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Synthesis of a Nucleoside Phosphoramidate Prodrug Inhibitor of HCV NS5B Polymerase: Phenylboronate as a Transient Protecting Group

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ABSTRACT: A synthetic process for 2′-*C*-methylcytidine-5′-[2-[(3-hydroxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzyl-phosphoramidate], a nucleotide prodrug inhibitor of hepatitis C virus NS5B polymerase, is described. The route developed was demonstrated on 100 g scale and featured the key application of phenylboronic acid as an effective transient means to protect the 2′,3′-hydroxyls of 2′-*C*-methylcytidine. This synthetic methodology resulted in a reduction in the number of isolations from five to two and an increase in the overall yield by 50% relative to the original unscalable discovery route. The synthesis and characterization of 2′-*C*-methylcytidine-2′,3′-*O*-phenylboronate is also provided.

INTRODUCTION

Hepatitis C virus (HCV) is blood-borne pathogen chronically infecting an estimated 3% of the global population, for which a vaccine is not available.[1] Treatment with boceprevir, telaprevir, or simeprevir, the first direct acting antivirals (DAAs) to be introduced, in combination with PEGylated interferon-α (IFN-α) and ribavirin is limited to those patients presenting with HCV genotype 1 infection.[2] Greater potency, broader genotype coverage, higher barrier to resistance, and improved safety profile are clinical requirements which have driven efforts to develop novel and more effective DAAs.[3] Beyond these HCV NS3/4A protease inhibitors, one potential therapeutic target is the HCV NS5B RNA polymerase, of which 2′-*C*-methylcytidine-5′-triphosphate is an effective inhibitor.[4,5] Valopicitabine **1**, an early prodrug of the parent nucleoside 2′-*C*-methylcytidine **2**, utilized a 3′-*O*-L-valine ester to improve bioavailability and achieved proof of concept in the clinic (Figure 1).[6] Several subsequent prodrugs of 2′-*C*-methylcytidine have employed phosphoramidate monoesters, diesters, or cyclic phosphoramidate diesters with the intention of delivering the key nucleoside-5′-monophosphate to the liver, thus bypassing its inefficient initial formation from the free nucleoside.[4,7] 2′-*C*-Methylcytidine prodrug **3** was identified as one such candidate of interest.[8] Bearing both *S*-acyl thioethyl and benzyl phosphoramidate moieties, **3** is a mixture of phosphorus diastereomers in a 1:1 ratio. A scalable synthetic route was required for prodrug **3** to enable the rapid onset of further preclinical studies. This article details the novel chemical synthesis developed toward achieving this objective; in particular, the use of phenylboronic acid (PBA) as an efficient, transient protecting group for 2′-*C*-methylcytidine is reported.

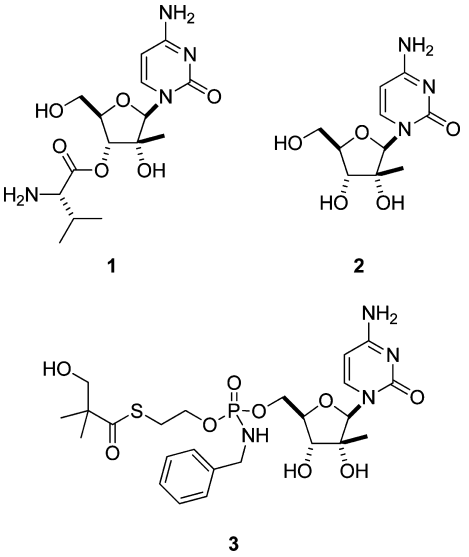

Figure 1. Valopicitabine **1**, 2′-*C*-methylcytidine **2**, and target phosphoramidate **3**.

RESULTS AND DISCUSSION

Route 1: Original Discovery Synthesis. The phosphoramidate prodrug **3** was initially synthesized using the discovery chemistry procedure on milligram scale in 20% overall yield from 2′-*C*-methylcytidine **2** (Scheme 1).[8a] The strategy involved (1) sequential protection of the 2′,3′-OH and NH_2 groups on the ribonucleoside with acetonide and dimethoxytrityl (DMTr), respectively, to avoid competitive phosphorylation and to improve the limited solubility of **2** in organic solvents;[7a] (2) coupling the protected nucleoside **5** with the H-phosphonate salt intermediate **6**, a convergent building block which had been used to access prodrugs of a variety of nucleosides; (3) transformation of the H-phosphonate **7** to phosphoramidate **8**; and (4) global

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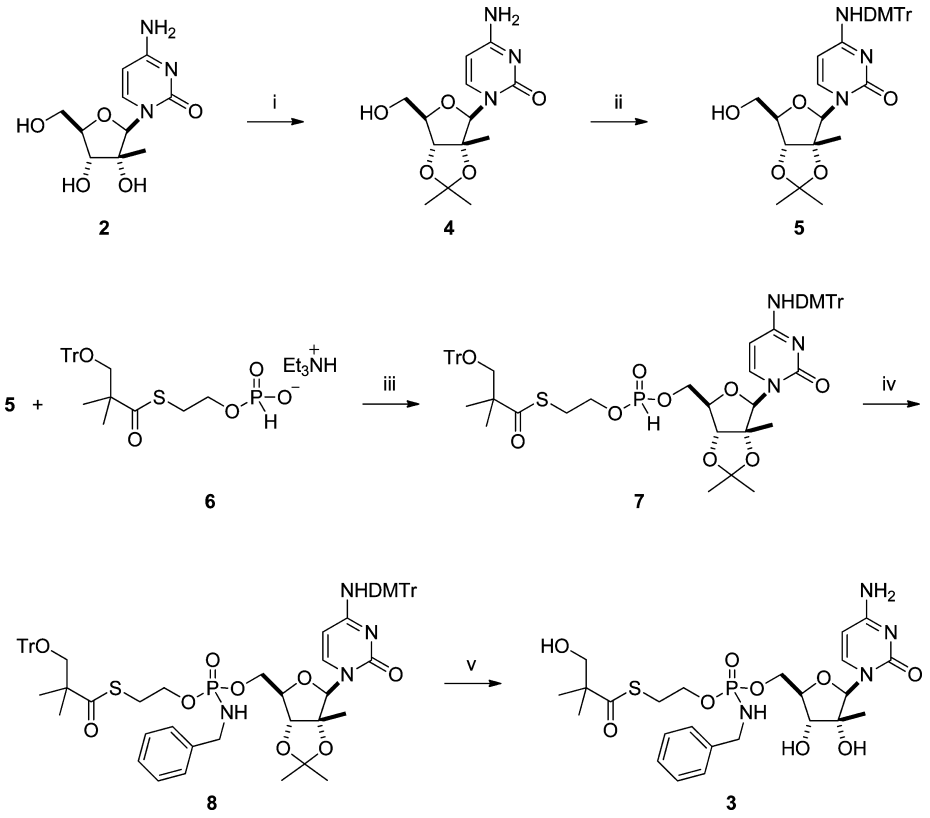
Scheme 1. Discovery Chemistry Synthesis of Prodrug 3 (Route 1)[a]



