Supplementary information

Identification of an Active Metabolite of PAR-1 Antagonist RWJ-58259 and Synthesis of Analogues to Enhance its Metabolic Stability

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General Experimental

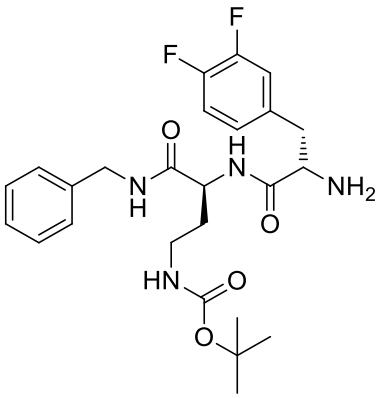
Reactions carried out under dry conditions were done in oven-dried glassware under an argon atmosphere. Where addition of solids was required reactions were carried out in three-necked round-bottom flasks and the addition done under a positive pressure of argon. Reactions were stirred magnetically and monitored by thin-layer chromatography (TLC) using aluminium sheets coated with silica gel 60 F_{254} purchased from Merck, which were then viewed under UV irradiation (λ = 254 nm) and developed further with a potassium permanganate, ninhydrin or 4-nitrophenylhydrazine dip. Silica gel 60 (40-63 μm) from Merck was used to purify compounds by flash column chromatography. All solvents were of commercial grade, purchased from Fisher Scientific, VWR or Sigma-Aldrich and used without further purification. Pet refers to petroleum ether (b.p. 40-60 °C). Reagents were purchased from Sigma-Aldrich, Alfa Aesar, Iris Biotech or Acros Organics and used as received from the supplier.

Instrumentation

Purification by preparative scale HPLC was carried out on a Varian Prostar (column: Phenomenex Jupiter 300 A C18, 250 x 4.6 mm, 10 μm; mobile phase gradient: 0-2 min (5%), 2-8 min (5-95%), 8-14 min (95%), 14-15 min (95-5%), 15-20 min (5%) MeCN in H_2O with 0.1% TFA). Melting points were measured using a Gallenkamp

apparatus and are uncorrected. A Perkin Elmer Spectrum 100 FT-IR spectrometer or Bruker Alpha FT-IR spectrometer was used to obtain infrared spectra. ^{1}H NMR spectra of pure samples dissolved in either $CDCl_3$ or DMSO-d_6 were recorded at 400 MHz, 500 MHz and 600 MHz and ^{13}C NMR at 125 MHz and 150 MHz on a Bruker AMX400, Avance 500 and Avance III 600 spectrometer at 25 °C. Chemical shifts (δ) for ^{1}H and ^{13}C nuclei are reported relative to the residual solvent signal on a parts per million (ppm) scale. Coupling constants (*J* values) are reported in Hertz (Hz) and are reported as $J_{H\text{-}H}$ couplings unless otherwise stated. High resolution mass spectra were acquired for pure samples on either a Thermo Finnigan MAT900Xp (EI and CI) or Water LCT Premier XE (ES) mass spectrometer. LC/MS analysis was carried out using a Waters Acquity UPLC (column: C18 BEH 50 x 2.1 mm, 1.7 μm; mobile phase gradient (5 min): 5-95% MeCN in H_2O with 0.1% formic acid).

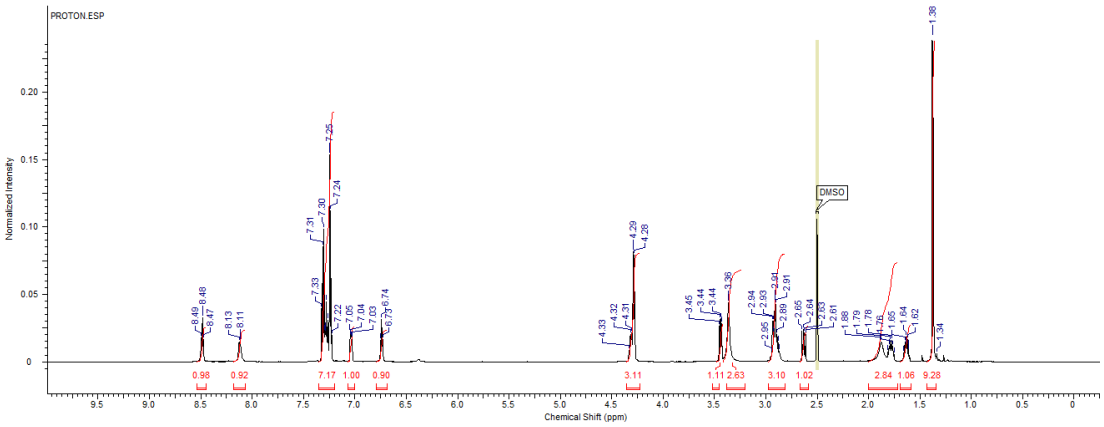
***tert*-Butyl {(*S*)-3-[(*S*)-2-amino-3-(3,4-difluorophenyl)propanamido]-4-(benzylamino)-4-oxobutyl}carbamate (2)**



i) To a stirred solution of (*S*)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-4-[(tert-butoxycarbonyl)amino]butanoic acid (540 mg, 1.24 mmol), HBTU (430 mg, 1.14 mmol) and HOBt (152 mg, 1.14 mmol) in DMF (25 mL) was added DIPEA (0.20 mL, 1.14 mmol). After 3 min of stirring, benzylamine (0.11 mL, 1.04 mmol) was added and stirring continued at 18 °C for 5 h. The reaction mixture was concentrated *in vacuo* and the white residue taken up in $CHCl_3$/EtOAc (60 mL, 1:1) and washed with H_2O (15 mL), 10% citric acid solution (15 mL), sat. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (25% EtOAc/Pet) to give the Fmoc-protected amide intermediate (Rf 0.13 (45% EtOAc/Pet)) as a colourless oil. This was dissolved in DMF (10 mL) and stirred with Et_2NH (1.28 mL, 12.4 mmol) at 18 °C for 3 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in $CHCl_3$/EtOAc (40 mL, 1:1) and washed with H_2O (2 x 15 mL), brine (15 mL) and sat. LiCl solution (4 x 15 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (0-2% MeOH/CH_2Cl_2) to give the deprotected amide intermediate (**1**) (259 mg, 81%) (Rf 0.2 (10% MeOH/CH_2Cl_2)).

ii) To a stirred solution of (*S*)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-(3,4-difluorophenyl)propanoic acid (410 mg, 0.97 mmol), HBTU (338 mg, 0.89 mmol) and HOBt (120 mg, 0.89 mmol) in DMF (20 mL) was added DIPEA (0.16 mL, 0.89 mmol). After 3 min of stirring, a solution of the deprotected amide intermediate (**1**) (250 mg, 0.81 mmol), from step i), in DMF (5 mL) was added and

stirring continued at 18 °C for 16 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (30 mL) and washed with H_2O (10 mL), 10% citric acid solution (10 mL), sat. $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (25-80% EtOAc/Pet) to give the Fmoc-protected title compound (Rf 0.19 (50% EtOAc/Pet)), which was dissolved in DMF (10 mL) and stirred with Et_2NH (1.00 mL, 9.69 mmol) at 18 °C for 3 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (30 mL) and washed with H_2O (2 x 10 mL), brine (10 mL) and sat. LiCl solution (4 x 15 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (0-2% MeOH/CH_2Cl_2) to give the title compound (227 mg, 57% (84% crude[1])); as a white solid; mp 218-224 °C (decomp.); Rf 0.56 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3286 (N-H), 3062-2926 (C-H), 1678 (C=O), 1634 (C=O), 1515 (ArC=C), 1453-1365 (C-H), 1279 (C-O), 1165 (C-N), 1116 (C-F), 770-694 (ArC-H) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 8.48 (1H, t, *J* = 6.0, N*H*CH_2Ph), 8.12 (1H, d, *J* = 7.5, CHN*H*COCH), 7.33-7.22 (7H, m, ArH), 7.04 (1H, m, ArH), 6.74 (1H. t, *J* = 5.7, N*H*CH_2CH_2), 4.32 (1H, m (obscured), C*H*NHCOCH), 4.29 (2H, d, *J* = 6.0, C*H*$_2$NHCO), 3.44 (1H, dd, *J* = 8.3, 4.9, C*H*NH_2), 2.96-2.86 (2H, m, C*H*$_2CH_2$CHNH & 1H, apparent dd, *J* = 13.6, 4.9, C(H)*H*$CHNH_2$), 2.64 (1H, dd, *J* = 13.6, 8.3, C(*H*)HCHNH_2), 1.88 (2H, bs, N*H*$_2$), 1.78 (1H, m, CH_2C(H)*H*CHNH), 1.63 (1H, m, CH_2C(*H*)HCHNH), 1.38 (9H, s, 3 x C*H*$_3$); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.1 (NH*C*OC), 171.2 (NH*C*OC), 155.5 (NH*C*OO), 149.9-148.9 (dd, *J* = 137.1, 12.5, *C*F), 148.3-147.3 (dd, *J* = 135.9, 12.5, *C*F), 139.2 (Ar*C*), 136.6 (Ar*C*), 128.3 (Ar*C*H), 127.1 (Ar*C*H), 126.9 (Ar*C*H), 126.1 (Ar*C*H), 118.3 (Ar*C*H), 118.7 (Ar*C*H), 116.9 (Ar*C*H), 116.8 (Ar*C*H), 77.7 (*C*$(CH_3)_3$), 55.8 (*C*HNH_2), 50.3 (*C*HNHCOCH), 42.0 (Ph*C*H_2NH), 39.8 (*C*H_2CHNH_2), 36.8 (*C*H_2CH_2CHNH), 32.9 (*C*H_2CHNHCOCH), 28.3 (3 x *C*H_3); m/z (CI+) 491 (20%, $[M+H]^+$), 391 (100%, $[M+2H\text{-}Boc]^+$); HRMS $C_{25}H_{33}F_2N_4O_4$ ($[M+H]^+$) calcd. 491.2470, found. 491.2467.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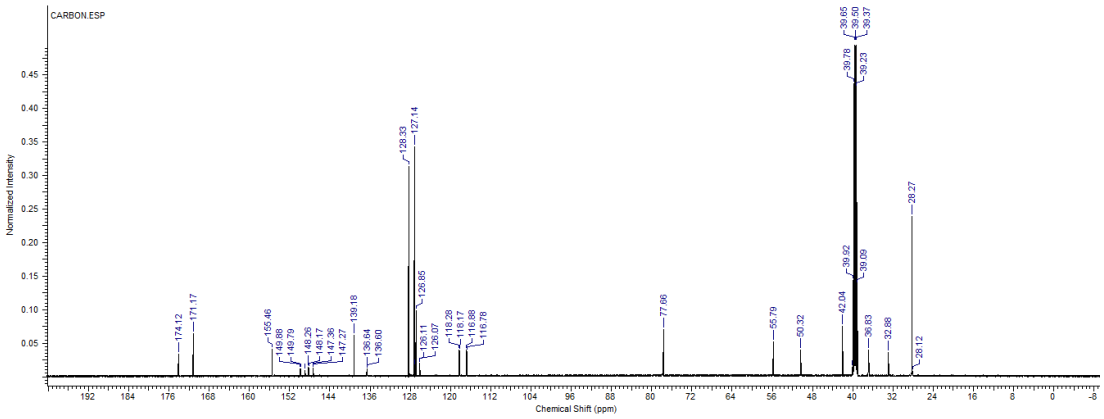
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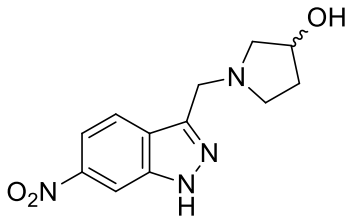
39.50
39.37
39.78
39.23
36.83
32.88
28.27
28.12

Racemic mixture of (*S*)-1-[(6-nitro-1*H*-indazol-3-yl)methyl]pyrrolidin-3-ol & (*R*)-1-[(6-nitro-1*H*-indazol-3-yl)methyl]pyrrolidin-3-ol

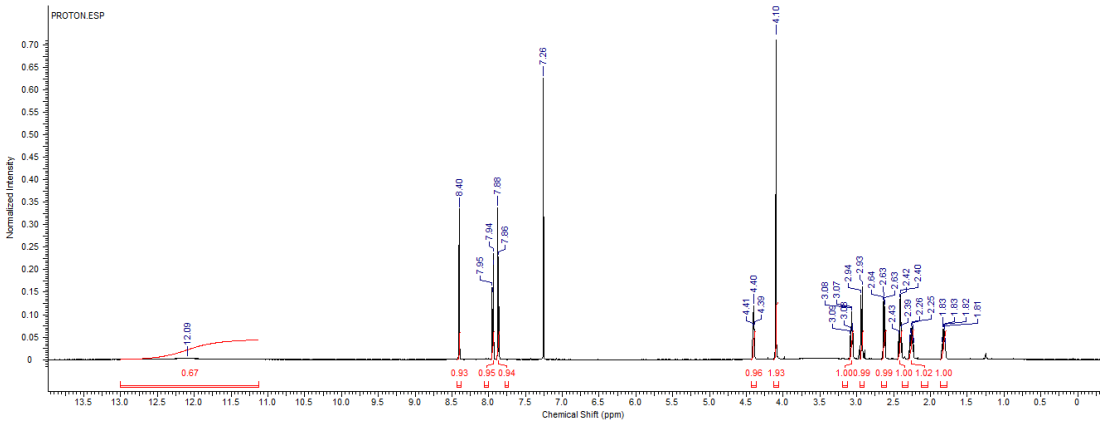


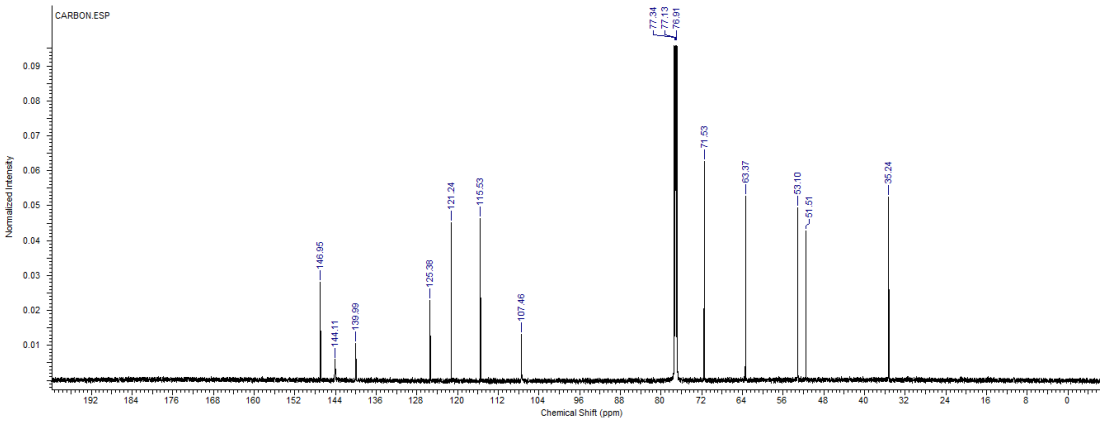
i) 6-Nitroindole (500 mg, 3.08 mmol) was added to a stirred solution of sodium nitrite (2.13 g, 30.8 mmol) in H_2O (25 mL) and DMF (2 mL) at 18 °C. 6 M HCl (4.6 mL, 27.8 mmol) was added dropwise over 10 min and stirring continued for 3 h before diluting with EtOAc (30 mL) and extracting. Aqueous layer extracted further with EtOAc (2 x 15 mL). Organic extracts combined, washed with H_2O (15 mL) and brine (15 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to leave the indazole-3-carbaldehyde intermediate as a dark brown solid.

ii) HCl (g), generated by the dropwise addition of conc. H_2SO_4 (65 mL, 1.20 mol) to NaCl (s) (58.0 g, 1.07 mol), was bubbled through a stirred solution of 1-Boc-3-pyrrolidinol (1.00 g, 5.34 mmol) in Et_2O (50 mL) for 3 h. Stirring was then continued for 20 h before concentrating *in vacuo* and drying further under high vacuum to leave the hydrochloride salt of 3-pyrrolidinol as a pale pink solid.

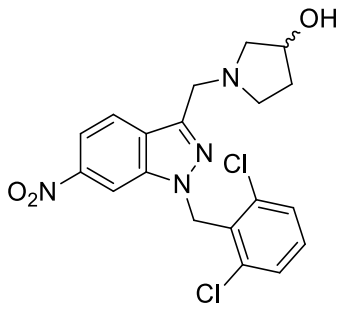
iii) The indazole-3-carbaldehyde intermediate was dissolved in CH_2Cl_2/DMF/AcOH (45/4.5/0.5 mL), to which was added the 3-pyrrolidinol (660 mg, 5.36 mmol) and the reaction mixture was stirred at 18 °C for 20 min. Sodium triacetoxyborohydride (1.63 mg, 7.70 mmol) was added portionwise over 10 min and stirring continued for 3 h before diluting with EtOAc (100 mL) and quenching with sat. aqueous $NaHCO_3$ (60 mL). Aqueous layer separated and extracted further with EtOAc (2 x 30 mL). Organic extracts combined and washed with sat. $NaHCO_3$ (aq) (2 x 25 mL), H_2O (2 x 25 mL) and brine (2 x 25 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to leave brown residue. Purification by flash column chromatography (0-10% MeOH/CH_2Cl_2) gave target compound (406 mg, 50%); as a brown solid; mp 166-168 °C; Rf 0.13 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3122 (O-H), 3068 (N-H), 2959-2835 (C-H), 1513 (NO_2), 1341 (NO_2), 1125-1059 (C-N), 874-730 (ArC-H) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 12.09 (1H, bs, N*H*), 8.40 (1H, d,

J = 1.9, CC*H*C), 7.94 (1H, dd, *J* = 9.0, 1.9, CHC*H*CNO$_2$), 7.87 (1H, d, *J* = 9.0, C*H*CHCNO$_2$), 4.40 (1H, m, C*H*OH); 4.10 (2H, s, CC*H*$_2$N); 3.07 (1H, td, *J* = 8.7, 4.1, NC*H*HCH$_2$), 2.93 (1H, d, *J* = 10.2, NC*H*HCHOH), 2.63 (1H, dd, *J* = 10.2-10.5, 5.3-5.7, NCH*H*CHOH), 2.41 (1H, q, *J* = 8.3-8.7, NCH*H*CH$_2$), 2.29-2.23 (1H, m, NCH$_2$CH*H*), 1.84-1.79 (1H, m, NCH$_2$C*H*H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 147.0 (Ar*C*), 144.1 (Ar*C*), 140.0 (Ar*C*), 125.4 (Ar*C*), 121.2 (*C*HCHCNO$_2$), 115.5 (CH*C*HCNO$_2$), 107.5 (C*C*HC), 71.5 (*C*HOH), 63.4 (N*C*H$_2$CHOH), 53.1 (N*C*H$_2$CH$_2$), 51.5 (*C*H$_2$CNN), 35.2 (NCH$_2$*C*H$_2$); m/z (CI+) 263 (100%, [M+H]$^+$); HRMS $C_{12}H_{15}N_4O_3$ ([M+H]$^+$) calcd. 263.1144, found. 263.1143.

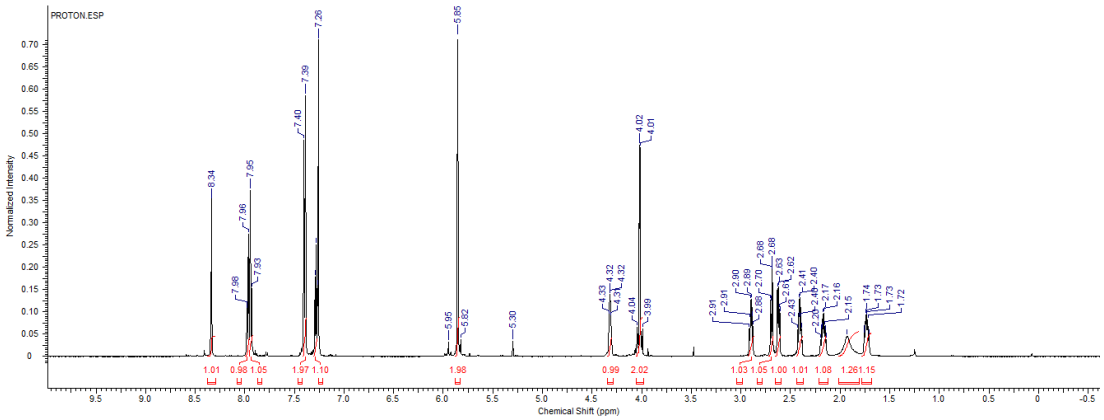




Racemic mixture of (*S*)-1-{[1-(2,6-dichlorobenzyl)-6-nitro-1*H*-indazol-3-yl]methyl}pyrrolidin-3-ol & (*R*)-1-{[1-(2,6-dichlorobenzyl)-6-nitro-1*H*-indazol-3-yl]methyl}pyrrolidin-3-ol



To a stirred solution of *rac*-1-[(6-nitro-1*H*-indazol-3-yl)methyl]pyrrolidin-3-ol (319 mg, 1.22 mmol) in dry THF (30 mL) under argon was added 2,6-dichlorobenzyl bromide (293 mg, 1.22 mmol) followed by portionwise addition over 10 min of caesium carbonate (396 mg, 1.22 mmol). The reaction mixture was stirred at 16 °C for 20 h then diluted with H_2O (10 mL) and extracted with EtOAc (2 x 20 mL). Organic extracts were washed with H_2O (2 x 10 mL) then brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash column chromatography (0-1.5% MeOH/CH_2Cl_2) gave the title compound (292 mg, 57%); as a light brown solid; mp 161-163 °C; Rf 0.45 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3083 (O-H), 2963-2839 (C-H), 1579 (ArC=C), 1524 (NO_2), 1437 (C-H), 1345 (NO_2), 1298-1089 (C-N), 874-732 (ArC-H), 666 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34 (1H, d, *J* = 1.9, NCC*H*C), 7.97 (1H, dd, *J* = 8.7, 1.9, NCC*H*CHC), 7.94 (1H, d, *J* = 8.7, NCCHC*H*C), 7.40 (2H, d, *J* = 7.9, 2 x C*H*CCl), 7.28 (1H, t, *J* = 7.9, C*H*CHCCl), 5.85 (2H, s, CH_2NN), 4.32 (1H, m, C*H*OH), 4.02 (2H, s, NCH_2CN), 2.90 (1H, td, *J* = 8.7, 5.3, C*H*HCH_2CHOH), 2.69 (1H, d, *J* = 10.2, NC*H*HCHOH), 2.62 (1H, dd, *J* = 10.2, 4.9, NCH*H*CHOH), 2.40 (1H, dt, *J* = 6.0-6.4, 8.7-9.4, CH*H*CH_2CHOH), 2.20-2.14 (1H, m, CH_2C*H*HCHOH), 1.93 (1H, bs, CHO*H*), 1.76-1.71 (1H, m, CH_2CH*H*CHOH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.7 (Ar*C*), 143.6 (Ar*C*), 139.5 (Ar*C*), 137.0 (2 x *C*Cl), 130.8 (Ar*C*), 130.6 (*C*HCHCCl), 129.0 (2 x *C*HCCl), 126.5 (Ar*C*), 121.9 (NCCH*C*HC), 115.2 (NC*C*HCHC), 106.3 (NC*C*HC), 71.7 (*C*HOH), 62.9 (N*C*H_2CHOH), 52.4 (*C*H_2CH_2CHOH), 51.5 (N*C*H_2CN), 48.8 (N*C*H_2CCCl), 35.2 (CH$_2$*C*H_2CHOH); m/z (CI+) 421 (100%, $[^{35,35}M+H]^+$), 423 (55%, $[^{35,37}M+H]^+$); HRMS $C_{19}H_{19}{}^{35}Cl_2N_4O_3$ ($[M+H]^+$) calcd. 421.0834, found. 421.0831.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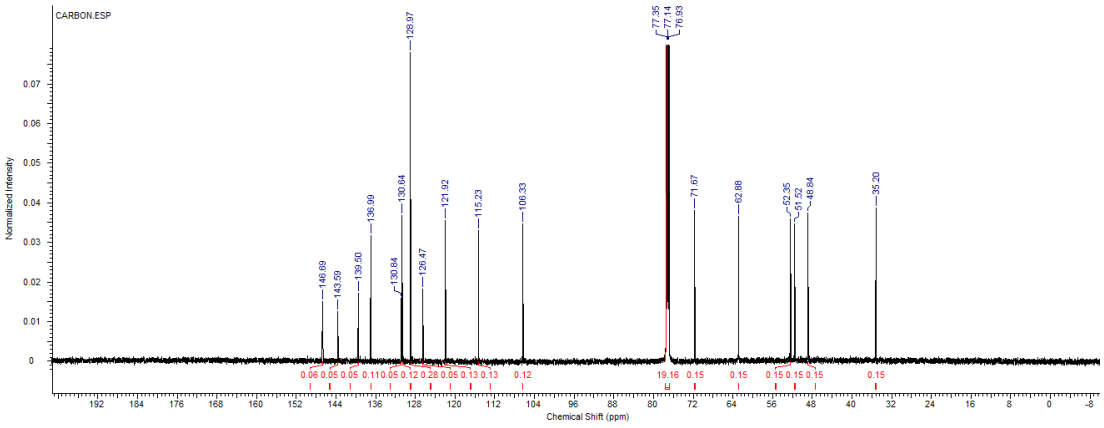
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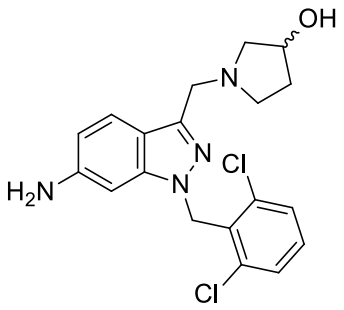
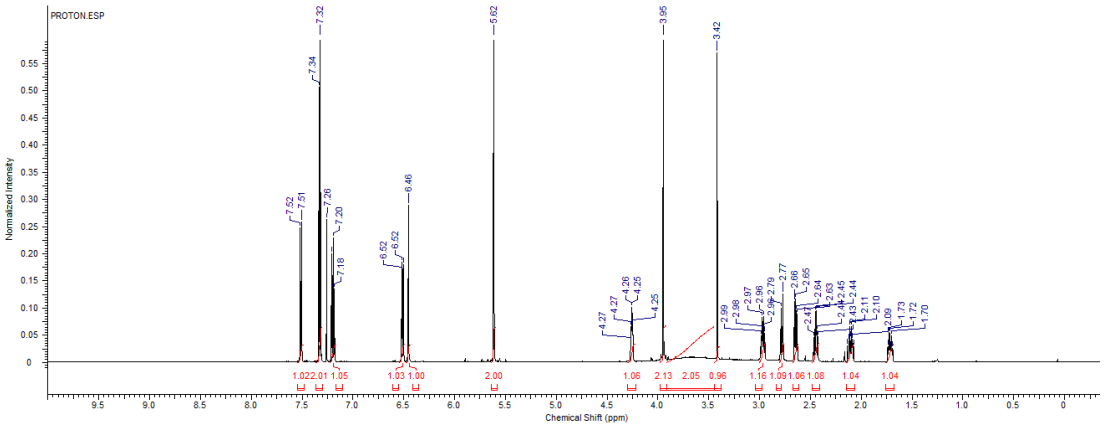
Racemic mixture of (*S*)-1-{[6-amino-1-(2,6-dichlorobenzyl)-1*H*-indazol-3-yl]methyl}pyrrolidin-3-ol & (*R*)-1-{[6-amino-1-(2,6-dichlorobenzyl)-1*H*-indazol-3-yl]methyl}pyrrolidin-3-ol (3)



