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C−F···H−C Hydrogen Bonds in Ribonucleic Acids

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Abstract: We report the synthesis of 1'-deoxy-1'-(benzimidazol-1-yl)-β-D-ribofuranose **7** and 1'-deoxy-1'-phenyl-β-D-ribofuranose **2**. With these two ribonucleoside analogues we have a set of nine different RNA building blocks in hand, which are isostere to the natural bases. Now it is possible to investigate their duplex stabilizing forces. These forces are hydrogen bonds, base stacking, and solvation. The phosphoramidites of all building blocks were incorporated into a 12mer RNA, and the resulting RNA duplexes were investigated by UV- and CD-spectroscopy. We found that some of the RNA analogues are universal bases. The best universal bases with the lowest destabilization and the smallest discrimination between the natural bases are **1** (**B**) and **9** (**E**). On the basis of UV measurements we determined the melting points and the thermodynamic data. We were able to show that there are no hydrogen bonds between the natural bases and the RNA analogues. From thermodynamic data we calculated the contributions for base stacking and solvation of all modified building blocks. Comparison of calculated and measured data of double modified base pairs in 12mer RNA duplexes showed a further duplex stabilizing force in base pairs containing fluorine atoms at the Watson−Crick binding site. This stabilizing force can be defined as C−F···H−C hydrogen bond as is observed in crystal structures of 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose.

Introduction

There are three predominant forces which are responsible for the stability of the secondary structure of nucleic acids. These forces are hydrogen bonds, base stacking, and solvation.[1−3] It is very difficult to investigate one of these forces without changing parameters which are also important for the other ones.[4] Consequently, in many articles only one of these predominant forces was investigated, and the interactions between them were ignored.

However, since these interactions are very important, it is thus necessary to have a series of molecules to investigate hydrogen bonding, base stacking, and solvation effects. In this series the molecules cannot be the natural U (T), C, G, or A. It must be a series of modified ribonucleosides. It is also important to investigate a change in RNA secondary structure when modified nucleotides are incorporated into oligonucleotides. These nucleosides should be designed so that they do not change the secondary structure. Therefore, molecules which are the closest steric mimics of the natural nucleosides have to be designed.[5]

To address this problem, we decided to synthesize some novel nucleic acid analogues in which the nucleobases are replaced by fluorobenzenes or fluorobenzimidazoles.[6] We prepared nine protected phosphoramidites, eight of them with base modifications and one abasic site (Figure 1).

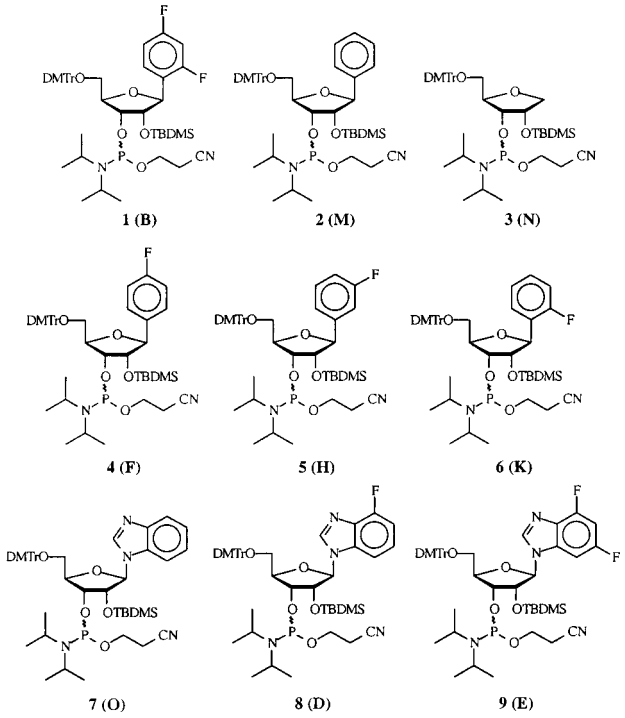

Figure 1. Synthesized modified phosphoramidites and the one-letter abbreviations of the nucleoside "bases".

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1′-Deoxy-1′-(2,4-difluorophenyl)-β-D-ribofuranose **1** is an isostere of the natural uridine, and 1′-deoxy-1′-(4-fluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose **8** is isosteric to inosine. The aromatic ring moiety was designed to be the closest possible steric mimic of the natural nucleobases, avoiding the presence of hydrophilic O- or N-containing groups. The best isosteric replacement of the C=O functionality is argued to be the C−F group, because of nearly identical bond lengths.[7,8] In the parent nucleosides of **4**−**6**, the natural bases are substituted by monofluorobenzenes. The fluorine atom has been introduced in all three possible positions on the benzene ring so that the influence of the fluorine position can be investigated. To evaluate the contribution of the fluorine atom on base-stacking effects for stability of duplex RNA we also synthesized the phosphoramidites **2**[9] and **7** without fluorine and the phosphoramidite **3** of the abasic site.

It is very important to investigate the influence of the F···H hydrogen bonds because little is known about hydrogen bonds containing fluorine as one of the acting atoms. In the literature there is some controversy whether "organic fluorine" can act as a hydrogen bond acceptor.[10,11] It is no question that the fluoride ion acts as a very strong proton acceptor. On one hand, the hydrogen bond of the bifluoride ion is one of the strongest known hydrogen bonds.[12] On the other hand, the C−F group, the so-called "organic fluorine", shows only a little or no ability to develop hydrogen bonds. On the basis of Cambridge Structural Database (CSD) studies, Dunitz and Taylor[11] maintained that the C−F group is a very poor acceptor, hardly ever forming hydrogen bonds. They compare the acceptor capabilities of the C−F group with −OH and −NH donors, but they do not consider a C−F···H−C hydrogen bond. The C−H group is known to be a hydrogen bond donor which can interact with oxygen, nitrogen, or chloride.[13,14] This raises the question concerning the existence and the nature of a C−F···H−C hydrogen bond. In contrast to that by Thalladi et al.,[15] who investigated fluorbenzenes and their crystal structures, this study deals with molecules which bear (apart from the C−F group) also O−H groups, which are more able to form hydrogen bonds.

Chemical Syntheses

The syntheses of **1**, **3**−**6**, **8**, and **9** have been discussed elsewhere.[6] Here we describe the syntheses of 1′-deoxy-1′-phenyl-β-D-ribofuranose phosphoramidite **2** and 1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose phosphoramidite **7**.

The synthesis of 1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose **13** (Figure 2) followed the glycosylation procedure of Vorbrüggen.[16] Refluxing 2 equiv of benzimidazole **10** with *N*,*O*-bis(trimethylsilyl)acetamide and subsequent reaction of the persilylated base with 1 equiv of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose **11** in the presence of the Lewis acid trimethylsilyl trifluoromethanesulfonate afforded the desired 2′,3′,5′-tri-*O*-acetyl-1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose **12** in 56% yield. As a byproduct a bis-glycosylated benzimidazol was obtained. Deprotection[17] of the acetylated nucleoside **12** furnished 1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose **13** in 90% yield.

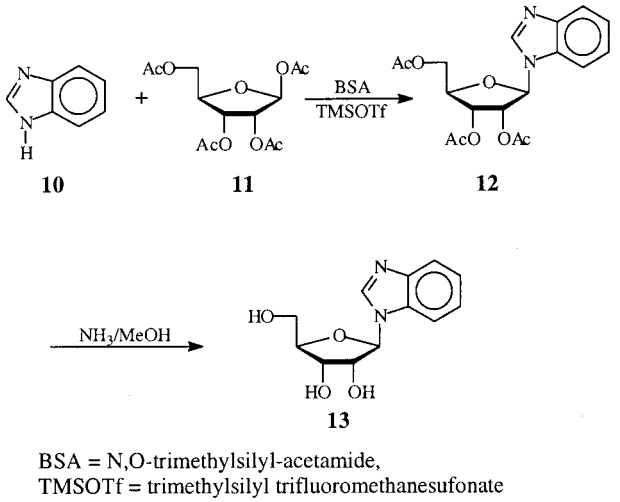

Figure 2. Synthesis of 1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose **13**.

