Electronic Supplementary Information for:

"Structural features localizing the ferroptosis inhibitor GIF-2197-r to lysosomes**"**

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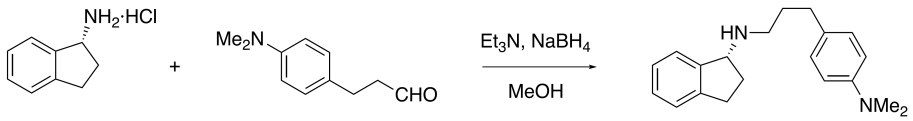
Syntheses of compounds

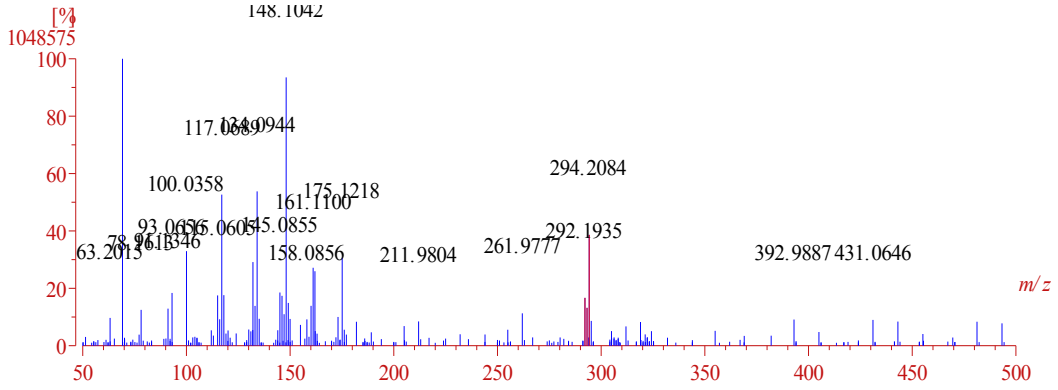


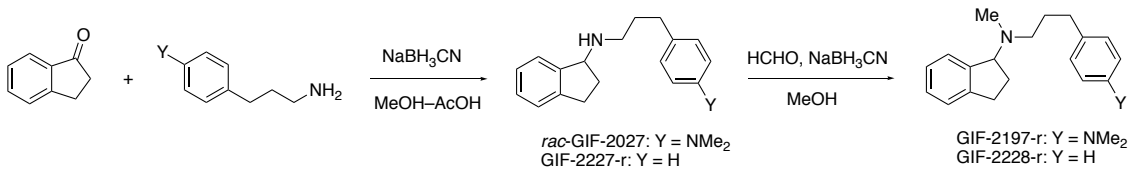
GIF-2027

GIF-2027: To a solution of 3-[(4-dimethylamino)phenyl]propanal (20 mg, 112 μmol) and (*R*)-1-aminoindane hydrochloride (20 mg, 118 μmol) in methanol (0.5 mL) was added triethylamine (16 μL, 115 μmol) at r.t., and the mixture was stirred for 2 h. Then, the reaction mixture was cooled to 0 °C, sodium borohydride (6.4 mg, 169 μmol) was added to the solution, and stirring was continued at 0 °C for 30 min. The reaction mixture was poured into water and the product was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate,

filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, AcOEt/Et_3N = 100/1) to give GIF-2027 as a pale-yellow oil (23 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.75–1.87 (complex, 3H, $ArCH_2C\underline{H_2}CH_2$ and $ArCH_2\underline{CH_2}CHN$), 2.32–2.42 (m, 1H, $ArCH_2\underline{CH_2}CHN$), 2.52–2.64 (m, 2H, $ArCH_2$), 2.7–3.02 (complex, 4H, $ArCH_2$ and NCH_2), 2.90 (s, 6H, $N(CH_3)_2$), 4.20 (t, J = 6.6 Hz, 1H, ArCHN), 6.69 (d, J = 8.6 Hz, 2H, ArH), 7.06 (d, J = 8.6 Hz, 2H, ArH), 7.15–7.26 (complex, 3H, ArH), 7.27–7.32 (m, 1H, ArH).

MS (EI, 70 eV) m/z 294.2084.

