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A Stereocontrolled Synthesis of a Phosphorothioate Cyclic Dinucleotide-Based STING Agonist

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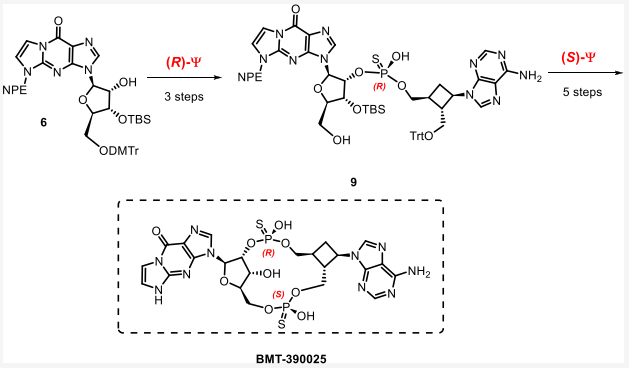
ABSTRACT: We describe a stereodefined synthesis of the newly identified non-natural phosphorothioate cyclic dinucleotide (CDN) STING agonist, BMT-390025. The new route avoids the low-yielding racemic approach using P(III)-based reagents, and the stereospecific assembly of the phosphorothioate linkages are forged via the recently invented P(V)-based platform of the so-called PSI (Ψ) reagent system. This P(V) approach allows for the complete control of chirality of the P-based linkages and enabled conclusive evidence of the absolute configuration. The new approach offers robust procedures for preparing the stereodefined CDN in eight steps starting from advanced nucelosides, with late-stage direct drop isolations and telescoped steps enabling an efficient scale-up that proceeded in an overall 15% yield to produce multigram amounts of the CDN.



INTRODUCTION

The stimulator of interferon genes (STING) targeted immunotherapies have gained much attention in drug discovery since the adaptor protein was discovered in 2008.[1] 2′,3′-Cyclic guanosine monophosphate-adenosine monophosphate (2′,3′-cGAMP) (Figure 1) is the representative structure of one of the four known human STING natural agonists and corresponds to the cyclic dinucleotide (CDN) class of natural products.[2]

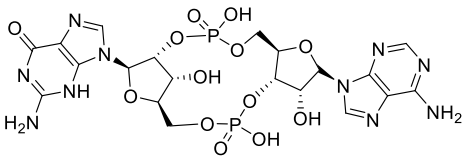


Figure 1. 2′,3′-cGAMP.

Much of the medicinal chemistry effort over the past decade has been characterized by modifications originating from these known CDNs with novel base, sugar, and phosphorus linkages explored in an effort to produce new CDNs that influence STING-pathway agonism in addition to exhibiting favorable drug-like properties.[3−5] One such optimization effort has been successful at the identification of the potent non-natural STING agonist, BMT-390025 (Figure 2).[6] Interestingly,

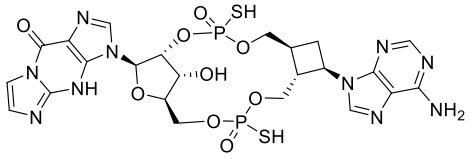


Figure 2. BMT-390025.

BMT-390025 is characterized as a CDN bearing two bridging phosphorothioate linkages, which impart four potential phosphorus diastereoisomers. Seven additional carbon-based stereocenters come from the "linear" tricyclic guanosine-derived nucleoside and the lobucavir-inspired[7] cyclobutane, which constitute the novel design elements of this complex molecule.

With the successful identification of BMT-390025, further supplies of material were required to support toxicology studies, with access to multigram quantities needed. The original synthesis had been developed by relying on the

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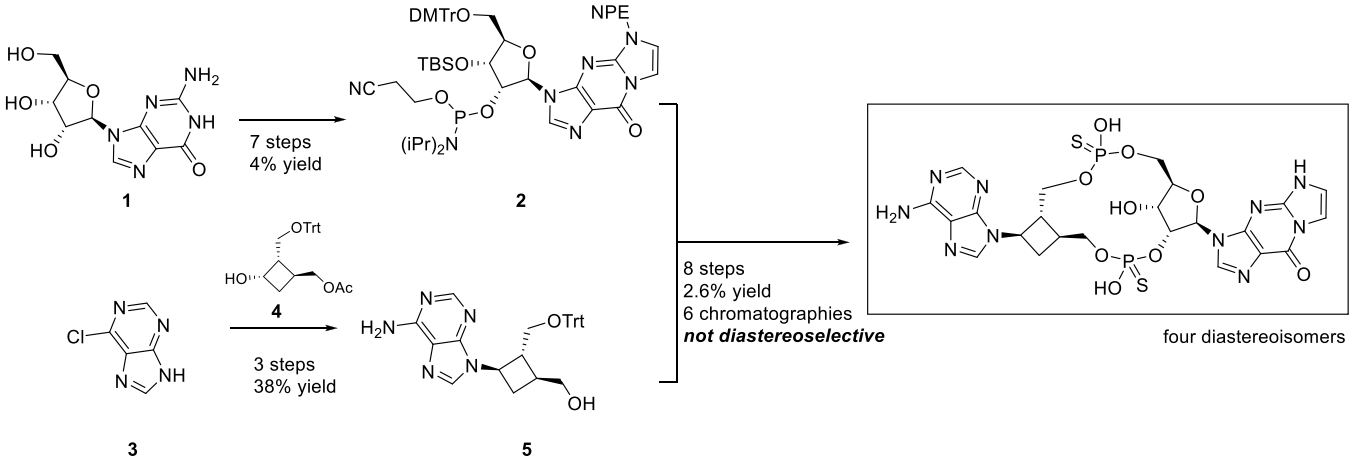
Scheme 1. Construction of the Phosphorothioate via the Original P(III) Route



classical phosphoramidite chemistry that first appeared in the literature during the 1970s and which has more recently found application in the synthesis of c-di-GMP thiophosphate.[8] Our strategy involved the loading of an appropriately protected nucleoside to give the low valent phosphoramidite **2**, as depicted in Scheme 1. Subsequent coupling of advanced nucleoside **5** followed by oxidative sulfurization generated the first phosphorothioate linkage as a mixture of epimers. Protecting group manipulations to allow for an analogous P(III)-sulfurization sequence then closed the CDN ring and delivered a mixture of four phosphorus-centered diastereoisomers that were separated by HPLC chromatography. These efforts produced an initial milligram amount of BMT-390025 having unknown stereochemistry at the phosphorus centers. In addition to the challenges associated with the diastereomeric phosphorothioate construction, the synthesis suffered from poor overall yields with the protected phosphoramidite monomer **2** and cyclobutane coupling partner **4**, which proved to be difficult to synthesize on a scale. Despite the P(III)-based coupling being rapid, it was highly moisture-sensitive, leading to variable results even after repeated azeotropes. The use of hazardous reagents such as H_2S gas, 1*H*-tetrazole, and CCl_4, together with the significant purification difficulties, made this route unlikely to be scaled beyond milligram quantities.

Due to the aforementioned challenges of phosphorothioate synthesis via low valent P(III), we were intrigued by the recent discovery of a P(V)-based reagent platform called the PSI (Ψ) reagent.[9] This reagent has already been utilized in the stereocontrolled synthesis of various nucleotidic architectures, including antisense oligonucleotides (ASOs) and CDNs, and most recently has found further application in the stereospecific synthesis of chiral phosphines and methylphosphonate nucleotides.[10] As such, BMT-390025 presented itself as an ideal candidate on which to test the scalability of such an approach. Reported herein are our efforts culminating in a stereocontrolled and scalable synthesis of this non-natural CDN utilizing this novel reagent system.

RESULTS AND DISCUSSION

Synthesis Design Using Ψ-Reagents. Scheme 2 shows the proposed modular stereocontrolled CDN synthesis, which entails a marked divergence from the P(III) approach, requiring only four steps ((a) 2′-load and couple; (b) 5′-load and couple) from starting nucleoside **6** to construct the stereopure phosphorothioate linkages of the CDN. We decided to use a stepwise approach to CDN synthesis as this has previously been reported to ensure the complete transfer of stereochemical information from the Ψ-reagent by forging the CDN macrocycle one P−O bond at a time. As such, we proposed loading of the 2′-position in nucleoside **6** with the (*R*)-Ψ reagent to give the (*S*)-loaded **7**. Coupling of the second nucleoside **5** was then envisioned to deliver the first phosphorothioate linkage **8** with a stereochemically pure (*R*)-configuration (Scheme 2a). A second loading and iteration using the (*S*)-Ψ reagent through a 5′-loading would then generate the second stereodefined phosphorus with an (*R*)-configuration **10** (Scheme 2b). Subjecting **10** to macrocyclization and a deprotection sequence would provide enantiomerically and diastereomerically pure BMT-390025.

At the outset of this proposed new synthesis, the stereochemistry of the phosphorothioate linkages in BMT-390025 arising from the P(III) synthesis was unknown; a tentative assignment was made based on the phosphorothioate stereochemistry of related CDN compounds having single-crystal X-ray data. The order of assembly and selection of Ψ-reagents would not only allow for the interception of common nucleoside intermediates used in the previous P(III) synthesis but would also allow us to confirm the proposed stereochemical assignment via this programmed approach.

