Synthesis of Fluorobenzene and Benzimidazole Nucleic-Acid Analogues and Their Influence on Stability of RNA Duplexes

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Dedicated to Prof. Dr. *Albert Eschenmoser* on the occasion of his 75th birthday

Six different ribonucleoside phosphoramidites with fluorobenzenes or fluorobenzimidazoles as base analogues, one abasic site, and inosine were synthesized and incorporated into oligoribonucleotides. The oligomers were investigated by means of UV and CD spectroscopy to assess the contribution of H-bonding, base stacking, and solvation to the stability of the RNA duplex. CD Spectra show that the incorporation of modified nucleosides does not lead to changes in the structure of RNA. The T_m differences determined are based on changes in base stacking and solvation. Individual contributions of base stacking and solvation of the modified nucleosides could be determined. In fluorobenzene · fluorobenzimidazole-modified base pairs, a duplex-stabilizing force was found that points to a weak F ··· H H-bond.

1. Introduction. – RNA shows a great variety of secondary structures, like double helices, hairpin loops, bulges, and internal loops. All these secondary structures are stabilized mainly by interactions of the bases with their neighbours [1][2]. The most important interaction in the stability of RNA is H-bonding. In addition, recent studies of RNA helices [3] suggest that stacking of the aromatic bases plays a significant role in the stability of duplex RNA. The origins of base-stacking stability [4] in nucleic acids are still discussed controversially, and there are relatively little experimental data that can help to settle the question of the origin of this phenomenon.

In natural RNA, bases are limited to the four predominant structures U, C, A, and G, so the number of compounds that can be used to investigate the parameters of base pairing and base stacking is limited. To address this problem, we decided to synthesize some new nucleic-acid analogues in which the nucleobases are replaced by fluorobenzimidazoles or fluorobenzenes. Thus, we prepared eight protected phosphoramidites, six of them with base modifications, one abasic site, and one derived from inosine (*Fig. 1*), *i.e.*, the monomer building blocks **1** for inosine **2**, for 1′-deoxy-1′-(4-fluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose **3** for 1′-deoxy-1′-(4,6-difluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose, **4** for 1′-deoxy-1′-(4-fluorophenyl)-β-D-ribofuranose, **5** for 1′-deoxy-1′-(3-fluorophenyl)-β-D-ribofuranose, **6** for 1′-deoxy-1′-(2-fluorophenyl)-β-D-ribofuranose, **7** for 1′-deoxy-1′-(2,4-difluorophenyl)-β-D-ribofuranose, and **8** for 1-deoxy-ribofuranose incorporation into oligoribonucleotides.

The parent nucleoside of **2** (*i.e.*, **13**) is an isostere of the natural inosine, and that of **7** (*i.e.*, **31**) is isosteric to uridine. The aromatic-ring moiety of **1**–**7** was designed to be the closest possible steric mimic of the natural nucleobases avoiding the presence of

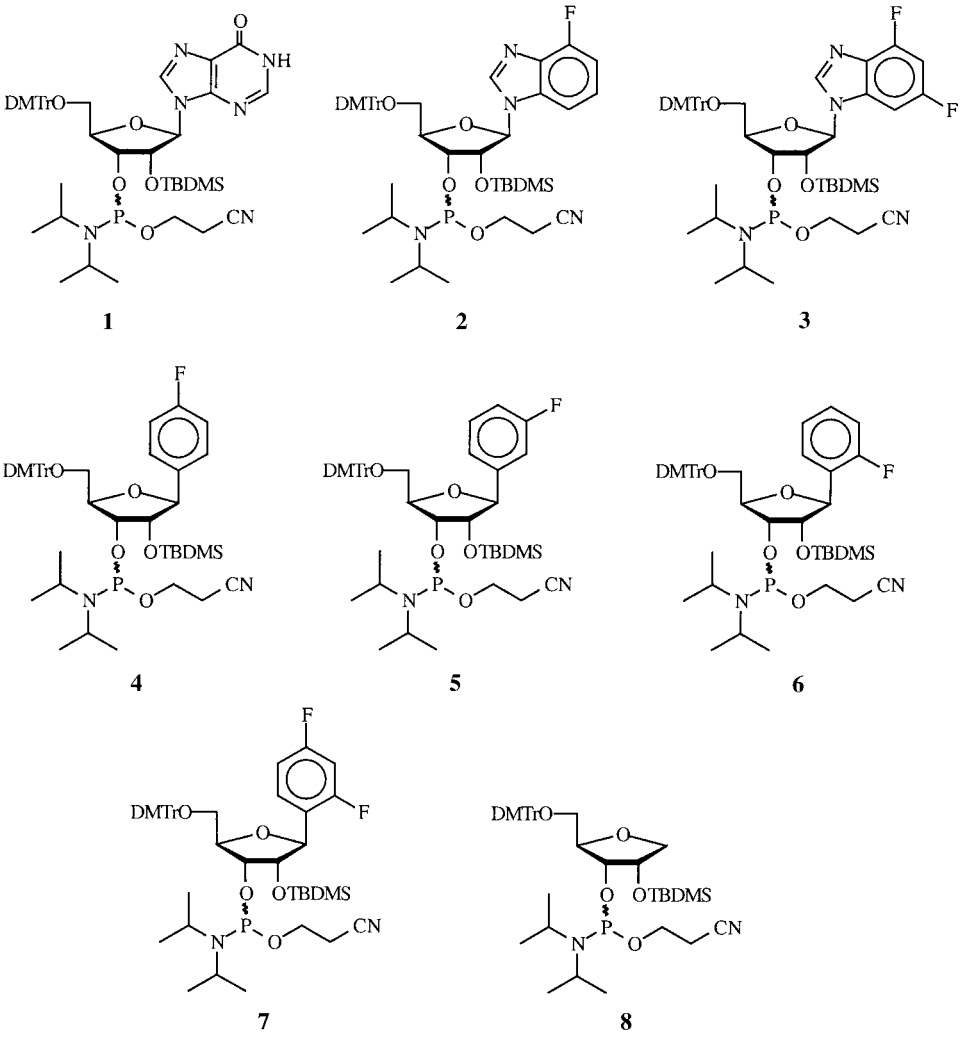


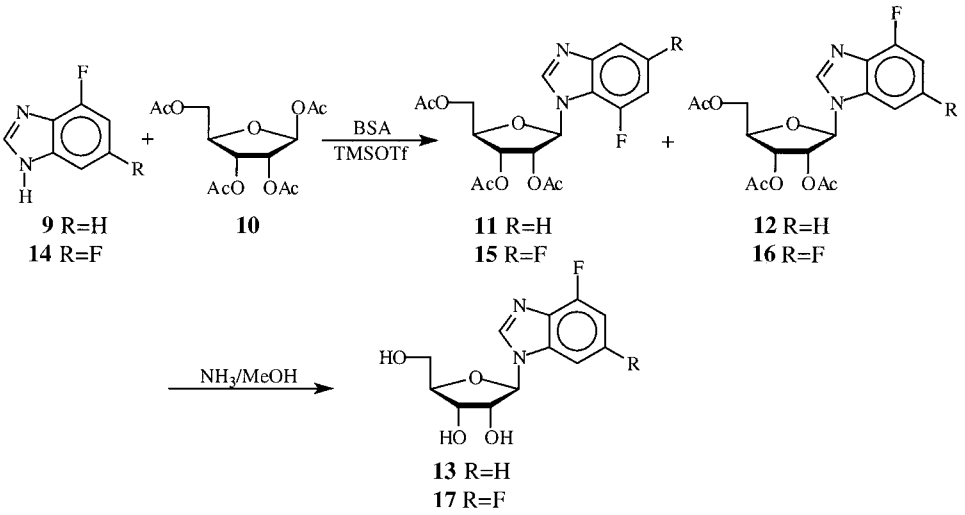
Fig. 1. *Monomer building blocks* **1**–**8** *for the incorporation into RNA oligonucleotides by solid-phase synthesis*

hydrophilic O- or N-containing groups [5]. The best isosteric replacement of the C=O functionality is the C–F group, because of nearly identical bond lengths [6]. In the parent nucleosides of **4**, **5**, and **6** (*i.e.* **28**, **29**, and **30**, resp.), the natural bases are substituted by monofluorobenzenes [7]. These compounds should show the influence of F···H H-bonding in duplex RNA. The F-atom has been introduced in all three possible positions on the benzene ring so that the influence of the F-position can be investigated as well. It is very interesting to investigate the influence of the F···H H-bonding because, in its crystal structure, **28** shows a very short C–F···H–C distance of 230 pm [8]. This is significantly shorter than the sum of the *van der Waals* radii of the F- and

H-atoms. In contrast, *Schweitzer* and *Kool* [9] have shown that 1′,2′-dideoxy-1′-(2,4-difluorotolyl)-β-D-ribofuranose, an isostere of the natural DNA base thymidine, develops no H-bonds to other natural bases or base analogues in duplex DNA. To evaluate the contribution of base-stacking effects to the stability of duplex RNA and the influence of the F-atom, we also synthesized the phosphoramidite **8** of the parent abasic site **20**.

2. Results and Discussion. – 2.1. *Chemical Syntheses.* The synthesis of 1′-deoxy-1′-(4-fluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose (**13**) was performed by the glycosylation procedure of *Vorbrüggen* [10] (*Scheme 1*). Refluxing 4-fluoro-1*H*-benzimidazole (**9**) [11] with *N*,*O*-bis(trimethylsilyl)acetamide and subsequent reaction of the persilylated base with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**10**) in the presence of the *Lewis* acid trimethylsilyl trifluoromethanesulfonate afforded the desired 2′,3′,5′-tri-*O*-acetyl-1′-deoxy-1′-(4-fluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose (**12**) in 65% yield. The undesired N^3-isomer **11** was obtained in only 8% yield. In the case of 4,6-difluoro-1*H*-benzimidazole (**14**) [12], the desired 2′,3′,5′-tri-*O*-acetyl-1′-deoxy-1′-(4,6-difluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose (**16**) was obtained in 67% yield, besides 11% of the corresponding N^3-isomer **15**. Deprotection of the acetylated nucleosides **12** and **16** in methanolic ammonia furnished 1′-deoxy-1′-(4-fluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose (**13**) and 1′-deoxy-1′-(4,6-difluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose (**17**) in 89 and 94% yield, respectively.

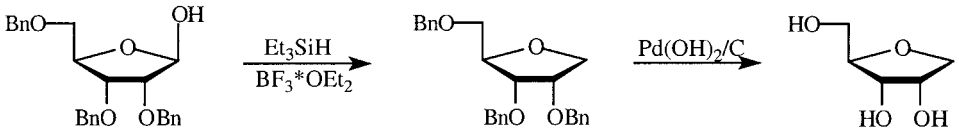
Scheme 1. *Synthesis of 1′-Deoxy-1′-(4-fluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose* (**13**) *and 1′-Deoxy-1′-(4,6-difluoro-1*H*-benzimidazol-1-yl)-β-D-ribofuranose* (**17**)



BSA = *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf = trimethylsilyl trifluoromethanesulfonate

The synthesis of 1-deoxy-D-ribofuranose (=1,4-anhydro-D-ribitol; **20**) was performed according to *Purdy et al.* [13] (*Scheme 2*). Thus, 2,3,5-tri-*O*-benzylribofuranose **18** [14] was dehydroxylated to **19** (84%) by treatment with triethylsilane and $BF_3 \cdot OEt_2$. Subsequent deprotection with 20% $Pd(OH)_2$/C in the presence of cyclohexene as the hydrogen donor [15] in EtOH gave **20** in 95% yield.

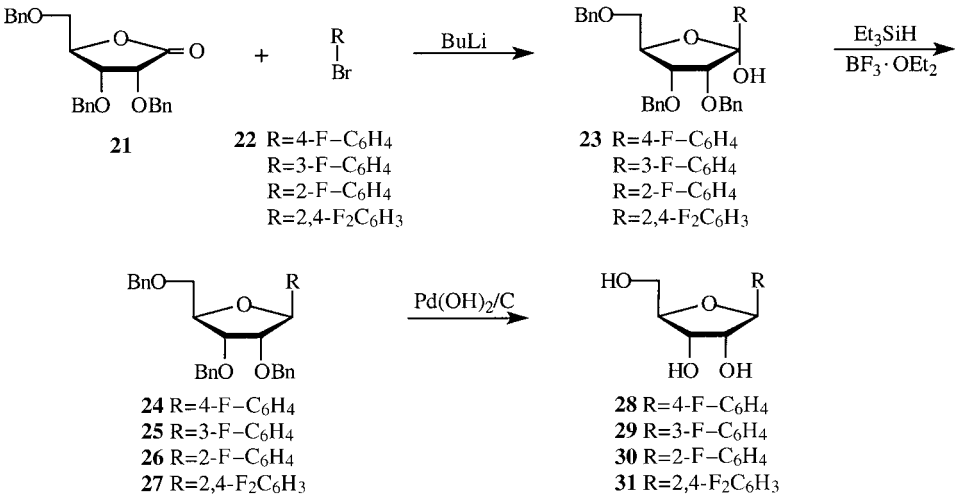
Scheme 2. *Synthesis of 1′-Deoxy-*D*-ribofuranose* **20**



18 **19** **20**

The *C*-glycosylated nucleosides **24**–**27** were all obtained in a similar way [16] (*Scheme 3*). We describe the synthesis of nucleoside **24** as an example. Lithiation of 1-bromo-4-fluorobenzene **22** with BuLi in THF at $-78°$ followed by addition of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**21**) [17] gave the intermediate lactol **23**, which was directly dehydroxylated with triethylsilane and $BF_3 \cdot OEt_2$ to afford stereoselectively **24** in 75% yield. Interestingly, all monofluorobenzenes were glycosylated in THF as the solvent of choice leading to the highest yield. In the case of the glycosylation of difluorobenzene in THF, the protected nuceloside **27** was obtained in only 31% yield [18]; by changing the solvent to Et_2O, the yield increased to 84%. Deprotection of the benzylated nucleoside **24** with 20% $Pd(OH)_2/C$ in the presence of cyclohexene in EtOH yielded **28** (99%). Similarly, **29**–**31** were obtained.

