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# A P(V)−N Activation Strategy for the Synthesis of Nucleoside Polyphosphates

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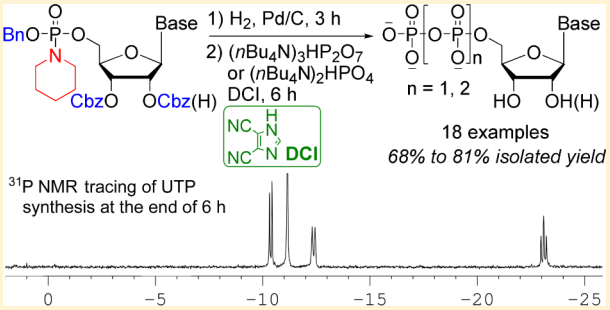
Supporting Information

**ABSTRACT:** A general and high-yielding synthesis of nucleoside 5′-triphosphates (NTPs) and nucleoside 5′-diphosphates (NDPs) from protected nucleoside 5′-phosphoropiperidates promoted by 4,5-dicyanoimidazole (DCI) has been developed. $^{31}$P NMR tracing experiments showed that the sequential deprotection and coupling reactions were exceptionally clean. The phosphoropiperidate exhibited superior reactivity to the conventional phosphoromorpholidate toward DCI-promoted NTP/NDP synthesis. The experimental results suggested that the mechanism of DCI activation could be distinctive for NTP and NDP synthesis, depending on the different nucleophilicity of pyrophosphate and phosphate.




## INTRODUCTION

Natural nucleoside polyphosphates (NPPs) including nucleoside 5′-triphosphates and 5′-diphosphates (NTPs and NDPs) play pivotal roles in numerous biological processes, such as energy transfer, gene replication and expression, intracellular signaling, and regulation of protein activity. Natural and modified NTPs are indispensable substrates for a myriad of techniques in modern biological research, ranging from polymerase chain reaction to DNA/RNA labeling, random mutagenesis, and DNA sequencing.[1] The high demand for NPPs in biology and medicine has always been a stimulus to establishment of general and practical synthetic routes for these compounds.[2]

Currently, the traditional "one-pot, three-step"[3] and salicyl phosphorochlorodite methods[4] are still in wide use, but their yields and generality are far from satisfactory. Although NTP synthesis experienced a low-tide period of nearly two decades, the past few years have witnessed revived interest in this area. Novel synthetic methods based on *H*-phosphonate,[5] cyclosaligenyl phosphate triester,[6] cyclic phosphite,[7] and sulfonyl imidazolium triflate[8] were brought to the arsenal. Among these new reports, the cyclic phosphite approach presented a straightforward and protection-free method, which is highly desired for NTP synthesis. But the reported isolated yields of dNTPs were low (19−46%). Moreover, the $^{1}$H and $^{31}$P NMR spectra of their dNTPs showed that the samples were contaminated with significant amount of impurities. In a recently published paper employing this method,[9] a very low yield of only 7% was obtained for a dNTP compound, and two distinctive sets of peaks on its $^{1}$H and $^{31}$P NMR spectra clearly indicated the existence of undesired 3′-triphosphate byproduct, which is contradictory to the high regioselectivity at the 5′-OH claimed by this method. On the other hand, the sulfonyl imidazolium triflate method also emphasized that no protection is required for the synthesis of nucleoside polyphosphate and related conjugates. However, this advantage is based on the prerequisite that nucleoside 5′-monophosphate (NMP) is available. In case that NTP synthesis has to start from specific nucleoside substrates, the high yielding and regioselective phosphorylation at the 5′-OH remains a bottleneck as exemplified by a recent report using this method (29% yield for a NMP synthesis from a nucleoside substrate).[9]

As a major type of NTP synthetic precursors, phosphoramidates, such as phosphoromorpholidates[10] and phosphorimidazolides,[11] have been utilized to synthesize NTPs in moderate yields, but these reactions are typically sluggish and require days to complete. To accelerate the reaction rate, more reactive zwitterionic phosphoramidate intermediates such as *N*-methylimidazolium,[12] *N*-methylpyrrolidinium,[13] and pyridinium phosphoramidates[5] were generated in situ to promote their coupling with pyrophosphate. But a concomitant issue was the formation of more polyphosphate byproducts.

Unlike the tetracoordinated phosphoramidates, tricoordinated phosphoramidites are much more reactive to nucleophilic displacement at the phosphorus center due to the free electron pair and trigonal pyramid geometry.[14] In oligonucleotide synthesis, phosphoramidites have been extensively used as phosphitylating regents to link two nucleoside units with a phosphite triester in the presence of an acidic activator.[15] In contrast, research on the activation of the more stable P(V)−N bond in phosphoramidate for coupling reactions has been

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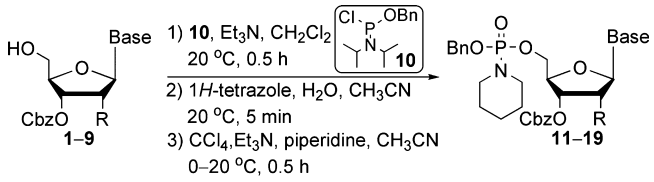
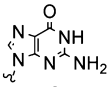
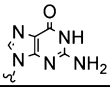
**Table 1. Synthesis of Protected Nucleoside 5′-Phosphoropiperidates (11−19)**




| Entry | Phosphoropiperidates (Substrates) | R | Base | Isolated yield (%) |
|---|---|---|---|---|
| 1 | **11** (**1**) | OCbz |  | 84 |
| 2 | **12** (**2**) | OCbz |  | 80 |
| 3 | **13** (**3**) | OCbz |  | 82 |
| 4 | **14** (**4**) | OCbz |  | 77 |
| 5 | **15** (**5**) | OCbz |  | 78 |
| 6 | **16** (**6**) | H |  | 85 |
| 7 | **17** (**7**) | H |  | 81 |
| 8 | **18** (**8**) | H |  | 80 |
| 9 | **19** (**9**) | H |  | 75 |

limited up to now. On the basis of the labile nature of phosphoramidates at low pH condition, we hypothesized that a proper acidic activator may weaken the P(V)−N bond in situ and promote its specific coupling with pyrophosphate/phosphate in the absence of $H_2O$. Furthermore, precedent reports[16] of the catalytic effects of 1*H*-tetrazole and *N*-methylimidazolium chloride on the synthesis of NDP-sugars, lipid polyphosphate-sugars, and lipid diphosphate conjugates strongly inspired our rationale for investigating this P(V)−N activation approach for NPP synthesis. We report here a novel and highly efficient method for the synthesis of both NTPs and NDPs from protected nucleoside 5′-phosphoropiperidates promoted by 4,5-dicyanoimidazole (DCI).

## RESULTS AND DISCUSSION

**Preparation of Protected Nucleoside 5′-Phosphoropiperidates.** As shown in Table 1, the protected phosphoropiperidates of eight natural ribo- and 2′-deoxyribonucleosides (**11**−**14**, **16**−**19**) and an antiviral nucleoside ribavirin (**15**) were rationally designed and synthesized. Compared to acetyl and benzoyl groups conventionally used in NTP synthesis, the combination of carboxybenzyl (Cbz) and benzyl ester enables simultaneous and quantitative deprotection of both nucleoside and phosphoramidate moieties by mild catalytic hydrogenation prior to the coupling reaction.

Moreover, the Cbz-protected nucleoside substrates (**1**−**9**) could be efficiently prepared in multigram quantities and excellent yields according to the reported procedures.[17] Phosphitylation of **1**−**9** with benzyl *N*,*N*-diisopropylchlorophosphoramidite (**10**), acid-catalyzed hydrolysis, and oxidative coupling with $CCl_4/Et_3N$/piperidine afforded **11**−**19** in excellent yields ranging from 75% to 85% over three consecutive steps. The preparation of **11**−**19** could be easily scaled up to multigram quantities without decrease in yields.

**Preparation of NTPs and NDPs.** The conversion of **11**−**19** to NPPs **23**−**40** was conducted in two consecutive steps (Table 2). First, hydrogenation with Pd/C yielded fully deprotected nucleoside 5′-phosphoropiperidates in 3 h. After Pd/C was removed by filtration, addition of 6 equiv of DCI (**20**) and 2 equiv of tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (**21**) or 8 equiv of DCI and 3 equiv of bis(tetra-*n*-butylammonium) hydrogen phosphate (**22**) converted the phosphoropiperidate intermediates to NPP products in 6 h at 20 °C. Ethanol precipitation followed by ion exchange chromatography afforded **23**−**40** in excellent isolated yields (68−81%) and high purity. It is noteworthy that this method exhibits no sensitivity to either furanose or nucleobase moieties, and the efficacy of coupling is equally high for both NTPs and NDPs.


