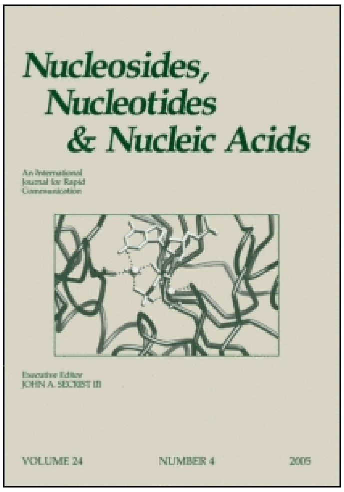

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Novel Types of Bicyclonucleosides: Isoxazolidinofuranosylthymines

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NOVEL TYPES OF BICYCLONUCLEOSIDES: ISOXAZOLIDINOFURANOSYLTHYMINES

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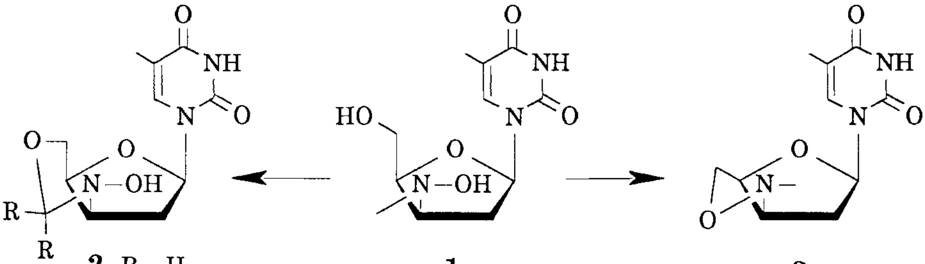
Abstract: Novel types of bicyclonucleosides (**3**, **9**) bearing a 3',5'-epoxyimino (or iminoepoxy) bridge have been prepared using intramolecular Mitsunobu reactions. Contrarily to close analogs, also of the β-D-*threo* configuration but oxidizable to nitrones (**2**, **2'**) which were found active against HIV, the new compounds exhibited no anti-HIV activity.

Compounds **1** and **2** (Scheme 1) have been shown[1,2] to represent the first examples of nucleoside analogs of the β-D-*threo* configuration exhibiting a significant activity against HIV-1 and HIV-2. Furthermore, **2** constituted the first example of an anti-HIV active bicyclonucleoside. The most interesting compound,[3] so far, in these series is the dimethyl derivative[4] **2'**. It is well known that deoxy-*N*-hydroxyamino compounds, e.g. **1**, spontaneously oxidize in the air to the corresponding aminoxyl free radical **1'** (for a review, see ref. 5).



