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SYNTHESIS AND ANTI-HIV ACTIVITY OF 3'-DEOXY-3'-(*N*-HYDROXYAMINO) ANALOGUES OF NUCLEOSIDES

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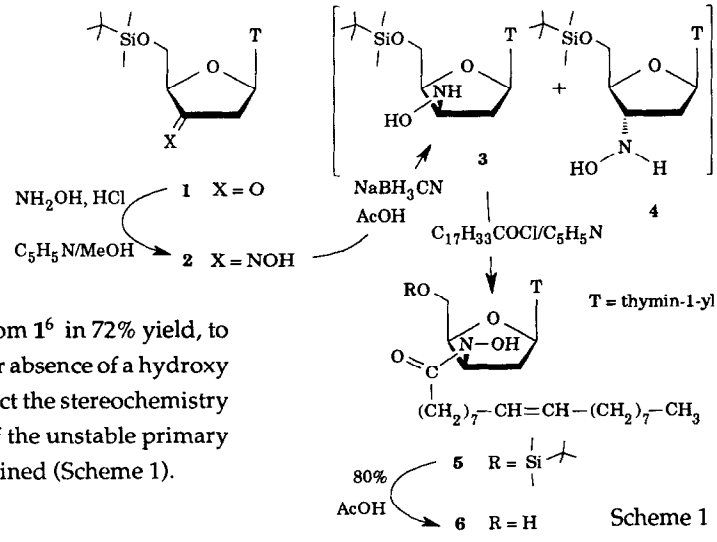
Abstract. 3'-Deoxy analogues of thymidine and uridine bearing a 3'-*N*-alkyl-(or *N*-aralkyl) -*N*-hydroxyamino group either on the α or the β face of the furanose ring have been prepared. One of these (**13**), exhibited a moderate anti-HIV activity.

Modified nucleosides still represent the most useful chemical series for the treatment of HIV viral infections. Such compounds, both endowed with anti-HIV activity and bearing a paramagnetic probe, could constitute efficient tools for the study of the mode of action of antiviral molecules. We have shown[1] that deoxy-*N*-hydroxyamino sugars, very close analogues of their natural oxygen-bearing counterparts, spontaneously oxidize in the air to the corresponding aminoxyl free radicals, thus affording spin labelled molecules. We describe here the preparation and properties of 3'-deoxy-3'-*N*-hydroxyaminonucleoside derivatives, one of which exhibits anti-HIV activity. Part of this work has been presented at a Carbohydrate Symposium.[2]

Two different synthetic pathways can be used to prepare sugar-modified nucleosides, one, direct, consisting in modifying the sugar moiety of an existing nucleoside, the other, indirect, implying the synthesis of a modified sugar then submitting it to a nucleosidation reaction. Both approaches will be described.

The most general and high-yielding routes to sugar hydroxylamines consist in the reduction of oximes[3] or nitrones.[4] The direct pathway, applied to the 3'-ketouridine oxime had led exclusively to the non natural *xylo* epimer.[5] We applied the same reaction to the oxime **2**, prepared from **1**[6] in 72% yield, to determine whether the presence or absence of a hydroxy group at the 2' position would affect the stereochemistry of the reaction. A *ca* 4:1 mixture of the unstable primary hydroxylamines **3** and **4** was obtained (Scheme 1).

