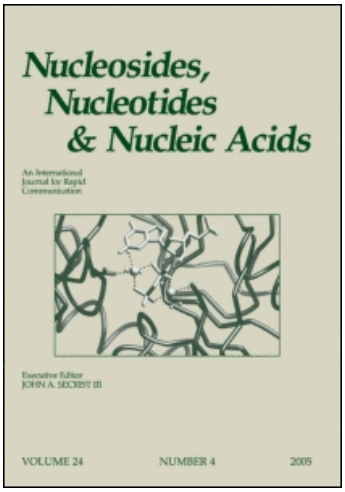

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^{19}F NMR OF RNA. THE STRUCTURAL AND CHEMICAL ASPECTS OF 5-FLUORO -CYTIDINE AND -URIDINE LABELLING OF OLIGORIBONUCLEOTIDES#

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Abstract: Results of PM3 semiempirical calculation revealed that energy and hydrogen bonds geometry of 1-methyl-5-fluoro-uracil and -cytosine base-pairs with 9-methyl-adenine and -guanine respectively are virtually the same as for the natural bases. Analysis of proton coupling constants proved that the sugar puckering of 5-fluorouridine and 5-fluorocytidine is analogous to non-modified ribonucleosides. 5-Fluorocytidine was regioselectively introduced to oligoribonucleotides, prepared using 2'-O-*tert*-dimethylsilyl protection, *via* post-synthetic quantitative ammonolysis of 4-O-methyl-5-fluorouridine derived precursor.

INTRODUCTION

Fluorine has attracted much attention as an NMR spin label in the study of biopolymers and biological systems[1]. The ^{19}F nucleus has the advantage of a 100% natural abundance, a high sensitivity of detection, 83.3% of that of ^{1}H, and a large chemical shift dispersion. The extreme sensitivity of the fluorine chemical shifts to the environment of the nucleus makes ^{19}F NMR ideal for interaction studies. In this application the small radius of fluorine atom implying little steric hindrance comparable to hydrogen, is also an advantage. However, one should be aware that the high electronegativity of the fluorine atom can cause changes in the electron densities of the molecule, thus affecting the pK_a values, the charge transfer properties, etc.

Dedicated to Professor Yoshihisa Mizuno on the occasion of his 75th birthday.

Additionally, the preparative methods are far from being general or versatile enough to provide access to the regioselectively labelled nucleic acids without multistep syntheses.

In the DNA field the application of both one- and two-dimensional {^{19}F-^{1}H} techniques was of interest for the ^{19}F NMR study of 5-fluorouracil-labelled DNA duplexes[2], hairpins[3] and repressor-operator interactions[1,4]. ^{1}H NMR was applied to analyze 5F-dU:dA[5,6] and 5F-dU:dG[7] associations and the influence of a 5-fluorouracil incorporation on local and global duplex conformations. The synthesis of DNA duplexes containing 5-fluorodeoxycytidine was advanced[8-12] but only limited data were reported[12,13] on the ^{19}F NMR of these structures.

Since the pioneering work[14,15] on the tRNA biologically labelled with 5-fluorouracil residues, limited progress has been made concerning an appropriate evaluation of the scope and limitation of ^{19}F NMR to study the structure, dynamics and interactions of RNA.

Our research program concerns an evaluation of the structural properties of regioselectively fluorinated oligoribonucleotides when taking into account such interactions as base-pairing, stacking and ligand binding with a view toward further application of ^{19}F NMR as a complementary method to ^{1}H NMR in studying the conformation and interactions of RNA domains. We have started with 5-fluoropyrimidines as labels for RNA ^{19}F NMR studies. When our work reached an advanced stage, the synthesis of oligoribonucleotides containing 5-fluorouridine[16] (5F-rU) and NMR data on its base pairing with adenosine on monomer[17] and duplex level[16] were reported. To our knowledge no results concerning the incorporation of 5-fluorocytidine (5F-rC) in RNA and its structural consequences have been published. Therefore, we have decided to focus on 5-fluorocytidine and to consider a 5-fluorouridine modification as an appropriate complement in this paper.

Here we present structural data concerning the influence of a 5-fluoro substitution on the uracil and cytosine base pairing properties and sugar puckering of the respective ribosides. Regioselective, post-synthetic modification route leading to the incorporation of 5-fluorocytidine to the corresponding oligoribonucleotides using a 2'-O-*tert*-butyldimethylsilyl (*t*BDMSi) protection will be described. Our results open the way to the application of 5-fluorocytidine as a ^{19}F NMR label in RNA studies.

