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EVIDENCE SUPPORTING THE ACTIVITY OF 2'-*C*-CYANO-2'-DEOXY-1-β-D-*arabino*-PENTAFURANOSYLCYTOSINE AS A TERMINATOR IN ENZYMATIC DNA-CHAIN ELONGATION

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Abstract: To investigate the stability of 2'-*C*-cyano-2'-deoxy-1-β-D-*arabino*-pentafuranosylcytosine 3'-phosphoric acid, its thymidine ester was prepared via the phosphoramidite method using allyl protection for the phosphate function. This ester is stable under acidic conditions but extremely labile under basic conditions, decomposing with a cleavage of the internucleotide bond even at pH 7.3.

2'-*C*-Cyano-2'-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC) is an antineoplastic nucleoside showing inhibitory actions in human tumor cells in vitro and antitumor effects on mouse leukemia P388 cells in vivo.[1] A mechanism, which is shown in Scheme I,[1b,c] has been proposed to explain the biological activity of CNDAC: when CNDAC is incorporated into a DNA chain by ligation with DNA polymerase in vivo to form a nucleotide containing a CNDAC 3'-phosphate linkage, the resulting nucleotide undergoes a β-elimination resulting in DNA-strand cleavage. Namely, CNDAC is considered to act as a chain terminator arresting DNA chain ligation. In order to confirm this proposed mechanism, preparation of CNDAC 3'-phosphate derivatives[1b] has been attempted thus far by existing methods using protectors such as 2-cyanoethyl and benzoyl for the phosphate moiety and the NH_2 group of the nucleoside base, respectively. Preparation of the derivatives, however, has not yet been successful, because basic treatments are necessary to remove the protecting groups, which in turn results in a decomposition of the target products. This paper describes the first preparation of a CNDAC 3'-phosphate derivative **1** as well as its stability.

Since CNDAC 3'-phosphates are predicted to be unstable under basic conditions, the choice of protecting groups are important in their synthesis. Accordingly we employed acetyl[1a] and allyl[2] protecting groups for the nucleoside base and the phosphate, respectively, which are removable by treatment with non-basic reagents such as diluted hydrochloric acid or an organopalladium complex. The condensation of **3**[1b] and the thymidine 5'-phosphoramidite **4**[3] assisted by 1*H*-tetrazole[4] in the presence of molecular sieves 3A in acetonitrile (5 min) followed by oxidation with *tert*-butyl hydrogen peroxide (TBHP)[5] or with bis(trimethylsilyl)peroxide (TMSOOTMS)[6] in the presence of trimethylsilyl triflate as a catalyst[5] (3 min) afforded the phosphotriester **5** (96% overall yield), showing ^{31}P NMR signals at –1.61 and –1.90 ppm (H_3PO_4 standard). In this process, when iodine in aqueous pyridine was employed for the oxidation, **5** was

