Oligonucleotides Containing Novel 4′-*C*- or 3′-*C*-(Aminoalkyl)-Branched Thymidines[1])

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

The synthesis of four novel 3′-*C*-branched and 4′-*C*-branched nucleosides and their transformation into the corresponding 3′-*O*-phosphoramidite building blocks for automated oligonucleotide synthesis is reported. The 4′-*C*-branched key intermediate **11** was synthesized by a convergent strategy and converted to its 2′-*O*-methyl and 2′-deoxy-2′-fluoro derivatives, leading to the preparation of novel oligonucleotide analogues containing 4′-*C*-(aminomethyl)-2′-*O*-methyl monomer **X** and 4′-*C*-(aminomethyl)-2′-deoxy-2′-fluoro monomer **Y** (*Schemes 2* and *3*). In general, increased binding affinity towards complementary single-stranded DNA and RNA was obtained with these analogues compared to the unmodified references (*Table 1*). The presence of monomer **X** or monomer **Y** in a 2′-*O*-methyl-RNA oligonucleotide had a negative effect on the binding affinity of the 2′-*O*-methyl-RNA oligonucleotide towards DNA and RNA. Starting from the 3′-*C*-allyl derivative **28**, 3′-*C*-(3-aminopropyl)-protected nucleosides and 3′-*O*-phosphoramidite derivatives were synthesized, leading to novel oligonucleotide analogues containing 3′-*C*-(3-aminopropyl)thymidine monomer **Z** or the corresponding 3′-*C*-(3-aminopropyl)-2′-*O*,5-dimethyluridine monomer **W** (*Schemes 4* and *5*). Incorporation of the 2′-deoxy monomer **Z** induced no significant changes in the binding affinity towards DNA but decreased binding affinity towards RNA, while the 2′-*O*-methyl monomer **Z** induced decreased binding affinity towards DNA as well as RNA complements (*Table 2*).

1. Introduction. – A large number of chemically modified oligonucleotides (ONs) have been synthesized and investigated to improve the binding affinity towards complementary RNA and the stability towards nucleases [1b][2]. As important examples, 2′-deoxy-2′-fluoro-modified ONs [3][4], and 2′-*O*-alkylated ONs, including 2′-*O*-methyl derivatives, have shown increased binding affinity towards RNA [5–8]. The increased binding affinity towards RNA has been attributed to the tendency of these nucleotides to adopt a C(3′)-*endo* conformation, giving thermally stable A-type duplexes [9].

ONs containing 4′-*C*-substituted nucleotide monomers have likewise been reported to show, in general, minor decreases in binding affinity towards RNA and small increases in binding affinity towards DNA [10–15], presumably originating from the C(2′)-*endo*-like conformation preferentially adopted by these nucleotides [16], in analogy with what is found in B-type DNA·DNA duplexes [9]. Furthermore, ONs containing 2′-*O*-alkylated or 4′-*C*-substituted nucleotides have shown increased

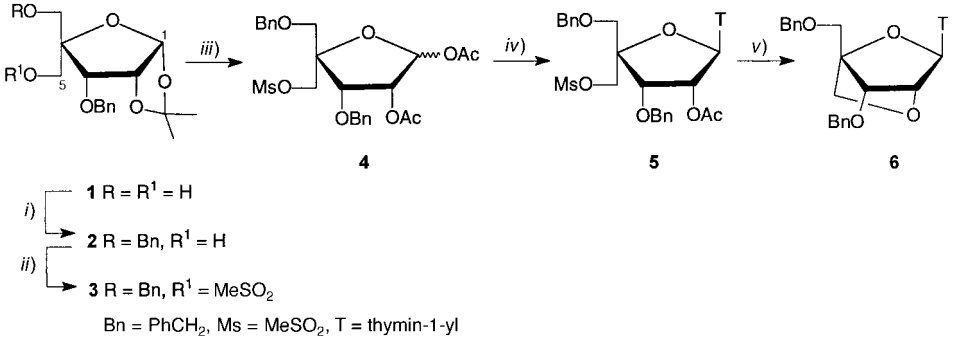
[1]) Part of this work has been published in a preliminary form [1a].

resistance towards enzymatic degradation, most pronounced for ONs containing modified nucleotides with an ionizable amino group in the sugar moiety, *e.g.*, 2′-*O*-(aminopropyl)- [17] and 4′-*C*-(aminoalkyl)-substituted thymidines [12][13][15]. Based on the results described above, we decided to evaluate the thermal stability of duplexes formed between ONs containing *2′,4′-dimodified* nucleotide monomers and complementary RNA and DNA. Thus, in an attempt to maximize the binding affinity of ONs containing 4′-*C*-substituted nucleotides towards RNA and to increase the nuclease stability, we decided to synthesize and evaluate ONs containing 4′-*C*-(aminomethyl)-2′-*C*-methoxy- and 4′-*C*-(aminomethyl)-2′-fluoro-modified thymidines.

In addition, we and others have been interested in obtaining modified ONs with a DNA-selective binding, *e.g.* for diagnostics applications. Thus, 2′-β-ethynyl-modified ONs were recently shown to preferentially hybridize with complementary DNA [18] which was explained by the tendency of these modified nucleotides to adopt a C(2′)-*endo*-type conformation. Likewise, we have reported the synthesis and evaluation of ONs containing 3′-*C*-(3-hydroxypropyl)- [19] and 3′-*C*-allyl-branched [20] thymidines showing better binding affinities towards DNA than towards RNA. However, this was not observed for ONs containing similar nucleotides with a C_1 3′-*C*-branch, *e.g.* 3′-*C*-(hydroxymethyl) [21], 3′-*C*-methyl [22][23], and 3′-*C*-(aminomethyl) [23]. A 3′-*C*-branch is expected to be oriented in a pseudoequatorial position, driving the sugar pucker towards a C(2′)-*endo* conformation. An explanation for the results described above could be that a larger C_3 3′-*C*-branch is not as well accommodated in the DNA · RNA duplex as a smaller C_1 branch. Herein we report the synthesis and evaluation of ONs containing 3′-*C*-(3-aminopropyl)thymidine and 3′-*C*-(3-aminopropyl)-2′-*O*,5-dimethyluridine in an attempt to improve the thermal stability of duplexes formed between ONs containing 3′-*C*-branched monomers and complementary DNA.

2. Results and Discussion. – 2.1. *2′-Substituted 4′-*C*-(Aminomethyl) Nucleosides and ONs.* For the synthesis of 4′-*C*-(aminomethyl)-2′-*C*-methoxy- and 4′-*C*-(aminomethyl)-2′-fluoro-derivatized thymidine nucleosides, a strategy starting from the known 3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-1,2-di-*O*-isopropylidene-β-L-lyxofuranose (**2**), which can be synthesized from 3-*O*-benzyl-4-*C*-(hydroxymethyl)-1,2-di-*O*-isopropylidene-β-L-lyxofuranose (**1**) *via* regioselective benzylation [24][25], was chosen (*Scheme 1*). In an attempt to improve the regioselectivity of the monobenzylation, furanose **1** was benzylated by the dibutyltin oxide method [26][27]. However, equal amounts of two dibenzylated regioisomers were obtained in moderate yields, and the reported [24][25] regioselective monobenzylation method was therefore used. It was decided to use an azido substituent as a group which could be converted to an amino functionality later at an appropriate step in the synthesis. For the introduction of this azido group, furanose **2** was mesylated under standard conditions to give furanose **3** in 92% yield, as reported [28]. However, treatment of **3** with NaN_3 in hot DMF was unsuccessful resulting in complete recovery of **3** that might be ascribed to the 'neopentylic' character of the mesyloxy group of **3** and its position on the concave face of the oxabicyclo[3.3.0]octane system [24]. Instead, both by isopropylidene group hydrolysis in 80% AcOH/H_2O followed by basic acetylation, and by direct one-pot acidic acetolysis [29], the 1,2-di-*O*-acetyl derivative **4** was obtained in 54% yield. Stereoselective reaction with silylated thymine [30][31] afforded in 75% yield the nucleoside **5**, which was heated under

Scheme 1



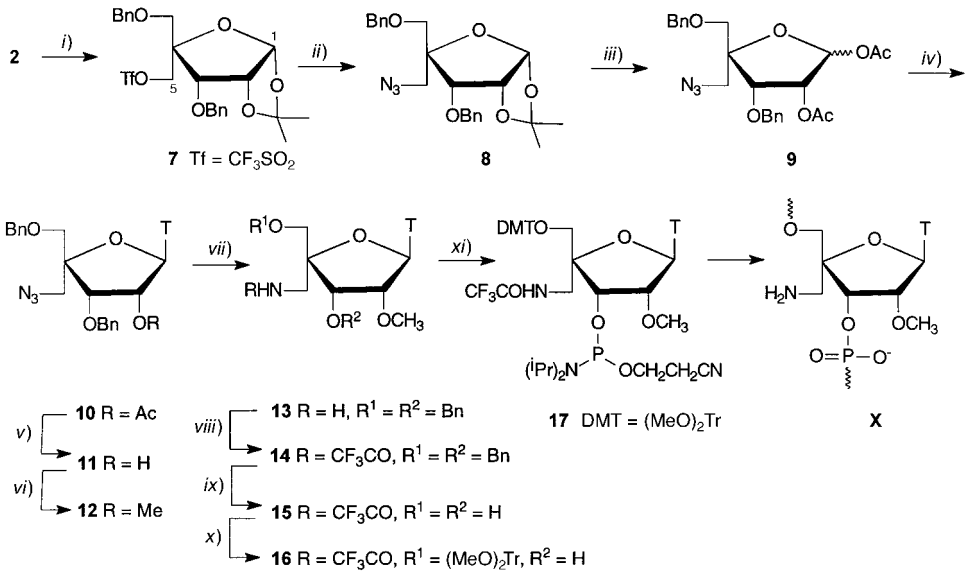
i) BnBr, NaH, DMF. *ii*) MsCl, Py. *iii*) 1. 50% AcOH; 2. Ac_2O, Py *or* AcOH/Ac_2O/conc. H_2SO_4 soln. 100 : 10 : 0.1. *iv*) Thymine, *N*,*O*-bis(trimethylsilyl)acetamide (BSA), $Me_3SiOSO_2CF_3$, MeCN. *v*) $LiEt_3BH$, THF.

reflux in DMF with NaN_3 to give several products of which none was identified. This disappointing result is consistent with another report describing the reaction of 4′-*C*-[(mesyloxy)methyl]thymidine derivatives with LiN_3 to give the desired 4′-*C*-(azidomethyl)nucleoside in only low yields [32]. At this point, it was realized that it was impossible to use the mesyloxy group as a leaving group with azide as the nucleophile. Instead, we used hydride as the nucleophile in an attempt to obtain a 4′-*C*-methylnucleoside. However, treatment of nucleoside **5** with lithium triethylborohydride yielded the 2,5-dioxabicyclo[2.2.1]heptane locked nucleic acid (LNA) monomer **6** [25][33] in 86% yield, presumably *via* reduction of the acetate followed by intramolecular attack of the resulting alkoxide on the 'neopentylic' C-atom.

In an attempt to introduce the azido group while taking advantage of the excellent leaving-group ability of the triflate ion, the 4-*C*-(triflyloxy)methyl derivative **7** was synthesized using standard chemistry and was reacted with NaN_3 in DMF at 60° to give furanose **8** in 59% yield (two steps) (*Scheme 2*). Acidic hydrolysis and basic acetylation (→ **9**) followed by coupling with silylated thymine as described above (→ **10**) gave, after deacetylation, nucleoside **11** in 83% yield from **8**. To obtain the 2′-*O*-methyl derivative **12**, nucleoside **11** was chemoselectively *O*-methylated in 90% yield by the reaction conditions described by *Wang* and *Seifert* [12]. We have successfully performed methylation of 3′-*C*-allyl-3′,5′-di-*O*-benzyl-5-methyluridine under similar conditions [34]. The structure of **12** was verified by ^{1}H- and ^{13}C-NMR data (NH of thymine at δ 9.35 and $CH_3O-C(2')$ at δ 59). Reduction of the azido group with *Lindlar* catalyst (→ **13**) followed by trifluoroacetylation of the resulting amino group afforded nucleoside **14** in 73% yield from **12**. Debenzylation to give diol **15** in 92% yield followed by dimethoxytritylation with $(MeO)_2TrCl$ to give the 5′-*O*-(4,4′-dimethoxytrityl)-protected analogue **16** in 91% yield, and subsequent phosphitylation afforded the phosphoramidite derivative **17** in 43% yield.

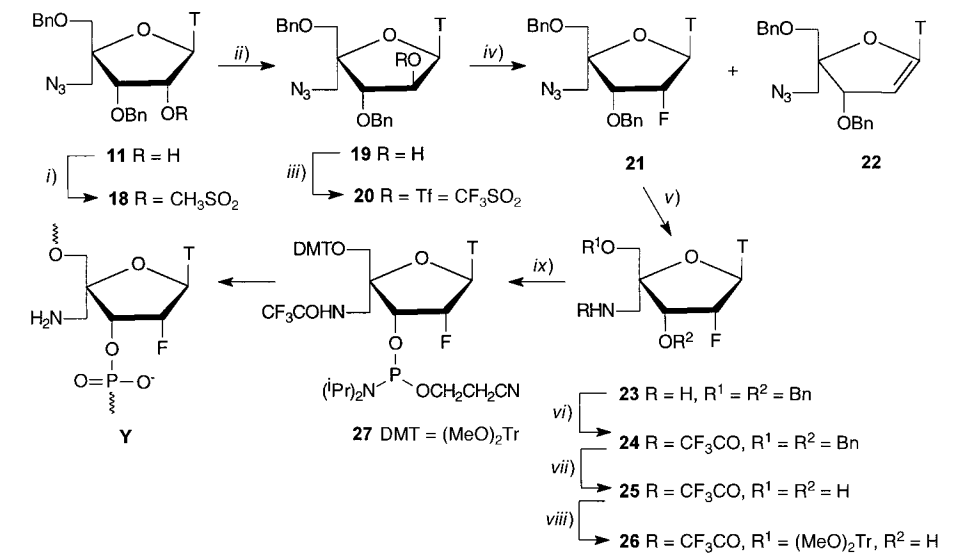
To obtain the analogous 4′-*C*-(aminomethyl)-2′-deoxy-2′-fluoro phosphoramidite derivative **27**, the configuration at C(2′) of nucleoside **11** was inverted by the anhydro approach [35]. Thus, mesylation of L-*lyxo*-configured **11** followed by reaction with aq. base afforded the L-*xylo*-configured nucleoside **19** in 94% yield from **11** (*Scheme 3*). Several attempts to prepare the 2′-fluoronucleoside **21** directly from **19** with

Scheme 2



i) $(CF_3SO_2)_2O$, Py, CH_2Cl_2. *ii*) NaN_3, DMF. *iii*) 1. 50% AcOH; 2. Ac_2O, Py. *iv*) Thymine, BSA, $Me_3SiOSO_2CF_3$, MeCN. *v*) NH_3, MeOH. *vi*) MeI, NaH, THF. *vii*), H_2, *Lindlar* catalyst, EtOH. *viii*) CF_3COOEt, Et_3N, CH_2Cl_2. *ix*) H_2, 5% Pd/C, EtOH. *x*) $(MeO)_2TrCl$, Py. *xi*) $NC(CH_2)_2OP(Cl)N^iPr_2$, $^iPr_2EtN$, CH_2Cl_2.

Scheme 3



i) $MeSO_2Cl$, Py. *ii*) NaOH, EtOH, H_2O. *iii*) Tf_2O, 4-(dimethylamino)pyridine (DMAP), Py, CH_2Cl_2. *iv*) Bu_4NF, THF. *v*) H_2, *Lindlar* catalyst, dioxane, EtOH. *vi*) CF_3COOEt, NEt_3, CH_2Cl_2. *vii*) H_2, 5% Pd/C, EtOH. *viii*) $(MeO)_2TrCl$, Py. *ix*) $NC(CH_2)_2OP(Cl)N^iPr_2$, $^iPr_2EtN$, CH_2Cl_2.