RESULTS AND DISCUSSION

Structural considerations.

To decide whether 5-fluoropyrimidines are suitable structural probes, one has to answer several questions. For example: (i)-Is the canonical base-pairing conserved both

in geometric aspects and in bond strength? (ii)-Is the sugar conformation of 5F-rU and 5F-rC preserved in order to facilitate substitution of natural U or C in the oligoribonucleotide to be studied? We decided to tackle both problems prior to the preparative work and subsequent NMR studies on oligoribonucleotide level. The problem reflected by the first question can be preliminarily viewed on the basis of semiempirical calculations. The second, often neglected question, was forwarded in view of data published[18] on the influence of 5-substituent on ribose pucker in pyrimidine ribonucleosides and can be answered by an analysis of the ^{1}H NMR spectra of mononucleosides.

It has been previously shown[19] that the PM3 semiempirical quantum mechanical method is capable of reproducing hydrogen bonds in base pairs. To investigate the gas-phase energetics of base-pairs formations we have performed calculations using the PM3 method[20] implemented in MOPAC 6.0[21]. N^1- and N^9-methylated analogues of pyrimidines and purines, respectively, were used as the models of nucleosides in these calculations.

The energy of the base-pair formation was obtained by subtracting the intramolecular energies of the individual bases from the energy of the base-pair. As shown in Table I, the 5-fluoro substitution only slightly disturbs the base-pair formation. The semiempirical calculations indicate (Table II) that while the 5F-rC:G base pair is less stable than its natural counterpart, 5F-rU is expected to form with A, a base-pair of enhanced stability relative to A:U. These results are in agreement with oligoribonucleotide T_m data[16] and the NMR results on the structure and stability of a 5F-rU:A base pair in deuterochloroform[17]. The difference in the calculated hydrogen bond distances and bond angle between the fluorinated and the natural base-pair is in the range of 0.01Å and 0.7°, respectively. These data provided a positive argument that 5-fluoropyrimidines can serve as structural probes when the base-pairing level of an interaction is considered.

The substitution of the C5 position of pyrimidine not only affects the heterocyclic base but may also influence the structure of the whole nucleoside including ribose puckering[18]. The conformational analysis of the sugar pucker in solution in terms of the geometry and the population of both the C2'-endo and the C3'-endo conformers rests upon the determination and interpretation of the proton -proton coupling data.

The ^{1}H NMR spectra of cytidine, uridine and their 5-fluoro derivatives were measured in D_2O under identical conditions at 22 °C. For 5F-rC the observed coupling constants are: $J_{1'2'}$=3.6 Hz, $J_{2'3'}$=5.3 Hz, $J_{3'4'}$=6.3 Hz and for C: $J_{1'2'}$=3.9 Hz, $J_{2'3'}$=5.2 Hz, $J_{3'4'}$=6.2 Hz. The corresponding values for 5F-rU and U were found to be identical: $J_{1'2'}$=4.5 Hz, $J_{2'3'}$=5.4 Hz, $J_{3'4'}$=5.4 Hz. The calculated fractional population of the C3'-

Table I. Hydrogen bond geometries in the minimized base-pair structures

hydrogen bond	r_{ha}	Θ_{dha}	hydrogen bond	r_{ha}	Θ_{dha}
O6(G)-N4(C)	1.80	173.7	N6(A)-O4(U)	1.82	175.7
N1(G)-N3(C)	1.78	170.8	N1(A)-N3(U)	1.78	175.3
N2(G)-O2(C)	1.84	177.6			
			N6(A)-O4(5F-rU)	1.83	176.2
O6(G)-N4(5F-rC)	1.80	174.0	N1(A)-N3(5F-rU)	1.77	175.4
N1(G)-N3(5F-rC)	1.79	171.2			
N2(G)-O2(5F-rC)	1.84	177.1	N6(A)-O4(T)	1.82	176.2
			N1(A)-N3(T)	1.78	176.0

r_{ha} - distance between hydrogen and acceptor atom [Å] , Θ_{dha} - the angle subtended at the hydrogen [°]

Table II. Energetics of base-pair formation (ΔH)

