Diastereoselective Synthesis of the Potent Antiviral Agent (−)-2′-Deoxy-3′-thiacytidine and Its Enantiomer

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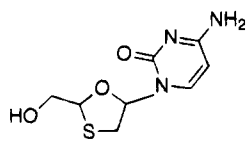
The discovery of the potent anti-human immunodeficiency virus (HIV) properties of 3′-azido-3′-deoxythymidine (AZT) has stimulated intensive efforts in the field of nucleosides and eventually led to the development of other anti-HIV nucleoside therapeutic agents such as 2′,3′-dideoxycytidine (ddC), 2′,3′-dideoxyinosine (ddI), and the recently approved drug 3′-deoxy-2′,3′-didehydrothymidine (d4T). Sometime ago, Soudeyns *et al.* disclosed a structurally novel potent anti-HIV dideoxy nucleoside analog BCH-189[1] (**1**) in which the methylene group at the 3′-position of the ribose ring is replaced by a sulfur atom. Subsequent extensive studies demonstrated that one enantiomer of BCH-189, namely (−)-2′-deoxy-3′-thiacytidine (3TC, lamivudine, **2**), is highly selective in vitro against HIV-1, HIV-2, and hepatitis B virus (HBV).[2,3] Currently, this compound is in the advanced stages of clinical evaluation for the treatment of both diseases. Recent clinical trials also demonstrated that 3TC in combination with AZT has a significant effect in controlling the progression of AIDS in patients.[4] Moreover, a concurrent trial of compound **2** on HBV patients showed nearly complete suppression of viral replication.[5] Critical clinical evaluation of the therapeutic potential of **2** requires that a large amount of material be readily accessible which translates into efficient synthetic protocols for its preparation.



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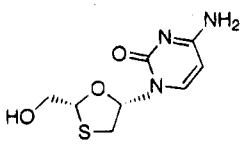
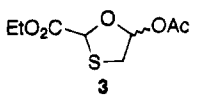


2 3TC



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In an earlier report, Choi *et al.*[6] have described a highly β-selective N-glycosylation procedure which was applied efficiently to the synthesis of BCH-189. This procedure was based on in situ complexation of an oxathiolane sugar moiety with stannic chloride. Subsequent independent attempts by Beach *et al.*[7] and Humber *et al.*[8] to synthesize **2** by exploiting this method led to some unexpected results. Indeed, the key glycosylation step of enantiomerically pure oxathiolane sugar moieties proceeded highly stereoselectively. Nevertheless, the major nucleoside product obtained was found to be optically inactive. Interestingly, replacement of stannic chloride with trimethylsilyl trifluoromethanesulfonate (TMSOTf)[7,9] or iodotrimethylsilane (TMSI)[8] as the Lewis acid promotor afforded a mixture of the cis and trans nucleosides which were optically active. Recently a number of enzymatic processes have been developed to generate 3TC from BCH-189. For example, cytidine deaminase derived from *E. coli* was found to selectively deaminate the (+) enantiomer of **1** to the corresponding uracil nucleoside which was readily separated from **2**.[10] 5′-Nucleotidase isolated from Crotalus atrox venom was utilized for the hydrolysis of the 5′-monophosphate derivative of **1**.[10,11] Futhermore, enantioselective hydrolysis of the 5′-butyrate ester of **1** using lipases has also been reported.[12] In this paper, we wish to report a practical and efficient synthesis of the clinically important nucleoside 3TC (**2**) based on a novel diastereoselective N-glycosylation reaction.

