Synthesis of enantiomerically pure (2′R,5′S)-(-)-1-[2-(hydroxymethyl)oxathiolan-5-yl]cytosine as a potent antiviral agent against Hepatitis B Virus (HBV) and Human Immunodeficiency...

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2, the best-fit trimer model fits well across the entire concentration range. In contrast, the best-fit dimer and tetramer models deviate substantially from the experimental data. Combined, these data suggest that 1 strongly aggregates in solution and that the aggregation state is that of cyclic trimer 2.

The trimerization constant, $K_3 = 20\,000\ M^{-2}$, obtained from the Saunders-Hyne analysis of 1, is very large, particularly given that quinolone 7 and isoquinolone 8 negligibly associate in 10% DMSO-d_6/$CDCl_3$.[18] For comparison, phenol is believed to form a cyclic hydrogen-bonded trimer in carbon tetrachloride with $K_3 = 4.78\ M^{-2}$.[17a] The robustness of the cyclic trimer under conditions where the individual binding contacts are weak provides strong support for the hydrogen bond mediated cyclic aggregation strategy. Efforts are underway to generate higher order cyclic aggregates and to stack these disk-shaped assemblies into tubes.

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(18) The equilibrium constant K_3 in the Saunders-Hyne analysis is the equilibrium constant for formation of the trimer from three monomers, not the equilibrium constant for addition of a third monomer onto an existing dimer.

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Synthesis of Enantiomerically Pure (2′*R*,5′*S*)-(−)-1-[2-(Hydroxymethyl)oxathiolan-5-yl]cytosine as a Potent Antiviral Agent against Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV)

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Summary: The synthesis of the enantiomerically pure 2′*R*,5′*S*-(−) form of BCH-189 from L-gulose has been accomplished, and this isomer has been found to exhibit the most potent anti-HIV and anti-HBV activities among the four possible isomers. This is the first example of an "L-like" nucleoside which is more potent than its "D-like" isomer.

BCH-189[2] 1 is an interesting nucleoside which exhibits a potent anti-HIV activity in vitro and is undergoing clinical trials in patients with AIDS and AIDS related complex. It is a member of an unusual class of nucleosides in which the 3′-CH_2 group has been replaced by a heteroatom such as sulfur[2-4] or oxygen.[2,5,6]

Since the β-D-isomers of nucleosides are in general the biologically active isomers, we have recently synthesized the 2′*S*,5′*R*-(+) 2 and 2′*S*,5′*S*-(−) 4 isomers of BCH-189 (Table I) from 1,6-thioanhydro-D-mannose[3a] and more recently from 1,6-thioanhydro-D-galactose.[3b] Surprisingly, it was found that the "D-like" isomer 2 was less potent than racemic BCH-189 1 in human peripheral blood mononuclear (PBM) cells. Furthermore, compound 2 was also less potent against hepatitis B virus (HBV) than the racemic BCH-189 1. Thus, it was of great interest to synthesize the antipodes ("L-like" isomers) and compare their activities to those of the compounds 2 and 4. Doong et al.[7] reported that racemic BCH-189 was a potent anti-HBV compound in 2.2.15 cells (clonal cells derived from HepG2 cells transfected with a plasmid containing HBV DNA that secrete hepatitis B virons). It was therefore important to determine if this unexpected result also applied to HBV. We now wish to report the enantiomeric synthesis of the (2′*R*,5′*S*)-(−)-BCH-189 3 and its α isomer, (2′*R*,5′*R*)-(+) 5 and their anti-HBV and anti-HIV[8] activities.

