Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 2-*exo*-2-(2'-Substituted-3'-phenyl-5'-pyridinyl)-7-azabicyclo-[2.2.1]heptanes. Novel Nicotinic Antagonist

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Supporting Information

Analysis Appendix							
compd	formula	Calcd.			Found		
		C	H	N	C	H	N
(±) **5a**	$C_{17}H_{18}ClFN_2 \bullet H_2O$	63.25	6.25	8.67	63.06	6.25	8.80
(-) **5a**	$C_{17}H_{18}ClFN_2 \bullet 0.5H_2O$	65.07	5.94	8.93	65.10	6.02	8.91
(+) **5a**	$C_{17}H_{18}ClFN_2 \bullet 0.33H_2O$	65.70	6.05	9.01	65.61	5.95	8.86
5b	$C_{17}H_{17}Cl_2N_2 \bullet 1.25H_2O$	59.40	6.01	8.15	59.28	5.86	8.12
5c	$C_{17}H_{24}N_3O \bullet 2.5HCl \bullet 1.25H_2O$	53.87	6.38	11.09	53.95	6.35	10.68
5d	$C_{19}H_{25}Cl_2N_3 \bullet 2.25H_2O$	56.09	7.31	10.33	55.96	7.40	9.85
7	$C_{16}H_{22}BrN_3O_2$	52.18	6.02	11.41	52.23	6.11	11.35
8	$C_{22}H_{27}N_3O$	72.30	7.45	11.50	71.74	7.45	11.26

7-*tert*-Butoxycarbonyl-2-*exo*-(2'-amino-3'-bromo-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (7). To a stirred solution of 968 mg (3.30 mmol) of 7-tert-Butoxycarbonyl-2-*exo*-(2'-amino-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (**6**) in methylene chloride (8 mL) and acetic acid (7 mL) under nitrogen at 0 °C was added bromine (0.260 mL, 5.05 mmol) followed by triethylamine (0.260 mL). After stirring the reaction for 16 h, the mixture was poured into 100 mL of NH_4OH:H_2O (1:2) solution and extracted 3 × with chloroform. The combined organic extracts were dried with magnesium sulfate and concentrated. The residue was purified by flash chromatography using 4:1 ether triethylamine to give 1.04 g (85%) of **7** as a colorless solid: mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.44 (s, 9H), 1.40–1.55 (m, 2H), 1.70–1.84 (m, 3H), 1.90 (dd, J = 9.0, 12.3 Hz, 1H), 2.70 (dd, J = 4.8, 8.8 Hz, 1H), 4.08 (br s, 1H), 4.33 (br s, 1H), 7.62 (s, 1H, pyridyl CH), 7.83 (s, 1H, pyridyl CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 28.3 (3C), 28.7, 29.7, 40.3, 44.6, 55.7, 62.0, 79.7, 104.6, 132.9, 138.8, 145.5, 154.0, 154.9. Anal. ($C_{16}H_{22}BrN_3O_2$) C, H, N.

7-*tert*-Butoxycarbonyl-2-*exo*-(2'-amino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (8). To a resealable reaction tube under nitrogen was added 403 mg (1.08 mmol) of 7-*tert*-butoxycarbonyl-2-*exo*-(2'-amino-3'-bromopyridinyl)-7-azabicyclo[2.2.1]heptane (**7**), $Pd(OAc)_2$ (25 mg, 0.011 mmol), P(o-tolyl)$_3$ (60 mg, 0.02 mmol), sodium carbonate (230 mg, 2.17 mmol), phenylboronicacid (210 mg, 1.72 mmol), degassed water (0.800 mL), and DME (4 mL). The reaction was heated at 80 °C for 1.5 h. The reaction mixture was poured into saturated sodium bicarbonate and extracted 3 × with ethyl acetate. The organic layers were dried with sodium sulfate, and concentrated. The residue was purified by flash chromatography using hexane:ethyl acetate (1:2) as eluent to provide 347 mg (88%) of **8** as a colorless solid: ^{1}H NMR ($CDCl_3$) δ (ppm) 1.38 (br s, 9H), 1.38–1.65 (m, 2H), 1.75–2.0 (m, 4H), 2.78 (dd, J = 5.2, 8.6 Hz, 1H), 4.16 (s, 1H), 4.35 (s, 1H), 4.60 (br s, 2 NH), 7.3–7.45 (m, 6H), 7.92 (d, J = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 28.2, 28.8, 29.7, 40.2, 44.8, 55.5, 62.1, 79.3, 121.6, 127.5, 128.6(2C), 128.8(2), 131.7, 136.5, 138.2, 145.6, 154.3, 154.8. Anal. ($C_{22}H_{27}N_3O_2$) C, H, N.

2-*exo*-(2'-Fluoro-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (5a) Hydrochloride. A solution of 150 mg (0.410 mmol) of 7-*tert*-butoxycarbonyl-2-*exo*-(2'-amino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (**8**) in concentrated hydrofluoric acid/pyridine (0.6 mL) was prepared in a plastic vessel. Sodium nitrite (110 mg, 1.6 mmol) was added and the reaction mixture stirred for 45 min at room temperature followed by heating to 100 °C for 1 h. The mixture was poured into a solution of 50 mL of NH_4OH:H_2O (1:1) and extracted with ethyl acetate. The combined organic layers were dried with magnesium sulfate and concentrated. The

residue was purified via flash chromatography using $CHCl_3$:CH_3OH:NH_4OH (45:9:1) to give 91 mg (83%) of **5a** as a colorless oil: ^1H NMR ($CDCl_3$) δ (ppm) 1.45–1.76 (m, 4H), 1.93 (dd, *J* = 9.3, 12.3 Hz, 2H), 2.04 (s, 1H), 2.83 (dd, *J* = 6.0, 9.3 Hz, 1H), 3.62 (br s, 1H), 3.80 (br s, 1H), 7.33–7.60 (m, 5H), 7.98 (dd, J_F = 2.4, 9.6 Hz, 1H), 8.07 (t, J_F = 1.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 29.97, 31.23, 40.32, 44.35, 56.36, 62.74, 123.01(d, J_{CF} = 28.5 Hz), 128.5 (m, 4C), 134.15 (d, J_{CF} = 5.1 Hz), 139.70 (d, J_{CF} = 4.2 Hz), 140.34 (d, J_{CF} = 18.9 Hz), 144.59 (d, J_{CF} = 57 Hz), 157.39, 160.55.

To a stirred solution of 91 mg (0.339 mmol) of **5a** in methylene chloride (2.5 mL) was added 1M HCl in ether (1.6 mL). After 30 min at room temperature, the solvent was removed under reduced pressure and the remaining solid was pumped overnight to give 90 mg (81%) of **5a**•HCl as a colorless solid. Anal. ($C_{17}H_{18}ClFN_2$•H_2O) C, H, N.

