PERKIN 1

Automated synthesis of branched oligodeoxynucleotide analogues using *arabino*-uridine as branching nucleotide

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Fully automated synthesis of branched oligodeoxynucleotides (branched ODNs) has been accomplished using 2′-*O*-levulinyl- or 5′-*O*-levulinyl-protected *arabino*-uridine derivatives as branching monomers. Selective removal of the levulinyl groups is accomplished using 0.5 M hydrazine hydrate in a pyridine–acetic acid–water (4:3:0.35, v/v/v) buffer. The affinity of the branched ODNs towards complementary DNA has been evaluated at 260 nm and 284 nm during thermal denaturing experiments. Enhanced affinity of a branched ODN compared with the corresponding linear reference is attributed to bimolecular triple helix formation.

Introduction

Recently, development of Y-shaped branched oligodeoxynucleotides (branched ODNs) as possible probes for high-affinity targeting of complementary ODNs has been attempted using the strategies outlined in Fig. 1A,[1–4] the rationale being that full-length duplex formation (Watson–Crick hydrogen bonds) between the branched DNA segment and the target is followed by alignment of the branch in the major groove of the duplex thus forming a triple helix (Hoogsteen hydrogen bonds). For entropic reasons, increased affinities and selectivities for such bimolecular complexes are envisaged in analogy with results reported for circular oligonucleotides.[5,6]

The hybridisation results obtained by Azhayeva *et al.*[2] employing 3′-deoxypsicothymidine (structure **A**, Fig. 2) as branching monomer were rather disappointing. Thus, two-phasic transitions and appreciable decreases in melting points (mps) compared with linear controls were obtained. These results were explained by the unnatural structure of the branching point nucleoside and destabilisation caused by the presence of the branch. We have used 1-(2-methyl-β-D-arabinofuranosyl)uracil (structure **B**, Fig. 2) as branching monomer[3,4] to give branched ODN analogues capable of hybridising to target ODNs as depicted in Fig. 1A. Increased thermal stabilities compared with the corresponding linear reference containing one modified monomer (structure **B**, 2′-*O*-unbranched, Fig. 2) were obtained in monophasic transitions.

However, to achieve thermal stabilities comparable to or even higher than those of unmodified controls, a more favourable branching-point geometry seems necessary. Earlier, we have shown an only moderately destabilising effect of incorporating one 2′-*O*-methylarabinonucleoside into 11-mer or 17-mer ODNs.[7] Decreases in mps of 1–6 °C per modification, compared with observed decreases of 11–14 °C for one unbranched 1-(2-methyl-β-D-arabinofuranosyl)uracil monomer **B**,[3,4] were achieved. Therefore, we decided to synthesize and evaluate the novel branched ODNs **B**–**M** (Tables 1–3) using *arabino*-uridine [1-(β-D-arabinofuranosyl)uracil] as branching monomer utilising the 2′-hydroxy group oriented into the major groove as attachment site for the branch. Besides the potential entropic advantages, the C-type branched ODNs (Fig. 1) might be capable of forming π-interactions at the ends of the two triplex-forming strands thereby inducing cooperativity between the two strands which has been reported to increase the stability of a related complex.[8]

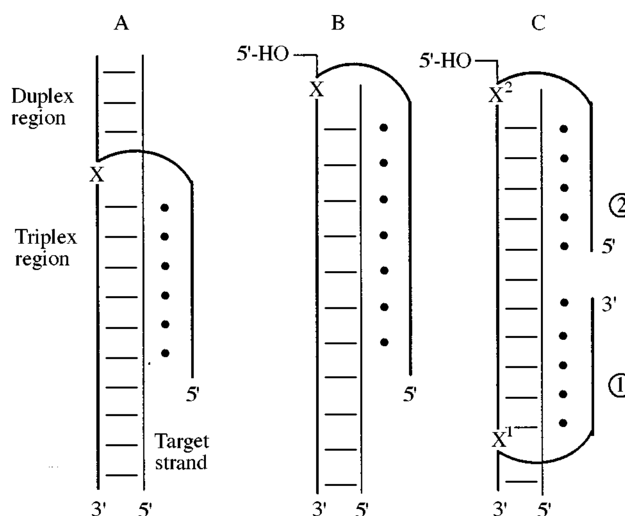

Fig. 1 Schematic representation of the structures of complexes formed between branched ODNs and complementary oligonucleotides

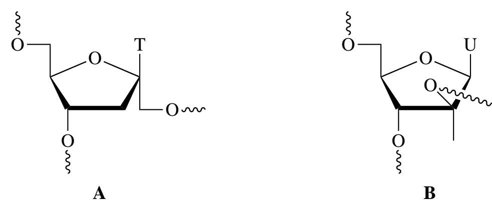

Fig. 2 Structures around branching nucleotides in previously described branched ODNs used in oligonucleotide targeting as depicted in Fig. 1A. T = thymin-1-yl, U = uracil-1-yl.

