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The Development of a Manufacturing Route to an MCHr1 Antagonist

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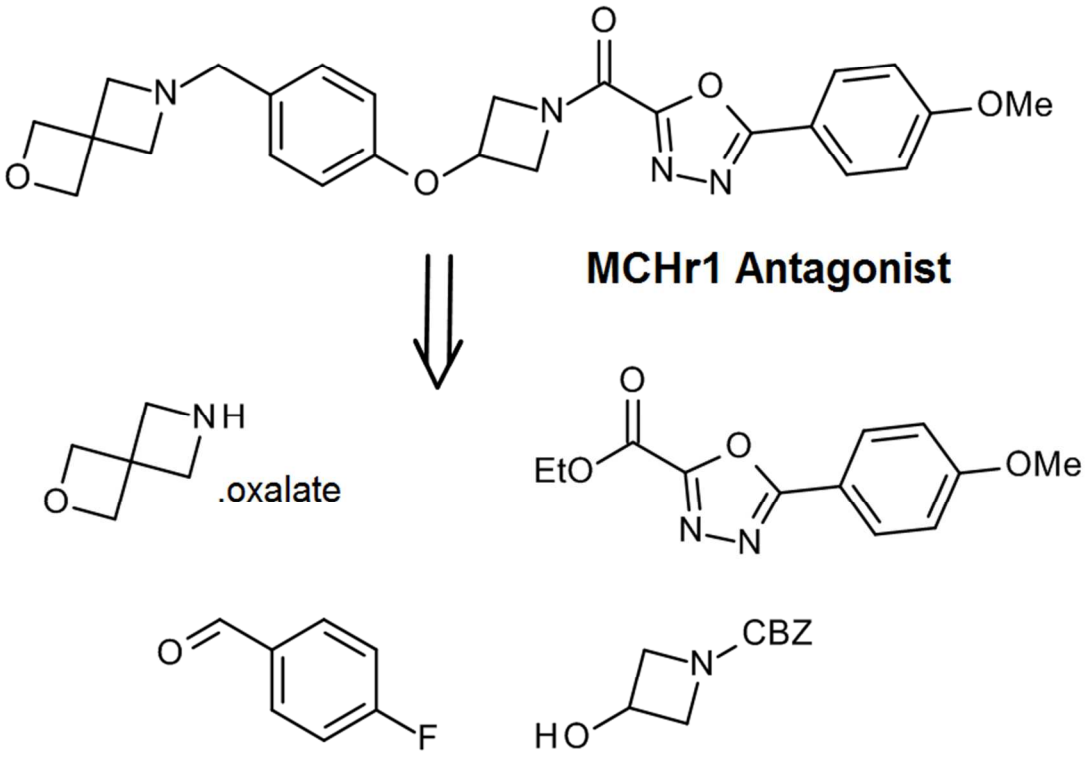


Abstract

Process development work to provide an efficient manufacturing route to a MCHr1 antagonist is presented herewith. Features of this development work include a scalable manufacturing route to the useful 6-oxa-2-azaspiro[3.3]heptane building block, and the use of a (soluble) alternative to sodium triacetoxyborohydride.

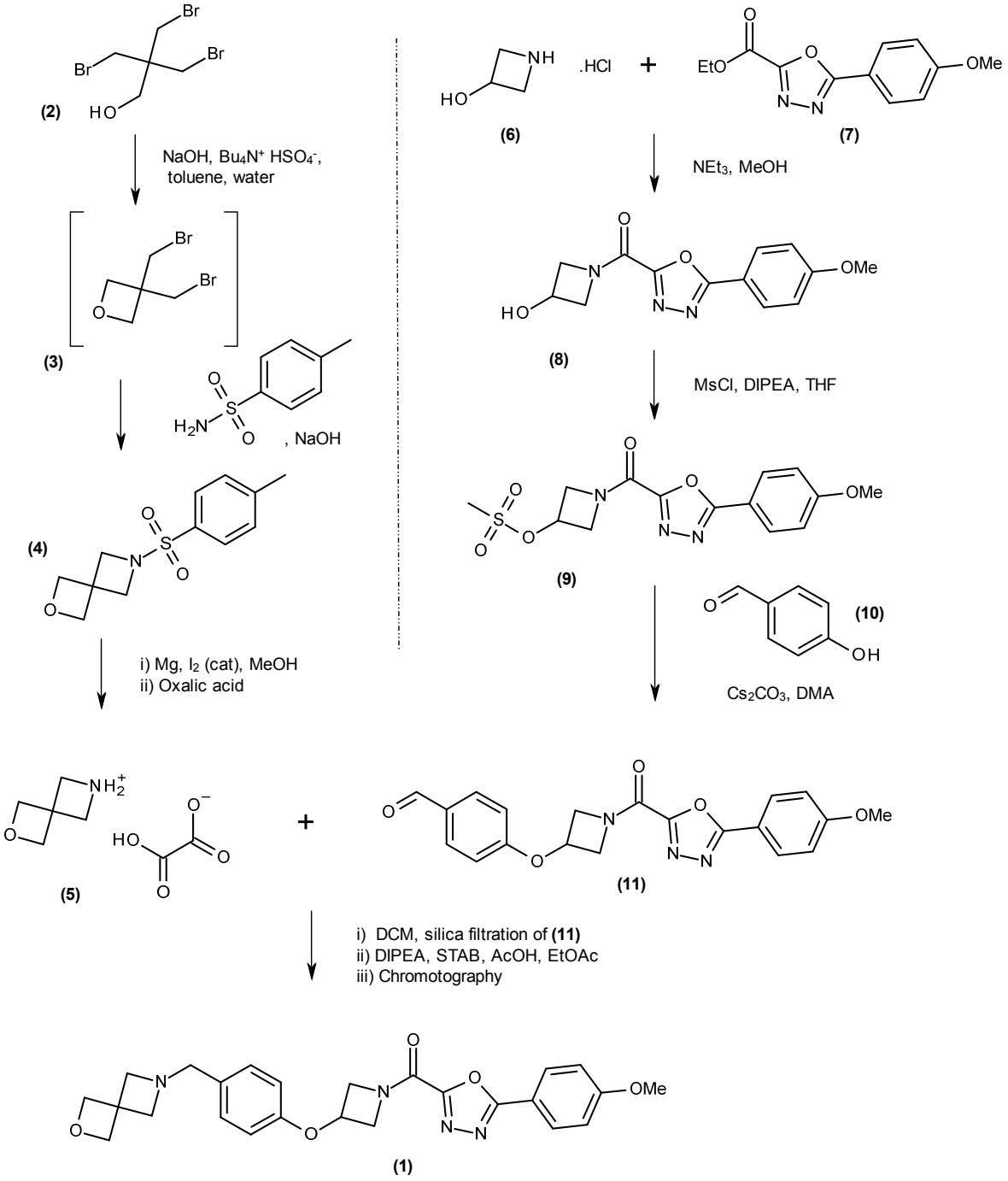
Keywords :
6-oxa-2-azaspiro[3.3]heptane
sodium triacetoxyborohydride
reductive amination
MCHr1 antagonist

