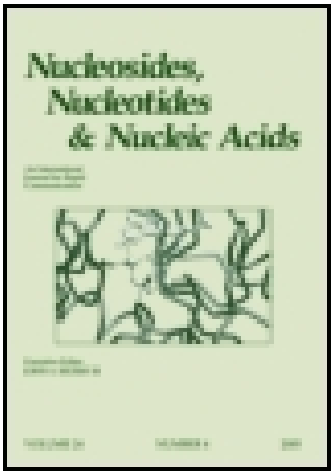

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Synthesis and Antiviral Activity of the Cyclopropano Homolog of 2,3-Didehydro-2,3-dideoxythymidine

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF THE CYCLOPROPANO HOMOLOG OF 2,3-DIDEHYDRO-2,3-DIDEOXYTHYMIDINE

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Abstract: 2,3-Didehydro-2,3-dideoxythymidine is among the nucleoside analogs which have been shown to be clinically useful as anti-HIV agents. Its 2,3-cyclopropano analog has now been synthesized and evaluated. However, the title compound is inactive against HIV.

Nucleoside analogs, beginning with 3'-azido-3'-deoxythymidine (AZT), have thus far shown the most promise as therapeutic agents against the human immunodeficiency virus (HIV). In addition to AZT, two other dideoxynucleosides, 2',3'-dideoxycytidine and 2',3'-dideoxyinosine have now been approved for human use. A number of other derivatives including 2',3'-didehydro-2',3'-dideoxy-thymidine (d4T) have shown promise in preliminary clinical trials.[1]

One problem with therapeutic use of dideoxynucleosides is their relative instability to glycosyl cleavage and thus inactivation. For example, the hydrolysis rate of 2',3'-dideoxyadenosine (ddA) is about 40,000 times that of adenosine. This lability is a result of the enhanced stability of the C-1' carbocation in ddA.[2] The stability of the *unsaturated* dideoxynucleosides is even lower. Under physiological conditions, d4T undergoes about 50% N-glycosyl bond cleavage to the corresponding free base within three days, while 2',3'-dideoxythymidine under these conditions is entirely stable.[3] This enhanced reactivity is undoubtedly due to the more stable C-1'-allylic carbocation which results from C-N bond cleavage in d4T.

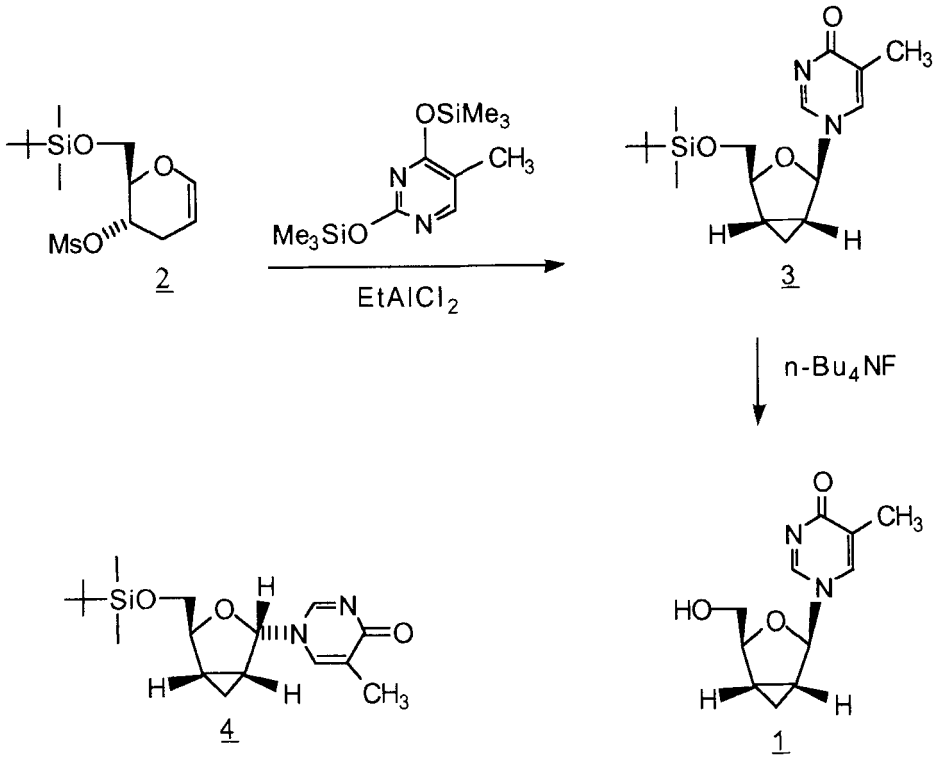
We sought to replace the 2',3'-double bond of d4T by an appropriate functional group which could inhibit glycosyl cleavage while maintaining antiviral activity. We expected that the 2',3'-cyclopropano derivative of d4T would fulfill these conditions, as the formation of a C-1' carbocation should be disfavored relative to d4T, while the similar electronic nature of the cyclopropano group, as compared to the C=C bond, as well as its minimal steric demand were anticipated to lead to retention of intrinsic antiretroviral activity.

