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Scalable Synthesis of a Nucleoside Phosphoramidate Prodrug Inhibitor of HCV NS5B RdRp: Challenges in the Production of a Diastereomeric Mixture

Benjamin A. Mayes,* Jingyang Wang, Jeevanandam Arumugasamy, Kannan Arunachalam, Erkan Baloglu, David Bauer, Alan Becker, Narayan Chaudhuri, Roberta Glynn, G. Mark Latham, Jie Li, Jinsoo Lim, Jia Liu, Steve Mathieu, F. Patrick McGarry, Elodie Rosinovsky, Adrien F. Soret, Alistair Stewart, and Adel Moussa

Idenix Pharmaceuticals Inc., 320 Bent Street, Cambridge, Massachusetts 02141, United States

Supporting Information

ABSTRACT: A scalable process is described for the synthesis of 2′-*C*-methylguanosine-5′-[2-[(3-hydroxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzylphosphoramidate], a nucleotide prodrug inhibitor of hepatitis C virus NS5B polymerase. The route features the use of phenylboronic acid to transiently protect the 2′,3′-hydroxyls of 2′-*C*-methylguanosine under mild conditions. The requirement to produce a 1:1 phosphorus diastereomeric mixture precluded the use of traditional crystallization techniques. High sensitivity of the drug substance to acidic and basic conditions and its preferred solubility in mixed aqueous−organic solvents presented additional processing challenges. The use of reverse phase chromatography for the final purification was eliminated by the development of a dual liquid−liquid extraction protocol, which removed both non-polar and polar impurities whilst maintaining the 1:1 diastereomeric ratio. Ethylene sulfide, a potential genotoxic impurity that was observed at significant levels in the original procedure, was controlled to <10 ppm in the final product. This process produced 20 kg of drug substance in 59% overall yield, with >99% purity in the requisite 1:1 diastereomeric ratio.

INTRODUCTION

2′-*C*-Methylguanosine-5′-[2-[(3-hydroxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzylphosphoramidate] (IDX184, **1**) is a nucleoside phosphoramidate prodrug inhibitor of hepatitis C virus (HCV) which has reached Phase 2 clinical trials (Figure 1).[1] Post oral administration, metabolism in the liver to the nucleoside monophosphate, followed by sequential phosphorylation by cellular kinases, produces 2′-*C*-methylguanosine triphosphate (2′-MeG-TP, **2**), which is a potent inhibitor of HCV NS5B RNA-dependent RNA polymerase (RdRp) *in vitro* (1b WT IC_{50} = 0.292 μM).[2] The etiology of hepatitis C infection and the development of direct-acting antiviral treatments, in particular nucleotide prodrug inhibitors of HCV RdRp, are thoroughly documented.[3] As an advanced clinical candidate, a practical synthetic route was required to enable production of IDX184 with high purity on a multikilogram scale.

An atypical active pharmaceutical ingredient (API), **1** presented multiple synthetic and processing challenges. Foremost, as an *S*-acyl-thioethyl benzylphosphoramidate prodrug of 2′-*C*-methylguanosine monophosphate, **1** is a mixture of phosphorus diastereomers in a 1:1 ratio. In addition to the anticipated differences in physical properties between the separate R_p and S_p isomers, **1** displayed a somewhat amphiphilic nature whereby the polar nucleoside was counterbalanced by the relatively more lipophilic prodrug. As a result, the solubility of **1** in either pure aqueous or organic systems was generally low, and aqueous/organic mixtures were required to achieve appreciable solubilites.[4] Notably, from a handling perspective, the API typically coagulated and became sticky prior to full solubilization on dissolution from dry powder.

Although the *in vitro* HCV replicon assay activities of the separate R_p and S_p diastereomers were similar (within 3-fold), the pure separated isomers did indeed display marked physical differences: for example, in contrast to the S_p isomer, the enriched R_p isomer was reluctant to crystallize in a range of solvent systems and was only ever isolated as an amorphous solid by evaporation to dryness; however, the relatively well behaved S_p isomer was amenable to the growth of single crystals suitable for structural determination by X-ray analysis (Figure 2).

In addition to these physical characteristics, the fragile nature of the prodrug moiety imparted problematic chemical characteristics, in particular, sensitivity to both acid and base. Even trace levels of acid or base were determined to be detrimental in the presence of a protic solvent, typically resulting in the formation of the free 2′-*C*-methylguanosine nucleoside **3**, the nucleoside 5′-monophosphate, the simple benzylphosphoramidate of 2′-*C*-methylguanosine, or a nucleoside phosphorus triester via solvolysis of one or both of the *S*-acyl-thioethyl or benzylphosphoramidate moieties.

Additionally, chemical degradation of **1** was accompanied by the generation of ethylene sulfide (ES), which under stressed conditions was observable even by ^{1}H NMR (10^3 ppm). ES is classified as a potential genotoxic impurity (PGI), and as such, its formation as a process impurity or degradant was a key issue

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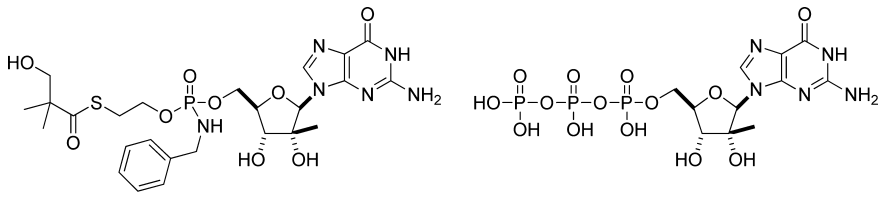


Figure 1. IDX184 (**1**) and 2′-MeG-TP (**2**).

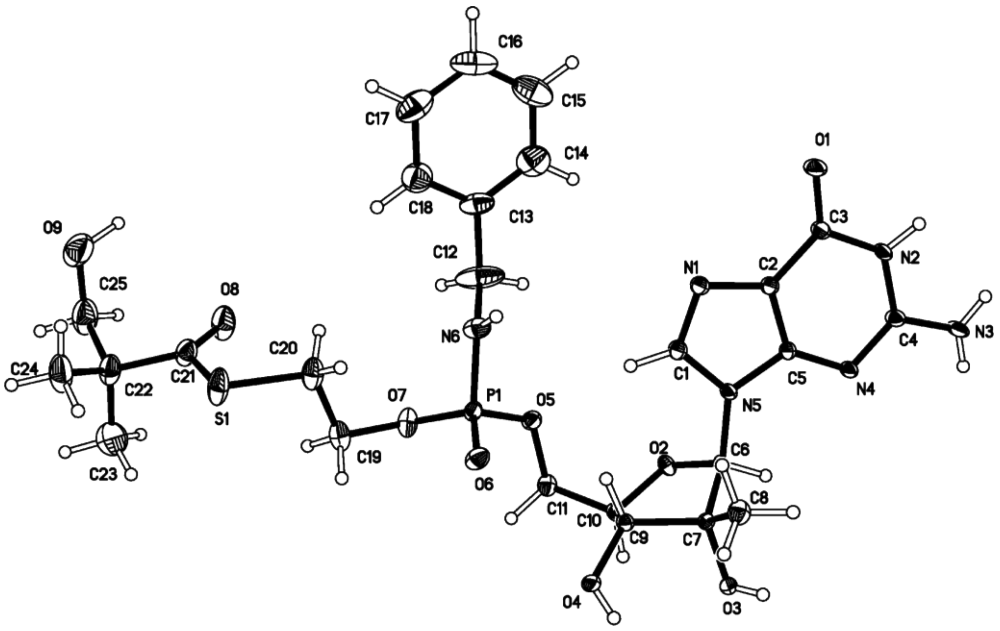
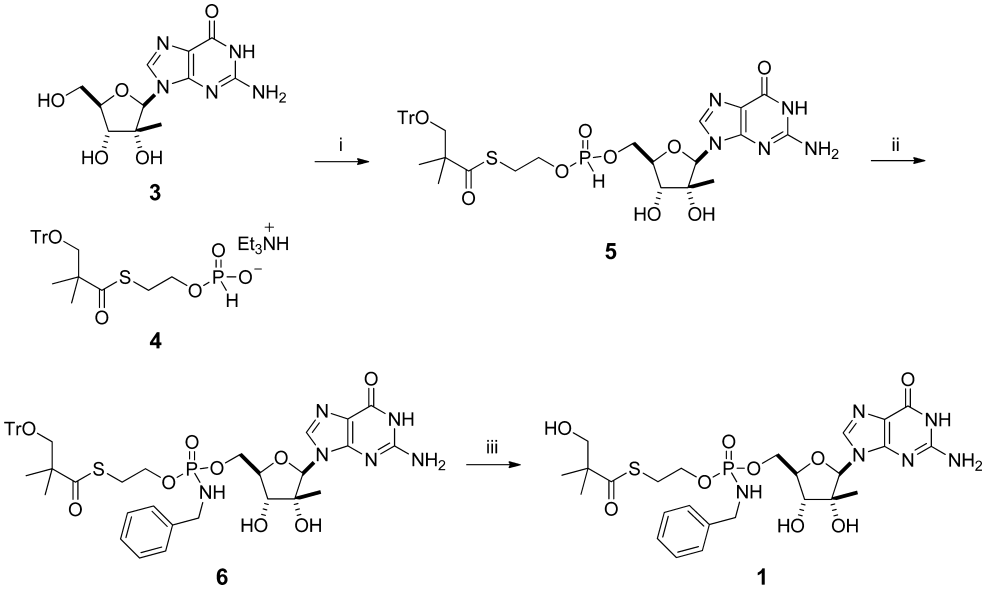


