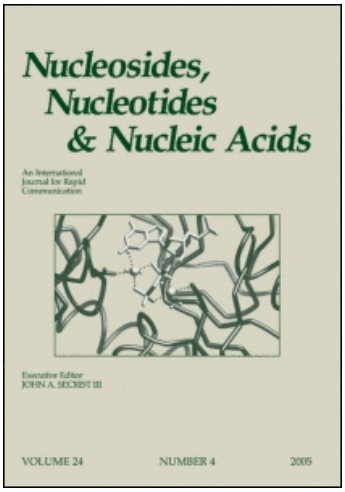

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Synthesis of Tetrazole Oxathiolane Nucleoside Analogues and Their Evaluation as HIV-1 Antiviral Agents

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SYNTHESIS OF TETRAZOLE OXATHIOLANE NUCLEOSIDE ANALOGUES AND THEIR EVALUATION AS HIV-1 ANTIVIRAL AGENTS

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ABSTRACT : The synthesis of 2-hydroxymethyl-5-[N_2-(5'-carboxamido tetrazolyl)]-1,3-oxathiolane (6a and 6b) and 2-hydroxymethyl-5-[N_2-(5'-aminotetrazolyl)]-1,3-oxathiolane (7a and 7b) is described. It involved the preparation of suitable 1,3-oxathiolane precursors via cyclocondensation between benzoyloxyacetaldehyde and 2-mercaptoacetaldehyde di-[2-methoxyethyl] acetal, followed by condensation of adequately substituted tetrazoles using trimethylsilyltriflate or titanium tetrachloride as acid catalysts. In a preliminary in vitro study these new tetrazole oxathiolane nucleoside analogues were found inactive against HIV-1 retrovirus.

Recent studies of a number of nucleosides in which the ribose moiety was replaced by five membered rings e.g. dioxolane or oxathiolane have demonstrated that these nucleoside analogues inhibit the *in vitro* replication and cytopathic effect of HIV-retrovirus, the ethiologic agent of AIDS (1-4). In addition, the broad spectrum antiviral activity of triazole ribonucleoside such as Ribavirin (5-7) have stimulated the interest of synthesizing ribonucleosides in which the triazole heterocycle was replaced by an isosteric heterocycle, the tetrazole ring (8,9). Based on these two biological observations, it was of interest to design new nucleoside analogues which structure incorporates an