(diethylamino)sulfur trifluoride (DAST) [36][37] and different temperatures and solvents (CH_2Cl_2 or DMF) were unsuccessful. An attempt to react tris(dimethylamino)sulfur (trimethylsilyl)difluoride (TASF) [38][39] with the (trifluoromethyl)sulfonyl derivative **20**, synthesized in 77% yield from **19** under standard conditions, was likewise unsuccessful. However, under strictly anhydrous conditions, 2′-fluoronucleoside **21** was obtained with dried 'anhydrous' tetrabutylammonium fluoride (Bu_4NF) [40][41]. Thus, nucleoside **20** was reacted with 10 equiv. of Bu_4NF in anh. THF to give **21** in 37% yield and ketene *N,O*-acetal **22** in 30% yield, the latter formed *via trans*-elimination of the 2′-*C*-(triflyloxy) group. The structure of **22** was verified by MS and NMR and by comparison with other structurally similar 1′,2′-unsaturated compounds [42][43]. This type of elimination reaction has been reported earlier [44], but the analogous reaction of the 4′-*C*-unbranched 2′-*O*-(triflyloxy) derivative of **20** afforded exclusively the desired 2′-fluoro product in a very high yield [45]. The *gauche* effect of the 2′-*O*-(triflyloxy) group positioned at the α-face of the furanose ring should drive the sugar pucker towards a C(2′)-*endo*-like conformation in both **20** and its 4′-*C*-unbranched analogue. However, also the 4′-*C*-(azidomethyl) group shifts the pentofuranose sugar pucker towards a C(2′)-*endo* conformation. Accordingly, the ^3J(H–C(1′), H_β–C(2′)) coupling constant of 4.3 Hz observed for **20**, corresponding to a H–C(1′)–C(2′)–H_β torsion angle of *ca.* 45°, as obtained from a simplified *Karplus* equation [46], is in accordance with a C(2′)-*endo*-like conformation. Thus, H–C(1′) and the 2′-*C*-(triflyloxy) group exist in a *trans*-diaxial geometry advantageous for *E*2 elimination, offering an explanation for the nearly equal amounts of substitution and elimination observed in the reaction. Following similar synthetic procedures as for the 2′-*O*-methylnucleosides (**12** → **16**, *Scheme 1*), 4′-*C*-(azidomethyl)-2′-deoxy-2′-fluoro derivative **21** was reduced to give the 4′-*C*-(aminomethyl)nucleoside **23**, trifluoroacetylated (→ **24**), debenzylated (→ **25**) and 5′-*O*-[$(MeO)_2$Tr]-protected to give **26** in an overall yield of 46% from **21**. Nucleoside **26** was further transformed into the 3′-*O*-phosphoramidite derivative **27** in 76% yield.

The phosphoramidites **17** and **27** were incorporated into ONs as monomers **X** and **Y**, respectively, on an automated DNA synthesizer. The stepwise coupling efficiency obtained for **17** was only 40% when 1*H*-tetrazole was used as activator (2 × 12 min coupling). With pyridinium hydrochloride as activator [47][48], the coupling efficiency was > 90% (10 min coupling). The ONs were deprotected by heating (55° for 12 h) in aqueous NH_3 solution followed by reversed-phase cartridge purification. Analysis by capillary gel electrophoresis verified the purity to be > 90%, and the composition of the ONs was confirmed by MALDI-MS. The phosphoramidite **17** was incorporated into three types of ONs: 14-mer oligothymidylates **II** – **IV**, mixed 9-mer sequences **VI** – **VIII**, and a sequence **XIII** containing 2′-*O*-methyl-modified ribonucleotides instead of unmodified 2′-deoxynucleotides. The hybridization properties of these **X**-modified oligonucleotides and of their reference strands **I**, **V**, and **XII** towards complementary DNA and RNA were measured both in a medium salt buffer and in a high salt buffer (*Table 1*).

Likewise, oligonucleotides **IX** – **XI** and **XIV** (*Table 1*), containing the monomer **Y**, were synthesized with a stepwise coupling efficiency for the amidite **27** of 80% with pyridinium hydrochloride as activator (10 min coupling). *Krug et al.* [49] have reported that when 2′-deoxy-2′-fluorouridine is incorporated as the 3′-terminal residue of an ON,

Table 1. *Melting Experiments of Oligonucleotides Containing Monomers* **X** *and* **Y**[a])

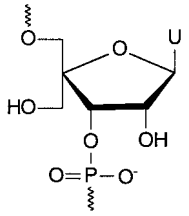
		Complementary ssDNA T_m/° or ΔT_m/° per mod. [b])		Complementary ssRNA T_m/° or ΔT_m/° per mod. [b])	
		[Na^+]: 150 mM[c])	[Na^+]: 750 mM[d])	[Na^+]: 150 mM[c])	[Na^+]: 750 mM[d])
I	5′-T_{14}	33.0	n.d.	31.0	n.d.
II	5′-T_7**X**T_6	−4.0	n.d.	−4.0	n.d.
III	5′-T_6**X**T**X**T_5	−3.0	n.d.	−3.0	n.d.
IV	5′-T_{12}**X**T	±0.0	n.d.	−1.0	n.d.
V	5′-G_dT$G_d$$A_dTA_dTG_d$$C_d$	31.0	36.5	29.7	34.0
VI	5′-G_dT$G_d$$A_d$**X**$A_dTG_d$$C_d$	+1.5	−0.5	+2.5	+2.5
VII	5′-G_dT$G_d$$A_d$**X**$A_d$**X**$G_d$$C_d$	+1.5	−0.3	+1.3	+1.3
VIII	5′-G_d**X**$G_d$$A_d$**X**$A_d$**X**$G_d$$C_d$	−0.3	−1.7	+0.8	+1.0
IX	5′-G_dT$G_d$$A_d$**Y**$A_dTG_d$$C_d$	+1.5	−0.5	+0.5	±0.0
X	5′-G_dT$G_d$$A_d$**Y**$A_d$**Y**$G_d$$C_d$	+1.8	±0.0	+1.5	+0.8
XI	5′-G_d**Y**$G_d$$A_d$**Y**$A_d$**Y**$G_d$$C_d$	+0.3	−0.8	+1.0	+0.7
XII	5′-$G^m$$U^m$$G^m$$A^m$$U^m$$A^m$$U^m$$G^m$C	31.5	37.0	42.5	50.5
XIII	5′-G^m**X**$G^m$$A^m$**X**$A^m$**X**$G^m$C	24.0	28.0	34.5	40.5
XIV	5′-G^m**Y**$G^m$$A^m$**Y**$A^m$**Y**$G^m$C	19.0	23.0	29.0	37.0

[a]) **X** = 1-[4-*C*-(aminomethyl)-2-*O*-methyl-α-L-lyxofuranosyl]thymine unit; **Y** = 1-[4-*C*-(aminomethyl)-2-deoxy-2-fluoro-α-L-lyxofuranosyl)thymine unit, G^m = 2′-*O*-methylguanosine unit, U^m = 2′-*O*-methyluridine unit, A^m = 2′-*O*-methyladenosine unit. [b]) T_m is shown for the oligonucleotides **I**, **V**, and **XII**–**XIV**. ΔT_m is shown for oligonucleotides **II**–**IV** with **I** as reference and for oligonucleotides **VI**–**XI** with **V** as reference. T_m = melting temperature determined as the maximum of the first derivative of the absorbance *vs.* temperature curve; ΔT_m/° per mod. = change in T_m per modification compared to the unmodified reference strand; n.d. = not determined. [c]) Measured at 260 nm in medium salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 140 mM sodium chloride, pH 7.1. [d]) Measured at 260 nm in high salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 740 mM sodium chloride, pH 7.1.

it is converted to arabinosyluracil *via* internal nucleophilic attack from the pyrimidine nucleobase and ring opening of the 2,2′-anhydro intermediate during the deprotection step with hot aqueous NH_3 solution. However, when 2′-deoxy-2′-fluorouridine was incorporated at other positions in an ON, a similar conversion to arabinosyluracil was not observed which is consistent with results reported by others [50]. A similar kind of degradation was reported for oligophosphoramidates containing 2′-deoxy-2′-fluorouridine monomers [51]. Furthermore, *Kawasaki et al.* [3] reported a significant degradation of 2′-deoxy-2′-fluoropyrimidine nucleosides under the usual ON deprotection conditions. They suggested the alternative use of methanolic NH_3 solution for 24 h at room temperature for ON deprotection. However, when oligonucleotide **XI** was deprotected under the latter conditions, MALDI-MS showed that *ca.* 50% of the ON products still contained the trifluoroacetyl protecting group. Furthermore, the N^2-isobutyryl protecting group of the guanine bases was not removed. Substitution of methanolic NH_3 with aqueous NH_3 solution did induce removal of the trifluoroacetyl protecting group after 24 h, but only *ca.* 50% of the N^2-isobutyryl protecting groups. Therefore, the usual deprotecting conditions (aqueous NH_3 solution at 55° for 12 h) were used in the present study. In fact, MALDI-MS did not indicate any kind of degradation of the ONs, as further verified by capillary gel electrophoresis showing the ONs to be > 90% pure. The MALDI-MS data were determined with an experimental error of less than 0.4 Da with internal standards. The hybridization properties of the

modified oligonucleotides **IX**–**XI** and **XIV** and of their reference strands **V** and **XII** towards complementary DNA and RNA were measured both in a medium salt buffer and in a high salt buffer (*Table 1*).

Upon single and two-fold incorporation of 2′-*O*-methyl monomer **X** in the middle of a 14-mer oligothymidylate (see ONs **II** and **III**, resp.), the stability of the duplexes with DNA and RNA was significantly decreased (ΔT_m/mod. $= -3$ and $-4°$) compared to the unmodified reference strand **I** when measured in a medium salt buffer, whereas the presence of a single **X** towards the 3′-end of sequence **IV** had no significant effect on the binding affinity towards complementary DNA and RNA. These results are consistent with results obtained with ONs containing 4′-*C*-(hydroxymethyl)uridine [52], although this analogue (see **U**) displayed even lower binding affinity towards complementary DNA (ΔT_m/mod. $= -8°$ for the sequence 5′-T_7**U**T_6). Due to the disappointing melting results, the hybridization properties of ONs **II**–**IV** were not evaluated in a high salt buffer.



U

More encouraging results were obtained when monomers **X** and **Y** were incorporated into 9-mer mixed oligonucleotide sequences (*Table 1*, **VI**–**XI**). Thus, the presence of a single **X** or **Y** (ONs **VI** and **IX**) or of two **X** or **Y** (ONs **VII** and **X**) in the 9-mer caused an increase (ΔT_m/mod. $\approx +1.5°$) in the stability of the resulting duplex with DNA when measured in a medium salt buffer. Incorporation of monomer **X** three times (ON **VIII**) caused a minor decrease (ΔT_m/mod. $= -0.3°$) in the thermal stability of the resulting duplex with DNA compared with the reference strand **V**, while the presence of **Y** in the same sequence (ON **XI**) caused a small increase (ΔT_m/mod. $= +0.3°$). When the corresponding ONs containing 2′-*O*-methyl derivative **X** (ONs **VI**–**VIII**) were hybridized with complementary RNA in a medium salt buffer, increases in the thermal stability of the resulting duplexes (ΔT_m/mod. $= +2.5$, $+1.3$, and $+0.8°$) were observed. Similar results were obtained with ONs containing the 2′-deoxy-2′-fluoro derivative **Y** though the presence of **Y** in the singly modified ON **IX** caused a significant smaller increase in T_m compared to **VI** containing the 2′-*O*-methyl monomer **X**. In relation to the main purpose of this work, namely evaluation of the possibility of maximizing the thermal affinity of *C*-branched ONs, comparison with results obtained with the 4′-*C*-(hydroxymethyl)uridine monomer **U** appeared relevant. In the mixed-sequence 9-mer context, monomer **U** in general induced decreased T_m values compared to the reference ON **V** with both DNA and RNA complements (ΔT_m/mod. against DNA $= 0$, -2.0 and $-2.0°$ and ΔT_m/mod. against RNA $= +2.0$, 0 and $-0.3°$ for the 9-mer sequences containing one, two or three **U** monomers, respectively). It is thus evident that the combined exchange of the HO–C(2′) and

the $HOCH_2-C(4')$ groups with a $F-C(2')$/$MeO-C(2')$ group and a $NH_2CH_2-C(4')$ group leads to increased thermal stability of duplexes with both DNA and RNA complements. The apparent sequence dependence on the influence of the different modifications on the thermal stability of the resulting duplexes stresses the importance of comparing only in identical sequence contexts and excludes direct comparison of the effect induced by, *e.g.*, monomers **X** and **U** with effects reported in the literature for, *e.g.*, 2′-*O*-alkyl-substituted ONs [5–8] or 4′-*C*-(aminomethyl)-modified ONs [23].

For ONs **VI**–**VIII** containing the 2′-*O*-methyl monomer **X**, ΔT_m/mod. for duplexes with DNA as well as RNA was reduced with increasing number of **X** monomers incorporated (medium salt buffer). The same tendency, though less pronounced, could be seen for ONs **IX**–**XI** containing the 2′-deoxy-2′-fluoro monomer **Y** when hybridized with complementary DNA in a medium salt buffer. The 2′-*O*-methyl group and the 2′-fluoro substituent both direct the sugar pucker towards a C(3′)-*endo* conformation (indicated by $^3J(H-C(1'), H-C(2'))$ values of *ca.* 1.5–4.5 Hz), which in general is expected to induce an increased thermal stability of duplexes with an RNA complement. However, compared to other 2′-*O*-methyl- and 2′-deoxy-2′-fluorouridines, 2′-modified 4′-*C*-(aminomethyl) nucleotides **X** and **Y** probably display a less pronounced tendency towards adopting a C(3′)-*endo*-like conformation, as indicated by the relatively high $^3J(H-C(1'), H-C(2'))$ values. Thus, the increase in stability of the duplexes **VI**, **VII**, and **IX**–**XI** with DNA is, most likely, caused by a reduction in the electrostatic repulsion between phosphate moieties due to the presence of the positively charged aminomethyl group under the experimental conditions used. To confirm an effect of the protonated aminomethyl group, the duplex stabilities were measured under conditions of high ionic strength ($[Na^+] = 750$ mM, *Table 1*). As anticipated, the stabilities of the duplexes involving ONs **VI**–**VIII** containing monomer **X** or ONs **IX**–**XI** containing monomer **Y** and complementary DNA were decreased relative to the stability of the duplex involving the unmodified reference strands **V** under high ionic strength conditions. This confirms the positive effect of the protonated aminomethyl group regarding duplex stability with complementary DNA. However, for ONs **VI** and **VII** in experiments towards complementary RNA, the presence of the positively charged aminomethyl group does not seem to affect the duplex stability.

Due to the larger *gauche* effect of the 2′-F-atom compared to the 2′-*O*-methyl group, we anticipated that substitution of the 2′-*O*-methyl monomer **X** with the 2′-deoxy-2′-fluoro monomer **Y** would cause an increase in binding affinity towards RNA. However, comparable binding affinities towards RNA were obtained for ONs **VII** and **VIII** containing 2′-*O*-methyl monomer **X** and ONs **X** and **XI** containing 2′-deoxy-2′-fluoro monomer **Y** ($[Na^+] = 150$ mM). When the hybridization affinities were measured under conditions of high ionic strength, the increases in binding affinity towards RNA for ONs **IX**–**XI** containing **Y** were somewhat reduced compared to the corresponding oligonucleotides **VI**–**VIII** containing **X**.

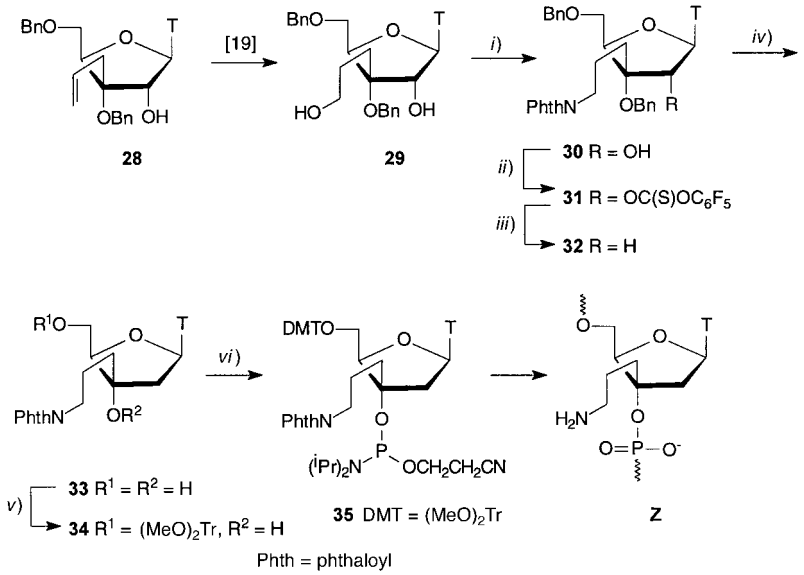
We also evaluated the effect of the presence of the 4′-*C*-(aminomethyl) group in an otherwise uniformly modified 2′-*O*-methyl-RNA ON. For the 2′-*O*-methyl-RNA reference strand **XII** (*Table 1*), containing uracil as nucleobase instead of thymine, no change in the thermal stability towards a DNA complement was observed compared to

the 2′-deoxy counterpart **V** confirming results reported earlier [5] [53]. However, when monomer **X** was incorporated three times in the 9-mer 2′-*O*-methyl-RNA strand (ON **XIII**), the thermal stability of the duplex with DNA was reduced significantly (ΔT_m value $-7.5°$). Thus, the positive effect of the 4′-*C*-(aminomethyl) substituent in the thymine monomer **X** observed earlier for ONs **VI**–**VIII** regarding binding affinity towards DNA is not seen with ON **XIII**. In fact, substitution of a thymine base with a uracil base generally results in a decrease in T_m of *ca.* 0.5° per substitution [54], which further emphasizes the detrimental effect of the 4′-*C*-(aminomethyl) group on the T_m value obtained for **XIII**. As expected, 2′-*O*-methyl-modified oligonucleotide **XII** showed increased binding affinity towards RNA (ΔT_m/2′-*O*-methylnucleotide $= +1.6°$) compared to the 2′-deoxy analog **V**. ON **XIII** showed significantly reduced binding affinity towards RNA in both salt buffers compared to the reference strand **XII**, but still increased binding affinity towards RNA compared to the completely unmodified 2′-deoxy analog **V**. Likewise, the 2′-deoxy-2′-fluoro monomer **Y** was incorporated in an otherwise 2′-*O*-methyl modified ON (**XIV**, *Table 1*). The negative effect of the presence of the 2′-deoxy-2′-fluoro monomer **Y** on the thermal stability of the 2′-*O*-methyl-RNA strand was even more pronounced than observed for the similar oligonucleotide containing **X** with resulting ΔT_m values of *ca.* $-13°$ for duplexes with complementary DNA and RNA. Based on these results, it appears that the incorporation of branched 4′-*C*-alkylnucleotide monomers in a 2′-*O*-methyl-RNA ON causes a detrimental disruption of the regular duplex structure.