Scheme 3. *Synthesis of Unprotected* C*-Nucleosides* **28**–**31**



21

22 R=4-F–C_6H_4
R=3-F–C_6H_4
R=2-F–C_6H_4
R=2,4-$F_2C_6H_3$

23 R=4-F–C_6H_4
R=3-F–C_6H_4
R=2-F–C_6H_4
R=2,4-$F_2C_6H_3$

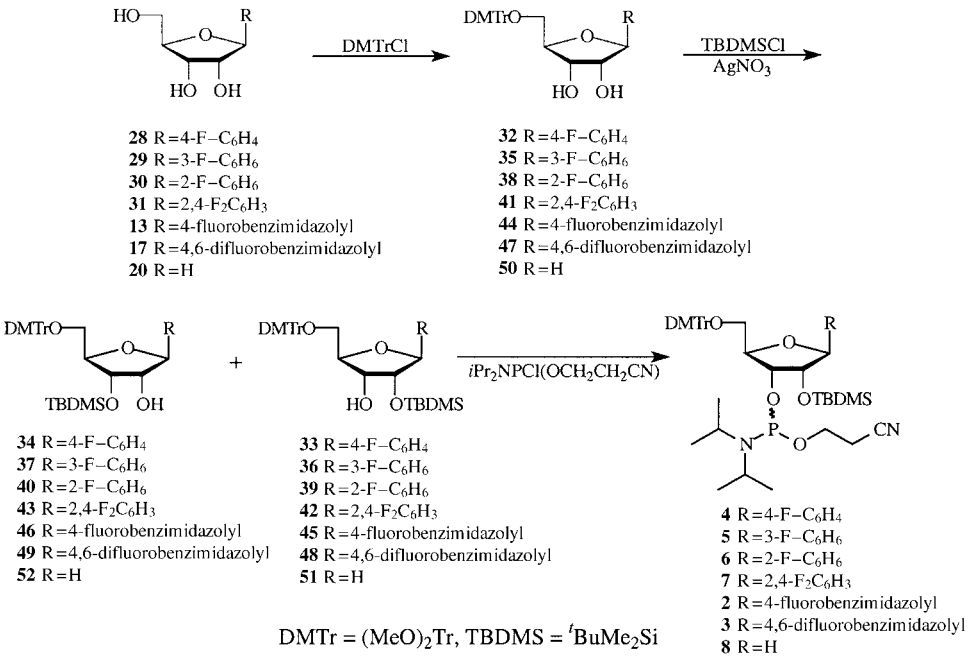
24 R=4-F–C_6H_4
25 R=3-F–C_6H_4
26 R=2-F–C_6H_4
27 R=2,4-$F_2C_6H_3$

28 R=4-F–C_6H_4
29 R=3-F–C_6H_4
30 R=2-F–C_6H_4
31 R=2,4-$F_2C_6H_3$

The conversions of the unprotected nucleosides **13**, **17**, and **28**–**31** or the unprotected sugar **20** to the corresponding phosphoramidites **2**–**8** are very similar. In the following, only the synthesis of phosphoramidite **4** is described (*Scheme 4*; for all other phosphoramidites, see *Exper. Part*). The 5′-OH function of **28** was protected with 4,4′-dimethoxytrityl chloride ($(MeO)_2TrCl$) in dry pyridine and Et_3N to afford the 5′-*O*-(4,4′-dimethoxytrityl) derivative **32** in 79% yield. To protect the 2′-OH function, **32** in THF/pyridine 1:1 was treated with $AgNO_3$ and a 1M (*tert*-butyl)dimethylsilyl chloride (^{t}BuMe$_2$SiCl) ($\rightarrow$ **33** in 43% yield). In all fluorinated compounds, the ^{t}BuMe$_2$Si at C(2′)

tended to move to the 3′-position in polar solvents, resulting often in low yields of the desired 2′-protected nucleosides. The final phosphitylation of **33** gave **4** in 67% yield. To prevent the isomerization of the silyl protecting group, *sym*-collidine and 1-methyl-1*H*-imidazole were used instead of $^{i}Pr_2EtN$ [19].

Scheme 4. *Synthesis of Phosphoramidites* **2**–**8**



The inosine phosphoramidite **1** was synthesized according to literature procedures [20].

2.2. *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 [21]. The fully protected dodecamers were cleaved from the controlled-pore-glass (CPG) support with 32% aqueous NH_3 solution at 55° overnight. The 2′-silyl groups were deprotected with $Et_3N \cdot 3$ HF within 24 h at room temperature [22]. The crude RNA oligomer was precipitated with BuOH at −20°, 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.

2.3. *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 [23]. Each melting curve was determined twice. The temperature range was 0–70° with a heating rate of 0.5°/min. A lower heating rate of 0.2°/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 [24].

2.4. *Thermodynamic Data*. The modified nucleosides were tested in a defined RNA sequence. This A,U-rich sequence was chosen in order to be able to compare our results with the those of *Schweitzer* and *Kool* [9], who incorporated modified nucleosides in the corresponding DNA. In the 12-mer oligoribonucleotides (5′-CUU UUC XUU CUU-3′ paired with 3′-GAA AAG YAA GAA-5′), only one position was modified, marked as X and Y, respectively. First, we measured only RNA duplexes containing natural bases (*Table 1*, *Entry 1*). The wobble base pair U · G shows the highest T_m (38.6°). This is 0.8° higher than the natural U · A base pair (T_m 37.8°). The U · C and U · U mismatches show nearly the same stability (T_m 30.4° and 30.1°).

Table 1. *Synthesized Non- or Mono-Modified Duplex RNA* (5′-CUU UUC XUU CUU-3′ paired with 3′-GAA AAG YAA GAA-5′) *and Their Thermodynamic Properties*. For X, see *Scheme 4*. ΔH^0, $T \cdot \Delta S^0$, and ΔG^0, in kcal/mol (T 298 K). Errors: T_m, ±0.2°; ΔH^0 and $T \cdot \Delta S^0$, ±5%; ΔG^0, ±2%.

Entry	X	Y = A				Y = C				Y = G				Y = U			
		T_m [°]	ΔH^0	$T \cdot \Delta S^0$	ΔG^0	T_m [°]	ΔH^0	$T \cdot \Delta S^0$	ΔG^0	T_m [°]	ΔH^0	$T \cdot \Delta S^0$	ΔG^0	T_m [°]	ΔH^0	$T \cdot \Delta S^0$	ΔG^0
1	U	*37.8*	87.8	75.9	11.9	*30.4*	84.5	74.8	9.8	*38.6*	83.0	71.1	11.9	*30.1*	89.5	79.8	9.7
2	**31** (R = 2,4-$F_2C_6H_3$)	*27.4*	88.6	79.6	9.0	*27.3*	84.8	75.9	8.9	*27.6*	83.6	74.6	9.0	*27.9*	91.2	82.1	9.1
3	**28** (R = 4-F–C_6H_4)	*23.8*	81.6	73.7	7.9	*24.1*	83.0	75.0	8.0	*24.2*	80.2	72.2	8.0	*25.6*	85.8	77.4	8.4
4	**29** (R = 3-F–C_6H_4)	*24.7*	79.3	71.1	8.2	*25.0*	82.6	74.4	8.2	*25.0*	80.0	71.8	8.2	*25.7*	84.1	75.7	8.4
5	**30** (R = 2-F–C_6H_4)	*27.3*	89.9	81.0	8.9	*25.1*	83.3	75.0	8.3	*27.4*	91.2	82.2	9.0	*26.5*	87.1	78.4	8.7
6	I	*31.2*	91.8	81.7	10.1	*41.7*	97.9	84.5	13.4	*31.7*	89.7	79.5	10.2	*34.2*	91.1	80.1	11.0
7	**13** (R = 4-fluoro-benzimidazol-1-yl)	*28.0*	86.7	77.6	9.1	*27.5*	81.8	72.9	8.9	*28.7*	84.1	74.8	9.3	*28.5*	85.8	76.6	9.2
8	**17** (R = 4,6-difluoro-benzimidazol-1-yl)	*28.4*	81.4	72.2	9.2	*28.7*	81.5	72.3	9.2	*29.4*	84.8	75.3	9.5	*29.3*	85.3	75.8	9.5
9	**20** (R = H; abasic site)	*20.6*	67.2	60.0	7.2	*18.6*	51.3	44.6	6.7	*20.9*	65.1	57.8	7.3	*18.2*	58.9	52.3	6.6

In a second series, we measured the fluorobenzene nucleosides **28**–**31** paired against natural bases (*Table 1*, *Entries 2–5*). In these cases, all T_m values are lower than those for the natural bases. Possible explanations for these findings are: *i*) there are no H-bonds between the modified and the natural bases and *ii*) the modified bases are less solvated by H_2O molecules. The absence of differences in T_m values by pairing for example 2,4-difluorobenzene against a purine or a pyrimidine indicates that there are no H-bonds; *Table 1*, *Entry 2*, shows that all T_m values are nearly identical (27.3–27.9°). As for the 2,4-difluorobenzene nucleoside **31**, which is isosteric to uridine, we found a new universal base [25] that paired with all natural bases without energy discrimination. An explanation for our second hypothesis is that the destabilization of the RNA duplex arises from the cost of desolvation of the H-bond donors or acceptors of the natural bases during formation of the corresponding modified-natural base pair [9]. So, we have two destabilizing effects that lower the stability of the RNA duplexes by *ca.* 8–14°.

Interestingly, when an F-atom is in the 2-position of the benzene ring, the duplex is 2–3° more stable than with a H-atom at the same position. So there seems to be an interaction between this F-atom and a further atom. Possibly this F-atom can form a H-bond to the H-atoms at C(5′) of its own sugar moiety. This requires the benzene ring to

take up a *syn*-conformation. A weak interaction between the F-atom and a H-atom at C(5′) observed in the corresponding crystal structures [7a,b] supports this hypothesis.

The fluorobenzimidazole-modified nucleosides **13** and **17** were compared to inosine (I) (*Table 1*, *Entries 6–8*). Both modified nucleosides show a destabilization of the duplex of *ca.* 4–14°. The destabilization between an inosine mismatch base pair and a fluorobenzimidazole · natural base pair is lower than in the case of the fluorobenzenes (*Table 1*, *Entries 2–5*). All RNA duplexes with the 4,6-difluorobenzimidazole-modified nucleoside are by 0.4–1.2° more stable than the ones with the 4-fluorobenzimidazole (*Table 1*, *Entries 7* and *8*).

Table 2 shows the results of pairing modified nucleosides aginst each other. We paired the 2,4-difluorobenzene-modified nucleoside **31** (uridine analogue) and the two fluorobenzimidazole-modified nucleosides **13** and **17** against the fluorobenzene-modified nucleosides **28–31** and the abasic site **20**. The RNA duplexes with the 4,6-difluorobenzimidazole nucleoside **17** are even *ca.* 1° more stable than those with the 4-fluorobenzimidazole nucleoside **13** (*cf.* the last two columns in *Table 2*).

Table 2. *Synthesized Doubly Modified Duplex RNA* (5′-CUU UUC XUU CUU-3′ paired with 3′-GAA AAG YAA -GAA-5′) *and Their Thermodynamic Properties.* For X and Y, see *Scheme 4.* ΔH^0, $T\cdot\Delta S^0$, and ΔG^0 in kcal/mol (T = 298 K). Errors: T_m ±0.2°; ΔH^0 and $T\cdot\Delta S^0$, ±5%; ΔG^0, ±2%.

Entry	X	Y = **31** (R = 2,4-$F_2C_6H_3$)				Y = **13** (R = 4-fluoro-1*H*-benzimidazol-1-yl)				Y = **17** (R = 4,6-difluoro-1*H*-benzimidazol-1-yl)			
		T_m [°]	ΔH^0	$T\cdot\Delta S^0$	ΔG^0	T_m [°]	ΔH^0	$T\cdot\Delta S^0$	ΔG^0	T_m [°]	ΔH^0	$T\cdot\Delta S^0$	ΔG^0
1	**31** (R = 2,4-$F_2C_6H_3$)	*32.5*	82.4	72.2	10.2	*33.5*	88.1	77.4	10.7	*34.6*	94.4	83.2	11.2
2	**28** (R = 4-F–C_6H_4)	*29.9*	84.1	74.5	9.6	*30.6*	82.2	72.4	9.8	*31.3*	85.0	75.0	10.0
3	**29** (R = 3-F–C_6H_4)	*31.3*	77.5	67.7	9.8	*30.3*	76.8	67.2	9.6	*31.4*	81.8	71.8	10.0
4	**30** (R = 2-F–C_6H_4)	*31.9*	83.5	73.4	10.1	*32.8*	86.9	76.4	10.5	*33.6*	87.6	76.9	10.7
5	**20** (R = H; abasic site)	*22.6*	59.7	52.0	7.7	*25.3*	58.1	49.8	8.3	*26.3*	55.1	44.6	8.5

What is the individual contribution of base stacking, solvation, and H-bonding to the stability of duplex RNA? Calculating the incorporation of the 2,4-difluorobenzene against the abasic site (T_m 22.6°, *Table 2*, *Entry 5*) gives a 4.4° (1.1 kcal/mol) more stable duplex RNA than the one with a uridine · abasic site base pair (T_m 18.2°; *Table 1*, *Entry 9*). This shows the contribution of stacking of the 2,4-difluorobenzene nucleoside compared with uridine. A U · U base pair in duplex RNA (T_m 30.1°; *Table 1*, *Entry 1*) is 2.2° more stable than a U · 2,4-difluorobenzene base pair (T_m 27.9°; *Table 1*, *Entry 2*). The incorporation of one 2,4-difluorobenzene stabilizes the duplex by *ca.* 4.4° (1.1 kcal/mol) by stronger stacking, but the U · 2,4-difluorobenzene base pair is 2.2° less stable than a U · U base pair. Thus, the contribution of solvation is 6.6° (1.7 kcal/mol) in destabilization of the RNA duplex per base pair. A RNA duplex with a 2,4-difluorobenzene · 2,4-difluorobenzene base pair should be 2.2° more stable (+2 · 4.4° (2 · 1.1 kcal/mol; stronger stacking), −6.6° (−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° (9.7 kcal/mol; *Table 1*, *Entry 1*) and a 2,4-difluorobenzene · 2,4-difluorobenzene base pair a T_m of 32.5° (10.2 kcal/mol; *Table 2*, *Entry 1*), confirming our calculations. In the same comparison for 4-fluorobenzimidazole and 4,6-difluorobenzimidazole, the destabilization of solvation is 6.3° (1.6 kcal/mol) and 6.5° (1.5 kcal/mol), respectively. The

stabilization of the duplex incorporating the 4-fluorobenzimidazole or 4,6-difluorobenzimidazole nucleoside by stacking effects are *ca.* 4.4° (1.0 kcal/mol) and 5.4° (1.2 kcal/mol) with regard to guanosine, respectively. A U · 4-fluorobenzimidazole base pair shows a T_m of 28.5 (9.2 kcal/mol; *Table 1*, *Entry 7*) and a 2,4-difluorobenzene · 4-fluorobenzimidazole base pair a T_m of 33.5° (10.7 kcal/mol; *Table 2*, *Entry 1*). The exchange of uridine by the 2,4-difluorobenzene nucleoside stabilizes the duplex by 4.4° (1.1 kcal/mol). The exchange of the second natural base by the 2,4-difluorobenzene nucleoside adds no further energy of solvation to the duplex RNA. So the corresponding RNA duplex with the 2,4-difluorobenzene · 4-fluorobenzimidazole base pair is 0.6° (0.4 kcal/mol) more stable than calculated. A similar result was obtained for the 2,4-difluorobenzene · 4,6-difluorobenzimidazole base pair. The corresponding RNA duplex with the 2,4-difluorobenzene · 4,6-difluorobenzimidazole base pair is 0.9° (0.6 kcal/mol) more stable than calculated. Thus, there seems to be another stabilizing force that increases T_m. It may be possible that this increase of T_m results from a weak F···H H-bond between the modified nucleosides. The existence of such F···H H-bonds in this class of molecules has been shown in the crystal structure of **28** with a F···H distance of 230 pm [7a]. This H-bond would be one of the first F···H H-bonds of so-called 'organic fluorine' [26] in aqueous solution. Further investigations to characterize this effect are under way.