To a solution of *rac*-1-{[1-(2,6-dichlorobenzyl)-6-nitro-1*H*-indazol-3-yl]methyl}pyrrolidin-3-ol (206 mg, 0.489 mmol) in MeOH (30 mL) was added $FeCl_3.6H_2O$ (31.0 mg, 0.114 mmol) and activated charcoal (248 mg), followed by *N*,*N*-dimethylhydrazine (0.74 mL, 9.78 mmol). The reaction mixture was heated to reflux for 5 h then allowed to cool to ambient temperature before filtering through celite, washing through with a cold mixture of CH_2Cl_2/MeOH (90 mL, 4:1). The filtrate was concentrated *in vacuo* to leave a brown oily residue, which was purified by flash column chromatography (0-6% MeOH/CH_2Cl_2) to give desired product **3** (120 mg, 63%); as a light brown/pink solid; mp 148-152 °C; Rf 0.25 (20% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3432 (O-H), 3344-3230 (N-H), 2958-2848 (C-H), 1626 (C=N), 1564-1495 (ArC=C), 1434 (C-H), 1292-1090 (C-N), 767-691 (ArC-H), 631 (C-Cl) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.51 (1H, d, *J* = 8.7, C*H*CHCNO$_2$), 7.33 (2H, d, *J* = 8.3, 2 x C*H*CCl), 7.20 (1H, t, *J* = 8.3, C*H*CHCCl), 6.51 (1H, dd, *J* = 8.7, 1.9, CHC*H*CNO$_2$), 6.45 (1H, d, *J* = 1.9, CC*H*C), 5.62 (2H, s, NNC*H*$_2$C), 4.26 (1H, m, C*H*OH), 3.95 (2H, s, C*H*$_2$CNN), 3.42 (1H, s, CHO*H*), 2.97 (1H, dt, *J* = 5.7, 8.7, C*H*HCH$_2$CHOH), 2.78 (1H, d, *J* = 10.5, NC*H*HCHOH), 2.62 (1H, dd, *J* = 10.4, 5.3, NCH*H*CHOH), 2.40 (1H, dt, *J* = 6.4, 9.0, CH*H*CH$_2$CHOH), 2.13-2.08 (1H, m, CH$_2$C*H*HCHOH), 1.74-1.69 (1H, m, CH$_2$CH*H*CHOH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.9 (Ar*C*), 142.4 (Ar*C*), 141.7 (Ar*C*), 136.9 (2 x *C*Cl), 131.8 (Ar*C*), 130.0 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 121.6 (*C*HCHCNO$_2$), 117.5 (Ar*C*), 112.3 (CH*C*HCNO$_2$), 92.5 (C*C*HC), 71.5 (*C*HOH), 62.5 (N*C*H$_2$CHOH), 52.0 (*C*H$_2$CH$_2$CHOH), 50.9 (*C*H$_2$CNN), 48.3 (*C*H$_2$NN), 35.1 (CH$_2$*C*H$_2$CHOH); m/z (CI+) 391 (100%, $[^{35,35}M+H]^+$), 393 (64%, $[^{35,37}M+H]^+$), 395 (9%, $[^{37,37}M+H]^+$); HRMS $C_{19}H_{21}{}^{35}Cl_2N_4O$ ($[M+H]^+$) calcd. 391.1092, found. 391.1091.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2.45
2.44
2.43
2.10
2.09
1.73
1.72
1.70
1.02 2.01 1.05
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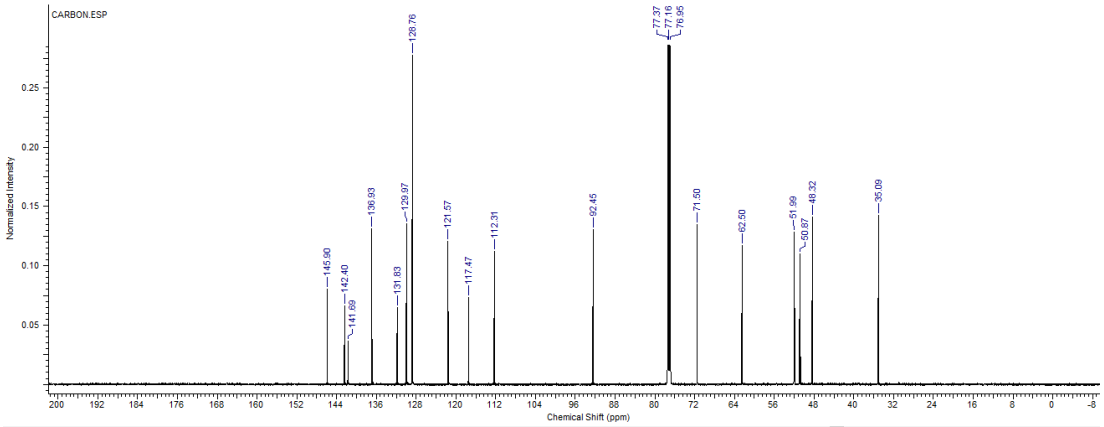
1.04
1.04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Chemical Shift (ppm)
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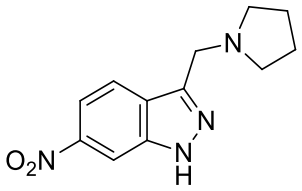
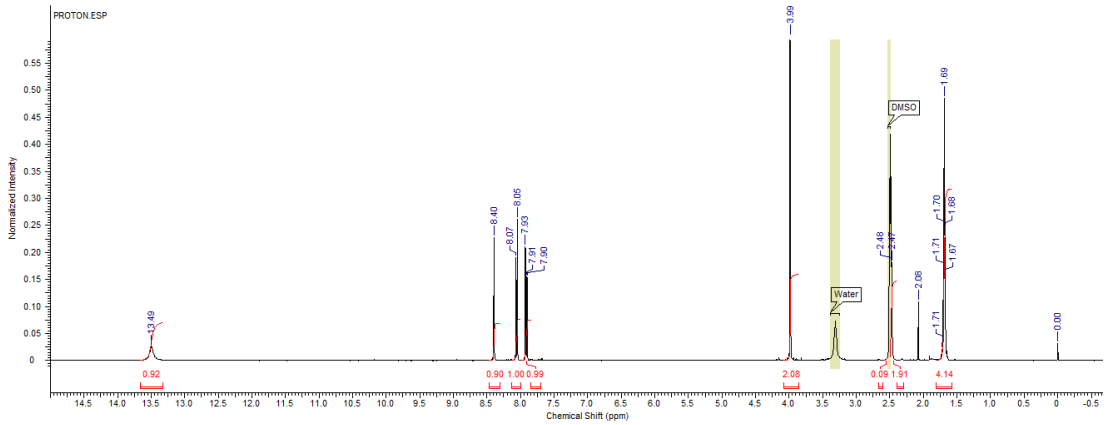
112.31
92.45
77.37
77.16
76.95
71.50
62.50
51.99
50.87
48.32
35.09

6-Nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole [1]



6-Nitroindole (500 mg, 3.08 mmol) was added to a stirred solution of sodium nitrite (2.13 g, 30.8 mmol) in H_2O (25 mL) and DMF (2 mL) at 18 °C. 6 M HCl (4.6 mL, 27.8 mmol) was added dropwise over 10 min and stirring continued for 3 h before diluting with EtOAc (30 mL) and extracting. Aqueous layer extracted further with EtOAc (2 x 15 mL). Organic extracts were combined and washed with H_2O (15 mL) and brine (15 mL), then dried ($MgSO_4$) and concentrated *in vacuo*. Residual dark brown solid was dissolved in CH_2Cl_2/DMF/AcOH (54/5.4/0.6 mL), to which was added pyrrolidine (548 mg, 0.643 mL, 7.70 mmol) and the reaction mixture was stirred at 18 °C for 20 min. Sodium triacetoxyborohydride (1.63 g, 7.70 mmol) was added portionwise over 10 min and stirring continued for 3 h before diluting with EtOAc (100 mL) and quenching with saturated aqueous $NaHCO_3$ (60 mL). Aqueous layer separated and extracted further with EtOAc (2 x 20 mL). Organic extracts were combined and washed with sat. $NaHCO_3$ (aq) (2 x 25 mL), H_2O (2 x 25 mL) and brine (2 x 25 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to leave dark brown viscous oil. Purification by flash column chromatography (0-2% MeOH/CH_2Cl_2) gave the title compound (474 mg, 62% (88% crude[1])) as a dark brown oil; Rf 0.18 (10% MeOH/CH_2Cl_2); IR ν_{max} (thin film from $CHCl_3$) 3398 (N-H), 1649 (C=N), 1524 (NO_2), 1348 (NO_2), 1048-994 (C-N), 824 (ArC-H), 762 (ArC-H) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 13.49 (1H, bs, N*H*), 8.39 (1H, d, *J* = 1.3, CC*H*CNH), 8.06 (1H, d, *J* = 8.8, C*H*CHCNO$_2$), 7.91 (1H, dd, *J* = 8.8, 2.0, CHC*H*CNO$_2$), 3.99 (2H, s, C*H*$_2$CNN), 2.51-2.47 (4H, m, 2 x C*H*$_2$N(CH$_2$)CH$_2$C), 1.71-1.67 (4H, m, 2 x C*H*$_2$CH$_2$N(CH$_2$)CH$_2$); ^{13}C NMR (125 MHz, DMSO-d6) δ 146.3 (Ar*C*), 144.4 (Ar*C*), 140.0 (Ar*C*), 125.5 (Ar*C*), 122.3 (*C*HCHCNO$_2$), 114.8 (CH*C*HCNO$_2$), 107.4 (C*C*HCNO$_2$), 54.0 (2 x *C*H$_2$N(CH$_2$)CH$_2$C), 51.8 (*C*H$_2$CNN), 23.7 (2 x *C*H$_2$CH$_2$N(CH$_2$)CH$_2$C); m/z (FAB+) 247 (100%, $[M+H]^+$); HRMS $C_{12}H_{15}N_4O_2$ calcd. 247.1195, found 247.1198.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2.47
2.08
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1.70
1.69
1.68
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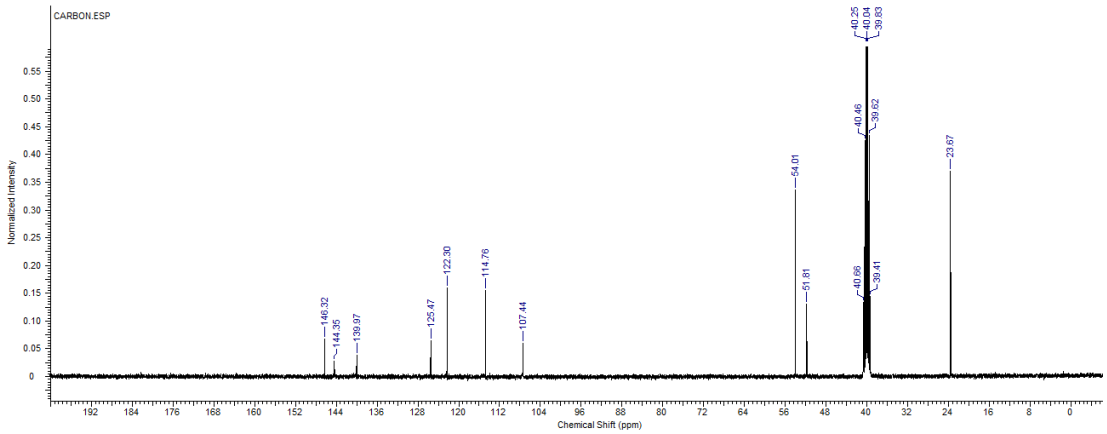
0.00
0.92
0.90
1.00
0.99
2.08
0.09
1.91
4.14


CARBON.ESP
Normalized Intensity
Chemical Shift (ppm)
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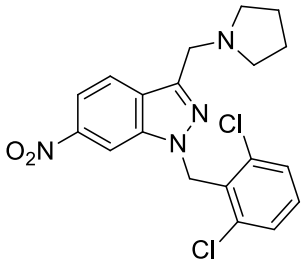
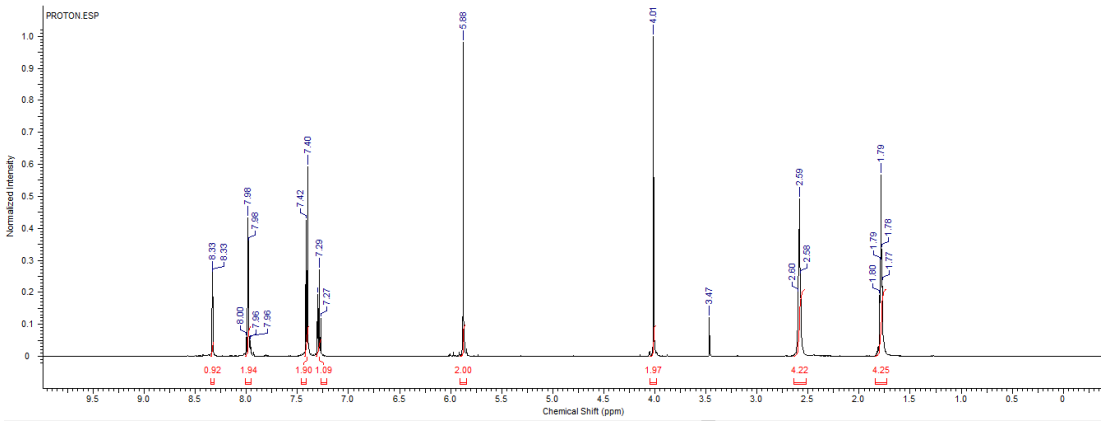
51.81
40.25
40.04
39.83
40.46
39.62
40.66
39.41
23.67

1-(2,6-Dichlorobenzyl)-6-nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole [2]



To a stirred solution of 6-nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole (468 mg, 1.87 mmol) in dry THF (20 mL) under argon was added 2,6-dichlorobenzyl bromide (449 mg, 1.87 mmol) followed by portionwise addition over 10 min of caesium carbonate (609 mg, 1.87 mmol). The reaction mixture was stirred at 16 °C for 24 h then diluted with H_2O (10 mL) and extracted with EtOAc (2 x 20 mL). Organic extracts were washed with H_2O (2 x 10 mL) then brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a dark brown solid residue. Purification by flash column chromatography (0-1% MeOH/CH_2Cl_2) gave the title compound (453 mg, 60% (66%[2])) as a brown solid; mp 136-138 °C (138-140 °C[2]); Rf 0.25 (5% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3081-2789 (C-H), 1521 (NO_2), 1438 (C-H), 1342 (NO_2), 1090 (C-N), 806-765 (ArC-H), 733 (C-Cl) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.33 (1H, d, *J* = 1.6, CC*H*CNH), 8.00-7.96 (1H, d, *J* = 8.8, C*H*CHCNO$_2$ & 1H, dd, *J* = 8.8, 1.6, CHC*H*CNO$_2$), 7.41 (2H, d, *J* = 7.9, 2 x C*H*CCl), 7.29 (1H, m, C*H*CHCCl), 5.88 (2H, s, C*H*$_2$NN), 4.01 (2H, s, C*H*$_2$CNN), 2.59 (4H, m, 2 x C*H*$_2$N(CH$_2$)CH$_2$C), 1.79 (4H, m, 2 x C*H*$_2$CH$_2$N(CH$_2$)CH$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.9 (Ar*C*), 144.5 (Ar*C*), 139.8 (Ar*C*), 137.3 (2 x *C*Cl), 131.2 (Ar*C*), 130.9 (*C*HCHCCl), 129.2 (2 x *C*HCCl), 126.9 (Ar*C*), 122.4 (*C*HCHCNO$_2$), 115.3 (CH*C*HCNO$_2$), 106.5 (C*C*HCNO$_2$), 54.6 (2 x *C*H$_2$N(CH$_2$)CH$_2$C), 52.3 (*C*H$_2$CNN), 49.1 (*C*H$_2$NN), 24.1 (2 x *C*H$_2$CH$_2$N(CH$_2$)CH$_2$C); m/z (CI+) 405 (100%, [^{35,35}M+H]$^+$), 407 (63%, [^{35,37}M+H]$^+$), 409 (12%, [^{37,37}M+H]$^+$); HRMS $C_{19}H_{19}{}^{35}Cl_2N_4O_2$ ([M+H]$^+$) calcd. 405.0885, found 405.0889.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1.80
1.79
1.79
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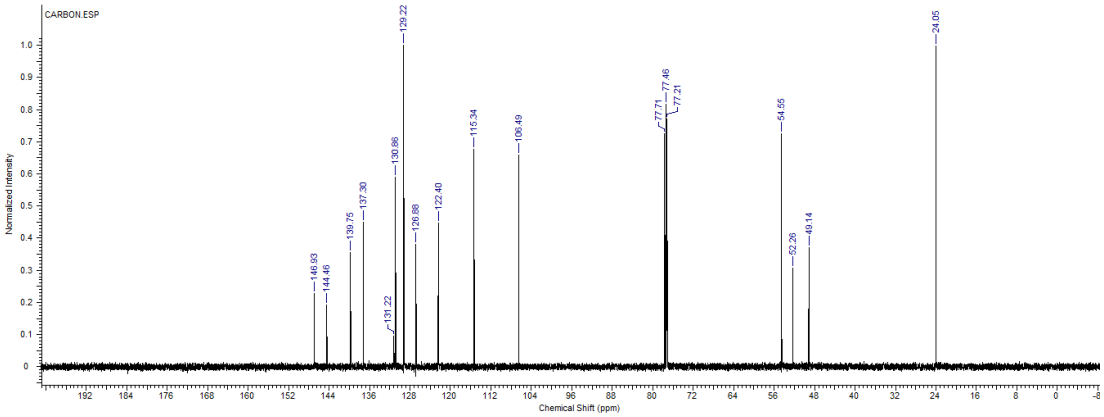
4.25
Chemical Shift (ppm)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122.40
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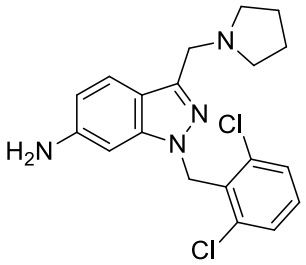
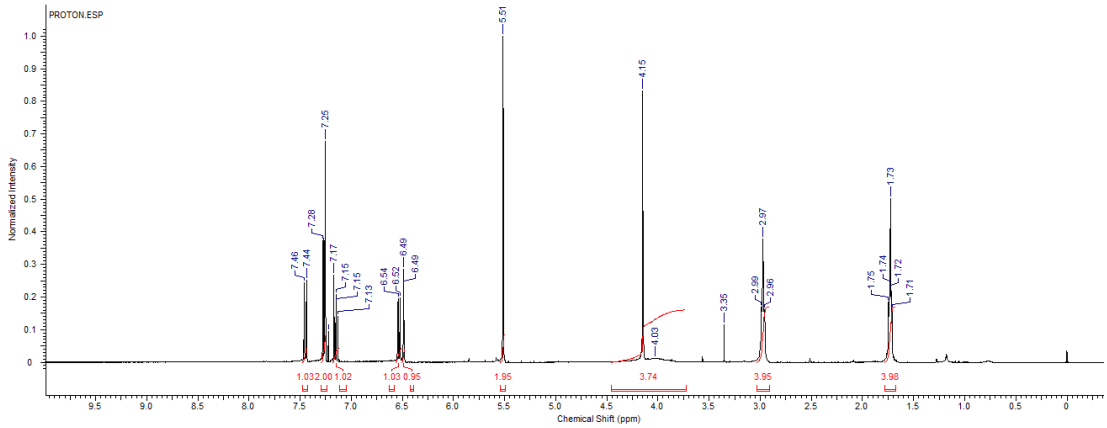
106.49
77.71
77.46
77.21
54.55
52.26
49.14
24.05
Chemical Shift (ppm)

1-(2,6-Dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazol-6-amine (4)[1]



To a solution of 1-(2,6-dichlorobenzyl)-6-nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole (539 mg, 1.33 mmol) in MeOH (40 mL) was added $FeCl_3.6H_2O$ (83.0 mg, 0.310 mmol) and activated charcoal (647 mg), followed by *N*,*N*-dimethylhydrazine (2.02 mL, 26.60 mmol). The reaction mixture was heated to reflux for 2 h then allowed to cool to ambient temperature before filtering through celite, washing through with a cold mixture of CH_2Cl_2/MeOH (90 mL, 4:1). The filtrate was concentrated *in vacuo* to leave a brown solid residue, which was purified by flash column chromatography (0-4% MeOH/CH_2Cl_2) to give target compound **4** (370 mg, 74% (60%[2])) as a light brown solid; mp 177-179 °C; Rf 0.1 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3323-3214 (N-H), 2961-2480 (C-H), 1626 (C=N), 1581-1498 (ArC=C), 1438 (C-H), 1090 (C-N), 816-760 (ArC-H), 730 (C-Cl) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (1H, d, *J* = 8.6, C*H*CHCNH$_2$), 7.27 (2H, m, 2 x C*H*CCl), 7.15 (1H, m, C*H*CHCl), 6.53 (1H, dd, *J* = 8.6, 1.8, CHC*H*CNH$_2$), 6.49 (1H, d, *J* = 1.5, CC*H*CNH$_2$), 5.51 (2H, s, C*H*$_2$NN), 4.15 (2H, s, C*H*$_2$CNN), 4.03 (2H, bs, N*H*$_2$), 2.97 (4H, m, 2 x C*H*$_2$N(CH$_2$)CH$_2$C), 1.73 (4H, bs, 2 x C*H*$_2$CH$_2$N(CH$_2$)CH$_2$C); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.6 (Ar*C*), 142.4 (Ar*C*), 136.82 (Ar*C*), 136.78 (2 x *C*Cl), 131.5 (Ar*C*), 130.1 (*C*HCHCCl), 128.6 (2 x *C*HCCl), 120.9 (*C*HCHCNH$_2$), 117.4 (Ar*C*), 113.4 (CH*C*HCNH$_2$), 91.9 (C*C*HCNH$_2$), 52.3 (2 x *C*H$_2$N(CH$_2$)CH$_2$C), 48.7 (*C*H$_2$CNN), 47.9 (*C*H$_2$NN), 23.7 (2 x *C*H$_2$CH$_2$N(CH$_2$)CH$_2$C); m/z (CI+) 375 (27%, $[^{35,35}M+H]^+$), 377 (17%, $[^{35,37}M+H]^+$), 379 (3%, $[^{37,37}M+H]^+$), 84 (100%, [1-methylpyrrolidine]$^+$), 305 (8%, [M+H-C_4H_8N]$^+$); HRMS $C_{19}H_{21}{}^{35}Cl_2N_4$ ([M+H]$^+$) calcd. 375.1143, found 375.1137.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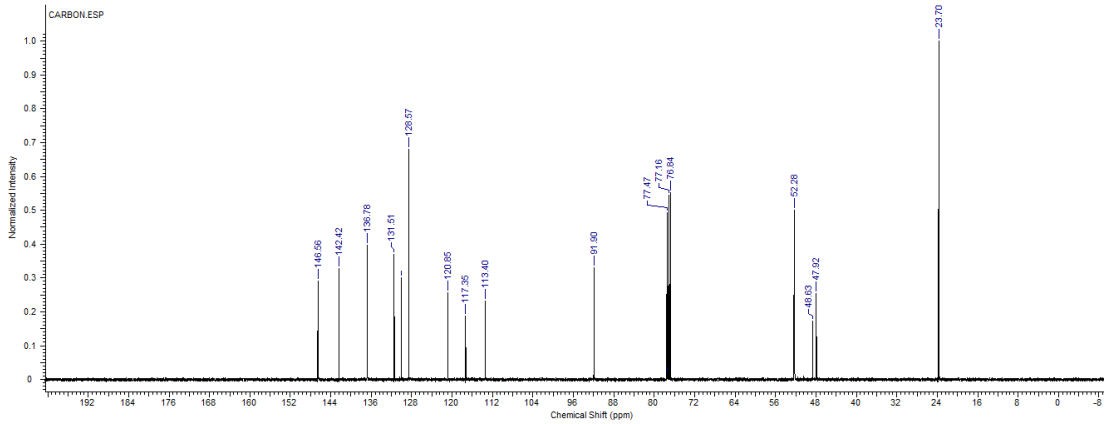
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Normalized Intensity
Chemical Shift (ppm)
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77.16
76.84
52.28
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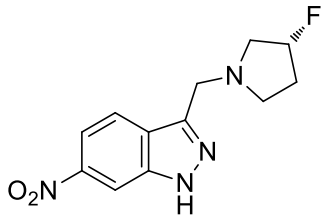
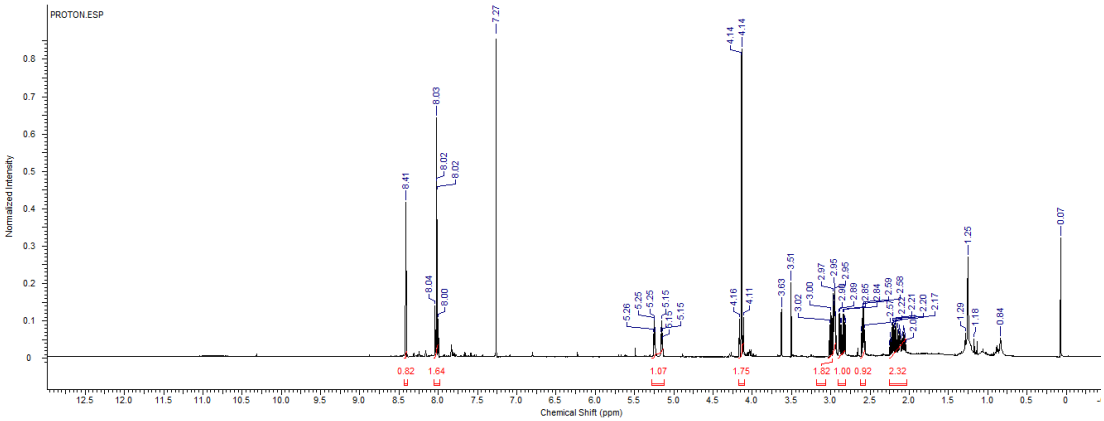
47.92
23.70