2.2. *3′-C-(Aminoalkyl)-Substituted Nucleosides and ONs.* We have recently published the synthesis and evaluation of ONs containing 3′-*C*-(3-hydroxypropyl)thymidine [19]. We have also been interested in evaluating ONs containing the corresponding amino derivative, 3′-*C*-(3-aminopropyl)thymidine, which could potentially increase the stability of the DNA · DNA and DNA · RNA duplexes by lowering the electrostatic repulsion between the two anionic strands. Thus, 3′-*C*-(hydroxypropyl)nucleoside **29** was synthesized *via* hydroboration/oxidation of the 3′-*C*-allyl derivative **28** as reported earlier [19] (*Scheme 4*). Standard *Mitsunobu* reaction [55] afforded the phthaloyl(Phth)-protected primary-amino-substituted nucleoside derivative **30** contaminated with triphenylphosphine oxide. Several attempts to remove the phosphine oxide by-product, *e.g.*, trituration with toluene, precipitation in Et_2O and extensive column chromatography, failed. Impure **30** was, therefore, thioacylated to give 2′-*O*-[(pentafluorophenoxy)thiocarbonyl]nucleoside **31** in 27% yield (two steps). Deoxygenation afforded the 2′-deoxynucleoside **32** in disappointing 25% yield (after extensive column-chromatographic purification and prep. TLC to remove tin impurities) and nucleoside **30** in 19% yield. Removal of the benzyl protecting groups of **32** by hydrogenation afforded the debenzylated diol **33** in only 18% yield. TLC showed the formation of several products upon addition of additional $Pd(OH)_2$/C during the reaction. Nevertheless, the small amount of diol **33** was 5′-*O*-$(MeO)_2$Tr-protected to give nucleoside **34**, which was subsequently phosphitylated to give the 3′-*O*-phosphoramidite building block **35** [20] in 23% yield (two steps).

The corresponding 3′-*C*-(3-aminopropyl)-2′-*O*-methylnucleoside derivative **42** was likewise synthesized and evaluated as a monomeric substitution in ONs. As the first step, by the same procedure as for the methylation of **11**, nucleoside **28** was methylated

Scheme 4

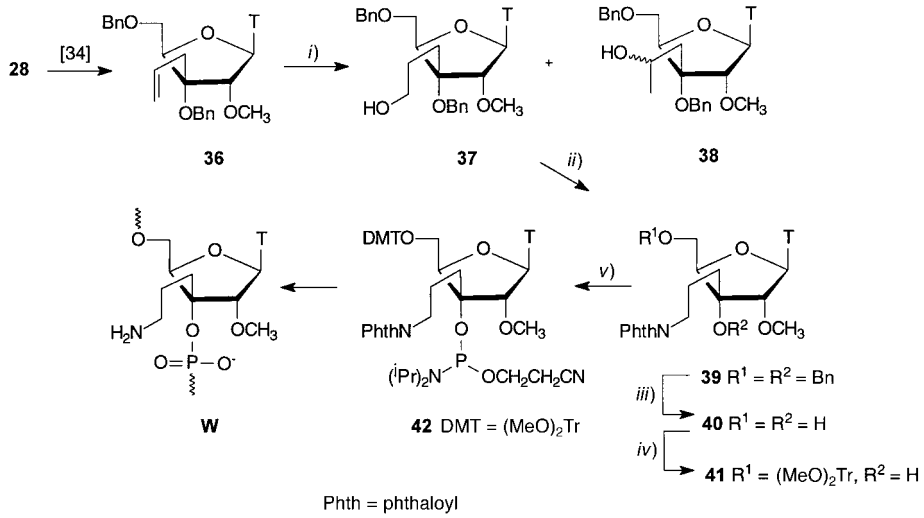


i) Phthalimide, PPh_3, diethyl diazenedicarboxylate (DEAD), THF. *ii*) $C_6F_5OC(S)Cl$, DMAP, CH_2Cl_2. *iii*) 2,2′-azobis[isobutyronitrile] (AIBN), Bu_3SnH, benzene. *iv*) H_2, 20% $Pd(OH)_2/C$, EtOH, dioxane. *v*) $(MeO)_2TrCl$, Py. *vi*) $NC(CH_2)_2OP(Cl)N^iPr_2$, $^iPr_2EtN$, CH_2Cl_2.

to give the 2′-*O*-methyl derivative **36** in 84% yield as reported earlier [34] (*Scheme 5*). Nucleoside **36** was subjected to a hydroboration/oxidation reaction sequence to afford the desired primary alcohol **37** in 54% yield. Furthermore, we isolated a mixture of the *Markovnikov* diastereoisomers **38** in 13% yield. *Mitsunobu* reaction of **37** with phthalimide [55] afforded the phthaloyl-protected derivative **39** in 20% yield in addition to a fraction contaminated with triphenylphosphine oxide. Subsequent debenzylation with BCl_3 afforded diol **40** whereupon derivative **41** was obtained by standard 5′-*O*-$(MeO)_2Tr$ protection in 65% yield (two steps). Eventual phosphitylation yielded the 3′-*O*-phosphoramidite derivative **42** in 33% yield after extensive column-chromatographic purification.

ONs containing 3′-*C*-(3-aminopropyl)thymidine monomer **Z** were synthesized from the phosphoramidite **35**. With pyridinium hydrochloride as activator and *t*-BuOOH [56] [57] as oxidizing agent, **35** was coupled in >95% stepwise yield (10 min coupling time). Standard deprotection with aq. NH_3 solution at 55° and cartridge purification afforded the ONs **XVI** and **XIX** (*Table 2*). The purity (>85%) and composition of the ONs was verified as described earlier for the ONs containing monomers **X** and **Y**. To avoid formation of ONs containing partially ring-opened phthaloyl functionalities under standard deprotection conditions, the use of methylamine for deprotection has been reported [17]. However, according to MALDI-MS this was not a problem for ONs **XVI** and **XIX**. Instead, a minor peak at $[M+54]^+$ in the MALDI-MS of oligomer **XVI** was seen, presumably originating from *Michael* addition of the amino group to acrylonitrile during deprotection. Similarly, ONs **XVII** and **XX**

Scheme 5



i) 1. BH_3 in 1,4-oxathiane, THF; 2. aq. NaOH soln., H_2O_2. *ii*) Phthalimide, PPh_3, DEAD, THF. *iii*) BCl_3 in hexane, CH_2Cl_2. *iv*) $(MeO)_2TrCl$, Py. *v*) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $^iPr_2EtN$, CH_2Cl_2.

containing monomer **W** were synthesized with a stepwise coupling efficiency for the phosphoramidite **42** of > 80% (10 min coupling) with pyridinium hydrochloride as activator and *t*-BuOOH as oxidizing agent. Deprotection, purification, capillary gel electrophoresis, and MALDI-MS were performed as described earlier to yield ONs **XVII** and **XX** with a purity of > 85%.

The hybridization of the modified ONs **XVI** and **XIX** containing the 3′-*C*-(3-aminopropyl) nucleoside monomer **Z** were evaluated in comparison to the reference strands **XV** and **XVIII**, respectively, in a medium salt buffer, and for duplexes involving a DNA complement furthermore in a high salt buffer (*Table 2*). Incorporation of 3′-*C*-

Table 2. *Melting Experiments of Oligonucleotides Containing Monomers* **Z** *and* **W**[a])

		Complementary ssDNA [Na^+] = 150 mM[b])		[Na^+] = 750 mM[c])		Complementary ssRNA [Na^+] = 150 mM[b])	
		T_m/°[d])	ΔT_m/° per mod.[d])	T_m/°[d])	ΔT_m/° per mod.[d])	T_m/°[d])	ΔT_m/° per mod.[d])
XV	5′-T_{14}	33.0	reference	42.5	reference	31.0	reference
XVI	5′-T_7**Z**T_6	33.0	±0.0	41.5	−1.0	28.5	−2.5
XVII	5′-T_7**W**T_6	23.5	−9.5	n.d.	n.d.	n.d.	n.d.
XVIII	5′-$G_dTG_dA_dTA_dTG_dC_d$	31.0	reference	36.5	reference	29.5	reference
XIX	5′-$G_dTG_dA_d$**Z**$A_dTG_dC_d$	31.5	+0.5	36.5	±0.0	25.5	−4.0
XX	5′-$G_dTG_dA_d$**W**$A_dTG_dC_d$	27.5	−3.5	n.d.	n.d.	26.0	−3.5

[a]) **Z** = 3′-*C*-(3-aminopropyl)thymidine unit; **W** = 3′-*C*-(3-aminopropyl)-2′-*O*-methyl-5-methyluridine unit. [b]) Measured at 260 nm in medium salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 140 mM sodium chloride, pH 7.1. [c]) Measured at 260 nm in high salt buffer: 1 mM EDTA, 10 mM sodium phosphate, 740 mM sodium chloride, pH 7.1. [d]) T_m = melting temperature determined as the maximum of the first derivative of the absorbance *vs.* temperature curve; ΔT_m/° per mod. = change in T_m per modification compared to the unmodified reference strand; n.d. = not determined.

(3-aminopropyl)thymidine monomer **Z** in the middle of an oligothymidylate (ON **XVI**) or in the middle of a mixed sequence 9-mer (ON **XIX**) induced no significant change in the thermal stability involving a DNA, while the presence of **Z** had a destabilizing effect on the duplexes involving complementary RNA (ΔT_m/mod. $= -2.5$ and $-4.0°$) when measured in a medium salt buffer. The hybridization results with DNA are in contrast to the results obtained with ONs containing 3′-*C*-(3-hydroxypropyl)thymidine [19] which showed small decreases in binding affinity towards DNA. An interaction between the positively charged amino group of **Z** and the anionic phosphate backbone could be an immediate explanation of the better hybridization results towards DNA obtained for ONs containing the 3′-*C*-(aminopropyl) branch. We, therefore, measured the T_m values of ONs **XVI** and **XIX** in a buffer with high salt concentration (*Table 2*). As the resulting changes in the T_m values for ONs **XVI** and **XIX** ($+8.5$ and $+5.0°$, resp.) are slightly smaller than for the reference strands **XV** and **XIII** ($+9.5$ and $+5.5°$, resp.), an electrostatic interaction between the amino group in **Z** and the phosphate backbone appears likely. Compared with the few reports describing ONs containing 3′-*C*-branched monomers, ONs containing the 3′-*C*-(aminopropyl)-branched monomer **Z** show quite promising hybridization properties towards DNA. Thus, in general, ONs containing 3′-*C*-(hydroxymethyl)- [21], 3′-*C*-methyl- [22][23], 3′-*C*-(aminomethyl)- [23], and 3′-*C*-allylnucleotide [20] monomers hybridize with complementary DNA with ΔT_m/mod. values of approximately 0 to $-1.0°$ under medium salt conditions.

The significant decrease in binding affinity of mid-modified ONs containing monomer **Z** towards RNA compared to DNA is noticeable (*Table 2*). Similar results were observed with ONs containing 3′-*C*-allylthymidine [20] or 3′-*C*-(3-hydroxypropyl)thymidine [19] and can be explained by the conformation of the sugar moiety. Thus, the presence of a 3′-β-substituent on the pentofuranose ring shifts the sugar pucker towards a C(2′)-*endo* type conformation through its preference to adopt a pseudo-equatorial orientation [58]. For monomer **Z**, coupling constants between H−C(1′) and H_α−C(2′)/H_β−C(2′) are *ca.* 5.5 and *ca.* 9.5 Hz, respectively, as seen, *e.g.*, for **34**, confirming the C(2′)-*endo*-type conformation, as these coupling constants correspond to a H−C(1′)−C(2′)−H_α torsion angle of *ca.* 41° and a H−C(1′)−C(2′)−H_β torsion angle close to 160° according to a simplified *Karplus* equation [46]. The presence of the monomer **Z** with C(2′)-*endo* conformation in the otherwise unmodified DNA strand might cause a rather significant distortion of the structure of the DNA · RNA duplex. For ONs containing 3′-*C*-(hydroxymethyl)- [21][59], 3′-*C*-methyl- [22][23], and 3′-*C*-(aminomethyl)thymidine [23] monomers, smaller decreases in ΔT_m/mod. values were in general observed for duplexes towards complementary RNA. The C_3-alkyl branch in **Z**, 3′-*C*-(3-hydroxypropyl)thymidine [19], and 3′-*C*-allylthymidine [20] monomers might be involved in some unfavorable steric interactions which are less pronounced for the modified monomers with only one C-atom in the 3′-*C*-branch. Thus, apparently a more selective DNA binding is observed when the 3′-*C*-branch contains three C-atoms. However, the limited amount of experimental data should be kept in mind before any general conclusions are drawn.

The hybridization properties of the modified oligonucleotides **XVII** and **XX**, containing monomer **W**, towards complementary DNA and RNA measured in a medium salt buffer are depicted in *Table 2*. Incorporation of a single 3′-*C*-(3-

aminopropyl)-2′-*O*,5-dimethyluridine monomer **W** in a 14-mer oligothymidylate induces a major decrease in the thermal stability of the duplex formed with complementary DNA with a ΔT_m/mod. value of $-9.5°$. More encouraging results were obtained when a single **W** was incorporated in the mixed 9-mer ON **XX**, inducing a ΔT_m/mod. value of $-3.5°$ towards both complementary DNA and RNA. The binding affinities towards complementary DNA obtained for **XVII** and **XX** are in a striking contrast to the results obtained with the 2′-deoxy analogue **Z**. However, ONs containing 3′-*C*-allyl-2′-*O*,5-dimethyluridine [34], 3′-*C*,2′-*O*,5-trimethyluridine [22], the conformationally restricted 2′-*O*-methyl-6,3′-ethanouridine [60], and 2′-*O*-methyl-3′-*C*,2′-*O*-linked bicyclic nucleosides [34] displayed similar pronounced decreases in the thermal stability of duplexes towards DNA and RNA.

Despite the finding that the 2′-*O*-methyl modification in general has shown RNA-affinity-enhancing properties, probably by shifting the pentofuranose pucker towards the C(3′)-*endo* conformation due to the *gauche* effect, the presence of the 2′-*O*-methyl modification in monomer **W** does not lead to an increased affinity of the oligonucleotides towards RNA compared to DNA. An explanation might be found in the conformation of the sugar ring since the coupling constant $^3J(H-C(1'),H-C(2'))$ for both monomers (as seen, *e.g.*, for nucleoside **41**) is *ca.* 8 Hz, thus indicating a C(2′)-*endo* conformation of the pentofuranose ring and additionally highlighting the dominating effect of the 3′-*β*-branch on the sugar conformation. Consequently, the 2′-*O*-methyl group is oriented in a pseudoequatorial position, probably causing a significant distortion of the structure of the duplexes with DNA as well as RNA, as seen in molecular-modeling studies of oligonucleotides containing 3′-*C*-allyl-2′-*O*,5-dimethyluridine [34].

