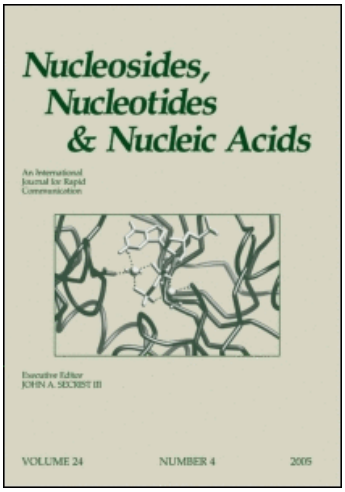

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The Resolution and Absolute Stereochemistry of the Enantiomers of *cis*-1-[2-(Hydroxymethyl)-1,3-oxathiolan-5-yl)cytosine (BCH189): Equipotent Anti-HIV Agents

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THE RESOLUTION AND ABSOLUTE STEREOCHEMISTRY OF THE ENANTIOMERS OF *cis*-1-[2-(HYDROXYMETHYL)-1,3-OXATHIOLAN-5-YL)CYTOSINE (BCH189): EQUIPOTENT ANTI-HIV AGENTS.

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Abstract: Enzymic resolution of the monophosphate derivative of (±)-*cis*-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine using the 5′-nucleotidase from *Crotalus atrox* venom has allowed facile access to the individual enantiomers. The absolute stereochemistry of the preferred (-)-enantiomer (8b) has been determined by X-ray crystallography of the bromine-containing derivative (10).

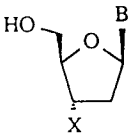
INTRODUCTION

In recent years nucleoside analogues have figured prominently in the search for effective antiviral agents particularly for the treatment of infection caused by the the Herpes Virus family and Human Immunodeficiency Virus[1,2]. Those analogues available by modification of a natural nucleoside or from a natural sugar have generally been prepared and tested in the single optical form dictated by the absolute stereochemistry of the starting-material. In other cases encompassing more fundamental modification to the sugar ring, e.g. carbocyclic analogues[3], compounds have in most cases been prepared and tested initially in racemic form. Where the

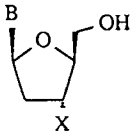
enantiomers of such compounds have been examined, useful biological activity shown by the racemate has been found to reside principally or exclusively in that having the pseudo-"natural" absolute stereochemistry i.e. corresponding to that of the natural nucleosides[4,5,6]. Whilst AZT (**1a**) and other dideoxynucleoside analogues such as ddC (**1b**), ddA (**1c**), d_4T (**2a**) and d_4C (**2b**) show potent activity against HIV, their corresponding "unnatural" optical forms (**3a-c**) and (**4a-b**) have been found to be inactive or to show substantially reduced potency[7,8]. In the *iso*-nucleoside series, whilst the "natural" enantiomers of *iso*-ddA (**5a**) and *iso*-ddG (**5b**) show substantial activity against HIV[9], the "unnatural" enantiomers (**6a-b**) are devoid of activity[10] and it has recently been demonstrated[11] that the anti-HIV activity of (±)-dioxolane-T [12] appears to reside in the "natural" (+)-enantiomer (**7**). Other examples may be found in the literature and the general trend appears well founded.

We have recently reported[13] what we believe to be the first example in the antiviral nucleoside analogue field where the individual optical forms show comparable biological effects. The separated enantiomers of the racemic oxathiolane nucleoside analogue (**8**),[14,15,16] obtained by preparative chiral HPLC, were shown to have equivalent potency *in vitro* against HIV-1 and HIV-2. In cytotoxicity testing however, the (+)-enantiomer was found to be significantly more toxic to cultured human lymphocytes than the (-)-form and it was imperative to determine the absolute stereochemistry of the two forms prior to establishing a chiral synthesis of the preferred (-)-enantiomer. Chiral HPLC did not allow access to sufficient material for a definitive stereochemical assignment to be made and other means were required to that end.

