Tetrahedron Letters, Vol.32, No 31, pp 3791-3794, 1991
Printed in Great Britain

0040-4039/91 $3.00 +
Pergamon Press plc

Asymmetric Synthesis of Enantiomerically Pure (-)-(1'R,4'R)-Dioxolane-thymine and Its Anti-HIV Activity.

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Key Words Asymmetric synthesis, Anti-HIV, Dioxolane- thymine

Abstract: An asymmetric synthesis leading to the enantiomerically pure dioxolane-T has been achieved and its crystal structure has been determined and compared to the previously reported racemate. (-)-(1'R,4'R)-Dioxolane-T was found to have potent and selective anti-HIV activity in primary human lymphocytes.

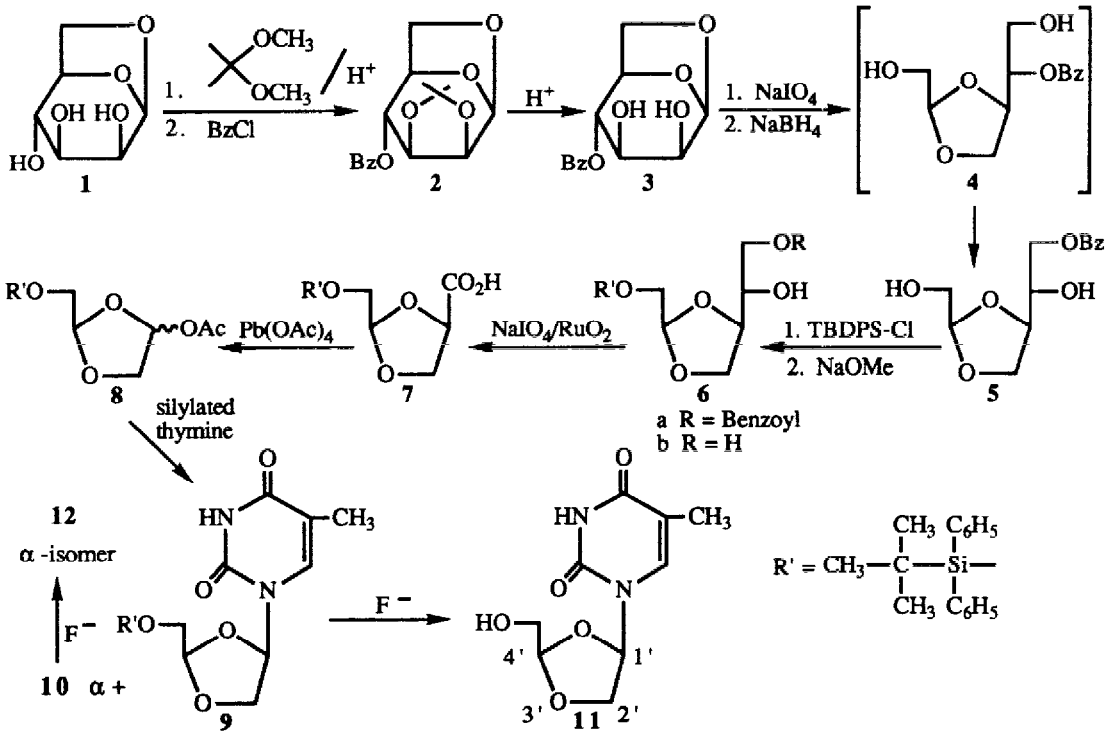
Since the discovery of AZT[1] as a potent inhibitor of HIV a number of other 2',3'-dideoxynucleosides such as AZDU (CS-87),[2,3] DDI,[4] DDC,[4] D4T,[5] and 2'-ara-fluoro-DDC[6] have also been found to be potent inhibitors of the HIV and are currently undergoing various stages of clinical trials.

