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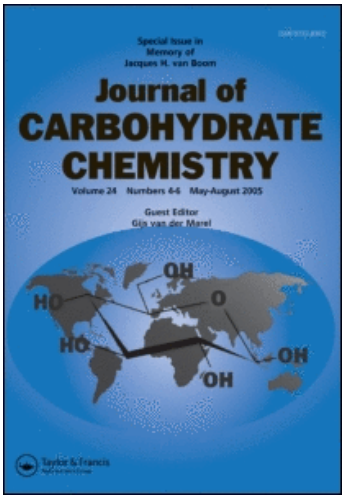

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Hiv-1 Specific Reverse Transcriptase Inhibitors: why are Tsao-Nucleosides so Unique?

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REVIEW

HIV-1 SPECIFIC REVERSE TRANSCRIPTASE INHIBITORS: WHY ARE TSAO-NUCLEOSIDES SO UNIQUE?

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1. INTRODUCTION

AIDS will still be one of the most important challenges for the Scientific Community in the approaching new century. Since the identification, in 1983-84,[1,2] of human immunodeficiency virus (HIV) as the etiological agent of AIDS, significant

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progress has been made in the treatment of HIV-infected patients. This has been in part due to the discovery and clinical use of an increasing number of anti-HIV drugs. However, while highly active antiretroviral therapy (HAART)[3] approaches have reduced the morbidity and mortality, the intertwined problems of drug induced viral resistance, poor compliance with complex regimens and therapy failure continue. Therefore, there remains a pressing need for the development of new antiviral agents that can be used not only as first line therapeutic candidates, but also in the antiretroviral-experienced patient population.

Key targets in the search for effective drugs useful for AIDS therapy are the viral enzymes that have critical roles in the life cycle of the virus. One such essential enzyme is reverse transcriptase (RT).[4,5] The enzyme catalyses the synthesis of proviral DNA from genomic viral RNA, a crucial step in the replication cycle of HIV. HIV RT is a heterodimer consisting of two subunits, p66 and p51.[6] The p66 subunit contains the polymerase domain and the RNAse domains, which degrades the RNA used as template. The catalytic site where polymerisation occurs contains a triad of aspartic acid residues at positions 110, 185 and 186.[7] At least two Mg^{2+} ions may assist with polymerisation by binding to the carboxyl groups of these aspartic acids.[8,9]

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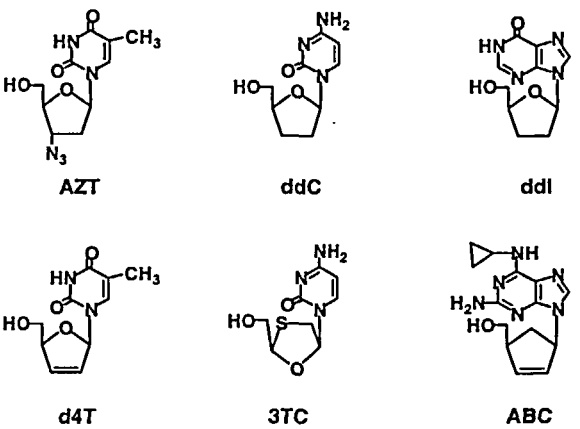


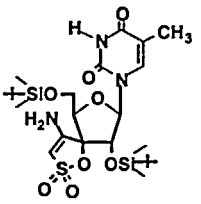
Figure 1. 2',3'- Dideoxynucleosides approved for AIDS patients

Two classes of RT inhibitors have been developed. The first group of inhibitors are the nucleoside RT inhibitors (NRTIs) such as 3'-azido-2',3'-dideoxythymidine (AZT), 2',3'-dideoxyinosine (ddI), 2',3'-dideoxycytidine (ddC), 2',3'-didehydro-2',3'-dideoxythymidine (d4T), (-)2',3'-dideoxy-3'-thiacytidine (3TC) and Abacavir (ABC) (Figure 1), all approved for the treatment of HIV-infected individuals.[10,11] All these drugs represent 2',3'-dideoxynucleoside analogues and require metabolic activation to their

corresponding 5'-triphosphates, acting then as competitive inhibitors of the natural substrates or/and as DNA chain terminators.[12]

The second group of RT inhibitors is the so-called non-nucleoside RT inhibitors (NNRTIs),[11,13,14] of which nevirapine, delavirdine and efavirenz have been formally approved for the treatment of HIV-1 infection in combination with NRTIs and/or protease inhibitors.[11,13] NNRTIs are structurally diverse but they all bind to a hydrophobic pocket, that is distinct from, but functionally and also spatially associated with the catalytic site.[6,15,16] NNRTIs are highly potent and specific inhibitors of HIV-1 replication.[11,14] The main characteristic of NNRTIs is their high potency and their specificity for HIV-1 replication.

Among NNRTIs, TSAO derivatives represent a particular and peculiar group of specific HIV-1 RT inhibitors, discovered in our laboratories in 1992.[17,18] Mechanistically TSAO derivatives behave as all the other NNRTIs, but structurally they are highly functionalised nucleosides. This can be seen as the first feature of their "unique" character. The prototype compound is [1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) designated as TSAO-T (Figure 2).[17b]



TSAO-T

Figure 2. Structure of TSAO-T

These compounds exert their unique selectivity for HIV-1 through a specific interaction with the HIV-1 RT that, unlike the interaction of the nucleoside RT inhibitors, is non-competitive with regard to the natural substrates.[19,20] Our experimental data strongly suggest a specific interaction of the amino group of the 3'-spiro moiety of TSAO molecules with a glutamic acid residue at position 138 (Glu-138) of the p51 subunit of HIV-1 RT.[21,22,23] There is crystallographic evidence that Glu-138 is located at the top of the finger domain of the p51 subunit of HIV-1 RT, that closely approaches the binding pocket of the HIV-1-specific RT inhibitors at the p66 subunit, and/or may even be part of this pocket.[6a,15,16] Moreover, other aminoacids in the NNRTIs binding pocket (Tyr-181, Tyr-188 and Val-106) at the level of p66 RT subunit are also needed for an optimum interaction of TSAO derivatives with HIV-1 RT.[18,21,22,24] TSAO derivatives are the only

