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A Convergent Synthesis of 2'-O-Methyl Uridine

Gilles PARMENTIER, Gaby SCHMITT, Frédéric DOLLE & Bang LUU*

Laboratoire de Chimie Organique des Substances Naturelles, associé au CNRS,
Université Louis Pasteur, Centre de Neurochimie, 5 rue Blaise Pascal, F-67084 Strasbourg, France.

Abstract - A convergent synthesis of 2'-O-methyl uridine (**1**) is described. The key steps in our synthesis are : (1) a facile obtention of the 2'-O-methyl sugar synthon using totally selective and efficient methylation conditions; (2) a stereoselective high-yield condensation with an uracil derivative, yielding the desired β-form.

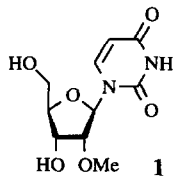
Introduction

During the last decade, ribozymes have become an interesting biomolecular tool[1]. They especially cleave efficiently target RNAs in a sequence-specific way, but are very sensitive to nuclease degradation and are unstable in serum. Synthetic ribozymes, using partially modified ribonucleotides such as 2'-O-methyl ribonucleotides, become nuclease-resistant, stable and can conserve their catalytic activity[1]. These properties make such artificial polymers potentially useful for *in vivo* gene expression studies and therapeutic applications for instance as anti-HIV drugs.

The synthesis of such synthetic ribozymes involves large quantities of the corresponding modified ribonucleosides as building blocks for classical phosphoramidite solid-phase oligonucleotide synthesis[2].

Syntheses of 2'-O-methyl ribonucleotides, starting from appropriately protected ribonucleosides, are extensively described in the literature[3-8] : they are all linear syntheses (starting from the corresponding natural adenosine, guanosine, cytosine and uracil), they show poor overall yields and they use in most cases non-selective and poorly-efficient methylation conditions of the 2'-hydroxyl function. They also describe identical sugar transformations, which are base-invariant.

These facts strongly suggested to us to develop a convergent synthesis of these nucleotides. This should avoid the restrictions due to the base-part, such as particular reactivity, efficient obligatory protection and low solubility in most organic solvents. Furthermore, the steric hindrance induced by the 2'-O-methyl group in our sugar moiety should favor the obtention of the desired β-anomeric form in the final condensation reaction with the base-derivative.

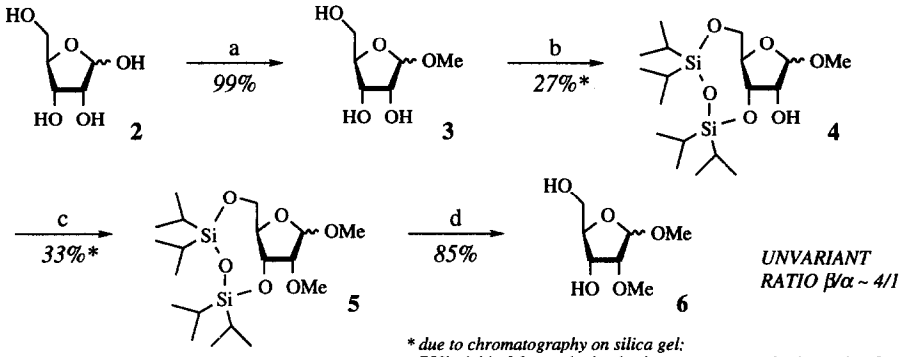


1

This paper describes the convergent synthesis of 2'-O-methyl uridine (**1**), chosen as a first model. The key steps in our synthesis are : (1) a facile obtention of the 2'-O-methyl sugar synthon using totally selective and efficient methylation conditions; (2) a stereoselective high-yield condensation with an uracil derivative, yielding the desired β-form with a satisfactory anomeric excess.

Discussion and results

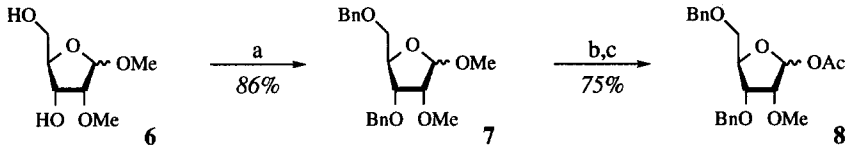
1'-O-Methyl ribofuranoside (**3**) was obtained in 99% yield from commercial ribose (**2**) using standard procedures (0.25% HCl in MeOH)[9].



Reagents and conditions: (a) HCl 0.25% (w/w), MeOH, 25°C, 90 mn; (b) $TIPDSCl_2$, Py, 25°C, 30 mn; (c) NaH (2.2 eq), MeI/HMPA 9/1 (v/v), 25°C, 30 mn; (d) n-$Bu_4N^+F^-$ (3.0 eq), THF, 25°C, 60 mn.

Reaction with 1,3-dichloro-1,1,3,3-*tetra*isopropyldisiloxane [$TIPDSCl_2$; Markiewicz disiloxane reagent[10]] in pyridine afforded cleanly compound **4** in 27% yield after flash chromatography on silica gel. Subsequent methylation after deprotonation with sodium hydride using an original 9/1 mixture of CH_3I and HMPA as solvents gave the 2'-O-methyl derivative **5** (33% yield after chromatography). Deprotection of the TIPDS using *tetra*-butyl ammonium fluoride[7] (TBAF) in THF generated the diol **6** (85% yield).

