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Route Selection and Process Development of a Multikilogram Route to the Inhaled A_{2a} Agonist UK-432,097

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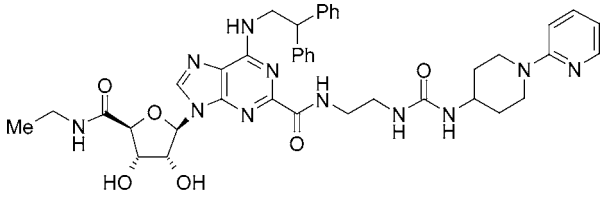
ABSTRACT: This article describes the selection, process development, and scale-up of a synthetic route to a complex nucleoside analogue, the A_{2a} agonist UK-432,097 (**1**), that culminated in the manufacture of over 25 kg of the API. The key steps in the process were (1) a stereoselective glycosidation reaction; (2) a scalable bleach−TEMPO oxidation; and (3) an unusual elevated temperature crystallization process for the final API. The problems that were encountered with the scale-up of the route together with how they were overcome are also presented.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death in the United States,[1] affects between 10 to 24 million of the U.S. population, and is predicted to become the fourth leading cause of death worldwide by 2030.[2] COPD is a progressive degenerative disease that is described as reduced capacity to breathe due to inflammation and damage of the lung tissues.[3] It is a severely debilitating disease that reduces quality of life, and the high level of patient aftercare results in a high economic burden. Treatments are limited, and as such there is a growing need for alternative therapies.[4]

UK-432,097 (**1**) (Figure 1) is a potent adenosyl subtype 2a agonist (A_{2a} agonist)[5] that offers a potential inhaled treatment for the inflammatory symptoms of COPD.[4,6] To support development of **1**, a series of demands for active pharmaceutical ingredient (API) were received. Campaign 1 (300 g), Campaign 2 (2.5 kg), and Campaign 3 (25 kg) would support the project through preclinical toxicology testing, drug product development in a solution and dry powder inhaler, and in phases 1 and 2 clinical programs.



1, UK-432,097

Figure 1. Structure of UK-432,097.

In this article, we describe the selection of the route used for scale-up, together with how it was initially developed and implemented in Campaigns 1 and 2 and further developed and refined for the manufacture of 25 kg of **1** in Campaign 3.

ROUTE SELECTION

The final stages of the route used in the medicinal chemistry synthesis of **1** (Scheme 1) involved the stereoselective glycosidation reaction between the uronamide **2** and the adenine core **3** under Vorbrüggen conditions[7] to give **4** followed by elaboration of the methyl ester on the heteroaryl group into the complete aminopyridine-containing side chain via the union of **5** and **6**.[8a]

From the perspective of developing a multikilo synthesis of **1**, we wished to identify a robust and efficient synthetic route that would be amenable to scale-up. We investigated the key bond-forming steps in the medicinal chemistry route, and identified major problems with the uronamide **2** that precluded it being used in a large scale manufacturing process. The synthesis of the uronamide **2** started from D-ribose as shown in Scheme 2 and utilised classical methods, including a ruthenium-catalysed oxidation[10a] of **7** to give **8**[10b] prior to installing the amide in **9** together with a protection−deprotection-reprotection sequence and the intermediacy of oils (**7**, **10**) and diastereomeric mixtures.

While the reagents used in this route were not particularly attractive for the development of a large scale process, it was the stability of the dibenzoate **2** that caused most concern. This material existed in its pure form as a gum despite extensive efforts to find a crystalline form, and required chromatographic purification. However, even when pure, the dibenzoate **2** was unstable and degraded quite quickly via what was presumed to be a β-elimination mechanism that ultimately led to what we believed to be furan-derivatives. Degradation of **2** even occurred during solvent strip-down of solutions, and also upon storage of the material as a gum. These factors led us to conclude that **2** was not a viable intermediate in a scalable synthesis of **1**.

The lack of stability of **2** highlighted to us the importance of selecting the right protecting group strategy when designing a new route. The amide oxidation state at C-5 of the sugar moiety in **1** renders the methine hydrogen at C-4 relatively acidic and prone to the observed β-elimination of the protected oxygen atom at C-3, as well as epimerization at C-4. For the β-elimination pathway, having a good leaving group (e.g., a

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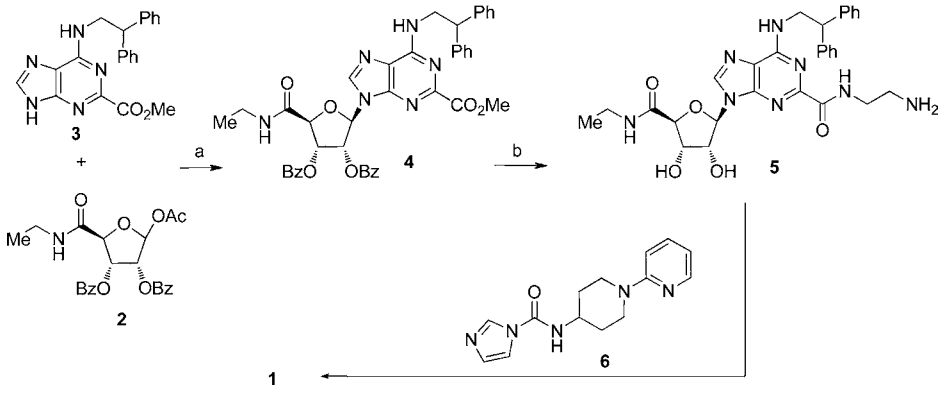
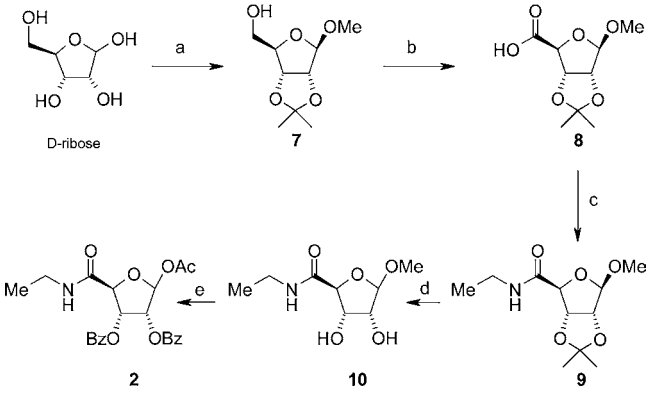
Scheme 1. Final stages of the medicinal chemistry route to 1[a]



[a]Reagents and conditions: (a) **3**, *N*,*O*-bis(trimethylsilyl)acetamide, PhMe then **2**, reflux; (b) (i) Na_2CO_3, MeOH, RT; (ii) ethylenediamine, 105 °C; (c) PhMe/^{i}PrOH, reflux.

Scheme 2. Synthesis of uronamide 2[a]



[a]Reagents and conditions: (a) HCl, MeOH, acetone; (b) KIO_4, $RuCl_3$, acetone (aq); (c) CDI, THF, $EtNH_2$; (d) PPTS, MeOH, reflux; (e) (i) Bz_2O, TEA, DMAP, DCM; (ii) AcOH, Ac_2O, H_2SO_4, DCM.

benzoate ester as in **2**) would promote this pathway in comparison with a poorer leaving group (e.g. alkoxy), although epimerization could still be a problem. These considerations led us to identify an alkoxy-like protecting group, specifically acetonide, as this has the additional advantage of also protecting the hydroxyl at C-2. To maintain as much convergency as possible, we wished to install the complete amide side chain present in **1** as late in the synthesis as possible via an amide bond-forming reaction. Taking these factors into account together with the strategy for the sugar unit, led us to the retrosynthetic analysis for the new route to **1** as depicted in Figure 2. Here, the nucleoside core **11** and the amine side chain **12** are coupled together followed by a final deprotection reaction to give **1**. Given the earlier discussion, the nucleoside **11** would logically be prepared via oxidation of the primary alcohol **13** followed by ethylamide formation.

However, stereoselective installation of the preferred nucleoside anomer **13** in the presence of the acetonide would be challenging. It is known that the presence of an acyl-protected hydroxyl group at C-2 is necessary for anchimeric assistance to impart high levels of stereoselectivity in glycosidation reactions;[7,8b] thus, having the acetonide present during the reaction would most likely lead to poor diastereoselectivity. Additionally it was unlikely that the acetonide would be compatible with the reaction conditions used in glycosidation reactions (e.g., TMS triflate or other Lewis acids). It therefore seemed logical to prepare the fully protected nucleoside **14** via a glycosidation reaction between cheap and commercially available 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**15**) and a suitably functionalized adenine unit **16**. In our previous contribution we described the synthesis of the related nucleoside analogue UK-371,104 which utilized a similar glycosidation reaction to unify the sugar and adenine units.[8b]

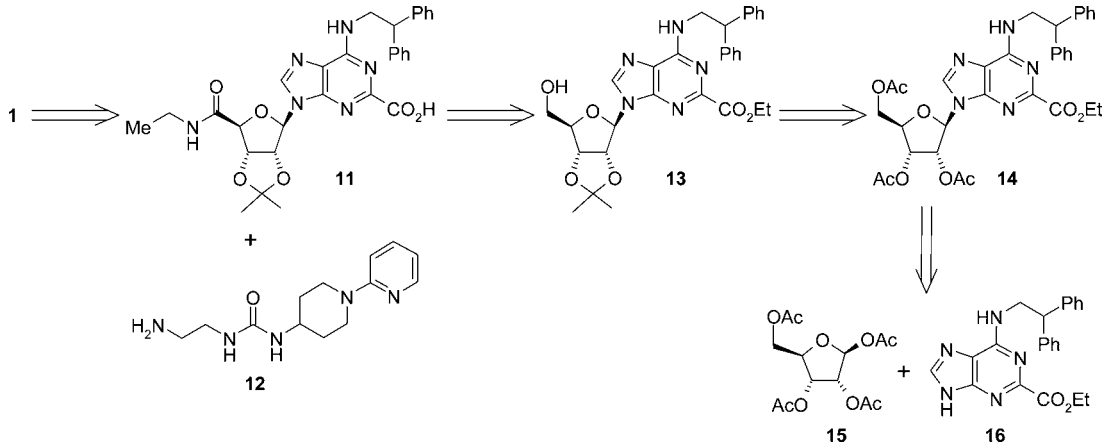


Figure 2. Retrosynthetic analysis for process chemistry route to **1**.

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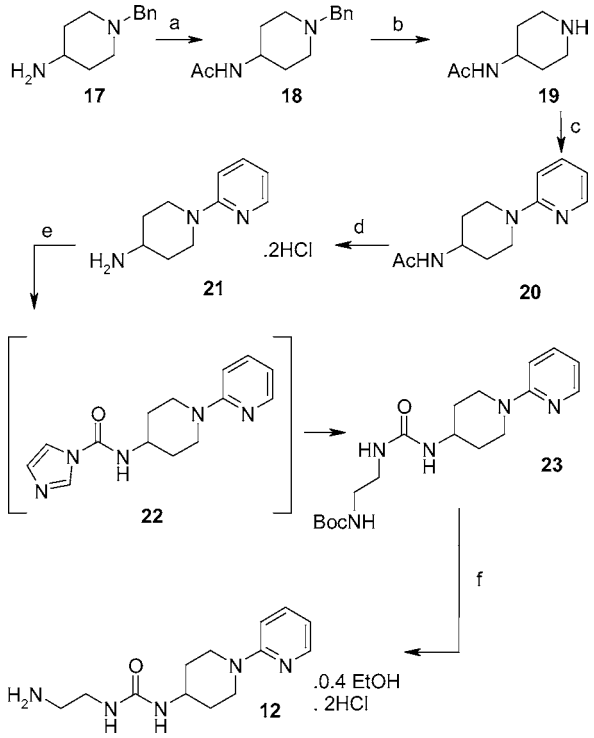
We anticipated that similar reaction conditions would be applicable for the glycosidation involved in the new route to **1**.