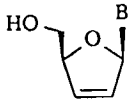
Enantiospecific hydrolysis of the 5′-monophosphate of the racemic form of carbocyclic adenosine has been achieved using the 5′-ribonucleotide phosphohydrolase (5′-nucleotidase) from *Crotalus atrox* venom[17] and this method was subsequently applied to the resolution of other carbocyclic nucleoside analogues[5]. The scope of this procedure has now been further extended by its successful application in the resolution of the enantiomers (**8a**) and (**8b**).[18] Treatment of racemic (**8**) with phosphorus oxychloride in trimethyl phosphate at 0° followed by appropriate work-up and sequential chromatography on charcoal and DEAE Sephadex (HCO_3- form) afforded the racemic monophosphate (**9**) as the ammonium salt (94%). A solution of



(1a) X = N_3 B = T
(1b) X = H B = C
(1c) X = H B = A



(3a)
(3b)
(3c)



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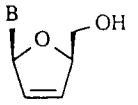
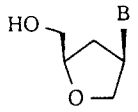
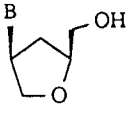
(2b) B = C



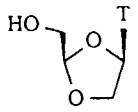
(4a)
(4b)



(5a) B = A
(5b) B = G

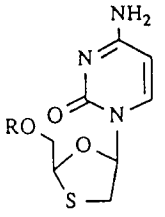


(6a)
(6b)

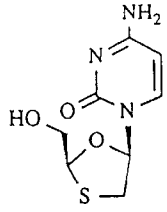


(7)

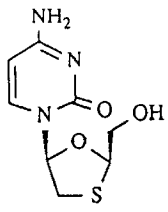
T = Thymine C = Cytosine A = Adenine G = Guanine



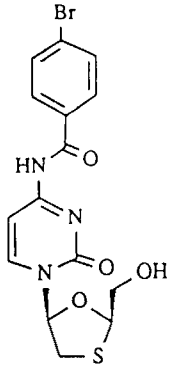
(8) R = H
(9) R = PO_3H_2



(8a)



(8b)



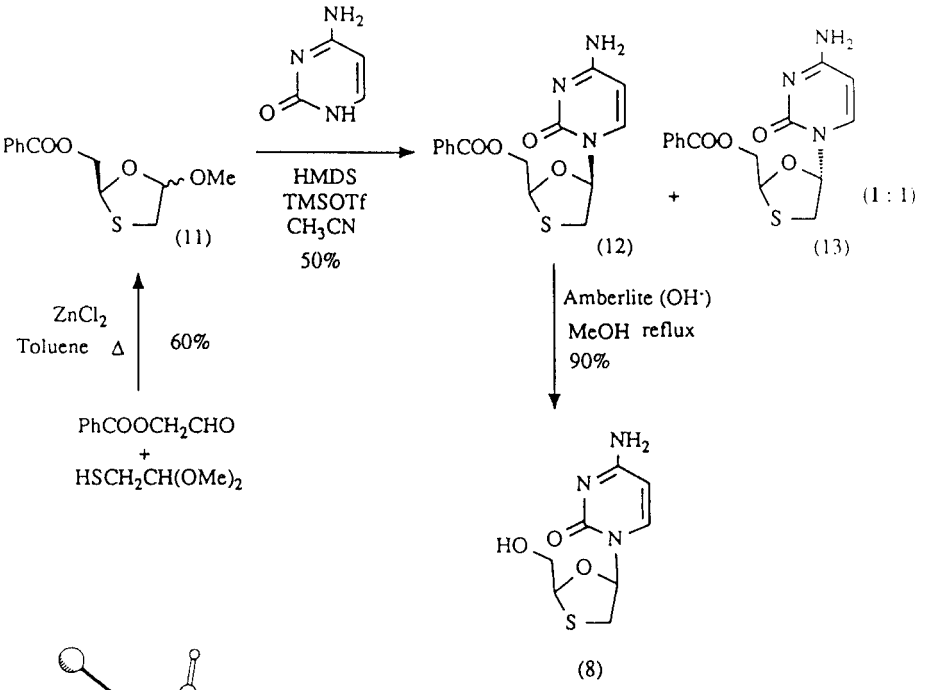
(10)

(**9**) at 37° in aqueous buffer prepared from glycine and magnesium chloride was treated with the 5′-nucleotidase [EC 3.1.3.5] and the resulting two-component mixture was separated by chromatography. The first eluted component was further purified by chromatography on silica gel to afford the (+)-nucleoside enantiomer in 33% yield. An aqueous solution of the second eluted product [the (-)-nucleoside enantiomer monophosphate] was treated with alkaline phosphatase from E.*coli* [EC 3.1.3.1]. Elaboration of the product and purification by chromatography afforded the (-)-nucleoside enantiomer in 35% yield. The optical purity of the two enantiomers as evidenced by chiral HPLC was > 99% and spectral data were identical to those of the racemate.