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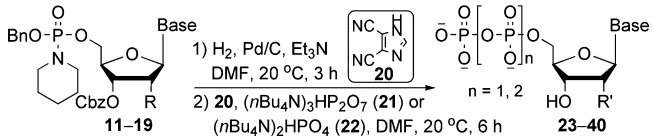
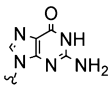
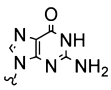
**Table 2. A DCI-Promoted Methodology for Conversion of 11−19 to NPPs 23−40**




| Entry | NPPs | n | R′ | Base | Isolated yield (%) |
|---|---|---|---|---|---|
| 1 | UTP (**23**) | 2 | OH |  | 81 |
| 2 | UDP (**32**) | 1 | OH | | 79 |
| 3 | CTP (**24**) | 2 | OH |  | 70 |
| 4 | CDP (**33**) | 1 | OH | | 69 |
| 5 | ATP (**25**) | 2 | OH |  | 75 |
| 6 | ADP (**34**) | 1 | OH | | 73 |
| 7 | GTP (**26**) | 2 | OH |  | 73 |
| 8 | GDP (**35**) | 1 | OH | | 73 |
| 9 | RTP (**27**) | 2 | OH |  | 73 |
| 10 | RDP (**36**) | 1 | OH | | 70 |
| 11 | dTTP (**28**) | 2 | H |  | 78 |
| 12 | dTDP (**37**) | 1 | H | | 76 |
| 13 | dCTP (**29**) | 2 | H |  | 70 |
| 14 | dCDP (**38**) | 1 | H | | 68 |
| 15 | dATP (**30**) | 2 | H |  | 75 |
| 16 | dADP (**39**) | 1 | H | | 72 |
| 17 | dGTP (**31**) | 2 | H |  | 72 |
| 18 | dGDP (**40**) | 1 | H | | 73 |

**Proof of Concept and Optimization of the P(V)−N Activation Strategy for NTP Synthesis.** To prove the concept of P(V)−N activation strategy for NTP synthesis, protected uridine 5′-phosphoromorpholidate (**41**) was first synthesized as a prototype compound due to the precedents of phosphoromorpholidate[10,16] in the synthesis of NTPs and NDP-sugars. $^{31}$P NMR tracing experiments showed that **41** was quantitatively transformed into uridine 5′-phosphoromorpholidate (**42**) by catalytic hydrogenation in 3 h. However, to our surprise, addition of 1 equiv of pyrophosphate **21** according to Moffatt's method[10b] yielded no UTP (**23**) at all, even after 5 days. At this point, 4 equiv of 1*H*-tetrazole was added to the unreacted **42** and **21** according to the method described by Wong et al. for NDP-sugar synthesis.[16a] **42** gradually disappeared in 72 h, and the desired triphosphate **23** was obtained in 57% yield (Figure 1).

Inspired by this result, a series of weakly acidic activators commonly used in phosphoramidite methodology ($pK_a$ ($H_2O$) = 4.9−9.2) was screened under the same conditions.[15] When DCI and pyridinium chloride were applied, significantly shortened reaction time (6 h) and comparable yields (67% for DCI and 56% for pyridinium chloride) were obtained. The precipitation of pyrophosphate upon addition of HCl salts of imidazole, *N*-methylimidazole, and DMAP precluded their application as activators. Due to its excellent solubility in organic solvents, nonhydroscopic nature, and commercial availability, DCI was selected as the best activator for further optimization.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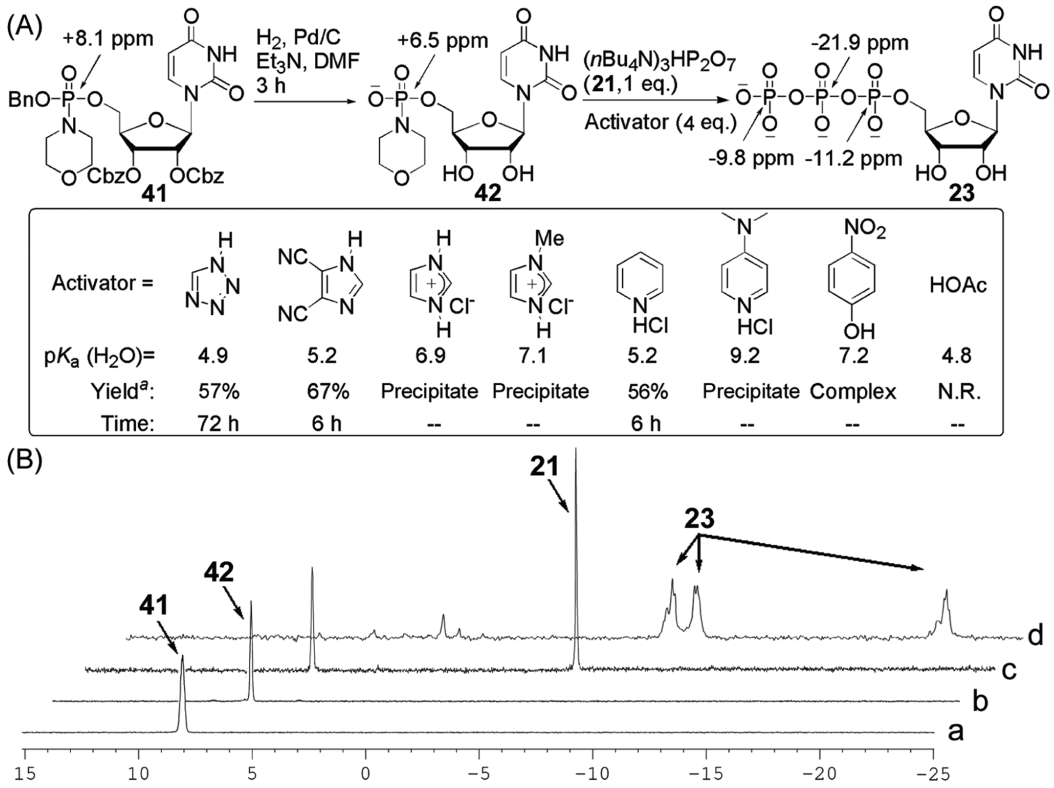


**Figure 1.** Proof of concept of the P(V)−N activation strategy for NTP synthesis (A) and $^{31}$P NMR tracing of the reaction with 1*H*-tetrazole as an activator (B). (a) **41**; (b) **42**, 3 h after hydrogenation; (c) 120 h after addition of **21**; (d) 72 h after addition of 1*H*-tetrazole. [a] $^{31}$P NMR yield.

Analysis of the $^{31}$P NMR spectrum of DCI-catalyzed coupling reaction with 1 equiv of **21** showed that $Up_4U$ was generated because of the competition of triphosphate product **23**. Thus, a higher excess of **21** was added to improve coupling specificity. As shown in Figure 2A, 2 equiv of **21** completely suppressed the side reaction and increased the yield of **23** to 88%. The only byproduct was a small amount of uridine 5′-monophosphate (UMP) derived from the hydrolysis of **42**.

Meanwhile, it was noticed that if the amount of **21** was increased to 2 equiv but that of DCI remained unchanged (4 equiv), the reaction rate dropped remarkably (10 h). This was possibly due to the neutralization of more DCI by additional **21**. The data in Figure 2B showed that 6 equiv of DCI restored the reaction time to 6 h, but further increasing the amount of DCI exhibited no significant improvement.

Our design of the protected P(V)−N precursors enabled diversity-oriented synthesis of a series of phosphoramidates with different amino leaving groups (**11**, **41**, **43**−**45**) in good yields (Figure 3A). As shown in Figure 3B, side by side comparison of their couplings with **21** (2 equiv) in the presence of DCI (6 equiv) showed that the reaction of phosphoropiperidate **11** with **21** (93% yield, 6 h) resulted in a yield higher than that of the conventional phosphoromorpholidate **41** (88% yield, 6 h), which was possibly due to the stronger protonation capability of the N atom of piperidine ($pK_a$ ($H_2O$) = 11.22) than that of morpholine ($pK_a$ ($H_2O$) = 8.36). Though Moffatt and Khorana also found that phosphoropiperidate exhibited reactivity better than that of phosphoromorpholidate, the very low yield of phosphoropiperidate by the DCC coupling method (20%) precluded its application as a P(V)−N precursor in their method.[10a] It is worth noting that all three phosphoramidates with a cyclic secondary amino leaving group (**11**, **41**, and **45**) achieved high NTP conversion (>80% yield). In contrast, the reactions of the ones with a noncyclic amino leaving group (**43** and **44**) were sluggish (<60%, 48 h) regardless of their $pK_a$s.

The effect of temperature was also evaluated for this reaction. **11** was first subjected to catalytic hydrogenation. Then, the reactions of uridine 5′-phosphoropiperidate (**46**) with **21** (2 equiv) and DCI (6 equiv) were performed at 20 °C, 30 °C, 40 °C, and 50 °C, respectively. The consumption of **46** was much faster at higher temperature (50 °C, 30 min), but the formation of UMP (hydrolysis of **46**), UDP (decomposition of **23**), and $Up_4U$ (competition reaction of **23** with **46**) became pronounced at the same time (Figure 4). The best compromise between reaction time and yield was found at 20 °C.

