Improved synthesis of cytidine diphosphate choline (CDP-choline) *via* selective phosphorylation

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An improved, three-step synthesis of cytidine diphosphate choline (CDP-choline) from cytidine was achieved in 68% overall yield. Selective phosphorylation of cytidine was accomplished by the use of morpholinophosphodichloridate to give cytidine-5'-phosphomorpholide, which was condensed with choline phosphate chloride in the presence of a catalytic amount of H_2SO_4 to give CDP-choline. The intermediates and products could be efficiently purified by recrystallisation, thus avoiding the use of chromatography at all stages. The reaction could be scaled up to 200 g in 64% overall yield, making this route attractive for industrial application.

Keywords: cytidine diphosphate choline, phosphorylation, scalable synthesis, choline phosphate chloride

Cytidine diphosphate choline (CDP-choline **1**) is a nucleotide coenzyme and serves as a choline donor in the biosynthesis of lipids,[1] lecithins,[2] and sphingomyelin.[3] It is a clinical drug for the treatment of several illnesses involving disturbance of the central nervous system, in particular, for regaining a patient's consciousness and for treatment of neuropsychic symptoms occurring during skull traumas and brain surgery.[4]

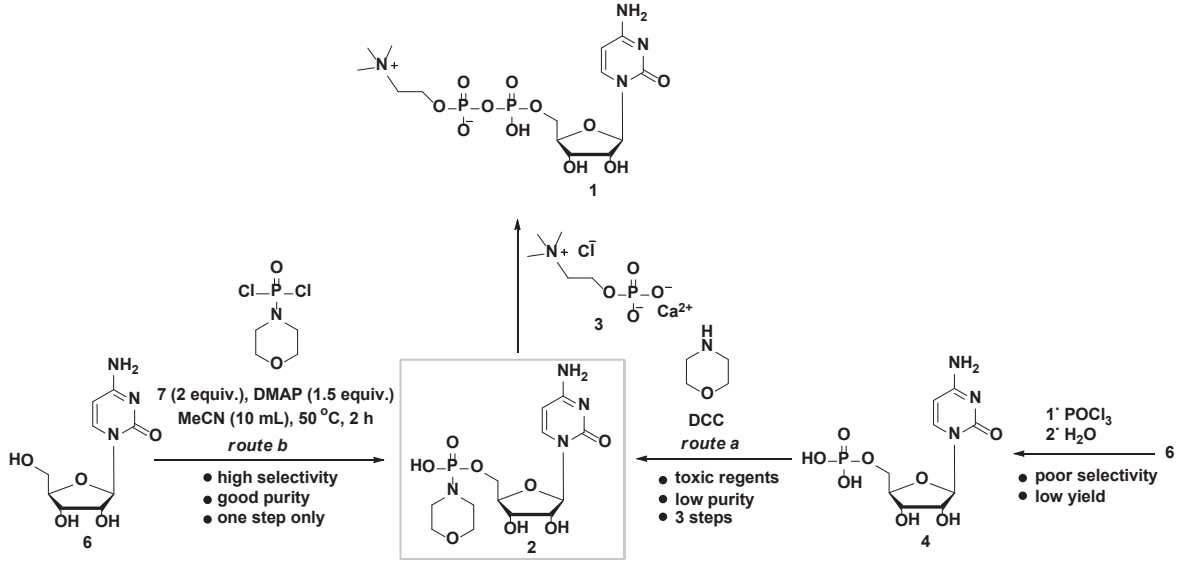
Among various methods for the synthesis of CDP-choline in the literature, the current preferred method is *via* the condensation of cytidine-5'-phosphomorpholide (**2**) with choline phosphate chloride (**3**) under mild reaction conditions.[5–7] Compound **2** was synthesised from 5'-cytidine monophosphate (**4**) and morpholine in the presence of DCC (*N*,*N*'-dicyclohexylcarbodiimide)[8] or *via* the controlled hydrolysis of cytidine-5'-phosphodichloride (**5**) followed by P–N bond formation with morpholine (Scheme 1, route a).[7] However, DCC is toxic and converted into urea which is difficult to separate from the mixture, thus leading to poor purity of product. Furthermore, phosphorylation with $POCl_3$ always meets with side reactions from the 2' or 3' hydroxyls and detracts from the acceptance of this method in industry.[9]

In the context of ongoing projects on the synthesis of nucleoside drugs,[10–14] herein we report the synthesis of CDP-choline *via* the selective phosphorylation of **6** with morpholinophosphordichlorodate (**7**) (Scheme 1, route b).

