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Development of a Scaleable Process for the Synthesis of the A2a Agonist, UK-371,104

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Abstract:

The development and utilization of a scaleable process for the manufacture of the A2a agonist UK-371,104 (1) is described. Key steps in the synthesis include (i) a palladium-catalyzed cyanation reaction to prepare the nitrile 10, (ii) a telescoped conversion of the acid 11 to the glycosidation substrate 9, and (iii) the stereoselective coupling of 8 with 9 in a glycosidation reaction mediated by TMS triflate in 1,2-dimethoxyethane followed by conversion through to 1.

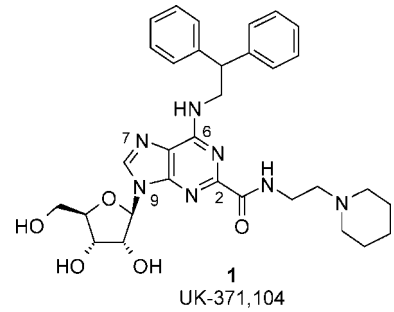

Figure 1. **Structure of UK-371,104 (1).**

Introduction

The search for novel anti-inflammatory agents has led to the discovery of a wide variety of biological targets that have provided medicinal chemists with opportunities to develop novel synthetic ligands of therapeutic relevance. The adenosine receptor is one such target. The A2a subtype has been the subject of significant interest in the search for drugs that agonize this receptor which could lead to new therapies for the treatment of diseases such as chronic obstructive pulmonary disease (COPD), for which there is a high unmet medical need.[1]

Colleagues in the Pfizer Discovery Chemistry Department at Sandwich undertook a program of research to identify A2a agonists for the inhaled treatment of COPD. Such compounds were designed to selectively agonize the A2a receptor sites on the surface of neutrophils in the lungs of patients. The delivery of the active agent directly to the site of action would require the successful candidate to be formulated for inhaled delivery, using dry-powder technology. A fundamental requirement for this formulation approach was for the active pharmaceutical ingredient (API) to possess a suitable crystalline solid form. Research in our Discovery Chemistry Department resulted in the identification of UK-371,104 (**1**, Figure 1), a crystalline nucleoside derivative, as a development candidate.[2]

UK-371,104 (**1**) is a functionalized nucleoside that is structurally related to adenosine and contains an unusual amide function at position 2 of the adenine ring together with a diphenylethylamino substituent at position 6. As the free base, UK-371,104 is a crystalline material with a melting point of 182 °C, and its physical properties are suitable for development as a dry-powder formulation without the need to resort to the preparation of a salt form. In order to supply preclinical and phase 1 clinical trials, it was necessary for us to find a scaleable process for the manufacture of multikilogram quantities of **1**. Initially we conducted a paper evaluation of the synthetic route used by our Discovery Chemistry colleagues (Scheme 1), considering key attributes and potential vulnerabilities if it were to be used as a basis for a large-scale manufacturing process.

The route was a linear sequence, using guanosine (**2**) as the starting material, that elegantly installed the key carbonyl function at position 2 on the adenine ring *via* palladium-catalyzed aminocarbonylation of the iodide **7** in the final step. The conversion of **2** to the 2-amino-6-chloro derivative **4** *via* the 2-amino-6-hydroxy derivative **3** was accomplished using the procedure published by Robins and Uznański.[3] Formation of the 2-iodo intermediate **5** followed the method described by Matsuda et al., employing a diazotization reaction.[4] The 6-chloro substituent of the iodide **5** was then selectively displaced by 2,2-diphenylethylamine to give the triacetate **6**, which was then deprotected to the carbonylation substrate **7**. This procedure was used by the Discovery Chemistry Department to provide UK-371,104 on multigram scale.

When considering the Discovery Chemistry route as a possible way to manufacture multikilogram amounts of **1** to supply clinical development, we felt that several aspects would cause significant problems on scale-up. The route lacked convergency, utilized potentially hazardous diazotization chemistry, and carried a potentially labile aryl iodide through two stages of the synthesis. In addition, the last stage of the process involved a palladium-catalyzed carbonylation reaction, which would have been technically challenging for us to carry out

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(1) Sullivan, G. W. *Curr. Opin. Invest. Drugs* **2003**, *4* (11), 1313.
(2) Mantell, S. J.; Monaghan, S. M. WO 0077018, 2000.
(3) Robins, M. J.; Uznański, B. *Can. J. Chem.* **1981**, *59*, 2601.
(4) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. *J. Med. Chem.* **1992**, *35*, 241.

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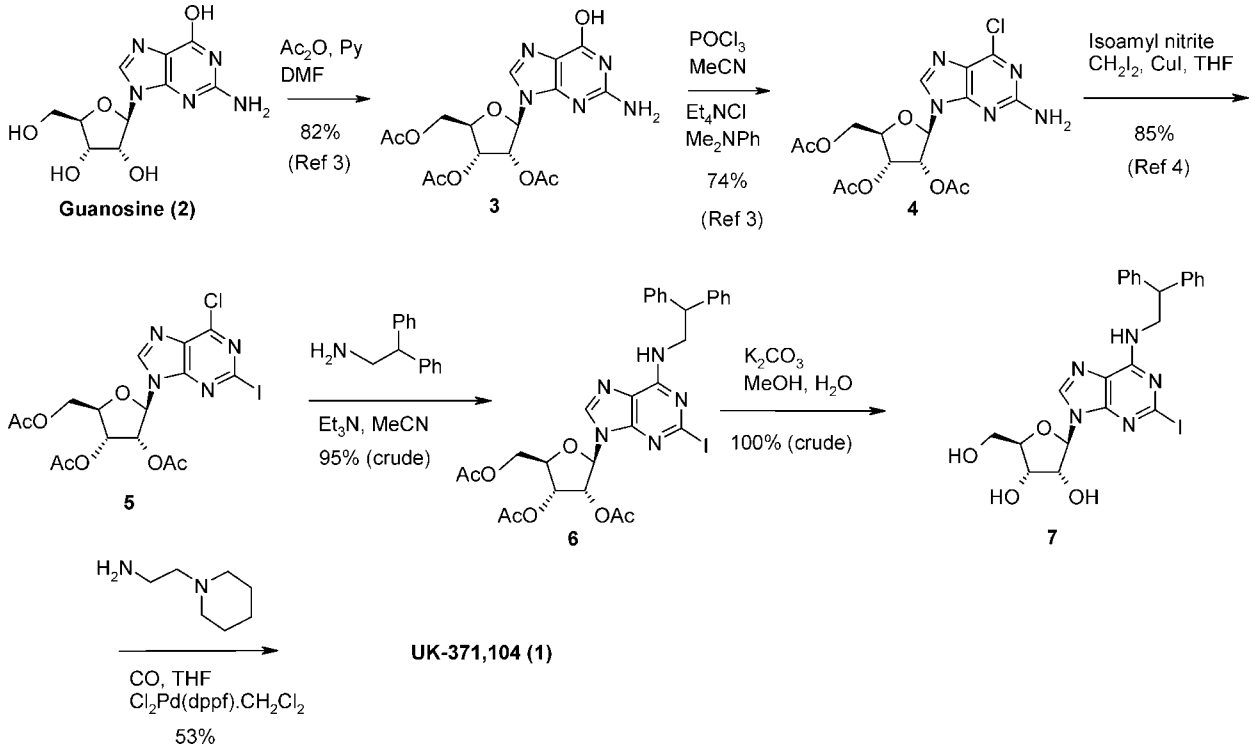
Scheme 1. **Discovery Chemistry synthesis of UK-371,104**



