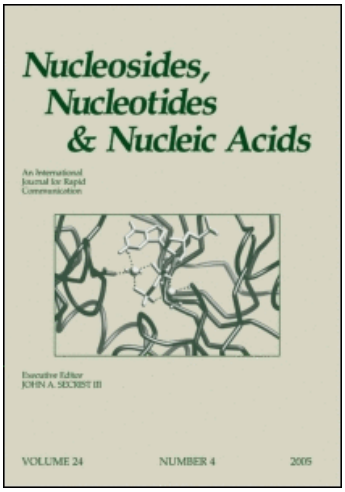

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4″-H-TSAO-T, A NOVEL PROTOTYPE IN THE HIV-1 SPECIFIC TSAO FAMILY

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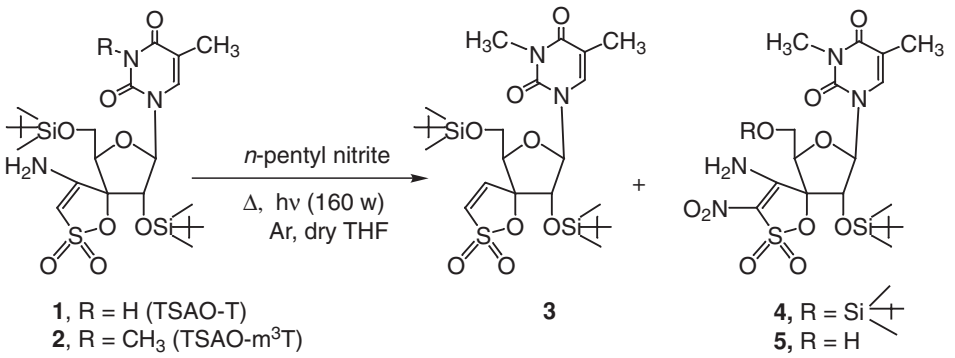
ABSTRACT

The first TSAO derivative that lacks the amino group at the 3′-spiro moiety has been prepared. This molecule retained its HIV-1 specificity (NNRTI characteristic) but did not select for any of the classical NNRTI-specific mutations in the NNRTI binding pocket, including 138-Lys (TSAO resistant strain).

AIDS treatment is seriously compromised by the relatively fast emergence of drug resistant virus variants. A key target in the search for effective drugs useful for AIDS therapy is the viral reverse transcriptase (RT). Among the different inhibitors of HIV RT, the nonnucleoside RT inhibitors (NNRTIs) represent a group of highly potent and specific inhibitors of HIV-1 replication (1). Given the exquisite potency of the NNRTIs to suppress virus replication in cell culture (2) and their capacity to lower by several orders of magnitude the virus load in HIV-1 infected individuals (3), the NNRTIs may become the cornerstone of future HIV treatment modalities when the resistance issue can be adequately addressed.

Among NNRTIs, TSAO nucleosides (4,5) occupy a unique position because they need to interact with the p51 subunit of the HIV-1 RT heterodimer (6). The prototype compound of this family is the thymine derivative designed as TSAO-T (**1**) and the most selective compound is TSAO-m^{3}T (**2**, Scheme 1). Recently, it has been proposed that TSAO molecules seem to interfere with the dimerisation process of the enzyme (7–9), resulting in loss of viral DNA binding affinity. This suggests a completely new and different mechanism of inhibition of HIV-RT with regard to the other known NNRTIs.



Scheme 1.

TSAO derivatives consistently select, in cell culture, for Glu-138-Lys mutated virus strains that are highly resistant to the inhibitory effect of TSAO but that retain sensitivity to the other NNRTIs as well as to nucleoside RT inhibitors (NRTIs). Our experimental data together with molecular modelling studies, strongly suggest a specific interaction between the 138-Glu of the p51 subunit of HIV-RT and the 4″-amino group of the 3′-spiro moiety of TSAO molecules. Based on this hypothesis, we were interested in removing this amino group and evaluate the effect on the activity and resistance profile of the corresponding deaminated TSAO derivative **3** (Scheme 1).

The synthesis of **3** was carried out by anhydrous diazotization/radical deamination procedure (10) using *n*-pentyl nitrite as the nitrosating agent. This non-aqueous, non-acidic reductive deamination conditions were selected to be compatible with the crucial TBDMS groups. Thus, when TSAO-m^{3}T (**2**) was treated with *n*-pentyl nitrite in THF, as hydrogen atom donating solvent, and the resulting mixture was warmed and photolyzed with visible light, the corresponding deaminated TSAO derivative **3** was isolated in low yield, together with the 3″-nitro-4″-amino spiro derivative **4** and its 5′-deprotected compound **5**.

The deaminated compound **3** proved inhibitory to HIV-1 replication in CEM ($EC_{50} = 0.15\ \mu M$) and in MT-4 ($EC_{50} = 0.53\ \mu M$) cell cultures. The compound was also inhibitory against HIV-1 RT ($IC_{50} = 3.32\ \mu M$). However, it was devoid of any activity either against HIV-2 RT or HIV-2 replication. On the other hand, the 3″-nitro-derivative **4** showed moderate activity against HIV-1 in cell culture ($EC_{50} = 7.0\ \mu M$ in CEM and $5.99\ \mu M$ in MT-4).

Compound **3** was able to select for virus resistant strains. The virus strain that emerged under escalating drug pressure of **3** was used for sensitivity/resistance testing against a variety of NNRTIs (including TSAO derivatives) and NRTIs. Interestingly, this drug resistant strain kept full sensitivity to all NRTI and NNRTIs, including TSAO compounds. Resistance was observed only against the deaminated TSAO derivative **1** in the presence of which it was selected. Even more intriguing is the observation that the drug also lost inhibitory potential against virus mutants

that contain NNRTIs specific mutations in the reverse transcriptase. The latter observations point to an interaction with the NNRTI pocket of the RT. However, when sequencing the RT gene of the drug resistant virus strain, we could not detect any of the well known NNRTI specific mutations in the RT gene (11). It is, still, unclear whether a mutation has appeared downstream of the region where the NNRTI mutations are located (i. e. RNAse H domain). These studies are currently ongoing.

In conclusion, we have synthesized the first TSAO molecule that lacks the amino group at the 3′-spiro moiety. This deaminated TSAO molecule showed a novel resistance spectrum. It kept its HIV-1 specificity (NNRTI characteristic) but did not select for any of the classical NNRTI-specific mutations, including 138-Lys (TSAO resistant strain). Also, the resistant viruses that emerged under deaminated TSAO pressure, were only resistant to this drug, but not to other NNRTIs including any other TSAOs that contain the amino group on the spiro moiety. So, the deaminated TSAO derivative represent a new type of molecule with HIV-1 specificity, but most likely with another mechanism of action than the classical NNRTIs.

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