The synthesis of the aminopyridine containing side-chain unit **12** from cheap and readily available *N*-benzyl-4-aminopiperidine (**17**) is shown in Scheme 3. Protection of the primary amine in **17** as an acetamide to give **18** was followed by hydrogenolysis of the benzyl group to give 4-acetamidopiperidine (**19**) as a crystalline solid. Thermally induced reaction of **19** with 2-bromopyridine gave **20** and hydrolysis of the acetamide group afforded the aminopiperidine **21** that was isolated as its dihydrochloride salt.[5c,9] Formation of the urea using 1,1′-carbonyldiimidazole (CDI) initially posed some problems due to the formation of the symmetrical urea derivative via reaction of the carbamoylimidazolide derivative **22** with **21**. This problem was overcome by slowly adding diisopropylethylamine (DIPEA) to a cooled suspension of **21** dihydrochloride and CDI in acetonitrile (in which DIPEA·HCl is soluble), resulting in slow release of **21** free base to generate the required carbamoyl imidazolide **22** with minimal formation of the undesired symmetrical urea side product. Subsequent addition of BOC-protected ethylenediamine and reaction at reflux furnished the desired urea **23** that, following workup, was crystallized from ethyl acetate. Deprotection of **23** to provide **12** was accomplished with anhydrous HCl in ethanol generated from acetyl chloride addition to the ethanol solution of **23**. Interestingly, the only crystalline form of **12** dihydrochloride that could be isolated was a solvate containing 0.4 mol of ethanol per mole of **12**. Over 35 kg of **12** dihydrochloride ethanolate was prepared at external vendors using this route.

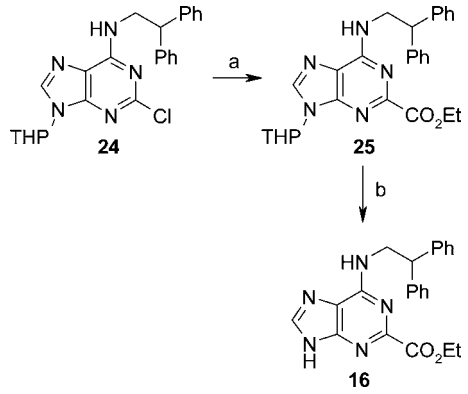
Scheme 3. Synthesis of amine 12[a]



[a]Reagents and conditions: (a) (i) AcCl, TEA, THF; (ii) EtOAc, 77%; (b) (i) H_2/Pd/C, EtOH; (ii) EtOAc, 83%; (c) AmOH, Na_2CO_3, 2-bromopyridine, 82%; (d) HCl (6 N), EtOH, 88%; (e) (i) CDI, MeCN, DIPEA; (ii) *tert*-butyl (2-aminoethyl)carbamate; (iii) EtOAc, 94%; (f) AcCl, EtOH, 94%.

The adenine unit **16** is related to an ester of the carboxylic acid used in the synthesis of UK-371,104 reported previously.[8b] However, the first synthesis of the carboxylic acid via palladium-catalysed cyanation of the respective chloride **24** followed by nitrile hydrolysis proved to be quite troublesome, and therefore an alternative method was developed that utilized palladium-catalysed ethoxycarbonylation of the chloride **24** to give the ethyl ester **25** (Scheme 4). The chloride substrate **24** was prepared using the published procedure,[5c,8b] and reaction with ethanol and carbon monoxide in the presence of $Pd(OAc)_2$/dppf and aqueous sodium carbonate afforded the desired ester **25** in 76% yield. Occasional venting and repressurization of the autoclave with carbon monoxide was necessary to remove the carbon dioxide generated in the reaction, as this diluted the carbon monoxide and slowed the reaction rate. Finally, removal of the THP protecting group was accomplished in almost quantitative yield using trifluoroacetic acid (TFA) in ethanol to provide **16**. This route was used by external vendors to prepare a total of 11 kg of **16** to use in Campaigns 1 and 2.

Scheme 4. Ethoxycarbonylation route to 16[a]



[a]Reagents and conditions: (a) 0.4 mol % $Pd(OAc)_2$, 0.8 mol % dppf, Na_2CO_3 (aq), EtOH, 300 psi CO, 105 °C, 76%; (b) TFA, EtOH, 50 °C, 99%.

■ ASSEMBLY OF BUILDING BLOCKS AND CONVERSION TO 1

Glycosidation of **16** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**15**) was accomplished, using conditions similar to those published[8b] for UK-371,104, and afforded the noncrystalline product **14** (Scheme 5).

When the published conditions were repeated exactly with **15** and **16**, with no external base, a much poorer reaction profile was obtained compared to the UK-371,104 substrate which contained a basic piperidine moiety. In the presence of an amine such as *N*-methylmorpholine (NMM), the reaction of **15** and **16** was much cleaner, and it was found that a slight excess of TMS triflate over NMM was necessary to achieve good reaction conversions. We suspect that this observation was due to the reaction of TMS triflate with NMM to give the silylated quaternary ammonium triflate salt **26** (Figure 3) which, while being reactive enough to silylate the adenine unit **16** to give **27** as required for the reaction, is presumably not a sufficiently reactive enough silyl transfer reagent to promote ionization of **15** to give the stabilized oxonium ion **28**. The slight excess of TMS triflate (1.3 equiv) relative to NMM (1.1

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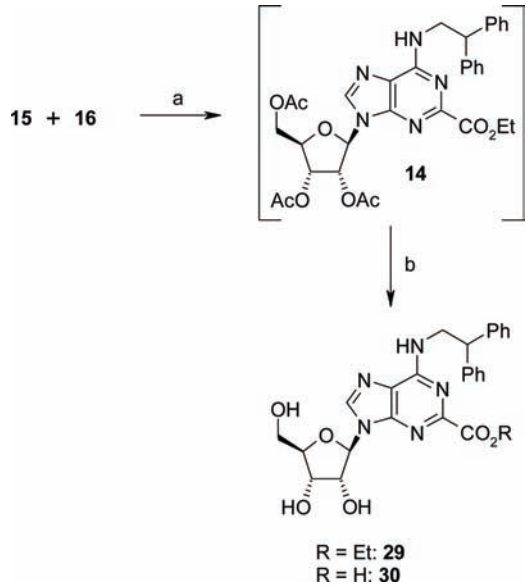
Scheme 5. Glycosidation and conversion to triol 29 (Campaigns 1 and 2)[a]



[a]Reagents and conditions: (a) TMS-OTf, DME, NMM, 60 °C; (b) EtOH, DBU. 90% over two steps.

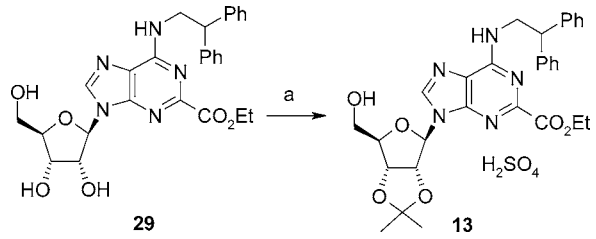
equiv) provides sufficient reactive catalyst to enable the glycosidation reaction to proceed.

The product **14** from the glycosidation reaction was not crystalline; thus, following workup the solution of **14** was taken directly into the next step where the acetate groups were removed in the presence of the ethyl ester attached to the adenine unit to give the triol **29** (Scheme 5). This was initially accomplished using transesterification in ethanol with 0.1 equiv of sodium ethoxide as the catalyst. However, these conditions invariably led to the formation of several percent of the carboxylic acid **30**, presumably due to the presence of traces of water and sodium hydroxide in the reaction system.

Efforts to find conditions that minimized the formation of **30** culminated in using a stoichiometric amount of DBU in ethanol, together with minimization of water to below 0.1 w/w % in the reaction medium. Even with these optimal conditions it was not possible to completely prevent formation of **30** and to avoid this impurity from interfering in the downstream chemistry, it was removed at this stage by washing the product solution with aqueous 0.1 M $NaHCO_3$ solution. Interestingly, the use of saturated solutions resulted in no purge of this impurity, probably because the increased ionic strength of the aqueous phase prevented the sodium salt of **30** from partitioning into that phase. The triol **29** was finally crystallized and isolated in good yield over the two steps. The corresponding oppositely configured α-anomer was not detected in either crude mixtures of **14** and **29** or in isolated solid **29**, the anomeric centre essentially being perfectly stereo controlled by the neighboring 2-acetate group in cation **28**. This process was run internally within Pfizer lab and kilo lab facilities for Campaigns 1 and 2, with totals of 976 g and 11.0 kg of **29** being made respectively. For Campaign 3, the process to **29** was outsourced, and 52 kg was received for subsequent processing.

Having coupled the sugar and adenine units together, the next phase of the synthesis required elaboration of the primary hydroxyl in **29** to an ethyl amide. This required selective protection of the two secondary alcohol groups in **29** to enable oxidation of the primary hydroxyl group. Formation of the acetonide **13** was accomplished with sulphuric acid in a mixture of acetone and 2 equiv of 2,2-dimethoxypropane (Scheme 6).

Scheme 6. Acetonide formation Campaign 3[a]



[a]Reagents and conditions: (a) Acetone, 2,2-DMP, H_2SO_4 (0.95 equiv), 88%.

Successful reactions required the use of 1.0 equiv, rather than a catalytic amount, of concentrated sulphuric acid, and the product **13** was isolated in a direct drop process from the reaction mixture as the hydrogen sulphate salt. It is likely that the driving force for this equilibrium reaction was the crystallization of the product. Although the free base of **13** was also crystalline, we elected to isolate and use the hydrogen sulphate salt directly in the subsequent step. For Campaigns 1 and 2, this process afforded 1.1 and 10 kg of the acetonide hydrogen sulphate salt **13**, respectively.

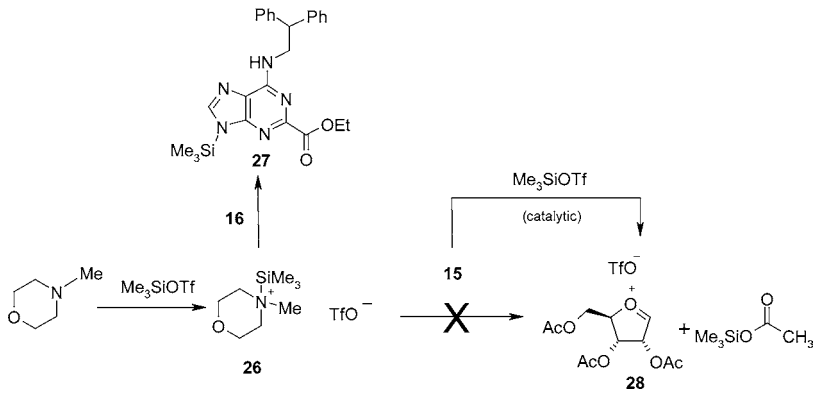


Figure 3. Rationalization of the stoichiometries of NMM and TMS triflate.

