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Development of a Diastereoselective Phosphorylation of a Complex Nucleoside via Dynamic Kinetic Resolution

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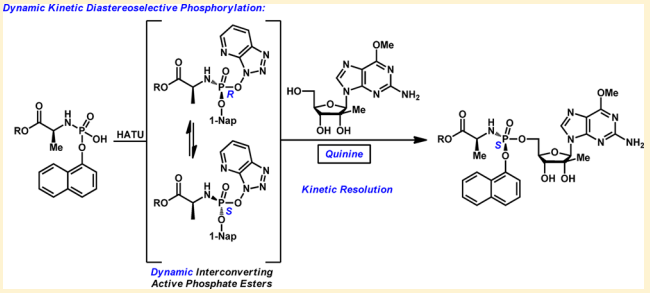
Supporting Information

ABSTRACT: The development of a diastereoselective nucleoside phosphorylation is described, which produces a single isomer of a complex nucleoside monophosphate pro-drug. A stable phosphoramidic acid derivative is coupled to the nucleoside, in a process mediated by HATU and quinine, to deliver the coupled product in high chemical yield and good diastereoselectivity. This unusual process was shown to proceed through a dynamic kinetic resolution of a 1:1 mixture of activated phosphonate ester diastereoisomers. The optimized conditions afforded the product with a combined [*S,S(P)*] and [*S,R(P)*] in-process yield of 89% and a ~7:1 [*S,S(P)*:*S,R(P)*] diastereomeric ratio. Isolation of the major isomer was facilitated by single crystallization from anisole, where the product was obtained in 57% isolated yield, excellent purity (>95%), and a high diastereomeric ratio (>50:1).



INTRODUCTION

Recently, phosphoramidate analogues of nucleosides have been investigated as potential antiviral therapeutics for the treatment of viral diseases (such as HBV,[1] HCV,[2] and influenza).[3] The parent nucleosides generally suffer from poor activity, caused by rate limiting *in vivo* monophosphorylation, a required step in the formation of the active nucleoside triphosphate.[4] Thus, preformation of the phosphoramidate acts as a pro-drug strategy, overcoming this limitation and releasing the monophosphate directly, aiding the formation of the active triphosphate. While this approach has been successful in the clinical setting, the newly introduced stereogenic center creates additional synthetic complexity; the stereogenic center located on phosphorus is exceptionally challenging to prepare in a selective fashion.[5] As a result, many phosphoramidate derivatives are isolated as mixtures of *P*-diastereoisomers, which presents challenges in terms of the uniformity, crystallinity, and stability (among others) of the potential therapeutic agent.[6] In several instances, isolation of the phosphorus diastereomers has revealed significant differences in biological activity between the two isomeric phosphate esters,[7] clearly demonstrating the importance of methodologies capable of delivering chiral phosphates as single isomers. Unfortunately, limited methods exist to address this problem. In one notable example, Miller and co-workers have shown that asymmetric phosphorylations are indeed possible with a peptide-based catalyst.[8] More commonly, however, either extensive chromatography or fractional crystallization is used.[9]

A recent example of the crystallization strategy was developed by Cho and co-workers, where a single isomer of a chiral phosphoramidate electrophile was utilized.[7,10] This approach leveraged the known stereospecific displacement of P(V) electrophiles;[11] in this example an optically pure phosphoramidate, containing a *p*-nitrophenol leaving group, was isolated by fractional crystallization. The desired isomer was reacted with the C5′−OH of the nucleoside, delivering a diastereomerically pure phosphoramidate with inversion of the stereochemistry at phosphorus. While effective in the described example, this approach is only practical if the electrophile is crystalline, stable, and utilizes readily available materials. As this may not always be the case, an additional method for the direct preparation and isolation of a single phosphoramidate nucleoside diastereomers is highly desirable.

In the context of a recent program, we sought to join a phosphoramidate (of type **1**) with nucleoside **2** to prepare phosphates **3**. During the discovery of these molecules, chlorophosphoramidate **1** had been employed with the magnesium alkoxide of **2**, in a manner analogous to the work of Noyori and co-workers (Scheme 1).[12] While this reaction was successful, it was plagued by low solution yields (<40%) and a correspondingly poor mass balance (~50%), attributed to the instability of the chlorophosphoramidate **1**.[13] In addition to these problems, phosphate **3** was isolated as an oil, a 1:1 mixture of diastereomers and in only ~27% yield after extensive chromatography.[14] While prior research had shown that the P(*S*) diastereomer (*S,S*)-**3** could be crystallized from anisole as its hemisolvate, the poor yield made the direct use of this method unfeasible.[13] Alternatively, and analogous to the work of Cho, either diastereomer could be accessed via phosphor-

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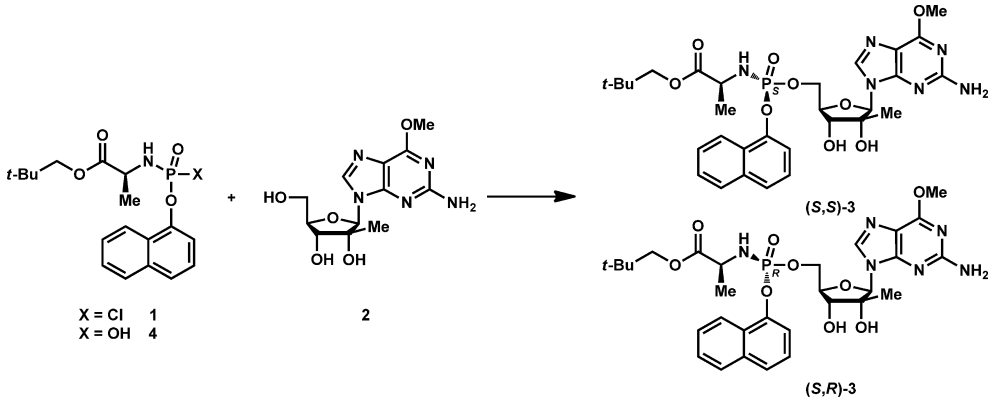
Scheme 1