Introduction

1 is a melanin-concentrating hormone receptor 1 (MCHr1) antagonist, thought to be involved in the regulation of appetite.[1a] MCHR1 antagonists are a class of agents that show promise for treating obesity, that have been well-validated in animal models.[1b]

1 was first manufactured using the 1st generation route (Scheme 1), which provided material for the pre-clinical and initial Phase I studies. However, several problems were associated with this route. The use of mesylation to activate the alcohol **8** gave rise to a genotoxic intermediate **9** and related (potentially genotoxic) impurities, which would be difficult to fully control if chromatography were to be removed from the final API stage. The aldehyde **11** was impure and required filtration through a silica plug before undertaking reductive amination, Due to the poor solubility of the aldehyde **11**, dichloromethane was found to be the only suitable solvent to achieve this. The deprotection of the sulphonamide **4** using magnesium in methanol resulted in a very difficult work-up and low yield (<35%) of the resulting 6-oxa-2-azaspiro[3.3]heptane **5** compound. Additionally, the route was low yielding, the API **1** was isolated with low purity, and chromatography was required for clinical use.

Scheme 1 – 1st generation route



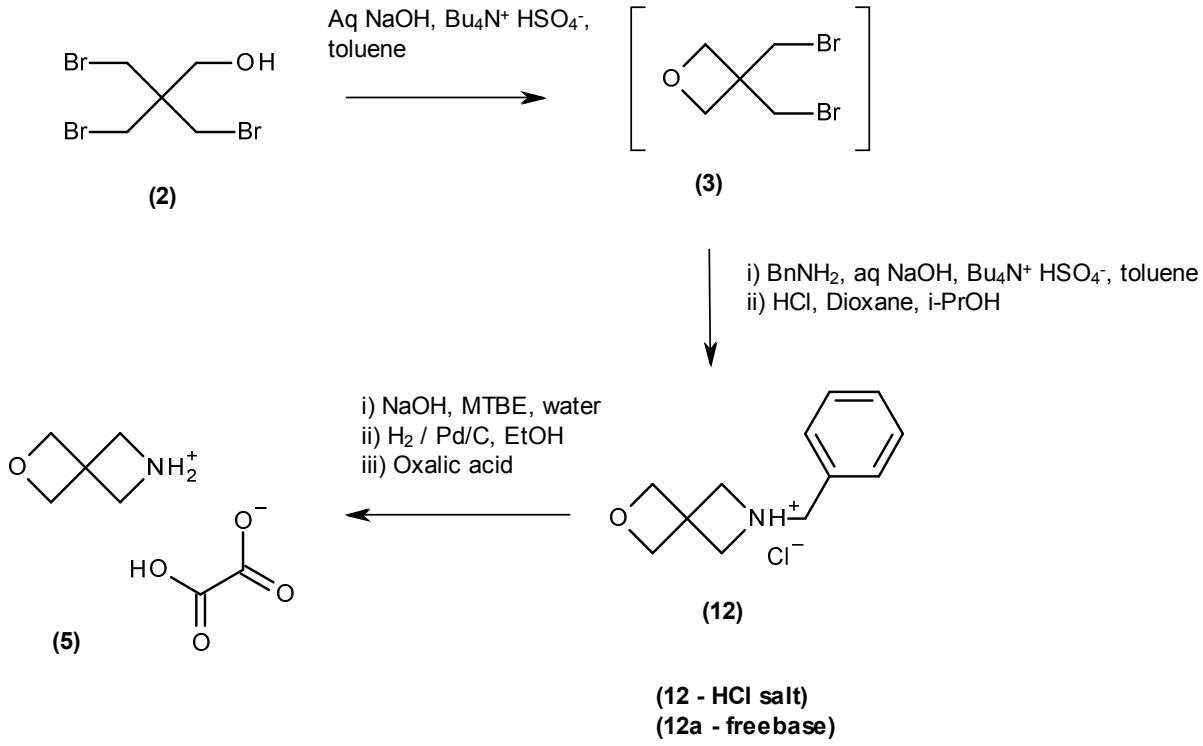
Results and Discussion

There is significant interest in the literature in the 6-oxa-2-azaspiro[3.3]heptane **5** fragment, which is incorporated into a number of drug-like molecules,[2] and is reported as a structural surrogate for morpholine.[3] Traditionally this molecule has been synthesised following the methodology first described by Carreira[4] *via* formation of a tosyl protected species **4** from tribromopentaerythritol **(2)** (a commercially available flame retardant). This procedure, followed by deprotection using magnesium/methanol was employed in the 1st generation synthesis (Scheme 1). Whilst small scale laboratory preparation was fit for purpose, scaling the deprotection procedure (to 100 L scale) was problematic, specifically the filtration of the magnesium salts. Though conversion was good, much material was lost during the work-up, and low yields (<35%) of poor quality material were observed. Future demands would require the development of a more scaleable procedure. Switching to a benzyl protecting group offered a more operable deprotection and work-up (Scheme 2), and ultimately this chemistry was proven on scale, to make a total of 65 kg of **5** as the oxalate salt in two batches.

The freebase (if required) may be conveniently liberated on laboratory scale, from the oxalate salt by treatment with ammonia in methanol, followed by filtration of the insoluble ammonium oxalate by-product and distillation to remove ammonia and solvent.

Differential Scanning Calorimetry (DSC), and further investigational testing in a more sensitive calorimeter (Setaram C80), indicated that both the oxalate salt **5** (and its freebase) have relatively low thermal stability (93°C measured in DSC (sealed gold pan at 5K/min) (oxalate salt) and 46°C measured in C80 (at 0.5K/min) with gas evolution from 60°C (freebase)). The decomposition noted gave significant heat release in both cases (>1000 J/g). Ensuring safe operating conditions on appropriate scale up is imperative.

