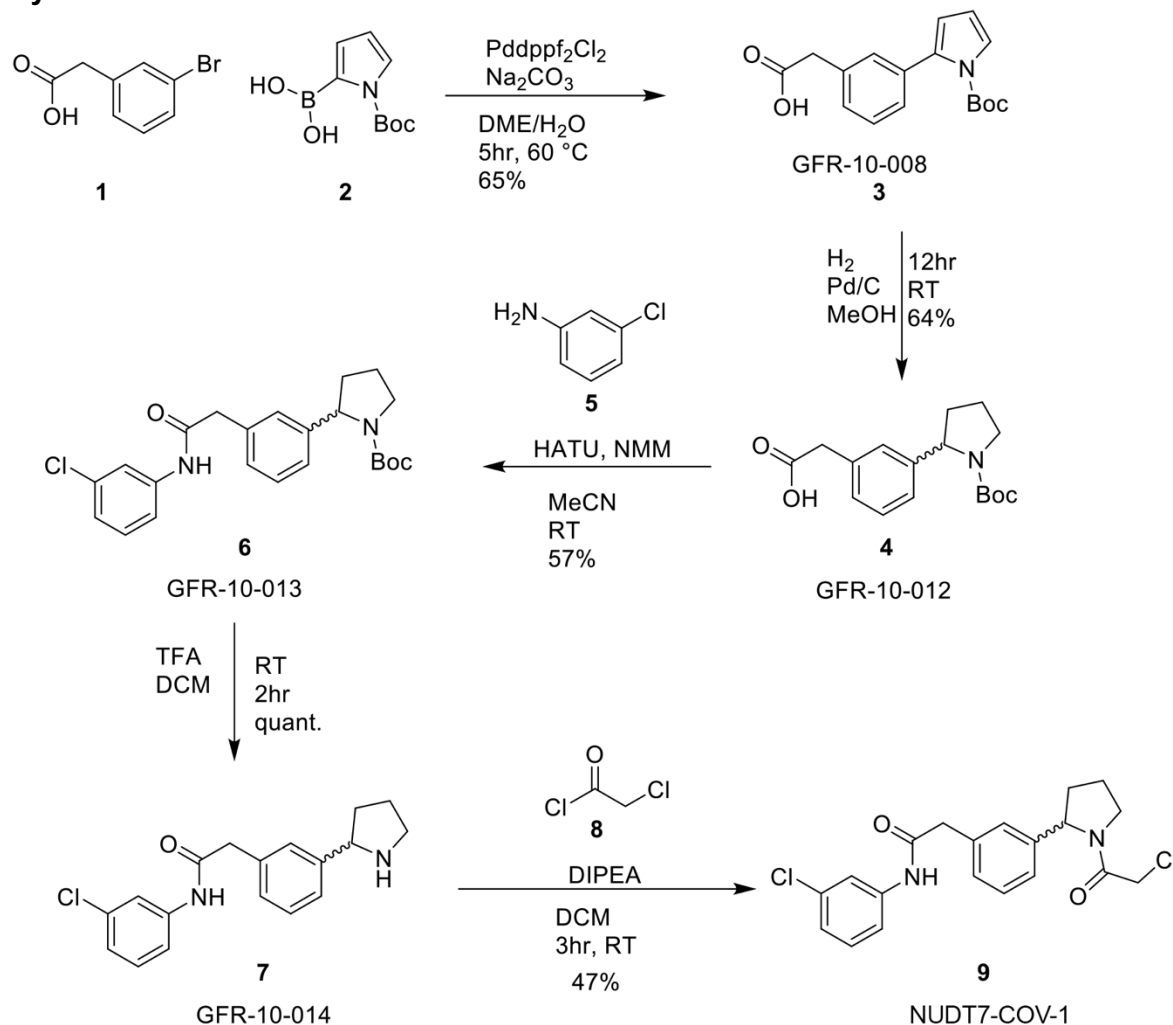


Synthesis of NUDT7 covalent inhibitor

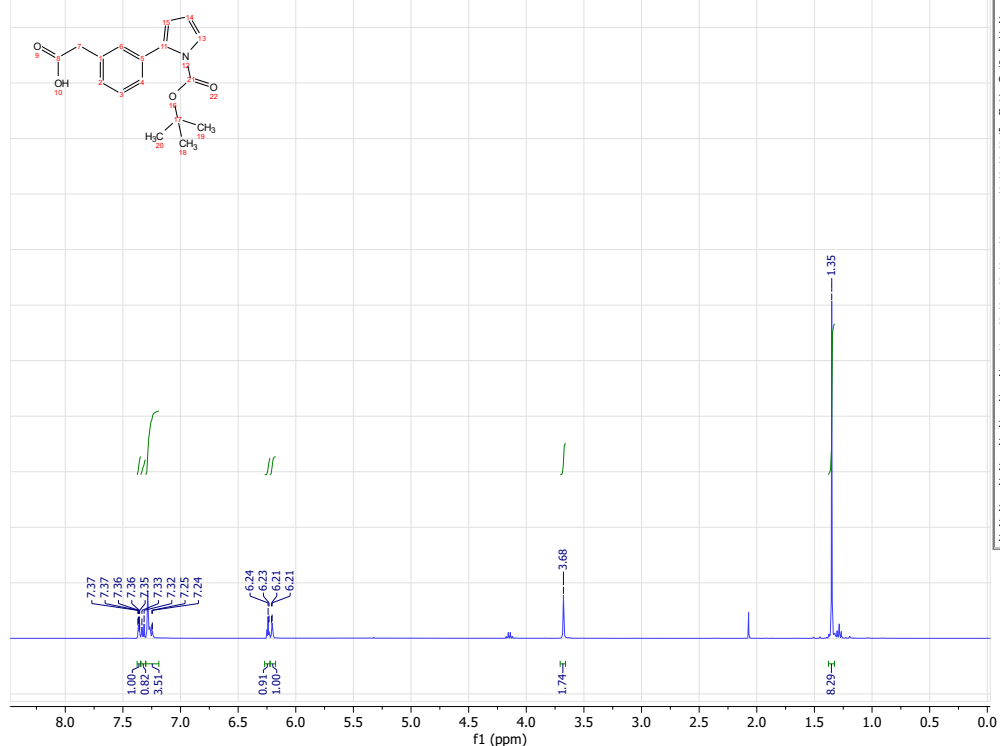


Synthesis of 2-(3-(1-(*tert*-butoxycarbonyl)-1H-pyrrol-2-yl)phenyl)acetic acid 3 (GFR-10-008)

A round bottom flask was loaded with 2-(3-bromophenyl)acetic acid (1 g, 4.65 mmol), 1-(*tert*-butoxycarbonyl)-1H-pyrrol-2-ylboronic acid (1.276 g, 6.05 mmol), Na_2CO_3 (1.971 g, 18.60 mmol) and PddppfCl_2 (0.171 g, 0.233 mmol). The air in the flask was displaced with nitrogen (3x) and to the mixture was added a previously degassed mixture of 1,2-Dimethoxyethane (20 mL)/Water (10 mL). The reaction was heated 3hr at 60 $^\circ\text{C}$. The mixture was filtered through a celite pad and concentrated to dryness. The crude was loaded on silica and purified by flash chromatography eluting with a gradient of 10 to 60% EtOAc in Cyclohexane, then with 10% MeOH in DCM. The title compound was isolated as brown oil (1.045g, 75%).