Nucleoside Monomer Synthesis. *"Linear" Tricyclic Guanosine.* The advanced tricyclic guanosine **6** was prepared in 6 steps according to literature precedent.[11,6] Orchestrating the appropriate protecting group strategy for this triply protected fragment presented several challenges when performed on-scale. The nonselective OTBS protection of diol **11** using the standard conditions of TBSCl and imidazole in DMF at room temperature gave a ∼1:1 regioisomeric mixture of protected 2′- and 3′-alcohols, **12** and **6**, respectively (Scheme 3). Separation by silica gel chromatography was sufficient for accessing the desired 3′-protected compound **6** on a gram scale, albeit in a modest yield of 31%. Increasing the scale of this reaction to >10 g led to the observation of significant (5−10%) silyl group migration back to **12**, which was occurring post purification during the evaporation of purified fractions.

This undesired scrambling hampered our ability to control key impurities and exacerbated downstream purification difficulties when this mixture was used in the subsequent Ψ-

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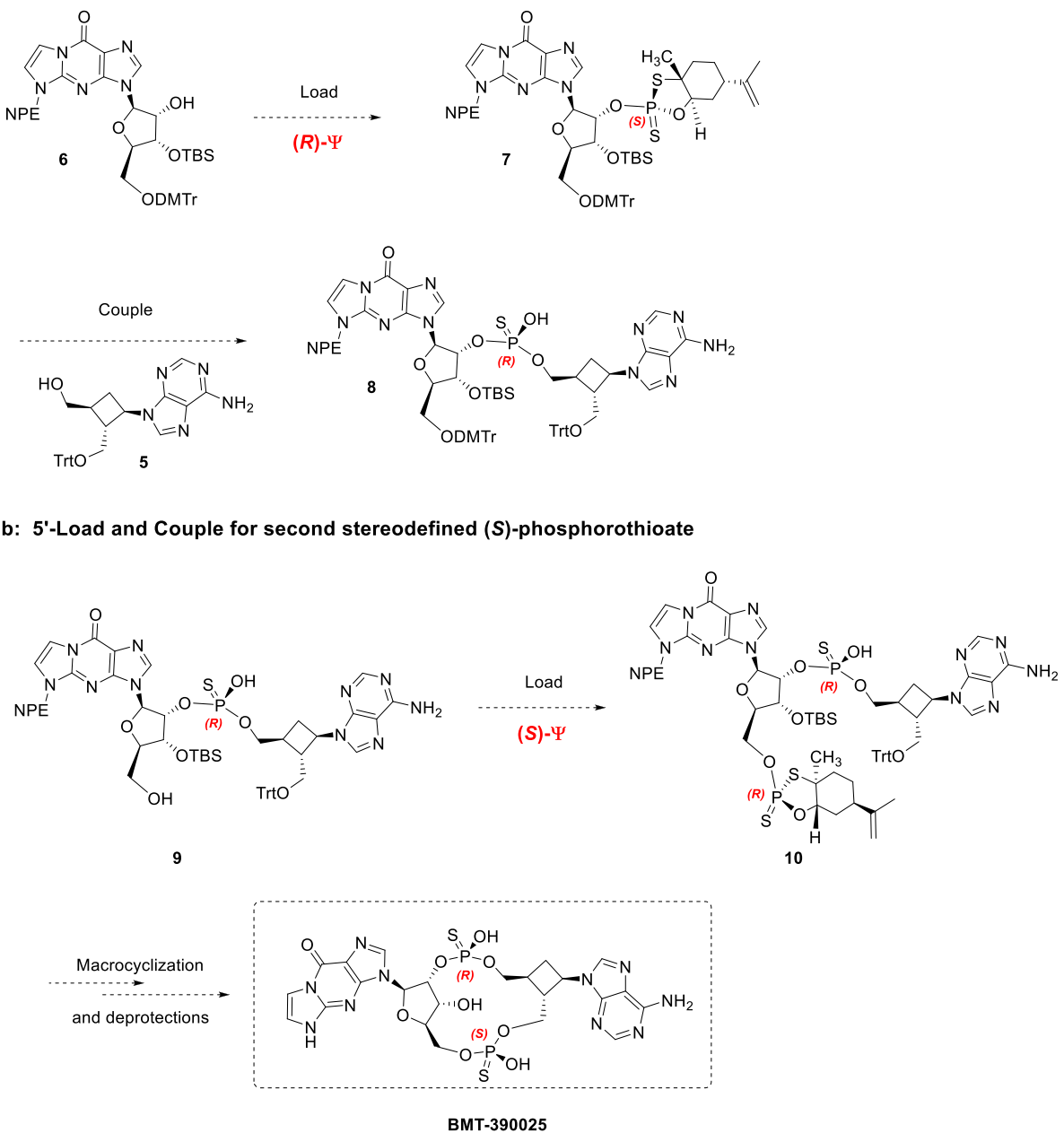
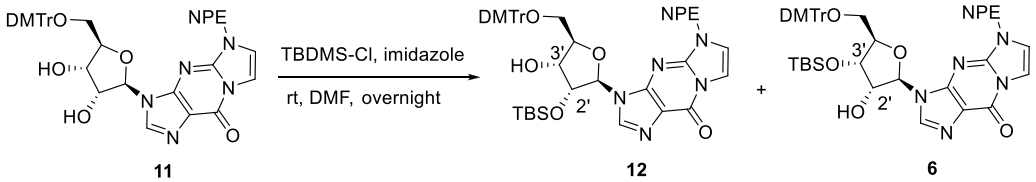
Scheme 2. Proposed Stepwise Ψ-Loading and Coupling Steps for Stereocontrolled Phosphorothioate Synthesis



Scheme 3. Nonselective Mixture of 2′- and 3′-Protected Nucleosides[a]



[a]NPE = *p*-nitrophenethyl.

loading step.[12] Attempts to leverage existing chemistry around selective protection methods included the use of silver nitrate reported by Ogilvie and the use of a scaffolding catalyst reported by Tan; however, both failed to show any improvement.[13,14] Upon the basis of a report by Usman,[15] in which they had subjected a 3′-silyl isomer to 1% Et_3N in MeOH in order to isomerize to a 1:1 mixture of 2′- and 3′-isomers, we decided to focus on surveying solvents for the already observed partial migration. Methanol alone was a suitable solvent to affect the migration, although extended

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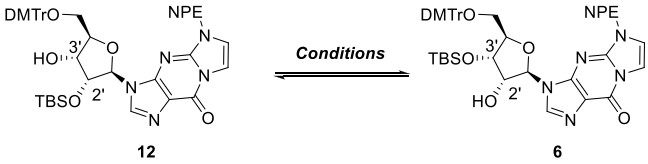
times (48 h) were needed to achieve 50% migration (Table 1). Following the Usam protocol, the addition of 1% Et_3N further

Table 1. Solvent Screen Used for TBDMS Migration and the Recycling of Undesired Regioisomer 12



12 6

conditions	results
dichloromethane, rt, 48 h	no significant migration observed
methanol, rt, 48 h	50% conversion
acetonitrile, rt, 48 h	10% conversion
ethyl acetate, rt, 48 h	10% conversion
THF, rt, 48 h	20% conversion
methanol, Et_3N, 50 °C, 6 h	50% conversion, decomposition evident
IPA, Et_3N, 50 °C, 4 h	50% conversion, clean profile
IPA, Et_3N, 80 °C, 1 h	complex mixture

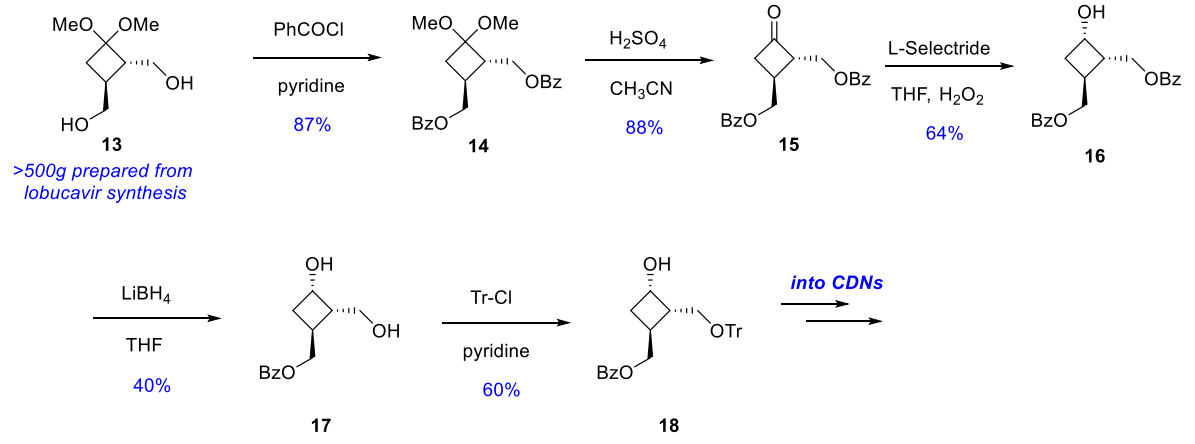
accelerated the migration, with 50% Et_3N in isopropanol (IPA) at 50 °C ultimately selected as the optimal combination to effect clean migration within 4 h. In the event, we developed a practical supercritical flow chromatography (SFC)[16] method for this separation, which allowed for rapid purification[16c] on a multigram scale and was combined with continuous and immediate evaporation[17] postpurification, resulting in <1% observed migration on a scale greater than 100 g. The undesired regioisomer was subjected to the IPA-Et_3N conditions to effectively "recycle" into a 1:1 mixture within 5 h on a 100 g scale. Evaporation and resubjecting the material to SFC allowed the effective yield for this transformation to be increased from the aforementioned 31% on a gram scale to 65−75% on a scale greater than100 g, dependent on the number of times the SFC purification−recycle protocol was repeated.