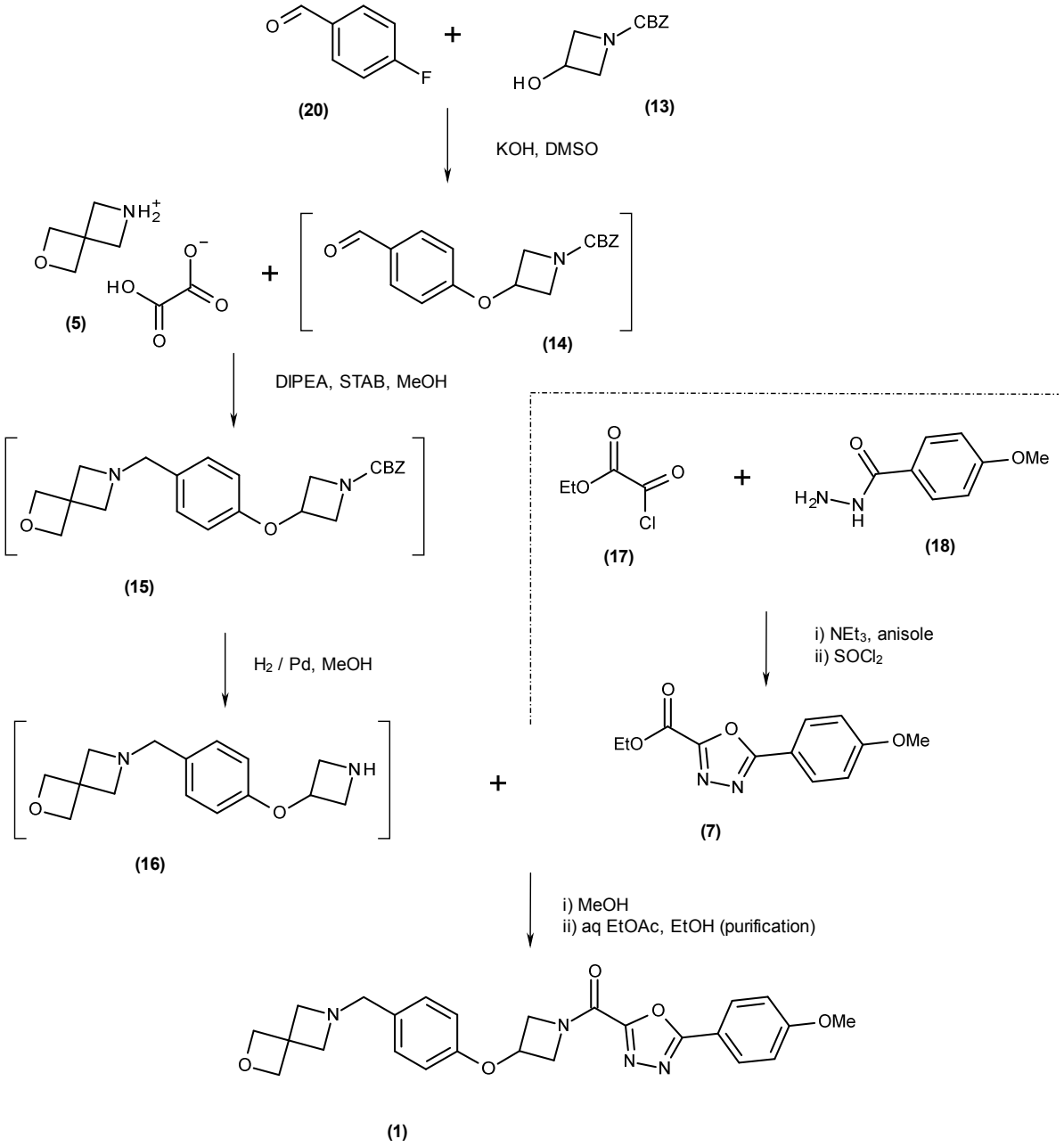
Scheme 2 – Synthesis of 6-oxa-2-azaspiro[3.3]heptane (5)



Scale up of the existing literature process[5] to form the oxadiazole **7** (Scheme 3) was also successful, ultimately producing 93 kg of material, and with scaleable routes to the key spirocyclic oxetane **5** and oxadiazole **7** fragments in hand, different approaches to the main branch of the synthetic pathway were explored. An alternative route producing improved quality **1**, with increased yield, reduced PGI risk and without chromatography was developed (Scheme 3). The commercially available coupling partners 4-fluorobenzaldehyde **20** and CBZ-Acetidinol **13** undergo a straightforward SNAr reaction in good yield (approximately 90% solution yield). Although the product **14** can be isolated as a solid, the kinetics of the crystallisation are very slow, and **14** has a low melting point (<50°C), making isolation difficult. Formation of the bisulphite adduct is also possible to achieve an isolation, but the coupling reaction is clean and can be telescoped through to the reductive amination step more efficiently. The second step

proceeds via liberation of **5** as the freebase, and formation of the iminium species *in situ*. A slow sodium triacetoxyborohydride addition favours reduction of the iminium species over reduction of **14** to the alcohol. Subsequent hydrogenation to remove the CBZ-protecting group was found to be trivial (though a high loading of the palladium catalyst was required to ensure completion) with subsequent coupling with the oxadiazole species **7** proceeding after an overnight stir in methanol. The product **1** can be crystallised on solvent swapping to ethanol, in approximately 60-65% yield, and this reaction sequence (from **20** and **13** through to **1)** was proven on 100 L scale. A two step purification procedure, using aqueous ethyl acetate recrystallisation, followed by a hot ethanol re-slurry (to ensure the correct polymorph) was used to supply further toxicological work.