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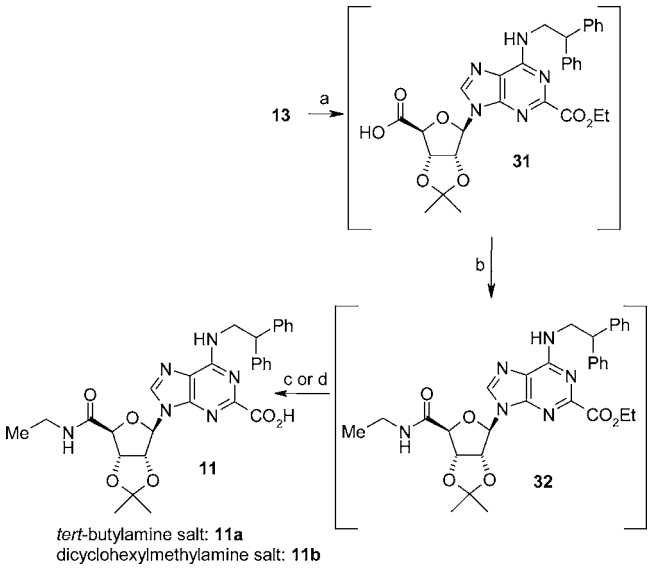
Further scale-up for Campaign 3 required a minor development of the acetonide-forming step. During Campaigns 1 and 2, variations in the colour and long-term stability of the acetonide hydrogen sulphate salt **13** were noted, and it was rationalised that traces of residual sulphuric acid could potentially be entrained within the isolated product giving rise to the instability and colouring on storage. Reducing the charge of sulphuric acid to 0.95 equiv gave a reproducibly stable hydrogen sulphate salt **13** that was again isolated by direct drop crystallization from the reaction medium in 88% yield. In Campaign 3, a total of 55 kg of hydrogen sulphate salt **13** was isolated in two lots (Scheme 6).

The next stage of the synthesis was the oxidation of the primary hydroxyl group in **13** (Scheme 7). Oxoammonium salts

Scheme 7. Oxidation, amide formation, and saponification sequence, Campaign 3[a]



[a]Reagents and conditions: (a) (i) NaClO (aq), TEAB, Na_2CO_3, TEMPO, DCM; (ii) Na_2SO_3 quench; (iii) HCl (aq); (b) (i) CDI, DCM, −10 °C; (ii) $EtNH_2$·HCl, DCM (reverse addition); (iii) Swap to MeOH; (c) [to **11a**] (i) NaOH (aq), MeOH; (ii) Swap to ^{i}PrOAc, then 1 N HCl (aq); (iii) 2 N HCl, water; (iv) ^{t}BuNH$_2$, ^{i}PrOAc; (v) Azeo dry; (vi) ^{t}BuNH$_2$, ^{i}PrOAc, 83% from **13**; (d) [to **11b**] (i) NaOH (aq), MeOH; (ii) Swap to ^{i}PrOAc, then citric (aq); (iii) MEK, dicyclohexylmethylamine, 68% from **13**.

are known for their ability to oxidise functional groups including alcohols,[11] and their in situ preparation from stable radical precursors with various oxidants is well documented.[12] In Campaigns 1 and 2, oxidation of the primary hydroxyl group in **13** was carried out using 1,1,5,5-tetramethylpiperidinyloxyl (TEMPO) and bleach in a two-phase system[13a,14,15] utilizing DCM and aqueous sodium bicarbonate in the presence of tetrabutylammonium bromide (TBAB).[13d] We also evaluated the sodium chlorite conditions that have been successfully scaled up[13b,c] but concluded that the bleach−TEMPO−TBAB conditions were safer to scale since the use and handling of hazardous sodium chlorite was avoided, and accordingly these conditions were used for the oxidation of 1 kg and 9.9 kg of **13** hydrogen sulphate salt during Campaigns 1 and 2, respectively. The product **31** was not crystalline, so solutions of **31** in DCM were obtained from this stage, and were used as such for the next step. Full safety testing consisting of DSC testing of TEMPO, the starting material, and product was carried out prior to scale-up as was full reaction calorimetry and TSU testing of intermediate solutions. No significant barriers were found to progression of this step or any other step described herein. Details of the testing for this and subsequent Campaign 3 steps are given in the Experimental Section.

For Campaign 3 further optimization of the reaction and workup was investigated as the phase separations during the workup operations were slow and thought to be due to TBAB acting as a surfactant. Tetraethylammonium bromide (TEAB) is approximately 4.5 times more water soluble than TBAB,[16] and TEAB gave an acceptable rate of reaction and a moderately improved rate of phase separation during workup compared to TBAB. Conversely, but as expected, trials of NaBr and KBr gave better workup phase separations but drastically reduced reaction rates due to negligible bromide levels in the organic phase. In Campaign 3, TBAB was replaced with TEAB, and the DCM solvent volume was reduced by 33%, giving approximately 48 kg of acid **31** in two batches as a DCM solution (Scheme 7).

In Campaigns 1 and 2, the DCM solutions of **31** obtained after workup were exchanged into THF by distillation, and the solution was azeotropically dried in readiness for the subsequent step. Formation of the ethylamide **32** proved to be relatively straightforward and was accomplished by activation of the carboxylic acid in THF using CDI followed by reaction with a commercial solution of ethylamine in THF. It was surprising to find that commercially available solutions of ethylamine in THF contained large amounts of water and hydrolysis of the acylimidazolide back to **31** was a significant competing reaction when the ethylamine solution was added to the acylimidazolide. We were unable to secure a source of anhydrous ethylamine in THF, and it would have been challenging to accurately and safely charge controlled amounts of anhydrous ethylamine (bp 17 °C). In the event we found that a reverse addition protocol, carried out by adding the solution of the acylimidazolide derived from **31** to a solution of 1.2 equiv of 70% w/w aqueous ethylamine in THF at −10 °C, suppressed hydrolysis, back to **31**, to an acceptable few percent. After quenching the reaction with aqueous citric acid and switching solvents to ethyl acetate, residual **31** was removed by washing with 1% w/w aqueous triethanolamine. Once again, the product was not crystalline; thus, the solution of **32** in ethyl acetate was exchanged into methanol in readiness for the next step.

The use of aqueous ethylamine in Campaign 2 not only imparted operational complexity of solvent exchanges but also carried a high risk that competing imidazolide hydrolysis might become a significant factor as the process was scaled up. The solvent exchanges were reduced in Campaign 3 by the discovery that the amide formation could be performed in DCM with a base and solid ethylamine hydrochloride, and as such, a solvent swap from that used in the oxidation step was not required. In laboratory trials the use of TEA as the liberating base gave poorly stirred mixtures as TEA hydrochloride formed a gum, whereas DIPEA gave good stirring as it gave a soluble hydrochloride salt. Critically, when using amine bases to liberate the ethylamine, an impurity, poorly resolved by HPLC, was seen at up to 15% by ^{1}H NMR spectroscopy and was identified as the C-4 epimer, **33** (Figure 4).

Retrospectively, when using a modified analytical HPLC method, the level of **33** in the original Campaign 2 intermediate **32** was found to be 5%, so this gave confidence that **33** could

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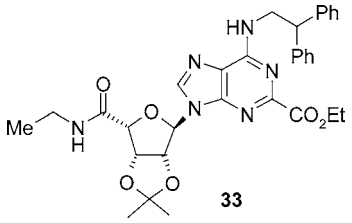


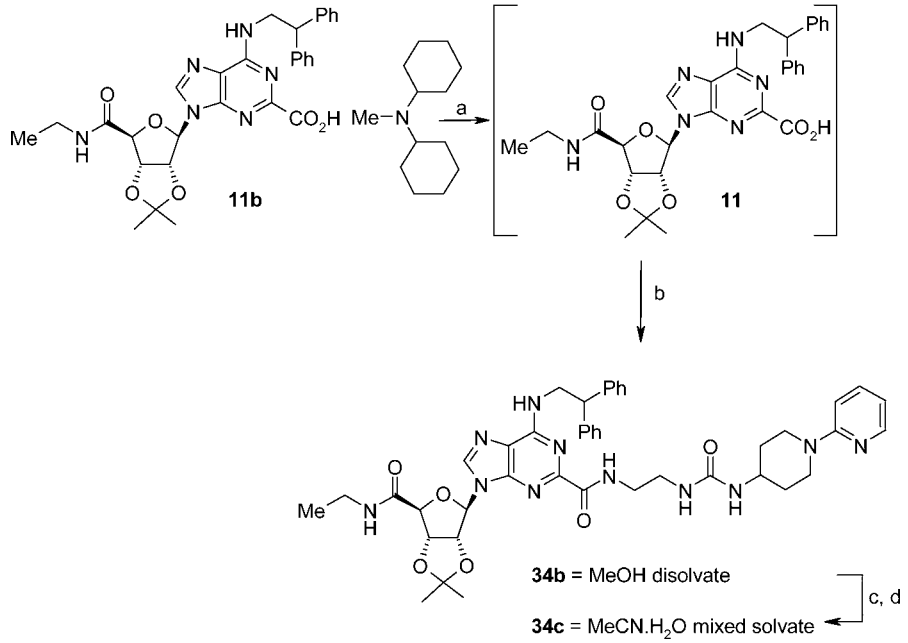
Figure 4. Structure of C-4 epimer **33**.

also be purged downstream in Campaign 3 if the initial level could be minimized. This was achieved by using no additional base, a −10 °C operating temperature, and an inverse addition of the imidazolide solution to a slurry of ethylamine hydrochloride in DCM. Use of these conditions resulted in a reproducible 7% of epimer **33** being obtained in the product **32** solution. Approximately 50 kg of **32** was produced in this way as a methanol solution in a single batch for Campaign 3 (Scheme 7).

Hydrolysis of the ethyl ester in **32** was accomplished using sodium hydroxide. It was found that this reaction was also quite sensitive to epimerization of the acidic chiral centre α to the amide, and also β-elimination. Indeed, an inadvertent overcharge of sodium hydroxide in Campaign 2 resulted in significant levels of impurity formation that, although gave a diminished yield, fortunately did not impact the downstream chemistry greatly. We found that **11**, whilst not being as crystalline as the free acid, formed a crystalline salt **11a** with *tert*-butylamine, and thus an isolation process was developed. The reaction mixture was acidified with hydrochloric acid at 40 °C, needing the slightly elevated temperature to avoid the deposition of an immobile gum of the free-acid form of **11** in the reactor. After removing most of the methanol by distillation, the free acid was then extracted into isopropyl acetate, and *tert*-butylamine was added to the wet organic phase. At this stage, the salt **11a** was still soluble, but crystallization was achieved by azeotropically drying the solution. Whilst this process worked well on 300-g scale in the lab for Campaign 1, on scaling this process to 4 kg into a kilo lab for Campaign 2 we observed a substoichiometric amount of *tert*-butylamine in the salt present in the slurry which we attributed to instability of the salt at the distillation temperature, resulting in some decomposition back to volatile *tert*-butylamine (bp 46 °C) and the free acid. This issue was overcome by addition of further *tert*-butylamine to the slurry after cooling, but was a concern for further scale-up in Campaign 3. Additionally, it proved impossible to remove all of the isopropyl acetate from the isolated and dried salt **11a**, although we did not determine whether this was due to a solvate being formed. In Campaigns 1 and 2, 630 g and 7.1 kg of **11a** were prepared, respectively.