[a]Conditions: (i) $HC(OEt)_3$, acetone, *p*-TSA (86%); (ii) (a) TMSCl, pyridine, (b) DMTrCl, DMAP, (c) NH_4OH, dioxane (81%); (iii) Me_3CCOCl, pyridine (94%); (iv) $BnNH_2$, CCl_4 (87%); (v) TFA, DCM (36%).

deprotection of DMTr, acetonide, and trityl groups to give the free nucleoside phosphoramidate **3**.

Critical evaluation of this route highlighted several major issues which were deemed to be unsuitable or impractical for scale up. First, the two-step protection protocol of 2′-*C*-methylcytidine resulted in a 30% loss of this valuable starting material, and the use of DMTr protection was poor in terms of atom economy. The additional expensive reagents, large amounts of solvents and waste associated with these reactions, and tedious chromatographic purifications made this procedure unfavorable. Second, the H-phosphonate **7** was determined to have poor thermal and hydrolytic stability. Third, carbon tetrachloride was used in large quantity, functioning as both reagent and solvent in the Atherton−Todd[9] reaction to install the benzylamine group to give **8**. The target nucleoside phosphoramidate **3** was isolated in a mere 36% yield by sequential silica gel chromatography, reverse-phase silica gel chromatography, then lyophilization. Alarmingly, multiple attempts to scale up this deprotection reaction beyond 1 g failed as the compound disintegrated to a complex mixture before the acetonide was completely removed. Additional identifiable side products observed by mass spectrometry were found to have incorporated a trifluoroacetate ester at one of the hydroxyl positions or to have displaced the benzylamine moiety, giving a trifluoroacetic phosphoric anhydride. Attempts to use other acids for the deprotection were also ineffective in producing the desired phosphoramidate **3**.[10]

Route 2: Synthesis with 2′- and 3′-Hydroxyls Unprotected. Although there are numerous reports of the formation of nucleoside phosphoramidates, there are relatively few examples specifically concerning prodrugs of 2′-*C*-methylcytidine **2**. The two main synthetic approaches, utilizing either P^{III} or P^{V} chemistry, have employed **2** protected with acetonide or as the 2′,3′-hydroxyl-unprotected nucleoside to form 2′-*C*-methylcytidine aryl phosphoramidates. The P^{III} coupling methodology has been exemplified using diphenylphosphite at 0 °C followed by installation of a functionalized amine using Atherton−Todd conditions in 40% yield from the acetonide-protected nucleoside **4** to give a 1:1 mixture of phosphorus diastereomers.[4,7a,11] The second approach has typically employed a classical 5′-hydroxyl activation with ^{t}BuMgCl followed by reaction with a functionalized electrophilic P^{V} phosphorochloridate.[12] In addition to being performed at −78 °C, under high dilution conditions (<0.1 M), the isolated phosphorus diastereomer ratios reportedly varied from 1:1 to 1:7.[4,13] Relatively modest yields obtained (5−36%) have been attributed to the poor solubility of unprotected nucleoside **2** under the reaction conditions.[7b]

Related P^{V} chemistry has also been applied to produce similar aryl aminoester phosphoramidate prodrugs of other nucleosides. In the case of 6-*O*-methyl-2′-*C*-methylguanosine, coupling of the nucleoside and phosphorochloridate using ^{t}BuMgCl or *N*-methylimidazole produced 1:1 phosphorus diastereomer ratios; however, the yields ranged from 22 to 25% and 11−28% under the two respective conditions.[14,15] With the aim of forming a single phosphorus diastereomer, the use of a phosphorochloridate reagent was replaced by an isolable pentafluorophenyl analogue in the ^{t}BuMgCl-mediated coupling to form the aryl phosphoramidate prodrug of 2′-deoxy-2′-fluoro-2′-*C*-methyluridine.[16] Although the chemistry

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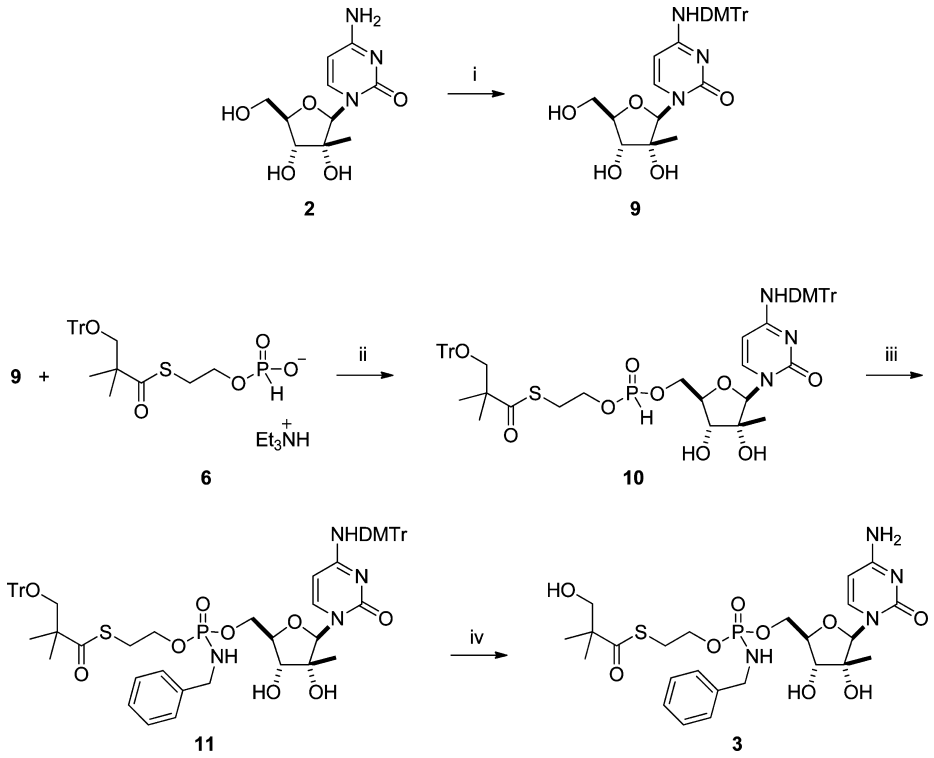
Scheme 2. Synthesis of 3 with 2′- and 3′-Hydroxyls Unprotected (Route 2)[a]



