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Synthesis of gemcitabine triphosphate (dFdCTP) as a tris(triethylammonium) salt

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Abstract—First synthesis of gemcitabine triphosphate (dFdCTP) as a tris(triethylammonium) salt is reported.
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Gemcitabine (2′, 2′-difluoro-2′-deoxycytidine) is registered as a clinical anti-cancer agent for the treatment of a number of solid tumor types including pancreatic, non-small cell lung (NSCL), ovary, bladder, and breast cancer.[1] Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleoside metabolites.[1] The NCI Cancer Imaging Program needed gemcitabine triphosphate (dFdCTP), to be used as a standard in imaging studies using a radiolabeled probe for assessing the uptake and retention of gemcitabine in tumors and potentially identifying tumors sensitive to the drug. To facilitate such biochemical, pharmacological, and other detailed studies of the metabolism of gemcitabine, a steady supply of authentic gemcitabine triphosphate (dFdCTP) was essential.[2]

Here, we wish to report the first synthesis of gemcitabine triphosphate (dFdCTP), as shown in Scheme 1. Although several methods have been reported for the general synthesis of nucleoside triphosphates,[3] many of these approaches did not produce the desired triphosphate (dFdCTP) in our hands. Our synthesis incorporates the method described by Bogachev,[4] using a modified purification procedure to give gemcitabine triphosphate as the tris(triethylammonium) salt.

Gemcitabine hydrochloride was phosphorylated by portionwise addition to a mixture of phosphorus oxychloride ($POCl_3$) in trimethyl phosphate at 5 °C.[5] The reaction was warmed to room temperature and stirred for 2 h. The progress of the reaction was followed by HPLC (small aliquots were worked up by treatment with aqueous $NaHCO_3$). Workup of the reaction afforded monophosphate **2** (dFdCMP), as a white solid (105%). This material was used in the next step without further purification. ^{1}H NMR (500 MHz, CD_3OD): δ 7.95–7.93 (d, 1H); 6.25–6.22 (m, 1H); 6.01 (m, 1H); 4.39–4.32 (m, 1H); 4.25–4.23 (m, 1H); 4.14–4.10 (m, 1H); 4.00–3.98 (m, 1H). An impurity related to trimethyl phosphate is seen at δ 3.57 (s) and 3.55 (s). MS: Electrospray (negative), Calcd for $C_9H_{12}F_2N_3O_7P$ = 343. Found: $(M-H)^-$ 342 (100%). HPLC: Luna C-18 column (4.6 × 150 mm, 5 μ), isocratic 10% MeOH/90% 20 mM NH_4OAc, flow rate: 1 mL/min at 20 °C, detection: UV at 254 nm, results: the compound elutes at 1.837 min with 92.77% area.

Monophosphate **2** (dFdCMP) was treated with trifluoroacetic anhydride in the presence of triethylamine and *N*, *N*-dimethylaniline in acetonitrile at −5 °C. The reaction mixture was warmed to room temperature and stirred for 20 min until the formation of a clear solution indicating the completion of the reaction. This reaction mixture was then evaporated in vacuo to complete dryness (TFA free) to yield crude **3**, which was used in the next step without further purification. Crude **3** was dissolved in acetonitrile containing 1-methylimidazole and triethylamine. The mixture was stirred at 0 °C for 5 min,

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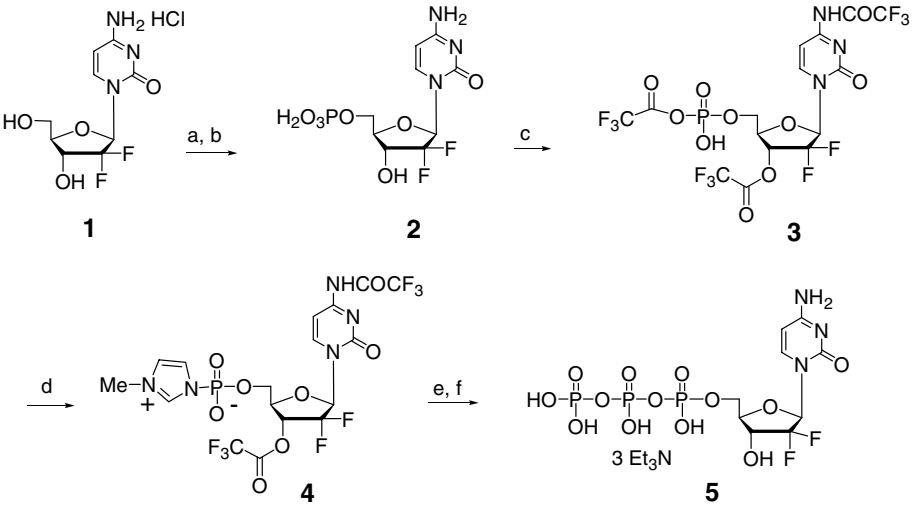