While the title compound, 2',3'-cyclopropano-2',3'-dideoxythymidine, 1, to our knowledge has not been previously synthesized, several 2',3'-cyclopropano nucleoside derivatives have been reported, including the 2',3'-cyclopropano cytidine[4] and uridine[5] derivatives. Both of these compounds were prepared by multistep routes. We initially attempted to prepare 1 in only a few synthetic steps, although we eventually found it necessary to employ a common intermediate which was developed for the synthesis of 2',3'-cyclopropano-2',3'-dideoxycytidine.[4]

Use of this intermediate, 2, for the sucessful synthesis of 1 is shown in Scheme 1.

Mesylate, 2, has been prepared by Okabe and Sun[4] in six steps and 60% overall yield starting from tri-o-acetyl-D-glucal. We have found that gram quantities of 2 are readily prepared following this procedure. The crucial coupling reaction was carried out using freshly distilled bis(trimethylsilyl)thymine[13] and 2 with two equivalents of $EtAlCl_2$ in refluxing acetonitrile. Under these conditions, the reaction was complete within one hour (the reaction with bis(trimethylsilyl)cytosine requires 48 hours at reflux[4]), providing a mixture of anomeric products. Purification by column chromatography afforded the desired β-anomer, 3, in 19% yield, mp 50-53 °C, as well as the α-anomer, 4, in 29% yield, mp 118-120 °C. When this reaction was instead run overnight at 25 °C, neither the yield of 3 nor the ratio of 3 to 4 was improved. Other reaction conditions have not been examined for this transformation.

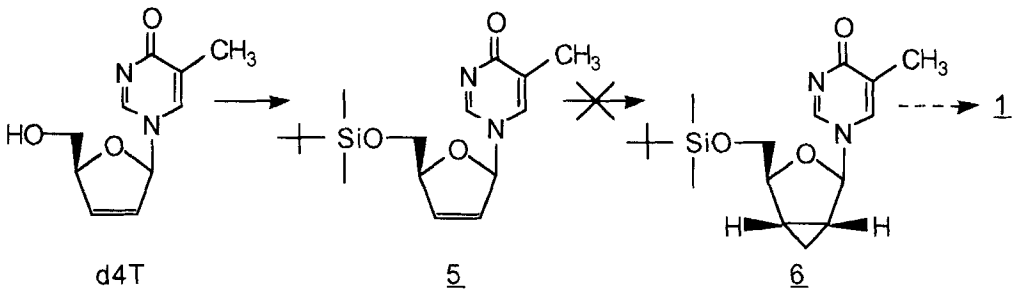
The final desilylation of 3 was efficiently carried out using n-Bu_4NF in THF at 25 °C, for ten minutes. Column chromatography gave the product, 1, as an off-white solid in 92% yield, mp 167-176 °C.