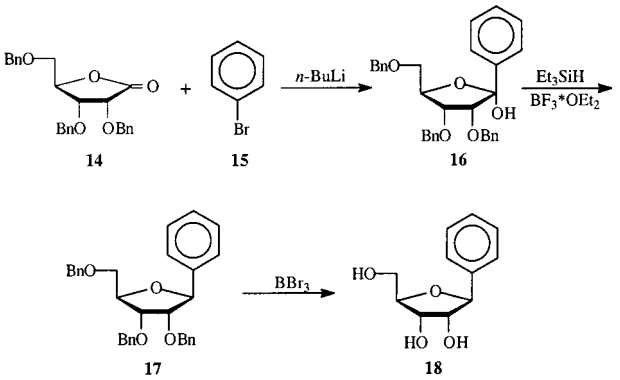

Figure 3. Synthesis of 1′-deoxy-1′-phenyl-β-D-ribofuranose **18**.

The synthesis of 1′-deoxy-1′-phenyl-β-D-ribofuranose **18** (Figure 3) starts with a C-glycosylation.[18] Lithiation of bromobenzene **15** with BuLi in THF at −78 °C followed by addition of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone[19] **14** gave the intermediate lactole **16**, which was directly dehydroxylated with triethylsilan and $BF_3 \cdot OEt_2$ to afford stereoselectively **17** in 75% yield. The deprotection of the benzylated nucleoside **17** with BBr_3 afforded 1′-deoxy-1′-phenyl-β-D-ribofuranose **18** in 69% yield.

The 5′-OH function was protected with 4,4′-dimethoxytrityl chloride (DMTrCl)[20,21] in dry pyridine to afford the 5′-*O*-(4,4′-dimethoxytrityl) protected nucleosides **19** and **20** in 73 and 75% yield, respectively (Figure 4). To protect the 2′-OH functions the nucleosides **19** and **20** were dissolved in THF/pyridine 1:1, treated with $AgNO_3$ and a 1 M (*tert*-butyl)dimethylsilyl chloride

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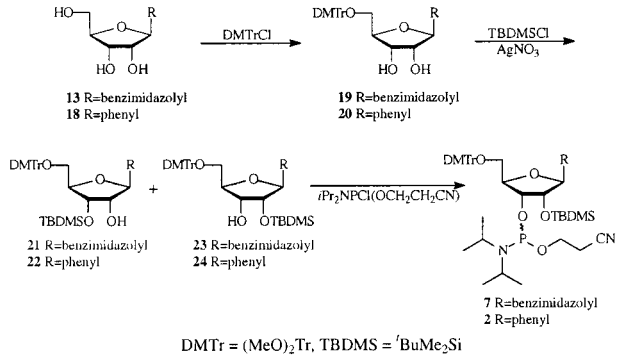

Figure 4. Synthesis of phosphoramidites **2** and **7**.

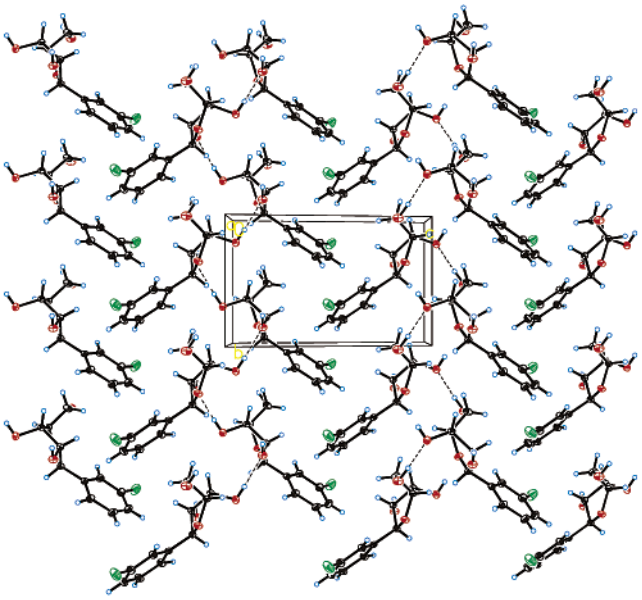

Figure 5. Crystal packing of 1′-deoxy-1′-(3-fluorophenyl)-β-D-ribofuranose.

solution in THF.[22,23] The 2′-TBDMS-protected nucleosides **23** and **24** were obtained in 27 and 29% yield, respectively. In both compounds the ^{t}BuMeSi group at C2′ tended to move to the 3′-position in polar solvents, resulting in low yields of the desired 2′-protected nucleosides. The final phosphitylation of **23** and **24** with *sym*-collidine, 1-methyl-1*H*-imidazole and 2-cyanoethyl diisopropyl-phosphoramidochloridite in acetonitrile afforded the phosphoramidites **7** and **2** in 54 and 57% yield, respectively.

Results and Discussion

All unprotected C-nucleosides were crystallized from methanol or water. 1′-Deoxy-1′-(3-fluorophenyl)-β-D-ribofuranose shows a distinct herringbone pattern (Figure 5).[24]

The crystal packings of 1′-deoxy-1′-(2-fluorophenyl)-β-D-ribofuranose,[25] 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose (Figure 6)[8] and 1′-deoxy-1′-(2,4-difluorophenyl)-β-D-ribofuranose[8] showed a different orientation of the nucleosides. In these structures the aromatic fluorophenyl rings are orientated to each other.

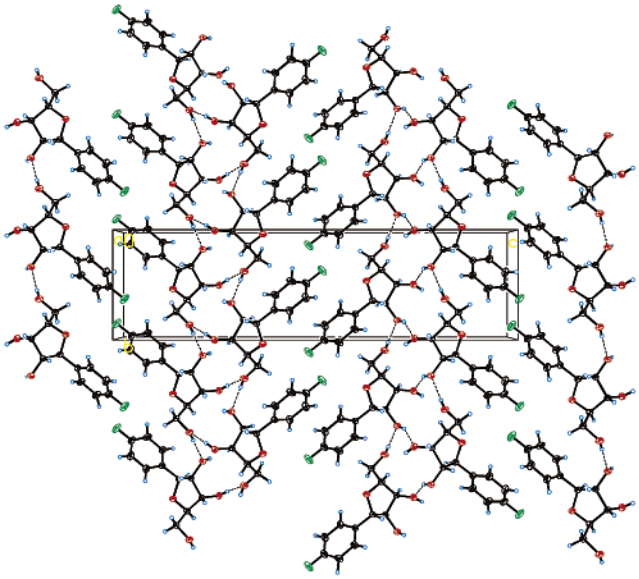

Figure 6. Crystal packing of 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose.

