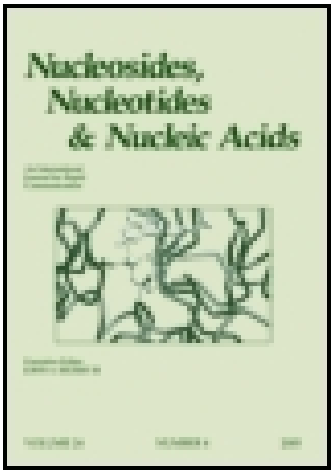

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SYNTHESIS, SOLUTION CONFORMATION AND BIOLOGICAL PROPERTIES OF 2',3'-DIDEOXY-3'-FLUORO-D-*ERYTHRO*-PENTOFURANOSIDES OF 2-THIOURACIL AND 2-THIOTHYMINE

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Abstract. The synthesis of the α- and ß-anomers of 2',3'-dideoxy-3'-fluoro-2-thiouridine and 2',3'-dideoxy-3'-fluoro-2-thiothymidine *via* Lewis acid catalysed nucleoside condensation is described. High resolution ^{1}H NMR data, solution conformations and biological properties are also presented.

2',3'-Dideoxy-3'-fluorothymidine (FLT) is one of the more potent *in vitro* inhibitors of HIV and its reverse transcriptase, but exhibits marked cytotoxicity. Its 4-thio derivative exhibits an enhanced therapeutic index in MT-4 cells,[1] prompting us to prepare the corresponding 2-thio analogues 2',3'-dideoxy-3'-fluoro-2-thiouridine (**5b**) and 2',3'-dideoxy-3'-fluoro-2-thiothymidine (**7b**) and their α-anomers **5a** and **7a**.

Condensation of silylated 2-thiouracil (**1**) with 1,5-di-O-benzoyl-2,3-dideoxy-3-fluoro-ß-D-*erythro*-pentofuranose (**3b**), synthesized as previously described,[2] in the presence of $SnCl_4$ (molar ratio of reagents 2:1:3) was conducted in 1,2-dichloroethane-acetonitrile (1:1, v/v) for 2 h at 30°C, followed by standard work-up. From a mixture of the reaction products in methanol, the α-anomer **4a** was deposited as crystals. Column chromatography of the remaining mixture yielded an additional quantity of **4a** (total, 47%), and the ß-anomer **4b** (16%). The overall yield of products was improved when the amount of $SnCl_4$ was increased to 4 molar equivalents, but this lead to a decrease in the ratio ß/α. The same trend was observed on prolongation of the reaction time. Deblocking of **4a** and **4b** with methanolic ammonia yielded the free nucleosides **5a** and **5b**, respectively.

Quite unexpectedly, replacement of silylated 2-thiouracil by silylated 2-thiothymine in the condensation reaction led to a complex mixture of products. Reaction of **2** with **3b** in the presence of trimethylsilyl triflate (TMS-Tfl), with a molar ratio of reactants of 2:1:1.5, in

TABLE 1

^{1}H NMR (500 Mhz) data of 2',3'-dideoxy-3'-fluoro-2-thiouridines **5a, b** and **7a, b** and their analogues

Compound	Chemical shifts (ppm) in D_2O vs internal DSS								
	1'	2'	2"	3'	4'	5'	5"	5	6
ß-3'F dT	6.35	2.39	2.65	5.36	4.38	3.38	3.82		7.68
ß-3'F dU	6.42	2.46	2.77	5.42	4.48	3.88	3.88		7.94
5a	6.74	2.81	2.55	5.25	4.80	3.63	3.66	5.96	7.77
7a	6.67	2.73	2.43	5.17	4.80	3.59	3.55		7.50
α-2 S dU	6.78	2.90	2.41	4.51	4.64	3.80	3.72	6.78	8.09
5b	7.01	2.11	2.81	5.27	4.33	3.80	3.80	5.97	8.16
7b	6.95	2.03	2.67	5.19	4.23	3.73	3.73		7.97
ß-2 S dU	7.03	2.38	2.70	4.52	4.19	3.97	3.88	7.03	8.12