Scheme 2. Synthesis of Phosphoramidic Acid

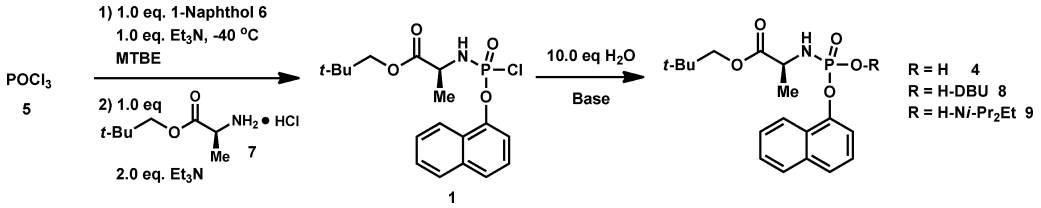
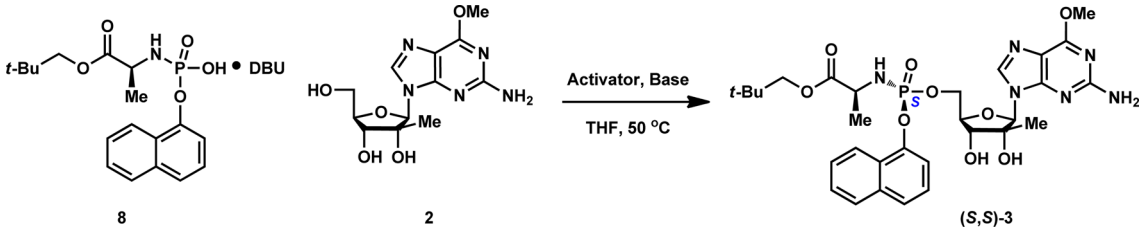


Table 1. Optimization of Bases



entry[a]	base	solution yield	solution yield of P(*S*)	ratio of P(*S*)/P(*R*)
1	DMAP	21.9	16.2	2.8
2	(*S*)-C_5Ph_5-DMAP	6.1	4.0	1.9
3	(*R*)-C_5Me_5-DMAP	7.5	5.0	2.0
4	quinidine	19.3	15.2	3.7
5	quinine	21.9	18.8	6.0
6	TEA	14.5	11.2	3.4
7	DBU	16.3	12.9	3.9
8	TMEDA	17.6	13.7	3.4
9	imidazole	2.6	1.9	3.0
10	K_2CO_3	0.0	0.0	0.0

[a]1.0 equiv nucleoside (1.0 g scale), 2.0 equiv DBU phosphate **8**, 1.0 equiv of HBPyU, 1.0 equiv base, and 2:1 CH_3CN/THF, 50 °C.

ylation with a diastereomerically pure *p*-nitrophenol phosphoramidate.[15] Unfortunately, fractional crystallization of the *p*-nitrophenol ester was not practical in this case. We therefore interrogated new approaches for the diastereoselective phosphorylation of nucleoside **2**.

After exploring several options, we decided to focus on a strategy employing standard coupling reagents to directly couple the phosphoramidic acid **4** (which contains no stereochemical information at the phosphorus) with the nucleoside (Scheme 1). We hoped to leverage the inherent stereochemistry of the nucleoside partner **2** to bias the coupling process and control the phosphorus stereochemistry.[16] While little precedence exists for this type of coupling, Ikehara and coworkers had reported the synthesis of dinucleotide phosphate esters using (mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT), starting from a phosphoramidic acid and obtaining a 1.3:1 mixture of diastereomers.[17] In contrast to phosphorus, many protocols exist for generating stereochemistry centered at the sulfur,[18] including both direct enantioselective methods[19] along with approaches such as dynamic kinetic resolution.[20] Herein, we describe the development of a diastereoselective coupling of a crystalline calcium phosphoramidic acid derivative and a nucleoside via a dynamic kinetic resolution. In this coupling process, quinine (a readily available compound) was found to significantly improve the observed diastereoselectivity, enabling the product to be isolated in high purity with a > 50:1 diastereomeric ratio.

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RESULTS AND DISCUSSION

The synthesis of the requisite phosphoramidic acid **4** was easily achieved by hydrolysis of the known chlorophosphoramidate **1** (Scheme 2).[10] However, attempts to purify and isolate the free acid **4** were inhibited by instability, with a significant self-catalyzed dehydration being observed to form the anhydride **4** (*vide infra*). Solutions of **4** were slightly more stable; however, decomposition within 24 h made direct use in the exploration of the key coupling reaction challenging. Gratifyingly, we found that acid **4** could be stabilized by the addition of amine bases such as DBU or diisopropylethylamine.[21] These amine salts could be stored and isolated, allowing us to explore the feasibility of the diastereoselective coupling process.

The key coupling reaction was initially investigated with DBU salt **8** and nucleoside **2** in the presence of a variety of different peptide coupling reagents.[22] After extensive screening, the product was obtained in low yield using HBPyU and DMAP in CH_3CN/THF at 40 °C; encouragingly, product **3** was observed as a 3:1 mixture of isomers, favoring the desired P(*S*) stereochemistry (Table 1, entry 1). In an effort to improve the diastereoselectivity of the coupling, a screen of chiral and achiral bases was performed (Table 1). Quinine was quickly identified as the optimal additive, providing an improvement in selectivity, to a moderate 6:1 d.r. (Table 1, entry 5). To address the low conversion of this reaction, we increased the amount of coupling agent to 2.0 equiv and screened additional reagents to further optimize the yield (Table 2). This screen resulted in some clear trends for both overall yield and selectivity. Coupling agents containing benzotriazole (Table 2, entries 1−4) and azobenzotriazole (Table 2, entries 5−7) afforded the product in ∼60% yield and 5−8:1 d.r. The counterion of the reagent had little effect on the diastereoselectivity; however, in general hexafluorophosphate outperformed tetrafluoroborates. Interestingly, reagents containing Oxyma (Table 2, entries 8−9)[23] gave the P-(*S*) product with very high diastereomeric ratios but with significantly lower conversion. The combination of HATU and quinine in THF provided the best balance of diastereoselectivity and reactivity, resulting in a 62% solution yield of **3** in a 7.8:1 diastereomeric ratio (Table 2, entry 6).

Table 2. Optimization of Activators

entry[a]	activator	solution yield	solution yield of P(*S*)	ratio of P(*S*)/P(*R*)
1	HBPyU	59.4	50.4	5.6
2	PyBOP	56.4	46.7	4.9
3	BOP	58.6	48.8	5.0
4	TBTU	40.2	32.8	4.5
5	TATU	47.5	41.6	7.1
6	**HATU**	**61.8**	**54.8**	**7.8**
7	PyAOP	59.3	51.4	6.5
8	HOTU	35.6	34.2	24.4
9	TOTU	20.2	19.3	23.3

[a]1.0 equiv nucleoside (1.0 g scale), 2.0 equiv DBU phosphate **8**, 2.0 equiv of activator, 2.0 equiv quinine, and THF, 50 °C.