Scheme 1

The structure and purity of this material are supported by all spectral and analytical data. An nOe difference experiment was carried out on 1, which further supports the assigned stereochemistry (See Experimental Section for details).

Another route examined for preparation of 1 is shown in Scheme 2. Commercially available d4T (Pharmatech) was silylated (DMF, imidazole, t-BuMe$_2$SiCl, 17 h, 50 °C) to afford silylether, 5, in 90% yield after chromatography, mp 172.5-174.5 °C. Cyclopropanation of 5 was attempted using methylene iodide and dimethylzinc[6] in ether or THF, however only starting material was recovered. Additionally, the same transformation was attempted using chloroiodomethane and dimethylzinc in 1,2-dichloroethane.[7] Once again only starting material was obtained. The failure of



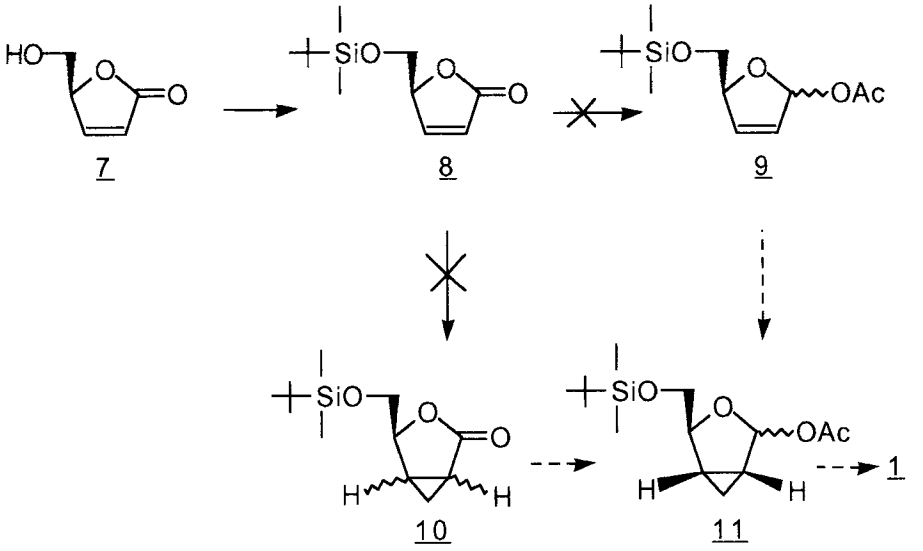
d4T 5 6

Scheme 2

Simmons-Smith and related methods for cyclopropanation of a similar substrate has recently been reported.[8]

We also examined application of a method reported for the synthesis of 3-alkyl nucleosides[9] for the synthesis of 1. This route is shown below in Scheme 3.

We expected that exposure of the key intermediate, 11, to bis(trimethylsilyl)thymine would give, after desilylation, the target, 1, as a mixture of anomers. Two routes to 11 were examined, both involving the intermediacy of furanone, 8. Commercially available lactone, 7, was silylated (DMF, imidazole, t-BuMe$_2$SiCl, 2 h, 50 °C) to afford 8 in 84% yield after chromatography, mp 31.5-33.0 °C.[10] Reduction and acetylation proceeds in good yield for the 2',3'-saturated analog of 8 to afford the corresponding lactol acetate.[9] However, attempted conversion of 8 to the *allylic* acetate, 9, using DIBAH at -78 °C, followed by acetylation, gave a complex mixture in which the C=C and C=O bonds had both been reduced. Apparently the presence of the 2',3'-double bond in 8 causes this route to fail. We then attempted cyclopropanation of 8 using dimethyloxosulfonium methylide.[11] A structurally similar α,β-unsaturated furanone has been converted to the cyclopropyllactone using this reagent.[12] Unfortunately, when 8 was treated with dimethyloxosulfonium methylide in DMSO, rapid consumption of the starting material occurred, providing a complex mixture of polar products.