At the planning stage of our synthesis, we recognized that there were two major obstacles that one has to surmount: (1) efficient preparation of the chiral oxathiolane sugar moiety and (2) a stereoselective N-glycosylation process that is compatible with enantiomerically pure substrate. Initially, we focused our effort on developing diastereoselective routes for the preparation of BCH-189 that do not require the use of metal salts. It was then discovered that coupling of an isomeric mixture (trans:cis ~2:1) of the oxathiolane **3** with persilylated cytosine using TMSI as the promotor proceeded stereoselectively to give the cis nucleoside (>15:1 cis:trans). Fractional recrystallization of the crude product mixture provided the major product which was converted to BCH-189 in excellent overall yield by a simple hydride reduction. To investigate the feasibility of adopting this route to synthesize compound **2**, enantiomerically pure oxathi-

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(11) The stereochemistries of compounds **2** and **8** were confirmed by a single crystal X-ray crystallography study performed by the structural chemistry group at Glaxo Group Research, U.K. The data on **2** has been reported in ref 10.

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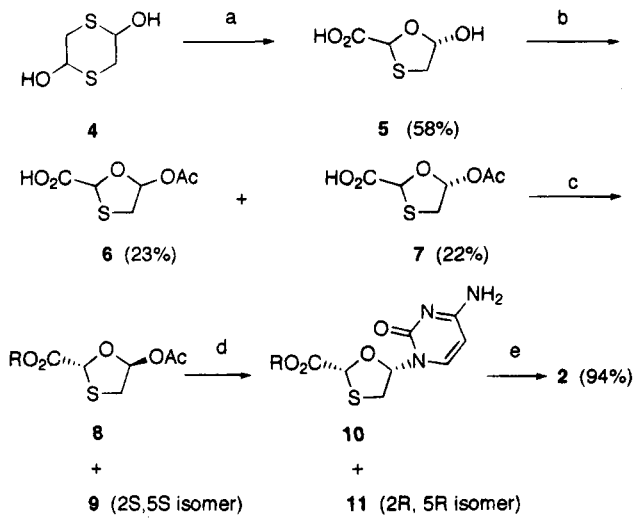
0022-3263/95/1960-2621$09.00/0

Scheme 1[a]



[a] R = (−)-L-menthyl: (a) glyoxylic acid hydrate, *t*-BuOMe, reflux; (b) (i) Ac_2O, $MeSO_3H$; (ii) recrystallization; (c) (i) (−)-L-menthol, DCC, DMAP, CH_2Cl_2; (ii) recrystallization; (d) $(TBDMS)_2$-cytosine, TMSI, CH_2Cl_2; (e) $LiAlH_4$, THF.

olane sugar was required. It was decided that the most straightforward way of generating the optically pure glycosyl donor would be by chemical resolution. After substantial experimentation, a short synthetic sequence was developed for the preparation of the key intermediate **8** (Scheme 1).

Reaction of glyoxylic acid monohydrate and **4** in refluxing *tert*-butyl methyl ether under azeotropic condition gave the trans hydroxy acid **5** in 58% yield. Treatment of **5** with acetic anhydride in the presence of a catalytic amount of methanesulfonic acid provided a 1:2 mixture (^1H NMR) of the *cis*- (**6**) and *trans*-acetoxy acid (**7**) (combined yield 83%). The trans isomer **7** was isolated as white crystals from this mixture by fractional recrystallization from benzene (22% based on a single isomer). After extensive effort, we found that resolution of compound **7** could be achieved readily *via* its menthyl ester derivatives. Reaction of compound **7** with L-menthol afforded a 1:1 diastereomeric mixture of the corresponding esters **8** and **9** in quantitative yield. Spectroscopic and chiral HPLC analyses[13] confirmed the absence of any cis isomer. Low temperature (−78 °C) recrystallization of this mixture from petroleum ether (40−60 °C) containing a minimum amount of diethyl ether provided compound **8** (46% based on one enantiomer). The absolute configuration of **8** [(1′*R*,2′*S*,5′*R*)-menthyl 5(*R*)-acetoxy-1,3-oxathiolane-2(*R*)-carboxylate)] was unequivocally established by single crystal X-ray crystallography.[11] With this compound available, the initial objective of preparing a chiral oxathiolane sugar suitable for the synthesis was accomplished and the stage was set for the crucial glycosylation step. Subjection of the acetoxy ester **8** to reaction with persilylated cytosine in dichloromethane in the presence of TMSI (1.1 equiv) afforded the expected cis and trans nucleosides in 75% yield. Analysis of the coupling products revealed that it was a 23:1 mixture of the cis (**10**) and trans (**11**) isomers. More importantly, it was evident from the ^1H NMR spectrum that the nucleosides formed were enantiomerically pure. The desired product **10** was readily obtained in pure form by recrystallization of this mixture from EtOAc/hexanes containing a small amount of MeOH. Finally, reduction of compound **10** with $LiAlH_4$ in THF provided 3TC (>98% ee based on chiral HPLC).[2a,10] The physical data derived from this material were in total agreement with those reported previously.[8,10] Additionally, we have also synthesized the (+) enantiomer of BCH-189 in an identical manner from (1′*S*,2′*R*,5′*S*)-menthyl 5(*S*)-acetoxy-1,3-oxathiolane-2(*S*)-carboxylate which was obtained by resolving acetoxy acid **7** with D-menthol.