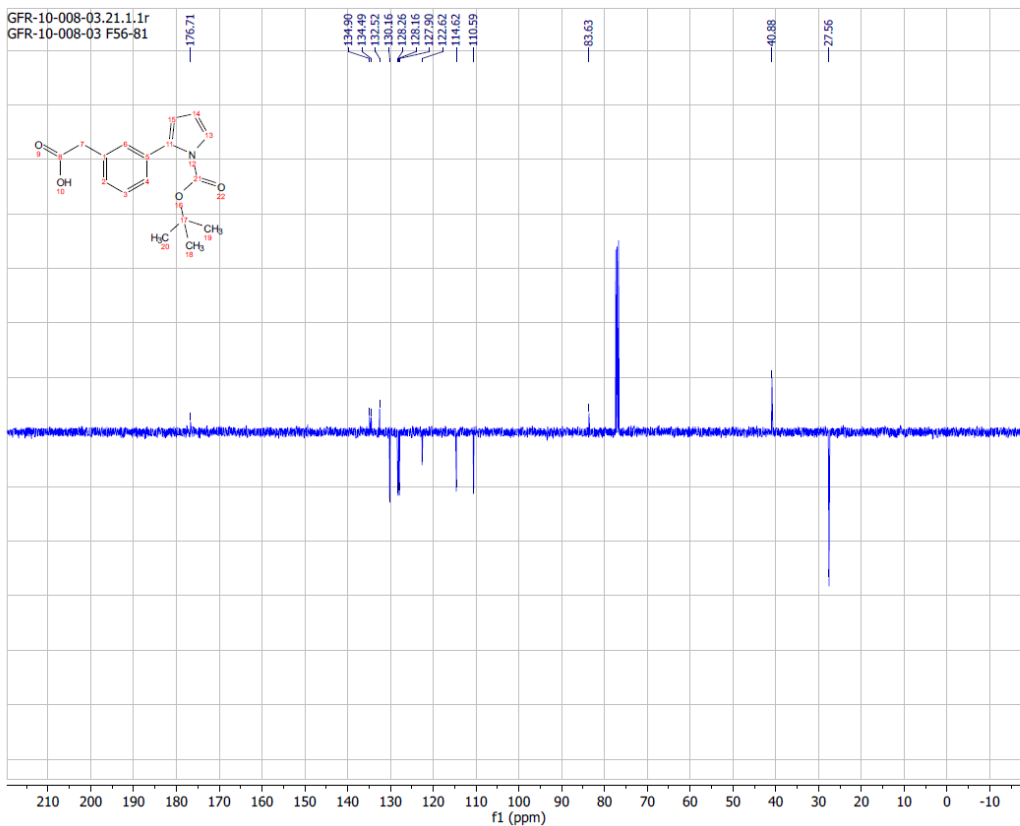
^1H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.18 (m, 5H), 6.27 – 6.16 (m, 2H), 3.68 (s, 2H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 176.71, 149.35, 134.90, 134.49, 132.52, 130.16, 128.26, 127.90, 122.62, 114.62, 110.59, 83.63, 40.88, 27.56.

GFR-10-008.10.1.1r
GFR-10-008 F12-36



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8 Author	
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GFR-10-008-03.21.1.1r
GFR-10-008-03 F56-81



