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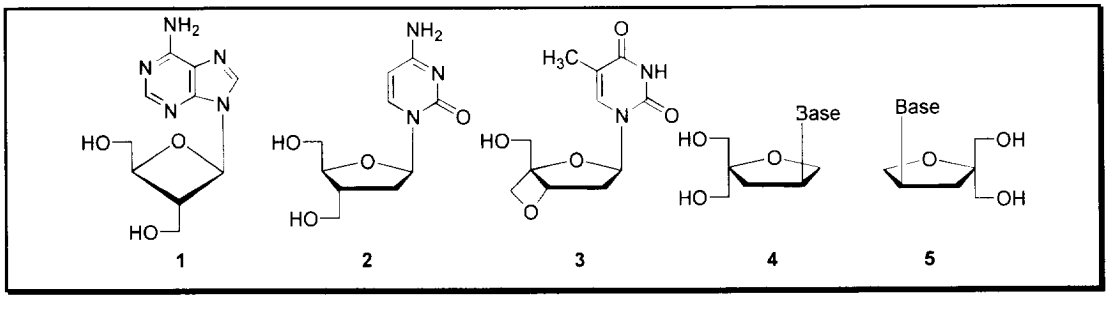
SYNTHESIS AND ANTIVIRAL EVALUATION OF 4'-HYDROXYMETHYL-2', 3'-DIDEOXY-3'-THIANUCLEOSIDES AND THEIR CYCLIC MONOPHOSPHATES

Qi Chao and Vasu Nair*
Department of Chemistry, The University of Iowa, Iowa City, IA 52242

Abstract: Novel 4'-hydroxymethyl-2',3'-dideoxy-3'-thianucleosides and their cyclic monophosphates have been synthesized and evaluated for anti-HIV activity. This is the first report of the use of the phosphorodiamidite methodology to prepare cyclic nucleoside monophosphates. © 1997 Elsevier Science Ltd.

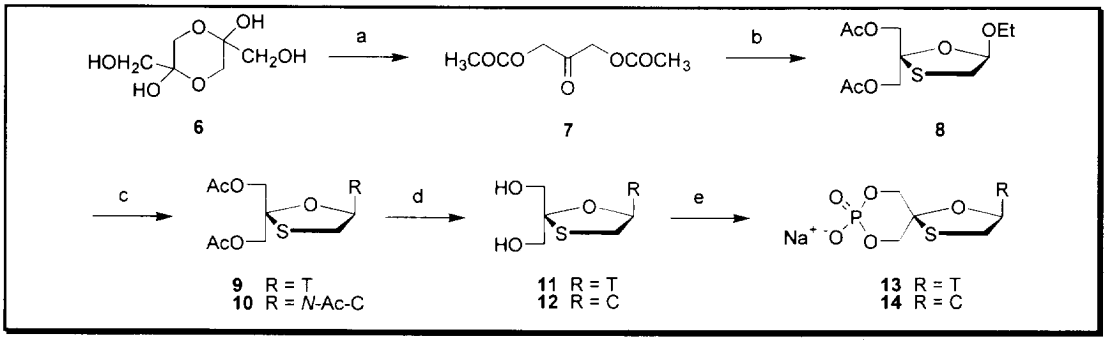
Since the discovery that some dideoxynucleosides possess the ability to inhibit the replication of HIV, a considerable number of modified dideoxynucleoside analogues have been synthesized and evaluated as anti-HIV agents. The mechanism for anti-HIV activity requires that the dideoxynucleosides be phosphorylated by cellular kinases to the corresponding triphosphates which then act as competitive inhibitors of HIV reverse transcriptase and/or chain terminators of viral DNA synthesis.[1-3] Five such nucleoside analogues (AZT, ddI, ddC, d4T, and 3TC) have been approved for the clinical treatment of HIV infections.[1] In these modifications, the carbohydrate ring has been extensively studied in terms of alterations at the 2'- and 3'-positions. Much less attention has been focused on 4'-substituted deoxy- and dideoxynucleosides. However, a few 4'-substituted nucleosides have been found to possess antiviral activity [e.g. anti-HIV data for the 4'-azidothymidine: IC_{50} 0.01 μM and CC_{50} 8 μM in CD-4+ (A 3.01) cell line].[4-6] Also, hydroxymethyl-branched nucleosides, such as oxetanocin A (**1**),[7] its analogue (**2**),[8] and the oxetanyl compound (**3**),[4] have been reported to exhibit potent antiviral activity (e.g. anti-HIV data for compound **2**: IC_{50} 0.01 μM in H9 cells). Regioisomers of 4'-hydroxymethyl dideoxynucleosides (e.g. **4** and **5**), with *S* and *R* absolute stereochemistry, are known.[9] As part of our continuing search for new 4'-substituted dideoxynucleosides with more potent anti-HIV activity, we wish to report on the development of synthetic approaches to novel 4'-hydroxymethylated 2',3'-dideoxy-3'-thianucleosides, their spirocyclic monophosphates, and the anti-HIV studies on these compounds. The rationale for selection of these compounds for study was based in