under cGMP in the facilities available to us at the time, and could also carry toxic palladium residues into the final API. We therefore elected to pursue a more convergent strategy for the large-scale manufacture of **1**, avoiding the issues associated with the Discovery Chemistry route, in order to support bulk demands for toxicology and clinical trials.

Results and Discussion

Retrosynthetic Analysis. Our retrosynthetic analysis for **1** is shown in Figure 2 and involves a late-stage, convergent coupling of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**) with an adenine unit **9** that is suitably functionalized at C-2 and C-6. Such glycosidation reactions are well-known in the literature to proceed with silylated heterocyclic bases under the influence of Lewis acids to afford products with high levels of stereoselectivity at the anomeric position on the sugar.[5] We elected to utilize the tetrahydropyranyl (THP)-protected adenine nitrile **10** as a precursor to **9** since a synthesis to this compound was known from our colleagues in Discovery Chemistry,[2] and its hydrolysis to the derived carboxylic acid **11** was expected to be straightforward. Disconnection of **10** then led back to commercially available 2,6-dichloropurine (**12**).

Synthesis of the Nitrile 10. In common with work carried out in the Discovery Chemistry Department on analogues related to **1**, we began our synthesis from 2,6-dichloropurine (**12**) as shown in Scheme 2. The steps to the protected chloroadenine **14** did not require any significant development prior to scale-up. Protection of the nitrogen at *N*-9 of the purine ring using a tetrahydropyran group was accomplished using the method of Robins et al. by treating 2,6-dichloropurine (**12**) with 3,4-dihydro-2*H*-pyran in ethyl acetate in the presence of catalytic *p*-toluenesulfonic acid at 50 °C.[6] The product **13** was isolated in 85% yield following workup and crystallization from ethyl acetate. Regioselective displacement of the 6-chloro substituent in **13** was accomplished by adaptation of the method of Bhakuni et al. by treating **13** with 2,2-diphenylethylamine at reflux in 2-propanol in the presence of Hünig's base.[7] The chloride **14**

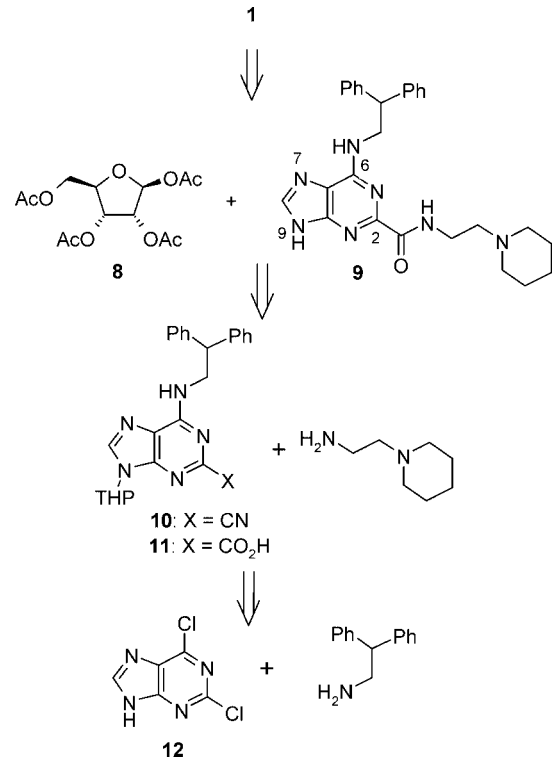
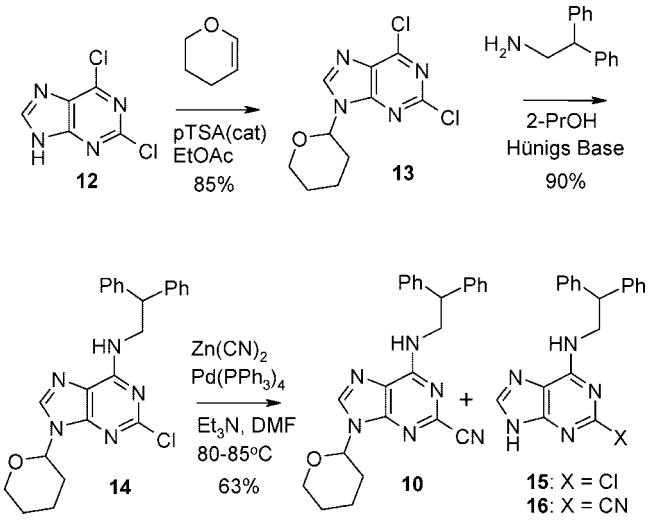


Figure 2. **Retrosynthetic analysis for UK-371,104 (1).**

(5) (a) Vorbrüggen, H; Ruh-Pohlenz, C. *Org. React.* **2000**, *55*, 1. (b) Vorbrüggen, H *Acta Biochim. Pol.* **1996**, *43* (1), 25. (c) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256.

Scheme 2. **Synthesis of the Nitrile 10**



was isolated in 90% yield by direct crystallization from the reaction mixture on cooling.

The method used by our colleagues in Discovery Chemistry to convert the chloride **14** to the nitrile **10** utilized a protracted sequence involving displacement of the chloro substituent by methanethiolate, followed by oxidation to the sulfone and subsequent displacement with cyanide.[2] We chose to follow a more direct method to convert the chloride **14** to the nitrile **10** by employing a palladium-catalyzed reaction with zinc cyanide using the method described by Gundersen.[8] We found that the best conditions were to use $Pd(PPh_3)_4$ (3 mol%), zinc cyanide (0.60 equiv) in DMF at 80−85 °C with triethylamine as a base to deactivate the acidic byproduct generated during the reaction. It was necessary to strike a balance between reaction conversion and the formation of impurities **15** and **16** arising from loss of the THP group from the starting material and product, respectively. A screen of alternative bases failed to lead to any improvement. Under optimum conditions on scale, the nitrile **10** was isolated in 63% yield as a crystalline solid. The key impurities present in the product were the chloride starting material **14** together with the THP-deprotected compounds **15** and **16**. The latter impurities in particular had the potential to negatively impact the phase separations carried out during aqueous workup of the subsequent step because of their very low solubility; thus, their presence in **10** required some degree of control.