	T:A	U:A	5F-rU:A	C:G	5F-rC:G
ΔH(kcal/mol)	-5.6	-5.7	-6.0	-11.9	-11.3

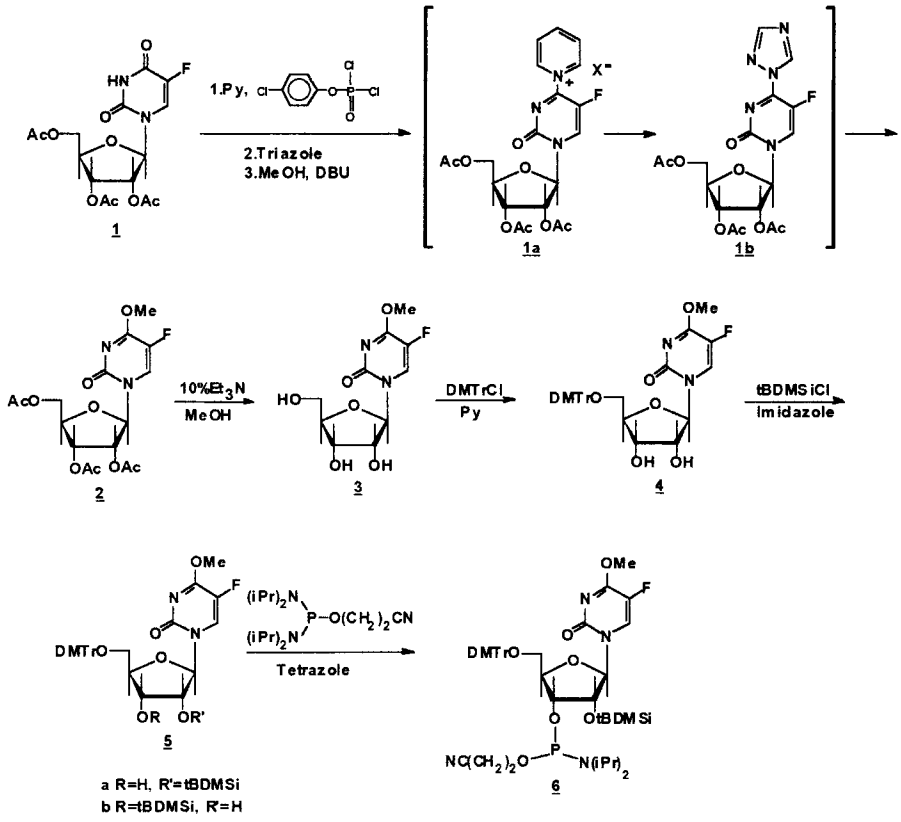
endo form is 0.57 for uridine and 5-fluorouridine. 5-Fluorocytidine prefered the C3'-endo pucker to a slightly greater extent (0.67) than cytidine (0.64). Taking into account the above data, it is reasonable to assume that the introduction of a fluorine atom at the C5 position of the pyrimidines does not influence the conformation of ribose moiety in uridine and in the case of cytidine.

These data and published results on 5F-rU[14-17] encouraged us to prepare oligoribonucleotides labelled with 5-fluorocytidine and 5-fluorouridine.

Synthesis of oligoribonucleotides labelled with 5F-rC and 5F-rU

We intend to study the conformation and the dynamics of RNA duplexes and bulge loops using ^{1}H and ^{19}F NMR. For this purpose the preparation of the oligomers containing 5F-rC and 5F-rU as fluorine labels was of interest. Recently a report on the synthesis of 5-fluorouridine containing oligoribonucleotides using 2'-O-*t*BDMSi protection appeared[16].

Synthetic problems arise in the case of 5-fluorocytidine. The electron withdrawing effect of fluorine is manifested in the lability of the 4-acylamino group in



protected 5F-rC; the application of 5F-rC with no exo-NH_2 group protection[10] resulted in low yields. A post-synthetic ammonolysis of an appropriate 4-substituted 5-fluorouracil derivative can generate 5-fluorocytosine on oligomer level. Two known examples of such an approach in the deoxy series apply either a 4-methylthio derivative (a concomitant hydrolysis[8]) or a 4-O-2,4,6-trimethylphenyl analogue[9].