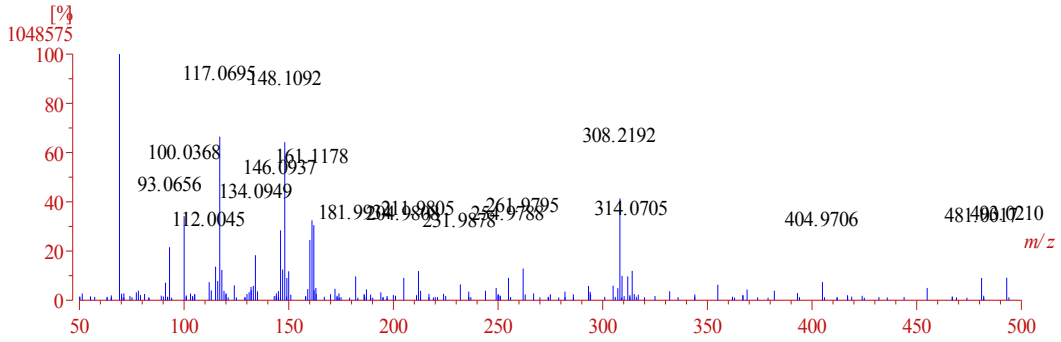




***N*-[3-(4-Dimethylamino)phenyl]propyl-2,3-dihydro-1*H*-inden-1-amine (*rac*-GIF-2027)**: To a solution of 4-(3-aminopropyl)-*N*,*N*-dimethylaniline (214 mg, 1.20 mmol) and 1-indanone (132 mg, 1.00 mmol) in methanol (5 mL) were added acetic acid (69 μL, 1.20 mmol) and sodium cyanoborohydride (126 mg, 2.00 mmol) at room temperature, and the mixture was heated under reflux for 24 h. The solution was poured into saturated sodium hydrogen carbonate solution and the product was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/Et_3N = 90/10/1) to give the title compound as a pale-yellow oil (230 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.75–1.90 (complex, 3H, $ArCH_2C\underline{H_2}CH_2$ and $ArCH_2\underline{CH_2}CHN$), 2.33–2.43 (m, 1H, $ArCH_2\underline{CH_2}CHN$), 2.54–2.67 (m, 2H, $ArCH_2$), 2.73–3.04 (complex, 4H, $ArCH_2$ and NCH_2), 2.91 (s, 6H, $N(CH_3)_2$), 4.23 (t, J = 6.6 Hz, 1H, ArCHN), 6.70 (d, J = 8.6 Hz, 2H, ArH), 7.08 (d, J = 8.6 Hz, 2H, ArH), 7.15–7.26 (complex,

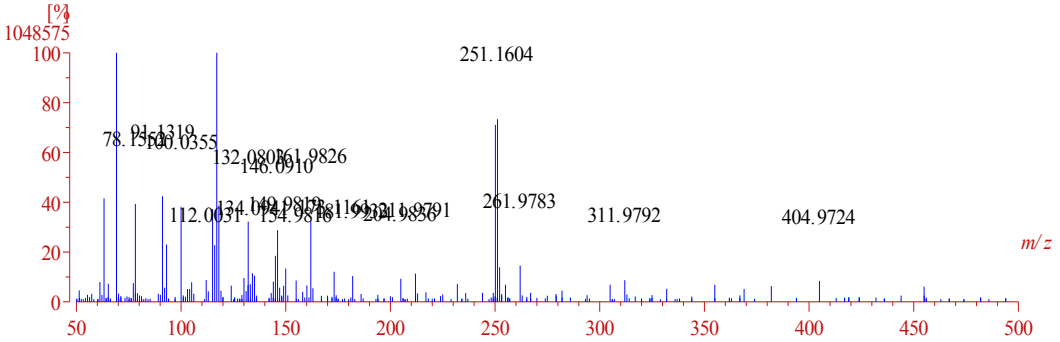
3H, ArH), 7.31–7.34 (m, 1H, ArH).