(+)- and (–)-2-*exo*-(2'-Fluoro-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1] heptane (+)- and (–)-5a. To a mixture of 520 mg (1.94 mmol) of racemic 2-*exo*-(2'-fluoro-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1] heptane (**5a**) and 0.80 g (0.0020 mol) of di-p-toluoyl-D-tartaric acid was added 45 mL of (4:1) isopropanol/water. The resulting mixture was warmed to dissolution and allowed to stand at room temperature overnight. The resulting crystals were filtered to give 0.83 g of the di-p-toluoyl salt of **5a**. This salt was recrystallized twice from isopropanol/water (4:1) mixtures to give 0.27 g of the tartrate salt. This salt was neutralized with aqueous Na_2CO_3 and extracted with CH_2Cl_2 to give 100 mg of the free base. The free base was converted to the hydrochloride salt and recrystallized from EtOAc/MeOH mixtures to give 42 mg of **(–)-**2-*exo*-(2'-fluoro-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1] heptane hydrochloride: mp 239–242 °C; $[\alpha]_D$ = -11.4 (c 0.35, MeOH). Anal. ($C_{17}H_{17}FN_2$ HCl) C, H, N.

The mother liquors from above were combined and evaporated to dryness, dissolved in water, basified with Na_2CO_3, and extracted with CH_2Cl_2. The dried extracts (Na_2SO_4) were evaporated to give 0.25 g of solid. To the solids was added 0.378 g of di-p-toluoyl-L-tartaric acid and 20 mL of (4:1) isopropanol/water and crystallized as above to afford 0.32 g of the tartrate salt of **5a**. The salt was converted to its free base as above to afford 0.13 g. The free base was converted to the hydrochloride salt and recrystallized from EtOAc/MeOH mixtures to afford 56 mg of (+)- 2-*exo*-(2'-fluoro-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1] heptane hydrochloride hydrate: mp 240–242 °C; $[\alpha]_D$ = +11.1 (c = 0.28, MeOH). Anal. ($C_{17}H_{18}ClFN_2$•0.33H_2O) C, H, N.

2-*exo*-(2'-Amino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]-heptane (5c) Hydrochloride. A solution of 165 mg (0.451 mmol) of 7-*tert*-butoxycarbonyl-2-*exo*-(2'-amino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (**8**) in methylene chloride (1.0 mL) and trifluoroacetic acid (1.0 mL) was allowed to stir at room temperature for 1 h. The reaction was decanted into a saturated $NaHCO_3$ solution and extracted 3 × with chloroform. The combined organic extracts were dried with sodium sulfate and concentrated. The residue was purified by flash chromatography using $CHCl_3$:CH_3OH:NH_4OH (45:9:1) as eluent to give 116 mg (97%) of **5c** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ (ppm) 1.38–1.82 (m, 4H), 1.87 (dd, *J* = 9.0, 12.2 Hz, 1H), 2.75 (dd, *J* = 5.1, 8.67 Hz, 1H), 3.54 (br s, 1H), 3.73 (br s, 1H), 4.61 (br s, 2 H), 7.30–7.47 (m, 6H), 7.93 (s, 1H, pyridyl CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 29.71, 30.82, 39.91, 44.68, 56.28, 62.85, 121.55, 127.48, 128.61(2C), 128.82(2C), 132.28, 136.85, 138.23, 145.51, 154.15.

To a stirred solution of 96 mg (0.362 mmol) of **5c** in methylene chloride (1.5 mL) was added 1M HCl in ether (3 mL). After 1 h. at room temperature, the solvent was removed under reduced pressure, and the remaining solid was pumped overnight to give 127 mg (92%) of **5c**•HCl as a colorless solid: mp decomposed >200 °C. Anal. ($C_{17}H_{24}N_3O$•2.5HCl•1.25H_2O) C, H, N.

2-*exo*-(2'-Chloro-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (5b) Hydrochloride. To a solution of 217 mg (0.594 mmol) of 7-*tert*-butoxycarbonyl-2-*exo*-[5'-(3'-phenyl-2'-aminopyridinyl)]-7-azabicyclo[2.2.1]heptane (**8**) in concentrated hydrochloric acid (1.5 mL) was added sodium nitrite (800 mg, 11.6 mmol). Copper (I) chloride (800 mg, 8.1 mmol) was then added in small portions and stirring continued for 30 min at 0 °C. The mixture was poured into a solution of 50 mL of NH_4OH:H_2O (1:1) and extracted 3 × with ethyl acetate. The combined organic layers were dried with magnesium sulfate and concentrated. The residue was purified via flash chromatography using $CHCl_3$:CH_3OH:NH_4OH (45:9:1) to give 100 mg (59%) of **5b** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ (ppm) 1.45–1.78 (m, 5H), 1.93 (dd, *J* = 9.0, 12.1 Hz, 1H), 2.81 (dd, *J* = 4.9, 8.9 Hz, 1H), 3.61 (br s, 1H), 3.78 (br s, 1H), 7.36–7.48 (m, 5H), 7.76 (s, pyridyl 1 CH), 8.29 (s, pyridyl 1 CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 30.07, 31.30, 40.27, 44.45, 56.28, 62.64, 127.98, 128.11(2C), 129.23(2C), 136.19, 137.69, 138–54, 141.41, 146.92, 147.33.

To a stirred solution of 70 mg (0. 246 mmol) of **5b** in ether (1.5 mL) was added 1M HCl in ether (1.5 mL). After 30 min at room temperature, the solvent was removed under reduced pressure and the remaining solid was pumped overnight to give 80 mg (97%) of **5b**•HCl as a colorless solid: mp 144–147 °C; Anal. ($C_{17}H_{17}Cl_2N_2$•1.25H_2O) C, H, N.

7-tert-Butoxycarbonyl-2-*exo*-(2'-dimethylamino-3'-phenyl-5-pyridinyl)-7-azabicyclo[2.2.1]heptane (9). A mixture of 123 mg (0.337 mmol) of 7-*tert*-butoxycarbonyl-2-*exo*-(2'-amino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (**8**), acetonitrile (9mL), 37% formaldehyde (1.2 mL), and $NaCNBH_3$ (380 mg, 7.1 mmol) was allowed to stir for 3 h at room temperature. Glacial acetic acid (0.5 mL) was added and stirring continued overnight. After 24 h the reaction mixture was decanted into NH_4OH:H_2O (1:1) and extracted 3 × with chloroform. The combined organic extracts were dried with sodium sulfate and concentrated. The residue was purified by flash chromatography using hexane:ethyl acetate (1:2) as eluent to give 99 mg (75%) of **9** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ (ppm) 1.38 (s, 9H), 1.5–1.6 (m, 2H), 1.7–2.0 (m, 4H), 2.68 (s, 6H), 2.82 (dd, *J* = 6.0, 9.0 Hz, 1H), 4.18 br s, 1H) 4.34 (br s, 1H), 7.20–7.55 (m, 6H), 8.05 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 28.22 (3C), 28.72, 29.75, 40.23, 41.28 (2C), 44.77, 55.81, 62.03, 79.35, 125.43, 126.73 127.94 (2C), 128.45 (2C), 132.52, 138.28, 140.88, 144.51, 154.5, 158.74.