There are also very short F···H distances. In the crystal of 1′-deoxy-1′-(2-fluorophenyl)-β-D-ribofuranose the shortest C−F···H−C distance is exact the sum of the van der Waals radii of 255 pm of fluorine and hydrogen[26,27] between the fluorine and H5′ of the sugar. In the crystal of 1′-deoxy-1′-(2,4-difluorophenyl)-β-D-ribofuranose there are two short F···H distances. The shortest with 257 pm between F2 and H5′B of the sugar and another one with 260 pm between F4 and H10 (an ortho hydrogen to F4 of an opposite molecule). Both distances are larger than the sum of the van der Waals radii. Thus, it seems that there is only a very weak interaction between fluorine and hydrogen and no distinct hydrogen bond. In the case of ribonucleoside 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose there is a difference. Here is the shortest C−F···H−C (crystallized from methanol) distance 230 pm between fluorine and H10 (an ortho hydrogen to F of an opposite molecule). This is significantly shorter than the sum of the van der Waals radii of fluorine and hydrogen. The C−F···H−C hydrogen bond shows a nearly linear conformation with an angle of 158°. 1′-Deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose crystallized from water shows the shortest F···H distance of 238 pm. The difference is caused by the incorporation of a water molecule into the elementary cell. The water is placed between 2′-OH and 5′-OH which makes the F···H distance longer, but it is still shorter than the van der Waals radii of fluorine and hydrogen of 255 pm. Thus, 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose is one of the first examples of a molecule which shows a C−F···H−C hydrogen bond in its crystal pattern (Figure 7).[28]

The modified nucleosides were tested in a defined RNA sequence. In the 12 mer oligoribonucleotides (5′-CUU UUC XUU CUU paired with 3′-GAA AAG YAA GAA) only one position was modified, marked as X and Y, respectively. All

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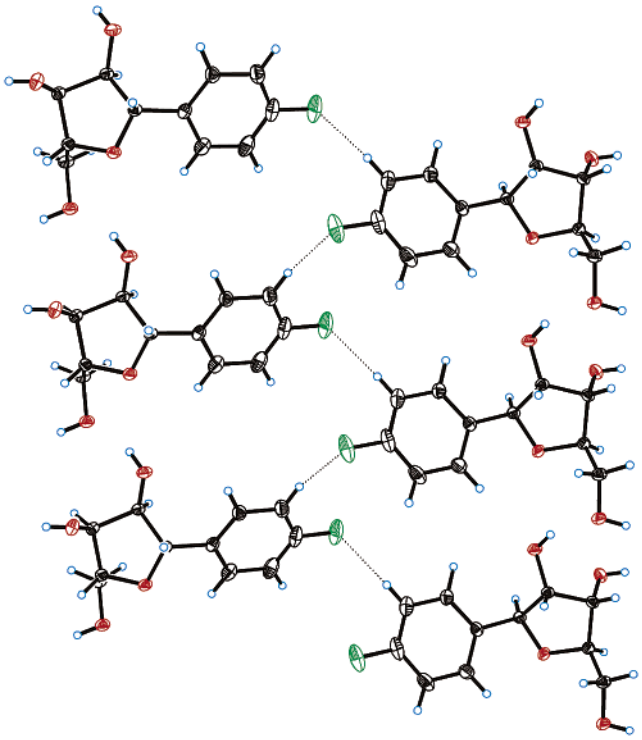

Figure 7. C−F···H−C hydrogen bonds in crystal of 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose.

Table 1. Synthesized Un- or Single Modified Duplex RNA (5′-CUU UUC XUU CUU Paired with 3′-GAA AAG YAA GAA) and Their Thermodynamic Properties[a]

	Y = A		Y = C		Y = G		Y = U	
X	T_m	ΔG^0	T_m	ΔG^0	T_m	ΔG^0	T_m	ΔG^0
U	37.8	11.9	30.4	9.8	38.6	11.9	30.1	9.7
B	27.4	9.0	27.3	8.9	27.6	9.0	27.9	9.1
F	23.8	7.9	24.1	8.0	24.2	8.0	25.6	8.4
H	24.7	8.2	25.0	8.2	25.0	8.2	25.7	8.4
K	27.3	8.9	25.1	8.3	27.4	9.0	26.5	8.7
M	23.0	7.7	22.6	7.6	23.5	7.9	23.1	7.7
I	31.2	10.1	41.7	13.4	31.7	10.2	34.2	11.0
D	28.0	9.1	27.5	8.9	28.7	9.3	28.5	9.2
E	28.4	9.2	28.7	9.2	29.4	9.5	29.3	9.5
O	28.7	9.2	25.6	8.5	28.9	9.4	29.4	9.3
N	20.6	7.2	18.6	6.7	20.9	7.3	18.2	6.6

[a] Phosphate buffer (140 mM NaCl, 10 mM Na_2HPO_4, 10 mM NaH_2PO_4); T_m: [°C]; ΔG^0: [kcal/mol] (T = 298 K). Errors: T_m: ±0.2 °C; ΔG^0: ±2%.

measurements were done in a phosphate buffer containing 140 mM NaCl, 10 mM Na_2HPO_4, 10 mM NaH_2PO_4.

First we measured only RNA duplexes containing natural bases. The wobble base pair U·G shows the highest T_m (38.6 °C, Table 1). This is 0.8 °C higher than the natural U·A base pair (T_m=37.8 °C). The U·C and U·U mismatches show nearly the same stability (T_m=30.4 °C and 30.1 °C).

In a second series we measured the benzene and the fluorobenzene nucleosides paired with natural bases (Table 1). In these cases all T_m values are lower than those for the natural bases. Possible explanations for these findings are the lack of hydrogen bonds between the modified and the natural bases and that the modified bases are less solvated by water molecules than the natural ones. The absence of differences in T_m values by pairing for example 2,4-difluorobenzene (**B**) against a purine or a pyrimidine indicates that there are no hydrogen bonds. Table 1 shows, that all T_m values are nearly identical (27.4−27.9 °C).

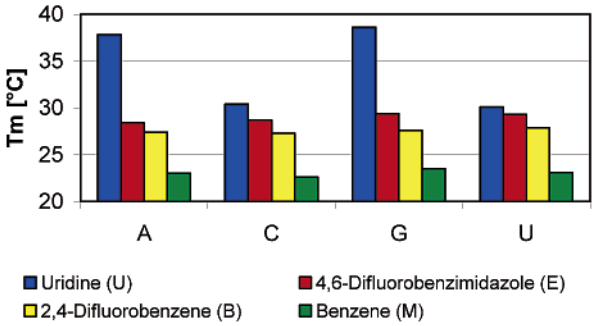

Figure 8. Pairing of nucleosides **U**, **B**, **E**, and **M** with the natural bases in the center of a 12-base pair RNA duplex measured by thermal melting temperature.

As for the 2,4-difluorobenzene (**B**), the uridine analogue, we found a new universal base,[29−32] which paired with all natural bases without energy discrimination. The other modified nucleosides are also universal bases, but the T_m ranges are greater than this one of **B**. In addition to **B** the nucleosides **E** and **M** are the bases with the least energy discrimination. The fluorinated nucleosides **B** and **E** are the better universal bases because they destabilize the RNA duplex 4.5−5.5 °C less than the benzene nucleoside **M** (Figure 8).

A second hypothesis that would further explain the lower T_m values for modified bases is that the destabilization of RNA duplex arises from the cost of desolvation of the hydrogen bond donors or acceptors of the natural bases during formation of the corresponding modified natural base pair.[33] Consequently, we have two destabilizing effects, which lower the stability of the RNA duplexes between 8 and 14 °C.

Interestingly, when a fluorine atom is in the 2-position of the benzene ring, the duplex is 2−3 °C more stable than with a hydrogen at the same position. There seems to be an interaction between this fluorine atom and an additional atom. Possibly this fluorine atom can form a hydrogen bond to the 5′-hydrogen of its own sugar moiety. This requires the benzene ring to take up a *syn*-conformation. Supporting this hypothesis is that a weak interaction between the fluorine and a 5′-hydrogen is observed in the corresponding crystal structures.

The fluorobenzimidazole-modified nucleosides were also compared to inosine (Table 1). Both modified nucleosides showed a destabilization of the duplex between 4 and 14 °C. The destabilization between an inosine mismatch base pair and a fluorobenzimidazole·natural base pair is lower than in the case of the fluorobenzenes. All RNA duplexes with the 4,6-difluorobenzimidazole (**E**)-modified nucleoside were between 0.4 and 1.2 °C more stable than the ones with the 4-fluorobenzimidazole (**D**) (Table 1).

