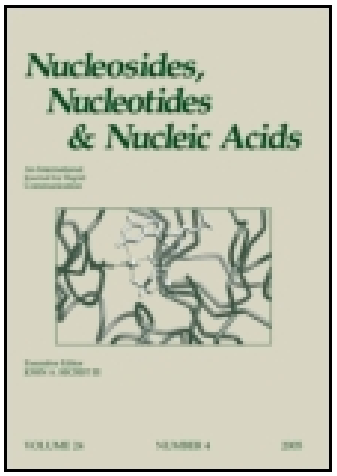

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Efficient synthesis of cytidine diphosphate choline (CDP-choline) and its analogs

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EFFICIENT SYNTHESIS OF CYTIDINE DIPHOSPHATE CHOLINE (CDP-CHOLINE) AND ITS ANALOGS

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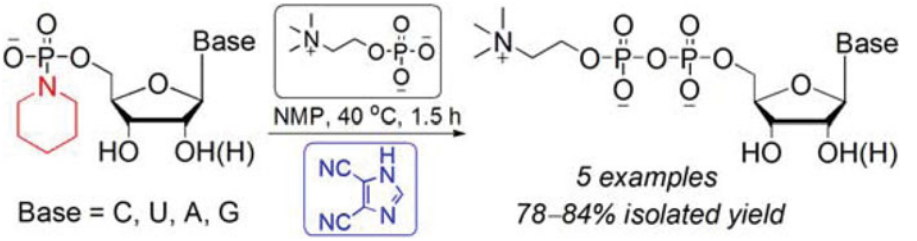
GRAPHICAL ABSTRACT



□ *An efficient P(V)–N activation approach for the synthesis of cytidine diphosphate choline (CDP-choline) and related ribo- and deoxyribonucleotide analogs has been established.*

Keywords CDP-choline; phosphoropiperidate; phosphocholine; 4,5-dicyanoimidazole; P(V)–N activation

INTRODUCTION

Cytidine diphosphate choline, also known as CDP-choline or citicoline, is an essential biomolecule in cellular metabolism.[1] It has been elucidated that CTP-phosphocholine cytidylyltransferase (CCT)-mediated formation of CDP-choline is the rate-limiting step in phosphatidylcholine (PC) biosynthetic pathway. In the biosynthesis of one of the key neurotransmitters, acetylcholine (ACh), CDP-choline serves as the choline donor.[2] Due

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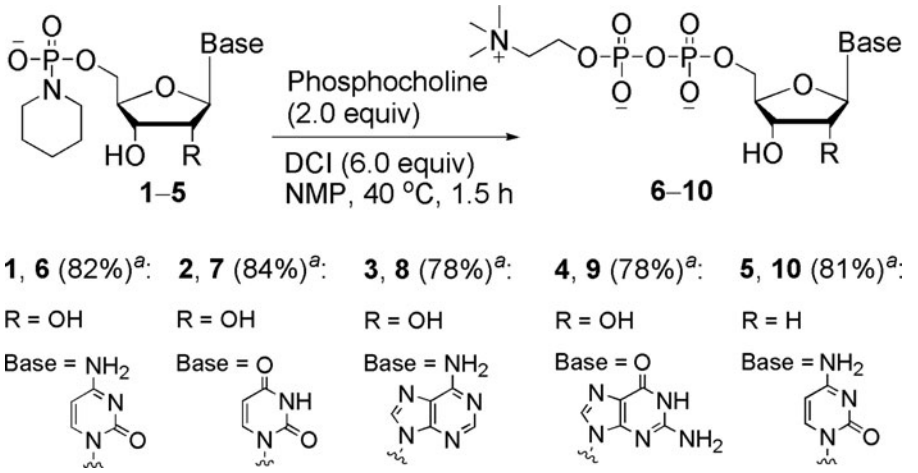
to its pivotal role in PC and ACh synthesis, CDP-choline has been extensively investigated as a neuroprotective agent in a large number of in vitro and in vivo studies.[1b,3] It has been postulated that CDP-choline restores the neuronal membrane integrity and functions by increasing the production of both PC and ACh and attenuating the formation of reactive oxygen species (ROS) at the nerve damage site.[3a] Clinical studies have established that administration of CDP-choline has beneficial effects on central nerve system disorders (memory impairment, Alzheimer's disease, and Parkinson's disease)[4] and injuries (stroke and brain/spinal cord trauma),[5] substance abuse,[6] and eye conditions (glaucoma and amblyopia).[7] Deoxycytidine diphosphate choline (dCDP-choline) has also been isolated from both human cells and sea urchin eggs, but its biological functions are still elusive.[8]