Results and discussion

Nucleoside **1**[9] was levulinated using levulinic anhydride and 4-(dimethylamino)pyridine (DMAP) in anhydrous diethyl ether

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Table 1 Sequences of synthesized branched ODNs (plus non-branched reference strand **A**) and measured mps (T_m) from hybridisation with complementary dA_{12}

	Sequence [a]	T_m/°C [b]	Triple helix [c]
A	3′-TTTTTTTT-$_{3'}$X$_{5'}$-TTT-5′	30	
B	3′-TTTTTTTT-$_{3'}$X$_{5'}$-TTT-5′ 5′-TTTTTTTCC-$^{2'}$	27	+
C	3′-TTTTTTTT-$_{3'}$X$_{5'}$-TTT-5′ 5′-TTTTTTTCCC-$^{2'}$	27	+
D	3′-TTTTTTTT-$_{3'}$X$_{5'}$-TTT-5′ 5′-TTTTTTTCCCC-$^{2'}$	27	+
E	3′-TTTTTTTT-$_{3'}$X$_{5'}$-TTT-5′ 5′-TTTGTTTCC-$^{2'}$	24	
F	3′-TTTTTTTT-$_{3'}$X$_{5'}$-TTT-5′ 5′-TGTGTGTCC-$^{2'}$	23	

[a] C = 2′-Deoxycytidine; G = 2′-deoxyquanosine; T = thymidine; X = modified ara-U nucleotide. [b] Mps were determined as the maximum of the first derivative of the absorption *vs.* temperature curve at 260 nm. [c] + Denotes confirmation of transition at 284 nm.

Table 2 Sequences of synthesized branched ODNs of the B-type and measured mps (T_m) from hybridisation with complementary dA_{12}

	Sequence [a]	T_m/°C [b]	Triple helix [c]
G	3′-CATTTTTTTTTTT-$_{3'}$X 5′-TTTTTT-$^{2'}$	37.6	
H	3′-CATTTTTTTTTTT-$_{3'}$X 5′-TTTTTTTTTT-$^{2'}$	41.2	+
I	3′-CATTTTTTTTTTT-$_{3'}$X 5′-TTTTTTCCCCC-$^{2'}$	35.8	+
J	3′-CATTTTTTTTTTT-$_{3'}$X 5′-TTTTTTTTTTCCCCC-$^{2'}$	41.0	+
K	3′-CATTTTTTTTTTT-$_{3'}$X 5′-TTTTTGTTTT-$^{2'}$	29.8	

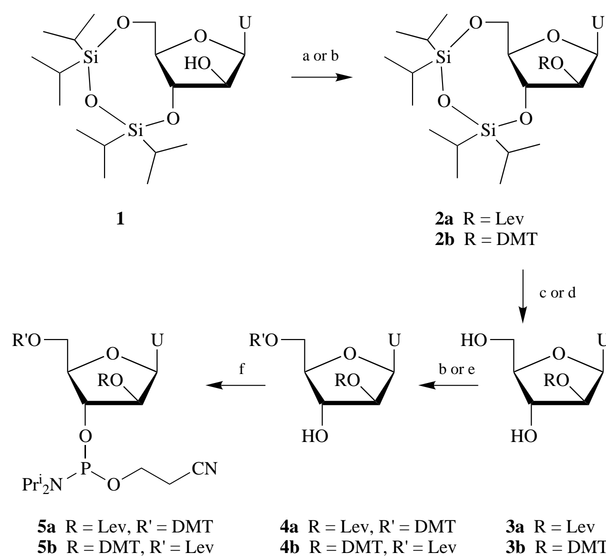
[a] C = 2′-Deoxycytidine; A = 2′-deoxyadenosine; G = 2′-deoxyguanosine; T = thymidine; X = modified ara-U nucleotide. [b] Mps were determined as the maximum of the first derivative of the absorption *vs.* temperature curve at 260 nm. [c] + Denotes confirmation of transition at 284 nm.