3. Conclusion. – 4′-*C*-(Aminomethyl)-2′-deoxy-2′-fluoro- and 4′-*C*-(aminomethyl)-2′-*O*-methylnucleosides have been efficiently synthesized by a convergent approach involving substitution of a 2′-[(methylsulfonyl)oxy] group or chemoselective 2′-*O*-methylation, respectively. The corresponding 3′-*O*-phosphoramidite derivatives were used in syntheses of partly modified 14-mer oligothymidylates and 9-mer mixed sequences by means of an automated DNA synthesizer. Compared with the corresponding 4′-*C*-branched 2′-deoxy derivatives, increased binding affinities towards complementary DNA and RNA were in general obtained, and a positive effect of the positively charged aminomethyl group was indicated at a physiologically relevant ionic strength. The introduction of the 2′-fluoro or 2′-methoxy substituents induces a conformational shift towards a C(3′)-*endo*-type furanose conformation. However, no additional effect was observed after incorporation of the 2′-substituted 4′-*C*-(aminomethyl) monomers in a 9-mer 2′-*O*-methyl-RNA strand. On the contrary, significantly reduced binding affinities resulted, indicating an unfavorable steric interaction involving the 4′-*C*-(aminomethyl) branch. The 3′-*C*-(3-aminopropyl)-2′-deoxy- and 4′-*C*-(aminomethyl)-2′-*O*-methylnucleosides were likewise synthesized by a convergent approach involving either free-radical deoxygenation at C(2′) or chemoselective 2′-*O*-methylation, respectively. The corresponding 3′-*O*-phosphoramidite derivatives were used for syntheses of partly modified 14-mer oligothymidylates and 9-mer mixed sequences. Compared with the corresponding unmodified reference ONs, the 2′-unsubstituted and 2′-substituted 3′-*C*-(3-aminopropyl)thymidine monomers induce

unchanged or decreased thermal stabilities of duplexes towards complementary DNA and decreased thermal stabilities towards RNA. Thus, one of the aims of the present research, namely to develop oligonucleotide analogues displaying DNA-selective hybridization, has been accomplished, but neither here nor earlier has it been possible to obtain significantly increased binding affinities for 3′-*C*-branched ONs. Noteworthy are, however, the affinity-enhancing properties of the 2′-substituted 4′-*C*-(aminomethyl)thymidine monomers reported herein. The somewhat troublesome synthetic routes devised for the relevant nucleosides and the nonideal oligomerizations of the corresponding phosphoramidite derivatives on automated synthesizers have so far precluded the evaluation of fully modified 3′-*C*- and 4′-*C*-branched ON derivatives. However, 4′-*C*- and 3′-*C*-branched ON analogues are likely to find utility as monomeric constituents, *e.g.*, conjugation sites, in antisense oligonucleotides.

Ms. *Britta M. Dahl* is thanked for oligonucleotide synthesis. *The Danish Natural Science Research Council* and *The Danish Technical Research Council* are thanked for financial support.

Experimental Part

General. Reactions were conducted under Ar when anh. solvents were used. Petroleum ether was of distillation range 60–80°. On workup, org. phases were dried (Na_2SO_4) and filtered. Column chromatography (CC): glass columns; silica gel *60* (0.040–0.063 mm). NMR spectra: chemical shift values δ in ppm rel. to $SiMe_4$ as internal reference (^1H and ^{13}C) and rel. to 85% H_3PO_4 soln. as external reference (^{31}P). MS: in *m*/*z*. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

3-O-*Benzyl-4*-C-*[(benzyloxy)methyl]-1,2-di*-O-*isopropylidene-β*-L-*lyxofuranose* (**2**). To a soln. of **1** (570 mg, 1.83 mmol) in anh. MeOH (50 ml), dibutyltin oxide (478 mg, 1.92 mmol) was added. The mixture was heated under reflux for 1 h, and the resulting clear soln. was cooled to r.t. and evaporated to give a yellow/white solid material which was redissolved in anh. toluene (25 ml). BnBr (0.65 ml, 5.5 mmol) was added to the mixture followed by Bu_4NBr (295 mg, 0.92 mmol), and the mixture was heated under reflux for 15 h. Additional Bu_4NBr (325 mg, 1.01 mmol) was added followed by BnBr (0.65 ml, 5.5 mmol), but no further conversion was observed according to anal. TLC. After additional heating under reflux for 24 h, the mixture was cooled to r.t. CH_2Cl_2 (30 ml) was added followed by a sat. aq. $NaHCO_3$ soln. (30 ml). The resulting precipitate was filtered off and the separated org. phase washed with sat. aq. NaCl soln. (3 × 20 ml), dried (Na_2SO_4), and evaporated. Purification by CC (10–50% AcOEt/petroleum ether, then 5% MeOH/CH_2Cl_2) afforded **2** (139 mg, 19%), another dibenzyl derivative (151 mg, 21%), and a tribenzyl derivative (206 mg, 23%) as oils, in addition to starting material **1** (*ca*. 128 mg, *ca*. 23% (contaminated with Sn-alkyls)) as a yellowish solid material. **2**: ^1H- and ^{13}C-NMR: in accordance with [24].

1,2-Di-O-*acetyl-3*-O-*benzyl-4*-C-*[(benzyloxy)methyl]-5*-O-*(methylsulfonyl)*-L-*lyxofuranose* (**4**). *Method A:* Furanose **3** [28] (630 mg, 1.32 mmol) was heated in 50% aq. AcOH soln. (5.5 ml) at 90° for 3 h and subsequently cooled to r.t. Evaporation followed by co-evaporation with abs. EtOH, toluene, and pyridine afforded an oil which was redissolved in anh. pyridine (3 ml). Ac_2O (2 ml) was added and the mixture stirred under Ar for 12 h at r.t. The mixture was poured into ice-cold H_2O (10 ml), and sat. aq. $NaHCO_3$ soln. (10 ml) was added followed by AcOEt (20 ml). The org. phase was washed several times with sat. aq. $NaHCO_3$ soln. The aq. phase was extracted with AcOEt (2 × 10 ml) and the combined org. phase dried (Na_2SO_4) and evaporated. Purification by CC (0–2% MeOH/CH_2Cl_2) afforded **4** (α/β-L 3:1; 370 mg, 54%).

Method B: To a soln. of **3** [28] (118 mg, 0.25 mmol) in AcOH (2 ml) and Ac_2O (0.2 ml), conc. H_2SO_4 (2 µl) was added. The soln. was stirred at r.t. for 12 h, poured into ice-cold H_2O (5 ml), and extracted with CH_2Cl_2 (3 × 10 ml). The org. phase was washed successively with sat. aq. $NaHCO_3$ soln. (3 × 10 ml) and sat. aq. NaCl soln. (10 ml), dried ($MgSO_4$), and evaporated. Purification by CC (0–2% MeOH/CH_2Cl_2) afforded furanose **4** (α/β-L 4:1) (70 mg, 54%). Oil. ^1H-NMR ($CDCl_3$): 7.37–7.21 (*m*, Bn); 6.38 (*d*, *J* = 4.7, H–C(1)(β-L)); 6.14 (*s*, H–C(1)(α-L)); 5.35 (*d*, *J* = 5.0, H–C(2)(α-L)); 5.17 (*dd*, *J* = 4.6, 6.3, H–C(2)(β-L)); 4.69–4.35 (*m*, Bn, H–C(3)); 4.25 (*d*, *J* = 11.3, Bn); 3.64, 3.52, 3.45, 3.39 (4*d*, *J* = 9.7, 10.0, 9.7, 10.0, 2 H–C(5), CH_2–C(4)); 2.98, 2.95 (2*s*, 2 $MeSO_2$), 2.13, 2.06, 2.04, 1.90 (4*s*, 4 MeCO). ^{13}C-NMR ($CDCl_3$): 170.1, 170.0, 169.6, 169.0 (4 C=O); 137.7,

137.0, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8 (Bn); 97.5 (C(1)(α-L)); 94.2 (C(1)(β-L)); 86.3 (C(4)(β-L)); 84.3 (C(4)(α-L)); 79.1, 74.5, 74.1, 73.7, 73.5, 71.6, 70.6, 70.2, 69.8 (C(2), C(3), C(5), CH_2–C(4), Bn); 37.6, 37.4 (2 $MeSO_2$); 21.0, 20.7, 20.6, 20.4 (4 MeCO). FAB-MS: 545 ($[M+Na]^+$). Anal. calc. for $C_{25}H_{30}O_{10}S$: C 57.46, H 5.79; found: C 57.72, H 5.74.

*1-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-*O*-(methylsulfonyl)-α-*L*-lyxofuranosyl)thymine* (**5**). To a soln. of **4** (460 mg, 0.88 mmol) in anh. MeCN (2 ml), thymine (0.20 g, 1.59 mmol) was added followed by *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 1.31 ml, 5.30 mmol). The mixture was heated under reflux until the soln. became clear (5 min). After cooling to 0°, $Me_3SiOSO_2CF_3$ (Me_3SiOTf; 0.27 ml, 1.50 mmol) was added dropwise, and the soln. was stirred for 12 h at r.t. As anal. TLC showed that the reaction was not complete, identical amounts as specified above of more thymine and BSA were further added, and the soln. was heated under reflux for 15 min, whereupon Me_3SiOTf (0.54 ml, 3.0 mmol) was added dropwise at 0°. After stirring at r.t. for 1.5 h, sat. aq. $NaHCO_3$ soln. (10 ml) was added, the mixture extracted with CH_2Cl_2 (3 × 20 ml), and the org. phase washed with sat. aq. $NaHCO_3$ soln. (2 × 10 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–2% MeOH/CH_2Cl_2) afforded **5** (386 mg, 75%). White solid material. ^{1}H-NMR ($CDCl_3$): 8.92 (br. *s*, NH); 7.40–7.26 (*m*, 11 H, H–C(6), Bn); 6.14 (*d*, *J* = 5.0, H–C(1′)); 5.48 (*t*, *J* = 5.4, H–C(2′)); 4.62 (*d*, *J* = 11.1, 1 H, Bn); 4.58 (*m*, 5 H, H–C(3′), 2 H–C(5′), Bn); 4.27 (*d*, *J* = 11.3, 1 H, Bn); 3.89 (*d*, *J* = 10.1, 1 H, CH_2–C(4′)); 3.59 (*d*, *J* = 10.2, 1 H, CH_2–C(4′)); 2.92 (*s*, $MeSO_2$); 2.10 (*s*, COMe); 1.59 (*d*, *J* = 0.5, Me). ^{13}C-NMR ($CDCl_3$): 170.2 (C=O); 163.6 (C(4)); 150.4 (C(2)); 137.0, 136.9, 136.1, 128.9, 128.8, 128.7, 128.5, 128.3, 128.2, 127.8 (C(6), Bn); 111.6 (C(5)); 87.8, 85.5 (C(1′), C(4′)); 77.8, 74.8, 74.4, 73.8, 70.8, 69.0 (C(2′), C(3′), C(5′), CH_2–C(4′), Bn); 37.2 ($MeSO_2$); 20.6 (*Me*CO); 11.9 (Me). FAB-MS: 589 ($[M+H]^+$). Anal. calc. for $C_{28}H_{32}N_2O_{10}S$: C 57.13, H 5.48, N 4.76; found: C 57.17, H 5.38, N 4.36.

*(1*S*,3*R*,4*R*,7*S*)-7-(Benzyloxy)-1-[(benzyloxy)methyl]-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane* (**6**) [25]. A soln. of **5** (73 mg, 0.124 mmol) in anh. THF (3 ml) was cooled to 0°. Then, 1.0M $LiEt_3BH$ in THF (0.25 ml, 0.25 mmol) was added dropwise, and the mixture was stirred at r.t. for 3 h and then cooled to 0°. An identical amount as specified above of $LiEt_3BH$ was additionally added dropwise, and the mixture was stirred for 12 h at r.t., and cooled to 0°. The reaction was quenched by slow addition of 35% aq. H_2O_2 soln./2N NaOH 1:1 (0.5 ml). The mixture was diluted with CH_2Cl_2 (10 ml) and washed with sat. aq. NaCl soln. (3 × 10 ml). The org. phase was dried (Na_2SO_4) and evaporated. Purification by CC (1% MeOH/CH_2Cl_2) afforded **6** (48 mg, 86%). White solid material. ^{1}H-NMR, ^{13}C-NMR, and FAB-MS: in accordance with [25].

*3-*O*-Benzyl-4-*C*-[(benzyloxy)methyl]-5-*O*-[(trifluoromethyl)sulfonyl]-1,2-*O*-isopropylidene-β-*L*-lyxofuranose* (**7**). To a soln. of **2** (6.34 g, 15.8 mmol) in anh. CH_2Cl_2 (60 ml), anh. pyridine (3.83 ml, 47.5 mmol) was added. The mixture was cooled to −50° and Tf_2O (4.15 ml, 25.3 mmol) added dropwise. The mixture was stirred for 1 h, then allowed to warm up to 0°, and stirred for 1 h, whereupon the reaction was quenched by slow addition of sat. aq. $NaHCO_3$ soln. (15 ml). Additional CH_2Cl_2 (40 ml) and sat. aq. $NaHCO_3$ soln. (50 ml) were added. The org. phase was successively washed with ice-cold 1M HCl (2 × 75 ml) and sat. aq. $NaHCO_3$ soln. (75 ml). The aq. phases were separately extracted with CH_2Cl_2 (25 ml each), and the combined org. phase was dried (Na_2SO_4) and evaporated: **7** as a white solid material which was used directly in the next step without further purification. ^{1}H-NMR ($CDCl_3$): 7.38–7.26 (*m*, 10 H, Bn); 5.74 (*d*, *J* = 3.6, H–C(1)); 5.00 (*d*, *J* = 10.7, 1 H, Bn); 4.81 (*d*, *J* = 10.7, 1 H, Bn); 4.75 (*d*, *J* = 12.1, 1 H, Bn); 4.61–4.54 (*m*, H–C(2), 2 H–C(5), Bn); 4.22 (*d*, *J* = 5.2, H–C(3)); 3.54 (*d*, *J* = 10.0, 1 H, CH_2–C(4)); 3.49 (*d*, *J* = 10.3, 1 H, CH_2–C(4)); 1.64, 1.35 (2*s*, Me_2C). ^{13}C-NMR ($CDCl_3$): 137.8, 137.3, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0 (Bn); 118.8 (*q*, *J* = 320, CF_3); 114.1 (Me_2C); 104.5 (C(1)); 84.2 (C(4)); 79.5, 78.8 (C(2), C(3)); 76.4, 74.0, 72.8, 70.5 (C(5), CH_2–C(4), Bn); 26.5, 25.9 (Me_2C). Anal. calc. for $C_{24}H_{27}F_3O_8S \cdot 0.25\,H_2O$: C 53.67, H 5.12; found: C 53.73, H 4.91.

*5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-1,2-*O*-isopropylidene-β-*L*-lyxofuranose* (**8**). To a soln. of **7** in anh. DMF (230 ml), NaN_3 (8.22 g, 0.126 mol) was added in one portion and the mixture stirred at 60° for 6 h. The soln. was cooled to r.t., filtered, and evaporated to give an oil which was redissolved in AcOEt (120 ml). The org. phase was washed with sat. aq. NaCl soln. (3 × 75 ml), dried (Na_2SO_4), and evaporated. Purification by CC (10–20% AcOEt/petroleum ether) afforded **8** (3.99 g, 59% from **2**). Clear oil. IR (NaCl): 2103 ($-N_3$). ^{1}H-NMR ($CDCl_3$): 7.38–7.26 (*m*, 10 H, Bn); 5.79 (*d*, *J* = 3.8, H–C(1)); 4.76 (*d*, *J* = 12.0, 1 H, Bn); 4.63 (*dd*, *J* = 3.8, 5.1, H–C(2)); 4.58 (*s*, 1 H, Bn); 4.54 (*s*, 1 H, Bn); 4.48 (*d*, *J* = 12.0, 1 H, Bn); 4.20 (*d*, *J* = 5.5, 1 H, H–C(3)); 4.05 (*d*, *J* = 13.4, 1 H, CH_2–C(4)); 3.58 (*d*, *J* = 10.2, 1 H, H_b–C(5)); 3.46 (*d*, *J* = 10.2, 1 H, H_a–C(5)); 3.33 (*d*, *J* = 13.4, 1 H, CH_2–C(4)); 1.67, 1.36 (2*s*, Me_2C). ^{13}C-NMR ($CDCl_3$): 138.0, 137.6, 128.5, 128.4, 128.0, 127.9, 127.7 (Bn); 113.5 (Me_2C); 104.2 (C(1)); 86.4 (C(4)); 78.9, 78.3, 73.6, 72.4, 71.3 (C(2), C(3), C(5), Bn); 52.1 (CH_2–C(4)); 26.6, 25.8 (Me_2C). FAB-MS: 448 ($[M+Na]^+$). Anal. calc. for $C_{23}H_{27}N_3O_5$: C 64.93, H 6.40, N 9.88; found: C 64.76, H 6.32, N 9.72.

