Supporting Information

The Design, synthesis and evaluation of tetra-substituted pyridines as potent 5-HT_{2C} receptor agonists

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Contents

1. Abbreviations - The following abbreviations and definitions have been used:

APCI	Atmospheric Pressure Chemical Ionisation
br	broad
CBz	Benzyloxycarbonyl
$CDCl_3$	Chloroform-d_1
CD_3OD	Methanol-d_4
Celite®	Filtration agent
CH_2Cl_2	Dichloromethane
δ	Chemical shift
d	Doublet
DCE	Dichloroethane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
ee	Enantiomeric excess
EI	Electron ionisation
ELSD	Evaporative Light Scattering Detector
eq	Equivalent
ESI	Electrospray ionisation (positive scan)
EtOAc	Ethyl acetate
Et_2O	Diethylether
EtOH	Ethanol
h	Hour(s)
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
HPLC	High Performance Liquid chromatography
HRMS	High resolution mass spectrum
IPA	Isopropyl alcohol
LRMS	Low resolution mass spectrum
M	Molarity

m	Multiplet
Me	Methyl
MeOH	Methanol
mg	Milligram
min(s)	Minute(s)
MHz	Megahertz
mL	Milliliter
mmol	Millimole
m/z	Mass spectrum peak
N	Normal concentration
NaOMe	Sodium methoxide
nm	Nanometer
NMR	Nuclear Magnetic Resonance
R_t	Retention time
s	Singlet
t	Triplet
Tf	(Trifluoromethyl)sulfonyl
THF	Tetrahydrofuran
UV-TIC	Ultraviolet-total ion count

2. General Chemistry Experimental Section

Experimental section

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions. Commercial reagents were purchased from Sigma Aldrich. Analytical thin layer chromatography (TLC) was performed using silica gel 60 F254 precoated glass-backed plates and visualized by ultraviolet radiation (254 nm) and potassium permanganate. Flash column chromatography was performed using silica gel (particle size 40−63 nm) under air pressure. ^{1}H NMR spectra were recorded on 600 MHz or 400 MHz spectrometers. The following abbreviations have been used for common solvents: $CDCl_3$, deuterochloroform; DMSO-d_6, deuterodimethylsulphoxide; CD_3OD, deuteromethanol Chemical shifts are reported in ppm with the resonance resulting from incomplete deuteration of the solvent as the internal standard ($CDCl_3$: 7.26 ppm, s), (CD_2Cl_2: 5.32 ppm, s), (DMSO-d_6: 2.5 ppm, s). ^{13}C NMR spectra were recorded on 150 MHz or 100 MHz spectrometers with complete proton decoupling. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($^{13}CDCl_3$: 77.16 ppm, t), ($^{13}CD_2Cl_2$: 54 ppm, qt), (DMSO-d_6: 39.52 ppm, sept). The mass spectra (*m/z*) were recorded using either electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI). HRMS was performed using electrospray ionization with time-of-flight mass analysis. HRMS signals are reported to 4 decimal places and are within ±5 ppm of theoretical values. Infrared spectra were recorded neat as thin films and only selected peaks are reported.

HPLC, unless indicated otherwise, was performed by one of the following methods:
A: Column: Sunfire C18 4.6 x 50mm; Mobile Phase A: 0.05% formic acid in water; Mobile Phase B: 0.05% formic acid in acetonitrile.
B: Column: Xterra 4.6 x 50mm; Mobile Phase A: 0.05% ammonia in water; Mobile Phase B: 0.05% ammonia in acetonitrile
C: Column: Luna C8 4.6 x 50mm; Mobile Phase A: 10mM ammonium acetate in water; Mobile Phase B: 10mM ammonium acetate in acetonitrile
D: Column: C18 4.6 x 50mm; Mobile Phase A: 0.1% formic acid in water; Mobile Phase B: 0.1% formic acid in acetonitrile.
E: Column: XBridge C18 4.6 x 150 mm; Mobile Phase A: 0.1% TFA in water; Mobile Phase B: 0.1% TFA in acetonitrile.

General procedure A: Sonogashira reactions

Copper iodide (19 mg, 0.1 mmol, 20 mol %) and bis(triphenylphosphine)palladium(II) dichloride (35 mg, 0.05 mmol, 10 mol %)[a] were added to a mixture of triflate or iodide derivative (0.5 mmol), alkyne (1 mmol, 2 eq.) and N,N-diisopropylethylamine (129 mg, 1 mmol, 2 eq.) in DMF (2.5 mL), The resulting mixture was then stirred for 2 h at room temperature.[b] Saturated NH_4Cl_{aq} (2 mL), water (5 mL) and Et_2O (10 mL) were added to the reaction mixture. The organic layer was separated and the aqueous layer extracted with Et_2O (2x). The combined organics were washed with brine (10 mL), dried over $MgSO_4$, filtered and the solvent removed under vacuum. Flash column chromatography over silica gel afforded the alkyne product.

[a] In the case of ethyl (Z)-3-(((trifluoromethyl)sulfonyl)oxy)but-2-enoate, used in the formation of **22e**, tetrakis(triphenylphosphine)palladium(0) (58 mg, 0.05 mmol, 10 mol %) was used as catalyst.

[b] When iodide derivatives were used as starting materials, the reaction mixture was stirred for 2 h at 50 ^{o}C.

General procedure B: Synthesis of amino pyridines

To the pure alkyne (0.5 mmol) obtained *via* the general procedure **A**, in a sealed tube, was added a solution of NH_3 in methanol (7M, 5.7 mL, 80 eq.). The mixture was stirred for 15 h at 80 ^{o}C. The reaction mixture was cooled to room temperature and the solvent removed in *vacuo*. Flash column chromatography over silica gel afforded the expected amino pyridine.

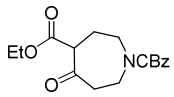
General procedure C: Reductive amination of aldehydes with aminopyridine 13.

Benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 84 mg, 0.22 mmol) was dissolved in DCE (4.4 mL, 0.05 M), then aldehyde (0.88 mmol) added and the resulting solution stirred at 23 °C for 30 min. PS-cyanoborohydride (124 mg, 3.5 mmol/g, 0.44 mmol) was added and the suspension heated with stirring to 55 °C for 17 h. Another portion of aldehyde (0.44 mmol) was added and the mixture stirred at 55 °C for a further 24 h then cooled to room temperature, filtered and the filtrate concentrated under reduced pressure. The crude product was purified by flash column chromatography to afford the title compound.

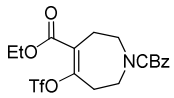
General procedure D: Removal of the CBz (Z) azepine protecting group.

CBz (Z) protected azepine (0.07mmol) was dissolved in EtOH (1.3 mL, 0.05M) and 10% Pd/C (10 mg) was added. The reaction vessel was pressurized with H_2 (45 psi) and then shaken at 23 °C for 3 h. The mixture was filtered through celite and the filtrate concentrated under reduced pressure to yield the title compound.

Experimental Data



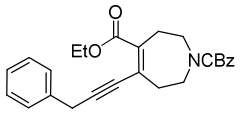
1-Benzyl 4-ethyl 5-oxoazepane-1,4-dicarboxylate (10). β-Ketoester **10** was synthesized in a quantitative yield according to a reported procedure.[1]



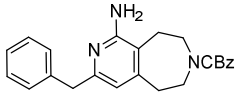
1-Benzyl 4-ethyl 5-(((trifluoromethyl)sulfonyl)oxy)-2,3,6,7-tetrahydro-1H-azepine-1,4-dicarboxylate (11). Vinyl triflate **11** was synthesized according to a reported procedure[1] from 1-benzyl 4-ethyl 5-oxoazepane-1,4-dicarboxylate (**10**, 5.8 g, 18.1 mmol). Flash column chromatography over silica gel (petroleum ether/EtOAc : 80/20) afforded the title compound as a yellow oil (6.5 g, 80% yield).

The following procedure was also used: A gum of 1-Benzyl 4-ethyl 5-oxoazepane-1,4-dicarboxylate (**11**, 3.0 g, 9.4 mmol) was dissolved in CH_2Cl_2 (100 mL, 0.10M). Solid NaO^tBu (1.4 g, 15.0 mmol) was added portionwise and the resulting mixture stirred for 30 min at 23 °C. A solution of the triflic anhydride (4.0 g, 2.3 mL, 14.1 mmol) in CH_2Cl_2 (100 mL) was added slowly dropwise at 23 °C with rapid stirring. The resulting solution was stirred for 2 h then quenched by addition of sat. sodium bicarbonate and extracted into CH_2Cl_2. The combined organics were dried over magnesium sulfate, filtered and concentrated to yield the crude product as orange oil. The product was purified by flash column chromatography eluting with 0-30% EtOAc in pentane to yield the title compound as a yellow oil (3.44 g, 81% yield). *Rf* 0.3 (petroleum ether/EtOAc : 80/20). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.26 (m, 5H), 5.15 (s, 2H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.75-3.62 (m, 4H), 2.81-2.64 (m, 4H), 1.32 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.5, 165.4, 155.5, 155.3, 152.0, 151.4, 136.5, 128.7, 128.3, 128.1, 128.0, 127.3, 126.9, 118.4 (q, *J* = 320.1 Hz), 67.7, 62.3, 45.5, 45.3, 42.5, 42.4, 34.9, 34.8, 28.3, 28.2, 14.0 (some ^{13}C signals due to CBz rotamers). FTIR (υ_{max} cm^{-1}) 2982, 1698, 1418, 1247, 1203, 1136, 1065, 822, 763. HPLC (4 min) t_R = 3.0 min, UV (DAD/TIC) >95% purity. LRMS (ESI) *m/z* 452 $[M+H]^+$. HRMS Calcd for $C_{18}H_{21}F_3NO_7S$: 452.1029; Found $(M+H)^+$: 452.1031.

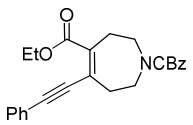
[1] Patent WO 2008/039420 A2



1-Benzyl 4-ethyl 5-(3-phenylprop-1-yn-1-yl)-2,3,6,7-tetrahydro-1H-azepine-1,4-dicarboxylate (12). Alkynylester **12** was synthesized according to general procedure **A** with 3-phenyl-1-propyne as alkyne (1 mmol, 116 mg). Column chromatography over silica gel (petroleum ether/EtOAc : 80/20) afforded the title compound as a yellow oil (185 mg, 89%). *Rf* 0.35 (petroleum ether/EtOAc : 80/20). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.10 (m, 10H), 5.15 (s, 2H), 4.17 (q, *J* = 7.0 Hz, 2H), 3.79 (s, 2H), 3.77-3.49 (m, 4H), 2.86-2.56 (m, 4H), 1.22 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 155.5, 137.9, 137.5, 136.7, 136.3, 132.0, 130.5, 128.5, 128.5, 128.0, 127.9, 127.8, 126.7, 96.6, 96.3, 83.4, 67.2, 60.9, 45.3, 44.4, 38.1, 30.9, 26.2, 14.1 (some ^{13}C signals due to CBz rotamers). FTIR (υ_{max} cm^{-1}) 2977, 2900, 1693, 1422, 1247, 1207, 1101, 729. HPLC (6 min) t_R = 3.73 min, ELSD >95% purity. LRMS (ESI) *m/z* 418 $[M+H]^+$. HRMS Calcd for $C_{26}H_{28}NO_4$: 418.2098; Found $(M+H)^+$: 418.2098.

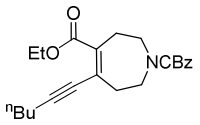


Benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (13). Aminopyridine **13** was synthesized according to general procedure **B** from 1-benzyl 4-ethyl 5-(3-phenylprop-1-yn-1-yl)-2,3,6,7-tetrahydro-1H-azepine-1,4-dicarboxylate (**12**, 208 mg, 0.5 mmol). Column chromatography over silica gel (EtOAc/CH_2Cl_2 : 50/50 + 1% MeOH) afforded the title compound as a light brown solid (151 mg, 78%). *Rf* 0.26 (EtOAc/CH_2Cl_2 : 50/50). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47-7.07 (m, 10H), 6.23 (s, 1H), 5.64 (s, 1H), 5.09 (s, 2H), 3.78 (s, 2H), 3.61-3.40 (m, 4H), 2.83-2.61 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.4, 156.1, 154.9, 149.6, 149.5, 140.3, 137.0, 128.8, 128.40, 128.2, 127.7, 127.4, 125.9, 114.9, 113.8, 66.2, 45.5, 45.3, 44.6, 43.3, 35.1, 35.0, 28.3, 28.0 (some ^{13}C signals due to CBz rotamers). FTIR (υ_{max} cm^{-1}) 3476, 3355, 3186, 2920, 1686, 1416, 1243, 1210, 1103, 696. HPLC (12 min) t_R = 7.55 min, UV (DAD/TIC) 95% purity. LRMS (ESI) *m/z* 388 $[M+H]^+$. HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{24}H_{26}N_3O_2$ 388.2026; found 388.2025.

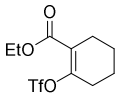


1-Benzyl 4-ethyl 5-(phenylethynyl)-2,3,6,7-tetrahydro-1H-azepine-1,4-dicarboxylate (14a). Alkynylester **14a** was synthesized according to the general procedure **A** with phenyl acetylene as alkyne (1 mmol, 102 mg). Column chromatography over silica gel (petroleum ether/EtOAc : 80/20) afforded the title compound as a yellow oil (179 mg, 89%). *Rf* 0.4 (petroleum ether/AcOEt = 80/20). ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.56-7.14 (m, 10H), 5.17 (s, 2H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.77-3.51 (m, 4H), 2.88-2.62 (m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 155.4, 138.4, 138.0, 136.7, 131.5, 131.1, 130.5, 128.6, 128.5, 128.3, 128.0, 127.8, 123.0, 97.8, 97.5, 90.2, 67.2, 61.0, 45.3, 44.4, 37.9, 31.0, 14.3. FTIR (υ_{max} cm^{-1}) 2983, 2936, 1692, 1423, 1246, 1210, 1076, 908, 727, 690. HRMS Calcd for $C_{25}H_{26}NO_4$: 404.1861; Found $[M+H]^+$: 404.1862.

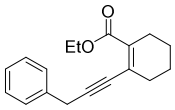


1-Benzyl 4-ethyl 5-(hex-1-yn-1-yl)-2,3,6,7-tetrahydro-1H-azepine-1,4-dicarboxylate (14b). Alkynylester **14b** was synthesized according to the general procedure **A** with 1-hexyne as alkyne (1 mmol, 82 mg). Column chromatography over silica gel (petroleum ether/EtOAc : 80/20) afforded the title compound as a yellow oil (163 mg, 85%). *Rf* 0.5 (petroleum ether/EtOAc = 80/20). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.28 (m, 5H), 5.15 (s, 2H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.67-3.49 (m, 4H), 2.76-2.56 (m, 4H), 2.37 (t, *J* = 7.0 Hz, 2H), 1.57-1.46 (m, 2H), 1.42 (dq, *J* = 14.1, 7.1 Hz, 2H), 1.31 (t, *J* = 7.1 Hz, 3H), 0.91 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 155.4, 137.0, 136.7, 136.6, 131.5, 130.9, 128.4, 127.9, 127.7, 99.6, 99.3, 81.5, 67.1, 60.7, 45.3, 44.2, 38.1, 30.7, 30.5, 21.9, 19.4, 14.1, 13.5. FTIR (υ_{max} cm^{-1}) 2960, 2932, 1694, 1423, 1247, 1205, 1100, 1055, 955, 732. HRMS Calcd for $C_{23}H_{30}NO_4$: 384.2216; Found $[M+H]^+$: 384.2220.

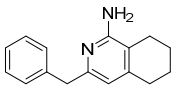


Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)cyclohex-1-ene-1-carboxylate (Precursor to 14c). The vinyl triflate was synthesized according to a reported procedure[2] from ethyl 2-oxocyclohexanecarboxylate (2.04 g, 12 mmol). Flash column chromatography over silica gel (petroleum ether/CH_2Cl_2 = 80/20) afforded the title compound as a colourless liquid (3.26 g, 90% yield). *Rf* 0.3 (petroleum ether/CH_2Cl_2 = 80/20). Colourless liquid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.27 (q, *J* = 7.1 Hz, 2H), 2.50-2.45 (m, 2H), 2.42-2.37 (m, 2H), 1.81-1.75 (m, 2H), 1.69-1.64 (m, 2H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.9, 151.4, 123.4, 118.4 (q, *J* = 319.9 Hz), 61.7, 28.6, 26.3, 22.4, 21.1, 14.1. FTIR (υ_{max} cm^{-1}) 2949, 1722, 1420, 1202, 1138, 1040, 912, 824, 611. HRMS Calcd for $C_{10}H_{14}F_3O_5S$: 303.0523; Found $[M+H]^+$: 303.0514.

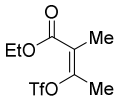
[2] Babinski, D.; Soltani, O.; Frantz, D. E. *Org. Lett.* **2008***, 10*, 2901-2904.



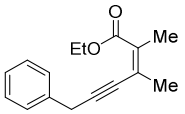
Ethyl 2-(3-phenylprop-1-yn-1-yl)cyclohex-1-ene-1-carboxylate (14c). Alkynylester **14c** was synthesized according to general procedure **A** from Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)cyclohex-1-ene-1-carboxylate (151 mg, 0.5 mmol) and 3-phenyl-1-propyne (116 mg, 1mmol) using bis(triphenylphosphine)palladium(II) dichloride (35 mg, 0.05 mmol) as catalyst. Flash column chromatography over silica gel (petroleum ether / CH_2Cl_2 : 60/40) afforded the title compound as a yellow oil (109 mg, 81% yield). Rf 0.21 (petroleum ether/ CH_2Cl_2 = 70/30). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.81 (s, 2H), 2.36 (dd, *J* = 9.1, 4.1 Hz, 4H), 1.73 – 1.58 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 136.8, 133.9, 128.6, 128.5, 128.1, 126.7, 94.8, 82.9, 60.5, 32.7, 26.3, 26.3, 22.0, 21.8, 14.3. FTIR (υ_{max} cm^{-1}) 2935, 1716, 1695, 1453, 1371, 1233, 1208, 1048, 728. HRMS Calcd for $C_{18}H_{21}O_2$: 269.1551; Found $[M+H]^+$: 269.1554.



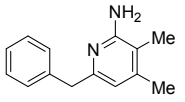
3-Benzyl-5,6,7,8-tetrahydroisoquinolin-1-amine (16c). Aminopyridine **16c** was synthesized according to general procedure **B** from Ethyl 2-(3-phenylprop-1-yn-1-yl)cyclohex-1-ene-1-carboxylate (**14c**, 134 mg, 0.5 mmol). Column chromatography over silica gel (petroleum ether/EtOAc = 50/50 + 1%MeOH) afforded the title compound as a yellow solid (108 mg, 91% yield). Rf 0.22 (petroleum ether/EtOAc : 50/50). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.40-7.03 (m, 5H), 6.28 (s, 1H), 4.41 (s, 2H), 3.85 (s, 2H), 2.59 (t, *J* = 6.1 Hz, 2H), 2.32 (t, *J* = 6.3 Hz, 2H), 1.94-1.79 (m, 2H), 1.75-1.63 (m, 2H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 157.2, 155.8, 147.7, 141.3, 129.5, 128.8, 126.5, 114.8, 114.0, 44.5, 29.8, 24.0, 23.4, 22.8. FTIR (υ_{max} cm^{-1}) 3429, 3390, 3334, 3195, 2931, 1592, 1572, 1407, 842. HRMS Calcd for $C_{16}H_{19}N_2$: 239.1548; Found $[M+H]^+$: 239.1546.



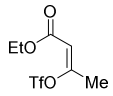
Ethyl (Z)-2-methyl-3-(((trifluoromethyl)sulfonyl)oxy)but-2-enoate (Precursor to 14d). The vinyl triflate was synthesized according to a reported procedure[2] from ethyl 2-methyl-3-oxobutanoate (100 mg, 0.69 mmol). Flash column chromatography over silica gel (CH_2Cl_2:pentane 50/50) afforded the title compound as a yellow oil (123 mg, 64% yield). ^{1}H- and ^{13}C-NMR matched with those previously reported in literature.[2] HPLC (2 min) t_R = 1.6 min, UV (DAD:TIC) 93% purity. LRMS (ESI) *m/z* 277 $[M+H]^+$.



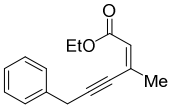
Ethyl (Z)-2,3-dimethyl-6-phenylhex-2-en-4-ynoate (14d). Alkynylester **14d** was synthesized according to general procedure **A** from ethyl (Z)-2-methyl-3-(((trifluoromethyl)sulfonyl)oxy)but-2-enoate (106 mg, 0.38 mmol). Column chromatography over silica gel (pentane/CH_2Cl_2 = 50/50) afforded the title compound as a colourless oil (86 mg, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.20 (m, 5H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.79 (s, 2H), 1.99 (s, 3H), 1.93 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H) ; HPLC (2 min) t_R = 1.73 min, UV (DAD:TIC) 95% purity. LRMS (ESI) *m/z* 243 $[M+H]^+$.



6-Benzyl-3,4-dimethylpyridin-2-amine (16d). Aminopyridine (**16d**) was synthesized according to general procedure **B** from ethyl (Z)-2,3-dimethyl-6-phenylhex-2-en-4-ynoate (**14d**, 86 mg, 0.36 mmol). Column chromatography over silica gel (CH_2Cl_2 to CH_2Cl_2/MeOH/NH_3 = 95/5/0.5) afforded the title compound as a yellow solid (30 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.04 (m, 5H), 6.35 (s, 1H), 3.91 (s, 2H), 2.18 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 155.5, 146.8, 140.2, 129.1, 128.8, 126.3, 116.5, 112.8, 43.8, 19.9, 12.4; HPLC (1.07 min) t_R = 1.73 min, ELSD 94% purity. LRMS (ESI) *m/z* 213 $[M+H]^+$.