Since **15** and **16** were much less soluble than **10**, they could not be easily removed by selective solvent extractions or recrystallization. The most effective way to remove these impurities from **10** was to dissolve the impure material in refluxing 2-propanol, then add a filter aid and filter the hot mixture. By carrying out this procedure, the amount of **15** and **16** in **10** could be controlled to acceptable levels.

Conversion of the Nitrile 10 to the Glycosidation Substrate 9. With the nitrile **10** in hand, we examined its conversion to the glycosidation substrate **9** (Scheme 3). Hydrolysis of the nitrile **10** to the carboxylic acid **11** using lithium hydroxide in a refluxing mixture of water and Industrial Methylated Spirit (IMS) gave the desired compound, but the formation of a very thick suspension of the lithium salt of the product made agitation difficult. Fortunately, substitution of lithium hydroxide for sodium or potassium hydroxide avoided this problem, and since sodium hydroxide gave a slightly cleaner reaction profile, this base was selected for further development.

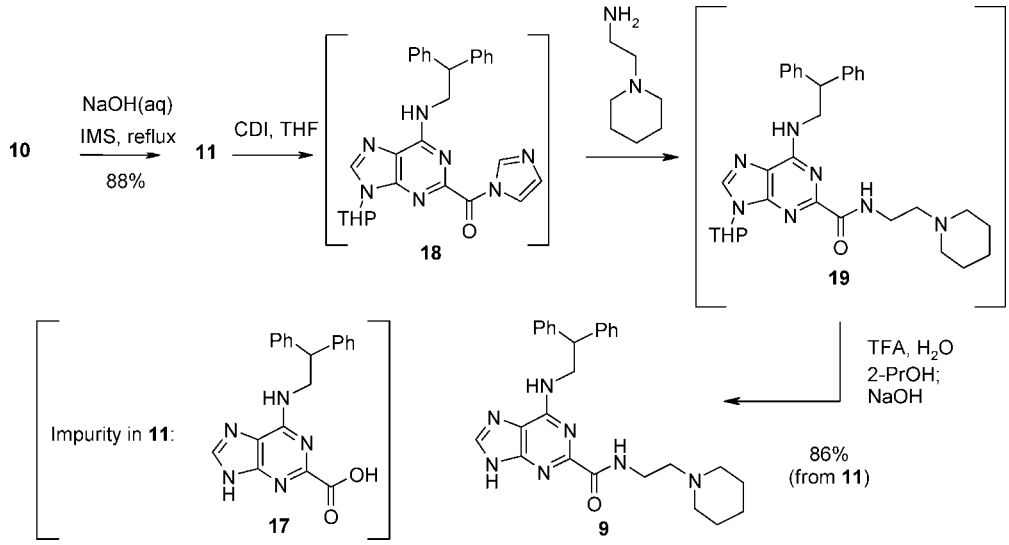
It was necessary to convert the sodium salt of the product **11** into the crystalline free acid since direct processing of a metal salt in the next step was found to be unsuccessful. In breaking the salt, we were concerned that prolonged contact of **11** with the low pH conditions required to generate the free acid could potentially result in loss of the THP group in the product, and we reasoned that this would be more likely in the presence of the IMS reaction solvent. Thus, we thought that it would be advantageous to isolate the sodium salt of the product **11** from the reaction mixture in order to remove it from the alcoholic solvent prior to acid treatment. We found that once the nitrile hydrolysis was complete, the solvent could be exchanged with water, leading to direct crystallization of the sodium salt of **11**, which could then be isolated by filtration and fed into the salt-break stage of the workup process.

The isolated sodium salt of **11** was then converted to the free acid of **11** which could be crystallized from ethyl acetate. Initially, it was found that suspending the sodium salt between water and dichloromethane and then adjusting the pH with hydrochloric acid effectively gave a solution of the free acid which could then simply be isolated *via* a solvent exchange from dichloromethane into ethyl acetate followed by crystallization. However, emulsions were commonly encountered during the extractive part of the process, leading to poor and time-consuming phase separations. The cause of this problem was found to be the presence of small amounts of solid material that was present in the extraction mixture. This solid was found to be the highly insoluble unprotected carboxylic acid **17**, which was thought to be derived from unprotected nitrile **15** present in the starting material **10** or from loss of the THP group in **11** during the acid treatment. As detailed earlier, we were able to suppress the level of the impurity **15** during the synthesis of the nitrile **10** but were unable to eliminate it completely. A screen of extraction solvents failed to find anything that was superior to dichloromethane in terms of phase separation. In cases where phase separation was impossible, we found that filtration of the mixture through a filter aid removed a sufficient amount of the insoluble fine solid to allow a stable interface to form.

Scale-up of this process in the laboratory using up to 180 g of the nitrile **10** gave the carboxylic acid **11** in 84−88% yield. Some problems were encountered during the crystallization of the sodium salt of the product where oiling was observed, but these were overcome by carrying out the initial crystallization at 50 °C following a slow cooling ramp from reflux temperature. Since the two batches of the nitrile **10** used contained relatively high levels of the insoluble impurity **16**, phase separations during the aqueous workup presented some challenges, and it was found necessary to filter mixtures through a filter aid in order to remove the insoluble materials. In one case this resulted

(6) Cassidy, F.; Olsen, R. K.; Robins, R. K. *J. Heterocycl. Chem.* **1968**, *5*, 461.
(7) Bhakuni, D. S.; Gupta, P. K.; Chowdhury, B. L. *Ind. J. Chem., Sect. B.* **1984**, *23*, 1286.
(8) Gundersen, L.-L. *Acta Chem. Scand.* **1996**, *50*, 58.

Scheme 3. **Conversion of nitrile 10 to glycosidation substrate 9**



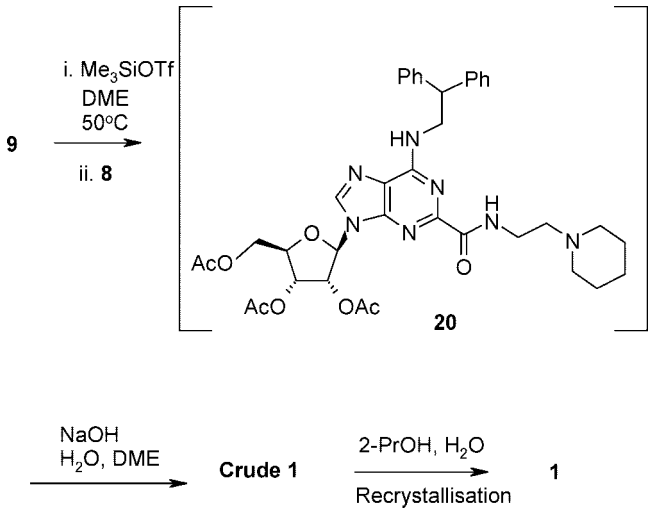
in initial loss of product on the filter aid, but we were able to recover this material by rewashing the filter cake. We also found that during the crystallization of the free acid of **11** from ethyl acetate, a very thick slurry was produced that was very difficult to stir. Fortunately, dilution with additional ethyl acetate gave mobile mixtures without negatively impacting the yield. Further scale-up in our kilo laboratory facility proceeded without major issue, leading to the isolation of two 1.7-kg batches of **11**, both in 86% yield, although the product was found to be less pure than previous batches made in the laboratory. We attributed this to the different source of the starting nitrile **10**, which was subsequently found to be impure. However, the impurities were found to purge well in the next stage of the synthesis. Once again the main processing problem was the phase separation, which took longer than expected upon scale-up to reveal a clean interface, but it was not necessary to pass the mixture through a filter aid.