Table 3 Sequences of synthesized branched ODNs of the C-type and measured mps (T_m) from hybridisation with complementary dA_{12}

	Type [a]	① [a,b]	② [a,b]	T_m/°C [c]	Triple helix [d]
L	C	T_4-3′	T_4-5′	29.6	
M	C	T_8-3′	T_8-5′	34.0	+

[a] Refers to Fig. 1. [b] T = Thymidine. [c] Mps were determined as the maximum of the first derivative of the absorption *vs.* temperature curve at 260 nm. [d] + Denotes confirmation of transition at 284 nm.

and anhydrous pyridine to give 1-[2-*O*-levulinyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil **2a** in 87% yield. Desilylation using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) to give compound **3a** in 76% yield followed by selective protection of the 5′-hydroxy functionality by reaction with 4,4′-dimethoxytrityl chloride (DMTCl) and silver nitrate in anhydrous pyridine afforded 1-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*-levulinyl-β-D-arabinofuranosyl]uracil **4a** in 96% yield. Subsequent phosphitylation of the 3′-hydroxy group using 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite and *N,N*-diisopropylamine in dichloromethane gave the desired amidite **5a** in 67% yield after precipitation from light petroleum (Scheme 1). 4,4′-Dimethoxytritylation of compound **1** using silver nitrate in anhydrous THF afforded nucleoside **2b** in quantitative yield. Subsequent desilylation to give compound **3b** was achieved in 97% yield using $KF \cdot 2H_2O$ in THF. Selective levulination of the primary hydroxy group using the enzyme Novozym 435® under conditions described earlier[10] afforded derivative **4b** in 93% yield. The corresponding acylation with pyridine as base afforded the undesired diacylated by-product in addition to compound **4b**.



Scheme 1 *Reagents:* (a) Levulinic anhydride, DMAP, pyridine; (b) DMTCl, $AgNO_3$, THF, pyridine; (c) TBAF, THF; (d) $KF \cdot 2H_2O$, 18-crown-6, THF; (e) levulinic anhydride, Novozym 435®, 1,4-dioxane; (f) 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite, *N,N*-diisopropylethylamine, CH_2Cl_2. DMT = 4,4′-dimethoxytrityl: Lev = levulinyl; U = uracil-1-yl.

Nucleoside **4b** was phosphitylated as described above for synthesis of compound **5a** to give phosphoramidite **5b** in 64% yield.

All ODNs were synthesized using standard phosphoramidite chemistry[11] with cycles including detritylation, coupling, capping and oxidation. Synthesis of the type A branched ODNs (Fig. 1) was initiated by constructing the linear strand using standard amidite concentration (0.1 M) and coupling time (2 min). However, when incorporating the modified nucleotide **X**, a 0.15 M solution of amidite **5a** and a coupling time of 12 min were applied. A separate end-capping cycle (consisting of detritylation and acetylation) was used to block further linear elongation at the 5′-end, thus preparing for branching. The support was treated with 0.5 M hydrazine hydrate in pyridine–acetic acid (4:1, v/v) for 90 min to remove the 2′-*O*-levulinyl protecting group. The first nucleotide in the branch was subsequently attached at the 2′-hydroxy group of nucleotide **X** by 12 min couplings at standard amidite concentration whereupon the remaining part of the branch was synthesized using standard conditions. The ODNs of the B- and C-type (Fig. 1) were synthesized by using compound **5b** at standard concentration and a coupling time of 2 × 12 min (coupling, washing, coupling with a fresh solution of amidite, capping, oxidation and detritylation). This procedure increased the coupling efficiency of amidite **5b** from ~65% (single coupling) to ~95%. As the next step, the first nucleotide in the branch originating from the 2′-hydroxy group was attached using a coupling time of 2 × 12 min. After finishing this branch, the looped B-type ODNs were subjected to standard ammonolytic deprotection which, besides nucleobase and phosphate deprotection and cleavage from the support, effected delevulination, thus liberating the 5′-hydroxy group of monomer **X**. Synthesis of the C-type branched and looped ODNs was accomplished using commercially available 'reversed' (5′-*O*-phosphitylated and 3′-*O*-4,4′-dimethoxytritylated) phosphoramidites in branch ① (Fig. 1C). After completion of branch ①, end-capping at the 3′-end was performed as described above. Subsequently, the support was treated with a freshly prepared 0.5 M solution of hydrazine hydrate in a buffer consisting of pyridine–acetic acid–water (4:3:0.35, v/v/v) for 5 min in order selectively to 5′-*O*-delevulinate at the branching point $\mathbf{X^1}$. The remaining part of the type-C ODNs were synthesized using amidite **5b** (to incorporate monomer $\mathbf{X^2}$) and standard phosphoramidites. The