Although the bond forming chemistry had worked well we wished to streamline the processing unit operations involved in this step for Campaign 3 notably, removing a recharge of amine to fully form the salt, and remove a break back to the free acid before the penultimate amide bond formation (vide infra). A salt screen was performed on **11** that focused initially on both tertiary amines and metal salts with the intent of directly using the salt in the following amide-forming step. The dicyclohexylmethylamine salt **11b** and the lithium salt were identified as suitable candidates, but during laboratory trials the dicyclohexylmethylamine salt **11b** gave the cleanest downstream processing and was selected for scale up in Campaign 3 (Scheme 7). After the ester was hydrolysed in NaOH/methanol, as described above, the mixture was worked up with citric acid and isopropyl acetate. After brine washing, the isopropyl acetate was distilled and replaced into the crystallization solvent methyl ethyl ketone (MEK), and dicyclohexylmethylamine was added to yield, after filtration and drying, a single batch of 44 kg of **11b** equating to a yield of

Scheme 8. Penultimate step, amide solvate preparations, Campaign 3[a]



[a]Reagents and conditions: (a) **11b**, HCl (aq), DCM; (b) (i) CDI, DCM, −10 °C; (ii) **12**, TEA, DCM, −10 °C; (iii) MeOH, water, 81%; (c) MeCN, water, 95%; (d) (i) MeCN, 65%; (ii) Second crop MeCN, water, 29%.

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68% over the three steps from the acetonide hydrogen sulphate salt **13**. Importantly, the level of the analogous C-4 epimer had reduced to 4% in salt **11b** from 7% in the ethyl ester **32**. Although the changes made to the transformation from **32** to salt **11b** meant that no extra charge of amine for salt formation was required, it did not reduce the number of solvent swaps in that part of the process. The main reduction in complexity was designed to come from using the new salt **11b** directly in the amide formation (vide infra), removing the need for a salt break and azeotropic drying of the resultant solution of the free acid **11**.

With the key units **11** and **12** in hand, the final stages of the synthesis were to couple them together via an amide formation (Scheme 8). In developing the coupling process for Campaigns 1 and 2, using the *tert*-butylamine salt **11a**, the main challenge was finding solvents that enabled sufficient solubility of all components, avoiding the reformation of the ethyl ester **32** by reaction of an activated derivative of **11** with the ethanol present in the solid form of **12**. Initially, the reaction was carried out in DCM, but it was found that the salt break of the *tert*-butylamine salt **11a** using aqueous hydrochloric acid gave poor phase separations that were attributed to the presence of low levels of insoluble impurities. This problem was overcome by carrying out the salt break using ethyl acetate as the organic phase, and this had the added advantage that the solution of the free acid could be easily dried by azeotropic distillation prior to the amide-formation stage. Unfortunately, cooling the dry ethyl acetate solution resulted in the deposition of **11** as a viscous gum in the vessel. This was avoided by the addition of some DCM to the solution once it had cooled to 35 °C. For the dihydrochloride **12** the situation was more complicated since the free base of **12** is highly soluble in aqueous systems even at high ionic strengths, and is also quite insoluble in organic solvents apart from DCM in which it is only modestly soluble. Therefore, it was necessary to use the dihydrochloride salt **12** directly in the amide-forming step using an organic solvent and an amine base to release the free base form of **12** in situ. This was achieved by combining **12** with DIPEA in DCM, and then slowly adding the ethyl acetate solution of the acyl imidazolide derived from **11** at −10 °C. These conditions also suppressed the formation of the ethyl ester from ethanol present in **12**, due to the slow reaction rate of ethanol with the acylimidazolide at the low temperature used.

At the outset of development work for this step, the coupled product **34** was not crystalline and we had planned to purify the product using chromatography during Campaign 1. However, we were unable to find a suitable and practical chromatography system for purification of **34** prior to the final stage of the synthesis. At the time the initial development work was carried out, a crystalline form of the final API had also not been found; thus, given our inability to find a suitable purification process for the final intermediate **34**, we planned to purify (by chromatography) the API **1** itself and then isolate it as an amorphous solid via precipitation in Campaign 1.

During the first lab scale-up, the coupling of **11** and **12** was carried out in two batches using DCM alone. After aqueous workup, the wet DCM solution containing the first batch of **34** was stored for a few days in a bottle whilst the second batch was brought through. When we returned to the stored material, we found that a large amount of solid had crystallized from the wet DCM solution. A sample was collected, dried and found to be a crystalline form of **34** that had crystallized as a DCM-solvate monohydrate **34a**, and the structure was confirmed by single-crystal X-ray analysis (Figure 5). Gratifyingly, the crystallization of the DCM solvate monohydrate **34a** resulted

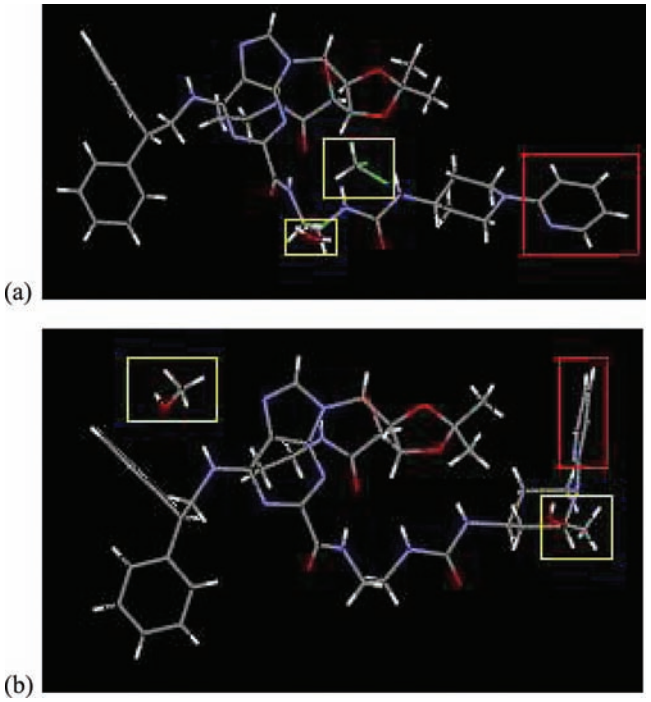

Figure 5. Single-crystal X-ray structures for **34** crystallized from (a) DCM giving **34a** and (b) methanol giving **34b**. Solvents and water molecules are boxed in yellow. Pyridyl substituent is boxed in red.

in an excellent purge of impurities, and enabled us to complete the Campaign 1 lab synthesis of 620 g of **34a** without the need to resort to chromatography. Furthermore, we found that recrystallization of **34a** using different solvents afforded two different crystalline families of solvated forms, either monosolvate monohydrates (from DCM, isopropanol, acetone, acetonitrile) or disolvates (from EtOH, MeOH) as determined by single-crystal X-ray crystallography. Examination of the structures showed that the conformation and internal H-bonding arrays were similar for each family except for the pyridine-bearing piperidine moiety. In both series, the piperidine moiety adopted a pseudochair conformation, but in the monosolvate monohydrate series, the pyridyl substituent took an equatorial position (Figure 5a) whereas in the disolvate family the piperidine adopted an axial position (Figure 5b). Within each family of solvates the location of solvent molecules was consistent, but the location did differ between each family.

Whilst the isolation of the DCM monosolvate monohydrate **34a** proved to be advantageous for Campaign 1, the DCM solutions of **34** generated during the workup of the coupling process would have been supersaturated and potentially could undergo undesirable crystallization from process solutions during the workup stages if used again. For further scale-up in Campaign 2, our attention focused on forming and crystallizing the methanol disolvate **34b** since this offered the potential to significantly simplify the work up of the coupling reaction as all the reaction byproduct (imidazole, DIPEA free base and hydrochloride), residual starting materials, solvents and many process-related impurities were soluble in methanol and would potentially be purged. Once the coupling reaction had reached completion, a simple exchange of solvents present

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in the reaction mixture into methanol by atmospheric pressure distillation resulted in the crystallization of the methanol disolvate **34b** which was then isolated by filtration. During Campaign 2, this process worked effectively to deliver a total of 4.2 kg of **34b**, but we found that some process-related impurities were not purged to the extent needed, and thus it was necessary to recrystallize this material twice from methanol to remove these impurities, giving 3.7 kg of **34b** ready for the final step.

The presence of methanol in **34b** was a potential problem since the conditions ultimately selected for the final step used methanesulphonic acid (vide infra), and we were concerned about the possible formation of the potentially genotoxic methyl methanesulphonate.[17] We found the methanol present in the isolated **34b** to be relatively loosely bound and could be removed during the drying process without adversely impacting the physical form of the solid, although the drying conditions were prolonged and required high humidity in the drying oven. This suggests that the methanol is replaced in the crystal lattice by water, although this was not proven. Whilst using the described process enabled the synthesis of 3.7 kg of methanol-free **34** ready for the final step of Campaign 2, the multiple recrystallizations and prolonged drying process would be impractical on larger scales and required further development.

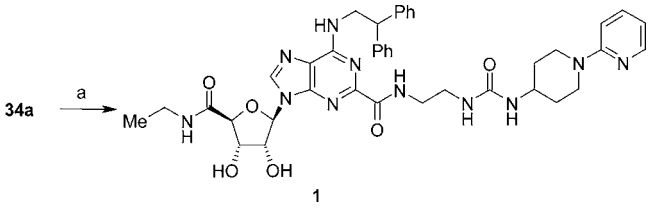
For Campaign 3, direct amide-coupling reactions of the dicyclohexylmethylamine salt **11b** with **12** on laboratory scale all indicated that this would be a viable process; however, when a sample of the bulk **11b** was piloted in the laboratory, a good conversion to amide **34** was observed, but crucially the crystallization from methanol was impaired, giving a poor recovery. In the time available the root cause of this failure was not identified, and a pragmatic way forward had to be found. As is very often the case, the supply of API was on critical path for the program; thus, for expediency the salt break was reintroduced as shown in Scheme 8, with all the attendant solvent exchanges and drying stages. The salt **11b** was broken with aqueous 1 N HCl and extracted into DCM for direct use in the CDI activation step. The side-chain hydrochloride salt **12** and TEA were then added to the acylimidazolide at 20 °C. A final solvent exchange of the DCM to methanol and seeding gave 40 kg of methanol disolvate **34b** isolated in a single batch in 81% yield.

In Campaign 2 the methanol disolvate **34b** required desolvation before being taken into the methanesulphonic acid-mediated acetonide hydrolysis. The desolvation was potentially an operational area of concern on the 10-fold scale up for Campaign 3, and thus a recrystallization of **34b** from acetonitrile and water was introduced to give the product as an acetonitrile monosolvate monohydrate **34c** in 95% recovery (Scheme 8).

To reach the desired purity specification a second recrystallization from acetonitrile was required during which extra water was not added. This recrystallization gave a poor 65% recovery, and a second crop was obtained, this time by reintroducing a small amount of water to the mother liquor, thereby increasing the water activity, to crystallize a further 29% material. In total **34c** was isolated in 94% yield over the two crops (see Experimental Section for details). Importantly, at this stage the analogous C-4 ribose epimer was not detectable, having been purged as anticipated during the downstream processing from **11**. Approximately 44 kg of acid salt **11b** was taken through the amide formation, methanol disolvate **34b** isolation, and acetonitrile−water recrystallizations to give 36.5 kg of **34c** in 78% overall yield from acid salt **11b** (Scheme 8).