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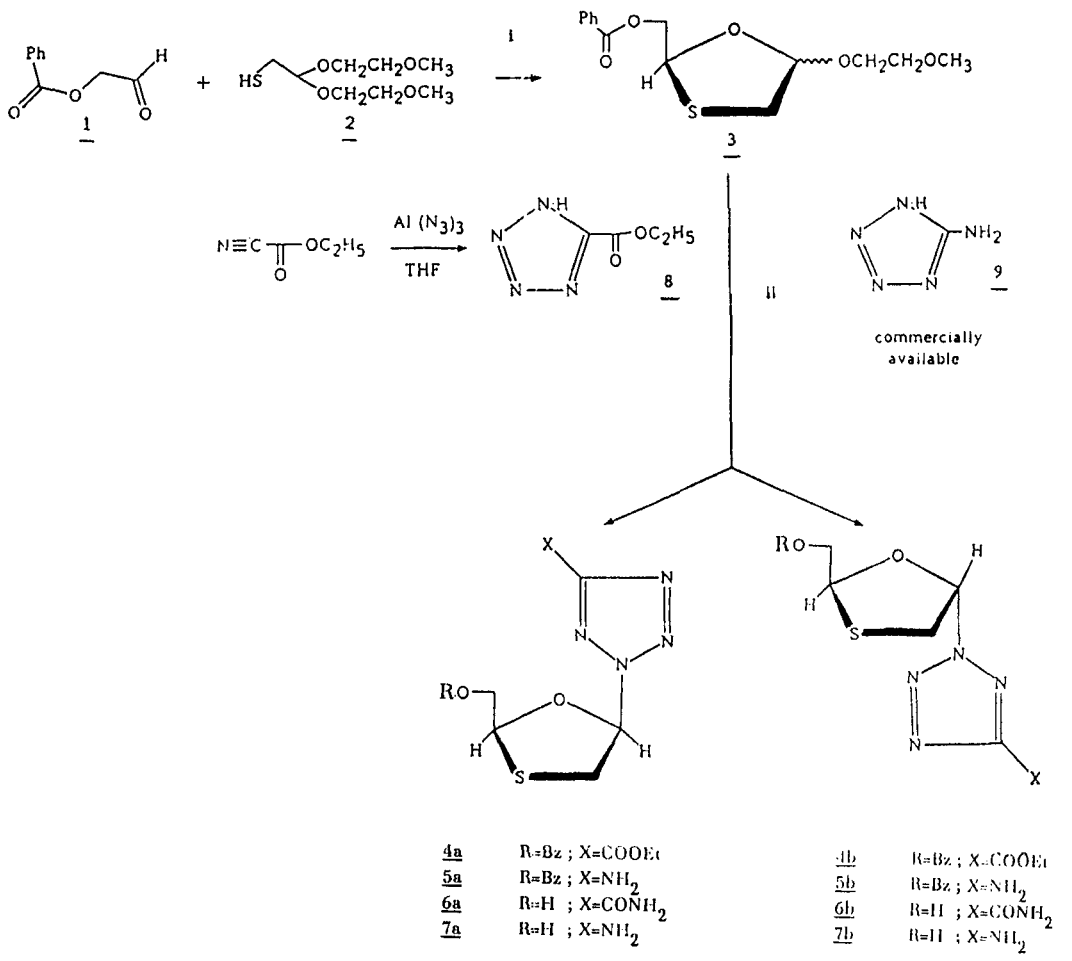


I. reflux 4h. in toluene, Ac pTs catalytic amount
II. TMS-Tf O° C, CH_3CN or $TiCl_4$ O°C, CH_3CN

scheme 1

1,3-oxathiolane ring substituted by suitable tetrazole moieties. The present work describes the synthesis of the cis and trans isomers of 2-hydroxymethyl-5-[N_2-(5'-carboxamidotetrazolyl)]-1,3-oxathiolane (6b and 6a) and 2-hydroxymethyl-5-[N_2-(5'-aminotetrazolyl)]-1,3-oxathiolane (7b and 7a) and their antiviral evaluation against HIV-1 virus.

RESULTS AND DISCUSSION

The synthetic strategy used for the preparation of these new nucleoside analogues is summarized in scheme 1. It involved in the first step the

Table 1

compound	C-5 of tetrazole moiety
5-aminotetrazole	157.0
5a	166.7
5b	166.6
5-carboxamidotetrazole	151.8
6a	164.3
6b	165.8

cyclocondensation of benzoyloxyacetaldehyde 1 with 2-mercaptoacetaldehyde di-[2-methoxyethyl]acetal 2 (10,11) at reflux in toluene with p-Toluenesulfonic acid as catalyst, leading to 2-benzoyloxymethyl-5-[2-methoxyethyloxy]-1,3-oxathiolane 3 in 55% yield.

The second step involved the condensation of suitable tetrazoles with the 1,3-oxathiolane intermediate 3. 5-aminotetrazole 9 is a commercially available compound, 5-carboethoxytetrazole 8 was prepared by addition of aluminium azide on ethylcyanoformate, followed by acidic hydrolysis (12). After silylation of tetrazole derivatives using 1,1,1,3,3,3-hexamethyldisilazane, the condensation on the 1,3-oxathiolane intermediate 3 was catalyzed using trimethylsilyltriflate or titanium tetrachloride in anhydrous CH_3CN. Due to the structure of the tetrazole ring, one might expect 4 isomers (N_1 cis, N_1 trans, N_2 cis and N_2 trans) which could possibly be formed during the condensation. The resulting tetrazole oxathiolane nucleoside analogue mixture contained in each case two predominant isomers, which were identified as the N_2 cis and N_2 trans isomers. According to the observations reported by Poonian et al. (9), it appears that the chemical shift of the carbon-5 of the tetrazole ring for N_1-substituted tetrazole is identical to the one of the tetrazole moiety alone, while for N_2-substituted tetrazole, the chemical shift of the carbon-5 is shifted downfield by approximately 10 ppm. Table 1 summarized the ^{13}C NMR data observed for the C_5 chemical shift, in the case of compound 5a, 5b, 6a, 6b and the parent tetrazole, 5-aminotetrazole and 5-carboxamidotetrazole.