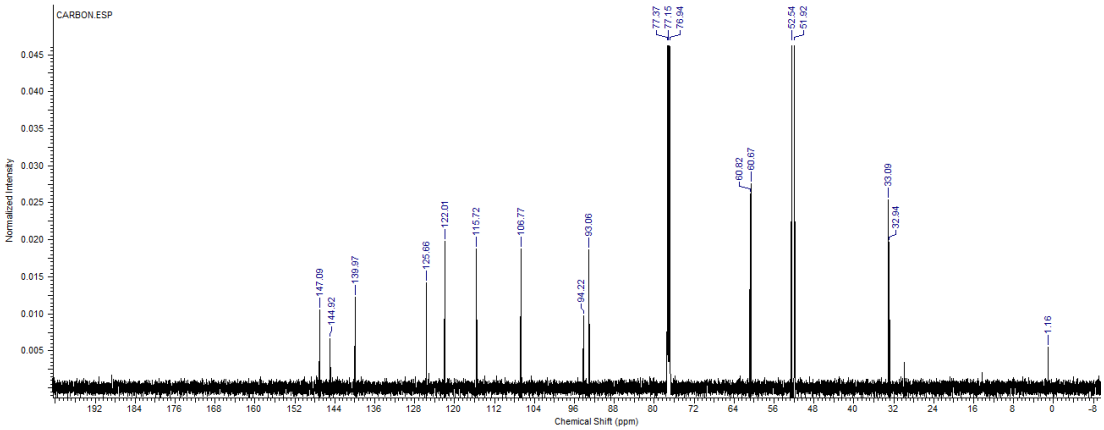
3-{[(3*R*)-3-Fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole



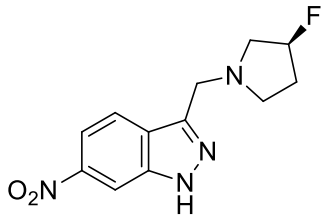
6-Nitroindole (500 mg, 3.08 mmol) was added to a stirred solution of sodium nitrite (2.13 g, 30.8 mmol) in H_2O (25 mL) and DMF (2 mL) at 18 °C. 6 M HCl (4.6 mL, 27.8 mmol) was added dropwise over 10 min and stirring continued for 3 h before diluting with EtOAc (30 mL) and extracting. Aqueous layer extracted further with EtOAc (2 x 15 mL). Organic extracts combined and washed with H_2O (15 mL) and brine (15 mL), then dried ($MgSO_4$) and concentrated *in vacuo*. Residual dark brown solid was dissolved in CH_2Cl_2/DMF/AcOH (54/5.4/0.6 mL), to which was added 3-*R*-(-)-fluoropyrrolidine.HCl (968 mg, 7.71 mmol) and the reaction mixture was stirred at 18 °C for 20 min. Sodium triacetoxyborohydride (1.63 g, 7.70 mmol) was added portionwise over 10 min and stirring continued for 3 h before diluting with EtOAc (100 mL) and quenching with sat. aqueous $NaHCO_3$ (60 mL). Aqueous layer separated and extracted further with EtOAc (2 x 20 mL). Organic extracts combined and washed with sat. $NaHCO_3$ (aq) (2 x 25 mL), H_2O (2 x 25 mL) and brine (2 x 25 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to leave dark brown oil. Purified by flash column chromatography (0.75% MeOH/CH_2Cl_2) gave the title compound (505 mg, 62%); as a dark brown oil; Rf 0.19 (5% MeOH/CH_2Cl_2); IR ν_{max} (thin film from $CHCl_3$) 3290 (N-H), 3059-2921 (C-H), 1664 (C=N), 1548 (NO_2), 1345 (NO_2), 1238 (C-N), 1114 (C-F), 1086 (C-N), 762-697 (ArC-H) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.41 (1H, d, *J* = 1.3, CC*H*CNO$_2$), 8.04-8.00 (1H, d, *J* = 8.9, C*H*CHCNO$_2$ & 1H, dd, *J* = 8.9, 1.5, CHC*H*CNO$_2$), 5.20 (1H, m, C*H*F), 4.15 & 4.12 (2H, ABq, *J* = 13.7, C*H*$_2$CNN), 3.02-2.94 (1H, m, NC(H)*H*CHF & 1H, m, NC(H)*H*CH$_2$CHF), 2.90-2.82 (1H, ddd, *J* = 29.9, 11.5, 5.1, NC(*H*)HCHF), 2.59 (1H, dt, *J* = 5.8, 8.3, NC(*H*)HCH$_2$CHF), 2.25-2.04 (2H, m, NCH$_2$C*H*$_2$CHF); ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.1 (Ar*C*), 144.9 (Ar*C*), 140.0 (Ar*C*), 125.7 (Ar*C*), 122.0 (*C*HCHCNO$_2$), 115.7 (CH*C*HCNO$_2$), 106.8 (C*C*HCNO$_2$), 94.2-93.1 (d, *J* = 176.1, *C*HF), 60.7 (d, *J* = 23.1, NCH$_2$CHF), 52.5 (NCH$_2$CH$_2$CHF), 51.9 (*C*H$_2$CNN), 33.0 (d, *J* = 22.0, NCH$_2$*C*H$_2$CHF); m/z (ESI+) 265 (30%, $[M+H]^+$), 217 (100%,

$[M+H+MeCN-C_4H_7FN]^+$; HRMS $C_{12}H_{14}FN_4O_2$ ($[M+H]^+$) calcd. 265.1101, found 265.1091.



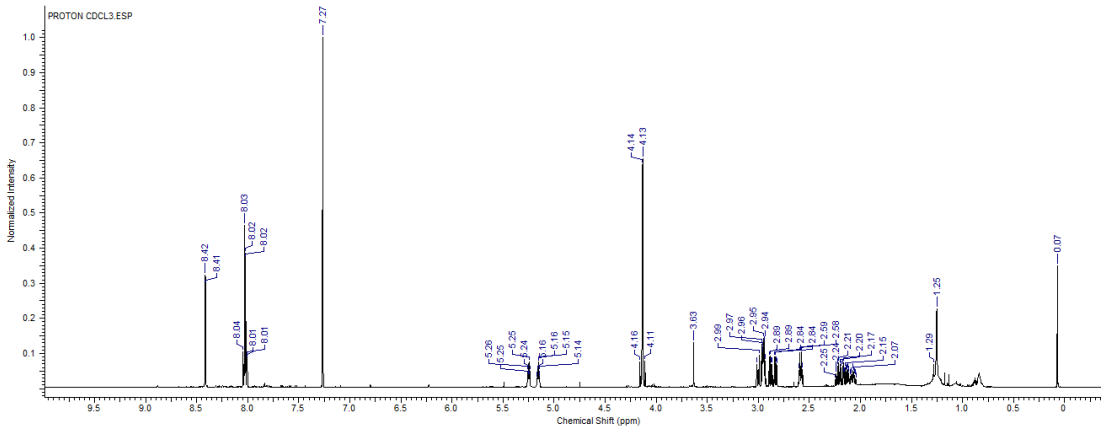


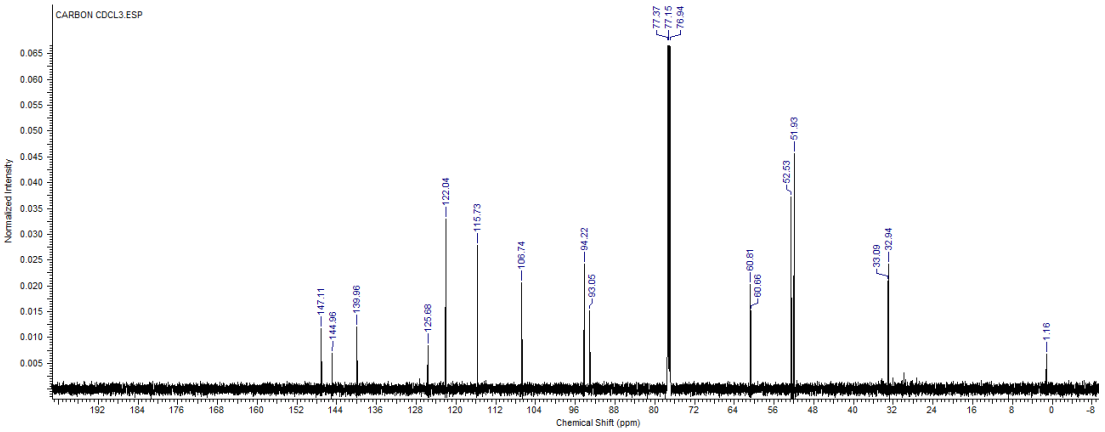
3-{[(3*S*)-3-Fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole



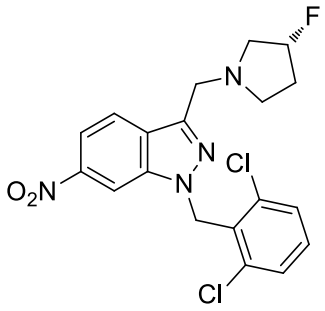
6-Nitroindole (500 mg, 3.08 mmol) was added to a stirred solution of sodium nitrite (2.13 g, 30.8 mmol) in H_2O (25 mL) and DMF (2 mL) at 18 °C. 6 M HCl (4.6 mL, 27.8 mmol) was added dropwise over 10 min and stirring continued for 3 h before diluting with EtOAc (30 mL) and extracting. Aqueous layer extracted further with EtOAc (2 x 15 mL). Organic extracts combined and washed with H_2O (15 mL) and brine (15 mL), then dried ($MgSO_4$) and concentrated *in vacuo*. Residual dark brown solid was dissolved in CH_2Cl_2/DMF/AcOH (54/5.4/0.6 mL), to which was added 3-S-(+)-fluoropyrrolidine.HCl (968 mg, 7.71 mmol) and the reaction mixture was stirred at 18 °C for 20 min. Sodium triacetoxyborohydride (1.63 g, 7.70 mmol) was added portionwise over 10 min and stirring continued for 3 h before diluting with EtOAc (100 mL) and quenching with sat. aqueous $NaHCO_3$ (60 mL). Aqueous layer separated and extracted further with EtOAc (2 x 20 mL). Organic extracts combined and washed with sat. $NaHCO_3$ (aq) (2 x 25 mL), H_2O (2 x 25 mL) and brine (2 x 25 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to leave dark brown oil. Purified by flash column chromatography (0.75% MeOH/CH_2Cl_2) gave the title compound (483 mg, 59%); as a dark brown oil; Rf 0.19 (5% MeOH/CH_2Cl_2); IR ν_{max} (thin film from $CHCl_3$) 3343 (N-H), 2947-2848 (C-H), 1710 (C=N), 1522 (NO_2), 1345 (NO_2), 1296-1173 (C-N), 1111 (C-F), 1079 (C-N), 794-696 (ArC-H) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.42 (1H, d, *J* = 1.5, CC*H*CNO_2), 8.05-8.01 (1H, d, *J* = 8.9, C*H*CHCNO_2 & 1H, dd, *J* = 8.9, 1.7, CHC*H*CNO_2), 5.20 (1H, m, C*H*F), 4.15 & 4.12 (2H, ABq, *J* = 13.8, CH_2CNN), 3.01-2.93 (1H, m, NC(H)*H*CHF & 1H, m, NC(H)*H*CH_2CHF), 2.89-2.82 (1H, ddd, *J* = 30.1, 11.7, 5.1, NC(*H*)HCHF), 2.59 (1H, dt, *J* = 5.8, 8.5, NC(*H*)HCH_2CHF), 2.25-2.04 (2H, m, NCH_2CH_2CHF); ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.1 (Ar*C*), 145.0 (Ar*C*), 140.0 (Ar*C*), 125.7 (Ar*C*), 122.0 (*C*HCHCNO_2), 115.7 (CH*C*HCNO_2), 106.7 (C*C*HCNO_2), 94.2-93.1 (d, *J* = 176.1, *C*HF), 60.7 (d, *J* = 23.1, N*C*H_2CHF), 52.5 (N*C*$H_2$$CH_2$CHF), 51.9 (*C*$H_2$CNN), 33.0 (d, *J* = 22.0, NCH_2*C*H_2CHF); m/z (ESI+) 265 (32%, $[M+H]^+$), 217 (100%,

[M+H+MeCN-C_4H_7FN]$^+$); HRMS $C_{12}H_{13}FN_4O_2$ ([M+H]$^+$) calcd. 265.1101, found 265.1111.

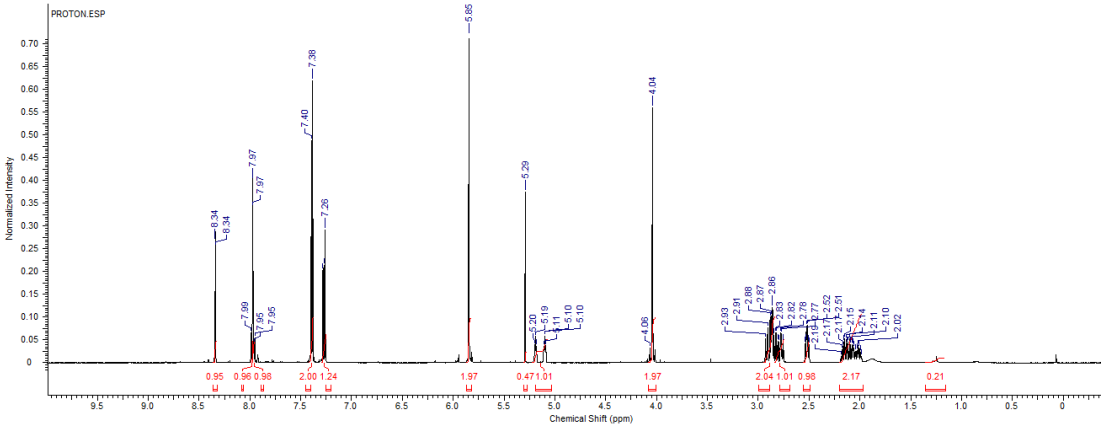




1-(2,6-Dichlorobenzyl)-3-{[(3*R*)-3-fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole



To a stirred solution of 3-{[(3*R*)-3-fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole (505 mg, 1.91 mmol) in dry THF (30 mL) under argon was added 2,6-dichlorobenzyl bromide (454 mg, 1.89 mmol) followed by portionwise addition over 10 min of caesium carbonate (616 mg, 1.89 mmol). The reaction mixture was stirred at 16 °C for 24 h then diluted with H_2O (10 mL) and extracted with EtOAc (2 x 20 mL). Organic extracts were washed with H_2O (2 x 10 mL) then brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo.* The residue was purified by flash column chromatography (0-0.25% MeOH/CH_2Cl_2) gave the title compound (630 mg, 79%); as a pale yellow solid; mp 134-136 °C; Rf 0.63 (5% MeOH/CH_2Cl_2); IR ν_{max} (neat) 2957-2812 (C-H), 1578 (C=N), 1515 (NO_2), 1427 (C-H), 1347 (NO_2), 1202 (C-N), 1139 (C-F), 1085 (C-N), 821-627 (ArC-H), 500 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34 (1H, d, *J* = 1.9, CC*H*CNO_2), 7.98 (1H, d, *J* = 8.7, C*H*CHCNO_2), 7.96 (1H, dd, *J* = 8.7, 1.9, CHC*H*CNO_2), 7.39 (1H, d, *J* = 7.9, C*H*CCl), 7.27 (1H, t, *J* = 8.3, C*H*CHCCl), 5.85 (2H, s, CH_2NN), 5.20-5.09 (1H, m, C*H*F), 4.04 (2H, d, *J* = 1.1, NCH_2CN), 2.93-2.84 (1H, m, NC(*H*)HCHF & 1H, m, NC(*H*)HCH_2), 2.83-2.75 (1H, ddd, *J* = 29.7, 11.7, 4.9, NC(H)*H*CH_2), 2.52 (1H, dt, *J* = 5.7, 8.7, NC(H)*H*CHF), 2.19-1.98 (2H, m, CH_2CH_2CHF); ^{13}C NMR (150 MHz; $CDCl_3$) δ 146.7 (Ar*C*), 143.5 (Ar*C*), 139.5 (Ar*C*), 137.0 (2 x *C*Cl), 130.9 (Ar*C*), 130.6 (*C*HCHCCl), 129.0 (2 x *C*HCCl), 126.4 (Ar*C*), 122.1 (*C*HCHCNO_2), 115.2 (CH*C*HCNO_2), 106.3 (C*C*HCNO_2), 94.2-93.1 (d, $J_{C\text{-}F}$ = 175.8, *C*HF), 60.5-60.4 (d, $J_{C\text{-}F}$ = 23.3, N*C*H_2CHF), 52.3 (N*C*H_2CH_2CHF), 51.6 (N*C*H_2CN), 48.8 (*C*H_2NN), 33.1-32.9 (d, $J_{C\text{-}F}$ = 22.1, CH_2*C*H_2CHF); m/z (CI+) 423 (100%, $[^{35,35}M+H]^+$), 425 (62%, $[^{35,37}M+H]^+$), 427 (10%, $[^{37,37}M+H]^+$); HRMS $C_{19}H_{18}{}^{35}Cl_2FN_4O_2$ ($[M+H]^+$) calcd. 423.0791, found. 423.0793.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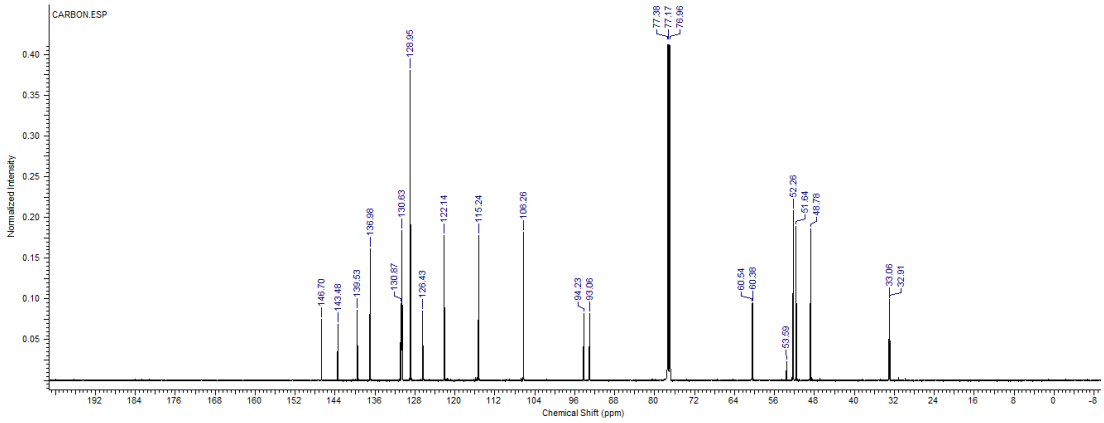
CARBON.ESP
Normalized Intensity
Chemical Shift (ppm)
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93.06
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77.17
76.96
60.54
60.36
53.59
52.26
51.64
48.78
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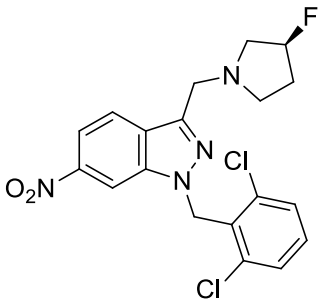
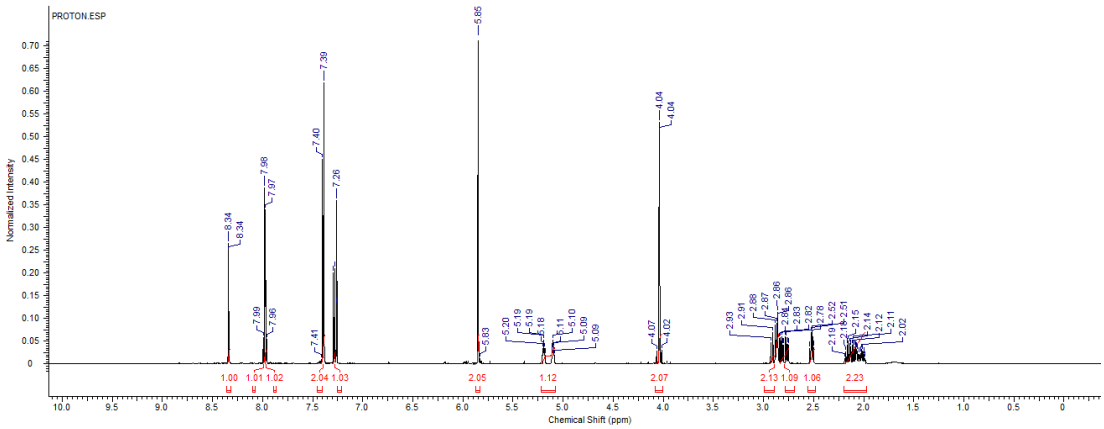
32.91

1-(2,6-Dichlorobenzyl)-3-{[(3*S*)-3-fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole



To a stirred solution of 3-{[(3*S*)-3-fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole (483 mg, 1.83 mmol) in dry THF (30 mL) under argon was added 2,6-dichlorobenzyl bromide (454 mg, 1.89 mmol) followed by portionwise addition over 10 min of caesium carbonate (616 mg, 1.89 mmol). The reaction mixture was stirred at 16 °C for 24 h then diluted with H_2O (10 mL) and extracted with EtOAc (2 x 20 mL). Organic extracts were washed with H_2O (2 x 10 mL) then brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash column chromatography (0-0.25% MeOH/CH_2Cl_2) gave the title compound (536 mg, 69%); as a yellow solid; mp 134-136 °C; Rf 0.63 (5% MeOH/CH_2Cl_2); IR ν_{max} (neat) 2955-2811 (C-H), 1562 (C=N), 1515 (NO_2), 1427 (C-H), 1346 (NO_2), 1202 (C-N), 1139 (C-F), 1085 (C-N), 802-627 (ArC-H), 500 (C-Cl) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.34 (1H, dd, *J* = 1.7, 0.8, NCC*H*C), 7.99 (1H, dd, *J* = 8.7, 0.8, NCCHC*H*C), 7.97 (1H, dd, *J* = 8.7, 1.7, NCC*H*CHC), 7.39 (2H, d, *J* = 8.1, 2 x C*H*CCl), 7.27 (1H, dd, *J* = 8.3, 7.7, C*H*CHCCl), 5.85 (2H, s, CH_2NN), 5.20-5.09 (1H, m, C*H*F), 4.04 (2H, d, *J* = 1.5, NCH_2CN), 2.93-2.84 (1H, m, NC(H)*H*CHF & 1H, m, C(H)*H*CH_2CHF), 2.83-2.75 (1H, ddd, *J* = 29.7, 11.7, 5.1, NC(*H*)HCHF), 2.52 (1H, dt, *J* = 5.7, 8.7, C(*H*)HCH_2CHF), 2.19-1.99 (2H, m, CH_2CH_2CHF); ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.7 (Ar*C*), 143.5 (Ar*C*), 139.5 (Ar*C*), 137.0 (2 x *C*Cl), 130.9 (Ar*C*), 130.6 (*C*HCHCCl), 129.0 (2 x *C*HCCl), 126.4 (Ar*C*), 122.1 (*C*HCHCNO$_2$), 115.2 (CH*C*HCNO$_2$), 106.3 (C*C*HCNO$_2$), 94.2-93.1 (d, $J_{C\text{-}F}$ = 175.8, *C*HF), 60.5 (d, $J_{C\text{-}F}$ = 23.3, N*C*H$_2$CHF), 52.3 (N*C*H$_2$CH$_2$CHF), 51.7 (N*C*H$_2$CN), 48.8 (*C*H$_2$NN), 33.0 (d, $J_{C\text{-}F}$ = 22.1, CH$_2$*C*H$_2$CHF); m/z (ESI+) 423 (100%, $[^{35,35}M+H]^+$), 425 (75%, $[^{35,37}M+H]^+$), 427 (15%, $[^{37,37}M+H]^+$); HRMS $C_{19}H_{18}{}^{35}Cl_2FN_4O_2$ ($[M+H]^+$) calcd. 423.0791, found 423.0810.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4.04
4.04
4.02
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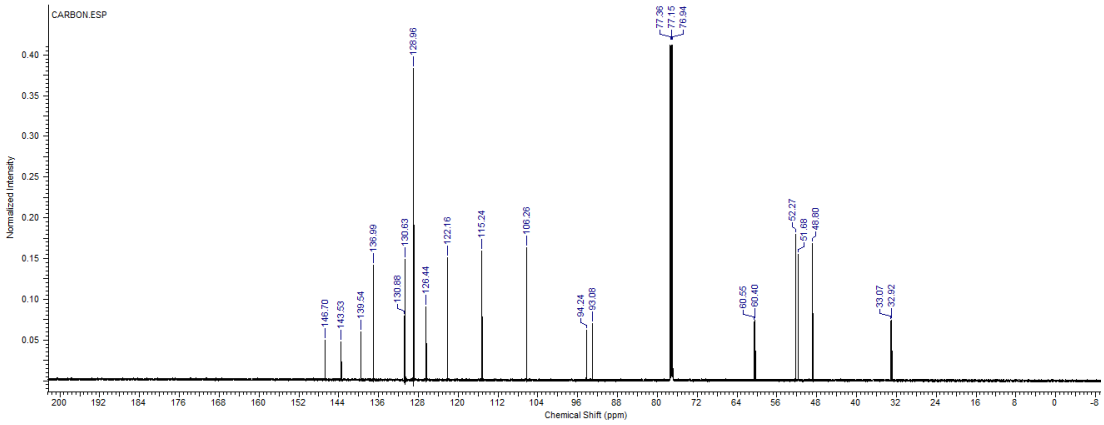
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Chemical Shift (ppm)
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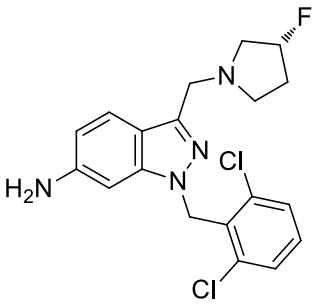
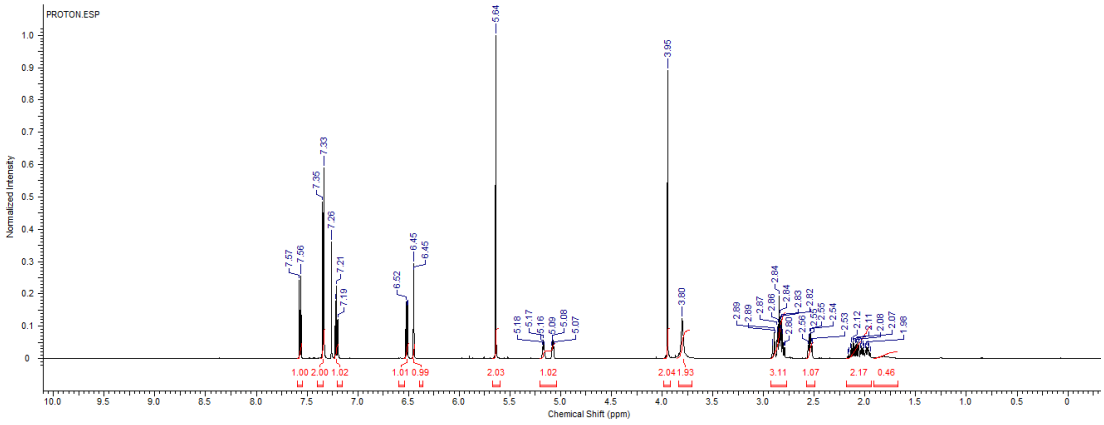
94.24
93.08
77.36
77.15
76.94
60.55
60.40
52.27
51.68
48.80
33.07
32.92