Cyclobutane Fragment. A diastereoselective [2+2] cycloaddition reported on a kilogram scale was successfully employed to deliver the known intermediate **13** on a scale greater than 500 g.[18] However, in contrast to the synthesis of lobucavir, this key fragment now necessitated a late-stage orthogonal protecting group strategy for the successful incorporation into the CDN class of molecules. To that end, an initial route was developed that comprised 5 steps from **13** to the advanced intermediate **18**, which proceeded in 11% overall yield (Scheme 4). The lowest yielding reaction was the nonselective $LiBH_4$-mediated deprotection of the benzoylated alcohol proximal to the hydroxycyclobutane, **16** → **17**. Attempts to improve on this deprotection centered around invoking the previously reported[18b] bicyclic *syn*-Alanate transition states encountered during the ketone reduction step, although all attempts at selective deprotection, including $LiAlH_4$ and BH_3,[19] failed.

Our focus to avoid this problematic step led us to the work of Lee-Ruff on the regioselective porcine pancreatic lipase (PPL) enzyme-catalyzed acylation and hydrolysis of cyclobutanone derivatives.[20] Initial investigations began with the PPL enzyme-catalyzed hydrolysis performed on diacetate, **19**, which was formed from the bis-acetylation of diol **13** with acetyl chloride in pyridine (Scheme 5). Well-aligned with Lee-Ruff's observation, the PPL-catalyzed bioconversion resulted in selective hydrolysis of the C-3 acetoxymethyl group leaving the C-2 acetoxymethyl intact, although isolated yields of **20** remained low due to emulsion issues encountered during workup, particularly with gram-scale reactions (Scheme 5). Attempts to mitigate these issues included the addition of MeOH to denature the enzyme but always resulted in a loss of the product to the aqueous layer during the subsequent extractive workup. Salting-out (K_2CO_3 or Na_2SO_4) and filtration-based (addition of Celite) workup procedures failed to improve on the low isolated yield.

A nonaqueous, selective enzyme-mediated acylation strategy was the next option. To our delight, PPL-mediated acetylation of diol **13** with vinyl acetate and toluene as the solvent mixture resulted in the regioselective acetylation of the C-3 hydroxymethyl group, giving monoacetate **21** (Scheme 6). Workup now consisted of a simple filtration to remove the enzyme and evaporation of the filtrate prior to purification by silica gel chromatography. Optimal reaction conditions necessitated the use of 150 mol % PPL in order to afford 100% conversion in 12 h. Yields of >90% were obtained after isolation, and the reaction scaled well (>100 g), utilizing the same conditions. To complete the synthesis of nucleoside **5**, a modified order of previously utilized transformations allowed the interception of the appropriately protected cyclobutane **4** (Scheme 6). This new four-step sequence delivered **4** in an overall 67% yield from diol **13** and represented a significant improvement over the previous five steps, 11% overall yield of

Scheme 4. Original Protecting Group Strategy Developed from the Lobucavir Common Intermediate



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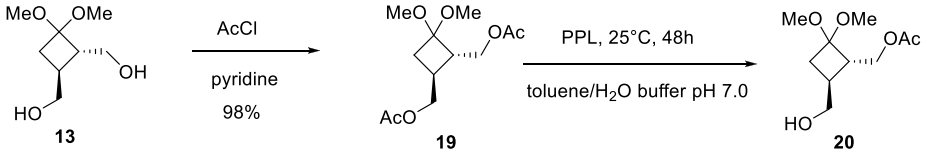
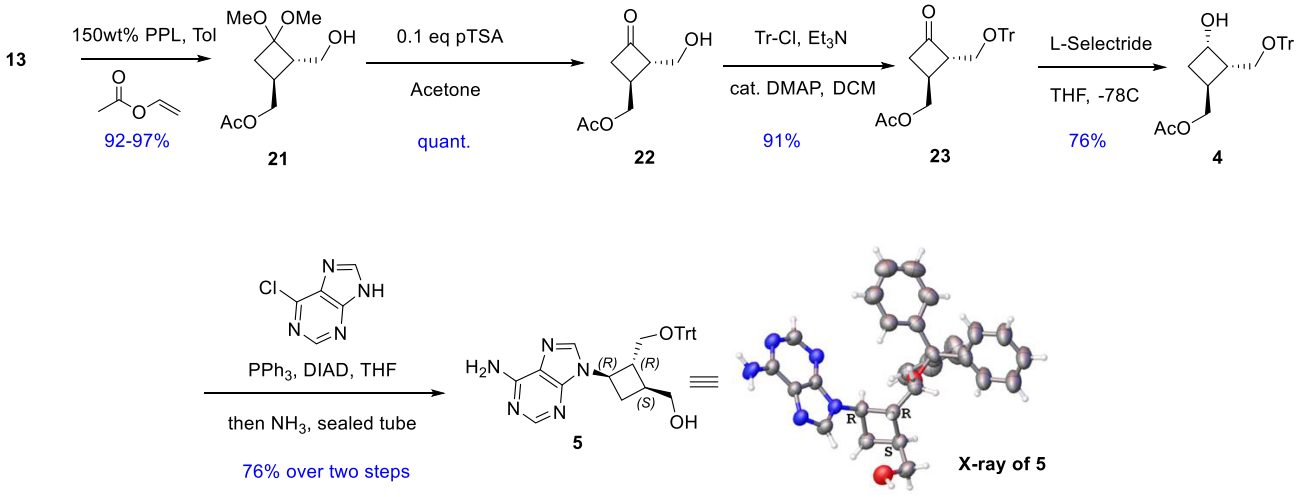
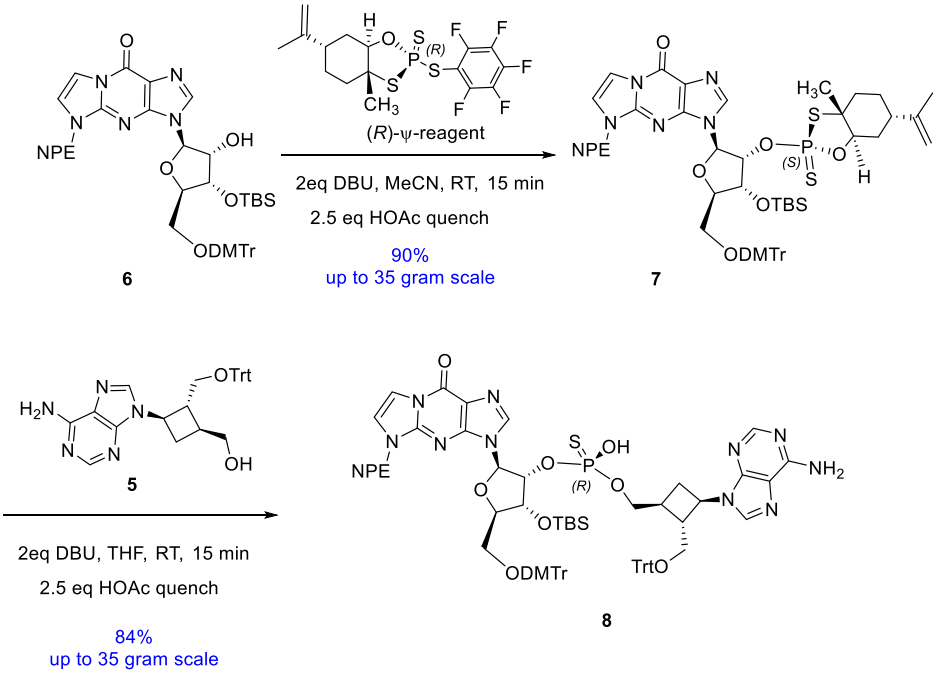
Scheme 5. PPL-Mediated Hydrolysis of Diacetate, 19



Scheme 6. Selective Enzyme-Mediated Acylation and Advancement to a Key Nucleoside, 5



Scheme 7. Stereoselective Phosphorothioate Construction via First Load and Couple Sequence with (*R*)-Ψ Reagent



18 from the same diol intermediate (Scheme 4). An analogous Mitsunobu and aminolysis sequence delivered the advanced nucleoside **5** in 75% yield for the two-step procedure on a multigram scale. The regiochemical and stereochemical structural integrity of **5** was confirmed by a single-crystal X-ray structure determination.