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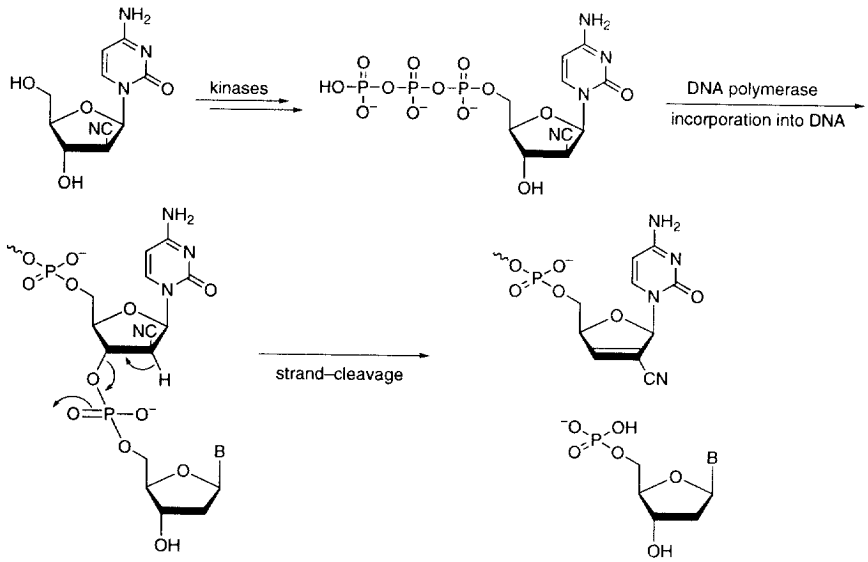
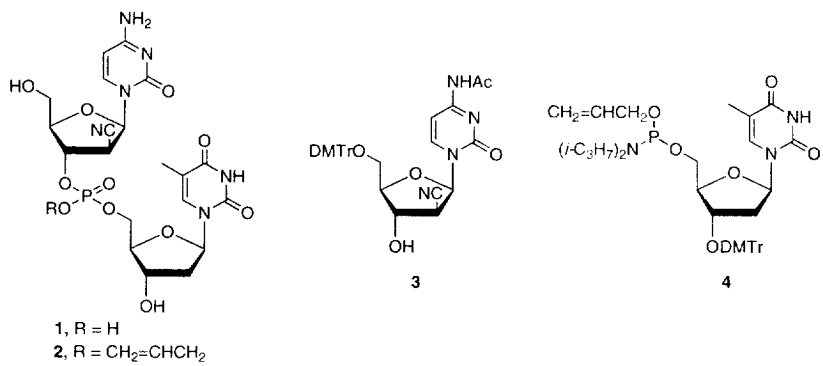


Scheme I. A possible pathway of the cleavage of CNDAC-incorporated DNA.

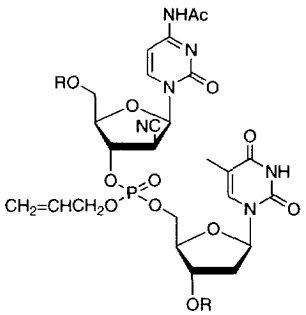
decomposed to a considerable extent under such basic conditions to afford a mixture of **7** and **9**. Subsequently, **5** was treated with 90% aqueous acetic acid (25 °C, 5 h)[1b] to form **6** (75% yield), which was converted to **2** by the reaction with a 1% HCl/methanol solution (25 °C, 1 h).[1a] Finally, the allyl protecting group was eliminated by treatment with a 1.0 equiv[7] of $Pd_2[(C_6H_5CH{=}CH)_2CO]{\cdot}CHCl_3$[8] in a 1:1 mixture of methanol and THF to give the target nucleotide **1** (almost quantitative yield from **6**). The product **1** showed a ^{31}P NMR signal at δ –1.09 ppm (D_2O) consistent with the phosphodiester. In the ^1H NMR spectrum in D_2O, **1** indicated signals due to 2'- and 3'-methine protons of the CNDAC sugar moiety at 4.0–4.1 and 4.84 ppm, confirming the existence of the CNDAC 3'-phosphate linkage. Further, the structure was confirmed by the ^{31}P–^1H COSY NMR spectrum shown in Figure 1, which exhibited only two cross peaks at δ –1.09/4.04 and –1.09/4.81 ppm due to the coupling of the phosphorus atom and the 5'-methylene protons of thymidine and the 3'-methine proton of CNDAC, respectively.