There are a few interesting features of this synthesis that we would like to point out. The high stereoselectivity of the N-glycosylation reaction involving **3** or **8** was observed only when TMSI was used as the promotor. Utilization of traditional-metal containing Lewis acids such as $SnCl_4$ or $TiCl_4$ did not give any nucleoside products. Interestingly, TMSI-mediated coupling of a chiral oxathiolane sugar possessing a protected hydroxymethyl substituent at the 2-position with persilylated cytosine was nonstereoselective.[7,8] Thus, it appears that the carbonyl moiety at the C-2 position is playing an important role in effecting the observed stereoselectivity. Since we have synthesized BCH-189 by employing the same reaction sequence depicted in Scheme 1 using an isomeric mixtures (trans:cis ~ 2:1) of the oxathiolane **3** with high stereoselectivity (cis:trans >15:1), it would seem that the geometric configuration at the anomeric center of **3** is unimportant in determining the stereochemical outcome of the glycosylation. At this point, we do not have sufficient data to determine whether the glycosylation occurred *via* the intermediacy of an oxonium ion or an anomeric iodide species.

In conclusion, we have presented the first diastereoselective synthesis of the clinically important compound 3TC and its (+) enantiomer based on a novel stereoselective N-glycosylation reaction using inexpensive starting materials and without recourse to tedious chromatographic separation of isomers. Although 3TC has been produced by enzymatic resolution of BCH-189[3,10,12] which was prepared stereoselectively, the overall efficiency is low as compared to our synthetic sequence. Moreover, our approach to the potent antiviral agent (+) BCH-189[2] is much more efficient as compared to those previously reported which utilized naturally occurring sugars as starting materials.[14,15] Currently, studies on this mechanistically intriguing glycosylation process regarding its scope and limitations, as well as its mechanistic aspects, are being conducted. We are particularly interested in the possibility of applying this synthetic approach to the preparation of furanose-derived 2′,3′-dideoxy nucleosides. The results of these investigations will be reported in due course.

Experimental Section

General Methods. All reagents were of commercial quality (Aldrich). Compound **4** was purchased from Wacker-Chemie GMBH FRG. Both L- and D-menthol were bought from Lancaster. Solvents used in reactions were dried and distilled before use according to standard procedures. Solvents used in extractions and recrystallizations were of reagent grade and used as received. The ^1H and ^{13}C NMR spectra were recorded using a

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Varian Gemini 300 spectrometer. The melting points were determined on a Buchi SMP-20 apparatus and were uncorrected. The optical rotation readings were obtained on a Autopol III automatic polarimeter made by Rudolph Research. Chiral HPLC analyses were performed on Pirkle β-Gem I (4.6 × 250 mm) and Cyclobond I Ac (4.6 × 250 mm) columns.