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compounds for which aminoacids at both subunits (p51 and p66) of HIV-1 RT are needed for optimal interaction with the enzyme. This can be seen as the second feature of their "unique" character. A recent biological study has revealed that TSAO-T seems to interfere with the dimerisation process of the enzyme, resulting in loss of viral DNA binding affinity.[25,26] This suggests a completely new and different mechanism of inhibition of HIV-1 RT with regard to the other known NNRTIs. This can be seen as the third feature of their "unique" character. So far very little is known in the literature about the dimerisation process of HIV-1 RT. This makes of TSAO derivatives a valuable and "unique" tool for the study of the dimerisation process.

2. STRUCTURE-ACTIVITY RELATIONSHIP (SAR) STUDIES

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TSAO-T is endowed with potent anti-HIV-1 activity, this antiviral activity does not vary markedly from one cell line to another (EC_{50} range: 0.017-0.058μM).[17a] TSAO derivatives are not inhibitory to HIV-2 strains, simian immunodeficiency virus (SIV), moloney murine sarcoma virus and a broad range of DNA and RNA viruses at subtoxic concentrations.[17] TSAO-T does not act as a DNA chain terminator. Interaction of TSAO-T with the enzyme is noncompetitive with respect to both the natural substrate (dGTP) and the template/primer [poly(rC).oligo(dG)]. TSAO-T, like the other NNRTIs, interferes with a non-substrate binding site at HIV-1 RT that seems not to be present at other DNA polymerases including HIV-2 RT.[20]

From the compounds synthesised, extensive SAR studies have been conducted, which have revealed the structural determinants for optimal anti-HIV activity in cell culture, within this class of compounds.

The analysis revealed that the structural requirements that the TSAO molecules must fulfil to inhibit HIV-1 replication are very strict with regard to the sugar part. Thus, the presence of *tert*-butyldimethylsilyl (TBDMS) groups at both 2' and 5' positions of the sugar moiety is a prerequisite for anti-HIV-1 activity. Removal of these lipophilic groups, either at 2', 5' or both positions, rendered the TSAO derivatives completely inactive at subtoxic concentrations (EC_{50}: 30μM-1000 μM).[17b] The 5'-silyl protecting group seems to be more critical for activity than the 2'-silyl protecting group. Thus, replacement of the silyl moiety at the 2' position by other moieties that mimic either the lipophilic or the steric properties of TBDMS led to compounds with only 2- to 10- fold reduced anti-HIV-1 activity.[27] However, the situation for the 5'-silyl moiety is drastically different. Other lipophilic groups, including a good variety of acyl derivatives, at the 5' position resulted in antivirally inactive TSAO compounds, both in cell culture and in inhibition studies

versus the enzyme. The only group that restored some activity, although drastically reduced, is the *t*-hexyldimethylsilyl group.[27]

The presence of the unique 3'-spiro moiety [3'-spiro-5"-(4'-amino-1",2"-oxathiole-2",2"-dioxide)], in nucleosides having a *ribo* configuration, is also a prerequisite for antiviral activity. The *xylo* isomer rendered the TSAO-T molecule completely inactive (EC_{50} >10 μM).[17b] Introduction of this spiro ring at position 2' instead of 3' of the sugar moiety, resulted in annihilation of the antiviral activity.[28] Replacement of the 3'-spiro moiety by other 3'-spiro rings such as 4-amino-2-oxazolone or 4-amino-1,2,3-oxatiazole-2,2-dioxide, resulted in a decrease of anti-HIV-1 activity of two orders of magnitude.[29]

Other sugar modified 3'-spiro-TSAO derivatives, i.e., allofuranosyl-TSAO analogues,[30] compounds with inverted configuration of the C-4' stereocenter[31] or bearing L-sugars[32] resulted in completely inactive compounds (EC_{50} > 10 μM).

In contrast to the stringent structural requirements of the sugar part of the TSAO derivatives to be recognised by HIV-1 RT, the base part is the fragment that allows more modifications being less critical for activity. The thymine moiety of TSAO-T can be replaced by a number of other pyrimidines, purines or 1,2,3-triazoles without marked decrease of antiviral efficacy.[17,18,33-36] The TSAO-purine derivatives are in general 3- to 5-fold less effective than the most active TSAO-pyrimidine derivatives. Among the TSAO-1,2,3-triazole compounds several derivatives with various substitutions at C-4 or C-5 of the triazole ring, show potent anti-HIV-1 activities.[36] The 5-substituted amido, methylamido- and dimethylamido derivatives were the most potent inhibitors among this series (EC_{50} = 0.056-0.52 μM), being comparable to TSAO-T. Interestingly, the cytotoxicity of the TSAO-pyrimidine or TSAO-purine analogues becomes significantly attenuated (10- to 20- fold), without affecting the anti-HIV-1 activity, by introduction of an alkyl or alkenyl group at N-3 (pyrimidines) or N-1 (purines) of the base moiety.[17,19,33-35] More recent data have shown that the thymine of TSAO-T can be replaced by nonaromatic cyclic entities or even acyclic substituents that mimic parts of the thymine ring,[37] keeping significant anti-HIV-1 activity.