Scheme 3

Antiviral testing of 1 was carried out at the Burroughs Wellcome Co., Research Triangle Park, North Carolina. The anti-HIV screening was run following the method of Everett.[14] Unfortunately, although 1 was non-toxic up to 200nM, no anti-HIV activity was found.[15]

Screening of 1 against HSV1, HSV2, HCMV, VZV, Hepatitis B and Flu A also showed no activity.

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus, and are uncorrected. ^{1}H-NMR were recorded at 400 MHz (Varian XL400) or 300MHz (Bruker 300). Coupling constants are measured in hertz. Elemental analyses were performed by Atlantic Microlab. Thin-layer chromatography was carried out on Baker Si 250F plates. Visualization was accomplished with UV exposure or treatment with phosphomolybdic acid. Flash chromatography was carried out on Baker silica gel (40mM).

5'-0-(t-Butyldimethylsilyl)-2',3'-cyclopropano-2',3'-dideoxy-β-thymidine, (3), and 5'-0-(t-butyldimethylsilyl)-2',3'-cyclopropano-2',3'-dideoxy-α-thymidine, (4):

A solution of the mesylate, 2[4] (0.507 g, 1.57 mmol) was dissolved in 20 ml of CH_3CN (dist. from CaH_2) under nitrogen, followed by bis(trimethylsilyl)thymine[13] (0.470 g, 1.74 mmol, 1.1 equiv.), and ethylaluminum dichloride (3.46 ml of a 1M solution in hexanes, 3.46 mmol, 2.2 equiv.), dropwise, at 25 °C. The homogeneous reaction mixture was heated at reflux for 1 hour (TLC showed that almost none of 2 remained), and cooled to 25 °C. Ethyl acetate and water were added, the layers were separated, and the organic phase was dried over sodium sulfate and concentrated to afford a mixture of 3 and 4. This mixture was separated by flash chromatography on silica gel (25% EtOAc/Hexanes to 50% EtOAc/Hexanes) to afford first 0.159 g (29%) of the α-isomer, 4, as an off-white solid, followed by 0.106 g (19%) of the β-isomer, 3, as an orange oil which solidified.

For 4: mp 118-120 °C; TLC (50% EtOAc/Hexanes): R_f = 0.27; ^{1}H-NMR (400MHz, $CDCl_3$): 8.68 (br s, 1H, NH); 7.32 (s, 1H, H-6); 6.17 (d, 1H, J = 2.7, H-1'); 4.17 (t, 1H, J = 4.2, H-4'); 3.67-3.70 (m, 2H, H-5'); 2.10-2.13 (m, 1H, H-2' or 3'); 1.90 (s, 3H, 5-CH_3); 1.70-1.74 (m, 1H, H-2' or 3'); 0.89 (s, 9H, $(CH_3)_3$); 0.48-0.94 (m, 2H, CH_2 of cyclopropyl); 0.06 and 0.07 (2s, 6H, CH_3Si).

For 3: mp 50-53 °C, TLC (50% EtOAc/Hexanes): R_f = 0.18; ^{1}H-NMR (400MHz, $CDCl_3$): 8.53 (br s, 1H, NH); 7.53 (s, 1H, H-6); 5.89 (s, 1H, H-1'); 4.08 (t, 1H, J = 6, H-4'); 3.61-3.64 (m, 2H, H-5'); 1.88-1.94 (m, 1H, H-2' or 3'); 1.92 (s, 3H, 5-CH_3); 1.65-1.70 (m, 1H, H-2' or 3'); 1.00-1.05 (m, 1H, CH_2 of cyclopropyl); 0.88 (s, 9H, $(CH_3)_3$); 0.47-0.49 (m, 1H, CH_2 of cyclopropyl); 0.06 (s, 6H, CH_3Si).

The coupling constants for $J_{1',2'}$ in 3 (0.0) and 4 (2.7) are very similar to those reported for the cytidine analogs[4] (β: 0.0; α: 3.1).