*1,2-Di-*O*-acetyl-5-azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-*L*-lyxofuranose* (**9**). Furanose **8** (5.93 g, 13.9 mmol) was heated in 50% aq. AcOH soln. (120 ml) at 100° for 1 h and cooled to r.t. Evaporation followed by co-evaporation successively with abs. EtOH, toluene, and pyridine afforded an oil which was redissolved in anh. pyridine (30 ml). Ac_2O (20 ml) was added, and the mixture was stirred under Ar for 12 h at r.t. The mixture was cooled to 0° and poured into ice-cold H_2O (50 ml). Sat. aq. $NaHCO_3$ soln. (50 ml) was added followed by AcOEt (100 ml). The org. phase was washed with sat. aq. $NaHCO_3$ soln., the aq. phase extracted with AcOEt (2 × 50 ml), and the combined org. phase dried (Na_2SO_4) and evaporated. Purification by CC (CH_2Cl_2) afforded **9** (α/β-L 1:4; 5.86 g, 90%). Clear oil. ^{1}H-NMR ($CDCl_3$): 7.39–7.24 (*m*, Bn); 6.42 (*d*, *J* = 4.7, H–C(1)(α-L)); 6.18 (*s*, H–C(1)(β-L)); 5.35 (*d*, *J* = 4.8, H–C(2)(β-L)); 5.25 (*dd*, *J* = 4.8, 6.6, H–C(2)(α-L)); 4.63–4.48 (*m*, Bn); 4.36 (*d*, *J* = 5.0, H–C(3)(β-L)); 4.29 (*d*, *J* = 6.5, H–C(3)(α-L)); 3.74 (*d*, *J* = 13.1, 1 H, CH_2–C(4)(α-L)); 3.64–3.43 (*m*, H–C(5), 2 H–C(4)); 3.37 (*d*, *J* = 13.3, 1 H, CH_2–C(4)(α-L)); 2.13, 2.12, 2.05, 1.90 (4*s*, 4 MeCO). ^{13}C-NMR ($CDCl_3$): 169.7, 169.6, 168.9 (4 C=O); 137.8, 137.3, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6 (Bn); 97.5 (C(1)(β-L)); 94.3 (C(1)(α-L)); 87.8 (C(4)(α-L)); 86.1 (C(4′)(β-L)); 79.0, 77.4, 74.3, 74.2, 73.7, 73.5, 73.4, 71.6, 71.5, 71.0 (2 C(2), 2 C(3), 2 C(5), Bn); 53.0 (*C*H_2–C(4)(β-L)); 52.7 (*C*H_2–C(4)(α-L)); 21.0, 20.7, 20.6, 20.3 (4 *Me*CO). FAB-MS: 492 ($[M+Na]^+$). Anal. calc. for $C_{24}H_{27}N_3O_7$: C 61.40, H 5.80; N 8.95; found: C 61.64, H 5.81, N 8.96.

*1-{2-*O*-Acetyl-5-azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-α-*L*-lyxofuranosyl}thymine* (**10**). To a soln. of **9** (5.86 g, 12.5 mmol) in anh. MeCN (50 ml), thymine (3.15 g, 25 mmol) and BSA (24.7 ml, 0.1 mol) were added. The soln. was heated under reflux for 30 min, cooled to 0°, and Me_3SiOTf (4.5 ml, 25 mmol) was added dropwise. The mixture was heated under reflux for another 30 min and then cooled to 0°. The reaction was quenched by addition of a sat. aq. $NaHCO_3$ soln. (60 ml). The mixture was filtered and evaporated, the oily residue dissolved in CH_2Cl_2 (75 ml), and the soln. washed with sat. aq. $NaHCO_3$ soln. (3 × 50 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–1% MeOH/CH_2Cl_2) afforded **10** (6.22 g, 93%). White solid material. ^{1}H-NMR ($CDCl_3$): 9.22 (br. *s*, NH); 7.40 (*d*, *J* = 1.3, H–C(6)); 7.39–7.26 (*m*, 10 H, Bn); 6.21 (*d*, *J* = 5.1, H–C(1′)); 5.46 (*t*, *J* = 5.6, H–C(2′)); 4.63 (*d*, *J* = 11.3, 1 H, Bn); 4.56 (*m*, 4 H, H–C(3′), Bn); 3.77 (*d*, *J* = 9.9, H_b–C(5′)); 3.65 (*d*, *J* = 13.0, 1 H, CH_2–C(4′)); 3.51 (*d*, *J* = 10.3, H_a–C(5′)); 3.38 (*d*, *J* = 13.1, 1 H, CH_2–C(4′)); 2.10 (*s*, COMe); 1.57 (*d*, *J* = 1.1, Me). ^{13}C-NMR ($CDCl_3$): 170.2 (C=O); 163.8 (C(4)); 150.5 (C(2)); 137.2, 137.1, 135.8 (C(6), Bn); 128.7, 128.6, 128.3, 128.2, 128.1, 127.8 (Bn); 111.5 (C(5)); 87.1 (C(1′), C(4′)); 77.5, 74.8, 74.6, 73.7, 71.4 (C(2′), C(3′), C(5′), Bn); 52.6 (*C*H_2–C(4′)); 20.5 (*Me*CO); 11.9 (Me). FAB-MS: 536 ($[M+H]^+$). Anal. calc. for $C_{27}H_{29}N_5O_7$: C 60.55, H 5.46, N 13.08; found: C 60.34, H 5.39, N 12.87.

*1-{5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-α-*L*-lyxofuranosyl}thymine* (**11**). Nucleoside **10** (6.18 g, 11.54 mmol) was stirred in a sat. NH_3 soln. in MeOH (100 ml) at r.t., and after 5 h, the mixture was evaporated. Purification by CC (1% MeOH/CH_2Cl_2) afforded **11** (6.18 g, 97%). White solid material. ^{1}H-NMR ($CDCl_3$): 10.3 (br. *s*, NH); 7.54 (*d*, *J* = 1.1, H–C(6)); 7.38–7.26 (*m*, 10 H, Bn); 5.99 (*d*, *J* = 4.4, H–C(1′)); 4.91 (*d*, *J* = 11.8, 1 H, Bn); 4.61 (*d*, *J* = 11.5, 1 H, Bn); 4.54–4.49 (*m*, 3 H, H–C(2′), Bn); 4.31 (*d*, *J* = 5.5, H–C(3′)); 3.81 (*d*, *J* = 13.5, 1 H, CH_2–C(4′)); 3.80 (*d*, *J* = 10.3, H_b–C(5′)); 3.52 (*d*, *J* = 10.1, H_a–C(5′)); 3.31 (*d*, *J* = 13.3, 1 H, CH_2–C(4′)); 1.50 (*d*, *J* = 0.8, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 151.4 (C(2)); 137.5, 137.3, 136.1 (C(6), Bn); 128.7, 128.5, 128.2, 128.1, 127.8 (Bn); 110.7 (C(5)); 90.3, 87.2 (C(1′), C(4′)); 77.9, 74.7, 73.9, 73.6, 71.4 (C(2′), C(3′), C(5′), Bn); 52.9 (*C*H_2–C(4′)); 11.9 (Me). FAB-MS: 494 ($[M+H]^+$). Anal. calc. for $C_{25}H_{27}N_5O_6$: C 60.84, H 5.51, N 14.19; found: C 60.80, H 5.38, N 14.10.

*1-{5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-2-*O*-methyl-α-*L*-lyxofuranosyl}thymine* (**12**). A soln. of **11** (0.99 g, 2.01 mmol) in anh. THF (20 ml) was cooled to 0°. NaH (0.241 g of a 60% dispersion in mineral oil, 6.03 mmol) was added and the mixture stirred for 30 min. MeI (0.63 ml, 10.1 mmol) was added dropwise, and the mixture was stirred at 0° for 10 h. Ice-cold H_2O (10 ml) was added followed by AcOEt (25 ml). The org. phase was washed with sat. aq. $NaHCO_3$ soln. (3 × 30 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0.5% MeOH/CH_2Cl_2) afforded **12** (0.92 g, 90%). White solid material. ^{1}H-NMR ($CDCl_3$): 9.35 (br. *s*, NH); 7.67 (*d*, *J* = 1.3, H–C(6)); 7.41–7.22 (*m*, 10 H, Bn); 6.07 (*d*, *J* = 2.4, H–C(1′)); 4.74 (*d*, *J* = 11.7, 1 H, Bn); 4.68 (*d*, *J* = 11.4, 1 H, Bn); 4.52 (*s*, 1 H, Bn); 4.49 (*d*, *J* = 11.7, 1 H, Bn); 4.35 (*d*, *J* = 5.5, 1 H, H–C(3′)); 3.94 (*d*, *J* = 10.4, H_b–C(5′)); 3.93 (*d*, *J* = 13.6, 1 H, CH_2–C(4′)); 3.87 (*dd*, *J* = 2.4, 5.7, H–C(2′)); 3.58 (*s*, MeO); 3.55 (*d*, *J* = 10.0, H_a–C(5′)); 3.34 (*d*, *J* = 13.7, 1 H, CH_2–C(6′)); 1.48 (*d*, *J* = 1.2, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 150.3 (C(2)); 137.3, 137.2, 135.8 (C(6), Bn); 128.7, 128.6, 128.2, 127.9, 127.8 (Bn); 110.8 (C(5)); 88.5, 87.2, 83.4 (C(1′), C(2′), C(4′)); 75.9, 73.6, 73.1, 70.2 (C(3′), C(5′), Bn); 59.2 (MeO); 53.0 (*C*H_2–C(4′)); 11.7 (Me). FAB-MS: 508 ($[M+H]^+$). Anal. calc. for $C_{26}H_{29}N_5O_6$: C 61.53, H 5.76, N 13.80; found: C 61.26, H 5.78, N 13.62.

*1-{5-Amino-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-2-*O*-methyl-α-*L*-lyxofuranosyl}thymine* (**13**). To a soln. of **12** (440 mg, 0.87 mmol) in abs. EtOH (13 ml), *Lindlar* catalyst (130 mg) was added. The mixture was stirred at r.t. under H_2 for 8 h, filtered through a thin layer of silica gel, and evaporated. Purification by

CC (2–5% MeOH/CH_2Cl_2) afforded **13** (316 mg, 75%). White solid material. ^{1}H-NMR ($CDCl_3$): 7.60 (*d*, *J* = 0.9, H–C(6)); 7.38–7.21 (*m*, 10 H, Bn); 6.12 (*d*, *J* = 3.3, H–C(1′)); 4.79 (*d*, *J* = 11.9, 1 H, Bn); 4.56–4.46 (*m*, 2 H, Bn); 4.48 (*d*, *J* = 11.7, 1 H, Bn); 4.33 (*d*, *J* = 5.9, H–C(3′)); 3.88 (*dd*, *J* = 3.6, 5.9, H–C(2′)); 3.82 (*d*, *J* = 10.4, H_b–C(5′)); 3.59 (*d*, *J* = 10.5, H_a–C(5′)); 3.50 (*s*, MeO); 3.12 (*d*, *J* = 13.8, 1 H, CH_2–C(4′)); 2.83 (*d*, *J* = 13.8, 1 H, CH_2–C(4′)); 1.49 (*d*, *J* = 0.5, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 150.5 (C(2)); 137.7, 137.4, 135.9 (C(6), Bn); 128.5, 128.1, 128.0, 127.8, 127.7 (Bn); 111.0 (C(5)); 88.0, 87.8, 83.9 (C(1′), C(2′), C(4′)); 76.8, 73.6, 73.4, 71.8 (C(3′), C(5′), Bn); 59.1 (MeO); 44.2 (*C*H_2–C(4′)); 11.8 (Me). FAB-MS: 482 ([*M* + H]$^+$). Anal. calc. for $C_{26}H_{31}N_3O_6 \cdot 0.25\,H_2O$: C 64.25, H 6.53, N 8.65; found: C 64.27, H 6.38, N 8.55.

*1-{3-*O*-Benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-2-*O*-methyl-5-[(trifluoroacetyl)amino]-α-*L*-lyxofuranosyl}-thymine* (**14**). To a soln. of nucleoside **13** (300 mg, 0.62 mmol) in anh. CH_2Cl_2 (8 ml), ethyl trifluoroacetate (0.15 ml, 1.25 mmol) and Et_3N (0.086 ml, 0.62 mmol) were added. The mixture was stirred for 12 h at r.t. and then evaporated. Purification by CC (1% MeOH/CH_2Cl_2) afforded **14** (346 mg, 97%). White solid material. ^{1}H-NMR ($CDCl_3$): 8.86 (br. *s*, NH); 7.52 (*d*, *J* = 1.3, H–C(6)); 7.39–7.18 (*m*, 11 H, NHCOCF_3, Bn); 5.90 (*d*, *J* = 1.6, H–C(1′)); 4.72 (*d*, *J* = 11.5, 1 H, Bn); 4.54–4.44 (*m*, 3 H, Bn); 4.43 (*d*, *J* = 6.1, H–C(3′)); 3.95 (*dd*, *J* = 1.7, 6.3, H–C(2′)); 3.77–3.68 (*m*, CH_2–C(4′)); 3.55–3.48 (*m*, 2 H–C(5′)); 3.58 (*s*, MeO); 1.51 (*d*, *J* = 1.0, Me). ^{13}C-NMR ($CDCl_3$): 164.1 (C(4)); 157.4 (*q*, *J* = 37, *C*OCF_3); 150.2 (C(2)); 136.8, 136.1 (C(6), Bn); 128.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.7 (Bn); 115.9 (*q*, *J* = 288, CO*C*F_3); 110.9 (C(5)); 90.2, 86.8, 83.0 (C(1′), C(2′), C(4′)); 76.0, 73.6, 73.2, 70.8 (C(3′), *C*H_2–C(4′), Bn); 59.3 (MeO); 41.4 (C(5′)); 11.8 (Me). FAB-MS: 578 ([*M* + H]$^+$). Anal. calc. for $C_{28}H_{30}F_3n_3O_7$: C 58.23, H 5.23, N 7.28; found: C 58.27, H 5.21, N 7.34.

*1-{5-Deoxy-2-*O*-methyl-5-[(trifluoroacetyl)amino]-α-*L*-lyxofuranosyl}thymine* (**15**). To a soln. of **14** (346 mg, 0.60 mmol) in abs. EtOH (10 ml), 5% Pd/C catalyst (150 mg) was added. The mixture was stirred at r.t. under H_2 for 1.5 h, filtered through a thin layer of silica gel, and evaporated. Purification by CC (3% MeOH/CH_2Cl_2) afforded **15** (223 mg, 92%). White solid material. ^{1}H-NMR (CD_3OD): 7.86 (*d*, *J* = 1.1, H–C(6)); 6.05 (*d*, *J* = 4.6, H–C(1′)); 4.51 (*d*, *J* = 5.8, H–C(3′)); 4.03 (*dd*, *J* = 4.8, 5.8, H–C(2′)); 3.70–3.62 (*m*, CH_2–C(4′), 2 H–C(5′)); 3.50 (*s*, MeO); 1.49 (*d*, *J* = 1.0, Me). ^{13}C-NMR (CD_3OD): 166.3 (C(4)); 157.4 (*q*, *J* = 35, *C*OCF_3); 152.4 (C(2)); 138.4 (C(6)); 117.5 (*q*, *J* = 286, CO*C*F_3); 111.7 (C(5)); 89.0, 88.9, 84.9 (C(1′), C(2′), C(4′)); 71.1, 64.3 (C(3′), *C*H_2–C(4′)); 59.1 (MeO); 42.1 (C(5′)); 12.4 (Me). EI-MS: 397 (M^+). Anal. calc. for $C_{14}H_{18}F_3N_3O_7 \cdot 0.25\,H_2O$: C 41.85, H 4.64, N 10.58, found: C 41.93, H 4.42, N 10.40.

*1-{5-Deoxy-4-*C*-[[(4,4′-dimethoxytrityl)oxy]methyl]-2-*O*-methyl-5-[(trifluoroacetyl)amino]-α-*L*-lyxofuranosyl}thymine* (**16**). To a soln. of **15** (223 mg, 0.56 mmol) in anh. pyridine (8 ml), $(MeO)_2$TrCl (361 mg, 1.07 mmol) was added and the mixture stirred at r.t. for 6 h. MeOH (2 ml) was added and the mixture evaporated. The oily residue was redissolved in CH_2Cl_2 (20 ml) and the soln. washed with sat. aq. NaCl soln. (3 × 15 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0.5–1.5% MeOH/CH_2Cl_2 containing 0.5% pyridine) afforded **16** (382 mg, 91%). Yellowish solid material. ^{1}H-NMR ($CDCl_3$): 9.54 (br. *s*, NH); 7.45 (*d*, *J* = 0.9, H–C(6)); 7.39–7.08 (*m*, 10 H, NHCOCF_3, $(MeO)_2$*Tr*); 6.84 (*dd*, *J* = 1.7, 9.0, 4 H, $(MeO)_2$*Tr*); 6.10 (*d*, *J* = 5.6, H–C(1′)); 4.49 (*d*, *J* = 5.1, H–C(3′)); 4.20 (*t*, *J* = 5.8, H–C(2′)); 3.82–3.75 (*m*, CH_2–C(4′)); 3.63–3.61 (*m*, 2 H–C(5′)); 3.79, 3.78 (2*s*, (*Me*O)$_2$Tr); 3.42 (br. *s*, OH–C(3′)); 3.36 (*s*, MeO); 1.48 (*d*, *J* = 1.0, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 159.0 ((MeO)$_2$*Tr*); 157.5 (*q*, *J* = 37, *C*OCF_3); 150.7 (C(2)); 143.9 ((MeO)$_2$*Tr*); 137.9, 135.7, 134.8, 134.7 (C(6), (MeO)$_2$*Tr*); 130.2, 130.1, 129.1, 128.3, 128.1, 127.4 ((MeO)$_2$*Tr*); 115.7 (*q*, *J* = 288, CO*C*F_3); 113.4 ((MeO)$_2$Tr); 112.0 (C(5)); 87.7, 87.2, 87.0, 83.0 (C(1′), C(2′), C(4′), *C*(Ar$_3$)); 71.1, 65.9 (C(3′), *C*H_2–C(4′)); 59.0 (MeO); 55.1 ((*Me*O)$_2$Tr); 41.3 (C(5′)); 11.6 (Me). FAB-MS: 699 (M^+). Anal. calc. for $C_{35}H_{36}F_3N_3O_9 \cdot 0.5\,C_7H_8$: C 62.01, H 5.41, N 5.63; found: C 62.04, H 5.50, N 5.50.