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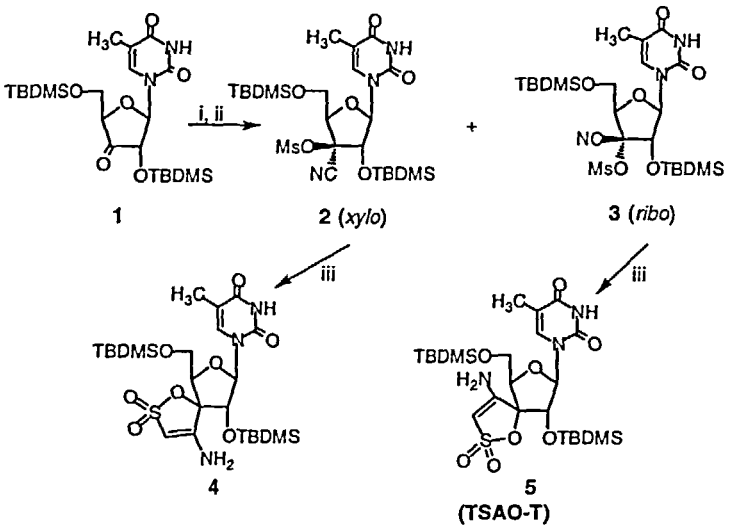
3. SYNTHESIS OF TSAO COMPOUNDS

3.1 Synthesis of the prototype compound (TSAO-T)

In previous works of our group concerning the synthesis of highly functionalised branched-chain sugars, we reported the unexpected behaviour of tertiary cyanomesylates,[38,39] prepared from furanos-3-uloses, pyranos-3-uloses or pyranos-2-

uloses, which under basic conditions underwent intramolecular aldol-type cyclocondensation to afford *C*-branched spiro derivatives having a 3(2)-spiro-5'-(4'-amino-1',2'-oxathiole-2',2'-dioxide) ring at the branching point. Formation of the spirooxathioledioxide ring can be explained by abstraction of one proton from the mesylate methyl group, followed by nucleophilic attack of the resulting carbanion at the nitrile carbon atom.[38,39] Extension of this procedure to α-mesyloxinitriles of nucleosides led us to discover a novel class of potent HIV-1 specific inhibitors whose prototype compound is the thymine derivative designated as TSAO-T (**5**).[17]



Reaction conditions: (i) NaCN/$NaHCO_3$; (ii) mesyl chloride/pyridine; (iii) Cs_2CO_3

Scheme 1

Hence, treatment (Scheme 1) of 2',5'-bis-*O*-*tert*-butyldimethylsilyl 3'-ketonucleoside **1**,[17b] with sodium cyanide followed by mesylation (mesyl chloride/pyridine) of the corresponding 3'-cyanohydrin epimers obtained, gave the respective 3'-*C*-cyano-3'-*O*-mesyl-β-D-*xylo*- and -*ribo*-furanosyl thymine nucleosides **2** and **3**. The major diastereomer was the *xylo* nucleoside **2**, resulting from the attack of the CN^- ion from the sterically less hindered α-face of the furanose ring.[17b,40] Treatment of these cyanomesylates **2** and **3** under basic non-nucleophilic conditions (Cs_2CO_3) gave the *xylo*- and *ribo*-spiro nucleosides **4** and **5**, respectively in a 5:1 ratio.[17b]

Following standard procedures, *xylo*- and *ribo*-spiro nucleosides afforded the fully deprotected compounds, the 2'- or 5'-partially silylated derivatives and the 2' deoxy derivatives.[17b]

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3.2 Synthesis of base modified analogues of TSAO-T

From the initially synthesised compounds, it was clear that the 3'-*ribo* configuration of the sugar, obtained as minor isomer, was crucial for the anti-HIV-1 activity. Therefore, a new synthetic strategy was devised.[33] This strategy (Scheme 2) makes use of the sugar synthon **6** which has the required configuration at the C-3' (*ribo*) and a 2'-*O*-acyl participating group, to obtain the β-nucleosides in the glycosylation reaction. So, glycosylation of **6** with a variety of persilylated heterocyclic bases gave the β-cyanomesyl nucleosides **7** from which the spironucleosides **8** were readily accessible by basic treatment (Cs_2CO_3). One of the advantages of this strategy is not only a full control of the stereochemistry, but also the possibility of introducing different natural and modified pyrimidines and purines.[33-35] The *ribo* configuration of the TSAO nucleosides (**7** and **8**) was determined by the configuration of the starting cyanohydrin used in the preparation of the cyano mesylate of ribose **6**.[34,38] Subsequent deprotection and silylation of these derivatives **8** gave the *ribo* TSAO-analogues **9**. The alkylation of the base[33] resulted in a marked decrease of the toxicity of the compounds, and in particular, the N-3 methyl derivative of TSAO-T (TSAO-m^{3}T, **10**) become the most selective compound of this series.[33,34] These results suggested that the N-3 position of TSAO-T should be further explored.