Figure 2. ORTEP drawing of the X-ray crystal structure of S_p diastereomer, showing crystallographic numbering system. Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

Scheme 1. Discovery Chemistry Synthesis of Prodrug 1[a]



[a]Conditions: (i) a) Me_3CCOCl, pyridine, −15 °C; b) SiO_2 chromatography (32%); (ii) a) $BnNH_2$, CCl_4, 5 °C; b) SiO_2 chromatography (99%); (iii) a) TFA, DCM, 25 °C; b) SiO_2 chromatography; c) C-18 chromatography; d) lyophilization (39%).

in the isolation of final API suitable for use in prolonged clinical trials.

This article describes the development of the synthetic process utilized to produce **1** on a 20 kg scale. Various

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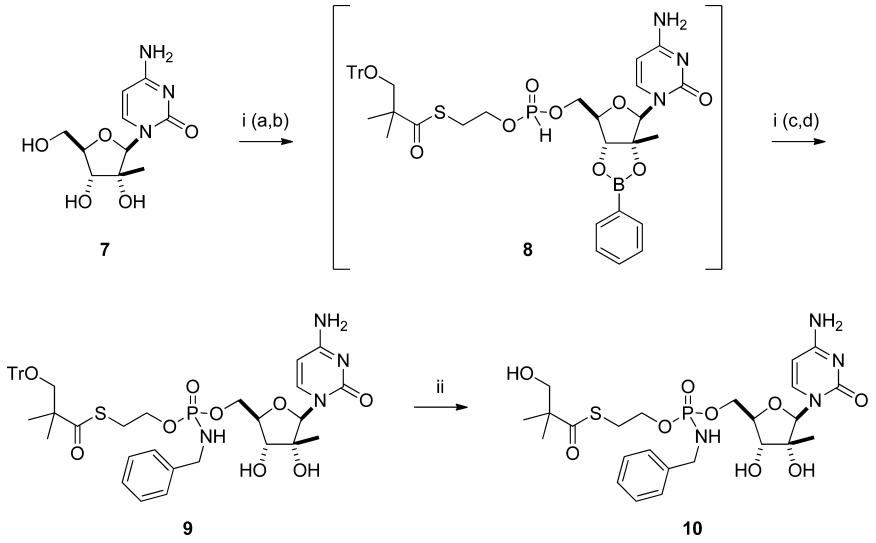
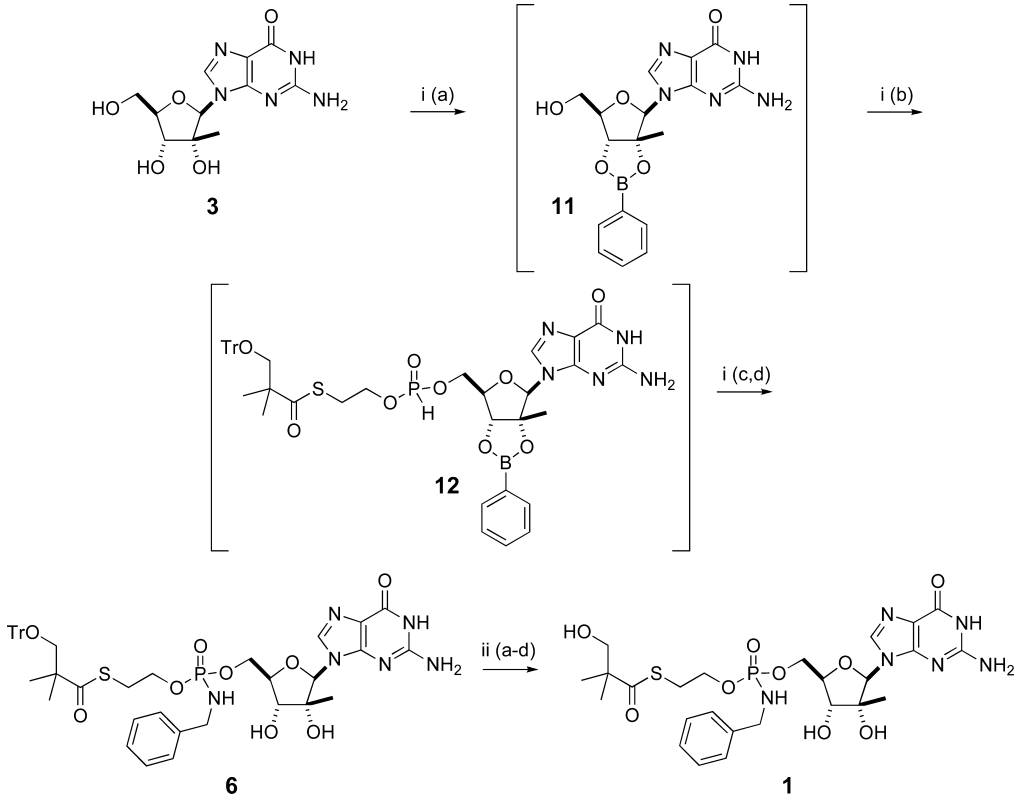
Scheme 2. Synthesis of Analogous 2′-*C*-Methylcytidine Prodrug 10 Using PBA Transient Protection[a]



[a]Conditions: (i) a) $PhB(OH)_2$, pyridine, reflux; b) H-phosphonate **4**, EDCI·HCl, MeCN; c) $BnNH_2$, CCl_4; d) 20% w/w citric acid (aq) (53%); (ii) AcCl, EtOH (61%).

Scheme 3. Synthesis of Prodrug 1 on Small Scale Using PBA Transient Protection[a]



[a]Conditions: (i) a) $PhB(OH)_2$, MeCN, Na_2SO_4 (s, 3.5 equiv), reflux; b) H-phosphonate **4**, Me_3CCOCl, pyridine; c) $BnNH_2$, CCl_4; d) 22% w/v citric acid (aq) (66%); (ii) a) AcCl (2 equiv), EtOH, 60 °C; b) $NaHCO_3$(s) neutralization; c) MTBE trituration; d) C-18 chromatography (65%).

challenges encountered with this unusual API are highlighted, along with the methods which were implemented for their circumvention.

RESULTS AND DISCUSSION

Synthetic Approaches. Two syntheses of phosphoramidate prodrug **1** or its separate diastereomers have been reported on a small scale (<1 g). The original procedure used in discovery allowed isolation of the first milligram quantities of **1** in three steps from 2′-*C*-methylguanosine **3** (Scheme 1).[5] The unprotected nucleoside was initially coupled with H-phosphonate salt intermediate **4** in low yield (32%). Although transformation of H-phosphonate **5** to phosphoramidate **6** was relatively efficient, subsequent terminal trityl group deprotection with TFA was not, and the final prodrug **1** was obtained

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in a mere 12% overall yield from **3**. Silica gel chromatography was employed at all steps, and additionally, the target phosphoramidate **1** underwent chromatography on reverse phase silica gel to achieve acceptable purity, followed by lyophilization.