2.5. *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° to ensure that only duplex RNA was present.

The CD spectra of a RNA duplex with only one modified base shows a typical curve for an A-type helix (*Fig. 2*). There is a strong maximum at *ca.* 270 nm, a weak minimum at *ca.* 245 nm, a weak maximum at *ca.* 225 nm and a strong minimum at *ca.* 210 nm.

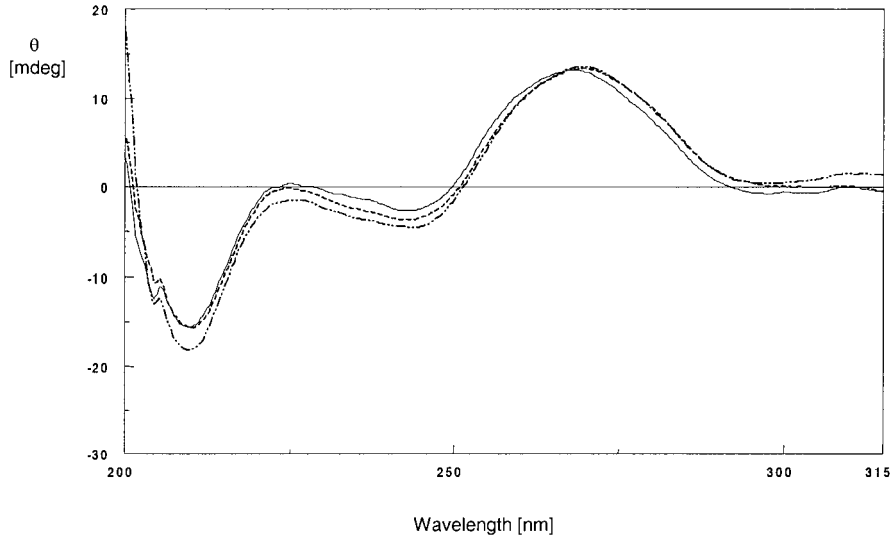


Fig. 2. *CD Spectra of dodecamer RNA duplex with one inosine · cytosine* (—), *one 4-fluorobenzimidazole · cytosine* (- · - · -), *or one 4,6-difluorobenzimidazole · cytosine* (- - -) *base pair*

Fig. 2 shows the CD spectra of three different RNA duplexes with the base pairs inosine · cytosine, 4-fluorobenzimidazole · cytosine, and 4,6-difluorobenzimidazole · cytosine. In comparison with the modified RNA, the unmodified RNA inosine · cytosine shows a small shift of *ca*. 2 nm for the strong maximum to 268 nm. The intensity at 270 nm corresponds to the number of paired and unpaired bases as well as to the extent of base-stacking interactions [27]. At this maximum, intensity differences are only small, so that the effect of missing H-bonds is compensated by stronger stacking interactions of the modified bases. The CD spectra of duplex RNA modified with fluorobenzenes have, in comparison with a U · A base pair, no significant differences. *Fig. 3* shows the CD spectra of duplex RNA with one base pair of modified nucleosides. All spectra are almost identical. Only in the region of 270 nm are small differences observed that could be explained by different stacking abilities of the individual bases.

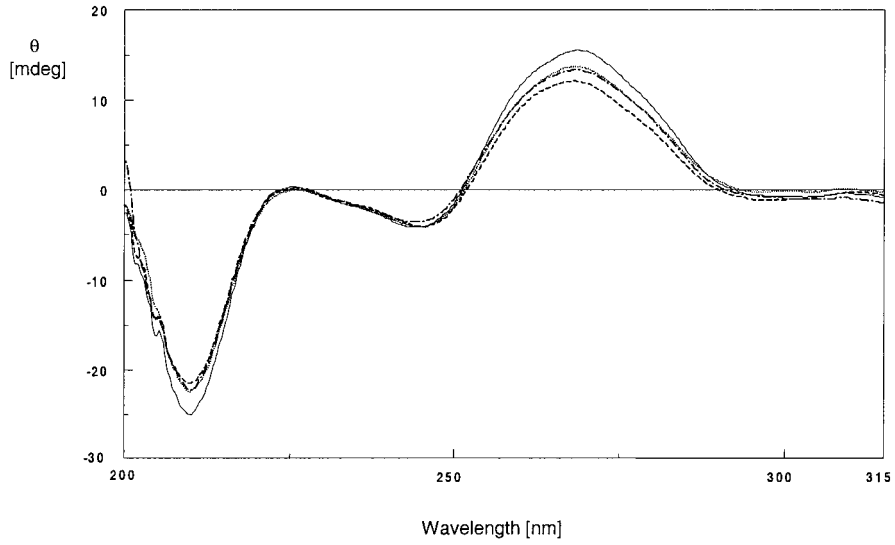


Fig. 3. *CD Spectra of dodecamer RNA duplex with one 2,4-difluorobenzene · 4,6-difluorobenzimidazole* (—), *one 4-fluorobenzene · 4,6-difluorobenzimidazole* (- - -), *one 3-fluorobenzene · 4,6-difluorobenzimidazole* (· ⋯), *or one 2-fluorobenzene · 4,6-difluorobenzimidazole* (- · - ·) *base pair*

All CD spectra indicate that the structure of duplex RNA is 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. Thus, all differences determined by UV measurements are a consequence of changes in stacking, solvation, or the ability to form H-bonds, and not of structural changes of the RNA duplex.

We thank *Thorsten Strube* for supporting synthesis and purification of oligonucleotides and Dr. *Gerd Dürner* for prep. HPLC purification of ^{t}BuMe$_2$Si-protected nucleosides. *J.P.* wishes to thank the Graduiertenkolleg *Chemische und Biologische Synthese von Wirkstoffen* for a Ph.D. fellowship.

Experimental Part

General. The fluorobenzenes and the fluoroacetanilides were obtained from *Lancaster*. The anh. solvents, *e.g.* THF, CH_2Cl_2, pyridine, or Et_2O 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; for convenience, primed locants are given to the monosaccharide moiety and unprimed ones to the 'nucleobase' moiety. MS: *PerSeptive Biosystems* MALDI-TOF spectrometer *Voyager DE*; ESI = electrospray ionization.

2′-O-*[(*tert-*Butyl)dimethylsilyl]-5′*-O-*(4,4′-dimethoxytrityl)inosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite*) (**1**). Synthesis according to [19].

2,3,5-Tri-O-*benzyl-β*-D-*ribofuranose* (**18**). Synthesis according to [14].

2,3,5-Tri-O-*benzyl*-D-*ribono-1,4-lactone* (**21**). Synthesis according to [16].

*4-Fluoro-1*H-*benzimidazole* (**9**). Synthesis according to [11].

*4,6-Difluoro-1*H-*benzimidazole* (**14**). Synthesis according to [12].

2,3,5-Tri-O-*acetyl-1-deoxy-1-(4-fluoro-1*H-*benzimidazol-1-yl)-β*-D-*ribofuranose* (**12**). To a suspension of **9** (3.2 g, 23.5 mmol) in MeCN (80 ml), *N,O*-bis(trimethylsilyl)acetamide (8.8 ml, 36 mmol) was added and heated under reflux for 15 min. After cooling to r.t., 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose (**10**) (7.6 g, 23.9 mmol) in MeCN (35 ml) and trimethylsilyl trifluoromethanesulfonate (5.4 ml, 29.8 mmol) were added and heated under reflux for 2.5 h. The mixture was treated with 5% $NaHCO_3$ soln. and extracted with CH_2Cl_2, the org. phase dried ($MgSO_4$) and evaported, and the residue purified by FC (CH_2Cl_2/MeOH 98:2): **12** (6.2 g, 65.7%). White foam. TLC (CH_2Cl_2/MeOH 98:2): R_f 0.41. ^{1}H-NMR (250 MHz, (D_6)DMSO): 8.54 (*s*, H–C(2)); 7.60 (*d*, J = 7.8, H–C(7)); 7.30 (*dt*, J = 4.9, 8.1, H–C(6)); 7.10 (*dd*, J = 7.8, 11.0, H–C(5)); 6.34 (*d*, J = 6.2, H–C(1′)); 5.66 (*t*, J = 6.3, H–C(2′)); 5.43 (*dd*, J = 4.6, 6.3, H–C(3′)); 4.42 (*m*, H–C(4′)); 4.37 (*m*, 2 H–C(5′)); 2.13, 2.07, 2.03 (3*s*, Me). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 170.01, 169.52, 169.22 (C=O); 143.04 (C(2)); 135.37 (*d*, J = 8.7, C(6)); 132.24 (*d*, J = 14.3, C(5)); 123.94 (*d*, J = 7.2, C(8)); 108.02 (*d*, J = 18.8, C(9)); 107.87 (C(7)); 86.47 (C(1′)); 79.47 (C(4′)); 71.75 (C(2′)); 69.53 (C(3′)); 62.94 (C(5′)); 20.50, 20.36, 20.15 (Me). ESI-MS: 395.1 ([M + H]$^+$).

*1-Deoxy-1-(4-fluoro-1*H-*benzimidazol-1-yl)-β*-D-*ribofuranose* (**13**). A soln. of **12** (5.46 g, 13.8 mmol) in NH_3-sat. MeOH (150 ml) was stirred for 20 h and then evaporated. The residue was purified by FC (CH_2Cl_2/MeOH 4:1): **13** (3.58 g, 96.4%). Yellow oil. TLC (CH_2Cl_2/MeOH 4:1): R_f 0.42. ^{1}H-NMR (250 MHz, (D_6)DMSO): 8.51 (*s*, H–C(2)); 7.60 (*d*, J = 8.2, H–C(7)); 7.26 (*dt*, J = 5.0, 8.0, H–C(6)); 7.06 (*dd*, J = 8.0, 11.0, H–C(5)); 5.89 (*d*, J = 6.2, H–C(1′)); 5.51 (*d*, J = 6.4, OH–C(2′)); 5.23 (*d*, J = 4.7, OH–C(3′)); 5.14 (*t*, J = 5.2, OH–C(5′)); 4.36 (*q*, J = 5.9, H–C(2′)); 4.13 (*m*, H–C(3′)); 3.99 (*q*, J = 3.4, H–C(4′)); 3.64 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 142.81 (C(2)); 136.04 (*d*, J = 10.1, C(6)); 132.46 (*d*, J = 16.8, C(5)); 123.28 (*d*, J = 7.3, C(8)); 108.05 (C(7)); 107.37 (*d*, J = 17.5, C(9)); 88.89 (C(1′)); 85.62 (C(4′)); 73.77 (C(2′)); 70.04 (C(3′)); 61.13 (C(5′)). ^{19}F-NMR (254.2 MHz, (D_6)DMSO): –128.92 (*m*, F–C(4)). ESI-MS: 267.1 ([M – H]$^-$).

1-Deoxy-5-O-*(4,4′-dimethoxytrityl)-1-(4-fluoro-1*H-*benzimidazol-1-yl)-β*-D-*ribofuranose* (**44**). To a soln. of **13** (1.08 g, 4 mmol) in anh. pyridine (20 ml) and Et_3N (740 μl, 6 mmol), 4,4′-dimethoxytrityl chloride ($(MeO)_2$TrCl; 1.44 g, 4.3 mmol) was added and the mixture stirred for 6.5 h (TLC monitoring) under Ar at r.t. The reaction was quenched by addition of MeOH (3 ml). The mixture was evaporated, the residue dissolved in CH_2Cl_2, the soln. extracted with 5% $NaHCO_3$ soln., dried ($MgSO_4$), evaporated, and co-evaporated twice with toluene, and the crude product purified by FC (CH_2Cl_2/MeOH 95:5); **44** (2.04 g, 89.1%). Yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.40. ^{1}H-NMR (250 MHz, (D_6)DMSO): 8.42 (*s*, H–C(2)); 7.54 (*d*, J = 9.0, H–C(7)); 7.39–6.81 (*m*, 13 arom. H); 7.07 (*m*, H–C(5), H–C(6)); 5.94 (*d*, J = 5.4, H–C(1′)); 5.66 (*d*, J = 6.0, OH–C(2′)); 5.30 (*d*, J = 5.5, OH–C(3′)); 4.52 (*q*, J = 5.6, H–C(2′)); 4.23 (*q*, J = 5.1, H–C(3′)); 4.13 (*m*, H–C(4′)); 3.71 (*s*, 2 MeO); 3.24 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 158.12 ($(MeO)_2$*Tr*); 153.34 (*d*, J = 250.2, C(4)); 144.76 ($(MeO)_2$*Tr*); 142.65 (C(2)); 135.88 (*d*, J = 8.6, C(6)); 135.42, 135.30 ($(MeO)_2$*Tr*); 132.29 (*d*, J = 16.85, C(5)); 129.75, 127.86, 127.73, 126.75 ($(MeO)_2$*Tr*); 123.33 (*d*, J = 7.3, C(8)); 113.20 ($(MeO)_2$*Tr*); 108.38 (C(7)); 107.62 (*d*, J = 17.5, C(9)); 89.18 (C(1′)); 85.72 ($(MeO)_2$*Tr*); 83.53 (C(4′)); 73.25 (C(2′)); 70.10 (C(3′)); 63.59 (C(5′)); 55.05 (MeO). ESI-MS: 569.4 ([M – H]$^-$).