Table 2 gives the results of pairing modified nucleosides against each other. We paired the 2,4-difluorobenzene (**B**) and the two fluorobenzimidazoles (**D** and **E**) against the fluorobenzene-modified nucleosides and the abasic site (**N**). The RNA duplexes with the 4,6-difluorobenzimidazole nucleoside

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Table 2. Synthesized Double Modified Duplex RNA (5′-CUU UUC XUU CUU Paired with 3′-GAA AAG YAA GAA) and Their Thermodynamic Properties[a]

	Y = B		Y = F		Y = H		Y = K	
X	T_m	ΔG^0	T_m	ΔG^0	T_m	ΔG^0	T_m	ΔG^0
B	32.5	10.2	29.9	9.6	31.3	9.8	31.9	10.1
D	33.5	10.7	30.6	9.8	30.3	9.6	32.8	10.5
E	34.6	11.2	31.3	10.0	31.4	10.0	33.6	10.7

[a] T_m: [°C]; ΔG^0: [kcal/mol] (T = 298 K). Errors: T_m: ±0.2 °C; ΔG^0: ±2%.

Table 3. Synthesized Double Modified Duplex RNA (5′-CUU UUC XUU CUU Paired with 3′-GAA AAG YAA GAA) and Their Thermodynamic Properties[a]

X	Y	T_m [°C]	$\Delta G°$ [kcal/mol]
N	**D**	25.3	8.3
N	**E**	26.3	8.5
N	**O**	26.2	8.5
N	**B**	22.6	7.7
N	**F**	20.9	7.1
N	**H**	21.0	7.2
N	**K**	21.3	7.3
N	**M**	19.5	6.8
O	**M**	28.9	9.1

[a] Errors: T_m: ±0.2 °C; ΔG^0: ±2%.

(**E**) are even approximately 1 °C more stable than the ones with the 4-fluorobenzimidazole nucleoside (**D**) (Table 2).

What are the individual contributions of base stacking, solvation, and hydrogen bonding to the stability of duplex RNA? Calculating the incorporation of the 2,4-difluorobenzene (**B**) against the abasic site (**N**) (T_m = 22.6 °C, Table 3) gives a 4.4 °C (1.1 kcal/mol) more stable duplex RNA than the one with an uridine·abasic site (**N**) base pair (T_m = 18.2 °C, Table 1). This indicates the contribution of stacking of the 2,4-difluorobenzene nucleoside (**B**) compared with uridine. An U·U base pair in duplex RNA (T_m = 30.1 °C, Table 1) is 2.2 °C more stable than a U·2,4-difluorobenzene (**B**) base pair (T_m = 27.9 °C, Table 1). An incorporation of one 2,4-difluorobenzene (**B**) stabilizes the duplex about 4.4 °C (1.1 kcal/mol) by increased stacking, but the U·2,4-difluorobenzene (**B**) base pair is 2.2 °C less stable than a U·U base pair. Thus, the contribution of solvation is 6.6 °C (1.7 kcal/mol) in destabilization of the RNA duplex per base pair. A RNA duplex with a 2,4-difluorobenzene (**B**)·2,4-difluorobenzene (**B**) base pair should be 2.2 °C more stable (+2 · 4.4 °C (2 · 1.1 kcal/mol, stronger stacking), −6.6 °C (−1.7 kcal/mol, less solvation)) than a U·U base pair. In our measurement a U·U base pair has a T_m of 30.1 °C (9.7 kcal/mol, Table 1), and a 2,4-difluorobenzene (**B**)· 2,4-difluorobenzene (**B**) base pair, has a T_m of 32.5 °C (10.2 kcal/mol, Table 2), proving our calculations.

In the same way we calculated the contributions of base stacking and solvation for the other modified nucleosides (Table 4).

The contributions of base stacking and solvation of the pyrimidine analogues **B**, **F**, **H**, **K**, and **M** were calculated against the complementary base uridine, the contributions of the purine analogues **D**, **E**, and **O** against the complementary base guanosine. All data were obtained only from the named RNA sequences.

In the case of the fluorobenzene nucleosides the number of fluorines in the aromatic ring shows a significant influence of the strength of base stacking. **B** with its two fluorine atoms is the best base-stacking nucleosides while the ones with a single fluorine atom show nearly the same results. The benzene nucleoside **M** has the smallest ability for strong base stacking (+1.3 °C) and the greatest destabilization by solvation effects (−8.3 °C). This explains why **M** is the universal base with the greatest destabilization effects of all investigated modified nucleosides.

Table 4. Contributions of Base-Stacking and Solvation of Modified Nucleosides[a]

	gain of stability through better base stacking	loss of stability through solvation
B	4.4 °C; 1.1 kcal/mol	−6.6 °C; −1.7 kcal/mol
F	2.7 °C; 0.5 kcal/mol	−7.2 °C; −1.8 kcal/mol
H	2.8 °C; 0.6 kcal/mol	−7.2 °C; −1.9 kcal/mol
K	3.1 °C; 0.7 kcal/mol	−6.7 °C; −1.7 kcal/mol
M	1.3 °C; 0.2 kcal/mol	−8.3 °C; −2.2 kcal/mol
D	4.4 °C; 1.0 kcal/mol	−6.3 °C; −1.6 kcal/mol
E	5.4 °C; 1.2 kcal/mol	−6.5 °C; −1.5 kcal/mol
O	5.3 °C; 1.2 kcal/mol	−6.3 °C; −1.7 kcal/mol

[a] Complementary base for the pyrimidine analogues is uridine, for the purine analogues, guanosine

With these results, the exact contributions for base stacking and solvation of all of the modified nucleosides, it is now possible to calculate the melting points of doubly modified base pairs. A G·benzene (**M**) base pair shows a T_m of 23.5 °C (7.9 kcal/mol, Table 1) and a benzimidazole (**O**)·benzene (**M**) base pair a T_m of 28.9 °C (9.1 kcal/mol, Table 3). The exchange of guanosine for benzimidazole (**O**) stabilizes the duplex by 5.3 °C (1.2 kcal/mol, Table 4). The exchange of the second natural base by benzimidazole adds no further energy of solvation to the duplex RNA.[33] Thus, the corresponding RNA duplex with the benzimidazole (**O**)·benzene (**M**) base pair should show a melting temperature of 28.8 °C and a free enthalpy ΔG^0 of 9.1 kcal/mol. The measured result of this base pair is a melting temperature of 28.9 °C and a free enthalpy ΔG^0 of 9.1 kcal/mol (Table 3). The calculated and the measured results are in agreement with each other. We also calculated the melting temperature of a 2,4-difluorobenzene (**B**)·4-fluorobenzimidazole (**D**) base pair. A U·4-fluorobenzimidazole (**D**) base pair shows a T_m of 28.5 °C (9.2 kcal/mol, Table 1) and a 2,4-difluorobenzene (**B**)·4-fluorobenzimidazole (**D**) base pair a T_m of 33.5 °C (10.7 kcal/mol, Table 2). The exchange of uridine for 2,4-difluorobenzene (**B**) stabilizes the duplex by 4.4 °C (1.1 kcal/mol, Table 4). Thus, the corresponding RNA duplex with the 2,4-difluorobenzene (**B**)·4-fluorobenzimidazole (**D**) base pair is 0.6 °C (0.4 kcal/mol) more stable than calculated (calculated: 32.9 °C; 10.3 kcal/mol; measured: 33.5 °C; 10.7 kcal/mol, Table 2). A similar result is obtained for the 2,4-difluorobenzene (**B**)·4,6-difluorobenzimidazole (**E**) base pair. The corresponding RNA duplex with the 2,4-difluorobenzene (**B**) ·4,6-difluorobenzimidazole (**E**) base pair is 0.9 °C (0.6 kcal/mol) more stable than calculated. In conclusion there seems to be a further stabilizing force which increases the T_m. It may be possible that this increase of T_m results from a weak F···H hydrogen bond between the modified nucleosides. The existence of such F···H hydrogen bonds in this class of molecules has been shown in the crystal structure of 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose with a F···H distance of 230 pm[8]. This hydrogen bond constitutes one of the first F···H hydrogen bonds of so-called "organic fluorine" in aqueous solution. For the orientation of the nucleobases in this double