End-Game Synthesis Enabled by Ψ-Reagents. With nucleosides **5** and **6** in hand, we commenced with the construction of the first stereocontrolled phosphorothioate linkage by loading the 2′-alcohol of **6** with the (*R*)-Ψ reagent (Scheme 7). Rapid addition of DBU to an acetonitrile solution of **6** and the (*R*)-Ψ reagent yielded S_p-loaded building block **7** within 5 min. Quenching with acetic acid followed by

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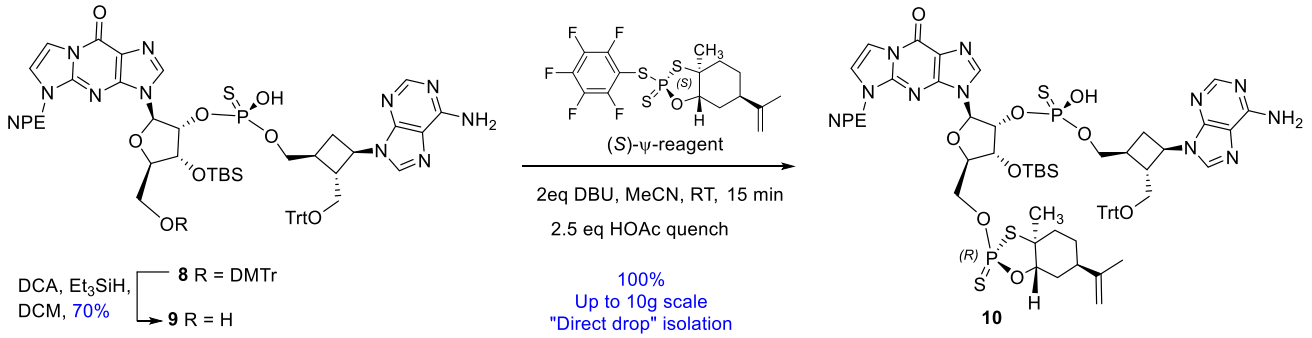
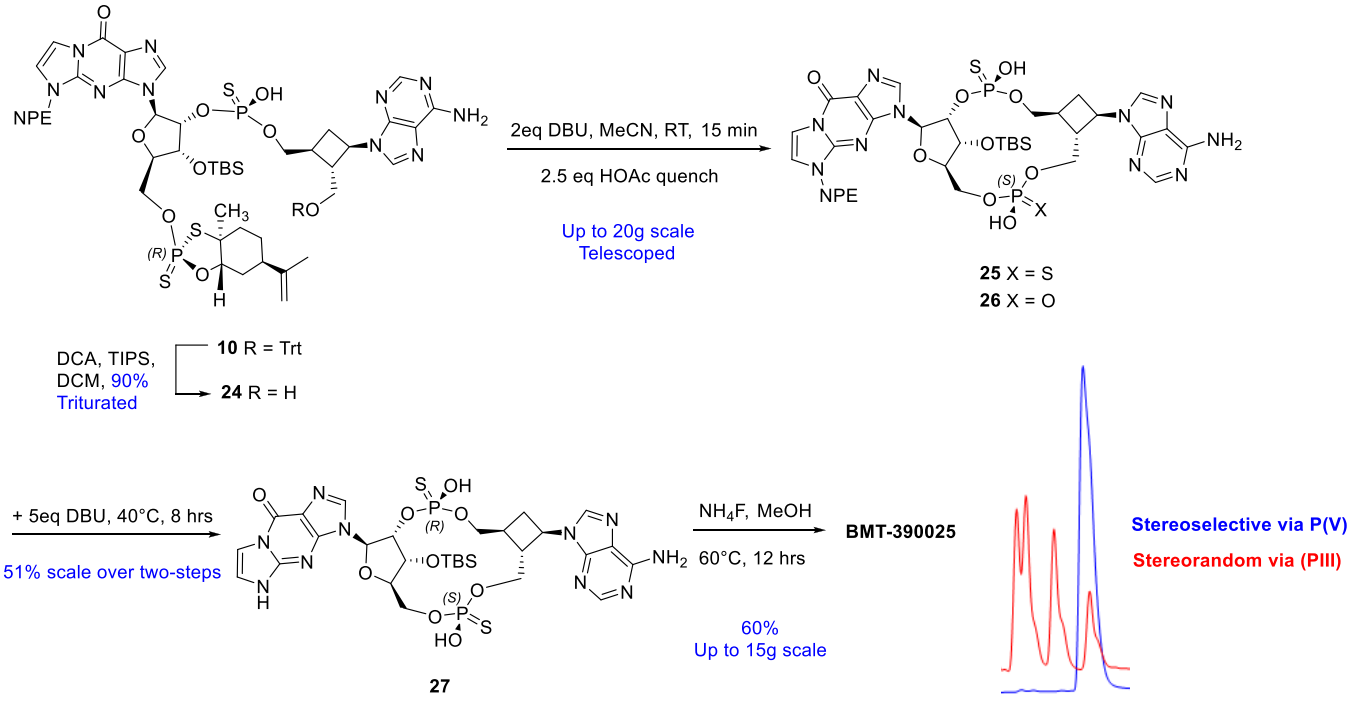
Scheme 8. Deprotection and Second Loading Step with (*S*)-Ψ Reagent



Scheme 9. End Game to Achieve BMT-390025



evaporation and purification by column chromatography afforded **7** in 90% isolated yield as a single S_p diastereoisomer as confirmed by ^{31}P NMR. By virtue of the rapid loading step, large-scale reactions (>30 g) performed equally well, with no observation of the previously observed OTBS migration. The subsequent coupling step between **7** and **5** proved equally as facile and was complete within 10 min to give dinucleotide **8** as a single R_p diastereoisomer in an excellent 84% isolated yield following column chromatography.

Selective deprotection of the 5′-DMTr in **8** was achieved under standard dichloroacetic acid (DCA) and triethylsilane (TES) conditions, which gave a 70% isolated yield of **9** after 6 h at rt along with unreacted starting material (Scheme 8). Attempts to further increase the conversion to **9** with longer reaction times or increased DCA concentrations led to concomitant Trt deprotection. The second loading step with the (*S*)-Ψ reagent produced R_p-loaded **10** in a similar manner to the first loading step (Scheme 8). However, despite an HPLC profile for the reaction showing complete and clean transformation to **10** within minutes of DBU addition, workup via an AcOH quench and evaporation followed by column chromatography gave only a 35% isolated yield. It became evident that, over prolonged reaction times (>10 min) and during the evaporation process, the impurity profile of the reaction mixture accumulated byproducts related to the P(V)-reagent, which contributed to product decomposition.[10] Methanolysis was also observed postcolumn chromatography, so efforts were focused on rapid, nonchromatographic isolation of loaded intermediate **10**. A direct drop isolation method was developed in which the reaction was dropped onto a 10-fold volume excess of MTBE to effectively precipitate the desired product. Removal of the supernatant and a citric acid wash of the DCM-dissolved product was effective at removing the residual DBU. This protocol gave consistent results and allowed for a 100% isolated yield of **10** that was suitable for use in the next deprotection step.

Trityl deprotection of **10** was complete within 1 h using a 2-fold excess of DCA (Scheme 9). Given the previously observed sensitivity of the auxiliary toward ring-opening, we decided to forego purification and telescope **24** into the macrocyclization step through a simple solvent switch from DCM to MeCN followed by the addition of DBU. Accompanying the desired cyclization to **25** was a significant quantity (~35−40%) of desulfurized product **26**. Beaucage has reported similar

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desulfurization of oligonucleoside phosphorothioates as a problem emanating from the secondary decomposition products of trichloroacetic acid (TCA) used in their DMTr deprotections.[21] This prompted us to perform subsequent trityl deprotections of **10** using only fresh DCA solutions with an intermediate isolation of **24** implemented from diethyl ether to give **24** as a crude white solid in 90% yield from **10**. Gratifyingly, cyclization using this material significantly reduced the observed desulfurization product **26** to only 1−2%, which was readily removed by SFC to give **25** as a single S_p-diastereoisomer at the newly formed phosphorothioate linkage. Further optimization allowed for NPE deprotection to occur in one pot directly after cyclization by the addition of 5 equiv of DBU and gentle heating (40 °C). SFC purification allowed penultimate **27** to be isolated, with the effective crude isolation and telescoping of 4 reactions generating a single R_p,S_p-diastereoisomer in 46% yield from **9**.

Final deprotection using triethylamine trihydrogen fluoride ($Et_3N{\cdot}3HF$) provided clean conversion to BMT-390025; however, difficulties associated with the workup and purification of the resulting triethylammonium salt contributed to significant product loss. Optimal conditions using the readily available and less toxic ammonium fluoride resulted in complete deprotection after heating in anhydrous MeOH overnight.[22] SFC purification conditions were developed using "matched" counterion ammonium acetate as the mobile phase, and direct injection of the final deprotection solution led to an efficient final purification delivering the target compound in 60% yield.