Control experiments showed that quinine was crucial for maximizing the observed diastereomeric ratio. In an effort to understand the role of quinine, a structure−activity study using TOTU was conducted (Table 3). Although TOTU was not identified as the optimal coupling reagent, Oxyma based systems, which provided the highest levels of diastereoselectivity, were ideal for probing quinine's role in the phosphoramidic acid coupling. Interestingly, quinidine, the pseudoenantiomer of quinine, provides the same phosphoramidate diastereomer (*S*,*S*)-**3**, but with a reduced level of selectivity (5.5:1 d.r., Table 3, entry 2), whereas quinine yields the desired compound in 16.3:1 d.r. under these conditions (Table 3, entry 1). This implies that the primary source of stereoinduction is provided by either the chiral environment of the nucleoside itself or the amino acid center on the electrophile and that quinine merely enhances the innate diastereoselectivity. Protection of the hydroxyl group in quinine leads to a dramatic loss in selectivity (entry 3). Further investigation of truncated forms of quinine showed the same sense of stereoinduction (entries 4−6) but at a consistently lower level when compared to that of quinine itself. These results suggested that quinine may be enhancing diastereoselectivity via preorganization through a hydrogen bond donor−acceptor complex.

Mechanism. Concurrently, a mechanistic study was undertaken in an effort to assist in the reaction optimization and to understand how diastereoselection was being achieved. We explored the possibility of a kinetic (or potentially dynamic kinetic) resolution of the activated phosphate esters (*S*,*S*)-**10** and (*S*,*R*)-**10** (Table 4). Assuming a mechanism analogous to amide bond formation with uronium salts such as HATU, reaction with phosphoramidic acid **8** would generate two activated phosphate esters (*S*,*S*)-**10** and (*S*,*R*)-**10**. On the basis of prior work by Cho and co-workers,[7] an invertive displacement by nucleoside **2** at phosphate ester (*S*,*S*)-**10** would then provide the observed phosphoramidate (*S*,*S*)-**3**. In this scenario, the stereogenic center at phosphorus could be formed either through (1) the activation step with HATU, preferentially generating (*S*,*R*)-**10**, or (2) from different rates of displacement by **2** on a 1:1 mixture of activated esters (*S*,*S*)-**10** and (*S*,*R*)-**10**. In this second case, a dynamic kinetic resolution may exist if the rate of interconversion between (*S*,*S*)-**10** and (*S*,*R*)-**10** exceeds the rate of coupling. To determine which pathway was operating, and the best approach to optimize this transformation, a series of ^{31}P NMR experiments was designed to investigate the reactivity of the proposed, activated esters **10**.

Examination of mixtures of the DBU salt **8** and HATU revealed the rapid formation of two new species in a 1:1 ratio. These were assigned as the diastereomeric activated ester intermediates **10**.[24] In spite of the presence of the stereogenic center in the alanine side chain, the two activated esters **10** were formed with little or no selectivity, regardless of the structure of the activator (Table 4).[25] This result is particularly informative since we had observed significant differences in selectivity between these reagents (see Table 2, entries 1, 6, and 9). On the basis of these observations, selective formation of a single diastereomer of the active ester intermediate **10** is not controlling the diastereoselectivity of the coupling reaction.

We then monitored the fate of these two species during the reaction via ^{31}P NMR. Upon addition of quinine and nucleoside **2**, a steady decrease in these signals was observed, but the ratio of these intermediates remained unchanged throughout the reaction (Figure 1). Additionally, the diastereomeric ratio of the product (*S*,*S*)-**3** is constant with respect to conversion. This data strongly supports a mechanism where two rapidly interconverting diastereomers of the activated esters (*S*,*S*)-**10** and (*S*,*S*)-**10** are formed. The stereodefining event would therefore be the final phosphorus−oxygen bond formation where the nucleoside **2** reacts at different rates with the two diastereomeric active esters **10**. On the basis of these results, the reaction is a dynamic kinetic resolution, and >50% yield of

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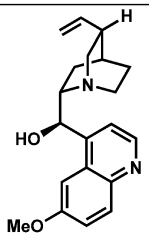
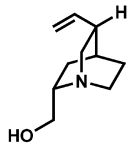
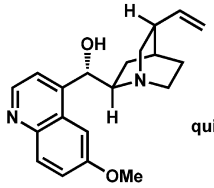
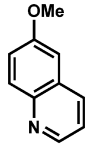
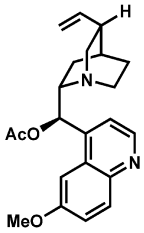
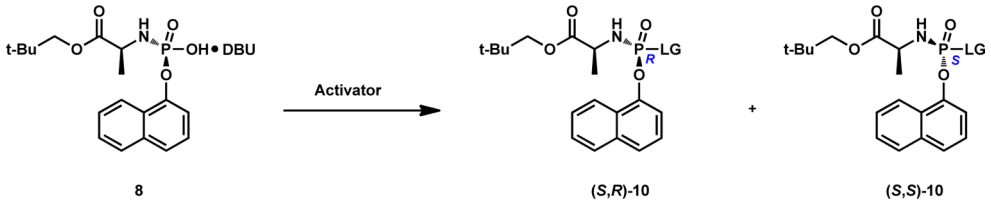
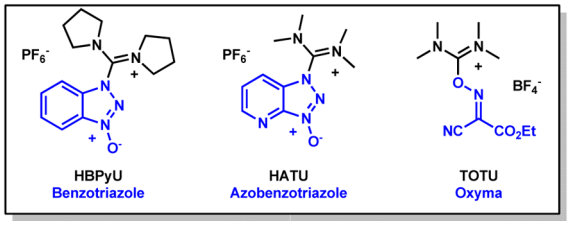
Table 3. SAR of Quinine Using TOTU as Activator[a]

Entry	Additive	Ratio P(*S*)/P(*R*)	Entry	Additive	Ratio P(*S*)/P(*R*)
1	 quinine	16.3	4	 QCl	8.4
2	 quinidine	5.5	5	 methoxy isoquinoline	6.4
3	 acetoxy quinine	4.3	6	 quinuclidine	6.7

[a]1.0 equiv nucleoside (1.0 g scale), 2.0 equiv DBU phosphate **9**, 2.0 equiv of TOTU, 2.0 equiv additive, and THF, 50 °C.