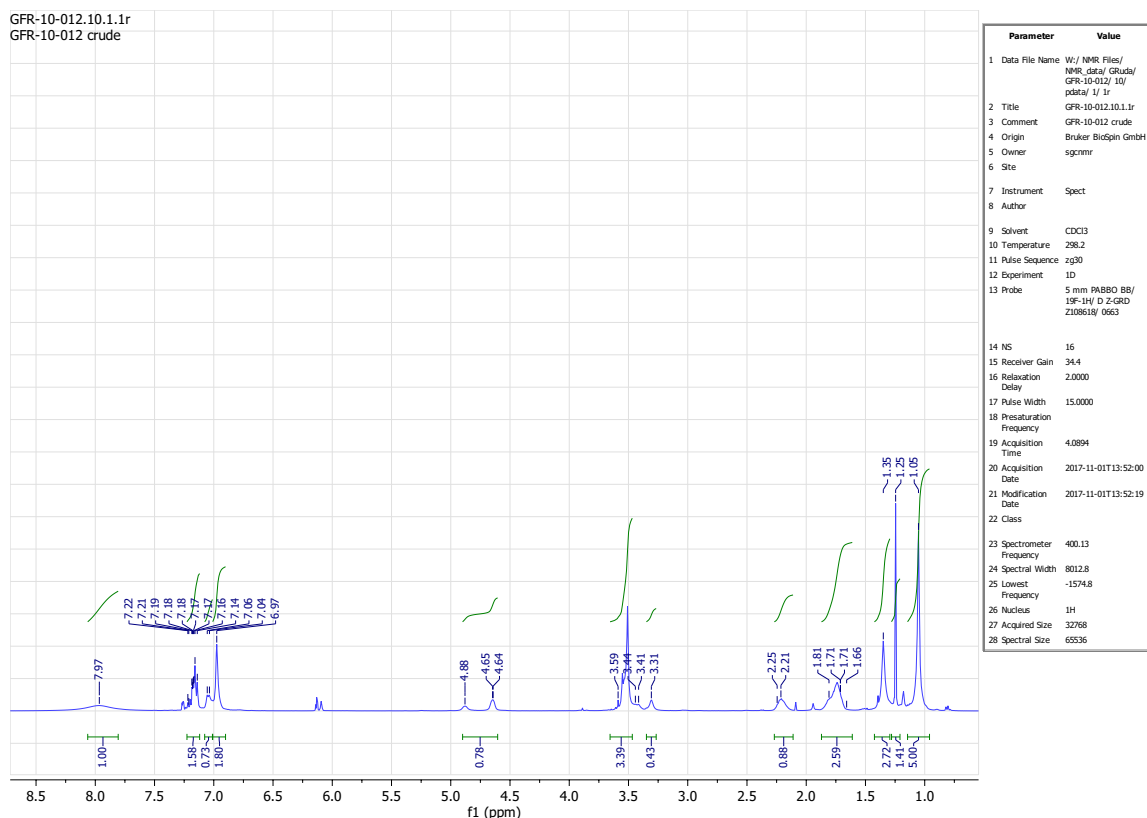
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LCMS: Rt 1.32, m/z 300.14 (ES-)

2-(3-(1-(tert-butoxycarbonyl)pyrrolidin-2-yl)phenyl)acetic acid **4 (GFR-10-012)**

Compound **3** was hydrogenated for 24hr on 10% Pd/C in MeOH at RT. The catalyst was filtered on a celite pad and the filtrate was concentrated under reduced pressure. The crude was used for the next step without further purification.

¹H NMR (400 MHz, Chloroform-d) δ 7.97 (s, 2H), 7.26 – 7.15 (m, 1H), 7.15 (d, J = 7.7 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.97 (s, 2H), 4.68 – 4.61 (m, 1H), 3.61 – 3.49 (m, 3H), 3.43 (d, J = 12.1 Hz, 1H), 3.31 (s, 1H), 2.27 – 2.17 (m, 1H), 1.82 – 1.66 (m, 1H), 1.35 (s, 3H), 1.25 (s, 1H), 1.05 (s, 5H).