Scheme 3 – 2nd generation route

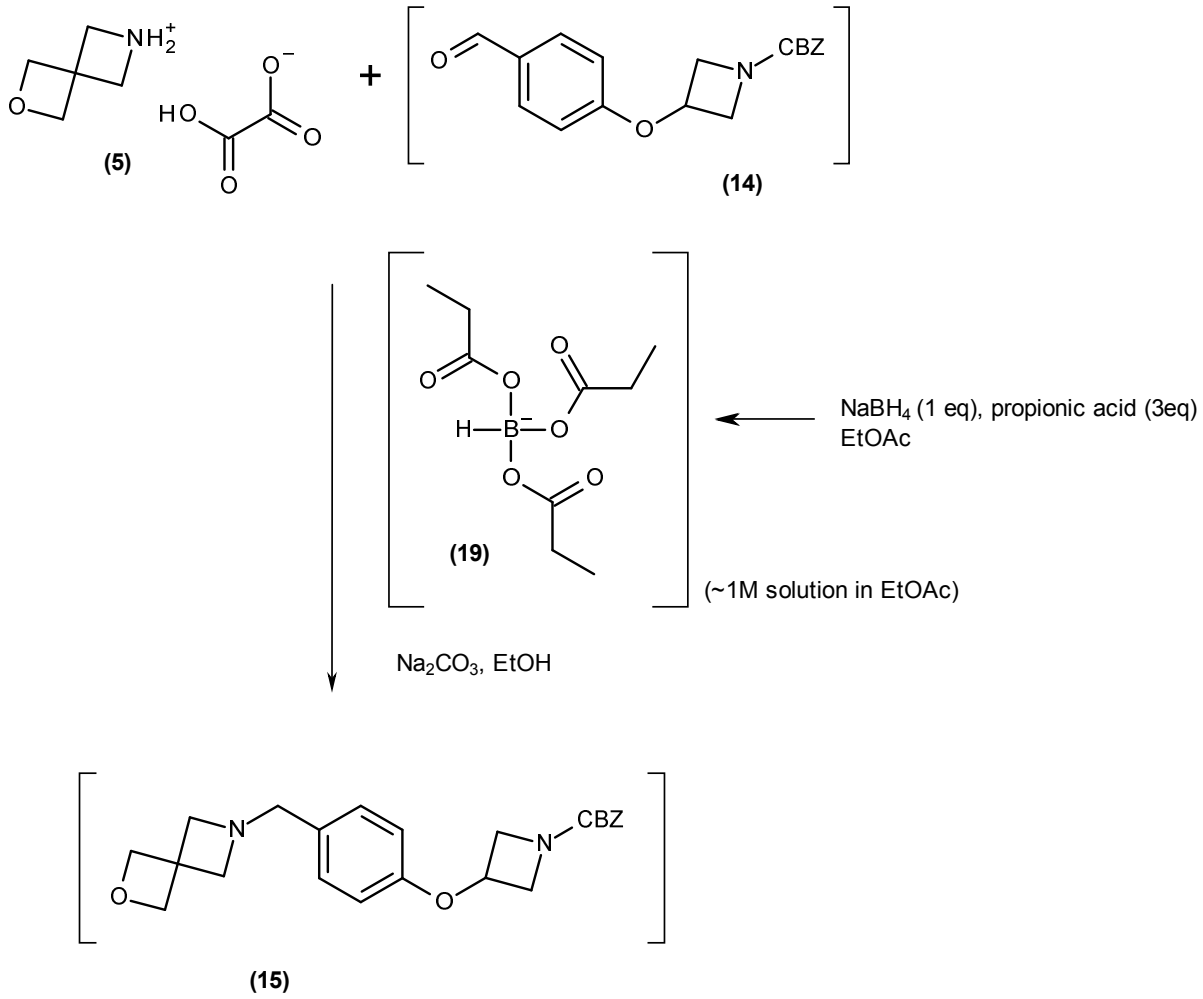


Subsequently, in anticipation of further scale up to the pilot plant, a number of improvements were made to this synthesis. Addition of solid sodium triacetoxyborohydride reagent into a mixture of **5** and **14** in methanol generates a potentially hazardous environment for an operator (hydrogen evolution during an addition of a solid) and, additionally, a large excess of solid sodium triacetoxyborohydride (3 mol eq) is required due to the competing degradation by

reaction with methanol. Whilst sodium triacetoxyborohydride is insoluble in most organic solvents, the analogous propionate [6] species **19** (Scheme 4) is soluble in a variety of solvents, including ethyl acetate, already used in the subsequent processing step to extract the product **15**. Therefore, an approximately one molar solution of **19** in ethyl acetate was pre-made (using controlled addition of propionic acid into a sodium borohydride/ethyl acetate mixture), which could then be more controllably added into the iminium mixture as a solution. The solution of **19** can be held for at least 24 hours at ambient temperature before use. As well as the operational advantages that this method presents, an improvement in quality was also observed; the alcohol by-product (from reduction of **14**) was suppressed, presumably by the more controlled addition method. Replacement of methanol (with ethanol) to dissolve the iminium precursors also reduced the molar amount of reductant required, presumably through reduced degradation of the reductant. The amount of catalyst required for the subsequent hydrogenation was also optimised and a 3 L trial reaction demonstrated the described modifications prior to the intended scale-up.

Improvements to the purification procedure (formation of an oxalate salt of **1**, and subsequent liberation of the freebase) were also demonstrated in the laboratory, which gave further improvement in the quality of the API.

Scheme 4 – Reductive amination using (soluble) propionoate anologue of sodium triacetoxyborohydride.



Conclusion

6-oxa-2-azaspiro[3.3]heptanes **5** is a useful synthetic building block, which features as a key structural motif in a number of drug molecules in the literature. An alternative more scaleable approach to this useful synthetic building block has been proven on scale. Additionally, the use of the propionoate analogue of sodium triacetoxyborohydride, as a means of facilitating a solution addition of a mild reductant to replace a potentially hazardous addition of solid sodium triacetoxyborohydride is also described as part of the development work performed to produce an alternative, efficient synthetic route to **1**.

Experimental Section

Preparation of crude 2-benzyl-6-oxa-2-azaspiro[3.3]heptane (12a)

3-bromo-2,2-bis(bromomethyl)propan-1-ol **(2)** (1.80 kg, 5.54 mol, 1.0 mol eq) and tetrabutylammonium hydrogensulfate (94.1 g, 0.27 mol, 0.05 mol eq) were charged to a 20 L jacketed vessel, under nitrogen. Toluene (9.0 L, 5.0 rel vols) was charged with stirring and the solution was warmed to 22°C. 50% aqueous sodium hydroxide (576 mL, 2.0 mol eq) was then charged. The mixture was then cooled to 23°C and stirred for 23 hours. Purified water (1.8 L, 1 rel vols) was added and the reaction mixture was stirred for 30 mins. The phases were allowed to settle for 30 mins and then separated. To this solution was charged tetrabutylammonium hydrogensulfate (188.2 g, 0.55 mol, 0.1 mol eq) and 50% aqueous sodium hydroxide (1.44 L, 5.0 mol eq). The mixture was heated to 73°C and benzylamine (578.6 g, 5.40 mol, 1 mol eq) was added over 10 mins by dropping funnel. The mixture was heated to 83°C and stirred for 20 hours. The batch was cooled to 20°C and purified water (3.30 L, 2.5 rel vols) was added with stirring. The mixture was stirred for 30 mins and allowed to settle and the phases were separated. The organic layer was extracted with 2 M acetic acid (4.62 L, 3.5 rel vols then 920 mL, 0.7 rel vols). Toluene (6.6 L, 5 rel vols) was added to the combined aqueous layers and the batch was basified using 50% aqueous sodium hydroxide (528 mL, 0.4 rel vols). The phases were separated and the aqueous layer was extracted with toluene (920 mL, 0.7 rel vols). The combined organic layers were concentrated under vacuum at 40-50°C to afford crude 2-benzyl-6-oxa-2-azaspiro[3.3]heptane **(12a)** (942.7 g, 90% yield) as a pale yellow oil.

