XIAP-BIR3 protein production for bioassay, crystallography and NMR

DNA encoding residues 252-350 or 250-354 of XIAP-BIR3 was cloned into a pET28B expression vector, Protein expression was performed using E.coli BL21 (DE3). A freshly transformed colony was transferred to 1 L of Terrific Broth media and grown overnight at 37 ºC, 200 rpm. 50 ml volumes of this overnight culture was then transferred into fresh 1 L flasks of Terrific Broth and grown for 4 hours at 37 ºC, 200 rpm before the temperature was dropped to 18 ºC. After two hours the expression was induced by the addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM and zinc acetate to a final concentration of 0.1 mM. Cells were grown overnight and then harvested by centrifugation. Bacterial cell pellets were resuspended in 50 mM Tris-HCl pH 7.5, 300 mM NaCl, 10% glycerol, 25 mM Imidazole-HCl pH 7.5 and lysed by sonication. The clarified lysate was incubated with Ni-NTA resin and tumbled overnight at 4 ºC. XIAP-BIR3 was eluted from the resin by the addition of 50 mM Tris-HCl pH 7.5, 300 mM NaCl, 10% glycerol, 500 mM Imidazole-HCl 7.5. Fractions containing XIAP-BIR3 were dialyzed overnight with thrombin to remove the histidine affinity against 25 mM Tris-HCl pH 8.0, 100 mM NaCl, and then concentrated prior to application to a S75 26/60 size exclusion column equilibrated in 25 mM Tris-HCl pH 8.0, 100 mM NaCl. The gel filtered protein was then concentrated to ~ 10 mg/ml for crystallization.

cIAP1 protein production for FP assay

DNA encoding residues 267-363 of cIAP1-BIR3 was cloned into a pGEX-4T expression vector. Protein expression was performed using E.coli BL21 (DE3). A freshly transformed colony was transferred to 1 L of Terrific Broth media and grown overnight at 37 ºC, 200 rpm. 50 ml volumes of this overnight culture was then transferred into fresh 1 L flasks of Terrific Broth and grown for 4 hours at 37 ºC, 200 rpm before the temperature was dropped to 18 ºC. After two hours the expression was induced by the addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM and zinc acetate to a final concentration of 0.1 mM. Cells were grown overnight and then harvested by centrifugation. Bacterial cell pellets were resuspended in 25 mM Tris-HCl pH 8.0, 200 mM NaCl and lysed by sonication. The clarified lysate was incubated with 10 ml of GST resin and tumbled overnight at 4 ºC. cIAP1-BIR3 was eluted from the resin by the addition of 25 mM Tris-HCl

pH 8.0, 200 mM NaCl, 25 mM reduced glutathione, pH 8.0. Fractions containing cIAP1-BIR3 were dialysed overnight with thrombin to remove the GST tag against 25mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP, and then concentrated prior to application to a S75 26/60 size exclusion column equilibrated in 25 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP.

Chemistry

All solvents employed were commercially available anhydrous grade, and reagents were used as received unless otherwise noted. Hereafter, petrol denotes the petroleum ether fraction boiling at 40 – 60 ^{o}C. Flash column chromatography was performed on a Biotage SP1 system (32–63 μm particle size, KP-Sil, 60 Å pore size). NMR spectra were recorded on a Bruker AV400 (Avance 400 MHz) spectrometer. Analytical LC–MS was conducted using an Agilent 1200 series with Mass Spec Detector coupled with an Agilent 6140 single quadrupole mass detector and an Agilent 1200 MWD SLUV detector. LC retention times, molecular ion (m/z) and LC purity (by UV) were based on the method below. Purity of compounds (as measured by peak area ratio) was >95%.

LC Method (BASIC)

Eluent A: 95:5 10 mM NH_4HCO_3+NH_4OH:CH_3CN (pH = 9.2)

Eluent B: CH_3CN

Gradient: 5-95% eluent B over 1.1 minutes

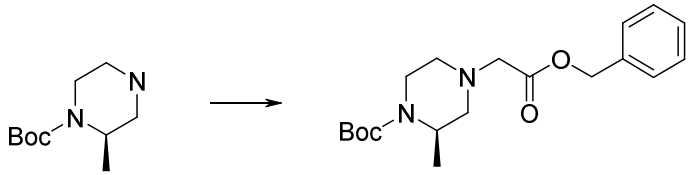
Flow: 0.9 ml/min

Column: Waters Acquity UPLC BEH C18; 1.7μ; 2.1x50 mm

Column T: 50°C

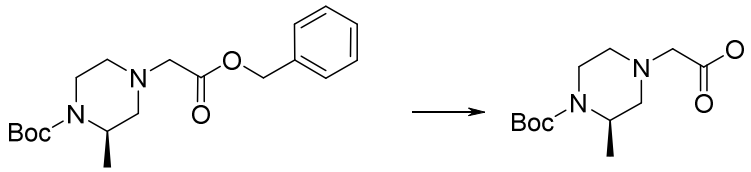
Compound 22 (Scheme 1): (*R*)-4-Carboxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester

Step 1: (*R*)-4-Benzyloxycarbonylmethyl-2-methyl-piperazine-1-carboxylic acid tert-butyl ester



Benzyl bromoacetate (807 µL, 5.0 mmoL) was added dropwise to a solution of (*R*)-2-methyl-piperazine-1-carboxylic acid tert-butyl ester (1.0 g, 5.0 mmol) in acetonitrile (5 mL) containing K_2CO_3 (760 mg, 5.5 mmol). The reaction was stirred at room temperature for 18 hours. The mixture was diluted with chloroform and quenched with brine. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica (gradient 0-50% EtOAc in petrol) to give the title compound (1.43g, 82%). ^{1}H NMR (400 MHz, Me-d3-OD): 7.48-7.13 (5H, m), 5.27-5.08 (2H, m), 4.18 (1H, s), 3.79 (1H, d), 3.33-3.19 (2H, m), 3.19-3.02 (1H, m), 2.83 (1H, d), 2.74 (1H, d), 2.36 (1H, dd), 2.29-2.14 (1H, m), 1.47 (9H, s), 1.26 (3H, d); LCMS: $[M+H]^+ = 349$.