Table 5. Partition Coefficients and HPLC Retention Times of the Modified Nucleosides

nucleoside	octanol–water partition coefficient log P	HPLC retention time [min]
uridine	0.022	n.d.
inosine	0.019	n.d.
1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose (**O**)	0.152	16.39
1′-deoxy-1′-(4-fluorobenzimidazol-1-yl)-β-D-ribofuranose (**D**)	1.782	16.82
1′-deoxy-1′-(4,6-difluorobenzimidazol-1-yl)-β-D-ribofuranose (**E**)	4.235	24.64
1′-deoxy-1′-phenyl-β-D-ribofuranose (**M**)	1.052	10.10
1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose (**F**)	1.497	14.95
1′-deoxy-1′-(3-fluorophenyl)-β-D-ribofuranose (**H**)	1.369	13.65
1′-deoxy-1′-(2-fluorophenyl)-β-D-ribofuranose (**K**)	0.809	12.49
1′-deoxy-1′-(2,4-difluorophenyl)-β-D-ribofuranose (**B**)	1.683	16.45

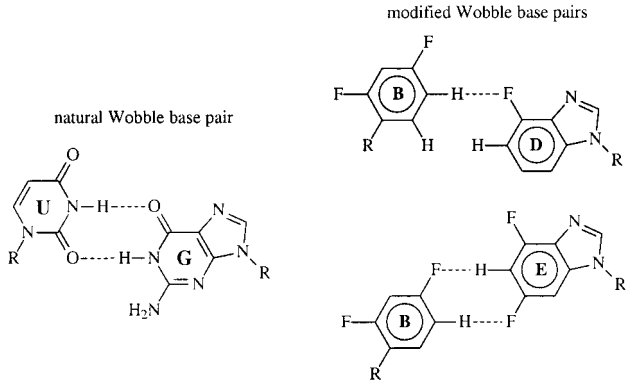

Figure 9. Natural U·G wobble base pair and postulated double modified base pairs with one or two F···H hydrogen bonds.

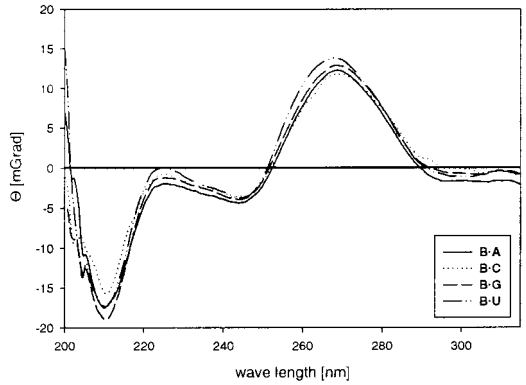

Figure 10. CD spectra of dodecamer RNA duplex with one B·natural base base pair.

modified base pair we postulate a formation as it is known from wobble base pairs in RNA (Figure 9).

The CD spectra of a RNA duplex with only one modified base follows a typical curve for an A-type helix (Figure 10). There is a strong maximum at approximately 270 nm, a weak minimum at approximately 245 nm, a weak maximum at approximately 225 nm, and a strong minimum at approximately 210 nm. Figure 10 shows the CD spectra of four different RNA duplexes with base pairs of the 2,4-difluorobenzene (**B**) base and the natural bases. The CD spectra of these four RNA duplexes with the universal base **B** show no significant differences.

Figure 11 shows the CD spectra of four different RNA duplexes. In comparison with the unmodified RNA (the U·A base pair in Figure 11) the modified ones show the greatest differences at the maximum at ca. 270 nm. In this region the differences could be explained by different stacking abilities

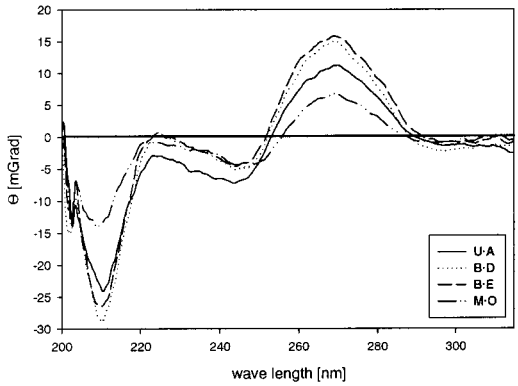

Figure 11. CD spectra of dodecamer RNA duplex with one modified base pair.

of the individual bases.[34] The results of stacking abilities obtained by calculation from the UV melting curves fit to the results obtained by CD spectroscopy. The small shift of maxima and minima in the CD spectra of the RNA duplex with the M·O base pair could be explained by a small horizontal stretch of the corresponding duplex. Initial results of molecular modeling point to this direction.

All CD spectra indicate that the structure of duplex RNA is only little or not disturbed by incorporation of one of our modified nucleic acid analogues. A base pair of modified nucleic acid analogues does not alter the A-type RNA structure. So all differences determined by UV measurements are a consequence of changes in stacking, solvation, or the ability to form hydrogen bonds and not of structural changes of the RNA duplex.

Besides improving the stabilitys these modifications have a pronounced influence on the lipophilicity of the RNA duplex. The partition coefficients between 1-octanol and water and the HPLC retention times reflect the change in lipophilicity (Table 5).[35,36] The modified nucleosides are between 40 and 200 times more lipophilic than the natural bases. This explains the reduced solvation of the bases by water molecules and the loss of stability of RNA duplexes containing fluoro-modified nucleosides.

Methods

Oligonucleotide Synthesis. The RNA oligomers were synthesized on an Eppendorf-D300+ synthesizer by phosphoramidite chemistry, with a coupling time for the modified monomers of 12 min.[37] The fully protected dodecamers were

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(35) Lien, E. J.; Goa, H.; Prabhakar, H. *J. Pharm. Sci.* **1991**, *80*, 517.
(36) Brinck, T.; Murray, J. S.; Politzer, P. *J. Org. Chem.* **1993**, *58*, 7070.
(37) Lyttle, H. M.; Wright, P. B.; Sinha, N. D.; Bain, J. D.; Chamberlin, A. R. *J. Org. Chem.* **1991**, *56*, 4608.

cleaved from the controlled-pore-glass (CPG) support with 32% aqueous NH_3 solution at 55 °C overnight. The 2′-silyl groups were deprotected with $Et_3N{\cdot}3$ HF within 24 h at room temperature.[38] The crude RNA oligomer was precipitated with BuOH at −20 °C, and the fully deprotected RNA was purified by means of anion-exchange HPLC (NucleoPac-PA-100). The pure oligomer was subsequently desalted (Sephadex-G25). All oligoribonucleotides were characterized by MALDI-TOF-MS, and the masses obtained were in good agreement with the calculated ones.