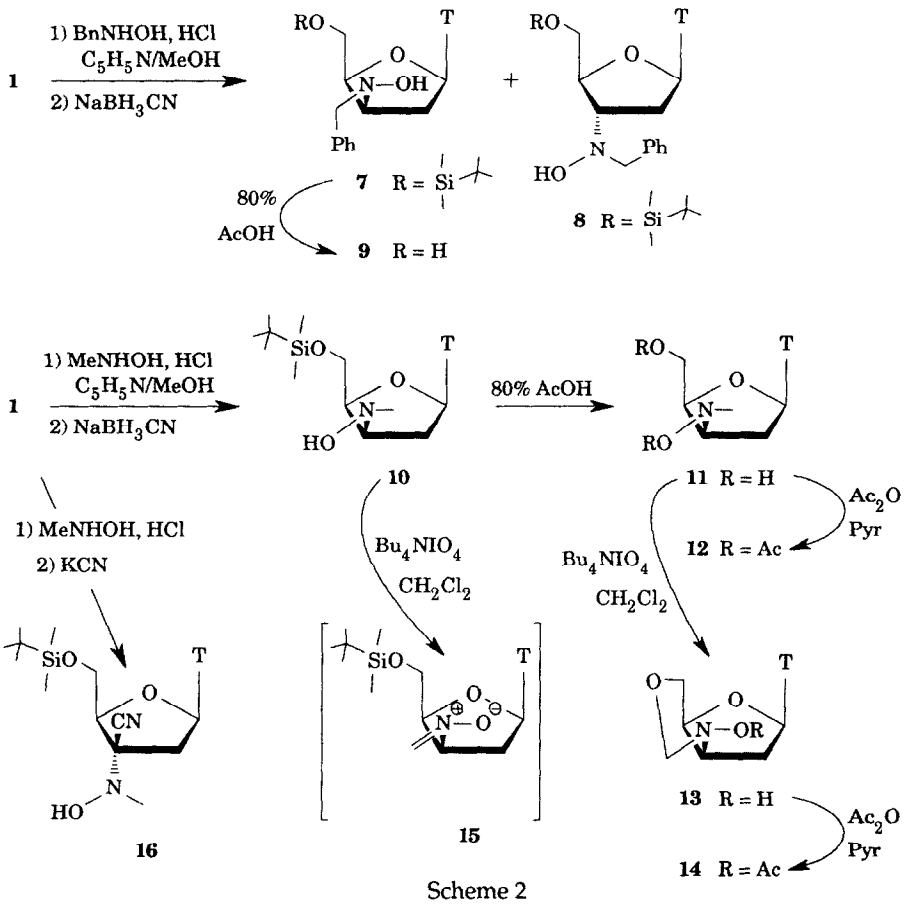


Scheme 1

+ Deceased September the 6th, 1992

The epimeric mixture, further enriched[7] in **3**, was acylated to the hydroxamic acid **5**[8] (67% yield) which was de-*O*-silylated to **6** (70%). More lipophilic than natural nucleosides, compounds like **6** constitute a novel class of potential antiviral agents. They spontaneously oxidize to amidoxyl free radicals, longer-lived than the corresponding aminoxyls owing to electron delocalization on the carbonyl group.

The reactions *via* nitrones led to different stereochemical fates depending on the nature of the reacting hydroxylamine. Treated with *N*-benzylhydroxylamine, **1** gave the expected nitrone which was not isolated[9] but immediately reduced to a 3:1 resolvable mixture of hydroxylamines **7** and **8** (cumulated 67% yield from **1**). The major epimer **7** was almost quantitatively de-*O*-silylated to **9**[10] (Scheme 2).



Scheme 2

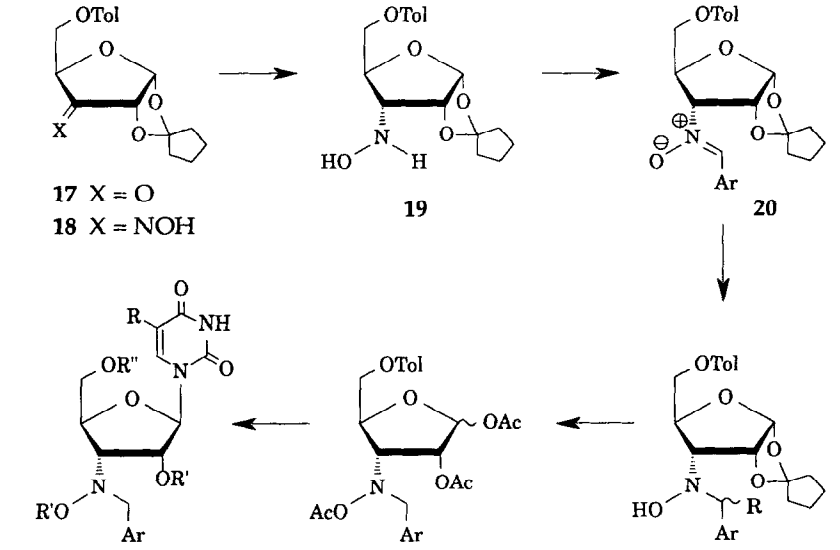
Reaction of **1** with *N*-methylhydroxylamine then sodium cyanoborohydride led stereospecifically to **10** (86%) which was de-*O*-silylated to **11**, itself quantitatively di-*O*-acetylated to **12**. Periodate oxidation of **11** gave **13**[11] (63% yield) which was quantitatively *O*-acetylated to **14**. The β-*threo* configuration of **13** was assigned from its NMR spectrum[11] as well as its conformation, shown to consist in a $^{3'}T_{4'}$ form for the furanose ring and a somewhat flattened $^{O(5')}C_{3'}$ chair for the perhydrooxazine ring.