GIF-2197-r: A solution of *N*-[3-(4-dimethylamino)phenyl]propyl-2,3-dihydro-1*H*-inden-1-amine (127 mg, 0.431 mmol), paraformaldehyde (66 mg) and sodium cyanoborohydride (45 mg, 0.72 mmol) in methanol (1.5 mL) was stirred at r.t. for 15 h. The solution was poured into saturated sodium hydrogen carbonate solution and the product was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, ethyl acetate) to give GIF-2197-r as a pale-yellow oil (75 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72–1.85 (m, 2H, $ArCH_2C\underline{H_2}CH_2$), 1.96–2.08 (complex, 2H, $ArCH_2C\underline{H_2}CHN$), 2.17 (s, 3H, NCH_3), 2.39–2.63 (complex, 4H, $ArCH_2$ and NCH_2), 2.72–2.82 (m, 1H, $ArCH_2$), 2.85–2.94 (m, 1H, $ArCH_2$), 2.89 (s, 6H, $N(CH_3)_2$), 4.42 (t, *J* = 7.2 Hz, 1H, ArCHN), 6.68 (d, *J* = 8.8 Hz, 2H, ArH), 7.06 (d, *J* = 8.8 Hz, 2H, ArH), 7.16–7.20 (complex, 3H, ArH), 7.32–7.37 (br m, 1H, ArH).
MS (EI, 70 eV) m/z 308.2192.



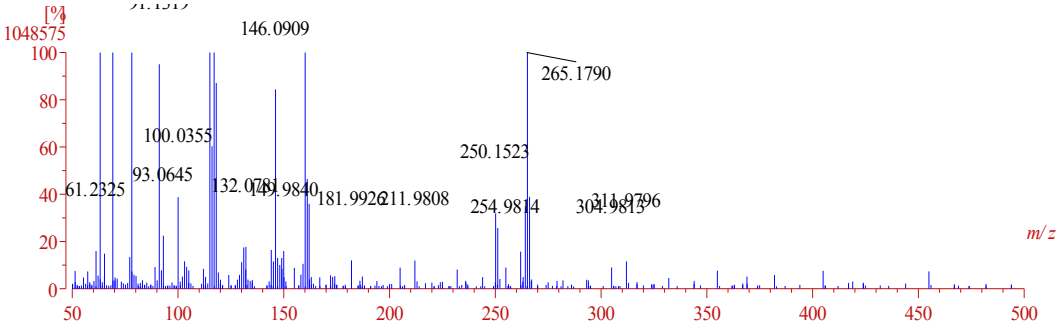
GIF-2227-r and GIF-2228-r were individually synthesized in a similar manner using 3-phenylpropan-1-amine in place of 4-(3-aminopropyl)-*N*,*N*-dimethylaniline.

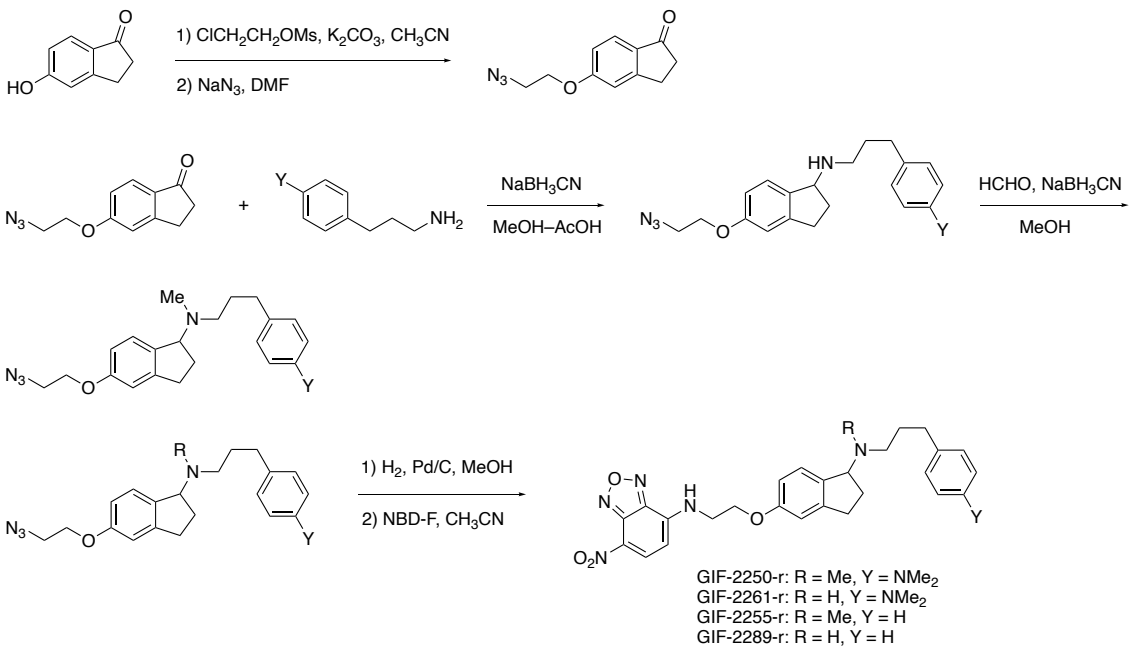
GIF-2227-r: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.76–1.92 (complex, 3H, $ArCH_2C\underline{H_2}CH_2$ and $ArCH_2\underline{CH_2}CHN$), 2.33–2.43 (m, 1H, $ArCH_2\underline{CH_2}CHN$), 2.63–2.84 (complex, 5H, $ArCH_2$ and NCH_2), 2.953–3.03 (m, 1H, $ArCH_2$), 4.22 (t, *J* = 6.6 Hz, 1H, ArCHN), 7.15–7.33 (complex, 9H, ArH).
MS (EI, 70 eV) m/z 251.1604