**Optimization of the P(V)−N Activation Strategy for NDP Synthesis.** On the basis of the newly established phosphoropiperidate/DCI system for NTPs, its application in NDP synthesis was explored. $^{31}$P NMR tracing experiments determined that 8 equiv of DCI and 3 equiv of **22** were optimal to maximize the reaction rate and eliminate the formation of $Up_3U$ byproduct (Figure S1 and S2, Supporting Information). Comparison of the reactions of **11** and **41** showed that the difference in the reactivity of phosphoropiperidate (91% yield, 6 h) and phosphoromorpholidate (76% yield, 12 h) became pronounced in the presence of less nucleophilic phosphate (Figure S3, SI).

**Mechanisms of DCI-Promoted NTP and NDP Synthesis.** As illustrated in the proposed reaction path for **23** (Figure 5A), protonation of the inactive monoionic phosphoramidate **46** ($P-O^-/P-NR_2$) generates the neutral phosphoramidate **47a** ($P-OH/P-NR_2$) and its zwitterionic tautomer **47b** ($P-O^-/P-NH^+R_2$).[18] Based on Michielssens and Lönnberg's research on the hydrolysis of nucleoside phosphoramidates,[19] the subsequent P−O coupling with **21** is proposed to proceed via an associative pathway with the more reactive


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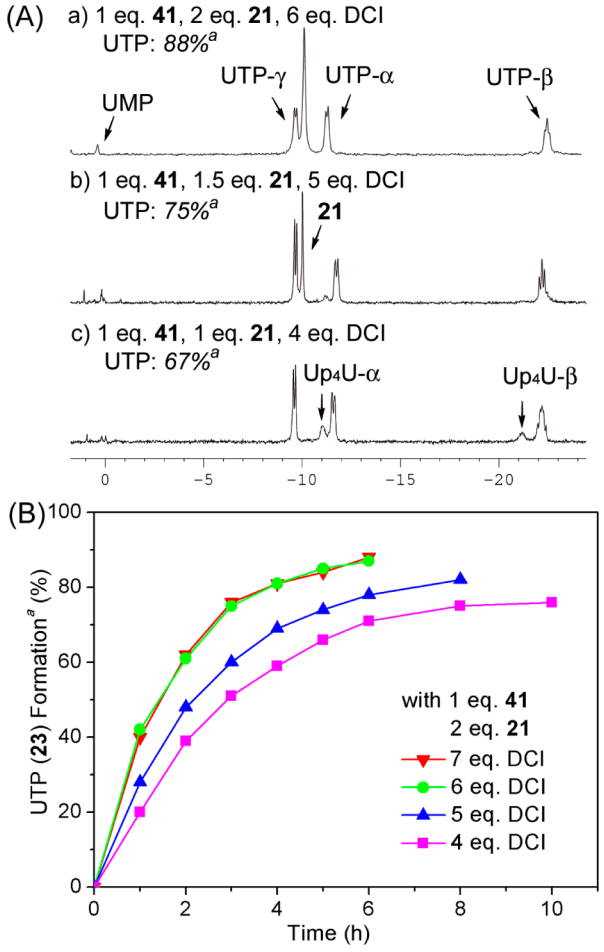


**Figure 2.** Effects of the amounts of **21** (A) and DCI (B) on UTP (**23**) formation. [a] $^{31}$P NMR yield.

tautomer **47b**. It was observed that if $Et_3N$ was not added in hydrogenation, the resulting **47** was highly susceptible to hydrolysis during filtration. In a control experiment, addition of DCI to the mixture of **11** (P−OBn/P−$NR_2$) and **21** exerted no activation effect, indicating that zwitterionic **47b** is essential for P(V)−N bond activation.

In addition to acid catalysis, nucleophilic catalysis of the conjugate base of DCI may also be involved.[15c−f,20] But during the reactions, no peaks other than those of the starting materials (**47**, **21**/**47**, **22**) and product (**23**/**32**) were observed on the $^{31}$P NMR spectra of the tracing experiments (Figure 5B and 5C), which may possibly be ascribed to the overlap of the peak of phosphorodicyanoimidazolide intermediate (**48**) with that of the $\alpha$-P of **23** and **32** (−11.5 ppm).[21] Therefore, 2,6-lutidinium chloride, which has a non-nucleophilic conjugate base, was tested for the synthesis of UTP (**23**) and UDP (**32**), respectively, to elucidate the role of the conjugate base of DCI. While 2,6-lutidinium chloride-promoted synthesis of **23** (91% yield, 6 h) was fairly close to that of DCI, the synthesis of **32** was dramatically affected (37%, 48 h) when 2,6-lutidinium chloride was used instead of DCI. The above experimental results indicated that nucleophilic catalysis of the conjugate base of DCI is trivial to the coupling reaction with highly nucleophilic pyrophosphate **21**. In contrast, nucleophilic catalysis via intermediate **48** is essential to promote the formation of diphosphate **32** when less nucleophilic phosphate **22** was used (Figure 5A).

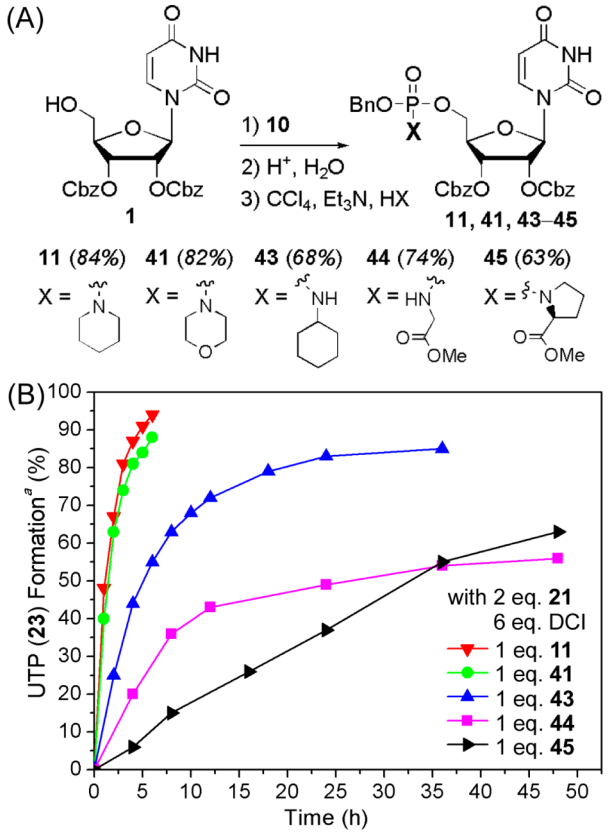


**Figure 3.** Synthesis of protected uridine 5′-phosphoramidates **11**, **41**, **43**−**45** (A) and effect of the amino leaving groups on UTP (**23**) formation (B). [a] $^{31}$P NMR yield.

## CONCLUSION

In summary, our P(V)−N activation strategy presents a novel and highly efficient approach for the synthesis of NTPs and NDPs from nucleoside 5′-phosphoropiperidates promoted by 4,5-dicyanoimidazole (DCI). This novel method features (1) easily accessible Cbz/Bn-protected nucleoside phosphoropiperidate precursors, which could be prepared in excellent yields (75−85%) over three consecutive steps on multigram scale, (2) one-pot removal of all protecting groups by catalytic hydrogenation before coupling with pyrophosphate and phosphate, (3) no sensitivity to nucleobase and furanose (18 ribo- and 2′-deoxyribonucleoside examples, including ribavirin triphosphate and diphosphate), (4) short reaction time and mild conditions (6 h at 20 °C), and (5) excellent isolated yields (68−81%) and high purity (as exemplified by the NMR spectra and HPLC of all 18 examples in Supporting Information).

Moreover, $^{31}$P NMR tracing spectra revealed that the conversion of fully deprotected nucleoside 5′-phosphoropiperidates to the desired NPPs in the presence of DCI were exceptionally smooth and clean (e.g., 93% for UTP, 91% for UDP). Our experimental results showed that phosphoropiperidate has superior reactivity toward the DCI-promoted P(V)−N bond activation and subsequent P(V)−O bond coupling reactions compared with that of the conventional phosphoromorpholidate and suggested that, other than acid catalysis, nucleophilic catalysis of the conjugate base of DCI may also play an important role in NPP synthesis, depending on the distinctive nucleophilic nature of pyrophosphate and phosphate. The application of this method to improve the synthesis


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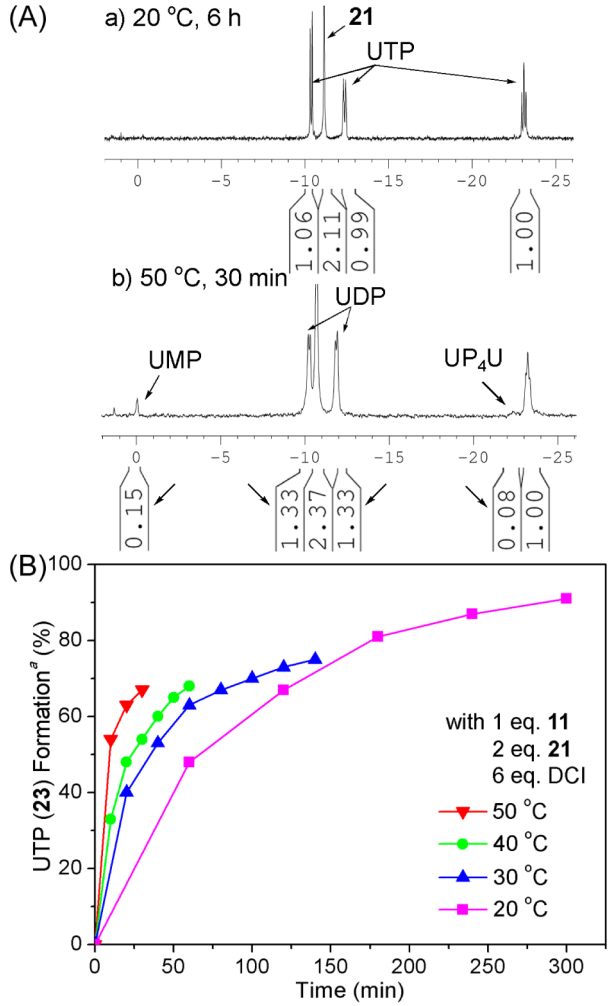


**Figure 4.** $^{31}$P NMR spectra of UTP (**23**) synthesis at 20 °C and 50 °C (A) and effect of reaction temperature on UTP (**23**) formation (B). [a] $^{31}$P NMR yield.

of NDP-sugars and dinucleoside polyphosphates is currently underway and will be reported in due course.