Since we had successfully utilized D-mannose as the starting material for the key intermediate, 1,6-thioanhydro-D-mannose in the synthesis of 2′*S*,5′*R*-(+)-BCH-189 2,[3a] L-mannose was considered as a carbohydrate template for the 2′*R*-isomers. However, it was found that L-mannose is too expensive to use as the starting sugar for a practical synthesis of the 2′*R*-isomers. In search of a more useful L-series sugar, it was determined that L-gulose (6) would serve well as a starting material. L-Gulose (6) can easily be prepared from the commercially available

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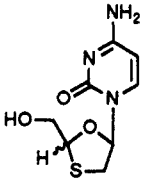
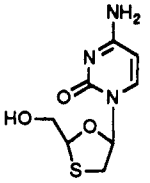
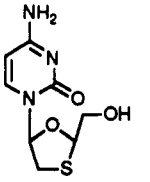
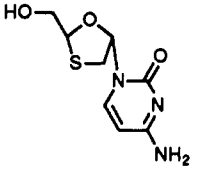
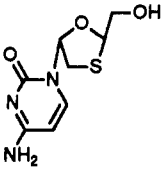
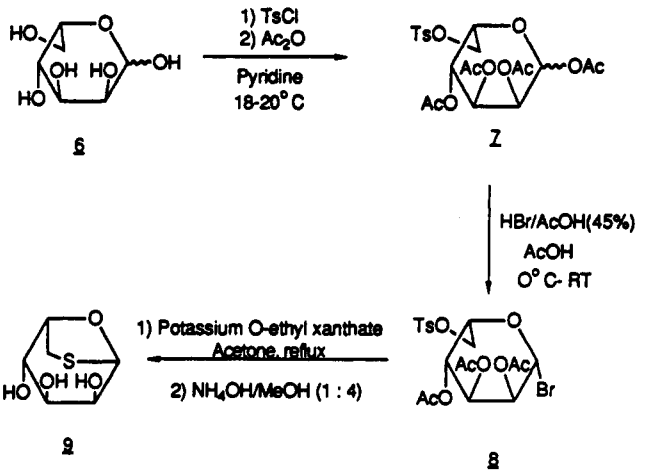
Table I. Optical Rotations and Anti-HBV Activity of Oxathiolane Nucleosides

	compds		optical rotation $[\alpha]^{25}_D$	anti-HBV activity[a] EC_{50}	cytotoxicity in CEM cells IC_{50}
	(±)-2′*RS*,5′*RS*	1		0.05 μM ref 7	20–40 μM ref 7
	(+)-2′*S*,5′*R*	2	+120.96° (*c* 1.04, MeOH)	0.5 μM	2 μM
	(−)-2′*R*,5′*S*	3	−121.6° (*c* 1.1, MeOH)	0.01 μM	>50 μM
	(−)-2′*S*,5′*S*	4	−143.2° (*c* 0.62, MeOH)	>5 μM	>100 μM
	(+)-2′*R*,5′*R*	5	+146.6° (*c* 0.55, MeOH)	>5 μM	nd

[a] Mean of triplicate assays, standard deviation <10%.

Scheme I



L-gulono-γ-lactone[9] which in turn can be prepared from L-ascorbic acid[10] or D-glucurono-6,3-lactone.[11]

Synthesis of the key intermediate, 1,6-thioanhydro-L-gulose (**9**) was accomplished in four steps from L-gulose (**6**) (Scheme I). Selective 6-O-tosylation followed by acetylation gave 1,2,3,4-tetra-*O*-acetyl-6-*O*-tosyl-L-gulose (**7**) as a foam in 96.7% yield. Treatment of **7** with 3 molar equiv of HBr/AcOH (45%, w/v) using acetic acid as solvent gave the bromo sugar **8** (99%). Reaction of the bromo sugar **8** with 3.3 molar equiv of potassium *O*-ethylxanthate in refluxing acetone[12] gave 2,3,4-tri-*O*-acetyl-1,6-thioanhydro-L-gulose, which was not isolated, but was deacetylated using NH_4OH/MeOH to give 1,6-thioanhydro-L-gulose[13] (**9**) (72% from **8**) as a crystalline solid after silica gel column chromatography. In contrast to the synthesis of the 2′*S*,5′*R*-(+) isomer from 1,6-thioanhydro-D-mannose in which a number of protection and deprotection steps were used prior to cleavage of the 2,3-cis diol with $Pb(OAc)_4$, it was found that direct cleavage of the cis diol of the unprotected 1,6-thioanhydro-L-gulose **9** could be effected using $NaIO_4$ and controlled conditions (Scheme II). Thus, treatment of **9** with $NaIO_4$ (1.4 equiv) at −10 °C to cleave the 2,3-cis diol followed by reduction of the resulting aldehyde with $NaBH_4$ and protection of