(*R*)-1-(2,6-Dichlorobenzyl)-3-[(3-fluoropyrrolidin-1-yl)methyl]-1H-indazol-6-amine (5)



To a solution of 1-(2,6-dichlorobenzyl)-3-{[(3*R*)-3-fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole (610 mg, 1.44 mmol) in MeOH (40 mL) was added $FeCl_3.6H_2O$ (78.0 mg, 0.290 mmol) and activated charcoal (784 mg), followed by *N*,*N*-dimethylhydrazine (2.19 mL, 28.8 mmol). The reaction mixture was heated to reflux for 2 h then allowed to cool to ambient temperature before filtering through celite, washing through with a cold mixture of CH_2Cl_2/MeOH (90 mL, 4:1). The filtrate was concentrated *in vacuo* to leave a brown solid residue, which was purified by flash column chromatography (0-1% MeOH/CH_2Cl_2) to give desired product **5** (408 mg, 72%); as a pale yellow solid; mp 158-160 °C; Rf 0.09 (5% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3438-3209 (N-H), 2956-2786 (C-H), 1625 (C=N), 1564-1496 (ArC=C), 1435 (C-H), 1292-1088 (C-N), 803-688 (ArC-H), 594 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (1H, d, *J* = 8.7, C*H*CHCNH$_2$), 7.34 (2H, d, *J* = 8.3, 2 x C*H*CCl), 7.21 (1H, t, *J* = 7.9, C*H*CHCCl), 6.52 (1H, dd, *J* = 8.7, 1.9, CHC*H*CNH$_2$), 6.45 (1H, d, *J* = 1.9, CC*H*CNH$_2$), 5.64 (2H, s, C*H*$_2$NN), 5.18-5.07 (1H, m, C*H*F), 3.95 (2H, s, NC*H*$_2$CN), 3.80 (2H, bs, N*H*$_2$), 2.91-2.79 (2H, m, NC*H*$_2$CHF & 1H, m, NC(*H*)HCH$_2$), 2.54 (1H, m, NC(H)*H*CH$_2$), 2.17-1.95 (2H, m, CH$_2$C*H*$_2$CHF); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.7 (Ar*C*), 142.4 (Ar*C*), 142.3 (Ar*C*), 137.0 (2 x *C*Cl), 131.9 (Ar*C*), 129.9 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 121.9 (*C*HCHCNH$_2$), 117.6 (Ar*C*), 112.1 (CH*C*HCNH$_2$), 93.3-94.4 (d, $J_{C\text{-}F}$ = 175.2, *C*HF), 92.5 (C*C*HCNH$_2$), 60.3 (d, $J_{C\text{-}F}$ = 23.3, NCH$_2$CHF), 52.0 (NCH$_2$CH$_2$CHF), 51.4 (NCH$_2$CN), 48.4 (*C*H$_2$NN), 33.0 (d, $J_{C\text{-}F}$ = 22.1, CH$_2$*C*H$_2$CHF); m/z (ESI+) 415 (20%, $[^{35,35}M+Na]^+$), 304 (100%, $[^{35,35}M\text{-}C_4H_7FN]^+$) , 306 (70%, $[^{35,37}M\text{-}C_4H_7FN]^+$) , 308 (13%, $[^{37,37}M\text{-}C_4H_7FN]^+$); HRMS $C_{19}H_{19}{}^{35}Cl_2FN_4Na$ ($[M+Na]^+$) calcd. 415.0869, found 415.0851.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2.82
2.55
2.54
2.53
2.12
2.11
2.08
2.07
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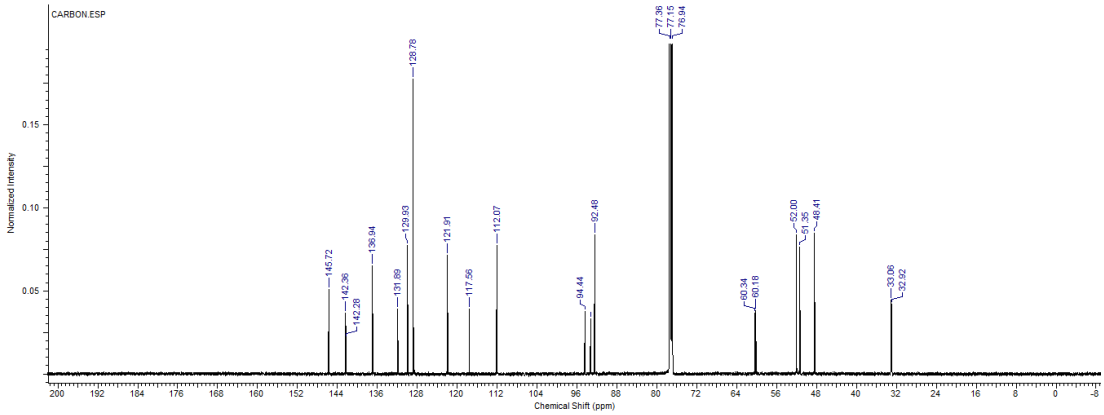
CARBON.ESP
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Chemical Shift (ppm)
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129.93
121.91
117.56
112.07
92.48
84.44
60.34
60.18
52.00
51.35
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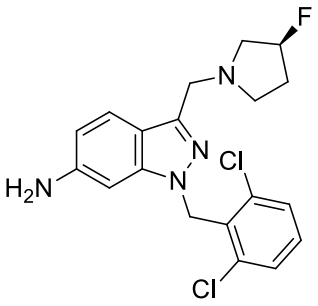
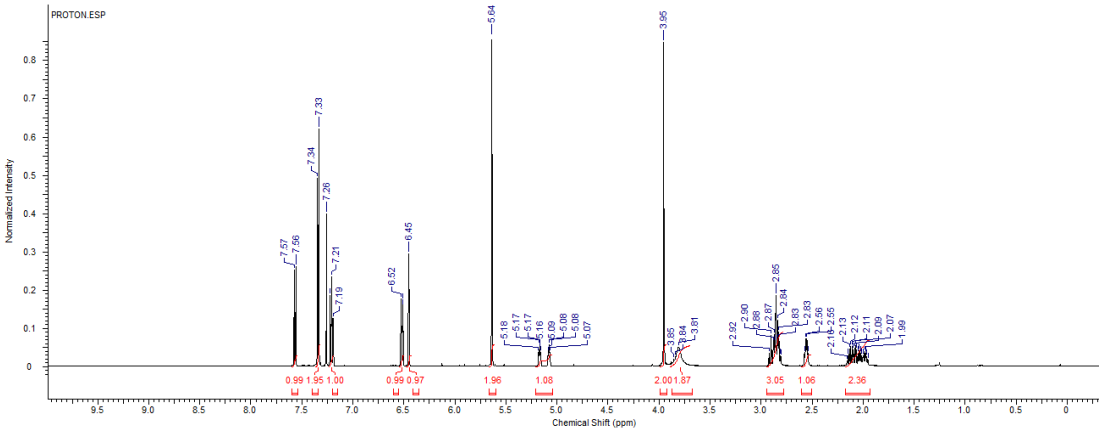
(*S*)-1-(2,6-Dichlorobenzyl)-3-[(3-fluoropyrrolidin-1-yl)methyl]-1H-indazol-6-amine (6)



To a solution of 1-(2,6-dichlorobenzyl)-3-{[(3*S*)-3-fluoropyrrolidin-1-yl]methyl}-6-nitro-1*H*-indazole (520 mg, 1.23 mmol) in MeOH (40 mL) was added $FeCl_3.6H_2O$ (68.0 mg, 0.250 mmol) and activated charcoal (669 mg), followed by *N*,*N*-dimethylhydrazine (1.87 mL, 24.6 mmol). The reaction mixture was heated to reflux for 2 h then allowed to cool to ambient temperature before filtering through celite, washing through with a cold mixture of CH_2Cl_2/MeOH (90 mL, 4:1). The filtrate was concentrated *in vacuo* to leave a brown solid residue, which was purified by flash column chromatography (0-1% MeOH/CH_2Cl_2) to give desired product **6** (375 mg, 78%); as a pale yellow solid; mp 158-160 °C; Rf 0.09 (5% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3438-3209 (N-H), 2956-2786 (C-H), 1625 (C=N), 1564-1496 (ArC=C), 1435 (C-H), 1292-1087 (C-N), 803-689 (ArC-H), 594 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (1H, d, *J* = 8.7, C*H*CHCNH$_2$), 7.34 (2H, d, *J* = 7.9, 2 x C*H*CCl), 7.21 (1H, t, *J* = 8.1, C*H*CHCCl), 6.52 (1H, dd, *J* = 8.5, 1.7, CHC*H*CNH$_2$), 6.45 (1H, d, *J* = 1.5, CC*H*CNH$_2$), 5.64 (2H, s, C*H*$_2$NN), 5.18-5.07 (1H, m, C*H*F), 3.95 (2H, s, NC*H*$_2$CN), 3.81 (2H, bs, N*H*$_2$), 2.92-2.80 (2H, m, NC*H*$_2$CHF & 1H, m, NC(*H*)HCH$_2$), 2.56 (1H, m, NC(H)*H*CH$_2$), 2.16-1.95 (2H, m, CH$_2$C*H*$_2$CHF); ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.7 (Ar*C*), 142.4 (Ar*C*), 142.3 (Ar*C*), 137.0 (2 x *C*Cl), 131.9 (Ar*C*), 129.9 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 121.9 (*C*HCHCNH$_2$), 117.6 (Ar*C*), 112.1 (CH*C*HCNH$_2$), 94.5-93.3 (d, $J_{C\text{-}F}$ = 175.2, *C*HF), 92.5 (C*C*HCNH$_2$), 60.3 (d, $J_{C\text{-}F}$ = 22.7, NCH$_2$CHF), 52.0 (NCH$_2$CH$_2$CHF), 51.4 (NCH$_2$CN), 48.4 (*C*H$_2$NN), 33.0 (d, $J_{C\text{-}F}$ = 21.5, CH$_2$*C*H$_2$CHF); m/z (ESI+) 415 (25%, [^{35,35}M+Na]$^+$), 304 (100%, [^{35,35}M-C_4H_7FN]$^+$) , 306 (87%, [^{35,37}M-C_4H_7FN]$^+$) , 308 (8%, [^{37,37}M-C_4H_7FN]$^+$); HRMS $C_{19}H_{19}{}^{35}Cl_2FN_4Na$ ([M+Na]$^+$) calcd. 415.0869, found 415.0865.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2.85
2.84
2.83
2.63
2.56
2.55
2.13
2.12
2.11
2.09
2.07
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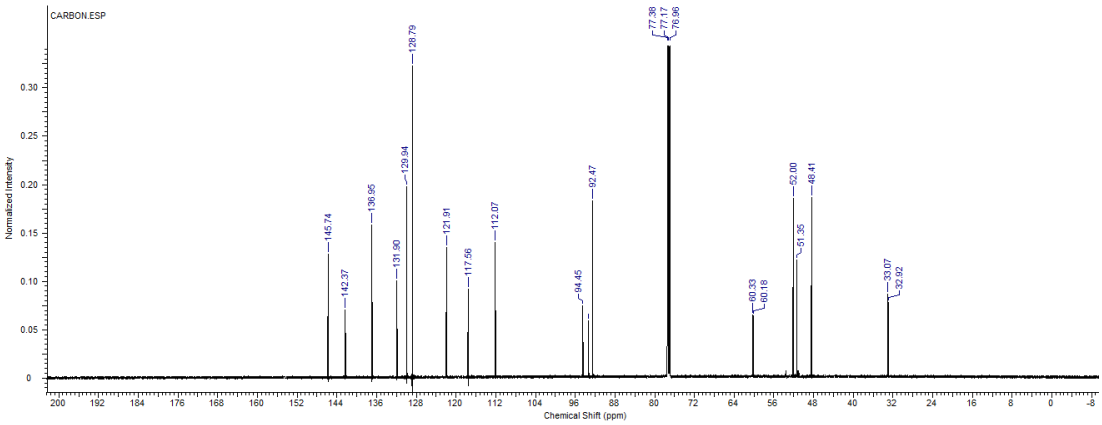
1.06
2.36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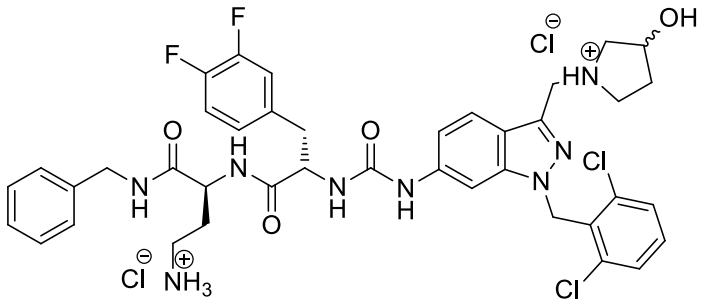
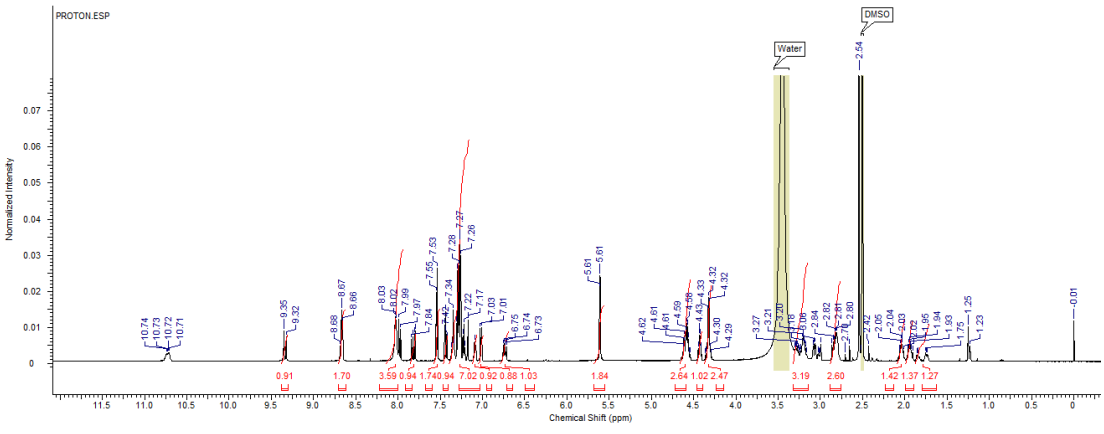
92.47
60.53
60.18
52.00
51.35
48.41
33.07
32.92

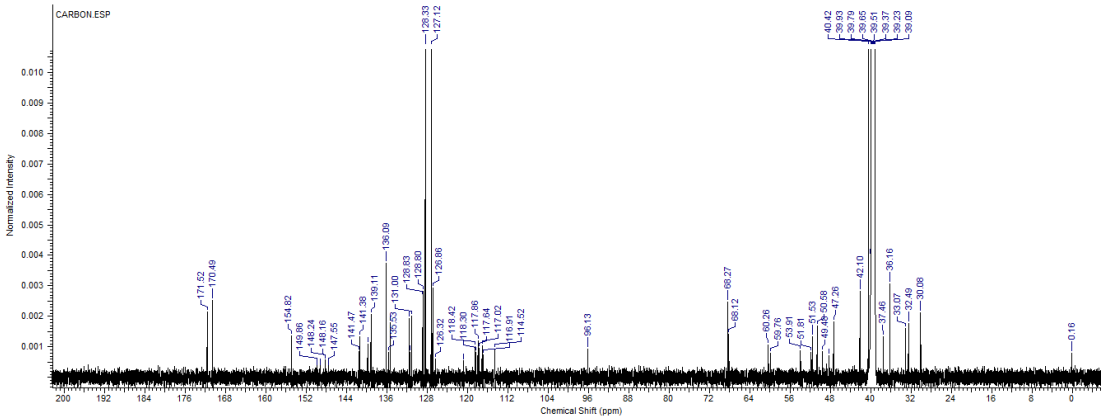
Racemic mixture of (*S*)-4-amino-*N*-benzyl-2-{[*S*]-2-[3-(1-{2,6-dichlorobenzyl}-3-{[(*S*)-3-hydroxypyrrolidin-1-yl]methyl}-1*H*-indazol-6-yl)ureido]-3-[3,4-difluorophenyl]propanamido}butanamide dihydrochloride salt & (*S*)-4-amino-*N*-benzyl-2-{[*S*]-2-[3-(1-{2,6-dichlorobenzyl}-3-{[(*R*)-3-hydroxypyrrolidin-1-yl]methyl}-1*H*-indazol-6-yl)ureido]-3-[3,4-difluorophenyl]propanamido}butanamide dihydrochloride salt (7)



To a sonicated (5 min), then stirred solution of **3** (60.0 mg, 0.153 mmol) in dry THF (8 mL), under dry conditions and cooled to 0 °C, was added triphosgene (16.0 mg, 54.0 μmol) and DMAP (37.0 mg, 306 μmol) under argon. After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **2** (38.0 mg, 77.0 μmol) in dry THF (4 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under argon then concentrated *in vacuo* and the residue purified by flash column chromatography (0-3% MeOH/CH_2Cl_2) to give the Boc-protected intermediate (27.0 mg). The off-white solid was suspended in a solution of HCl (4 N) in dioxane and stirred for 2.5 h before concentrating *in vacuo* and purifying by preparative RP-HPLC (retention time: 7.81 min). The isolated product was taken up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **7** (12.0 mg, 19%); as a white solid; mp 182-186 °C (decomp.); Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3273 (O-H), 3080 (N-H), 2958-2848 (C-H), 1634 (C=O), 1584 (C=N), 1539-1493 (ArC=C), 1434 (C-H), 1280-1086 (C-N), 769-696 (ArC-H), 608 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.72 (1H, m, N^+HCH_2CNN), 9.35 & 9.32 (1H, 2 x s, N*H*CONHCH), 8.68-8.66 (1H, m, N*H*CHCH$_2$CH$_2$ & 1H, m, N*H*CH$_2$Ph), 8.03 (3H, m, N^+H_3), 7.99 & 7.97 (1H, 2 x s, CC*H*CNH), 7.83 & 7.80 (1H, 2 x d, *J* = 8.9 & 8.7, CC*H*CHCNH), 7.54 (2H, m, 2 x C*H*CCl), 7.43 (1H, m, C*H*CHCCl), 7.37-7.17 (7H, m, ArH), 7.09 (1H, m, ArH), 7.02 & 7.01 (1H, 2 x d, *J* = 8.9 & 8.7, CCHC*H*CNH), 6.75 & 6.72 (1H, 2 x d, *J* = 7.7 &

7.7, N*H*CONHC), 5.61 (2H, m, CH_2NN), 4.65-4.54 (2H, m, CH_2CNN & 1H, m, C*H*NHCONH), 4.43 (1H, dt, *J* = 6.0, 7.9, C*H*CH_2CH_2), 4.36-4.29 (2H, m, NHCH_2Ph & 1H, m C*H*OH), 3.48 & 3.20 (0.5H & 0.5H, m, Pyr-C(5)H_2), 3.47 & 3.01 (0.5H & 0.5H, m, Pyr-C(2)H_2), 3.41 & 3.26 (0.5H & 0.5H, m, Pyr-C(5')H_2), 3.19 (0.5H & 0.5H, m, Pyr-C(2')H_2), 3.07 (1H, m, C(H)*H*CHNHCONH), 2.86-2.78 (1H, m, C(*H*)HCHNHCONH & 2H, m CH_2CH_2CHNH), 2.08-1.91 (2H, m, CH2CHNHCONH), 2.02 & 1.74 (0.5H & 0.5H, m, Pyr-C(4)H_2), 1.94 & 1.84 (0.5H & 0.5H, m, Pyr-C(4')H_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.5 (NH*C*OC), 170.5 (NH*C*OC), 154.82 & 154.81 (NH*C*ONH), 149.9-149.1 (dd, *J* = 104.5, 12.1, *C*F), 148.2-147.5 (dd, *J* = 104.5, 12.1, *C*F), 141.5 & 141.4 (Ar*C*), 139.7 (Ar*C*), 139.1 (Ar*C*), 136.1 (2 x *C*Cl), 135.5 & 135.3 (Ar*C*), 131.5 & 131.4 (Ar*C*), 131.0 (*C*HCHCCl), 128.83 (*C*HCCl), 128.80 (*C*HCCl), 128.3 (2 x Ar*C*H), 127.1 (2 x Ar*C*H), 126.9 (Ar*C*H), 126.3 (Ar*C*H), 120.8 & 120.7 (C*C*HCHCNH), 118.4 (d, *J* = 17.6, Ar*C*), 117.9 (Ar*C*), 117.6 (Ar*C*), 117.0 (d, *J* = 16.5, Ar*C*), 114.5 (CCH*C*HCNH), 96.1 (C*C*HCNH), 68.3 (Pyr-*C*(3)HOH), 68.1 (Pyr-*C*(3')HOH), 60.3 (Pyr-*C*(2')H_2), 59.8 (Pyr-*C*(2)H_2), 53.9 (*C*HNHCONH), 51.8 (Pyr-*C*(5')H_2), 51.5 (Pyr-*C*(5)H_2), 50.6 (*C*HCH_2CH_2), 49.5 & 48.3 (*C*H_2CNN), 47.33 & 47.26 (*C*H_2NN), 42.1 (*C*H_2Ph), 37.5 (*C*H_2CHNHCONH), 36.2 (*C*H_2CH_2CH), 33.1 (Pyr-*C*(4)H_2), 32.5 (Pyr-*C*(4')H_2), 30.1 (*C*H_2CHNHCOCH); m/z (ESI+) 807 (100%, [^{35,35}M+H]$^+$), 809 (71%, [^{35,37}M+H]$^+$), 811 (15%, [^{37,37}M+H]$^+$), 829 (20%, [M+Na]$^+$).

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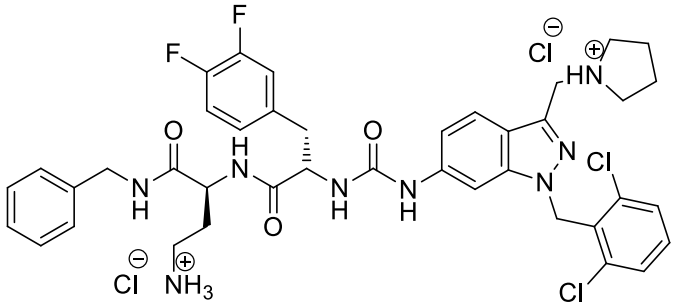
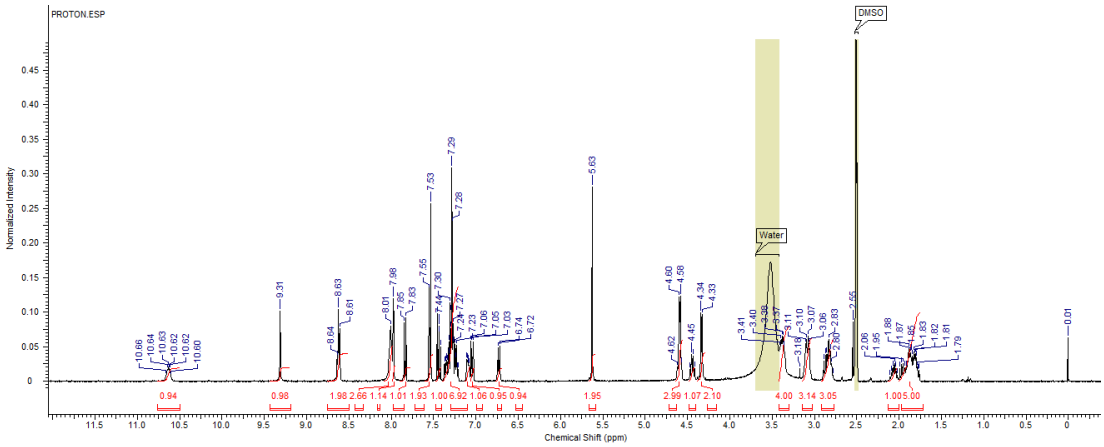
50.58
49.48
47.26
42.10
37.46
36.16
33.07
32.49
30.08
0.16

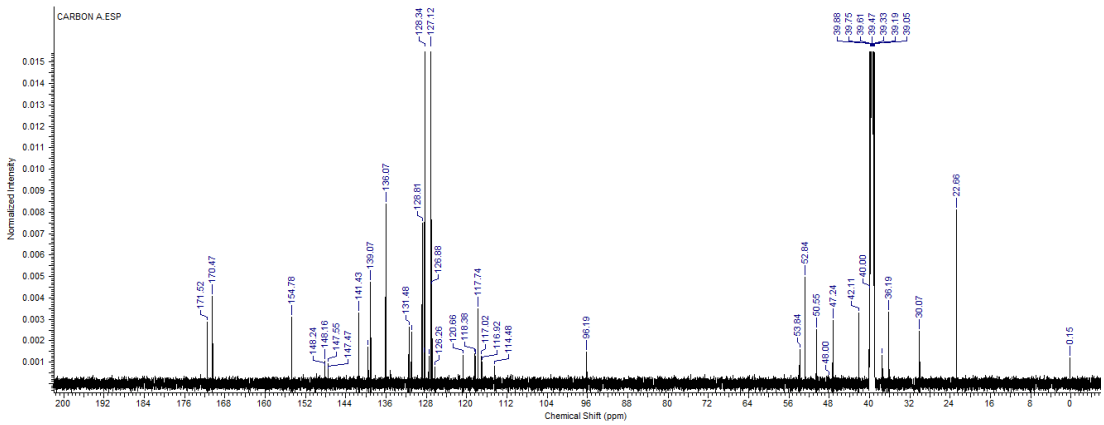
(*S*)-4-Amino-*N*-benzyl-2-({*S*}-2-{3-[1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazol-6-yl]ureido}-3-{3,4-difluorophenyl}propanamido)butanamide dihydrochloride salt (8)



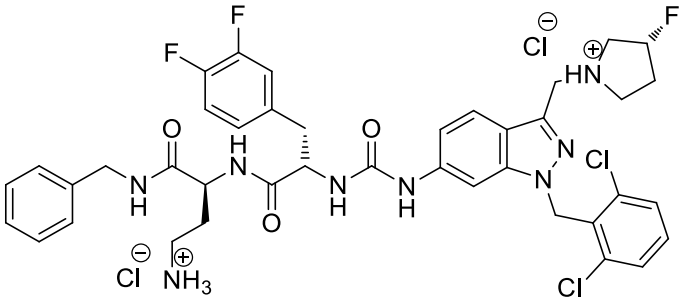
To a sonicated (5 min), then stirred solution of **4** (40.0 mg, 0.106 mmol) in dry THF (9 mL), under dry conditions and cooled to 0 °C, was added together triphosgene (11.0 mg, 37.1 μmol) and DMAP (26.0 mg, 212 μmol). After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min under argon. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **2** (26.0 mg, 53.0 μmol) in dry THF (4 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under argon then concentrated *in vacuo* and the residue purified by flash column chromatography (3-5% MeOH.NH_3/CH_2Cl_2) to give the Boc-protected intermediate (25.0 mg), Rf 0.35 (10% MeOH/CH_2Cl_2). The off-white solid was suspended in a solution of HCl (4N) in dioxane (2 mL) and stirred for 2.5 h before concentrating *in vacuo*, taking up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **8** (20.0 mg, 53%); as a white solid; mp 176-184 °C; Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3280 (N-H), 3059 (N-H), 2926 (C-H), 1634 (C=O), 1585 (C=O), 1537 (C=O), 1435 (C-H), 1209 (C-N), 769 (C-Cl), 696 (ArC-H) cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.43 (1H, bs, N^+*H*CH_2CNN), 9.26 (1H, s, N*H*CONHCH), 8.64-8.63 (1H, m, N*H*CH_2Ph & 1H, m, CHN*H*COCH), 7.97 (1H, s, CC*H*CNH), 7.95 (3H, bs, N^+H_3), 7.80 (1H, d, *J* = 8.7, CC*H*CHCNH), 7.54 (2H, d, *J* = 8.1, 2 x C*H*CCl), 7.43 (1H, m, C*H*CHCCl), 7.35-7.21 (7H, m, ArH), 7.09-7.07 (1H, m, ArH), 7.02 (1H, dd, *J* = 8.7, 1.1, CHC*H*CNH), 6.67 (1H, d, *J* = 7.7, CHN*H*CONH), 5.61 (2H, s, CH_2NN), 4.60-4.55 (1H, m, C*H*NHCONH & 2H, m, CH_2CNN), 4.42 (1H, apparent dt, *J* = 6.0, 7.9, C*H*NHCOCH), 4.33 (2H, m, CH_2NHCO), 3.38 (2H distorted by H_2O peak, m, 2 x C(H)*H*N^+H(CH_2)CH_2C), 3.11-3.06 (2H, m, 2 x C(*H*)HN^+H(CH_2)CH_2C & 1H, m, C(H)*H*CHNHCONH), 2.89-2.78 (1H, m, C(*H*)HCHNHCONH & 2H, m, C$H_2$$CH_2$CHNH), 2.11-1.76 (2H, m, CH_2CHNHCOCH & 4H, m, 2 x

C*H*$_2$CH$_2$N$^+$H(CH$_2$)CH$_2$C); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 171.5 (NH*C*OCH), 170.5 (NH*C*OCH), 154.8 (NH*C*ONH), 148.2 (d, J_{CF} = 12.5, *C*F), 147.5 (d, J_{CF} = 12.5, *C*F), 141.4 (Ar*C*), 139.6 (Ar*C*), 139.1 (Ar*C*), 136.1 (2 x *C*Cl), 131.5 (Ar*C*), 131.0 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 128.5 (Ar*C*), 128.3 (2 x Ar*C*H), 127.5 (Ar*C*), 127.1 (2 x Ar*C*H), 126.9 (Ar*C*H), 126.3 (Ar*C*H), 120.7 (*C*HCHCNH), 118.3 (d, *J* = 16.7, Ar*C*H), 117.7 (Ar*C*), 117.0 (d, *J* = 16.1, Ar*C*H), 114.5 (CH*C*HCNH), 96.2 (C*C*HCNH), 53.8 (*C*HNHCONH), 52.8 (2 x *C*H$_2$N$^+$H(CH$_2$)CH$_2$C), 50.6 (*C*HNHCOCH), 48.0 (*C*H$_2$CNN), 47.2 (*C*H$_2$NN), 42.1 (*C*H$_2$NHCO), 37.5 (*C*H$_2$CHNHCONH), 36.2 (*C*H$_2$CH$_2$CHNH), 30.1 (*C*H$_2$CHNHCOCH), 22.7 (2 x *C*H$_2$CH$_2$N$^+$H(CH$_2$)CH$_2$C); m/z (ESI+) 791 (100%, [^{35,35}M+H]$^+$), 793 (80%, [^{35,37}M+H]$^+$), 795 (9%, [^{37,37}M+H]$^+$); HRMS $C_{40}H_{43}{}^{35}Cl_2F_2N_8O_3$ calcd. 791.2803, found 791.2819.



