- Supporting Information -

Structure-Guided Design of Nurr1 Agonists Derived from the Natural Ligand Dihydroxyindole

Minh Sai[1,#], Jan Vietor[1,#], Moritz Kornmayer[1], Markus Egner[1], Úrsula López-García[1], Georg Höfner[1], Jörg Pabel[1], Julian A. Marschner[1], Thomas Wein[1], Daniel Merk[1]*

[1] Ludwig-Maximilians-Universität München, Department of Pharmacy, 81377 Munich, Germany

[#] M.S. and J.V. contributed equally to this work

* daniel.merk@cup.lmu.de

Table of Contents

Supplementary Figures

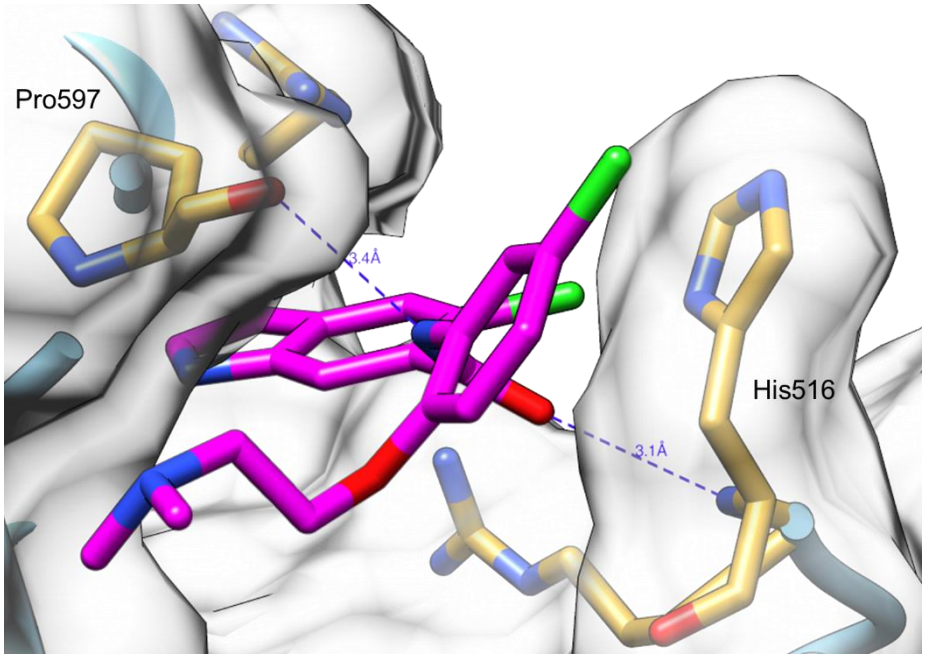


Figure S1. Initial evaluation by docking experiments suggested interactions of a carboxamide in 5- or 6-position of the 1*H*-indole scaffold with the backbone of His516 and Pro597 of the Nurr1 LBD (PDB ID 6dda[1], **5o** shown as example ligand) thus indicating potential of *N*-substituted carboxamides for extension of the natural ligand DHI.

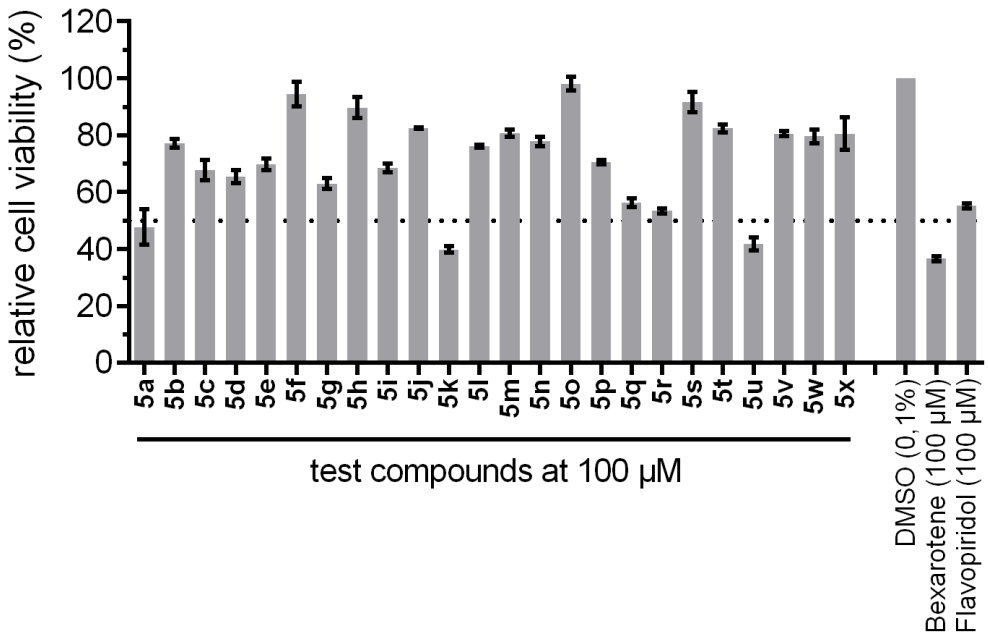


Figure S2. Toxicity evaluation of the screening compounds in a WST-8 assay in HEK293T cells at 100 µM (data are the mean±S.E.M.; n=4). Compounds **5a**, **5k** and **5u** causing <50% cell viability were excluded from follow-up studies. Like **5a**, **5k** and **5u**, compound **5q** diminished Renilla luciferase activity in the reporter gene assay to <50% and was also excluded despite slightly lower toxicity in the WST-8 assay.

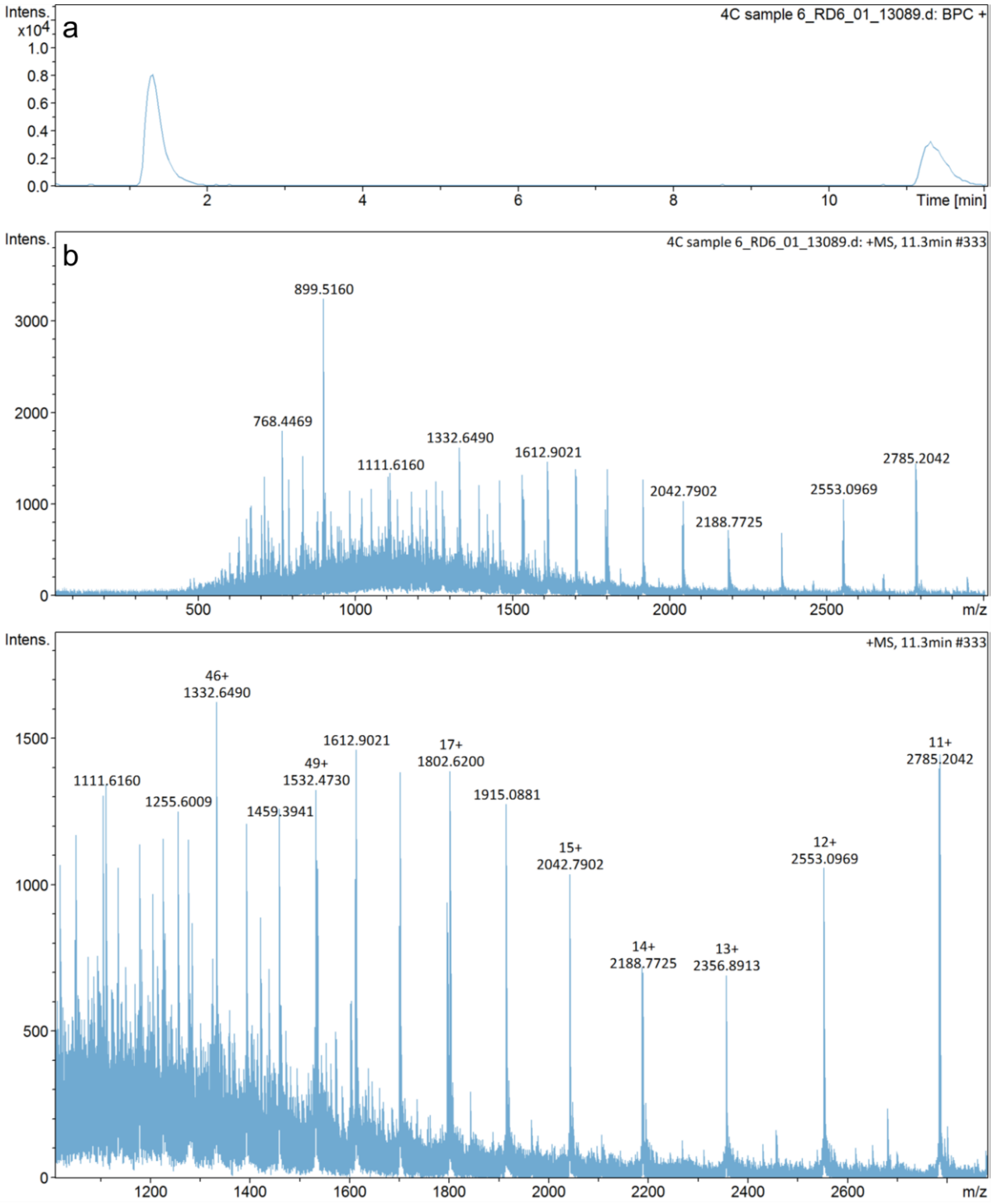


Figure S3. LC-UV (280 nm, a) and MS pattern (ESI+, b) of the Nurr1 LBD (10 µM, M = 30.627 kDa) without ligand.