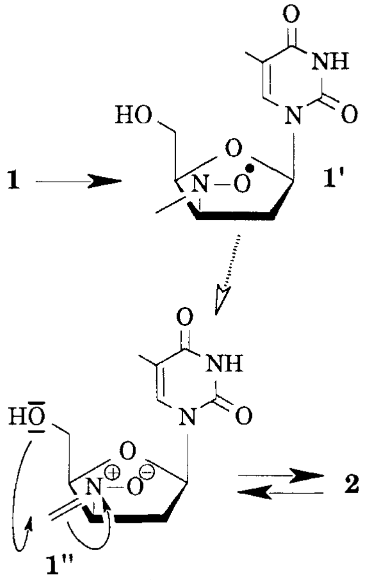
2 R = H
2' R = Me

1

3

Scheme 1

The intermediate aminoxyl further oxidizes to a nitrone, a methylenic nitrone[4-7,2] **1''** with a variety of oxidizing agents (Scheme 2). The methylenic nitrone **1''** undergoes a ring-chain tautomerism with **2**. Thus, all the active compounds in these series can give rise to a nitrone and moreover, the most active of all, **2'** is the one for which the open nitrone form is the more abundant at equilibrium. One working hypothesis, which is being tested using experimental and computational means, would be that the electrophilic methylidene group of **1''** could function as a surrogate of the 5'-*O*-phosphoryl group of an incoming nucleoside triphosphate about to be incorporated into the new DNA strand by HIV reverse transcriptase (RT). Alternatively, some nucleophilic site of the HIV RT could be blocked by the same electrophilic methylene group. In order to assess that compounds unable to generate an electrophilic methylene group, but having some structural resemblance with these active nucleosides - a β-D-*threo* configuration, an aminoxy or *N*-hydroxyamino group at the 3' position - were inactive, we prepared some examples of such compounds, in particular both possible topoisomers of nucleoside analogs bearing an oxazaethano 3',5' bridge. It should be noted, however, that compound **2** was found[8] inactive against HIV-2 in CEM/TK⁻ cells (deficient for thymidine kinase) indicating the necessity of a prior phosphorylation of this compound to promote its antiviral activity. Some of the new analogs prepared cannot be phosphorylated without prior (bio)modification.



Scheme 2

An intramolecular Mitsunobu reaction[9] applied to **1**, afforded **3** in 71% yield. A small amount (*ca* 10%) of an oxidized 1:1 adduct of **1** and diethyl azodicarboxylate was also formed, but was not isolated in pure state. The structure of **3** was established from its spectroscopic data, in particular a shielded H-3' (δ 3.58, TABLE 1) confirming the presence of a nitrogen atom and a large value of $J_{3',4'}$ (6.5 Hz, TABLE 2) indicating the presence of a fused ring. Moreover, the H-3' NMR signals, poorly resolved at room temperature, confirmed the presence of a slowly inverting nitrogen atom at that position.

TABLE 1. ^{1}H NMR Data of Nucleosides Analogs. Chemical Schifts ($CDCl_3$, 25 °C, 200 MHz, δ in ppm).

Cmpd	H-1'	Hα-2'	Hβ-2'	H-3'	H-4'	Ha-5'	Hb-5'
3[a]	6.36	2.67	2.01	3.58	5.00	4.02	4.13
5	6.29	2.62	2.52	5.01	4.17	4.10	4.32
6	6.21	2.57	2.30	4.32	~4	~4	~4
7	6.20	2.54	2.62	4.60	~4.08	~4.08	4.21
8	6.07	2.48	2.16	4.13	3.88	3.61	3.72
9[a]	6.15	2.73	2.23	4.70	4.91	3.20	3.42
10	6.36	2.73	2.32	4.80	4.90	3.91	4.08

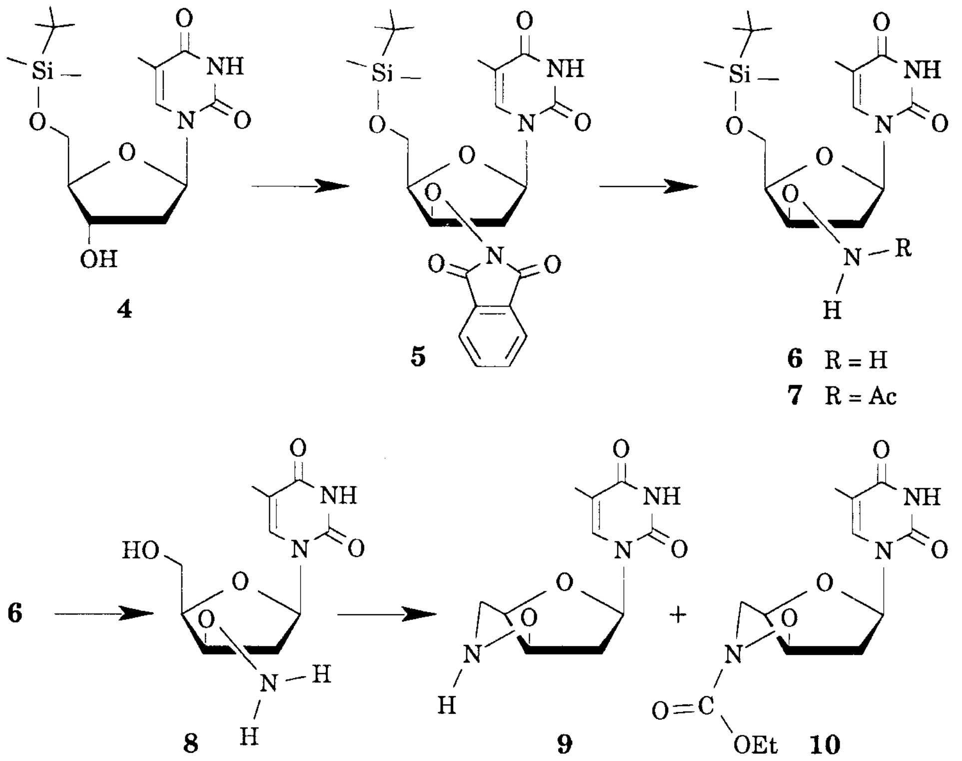
[a] t = 55 °C.