2-*exo*-(2'-Dimethylamino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (5d) Dihydrochloride. A solution of 98 mg (0.249 mmol) of 7-*tert*-butoxycarbonyl-2-*exo*-(2'-dimethylamino-3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane (**9**) in methylene chloride (1.5 mL) was stirred at 0 °C for 15 min. Trifluoroacetic acid (1.5 mL) was added and the reaction mixture was allowed to stir at room temperature for 30 min. The reaction mixture was decanted into a NH_4OH:H_2O (1:1) solution and extracted 3 × with chloroform. The combined organic extracts were dried with sodium sulfate and concentrated. The residue was purified by flash chromatography using $CHCl_3$:CH_3OH:NH_4OH (80:18:2) as eluent to give (64 mg, 88%) of **5d** as a colorless oil: ^{1}H NMR ($CDCl_3$ δ (ppm) 1.3–1.8 (m, 5H), 1.88 (dd, *J* = 9.0, 12.3 Hz, 1H), 2.04 (br s, 1H), 2.68 (s, 6H), 2.80 (dd, *J* = 6.0, 9.0 Hz, 1H), 3.57 (br s, 1H), 3.74 (br s, 1H), 7.25–7.55 (m, 6H), 8.07 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 29.75, 30.77, 39.84, 41.27 (2C), 44.78, 56.38; 62.78, 125.40, 126.72, 127.96 (2C), 128.49 (2C), 133.05, 138.67 140.91, 144.40, 158.56.

To a stirred solution of 61 mg (0.208 mmol) of **5d** in methylene chloride (2.0 mL) was added 1M HCl in ether (2.0 mL). After 30 min at room temperature, the solvent was removed under reduced pressure and the remaining solid was pumped overnight to give 76 mg (99%) of **5d**•2HCl as a colorless solid: mp 176–181 °C. Anal. ($C_{19}H_{25}Cl_2N_3$•2.25H_2O) C, H, N.

[^{3}H]Epibatidine Binding Assay. Adult male rat cerebral cortices (Pelfreeze Biological, Rogers, AK) were homogenized in 39 volumes of ice-cold 50 mM Tris buffer (pH 7.4 at 4 °C)

containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, and 1 mM MgCl2 and sedimented at 37,000 g for 10 min at 4 °C. The supernatant was discarded, the pellet resuspended in the original volume of buffer, and the wash procedure repeated twice more. After the last centrifugation, the pellet was resuspended in 1/10 its original homogenization volume and stored at -80 °C until needed. In a final volume of 0.5 mL, each assay tube contained 3 mg wet weight male rat cerebral cortex homogenate (added last), 0.5 nM [^{3}H]epibatidine (NEN Life Science Products, Wilmington, DE) and one of 10–12 different concentrations of test compound dissolved in buffer (pH 7.4 at room temperature) containing 10% DMSO resulting in a final DMSO concentration of 1%. Total and nonspecific bindings were determined in the presence of vehicle and 300 uM (–)-nicotine, respectively. After a 4-h incubation at room temperature, the samples were vacuum-filtered over GF/B filter papers presoaked in 0.03% polyethylenimine using a Brandel 48-well harvester and washed with 6 mL of ice-cold buffer. The amount of radioactivity trapped on the filter was determined by standard liquid scintillation techniques in a TriCarb 2200 scintillation counter (Packard Instruments, Meriden, CT) at approximately 50% efficiency. The binding data were fit using the nonlinear regression analysis routines in Prism (Graphpad, San Diego, CA). The K_i values for the test compounds were calculated from their respective IC50 values using the Cheng-Prusoff equation.

[^{125}I]iodo-MLA Binding Assay. Adult male rat cerebral cortices (Pel-Freez Biologicals, Rogers, AK) were homogenized (polytron) in 39 volumes of ice-cold 50 mM Tris buffer (assay buffer; pH 7.4 at 4 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, and 1 mM MgCl2. The homogenate was centrifuged at 35,000 g for 10 min at 4 °C and the supernatant discarded. The pellet was resuspended in the original volume of buffer and the wash procedure repeated twice more. After the last centrifugation step, the pellet was resuspended in one-tenth the original homogenization volume and stored at -80 °C until needed. Triplicate samples were run in 1.4-mL polypropylene tubes (Matrix Technologies Corporation, Hudson, NH). Briefly, in a final volume of 0.5 mL, each assay sample contained 3 mg wet weight rat cerebral cortex (added last), 40–50 pM [^{125}I]MLA and 50 nM final concentration of test compound dissolved in buffer containing 10% DMSO, giving a final DMSO concentration of 1%. Total and nonspecific binding were determined in the presence of vehicle and 300 uM (–)-nicotine, respectively. After a 2-h incubation on ice, the samples were vacuum-filtered using a Multimate 96-well harvester (Packard Instruments, Meriden, CT) onto GF/B filters presoaked for at least 30 min in assay buffer containing 0.15% bovine serum albumin. Each well was then washed with approximately 3.0 mL of ice-cold buffer. The filter plates were dried, and 30 uL of Microscint20 (Packard) was added to each well. The amount of radioligand remaining on each filter was determined using a

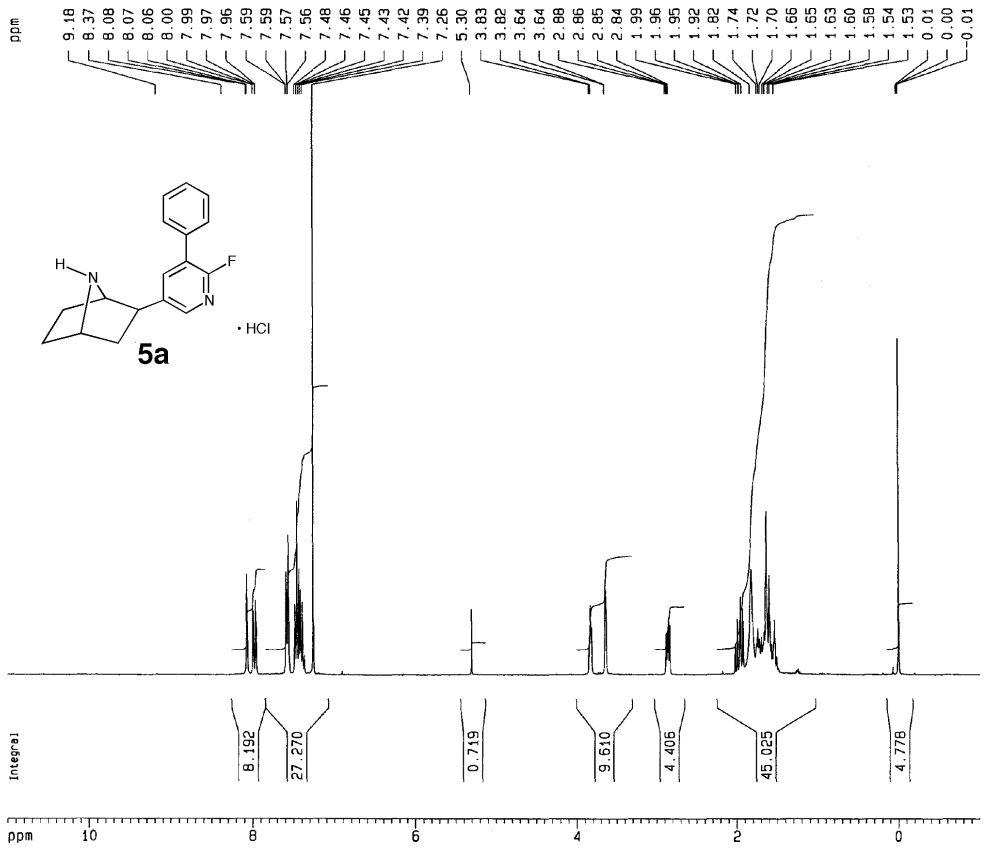
TopCount 12-detector (Packard) microplate scintillation counter at approximately 70% efficiency.