DMT group of the last nucleotide incorporated was in all syntheses left on for the purpose of purification. The coupling yields determined by a VIS spectrophotometer as the amount of the DMT carbenium ion released during each detritylation step was >99% for commercial amidites and ~60% for the modified amidite **5a**. The branched ODNs were cleaved from the solid support and deprotected by treatment with 32% aq. ammonia for 72 h at room temperature. The crude ODNs were desalted, purified and detritylated on disposable reversed-phase cartridges, taking advantage of the hydrophobicity of the DMT group. The composition of ODNs **C**, **F**, **H**, **J** and **M**, chosen as representative probes, was confirmed by matrix-assisted laser desorption mass spectrometry (**C**: Found, 6589 Da. Calc., 6587 Da; **F**: Found, 6374 Da. Calc., 6373 Da; **H**: Found, 7237 Da. Calc., 7235 Da; **J**: Found, 8678 Da. Calc., 8681 Da; **M**: Found, 9060 Da. Calc., 9062 Da. The purity was confirmed for all ODNs using capillary gel electrophoresis.

The ODNs were designed to orient the additional branches parallel to the complementary dA_{12} strand which should allow the formation of stable triple helices.[12] The sequence of branching in the A-type oligodeoxynucleotides was varied to evaluate the effect of the length of the cytidine linker. Furthermore, thymidines in the branch were replaced with guanosines to test the effect of mismatches on triple-helix formation and thermal stability. Mp (T_m) determinations were carried out in medium salt buffer as described earlier.[4] The strand dissociations (Tables 1–3) were detected through hyperchromicities at 260 nm and 284 nm, the latter being characteristic for triple-helix dissociations.[13,14] The insertion of the modified nucleotide **X** without attaching an additional branch (ODN **A**) causes a drop in T_m of 6 °C compared with unmodified controls (T_{12} and 3′-T_8dUT_3-5′). As expected, no transition was detected at 284 nm with the linear ODN **A**. For the branched ODNs **B**–**D**, triple-helix dissociations were confirmed, and, as expected, ODNs **E** and **F** having mismatches in the branch showed no sign of dissociation when measured at 284 nm. It may therefore be concluded that the T_8-branches in ODNs **B**–**D** are involved in complexes with dA_{12} as depicted in Fig. 1A. The mp of mismatched ODNs **E** and **F** reveal that a non-binding branch has a significant destabilising effect on the duplex (decrease in T_m compared with that of **A**: ~7 °C). This could, at least in part, be due to unfavourable steric interactions at the branching point. This effect is partly compensated for by the stabilising effect of triple-helix formation as seen for ODNs **B**–**D**, but the mps are still lower than for the linear reference **A**. The results for the different linkers indicate that the length of the cytidine linker (C_2–C_4) is of no importance.

The looped ODNs **G**–**K** (Fig. 1B, Table 2) show, in contrast to earlier results for unmodified triplex-forming back-looped ODNs,[15] that there seems to be no preference for the sequence with a five-nucleotide loop *versus* the corresponding sequence without a connecting loop (ODN **J** *versus* ODN **H**). This supports our original idea that direct entropically favoured back-folding should be possible having an attachment site oriented into the major groove. However, incorporation of the modification causes a decrease in the mp of 4 °C (3′-CAT_{12}-5′: 28.2 °C, 3′-CAT_{11}X-5′: 24.0 °C), a decrease that can be overcome by bimolecular triplex formation (Table 2, ONDs **H** and **J**). The looped ODNs form triplexes of comparable stability to complexes formed between a target strand and unmodified ODNs with a loop consisting of five nucleotides (3′-$CAT_{12}C_5T_6$-5′: 36.0 °C, 3′-$CAT_{12}C_5T_{10}$-5′: 40.4 °C). Incorporation of one or two mismatches in the triplex-forming strand causes a decrease in the mp and no detectable transition at 284 nm, indicating the expected selectivity in the binding of the third strand, and also showing that binding involving the third strand causes stabilisation of the complex.

The looped and branched ODNs **L** and **M** (Fig. 1C, Table 3) show that eight nucleotides in the third strand are sufficient for triplex formation, whereas four are not. Triplex formation for ODN **M** causes an increase in the T_m to 34 °C compared with 28.5 °C for the corresponding linear ODN (3′-$CAXT_{10}$X-5′). The results indicate that the two branches do not interact cooperatively and do not stabilise the triplexes by π-interactions. In contrast to the A-type ODNs, there seems to be no destabilising effect in the B- and C-type ODNs due to branches not involved in Hoogsteen hydrogen bonding. This may originate from the difference in incorporating **X** in the middle (in A-type ODNs) or in the ends, the latter generally known to be less detrimental to duplex formation. In all cases where triplex structures were found, concomitant dissociation of the triplex and duplex complexes was observed (monophasic bimolecular transitions), indicating a cooperativity between the Watson–Crick bonds and the Hoogsteen bonds.