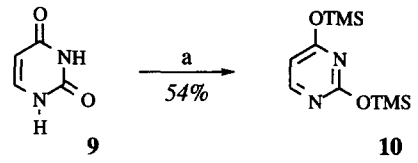
The poor overall yield (7-8%) of 1',2'-O,O-dimethyl-D-ribofuranose (**5**) from 1'-O-methyl ribofuranose (**3**) led us to develop a one-pot synthesis using the same reactions and above all avoiding intermediate purifications. A 75% overall yield was now obtained using this method for the 3 steps.



Reagents and conditions: (a) NaH (2.2 eq), BnBr (2.2 eq), CH_3CN, 25°C, 120 mn; (b) TFA/H_2O 1/1 (v/v), 25°C, 60 mn; (c) Ac_2O, Py, 25°C, 16 h.

The two hydroxyl functions of ribofuranose **6** were cleanly protected using benzyl bromide in CH_3CN (86% yield). Benzylation was prefered to benzoylation for two reasons : (1) compatibility with building-block chemistry[2] (for A, G, C especially); (2) avoidance of the undesired anomeric α-form, which is mostly obtained when 5'-O-benzoyl ribose is condensed with bases, due to stabilisation of the intermediate transition state[11]. Subsequent hydrolysis (TFA/H_2O) followed by acetylation[12] (Ac_2O, pyridine) afforded 3',5'-O,O-dibenzyl-1'-O-acetyl-2'-O-methyl-D-ribofuranose (**8**).

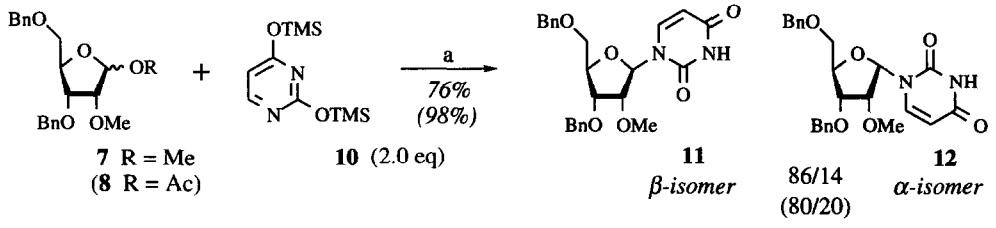
Bis-TMS uracil (**10**) was obtained from commercial uracil (**9**) using trimethylsilyl chloride[13] (TMSCl) in dioxane containing triethylamine (54% after distillation).



9 **10**

Reagents and conditions: (a) TMSCl (2.0 eq), Et_3N (2.0 eq), Dioxane, 25°C, 16 h.

Condensation of the protected 2'-O-methyl furanose (**7**, respectively **8**) and bis-TMS uracil (**10**) using trimethylsilyl triflate[14-17] [TMSOTr method of Vorbrüggen[18]] in CH_3CN was stereoselective and gave an anomeric mixture of the desired compound (76% yield from **7**, 98% yield from **8**). These reactions certainly involve an oxonium ion intermediate, giving mixtures of nucleoside anomers, regardless of the anomeric composition of the sugar derivatives[19]. The two adducts were easily separated by chromatography on silica gel and their anomeric identity (α or β) was assigned using ^1H-two-dimensional NMR experiments (in the NOESY the cross-peak between the H-1' and H-4' undoubtedly designed the β-anomeric form). The desired β-anomer was in both cases largely predominant (β/α ratio > 4).



7 R = Me
(**8** R = Ac)

10 (2.0 eq)

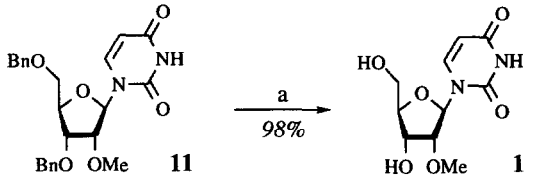
11
β-isomer

86/14
(80/20)

12
α-isomer

Reagents and conditions: (a) TMSOTr (2.0 eq), CH_3CN, 25°C, 2h to 3h.

Other condensation methods using $SnCl_4$ or HgCN as catalysts[20] were tried without success [low yield (25%) in the first case and no reaction at all in the second one].



11 **1**

Reagents and conditions: (a) H_2 (1 Atm), Pd/C 10%, EtOAc, 25°C, 30 mn.

The debenzylation of **11** (pure β-form) using hydrogen gas and Pd/C in EtOAc yielded the known 2'-O-methyl uridine (**1**). Its spectroscopic data were strictly identical to those described for **1** obtained through a linear synthesis starting from natural uridine (pure β-form)[6,21,22].

Conclusion

2'-O-Methyl uridine (**1**) was synthesized *via* a convergent synthesis. The 2'-O-methyl sugar synthon was prepared in 5 to 7 steps from commercial available D-ribofuranose in 48% to 64% overall yield, high yield due to optimal methylation conditions. Condensation with uracile derivative was successful and gave with satisfactory anomeric excess ($\beta/\alpha > 4/1$) the desired β-form (76% to 98% yield).

This convergent approach opens routes to the obtention of the 2'-O-methyl-adenosine, -guanosine and -cytidine, as well as other modified 2'-O-methyl nucleosides.