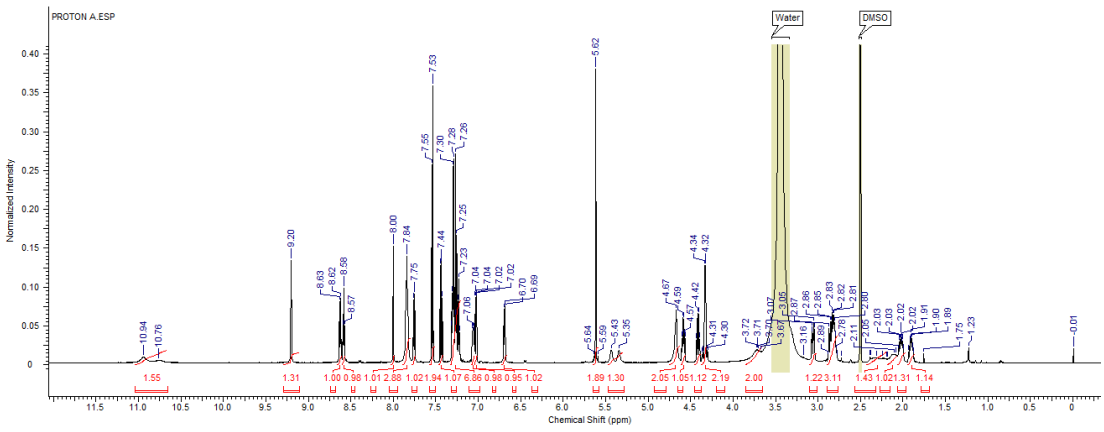


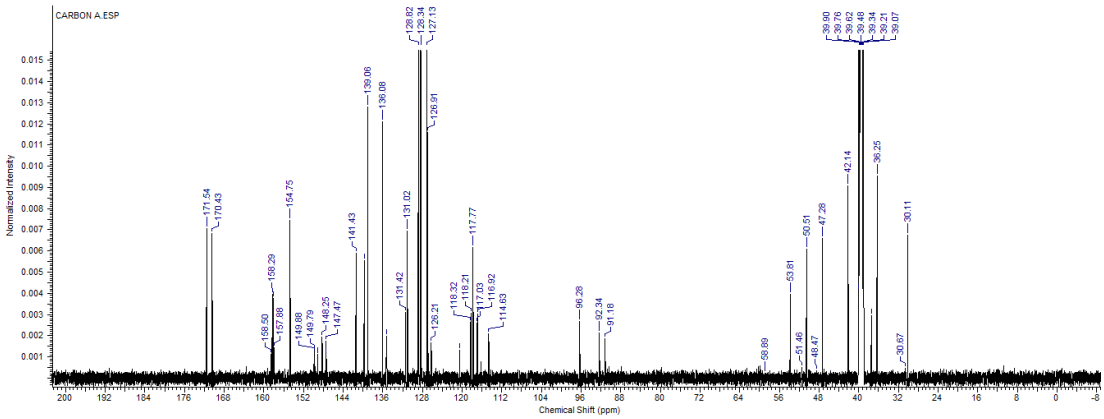
(*S*)-4-Amino-*N*-benzyl-2-{[*S*]-2-[3-(1-{2,6-dichlorobenzyl}-3-{[(*R*)-3-fluoropyrrolidin-1-yl]methyl}-1*H*-indazol-6-yl)ureido]-3-[3,4-difluorophenyl]propanamido}butanamide dihydrochloride salt (9)



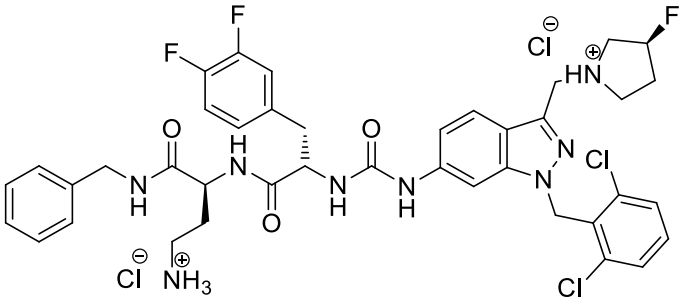
To a sonicated (5 min), then stirred solution of **5** (50.0 mg, 0.127 mmol) in dry THF (5 mL), under dry conditions and cooled to 0 °C, was added triphosgene (13.0 mg, 44.5 μmol) and DMAP (31.0 mg, 254 μmol). After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min under argon. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **2** (31.0 mg, 63.6 μmol) in dry THF (1 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under argon then concentrated *in vacuo* and the residue purified by flash column chromatography (0-2% MeOH/CH_2Cl_2) to give the Boc-protected intermediate (42.0 mg), Rf 0.31 (10% MeOH/CH_2Cl_2). The off-white solid was suspended in a solution of HCl (4 N) in dioxane and stirred for 2.5 h before concentrating *in vacuo* and purifying by preparative RP-HPLC (retention time: 8.68 min). The isolated product was taken up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **9** (6.70 mg, 17%); as a white solid; mp 169-172 °C; Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3273 (N-H), 3060 (N-H), 2926 (C-H), 1633 (C=O), 1585 (C=N), 1536 (C=O), 1512 (C=O), 1434 (C-H), 1209 (C-N), 1162 (C-F), 1027 (C-N), 770-696 (ArC-H), 608 (C-Cl) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 10.94-10.75 (1H, m, $CH_2N^+H(C_4H_7F)$), 9.20 (1H, s, N*H*CONHCH), 8.63 (1H, d, *J* = 7.9, N*H*CHCH$_2$CH$_2$), 8.58 (1H, t, *J* = 5.9, N*H*CH$_2$Ph), 8.00 (1H, s, CC*H*CNH), 7.84 (3H, bs, N^+H_3), 7.75 (1H, d, *J* = 8.7, CC*H*CHCNH), 7.54 (2H, d, *J* = 8.1, 2 x C*H*CCl), 7.44 (1H, m, C*H*CHCCl), 7.32-7.22 (7H, m, ArH), 7.06 (1H, m, ArH) 7.03 (1H, dd, *J* = 8.9, 1.5, CCHC*H*CNH), 6.69 (1H, d, *J* = 7.7, N*H*CONHC), 5.62 (2H, s, C*H*$_2$NN), 5.39 (1H, m, C*H*F), 4.67 (2H, bs, C*H*$_2N^+H(C_4H_7F)$), 4.58 (1H, dt, *J* = 4.9, 8.1, C*H*NHCONH), 4.42 (1H, dt, *J* = 6.2, 7.7, C*H*CH$_2$CH$_2$), 4.33 (2H, m, NHC*H*$_2$Ph), 3.80-3.25 (4H distorted by H_2O

peak, m, 2 x CH_2N$^+$H(CH_2)CH_2C), 3.06 (1H, dd, *J* = 13.9, 4.5, C(H)*H*CHNHCONH), 2.89-2.77 (1H, m, C(*H*)HCHNHCONH & 2H, m, CH_2CH_2CHNH), 2.40-2.24 (1H distorted by solvent peak, m, CHFC(H)*H*CH_2), 2.18-2.05 (1H, m, CHFC(*H*)HCH_2), 2.05-1.86 (2H, m, CH_2CH_2CHNH); ^{13}C NMR (600 MHz, DMSO-d$_6$) δ 171.5 (NHCOC), 170.4 (NHCOC), 158.5-157.9 (q, *J* = 31.9, Ar*C*), 154.8 (NHCONH), 149.9-149.1 (dd, *J* = 105.6, 12.1, *C*F), 148.3-147.5 (dd, *J* = 105.6, 12.1, *C*F), 141.4 (Ar*C*), 139.7 (Ar*C*), 139.1 (Ar*C*), 136.1 (2 x *C*Cl), 135.3 (Ar*C*), 131.4 (Ar*C*), 131.0 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 128.3 (Ar*C*), 127.1 (Ar*C*), 126.9 (Ar*C*), 126.2 (Ar*C*), 120.5 (C*C*HCHCNH), 118.3 (d, *J* = 17.6, Ar*C*), 117.8 (Ar*C*), 117.0 (d, *J* = 16.5, Ar*C*), 114.6 (CCH*C*HCNH), 96.3 (C*C*HCNH), 92.3-91.2 (d, J_{C-F} = 175.2, *C*HF), 58.9 (NH$^+$*C*H$_2$CHF), 53.8 (*C*HCH$_2$C), 51.5 (N$^+$HCH$_2$CH$_2$CHF), 50.5 (*C*HCH$_2$CH$_2$), 48.5 (*C*H$_2$N$^+$H(C$_4$H$_7$F)), 47.3 (*C*H$_2$NN); 42.1 (*C*H$_2$Ph), 37.5 (CH*C*H$_2$C), 36.3 (*C*H$_2$N$^+$H$_3$), 30.6 (CHF*C*H$_2$CH$_2$), 30.1 (CH*C*H$_2$CH$_2$N$^+$H$_3$); m/z (ESI+) 809 (100%, [^{35,35}M+H]$^+$), 811 (55%, [^{35,37}M+H]$^+$), 813 (5%, [^{37,37}M+H]$^+$); HRMS $C_{40}H_{42}{}^{35}Cl_2F_3N_8O_3$ ([M+H]$^+$) calcd. 809.2709, found. 809.2719.



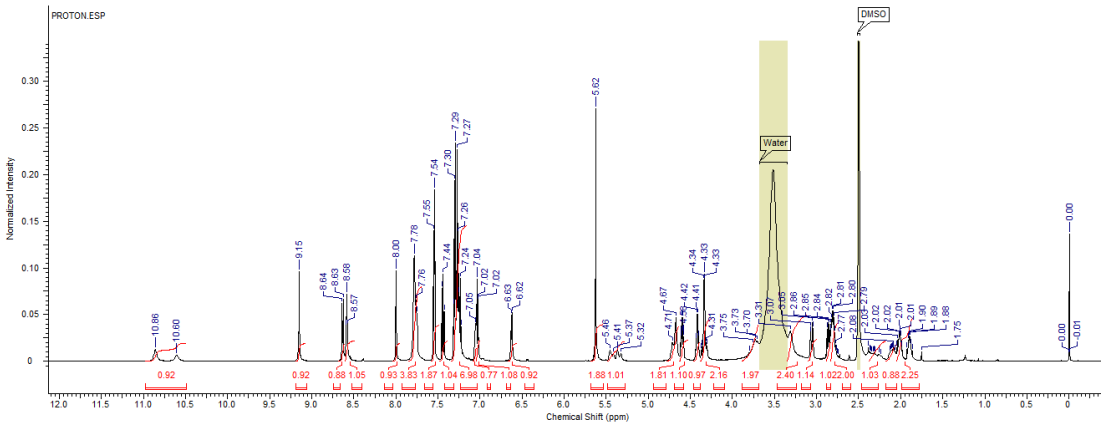


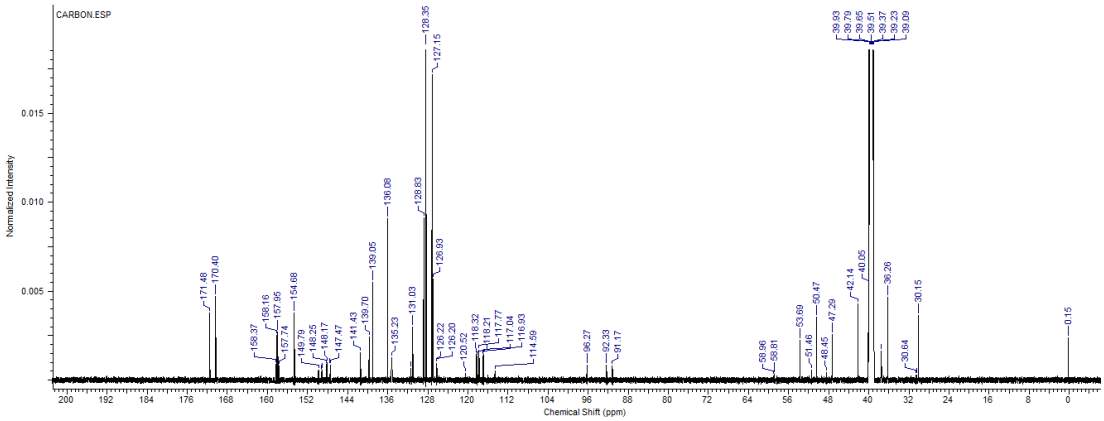
(*S*)-4-Amino-*N*-benzyl-2-{[*S*]-2-[3-(1-{2,6-dichlorobenzyl}-3-{[(*S*)-3-fluoropyrrolidin-1-yl]methyl}-1*H*-indazol-6-yl)ureido]-3-[3,4-difluorophenyl]propanamido}butanamide dihydrochloride salt (10)



To a sonicated (5 min), then stirred, solution of **6** (60.0 mg, 0.153 mmol) in dry THF (8 mL), under dry conditions and cooled to 0 °C, was added triphosgene (16.0 mg, 54.0 μmol) and DMAP (37.0 mg, 306 μmol) under argon. After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **2** (38.0 mg, 77.0 μmol) in dry THF (4 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under argon then concentrated *in vacuo* and the residue purified by flash column chromatography (0-3% MeOH/CH_2Cl_2) to give the Boc-protected intermediate (45.0 mg), Rf 0.31 (10% MeOH/CH_2Cl_2). The off-white solid was suspended in a solution of HCl (4 N) in dioxane and stirred for 2.5 h before concentrating *in vacuo* and purifying by preparative RP-HPLC (retention time: 8.84 min). The isolated product was taken up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **10** (20.0 mg, 46%) as a white solid; mp 154-156 °C; Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3267 (N-H), 3061 (N-H), 2943 (C-H), 1633 (C=O), 1585 (C=N), 1537 (C=O), 1512 (C=O), 1434 (C-H), 1209 (C-N), 1161 (C-F), 1025 (C-N), 769-695 (ArC-H), 607 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.86-10.60 (1H, m, $CH_2N^+H(C_4H_7F)$), 9.15 (1H, s, N*H*CONHCH), 8.63 (1H, d, *J* = 7.9, N*H*CHCH$_2$CH$_2$), 8.58 (1H, t, *J* = 6.0, N*H*CH$_2$Ph), 8.00 (1H, s, CC*H*CNH), 7.78 (3H, bs, N^+H_3), 7.75 (1H, d, *J* = 8.7, CC*H*CHCNH), 7.55 (2H, d, *J* = 8.3, 2 x C*H*CCl), 7.44 (1H, t, *J* = 8.7, C*H*CHCCl), 7.32-7.22 (7H, m, ArH), 7.05 (1H, m, ArH), 7.03 (1H, dd, *J* = 9.0, 1.5, CCHC*H*CNH), 6.62 (1H, d, *J* = 7.9, N*H*CONHC), 5.62 (2H, s, CH_2NN), 5.39 (1H, dd, *J* = 53.8, 25.6, C*H*F), 4.70 (2H, d, *J* = 25.2, C$H_2N^+H(C_4H_7F)$), 4.60 (1H, dt, *J* = 4.9, 8.3, C*H*CH$_2$C), 4.42 (1H, apparent dt, *J* = 6.4, 7.9, C*H*CH$_2$CH$_2$), 4.35 (2H, ddd,

J = 21.1, 15.4, 6.0, NHC*H*$_2$Ph), 3.80-3.25 (4H distorted by H_2O peak, m, 2 x $CCH_2N^+HCH_2$), 3.06 (1H, dd, *J* = 13.9, 4.5, CHC(H)*H*C), 2.85 (1H, dd, *J* = 13.9, 8.3, CHC(*H*)HC), 2.83-2.74 (2H, m, $CH_2N^+H_3$), 2.37-2.23 (1H distorted by solvent peak, m, CHFC(H)*H*CH_2), 2.13-2.07 (1H, m, CHFC(*H*)HCH_2), 2.05-1.86 (2H, m, $CHCH_2CH_2N^+H_3$); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.5 (NH*C*OC), 170.4 (NH*C*OC), 158.4-157.7 (dd, *J* = 63.8, 31.6, Ar*C*), 154.7 (NH*C*ONH), 149.9-149.1 (dd, *J* = 104.9, 11.9, *C*F), 148.3-147.5 (dd, *J* = 104.3, 12.5, *C*F), 141.4 (Ar*C*), 139.7 (Ar*C*), 139.1 (Ar*C*), 136.1 (2 x *C*Cl), 135.2 (Ar*C*), 131.4 (Ar*C*), 131.0 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 128.3 (Ar*C*), 127.2 (Ar*C*), 126.9 (Ar*C*), 126.2 (Ar*C*), 120.5 (C*C*HCHCNH), 118.3 (d, *J* = 16.7, Ar*C*), 117.8 (Ar*C*), 117.0 (d, *J* = 16.7, Ar*C*), 114.6 (CCH*C*HCNH), 96.3 (C*C*HCNH), 92.3-91.2 (d, $J_{C\text{-}F}$ = 175.2, *C*HF), 58.9 (d, $J_{C\text{-}F}$ = 22.7 NH^+CH_2CHF), 53.7 (*C*HCH_2C), 51.5 ($N^+HCH_2CH_2CHF$), 50.5 (*C*HCH_2CH_2), 48.5 ($CH_2N^+H(C_4H_7F)$), 47.3 (CH_2NN); 42.1 (CH_2Ph), 37.5 (CH*C*H_2C), 36.3 ($CH_2N^+H_3$), 30.6 (d, $J_{C\text{-}F}$ = 21.5, CHF*C*H_2CH_2), 30.1 ($CHCH_2CH_2N^+H_3$); m/z (ESI+) 809 (100%, $[^{35,35}M+H]^+$), 811 (40%, $[^{35,37}M+H]^+$), 813 (6%, $[^{37,37}M+H]^+$); HRMS $C_{40}H_{42}{}^{35}Cl_2F_3N_8O_3$ ($[M+H]^+$) calcd. 809.2709, found. 809.2718.




CARBON.ESP
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147.47
141.43
139.70
139.05
136.08
135.23
131.03
128.83
128.35
127.15
129.93
126.22
126.20
120.52
118.32
118.27
117.77
117.04
116.93
114.59
96.27
92.33
91.17
59.96
59.81
53.69
51.46
50.47
48.45
47.29
42.14
40.05
39.93
39.79
39.65
39.51
39.37
39.23
39.09
36.26
30.64
30.15
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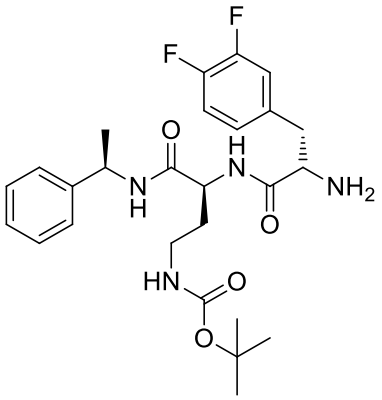
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

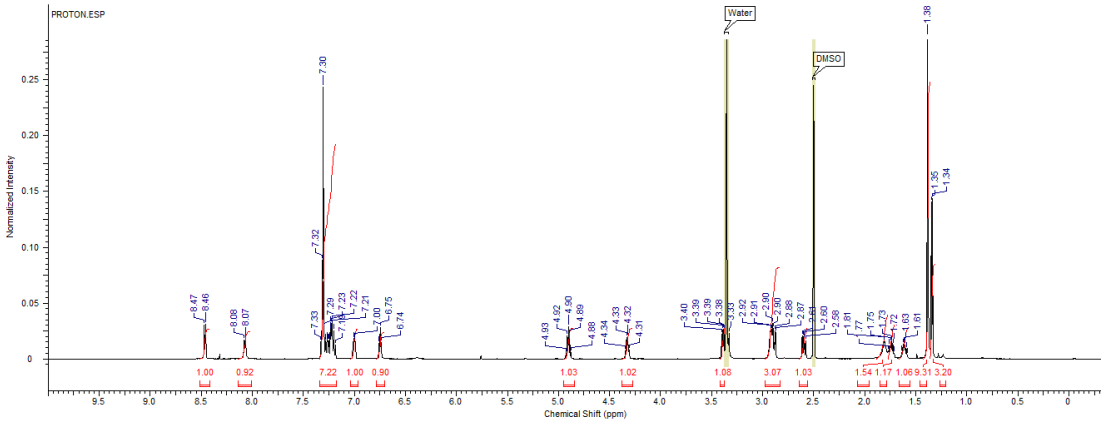
***tert*-Butyl ([*S*]-3-[(*S*)-2-amino-3-(3,4-difluorophenyl)propanamido]-4-oxo-4-{[(*R*)-1-phenylethyl]amino}butyl)carbamate (13)**

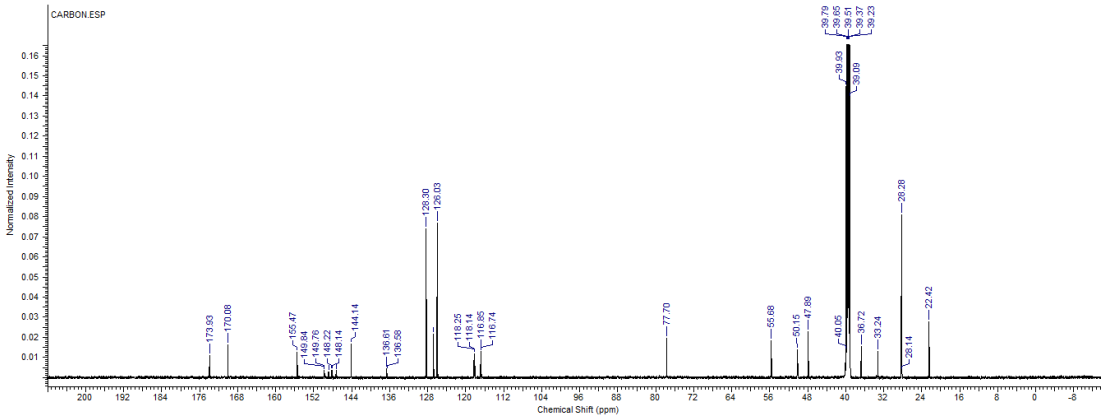


i) To a stirred solution of (*S*)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-4-[(tert-butoxycarbonyl)amino]butanoic acid (100 mg, 0.23 mmol), HBTU (80.0 mg, 0.21 mmol) and HOBt (28.0 mg, 0.21 mmol) in DMF (5 mL) was added DIPEA (37.0 µL, 0.21 mmol). After 3 min of stirring, (*R*)-1-phenylethanamine (24.0 µL, 0.19 mmol) was added and stirring continued at 18 °C for 5 h. The reaction mixture was concentrated *in vacuo* and the white residue taken up in $CHCl_3$/EtOAc (60 mL, 1:1) and washed with H_2O (15 mL), 10% citric acid solution (15 mL), sat. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was concentrated *in vacuo* and the residue dissolved in DMF (5 mL) and stirred with Et_2NH (0.24 mL, 2.30 mmol) at 18 °C for 3 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in $CHCl_3$/EtOAc (40 mL, 1:1) and washed with H_2O (2 x 15 mL), brine (15 mL) and sat. LiCl solution (4 x 15 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (0-2% MeOH/CH_2Cl_2) to give the deprotected amide intermediate (**11**) (48.0 mg, 79%) (Rf 0.63 (10% MeOH/CH_2Cl_2).