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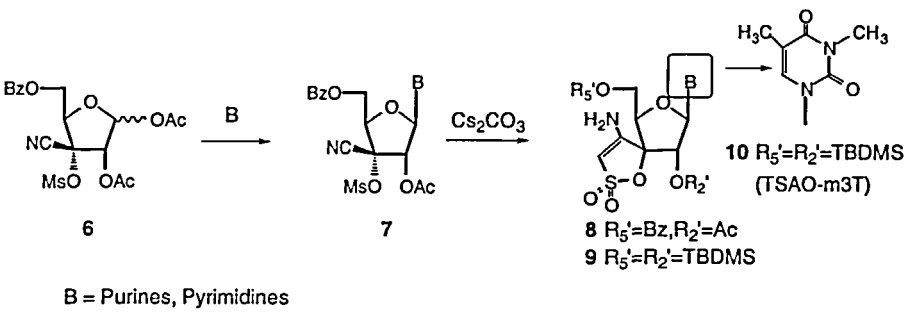
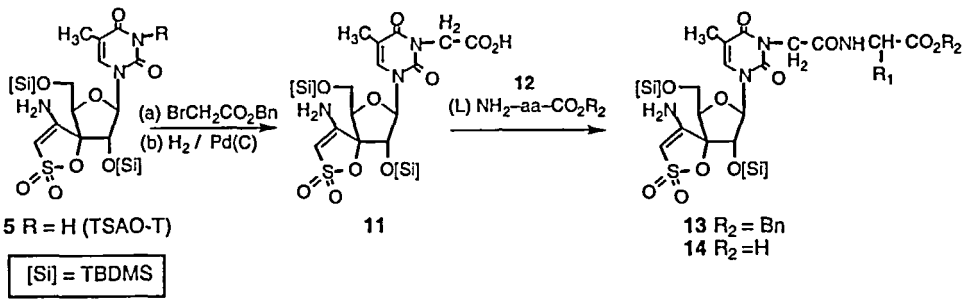


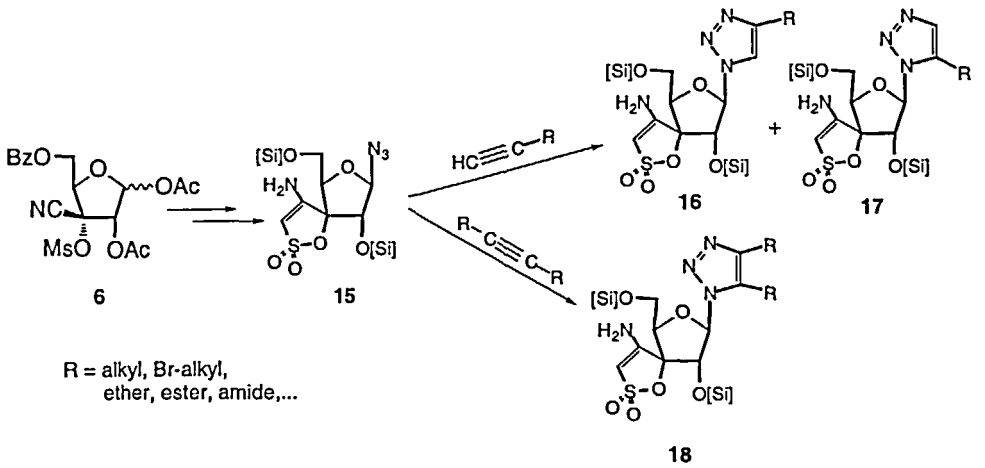
Scheme 2

Hence, a series of analogues of the prototype compound TSAO-T substituted at position N-3 of thymine with amino acids[41] have also been prepared, with the aim to introduce additional functional groups that can interact with the HIV-1 RT. Thus, coupling of the appropriated TSAO intermediate **11** (Scheme 3) with a conveniently protected (L) amino acid **12** in the presence of BOP and triethylamine gave the protected TSAO-N-3-aminoacid derivatives (**13**). Deprotection of the amino acid moiety of **13** gave the target TSAO-aminoacid derivatives **14** in good yields.[41]



Scheme 3

Not only purines and pyrimidines were introduced in the sugar part of TSAO molecules. A series of 1,2,3-triazole-TSAO derivatives (Scheme 4) were prepared[36] by 1,3-dipolar cycloaddition of the ribofuranosyl azide **15** to differently substituted acetylenes. The azide was obtained from synthon **6** by reaction with trimethylsilyl azide and stannic chloride followed by replacement of the protecting groups by TBDMS. Cycloaddition of azide **15** to unsymmetrical acetylenes gave a mixture of the corresponding 4- and 5-substituted isomers **16** and **17**, whereas cycloaddition of azide **15** to symmetric acetylenes gave 4,5-disubstituted 1,2,3-triazole nucleosides **18**.



Scheme 4

The major feature of these TSAO-1,2,3-triazole derivatives is the potent anti HIV-1 activity of the 5-substituted carbamoyl derivatives, comparable to that of TSAO-T, while the respective 4-substituted isomers were 10-fold less active.[36]

A theoretical study[42] in which an extensive conformational search of possible binding modes of these TSAO-triazole molecules with HIV-1 RT highlighted some clear

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differences between the 4- and 5-substituted families of compounds, giving credence to a model in which the substituents on the amide at C-5 are located in two adjacent hydrophobic zones separated by Phe-A227, a binding mode which is not accessible to the 4-isomers.[42]

Thus, novel series of 1,2,3-analogues possessing different hydrocarbon shapes capping the amide at C-5 were synthesised in attempts to exploit the compound's interactions with this region. The procedure for the synthesis of the carbamoyl substituted-1,2,3-triazole-TSAO analogues,[36] indicated above (Scheme 4), gave the most active 5-isomers as minor products in low yields, due to steric and electronic factors in the cycloaddition reaction. Therefore, in order to obtain exclusively the most active 5-isomers, we devised a new synthetic strategy (Scheme 5) based on the cycloaddition of glycosylazides[43,44] to 2-oxo-alkylidenetriphenyl phosphoranes, which, upon concomitant elimination of triphenylphosphane oxide, yielded the 5-substituted ester derivative **19** exclusively.[42] Aminolysis of **19** with primary or secondary (a)cyclic alkyl amines gave the target 5-substituted alkyl- or dialkylcarbamoyl-1,2,3-triazoles **20** and **21**.