## EXPERIMENTAL SECTION

**General Methods.** Chemical reagents and solvents were obtained from commercial suppliers. Natural ribonucleosides (A, C, G, U), 2′-deoxyribonucleosides (dA, dC, dG, dT), and ribavirin (R) were protected with Cbz groups as previously reported.[1,2] Tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (**21**) and bis(tetra-*n*-butylammonium) hydrogen phosphate (**22**) were prepared according to known procedures.[3,4] All reactions were performed under an atmosphere of dry argon and monitored by analytical thin-layer chromatography on plates coated with 0.25 mm silica gel 60 F254. TLC plates were visualized by UV irradiation (254 nm). Flash column chromatography employed silica gel (particle size 32−63 μm). Ion exchange chromatography employed DEAE A-25 exchanger. All NMR spectra were obtained with a 400 MHz instrument with chemical shifts reported in parts per million (ppm, $\delta$) referenced to $CDCl_3$ or $D_2O$. IR spectra were recorded on a FT-IR spectrometer. Low-resolution and high-resolution mass spectra were obtained with an ion trap and a TOFQ mass spectrometer, respectively, and reported as $m/z$. HPLC traces of **23** to **40** were recorded on an analytical instrument equipped with a C18 analytical column (4.6 × 150 mm, 5 μm) [flow rate = 1.0 mL/min; linear gradient of 5% to 80% MeOH in TEAB buffer (10 mM, pH 8.5) over 20 min; UV detection at 230 and 254 nm].

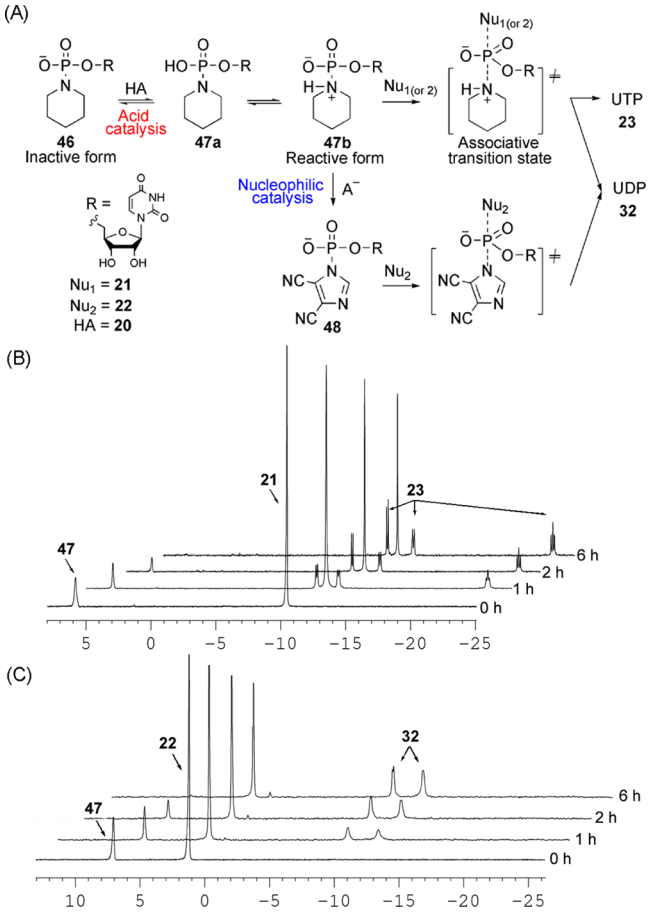


**Figure 5.** Proposed reaction mechanisms (A) and $^{31}$P NMR tracing spectra of UTP (**23**) synthesis (B) and UDP (**32**) synthesis (C).

**Benzyl *N,N*-Diisopropylchlorophosphoramidite (10).** To a solution of $PCl_3$ (59.2 mL, 682 mmol) in $CH_2Cl_2$ (200 mL) at 0 °C was added a solution of benzyl alcohol (14.2 mL, 136 mmol) in $CH_2Cl_2$ (50 mL) dropwise over 3 h. The reaction was stirred at 20 °C for 2 h. Concentration in vacuo afforded benzyl dichlorophosphite as light yellow oil (28.5 g, 99%). To a solution of benzyl dichlorophosphite (28.5 g, 134 mmol) in ether (120 mL) was added a solution of diisopropylamine (19.3 mL, 136 mmol) and triethylamine (19.0 mL, 136 mmol) in ether (50 mL) dropwise at −20 °C over 3 h. The reaction was stirred overnight at 20 °C. The triethylammonium chloride was removed by filtration, and the filtrate was concentrated in vacuo. Vacuum distillation (90 °C, 8 mmHg) of the residue oil afforded **10** (28.4 g, 82%) as colorless oil; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.37 (s, 4H), 7.33−7.32 (m, 5H), 4.99−4.91 (m, 2H), 3.90−3.84 (m, 2H), 1.35 (d, $J$ = 6.0 Hz, 6H), 1.27 (d, $J$ = 5.6 Hz, 6H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 137.5, 128.5, 127.9, 127.3, 67.6, 46.1, 46.0, 24.1, 23.3 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 181.57 ppm.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′,3′-*O*-bis-(benzyloxycarbonyl)uridine (11).** General procedure for the synthesis of protected nucleoside 5′-phosphoramidates (**11**−**19**, **41**, **43**−**45**): To a solution of Cbz-protected uridine (**1**, 500 mg, 0.98 mmol) and $Et_3N$ (0.42 mL, 3.0 mmol) in $CH_2Cl_2$ (20 mL) was added a solution of benzyl *N,N*-diisopropylchlorophosphoramidite (**10**, 548 mg, 2.0 mmol) in $CH_2Cl_2$ (10 mL) dropwise. The reaction was stirred at ambient temperature for 30 min and concentrated in vacuo. The residue was coevaporated with $CH_3CN$ (10 mL × 2) and redissolved in EtOAc (5 mL). $Et_3N$·HCl salt was removed by filtration, and the filtrate was concentrated to afford the crude phosphoramidite intermediate. To a solution of the intermediate in $CH_3CN$ (20 mL) were added 1*H*-tetrazole (140 mg, 2.0 mmol) and deionized $H_2O$ (0.2 mL). The reaction was stirred for 5 min and concentrated in vacuo.