Currently, a large portion of the commercially available CDP-choline is produced through biotransformation, which is limited by low reaction concentration, moderate overall conversion yield, and complicated purification procedures.[9] The chemical synthesis of CDP-choline is complementary to microbial fermentation-based methods, and especially useful in the preparation of modified or labeled CDP-choline analogs. The major chemical synthetic method is the coupling of cytidine 5′-phosphates (CMP) with phosphocholine using different condensing reagents, such as dicyclohexylcarbodiimide (DCC),[10] *p*-toluenesulfonyl chloride,[11] and DMF/$SOCl_2$[12] in low to moderate yields. In addition, CMP has also been converted to the activated phosphate[13] or phosphoramidate[14] to improve the efficacy of the coupling reactions. However, the yields of all these reported methods rarely exceeded 60%. On the basis of the P(V)–N activation strategy that we established for the synthesis of nucleoside 5′-polyphosphates (NPPs),[15] nucleoside diphosphate sugars (NDP-sugars),[16] and dinucleoside polyphosphates,[17] we report in this paper an efficient approach for the preparation of CDP-choline and related nucleotide analogs from nucleoside 5′-phosphoropiperidates in high yields.

RESULTS AND DISCUSSION

As shown in Scheme 1, CDP-choline (**6**) and related nucleotide analogs were efficiently prepared by treating nucleoside 5′-phosphoropiperidates (**1–5**) with 2.0 equiv of bis(tetra-*n*-butylammonium) phosphocholine and 6.0 equiv of DCI in anhydrous *N*-methylpyrrolidone (NMP) at 40°C for 1.5 hours. After the reaction solutions were cooled to ambient temperature, the precipitated crude products were separated by filtration. Ethanol precipitation of the sodium salts followed by ion exchange chromatography afforded **6–10** in high isolated yields ranging from 78–84%.



SCHEME 1 The P(V)–N activation method for the synthesis of CDP-choline and related nucleotide analogs (**6–10**). [a]Isolated yield.

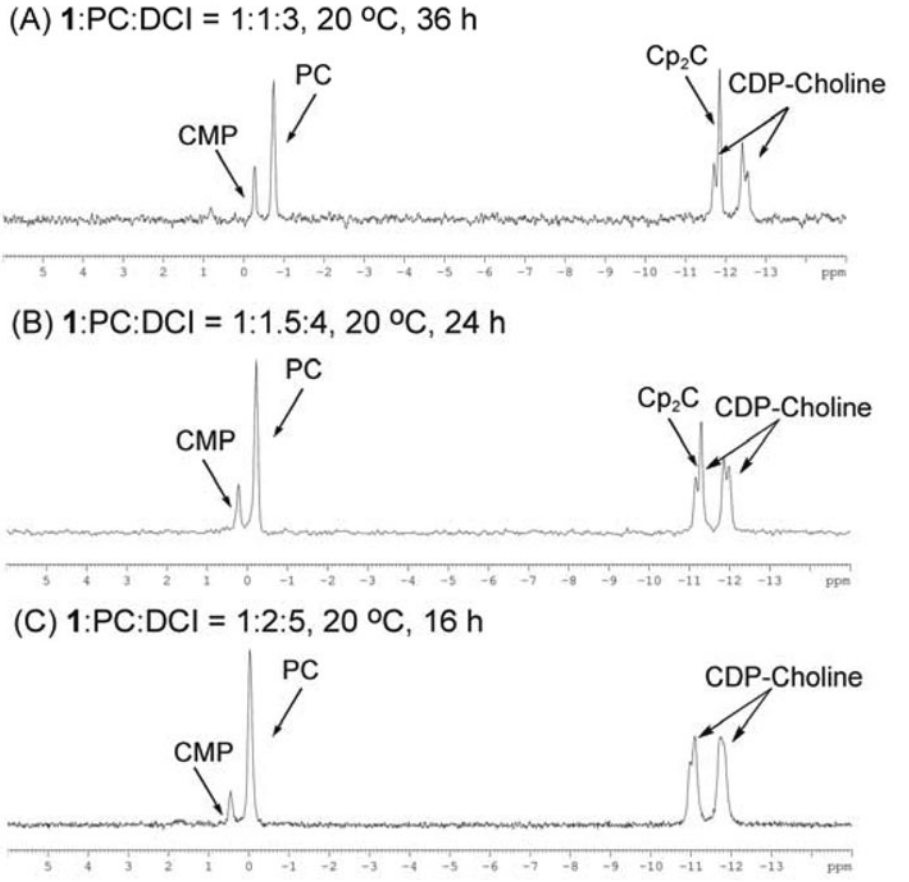
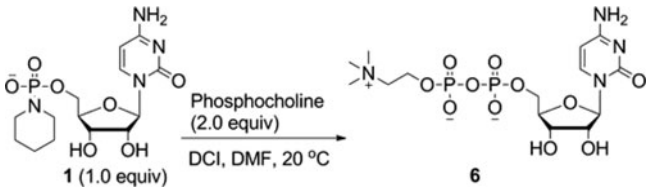


