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A Stereospecific Synthesis of 2',3'-Dideoxy-β-L-cytidine (β-L-ddC), A Potent Inhibitor Against Human Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV)

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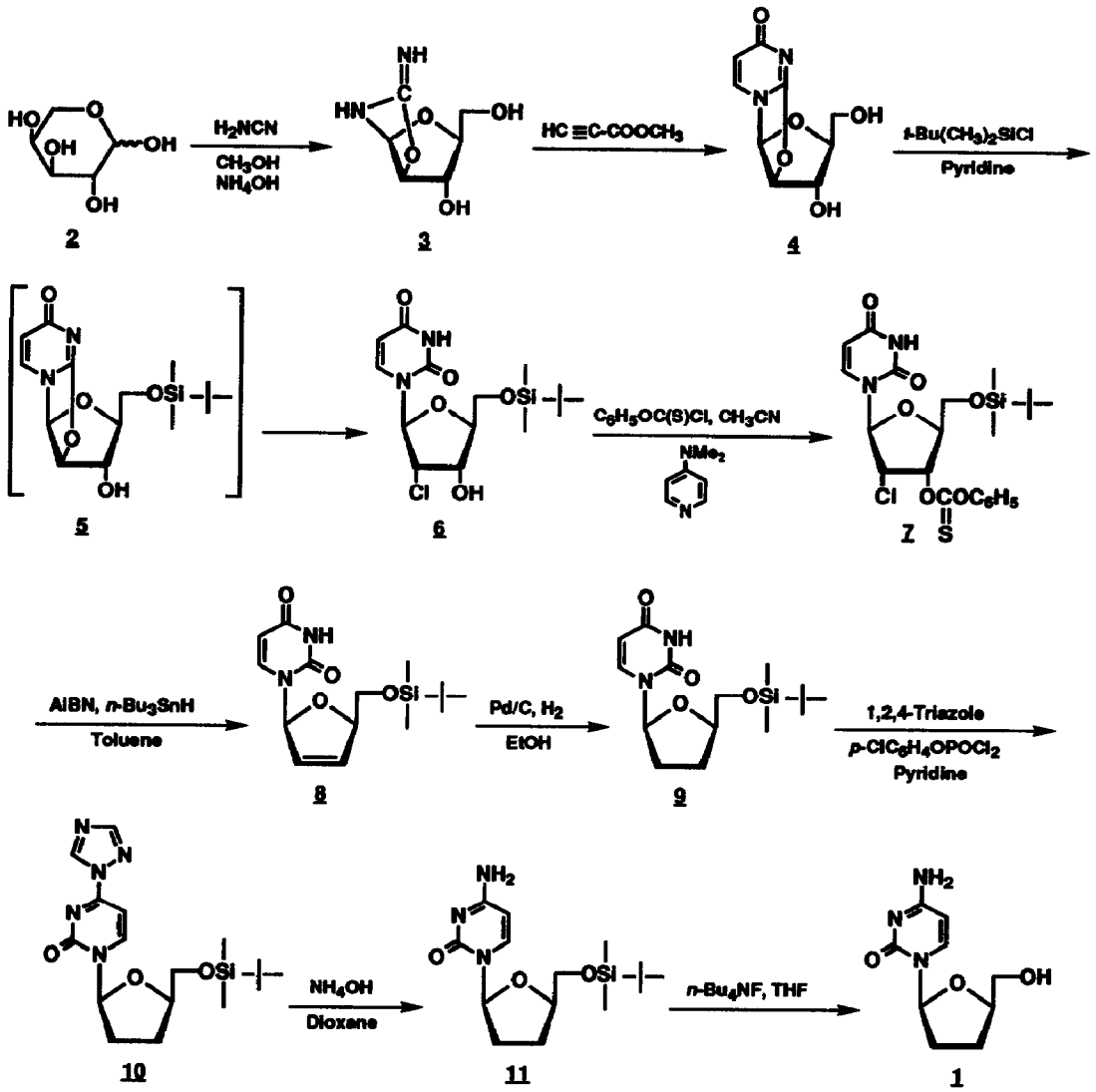
Abstract: 2',3'-Dideoxy-β-L-cytidine (β-L-ddC), a potent inhibitor against human hepatitis B virus (HBV) and human immunodeficiency virus (HIV), has been stereospecifically synthesized from L-arabinose in 9 steps.