The next stage of the process was to convert the carboxylic acid **11** to the glycosidation substrate **9**. At the outset of this work, we envisioned that the coupling of **11** with 1-(2-piperidinyl)ethylamine could be simply achieved by using *N,N′*-carbonyldiimidazole to activate the carboxyl function to give the intermediate acyl imidazolide **18** followed by addition of the amine. Using dry THF as the solvent, we found that this protocol worked well, but we did find that monitoring the formation of the acyl imidazolide **18** was somewhat troublesome. Initially, after screening various reagents to quench the active species prior to HPLC analysis, we elected to quench samples of the reaction mixture with concentrated aqueous ammonium hydroxide, and then examined the resultant carboxamide by reverse-phase HPLC. It is interesting to note that we did not find the competing reaction of the acyl imidazolide with water to be a significant problem using this method.

Unfortunately, the coupled product **19** was not crystalline and could only be isolated by evaporation to dryness to give a gum. However, the glycosidation substrate **9** was crystalline, and since this is the product of the next stage of the synthetic sequence, we elected to telescope the two steps together. Deprotection of THP groups in compounds such as **19** is generally carried out under aqueous acidic conditions. Using excess aqueous hydrochloric acid in IMS gave the desired product **9** as a hydrochloride salt, but this salt-form proved to have poor filtration properties and to be insufficiently soluble in organic solvents to make it useable directly in the subsequent step. Use of 2 equivalents of *p*-toluenesulfonic acid in aqueous ethanol also worked but suffered from similar issues. We also had concerns about the reaction of such strong acids with alcohols leading to the presence of genotoxic impurities[13] in the product **9**; thus, we proceeded to examine trifluoroacetic acid in aqueous 2-propanol. Whilst these conditions gave the monotrifluoroacetate salt of **9** as a monohydrate by a direct-drop process in 76% yield, we found that this form of **9** failed to react in the next step of the synthetic sequence. We therefore chose to directly break the salt and isolate the free base form from the reaction. This was accomplished by simple basification of the reaction mixture to approximately pH 10 using aqueous sodium hydroxide to give a slurry of the free base which could be filtered directly. Care was taken since under more basic conditions the solubility of the product in the solvent mixture increased, which we attributed to deprotonation at the ring nitrogen in **9**. Since the filtration rates of the slurries generated in this manner were slow, we found it to be advantageous to subject the slurry to a heat−cool cycle. This had the effect of modestly improving the rate of filtration, presumably through slightly increasing the particle size of the bulk material *via* an Ostwald ripening process.

Whilst it was pleasing to find that the acid **11** could be converted through to the glycosidation substrate **9** in good yield, we subsequently found that the free base of **9** was actually a monohydrate based on Karl Fischer analysis of the product. Since the next step in the synthetic route required the use of a very moisture-sensitive reagent (TMS triflate), we needed to find a way to remove the water from **9** prepared using this process. Prolonged drying reduced the water content in **9**, but not sufficiently to be useful. Poor solubility of **9** in water-immiscible organic solvents limited the opportunity to control water through an extractive workup. However, we found that recrystallizing hydrated **9** from *n*-propanol, incorporating azeotropic removal of water from the system by distillation, gave crystalline **9** free base that was suitable for use in the next

Scheme 4. **Conversion of the glycosidation substrate 9 to UK-371,104 (1)**



reaction. Testing of the product by Karl Fischer analysis indicated that the product still contained low levels of water (up to 1% w/w), which suggests that either **9** crystallizes from *n*-butanol with some retained water or that it slowly absorbs water on exposure to a moist atmosphere prior to testing. Although we were unable to fully examine the propensity of **9** to hydrate, low levels of water in **9** could be tolerated, and material made in this way performed acceptably well in the subsequent step, enabling us to proceed with our synthesis.

Scale-up of the conversion of the acid **11** to the glycosidation substrate **9** in the laboratory proceeded without issue, and two 249-g batches of **11** were processed to give a total of 457 g of **9** in an average yield of 77%. Two 1.7-kg batches of **11** were then processed in our kilo laboratory facility to give two 1.1-kg batches of **9** in an average yield of 62%. The lower yield obtained compared to the yields from the laboratory batches was attributed to the higher impurity burden present in the starting material (as mentioned previously), with the impurities being purged in this step.

Conversion of the Glycosidation Substrate 9 to UK-371,104 (1). The coupling of **9** with the peracetylated sugar **8** was viewed at the outset as being the most challenging step for the development of a scaleable process. Moreover, we had found that the coupled product **20** (Scheme 4) was not crystalline and could not be purified by nonchromatographic methods. Therefore a high-yielding and clean glycosidation process was sought so that this intermediate could be generated and then processed directly to **1** without any intervening purification.

There are many methods that have been used for the stereo- and regioselective attachment of sugar moieties to heterocyclic bases to give nucleoside derivatives, and they generally rely on the formation of an oxonium ion (or equivalent) through ionization at the anomeric position of a suitably activated sugar followed by addition of the heterocycle, often being rendered more nucleophilic via silylation.[5] The level of stereoselectivity formed at the new anomeric centre can be a problem in these reactions, but we were fortunate that peracylated ribose derivatives such as the commercially available tetraacetate **8** are well-precedented to undergo highly stereoselective addition of silylated heterocyclic bases under the influence of Lewis acids. With regard to regioselectivity (i.e., alkylation at *N*-7 vs *N*-9 of **9**) experience from our colleagues in Discovery Chemistry highlighted that selectivity for *N*-9 alkylation was complete in the glycosidation reactions of related adenine derivatives. In such cases, it appears that regioselectivity is controlled by the bulky diphenylethylamino substituent at the C-6 position sterically blocking the *N*-7 position towards reaction with the oxonium ion.

