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Studies on the syntheses and biological activities of isonucleosides

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Abstract: Isonucleosides were synthesized by the reaction of suger epoxide and nucleobase in the presence of K_2CO_3 and crown ether. The substitution is regioselective. Some of isonucleoside derivatives showed significant activities of cytotoxcity in HL-60 cells.Oligodeoxynucleotides incorporated with isonucleoside have a increase in stability towardes nuclease S1.

A number of analogues of nucleosides have been found to possess anti-cancer and antiviral activities.[1-4] Isonucleoside is a new class of nucleoside analogues in which the nucleobase is linked to the position of ribose other than $C_{1'}$. Therefore isonucleoside attracted much attention owing to their chemical and enzymatic stability and potential antiviral activities[5]. A series of isomeric of 2',3'-dideoxy-nucleosides which contain a modified carbohydrate moiety have been synthesized and some of the compounds exhibited significant and selective anti-HIV activity[6,7]. New regioisomer of AZT, AZU, BVDU and IDU have also been investigated[8]. Many isonucleosides syntheses have made use of epoxide opening by the azide anion, subsequent reduction furnishes the amine which is used to build up the heterocyclic moiety. An alternative synthesis involved the nucleophilic substitution of leaving group in the sugar ring by heterocyclic moiety under basic condition. In order to avoid the lengthy synthetic routes, we synthesized the isonucleosides using epoxide opening by the nucleobase itself in the basic condition.[9] The desired epoxide can be obtained from corresponding sugar.

3-(S)-Hydroxy-4-(S)-O-tosyl-5-(S)-dimethoxymethyl-tetrahydrofuran 2 prepared from 1',2'-O-isopropylidene-α-D-xylose 1 in very good yield was treated with potassium carbonate in methanol at room temperature to yield 95% of 3,4-epoxy-5-(S-trans)-dimethoxymethyl-tetrahydrofuran 3. The substituted nucleobase was found by the reaction of the epoxide 3 with corresponding nucleobase in the presence of potassium tert-butoxide and crown ether. Due to the bulky dimethoxymethyl group at C-5', the substitution of epoxide 3 is regioselective, two regioisomers 4 and 5 were obtained and separated with 4 as the main product(4:5=18:1). The structure of compounds 4 and 5 were identified by ^{1}H NMR COSY and NOESY spectra. The dimethoxymethyl group in 4 was hydrolyzed in 3% TFA at 80°C and reduced by $NaBH_4$ at room temperature to give compound 6 in good yield. Same strategy could be used for the synthesis of compound 10 in which the configuration of sugar ring was inverted. Intermediate epoxide 7 was obtained from L-xylose in 91.3% yield by same procedure. Due to the steric effect of the dimethylacetal group at C-5, nucleobase favors to attack at C-2 position of epoxide 7, 9 and 8 were obtained in the ratio of 26 to 1. The structure of 10a was identified as the enantiomer of 6a (6a: $[\alpha]_D^{30}$ = +37.1(c: 0.24,CH_3OH), 10a: $[\alpha]_D^{30}$ = −37.3(c: 0.22, CH_3OH)).

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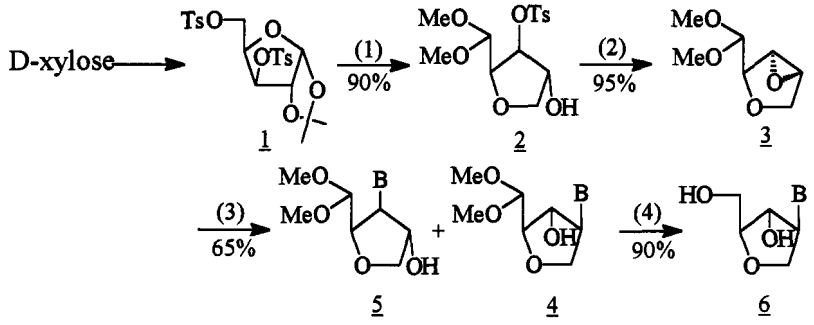
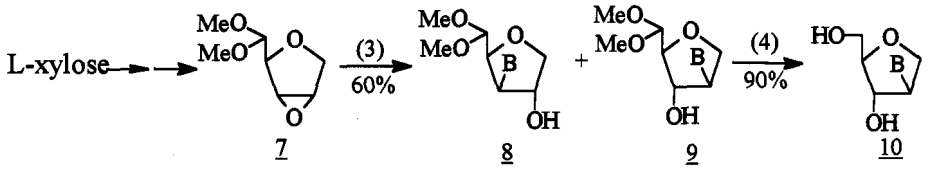