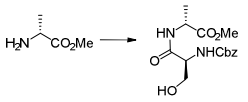
Step 2: (*R*)-4-Carboxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester **22**



Pd/C (435 mg, 0.4 mmol) was added to a solution of (*R*)-4-benzyloxycarbonylmethyl-2-methyl-piperazine-1-carboxylic acid tert-butyl ester (1.4 g, 4.1 mmol) in MeOH (41 mL). The reaction was stirred under H_2 (~1 bar) for 30 minutes. The catalyst was removed by filtration through Celite and the filtrate evaporated in vacuo to give the title compound (1.0 g, 94%). ^{1}H NMR (400 MHz, Me-d3-OD): 4.51-4.42 (1H, m), 4.10-3.99 (1H, m), 3.58 (1H, d), 3.53-3.39 (2H, m), 3.28 (1H, s), 2.99-2.82 (2H, m), 1.49 (9H, s), 1.36 (3H, d).

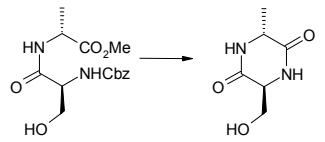
Compound 23 (Scheme 1): (2*R*,5*R*)-4-Carboxymethyl-5-methoxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester

Step 1: (*R*)-2-((*S*)-2-Benzyloxycarbonylamino-3-hydroxy-propionyl-amino)-propionic acid methyl ester



Diisopropylethylamine (375 mL) was added dropwise to a cooled mixture of (*R*)-2-amino-propionic acid methyl ester hydrochloride (100 g, 0.716 mol), EDC (165 g, 0.86 mol), carbobenzyloxy-L-serine (171.4 g, 0.716 mol) and dichloromethane (3.6 L). The resulting mixture was stirred under nitrogen at ambient temperature for 16 h. After removing solvent *in vacuo* at 40 ^{o}C, the residue was diluted with saturated sodium carbonate (1 L), water (1 L) and extracted with EtOAc (2 L, 2 x 1 L). The combined organic phases were washed with 2 M hydrochloric acid (1 L), saturated brine solution (1 L), dried over magnesium sulfate and concentrated *in vacuo* at 40 ^{o}C, to give the title compound (172 g, 74%) as a colourless solid. ^{1}H NMR (400 MHz, Me-d3-OD): 7.44-7.28 (6H, m), 5.13 (2H, s), 4.46 (1H, d), 4.43 (1H, d), 4.25 (1H, t), 3.82-3.68 (5H, m), 1.39 (3H, d).

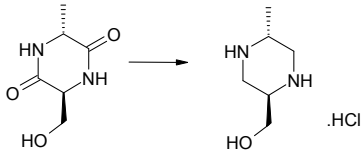
Step 2: (*3S,6R*)-3-Hydroxymethyl-6-methyl-piperazine-2,5-dione



To (*R*)-2-((*S*)-2-benzyloxycarbonylamino-3-hydroxy-propionylamino)-propionic acid methyl ester (172 g, 0.53 mol) was added 10% Pd / C (8.6 g), MeOH (530 mL) and cyclohexene (344 mL) under nitrogen. The mixture was heated to reflux for 17 h. MeOH (500 mL) was added and the reflux continued for 1 h. The hot reaction mixture was filtered through a pad of celite, cake washing with hot MeOH (2 x 500 mL). The combined filtrates were concentrated and the resulting solid was slurried in 2-butanone (400 mL), then petrol (400 mL) was added gradually over 10 min. After stirring for 30 min, the solids were filtered and cake washed with 2:1 petrol / 2-butanone (300 mL). The filter cake was dried *in vacuo* at 40 ^{o}C, to give the title compound (68.3 g, 81%) as an off white

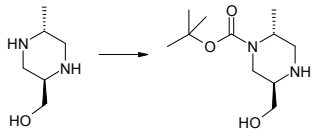
solid. ^{1}H NMR (400 MHz, DMSO-d6): 8.08 (1H, s), 7.90 (1H, s), 5.11 (1H, t), 3.92 (1H, q), 3.80-3.71 (1H, m), 3.71-3.60 (1H, m), 3.58-3.47 (1H, m), 1.24 (3H, d).

Step 3: ((2*R*,5*R*)-5-Methyl-piperazin-2-yl)-methanol hydrochloride



To (*3S,6R*)-3-hydroxymethyl-6-methyl-piperazine-2,5-dione (34 g, 0.215 mol) was added a solution of borane in THF (1 M, 1.6 L, 1.6 mol) and the mixture was heated to 70 ^{o}C for 18 h. The solution was cooled in ice, then MeOH (425 mL) was gradually added, followed by 5 M hydrochloric acid (113 mL). The mixture was heated to 70 ^{o}C for 2 h and then cooled to ambient temperature. The resulting solid was filtered, cake washed with THF (200 mL) and dried *in vacuo* at 40 ^{o}C, to give the title compound (39.3 g, quant.) as a colourless solid. ^{1}H NMR (400 MHz, DMSO-d6): 9.79 (3H, s), 5.59 (1H, s), 3.76-3.40 (5H, m), 3.19-2.94 (2H, m), 1.28 (3H, d).

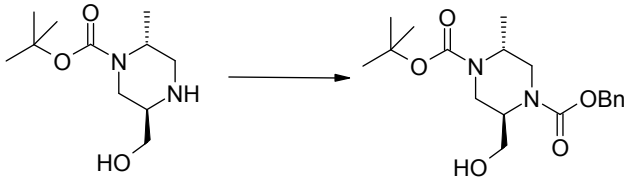
Step 4: (2*R*,5*R*)-5-Hydroxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester



To the piperazine dihydrochloride (20 g, 119 mmol) in MeOH (96 mL) at 0 °C (ice bath) was added triethylamine (48.7 mL, 357 mmol). *tert*-Butyl dicarbonate (61 g, 280 mmol) in MeOH (145 mL) was added over 30 min. The reaction temperature was maintained at <10 ^{o}C for 1 h, then mixture was warmed to ambient temperature over 1 h and subsequently heated to 50 ^{o}C for 18 h. The reaction was concentrated and the residue dissolved in ethanol (397 mL). A solution of NaOH (23.8 g, 595 mmol) in water (397 mL) was added and the reaction heated to 100 ^{o}C for 18 h, then cooled to ambient temperature. Mixture was neutralized with 1M HCl (~300 mL) to pH 9 (using a pH meter), then extracted with chloroform (3 x 700 mL), dried over sodium sulfate, filtered and concentrated. The