part on the potent anti-HIV activity of their parent compound, 3TC [e.g., for (±) 3TC: IC_{50} 0.37 μM and CC_{50} 405 μM in MT-4 cells; for (-)3TC: IC_{50} 0.61 μM (MT-4 cells) and CC_{50} 363 μM (CEM cells) and IC_{50} 0.002 μM and CC_{50} >100 μM in PBM cells; for (+) 3TC: IC_{50} 0.21 μM and CC_{50} >100 μM in PBM cells],[10-12] and on the anti-HSV and anti-HCMV activities of 1-[(*S*)-2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine (cyclic HPMPC) and ganciclovir cyclic phosphonate.[13,14]

RESULTS AND DISCUSSION

Our synthetic strategy involved the cyclocondensation of 1,3-diacetoxyacetone (**7**) with 2-mercaptoacetaldehyde diethyl acetal,[15] in the presence of an acid catalyst, to form the 2,2-disubstituted 1,3-oxathiolane derivative (**8**), which is the appropriate nucleoside precursor.[16] However, our initial attempt to produce the starting compound, 1,3-diacetoxyacetone (**7**), from 1,3-dichloroacetone[17,18] by treatment with excess potassium acetate in acetic acid, resulted in two products, 1-acetoxy-3-chloroacetone (major) and **7** (minor). A more efficient method for producing **7** (85% yield) was developed from the 1,3-dihydroxyacetone dimer (**6**) and acetic anhydride in pyridine (Scheme 1). Cyclocondensation was accomplished by treatment of **7** with mercaptoacetaldehyde



Scheme 1. Reagents: (a) Ac_2O, pyridine; (b) $HSCH_2CH(OEt)_2$, *p*-toluenesulfonic acid, benzene; (c) silylated base, TMS triflate, CH_3CN; (d) NH_3/MeOH; (e) (1) 1*H*-tetrazole, 2-cyanoethyl tetraisopropylphosphorodiamidite, CH_3CN; (2) I_2, THF, H_2O, 2,6-lutidine; (3) NH_3/MeOH: (4) Dowex 50W×4–400 (Na^+).

diethyl acetal and *p*-toluenesulfonic acid in benzene to produce the key intermediate **8**, (±)-2,2-*bis*(acetoxymethyl)-5-ethoxyl-1,3-thioxalane (only one enantiomer shown) in 56% isolated yield. The condensation of **8** with silylated thymine in acetonitrile in the presence of trimethylsilyl trifluoromethanesulfonate produced the diacetyl thymidine derivative **9** (56%). Deacetylation of **9** with NH_3/CH_3OH gave the desired target, (±)-3'-deoxy-4'-hydroxymethyl-3'-thiathymidine (**11**, mp 168-169 °C, 92% yield). Treatment of the thymidine derivative **11** with 1 equivalent of

2-cyanoethyl tetraisopropylphosphorodiamidite in the presence of 1*H*-tetrazole in acetonitrile, followed by oxidation with iodine and deprotection with NH_3/CH_3OH, gave the thianucleoside cyclic monophosphate **13** (mp 223-224 °C, 68 % yield for three steps). The thiacytidine **12** and its cyclic monophosphate **14** were prepared by procedures similar to those used for **11** and **13**, except, for solubility reasons, the solvent for the phosphorylation was DMF.[19] Both cyclic nucleotides, **13** and **14**, were converted to their sodium salt and purified by preparative reversed phase HPLC. The ^{13}C and ^{31}P NMR spectra confirmed the cyclic monophosphate structures for both nucleotides and also showed appropriate ^{13}C -^{31}P splitting patterns.[19]

Anti-HIV evaluation of compounds **11–14** in the CEM-SS cell line revealed that they were inactive. Other antiviral studies are in progress.