Purification of (12a) to produce 2-benzyl-6-oxa-2-azaspiro[3.3]heptane hydrochloride (12)

A solution of 2-benzyl-6-oxa-2-azaspiro[3.3]heptane **(12a)** (926.5 g, 4.90 mol, 1.0 mol eq) in isopropanol (2.78 L, 3 rel vols) was charged to a 10 L jacketed vessel, under nitrogen. The mixture was cooled to 4°C with stirring and 4 M HCl in dioxane (1.23 L, 4.90 mol, 1.0 mol eq) was added by dropping funnel over 30 mins whilst maintaining the batch temperature below 21°C. The batch was cooled back to 0-5°C and stirred in this temperature range for 40 mins. The slurry was filtered and the flask and filter cake were washed with methyl tert-butyl ether (2 × 927 mL, 1 rel vols). The damp solid was dried in a vacuum oven at ambient temperature to afford 2-benzyl-6-oxa-2-azaspiro[3.3]heptane hydrochloride **(12)** (605.6 g, 55% yield) as a white solid.

^{1}H NMR (400 MHz, D_2O): 7.43-7.35 (m, 3H), 7.34-7.30 (m, 2H), 4.75 (br s, 1H), 4.69 (s, 4H), 4.27 (s, 4H), 4.20 (s, 2H). ^{13}C NMR (100 MHz, D_2O) δ ppm 37.53 (s, 1C) 57.79 (s, 2C) 61.27 (s, 2C) 78.82 (s, 1C) 80.16 (s, 1C) 129.31 (s, 1C) 129.39 (s, 2C) 129.89 (s, 2C) 130.04 (s, 1C).

Preparation of 6-oxa-2-azaspiro[3.3]heptane (5)

Step A (freebase) - 2-benzyl-6-oxa-2-azaspiro[3.3]heptane hydrochloride **(12)** (564.2 g, 2.51 mol, 1.0 mol eq) was charged to a 10 L jacketed vessel. Purified water (1.13 L, 2 rel vols), methyl tert-butyl ether (1.97 L, 3.5 rel vols) and saturated aqueous brine solution (395 mL, 0.7 rel vols) were added with stirring at 20-25°C and the batch was basified using 5 M aqueous sodium hydroxide (1.09 L, 1.2 rel vols) until the pH of the reaction mixture was 13.0. The mixture was stirred for 30 mins at this pH and the phases were separated. The aqueous layer was extracted with methyl tert-butyl ether (959 mL, 1.7 rel vols). The combined organic layers were concentrated to afford 2-benzyl-6-oxa-2-azaspiro[3.3]heptane **(12a)** (471.3 g, 99% yield from HCl salt) as a pale yellow oil.

Step B (hydrogenation) - 2-benzyl-6-oxa-2-azaspiro[3.3]heptane **(12a)** (419.3 g, 2.22 mol, 1.0 mol eq), ethanol (2.93 L, 7 rel vols.) and 5% Pd/C (83.9 g, 20 wt%, Johnson Matthey Type 394) were charged to a 7.5 L stainless steel hydrogenation vessel, which was sealed. The vessel was purged with nitrogen then hydrogen. The reaction mixture was heated to 45°C with stirring and the vessel was charged with 4 bar hydrogen. The reaction mixture was stirred for 16 hours, and then vented and purged with nitrogen. The batch was filtered on a dicalite bed. The vessel was rinsed with ethanol (839 mL, 2 rel vols) and the wash was also filtered. The filter cake was washed with additional ethanol (839 mL, 2 rel vols). The combined organic solution was vacuum distilled (100-135 mbar, 35-50°C) to afford 6-oxa-2-azaspiro[3.3]heptane **(5 - as the freebase)** (264.8 g, ~21.6 wt% ethanol by 1H NMR, corrected weight = 207.6 g, 94.3% yield) as a pale yellow oil.

Step C (salt formation) - 6-oxa-2-azaspiro[3.3]heptane **(5)** (21.86 g @ 90.0%w/w, 0.198 mol, 1.0 mol eq), isopropanol (78.7 mL, 4 rel vols) and water (19.7 mL, 1.0 rel vols) were charged to a 500 mL round bottom flask under nitrogen. The reaction mixture was cooled to 5°C with stirring and a solution of oxalic acid (18.76 g, 0.208 mol, 1.05 mol eq) in isopropanol (98.4 mL, 5 rel vols) was added over approx 15 mins. The reaction mixture was warmed to 20-25°C, stirred for 1 hr and filtered. The damp solid (53.7 g) was then recharged to the round bottom flask along with IPA (167 mL, 8.5 rel vols) and water (29.5 mL, 1.5 rel vols). The white slurry was stirred at 40-45°C for 1 hr, cooled to 20-25°C and filtered. The flask and filter cake were washed with isopropanol (2 × 39.4 mL, 2 rel vols). The filter cake was washed with additional isopropanol (39.4 mL, 2 rel vols) and the damp solid was dried in a vacuum oven at 50°C to afford 6-oxa-2-azaspiro[3.3]heptane **(5 – as the oxalate salt)** (33.35 g, 89% yield, 95.8% w/w by ^{1}H NMR assay) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ: 4.28 (s, 4 H) 4.80 (s, 4 H). ^{13}C NMR (101 MHz, D_2O) δ ppm 40.11 (s, 1 C) 54.65 (s, 2 C) 80.07 (s, 2 C) 165.62 (s, 2 C).

Preparation of ethyl 5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-carboxylate (7)

Anisole (650 L, 10.0 rel vols), 4-methoxybenzhydrazide **(18)** (65.0 kg, 1.0 mol eq) and triethylamine (98.9 kg, 2.5 mol eq) were charged into the reactor at 25°C. Ethyl chloroxoacetate **(17)** (64.7 kg, 1.2 mol eq) was slowly added into the reactor lot wise at 25°C. The mixture was stirred at 25°C for 10 mins and then the temperature was raised to 50°C. The reaction mixture was stirred at 50°C until reaction was complete. Thionyl chloride (55.8 kg, 1.2 mol eq) was charged at <70°C into the reactor. The temperature was raised to 90°C and maintained at 90°C until reaction was complete. Magnesium sulphate (32.5 kg) in water (325.0 L) was slowly added to the reactor at 40°C. The reaction temperature was maintained at 40°C for 30 mins and then the contents were allowed to settle for 30 min. The bottom aqueous layer was separated off and discarded. The organic layer was washed twice with a solution of sodium carbonate (62.2 kg) in water (325 L) at 40°C. The organic layer was concentrated to 3.0 rel vols level under vacuum then cooled to 55°C. N-heptane (325 L) was charged into the reactor and the mixture stirred for 1 hour at 55°C. The reaction mass was cooled to 10°C over a period of 4 hours and then stirred at 10°C for 1 hour. The reaction slurry was filtered and washed the wet cake twice with n-heptane (130 L, 2.0 rel vols), followed by slurry wash with ethanol (325 L, 5.0 rel vols). The product was dried under vacuum at 40°C to yield an off white solid **(7)** (60.5 kg, 62%).