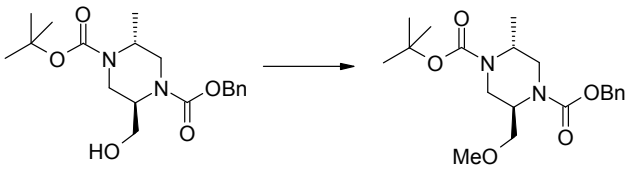
residue was re-dissolved in MeOH and concentrated, then dried *in vacuo* at 40 °C, to give the title compound (21 g, 75%) as a colourless solid. ^{1}H NMR (400 MHz, Me-d3-OD): 4.20-4.07 (1H, m), 3.79 (1H, dd), 3.71-3.58 (2H, m), 3.54 (1H, dd), 3.24 (1H, dd), 3.18-3.01 (1H, m), 3.01-2.89 (1H, m), 2.55 (1H, dd), 1.48 (9H, s), 1.25 (3H, s).

Step 5: (2*R*,5*R*)-2-Hydroxymethyl-5-methyl-piperazine-1,4-dicarboxylic acid 1-benzyl ester 4-*tert*-butyl ester



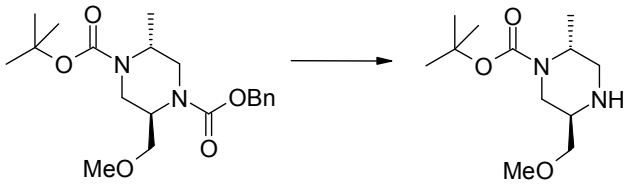
To (2*R*,5*R*)-5-hydroxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (21 g, 90 mmol) in THF (210 mL) at 3-4 °C (external ice bath cooling) was added 1 M aqueous NaOH (99.5 mL) and benzyl chloroformate (12.9 mL, 90.43 mmol). After stirring for 1 h at the same temperature, the mixture was left to stir for another 1 h and then warmed to ambient temperature. The organic layer was separated and the aqueous phase extracted with EtOAc (2 x 50 mL). The combined organic extracts were washed with saturated brine solution (150 mL), then dried over sodium sulfate, filtered and concentrated. The crude oil was purified by column chromatography on silica gel (gradient elution, 0 - 100%, EtOAc/petrol), to give the title compound (26 g, 76%) as a pale yellow oil which was used without further purification. ^{1}H NMR (400 MHz, Me-d3-OD): 7.47-7.15 (5H, m), 5.26-5.07 (2H, m), 4.25 (2H, s), 4.04-3.88 (1H, m), 3.83 (1H, d), 3.61 (2H, bs), 3.31-3.09 (2H, m), 1.48 (9H, s), 1.14 (3H, t).

Step 6: (2*R*,5*R*)-2-Methoxymethyl-5-methyl-piperazine-1,4-dicarboxylic acid 1-benzyl ester 4-*tert*-butyl ester



(2R,5R)-2-Hydroxymethyl-5-methyl-piperazine-1,4-dicarboxylic acid 1-benzyl ester 4-*tert*-butyl ester (25 g, 68 mmol) in dichloromethane (823 mL) was cooled to 4 ^{o}C (ice bath). 1,8-bis(dimethylamino)naphthalene (72 g, 337 mmol) was added in one portion followed by trimethyl oxonium tetrfluoroborate (50 g, 337 mmol) portionwise over 5 minutes. The mixture was stirred for 35 min at 5 ^{o}C, the ice bath removed, then warmed from 5-17 ^{o}C over 1 h. After stirring for a further 20 minutes, saturated aqueous ammonium chloride (300 mL) was added slowly and the reaction stirred for 10 minutes. The organic layer was separated and the aqueous phase extracted with dichloromethane (2 x 300 mL). The combined organic extracts were washed with 1.0 M HCl (3 x 1.5 L), saturated sodium bicarbonate solution (3 x 1.5 L), dried over sodium sulfate, filtered and concentrated. The oil was dissolved in dichloromethane and petrol added until precipitation of 1,8-bis(dimethylamino)naphthalene occurred. Purification by column chromatography (silica gel, 0-100%, EtOAc/petrol) gave an oil which contained 1,8-bis(dimethylamino)naphthalene as a contaminant. The remaining 1,8-bis(dimethylamino)naphthalene was captured during filtration through a SCX-2 column eluting with MeOH, to give the title compound (18 g, 65%) as a pale yellow oil. ^{1}H NMR (400 MHz, Me-d3-OD): 7.46-7.26 (5H, m), 5.26-5.07 (2H, m), 4.31 (2H, d), 4.04-3.85 (1H, m), 3.79 (1H, d), 3.46 (2H, s), 3.28-3.09 (2H, m), 1.48 (9H, s), 1.15 (3H, dd).

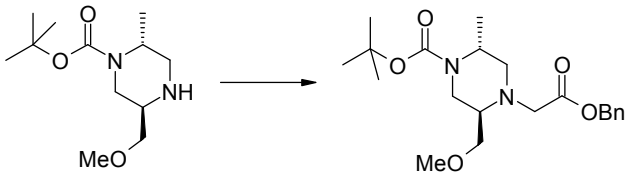
Step 7: (2*R*,5*R*)-5-methoxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester



10% Pd / C (5.0 g, 4.8 mmol) and (2*R*,5*R*)-2-methoxymethyl-5-methyl-piperazine-1,4-dicarboxylic acid 1-benzyl ester 4-*tert*-butyl ester (18.0 g, 48.0 mmol) were mixed with MeOH (190 mL) at ambient temperature and the mixture was hydrogenated at ambient temperature and ~1 bar for 3 h. The mixture was filtered through Celite and the filtrate concentrated, to give the title compound (11 g, 99%) as a colourless oil. ^{1}H NMR (400 MHz, Me-d3-OD): 4.16 (1H, dd), 3.80 (1H, d), 3.71-3.47

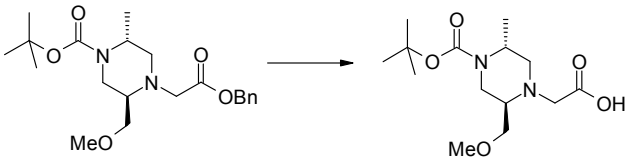
(1H, m), 3.47-3.40 (1H, m), 3.39 (3H, s), 3.30-3.17 (2H, m), 3.17-3.00 (2H, m), 2.60 (1H, dd), 1.48 (9H, s), 1.25 (3H, d).