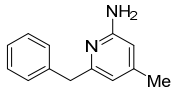


Ethyl (Z)-3-(((trifluoromethyl)sulfonyl)oxy)but-2-enoate (Precursor to 14e). The vinyl triflate was synthesized according to a reported procedure[1] from Ethyl acetoacetate (2.6 g, 20 mmol). Flash column chromatography over silica gel (petroleum ether/CH_2Cl_2 = 80/20) afforded the title compound as a colourless liquid (4.45 g, 85% yield). ^{1}H- and ^{13}C-NMR matched with those previously reported in literature.[1]

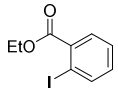


Ethyl (Z)-3-methyl-6-phenylhex-2-en-4-ynoate (14e). Alkynylester **14e** was synthesized according to general procedure **A** from ethyl (Z)-3-(((trifluoromethyl)sulfonyl)oxy)but-2-enoate (131 mg, 0.5 mmol). Column chromatography over silica gel (petroleum ether/ CH_2Cl_2 : 60/40) afforded the title compound as a

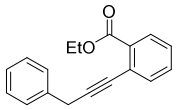
yellow oil (77.6 mg, 68% yield). Rf 0.26 (petroleum ether/CH_2Cl_2 = 60/40). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 7.7 Hz, 2H), 7.24 (t, *J* = 7.4 Hz, 1H), 5.98 (d, *J* = 1.2 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.88 (s, 2H), 2.06 (d, *J* = 1.4 Hz, 3H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.3, 136.2, 135.3, 128.6, 128.1, 126.8, 124.0, 99.7, 81.8, 60.1, 26.4, 25.8, 14.4. FTIR (υ_{max} cm^{-1}) 3030, 2980, 1720, 1699, 1620, 1375, 1219, 1149, 1048, 851, 726. HRMS Calcd for $C_{15}H_{17}O_2$: 229.1248; Found $[M+H]^+$: 229.1252.



6-Benzyl-4-methylpyridin-2-amine (16e). Aminopyridine **16e** was synthesized according to general procedure **B** from ethyl (Z)-3-methyl-6-phenylhex-2-en-4-ynoate (**14e**, 114 mg, 0.5 mmol). Column chromatography over silica gel (petroleum ether/EtOAc : 30/70 + 1% MeOH) afforded the title compound as a yellow solid (63 mg, 64% yield). Rf 0.33 (petroleum ether/EtOAc : 30/70). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (dt, *J* = 6.9, 6.0 Hz, 4H), 7.21 (t, *J* = 7.1 Hz, 1H), 6.27 (s, 1H), 6.15 (s, 1H), 4.42 (s, 2H), 3.94 (s, 2H), 2.15 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.1, 158.3, 149.5, 139.8, 129.3, 128.5, 126.3, 114.8, 106.7, 44.3, 21.1. FTIR (υ_{max} cm^{-1}) 3453, 3304, 3158, 1633, 1439, 1231, 840, 700. HRMS Calcd for $C_{13}H_{15}N_2$: 199.1235; Found $[M+H]^+$: 199.1240.

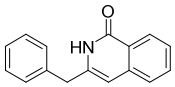


Ethyl 2-iodobenzoate (precursor to 14f). Synthesized according to a reported procedure from 2-iodo benzoic acid.[3]

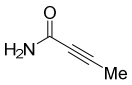


Ethyl 2-(3-phenylprop-1-yn-1-yl)benzoate (14f). Alkynylester **14f** was synthesized according to general procedure **A** from ethyl 2-iodobenzoate (138 g, 0.5 mmol) and 3-phenyl-1-propyne (116 mg, 1 mmol). Column chromatography over silica gel (petroleum ether/CH_2Cl_2 : 70/30) afforded the title compound as a yellow oil (103 mg, 78% yield). Rf 0.25 (petroleum ether/ CH_2Cl_2 : 70/30). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.56 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 2H), 7.43 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.37-7.30 (m, 3H), 7.25 (d, *J* = 7.4 Hz, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 3.91 (s, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 136.7, 134.4, 132.5, 131.5, 130.3, 128.6, 128.2, 127.6, 126.7, 124.1, 92.9, 81.5, 61.3, 26.2, 14.4. FTIR (υ_{max} cm^{-1}) 3029, 2981, 1723, 1707, 1453, 1288, 1247, 1077, 755. HRMS Calcd for $C_{18}H_{17}O_2$: 265.1229; Found $[M+H]^+$: 265.1232.

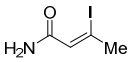
[3] Penhoat, M.; Levacher, V.; Dupas, G.; *J. Org. Chem.* **2003**, *68*, 9517-9520.



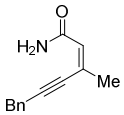
3-Benzylisoquinolin-1(2H)-one (15f). Pyridinone (**15f**) was synthesized according to general procedure **B** from ethyl 2-(3-phenylprop-1-yn-1-yl)benzoate (**14f**, 132 mg, 0.5 mmol). The reaction mixture was stirred for 2 days at 90 ^{o}C. Column chromatography over silica gel (petroleum ether/EtOAc : 70/30) afforded the title compound as a yellow solid (77 mg, 66% yield). *Rf* 0.3 (petroleum ether/EtOAc : 70/30). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.73-7.54 (m, 1H), 7.45 (dd, *J* = 16.3, 7.9 Hz, 2H), 7.38-7.15 (m, 5H), 6.32 (s, 1H), 3.95 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 140.1, 138.4, 136.4, 132.7, 129.3, 129.0, 127.4, 127.4, 126.3, 126.0, 124.7, 105.1, 39.8. FTIR (υ_{max} cm^{-1}) 3166, 3027, 2920, 2855, 1659, 1641, 1454, 1259, 1029, 753, 703. HRMS Calcd for $C_{16}H_{14}NO$: 236.1080; Found $[M+H]^{+}$: 236.1083.



But-2-ynamide (precursor to 14e). Synthesized in a 51% yield according to a known procedure[4]

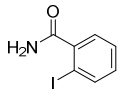


(Z)-3-Iodobut-2-enamide (precursor to 14e). Synthesized in a 58% yield according to a known procedure[4]

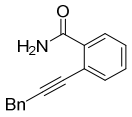


(Z)-3-Methyl-6-phenylhex-2-en-4-ynamide (14e). Alkynylamide **14e** was synthesized according to general procedure **A** from (Z)-3-iodobut-2-enamide (105 mg, 0.5 mmol). Column chromatography over silica gel (EtOAc/Petroleum ether : 70/30) afforded the title compound as a white solid (71 mg, 71% yield). *Rf* 0.38 (EtOAc/Petroleum ether : 70/30). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.44-7.16 (m, 5H), 6.90 (s, 1H, NH), 5.93 (s, 1H), 5.93 (s, 1H, NH), 3.86 (s, 2H), 2.05 (d, *J* = 0.9 Hz, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 167.4, 136.3, 129.4, 129.3, 129.0, 128.4, 127.5, 100.4, 81.4, 26.5, 25.7. FTIR (υ_{max} cm^{-1}) 3316, 3140, 1671, 1610, 1446, 1322, 1140, 844. HRMS Calcd for $C_{13}H_{14}NO$: 200.1084; Found $[M+H]^{+}$: 200.1083.

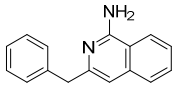
[4] Bair, J. S.; Palchaudhuri, R.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2010**, *132*, 5469-5478



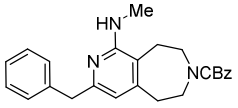
2-iodobenzamide (Precursor to 17f). 1 was synthesized in 50% yield from 2-iodo benzoic acid according to a reported procedure.[5]



2-(3-Phenylprop-1-yn-1-yl)benzamide (17f). Alkynylamide **17f** was synthesized according to general procedure **A** using 3-phenyl-1-propyne as alkyne (1 mmol, 116 mg). Column chromatography over silica gel (petroleum ether/EtOAc : 50/50) afforded the compound as a white solid (93 mg, 79%). *Rf* 0.4 (petroleum ether/EtOAc : 50/50). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18-7.95 (m, 1H), 7.60-7.27 (m, 9H), 5.71 (s, 1H), 3.90 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 135.8, 134.6, 133.8, 130.9, 130.2, 128.8, 128.4, 128.0, 127.1, 120.6, 94.9, 81.5, 26.0. FTIR (υ_{max} cm^{-1}) 3366, 3169, 1642, 1400, 1114, 700. HRMS Calcd for $C_{16}H_{14}NO$: 236.1089; Found $[M+H]^+$: 236.1091.



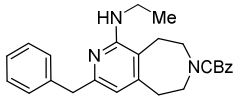
3-Benzylisoquinolin-1-amine (16f). Aminopyridine **16f** was synthesized according to general procedure **B** from 2-(3-phenylprop-1-yn-1-yl)benzamide (**17f**, 117 mg, 0.5 mmol). Column chromatography over silica gel (petroleum ether/EtOAc : 50/50 + 1%MeOH) afforded the title compound as a yellow solid (45 mg, 39% yield). *Rf* 0.3 (petroleum ether/EtOAc : 50/50). yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.3 Hz, 1H), 7.65-7.52 (m, 2H), 7.46-7.38 (m, 1H), 7.31 (dd, *J* = 7.4, 6.1 Hz, 4H), 7.23 (td, *J* = 5.4, 2.9 Hz, 1H), 6.77 (s, 1H), 5.43 (s, 2H), 4.10 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.0, 152.5, 139.9, 138.2, 130.3, 129.5, 128.5, 127.0, 126.3, 125.6, 122.6, 116.4, 110.9, 44.1. FTIR (υ_{max} cm^{-1}) 3496, 3295, 3130, 3056, 1633, 1436, 1072, 837, 733. HRMS Calcd for $C_{16}H_{15}N_2$: 235.1270; Found $[M+H]^+$: 235.1267.



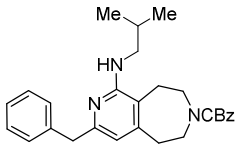
Benzyl 3-benzyl-1-(methylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20i, precursor to **7)**. Synthesized via general procedure **C** for reductive amination from benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 84 mg, 0.22 mmol) and formaldehyde

[5] Jithunsa, M.; Ueda, M.; Miyata, O.; *Org. Lett.*, **2011**, *13*, 518-521. (a solution of NH_3 in dioxane was used instead of gaseous NH_3).

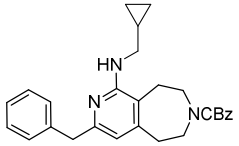
(70mg, 0.88 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-35% EtOAc in heptane to afford the title compound as a white solid (27 mg, 31% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.36-7.08 (m, 10H), 6.18-6.10 (m, 1H), 5.05 (s, 2H), 3.86 (s, 2H), 3.64-3.51 (m, 4H), 2.90-2.84 (m, 3H), 2.79-2.63 (m, 4H). HPLC (4 min) t_R = 2.1 min, UV (DAD/TIC) >95% purity. LRMS (ESI) *m/z* 402 $[M+H]^+$.



Benzyl 3-benzyl-1-(ethylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20a). Synthesized via general procedure **C** for reductive amination from benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 64 mg, 0.17 mmol) and acetaldehyde (8.8 mg, 0.20 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-30% EtOAc in heptane to afford the title compound as a white solid (41 mg, 60% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.37-7.15 (m, 9H), 7.12-7.08 (m, 1H), 6.18-6.11 (m, 1H), 5.05 (s, 2H), 3.83 (s, 2H), 3.68-3.50 (m, 4H), 3.43-3.31 (m, 2H), 2.78-2.63 (m, 4H), 1.18-1.09 (m, 3H). HPLC (4 min) t_R = 2.2 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 416 $[M+H]^+$.

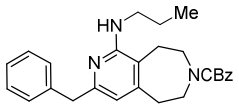


Benzyl 3-benzyl-1-(isobutylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20b). Synthesized via the general procedure for reductive amination from benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 50 mg, 0.13 mmol) and isobutyraldehyde (9.6 mg, 0.13 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-30% EtOAc in heptane to afford the title compound as a white solid (17 mg, 30% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.35-7.16 (m, 9H), 7.15-7.09 (m, 1H), 6.18-6.10 (m, 1H), 5.05 (s, 2H), 3.82 (s, 2H), 3.66-3.50 (m, 4H), 3.19-3.13 (m, 2H), 2.79-2.66 (m, 4H), 1.92-1.78 (m, 1H), 0.92-0.78 (m, 6H). HPLC (4 min) t_R = 2.5 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 444 $[M+H]^+$.

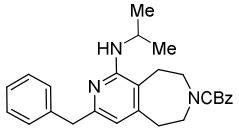


Benzyl 3-benzyl-1-((cyclopropylmethyl)amino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20c). Synthesized via the general procedure **C** for reductive amination from benzyl 1-amino-

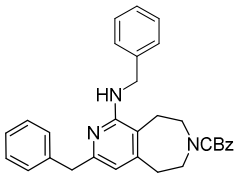
3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 60 mg, 0.16 mmol) and cyclopropanecarbaldehyde (44 mg, 0.62 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-30% EtOAc in heptane to afford the title compound as a white solid (46 mg, 67% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.33-7.17 (m, 9H), 7.16-7.08 (m, 1H), 6.19-6.12 (m, 1H), 5.05 (s, 2H), 3.82 (s, 2H), 3.68-3.51 (m, 4H), 3.22-3.18 (m, 2H), 2.79-2.67 (m, 4H), 1.12-1.01 (m, 1H), 0.42-0.36 (m, 2H), 0.20-0.12 (m, 2H). HPLC (4 min) t_R = 2.2 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 442 $[M+H]^+$.



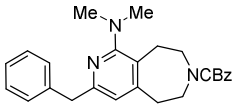
Benzyl 3-benzyl-1-(propylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20d). Synthesized via the general procedure **C** for reductive amination from benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 65 mg, 0.17 mmol) and propionaldehyde (39 mg, 0.68 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-40% EtOAc in heptane to afford the title compound as a white solid (37 mg, 51% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.32-7.15 (m, 9H), 7.14-7.08 (m, 1H), 6.18-6.10 (m, 1H), 5.04 (s, 2H), 3.83 (s, 2H), 3.64-3.49 (m, 4H), 3.38-3.29 (m, 2H), 2.78-2.61 (m, 4H), 1.60-1.48 (m, 2H), 0.94-0.85 (m, 3H). HPLC (4 min) t_R = 2.3 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 430 $[M+H]^+$.



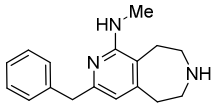
Benzyl 3-benzyl-1-(isopropylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20e). Synthesized via the general procedure **C** for reductive amination from benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 63 mg, 0.16 mmol) and acetone (38 mg, 0.65 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-40% EtOAc in heptane to afford the title compound as a white solid (18 mg, 26% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.32-7.16 (m, 9H), 7.15-7.08 (m, 1H), 6.19-6.12 (m, 1H), 5.04 (s, 2H), 4.29-4.19 (m, 1H), 3.83 (s, 2H), 3.64-3.52 (m, 4H), 2.79-2.63 (m, 4H), 1.18-1.09 (m, 6H). HPLC (4 min) t_R = 2.3 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 430 $[M+H]^+$.



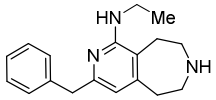
Benzyl 3-benzyl-1-(benzylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20f). Synthesized via the general procedure **C** for reductive amination from benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 22 mg, 0.06 mmol) and benzaldehyde (27 mg, 0.25 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-50% EtOAc in heptane to afford the title compound as a white solid (16 mg, 56% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.32-7.05 (m, 15H), 6.20 (s, 1H), 4.89 (s, 2H), 4.59 (s, 2H), 3.78 (s, 2H), 3.59-3.43 (m, 4H), 2.79-2.71 (m, 4H). HPLC (4 min) t_R = 2.5 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 444 $[M+H]^+$.



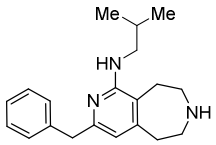
Benzyl 3-benzyl-1-(dimethylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20g). Potassium carbonate (46 mg, 0,33 mmol) and iodomethane (71 mg, 0.49 mmol) were added to a solution of benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrid[3,4d]azepine-7-carboxylate (**13**, 64 mg, 0.16 mmol) in DMF (1.7 mL, 0.1M). The mixture was heated to 80 °C for 16 h. More iodomethane (71 mg, 0.49 mmol) was added and heating continued for 6 h before the mixture was cooled to room temperature, concentrated and extracted with CH_2Cl_2. The combined organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography eluting with a gradient of 1-40% EtOAc in heptane to afford the title compound as a white solid (31 mg, 46% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.36-7.19 (m, 9H), 7.18-7.09 (m, 1H), 6.62-6.54 (m, 1H), 5.06 (s, 2H), 3.92 (s, 2H), 3.60-3.49 (m, 4H), 2.99-2.89 (m, 2H), 2.81-2.71 (m, 2H), 2.70-2.62 (m, 6H). HPLC (4 min) t_R = 2.6 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 416 $[M+H]^+$.



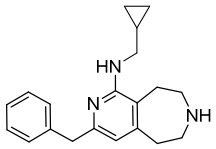
3-Benzyl-N-methyl-6,7,8,9-tetrahydro-5H-pyrido [3,4-d] azepin-1-amine (7). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(methyl amino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20i**, 27 mg, 0.07 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-10% MeOH in 1:1 CH_2Cl_2:EtOAc to afford the title compound as a white solid (16 mg, 91% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.27-7.19 (m, 4H), 7.17-7.11 (m, 1H), 6.15 (s, 1H), 3.88 (s, 2H), 2.88 (s, 3H), 2.82-2.77 (m, 4H), 2.77-2.69 (m, 4H). HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{17}H_{22}N_3$ 268.1802; found 268.1808.



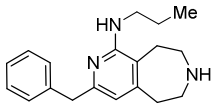
3-Benzyl-N-ethyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21a). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(ethylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20a**, 41 mg, 0.10 mmol). The crude product was purified by flash column chromatography eluting with a gradient of 1-10% MeOH in 1:1 CH_2Cl_2:EtOAc to afford the title compound as a white solid (16 mg, 57% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.26-7.18 (m, 4H), 7.17-7.09 (m, 1H), 6.17 (s, 1H), 3.83 (s, 2H), 3.39 (q, *J* = 7.8 Hz, 2H), 2.82-2.77 (m, 4H), 2.76-2.65 (m, 4H), 1.16 (t, *J* = 7.8 Hz, 3H). HPLC (4 min) t_R = 0.5 min, UV (DAD:TIC) >95% purity. HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{18}H_{24}N_3$ 282.1965; found 282.1959.



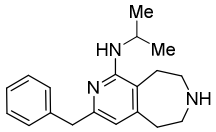
3-Benzyl-N-isobutyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21b). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(isobutylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20b**, 17 mg, 0.04 mmol). The crude product was purified by preparative TLC eluting with 20% MeOH in CH_2Cl_2 to afford the title compound as a white solid (10 mg, 80% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.29-7.18 (m, 4H), 7.17-7.10 (m, 1H), 6.22 (s, 1H), 3.83 (s, 2H), 3.19 (d, *J* = 6.8 Hz, 2H, 3.18-3.07 (m, 4H), 2.93-2.87 (m, 4H), 1.93-1.81 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 6H). HPLC (4 min) t_R = 1.0 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 310 $[M+H]^+$.



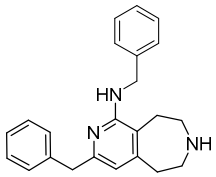
3-Benzyl-N-(cyclopropylmethyl)-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21c). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-((cyclopropylmethyl)amino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20c**, 46 mg, 0.10 mmol). The crude product was purified by preparative TLC eluting with 1% NH_3/10% MeOH in CH_2Cl_2 to afford the title compound as a white solid (22 mg, 69% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.28-7.18 (m, 4H), 7.17-7.10 (m, 1H), 6.22 (s, 1H), 3.83 (s, 2H), 3.22 (d, *J* = 6.9 Hz, 2H), 3.10-3.03 (m, 4H), 2.92-2.83 (m, 4H), 1.16-1.02 (m, 1H), 0.47-0.39 (m, 2H), 0.21-0.18 (m, 2H). HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{20}H_{26}N_3$ 308.2121; found 308.2123.



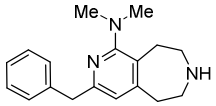
3-Benzyl-N-propyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21d). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(propylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20d**, 37 mg, 0.09 mmol). The crude product was purified by preparative TLC eluting with 1% NH_3/10% MeOH in CH_2Cl_2 to afford the title compound as a white solid (22 mg, 87% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.27-7.18 (m, 4H), 7.17-7.09 (m, 1H), 6.18 (s, 1H), 3.84 (s, 2H), 3.36-3.28 (m, 2H), 2.97-2.85 (m, 4H), 2.81-2.74 (m, 4H), 1.57 (sextet, *J* = 7.8 Hz, 2H), 0.89 (t, *J* = 7.8 Hz, 3H). HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{19}H_{26}N_3$ 296.2121; found 296.2121.



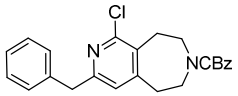
3-Benzyl-N-isopropyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21e). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(isopropylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20e**, 18 mg, 0.04 mmol). The crude product was purified by preparative TLC eluting with 1% NH_3/10% MeOH in CH_2Cl_2 to afford the title compound as a white solid (9 mg, 56% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.27-7.18 (m, 4H), 7.17-7.10 (m, 1H), 6.27 (s, 1H), 4.25 (septet, *J* = 6.9 Hz,1H), 3.85 (s, 2H), 3.23-3.17 (m, 4H), 2.98-2.90 (m, 4H), 1.17 (d, *J* = 6.9 Hz, 6H). HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{19}H_{26}N_3$ 296.2121; found 296.2121.



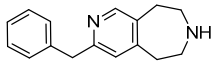
N,3-Dibenzyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21f). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(benzylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20f**, 16 mg, 0.04 mmol). The crude product was purified by alumina column chromatography eluting with a gradient of 1-20% MeOH in 1:1 CH_2Cl_2:EtOAc to afford the title compound as a white solid (12 mg, 55% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.26-7.04 (m, 10H), 6.19 (s, 1H), 4.58 (s, 2H), 3.79 (s, 2H), 2.86-2.70 (m, 8H). HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{23}H_{26}N_3$ 344.2121; found 344.2124.