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The residue was dissolved in $CH_2Cl_2$ (50 mL) and washed with HCl aqueous solution (0.5 M, 30 mL) and deionized $H_2O$ (30 mL). The organic phase was dried with anhydrous $Na_2SO_4$ and concentrated in vacuo to give the crude *H*-phosphonate diester intermediate. To an ice-cooled solution of piperidine (120 $\mu$L, 1.2 mmol), $Et_3N$ (0.05 mL), and $CCl_4$ (0.15 mL) in $CH_3CN$ (20 mL) were added a solution of the intermediate in $CH_3CN$ (5 mL) dropwise. The reaction was stirred at ambient temperature for 30 min and concentrated in vacuo. The residue was dissolved in EtOAc (5 mL). $Et_3N$·HCl salt was removed by filtration, and the filtrate was concentrated to give the crude product. Flash column chromatography (petroleum ether/EtOAc 1:2 to $CH_2Cl_2$/MeOH 50:1) on silica gel afforded **11** (615 mg, 84%) as a white foam, mp: 44−45 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.02 (s, 1H), 7.59, 7.54 (d, $J$ = 8.2 Hz, 1H), 7.37−7.33 (m, 15H), 6.18, 6.13 (d, $J$ = 6.0 Hz, 1H), 5.64, 5.49 (d, $J$ = 8.0 Hz, 1H), 5.36 (s, 1H), 5.23 (m, 1H), 5.14−4.96 (m, 6H), 4.36 (s, 1H), 4.27−4.17 (m, 2H), 3.06 (s, 4H), 1.53 (s, 2H), 1.45 (s, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 161.8, 152.9, 152.8, 149.0, 138.5, 135.4, 135.0, 133.5, 127.7, 127.6, 127.5, 127.4, 126.9, 102.3, 102.1, 85.5, 85.2, 79.6, 79.5, 76.2, 74.9, 74.8, 72.4, 69.5, 69.3, 67.2, 63.8, 63.7, 44.4, 25.0, 23.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.37, 8.96 ppm; IR (KBr): $\nu_{max}$ 3441, 2933, 2854, 2354, 1759, 1693, 1636, 1497, 1458, 1386, 1264, 1118, 1057, 1015, 966, 905, 812, 782 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{37}H_{41}N_3O_{12}P$ $[M + H]^+$ 750.2422; found 750.2411.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-*N*$^4$-2′,3′-*O*-tris(benzyloxycarbonyl)cytidine (12).** Starting from Cbz-protected cytidine (**2**, 600 mg), flash column chromatography afforded **12** (649 mg, 80%) as a white foam, mp: 46−47 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.05, 8.01 (d, $J$ = 7.8 Hz, 1H), 7.76 (s, 1H), 7.39−7.31 (m, 20H), 7.18 (t, $J$ = 8.0 Hz, 1H), 6.16, 6.11 (d, $J$ = 2.8 Hz, 1H), 5.38−5.33 (m, 2H), 5.22−4.95 (m, 8H), 4.42−4.22 (m, 3H), 3.04 (m, 4H), 1.52 (m, 2H), 1.43 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.5, 154.6, 153.8, 152.0, 144.1, 136.1, 134.9, 134.6, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 95.2, 89.0, 88.8, 80.1, 77.2, 76.8, 72.7, 70.4, 68.2, 68.0, 64.9, 45.4, 26.0, 24.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.50, 9.15 ppm; IR (KBr): $\nu_{max}$ 3424, 2939, 2854, 2349, 2317, 1755, 1675, 1629, 1558, 1382, 1266, 1233, 1121, 1058, 966, 908, 844, 785, 742, 697 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{45}H_{48}N_4O_{13}P$ $[M + H]^+$ 883.2950; found 883.2955.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′,3′-*O*-bis(benzyloxycarbonyl)adenosine (13).** Starting from Cbz-protected adenosine (**3**, 500 mg), flash column chromatography afforded **13** (589 mg, 82%) as a white foam, mp: 46−47 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.28, 8.26 (s, 1H), 8.02, 7.99 (s, 1H), 7.40−7.31 (m, 10H), 6.23, 6.19 (d, $J$ = 5.2 Hz, 1H), 5.98−5.94 (m, 3H), 5.72−5.68 (m, 1H), 5.13−5.00 (m, 6H), 4.47 (s, 1H), 4.37−4.26 (m, 2H), 3.02 (s, 4H), 1.48 (s, 2H), 1.39 (s, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 155.5, 153.9, 153.7, 153.4, 149.9, 149.8, 139.2, 139.1, 136.4, 136.3, 134.6, 134.5, 128.7, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 120.1, 120.0, 86.0, 85.6, 80.8, 77.2, 76.1, 76.0, 73.8, 70.5, 70.4, 68.1, 68.0, 64.7, 64.4, 45.3, 25.9, 24.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.27, 9.11 ppm; IR (KBr): $\nu_{max}$ 3457, 3207, 3035, 2937, 2853, 2377, 2316, 1759, 1644, 1503, 1460, 1370, 1278, 1241, 1059, 1006, 965, 905, 845, 741 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{38}H_{42}N_6O_{10}P$ $[M + H]^+$ 773.2695; found 773.2703.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′,3′-*O*-bis(benzyloxycarbonyl)guanosine (14).** Starting from Cbz-protected guanosine (**4**, 500 mg), flash column chromatography afforded **14** (552 mg, 77%) as a white foam, mp: 54−55 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 11.86 (d, $J$ = 24.0 Hz, 1H), 7.33−7.13 (m, 15H), 6.58 (m, 1H), 6.33−6.26 (m, 2H), 6.04−6.00 (m, 2H), 5.18−5.10 (m, 4H), 4.99 (d, $J$ = 12.0 Hz, 2H), 4.64−4.42 (m, 2H), 4.24, 4.22 (m, 1H), 2.99 (m, 4H), 1.45 (m, 2H), 1.39 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 157.9, 153.9, 153.7, 153.6, 152.2, 136.6, 134.6, 134.5, 133.7, 128.6, 128.5, 128.3, 128.2, 127.8, 115.9, 87.0, 86.6, 80.1, 75.3, 74.8, 74.1, 73.6, 70.5, 68.1, 64.0, 45.3, 25.9, 24.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.70 ppm; IR (KBr): $\nu_{max}$ 3324, 3169, 2940, 2854, 1760, 1691, 1638, 1590, 1278, 1002, 965, 843, 783, 697 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{38}H_{42}N_6O_{11}P$ $[M + H]^+$ 789.2644; found 789.2635.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′,3′-*O*-bis(benzyloxycarbonyl)ribavirin (15).** Starting from Cbz-protected ribavirin (**5**, 500 mg), flash column chromatography afforded **15** (573 mg, 78%) as a white foam, mp: 44−45 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.41 (d, $J$ = 8.0 Hz, 1H), 7.35−7.32 (m, 15H), 6.11−6.07 (m, 2H), 5.74 (m, 1H), 5.57 (m, 1H), 5.11 (m, 4H), 4.99 (m, 2H), 4.50 (s, 1H), 4.22 (m, 2H), 3.00 (m, 4H), 1.49 (m, 2H), 1.40 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 160.4, 157.7, 153.8, 153.5, 144.7, 136.3, 134.5, 128.8, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 89.6, 81.4, 77.3, 73.6, 70.7, 70.5, 68.1, 64.4, 45.3, 25.9, 24.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.11, 8.84 ppm; IR (KBr): $\nu_{max}$ 3459, 3067, 2961, 2857, 2377, 2316, 1760, 1696, 1608, 1500, 1460, 1141, 1112, 1065, 1008, 974, 910, 828, 783, 746, 699, 586 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{36}H_{41}N_5O_{11}P$ $[M + H]^+$ 750.2535; found 750.2539.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′-deoxy-3′-*O*-(benzyloxycarbonyl)thymidine (16).** Starting from Cbz-protected thymidine (**6**, 400 mg), flash column chromatography afforded **16** (552 mg, 85%) as a white foam, mp: 39−40 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.02 (s, 1H), 7.48 (s, 1H), 7.38−7.35 (m, 10H), 6.37−6.29 (m, 1H), 5.22 (d, $J$ = 5.9 Hz, 1H), 5.17 (s, 2H), 5.09−4.95 (m, 2H), 4.27−4.20 (m, 3H), 3.05 (m, 4H), 2.50, 2.43 (dd, $J_1$ = 6.9 Hz, $J_2$ = 14.4 Hz, 1H), 2.07−1.92 (m, 1H), 1.86, 1.83 (s, 3H), 1.54 (m, 2H), 1.45 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.6, 154.3, 150.2, 136.1, 134.9, 134.6, 128.8, 128.7, 128.6, 128.5, 128.4, 128.0, 127.8, 111.5, 111.4, 84.9, 84.6, 82.8, 78.2, 78.0, 77.2, 70.2, 68.1, 65.7, 45.4, 45.3, 37.7, 37.6, 26.0, 24.2, 12.3 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.49, 9.24 ppm; IR (KBr): $\nu_{max}$ 3425, 3067, 2939, 2852, 2377, 2348, 2317, 2251, 1749, 1691, 1543, 1460, 1403, 1262, 1123, 1058, 1006, 964, 912, 738, 697 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{30}H_{37}N_3O_9P$ $[M + H]^+$ 614.2262; found 614.2269.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′-deoxy-*N*$^4$-3′-*O*-bis(benzyloxycarbonyl)cytidine (17).** Starting from Cbz-protected 2′-deoxycytidine (**7**, 500 mg), flash column chromatography afforded **17** (599 mg, 81%) as a white foam, mp: 45−46 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.13 (m, 2H), 7.36−7.34 (m, 14H), 7.17 (m, 1H), 6.23 (m, 1H), 5.19−5.15 (m, 5H), 5.02−4.92 (m, 2H), 4.36 (s, 1H), 4.24 (m, 2H), 3.00 (m, 4H), 2.83 (m, 1H), 1.96 (m, 1H), 1.50 (m, 2H), 1.40 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.4, 154.7, 154.3, 152.3, 143.8, 136.1, 135.1, 134.7, 128.8, 128.7, 128.5, 128.4, 128.3, 127.9, 127.8, 94.9, 87.3, 83.6, 78.1, 70.1, 68.1, 67.9, 65.5, 45.3, 39.2, 26.0, 24.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.35, 9.14 ppm; IR (KBr): $\nu_{max}$ 3459, 1747, 1660, 1562, 1501, 1450, 1392, 1331, 1258, 1120, 966, 742, 697 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{37}H_{42}N_4O_{10}P$ $[M + H]^+$ 733.2633; found 733.2621.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′-deoxy-3′-*O*-(benzyloxycarbonyl)adenosine (18).** Starting from 2′-deoxyadenosine (**8**, 400 mg), flash column chromatography on silica gel afforded **18** (517 mg, 80%) as a white foam, mp: 46−47 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.28 (s, 1H), 8.07 (d, $J$ = 4.0 Hz, 1H), 7.34−7.29 (m, 10H), 6.71 (s, 2H), 6.42 (m, 1H), 5.43, 5.36 (d, $J$ = 4.0 Hz, 1H), 5.15 (s, 2H), 5.13−4.93 (m, 2H), 4.36 (m, 1H), 4.26−4.21 (m, 2H), 2.98 (m, 4H), 2.89−2.58 (m, 2H), 1.44 (m, 2H), 1.36 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 155.7, 154.3, 153.2, 149.7, 138.7, 136.3, 134.7, 128.8, 128.7, 128.6, 128.5, 128.3, 127.9, 127.8, 119.9, 84.6, 84.1, 83.2, 78.4, 70.2, 68.0, 65.4, 45.3, 37.8, 26.0, 24.2 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 9.35 ppm; IR (KBr): $\nu_{max}$ 3337, 2924, 2854, 1747, 1648, 1595, 1463, 1380, 1255, 1064, 741, 697, 651, 567 cm$^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{30}H_{36}N_6O_7P$ $[M + H]^+$ 623.2378; found 623.2388.