To summarise, synthesis of branched ODNs using *arabino*-uridine as branching point **X** has been accomplished. The synthetic strategy allows synthesis of branched ODNs with sequences of arbitrary length and base composition. The thermal stability of branched ODNs is increased compared with the branched mismatched references. The melting results for the looped and branched ODNs showed that eight nucleotides in the additional branch are sufficient to achieve a stable triplex structure. However, compared with the corresponding unmodified linear reference, no significant increase in T_m could be obtained. This is probably due to disruption of the Watson–Crick duplex structure caused by the modification **X** as well as unfavourable steric interactions in the branching point, *e.g.* between the nucleobase and the 2′-phosphate group. This suggests that arabinonucleosides containing short, non-phosphorus 2′-*O*-linkers should be examined as the next class of *arabino*-configurated branched ODNs.

Experimental

NMR spectra were obtained on a Bruker AC250 spectrometer at 250 MHz for ^{1}H NMR, 63 MHz for ^{13}C NMR and 101 MHz for ^{31}P NMR spectroscopy. δ-Values are reported in ppm relative to internal $SiMe_4$ for ^{1}H and ^{13}C and relative to external 85% H_3PO_4 for ^{13}P spectra. All coupling constants are in Hz. Fast-atom bombardment mass spectrometry (FAB-MS) was performed on a Kratos MS 50 RF spectrometer. The enzyme Novozym 435® is commercially available from Novo Nordisk A/S, Bagsvaerd, Denmark. Unmodified and reversed (5′-*O*-phosphitylated and 3′-*O*-4,4′-dimethoxytritylated) amidites were purchased from Cruachem. Matrix-assisted laser desorption mass spectrometry was performed using a Micromass TofSpec E mass spectrometer using a matrix of diammonium hydrogen citrate and 2,6-dihydroxyacetophenone. Capillary gel electrophoresis was performed using a Beckman P/ACE System 5000 (ss DNA 100 Gel Column). Light petroleum refers to the fraction with distillation range 60–80 °C.

1-[2′-*O*-Levulinyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil 2a

Compound **1**[9] (497 mg, 1.02 mmol) was dissolved in anhydrous pyridine (10 cm^3) and DMAP (6 mg, 0.05 mmol) was added. The mixture was kept under N_2 and cooled to 0 °C. An ethereal solution of levulinic anhydride (3.1 cm^3, 3.1 mmol) was added dropwise. After being stirred for 1.5 h, the reaction was quenched with saturated aq. $NaHCO_3$ (45 cm^3). The mixture was extracted with ethyl acetate (2 × 100 cm^3) and the organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was purified by column chromatography (5% MeOH in CH_2Cl_2, v/v) to give compound **2a** as a foam (522 mg, 87%), δ_H($CDCl_3$) 0.9–1.2 (m, 28 H, 4 × Pr^i), 2.2 (s, 3 H, CH_3), 2.4–2.8 (m, 4 H, CH_2CH_2), 3.8 (m, 1 H, 4′-H), 4.0–4.2 (m, 2 H, 5′-H_2), 4.4 (t, *J* 8.3, 1 H, 3′-H), 5.6 (dd, *J* 6.3 and 7.9, 1 H, 2′-H), 5.7 (d, *J* 8.2, 1 H, 5-H), 6.2 (d, *J* 6.2, 1 H, 1′-H), 7.6 (d, *J* 8.2, 1 H, 6-H) and 8.8 (br, 1 H, NH); δ_C($CDCl_3$) 12.3–17.4 (TIPDS), 27.4 (CH_3), 29.7 (CH_2), 37.5 (CH_2), 60.3 (C-5′), 71.1

(C-3′), 75.9 (C-2′), 80.6 and 81.7 (C-1′, C-4′), 101.9 (C-5), 140.3 (C-6), 149.9 (C-2), 163.0 (C-4), 171.3 (C=O) and 205.7 (C=O).