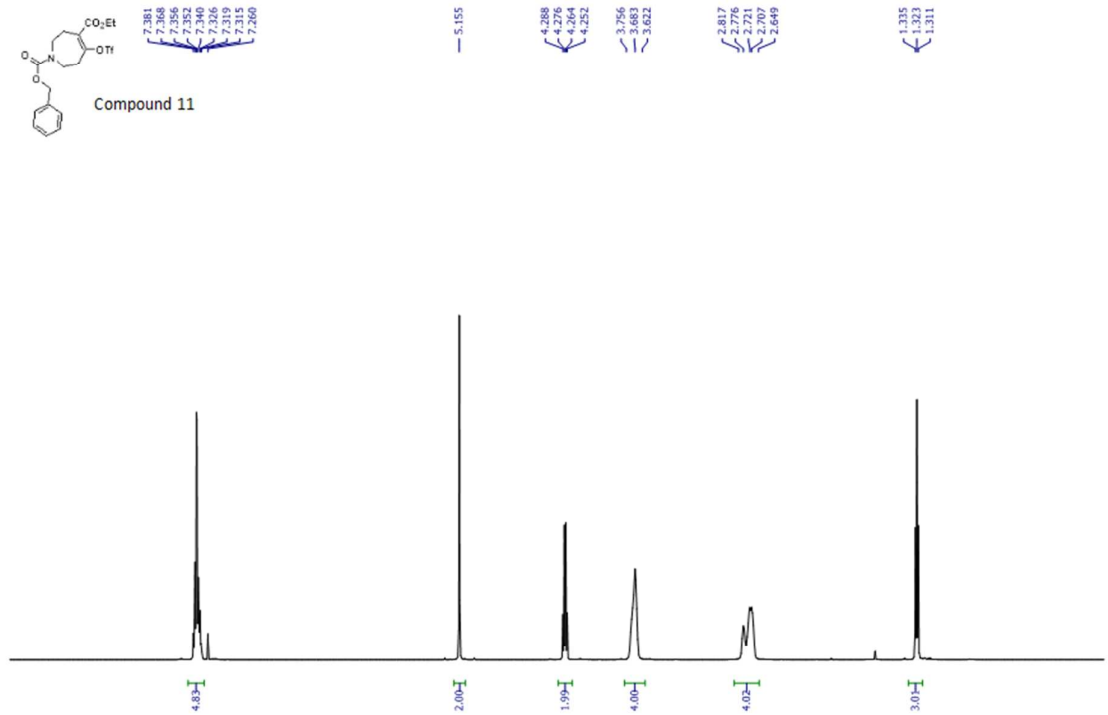
3-Benzyl-N,N-dimethyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepin-1-amine (21g). Synthesized via the general procedure **D** for CBz deprotection from benzyl 3-benzyl-1-(dimethylamino)-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20g**, 31 mg, 0.07 mmol). The crude product was purified by preparative TLC eluting with 1% NH_3/10% MeOH in CH_2Cl_2 to afford the title compound as a white solid (17 mg, 82% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.28-7.20 (m, 4H), 7.18-7.11 (m, 1H), 6.62 (s, 1H), 3.94 (s, 2H), 3.09-2.98 (m, 6H), 2.91-2.86 (m, 2H), 2.71 (s, 6H). HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{18}H_{24}N_3$ 282.1965; found 282.1970.



Benzyl 3-benzyl-1-chloro-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (20h). Copper(II)chloride (208 mg, 1.6 mmol) and isoamyl nitrite (227 mg, 1.9 mmol) were suspended in DCE (6.4 mL) and heated to 65 °C. A solution of benzyl 1-amino-3-benzyl-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**13**, 500 mg, 1.2 mmol) in DCE (2.7 mL) was added dropwise over 20 min. The resulting mixture heated with stirring for 1 h then cooled to room temperature and stirred for a further 12 h. The mixture was extracted with Et_2O and the combined organics dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography eluting with 1-40% EtOAc in heptane afforded the title compound as a white solid (214 mg, 41% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.35-7.12 (m, 10H), 7.00-6.92 (m, 1H), 5.05 (s, 2H), 3.98 (s, 2H), 3.64-3.52 (m, 4H), 3.18-3.05 (m, 2H), 2.91-2.83 (m, 2H). HPLC (6 min) t_R = 3.59 min, ELSD >95% purity. LRMS (ESI) *m/z* 407 $[M+H]^+$.



3-Benzyl-6,7,8,9-tetrahydro-5H-pyrido[3,4-d]azepine (21h). A suspension of 10% Pd/C (10 mg) was added to a solution of benzyl 3-benzyl-1-chloro-5,6,8,9-tetrahydro-7H-pyrido[3,4-d]azepine-7-carboxylate (**20h**, 50 mg, 0.12 mmol) in EtOH (1.0 mL) and an atmosphere of H_2 gas (45 psi) applied. The mixture was shaken at room temperature for 4 h, then filtered and concentrated under reduced pressure to yield. Purification by preparative TLC eluting with 1% NH_3/10% MeOH in CH_2Cl_2 to afforded the title compound as a white solid (7 mg, 25% yield). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.26 (s, 1H), 7.30-7.13 (m, 6H), 4.08 (s, 2H), 3.32-3.21 (m, 4H), 3.18-3.09 (m, 4H). HPLC (4 min) t_R = 0.3 min, UV (DAD:TIC) >95% purity. LRMS (ESI) *m/z* 239 $[M+H]^+$. HRMS (EI) *m/z*: $[M+H]^+$ calcd for $C_{16}H_{19}N_2$ 239.1543; found 239.1540.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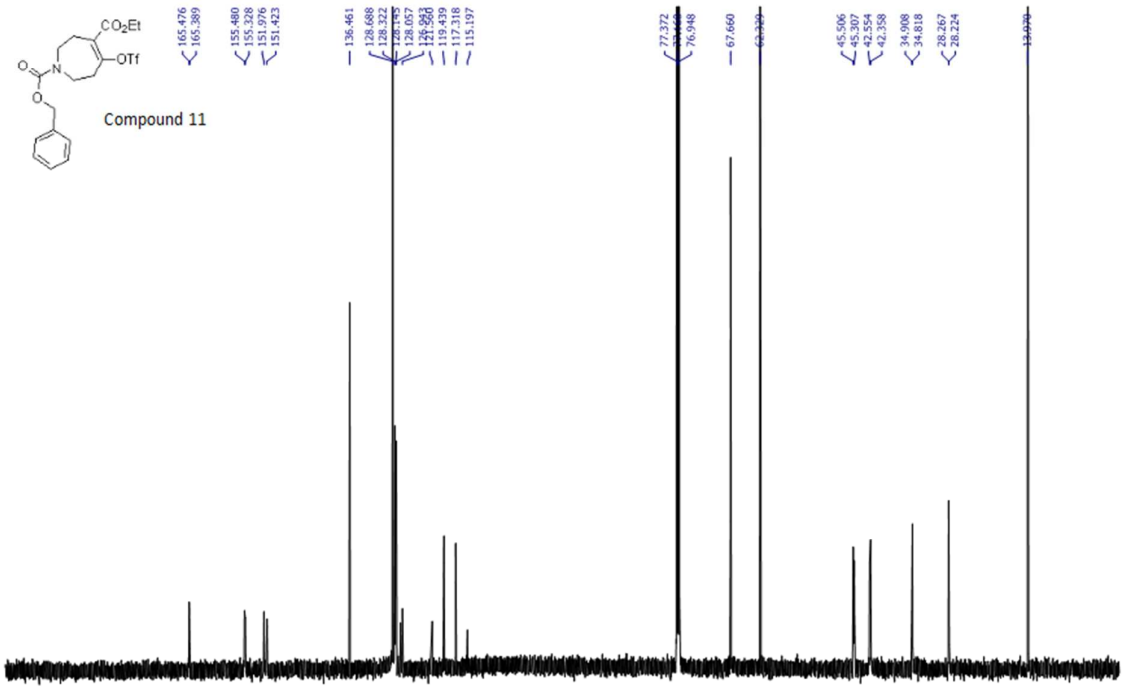
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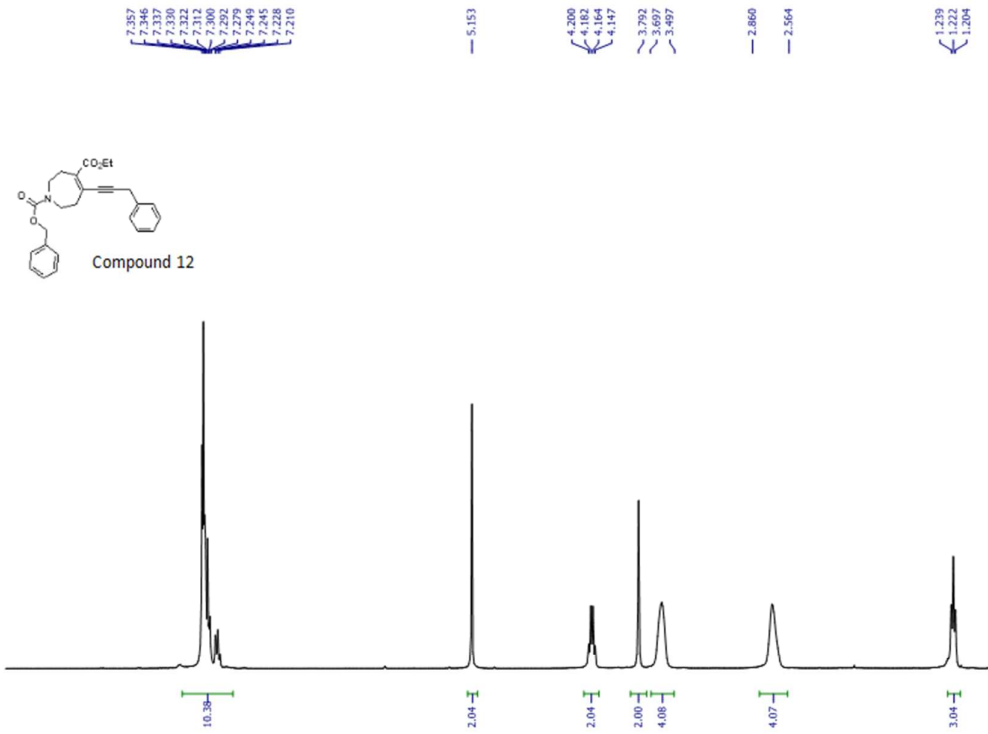
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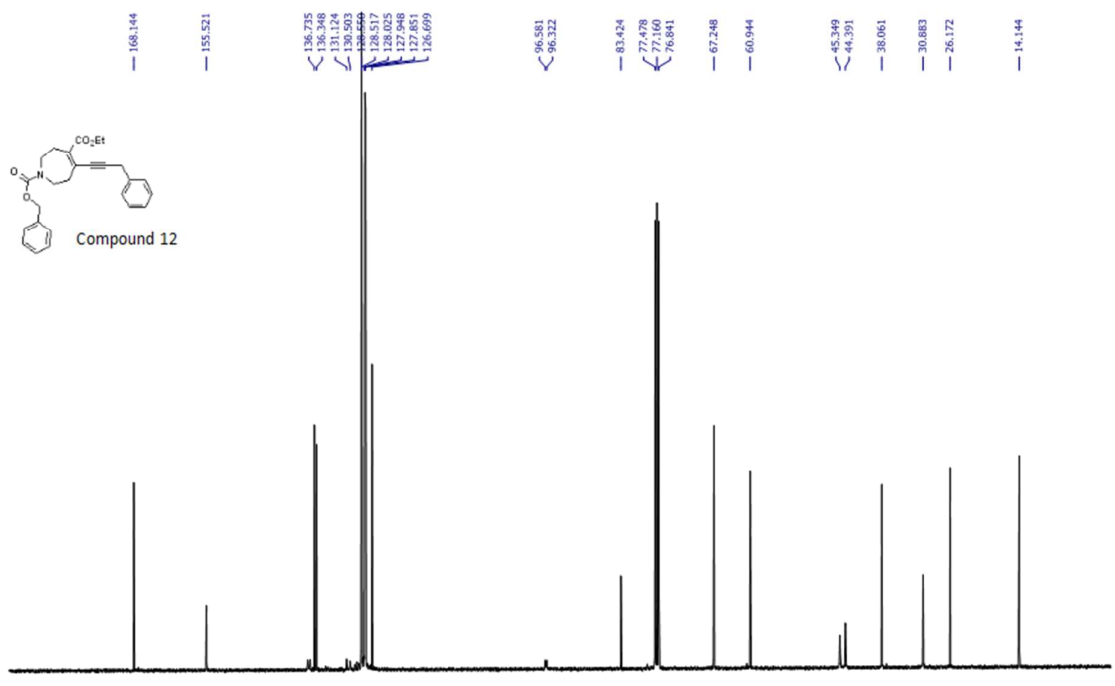
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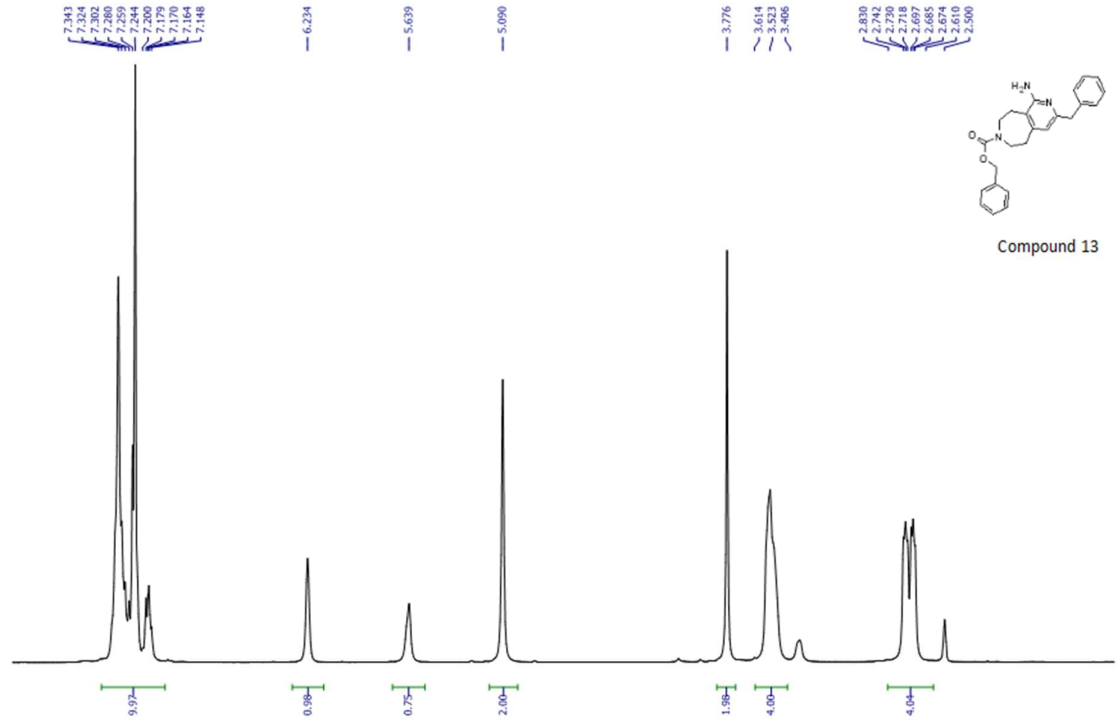
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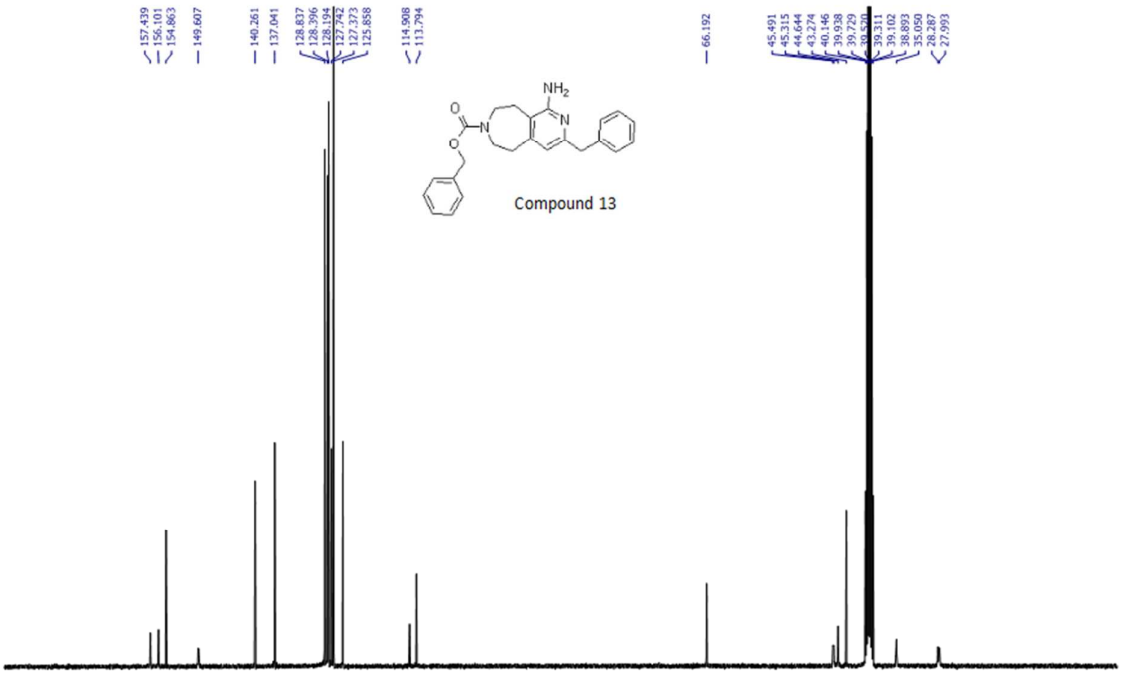
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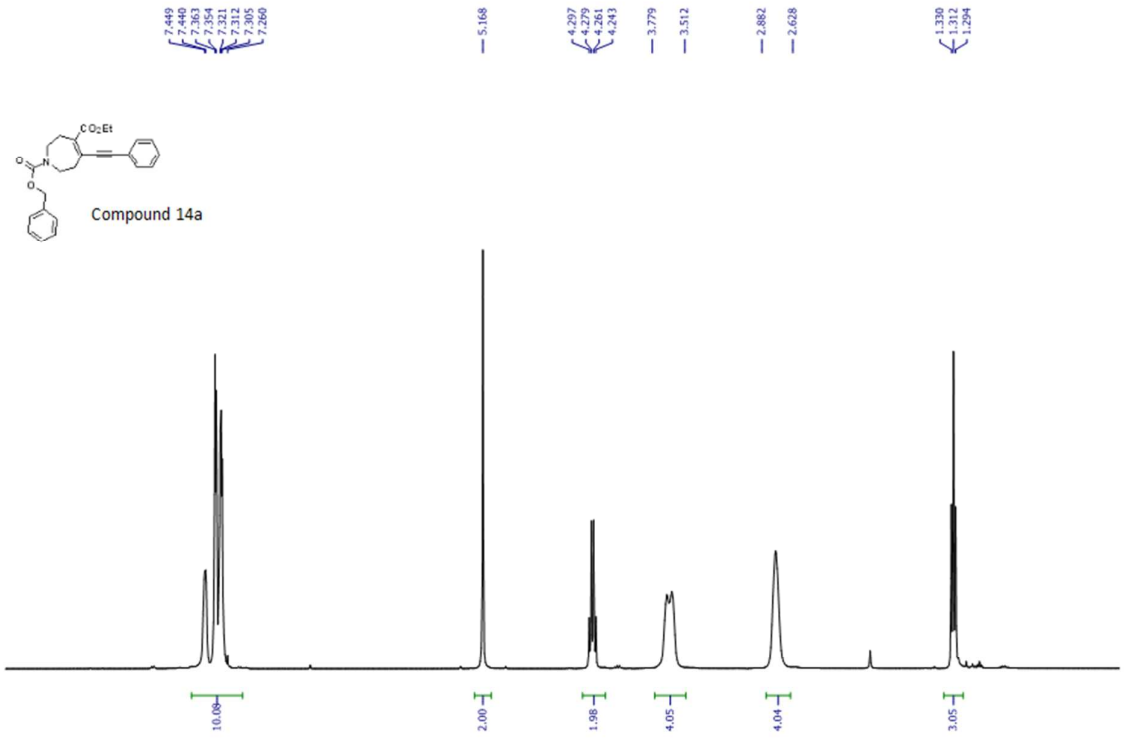
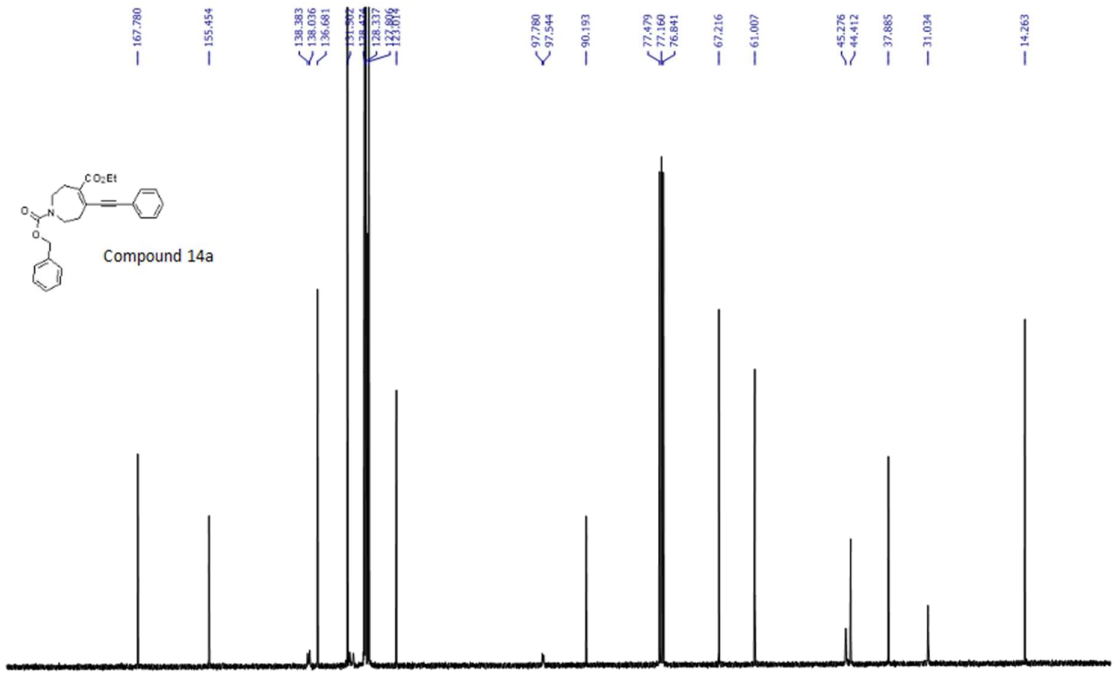
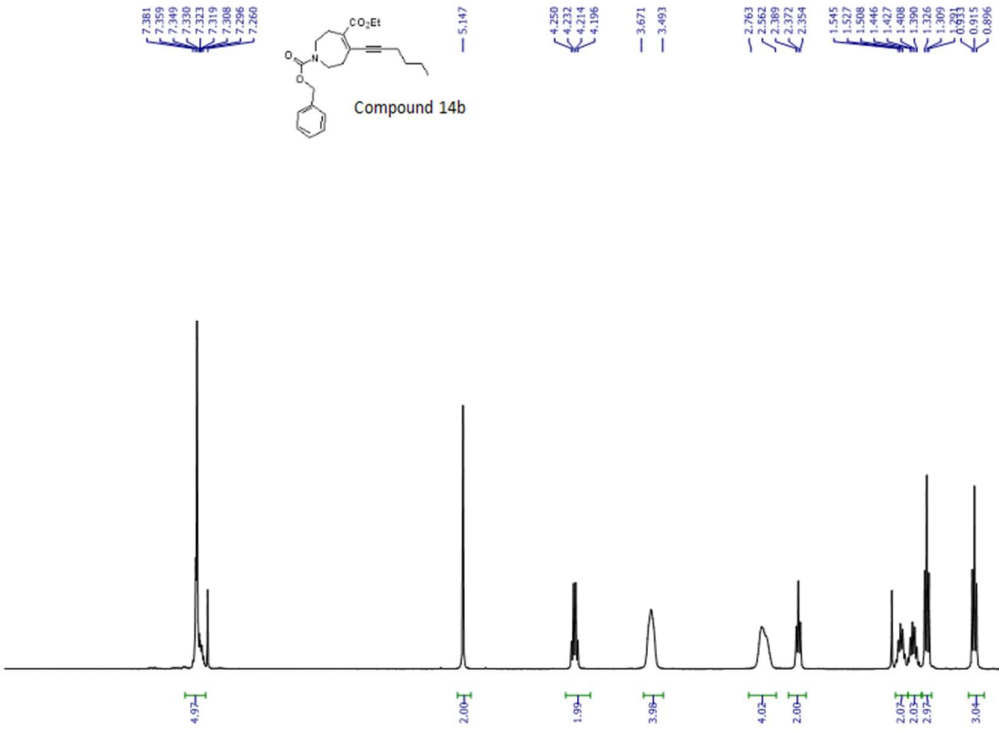
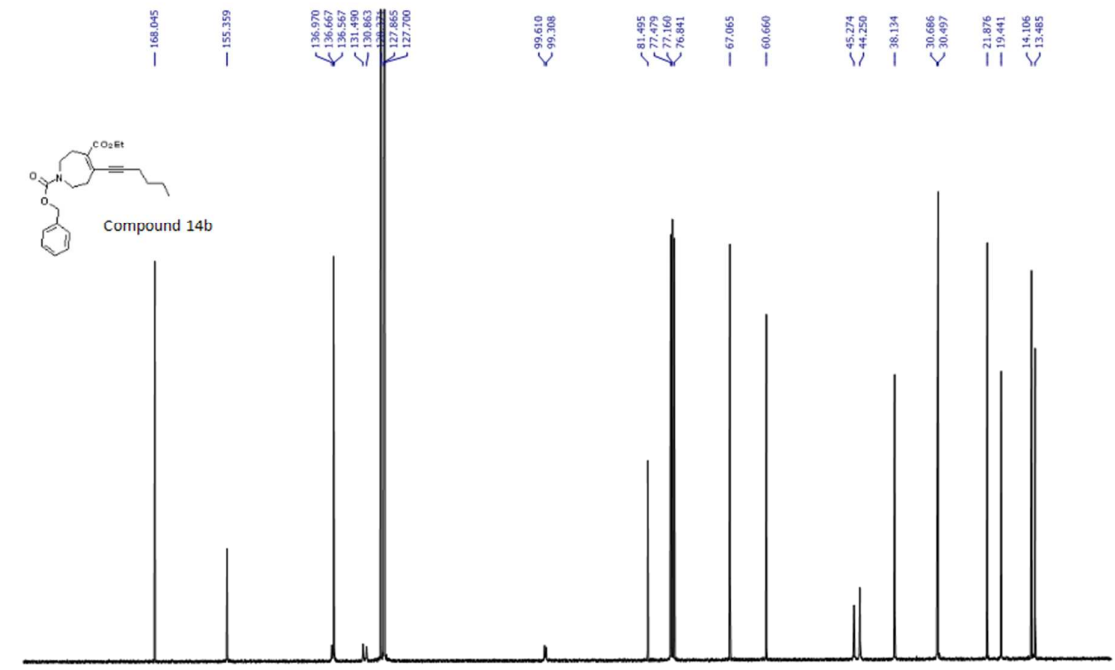
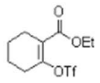
125.658
114.908
113.794
66.192
45.491
45.315
44.644
43.274
40.146
39.938
39.729
39.520
39.311
39.102
38.893
35.050
28.287
27.993
Compound 13