UV Melting Curves. UV melting profiles of the RNA duplexes were recorded in a phosphate buffer containing NaCl (140 mmol, pH 7.0) at oligonucleotide concentrations of 2 μM for each strand at wavelengths of 260 and 274 nm.[39] Each melting curve was determined four times. The errors of T_m and thermodynamic data resulted from the standard deviation of the four measurements of each duplex. The temperature range was 0−70 °C with a heating rate of 0.5 °C/min. A lower heating rate of 0.2 °C/min led to identical results. The thermodynamic data were extracted from the melting curves by means of a two-state model for the transition from duplex to single strands.[40]

CD Spectra. CD spectra of RNA duplexes were recorded at 315−200 nm with oligonucleotide concentration of 2 μM of each strand in a phosphate buffer containing NaCl (140 mmol, pH 7). The measurement was performed at 10 °C to ensure that only duplex RNA was present.

HPLC Retention Times. HPLC retention times were measured with the unprotected nucleosides with a RP-18 column (LiChrospher EcoCART 125-3). The eluation detergent was water with 5% acetonitrile and a flow of 0.6 mL/min.

Experimental Section

The anhydrous solvents, for example, THF, CH_2Cl_2, pyridine, and diethyl ether, were obtained from Fluka and used without further purification. Dry MeCN (H_2O <30 ppm) for the phosphitylation reaction was purchased from Perseptive Biosystems. Flash column chromatography (FC): silica gel 60 (40−63 μm) from Merck. TLC: silica gel 60 F_{254} plates from Merck; HPLC: anion-exchange column NucleoPac PA-100 from Dionex; desalting with a Sephadex-G25 column from Pharmacia. UV/melting profiles: Varian-Cary-1-UV/vis spectrophotometer, Cary temperature controller, 10-mm cuvette. CD spectra: Jasco-710 spectropolarimeter. NMR: Bruker-AM250 and Bruker-WH270 (^{1}H,^{13}C) and Bruker-AMX400 (^{1}H,^{13}C,^{31}P) spectrometers; δ in ppm, J in Hz. MS: PerSeptive Biosystems MALDI-TOF spectrometer Voyager DE: ESI = electrospray-ionization.

2′,3′,5′-Tri-*O*-acetyl-1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose (12). To a suspension of benzimidazole (5.2 g, 44 mmol) **10** in MeCN (80 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (10.75 mL, 44 mmol) and heated under reflux for 15 min. After the mixture was cooled to room temperature, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (7.0 g, 22 mmol) **11** in MeCN (80 mL) and trimethylsilyl trifluoromethanesulfonate (5 mL, 27.6 mmol) were added and heated under reflux for 2.5 h. The mixture was treated with 5% $NaHCO_3$ solution and extracted with CH_2Cl_2, the organic phase was dried and evaporated, and the residue was purified by FC (CH_2Cl_2/MeOH, 98:2). The product was obtained as a white foam in 56% yield (4.6 g, 12.2 mmol). TLC (CH_2Cl_2/MeOH, 95:5): R_f = 0.43; ^{1}H NMR (400 MHz d_6-DMSO, ppm) 8.49 (1H, s, H2), 7.73 (2H, m, arom H), 7.29 (2H, m, arom H), 6.32 (1H, d, J = 6.4 Hz, H1′), 5.68 (1H, t, J = 6.3 Hz, H2′), 5.43 (1H, dd, J = 4.6 Hz, H3′), 4.41 (1H, m, H4′), 4.37 (2H, m, H5′), 2.13 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.03 (3H, s, CH_3); ^{13}C NMR (100.6 MHz d_6-DMSO, ppm) 169.55, 169.06, 168.76 (C=O), 143.36 (C2), 142.36, 131.93, 122.65, 119.06, 110.85 (arom C), 85.76 (C1′), 78.11 (C4′), 71.10 (C2′), 69.09 (C3′), 62.53 (C5′), 20.04, 19.90, 19.69 (CH_3); ESI-MS: 377.2 ([M + H]$^+$).

1′-Deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose (13). A solution of **12** (3 g, 7.9 mmol) in NH_3-saturated MeOH (175 mL) was stirred for 20 h and then evaporated. The residue was purified by FC (CH_2Cl_2/MeOH 4:1). The product was obtained as a white solid in 90% yield (1.79 g, 7.1 mmol). TLC (CH_2Cl_2/MeOH, 4:1): R_f = 0.53; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 8.45 (1H, s, H2), 7.70 (2H, m, arom H), 7.24 (2H, m, arom H), 5.86 (1H, d, J = 6.2 Hz, H1′), 5.46 (1H, d, J = 6.5 Hz, OH-2′), 5.21 (1H, d, J = 4.8 Hz, OH-3′), 5.10 (1H, t, J = 5.2 Hz, OH-5′), 4.36 (1H, q, J = 5.3 Hz, H2′), 4.12 (1H, m, H3′), 3.96 (1H, q, J = 3.4 Hz, H4′), 3.63 (2H, m, H5′); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 143.82 (C2), 142.42, 132.98, 122.62, 122.02, 119.54, 111.54 (arom C), 88.64 (C1′), 85.43 (C4′), 73.58 (C2′), 70.13 (C3′), 61.26 (C5′); ESI-MS: 251.1 ([M + H]$^+$).

1′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-1′-(benzimidazol-1-yl)-β-D-ribofuranose (19). To a solution of **13** (2.73 g, 10.9 mmol) in anhydrous pyridine (100 mL) was added DMTrCl (5.2 g, 15.4 mmol), and the mixture was stirred for 4 h under argon at room temperature. The reaction was quenched by addition of MeOH (3 mL). The mixture was evaporated, the residue was dissolved in CH_2Cl_2, and the solution was extracted with 5% $NaHCO_3$ solution, dried ($MgSO_4$), evaporated, and coevaporated twice with toluene. The crude product was purified by FC (CH_2Cl_2/MeOH, 95:5). The product was obtained as a yellow foam in 73% yield (4.43 g, 8 mmol). TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.68; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 8.35 (1H, s, H2), 7.68 (2H, m, arom H), 7.38−6.81 (15H, m, arom H), 5.91 (1H, d, J = 5.5 Hz, H1′), 5.59 (1H, d, J = 6.1 Hz, OH-2′), 5.27 (1H, d, J = 5.4 Hz, OH-3′), 4.36 (1H, q, J = 5.7 Hz, H2′), 4.19 (1H, q, J = 5.2 Hz, H3′), 4.10 (1H, q, J = 5.2 Hz, H4′), 3.73 (6H, s, OCH_3), 3.23 (2H, m, H5′); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 158.08, 144.78 (DMTr), 143.94 (C2), 142.24 (arom C), 135.42, 135.33 (DMTr), 132.84 (arom C), 129.77, 127.84, 127.71, 126.71 (DMTr), 122.57, 122.13, 119.65 (arom C), 113.19 (DMTr), 111.75 (arom C), 88.95 (C1′), 85.68 (DMTr), 83.33 (C4′), 73.07 (C2′), 70.12 (C3′), 63.70 (C5′), 55.02 (OCH_3); ESI-MS: 553.2 ([M + H]$^+$).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-*tert*-butyldimethylsilyl-1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose (23). To a solution of **19** (0.95 g, 1.7 mmol) in anhydrous THF/pyridine 1:1 (20 mL) were added $AgNO_3$ (380 mg, 2.2 mmol) and 1 M ^{t}BuMe$_2$SiCl in THF (2.2 mL, 2.2 mmol) and were stirred for 20 h under argon at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution. The suspension was filtered, the filtrate was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The residue was coevaporated twice with toluene and purified by FC (CH_2Cl_2/^{i}PrOH, 98:2). The product was obtained as a white foam in 27% yield (310 mg, 0.46 mmol). TLC (CH_2Cl_2/^{i}PrOH, 98:2): R_f = 0.35; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 8.35 (1H, s, H2), 7.66 (2H, m, arom H), 7.41−6.82 (15H, m, arom H), 5.92 (1H, d, J = 6.5 Hz, H1′), 5.19 (1H, d, J = 5.5 Hz, OH-3′), 4.58 (1H, t, J = 5.7 Hz, H2′), 4.18 (1H, m, H3′), 4.13 (1H, m, H4′), 3.71 (6H, s, OCH_3), 3.29 (2H, m, H5′), 0.68 (9H, s, $SiC(CH_3)_3$), −0.15, −0.31 ($SiCH_3$);^{13}C NMR (100.6 MHz d_6-DMSO, ppm) 158.10, 144.72 (DMTr), 143.95 (C2), 142.59 (arom C), 135.21, 135.11 (DMTr), 132.40 (arom C), 129.78, 129.72, 127.79, 127.61, 126.73 (DMTr), 122.37, 122.12, 119.65 (arom C), 113.16 (DMTr), 111.92 (arom C), 88.61 (C1′), 85.82 (DMTr), 83.99 (C4′), 74.51 (C2′), 69.94 (C3′), 63.50 (C5′), 55.00 (OCH_3), 25.43 ($SiC(\underline{C}H_3)_3$), 17.69 ($Si\underline{C}(CH_3)_3$), −5.12, −5.61 ($SiCH_3$); ESI-MS: 667.6 ([M + H]$^+$).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-*tert*-butyldimethylsilyl-1′-deoxy-1′-(benzimidazol-1-yl)-β-D-ribofuranose (21) was obtained from the