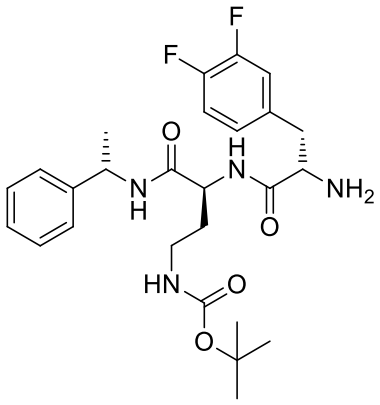
ii) To a stirred solution of (*S*)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-(3,4-difluorophenyl)propanoic acid (74.0 mg, 0.17 mmol), HBTU (61.0 mg, 0.16 mmol) and HOBt (22.0 mg, 0.16 mmol) in DMF (5 mL) was added DIPEA (28.0 µL, 0.16 mmol). After 3 min of stirring, a solution of the deprotected amide intermediate (**2**) (47.0 mg, 0.15 mmol), from step i), in DMF (5 mL) was added and stirring continued at 18 °C for 16 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (30 mL) and washed with H_2O (10 mL), 10%

citric acid solution (10 mL), sat. $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layer was concentrated *in vacuo* and the residue dissolved in DMF (5 mL) and stirred with Et_2NH (0.18 mL, 1.74 mmol) at 18 °C for 3 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (30 mL) and washed with H_2O (2 x 10 mL), brine (10 mL) and sat. LiCl solution (4 x 15 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (0-2% MeOH/CH_2Cl_2) to give the title compound (55.0 mg, 75%); as a white solid; mp 118-120 °C; Rf 0.38 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3293 (N-H), 3061 (N-H), 2971-2871 (C-H), 1673 (C=O), 1634 (C=O), 1514 (C=O), 1446-1365 (C-H), 1280 (C-O), 1250 (C-N), 1209 (C-N), 1115 (C-F), 696 (ArC-H) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 8.46 (1H, d, *J* = 7.9, N*H*CH(CH_3)Ph), 8.07 (1H, d, *J* = 7.9, N*H*CHCH_2CH_2), 7.33-7.19 (7H, m, ArH), 7.00 (1H, m, ArH), 6.75 (1H, t, *J* = 5.7, N*H*CH_2CH_2), 4.90 (1H, quintet, *J* = 7.2, C*H*CH_3), 4.32 (1H, apparent dt, *J* = 6.8, 7.5, NHC*H*CH_2CH_2), 3.39 (1H, dd, *J* = 8.3, 4.9, C*H*NH_2), 2.94-2.87 (2H, m, NHCHCH_2C*H*$_2$ & 1H, apparent dd, *J* = 13.6, 4.5, C(H)*H*CHNH_2), 2.60 (1H, dd, *J* = 13.6, 8.3, C(*H*)HCHNH_2), 1.81 (2H, bs, N*H*$_2$), 1.74 (1H, m, NHCHC(H)*H*CH_2), 1.61 (1H, m, NHCHC(*H*)HCH_2), 1.38 (9H, s, (C*H*$_3$)$_3$C), 1.34 (3H, d, *J* = 7.2, C*H*$_3$CH); ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.9 (NH*C*OC), 170.1 (NH*C*OC), 155.5 (NH*C*OO), 149.8-148.8 (dd, J_{C-F} = 137.7, 12.5, *C*F), 148.2-147.2 (dd, J_{C-F} = 136.5, 12.5, *C*F), 144.1 (Ar*C*), 136.6 (Ar*C*), 128.3 (2 x Ar*C*H), 126.8 (Ar*C*H), 126.1 (Ar*C*H), 126.0 (2 x Ar*C*H), 118.2 (d, J_{C-F} = 16.7, *C*HCF), 116.8 (d, J_{C-F} = 16.7, *C*HCF), 77.7 (*C*(CH_3)$_3$), 55.7 (*C*HNH_2), 50.2 (NH*C*HCH_2CH_2), 47.9 (*C*HCH_3), 40.1 (*C*H_2CHNH_2), 36.7 (*C*H_2CH_2NHCO), 33.2 (CH_2*C*H_2NHCO), 28.3 (C(*C*H_3)$_3$), 22.4 (*C*H_3CH); m/z (CI+) 505 (88%, $[M+H]^+$), 405 (100%, $[M+H\text{-Boc}]^+$); HRMS $C_{26}H_{35}F_2N_4O_4$ ($[M+H]^+$) calcd. 505.2626, found. 505.2623.


PROTON.ESP
Water
DMSO
Normalized Intensity
Chemical Shift (ppm)


CARBON.ESP
Normalized Intensity
Chemical Shift (ppm)

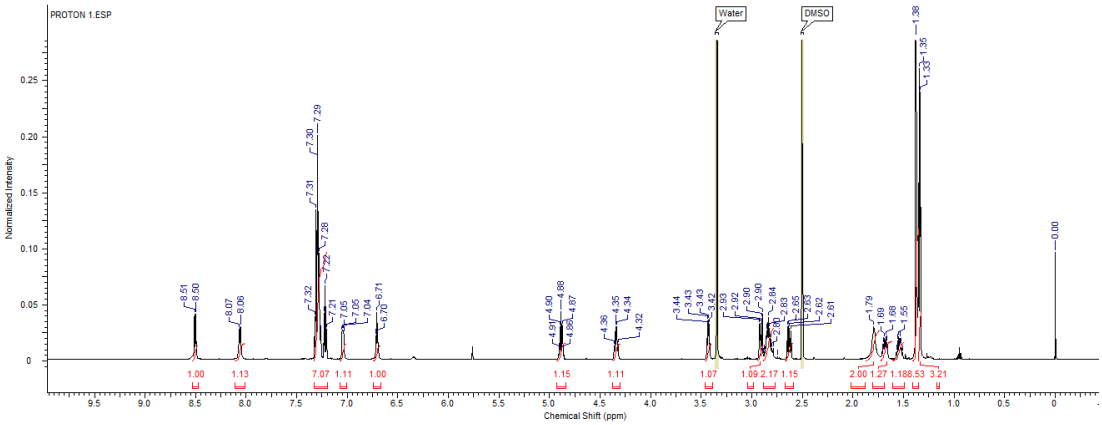
***tert*-Butyl ([*S*]-3-[(*S*)-2-amino-3-(3,4-difluorophenyl)propanamido]-4-oxo-4-{[(*S*)-1-phenylethyl]amino}butyl)carbamate (14)**

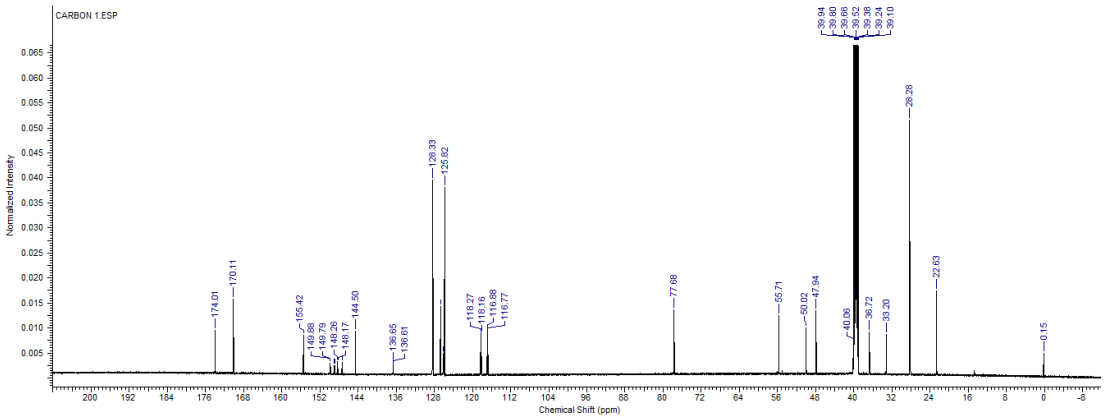


i) To a stirred solution of (*S*)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-4-[(tert-butoxycarbonyl)amino]butanoic acid (200 mg, 0.46 mmol), HBTU (160 mg, 0.42 mmol) and HOBt (56.0 mg, 0.42 mmol) in DMF (5 mL) was added DIPEA (74.0 µL, 0.42 mmol). After 3 min of stirring (*S*)-1-phenylethanamine (48.0 µL, 0.38 mmol) was added and stirring continued at 18 °C for 5 h. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (25% EtOAc/Pet) to give the Fmoc-protected amide intermediate (Rf 0.75 (50% EtOAc/Pet)), which was dissolved in DMF (5 mL) and stirred with Et_2NH (0.48 mL, 4.60 mmol) at 18 °C for 5 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in $CHCl_3$/EtOAc (40 mL, 1:1) and washed with H_2O (2 x 15 mL), brine (15 mL) and sat. LiCl solution (4 x 15 mL). The organic layer was concentrated *in vacuo* and the residue purified by flash column chromatography (0-10% MeOH/CH_2Cl_2) to give the deprotected amide intermediate (**12**) (90.0 mg, 74%) (Rf 0.25 (10% MeOH/CH_2Cl_2).

ii) To a stirred solution of (*S*)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-(3,4-difluorophenyl)propanoic acid (133 mg, 0.31 mmol), HBTU (111 mg, 0.29 mmol) and HOBt (38.0 mg, 0.29 mmol) in DMF (5 mL) was added DIPEA (51.0 µL, 0.29 mmol). After 3 min of stirring, a solution of the deprotected amide intermediate (**3**) (88.0 mg, 0.27 mmol), from step i), in DMF (2mL) was added and stirring continued at 18 °C for 16 h. The reaction mixture was concentrated *in vacuo* and the residue taken up in EtOAc (30 mL) and washed with H_2O (10 mL), 10% citric acid solution (10 mL), sat. $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layer was concentrated *in vacuo* and the residue dissolved in DMF (5 mL)

and stirred with Et_2NH (0.33 mL, 3.14 mmol) at 18 °C for 5 h. The reaction mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (0-3% MeOH/CH_2Cl_2) to give the title compound (106 mg, 77%); as a white solid; mp 106-112 °C; Rf 0.38 (10% MeOH/CH_2Cl_2; IR ν_{max} (neat) 3291 (N-H), 3061 (N-H), 2973-2873 (C-H), 1676 (C=O), 1633 (C=O), 1515 (C=O), 1446-1365 (C-H), 1280 (C-O), 1249 (C-N), 1210 (C-N), 1116 (C-F), 696 (ArC-H) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 8.50 (1H, d, *J* = 7.9, N*H*CH(CH_3)Ph), 8.06 (1H, d, *J* = 8.3, N*H*CHCH_2CH_2), 7.32-7.20 (7H, m, ArH), 7.04 (1H, m, ArH), 6.71 (1H, t, *J* = 5.7, N*H*CH_2CH_2), 4.88 (1H, quintet, *J* = 7.3, C*H*CH_3), 4.34 (1H, apparent dt, *J* = 6.0, 8.1, NHC*H*CH_2CH_2), 3.43 (1H, dd, *J* = 8.3, 4.9, C*H*NH_2), 2.91 (1H, dd, *J* = 13.6, 4.9, C(H)*H*CHNH_2), 2.88-2.79 (2H, m, NHCHCH_2C*H*$_2$), 2.63 (1H, dd, *J* = 13.6, 8.3, C(*H*)HCHNH_2), 1.79 (2H, bs, N*H*$_2$), 1.71-1.65 (1H, m, NHCHC(H)*H*CH_2), 1.57-1.51 (1H, m, NHCHC(*H*)HCH_2), 1.38 (9H, s, (C*H*$_3$)$_3$C), 1.34 (3H, d, *J* = 7.0, C*H*$_3$CH); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.0 (NH*C*OC), 170.1 (NH*C*OC), 155.4 (NH*C*OO), 149.9-148.9 (dd, $J_{C\text{-}F}$ = 137.7, 12.5, *C*F), 148.3-147.3 (dd, $J_{C\text{-}F}$ = 136.5, 12.5, *C*F), 144.5 (Ar*C*), 136.7 (Ar*C*), 128.3 (2 x Ar*C*H), 126.7 (Ar*C*H), 126.1 (Ar*C*H), 125.8 (2 x Ar*C*H), 118.2 (d, $J_{C\text{-}F}$ = 16.7, *C*HCF), 116.8 (d, $J_{C\text{-}F}$ = 16.7, *C*HCF), 77.7 (*C*(CH_3)$_3$), 55.7 (*C*HNH_2), 50.0 (NH*C*HCH_2CH_2), 47.9 (*C*HCH_3), 40.1 (*C*H_2CHNH_2), 36.7 (CHCH_2*C*H_2NH), 33.2 (CH*C*H_2CH_2NH), 28.3 (C(*C*H$_3$)$_3$), 22.6 (*C*H$_3$CH); m/z (ESI+) 505 (75%, $[M+H]^+$), 527 (100%, $[M+Na]^+$); HRMS $C_{26}H_{35}F_2N_4O_4$ ($[M+H]^+$) calcd. 505.2626, found. 505.2621.

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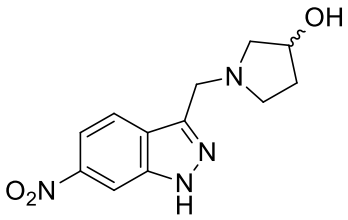
40.06
39.94
39.80
39.66
39.52
39.38
39.24
39.10
36.72
33.20
28.28
22.63
0.15

Racemic mixture of (*S*)-1-[(6-nitro-1*H*-indazol-3-yl)methyl]pyrrolidin-3-ol & (*R*)-1-[(6-nitro-1*H*-indazol-3-yl)methyl]pyrrolidin-3-ol

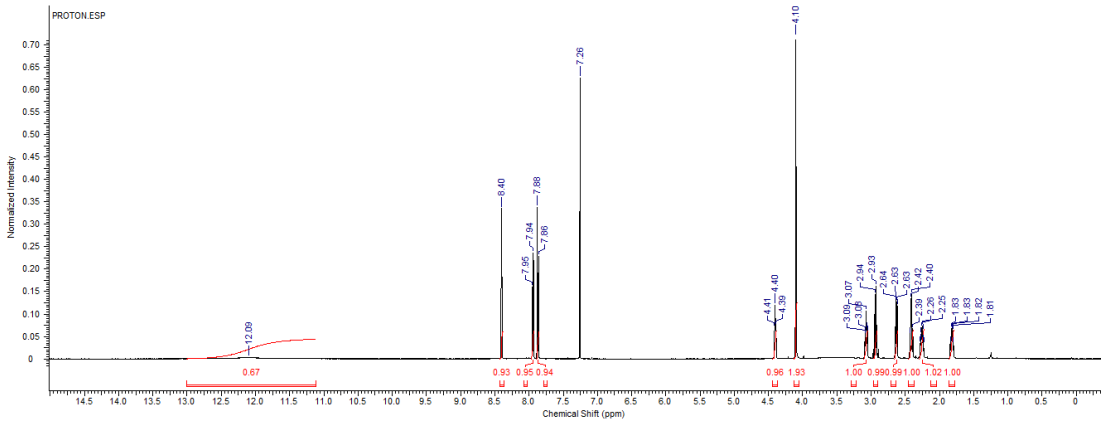


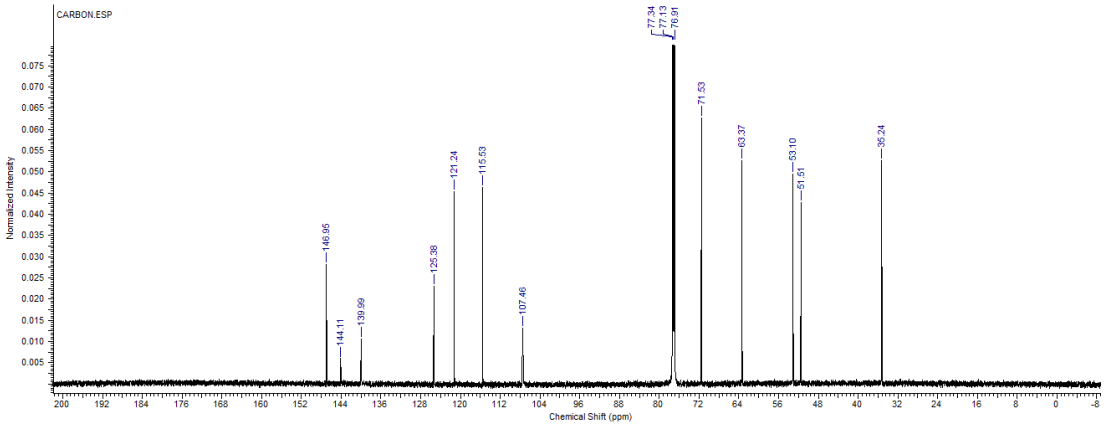
i) 6-Nitroindole (500 mg, 3.08 mmol) was added to a stirred solution of sodium nitrite (2.13 g, 30.8 mmol) in H_2O (25 mL) and DMF (2 mL) at 18 °C. 6 M HCl (4.6 mL, 27.8 mmol) was added dropwise over 10 min and stirring continued for 3 h before diluting with EtOAc (30 mL) and extracting. Aqueous layer extracted further with EtOAc (2 x 15 mL). Organic extracts combined, washed with H_2O (15 mL) and brine (15 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to leave the indazole-3-carbaldehyde intermediate as a dark brown solid.

ii) HCl (g), generated by the dropwise addition of conc. H_2SO_4 (65 mL, 1.20 mol) to NaCl (s) (58.0 g, 1.07 mol), was bubbled through a stirred solution of 1-Boc-3-pyrrolidinol (1.00 g, 5.34 mmol) in Et_2O (50 mL) for 3 h. Stirring was then continued for 20 h before concentrating *in vacuo* and drying further under high vacuum to leave the hydrochloride salt of 3-pyrrolidinol as a pale pink solid.

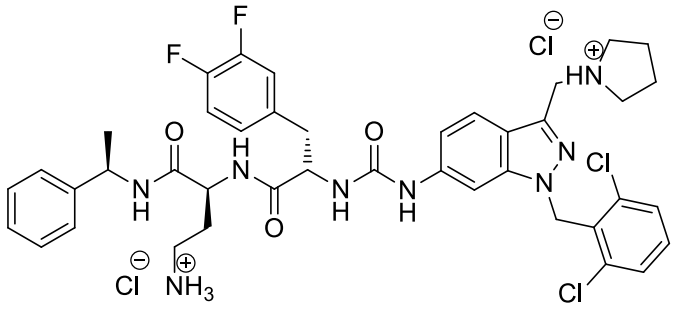
iii) The indazole-3-carbaldehyde intermediate was dissolved in CH_2Cl_2/DMF/AcOH (45/4.5/0.5 mL), to which was added the 3-pyrrolidinol (660 mg, 5.36 mmol) and the reaction mixture was stirred at 18 °C for 20 min. Sodium triacetoxyborohydride (1.63 mg, 7.70 mmol) was added portionwise over 10 min and stirring continued for 3 h before diluting with EtOAc (100 mL) and quenching with sat. aqueous $NaHCO_3$ (60 mL). Aqueous layer separated and extracted further with EtOAc (2 x 30 mL). Organic extracts combined and washed with sat. $NaHCO_3$ (aq) (2 x 25 mL), H_2O (2 x 25 mL) and brine (2 x 25 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to leave brown residue. Purification by flash column chromatography (0-10% MeOH/CH_2Cl_2) gave target compound (406 mg, 50%); as a brown solid; mp 166-168 °C; Rf 0.13 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3122 (O-H), 3068 (N-H), 2959-2835 (C-H), 1513 (NO_2), 1341 (NO_2), 1125-1059 (C-N), 874-730 (ArC-H) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 12.09 (1H, bs, N*H*), 8.40 (1H, d,

J = 1.9, CC*H*C), 7.94 (1H, dd, *J* = 1.9, 9.0, CHC*H*CNO$_2$), 7.87 (1H, d, *J* = 9.0, C*H*CHCNO$_2$), 4.40 (1H, m, C*H*OH); 4.10 (2H, s, CC*H*$_2$N); 3.07 (1H, td, *J* = 8.7, 4.1, NC*H*HCH$_2$), 2.93 (1H, d, *J* = 10.2, NC*H*HCHOH), 2.63 (1H, dd, *J* = 10.2-10.5, 5.3-5.7, NCH*H*CHOH), 2.41 (1H, q, *J* = 8.3-8.7, NCH*H*CH$_2$), 2.29-2.23 (1H, m, NCH$_2$CH*H*), 1.84-1.79 (1H, m, NCH$_2$C*H*H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 147.0 (Ar*C*), 144.1 (Ar*C*), 140.0 (Ar*C*), 125.4 (Ar*C*), 121.2 (*C*HCHCNO$_2$), 115.5 (CH*C*HCNO$_2$), 107.5 (C*C*HC), 71.5 (*C*HOH), 63.4 (N*C*H$_2$CHOH), 53.1 (N*C*H$_2$CH$_2$), 51.5 (*C*H$_2$CNN), 35.2 (NCH$_2$*C*H$_2$); m/z (CI+) 263 (100%, [M+H]$^+$); HRMS $C_{12}H_{15}N_4O_3$ ([M+H]$^+$) calcd. 263.1144, found. 263.1143.





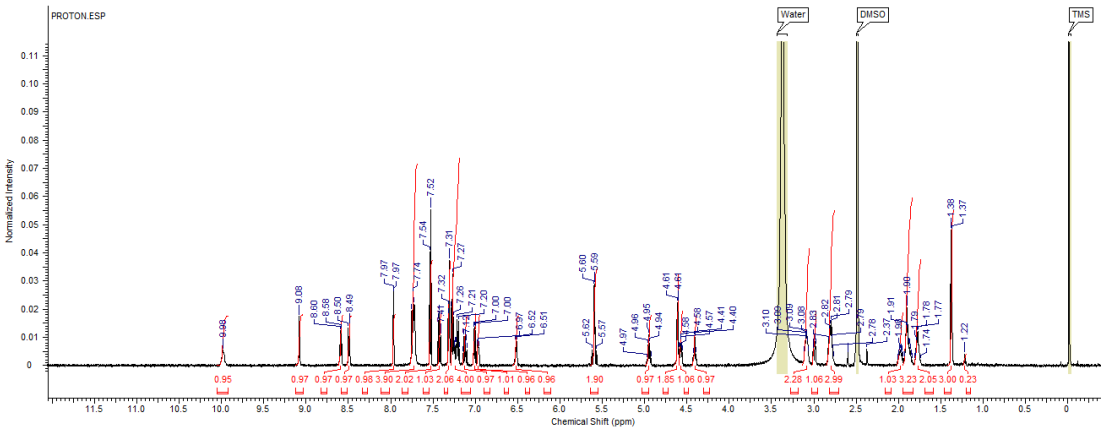
(*S*)-4-Amino-2-({*S*}-2-{3-[1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazol-6-yl]ureido}-3-{3,4-difluorophenyl}propanamido)-*N*-[(*R*)-1-phenylethyl]butanamide dihydrochloride salt (15)

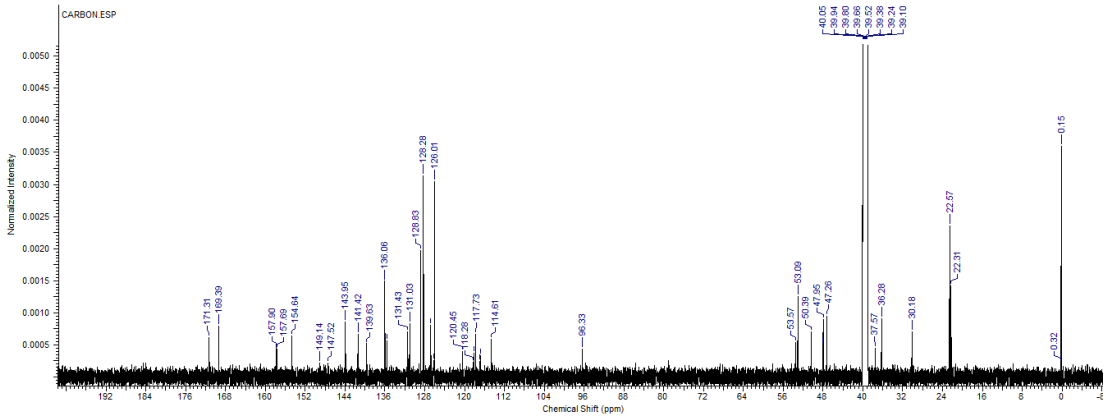


To a sonicated (5 min), then stirred, solution of **4** (50.0 mg, 133 μmol) in dry THF (5 mL), under dry conditions and cooled to 0 °C, was added together triphosgene (14.0 mg, 46.6 μmol) and DMAP (32.0 mg, 266 μmol). After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min under argon. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **13** (32.0 mg, 66.5 μmol) in dry THF (3 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under argon then concentrated *in vacuo* and the residue purified by flash column chromatography (2-5% MeOH/CH_2Cl_2) to give the Boc-protected intermediate, Rf 0.31 (10% MeOH/CH_2Cl_2). The off-white solid was suspended in a solution of HCl (4N) in dioxane (2 mL) and stirred for 2.5 h before concentrating *in vacuo* and purifying by preparative RP-HPLC (retention time: 10.82 min). The isolated product was taken up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **15** (3.70 mg, 6%[*]); as a white solid; mp 158-163 °C (decomp.); Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3274 (N-H), 3059 (N-H), 2973 (C-H), 1663 (C=O), 1647 (C=O), 1515 (C=O), 1436 (C-H), 1198 (C-N), 1177 (C-N), 1128 (C-F), 834-699 (ArC-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600MHz) δ 9.98 (1H, bs, N^+HCH_2CNN), 9.08 (1H, s, N*H*CONHCH), 8.59 (1H, d, *J* = 8.3, N*H*CHCH$_2$CH$_2$), 8.49 (1H, d, *J* = 8.3, N*H*CH(CH$_3$)Ph), 7.97 (1H, d, *J* = 1.1, CC*H*CNH), 7.75-7.70 (1H, d, *J* = 8.7, C*H*CHCNH & 3H, bs, N^+H_3), 7.53 (2H, d, *J* = 8.3, 2 x C*H*CCl), 7.42 (1H, t, *J* = 8.3, C*H*CHCCl), 7.32-7.10 (7H, m, ArH), 7.01 (1H, dd, *J* = 8.7, 1.1, CHC*H*CNH), 6.97 (1H, m, ArH), 6.52 (1H, d, *J* = 7.9, CHN*H*CONH), 5.62 & 5.57 (2H, ABq, *J* = 14.5, CH_2NN), 4.95 (1H, dq, *J* = 7.5, 7.2,

[*] Low yield was suspected to be a result of poor quality THF (dry)

C*H*CH_3), 4.61 (2H, d, *J* = 5.3, CH_2CNN), 4.57 (1H, apparent dt, *J* = 4.9, 7.9, C*H*NHCONH), 4.41 (1H, apparent dt, *J* = 7.5, 7.2, C*H*CH_2CH_2), 3.38 (2H distorted by H_2O peak, m, 2 x C(H)*H*$N^+H(CH_2)CH_2$C), 3.11-3.06 (2H, m, 2 x C(*H*)$HN^+H(CH_2)CH_2$C), 2.99 (1H, dd, *J* = 13.6, 4.1, C(H)*H*CHNHCONH), 2.83-2.77 (1H, m, C(*H*)HCHNHCONH & 2H, m, CH_2CH_2CHNH), 2.01-1.95 (1H, m, C(H)*H*CHNHCOCH), 1.93-1.84 (1H, m, C(*H*)HCHNHCOCH & 2H, m, 2 x C(H)*H*$CH_2N^+H(CH_2)CH_2$C), 1.81-1.74 (2H, m, 2 x C(*H*)$HCH_2N^+H(CH_2)CH_2$C), 1.37 (3H, d, *J* = 6.8, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3 (NH*C*OC), 169.4 (NH*C*OC), 157.8 (d, *J* = 31.6, Ar*C*), 154.6 (NH*C*ONH), 149.1 (Ar*C*), 147.5 (Ar*C*), 144.0 (Ar*C*), 141.4 (Ar*C*), 139.6 (Ar*C*), 136.1 (2 x *C*Cl), 135.6 (Ar*C*), 131.4 (Ar*C*), 131.0 (*C*HCHCCl), 128.8 (2 x *C*HCCl), 128.3 (2 x Ar*C*H), 126.8 (Ar*C*H), 126.2 (Ar*C*H), 126.0 (2 x Ar*C*H), 120.5 (C*C*HCHCNH), 118.2 (d, *J* = 16.7, Ar*C*H), 117.7 (Ar*C*), 116.9 (d, *J* = 16.7, Ar*C*H), 114.6 (CH*C*HCNH), 96.3 (C*C*HCNH), 53.6 (*C*HNHCONH), 53.1 (2 x *C*$H_2N^+H(CH_2)CH_2$C), 50.4 (*C*HCH_2CH_2), 48.0 (CH_2C*C*N), 47.95 (*C*HCH_3), 47.3 (*C*H_2NN), 37.6 (*C*H_2CHNHCONH), 36.3 (*C*H_2CH_2CHNH), 30.2 (*C*H_2CHNHCOCH), 22.6 (2 x *C*$H_2CH_2N^+H(CH_2)CH_2$C), 22.3 (*C*H_3); m/z (ESI+) 805 (100%, [^{35,35}M+H]$^+$), 807 (78%, [^{35,37}M+H]$^+$), 809 (12%, [^{37,37}M+H]$^+$); HRMS $C_{41}H_{45}{}^{35}Cl_2F_2N_8O_3$ ([M+H]$^+$) calcd. 805.2960, found. 805.2922.