In order to check that the formation of **13** from **11** proceeded *via* a "methylenic nitrone"[12] which then

underwent a nucleophilic cyclization, we ran the oxidation reaction on **10**, compound in which the blocked 5' position prevents the cyclization step. In these conditions, we obtained the unstable methylenic nitrone **15** which could not be isolated in a pure form. Structure of **15** was nevertheless clearly established from its MS (383, $M^{\cdot+}$, 368, $M^{\cdot+}$ - $Me^{\cdot}$, 326, $M^{\cdot+}$ - Me_3C) and ^{1}H-NMR spectra (δ 6.4 and 6.59, 2 *d*, *J* = 7.5 Hz, H_2C=N).

Treatment of **1** with *N*-methylhydroxylamine then potassium cyanide led to **16** (32%). The addition on the intermediate nitrone took place, in this case, from the β face, no trace of the second epimer being found. The C-3' configuration of **16** was established by NOE experiments that showed, in particular, nuclear spin population transfer between N-CH_3 and protons H-1', H-2'$_{pro\text{-}R}$ and H-4'.

The indirect method (Scheme 3) starts from the ketosugar derivative **17**,[13] the 1,2-*O*-cyclopentylidene blocking group having been chosen for its particular ease of hydrolysis. The oxime **18** (60%), obtained as a *ca* 1:1 unresolvable mixture of *E* and *Z* diastereoisomers, was stereospecifically reduced ($NaBH_3CN$, pH 3) to the *ribo* "first generation" hydroxylamine **19** in 54% yield. By using sodium cyanoborodeuteride, (3-$^2H_1$)**19** was obtained. Upon treatment with a series of aromatic aldehydes, **19** led to the nitrones **20** in yields ranging from 63 to 92%. Reduction of **20** ($NaBH_4$) gave the corresponding "second generation" hydroxylamines **21** (50-86% yields) whereas reducing **20** (Ar = *p*-methoxyphenyl) with sodium borodeuteride led stereoselectively to the epimeric mixture **21'** in which one epimer of unknown configuration was preponderant. Starting from (3-$^2H_1$)**19**, (3-$^2H_1$)**21** was also prepared. These specifically deuterated derivatives have proved useful for the interpretation of ESR spectra (*vide infra*). Hydrolysis (Dowex 50 H^+) of **21**, followed by acetylation, gave **22** (50-62% yields). Compounds **22** were submitted to nucleosidation using di-*O*-trimethylsilylated uracil, 5-bromouracil and 5-iodouracil. The β anomer of **23** (13 examples) was preferentially formed in moderate (30-40%) yields. In two cases, **23** has been deblocked to **24** (R = Br, Ar = *p*-FC_6H_4 and R =I, Ar = *p*-$MeOC_6H_4$).[14]