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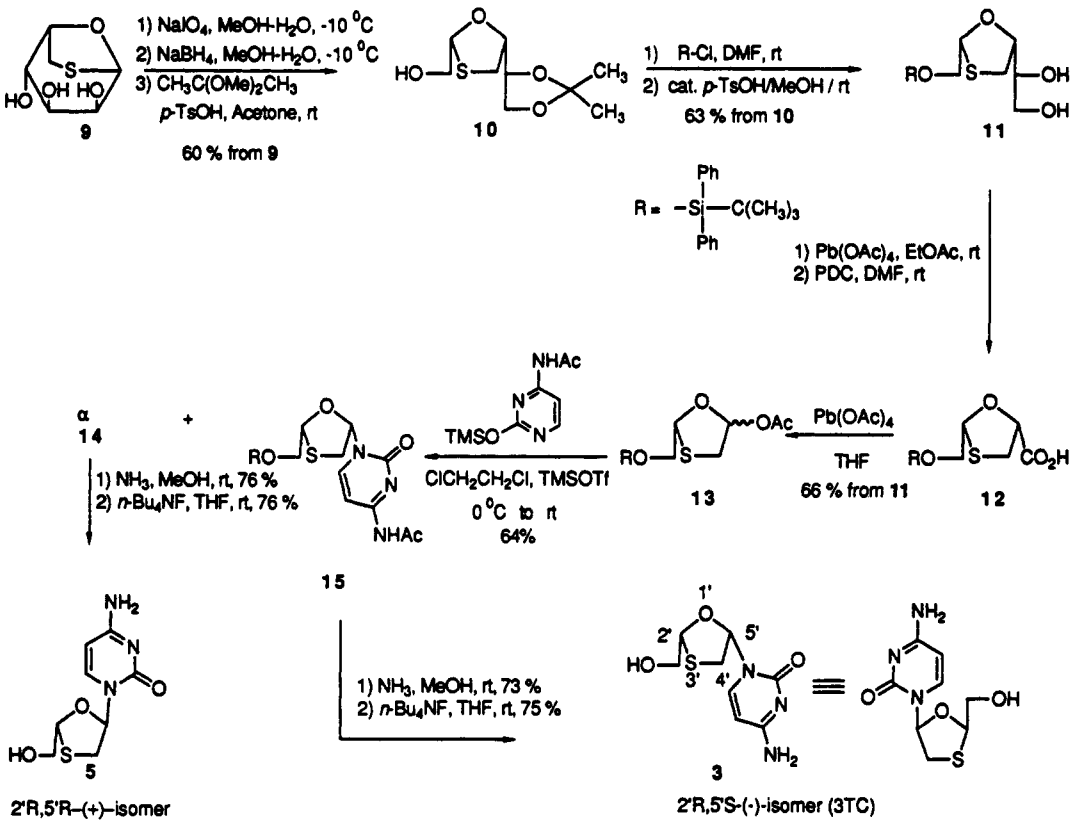
(13) Compound **9**: mp 142–144 °C (2-propanol); NMR (DMSO-d_6) δ 2.8 (dd, J = 6.6 and 10.1 Hz, 1 H, 6-H_a), 3.1 (d, J = 10.1 Hz, 1 H, 6-H_b), 3.4–3.8 (complex multip, 3 H, 2-, 3-, and 4-H), 4.5 (d, J = 5 Hz, 1 H, OH), 4.55 (dd, J = 5.94 and 6.37 Hz, 1 H, 5-H), 4.82 (d, J = 4.6 Hz, 1 H, OH), 5.1 (d, J = 4.4 Hz, 1 H, OH), 5.35 (d, J = 2.2 Hz, 1 H, 1-H); $[\alpha]^{25}_D$ −79.2° (*c* 1.06, MeOH). Anal. Calcd for $C_6H_{10}O_4S$: C, 40.4; H, 5.66; S, 17.99. Found: C, 40.42; H, 5.67; S, 17.89.