7.449
7.440
7.363
7.354
7.321
7.312
7.305
7.260
5.168
4.297
4.279
4.261
4.243
3.779
3.512
2.882
2.628
1.330
1.312
1.294
CO2Et
Compound 14a
10.00
2.00
1.98
4.05
4.04
3.05


167.780
155.454
138.383
138.036
136.681
128.337
97.780
97.544
90.193
77.479
77.160
76.841
67.216
61.007
45.276
44.412
37.885
31.034
14.263
CO2Et
Compound 14a

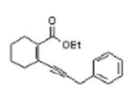

7.381
7.359
7.349
7.330
7.323
7.319
7.308
7.296
7.260
5.147
4.250
4.232
4.214
4.196
3.671
3.493
2.763
2.562
2.389
2.372
2.354
1.545
1.527
1.508
1.446
1.427
1.408
1.390
1.326
1.309
0.931
0.915
0.896
Compound 14b
4.97
2.00
1.99
3.98
4.02
2.00
2.07
2.03
2.97
3.04


168.045
155.359
136.970
136.667
136.567
131.490
130.863
127.865
127.700
99.610
99.308
81.495
77.479
77.160
76.841
67.065
60.660
45.274
44.250
38.134
30.686
30.497
21.876
19.441
14.106
13.485
Compound 14b

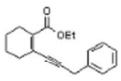


Precursor to Compound 14c

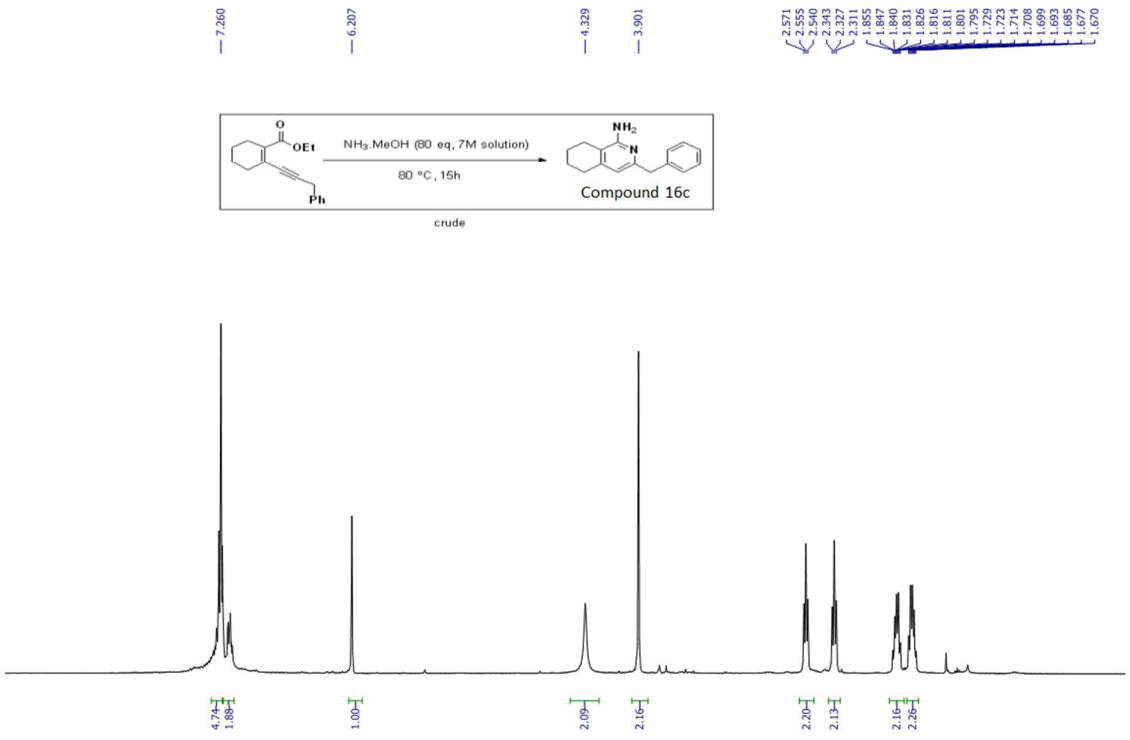
Precursor to Compound 14c

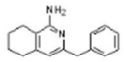


Compound 14c

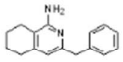


Compound 14c

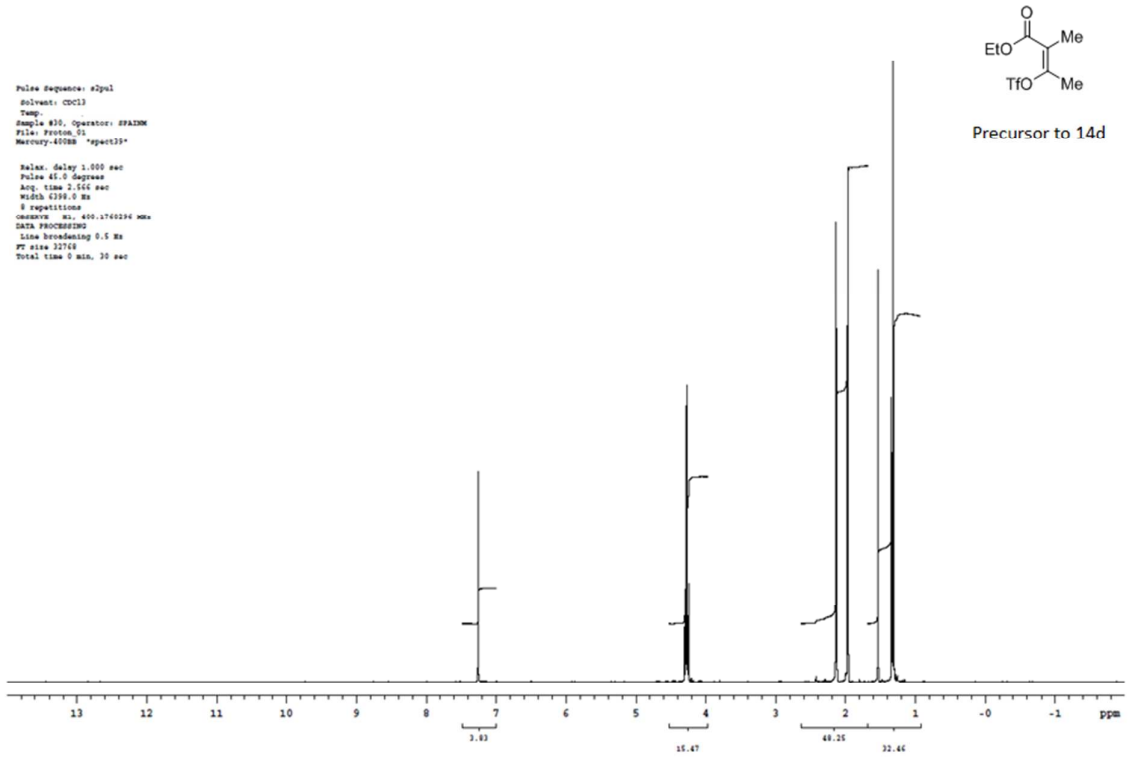

7.260
6.207
4.329
3.901
2.571
2.555
2.540
2.343
2.327
2.311
1.855
1.847
1.840
1.831
1.826
1.816
1.811
1.801
1.795
1.729
1.723
1.714
1.708
1.699
1.693
1.685
1.677
1.670
O
OEt
Ph
NH3.MeOH (80 eq, 7M solution)
80 °C, 15h
NH2
N
Compound 16c
crude
4.74
1.88
1.00
2.09
2.16
2.20
2.13
2.16
2.26

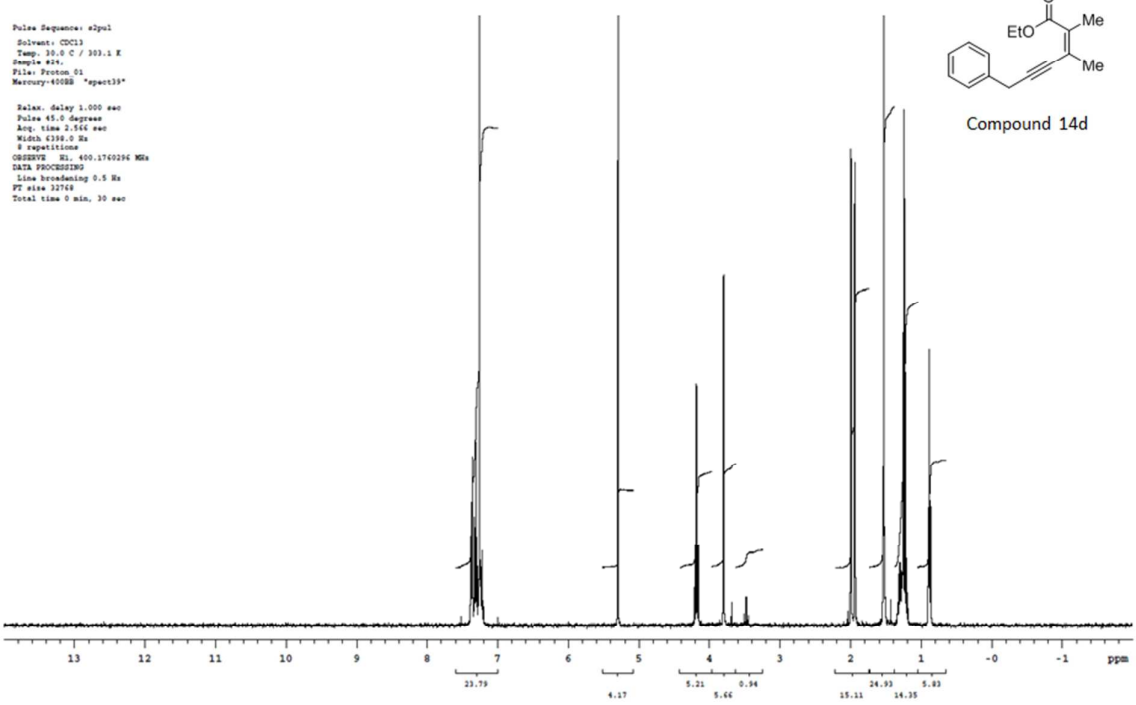


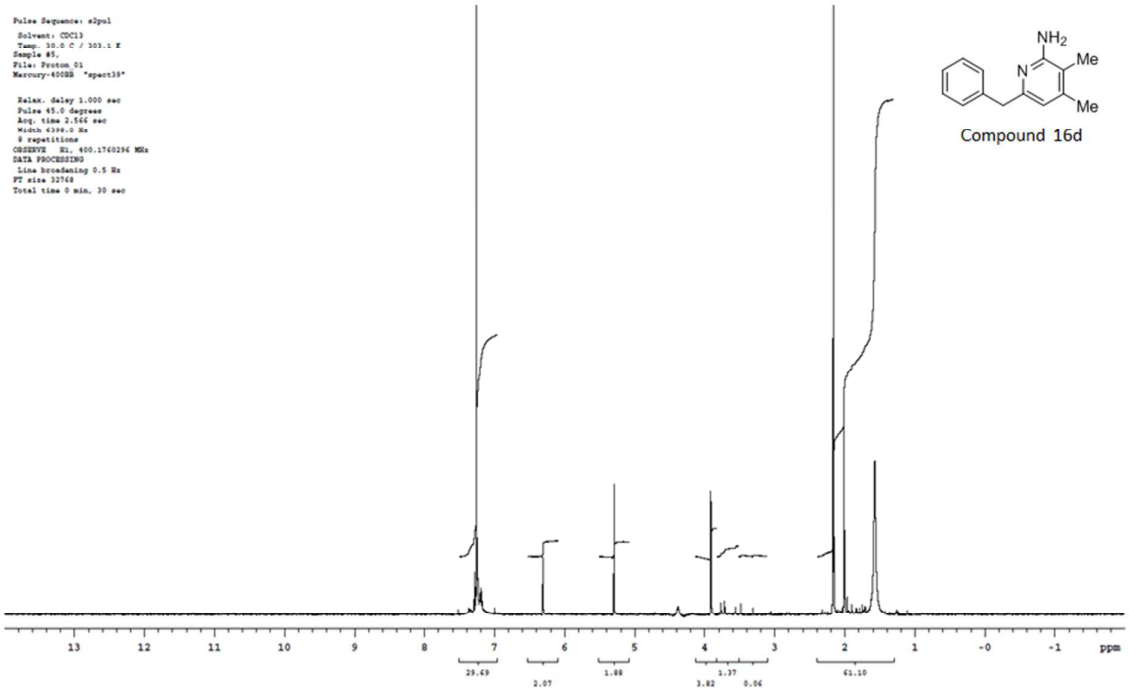
Compound 16c

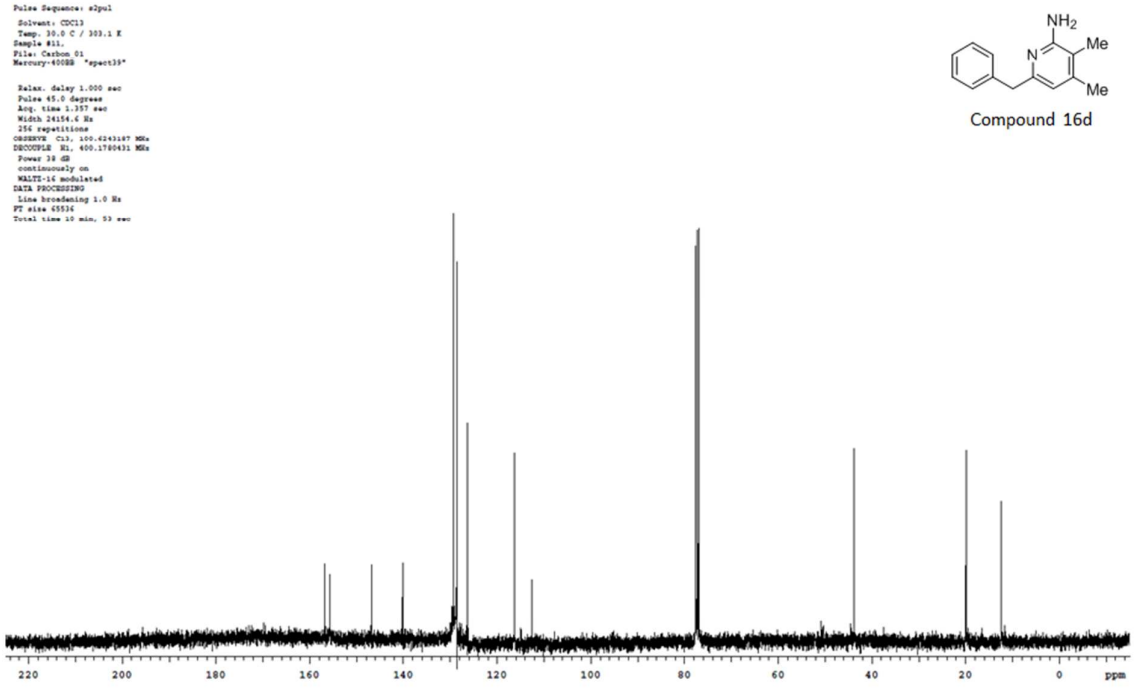


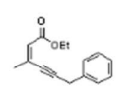
Compound 16c


Pulse Sequence: s2pul
Solvent: CDCl3
Temp:
Sample #30, Operator: SPAINM
File: Proton_01
Mercury-400BB "spect39"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.566 sec
Width 6398.0 Hz
8 repetitions
OBSERVE H1, 400.1740296 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min, 30 sec
EtO
Me
TfO
Me
Precursor to 14d
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm
3.03
15.47
48.25
32.46

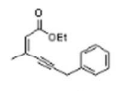

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Sample #24,
File: Proton_01
Mercury-400BB "spect39"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.566 sec
Width 6398.0 Hz
8 repetitions
OBSERVE H1, 400.1740296 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min, 30 sec
EtO
Me
Me
Compound 14d
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm
23.79
4.17
5.21
5.66
0.94
15.11
24.93
14.35
5.83


Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Sample #5,
File: Proton_01
Mercury-400BB "spect39"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.566 sec
Width 4399.0 Hz
8 repetitions
OBSERVE H1, 400.1740296 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min, 30 sec
NH2
Me
N
Me
Compound 16d
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm
29.69
2.07
1.88
1.37
3.02
0.06
61.10

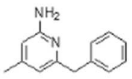

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Sample #11,
File: Carbon_01
Mercury-400BB "spect39"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.357 sec
Width 24154.6 Hz
256 repetitions
OBSERVE C13, 100.6243187 MHz
DECOUPLE H1, 400.1780431 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 10 min, 53 sec
NH2
Me
N
Me
Compound 16d
220
200
180
160
140
120
100
80
60
40
20
0
ppm



