-2H-[1,3']bipyridinyl (4.69 g, 22.6 mmol) was dissolved in THF (100 mL) to which Raney Nickel (1.08 g) was added. The reaction was shaken under a hydrogen atmosphere (50 psi) for 4 h. The catalyst was removed by filtration and the solvent

evaporated to give 3,4,5,6-tetrahydro-2H-[1,3']bipyridinyl-6'-ylamine as a purple solid (4.86 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 2.9, 8.8 Hz, 1H), 6.47 (dd, *J* = 0.7, 8.8 Hz, 1H), 4.18 (s, 2H), 2.97 (m, 4H), 1.71 (m, 4H), 1.53 (m, 2H).

2,6-Dimethyl-4-(6-nitro-pyridin-3-yl)-morpholine. 5-Bromo-2-nitropyridine (4.84 g, 23.84 mmol), tetra-*n*-butyl ammonium iodide (0.440 g, 1.19 mmol), 2,6-dimethyl-morpholine (3.02 g, 26.22 mmol) and potassium carbonate (3.62 g, 26.22 mmol) were mixed in DMSO (45 mL). The reaction mixture was warmed to 80 °C for 6 h. The reaction mixture was diluted with ethyl acetate and filtered. The volume of the filtrate was reduced to remove ethyl acetate, and the remaining solution was diluted with water (50 mL). A precipitate immediately formed and was collected by filtration then washed on the funnel with water to provide 2,6-dimethyl-4-(6-nitro-pyridin-3-yl)-morpholine as an orange solid (4.39 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 9.0 Hz, 1H), 8.11 (d, *J* = 2.9 Hz, 1H), 7.19 (dd, *J* = 2.9, 9.3 Hz, 1H), 3.77 (m, 2H), 3.65 (dd, *J* = 2.2, 12.9 Hz, 2H), 2.66 (dd, *J* = 10.7, 12.5 Hz, 2H), 1.29 (d, *J* = 6.4 Hz, 6H).

5-(2,6-Dimethyl-morpholin-4-yl)-pyridin-2-ylamine. 2,6-Dimethyl-4-(6-nitro-pyridin-3-yl)-morpholine (4.00 g, 16.86 mmol) was dissolved in THF (100 mL) to which Raney Nickel (3.10 g) was added. The reaction mixture was shaken under a hydrogen atmosphere (50 psi) for 4 h. The catalyst was filtered and the solvent evaporated to give 5-(2,6-dimethyl-morpholin-4-yl)-pyridin-2-ylamine as a purple solid (3.05 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 2.9, 8.8 Hz, 1H), 6.49

(dd, *J* = 0.7, 8.8 Hz, 1H), 3.79 (m, 2H), 2.34 (dd, *J* = 10.5, 10.5, 2H), 1.22 (d, *J* = 6.3 Hz, 6H).

***N,N*-Bis-(2-methoxy-ethyl)-benzene-1,4-diamine.** 1-Bromo-4-nitrobenzene (5 g, 0.025 mmol), potassium carbonate (3.7 g, 27 mmol), tetra-n-butylammonium iodide (0.45 g, 1.2 mmol) and bis-(2-methoxy-ethyl)-amine (4 mL, 27 mmol) were added to dry DMSO (50 mL) and heated to 90 °C under a nitrogen atmosphere for 6 h. The reaction mixture was allowed to cool and poured into ice water. A brown solid formed and was collected by filtration. This solid was dried overnight in vacuo at room temperature then purified by chromatography on silica gel eluting with 10 – 50% ethyl acetate in hexanes to give bis-(2-methoxy-ethyl)-(4-nitro-phenyl)-amine as a yellow oil. This oil was dissolved in THF (50 mL) and treated with Raney Nickel (0.5 g) under an atmosphere of hydrogen (500 psi) for 9 h. Removal of the catalyst by filtration through celite followed by evaporation of the filtrate *N,N*-bis-(2-methoxy-ethyl)-benzene-1,4-diamine as a purple liquid, which was used without further purification. ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.71 (dd, *J* = 3, 0.5 Hz, 1H), 6.96 (dd, *J* = 8, 2 Hz, 1H), 6.49 (d, *J* = 9 Hz, 1H), 3.65 (t, *J* = 6 Hz, 4H), 3.53 (t, *J* = 6 Hz, 4H), 3.33 (s, 6H).

6-Acetyl-2-amino-8-cyclopentyl-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (115). A solution of 8-cyclopentyl-6-(1-ethoxy-vinyl)-2-(4-methoxy-benzylamino)-5-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (0.10g, 0.23mmol; made from 6-bromo-8-cyclopentyl-2-(4-methoxy-benzylamino)-5-methyl-8H-pyrido[2,3-d]pyrimidin-7-one by

the procedure employed to prepare **130**) in TFA (4 mL) was heated under reflux for 5 h. The solution was concentrated to a gum that was triturated in slightly basic water. The solid was collected and purified by chromatography on silica gel eluting with ethyl acetate, giving **115** as a white solid (0.040g, 61%). MS (APCI) m/z 287.1 (M+1). ^{1}H NMR δ ($CDCl_3$) 8.64 (s, 1H), 5.84 (t, J=8.8 Hz, 1H), 5.27 (br s, 2H), 2.52 (s, 3H), 2.32 (s, 3H), 2.25 (m, 2H), 2.03 (m, 2H), 1.82 (m, 2H), 1.63 (m, 2H).

