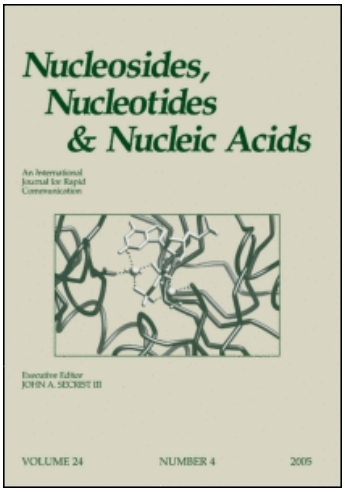

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AN EFFICIENT PROCESS FOR THE SYNTHESIS OF CYCLIC HPMPC

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ABSTRACT

1-[((*S*)-2-Hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl] cytosine (cyclic HPMPC) was readily synthesized in gram to multi-kilogram quantities by treating a DMF suspension of HPMPC with four molar equivalents of ethyl chloroformate. This dehydrative intramolecular cyclization process typically afforded cHPMPC in 94% isolated yield and high purity. Benign by-products and solvents were easily removed.

The anti-viral agent, cyclic HPMPC, **2**, (Fig. 1, cHPMPC) has generally been synthesized by treating HPMPC (**1**) dihydrate with dicyclohexyl carbodiimide (DCC) and morpholine to promote a dehydrative intramolecular cyclization reaction (1). This process involved the use of large quantities of the potentially dangerous reagent DCC and extensive processing was required to remove the by-product, dicyclohexyl urea (DCU), because it interfered with the drug product formulation of cHPMPC. In an attempt to develop a safe, efficient and scaleable process, many potential cyclization promoters were tested on a stable, anhydrous form of solid HPMPC (2).

Attempts to employ Ti (IV) chloride or triflate as a cyclization promoter in an organic solvent such as acetonitrile or dichloromethane proved unsuccessful primarily due to the poor solubility of HPMPC (3). Dehydrative cyclizations using alkyl sulfonyl chlorides 4, trifluoroacetic anhydride and triphosgene were only marginally successful and a high percentage of the dimer **5** (Fig. 1) was observed.

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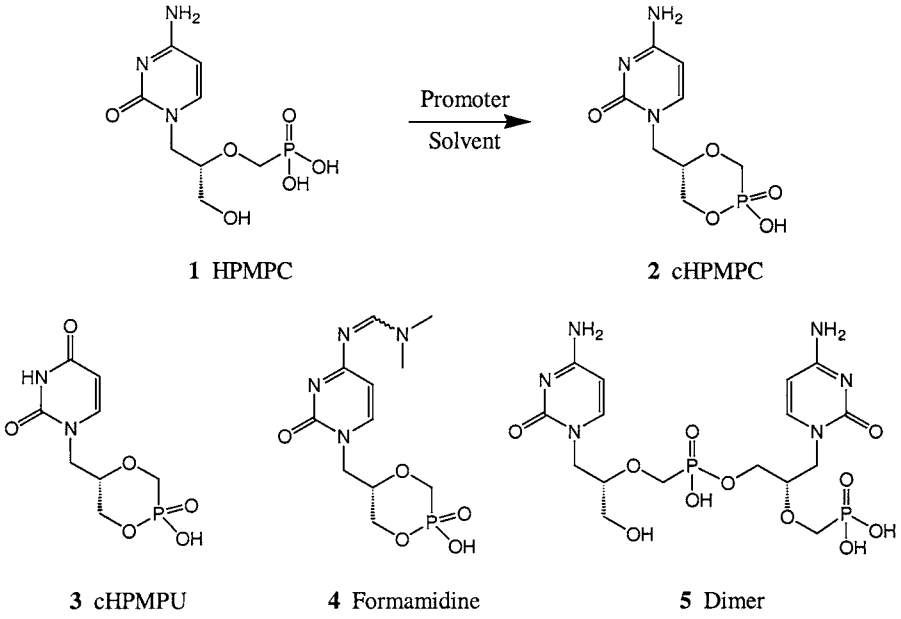

Figure 1.

The cyclization of anhydrous HPMPC was also studied using phosphoryl, thionyl, and oxalyl chloride as the promoters.

Although cHPMPC (**2**) was formed in 20–50% yield, major impurities including unreacted HPMPC, cyclic HPMPU, **3**, (uracil analog produced from cytosine hydrolysis) and large amounts of formamidine **4** were obtained in cases where DMF was used as the reaction solvent. The complex mixture of by-products made it difficult to effectively isolate cHPMPC.

Ethyl chloroformate has been used to promote the formation of cyclic phosphates of 2′,3′-nucleosides since the late 1950's (5). The use of isobutyl- and ethyl chlorformate in the solvent DMF were both studied as potential cyclization initiators. The former was desirable because the by-product, isobutyl alcohol, was slow to esterify cHPMPC presumably due to steric demands. Both of the chloroformates efficiently promoted the intramolecular cyclization of HPMPC to afford cHPMPC in greater than 92% isolated yield. However, the isobutyl moeity proved to be non-polar enough to cause separation between the two viscous liquid phases of the reaction mixture creating problems with mixing and reproducibility.