*1-{5-Deoxy-4-*C*-[[(4,4′-dimethoxytrityl)oxy]methyl]-2-*O*-methyl-5-[(trifluoroacetyl)amino]-α-*L*-lyxofuranosyl}thymine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**17**). To a soln. of **16** (160 mg, 0.23 mmol) in anh. CH_2Cl_2 (4 ml), ^{i}Pr$_2$EtN (0.16 ml, 0.92 mmol) was added, and the mixture was cooled to 0°. 2-Cyanoethyl diisopropylphosphoramidochloridite (0.11 ml, 0.46 mmol) was added dropwise, and the mixture was stirred for 4 h at r.t., diluted with AcOEt (25 ml), and washed with sat. aq. NaCl soln. (3 × 10 ml). The org. phase was dried (Na_2SO_4) and evaporated. After purification by CC (AcOEt/CH_2Cl_2/petroleum ether/Et_3N 15 : 50 : 35 : 1), the product was redissolved in anh. toluene and precipitated in petroleum ether (50 ml) at −30° under vigorous stirring. A second purification by CC (1% Et_3N/CH_2Cl_2) followed by precipitation in petroleum ether as described above afforded phosphoramidite **17** (89 mg, 43%). White solid material. ^{31}P-NMR ($CDCl_3$): 153.0, 152.5. FAB-MS: 900 (M^+).

*1-{5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-2-*O*-(methylsulfonyl)-α-*L*-lyxofuranosyl}thymine* (**18**). A soln. of **11** (1.16 g, 2.35 mmol) in anh. pyridine (6 ml) was cooled to 0°, and MsCl (0.36 ml, 4.7 mmol) was added dropwise. The mixture was stirred at r.t. for 2 h, poured into ice-cold H_2O (10 ml) and CH_2Cl_2 (50 ml), and a sat. aq. $NaHCO_3$ soln. (20 ml) was added. The org. phase was separated and washed successively

with sat. aq. $NaHCO_3$ soln. (3×25 ml) and ice-cold 1M HCl (3×25 ml), dried (Na_2SO_4), and evaporated to give **18** as a red foam which was used directly in the next step without further purification. ^{1}H-NMR ($CDCl_3$): 9.28 (br. *s*, NH); 7.52 (*d*, *J* = 1.2, H–C(6)); 7.35–7.22 (*m*, 10 H, Bn); 6.13 (*d*, *J* = 3.9, H–C(1′)); 5.35 (*dd*, *J* = 4.1, 5.5, H–C(2′)); 4.87 (*d*, *J* = 11.4, 1 H, Bn); 4.54–4.47 (*m*, 3 H, Bn); 4.41 (*d*, *J* = 5.7, H–C(3′)); 3.85 (*d*, *J* = 10.4, H_b–C(5′)); 3.73 (*d*, *J* = 13.4, 1 H, CH_2–C(4′)); 3.49 (*d*, *J* = 10.4, H_a–C(5′)); 3.39 (*d*, *J* = 13.5, 1 H, CH_2–C(4′)); 3.15 (*s*, $MeSO_2$); 1.49 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 163.7 (C(4)); 150.7 (C(2)); 136.9, 135.4 (Bn); 128.8, 128.7, 128.5, 128.4, 127.8 (C(6), Bn); 111.6 (C(5)); 87.7, 87.5 (C(1′), C(4′)); 79.5, 76.3, 74.1, 73.7, 70.8 (C(2′), C(3′), C(5′), Bn); 52.7 (*C*H_2–C(4′)); 38.8 ($MeSO_2$); 11.8 (Me). FAB-MS: 572 ($[M+H]^+$).

*1-{5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-α-*L*-xylofuranosyl}thymine* (**19**). Nucleoside **18** was stirred in 90% aq. EtOH soln. (80 ml), and 1N NaOH (5.17 ml, 5.17 mmol) was added. The mixture was heated under reflux for 30 min, cooled to r.t., neutralized with AcOH and evaporated. The resulting oil was redissolved in CH_2Cl_2 (50 ml), and a sat. aq. $NaHCO_3$ soln. (50 ml) was added. The org. phase was washed with sat. aq. $NaHCO_3$ soln. (3×30 ml), dried (Na_2SO_4), and evaporated. Purification by CC (1.5% MeOH/CH_2Cl_2) afforded **19** (1.09 g, 94%). White solid material. ^{1}H-NMR ($CDCl_3$): 10.8 (br. *s*, NH); 7.45 (*d*, *J* = 0.9, H–C(6)); 7.39–7.26 (*m*, 10 H, Bn); 6.24 (*d*, *J* = 3.8, H–C(1′)); 4.97 (*d*, *J* = 5.1, OH–C(2′)); 4.84 (*m*, H–C(2′)); 4.72 (*d*, *J* = 11.8, 1 H, Bn); 4.56 (*s*, 1 H, Bn); 4.55 (*s*, 1 H, Bn); 4.49 (*d*, *J* = 11.5, 1 H, Bn); 4.14 (*d*, *J* = 2.1, H–C(3′)); 3.73 (*d*, *J* = 9.8, H_b–C(5′)); 3.71 (*d*, *J* = 9.8, H_a–C(5′)); 3.66 (*d*, *J* = 12.8, 1 H, CH_2–C(4′)); 3.49 (*d*, *J* = 12.7, 1 H, CH_2–C(4′)); 1.61 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 165.7 (C(4)); 150.8 (C(2)); 138.7, 137.5, 137.3 (C(6), Bn); 128.5, 128.4, 128.1, 128.0, 127.7 (Bn); 108.2 (C(5)); 86.6, 86.0, 83.7 (C(1′), C(2′), C(4′)); 73.9, 73.5, 72.0, 69.9 (C(3′), C(5′), Bn); 51.7 (*C*H_2–C(4′)); 12.0 (Me). FAB-MS: 494 ($[M+H]^+$). Anal. calc. for $C_{25}H_{27}N_5O_6$: C 60.84, H 5.51, N 14.19; found: C 60.81, H 5.52, N 14.05.

*1-{5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-5-deoxy-2-*O*-[(trifluoromethyl)sulfonyl]-α-*L*-xylofuranosyl}thymine* (**20**). A soln. of **19** (450 mg, 0.911 mmol) in anh. CH_2Cl_2 (20 ml) was cooled to 0°, 4-(dimethylamino)pyridine (DMAP; 445 mg, 3.64 mmol) and anh. pyridine (0.74 ml, 9.1 mmol) were added, followed by dropwise addition of trifluoromethanesulfonic anhydride (0.33 ml, 2.0 mmol). The mixture was stirred at r.t. for 2 h and poured into an ice-cold sat. aq. $NaHCO_3$ soln. (10 ml). The org. phase was washed successively with 1M ice-cold HCl (2×20 ml) and sat. aq. $NaHCO_3$ soln. (2×20 ml), dried (Na_2SO_4), and evaporated. Purification by CC (silica gel, 1.5% MeOH/CH_2Cl_2) afforded **20** (437 mg, 77%). Yellowish solid material which was used directly in the next step without further purification. ^{1}H-NMR ($CDCl_3$): 8.71 (br. *s*, NH); 7.40–7.21 (*m*, 11 H, H–C(6), Bn); 6.39 (*d*, *J* = 4.4, H–C(1′)); 5.54 (*t*, *J* = 4.2, H–C(2′)); 4.81 (*d*, *J* = 12.0, 1 H, Bn); 4.56–4.47 (*m*, 3 H, H–C(3′), Bn); 4.52 (*d*, *J* = 11.6, 1 H, Bn); 3.67 (*d*, *J* = 12.7, 1 H, CH_2–C(4′)); 3.54–3.44 (*m*, 2 H–C(5′)); 3.43 (*d*, *J* = 12.9, 1 H, CH_2–C(4′)); 1.76 (*d*, *J* = 1.0, Me). FAB-MS: 626 ($[M+H]^+$).

*1-{5-Azido-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-2,5-dideoxy-2-fluoro-α-*L*-lyxofuranosyl}thymine* (**21**) *and 1-[4-*C*-(Azidomethyl)-3,5-di-*O*-benzyl-2,5-dideoxy-*D*-*erythro*-pent-1-enofuranosyl]thymine* (**22**). To a soln. of **20** (437 mg, 0.70 mmol) in anh. THF (10 ml) at 0°, Bu_4NF (2.38 g, dried for 48 h at 0.3 Torr at 40°, *ca.* 9.11 mmol) in anh. THF (4 ml) was added slowly, and the mixture was stirred for 24 h at 5°. The mixture was evaporated, the resulting oil redissolved in AcOEt (20 ml), and the soln. washed with sat. aq. NaCl soln. (3×20 ml), dried (Na_2SO_4), and evaporated. Purification by CC (1. 10–25% AcOEt/petroleum ether; 2. 0–2% MeOH/CH_2Cl_2) afforded **21** (130 mg, 37%). White solid material. ^{1}H-NMR ($CDCl_3$): 8.28 (br. *s*, NH); 7.43 (*d*, *J* = 1.1, H–C(6)); 7.40–7.22 (*m*, 10 H, Bn); 6.20 (*dd*, *J* = 3.5, 15.6, H–C(1′)); 5.18 (*ddd*, *J* = 3.6, 5.0, 52.9, H–C(2′)); 4.82 (*d*, *J* = 11.7, 1 H, Bn); 4.58–4.49 (*m*, 3 H, Bn); 4.37 (*dd*, *J* = 5.0, 14.9, H–C(3′)); 3.84 (*d*, *J* = 10.5, H_b–C(5′)); 3.69 (*d*, *J* = 13.3, 1 H, CH_2–C(4′)); 3.57 (*d*, *J* = 10.5, H_a–C(5′)); 3.43 (*d*, *J* = 13.3, 1 H, CH_2–C(4′)); 1.57 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 163.9 (C(4)); 150.4 (C(2)); 137.0, 136.9, 136.0 (C(6), Bn); 128.7, 128.6, 128.3, 128.2, 128.0, 127.8 (Bn); 111.3 (C(5)); 92.3 (*d*, *J* = 194, C(2′)); 88.3 (*d*, *J* = 34, C(1′)); 87.1 (C(4′)); 76.4 (*d*, *J* = 14.7, C(3′)); 73.7, 73.5, 70.9 (2*s* and *d* (73.5), *J* = 2.1, C(5′), Bn); 52.6 (*d*, *J* = 3.2, *C*H_2–C(4′)); 11.8 (Me). FAB-MS: ($[M+H]^+$). Anal. calc. for $C_{25}H_{26}FN_5O_5$: C 60.60, H 5.29, N 14.13; found: C 60.71, H 5.06, N 13.96.

In addition, the less polar derivative **22** was isolated as a yellowish gum (100 mg, 30%). ^{1}H-NMR ($CDCl_3$): 9.33 (br. *s*, NH); 7.50 (*d*, *J* = 0.9, H–C(6)); 7.39–7.26 (*m*, 10 H, Bn); 5.73 (*d*, *J* = 2.4, H–C(2′)); 4.78 (*d*, *J* = 2.7, H–C(3′)); 4.60 (*d*, *J* = 11.6, 1 H, Bn); 4.58 (*s*, 1 H, Bn); 4.56 (*s*, 1 H, Bn); 4.50 (*d*, *J* = 11.9, 1 H, Bn); 3.91 (*d*, *J* = 12.9, 1 H, CH_2–C(4′)); 3.74 (*d*, *J* = 9.5, H_b–C(5′)); 3.68 (*d*, *J* = 13.2, 1 H, CH_2–C(4′)); 3.40 (*d*, *J* = 9.3, H_a–C(5′)); 1.94 (*d*, *J* = 0.9, Me). ^{13}C-NMR ($CDCl_3$): 163.0 (C(4)); 148.1, 146.1 (C(2), C(1′)); 137.7, 137.3 (Bn); 134.4 (C(6)); 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6 (Bn); 111.5 (C(5)); 88.5, 88.4, 83.1 (C(2′), C(3′), C(4′)); 73.6, 71.3, 68.5 (C(5′), Bn); 50.3 (*C*H_2–C(4′)); 12.4 (Me). FAB-MS: 476 ($[M+H]^+$). Due to the instability of **22**, elemental analysis could not be obtained.

*1-{5-Amino-3-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-2,5-dideoxy-2-fluoro-α-*L*-lyxofuranosyl}thymine* (**23**). As described for **13**, with **21** (390 mg, 0.79 mmol), abs. EtOH/anh. dioxane 1:4 (11 ml), and *Lindlar* catalyst

(120 mg). Purification by CC (2–5% MeOH/CH_2Cl_2) afforded **23** (240 mg, 64%). White solid material. ^{1}H-NMR ($CDCl_3$): 7.45 (*d*, *J* = 1.4, H–C(6)); 7.38–7.23 (*m*, 10 H, Bn); 6.26 (*dd*, *J* = 4.0, 15.1, H–C(1′)); 5.28–5.08 (*ddd*, *J* = 4.4, 5.1, 52.1, H–C(2′)); 4.85 (*d*, *J* = 11.6, 1 H, Bn); 4.53–4.50 (*m*, 3 H, Bn); 4.36 (*dd*, *J* = 5.3, 12.8, H–C(3′)); 3.90 (br. *s*, NH_2); 3.77 (*d*, *J* = 10.5, H_b–C(5′)); 3.61 (*d*, *J* = 10.4, H_a–C(5′)); 3.00 (*d*, *J* = 13.7, 1 H, CH_2–C(4′)); 2.90 (*d*, *J* = 13.9, 1 H, CH_2–C(4′)); 1.58 (*d*, *J* = 0.8, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 150.6 (C(2)); 137.3, 135.9 (C(6), Bn); 128.7, 128.6, 128.2, 128.1, 127.9, 127.7 (Bn); 111.3 (C(5)); 92.7 (*d*, *J* = 195, C(2′)); 87.9 (C(4′)); 87.5 (*d*, *J* = 33, C(1′)); 77.1 (*d*, *J* = 14, C(3′)); 73.6, 72.2 (C(5′), Bn); 44.1 (CH_2–C(4′)); 11.9 (Me). FAB-MS: 470 ([*M* + H]$^+$). Anal. calc. for $C_{25}H_{28}FN_3O_5 \cdot 0.25\ H_2O$: C 63.35, H 6.06, N 8.86; found: C 63.46, H 5.95, N 8.85.

*1-{3-*O*-Benzyl-4-*C*-[(benzyloxy)methyl]-2,5-dideoxy-2-fluoro-5-[(trifluoroacetyl)amino]-α-*L*-lyxofuranosyl}-thymine* (**24**). As described for **14**, with **23** (230 mg, 0.49 mmol), anh. CH_2Cl_2 (6 ml), ethyl trifluoroacetate (0.18 ml, 1.48 mmol), and Et_3N (0.14 ml, 0.98 mmol). Purification by CC (1% MeOH/CH_2Cl_2) afforded **24** (248 mg, 89%). White solid material. ^{1}H-NMR ($CDCl_3$): 9.55 (br. *s*, NH); 7.40–7.22 (*m*, 11 H, H–C(6), Bn); 7.10 (*t*, *J* = 6.3, $NHCOCF_3$); 5.90 (*dd*, *J* = 2.3, 19.3, H–C(1′)); 5.42–5.21 (*ddd*, *J* = 2.3, 5.3, 54.0, H–C(2′)); 4.79 (*d*, *J* = 11.5, 1 H, Bn); 4.54–4.45 (*m*, 4 H, H–C(3′), Bn); 3.77–3.67 (*m*, 3 H, 2 H_b–C(5′), CH_2–C(4′)); 3.57 (*d*, *J* = 10.5, 1 H, CH_2–C(4′)); 1.67 (*d*, *J* = 1.2, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 157.8 (*q*, *J* = 37, *C*OCF_3); 150.3 (C(2)); 137.4, 136.9, 136.6 (C(6), Bn); 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.8 (Bn); 115.8 (*q*, *J* = 288, CO*C*F_3); 111.5 (C(5)); 92.0 (*d*, *J* = 192, C(2′)); 91.1 (*d*, *J* = 36, C(1′)); 86.3 (C(4′)); 77.6 (C(3′)); 73.7, 73.5, 72.1 (2*s* and *d* (73.5), *J* = 2.0, *C*H_2–C(4′), Bn); 41.4 (C(5′)); 11.8 (Me). FAB-MS: 566 ([*M* + H]$^+$). Anal. calc. for $C_{27}H_{27}F_4N_3O_6$: C 57.34, H 4.81, N 7.43; found: C 57.37, H 4.74, N 7.37.