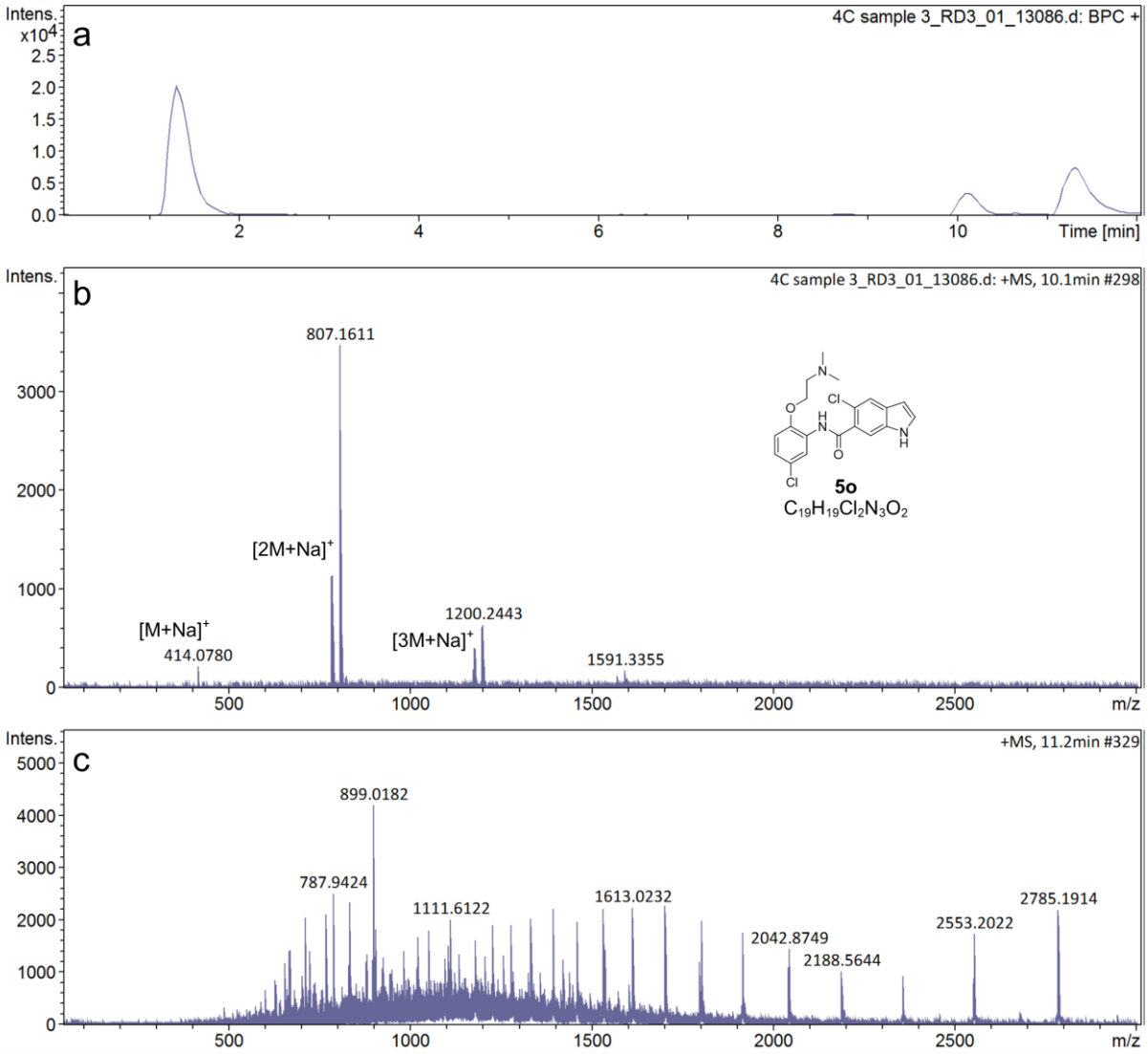


Figure S4. **5o** formed no covalent adduct with the Nurr1 LBD. (a) LC-UV (280 nm) of the Nurr1 LBD (10 µM) + **5o** (100 µM) mixture after 120 h incubation at 4 °C showed one signal for the ligand (10.1 min) and one for the intact Nurr1 LBD (11.2 min). (b) The MS pattern at 10.1 min corresponds to **5o**. (c) The MS pattern at 11.2 min corresponds to the intact Nurr1 LBD without covalent modification.

Experimental procedures

General. All chemicals were of reagent grade and used without further purification unless otherwise specified. All reactions were conducted in oven-dried Schlenk glassware under argon atmosphere and in absolute solvents. Other solvents, especially for work-up procedures, were of reagent grade or purified by distillation (*iso*-hexane, ethyl acetate, ethanol). Reactions were monitored by thin layer chromatography on TLC Silica gel 60 F_{254} aluminium sheets by Merck and visualized under ultraviolet light (254 nm). Purification by CC was performed on a puriFlash® XS520Plus system (Advion, Ithaca, NY, USA) using high performance spherical silica columns (SIHP, 50 µM) by Interchim and a gradient of *iso*-hexane to EtOAc, reversed-phase CC was performed on a puriFlash® 5.250 system (Advion) using C18HP columns (SIHP, 15 µM) by Interchim and a gradient of 0.1% formic acid (FA) in water with 10 to 100% acetonitrile (HPLC gradient grade). Preparative HPLC was also performed on the puriFlash® 5.250 system using a PREP-LC column (C18-HQ, 5 µM) by Interchim and gradient of 0.1% FA in water with 10 to 100% methanol (HPLC gradient grade). Mass spectra were obtained on a puriFlash®-CMS system (Advion) using atmospheric pressure chemical ionization (APCI). HRMS were obtained with a Thermo Finnigan LTQ FT instrument for electrospray ionization (ESI). NMR spectra were recorded on Bruker Avance III HD 400 MHz or 500 MHz spectrometers equipped with CryoProbe™ Prodigy broadband probe (Bruker). Chemical shifts are reported in δ values (ppm), coupling constants (*J*) in hertz (Hz). Purity was determined by quantitative ^{1}H NMR (qH NMR) according to a method described by Pauli et al. with internal calibration.[2] The qH NMR measurements were carried out under conditions allowing complete relaxation to assure the exact determination of peak area ratios. Used internal standards were Ethyl 4-(dimethylamino)benzoate (LOT# BCCC6657, purity 99.63%) and maleic acid (LOT# BCBM8127V, purity 99.94%) in $CDCl_3$, MeOD-d_4, DMSO-d_6 or acetone-d_6. All compounds for biological testing had a purity >95% according to qH NMR.

General procedures

General procedure for amide coupling with EDC·HCl (GP1)

5-Chloro-1*H*-indole-6-carboxylic acid (**3**, 1.1 eq.) was suspended in chloroform (~0.04 M) and EDC·HCl (1.2 eq) was added. The mixture was stirred at rt for 1 h, whereby the solution cleared up. Then the respective amine (**4e**,**m**,**o**,**r**,**t**,**v**, 1.0 eq) and triethylamine (0.1 eq) were added and the resulting mixture was stirred at rt for 18 h. The solution was diluted with aqueous NaOH (2 M) and the aqueous layer was extracted with DCM (3 x). The combined organic layer was washed with aqueous NaOH (2 M), dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by CC and preparative HPLC.

Synthesis and analytical characterization of **5e,m,o,r,t,v, 10-13**

5-Chloro-*N*-(1-isopropylpiperidin-4-yl)-1*H*-indole-6-carboxamide (5e) Preparation according to GP1, using 5-chloro-1*H*-indole-6-carboxylic acid (**3**, 108 mg, 550 µmol, 1.10 eq) and commercially available 4-amino-1-isopropylpiperidine (**4e**, 71.1 mg, 500 µmol, 1.00 eq) yielded amide **5e** as a yellow oil (50 mg, 31%). R_f (EtOAc/EtOH = 1:1 + 2% TEA) = 0.55. MS (+APCI): *m*/*z* 319.9 ([M+H]$^+$). HRMS (+ESI): *m*/*z* calculated 320.15242 for $[C_{17}H_{23}ClN_3O]^+$, found 320.15277 ([M+H]$^+$). ^{1}H NMR (400 MHz, MeOD-d_4): δ = 8.51 (s, 1H), 7.63 (s, 1H), 7.53–7.47 (m, 1H), 7.39 (d, *J* = 3.1 Hz, 1H), 6.47 (dd, *J* = 3.1, 0.9 Hz, 1H), 4.25–4.06 (m, 1H), 3.54–3.38 (m, 3H), 3.25–3.08 (m, 2H), 2.36–2.18 (m, 2H), 2.04–1.81 (m, 2H), 1.35 (d, *J* = 6.6 Hz, 6H) ppm. ^{13}C NMR (126 MHz, CD_2Cl_2): δ = 167.42, 134.44, 130.41, 128.77, 128.45, 121.85, 121.72, 113.30, 102.12, 56.20, 47.26, 46.38, 30.19, 17.24 ppm. qH NMR (400 MHz, MeOD-d_4, Ethyl 4-(dimethylamino)benzoate as reference): purity = 98.9%.

5-Chloro-*N*-[3-(4-methylpiperidin-1-yl)propyl]-1*H*-indole-6-carboxamide (5m) Preparation according to GP1, using 5-chloro-1*H*-indole-6-carboxylic acid (**3**, 68.9 mg, 352 µmol, 1.10 eq) and commercially available 3-(4-methylpiperidin-1-yl)propan-1-amine (**4m**, 50.0 mg, 320 µmol, 1.00 eq) yielded amide **5m** as a yellow solid (28 mg, 26%). R_f (EtOAc/EtOH = 1:1 + 2% TEA) = 0.51. MS (+APCI): *m*/*z* 333.8 ([M+H]$^+$). HRMS (+ESI): *m*/*z* calculated 334.16807 for $[C_{18}H_{25}ClN_3O]^+$, found 334.16844 ([M+H]$^+$). ^{1}H NMR (400 MHz, MeOD-d_4): δ = 8.45 (s, 1H), 7.64 (s, 1H), 7.56 (s, 1H), 7.41 (d, *J* = 3.1 Hz, 1H), 6.48 (d, *J* = 3.1 Hz, 1H), 3.61–3.44 (m, 4H), 3.26–3.15 (m, 2H), 3.06–2.87 (m, 2H), 2.14–1.99 (m, 2H), 1.99–1.86 (m, 2H), 1.81–1.64 (m, 1H), 1.59–1.41 (m, 2H), 1.02 (d, *J* = 6.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, MeOD-d_4): δ = 172.17, 135.42, 131.61, 129.47, 129.44, 122.09, 122.08, 112.95, 102.32, 55.69, 53.93, 37.90, 32.51, 29.95, 25.69, 21.27 ppm. qH NMR (400 MHz, MeOD-d_4, Ethyl 4-(dimethylamino)benzoate as reference): purity = 97.0%.