FIGURE 1 Effect of the amount of phosphocholine on the formation of CDP-choline (**6**).

TABLE 1 The effect of the amount of DCI on the formation of CDP-choline (**6**).



Equiv of DCI	Time (hours)	Yield of 6 (%)[a]
4.0	22	81
5.0	16	83
6.0	8	85
7.0	6	80

[a]^{31}P NMR yield.

In the preliminary experiment, cytidine 5′-phosphoropiperidate (**1**) was treated with phosphocholine in 1:1 molar ratio in DMF at 20°C with or without DCI activator. ^{31}P NMR tracing experiments showed that there was no reaction between the two reactants without DCI after 48 hours, which was in agreement with our previous observation that the reactivity of monoionic phosphoramidates toward phosphate nucleophiles was low. In contrast, the presence of 3.0 equiv of DCI promoted the reaction to complete in 36 hours. As we proposed in a previous report,[15a] the protonation of the inactive **1** ($P–O^-/P–NR_2$) by DCI generated the reactive zwitterionic phosphoramidate intermediate ($P–O^-/P–NH^+R_2$), and the subsequent P–O coupling with phosphocholine proceeded via an associative pathway. In addition to the acid catalysis mentioned above, nucleophilic catalysis of the conjugate base of DCI was also supposed to be involved in this reaction due to the low nucleophilicity of phosphocholine. However, the ^{31}P NMR yield of CDP-choline (**6**) was only 52% with 1 equiv of phosphocholine and 3 equiv of DCI. The low nucleophilicity of phosphocholine resulted in not only slow reaction rate but also pronounced side reactions, the hydrolysis (ca. 22%) and self-condensation (ca. 26%) of **1**. To solve this problem, higher equivalents of phosphocholine (1.5 and 2.0 equiv) were tested in the reaction. As shown in Figure 1, the self-condensation byproduct (Cp_2C) was almost completely

TABLE 2 The effect of temperature on the formation of CDP-choline (**6**)

Temperature (°C)	Time (hours)	Yield of **6** (%)[a]
20	8	85
30	3.5	83
40	1.5	84
50	0.7	80

[a]^{31}P NMR yield.

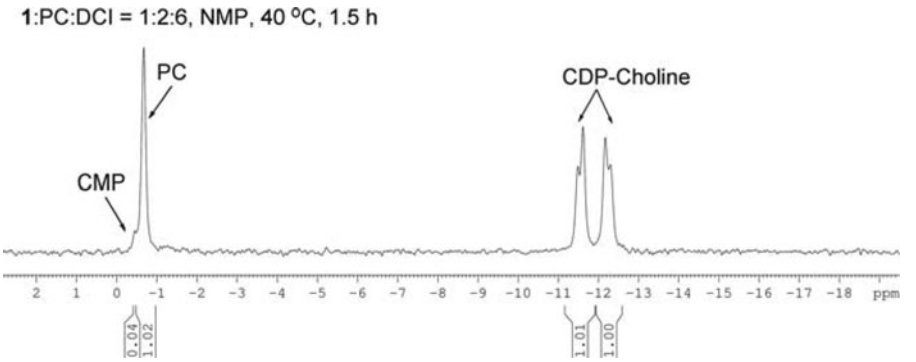


FIGURE 2 ^{31}P NMR of the crude reaction mixture of CDP-choline (**6**) in NMP.

suppressed and the yield of **6** was improved to 83%, when 2.0 equiv of phosphocholine was applied.

In the following research, the effect of the amount of DCI was investigated in detail. The data in Table 1 showed that the reaction time was significantly shortened from 22 to 8 hours, when the amount of DCI was increased from 4.0 to 6.0 equiv. Though further increment of the amount of DCI to 7.0 equiv slightly increased the reaction rate (6 hours), the self-condensation side reaction was also promoted.