CONCLUSION

In summary, we have developed an efficient and multigram synthesis of the novel CDN, BMT-390025. Key features included leveraging an observed silyl group migration to maximize the throughput of advanced nucleoside **6** and the use of an enzyme-catalyzed selective acylation to streamline the protecting group strategy to access the nucleoside coupling partner **5**. This route avoided the low-yielding racemic approach using P(III)-based reagents and the associated reaction sensitivity, multiple impurities, and intensive use of chromatographic separation, which further complicated the use of the original synthetic route on a scale. The P(V) approach with the recently disclosed Ψ-reagents allowed for the complete control of chirality of the P-based linkages and enabled conclusive evidence of the absolute configuration. The new approach offers robust procedures for preparing the stereodefined CDN in eight steps starting from the advanced nucleosides, with late-stage direct drop isolations and telescoped steps enabling an efficient scale-up that proceeded in an overall 15% yield.

EXPERIMENTAL SECTION

General Experimental Information. All reactions were performed under a nitrogen atmosphere using anhydrous techniques unless otherwise noted. Reagents were used as received from the vendors, unless otherwise noted. Quoted yields are for isolated material. Reactions were monitored by reversed-phase HPLC on a Shimadzu system using CH_3CN/H_2O/MeOH as the mobile phase (containing either 0.05% TFA or 0.1% NH_4OAc). Supercritical fluid chromatography (SFC) conditions for compound **6** were as follows: BEH 2-EP (25 cm × 5 cm, I.D. 5 μm), 35% CAN/IPA [1:1 (v/v)] with 0.1% NH_4OH in CO_2, 35 °C, 280 mL/min, 238 nm, 100 bar BPR, crude sample concentration at 312.3 mg/mL, 1.4 mL injection volume, 2.1 min injection cycle time. SFC conditions for compound **25** were as follows: BEH 2-ethylpyridine (25 cm × 5 cm, 5 μm), 40% $MeOH/H_2O$ (95:5) with 10 mM NH_4HCO_3 in CO_2, 35 °C, 350 mL/min, 220 nm, 100 bar BPR, 16.81 mg/mL, 3.5 mL/2.3 min. SFC conditions for compound **27** were as follows: BEH 2-ethylpyridine (25 × 5 cm, 5 μm), 40% $MeOH/H_2O$ (95:5) with 10 mM NH_4HCO_3 in CO_2, 35 °C, 350 mL/min, 220 nm, 100 bar BPR, 12.56 mg/mL, 3 mL/2 min. SFC conditions for BMT-390025 were as follows: BEH 2-EP (25 cm × 5 cm, 5 μm), 65% MeOH/ACN/water (47.5:47.5:5) with 20 mM ammonium acetate in CO_2, 230 mL/min, 220 nm, 47 °C, 100 bar BPR, 40 mg/mL, 3.75 mL/3.3 min. NMR samples were dissolved in approximately 0.65 mL of a suitable deuterated solvent (dimethyl sulfoxide-d_6, methanol-d_4, choroform-d, deuterium oxide). NMR spectra were recorded on a 400 MHz Avance III HD spectrometer equipped with a 5 mm BBFO probe, a 500 MHz Bruker Avance III HD NMR spectrometer equipped with a 5 mm BBO Prodigy cryoprobe, or a 700 MHz Bruker Avance III HD NMR spectrometer equipped with a 5 mm TCI cryoprobe (Bruker, Billerica, MA). One- and two-dimensional (1D and 2D) NMR spectra for structure elucidation were collected at 27 °C and include 1D proton, proton decoupled carbon, proton decoupled phosphorus, 2D COSY (correlation spectroscopy) multiplicity-edited heteronuclear single quantum coherence (1H-13C edited HSQC), and heteronuclear multiple-bond correlation ($^1H-^{13}C$ HMBC and $^1H-^{31}P$ HMBC) experiments. ACD/NMR prediction software (ACD/LABORATORIES Release 2015 Pack 2) and ACD NMR Spectrus Workbook (ACD/NMR Workbook 2019.1.1) from Advanced Chemistry Development Inc. (Toronto, ON, Canada) were used. NMR spectra are referenced to residual undeuterated solvents. Data for ^1H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublets, t = triplet, dt = doublet of triplets, td = triplet of doublets, q = quartet, quint = quintet, m = multiplet, b = broad), integration, and coupling constant (Hz). Data for ^{13}C NMR and ^{31}P NMR are reported in terms of chemical shift, and no special nomenclature is used for equivalent carbons. High-resolution mass spectra (HRMS) were acquired on a Thermo Fisher QExactive mass spectrometer operating in electrospray ionization (ESI) mode (positive or negative ion). Compounds **13**,[18] **21**,[20] **22**,[20] **23**,[6] and **4**[6] are known compounds and were prepared according to the literature-reported procedures. Compound **26** was assigned based on mass analysis but was not isolated and characterized. Compound **5** was obtained as crystalline material suitable for X-ray diffraction following recrystallization from absolute ethanol.

*3-((2R,3R,4S,5R)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)-4-((tert-butyldimethylsilyl)oxy)-3-hydroxytetrahydrofuran-2-yl)-5-(4-nitrophenethyl)-3,5-dihydro-9H-imidazo[1,2-a]purin-9-one (**6**).* To a 5 L round-bottom flask were added compound **11** (240 g, 288 mmol),[6] anhydrous DMF (1300 mL), 1*H*-imidazole (58.8 g, 863 mmol), and TBSCl (52.1 g, 345 mmol). The reaction mixture was stirred at room temperature, and the reaction progress was monitored by HPLC. After completion of the reaction (14 h), ice−water (1500 mL) was charged, and the resulting supernatant was decanted. EtOAc (2000 mL) was charged to dissolve the precipitated gum and gave a solution after stirring the mixture for 30 min. It was washed with aqueous LiCl solution (1000 mL) and then water (1000 mL), dried (Na_2SO_4), and evaporated to give the crude product as a dark red gum (308 g). The crude material was purified by column chromatography (silica gel, eluting with 0−20% MeOH in CH_2Cl_2) to give a mixture of two isomers **12** and **6** (204 g). The mixture was further purified by supercritical fluid chromatography to give compound **6** as a yellow foam (79 g, 89 mmol, yield 31%). Data for compound **6**: ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 8.7 Hz, 2H), 7.88 (s, 1H), 7.56 (d, J = 2.7 Hz, 1H), 7.39 (d, J = 8.6 Hz, 2H), 7.29 (m, 4H), 7.25 (d, J = 8.8 Hz, 2H), 7.23 (m, 2H), 7.17 (m, 1H), 6.77 (d, J = 8.3 Hz, 4H), 6.66 (d, J = 2.7 Hz, 1H), 5.97 (d, J = 4.8 Hz, 1H), 4.47 (q, J = 5.4 Hz, 1H), 4.40 (m, 1H), 4.28 (m, 2H), 4.15 (q, J = 4.0 Hz, 1H), 3.74 (s, 3H), 3.74 (s, 3H), 3.46 (dd, J = 3.3, 10.6 Hz, 1H), 3.28 (dd, J = 4.2, 10.6 Hz, 1H), 3.18 (t, J = 7.1 Hz, 2H), 0.87 (s, 9H), 0.07 (s, 3H), −0.02 (s, 3H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 158.6 (s, 2C), 151.8, 149.9, 147.1, 144.8, 144.8, 144.2, 136.54,

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135.6, 135.4, 130.0 (s, 2C), 130.0 (s, 2C), 129.7 (s, 2C), 128.1 (s, 2C), 127.9 (s, 2C), 127.0, 123.9 (s, 2C), 117.4, 116.8, 113.2 (s, 4C), 107.0, 88.3, 86.6, 83.9, 74.8, 71.9, 63.0, 55.2 (s, 2C), 46.1, 34.9, 25.7 (s, 3C), 18.0, −4.7, −4.8; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{47}H_{53}N_6O_9Si$ 873.3638, found 873.3622. The undesired compound **12** (95 g) was heated in triethylamine and isopropanol at 50 °C overnight to give a mixture of the two isomers, which were again separated by SFC. The recycle procedure was repeated again to give a total of 84 g of the desired isomer from the recycling of undesired product and an overall 65% yield of **6** (163 g, 186 mmol).