Table 4. ^{31}P NMR Investigation of the Reaction of 8 and Uronium Salts





entry	activator	ratio of active esters (10)	ratio of coupled product P(*S*):P(*R*) 3
1	HBPyU	54:46	85:15
2	HATU	54:46	89:11
3	TOTU	44:56	96:4

the desired diastereomer should be possible with optimized conditions.

During the course of the study, variable levels of the dimeric phosphoric acid anhydrides **11** (a mixture of three diastereoisomers) were observed during the coupling reaction.[26] Control experiments with isolated anhydride **11** showed poor activity for the phosphorylation of nucleoside **2**, and therefore, its formation needed to be minimized in order to optimize the yield. Two pathways to the formation of anhydride **11** were found. Counterintuitively, increasing the water content in the reaction during the activation resulted in the higher level of anhydrides **11** being observed (Table 5). Thus, careful control of water content during the reaction is critical for high conversion to the desired product. However, the amount of water present did not correlate with the amount of anhydride **11** formed. We considered that the formation of **11** could be due to a reaction of phosphoramidic acid **8** with activated esters **10** during the activation process (Scheme 3). When we examined the formation of **10** with a variety of coupling agents, it became clear that the intermediate uronium species **12** is a likely source for the formation of this inactive form of the phosphoramidic acid. It is presumed that reaction of phosphoramidic acid **8** with uronium-based coupling agents leads to initial formation of uronium ester **12**. This species then

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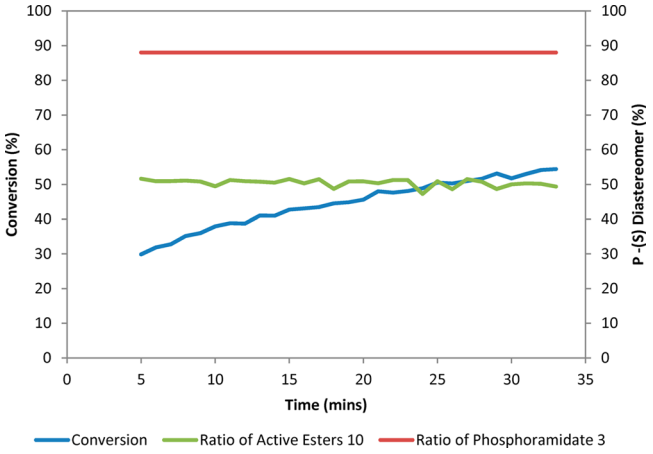


Figure 1. ^{31}P NMR monitoring of reaction of HATU, phosphate **8**, nucleoside **2**, and quinine.

Table 5. Effect of Water on Anhydride Formation

entry	water (equiv)	anhydride (%)
1	0.06	40.6
2	0.25	83.7
3	1.00	97.6

reacts with either residual **8**, to give the undesired anhydrides **11**, or the counterion (e.g., hydroxybenzotriazole) to give the activated ester intermediates **13**. Whereas the use of HATU in the presence of 0.06 equiv H_2O led to the formation of 40% **11**, the use of the coupling agent PyClU (X = Cl) led to exclusive formation of anhydride **11**. Reaction of phosphoramidic acid **8** and active ester **13** as a secondary pathway to the anhydride **11** may also be operative.

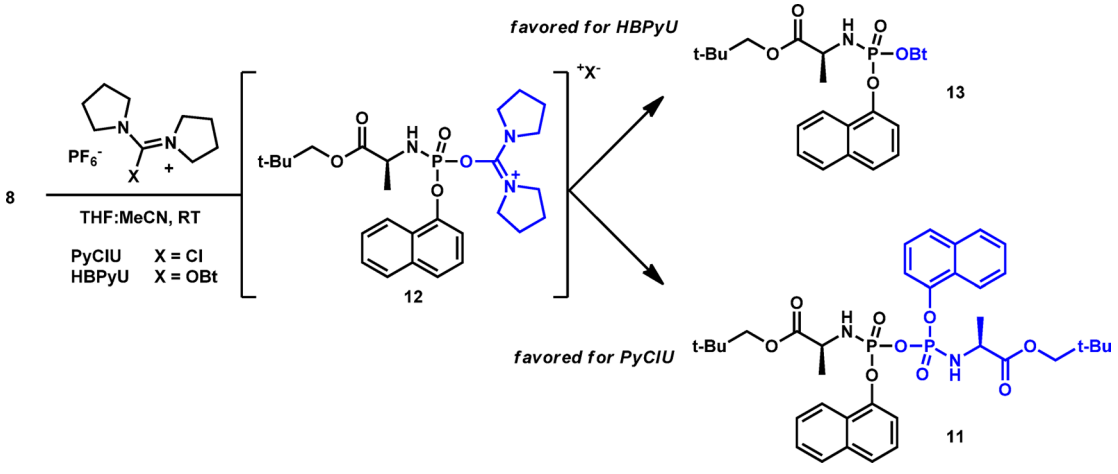
When the fate of the anhydrides **11** was examined under the reaction conditions with HATU and quinine, it was found to be active in the phosphorylation of nucleoside **2** (Figure 2), albeit at a much slower rate than the active esters **10**. To understand the rationale for the decreased reaction rate of anhydrides **11**, several control experiments were performed. Direct reaction of the mixture of anhydrides **11** with nucleoside **2** in the presence of quinine did not generate the desired phosphoramidate **3**, showing that the direct coupling with nucleoside **2** and anhydride **11** is not viable. Rather, it was found that in the presence of hydroxyazabenzotriazole (HOAt) and quinine, regeneration of a 1:1 mixture of the active ester **10** and the phosphoramidic acid **4** was observed, demonstrating that HOAt was capable of recycling the inactive anhydride back to activated ester **10**. Therefore, a pathway exists for the inactive anhydrides **11** to proceed on to the desired product but only after regeneration of the active esters **10** (Scheme 4).