***trans*-5-Hydroxy-1,3-oxathioalne-2-carboxylic Acid (5).** A suspension of dithiane-1,4-diol (**4**, 82.70 g, 0.54 mol) and glyoxylic acid monohydrate (100.0 g, 1.09 mol) in *tert*-butyl methyl ether (1.1 L) was stirred under a blanket of nitrogen and heated to reflux under Dean-Stark conditions. The reflux was continued for 8 h during which time 15.3 mL (0.85 mol) of water was collected. The slightly turbid mixture was filtered, and the solvent was distilled at atmospheric pressure until a volume of 600 mL remained. Cyclohexane (340 mL) was added, and the solution was cooled to 5 °C, seeded, and allowed to stir and crystallize. The suspension was stirred at 0−5 °C for 2 h. The product was isolated by filtration, washed with 100 mL of *tert*-butyl methyl ether−cyclohexane (2:1), and dried overnight *in vacuo* at room temperature (94.44 g): mp 94−95 °C; ^{1}H NMR (DMSO) δ 2.85 (d of d, 1H, J = 2.4, 10.5 Hz), 3.13 (d of d, 1H, J = 4.3, 10.5 Hz), 5.47 (s, 1H), 5.84 (br s, 1H), 6.95 (d, 1H, J = 4.7 Hz). Anal. Calcd for $C_4H_6O_4S$: C, 32.00: H, 4.02; S, 21.36. Found: C, 32.15; H, 4.06; S, 21.50.

***cis*-5-Acetoxy-1,3-oxathiolane-2-carboxylic Acid (6) and *trans*-5-Acetoxy-1,3-oxathiolane-2-carboxylic Acid (7).** One drop of concentrated H_2SO_4 was added to a stirred solution of *trans*-5-hydroxy-oxathiolane-2-carboxylic acid (**5**, 7.0 g, 46.7 mmol) in glacial acetic acid (40 mL) and acetic anhydride (15 mL, 15.9 mmol) at ambient temperature. The resultant clear solution was stirred for 1 h and then poured onto crushed ice and brine (20 mL). This mixture was extracted with CH_2Cl_2 (100 mL), and the combined extract was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure to give 8.5 g (95%) of a light yellow syrup which consisted of compound **6** and **7** in a 1:2 ratio. This mixture was dissolved in benzene (20 mL) and was left standing overnight during which time white crystals were formed that were collected by filtration and dried under vacuum to give 2 g (22%) of *trans*-5-acetoxy-1,3-oxathiolane-2-carboxylic acid (**7**): mp 111−112 °C; ^{1}H NMR (DMSO) δ 2.03 (s, 3H), 3.21 (d, 1H, J = 12.0 Hz), 3.32 (d of d, 1H, J = 3.0, 12.0 Hz), 5.65 (s, 1H), 6.65 (d, 1H, J = 4.0 Hz); ^{13}C NMR (DMSO) δ 20.91, 36.51, 78.86, 99.15, 169.36, 170.04. Anal. Calcd for $C_6H_8O_5S{\cdot}H_2O$: C, 33.97; H, 5.70. Found: C, 33.91; H, 5.69.

The mother liquor was concentrated under reduced pressure and then was redissolved in ether (20 mL). This solution was left standing at room temperature overnight, and the *cis*-acid **6** slowly crystallized out and was collected by filtration and dried *in vacuo* to afford *cis*-5-acetoxy-1,3-oxathiolane-2-carboxylic acid (**6**) (2.1 g, 23%): m.p. 111−112 °C; ^{1}H NMR (DMSO) δ 1.96 (s, 3H), 3.25−3.33 (m, 2H), 5.74 (s, 1H), 6.69 (d, 1H, J = 3.0 Hz); ^{13}C NMR (DMSO) δ 21.00, 37.16, 79.57, 98.58, 169.36, 170.69. Anal. Calcd for $C_6H_8O_5S{\cdot}H_2O$: C, 33.97; H, 5.70. Found: C, 33.93; H, 5.64.