Purity Data

	Formula	Calculated	Found	Purity by HPLC*	LCMS
17	$C_{21}H_{24}BrN_2O.1.25H_2O.2.2$ HCl	C, 44.01; H, 5.05; N, 17.11, Cl (ionic), 13.61; H_2O, 3.93	C, 43.74; H, 5.07; N, 16.78; Cl (ionic), 13.73 ; H_2O, 3.81		
18	C19H23N7O1		Not determined	93%	Calcd: 366.2 Found: 366.2
19	$C_{19}H_{21}N_7O.2.1$ HCl.1.5 H_2O	C, 48.87; H, 5.63; N, 20.99, Cl (ionic), 15.94	C, 49.23; H, 5.53; N, 20.68, Cl (ionic), 15.69		
20	$C_{21}H_{25}N_7O.2.0$ HCl. 2.5 H2O	C, 49.51; H, 6.33; N, 19.25	C, 49.64; H, 6.12; N, 19.23		
21	C22H27N7O.2.0 HCl.3.5 H2O	C, 48.88; H, 6.70; N, 18.11	C, 48.88; H, 6.39; N, 17.95		
22	$C_{21}H_{24}N_7O\cdot1.0$ $H_2O\cdot2.0$ HCl	C, 50.73; H, 5.75; N, 19.46, Cl (ionic), 13.77	C, 50.41; H, 5.64; N, 19.59; Cl (ionic), 14.16; H_2O, 3.60.		
23	$C_{21}H_{26}N_8O\cdot1.25$ $H_2O\cdot2$ HCl	C, 50.46; H, 6.02; N, 22.14, Cl (ionic), 15.98; H_2O, 4.58	C, 50.25; H, 6.13; N, 22.32; Cl (ionic), 14.13; H_2O, 4.49		
24	$C_{22}H_{27}N_7O\cdot2.85$ $H_2O\cdot2.2$ HCl	C, 49.20; H, 6.55; N, 18.26, Cl (ionic), 14.52; H_2O, 9.56	C, 49.43; H, 6.32; N, 17.87; Cl (ionic), 14.38 ; H_2O, 7.35		
25	$C_{34}H_{29}N_7O\cdot1.2$ $H_2O\cdot2.1$ HCl	C, 53.36; H, 6.52; N, 18.93, Cl (ionic), 14.38; H_2O, 4.17	C, 53.25; H, 6.43; N, 18.80; Cl (ionic), 14.36; H_2O, 3.87.		
26	$C_{22}H_{27}N_7O_2\cdot1.0$ $H_2O\cdot2.0$ HCl	C, 51.56; H, 6.10; N, 19.13, Cl (ionic), 13.84; H_2O, 3.51	C, 51.13; H, 5.95; N, 19.05; Cl (ionic), 13.70; H_2O, 0.67.		
27	C23H29N7O2		Not determined	95%	Calcd: 436.2 Found: 436.2
28	C23H29N7O2		Not determined	96%	Calcd: 450.2 Found: 450.2
29	$C_{25}H_{33}N_7O_2$ .2.16 HCl	C, 53.78; H, 6.35; N, 17.56	C, 54.03; H, 6.64; N, 17.17.		
30	$C_{25}H_{33}N_7O_2$ 2.6 HCl .0.35 H_2O	C, 52.26; H, 6.56; N, 16.88	C, 52.01; H, 6.96; N, 16.88		
31	$C_{25}H_{33}N_7O_3$ .2 HCl .3.44 H_2O	C, 48.87; H, 6.87; N, 15.96	C, 48.48; H, 6.66; N, 15.66		
32	$C_{25}H_{33}N_7O_2\cdot1.0$ $H_2O\cdot2.0$ HCl	C, 54.15; H, 6.72; N, 17.68, Cl (ionic), 12.78; H_2O, 3.25	C, 54.18; H, 6.98; N, 17.51; Cl (ionic), 12.15; H_2O, 2.60		
33	$C_{23}H_{27}N_7O_2$.4.25 HCl	C, 46.94; H, 5.35; N, 16.66	C, 46.77; H, 5.33; N, 16.30		
34	$C_{24}H_{29}N_7O_3\cdot0.75$ $H_2O\cdot2.0$ HCl	C, 52.41; H, 5.96; N, 17.83, Cl (ionic), 12.89; H_2O, 2.46	C, 52.25; H, 5.86; N, 17.85; Cl (ionic), 12.10 ; H_2O, 1.52		
38	C22H27N7O1		Not determined	97%	Calcd: 406.2 Found: 406.2
39	$C_{17}H_{16}BrN_5O.0.1$ H_2O	C, 52.62; H, 4.21; N, 18.05	C, 52.23; H, 4.10; N, 17.91		
40	$C_{18}H_{18}BrN_5O.0.33H_2O$	C, 53.22; H, 4.63; N, 17.24	C, 52.88; H, 4.38; N, 17.04		