Results and discussion

Central to our approach for the synthesis of CDP-choline is the selective phosphorylation of **6** using sterically-hindered **7** as phosphorylation regent. **7** was synthesised by the direct phosphorylation of morpholine with $POCl_3$, a compound whose utility for the conversion of alcohols and amines into various phosphorylation derivatives.[15] Due to the reactivity of three chloro atoms in $POCl_3$, gradually adding $POCl_3$ to excess morpholine avoids the bifunctional reaction exclusively. After reaction, **7** could be purified by fractional distillation to yield as a colourless oil (b.p. 124–126 °C at 1.33 KPa). Due to the presence of the electron-donating morpholino group, **7** displays lower reactivity than $POCl_3$ and could tolerate moisture and air better. Usefully, **7** could be synthesised on the 200 g scale and stored at 4 °C.

The major concern of utilising **7** as phosphorylation reagent is its selectivity for the 5' hydroxyl group. We therefore assessed the selectivity for 5' hydroxylation using **6** and **7** in the presence of various organic bases. After phosphorylation, H_2O was added to destroy the excess of **7**, and **2** was obtained in a single step. The solvent, the base, temperature and the ratio of substrates were evaluated and the results are summarised in Table 1.



Scheme 1

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Table 1 Optimisation of the reaction conditions for the base-catalysed synthesis of cytidine-5'-phosphomorpholide **2** from cytidine **6** and morpholinophosphordichlorodate **7** (Scheme 1, route b)[a,c]

Entry	n_6:n_7	Solvent	Temp/°C	Time/h	Base	Yield/%[b]
1	1:1	MeCN	r.t.	1	TEA	32
2	1:2	MeCN	r.t.	1	TEA	46
3	1:3	MeCN	r.t.	1	TEA	41
4	1:2	MeCN	50	1	TEA	39
5	1:2	MeCN	0	1	TEA	63
6	1:2	MeCN	0	2	TEA	72
7	**1:2**	**MeCN**	**0**	**2**	**DMAP**	**81**
8	1:2	MeCN	0	2	pyridine	68
9	1:2	dioxane	0	2	DMAP	64
10	1:2	pyridine	0	2	DMAP	76
11	1:2	DMF	0	2	DMAP	56
12	1:2	MeCN	0	3	DMAP	73

[a]Reaction conditions: morpholinophosphorodichloridate **7** (1, 2 or 3 equiv.) was added slowly to a stirred mixture of cytidine **6** (1 equiv.) and an organic base in various solvents for various times.

[b]Isolated yield.

[c]TEA = triethylamine, DMAP = 4-dimethylaminopyridine.

When the reaction of **6** and **7** (1:1) was conducted in MeCN (10 mL) at room temperature for 1 h using triethylamine TEA as the base, **2** was obtained in 32% yield (Table 1, entry 1). The yield was increased to 46% when 2 equiv. of **7** was employed (entry 2). Increasing the amount of **7** led to a lower yield of 41% due to the side reactions of the 2' or 3' hydroxyl groups (entry 3). A lower yield was also observed at 50 °C and this led us to conduct the reaction at a lower temperature to improve the yield (entry 4). Gratifyingly, the reaction at 0 °C gave a yield of 63% (entry 5). Doubling the reaction time improved the yield to 72% (entry 6). Other bases such as DMAP or pyridine were assessed and DMAP gave the best yield of 81% (entry 7). Other solvents such as dioxane, pyridine and DMF were also examined but failed to give better results (entries 9–11). Extending the reaction time to 3 h reduced the yield to 73% (entry 12). Thus, the optimised reaction conditions were determined as described in entry 7.

