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A NOVEL CLASS OF 1,3-OXATHIOLANE NUCLEOSIDE ANALOGUES HAVING POTENT ANTI-HIV ACTIVITY

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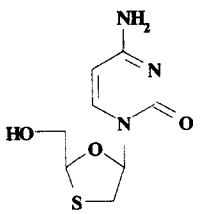
†Dedicated to the memory of the late professor Bernard Belleau.

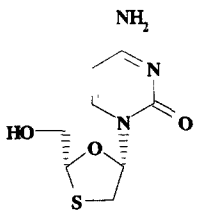
Abstract: We have developed a novel class of 1,3-oxathiolane nucleoside analogues which were evaluated for anti-HIV activity in the MT-4 cell line. **BCH-371**, the adenine derivative, has been found to exhibit significant anti-HIV activity.

Acquired Immunodeficiency Syndrome (AIDS) has become a modern day scourge. The number of AIDS and HIV positive cases have grown rapidly and relatively unchecked despite almost a decade of research efforts[1]. Currently, there are three approved drugs for the treatment of AIDS, but all of these drugs suffer from serious drawbacks such as bone marrow toxicity[2] (AZT), peripheral neuropathy and acute pancreatitis[3] (ddI and ddC) as well as rapid development of resistance[4]. Furthermore, ddI-resistant isolates of HIV-1 have been found to be cross-resistant[5] to ddC. Clearly the need for a better drug is of the utmost importance. In this regard we have recently designed a novel class of 2',3'-dideoxynucleoside analogues in which the 3'-carbon atom was replaced by a sulfur atom or by an oxygen atom[6]. This sugar modification influenced the biological and toxicological properties in an unusual manner and led to the discovery of **BCH-189,** a (±)-1,3-oxathiolane nucleoside analogue, which possessed favorable characteristics for further development[7]. In fact, **3TC**, the (-)-enantiomer of **BCH-189** having the unnatural sugar configuration is a highly selective RT inhibitor and has completed a multicenter Phase I / II clinical trials for the treatment of HIV infection and AIDS with an excellent safety profile[8].

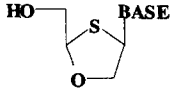
As part of our continuing antiviral program, it was of interest to study the role and effect of transposing the heteroatoms in the oxathiolane ring of **BCH-189** thus giving rise to a new class of (±)-1,3-oxathiolane nucleoside analogues[9] **1**.



BCH-189



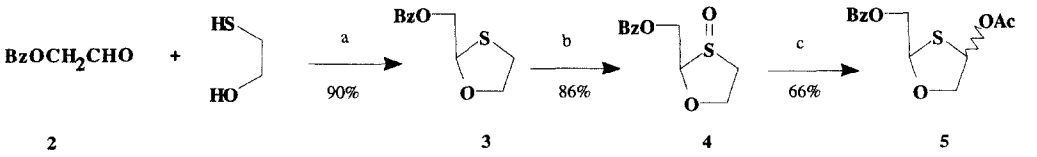
3TC



1

The new class of (±)-1,3-oxathiolane compounds was synthesized by coupling an oxathiolane moiety bearing a suitable leaving group at the 4-position with a persilylated heterocyclic base in the present of a Lewis acid to give a cis-trans mixture of nucleoside analogues[10]. The preparation of the 1,3-oxathiolane moiety (Scheme 1) began with the acid catalyzed reaction of benzoyloxyacetaldehyde **2** with mercaptoethanol giving 2-benzoyloxymethyl-1,3-oxathiolane **3** in 90% yield. The introduction of the 4-acetoxy leaving group in the 1,3-oxathiolane ring could be achieved by a Pummerer rearrangement of the sulfoxide **4** in acetic anhydride. Thus, oxidation of **3** with magnesium monoperoxyphthalate under phase transfer conditions gave the desired sulfoxide **4** as a mixture of isomers in quantitative yields. The Pummerer reaction was initially problematic, standard conditions[11] such as heating in acetic anhydride with or without sodium acetate gave irreproducible results especially on scale-up. Other procedures such as the use of trifluoroacetic anhydride[12] as catalyst gave a complex mixture. The Pummerer reaction was therefore studied in greater detail. We observed that the presence of a high concentration of acetate ions was essential for the reaction to proceed. Since sodium acetate was almost insoluble in acetic anhydride, we opted for a more soluble source of acetate ions. Indeed, when a solution of the sulfoxide **4** in acetic anhydride was heated in the presence of tetra-*n*-butylammonium acetate, the desired key-intermediate 2-benzoyloxymethyl-4-acetoxy-1,3-oxathiolane **5** was obtained in 66% yield as a mixture of cis and trans isomers in a ratio of 1:1. The reaction also gave consistent yields on scale-up.