Experimental

Melting points were measured on a Reichert hot stage microscope and are uncorrected. Optical rotations ($[\alpha]_D$) were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$. IR spectra were recorded in KBr on a Perkin-Elmer 881 infrared spectrophotometer. UV spectra were measured on a Kontron-Uvikon 810 UV-vis spectrophotometer. NMR spectra were recorded on Bruker SY (200 MHz) and Bruker AM (400 MHz) spectrometer using $CHCl_3$ (δ = 7.26 ppm), CD_3CN (δ = 1.95 ppm), CD_2Cl_2 (δ = 5.32 ppm) and DMSO-d_6 (δ = 2.55 ppm) as internal standards for ^1H NMR and $CDCl_3$ (δ = 77.0 ppm), CD_3CN (δ = 1.9 and 118.2 ppm) and DMSO-d_6 (δ = 39.5 ppm) as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS (+, ⁻, ', '', * ,° = interchangeable assignement ; [β,α] = β,α-anomer). The nature of the different carbons (C, CH, CH_2 or CH_3) was determined by ^{13}C to ^1H polarisation transfer (DEPT). Mass spectra (MS) were measured on a VG Analytical ZAB-HF apparatus in the FAB mode. Microanalyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS. TLC were run on pre-coated silica gel plates 60 F 254 (Merck, 0.25 mm). In order to reveal the compounds, TLC plates were exposed to UV-light, dipped in a solution of vanillin (1 g) in EtOH/H_2SO_4 (95/5, 1 l) and heated on a hot plate to reveal the compounds. Medium pressure chromatography (P = 0.5 - 1.1 bar) was carried out using silica gel (40 - 63 μm, Merck) columns. All solvents were freshly distilled before use. Air- or moisture- sensitive reactions were conducted in flame-dried glassware and under an inert atmosphere.

1'-O-Methyl-D-ribofuranose (**3**)

To a solution of D-erythro-pentafuranose (**2**) (25.0 g, 0.17 moles) in MeOH (350 ml) was added a 2% solution of hydrogen chloride in MeOH (50 ml). The mixture was stirred for 90 min. Ag_2CO_3 (15.0 g) was carefully added and the mixture was vigorously stirred for 15 min. $MgSO_4$ (30.0 g) was then added, the mixture was stirred for another 15 min and filtered. The clear filtrate was concentrated to dryness to give 1'-O-methyl-D-erythro-pentafuranose (**3**) (27.3 g) as a syrup (99 % yield).

3 **^1H NMR** 400 MHz (DMSO-d_6) δ : 3.23 (s, 3H, OCH_3 [β]) ; 3.27 (s, 3H, OCH_3 [α]) ; 3.25-3.82 (br, 5H, H-2', 3', 4', 5') ; 4.57 (t, 2H, J = 6.5 Hz, HO-5') ; 4.61 (s, 1H, H-1' [β]) ; 4.71 (d, 1H, J = 2.2 Hz, H-1' [α]) ; 4.78 (br, 1H, $w_{1/2}$ = 20.0 Hz, HO-3') ; 4.98 (br, 1H, $w_{1/2}$ = 15.0 Hz, HO-2'). **^{13}C NMR** 100 MHz (DMSO-d_6) δ : 56.3 (OMe [β]) ; 56.6 (OMe [α]) ; 63.8 (CH_2-5' [α]) ; 65.2 (CH_2-5' [β]) ; 71.4* (CH-3' [α]) ; 73.0* (CH-3' [β]) ; 73.3* (CH-2' [α]) ; 76.3* (CH-2' [β]) ; 85.6* (CH-4' [β]) ; 87.1* (CH-4' [α]) ; 104.8 (CH-1' [α]) ; 110.1 (CH-1' [β]). **Rf** (EtOAc) : 0.16. **Analysis** $C_6H_{12}O_5$ requires C, 43.92 ; H, 7.30 ; found C, 43.91 ; H, 7.27.

1',2'-O,O-Dimethyl-D-ribofuranose (**6**)

Long route

3',5'-O-(*Tetra*isopropyldisiloxane-1,3-diyl)-1'-O-methyl-D-ribofuranose (**4**)

1'-O-Methyl-D-ribofuranose (**3**) (2.6 g, 15.8 mmoles) was dried by several coevaporations with dry CH_3CN and then treated with 1,3-dichloro-1,1,3,3-*tetra*isopropyldisiloxane (5.0 ml, 15.8 mmoles, 1.0 eq) in dry Py (50 ml) at 25°C under Ar for 30 min.

The mixture was concentrated to dryness and redissolved in EtOAc, washed several times with water and brine. The organic layer was dried with Na_2SO_4 and filtered. The filtrate was concentrated to dryness and chromatographed on silica gel. Elution with Hexane/EtOAc (92/8) gave 3',5'-O-(*tetra*isopropyldisiloxane-1,3-diyl)-1'-O-methyl-D-ribofuranose (**4**), 0.6 g (27% yield#) as an oil.