Scheme 1. Reagents and conditions: (a) $POCl_3$, $(MeO)_3PO$; (b) H_2O; (c) $(CF_3CO)_2O$; (d) 1-methylimidazole; (e) $(nBu_4N)_3HP_2O_7$; (f) DEAE Sephadex A25, Et_3NHCO_3 (pH 7.5).

when TLC analysis of an aliquot showed no presence of **3**. The reaction mixture containing **4** was allowed to warm to 20 °C and then was treated with a solution of tris(tetrabutylammonium) hydrogen pyrophosphate in acetonitrile. After stirring for 7–10 min at room temperature, the reaction mixture was quenched by the addition of water. Following an aqueous workup, the crude gemcitabine triphosphate (dFdCTP) was purified by chromatography on DEAE Sephadex A25 (1.6×20 cm bed volume) eluted with deionized water (one column volume), followed by elution with a linear gradient from deionized water (500 mL) to 0.4 M triethylammonium bicarbonate (pH 7.5, 500 mL). Fractions containing pure dFdCTP as a tris(triethylammonium) salt were combined and concentrated in vacuo to give the desired triphosphate **5** (dFdCTP)(140 mg, 17%). The reproducibility of the above procedure was confirmed by repeating the reaction sequence several times.

^{1}H NMR (500 MHz, D_2O): δ 8.17–8.15 (d, 1H); 6.39–6.38 (d, 1H); 6.28–6.26 (m, 1H); 4.66–4.57 (m, 1H); 4.44–4.42 (m, 1H); 4.32–4.25 (m, 2H); 3.23–3.19 (q, 18H); 1.30–1.27 (t, 27H); ^{31}P NMR (202.456 MHz, D_2O): δ −10.35 to −10.54 (m, 1P); −10.94 to −11.03 (d, 1P); −22.79 (m, 1P). HRMS: TOF Electrospray (negative): Calcd for $M-H = C_9H_{13}F_2N_3O_{13}P_3$ = 501.9629, Found: *m/z* (relative intensity) 501.9611 (100%), $(M-H)^-$. HPLC: Bio Basic column and step gradient elution using ammonium acetate buffer and acetonitrile eluted **5**[2] with a retention time of 4.17 min showing 97.03% purity.

Milligram quantities of this material (dFdCTP, NSC 746306) for the use as a standard can be obtained from the NCI (http://dtp.nci.nih.gov).

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Supplementary data

Detailed experimental procedures for the synthesis of compounds **2**, **3**, **4**, and **5** are included in the Supporting Information section of this report. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.063.

References and notes

1. Norenberg, J. P. U.S. Patent 20070025910, 2007; *Chem. Abstr.* **2007**, *146*, 200935.
2. The following reference describes an assay of dFdCTP: Veltkamp, S. A.; Hillebrand, M. J. X.; Rosing, H.; Jansen, R. S.; Wickremsinha, E. R.; Perkins, E. J.; Schellens, J. H. M.; Beijnen, J. H. *J. Mass Spectrom.* **2006**, *41*, 1633, This reference describes obtaining dFdCTP from Eli Lilly and Company; however, the method of synthesis or isolation was not indicated.
3. Burgess, K.; Cook, D. *Chem. Rev.* **2000**, *100*, 2047.
4. Bogachev, V. S. *Russ. J. Bioorg. Chem.* **1996**, *22*, 599.
5. Bonjouklian, R.; Grindey, G. B.; Hertel, L. W. European Patent Application 0376518, 1990; *Chem. Abstr.* **1991**, *114*, 43489.