^{1}H NMR (400 MHz, DMSO-d6) 1.38 (t, J=7.11 Hz, 3 H) 3.88 (s, 3 H) 4.46 (q, J=7.11 Hz, 2 H) 7.18 (m, J=8.73 Hz, 2 H) 8.0 (d, J=8.73 Hz, 2 H). ^{13}C NMR (101 MHz, DMSO-d6) δ ppm 14.33

(s, 1 C) 56.07 (s, 1 C) 63.36 (s, 1 C) 115.15 (s, 1 C) 115.51 (s, 2 C) 129.55 (s, 2 C) 154.54 (s, 1 C) 156.51 (s, 1 C) 163.21 (s, 1 C) 165.85 (s, 1 C).

Preparation of [5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(2-oxa-6-azaspiro[3.3]heptan-6-ylmethyl)phenoxy]azetidin-1-yl]methanone (1) on 100 L scale.

CBZ Acetidinol **(13)** (2.250 kg, 1.00 mol eq) and 4-fluorobenzaldehyde **(20)** (1.32 kg, 1.00 mol eq) were dissolved in dimethylsulphoxide (13.5 L, 6.0 rel vols) at 20°C. Potassium hydroxide (0.625 kg, 11.1 mol, 1.05 mol eq) was charged to the solution in two lots, maintaining <28°C. The reaction mixture was held at 20°C for 3.5 hours, before adding ethyl acetate (13.5 L, 6.0 rel vols) and a solution of ammonium chloride (1.7 kg, 31.8 mols, 3.0 mol eq) in water (13.5L, 6.0 rel vols) whilst maintaining the temperature at ≤28°C. The layers were separated and the aqueous layer extracted with a further portion of ethyl acetate (6.75L, 3.0 rel vols). The organic phases were then combined and washed with a solution of sodium chloride (1.24 kg, 2.0 mol eq) in water (11.25 L, 5.0 rel vols). The organic solution was concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). Methanol (11.25 L, 5.0 rel vols) was added and the organic solution was concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). Further methanol (11.25 L, 5.0 rel vols) was added and the organic solution was again concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). The reaction mixture was diluted with methanol (12.4L, 5.5 rel vols), and then **(5)** (2.41 kg, 1.20 mol eq) and diisopropylethylamine (7.4 L, 4.00 mol eq) were added. Sodium triacetoxyborohydride (6.75 kg, 3.00 mol eq) was then added in five equal portions over a 2 hour period. Ethyl acetate (27 L, 12.0 rel vols) and then a solution of 23% aqueous ammonia solution (7.5 L, 3.0 rel wt) in water (225 L, 10 rel vols), were added maintaining <28°C. The layers were separated and the aqueous phase was extracted with further ethyl acetate (6.75L, 3.0 rel vols). The organic phases were combined and concentrated

under vacuum to a final volume of 6.75 L (3.0 rel vols). Methanol (11.25 L, 5.0 rel vols) was added and the organic solution concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). A further portion of methanol (11.25 L, 5.0 rel vols) was added and the organic solution again concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). The reaction was diluted with methanol (12.4L, 5.5 rel vols), and then 10% palladium on charcoal (50% wet) (1.05 kg, 0.46 rel wt) was added. The reaction mixture was thoroughly inerted with nitrogen before being exposed to 3 barg pressure of hydrogen for 3 hours. The reaction mixture was filtered through Celite and the catalyst residues washed with methanol (6.75 L, 3.0 rel vols). **(7)** (1.93 kg, 0.73 mol eq) was charged, followed by methanol (1.125 L, 0.5 rel vols) and the reaction mixture was stirred for 18 hours. The organic solution was concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). Ethanol (11.25 L, 5.0 rel vols) was added and the mixture concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). Further ethanol (11.25 L, 5.0 rel vols) was added and the mixture was again concentrated under vacuum to a final volume of 6.75 L (3.0 rel vols). The reaction mixture was diluted with ethanol (11.25 L, 5.0 rel vols) and then cooled to 10°C before filtering, washing with ethanol (4.5 L, 2.0 rel vols) and drying at 55°C. The product was isolated as an off white solid **(1)** (2.93 kg, 60% yield).

^{1}H NMR (500 MHz, DMSO-d6) δ: 3.24 (s, 4 H) 3.41 (s, 2 H) 3.87 (s, 3 H) 4.08 (dd, J=11.28, 3.61 Hz, 1 H) 4.53 (dd, J=10.72, 3.73 Hz, 1 H)) 4.59 (s, 4 H) 4.62 (m, 1 H) 5.07 (m, 1 H) 5.13 (m, 1 H) 6.82 (d, J=8.64 Hz, 2 H) 7.16 (d, J=8.96 Hz, 2 H) 7.19 (d, J=8.61 Hz, 2 H) 8.00 (d, J=9.08 Hz, 2 H). ^{13}C NMR (126 MHz, DMSO-d6) δ_H 38.46 (s, 1 C) 55.61 (s, 1 C) 55.66 (s, 1 C) 60.21 (s, 1 C) 61.80 (s, 1 C) 62.75 (s, 2 C) 66.10 (s, 1 C) 80.01 (s, 2 C) 114.38 (s, 2 C) 114.88 (s,1 C) 115.05 (s, 2 C) 129.00 (s, 2 C) 129.74 (s, 2 C) 131.34 (s, 1 C) 153.12 (s, 1 C) 155.12 (s, 1 C) 157.17 (s, 1 C) 162.59 (s, 1 C) 164.45 (s, 1 C)

Preparation of [5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(2-oxa-6-azaspiro[3.3]heptan-6-ylmethyl)phenoxy]azetidin-1-yl]methanone (1) on 3 L scale in preparation for pilot plant manufacture.