[a]Conditions: (i) (a) TMSCl, pyridine, (b) DMTrCl, DMAP, (c) TBAF, THF (93%); (ii) Me_3CCOCl, pyridine (27%); (iii) $BnNH_2$, CCl_4 (75%); (iv) TFA, DCM (71%).

was performed on the unprotected 2′-deoxynucleoside, acetonide-protected adenosine was utilized as a model substrate in the development to avoid the complications of a competing hydroxyl group. In practice, 5−8% of the 3′,5′-bisphosphorylated uridine side product was produced; however, this level was noted to be higher for the cytidine analogue.

Application of the benzyl phosphoramidate prodrug in **3** to 2′-*C*-methylguanosine has been reported using a related pentafluorophenyl phosphorus ester coupling reaction with ^{t}BuMgCl-mediated 5′-hydroxyl activation.[17] Although the 2′,3′-hydroxyls were unprotected, the use of 6-*O*-methyl-2′-*C*-methylguanosine was presumably required to improve solubility and avoid base phosphorylation.[12b] The racemic benzyl pentafluorophenyl phosphoramidate intermediate was formed in 38% yield, and the subsequent coupling yields with the two separate phosphorus enantiomers were a disparate 59 and 36%, respectively, a potential indication that a 1:1 phosphorus diastereomer prodrug ratio may not be trivial to obtain via this methodology.

Installation of the same benzyl phosphoramidate prodrug to an unnatural, diaminopurine nucleoside analogue has been reported using P^{III} chemistry with H-phosphonate salt intermediate **6**.[18] The 2′,3′-hydroxyls were unprotected during the coupling; however, the amino functions on the base were protected with DMTr groups which presumably improved solubility of the 2′-*C*-methyl ribonucleoside.

After consideration of these various alternate approaches, a second strategy to make prodrug **3**, which involved leaving the nucleoside 2′- and 3′- hydroxyls free, was investigated to circumvent the problematic acetonide removal of route 1, thereby also having the advantage of avoiding the initial acetonide protection step (Scheme 2). The solubility of 2′-*C*-methylcytidine was improved by maintaining the DMTr amino protection, while the P^{III} coupling/Atherton−Todd chemistry was retained from route 1 to facilitate a 1:1 phosphorus diastereomer ratio. This approach involved initial trimethylsilyl protection of the 2′-, 3′-, and 5′-OH groups, prior to introduction of DMTr onto the cytosine base, followed by silyl cleavage using TBAF to give **9**. Coupling of **9** with the H-phosphonate salt intermediate **6** using pivaloyl chloride and purification by silica gel chromatography resulted in a mere 27% yield of H-phosphonate **10**.[19] Similar isolated yields (25−27%) were obtained using EDCI in place of pivaloyl chloride. Introduction of the benzylamine group to give phosphoramidate **11** was performed using the original conditions in 75% yield. Finally, removal of the DMTr and trityl groups gave the desired nucleoside phosphoramidate **3** in a significantly increased 71% deprotection yield. However, despite this improved final deprotection step, the unprotected 2′,3′-OH groups led to a decrease in overall yield to 13%. As a result, it was apparent that protection of the 2′,3′-hydroxyl groups would be advantageous to obtain reasonable yields. It was envisioned that a highly labile moiety may potentially avoid similar problems associated with the acetonide removal and enable the efficient synthesis of the desired phosphoramidate prodrug on larger scale.

Route 3: Phenylboronic Acid Protection of 2′-*C*-Methylcytidine. Boric[20] and boronic acid[21] containing protecting groups have been used for the protection of carbohydrates and other 1,2-diols; however, their application in nucleoside chemistry has received only minimal attention.[22] It was envisaged that the hydrolytic lability of a 1,2-cyclic boronate would be an advantageous characteristic in this instance. Phenylboronic acid is a relatively inexpensive reagent

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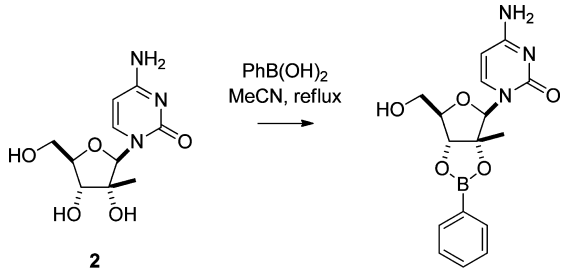
which has been shown to regioselectively form 2′,3′-cyclic boronates with unmodified nucleosides, including natural cytidine.[22a] It was reasoned that its introduction to give a 2′-*C*-methylcytidine-2′,3′-cyclic boronate would behave similarly, and that its facile removal in a one-pot process would provide an efficient, transient protection strategy, whilst beneficially improving the solubility of **2**. It was also anticipated that protection of the cytosine $-NH_2$ may be unnecessary, thus eliminating the requirement for the uneconomical DMTr group, as demonstrated with other 2′-*C*-methylcytidine phosphoramidate systems using P^{III} coupling reagents.[7a]

Formation of 2′-*C*-methylcytidine-2′,3′-*O*-phenylboronate **12** was initially achieved on a 1 g scale by refluxing 2′-*C*-methylcytidine **2** in acetonitrile with 2 equiv of PBA (Scheme 3). The resulting white suspension was cooled and filtered to