TABLE 2. ^{1}H NMR Data of Nucleosides Analogs. Interproton Couplings ($CDCl_3$, 25 °C, 200 MHz, J in Hz).

Cmpd	$J_{1',2'\alpha}$	$J_{1',2'\beta}$	$J_{2'\alpha,2'\beta}$	$J_{2'\alpha,3'}$	$J_{2'\beta,3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
3[a]	7.5	6.0	14.5	7.5	4.0	6.5	1.8	4.0	10.0
5	7.5	4.0	15.0	4.5	1.5	3.0	6.5	3.0	9.5
6	8.0	3.0	15.0	5.0	1.5	~2.0	?	?	?
7	7.0	3.5	16.0	4.7	~1.0	~3.0	?	?	13.0
8	8.0	3.0	15.0	5.5	0.5	4.0	7.0	5.0	11.5
9[a]	7.5	6.0	15.0	7.5	2.5	5.0	5.0	1.0	12.5
10	7.5	6.0	15.0	6.5	2.0	4.0	5.0	0.5	12.5

[a] t = 55 °C.

Submitted to a Mitsunobu reaction using *N*-hydroxyphthalimide as a nucleophile, compound **4**[10,2] led to **5** (85%) which was hydrazinolyzed (79%) to the *O*-aminonucleoside **6** (Scheme 3). This method of preparation of *O*-(*N*-phthalimido)sugars was introduced by Grochowski and Jurczak,[11] then used for the preparation of various kinds of *O*-aminosugars and modified disaccharides.[7,12] Two preliminary accounts reporting the synthesis of *O*-aminonucleosides have also been published.[13] These *O*-aminonucleosides



Scheme 3

constitute structurally close analogs (isosters of the superior homolog) of natural nucleosides but imparted with markedly different nucleophilic properties. Upon treatment with 2-acetoxypropenenitrile, **6** was almost quantitatively mono-*N*-acetylated to **7**, whereas its acidic methanolysis afforded **8** (75% yield). An intramolecular Mitsunobu reaction of **8** gave a resolvable mixture of **9** (75%) and **10** (10%). In the case of **9**, the 5' protons are the more affected by the presence of the inverting nitrogen (and exchanging NH group). The ^{1}H-NMR data of TABLES 1 and 2, in particular the δ values of H-3' and H_2-5', confirm the structure assignment.

Compounds **3**, **8** and **9** have been submitted to the NCI *in vitro* anti-AIDS Drug Discovery Program of the US National Cancer Institute where, following a described procedure,[14] they were found inactive. They were also found inactive against a model oncovirus SV_{40} following a procedure previously described for Polyoma virus.[15] These compounds are also devoid of cytotoxic activity (minimum molar cytotoxic concentration superior to 10^{-3}).