(1′*R*,2′*S*,5′*R*)-Menthyl 5(*R*)-Acetoxy-1,3-oxathiolane-2(*R*)-carboxylate (8). A solution of dicyclohexylcarbodiimide (1.362 g, 6.6 mmol) in dichloromethane (5 mL) was added to a 50 mL round bottom flask containing a solution of *trans*-5-acetoxy-1,3-oxathiolane-2-carboxylic acid (**7**, 1.16 g, 6.04 mmol), (1*R*,2*S*,5*R*)-(−)-menthol (1.038 g, 6.60 mmol), and 4-(dimethylamino)-pyridine (75 mg, 0.62 mmol) in dichloromethane (10 mL) at 0 °C. The resulting white slurry was stirred at room temperature for 3 h at which time methanol (0.2 mL) and glacial acetic acid (0.2 mL) were added. After being stirred for 10 min, the reaction mixture was diluted with hexanes (25 mL), filtered through Celite, and concentrated. The crude product thus obtained was redissolved in hexanes (25 mL), filtered through Celite, and concentrated to provide 1.98 g (100%) of (1′*R*,2′*S*,5′*R*)-menthyl 5(*R*)-acetoxy-1,3-oxathiolane-2(*R*)-carboxylate and (1′*R*,2′*S*,5′*R*)-menthyl 5(*S*)-acetoxy-1,3-oxathiolane-2(*S*)-carboxylate. This mixture of diastereoisomers was dissolved in petroleum ether (40−60 °C) containing a minimum amount of diethyl ether and was cooled in a dry ice−acetone bath. A white solid precipitate was formed which was collected (620 mg) immediately by suction filtration. This material was recrystallized again under the same conditions to yield 450 mg of compound **8** as a crystalline white solid: mp 105−106 °C; $[\alpha]^{22}_D$ −60° (*c* 0.51,$CHCl_3$); ^{1}H NMR (CDC_3) δ 0.77 (d, 3H, J = 7 Hz), 0.91 (d, 3H, J = 7.0 Hz), 0.92 (d, 3H, J = 7.0 Hz), 0.86−2.06 (m, 9H), 2.10 (s, 3H), 3.16 (d, 1H, J = 12.0 Hz), 3.44 (d of d, 1H, J = 4.0, 12.0 Hz), 4.74 (d of t, 1H, J = 5.0, 12.0 Hz), 5.63 (s, 1H), 6.79 (d, 1H, J = 4.0 Hz); ^{13}C NMR ($CDCl_3$) δ 16.16, 20.74, 21.11, 21.97, 23.29, 26.08, 31.38, 34.13, 37.24, 40.62, 47.07, 76.11, 79.97, 99.78, 168.60, 169.68. Anal. Calcd for $C_{16}H_{26}O_5S$: C, 58.16: H, 7.93; S, 9.70. Found: C, 57.85; H, 8.08; S, 9.49.