GIF-2228-r: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.76–1.88 (m, 2H, $ArCH_2C\underline{H_2}CH_2$), 1.95–2.08 (complex, 2H, $ArCH_2C\underline{H_2}CHN$), 2.18 (s, 3H, NCH_3), 2.39–2.5 (complex, 2H, $ArCH_2$), 2.55–2.72 (m, 2H, NCH_2), 2.74–2.94 (m, 2H, $ArCH_2$), 4.42 (t, J = 7.4 Hz, 1H, ArCHN), 7.14–7.21 (complex, 6H, ArH), 7.23–7.28 (complex, 2H, ArH), 7.32–7.36 (broad m, 1H, ArH).

MS (EI, 70 eV) m/z 265.1790





5-(2-Azidoethoxy)indan-1-one: A mixture of 5-hydroxy-1-indanone (1.48 g, 10 mmol), potassium carbonate (1.66 g, 12 mmol) and 2-chloroethyl mesylate (2.38 g, 15 mmol) in acetonitrile (30 mL) was heated under reflux for 22 h. Then, the mixture was poured into water and the product was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford an intermediate chloroethyl ether. This crude intermediate was dissolved in DMF (30 mL). To this solution was added sodium azide (1.95 g, 30 mmol) and the mixture was heated at 80 °C for 4 h. The reaction mixture was poured into water and the product was extracted with ethyl acetate/hexane (1/1). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, ethyl acetate/hexane = 1/1) to give the desired compound as a pale-yellow solid (1.85 g, 85% in 2 steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.66–2.7 (m, 2H, $COCH_2$), 3.09 (br t, J = 6 Hz, 2H, $ArCH_2$), 3.63 (t, J = 5 Hz, 2H, N_3CH_2), 4.21 (t, J = 5 Hz, 2H, OCH_2), 6.90–6.94 (br m, 2H, ArH), 7.70 (d, J = 9.2 Hz, 1H, ArH).

5-(2-Azidoethoxy)-*N*-{3-[4-(dimethylmino)phenyl]propyl}-2,3-dihydro-1*H*-inden-1-amine: To a solution of 4-(3-aminopropyl)-*N*,*N*-dimethylaniline (178 mg, 1.00 mmol) and 5-(2-azidoethoxy)indan-1-one (109 mg, 0.50 mmol) in methanol (2 mL) were added acetic acid (57 μL, 1.0 mmol) and sodium cyanoborohydride (63 mg, 1.0 mmol) at room temperature, and the mixture was heated under reflux for 24 h. The solution was poured into saturated sodium hydrogen carbonate solution and the product was extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1) to give the desired product as a pale-yellow oil (110 mg, 58%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.75–1.86 (complex, 3H, $ArCH_2C\underline{H}_2$), 2.35 (m, 1H, $ArCH_2C\underline{H}_2$), 2.57 (dt, J = 3.2 and 8.0 Hz, 2H, $ArCH_2$), 2.71 (t, J = 7.1 Hz, 2H, $ArCH_2$), 2.7–2.8 (m, 1H), 2.90 (s, 6H, $N(CH_3)_2$), 2.90–2.99 (m, 1H), 3.56 (t, J = 5 Hz, 2H, N_3CH_2), 4.12 (t, J = 5 Hz, 2H, OCH_2), 4.15 (t, J = 6.4 Hz, 1H, $ArC\underline{H}NH$), 6.68 (d, J = 8.4 Hz, 2H, ArH), 6.74 (d, J = 8.2 Hz, 1H, ArH), 6.77 (s, 1H, ArH), 7.05 (d, J = 8.4 Hz, 2H, ArH), 7.20 (d, J = 8.2 Hz, 1H, ArH).