1, R = H
2, R = CH_2=$CHCH_2$

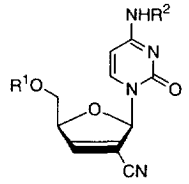
3

4

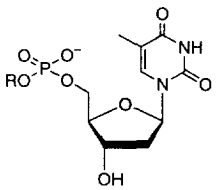


5, R = DMTr
6, R = H

DMTr = $C_6H_5(p\text{-}CH_3OC_6H_4)_2C$



7, R^1 = DMTr; R^2 = Ac
8, R^1 = R^2 = H



9, R = H
10, R = CH_2=$CHCH_2$

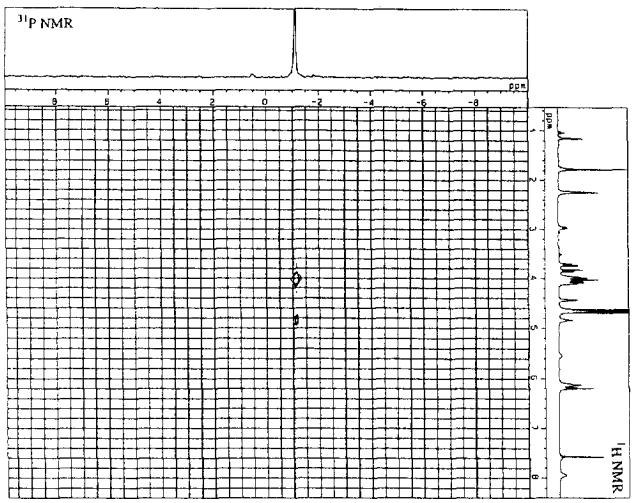


Figure 1. The ^{31}P-^{1}H COSY NMR spectrum of **1**.

The stability of **1** in aqueous solutions of various pHs at 25 °C was investigated by monitoring the ^{31}P NMR spectra. Figure 2 shows the results. The nucleotide **1** was quite stable in acidic media of pH <4, undergoing no decomposition for 24 h. On the other hand, **1** was labile in basic solutions and decomposed to give a mixture of several products, including primarily 2'-*C*-cyano-2',3'-didehydro-2',3'-dideoxycytosine (**8**)[1b,9] and thymidine 5'-monophosphate (**9**). For example, **1** was smoothly decomposed and disappeared within 60 min at 25 °C in a solution of pH 8.8 (enzymatic DNA ligation conditions).[1c] The decomposition was completed in solutions of pH 8.4 and 7.3 for 2 and 8 h, respectively.

We prepared thymidine CNDAC 3'-phosphate via the phosphoramidite approach, using acetyl and allyl groups for the protection of the cytosine base and the phosphate linkage, respectively. As expected, this ester is extremely unstable in basic media, including conditions for the enzymatic production of DNA. This result strongly supports the hypothetical mechanism of the self-strand cleavage of CNDAC-incorporated DNA shown in Scheme I. In addition, the present work has demonstrated the utility of allyl protection for the synthesis of such a base-labile nucleotide.

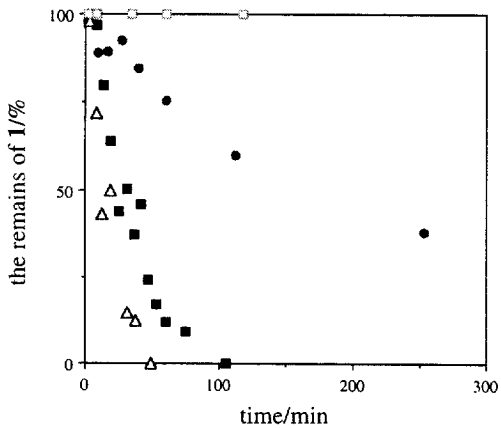


Figure 2. Profiles of the decomposition of **1** in aqueous solution of various pHs at 25 °C. △, pH 8.8; ■, pH 8.4; ●, pH 7.3; □, pH 4.0.

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(3) This amidite was prepared by the reaction of 3'-*O*-(*p*,*p*'-dimethoxytrityl)thymidine and $(CH_2{=}CHCH_2O)P[N(i\text{-}C_3H_7)2]2$ by the aid of 1*H*-tetrazole. See, Hayakawa, Y.; Harada, H.; Hirose, M.; Noyori, R.; Wakabayashi, S.; Miyazaki, K.; Kawase, Y.; Kato, I. *Nucleic Acids Res. Symp. Ser.* **1991**, *25*, 63.

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(7) Deallylation with a catalytic amount of the Pd(0) complex in the presence of a nucleophile such as diethylammonium hydrogencarbonate resulted in the failure. In this case, the undesired β-elimination of the internucleotide linkage took place to give a mixture of **8** and allyl thymidine 5'-phosphate (**10**).

(8) Ukai, T.; Kawazura, H.; Ishii, Y. *J. Orgmetal. Chem.* **1974**, *65*, 253. This compound is commercially available from Aldrich.

(9) The product **8** also decomposed under the basic conditions to produce several compounds.