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(39) Schweitzer, M.; Engels, J. W. In *Antisense: From Technology to Therapy*; Schlingensiepen, R., Brysch, W., Schlingensiepen, K.-H., Eds.; Blackwell Science: Cambridge, MA, 1997; Vol. 6, pp 78−103.
(40) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601.

reaction described above as the slower-migrating isomer. The product was obtained as a white foam in 36% yield (410 mg, 0.61 mmol). TLC (CH_2Cl_2/^{i}PrOH, 98:2): $R_f = 0.32$; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 8.40 (1H, s, H2), 7.69 (2H, m, arom H), 7.40−6.82 (15H, m, arom H), 5.90 (1H, d, $J = 6.1$ Hz, H1′), 5.50 (1H, d, $J = 6.5$ Hz, OH-2′), 4.52 (1H, q, $J = 5.8$ Hz, H2′), 4.35 (1H, m, H3′), 4.07 (1H, m, H4′), 3.72 (6H, s, OCH_3), 3.24 (2H, m, H5′), 0.82 (9H, s, $SiC(CH_3)_3$), 0.07, 0.02 ($SiCH_3$); ^{13}C NMR (100.6 MHz d_6-DMSO, ppm) 158.11, 144.58 (DMTr), 143.99 (C2), 142.52 (arom C), 135.26, 135.17 (DMTr), 132.64 (arom C), 129.69, 127.81, 127.63, 126.74 (DMTr), 122.47, 122.11, 119.64 (arom C), 113.16 (DMTr), 111.85 (arom C), 88.87 (C1′), 85.87 (DMTr), 83.82 (C4′), 72.47 (C2′), 71.92 (C3′), 63.14 (C5′), 55.03 (OCH_3), 25.73 ($SiC(\underline{C}H_3)_3$), 17.99 ($Si\underline{C}(CH_3)_3$), −4.50, −5.14 ($SiCH_3$); ESI-MS: 667.5 ($[M + H]^+$).

1′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-1′-(benzimidazol-1-yl)-β-D-ribofuranose Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (7). To a solution of **23** (150 mg, 0.22 mmol) in anhydrous MeCN (8 mL) were added collidine (=2,4,6-trimethylpyridine, 285 μL, 2.2 mmol), 1-methyl-1H-imidazole (9 μL, 0.11 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (72 μL, 0.32 mmol), and the mixture was stirred for 15 min at 0 °C and for 45 min at room temperature under argon. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The crude product was purified by FC (CH_2Cl_2/MeOH, 99:1). The product (diastereoisomer mixture) was obtained as a white foam in 54% yield (105 mg, 0.12 mmol). TLC (hexane/AcOEt, 4:1): $R_f = 0.09$; ^{1}H NMR (400 MHz d_6-DMSO, ppm) 8.12, 8.08 (2H, s, H2), 7.79 (2H, d, $J = 8.0$ Hz, arom H), 7.63 (2H, d, $J = 8.1$ Hz, arom H), 7.48−6.80 (30H, m, arom H), 5.92, 5.86 (2H, d, $J = 7.5$ Hz, $J = 7.0$ Hz, H1′), 4.76 (2H, m, H2′), 4.31 (2H, m, H3′), 3.95 (2H, m, H4′), 3.79, 3.78 (12H, s, OCH_3), 3.56 (8H, m, H5′, CH_2CN), 2.68 (4H, m, OCH_2), 1.20 (12H, m, $CH(CH_3)_2$), 0.81, 0.74 (18H, s, $SiC(CH_3)_3$), −0.14, −0.37, −0.40, −0.49 (12H, s, $SiCH_3$); ^{31}P NMR (162 MHz, $CDCl_3$, ppm) 150.58, 149.99; ESI-MS: 867.7 ($[M + H]^+$).

2′,3′,5′-Tri-*O*-benzyl-1′-deoxy-1′-phenyl-β-D-ribofuranose (17). A solution of bromobenzene (0.76 mL, 7.1 mmol) **15** in anhydrous THF (20 mL) was treated under argon at −78 °C within 10 min with 1.6 M BuLi in hexane (4.5 mL, 7.2 mmol). After 20 min at −78 °C a solution of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (2.0 g, 4.8 mmol) **14** in THF (20 mL) was added over 30 min, and the mixture was stirred for an additional hour and then warmed within 2 h to −30 °C (TLC control).The reaction was quenched by addition of water, the mixture was extracted with Et_2O, and the organic phase was dried ($MgSO_4$) and evaporated to afford an oil. The residue was dissolved in CH_2Cl_2 (20 mL) and treated at −78 °C with BF_3·Et_2O (1.2 mL, 9.5 mmol) and Et_3SiH (1.5 mL, 9.5 mmol). The mixture was stirred for 1 h at −78 °C and then warmed overnight to 10 °C. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by FC (hexane/AcOEt, 4:1). The product was obtained as an orange solid in 75% yield (1.71 g, 3.6 mmol). TLC (hexane/AcOEt, 4:1): $R_f = 0.45$; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 7.40−7.19 (20H, m, arom H), 4.88 (1H, d, $J = 6.5$ Hz, H1′), 4.61−4.43 (6H, m, $PhCH_2$), 4.24 (1H, q, $J = 4.0$ Hz, H4′), 4.06 (1H, t, $J = 4.4$ Hz, H3′), 3.90 (1H, m, H2′), 3.64 (2H, m, H5′); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 140.60, 138.30, 138.20, 138.07, 128.26, 128.21, 128.15, 127.84, 127.56, 127.50, 127.40, 126.25 (arom C), 83.42 (C1′), 81.90 (C4′), 81.07 (C2′), 77.28 (C3′), 72.42, 71.07, 70.98 ($PhCH_2$), 70.32 (C5′); ESI-MS: 498.4 ($[M + NH_3]^+$).