5-Chloro-*N*-{5-chloro-2-[2-(dimethylamino)ethoxy]phenyl}-1*H*-indole-6-carboxamide (5o) 5-Chloro-1*H*-indole-6-carboxylic acid (**3**, 150 mg, 767 µmol, 1.00 eq.) and oxalyl chloride (69.0 µL, 767 µmol, 1.00 eq.) were dissolved in 4 mL of a mixture of one drop of DMF in DCM (14 mL). After 2 h, the solvent was removed under reduced pressure. The remaining residue was dissolved in DCM (6 mL) and 5-chloro-2-[2-(dimethylamino)ethoxy]aniline (**4o**, 165 mg, 767 µmol, 1.00 eq.) was added to the solution. The reaction mixture was stirred for 18 h at rt. Then DCM (10 mL) and saturated $NaHCO_3$ solution (10 mL) were added. The separated organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by CC and subsequent recrystallization from methanol yielded compound **5o** as a colorless crystalline solid (23 mg, 8%). R_f (*iso*-hexane/EtOH = 1:1 + 2% TEA) = 0.24. MS (+APCI): *m*/*z* 391.3 ([M+H]$^+$). HRMS (+EI): *m*/*z* calculated 391.08488 for $[C_{19}H_{19}Cl_2N_3O_2]^{+\bullet}$, found: 391.08645 ([M]$^{+\bullet}$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 10.17 (s, 1H), 8.78 (s, 1H), 8.71–8.66 (m, 1H), 7.90–7.86 (m, 1H), 7.68 (s, 1H), 7.38–7.33 (m, 1H), 7.03 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.54–6.50 (m, 1H), 4.09 (t, *J* = 5.4 Hz, 2H), 2.55 (t, *J* = 5.0 Hz, 2H), 2.02 (s, 6H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.00, 146.62, 134.11, 131.66, 130.50, 129.03, 128.13, 128.09, 123.77, 122.13, 121.87, 120.81, 116.73, 113.63, 102.52, 69.45, 57.99, 45.18 ppm. qH NMR (400 MHz, MeOD-d_4, maleic acid as reference): purity = 95.4%.

5-Chloro-*N*-{2-[decahydroisoquinolin-2(1*H*)-yl]ethyl}-1*H*-indole-6-carboxamide (5r) Preparation according to GP1, using 5-chloro-1*H*-indole-6-carboxylic acid (**3**, 73.2 mg, 374 µmol, 1.10 eq) and commercially available 2-(decahydroisoquinolin-2-yl)ethan-1-amine (**4r**, mixture of diastereomers, 62.0 mg, 340 µmol, 1.00 eq) yielded amide **5r** as a colourless solid (mixture of stereoisomers, 39 mg, 32%). R_f (EtOAc/EtOH = 1:1 + 2% TEA) = 0.52. MS (+APCI):

m/*z* 359.9 ([M+H]$^+$). HRMS (+ESI): *m*/*z* calculated 360.18372 for [$C_{20}H_{27}ClN_3O$]$^+$, found 360.18412 ([M+H]$^+$). ^{1}H NMR (500 MHz, MeOD-d_4): δ = 8.53 (s, 1H), 7.64 (s, 1H), 7.59 (s, 1H), 7.41 (d, *J* = 3.1 Hz, 1H), 6.48 (d, *J* = 3.1 Hz, 1H), 3.72 (t, *J* = 6.2 Hz, 2H), 3.60–0.96 (m, 18H) ppm. ^{13}C NMR (126 MHz, MeOD-d_4) δ = 172.36, 135.40, 131.78, 129.61, 128.85, 122.20, 122.19, 113.21, 102.36, 59.86, 58.41, 55.04, 41.30, 36.98, 33.79, 33.33, 32.08, 30.89, 27.09, 26.74 ppm. qH NMR (400 MHz, MeOD-d_4, Ethyl 4-(dimethylamino)benzoate as reference): purity = 97.9%.

5-Chloro-*N*-[4-(diethylamino)cyclohex-1-yl]-1*H*-indole-6-carboxamide (5t) Preparation according to GP1, using 5-chloro-1*H*-indole-6-carboxylic acid (**3**, 108 mg, 550 µmol, 1.10 eq) and commercially available *N*,*N*-diethylcyclohexane-1,4-diamine (**4t**, mixture of diastereomers, 85.2 mg, 500 µmol, 1.00 eq) yielded amide **5t** as a colorless solid (mixture of diastereomers: 6:4 *trans/cis*, 34 mg, 20%). R_f (EtOAc/EtOH = 1:1 + 2% TEA) = 0.51. MS (+APCI): *m*/*z* 347.9 ([M+H]$^+$). HRMS (+ESI): *m*/*z* calculated 348.18372 for [$C_{19}H_{27}ClN_3O$]$^+$, found 348.18419 ([M+H]$^+$). ^{1}H NMR (500 MHz, MeOD-d_4): δ = 8.55 (s, 1H), 7.63 (s, 0.6·1H), 7.61 (s, 0.4·1H), 7.53 (s, 0.6·1H), 7.48 (s, 0.4·1H), 7.41–7.37 (m, 1H), 4.24 (quint, *J* = 3.3 Hz, 0.6·1H), 3.89 (tt, *J* = 11.8, 4.1 Hz, 0.4·1H), 3.27–2.97 (m, 5H), 2.26–2.04 (m, 3H), 1.90-1.81 (m, 4H), 1.56–1.41 (m, 1H), 1.38–1.25 (m, 6H) ppm. ^{13}C NMR (126 MHz, MeOD-d_4): δ = 171.23, 170.94, 135.52, 135.43, 131.44, 131.42, 130.24, 130.09, 129.23, 129.18, 122.20, 121.93, 121.84, 112.95, 112.71, 102.27, 62.09, 61.71, 46.22, 46.11, 45.93, 31.61, 29.45, 26.76, 23.00, 22.87, 11.04, 11.00 ppm. qH NMR (400 MHz, MeOD-d_4, Ethyl 4-(dimethylamino)benzoate as reference): purity = 97.9%.

5-Chloro-*N*-[(1-butylpyrrolidin-3-yl)methyl]-1*H*-indole-6-carboxamide (5v) Preparation according to GP1, using 5-chloro-1*H*-indole-6-carboxylic acid (**3**, 94.7 mg, 484 µmol, 1.10 eq) and commercially available (1-butylpyrrolidin-3-yl)methanamine (**4v**, mixture of enantiomers, 68.8 mg, 440 µmol, 1.00 eq) yielded amide **5v** as a yellow oil (11 mg, 8%). R_f (EtOAc/EtOH = 1:1 + 2% TEA) = 0.48. MS (+APCI): *m*/*z* 333.7 ([M+H]$^+$). HRMS (+ESI): *m*/*z* calculated 334.16807 for [$C_{18}H_{25}ClN_3O$]$^+$, found 334.16848 ([M+H]$^+$). ^{1}H NMR (500 MHz, MeOD-d_4): δ = 8.50 (s, 1H), 7.63 (s, 1H), 7.53 (s, 1H), 7.40 (d, *J* = 3.1 Hz, 1H), 6.47 (d, *J* = 2.9 Hz, 1H), 3.69–3.37 (m, 5H), 3.27–3.08 (m, 3H), 2.89–2.70 (m, 1H), 2.36–2.18 (m, 1H), 2.01–1.85 (m, 1H), 1.77–1.63 (m, 2H), 1.49–1.36 (m, 2H), 0.99 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, MeOD-d_4): δ = 171.94, 135.43, 131.54, 129.72, 129.39, 122.04, 122.02, 112.83, 102.30, 57.92, 56.39, 54.77, 42.74, 38.84, 28.95, 28.42, 20.88, 13.89 ppm. qH NMR (400 MHz, MEOD-d_4, Ethyl 4-(dimethylamino)benzoate as reference): purity = 95.4%.

6-Chloro-*N*-[5-chloro-2-{2-(dimethylamino)ethoxy]phenyl}-1*H*-indole-5-carboxamide (10) Preparation according to GP1, using 6-chloro-1*H*-indole-5-carboxylic acid (**3a**, 108 mg, 550 µmol, 1.10 eq) and commercially available 5-chloro-2-[2-(dimethylamino)ethoxy]aniline (**4o**, 107 mg, 500 µmol, 1.00 eq) yielded amide **3a** as a colorless solid (9 mg, 5%). R_f (*iso*-hexane/EtOAc = 1:1) = 0.36. MS (+APCI): *m*/*z* 391.8 ([M+H]$^+$). HRMS (+ESI): *m*/*z* calculated 392.09271 for [$C_{19}H_{20}Cl_2N_3O_2$]$^+$, found 392.09335 ([M+H]$^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.62 (s, 1H), 10.45 (s, 1H), 8.66 (d, *J* = 2.6 Hz, 1H), 7.95 (s, 1H), 7.63–7.58 (m, 1H), 7.52–7.46 (m, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 7.08 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.66–6.60 (m, 1H), 4.20–4.13 (m, 2H), 2.51–2.43 (m, 2H), 1.88 (s, 6H) ppm. ^{13}C NMR (101 MHz, acetone-d_6): δ = 166.92, 147.88, 138.03, 134.04, 128.87, 128.33, 128.14, 127.53, 124.60, 124.03, 122.56, 120.74, 119.93, 113.38, 103.28, 70.84, 58.37, 44.92 ppm. qH NMR (400 MHz, DMSO-d_6, Ethyl 4-(dimethylamino)benzoate as reference): purity = 95.5%.

