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CDP-Ethanolamine and CDP-Choline: one-pot synthesis and ^{31}P NMR study

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ABSTRACT

Herein we report a one-pot multi-step synthesis of the cofactors CDP-Ethanolamine and CDP-Choline starting from cytidine 5′-monophosphate and using commercially available and/or easily prepared reagents. While studying the ^{31}P NMR spectrum of CDP-Ethanolamine, an unexpected characteristic for a pyrophosphate diester was observed as it showed a singlet or two doublets depending upon the pH. Therefore, further NMR studies were undertaken to investigate the pH dependence of the peak splitting pattern and measure the acid dissociation constants of the compounds.

Nucleolipids are organic hydrid compounds that are made up of a nucleobase, or a nucleos(t)ide or an oligonucleotide, and a lipophilic moiety. Nucleolipids possessing both nucleic acid type recognition potentiality and lipophilic chains are emerging as valuable building blocks for generating supramolecular systems. Indeed, these amphiphiles possess a diversity of functional groups capable of cooperative noncovalent interactions combined with specific base recognition.[1] As part of our ongoing study on phospholipid metabolomics in *Plasmodium falciparum*,[2] we have been interested in the synthesis of CDP-Ethanolamine (CDP-Etn) and CDP-Choline (CDP-Cho), two nucleolipid building blocks used as essential standards in LC/MS/MS analytical studies. So far, only a few synthetic methods have been described in the literature for these two compounds. The reports for CDP-Etn (the last one appeared in 1972) indicate a long reaction time together with low yields.[3–8] Microbial and enzymatic productions of CDP-Etn have also been reported but require access to the biological materials together with specific technology.[9–12]

Eugene P. Kennedy has made an essential contribution to the study of phospholipid metabolism.[13] Thus, the eponymous pathway depicts de novo pathways of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). He was also the first to report the synthesis of their precursors, CDP-Etn and CDP-Cho.[3] Coupling of cytidine 5′-monophosphate (CMP) with either *O*-phosphoryl-choline or *O*-phosphoryl-ethanolamine in anhydrous pyridine or DMF was performed in the presence of a coupling agent leading to the desired compounds in very low yields, especially for CDP-Etn.[3,14–16] Then, these compounds have also been obtained either by reacting CDP with ethyleneimine in water (yielding CDP-Etn in 31%) followed by methylation with methyl iodide to give CDP-Cho,[4,5] or by condensation of activated CMP such as cytidine 5′-phosphoromorpholidate with phosphate esters.[6–8,17] Our initial attempts to synthesize CDP-Etn according to literature data failed to give the desired compound even in moderate yield. We also tried, unsuccessfully, a direct coupling of CMP-morpholidate with *O*-phosphorylethanolamine in pyridine, under conditions described for the synthesis of ADP-ethanolamine.[18] Consequently, we decided to set up a more practical reaction protocol for the synthesis of CDP-Etn and CDP-Cho. As regards the synthesis of nucleoside 5′-diphosphates, three multi-step procedures are widely used and involve (i) the displacement of a 5′-*O*-tosyl group with tris(tetra-*n*-butylammonium) pyrophosphate;[19] (ii) the use of *cyclo*Sal-nucleotides as intermediates;[20] (iii) the nucleophilic attack of tri-*n*-butylammonium phosphate on activated nucleoside 5′-phosphorimidazolates.[21] Taking into account this latter approach, we report an efficient one-pot synthesis of the target derivatives starting from CMP (Scheme 1). In addition, the physicochemical analysis of these compounds revealed similar apparent

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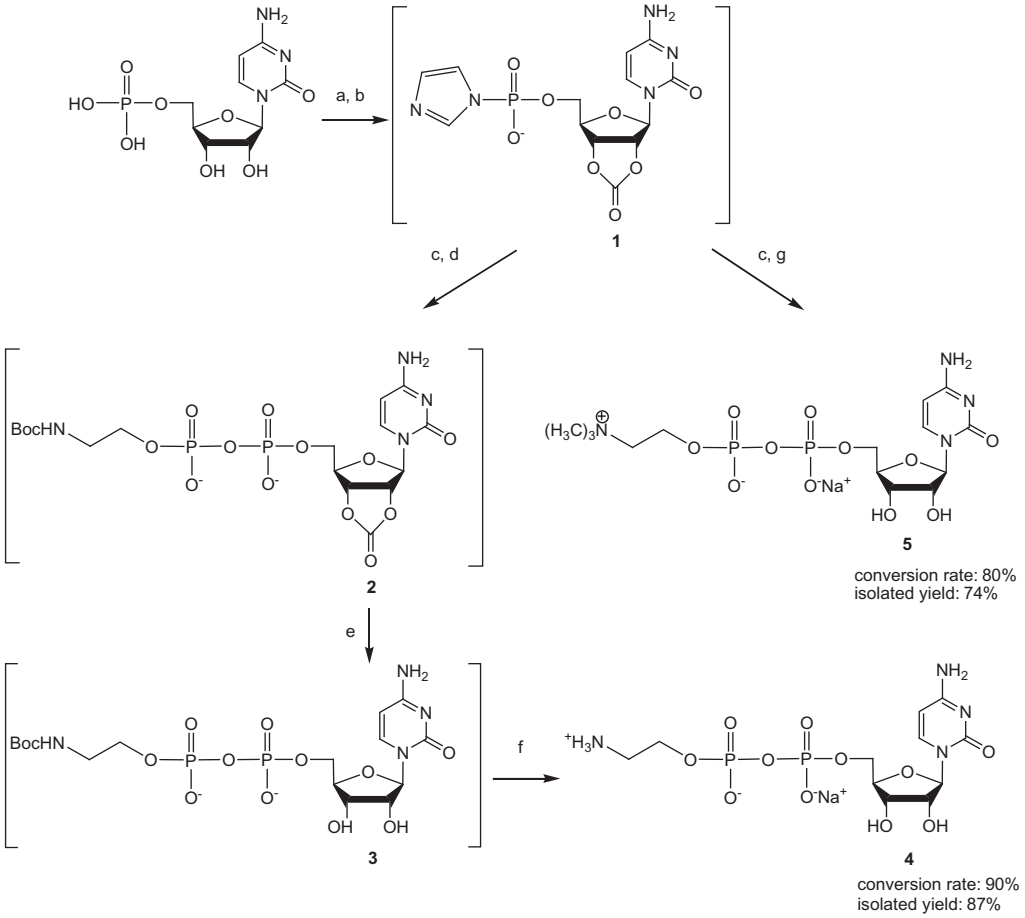

