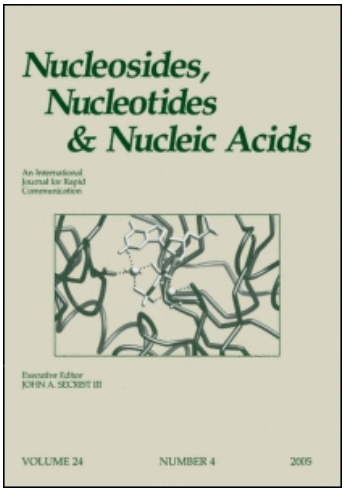

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

SYNTHESIS AND BIOLOGICAL ACTIVITY OF BRANCHED CHAIN-SUGAR NUCLEOSIDES[1]

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To cite this Article Ueda, Tohru , Matsuda, Akira , Yoshimura, Yuichi and Takenuki, Kenji(1989) 'SYNTHESIS AND BIOLOGICAL ACTIVITY OF BRANCHED CHAIN-SUGAR NUCLEOSIDES[1]', Nucleosides, Nucleotides and Nucleic Acids, 8: 5, 743 — 752
To link to this Article: DOI: 10.1080/07328318908054212
URL: http://dx.doi.org/10.1080/07328318908054212

NUCLEOSIDES & NUCLEOTIDES, 8(5&6), 743-752 (1989)

SYNTHESIS AND BIOLOGICAL ACTIVITY OF BRANCHED CHAIN-SUGAR NUCLEOSIDES[1]

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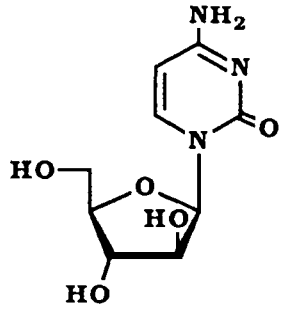
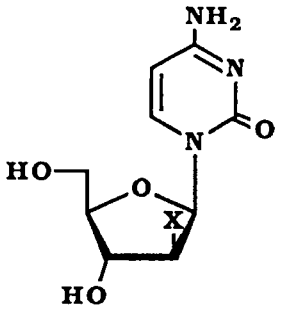
Abstract: 2'-Alkyl derivatives of cytidine and thymidine have been synthesized. 2'-Deoxy-6,2'-methanocytidine has also been prepared. Among them, 2'-deoxy-2'-methylidene-cytidine exhibited potent antitumor activities.

For the design of antitumor or antiviral nucleosides, manipulation of the 2'-substitution of 2'-deoxyribonucleosides may be important. As a best example, 1-β-D-arabinofuranosylcytosine (ara-C) is one of the most potent drugs for the treatment of acute human leukemia.[2] It is an antagonist, as a form of 5'-triphosphate, of dCTP incorporation into DNA by DNA polymerase thus inhibiting DNA synthesis. One can postulate that the presence of the 2'-hydroxyl group of 2'-deoxycytidine in arabino configuration may be important as a steric effector of the conformation around the glycosylic linkage; the 2'-hydroxyl group would restrict the ara-C conformation in more anti-form.

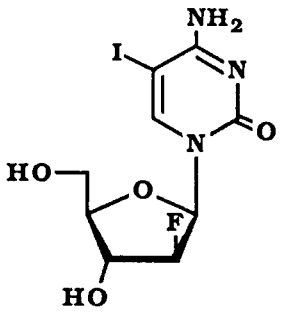
If we assume the 2'-substituents simply acting as the steric effector, one can expect similar activity for the 2'-deoxycytidine compounds having 2'-arabino-substituents of similar size. In fact, 2'-azido and 2'-amino arabino-cytidines were reported to be potent growth inhibitors of leukemic cells.[3] Various potent antiviral nucleosides reported so far also include several arabino-compounds, such as FIAC[4], FMAU[4], and BVAU.[5] (Fig. 1) These compiled results



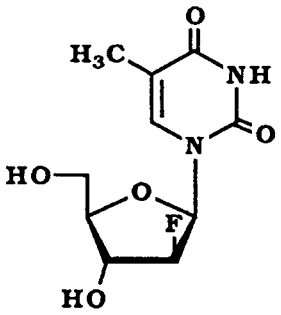
ara-C



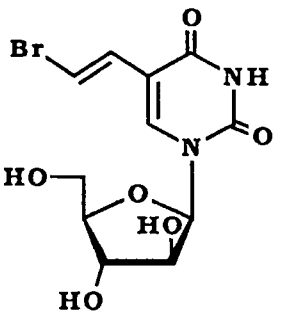
X = N_3, NH_2



FIAC



FMAU



BVAU

Figure 1

would alternatively indicate that the presence of the electronegative groups or atoms at the 2'-arabino position is important. In addition, if the syn-anti conformation of the nucleosides is simply crucial for exhibiting activities, the 2'-deoxyribonucleosides conformationally fixed in the anti-form would be expected to be also active provided that other molecular arrangement for the usual nucleosides is not influenced too much by fixation.