Scheme 3. Formation of 2′-*C*-Methylcytidine-2′,3′-phenylboronate 12



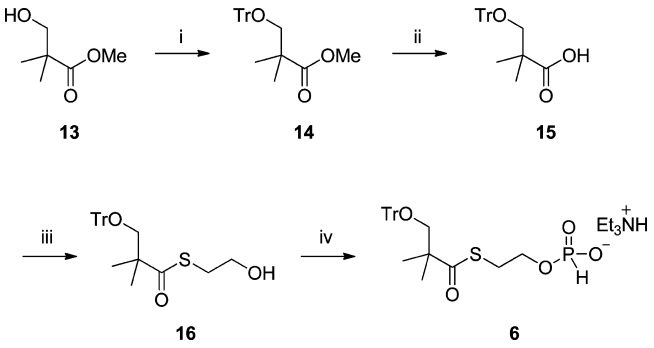
give the first example of a 2′-*C*-methylnucleoside-2′,3′-cyclic boronate, isolated as a white solid in 75% yield.[23] The regioselectivity of the 2′,3′-cyclic boronate was confirmed by 2D NMR spectrometry. Sensitivity of **12** to hydrolytic cleavage was apparent from specific rotation[24] data recorded at various time points; initially, a specific rotation of +15.6 was observed in DMSO containing 0.1% water. After 28 h, the same solution yielded a specific rotation under similar conditions of +78.1, corresponding to a slow but appreciable hydrolysis to 2′-*C*-methylcytidine, for which $[\alpha]_D^{20}$ = +137.9.[25]

Removal of the water byproduct was required to obtain full conversion of **2** to the boronate **12**. Either anhydrous sodium sulfate addition or by switching solvent to pyridine and subsequently distilling off the pyridine−water azeotrope resulted in 97−99% conversion, as indicated by ^{1}H NMR. For further development, the pyridine method was utilized and the boronate was taken directly to the next step.

Formation of H-Phosphonate Salt Intermediate 6. The medicinal chemistry synthesis,[8a] which was originally performed to give single gram quantities of H-phosphonate salt intermediate **6**, was modified and scaled to 7 kg batch size (Scheme 4).

Modifications for scale up to 1.8 kg of hydroxyester **13** included decreasing the amounts of dichloromethane by 74% to 10 volumes and trityl chloride by 20% to 0.95 equiv. One hour reaction time was found to be sufficient rather than 15 h, and the workup was simplified by using only a single aqueous wash. Dimethoxyethane was used in preference to the carcinogenic 1,4-dioxane in the subsequent saponification, with a 60% reduction in total reaction volume. After switching solvents to dichloromethane and acidification with 5 N HCl, crystallization from dichloromethane/*n*-heptane provided crystalline trityl acid **15** in 73% yield over two steps.

Scheme 4. Formation of H-Phosphonate Salt Intermediate 6[a]



[a]Conditions: (i) TrCl, DMAP, TEA, DCM; (ii) NaOH, H_2O, DME; (iii) (a) CDI, DCM, (b) $HO(CH_2)_2SH$; (iv) H_3PO_3, DCM, Me_3CCOCl, TEA (66% overall yield from **13**).

Modification of the original procedure used at 3 g scale to form thioester **16** began by reducing the molar equivalents of carbonyldiimidazole (CDI) by 20% to 1.05 and mercaptoethanol by 12% to 1.15 with no detriment to the reaction profile. The high-boiling DMF/toluene solvent system was replaced by dichloromethane which also enabled a reduction in volume of 60%. Addition of CDI was performed portionwise to allow for controlled evolution of CO_2 gas. Mercaptoethanol was then added at such a rate to control the exotherm below 25 °C. Workup consisted of sequential washing with acid, base, and water. The concentrated crude thioester was then taken up in dichloromethane and triethylamine, avoiding the use of pyridine in the H-phosphonate formation. The amount of phosphorous acid was reduced by 50% to 2 equiv and that of pivaloyl chloride by 73% to 1.5 equiv. The exotherm on addition of pivaloyl chloride was controlled via cooling to maintain an internal temperature below 25 °C. TEA salt formation was achieved using further triethylamine, thus eliminating the use of the expensive triethylammonium bicarbonate buffer, and workup was further simplified to a single water wash. Concentration of the organics and drying via acetonitrile azeotropic distillation provided H-phosphonate **6** with <0.4% water. As the next reaction solvent, residual acetonitrile presented no concern. This material was of satisfactory quality (~90% LCAP[26]) to be used directly in the coupling step, thereby eliminating the need for purification by silica gel chromatography. As a result, production of intermediate **6** was achieved with 66% overall yield from hydroxyester **13** in multi-kilogram batches.

Synthesis of Phosphoramidate Prodrug 3. The synthetic procedure developed to produce phosphoramidate **3** is outlined in Scheme 5. With access to H-phosphonate intermediate **6**, P^{III} chemistry was employed with the transiently 2′,3′-*O*-boronate-protected nucleoside: benzyl phosphoramidate formation under Atherton−Todd conditions was again anticipated to provide the desired 1:1 ratio of phosphorus diastereomers.

Protection of the high-value 2′-*C*-methylcytidine **2** with PBA was achieved in refluxing pyridine while removing the azeotrope. Evaporation of the pyridine gave a golden oil which was stable enough to be stored overnight under vacuum or inert atmosphere if required.[27] Analysis by proton NMR indicated greater than a 97:3 ratio of boronate **12** to starting material **2** on 150 g scale.

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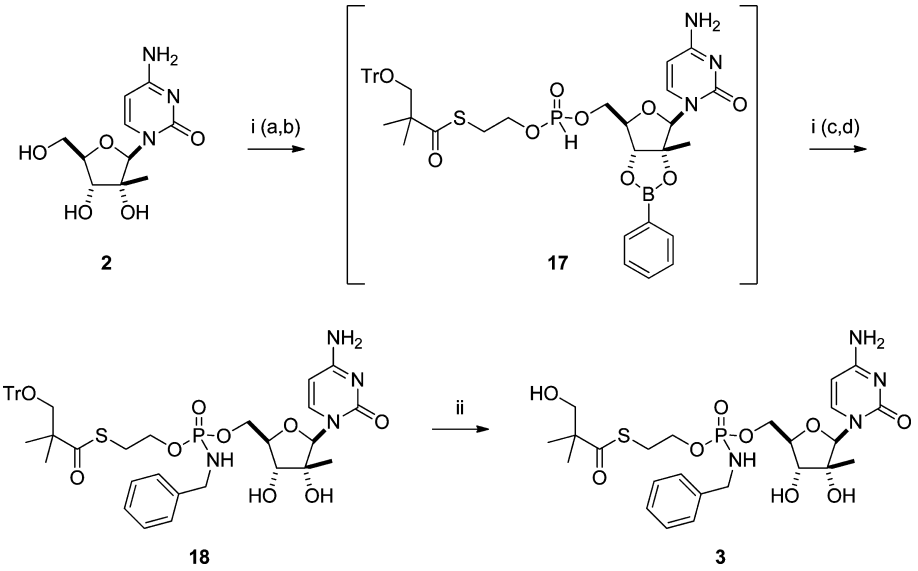
Scheme 5. Synthesis of Prodrug 3 Using PBA Transient Protection Strategy (Route 3)[a]



[a]Conditions: (i) (a) $PhB(OH)_2$, pyridine, reflux, (b) H-phosphonate **6**, EDCI·HCl, MeCN, (c) $BnNH_2$, CCl_4, (d) 20% w/w citric acid (aq) (53%); (ii) AcCl, EtOH (61%).