**5′-*O*-[Benzyloxy(piperidin-1-yl)]phosphinyl-2′-deoxy-3′-*O*-(benzyloxycarbonyl)guanosine (19).** Starting from Cbz-protected 2′-deoxyguanosine (**9**, 400 mg), flash column chromatography afforded **19** (479 mg, 75%) as a white foam, mp: 91−92 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.76 (d, $J$ = 12.0 Hz, 1H), 7.37−7.29 (m, 10H), 6.59−6.52 (m, 2H), 6.19 (m, 1H), 5.47, 5.36 (d, $J$ = 8.0, 1H), 5.17 (m, 2H), 5.01 (m, 2H), 4.56−4.31 (m, 2H), 4.24, 4.16 (m, 1H), 3.01 (m, 4H), 2.79−2.46 (m, 3H), 1.48 (m, 2H), 1.40 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 158.9, 154.2, 153.9, 151.3, 136.3, 135.8, 134.8, 128.7, 128.6, 128.5, 128.4, 127.8, 117.6, 84.8, 84.1, 82.9, 78.5, 70.1, 68.1, 65.2, 45.3, 36.7, 25.9, 24.2 ppm; $^{31}$P NMR (162 MHz,


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$CDCl_3$) $\delta$ 9.55 ppm; IR (KBr): $\nu_{max}$ 3426, 3197, 2940, 2864, 1748, 1690, 1638, 1535, 1383, 1264, 1171, 1069, 1001, 744, 698 $cm^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{30}H_{36}N_6O_8P$ $[M + H]^+$ 639.2327; found 639.2316.

**5′-*O*-[Benzyloxy(morpholin-4-yl)]phosphinyl-2′,3′-*O*-bis-(benzyloxycarbonyl)uridine (41).** Starting from Cbz-protected uridine (**1**, 200 mg), flash column chromatography afforded **41** (240 mg, 82%) as a white foam, mp: 54−55 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.40, 7.32 (d, $J$ = 8.2 Hz, 1H), 7.29−7.16 (m, 15H), 6.00, 5.93 (d, $J$ = 5.7 Hz, 1H), 5.56, 5.44 (d, $J$ = 8.1 Hz, 1H), 5.29, 5.18 (q, $J$ = 4.0 Hz, 1H), 5.04−4.93 (m, 6H), 4.25−4.10 (m, 3H), 3.48−3.40 (m, 4H), 3.01−2.93 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.7, 153.9, 153.8, 150.0, 139.8, 139.7, 135.8, 134.5, 134.4, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 103.2, 87.5, 86.9, 80.4, 80.3, 77.3, 75.8, 75.7, 73.2, 70.6, 70.5, 68.8, 68.7, 68.6, 66.9, 66.8, 64.9, 44.5 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 8.19, 7.79 ppm; IR (KBr): $\nu_{max}$ 3445, 2967, 2857, 2347, 2063, 1759, 1692, 1636, 1499, 1459, 1386, 1264, 1112, 1012, 974, 909, 815, 782, 741, 698, 608 $cm^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{36}H_{39}N_3O_{13}P$ $[M + H]^+$ 752.2215; found 752.2227.

**5′-*O*-[Benzyloxy(cyclohexylamino)]phosphinyl-2′,3′-*O*-bis-(benzyloxycarbonyl)uridine (43).** Starting from Cbz-protected uridine (**1**, 200 mg), flash column chromatography afforded **43** (202 mg, 68%) as a white foam, mp: 49−51 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.54, 7.48 (d, $J$ = 8.2 Hz, 1H), 7.37−7.33 (m, 15H), 6.14, 6.09 (d, $J$ = 7.8 Hz, 1H), 5.64, 5.49 (d, $J$ = 8.2 Hz, 1H), 5.38 (t, $J$ = 4.4 Hz, 1H), 5.25 (t, $J$ = 5.7 Hz, 1H), 5.14−5.00 (m, 6H), 4.34−4.21 (m, 3H), 2.97−2.88 (m, 2H), 1.64−1.86 (m, 6H), 1.25−1.12 (m, 4H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.4, 153.9, 153.8, 149.9, 139.6, 136.1, 136.0, 134.5, 128.8, 128.7, 128.6, 128.5, 128.4, 127.9, 127.8, 103.3, 103.2, 86.8, 86.4, 80.6, 80.5, 77.2, 75.8, 75.7, 73.4, 73.3, 70.6, 70.5, 68.4, 68.3, 68.2, 64.8, 64.7, 50.9, 50.8, 35.8, 35.7, 25.2, 24.9 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 8.93, 8.50 ppm; IR (KBr): $\nu_{max}$ 3734, 2932, 2855, 2377, 2316, 1758, 1695, 1500, 1457, 1265, 1013, 973, 910, 866, 815, 740, 698 $cm^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{38}H_{43}N_3O_{12}P$ $[M + H]^+$ 764.2579; found 764.2586.

**5′-*O*-{Benzyloxy[(methoxycarbonyl)methylamino]}-phosphinyl-2′,3′-*O*-bis(benzyloxycarbonyl)uridine (44).** Starting from Cbz-protected uridine (**1**, 200 mg), flash column chromatography afforded **44** (217 mg, 74%) as a white foam, mp: 48−50 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.45 (d, $J$ = 8.8 Hz, 1H), 7.51, 7.48 (d, $J$ = 8.2 Hz, 1H), 7.38−7.32 (m, 15H), 6.10, 6.06 (d, $J$ = 5.7 Hz, 1H), 5.65, 5.53 (d, $J$ = 8.1 Hz, 1H), 5.42−5.30 (m, 2H), 5.13−5.04 (m, 6H), 4.39−4.02 (m, 3H), 3.92, 3.82 (m, 1H), 3.72−3.63 (m, 5H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 171.4, 171.3, 162.9, 162.8, 153.9, 153.8, 153.7, 150.2, 150.1, 140.0, 139.9, 135.8, 135.7, 134.5, 134.4, 128.7, 128.6, 128.4, 128.3, 128.0, 127.9, 103.4, 103.2, 87.1, 86.8, 80.3, 80.2, 77.2, 75.7, 75.5, 73.1, 73.0, 70.5, 70.4, 68.6, 68.5, 64.9, 64.8, 64.7, 52.4, 42.7, 42.6 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 8.73, 8.42 ppm; IR (KBr): $\nu_{max}$ 3210, 2956, 2378, 2349, 2316, 1756, 1694, 1498, 1459, 1694, 1459, 1389, 1265, 1007, 906, 816, 783, 740, 697 $cm^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{35}H_{37}N_3O_{14}P$ $[M + H]^+$ 754.2008; found 754.2017.

**5′-*O*-{Benzyloxy[(2*S*)-methoxycarbonyl-pyrrolidin-1-yl]}-phosphinyl-2′,3′-*O*-bis(benzyloxycarbonyl)uridine (45).** Starting from Cbz-protected uridine (**1**, 200 mg), flash column chromatography afforded **45** (195 mg, 63%) as a white foam, mp: 46−48 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.66 (s, 1H), 7.76, 7.55 (d, $J$ = 8.2 Hz, 1H), 7.43−7.33 (m, 15H), 6.27, 6.14 (d, $J$ = 6.6 Hz, 1H), 5.70, 5.44 (d, $J$ = 8.2 Hz, 1H), 5.41, 5.36 (m, 1H), 5.29, 5.23 (t, $J$ = 6.2 Hz, 1H), 5.18−5.00 (m, 6H), 4.44−4.21 (m, 4H), 3.71, 3.68 (s, 3H), 3.31−2.99 (m, 2H), 2.18−1.62 (m, 5H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 174.2, 162.6, 162.4, 154.0, 153.8, 150.1, 150.0, 139.9, 139.6, 136.2, 136.1, 136.0, 134.6, 134.5, 128.7, 128.6, 128.4, 128.2, 128.1, 103.4, 103.2, 86.2, 85.4, 80.8, 80.7, 77.2, 75.7, 75.6, 73.7, 73.5, 70.6, 70.5, 70.4, 68.4, 64.9, 60.3, 60.2, 52.3, 47.2, 46.7, 31.3, 31.0, 25.1 ppm; $^{31}$P NMR (162 MHz, $CDCl_3$): $\delta$ 6.72, 5.85 ppm; IR (KBr): $\nu_{max}$ 3425, 3065, 3036, 2956, 2883, 2378, 2349, 2316, 1755, 1695, 1459, 1395, 1265, 1120, 1003, 783, 744, 698 $cm^{-1}$; HRMS (ESI+): $m/z$ calcd for $C_{38}H_{41}N_3O_{14}P$ $[M + H]^+$ 794.2321; found 794.2310.