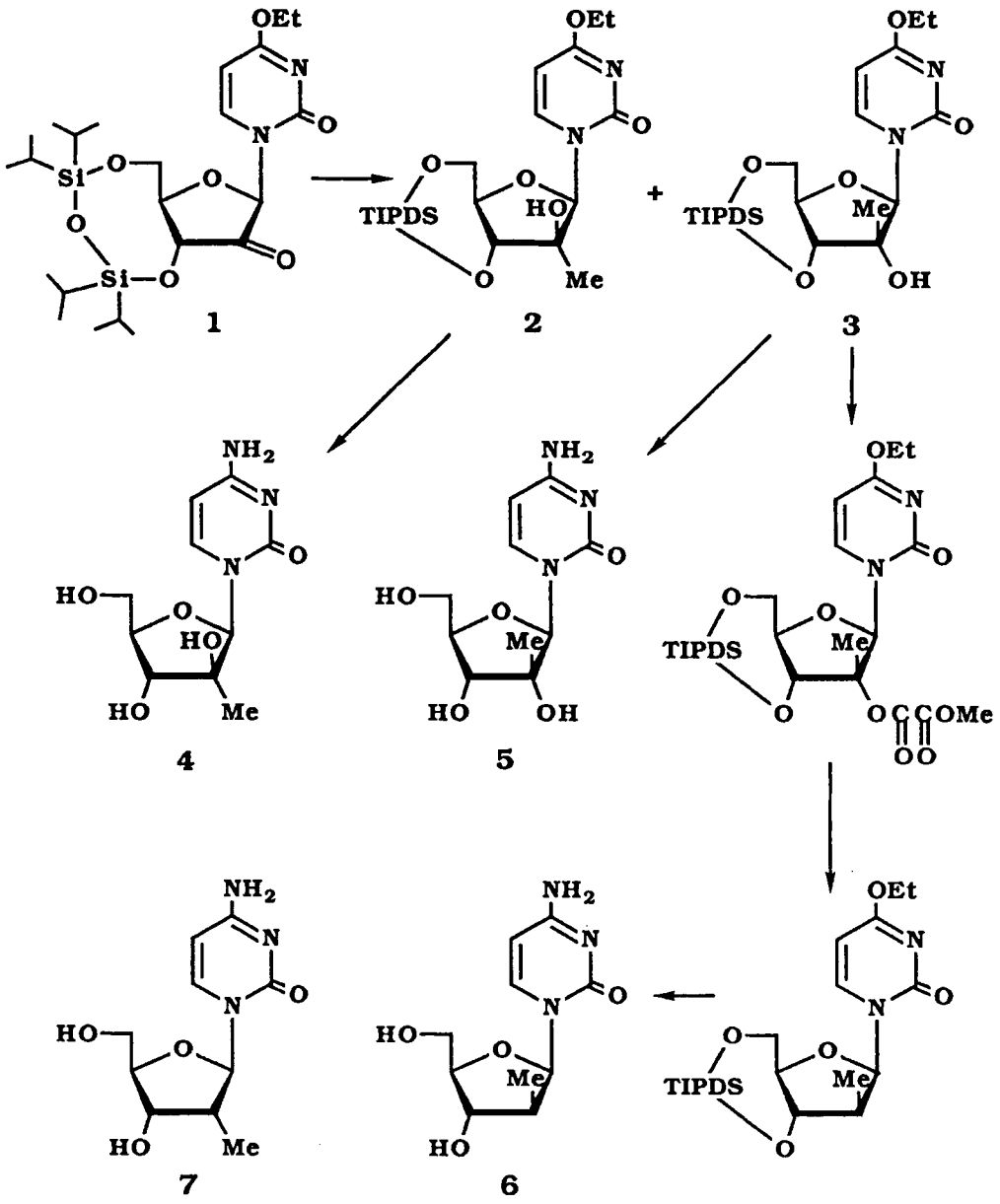
Although ara-C is very active for antileukemic agent, the serious drawback of this drug is that ara-C is very susceptible to hydrolytic deamination by cytidine deaminase to a chemotherapeutically inactive arabinosyluracil. Thus, it may be one reason that ara-C is not effective against solid tumors. Therefore, the modification of the 2'-position should be designed to provide new cytosine nucleosides resistant to cytidine deaminase.