Step 8: (2*R*,5*R*)-4-Benzyloxycarbonylmethyl-5-methoxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester



To a stirred suspension of (2*R*,5*R*)-5-methoxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (11.0 g, 45.0 mmol), potassium carbonate (6.8 g, 49.1 mmol) and acetonitrile (48 mL) was added benzyl bromoacetate (7.08 mL, 44.67 mmol) dropwise at ambient temperature. The reaction was stirred for 18 h at ambient temperature, then diluted with chloroform (150 mL), filtered and the filtrate concentrated. The resulting oil was purified by column chromatography on silica gel (gradient elution, 0 - 70%, EtOAc/petrol), to give the title compound (16 g, 92%) as a pale yellow oil. ^{1}H NMR (400 MHz, Me-d3-OD): 7.45-7.22 (5H, m), 5.21-5.12 (2H, m), 4.84 (2H, s), 4.16-4.03 (1H, m), 3.91 (1H, d), 3.62 (1H, d), 3.56-3.40 (2H, m), 3.31-3.19 (3H, m), 3.04-2.92 (1H, m), 2.80 (1H, dd), 2.68 (1H, dd), 1.48 (9H, s), 1.19 (3H, d).

Step 9: (2*R*,5*R*)-4-Carboxymethyl-5-methoxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester **23**



The title compound was prepared using a hydrogenolysis method analogous to that described for the synthesis of (2*R*,5*R*)-5-methoxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (see Step 7). ^{1}H NMR (400 MHz, Me-d3-OD): 4.35-4.17 (1H, m), 4.01 (1H, d), 3.85-3.55 (3H, m), 3.55-3.34 (6H, m), 3.21-2.94 (2H, m), 1.50 (9H, s), 1.28 (3H, d).

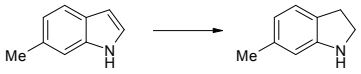
General procedure A: BH_3·THF reduction

6-Chloro-2,3-dihydro-1H-indole (Compound 24, Scheme 2)

6-Chloroindole (1.0 g, 6.6 mmol) was dissolved in a solution of borane in THF (1 M, 9.83 mmol) at 0 ^{o}C and stirred for 30 min. TFA (9.83 mL) was added dropwise and the solution stirred at 0 ^{o}C for 30 min. 6 M aqueous NaOH was added until the solution was basic (pH 11). The aqueous solution was extracted with dichloromethane (3 x 25 mL), dried over sodium sulfate, filtered and concentrated to give the title compound (864 mg, 86%) as a yellow oil. ^{1}H NMR (400 MHz, Me-d3-OD): 6.99 (1H, d), 6.64-6.55 (2H, m), 3.50 (2H, t), 2.95 (2H, t).

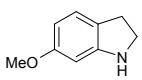
General procedure B: $NaBH_3CN$ reduction

6-Methyl-2,3-dihydro-1H-indole (Compound 25, Scheme 2)



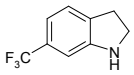
6-Methyl indole (0.50 g, 3.8 mmol) was dissolved in glacial acetic acid (19.1 mL) and cooled to 0 ^{o}C. Sodium cyanoborohydride (0.48 g, 7.6 mmol) was added portionwise and the mixture warmed to ambient temperature and stirred for 2 h. The reaction was diluted with water (8.0 mL), made alkaline with 40% aqueous NaOH and extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine (3 x 30 mL), dried over sodium sulfate, filtered and concentrated. Purification by column chromatography on silica gel (gradient elution, 0-100% EtOAc/petrol), gave the title compound (0.256 g, 51%) as a purple oil. ^{1}H NMR (400 MHz, Me-d3-OD): 6.96 (1H, d), 6.53 (2H, d), 3.44 (2H, t), 2.94 (2H, t), 2.23 (3H, s).

6-Methoxy-2,3-dihydro-1H-indole (Compound 26, Scheme 2)



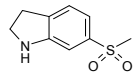
Starting with 6-methoxy indole, the title compound was prepared by using similar methods to those described in general procedure B. ^{1}H NMR (400 MHz, Me-d3-OD): 6.96 (1H, d), 6.37-6.18 (2H, m), 3.72 (3H, s), 3.47 (2H, t), 2.91 (2H, t).

6-Trifluoromethyl-2,3-dihydro-1H-indole (Compound 27, Scheme 2)



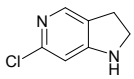
Starting with 6-trifluoromethyl indole, the title compound was prepared by using similar methods to those described in general procedure B. ^{1}H NMR (400 MHz, Me-d3-OD): 7.19 (1H, d), 6.89 (1H, d), 6.81 (1H, s), 3.55 (2H, t), 3.05 (2H, t).

6-Methylsulfonyl-2,3-dihydro-1H-indole (Compound 28, Scheme 2)



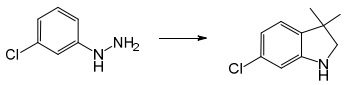
Prepared from 6-methylsulfonyl-1H-indole in an analogous manner to that described in general procedure A. MS: $[M+H]^+ = 198$.

6-Chloro-2,3-dihydro-1H-pyrrolo[3,2-c]pyridine (Compound 29, Scheme 2)



Under a nitrogen atmosphere, 6-chloro-5-aza indole (500 mg, 3.29 mmol) was dissolved in 2.0 M $BH_3{\cdot}SMe_2$ in THF (6.6 mL, 13.16 mmol) and heated gently to 68 ^{o}C for 2 h. After cooling to ambient temperature, MeOH (6.0 mL) was added slowly over 20 minutes. Once bubbling had stopped, the reaction was heated to 68 ^{o}C for 30 minutes, then cooled to ambient temperature and concentrated *in vacuo*. Chromatography on silica gel (gradient elution, 0-60% EtOAc/petrol), gave the title compound (209 mg, 41%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): 7.86 (1H, s), 6.43 (1H, s), 4.33 (1H, s), 3.80-3.65 (2H, m), 3.05 (2H, t).