Having gained a more complete picture of the overall reaction, we had a clear path to optimize the reaction. It was recognized that identification of an anhydrous form of the phosphoramidic acid, along with further adjustment to the charges of HATU and quinine, would be required to control the amounts of inactive anhydrides **11** and obtain higher levels of conversion. A key issue we wished to address was the salt form of phosphoramidic acid **4**. Although the DBU salt **8** was utilized to this point, it is difficult to purify and can only be isolated as a viscous gel. We recognized the need to identify a much more robust, crystalline form of the phosphoramidic acid coupling partner. Through a crystallization screen, we then identified the calcium diphosphoramidate dihydrate **15** salt (Scheme 5) as an ideal candidate for isolation. Treatment of the DABCO phosphate salt **14** with aqueous calcium chloride and isopropanol promoted the crystallization of **15** directly from the aqueous stream, producing easily filtered thin rods. This salt form was readily prepared on a multigram scale and was isolated in 69% yield from **1** with >98 area percent purity. With a stable, isolable salt form in hand, we then turned our attention to optimizing the coupling reaction.

Because of the inactivity of the calcium phosphate dihydrate **15** in common organic solvents, as well as the presence of water from the resulting dihydrate, calcium phosphate **15** cannot be used directly in the coupling reaction. Thus, we liberated the phosphoramidic acid for the coupling process. This was accomplished by partitioning **15** in aqueous hydrochloric acid (1.0 N) and 2-methyltetrahydrofuran (MeTHF). Diisopropylethylamine (1.0 equiv) was added to increase the stability of the solution of phophoramidic acid in MeTHF at elevated temperatures and to facilitate azeotropic removal of water following the acid wash. The use of diisopropylethylamine *in lieu* of DBU afforded greater stability during azeotropic drying and a cleaner reaction profile in the subsequent coupling.

The first parameter examined for the optimization of the coupling was the impact of quinine equivalents on diastereoselectivity (Table 6, entries 1−8). We found that at >1 equiv

Scheme 3. Formation of Anhydride 11



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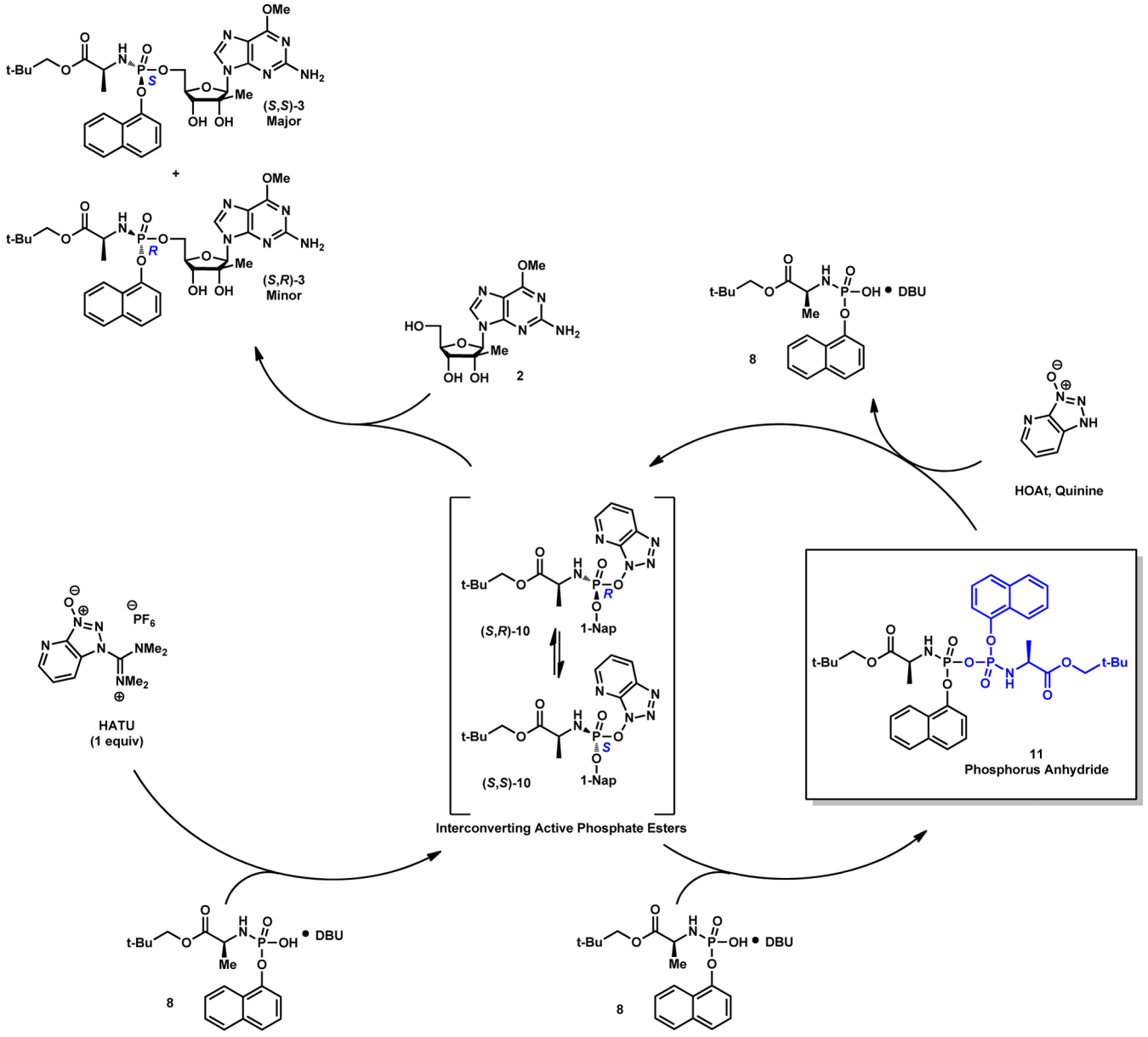
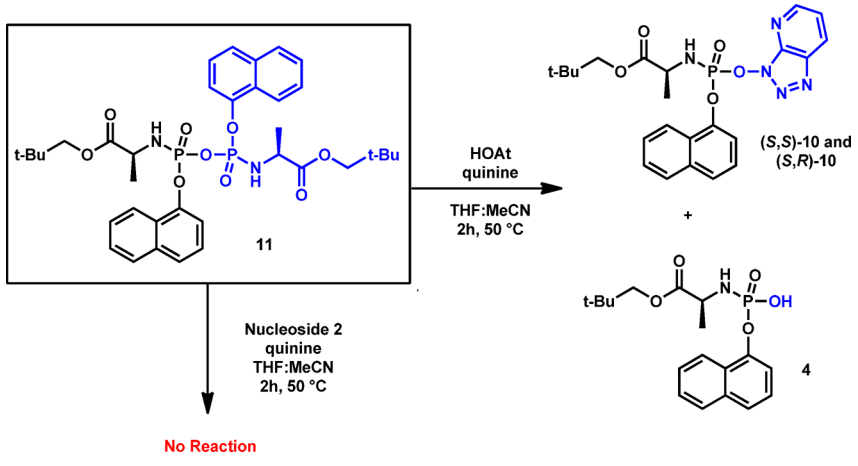


Figure 2. Proposed mechanism for the phosphorylation of **2**.