Tail-flick test. Antinociception was assessed by the tail-flick method of D'Amour and Smith.22 A control response (2–4 sec) was determined for each mouse before treatment, and a test latency was determined after drug administration. In order to minimize tissue damage, a maximum latency of 10 sec was imposed. Antinociceptive response was calculated as percent maximum possible effect (% MPE), where %MPE = [(test-control)/(10-control)] x 100. Groups of eight to twelve animals were used for each dose and for each treatment. The mice were tested 5 min after i. t. injections of epibatidine analogs for the dose-response evaluation. Eight to twelve mice were treated per dose and a minimum of 4 doses were performed for dose-response curve determination.

Hot-plate Test. Mice were placed into a 10 cm wide glass cylinder on a hot plate (Thermojust Apparatus) maintained at 55.0 C. Two control latencies at least ten min apart were determined for each mouse. The normal latency (reaction time) was 8 to 12 seconds. Antinociceptive response was calculated as percent maximum possible effect (% MPE), where %MPE = [(test-control)/40-control) x 100]. The reaction time was scored when the animal jumped or licked its paws. Eight mice per dose were injected s.c. with epibatidine analogs and tested 5 min thereafter in order to establish a dose-response curve.

Locomotor activity. Mice were placed into individual Omnitech photocell activity cages (28 x 16.5 cm) 5 min after s.c. administration of either 0.9% saline or epibatidine analogs. Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 10 min. Data were expressed as number of photocell interruptions.

Body temperature. Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Readings were taken just before and at different times after the s.c. injection of either saline or epibatidine analogs. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21–24°C from day to day.



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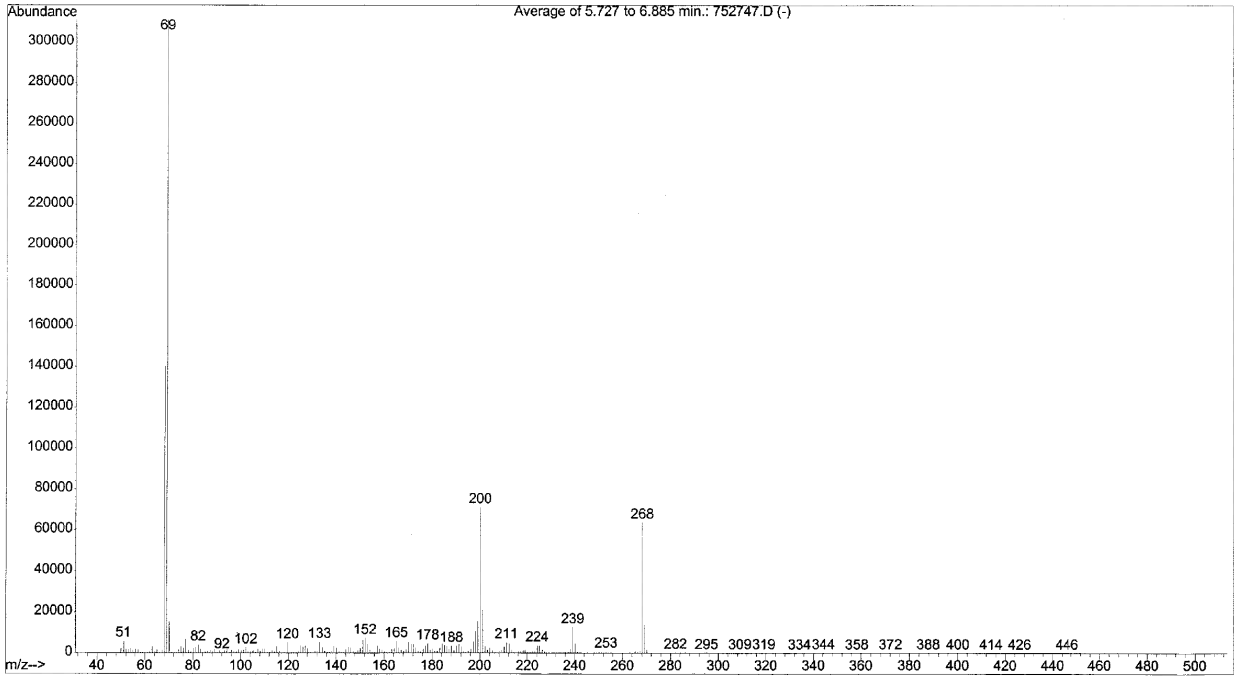
5a



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5a

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Method: Epibatidine
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Sampling Int: 0.1 Seconds

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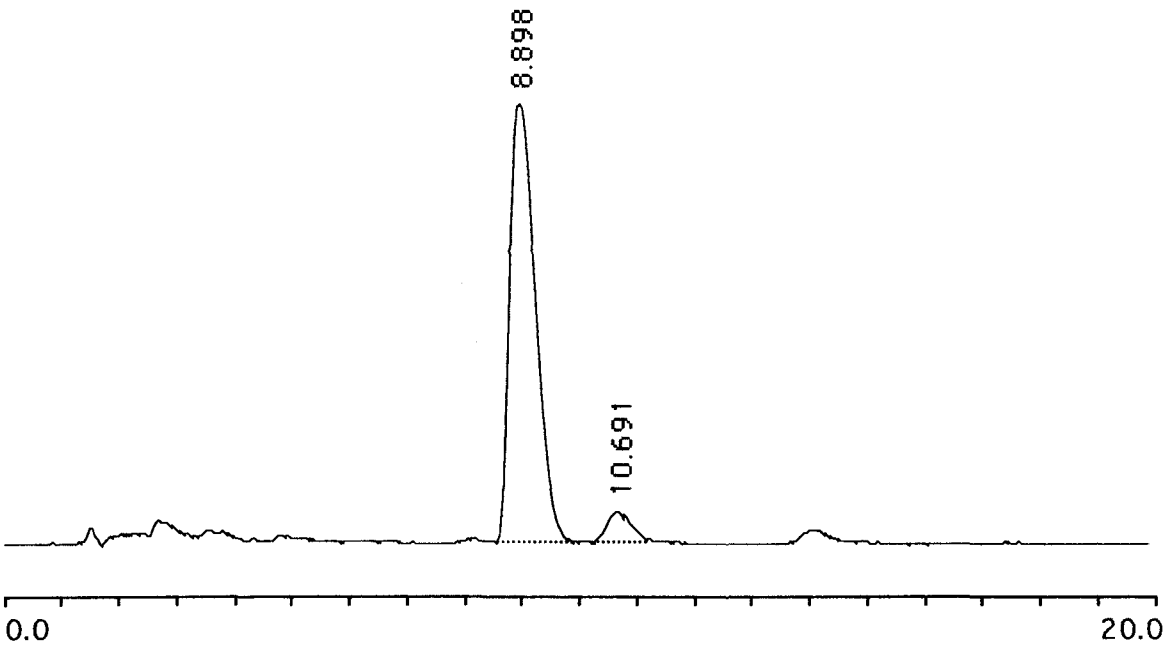