After redissolution in pyridine, the boronate was added to a solution of H-phosphonate salt **6** in acetonitrile and the carbodiimide coupling agent EDCI at 45 °C generated a 7:1 ratio of H-phosphonate diester to 2′-*C*-methylcytidine **2** by HPLC analysis.[28] Neither extended reaction time, elevated reaction temperature, nor the addition of further EDCI was beneficial, potentially due to cleavage of the PBA protection on prolonged coupling. Pivaloyl chloride was also effective as a coupling agent; however, as in the case of route 2, these reactions were less reproducible and robust, primarily due to higher sensitivity to temperature and moisture. Although H-phosphonate formation using boronate-protected **12** was inferior to using acetonide **5**, it was dramatically superior to using the 2′,3′-*O*-unprotected nucleoside **9**. This lower conversion was more than compensated, however, by avoiding both the initial inefficient protection of **2** and, ultimately, the problematic deprotection of acetonide **8**.

In order to avoid isolation of the H-phosphonate **17**, investigations were made into the possibility of executing the benzyl phosphoramidate formation directly on this reaction mixture. After 4 h reaction time, the H-phosphonate mixture was cooled to 15−20 °C and excess benzylamine (6.2 equiv) was added followed by 4.6 equiv of carbon tetrachloride, resulting in almost immediate conversion of the P−H to phosphoramidate. Addition of ethyl acetate and aqueous citric acid effected complete and rapid removal of the PBA protection to produce the 2′,3′-OH nucleoside phosphoramidate **18** in situ. Pyridine, benzylamine, PBA, and EDCI-related urea byproducts were successfully removed after consecutive citric acid and aqueous bicarbonate washes.[29] Purity of the crude product was upgraded by passing through a short silica plug (3:1 w/w silica/crude) to give **18** with 98.5% LCAP purity and the required 1:1 phosphorus diastereomer ratio in 53% yield from 2′-*C*-methylcytidine.[30]

Using this approach, removal of only a single, labile trityl group was required to obtain the final phosphoramidate prodrug **3**. The absence of the robust acetonide (and DMTr) protection allowed a critical switch of acid from 10% TFA in the discovery synthesis to 3 equiv of dry HCl, generated in situ using acetyl chloride in ethanol,[31] thereby avoiding the dramatic degradation of the final product which plagued the initial scale up efforts. Treatment of the phosphoramidate **18** with this HCl solution at 60 °C resulted in complete deprotection of the trityl group in <1 h. In contrast to the original acetonide, DMTr and trityl deprotection of **8** with TFA, which was unscalable over 1 g, this procedure was shown to be successful at as high as 250 g phosphoramidate batch size. After addition of solid sodium bicarbonate and subsequent trituration with TBME to remove the trityl byproduct, the residual crude product was purified by reverse-phase silica chromatography (8:1 w/w silica: crude) to give a 61% yield of prodrug **3**. Overall, this route 3 procedure required only two product isolations, at the penultimate and final compound stages, and was scaled up successfully to provide nucleoside phosphoramidate **3** in 32% yield from 2′-*C*-methylcytidine.[32]

Route Comparison via Selected Green Metrics. It is noteworthy to assess the improvements gained in synthetic efficiency of the scaled up methodology over the original discovery procedure. Fundamental green metrics[33] not only provide an assessment of the environmental impact of synthetic routes but also give an approximate relative indication of their associated throughput, cost and time—important factors in the rapid, first time scale up of early lead drug candidates. Selected green metrics are presented for the three routes to **3** (Table 1).

The original acetonide route actually out-performed the second in terms of overall yield primarily due to the poor reaction yield during the H-phosphonate coupling associated with the unprotected 2′,3′-hydroxyls. The cumulative process mass intensity[34] for route 1, however, was determined to be 30% higher than that for route 2. In contrast, despite the modest absolute values, the boronate route showed marked improvements over the original discovery method in all calculated metrics: the number of isolations was reduced from five to two; both the overall yield and atom economy[35] were increased by half, and the cumulative process mass intensity was reduced by 82%. Although the use of CCl_4 in the Atherton−Todd reaction was not completely obviated, a 97% reduction in the amount of CCl_4 required per gram of final

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Table 1. Comparison of Selected Green Metrics for the Three Routes to 3

route	number of isolations	overall yield (%)	atom economy (%)	process mass intensity[a] (g/g)
route 1, acetonide	5	20	25.5	511
route 2, free 2′,3′-OH	4	13	26.8	394
route 3, boronate	2	32	38.0	93

[a]Cumulative process mass intensity of substrates, reagents, and reaction solvents.

product **3** was achieved. In practice, relative to the unscalable discovery synthesis, route 3 demanded smaller quantities of raw materials, allowed for larger batch sizes, and generated less waste, resulting in a significantly quicker, cheaper, and greener preclinical production campaign.

CONCLUSION

A novel synthetic approach to 2′-*C*-methylcytidine phosphoramidate **3** was developed. In contrast to the original procedure, which was not viable beyond 1 g due to the harsh conditions required to remove the acetonide, the new scalable route featured the application of phenylboronic acid as an alternative, mild, efficient, and transient protecting group. Accordingly, the first example of a 2′-*C*-methylnucleoside-2′,3′-*O*-phenylboronate is reported. Major advantages of the new route included scalability, reduction in the number of steps from seven to five and number of isolations from five to two, increase in the overall yield by 50%, and significant improvement in basic green efficiency metrics. Additionally, synthesis of the H-phosphonate salt **6** was optimized and scaled up to provide this key intermediate in multi-kilogram batch sizes.

EXPERIMENTAL SECTION

General. 2′-*C*-Methylcytidine was obtained commercially or synthesized in-house.[36] All reactions were performed under an argon atmosphere using anhydrous solvents unless otherwise stated. Reduced pressure distillations were performed between 250 and 25 mbar. HPLC spectra were obtained on an Agilent 1100 series instrument equipped with a UV detector at 254 nm and an Agilent Zorbax Eclipse XDB C_8 reverse-phase column (4.6 mm × 75 mm, 3.5 μm) at 25 °C; flow rate 1.4 mL/min; mobile phase A, acetonitrile; B, 0.01 M ammonium acetate aqueous buffer; run time 10 min; gradient, 5 to 80% A over 5.5 min, hold at 80% A for 4.5 min.