Scheme 4. Fate of Phosphorus Anhydride 11



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Scheme 5. Generation of Crystalline Calcium Phosphate 15

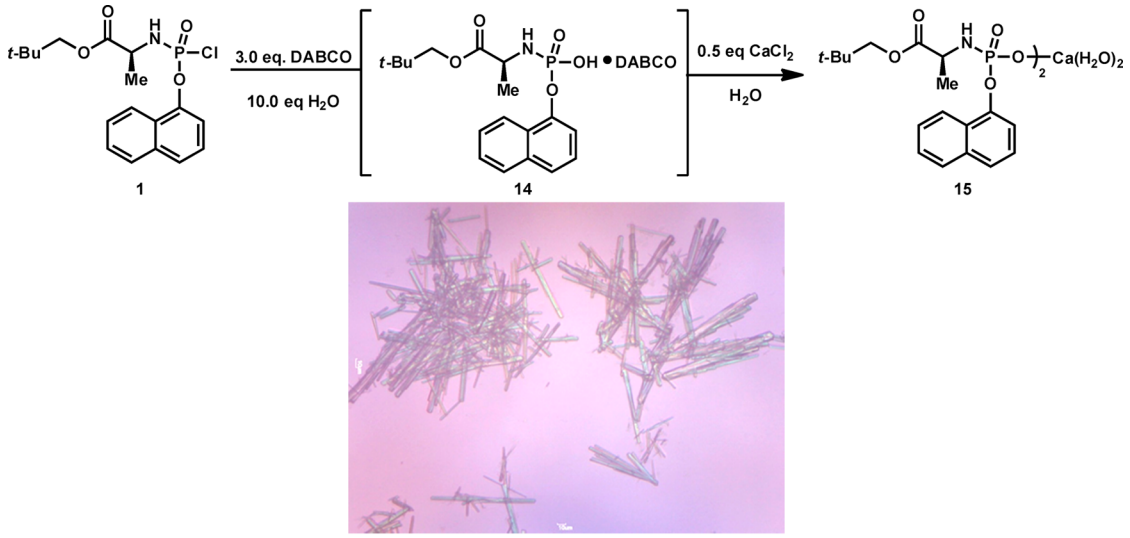
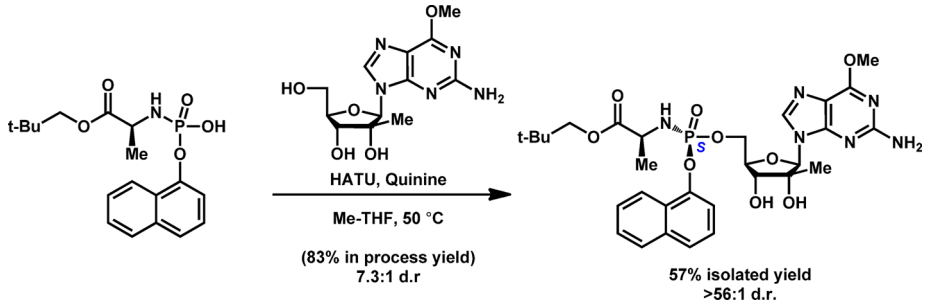


Table 6. Optimization of the Reaction



entry	quinine (equiv)	phosphoramidic acid (equiv)	solution yield[a]	ratio P(*S*)/P(*R*)
1[b]	2.0	2.00	85.7	5.9
2	1.0	2.00	97.4	6.9
3	0.9	2.00	98.7	6.7
4	0.8	2.00	94.4	6.6
5	0.7	2.00	93.1	6.4
6	0.6	2.00	95.2	6.2
7	0.5	2.00	74.4	6.0
8	0.1	2.00	73.2	3.8
9[c]	**0.9**	**1.50**	**82.8**	**7.3**
10	0.9	1.25	76.0	8.3
11	0.9	1.00	54.7	9.1

[a]In process yield of both (*S*,*S*)-**3** and (*S*,*R*)-**3** diastereomers. [b]Entries 1−8 were conducted in 13.4 mL/g 2-methyltetrahydrofuran at 50 °C. [c]Entries 9−11 were conducted in 15.0 mL/g 2-methyltetrahydrofuran at 50 °C.

and <0.6 equiv of quinine relative to nucleoside, that d.r. and yield were negatively impacted (entry 1, 6−8). The optimal quantity of quinine was between 1.0 and 0.9 equiv (entry 2,3). As a result of the challenges in removing phosphoramidic acid during the isolation, next, we sought to reduce the amount of the phosphoramidic acid relative to nucleoside **2** in the reaction. Gratifyingly, 1.5 equiv provided high yield and good in-process d.r. (~80% yield, 7:1 d.r.) while maintaining the desired purity of isolated (*S*,*S*)-**3**. Further reduction in the equivalents of acid **4** led to low conversion but interestingly higher d.r. (9:1). As a final observation, an additional charge of 0.75 equiv of diisopropylethylamine during the coupling was found to prevent reaction stalling. The final optimized set of conditions was 1.5 equiv of the phosphoramidic acid, 2.0 equiv HATU, 0.9 equiv quinine, and 1.0 equiv nucleoside in 2-methyltetrahydrofuran (15 mL/g of **2**) at 50 °C. Once complete, the reaction mixture was diluted with isopropyl acetate and washed with an aqueous 10 wt % solution of L-tartaric acid. This served to remove ~12% of the quinine; a key impurity that prevented efficient crystallization. A subsequent wash with an aqueous 5 wt % solution of sodium carbonate, then water served to neutralize the reaction mixture and remove remaining tetramethylurea and HOAt. The solvent was then partially exchanged to anisole (<10 vol % 2-methyltetrahydrofuran) which served to both azeotropically remove water and also facilitate crystallization of (*S*,*S*)-**3** as the anisole hemisolvate. The solution was seeded with the pure P(*S*) diastereomer, and the mixture was allowed to crystallize

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overnight. The desired product (*S*,*S*)-**3** was obtained as a white crystalline compound in 57% yield.