From our studies of 5-fluorodeoxycytidine[12] we found that the easily accessible 4-O-methyl-5-fluorodeoxyuridine can be smoothly transformed into 5F-dC with ammonia-dioxane. The 4-O-methyl derivative, contrary to 4-(1,2,4-triazol-1-yl)-5-fluorodeoxyuridine, is perfectly stable during an oligodeoxynucleotide chain assembly on solid support using phosphoramidite chemistry. As we have found the riboside 2 when treated with ammonia/methanol overnight at room temp. gave (TLC) 5-fluorocytidine in a quantitatative manner with the same physical data as reported[22].

The above results encouraged us to prepare 5'-O-dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-4-O-methyl-5-fluorouridine 3'-O-(2-cyanoethyl-N,N-diisopropyl) phosphoramidite 6 as the precursor building block for a post-synthetic regioselective introduction of 5-fluorocytidine to RNA (see scheme above).

In the course of the transformation of 1 to 2 the lactam system of 5-fluorouracil residue was functionalized using the procedure of 4-O-phosphorylation[23]. Both fluorescent 4-pyridinium salt 1a and 4-(1,2,4-triazol-1-yl)-derivative 1b were too labile to be isolated. When treated *in situ* with methanol in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), the 4-O-methyl analogue 2 was obtained with a 93% yield. The 2',3',5'-de-O-acetylation to form 3 was performed using triethylamine with methanol. Further transformations to phosphoramidite 6 were performed under standard conditions. The overall yield of 6 was 33% after the recycling of isomer 5b.

The following oligoribonucleotides were prepared:

7 CGCG**C**$^{\mathbf{F}}$G, 8 UAUA**U**$^{\mathbf{F}}$A, 9 GUCUCUCG

10 CAGAUCU$^{\mathbf{F}}$GAGC, 11 CAGAU**C**$^{\mathbf{F}}$UGAGC

The oligomers 7 and 8 are self-complementary. The association of oligomer 9 with 10 or 11 repeats part of the TAR RNA element of HIV-1 with the "bulge" formed by the functionally important three pyrimidines:

5'CAGA/U**C**$^{\mathbf{F}}$U\GAGC3' 5'CAGA/UCU$^{\mathbf{F}}$\GAGC3'

3'GUCU———CUCG5' 3'GUCU———CUCG5

A synthesis of oligoribonucleotides 8-10 was performed using the standard procedures described for the method applying the 2'-O-*t*BDMSi protection[16,24-26]. In the case of the preparation of 7 and 11, the post-synthetic transformation of the 4-O-methyl-5-fluorouridine precursor into 5-fluorocytidine occurred simultaneously with the oligomer cleavage from the solid support, the removal of the 2-cyanoethyl groups and deprotection of the exo-amine groups when treated with anhydr. ammonia/methanol for 80 hrs at room temp. The conversion to a 5-fluorocytidine unit went in a quantitative manner as proved by an oligoribonucleotide composition analysis. No 5-fluorouridine was detected when digesting 7 and 11. In all cases 2'-O-desilylation was achieved with tetra-*n*-butylammonium fluoride in tetrahydrofuran (TBAF/THF). The mixture after desilylation contained a large excess of the deblocking reagent, tetra-*n*-butylammonium fluoride. In view of further ^{1}H NMR and ^{19}F NMR applications, the tetra-*n*-butylammonium cations and the fluoride anions should be completely removed from the sample. This problem was not encountered earlier[27] since most of the RNA syntheses using a 2'-O-silyl protection were aimed at functional or crystallographic RNA studies. We tested the efficiency of the different routes to achieve this separation by estimating the concentration of remaining ions by ^{1}H and ^{19}F NMR. Gel filtration chromatography on Biogel P-2 turned out to be the purification method of choice. The sample after Biogel P-2 and Dowex 50Wx8 (ammonium form) chromatography was homogeneous with respect to the above. The oligoribonucleotide sample could then be finally purified by the reverse phase or ion-exchange HPLC and desalted. The use of ammonium

bicarbonate instead of an acetate buffer during reverse phase HPLC separation prevented acetate ions contamination of the oligoribonucleotide samples for ^{1}H NMR measurements.

A structural ^{1}H and ^{19}F NMR analysis of the RNA duplexes is in progress.