2′-*C*-Methylcytidine-2′,3′-*O*-phenylboronate (12). 2′-*C*-Methylcytidine **2** (1.0 g, 3.89 mmol) was suspended in anhydrous MeCN (10 mL) at 20−25 °C. Phenylboronic acid (0.948 g, 7.77 mmol) was charged in one portion, and the mixture was heated at reflux (82 °C) for 2 h. The suspension was cooled to 20−25 °C. The solids were collected by vacuum filtration and dried under vacuum at 30−35 °C to afford 1.01 g (75%) of phenylboronate **12** as a white solid: mp 245−246 °C; *m*/*z* (ESI$^-$, direct injection) 342.2 $[M - H]^-$ 100%; ν_{max} (KBr, cm^{-1}) 3424, 3213, 1651, 1611, 1355; $[\alpha]_D^{20}$ = +15.62 (*c* 1.0, DMSO containing 0.1% H_2O) at *t* = 0; $[\alpha]_D^{20}$ = +78.13 (*c* 1.0, DMSO containing 0.1% H_2O) at *t* = 28 h; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (3H, s, CH_3), 3.74 (1H, ddd, $J_{5',5''}$ 12.1 Hz, $J_{5',OH\text{-}5'}$ 5.6 Hz, $J_{5'',4'}$ 4.2 Hz, *H*-5′), 3.82 (1H, ddd, $J_{5'',5'}$ 12.1 Hz, $J_{5'',OH\text{-}5'}$ 4.5 Hz, $J_{5'',4'}$ 3.4 Hz, *H*-5″), 4.05 (1H, m, *H*-4′), 4.65 (1H, d, $J_{3',4'}$ 5.2 Hz, *H*-3′), 5.78 (1H, d, $J_{5,6}$ 7.5 Hz, *H*-5), 6.16 (1H, br s, *H*-1′), 7.30 (2H, 2 × br s, NH_2), 7.44 (2H, t, *J* 7.6 Hz, 2 × Ar-H_{meta}), 7.55 (1H, dt, *J* 7.6 Hz, *J* 1.3 Hz, Ar-H_{para}), 7.75 (2H, dd, *J* 7.6 Hz, *J* 1.3 Hz, 2 × Ar-H_{ortho}), 7.80 (1H, d, $J_{6,5}$ 7.5 Hz, *H*-6); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.23 (CH_3), 60.49 (*C*-5′), 84.84 (*C*-3′), 85.12 (*C*-4′), 91.23 (*C*-2′), 92.34 (*C*-1′), 93.75 (*C*-5), 127.89 (2 × Ar-C_{meta}), 131.77 (Ar-C_{para}), 134.52 (2 × Ar-C_{ortho}), 141.50 (*C*-6), 154.84, 165.53 (*C*-2, *C*-4). Ar-C_{ipso} not observed. Anal. Calcd for $C_{16}H_{18}N_3O_5B$: C, 56.00; H, 5.29; N, 12.25; B, 3.15. Found: C, 55.95; H, 5.37; N, 12.20; B, 2.99%.

2,2-Dimethyl-3-(trityloxy)propanoic Acid (15). To a reactor were charged methyl 2,2-dimethylhydroxypropionate **13** (1.82 kg, 13.77 mol), dichloromethane (18.2 L), trityl chloride (3.65 kg, 13.10 mol), DMAP (0.168 kg, 1.37 mol), and triethylamine (1.95 kg, 19.27 mol), and the mixture was heated to 40−45 °C. The mixture may be held at that temperature for 1−24 h without detriment to the reaction profile. After being cooled to 20−25 °C, dichloromethane (3.64 L) and water (7.28 L) were added and the biphasic mixture was stirred for 10 min. The organic layer was separated and distilled under vacuum at 38−42 °C. The crude golden oil methyl 2,2-dimethyl-3-(trityloxy)propanoate **14** (assumed 13.77 mol) was transferred using 1,2-dimethoxyethane (16 L) to a reactor containing NaOH (1.103 kg, 27.58 mol) dissolved in water (12.12 L). Further DME (3.6 L) was added, and the reaction mixture was heated to 75 °C for 15 h and monitored by TLC (5% MeOH in DCM). After concentration to approximately 17 L reaction volume and cooling to 15 °C, DCM (15 L) was added and the mixture was acidified with HCl (5 N, 7.32 kg) keeping the internal temperature <15 °C. The organic layer was separated and washed with water (10 L), and DCM was partially distilled at 40 °C to give a volume of approximately 15 L. Heptanes (30 L) were added, and the mixture was cooled from 40 to −10 °C at 3 °C/h. The solids were isolated by filtration on a Nutsche filter equipped with a 50 μm frit and dried under argon to give 3.61 kg of acid **15** as fine off-white crystals in 73% yield: *m*/*z* (ESI$^-$) 259.33 $[TrO]^-$ 100%, 359.28 $[M - H]^-$ 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (6H, s, $C(CH_3)_2$), 3.20 (2H, s, CH_2O), 7.22−7.32 (9H, m, 9 × Ar-*H*), 7.45−7.48 (6H, m, 6 × Ar-*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.52 ($C(CH_3)_2$), 43.50 ($C(CH_3)_2$), 69.52 (CH_2O), 86.46 (CPh_3), 127.00 (3 × Ar-C_{para}), 127.77, 128.75 (6 × Ar-C_{ortho}, 6 × Ar-C_{meta}), 143.82 (3 × Ar-C_{ipso}), 182.65 (COOH).