The second synthesis described access to the individual diastereomers of **1**, in which a racemic benzyl-pentafluorophenyl-phosphoramidate intermediate was formed prior to phosphorus enantiomer separation and subsequent coupling with 6-*O*-methyl-2′-*C*-methylguanosine using ^{t}BuMgCl-mediated 5′-hydroxyl activation.[6] In this case, coupling yields with the two separated enantiomeric intermediates were a disparate 59 and 36%, respectively, which potentially indicated that formation of **1** in a 1:1 ratio at phosphorus may be problematic via this approach. Additionally, the formation of the key racemic pentafluorophenyl intermediate in low yield (38%) and the use of 6-*O*-methyl-protected **3**, which necessitated an extra methoxypurine-to-guanine conversion, made this methodology unattractive to pursue.

Preceding investigations into this 2′-*C*-methylguanosine prodrug, an analogous nucleotide prodrug inhibitor of HCV NS5B RdRp, **10**, was under development, the synthesis of which employed the use of phenylboronic acid (PBA) as an effective transient means to protect the 2′,3′-hydroxyls of 2′-*C*-methylcytidine **7** (Scheme 2).[7] This approach was determined to be superior to the use of either unprotected or acetonide-protected 2′-*C*-methylcytidine. Although this PBA methodology was only demonstrated on a 100 g scale, its application to the synthesis of **1** was appealing: only two synthetic steps were required from two advanced building blocks, **3** and **4**, both of which were readily accessible on a multi-kilogram scale.[8]

PBA Route. The procedure developed to produce phosphoramidate **1** is shown in Scheme 3. Protection of nucleoside **3** with PBA was performed in refluxing MeCN in the presence of anhydrous sodium sulfate to sequester the 2 mol of water generated during the condensation. Whereas with 2′-*C*-methylcytidine **7** either MeCN or pyridine was utilized as solvent to achieve high conversions (>97% by ^{1}H NMR), in this case, MeCN was preferred in order to avoid the use and subsequent evaporation of large volumes of pyridine on scale. Regioselectivity of the boronate formation was confirmed by 2D NMR, and the hydrolytic behavior of 2′-*C*-methylguanosine-2′,3′-cyclic boronate was determined to be similar to that of the analogous cytidine, indicating its suitability for use as a transient protecting group. In order to avoid premature hydrolysis of the cyclic boronate **11**, an additional charge of Na_2SO_4 was performed prior to the addition of H-phosphonate salt **4** (1.5 equiv) dissolved in MeCN. Coupling was then effected via a mixed phosphorus anhydride intermediate on addition of a solution of pivaloyl chloride (5 equiv) in pyridine (10.5 equiv) to a chilled mixture of boronate-protected nucleoside and **4**, generating the H-phosphonate **12**. Coupling using pivaloyl chloride/pyridine at <15 °C, or alternatively the carbodiimide reagent EDCI at 40 °C, required in both cases similar stoichiometries (5 equiv) for good conversion: in contrast to the cytidine analogue, in this case the more cost-effective and simpler to handle acid chloride was preferred for use on scale.

Whereas in the original discovery synthesis the unprotected H-phosphonate intermediate **5** was isolated, here the boronate-protected H-phosphonate **12** was used *in situ*: the addition of benzylamine followed by carbon tetrachloride successfully resulted in rapid conversion to the benzylphosphoramidate via an Atherton−Todd reaction.[9] This switch in order of addition relative to the original route was determined to be highly beneficial, as charging the benzylamine second invariably led to significant levels (∼10%) of the undesired P-OH side product prior to installation of the phosphoramidate. Performed in this manner, the Atherton−Todd reaction efficiently and reproducibly provided a 1:1 mixture of diastereomers at phosphorus by in-process LC analysis.

On addition of aqueous citric acid and MTBE, the PBA protection was completely and rapidly hydrolyzed *in situ*, revealing the phosphoramidate **6**. Sequential mild aqueous washes at pH 4 and pH 8 allowed removal of pyridine, pivalic acid, benzylamine, and PBA-related byproducts whilst maintaining product integrity. Isolation of **6** from the resultant organic layer was then achieved on addition of further MTBE, which induced precipitation of both product diastereomers. It was typically observed that the R_p diastereomer was more reluctant to precipitate than the S_p; however, on prolonged stirring, the phosphorus isomer ratio invariably met the required 45:55 specification.[10] Filtration of the precipitated solids and washing with the next process solvent provided **6** with >95% LCAP[11] purity with a 1:1 diastereomer ratio in 66% yield from nucleoside **3**. As with 2′-*C*-methylcytidine **7**, this PBA transient protection methodology resulted in a 2-fold increase in coupling yield relative to the analogous coupling with the 2′,3′-hydroxyls unprotected.

It was immediately evident from the discovery chemistry procedures that removal of the terminal trityl protecting group using TFA/DCM was impractical due to a high level of product degradation. Similar to the process with the analogous 2′-*C*-methylcytidine prodrug **9**, dry HCl was instead found to be a superior alternative. Accordingly, treatment of **6** with 2 equiv of dry HCl, generated via reaction of acetyl chloride with ethanol, promoted complete deprotection of the trityl group at 60 °C in 0.5−1 h. Neutralization to pH 5−6 was performed on addition of solid $NaHCO_3$, thus avoiding aqueous systems, and after filtration through Celite, the filtrate was evaporated to a solid prior to trityl byproduct removal on trituration with MTBE.

Purification of the crude solid **1** (typically 80−85% LCAP) was then undertaken using chromatography on C-18 silica gel (11:1 w/w silica:crude ratio). The crude material was dissolved in a mixture of THF, water, and saturated aqueous $NaHCO_3$, to ensure the impure **1** was loaded onto the column at a relatively neutral pH.[12] Elution was performed stepwise with seven increasingly non-polar solvent systems from 1.5 to 25% MeCN/water (∼300 vol total).[13] A large volume of high aqueous content systems was required to remove multiple close-running impurities. Both diastereomers eluted in the same system (25% MeCN/water); however, the R_p diastereomer eluted somewhat earlier than the S_p, and accordingly, judicious fraction selection by LC analysis was required to achieve high chemical purity whilst maintaining the desired 1:1 phosphorus isomer ratio. After evaporation of the appropriate MeCN/water fractions, the target prodrug **1** was obtained in 65% yield with >98% LCAP purity in 1:1 diastereomer ratio.

The overall yield of **1** from nucleoside **3** of 40−45% compared favorably with the 12% yield via the original unprotected approach, and this PBA methodology was utilized to produce the first kilogram batches of **1**. Despite this improvement, multiple challenges were observed which needed to be addressed to enable production of larger quantities:

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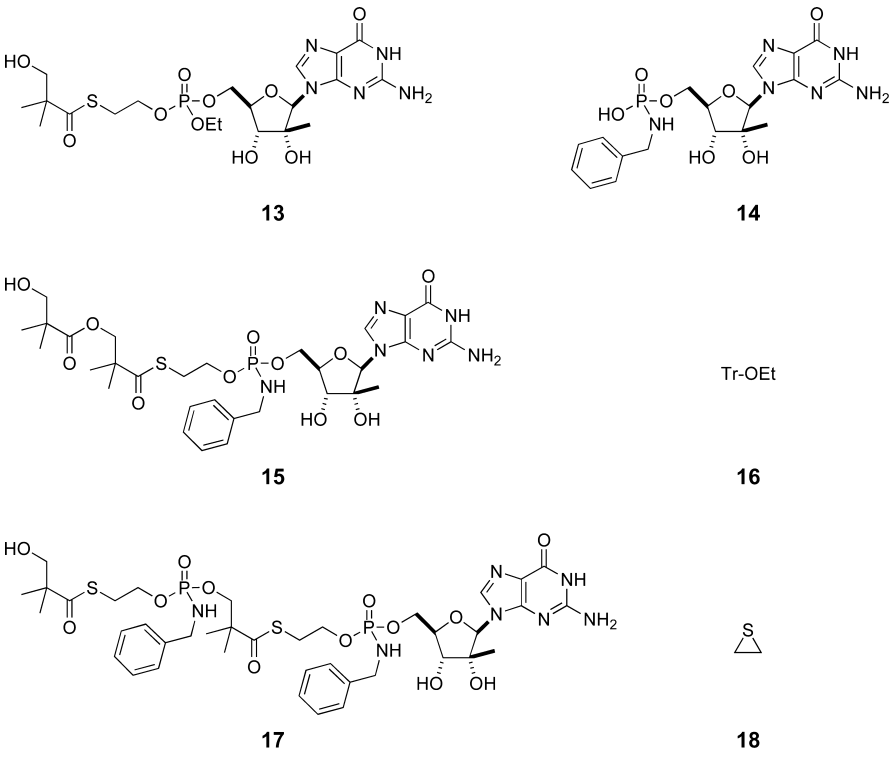

Figure 3. Major impurities observed in deprotection of **6**.