4 **^{1}H NMR** 400 MHz (DMSO-d_6) δ : 0.96-1.00 (br, 28H, CH_3-(*i*Pr)) ; 3.18 (s, 3H, OCH_3 [β]) ; 3.29 (s, 3H, OCH_3 [α]) ; 3.74-3.90 (br, 4H, H-3', 4', 5') ; 4.09 (br, 1H, H-2' [α]) ; 4.26 (dd, 1H, J = 4.5 and 7.5 Hz, H-2' [β]) ; 4.60 (s, 1H, H-1' [α]) ; 4.77 (d, 1H, J = 3.9 Hz, H-1' [β]) ; 4.94 (br, 1H, $w_{1/2}$ = 15.0 Hz, HO-2'). **^{13}C NMR** 100 MHz (DMSO-d_6) δ : 14.1-14.7 (CH-(*i*Pr)) ; 18.7-19.2 (CH_3-(*i*Pr)) ; 55.9 (OMe [β]) ; 56.1 (OMe [α]) ; 64.4 (CH_2-5' [α]) ; 65.7 (CH_2-5' [β]) ; 72.5* (CH-3' [α]) ; 75.0* (CH-3' [β]) ; 73.1* (CH-2' [α]) ; 76.6* (CH-2' [β]) ; 82.1* (CH-4' [β]) ; 84.1* (CH-4' [α]) ; 104.4 (CH-1' [α]) ; 109.1 (CH-1' [β]). **Rf** (hexane/EtOAc : 9/1) : 0.25.

3',5'-O-(*Tetra*isopropyldisiloxane-1,3-diyl)-1',2'-O,O-dimethyl-D-ribofuranose (**5**).

To a solution of 3,5-O-(*tetra*isopropyldisiloxane-1,3-diyl)-1-O-methyl-D-ribofuranose (**4**) (0.5 g, 1.2 mmoles) in a mixture of CH_3I (20 ml) and HMPA (2 ml) was carefully added NaH (65 mg, 2.6 mmoles, 2.2 eq, 95%). The mixture was stirred at 25°C under Ar for 30 min. The excess of NaH was neutralized by addition of H_2O (1 ml) and the mixture was concentrated. The residue was dissolved in a mixture of EtOAc (90 ml) and H_2O (10 ml). The organic layer was separated, washed several times with H_2O and brine, dried with Na_2SO_4 and filtered. The filtrate was concentrated to dryness and chromatographed on silica gel. Elution with Hexane/EtOAc (95/5) gave 3',5'-O-(*tetra*isopropyldisiloxane-1,3-diyl)-1',2'-O,O-dimethyl-D-ribofuranose (**5**), 167 mg (33% yield#) as an oil.

5 **^{1}H NMR** 200 MHz (CD_2Cl_2) δ : 0.91-1.30 (br, 28H, CH_3-(*i*Pr)) ; 3.28 (s, 3H, OMe-1' [β]) ; 3.52 (s, 3H, OMe-2' [β]) ; 3.56 (d, 1H, J = 4.3 Hz, H-2' [β]) ; 3.88 (br, 3H, H-4', 5' [β]) ; 4.45 (dd, 1H, J = 6.0 and 7.4 Hz, H-3' [β]) ; 4.70 (s, 1H, H-1' [β]). **^{13}C NMR** 100 MHz ($CDCl_3$) δ : 12.7-13.4 (CH-(*i*Pr)) ; 16.9-17.4 (CH_3-(*i*Pr)) ; 54.6 (OMe-1' [β]) ; 59.3 (OMe-2' [β]) ; 63.6 (CH_2-5' [β]) ; 73.6* (CH-3' [β]) ; 80.9* (CH-2' [β]) ; 84.4* (CH-4' [β]) ; 105.6 (CH-1' [β]). **Rf** (hexane/EtOAc : 9/1) : 0.43.

#due to deprotection of the silyloxane group during the chromatography on silica gel.

1',2'-O,O-Dimethyl-D-ribofuranose (**6**)

To a solution of 3',5'-O-(*tetra*isopropyldisiloxane-1,3-diyl)-1',2'-O,O-dimethyl-D-ribofuranose (**5**) (0.15 g, 0.35 mmoles) in THF (50 ml) was added a 1.0M solution of TBAF in THF (1.0 ml, 3 eq). The solution was stirred for 30 min at 25°C, concentrated to dryness and chromatographed on silica gel. Elution with pure EtOAc gave 1',2'-O,O-dimethyl-D-ribofuranose (**6**), 52.9 mg (85% yield) as an oil.

6 **^{1}H NMR**§ 200 MHz ($CDCl_3$) δ : 2.48* (br, 1H, HO-3') ; 2.86* (br, 1H, HO-5') ; 3.33 (s, 3H, OMe-1' [α]) ; 3.37 (s, 3H, OMe-1' [β]) ; 3.39 (s, 3H, OMe-2' [α]) ; 3.47 (s, 3H, OMe-2' [β]) ; 3.64 (d, 1H, J = 4.8 Hz, H-2' [β]) ; 3.69 (center of ABXY system, 2H, H-5') ; 3.88 (br, 1H, H-2' [α]) ; 3.95 (br, 1H, H-4') ; 4.16 (br, 1H, H-3' [α]) ; 4.19 (br, 1H, H-3' [β]) ; 4.79 (s, 1H, H-1' [α]) ; 4.83 (s, 1H, H-1' [β]). **^{13}C NMR** 50MHz ($CDCl_3$) δ : 55.0 (OMe-1' [α]) ; 55.6 (OMe-1' [β]) ; 58.5 (OMe-2' [β]) ; 59.2 (OMe-2' [α]) ; 63.2 (CH_2-5') ; 70.9* (CH-3' [β]) ; 72.5° (CH-3' [α]) ; 80.9* (CH-2' [β]) ; 81.8° (CH-2' [α]) ; 84.3* (CH-4') ; 85.1 (CH-2' [β]) ; 105.9 (CH-1' [β]) ; 108.4 (CH-1' [α]). **Rf** (EtOAc) : 0.23. **Analysis** $C_7H_{14}O_5$ requires C, 47.22 ; H, 7.81 ; found C, 47.42 ; H, 7.67.

