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Efficient synthesis of nucleoside 5′-triphosphates and their β,γ-bridging oxygen-modified analogs from nucleoside 5′-phosphates

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ABSTRACT

Thirteen nucleoside 5′-triphosphates (NTPs) and their β,γ-bridging oxygen-modified analogs (β,γ-CX_2-NTPs, X = H, F, Cl, and Br) have been efficiently synthesized from nucleoside 5′-phosphoropiperidates with 4,5-dicyanoimidazole as the activator. A high-yielding and chromatography-free protocol for the preparation of both natural and base-modified nucleoside 5′-phosphoropiperidates from the corresponding nucleoside 5′-phosphates was also developed.

Nucleoside 5′-triphosphates (NTPs) are a unique group of biomolecules that play key roles in a vast array of pivotal biological processes, such as energy metabolism, polymerization of DNA and RNA, signal transduction, and regulation of protein functions.[1] Due to the existence of the high-energy phosphoanhydride bonds, NTPs are highly susceptible to hydrolysis.[2] Replacement of the labile P–O–P linkage with an isosteric P–CXY–P (X, Y = H, F, Cl, Br, and OH) unit at β,γ-bridging position (Fig. 1) enhances the metabolic stability of the modified NTP analogs.[3] These hydrolysis-resistant NTP analogs have been extensively utilized to probe the catalytic mechanism of phosphoryl tranfer processes,[4] and investigated as enzyme inhibitors[5] and receptor agonists[6] in numerous medicinal studies.

Due to their important roles in biological research and potential pharmaceutical applications, various synthetic methods for NTPs have been adopted for the preparation of β,γ-bridging oxygen-modified NTP analogs (β,γ-CX_2-NTPs, X = H, F, Cl, and Br). The conventional 'one-pot, three-step',[6b–d] salicyl chlorophosphite,[5b] and phosphoromorpholidate[3d,4a,6e] methods have been employed to synthesize β,γ-CX_2-NTPs in moderate yields. These compounds have also been obtained from the coupling of nucleoside 5′-phosphates (NMPs) with diphosphonates in the presence of condensing reagents, such as diphenylphosphoryl chloride,[4e] CDI,[4d,7] $(CF_3CO)_2$-O/*N*-methylimidazole,[8] and sulfonyl imidazolium salts.[9] Moreover, a solid-phase method has been reported for the synthesis of β,γ-CH_2-NTPs.[5a] However, the preparation of special β,γ-CH_2-triphosphitylating reagent greatly limits its practical application.

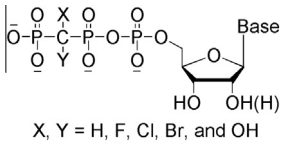

Figure 1. The structures of β,γ-bridging oxygen-modified NTP analogs.

More recently, we established a novel P(V)–N activation approach for the synthesis of nucleoside diphosphates (NDPs), triphosphates (NTPs),[10] and nucleoside diphosphate sugars (NDP-sugars)[11] from the fully protected phosphoropiperidates. Though the Bn and Cbz protecting groups of the phosphoropiperidate precursors could be quantitatively removed by catalytic hydrogenation, reducible functional groups, such as —N_3 and —C≡C— on ribose moiety and halogen atoms (e.g., F, Cl, Br, and I) on nucleobases could not be tolerated. Therefore, a direct and efficient access to the unprotected phosphoropiperidates may significantly simplify the original method and extend the applications of the P(V)–N activation strategy to more diversified nucleoside substrates. We report herein a high-yielding and chromatography-free method for the preparation of nucleoside 5′-phosphoropiperidates

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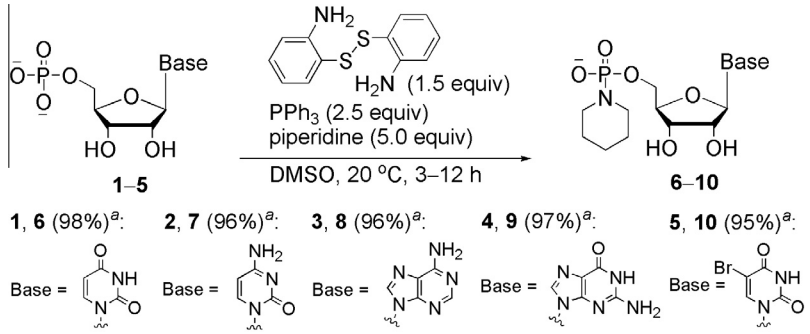