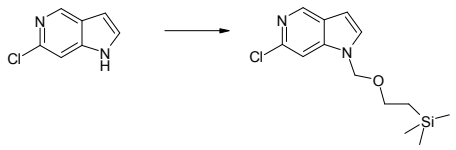
6-Chloro-3,3-dimethyl-2,3-dihydro-1H-indole (Compound 30, Scheme 2)



3-Chlorophenylhydrazine hydrochloride (5.0 g, 28 mmol) and isobutyraldehyde (2.56 mL, 28 mmol) in glacial acetic acid (93 mL) were heated to 60 ^{o}C for 3 h. The reaction mixture was cooled with a cold water bath and diluted with 1,2-dichloroethane (93 mL). $NaBH(OAc)_3$ (3.96 g, 18.7 mmol) was added in portions over 15 minutes and the reaction allowed to stir for 30 minutes. The solvent was removed *in vacuo* and the residue diluted with EtOAc (200 mL) and saturated sodium carbonate (200 mL). The mixture was extracted with EtOAc (200 mL) and the combined organic phases were washed with saturated brine solution (400 mL), dried over sodium sulfate, filtered and the solvent evaporated *in vacuo*. The two regioisomers were separated by chromatography on silica gel (gradient elution, 0-60% EtOAc/petrol then 0-100% Et_2O/petrol), to give the title compound (190 mg, 4%) as a yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): 6.93 (1H, d), 6.71 (1H, dd), 6.61 (1H, d), 3.34 (2H, s), 1.31 (6H, s).

Compound 31, Scheme 2: 6-Chloro-3,3-dimethyl-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine

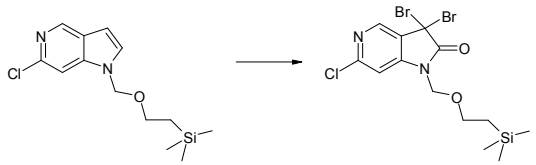
Step 1: 6-Chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1H-pyrrolo[3,2-c]pyridine



Sodium hydride (60%, 1.40 g, 35.0 mmol) was added in portions over 20 minutes to a solution of 6-chloro-5-aza-indole (4.45 g, 29.1 mmol) in DMF (17.2 mL) at 0 ^{o}C (ice bath cooling). The mixture was stirred for 1 h. (2-Chloromethoxy-ethyl)-trimethyl-silane (5.83 g, 35.0 mmol) was then added over 15 min. After stirring for 1 h, the reaction was quenched with water (100 mL) and the mixture extracted with dichloromethane (3 x 100 mL). The combined organic extracts were washed with brine (3 x 300 mL), dried over Na_2SO_4, filtered and concentrated. Chromatography on silica gel (gradient elution, 0 - 100%, EtOAc/petrol 40-60 ^{o}C), gave the title compound (6.7 g, 82%) as a yellow

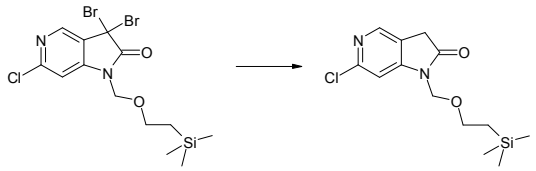
oil. ^{1}H NMR (400 MHz, Me-d3-OD): 8.61 (1H, s), 7.63 (1H, s), 7.50 (1H, d), 6.71 (1H, d), 5.57 (2H, s), 3.53 (2H, t), 0.88 (2H, t), -0.04--0.16 (9H, m).

Step 2: 3,3-Dibromo-6-chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one



A solution of 6-chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1H-pyrrolo[3,2-c]pyridine (6.70 g, 23.8 mmol) in anhydrous 1,4-dioxane (41 mL) was added to a solution of pyridium hydrobromide perbromide (38.0 g, 119 mmol) in anhydrous 1,4-dioxane (41 mL) over 30 minutes. After stirring for 1 h, water (100 mL) was added and the reaction stirred for 10 min, then the resulting mixture was extracted with EtOAc (100 mL x 3). The combined organic extracts were washed with water (3 x 100 mL) and brine (3 x 100 mL), then dried over Na_2SO_4, filtered and concentrated, to give the title compound (10.3 g, 58%) as a red oil. ^{1}H NMR (400 MHz, CDCl3): 8.56 (1H, s), 7.07 (1H, s), 5.21 (2H, s), 3.62 (2H, t), 0.99-0.92 (2H, m), 0.02--0.01 (9H, m). MS: $[M+H]^+ = 456$.

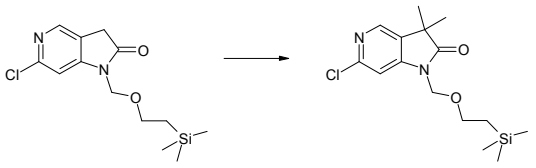
Step 3: 6-Chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one



Zinc powder (14.8 g, 226 mmol) was added to a biphasic mixture of 3,3-dibromo-6-chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one (10.3 g, 22.6 mmo) in THF (129 mL) and saturated ammonium chloride solution (33 mL). Due to delayed exotherm, the reaction was cooled in ice. The reaction was stirred for 3 h at ambient temperature, filtered and the filtrate concentrated. The residue was dissolved in EtOAc (20 mL) and water (20 mL) and passed through a short plug of celite, washing with EtOAc. The organic layer was separated and the aqueous phase

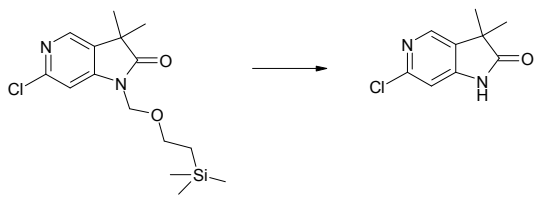
extracted with EtOAc (2 x 20 mL). The combined organic solutions were washed with brine, dried over magnesium sulfate, filtered and concentrated. Chromatography on silica gel (gradient elution, 0-100%, EtOAc/petrol), gave the title compound (5.08 g, 75%) as a pale yellow oil. ^{1}H NMR (400 MHz, Me-d3-OD): 8.14 (1H, s), 7.18 (1H, s), 5.17 (2H, s), 3.63 (2H, t), 0.94 (2H, t), 0.18-0.18 (9H, m).