Based on the precedent discussed earlier, it was expected that the (+)-enantiomer should have the "natural" absolute stereochemistry (**8a**). Treatment of the (-)-enantiomer with 4-bromobenzoyl chloride afforded the N^4-amide (**10**) together with some N,O-*bis* acyl derivative which was removed by chromatography. The presence of the heavy bromine atom allowed the absolute stereochemistry of (**10**) to be determined by X-ray crystallography (Figure 1) serving to confirm the "unnatural" absolute stereochemistry of the (-)-nucleoside enantiomer (**8b**).

The greater activity shown by the "natural" enantiomers of nucleoside analogues is understandable given the closer structural resemblance to their naturally-occurring counterparts. Preferential recognition by the enzymes (kinases) responsible for the activation of nucleosides or by the target enzymes (polymerases) at the active triphosphate level may account for such differences although in many cases this remains to be rigorously established. In the oxathiolane nucleoside case, the greater toxicity of the "natural" (+)-enantiomer (**8a**) may be similarly rationalized but it is remarkable that the "unnatural" (-)-enantiomer (**8b**) should show equivalent activity against HIV. Work to define the exact mechanism by which (**8b**) elicits the observed level of antiviral activity[19] is in progress. Preliminary studies point to a conventional mechanism of action in that investigation of the cellular metabolism[20] has shown that the compound is phosphorylated to the triphosphate and that this nucleotide is a selective inhibitor of the HIV-1 reverse transcriptase[21].

Synthesis of Racemate (8)



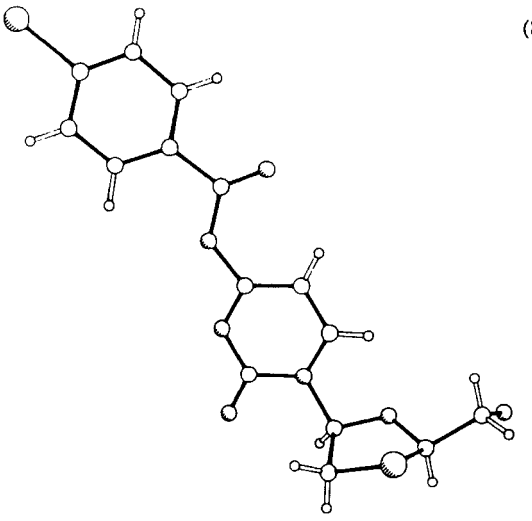


Figure 1.

X-ray structure of compound (10)

In the search for selective antiviral agents, particularly in the area of dideoxynucleoside analogues involving more fundamental modifications to the "sugar" ring, these findings serve to re-inforce the need for caution in drawing parallels with the established series. Where possible it will clearly be prudent to establish a comparative profile before targetting a particular enantiomer for chiral synthesis. The apparent generality of the enzymic resolution as evidenced by its successful extension to the oxathiolane series described herein may prove to be of particular benefit to that end.

EXPERIMENTAL

(±)-*cis*-1-[2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine dihydrogenphosphate ammonium salt (9)

Phosphorus oxychloride (2.44 mL) was added to a stirred suspension of (**8**) (1.0 g, 4.36 mM) in trimethylphosphate (20 mL) at 0°. After 35 min at 0° the resulting solution was poured into ice-chilled water (60 mL) and the pH was adjusted to 2.5 by addition of aqueous 1M-sodium hydroxide. The solution was applied to a charcoal column (DARCO; 10 g) which was eluted with water and then ethanol-aqueous ammonia. Appropriate fractions were combined, concentrated and the solution was applied to a column of DEAE Sephadex A25 (HCO_3^--form) (25 g). Elution was performed with water (120 mL) and a linear gradient from water to 0.4M aqueous ammonium hydrogen carbonate (total 1.0 L). Appropriate fractions were combined concentrated and freeze-dried to afford the title compound (1.37 g; 94%) as a white solid. ^{1}H NMR[22] (250MHz: D_2O) δ3.23 (dd,$J_{5',4'a}$=4.2Hz, $J_{4'a,4'b}$=11.6Hz, 1H, 4′ a-H), 3.55 (dd,$J_{5',4'b}$=5.8Hz, $J_{4'a,4'b}$=11.6Hz, 1H, 4′ b-H), 4.08 (m,1H,2′-CH_2O), 4.20 (m,1H,2′-CH_2O), 5.43 (m,1H,2′-H), 6.07 (d,$J_{5,6}$=7.82Hz, 1H, H-5), 6.33 (dd (overlapping),$J_{5',4'a}$=4.2Hz, $J_{5',4'b}$=5.8Hz, 1H, 5′-H), 8.08 (d, $J_{5,6}$=7.82Hz, 1H, H-6). ^{31}P NMR (400MHz:D_2O) δ31.38.