The final stage of the synthesis was to remove the acetonide protecting group in **34** and then to isolate the final API **1** (Scheme 9). The development of the workup stage was

Scheme 9. Isolation of amorphous API 1, Campaign 1[a]



[a]Reagents and conditions: (a) (i) TFA, H_2O, butan-2-ol, 70 °C; (ii) NaOH, H_2O; (iii) TBME, 53%.

constrained somewhat by the low solubility of **1** in all relatively volatile anhydrous organic solvents that might be useful for phase separations during an aqueous workup. Fortunately, polar, water-immiscible organic solvents that had the capability of retaining significant amounts of water in the organic phase (such as butan-2-ol, MEK, and methyl acetate) proved to be effective solvents for **1**, and thus development work focused around these systems. Initially, deprotection of **34** was achieved using hydrochloric acid in ethanol at 60 °C, but these conditions resulted in significant cleavage of the nucleoside bond and also had the risk of generating ethyl chloride, a potential genotoxic impurity. We therefore screened different conditions and found that aqueous TFA was an effective reagent that avoided the formation of potentially genotoxic side products when used with alcoholic solvents. These conditions were selected for use in Campaign 1. Heating the DCM solvate monohydrate **34a** in a mixture of 5.3 equiv of TFA in a mixture of water and butan-2-ol at 70 °C resulted in the deprotection of the acetonide to give **1** (Scheme 9), however we observed the formation of several impurities as the reaction proceeded (products from hydrolysis of the nucleoside bond, hydrolysis of both amide bonds, and solvolysis of the ethylamide with butan-2-ol being the main degradants) and purity became progressively worse with time. In addition, unsurprisingly, the formation of but-2-yl trifluoroacetate in significant quantity was observed. Following workup, which involved pH adjustment, multiple water washes, and azeotropic drying of the solution, a relatively pure solution of **1** in butan-2-ol was produced in Campaign 1. However, a method to isolate amorphous material from the butan-2-ol solution was required since a crystalline form of the product had not been found at this point. To this end, we screened the addition of the butan-2-ol solution to various antisolvents to determine whether precipitation and isolation of an amorphous solid was viable. By using *tert*-butyl methyl ether (TBME) as the antisolvent, a filterable precipitate was formed on gram scale that on drying afforded the product in reasonable yield and purity. When this method was applied to the combined solutions from Campaign 1, the precipitation, filtration, and *tert*-butyl methyl ether washing stages worked as expected, but when the material was dried in a vacuum oven, the solid collapsed to a gum that subsequently expanded to a voluminous foam. This was due to inefficient removal of butan-2-ol from the solid during the washing process. Indeed, it proved impossible to remove all traces of residual butan-2-ol from the product even in a very prolonged drying process, and

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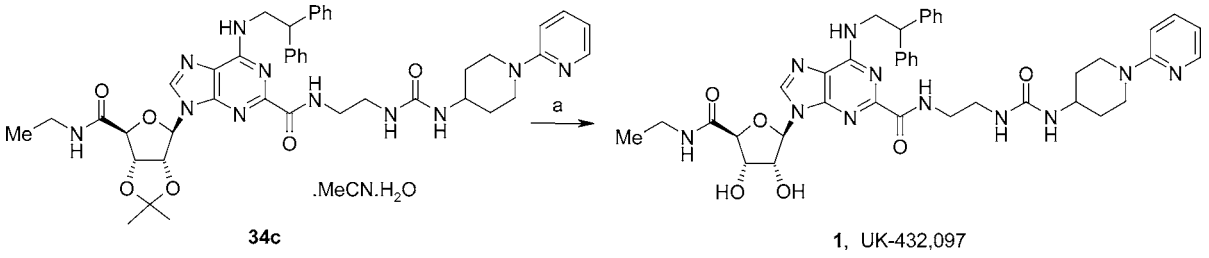
Scheme 10. Isolation of crystalline API 1, Campaign 3[a]



[a]Reagents and conditions: (a) (i) MSA (aq), 70 °C; (ii) Na_2HPO_4 (aq), MeOAc; (iii) Azeotropically dry, seed and crystallize at reflux, 78%.

the overall yield from **34a** was low (53%). Nevertheless, 313 g of **1** of suitable quality was prepared in Campaign 1, but it was recognized that the conditions used for the deprotection and isolation were far from ideal and required further development for Campaigns 2 and 3.

Fortunately, sometime after the completion of Campaign 1, a crystalline form of **1** was discovered,[5b] and we were able to develop a crystallization process for Campaign 2 as well as alternative conditions for the deprotection stage. To find alternative conditions for the acetonide deprotection, we were keen to avoid the use of organic solvents in the reaction stage since we were worried about reactions occurring between the acid promoter and a solvent, leading to potentially genotoxic impurities. We envisioned that, by utilising the basic 2-aminopyridine group in the molecule, it might be possible to combine superstoichiometric amounts of an acid with **34** to form a water-soluble salt that would enable the reaction to be carried out in water without the addition of an organic solvent. When this was tried, some acids (TFA, *p*-toluenesulphonic acid) resulted in the formation of gums, whereas reacting 1.1 equiv of aqueous methanesulphonic acid (MSA) with the acetonide **34** at 70 °C gave a clear solution that underwent deprotection over 24 h. To minimise degradation reactions occurring once the conversion was complete, the reaction was stopped by neutralization of the 0.1 M excess methanesulphonic acid by the addition of 0.12 equiv of aqueous solution of disodium hydrogen phosphate at 70 °C. On cooling the reaction mixture, the methanesulphonate salt of **1** remained in solution and enabled the workup to be undertaken without formation of unwanted gums or solids.

The extraction solvent used during the workup would necessarily depend on the solvent to be used for the crystallization of **1**. The discovery of a crystalline form of **1** was a major breakthrough for the project, but it took a considerable time to develop a workable crystallization process. After much experimentation, it emerged that the key features needed to crystallize **1** were a polar nonhydroxylic solvent containing a few percent of water, and conducting the crystallization at elevated temperature. The latter requirement is quite unusual, but without heating, supersaturated solutions would either remain as clear solutions or would precipitate amorphous gums. The first set of reliable conditions that were identified was to prepare a solution of **1** in 10 volumes of a 2% v/v solution of water in MEK and to heat this solution at 70 °C over several hours over which time **1** crystallized in reasonable yield. The use of MEK was tested for the workup of the deprotection step by taking the aqueous solution of the methanesulphonate salt of **1**, adding MEK, and neutralising the mixture with sodium hydrogen carbonate. The resulting wet MEK solution of **1**, after washing with water, was dried by azeotropic distillation with the aim of reducing the water level to 2%. However, when a sample of the dried solution was taken and examined by ^1H spectroscopy, new impurities, strongly suspected to be MEK self-condensation products, were evident. Moreover, it proved impossible to crystallize **1** from this solution, and MEK was abandoned and effort was focussed on other solvents. From previous screens it was known that **1** only dissolved in water-immiscible solvents (e.g., butan-2-ol, MEK) saturated with several percent of water. In addition, a solvent that could be azeotropically dried to adjust the water content to the correct level was needed. On this basis methyl acetate (dissolves approximately 8% w/w water when saturated, azeotrope contains 5% water at 56 °C) and ethyl acetate (dissolves approximately 3.3% w/w water when saturated, azeotrope contains 8% water at 71 °C) were selected for further investigation.[18] Wet ethyl acetate was immediately ruled out as **1** was insoluble, even in 50 volumes. However, water-saturated methyl acetate easily dissolved **1** in 10 volumes. Methyl acetate, given its low boiling point (57 °C) and flash point (−9 °C) is not a preferred solvent for large-scale use, but the significant advantages it offered to the workup and crystallization process outweighed the potential disadvantages, and so it was cautiously selected for further development.

Initial investigations for the use of methyl acetate in the workup of the deprotection reaction were hampered by poor phase separations, but we found that using 10% w/w disodium hydrogen phosphate for the salt break and extraction with methyl acetate followed by a wash of the organic phase using a 2% w/w disodium hydrogen phosphate solution provided sufficient ionic strength to enable reasonable phase separations to occur. The organic phase was then diluted with methyl acetate and azeotropically dried until the water content was approximately 2% w/w. Heating the resulting solution at reflux over 20 h then resulted in crystallization of **1** in good yield.

The final stages of the synthesis were scaled up in our kilo lab facility for Campaign 2, with the deprotection stage being run in two batches to give solutions of **1** in methyl acetate that were combined for the crystallization stage. The deprotection step worked largely as expected but some problems were encountered with the crystallization process with oiling occurring. Adjustment of the water content to 2.2% and the addition of seed crystals of **1** and heating the mixture for 20 h eliminated the oiling altogether, and following filtration, washing, and drying, Campaign 2 afforded a total of 2.52 kg of **1** in 72% yield from the desolvated acetonide **34b**.

For Campaign 3, the only significant change to the last chemical step and API crystallization was the use of **34c** as an input material in preference to desolvated **34b**. The mixed acetonitrile water solvate **34c** was taken up in aqueous MSA and heated at 70 °C for 10 h and worked up as before. The API

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(**1**) was crystallized by azeotropically drying the methyl acetate solution to 2.4% w/w water, seeding, and holding at reflux for at least 24 h. The final API, UK-432,097, **1** of appropriate purity and polymorphic form was isolated as a 25.3-kg single batch in 78% from **34c** (Scheme 10).

SUMMARY

We have identified and developed a new synthetic route to the structurally complex A_{2a} agonist UK-432,097 (**1**) that was used to prepare over 25 kg of API to support early phase development. The new route addressed the issues associated with the medicinal chemistry route by oxidizing the ribose C-5 hydroxyl position and elaborating it to the ethylamide, post nucleoside bond formation, and also resulted in the discovery of a robust crystalline form of the API. The key steps involved in the process were (1) a stereoselective glycosidation reaction; (2) a scalable bleach−TEMPO oxidation; and (3) an unusual elevated-temperature crystallization process for the final API.

EXPERIMENTAL SECTION

All materials obtained from commercial suppliers were used without further purification unless otherwise stated. All reactions were performed under an inert nitrogen atmosphere. Melting points are uncorrected. NMR spectra were recorded on a Jeol 400 MHz instrument: ^{1}H NMR at 70 °C in DMSO-d_6 as the solvent unless otherwise indicated. Chemical shifts are reported in ppm (δ) relative to residual protons in the deuterated solvent. HPLC chromatograms were recorded on Aligent 1100 instruments, and LCMS data were recorded on an Agilent 1100 series running a Waters Micromass ZQ mass spectrometer. Two HPLC methods were used.

Acidic method: Zorbax CB C18 column, 50 mm × 3 mm, 1.8 μm column. Flow rate = 1.2 mL/minute, wavelength 225 nm, temperature 70 °C. Eluent A = MeCN. Eluent B = 0.05% TFA in water. Eluent ramp (time, A:B ratio), 0 min, 5:95; 3.5 min, 100:0; 4.5 min, 100:0; 4.6 min, 5:95; 5 min, stop.

Basic method: Zorbax Extend CB C18 column, 50 mm × 3 mm, 1.8 μm column. Flow rate = 1.2 mL/minute, wavelength 225 nm, temperature 70 °C. Eluent A = MeCN. Eluent B = 0.1% NH_4OH in water. Eluent ramp (time, A:B ratio), 0 min, 5:95; 3.5 min, 100:0; 4.5 min, 100:0; 4.6 min, 5:95; 5 min, stop.