EXPERIMENTAL

General. Solvents were purified according to the usual procedures. All the 2'-O-*t*BDMSi-protected ribonucleosides and their 3'-(2-cyanoethyl)-(N,N-diisopropyl) phosphoramidites, including 5-fluorouridine synthon[16], were prepared according to standard procedures[24-26]. TLC was carried out on Merck silica gel HF_{254} glass plates, using the solvent systems: A (chloroform-methanol 9:1 v/v), B (chloroform-ethyl acetate 17:3 v/v), C (isopropanol-conc.ammonia-water 7:1:2 v/v) or on silanised silica gel plates (Merck #5747), using solvent system D (acetone-water 8:2 v/v). Short column chromatography was performed on Merck silica gel 60H. The UV absorption was measured on a Beckman DU70 spectrophotometer. The NMR spectra were measured on a Varian Unity 300 (^{1}H, 300 MHz) spectrometer. Chemical shifts are reported in ppm relative to TMS (internal standard) for ^{1}H and ^{13}C and 85% phosphoric acid or trifluoroacetic acid (external standards) for ^{31}P or ^{19}F respectively. The spectra were recorded in $CDCl_3$ solutions (^{1}H, ^{13}C and ^{19}F) or acetonitrile-1% triethylamine (^{31}P), unless otherwise specified. The gas-phase energetics of base-pair formation have been calculated on a PC using the PM3 method[20] implemented in MOPAC 6.0[21]. The PRECISE option with the EF routine was used throughout. For a visualization of the results (data not shown) a SGI Iris Indigo^{2} workstation and InsightII software from Biosym Technologies GmbH were used. HPLC separations were performed with a Waters 600E instrument equipped with a diode-array UV detector. Solid reagents were rendered dry prior to a reaction by a repeated evaporation of pyridine solutions. Unless otherwise specified, the reaction mixture work-up consisted of washing the organic solution with 3 volumes of sat. $NaHCO_3$, a volume of sat. NaCl and drying of the solution with Na_2SO_4. All of the operations with RNA oligomers after the ammonia deprotection step were performed with sterile water and buffers, using gloves and sterilized equipment.

2',3',5'-tri-O-Acetyl-4-O-methyl-5-fluorouridine 2. Dried 2',3',5'-tri-O-acetyl-5-fluorouridine 1 (990 mg, 2.5 mmole, 1 equiv.) and 1,2,4-triazole (528 mg, 7.65 mmole, 3 equiv.) were dissolved in anhydr. pyridine (13 ml) and 4-chlorophenyl-phosphorodichloride (620 µl; 3.8 mmole; 1.5 eqiv.) was added. The reaction mixture was stirred in the dark for 110 hrs. The course of the reaction was followed by HPLC (DeltaPak column, isocratic 0.1M ammonium acetate in 18.5% acetonitrile-water: 1

t_r=11.94 min, 1b t_r 13.84 min). The reaction was stopped by the dropwise addition of water (200 µl, 11.5 mmole, 3 eqiv.) with ice-cooling. The mixture was left for 1 hr at room temp. The solvents were evaporated, the resulted thick oil was dissolved in chloroform (100 ml) and was washed with sat. $NaHCO_3$ (25 ml). The water phase was back-extracted with chloroform and combined extracts were dried with Na_2SO_4 and evaporated. The resulting foam was dissolved in anhydr. methanol (10 ml) and DBU (10 µl; 0.06 mmole; 0.03 eqiv.) was added. After 15 min. at room temp. TLC showed a complete reaction. Acetic acid (50% aq. solution, 15 µl) was added to neutralize DBU and the reaction mixture was concentrated to a thick oil. A chromatography on silica gel (chloroform-methanol, stepwise gradient from 0 to 1% of methanol) gave pure 2 as a foam (956 mg; 2.37 mmole; 93%). TLC (A) 1b R_f=0.68 (blue fluorescence), 2 R_f=0.84. UV (methanol): λ_{max}=285 nm, ε=5880. ^{1}H NMR: 7.83 (d, J_{H6-F}=5.9 Hz; H6), 6.61 (dd, 1H, $J_{1'2'}$=4.3Hz, $J_{H1'-F}$=1.5Hz, H1'), 5.34 (dd, 1H, $J_{1'2'}$=4.3Hz, $J_{2'3'}$=5.3Hz, H2'), 5.30 (d, 1H, $J_{2'3'}$=5.3Hz, H3'), 4.43-4.38 (m, 3H, H4', H5', H5''), 2.18, 2.12, 2.11 (3s, 9H, OAc), 4.08 (s, 3H, OCH_3). ^{13}C NMR: 170.02, 169.58, 169.48 ($\underline{C}OCH_3$), 163.01 (C4), 153.29 (C2), 137.06 (C5), 126.37 (C6), 88.19 (C1'), 79.84 (C4'), 73.63 (C2'), 69.62 (C3'), 62.63 (C5'), 55.33 (OCH_3), 20.72, 2x20.48 ($CO\underline{C}H_3$). ^{19}F NMR: -92.00.