2',3'-Cyclopropano-2',3'-dideoxythymidine, (1):

A solution of 3 (0.152 g, 0.431 mmol) was dissolved in 5 ml of dry THF under N_2, followed by 1.3 ml of a 1M solution of n-Bu_4NF in

THF (1.3 mmol, 3 equiv.). After 10 min. at 25 °C, the reaction mixture was concentrated to an oil and purified by flash chromatography on silica gel (elution with 1% MeOH/EtOAc to 2% MeOH/EtOAc). Pure fractions were combined and concentrated to provide a tan solid which was triturated in ether and dried to give 0.094 g (92%) of 1 as an off-white solid. The same method was also used starting from 4 to provide the α-isomer of 1 in a similar yield.

For 1: mp 167-176°C; TLC (10% MeOH/$CHCl_3$): R_f = 0.31; ^{1}H-NMR (400MHz, CD_3OD): 7.97 (s, 1H, H-6); 6.07 (s, 1H, H-1'); 4.12 (t, 1H, J = 4.8, H-4'); 3.63-3.72 (m, 2H, H-5'); 2.04-2.12 (m, 2H, H-2' and 3'); 1.99 (s, 3H, 5-CH_3); 1.11-1.16 (m, 1H, CH_2 of cyclopropyl); 0.51-0.55 (m, 1H, CH_2 of cyclopropyl). Anal. Calcd. for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.35; H, 6.00; N, 11.65.

An nOe difference experiment on 1 was carried out. The H-1' proton at 6.07 ppm gave a 3% enhancement of the methylene proton at 0.51-0.55 ppm on the cycloprone ring. No enhancement was seen for the H-5' multiplet.

Additionally, the anomeric proton in the α-anomer of 1 (prepared from 4), which appears at 6.25 ppm as a doublet, J = 3, was irradiated. An nOe enhancement of the C-5' multiplet (appearing at 3.72 ppm) of 2% was observed, while no nOe effect was seen at the cyclopropyl methylene protons.

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REFERENCES

1. K. Murahami, T. Shirasaha, H. Yoshioka, E. Kojima, S. Aoki, H. Ford, Jr., J. Driscoll, J. Kelley, and H. Mitsuya J. Med. Chem. 34, 1606, 1991.
2. J. York J. Org. Chem. 46, 2171, 1981.

3. J. Balzarini, G.-J. Kang, M. Dalal, P. Herdewijn, E. DeClercq, S. Broder and D. Johns Molecular Pharmacology 32, 167, 1987.
4. M. Okabe and R.-C. Sun Tet. Letters 17, 2203, 1989.
5. J.-C. Wu and J. Chattopadhyaya Tetrahedron 46, 2587, 1990.
6. J. Furukawa, N. Kawabata, and J. Nishimura Tetrahedron 24, 53, 1968.
7. S. Denmark and J. Edwards J. Org. Chem. 56, 6974, 1991.
8. V. Samano and M. Robins J. Am. Chem. Soc. 114, 4007, 1992.
9. K. Agyei-Aye and D. Baker Carbohydrate Res. 183, 261, 1988.
10. The racemate of 8 has been reported, as an oil: M. Okabe, R. C.-Sun, S. Tam, L. Todaro, and D. Coffen. J. Org. Chem. 53, 4780, 1988. Our NMR data for 8 correspond closely to the published spectral data for rac-8.
11. E. J. Corey and M. Chaykovshy J. Am. Chem. Soc. 87, 1353, 1965.
12. T. Minami, M. Matsumoto, H. Suganuma, and T. Agawa J. Org. Chem. 43, 2149, 1978.
13. P. Garner and J. Park J. Org. Chem. 53, 3772, 1990.
14. D. Averett J. Virological Methods 23, 263, 1989.
15. A recent report (J. B. Rodriguez, V. E. Marquez, M. C. Nicklaus, and J. J. Barchi, Jr. Tet. Letters, 34, 6233, 1993 offers a theory which could explain the observed inactivity of 1 and analogs against HIV.

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