(1) It was imperative that the initial boronate protection proceed to completion. In one instance where the agitator speed was too high, a significant portion of the nucleoside **3**/MeCN suspension was determined to have coated the walls of the reactor prior to reaction with PBA, leading to a non-representative in-process protection sample. The subsequent coupling step consequently performed poorly (~30% LCAP), which negatively impacted both the quantity and quality of **6**.

(2) Although it is utilized only as a reagent (12 equiv), the use of the class 1 solvent CCl_4 in the Atherton–Todd reaction was not ideal from regulatory or environmental perspectives.

(3) The AcCl/EtOH deprotection was vastly superior to the original TFA protocol; however, it suffered from the concomitant generation of P-OEt impurity **13** at up to 10% LCAP, dependent upon the amount of acid, temperature, and reaction time. This impurity was not trivial to remove by chromatography, due to its polarity being similar to that of **1**, which partially dictated the methodical approach to the elution systems.

(4) Neutralization of the *in situ* generated HCl on completion of the deprotection reaction by solid $NaHCO_3$ was far from efficient, and the subsequent filtration through Celite on scale proved to be slow.

(5) Processing of the ethanolic filtrate by concentration to dryness and trituration of the resulting sticky solid to remove the trityl byproduct was not feasible for larger production equipment.

(6) The use of column chromatography as a final purification method presented multiple practical hurdles. Because it was determined that normal phase silica gel was not suitable to provide effective separation of impurities, chromatography on reverse phase (RP) silica gel was utilized, which required evaporation of large volumes of aqueous/organic product solutions at elevated temperatures. Even trace levels of acid, from incomplete $NaHCO_3$ neutralization, could induce product degradation on prolonged distillation of the elution solvents. The RP chromatography itself was not efficient nor reproducible, as based on the quality of the input crude material, product fraction selection was dictated by both chemical and diastereomeric purity to achieve a 1:1 isomer ratio. Indeed, in one instance, RP chromatography was completely ineffective, as the product was not retained on the column, eluting in the first 1.5% MeCN/water system. It was determined that the use of heptane in place of MTBE in the precipitation of **6**, in order to achieve a more precise 1:1 diastereomer ratio, had also precipitated an unidentified non-UV-active material which negatively impacted the performance of the final purification. Additionally, RP silica gel brand variability and accessibility on scale were further hindering factors, as was the fact that 50% of the estimated cost of goods for **1** was due to the RP silica gel itself.

(7) The existing chromatographic purification and subsequent evaporation to dryness of product fractions resulted in the observation of ES at significant levels (range 400–3000 ppm) in the final API **1**.[14] Because ES is a PGI, these levels prohibited the utilization of several batches of **1** for clinical trial durations longer than 1 month at the proposed 200 mg daily dose.[15] A protocol was therefore required to reduce the ES level whilst maintaining the overall chemical purity and 1:1 diastereomeric ratio. Based on the observation that ES levels did not increase over time on storage,[16] it was determined that, if **1** were produced with acceptable levels of ES at $t = 0$ h, then prolonged shelf life would not present an issue.

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(8) The final physical form of **1** obtained on evaporation to dryness presented some degree of diastereomeric inhomogeneity, presumably due to the different precipitation rates of the two isomers and their non-uniform distribution within the resultant amorphous API.

Accordingly, multiple modifications of the existing PBA methodology were required for scale up to fixed equipment.

Scale Up Challenges. *Purification and Isolation of 1.* It was apparent that a new deprotection, workup, and isolation protocol was required to remove various process impurities whilst avoiding the use of RP chromatographic purification, evaporation to dryness procedures, and the resultant formation of sticky non-homogeneous solids. Typical impurities observed after the standard trityl deprotection reaction conditions included various product-related species of polarity higher and lower than that of **1**, along with the three-membered ring ethylene sulfide **18**, which was the impurity of most immediate concern (Figure 3). International Agency for Reseach on Cancer (IARC) monographs on the evaluation of carcinogenic risks to humans categorize ES into Group 3, "the agent is not classifiable as to its carcinogenicity to humans"; however, for the purposes of this program, ES was treated as a confirmed genotoxic impurity (GTI).[17] The existing FDA[18] and EMA[19] guidances on approaches to deal with GTIs have recently been augmented by ICH guideline M7,[20] in which recommendations for assessment of actual and potential mutagenic impurities in final drug substances are outlined. Under this framework, ES was assigned to Class 3, categorized as an impurity which exhibited an alerting structure, unrelated to the drug substance, without mutagenicity data. In this case, a control strategy was pursued in order to produce **1** with ES at levels below the generic threshold of toxicological concern (TTC). The anticipated clinical trial duration for IDX184, as with related direct-acting antiviral HCV therapeutics, was 1−3 months, which dictated a maximum acceptable ES intake of 20 μg per day. At the intended 200 mg daily dose, this corresponded to an acceptable ES level of up to 100 ppm in the final drug substance **1**.

Initially, various potential methods to reduce ES content in **1** were investigated. Despite the volatility of ES (bp 56 °C), extended vacuum drying of the amorphous powder at 40 °C did not achieve target reductions. Silica-supported amine and thiol nucleophlic scavengers were unsatisfactory and, in the case of the amine functionality, actually promoted degradation of **1**. Conversely, either Ar or N_2 degassing of aqueous/organic product solutions at 40 °C, followed by repeated co-evaporation of **1** with EtOH/water 1:1 mixtures or spray-drying under mild conditions (outlet temperature 60−100 °C), resulted in acceptable ES levels (<100 ppm) on gram scale.

Additionally, a liquid−liquid extraction approach was explored whereby the poor solubility of **1** in aqueous or organic solvents was modulated on introduction of a cosolvent, which was selected to effect partition of **1** into the predominantly organic layer, whilst maintaining phase separation from the predominantly aqueous system. Accordingly, a mixture of EtOAc, MeCN and water in 6:5:3 volumetric ratio exhibited suitable properties and phase separation was observed to be rapid when conducted between 25 and 40 °C. Extraction of impure **1** (92% LCAP, 3000 ppm ES) was demonstrated on 100 g scale and after evaporation of the "organic" phase, which contained ∼20% of the input water, followed by coevaporation with EtOH/water, **1** was obtained with >90% mass recovery. Initial ES analysis indicated a substantial reduction (158 ppm) and subsequent further EtOH/water coevaporation actually surpassed target ES levels (30 ppm). An improvement in LCAP purity to 98.3% occurred, and importantly, the 1:1 phosphorus diastereomer ratio had been unaffected.

Encouraged by the 2-fold benefit of this extractive purification, namely a >95% reduction in ES and removal of polar impurities into the aqueous layer, this protocol was pursued. In the short term it was determined to be advantageous as an effective means to extract **1** from water/MeCN product fractions post RP chromatography. On addition of EtOAc, the organic phase containing EtOAc and MeCN (and water) was separated, thus reducing the water distillation volume by 80−90%. Based on the fact that **1** can remain dissolved in MeCN/water 1:1 mixtures at 10% w/v, it was envisioned that selection of a suitable low-polarity organic solvent may enable the inverse product partition, forcing **1** into the "aqueous" layer. Toluene or EtOAc/heptane were determined to be feasible, allowing extraction of multiple low-polarity impurities into the respective organic phases. Toluene was adjudged to be more effective, and on a small scale, a mixture of toluene, MeCN, and water in 2:3:2 volumetric ratio was demonstrated on neutralized crude **1** to produce a suitable phase separation, removing the low-polarity impurities **15**, **16**, and **17** and unreacted **6** whilst maintaining a 1:1 diastereomer ratio in the resultant aqueous layer. This second extractive purification eliminated the existing problematic MBTE trituration to remove the trityl group byproduct **16**, which had resulted in the formation of sticky solids with difficult handling properties.