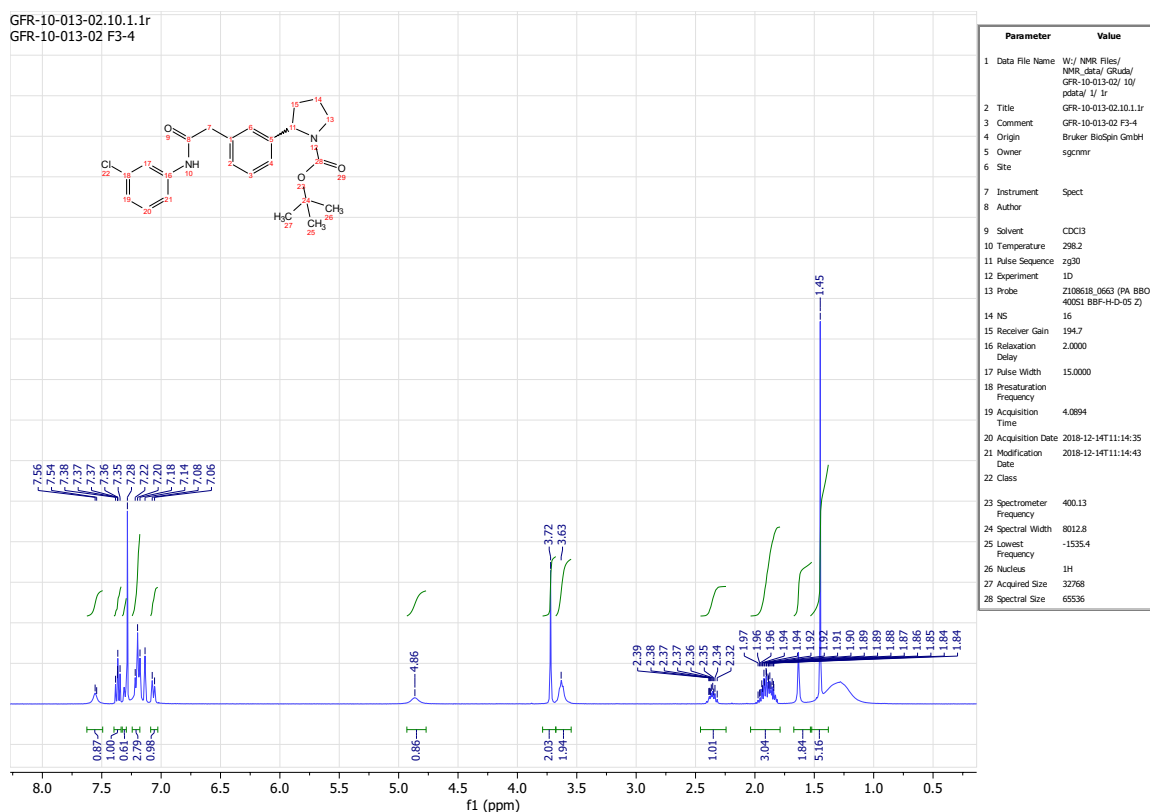


LCMS: Rt 1.23, m/z 304.19 (ES-)

Tert-butyl 2-(3-(2-((3-chlorophenyl)amino)-2-oxoethyl)phenyl)pyrrolidine-1-carboxylate **6 (GFR-10-013)**

N-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (0.163 g, 0.428 mmol) was added to a solution of 2-(3-(1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl)phenyl)acetic acid **4** (0.109 g, 0.357 mmol) in Acetonitrile (3 mL). To the mixture was added *N*-methylmorpholine (0.118 mL, 1.071 mmol) and the reaction was stirred 30 min at RT, then 3-chloroaniline (0.043 mL, 0.410 mmol) was added with a syringe. Upon consumption of the starting material the reaction was filtered, concentrated to dryness and purified by flash chromatography on Biotage SNAP ultra-cartridge eluting with a gradient from 5 to 95% EtOAc in Cyclohexane. The title product was isolated a light yellow oil (0.085 g, 57%)

^1H NMR (400 MHz, Chloroform- d) δ 7.60 (d, J = 17.0 Hz, 2H), 7.52 (s, 1H), 7.42 (s, 1H), 7.34 (t, J = 7.6 Hz, 2H), 7.31 (s, 2H), 7.16 (dd, J = 19.5, 11.6 Hz, 8H), 7.05 (d, J = 7.9 Hz, 2H), 4.92 (s, 1H), 4.79 (s, 1H), 3.68 (s, 7H), 3.57 (s, 1H), 2.35 (s, 1H), 2.01 – 1.78 (m, 5H), 1.45 (s, 2H), 1.43 (s, 8H), 1.18 (s, 8H).



