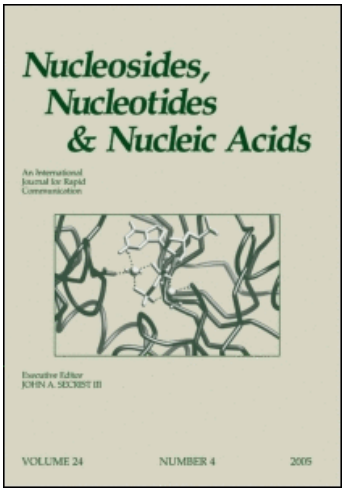


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Synthesis of a 2-Hydroxy-oxolane Derivative as a New Potential Crosslinking Agent of DNA

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**To cite this Article** Nzita, L. , Ladame, S. , Gomez, L. and Moreau, S.(1997) 'Synthesis of a 2-Hydroxy-oxolane Derivative as a New Potential Crosslinking Agent of DNA', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1781 — 1784
**To link to this Article: DOI:** 10.1080/07328319708006277
**URL:** http://dx.doi.org/10.1080/07328319708006277

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1781-1784 (1997)

# SYNTHESIS OF A 2-HYDROXY-OXOLANE DERIVATIVE AS A NEW POTENTIAL CROSSLINKING AGENT OF DNA

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**Abstract** : The design and the synthesis of a new potential crosslinking agent of DNA is reported. It is based on the alkylating properties of a natural toxin (botryodiplodin) and will be further linked to antisense oligonucleotides.

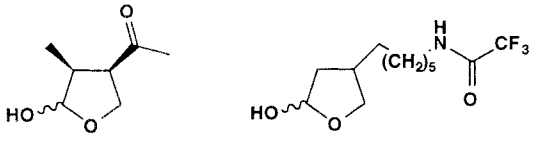


Antisense oligonucleotides (AS) designed to specifically bind to complementary mRNA can be used to inhibit gene expression or to control the development of viruses and parasites (see[1] for a review). Chemically modified oligonucleotides (phosphorothioates) are currently introduced in various clinical trials as antiviral or anticancer drugs[2]. Requirements for synthetic oligonucleotides as efficient inhibitors of gene expression include their ability to elicit RNase-H activity, a cellular enzyme which cleave the RNA strand of RNA-DNA hybrids[3]. Chemical modification of oligonucleotides[4] does not allow RNase H activity with the noticeable exception of phosphorothioates derivatives. This limitation can be circumvented by using chemically reactive AS able to covalently link the mRNA targeted region [5].

We report here the design and synthesis of a new compound based on the alkylating properties of botryodiplodin, a natural toxin which could be further linked to AS oligonucleotides.

Botryodiplodin (**1**) is a sesquiterpenic mycotoxin isolated from the culture medium of various fungal species (*Botryodiplodia theobramae, Penicillium roqueforti*)[6]. It has been shown to inhibit the growth of Gram-positive and Gram-negative bacteria[7]. It inhibits also DNA, RNA and protein synthesis in various strains of mammalian cells[8], and possesses a genotoxic activity in the *Salmonella thyphimurium* test described by Ames[9]. Furthermore the mycotoxin interacts with DNA in eucaryotic cells by introducing DNA-protein cross-links[10].It has been shown that all the biological properties, in particular the ability to induce DNA-protein cross-links, are related to the presence of the hemiacetalic function in the molecule.[10].

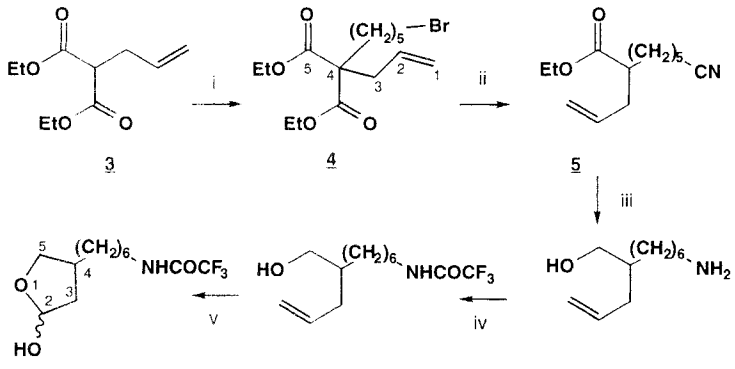
Structures of 2'-deoxynucleoside adducts with botryodiplodin revealed that the alkylating properties of this toxin were linked to the formation of amino-oxolane derivatives of exocyclic amino groups of nucleic acid bases[11].