(2′S,5′R-(+)- and 2′R,5′S-(-)-1-[2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (8a and 8b)

5′-Nucleotidase [EC 3.1.3.5] (obtained from Sigma, 1020 units) was added to a solution of (**9**) (1.35 g, 4.13 mM) in a buffer solution prepared from glycine (175 mg)

and magnesium chloride (85 mg) in water (30 mL). The mixture was maintained at 37 °C for 6 hours; a further addition of the enzyme (340 units) was made after 3.5 hours. The resulting mixture of two components was applied to a column of DEAE-Sephadex A-25 (HCO_3^--form, 20 g) and elution was effected with water (160 mL) and 0.1, 0.2, 0.3 and 0.4M aqueous ammonium hydrogen carbonate (200 mL each). Appropriate fractions containing the first eluted component were combined and evaporated. The residue was further purified by chromatography on silica gel (60 g, Merck 7734) using chloroform-methanol mixtures for elution. Crystallization from methanol/ethyl acetate afforded **(8a)** (300 mg; 31%) as white needles m.p. 159-161 °. $[\alpha]_D^{21}$: +137° (c 1.04, MeOH). Elemental analysis, $C_8H_{11}N_3O_3S$ requires C, 41.9; H, 4.8; N, 18.3: found C, 41.7; H, 4.8; N, 18.2%.

Fractions containing the second eluted component were combined and evaporated and the residue was dissolved in water (30 mL). The solution was treated with alkaline phosphatase from E.*coli* [EC 3.1.3.1] (obtained from Sigma, 624 units) and maintained at 37 °C for 1 hour. Solvent was evaporated under reduced pressure and the residue purified by chromatography on silica gel and crystallization as described for **(8a)** to afford compound **(8b)** (320mg 33%) as colourless needles m.p. 160-2 °C ^{1}H NMR[22] (DMSO-d_6) δ3.04 (dd,$J_{5',4'a}$=4.5Hz, $J_{4'a,4'b}$=11.9Hz,1H,4′ a-H), 3.40 (dd,$J_{5',4'b}$=5.25Hz, $J_{4'a,4'b}$=11.9Hz, 1H, 4′ b-H), 3.73 (m, 2H, 2′-CH_2OH), 5.18 (t, $J_{2',2'\text{-}CH2OH}$=4.2Hz, 1H, 2′-H), 5.29 (t, $J_{2'\text{-}CH2,OH}$=4.6Hz, 1H, OH), 5.73 (d, $J_{5,6}$=7.5Hz, 1H, H-5), 6.21 (dd (overlapping),$J_{5',4'a}$=4.5Hz, $J_{5',4'b}$=5.25Hz, 1H, 5′-H), 7.19 (br s,2H,NH_2), 7.81 (d,$J_{5,6}$=7.5Hz,1H,H-6). IR (nujol) 3372, 1663, 1613cm^{-1}. $[\alpha]_D^{21}$: -137° (c 1.00, MeOH). Elemental analysis, $C_8H_{11}N_3O_3S$ requires C, 41.9; H, 4.8; N, 18.3: found C, 41.8; H, 5.1; N, 18.0%.