(1′*R*,2′*S*,5′*R*)-Menthyl 5(*S*)-Cytosin-1″-yl-1,3-oxathiolane-2(*R*)-carboxylate (10). *tert*-Butyldimethylsilyl trifluoromethanesulfonate (1.1 mL, 4.79 mmol) was added to a suspension of cytosine (0.27 g, 2.5 mmol) in CH_2Cl_2 (2 mL) containing 2,4,6-collidine (0.65 ml, 4.92 mmol) at room temperature. The resultant mixture was stirred for 15 min, and a clear solution was produced. A solution of (1′*R*,2′*S*,5′*R*)-menthyl 5(*R*)-acetoxy-1,3-oxathiolane-2(*R*)-carboxylate (**8**, 0.66 g, 1.99 mmol) in methylene chloride (1.5 mL) was added to the mixture, and stirring was continued for 5 min. Iodotrimethylsilane (0.31 mL, 2.18 mmol) was introduced dropwise, and a white precipitate was produced when the addition was completed. The reaction mixture was allowed to stir for 18 h. The reaction was quenched by addition of a saturated aqueous solution of $Na_2S_2O_3$ (10 mL) and CH_2Cl_2 (30 mL). The organic layer was separated and washed with brine (2 × 10 mL). The solvent was removed *in vacuo* to give a viscous oil which was suspended in diethyl ether (30 mL). To this suspension was added a saturated aqueous solution of $NaHCO_3$ (20 mL) with vigorous stirring. A white precipitate appeared, and the resultant suspension was diluted with hexanes (10 mL). The precipitate was collected by filtration to give 0.57 g (75%) of a white solid. The ^{1}H NMR spectrum of this material indicated that it was a mixture of the *cis*- and *trans*-diastereomers of the expected nucleoside in a 23:1 ratio. To obtain compound **10**, this mixture was recrystallized from EtOAc−hexanes and a small amount of MeOH: $[\alpha]^{22}_D$ −144° (*c* 1.02, $CHCl_3$); mp 219 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3H, J = 7.0 Hz), 0.85−0.94 (m, 6H), 1.02−1.10 (m, 2H), 1.42−2.06 (m, 7H), 3.14 (d of d, 1H, J = 6.6, 12.1 Hz), 3.54 (d of d, 1H, J = 4.7, 12.1 Hz), 4.72−4.78 (m, 1H), 5.46 (s, 1H), 5.99 (d, 1H, J = 7.5 Hz), 8.43 (d, 1H, J = 7.6 Hz); ^{13}C ($CDCl_3$) δ 16.1, 20.7, 21.9, 23.2, 26.4, 31.4, 34.0, 36.3, 40.7, 47.1, 76.7, 78.4, 90.3, 94.6, 141.8, 155.4, 165.6, 169.8. Anal. Calcd for $C_{18}H_{27}N_3O_4S$: C, 56.67: H, 7.13; N, 11.01; S, 8.41. Found: C, 56.84; H, 7.24; N, 11.22; S, 8.70.

2(*R*)-(Hydroxymethyl)-5(*S*)-cytosin-1′-yl-1,3-oxathiolane (2, 3TC). A solution of (1′*R*,2′*S*,5′*R*)-menthyl 5(*S*)-cytosin-1″-yl-1,3-oxathiolane-2(*R*)-carboxylate (**10**, 67 mg, 0.18 mmol) in THF (1 mL) was slowly added to a stirred suspension of lithium aluminum hydride (19 mg, 0.5 mmol) in THF (2 mL) at ambient temperature under an argon atmosphere. Stirring was continued for 30 min. The reaction was quenched with methanol (3 mL), followed by the addition of silica gel (5 g). The resultant slurry was stirred for 30 min and then was transferred to a short column packed with Celite and silica gel and was eluted with a 1:1:1 mixture of EtOAc−hexane−methanol (50 mL). The eluate was concentrated and subjected to silica gel column chromatography (EtOAc−hexane−methanol, 1:1:1) to give a gummy solid. This solid was dried azeotropically with toluene to give 38 mg (94%) of the desired product. This material was further purified by recrystallisation from EtOAc−MeOH to give a crystalline white solid: $[\alpha]^{22}_D$ −135° (*c* 1.01, MeOH); mp 158−160 °C; ^{1}H NMR (CD_3OD) δ 3.05 (d of d, 1H, J = 4.3, 11.9 Hz) 3.42 (d of d, 1H, J = 5.3, 11.9 Hz), 3.76−3.89 (m, 2H), 5.19−5.21 (m, 1H), 5.81 (d, 1H, J = 7.6 Hz), 6.20−6.23 (m, 1H), 7.01−7.16 (br m, 2H, exchangeable), 7.98 (d, 1H, J = 7.5 Hz); ^{13}C (CD_3OD) δ 38.5, 64.1, 88.0, 88.9, 95.7, 142.8, 157.9, 167.7. Anal. Calcd for $C_8H_{11}N_3O_3S$: C, 41.91: H, 4.84; N, 18.33; S, 13.99. Found: C, 41.72; H, 4.90; N, 18.35; S, 13.91.

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