In an ongoing effort to find more potent and less toxic anti-HIV compounds than those above, a number of new 2',3'-dideoxynucleosides have been synthesized and tested against HIV. Among these, several interesting nucleosides modified by replacement of the C3'position by a heteroatom such as (±)-BCH-189[7] and dioxolane-thymine (dioxolane-T or D-T)[7,8] have recently been reported. (±)-BCH-189 exhibits an excellent anti-HIV activity (EC_{50}=0.3-0.4 CEM)[7] and is currently undergoing preclinical toxicology. (±)-Dioxolane-T has been reported to exhibit an anti-HIV activity of EC_{50}=20 μM in ATH8 cells.[8] Both BCH-189 and (±)-dioxolane-T are known only as a racemic mixture and no asymmetric synthesis leading to enantiomerically pure isomers of either has been reported. Therefore it was of interest to synthesize one of the enantiomers and compare the differences in activities between that enantiomer and the racemic mixture. We wish to report here an asymmetric synthesis of 1'S,4'R- and 1'R,4'R-dioxolane-T and their anti-HIV activity in human peripheral blood mononuclear (PBM) cells.[2]

The starting material for the synthesis (Scheme 1) was 1,6-anhydro-D-mannose[9,10] **1** which contains the necessary stereochemistry of the enantiomeric dioxolane-T **11**. Compound **1** was converted to the 2,3-isopropylidene derivative which, without isolation, was benzoylated to give **2**.[9] The isopropylidene protection was then removed using catalytic amounts of H_2SO_4 in 60% aqueous dioxane at 75°C to give **3** in 85% yield. Treatment of **3** with $NaIO_4$ in H_2O/EtOH (1:1) for 1 hr gave the dialdehyde as an intermediate which was then reduced to **4** by treatment with $NaBH_4$. Under the reaction conditions, **4** was converted to **5** in 82% yield *via* a

base catalyzed 2^0 to 1^0 benzoyl migration. Silyl protection of **5** followed by treatment with NaOMe gave the diol **6b** in 90% yield which was subsequently converted to the acid **7** by treatment with $NaIO_4/RuO_2$[11] in 77% yield. Conversion of **7** to the key intermediate **8** was affected by modified Hunsdiecker reaction[8,12] using $Pb(OAc)_4$ in EtOAc. Condensation of **8** with silylated thymine in the presence of trimethylsilyltriflate (TMSOTf) in CH_2Cl_2[13] gave a mixture of **9**(R,R) (45%) and **10**(S,R) (29%). It was later found that using $SnCl_4$ in place of TMSOTf produced the (R,R)-isomer **9** as the major isomer with only a trace of **10**(S,R). Desilylation of **9** and **10** with tetrabutylammonium fluoride gave the desired free nucleosides[14] **11** and **12**, respectively.

Scheme 1



Conformational analysis of various 2',3'-dideoxynucleosides has been studied by X-ray crystallography in order to gain insight into the molecular mechanism of anti-HIV activity.[15,14] The results indicate that active compounds tend to assume a 3'-exo ($^2T_3$) or similar carbohydrate conformation while inactive compounds prefer to have a 3'-endo ($^3T_2$) conformation. The solid state conformation, recently reported by Norbeck, *et.al*,[8] for (±)–dioxolane-T shows a $^3T_4$ conformation. This differs from that of $^2T_3$ which is the prevalent conformation observed for active anti-HIV nucleosides. It was of interest, therefore, to determine the solid state conformation of the enantiomeric (-)-(R,R)-dioxolane-T and compare it to that of the racemic mixture (Table 1).

TABLE 1. Molecular Conformation of (-)-(R,R)-Dioxolane-Thymine

Parameter	Unit	(-)-D-T A	(-)-D-T B	(±)-D-T
N1-C1'	Å	1.483(6)	1.471(6)	--
χ (C2-N1-C1'-O4')	deg.	-175.2(5)	-133.4(6)	-122
γ (O3'-C4'-C5'-O5')	deg.	48.9(6)	64.1(9)	66
P (pseudo rotation)	deg.	9.0	20.0	32.
ν_{max}	deg.	40.5(6)	36.0(6)	42.4
Dioxolane ring conf.		$^3T_2$	^3E	$^3T_4$

It was found that (-)-(R,R)-dioxolane-T has two independent conformations in the solid state. Both molecules assume the O3'-endo dioxolane ring conformation which is similar to that observed for the racemic mixture and contrary to the C3'-exo conformation observed for the ribose rings of anti-HIV nucleosides such as AZT, AZDU, and 3'-deoxy-3'-fluoro-thymidine.[15-17] This difference might originate from the flexibility of the dioxolane ring system.