Step 4: 6-Chloro-3,3-dimethyl-1-(2-trimethylsilanyl-ethoxymethyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one



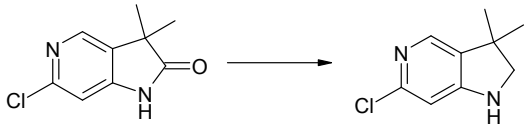
6-Chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one (5.08 g, 17.05 mmol) in anhydrous THF (85 mL) was cooled to -78 ^{o}C. 1 M LiHMDS in THF (37.5 mL) was added dropwise over 10 minutes and the reaction stirred for 30 minutes. Methyl iodide (3.18 mL, 51.1 mol) was added and the reaction stirred for 30 minutes then warmed to ambient temperature over 1.5 h. The mixture was quenched with saturated aqueous ammonium chloride (50 mL), then extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with water (3 x 150 mL), brine (3 x 150 mL), dried over sodium sulfate, filtered and concentrated. Chromatography on silica gel (gradient elution 0-70%, EtOAc/petrol), gave the title compound (2.83 g, 51%) as a pale yellow oil. ^{1}H NMR (400 MHz, Me-d3-OD): 8.22 (1H, s), 7.22 (1H, s), 5.19 (2H, s), 3.60 (2H, t), 1.44 (6H, s), 0.93 (2H, t), 0.16-0.20 (9H, m).

Step 5: 6-Chloro-3,3-dimethyl-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one



To a solution of 6-chloro-3,3-dimethyl-1-(2-trimethylsilanyl-ethoxymethyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one (7.25 g, 22.2 mmol) in dichloromethane (50 mL) was added TFA (50 mL) and resulting mixture was stirred at 20 °C for 1 h. The solution was evaporated *in vacuo* and the residue dissolved in MeOH and solvent re-evaporated to give a solid. To an aliquot of this material (2.40 g) in THF (53 mL) was added piperazine (4.56 g, 52.9 mmol). After stirring for 1 h, water (30 mL) and EtOAc (30 mL) were added and the organic layer separated. The organic layer was washed with water (30 mL x 2) and brine (30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Chromatography on silica gel (gradient elution, 0-100% EtOAc/petrol), gave the title compound (1.4 g, 67%) as a colorless solid. ^{1}H NMR (400 MHz, Me-d3-OD): 8.12 (1H, s), 6.99 (1H, s), 1.41 (6H, s).

Step 6: 6-Chloro-3,3-dimethyl-2,3-dihydro-1H-pyrrolo[3,2-c]pyridine **31**

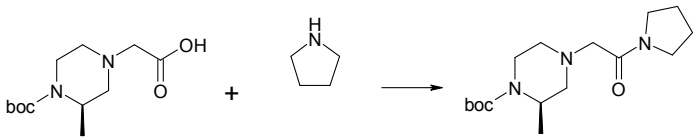


6-Chloro-3,3-dimethyl-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one (1.4 g, 7.14 mmol) was dissolved in a solution of borane – dimethyl sulfide complex in THF (2 M, 36 mL, 71.4 mmol) and heated to 65 °C for 3 h, then cooled to ambient temperature. MeOH was added and the reaction heated at reflux for 1.5 h. After overnight stirring, the solvent was removed *in vacuo*. Column chromatography on silica gel (gradient elution, 0-100% EtOAc/petrol), gave the title compound (1.23 g, 95%) as a colorless solid. ^{1}H NMR (400 MHz, Me-d3-OD): 7.65 (1H, s), 6.42 (1H, s), 3.43 (2H, s), 1.34 (6H, s).

Compound 5: 2-((R)-3-methyl-piperazin-1-yl)-1-pyrrolidin-1-yl-ethanone hydrochloride salt

Step 1: General procedure C: PyBroP coupling

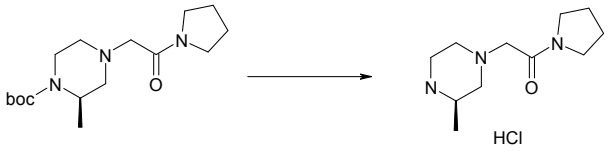
(*R*)-2-Methyl-4-(2-oxo-2-pyrrolidin-1-yl-ethyl)-piperazine-1-carboxylic acid tert-butyl ester



To a solution of (*R*)-4-carboxymethyl-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (200 mg, 0.77 mmol) and pyrrolidine (75 µL, 0.85 mmol) in dichloromethane (5 mL) were added PyBroP (400 mg, 0.85 mmol) and triethylamine (217 µL, 1.55 mmol). The reaction was stirred at room temperature for 16 hours, quenched with brine and the product was extracted with dichloromethane. The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified by column chromatography (gradient 0-100% EtOAc in Petrol) to give the title compound (220 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, CDCl3): 4.24 (1H, s), 3.84 (1H, d), 3.71-3.44 (4H, m), 3.26-3.03 (3H, m), 2.85 (1H, d), 2.75 (1H, d), 2.31 (1H, dd), 2.24-2.11 (1H, m), 2.02-1.92 (2H, m), 1.92-1.74 (2H, m), 1.48 (9H, s), 1.28 (3H, s).

Step 2: General procedure D: Boc deprotection

2-((*R*)-3-methyl-piperazin-1-yl)-1-pyrrolidin-1-yl-ethanone hydrochloride salt **5**



(*R*)-2-Methyl-4-(2-oxo-2-pyrrolidin-1-yl-ethyl)-piperazine-1-carboxylic acid tert-butyl ester (200 mg, 0.64 mmol) was dissolved in a saturated solution of HCl in EtOAc (20 mL) and the solution was stirred at room temperature for 2 hours. The solvent was removed in vacuo, the solid residue was washed with Et_2O (2x) and dried in the vacuum oven to give the desired product as a white solid (150 mg). ^{1}H NMR (400 MHz, Me-d_3-OD): 4.44 (2H, s), 4.05-3.85 (3H, m), 3.78 (1H, t), 3.74-3.63 (2H, m), 3.63-3.43 (5H, m), 2.24-1.99 (2H, m), 1.99-1.73 (2H, m), 1.49 (3H, d).