§ Spectra assigned using ^{1}H two-dimensional COSY & NOESY NMR experiments.

Short route

1'-O-Methyl-D-ribofuranose (**3**) (2.6 g, 15.8 mmoles) was dried by several coevaporation with dry CH_3CN and then treated with 1,3-dichloro-1,1,3,3-*tetra*isopropyldisiloxane (5.0 ml, 15.8 mmoles, 1.0 eq) in dry Py (50 ml) at 25°C under Ar for 30 min. NaH (1.2 g, 47.5 mmoles, 3.0 eq, 95%) was added carefully and the resulting grey mixture was stirred for 15 min and concentrated to dryness. The crude residue was suspended in a mixture of CH_3I (50 ml, large excess) and HMPA (2 ml). NaH (760 mg, 31.6

mmoles, 2.0 eq) was added and the mixture was stirred for 30 min at 25°C under Ar. The excess of NaH was neutralized by carefully adding MeOH (5 ml) and H_2O (5 ml) and the mixture was concentrated. The residue was suspended directly in a 1.0 M TBAF solution in THF (70 ml, 70 mmoles, 4.4 eq), the mixture was stirred for 2 h at 25°C and filtered. The filtrate was concentrated to dryness, and chromatographed on silica gel. Elution with pure EtOAc gave 1',2'-O,O-dimethyl-D-ribofuranose (**6**), 2.12 g (75% yield) as an oil.

3',5'-O-(Dibenzyl)-1',2'-O,O-dimethyl-D-ribofuranose (**7**)

1',2'-O,O-Dimethyl-D-ribofuranose (**6**) (2.0 g, 11.2 mmoles) was treated with benzyl bromide (2.9 ml, 24.6 mmoles, 2.2 eq) and NaH (848 mg, 33.6 mmoles, 3 eq) in dry CH_3CN (15 ml) at 25°C under Ar for 2 h. The excess of NaH was neutralized by addition of H_2O (5 ml). This mixture was then extracted 3 times with CH_2Cl_2 (50 ml). The organic layers were combined, washed with H_2O and brine, dried with Na_2SO_4 and filtered. The filtrate was concentrated to dryness and chromatographed on silica gel. Elution with hexane/EtOAc (75/25) gave 3',5'-O-(dibenzyl)-1',2'-O,O-dimethyl-D-ribofuranose (**7**), 3.97 g (86% yield) as an oil.

7 **^{1}H NMR** 200 MHz ($CDCl_3$) δ : 3.33 (s, 3H, OMe-1' [β]) ; 3.44 (s, 3H, OMe-2' [β]) ; 3.49 (br, 1H, H-2' [β]) ; 3.59 (br, 2H, H-5') ; 4.05 (br, 1H, H-3' [β]) ; 4.26 (br, 1H, H-4' [β]) ; 4.57 (br, 4H, CH_2-Ph) ; 4.90 (s, 1H, H-1' [β]) ; 7.29 (br, 10H, Ph). **^{13}C NMR** 50 MHz ($CDCl_3$) δ : 55.1 (OMe-1' [β], OMe-2' [α]) ; 58.3 (OMe-2' [β]) ; 59.2 (OMe-1' [α]) ; 72.4 (CH_2-5' [α]) ; 72.6 (CH_2-5' [β]) ; 72.6 (CH_2-Ph) ; 73.2 (CH_2-Ph) ; 78.4*° (CH-2') ; 79.8° (CH-3' [α]) ; 80.4* (CH-3' [β]) ; 82.3* (CH-4') ; 105.8 (CH-1' [β]) ; 106.5 (CH-1' [α]) ; 127.6-128.4 (Ph). **Rf** (hexane/EtOAc : 1/1) : 0.53. **Analysis** $C_{21}H_{26}O_5$ requires C, 70.39 ; H, 7.21 ; found C, 70.30 ; H, 7.41.

3',5'-O-Dibenzyl-1'-O-acetyl-2'-O-methyl-D-ribofuranose (**8**)

3',5'-O-Dibenzyl-1',2'-O,O-dimethyl-D-ribofuranose (**7**) (0.6 g, 1.67 mmoles) was treated in a mixture of TFA (18 ml) and H_2O (2 ml) at 25°C for 2 h. The mixture was concentrated to dryness to give 3',5'-O-dibenzyl-2'-O-methyl-D-ribofuranose as an oil.