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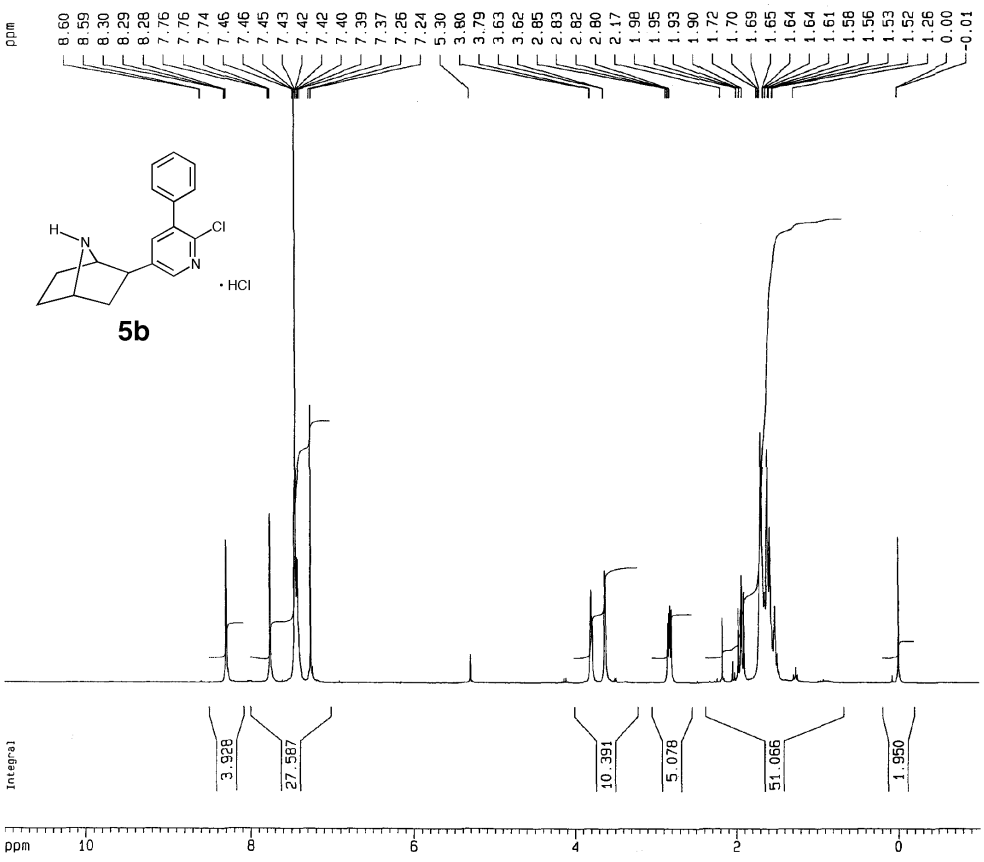

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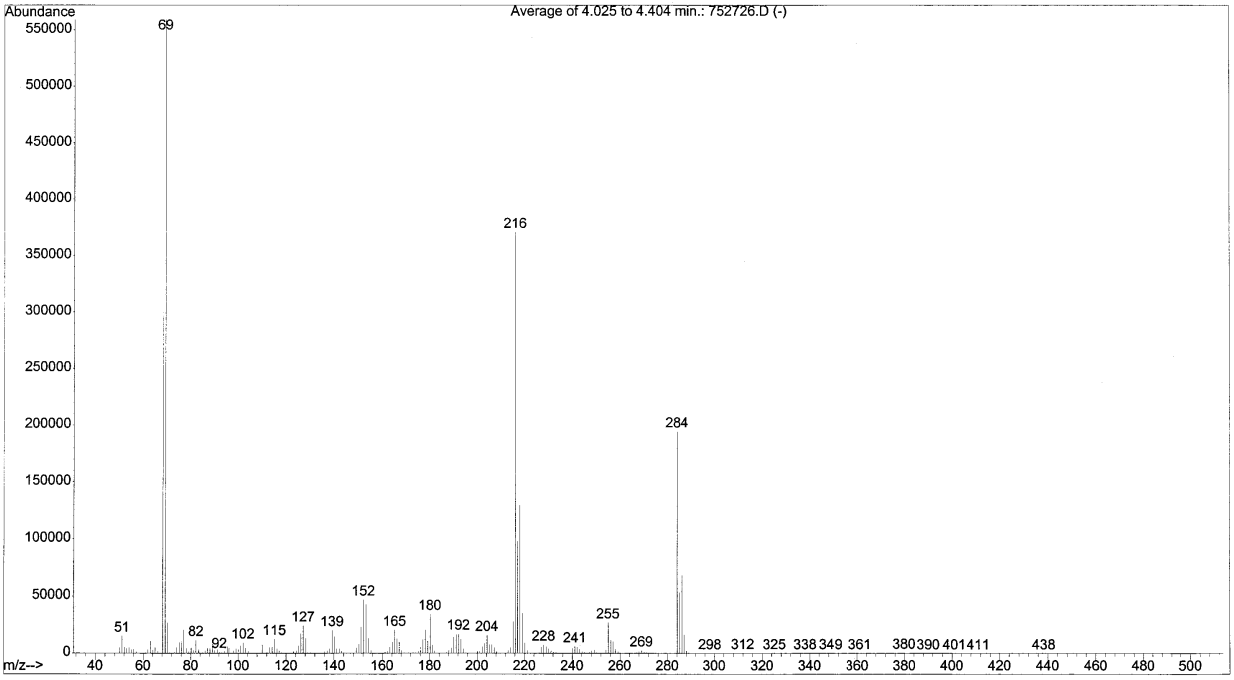
5b