5-(2-Azidoethoxy)-*N*-{3-[4-(dimethylamino)phenyl]propyl}-*N*-methyl-2,3-dihydro-1*H*-inden-1-amine: A solution of 5-(2-azidoethoxy)-*N*-{3-[4-(dimethylmino)phenyl]propyl}-2,3-dihydro-1*H*-inden-1-amine (50 mg, 0.13 mmol), paraformaldehyde (12 mg) and sodium cyanoborohydride (16 mg, 0.26 mmol) in methanol (1 mL) was stirred at r.t. for 18 h. The solution

was poured into saturated sodium hydrogen carbonate solution and the product was extracted with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, acetone) to give the methylated amine as a pale-yellow oil (27 mg, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72–1.84 (complex, 2H, $ArCH_2C\underline{H}_2$), 1.98–2.05 (m, 2H, $ArCH_2C\underline{H}_2$), 2.15 (s, 3H, NCH_3), 2.38–2.45 (m, 1H), 2.44–2.62 (complex, 2H), 2.7–2.8 (m, 1H), 2.89 (br, 8H), 3.57 (t, *J* = 5.0 Hz, 2H, N_3CH_2), 4.13 (t, *J* = 5.0 Hz, 2H, OCH_2), 4.35 (t, *J* = 7.4 Hz, 1H, ArCHN), 6.68 (d, *J* = 8.7 Hz, 2H, ArH), 6.75 (s, 1H, ArH), 6.75–6.78 (1H, ArH), 7.05 (d, *J* = 8.7 Hz, 2H, ArH), 7.25–7.26 (1H, ArH).

GIF-2250-r: To a solution of 5-(2-azidoethoxy)-*N*-{3-[4-(dimethylamino)phenyl]propyl}-*N*-methyl-2,3-dihydro-1*H*-inden-1-amine (22 mg, 56 μmol) in methanol (0.5 mL) was added Pd/C (10wt%, 4.2 mg) and the mixture was stirred at r.t. under hydrogen atmosphere (1 atm) for 6.5 h. The mixture was filtered through a pad of celite, and the filtrate was concentrated under reduced pressure to give the azido-reduced amine (17.2 mg). The crude amine was dissolved in acetonitrile (0.5 mL), and to this was added NBD-F (9.0 mg, 47 μmol) dissolved in acetonitrile (0.5 mL). The solution was stirred at r.t. for 5.5 h, and then concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, acetone) to give GIF-2250-r as a red-brown oil (19.7 mg, 79% in two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72–1.83 (complex, 2H, $ArCH_2C\underline{H}_2$), 1.98–2.07 (m, 2H, $ArCH_2C\underline{H}_2$), 2.15 (s, 3H, NCH_3), 2.38–2.62 (complex, 3H, NCH_2 and $ArCH_2$), 2.71–2.94 (complex, 3H, $ArCH_2$), 2.89 (s, 6H, $N(CH_3)_2$), 3.86–3.92 (m, 2H, $HNC\underline{H}_2$), 4.30 (t, *J* = 5 Hz, 2H, OCH_2), 4.36 (t, *J* = 6.7 Hz, 1H, ArCHN), 6.28 (d, *J* = 8.5 Hz, 1H, ArH), 6.52 (br, 1H, ArH), 6.67 (d, *J* = 8.5 Hz, 2H, ArH), 6.76 (s, 1H, ArH), 6.75–6.78 (1H, ArH), 7.05 (d, *J* = 8.5 Hz, 2H, ArH), 8.51 (d, *J* = 8.5 Hz, 1H, ArH).