We present here a summary of our recent studies on the synthesis of 2'-branched chain-sugar pyrimidine nucleosides including 2'-carbon-bridged cyclonucleosides.[6]

Synthesis of 2'-Alkyl-2'-deoxycytidines

We have set up the reaction introducing short-chain alkyl groups into the 2'-position of 2'-deoxycytidine. Especially, methyl and ethyl group may be suitable substituents in order to check the above-mentioned hypothesis for the design of ara-C equivalents. For this purpose, alkyl additions of suitably protected 2'-keto pyrimidine nucleosides was examined (Scheme 1). A 2'-keto pyrimidine nucleoside **1**, readily prepared from uridine, was subjected to alkyl addition under various conditions with alkyl lithium, aluminum and magnesium derivatives. While the addition of methyllithium or trimethylaluminum to **1** gave the adduct from the alpha side giving only 2'-methyl-arabinosyl derivative **2**, MeMgBr (3equiv.) gave a diastereomeric mixture of **2** and **3**. We found that the introduction of electron-donating substituent on C-5 of the pyrimidine gave a product ratio in favor of beta-addition. Perhaps the Grignard reagent would involve the 2-keto function in addition to the 2'-keto group for complex formation as the reactive intermediate. De-silylation of **2** and **3** followed by ammonolysis furnished a set of 2'-alkylcytidines **4** and **5**, respectively.[6c]

The deoxygenation of the 2'-position of **3** was next undertaken. Treatment of **3** with methyloxalyl chloride followed by tributyltin hydride reduction afforded the 2'-deoxy derivative in high yield, which was converted to (2'S)-2'-



Scheme 1

deoxy-2'-methylcytidine **6** as a crystalline hydrochloride.[6a] Similar treatment of 2 gave a mixture of 2'R and 2'S-methyl derivative which was separated to furnish the diastereomer **7**. By a similar approach, 2'-ethyl derivatives of cytidine and 2'-methyl derivative of thymidine were likewise prepared.