To further promote the coupling reaction, the reaction temperature was gradually elevated from 20 to 50°C. As shown in Table 2, the reaction time was remarkably shortened to 45 minutes, when the reaction was conducted at 50°C. But it was also noticed that when the reaction temperature was above 40°C, the yield of **6** began to drop due to the self-condensation of **1**.

Due to the limited solubility of phosphocholine in DMF, some other polar aprotic solvents, such as DMSO, pyridine, and NMP, were tested for this reaction. It was found that NMP exhibited the best solvency for phosphocholine. The ^{31}P NMR data (Figure 2) also showed that the yield of **6** was improved to 95% with unexpected suppression of the hydrolysis of **1** (<4%), when NMP was used as the solvent.

CONCLUSIONS

In summary, we developed an efficient method for the synthesis of CDP-choline and related ribo- and deoxyribonucleoside analogs. The detailed reaction conditions such as the ratio of reactants, amount of DCI, temperature, and solvent were systematically optimized for CDP-choline. Compared to the known methods, this new approach features easily accessible starting materials, short reaction time, and high isolated yields, and could be applied to the synthesis of a variety of nucleoside diphosphate analogs.

EXPERIMENTAL

General Methods. All reactions were performed in anhydrous solvents under an atmosphere of dry argon. Ion-exchange chromatography employed DEAE Sephadex A-25 exchanger. NMR spectra were obtained with a 400 MHz instrument with chemical shifts reported in parts per million (ppm, δ). IR spectra were recorded on a FT-IR spectrometer. Low-resolution mass spectra were obtained with an ion trap mass spectrometer and reported as *m/z*. The triethylammonium salts of nucleoside 5′-phosphoropiperidates were synthesized from the nucleoside 5′-phosphates according to the procedure described in our previous report in over 95% yield.[15b] The bis(tetra-*n*-butylammonium) phosphocholine was prepared according to a reported method.[18]

General procedure for the synthesis of nucleoside 5′-diphosphate choline (6–10): To a solution of nucleoside 5′-phosphoropiperidate (0.1 mmol) in NMP (2 mL) were added bis(tetra-*n*- butylammonium) phosphocholine (0.2 mmol) and DCI (0.6 mmol). The reaction was stirred at 40°C for 1.5 hours. The precipitation was collected by centrifuge. The solid residue was dissolved in NaOAc aqueous solution (3 M, 1 mL). Then, 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 0.05–0.2 M), combination of appropriate fractions, and lyophilization afforded NDP-choline 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 NDP-choline as sodium salt, a white solid.

Cytidine 5′-diphosphate choline, sodium salt (6): Starting from cytidine 5′-phosphoropiperidate (49 mg, 0.1 mmol), compound **6** was synthesized according to the general procedure. Ion-exchange chromatography afforded **6** (42 mg, 82%) as a white solid; ^{1}H NMR (400 MHz, D_2O): δ 7.90 (d, J = 7.6 Hz, 1H), 6.07 (d, J = 7.6 Hz, 1H), 5.94 (d, J = 4 Hz, 1H), 4.35−4.30 (m, 2H), 4.28−4.27 (m, 4H), 4.25−4.22 (m, 1H), 3.65−3.63 (m, 2H), 3.18 (s, 9H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 165.8, 157.3, 141.1, 96.1, 88.9, 82.2, 73.7, 68.9, 65.6, 64.4, 59.4, 53.6 ppm; ^{31}P NMR (162 MHz, D_2O): δ –11.1 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 20.8 Hz, 1P), –11.9 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 20.8 Hz, 1P) ppm; IR: ν_{max} 3671, 2988, 2899, 1645, 1488, 1392, 1234, 1063, 782, 634, 609, 552 cm^{-1}; HRMS (ESI–): *m/z* calcd for $C_{14}H_{25}N_4O_{11}P_2$ $[M–H]^-$ 487.1001; found 487.1010.

Uridine 5′-diphosphate choline, sodium salt (7): Starting from uridine 5′-phosphoropiperidate (49 mg, 0.1 mmol), compound **7** was synthesized according to the general procedure. Ion-exchange chromatography afforded **7** (43 mg, 84%) as a white solid; 7.86 (d, J = 8.0 Hz, 1H), 5.88−5.83 (m, 2H), 4.29−4.25 (m, 4H), 4.17−4.09 (m, 3H), 3.59 (m, 2H), 3.12 (s, 9H) ppm; ^{13}C

NMR (100 MHz, D_2O): δ 166.0, 151.6, 141.1, 102.2, 88.0, 82.8, 73.3, 69.2, 65.4, 64.5, 59.4, 53.5 ppm; ^{31}P NMR (162 MHz, D_2O): δ –11.6 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 21.0 Hz, 1P), –12.3 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 21.0 Hz, 1P) ppm; IR: ν_{max} 3673, 2983, 2904, 1689, 1463, 1395, 1237, 1082, 770, 608, 536 cm^{-1}; HRMS (ESI–): *m/z* calcd for $C_{14}H_{24}N_3O_{12}P_2$ $[M–H]^-$ 488.0841; found 488.0853.