1-{2,5-Dideoxy-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl}thymine (**25**). As described for **15**, with **24** (242 mg, 0.43 mmol), abs. EtOH (7 ml), and 5% Pd/C (110 mg). Purification by CC (2–5% MeOH/CH_2Cl_2) afforded **25** (154 mg, 92%). White solid material. ^{1}H-NMR (CD_3OD): 7.82 (*d*, *J* = 1.1, H–C(6)); 6.17 (*dd*, *J* = 3.1, 17.2, H–C(1′)); 5.31–5.10 (*ddd*, *J* = 3.2, 5.4, 53.8, H–C(2′)); 4.64 (*dd*, *J* = 5.6, 17.2, H–C(3′)); 3.75–3.38 (*m*, 2 H–C(5′), CH_2–C(4′)); 1.67 (*d*, *J* = 1.0, Me). ^{13}C-NMR (CD_3OD): 166.6 (C(4)); 159.5 (*q*, *J* = 37, *C*OCF_3); 152.4 (C(2)); 138.3 (C(6)); 117.6 (*q*, *J* = 287, CO*C*F_3); 111.8 (C(5)); 95.0 (*d*, *J* = 190, C(2′)); 89.7 (*d*, *J* = 34, C(1′)); 88.8 (C(4′)); 71.2 (*d*, *J* = 15, C(3′)); 63.7 (*C*H_2–C(4′)); 41.7 (*d*, *J* = 4.1, C(5′)); 12.3 (Me). FAB-MS: 386 ([*M* + H]$^+$). Anal. calc. for $C_{13}H_{15}F_4N_3O_6 \cdot 0.25\ H_2O$: C 40.06, H 4.01, N 10.78; found: C 40.09, H 3.89, N 10.48.

*1-{2,5-Dideoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl}thymine* (**26**). As described for **16**, with **25** (145 mg, 0.38 mmol), anh. pyridine (4 ml), and $(MeO)_2$TrCl (206 mg, 0.61 mmol). Purification by CC (1% MeOH/CH_2Cl_2 containing 0.5% of pyridine) afforded **26** (246 mg, 87%). Yellowish solid material. ^{1}H-NMR ($CDCl_3$): 9.69 (br. *s*, N H); 7.38–7.14 (10 H, $(MeO)_2$*Tr*, H–C(6)); 7.09 (*t*, *J* = 6.3, $NHCOCF_3$); 6.82 (*dd*, *J* = 3.6, 9.2, 4 H, $(MeO)_2$*Tr*); 6.05 (*dd*, *J* = 4.0, 16.8, H–C(1′)); 5.44–5.23 (*ddd*, *J* = 4.4, 4.6, 54.1, H–C(2′)); 4.65 (*dd*, *J* = 5.5, 11.9, H–C(3′)); 4.30 (br. *s*, OH–C(3′)); 3.76, 3.77 (2*s*, 2 MeO); 3.72–3.62 (*m*, 2 H–C(5′)); 3.41 (*d*, *J* = 10.8, 1 H, CH_2–C(4′)); 3.34 (*d*, *J* = 10.5, 1 H, CH_2–C(4′)); 1.60 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 164.0 (C(4)); 158.9 ($(MeO)_2$*Tr*); 159.5 (*q*, *J* = 37, *C*OCF_3); 150.6 (C(2)); 144.0 ($(MeO)_2$*Tr*); 136.9, 136.5, 134.8 (C(6), $(MeO)_2$Tr); 130.1, 129.1, 128.3, 128.1, 125.3 ($(MeO)_2$*Tr*); 115.7 (*q*, *J* = 288, CO*C*F_3); 113.3 ($(MeO)_2$*Tr*); 112.0 (C(5)); 92.7 (*d*, *J* = 191, C(2′)); 89.1 (*d*, *J* = 33, C(1′)); 87.6, 86.8 (C(4′), $C(Ar_3)$); 71.8 (*d*, *J* = 16, C(3′)); 66.0 (*C*H_2–C(4′)); 55.1 (2 MeO); 41.1 (C(5′)); 11.6 (Me). FAB-MS: 687 ([*M*]$^+$). Anal. calc. for $C_{34}H_{33}F_4N_3O_8 \cdot 0.6C_7H_8$: C 61.75, H 5.13, N 5.66; found: C 61.96, H 5.16, N 5.89.

*1-(5-Deoxy-4-*C*-{[(4,4′-dimethoxytrityl)oxy]methyl}-2-fluoro-5-[(trifluoroacetyl)amino]-α-L-lyxofuranosyl)-thymine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**27**). As described for **17**, with **26** (246 mg, 0.36 mmol), anh. CH_2Cl_2 (4 ml), ^{i}Pr_2EtN (0.16 ml, 0.94 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (0.11 ml, 0.47 mmol). After purification by CC (1% Et_3N/CH_2Cl_2), the product was redissolved in anh. toluene and precipitated in vigorously stirred petroleum ether (40 ml) at −30°. A second purification by CC (0.5% Et_3N/CH_2Cl_2) afforded **27** (245 mg, 76%). White solid material. ^{31}P-NMR ($CDCl_3$): 154.2 (*dd*, *J* = 9.4, 18.2); 152.9 (*d*, *J* = 18.7). FAB-MS: 888 ([*M* + H]$^+$).

*1-{3,5-Di-*O*-benzyl-3-*C*-[3-(phthalimido)propyl]-β-D-ribofuranosyl}thymine* (**30**). To a soln. of **29** [19] (3.47 g, 6.99 mmol) in anh. THF (10 ml), phthalimide (1.23 g, 8.39 mmol) and PPh_3 (2.29 g, 8.74 mmol) were added, and the mixture was cooled to 0°. A soln. of diethyl diazenedicarboxylate (1.37 ml, 8.67 mmol) in anh. THF (3 ml) cooled to 0° was added dropwise within 15 min, and the mixture was stirred for 12 h at r.t. AcOEt (60 ml) was added, and the mixture was washed successively with sat. aq. NaCl soln. (2 × 50 ml) and H_2O (2 × 50 ml). The aq. phases were extracted with AcOEt (2 × 50 ml), and the combined org. phase was dried (Na_2SO_4) and evaporated. Purification by CC (30–90% AcOEt/petroleum ether) afforded **30** (4.34 g), contaminated with PPh_3(O). ^{1}H-NMR ($CDCl_3$): 8.35 (*s*, NH); 7.81–7.19 (*m*, Bn, H–C(6), phth, PPh_3(O)); 6.10

(*d*, *J* = 7.8, H–C(1′)); 4.51 (*m*, 4 H, Bn); 4.37 (br. *s*, H–C(4′)); 4.16 (*dd*, *J* = 7.8, 11.5, H–C(2′)); 3.83 (*dd*, *J* = 2.9, 10.7, H_b–C(5′)); 3.75 (*t*, *J* = 6.4, 2 H–C(3″)); 3.52 (*d*, *J* = 9.9, H_a–C(5′)); 2.92 (*d*, *J* = 11.4, OH–C(2′)); 2.21–1.88 (*m*, 2 H–C(1″), 2 H–C(2″)); 1.44 (3 H, *d*, *J* = 0.8, Me). ^{13}C-NMR ($CDCl_3$): 168.6 (phth); 163.5 (C(4)); 151.1 (C(2)); 137.1, 136.7, 136.1, 134.1, 133.3 (Bn, C(6), phth); 132.2–131.9 (several signals, Bn, phth, PPh_3(O)); 128.8–127.4 (several signals, Bn, phth, PPh_3(O)); 123.3 (phth); 111.3 (C(5)); 87.3, 82.6, 81.0, 79.1 (C(1′), C(2′), C(3′), C(4′)); 73.6, 69.8, 65.6 (Bn, C(5′)); 38.1 (C(3″)); 27.6, 22.4 (C(1″), C(2″)); 11.7 (Me). FAB-MS: 626 ($[M+H]^+$). HR-FAB-MS: 626.2584 ($C_{35}H_{36}N_3O_8^+$; calc. 626.2502).

1-{3,5-Di-O*-benzyl-2*-O*-[(pentafluorophenoxy)thiocarbonyl]-3*-C*-[3-(phthalimido)propyl]-β-*D*-ribofuranosyl}thymine* (**31**). To a soln. of **30** (3.96 g, contaminated with PPh_3(O)) in anh. CH_2Cl_2 (20 ml) under Ar, DMAP (1.39 g, 11.4 mmol) was added and the soln. cooled to −15°. *O*-(Pentafluorophenyl) carbonochloridothioate (1.32 ml, 8.2 mmol) was added dropwise, and the soln. was stirred for 12 h at r.t. The mixture was poured into ice-cold H_2O (70 ml), stirred for 30 min, and filtered through a thin layer of *Celite*. The mixture was successively washed with H_2O (30 ml), sat. aq. $CuSO_4$ soln. (2 × 50 ml), and sat. aq. NaCl soln. (2 × 25 ml). All the aq. phases were extracted with CH_2Cl_2 (25 ml each), and the combined org. phase was dried (Na_2SO_4) and evaporated. Purification by CC (0–3% MeOH/CH_2Cl_2 containing 0.25% of pyridine) afforded **31** (1.23 g, 27%, two steps) as a yellowish solid material. In addition, a fraction (1.78 g) consisting of unreacted starting material and PPh_3(O) was isolated. **31**: ^{1}H-NMR ($CDCl_3$): 8.47 (*s*, NH); 7.78–7.62 (*m*, 5 H, H–C(6), phth); 7.38–7.24 (*m*, 10 H, Bn); 6.59 (*d*, *J* = 8.1, H–C(2′)); 6.16 (*d*, *J* = 8.0, H–C(1′)); 4.71 (*d*, *J* = 11.4, 1 H, Bn); 4.58 (*d*, *J* = 11.5, 1 H, Bn); 4.54 (*s*, 2 H, Bn); 4.42 (br. *s*, H–C(4′)); 3.92 (*dd*, *J* = 2.4, 11.2, H_b–C(5′)); 3.73–3.61 (*m*, H_a–C(5′), 2 H–C(3″)); 2.10–1.80 (*m*, 2 H–C(1″), 2 H–C(2″)); 1.35 (*d*, *J* = 0.7, Me). ^{13}C-NMR ($CDCl_3$): 191.8 (C=S); 168.4 (phth); 163.6 (C(4)); 150.6 (C(2)); 137.4, 136.4, 135.9, 134.1, 131.9, 128.7, 128.6, 128.3, 127.8, 127.4, 127.1 (Bn, C(6), phth); 123.2 (phth); 111.4 (C(5)); 87.6, 83.9, 83.4, 82.6 (C(1′), C(2′), C(3′), C(4′)); 73.6, 69.5, 65.1 (Bn, C(5′)); 37.7 (C(3″)); 26.9, 22.3 (C(1″), C(2″)); 11.5 (Me). FAB-MS: 852 ($[M+H]^+$).

3′,5′-Di-O*-benzyl-3′*-C*-[3-(phthalimido)propyl]thymidine* (**32**). Through a soln. of **31** (1.18 g, 1.39 mmol) in anh. benzene (4 ml), Ar was bubbled for 15 min with stirring. Then, 2,2′-azobis[isobutyronitrile] (AIBN; 0.114 g, 0.70 mmol) was added, and bubbling with Ar was continued for further 15 min. The temp. was raised to 90°, Bu_3SnH (0.56 ml, 2.1 mmol) added, and the mixture heated under reflux for 1 h, then allowed to cool to r.t., and subsequently evaporated. Purification by CC (1. petroleum ether, then 0–2% MeOH/CH_2Cl_2; 2. petroleum ether, then 0–1% MeOH/CH_2Cl_2) and prep. TLC (1. 2% MeOH/CH_2Cl_2; 2. 4% MeOH/CH_2Cl_2) afforded **32** (208 mg, 25%). White solid material. ^{1}H-NMR ($CDCl_3$): 8.52 (*s*, NH); 7.81–7.61 (*m*, 5 H, H–C(6), phth); 7.36–7.15 (*m*, 10 H, Bn); 6.39 (*dd*, *J* = 5.3, 9.5, H–C(2′)); 4.50–4.38 (*m*, 4 H, Bn); 4.27 (br. *s*, H–C(4′)); 3.82 (*dd*, *J* = 3.0, 11.0, H_b–C(5′)); 3.74–3.67 (*m*, 2 H–C(3″)); 3.56 (*d*, *J* = 9.9, H_a–C(5′)); 2.53 (*dd*, *J* = 5.0, 13.0, H_b–C(2′)); 2.00–1.77 (*m*, H_a–C(2′), 2 H–C(1″)); 1.48 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 168.5 (phth); 163.8 (C(4)); 150.4 (C(2)); 137.8, 136.9, 136.3, 134.1, 131.9, 128.7, 128.5, 128.1, 127.7, 127.4, 123.3 (Bn, C(6), phth)); 110.7 (C(5)); 86.4, 84.3, 83.1 (C(1′), C(3′), C(4′)); 73.5, 70.2, 64.1 (Bn, C(5′)); 41.6 (C(2′)); 37.8 (C(3″)); 27.6, 23.5 (C(1″), C(2″)); 11.8 (Me). HR-FAB-MS: 610.2505 ($C_{35}H_{36}N_3O_7^+$: calc. 610.2553).

3′-C*-[3-(Phthalimido)propyl]thymidine* (**33**). To a soln. of **32** (0.326 mg, 0.53 mmol) in abs. EtOH (1 ml) and anh. dioxane (0.5 ml), 20% Pd(OH)/C (100 mg) was added. The mixture was degassed and H_2 introduced *via* a balloon. The black soln. was stirred for 3 days under H_2 at r.t. (additional catalyst (100 mg) was added after 24 h and 48 h). Then 10% MeOH/CH_2Cl_2 was added and the mixture stirred for 12 h. After filtration through a *Celite* pad, which was washed thoroughly with MeOH, the filtrate was evaporated. Purification by CC (0–3% MeOH/CH_2Cl_2) afforded **33** (42 mg, 19%). White solid material. ^{1}H-NMR (CD_3OD): 8.04 (*d*, *J* = 1.0, H–C(6)); 7.83–7.75 (*m*, 4 H, phth); 6.30 (*dd*, *J* = 5.3, 9.3, H–C(1′)); 3.86 (*d*, *J* = 3.0, H–C(4′)); 3.74–3.63 (*m*, 2 H–C(5′), 2 H–C(3″)); 2.19 (*dd*, *J* = 5.4, 12.7, H_b–C(2′)); 2.03 (*dd*, *J* = 9.4, 12.6, H_a–C(2′)); 1.83 (*d*, *J* = 1.2, Me); 1.78–1.71 (*m*, 2 H–C(1″), 2 H–C(2″)). ^{13}C-NMR (CD_3OD): 170.1 (phth); 166.6 (C(4)); 152.7 (C(2)); 138.8, 135.5, 133.5 (C(6), phth); 124.2 (phth); 111.4 (C(5)); 90.4, 86.1, 81.8 (C(1′), C(3′), C(4′)); 62.5 (C(5′)); 44.0 (C(2′)); 39.1 (C(3″)); 33.6, 24.6 (C(1″), C(2″)); 12.4 (Me). FAB-MS: 430 ($[M+H]^+$).

5′-O*-(4,4′-Dimethoxytrityl)-3′*-C*-[3-(phthalimido)propyl]thymidine* (**34**) [20]. To a soln. of **33** (55 mg, 0.13 mmol) in anh. pyridine (1 ml) at r.t., $(MeO)_2$TrCl (95 mg, 0.28 mmol) was added in one portion, and the mixture was stirred at r.t. for 12 h. MeOH (0.20 ml) was added to quench the reaction, and the mixture was evaporated. The residue was redissolved in CH_2Cl_2 (5 ml) and the org. phase washed with sat. aq. NaCl soln. (3 × 5 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–2% MeOH/CH_2Cl_2 containing 1% of pyridine) afforded **34** (54 mg, 57%). White solid material. ^{1}H-NMR ($CDCl_3$): 9.06 (br. *s*, NH); 7.84–7.63 (2*m*, 5 H, H–C(6), phth); 7.38–7.22 (*m*, 9 H, $(MeO)_2$*Tr*); 6.83 (*dd*, *J* = 2.2, 9.0, 4 H, $(MeO)_2$*Tr*); 6.49 (*dd*, *J* = 5.0, 9.6, H–C(1′)); 4.05 (br. *s*, H–C(4′)); 3.78 (*s*, 2 MeO); 3.61 (*dd*, *J* = 3.5, 10.5, H_b–C(5′)); 3.58–3.47 (*m*, 2 H–C(3″)); 3.14 (*dd*, *J* = 2.4, 10.8, H_a–C(5′)); 2.38 (*dd*, *J* = 5.3, 12.8, H_b–C(2′)); 2.09 (*dd*, *J* = 9.8, 12.4,

H_a–C(2′)); 1.75–1.45 (*m*, 2 H–C(1″), 2 H–C(2″)); 1.21 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 168.6 (phth); 163.9 (C(4)); 158.9 ((MeO)$_2$*Tr*); 150.7 (C(2)); 143.8 ((MeO)$_2$*Tr*); 136.2, 136.1, 135.0, 134.8, 134.1, 132.0, 130.3, 130.3, 128.5, 128.0, 127.4, 123.3 (C(6), (MeO)$_2$*Tr*, phth); 113.3 ((MeO)$_2$Tr); 111.3 (C(5)); 87.9, 87.3, 84.2, 81.0 (C(1′), C(3′), C(4′), C(Ar)$_3$); 62.7 (C(5′)); 55.2 (2 MeO); 44.0 (C(2′)); 37.9 (C(3″)); 31.8, 23.4 (C(1″), C(2″)); 11.2 (Me). FAB-MS: 731 (M^+). NMR Data: in accordance with [20].