1-(2′-*O*-Levulinyl-β-D-arabinofuranosyl)uracil 3a
Compound **2a** (495 mg, 0.85 mmol) was dissolved in anhydrous THF (25 cm^3) under N_2 and a solution of TBAF in THF (2.5 cm^3, 2.5 mmol) was added. After being stirred for 15 min, the reaction was complete, and the mixture was loaded directly onto a silica gel column where it was eluted with 10% MeOH in CH_2Cl_2 (v/v) to give compound **3a** as a pale yellow foam (221 mg, 76%). $\delta_H(CD_3OD)$ 2.3 (s, 3 H, CH_3), 2.5–2.9 (m, 4 H, CH_2CH_2), 3.8–4.3 (m, 6 H, 3′- and 4′-H, 5′-H_2, 3′- and 5′-OH), 5.4 (dd, *J* 3.9 and 5.0, 1 H, 2′-H), 5.8 (d, *J* 8.1, 1 H, 5-H), 6.4 (d, *J* 5.0, 1 H, 1′-H) and 7.9 (d, *J* 8.1, 1 H, 6-H); $\delta_C(CD_3OD)$ 28.6 (CH_3), 29.5 (CH_2), 38.4 (CH_2), 61.8 (C-5′), 74.5 (C-3′), 79.1 (C-2′), 84.9 and 85.3 (C-1′ and -4′), 102.0 (C-5), 143.2 (C-6), 152.0 (C-2), 166.26 (C-4), 172.9 (C=O) and 208.7 (C=O).

1-[5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-levulinyl-β-D-arabinofuranosyl]uracil 4a
Compound **3a** (162.5 mg, 0.47 mmol) was dissolved in anhydrous THF (15 cm^3) under argon and anhydrous pyridine (1 cm^3) was added. Silver nitrate (89 mg, 0.52 mmol) and DMTCl (177 mg, 0.52 mmol) were added and the mixture was stirred at room temperature for 24 h. The mixture was filtered, poured into 5% aq. $NaHCO_3$ (10 cm^3) and was extracted with ethyl acetate (2 × 50 cm^3). The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was purified by column chromatography (5% MeOH in CH_2Cl_2, v/v) to give compound **4a** as a pale yellow foam (287 mg, 96%), $\delta_H(CDCl_3)$ 2.1 (s, 3 H, CH_3), 2.4–2.8 (m, 4 H, CH_2CH_2), 3.5 (m, 2 H, 5′-H_2), 3.6 (br, 1 H, 3′-OH), 3.8 (s, 6 H, OCH_3), 4.0 (m, 1 H, 4′-H), 4.4–4.5 (m, 1 H, 3′-H), 5.3 (dd, *J* 4.7 and 5.6, 1 H, 2′-H), 5.5 (d, *J* 8.1, 1 H, 5-H), 6.3 (d, *J* 5.7, 1 H, 1′-H), 7.1–7.5 (m, 13 H, ArH), 7.7 (d, *J* 8.2, 1 H, 6-H) and 9.0 (br, 1 H, NH); $\delta_H(CDCl_3)$ 27.7 (CH_3), 29.6 (CH_2), 37.7 (CH_2), 55.1 (OCH_3), 55.2 (OCH_3), 61.7 (C-5′), 74.2 (C-3′), 79.0 (C-2′), 81.9, 83.2 and 86.7 (C-1′ and -4′, *C*Ar_3), 101.6 (C-5), 113.2, 113.3, 127.0, 127.9, 128.1, 130.1, 130.1, 135.4 and 135.5 (aryl), 141.0 (C-6), 144.4 (aryl), 150.0 (C-2), 158.6 (aryl), 163.1 (C-4), 172.4 (C=O) and 206.1 (C=O).

1-{3′-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-levulinyl-β-D-arabinofuranosyl}-uracil 5a
Nucleoside **4a** (192 mg, 0.298 mmol) was coevaporated with anhydrous acetonitrile (3 × 2.5 cm^3) and dried overnight *in vacuo*. After dissolution of compound **4a** in anhydrous CH_2Cl_2 (1.5 cm^3) under argon, *N*,*N*-diisopropylethylamine (0.20 cm^3, 1.2 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.13 cm^3, 0.48 mmol) was added under stirring at room temperature. After 1 h, the reaction was quenched with MeOH (1 cm^3), dissolved in ethyl acetate (6 cm^3) and washed successively with saturated aq. $NaHCO_3$ (3 × 5 cm^3) and saturated aq. NaCl (3 × 5 cm^3). The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was dissolved in toluene (1 cm^3) and the product was precipitated from light petroleum (70 cm^3; cooled to −65 °C) under vigorous stirring. The precipitate was collected by filtration, re-dissolved and coevaporated with anhydrous acetonitrile (3 × 2.5 cm^3) to give compound **5a** as a foam (168 mg, 67%), $\delta_P(CDCl_3)$ 151.8 and 151.9.