Representative Examples of Campaign 3 Outsourced Chemistry. *tert-Butyl 2-{N′-[1-(2-pyridyl)-4-piperidyl]-ureido}ethylcarbamate (**23**).* Amine salt **21**[5c,9] (23.0 kg, 91.9 mol) and 1,1′-carbonyldiimidazole (16.4 kg, 101 mol) were suspended in acetonitrile (118 kg) and cooled to 5 °C. DIPEA (24.4 kg, 189 mol) was added, with a final acetonitrile (9 kg) line rinse, over 6 h with maximum stirring, keeping the temperature below 10 °C, giving a solution. Upon full consumption of **21**, the mixture was warmed to room temperature, and a solution of *N*-BOC-ethylene diamine (15.5 kg, 96.7 mol) in acetonitrile (9 kg) was added and then heated to reflux for 2 h. Upon reaction completion acetonitrile (110 kg) was removed in vacuo and replaced with DCM (230 kg). The organic phase was washed with water (2 × 92 L). The combined DCM phases were concentrated (145 kg solvent removed), further distilled, and replaced with ethyl acetate (5 × 139 kg) with a further 558 kg of solvent being removed. The ethyl acetate solution was cooled to 55 °C, and the resulting slurry was granulated at 55 °C for 6.5 h. The slurry was cooled to 5 °C over 6 h upon which the thick slurry was stirred at that temperature for 2 h, and the product was collected by filtration. The filtrate was used as a secondary cold wash of the reaction vessel and refiltered on the original cake cold. The cake was washed with chilled ethyl acetate (42 kg). The product was dried on the filter at 61 °C for 45 h under s stream of nitrogen, giving **23** as a white solid 30.2 kg (83.0 mol, 90%).

*N-(2-Aminoethyl)-N′-[1-(2-pyridyl)-4-piperidyl]urea Hydrochloride Ethanol Solvate (**12**).* Acetyl chloride (38.9 kg, 496 mol) was added to a solution of BOC protected amine **23** (30.0 kg, 82.5 mol) in absolute ethanol (236 kg) at 45 °C. The reaction mixture was heated to 45 °C for 2 h and was cooled to room temperature over 2 h during which time a suspension was formed. The mixture was stirred for 2 h, the solid was collected by filtration, and the filter cake was washed with cold (0−5 °C) absolute ethanol (2 × 47 kg). The solid was dried on the filter for 3 h and then under a stream of nitrogen for 96 h to give the ethanol solvate **12** as a colourless hygroscopic solid, (27.6 kg, 85%). ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.02 (3H, br s,), 7.94 (2H, m), 7.35 (1H, d, J = 11.8 Hz), 6.89 (1H, t, J = 6.4 Hz), 4.33 (br s), 4.14 (3H, br d, J = 13.9 Hz), 3.7 (1H, m), 3.40 (2H, t, J = 11.1 Hz), 3.23 (H, t, J = 6.1 Hz), 2.81 (2H, q, J = 5.9 Hz), 1.89 (2H, m), 1.46 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 158.5, 151.8, 144.5, 137.3, 113.3, 112.8, 45.9, 45.8, 37.9, 31.8.

*2-Chloro-N-(2,2-diphenylethyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**24**).* For representative experimental details of conversion of dichloropurine to chloropurine derivative **24** see reference 8b experimental section, compounds **13** and **14**.

*Ethyl 6-[(2,2-Diphenylethyl)amino]-9-(tetrahydro-2H-pyran-2-yl)-9H-purine-2-carboxylate (**25**).* 1,1′-Bis-(diphenylphosphino)ferrocene (7.00 g, 12.6 mmol) was added to a suspension of palladium acetate (1.50 g, 6.68 mmol) in absolute ethanol (1 L), and the resultant suspension was stirred under an atmosphere of nitrogen for 18 h to give the catalyst mixture. Anhydrous sodium carbonate (94 g, 887 mmol) was added to a solution of chloropurine 24 (700 g, 1.61 mol) in ethanol (4.5 L) in an autoclave, and the catalyst mixture was added under nitrogen. The autoclave was flushed twice with carbon monoxide and then pressurised to 20 bar with carbon monoxide. The mixture was then heated at 105 °C with stirring for 10 h, and the autoclave was then vented, flushed with carbon monoxide, and then repressurised to 20 bar of carbon monoxide. Heating at 105 °C was continued for a further 14 h upon which the mixture was cooled to 60 °C and filtered through a bed of warm Celite. The resultant filtrate was allowed to cool to ambient temperature, whereupon crystallization occurred, and after stirring at this temperature for 7 h, the resultant suspension was filtered. The filter cake was washed with cold ethanol (500 mL), and the solid was dried in vacuo for 24 h at 55 °C to give ethyl ester **25** as a cream-coloured solid (575 g, 76%), mp 138−140 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C) δ: 8.05 (1H, s), 7.45−7.15 (10H, m), 5.95−5.80 (2H, m), 4.60−4.30 (5H, m), 4.15 (1H, br d), 3.80 (1H, br t), 2.20−1.60 (6H, m), 1.50 (3H, t).

*Ethyl 6-[(2,2-Diphenylethyl)amino]-9H-purine-2-carboxylate (**16**).* Trifluoroacetic acid (73.5 g, 0.645 mol) was added to a suspension of ethyl ester **25** (250 g, 0.530 mol) in absolute ethanol (1.25 L), and the resultant mixture was heated at 50 °C for 20 h. The mixture was then cooled, and the solid was collected by filtration. The filter cake was washed with absolute ethanol (350 mL) and was dried in vacuo at 50 °C overnight to give purine derivative **16** (206.5 g, 99%) as a cream-coloured

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fine powder, mp >200 °C. ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ: 8.36 (1H, br s), 8.00 (1H, br t), 7.48−7.12 7 (10H, m), 4.80−4.00 (5H, br m), 1.48−1.22 (3H, br m).

*N-(2,2-Diphenylethyl)-2-(ethoxycarbonyl)adenosine (**29**).* *N*-Methylmorpholine (1.44 kg, 14.2 mol) was charged to a suspension of **16** (5.00 kg, 12.9 mol) in anhydrous DME (15 L) at 22 °C. The mixture was then heated to 50 °C, and a solution of trimethylsilyl trifluoromethanesulphonate (3.73 kg, 16.8 mol) in DME (6.25 L) was then added at such a rate as to maintain the reaction temperature between 50 and 60 °C. The temperature of the reaction mixture was then adjusted to 65 °C, and a solution of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**15**) (4.52 kg, 14.2 mol) in DME (17.5 L) was added over a period of 15 min. The reaction mixture was stirred at 65 °C for 90 min and upon reaction completion was quenched at 65 °C over 15 min with an aqueous solution of trisodium citrate monohydrate (1.90 kg, 6.46 mol) and citric acid monohydrate (1.31 kg) in water (12.5 L). The DME was mostly removed by distillation (initially under vacuum) and replaced with isopropyl acetate (25 L). After cooling to 25 °C the phases were separated, and the upper organic phase was washed with water (2 × 12.5 L) and saturated aqueous sodium bicarbonate solution (12.5 L). The organic phase was then diluted with isopropyl acetate (45 L), and the solution was azeotropically dried by distillation at atmospheric pressure until approximately 45 L of distillate had been collected, and the organic solution contained no more than 0.03% w/w water by Karl Fischer analysis. The mixture was then concentrated to a volume of approximately 18 L by distillation at atmospheric pressure, and absolute ethanol (60 L) was added. The mixture was then further concentrated by distillation at atmospheric pressure until the volume was approximately 43 L. The resultant solution of triacetate **14** was then cooled to 20 °C, and a solution of 1,8-diazabicyclo[5.4.0]-undec-7-ene (1.96 kg, 12.9 mol) in absolute ethanol (7 L) was added over 15 min and was stirred for 3 h upon which the mixture was slowly added to a solution of citric acid (2.71 kg, 12.9 mol) in water (37.5 L), and the equipment was rinsed through with ethanol (2 L). The resulting mixture was then concentrated to a volume of approximately 55 L by distillation (initially under vacuum). The mixture was then cooled to 25 °C, it was then extracted with isopropyl acetate (75 L), and the organic phase was washed with 0.1 M aqueous sodium bicarbonate solution (2 × 37.5 L). The solution was then diluted with isopropyl acetate (25 L) and then azeotropically dried by distillation at atmospheric pressure until the final volume was 32 L. The solution was then cooled to 20 °C over 3 h, seeded with crystals of **29** (10 g), aged at 20 °C for 18 h, and was then cooled to 2 °C for 2 h. The solids were collected by filtration, washed with cold isopropyl acetate (2 × 10 L), and were then dried in vacuo at 50 °C for 48 h to give **29** (6.05 kg, 11.64 mol, 90%) as an off-white solid that contained approximately 4% w/w of entrained residual isopropyl acetate. ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C) δ: 8.20 (1H, br s), 7.84 (1H, br s), 7.32 (4H, br d, J = 7.1 Hz), 7.23 (4H, br t, J = 7.3 Hz), 7.14 (2H, br t, J = 7.3 Hz), 5.89 (1H, d, J = 7.1 Hz), 5.22 (1H, d, J = 5.9 Hz), 4.94 (1H, d, J = 4.6 Hz), 4.81 (1H, t, J = 5.7 Hz), 4.62 (1H, br s), 4.56 (1H, dd, J = 11.1, 6.1 Hz), 4.32 (2H, m), 4.15 (1H, dd, J = 8.1, 4.9 Hz), 3.94 (1H, dd, J = 6.9, 3.7 Hz), 3.66 (1H, ddd, J = 12.0, 4.9, 4.3 Hz), 3.57 (1H, ddd, J = 12.0, 6.6, 4.2 Hz), 1.33 (3H, br t, J = 6.9 Hz). (Residual ^{i}PrOAc signals, 4.84 (hept, J = 6.3 Hz), 1.92 (s), 1.14 (d, J = 6.3 Hz)). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 164.4, 154.7, 151.1, 149.1, 143.1, 142.0, 128.9, 128.7, 126.9, 120.8, 87.9, 86.6, 74.2, 71.2, 62.1, 61.8, 50.1, 45.2, 14.7, (Residual ^{i}PrOAc signals, 67.5, 22.13, 21.6). HPLC (basic method) retention time 2.72′. LCMS m/z: 520.2 (MH^+).

Campaign 3 In-House Preparations. *N-(2,2-Diphenylethyl)-2-(ethoxycarbonyl)-2′,3′-O-isopropylidene Adenosine Hydrogen Sulphate (**13**).* Sulphuric acid (98% w/w; 5.73 kg, 58.5 mol, 0.95 equiv) was added over a period of 10 min to a suspension of triol **29** (32.0 kg, 61.6 mol, 1 equiv) in acetone (149 L) and 2,2-dimethoxypropane (12.83 kg, 123 mol, 2 equiv). The sulphuric acid addition line was rinsed through with acetone (10 L), and the resultant solution was stirred at 20 °C for 50 min after which a thick slurry had formed. The mixture was filtered and the cake washed with acetone (32 L). The solid was dried in vacuo at 50 °C overnight to yield sulphate salt **13** as a tan solid (35.6 kg, 54.1 mol, 88%). ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): 8.41 (1H, br s), 7.88 (1H, br s), 7.31 (4H, m), 7.24 (4H, m), 7.14 (2H, m), 6.14 (1H, d, J = 2.4 Hz), 5.28 (1H, dd, J = 6.1, 2.4 Hz), 4.99 (10H, br s), 4.62 (1H, br s), 4.32 (2H, m), 4.17 (1H, m), 3.57 (1H, dd, J = 11.7, 5.4 Hz), 3.52 (1H, dd, J = 11.8, 5.0 Hz), 1.52 (3H, s), 1.33 (3H, br t, J = 7.1 Hz), 1.30 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 164.3, 154.6, 151.2, 148.6, 143.1, 142.1, 128.9, 128.1, 126.9, 120.4, 113.6, 89.7, 87.8, 84.3, 81.9, 62.2, 61.8, 50.1, 45.2, 27.6, 25.8, 14.6 HPLC (basic method) retention time = 3.25′. LCMS m/z: 560.1 (Parent MH^+).