Scheme 1. Synthesis of CDP-Etn and CDP-Cho. Reagents and conditions: (a) tributylamine, DMF, 50 °C, 10 min; (b) CDI, rt, 30 min; (c) MeOH, 30 min; (d) *N*-Boc-2-aminoethylphosphate, $ZnCl_2$, DMF, rt, 24 h; (e) TEAB 1 M pH 9, rt, 3 h; (f) TFA/DCM (1/1, v/v), rt, 1 h then purification by chromatography; (g) choline phosphate salt, $ZnCl_2$, DMF, rt, 24 h then purification by chromatography.

acid dissociation constants (pK_a), but different ^{31}P NMR splitting patterns that depend on pH.

Our synthetic strategy began with the activation of CMP in the presence of 1,1′-carbonyldiimidazole (CDI). Interestingly, we found that heating commercial cytidylic acid, the acid form of CMP, with tributylamine in DMF at 50 °C for 10 min before addition of CDI, allowed the rapid formation of the corresponding nucleoside 2′,3′-carbonate 5′-phosphorimidazolate **1** (Scheme 1). Without this preheating step, no activation occurred. After 30 min of activation, the intermediate **1** was characterized by ^{31}P NMR (δ $^{31}P = -10.2$ ppm) and mass spectrometry, then the excess of reactant was quenched with methanol. The progress of the reaction was monitored by HPLC coupled with a Photo Diode Array detector since it is a lower material-consuming technique than ^{31}P NMR.[22] To circumvent the dual reactivity on both edges of *O*-phosphorylethanolamine as well as to increase its solubility in DMF, we protected the primary amino group of *O*-phosphorylethanolamine with the acid-labile Boc group.[23] Then, addition of *N*-Boc-2-aminoethylphosphate and zinc chloride as the catalyst[24] to the reaction mixture led to a complete conversion of phosphorimidazolate **1** into intermediate **2** after 24 h. In situ deprotection of the 2′,3′-carbonate group under basic conditions was quantitative after 3 h. The Boc protecting group of compound **3** was then removed with a TFA/DCM (1/1, v/v) mixture to give CDP-Etn **4**. We then applied the synthetic sequence for CDP-Cho. Treatment of the 2′,3′-carbonate 5′-phosphorimidazolate derivative **1** with choline phosphate[25] gave compound **5**. The conversion rates (determined by HPLC) for CDP-Etn **4** and CDP-Cho **5** were 90% and 80%, respectively. Purification by semi-preparative HPLC was used to isolate CDP-Etn. Alternatively, CDP-Etn and CDP-Cho could be purified by anion exchange chromatography using a gradient of triethylammonium hydrogen carbonate (TEAB) followed by another reversed-phase chromatography. Finally, exchange of the triethylammonium counter-ions by sodium led to compounds **4** and **5** as sodium salts in 87% and 74% yield, respectively. Thus, these derivatives were obtained in better yields than those reported previously for their synthesis. The final products were fully characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopic analyses and high resolution mass spectrometry.[26]

In our NMR experiments, the sample concentration was kept low enough to avoid intermolecular base stacking and to enable the detection of clear and sharp NMR signals.[27] We noticed that protons belonging to the nucleobase of CDP-Etn, when purified by HPLC under acidic conditions, display downfield shifts compared to those of the sodium salt of CDP-Etn ($\Delta\delta = 0.15$ ppm for H_5, $\Delta\delta = 0.23$ ppm for H_6). This shift has been attributed to the protonation of cytosine at low pH. Literature data report two spin AX-systems for CDP-Etn ($\delta P_\alpha = -7.95$ ppm; $\delta P_\beta = -8.10$ ppm, $^{2}J_{\alpha\beta} = 20.9$ Hz) and CDP-Cho ($\delta P_\alpha = -8.13$ ppm; $\delta P_\beta = -8.93$ ppm, $^{2}J_{\alpha\beta} = 21.1$ Hz) at pH 7.25.[28] However, Robitaille et al. describe a singlet for CDP-Etn at neutral pH.[29] In our case, the ^{31}P NMR spectrum of CDP-Etn, recorded in D_2O, showed only a singlet at −11.16 ppm (Fig. 1a). Upon addition of NaOH 1 N (10% of the final volume), this singlet was instantaneously split into two doublets ($\delta P_\alpha = -10.56$ ppm; $\delta P_\beta = -11.27$ ppm, $^{2}J_{\alpha\beta} = 20.9$ Hz) as shown in Figure 1b. Thus, compared to the ^{31}P NMR of CDP-Cho, where only