*5′-*O*-(4,4′-Dimethoxytrityl)-3′-*C*-[3-(phthalimido)propyl]thymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**35**). To a mixture of **34** (54 mg, 0.074 mmol), anh. CH_2Cl_2 (2 ml), and ^{i}Pr$_2$EtN (0.05 ml, 0.30 mmol) at 0°, 2-cyanoethyl diisopropylphosphoramidochloridite (0.036 ml, 0.15 mmol) was added dropwise, and the mixture was stirred for 12 h at r.t. The reaction was quenched with sat. aq. $NaHCO_3$ soln. (0.1 ml) and diluted with AcOEt (5 ml). The mixture was washed with sat. aq. $NaHCO_3$ soln. (4 × 5 ml) and the org. phase dried (Na_2SO_4) and evaporated. Purification by CC (AcOEt/CH_2Cl_2/petroleum ether 10:35:1:54) afforded **35** (28 mg, 41%). White solid material. ^{31}P-NMR ($CDCl_3$): 141.0, 140.3. FAB-MS: 932 (M^+).

*1-[3,5-Di-*O*-benzyl-3-*C*-(3-hydroxypropyl)-2-*O*-methyl-β-*D*-ribofuranosyl]thymine* (**37**) *and 1-{3,5-Di-*O*-benzyl-3-*C*-[(*RS*)-2-hydroxypropyl]-2-*O*-methyl-β-*D*-ribofuranosyl}thymine* (**38**). To a soln. of **36** [34] (703 mg, 1.43 mmol) in anh. THF (6 ml), 7.8M BH_3 in 1,4-oxathiane (0.22 ml, 1.72 mmol) was added dropwise at r.t., and stirring was continued for 90 min. After cooling to 0° 2M aq. NaOH (0.86 ml, 1.72 mmol) was dropwise added, followed by the addition of 35% aq. H_2O_2 soln. (w/w, 0.2 ml). The mixture was allowed to warm to r.t. and, after stirring for 1 h, poured into ice-cold H_2O (20 ml). The mixture was filtered and extracted with CH_2Cl_2 (3 × 10 ml). The combined org. phase was washed successively with H_2O (3 × 5 ml) and sat. aq. $NaHCO_3$ soln. (5 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–2.5% MeOH/CH_2Cl_2) afforded as the less polar product **38** (98 mg, 13%) as a diastereoisomer mixture and **37** (400 mg, 54%) as the most polar compound.

Data of mixture **38**: ^{1}H-NMR ($CDCl_3$): 8.91 (br. *s*); 7.82 (*d*, *J* = 1.2); 7.80 (*d*, *J* = 1.3); 7.42–7.26 (*m*); 6.54 (*d*, *J* = 7.8); 6.40 (*d*, *J* = 7.7); 4.85 (*d*, *J* = 11.5); 4.67–4.51 (*m*); 4.31 (br. *s*); 4.21–4.17 (*m*); 4.00 (*d*, *J* = 7.7); 3.81–3.77 (*m*); 3.66 (*dd*, *J* = 1.6, 11.0); 3.51–3.34 (*m*); 3.44 (*s*, MeO); 3.39 (*s*, MeO); 3.19 (*d*, *J* = 1.5); 2.05–1.91 (*m*); 1.83 (br. *s*); 1.62 (*d*, *J* = 1.0, Me); 1.57 (*d*, *J* = 0.9, Me); 1.23, 1.21 (2*s*, 2 H–C(3″)). ^{13}C-NMR ($CDCl_3$): 163.8, 163.7 (2 C(4)); 150.6 (2 C(2)); 138.0, 137.8, 136.8, 136.5, 136.2, 136.0 (Bn, 2 C(6)); 128.9, 128.8, 128.6, 128.5, 128.0, 127.8, 127.7, 127.6, 127.3, 126.9 (Bn); 111.7, 111.5 (2 C(5)); 86.4, 86.3, 85.5, 85.2, 84.7, 84.2, 82.6, 81.5 (2 C(1′), 2 C(2′), 2 C(3′), 2 C(4′)); 73.7, 73.6, 69.8, 69.5, 65.7, 64.9, 64.4, 62.7, 59.0, 58.2 (2 MeO, Bn, 2 C(5′), 2 C(2″)); 40.5, 37.9 (2 C(1″)); 23.6, 23.2, (2 C(3″)); 12.0, 11.9 (2 Me). FAB-MS: 511 ($[M+H]^+$). Anal. calc. for $C_{28}H_{34}N_2O_7 \cdot 0.25\ H_2O$: C 65.29, H 6.75, N 5.44; found: C 65.41, H 6.80, N 5.10.

Data of **37**: ^{1}H-NMR ($CDCl_3$): 8.75 (br. *s*, NH); 7.77 (*d*, *J* = 1.0, H–C(6)); 7.42–7.25 (*m*, 10 H, Bn); 6.36 (*d*, *J* = 7.9, H–C(1′)); 4.92 (*d*, *J* = 11.9, 1 H, Bn); 4.61 (*s*, 2 H, Bn); 4.59 (*d*, *J* = 9.8, 1 H, Bn); 4.20 (*d*, *J* = 2.6, H–C(4′)); 4.00 (*d*, *J* = 7.9, H–C(2′)); 3.78 (*dd*, *J* = 2.8, 11.0, H_b–C(5′)); 3.65 (*m*, H_a–C(5′), 2 H–C(3″)); 3.39 (*s*, MeO); 2.22–2.16 (*m*, H_b–C(2″)); 2.00–1.78 (*m*, 2 H–C(1″)); 1.62 (*m*, H_a–C(2″)); 1.57 (*d*, *J* = 1.0, Me). ^{13}C-NMR ($CDCl_3$): 163.8 (C(4)); 150.7 (C(2)); 138.9, 137.0, 136.5 (Bn, C(6)); 128.8, 128.4, 128.3, 127.8, 127.4, 127.1 (Bn); 111.3 (C(5)); 87.7, 85.7, 83.6, 83.5 (C(1′), C(2′), C(3′), C(4′)); 73.6, 69.6, 65.6, 62.6 (Bn, C(5′), C(3″)); 59.4 (MeO); 26.2, 26.1 (C(1″), C(2″)); 11.9 (Me). FAB-MS: 511 ($[M+H]^+$). Anal. calc. for $C_{28}H_{34}N_2O_7 \cdot 0.25\ H_2O$: C 65.29, H 6.75, N 5.44; found: C 65.36, H 6.66, N 5.32.

*1-{3,5-Di-*O*-benzyl-2-*O*-methyl-3-*C*-[3-(phthalimido)propyl]-β-*D*-ribofuranosyl}thymine* (**39**). To a soln. of **37** (560 mg, 1.10 mmol) in anh. THF (2 ml) under Ar, phthalimide (0.194 g, 1.32 mmol) and PPh_3 (0.362 g, 1.38 mmol) were added, and the mixture was cooled to 0°. A soln. of diethyl diazenedicarboxylate (0.24 ml, 1.36 mmol) in anh. THF (0.3 ml) cooled to 0° was added dropwise during 15 min, and the mixture was stirred for 12 h at r.t. AcOEt (10 ml) was added and the mixture washed successively with a sat. aq. NaCl soln. (2 × 10 ml) and H_2O (2 × 10 ml). The aq. phases were extracted with AcOEt (2 × 5 ml). The combined org. phase was dried (Na_2SO_4) and evaporated. Purification by CC (40–80% AcOEt/petroleum ether) afforded **39** (140 mg, 20%), a fraction (417 mg) consisting of **39** and $PPh_3(O)$, and a fraction (137 mg) consisting of unreacted **37** and $PPh_3(O)$. ^{1}H-NMR ($CDCl_3$): 8.69 (br. *s*, NH); 7.80–7.63 (*m*, 5 H, H–C(6), phth); 7.36–7.20 (*m*, 10 H, Bn); 6.36 (*d*, *J* = 8.0, H–C(1′)); 4.89 (*d*, *J* = 11.6, 1 H, Bn); 4.57 (*d*, *J* = 11.6, 1 H, Bn); 4.48 (*d*, *J* = 11.6, 1 H, Bn); 4.42 (*d*, *J* = 11.5, 1 H, Bn); 4.18 (br. *s*, H–C(4′)); 3.94 (*d*, *J* = 8.0, H–C(2′)); 3.73 (*m*, H_b–C(5′), 2 H–C(3″)); 3.57 (*d*, *J* = 10.8, H_a–C(5′)); 3.36 (*s*, MeO); 2.20–1.80 (*m*, 2 H–C(1″), 2 H–C(2″)); 1.48 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 168.5 (phth); 163.7 (C(4)); 150.6 (C(2)); 138.6, 136.8, 136.2, 134.1, 131.9, 128.8, 128.4, 128.3, 127.5, 127.4, 127.2 (Bn, C(6), phth); 123.3 (phth); 111.3 (C(5)); 87.5, 85.5, 83.3 (C(1′), C(2′), C(3′), C(4′)); 73.5, 69.8, 65.8 (Bn, C(5′)); 59.3 (MeO); 38.0 (C(3″)); 27.2, 22.5 (C(1″), C(2″)); 11.8 (Me). FAB-MS: 640 ($[M+H]^+$). Anal. calc. for $C_{36}H_{37}N_3O_8 \cdot 0.5\ H_2O$: C 66.65, H 5.90, N 6.48; found: C 66.69, H 5.84, N 6.53.

*1-{2-*O*-Methyl-3-*C*-[3-(phthalimido)propyl]-β-*D*-ribofuranosyl}thymine* (**40**). To a soln. of **39** (120 mg, 0.19 mmol) in anh. CH_2Cl_2 (5 ml) at −78°, 1M BCl_3 in hexane (0.75 ml, 0.75 mmol) was added dropwise during

15 min with stirring. After 2 h, the mixture was allowed to slowly warm up to $-20°$, and after further stirring for 6 h, MeOH (3 ml) was added. The mixture was stirred for 12 h at r.t. followed by evaporation and co-evaporation several times with MeOH. Purification by CC (0–4% MeOH/CH_2Cl_2) afforded **40** (73 mg, 85%). White solid material. ^{1}H-NMR ((D_6)DMSO): 11.3 (br. *s*, NH); 7.97 (*d*, *J* = 0.8, H–C(6)); 7.90–7.81 (*m*, 4 H, phth); 5.94 (*d*, *J* = 7.9, H–C(1′)); 5.24 (*t*, *J* = 3.8, OH–C(5′)); 4.81 (*s*, OH–C(3′)); 3.76 (*d*, *J* = 7.4, H–C(2′)); 3.73 (br. *s*, H–C(4′)); 3.61–3.53 (*m*, 2 H–C(5′), 2 H–C(3″)); 3.23 (*s*, MeO); 1.76 (*d*, *J* = 0.8, Me); 1.81–1.68 (*m*, 2 H–C(1″), 2 H–C(2″)). ^{13}C-NMR ((D_6)DMSO): 168.4 (phth); 164.0 (C(4)); 151.2 (C(2)); 136.9, 134.7, 132.0, 123.4 (C(6), phth); 110.2 (C(5)); 86.8, 85.6, 85.3, 78.4 (C(1′), C(2′), C(3′), C(4′)); 61.0, 58.6 (MeO, C(5′)); 38.4 (C(3″)); 31.0, 22.8 (C(1″), C(2″)); 12.7 (Me). HR-FAB-MS: 460.1780 ($C_{22}H_{26}N_3O_8^+$; calc. 460.1720).

*1-{5-*O*-(4,4′-Dimethoxytrityl)-2-*O*-methyl-3-*C*-[3-(phthalimido)propyl]-β-*D*-ribofuranosyl}thymine* (**41**). To a soln. of **40** (85 mg, 0.19 mmol) in anh. pyridine (1.5 ml) at r.t., $(MeO)_2$TrCl (125 mg, 0.37 mmol) was added in one portion, and the mixture was stirred for 12 h, whereupon MeOH (0.25 ml) was added to quench the reaction. After stirring for 10 min, the mixture was evaporated, the residue dissolved in CH_2Cl_2 (5 ml), and the soln. washed with sat. aq. NaCl soln. (3 × 5 ml), dried (Na_2SO_4), and evaporated. Purification by CC (0–1.5% MeOH in CH_2Cl_2/pyridine 99 : 1) afforded **41** (117 mg, 77%). Yellowish solid material. ^{1}H-NMR ($CDCl_3$): 8.73 (br. *s*, NH); 7.83–7.69 (*m*, 5 H, H–C(6), phth); 7.40–7.16 (*m*, 9 H, $(MeO)_2$*Tr*); 6.86 (*dd*, *J* = 1.6, 9.1, 4 H, $(MeO)_2$*Tr*); 6.19 (*d*, *J* = 6.6, H–C(1′)); 4.10 (br. *s*, H–C(4′)); 3.91 (*d*, *J* = 6.8, H–C(2′)); 3.80, 3.79 (2*s*, 2 MeO); 3.70 (*dd*, *J* = 3.0, 10.9, H_b–C(5′)); 3.51 (*s*, MeO); 3.41–3.24 (*m*, H_a–C(5′), 2 H–C(3″)); 2.87 (*s*, OH–C(3′)); 1.69–1.50 (*m*, 2 H–C(1″), 2 H–C(2″)); 1.14 (*d*, *J* = 0.8, Me). ^{13}C-NMR ($CDCl_3$): 168.4 (phth), 163.7 (C(4)); 159.1 ($(MeO)_2$*Tr*); 150.6 (C(2)); 143.5 ($(MeO)_2$*Tr*); 136.1, 134.6, 134.0, 132.1, 130.5, 129.1, 128.3, 128.1, 127.6, 136.1, 125.4, 123.3 (C(6), $(MeO)_2$*Tr*, phth); 113.3 ($(MeO)_2$*Tr*); 111.8 (C(5)); 87.7, 86.2, 85.7, 85.1, 78.7 (C(1′), C(2′), C(3′), C(4′), $C(Ar_3)$); 62.2 (C(5′)); 59.1 (MeO); 55.2 (2 MeO); 37.9 (C(3″)); 30.5, 22.6 (C(1″), C(2″)); 11.0 (Me). FAB-MS: 761 (M^+). Anal. calc. for $C_{43}H_{43}N_3O_{10} \cdot 0.6\ C_7H_8$: C 69.41, H 5.90, N 5.14; found: C 69.30, H 6.07, N 5.03.

*1-{5-*O*-(4,4′-Dimethoxytrityl)-2-*O*-methyl-3-*C*-[3-(phthalimido)propyl]-β-*D*-ribofuranosyl}thymine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**42**). To a soln. of **41** (109 mg, 0.14 mmol) in anh. CH_2Cl_2 (2 ml) and ^{i}Pr$_2$EtN (0.20 ml, 1.20 mmol) under Ar at 0°, 2-cyanoethyl diisopropylphosphoramidochloridite (0.14 ml, 0.58 mmol) was added dropwise, and the mixture was stirred for 12 h at r.t. The reaction was quenched by addition of sat. aq. $NaHCO_3$ soln. (0.1 ml), and the resulting mixture was diluted with AcOEt (5 ml) and washed with a sat. aq. $NaHCO_3$ soln. (4 × 5 ml). The org. phase was dried (Na_2SO_4) and evaporated. After purification by CC (1. 0–1% Et_3N/CH_2Cl_2; 2. AcOEt/CH_2Cl_2/Et_3N/petroleum ether 15 : 45 : 1 : 39; 3. AcOEt/CH_2Cl_2/Et_3N/petroleum ether 15 : 35 : 1 : 49), the residue was dissolved in anh. toluene (1 ml) and precipitated in vigorously stirred petroleum ether (250 ml) at $-50°$: **42** (45 mg, 34%). White solid material. ^{31}P-NMR ($CDCl_3$): 141.3, 141.2. FAB-MS: 984 ($[M + Na]^+$).

Oligonucleotide Synthesis. All ONs were prepared on a *Biosearch 8700* DNA synthesizer by the phosphoramidite approach as described earlier [61] with *tert*-butyl hydroperoxide as oxidant for ONs containing units **Z** or **W**, and with a mixture of I_2, pyridine, and H_2O as oxidant for ONs containing units **X** or **Y**. After completion of the sequences, deprotection with conc. NH_3 in MeOH (32% (*w*/*w*), 55°, 12 h) of 5′-*O*-$(MeO)_2$Tr-on ONs and reversed-phase cartridge purification yielded the final ON products, which by capillary gel electrophoresis were shown to be > 85% pure. The composition of the modified ONs was verified by MALDI-MS analysis.

Thermal Stability Studies. Melting temperatures (T_m values) were determined as described earlier [25]. A medium salt buffer and a high salt buffer with compositions as indicated in *Table 1* were used.

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