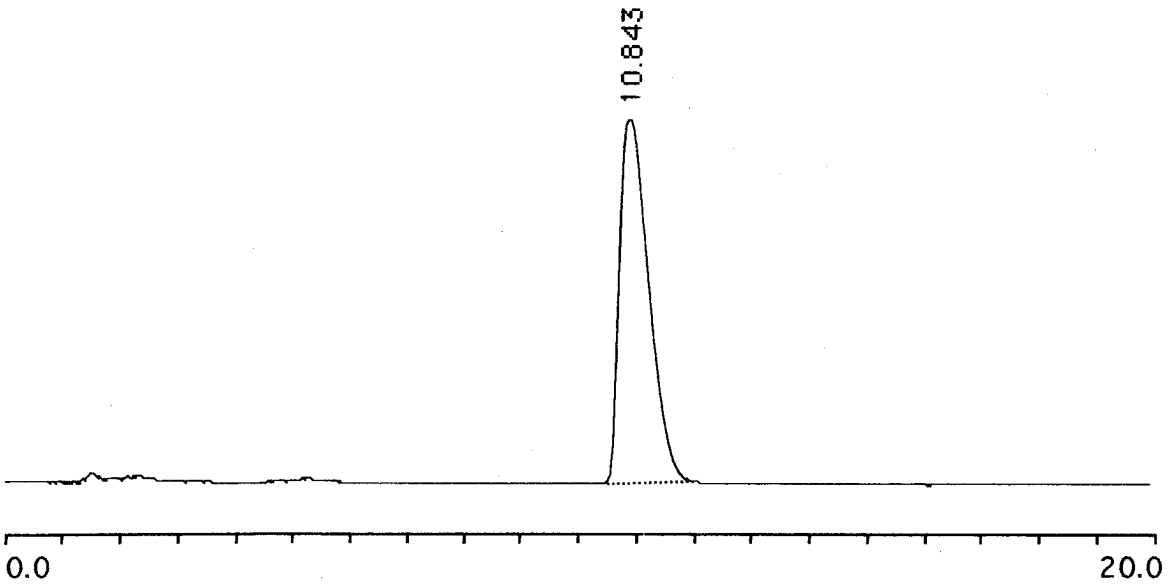


Date: Tue, Jul 17, 2001 1:37 PM **5b**
Data: Epibatidine-17JUL101-008

Sample: RTI-7527-26 (about 1 mg/ml CH3OH), A: [TFA-H2O], B: CH3CN, 25% B, 0.6 ml/min, 225 nm, Phenomenex Synergi 4μ Hydro-RP 80A (3*150 mm, 4 μm)

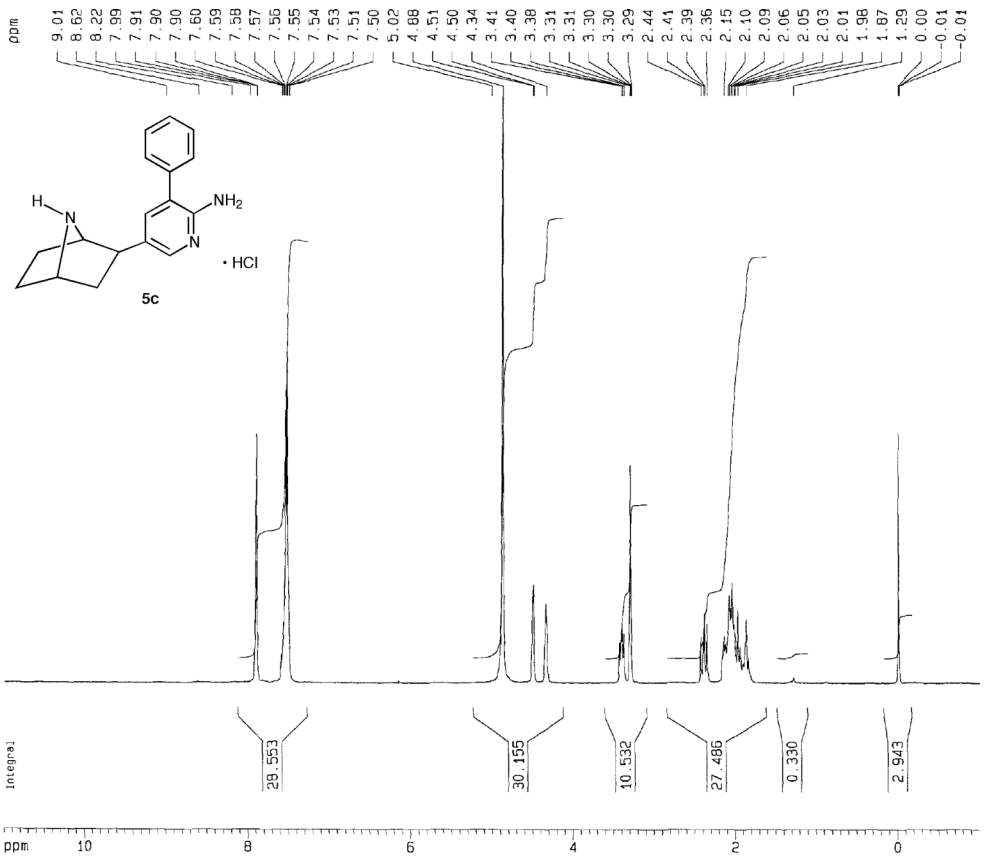
Method: Epibatidine
Inject Vol: 1
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	10.843	N	84079	2763672	100.000
Total Area				2763672	100.000

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Current Data Parameters
NAME            RT1752728
EXPNO                 190
PROCNO                  1

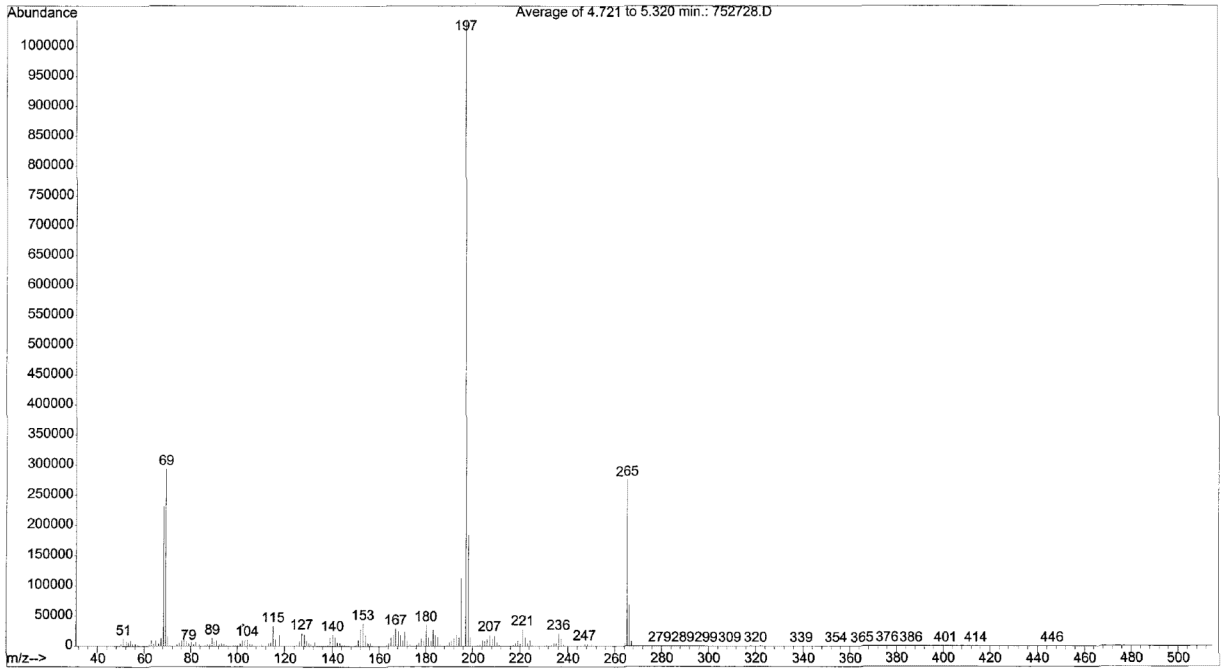
F2 - Acquisition Parameters
Date_             20010716
Time                15.19
INSTRUM             spect
PROBHD    5 mm QNP  1H/
PULPROG                zg
TD                  32768
SOLVENT              MeOH
NS                     16
DS                      2
SWH              6172.839 Hz
FIDRES           0.188380 Hz
AQ               2.6542580 sec
RG                  456.1
DW                 81.000 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
P1                  5.50 usec
SFO1          300.1318534 MHz
NUC1                   1H
PL1                -6.00 dB