Recently, 2',3'-dideoxy-β-D-cytidine (ddC) has been shown to be a potent inhibitor of the replication of both human immunodeficiency virus (HIV)[1] and human hepatitis B virus (HBV)[2,3] in vitro. However, long-term usage of ddC causes delayed toxicity such as peripheral neuropathy in patients, which was suggested to be as a result of the depletion of mitochondrial DNA (mt DNA) in cells treated with ddC.[4] L-Nucleosides, the enantiomers of natural D-nucleosides, were believed not to be recognized by normal cellular enzymes and, therefore, are not or very poorly metabolized in the host animals.[5] However, it was found that L-ribonucleoside 5'-diphosphates interacted with bacterial polynucleotide phosphorylase and nucleolytic enzymes.[6,7] Recently, Spadari et al.[8] reported that L-thymidine is not recognized by human thymidine kinase, but functions as specific substrates for the herpes simplex virus type 1 (HSV-1) viral enzyme and demonstrates anti-HSV-1 activity in HeLa cells. Based on these findings, various 2',3'-dideoxy-L-nucleoside analogues were synthesized and evaluated as potential anti-HIV and anti-HBV agents in our laboratory. Among these compounds, 2',3'-dideoxy-β-L-cytidine (**1**, β-L-ddC) was found to be 280 times more potent than 2',3'-dideoxy-β-D-cytidine against HBV, with respective ED_{50} values of 0.01 and 2.8 μM, and negligible inhibition to the host mitochondrial DNA synthesis.[9,10]

Since the coupling reaction of 1-*O*-acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-L-ribofuranose and silylated cytosine,[9-11] a key step in the synthesis of β-L-ddC, produced a mixture of α and β anomers, thereby reducing the yield of the desired β-anomer which was further diminished when isolated by repeated chromatography on a silica gel column, an efficient methodology for the synthesis of β-L-ddC was highly desirable. In this report, a stereospecific approach for the synthesis of β-L-ddC is described (Scheme I).

Scheme I


H2NCN
CH3OH
NH4OH
HC≡C-COOCH3
t-Bu(CH3)2SiCl
Pyridine
C6H5OC(S)Cl, CH3CN
AIBN, n-Bu3SnH
Toluene
Pd/C, H2
EtOH
1,2,4-Triazole
p-ClC6H4OPOCl2
Pyridine
NH4OH
Dioxane
n-Bu4NF, THF
2
3
4
5
6
7
8
9
10
11
1

2,2'-Anhydro-β-L-uridine (**4**), which has been previously reported by Holy[12] was selected as a logical precursor for the synthesis of β-L-ddC. Treatment of L-arabinose (**2**) with cyanamide in aqueous methanolic ammonia gave 2'-amino-1,2-oxazoline (**3**), which upon reaction with methylpropiolate afforded the anhydro derivative **4**.[12] Reaction of compound **4** with *tert*-butyldimethylsilyl chloride in anhydrous pyridine at room temperature for 3 days resulted in the regioselective formation of derivative **5**. However, when the reaction was allowed to proceed under reflux for an additional 2 h, the 2'-chloro derivative **6** was produced in 86% yield, via a back-side nucleophilic attack on the 2'-position of compound **5** by the chloride ion formed during the silylation reaction. Treatment of compound **6**[13] with phenyl chlorothionocarbonate and 4-dimethylaminopyridine in acetonitrile under nitrogen at room temperature yielded the 2'-chloro-3'-*O*-phenoxythiocarbonyl derivative **7**. Reduction of compound **7** with tri-*n*-butyltin hydride and azobisisobutyronitrile (AIBN) in dry toluene at 110 °C gave the 2',3'-unsaturated nucleoside **8**,[14] which upon reduction under 50 psi of hydrogen in the presence of 10% palladium on powdered carbon afforded 5'-protected 2',3'-dideoxy uridine derivative **9**.[15] Treatment of compound **9** with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in anhydrous pyridine at room temperature yielded the 4-triazolylpyrimidinone derivative **10**. Subsequent treatment of compound **10** with a mixture of ammonium hydroxide/dioxane (2:1, v/v) gave the 5'-protected 2',3'-dideoxy cytidine derivative **11**,[16] which was then deblocked by reaction with tetra-*n*-butylammonium fluoride in THF to afford the target compound 2',3'-dideoxy-β-L-cytidine (**1**, β-L-ddC).[17]