Compounds **7, 9-10** and **12-20** were prepared starting from the appropriate piperazine and the appropriate indoline or aza-indoline in a similar manner as that described in General procedures C and D. Analytical data obtained were as follows:

1-(2,3-Dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **7**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.16 (1H, d), 7.30 (1H, d), 7.22 (1H, t), 7.12 (1H, t), 4.55 (2H, s), 4.14 (2H, s), 3.93 (3H, d), 3.79 (1H, d), 3.65 (2H, s), 3.48 (1H, s), 3.34-3.32 (2H, m), 1.49 (3H, d); LCMS: $[M+H]^+$ = 260.

1-(6-Methoxy-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **9**

^{1}H NMR (400 MHz, Me-d_3-OD): 7.82 (1H, d), 7.11 (1H, d), 6.62 (1H, dd), 4.19 (2H, t), 3.78 (3H, s), 3.12 (2H, t), 3.03-2.82 (6H, m), 2.29-2.14 (1H, m), 1.89 (1H, t), 1.07 (3H, d); LCMS: $[M+H]^+$ = 290.

1-(6-Methyl-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **10**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.01 (1H, s), 7.11 (1H, d), 6.88 (1H, d), 4.18 (2H, t), 3.26-3.01 (2H, m), 3.00-2.83 (6H, m), 2.33 (3H, s), 2.28-2.13 (1H, m), 1.89 (1H, t), 1.07 (3H, d); LCMS: $[M+H]^+$ = 274.

1-(6-Fluoro-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **12**

^{1}H NMR (400 MHz, Me-d_3-OD): 7.89 (1H, dd), 7.19 (1H, t), 6.82-6.70 (1H, m), 4.24 (2H, t), 3.17 (2H, t), 3.04-2.73 (6H, m), 2.30-2.14 (1H, m), 1.90 (1H, t), 1.07 (3H, d); LCMS: $[M+H]^+$ = 278.

1-(6-Fluoro-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **13**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.16 (1H, s), 7.19 (1H, d), 7.03 (1H, dd), 4.22 (2H, t), 3.18 (2H, t), 3.06-2.73 (6H, m), 2.30-2.14 (1H, m), 1.90 (1H, t), 1.07 (3H, d); LCMS: $[M+H]^+$ = 294.

1-(6-Bromo-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **14**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.33 (1H, s), 7.24-7.10 (2H, m), 4.22 (2H, t), 3.37 (1H, s), 3.16 (2H, t), 3.04-2.77 (6H, m), 2.32-2.17 (1H, m), 1.93 (1H, t), 1.09 (3H, d); LCMS: $[M+H]^+$ = 340.

2-[(3*R*)-3-Methylpiperazin-1-yl]-1-[6-(trifluoromethyl)-2,3-dihydro-1H-indol-1-yl]ethan-1-one **15**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.46 (1H, s), 7.41 (1H, d), 7.34 (1H, d), 4.27 (2H, dd), 3.37-3.36 (2H, m), 3.32-3.25 (2H, m), 2.94 (5H, m), 2.31-2.14 (1H, m), 1.98-1.85 (1H, m), 1.08 (3H, d); LCMS: $[M+H]^+$ = 328.

1-(6-Methanesulfonyl-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **16**

^{1}H NMR (400 MHz, DMSO-d_6): 8.53 (1H, s), 7.57 (1H, dd), 7.51 (1H, d), 4.18 (2H, t), 3.47 (2H, s), 3.31-3.22 (4H, m), 3.13 (3H, s), 3.07-2.94 (3H, m), 2.65 (1H, t), 2.45 (1H, t), 1.19 (3H, d); LCMS: $[M+H]^+$ = 338.

1-{6-Chloro-1H,2H,3H-pyrrolo[3,2-c]pyridin-1-yl}-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **17**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.25 (1H, s), 8.11 (1H, s), 4.39 (2H, s), 4.35-4.22 (2H, m), 3.86-3.68 (4H, m), 3.62-3.41 (2H, m), 1.45 (3H, d); LCMS: $[M+H]^+$ = 295.

1-(6-Chloro-3,3-dimethyl-2,3-dihydro-1H-indol-1-yl)-2-[(3*R*)-3-methylpiperazin-1-yl]ethan-1-one **18**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.14 (1H, s), 7.20 (1H, d), 7.08 (1H, dd), 3.99 (2H, s), 3.04-2.76 (6H, m), 2.32-2.18 (1H, m), 1.94 (1H, t), 1.36 (6H, s), 1.09 (3H, d); LCMS: $[M+H]^+$ = 322.

1-(6-Chloro-3,3-dimethyl-2,3-dihydro-1H-indol-1-yl)-2-[(2*R*,5*R*)-2-(methoxymethyl)-5-methylpiperazin-1-yl]ethan-1-one **19**

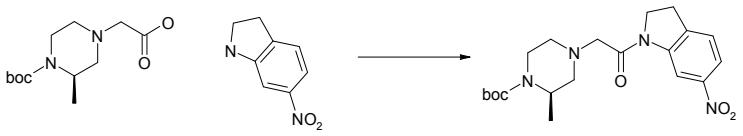
^{1}H NMR (400 MHz, Me-d_3-OD): 8.18 (1H, d), 7.27 (1H, d), 7.16 (1H, dd), 4.60-4.41 (2H, m), 4.16 (1H, s), 4.01-3.46 (11H, m), 1.50-1.40 (9H, m); LCMS: $[M+H]^+$ = 366.

1-{6-Chloro-3,3-dimethyl-1H,2H,3H-pyrrolo[3,2-c]pyridin-1-yl}-2-[(2*R*,5*R*)-2-(methoxymethyl)-5-methylpiperazin-1-yl]ethan-1-one **20**

^{1}H NMR (400 MHz, Me-d_3-OD): 8.44 (1H, s), 8.25 (1H, s), 4.62 (1H, d), 4.55 (1H, d), 4.36-4.02 (3H, m), 3.92-3.68 (5H, m), 3.68-3.51 (2H, m), 3.35 (3H, s), 1.53 (6H, s), 1.46 (3H, d); LCMS: $[M+H]^+$ = 367.