In summary, methodologies for the synthesis of racemic 4'-hydroxymethylated 2',3'-dideoxy-3'-thianucleosides and their cyclic monophosphates have been developed. The methodology includes the first reported use of phosphorodiamidites for the preparation of nucleoside cyclic monophosphates. Compounds **11–14** did not show any significant activity in an anti-HIV primary screen.

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19. Condensation of compound **8** (520 mg, 2.1 mmol) with 4-*N*-acetylcytosine (TMS derivative) (413 mg, 2.7 mmol) in the presence of TMS triflate in CH_3CN produced 676 mg (84%) of **10.** Deacetylation of **10** (280 mg, 0.78 mmol) with MeOH/NH_3 produced 184 mg (86%) of (±) **12** as white solid: mp 210–212 °C; UV (MeOH) λ_{max} nm 270 (9505); ^{1}H NMR (DMSO-d_6) δ 2.97 (dd, J = 6.1 Hz and 11.6 Hz, 1H), 3.39-3.51 (m, 2H), 3.59-3.75 (m, 3H), 5.13 (dd, J = 5.7 Hz and 6.3 Hz, 1H, exchange with D_2O), 5.19 (t, J = 5.8 Hz, 1H, exchange with D_2O), 5.74 (d, J = 7.5 Hz, 1H), 6.35 (pseudo t, 1H), 7.20 (br, 2H, exchangeable), 7.85 (d, J = 7.5 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 36.1 (CH_2), 64.0 (CH_2), 64.5 (CH_2), 87.2 (CH), 94.1 (CH), 97.3, 140.9 (CH), 154.7, 165.6. Anal. Calcd for $C_9H_{13}N_3O_4S$: C, 41.69; H, 5.05; N, 16.21. Found: C, 41.57; H, 4.77; N, 15.93.

Phosphorylation of compound **12** (100 mg, 0.38 mmol) was carried out by treatment first with 1*H*-tetrazole (80 mg, 1.4 mmol) and 2-cyanoethyl tetraisopropylphosphorodiamidite (114 mg, 0.38 mmol) in dry DMF (5 mL) for 2 h followed by oxidation with (I_2:H_2O:THF: 2,6-lutidine: 0.5 g:5 mL:10 mL:5 mL). The reaction mixture was worked up and chromatographed on silica gel plates (30% MeOH/$CHCl_3$). The isolated phosphate was treated (in 10 mL MeOH) with NH_3 /MeOH solution (4 mL) for 1 h and then worked up. The crude product (in H_2O) was passed through a Dowex 50W×4-400 (Na^+) column (H_2O as eluant). The product was further purified by HPLC on an Amberlite XAD-4 column (H_2O as eluant) to give (±) **14** as a white solid (32% overall yield): mp >230 °C; UV (MeOH) λ_{max} 270 nm (10193); ^{1}H NMR (D_2O) δ 3.26 (dd, J = 6.6 Hz and 11.8 Hz, 1H), 3.57 (dd, J = 4.9 Hz and 11.8 Hz, 1H), 4.30-4.40 (m, 3H), 4.50 (dd, J = 4.6 Hz and 12.4 Hz, 1H), 6.06 (d, J = 7.6 Hz, 1H), 6.50 (pseudo t, 1H), 7.85 (d, J = 7.6 Hz, 1H); ^{13}C NMR (D_2O) δ 37.5 (CH_2), 74.5 (d, J = 5.5 Hz, CH_2), 75.4 (d, J = 5.5 Hz, CH_2), 89.2 (d, J = 4.9 Hz), 90.6 (CH), 99.3 (CH), 143.9, 159.9, 168.9. ^{31}P NMR (D_2O) δ -3.93. Anal. calcd for $C_9H_{11}N_3NaO_6PS$: C, 31.49; H, 3.23; N, 12.24. Found: C, 31.13; H, 3.05; N, 11.78.

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