Scheme 1. A general and optimized method for the synthesis of nucleoside 5′-phosphoropiperidates (**6**–**10**) from nucleoside 5′-phosphates. [a]Isolated yield.

from the corresponding NMPs. The obtained nucleoside 5′-phosphoropiperidates exhibited excellent reactivity toward pyrophosphate and diphosphonate reagents in the presence of 4,5-dicyanoimidazole (DCI) and afforded NTP and β,γ-CX_2-NTP products in high isolated yields.

As shown in Scheme 1, the phosphoropiperidates of four natural nucleosides (**6**–**9**) and a base-modified nucleoside (5-bromouridine, **10**) were prepared from the corresponding NMP starting materials (**1**–**5**). In contrast to the DCC condensation method,[4a,12] the redox condensation approach based on 2,2′-dipyridyldisulfide/PPh_3 is amenable to amine nucleophiles with a wide range of pK_a values.[13] In precedent reports, 2.0–5.0 equiv of 2,2′-dipyridyldisulfide/PPh_3 in 1:1 molar ratio were used to give the phosphoromorpholidates and phosphoropiperidates of adenosine and guanosine in good yields (~70–80%).[14] In the present work, 2,2′-dithiodianiline, a less expensive disulfide reagent, was used

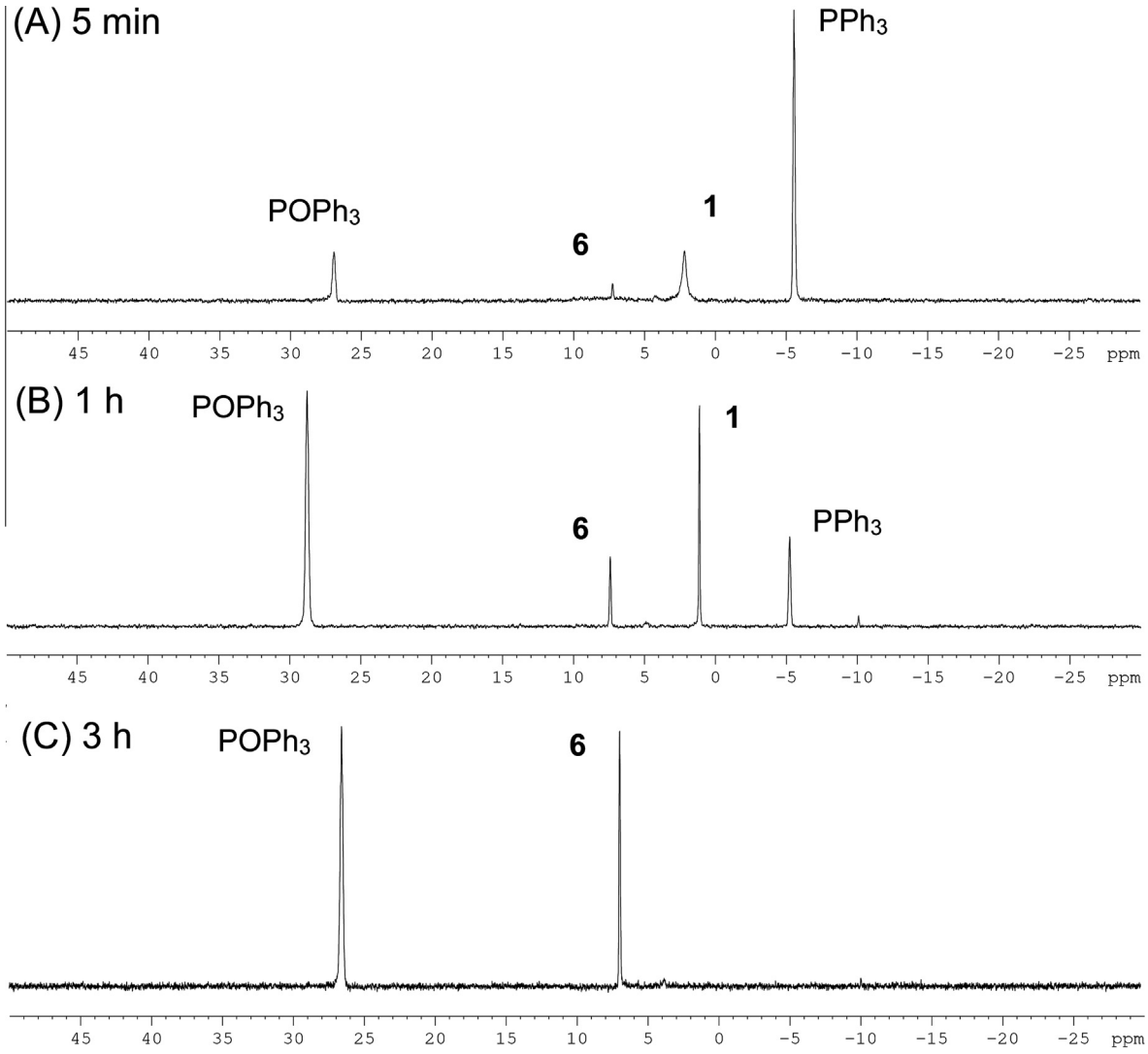


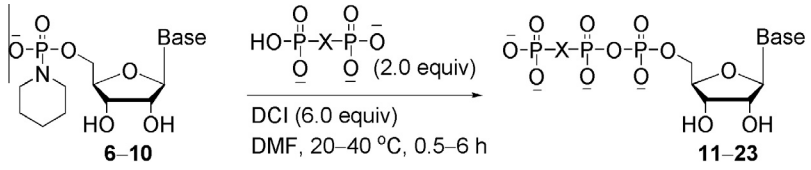
Figure 2. ^{31}P NMR tracing of the redox condensation reaction mixture for the synthesis of uridine 5′-phosphoropiperidate (**6**).