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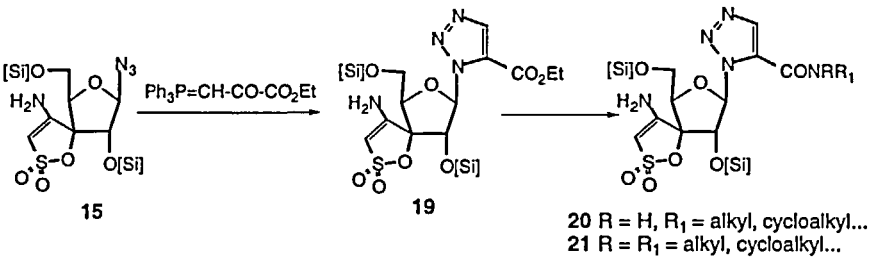
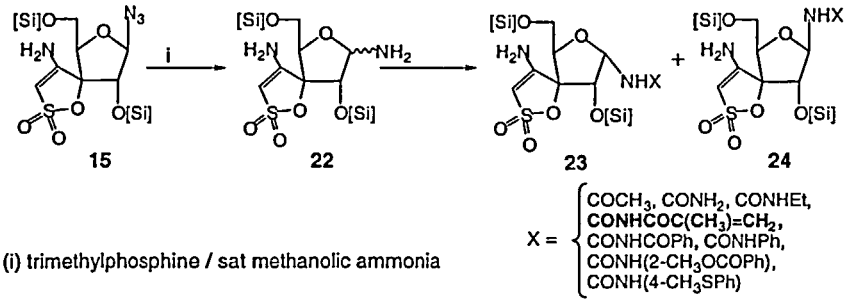


Scheme 5

Following our interest in further exploring the role that the nucleobase may play in the interaction of TSAO derivatives with the HIV-1 RT, we went a step ahead and tried to answer the following question: Is the base really necessary for this interaction? With this aim, a series of 3-spirosugar derivatives substituted at the anomeric position with amine, amide, urea or thiourea moieties derived from a systematic disassemblage of the molecular architecture of the thymine ring, that mimics parts or the whole thymine base of TSAO-T, were prepared.[37] The anomeric urea or thiourea substituted 3-spirosugar derivatives (**23** and **24**) were prepared (Scheme 6), in good yields, by reaction of the suitably substituted isocyanates or isothiocyanates with the 1-amino sugar intermediate **22**, prepared in quantitative yield by reduction of the ribosyl azide derivative **15**, according to the method of Staudinger.[45]

Those urea derivatives that mimic most closely the intact TSAO-T molecule retained the highest antiviral activity, among them the abasic *N'*-methacryloylurea spiro derivative [**24**; X = $CONHCOC(CH)_3=CH_2$] showed an activity just one order of magnitude lower than the prototype TSAO-T.



Scheme 6

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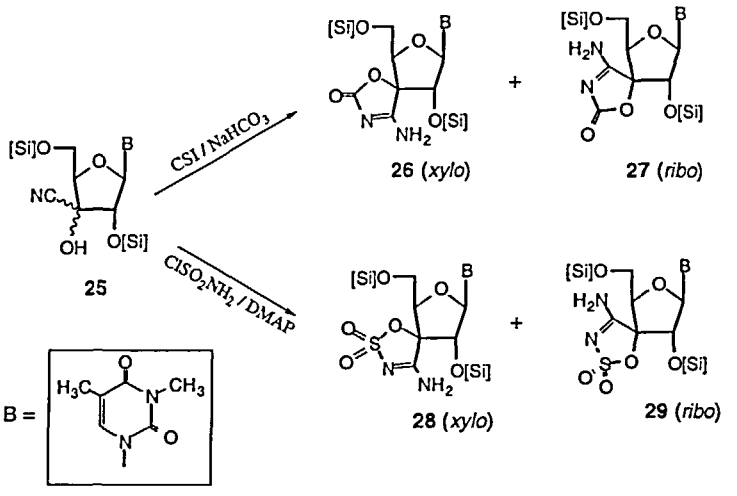
Molecular modelling studies[37] carried out with this prototype urea derivative indicate[37] that a heteroaromatic ring is not an absolute requirement for a favorable interaction between TSAO derivatives and HIV-1 RT. Urea derivatives, which can mimic to a large extent both the shape and the electrostatic potential of a thymine ring, can effectively replace this nucleic acid base when incorporated into a TSAO molecular framework with only moderate loss of activity. The compounds of this series represent the first examples of sugar derivatives that interact in a specific manner with HIV-1 RT.[37]

3.3 Other 3'-spiro nucleoside analogues of TSAO-T

As part of our program to further explore the importance of substituent effects on the anti-HIV-1 activity of TSAO derivatives, we focused our attention on modifications of the 3'-spiro moiety of TSAO compounds. This represents the part of the molecule closest to the interface between the p51 and p66 subunit domains. Novel TSAO analogues were prepared[29] in which the spiro aminooxathioledioxide was replaced by other spiro moieties that maintain an NH_2 group at the same position of the NH_2 of TSAO-T (this amino group has been proposed to be responsible for the interaction of the prototype compound TSAO-T with the Glu-138 of p51 subunit of the enzyme).[21,22,24]

Our strategy for the synthesis of these new series of 3'-spiro nucleosides has been based on the functionalisation and ring closure of the corresponding cyanohydrins (**25**) obtained from 3'-ketonucleosides.[17b] This strategy allowed to obtain in 2-3 steps such highly functionalised nucleosides starting from a common precursor. The nucleosides