Scheme II



the vicinal diol as its isopropylidine derivative gave **10** (60%). Silylation of the remaining primary alcohol followed by treatment with catalytic *p*-toluenesulfonic acid in MeOH at room temperature gave protected diol **11**. Oxidative cleavage of the diol **11** with $Pb(OAc)_4$ followed by further oxidation with pyridinium dichromate (PDC) in DMF[14] gave the acid **12** without oxidization of the sulfur atom to the sulfoxide. Oxidative decarboxylation of **12** using $Pb(OAc)_4$/pyridine[15] in dry THF gave the acetate **13** (66% from **11**). Condensation of **13** with silylated N^4-acetylcytosine in dry 1,2-dichloroethane using trimethylsilyl triflate[16] as the Lewis acid catalyst gave an α,β-mixture (1:2) of **14** and **15** (64%). Separation of the anomers by silica gel chromatography followed by deacetylation using NH_3/MeOH and desilylation using tetra-*n*-butylammonium fluoride gave the final compounds, 2′*R*,5′*R*-(+) isomer **5** and 2′*R*,5′*S*-(−) isomer **3**, respectively.[17]

Recently, it has been reported[18] that the use of stannic chloride in place of TMSOTf as the Lewis acid gives exclusively the β-isomer. The use of this reagent in the condensation of our chiral acetate **13** with silyated N^4-acetylcytosine also gave exclusively the β isomer. Unfortunately, it was found to give a racemic mixture based on optical rotation as well as HPLC analysis using a chiral column.[19]

The anti-HBV activity of compounds **2**–**5** was evaluated in 2.2.15 cells[7] and is summarized in Table I. The results indicate that 2′*R*,5′*S*-isomer **3** was the most potent among the four isomers. As expected, α-isomers **4** and **5** were not as potent as the β-isomers **2** and **3**. In addition, compound **3** was significantly less toxic than compound **2**. The anti-HIV activity of compounds **2**–**5** was evaluated in human peripheral blood mononuclear cells.[8] The results indicated that compound **3** (EC_{50} = 0.0018 μM) was also the most potent isomer (**2**: EC_{50} = 0.21 μM, **1**: EC_{50} = 0.02–0.06 μM). Interestingly, compound **5** (α-isomer of **3**) showed a moderate activity (EC_{50} = 10.1 μM), whereas compound **4** (α-isomer of **2**) was inactive (EC_{50} > 100 μM). Recently, Coates et al.[4] reported the separation of racemic BCH-189 into (+) and (−) isomers by HPLC and their anti-HIV activities in MT4 cells as well as other cell systems. The results indicate that in MT4 cells the (−) isomer (2′*R*,5′*S*) was also more potent than the (+)-isomer (2′*S*,5′*R*). Although both results agree that **3** is more potent than **2**, these results may not be quantitatively compared due to the biochemical differences of the systems which include the levels of kinases.

In summary, we have accomplished the enantiomeric synthesis of (2′*R*,5′*S*)-(−)-BCH-189 from L-gulose via 1,6-thioanhydro-L-gulose and evaluated the anti-HBV and anti-HIV activities, which indicated (2′*R*,5′*S*)-(−)-BCH-189 **3** to be more potent than (2′*S*,5′*R*)-(+)-BCH-189 **2** by at least 1 order of magnitude. The significance of this finding is the fact that this is the first example of an L-like nucleoside found to be more potent than a D-like nucleoside. The cause of this unusual finding as well as the exhaustive structure–activity relationships of pyrimidine and purine oxathiolane nucleosides are in progress in our laboratories.

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