Scheme 3

Among the described compounds, those bearing a *N*-hydroxyamino group spontaneously oxidized in solutions from which air was not thoroughly excluded, leading to a minute stationary concentration of the corresponding free radicals. The concentration of these paramagnetic species was sufficient to allow the obtention of good EPR spectra, but too small to significantly degrade the resolution of NMR spectra. These EPR data are collected in the Table. The influence of the spin density on nitrogen is apparent from the fact that the value of the a_N hyperfine coupling corresponding to **5**, compound bearing an α carbonyl group, is about half that of the other compounds. For aminoxyls in which the aminoxyl group is not conjugated with any π system, a_N depends mainly on the planarity of the aminoxyl group[15] measured as the angle (α) between the N-O bond and the C_α-N-C_α plane, a_N values of 14.5-15.2 G in diglyme corresponding to α values of 10 ± 5°. For planar (or almost planar) aminoxyls, conformational information can be obtain by using the empirical equation $a_{H\beta} = B_2\cos^2\theta$, with $B_2 = (25 \pm 1)$[16] of 26 G[17] where θ stands for the dihedral angle between a C-H_β bond and the axis of the p_z orbital on nitrogen. From the EPR data of the aminoxyl radicals generated from **7**, **8**, and **11** on the one hand and **21** and **21'** on the other hand, it appears that the conformation around the C_3-N bond was less affected by a configurational change at C-3 than by changes in the substitution at C-2. Aminoxyls from **7**, **8**, and **11** predominantly exist as eclipsed conformers in which the C-4 bond lies in the plane of the aminoxyl group (or close to that situation) whereas in the case of **21**, conformers corresponding to higher values of a_H are more represented. The conformational equilibrium corresponding to a rotation around the N-CH_2 bond clearly implies only species in which one of the C-H benzylic bonds lies in the aminoxyl plane[16] for **7** and **8**, whereas for **21**, besides one conformer of this type, the participation of another, almost staggered, conformer cannot be excluded. Moreover, the EPR spectrum of the aminoxyl generated from **13** confirmed the structure of its diamagnetic counterpart as established by NMR.

Table. EPR data (in diglyme, a values in G) of some representative free radicals spontaneously formed from the corresponding hydroxylamines or hydroxamic acids.

Starting diamagnetic species	temp	g	a_N	a_{H3}	a_{CH2N}		long-range a_H		
5	85 °C	2.0066	7.59	2.47					
7	120 °C	2.0058	14.50	3.10	9.50	7.90	1.15	0.80	0.80
8	100 °C	2.0060	15.00	3.40	8.60	8.60	0.50	0.50	0.50
11	100 °C	2.0058	14.60	3.60	3x12.90[a]		1.00	0.80	
13	70 °C	2.0060	15.10	20.00	4.75	15.80			
16	50 °C	2.0061	15.20		3x11.60[a]		0.50	0.50	0.50
21[b]	65 °C	2.0058	14.70	12.50	10.40	7.40	0.30		
(3-$^2H_1$)**21**[b]	60 °C	2.0060	14.65	1.90[c]	10.35	7.20	0.40		
21'[b]	62 °C	2.0064	14.60	12.90	1.55[c]	7.30			

a. CH_3-N. b. Ar = *p*-methoxyphenyl. c. Coupling with deuterium.

Most of the deblocked compounds cited have been tested for their antiviral activity against a model oncovirus SV_{40}.[18] Marginal activities have been measured for **9**, **11** and **16**. On the other hand, **9** and **13**

have been submitted to the NCI *in vitro* Anti-AIDS Drug Discovery Program of the US National Cancer Institute. Following a described procedure,[19] compound **9** was found inactive, but **13** rated as moderately active against HIV-1 with a molar EC_{50} and IC_{50} of $2.5.10^{-6}$ and $>2.10^{-4}$ respectively. These scores do not establish **13** as a clinically useful anti-AIDS drug but as a potential new lead compound, its bicyclic structure and *threo* configuration being different from those of the established anti-AIDS nucleosides.

Acknowledgements

The authors warmly thank Prof. M. Geoffroy for the measurements of the EPR spectra, Prof. A. Buchs and Dr F. Gulaçar for the MS, Dr F. Barbalat-Rey for the NMR, Dr H. Eder for the microanalyses, Dr N. Dolatshahi for antiviral tests with SV_{40}, and, Dr I. Komaromi and Miss A. Ricca for quantum and molecular mechanics computations. This work was supported by the Swiss National Science Foundation (grant # 20-31259.91).