EXPERIMENTAL

General Methods.[16]

1-[2'.3',5'-Trideoxy-5',3'-(epoxy-*N*-methylimino)-β-D-*threo*-pentofuranosyl]thymine (3). - To a solution of **1** (270 mg, 1 mmol) in THF (30 mL), triphenylphosphine (790 mg, 3 mmol) was added and the solution stirred for 10 min under nitrogen. Diethyl azodicarboxylate (DEAD, 0.32 mL, 2 mmol) was then added dropwise. After completion of the reaction (20 min, TLC), the reaction mixture was concentrated and submitted to a column chromatography (30:1 CH_2Cl_2/MeOH) affording **3** contaminated with about 10% of an impurity which was discarded using a second column chromatography separation (15:4:1 EtOAc/cyclohexane/MeOH). After a further chromatographic purification (14:5:1 chloroform/petroleum ether/MeOH), **3** (180 mg, 71%) was obtained as white crystals: mp 106.2-106.6 °C, R_F 0.19 (15:4:1 EtOAc/cyclohexane/MeOH); $[\alpha]_D^{22}$ -25.26° (*c* 0.9, $CHCl_3$); λ_{max}^{EtOH} 207 nm (ε 17900) and 266 (17000); ν_{max}^{KBr} 3560 (NH), 3160, 3020 (CH), and 1680 (C=O) cm^{-1}. ^{1}H-NMR, see TABLES 1 and 2; 8.30 (*bs*, 1 H, NH), 7.70 (*q*, 1 H, $J_{6,Me}$ = 1.0 Hz, H-6), 2.70 (*s*, 3 H, NMe), and 2.00 (*d*, 3 H, Me-5). MS: *m/z* (%) 85 (100), 69 (66), 128 (64, $M^{\cdot+}$ - thymine), 97 (54), 54 (36), 253 (22, $M^{\cdot+}$), 207 (4), 194 (3.6), 181 (1), and 223 (0.4, $M^{\cdot+}$ - CH_2O).

Anal. Calcd for $C_{11}H_{15}N_3O_4$ (253.26): C, 52.17; H, 5.97; N, 16.59. Found: C, 51.90; H, 5.95; N, 16.41.

1-(5'-*O*-*tert*-Butyldimethylsilyl-2'-deoxy-3'-*O*-phthalimido-β-D-*threo*-pentofuranosyl)thymine (5). - A solution of **4** (1 g, 2.82 mmol), triphenylphosphine (0.96 g, 3.67 mmol) and *N*-hydroxyphthalimide in dry THF (35 mL) was stirred for 10 min at 20 °C under N_2, then DEAD (0.62 mL, 4 mmol) was added dropwise. After completion of the reaction (30 min, TLC), the reaction mixture, concentrated, was submitted to two successive column chromatographies (1:1 $CHCl_3/Et_2O$) to afford **5** (1.2 g, 84.7%) as a white solid: mp 138.0-139.0 °C, R_F 0.19 (1:1 $CHCl_3/Et_2O$); $[\alpha]_D^{21}$ -3.45° (*c* 1.04, EtOH); λ_{max}^{EtOH} 220 nm (ε 39300), 242 (11300), 266 (10120), and 300 (2300); ν_{max}^{KBr}

3400 (NH), 3160, 2850, 2820 (CH), 1740, 1680, 1650, and 1640 (C=O) cm^{-1}. ^{1}H-NMR, see TABLES 1 and 2; 8.44 (*bs*, 1 H, NH), 7.75-7.90 (*m*, 5 H, Ar + H-6), 2.00 (*d*, 3 H, $J_{6,Me}$ = 1.0 Hz, Me-5), 0.89 (*s*, 9 H, Me_3C), 0.10 and 0.09 (2 *s*, 2x3 H, Me_2Si). MS: *m/z* (%) 81 (100), 73 (43), 89 (39), 220 (15), 155 (14), 59 (12, $Me_3C\cdot$), and 502 (0.3 $M^{\cdot+}$).

Anal. Calcd for $C_{24}H_{31}N_3O_7Si$ (501.62): C, 57.47; H, 6.23; N, 8.38. Found: C, 57.37; H, 6.35; N, 8.25.