F2 - Processing parameters
SI                  16384
SF            300.1300065 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00

1D NMR plot parameters
CX                  20.00 cm
F1P                11.000 ppm
F1                3301.43 Hz
F2P                -1.000 ppm
F2                -300.13 Hz
PPMCM             0.60000 ppm/cm
HZCM            180.07800 Hz/cm
```

```
File       : C:\HPCHEM\1\DATA\SAMPLES\752728.D
Operator   : R. Mitchell
Acquired   : 17 Jul 2001    8:57 am using AcqMethod EIDIPHED
Instrument :    GC/MS Ins
Sample Name: RTI-7527-28
Misc Info  : Abraham
Vial Number: 1
```

5c



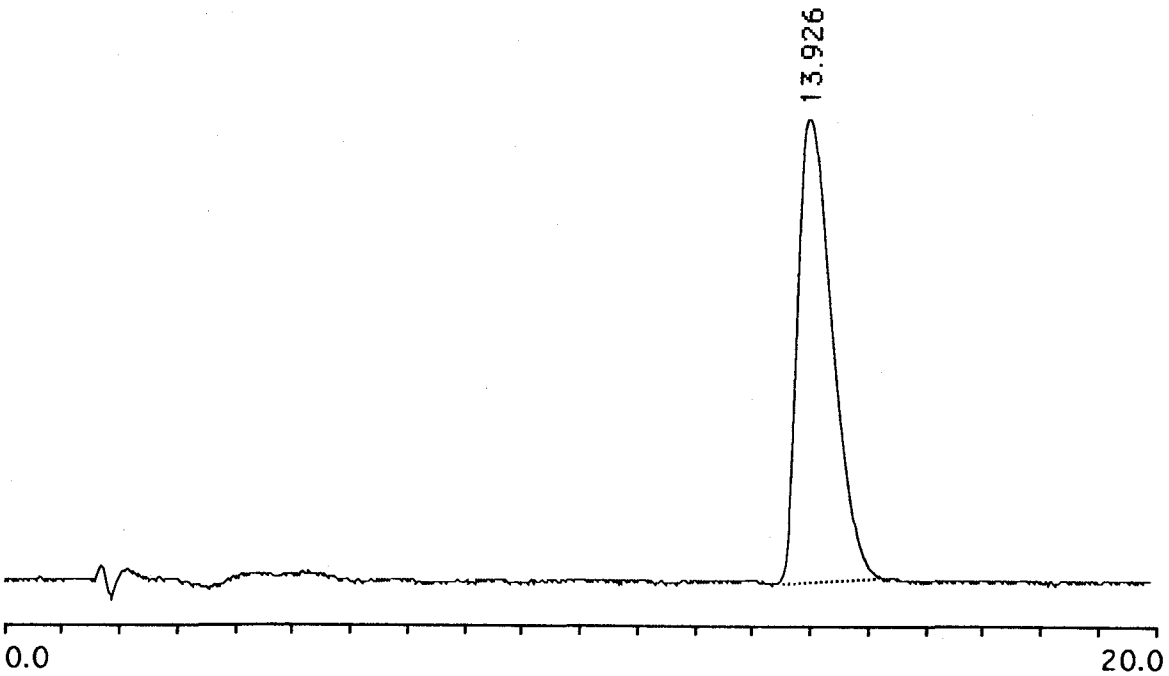
Date: Wed, Jul 18, 2001 9:20 AM
Data: Epibatidine-18JUL101-002

5c

Sample: RTI-7527-28 (about 1 mg/ml CH3OH), A: [TFA-H2O], B: CH3CN, 10% B, 0.6 ml/min, 225 nm, Phenomenex Synergi 4μ Hydro-RP 80A (3*150 mm, 4 μm)

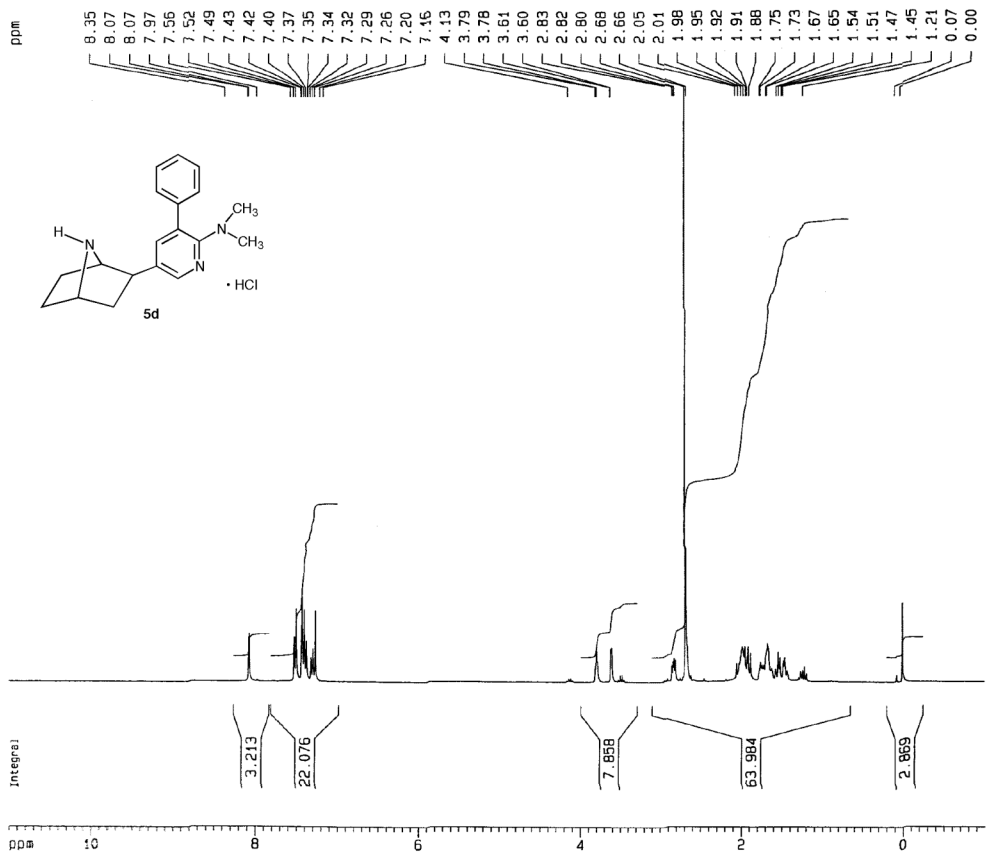
Method: Epibatidine
Inject Vol: 1
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	13.926	N	34311	1326864	100.000
Total Area				1326864	100.000



Current Data Parameters
NAME 1eb
EXPND 1BD
PROCNO 1

F2 - Acquisition Parameters
Date_ 20001206
Time 12.12
INSTRUM spect
PROBHD 5 mm QNP 1H/
PULPROG zg
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 456.1
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
P1 5.50 usec
SFO1 300.1318534 MHz
NUC1 1H
PL1 -6.00 dB

F2 - Processing parameters
SI 16384
SF 300.1300051 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 3301.43 Hz
F2P -1.000 ppm
F2 -300.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 180.07800 Hz/cm