5-Chloro-*N*-{5-chloro-2-[2-(dimethylamino)ethoxy]phenyl}-1-methyl-1*H*-indole-6-carboxamide (11) 5-Chloro-1-methyl-1*H*-indole-6-carboxylic acid (**3b**, 14 mg, 67 µmol, 1.00 eq.), 5-chloro-2-[2-(dimethylamino)ethoxy]aniline (**4o**, 19 mg, 87 µmol, 1.3 eq.), 1-

methylimidazole (18.6 µL, 234 µmol, 3.5 eq.) and chloro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate (22.5 mg, 80 µmol, 1.2 eq.) were dissolved in DMF (1 mL) and the reaction mixture was stirred at 80 °C overnight. Then the solution was diluted with 2N aqueous NaOH and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and evaporated. The resulting residue was purified by CC and yielded compound **11** as a colorless solid (7 mg, 26%). R_f (*iso*-hexane/EtOAc = 8:2 + 2% TEA) = 0.3. MS (+APCI): *m*/*z* found 405.6 ($[M+H]^+$). HRMS (+ESI): *m*/*z* calculated 406.10836 for $[C_{20}H_{22}Cl_2N_3O_2]^+$, found 406.10908 ($[M+H]^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.09 (s, 1H), 8.74–8.69 (m, 1H), 7.84 (s, 1H), 7.67 (s, 1H), 7.19 (d, *J* = 3.1 Hz, 1H), 7.03 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 6.49–6.43 (m, 1H), 4.11 (t, *J* = 5.5 Hz, 2H), 3.83 (s, 3H), 2.63–2.54 (m, 2H), 2.05 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.79, 146.40, 134.94, 132.47, 131.35, 130.81, 128.29, 127.85, 123.53, 121.85, 121.61, 120.64, 116.11, 112.00, 100.74, 69.08, 57.88, 45.10, 33.21 ppm. qH NMR (400 MHz, $CDCl_3$, Ethyl-4-(dimethylamino)benzoate as reference): purity = 95.9%.

5-Chloro-*N*-[(5-chloro-1*H*-indol-6-yl)methyl]-2-[2-(dimethylamino)ethoxy]aniline (12) 5-Chloro-1*H*-indole-6-carbaldehyde (**15**, 0.045 g, 1.00 eq.) and 5-chloro-2-[2-(dimethylamino)ethoxy]aniline (**4o**, 59 mg, 276 µmol, 1.1 eq.) was dissolved in a mixture of DCM (3mL) and DCE (1 mL). Acetic acid (28 µL, 502 µmol, 2.00 eq.) was added to the solution and the reaction mixture was stirred for 2 h at rt. Then sodium triacetoxyborohydride (90 mg, 425 µmol, 1.69 eq) was added and the solution was stirred for further 2 h at rt. The reaction was quenched with saturated $NaHCO_3$ solution and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and evaporated. The resulting residue was purified by reverse CC and yielded compound **12** as a beige solid. (34 mg, 36%). R_f (DCM/MeOH = 95:5) = 0.39. MS (+APCI): *m*/*z* 377.4 ($[M+H]^+$). HRMS (+ESI): *m*/*z* calculated 378.11344 for $[C_{19}H_{22}Cl_2N_3O]^+$, found 378.11344 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.33 (s, 1H), 7.65 (s, 1H), 7.48 (s, 1H), 7.40–7.33 (m, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.52 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.50–6.42 (m, 2H), 5.84 (s, 1H), 4.53 (d, *J* = 5.8 Hz, 2H), 4.10 (t, *J* = 5.7 Hz, 2H), 2.66 (t, *J* = 5.7 Hz, 2H), 2.23 (s, 6H).ppm. ^{13}C{^{1}H}-NMR (101 MHz, acetone-d_6) δ = 144.96, 140.56, 135.25, 128.73, 128.23, 126.60, 126.42, 123.88, 120.42, 114.92, 113.71, 111.27, 109.50, 101.04, 68.06, 58.25, 45.32, 45.15 ppm. qH NMR (400 MHz, acetone-d_6, Ethyl 4-(dimethylamino)benzoate as reference): purity = 96.9%.

7-Chloro-*N*-{5-chloro-2-[2-(dimethylamino)ethoxy]phenyl}quinolin-4-amine (13) 4-Bromo-7-chloro-chloroquinoline (**16**, 170 mg, 699 µmol, 3.00 eq.), $Pd(OAc)_2$ (5.3 mg, 23 µmol, 0.1 eq.), (+/-)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene (29 mg, 47 µmol, 0.2 eq.), K_3PO_4 (82.6 mg, 389 µmol,1.67 eq.) and 5-Chloro-2-[2-(dimethylamino)ethoxy]aniline (**5o**, 50.0 mg, 233 µmol, 1.00 eq.) were dissolved in 1,4-dioxane (10 mL). The suspension was stirred at 90 °C for 24 h. After completion of the reaction the mixture was filtered through Celite and purified by CC yielded compound **13** as a yellow solid (25 mg, 29%). R_f (DCM/MeOH = 95:5) = 0.13. MS (+APCI): *m*/*z* 375.7 ($[M+H]^+$). HRMS (+ESI): *m*/*z* calculated 376.09779 for $[C_{19}H_{20}Cl_2N_3O]^+$, found 376.09885 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6) δ = 8.57 (d, *J* = 5.2 Hz, 1H), 8.54 (s, 1H), 8.27 (d, *J* = 9.0 Hz, 1H), 7.95 (d, *J* = 2.2 Hz, 1H), 7.55 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.49 (d, *J* = 2.5 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 1H), 7.13 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.01 (d, *J* = 5.2 Hz, 1H), 4.22 (t, *J* = 5.3 Hz, 2H), 2.57 (t, *J* = 5.4 Hz, 2H), 2.23 (s, 6H) ppm. ^{13}C NMR (101 MHz, acetone-d_6) δ = 152.13, 150.06, 149.79, 147.27, 134.39, 132.89, 128.44, 126.28, 125.43, 124.07, 123.28, 121.72, 119.01, 117.67, 102.99, 68.52, 57.84, 44.8 ppm. qH NMR (400 MHz, acetone-d_6, Ethyl-4-(dimethylamino)benzoate as reference): purity = 95.2%.

Synthesis and analytical characterization of intermediates

5-Chloro-1*H*-indole-6-carboxylic acid (3) Methyl 5-chloro-1*H*-indole-6-carboxylate (**9**, 2.80 g, 13.4 mmol, 1.00 eq.) was dissolved in EtOH (25 mL) and water (25 mL). $LiOH \cdot H_2O$ (1.69 g, 40.2 mmol, 3.00 eq.) was added and the reaction mixture was stirred at rt for 18 h. After removal of the solvent, the resulting solid was dissolved in water. The alkaline solution (pH > 11) was extracted with EtOAc. The aqueous layer was acidified with aqueous hydrochloric acid (10%) and extracted with EtOAc. The latter organic layers were combined, washed with brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the residue was recrystallized from a mixture of DCM/diisopropylether (1:1) yielded compound **3** as a beige solid (2.47 g, 94 %). R_f (*iso*-hexane/EtOAc = 8:2 + 2% AcOH) = 0.22. MS (+APCI): *m*/*z* 195.7 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 11.15 (s, 1H), 10.69 (s, 1H), 8.13 (s, 1H), 7.70 (s, 1H), 7.62–7.58 (m, 1H), 6.56–6.53 (m, 1H) ppm. ^{13}C NMR (126 MHz, acetone-d_6): δ = 168.58, 134.20, 131.45, 129.74, 123.62, 122.39, 121.82, 115.47, 101.46 ppm.

5-Chloro-1-methyl-1*H*-indole-6-carboxylic acid (3b) Methyl 5-chloro-1-methyl-1*H*-indole-6-carboxylate (**9a**, 30.3 mg, 135 µmol, 1.00 eq.) was dissolved in 2 mL of a mixture of EtOH/water (1:1). Lithium hydroxide (9.73 mg, 406 µmol, 3.00 eq) was added and the mixture was stirred at rt overnight. The solvent was removed and water was added. The alkaline solution (pH > 11) was extracted with EtOAc. The aqueous layer was acidified with aqueous hydrochloric acid (10%) and extracted with EtOAc. The latter organic layers were combined, dried over $MgSO_4$ filtered and concentrated under reduced pressure giving compound **3b** as a colorless solid (28 mg, 98.6%). R_f (i-hexane/EtOAc = 8:2 + 2% AcOH) = 0.25. MS (+APCI): *m*/*z* 209.7 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 8.04 (s, 1H), 7.68 (s, 1H), 7.49 (d, *J* = 3.0 Hz, 1H), 6.49 (dd, *J* = 3.0, 0.8 Hz, 1H), 3.93 (s, 3H) ppm. ^{13}C NMR (101 MHz, acetone-d_6): δ = 166.58, 134.59, 133.86, 131.72, 123.57, 122.36, 122.02, 113.52, 100.34, 32.37 ppm.

2-Chloro-4-methyl-5-nitrobenzoic acid (7)[3] 2-Chloro-4-methylbenzoic acid (**6**, 10.0 g, 55.8 mmol, 1.00 eq.) was suspended in sulfuric acid (100 mL) and cooled to 5 °C. Over a period of 40 min nitric acid (3.39 mL, 52.7 mmol, 0.90 eq.) was added dropwise so that the reaction mixture did not exceed a temperature of 15 °C. After complete addition of nitric acid the reaction mixture was stirred at rt for 30 min and then poured into ice water. The solid that subsequently precipitated was filtered off, washed with cold water and dissolved in EtOH. Water was added dropwise to the solution, whereupon a colorless solid precipitated. The solid was filtered off, washed with cold water and dried under reduced pressure yielded compound **7** as a colorless solid (7.57 g, 59%). R_f (*iso*-hexane/EtOAc = 8:2 + 2% AcOH) = 0.33. MS (-APCI): *m*/*z* 214.5 ($[M-H]^-$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 8.54 (s, 1H), 7.72 (s, 1H), 2.65 (s, 3H) ppm. ^{13}C NMR (101 MHz, acetone-d_6): δ = 164.70, 148.50, 139.43, 138.57, 136.14, 129.76, 128.72, 20.03 ppm.

Methyl 2-chloro-4-methyl-5-nitrobenzoate (8)[3] Acetyl chloride (12.4 mL, 174 mmol, 5.00 eq.) was added dropwise to methanol (70 mL) at 5-10 °C. A solution of 2-chloro-4-methylnitrobenzoic acid (**7**, 7.50 g, 34.8 mmol, 1.00 eq.) in methanol (100 mL) was added in portions and the reaction mixture was stirred at 50 °C for 4 h. After cooling to rt the solvent was removed under reduced pressure. The yellow solid obtained was dissolved in DCM and washed with brine solution and water. The organic phase was dried over $MgSO_4$, filtered and evaporated to yield **8** as a colorless solid (7.66 g, 96 %). R_f (*iso*-hexane/EtOAc = 8:2) = 0.41. MS (+APCI): *m*/*z* 229.5 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 8.48 (s, 1H), 7.73 (s, 1Hl), 3.95 (s, 3H), 2.65 (s, 3H) ppm. ^{13}C NMR (101 MHz, acetone-d_6): δ = 167.44, 148.34, 139.57, 138.30, 136.08, 129.51, 128.51, 53.18, 20.05 ppm.