LCMS: Rt 1.80, m/z 413.2 (ES $^-$)

Synthesis *N*-(3-chlorophenyl)-2-(3-(pyrrolidin-2-yl)phenyl)acetamide trifluoroacetate **7** (GFR-10-014)

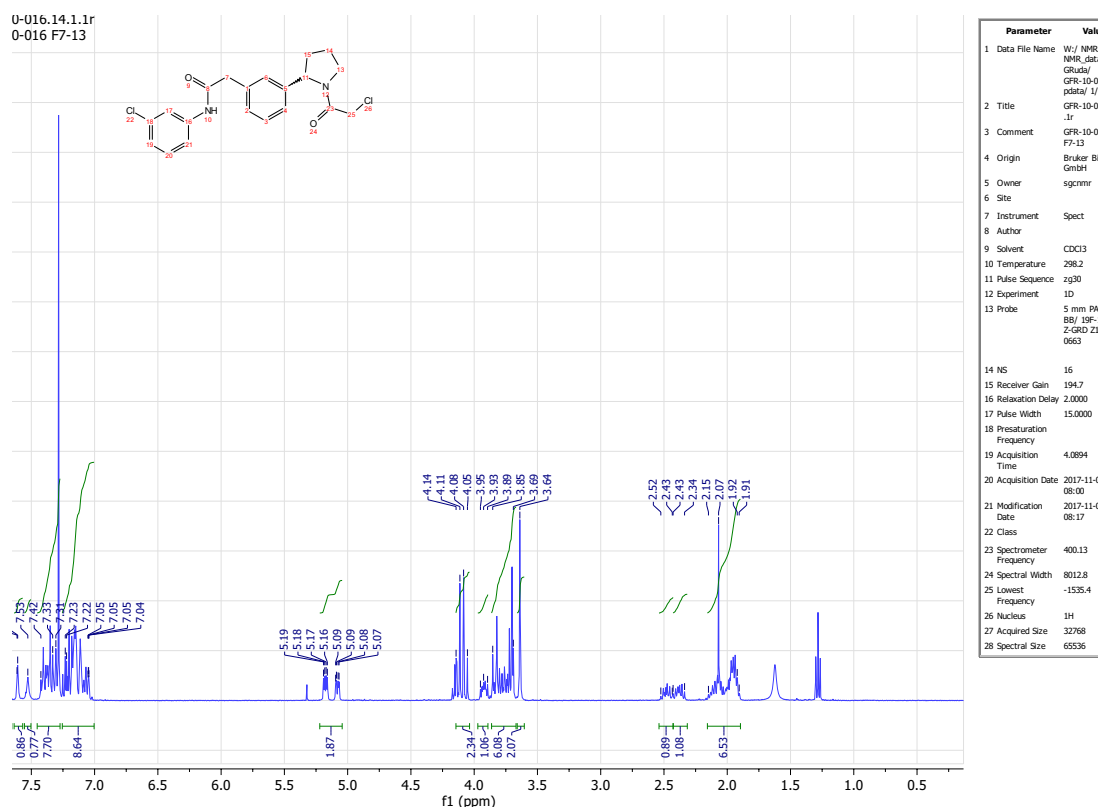
To a solution of *tert*-butyl 2-(3-(2-((3-chlorophenyl)amino)-2-oxoethyl)phenyl)pyrrolidine-1-carboxylate **0** (50 mg, 0.121 mmol) in CH_2Cl_2 (2 mL) was added trifluoroacetic acid (0.186 mL, 2.4 mmol) and the reaction was stirred 2hr at 25 °C. The volatiles were removed under reduced pressure and the crude residue was used in the next step without further purification. Yellow foam (quant.)

LCMS: Rt 1.46, m/z 313.2 (ES $^-$); m/z 315.2 (ES $^+$).