The P-OEt side product **13** was the remaining impurity of concern, as it was not efficiently purged by either the EtOAc/MeCN/water or the toluene/MeCN/water extractions, due to its polarity being similar to that of product **1**. Formed as a side product by acidic ethanol solvolysis of **1**, modification of the trityl deprotection reaction to be conducted under milder conditions was deemed to be the most feasible method to achieve the desired specification of <0.50% LCAP for the sum of both diastereomers.[21] Accordingly, the amount of dry HCl generated on addition of AcCl to EtOH was reduced from 2 to 1.5 equiv, and the amount of EtOH was substantially reduced by utilizing MeCN as a cosolvent in 1:1 MeCN/EtOH volumetric ratio. Lowering the reaction temperature from 60 to 40 °C substantially decreased the deprotection rate, accommodating longer processing times on increased scale. In place of solid $NaHCO_3$, the reaction mixture was treated with Dowex 66 basic resin, thus avoiding the slow filtration through Celite and reducing the potential for incomplete neutralization.

Altogether, these modifications to the deprotection reaction and workup, along with implementation of dual liquid−liquid extractive purifications, allowed isolation of **1** without RP chromatography on 50 g scale with 99.6% LCAP purity, 0.19% LCAP **13**, in 1:1 diastereomeric ratio. Subsequent trituration of the agglomerated solids in IPAc at 40 °C resulted in a significantly more homogeneous, easily filtered amorphous powder, which maintained the same 1:1 diastereomer ratio. Final analysis showed an ES level of 12 ppm; thus, this procedure formed the basis of a process for scale up to fixed equipment.

Production of 1 in Fixed Equipment. The initial boronate protection was performed on 8 kg of nucleoside **3**, with PBA and solid Na_2SO_4, suspended in refluxing MeCN. After 4.5 h,

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^{1}H NMR in-process analysis indicated 99% conversion to the desired cyclic boronate **11**. The second charge of solid Na_2SO_4 was omitted on this scale, as boronate hydrolysis was determined to be negligible on proceeding directly to the subsequent reaction with H-phosphonate **4**.

Coupling using pivaloyl chloride/pyridine was performed at 8−17 °C as for the smaller scale runs, and after 2.5 h, analysis indicated the LCAP ratio of P-H intermediate to **3** was 7.65:1, at which point the reaction was considered complete.[22] On addition of benzylamine, the batch thickened but remained stirrable, and after activation with CCl_4, the conversion of P−H intermediate **12** to the boronate-protected benzylphosphoramidate completed with >99% conversion.

Cleavage of the cyclic boronate with aqueous citric acid and dilution with MTBE yielded three phases, a lower aqueous phase, a middle phase containing solids, and an upper organic phase, from which the lower aqueous was separated. After being washed with aqueous $NaHCO_3$/NaCl, the organics were twice diluted with MTBE and partially distilled to remove residual water (and MeCN), which was determined to negatively impact the performance of the subsequent filtration. Addition of further MTBE induced precipitation of both phosphorus diastereomers: after 7 h, the R_p:S_p ratio was within specification at 0.459:0.540; however, on stirring for a further 25 h, the ratio of R_p:S_p was a more satisfactory 0.476:0.524. Filtration of the solids, washing with MTBE and EtOH, and vacuum drying at 45 °C produced 20.7 kg of trityl-protected **6** with 97.7% LCAP, 0.41 wt % water, 0.19 wt % MBTE and 1.4 wt % EtOH, in 85% purity adjusted yield.

Deprotection of **6** was performed on 20 kg scale using the new reaction conditions developed to minimize the level of P-OEt **13** side product, with the modification that the reaction temperature was further reduced as a conservative measure, from 40 to 25 °C. The LCAP ratio of **1** to **6** was 2.64:1 at 5 h, 6.55:1 at 8 h, and 11.3:1 at 11 h, and the P-OEt **13** LCAP increased from 0.24% to 0.38% to 0.52% over the same time points.[23] The reaction was quenched at 11 h to avoid further formation of this impurity. Two charges of Dowex 66 resin neutralized the mixture to target pH 6, and after filtration to recover the resin, the product filtrate purity was 88.1% LCAP.

Extractions with the toluene/MeCN/water system to remove impurities with RRT > 1, along with residual starting material **6**, were performed first, after distillation of 90% of the filtrate solvents. A 1.0:1.9:1.4 overall volumetric ratio of toluene/MeCN/water was utilized, and after separation of the lower aqueous phase, which contained ∼20% of the total MeCN charged, analysis indicated 97.7% LCAP purity with 0.465:0.535 R_p:S_p diastereomer ratio. A second extraction of lower polarity impurities from the aqueous layer was performed with a 1.0:1.4:2.4 overall volumetric ratio of toluene/MeCN/water. This second aqueous phase, which contained ∼25% of the total MeCN charged, was determined to have a slightly improved purity (97.9% LCAP). The R_p:S_p ratio after the second toluene extraction was unchanged at 0.465:0.535, as was the total amount of P-OEt impurity **13** at 0.55% LCAP, with contributions of 0.35% and 0.20% LCAP from its respective diastereomers. Analysis of the toluene/MeCN extracts indicated that the total amount of **1** contained in the first and second organic phases was 6.5% of the theoretical product yield.[24]

The MeCN/water product solution was reduced in volume by half prior to removal of impurities with RRT < 1 on extraction of the product **1** with the EtOAc/MeCN/water system. A 2.8:1.0:1.3 overall volumetric ratio of EtOAc/MeCN/water produced a clearly defined separation of phases, and an organic product layer, containing EtOAc and MeCN with an estimated 5% of the total water charged, was obtained with 98.9% LCAP and 0.466:0.534 R_p:S_p diastereomer ratio. The P-OEt **13** impurity was partially retained in the aqueous layer, reducing the total level to 0.38% LCAP. A second extraction of product from the aqueous phase was performed using a 2.8:1.0:1.1 overall volumetric ratio of EtOAc/MeCN/water, and a second organic product layer, which contained EtOAc and MeCN with approximately 10% of the total water, was separated. This product layer was significantly more dilute than the first and contained 0.70% LCAP of P-OEt **13**, 98.5% LCAP purity of **1** with 0.425:0.548 R_p:S_p diastereomer ratio. The two mixed organic product phases were combined, and analysis of the residual aqueous solutions indicated 4.9% of the theoretical product **1** yield remained unextracted.

A second deprotection reaction performed on the same scale of input **6** (21.7 kg) proceeded in an identical manner, and the two sets of organic extracts were combined to be further processed as one batch (98.7% LCAP average). At this point, prior reactions on smaller scale had involved distillation of the product solutions to dryness before co-evaporating with EtOH/water to ensure low ES content and triturating with IPAc. As these operations were not practical on larger scale, a solvent exchange of the EtOAc/MeCN[25] extracts to isopropanol was performed, which ensured complete removal of residual solvent and water, prior to a second solvent exchange into IPAc for the final trituration.

Accordingly, approximately 95% of the combined organic product solution was evaporated, and five sequential isopropanol charges and equivalent volume distillations were performed. By the final isopropanol charge, a thick product slurry was observed. Seven sequential IPAc charges and equivalent volume distillations were then performed to maximize recovery of **1** and maintain the desired 1:1 diastereomer ratio. Trituration of the resulting thick product slurry in IPAc at 35 °C, then slow cooling to 0 °C followed by filtration and tray-drying, allowed isolation of **1** with some agglomerated but non-sticky particulates. Subsequent pin-milling provided a uniform homogeneous amorphous powder with the desired particle size distribution (D_{90} = 200−300 μm). Residual solvent limits were determined to be <5000 ppm for MBTE, isopropanol, and IPAc,[26] and a <9 ppm value for ES was achieved. The final R_p:S_p diasteremeric ratio was 0.457:0.543, which was effectively unchanged throughout the purification processing. The constituent P-OEt **13** diastereomers represented the largest single impurities and were integrated at 0.26% and 0.13% LCAP, respectively. Accounting for impurities ≥0.05%, the final purity of **1** was determined to be 99.4% LCAP. The deprotection yield was improved on scale to 69%, purity adjusted. Overall, starting from two 8 kg batches of nucleoside **3**, 20.2 kg of **1** was obtained, representing a 59% two-step yield.