^{1}H NMR 400 MHz ($CDCl_3$) δ : 1.04 (br, 1H, HO-1') ; 3.47 (s, 3H, OMe-2' [α]) ; 3.35-3.70 (br, 3H, H-2', 5') ; 3.49 (s, 3H, OMe-2' [β]) ; 3.78* (br, 1H, H-3' [β]) ; 4.01° (br, 1H, H-3' [α]) ; 4.23* (br, 1H, H-4' [β]) ; 4.37° (br, 1H, H-4' [α]) ; 4.40-4.72 (br, 4H, CH_2-Ph) ; 5.27 (s, 1H, H-1'[α]) ; 5.32 (d, 1H, J = 3.9 Hz, H-1' [β]) ; 7.29 (m, 10H, Ph). **^{13}C NMR** 50 MHz ($CDCl_3$) δ : 58.3 (OMe-1' [α]) ; 58.4 (OMe-1' [β]) ; 69.4 (CH_2-5' [α]) ; 69.8 (CH_2-5' [β]) ; 72.6 (CH_2-Ph) ; 73.4 (CH_2-Ph) ; 77.0*° (CH-2' [α]) ; 77.1*° (CH-2' [β]) ; 80.0* (CH-3' [β]) ; 80.5° (CH-3' [α]) ; 80.8* (CH-4' [β]) ; 83.1° (CH-4' [α]) ; 95.9 (CH-1' [β]) ; 99.5 (CH-1' [α]) ; 127.5-128.3 (Ph). **Rf** (hexane/EtOAc : 1/1) : 0.25.

The crude 3',5'-O-dibenzyl-2'-O-methyl-D-ribofuranose was treated with a mixture of Ac_2O (10 ml) and Py (10 ml) at 25°C under Ar for 2 h. The mixture was neutralized by addition of cold H_2O (10 ml) at 4°C and extracted with CH_2Cl_2. The filtrate was concentrated to dryness and chromatographed on silica gel. Elution with Hexane/EtOAc (75/25) gave 3',5'-O-dibenzyl-1'-O-acetyl-2'-O-methyl-D-ribofuranose (**8**), 482 mg (75% yield from **7**) as an oil.

8 **^{1}H NMR** 400 MHz ($CDCl_3$) δ : 1.94 (s, 3H, Ac) ; 3.45* (s, 3H, OMe-2' [α]) ; 3.48* (s, 3H, OMe-2' [β]) ; 3.49 (br, 2H, H-5' [α]) ; 3.52 (br, 1H, H-2') ; 3.74 (br, 2H, H-5' [β]) ; 3.86 (br, 1H, H-2' [α]) ; 4.01 (br, 1H, H-4' [α]) ; 4.15 (br, 1H, H-3' [β]) ; 4.33 (br, 1H, H-4' [β]) ; 4.35 (br, 1H, H-3' [α]) ; 4.41-4.76 (br, 4H, CH_2-Ph) ; 6.15 (d, 1H, J = 3.9 Hz, H-1' [β]) ; 6.37 (d, 1H, J = 4.4 Hz, H-1' [α]) ; 7.31 (br, 10H, Ph). **^{13}C NMR** 50 MHz ($CDCl_3$) δ : 21.1' (CH_3 [α]) ; 21.6' (CH_3 [β]) ; 58.4" (OMe-1' [α]) ; 59.3" (OMe-1' [β]) ; 69.6$^+$ (CH_2-5' [α]) ; 69.9$^+$ (CH_2-5' [β]) ; 72.5-73.6 (CH_2-Ph) ; 75.2* (CH-2' [β]) ; 75.3* (CH-2' [α]) ; 80.6° (CH-3' [β]) ; 81.4° (CH-3' [α]) ; 81.7$^-$ (CH-4' [α]) ; 83.9$^-$ (CH-4' [β]) ; 94.6** (CH-1' [β]) ; 98.3** (CH-1' [α]) ; 127.7-128.5 (Ph) ; 137.5"" (CO [α]) ; 137.9"" (CO [β]). **Rf** (hexane/EtOAc : 1/1) : 0.49 and 0.55. **Analysis** $C_{22}H_{12}O_6$ requires C, 68.42 ; H, 6.70 ; found C, 68.80 ; H, 6.84.

Bis(trimethylsilyl)uracyl (**10**)

To a suspension of dry powdered uracil (11.3 g, 100 mmoles) and trimethylsilyl chloride (21.1 g, 190 mmoles, 1.9 eq) in dry dioxane (100 ml) was added dropwise the solution of triethylamine (19.5 g, 190 moles, 1.9 eq) in dry dioxane (20 ml) with stirring under Ar at 25°C. After addition, the stirring was continued for 16 h. The precipitate mixture of triethylamine hydrochloride and

uracil was filtered off and washed 3 times with dry dioxane (20 ml). Filtrate and washings were collected and the solvents were removed. The resulting viscous oily residue was distilled under reduced pressure (74°C/0.022 mm Hg) to give bis(trimethylsilyl)uracyl (**10**), 15.6 g (54%) as a colorless oil.

10 **^{1}H NMR** 200 MHz (DMSO-d_6) δ : 0.00 (s, 18H, TMS) ; 5.42 (d, 1H, J = 7.6 Hz, H-6) ; 7.37 (d, 1H, J = 7.6 Hz, H-56) ; 10.8 (s, 1H, NH). **Rf** : decomposes into uracyle on TLC.