5-Fluorocytidine. 2',3',5'-tri-O-Acetyl-4-O-methyl-5-fluorocytidine 2 (50 mg, 0,12 mmole) was treated with anhydr. ammonia/methanol (2 ml) overnight at room temperature. The solvents were evaporated and the residue chromatographed on RP silicagel (0-5% aceton/water) yielding 5-fluorocytidine as a crystalline solid (26 mg, 0.10 mmole, 81%). The recrystallization from ethanol gave a pure compound of m.p. 195°C [lit[22] m.p. 193°C]. TLC (C): R_f = 0.61. UV (water): λ_{max}=281nm, ε=8000. ^{1}H NMR (D_2O): 7.99 (d, J_{H6-F}=6.4, 1H, H6), 5.82 (dd, $JH_{1'2'}$=3.6, $JH_{H1'-F}$=1.6, 1H, H1'), 4.22 (m, 1H, H2'), 4.13 (m, 1H, H3'), 4.08 (m, 1H, H4'), 3.90-3.77 (m, 2H, H5',5''). ^{13}C NMR (D_2O): 156.59 (C2), 159.15 (C4), 138.34 (C5), 126.50 (C6), 91.05 (C1'), 75.01 (C2'), 69.86 (C3'), 84.62 (C4'), 61.28 (C5'). ^{19}F NMR (D_2O): -89.77.

4-O-Methyl-5-fluorouridine 3. 2',3',5'-tri-O-Acetyl-4-O-methyl-5-fluorouridine 2 (863 mg, 2.37 mmole) was dissolved in dry 10% triethylamine in methanol (v/v, 7ml) and left for 18 hrs at room temp. The solvents were evaporated and the residue was chromatographed on silica gel (chloroform-methanol from 0 to 7%) yielding 3 as a foam (510 mg, 1.8 mmole, 78%). TLC (A): R_f=0.32. UV (water): λ_{max}=285 nm, ε=6000. ^{1}H NMR (DMSO-d_6): 8.66 (d, 1H, J_{H6-F} 6.8Hz, H6), 5.70 (m, 1H, H1'), 4.02-3.96 (m, 2H, H2', H3'), 3.93 (s, 3H, OCH_3), 3.90 (m, 1H, H4'), 3.79-3.58 (m, 2H, H5', H5''). ^{13}C NMR (DMSO-d_6): 162.93 (C4), 152.47 (C2), 135.64 (C5), 128.92 (C6), 90.00 (C1'), 83.93 (C4'), 74.40 (C2'), 68.17 (C3'), 59.36 (C5'), 54.45 (OCH_3). ^{19}F NMR (DMSO-d_6): -92.50.

5'-O-Dimethoxytrityl-4-O-methyl-5-fluorouridine 4. Dried 4-O-methyl-5-fluorouridine 3 (452 mg, 1.63 mmole, 1 equiv.) was treated with dimethoxytrityl chloride (582 mg, 1.70 mmole, 1.05 equiv.) in anhydr. pyridine (7 ml). The mixture was left at room temp. overnight and the solvent was evaporated. The residue was dissolved in chloroform (50 ml) and worked-up. A chromatography on silica gel (chloroform-methanol from 0 to 1.5%) gave pure 4 as a foam (863 mg, 1.49 mmole, 91%). TLC (A) R_f=0.47. ^{1}H NMR: 7.95 (d, 1H, J_{H6-F}=5.6Hz, H6), 7.31-6.79 (m, 13H, trityl), 5.75 (dd, 1H, $J_{1'2'}$=4.5Hz, $J_{H1'-F}$=0.9Hz, H1'), 4.52 (m, 1H, H2'), 4.42 (m, 2H, H3', H4'), 4.07 (s, 3H, 4-OCH_3), 3.79 (s, 6H, trityl OCH_3). ^{13}C NMR: 162.93 (C2), 155.05 (C4), 136.82 (C5), 127.79 (C6), 93.50 (C1'), 86.55 (C4'), 77.44 (C2'), 72.63 (C3'), 63.20 (C5'), 55.28 (4-OCH_3). ^{19}F NMR: -92.63.