The protocol that is usually employed when attaching sugar units to heterocyclic bases is the presilylation of the heterocycle with a reagent such as hexamethyldisilazane or *N,O*-bis-trimethylsilylacetamide, followed by reaction with a peracylated sugar under the catalysis of a perfluoroalkyl triflate, which can be generated *in situ*.[9] As such, the Vorbrüggen modification of the Hilbert−Johnson method has found great utility in the preparation of nucleoside derivatives on preparative scale. We examined these conditions and other related methods for their potential to be used in the large-scale synthesis of **1**, but found that the reactions were capricious and often difficult to drive to completion. Of the conditions initially screened, we found that a combination of excess TMS triflate (to silylate **9** as well as promoting ionization of **8**) and DBU in acetonitrile[10] showed some promise, but further work highlighted that these conditions were not sufficiently robust to be considered as scaleable. A screen of a range of Lewis acids as alternatives to TMS triflate did not yield any useful alternatives.

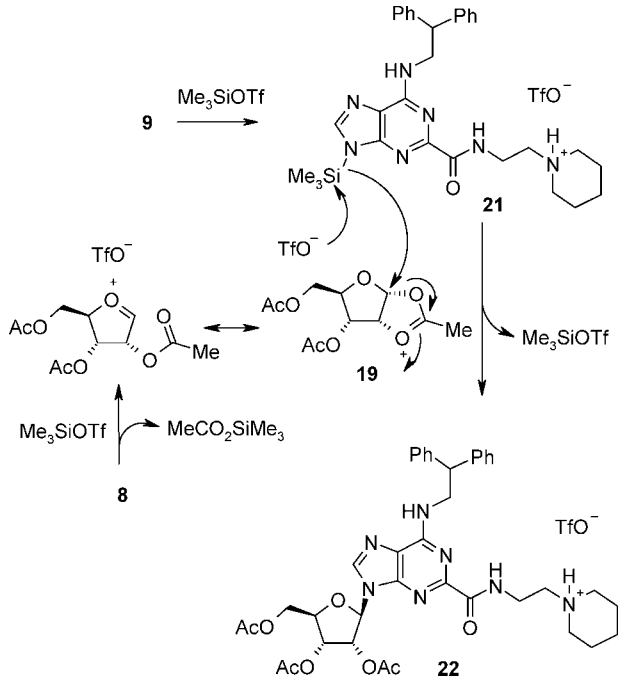
In a related project previously worked on by some colleagues in the Pfizer laboratories in Groton, U.S.A., a similar glycosidation process had been carried out by combining a heterocycle with stoichiometric amounts of TMS triflate in 1,2-dimethoxyethane (DME) followed by addition of an acetylated sugar derivative.[11] Our colleagues found that using DME as the solvent was uniquely good for this type of reaction. In our hands, the DME/TMS triflate combination performed extremely well, and despite DME being a nonideal solvent for large-scale manufacture, we found that its unique advantages outweighed the issues associated with its use on scale.

Treatment of a suspension of **9** in DME with 2 equiv of TMS triflate at 50−60 °C resulted in the formation of a clear yellow solution. Below 50 °C a solid crystallized out from the reaction mixture, which we presumed was the silylated triflate salt **21** (Scheme 5), although we did not isolate or characterize this intermediate. Subsequent addition of the peracetylated sugar **8** as a solution in DME resulted in the formation of the triflate salt **22** of the product over a period of a few hours at 50−60 °C. The reaction was very highly stereoselective, and we did not observe any of the α-anomer when analyzing the reaction mixtures or the downstream products in the synthetic route.

The use of alternative silylating agent/acid combinations in DME was briefly examined. Neither trimethylsilyl chloride/TMS triflate, hexamethyl disilazane/triflic acid, *N,O*-bis(trimethylsilyl)acetamide/$BF_3 \cdot OEt_2$ nor *N,O*-bis(trimethylsilyl)acetamide/$SnCl_4$ gave efficient reactions, but use of *N,O*-bis(trimethylsilyl)acetamide/triflic acid did give clean reaction

(9) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279.
(10) Kristinsson, H.; Nebel, K.; O'Sullivan, A. C.; Struber, F.; Winkler, T.; Yamaguchi, Y. *Tetrahedron* **1994**, *50*, 6825.
(11) Fox, D. E.; Scott, R. W. Unpublished results.

Scheme 5. **Working hypothesis for the mechanism of the glycosidation reaction**



product. However, since workup of such reactions would generate stoichiometric amounts of acetamide, a category 2B carcinogen that would need to be controlled to ppm levels in the API, we elected to develop and scale the process using TMS triflate alone.

Our working hypothesis for the mechanism of the glycosidation reaction is shown in Scheme 5. We assumed that **9** was silylated at *N*-9 to give the intermediate **21** which then reacts with the stabilized oxonium ion **19** to deliver the triflate salt **22**. The high level of stereoselectivity for the β-anomer obtained through the Lewis acid-promoted reaction of silylated heterocycles with acyl-protected ribose derivatives such as **8** is well precedented and is thought to be a consequence of the stabilizing acyloxy group shielding the α-face of the sugar ring.[5] Attack of the incoming nucleophile occurs from the top face to give the desired diastereoisomer, resulting in TMS triflate being regenerated. In theory, one equivalent of TMS triflate is required to silylate **9**, and then a catalytic amount would then be required to ionize **8** to the oxonium ion. However, in practice we found that charges of less than 2 equiv tended to lead to incomplete reactions, at least on laboratory scale. It was not clear whether the second equivalent of TMS triflate was necessary to install a second silyl group onto the purine heterocycle (e.g., on the diphenylethylamino nitrogen), or whether the extra reagent was required merely to overcome the presence of adventitious water that would inevitably be present in the small-scale reactions that were run during laboratory development of this step.

Following neutralization of the triflate salt **22**, we wished to develop a workup process that avoided the need to isolate the noncrystalline glycosidation product **20**. Concurrent with this work we were developing the final API isolation process and found that, whereas the final target **1** was poorly soluble in dry water-miscible organic solvents, the presence of small amounts of water dramatically increased solubility. Continued addition of water to the solution then resulted in recrystallization of **1**, even from solvents such as DME. This led us to consider the possibility that **20** could be hydrolyzed directly after the glycosidation step, thereby giving **1** that could be crystallized from the reaction mixture by the addition of water. It was found that **1** only dissolved sufficiently well in two solvents, *tert*-amyl alcohol and dichloromethane, to allow some sort of extractive workup to be considered. However, use of dichloromethane resulted in the formation of emulsions, and *tert*-amyl alcohol gave poor recoveries following workup and crystallization.