GIF-2261-r: To a solution of 5-(2-azidoethoxy)-*N*-{3-[4-(dimethylamino)phenyl]propyl}-2,3-dihydro-1*H*-inden-1-amine (33 mg, 87 μmol) in methanol (1.5 mL) was added Pd/C (10wt%, 10 mg) and the mixture was stirred at r.t. under hydrogen atmosphere (1 atm) for 7 h. The mixture was filtered through a pad of celite, and the filtrate was concentrated under reduced pressure to give the azido-reduced amine (30.3 mg). The crude amine was dissolved in acetonitrile (0.5 mL), and to this was added NBD-F (14.6 mg, 80 μmol) dissolved in acetonitrile (0.5 mL). The solution was stirred at r.t. for 0.5 h, and then poured into saturated sodium hydrogen carbonate solution. The product was extracted with ethyl acetate and the organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1) to give GIF-2261-r as a red-brown oil

(24.3 mg, 59% in two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.7–1.91 (br, 1H, $ArCH_2C\underline{H}_2$), 1.82 (quin, *J* = 7.4 Hz, 2H, $ArCH_2C\underline{H}_2$), 2.32–2.41 (m, 1H, $ArCH_2C\underline{H}_2$), 2.52–2.6 (complex, 2H, NCH_2 and $ArCH_2$), 2.72 (t, *J* = 7.4 Hz, 2H, $ArCH_2$), 2.74–2.81 (complex, 1H, $ArCH_2C\underline{H}_2$), 2.88–3.0 (1H), 2.89 (s, 6H, $N(CH_3)_2$), 3.86–3.92 (t, *J* = 5 Hz, 2H, $HNC\underline{H}_2CH_2O$), 4.19 (t, *J* = 6.4 Hz, 1H, ArCHN), 4.28 (t, *J* = 5 Hz, 2H, OCH_2), 6.28 (d, *J* = 8.7 Hz, 1H, ArH), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 6.74 (d, *J* = 8.7 Hz, 2H, ArH), 6.77 (s, 1H, ArH), 7.04 (d, *J* = 8.7 Hz, 2H, ArH), 8.50 (d, *J* = 8.7 Hz, 1H, ArH).

GIF-2255-r and GIF-2289-r were individually synthesized in a similar manner using 3-phenylpropan-1-amine in place of 4-(3-aminopropyl)-*N*,*N*-dimethylaniline.

GIF-2255-r: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.77–1.86 (complex, 2H, $ArCH_2C\underline{H}_2$), 1.98–2.07 (complex, 2H, $ArCH_2C\underline{H}_2$), 2.16 (s, 3H, NCH_3), 2.43 (br t, *J* = 7 Hz, 2H, NCH_2), 2.54–2.92 (complex, 4H, $ArCH_2$), 3.89 (br, 2H, $HNC\underline{H}_2$), 4.30 (t, *J* = 5 Hz, 2H, OCH_2), 4.36 (t, *J* = 7 Hz, 1H, ArCHN), 6.28 (d, *J* = 8.7 Hz, 1H, ArH), 6.75–6.78 (br, 2H, ArH), 7.13–7.18 (complex, 4H, ArH), 7.23–7.28 (complex, 2H, ArH), 8.51 (d, *J* = 8.7 Hz, 1H, ArH).

GIF-2289-r: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.76–1.92 (complex, 3H, $ArCH_2C\underline{H}_2CH_2$ and $ArCH_2C\underline{H}_2CHN$), 2.34–2.42 (m, 1H, $ArCH_2C\underline{H}_2CHN$), 2.63–2.84 (complex, 5H, NCH_2 and $ArCH_2$), 2.95–3.03 (m, 1H, $ArCH_2$), 3.89 (br, 2H, $HNC\underline{H}_2$), 4.23 (t, *J* = 6.4 Hz, 1H, ArCHN), 4.30 (t, *J* = 5 Hz, 2H, OCH_2), 6.29 (d, *J* = 8.7 Hz, 1H, ArH), 6.75–6.78 (br, 2H, ArH), 7.16–7.32 (complex, 6H, ArH), 8.52 (d, *J* = 8.7 Hz, 1H, ArH).