5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-4-O-methyl-5-fluorouridine 5a. Dried 5'-O-dimethoxytrityl-4-O-methyl-5-fluorouridine 4 (718 mg, 1.27 mmole, 1 equiv.) and imidazole (338 mg, 4.97 mmole, 4 equiv.) were dissolved in anhydr. pyridine (4 ml) and treated with *t*-butyldimethylchlorosilane (224 mg, 1.49 mmole, 1.2 equiv.) with stirring at room temp. overnight. Following the evaporation of pyridine, the residue was dissolved in chloroform (50 ml) and after the usual work-up chromatographed on silica gel (chloroform-ethyl acetate from 0 to 1%) giving pure 5a as a foam (347 mg, 0.51 mmole, 40%) together with the 3'-O-silyl isomer 5b (320 mg, 0.47 mmole). The compound 5b was isomerized in methanol containing 1% triethylamine to the mixture of 5a and 5b (approx. 1:1). After two isomerizations and separations the overall yield of 5a was raised to 589 mg (0.866 mmole, 69%). TLC (B); 5a R_f=0.59, 5b R_f=0.24. ^{1}H NMR: 5a: 8.20 (d, 1H, J_{H6-F}=5.9Hz, H6), 7.43-6.83 (m, 13H, trityl), 5.81 (s, 1H, H1'), 4.35 (m, 2H, H2', H3'), 4.1 (m, 1H, H4'), 4.05 (s, 3H, 4-OCH_3), 3.79 (s, 6H, trityl OCH_3), 3.52 (m, 2H, H5', H5''), 0.94 (s, 9H, *t*-butyl), 0.30, 0.20 (2s, 6H, Si-CH_3); 5b: 7.86 (d, 1H, J_{H6-F}=5.9Hz, H6), 7.43-6.83 (m, 13H, trityl), 5.97 (dd, 1H, $J_{1'2'}$=5.4Hz, $J_{H1'-F}$=1.5Hz, H1'), 4.22 (m, 1H, H2'), 4.31 (m, 1H, H3'), 4.07 (m, 1H, H4') 4.06 (s, 3H, 4-OCH_3), 3.79 (s, 6H, trityl OCH_3), 3.52-3.21 (m, 2H, H5', H5''), 0.88 (s, 9H, *t*-butyl), 0.09, 0.00 (2s, 6H, Si-CH_3). ^{13}C NMR: 5a 162.80, (C4), 153.37 (C2), 136.64 (C5), 127.44 (C6), 90.63 (C1'), 83.30 (C4'), 76.61 (C2'), 69.46 (C3'), 61.62 (C5'), 55.07 (4-OCH_3), 25.85 ($\underline{C}(CH_3)_3$), 18.11 ($C(\underline{C}H_3)_3$), -4.41, -5.42 (Si-CH_3); 5b: 162.68 (C4), 153.79 (C2), 136.79 (C5), 127.36 (C6), 90.93 (C1'), 84.30 (C4'), 76.06 (C2'), 71.57 (C3'), 62.16 (C5'), 55.09 (4-OCH_3), 25.63 ($\underline{C}(CH_3)_3$), 18.06 ($C(\underline{C}H_3)_3$), -4.76, -4.94 (Si-CH_3). ^{19}F NMR: 5a: -92.96; 5b: -92.85.

5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-4-O-methyl-5-fluorouridine 3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite 6. 5'-O-Dimethoxytrityl-2'-O-t-butyl-di-methylsilyl-4-O-methyl-5-fluorouridine 5a (350 mg, 0.50 mmole, 1 equiv.) and

tetrazole (33.6 mg, 0.48 mmole, 0.95 equiv.) were vacuum dried, dissolved in anhydr. acetonitrile (2.5 ml) and treated under argon with bis(N,N-diisopropyl)(2-cyanoethoxyphosphine) (159.8 mg, 0.53 mmole, 1.05 equiv.) at room temp. overnight. Triethylamine (0.4 ml) was added, followed by sat. NaCl (4.2 ml) and the resulting solution was extracted with ethyl acetate (2x30 ml). The extracts were dried with Na_2SO_4 and evaporated to a foam. A chromatography on silicagel (hexane-ethyl acetate containing 0.4% triethylamine from 0 to 70% of ethyl acetate) gave pure 6 as a foam (321 mg, 0.36 mmole, 71%). The purified phosphoramidite was dissolved in benzene, filtered through Millipore filter and freeze-dried before use. TLC (D) R_f=0.45. ^{31}P NMR: 150.13, 149.01. ^{19}F NMR: -93.35, -93.40.