The conversion of pure isolated **20** to **1** under transesterification conditions (e.g., EtOH/EtONa) was a clean reaction, and the UK-371,104 (**1**) could be crystallized directly from the reaction mixture, which suggested to us that this type of direct-drop approach might be useful on scale. However, for such a process to be used on reaction mixtures from the glycosidation stage, at least two additional equivalents of base would be needed to quench the triflic acid/TMS triflate present in the glycosidation reaction mixture. In addition, it was likely that removal of at least some of the DME would be required to ensure that the concentration of the alcohol solvent in the mixture was high enough to ensure reaction completion, given that transesterification is an equilibrium process. Disappointingly, when a reaction mixture from the glycosidation step was quenched with ethanol and then treated with sodium ethoxide, no crystallization of **1** occurred despite a clean reaction profile. These factors, together with a desire to subject the glycosidation reaction to some sort of aqueous quench and neutralization to deactivate any residual TMS triflate or triflic acid generated prior to subsequent processing led us to consider an alternative approach.

Conversion of purified **20** to **1** was clean and rapid under standard hydrolytic conditions in DME, requiring at least 3 equiv of dilute NaOH to remove the acetate groups. Interestingly, use of aqueous K_2CO_3 or aqueous Na_3PO_4 was not as effective as NaOH in these reactions. Following completion of the hydrolysis reaction, dilution of the resulting mixture in DME with water afforded crystalline UK-371,104 that was filtered from the mixture in approximately 60% yield over the two stages from **9**. When more concentrated NaOH (5 M) was used, it was noticed that two liquid phases formed, which was presumably a consequence of the high ionic strength of the aqueous phase. This suggested to us that it might be possible to remove aqueous-soluble impurities from the process by carrying out a phase separation, and that this might lead to a more reliable crystallization step when the organic phase was diluted with water.

Having demonstrated that this process was viable using isolated **20**, it was then assessed using unquenched reaction mixtures obtained from the glycosidation step. Once the formation of **20** was complete, the reaction was quenched by the addition of 5 M NaOH. It was found that 7.5 equiv of NaOH were required, and measurement of the pH of the reaction mixture demonstrated that a pH greater than 13 units was required in order for the reaction to reach completion. We were initially anticipating that approximately 5 equiv of NaOH would be required, calculated from the 3 mol needed for the hydrolysis of the three acetate groups plus 2 mol required to quench the triflic acid generated from the 2 equiv of TMS triflate used in

the glycosidation reaction. The extra two equiv of NaOH appear to be consumed by the two of trimethylsilyl alcohol (pK_a = 11) that are also formed in the reaction.[12]

After stirring the reaction until the hydrolysis was complete, the aqueous phase (which was a relatively small volume compared to the organic phase) was removed, and the organic solution was diluted with 2 vol of water (relative to the charge of DME). After the mixture warmed to 50 °C with stirring, UK-371,104 (**1**) crystallized from the mixture and was isolated by filtration and was dried to give the product in 75−95% yield over the two steps from the glycosidation substrate **9**, with acceptable quality. We therefore concluded that this approach was viable for scale-up, and our attention turned to developing a method to purify crude **1** generated by this process to give clinical quality API.

Purification of UK-371,104 API. Although we had no scale-up experience and no detailed purity data for the formation of crude **1** as detailed above, we considered that it was unlikely that the material so-formed would be of suitable quality for use in regulatory toxicology studies and clinical trials. We were concerned about residual levels of the DME together with low-level impurities and the potential presence of insoluble particulates. We therefore devised a method to dissolve crude **1**, filter the resulting solution, and then crystallize the product to give clinical quality material.

The solubility of **1** was low in all ICH class 3 solvents. However, the observation that the addition of water to water-miscible solvents dramatically increased solubility led us to devise a system whereby crude **1** was dissolved in a 20% v/v mixture of water in 2-propanol. The resulting solution was filtered to remove particulate matter, and the water was removed from the mixture by azeotropic distillation. On cooling, the product crystallized and, following filtration and drying, afforded clinical-grade **1** in approximately 85% isolated yield on laboratory scale. Use of other solvents in this step was found to give less reproducible crystallizations, and we therefore selected this process for use on scale.

The conversion of the glycosidation substrate **9** through to UK-371,104 **1** was scaled-up, first in the laboratory and then in our kilo laboratory facility. In the laboratory, the process successfully gave two 200-g batches of crude **1** in an average 77% yield from **9**, with no major processing issues. However, two minor problems did arise during the reaction. First there was some precipitation of inorganic materials during the hydrolysis stage, although these were easily removed with the aqueous phase. Second, the crystallization of **1** was problematic, due to initial product deposition as an oil on cooling to 30 °C. Fortunately, reheating the mixture to 50 °C resulted in crystallization, and the product was isolated without any impact on yield.

Upon scale-up into our kilo laboratory facility further problems were encountered, which we largely attributed to the lower quality of the TMS triflate that was used. During the development of the process in the laboratory we had always used TMS triflate that was purchased in sealed glass ampoules, and this material was always a colorless or pale-yellow liquid. However, the reagent used for kilo-scale manufacture was received in a dark amber bottle and was found to be a black liquid just prior to being used in the manufacturing campaign. A sample of this material was tested in a laboratory-scale reaction and was found to work as expected, with no obvious impact on impurity profile although, not surprisingly, the reaction mixtures were significantly darker than was usual. In the plant, the bond-forming chemistry proceeded as expected using the black TMS triflate, apart from the dark reaction color, but additional problems were encountered during the workup. In the first batch that was run, no liquid−liquid phase separation occurred following the hydrolysis with 5 M NaOH, although it was noticed that some solid had precipitated on the walls of the reactor vessel. After removing the solids, the solution was diluted with water and was heated, but the product did not crystallize over the time period expected. Fortunately, prolonged agitation of the mixture did result in crystallization, and the crude **1** was isolated by filtration. In the second batch, the liquid−liquid phase separation this time did occur, but again crystallization of the product required prolonged stirring. A total of 1.7 kg of crude **1** was isolated in an average 61% yield, but both batches were a dark brown color and a new impurity was visible by thin-layer chromatography. Since the quality of these two batches was so poor, we did not anticipate that taking it directly into the API recrystallization step would provide sufficient cleanup. We therefore decided to reslurry the combined batches in a 1:1 mixture of DME and water, and this procedure returned 1.5 kg of **1** in 94% yield with significant purge of impurities and improvement to the color of the product. This material was then processed without issue through the aqueous 2-propanol filtration/recrystallization process to give 1.28 kg of clinical quality **1** with 85% recovery.

Summary

We have developed a scaleable route to the prototype inhaled A2a agonist UK-371,104 (**1**) that overcame the issues associated with the method used during medicinal chemistry research. Key steps in the synthesis are (i) a palladium-catalyzed cyanation reaction to prepare the nitrile **10**, (ii) a telescoped conversion of the acid **11** to the glycosidation substrate **9**, and (iii) the highly stereoselective coupling of **8** with **9** mediated by TMS triflate in DME and conversion through to clinical quality **1**. The route was developed on laboratory scale, and then scaled into a kilo-scale facility where 1.28 kg of API was manufactured. Further scale-up of this process beyond a few kilograms of **1** would require additional research in order to develop more effective solutions to some of the problems described in this account. An improved synthesis of the acid **11** that avoided the processing issues associated with the cyanation/hydrolysis protocol would be advantageous. In addition, further process development of the glycosidation and API crystallization steps would further enhance process robustness and efficiency for larger-scale manufacture.