*((1S,2R,3R)-3-(6-Amino-9H-purin-9-yl)-2-((trityloxy)methyl)-cyclobutyl)methanol (**5**).* A 1 L steel bomb was charged with ((1*S*,2*R*,3*R*)-3-(6-chloro-9*H*-purin-9-yl)-2-((trityloxy)methyl)-cyclobutyl)methyl acetate (27g, 48.8 mmol)[6] and 1,4-dioxane (135 mL), and the mixture was stirred for 20 min until all starting material dissolved. NH_4OH (279 mL, 2148 mmol) was added before sealing the bomb and heating using an oil bath at 70 °C for 2 days. After completion of the reaction, the temperature was allowed to cool to 20 °C before concentrating to dryness. The crude material was dissolved in EtOH (100 mL) and evaporated; this process was repeated two more times. The resulting crude material was dissolved in minimal EtOH before adding CH_2Cl_2 (500 mL) and the precipitate collected by filtration, and EtOH (500 mL) was added to the cake (34 g). The suspension was sonicated for 30 min, and the insoluble solid was removed by filtration. The filtrate was concentrated to dryness to give compound **5** as a white solid (21.7 g, 90% yield). Data for compound **5**: ^1H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.13 (s, 1H), 7.28−7.15 (m, 18H), 4.78 (q, J = 9.0 Hz, 1H), 4.58 (t, J = 5.3 Hz, 1H), 3.48 (t, J = 5.5 Hz, 2H), 3.10 (m, 2H), 2.97 (m, 1H), 2.45 (td, J = 7.9, 10.3 Hz, 1H), 2.21 (q, J = 10.1 Hz, 1H), 2.09 (m, 1H); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 156.0, 152.2, 149.5, 143.7 (s, 3C), 139.4, 128.1 (s, 6C), 127.7 (s, 6C), 126.8 (s, 3C), 119.0, 85.6, 64.0, 63.5, 47.7, 45.9, 33.3, 30.2; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{30}H_{30}N_5O_2$ 492.2394, found 492.2388.

*3-((2R,3R,4R,5R)-5-((Bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)-4-((tert-butyldimethylsilyl)oxy)-3-(((2S,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]-oxathiaphosphol-2-yl)oxy)tetrahydrofuran-2-yl)-5-(4-nitrophenethyl)-3,5-dihydro-9H-imidazo[1,2-a]purin-9-one (**7**).* To a 1 L round-bottom flask was added compound **6** (33.0 g, 37.8 mmol), dissolved in anhydrous CH_3CN (150 mL), and the mixture was concentrated. This was repeated twice, and the residual solid was placed under a high vacuum for 60 min. The solid was dissolved in anhydrous CH_3CN (329 mL), and the (*R*)-Ψ-reagent (33.8 g, 76.0 mmol) and DBU (11.4 mL, 76.0 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature with progress monitored by HPLC. After completion of the reaction (20 min), acetic acid (5.41 mL, 94.0 mmol) was charged, and the mixture was concentrated. The crude solid was purified by column chromatography (silica gel, eluting with 0−50% EtOAc in CH_2Cl_2) to give **7** as a yellow foam (38.2 g, 90% yield). Data for compound **7**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, J = 8.7 Hz, 2H), 7.70 (s, 1H), 7.57 (d, J = 2.7 Hz, 1H), 7.36 (d, J = 8.7 Hz, 2H), 7.32−7.31 (ov, 5H), 7.15 (d, J = 8.9 Hz, 4H), 6.83−6.78 (m, 4H), 6.60 (d, J = 2.7 Hz, 1H), 6.13 (ddd, J = 4.8, 8.0, 16.1 Hz, 1H), 5.94 (d, J = 8.1 Hz, 1H), 5.02 (s, 1H), 4.89 (s, 1H), 4.60 (m, 1H), 4.53 (d, J = 4.8 Hz, 1H), 4.39 (td, J = 3.2, 12.3 Hz, 1H), 4.23 (br s, 1H), 4.20 (td, J = 7.6, 14.5 Hz, 1H), 3.93 (dd, J = 1.9, 12.7 Hz, 1H), 3.77 (s, 6H), 3.73 (m, 1H), 3.28 (m, 2H), 2.53 (m, 1H), 2.20 (m, 1H), 1.97 (m, 1H), 1.91(m, 1H), 1.82 (m, 1H), 1.77 (ov, 4H), 1.68 (m, 1H), 1.52 (s, 3H), 0.94 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 158.6, 151.6, 149.0, 147.3, 147.1, 145.2, 144.9, 144.3, 139.4 (s, 2C), 139.1, 130.0 (s, 2C), 129.1 (s, 4C), 127.8 (s, 2C), 127.7 (s, 2C), 127.0, 123.9 (s, 2C), 118.4, 118.3, 113.1 (s, 4C), 111.8, 107.2, 88.8, 87.8 (d, J = 5.5 Hz, 1C), 86.7, 81.4, 76.1 (d, J = 5.4 Hz, 1C), 72.8 (d, J = 4.5 Hz, 1C), 66.2, 62.8, 55.2, 46.8, 38.9, 35.1, 33.8 (d, J = 8.2 Hz, 1C), 27.6 (d, J = 16.3 Hz, 1C), 25.7 (s, 3C), 23.4, 22.7, 21.5, 18.3, −4.5, −4.9; ^{31}P NMR (202 MHz, $CDCl_3$) δ 101.75 (s, 1P); HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{57}H_{68}N_6O_{10}PS_2Si$ 1119.3940, found 1119.3921

*O-(((1S,2R,3R)-3-(6-Amino-9H-purin-9-yl)-2-((trityloxy)methyl)-cyclobutyl)methyl) O-((2R,3R,4R,5R)-5-((Bis(4-methoxyphenyl)-(phenyl)methoxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-2-(5-(4-nitrophenethyl)-9-oxo-5,9-dihydro-3H-imidazo[1,2-a]purin-3-yl)-tetrahydrofuran-3-yl) O-Hydrogen Phosphorothioate (**8**).* To a 1 L round-bottom flask were added **7** (31.0 g, 27.7 mmol), **5** (16.3 g, 33.2 mmol), and anhydrous 1:1 MeCN/THF (200 mL). The mixture was stirred for 30 min at 20 °C to give a homogeneous solution, which was then evaporated. This azeotroping procedure was repeated two more times before the addition of anhydrous THF (310 mL). DBU (12.52 mL, 83 mmol) was added dropwise over the course of 10 min. The initial slurry completely dissolved after the addition of DBU, and the color of the reaction mixture changed to a light yellow solution. The reaction mixture was stirred at room temperature with progress monitored by HPLC. After completion of the reaction (15 min), the crude solution was dropped onto MTBE (1000 mL) over the course of 10 min. The resulting precipitate was collected and purified by column chromatography (silica gel, eluting with 0−10% MeOH in CH_2Cl_2) to give **8** as a white foam (33.6 g, 23.3 mmol, 84% yield). Data for **8**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.16 (s, 1H), 8.06 (d, J = 8.7 Hz, 2H), 7.88 (s, 1H), 7.46 (d, J = 2.7 Hz, 1H), 7.39 (m, 2H), 7.28−7.22 (m, ov, 12H), 7.19−7.05 (m, 12H), 6.70 (br d, J = 8.9 Hz, 2H), 6.69 (br d, J = 8.9 Hz, 2H), 6.50 (d, J = 2.7 Hz, 1H), 6.22 (d, J = 7.1 Hz, 1H), 5.69 (ddd, J = 4.8, 7.2, 12.4 Hz, 1H), 4.69 (q, J = 8.7 Hz, 1H), 4.60 (dd, J = 2.1, 4.6 Hz, 1H), 4.24−4.06 (m, 3H), 3.69−3.65 (m, 8H), 3.35 (m, 2H), 3.17−3.01 (m, 4H), 2.84 (m, 1H), 2.38 (td, J = 8.1, 10.7 Hz, 1H), 2.15 (m, 1H), 2.05 (m, 1H), 0.88 (s, 9H), 0.19 (s, 3H), 0.11 (s, 3H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 158.6, 158.4, 158.4, 154.b (br s, 1C), 151.9, 150.5, 149.7, 147.0, 145.1, 144.6, 144.5, 143.7 (s, 3C), 140.1 (br s, 1C), 138.5, 135.8, 135.6, 130.1 (s, 2C), 130.0 (s, 2C), 129.8 (s, 2C), 128.6 (s, 6C), 128.1 (s, 2C), 127.8 (s, 2C), 127.7 (s, 6C), 126.9 (s, 3C), 126.7, 123.9 (s, 2C), 119.3, 117.6, 116.8, 113.1 (br s, 2C), 113.1 (br s, 2C), 106.6, 86.5, 86.4, 85.5 (br d, J = 4.5 Hz, 1C), 85.3, 75.2 (br d, J = 5.4 Hz, 1C), 72.6 (br d, J = 3.6 Hz, 1C), 67.0 (br d, J = 5.4 Hz, 1C), 64.0, 63.4, 55.1, 47.5, 46.1, 45.8, 34.7, 31.64 (d, J = 7.3 Hz, 1C), 30.0, 25.9 (s, 3C), 18.2, −4.0, −4.9; ^{31}P NMR (202 MHz, $CDCl_3$) δ 58.20 (s, 1P); HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{77}H_{81}N_{11}O_{12}PSSi$ 1442.5288, found 1442.5284.