Scheme 1

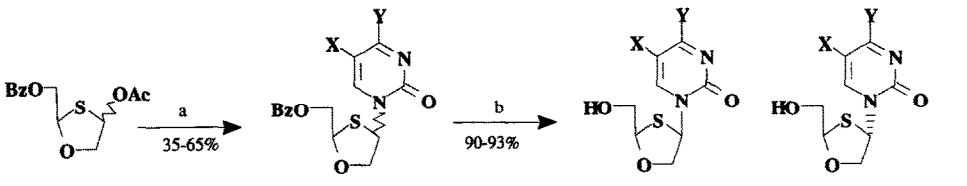


2 3 4 5

a) PTSA, toluene, reflux, b) MMPP, CH_2Cl_2-H_2O, c) Ac_2O, $(nBu)_4NOAc$

The coupling of the acetoxy derivative **5** with persilylated cytosine or 5-fluorocytosine (Scheme 2) in the presence of tin tetrachloride gave the desired nucleoside analogue **6** or **7** as an mixture of 1:1 ratio of cis and trans isomers in modest yields (35-50%). The use of trimethylsilyl trifluoromethanesulfonate as catalyst failed to give any product. The isomers of **6** or **7** were then separated either directly by flash chromatography on silica gel (in case of 5-fluorocytosine analogues) or indirectly through acetylation of the amino group (in case of cytosine analogues). Removal of the protecting groups with methanolic ammonia afforded the desired nucleosides **10-13** in good yields. Similar condensations of **5** with silylated thymine and uracil in the presence of TMS-triflate gave **8** and **9** in good yield (60-65%), which were deprotected under basic conditions, followed by separation of isomers on silica gel giving **14-17** in pure form.

Scheme 2



5

6 Y=NH_2, X=H
7 Y=NH_2, X=F
8 Y=OH, X=CH_3
9 Y=OH, X=H

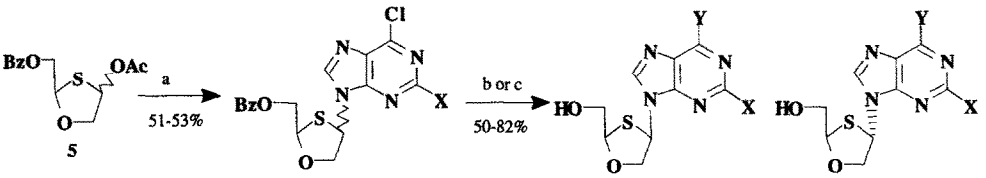
10 Y=NH_2, X=H
12 Y=NH_2, X=F
14 Y=OH, X=CH_3
16 Y=OH, X=H

11 Y=NH_2, X=H
13 Y=NH_2, X=F
15 Y=OH, X=CH_3
17 Y=OH, X=H

a) $(TMS)_2$pyrimidine, CH_2Cl_2 , $SnCl_4$, or TMS-triflate, RT 16h, b) NH_3/MeOH.

Adenine and guanine derivatives were prepared via coupling of **5** with persilylated 6-chloropurine and 2-amino-6-chloropurine under refluxing conditions in 1,2-dichloroethane using TMS-triflate as acid catalyst (Scheme 3) giving a mixture of 1:1 ratio of requisite cis and trans N-9 regioisomers **18** and **19** which were separated to single isomers by chromatography on silica gel in good yield (51-53%). Individual isomers of **18** were converted to adenine analogues **20** and **21** by heating with ethanolic ammonia in a steel bomb while the cis isomer of **19** was transformed to guanine analogue **22** by heating with sodium hydroxide[13] in wet methanol. The relative stereochemistry of these products was assigned by difference NOE spectra[14].