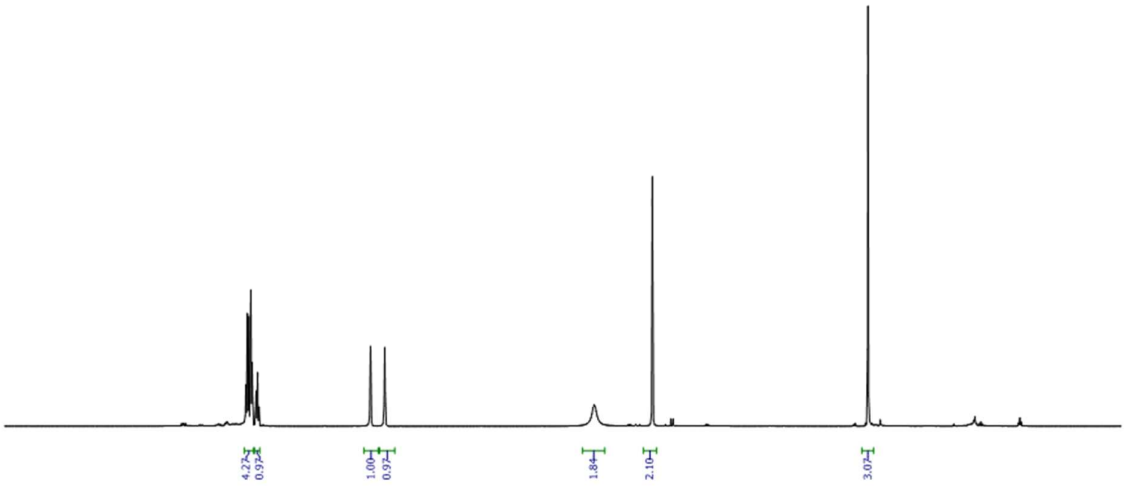
Compound 14e

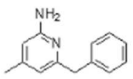


Compound 14e

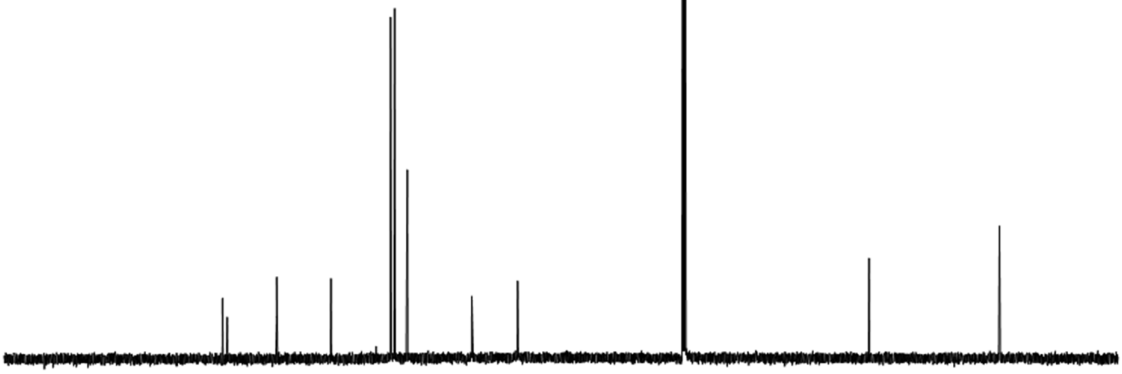


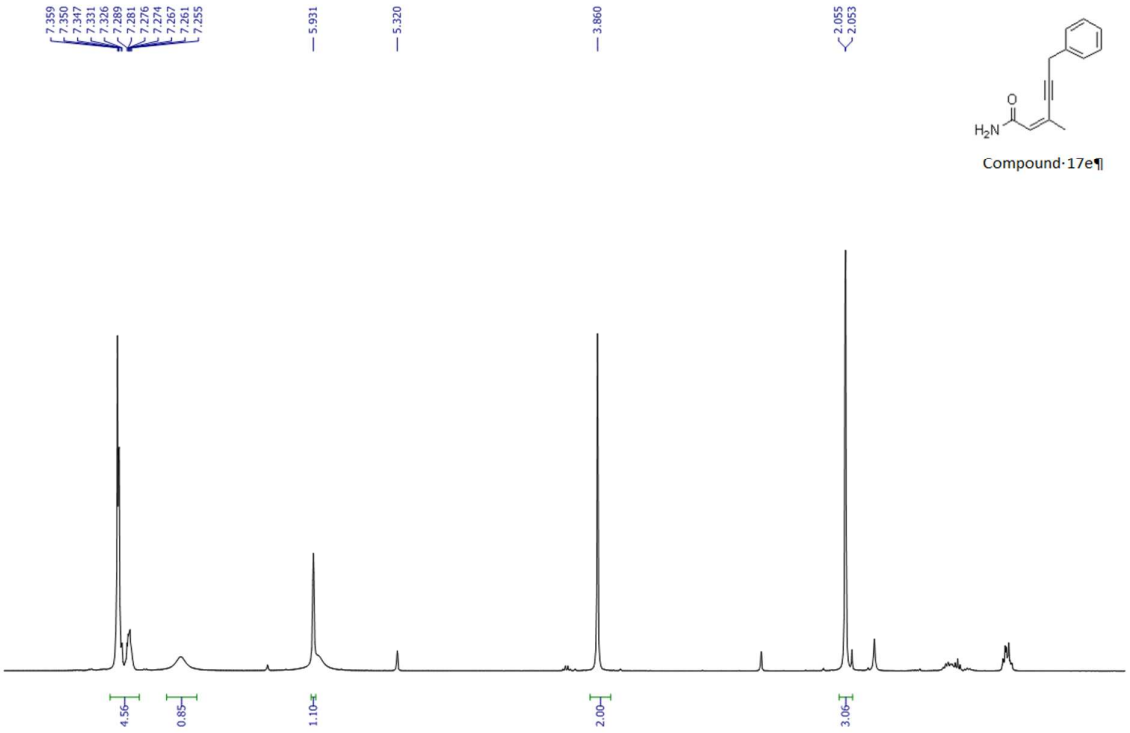
Compound 16e

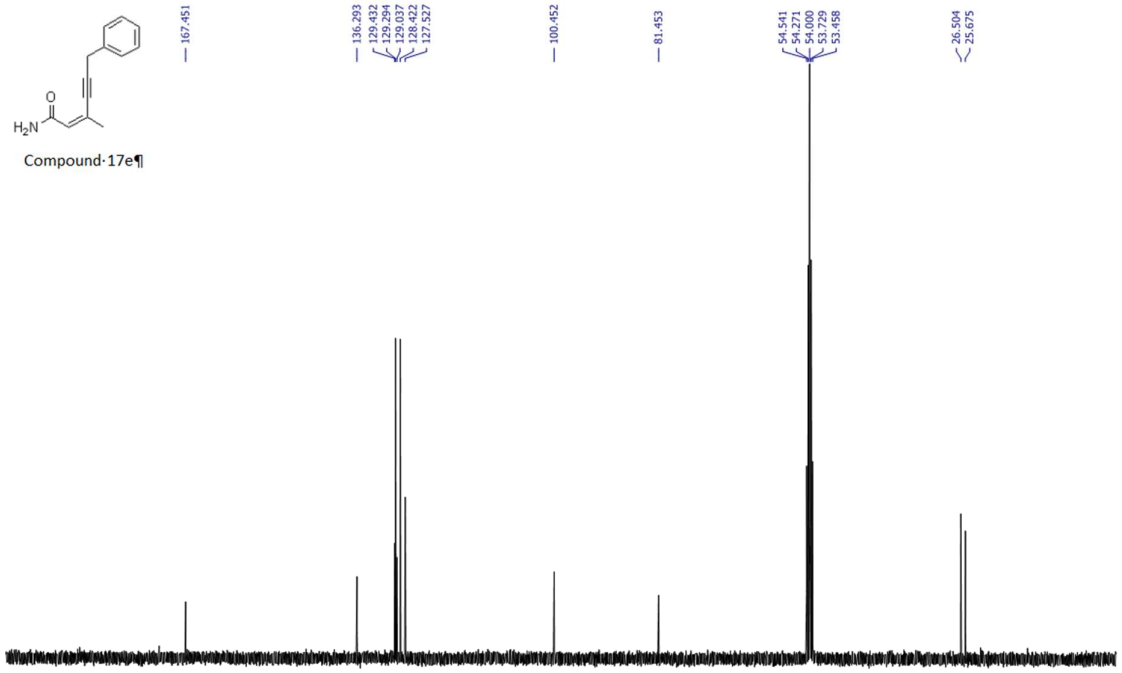


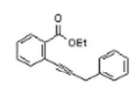


Compound 16e

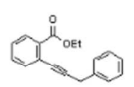



7.359
7.350
7.347
7.331
7.326
7.289
7.281
7.276
7.274
7.267
7.261
7.255
5.931
5.320
3.860
2.055
2.053
O
H2N
Compound·17e¶
4.56
0.85
1.10
2.00
3.06

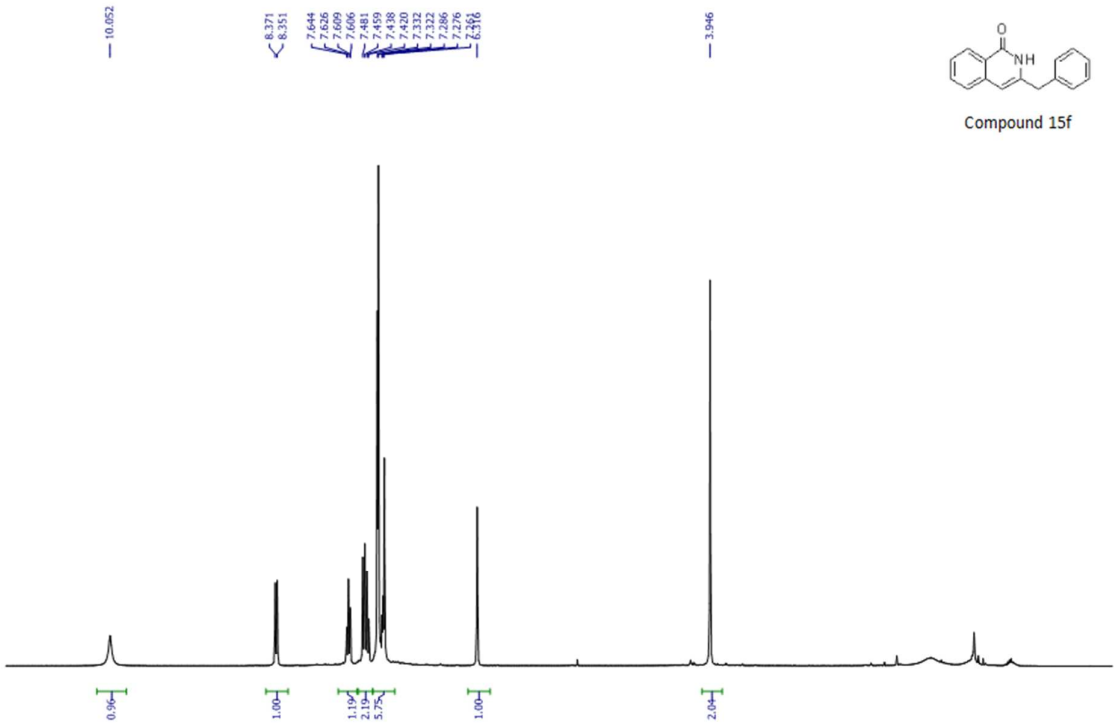

167.451
136.293
129.432
129.294
129.037
128.422
127.527
100.452
81.453
54.541
54.271
54.000
53.729
53.458
26.504
25.675
O
H2N
Compound·17e¶

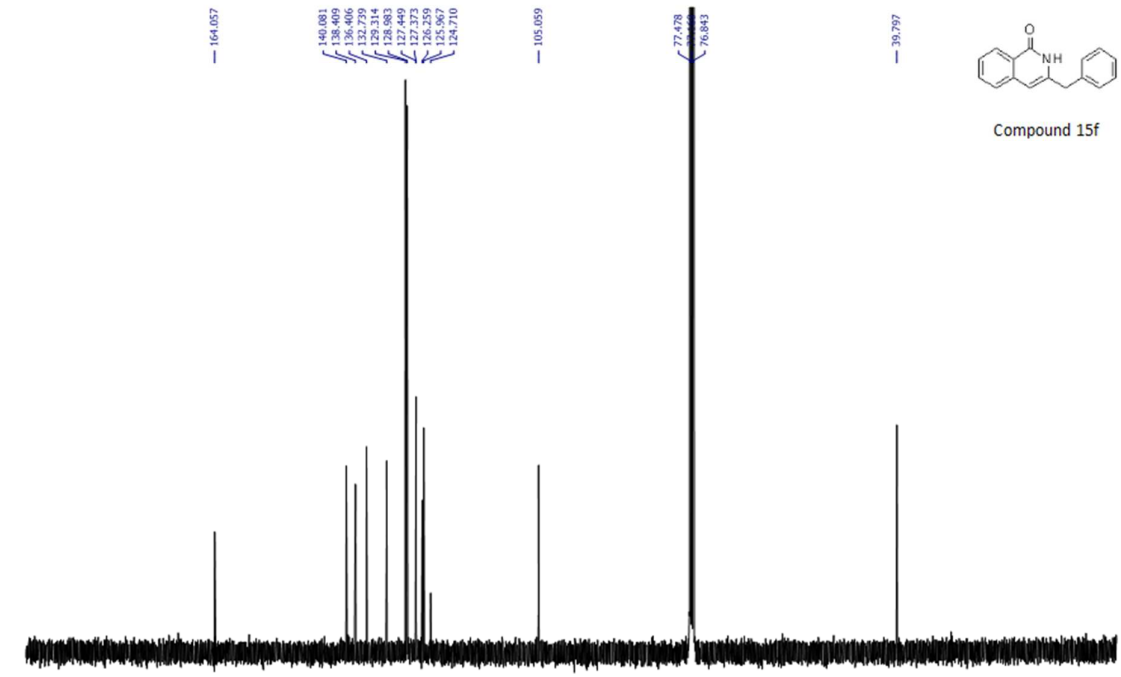


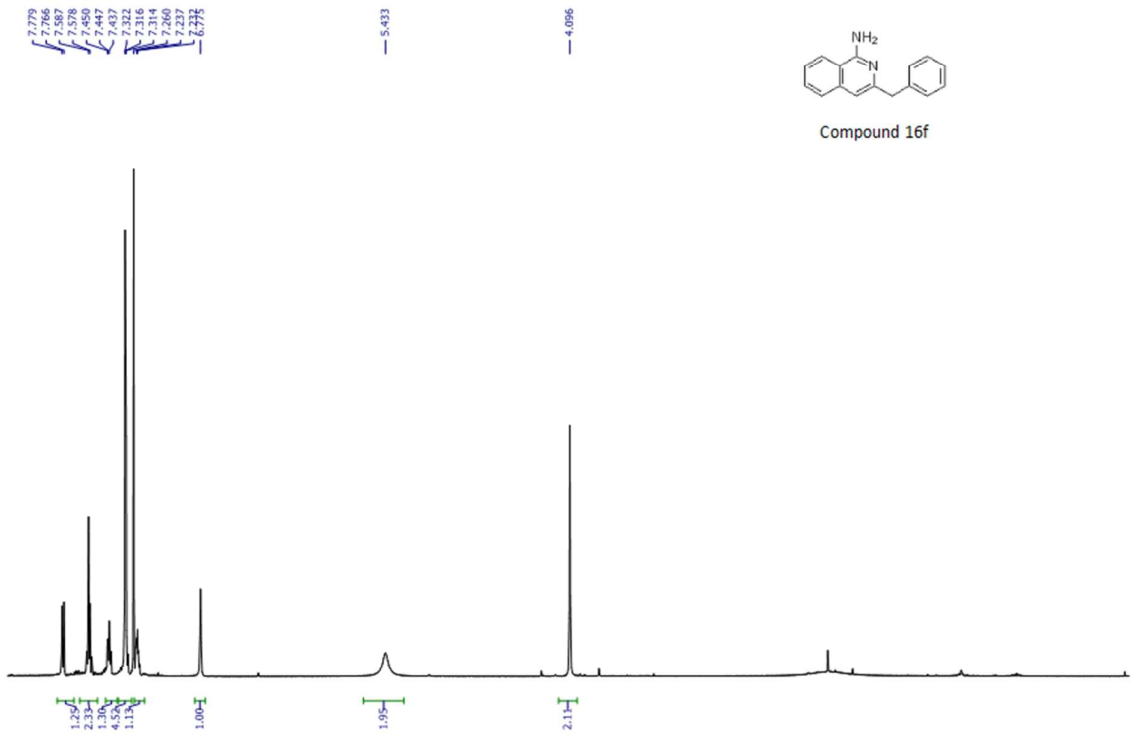
Compound 14f



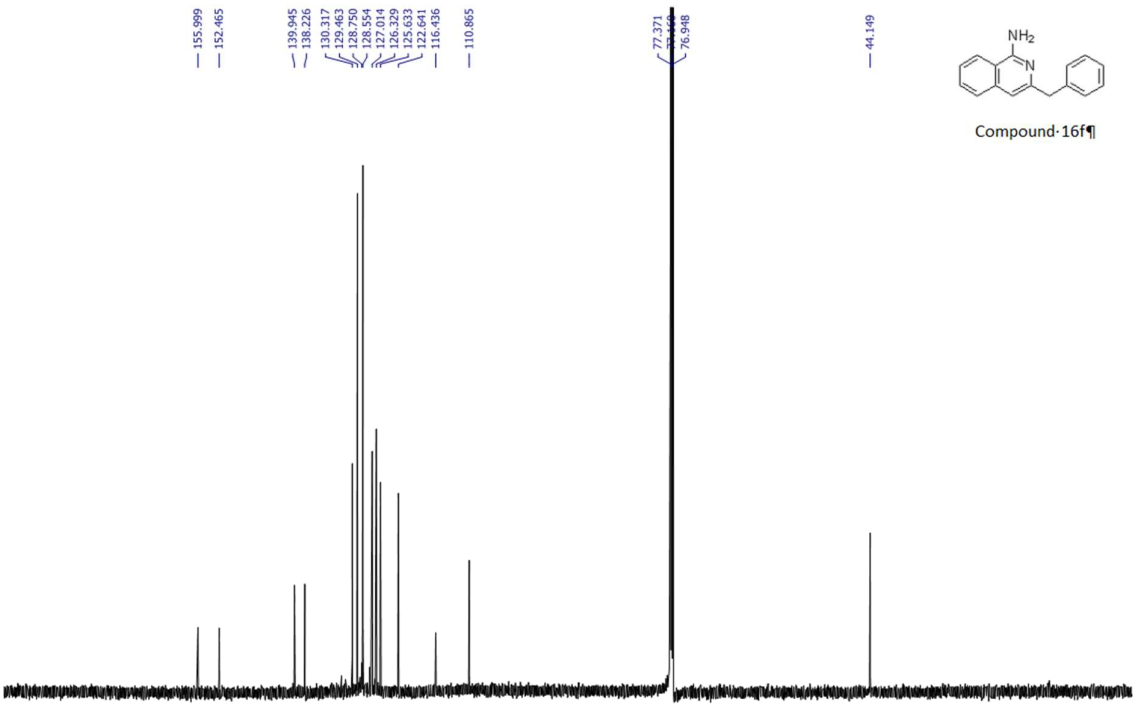
Compound 14f


10.052
8.371
8.351
7.644
7.626
7.609
7.606
7.481
7.459
7.438
7.430
7.332
7.322
7.286
7.276
7.261
6.316
3.946
0.96
1.00
1.19
2.19
5.75
1.00
2.04
Compound 15f

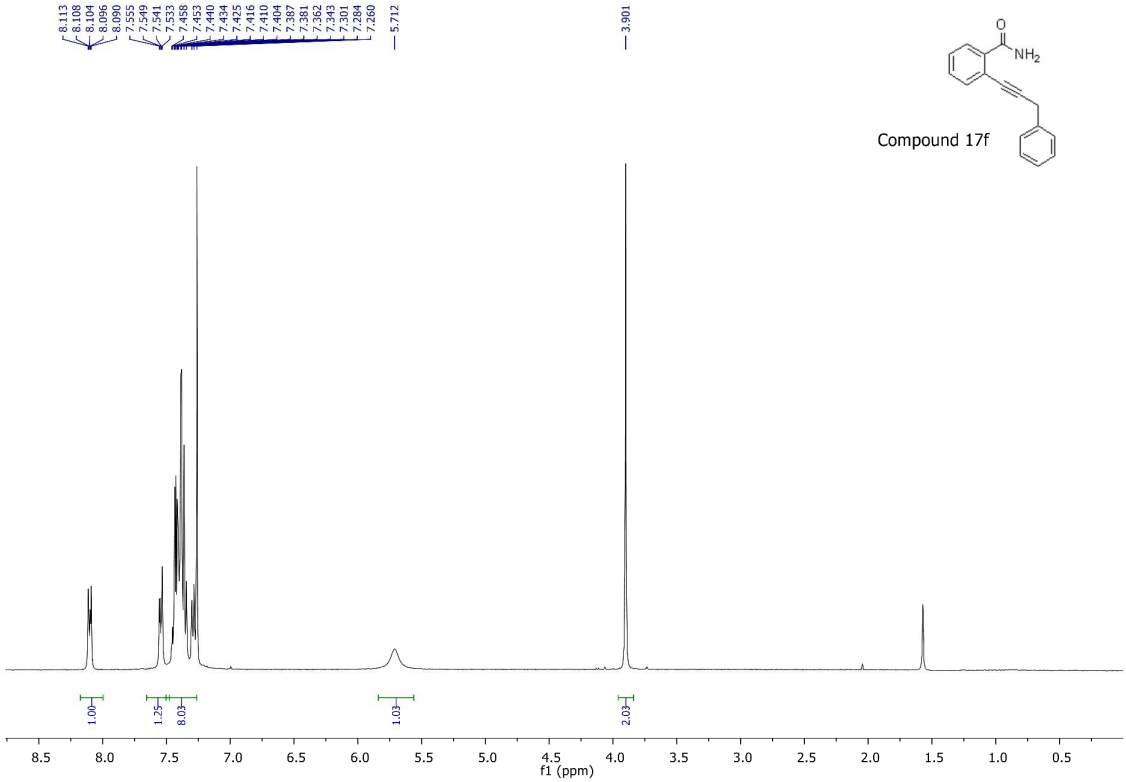

164.057
140.081
138.409
136.406
132.739
129.314
128.983
127.449
127.373
126.259
125.967
124.710
105.059
77.478
76.843
39.797
Compound 15f