Scheme 7

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bearing a spiro 4-amino-2-oxazolone moiety at 3' position (**26** and **27**) were prepared (Scheme 7) by reaction of cyanohydrins **25**[17b] with chlorosulfonylisocyanate (CSI)[46,47] to give, after basic treatment (aq $NaHCO_3$), the target 3'-spiro nucleosides. The 3'-spiro 4-amino-1,2,3-oxathiazole-2,2-dioxide nucleosides **28** and **29** were prepared by reaction of cyanohydrins (**25**) with sulfamoyl chloride in the presence of DMAP.[48] Surprisingly, although the novel *ribo*-TSAO analogues (**27** and **29**) fulfilled the structural requirements of the TSAO family for activity, their anti-HIV-1 activity was at least 2 orders of magnitude lower (EC_{50}: 5.8-13 μM and 5.3 μM for **27** and **29**, respectively) than that of the parent TSAO-m^3T derivative (**10**) (EC_{50}: 0.04-0.06 μM).[17b,29]

A comparative study based on NMR studies in solution and on theoretical calculations[49] of the hydrophobicity, the solvation free energies and molecular electrostatic potentials (MEP) of the three compounds (**10**, **27** and **29**) showed that no significant conformational differences were detected in solution between TSAO-m^3T and its analogues. The calculated hydrophobicity (log P) values, dipole moments and the electrostatic contributions to the solvation free energies of the three spiro ring systems were also similar. However, the differences found in the calculated MEPs of the spiro systems, among the three compounds suggested that the different electrostatic surroundings of the 4"-amino group of the spiro moiety in the TSAO analogues with respect to that of the parent TSAO-m^3T may be responsible for a detrimental electrostatic interaction of the spiro rings of these new analogues (**27** and **29**) with the Glu-B138 of the enzyme HIV-1 RT.[49]

4. INCORPORATION OF TSAO-T IN POTENTIAL BIDENTATE INHIBITORS OF HIV-1 RT

4.1 [NRTI]-[NNRTI] heterodimers

The binding of the NNRTIs to their hydrophobic pocket of the HIV-1 RT slows down the rate of incorporation of the dNTPs (as dNMPs) in the DNA product.[50] Because of the cooperative interaction between the substrate-binding site and nonsubstrate (NNRTI)-binding-site, combination of the functionalities of a non-nucleoside (NNRTI) and a nucleoside (NRTI) type RT inhibitor has been postulated to result in a very tight binding to the HIV-1 RT.[50]

On the other hand, crystallographic studies revealed that the NRTI binding site and the NNRTI binding pocket are close enough (aprox. 10-19Å)[15] to be reached by one single molecule that combines in its structure the functionalities of both kinds of inhibitors.

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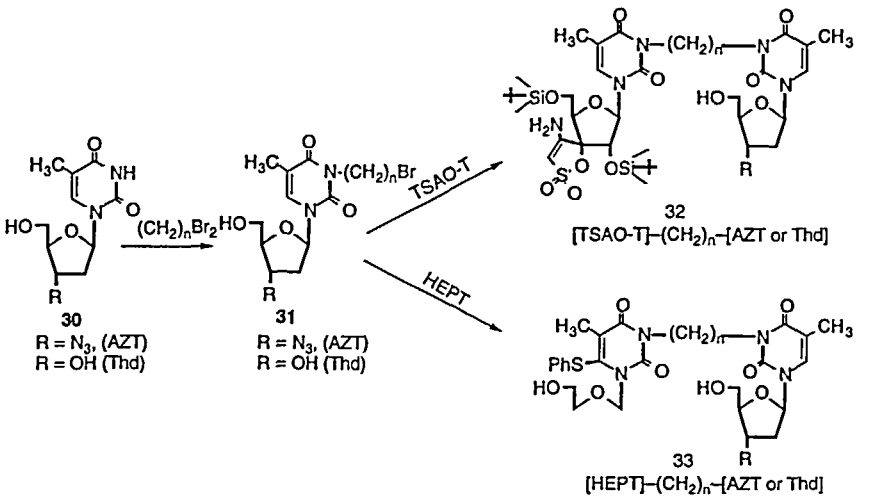
With this aim, and in an attempt to combine the HIV-inhibitory capacity of NRTI analogues and NNRTI, we have prepared and evaluated for their anti-HIV-activity several heterodimers of general formula [NRTI]-$(CH_2)_n$-[NNRTI], which combine in their structure a NRTI such as AZT and a NNRTI such as TSAO-T or HEPT.[51] These two classes of inhibitors (NRTI and NNRTI) were linked at the N-3 of the thymine base of each compound by an appropriate spacer. As spacer, we used aliphatic chains of different lengths in order to obtain a heterodimer possessing an optimum distance between both active principals (NRTI and NNRTI).

The heterodimers were prepared by basic treatment of the first key nucleoside **30** (Scheme 8) (AZT or dThd) with the appropriate dibromoalkyl reagent followed by reaction of the N-3-bromoalkyl-AZT or N-3-bromoalkyl-dThd nucleoside intermediates (**31**) with the NNRTI (TSAO-T or HEPT), to give the N-3,N-3-alkyl heterodimers (**32** or **33**).[51]

The [TSAO-T]-$(CH_2)_n$-[AZT] heterodimers (**32**) proved markedly inhibitory to HIV-1. The best compound of this series was the [AZT]-$(CH_2)_3$-[TSAO-T] heterodimer. However, the compound was a less potent inhibitor than the parent compound from which it was derived.[51]

In order to obtain better insights in the feasibility of this approach and to increase the inhibitory efficacy of the test compounds against the HIV-1 RT, novel series of [NRTI]-spacer-[TSAO-T] heterodimers were prepared.[52] Four types of modifications were carried out (Scheme 9) in the model heterodimers [AZT]-$(CH_2)_3$-[TSAO-T].