Scheme 3



5

18 X=H
19 X=NH_2

20 Y=NH_2 , X=H
22 Y=OH, X=NH_2

21 Y=NH_2 , X=H

a) Persilylated 6-chloropurine or 2-amino-6-chloropurine, TMS-triflate, $(CH_2Cl)_2$, reflux 2h, b) NH_3/EtOH, 110°C 16h, c) NaOH, MeOH-H_2O , reflux 5h.

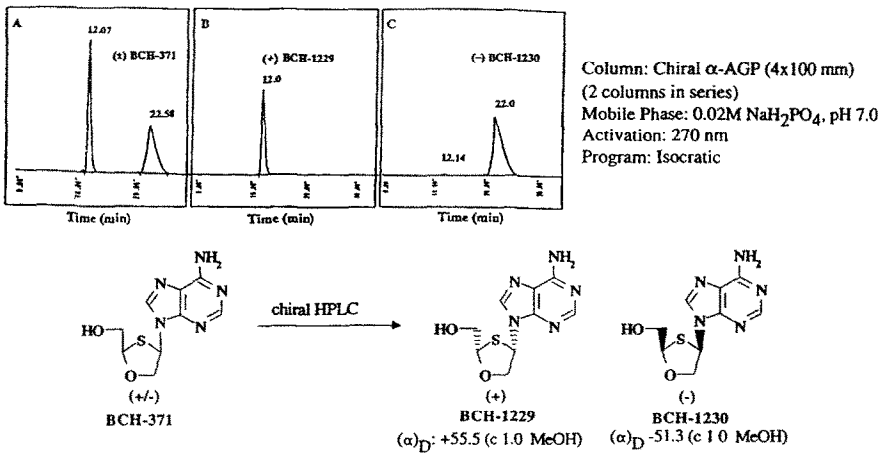
The prepared (±)-1,3-oxathiolane nucleoside analogues were evaluated for anti-HIV activity in MT-4 cells at concentrations up to 100 μg/ml and the results are summarized in Table 1. All of the trans isomers were found to be inactive and non-toxic. While the cis cytosine derivative **10 (BCH-270)** was cytotoxic at 10 μg/ml, cis 5-fluorocytosine and adenine derivatives **12 (BCH-1081)** and **20 (BCH-371)** exhibited a good anti-HIV activity of 2.5 and 2.1μg/ml.

Table 1: Anti-HIV-1 Activity of (±)-1,3-oxathiolane nucleoside analogues in MT-4 cell line.

Compound	BCH	Base	Stereochemistry	ID_{50}, µg/mL	CD_{50}, µg/mL
10	**BCH-270**	cytosine	cis	>10	10
11		cytosine	trans	>100	>100
12	**BCH-1081**	5-F-cytosine	cis	2.5	>100
13		5-F-cytosine	trans	>100	>100
14		thymine	cis	>100	>100
15		thymine	trans	>100	>100
16		uracil	cis	>100	>100
17		uracil	trans	>100	>100
20	**BCH-371**	adenine	cis	2.1	100
21		adenine	trans	>100	>100
22		guanine	cis	>100	>100
	BCH-189			0.13	>100
AZT				0.06	>1

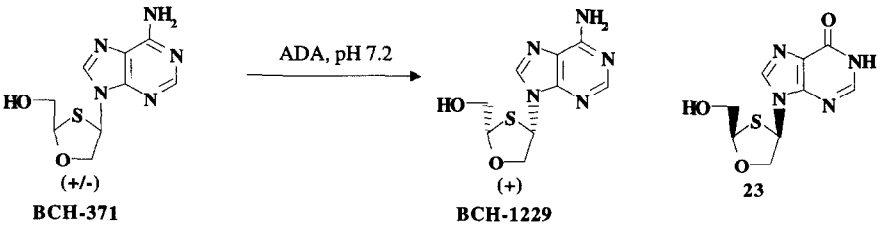
Since **BCH-371** is racemic it was therefore interesting to find out which of the enantiomers carries the antiviral activity, because as we have shown previously in thia-nucleoside analogues the antiviral activity can be separated from cytotoxic effects through resolution[7c]. **BCH-371** was resolved by two methods: The first approach involved HPLC using a chiral support column. Under the conditions in Figure 1 the separation of **BCH-371** was easily achieved giving the (+)-enantiomer **BCH-1229** at a retention time of 12.0 min and the (-)-enantiomer **BCH-1230** at a retention time of 22.0 mın. At this point the absolute configuration of either enantiomers could not be determined.