instead of 2,2′-dipyridyldisulfide. We found that when **1**–**5** were treated with 1.5 equiv of the disulfide, 2.5 equiv of PPh_3, and 5.0 equiv of piperidine in DMSO at 20 °C, the phosphoropiperidates **6**–**10** could be obtained almost quantitatively on ^{31}P NMR in 3–12 h (Fig. 2). Acetone precipitation afforded the sodium salts of **6**–**10** in 95–98% isolated yields and high purity.

To improve the solubility of P(V)–N precursors in DMF, the sodium salts of **6**–**10** were first converted to the triethylammonium salts. Treatment of the phosphoropiperidates **6**–**10** with 2.0 equiv of tris(tetra-*n*-butylammonium) hydrogen pyrophosphate and 6.0 equiv of DCI in DMF at 20 °C according to our previous report[10] gave the desired NTPs in high isolated yields (Table 1), indicating that the unprotected phosphoropiperidates synthesized by the redox condensation method were as reactive as those obtained in situ from the catalytic hydrogenation of the protected phosphoropiperidates. It is worth noting that 5-bromouridine 5′-triphosphate (**15**), which could not stand the catalytic hydrogenation condition in the original method, was afforded in 80% yield via this approach. Due to the higher stability of β,γ-CX_2-NTPs, the DCI-promoted coupling reactions were conducted in DMF at 40 °C with 2.0 equiv of tris(tetra-*n*-butylammonium) hydrogen diphosphonate reagents and 6.0 equiv of DCI. The β,γ-CX_2-NTP products were obtained in excellent isolated yields ranging from 71–84% in only 0.5–1 h.