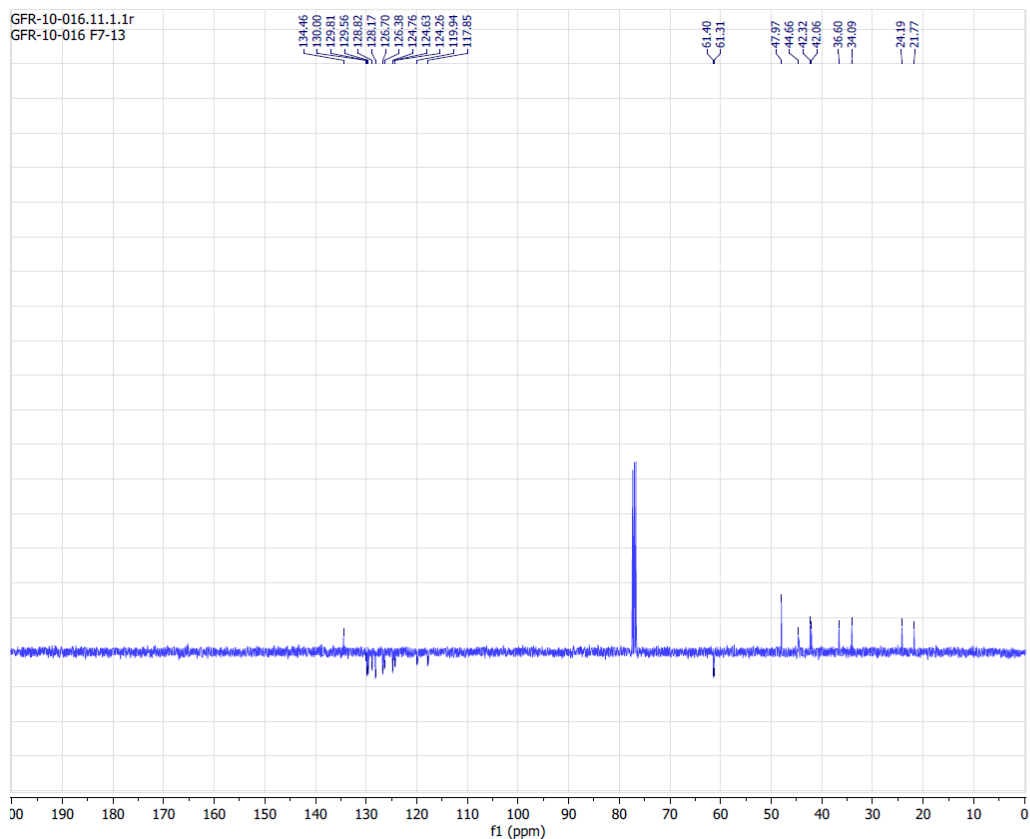
Synthesis of 2-(3-(1-(2-chloroacetyl)pyrrolidin-2-yl)phenyl)-*N*-(3-chlorophenyl)acetamide **9** (NUDT7-COV-1)

Chloroacetyl chloride (0.019 mL, 0.238 mmol) was added to a solution of *N*-(3-chlorophenyl)-2-(3-(pyrrolidin-2-yl)phenyl)acetamide trifluoroacetate **7** (0.085 mg, 0.198 mmol) and DIPEA (0.138 mL, 0.793 mmol) in dry CH_2Cl_2 (2 mL). The reaction was stirred at RT for 3hr. The mixture was concentrated to dryness and the crude residue was adsorbed on silica and purified by flash chromatography on a Biotage SNAP ultra column eluting with a gradient of 5

¹H NMR (400 MHz, Chloroform-*d*) δ 7.68 (t, *J* = 2.0 Hz, 1H), 7.61 (t, *J* = 1.9 Hz, 1H), 7.51 (s, 1H), 7.37 (ddd, *J* = 20.9, 17.1, 9.3 Hz, 5H), 7.28 (s, 3H), 7.27 – 7.02 (m, 10H), 5.18 (dd, *J* = 8.1, 3.9 Hz, 1H), 5.08 (dd, *J* = 7.9, 2.8 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 1H), 3.92 (ddd, *J* = 10.1, 7.6, 5.0 Hz, 1H), 3.90 – 3.62 (m, 9H), 2.55 – 2.32 (m, 2H), 2.17 – 2.06 (m, 1H), 2.10 – 1.98 (m, 1H), 2.01 – 1.88 (m, 3H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 194.25, 188.56, 165.17, 134.45, 129.99, 129.86, 129.80, 129.55, 128.81, 126.69, 126.37, 124.75, 124.62, 124.24, 119.93, 119.89, 117.84, 117.70, 61.39, 47.96, 44.66, 42.31, 42.05, 36.60, 34.09, 24.19, 21.77.



GFR-10-016.11.1.1r
GFR-10-016 F7-13



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