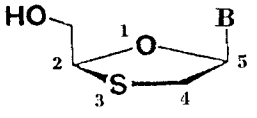
Moreover, we observed by TLC that under the experimental conditions used, N_1 tetrazole nucleoside analogues were present only in trace amounts. This result seems to be in good agreement with the observations reported by Poonian et al. (9), who observed that acid-catalyzed condensation between 5-aminotetrazole and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose led to the $N_2\beta$ adduct as the predominant component. The two isomers 4a and 4b were easily separated by flash column chromatography. However it was not possible at this step to separate the compounds 5a and 5b.

In the final step of the synthesis, direct aminolysis using ammonia in methanol led directly to the fully deprotected 5-carboxamidotetrazole

Table 2

	cis			trans		
	6a	7a	BCH-189	6b	7b	BCH-189
H_2	5.35	5.09	5.25	5.48	5.27	..5.65
H_5	6.18	6.41	6.30	6.30	6.62	..6.48

nucleoside analogues 6a and 6b through a one pot reaction, while similarly the final 5-aminotetrazole nucleoside analogues 7a and 7b were obtained. It should be mentioned that all the obtained analogues are diastereoisomer mixtures. The structure of the final compounds was established through ^1H NMR data. Indeed the chemical shifts for the protons H_2 and H_5 of the 1,3-oxathiolane ring are known to be characteristic of the cis/trans conformation. The chemical shifts for H_2 and H_5 protons in compounds 6a, 6b, 7a and 7b were found to be very similar to the corresponding protons of the wellknown parent nucleoside BCH-189 already reported by Belleau et al. (1,2). Table 2 summarized the different chemical shifts for H_2 and H_5 protons in all cases.



6a, 6b: B= 5-carboxamidotetrazole
7a, 7b: B= 5-aminotetrazole
BCH-189: B= cytosine

The antiviral activity was tested by the Syncytium formation assay. The assessment of antiviral potency was based on the cytopathogenic effect of HIV-1 (HIV-1 BRU prototype) on MT_4 cells. Syncytium formation was evaluated under an inverted optical microscope, 4 to 7 days after HIV-1 injection. However, these new tetrazole 1,3-oxathiolane nucleoside analogues failed to show significant antiviral activity against MT4 cells infected by HIV-1. Moreover these new compounds showed significant toxicity.

In conclusion, a strategy for the synthesis of new tetrazole 1,3-oxathiolane nucleoside analogues has been achieved. The lack of significant activity against HIV-1 clearly indicates that the introduction of a tetrazole ring as a pseudo nucleic base abolishes the antiviral activity. This observation appears to be of interest since on the one hand, the triazole ring in the case of ribofuranose serie (Ribavirin) was found active on the replication of HIV in human adult T lymphocytes (13,14,15) and on the other hand 1,3-oxathiolane ring substituted with cytosine was found of therapeutic interest against HIV-1 (1,2,3).

EXPERIMENTAL SECTION

Chemistry

^1H NMR spectra were recorded on a BRUKER AM200. The chemical values δ were expressed in ppm relative to tetramethylsilane. IR absorption

spectra were recorded with Perkin-Elmer Infracord 357 Instrument. TLC analysis were carried out using Merck pre-coated silicagel 60 F_{254} plates. Flash column chromatography separations were performed using Merck G60 silicagel (230-400 mesh) under pressure. Melting points were taken in a Buchi SMP-20 apparatus in sealed tubes and are uncorrected.

2-benzoyloxymethyl-5-[2-methoxyethyloxy]-1,3-oxathiolane 3 has been synthesized as earlier described (11).