B=a: adenine; b: uracil; c: thymine; d: cytosine;
e: hypoxanthine; f: 8-Cl-adenine; g: 5-I-uracil



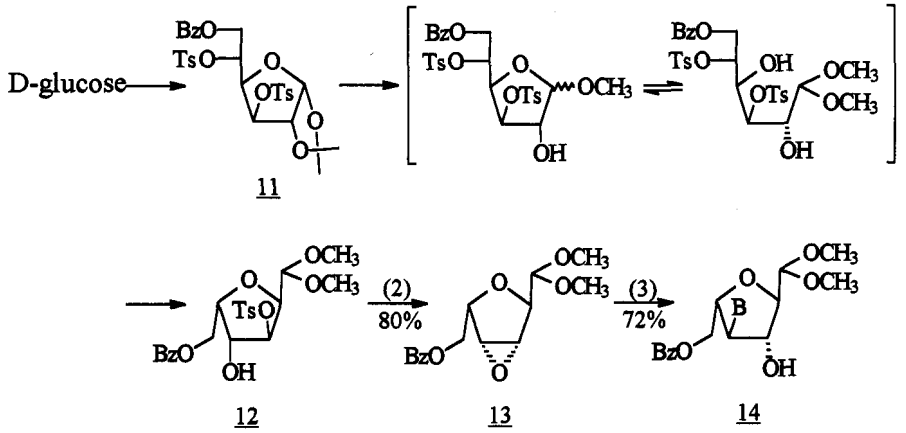
B=a: adenine; b: uracil; c: thymine

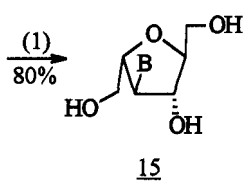
(1) TsCl, 20°C, 4days; 1%TFA, 75°C, 8h (2) K_2CO_3/CH_3OH

(3) Nucleobase/tBuOK/18-crown-6/DMF, 80 °C, 30min

(4) 0.3%TFA, 80°C, 3h; $NaBH_4$, r.t., 40min

One further structural modification was made possible by the availability of tosylhexose. Protected D-glucose 11 was treated by TFA in methanol to give a mixture of methylglucoside which was cyclized to give the desired intermediate 12. Epoxide 13 was formed from 12 in 80% yield. A regioselective epoxide opening took place in the presence of K_2CO_3 and crown ether to yield 14 in 72%. The corresponding isonucleosides 15 were obtained from 14. Another example of such a ring-opening of epoxide has been accomplished. As is predictable from the presence of tosyl group in the moleculer, three new isonucleoside derivatives 17, 18, 19 were obtained from epoxide 16. The mechanism for the formation of 19 was suggested.