2-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-5*-O-*(4,4′-dimethoxytrityl)-1-(4-fluoro-1*H-*benzimidazol-1-yl)-β*-D-*ribofuranose* (**45**). To a soln. of **44** (2.52 g, 4.4 mmol) in anh. THF/pyridine 1:1 (40 ml), $AgNO_3$ (900 mg, 5.3 mmol) and 1M ^{t}BuMe$_2$SiCl in THF (6.2 ml, 6.2 mmol) were added and stirred for 20 h under Ar at r.t. The reaction was quenched by addition of sat. aq. $NaHCO_3$ soln. The suspension was filtered, the filtrate extracted with CH_2Cl_2, the org. phase dried ($MgSO_4$) and evaporated, and the residue co-evaporated twice with toluene and purified by prep. HPLC (*MN Nucleoprep 100-20* from *Macherey-Nagel*, hexane/dioxane 5:2): faster-migrating isomer **45** (1.23 g, 40.7%). Colourless foam. TLC (hexane/AcOEt 4:1): R_f 0.26. ^{1}H-NMR (270 MHz, (D_6)DMSO): 8.45 (*s*, H–C(2)); 7.57 (*d*, J = 7.6, H–C(7)); 7.41–6.83 (*m*, 13 arom. H); 6.98 (*m*, H–C(5),

H–C(6)); 5.97 (*d*, *J* = 6.3, H–C(1′)); 5.26 (*d*, *J* = 5.3, OH–C(3′)); 4.60 (*q*, *J* = 6.0, H–C(2′)); 4.35 (*m*, H–C(3′)); 4.09 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.30 (*m*, 2 H–C(5′)); 0.82 (*s*, ^{t}BuSi); 0.07, 0.02 (2*s*, Me_2Si). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 158.10 ($(MeO)_2$*Tr*); 153.27 (*d*, *J* = 250.9, C(4)); 144.47 ($(MeO)_2$*Tr*); 142.78 (C(2)); 135.63 (*d*, *J* = 8.4, C(6)); 135.21, 135.14 ($(MeO)_2$*Tr*); 132.28 (*d*, *J* = 17.3, C(5)); 129.62, 127.74, 127.60, 126.69 ($(MeO)_2$*Tr*); 123.15 (*d*, *J* = 7.1, C(8)); 113.13 ($(MeO)_2$*Tr*); 108.36 (C(7)); 107.52 (*d*, *J* = 17.3, C(9)); 89.07 (C(1′)); 85.85 ($(MeO)_2$*Tr*); 83.95 (C(4′)); 72.64 (C(2′)); 71.81 (C(3′)); 62.99 (C(5′)); 54.98 (MeO); 25.67 (Me_3CSi); 17.90 (Me_3*C*Si); –4.56, –5.19 (Me_2Si). ESI-MS: 685.5 ($[M+H]^+$).

3-O-*[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5*-O-*(4,4′-dimethoxytrityl)-1-(4-fluoro-1*H*-benzimidazol-1-yl)-β-*D*-ribofuranose* (**46**) was obtained from the reaction described above as the slower-migrating isomer (1.48 g, 49.0%). Colourless foam. TLC (hexane/AcOEt 4:1): R_f 0.26. ^{1}H-NMR (270 MHz, (D_6)DMSO): 8.46 (*s*, H–C(2)); 7.56 (*d*, *J* = 9.0, H–C(7)); 7.38–6.82 (*m*, 13 arom. H); 7.07 (*m*, H–C(5), H–C(6)); 5.92 (*d*, *J* = 5.9, H–C(1′)); 5.53 (*d*, *J* = 6.5, OH–C(2′)); 4.51 (*q*, *J* = 6.0, H–C(2′)); 4.35 (*m*, H–C(3′)); 4.09 (*m*, H–C(4′)); 3.72 (2*s*, Me); 3.30 (*m*, 2 H–C(5′)); 0.82 (*s*, ^{t}BuSi); 0.07, 0.02 (2*s*, Me_2Si). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 158.10 ($(MeO)_2$*Tr*); 153.27 (*d*, *J* = 250.9, C(4)); 144.47 ($(MeO)_2$*Tr*); 142.78 (C(2)); 135.63 (*d*, *J* = 8.4, C(6)); 135.21, 135.14 ($(MeO)_2$*Tr*); 132.28 (*d*, *J* = 17.3, C(5)); 129.62, 127.74, 127.60, 126.69 ($(MeO)_2$*Tr*); 123.15 (*d*, *J* = 7.1, C(8)); 113.13 ($(MeO)_2$*Tr*); 108.36 (C(7)); 107.52 (*d*, *J* = 17.3, C(9)); 89.07 (C(1′)); 85.85 ($(MeO)_2$*Tr*); 83.95 (C(4′)); 72.64 (C(2′)); 71.81 (C(3′)); 62.99 (C(5′)); 54.98 (MeO); 25.67 (Me_3CSi); 17.90 (Me_3*C*Si); –4.56, –5.19 (Me_2Si). ESI-MS: 685.5 ($[M+H]^+$).

2-O-*[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5*-O-*(4,4′-dimethoxytrityl)-1-(4-fluoro-1*H*-benzimidazol-1-yl)-β-*D*-ribofuranose 3-(2-Cyanoethyl Diisopropylphosphoramidite*) (**2**). To a soln. of **45** (200 mg, 0.29 mmol) in anh. MeCN (10 ml), collidine (= 2,4,6-trimethylpyridine; 380 µl, 2.9 mmol), 1-methyl-1*H*-imidazole (12 µl, 0.15 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (96 µl, 0.43 mmol) were added and the mixture stirred for 15 min at 0° and for 45 min at r.t. (TLC monitoring) under Ar. The reaction was quenched by addition of sat. aq. $NaHCO_3$ soln., the mixture extracted wtih CH_2Cl_2, the extract dried ($MgSO_4$) and evaporated, and the crude product purified by FC (CH_2Cl_2/MeOH 99:1): **2** (135 mg, 52.3%; diastereoisomer mixture). Colourless foam. TLC (CH_2Cl_2/MeOH 98:2): R_f 0.33, 0.41. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.12, 8.08 (2*s*, H–C(2)); 7.49–6.81 (*m*, 13 arom. H, H–C(5), H–C(6), H–C(7)); 5.92, 5.84 (2*d*, *J* = 7.7, 5.8, H–C(1′)); 4.75 (*m*, H–C(2′)); 4.40 (*m*, H–C(3′)); 4.32 (*m*, H–C(4′)); 3.80, 3.79 (2*s*, 2 MeO); 3.48 (*m*, 2 H–C(5′)); 2.66 (*m*, CH_2O); 1.20 (*m*, 2 Me_2CH); 0.75 (*s*, ^{t}BuSi); –0.12, –0.38 (2*s*, Me_2Si). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 152.49, 149.32; ratio 1:2.7. ESI-MS: 885.6 ($[M+H]^+$).

2,3,5-Tri-O-*acetyl-1-deoxy-1-(4,6-difluoro-1*H*-benzimidazol-1-yl)-β-*D*-ribofuranose* (**16**). As described above for **12**, with 4,6-difluoro-1*H*-benzimidazole (**14**; 3.1 g, 20 mmol) MeCN (80 ml), *N*,*O*-bis(trimethylsilyl)acetamide (7.4 ml, 30 mmol), **10** (6.4 g, 20 mmol), MeCN (35 ml), and trimethylsilyl trifluoromethanesulfonate (4.5 ml, 25 mmol): **16** (5.6 g, 67.8%). White foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.49. ^{1}H-NMR (250 MHz, (D_6)DMSO): 8.56 (*s*, H–C(2)); 7.57 (*dd*, *J* = 9.0, 2.1, H–C(7)); 7.19 (*dt*, *J* = 10.5, 2.0, H–C(5)); 6.33 (*d*, *J* = 6.1, H–C(1′)); 5.64 (*t*, *J* = 6.3, H–C(2′)); 5.43 (*dd*, *J* = 6.3, 4.3, H–C(3′)); 4.40 (*m*, H–C(4′), 2 H–C(5′)); 2.14, 2.08, 2.04 (3*s*, Me). ^{13}C-NMR (63.9 MHz, (D_6)DMSO): 170.00, 169.52, 169.26 (C=O); 158.48 (*dd*, *J* = 11.2, 238.9, C(4)); 152.67 (*dd*, *J* = 15.1, 253.2, C(6)); 143.67 (C(2)); 134.67 (*dd*, *J* = 5.5, 16.5, C(9)); 128.94 (*d*, *J* = 18.1, C(8)); 98.32 (*dd*, *J* = 22.5, 7.5, C(5)); 95.09 (*dd*, *J* = 28.4, 4.4, C(7)); 86.43 (C(1′)); 79.57 (C(4′)); 71.69 (C(3′)); 69.43 (C(2′)); 62.95 (C(5′)); 20.48, 20.38, 20.18 (CH_3). ESI-MS: 413.0 ($[M+H]^+$).

*1-Deoxy-1-(4,6-difluoro-1*H*-benzimidazol-1-yl)-β-*D*-ribofuranose* (**17**). As described for **13**, with **16** (5.59 g, 13.5 mmol) and NH_3-sat. (150 ml): **17** (3.66 g, 94.2%). Yellow solid. TLC (CH_2Cl_2/MeOH 4:1): R_f 0.64. ^{1}H-NMR (250 MHz, (D_6)DMSO): 8.52 (*s*, H–C(2)); 7.65 (*dd*, *J* = 9.1, 2.2, H–C(7)); 7.13 (*dt*, *J* = 2.2, 10.5, H–C(5)); 5.87 (*d*, *J* = 6.4, H–C(1′)); 5.51 (*d*, *J* = 6.5, OH–C(2′)); 5.25 (*d*, *J* = 4.6, OH–C(3′)); 5.22 (*t*, *J* = 5.0, OH–C(5′)); 4.34 (*q*, *J* = 6.3, H–C(2′)); 4.12 (*m*, H–C(3′)); 4.00 (*q*, *J* = 3.1, H–C(4′)); 3.66 (*m*, 2 H–C(5′)). ^{13}C-NMR (63.9 MHz, (D_6)DMSO): 158.13 (*dd*, *J* = 238.3, 11.3, C(4)); 152.53 (*dd*, *J* = 252.7, 15.2, C(6)); 143.70 (C(2)); 134.95 (*dd*, *J* = 10.7, 5.4, C(9)); 129.01 (*d*, *J* = 17.7, C(8)); 97.66 (*dd*, *J* = 22.1, 7.5, C(5)); 95.43 (*d*, *J* = 28.4, C(7)); 89.07 (C(1′)); 85.83 (C(4′)); 73.63 (C(2′)); 70.04 (C(3′)); 61.08 (C(5′)). ^{19}F-NMR (254.2 MHz, (D_6)DMSO): –116.40 (*m*, F–C(6)); –125.25 (*m*, F–C(4)). ESI-MS: 285.1 ($[M-H]^-$).

*1-Deoxy-1-(4,6-difluoro-1*H*-benzimidazol-1-yl)-5*-O-*(4,4′-dimethoxytrityl)-β-*D*-ribofuranose* (**47**). As described above for **44**, with **17** (640 mg, 2.2 mmol), anh. pyridine (10 ml), Et_3N (470 µl, 3.3 mmol), $(MeO)_2$TrCl (0.91 g, 2.6 mmol) (20 h): **47** (0.98 g, 74.8%). Yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.52. ^{1}H-NMR (270 MHz, (D_6)DMSO): 8.43 (*s*, H–C(2)); 7.47 (*dd*, *J* = 8.8, 1.9, H–C(7)); 7.35–6.81 (*m*, 13 arom. H, H–C(5)); 5.93 (*d*, *J* = 5.1, H–C(1′)); 5.69 (*d*, *J* = 5.9, OH–C(2′)); (*d*, *J* = 5.5, OH–C(3′)); 4.49 (*q*, *J* = 5.5, H–C(2′)); 4.21 (*q*, *J* = 5.2, H–C(3′)); 4.12 (*m*, H–C(4′)); 3.72 (*s*, 2 MeO); 3.23 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 158.22 (*dd*, *J* = 239.2, 11.3 Hz, C(4)); 158.04 ($(MeO)_2$*Tr*); 152.57 (*dd*, *J* = 253.3, 15.3,

C(6)); 146.82 (C(2)); 144.69, 135.36, 135.31 ((MeO)$_2$*Tr*); 135.13 (*dd*, *J* = 10.8, 5.3, C(9)); 129.05 ((MeO)$_2$*Tr*); 128.94 (*d*, *J* = 15.5, C(8)); 127.75, 127.59, 126.66, 113.11 ((MeO)$_2$*Tr*); 97.83 (*dd*, *J* = 22.1, 7.3, C(5)); 95.17 (*d*, *J* = 28.1, C(7)); 89.09 (C(1′)); 85.62 ((MeO)$_2$*Tr*); 83.50 (C(4′)); 73.26 (C(2′)); 69.90 (C(3′)); 63.40 (C(5′)); 54.95 (MeO). ESI-MS: 587.4 ([*M* − H]$^-$).