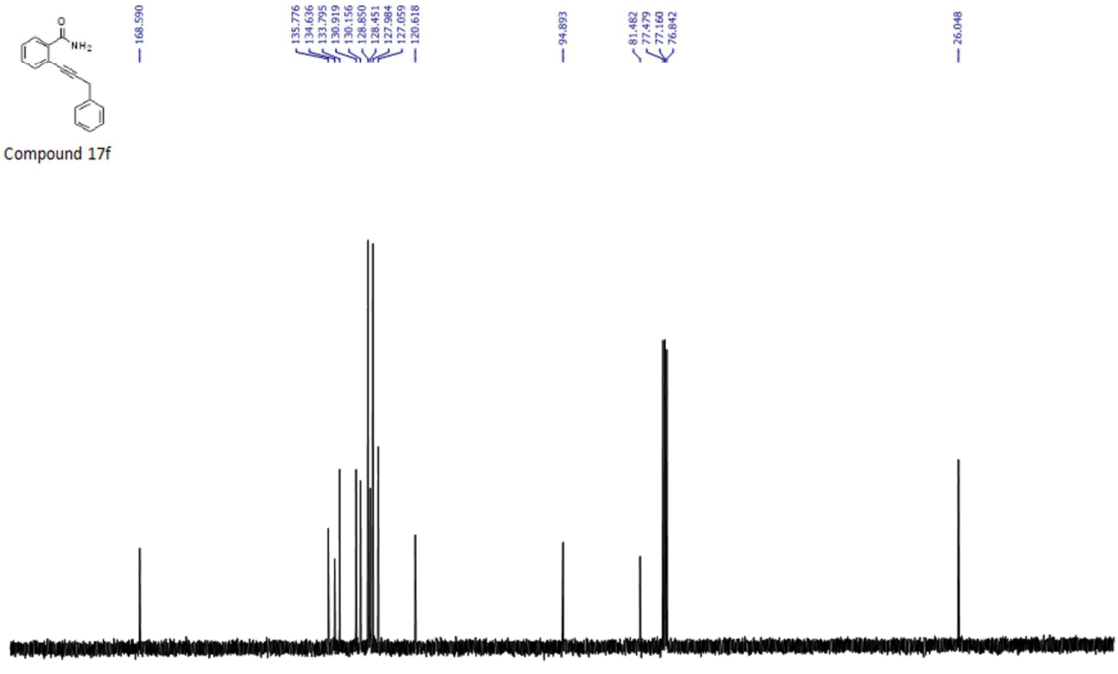


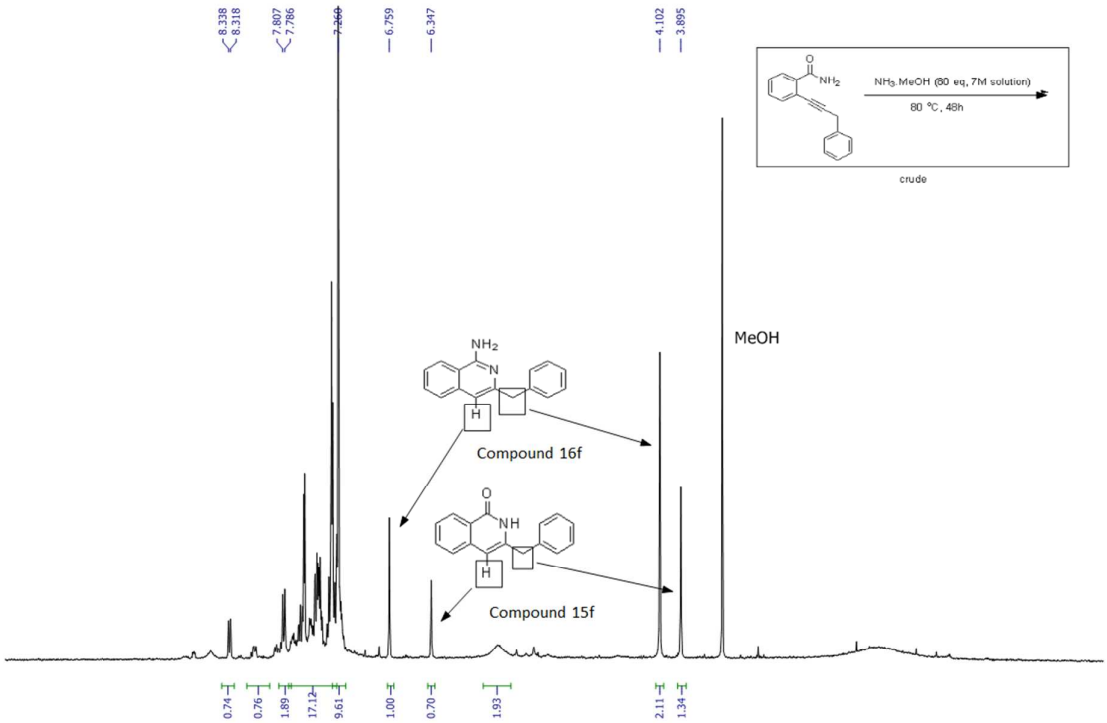
Compound 16f

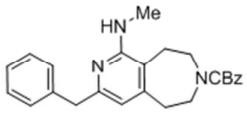


Compound·16f¶

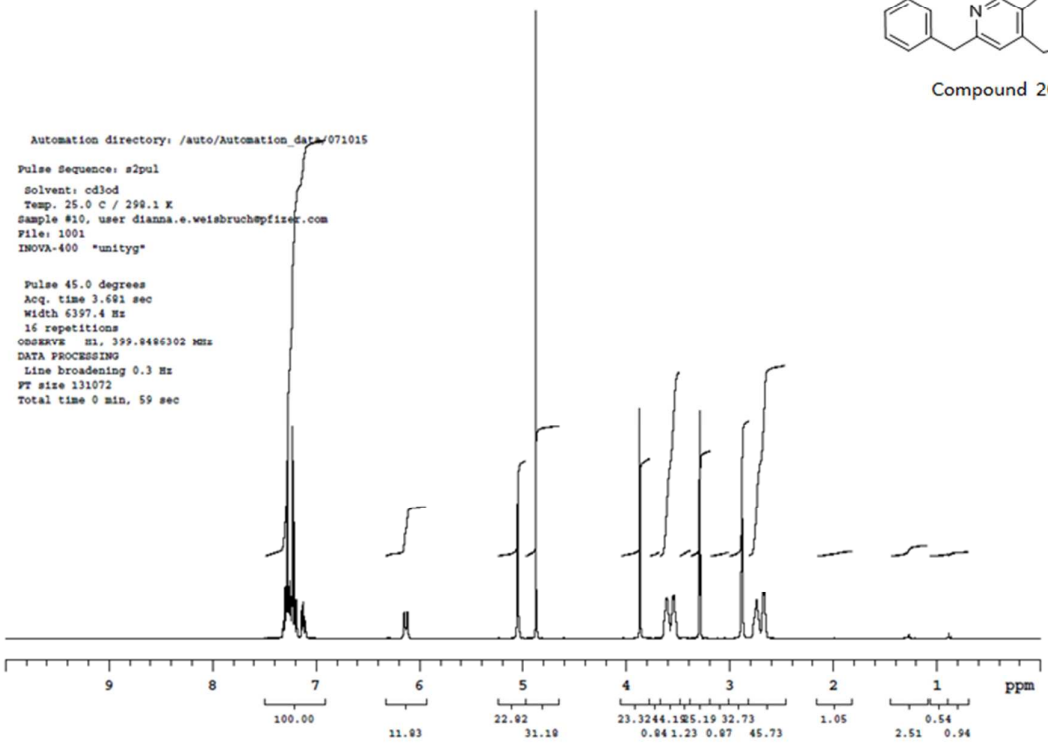

8.113
8.108
8.104
8.096
8.090
7.555
7.549
7.541
7.533
7.458
7.453
7.440
7.434
7.425
7.416
7.410
7.404
7.387
7.381
7.362
7.343
7.301
7.284
7.260
5.712
3.901
NH2
O
Compound 17f
1.00
1.25
8.03
1.03
2.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

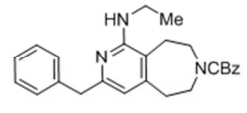

168.590
135.776
134.656
133.705
130.919
130.156
128.850
128.451
127.984
127.059
120.618
94.893
81.462
77.479
77.160
76.842
26.048
NH2
O
Compound 17f


8.338
8.318
7.807
7.786
7.260
6.759
6.347
4.102
3.895
NH3.MeOH (60 eq, 7M solution)
80 °C, 48h
crude
MeOH
Compound 16f
Compound 15f
0.74
0.76
1.89
17.12
9.61
1.00
0.70
1.93
2.11
1.34

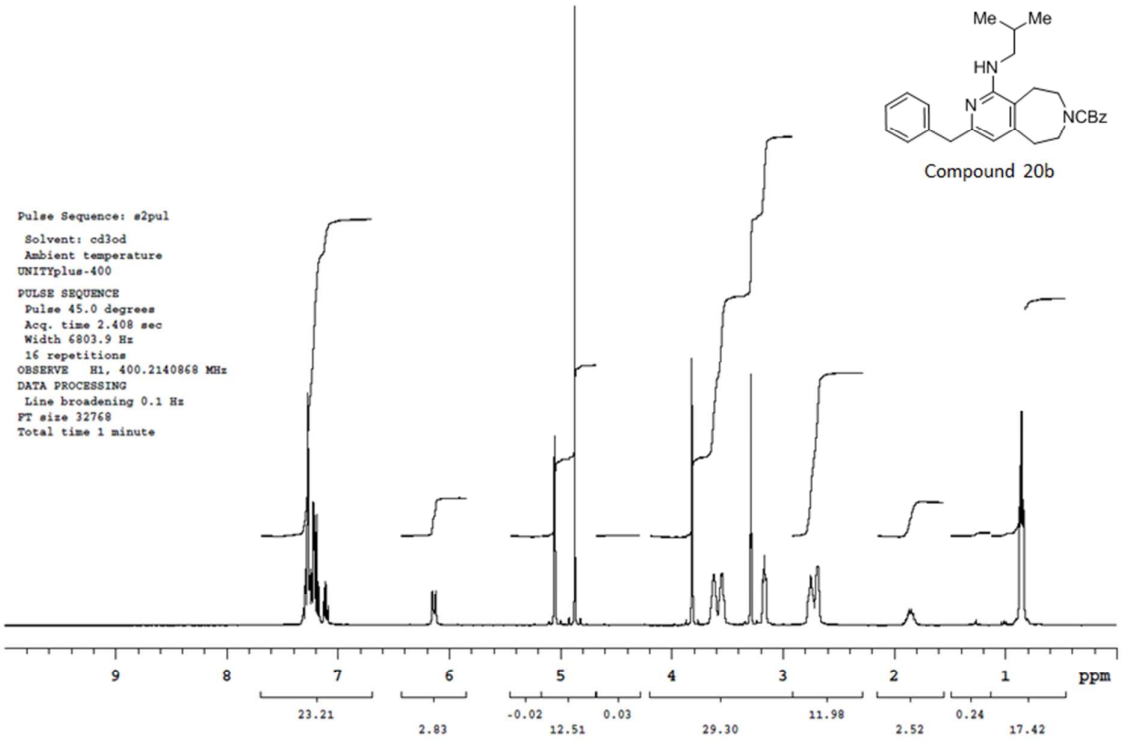


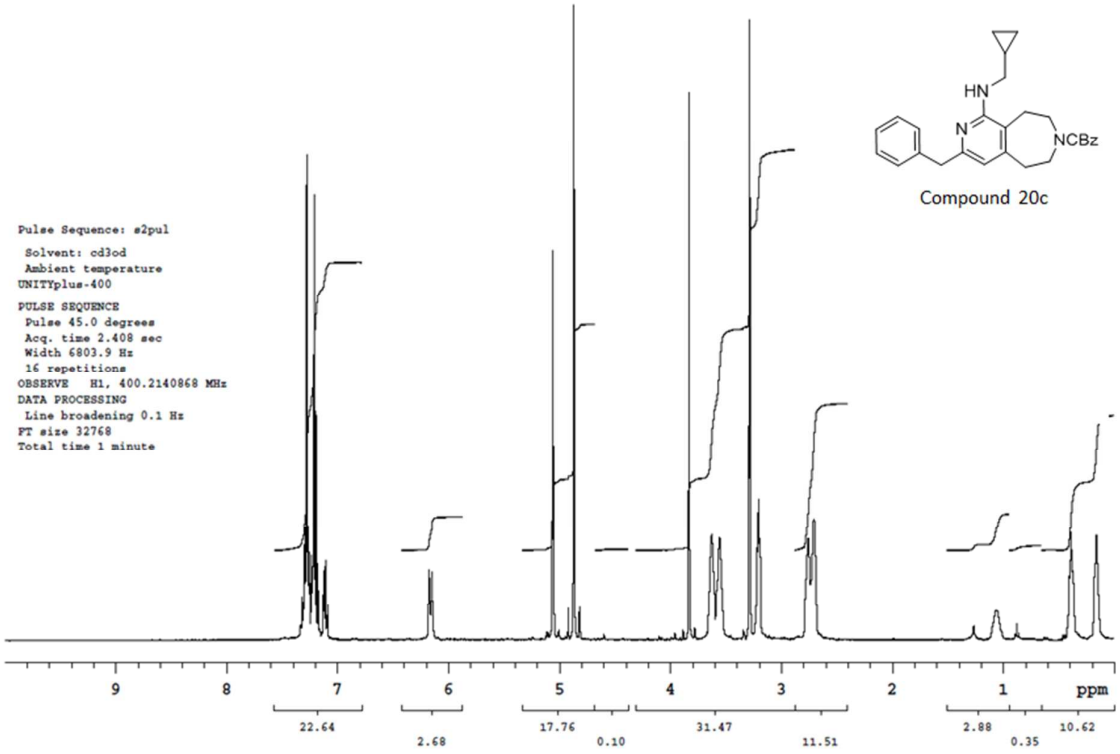
Compound 20i

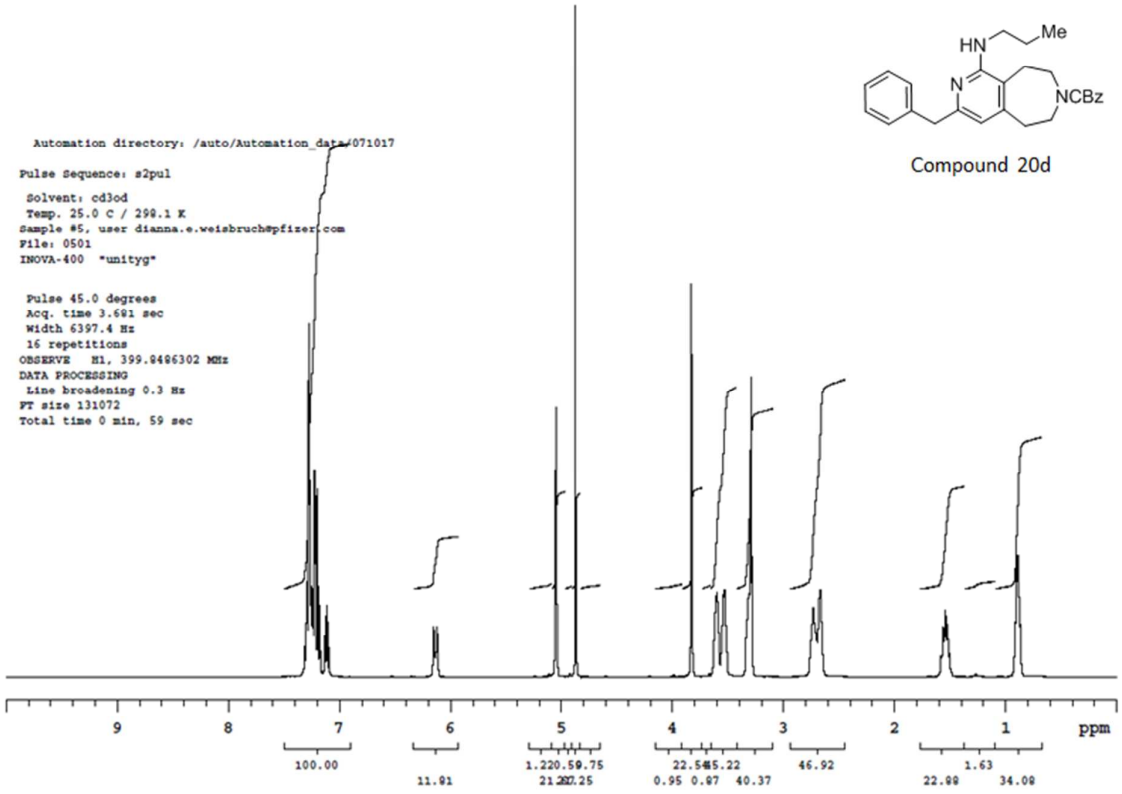




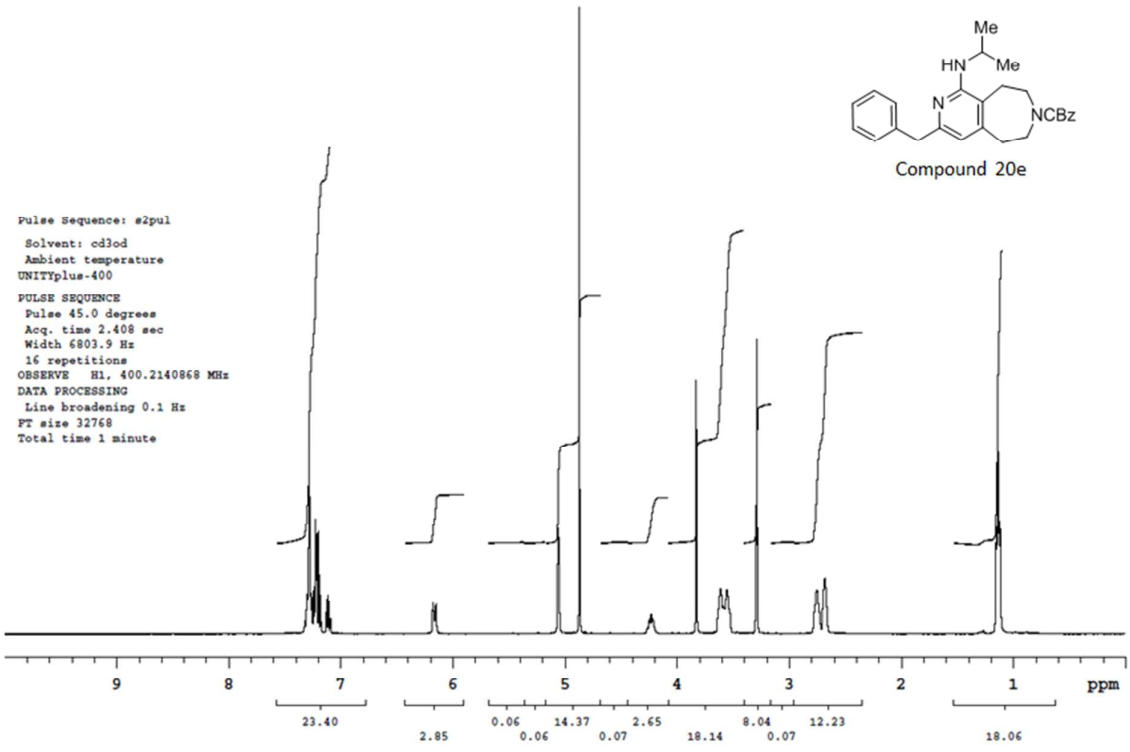
Compound 20a


Me
Me
HN
N
NCBz
Compound 20b
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
9
8
7
6
5
4
3
2
1
ppm
23.21
2.83
-0.02
0.03
12.51
29.30
11.98
2.52
0.24
17.42

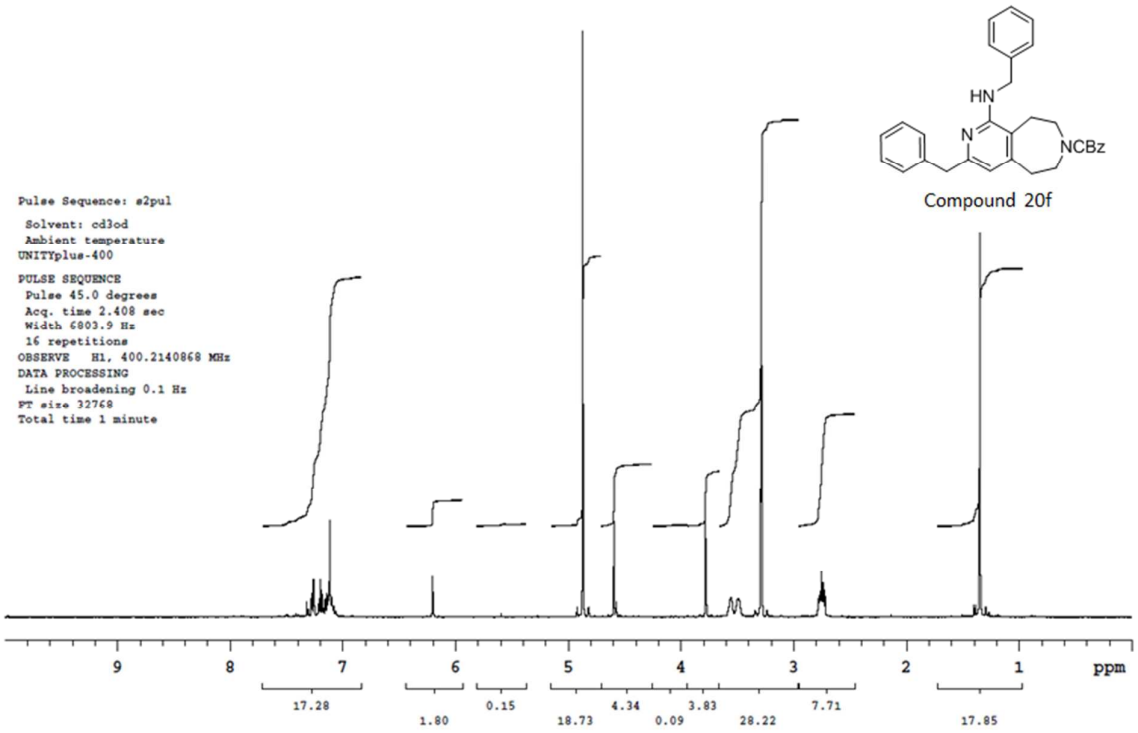

HN
N
NCBz
Compound 20c
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
9
8
7
6
5
4
3
2
1
ppm
22.64
2.68
17.76
0.10
31.47
11.51
2.88
0.35
10.62