Figure 1: Chromatograph of chiral HPLC resolution of **BCH-371**



The second approach, which also provided a clue about the absolute configuration of the two enantiomers, was enzymatic resolution using adenosine deaminase enzyme. This enzyme will enantiospecifically deaminate most adenine nucleoside analogues provided that the base and the hydroxymethyl group are in the natural sugar or D-sugar configuration[15]. Thus treatment of **BCH-371** with adenosine deaminase (Scheme 4) gave the hypoxanthine derivative **23** in the natural sugar configuration and the adenine derivative **BCH-1229** having unnatural configuration was left untouched. By comparison of the HPLC retention time and rotation value of this adenine derivative with the one obtained previously, we concluded that the (+)-enantiomer, **BCH-1229**, has the unnatural configuration and the (-)-enantiomer, **BCH-1230**, has the natural configuration. Our hypothesis has been later confirmed by a total synthesis of **BCH-1230** starting from a sugar of known absolute configuration[16].

Scheme 4



Both enantiomers were submitted for anti-HIV evaluation (Table 2). **BCH-1229**, the (+)-enantiomer was found to possess weak activity at 50μg/ml, while **BCH-1230**, the (-)-enantiomer having the natural configuration exhibited significant activity at 1.1μg/ml. Both enantiomers were also found to be slightly cytotoxic at concentration of 100μg/ml. As a comparision, ddI shows anti-HIV activity at 2.3μg/ml.

Table 2: Anti-HIV-1 activity of enantiomers in MT-4 cell line.

Compound	Enantiomer	ID_{50}, μg/mL	CD_{50}, μg/mL
BCH-371	(±)	2.1	100
BCH-1229	(+)	50	100
BCH-1230	(-)	1.1	100
ddI		2.3	100

In summary, a novel class of anti-HIV nucleoside analogues has been discovered. **BCH-1230**, the adenine derivative having a natural configuration, was found to be twice as active as ddI.

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References and Notes:

1. WHO Weekly Epidemiological Record **1991**, 66 (23), 165.

2. Sommadossi, J.-P.; Carlisle, R., *Antimicrob. Agents Chemother.* **1987**, 31, 452.
3. a)Yarchoan, R.; Thomas, R. V.; Allain, J. P.; McAtee, N.; Dubinsky, R.; Mitsuya, H.; Lawhy, T. J.; Safai, B.; Myers, C. E.; McNeely, M. C.; Pluda, J. M.; Leuther, M.; Collins, J. M.; Broder, S., *Lancet,* **1988**, 1, 76. b) Lambert, J.S.; Seidlin, M.; Reichman, R.C.; Plank, C. S.; Laverty, M.; Morse, G.D.; Pharm, D.; Knupp, C.; McLaren, C.; Pettinelli, C.; Valentine, F.T.; Dolin, R., *N. Engl. J. Med.* **1990,** 322, 1333. c) Cooley, T. P.; Kunches, L.M.; Saunders, C.A.; Ritter, J.K.; Perkins, C.J.; McLaren, C.; Path, M.R.C.; McCaffery, R.P.; Liebman, H.A., *N. Engl. J. Med.* **1990,** 322, 1340.
4. a)Larder, B. A.; Darby, G.; Richmann, D. D., *Science,***1989**, 243, 1731. b) Land. S.; Treloar, G.; McPhee, D.; Birch, C.; Doherty, R.; Cooper, D.; Gust, I., *J. Infect. Dis.*, **1990,** 161, 326.
5. St-Clair, M. H.; Martin, J. L.; Tudor-Williams, G.; Bach, M. C.; Vavro, C. L.; King, D. M.; Kellam, P.; Kemp, S. D.; Larder, B. A., *Science,* **1991,** 253, 1557.
6. a) Belleau, B.; Dixit, D.; Nguyen-Ba, N.; and Kraus, J-J.; *Abstracts of Papers, Fifth International Conference on AIDS, Montreal, Canada,* **1989,** Abtract No. T.C.01, p. 515 . b) Belleau B. and Nguyen-Ba N. *US. Pat. No.: 5,047,407.*
7. a) Soudeyns, H.; Yao, X-J.; Gao, Q.; Belleau, B.; Kraus, J-L; Nguyen-Ba, N.; Spira, B.; and Wainberg, M. A., *Antimicrob. Agents Chemother.,* **1991**, 35, 1386. b)Coates, J. A. V.; Cammack, N.; Jenkinson, H. J.; Mutton. I. M.; Pearson, B. A.; Storer, R.; Cameron, J. M.; and Penn, C. R., *Antimicrob. Agents Chemother.,* **1992**, 36, 202. c) Coates, J. A. V.; Cammack, N.; Jenkinson, H. J.; Jowett, A.J.; Jowett, M.I.; Pearson, B. A.; Penn, C. R.; Rouse, P.L.; Viner, K.C.; and Cameron, J. M. , *Antimicrob. Agents Chemother.,* **1992**, 36, 733.
8. Van Leeuwen, R.; Boucher, C.; Katlama, C.; Ingrand, D.; Kitchen, V.; Weber, J.; McDade, H., *Final Program & Oral Abstracts, VIII International Conference on AIDS, Amsterdam, The Netherlands,* **1992,** Abstract No. WeB 1014.
9. Belleau, B.; Brasili, L; Nguyen-Ba, N.; and Chan, L, *Patent Application US. 612840*
10. Vorbruggen, H.; Krolikiewicz, K.; and Bennua B., *Chem. Ber.,* **1981**, 114, 1234.
11. a) Parham, W.E.; and Edwards, L.D., *J. Org. Chem.*, **1968**, 33, 4150. b) Imichıjima, S.; Maniwa, K.; and Tsuchihashi, G., *J. Am.Chem. Soc.*, **1974,** 96, 4280.
12. Tamıkaga, R.; Yabukı, Y.; Ono, N.; and Kaji, A., *Tetrahedron Lett.*, **1976**, 2257.
13. Ogilvie, K. K.; and Hanna, H. R., *Can. J. Chem.,* **1984**, 62, 2702.
14. Compound **20**: m.p.: 200-202°C,^{1}H-NMR: (300 MHz, DMSO-d_6): δ in ppm: 8.31 (s, 1H, H-8'), 8.16 (s, 1H, H-2'), 7.31 (bs, 2H, -NH_2, D_2O-exchangble), 6.36 (d, 1H, H-4, J=3.8 Hz), 5.41 (t, 1H, H-2, J=3.8 Hz), 5.34 (t, 1H, OH, D_2O- exchangble), 4.66 (d, 1H, H-5, J=11.4 Hz), 4.09 (dd, 1H, H-5, J=4.1 and 10.4 Hz), and 3.83 (m, 2H, CH_2OH). Compound **21:** m.p.: 185-187°C, ^{1}H-NMR: (300 MHz, DMSO-d_6): δ in ppm: 8.23 (s, 1H, H-8'), 8.17 (s, 1H, H-2'), 7.32 (bs, 2H, -NH_2, D_2O-exchangble), 6.36 (d, 1H, H-4, J=3.8 Hz), 5.75 (t, 1H, H-2, J=5.7 Hz), 5.32 (t, 1H, OH, D_2O- exchangble), 4.50 (d, 1H, H-5, J=10.2 Hz), 4.35 (dd, 1H, H-5, J=4.1 and 10.0 Hz), 3.73 (m, 1H, CH_2OH) and 3.43 (m, 1H, CH_2OH). Assignment of the cis configuration ın **20** was based on the NOE enhancement for the H-4 proton upon irradiation of the H-2. Furthermore, NOE enhancement of the H-8' proton was also observed when the $\underline{CH_2}OH$ protons were irradiated. In contrast, ırradation of the H-2 proton ın the trans isomer **21** led to an enhancement of the H-8' proton .
15. Gala, D.; and Schuhmacher, D. P., *Synlett,* **1992**, 61.
16. Jin, H-L.; Wang, W.; and Mansour, T. , *unpublished results.*