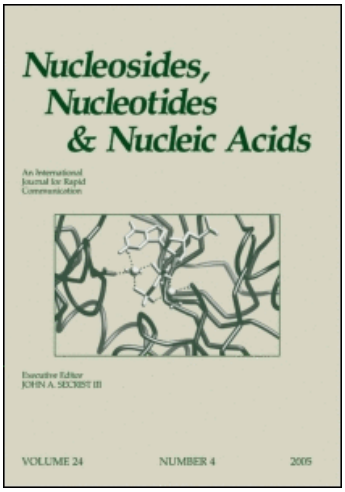


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Novel Analogues of the Anti-HIV-1 Agent TSAO-T Modified at the 3'-Spiro Moiety

Rosa Alvarez[a]; María-Luisa Jimeno[a]; Erik De Clercq[b]; Jan Balzarini[b]; María-José Camarasa[a]
[a] Instituto de Química Médica (C.S.I.C.), Madrid, Spain [b] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

To cite this Article Alvarez, Rosa , Jimeno, María-Luisa , De Clercq, Erik , Balzarini, Jan and Camarasa, María-José(1997) 'Novel Analogues of the Anti-HIV-1 Agent TSAO-T Modified at the 3'-Spiro Moiety', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1033 — 1036
To link to this Article: DOI: 10.1080/07328319708006126
URL: http://dx.doi.org/10.1080/07328319708006126

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1033-1036 (1997)

NOVEL ANALOGUES OF THE ANTI HIV-1 AGENT TSAO-T MODIFIED AT THE 3'-SPIRO MOIETY.

Rosa Alvarez,[§] María-Luisa Jimeno,[§] Erik De Clercq,[+] Jan Balzarini [+] and María-José Camarasa*[§]

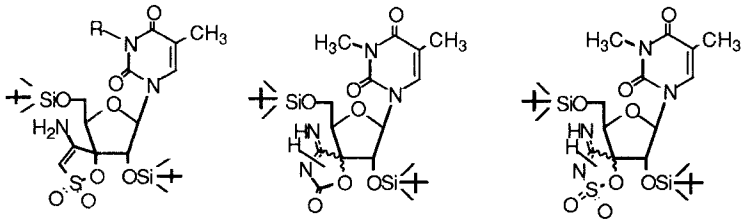
Instituto de Química Médica (C.S.I.C.). Juan de la Cierva, 3. 28006 Madrid, Spain[§]
Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium[+]

Abstract: Novel TSAO-T analogues, in which the 3'-spiroaminooxatioledioxide moiety has been replaced by other 3'-spiro moieties bearing a NH group at the same position as the 4"-NH_2 of TSAO-T have been prepared and evaluated for their inhibitory effect on HIV replication in cell culture. In contrast to the prototype compound TSAO-T, the novel TSAO derivatives were inactive at subtoxic concentrations.

INTRODUCTION

TSAO derivatives are potent and highly specific inhibitors of human immunodeficiency virus type 1 (HIV-1) replication.[1] They do not require intracellular metabolism. They interact with the HIV-1 reverse transcriptase (RT) at a non-substrate binding site.[1,2] TSAO derivatives are not antivirally effective agaisnt HIV-2 or other retroviruses.[1] The prototype compound is [1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribo-furanosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) designated TSAO-T (**1**). Extensive structure-activity relationship studies have shown that the thymine moiety of TSAO-T can be replaced by other nucleobases or heterocycles without marked decrease of antiretroviral efficacy.[1] Moreover, alkylation of N-3 of thymine markedly reduces cytotoxicity without affecting the antiviral activity.[3] Consequently, the 3-*N*-methyl-substituted TSAO-T derivative (TSAO-m^3T, **2**) is the most selective compound of this series.[3] However, the sugar part turns out to be very stringent in its structural requirements, and only those TSAO derivatives having the 3'-spiro moiety in nucleosides with a *ribo* configuration possess antiviral activity.[1,2] Also, the presence of a lipophilic 5'-substituent of the ribose is a critical determinant for antiviral efficacy. The nature of the 2'-substituent appears to be much less stringent.[4]