Triethylammonium 2-(2,2-dimethyl-3-(trityloxy)-propanoylthio)ethyl Phosphonate (6). To a reactor were charged 2,2-dimethyl-3-(trityloxy)propanoic acid **15** (3.61 kg, 10.02 mol) and dichloromethane (21.3 L). A slurry of 1,1′-carbonyldiimidazole (1.706 kg, 10.52 mol) in DCM (2.6 L) was charged portionwise (CO_2(g) evolution). The reaction mixture was stirred not less than 1 h at 20−25 °C. The mixture was cooled to 15−20 °C, and mercaptoethanol (0.90 kg, 11.52 mol) was added to maintain internal temperature at <25 °C. The mixture was stirred for 1.5 h at which point **15** was not observed by TLC analysis (5% MeOH in DCM). The mixture was washed with HCl (5 N, 2 × 7 L) followed by aqueous K_2CO_3 (10%, 10 L) and water (10 L). The lower organic layer was distilled under vacuum at 38−42 °C to give thioester **16**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (6H, s, $C(CH_3)_2$), 3.06 (2H, t, CH_2S), 3.17 (2H, s, CH_2OTr), 3.70 (2H, t, CH_2OH), 7.21−7.30 (9H, m, 9 × Ar-*H*), 7.40−7.42 (6H, m, 6 × Ar-*H*). The crude golden oil **16** (assumed 10.02 mol) was transferred to a reactor with dichloromethane (12.7 L). Phosphorous acid

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(1.65 kg, 20.06 mol) was charged followed by triethylamine (3.0 L, 21.56 mol), keeping the internal temperature at <25 °C. A solution of pivaloyl chloride (1.86 L, 15.10 mol) in DCM (1.8 L) was added to maintain internal temperature at <25 °C, and the mixture was stirred at 20−25 °C for 2 h. Additional triethylamine (2.79 L, 20.06 mol) was charged to the reaction. After 10 min, water (5 L) was added. The settled phases were separated, and the organic layer was distilled under vacuum at 38−42 °C. After coevaporation with acetonitrile (2 × 5 L), H-phosphonate salt **6** containing residual acetonitrile (7.7 kg, 132%) was obtained as a pourable golden oil of sufficient quality (>90% LCAP, <0.4% water content) to be used in the next step. An analytical sample of **6** was obtained after washing 1% TEA in DCM solution of **6** with an aqueous mixture of 3% TEA in brine followed by drying (Na_2SO_4), filtration, and concentration to an oil. Purification using silica gel chromatography (1% TEA in DCM then 1% TEA, 10% MeOH in DCM as eluent) gave **6** as a clear, colorless, viscous oil: m/z (ESI^-) 483.1 $[M - NEt_3 - H]^-$ 100%; 967.4 $[2(M - NEt_3) - H]^-$ 25%; ν_{max} (film, cm^{-1}) 3388 (br), 2980, 1647 (br), 2603, 1676 (s); ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (6H, s, $(CH_3)_2C$), 1.30 (9H, t, J 7.3 Hz, $N(CH_2CH_3)_3$), 3.02 (6H, q, J 7.3 Hz, $N(CH_2CH_3)_3$), 3.15 (2H, s, CH_2OTr), 3.18 (2H, t, J 6.9 Hz, CH_2S), 3.96 (2H, dt, J 8.6 Hz, J 6.9 Hz, CH_2OP), 6.87 (1H, d, $^1J_{H-P}$ 617 Hz, P-H), 7.20−7.24 (3H, m, 3 × Ar-H_{para}), 7.27−7.31 (6H, m, 6 × Ar-H_{meta}), 7.39−7.43 (6H, m, 6 × Ar-H_{ortho}), 12.71 (1H, br s, Et_3N^+-H); contains 0.4 mol MeOH by proton NMR; ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.50 (3 × NCH_2CH_3), 22.87 ($C(CH_3)_2$), 29.55 ($^3J_{C-P}$ 6.8 Hz, CH_2S), 45.38 (3 × NCH_2CH_3), 50.86 ($C(CH_3)_2$), 62.32 ($^2J_{C-P}$ 4.3 Hz, CH_2OP), 69.87 (CH_2OTr), 86.35 (CPh_3), 126.93 (3 × Ar-C_{para}), 127.71 (3 x Ar-C_{meta}), 128.78 (3 × Ar-C_{ortho}), 143.86 (Ar-C_{ipso}), 204.52 (C═OS); ^{31}P NMR (162 MHz, $CDCl_3$) δ 4.33 (1P, s). Anal. Calcd for $[C_{32}H_{44}NO_5PS.(0.4\ CH_3OH)]$: C, 65.01; H, 7.68; N, 2.34; S, 5.36; P, 5.29. Found: C, 64.57; H, 7.56; N, 2.10; S, 5.12; P, 5.30.

2′-*C*-Methylcytidine-5′-[2-[(3-trityloxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzylphosphoramidate] (18). Pyridine (2.5 L) was charged to 2′-*C*-methylcytidine **2** (150 g, 0.583 mol) at 20−25 °C. Phenylboronic acid (78 g, 0.626 mol) was added, and the mixture was heated at reflux (115 °C) for 3 h. The pyridine−water azeotrope was then distilled at atmospheric pressure, removing 1.2 L of distillate. The mixture was cooled to 20−25 °C, and the pyridine was evaporated under vacuum to obtain a golden oil. Analysis of the crude material by ^1H NMR indicated greater than 97% conversion to the desired phenylboronate **12**, which was stable stored under argon at 20−25 °C for >15 h. H-Phosphonate salt intermediate **6** (0.615 kg, 1.049 mol) was dissolved in acetonitrile (3 L) at 20−25 °C. A solution of **12** (assumed 0.583 mol) in pyridine (250 mL) was charged to the mixture followed by EDCI·HCl (0.570 kg, 2.973 mol), and the reaction was stirred at 41−46 °C for 4 h. The reaction was cooled to <20 °C, and benzylamine (395 mL, 3.61 mol) was added followed by carbon tetrachloride (260 mL, 2.69 mol) controlling the internal temperature to <20 °C. After 1 h, EtOAc (1 L) was charged to the mixture and acidified to pH 4 with aqueous citric acid (20% w/w, 3 L) to effect cleavage of the cyclic boronate. The separated aqueous phase was extracted with EtOAc (2.5 L). The combined organics were washed with aqueous citric acid (10% w/w, 3 L) then twice with aqueous $NaHCO_3$ (saturated, 5 L then 2 L). The organic phase was dried (Na_2SO_4), filtered, and evaporated at 25−35 °C to give 712 g of crude **18** as a yellow foam. The crude residue was dissolved in DCM (1 L) and purified on a silica gel (2.3 kg) plug. Elution was carried out using an increasing gradient of MeOH in DCM; 4% (7 L), 5% (3 L), 6% (2 L), 7% (10 L). Evaporation of the appropriate fractions gave 254 g (53% yield; 98.5% LCAP) and 73 g (13% yield; 87.6% LCAP, repurified with subsequent batches) of trityl phosphoramidate **18** with combined yield 65%: ^1H NMR (400 MHz, DMSO-d_6) two diastereomers δ 0.95 (3H, s, CH_3), 1.14 (6H, s, $(CH_3)_2C$), 3.06 (2H, s, CH_2OTr), 3.10 (2H, m, CH_2S), 3.59 (1H, br m, H-3′), 3.83−4.02 (5H, m, H-4′, CH_2O, CH_2Ph), 4.09−4.13 (1H, m, H-5′), 4.14−4.28 (1H, m, H-5″), 5.11 (1H, s, OH-2′), 5.30 (1H, br d, J 6.6 Hz, OH-3′), 5.70 (1H, d, J 7.4 Hz, H-5), 5.72−5.79 (1H, m, P-N-H), 5.94 (1H, br s, H-1′), 7.19−7.35 (22H, m, 20 × Ar-H, NH_2), 7.55, 7.59 (2 × 0.5H, 2d, J 7.5 Hz, H-6); ^{31}P NMR (162 MHz, DMSO-d_6) two diastereomers δ 9.68−9.95 (1P, m).