Scheme 8

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First, we expanded the heterodimer approach to other approved NRTIs and therefore, we replaced the AZT in the model heterodimer by d4T (**34** and **35**). Second, we focused on modifications of the spacer, and prepared a series of [AZT]-spacer-[TSAO-T] heterodimers in which the flexible polymethylene spacer of the heterodimer prototype was replaced by an expanded range of spacers of different length (**36** and **37**), conformational freedom (**38** and **39**) or of aromatic nature (**40**). Third, in order to circumvent the dependence of the NRTI moiety of the heterodimer on activation by nucleoside kinases, we prepared heterodimers of general formula [5'-MMP-NRTI]-$(CH_2)_n$-[TSAO-T] in which the NRTI bore a masked monophosphate group at the 5'-position (**41-46**).

[NRTI]N^3–$(CH_2)_n$–N^3[TSAO-T]

34 NRTI = d4T; n = 3
35 NRTI = d4T; n = 6
36 NRTI = AZT ; n = 6
37 NRTI = dThd ; n = 6

[AZT]N^3–Spacer–N^3[TSAO-T]

38 Spacer = $-(CH_2)_2-O-(CH_2)_2-$
39 Spacer = $-CH_2-C{\equiv}C-CH_2-$
40 Spacer = $-CH_2-Ph-CH_2-$

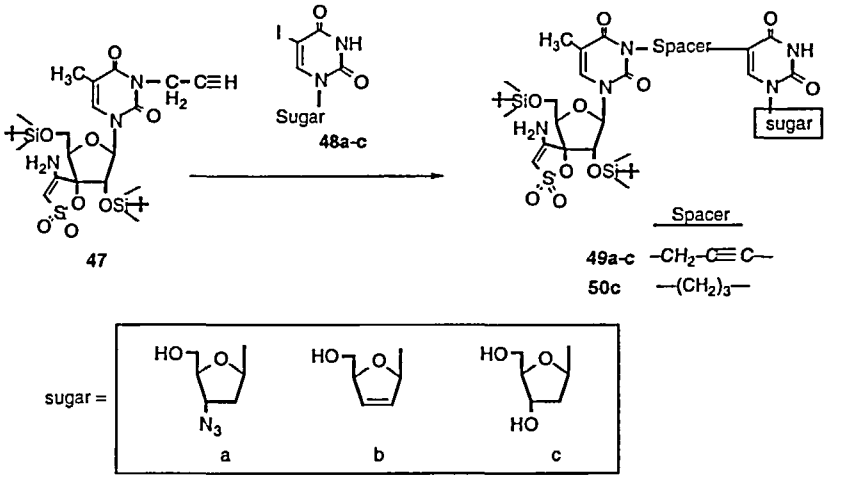
[5'-MMP-NRTI]N^3–$(CH_2)_3$–N^3[TSAO-T]

41 NRTI = AZT; n = 3
42 NRTI = AZT; n = 6
43 NRTI = d4T; n = 3
44 NRTI = d4T; n = 6
45 NRTI = dThd; n = 3
46 NRTI = dThd; n = 6

Scheme 9

Finally, we explored other attachment points of the spacer, by anchoring the linker at the C-5 position of the thymine base of the NRTI and at the N-3 position of the

thymine base of TSAO-T. For the synthesis of this latest series of heterodimers (**49** and **50**), we developed a facile and straightforward two-step procedure outlined in Scheme 10.



Scheme 10

The key step for the synthesis involves the palladium cross-coupling reaction[53-55] of terminal alkyne **47**[52b] with 5-iodo nucleosides **48a-c**, in DMF with tetrakis (triphenylphosphine)palladium(0), copper(I)iodide and triethylamine, to give heterodimers **49a-c**. The alkyne **47** was easily prepared in excellent yield (90%) by reaction of TSAO-T with propargyl bromide in the presence of K_2CO_3. Finally, hydrogenation of **49c** on Pd/C gave heterodimer **50c** with a flexible polymethylene linker.

Among the novel heterodimers, several derivatives show potent anti-HIV-1 activity, which proved comparable, or even superior, to that of the AZT heterodimer prototype. The nature of the NRTI was important for the eventual anti-HIV-1 activity. In particular, the d4T heterodimer derivative containing a propyl linker between the N-3 positions of the base of TSAO-T and d4T (**34**) was ~ 5- to 10- fold more inhibitory to HIV-1 than the corresponding AZT heterodimer prototype.[52b] The nature of the linker seems important for the activity of the heterodimers. Ether type **38** or double bond (**39**) linkers gave compounds with similar activity to the parent heterodimer. In contrast, introduction of an aromatic spacer (**40**) resulted in a markedly decreased activity. Change in the position of the linker in the ddN from N-3 to C-5 (**49a-c** and **50c**) gave compounds with similar or 5-9 fold lower activity than the parent N^3-N^3-heterodimer.[52b]

4.2 Metal-chelating TSAO-T derivatives

It has been reported that there is a measurable interaction between the inhibitors

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binding pocket and the Mg^{2+} binding site.[50] Therefore, attaching an NNRTI to molecules that should bind specifically to the nearby metal ion-binding site might create bidentate inhibitors with a stronger affinity, even for mutant enzyme forms. With this aim, we prepared, as potential bidentate inhibitors,[56] several TSAO derivatives which combine in their structure the functionality of a NNRTI (TSAO-T) and a metal chelating moiety linked by an appropriate spacer at the N-3 position of the thymine. As a chelating moiety we chose a β-diketone group due to its known properties to form strong complexes with divalent metal ions.[57,58] As spacer, we used polymethylene linkers of different lengths.