1'-(3',5'-O-Benzyl-2'-O-methyl-β-D-ribofuranosyl) uracil (**11**) and 1'-(3',5'-O-benzyl-2'-O-methyl-α-D-ribofuranosyl) uracil (**12**)

To a stirred solution of 3',5'-O-dibenzyl-1',2'-O,O-dimethyl-D-ribofuranose (**7**) (0.5 g, 1.4 mmol) and O,O-bis-(trimethylsilyl)-uracil (**10**) (0.8 g, 2.8 mmol, 2.0 eq) in dry CH_3CN (20 ml) with molecular sieves (4 Å) was added dropwise trimethylsilyl triflate (0.55 ml, 2.8 mmol, 2.0 eq) in CH_3CN (5 ml) at -30°C. After 30 min, the reaction mixture was warmed to 25°C and stirred for 2 h under Ar. The mixture was then diluted with CH_2Cl_2 (100 ml) and extracted once with ice cold sat. aq. $NaHCO_3$ (20 ml). The aqueous solution was then re-extracted twice with CH_2Cl_2 (50 ml). The organic layers were combined, washed with H_2O, dried with Na_2SO_4, evaporated under reduced pressure and chromatographed on silica gel. Elution with hexane/EtOAc (60/40) gave 1'-(3',5'-O-benzyl-2'-O-methyl-β-D-ribofuranosyl) uracil (**11**), 466 mg (65% yield) and 1'-(3',5'-O-benzyl-2'-O-methyl-α-D-ribofuranosyl) uracil (**12**), 61 mg (11% yield).

The procedure described above was carried out with 3',5'-O-dibenzyl-1'-O-acetyl-2'-O-methyl-D-ribofuranose (**8**) (0.55 g, 1.4 mmol) to give 1'-(3',5'-O-benzyl-2'-O-methyl-β-D-ribofuranosyl) uracil (**11**), 480 mg (79% yield) and 1'-(3',5'-O-benzyl-2'-O-methyl-α-D-ribofuranosyl) uracil (**12**), 116 mg (19% yield).

11 **^{1}H NMR** 400 MHz ($CDCl_3$) δ : 3.55 (s, 3H, OMe-2') ; 3.69 (br, 1H, H-5') ; 3.93 (d, 1H, J = 13.2 Hz, H-2') ; 4.06 (d, 1H, J = 12.7 Hz, H-3') ; 4.27 (d, 1H, J = 8.0 Hz, H-4') ; 4.57 (br, 4H, CH_2-Ph) ; 5.21 (d, 1H, J = 8.2 Hz, H-5) ; 5.94 (d, 1H, J = 1.5 Hz, H-1') ; 7.29 (br, 10H, Ph) ; 7.95 (d, 1H, J = 8.2 Hz, H-6) ; 8.68 (s, 1H, N-H). **^{13}C NMR** 50MHz ($CDCl_3$) δ : 58.3 (OMe-2') ; 67.5 (CH-2') ; 72.4 (CH_2-5') ; 73.5 (CH_2-Ph) ; 74.0 (CH_2-Ph) ; 80.9 (CH-3') ; 82.1 (CH-4') ; 87.8 (CH-1') ; 101.4 (CH-6) ; 128.0-128.5 (Ph) ; 140.2 (CH-5) ; 150.1 (C-2) ; 163.7 (C-4). **Rf** (hexane/EtOAc : 1/1) : 0.12. **Analysis** $C_{24}H_{26}O_6N_2$ requires C, 65.70 ; H, 6.11 ; N, 6.39 ; found C, 66.55 ; H, 6.28 ; N, 6.32.

12 **^{1}H NMR** 400 MHz ($CDCl_3$) δ : 3.55 (s, 3H, OMe-2') ; 3.69 (br, 1H, H-5') ; 3.93 (d, 1H, J = 13.2 Hz, H-2') ; 4.06 (d, 1H, J = 12.7 Hz, H-3') ; 4.27 (d, 1H, J = 8.0 Hz, H-4') ; 4.57 (br, 4H, CH_2-Ph) ; 5.21 (d, 1H, J = 8.2 Hz, H-5) ; 5.94 (d, 1H, J = 1.5 Hz, H-1') ; 7.29 (br, 10H, Ph) ; 7.95 (d, 1H, J = 8.2 Hz, H-6) ; 8.68 (s, 1H, N-H). **^{13}C NMR** 50MHz ($CDCl_3$) δ : 59.8 (OMe-2') ; 69.4 (CH-2') ; 73.0 (CH_2-5') ; 76.3 (CH_2-Ph) ; 77.0 (CH_2-Ph) ; 79.2 (CH-3') ; 81.5 (CH-4') ; 84.8 (CH-1') ; 101.0 (CH-6) ; 128.0-128.5 (Ph) ; 142.3 (CH-5) ; 150.7 (C-2) ; 163.7 (C-4).

1'-(2'-O-Methyl-β-D-ribofuranosyl) uracil (**1**)

A solution of 1'-(3',5'-O-benzyl-2'-O-methyl-β-D-ribofuranosyl) uracil (**11**) (0.4 g, 0.91 mmoles) in EtOAc (50ml) was treated with 10% palladium on charcoal (0.05 g) and the suspension was shaken with hydrogen at 25 °C for 30 min, then the catalyst was removed by filtration. The solution was evaporated under reduced pressure to give 1'-(2'-O-methyl-β-D ribofuranosyl) uracil (**1**), 233 mg (99% yield).