(2′R,5′S)-N^4-(4-Bromobenzoyl)-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (10)

A suspension of 4-bromobenzoyl chloride (254 mg, 1.16 mM) in pyridine (5 mL) was added dropwise with stirring to an ice-chilled solution of **(8b)** (267 mg, 1.16 mM) in pyridine (10 mL). After 30 min. the mixture was allowed to attain room temperature and ethanol was added to quench residual acid chloride. Solvents were evaporated, the residue was taken up in ethyl acetate and the solution washed

successively with 1M-HCl and brine. The solvent was evaporated and the residue purified by chromatography on silica gel (as for compound **8a**) to afford (**10**) (99 mg, 21%). Recrystallization from methanol afforded colourless plates suitable for X-ray analysis m.p. 199-201°. ^{1}H NMR[22] (DMSO-d6) δ3.24 (dd,$J_{5',4'a}$=3.5Hz, $J_{4'a,4'b}$=12.1Hz,1H,4′ a-H), 3.69 (dd,$J_{5',4'b}$=5.25Hz, $J_{4'a,4'b}$=12.1Hz,1H,4′ b-H), 3.84 (t,$J_{2',2'\text{-}CH2OH}$=4.87Hz,2H,2′ -CH_2OH), 5.27 (t,$J_{2',2'\text{-}CH2OH}$=4.87Hz,1H,2′ -H), 5.46 (t,$J_{2'\text{-}CH2,OH}$=4.5Hz,1H,OH), 6.25 (dd,$J_{5',4'a}$=3.5Hz, $J_{5',4'b}$=5.25Hz,1H,5′ -H), 7.32 (br d,$J_{5,6}$=7.5Hz,1H,5-H), 7.73 (d,$J_{Ar\text{-}Ha,Ar\text{-}Hb}$=8.25Hz,2H,Ar-Ha), 7.93 (d,$J_{Ar\text{-}Ha,Ar\text{-}Hb}$=8.25Hz,2H Ar-$H_b$), 8.47 (d,$J_{5,6}$=7.5Hz,1H,6-H), 11.39 (br s,1H,NH). IR (nujol) 1690, 1634 cm^{-1}. $[\alpha]_D^{21}$: -126° (c 0.4, MeOH). Elemental analysis, $C_{15}H_{14}BrN_3O_4S$ requires C, 43.7; H, 3.4; N, 10.2; S, 7.8: found C, 43.8; H, 3.5; N, 10.1; S, 7.6%.

X-ray Crystal Structure Analysis of (10)

A suitable crystal of (**10**) ($C_{15}H_{14}BrN_3O_4S$, MW = 412.26) with approximate dimensions of 0.48 x 0.40 x 0.02 mm was obtained by crystallization from methanol and belongs to the monoclinic space group $P2_1$ (No. 4), Z = 2, D_c = 1.64 g cm^{-3}. The cell dimensions were determined as a = 4.812(2), b = 11.638(4) and c = 14.967(6) Å, β = 93.42(3)°, V = 837(1) Å^3 by least squares refinement on diffractometer angles for 15 automatically centred reflections, λ = 1.54184 Å, μ = 47.4 cm^{-1}, F(000) = 416. Three-dimensional X-ray intensity data were measured at 295K on a Nicolet R3m/V diffractometer with monochromatised Cu-Kα radiation. Of the 2254 unique reflections ($0 < 2\theta < 115°$), 2108 had intensities ≥ 3.0 σ (I) and no absorption correction was applied. Two control reflections monitored every 98 reflections showed no appreciable decay during 31.8 hours exposure of the crystal to X-rays. Direct methods resulted in the location of all non-hydrogen atoms. These were refined by full-matrix least squares with anisotropic thermal parameters. Hydrogen atoms bonded to carbon were refined in riding mode, hydrogen atoms bonded to nitrogen or oxygen were omitted from the refinement. Individual weights were applied according to the scheme:

$$w = [\sigma^2(F_0) + 0.0035\ |F_0|^2]^{-1}$$

and refinement converged at R = 0.080, R_w = 0.090; goodness-of-fit 1.88. The absolute configuration was determined using Rogers's eta test[23] η = 1.03(9).

All computations were carried out using the SHELXTL PLUS (μ-VAX II) system of programs[24].

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The synthesis of the racemic form (8) of the oxathiolane nucleoside analogue described in this reference is shown in the Scheme. The oxathiolane (11) was obtained as a 1:1 mixture of anomers from reaction of mercaptoacetaldehyde

dimethylacetal and benzoyloxyacetaldehyde in the presence of zinc chloride in toluene at reflux. Treatment of (11) with silylated cytosine in acetonitrile in the presence of TMS-triflate gave a 1:1 mixture of the β- (12) and α- (13) anomers from which the required β-anomer was obtained by crystallisation from aqueous ethanol. Deprotection of (12) to give the desired racemic compound (8) was accomplished using Amberlite (OH^-) resin in methanol at reflux.

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