CBZ Acetidinol **(13)** (100 g, 1.00 mol eq) and 4-fluorobenzaldehyde **(20)** (59.9 g, 1.00 mol eq) were dissolved in dimethylsulphoxide (600 ml, 6.0 rel vols) at 20°C. Potassium hydroxide (31.9 g, 1.00 mol eq) was charged in two lots, maintaining <28°C. The reaction mixture was held at 20°C for 3.5 hours, before ethyl acetate (600 ml, 6.0 rel vols) and a solution of ammonium chloride (77.4 g, 3.0 mol eq) in water (600 ml, 6.0 rel vols) were added maintaining the temp at ≤28°C. The layers were separated and the organic phase was washed twice with a solution of sodium chloride (50 g, 0.5 rel wt) in water (500 ml, 5.0 rel vols). The organic solution was concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). Ethanol (600 ml, 6.0 rel vols) was added and and the organic solution concentrated under vacuum to a final volume of 400 ml (4.0 rel vols). Further ethanol (600 ml, 6.0 rel vols) was added and the organic solution concentrated under vacuum to a final volume of 400 ml (4.0 rel vols). The reaction mixture was diluted with ethanol (450 ml, 4.5 rel vols), and then **(5)** (120.0 g, 1.30 mol eq) and sodium carbonate (66.5 g, 1.3 mol eq) were added. A pre-prepared solution of sodium borohydride (36.5 g, 2.0 mol eq), propionic acid (214 g, 6.0 mol eq) and ethyl acetate (950 ml, 9.5 rel vols) was added over 4 hours (preparation was by addition of propionic acid to the sodium borohydride/ethylacetate mixture over 1 hour, maintaining <20°C, followed by overnight hold at 20°C). Ethyl acetate (250 ml, 2.5 rel vols), water (700 ml, 7.0 rel vols) and then a solution of 29% aqueous ammonia solution (300 ml, 3.0 rel vols), were added maintaining <28C. The layers were then separated by dipleg (set to remove liquid above 12.5 rel vols) retaining the upper portion. Water (400 ml, 4.0 rel vols) was added to the lower aqueous phase and the aqueous phase extracted with further ethyl acetate (600 ml, 6.0 rel vols), by dipleg (set to remove liquid

above 12.5 rel vols). The organic extracts were combined and the small amount of lower aqueous phase separated off and discarded. The organic solution was concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). Methanol (500 ml, 5.0 rel vols) was added and the organic solution was concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). Further methanol (500 ml, 5.0 rel vols) was added and the organic solution again concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). The reaction mixture was diluted with methanol (350 ml, 3.5 rel vols), and then 10% palladium on charcoal (50% wet) (10.0 g, 0.1 rel wt) was added. The reaction mixture was thoroughly inerted with nitrogen, before being exposed to 3 barg pressure of hydrogen for 3 hours. The reaction mixture was then filtered and the catalyst residues were washed with methanol (300 ml, 3.0 rel vols). **(7)** (95.6 g, 0.75 mol eq) was charged and the mixture stirred for 18 hours. The organic solution was concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). Ethanol (500 ml, 5.0 rel vols) was added and the organic solution was concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). Further ethanol (500 ml, 5.0 rel vols) was added and the organic solution again concentrated under vacuum to a final volume of 500 ml (5.0 rel vols). The reaction mixture was diluted with ethanol (500 ml, 5.0 rel vols) and cooled to 20°C before filtering, washing with ethanol (4.5 L, 2.0 rel vols) and drying at 40°C. The product was isolated as an off white solid **(1)** (137.7 g, 62% yield).

^{1}H NMR (500 MHz, DMSO-d6) δ: 3.24 (s, 4 H) 3.41 (s, 2 H) 3.87 (s, 3 H) 4.08 (dd, J=11.28, 3.61 Hz, 1 H) 4.53 (dd, J=10.72, 3.73 Hz, 1 H)) 4.59 (s, 4 H) 4.62 (m, 1 H) 5.07 (m, 1 H) 5.13 (m, 1 H) 6.82 (d, J=8.64 Hz, 2 H) 7.16 (d, J=8.96 Hz, 2 H) 7.19 (d, J=8.61 Hz, 2 H) 8.00 (d, J=9.08 Hz, 2 H). ^{13}C NMR (126 MHz, DMSO-d6) δ_H 38.46 (s, 1 C) 55.61 (s, 1 C) 55.66 (s, 1 C) 60.21 (s, 1 C) 61.80 (s, 1 C) 62.75 (s, 2 C) 66.10 (s, 1 C) 80.01 (s, 2 C) 114.38 (s, 2 C) 114.88

(s,1 C) 115.05 (s, 2 C) 129.00 (s, 2 C) 129.74 (s, 2 C) 131.34 (s, 1 C) 153.12 (s, 1 C) 155.12 (s, 1 C) 157.17 (s, 1 C) 162.59 (s, 1 C) 164.45 (s, 1 C).

Purification of [5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(2-oxa-6-azaspiro[3.3]heptan-6-ylmethyl)phenoxy]azetidin-1-yl]methanone (1)

[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(2-oxa-6-azaspiro[3.3]heptan-6-ylmethyl)phenoxy]azetidin-1-yl]methanone **(1)** (2.89 kg, 1.0 mol eq), ethyl acetate (29 L, 10.0 rel vols) and water (43.3 L, 15.0 rel vols) were added to the reaction vessel. The mixture was heated to 60°C for 1 hour, and then cooled to 50°C before a screening filtration. The reaction solution was cooled the reaction to 40°C and **(1)** was added as seed (2.5 g, 0.001 rel wt). The reaction was cooled over 2 hours to 20°C, and held for 1 hour. The organic phase was washed with water (4.3 L, 1.5 rel vols) and dried at 55°C. The product was then slurried with ethanol (27.4 L, 12.0 rel vols) at 60°C for 1 hour, before being filtered and washed with ethanol (3.4 L, 1.5 rel vols). The product was dried at 55°C to yield the product as a white solid **(1)** (2.12 kg, 73%).
^{1}H NMR (500 MHz, DMSO-d6) δ: 3.24 (s, 4 H) 3.41 (s, 2 H) 3.87 (s, 3 H) 4.08 (dd, J=11.28, 3.61 Hz, 1 H) 4.53 (dd, J=10.72, 3.73 Hz, 1 H)) 4.59 (s, 4 H) 4.62 (m, 1 H) 5.07 (m, 1 H) 5.13 (m, 1 H) 6.82 (d, J=8.64 Hz, 2 H) 7.16 (d, J=8.96 Hz, 2 H) 7.19 (d, J=8.61 Hz, 2 H) 8.00 (d, J=9.08 Hz, 2 H). ^{13}C NMR (126 MHz, DMSO-d6) δ_H 38.46 (s, 1 C) 55.61 (s, 1 C) 55.66 (s, 1 C) 60.21 (s, 1 C) 61.80 (s, 1 C) 62.75 (s, 2 C) 66.10 (s, 1 C) 80.01 (s, 2 C) 114.38 (s, 2 C) 114.88 (s,1 C) 115.05 (s, 2 C) 129.00 (s, 2 C) 129.74 (s, 2 C) 131.34 (s, 1 C) 153.12 (s, 1 C) 155.12 (s, 1 C) 157.17 (s, 1 C) 162.59 (s, 1 C) 164.45 (s, 1 C). HRMS Calcd for $C_{25}H_{27}N_4O_5$: 463.1976; HRMS found [M+H]+: 463.1978