1′-Deoxy-1′-β-D-phenylribofuranose (18). A solution of **17** (0.2 g, 0.42 mmol) in anhydrous CH_2Cl_2 was treated with 1 M BBr_3 in CH_2Cl_2 (1 mL, 1 mmol) at −78 °C and stirred for 1.5 h under argon. The reaction was quenched by addition of CH_2Cl_2/MeOH, 1:1 (5 mL) and evaporated. The residue was purified by FC (CH_2Cl_2/MeOH, 9:1). The product was obtained as a white solid in 69% yield (60 mg, 0.29 mmol). TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.24$; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 7.41−7.22 (5H, m, arom H), 4.93 (1H, d, $J = 6.8$ Hz, H1′), 4.86 (1H, d, $J = 4.7$ Hz, OH-3′), 4.77 (1H, t, $J = 5.5$ Hz, OH-5′), 4.54 (1H, d, $J = 7.1$ Hz, OH-2′), 3.88 (1H, m, H4′), 3.80 (1H, m, H3′), 3.68 (1H, q, $J = 5.6$ Hz, H2′), 3.53 (2H, m, H5′); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 141.42, 127.96, 127.23, 126.24 (arom C), 85.06 (C1′), 82.99 (C4′), 77.63 (C2′), 71.44 (C3′), 62.06 (C5′); ESI-MS: 209.0 ($[M + H]^-$).

5′-*O*-(4,4′-Dimethoxytrityl)-1′-deoxy-1′-phenyl-β-D-ribofuranose (20). To a solution of **18** (1.0 g, 4.75 mmol) in anhydrous pyridine (25 mL) and Et_3N (1.0 mL, 7.2 mmol) was added DMTrCl (1.93 g, 5.7 mmol), and the mixture was stirred for 4 h under argon at room temperature. The reaction was quenched by addition of MeOH (3 mL). The mixture was evaporated, the residue was dissolved in CH_2Cl_2, and the solution was extracted with 5% $NaHCO_3$ solution, dried ($MgSO_4$), evaporated, and coevaporated twice with toluene. The crude product was purified by FC (CH_2Cl_2/MeOH, 98:2). The product was obtained as a yellow foam in 75% yield (1.83 g, 3.57 mmol). TLC (CH_2Cl_2/MeOH, 98:2): $R_f = 0.23$; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 7.47−6.86 (18H, m, arom H), 5.07 (1H, d, $J = 6.5$ Hz, H1′), 4.93 (1H, d, $J = 5.2$ Hz, OH-3′), 4.66 (1H, d, $J = 6.3$ Hz, OH-2′), 3.99 (1H, m, H4′), 3.88 (1H, q, $J = 4.9$ Hz, H3′), 3.74 (1H, m, H2′), 3.73 (6H, s, OCH_3), 3.18 (2H, m, H5′); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 158.08 (DMTr), 149.62 (arom C), 144.98 (DMTr), 141.29, 136.11 (arom C), 135.69, 129.77, 128.06, 127.79 (DMTr), 127.29, 126.66, 125.96, 123.89 (arom C), 113.17, 85.40 (DMTr), 83.56 (C1′), 82.99 (C4′), 77.60 (C2′), 71.41 (C3′), 64.18 (C5′), 55.03 (OCH_3); ESI-MS: 511.4 ($[M + H]^-$).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-*tert*-butyldimethylsilyl-1′-deoxy-1′-phenyl-β-D-ribofuranose (24). To a solution of **20** (1.09 g, 2.1 mmol) in anhydrous THF/pyridine, 1:1 (20 mL) were added $AgNO_3$ (430 mg, 2.5 mmol) and 1 M ^{t}BuMe$_2$SiCl in THF (2.5 mL, 2.5 mmol) and stirred for 20 h under argon at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution. The suspension was filtered, the filtrate was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The residue was coevaporated twice with toluene and purified by FC (CH_2Cl_2 → CH_2Cl_2/^{i}PrOH, 95:5). The product was obtained as a white foam in 29% yield (390 mg, 0.62 mmol). TLC (CH_2Cl_2): $R_f = 0.24$; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 7.46−6.82 (18H, m, arom H), 4.74 (1H, d, $J = 5.2$ Hz, OH-3′), 4.67 (1H, d, $J = 6.4$ Hz, H1′), 3.95 (3H, m, H2′, H3′, H4′), 3.73 (6H, s, OCH_3), 3.23 (2H, m, H5′), 0.78 (9H, s, $SiC(CH_3)_3$), −0.12, −0.17 (3H, s, $SiCH_3$); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 158.06, 145.02 (DMTr), 140.54, 140.21 (arom C), 135.54, 135.47, 129.75 (DMTr), 128.88, 128.17 (arom C), 128.03, 127.76 (DMTr), 127.37, 126.65 (arom C), 126.22, 113.13, 85.43 (DMTr), 83.66 (C1′), 83.13 (C4′), 79.66 (C2′), 71.55 (C3′), 63.88 (C5′), 55.01 (OCH_3), 25.63 ($SiC(\underline{C}H_3)_3$), 17.89 ($Si\underline{C}(CH_3)_3$), −4.98, −5.28 ($SiCH_3$); ESI-MS: 625.6 ($[M + H]^-$).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-*tert*-butyldimethylsilyl-1′-deoxy-1′-phenyl-β-D-ribofuranose (22) was obtained from the reaction described above as the faster-migrating isomer. The product was obtained as a white foam in 42% yield (560 mg, 0.89 mmol). TLC (CH_2Cl_2): $R_f = 0.27$; ^{1}H NMR (250 MHz d_6-DMSO, ppm) 7.42−6.82 (18H, m, arom H), 4.89 (1H, d, $J = 7.2$ Hz, OH-2′), 4.67 (1H, d, $J = 6.4$ Hz, H1′), 4.01 (1H, m, H3′), 3.94 (1H, m, H2′), 3.74 (1H, m, H4′), 3.72 (6H, s, OCH_3), 3.22 (2H, m, H5′), 0.78 (9H, s, $SiC(CH_3)_3$), −0.01, −0.06 (3H, s, $SiCH_3$); ^{13}C NMR (62.9 MHz d_6-DMSO, ppm) 157.78, 145.01 (DMTr), 140.54, 140.22 (arom C), 135.44, 135.48, 129.75 (DMTr), 128.89, 128.04 (arom C), 127.75, 127.64 (DMTr), 127.37, 126.39 (arom C), 126.22, 112.73, 85.43 (DMTr), 83.72 (C1′), 83.38 (C4′), 79.88 (C2′), 71.63 (C3′), 63.84 (C5′), 54.98 (OCH_3), 25.73 ($SiC(\underline{C}H_3)_3$), 17.88 ($Si\underline{C}(CH_3)_3$), −5.01, −5.25 ($SiCH_3$); ESI-MS: 625.5 ($[M + H]^-$).

1′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-1′-phenyl-β-D-ribofuranose Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (2). To a solution of **24** (200 mg, 0.32 mmol) in anhydrous

MeCN (10 mL) were added collidine (=2,4,6-trimethylpyridine, 430 μL, 3 mmol), 1-methyl-1H-imidazole (13 μL, 0.17 mmol), and 2-cyanoethyl diisopropyl-phosphoramidochloridite (110 μL, 0.5 mmol), and the mixture stirred for 15 min at 0 °C and for 30 min at room temperature under argon. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The crude product was purified by FC (hexane/AcOEt, 4:1). The product (diastereoisomer mixture) was obtained as a white foam in 57% yield (152 mg, 0.18 mmol). TLC (hexane/AcOEt, 4:1): $R_f = 0.39$; ^{1}H NMR (400 MHz d_6-DMSO, ppm) 7.56−6.83 (36H, m, arom H), 4.77 (2H, m, H1′), 4.16 (4H, m, H2′, H3′), 3.95 (2H, m, H4′), 3.79, 3.78 (12H, s, OCH_3), 3.55 (8H, m, H5′, CH_2CN), 2.66 (4H, m, OCH_2), 1.16 (12H, m, $CH(CH_3)_2$), 0.80, 0.79 (9H, s, $SiC(CH_3)_3$), −0.13, −0.15, −0.29, −0.30 (3H, s, $SiCH_3$); ^{31}P NMR (162 MHz, $CDCl_3$, ppm) 151.88, 149.35; ESI-MS: 827.6 ($[M + H]^+$).

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