5-ethoxycarbonyl tetrazole 8.

To an heterogeneous solution of $AlCl_3$ (2.680 g, 0.02mol), NaN_3(5.842 g, 0.089mol.) in anhydrous THF, ethylcyanoformate (2.0g, 0.02 mole) was added. The solution was refluxed for 18h. After acid hydrolysis (HCl 15%, 40 ml), the aqueous layer was extracted with EtOAc (3x30 ml) and dried over $MgSO_4$. After solvent evaporation, the tetrazole 8 was isolated in 55% yield; mp = 90°C
^{1}H NMR ($CDCl_3$)- 12.8 (broad s,1H,NH), 4.52(q,2H,CH_2), 1.4(t,3H,CH_3)
IR (cm^{-1}) 2300-3200 tetrazole ring.

cis and trans isomers of 2-benzoyloxymethyl-5-[N_2-(5'-ethoxycarbonyl tetrazolyl)]-1,3-oxathiolane 4a and 4b.

After silylation using 1,1,1,3,3,3-hexamethyldisilazane, 5-ethoxycarbonyl tetrazole 8 (0.150 g, 1.1 mmol) was dissolved in dry acetonitrile (5 ml). 1 equivalent (0.250 g, 1 mmole) of the corresponding 1,3-oxathiolane 3 in CH_3CN was added to this solution, at 0°C under nitrogen. After half an hour, trimethylsilyltriflate (0.130 ml, 0.71 mmol) was added via a syringe. The reaction was kept 5 h at 0°C, then 15h at room temperature, and finally quenched with 5% hydrogen sodium carbonate solution (5 ml). After solvent extraction (EtOAc, 3x10 ml) and drying over $MgSO_4$, the mixture of cis and trans isomers was obtained in 50% yield in a ratio cis/trans = 1/4. The two isomers were separated by flash column chromatography using mixture of EtOAc-Toluene [0.5 : 9.5] as eluent.
trans isomer : Rf : 0.50 [EtOAc-Toluene, 1:4] 40% yield
4b : ^{1}H NMR ($CDCl_3$) - 7.4-8.0(m,5H,arom), 6.97(dd,1H,O-C<u>H</u>-N), 5.92(t,1H,O-C<u>H</u>-S), 4.55(q,2H,C<u>H</u>$_2$-O), 4.46(d,2H,C<u>H</u>$_2$-O), 3.70(dd,1H,S-C<u>H</u>$_2$), 3.32(dd,1H,S-C<u>H</u>$_2$), 1.44(t,3H,C<u>H</u>$_3$)
cis isomer : Rf : 0.39 [EtOAc-Toluene, 1:4] 10% yield
4a : ^{1}H NMR ($CDCl_3$) - 7.34-8.16(m,5H,arom), 6.76(dd,1H,O-C<u>H</u>-N), 5.75(t,1H,O-C<u>H</u>-S), 4.60(q,2H,C<u>H</u>$_2$-O), 4.51(m,2H,C<u>H</u>$_2$-O), 3.77(dd,1H,S-C<u>H</u>$_2$), 3.35(dd,1H,S-C<u>H</u>$_2$), 1.45(t,3H,C<u>H</u>$_3$)

cis isomer of 2-hydroxymethyl-5-[N_2-(5'-carboxamido tetrazolyl)]-1,3-oxathiolane 6a

The cis isomer 4a (0.013 g, 0.035 mmol) was stirred for 15h in methanolic ammonia solution (5 ml) and after purification by flash column

chromatography [EtOAc-Hexane , 7 : 1], compound 6a was obtained in 50% yield.
Rf : 0.16 [EtOAc-Toluene, 7:1]
^1H NMR (CD_3OD) - 6.18(dd,1H,O-C<u>H</u>-N), 5.36(t,1H,O-C<u>H</u>-S), 3.71(m,2H,C<u>H</u>$_2$-OH), 3.40(dd,1H,S-C<u>H</u>$_2$), 2.99(dd,1H,S-C<u>H</u>$_2$)

trans isomer of 2-hydroxymethyl-5-[N_2-(5'-carboxamido tetrazolyl)]-1,3-oxathiolane 6b