2′-*C*-Methylcytidine-5′-[2-[(3-hydroxy-2,2-dimethyl-1-oxopropyl)thio]ethyl-*N*-benzylphosphoramidate] (3). Trityl phosphoramidate **18** (246 g, 0.296 mol) was dissolved in anhydrous EtOH (3.5 L), and acetyl chloride (62.6 mL, 0.88 mol) was added resulting in an exotherm from 18 to 27 °C. The mixture was heated to 60−65 °C for 45 min at which point it was cooled to <25 °C. Solid sodium bicarbonate (1.04 kg) was added portionwise, bringing the pH to 5.5−6. The mixture was filtered through Celite and washed with EtOH (7 L) prior to concentration of the filtrate at 35 °C. The residue was triturated with TBME (3 L) for 1 h and then filtered to obtain 185 g of crude product **3** (93% LCAP). The crude material was dissolved in MeCN (58 mL), water (164 mL), and aqueous $NaHCO_3$ (saturated, 170 mL) and purified by chromatography on reverse-phase silica (octadecyl, 40 μm, 1.5 kg, prewashed with a gradient of 100% MeCN to 100% water). Elution was carried out using a stepwise gradient of MeCN in water: 3, 10, 15, and held at 25% to obtain the product. Evaporation of the appropriate fractions gave 106 g (61% yield; 98.5% LCAP) of phosphoramidate **3** as a white solid: m/z (ESI^+) 587.12 $[M + H]^+$ 100%; 1173.62 $[2M + H]^+$ 80%; ν_{max} (KBr, cm^{-1}) 3343 (br), 1647 (br), 1616, 1495; $[\alpha]_D^{20}$ = +55.01 (c 1.0, DMSO); ^1H NMR (400 MHz, DMSO-d_6) two diastereomers δ 0.94 (3H, 2s, CH_3), 1.11 (6H, s, $(CH_3)_2C$), 3.04 (2H, m, J 6.4 Hz, CH_2S), 3.44 (2H, d, J 5.0 Hz, CH_2OH), 3.60 (1H, br m, H-3′), 3.82−4.01 (5H, m, H-4′, CH_2O, CH_2Ph), 4.07−4.12 (1H, m, H-5′), 4.13−4.24 (1H, m, H-5″), 4.94 (1H, t, J 5.0 Hz, CH_2OH), 5.07 (2 × 0.5H, 2s, OH-2′), 5.26 (1H, t, J 6.8 Hz, OH-3′), 5.64−5.76 (1H, m, P-N-H), 5.69, 5.70 (2 × 0.5H, 2d, 2 × J 7.6 Hz, H-5), 5.93 (1H, br s, H-1′), 7.13−7.20 (2H, 2 × br s, NH_2), 7.20−7.25 (1H, m, Ar-H), 7.28−7.35 (4H, m, 4 × Ar-H), 7.53, 7.57 (2 × 0.5H, 2d, J 7.6 Hz, H-6); ^{13}C NMR (100 MHz, DMSO-d_6) two diastereomers δ 19.81 (CH_3), 21.79 ($C(CH_3)_2$), 28.17, 28.24 (CH_2S), 44.18 ($PhCH_2$), 51.62 ($C(CH_3)_2$), 63.74, 63.79 (CH_2O), 64.21, 64.51 (C-5′), 68.29 (CH_2OH), 72.41, 72.57 (C-3′), 77.80, 77.85 (C-2′), 79.47, (C-4′), 91.66, (C-1′), 93.82 (C-5), 126.68, 127.09, 128.08, 128.09 (5 x Ar-C), 140.34, 140.38, 140.40 (Ar-C_{ipso}, C-6), 155.12, 165.21 (C-2, C-4), 203.85 (C═OS); ^{31}P NMR (162 MHz, DMSO-d_6) two diastereomers δ 9.71, 9.91 (1P, 2s, ratio 1.00:1.07). Anal. Calcd for $C_{24}H_{35}N_4O_9PS$: C, 49.14; H, 6.01; N, 9.55; S, 5.47; P, 5.28. Found: C, 48.74; H, 5.83; N, 9.41; S, 5.81; P, 5.33.

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Notes

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(23) PBA protection of other unnatural nucleosides has also been performed and will be reported in due course.

(24) Values of $[\alpha]_D^{20}$ (*c* 1.0, DMSO containing 0.1% H_2O).

(25) Data qualitatively illustrate the general trend for the specific rotation to tend toward the value for 2′-*C*-methylcytidine over time. Concentrations differ.

(26) Liquid chromatography area percentage.

(27) Although not performed, concentration to 1/10th volume and telescoping directly to the coupling reaction is anticipated to be feasible.

(28) Boronates **12** and **17** were completely deprotected during the reverse-phase HPLC analysis; therefore, the respective 2′,3′-diols were monitored.

(29) It remains to be determined whether arylboronic acids in general represent a genotoxic hazard. O'Donovan, M. R.; Mee, C. D.; Fenner, S.; Teasdale, A.; Phillips, D. H. *Mutat. Res.* **2011**, *724*, 1−6. Beyond preclinical development, the potential for PBA to be a genotoxic impurity in **3** would need to be considered. Methods to control residual arylboronic acids in APIs are discussed in http://www.andersonsprocesssolutions.com/controlling-residual-arylboronic-acids-as-potential-genotoxic-impurities-in-apis/#_ednref1 (Accessed 01 April 2014).

(30) A further 13% yield with 88% LCAP purity was also obtained (which was combined and repurified with subsequent batches) to give a total yield of 65% from 2′-*C*-methylcytidine.

(31) Highly exothermic.

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