**Uridine-5′-triphosphate, Tetrasodium Salt (23).** General procedure for the synthesis of NTPs (**23**−**31**): To a solution of **11** (50 mg, 0.067 mmol) and $Et_3N$ (9 $\mu$L, 0.067 mmol) in DMF (1.5 mL) was added 5% Pd/C (5 mg). The reaction was stirred under a hydrogen atmosphere at 20 °C for 3 h. The catalyst was removed with a syringe filter (0.45 $\mu$m pore size) and washed with DMF (0.2 mL × 2) under an atmosphere of argon. To the combined DMF solution were added tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (**21**, 123 mg, 0.136 mmol) and 4,5-dicyanoimidazole (DCI, 48 mg, 0.41 mmol). The reaction was stirred at 20 °C for 6 h and concentrated in vacuo. The residue was dissolved in NaOAc aqueous solution (10 M, 0.5 mL), and EtOH (50 mL) was added. The resulting white precipitate was collected by centrifuge. The crude product was dissolved in deionized $H_2O$ (0.5 mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with $NH_4HCO_3$ buffer (linear gradient 0.2 to 0.6 M), combination of appropriate fractions, and lyophilization afforded the product in ammonium salt form. Passage of the solution of the ammonium salt in deionized $H_2O$ through a bed of Dowex 50W-X8 ion-exchange resin ($Na^+$ form) and lyophilization afforded **23** (31 mg, 81%) as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.95 (d, $J$ = 8.1 Hz, 1H), 5.97−5.94 (m, 2H), 4.41−4.37 (m, 2H), 4.26−4.23 (m, 3H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 165.3, 151.0, 140.8, 101.8, 87.4, 82.5, 72.8, 68.7, 64.0 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −7.89 (d, $J_{P,P}$ = 17.4 Hz, 1P), −11.1 (d, $J_{P,P}$ = 17.4 Hz, 1P), −22.0 (t, $J_{P,P}$ = 17.4 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_9H_{11}N_2O_{15}P_3$ $[M - H]^-$ 483.0; found 483.1.

**Cytidine-5′-triphosphate, Tetrasodium Salt (24).** Starting from **12** (60 mg), CTP (27 mg, 70%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.95 (d, $J$ = 8.0 Hz, 1H), 6.10 (d, $J$ = 8.0 Hz, 1H), 5.94 (d, $J$ = 4.0 Hz, 1H), 4.33 (m, 1H,), 4.27 (m, 1H), 4.21 (m, 3H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 165.3, 156.7, 141.7, 96.5, 89.0, 82.9, 74.2, 69.2, 64.7 ppm; $^{31}$P NMR ($D_2O$, 162 MHz): $\delta$ −10.55 (d, $J_{P,P}$ = 19.4 Hz, 1P), −11.31 (d, $J_{P,P}$ = 19.4 Hz, 1P), −22.94 (t, $J_{P,P}$ = 19.4 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_9H_{15}N_3O_{14}P_3$ $[M - H]^-$ 482.0; found 482.1.

**Adenosine-5′-triphosphate, Tetrasodium Salt (25).** Starting from **13** (52 mg), ATP (30 mg, 75%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.45 (s, 1H), 8.17 (s, 1H), 6.04 (d, 1H), 4.68 (t, 1H,), 4.50 (m, 1H), 4.34 (m, 1H), 4.20 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 154.1, 150.8, 148.9, 140.6, 118.6, 87.1, 84.1, 74.5, 70.4, 65.3 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −6.26 (d, $J_{P,P}$ = 19.4 Hz, 1P), −11.00 (d, $J_{P,P}$ = 19.4 Hz, 1P), −21.80 (t, $J_{P,P}$ = 19.4 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_{10}H_{15}N_5O_{13}P_3$ $[M - H]^-$ 506.0; found 506.1.

**Guanosine-5′-triphosphate, Tetrasodium Salt (26).** Starting from **14** (53 mg), GTP (30 mg, 73%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.01 (s, 1H), 5.81 (d, $J$ = 4.0 Hz, 1H), 4.63 (t, $J$ = 4.0 Hz, 1H), 4.48 (dd, 1H), 4.27 (s, 1H), 4.16 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 158.8, 153.8, 151.6, 137.5, 116.1, 86.8, 83.8, 73.9, 70.2, 65.2 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −8.98 (d, $J_{P,P}$ = 17.8 Hz, 1P), −11.13 (d, $J_{P,P}$ = 17.8 Hz, 1P), −22.54 (t, $J_{P,P}$ = 17.8 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_{10}H_{15}N_5O_{14}P_3$ $[M - H]^-$ 522.0; found 522.2.

**Ribavirin-5′-triphosphate, Tetrasodium Salt (27).** Starting from **15** (50 mg), RTP (28 mg, 73%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.74 (s, 1H), 5.96 (d, $J$ = 4.0 Hz, 1H), 4.62 (s, 1H), 4.51 (s, 1H), 4.30 (s, 1H), 4.12 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 162.9, 156.4, 145.9, 92.0, 84.2, 74.8, 70.2, 65.3 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −8.49 (d, $J_{P,P}$ = 19.4 Hz, 1P), −9.08 (d, $J_{P,P}$ = 19.4 Hz, 1P), −20.68 (t, $J_{P,P}$ = 19.4 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_8H_{14}N_4O_{14}P_3$ $[M - H]^-$ 483.0; found 483.1.

**Thymidine-5′-triphosphate, Tetrasodium Salt (28).** Starting from **16** (40 mg), dTTP (29 mg, 78%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.72 (s, 1H), 6.32 (t, $J$ = 8.0 Hz, 1H), 4.64 (d, 1H), 4.17 (m, 3H), 2.36−2.31 (m, 2H), 1.89 (s, 3H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 166.6, 151.8, 137.3, 111.8, 85.4, 84.9, 70.8, 65.4, 38.5, 11.6 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −8.54 (d, $J_{P,P}$ = 17.8 Hz, 1P), −11.38 (d, $J_{P,P}$ =


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17.8 Hz, 1P), −22.58 (t, $J_{P,P}$ = 17.8 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_{10}H_{16}N_2O_{14}P_3$ $[M - H]^-$ 481.0; found 481.1.

**2′-Deoxycytidine-5′-triphosphate, Tetrasodium Salt (29).** Starting from **17** (49 mg), dCTP (26 mg, 70%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.89 (d, $J$ = 8.0 Hz, 1H), 6.24 (m, 1H), 6.06 (d, $J$ = 8.0 Hz, 1H), 4.54 (s, 1H), 4.13 (s, 3H), 2.35−2.31 (m, 1H), 2.26−2.21 (m, 1H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 165.7, 157.1, 141,7, 96.5, 85.9, 85.5, 70.5, 65.2, 39.3 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −9.26 (d, $J_{P,P}$ = 17.8 Hz, 1P), −10.95 (d, $J_{P,P}$ = 17.8 Hz, 1P), −22.32 (t, $J_{P,P}$ = 17.8 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_9H_{15}N_3O_{13}P_3$ $[M - H]^-$ 466.0; found 466.1.

**2′-Deoxyadenosine-5′-triphosphate, Tetrasodium Salt (30).** Starting from **18** (42 mg), dATP (29 mg, 75%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.42 (s, 1H), 8.15 (s, 1H), 6.44 (t, $J$ = 4.0 Hz, 1H), 4.71 (s, 1H), 4.24 (s, 1H), 4.20−4.08 (m, 2H), 2.80−2.73 (m, 1H), 2.55−2.52 (m, 1H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 155.6, 152.7, 148.8, 140.1, 118.7, 85.9, 83.7, 70.9, 65.3, 39.1 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −7.78 (d, $J_{P,P}$ = 19.4 Hz, 1P), −10.61 (d, $J_{P,P}$ = 19.4 Hz, 1P), −21.89 (t, $J_{P,P}$ = 19.4 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_{10}H_{15}N_5O_{12}P_3$ $[M - H]^-$ 490.0; found 490.1.