Alternative purification procedure - preparation of [5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(2-oxa-6-azaspiro[3.3]heptan-6-ylmethyl)phenoxy]azetidin-1-yl]methanone (1) (*via* the oxalate salt)

Step A - [5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(6-oxa-2-azaspiro[3.3]heptan-2-ylmethyl)phenoxy]azetidin-1-yl]methanone **(1)** (10.0 g) was added to dimethylsulphoxide (100 ml, 10.0 rel vols) and heated to 60°C to give a solution. The contents were then cooled to 20°C. A solution of oxalic acid (1.84 g 1.0 mol eq) in dimethylsulphoxide (10 ml, 1.0 rel vol) was added at 20°C. Ethanol (200 ml, 20.0 rel vol) was added and the contents stirred for 16 hours. The precipitated solid was filtered and washed with ethanol (50 ml, 5.0 rel vol). The solid was dried at 40°C to give the oxalate salt of **(1)** as an off white solid (8.58g, 76% yield)

Step B - [5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-[3-[4-(6-oxa-2-azaspiro[3.3]heptan-2-ylmethyl)phenoxy]azetidin-1-yl]methanone; oxalic acid (oxalate salt of **(1)**) (6.0g, 1.0 mole eq) was added to ethyl acetate (60 ml, 10.0 rel vol) at 20°C and stirred to give an off white biphasic slurry. 15.5% aqueous ammonia (7.5 mol eq, 35.5ml) was charged over 3 minutes. The contents were heated to 60°C, to form a biphasic solution. The solution was screened and the lower aqueous layer separated and discarded. The organic layer was washed with water (30 ml 5.0 rel vols) and the lower aqueous layer was separated. The organic layer was cooled to 20°C and the slurry distilled under vacuum to 5.0 rel vols (30 ml). Ethanol (48ml, 8.0 rel vol) was added and the mixture concentrated under vacuum to 5.0 rel vols (30ml). Further ethanol (48ml, 8.0 rel vol) was added and the mixture again concentrated under vacuum to 5.0 rel vols (30ml). The mixture was diluted with ethanol (18ml, 3.0 rel vol) to make up to 8.0 rel vol, cooled to 20°C and stirred for 80 minutes. The solid was filtered and washed with ethanol (24ml, 4 rel vol), then discharged **(1)** to a vacuum oven and dried at 40°C. (Yield = 4.43g, 88%).

^{1}H NMR (500 MHz, DMSO-d6) δ: 3.24 (s, 4 H) 3.41 (s, 2 H) 3.87 (s, 3 H) 4.08 (dd, J=11.28, 3.61 Hz, 1 H) 4.53 (dd, J=10.72, 3.73 Hz, 1 H)) 4.59 (s, 4 H) 4.62 (m, 1 H) 5.07 (m, 1 H) 5.13 (m, 1 H) 6.82 (d, J=8.64 Hz, 2 H) 7.16 (d, J=8.96 Hz, 2 H) 7.19 (d, J=8.61 Hz, 2 H) 8.00 (d, J=9.08 Hz, 2 H). ^{13}C NMR (126 MHz, DMSO-d6) δ_H 38.46 (s, 1 C) 55.61 (s, 1 C) 55.66 (s, 1 C) 60.21 (s, 1 C) 61.80 (s, 1 C) 62.75 (s, 2 C) 66.10 (s, 1 C) 80.01 (s, 2 C) 114.38 (s, 2 C) 114.88 (s,1 C) 115.05 (s, 2 C) 129.00 (s, 2 C) 129.74 (s, 2 C) 131.34 (s, 1 C) 153.12 (s, 1 C) 155.12 (s, 1 C) 157.17 (s, 1 C) 162.59 (s, 1 C) 164.45 (s, 1 C)

HRMS Calcd for $C_{25}H_{27}N_4O_5$: 463.1976; HRMS found [M+H]+: 463.1978

References

1. (a) Johansson, A.; Löfberg, C; Antonsson, M.; von Unge, S.; Hayes, M.; Judkins, R.; Ploj, K.; Benthem, L.; Lindén, D.; Brodin, P.; Wennerberg, M.; Fredenwall, M.; Li, L.; Persson, J.; Bergman, R.; Pettersen, A.; Gennemark, P.; Hogner, A. *J. Med. Chem. http://dx.doi.org/10.1021/acs.jmedchem.5b01654* (Manuscript just accepted) (b) Luthin, D.R.; Life Sciences Volume 81, Issue 6, (2007), Pages 423–440
2. (a) Casaubon, R.L.; Narayan, R.; Oalmann, C.; Vu, C.B.; PCT Int. Appl., 2013059587, 25 Apr 2013. (b) Burns, C.J ; PCT Int. Appl., 2014000032, 3 Jan 2014 (*Phenyl amino pyrimidine bicyclic compounds and uses thereof*).
3. Wuitschik, G.; Rogers-Evans, M; Buckl, A, Bernasconi, M.; Märki, M.; Godel T.; Fischer H.; Wagner B.; Parrilla, I.; Schuler, F.; Schneider, J.; Alker, A.; Schweizer, W.B.; Müller, K. and Carreira, E.M. *Angew. Chem., Int. Ed.* **2008,** *47*, 4512–4515.
4. Burkhard, J.; Carreira, E.M. *Org. Lett.* **2008**, *16*, 3525-3526.
5. Dost, J.; Heschel, M.; Stein, J. *J. Prakt. Chem. (Leipzig)* **1985**, 327(1), 109-116.

6. (a) McGill, J. M.; Labell, E. S.; Williams, M. A. *Tetrahedron Lett.* **1996**, *37*, 3977. (b) McGill, J. M.; Burks, J. E.; Espinosa, L.; Labell, E. S.; Ritter, A. R.; Speakman, J. L.; Williams, M. A.; Bradley, D. A.; Haenl, M. G.; Schmid, C. R. *Org. Process Res. Dev.* **1997**, *1*, 198.