Based on our SAR studies, molecular characterisation of TSAO-resistant HIV-1 strains and enzymatic studies on different mutated HIV-RT, we postulated that the amino group of the 3'-spiro moiety is most likely responsible for the interaction of the TSAO molecule with a glutamic acid residue at position 138 (Glu-138) of the p51 subunit of HIV-1-RT.[5,6]



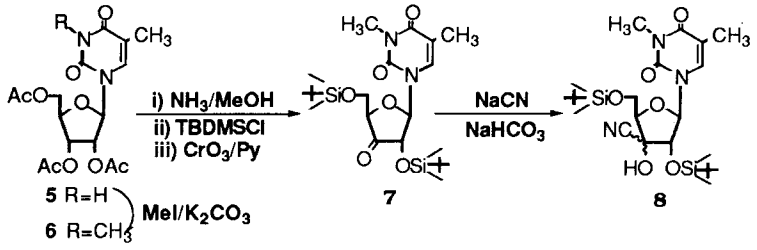
R = H; TSAO-T (**1**)
R = CH_3; TSAO-m^{3}T (**2**)

3 **4**

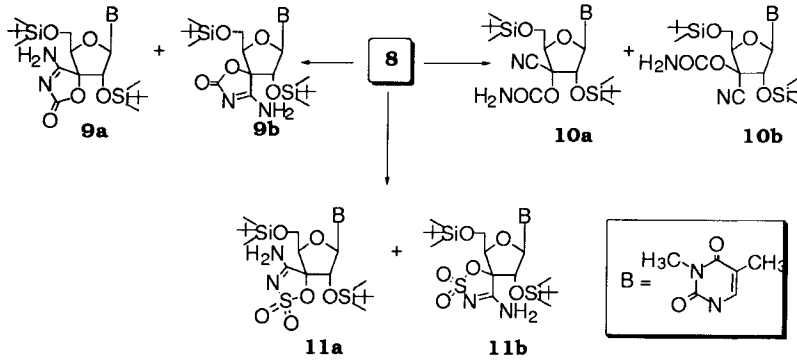
As a part of our program to further explore the importance of substituent effects on the HIV-1 activity of TSAO derivatives, we have designed, prepared and evaluated as inhibitors of HIV replication, novel TSAO analogues (**3** and **4**) in which the 3'-spiroaminooxathioledioxide moiety was replaced by other spiro moieties that maintain a NH group at the same position as the 4"-NH_2 in the prototype compound TSAO-T (**1**), to allow the interaction with Glu-138 of the enzyme. Moreover, compound **3** having a 3'-spirooxazolone moiety with a -CONH- grouping nearby the 4"-NH group may also interact with asparagine (Asn) residues at positions 136 and 137 of the RT. These residues are highly conserved in all HIV-1, HIV-2 and SIV strains. Therefore, this additional interaction may render TSAO derivatives with a broaden antiretroviral spectrum. In both series the substituents at 2' and 5' positions of the sugar moiety and at N-3 of the thymine base were chosen as the ones of the more selective TSAO compound (TSAO-m^{3}T, **2**).

RESULTS AND DISCUSSION

The synthesis of the TSAO analogues was carried out as outlined in Schemes 1 and 2. The spironucleosides **9a**, **9b**, **11a** and **11b** were prepared from the corresponding keto nucleosides via cyanohidrins. Cyanohidrin **8** was prepared as depicted in Scheme 1, following our previously reported method,[7] by reaction of 3'-ketonucleoside **7** with sodium cyanide. The methyl group at the N-3 position of the thymine base was introduced at the first step of the synthesis. Reaction of the cyanohidrin mixture **8** with chlorosulfonyl isocyanate (Scheme 2) followed by treatment with saturated aqueous $NaHCO_3$ solution gave the respective 3'-spiro *xylo*- and *ribo*-furanosyl nucleosides **9a**,



Scheme 1



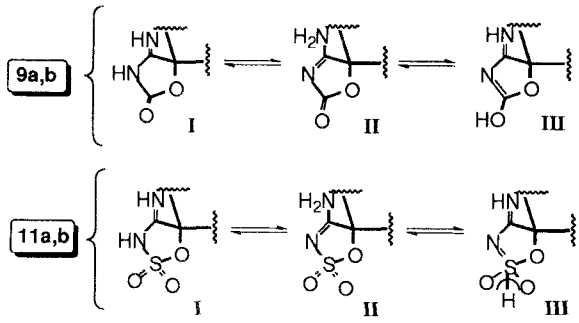
Scheme 2

and **9b** being the *xylo* nucleoside (**9b**) the major compound of the reaction. When the reaction was carried out using water instead of $NaHCO_3$, the carbamates **10a** and **10b** were obtained.