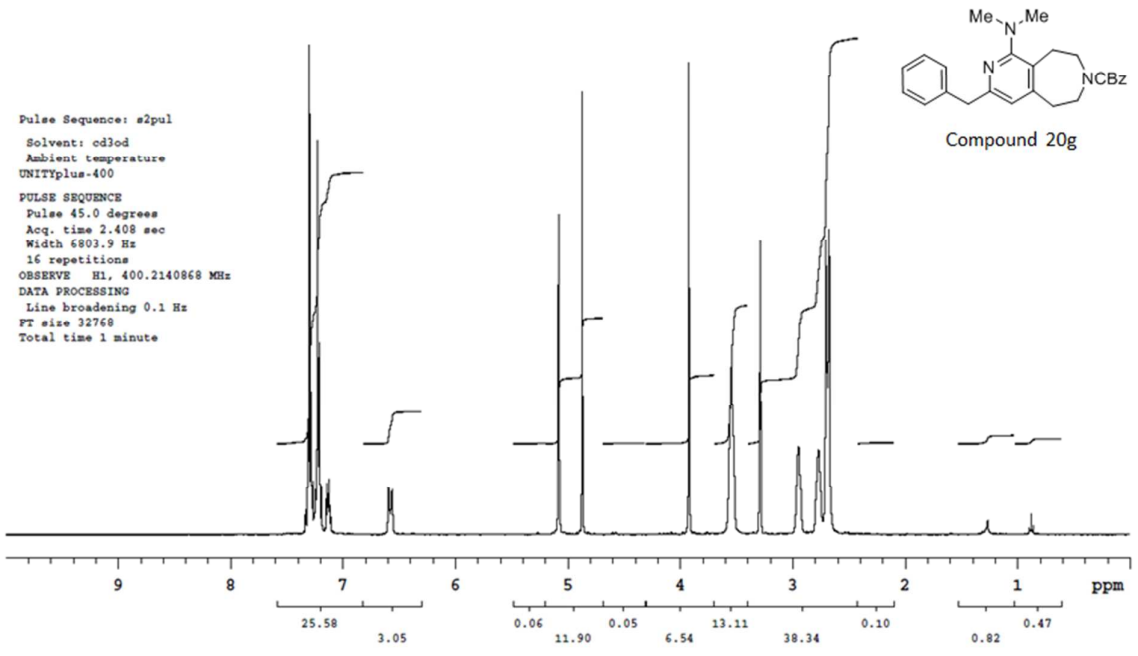


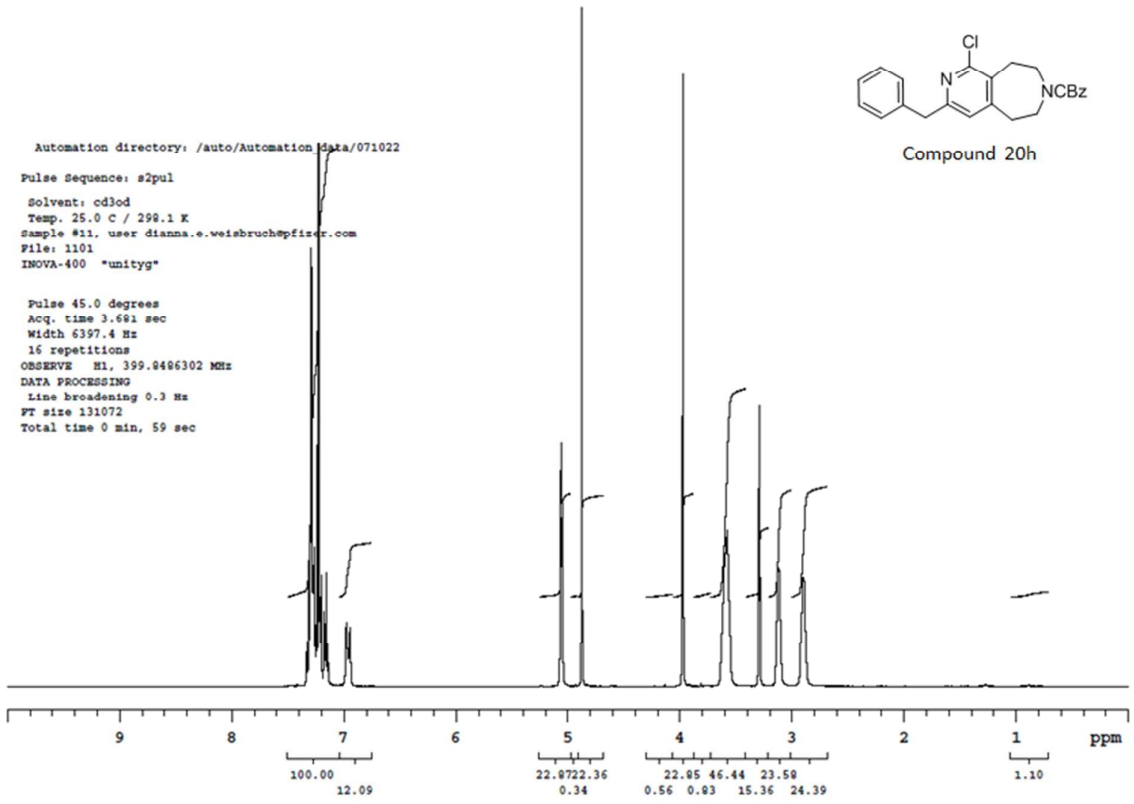
Compound 20d

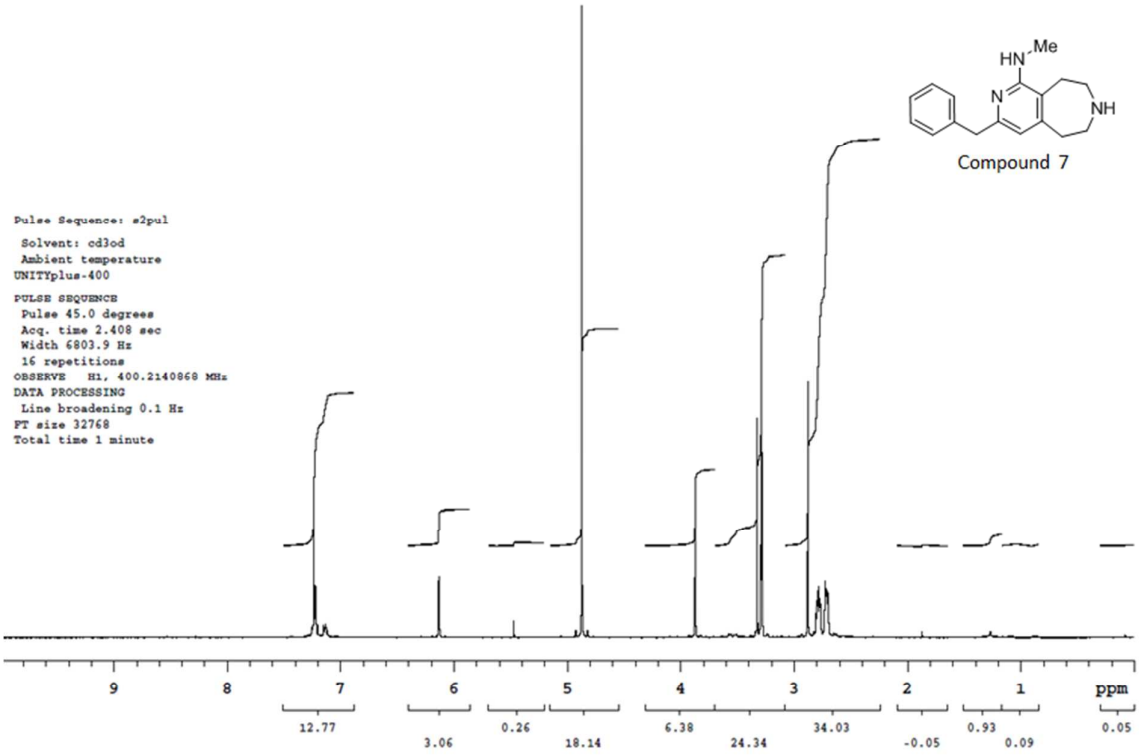


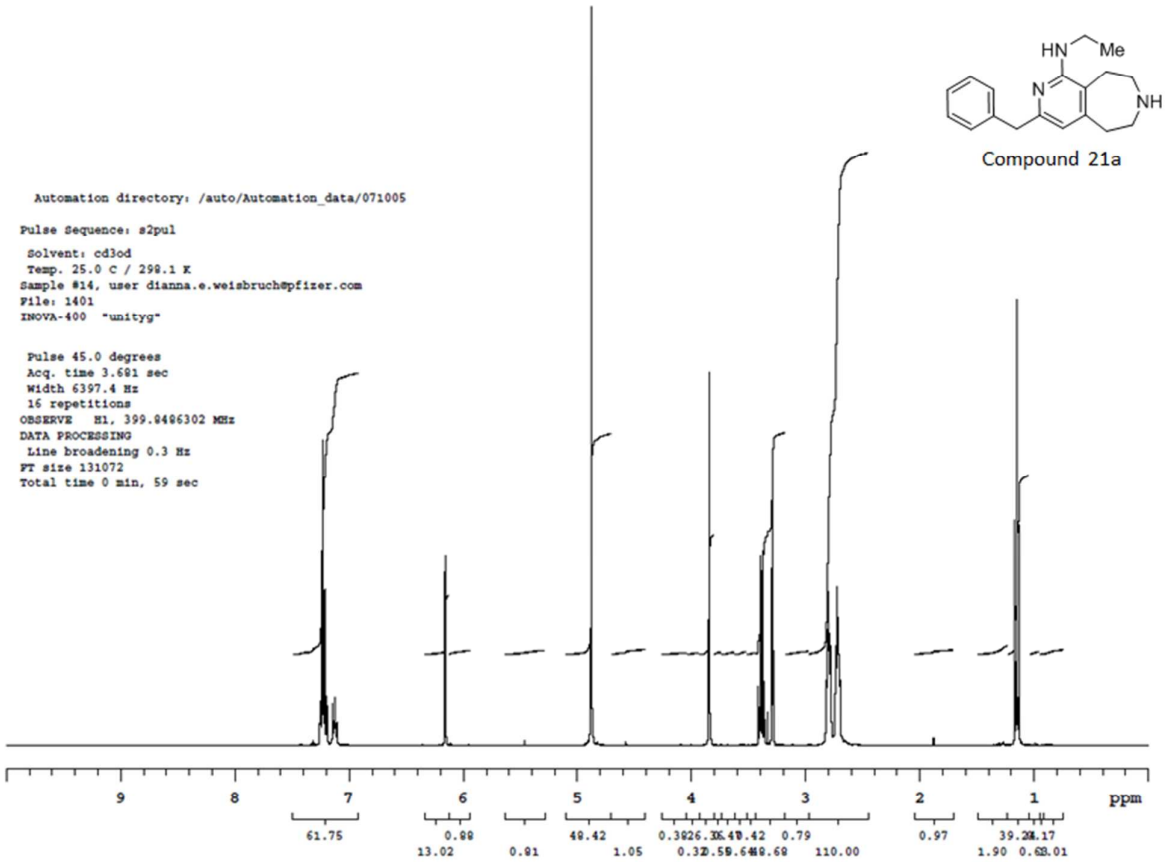
Compound 20e


HN
N
NCBz
Compound 20f
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
9
8
7
6
5
4
3
2
1
ppm
17.28
1.80
0.15
18.73
4.34
0.09
3.83
28.22
7.71
17.85

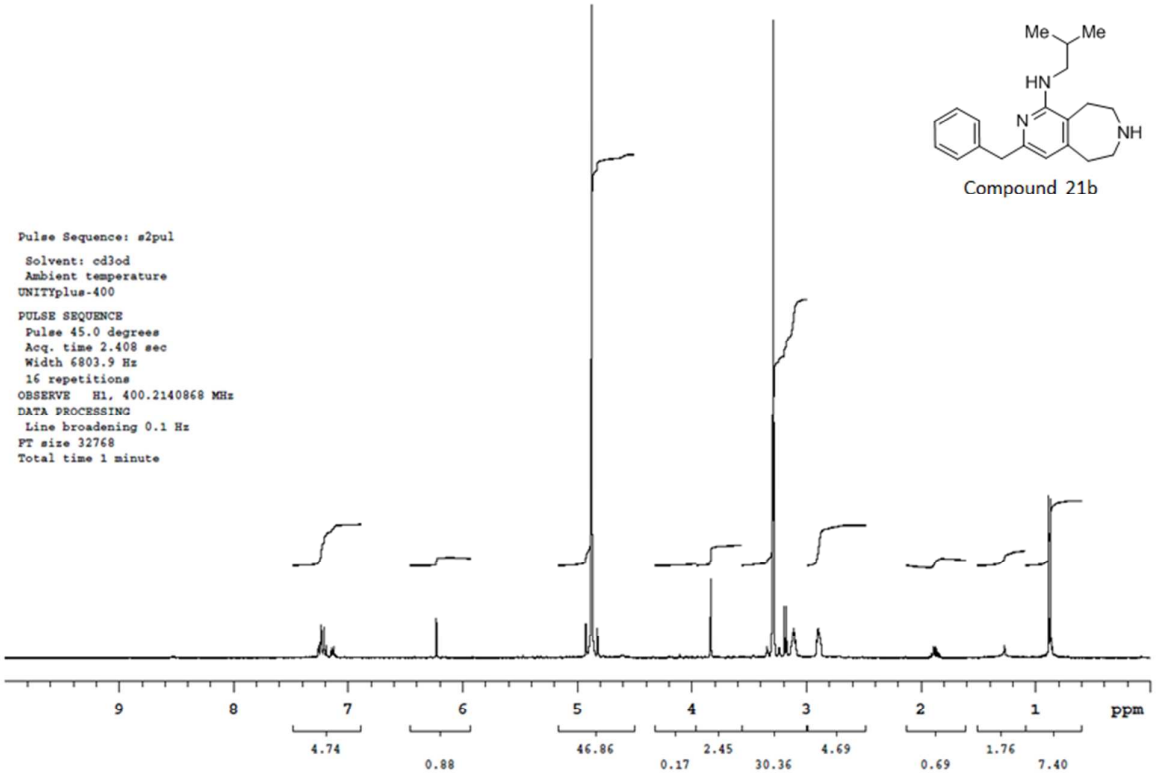

Me
N
Me
N
NCBz
Compound 20g
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
9
8
7
6
5
4
3
2
1
ppm
25.58
3.05
0.06
11.90
0.05
6.54
13.11
38.34
0.10
0.82
0.47


Cl
N
NCBz
Compound 20h
Automation directory: /auto/Automation_data/071022
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.0 C / 298.1 K
Sample #11, user dianna.e.weisbruch@pfizer.com
File: 1101
INOVA-400 "unityg"
Pulse 45.0 degrees
Acq. time 3.681 sec
Width 6397.4 Hz
16 repetitions
OBSERVE H1, 399.9496302 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 131072
Total time 0 min, 59 sec
9
8
7
6
5
4
3
2
1
ppm
100.00
12.09
22.97
22.36
0.34
22.95
46.44
23.50
0.56
0.83
15.36
24.39
1.10

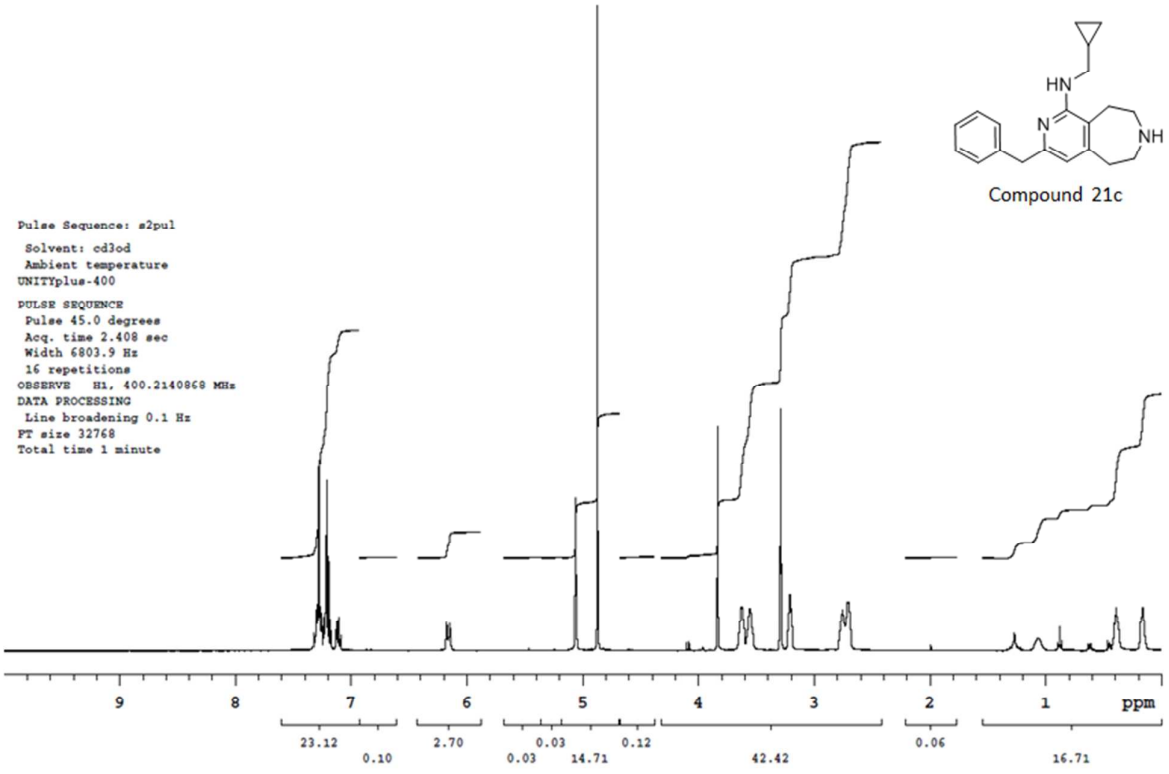

HN
Me
N
NH
Compound 7
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
9
8
7
6
5
4
3
2
1
ppm
12.77
3.06
0.26
18.14
6.38
24.34
34.03
-0.05
0.93
0.09
0.05