1 2

These data led us to prepare a synthetic compound (2) able to retain the alkylating properties of botryodiplodin and which could be then conjugated to antisense oligonucleotides. The reactive five membered ring hemiacetal is substituted with an amino-alkyl chain (with a transient protection) which will allow a covalent linkage to various AS oligonucleotides. The synthetic scheme is decribed below :

The key step in this synthesis lies in the introduction of the hemiacetal ring through the oxidative cleavage of a terminal olefin 7[12].



3 4 5

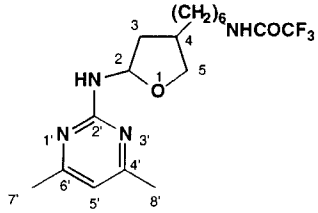
2 7 6

i : 1)NaH/DMF 2)1,5-Dibromopentane ; ii : NaCN/DMSO, 160°C ; iii : LAH /THF; iv : Ethyltrifluoroacetate/$CH_3OH$ ; v : $OsO_4$, $NaIO_4$ /THF,$H_2O$ (2/1).

The commercially available diethylallyl malonate 3 was alkylated with a five fold excess of 1,5-dibromopentane to provide the bromo derivative 4 in 70% yield. Treatment of 4 with sodium cyanide in DMSO[13] led to the deethoxycarbonylation and the simultaneus substitution of the terminal bromine by a nitrile group to give 5 in 60% after flash chromatography. A one step LAH reduction of 5 led to the amino alcohol 6 which was subsequently N-trifluoroacetylated, without further purification, with ethyl trifluoroacetate to afford 7 in 70% global yield. Satisfactory spectroscopic data ($^1$H-NMR, $^{13}$C-NMR) were obtained for these compounds[14]. Osmium tetroxide and sodium periodate oxidation of the double bond in aqueous THF allowed us to get the oxolane derivative 2. The cyclic structure of 2 can be easily deduced from $^1$H and $^{13}$C NMR data through the detection of the hemiacetalic proton at 5.4 ppm (m) and carbon at

98.9 and 98.4 ppm. Furthermore these spectroscopic data demonstrated the presence of equilibrium between the two stereoisomers well characterized on natural botryodiplodin. H-1 and H-5 resonance lines of **2** exhibit a similar pattern as the one observed for botryodiplodin [12,6]. High resolution mass spectra of the acetyl derivative of **2** gave m/z 266.1359 [calc. for $(M\text{-}AcOH)^+$ : $C_{12}H_{19}O_2NF_3$ , 266.1367]

The alkylating properties of **2** were then checked on a pyrimidine derivative as previously carried out on botryodiplodin[11]. To allow for easier interpretation of spectroscopic data on an unresolvable mixture of 2-amino-oxolane isomers, we choosed the 2-amino-4,6-dimethyl pyrimidine as a nucleic acid base model. Stoechiometric amount of compounds **2** and the aminopyrimidine were allowed to react in DMF at 60°C for 48 h Purification by silicagel flash chromatography using a $CH_3OH/CHCl_3$ gradient as elution solvent lead to the isolation of a diastereoisomeric mixture **8** in 30% yield.




**8**

Structural determination was reached by comparison with previously published data[11]. Mass spectrum of **8** (CI) gave a molecular ion at 389 ($MH^+$; $C_{18}H_{28}N_4O_2F_3$ (M+1) = 389.20 ). The substitution at C2 of the oxolane ring by a nitrogen was shown by the proton chemical shift (H2 : 5.95, m) and the corresponding carbon chemical shift (82.77 and 82.31). Furthermore $^{13}C$ and $^1H$ NMR data clearly revealed a 1/1 mixture of diastereoisomers. Numerous lines in the C13 spectrum appeared as doublets (C-5 : 72.07, 71.81 ; C-4 : 39.26, 38.05 ; C-2' : 161. 29, 161.19 ; C-5' 111.28, 111.14) The complex pattern of $^1H$ NMR spectrum prevented the full assignment of NMR peaks. Nevertheless two multiplets centered at 3.92 and 3.40 ppm assigned to H-5 protons, revealed overlapping multiplets as expected for a 1/1 mixture of streoisomers and as already observed for compound **2**.