	Formula	Calculated	Found	Purity by HPLC*	
41	$C_{23}H_{26}N_7OBr \cdot 2.64\ H_2O \cdot 2.0\ HCl$	C, 43.68; H, 5.55; N, 16.21, Cl (ionic), 11.72	C, 44.08; H, 5.32; N, 15.23, Cl (ionic), 11.65.		
42			Not determined	92% (single minor impurity)	
43	$C_{24}H_{29}N_7O_2 \cdot 2.4\ H_2O \cdot 1.85\ HCl$	C, 51.64; H, 6.44; N, 17.56, Cl (total), 11.75	C, 51.31; H, 6.41; N, 17.20; Cl (total), 12.11		
44	C25H31N7O3		Not determined	95%	Calcd: 478.25 Found: 478.2
45	$C_{24}H_{32}N_6O_3Br_1\ 0.13\ H_2O$	C, 54.00; H, 5.90; N, 15.74	C, 53.61; H, 5.68; N, 15.60.		
46	C24H30Br1N7O1		Not Determined	95%	Calcd: 512.17 Found: 512.1
47	$C_{23}H_{28}BrN_7O_1\ 3.00\ H_2O\ 1.65$ HCl $0.60\ C_2H_5OH$	C, 43.70; H, 5.74; N, 14.74	C, 43.76; H, 5.79; N, 14.39.		
48	$C_{23}H_{28}Br_1N_7O_1$, 0.15 HCl, 2.55 C_2H_5OH . 0.45 CHCl3	C, 50.79; H, 6.55; N, 14.52	C, 50.83; H, 5.69; N, 14.21		
49	$C_{23}H_{27}BrN_6O_1$				
50	$C_{23}H_{27}Br_1N_6O_2$, 0.45 H_2O	C, 54.43; H, 5.54; N, 16.56	C, 54.04; H, 5.23; N, 16.33		
51	$C_{22}H_{25}BrN_6O_2$.1.37 H_2O	C, 51.80 H, 5.48 N, 16.48	C, 51.42 H, 5.00 N, 16.08.		
52	$C_{26}H_{34}N_6O_4$	C, 63.14; H, 6.93; N, 16.99	C, 63.04; H, 6.77; N, 16.86		
53	$C_{26}H_{33}N_7O_2$ .2.89 HCl .3.0 H2O	C, 49.18; H, 6.65; N, 15.44	C, 49.55; H, 6.80; N, 14.76		
54	$C_{26}H_{33}N_7O_2$ 2.70 HCl, 0.10 H_2O	C, 54.23, H, 6.28, N, 17.03	C, 54.60; H, 6.68; N, 16.57		
55	$C_{25}H_{31}N_7O_2$, 2.70 HCl, 1.05 C_2H_5OH	C, 53.50; H, 6.63; N, 16.12	C, 53.45; H, 6.47; N, 15.85.		
56	$C_{24}H_{29}N_7O_2$, 2.10 HCl, 2.85 H_2O, 0.45 C_2H_5OH	C, 50.16; H, 6.68; N, 16.45; Cl^-, 12.49	C, 50.37; H, 6.90; N, 16.45; Cl^-, 12.61.		
57	$C_{25}H_{31}N_7O_2$, 2.80 HCl, 0.45 $C_3H_8O_2$	C, 53.35; H, 6.25; N, 16.25	C, 52.96; H, 6.62; N, 15.95		
58	$C_{25}H_{30}N_6O_2\ 0.35\ H_2O$	C, 66.31; H, 6.83; N, 18.56	C, 66.68; H, 6.76; N, 18.07.		
59	$C_{25}H_{30}N_6O_3$, 1.76 $C_3H_8O_1$, 0.36 $CHCl_3$	C, 60.20; H, 7.33; N, 13.75	C, 60.48; H, 6.97; N, 13.35.		
60	C24H28N6O3		Not determined	100%	Calcd: 449.23 Found: 449.2
61	$C_{24}H_{29}BrN_6O_2\ 1.45\ H_2O\ 0.15$ EtOAc	C, 61.93, H, 7.05, N, 16.29	C, 61.85; H, 7.10; N, 15.91.		

* Reversed phase HPLC was performed on a Vydac C18 column (4.6 x 25 mm) eluting with a gradient of acetonitrile in water containing 0.1% trifluoroacetic acid with a flow rate of 1 mL/min. Detection was performed at 254 nm.