Methyl 5-chloro-1*H*-indole-6-carboxylate (9)[3] Methyl 2-chloro-4-methyl-5-nitrobenzoate (**8**, 7.66 g, 33.4 mmol, 1.00 eq.) was dissolved in DMF (50 mL). *N,N*-dimethylformamide dimethylacetal (10.4 mL, 49.9 mmol, 1.50 eq.) was added to the solution and the reaction

mixture was stirred for 2 h at 130 °C. The reaction mixture was concentrated under vacuum, dissolved in EtOAc, washed with brine and water. The aqueous phases were extracted with EtOAc and the combined organic phases were dried over $MgSO_4$. After removing the solvent, the obtained purple solid was dissolved in acetic acid (100 mL) and water (20 mL). Over a period of 40 min, zinc powder (13.06 g, 65.34mmol, 6.0 eq.) was added in portions and the reaction mixture was heated to 80 °C for 2 h. After cooling to rt, the mixture was diluted with EtOAc and filtered. The organic phase was separated and washed with saturated $NaHCO_3$ solution and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The brown oil was purified by CC and yielded compound **9** as a green solid (2.80 g, 40 %). R_f (*iso*-hexane/EtOAc = 8:2) = 0.23. MS (+APCI): *m*/*z* 209.7 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.69 (s, 1H), 8.03 (s, 1H), 7.71 (s, 1H), 7.60 (t, *J* = 2.8 Hz, 1H), 6.54 (m, 1H), 3.87 (s, 3H) ppm. ^{13}C NMR (126 MHz, acetone-d_6): δ = 167.20, 134.86, 132.86, 130.68, 124.13, 123.34, 122.65, 116.02, 102.38, 52.26 ppm.

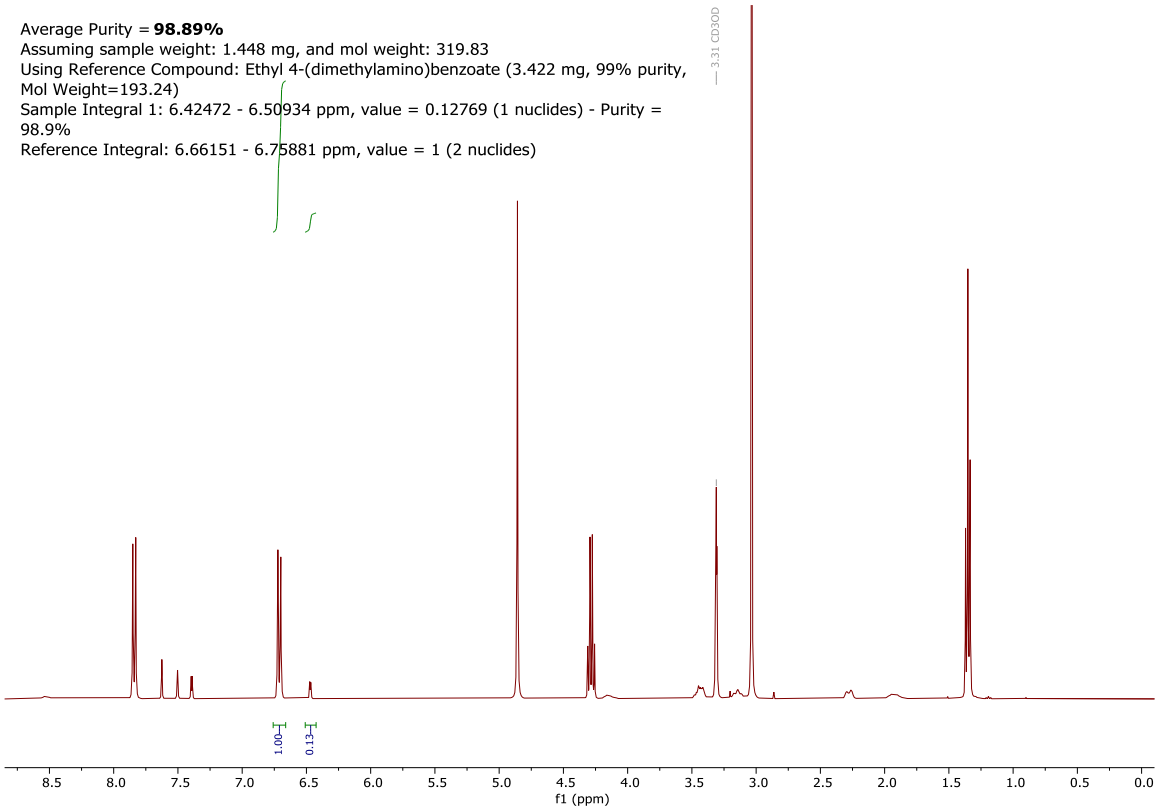
Methyl 5-chloro-1-methyl-1*H*-indole-6-carboxylate (9a) Sodium hydride (19.6 mg, 490 µmol,1.20 eq.) was added portionwise to methyl 5-chloro-1*H*-indole-6-carboxylate (**9**, 85.6 mg, 0.408 mmol, 1.00 eq.) in DMF (1.5 mL) cooled to 0 °C over a period of 10 min. The resulting solution was stirred at 0 °C for further 10 min. Methyl iodide (30 µL, 49 µmol, 1.20 eq.) was added and the solution was stirred at rt for 2 h. The reaction mixture was quenched with saturated NH_4Cl solution and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by CC to yield compound **9a** as a colorless solid (30 mg, 33%). R_f (*iso*-hexane/EtOAc = 8:2) = 0.3. MS (+APCI): *m*/*z* 223.6 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 7.96 (s, 1H), 7.68 (s, 1H), 7.49 (d, *J* = 3.1 Hz, 1H), 6.52–6.48 (m, 1H), 3.92 (s, 3H), 3.89 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 167.12, 134.47, 133.09, 131.73, 124.33, 122.65, 122.17, 113.29, 100.83, 52.23, 33.18 ppm.

5-Chloro-1*H*-indole-6-ylmethanol (14) Methyl 5-chloro-1H-indole-6-carboxylate (**3**, 0.5 g, 2.39 mmol, 1.00 eq.) was dissolved in THF (4 mL) under nitrogen. The solution was stirred at 0 °C and a solution of lithium aluminium hydride (596 µL, 2.39 mmol, 1.0 eq., 4 M in diethyl ether) was added dropwise. The reaction mixture was stirred for 1h and quenched with EtOAc and water. The aqueous layer was extracted with EtOAc and the combined organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by CC yielded compound **14** as a colorless solid (115 mg, 71%). R_f (*iso*-hexane/EtOAc = 2:1) = 0.2. MS (+APCI): *m*/*z* 181.6 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.35 (s, 1H), 7.72–7.65 (m, 1H), 7.57 (s, 1H), 7.40–7.31 (m, 1H), 6.48–6.41 (m, 1H), 4.81–4.73 (m, 2H), 4.25 (t, *J* = 5.7 Hz, 1H) ppm. ^{13}C NMR (101 MHz, acetone-d_6): δ = 135.25, 132.31, 127.92, 126.12, 123.00, 119.82, 110.65, 100.95, 61.77 ppm.

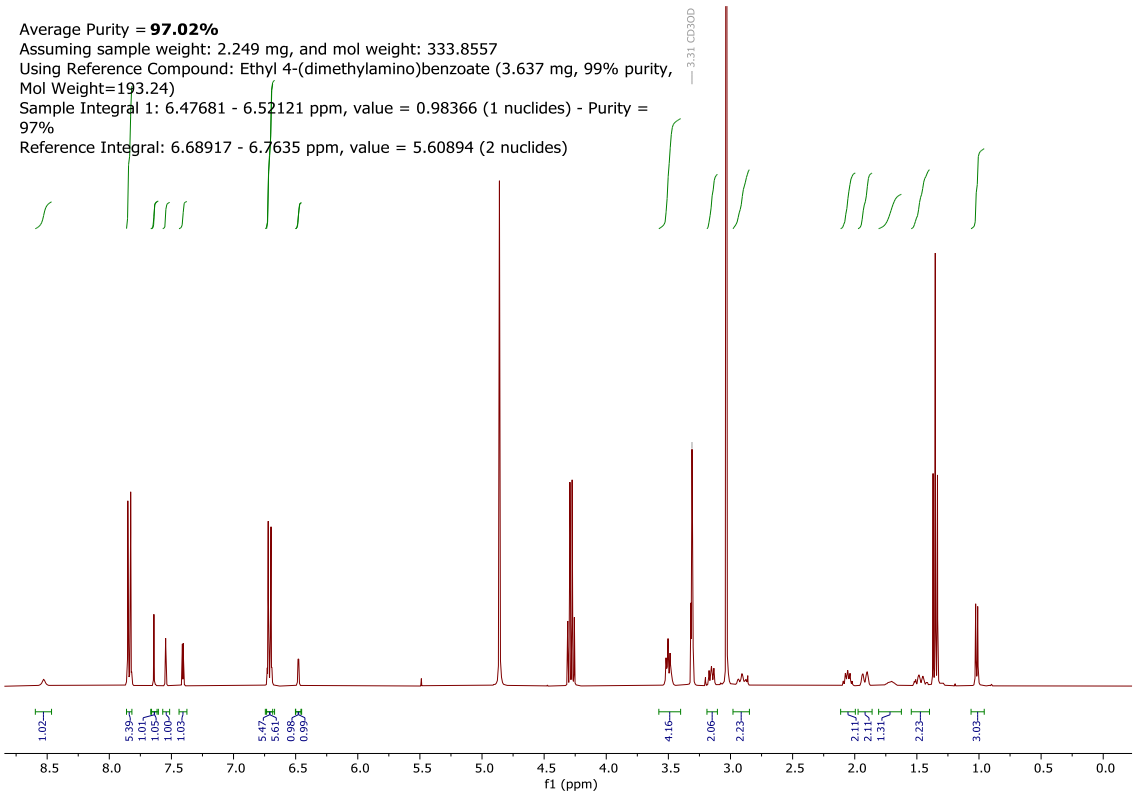
5-Chloro-1*H*-indole-6-carbaldehyde (15) 5-Chloro-1*H*-indole-6-ylmethanol (**14**, 0.091 g, 496 µmol, 1.00 eq.) was dissolved in a mixture of DCM (4 mL) and DMF (2mL) at 0 °C. Dess-Martin-Periodinan (0.231 g, 546 µmol, 1.10 eq.) was added to the solution and the reaction mixture was allowed to warm to rt and stirred for 1 h. A solution of 1 N NaOH was added and the biphasic mixture was filtered through Celite. The biphasic mixture was extracted with EtOAc and the organic phase was dried over $MgSO_4$. The organic solvent was removed under reduced pressure and the residue was purified by CC to yield compound **14** as a brown solid (mixture of tautomers, 89 mg, 100%). R_f (*iso*-hexane/EtOAc = 2:1) = 0.5. MS (+APCI): *m*/*z* 179.7 ($[M+H]^+$). ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.91 (s, 1H), 10.48 (s, 1H), 8.07 (s, 1H), 7.75–7.67 (m, 2H), 6.63–6.56 (m, 1H) ppm. ^{13}C NMR (101 MHz, acetone-d_6): δ = 190.14, 135.53, 135.38, 134.44, 134.40, 132.52, 132.35, 128.07, 126.82, 121.90, 113.88, 113.83, 102.87, 102.82 ppm.