1-(3'-*O*-Amino-2'-deoxy-5'-*O*-*tert*-butyldimethylsilyl-β-D-*threo*-pentofuranosyl)thymine (6). - To a solution of **5**, (0.67 g, 1.34 mmol) in EtOH, hydrazine hydrate (0.2 mL, 4 mmol) was added. After 10 min stirring at 20 °C, the reaction mixture, concentrated, was submitted to a column chromatography (19:1 CH_2Cl_2/MeOH) to afford **6** (0.39 g, 78.6%): mp 171.7-172.5 °C, R_F 0.21 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D^{27}$ -81° (*c* 1.05, $CHCl_3$); λ_{max}^{EtOH} 209 nm (ε 9350), and 267 (8860); ν_{max}^{KBr} 3300, 3200 (NH), 3140, 3010, 2920, 2900 (CH), 1680, and 1640 (C=O) cm^{-1}. ^{1}H-NMR: see TABLES 1 and 2; 8.23 (*bs*, 1 H, NH), 7.48 (*q*, 1 H, $J_{6,Me}$ = 1.5 Hz, H-6), 5.51 (*bs*, 2 H, ONH_2), 1.94 (*d*, 3 H, Me-5), 0.93 (*s*, 9 H, Me_3C), and 0.16 (*s*, 6 H, Me_2Si). MS: *m/z* (%) 75 (100), 81 (63), 155 (55), 117 (20, Me_3CSiMe_2), 125 (12, thymine), 219 (5), 234 (2.5), 246 (2, $M^{\cdot+}$ - thymine), and 372 (0.2, $M^{\cdot+}$).

Anal. Calcd for $C_{16}H_{29}N_3O_5Si$ (371.51): C, 51.73; H, 7.87; N, 11.31. Found: C, 51.70; H, 7.83; N, 11.37.

1-(3'-*O*-Acetamido-2'-deoxy-5'-*O*-*tert*-butyldimethylsilyl-β-D-*threo*-pentofuranosyl)thymine (7). - A solution of **6** (370 mg, 1 mmol) and 2-acetoxypropenenitrile (0.22 mL, 2 mmol) in THF (10 mL) was refluxed for 2 h. The reaction mixture, concentrated, was submitted to a column chromatography (19:1 CH_2Cl_2/MeOH) which afforded **7** (380 mg, 92%) as a white solid: mp 91.0-92.0 °C, R_F 0.17 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D^{22}$ -90.9° (*c* 0.88, $CHCl_3$); λ_{max}^{EtOH} 212 nm (ε 5180), and 266 (8590); ν_{max}^{KBr} 3460 (NH), 3200, 2960, 2840 (CH), 1700, 1680, and 1650 (C=O) cm^{-1}. ^{1}H-NMR: see TABLES 1 and 2; 9.66 (*bs*, 1 H, NHAc), 9.50 (*bs*, 1 H, NH), 7.58 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 1.95 (*s*, 3 H, HNCOMe), 1.88 (*d*, 3 H, Me-5), 0.93 (*s*, 9 H, Me_3C) and 0.12 (*s*, 6 H, Me_2Si). MS: *m/z* (%) 81 (100), 73 (88, ONHAc), 132 (61), 127 (55, thymine), 188 (44), 156 (32), 230 (20), 414 (7.9, $M^{\cdot+}$), 288 (4.5, $M^{\cdot+}$ - thymine), 356 (3.5, $M^{\cdot+}$ - Me_3C), 299 (0.4, $M^{\cdot+}$ - Me_3CSiMe_2), and 339 (0.2, $M^{\cdot+}$ - ONHAc).

Anal. Calcd for $C_{18}H_{31}N_3O_6Si$ (413.55): C, 52.28; H, 7.56; N, 10.16. Found: C, 52.14; H, 7.67; N, 10.09.