The experimental results of **11**–**15** in Table 1 well demonstrated the generality and reproducibility of the reported P(V)–N activation strategy for the synthesis of NTPs. To explore its application in the preparation of β,γ-CX_2-NTPs, uridine 5′-phosphoropiperidate (**6**) was treated with 2.0 equiv of tris(tetra-*n*-butylammonium) hydrogen methylenediphosphonate and 6.0 equiv of acidic activators in DMF at 20 °C. As listed in Table 2, the data of ^{31}P NMR tracing experiments were in good accordance with those obtained for NTPs. In the negative control experiment, no reaction was observed in the absence of the acidic activator even after 72 h. While DCI exhibited the highest reactivity and efficacy in the activation of P(V)–N bond (96%, 3 h, Fig. 3), 2,6-lutidinium chloride yielded a comparable result (90%, 4 h). Though the reaction promoted by pyridinium chloride was as fast as that by 2,6-lutidinium chloride, more byproducts were observed (75%, 4 h). In contrast, the reaction with 1*H*-tetrazole achieved high yield, but it took much longer to finish (95%, 24 h). Meanwhile, addition of *N*-methylimidazolium chloride and imidazolium chloride resulted in the precipitation of methylenediphosphonate.

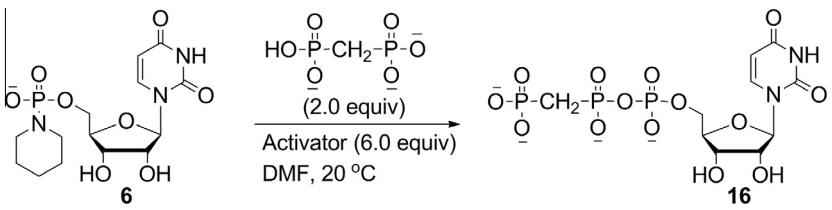
Table 1
DCI-promoted conversion of nucleoside 5′-phosphoropiperidates to NTPs (**11**–**15**) and β,γ-CX_2-NTPs (**16**–**23**)



Compd	X	Base	Temp (°C)	Time (h)	Yield[a] (%)
11	O	U	20	6	81
12	O	C	20	6	74
13	O	A	20	6	77
14	O	G	20	6	76
15	O	5-BrU	20	6	80
16	CH_2	U	40	1	79
17	CH_2	C	40	1	71
18	CH_2	A	40	1	72
19	CH_2	G	40	1	74
20	CH_2	5-BrU	40	1	78
21	CF_2	U	40	1	71
22	CCl_2	U	40	1	78
23	CBr_2	U	40	0.5	84

[a] Isolated yield.

Table 2
Effect of the acidic activator on the formation of β,γ-CH_2-UTP (**16**)



Activator	Time (h)	Yield[a] (%)
No activator	72	No reaction
DCI	3	96
1*H*-Tetrazole	24	95
2,6-Lutidine·HCl	4	90
Pyridine·HCl	4	75
N-Methylimidazole·HCl	N.A.	Precipitation
Imidazole·HCl	N.A.	Precipitation

[a] ^{31}P NMR yield.

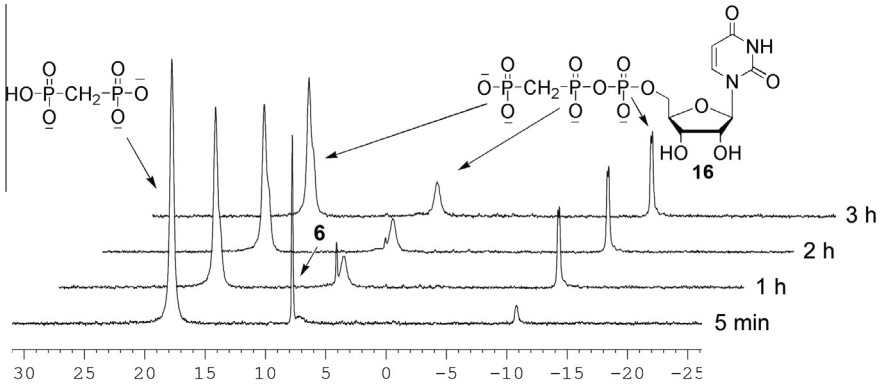


Figure 3. The stacked ^{31}P NMR tracing spectra of the DCI-promoted synthesis of β,γ-CH_2-UTP (**16**) at 20 °C.