Ethyl chloroformate was found to be the most efficient promoter of the dehydrative intramolecular cyclization of HPMPC (Fig. 2). Four molar equivalents of ethyl chloroformate were added dropwise to a rapidly stirring suspension of anhydrous HPMPC in DMF (1g of HPMPC per 2 mL of DMF) heated to 40°C. A vigorous evolution of CO_2 gas commenced during the chloroformate addition. The addition rate of chloroformate was adjusted to keep the temperature of the reaction mixture at 45–60°C. It was presumed that HPMPC reacted with ethyl chloroformate

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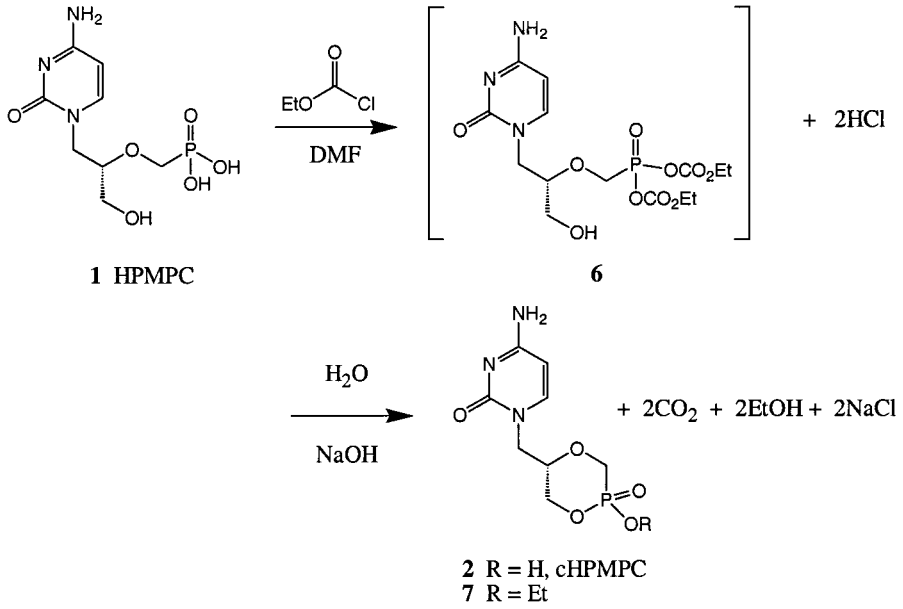

Figure 2.

to produce phosphono-formyl dianhydride **6** (Fig. 2) which underwent intramolecular displacement by the primary hydroxyl group. Concommitant elimination of CO_2 and ethanol drove the formation of the six-membered ring. After the addition of ethyl chloroformate was completed, the resulting viscous solution was stirred with heating to maintain a reaction temperature of 60–65°C for 30 mins. Prolonged exposure of cHPMPC to the by-product, ethanol, under acidic conditions will result in the formation of undesired cyclic ester **7** (Fig. 2). The reaction was quenched by adding methanol (1.6 mL per gram of **1**) and H_2O (0.4 mL per gram of **1**) and stirring for 5 mins keeping the temperature of the solution between 55–60°C. A solution of 50% (w/w) aqueous NaOH (0.23 g per gram of **1**) was added to the warm crude reaction mixture to neutralize the HCl and raise the pH to ~1.5. The mixture was cooled to room temperature and the pH drifted downward as cHPMPC precipitated, but more NaOH (aq) was added to raise the pH to ~3.5. The crude solid cHPMPC was filtered, rinsed with MeOH and air dried.

The isolated crude cHPMPC was re-dissolved in a mixture of MeOH (5.6 mL per gram of **1**) and H_2O (0.32 mL per gram of **1**) at 40°C and filtered to remove any mechanical impurities. The filtration apparatus was rinsed forward with MeOH (0.4 mL per gram of **1**). The combined filtrates were heated to 60°C and HCl (aq, conc., 0.32 g per gram of **1**) was added dropwise to lower the pH to ~1.9 (solution temp. was 62–68°C). The pH drifted upward as cHPMPC started to precipitate at ~65°C. After cooling the mixture to room temperature, additional HCl (aq, conc.) was added to lower the pH to ~3.0. The mixture was stirred gently for one hour before filtering and the solid cHPMPC was rinsed with methanol and acetone and air

dried. The typical yield for this process was 94% of theoretical and purity generally greater than 99% by HPLC analysis (6). Residual solvent levels were generally lower than 0.01% or non-detectable by GC analysis (6).

The use of readily available, inexpensive ethyl chloroformate as a dehydrative intramolecular cyclization promoter to convert HPMPC (**1**) to cHPMPC (**2**) proved to be facile, cost effective and safe. There were no major side reactions and the by-products: NaCl, EtOH and CO_2 were benign and easily removed. Typically, 100 grams of cHPMPC were synthesized in a one-liter vessel demonstrating the efficiency of this method. This process was used to produce "clinical-grade" cHPMPC drug substance in scales ranging from 100–3000 grams.

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6. HPLC and GC analysis were performed at Gilead Sciences, Analytical Chemistry Department by Mark Kenney and Theresa Lynch.

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