**2′-Deoxyguanosine-5′-triphosphate, Tetrasodium Salt (31).** Starting from **19** (43 mg), dGTP (29 mg, 72%) was obtained as the tetrasodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.97 (s, 1H), 6.15 (d, 1H), 4.66 (s, 1H), 4.14−4.04 (m, 3H), 2.65 (m, 1H), 2.42−2.36 (m, 1H); $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 159.0, 153.9, 151.4, 137.8, 116.3, 85.7, 83.6, 71.1, 65.4, 38.5; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −8.33 (d, $J_{P,P}$ = 19.4 Hz, 1P), −11.02 (d, $J_{P,P}$ = 19.4 Hz, 1P), −22.32 (t, $J_{P,P}$ = 19.4 Hz, 1P); LRMS (ESI−): $m/z$ calcd for $C_{10}H_{15}N_5O_{13}P_3$ $[M - H]^-$ 506.0; found 506.1.

**Uridine-5′-diphosphate, Trisodium Salt (32).** General procedure for the synthesis of NDPs (**32**−**40**): To a solution of **11** (50 mg, 0.067 mmol) and $Et_3N$ (9 $\mu$L, 0.067 mmol) in DMF (1.0 mL) was added 5% Pd/C (5 mg). The reaction was stirred under a hydrogen atmosphere at 20 °C for 3 h. The catalyst was removed with a syringe filter (0.45 $\mu$m pore size) and washed with DMF (0.1 mL × 2) under an atmosphere of argon. To the combined DMF solution were added a solution of bis(tetra-*n*-butylammonium) hydrogen phosphate (**22**, 58 mg, 0.2 mmol) in DMF (1.0 mL) and 4,5-dicyanoimidazole (DCI, 63 mg, 0.54 mmol). The reaction was stirred at 20 °C for 6 h and concentrated in vacuo. The residue was dissolved in NaOAc aqueous solution (10 M, 0.5 mL), and EtOH (50 mL) was added. The resulting white precipitate was collected by centrifuge. The crude product was dissolved in deionized $H_2O$ (0.5 mL) and loaded on a DEAE Sephadex A-25 ion exchange column (1.6 × 25 cm). Elution with $NH_4HCO_3$ buffer (linear gradient 0.2 to 0.6 M), combination of appropriate fractions, and lyophilization afforded the product in ammonium salt form. Passage of the solution of the ammonium salt in deionized $H_2O$ through a bed of Dowex 50W-X8 ion-exchange resin ($Na^+$ form) and lyophilization afforded **32** (25 mg, 79%) as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.92 (d, $J$ = 8.0 Hz, 1H), 5.89−5.87 (m, 2H), 4.35−4.30 (m, 2H), 4.18−4.14 (m, 3H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 166.4, 151.9, 141.9, 102.8, 88.7, 83.3, 74.0, 69.4, 64.4 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −6.30 (d, $J_{P,P}$ = 21.1 Hz, 1P), −10.40 (d, $J_{P,P}$ = 21.1 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_9H_{13}N_2O_{12}P_2$ $[M - H]^-$ 403.0; found 403.2.

**Cytidine-5′-diphosphate, Trisodium Salt (33).** Starting from **12** (60 mg), CDP (22 mg, 69%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.92 (d, $J$ = 8.0 Hz, 1H), 6.05 (d, $J$ = 8.0 Hz, 1H), 5.91 (d, $J$ = 4.0 Hz, 1H) 4.30 (d, 1H), 4.24 (s, 1H), 4.19−4.15 (m, 3H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 166.0, 157.5, 141.7, 96.6, 89.3, 82.8, 74.3, 69.2, 64.4 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −9.52 (d, $J_{P,P}$ = 19.4 Hz, 1P), −11.13 (d, $J_{P,P}$ = 19.4 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_9H_{13}N_2O_{12}P_2$ $[M - H]^-$ 402.0; found 402.2.

**Adenosine-5′-diphosphate, Trisodium Salt (34).** Starting from **13** (52 mg), ADP (24 mg, 73%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.38 (s, 1H), 8.04 (s, 1H), 6.00 (d, $J$ = 4.0 Hz, 1H), 4.64 (dd, 1H), 4.48 (s, 1H), 4.29 (s, 1H), 4.14 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 155.6, 152.8, 149.1, 140.0, 118.7, 87.1, 84.0, 74.5, 70.2, 64.9 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −8.52 (d, $J_{P,P}$ = 19.4 Hz, 1P), −10.88 (d, $J_{P,P}$ = 19.4 Hz, 1P) ppm; LRMS (ESI−) $m/z$ calcd for $C_{10}H_{14}N_5O_{10}P_2$ $[M - H]^-$ 426.0; found 426.2.

**Guanosine-5′-diphosphate, Trisodium Salt (35).** Starting from **14** (53 mg), GDP (25 mg, 73%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.01 (s, 1H), 5.82 (d, $J$ = 8.0 Hz, 1H), 4.64 (m, 1H), 4.45 (t, $J$ = 4.0 Hz, 1H), 4.27 (s, 1H), 4.14 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 158.8, 153.9, 151.6, 137.5, 116.1, 86.9, 83.8, 73.8, 70.3, 65.1 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −10.53 (d, 1P), −11.32 (d, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_{10}H_{14}N_5O_{11}P_2$ $[M - H]^-$ 442.0; found 442.2.

**Ribavirin-5′-diphosphate, Trisodium Salt (36).** Starting from **15** (50 mg), RDP (22 mg, 70%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.75 (s, 1H), 5.97 (s, 1H), 4.64 (dd, 1H), 4.51 (d, 1H), 4.31 (s, 1H), 4.13 (m, 2H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 162.9, 156.4, 145.8, 92.1, 84.0, 74.7, 70.0, 64.8 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −9.30 (d, 1P), −10.78 (d, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_8H_{13}N_4O_{11}P_2$ $[M - H]^-$ 403.0; found 403.2.

**Thymidine-5′-diphosphate, Trisodium Salt (37).** Starting from **16** (40 mg), dTDP (23 mg, 76%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.66 (s, 1H), 6.26 (t, 1H), 4.56 (s, 1H), 4.08 (m, 3H), 2.28 (m, 2H), 1.84 (s, 3H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 166.6, 151.8, 137.5, 111.8, 85.5, 85.0, 70.8, 65.1, 38.5, 11.7 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −9.04 (d, $J_{P,P}$ = 21.1 Hz, 1P), −10.91 (d, $J_{P,P}$ = 21.1 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_{10}H_{15}N_2O_{11}P_2$ $[M - H]^-$ 401.0; found 401.2.

**2′-Deoxycytidine-5′-diphosphate, Trisodium Salt (38).** Starting from **17** (49 mg), dCDP (21 mg, 68%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.90 (d, $J$ = 4.0 Hz, 1H), 6.22 (dd, 1H), 6.03 (d, $J$ = 4.0 Hz, 1H), 4.51 (d, 1H), 4.10−4.07 (m, 3H), 2.34−2.29 (m, 1H), 2.25−2.20 (m, 1H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 165.5, 156.7, 141.9, 96.4, 86.0, 85.5, 70.7, 65.1, 39.4 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −9.99 (d, $J_{P,P}$ = 21.1 Hz, 1P), −11.12 (d, $J_{P,P}$ = 21.1 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_9H_{14}N_3O_{10}P_2$ $[M - H]^-$ 386.0; found 386.2.

**2′-Deoxyadenosine-5′-diphosphate, Trisodium Salt (39).** Starting from **18** (42 mg), dADP (23 mg, 72%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 8.32 (s, 1H), 8.03 (s, 1H), 6.33 (dd, 1H), 4.66 (d, 1H), 4.20 (s, 1H), 4.07 (m, 2H), 2.68 (m, 1H), 2.50 (m, 1H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 154.9, 151.9, 148.5, 140.1, 118.4, 85.8, 83.8, 71.2, 65.3, 39.2 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −10.18 (d, $J_{P,P}$ = 21.1 Hz, 1P), −10.96 (d, $J_{P,P}$ = 21.1 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_{10}H_{14}N_5O_9P_2$ $[M - H]^-$ 410.0; found 410.2.

**2′-Deoxyguanosine-5′-diphosphate, Trisodium Salt (40).** Starting from **19** (43 mg), dGDP (24 mg, 73%) was obtained as the trisodium salt, a white solid. $^1$H NMR (400 MHz, $D_2O$): $\delta$ 7.97 (s, 1H), 6.16 (t, 1H), 4.65 (s, 1H), 4.13 (s, 1H), 4.03 (m, 2H), 2.65 (m, 1H), 2.41 (m, 1H) ppm; $^{13}$C NMR (100 MHz, $D_2O$): $\delta$ 158.8, 153.8, 151.3, 137.6, 116.1, 85.6, 83.4, 71.1, 65.1, 38.6 ppm; $^{31}$P NMR (162 MHz, $D_2O$): $\delta$ −6.30 (d, $J_{P,P}$ = 21.1 Hz, 1P), −10.40 (d, $J_{P,P}$ = 21.1 Hz, 1P) ppm; LRMS (ESI−): $m/z$ calcd for $C_{10}H_{14}N_5O_{10}P_2$ $[M - H]^-$ 426.0; found 426.2.

## ■ ASSOCIATED CONTENT

### Supporting Information

Optimization data for NDP synthesis, NMR spectra for all compounds, and HPLC traces for all 18 NPPs. 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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