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39.80
39.66
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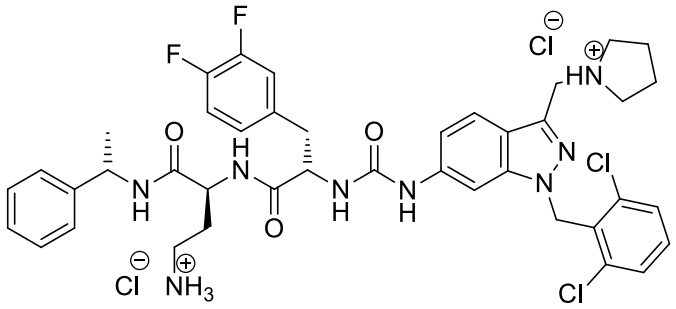
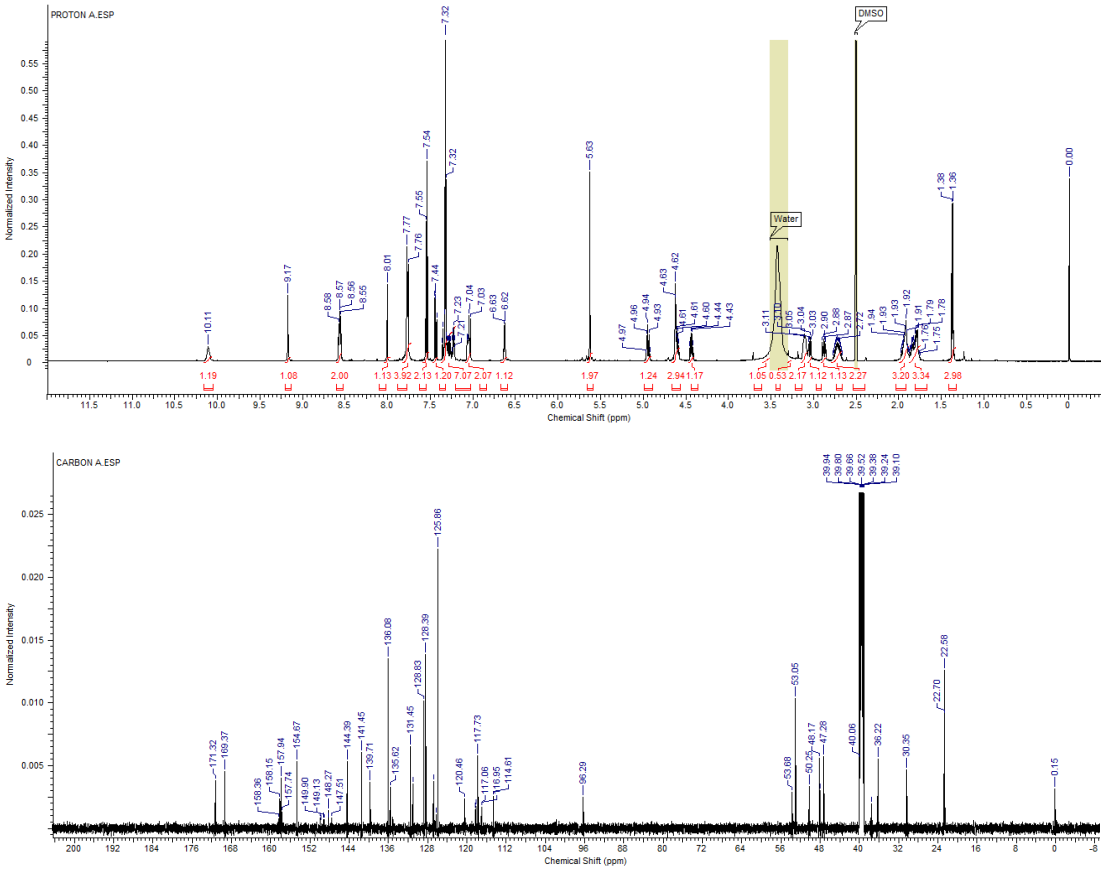
39.38
39.24
39.10
37.57
36.28
30.18
22.57
22.31
0.32
0.15

(*S*)-4-amino-2-({*S*}-2-{3-[1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazol-6-yl]ureido}-3-{3,4-difluorophenyl}propanamido)-*N*-[(*S*)-1-phenylethyl]butanamide dihydrochloride salt (16)

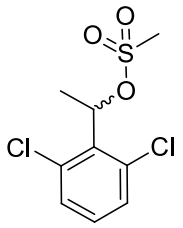


To a sonicated (5 min), then stirred, solution of **4** (60.0 mg, 133 μmol) in dry THF (8 mL), under dry conditions and cooled to 0 °C, was added together triphosgene (16.0 mg, 54.0 μmol) and DMAP (37.0 mg, 306 μmol). After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min under argon. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **14** (39.0 mg, 77.0 μmol) in dry THF (3 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under argon then concentrated *in vacuo*. The residue was suspended in a solution of HCl (4N) in dioxane (2 mL) and stirred for 2.5 h before concentrating *in vacuo* and purifying by preparative RP-HPLC (retention time: 8.56 min). The isolated product was taken up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **16** (22.5 mg, 33%); as a white solid; mp 174-178 °C (decomp.); Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3275 (N-H), 3061 (N-H), 2972 (C-H), 1665 (C=O), 1633 (C=O), 1514 (C=O), 1436 (C-H), 1198 (C-N), 1179 (C-N), 1129 (C-F), 834-699 (ArC-H) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600MHz) δ 10.11 (1H, bs, N^+*H*CH_2CNN), 9.17 (1H, s, N*H*CONHCH), 8.58-8.55 (overlapping 1H, d, *J* = 7.9, N*H*CHCH_2CH_2 & 1H, d, *J* = 7.9, N*H*CH(CH_3)Ph), 8.01 (1H, d, *J* = 0.9, CC*H*CNH), 7.78-7.76 (3H, bs, N^+H_3 & 1H, d, *J* = 8.5, C*H*CHCNH), 7.55 (2H, d, *J* = 8.3, 2 x C*H*CCl), 7.44 (1H, m, C*H*CHCCl), 7.36-7.21 (7H, m, ArH), 7.07-7.03 (1H, m, ArH & 1H, dd, *J* = 8.9, 1.5, CHC*H*CNH), 6.63 (1H, d, *J* = 7.7, CHN*H*CONH), 5.63 (1H, s, CH_2NN), 4.94 (1H, overlapping dq, *J* = 7.0, 7.3, C*H*CH_3), 4.63-4.58 (2H m, CH_2CNN & 1H, m, C*H*NHCONH), 4.43 (1H, overlapping dt, *J* = 6.2, 7.9, C*H*CH_2CH_2), 3.39 (2H, obscured by H_2O peak, 2 x C(H)*H*N^+H(CH_2)CH_2C), 3.10 (2H, m, 2 x C(*H*)HN^+H(CH_2)CH_2C), 3.04 (1H, dd, *J* = 13.9, 4.7, C(H)*H*CHNHCONH), 2.88 (1H, dd, *J* = 13.9, 8.1, C(*H*)HCHNHCONH),

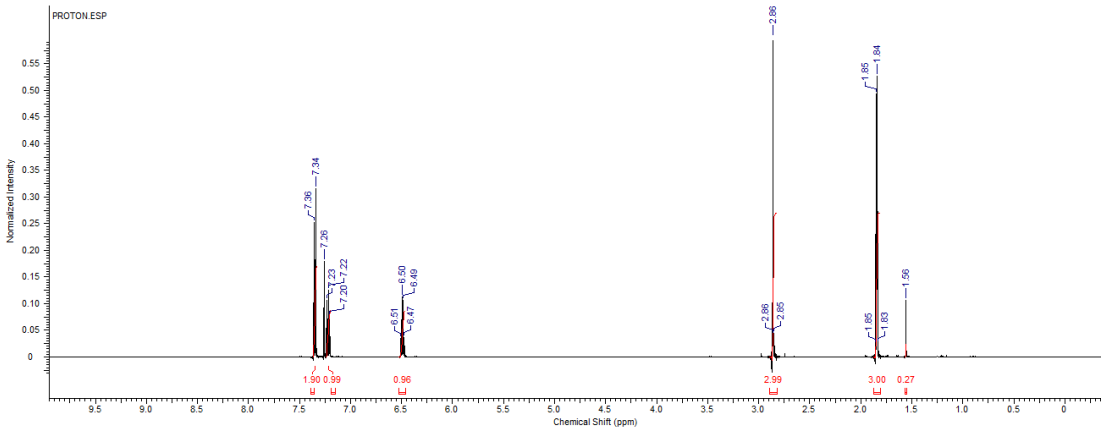
2.77-2.67 (2H, m, CH_2CH$_2$CHNH), 1.98-1.88 (2H, m, 2 x C(H)HCH$_2$N$^+$H(CH$_2$)CH$_2$C & 1H C(H)HCHNHCOCH), 1.87-1.75 (2H, m, 2 x C(H)HCH$_2$N$^+$H(CH$_2$)CH$_2$C & 1H C(H)HCHNHCOCH), 1.37 (3H, d, J = 7.0, CH_3); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 171.3 (NHCOC), 169.4 (NHCOC), 158.0 (q, J = 30.8, ArC), 154.7 (NHCONH), 149.9-149.2 (dd, J = 104.5, 13.2, CF), 148.2-147.5 (dd, J = 103.4, 13.2, CF), 144.4 (ArC), 141.5 (ArC), 139.7 (ArC), 136.1 (2 x CCl), 135.6 (ArC), 131.5 (ArC), 131.0 (CHCHCCl), 128.8 (2 x CHCCl), 128.4 (2 x ArCH), 126.8 (ArCH), 126.2 (ArCH), 125.9 (2 x ArCH), 120.5 (CCHCHCNH), 118.3 (d, J = 17.6, CHCF), 117.7 (ArC), 117.0 (d, J = 16.5, CHCF), 114.6 (CHCHCNH), 96.3 (CCHCNH), 53.6 (CHNHCONH), 53.1 (2 x CH$_2$N$^+$H(CH$_2$)CH$_2$C), 50.3 (CHCH$_2$CH$_2$), 48.2 (CH$_2$CNN), 48.05 (CHCH$_3$), 47.3 (CH$_2$NN), 37.6 (CH$_2$CHNHCONH), 36.2 (CH$_2$CH$_2$CHNH), 30.4 (CH$_2$CHNHCOCH), 22.7 (2 x CH$_2$CH$_2$N$^+$H(CH$_2$)CH$_2$C), 22.6 (CH$_3$); m/z (ESI+) 805 (100%, [^{35,35}M+H]$^+$), 807 (64%, [^{35,37}M+H]$^+$), 809 (11%, [^{37,37}M+H]$^+$); HRMS C$_{41}$H$_{45}$^{35}Cl$_2$F$_2$N$_8$O$_3$ ([M+H]$^+$) calcd. 805.2960, found. 805.2968.



Racemic mixture of (*S*)-1-(2,6-dichlorophenyl)ethyl methanesulfonate & (*R*)-1-(2,6-dichlorophenyl)ethyl methanesulfonate (17)



To a solution of 2,6-dichlorobenzaldehyde (2.00 g, 11.4 mmol) in THF (dry) (50 mL) at 0 °C under dry conditions was added a solution of methyl magnesium bromide (3 M) in Et_2O (11.4 mL, 34.3 mmol) in two portions. The reaction mixture was stirred for 1.5 h before quenching with cold sat. NH_4Cl (aq) solution (100 mL) and extracting with Et_2O (3 x 80 mL). Organics were combined, dried ($MgSO_4$) and concentrated *in vacuo* to leave a clear yellow oil, which was taken up in CH_2Cl_2 (30 mL) and stirred at 0 °C. To this solution was added triethylamine (3.25 mL, 22.2 mmol) followed by methanesulfonyl chloride (1.17 mL, 14.4 mmol). The reaction mixture was stirred for 1.5 h before quenching with H_2O (30 mL) and extracting with CH_2Cl_2 (3 x 20 mL). Organics were combined and washed with H_2O (20 mL), HCl (1 M) (20 mL), sat. $NaHCO_3$ (aq) solution (20 mL) and brine (20 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to leave clear, colourless oil. Purified by flash column chromatography (5% Et_2O/Pet) and dried under high vacuum to give the title compound (**17**) (2.72 g, 89%); as a white solid; mp 49-50 °C; Rf 0.06 (20% Et_2O/Pet); IR ν_{max} (neat) 3025-2934 (C-H), 1580 (ArC=C), 1562 (ArC=C), 1435 (C-H), 1349 (SO_2), 1171 (SO_2), 1084 (C-O), 906-763 (ArC-H), 518 (C-Cl) cm^{-1}; H NMR (600 MHz, $CDCl_3$) δ 7.35 (2H, d, *J* = 8.3, C*H*CCl), 7.22 (1H, t, *J* = 8.3, C*H*CHCCl), 6.49 (1H, q, *J* = 6.8, C*H*CH_3), 2.86 (3H, s, C*H*$_3$S), 1.84 (3H, d, *J* = 6.8, C*H*$_3$CH); ^{13}C NMR δ 134.8 (2 x *C*Cl), 133.8 (Ar*C*), 130.4 (*C*HCHCCl), 129.7 (*C*HCCl), 75.9 (*C*HCH_3), 38.5 (*C*H$_3$S), 19.6 (*C*H$_3$CH); m/z (CI+) 268 (2%, $^{35,35}M^+$), 173 (100%, $[^{35,35}$M-OMs$]^+$), 175 (66%, $[^{35,37}$M-OMs$]^+$) , 177 (11%, $[^{37,37}$M-OMs$]^+$); HRMS $C_9H_{10}{}^{35}Cl_2O_3S$ (M^+) calcd. 267.9728, found. 267.9727.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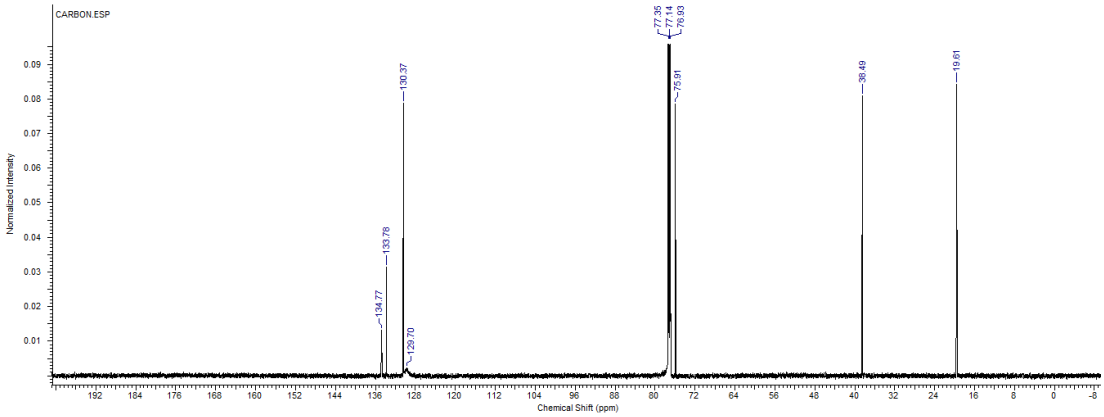
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Chemical Shift (ppm)
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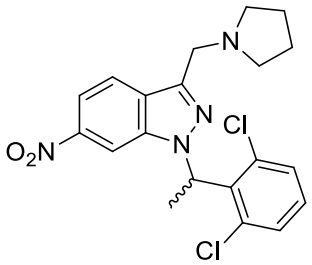
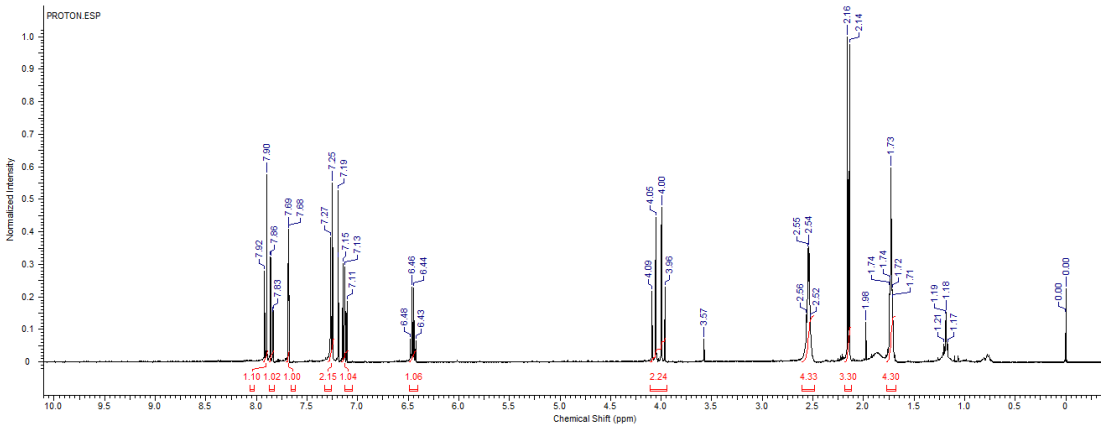
38.49
19.61

Racemic mixture (*S*)-1-[1-(2,6-dichlorophenyl)ethyl]-6-nitro-3-(pyrrolidin-1-ylmethyl)-1H-indazole & (*R*)-1-[1-(2,6-dichlorophenyl)ethyl]-6-nitro-3-(pyrrolidin-1-ylmethyl)-1H-indazole



To a stirred solution of 6-nitro-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole (183 mg, 0.743 mmol) in DMF (20 mL), under dry conditions, was added *rac*-1-(2,6-dichlorophenyl)ethyl methanesulfonate (200 mg, 0.743 mmol) and caesium carbonate (242 mg, 0.743 mmol). The reaction mixture was stirred under argon at 100 °C for 20 h before allowing to reach ambient temperature then diluted with H_2O (10 mL) and extracted with EtOAc (3 x 20 mL). The organics were combined and washed with H_2O (10 mL) then brine (2 x 10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to leave an oily brown residue. Purification by flash column chromatography (0-1.5% MeOH in CH_2Cl_2) gave target compound (171 mg, 55%); as a light brown solid; mp 92-96 °C; Rf 0.44 (10% MeOH in CH_2Cl_2); IR ν_{max} (neat) 3063-2783 (C-H), 1579 (ArC=C), 1561 (ArC=C), 1521 (NO_2), 1438 (C-H), 1339 (NO_2), 1210 (C-N), 1122 (C-N), 1067 (C-N), 870-734 (ArC-H), 593 (C-Cl) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (1H, dd, *J* = 8.8, 0.8, C*H*CHCNO$_2$), 7.85 (1H, ddd, *J* = 8.8, 2.0, 0.8, CHC*H*CNO$_2$), 7.68 (1H, dd, *J* = 1.8, 0.8, CC*H*CNO$_2$), 7.26 (2H, d, *J* = 8.1, 2 x C*H*CCl), 7.13 (1H, m, C*H*CHCCl), 6.45 (1H, q, *J* = 7.1, C*H*CH$_3$), 4.07 & 3.98 (2H, ABq, *J* = 13.5, C*H*$_2$CNN), 2.55 (4H, m, 2 x C*H*$_2$N(CH$_2$)CH$_2$C), 2.15 (3H, d, *J* = 7.1, C*H*$_3$), 1.73 (4H, m, 2 x C*H*$_2$CH$_2$N(CH$_2$)CH$_2$C); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.1 (Ar*C*), 142.5 (Ar*C*), 138.8 (Ar*C*), 135.2 (Ar*C*), 134.6 (2 x *C*Cl), 129.9 (2 x *C*HCCl), 129.7 (*C*HCHCCl), 127.4 (Ar*C*), 121.9 (*C*HCHCNO$_2$), 115.1 (CH*C*HCNO$_2$), 105.9 (C*C*HCNO$_2$), 56.2 (*C*HCH$_3$), 54.1 (2 x *C*H$_2$N(CH$_2$)CH$_2$C), 52.0 (*C*H$_2$CNN), 23.7 (2 x *C*H$_2$CH$_2$N(CH$_2$)CH$_2$C), 17.0 (*C*H$_3$); m/z (ESI+) 419 (100%, $[^{35,35}M+H]^+$), 421 (95%, $[^{35,37}M+H]^+$), 423 (19%, $[^{37,37}M+H]^+$); HRMS $C_{20}H_{21}{}^{35}Cl_2N_4O_2$ ($[M+H]^+$) calcd. 419.1042, found. 419.1046.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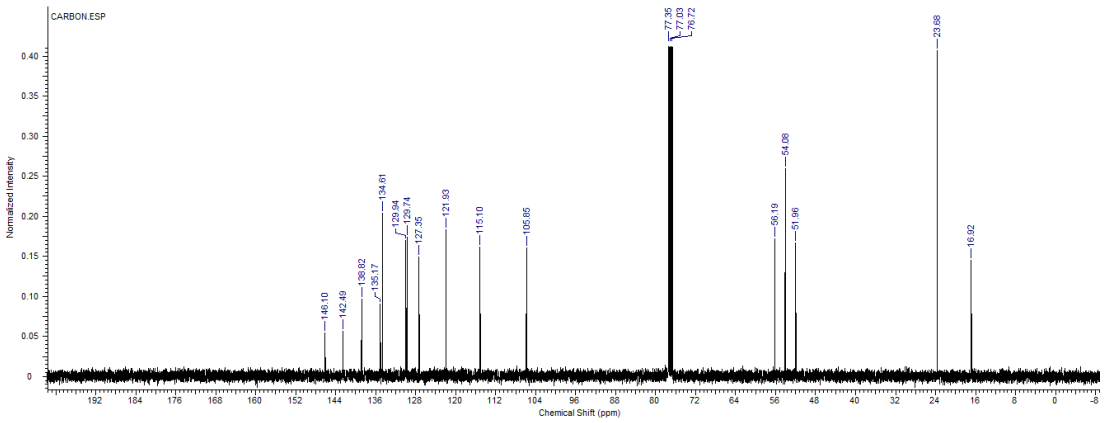
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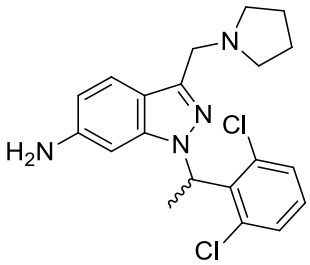
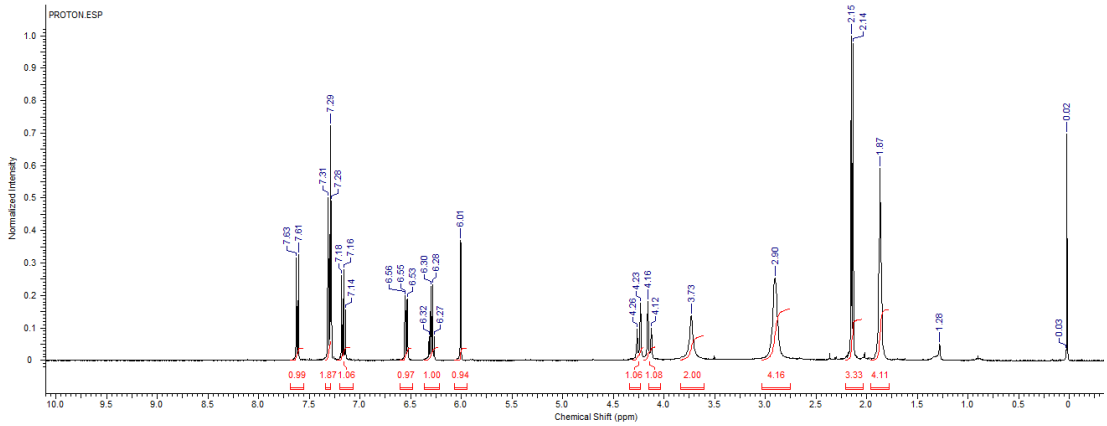
121.93
115.10
105.85
56.19
54.08
51.96
23.68
16.92

Racemic mixture of (*S*)-1-[1-(2,6-dichlorophenyl)ethyl]-3-(pyrrolidine-1-ylmethyl)-1*H*-indazol-6-amine & (*R*)-1-[1-(2,6-dichlorophenyl)ethyl]-3-(pyrrolidine-1-ylmethyl)-1*H*-indazol-6-amine (18)