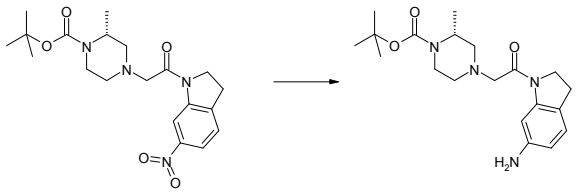
Compound 8, Scheme 4: 1-(6-amino-2,3-dihydro-indol-1-yl)-2-((*R*)-3-methyl-piperazin-1-yl)-ethanone

Step1: (*R*)-4-[2-(6-Nitro-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester



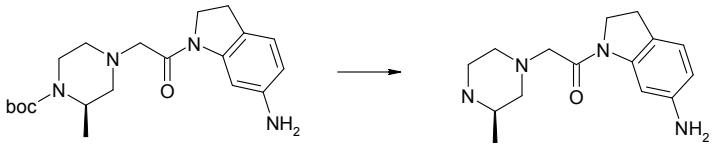
The title compound was prepared starting from carboxylic acid **22** and 6-nitro-2,3-dihydro-1H-indole in 50% yield following a procedure similar to that described in General procedure C. ^{1}H NMR (400 MHz, Me-d3-OD): 8.96 (1H, s), 7.97 (1H, dd), 7.45 (1H, d), 4.39 (2H, t), 4.24 (1H, s), 3.83 (1H, d), 3.36 (4H, s), 3.20 (1H, t), 2.94 (1H, d), 2.84 (1H, d), 2.35 (1H, dd), 2.25-2.12 (1H, m), 1.48 (9H, s), 1.31 (3H, d); LCMS: $[M+H]^+ = 405$.

Step 2: (*R*)-4-[2-(6-Amino-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester



(*R*)-2-Methyl-4-[2-(6-nitro-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-piperazine-1-carboxylic acid *tert*-butyl ester (390 mg, 0.96 mmol) and 10% Pd/C (0.102 mg, 0.10 mmol) were mixed with MeOH (9.6 mL). The reaction was hydrogenated at ~ 1 bar for 30 minutes at ambient temperature then filtered under vacuum and concentrated, to give the title compound (360 mg, 100%) as an off white glass. ^{1}H NMR (400 MHz, Me-d3-OD): 7.64 (1H, d), 6.97 (1H, d), 6.47 (1H, dd), 4.32-4.17 (3H, m), 3.82 (1H, d), 3.28 (2H, s), 3.24-3.14 (1H, m), 3.07 (2H, t), 2.92 (1H, d), 2.81 (1H, d), 2.32 (1H, dd), 2.24-2.07 (1H, m), 1.48 (9H, s), 1.30 (3H, d).

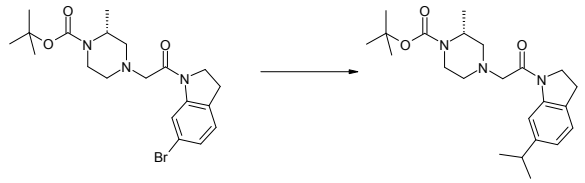
Step 3: 1-(6-Amino-2,3-dihydro-indol-1-yl)-2-((*R*)-3-methyl-piperazin-1-yl)-ethanone **8**



(*R*)-4-[2-(6-Amino-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester was deprotected following similar methods to those described in General procedure D to give the desired product as pale yellow solid (82% yield). ^{1}H NMR (400 MHz, Me-d_3-OD): 7.65 (1H, d), 6.97 (1H, d), 6.47 (1H, dd), 4.14 (2H, t), 3.37 (1H, s), 3.27-2.90 (8H, m), 2.47-2.33 (1H, m), 2.13 (1H, q), 1.19 (3H, d); LCMS: $[M+H]^+ = 275$.

Compound 11, Scheme 4: 1-(6-Isopropyl-2,3-dihydro-indol-1-yl)-2-((*R*)-3-methyl-piperazin-1-yl)-ethanone

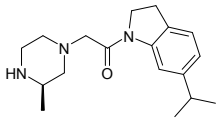
Step 1: (*R*)-4-[2-(6-Isopropyl-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester



$Pd(PPh_3)_4$ (32 mg, 0.03 mmol), (*R*)-4-[2-(6-bromo-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (246 mg, 0.56 mmol) and 2-isopropenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (113 mg, 0.67 mmol) were dissolved in DMF (1.87 mL). Cs_2CO_3 (549 mg, 1.7 mmol) dissolved in water (0.37 mL) was added to the DMF solution. The reaction was degassed and heated to 85 ^{o}C for 18 h then was filtered and concentrated. Column chromatography on silica gel (gradient elution, 0-100% EtOAc/petrol), gave (*R*)-4-[2-(6-isopropenyl-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (0.18 g) as a yellow

oil. 10% Pd/C (48 mg, 0.05 mmol) and (*R*)-4-[2-(6-isopropenyl-2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-2-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (0.18 g) were mixed with MeOH (4.5 mL). The reaction was hydrogenated at ~ 1 bar for 30 min at ambient temperature then filtered under vacuum and concentrated, to give the title compound (157 mg, 86%) as a colorless oil. ^{1}H NMR (400 MHz, Me-d3-OD): 8.08 (1H, s), 7.15 (1H, d), 6.95 (1H, d), 4.35-4.18 (3H, m), 3.83 (1H, d), 3.30 (2H, s), 3.25-3.12 (3H, m), 2.97-2.76 (3H, m), 2.33 (1H, dd), 2.20-2.08 (1H, m), 1.48 (9H, s), 1.31 (3H, d), 1.25 (6H, d).

Step 2: 1-(6-Isopropyl-2,3-dihydro-indol-1-yl)-2-((R)-3-methyl-piperazin-1-yl)-ethanone **11**



The title compound was prepared in a similar manner as that described in General procedure D. ^{1}H NMR (400 MHz, Me-d3-OD): 8.10 (1H, s), 7.15 (1H, d), 6.94 (1H, d), 4.19 (2H, t), 3.25-3.02 (2H, m), 3.02-2.74 (6H, m), 2.30-2.15 (1H, m), 1.91 (1H, t), 1.25 (6H, d), 1.08 (3H, d). LCMS: $[M+H]^+$ = 302.