*Ethyl 6-[(2,2-Diphenylethyl)amino]-9-(2,3-O-isopropylidene-β-D-ribofuranuronosyl)-9H-purine-2-carboxylate (**31**).* **Safety testing data.** DSC testing of TEMPO showed strong exothermic decomposition from 196 °C, whereas sulphate salt **13** showed only a melting endotherm from 142 °C. Reaction calorimetry showed no accumulation at any stage, but the addition of bleach solution showed a dose-controlled exotherm of −275 kJ/mol. TSU screening of the reaction mixture after addition of bleach showed no thermal events up to 176 °C, and screening of the final DCM solution before azeotropic drying showed no thermal events up until 200 °C.

An aqueous sodium hydrogen carbonate solution (13.7 kg dissolved in 164 L water) was added to a stirred slurry of sulphate salt **13** (35.6 kg, 54.06 mol, 1 equiv), TEAB (11.4 kg, 54.06 mol, 1 equiv), and TEMPO (144 g, 0.922 mol, 1.7 mol %) in DCM (355 L). The mixture was cooled to 2 °C, and aqueous sodium hypochlorite (13% w/w; 71.8 kg, 127 mol, 2.35 equiv) was added over 60 min at such a rate that the internal temperature did not rise above 5 °C, and the mixture was stirred for 2 h. Upon reaction completion the mixture was warmed to 20 °C, and aqueous sodium sulphite (35.6 kg in 356 L of water) was added and stirred overnight. Upon a negative test for active oxidant the phases were separated, and the retained organic phase was washed with 1 M hydrochloric acid (360 L). A subsequent wash with aqueous sodium chloride (71 kg NaCl in 356 L water) gave an emulsion that required overnight separation and settling. The aqueous sodium chloride phase was back extracted with DCM (178 L), requiring another slow separation. The DCM extracts were combined and azeotropically distilled until the water content was not more than 0.05% w/w and the product held as a solution. A small sample of the solution of **31** was evaporated to a gum for data collection. ^{1}H NMR (400 MHz, DMSO-d_6, 90 °C): δ 8.30 (1H, s), 7.63 (1H, br s), 7.31 (4H, br d, J = 7.6 Hz), 7.25 (4H, m), 7.15 (2H, m), 6.32 (1H, s), 5.66 (1H, dd, J = 6.1, 2.2 Hz), 5.46 (1H, br d, J = 6.1 Hz), 4.61 (2H, m), 4.31 (2H, br q, J = 7.1 Hz), 4.23 (2H, br m), 3.00 (br s), 1.51 (3H, s), 1.34 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 171.1, 164.0, 154.7,

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150.4, 148.9, 143.1, 143.0, 128.9, 128.7, 126.9, 119.4, 113.0, 89.9, 86.6, 84.6, 84.3, 61.6, 50.0, 45.3, 27.0, 25.4, 14.6. HPLC (acidic method) retention time = 3.28′;. LCMS m/z: 574.4 (MH^+).

*Ethyl 6-[(2,2-Diphenylethyl)amino]-9-(N-ethyl-2,3-O-isopropylidene-β-D-ribofuranosyluronamide)-9H-purine-2-carboxylate (**32**).* **Safety testing data.** DSC testing of ethylamine hydrochloride showed a melting endotherm from 77 °C and a further endotherm from 313 °C, whereas acid **31** showed an endotherm from 56 °C followed by a strong exotherm from 185 °C. Reaction calorimetry showed no accumulation at any stage and no significant energy release at any stage. **TSU screening of the reaction mixtures:** after addition of CDI, after addition of the imidazolide to the amine salt, and after the final solution was concentrated, all showed no thermal events up to 185 °C.

1,1′-Carbonyldiimidazole (16.28 kg, 100.4 mol, 1.2 equiv) was added to a stirred solution of acid **31** (∼48.0 kg, 83.6 mol, 1 equiv) in DCM (480 L) at room temperature. Upon completion of acylimidazolide formation (approximately 3 h) the mixture was cooled to 5 °C. In a separate vessel a slurry of ethylamine hydrochloride (8.2 kg, 100 mol, 1.2 equiv) in DCM (144 L) was prepared and cooled to −15 °C. The acylimidazolide solution was then added, followed by DCM (30 L) line rise to the cooled salt slurry at such a rate to maintain the internal temperature below 0 °C. The reaction mixture was then stirred at 0 °C for 16 h, whereupon the mixture was warmed to 20 °C and diluted with methanol (360 L). The mixture was distilled at ambient pressure to leave approximately 480 L vessel volume, and the solvent was replaced with fresh methanol (360 L). The vessel contents were again distilled to leave approximately 480 L volume reaching a final internal temperature of 63 °C. The methanol solution was held in drums in readiness for the preparation of salts of acid **11** (vide infra). A small sample was evaporated to a gum for data collection. ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.33 (1H, br s), 7.86 (1H, br s), 7.30 (4H, d, J = 7.6 Hz), 7.24 (4H, m), 7.14 (2H, m), 6.33 (1H, br s), 5.48 (1H, br d, J = 6.1 Hz), 5.37 (1H, br d, J = 6.1 Hz), 4.62 (1H, br s), 4.48 (1H, s), 4.30 (2H, m), 4.15 (2H, br m), 2.74 (2H, m), 1.51 (3H, s), 1.32 (6H, m), 0.57 (3H, br t, J = 7.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 168.6, 164.2, 154.7, 150.9, 148.7, 143.1, 142.6, 128.9, 128.7, 126.8, 120.5, 113.2, 89.6, 87.2, 84.2, 83.9, 61.6, 50.0, 45.2, 33.5, 27.2 25.6, 14.6, 14.3. HPLC (acidic method) retention time = 3.36′; (basic method) retention time = 3.26′. LCMS m/z: 601.4 (MH^+).

*6-[(2,2-Diphenylethyl)amino]-9-(N-ethyl-2,3-O-isopropylidene-β-D-ribofuranosyluronamide)-9H-purine-2-carboxylic Acid tert-Butylamine Salt (**11a**).* Sodium hydroxide, 1 M solution (8.4 L), was charged over 30 min to a 5 °C solution of ester **32** (4.57 kg, 7.61 mol) in methanol (∼23 L) and stirred for 1 h. Upon reaction completion the reaction was warmed to 40 °C and slowly neutralized to pH 6−7 with 1 M hydrochloric acid (2 L) and solvent removed (20 L) by distillation at ambient pressure. Whilst at reflux isopropyl acetate (23 L) was added, and a further 23 L of solvent removed by distillation. Fresh isopropyl acetate (23 L) was added whilst the mixture was above 50 °C; the mixture was cooled to 20 °C, and water (9 L) was added. The mixture was acidified to pH 1−2 with 2 M hydrochloric acid (3 L). The phases were separated, and the organic phase was washed with water (14 L). The organic phase was returned to the reaction vessel, and a solution of *tert*-butylamine (670 g, 9.3 mol) in isopropyl acetate (4.5 L) was added over 15 min and washed through with isopropyl acetate (11 L). The solution was dried and ambient pressure distilled, and solvent was replaced; 16.5 L was removed and replaced with fresh isopropyl acetate (11 L), and then a further 9 L of solvent was removed. At this point a check of the reaction mixture showed *tert*-butylamine had been lost during the distillation, so a further charge of *tert*-butylamine (320 mL) in isopropyl acetate (1 L) was made. The mixture was cooled to 20 °C over 3 h, then to 0 °C over 2 h to give a stirrable slurry. The product was filtered, washed on the filter with chilled isopropyl acetate (4.4 L), and dried in vacuo at 50 °C for 48 h to give salt **11a** as a white solid (4.1 kg, 6.35 mol, 83%). ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.18 (1H, br s), 7.75 (1H, br s), 7.30 (4H, br d, J = 7.6 Hz), 7.24 (4H, br t, J = 7.6 Hz), 7.14 (2H, br t, J = 7.1 Hz), 6.23 (1H, d, J = 2.5 Hz), 5.29 (1H, dd, J = 6.0, 2.8 Hz), 5.25 (1H, dd, J = 6.0, 2.8 Hz), 4.56 (1H, br t, J = 7.6 Hz), 4.47 (1H, d, J = 2.4 Hz), 4.21 (2H, br s), 2.95, (2H, br quin, J = 7.1 Hz), 1.52 (3H, s), 1.31 (3H, s), 1.18 (9H, s), 0.76 (3H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 170.0, 168.7, 161.5, 154.7, 149.1, 143.4, 140.0, 128.9, 128.6, 126.8, 114.0, 89.5, 85.4, 83.5, 83.3, 50.8, 50.1, 44.7, 33.9, 28.0, 27.4, 25.7, 14.9. HPLC (basic method) retention time 2.08′; LCMS m/z: 573.3 (Parent MH^+).

*6-[(2,2-Diphenylethyl)amino]-9-(N-ethyl-2,3-O-isopropylidene-β-D-ribofuranosyluronamide)-9H-purine-2-carboxylic Acid Dicyclohexylmethylamine Salt (**11b**).* **Safety testing data.** DSC testing of the solution post brine washing showed only endothermic activity past 313 °C. Product salt **11b** showed an endothermic melt from 166 °C and further endothermic activity past 319 °C. Reaction calorimetry showed no accumulation at any stage and the only significant energy release of −246 kJ/mol on neutralization of the sodium salt with aqueous citric acid, but the addition of bleach solution showed a dose-controlled exotherm of −275 kJ/mol. TSU screening of the organic phase after addition of aqueous citric acid showed no thermal events up to 179 °C, and screening of the organic solution before distillative removal of methanol showed no thermal events up until 199 °C.

A solution of aqueous sodium hydroxide (20.9 kg, 208.9 mol, 2.5 equiv) in water (213 L) was cooled to 10 °C and added slowly to a solution of ester **32** (∼50.2 kg, 83.6 mol, 1.0 equiv) in methanol (∼502 L), maintaining the temperature at or below 0 °C. The solution was warmed to 20 °C and stirred for 3 h. The reaction mixture was diluted with isopropyl acetate (251 L), and aqueous citric acid (10% w/vol, 250 L) was added, whereupon the mixture was heated to reflux and 540 L of distillate was removed. Isopropyl acetate (250 L) was added to the remaining solution which was cooled to 20 °C, and the phases were separated. The organic phase was washed with sodium chloride solution (20% w/vol, 250 L). The combined aqueous washes were back extracted with isopropyl acetate (250 L), and subsequently washed with citric acid solution (10% w/vol, 250 L). The isopropyl extracts were combined and distilled under reduced pressure to low volume (∼100 L), and methyl ethyl ketone (251 L) was added. This distillation and replacement was repeated with the final volume made up to approximately 275 L. The solution was held at 50 °C and dicyclohexylmethylamine (13.1 kg, 66.9 mol, 0.8 equiv) was added and the mixture cooled to 18 °C and stirred for 16 h. The mixture was filtered and the solid washed with methyl ethyl ketone (100 L) and dried at 50 °C in vacuo to give salt **11b** as a white solid (43.9 kg, 57.2 mol, 68%). ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.29 (1H, br s), 7.63 (1H, br m),

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7.50 (1H, br m), 7.30 (4H, d, J = 7.2 Hz), 7.24 (4H, t, J = 7.2 Hz), 7.13 (2H, t, J = 7.2 Hz), 6.30 (1H, br s), 5.37 (1H, br d, J = 6.1 Hz), 5.33 (1H, br d, J = 6.1 Hz), 4.58, (1H, br m), 4.50 (br s), 4.21 (2H, br s), 2.83 (2H, m), 2.61 (2H, m), 2.23 (3H, s), 1.71 (8H, m), 1.54 (2H, br s), 1.52 (3H, s), 1.32 (3H, s), 1.21 (8H, m), 1.05 (2H, m), 0.63 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 168.8, 168.3, 157.5, 154.7, 148.9, 143.3, 141.0, 128.9, 128.6, 126.8, 119.3, 113.7, 89.6, 86.0, 83.7, 83.5, 60.3, 50.1, 44.8, 33.8, 32.4, 29.2, 28.6, 27.3, 25.7, 25.5, 24.7, 14.7. HPLC (basic method) retention time 2.07′. LCMS m/z: 573.4 (Parent MH$^+$).