Anti-HIV activity and toxicity of **11** and **12** was evaluated in PBM cells.[2] In contrast to the previous report of (±)-dioxolane-T as a moderate anti-HIV agent in ATH8 cells, the (-)-(R,R)-form **11** exhibited a potent anti-HIV activity (EC_{50}= 0.3 μM) in primary human lymphocytes. This difference might be explained based on the rate of phosphorylation of **11** in these two different cell culture systems. The (S,R)-isomer **12** did not exhibit any significant anti-HIV activity (EC_{50}>100 μM) Neither of the compounds were toxic in uninfected PBM cells or rapidly dividing Vero cells evaluated up to 100 μM.

In summary, a carbohydrate chiral synthon has been successfully utilized to synthesize an enantiomerically pure nucleoside of biological interest. A similar approach utilizing other carbohydrate chiral synthons is currently being explored as a general approach for asymmetric syntheses of biologically important nucleosides including BCH-189.

Acknowledgement. This research was partially supported by the Public Health Research Grants from the National Institute of Allergy, Infectious Diseases (AI26055, AI25899), the Department of Veterans Affairs, and the Father Vincent Grant for AIDS Research (PVR).

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14. In order to avoid complications the furanose numbering system was used for interpretation of the NMR data. (**11**)A white solid: $[\alpha]^{25}_D$ - 18 8⁰ (c 0.17, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.75 (d, J=1.2 Hz, 3H, CH_3), 3.63 (dd, J=6.0, 2.6 Hz, 2H, 5'-H), 4.03 (dd, J=9.9, 5.5 Hz, 1H, 2'-H), 4.22 (dd, J=9.9, 2 0 Hz, 1H, 2'-H), 4.90 (t, J=2.6 Hz, 1H,4'-H), 5.16 (t, J=6.0 Hz, 1H, OH), 6.21 (dd, J=5.5, 2.0 Hz, 1H, 1'-H), 7.67 (d, J=1.2 Hz, 1H, 6-H), 11.27 (br s, 1H, NH); UV (H_2O) λ_{max} 266.0 (ε 10757), (pH 2) 266.5 (ε 9894), (pH 11)266.3 (ε 8397); Anal. Calcd for $C_9H_{12}O_5N_2$: C, 47.36; H, 5.31; N, 12.28. Found: C, 47.28; H, 5.34; N, 12.29. (**12**) A white foam: $[\alpha]^{25}_D$ + 10.7⁰ (c 0.15, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.79 (d, J=1.2 Hz, 3H, CH_3), 3.43 (dd, J=6.0, 3.7 Hz, 2H, 5'-H), 4.02 (dd, J=9.5, 3.3 Hz, 1H, 2'-H), 4.28 (dd, J=9.5, 5.6 Hz, 1H, 2'-H), 5.00 (t, J=6.0 Hz, 1H, OH), 5.47 (t, J=3.7 Hz, 1H, 4'-H), 6.17 (dd, J=5.6, 3.3 Hz, 1H, 1'-H), 7.43 (d, J=1.2 Hz, 1H, 6-H), 11.32 (br s, 1H, NH); UV (H_2O) λ_{max} (pH 7) 266.5 (ε 9454), (pH 2) 266.5 (ε 9199), (pH 11) 266.3 (ε 6925); Anal. Calcd for $C_9H_{12}O_5N_2$: C, 47.36; H, 5.31; N, 12.28. Found: C, 47.22; H, 5.32; N, 12.16.
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(Received in USA 10 April 1991)