```
File       : C:\HPCHEM\1\DATA\SAMPLES\752748.D
Operator   : R. Mitchell
Acquired   : 17 Jul 2001  10:01 am using AcqMethod EIDIPHED
Instrument :   GC/MS Ins
Sample Name: RTI-7527-48
Misc Info  : Abraham
Vial Number: 1
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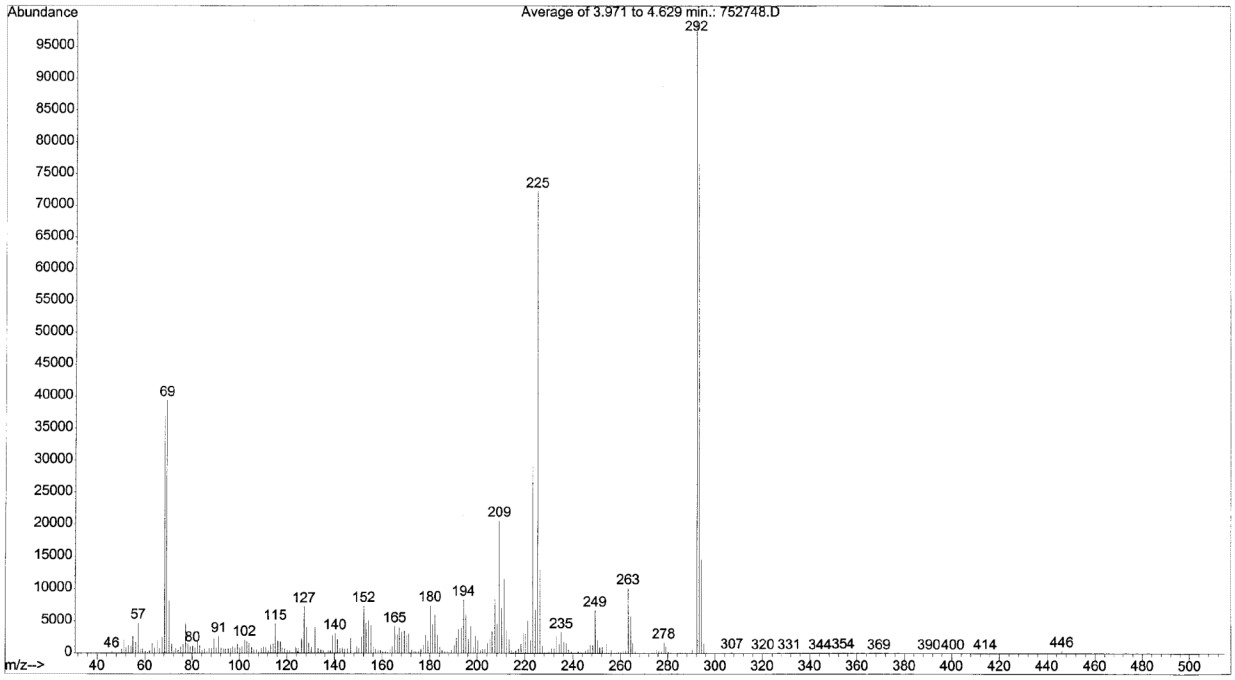
5d

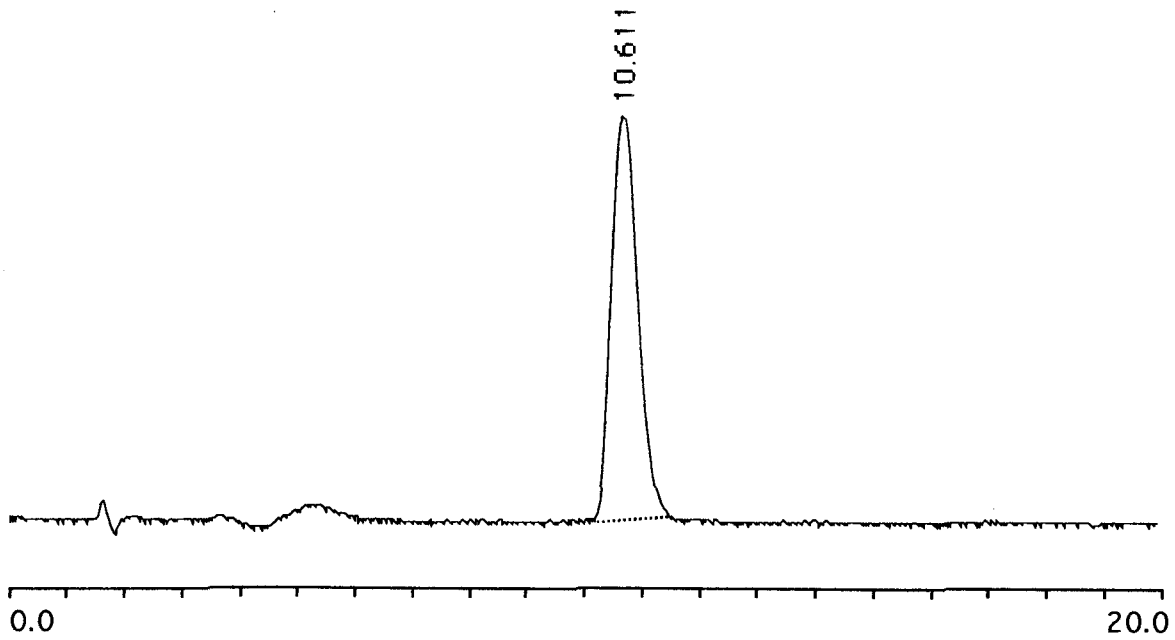


Date: Wed, Jul 18, 2001 10:07 AM **5d**
Data: Epibatidine-18JUL101-003

Sample: RTI-7527-48 (about 1 mg/ml CH3OH), A: [TFA-H2O], B: CH3CN, 15% B, 0.6 ml/min, 225 nm, Phenomenex Synergi 4μ Hydro-RP 80A (3*150 mm, 4 μm)

Method: Epibatidine
Inject Vol: 1
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	10.611	N	16462	484919	100.000
Total Area				484919	100.000