1-[2′-*O*-(4,4′-Dimethoxytrityl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil 2b
Compound **1** [9] (1.62 g, 3.33 mmol) was coevaporated with anhydrous pyridine (2 × 5 cm^3) and dissolved in a mixture of anhydrous THF (30 cm^3) and anhydrous pyridine (3 cm^3) under argon. Silver nitrate (2.64 g, 15.5 mmol) was added followed by DMTCl (5.33 g, 15.7 mmol) and the mixture was stirred overnight. The reaction mixture was subsequently filtered through a layer of silica gel, and evaporated *in vacuo*. The residue was taken up in ethyl acetate and washed successively with saturated aq. $NaHCO_3$ (3 × 20 cm^3) and saturated aq. NaCl (2 × 20 cm^3), and the organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was purified by column chromatography (20–40% ethyl acetate–light petroleum, 1% pyridine) to give compound **2b** as a yellow foam (2.62 g, 100%), $\delta_H(CDCl_3)$ 1.0–1.1 (m, 28 H, Pr^i), 3.7 (m, 1 H, 4′-H), 3.8 (s, 6 H, OCH_3), 3.9–4.6 (m, 4 H, 2′- and 3′-H, 5′-H_2), 5.5 (d, *J* 5.0, 1 H, 1′-H), 5.6 (d, *J* 8.2, 1 H, 5-H), 6.8 (m, 4 H, ArH), 7.1–7.4 (m, 9 H, ArH), 7.5 (d, *J* 8.2, 1 H, 6-H) and 8.4 (s, 1 H, NH); $\delta_C(CDCl_3)$ 12.8–17.3 (Pr^i), 55.1 (OCH_3), 61.8 (C-5′), 75.1 (C-2′), 77.4 (C-3′), 80.7, 81.8 and 88.4 (C-1′ and -4′, *C*Ar_3), 101.0 (C-5), 113.2, 127.2–135.1, 142.8 and 144.2 (aryl), 149.6 (C-2), 158.9 (aryl) and 162.7 (C-4); FAB-MS: *m/z* 811 (M + 23).

1-[2′-*O*-(4,4′-Dimethoxytrityl)-β-D-arabinofuranosyl]uracil 3b
Compound **2b** (1.97 g, 2.50 mmol) was dissolved in THF (36 cm^3). KF·2H_2O (1.42 g, 15 mmol) and 18-crown-6 (233 mg, 0.88 mmol) was added to the vigorously stirred solution at room temperature. After 48 h, the reaction mixture was filtered, evaporated *in vacuo* and the residue was purified by column chromatography (2–5% MeOH in CH_2Cl_2 containing 1% pyridine, v/v/v) to give compound **3b** as a foam (1.33 g, 97%); $\delta_H(CD_3OD)$ 3.4–3.5 (m, 1 H, 4′-H), 3.7–3.9 (m, 3 H, 3′-H and 5′-H_2)), 3.9 (s, 6 H, OCH_3), 4.4 (dd, *J* 3.1 and 4.6, 1 H, 2′-H), 5.8 (d, *J* 8.1, 1 H, 5-H), 6.1 (d, *J* 4.4, 1 H, 1′-H), 6.9–7.5 (m, 13 H, ArH) and 8.1 (d, *J* 7.0, 1 H, 6-H); $\delta_C(CD_3OD)$ 56.0 (OCH_3), 63.1 (C-5′), 76.8 (C-2′), 79.3 (C-3′), 85.9, 86.0 and 89.9 (C-1′ and -4′, *C*Ar_3), 101.8 (C-5), 114.6, 128.5–132.0 and 136.9 (aryl), 145.7 (C-6), 146.5 (aryl), 152.1 (C-2), 160.8 (aryl) and 166.5 (C-4); FAB-MS: *m/z* 569 (M + 23).