qH NMR of **5e,m,o,r,t,v, 10-13** for purity analysis

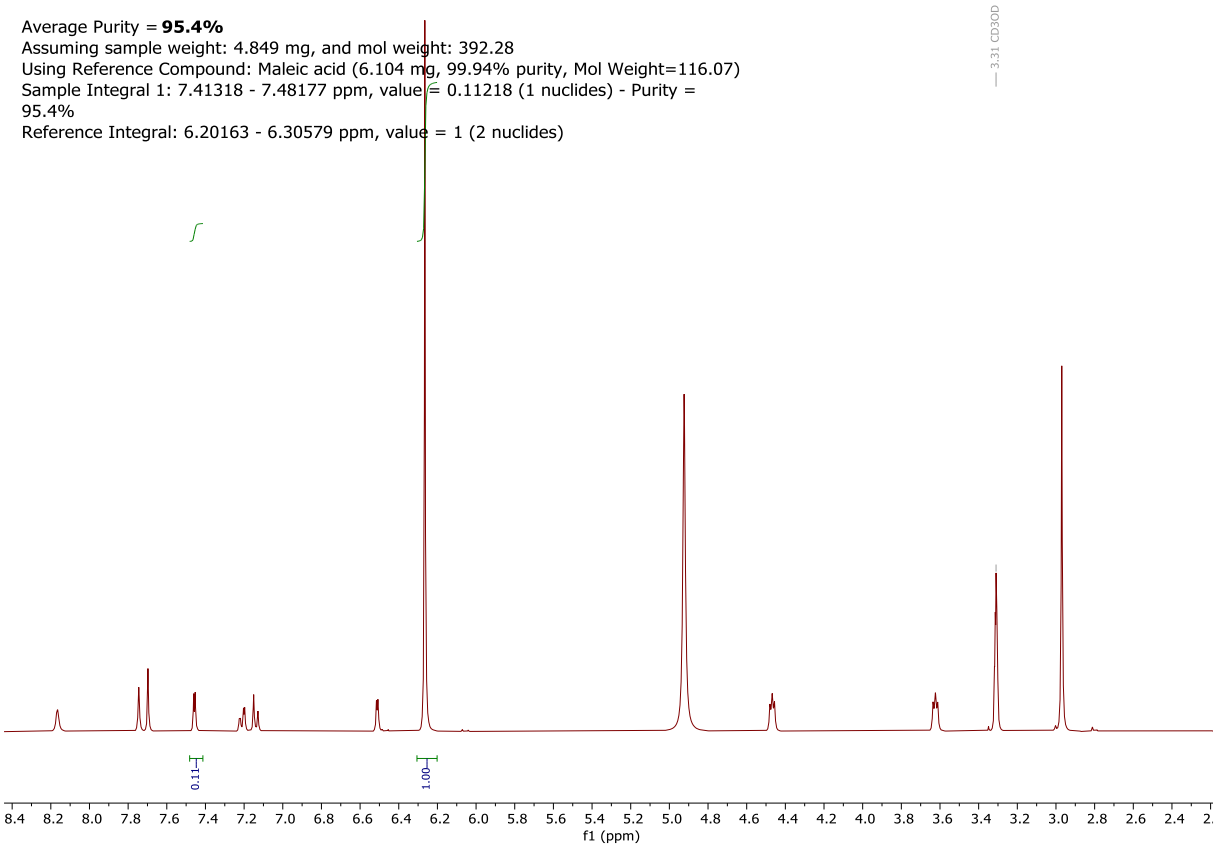
Compound **5e**:



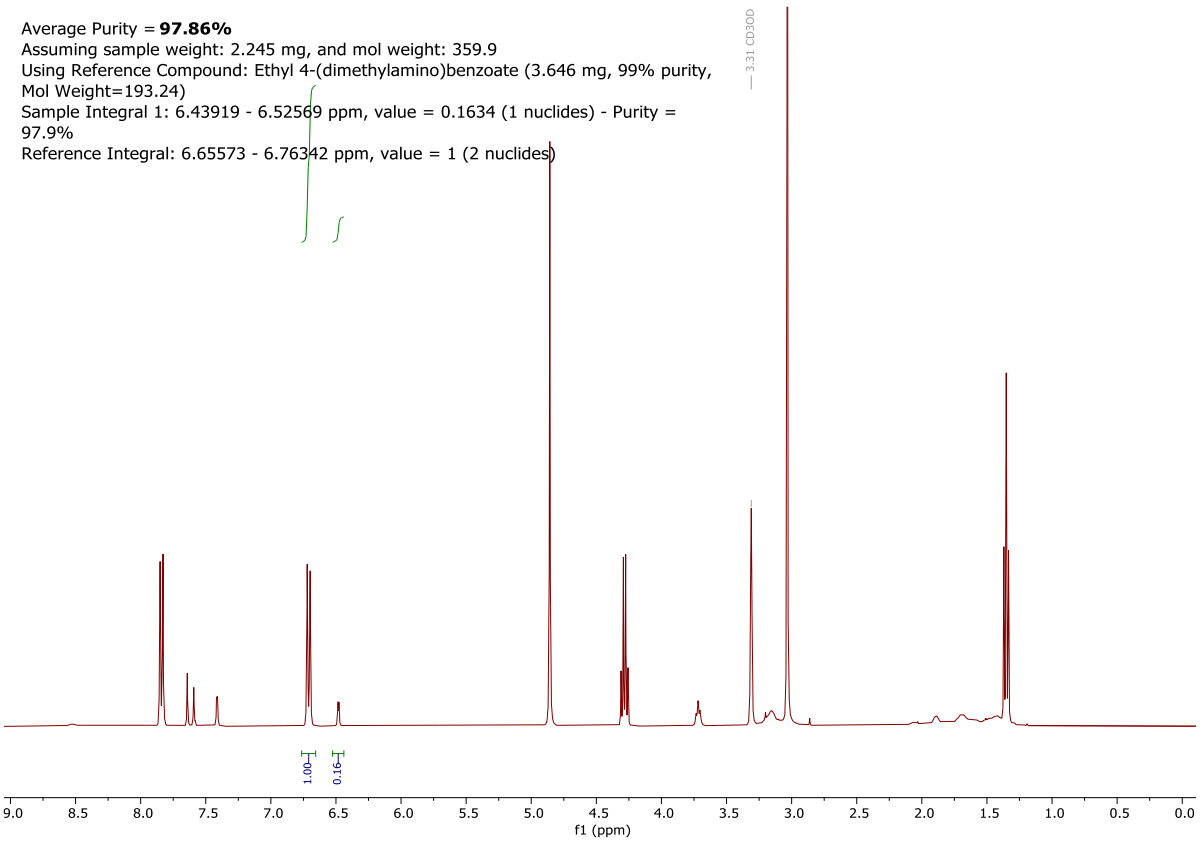
Compound **5m**:



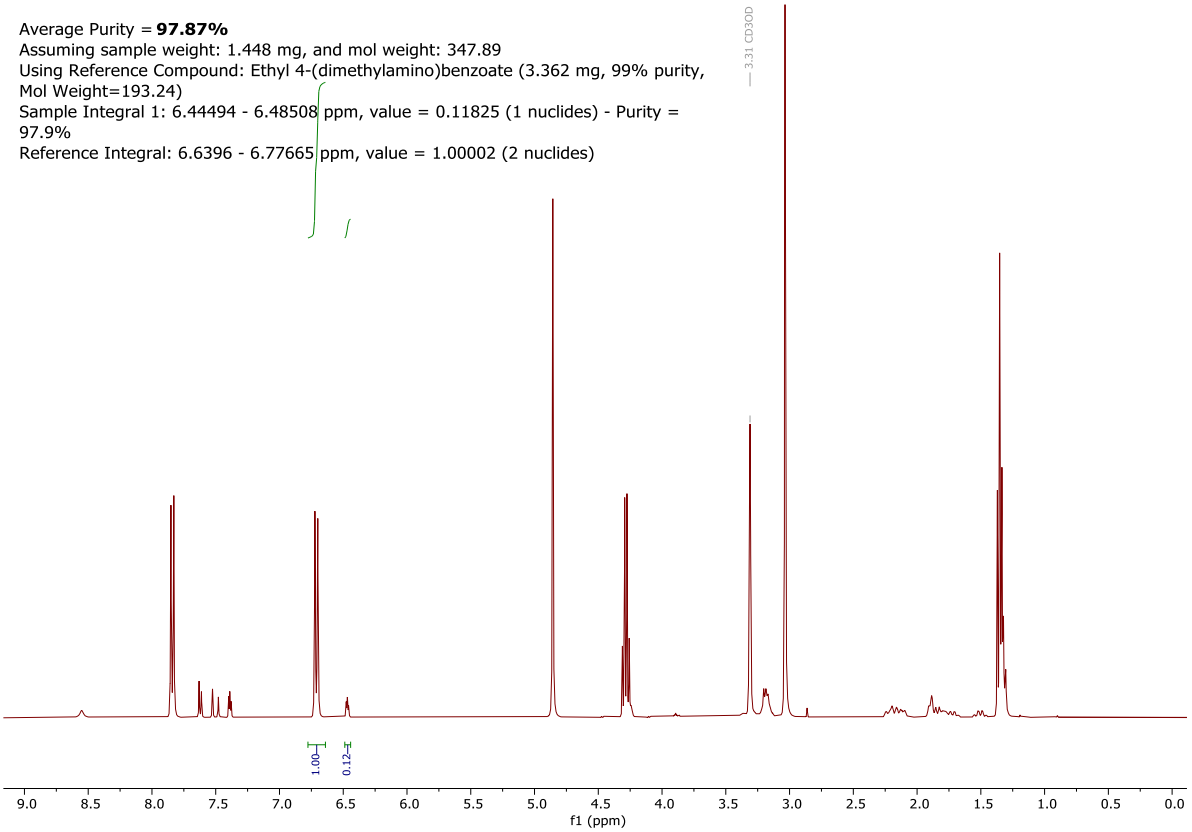
Compound **5o**:



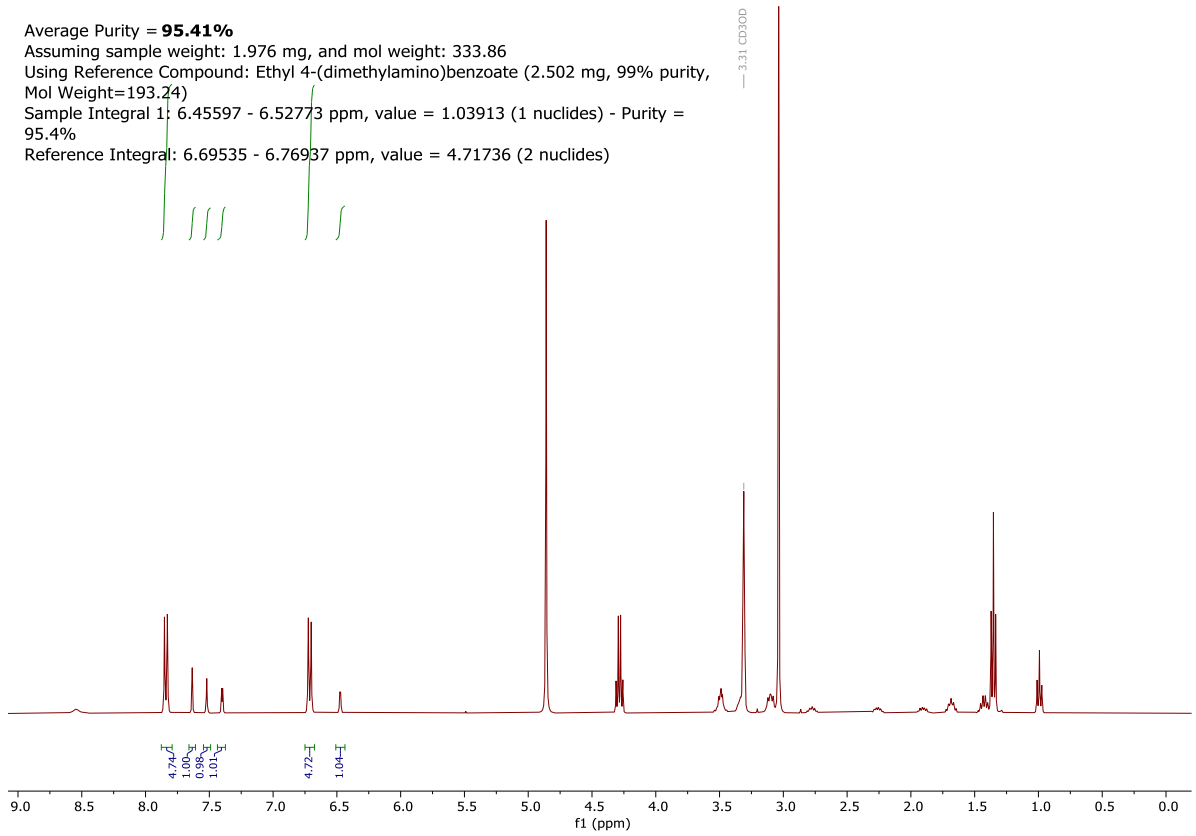
Compound **5r**:



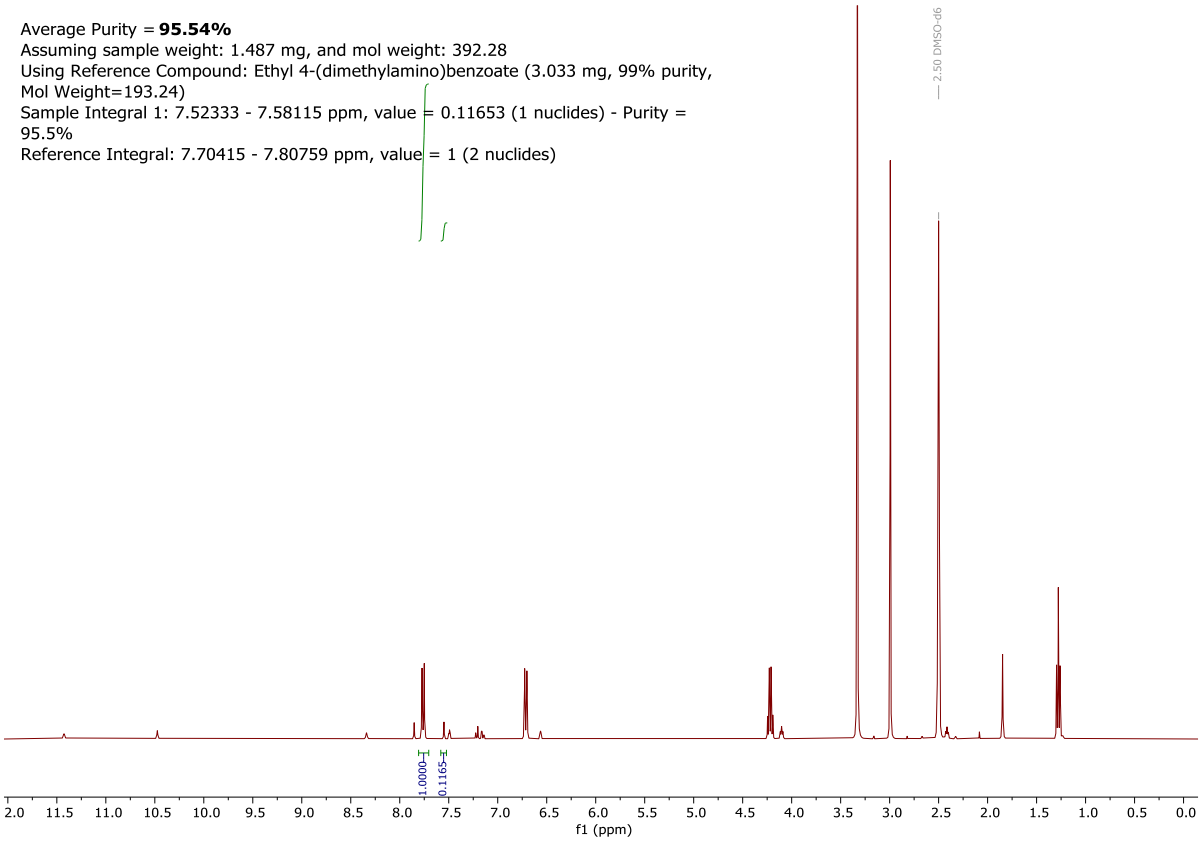
Compound **5t**:



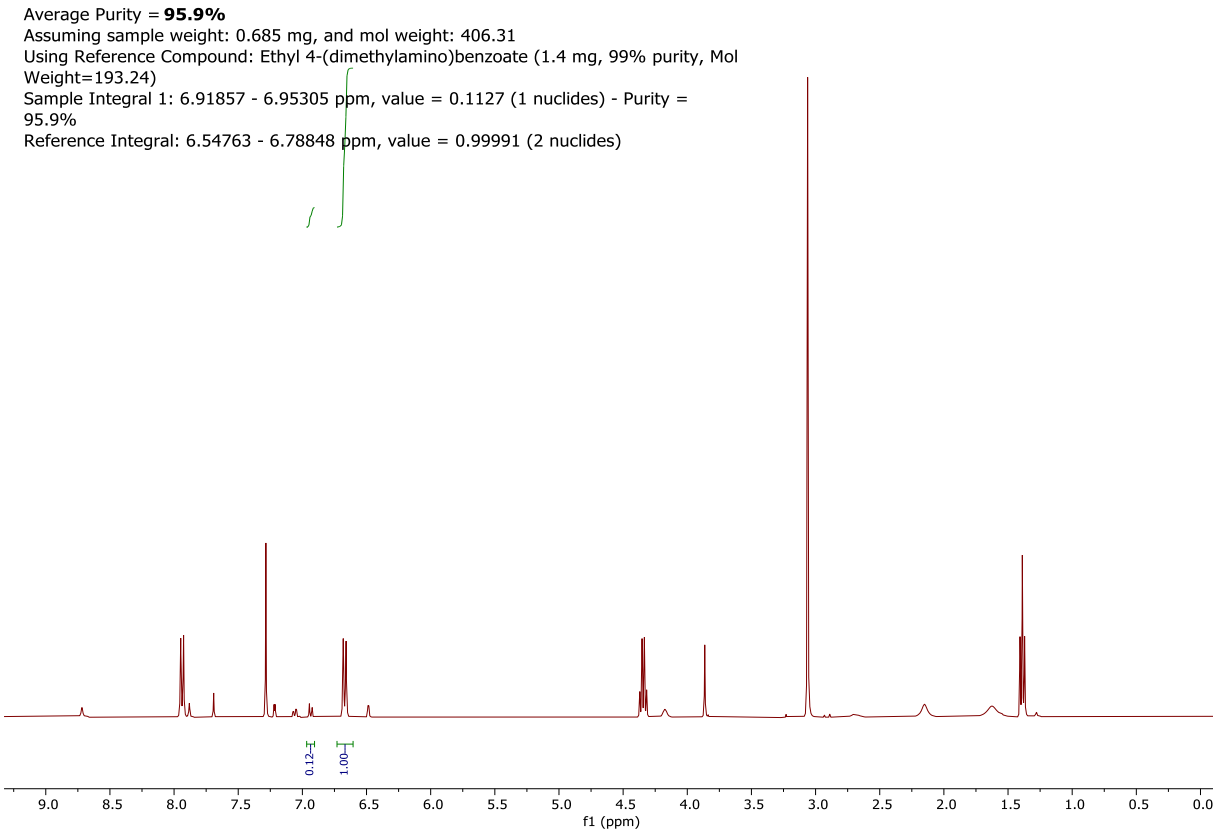
Compound **5v**:



Compound **10**:

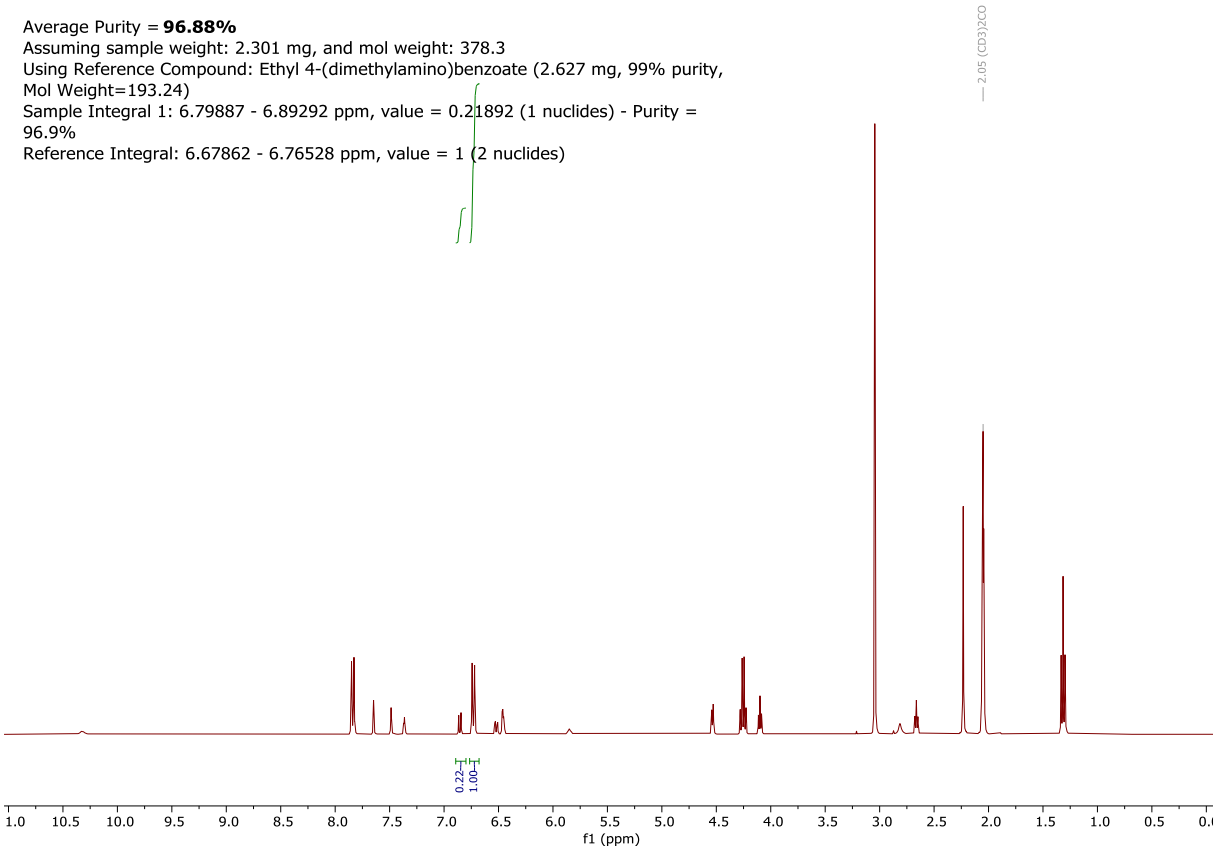


Compound **11**:



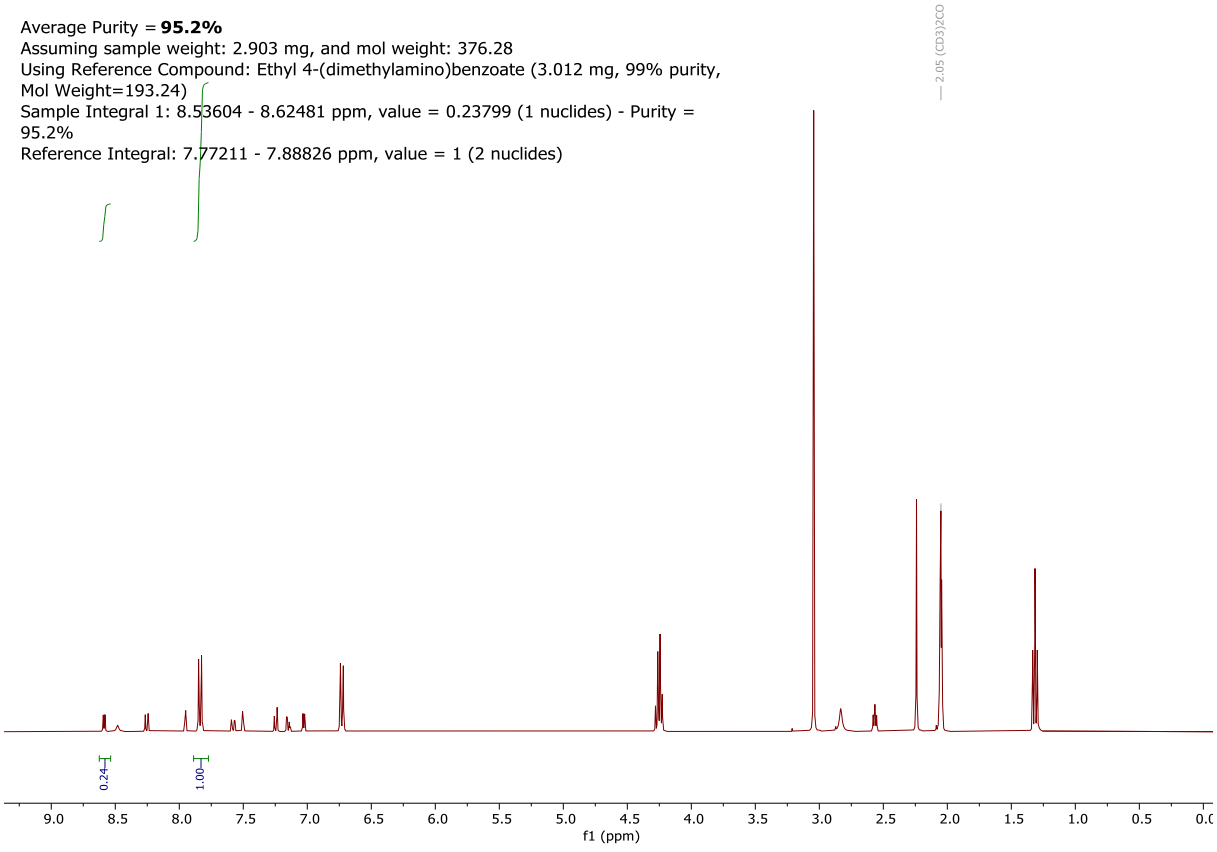
Compound **12**:

Average Purity = **96.88%**
Assuming sample weight: 2.301 mg, and mol weight: 378.3
Using Reference Compound: Ethyl 4-(dimethylamino)benzoate (2.627 mg, 99% purity, Mol Weight=193.24)
Sample Integral 1: 6.79887 - 6.89292 ppm, value = 0.21892 (1 nuclides) - Purity = 96.9%
Reference Integral: 6.67862 - 6.76528 ppm, value = 1 (2 nuclides)



Compound **13**:

Average Purity = **95.2%**
Assuming sample weight: 2.903 mg, and mol weight: 376.28
Using Reference Compound: Ethyl 4-(dimethylamino)benzoate (3.012 mg, 99% purity, Mol Weight=193.24)
Sample Integral 1: 8.53604 - 8.62481 ppm, value = 0.23799 (1 nuclides) - Purity = 95.2%
Reference Integral: 7.77211 - 7.88826 ppm, value = 1 (2 nuclides)



Supplementary References

(1) Bruning, J. M.; Wang, Y.; Oltrabella, F.; Tian, B.; Kholodar, S. A.; Liu, H.; Bhattacharya, P.; Guo, S.; Holton, J. M.; Fletterick, R. J.; Jacobson, M. P.; England, P. M. Covalent Modification and Regulation of the Nuclear Receptor Nurr1 by a Dopamine Metabolite. *Cell Chem. Biol.* **2019**, *26* (5), 674-685.e6.

(2) Pauli, G. F.; Chen, S. N.; Simmler, C.; Lankin, D. C.; Gödecke, T.; Jaki, B. U.; Friesen, J. B.; McAlpine, J. B.; Napolitano, J. G. Importance of Purity Evaluation and the Potential of Quantitative 1H NMR as a Purity Assay. *J. Med. Chem.* **2014**, *57* (22), 9220–9231.

(3) McCoull, W.; Barton, P.; Brown, A. J. H.; Bowker, S. S.; Cameron, J.; Clarke, D. S.; Davies, R. D. M.; Dossetter, A. G.; Ertan, A.; Fenwick, M.; Green, C.; Holmes, J. L.; Martin, N.; Masters, D.; Moore, J. E.; Newcombe, N. J.; Newton, C.; Pointon, H.; Robb, G. R. et al. Identification, Optimization, and Pharmacology of Acylurea GHS-R1a Inverse Agonists. *J. Med. Chem.* **2014**, *57* (14), 6128–6140.