*6-[(2,2-Diphenylethyl)amino]-9-(N-ethyl-2,3-O-isopropylidene-β-D-ribofuranosyluronamide)-N-(2-{N′-[1-(2-pyridyl)-4-piperidyl]ureido}ethyl)-9H-purine-2-carboxamide Methanol Disolvate (**34b**).* **Safety testing data.** DSC testing of the starting material salt **11b** showed endothermic melt from 188 °C and a small broad exotherm from 306 °C. Testing of salt **12** showed an endothermic melt from 127 °C and two small exotherms from 176 °C. Product solvate **34b** showed an endothermic melt from 119 °C and a small exotherm past 286 °C. Reaction calorimetry showed no accumulation at any stage; however, peak gas evolution of 3.4 L/min/mol was seen during CDI addition, and a further peak flow of 1.8 L/min/mol was seen on addition of salt **12**. A dose-controlled exotherm of −42 kJ/mol was seen upon addition of TEA. TSU screening of reaction after addition of CDI showed a small exotherm from 140 °C. TSU testing post TEA addition showed no thermal events up to 200 °C.

Dicyclohexylmethylamine salt **11b** (43.9 kg, 57.2 mol, 1.0 equiv) was dissolved in DCM (176 L) at 20 °C, and hydrochloric acid (1M, 132 L) was added. The layers were separated, and the aqueous phase was extracted with DCM (44 L). The initial organic phase was washed with water (132 L), and the two DCM extracts of acid 20 were combined and azeotropically dried until below 0.1% w/w water by Karl Fischer analysis to give a dry DCM solution of free acid **11**. 1,1′-Carbonyldiimidazole (11.13 kg, 68.6 mol, 1.2 equiv) was added portionwise to the free acid **11** solution in DCM (~220 L). The reaction was stirred for 2 h at 20 °C, and then amine hydrochloride salt **12** (23.2 kg, 68.6 mol, 1.2 equiv) was added as a solid followed by triethylamine (6.9 kg, 68.6 mol, 1.2 equiv). The reaction mixture was stirred overnight upon which the DCM was distilled and replaced with methanol at ambient pressure. The solution was cooled to 20 °C and seeded with product amide **34b**, stirred for 8 h at 20 °C, and then cooled to 0 °C and held overnight. The mixture was filtered, washed with chilled methanol (33 L), and dried at 50 °C in vacuo to give amide methanol disolvate **34b** as a white powder (40.7 kg, 46.2 mol, 81%). ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.35 (1H, br s), 8.29 (1H, br s), 8.04 (1H, dd, J = 4.9, 1.9 Hz), 7.70 (1H, br s), 7.43 (2H, m), 7.33 (4H, m), 7.23 (4H, m), 7.13 (2H, m), 6.71 (1H, d, J = 8.6 Hz), 6.53 (1H, dd, J = 6.9, 4.9), 6.36 (1H, br s), 5.87 (1H, br m), 5.78 (1H, d, J = 7.9 Hz), 5.53 (1H, dd, J = 6.1, 2.1 Hz), 5.35 (1H, d, J = 5.8 Hz), 4.58 (1H, m), 4.55 (1H, d, J = 2.0 Hz), 4.24 2H, (br s), 4.01 (2H, m), 3.59 (1H, m), 3.34 (2H, m), 3.27 (2H, m), 2.88 (2H, m), 2.73 (2H, m), 1.75 (2H, m), 1.50 (3H, s), 1.32 (3H, s), 1.27 (2H, m), 0.51 (3H, t, J = 7.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 169.4, 164.1, 159.3, 158.3, 154.6, 153.3, 148.5, 148.1, 143.4, 143.3, 142.6, 138.0, 128.8, 128.7, 126.8, 120.2, 113.3, 112.9, 107.5, 90.1, 87.2, 84.2, 83.8, 50.4, 47.2, 44.9, 44.1, 41.0, 33.6, 32.2, 27.1, 25.6, 14.2. HPLC (basic method) retention time 3.02′. LCMS m/z: 818.5 (nonsolvated MH$^+$).

*6-[(2,2-Diphenylethyl)amino]-9-(N-ethyl-2,3-O-isopropylidene-β-D-ribofuranosyluronamide)-N-(2-{N′-[1-(2-pyridyl)-4-piperidyl]ureido}ethyl)-9H-purine-2-carboxamide Acetonitrile Monosolvate Monohydrate (**34c**).* Methanol disolvate **34b** (40.7 kg, 46.1 mol) was dissolved in acetonitrile (204 L) at reflux and water (2 L, 99.5 mol). The mixture was cooled to 2 °C over 2 h to give a yellow slurry which was stirred for 48 h and then filtered; the cake was washed with chilled acetonitrile (41 L) and pulled dry. The solid was dried in vacuo overnight at 50 °C to yield acetonitrile monosolvate monohydrate **34c** as a white solid (38.8 kg, 44.2 mol, 96%).

*Recrystallization of **34c**.* **Safety Testing Data.** DSC testing of the starting material salt **34c** showed endothermic melt from 120 °C and a small broad exotherm from 286 °C. Reaction calorimetry showed an exotherm of −67.5 kJ/mol.

Acetonitrile monosolvate monhydrate **34c** (38.8 kg, 44.2 mol) was recrystallized from acetonitrile (194 L) in the absence of water to give an inferior yield (25.45 kg, 29.0 mol, 65%). The filtrate was distilled to a total volume of approximately 65 L, water (0.6 L, 33.2 mol) was added, and the mixture was cooled to 70 °C and seeded with **34c**. The mixture was cooled to 2 °C to give a yellow slurry that was stirred for 14 h and filtered, and the cake was washed with chilled acetonitrile (14 L) and pulled dry. The solid was dried in vacuo overnight at 50 °C to yield acetonitrile monosolvate monohydrate **34c** as a white solid (11.25 kg, 12.83 mol, 29%). Total yield of 36.7 kg, 94% over the two crops. ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.35 (1H, br s), 8.29 (1H, br s), 8.04 (1H, ddd, J = 4.9, 2.1, 1.1 Hz), 7.70 (1H, br s), 7.43 (2H, m), 7.33 (4H, m), 7.23 (4H, m), 7.13 (2H, m), 6.71 (1H, d, J = 8.5 Hz), 6.53 (1H, dd, J = 6.1, 5.3), 6.36 (1H, br s), 5.87 (1H, br m), 5.78 (1H, d, J = 7.5 Hz), 5.53 (1H, dd, J = 6.1, 1.9 Hz), 5.35 (1H, d, J = 6.1 Hz), 4.58 (1H, m), 4.55 (1H, d, J = 2.3 Hz), 4.25 (2H, br s), 4.01 (2H, m), 3.60 (1H, m), 3.34 (2H, m), 3.27 (2H, m), 2.88 (2H, m), 2.73 (2H, m), 2.01 (MeCN, s), 1.75 (2H, m), 1.50 (3H, s), 1.32 (3H, s), 1.26 (2H, m), 0.51 (3H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 169.5, 164.1, 159.3, 158.3, 154.6, 153.4, 148.5, 148.1, 143.4, 143.3, 142.6, 138.0, 128.8, 128.7, 126.8, 120.2, 118.5 (MeCN), 113.3, 112.9, 107.5, 90.2, 87.2, 84.2, 83.8, 50.4, 47.2, 44.9, 44.1, 41.0, 33.7, 32.2, 27.1, 25.6, 14.2, 1.6 (MeCN). HPLC (basic method) retention time 3.02′. LCMS m/z: 818.5 (nonsolvated MH$^+$).

*6-[(2,2-Diphenylethyl)amino]-9-(N-ethyl-β-D-ribofuranosyluronamide)-N-(2-{N′-[1-(2-pyridyl)-4-piperidyl]ureido}ethyl)-9H-purine-2-carboxamide (**1**).* Methanesulphonic acid (4.72 kg, 49.1 mol) was added to a stirred slurry of acetonide **34c** (36.5 kg, 41.6 mol) in water (183 L), and the solution was heated at 70 °C for 10 h. Upon reaction completion, 1.5% residual starting material as monitored by acidic HPLC method, the reaction mixture was cooled to 20 °C and diluted with methyl acetate (355 L). The reaction mixture was washed with 5% w/w aqueous disodium hydrogen phosphate solution. The phases were separated, and the organic phase was washed once more with 0.5% w/w aqueous disodium hydrogen phosphate solution. The resultant water-wet methyl acetate solution of **1** was transferred to a speck-free vessel via a 1 μm suprapore polypropylene inline filter and was then azeotropically dried by distillation and replacement of the solvent (815 L in total) with portions of fresh methyl acetate until the residual water level was 2.2% w/w by Karl Fisher analysis. The crystallization solution was seeded and heated at reflux for a further 24 h whereupon crystallization occurred. The slurry was cooled to 22 °C and filtered, and the cake was washed with 2.4% water-

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wet methyl acetate (35 L) and pulled dry. The solid was transferred to a vacuum oven and dried in vacuo overnight at 50 °C to yield **1** as a white solid (25.3 kg, 32.5 mol, 78%), mp 190−192 °C; ^{1}H NMR (400 MHz, DMSO-d_6, 70 °C): δ 8.58 (1H, br s), 8.43 (1H, br s), 8.10 (1H, br t, J = 5.9 Hz), 8.04 (1H, dd, J = 5.1, 2.0 Hz), 7.73 (1H, br s), 7.43 (1H, ddd, J = 8.8, 6.9, 2.0 Hz), 7.37 (4H, d, J = 7.5 Hz), 7.24 (4H, t, J = 7.3 Hz), 7.12 (2H, t, J = 7.3 Hz), 6.71 (1H, d, J = 8.5 Hz), 6.53 (1H, dd, J = 7.1, 5.1 Hz), 6.00 (1H, d, J = 6.8 Hz), 5.85 (1H, br m), 5.81 (1H, d, J = 7.5 Hz), 5.41 (1H, d, J = 4.6 hz), 5.34 (1H, d, J = 5.9 Hz), 4.64 (1H, m), 4.57 (1H, t, J = 7.4 Hz), 4.29 (2H, m), 4.21 (1H, m), 4.01 (2H, br dt, J = 13.4, 3.4 Hz), 3.60 (1H, m), 3.33 (2H, br m), 3.24 (2H, br m), 3.19 (2H, m), 2.86 (2H, br t, J = 13.6 Hz), 1.74 (2H, m), 1.24 (2H, br ddq, J = 10.8, 3.4 Hz), 0.97 (3H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): δ 169.7, 163.9, 159.3, 158.5, 154.3, 153.1, 149.5, 148.1, 143.5, 142.1, 138.0, 128.8, 128.8, 126.8, 120.6, 112.9, 107.5, 87.9, 85.0, 73.5, 73.4, 50.8, 47.2, 44.8, 44.1, 41.5, 39.5, 34.0, 32.2, 15.2. HPLC (acidic method) retention time 2.36′. High res MS, ES m/z: 778.3776 obs (calc 778.3783) (MH^+).

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Notes

The authors declare no competing financial interest.

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