The synthesis of the target structures[56] was carried out (Scheme 11) by a two step procedure that consisted of alkylation of β-diketones (**51**) with the appropriate dibromoalkyl reagent to give a mixture of bromo-alkyl-β-diketones (**52**) and bromo-alkylenol ethers (**53**). Coupling of the bromoalkyl intermediates thus obtained (**52** and **53**), with TSAO-T in the presence of K_2CO_3 gave the TSAO-T-alkyl-chelating compounds **54** and **55**.

Some of the new compounds preserved their anti-HIV-1 activity being comparable to that of the parent compound TSAO-T, but display a markedly increased antiviral selectivity.[56] There was a clear trend between antiviral activity and length of the spacer that links the TSAO molecule with the chelating moiety. A shorter spacer invariably resulted in an increased antiviral potency of the compounds.

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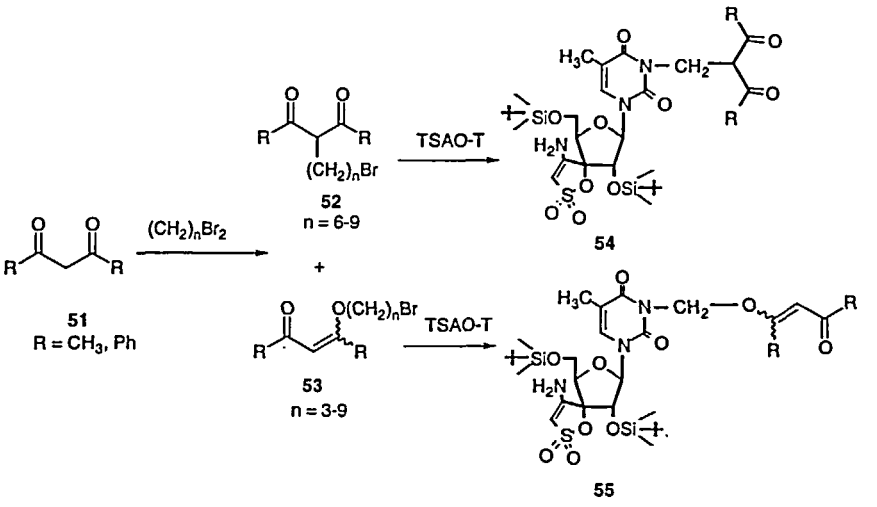

Scheme 11

5. MODEL OF INTERACTION OF TSAO-T WITH HIV-1 RT

Despite the similar binding geometry observed for several chemically diverse inhibitors in their complexes with HIV-1 RT, considerable variation exists in the non-

nucleoside binding pocket of the protein.[59] Co-crystallisation attempts with TSAO derivatives have been unsuccessful so far. This fact, together with the unusual drug resistance profile of TSAO (Glu138→Lys) and recent experimental results that suggest RT dimer disruption upon binding of TSAO derivatives,[25,26] strongly suggest a binding mode for TSAO that may deviate substantially from that of other non-nucleoside inhibitors.

Our working model is based on the coordinates of RT inhibitor complexes and extensive conformational analyses and molecular dynamics simulations. Particular attention is being paid to those aminoacid residues in the binding site that are known, by sensitivity studies of resistant HIV-1 strains, to be important for the interaction of TSAO derivatives, and also to the structure-activity relationships. In our preliminary model, TSAO binds at the interface between the p51 and p66 subunits of RT in such a way that most of the molecule lies within the non-nucleoside binding pocket and only the spiro moiety slightly protrudes toward the p51 subunit so that the exocyclic amino group is placed in a suitable position for hydrogen bonding to the side chain of Glu138. This TSAO fragment is known to be crucial for activity, and this protein residue is the one that changes first under the selective pressure of TSAO derivatives being replaced by a lysine. The spiro ring and the amino group of TSAO in its complex with RT are thus nearly superimposable on the phenyl ring and the OH group of Tyr A181, respectively, in the structure of the apoenzyme or the catalytically active complex.[60]

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The bulky 5'-*tert*-butyldimethylsilyl substituent is docked into the cavity delineated by the aromatic rings of Tyr-A181, Tyr-A188 and Trp-A229 and the hydrophobic rings of Pro-A95 and Pro-A97. The numerous contacts may account for the rather stringent requirements for this group at this position. On the other hand, the substituent on the 2' position, which is also a *tert*-butyldimethylsilyl group in TSAO-T, interacts more loosely with the side chains of Val-A106 and Val-A179, and can therefore accommodate more structural variation albeit at a cost in affinity. The thymine ring is situated immediately above the plane of Tyr-A318, in a cavity delineated by the side chains of residues Leu-A100, Lys-A103, Val-A106, and Leu-A234 and the carbonyl groups of Lys-A101, His-A235 and Pro-A236[37] so that the different N-3 substituents are placed facing the proposed entrance to the pocket, namely, the channel between Pro-A236 and the A225-A226 and A105-A106 loops.

6. CONCLUSIONS

Since the discovery of TSAO-T, more than 400 derivatives have been prepared. This overview is mostly focused on their syntheses, that have not always been straight-

forward, due to the high degree of functionalisation, stereogenic centres and the presence of both acid and base sensitive groups. The evaluation of all these derivatives has allowed a better understanding of the interaction of the TSAO-family with its target enzyme (HIV-1 RT), and from this evaluation it can be concluded that the sugar template of TSAO-derivatives is crucial to place the different substituents (at positions 1', 2',3' and 5') in an optimal position to correctly interact with the enzyme.

The differences found in TSAO derivatives, when compared with the rest of NNRTIs, in the selection of HIV-1 resistant strains, in the amino acids involved in their interaction and on a possible interference with the dimerisation process makes of TSAO molecules valuable and "unique" tools among HIV-1 RT inhibitors.

7. ACKNOWLEDGEMENTS

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