Table 3
Effect of reaction temperature on the formation of β,γ-CH_2-UTP (**16**)

Temp (°C)	Time (h)	Yield[a] (%)
20	3	96
30	2	96
40	1	94
50	0.5	89

[a] ^{31}P NMR yield.

In the following research, we tested the possibility to reduce the amount of methylenediphosphonate to 1.5 equiv without causing the formation of symmetrical dinucleoside polyphosphate byproduct (NppCH_2ppN). But the experimental result showed that significant amount of **6** (ca. 11%) was transformed into UppCH_2ppU, indicating that β,γ-CH_2-UTP (**16**) was highly reactive toward the P(V)–N phosphorylating reagent and 2.0 equiv of methylenediphosphonate was the minimum amount required to suppress the formation of NppCH_2ppN.

Due to the enhanced stability of β,γ-CH_2-NTPs, the reaction temperature was elevated to accelerate the reaction rate. As shown in Table 3, the reaction time for the synthesis of **16** was significantly shortened with increasing temperature (20–50 °C). But it was also observed that when the reaction was performed at 50 °C, the amount of phosphate and polyphosphate byproducts began to increase due to the hydrolysis of **6**. Therefore, 40 °C is optimal to maximize both the reaction rate and yield of β,γ-CH_2-NTPs.

With the optimized reaction conditions, the P(V)–N activation method was applied to the coupling of **6** with difluoro-, dichloro-, and dibromomethylenediphosphonate. It has been reported that the reactions of halogen-substituted methylenediphosphonates with phosphorylating reagents were typically slower than those with methylenediphosphonate due to the electron-withdrawing effect of halogen atoms.[8] But in our experiments, we observed that difluoro- and dichloromethylenediphosphonate generated the β,γ-CX_2-NTPs in comparable reaction time and yield to methylenediphosphonate at 40 °C (1 h). A plausible explanation was that the elevated reaction temperature diminished the difference in the nucleophilicity of the diphosphonate reagents. Surprisingly, the reaction rate of dibromomethylenediphosphonate was even faster at 40 °C (0.5 h, Fig. 4).

In summary, we developed an efficient method for the synthesis of NTPs and β,γ-CX_2-NTPs from NMP starting materials. The modified redox condensation method based on 2,2′-dithiodianiline/PPh_3 provided a general, high-yielding, and chromatography-free protocol for the preparation of unprotected nucleoside 5′-phosphoropiperidate intermediates and extended the applicable scope of the P(V)–N activation strategy to more nucleoside analogs with reducible functional groups. While the subsequent DCI-promoted coupling of the phosphoropiperidates with pyrophosphate furnished clean and smooth transformation into the corresponding NTPs, the reactions with different diphosphonates at elevated temperature (40 °C) afforded the β,γ-bridging oxygen-modified NTP analogs within 1 h in high isolated yields.

Acknowledgments

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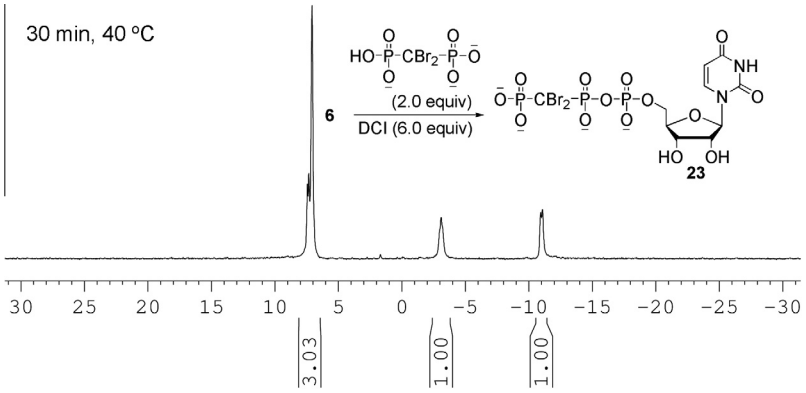

Figure 4. The ^{31}P NMR spectrum of the crude reaction mixture of β,γ-CBr_2-UTP (**23**).

Supplementary data

Supplementary data (experimental procedures and characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.02.031.

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