*O-(((1S,2R,3R)-3-(6-Amino-9H-purin-9-yl)-2-((trityloxy)methyl)-cyclobutyl)methyl) O-((2R,3R,4R,5R)-4-((tert-Butyldimethylsilyl)-oxy)-5-(hydroxymethyl)-2-(5-(4-nitrophenethyl)-9-oxo-5,9-dihydro-3H-imidazo[1,2-a]purin-3-yl)tetrahydrofuran-3-yl) O-Hydrogen (R)-Phosphorothioate (**9**).* To a 500 mL round-bottom flask were added **8** (31.5 g, 21.8 mmol), anhydrous CH_2Cl_2 (150 mL), and triethylsilane (10.5 mL, 65.5 mmol). Dichloroacetic acid (5.4 mL, 65.5 mmol) was charged dropwise over a course of 10 min. The reaction mixture was stirred at room temperature with progress monitored by HPLC. After completion of the reaction (14 h), the crude solution was dropped onto MTBE (200 mL) over the course of 10 min. The resulting precipitate was collected and purified by column chromatography (silica gel, eluting with 0−20% MeOH in CH_2Cl_2) to give **9** as a light yellow foam (17.3 g, 15.2 mmol, 70% yield). Data for **9**: ^1H NMR (500 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.14 (s, 1H), 8.10 (s, 1H), 8.06 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 2.7 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 2.7 Hz, 1H), 7.26−7.17 (m, 14H), 7.17−7.12 (m, 3H), 5.97 (d, J = 5.9 Hz, 1H), 5.34 (q, J = 5.7 Hz, 1H), 4.71 (q, J = 8.8 Hz, 1H), 4.45 (dd, J = 3.9, 4.6 Hz, 1H), 4.32 (m, 2H), 3.98 (q, J = 3.9 Hz, 1H), 3.84 (m, 1H), 3.72 (m, 1H), 3.69 (br dd, J = 3.9, 11.9 Hz, 1H), 3.60 (br dd, J = 4.0, 11.9 Hz, 1H), 3.24 (m, 2H), 3.01 (m, 2H), 2.88 (m, 1H), 2.36 (m, 1H), 2.22−2.15 (m, 2H), 0.83 (s, 9H), −0.05 (s, 6H); $^{13}C\{^1H\}$ NMR (126 MHz, DMSO-d_6) δ 154.4 (br s, 1C), 151.0, 150.3 (br s, 1C), 149.5, 149.0, 146.2, 146.0 (s, 2C), 144.2, 143.6 (s, 3C), 140.1, 138.2, 130.2 (s, 2C), 128.0 (s, 6C), 127.7 (s, 6C), 126.7 (s, 3C), 123.3 (s, 2C), 119.3, 118.6, 115.8, 105.9, 86.6 (br d, J = 10.9 Hz, 1C), 85.5, 85.3, 76.2 (br s, 1C), 70.0, 67.3 (br s, 1C), 63.2, 61.5, 47.2, 45.8, 45.2, 33.8, 31.1 (br d, J = 7.3 Hz, 1C), 30.4, 25.7 (s, 3C), 17.7, −3.2 (s, 2C); ^{31}P NMR (202 MHz, DMSO-d_6) δ 57.0 (br s, 1P); HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{56}H_{63}N_{11}O_{10}PSSi$ 1140.3987, found 1140.3981.

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*O-(((1S,2R,3R)-3-(6-Amino-9H-purin-9-yl)-2-((trityloxy)methyl)-cyclobutyl)methyl) O-((2R,3R,4R,5R)-4-((tert-Butyldimethylsilyl)-oxy)-5-((((2R,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)-methyl)-2-(5-(4-nitrophenethyl)-9-oxo-5,9-dihydro-3H-imidazo-[1,2-a]purin-3-yl)tetrahydrofuran-3-yl) O-Hydrogen Phosphoro-thioate (**10**).* To a 1 L round-bottom flask were added **9** (8.7 g, 7.6 mmol) and anhydrous 1:1 MeCN/THF (160 mL). The mixture was stirred for 30 min at 20 °C to give a homogeneous solution, which was then evaporated. This azeotroping procedure was repeated two more times, and the residual solid was placed under a high vacuum for 60 min. The (*S*)-Ψ-reagent (5.1 g, 11.4 mmol) and anhydrous THF (100 mL) were then charged, and the slurry was cooled to 0 °C. DBU (5.7 mL, 37.9 mmol) was added over the course of 1 min. The reaction mixture was stirred at 0 °C with progress monitored by HPLC. After completion of the reaction (5 min), acetic acid (4.3 mL, 76.0 mmol) was added, and the crude solution was dropped onto cold (0 °C) MTBE (500 mL) over the course of 10 min. The resulting precipitate was allowed to settle for 10 min before decanting the MTBE solution. The precipitate was dissolved in CH_2Cl_2 (600 mL) and washed with citric acid (20% w/v aq solution, 3 × 200 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a solid, which was slurried in MTBE (200 mL) and filtered to give **10** as a white powder (10.5 g, 7.6 mmol, 100% yield). Data for **10**: ^{1}H (500 MHz), ^{13}C{^{1}H} (126 MHz), ^{31}P (202 MHz), $CDCl_3$. The structure of **10** in this crude sample was not fully elucidated. Many resonances in the various 1D spectra acquired were broad. Two-dimensional NMR allowed the assignment of some resonances in the ribose and cyclobutyl rings, the *tert*-butyldimethylsilyl ester, ethyl nitrobenzene, and limonine moiety. Please see Supporting Information for spectra. HRMS (ESI-TOF): m/z [M + H]$^+$ calcd for $C_{66}H_{78}N_{11}O_{11}P_2S_3Si$ 1386.4283, found 1386.4295.

*O-(((1S,2R,3R)-3-(6-Amino-9H-purin-9-yl)-2-(hydroxymethyl)-cyclobutyl)methyl) O-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)-oxy)-5-((((2R,3aR,6S,7aR)-3a-methyl-6-(prop-1-en-2-yl)-2-sulfidohexahydrobenzo[d][1,3,2]oxathiaphosphol-2-yl)oxy)-methyl)-2-(5-(4-nitrophenethyl)-9-oxo-5,9-dihydro-3H-imidazo-[1,2-a]purin-3-yl)tetrahydrofuran-3-yl) O-Hydrogen (R)-Phosphor-othioate (**24**).* To a 1 L round-bottom flask were added **10** (20.1 g, 14.5 mmol), anhydrous CH_2Cl_2 (240 mL), and triisopropylsilane (31.5 mL, 145.0 mmol). The mixture was stirred for 30 min at 20 °C to give a homogeneous solution. Dichloroacetic acid (7.62 mL, 116 mmol) in CH_2Cl_2 (39.8 mL) was added over 5 min, during which time the mixture went from light yellow to orange to pale yellow. The reaction progress was monitored by HPLC, and after completion of the reaction (1.25 h), the mixture was evaporated. The residue was treated with Et_2O (1000 mL), and the resulting precipitate was collected to give **24** as a white powder (14.9 g, 13.1 mmol, 90% yield). Data for compound **24**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.23 (s, 1H), 8.12 (s, 1H), 8.08 (m, 2H), 7.55 (d, J = 2.7 Hz, 1H), 7.52 (br d, J = 8.8 Hz, 2H), 7.31 (d, J = 2.7 Hz, 1H), 6.09 (d, J = 6.6 Hz, 1H), 5.72 (td, J = 6.1, 11.8 Hz, 1H), 4.72 (br s, 2H), 4.56 (m, 1H), 4.55 (m, 1H), 4.42 (m, 1H), 4.40 (m, 1H), 4.31 (m, 1H), 4.27 (m, 1H), 4.15 (dt, J = 2.5, 5.7 Hz, 1H), 3.49 (m, 2H), 3.40 (m, 1H), 3.33 (m, 1H), 3.32 (m, 2H), 3.30 (m, 4H), 2.62 (m, 1H), 2.18 (m, 1H), 2.13 (m, 1H), 1.98 (q, J = 9.9 Hz, 1H), 1.91 (m, 1H), 1.87 (m, 1H), 1.84 (m, 1H), 1.78 (m, 1H), 1.71 (m, 1H), 1.63 (s, 3H), 1.58 (s, 3H), 0.89 (s, 9H), 0.18 (s, 3H), 0.17 (s, 3H); ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ 151.1, 149.7, 148.7 (m, 1C), 148.7 (br s, 1C), 146.3, 146.1, 144.9, 144.2, 141.0 (br s, 1C), 139.1, 130.2 (s, 2C), 123.4 (s, 2C), 119.4 (br s, 1C), 118.6, 116.2, 111.3, 105.9, 86.2 (br d, J = 5.5 Hz, 1C), 85.8, 83.0 (br d, J = 9.1 Hz, 1C), 74.0 (br s, 1C), 71.7 (br s, 1C), 65.8, 60.75, 47.5, 47.00, 45.4 (br s, 1C), 38.2, 33.8, 33.1, 30.5 (br d, J = 6.4 Hz, 1C), 29.2 (br s, 1C), 25.8 (s, 3C), 22.6, 22.3, 17.9, −4.3, −5.2; ^{31}P NMR (202 MHz, DMSO-d_6) δ 100.8 (s, 1P), 57.94 (br s, 1P); HRMS (ESI-TOF) m/z [M + 2H]$^{2+}$/2 calcd for $C_{47}H_{65}N_{11}O_{11}P_2S_3Si/2$ 572.6636, found 572.6623.