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13) Compound **6**: as a foam; ^{1}H NMR ($CDCl_3$) δ 0.15 (s, 6 H, $SiMe_2$), 0.95 (s, 9 H, $SiCMe_3$), 3.03-3.15 (br s, 1 H, 3'-OH, D_2O exchangeable), 3.90-4.00 (d, 2 H, 5'-H), 4.15-4.25 (m, 1 H, 4'-H), 4.30-4.40 (m, 2 H, 2'-H and 3'-H), 5.70 (d, 1 H, 5-H), 6.15-6.20 (m, 1 H, 1'-H), 7.90 (d, 1 H, 6-H), 9.50 (br s, 1 H, 3-NH, D_2O exchangeable).

14) Compound **8**: yield: 62% as a foam; ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $SiMe_2$), 0.95 (s, 9 H, $SiCMe_3$), 3.90- 3.95 (d, 2 H, 5'-H), 4.90-5.00 (m, 1 H, 4'-H), 5.70 (d, 1 H, 5-H), 5.80-5.90 (d, 1 H, 2'-H), 6.25-6.36 (d, 1 H, 3'-H), 7.05-7.10 (m, 1 H, 1'-H), 7.80 (d, 1 H, 6-H), 9.55 (br s, 1 H, 3-NH, D_2O exchangeable).

15) Compound **9**: yield: 91% as a white foam; TLC, R_f 0.50 (CH_2Cl_2/EtOAc, 1:1, v/v); ^{1}H NMR ($CDCl_3$) δ 0.08 [s, 6 H, $Si(CH_3)_2$], 0.95 [s, 9 H, $SiC(CH_3)_3$], 1.90-2.22 (m, 4 H, 2'-H and 3'-H), 3.70 (m, 1 H, 5'-H_A), 4.07 (m, 1 H, 5'-H_B), 4.10 (m, 1 H, 4'-H), 5.65 (d, 1 H, 5-H), 6.10 (m, 1 H, 1'-H), 8.05 (d, 1 H, 6-H), 9.45 (br s, 1 H, 3-NH, D_2O exchangeable).

16) Compound **11**: yield: 52% (based on compound **9**) as a white foam; ^{1}H NMR ($CDCl_3$) δ 0.10 [s, 6 H, $Si(CH_3)_2$], 0.90 [s, 9 H, $SiC(CH_3)_3$], 1.80-2.60 (m, 4 H, 2'-H and 3'-H), 3.60-4.00 (m, 2 H, 5'-H), 4.05-4.15 (m, 1 H, 4'-H), 5.65 (d, 1 H, 5-H), 6.05 (m, 1 H, 1'-H), 7.70-8.00 (br s, 1 H, 4-NH_2, D_2O exchangeable), 8.05 (d, 1 H, 6-H).

17) Compound **1**: yield: 80% as white solid; mp 194-196 °C; TLC, R_f 0.23 (EtOAc/EtOH, 2:1, v/v); $[\alpha]_D$ -90.3° (*c* = 0.14, MeOH); UV (MeOH) λ_{max} 270 nm (ε 6979), λ_{min} 248 nm; UV (0.01 N HCl) λ_{max} 282 nm (ε 11965), λ_{min} 242 nm; UV (0.01 N NaOH) λ_{max} 273 nm (ε 8340), λ_{min} 250 nm; ^{1}H NMR (Me_2SO-d_6) δ 1.74-2.24 (m, 4 H, 2'-H and 3'-H), 3.49-3.65 (m, 2 H, 5'-H), 3.98-4.04 (m, 1 H, 4'-H), 4.96-5.00 (t, 1 H, 5'-OH, D_2O exchangeable), 5.67 (d, 1 H, 5-H, *J* = 7.4 Hz), 5.91 (m, 1 H, 1'-H), 7.01-7.06 (br s, 2 H, 4-NH_2, D_2O exchangeable), 7.87-7.90 (d, 1 H, 6-H, *J* = 7.4 Hz).

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