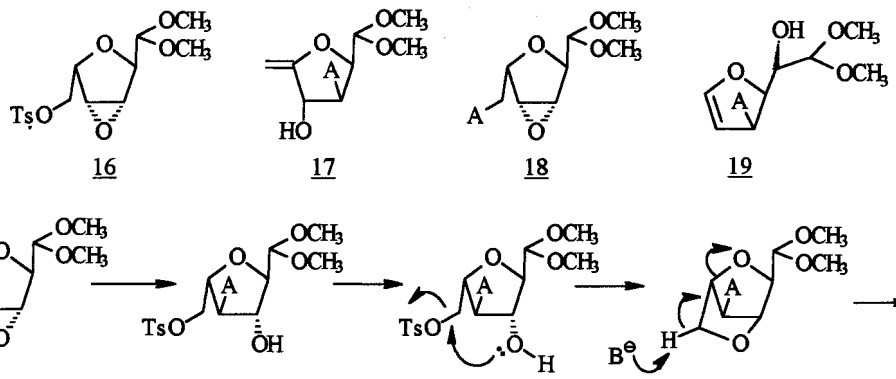
B= a: adenine; b: uracil; c: thymine

(1) 1% TFA/MeOH, reflux

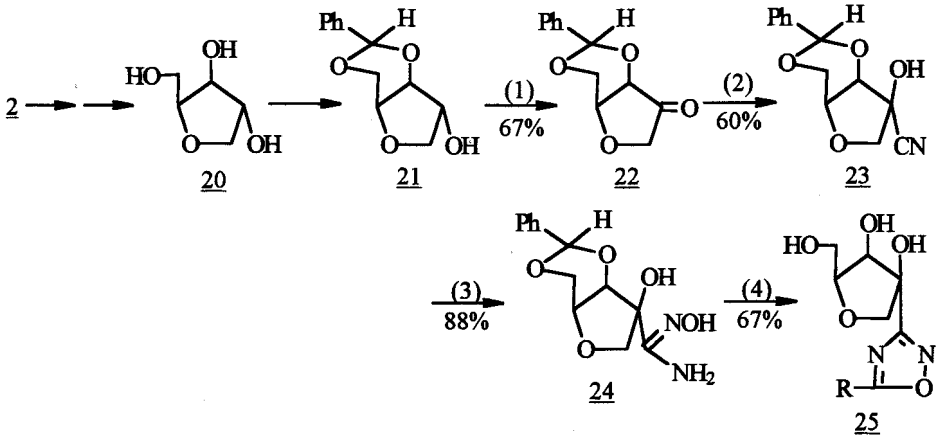
(2) K_2CO_3 /MeOH

(3) K_2CO_3 /18-crown-6/DMF/nucleobase, 85 °C

(4) 0.3% TFA/H_2O; $NaBH_4$



An attempt was made to modify the structure of hydroxytetrahydrofuran derivative 20, thus introducing the cyanide and providing compound 25, a new derivative of isonucleoside in which heterocyclic moiety is linked with sugar by C-C bond. Therefor, protected hydroxytetrahydrofuran 21 was oxidized by Jones reagent to give 22 in 67% yield. The cyanide group was introduced into the tetrahydrofuran ring by the nucleophilic addition of carbonyl group in 22. Cyanide anion attacked the carbonyl group at the direction trans to the benzylidene group. Intermediate 24 can be cyclized with acid anhydride to give the isonucleoside 25.



(1) CrO_3 / Py / Ac_2O / CH_2Cl_2, r.t.
(2) KCN / EtOAc / H_2O, r.t.
(3) NH_2OH / CH_3OH, 80°C
(4) $(RCO)_2O$, $CHCl_3$, 70°C; 80% AcOH, 70°C

The isonucleosides were evaluated in vitro for cytotoxicity in HL-60 cells and inhibitory effect on HSV-1 and HSV-2. Some of isonucleosides showed significant activities of cytotoxicity in HL-60 cells. In order to investigate the relationship between the biological activities and structure of isonucleosides, the conformations of 6 in solution have been studied by ^1H NMR spectroscopy. Our result indicated that all the compounds adopted predominantly the $C_{2'\text{-endo}}/C_{3'\text{-exo}}$ conformations. It seems that the cytotoxicity for HL-60 cells is reduced with an increase in the population of $C_{2'\text{-endo}}/C_{3'\text{-exo}}$ conformer.

Recently oligonucleotides incorporated with hexose nucleoside analogues were reported to have a significant increase in stability towards phosphodiesterases and also retained hybridization properties[10]. By means of computer molecular modeling, the interactions between trinucleotide incorporated with isonucleosides and normal trimer was studied. It was found that the plane of base-pairing remained parallel and the hydrogen bonds had no significant changes. But a great change has been observed in the

torsion angles in sugar phosphate backbond of the oligomer incorporated with isonucleoside. These alterations in torsion angles might effect the recognition of endonuclase to the oligodeoxynucleotide. It would be interesting to study the synthesis and characters of oligodeoxynucleotide incorporated with isonucleoside.

Therefore, trimer d(TTT*) 26 and d(TT*T) 27 (T*=6c)were synthesized via phosphotriester method in solution. 26(^{31}P NMR(D_2O,δ):-0.62,-1.07ppm,FABMS$^-$:849(M-H)), 27(^{31}P NMR (D_2O, δ): -0.69, -0.99ppm, FABMS$^-$:849 (M-H)). The stabilities of 26 and 27 against Nuclease S1 at 37°C, 30min were determined. The percentage of remaining trimer was analyzed by HPLC. It was found that 97.3% of 27 had no change and 28% of 26 was degraded, whereas 55% of normal trithymidine diphosphate was hydrolyzed in the same condition. These characters prompt us for further research on the application of isodeoxynucleoside in antisense oligodeoxynucleotide. Heptamer d(T*T*T*T*T*T*T*C) 28 and d(T*TTTTT*C) 29 were obtained by solid synthesis. The phosphorylated isonucleoside was synthesized by general procedure and the yields of coupling reaction are quantitative.

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