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7. Flash column chromatography (silica gel, 19:1 Et2O/MeOH) did not allow a perfect separation.
8. All new isolated compounds gave satisfactory data (^{1}H-NMR, IR, UV, MS, combustion analysis) consistent with the assigned structure.
9. Nitrones of 3'-ketothymidine are very unstable, still more prone to E_{1cB} elimination of the base than 3'-ketouridine itself.
10. Mp 97.1-98.4 °C; $[\alpha]_D^{22}$ +31° (*c* =1, $CHCl_3$).
11. Compound **13** was obtained as white crystals: mp 197.6-198.5 °C, R_F 0.28 (9:1 CH_2Cl_2/MeOH), $[\alpha]_D^{23}$ +168° (*c* 0.45, $CHCl_3$) ; λ_{max}^{EtOH} 206 nm (ε 8982), 266 (8482); ν_{max}^{KBr} 3488 (NH), 3392 (OH), 1296, 1661 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 9.96 (*bs*, 1 H, NH), 8.03 (*s*, 1 H, H-6), 7.75 (*bs*, 1 H, N-OH), 6.08 (*d*, 1 H, $J_{1',2'pro-R}$ = 7.2 Hz, $J_{1',2'pro-S}$ = 0, H-1'), 4.62, 3.85 (2 *d*, 2x1 H, *J* = 7.5 Hz, NCH_2), 4.30 (*d*, 1 H, $J_{5'pro-R,5'pro-S}$ = 13 Hz, $J_{5'pro-S,4'}$ = 0, H-$5'_{pro-S}$), 4.15 (*dd*, 1 H, $J_{5'pro-R,4'}$ = 1.8 Hz, H-4'), 3.75 (*dd*, 1 H, H-$5'_{pro-R}$), 3.23 (*dd*, 1 H, $J_{2'pro-R,3'}$ = 4.5 Hz, $J_{2'pro-S,3'}$ = 0, $J_{3',4'}$ = 3.5, H-3'), 2.85 (*d*, 1 H, $J_{2'pro-R,2'pro-S}$ = 14.5 Hz, H-$2'_{pro-S}$), 2.40 (*ddd*, 1 H, H-$2'_{pro-R}$), 1.85 (*s*, 3 H, CH_3),. MS: *m/z* (%) 81 (100), 127 (64, thymine), 68 (38),

143 (21, $M^{\cdot+}$ - thymine), 99 (18), 196 (1.2), 252 (0.6, $M^{\cdot+}$ - OH), 269 (0.5, $M^{\cdot+}$).

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14. Properties of **24** (R = Br, Ar = *p*-F-C_6H_4): mp 85.5-87.5 °C, R_F 0.4 (6:1 $CHCl_3$/MeOH), $[\alpha]_D^{21}$ -43.2° (*c* 0.8, MeOH); λ_{max}^{EtOH} 208 nm (ε 16500), 280 (8400); ν_{max}^{KBr} 3404 (OH), 3200 (NH), 1703 (CO) cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.68 (*bs*, 1 H, NH), 8.13 (*s*, 1 H, H-6), 7.40 (*m*, 2 H, H-3'' + H-5''), 7.14 (*m*, 2 H, H-2'' + H-6''), 5.90 (*bs*, 1 H, N-OH), 5.62 (*d*, 1 H, H-1'), 5.31 (*bs*, 1 H, OH), 4.69 (*dd*, 2 H, $J_{1',2'}$ = 1 Hz, H-2' + OH), 4.31, 3.81 (AB, 2 H, J_{CH2} = 14.5 Hz, NCH_2), 3.75 (*ddd*, 1 H, H-4'), 3.45 (*dd*, 1 H, $J_{4',5'b}$ = 6 Hz, H-5'b), 3.41 (*dd*, 1 H, $J_{4',5'a}$ = 7 Hz, $J_{5'a,b}$ = 11, H-5'a), 2.88 (*dd*, 1 H, $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 2, H-3'). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 162.40 (C-4''), 149.99-159.19 (C=O), 141.36 (C-6); 133.72 (C-1''), 130.43 (C-2''), 115.08 (C-3''), 94.65 (C-5), 89.98 (C-1'), 79.65 (C-2'), 75.54 (C-3'), 68.39 (C-4'), 63.23 (C-5'), 59.5 (N-CH_2). MS: *m/z* (%) 109 (100), 147 (18), 149 (17), 190 (16), 192 (16), 122 (10), 120 (11), 100 (9), 255 (0.3, $M^{\cdot+}$ - base).
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