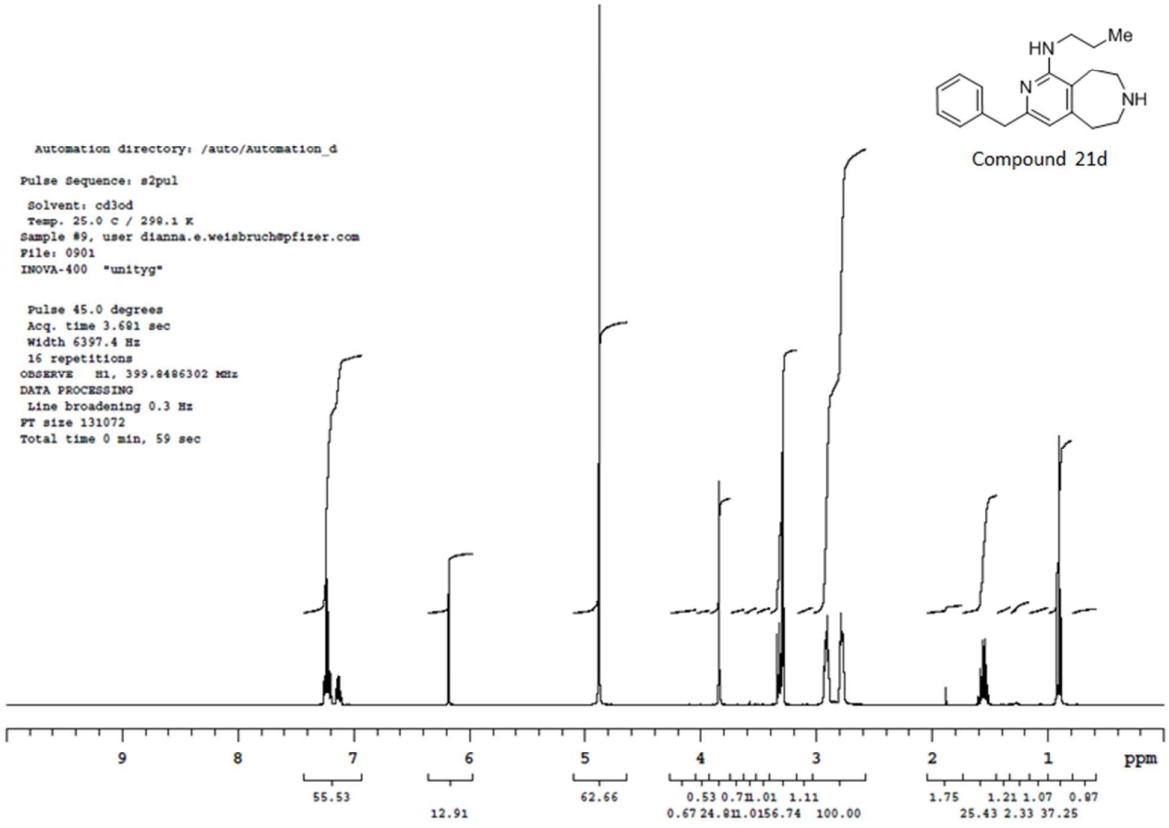


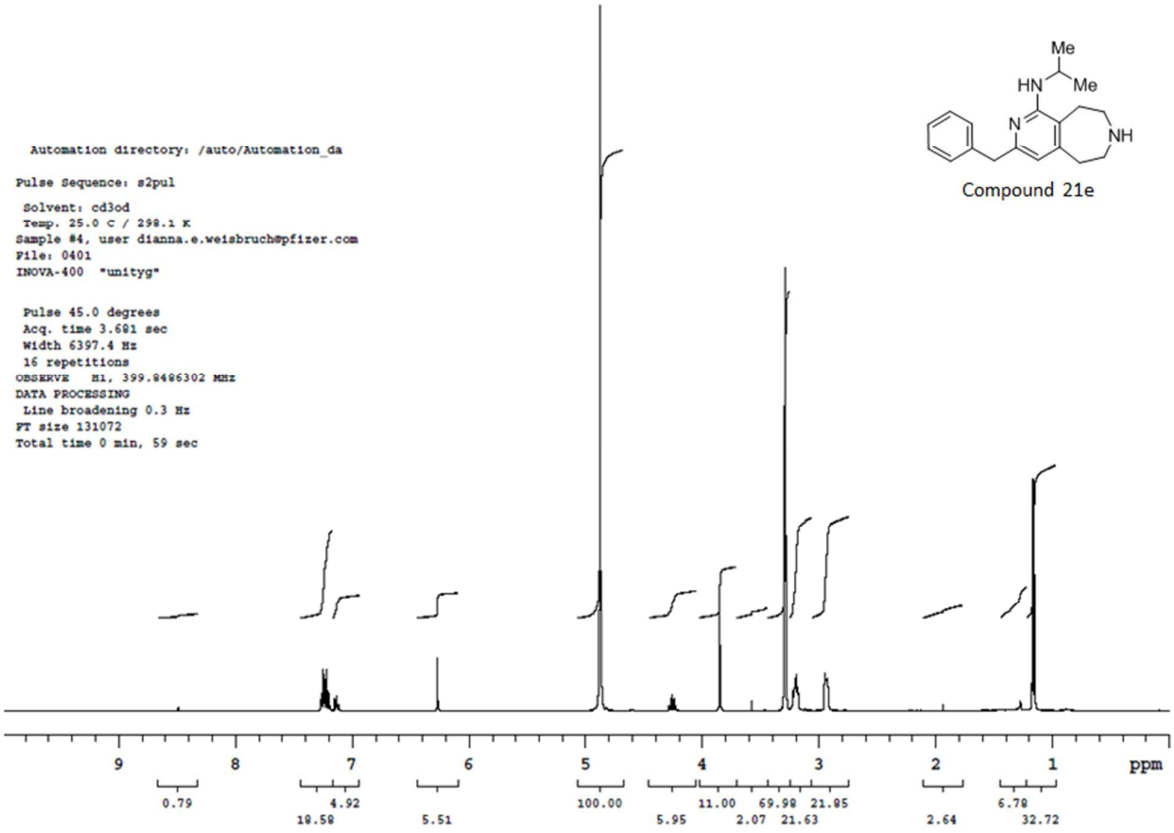
Compound 21a

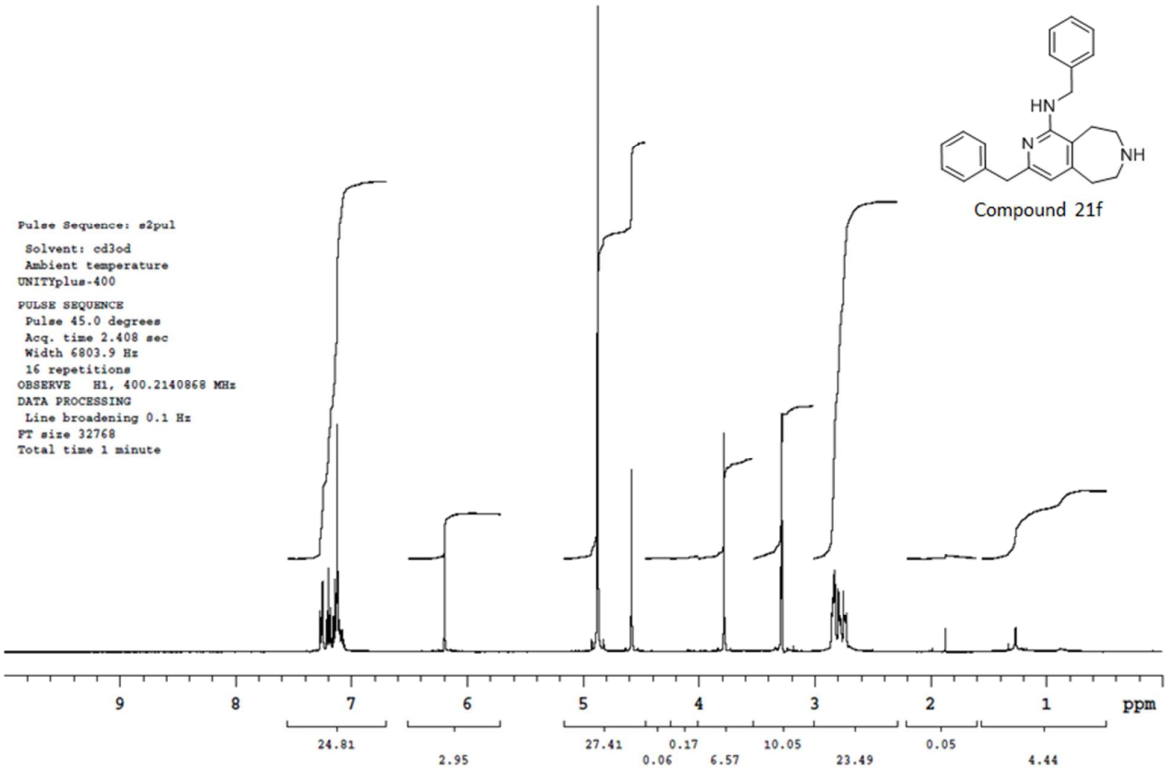


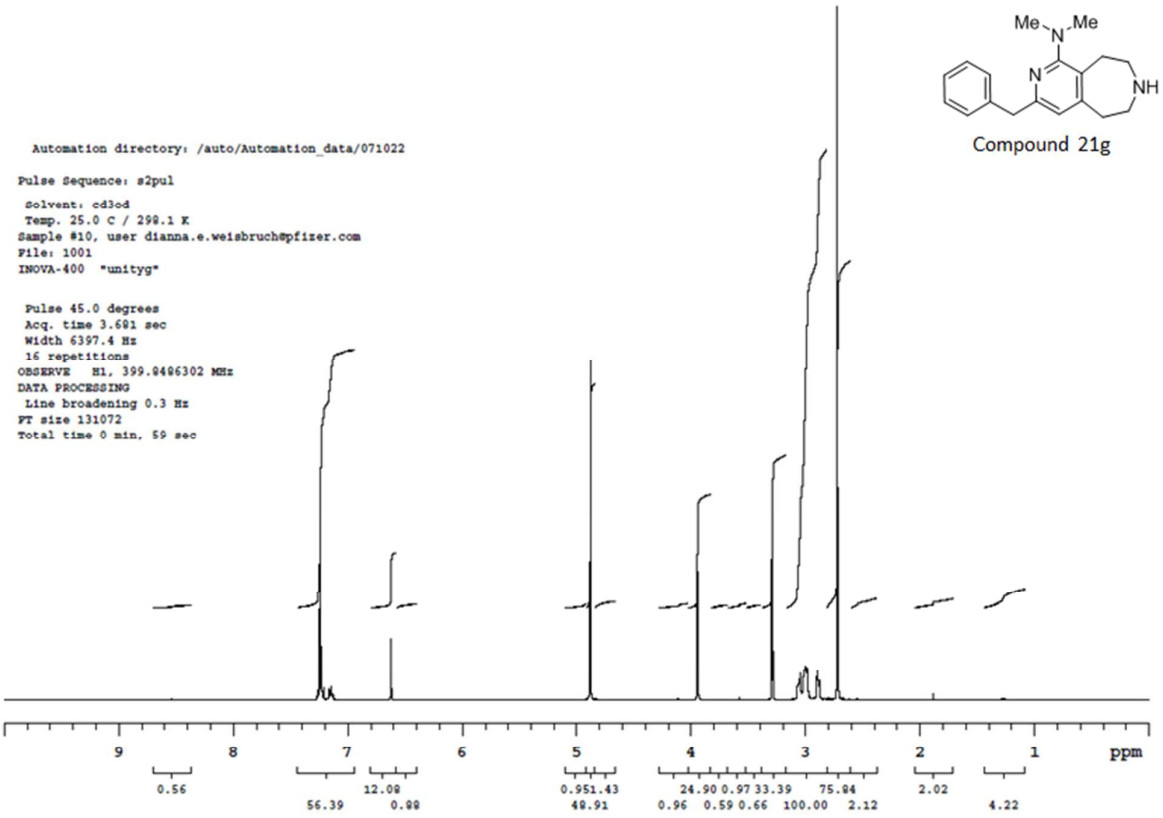
Compound 21b


HN
N
NH
Compound 21c
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
9
8
7
6
5
4
3
2
1
ppm
23.12
0.10
2.70
0.03
0.03
14.71
0.12
42.42
0.06
16.71

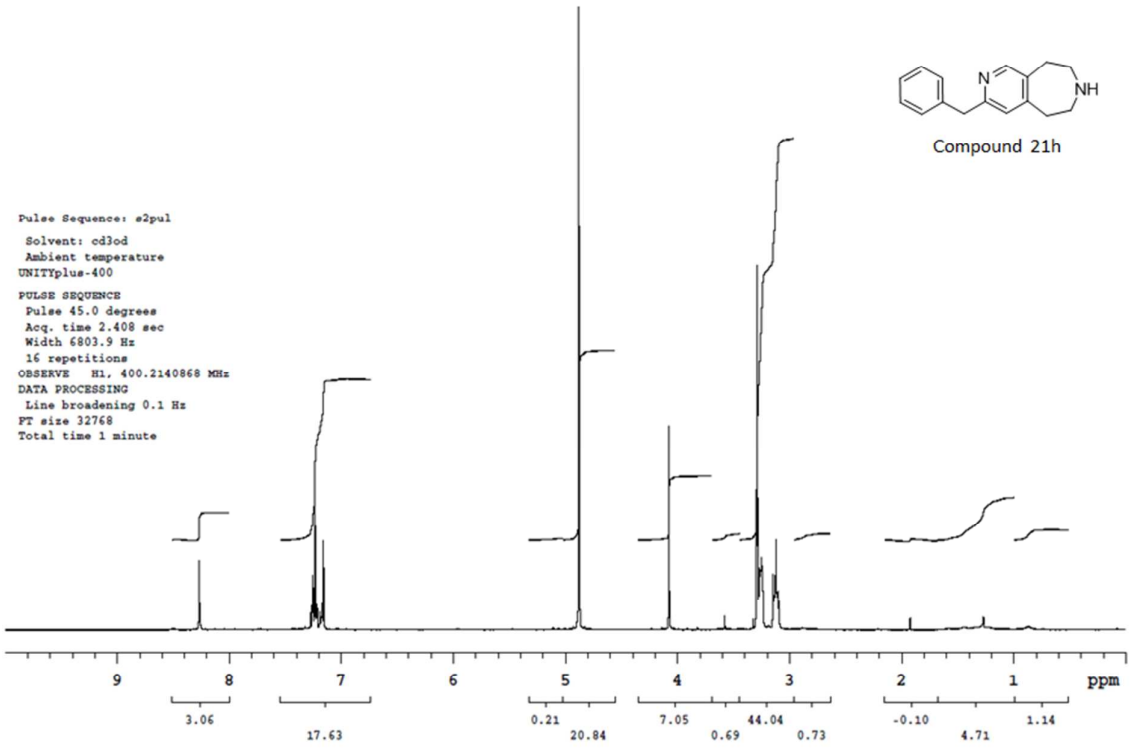

HN
Me
N
NH
Compound 21d
Automation directory: /auto/Automation_d
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.0 C / 298.1 K
Sample #9, user dianna.e.weisbruch@pfizer.com
File: 0901
INOVA-400 "unityg"
Pulse 45.0 degrees
Acq. time 3.691 sec
Width 6397.4 Hz
16 repetitions
OBSERVE H1, 399.8486302 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 131072
Total time 0 min, 59 sec
9
8
7
6
5
4
3
2
1
ppm
55.53
12.91
62.66
0.53 0.7
0.67 24.9
1.01 1.11
1.0156.74 100.00
1.75 1.21 1.07 0.87
25.43 2.33 37.25


Automation directory: /auto/Automation_da
Pulse Sequence: s2pul
Solvent: cd3od
Temp. 25.0 C / 298.1 K
Sample #4, user dianna.e.weisbruch@pfizer.com
File: 0401
INOVA-400 "unityg"
Pulse 45.0 degrees
Acq. time 3.691 sec
Width 6397.4 Hz
16 repetitions
OBSERVE H1, 399.9496302 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 131072
Total time 0 min, 59 sec
Me
HN
Me
N
NH
Compound 21e
9
8
7
6
5
4
3
2
1
ppm
0.79
4.92
10.59
5.51
100.00
5.95
11.00
69.98
21.85
2.07
21.63
2.64
6.79
32.72


Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.408 sec
Width 6803.9 Hz
16 repetitions
OBSERVE H1, 400.2140868 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
HN
N
NH
Compound 21f
9
8
7
6
5
4
3
2
1
ppm
24.81
2.95
27.41
0.06
0.17
6.57
10.05
23.49
0.05
4.44



Compound 21g



Compound 21h

5. Biological assay experimental procedures

Fluorescence Polarisation Assay

Cell Culture: Swiss 3T3 stably transfected with the 5-HT_{2C} receptor were cultured under standard cell culture techniques. Specifically, cells were grown in 50mL growth medium (Dulbecco's Modified Eagle's Medium (DMEM) culture media supplemented with 10% dialysed foetal calf serum (FCS), 2mM penicillin/streptomycin and 20µg/mL geneticin) in 225 cm^2 flasks at 37 °C and 5% CO_2. Cells were grown to 60-80% confluency, harvested using trypsin-EDTA and pelleted by centrifugation for long-term storage at -80 °C.

Membrane Preparation: Cell pellets were thawed on ice and resuspended in 3 mL of membrane preparation buffer (see Media and Buffers for composition) per 1 mL of packed cell. The suspension was homogenised on ice for several 5s intervals using a hand-held homogeniser. The homogenate was then centrifuged at 1,000rpm for 5 min at 4 °C.

The supernatants were then collected and retained. Initial cell & nuclei pellets (P1) were subsequently re-homogenised and centrifuged using the conditions cited above, and the supernatants collected and pooled with those retained from the first spin.

The pooled supernatants were centrifuged at 19,500 rpm for 45 min at 4 °C, and the supernatants discarded. The pellets (P2) were then resuspended in 3 mL of membrane preparation buffer per 1 mL of the original packed cell volume. Protein concentrations were subsequently measured and the membrane suspension was finally frozen in aliquots of set volume and stored at -80 °C prior to use in assays.

Assay Protocol: Assays were conducted in Corning Costar 384-well black, flat-bottomed plates. Assays were performed in 40 µL total volume consisting of 20 µL Cy3b-labeled [FP] ligand (final concentration = 1 nM), 2 µL competitor, and 20 µL membrane (2.5-5 µg/well) in binding buffer (50 mM Tris-HCl (pH 7.7) containing 10 mM MgCl2, 3 mM CaCl2, 1 mM EDTA, 0.1mM Pargyline, 0.1% Ascorbic Acid and 0.05% Pluronic), combined in the order listed. Total binding data points were measured in the absence of unlabeled ligand. Nonspecific binding data points were measured in the presence of 10 µM mianserin. Assay constituents were subsequently incubated for 60 min at 37 °C. Fluorescence polarization was measured on an EnVision microplate reader (PerkinElmer) using a 555/38-nm Texas Red FP excitation filter, 632-nm Texas Red FP P-pol emission filter, 632-nm Texas Red FP S-pol second emission filter, and 485-nm Texas Red FP dual mirror.

Data Analysis: The assay window (specific binding) per plate was calculated by subtracting the mean NSB readings from the mean of total binding readings. Subsequently specific binding read per well (with mean NSB subtracted) was expressed as a percentage of the plate window to determine the amount of bound FP-ligand. These values were plotted against the concentration of the compound tested and a sigmoidal inhibitory concentration effect curve was fitted to the data using a four-parameter logistic equation and free-fitting parameters to give an IC_{50} value (the concentration of compound required to inhibit 50% of the specific binding at the 5-HT_{2C} receptor).

The inhibitory dissociation constant (K_i) value was then calculated from the IC_{50} value using the Cheng-Prusoff equation. Following determination of individual K_i values for compounds tested, an overall geometric mean was calculated together with 95% confidence intervals and n values, where n is the total number of individual K_i values.

GTPγS Assay

Cell Culture: Swiss 3T3 stably transfected with the 5-HT_{2C} receptor were cultured under standard cell culture techniques. Specifically, cells were grown in 50mL growth medium (Dulbecco's Modified Eagle's Medium (DMEM) culture media supplemented with 10% dialysed foetal calf serum (FCS), 2mM penicillin/streptomycin and 20µg/mL geneticin) in 225 cm^2 flasks at 37 °C and 5% CO_2. Cells were grown

to 60-80% confluency, harvested using trypsin-EDTA and pelleted by centrifugation for long-term storage at -80 °C.

Membrane Preparation: Cell pellets were thawed on ice and resuspended in 3 mL of membrane preparation buffer (see Media and Buffers for composition) per 1 mL of packed cell. The suspension was homogenised on ice for several 5s intervals using a hand-held homogeniser. The homogenate was then centrifuged at 1,000rpm for 5 min at 4 °C.

The supernatants were then collected and retained. Initial cell & nuclei pellets (P1) were subsequently re-homogenised and centrifuged using the conditions cited above, and the supernatants collected and pooled with those retained from the first spin.

The pooled supernatants were centrifuged at 19,500 rpm for 45 min at 4 °C, and the supernatants discarded. The pellets (P2) were then resuspended in 3 mL of membrane preparation buffer per 1 mL of the original packed cell volume. Protein concentrations were subsequently measured and the membrane suspension was finally frozen in aliquots of set volume and stored at -80 °C prior to use in assays.

Assay protocol: The PerkinElmer DELFIA GTP binding kit was used for GTPγS binding functional assay. The DELFIA guanosine triphosphate (GTP)– binding assay is a time-resolved fluorometric assay based on guanosine diphosphate (GDP)–GTP exchange on G-protein subunits followed by G-protein-coupled receptor (GPCR) activation by an agonist. Then, 60 µL of assay buffer that contained 50 mM HEPES (pH 7.4), 100 mM NaCl2, 5 mM MgCl2, and 1 µM GDP was added to a 96-well AcroWell filtration plate. Then, 20 µL of buffer was added to wells designated as basal, 20 µL of 10 µM 5-HT (final concentration) was added to wells designated as maximal stimulation, 20 µL of each concentration of testing compound (in triplicate) was added to the remaining wells, and 20 µL of 0.5 mg/mL of membrane (10 µg/ well) was added to all wells. The AcroWell filtration assay plate was placed on a plate rocker for 45 min at room temperature. Following this incubation, 10 µL of 100 nM Europium labelled GTP (9 nM final concentration) was added to all wells. The plate was returned to the plate rocker for 30 to 40 min. After incubation, the plate was placed on a vacuum manifold and washed 3 times with 300 µL/well of ice-cold wash buffer, after which the plate was read on a Victor fluorescent plate reader (340 nm excitation, 615 nm emission) within 30 min after washing.

Data Analysis: Average signal obtained in the basal wells was subtracted from the average signal obtained in 5-HT stimulated wells to obtain the maximal GTP-Eu binding signal caused by 10 µM 5-HT stimulation. The basal mean was also subtracted from values obtained for each known dilution. These values were then divided by the mean 5-HT-stimulated value–mean basal value to obtain a percentage. These values were graphed using GraphPad Prism. EC_{50}s were then calculated from the sigmoidal dose-response curves. Efficacy was determined as a percentage increase above basal at 10 µM as compared to 10 µM 5-HT. An overall geometric mean was calculated together with 95% confidence intervals.

Calcium Mobilisation Assay

The agonist potency and efficacy of the compounds were tested by measuring their ability to induce a fluorescent based Ca^{2+} mobilization signal in a FLIPR assay using CHO K1 cells expressing recombinant human 5-HT_{2B} receptor. Both agonist affinity (EC_{50}) and efficacy (E_{max}) were determined.

Cell culture

Chinese hamster ovary cells (CHO K1) stably transfected with human 5-HT_{2C} or 5-HT_{2B} receptor were cultured under standard cell culture techniques. Specifically, cells were grown at 37 °C and 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) culture media supplemented with 10% dialysed foetal calf serum (FCS), 1% non-essential amino acids, 1mM sodium pyruvate, 800µg/mL geneticin and 50µg/ml zeocin. Cells were harvested for passaging and storage using trypsin-EDTA, centrifugation and

resuspension in culture medium. Cells were grown to 60-80% confluency, harvested and adjusted to 15-20 x 10^6 cells/ml/vial in medium/10% DMSO and stored long-term at -80 °C.

Preparation of cell plates

Cells were seeded into black-walled clear-bottomed 384 well plates 24h before use. Frozen cells were defrosted in a 37 °C water bath and immediately transferred into 1mL/vial 37 °C culture medium, diluted to 10ml and DMSO removed by centrifugation. The cells were re-suspended in 15ml/vial of cell culture medium, counted and adjusted to give 500,000 cells/mL (10,000 cells/well). 20µL/well of cell suspension was added to the 384 well plates which were then incubated overnight at 37 °C.

Preparation of compound plates

Test compounds were prepared at 4mM in 100% dimethyl sulphoxide (DMSO) and diluted in Dulbeccos PBS (+$CaCl_2$, +$MgCl_2$) with 0.9% DMSO and 0.05% pluronic F-127 to give appropriate test concentrations. The maximum agonist response was determined with 5-HT at a final assay concentration of 10µM in the diluent above. The minimum response was determined with Dulbeccos PBS (+$CaCl_2$, +$MgCl_2$) with 0.9% DMSO and 0.05% pluronic F-127. Test compounds, maximum and minimum controls were added to a 384 well polypropylene plate.

Preparation of FLIPR dye

The FLIPR calcium assay reagent was diluted with assay buffer (Hank's Balanced Salt Solution (HBSS))/20mM HEPES and 2.5mM probenecid (diluted with 1M aqueous sodium hydroxide and DPBS (+$CaCl_2$, +$MgCl_2$)).

Running the assay using FLIPR

20µL/well FLIPR calcium assay reagent was added to the cell plates, which were incubated for 1h at 37 °C. Cell plates and compound plates were then transferred onto the FLIPR. The assay was run using the appropriate FLIPR program, which initiates the reaction by transferring 15µL compound into the corresponding well of the cell plate.

Data analysis

The statistical parameter exported from each well was the max peak height of the response. The mean minimum was subtracted from all values and then the activity was expressed as a percentage of the mean maximal response to 10µM 5-HT and dose-response curves plotted from which both agonist affinity (EC_{50}) and efficacy (E_{max}) were determined.

6. X-ray Structure of Compound 13

A sample of intermediate **13** was crystallised from CD_3OD, enabling an X-ray structure to be obtained to further confirm structure assignment. This structure has been deposited in the Cambridge Crystallographic Database (CCDC 1024393):