The use of CCl_4 as a reagent (12 equiv) in the Atherton−Todd transformation of P-H intermediate to the benzylphosphoramidate **6**, although efficient, was undesirable.[27] During development and production, analysis of multiple batches of **1** indicated that the required concentration limit (<4 ppm) was consistently achieved. With the intention of switching CCl_4 for an alternative chlorinating agent in the Atherton−Todd reaction for the production of subsequent clinical batches, small feasibility scale assessments were

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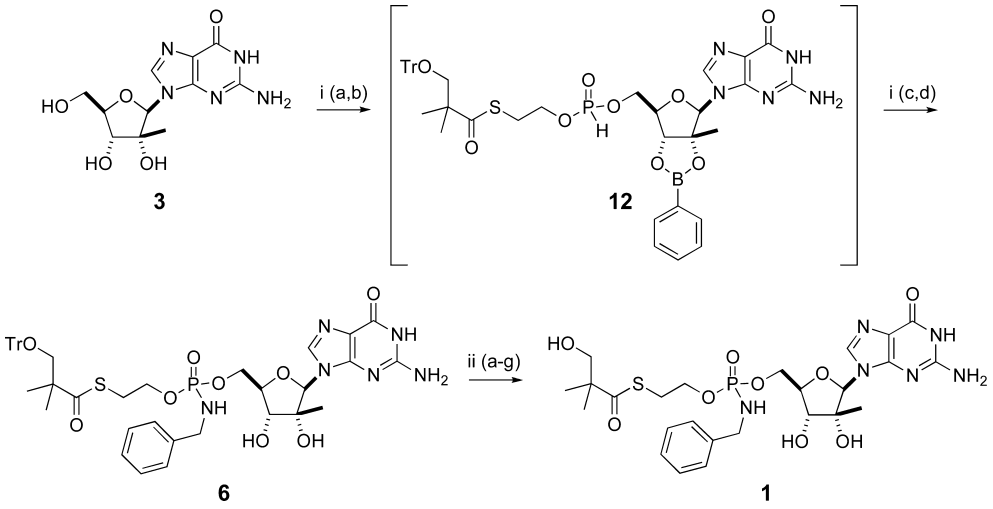
Scheme 4. Optimized Process for the Production of Prodrug 1 on a Multi-kilogram Scale[a]



[a]Conditions: (i) a) $PhB(OH)_2$, MeCN, Na_2SO_4 (s, 2.5 equiv), reflux; b) H-phosphonate **4**, Me_3CCOCl, pyridine, 8−17 °C; c) $BnNH_2$, CCl_4, 5 to 25 °C; d) 21.3% w/w citric acid (aq) (85%); (ii) a) AcCl (1.5 equiv), EtOH: MeCN 1:1, 25 °C; b) Dowex 66 resin neutralization; c) toluene/MeCN/H_2O extraction; d) EtOAc/MeCN/H_2O extraction; e) IPA solvent exchange; f) IPAc solvent exchange; g) IPAc trituration (69%).

undertaken. *N*-Chlorosuccinimide and 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) were determined to be suitable alternatives, and proof of concept was successfully achieved on 5 g scale using 0.6 equiv of DCDMH. Importantly, the reaction workup behaved similarly to that using CCl_4, resulting in trityl-protected **6** with a 1:1 diastereomer ratio.

It remains to be determined whether arylboronic acids and/or esters in general represent a genotoxic hazard.[28] Various methods to control residual arylboronic acids in APIs have been proposed,[29] and the use of the *N*-methylglucamine-containing polystyrene resin Amberlite IRA743[30] was particularly effective at removing PBA in related chemical processes in-house. In the case of PBA itself and the cyclic boronate **11**, no specific Ames tests were conducted for these compounds; however, the boron content of the final API **1** produced on 20 kg scale was measured by ICP-OES to be 6 ppm, indicating a low risk of residual PBA being present at meaningful concentrations.[31]

It is illuminating to calculate the overall process mass intensity (PMI) metric for the production of **1**, where PMI is defined as the total mass of all reactants, reagents, and solvents input during both reaction and purification per kg of product output.[32] Initial campaigns starting with 500 g−1 kg of nucleoside **3** using the PBA transient protection, subsequent deprotection of **6**, and purification of **1** using RP silica gel chromatography (Scheme 3) resulted in a cumulative PMI of 4831 kg/kg. The corresponding optimized process, developed on 8 kg batch sizes of **3**, which utilized extractive purifications to avoid the use of RP silica (Scheme 4), achieved a cumulative PMI of 562 kg/kg. While still relatively modest in absolutely terms, these modifications represented a reduction in PMI of almost 90%.

CONCLUSION

A novel, scalable process for the synthesis of 2′-*C*-methylguanosine-5′-[2-[(3-hydroxy-2,2-dimethyl-1-oxopropyl)-thio]ethyl-*N*-benzylphosphoramidate] (IDX184, **1**) via the use of mild, PBA transient protection was developed. The physical and chemical characteristics of **1** presented a variety of challenges, notably its preferred solubility in organic/aqueous solvent mixtures and sensitivity to acidic and basic conditions. The requirement to produce **1** as a 1:1 mixture of R_p:S_p diastereomers precluded the use of typical crystallization techniques. The original use of impractical, non-reproducible, and expensive final purification via RP chromatography was obviated. In its place, a dual extraction protocol was developed to remove first non-polar and then polar impurities, and judicious use of solvent exchanges avoided the formation of sticky solids, whilst maintaining the 1:1 diastereomer ratio.

Additionally, the GTI ethylene sulfide was controlled to levels <10 ppm, which allowed progression of the program into clinical trials of the desired 1−3 month duration. Overall, 20 kg of **1** was produced in one campaign from two 8 kg nucleoside batches with 99.4% LCAP purity, in 0.46:0.54 R_p:S_p ratio, with 59% two-step yield. The associated PMI was reduced by 90%, to 562 kg/kg, relative to the prior unscalable methodology.

EXPERIMENTAL SECTION

General. 2′-*C*-Methylguanosine was obtained commercially. HPLC was performed on a Waters 2965 Alliance separation module equipped with a 2996 PDA detector, an Agilent 1100 series instrument equipped with a UV detector or equivalent LC instrument. Method for **6**: Zorbax Eclipse XDB C_8 reverse phase column (4.6 mm × 75 mm, 3.5 μm) at 28 °C; flow rate 1.4 mL/min; injection volume 8 μL; mobile phase A = 0.01 M ammonium acetate aqueous pH 4.4 buffer, B = MeCN; run time 20 min; gradient 5% to 80% B over 5.5 min, hold at 80% B for 4.5 min; gradient 80% to 5% B over 3 min, hold at 5% B for 7 min; monitoring 254 nm. Method for **1**: Waters XBridge C_{18} reverse phase column (4.6 mm × 100 mm, 3.5 μm) at 30 °C; flow rate 1.0 mL/min; injection volume 10 μL; mobile phase A = 95:5 H_2O/MeCN, B = 95:5 MeOH/MeCN; run time 65 min; gradient 5% to 30% B over 10 min, hold at 30% B for 20 min; gradient 30% to 100% B over 25 min, hold at 100% B for 2 min, gradient 100% to 5% B over 8 min; monitoring 255 nm.

2′-*C*-Methylguanosine-2′,3′-*O*-phenylboronate 11. 2′-*C*-Methylguanosine **3** (1.0 g, 3.36 mmol, 1.00 equiv) was suspended in anhydrous MeCN (10 mL) between 20 and 25 °C under argon. PBA (0.43 g, 3.53 mmol, 1.05 equiv) was charged in one portion, and the mixture was heated at reflux (82 °C) for 3 h. The suspension was then cooled to 20−25 °C