CONCLUSIONS

A short sequence was developed and demonstrated to allow rapid and efficient access to a single diastereomer of a highly functionalized nucleoside phosphoramidate without chromatography or a selective fractional crystallization of an intermediate. Key enabling findings to the success of this strategy include (1) the identification of a stable, crystalline form of the fully elaborated phosphate and (2) the discovery of a highly efficient, diastereoselective coupling of the nucleoside and the phosphate, with HATU, in the presence of quinine, which enhances both conversion and diastereoselectivity.[27] While only previously reported in the patent literature,[19] this methodology has already found application in the synthesis of other important nucleoside phosphoramidates.[28]

EXPERIMENTAL SECTION

General. All reagents were used as received. The proton and carbon nuclear magnetic resonance shifts are reported in ppm. high resolution mass spectrometry (HRMS) was collected using ESI+ with an LTQ-Oribtrap mass analyzer. The reactions involving the synthesis of **15** were monitored by reverse phase HPLC using a Shimadzu Prominence LC equipped with a Supelco Acentis Express C18 (2.7 μm, 4.6 × 50 mm) column at a 1.0 mL/min flow rate and 220 nm detector wavelength. The mobile phases consisted of (A) 0.05% TFA in 95:5 water/acetonitrile and (B) 0.05% TFA in 5:95 water/acetonitrile. A gradient of t = 0 min, 0% B, and t = 30 min, 100% B was used. Approximate retention times are (**15**) 15.0 min. The reactions involving the synthesis of **3** were monitored by reverse phase HPLC using a Shimadzu Prominence LC equipped with a Phenomenex Kinetex C8 (2.6 μm, 4.6 × 150 mm) column at a 1.0 mL/min flow rate and 220 nm detector wavelength. The mobile phases consisted of (A) 0.05% TFA in 20:80 MeOH/water and (B) 0.05% TFA in 20:80 MeOH/acetonitrile. A gradient of t = 0 min, 0% B; t = 5 min, 30% B; t = 25 min, 50% B; t = 30 min, 100% B; and t = 35 min, 100% B was used. Approximate retention times are (**2**), 3.6 min, (**15**) 19.0 min, (P(*R*) diastereomer of **3**) 20.6 min, and (P(*S*) diastereomer of **3**) 21.5 min.

*(2S)-Neopentyl 2-((chloro(naphthalen-1-yloxy)phosphoryl)-amino)propanoate (**1**).*[10] To a cooled (−40 °C) 10 L reactor containing MTBE (4.13 L) was added $POCl_3$ (100 g, 0.655 mol), followed by 1-naphthol (93.7 g, 0.655 mol). The mixture was agitated for 5 min, then triethylamine (90.6 mL, 0.650 mol) was added slowly maintaining internal temperature below −25 °C throughout the addition. The resulting white slurry was agitated for an additional 30 min. Amino acid **7** (127 g, 0.650 mol) was charged in one portion, followed by slow addition of triethylamine (181 mL, 1.30 mol) over the course of 30 min. The slurry was agitated for an additional 2 h before warming up to 0 °C. The mixture was filtered, and the filter cake was rinsed with MTBE (2 × 50.0 mL). The filtrate was collected and used as is for the next step without further purification (5.54 kg, 10.3 wt % in MTBE, 77% solution yield).

*Calcium Bis(S)-naphthalen-1-yl-(1-(neopentyloxy)-1-oxopropan-2-yl)phosphoramidate Dihydrate (**15**).* A solution of **1** in MTBE (5.54 kg, 10.3 wt %, 16.3 mol) was cooled to 5 °C, and DABCO (548 g, 16.3 mol) was added in one portion. The mixture was agitated for 30 min, and then water (294 g, 16.3 mol) was added over a course of 10 min, maintaining the internal temperature below 10 °C. The reaction was aged for 3 h before the phases were separated retaining the lower aqueous layer. The solution was diluted with water (1.90 g, 10 wt % of phosphate final volume) and further diluted with isopropanol (1.57 L). A solution of calcium chloride dihydrate (1.27 kg, 8.61 mol, 0.53 equiv) and water (5.06 g) was added over 30 min at 20 °C, seeded (5.60 g, 0.2 wt %), and held at this temperature over 12 h. The white slurry was filtered, rinsed with a 20% v/v solution of IPA in water (2 × 50.0 mL), and dried in a vacuum oven at 50 °C. Calcium salt **15** was obtained as a white crystalline solid (478 g, 89 wt %, 69% yield).

^{1}H NMR (500 MHz, DMSO-d_6, 23 °C) δ: 8.17 (d, J = 8.2 Hz, 2H), 7.77 (dd, J = 7.8, 1.5 Hz, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.38−7.44 (m, 6H), 7.30 (t, J = 7.3 Hz, 2H), 4.00−4.05 (m, 2H), 3.68 (br m, 2H), 3.62 (d, J = 10.4 Hz, 2H), 3.49 (d, J = 10.4 Hz, 2H), 1.20 (d, J = 6.9 Hz, 6H), 0.78 (s, 18H).

^{13}C NMR (125.8 MHz, DMSO-d_6, 23 °C): δ 174.7 (d, J = 6.3 Hz), 149.8, 134.1, 127.2, 127.0 (d, J = 7.5 Hz), 126.1, 125.6, 124.8, 122.4, 120.7, 114.1, 72.8, 50.2, 31.0, 26.0, 21.5 (d, J = 3.8 Hz).

^{31}P NMR (500 MHz, DMSO-d_6, 23 °C) δ = −1.89 (br s).

IR (KBr, cm^{-1}): 3397 (br m), 2959 (m), 1731 (m), 1395 (m), 1238 (s), 1085 (s).

HRMS (ESI, m/z): Calcd for $C_{18}H_{25}O_5NP$ (M + H)$^+$: 366.1465. Found: 366.1461.

$[\alpha]_D^{20}$ (c = 2.00 mg/mL, i-PrOH): −12.80°.