Similarly, compound 4b (0.035 g, 0.096 mmol) was stirred for 24h in methanolic ammonia solution (7ml) and after purification by flash column chromatography [EtOAc-Hexane, 4:1], compound 6b was obtained in 70% yield.
Rf : 0.10 [EtOAc-Hexane, 4:1]
^1H NMR (CD_3OD) - 6.30(dd,1H,O-C<u>H</u>-N), 5.48(t,1H,O-C<u>H</u>-S), 3.45(m,3H,C<u>H</u>$_2$-OH and S-C<u>H</u>$_2$), 3.06(dd,1H,S-C<u>H</u>$_2$)

cis and trans isomers of 2-benzoyloxymethyl-5-[N_2-(5'-aminotetrazolyl]-1,3-oxathiolane 5a, 5b

After silylation with 1,1,1,3,3,3-hexamethyldisilazane, 5-aminotetrazole (0.172 g, 2.14 mmole) was dissolved in dry acetonitrile (5 ml). The corresponding 1,3-oxathiolane 3 (0.408 g, 1.63 mmole) dissolved in dry CH_3CN (5ml) was added to this solution at 0°C under nitrogen . 0.155 ml (1.12 mmol) of titanium tetrachloride was added via a syringe to this cold mixture. The reaction mixture was kept at 0°C for 2h, then 24h at room temperature, and finally quenched with 5% hydrogen sodium carbonate solution (7ml). After solvent extraction (EtOAc, 3x15 ml) and drying over $MgSO_4$, the mixture of cis and trans isomers was obtained in 30% yield (crude mixture). Purification by flash column chromatography using EtOAc-Toluene mixture as eluent did not led to a separation of the two isomers but a clean mixture of cis and trans isomers [1/1] was isolated (27 mg).
cis/trans isomers mixture : Rf : 0.23 [EtOAc-Toluene , 1:4]
^1H NMR ($CDCl_3$)- 10.58(s broad,2H,NH_2), 7.90-7.95(m,2H,arom), 7.41-7.60(m,3H,arom), 6.78[dd,1H,O-C<u>H</u>-N,(trans)], 6.55[dd,1H,O-C<u>H</u>-N,(cis)], 6.13[dd,1H,O-C<u>H</u>-S,(trans)], 5.89[dd,1H,O-C<u>H</u>-S,(cis)], 4.7-4.5[m,2H,COOC<u>H</u>$_2$,(cis+trans)], 3.90-3.80[dd,1H,SC<u>H</u>$_2$(cis+trans)], 3.2-3.35[dd,1H,SC<u>H</u>$_2$,(cis+trans)]

2-hydroxymethyl-5-[N_2-(5'-aminotetrazolyl)]-1,3-oxathiolane 7a and 7b

A mixture of isomers 5a, 5b (0.015g , 0.050 mmole) was stirred for 24h in a methanolic ammonia solution (5ml). After evaporation and purification by flash column chromatography using EtOAc-Methanol [1:0.05] as eluent, the two isomers were separated in a 1 to 1 ratio.
trans isomer 7b : Rf : 0.36 [EtOAc-Methanol, 4:1] 45% yield

^{1}H NMR (DMSO-d_6)- 6.62(dd,1H,O-C<u>H</u>-N), 5.27(dd,1H,O-C<u>H</u>-S), 4.02(m,2H,C<u>H$_2$</u>-OH), 3.32(dd,1H,S-C<u>H$_2$</u>), 3.06(dd,1H,S-C<u>H$_2$</u>)

cis isomer <u>7a</u> : Rf : 0.18 [EtOAc-Methanol, 4:1] 46% yield

^{1}H NMR (DMSO-d_6)- 6.41(dd,1H, O-C<u>H</u>-N), 5.09(dd,1H,O-C<u>H</u>-S), 3.80(m,2H,C<u>H$_2$</u>-OH), 3.30(dd,1H,S-C<u>H$_2$</u>), 3.0(dd,1H,S-C<u>H$_2$</u>)

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