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under argon. The solids were collected by filtration under reduced pressure and dried under vacuum between 30 and 35 °C to afford 999 mg (77%) of 2′-*C*-methylguanosine-2′,3′-*O*-phenylboronate **11** as a white solid: mp 282−284 °C dec; *m*/*z* (ESI−, direct injection) 382.0 $[M-H]^-$ 100%; ν_{max} (KBr disc, cm^{-1}) 3575, 3405, 3196 (br), 1690, 1638, 1604, 1354; $[\alpha]_D^{20}$ = −27.49 (*c* 1.0, DMSO containing 0.1% H_2O) at *t* = 0 h; $[\alpha]_D^{20}$ = −2.31 (*c* 1.0, DMSO containing 0.1% H_2O) at *t* = 28 h; ^{1}H NMR (400 MHz, DMSO-d_6) 1.23 (3H, s, CH_3), 3.78 (1H, ddd, $J_{5',5''}$ = 12.1 Hz, $J_{5',OH\text{-}5'}$ = 6.1 Hz, $J_{5',4'}$ = 4.4 Hz, *H*-5′), 3.84 (1H, ddd, $J_{5'',5'}$ = 12.1 Hz, $J_{5'',OH\text{-}5'}$ = 5.1 Hz, $J_{5'',4'}$ = 3.6 Hz, *H*-5″), 4.15 (1H, m, *H*-4′), 4.83 (1H, d, $J_{3',4'}$ = 4.7 Hz, *H*-3′), 5.24 (1H, a-t, $J_{OH\text{-}5',5'/5''}$ = 5.6 Hz, O*H*-5′), 6.13 (1H, br-s, *H*-1′), 6.55 (2H, s, NH_2), 7.45 (2H, t, *J* = 7.2 Hz, 2 × Ar-H_{meta}), 7.57 (1H, dt, *J* = 7.2 Hz, *J* = 1.3 Hz, Ar-H_{para}), 7.77 (2H, dd, *J* = 7.2 Hz, *J* = 1.3 Hz, 2 × Ar-H_{ortho}), 8.00 (1H, s, *H*-8), 10.71 (1H, s, *N*-*H*); ^{13}C NMR (100 MHz, DMSO-d_6) 20.28 (CH_3), 60.90 (*C*-5′), 85.24 (*C*-3′), 85.79 (*C*-4′), 91.02, 91.04 (*C*-1′, *C*-2′), 116.35, 150.72, 153.75, 156.62 (*C*-2, *C*-4, *C*-5, *C*-6), 127.94 (2 × Ar-C_{meta}), 131.84 (Ar-C_{para}), 134.56 (2 × Ar-C_{ortho}), 135.57 (*C*-8) (Ar-C_{ipso} not observed). Anal. Calcd for $C_{17}H_{18}N_5O_5B$: C, 53.29; H, 4.73; N, 18.28; B, 2.82. Found: C, 53.53; H, 4.74; N, 18.01; B, 2.74.

2′-*C*-Methylguanosine-5′-[2-[(3-trityloxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzylphosphoramidate] (6). 2′-*C*-Methylguanosine **3** (8.0 kg, 26.91 mol, 1.00 equiv), anhydrous sodium sulfate (9.60 kg, 67.59 mol, 2.51 equiv), and PBA (3.44 kg, 28.21 mol, 1.05 equiv) were charged to a reactor. MeCN (48.9 L) was charged to the reactor, and agitation commenced (100 rpm). The batch was heated to reflux (80 °C) and held for 4.5 h. Analysis by ^{1}H NMR indicated 99% conversion to the desired phenylboronate **11**, and the batch was cooled to 22 °C. Triethylammonium 2-(2,2-dimethyl-3-(trityloxy)propanoylthio)ethyl phosphonate[33] **4** (22.60 kg, 88% LCAP, 33.97 mol, 1.26 equiv) was dissolved in MeCN (17.6 L) and charged to the reactor. After rinsing forward with MeCN (6.4 L), the batch was cooled to −2 °C. In a separate vessel, a mixture of pyridine (22.96 L, 283 mol, 10.55 equiv) and trimethylacetyl chloride (16.38 kg, 135.84 mol, 5.05 equiv) was prepared. This mixture was charged over 2.5 h to the batch, maintaining an internal temperature <8 °C. The charge vessel was rinsed forward with MeCN (12.8 L), and the batch was warmed to 10 °C over 1.5 h and then held between 10 and 17 °C. After 1 h, analysis by HPLC indicated the LCAP ratio of P-H intermediate to **3** was 7.65:1. The reaction mixture was diluted with MeCN (25.2 L) and cooled to −3 °C. Benzylamine (42.00 kg, 391.97 mol, 14.56 equiv) was charged over 1.75 h, maintaining the batch temperature <8 °C, followed with a MeCN (6.5 L) rinse. Carbon tetrachloride (48.27 kg, 313.81 mol, 11.7 equiv) was added over 1.75 h, maintaining the batch temperature <10 °C, followed with a MeCN (6.4 L) rinse. After 2.5 h, analysis by HPLC indicated 0.60% LCAP P-H intermediate remaining. MTBE (240 L) was charged, and the batch was warmed to 25 °C. Aqueous citric acid solution (21.3% w/w, 376.1 kg, 15.5 equiv) was added, and cleavage of the cyclic boronate was effected over 1 h. The phases were allowed to separate for 3 h. The lower aqueous phase was discarded. Additional MeCN (6.2 L) was added, and the organics were washed with an aqueous solution (236.0 kg) of $NaHCO_3$ (3.4% w/w) and NaCl (8.4% w/w). The batch was agitated for 0.25 h before the phases were allowed to separate over 2.5 h. The lower aqueous phase was discarded. MTBE (118 L) was charged to the organics, and 118.7 kg of solvent was removed by distillation under vacuum with a maximum jacket temperature of 33 °C. A second charge of MTBE (118 L) was performed, and a further 88.7 kg of distillate was recovered. Distillation ceased, and a third charge of MTBE (471 L) was made prior to cooling the mixture to 21 °C. After 7 h, the diastereomer ratio by ^{31}P NMR was 0.459:0.540. After 32 h, the diastereomer ratio was 0.476:0.524. The solids were recovered by filtration, and the wet cake was washed with MTBE (64 L) and EtOH (64 L) before the filter was purged with nitrogen. Drying under vacuum at 45 °C with a nitrogen purge gave trityl-protected phosphoramidate **6** as a white solid (20.7 kg, 85% purity adjusted yield, 97.7% LCAP).

A second reaction using 8.0 kg of **3** was processed similarly to provide additional trityl-protected phosphoramidate **6** (21.7 kg, 85% purity adjusted yield, 97.0% LCAP, diastereomer ratio 0.457:0.543): *m*/*z* (ESI+) 869.40 $[M+H]^+$ 100%; *m*/*z* (ESI−) 867.46 $[M-H]^-$ 100%; ^{1}H NMR (400 MHz, DMSO-d_6) two diastereomers 0.83 (3H, s, CH_3), 1.13 (6H, s, $(CH_3)_2C$), 3.00−3.11 (4H, m, CH_2S, CH_2OTr), 3.83−4.01 (6H, m, *H*-3′, *H*-4′, CH_2O, CH_2Ph), 4.12−4.28 (2H, m, *H*-5′, *H*-5″), 5.16 (1H, s, O*H*-2′), 5.39, 5.41 (2 × 0.5H, 2*d*, 2 × *J* = 6.4 Hz, O*H*-3′), 5.65 (1H, m, P-N-*H*), 5.77 (1H, s, *H*-1′), 6.52 (2H, br-s, NH_2), 7.17−7.43 (20H, m, 20 × Ar-*H*), 7.77, 7.78 (2 × 0.5H, 2*s*, *H*-8), 10.40 (1H, br-s, N-*H*).

2′-*C*-Methylguanosine-5′-[2-[(3-hydroxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzylphosphoramidate] (1). Trityl-protected phosphoramidate **6** (20.7 kg, assumed 23.82 mol, 1.00 equiv), MeCN (166 L), and EtOH (166 L) were charged to a reactor, and agitation commenced (100 rpm). In a separate vessel, a dry HCl solution was prepared by charging acetyl chloride (2.81 kg, 35.80 mol, 1.50 equiv) to a mixture of EtOH (20.5 L) and MeCN (20.5 L) over 0.5 h, maintaining internal temperature <35 °C. A line rinse with MeCN (3.9 L) was added to the vessel and stirred for 0.5 h. The HCl solution (37.5 kg) was charged to the reactor over 8 min and rinsed forward with MeCN (6.4 L). The reaction was held at 25 °C for 11.5 h, at which point analysis by HPLC indicated the LCAP ratio of **1** to **6** was 11.3:1. Dowex 66 basic resin (45 kg) was washed with a mixture of MeCN (150 L) and water (150 L). The washed resin (35.2 kg) was charged to the reactor over 8 min, during which time no temperature change was observed. After 1 h, the solution pH was 4. Additional Dowex 66 resin (3.2 kg) was added, and after a further 0.5 h, the measured pH was 6. The Dowex resin was removed by filtration and washed with a mixture of MeCN (43 L) and water (43 L). A clear, colorless solution of crude **1** was obtained (392 kg, 88.1% LCAP).