The traditional method for the synthesis of **2** with $POCl_3$ gave only a 40% yield and needed three steps. We surmised that the better yield of our method was due to the good selectivity for 5' hydroxylation rather than 2' or 3' hydroxylation because of the steric hindrance of morpholino group.

With **2** in hand, next, we conducted the reaction of **2** and **3** to give the final product CDP-choline. After intensive experiment and optimisation, CDP-choline was synthesised in 85% yield catalysed by 10 mol% H_2SO_4 at 50 °C for 3 h.

To evaluate the reproducibility and the stability of the reaction, the synthesis of **2** and CDP-choline on a larger scale was performed. The synthesis of **2** by the selective phosphorylation on a 200 g scale was carried out and the reaction still worked well in 80% yield. The synthesis of CDP-choline on a 200 g scale also gave a yield of 80%. An added virtue is that CDP-choline could be purified by recrystallisation, thus chromatography or a lengthy purification process was not required, which made this route more attractive for industrial applications.

Experimental

Melting points were recorded with a micro melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AV/400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Chemical shifts δ are given in ppm relative to tetramethylsilane as internal standard, or residual DMSO-d_6 or D_2O for ^{1}H or ^{13}C NMR spectroscopy. Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q) and multiplet (m). High resolution mass spectra were obtained with a 3000 mass spectrometer, using a Waters Q-TofMS/MS system. All reagents and solvents were purchased from commercial sources and purified before use.

Cytidine-5'-phosphomorpholide (**2**): Cytidine (0.243 g, 1.0 mmol) and DMAP (0.183 g, 1.5 mmol) in MeCN (10 mL) were stirred slowly and cooled to 0 °C, and **7** (2.0 mmol) was added slowly. The mixture was heated to 50 °C and kept at this temperature for 2 h. The solvent was removed *in vacuo* and the residue was purified by recrystallisation from EtOH to give **2** as a white semi-solid (0.318 g); yield 81%; m.p. 62–64 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, J = 7.6 Hz, 1H), 7.39 (s, 2H), 7.19 (d, J = 7.6 Hz, 1H), 5.77 (d, J = 2.8 Hz, 1H), 5.51 (d, J = 4.8 Hz, 1H), 5.18 (t, J = 5.2 Hz, 1H), 5.08 (d, J = 5.6 Hz, 1H), 3.76–3.71 (m, 1H), 3.61–3.56 (m, 1H), 3.45–3.42 (m, 4H), 3.03–2.99 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 157.8, 145.9, 141.6, 88.1, 86.2, 74.3, 70.7, 65.1, 65.0, 60.3, 60.2; HRMS calcd for $C_{13}H_{22}N_4O_8P$ $[M + H]^+$ 393.1170, found: 393.1172.

CDP-choline (**1**): **2** (0.392 g, 1.0 mmol) was added to MeOH (10 mL) followed by the addition of **3** (0.310 g, 1.2 mmol) and was stirred at room temperature for 10 min. Then 98% H_2SO_4 (0.005 mL, 10 mol%) was added. The mixture was kept at 50 °C for 3 h. The solvent was removed *in vacuo* and the residue was purified by recrystallisation from EtOH to give **1** as a white solid (0.410 g); yield 85%. ^{1}H NMR (400 MHz, D_2O) δ 7.86 (s, 2H), 6.04 (d, J = 5.2 Hz, 1H), 5.91 (d, J = 5.2 Hz, 1H), 4.32 (brs, 2H), 4.26–4.22 (m, 2H), 4.18 (brs, 2H), 4.11 (t, J = 3.2 Hz, 1H), 3.60 (t, J = 2.4 Hz, 2H), 3.14 (s, 9H); ^{13}C NMR (100 MHz, D_2O) δ 166.1, 157.7, 141.5, 96.6, 89.3, 82.6, 74.1, 69.3, 66.0, 65.9, 64.8, 59.9, 54.0; HRMS calcd for $C_{14}H_{27}N_4O_{11}P_2$ $[M + H]^+$ 489.1146, found: 489.1140.

For the synthesis of **1** from **6** on a 200 g scale, see the Electronic Supplementary Information (ESI).

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Electronic Supplementary Information

The ESI is available through:
stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

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