Experimental Section

All reactions involving air-sensitive reagents were performed under dry nitrogen. Melting points are uncorrected. 300 MHz ^{1}H NMR spectra were recorded using $CDCl_3$ as the solvent

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unless otherwise indicated. Chemical shifts are reported in ppm (δ) relative to residual protons in the deuterated solvent. All materials obtained from commercial suppliers were used without further purification.

2,6-Dichloro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (13). To a suspension of 2,6-dichloropurine (1.00 kg, 5.29 mol) in ethyl acetate (10 L) was added *p*-toluenesulfonic acid monohydrate (8.0 g, 0.042 mol), and the resultant mixture was heated to 50 °C under an atmosphere of nitrogen. To the stirred mixture was added 3,4-dihydro-2*H*-pyran (750 mL, 8.25 mol) over a period of 2 h causing the reaction temperature to increase from 50 to 62 °C. During the addition, the solids dissolved to give a clear solution. The reaction was then stirred for a further 30 min, and was then allowed to cool to ambient temperature over 18 h. The resultant solution was washed with water (2 L then 1 L), concentrated to a low volume, and was seeded to induce crystallization. After being left to crystallize at ambient temperature overnight, the solid was collected by filtration and was dried at 50 °C under vacuum to give **13** (1.27 kg, 88%) as a colorless solid, mp 122−124 °C. A second crop of **13** (0.14 kg, 10%) was obtained on cooling the filtrate. ^{1}H NMR δ: 8.34 (1H, s), 5.77 (1H, dd), 4.21 (1H, br d), 3.77 (1H, br t), 2.25−1.58 (6H, m).

2-Chloro-*N*-(2,2-diphenylethyl)-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-6-amine (14). To a suspension of **13** (0.93 kg, 3.42 mol) in 2-propanol (10.3 L) under an atmosphere of nitrogen was added diisopropylethylamine (0.96 mL, 5.5 mol) and 2,2-diphenylethylamine (0.705 kg, 3.57 mol), and the resultant suspension was heated to reflux for 3 h to give a clear solution. The solution was then cooled to ambient temperature, and some seed crystals were added to induce crystallization. The mixture was then stirred at −10 °C for 18 h, and the solid was collected by filtration. The filter cake was washed with 2-propanol (2 × 1 L), and the solid was dried at 50 °C under vacuum to give **14** (1.33 kg, 90%) as a colourless solid, mp 117−119 °C. ^{1}H NMR δ: 7.90 (1H, br s), 7.42−7.16 (10H, m), 5.90 (1H, br s), 5.69 (1H, d), 4.43−4.07 (4H, m), 3.78 (1H, br t), 2.19−1.52 (6H, m).

6-[(2,2-Diphenylethyl)amino]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine-2-carbonitrile (10). The chloride **14**, (1.10 kg, 2.54 mol), *N,N*-dimethylformamide (4 L), triethylamine (0.814 L, 5.84 mol), zinc cyanide (0.184 kg, 1.57 mol), and tetrakis(triphenylphosphine)palladium(0) (0.092 kg, 0.08 mol) were charged to a stirred autoclave, and the contents were purged with argon and then pressurized with argon to 10 bar. The contents were heated to 80−82 °C with stirring for 21 h, and the reaction was cooled to ambient temperature. The reaction mixture was added to deionized water (20 L) over 15 min with stirring to give a suspension. Stirring was continued for a further 0.5 h, after which time, the solid was collected by filtration. The damp filter cake was suspended in 2-propanol (10 L), and the suspension was heated to reflux with stirring. The mixture was then filtered through a filter aid pad whilst still hot, and the filtrate was allowed to cool, with stirring, to ambient temperature over 16 h, during which time crystallization occurred. The solid was collected by filtration, washed with 2-propanol (2 L), and was dried *in vacuo* at 50 °C. The solid so obtained was combined with material obtained from similar experiments, and the combined material was recrystallized from 2-propanol, including a hot filtration step, in a similar fashion to that described above. A total of 7.10 kg (16.4 mol) of the chloride **14** was converted to a total of 4.40 kg (63%) of **10** using this process. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (1H, s), 7.40−7.20 (10H, m), 6.00−5.75 (1H, br s), 5.70 (1H, d), 4.40−4.20 (3H, m), 4.20−4.10 (1H, m), 3.80−3.70 (1H, m), 2.20−1.90 (3H, m), 1.90−1.60 (3H, m); LRMS (AP−) *m/z* $[M - H]^-$ 423.

6-[(2,2-Diphenylethyl)amino]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine-2-carboxylic Acid (11). To a solution of **10** (1.89 kg, 4.45 mol) in industrial methylated spirits (IMS, 8.3 L) was added aqueous sodium hydroxide (1.9 L of a 6.6 M solution, 12.5 mol), and the mixture was heated at reflux for 2 h at which point the reaction was complete. Approximately 4.3 L was distilled from the reaction mixture at atmospheric pressure, and deionized water (4.3 L) was then added. This operation was repeated, removing 4 L of distillate and then adding 4.5 L of water. The mixture was cooled to 25 °C over a period of 5 h during which time the sodium salt of the product crystallized to give a thick slurry. The mixture was stirred at 25 °C for 9.5 h and cooled to 5 °C, and the solid product was isolated by filtration and washed with a mixture of water (1.4 L) and IMS (0.2 L). The damp product was suspended in a stirred mixture of dichloromethane (10.6 L) and water (10.6 L), and the pH of the aqueous phase was adjusted to a stable pH of between 1.2 and 1.4 by the addition of concentrated hydrochloric acid (approximately 0.55 L) whilst maintaining good agitation. The phases were allowed to separate, and the aqueous layer was extracted with dichloromethane (2.5 L). The combined organic phases were washed with water (4 L) and were concentrated to an approximate volume of 5 L by distillation at atmospheric pressure. Ethyl acetate (11.5 L) was added, and distillation was continued until the vapour temperature reached 73 °C during which time the mixture became a thick slurry. Additional ethyl acetate (8 L) was then added, and the mixture was cooled to 25 °C over 14 h. The mixture was then cooled to 6 °C, and the solid product was collected on a filter, washed with ethyl acetate (1.5 L), and dried at 60 °C under vacuum to give **11** as a colourless solid (1.70 kg, 86%), mp 155 °C dec. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10 (1H, s), 7.40−7.10 (10H, m), 6.30 (1H, br s), 5.90 (1H, d), 4.50−4.20 (3H, m), 4.15 (1H, br d), 3.80 (1H, br t), 2.20−1.60 (6H, m). LRMS (AP−) *m/z* $[M - H]^-$ 442.