1 **Mp** : 158-160°C [Litt.[6] 159-161°C]. $[\alpha]_D^{25}$ (DMSO, 1.5) : + 8.4 [Litt.[21] (H_2O, 1.6) + 41]. **UV** (MeOH) λ_{max} : 261 nm (10900), [Litt.[22] (EtOH) 263 nm (10100)]. **IR** (KBr) ν (cm^{-1}) : 3428 ; 2936 ; 1720 ; 1466 ; 1391 ; 1270 ; 1208 ; 1112 ; 1085 ; 1058. **^{1}H NMR** 400 MHz (DMSO-d_6) δ : 3.35 (s, 3H, OMe-2') ; 3.49-4.15 (br, 5H, H-2' ,3' ,4' ,5') ; 5.14 (br, 2H, OH-3', 5') ; 5.65 (d, 1H, J = 8.1 Hz, H-5) ; 5.86 (d, 1H, J = 5.0 Hz, H-1') ; 7.93 (d, 1H, J = 8.1 Hz, H-6) ; 11.34 (s, 1H, N-H). **^{1}H NMR** 400 MHz (CD_3CN) δ : 3.47 (s, 3H, OMe-2') ; 3.69-3.90 (br, 4H, H-2' ,3' ,5') ; 4.17 (br, 1H, H-4') ; 5.61 (d, 1H, J = 8.2 Hz, H-5) ; 5.84 (d, 1H, J = 3.9 Hz, H-1') ; 7.86 (d, 1H, J = 8.2 Hz, H-6). **^{13}C NMR** 100MHz (CD_3CN) δ : 59.0 (OMe-2') ; 61.7 (CH_2-5') ; 69.7 (CH-2') ; 84.0 (CH-3') ; 86.0 (CH-4') ; 88.4 (CH-1') ; 102.8 (CH-6) ; 141.7 (CH-5) ; 151.7 (C-2) ; 164.2 (C-4). **MS** (FAB$^+$) : 281.0 [M+Na$^+$] ; 259.1 [MH$^+$] ; 176.0 ; 147.0. **Rf** (EtOAc) : 0.05. **Analysis** $C_{10}H_{14}O_6N_2$ requires C, 46.53 ; H, 5.44 ; N, 10.79 ; found C, 46.72 ; H, 5.67 ; N, 10.32.

References

1. Paolella, G.; Sproat, B.S. and Lamond, A.I., *EMBO J.* **1992**, *11*, 1913-1919.
2. Usman, N.; Ogilvie, K.K.; Jiang, M.-Y. and Cedergren R.J., *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854.
3. Broom, A.D. and Robins, R.K., *J. Am. Chem. Soc.* **1964**, *87*, 1145-1146.
4. Robins, M.J.; Naik, S.R. and Lee, A.S.K., *J. Org. Chem.* **1974**, *39*, 1891-1899.
5. Hisanaga, Y.; Tanabe, T.; Yamauchi, K. and Kinoshita, M., *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1569-1570.
6. Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S. Miura, K. and Ohtsuka, E., *Nucleic Acids Research* **1987**, *15*, 6131-6148.
7. Sproat, B.S.; Beijer B. and Iribarren, A., *Nucleic Acids Research* **1990**, *18*, 41-49.
8. Beijer B.; Sulston, I.; Sproat, B.S.; Rider, P.; Lamond, A.I. and Neuner P., *Nucleic Acids Research* **1990**, *18*, 5143-5151.
9. Fox, J.J.; Yung, N.C.; Wempen, I. and Hoffer, M., *J. Am. Chem. Soc.* **1961**, *83*, 4066-4070.
10. Markiewicz, W.T., *J. Chem. Res.* **1979**, *S*, 24-25.
11. Prystas, M. and Sorm, F., *Collection Czech. Chem. Commun.* **1965**, *30*, 2960-2975.
12. Guthrie, R.D. and Smith, S.C., *Chem. Ind. (London)* **1968**, 547-550.
13. Nishimura, T. and Iwai, I., *Chem. Pharm. Bull.* **1964**, *12*, 352-356.
14. Abdel-Megied, A.E.-S.; Pedersen, E.B. and Nielsen, C.M., *Synthesis* **1991**, 313-317.
15. Pedersen, E.B. and Wengel, J., *Synthesis* **1991**, 451-453.
16. Jesper, L.; Wengel, J.; Pedersen, E.B. and Vestergaard B.F., *Synthesis* **1991**, 1183-1190.
17. Wolf, J.; Jarrige, J.-M., Florent, J.-C., Grierson, D.S. and Monneret, C., *Synthesis* **1992**, 773-778.
18. Vorbrüggen, H.; Krolikiewicz, K. and Bennua, B, *Chem. Ber.* **1981**, *114*, 1234-1255.
19. Chou, T.S.; Heath, P.C.; Patterson, L.E.; Poteet, L.M.; Lakin, R.E. and Hunt, A.H., *Synthesis* **1992**, 565-570.
20. Niedballa, U. and Vorbrüggen, H., *J. Org. Chem.* **1974**, *39*, 3654-3660.
21. Furukawa, Y.; Kobayashi, Y.; Kanai, Y. and Honjo, M., *Chem. Pharm. Bull. Japan* **1965**, *13*, 1273-1278.
22. Martin, D.M.G.; Reese, C.B. and Stephenson, G.F., *Biochemistry* **1968**, *7*, 1406-1409.

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