Synthesis of 2'-Deoxy-6,2'-methanocytidine

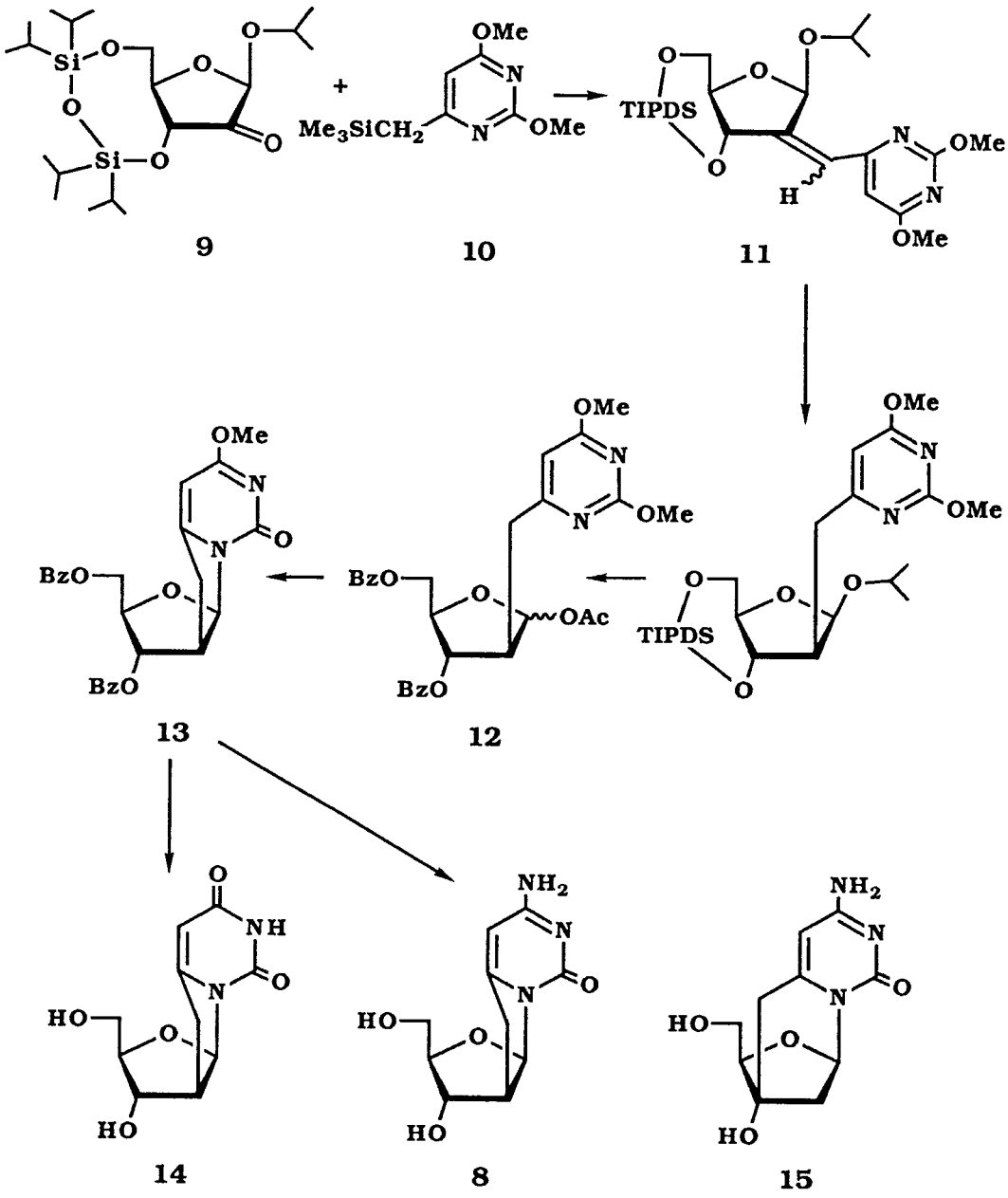
The above-mentioned 2'-methyl derivative **6** has a methyl group in place of 2'-hydroxyl group of ara-C. If we are to fix completely the rotation around glycosyl linkage, the best way is to connect between the C-6 and the methyl group at the 2'-position, that is 2'-deoxy-6,2'-methanocytidine **8** (Scheme 2). We have already synthesized this type of nucleosides from 2'-methylideneuridines by way of intramolecular radical or ionic cyclization of suitable intermediates.[7] We have now explored the third method to reach **8** involving the intramolecular glycosylation.

Isopropyl 3,5-O-silyl-protected β-D-erythro-2-pentulofuranoside **9** was prepared from D-ribose. Condensation of **9** with 2,4-dimethoxy-6-trimethylsilylmethylpyrimidine **10** in the presence of BuLi in THF gave the 2-benzylidene derivative **11** in high yield. Hydrogenolysis of **11** followed by transformation of the protecting group provided the 1-O-acetyl-2-deoxy-2-(6-pyrimidinylmethyl)ribose derivative **12**. Intramolecular ribosylation of **12** in the presence of stannic chloride afforded the 6,2'-methano derivative **13**. Hydrolysis or ammonolysis of **13** furnished 2'-deoxy-6,2'-methanouridine (**14**) or 2'-deoxy-6,2'-methanocytidine (**8**), respectively. The physical properties of the former was identical with those of the authentic specimen.[7a]

The regio-isomer of **8**, 2'-deoxy-6,3'-methanocytidine **15**, has already been prepared in our laboratory by a similar approach.[8]

Synthesis of 2'-Deoxy-2'-methylidenecytidine and Its Derivatives

The 2'-substituents so far reported occupy either ribo or arabino configuration. However, there is a third choice