6-[(2,2-Diphenylethyl)amino]-*N*-[2-(1-piperidinyl)ethyl]-9*H*-purine-2-carboxamide (9). To a mixture of **11** (1.70 kg, 3.83 moles) in dry THF (17 L) was added *N,N′*-carbonyldiimidazole (0.75 kg, 4.60 moles), and the mixture was stirred for approximately 3 h at 22 °C. The mixture was then cooled to 10 °C, and a solution of 1-(2-aminoethyl)piperidine (0.59 kg, 4.60 mol) in dry THF (0.7 L) was added to the reaction mixture over a period of 0.5 h whilst maintaining the temperature below 20 °C. The mixture was stirred for approximately 1 h at 20 °C, treated with deionized water (0.1 L), and then concentrated by distillation under a slight vacuum to remove approximately 12 L of solvent. To the residue was then added 2-propanol (22 L), and distillation was continued until the reaction temperature reached 83 °C. The volume of the mixture

was then adjusted to approximately 21 L by the addition of 2-propanol (4 L), and the mixture was cooled to 20 °C. Deionized water (10.6 L) and trifluoroacetic acid (2.6 kg, 18 mol) were added, and the resultant mixture was heated at reflux for approximately 2 h. The reaction mixture was cooled to 20 °C, and then the pH of the mixture was adjusted to between pH 9.8 and 10.0 by the addition of aqueous sodium hydroxide (1.5 L of 40% w/w solution) with the temperature below 30 °C and good agitation. The resultant slurry was heated to reflux, cooled to 25 °C over 8 h, and then cooled further to 8 °C. The solid was collected by filtration, and the filter cake was washed twice with 2-propanol (2 L each wash). The damp product was then suspended in 1-propanol (54 L), and the mixture was heated to reflux to give a solution. Approximately 27 L of the solvent was then removed from the mixture by distillation at atmospheric pressure. The mixture was then cooled to 25 °C over approximately 4 h, and then cooled further to 6 °C. The solid was collected by filtration, and the filter cake was washed with 1-propanol (6 L). The solid was dried at 60 °C under vacuum to give **9** as a colourless solid (1.14 kg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 15.25 (1H, br s), 8.55 (1H, br s), 8.30 (1H, s), 7.40−7.15 (10H, m), 5.90 (1H, br s), 4.50−4.25 (3H, m), 3.60 (2H, q), 2.55 (2H, t), 2.50−2.30 (4H, m), 1.50−1.20 (6H, m). LRMS (AP+) *m*/*z* [MH^+] 470.

6-[(2,2-Diphenylethyl)amino]-*N*-[2-(1-piperidinyl)ethyl]-9-(β-D-ribofuranosyl)-9*H*-purine-2-carboxamide (1). To a stirred suspension of **9** (200 g, 0.426 mol) in anhydrous 1,2-dimethoxyethane (DME, 800 mL) under an atmosphere of nitrogen was added a solution of trimethylsilyl trifluoromethanesulfonate (200 g, 0.900 mol) in anhydrous DME (200 mL) over a period of 15 min. During the addition, the solid dissolved to give a deep red/amber solution, and the reaction temperature rose from 20 to 31.5 °C. The resultant mixture was heated to 55−60 °C, and a solution of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**, 163 g, 0.512 mol) in anhydrous DME (400 mL) was added over a period of 40 min, followed by a rinse with anhydrous DME (200 mL). The reaction mixture was heated at 60 °C for 3 h, and was then allowed to cool to ambient temperature. This crude reaction solution, containing the triacetate **20**, was held at ambient temperature for 18 h, and then treated with an aqueous solution of sodium hydroxide (640 mL of a 5 M solution, 3.2 mol) over a 45 min period with cooling. The resultant mixture was stirred at ambient temperature for 3 h, and the layers were separated. The stirred organic phase was diluted with deionized water (1800 mL) with cooling, and the resultant mixture was then heated to 50−55 °C whereupon crystallization started. To this heated and stirred suspension was added more deionized water (1800 mL) over a period of 50 min. Once the addition was complete, the resultant slurry was cooled to 10 °C over a period of 45 min, and the solid was collected by filtration. The filter cake was then washed with a solution made up from DME (400 mL) and deionized water (800 mL), and was then dried at 55 °C *in vacuo* to give the crude **1** as a brown solid (203 g). This material was combined with material obtained from similar processes and was purified in the following manner. To a suspension of crude **1** (398 g, 0.661 mol) in 2-propanol (7.05 L) was added deionized water (1.76 L), and the resultant mixture was stirred and warmed until a clear solution was obtained. The resultant solution was filtered, and the filtrate was distilled under nitrogen at atmospheric pressure with periodic addition of filtered 2-propanol to maintain the reaction volume. Over the course of the distillation, a total of 29.1 L of distillate was collected, and a total of 26.1 L of filtered 2-propanol was added. Towards the end of the distillation, the amount of water present in the distillate was measured by Karl Fischer analysis to be <0.5% w/w. The mixture was allowed to cool to 40 °C over 3.5 h with stirring, during which time crystallization occurred. The resultant slurry was stirred at ambient temperature for 12.5 h, and cooled to 2 °C in an ice bath over 5.5 h. The solid was collected by filtration, washed with chilled, filtered 2-propanol (2 × 1.5 L), and dried at 60 °C *in vacuo* to give **1** as a pale beige-coloured solid (306 g), mp 182 °C, that was found to contain 0.63% w/w (20 mol %) of water by Karl Fischer analysis. ^{1}H NMR (500 MHz, d^6-DMSO) δ: 8.50 (1H, br t), 8.40 (1H, s), 8.00 (1H, br t), 7.35 (4H, d), 7.26 (4H, t), 7.15 (2H, t), 5.91 (1H, d), 5.39 (1H, d), 5.14 (1H, d), 5.06 (1H, t), 4.64−4.50 (2H, m), 4.28−4.18 (2H, m), 4.18−4.10 (1H, m), 3.96−3.90 (1H, m), 3.70−3.61 (1H, m), 3.60−3.50 (1H, m), 3.46−3.37 (2H, m), 2.50−2.44 (2H, m, partly obscured by DMSO peak), 2.40−2.32 (4H, m), 1.46−1.38 (4H, m), 1.36−1.28 (2H, m). LRMS (AP+) *m*/*z* [MH^+] 602. Anal. Calcd for $C_{32}H_{39}N_7O_5 \cdot 0.2\ H_2O$: C, 63.50; H, 6.56; N, 16.20. Found: C, 63.55; H, 6,56; N, 16.27.

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