Treatment of the cyanohidrin mixture **8** with sulfamoyl chloride and DMAP (Scheme 2) afforded the cyclized spiro nucleosides **11a** and **11b**.

The spiro moieties at 3'-position of spironucleosides **9a**, **9b**, **11a** and **11b** could exist in different tautomeric forms (Scheme 3). Unequivocal assignment of the predominant tautomer (in acetone) was carried out by inverse detection ^{1}H-^{15}N correlation spectroscopy experiments on compounds **9a** and **11a**. The results of these experiments were only compatible with an enamine structure[8] for the spiro moieties and therefore with tautomer II.

The absolute configuration at C-3' for nucleosides **9a**, **9b**, **10a**, **10b**, **11a** and **11b** was assumed to be the same as that of the corresponding starting cyanohydrins **8** and were assigned as *xylo* for the major compounds **9b**, **10b** and **11b** and *ribo* for the minor compounds **9a**, **10a** and **11a**. The *xylo* configuration of the major compounds (**9b**, **10b** and **11b**) resulted from the approach of the CN^- ion from the sterically less hindered α-



Scheme 3

face of the furanose ring.[9] These assignments were confirmed by NOESY experiments carried out on the spiro derivatives **9a** and **11a** showing , both compounds, correlation peaks between the NH-4" and H-2' and H-5' that were only compatible with a *ribo* configuration.

Spironucleosides **9a**, **9b**, **11a**, and **11b** and carbamates **10a** and **10b** were evaluated on their anti-HIV-1 and -HIV-2 activity in CEM and MT-4 cell cultures. In contrast with TSAO-T and TSAO-m^{3}T, none of the compounds showed antiviral activity at subtoxic concentrations.

Acknowledgements. We thank the Spanish CICYT, the Plan Regional de Investigación de la Comunidad de Madrid, the NATO (Collaborative Research Programme) and the Biomedical Research Programme and tha Human Capital and Mobility Program of the European Community for financial support.

REFERENCES

1 For a review see Balzarini, J.; Camarasa, M.J.; Karlsson, A. *Drugs of the Future* **1993**, *18* , 1043-1055. and Camarasa, M.J.; Pérez-Pérez, M.J.; Velázquez, S.; San-Félix, A.; Alvarez, R.; Ingate, S.; Jimeno, M.L.; Karlsson, A.; De Clercq, E.; Balzarini, J. Nucleosides & Nucleotides **1995**, *14*, 585-594.

2 Balzarini, J.; Pérez-Pérez, M.J.; San-Félix, A.; Camarasa, M.J.; Bathurst, I.C.; Barr, P.J.; De Clercq, E. *J. Biol. Chem.*, **1992**, *267*, 11831-11838.

3 Pérez-Pérez, M.J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M.J. *J. Med. Chem.* **1992**, *35*, 2988-2995.

4 Ingate, S.; Pérez-Pérez, M.J.; De Clercq, E.; Balzarini, J.; Camarasa, M.J. *Antiviral Res.*, **1995**, *27*, 281-299.

5 Balzarini, J.; Karlsson, A.; Vandamme, A.M.; Pérez-Pérez, M.J.; Vrang,, L.; Öberg,, B.; San-Félix, A.; Velázquez, S.; Camarasa, M.J.; De Clercq, E. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 6952-6956.

6 Balzarini, J.; Kleim, J.P.; Riess, G.; Camarasa, M.J.; De Clercq, E.; Karlsson, A. *Biochem. Biophys. Res. Commun.*, **1994**, *201*, 1305-1312.

7 Pérez-Pérez, M.J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M.J. *J. Med. Chem.* **1992**, *35*, 2988-2995.

8 Webb, G.A. (Ed.) *Annual reports on NMR spectroscopy*, **1993**, *25* .

9 Hayakawa, H.; Tanaka, H.; Itoh, N.; Nakajina, M.; Miyasaka, T.; Yamaguchi, K.; Iitaka, Y. *Chem. Pharm. Bull.*, 1987, **35**, 2605.