Adenosine 5′-diphosphate choline, sodium salt (8): Starting from adenosine 5′-phosphoropiperidate (52 mg, 0.1 mmol), compound **8** was synthesized according to the general procedure. Ion-exchange chromatography afforded **8** (42 mg, 78%) as a white solid; ^1H NMR (400 MHz, D_2O): δ 8.40 (s, 1H), 8.15 (s, 1H), 6.05 (d, J = 4 Hz, 1H), 4.54–4.44 (m, 2H), 4.43–4.42 (m, 4H), 4.30–4.28 (m, 2H), 3.55 (s, 2H), 3.10 (s, 9H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 155.2, 152.5, 148.7, 139.3, 118.1, 86.4, 83.4, 73.7, 69.9, 65.4, 64.8, 59.4, 53.4 ppm; ^{31}P NMR (162 MHz, D_2O): δ –11.5 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 20.9 Hz, 1P), –12.3 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 20.9 Hz, 1P) ppm; IR: ν_{max} 3332, 2981, 2491, 1651, 1477, 1409, 1331, 1235, 1118, 944, 873, 817, 784, 710, 646, 561 cm^{-1}; HRMS (ESI–): *m/z* calcd for $C_{15}H_{25}N_6O_{10}P_2$ $[M–H]^-$ 511.1113; found 511.1126.

Guanosine 5′-diphosphate choline, sodium salt (9): Starting from guanosine 5′-phosphoropiperidate (53 mg, 0.1 mmol), compound **9** was synthesized according to the general procedure. Ion-exchange chromatography afforded **9** (43 mg, 78%) as a white solid; ^1H NMR (400 MHz, D_2O): 8.10 (s, 1H), 5.93 (s, 1H), 4.51 (s, 1H), 4.25–4.40 (m, 4H), 4.20 (s, 2H), 3.62 (s, 2H), 3.17 (s, 9H); ^{13}C NMR (100 MHz, D_2O): δ 159.9, 154.7, 151.9, 137.4, 116.4, 86.7, 83.7, 73.6, 70.4, 66.0, 65.3, 59.8, 53.9 ppm; ^{31}P NMR (162 MHz, D_2O): δ –11.3 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 20.8 Hz, 1P), –12.1 (d, $J_{P\text{-}\alpha,P\text{-}\beta}$ = 20.8 Hz, 1P) ppm; IR: ν_{max} 3668, 2986, 2313, 1667, 1530, 1481, 1237, 1080, 1047, 939, 917, 778, 642, 571 cm^{-1}; HRMS (ESI–): *m/z* calcd for $C_{15}H_{25}N_6O_{11}P_2$ $[M–H]^-$ 527.1062; found 527.1077.

2′-Deoxycytidine 5′-diphosphate choline, sodium salt (10): Starting from 2′-deoxycytidine 5′-phosphoropiperidate (48 mg, 0.1 mmol), compound **10** was synthesized according to the general procedure. Ion exchange chromatography afforded **10** (40 mg, 81%) as a white solid; ^1H NMR (400 MHz, D_2O): δ 7.81 (d, J = 8.1 Hz, 1H), 6.21 (t, J = 7.3 Hz, 1H), 5.98 (d, J = 7.2 Hz, 1H), 4.45 (s, 1H), 4.26 (s, 2H), 4.07–4.05 (m, 3H), 3.55 (s, 2H), 3.09 (s, 9H), 2.32–2.26 (m, 1H), 2.21–2.14 (m, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 166.3, 157.7, 141.7, 96.6, 86.1, 85.5, 70.9, 66.1, 65.5, 60.0, 54.1, 39.5 ppm; ^{31}P NMR (162 MHz, D2O): δ –10.9, –11.7 ppm; IR: ν_{max} 3680, 2979, 2909, 1682, 1498, 1402, 1248, 1078, 794, 653, 628, 564 cm^{-1}; HRMS (ESI–): *m/z* calcd for $C_{14}H_{25}N_4O_{10}P_2$ $[M–H]^-$ 471.3172; found 471.3165.

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