*(S)-Neopentyl 2-(((S)-(((2R,3R,4R,5R)-5-(2-Amino-6-methoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-(naphthalen-1-yloxy)phosphoryl)amino)propanoate Hemi-Anisole Solvate ((S,S)-**3**).* To a 10 L reactor was charged 2-methyltetrahydrofuran (2.50 L) and calcium salt **15** (530 g, 0.605 mol, 1.57 equiv). The reactor was rinsed with additional 2-methyltetrahydrofuran (2.08 L), and the solution was washed with an aqueous solution of HCl (1 N, 2 × 2.50 L), then brine (2.50 L). Diisopropylethylamine (200 mL, 1.49 equiv) was added, and the solution was distilled to ∼7 L. The solution was dried by azeotropic distillation with 2-methyltetrahydrofuran until a KF was <0.1 wt % (9.35 L). HATU (585 g, 1.54 mol, 2.00 equiv) was added followed by diisopropylamine (101 mL, 0.58 mol, 0.75 equiv), quinine (225 g, 0.695 mol, 0.90 equiv), then nucleoside **2** (240 g, 0.770 mol, 1.00 equiv). The reaction was heated to 50 °C and stirred for 5 h, then cooled to room temperature, and quenched by the addition of an aqueous solution of L-tartaric acid (10 wt %, 2.4 kg) and diluted with isopropyl acetate (2.38 L). The phases were split, and the organic layer was washed with an aqueous solution of sodium carbonate (5 wt %, 2.40 kg), then water (2.50 L). The reaction mixture was filtered and concentrated to 4.5 L, then anisole (4.5 L) was added. The solution was further concentrated to 6.5 L, cooled to 20 °C, then seeded with **3** (2.50 g) and held for 15 h. The crystals were isolated by filtration, washed with anisole (2 × 0.50 L), and dried in a vacuum oven at 50 °C for 24 h to afford the hemianisole solvate **3** as white crystals (313 g, 57% yield, 95.7 wt %, 56:1 d.r.).

^{1}H NMR (500 MHz, DMSO-d_6, 23 °C): δ 8.15 (app d, J = 7.6 Hz, 1H), 7.97 (s, 1H), 7.93−7.95 (m, 1H), 7.73 (d, J = 8.2 Hz, 1H), 7.54−7.57 (m, 2 H), 7.49−7.51 (m, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.27−7.30 (m, 1H), 6.91−6.93 (m, 1.5H), 6.52 (s, 2H), 6.20 (dd, J = 15.0, 10.0 Hz, 1H), 5.88 (s, 1H), 5.41 (m, 1H), 5.24 (s, 1H), 4.37−4.47 (m, 2H), 4.10 (br m, 2H), 3.96 (m, 4H), 3.74 (s, 1.5H), 3.69 (d, 10.4 Hz, 1H), 3.55 (d, J = 10.4 Hz, 1H), 1.26 (d, 7.3 Hz, 3H), 0.82 (s, 3H), 0.80 (s, 9H).

^{13}C NMR (125.8 MHz, DMSO-d_6, 23 °C): δ 173.0 (d, J = 5.0 Hz), 160.7, 159.9, 159.2, 153.6, 146.5 (d, J = 6.3 Hz), 137.6 (br s), 134.2, 129.4, 127.6, 126.6, 126.4, 126.0 (d, J = 6.3 Hz), 125.7, 124.1, 121.6, 120.4, 114.7 (d, J = 3.8 Hz), 113.84, 113.77, 90.7 (br s), 80.3 (d, J = 7.5 Hz), 78.1, 73.2, 73.1, 66.6 (d, J = 3.8 Hz), 54.9. 53.2, 49.9, 31.1, 26.0, 20.0 (d, J = 7.5 Hz), 19.9.

^{31}P NMR (202.4 MHz, DMSO-d_6, 23 °C): δ 4.24.

IR (KBr, cm^{-1}): 3331 (br s), 1728 (m), 1607 (s), 1587 (s), 1393 (s), 1259 (s).

HRMS (ESI, m/z): Calcd for $C_{30}H_{40}O_9N_6P$ (M + H)$^+$: 659.2589. Found: 659.2586.

$[\alpha]_D^{20}$ (c = 6.89 mg/mL, i-PrOH): +16.80°.

*(S)-Neopentyl 2-(((R)-(((2R,3R,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)-(naphthalen-1-yloxy)phosphoryl)amino)propanoate ((S,R)-**3**).* The generation of (*S*,*R*)/(*S*,*S*)-**3** as a mixture of diastereomers[13] and subsequent isolation of (*S*,*R*)-**3** was accomplished using a previously reported procedure.[14]

^{1}H NMR (500 MHz, DMSO-d_6, 23 °C): δ 8.13 (app d, J = 7.6 Hz, 1H), 7.97 (s, 1H), 7.93−7.95 (m, 1H), 7.73 (d, J = 8.2 Hz, 1H), 7.54−7.57 (m, 2 H), 7.49−7.51 (m, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.27−7.30

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(m, 1H), 6.91−6.93 (m, 1.5H), 6.52 (s, 2H), 6.20 (dd, J = 15.0, 10.0 Hz, 1H), 5.88 (s, 1H), 5.41 (m, 1H), 5.24 (s, 1H), 4.37−4.47 (m, 2H), 4.10 (br m, 2H), 3.96 (m, 4H), 3.74 (s, 1.5H), 3.69 (d, 10.4 Hz, 1H), 3.55 (d, J = 10.4 Hz, 1H), 1.26 (d, 7.3 Hz, 3H), 0.82 (s, 3H), 0.80 (s, 9H).

^{13}C NMR (125.8 MHz, DMSO-d_6, 23 °C): δ 173.2 (d, J = 4.6 Hz), 160.7, 159.9, 153.7, 146.5 (d, J = 6.4 Hz), 137.4 (br s), 134.3, 127.6, 126.6, 126.2, 126.0 (d, J = 6.4 Hz), 125.6, 124.1, 121.6, 114.6 (d, J = 2.7 Hz), 113.8, 90.6 (br s), 80.2 (d, J = 7.3 Hz), 78.2, 73.2, 73.2, 66.1, 53.2, 50.0, 31.1, 26.0, 19.9 (d, J = 7.5 Hz), 20.0, 19.9.

^{31}P NMR (202.4 MHz, DMSO-d_6, 23 °C): δ 3.9.

IR (KBr, cm^{-1}): 3349 (br s), 2959 (m), 1740 (m), 1612 (s), 1587 (s), 1481 (m), 1395 (s), 1259 (s), 1085 (m), 1043 (s).

HRMS (ESI, m/z): Calcd for $C_{30}H_{40}O_9N_6P$ (M + H)$^+$: 659.2589. Found: 659.2577.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H, ^{13}C, and ^{31}P NMR spectra and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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