Oligoribonucleotides 7-11. The general protocol.

Synthesis. The oligoribonucleotides were synthesised on a Applied Biosystems 391PCR-Mate DNA/RNA synthesizer. The standard trityl-off protocol (Applied Biosystems Manual) for 1μmol scale was used applying a condensation time of 600s. The average coupling yield (judged from the extinction of trityl cation released) was 97% per step. For scaling-up the 1μmol scale was repeated 8-10 times. Two initial deprotection protocols were used. In the case of oligomers 8, 9 and 10: after the synthesis LCAA-CPG support was placed in a vial, 35% aq. ammonia/ethanol (3:1, 1.5 ml) was added, the vial was tightly capped and left at 55°C for 16 h. The supernatant was transferred to an Eppendorf tube, the remaining support was washed with water (2x0.5 ml) and the combined extracts were evaporated to dryness. In the case of oligomers 7 and 11: the LCAA-CPG support was placed in a vial and treated with saturated ammonia in methanol (1.5 ml) at room temp. for 80 hrs. Subsequently, for all cases the residue was dissolved in 1 M TBAF/THF (1 ml). After 16 hrs the reaction was quenched with water (0.5 ml).

Purification. The reaction mixture was immediately applied to a column of Biogel P2 (34x2 cm), and chromatographed with water. The UV-absorbing fractions (12 ml) were concentrated (3 ml) and passed through a column of Dowex 50Wx8 (NH_4^+ form, 1x6 cm) and washed with water. Fractions containing crude oligomer (approx. 9 ml, 55-60 O.D. for 7, 8 and 9, 55-80 O.D. for 10 and 11) were evaporated to dryness. The final purification was achieved by HPLC:

(i)-for octamer 9: a RP μBondapak column (7.8x300 mm), a solvent gradient of 5 - 7.5% of acetonitrile in 0.1 M NH_4HCO_3 during 30 min, a solvent flow 1.5 ml/min. The yield from one 1μmole synthesis after Biogel P2 was 25-30 O.D.

(ii)-for heksamers 7 and 8 and undecamers 10 and 11: a SAX Waters column (8x100 mm), a solvent gradient of 0.001M - 0.3M KH_2PO_4 (pH 6.3) in 60% formamide during 45 min, a solvent flow of 0.8 ml/min, at room temperature. The purified oligomers were

freed from formamide by filtration through a NAP25 cartridges (Pharmacia). Typical yield from one 1μmole synthesis was 10-16 O.D.
All oligoribonucleotide of declared (see above) purity were kept as lyophilisates at the temp. of -20°C.

Composition analysis. An aliquot of oligomer (0.6-1 O.D.) was dissolved in distilled water (20 μl) and nuclease P1 (Pharmacia, activity 800 u/mg) was added (20 μl) of the solution containing 0.5 mg/ml in 0.06 M NaOAc, pH 5.5). The solution was incubated at 37°C for 18 hrs, a Tris-HCl buffer (0.6M, pH 8.7, 20μl) was added, followed by bovine alkaline phosphatase (Boeringer, 1 μl, equiv. to 5 μg) and the incubation was continued for 2 hrs. The solution was centrifuged and a sample injected on the HPLC column (Waters C-18 Resolve cartrige, 8x100 mm, solvent gradient 0.1 M NH_4OAc for 8 min., then a rise to 12.5% acetonitrile in 0.1 NH_4OAc during 5 min., then isocratic for 15 min.). In these conditions A, C, G, U and 5F-rU are well resolved. In the case of oligomer 11 5F-rC and U comigrate. Digests from oligomers containing 5F-rC and U were additionally chromatographed using a gradient of 5% acetonitrile in 0.1 AcOH for 15 min., then rising to 12.5% acetonitrile and 0.1 M NH_4OAc during 5 min. and isocratic for 10 min. In these conditions 5F-rC and U are well resolved. All the digestions gave the correct nucleoside ratios within a 5-10% error margin.

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