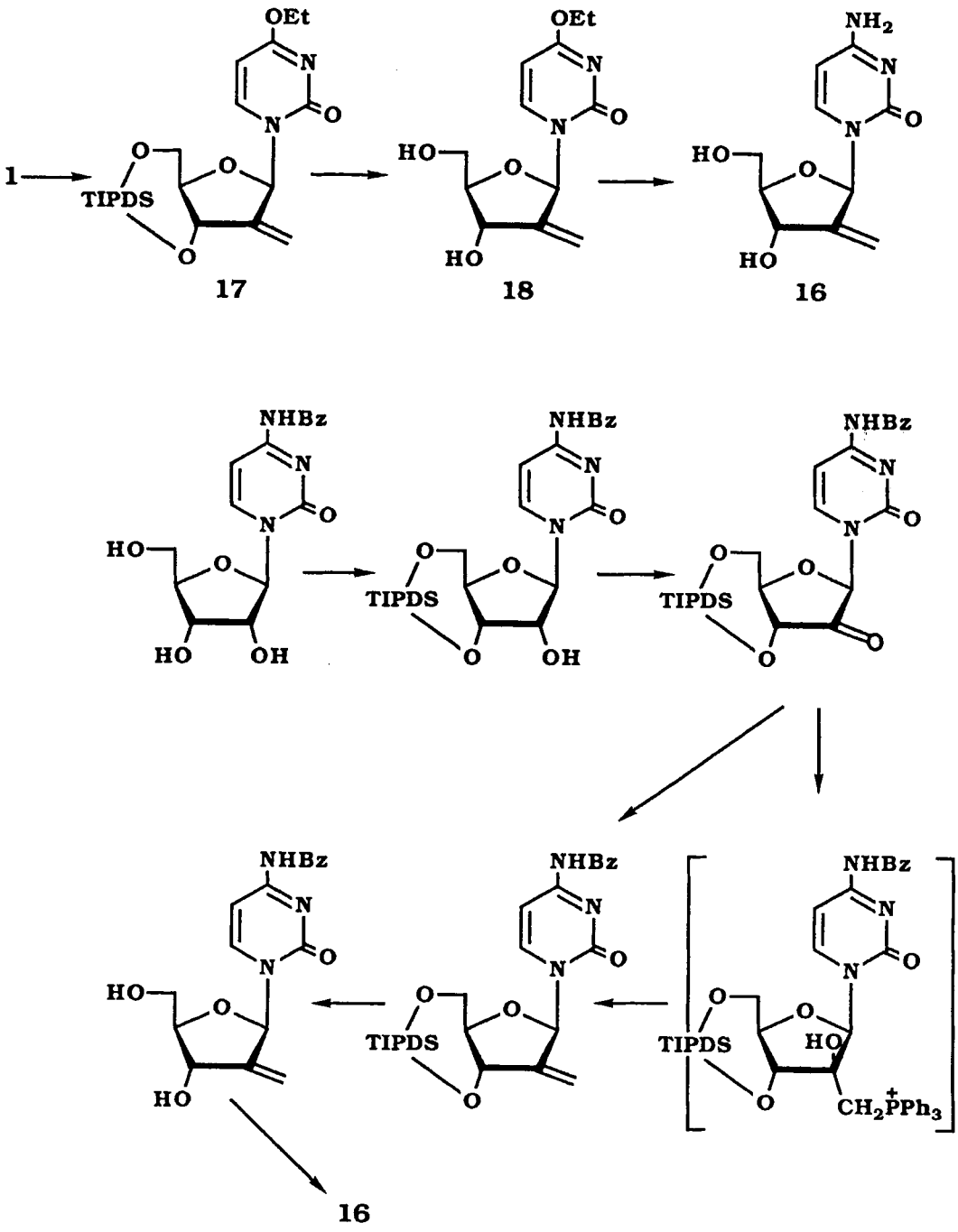
Scheme 2

of substitution at the 2'-position which occupies in-plane orientation. Such substitution can be achieved by introduction of the methylidene group at the 2'-position. We have already synthesized this type of compounds in pyrimidine and purine nucleosides, such as 2'-deoxy-2'-methylidene-uridine[7b] (and -adenosine[9]), for the purpose of construction of C-cyclo linkage from them. However, no significant biological activities have so far been detected with these compounds. Nevertheless, it is well expected that the introduction of the 2'-methylidene group in nucleosides should alter the nature of the 3'-hydroxyl group. Thus, the 3'-hydroxyl group would now be of an allyl alcohol. If this nucleoside is incorporated in polynucleotides, the nature of the internucleotidic linkages involving the allyl alcohol in one part would be remarkably unstable toward various nucleophiles which makes the polynucleotides very fragile.