1-[2′-*O*-(4,4′-Dimethoxytrityl)-5′-*O*-levulinyl-ß-D-arabinofuranosyl]uracil 4b
Compound **3b** (306 mg, 0.56 mmol) was dissolved in anhydrous dioxane (3 cm^3) under argon. Novozym 435® (200 mg) was added followed by a freshly prepared solution of levulinic anhydride in 1,4-dioxane (10 cm^3, 3.68 mmol), and the mixture was stirred overnight at room temperature. The mixture was filtered, washed with saturated aq. $NaHCO_3$ (3 × 5 cm^3), and the organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was purified by column chromatography (1–5% MeOH in CH_2Cl_2 containing 1% pyridine, v/v/v) to give compound **4b** as a pale yellow foam (332 mg, 93%), $\delta_H(CDCl_3)$ 2.2 (s, 3 H, CH_3), 2.6–2.8 (m, 4 H, CH_2CH_2), 3.5 (br, 1 H, 3′-OH), 3.8 (s, 6 H, OCH_3), 3.8–4.4 (m, 5 H, 2′-, 3′- and 4′-H and 5′-H_2), 5.8 (d, *J* 8.1, 1 H, 5-H), 6.1 (br, 1 H, 1′-H), 6.8–6.9 (m, 4 H, ArH), 7.2–7.3 (m, 9 H, ArH), 7.8 (br, 1 H, 6-H) and 9.1 (s, 1 H, NH); $\delta_C(CDCl_3)$ 27.8 and 29.7 (CH_2CH_2), 37.8 (CH_3), 55.1 (OCH_3), 63.1 (C-5′), 76.0 and 78.0 (C-2′ and -3′), 80.2, 84.0 and 88.3 (C-1′ and -4′, *C*Ar_3), 101.4 (C-5), 113.3 and 127.3–135.3 (aryl), 143.0 (C-6), 144.2 (aryl), 150.0 (C-2), 158.8 (aryl), 163.0 (C-4), 172.6 (C=O) and 206.7 (C=O); FAB-MS: *m/z* 667 (M + 23).

1-{3′-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-2′-*O*-(4,4′-dimethoxytrityl)-5′-*O*-levulinyl-β-D-arabinofuranosyl}-uracil 5b
Nucleoside **4b** (291 mg, 0.45 mmol) was coevaporated with anhydrous acetonitrile (3 × 2 cm^3) and re-dissolved in anhydrous CH_2Cl_2 (3.6 cm^3) under argon. *N*,*N*-Diisopropylethylamine (1.7 cm^3, 9.9 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.18 cm^3, 0.67 mmol) were added to the stirred mixture at room temperature. After 1 h, the reaction was quenched with anhydrous methanol (1 cm^3), diluted with ethyl acetate and washed successively with saturated aq. $NaHCO_3$ (3 × 5 cm^3) and NaCl (3 × 5 cm^3).

The organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was purified by column chromatography [a 1:1 (v/v) mixture of light petroleum and a mixture of CH_2Cl_2, ethyl acetate and Et_3N (9:9:2, v/v/v)], evaporated, re-dissolved in toluene (2 cm^3) and precipitated from light petroleum (65 cm^3; −65 °C) under vigorous stirring. The precipitate was collected by filtration as a solid, re-dissolved, and coevaporated with anhydrous acetonitrile (3 × 5 cm^3) to give compound **5b** as a pale yellow foam (243 mg, 64%), δ_P($CDCl_3$) 149.5 and 150.6.

Oligonucleotide synthesis

All oligonucleotides were synthesized on a Pharmacia Gene Assembler Special® synthesizer. Solid supports on a 0.2 μmol scale were obtained from Pharmacia or Cruachem. The amidite solution volume applied for all couplings was 75 μl. The commercial amidites were used in 0.1 M concentration with 2 min coupling time. The modified *arabino*-uridine building block was used in 0.1–0.2 M concentrations and the coupling time was extended to 12 min or 2 × 12 min as described in the Results and discussion section. The levulinyl protection group was removed by treatment of the solid support either with a 0.5 M solution of hydrazine hydrate in pyridine–acetic acid (4:1, v/v) for 90 min or with a 0.5 M solution of hydrazine hydrate in a buffer consisting of pyridine–acetic acid–water (4:3:0.35, v/v/v). The branching step was performed with a 0.1 M amidite solution using 12 min or 2 × 12 min coupling time. For all modified oligonucleotides the DMT group of the latest incorporated nucleotide was left on for purification, but for the unmodified references it was removed as the last step on the synthesizer. The oligonucleotides were deprotected and cleaved from solid support by incubation in 32% aq. ammonia at room temperature for 72 h. All oligonucleotides were desalted through size-exclusion chromatography (NAP™-10-columns, Sephadex® G-25 medium, Pharmacia). Purification of the modified oligonucleotides was achieved by use of disposable reversed-phase columns (COP™-columns, Cruachem; the procedure includes detritylation). The procedures were carried out according to manufacturer's protocols.

Determination of T_m

Mps (T_m) were determined as described earlier.[4]

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