*3-((1R,3R,6S,8R,9R,12S,15R,17R,18R)-8-(6-Amino-9H-purin-9-yl)-18-((tert-butyldimethylsilyl)oxy)-3,12-dihydroxy-3,12-disulfido-2,4,11,13,16-pentaoxa-3,12-diphosphatricyclo[13.2.1.0^{6,9}]-]octadecan-17-yl)-5-(4-nitrophenethyl)-3,5-dihydro-9H-imidazo-[1,2-a]purin-9-one (**12**) and 3-((1R,3R,6S,8R,9R,12S,15R,17R,18R)-8-(6-Amino-9H-purin-9-yl)-18-((tert-butyldimethylsilyl)oxy)-3,12-dihydroxy-3,12-disulfido-2,4,11,13,16-pentaoxa-3,12-diphosphatricyclo[13.2.1.0^{6,9}]octadecan-17-yl)-3,5-dihydro-9H-imidazo[1,2-a]purin-9-one (**27**).* To a 2 L round-bottom flask were added **24** (16.0 g, 14.0 mmol) and anhydrous 1:1 MeCN/THF (200 mL). The mixture was stirred for 30 min at 20 °C to give a homogeneous solution, which was evaporated. This azeotroping procedure was repeated two more times, and the solid was placed under a high vacuum for 60 min. Anhydrous THF (1400 mL) was charged followed by DBU (21.1 mL, 140.0 mmol) in one portion. The reaction mixture was stirred at room temperature with progress monitored by HPLC. After completion of the reaction (2 min) to give **25**, the reaction mixture was evaporated to remove the THF, and the flask was charged with CH_3CN (1400 mL) and heated to 40 °C using a heating mantle. The deprotection reaction progress was monitored by HPLC, and after completion of the reaction (14 h), the mixture was evaporated and purified by supercritical fluid chromatography to give **27** as a white solid (5.9 g, 7.1 mmol, 51% yield over two steps). Data for **25** after SFC purification of an aliquot: ^{1}H NMR (500 MHz, CD_3OD) δ 8.47 (s, 1H), 8.28 (br s, 1H), 8.17 (s, 1H), 8.07 (d, J = 8.7 Hz, 2H), 7.50 (br d, J = 1.7 Hz, 1H), 7.41 (d, J = 8.7 Hz, 2H), 7.16 (br d, J = 1.7 Hz, 1H), 6.17 (d, J = 8.2 Hz, 1H), 5.34 (m, 1H), 4.82−4.74 (m, 2H), 4.49−4.33 (m, 3H), 4.29−4.20 (m, 3H), 4.12−4.03 (m, 2H), 3.89 (m, 1H), 3.35 (m, 1H), 3.26 (m, 1H), 2.60 (td, J = 8.1, 10.4 Hz, 1H), 2.47 (m, 1H), 2.29 (q, J = 10.0 Hz, 1H), 1.04 (s, 9H), 0.35 (s, 3H), 0.30 (s, 3H); ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 155.8 (br s, 1C), 153.6 (br s, 1C), 152.6, 151.5 (br s, 1C), 150.6, 148.3, 147.6, 146.3, 142.4 (br s, 1C), 140.0 (br s, 1C), 131.3 (s, 2C), 124.5 (s, 2C), 120.6, 120.1, 116.4 (br s, 1C), 107.1, 87.7 (br d, J = 9.1 Hz, 1C), 86.2 (br s, 1C), 77.3 (br s, 1C), 75.1 (br d, J = 3.6 Hz, 1C), 69.5 (br d, J = 3.6 Hz, 1C), 68.2 (d, J = 3.3 Hz, 1C), 66.6 (br s, 1C), 51.0 (br s, 1C), 47.4, 36.0, 33.5 (br d, J = 8.2 Hz, 1C), 30.2 (br s, 1C), 26.7 (s, 3C), 19.3, −3.5, −4.6; ^{31}P NMR (202 MHz, CD_3OD) δ 58.0 (br s, 1P), 55.8 (br s, 1P); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{37}H_{48}N_{11}O_{11}P_2S_2Si$ 976.2215, found 976.2208. Data for **27**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.51 (s, 1H), 8.37 (s, 1H), 8.24 (s, 1H), 8.15 (s, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.41 (br d, J = 2.2 Hz, 2H), 5.97 (d, J = 8.6 Hz, 1H), 5.35 (m, 1H), 4.63 (br s, 1H), 4.67−4.59 (m, 2H), 4.14 (dt, J = 4.9, 10.7 Hz, 1H), 4.08 (m, 1H), 4.04 (m, 1H), 3.89 (m, 1H), 3.85 (m, 1H), 3.76 (m, 1H), 3.71 (m, 2H), 3.02 (m, 2H), 2.49 (m, 1H), 2.24 (m, 1H), 2.12 (q, J = 9.9 Hz, 1H), 0.93 (s, 9H), 0.20 (s, 3H), 0.19 (s, 3H); ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ 155.4 (br s, 1C), 151.6 (br s, 1C), 151.3, 151.1, 149.3, 146.0, 139.7 (br s, 1C), 137.3 (br s, 1C), 118.9, 116.4, 114.1, 106.8, 85.5 (br d, J = 7.3 Hz, 1C), 83.0 (br s, 1C), 74.7 (br s, 1C), 73.8 (br s, 1C), 67.3 (br s, 1C), 66.8 (br s, 1C), 65.5 (br s, 1C), 48.9 (br s, 1C), 47.2 (br s, 1C), 33.3 (br s, 1C), 28.8 (br s, 1C), 26.0 (s, 3C), 18.1, −4.0, −5.2; ^{31}P NMR (202 MHz, DMSO-d_6) δ 55.6 (br s, 1P); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{29}H_{41}N_{10}O_9P_2S_2Si$ 827.1738, found 827.1742

3-((1R,3R,6S,8R,9R,12S,15R,17R,18R)-8-(6-Amino-9H-purin-9-yl)-3,12,18-trihydroxy-3,12-disulfido-2,4,11,13,16-pentaoxa-3,12-diphosphatricyclo[13.2.1.0^{6,9}]octadecan-17-yl)-3,5-dihydro-9H-imidazo[1,2-a]purin-9-one, BMT-390025. To a 1 L round-bottom flask were added **27** (14.0 g, 16.9 mmol), anhydrous MeOH (170 mL), and NH_4F (3.1 g, 85.0 mmol). The mixture was heated to 60 °C using a heating mantle, and the reaction progress was monitored by HPLC. After completion of the reaction (14 h), the mixture was purified by supercritical fluid chromatography to give BMT-390025 as a white solid (7.6 g, 10.1 mmol, 60% yield): ^{1}H NMR (700 MHz, D_2O) δ 8.21 (s, 2H), 8.15 (s, 1H), 7.52 (d, J = 2.5 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1H), 6.16 (d, J = 7.9 Hz, 1H), 5.46 (dt, J = 4.5, 8.6 Hz, 1H), 4.81 (d, J = 4.3 Hz, 1H), 4.75 (ov, 1H), 4.53 (m, 1H), 4.31 (m, 1H), 4.28−4.21 (m ov, 3H), 4.04 (m, 1H), 3.86 (td, J = 4.9, 10.2 Hz, 1H), 3.01 (m, 1H), 2.65 (td, J = 8.5, 11.2 Hz, 1H), 2.48 (m, 1H), 2.31 (m, 1H); ^{13}C{^{1}H} NMR (176 MHz, D_2O) δ 153.2 (br s, 1C), 153.1, 150.5, 148.9 (br s, 1C), 148.5, 145.3, 141.76, 140.0 (br s, 1C), 118.3, 116.7, 115.7, 107.0, 86.7 (br d, J = 6.4 Hz, 1C), 84.4 (d, J = 10.2 Hz, 1C), 75.8 (d, J = 7.6 Hz, 1C), 71.2 (d, J = 2.5 Hz, 1C), 68.1 (d, J = 6.4

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Hz, 1C), 67.3 (d, J = 5.1 Hz, 1C), 64.9 (d, J = 5.1 Hz, 1C), 49.1, 46.6 (d, J = 7.6 Hz, 1C), 31.4 (d, J = 11.4 Hz, 1C), 28.0; ^{31}P NMR (202 MHz, D_2O) δ 55.9 (s, 1P), 52.3 (s, 1P); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{23}H_{27}N_{10}O_9P_2S_2$ 713.0879, found 713.0897; $[\alpha]_D^{23}$ −41.71 (c 0.556, H_2O)

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00784.

^{1}H and ^{13}C{^{1}H} NMR spectra for all new compounds; ^{31}P NMR spectra for compounds **7**, **8**, **9**, **10**, **24**, **25**, **27**, and BMT-390025; X-ray crystallography data for compound **5** (PDF)

Accession Codes

CCDC 2074384 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

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