2-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-1-(4,6-difluoro-1*H*-benzimidazol-1-yl)-5*-O-*(4,4′-dimethoxytrityl)-β-*D*-*ribofuranose* (**48**). As described above for **45**, with **47** (2.53 g, 4.3 mmol). HPLC (*MN Nucleoprep 100-20* from *Macherey-Nagel*, hexane/AcO^{i}Pr 3:2): slower-migrating isomer **48** (0.97 g, 32.1%). Colourless foam. TLC (CH_2Cl_2/MeOH 99:1): R_f 0.55. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.47 (*s*, H−C(2)); 7.47 (*dd*, *J* = 8.9, 2.0, H−C(7)); 7.39–6.84 (*m*, 13 arom. H); 7.12 (*dt*, *J* = 8.5, 2.0, H−C(5)); 5.97 (*d*, *J* = 6.3, H−C(1′)); 5.24 (*d*, *J* = 5.5, OH−(3′)); 4.59 (*t*, *J* = 5.8, H−C(2′)); 4.19 (*m*, H−C(3′)); 4.14 (*m*, H−C(4′)); 3.73 (*s*, 2 MeO); 3.31 (*m*, 2 H−C(5′)); 0.72 (*s*, ^{t}BuSi); −0.08, −0.24 (2*s*, Me_2Si). ^{13}C-NMR (100.6 MHz, (D_6)DMSO): 158.08 (*dd*, *J* = 250.9, 11.5, C(4)); 158.02 ((MeO)$_2$*Tr*); 152.58 (*dd*, *J* = 252.4, 15.4, C(6)); 144.63 ((MeO)$_2$*Tr*); 143.52 (C(2)); 135.10, 135.05 ((MeO)$_2$*Tr*); 134.45 (*d*, *J* = 10.2, C(9)); 129.64 ((MeO)$_2$*Tr*); 128.95 (*d*, *J* = 16.7, C(8)); 127.67, 127.43, 126.64, 113.07 ((MeO)$_2$*Tr*); 97.91 (C(5)); 95.31 (*d*, *J* = 32.6, C(7)); 88.56 (C(1′)); 85.72 ((MeO)$_2$*Tr*); 84.23 (C(4′)); 74.59 (C(2′)); 69.67 (C(3′)); 63.28 (C(5′)); 54.88 (MeO); 25.31 (*Me*$_3$CSi); 17.61 (Me$_3$*C*Si); −5.16, −5.76 (Me_2Si). ESI-MS: 701.5 ([*M* − H]$^-$).

3-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-1-(4,6-difluoro-1*H*-benzimidazol-1-yl)-5*-O-*(4,4′-dimethoxytriphenylmethyl)-β-*D*-*ribofuranose* (**49**) was obtained from the reaction described above as the faster-migrating isomer (1.55 g, 51.3%). Colourless foam. TLC (CH_2Cl_2/MeOH 99:1): R_f 0.55. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.47 (*s*, H−C(2)); 7.48 (*dd*, *J* = 8.7, 2.0, H−C(7)); 7.36–6.82 (*m*, 13 arom. H, H−C(5)); 5.90 (*d*, *J* = 5.6, H−C(1′)); 5.53 (*d*, *J* = 6.4, OH−C(2′)); 4.50 (*q*, *J* = 5.8, H−C(2′)); 4.32 (*t*, *J* = 4.7, H−C(3′)); 4.07 (*q*, *J* = 4.0, H−C(4′)); 3.72 (*s*, 2 MeO); 3.26 (*m*, 2 H−C(5′)); 0.81 (*s*, ^{t}BuSi); 0.06, −0.01 (2*s*, Me_2Si). ^{13}C-NMR (100.6 MHz, (D_6)DMSO): 158.20 (*dd*, *J* = 239.5, 11.5, C(4)); 158.11 ((MeO)$_2$*Tr*); 152.59 (*dd*, *J* = 272.9, 15.0, C(6)); 144.55 ((MeO)$_2$*Tr*); 143.32 (C(2)); 135.22, 135.19 ((MeO)$_2$*Tr*); 134.86 (*d*, *J* = 10.7, C(9)); 129.64 ((MeO)$_2$*Tr*); 129.04 (*d*, *J* = 16.8, C(8)); 127.77, 127.57, 126.72, 113.13 ((MeO)$_2$*Tr*); 97.87 (*dd*, *J* = 20.6, 7.4, C(5)); 95.40 (*d*, *J* = 27.9, C(7)); 89.11 (C(1′)); 85.86 ((MeO)$_2$*Tr*); 84.00 (C(4′)); 72.71 (C(2′)); 71.65 (C(3′)); 62.87 (C(5′)); 54.97 ((MeO); 25.69 (*Me*$_3$CSi); 17.93 (Me$_3$*C*Si); −4.53, −5.19 (Me_2Si). ESI-MS: 701.5 ([*M* − H]$^-$).

2-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-1-(4,6-difluoro-1*H*-benzimidazol-1-yl)-5*-O-*(4,4′-dimethoxytrityl)-β-*D*-*ribofuranose-3-(2-Cyanoethyl Diisopropylphosphoramidite)* (**3**). As described above for **2**, with **48** (200 mg, 0.29 mmol) (15 min at 0° and 25 min at r.t.): **3** (180 mg, 70.0%; diastereoisomer mixture). Colourless foam. TLC (CH_2Cl_2/MeOH 98:2): R_f 0.65. ^{1}H-NMR (270 MHz, $CDCl_3$): 8.12, 8.11 (2*s*, H−C(2)); 7.46–6.73 (*m*, 13 arom. H, H−C(5), H−C(7)); 5.85, 5.78 (2*d*, *J* = 7.6, 7.2, H−C(1′)); 4.67 (*m*, H−C(2′)); 4.41 (*m*, H−C(3′)); 4.32 (*m*, H−C(4′)); 3.79, 3.78 (2*s*, 2 MeO); 3.52 (*m*, 2 H−C(5′)); CH_2CN); 2.67 (*m*, CH_2O); 1.19 (*m*, 2 Me_2CH); 0.82, 0.76 (2*s*, ^{t}BuSi); −0.09, −0.10, −0.32, −0.34 (4*s*, Me_2Si). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 152.62, 149.33; ratio 1:2.7. ESI-MS: 903.6 ([*M* + H]$^+$).

2,3,5-Tri-O-*benzyl-1-deoxy*-D-*ribofuranose* (**19**). A soln. of **18** (1.0 g, 2.4 mmol) in MeCN (10 ml) was treated at r.t. with Et_3SiH (1.52 ml, 9.6 mmol) and $BF_3 \cdot Et_2O$ (0.6 ml, 4.8 mmol). The mixture was stirred for 1.5 h at r.t. under Ar, then quenched by addition of sat. aq. $NaHCO_3$ soln., and extracted with CH_2Cl_2. The org. phase was dried ($MgSO_4$) and evaporated and the residue purified by FC (hexane/AcOEt 4:1): **19** (850 mg, 88.4%). Colourless oil. TLC (CH_2Cl_2/MeOH 99:1): R_f 0.49. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.29–7.17 (*m*, 15 arom. H); 4.58–4.41 (*m*, Ph*CH*$_2$); 4.09 (*m*, H−C(4)); 3.89 (*m*, 2 H−C(1), H−C(2), H−C(3)); 3.50 (*m*, 2 H−C(5)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 138.05, 137.88, 137.80, 128.25, 128.21, 128.18, 127.80, 127.72, 127.61, 127.49, 127.42 (arom. C); 80.35 (C(4)); 78.18 (C(1)); 76.42 (C(2)); 73.28, 72.05, 71.66 (Ph*C*H$_2$); 70.45 (C(3)); 69.93 (C(5)). ESI-MS: 422.2 ([*M* + NH_4]$^+$).

1-Deoxy-D-*ribofuranose* (=*1,4-Anhydro*-D-*ribitol*; **20**). To a soln. of **19** (3.9 g, 9.6 mmol) in EtOH (70 ml) and cyclohexene (35 ml), 20% $Pd(OH)_2$/C (800 mg) was added, and the suspension was stirred under reflux for 4 h (TLC monitoring). The catalyst was removed by filtration, the filtrate evaporated and the crude product purified by FC (CH_2Cl_2/MeOH 9:1): **20** (1.23 g, 95.3%). Colourless solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.22. ^{1}H-NMR (250 MHz, (D_6)DMSO): 4.69 (*m*, OH−C(2), OH−C(3)); 4.57 (*t*, *J* = 5.7, OH−C(5)); 3.96 (*m*, H−C(4)); 3.83 (*m*, H−C(2)); 3.74 (*q*, *J* = 5.5, H−C(3)); 3.52 (*m*, 2 H−C(1), H−C(5)); 3.35 (*m*, 1 H−C(5)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 83.20 (C(4)); 71.99 (C(1)); 71.55 (C(2)); 70.41 (C(3)); 61.76 (C(5)). ESI-MS: 133.0 ([*M* − H]$^-$).

1-Deoxy-5-O-*(4,4′-dimethoxytrityl)*-D-*ribofuranose* (**50**). As described above for **44**, with **20** (1.22 g, 9.1 mmol), pyridine (40 ml), Et_3N (1.9 ml, 19 mmol), and (MeO)$_2$TrCl (3.7 g, 10.9 mmol). FC (CH_2Cl_2/MeOH 98:2) gave **50** (3.41 g, 85.9%). White foam. TLC (CH_2Cl_2/MeOH 98:2): R_f 0.13. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.42–6.86 (*m*, 13 arom. H); 4.77 (*d*, *J* = 4.5, OH−C(2)); 4.73 (*d*, *J* = 6.2, OH−C(3)); 4.01

(*m*, H–C(2)); 3.94 (*m*, H–C(1)); 3.77 (*m*, H–C(3), H–C(4)); 3.73 (*s*, 2 MeO); 3.60 (*dd*, *J* = 8.9, 3.1, H–C(1)); 3.02 (*m*, 2 H–C(5)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 158.02, 145.06, 135.79, 129.70, 127.74, 126.58, 113.14, 85.15 ($(MeO)_2$*Tr*); 80.97 (C(4)); 72.50 (C(1)); 72.24 (C(2)); 70.30 (C(3)); 64.45 (C(5)); 55.01 (MeO). ESI-MS: 435.2 ($[M-H]^-$).

2-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-5*-O-*(4,4′-dimethoxytrityl)*-D-*ribofuranose* (**51**). As described above for **45**, with **50** (3.2 g, 7.3 mmol), THF/pyridine 1:1 (60 ml), $AgNO_3$ (1.5 g, 13.3 mmol), and 1M ^{t}BuMe$_2$SiCl in THF (10.2 ml, 10.2 mmol). FC (CH_2Cl_2): slower-migrating isomer **51** (2.43 g, 60.3%). Colourless foam. TLC (CH_2Cl_2): R_f 0.43. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.40–6.83 (*m*, 13 arom. H); 4.49 (*d*, *J* = 6.0, OH–C(3)); 4.19 (*m*, H–C(2)); 3.96 (*m*, H–C(1)); 3.77 (*m*, H–C(3), H–C(4)); 3.73 (*s*, 2 MeO); 3.58 (*m*, H–C(1)); 3.00 (*m*, 2 H–C(5)); 0.88 (*s*, ^{t}BuSi); 0.07, 0.06 (2*s*, Me$_2$Si). ^{13}C-NMR (100.6 MHz, (D_6)DMSO): 158.05, 145.09, 135.75, 129.73, 127.78, 127.73, 127.42, 126.61, 113.16, 85.17 ($(MeO)_2$*Tr*); 80.92 (C(4)); 73.56 (C(1)); 72.57 (C(2)); 70.24 (C(3)); 64.33 (C(5)); 55.05 (MeO); 25.88 (*Me*$_3$CSi); 18.11 (Me$_3$*C*Si); –4.54, –4.87 (Me$_2$Si). ESI-MS: 549.4 ($[M-H]^-$).

3-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-5*-O-*(4,4′-dimethoxytrityl)*-D-*ribofuranose* (**52**) was obtained from the reaction described above as the faster-migrating isomer (1.1 g, 27.3%). Colourless foam. TLC (CH_2Cl_2): R_f 0.64. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.42–6.82 (*m*, 13 arom. H); 4.53 (*d*, *J* = 6.0, OH–C(2)); 4.17 (*m*, H–C(2)); 3.95 (*m*, H–C(1)); 3.89 (*m*, H–C(3)); 3.79 (*m*, H–C(4)); 3.72 (*s*, 2 MeO); 3.57 (*m*, H–C(1)); 3.02 (*m*, 2 H–C(5)); 0.72 (*s*, ^{t}BuSi); –0.04, –0.15 (*s*, Me$_2$Si). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 158.07, 144.94, 135.56, 129.68, 127.76, 127.65, 126.61, 113.82, 85.33 ($(MeO)_2$*Tr*); 81.42 (C(4)); 73.36 (C(1)); 72.13 (C(2)); 71.77 (C(3)); 63.85 (C(5)); 25.61 (*Me*$_3$CSi); 17.65 (Me$_3$*C*Si); –4.71, –5.29 (Me$_2$Si). ESI-MS: 550.7 ($[M-H]^-$).

2-O-*[(*tert-*Butyl)dimethylsilyl]-1-deoxy-5*-O-*(4,4′-dimethoxytrityl)*-D-*ribofuranose 3-(2-Cyanoethyl Diisopropylphosphoramidite)* (**8**). As described above for **2**, with **51** (200 mg, 0.36 mmol), MeCN (11 ml), collidine (480 µl, 3.7 mmol), 1-methyl-1*H*-imidazole (15 µl, 0.19 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (122 µl, 0.54 mmol) (15 min. at 0° and 35 min. at r.t.): **8** (130 mg, 47.8%; diastereoisomer mixture). Colourless foam. TLC (CH_2Cl_2/MeOH 99:1): R_f 0.49, 0.55. ^{1}H-NMR (270 MHz, $CDCl_3$): 7.40–6.72 (*m*, 13 arom. H); 4.36, 4.30 (2*q*, *J* = 4.9, 5.3, H–C(2)); 4.07 (*m*, 1 H–C(1)); 4.00 (*m*, H–C(3), H–C(4)); 3.72, 3.71 (2*s*, 2 MeO); 3.46 (*m*, CH_2CN, 1 H–C(1)); 3.28 (*m*, 2 H–C(5)); 2.53 (*m*, CH_2O); 1.05 (*m*, Me$_2$CH); 0.85, 0.83 (2*s*, ^{t}BuSi); 0.05, 0.04, 0.02, 0.01 (4*s*, MeSi). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 149.67, 149.15; ratio 1:1.3. ESI-MS: 751.1 ($[M+H]^+$).