To a solution of *rac*-1-[1-(2,6-dichlorophenyl)ethyl]-6-nitro-3-(pyrrolidin-1-ylmethyl)-1H-indazole (138 mg, 0.33 mmol) in MeOH (30 mL) was added $FeCl_3.6H_2O$ (21.0 mg, 0.077 mmol) and activated charcoal (168 mg), followed by *N*,*N*-dimethylhydrazine (0.50 mL, 6.58 mmol). The reaction mixture was heated to reflux for 2 h then allowed to cool to ambient temperature before filtering through celite, washing through with a cold mixture of CH_2Cl_2/MeOH (60 mL, 4:1). The filtrate was concentrated *in vacuo* to leave a brown solid residue. Purification by flash column chromatography (0-5% MeOH/CH_2Cl_2) gave desired product **18** (107 mg, 84%); as a light brown solid; mp 196-199 °C (decomp.); Rf 0.25 (10% MeOH in CH_2Cl_2); IR ν_{max} (neat) 3310-3202 (N-H), 2953-2789 (C-H), 1619 (C=N), 1560-1514 (ArC=C), 1434 (C-H), 1086 (C-N), 812-778 (ArC-H), 608 (C-Cl) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (1H, d, *J* = 8.6, C*H*CHCNH$_2$), 7.30 (2H, d, *J* = 8.1, 2 x C*H*CCl), 7.16 (1H, t, *J* = 8.1, C*H*CHCCl), 6.54 (1H, dd, *J* = 8.6, 1.8, CHC*H*CNH$_2$), 6.29 (1H, q, *J* = 7.1, C*H*CH$_3$), 5.98 (1H, d, *J* = 1.8, CC*H*CNH), 4.24 (1H, d, *J* = 13.6, C(H)*H*CCC), 4.14 (1H, d, *J* = 13.6, C(*H*)HCCC), 3.73 (2H, s, N*H*$_2$), 2.90 (4H, bs, 2 x C*H*$_2$NCH$_2$C), 2.14 (3H, d *J* = 7.1, C*H*$_3$), 1.87 (4H, bs, 2 x C*H*$_2$CH$_2$N); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.1 (Ar*C*), 145.4 (Ar*C*), 141.8 (Ar*C*), 136.0 (Ar*C*), 134.6 (2 x *C*Cl), 129.7 (2 x *C*HCCl), 129.0 (*C*HCHCCl), 121.4 (*C*HCHCNH$_2$), 118.3 (Ar*C*), 112.6 (CH*C*HCNH$_2$), 92.2 (C*C*HCNH$_2$), 55.4 (*C*HCH$_3$), 53.0 (2 x *C*H$_2$NCH$_2$), 50.4 (C*C*H$_2$N), 23.7 (2 x *C*H$_2$CH$_2$N), 17.0 (*C*H$_3$); m/z (ESI+) 389 (100%, $[^{35,35}M+H]^+$), 391 (91%, $[^{35,37}M+H]^+$), 393 (15%, $[^{37,37}M+H]^+$); HRMS $C_{20}H_{23}{}^{35}Cl_2N_4$ ($[M+H]^+$) calcd. 389.1300, found. 389.1302.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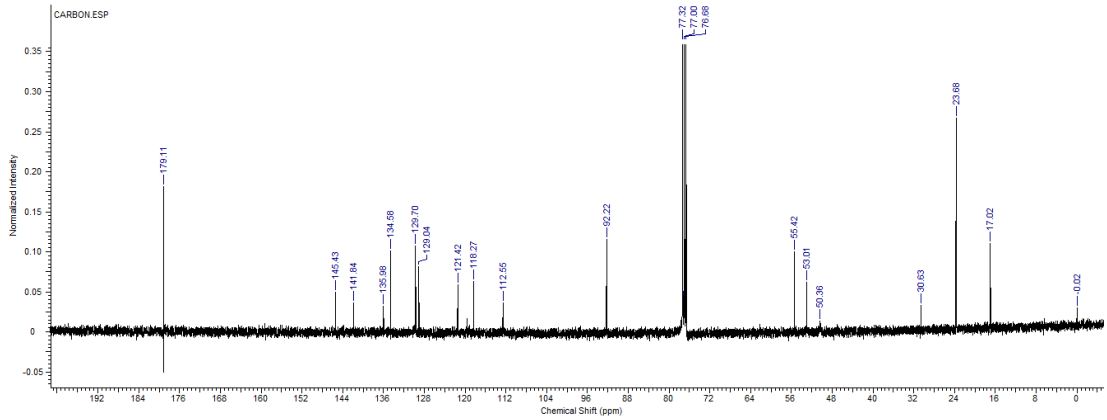
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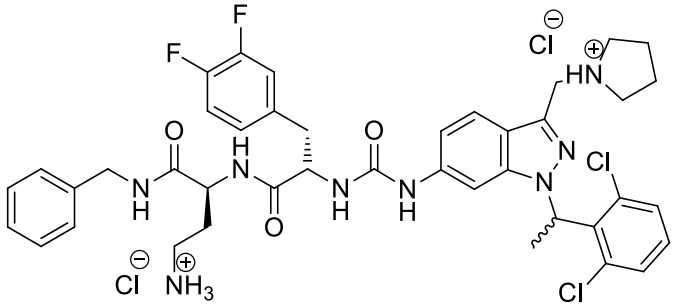
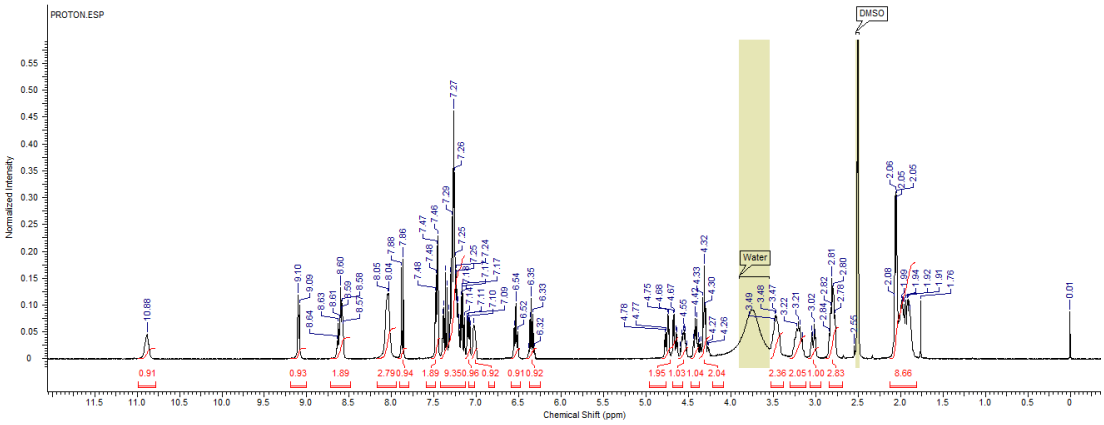
55.42
53.01
50.36
30.63
23.68
17.02
-0.02

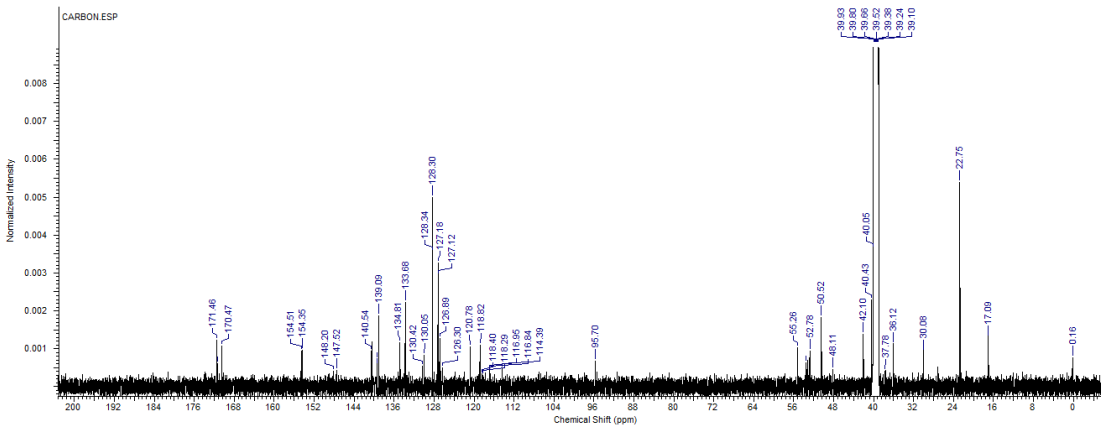
Racemic mixture of (*S*)-4-amino-*N*-benzyl-2-[(*S*)-2-(3-{1-[(*R*)-1-(2,6-dichlorophenyl)ethyl]-3-[pyrrolidin-1-ylmethyl]-1*H*-indazol-6-yl}ureido)-3-(3,4-difluorophenyl)propanamido]butanamide dihydrochloride salt & (*S*)-4-amino-*N*-benzyl-2-[(*S*)-2-(3-{1-[(*S*)-1-(2,6-dichlorophenyl)ethyl]-3-[pyrrolidin-1-ylmethyl]-1*H*-indazol-6-yl}ureido)-3-(3,4-difluorophenyl)propanamido]butanamide dihydrochloride salt (19)



To a sonicated (5 min), then stirred, solution of **18** (50.0 mg, 129 μmol) in dry THF (5 mL), under dry conditions and cooled to 0 °C, was added together triphosgene (13.0 mg, 45.0 μmol) and DMAP (31.0 mg, 257 μmol). After 5 min the reaction mixture was allowed to reach ambient temperature and stirred for 30 min under nitrogen. The reaction mixture was cooled again to 0 °C for 5 min and a solution of **2** (32.0 mg, 64.3 μmol) in dry THF (3 mL) was added. The reaction mixture was left to reach ambient temperature and stirred for 2 h under nitrogen then concentrated *in vacuo*. The residue was suspended in a solution of HCl (4 N) in dioxane (2 mL) and stirred for 2.5 h before concentrating *in vacuo* and purifying by preparative RP-HPLC (retention time: 8.92 min). The isolated product was taken up in a 0.1 M HCl (aq) solution (2 mL) and lyophilised to give title compound **19** (22.5 mg, 33%); as a white solid; mp 172-175 °C (decomp.); Rf 0.06 (10% MeOH/CH_2Cl_2); IR ν_{max} (neat) 3258 (N-H), 3084 (N-H), 2920-2850 (C-H), 1649 (C=O), 1547 (C=O), 1512 (C=O), 1434 (C-H), 1158 (C-N), 1116 (C-F), 1080 (C-N), 770 (ArC-H), 576 (C-Cl) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.88 (1H, bs, N^+HCH_2CNN), 9.10 (1H, s, N*H*CONHCH), 8.64-8.57 (1H, m, N*H*CH_2Ph & 1H, m, CHN*H*COCH), 8.05 (3H, bs, N^+H_3), 7.87 (1H, d, *J* = 8.8, C*H*CHCNH), 7.47 (2H, m, 2 x C*H*CCl), 7.40-7.14 (9H, ArH), 7.10 (1H, ddd, *J* = 8.8, 4.3, 1.5, CHC*H*CNH), 7.05-7.00 (1H, m, ArH), 6.54 (1H, t, *J* = 7.8-8.1, CHN*H*CONH), 6.35 (1H, quintet, *J* = 7.1-7.6, C*H*CH_3), 4.78-4.64 (2H, m, CH_2CNN), 4.56 (1H, m, C*H*NHCONH), 4.42 (1H, apparent dt, *J* = 6.0, 7.9, C*H*NHCOCH), 4.32 (2H, m, CH_2NHCO), 3.47 (2H, m, 2 x C(H)*H*N^+H(CH_2)CH_2C),

3.21 (2H, m, 2 x C(*H*)HN^{+}H(CH$_2$)CH$_2$C), 3.03 (1H, dt, *J* = 13.9, 4.0, C(H)*H*CHNHCONH), 2.84-2.78 (1H, m, C(*H*)HCHNHCONH & 2H, m, C*H*$_2$CH$_2$CHNH), 2.07-1.89 (3H, dd, *J* = 7.1, 2.0, C*H*$_3$CH & 2H, m, C*H*$_2$CHNHCOCH & 4H, m, 2 x C*H*$_2$CH$_2$N^{+}H(CH$_2$)CH$_2$C); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ 171.5 (NH*C*OCH), 171.3 (Ar*C*), 170.5 (NH*C*OCH), 154.5 (NH*C*ONH), 154.4 (Ar*C*), 148.2 (Ar*C*), 147.5 (Ar*C*), 140.5 (Ar*C*), 139.4 (Ar*C*), 139.1 (Ar*C*), 134.8 (Ar*C*), 133.7 (2 x *C*Cl), 130.4 (*C*HCHCCl), 130.1 (2 x *C*HCCl), 128.34 (Ar*C*H), 128.3 (Ar*C*H), 127.2 (Ar*C*H), 127.1 (Ar*C*H), 126.9 (Ar*C*H), 126.3 (Ar*C*H), 120.8 (*C*HCHCNH), 118.8 (Ar*C*), 118.3 (d, *J* = 16.5, Ar*C*H), 116.9 (d, *J* = 16.5, Ar*C*H), 114.4 (CH*C*HCNH), 95.7 (C*C*HCNH), 55.3 (*C*HCH$_3$), 53.5 (*C*HNHCONH), 52.8 (2 x *C*H$_2$N^{+}H(CH$_2$)CH$_2$C), 50.5 (*C*HNHCOCH), 48.1 (*C*H$_2$CNN), 42.1 (*C*H$_2$NHCO), 37.8 (*C*H$_2$CHNHCONH), 36.1 (*C*H$_2$CH$_2$CHNH), 30.1 (*C*H$_2$CHNHCOCH), 22.8 (2 x *C*H$_2$CH$_2$N(CH$_2$)CH$_2$C), 17.1 (*C*H$_3$CH); m/z (ESI+) 805 (100%, [^{35,35}M+H]$^{+}$), 807 (88%, [^{35,37}M+H]$^{+}$), 809 (22%, [^{37,37}M+H]$^{+}$); HRMS C$_{41}$H$_{45}$^{35}Cl$_2$F$_2$N$_8$O$_3$ ([M+H]$^{+}$) calcd. 805.2960, found. 805.2903.





Biology

Biological testing of compounds was done in collaboration with Prof Rachel Chambers' research group at the Centre for Inflammation and Tissue Repair (UCL, Division of Medicine). Calcium mobilisation assay work was assisted by Dr Natalia Smoktunowicz and CCL2 ELISA work was also carried out by Natalia. *In vivo* work was carried out by Dr Ricardo José.

Materials and methods

Reagents:

Thrombin, extracted from human plasma, was purchased from Calbiochem (Merck Biosciences, UK). PAR-1 agonist peptide and reverse peptide were supplied by Bachem. The original RWJ-58259 sample used as standard was a gift from Dr Claudia Derian (Johnson & Johnson Pharmaceutical Research & Development, USA).

Cell culture:

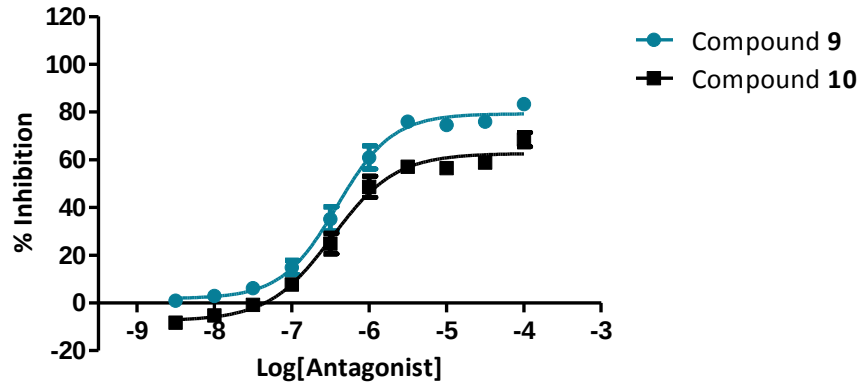
Primary human lung fibroblasts (pHLFs), grown from explant cultures of normal adult lung tissue, were a gift from Dr Robin J McAnulty (University College London). Cells were maintained in DMEM at 37 °C (10% CO_2) supplemented with glutamine (4 mM), penicillin, streptomycin, and 10% (v/v) FBS (all from Invitrogen) and were below passage 10 when used for experiments.

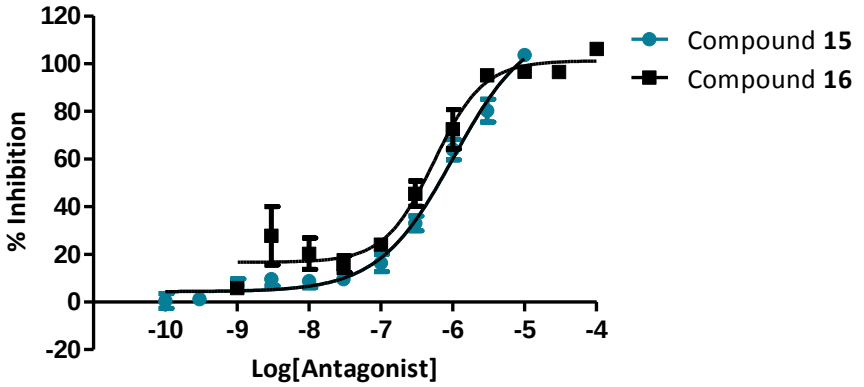
Measurement of intracellular Ca^{2+} levels:

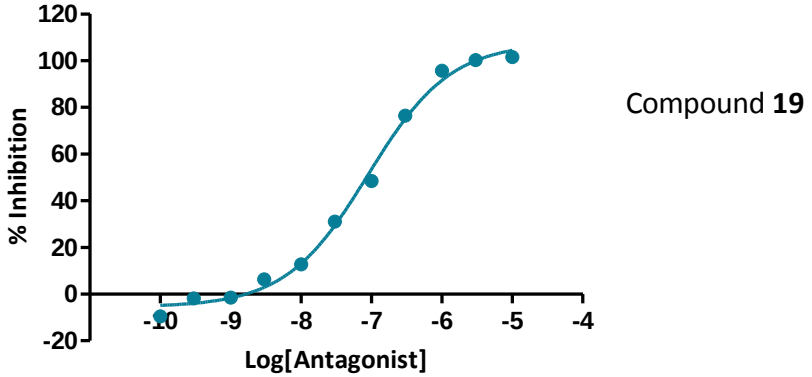
pHLFs were seeded in clear-bottom, black, 96-well microplates at a density of 10,000 cells/well for 48 h and then quiesced without FBS for 24 h. Intracellular Ca^{2+} levels were assessed using the Fluo-4 NW kit (Invitrogen, UK) according to the manufacturer's instructions. The dye was dissolved in assay buffer (20 mM HEPES in HBSS) supplemented with 2.5 mM probenecid. Wells were aspirated and solutions of test compounds (3mM in DMSO), diluted in the dye buffer solution to give a final concentration range of 30-0.0001 μM, were added to the relevant wells (100 μL per well). For positive controls, the equivalent volume of DMSO only was diluted in the

dye buffer solution and added to the relevant wells. The cells were then incubated again at 37 °C (10% CO_2) for 30 min and at ambient conditions, in the dark, for 30 min. Cells were then stimulated by addition of a solution of thrombin in assay buffer (50 µL, 10 nM) and changes in intracellular Ca^{2+} concentration were monitored in real-time using a fluorescent imaging plate reader (FLIPR® Tetra System, Molecular Devices, Inc). Measurements were taken every 1 sec for 1 min, then every 6 sec for a further 2 min. Experiments were performed in three replicates on the same plate and the data plotted as change in relative fluorescence units (RFU).[3]

Dose-inhibition curves for compounds **9, 10, 15, 16 & 19**:







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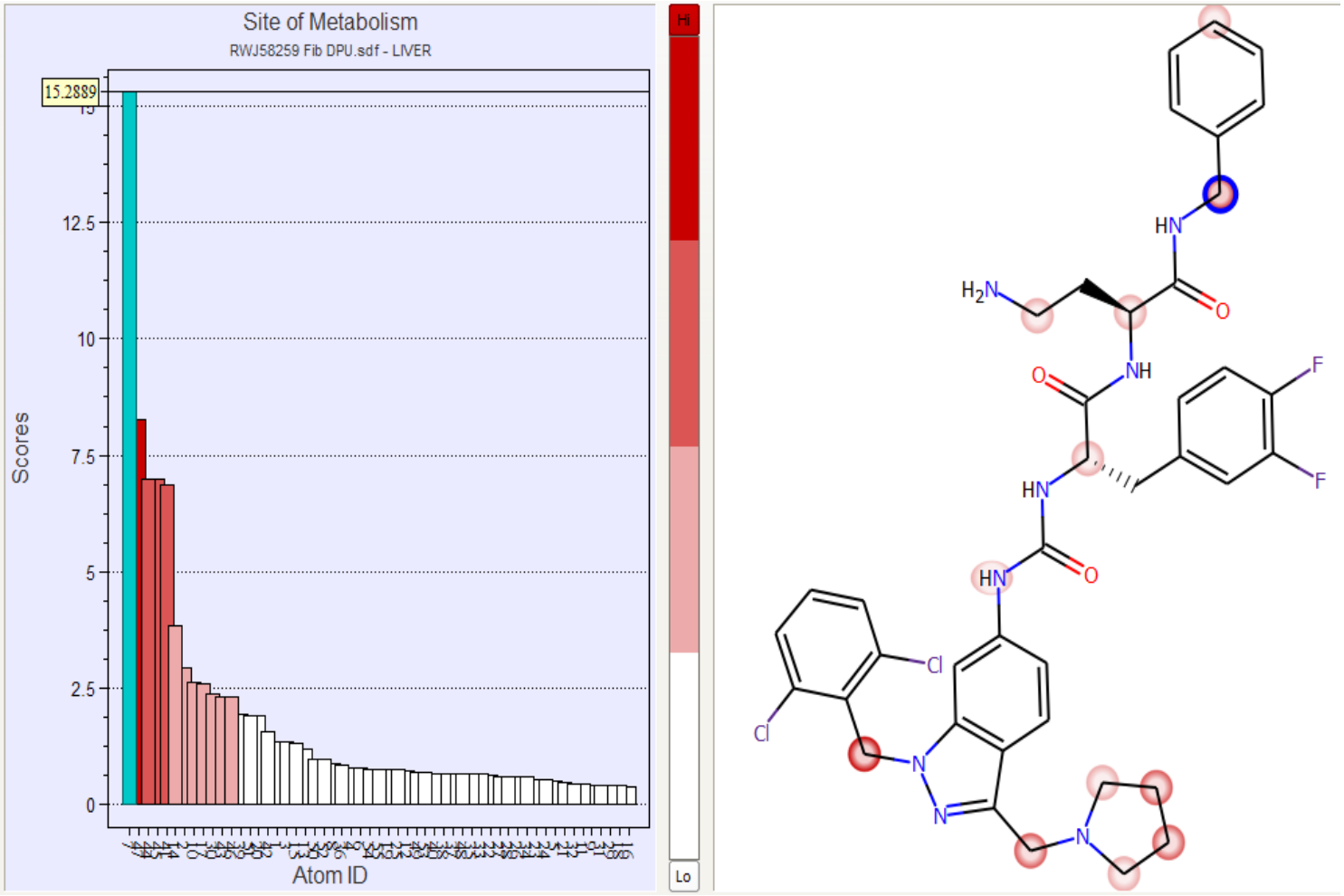
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2.5
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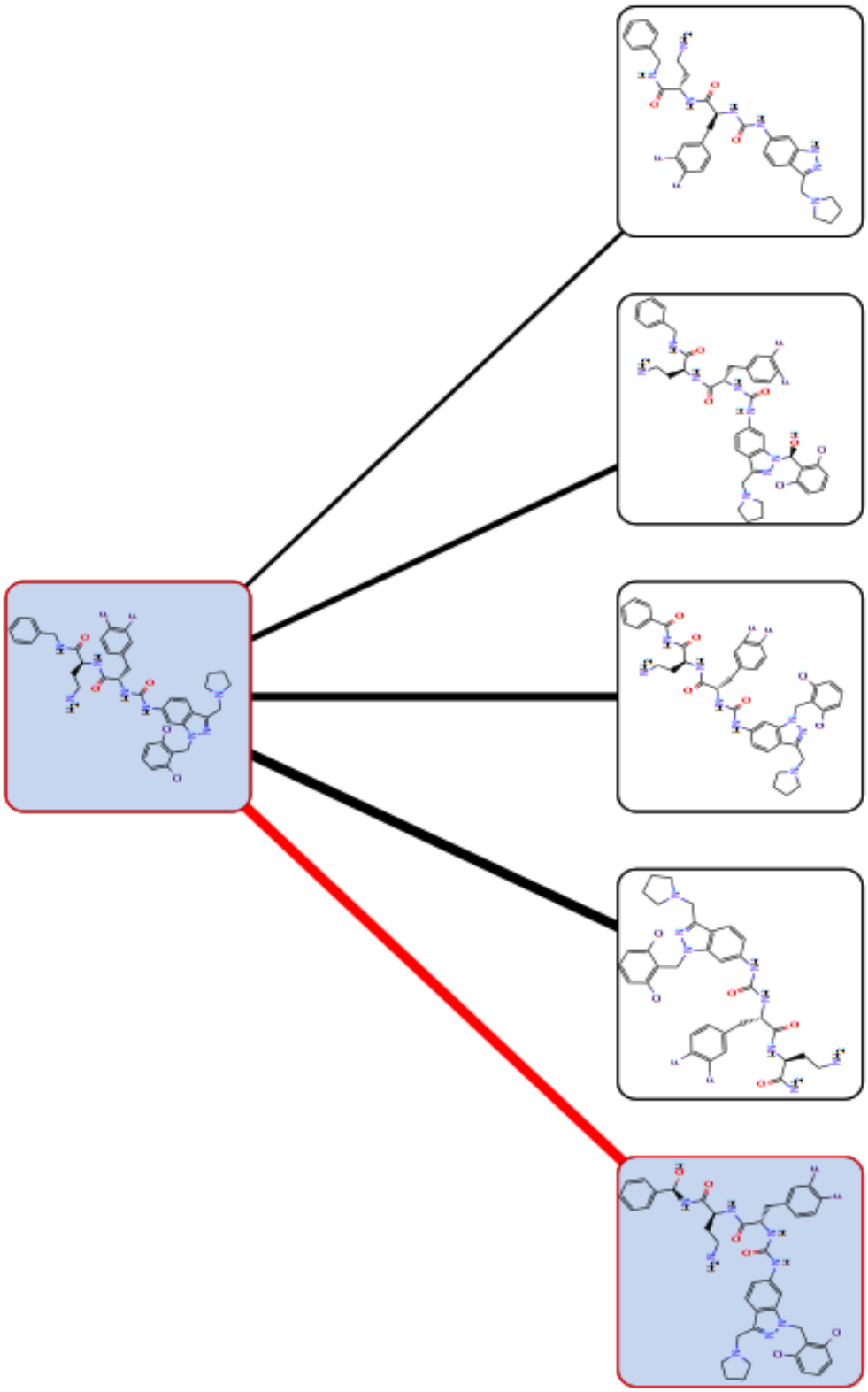

References:

1. H. C. Zhang, C. K. Derian, P. Andrade-Gordon, W. J. Hoekstra, D. F. McComsey, K. B. White, B. L. Poulter, M. F. Addo, W. M. Cheung, B. P. Damiano, D. Oksenberg, E. E. Reynolds, A. Pandey, R. M. Scarborough and B. E. Maryanoff, *Journal of Medicinal Chemistry*, 2001, **44**, 1021-1024.
2. A. M. Valdivielso, M. T. Garcia-Lopez and R. Herranz, *Arkivoc*, 2008, 287-294.
3. A. Ortiz-Stern, X. Deng, N. Smoktunowicz, P. F. Mercer and R. C. Chambers, *Journal of Cellular Physiology*, 2012, **227**, 3575-3584.