Compound	Proton-proton coupling constant (Hz)							
	1',2'	1',2"	2',2"	2',3'	2",3'	3',4'	4',5'	4',5"
ß-3'F dT	9.29	5.81	-14.84	5.31	0.60	0.87	4.43	4.16
ß-3'F dU	9.13	5.86	-14.97	5.01	0.55	0.75	4.37	4.37
5a	7.09	1.22	-15.87	4.71	0.80	0.80	4.57	3.62
7a	7.13	1.14	-15.86	4.78	1.00	1.00	4.36	3.63
α-2 S dU	6.90	1.80	-15.44	5.93	1.80	1.80	4.02	5.42
5b	8.73	5.51	-14.60	5.00	1.00	1.00	3.14	3.14
7b	8.73	5.54	-14.64	5.15	1.00	1.00	2.78	2.78
ß-2 S dU	6.59	6.28	-14.25	6.62	4.43	4.05	3.37	5.00

acetonitrile for 4 h at room temperature, resulted in only slow formation of nucleoside products (monitored by TLC). Addition to this mixture of a further 1.5 molar equivalent of TMS-Tfl, and continuation of the reaction for 2 h, led to isolation, after standard work-up and column chromatography, of a non-separable mixture of **6a, b** (45% relative to **3b**, ß/α 1:3 by ^{1}H NMR), the α-anomer **3a** of the starting sugar (11%), identified with an authentic sample, unaltered **3b** (20%), and other unidentified products. Subsequent condensation of **2** with **3b** in the presence of TMS-Tfl (molar ratio 2:1:3) in acetonitrile for 24 h at room temperature afforded a mixture of **6a, b** (23%, ß/α 1:3), and an enhanced proportion of unidentified products, but not including **3a, b** (TLC). Replacement of TMS-Tfl by $SnCl_4$ did not lead to an improvement in either the yield of **6a, b** (40%) or the ß/α ratio (1:4 by ^{1}H NMR). These and the blocked anomers **6a, b** could not be separated. However, standard deprotection of the mixture of blocked anomers, followed by column chromatography, afforded the desired free

TABLE 2
Conformer populations in D_2O

Compound	S	g+	t	g-	Ng+	Nt	Sg+	St	Sg-
ß-3'F dT	1.02	0.51	0.24	0.24			0.53	0.25	0.24
ß-3'F dU	1.04	0.50							
5a	1.03	0.56	0.18	0.26			0.58	0.19	0.26
7a	1.00	0.58	0.19	0.23			0.58	0.19	0.23
α-2 S dU	0.90	0.42	0.40	0.19	0.06	0.04	0.35	0.36	0.19
5b	1.00	0.75							
7b	1.00	0.81							
ß-2 S dU	0.60	0.52	0.37	0.11	0.25	0.15	0.27	0.22	0.11

nucleosides **7a** and **7b**, whose structure was confirmed by high resolution ^1H NMR (Table 1), CD and MS spectroscopy.

Their conformational properties in aqueous medium are of some interest (Table 2). Both anomers of **5** and **7** exhibit a virtually 100% population of the S conformer of the pentose moiety. By contrast, in the absence of the 3'-fluoro-, the conformation of the pentose ring is dependent on the anomeric form, *viz.* about 60% S for 2-thio-2'-deoxyuridine and 90% for its α-anomer, and 60% S for 2-thiothymidine and 80% S for its α-anomer.

Biological Results

Nucleosides **5b** and **7b** were evaluated against HIV-1(III_B) and HIV-2(ROD) strains in CEM cells, and were found to be inhibitory at an EC_{50} of 3 μg/ml for **5b**, and 0.07 to 0.10 μg/ml for **7b**. CC_{50}in CEM cells was 100 μg/ml for **5b** and 58 μg/ml for **7b**.

Nucleosides **5b** and **7b** were shown to be phosphorolytically cleaved by thymidine phosphorylase (dThd Pase) from *E.coli* in contrast to FLT which was not a substrate for dThd Pase at the conditions used (4 mM substrate concentration, 50 mM phosphate buffer pH 7.4 and dThd-Pase, 1.66 U/100 μl).

Catabolism of **5b** and **7b** as well as the lack of thymidine kinase and/or of reverse transcriptase affinity may be the reason for the lack of activity of these compounds. Further biological investigations are underway.

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