Clearly compound **2** exhibits alkylating properties similar to botryodiplodin. Work in progress deals with the synthesis of oligonucleotide conjugates of **2** and the evaluation of their ability for crosslinking to a complementary sequence.

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14 Compound **4** : $C_{15}H_{25}O_4Br$ MS (CI) : 351.3 ($MH^+$), calc. M = 350.09; $^1$H-NMR(200MHz, $CDCl_3$): d 5.5 (1H,m,H-2), 5.0 (2H,m,H-1), 4.11 (4H,q J=7 Hz, Et-O) 3.32(2H,t J=6.6 Hz,$\underline{CH_2}$-Br), 2.57 (2H, broad d,H-3) 1.85-1.34 (8H, broad m), 1.18 (6H, t, J=7 Hz Et-O); 13C-NMR(50.32. MHz,$CDCl_3$): d 171.00, 132.36, 118.75, 61.05, 57.10, 36.79, 33.49, 32.21, 28.15, 22.87, 14.00.

Compound **5** : $C_{13}H_{21}O_2N$ MS (EIHR) : 223.1592 (M), calc. M = 223.1572 ; $^1$H-NMR(200MHz, $CDCl_3$): d 5.61(1H,m,H-2), 4,90 (2H,m,H-1) 4.0 (2H,q,J=7 Hz Et-O), 2.21 (2H,t J=7.1 Hz,$\underline{CH_2}$CN) 1.55-1.21 (broad m,alkylchain); $^{13}$C-NMR(50.32 MHz,$CDCl_3$): d 174.96, 135.00, 119.35, 116.40, 59.80, 44.74, 36.18, 30.96, 28.12, 26.06, 24.82,13.99.

Compound **7** : $^1$H-NMR(200MHz, CDCl3): d 7.44 (NH), 5.68 (1H,m,H-2), 4.90,(2H,m,H_1), 3.42 (2H, broad d,H-3), 3.20 (2H,broad q,$\underline{CH_2}$NH), 2.26(2H,t,H-3), 1.5-1,15 (11H,m) ; $^{13}$C-NMR(50.32 MHz,$CDCl_3$): d 157.30(q, J=36.7 Hz, $^2$JC-F), 136.83, 115.50(q, J=286.8 Hz, $^1$JC-F), 115.97, 65.06, 40.14, 39.83, 29.90, 29.00, 28.30, 26.24;

Compound **2** : $C_{12}H_{20}NO_3F_3$ MS(CI) : 266.2 (M-18,$H^+$) ; $^1$H-NMR(200MHz, $CDCl_3$): d 7.28 (1H,t,NH), 5.41 (1H,m,H-2), 4.29 (1H,bd,O$\underline{H}$), 4.04, 3.84, 3.48, 3.31 (2H,4t,H-5) 3.20 (2H,q,$\underline{CH_2}$NH), 1.90-1.15 (13H,multiplets); $^{13}$C-NMR (50.32 MHz,DMSO-d6): d 157.30 (q,$^2$JC-F=36.7 Hz, ), 115.81 (q, $^1$JC-F,=286.8 Hz, ) 98.87 (C-2), 98.38 (C-2), 72.64 (C-5), 71.79 (C-5), 39.80, 38.71 (C-4), 36.47 (C-4), 34.35, 33.07, 32.71, 29.04, 28.59, 28.22, 28.10, 27.10, 26.40.

Compound **8** : $C_{12}H_{20}NO_3F_3$ MS(CI) :389.0 (MH+) cacl. M=388.20 NMR(200MHz, $CDCl_3$): d 6.80(NH), 6.35(1H,d,H-Pyrimidine)), 5.99(1H,m,H-2), 5.5(1H,bt,NH), 3.99-3.90 and 3.43-3.39 (2H,m,H-5) 3.34 (2H,bq,$\underline{CH_2}$NH)2.35(1H,m), 2.24 (6H,s,CH3.C-7',8') 1.88-1.27( 12H,multiplets); 13C-NMR(50.32 MHz,DMSO-d6): d 167.56, 161.20, 157.00(q, $^2$JC-F=36.6 Hz, ), 115.80 (q, J=286.8 Hz, $^1$JC-F,), 111.26 and 111.18(C-5'), 82.76 and 82.30(C-2), 71.20 and 71.11(C-5), 39.79, 39.25, 39.00, 38.28, 38.03, 32.97, 32.55, 29.06, 28.73, 28.18, 28.03, 26.45, 23.72.