2,3,5-Tri-O-*benzyl-1-deoxy-1-(4-fluorophenyl)-β*-D-*ribofuranose* (**24**). A soln. of 1-bromo-4-fluorobenzene (**22**); 150 µl, 1.3 mmol) in anh. THF (80 ml) was treated under Ar at –78° within 10 min with 1.5M BuLi in hexane (15.6 ml, 23.4 mmol). After 20 min at –78°, a soln. of **21** (7.0 g, 16.7 mmol) in THF (50 ml) was added over 30 min, and the mixture was stirred for an additional hour and then warmed within 2 h to –30° (TLC control). The reaction was quenched by addition of H_2O, the mixture extracted with Et_2O, and the org. phase dried ($MgSO_4$) and evaporated to afford an oil. The residue was dissolved in CH_2Cl_2 (100 ml) and treated at –78° with $BF_3 \cdot Et_2O$ (4.2 ml, 33.4 mmol) and Et_3SiH (5.3 ml, 33.4 mmol). The mixture was stirred for 1 h at –78° and then warmed overnight to 10°. The reaction was quenched by addition of sat. aq. $NaHCO_3$ soln., the mixture extracted with CH_2Cl_2, the org. phase dried ($MgSO_4$) and evaporated, and the residue purified by FC (hexane/AcOEt 4:1): **24** (6.31 g, 75.8%). Orange solid. TLC (hexane/AcOEt 4:1): R_f 0.35. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.44–7.08 (*m*, 19 arom. H); 4.87 (*d*, *J* = 6.8, H–C(1′)); 4.61–4.42 (*m*, PhC*H*$_2$); 4.24 (*q*, *J* = 3.8, H–C(4′)); 4.07 (*dd*, *J* = 4.8, 3.8, H–C(3′)); 3.87 (*dd*, *J* = 5.0, 6.8, H–C(2′)); 3.64 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 138.19, 137.95 (arom. C); 136.63 (C(1)); 128.18, 128.11, 128.05, 127.74, 127.43, 127.36 (arom. C); 123.99 (C(5)); 114.95 (C(2)); 114.64 (C(6)); 83.41 (C(1′)); 81.23 (C(4′)); 81.08 (C(2′)); 77.27 (C(3′)); 72.41 (PhCH$_2$); 71.10 (PhCH$_2$); 70.96 (PhCH$_2$); 70.27 (C(5′)). ESI-MS: 516.2 ($[M+H]^+$).

1-Deoxy-1-(4-fluorophenyl)-β-D-*ribofuranose* (**28**). As described above for **20**, with **24** (3 g, 6 mmol), EtOH (60 ml), cyclohexene (30 ml), and 20% $Pd(OH)_2$/C (600 mg) (5 h): **28** (1.36 g, 99.5%). Colourless solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.39. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.42 (*m*, 2 arom. H); 7.14 (*m*, 2 arom. H); 4.97 (*d*, *J* = 7.0, H–C(1′)); 4.91 (*d*, *J* = 4.7, OH–C(3′)); 4.82 (*t*, *J* = 5.5, OH–C(5′)); 4.56 (*d*, *J* = 7.3, OH–C(2′)); 3.89 (*m*, H–C(3′)); 3.81 (*m*, H–C(4′)); 3.66 (*m*, H–C(2′)); 3.54 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 137.72 (C(1)); 128.06 (*d*, *J* = 11.5, C(3)); 114.79 (C(2)); 114.47 (C(6)); 85.18 (C(1′)); 82.21 (C(4′)); 77.55 (C(2′)); 71.35 (C(3′)); 61.97 (C(5′)). ^{19}F-NMR (254.2 MHz, (D_6)DMSO): –115.87 (*sept*. *J* = 5.1, F–C(4)). ESI-MS: 227.0 ($[M-H]^-$). Anal. calc. for $C_{11}H_{13}FO_4$ (228.21): C 57.89, H 5.74; found: C 57.88, H 5.84.

1-Deoxy-5-O-*(4,4′-dimethoxytrityl)-1-(4-fluorophenyl)-β*-D-*ribofuranose* (**32**). As described above for **44**, with **28** (1.37 g, 6 mmol), pyridine (30 ml), Et_3N (1.25 µl, 9 mmol), and $(MeO)_2$TrCl (2.44 g, 7.2 mmol) (4.5 h).

FC (CH_2Cl_2/MeOH 98:2): **32** (2.52 g, 79.1%). Yellow foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.37. ^{1}H-NMR (150 MHz, (D_6)DMSO): 7.48–6.87 (*m*, 17 arom. H); 5.13 (*d*, *J* = 6.7, H–C(1′)); 5.01 (*d*, *J* = 5.1, OH–C(3′)); 4.66 (*d*, *J* = 6.6, OH–C(2′)); 3.98 (*m*, H–C(4′)); 3.89 (*q*, *J* = 4.9, H–C(3′)); 3.74 (*s*, H–C(2′), 2 MeO); 3.18 (*m*, 2 H–C(5′)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 158.07 ((MeO)$_2$*Tr*); 149.63 (C(4)); 144.95 ((MeO)$_2$*Tr*); 137.44 (C(1)); 135.63, 129.76, 127.84, 127.78, 126.68 ((MeO)$_2$*Tr*); 123.91 (C(5)); 115.03 (C(2)); 114.70 (C(6)); 113.18, 85.40 ((MeO)$_2$*Tr*); 83.19 (C(1′)); 82.69 (C(4′)); 77.60 (C(2′)); 71.42 (C(3′)); 64.14 (C(5′)); 55.03 (MeO). ESI-MS: 529.2 ([*M* – H]$^-$).

*2-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(4-fluorophenyl)-β-*D*-ribofuranose* (**33**). As described above for **45**, from **32** (530 mg, 1 mmol), THF/pyridine 1:1 (10 ml), $AgNO_3$ (204 mg, 1.2 mmol), and 1M ^{t}BuMe$_2$SiCl in THF (1.4 ml, 1.4 mmol). FC (CH_2Cl_2): slower-migrating isomer **33** (280 mg, 43.5%). Colourless foam. TLC (CH_2Cl_2): R_f 0.22. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.49–6.83 (*m*, 17 arom. H); 4.77 (*d*, *J* = 4.4, OH–C(3′)); 4.67 (*d*, *J* = 6.4, H–C(1′)); 4.00 (*m*, H–C(4′)); 3.93 (*m*, H–C(2′), H–C(3′)); 3.73 (*s*, 2 MeO); 3.28 (*m*, 1 H–C(5′)); 3.15 (*m*, 1 H–C(5′)); 0.78 (*s*, ^{t}BuSi); –0.11, –0.17 (2*s*, Me$_2$Si). ^{13}C-NMR (100.6 MHz, (D_6)DMSO): 158.07 ((MeO)$_2$*Tr*); 144.96 (C(4)); 144.75 ((MeO)$_2$*Tr*); 137.22 (C(1)); 135.52, 135.44, 129.74, 129.71, 129.66, 128.88, 128.19, 127.89, 127.61 ((MeO)$_2$*Tr*); 126.67 (C(3)); 114.94 (C(2)); 114.73 (C(6)); 113.14, 85.57 ((MeO)$_2$*Tr*); 83.50 (C(1′)); 82.68 (C(4′)); 79.59 (C(2′)); 71.56 (C(3′)); 63.83 (C(5′)); 55.00 (MeO); 25.57 (*Me*$_3$CSi); 17.85 (Me$_3$*C*Si); –5.07, –5.30 (Me$_2$Si). ESI-MS: 643.0 ([*M* – H]$^-$).

*3-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(4-fluorophenyl)-β-*D*-ribofuranose* (**34**) was obtained from the reaction described above as the faster-migrating isomer (270 mg, 42.0%). Colourless foam. TLC (CH_2Cl_2): R_f 0.42. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.49–6.87 (*m*, 17 arom. H); 4.89 (*d*, *J* = 7.2, OH–C(2′)); 4.66 (*d*, *J* = 6.7, H–C(1′)); 4.01 (*m*, H–C(3′)); 3.94 (*m*, H–C(4′)); 3.75 (*m*, H–C(2′)); 3.73 (*s*, 2 MeO); 3.29 (*m*, 1 H–C(5′)); 3.09 (*m*, 1 H–C(5′)); 0.79 (*s*, ^{t}BuSi); 0.01–0.05 (2*s*, Me$_2$Si). ^{13}C-NMR (100.6 MHz, (D_6)DMSO): 158.07 ((MeO)$_2$*Tr*); 144.96 (C(4)); 144.75 ((MeO)$_2$*Tr*); 137.21 (C(1)); 135.51, 135.44, 129.74, 129.70, 129.66, 128.19, 127.77, 127.66 ((MeO)$_2$*Tr*); 126.67 (C(3)); 114.94 (C(2)); 114.73 (C(6)); 113.14, 85.46 ((MeO)$_2$/*Tr*); 83.93 (C(1′)); 82.27 (C(4′)); 77.27 (C(2′)); 73.29 (C(3′)); 63.49 (C(5′)); 54.99 (MeO); 25.74 (*Me*$_3$CSi); 17.95 (Me$_3$*C*Si); –4.51, –5.09 (Me$_2$Si). ESI-MS: 643.4 ([*M* – H]$^-$).

*2-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(4-fluorophenyl)-β-*D*-ribofuranose 3-(2-Cyanoethyl Diisopropylphosporamidite)* (**4**). As described above for **2**, with **33** (200 mg, 0.31 mmol), MeCN (10 ml), collidine (440 µl, 3.1 mmol), 1-methyl-1*H*-imidazole (14 µl, 0.18 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (112 µl, 0.5 mmol) (15 min at 0° and 15 min at r.t.). FC (hexane/AcOEt 4:1): **4** (176 mg, 67.2%; diastereoisomer mixture). Colourless foam. TLC (hexane/AcOEt 4:1): R_f 0.42. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.51–6.81 (*m*, 17 arom. H); 4.75, 4.70 (2*d*, *J* = 8.2, 8.1, H–C(1′)); 4.17 (*m*, H–C(3′)); 4.12 (*m*, H–C(2′)); 3.98 (*m*, H–C(4′)); 3.79, 3.78 (2*s*, 2 MeO); 3.54 (*m*, 1 H–C(5′), CH$_2$CN); 3.19 (*m*, 1 H–C(5′)); 2.66 (*m*, CH$_2$O); 1.17 (*m*, 2 Me$_2$CH); 0.80, 0.79 (2*m*, ^{t}BuSi); –0.10, –0.12, –0.20, –0.28 (4*s*, Me$_2$Si). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 151.37, 148.68; ratio 1:3.2. ESI-MS: 845.6 ([*M* + H]$^+$).

*2,3,5-Tri-*O*-benzyl-1-deoxy-1-(3-fluorophenyl)-β-*D*-ribofuranose* (**25**). As described above for **24**, with 1-bromo-3-fluorobenzene (395 µl, 3.6 mmol), THF (12 ml), 1.5M BuLi in hexane (2.25 ml, 3.4 mmol) **21** (1.0 g, 2.4 mmol), and THF (8 ml). Subsequent treatment in CH_2Cl_2 (15 ml) with $BF_3 \cdot Et_2O$ (600 µl, 4.8 mmol) and Et_3SiH (760 µl, 4.8 mmol): **25** (0.89 g, 74.8%). Orange solid. TLC (hexane/AcOEt 4:1): R_f 0.26. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.38–7.05 (*m*, 19 arom. H); 4.92 (*d*, *J* = 6.4, H–C(1′)); 4.50 (*m*, PhC*H*$_2$); 4.26 (*q*, *J* = 3.9, H–C(4′)); 4.08 (*t*, *J* = 4.5, H–C(3′)); 3.91 (*m*, H–C(2′)); 3.65 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 162.14 (*d*, *J* = 243.3, C(3)); 143.71 (*d*, *J* = 7.0, C(1)); 138.21, 138.15, 137.97 (arom. C); 130.08 (*d*, *J* = 8.4, C(5)); 128.22, 128.18, 128.13, 127.80, 127.53, 127.41, 127.34 (arom. C); 122.26 (*d*, *J* = 2.6, C(6)); 114.30 (*d*, *J* = 20.9, C(2)); 112.74 (*d*, *J* = 22.2, C(4)); 83.37 (C(1′)); 81.22 (C(4′)); 81.17 (C(2′)); 77.17 (C(3′)); 72.42 (Ph*C*H$_2$); 71.13 (Ph*C*H$_2$); 70.99 (Ph*C*H$_2$); 70.14 (C(5′)). ESI-MS: 516.5 ([*M* + NH$_4$]$^+$).

*1-Deoxy-1-(3-fluorophenyl)-β-*D*-ribofuranose* (**29**). As described above for **20**, with **25** (0.86 g, 1.7 mmol), EtOH (20 ml), cyclohexene (10 ml), and 20% Pd(OH)$_2$/C (200 mg) (3 h): **29** (0.38 g, 96.7%). Colourless solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.32. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.36, 7.23, 7.07 (*m*, arom. H); 5.03 (*d*, *J* = 7.1, OH–C(3′)); 4.94 (*d*, *J* = 4.7, H–C(1′)); 4.85 (*t*, *J* = 5.5, OH–C(5′)); 4.58 (*d*, *J* = 7.2, OH–C(2′)); 3.90 (*m*, H–C(3′)); 3.82 (*q*, *J* = 3.5, H–C(4′)); 3.65 (*m*, H–C(2′)); 3.54 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 162.11 (*d*, *J* = 242.8, C(3)); 144.60 (*d*, *J* = 7.1, C(1)); 129.84 (*d*, *J* = 8.0, C(5)); 122.12 (*d*, *J* = 2.2, C(6)); 113.83 (*d*, *J* = 20.9, C(2)); 112.57 (*d*, *J* = 22.0, C(4)); 85.22 (C(1′)); 82.16 (C(4′)); 77.66 (C(2′)); 71.33 (C(3′)); 61.85 (C(5′)). ^{19}F-NMR (254.2 MHz, (D_6)DMSO): –113.87 (*m*, F–C(3)). ESI-MS: 227.1 ([*M* – H]$^-$). Anal. calc. for $C_{11}H_{13}FO_4$ (228.21): C 57.89, H 4.74; found: C 57.94, H 5.81.