Solvent (351 kg) was partially removed by distillation under vacuum with a maximum jacket temperature of 40 °C. Water (414 L) and MeCN (621 L) were charged to the concentrate, and a solution was obtained on stirring at 100 rpm for 1.5 h at 42 °C. After the mixture cooled to 26 °C, toluene (357 L) was charged. A light slurry was observed, at which point additional water (45 L) and MeCN (57 L) were added. Dissolution was complete after a further 1.5 h, at which point the phases were allowed to separate at 25 °C for 1 h. The organic phase (733 kg) was discarded. Analysis of the aqueous phase (598 kg) by HPLC indicated 97.7% LCAP with a 0.465:0.535 diastereomer ratio. The aqueous phase was charged with MeCN (164 L) and toluene (207 L), and the mixture was agitated at 100 rpm for 0.25 h. The phases were allowed to separate at 25 °C for 1 h. The organic phase (340 kg) was discarded. Analysis of the aqueous phase (564 kg) by HPLC indicated 97.9% LCAP with

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a 0.465:0.535 diastereomer ratio. Solvent (305 kg) was partially removed from the aqueous phase by distillation under vacuum with a maximum jacket temperature of 45 °C. MeCN (202 L) and EtOAc (574 L) were charged to the concentrate, and the mixture was agitated (100 rpm) for 0.25 h. The phases were allowed to separate at 25 °C for 4.5 h. Analysis of the organic phase (699 kg) by HPLC indicated 98.9% LCAP with a 0.466:0.534 diastereomer ratio. MeCN (202 L) and EtOAc (574 L) were charged to the aqueous phase (216 kg), and the mixture was agitated (100 rpm) for 2 h. The phases were allowed to separate at 25 °C for 4 h. The second aqueous phase (147 kg) was discarded. Analysis of the second organic phase (744 kg) by HPLC indicated 98.5% LCAP with a 0.452:0.548 diastereomer ratio. Combination of the two organic phases provided a total product solution of 1443 kg.

A second deprotection reaction of **6** (21.7 kg, assumed 24.97 mol, 1.00 equiv) was performed in a similar manner: analysis of the first organic product phase (748.5 kg) by HPLC indicated 98.8% LCAP with a 0.464:0.536 diastereomer ratio, and analysis of the second organic product phase (798.9 kg) by HPLC indicated 98.5% LCAP with a 0.443:0.557 diastereomer ratio. Combination of the two organic phases provided a total product solution of 1547 kg.

The two product solutions (combined 2990 kg, average 98.7% LCAP) were charged into the same reactor, and solvent (2868 kg) was partially removed by distillation under vacuum with a maximum jacket temperature of 40 °C. 2-Propanol (115 L) was charged to the concentrate, and solvent (90 kg) was partially removed by distillation under vacuum with a maximum jacket temperature of 40 °C. This 2-propanol/distillation process was repeated four more times, removing a total of 438 kg of distillate. Isopropyl acetate (93 L) was charged to the concentrate, and solvent (82 kg) was partially removed by distillation under vacuum with a maximum jacket temperature of 40 °C. This isopropyl acetate/distillation process was repeated six more times, removing a total of 558 kg of distillate. An eighth charge of isopropyl acetate (103 L) was performed, and the batch was held at 35 °C for 0.75 h. The slurry was cooled to 0 °C over 4 h and held at 0 °C for 2 h. The solids were filtered under vacuum and washed with isopropyl acetate (77 L), and the filter was purged with nitrogen. Drying under vacuum at 45 °C with a nitrogen purge gave phosphoramidate **1** as a white solid (20.2 kg, 69% purity adjusted yield, 99.4% LCAP[34] with a 0.457:0.543 diastereomer ratio): *m/z* (ESI+) 627.05 $[M + H]^+$ 100%, 1253.55 $[2M+H]^+$ 20%; ν_{max} (KBr disc, cm^{-1}) 3334 (br), 1685, 1635, 1598, 1571; ^{1}H NMR (400 MHz, DMSO-d_6) two diastereomers 0.85 (3H, s, CH_3), 1.11 (6H, s, $(CH_3)_2C$), 3.05 (2H, m, CH_2S), 3.44 (2H, d, J = 5.5 Hz, CH_2OH), 3.89−4.02 (6H, m, *H*-3′, *H*-4′, CH_2O, CH_2Ph), 4.14−4.20 (1H, m, *H*-5′), 4.22−4.30 (1H, m, *H*-5″), 4.97 (1H, t, J = 5.5 Hz, CH_2OH), 5.22 (1H, s, O*H*-2′), 5.43, 5.46 (2 × 0.5H, 2d, 2 × J = 6.4 Hz, O*H*-3′), 5.68 (1H, m, P-N-*H*), 5.78 (1H, s, *H*-1′), 6.56 (2H, br-s, NH_2), 7.20−7.24 (1H, m, Ar-*H*), 7.28−7.34 (4H, m, 4 × Ar-*H*), 7.80, 7.81 (2 × 0.5H, 2s, *H*-8), 10.69 (1H, br-s, N-*H*); ^{13}C NMR (100 MHz, DMSO-d_6) two diastereomers 19.35 (CH_3), 21.27 ($C(CH_3)_2$), 27.63, 27.70 (CH_2S), 43.65, 43.69 ($PhCH_2$), 51.11 ($C(CH_3)_2$), 63.24, 63.28 (CH_2O), 64.41, 64.71 (*C*-5′), 67.78 (CH_2OH), 72.01, 72.21 (*C*-3′), 77.61, 77.63 (*C*-2′), 79.53, 79.61 (*C*-4′), 89.81, 89.90 (*C*-1′), 115.87, 115.91 (*C*-5), 126.14 (Ar-C_{para}), 126.60, 126.61, 127.55, 127.56 (4 × Ar-*C*), 134.42, 134.58 (*C*-8), 139.82, 139.88 (Ar-C_{ipso}), 150.19, 150.22 (*C*-4), 153.07, 153.09, 156.18 (*C*-2, *C*-6), 203.39 (*C*═OS); ^{31}P NMR (162 MHz, DMSO-d_6) two diastereomers 9.78, 9.92 (1P, 2*s*); HRMS (ESI) $[M+H]^+$ *m/z* calcd for $C_{25}H_{36}N_6O_9PS$ 627.2002, found 627.2010. Anal. Calcd for $C_{25}H_{35}N_6O_9PS$: C, 47.92; H, 5.63; N, 13.41; P, 4.94. Found: C, 48.18; H, 5.76; N, 13.30; P, 4.98.

■ ASSOCIATED CONTENT

Supporting Information

Crystallographic information for the X-ray structure determination of the S_p diastereomer of IDX184 and experimental details for the formation of phosphoramidate **6** using DCDMH. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mayes.ben@idenix.com.

Notes

The authors declare no competing financial interest.

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(4) For example: solubility of **1** in water = 0.008 mol L^{-1}; in EtOH = 0.04 mol L^{-1}; in water/EtOH 1:1 = 0.16 mol L^{-1}.

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(8) Discussion of other methodologies to couple nucleosides to form phosphoramidate prodrugs is provided in ref 7. For a full account, see: Padere, U.; Garnier-Amblard, E. C.; Coats, S. J.; Amblard, F.; Schinazi, R. F. *Chem. Rev.* **2014**, *114*, 9154−9218.

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(10) The acceptable range for the 1:1 diastereomer specification was a R_p:S_p ratio of 45:55 to 55:45.

(11) Liquid chromatography area percentage.

(12) THF/water/saturated aqueous $NaHCO_3$ vol ratio was 1:0.25:0.25.

(13) A typical elution profile consisted of 1.5% MeCN/water, 21 vol; 5% MeCN/water, 16 vol; 10% MeCN/water, 35 vol; 15% MeCN/water, 35 vol; 18% MeCN/water, 33 vol; 20% MeCN/water, 33 vol; 25% MeCN/water, 118 vol.

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(14) ES was quantified by a validated headspace gas chromatographic analytical method.
(15) *Vide infra*.
(16) At three ICH storage conditions: 5 °C; 25 °C /40% relative humidity (RH); 40 °C /75% RH.
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(21) Impurity **13** was qualified in preclinical toxicology studies allowing this specification to be set for the final API.
(22) The cyclic boronates were completely hydrolyzed under the HPLC conditions; therefore, the respective deprotected species were monitored. Conversion of 7:1 at this stage was typical, and addition of further PivCl/pyridine did not improve isolated yields.
(23) LCAP represents the sum of both diastereomers of P-OEt **13**.
(24) The first and second organic layers contained a 10:1 ratio of product **1** content.
(25) The extracts contained EtOAc, MeCN, and ∼7 wt% water.
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(31) Based on a 200 mg daily dose of **1**, 6 ppm equated to 1.2 μg, which was 16-fold below the 20 μg per day limit for PGIs based on a 1−3 month clinical trial duration.
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(33) *Vide supra*, see ref 7.
(34) 99.4% LCAP integrating all impurities ≥0.05%.

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