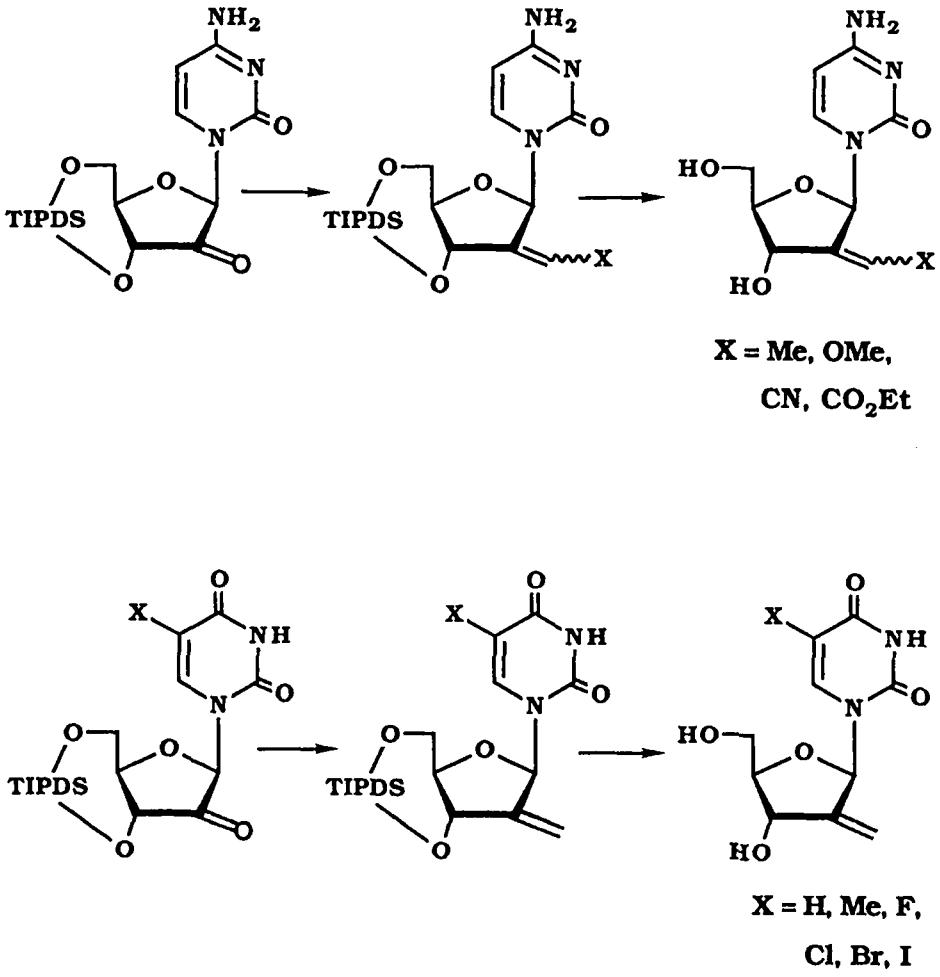
Therefore, we set up the synthesis of 2'-deoxy-2'-methylidenecytidine (**16**) and its derivatives (Scheme 3). Treatment of **1** with methylenetriphenylphosphorane in DMF gave the 2'-methylidene derivative (**17**), which was sugar-deprotected to give **18**, then ammonolyzed to furnish **16** as a crystalline hydrochloride. The same compound was readily prepared from cytidine by the similar route shown in Scheme 3. For comparison, various 2'-(substituted methylidene) derivatives of cytidine have been prepared (Scheme 4). The 2'-methylidene derivatives of 5-substituted 2'-deoxyuridine were also prepared (Scheme 4).

Biological Properties of 2'-Alkyl and 2'-Alkylidene Nucleosides

Among 2'-methyl and 2'-ethyl derivatives of cytidine and thymidine, (2'S)-2'-deoxy-2'-methylcytidine (**6**) with a methyl function in arabino configuration showed strong *in vitro* cytotoxicity (IC_{50}, 0.26 μg/ml) for L1210 cells like ara-C. However, it was not active against solid tumors again like ara-C. The result of antitumor activity of C-cyclocytidines (**14** and **15**) was rather disappointing. On the other



Scheme 3



Scheme 4

hand, 2'-deoxy-2'-methylidenecytidine (16) showed remarkably potent growth inhibitory activity for leukemic cells (IC_{50}, 0.11 μg/ml) as well as several solid tumor cells (for example, IC_{50}, 3.8 μg/ml for SW480). Other 2'-methylidene derivatives including that of 5-fluorouracil or that having substituent at the methylidene group showed weak or no activities.

It should be worth to note that **16** was resistant to deamination by cytidine deaminase unlike ara-C. The molecular structure of **16** in solid state by X-ray analysis showed that it retains a similar overall conformation to that of 2'-deoxycytidine having anti-form with g,g-orientation of the 4'-hydroxymethyl group.

A preliminary result on the in vitro antiherpes test showed that 2'-methylidenethymidine exhibited potent activity for HSV-1 so did the 5-iodo derivative.

In conclusion, 2'-deoxy-2'-methylidenecytidine (**16**) may have a new mechanistic mode of exhibiting biological activities and it seems to be a nucleoside valid for further investigation as a new antitumor agent.

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