*1-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(3-fluorophenyl)-β-*D*-ribofuranose* (**35**). As described above for **44**, with **29** (600 mg, 2.6 mmol), with pyridine (20 ml), Et_3N (560 µl, 4 mmol), and (MeO)$_2$TrCl (1.06 g, 3.1 mmol)

(4 h). FC (CH_2Cl_2/MeOH 98:2): **35** (1.34 g, 96.4%). Yellow foam. TLC (CH_2Cl_2/MeOH 98:2): R_f 0.24. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.45–6.87 (*m*, 19 arom. H); 5.17 (*d*, *J* = 6.7, OH–C(3′)); 5.00 (*m*, *J* = 5.1, H–C(1′)); 4.70 (*d*, *J* = 6.4, OH–C(2′)); 4.01 (*m*, H–C(4′)); 3.89 (*q*, *J* = 4.7, H–C(3′)); 3.79 (*m*, H–C(2′)); 3.74 (*s*, 2 MeO); 3.19 (*m*, 2 H–C(5′)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 158.26 (*d*, *J* = 248.8, C(3)); 158.03, 144.91 ((MeO)$_2$*Tr*); 144.38 (*d*, *J* = 7.0, C(1)); 135.61, 135.55 ((MeO)$_2$*Tr*); 129.93 (*d*, *J* = 19.7, C(5)); 129.70, 127.75, 127.68, 126.62 ((MeO)$_2$*Tr*); 123.85 (C(6)); 113.88 (*d*, *J* = 13.0, C(2)); 113.13 ((MeO)$_2$*Tr*); 112.32 (*d*, *J* = 21.8, C(4)); 85.37 ((MeO)$_2$*Tr*); 83.17 (C(1′)); 82.63 (C(4′)); 77.57 (C(2′)); 71.36 (C(3′)); 64.10 (C(5′)); 54.97 (MeO). ESI-MS: 529.4 ([*M* – H]$^-$).

*2-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-1-(3-fluorophenyl)-β-*D*-ribofuranose* (**36**). As described above for **45**, with **35** (1.32 g, 2.5 mmol), THF/pyridine 1:1 (20 ml), $AgNO_3$ (508 mg, 3 mmol), and 1M ^{t}BuMe$_2$SiCl (3.5 ml, 3.5 mmol). FC (CH_2Cl_2): slower-migrating isomer **36** (530 mg, 33.1%). Colourless foam. TLC (CH_2Cl_2): R_f 0.36. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.45–6.82 (*m*, 17 arom. H); 4.85 (*d*, *J* = 5.2, OH–C(3′)); 4.70 (*d*, *J* = 6.8, H–C(1′)); 4.00 (*m*, H–C(4′)); 3.92 (*m*, H–C(2′), H–C(3′)); 3.73 (*s*, 2 MeO); 3.22 (*m*, 2 H–C(5′)); 0.78 (*s*, ^{t}BuSi); –0.10, –0.16 (*s*, Me$_2$Si). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 163.50 (*d*, *J* = 257.7, C(3)); 158.10, 145.05 ((MeO)$_2$*Tr*); 144.83 (C(1)); 135.51, 135.41 ((MeO)$_2$*Tr*); 130.10 (*d*, *J* = 9.0, C(5)); 129.77, 127.81, 127.62 ((MeO)$_2$*Tr*); 126.73 (C(6)); 113.77 (C(2)); 113.17 ((MeO)$_2$*Tr*); 112.54 (C(4)); 85.51 ((MeO)$_2$*Tr*); 84.08 (C(1′)); 82.27 (C(4′)); 79.68 (C(2′)); 71.65 (C(3′)); 64.11 (C(5′)); 55.02 (MeO); 25.63 (*Me*$_3$CSi); 17.91 (Me$_3$*C*Si); –4.94, –5.31 (Me$_2$Si). ESI-MS: 643.4 ([*M* – H]$^-$).

*3-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(3-fluorophenyl)-β-*D*-ribofuranose* (**37**) was obtained from the reaction described above as the faster-migrating isomer (680 mg, 42.5%). Colourless foam. TLC (CH_2Cl_2): R_f 0.48. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.43–6.85 (*m*, 17 arom. H); 5.00 (*d*, *J* = 7.3, OH–C(2′)); 4.68 (*d*, *J* = 6.5, H–C(1′)); 3.97 (*m*, H–C(3′), H–C(4′)); 3.77 (*m*, H–C(2′)); 3.73 (*s*, 2 MeO); 3.18 (*m*, 2 H–C(5′)); 0.78 (*s*, ^{t}BuSi); –0.01, –0.06 (*s*, Me$_2$Si). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 162.22 (*d*, *J* = 243.0, C(3)); 158.11, 144.83 ((MeO)$_2$*Tr*); 144.60 (*d*, *J* = 7.0, C(1)); 135.55, 135.44 ((MeO)$_2$*Tr*); 130.09 (*d*, *J* = 8.1, C(5)); 129.70, 127.95, 127.67, 126.70 ((MeO)$_2$*Tr*); 121.94 (C(6)); 114.05 (*d*, *J* = 19.7, C(2)); 113.16 ((MeO)$_2$*Tr*); 112.43 (*d*, *J* = 18.0, C(4)); 85.61 ((MeO)$_2$*Tr*); 83.57 (C(1′)); 82.74 (C(4′)); 77.39 (C(2′)); 73.39 (C(3′)); 63.50 (C(5′)); 55.00 (MeO); 25.75 (*Me*$_3$Si); 17.99 (Me$_3$*C*Si); –4.51, –5.10 (Me$_2$Si). ESI-MS: 643.4 ([*M* – H]$^-$).

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(3-fluorophenyl)-β-*D*-ribofuranose 3-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5**). As described above for **2**, with **36** (200 mg, 0.31 mmol), MeCN (10 ml), collidine (440 µl, 3.1 mmol), 1-methyl-1*H*-imidazole (14 µl, 0.18 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (112 µl, 0.5 mmol) (15 min at 0° and 15 min at r.t.). FC (hexane/AcOEt 4:1): **5** (170 mg, 64.9%; diastereoisomer mixture). Colourless foam. TLC (hexane/AcOEt 4:1): R_f 0.31. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.52–6.81 (*m*, 17 arom. H); 4.77, 4.73 (2*d*, *J* = 8.4, 7.2, H–C(1′)); 4.20 (*m*, H–C(3′)); 4.14 (*m*, H–C(2′)); 4.01 (*m*, H–C(4′)); 3.79, 3.78 (2*s*, 2 MeO); 3.52 (*m*, 1 H–C(5′), CH_2CN); 3.25 (*m*, 1 H–C(5′)); 2.64 (*m*, CH_2O); 1.17 (*m*, 2 Me$_2$CH); 0.87, 0.81 (2*m*, ^{t}BuSi); –0.09, –0.11, –0.17, –0.27 (4*s*, Me$_2$Si). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 151.47, 148.69; ratio 1:3.7. ESI-MS: 845.6 ([*M* + H]$^+$).

*2,3,5-Tri-*O*-benzyl-1-deoxy-1-(2-fluorophenyl)-β-*D*-ribofuranose* (**26**). As described above for **24**, with 1-bromo-2-fluorobenzene (1.95 ml, 18 mmol), THF (50 ml), 1M BuLi in hexane (11.3 ml, 17 mmol), **21** (5.0 g, 12 mmol), and THF (25 ml). Subsequent treatment in CH_2Cl_2 (50 ml) with $BF_3 \cdot Et_2O$ (3.0 ml, 24 mmol) and Et_3SiH (3.8 ml, 24 mmol): **26** (4.76 g, 80.0%). Orange solid. TLC (hexane/AcOEt 4:1): R_f 0.35. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.57 (*dt*, *J* = 7.6, 1.5, H–C(5)); 7.39–7.26 (*m*, 16 arom. H, H–C(3)); 7.19 (*m*, H–C(6)); 7.08 (*dt*, *J* = 7.5, 0.8, H–C(4)); 5.23 (*d*, *J* = 3.8, H–C(1′)); 4.55 (*m*, PhCH_2 benzyl); 4.23 (*q*, *J* = 4.0, H–C(4′)); 4.06 (*m*, H–C(2′), H–C(3′)); 3.69 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 159.55 (*d*, *J* = 245.3, C(2)); 138.21, 138.09 (arom. C); 129.55 (*d*, *J* = 8.5, C(6)); 128.12 (C(4)); 128.08, 127.86, 127.66, 127.66, 127.48, 127.41 (arom. C); 127.23 (*d*, *J* = 12.8, C(1)); 124.26 (*d*, *J* = 3.0, C(5)); 115.05 (*d*, *J* = 21.3, C(3)); 82.13 (C(1′)); 80.52 (C(4′)); 76.88 (C(2′)); 76.43 (C(3′)); 72.37 (Ph*C*H$_2$); 71.02 (Ph*C*H$_2$); 69.76 (C(5′)). ESI-MS: 516.5 ([*M* + NH$_4$]$^+$).

*1-Deoxy-1-(2-fluorophenyl)-β-*D*-ribofuranose* (**30**). As described above for **20**, with **26** (4.76 g, 9.5 mmol), EtOH (80 ml), cyclohexene (40 ml), and 20% Pd(OH)$_2$/C (900 mg) (2 h): **30** (2.09 g, 95.9%). Colourless solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.41. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.60 (*dt*, *J* = 6.0, 1.8, H–C(5)); 7.31 (*m*, H–C(3)); 7.15 (*m*, H–C(4), H–C(6)); 5.02 (*d*, *J* = 5.9, H–C(1′)); 4.88 (*m*, OH–C(2′), OH–C(3′)); 4.81 (*t*, *J* = 5.5, OH–C(5′)); 3.85 (*m*, H–C(2′), H–C(3′), H–C(4′)); 3.58 (*m*, 2 H–C(5′)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 159.97 (*d*, *J* = 245.2, C(2)); 129.23 (*d*, *J* = 8.2, C(6)); 128.44 (*d*, *J* = 4.3, C(4)); 128.04 (*d*, *J* = 12.9, C(1)); 124.27 (*d*, *J* = 3.3, C(5)); 115.00 (*d*, *J* = 21.5, C(3)); 84.44 (C(1′)); 77.58 (C(4′)); 76.72 (C(2′)); 71.00 (C(3′)); 61.69 (C(5′)). ESI-MS: 227.0 ([*M* – H]$^-$).

*1-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(2-fluorophenyl)-β-*D*-ribofuranose* (**38**). As described above for **44**, with **30** (1.81 g, 7.9 mmol), pyridine (50 ml), Et_3N (1.2 ml, 12 mmol), and $(MeO)_2TrCl$ (3.22 g, 9.5 mmol) (3 h). FC (CH_2Cl_2/MeOH 98:2): **38** (4.11 g, 90.2%). Yellow foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.37. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.57 (*t*, *J* = 6.1, H–C(5)); 7.46–6.85 (*m*, 13 arom. H, H–C(3), H–C(4), H–C(6)); 5.18 (*d*, *J* = 5.1, H–C(1′)); 4.97 (*m*, OH–C(2′), OH–C(3′)); 3.98 (*q*, *J* = 2.9, H–C(4′)); 3.90 (*m*, H–C(2′), H–C(3′)); 3.74 (*s*, 2 MeO); 3.22 (*m*, 2 H–C(5′)). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 159.74 (*d*, *J* = 245.4, C(2)); 158.08, 144.96, 135.67, 135.60, 129.76 ((MeO)$_2$*Tr*); 129.32 (*d*, *J* = 8.2, C(6)); 128.87 ((MeO)$_2$*Tr*); 128.14 (*d*, *J* = 4.6, C(4)); 127.90, 127.76, 126.64 ((MeO)$_2$*Tr*); 125.29 (C(1)); 124.14 (C(5)); 115.13 (*d*, *J* = 21.3, C(3)); 113.14, 85.40 ((MeO)$_2$*Tr*); 81.97 (C(1′)); 78.61 (C(4′)); 76.55 (C(2′)); 70.93 (C(3′)); 63.82 (C(5′)); 55.00 (MeO). ESI-MS: 529.0 ([*M* – H]$^-$).

*2-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(2-fluorophenyl)-β-*D*-ribofuranose* (**39**). As described above for **45**, with **38** (3.5 g, 6.6 mmol), THF/pyridine 1:1 (50 ml), $AgNO_3$ (1.35 g, 7.9 mmol), and 1M ^{t}BuMe$_2$SiCl in THF (9.2 ml, 9.2 mmol). Prep. HPLC (*MN Nucleoprep 100-20* from *Macherey-Nagel*, hexane/AcOEt/CH_2Cl_2 47:3:50): slower-migrating isomer **39** (1.33 g, 31.3%). Colourless foam. TLC (CH_2Cl_2): R_f 0.30. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.62 (*t*, *J* = 6.3, H–C(5)); 7.47–6.87 (*m*, 13 arom. H, H–C(3), H–C(4), H–C(6)); 5.01 (*d*, *J* = 5.3, H–C(1′)); 4.84 (*d*, *J* = 5.7, OH–C(3′)); 4.03 (*m*, H–C(2′), H–C(4′)); 3.90 (*m*, H–C(3′)); 3.74 (*s*, 2 MeO); 3.23 (*m*, 2H–C(5′)); 0.80 (*s*, ^{t}BuSi); –0.06, –0.11 (*s*, Me$_2$Si). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 159.70 (*d*, *J* = 245.1, C(2)); 158.08, 144.96, 135.51, 129.76 ((MeO)$_2$*Tr*); 129.30 (*d*, *J* = 8.1, C(6)); 128.14 (*d*, *J* = 4.6, C(4)); 127.76, 127.67, 126.65 ((MeO)$_2$*Tr*); 125.27 (C(1)); 124.15 (C(5)); 115.15 (*d*, *J* = 21.4, C(3)); 113.13, 85.45 ((MeO)$_2$*Tr*); 82.61 (C(1′)); 79.00 (C(4′)); 77.76 (C(2′)); 70.76 (C(3′)); 63.85 (C(5′)); 54.99 (MeO); 25.56 (*Me*$_3$CSi); 17.85 (Me$_3$*C*Si); –4.89, –5.42 (Me$_2$Si). ESI-MS: 643.6 ([*M* – H]$^-$).