1-(3'-*O*-Amino-2'-deoxy-β-D-*threo*-pentofuranosyl)thymine (8). - A solution of **6** (0.58 g, 1.56 mmol) in MeOH (20 mL) was brought to pH 1-2 by addition of 6M methanolic HCl. After 10 min at 20 °C, the reaction mixture was neutralized (10% NaOH in saturated NaCl aqueous solution), concentrated, then submitted to column chromatography (9:1 $CHCl_3$/MeOH) to afford **8** (0.3 g, 75%) as a solid: mp 132.3-133.2 °C, R_F 0.45 (9:1 $CHCl_3$/MeOH); $[\alpha]_D^{22}$ -20.4° (*c* 0.9, MeOH); λ_{max}^{EtOH} 209 nm (ε 8900), and 266 (8630); ν_{max}^{KBr} 3500-3100 (NH_2, NH, OH), 3010, 2900 (CH), 1690, and 1640 (C=O) cm^{-1}. ^{1}H-NMR: see Tables 1 and 2, 11.21 (*bs*, 1 H, NH), 7.48 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 6.20 (*bs*, 2 H, ONH_2), 4.80 (*t*, 1 H, $J_{5',OH}$ = 5.5 Hz, OH), and 1,75 (*d*, 3 H, Me-5). MS: *m*/*z* (%) 69 (100), 132 (88, $M^{\cdot+}$ - thymine), 81 (82), 127 (75, thymine), 55 (47), 172 (3.5), 258 (3.2, $M^{\cdot+}$ + 1), 207 (2.6), and 225 (2.1, $M^{\cdot+}$ ONH_2).

Anal. Calcd for $C_{10}H_{15}N_3O_5$ (257.25): C, 46.69; H, 5.88; N, 16.33. Found: C, 46.44; H, 5.91; N, 16.31.

1-[2',3',5'-Trideoxy-3',5'-(epoxyimino)-β-D-*threo*-pentofuranosyl]thymine (9). - To a mixture of **8** (0.32 g, 11.24 mmol), triphenylphosphine (0.9 g, 3.4 mmol) and THF (30 mL), DEAD (0.35 mL, 2.25 mmol) was added dropwise under N_2. After 20 min stirring at 20 °C, the concentrated reaction mixture was submitted to column chromatography (9:1 $CHCl_3$/MeOH) to give **9** (0.22 g, 74%) and **10** (0.04 g, 10%). Properties of **9** are the following: mp 196.0-197.0 °C, R_F 0.4 (9:1 $CHCl_3$/MeOH); $[\alpha]_D^{26}$ +47° (*c* 0.95, EtOH): λ_{max}^{EtOH} 261 nm (ε 3800) and 265 (3890); ν_{max}^{KBr} 3480 (NH), 3160, 3040, 2940 (CH), 1680, and 1640 (C=O) cm^{-1}. ^{1}H-NMR: see TABLES 1 and 2, 8.15 (*bs*, 1 H, NH), 7.40 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 6.00 (*bs*, 1 H, NH-O), and 1.98 (*d*, 3 H, Me-5). MS: *m*/*z* (%) 55 (100), 126 (87, thymine), 70 (58), 81 (35), 96 (28), 113 (18, $M^{\cdot+}$ - thymine), 152 (15), 181 (7), 223 (3), and 239 (2, $M^{\cdot+}$).

Anal. Calcd for $C_{10}H_{13}N_3O_4$ (239.23): C, 50.21; H, 5.48; N, 17.56. Found: C, 49.94; H, 5.45; N, 17.31.

1-[2',3',5'-Trideoxy-3',5'-(epoxy-(*N*-ethoxycarbonyl)imino)-β-D-*threo*-pentofuranosyl]thymine (10). - Obtained as described for the preparation of **9**: mp 143.1-144.2 °C, R_F 0.7 (9:1 CH_3Cl_3/MeOH); ν_{max}^{KBr} 3400 (NH), 3200, 3100, 3040 (CH, 1710, and 1650 (C=O) cm^{-1}. ^{1}H-NMR: see TABLES 1 and 2, 8.41 (*bs*, 1 H, NH), 7.40 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 4.28 (*q*, 2 H, $J_{CH2,CH3}$ = 7 Hz, COOCH2), 1.97 (*d*, 3 H, Me-5), and 1.33 (*t*, 3 H, COOCH2CH3). MS: *m*/*z* (%) 46 (10, OCH_2Me), 81 (40), 70 (28), 55 (25), 127 (25, thymine), 118 (26), 142 (23), 157 (9), 186 (5.7, $M^{\cdot+}$ - thymine), and 312 (0.4, $M^{\cdot+}$ + 1).

Anal. Calcd for $C_{13}H_{17}N_3O_6$ (311.30): C, 50.16; H, 5.50; N, 13.50. Found: C, 50.07; H, 5.43; N, 13.46.

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