*3-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(2-fluorophenyl)-β-*D*-ribofuranose* (**40**) was obtained from the reaction described above as the faster-migrating isomer (1.26 g, 29.6%). Colourless foam. TLC (CH_2Cl_2): R_f 0.30. ^{1}H-NMR (250 MHz, (D_6)DMSO): 7.63 (*t*, *J* = 6.2, H–C(5)); 7.45–6.85 (*m*, 13 arom. H, H–C(3), H–C(4), H–C(6)); 4.98 (*m*, H–C(1′), OH–C(2′)); 4.05 (*t*, *J* = 5.2, H–C(3′)); 3.93 (*m*, H–C(2′), H–C(4′)); 3.73 (*s*, 2 MeO); 3.19 (*m*, 2 H–C(5′)); 0.75 (*s*, ^{t}BuSi); –4.56, –5.19 (2*s*, Me$_2$Si). ^{13}C-NMR (62.9 MHz, (D_6)DMSO): 159.72 (*d*, *J* = 245.2, C(2)); 158.10, 144.86, 135.49 ((MeO)$_2$*Tr*); 129.31 (*d*, *J* = 8.3, C(6)); 128.15 (*d*, *J* = 4.7, C(4)); 127.92, 127.75, 126.69 ((MeO)$_2$*Tr*); 125.29 (C(1)); 124.19 (C(5)); 115.19 (*d*, *J* = 20.9, C(3)); 113.13, 85.57 ((MeO)$_2$*Tr*); 82.18 (C(1′)); 78.89 (C(4′)); 76.15 (C(2′)); 72.54 (C(3′)); 63.20 (C(5′)); 54.99 (MeO); 25.67 (*Me*$_3$CSi); 17.86 (Me$_3$*C*Si); –4.56, –5.19 (Me$_2$Si). ESI-MS: 643.4 ([*M* – H]$^-$).

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-5-*O*-(4,4′-dimethoxytrityl)-1-(2-fluorophenyl)-β-*D*-ribofuranose 3-(2-Cyanoethyl Diisopropylphosphoramidite)* (**6**). As described above for **2**, with **39** (200 mg, 0.31 mmol), MeCN (10 ml), collidine (440 µl, 3.1 mmol), 1-methyl-1*H*-imidazole (14 µl, 0.18 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (112 µl, 0.5 mmol) (15 min at 0° and 1 h at r.t.). FC (hexane/AcOEt 4:1): **6** (150 mg, 57.3%; diastereoisomer mixture). Colourless foam. TLC (hexane/AcOEt): R_f 0.29. ^{1}H-NMR (270 MHz, $CDCl_3$): 7.73–6.79 (*m*, 17 arom. H); 5.19, 5.17 (2*d*, *J* = 7.8, H–C(1′)); 4.25 (*m*, H–C(3′)); 4.16 (*m*, H–C(2′)); 3.96 (*m*, H–C(4′)); 3.79, 3.78 (2*s*, 2 MeO); 3.65 (*m*, 1 H–C(5′), CH_2CN); 3.22 (*m*, 1 H–C(5′)); 2.66 (*m*, CH_2O); 1.14 (*m*, 2 Me$_2$CH); 0.81, 0.80 (2*m*, ^{t}BuSi); –0.05; –0.09, –0.15, –0.19 (4*s*, Me$_2$Si). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 150.47, 148.83; ratio 1:2.7. ESI-MS: 845.6 ([*M* + H]$^+$).

*2,3,5-Tri-*O*-benzyl-1-deoxy-1-(2,4-difluorophenyl)-β-*D*-ribofuranose* (**27**). As described above for **24**, with 1-bromo-2,4-difluorobenzene (150 µl, 1.3 mmol), anh. Et_2O (10 ml), 1.5M BuLi in hexane (2.4 ml, 3.6 mmol), **21** (1.0 g, 2.4 mmol), and Et_2O (5 ml). Subsequent treatment in CH_2Cl_2 (10 ml) with $BF_3 \cdot Et_2O$ (600 µl, 4.8 mmol) and Et_3SiH (760 µl, 4.8 mmol): **27** (1.04 g, 84.6%). Orange solid. TLC (hexane/AcOEt 4:1): R_f 0.35. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.59 ('*q*', *J* = 6.9, H–C(6)); 7.29 (*m*, 16 arom. H, H–C(3)); 6.93 (*dt*, *J* = 8.4, 2.2, H–C(5)); 5.17 (*d*, *J* = 4.5, H–C(1′)); 4.55 (*m*, PhC*H*$_2$); 4.22 (*q*, *J* = 3.9, H–C(4′)); 4.05 (*m*, H–C(2′), H–C(3′)); 3.67 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 162.51 (*dd*, *J* = 141.7, 12.6, C(4)); 158.87 (*dd*, *J* = 143.7, 12.4, C(2)); 138.18, 138.10, 137.96 (arom. C); 129.36 (C(6)); 128.22, 128.09, 127.70, 127.50, 127.42, 127.11 (arom. C); 123.61 (C(1)); 111.30 (*d*, *J* = 20.8, C(5)); 103.62 (*t*, *J* = 25.7, C(3)); 82.10 (C(1′)); 80.72 (C(4′)); 76.84 (C(2′)); 75.96 (C(3′)); 72.40 (Ph*C*H$_2$); 71.04 (Ph*C*H$_2$); 69.73 (C(5′)). ESI-MS: 534.4 ([*M* + NH_4]$^+$).

*1-Deoxy-1-(2,4-difluorophenyl)-β-*D*-ribofuranose* (**31**). As described above for **20** with **27** (6.5 g, 12.6 mmol), EtOH (100 ml), cyclohexene (50 ml) and 20% Pd(OH)$_2$/C (1 g) (3 h): **31** (3.08 g, 99.3%). Colourless solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.42. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.65 ('*q*', *J* = 6.9, H–C(6)); 7.18 (*m*, H–C(3)); 7.07 (*dt*, *J* = 8.6, 2.4, H–C(5)); 5.04 (*d*, *J* = 6.0, OH–C(2′)); 4.91 (*d*, *J* = 5.0, OH–C(3′)); 4.84 (*m*, H–C(1′), OH–C(5′)); 3.85 (*m*, H–C(2′), H–C(3′), H–C(4′)); 3.57 (*m*, 2 H–C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 162.58 (*dd*, *J* = 109.2, 12.1, C(4)); 158.94 (*dd*, *J* = 111.7, 12.1, C(2)); 129.67 (C(6)); 124.40 (*dd*, *J* = 13.1, 3.5, C(1)); 114.74 (*dd*, *J* = 20.9, 3.5, C(5)); 103.42 (*t*, *J* = 25.9, C(3)); 84.59 (C(1′));

77.08 (C(4′)); 76.65 (C(2′)); 70.94 (C(3′)); 61.59 (C(5′)). ^{19}F-NMR (254.2 MHz, (D_6)DMSO: −112.0 (*quint.*, J = 7.6, F−C(4)); −114.74 (*q*, J = 8.9, F−C(2)). ESI-MS: 245.0 ([M − H]$^-$). Anal. calc. for $C_{11}H_{12}F_2O_4$ (246.2): C 53.66, H 4.91; found: C 53.65, H 4.94.

*1-Deoxy-1-(2,4-difluorophenyl)-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranose* (**41**). As described above for **44**, with **31** (1 g, 4 mmol), pyridine (25 ml) Et_3N (840 µl, 6 mmol), and $(MeO)_2$TrCl (1.63 g, 4.8 mmol) (2.5 h). FC (CH_2Cl_2/MeOH 98:2): **41** (1.86 g, 83.4%). Yellow foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.48. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.56 (‘*q*’, J = 6.8, H−C(6)); 7.45–6.86 (*m*, 13 arom. H, H−C(3), H−C(5)); 5.19 (*d*, J = 5.4, OH−C(2′)); 4.98 (*d*, J = 5.2, OH−C(3′)); 4.92 (*d*, J = 4.3, H−C(1′)); 3.98 (*q*, J = 5.2, H−C(2′)); 3.90 (*m*, H−C(3′), H−C(4′)); 3.74 (*s*, 2 MeO); 3.20 (*m*, 2 H−C(5′)). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 161.71 (*d*, J = 246.1, C(4)); 161.53 (*d*, J = 246.4, C(2)); 158.04, 144.87, 135.63, 135.54, 129.71 ((MeO)$_2$*Tr*); 129.11 (*dd*, J = 9.8, 6.1, C(6)); 127.76, 127.72, 126.63 ((MeO)$_2$*Tr*); 124.28 (*dd*, J = 13.1, 3.2, C(1)); 113.13 ((MeO)$_2$*Tr*); 111.12 (*dd*, J = 20.8, 3.2, C(5)); 103.69 (*t*, J = 25.9, C(3)); 85.40 ((MeO)$_2$*Tr*); 82.25 (C(1′)); 78.10 (C(4′)); 76.36 (C(2′)); 70.93 (C(3′)); 63.77 (C(5′)); 54.98 (MeO). ESI-MS: 547.2 ([M − H]$^-$).

*2-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-1-(2,4-difluorophenyl)-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranose* (**42**). As described above for **45**, with **41** (2.35 g, 4.3 mmol), anh. THF/pyridine 1:1 (40 ml), $AgNO_3$ (870 mg, 5.2 mmol), and 1M ^{t}BuMe$_2$SiCl (6 ml, 6 mmol). FC (CH_2Cl_2): slower-migrating isomer **42** (1.01 g, 35.5%). Colourless foam. TLC (CH_2Cl_2): R_f 0.18. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.59 (*q*, J = 6.7, H−C(6)); 7.45–6.82 (*m*, 15 arom. H, H−C(3), H−C(5)); 4.96 (*d*, J = 5.7, OH−C(3′)); 4.84 (*d*, J = 5.7, H−C(1′)); 4.02 (*m*, H−C(3′), H−C(4′)); 3.93 (*t*, J = 5.2, H−C(2′)); 3.74 (*s*, 2 MeO); 3.21 (*m*, 2 H−C(5′)); 0.79 (*s*, ^{t}BuSi); −0.05, −0.11 (2*s*, Me$_2$Si). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 161.69 (*d*, J = 246.3, C(2)); 161.51 (*d*, J = 246.5, C(4)); 158.04, 144.87, 135.46, 135.41, 129.71, 129.68 ((MeO)$_2$*Tr*); 129.07 (*dd*, J = 11.6, 7.8, C(6)); 127.73, 127.62 ((MeO)$_2$*Tr*); 123.85 (*dd*, J = 15.7, 3.4, C(1)); 113.12 ((MeO)$_2$*Tr*); 111.12 (*dd*, J = 20.8, 3.4, C(5)); 103.61 (*t*, J = 26.1, C(3)); 85.45 ((MeO)$_2$*Tr*); 82.94 (C(1′)); 78.79 (C(4′)); 77.20 (C(2′)); 70.20 (C(3′)); 63.51 (C(5′)); 54.97 (MeO); 25.50 (*Me*$_3$CSi); 17.78 (Me$_3$*C*Si); −4.93, −5.47 (Me$_2$Si). ESI-MS: 661.4 ([M − H]$^-$).

*3-*O*-[(*tert*-Butyl)dimethylsilyl]-1-deoxy-1-(2,4-difluorophenyl)-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranose* (**43**) was obtained from the reaction described above as the faster-migrating isomer (0.89 g, 31.4%). Colourless foam. TLC (CH_2Cl_2): R_f 0.21. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.61 (*q*, J = 8.2, H−C(6)); 7.45–6.84 (*m*, 15 arom. H, H−C(3), H−C(5)); 4.96 (*d*, J = 6.6, OH−C(2′)); 4.83 (*d*, J = 5.6, H−C(1′)); 4.02 (*m*, H−C(3′)); 3.94 (*m*, H−C(2′)); 3.73 (*s*, 2 MeO); 3.29 (*m*, H−C(4′)); 3.15 (*m*, 2 H−C(5′)); 0.76 (*s*, ^{t}BuSi); −0.01, −0.08 (2*s*, Me$_2$Si). ^{13}C-NMR (67.9 MHz, (D_6)DMSO): 161.65 (*d*, J = 246.1, C(2)); 161.49 (*d*, J = 246.3, C(4)); 158.05, 144.67, 135.46, 135.42, 129.67 ((MeO)$_2$*Tr*); 129.13 (*dd*, J = 11.9, 8.0, C(6)); 127.71, 127.63, 126.63 ((MeO)$_2$*Tr*); 123.82 (*dd*, J = 12.4, 3.9, C(1)); 113.11 ((MeO)$_2$*Tr*); 111.30 (*dd*, J = 24.6, 3.7, C(5)); 103.59 (*t*, J = 26.7, C(3)); 85.46 ((MeO)$_2$*Tr*); 82.52 (C(1′)); 78.29 (C(4′)); 75.94 (C(2′)); 72.63 (C(3′)); 63.21 (C(5′)); 54.97 (MeO); 25.64 (*Me*$_3$CSi); 17.82 (Me$_3$*C*Si); −4.59, −5.21 (Me$_2$Si). ESI-MS: 661.4 ([M − H]$^-$).

*2-*O*-[(*tert*-Butyl)dimethylsilyl)-1-deoxy-1-(2,4-difluorophenyl)-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribofuranose 3-(2-Cyanoethyl Diisopropylphosphoramidite)* (**7**). As described above for **2**, with **42** (250 mg, 0.38 mmol), MeCN (12 ml), collidine (500 µl, 3.8 mmol), 1-methyl-1*H*-imidazole (15 µl, 0.19 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (128 µl, 0.57 mmol) (15 min at 0° and 1 h at r.t.). FC (hexane/AcOEt 4:1): **7** (202 mg, 62.1%, diastereoisomer mixture). Colourless foam. TLC (hexane/AcOEt 4:1): R_f 0.36, 0.34. ^{1}H-NMR (270 MHz, (D_6)DMSO): 7.67 (*m*, H−C(6)); 7.53–6.77 (*m*, 13 arom. H, H−C(3), H−C(5)); 5.14, 5.12 (2*d*, J = 7.7, 6.8 Hz, H−C(1′)); 4.23 (*m*, H−C(2′), H−C(3′), H−C(4′)); 3.80, 3.79 (2*s*, 2 MeO); 3.54 (*m*, 2 H−C(5′)); 1.17 (*m*, 2 Me$_2$CH); 0.82 (*s*, ^{t}BuSi); −0.05, −0.17 (2*s*, Me$_2$Si). ^{31}P-NMR (161.98 MHz, $CDCl_3$): 150.70, 148.85; ratio 1:3.8. ESI-MS: 863.6 ([M + H]$^+$).

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