Pergamon

Tetrahedron 54 (1998) 3607–3630

TETRAHEDRON

LNA (Locked Nucleic Acids): Synthesis of the Adenine, Cytosine, Guanine, 5-Methylcytosine, Thymine and Uracil Bicyclonucleoside Monomers, Oligomerisation, and Unprecedented Nucleic Acid Recognition

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Received 31 December 1997; accepted 29 January 1998

Abstract: LNA (Locked Nucleic Acids), consisting of 2'-*O*,4'-*C*-methylene bicyclonucleoside monomers, is efficiently synthesized and its nucleic acid recognition potential evaluated for six different nucleobases, namely adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil. Unprecedented increases (+3 to +8 °C per modification) in the thermal stability of duplexes towards both DNA and RNA were obtained when evaluating mixed sequences of partly or fully modified LNA. Studies of mis-matched sequences show that LNA obey the Watson-Crick base pairing rules with generally improved selectivities compared to the corresponding unmodified reference strands.

INTRODUCTION

During the last decade, partly stimulated by the potential of developing nucleic acid targeted drugs, a large number of nucleic acid analogues have been chemically synthesized,[1-4] *e.g.*, with the goal of developing superior agents for selective high-affinity recognition of single stranded DNA and RNA. A number of these analogues are capable of hybridizing with complementary DNA or RNA (**I**) with increased thermal stabilities compared to the parent DNA:DNA and DNA:RNA duplexes. As illuminating examples, peptide nucleic acids[5,6] (PNA, **II**), 2'-fluoro N3'-P5'-phosphoramidates[7] (**III**) and 1',5'-anhydrohexitol nucleic acids[8,9] (HNA, **IV**) are depicted in Figure 1. These three analogues induce significant positive changes in the melting temperature per modification (ΔT_m, see caption Figure 1) compared to the parent duplexes. However, none of the analogues **II**-**IV**, or any other oligonucleotide (ON) analogue synthesized so far, can be considered ideal with respect to nucleic acid recognition. Thus, parent PNA is only sparingly soluble in an aqueous medium, and the formation of PNA_2:ON triplexes is required for the formation of complexes of thermal stabilities comparable to those of **III** and **IV**.[5-10] Though HNA (**IV**) generally induces excellent thermal stabilities of duplexes towards complementary RNA, the results towards DNA are less convincing, and incorporation of a single HNA monomer

0040-4020/98/$19.00
PII: S0040-4020(98)00094-5

into an otherwise unmodified sequence leads to decreases in melting temperature (T_m). 2'-Fluoro N3'-P5'-phosphoramidates (**III**) are capable of high affinity recognition of both DNA and RNA. However, rather drastic structural changes compared to the natural nucleic acids counterparts, namely introduction of a 2'-fluoro group and a 3'-amino group, are necessary to induce sufficient preorganisation of the pentofuranose ring into an N-type conformation (3'-*endo* conformation) known to predominate for A-type DNA:RNA duplexes (Figure 2). Due to the backbone modification in **III**, standard oligomerization chemistry is not directly applicable which necessitates the use of either dimeric building blocks or slightly changed coupling procedures.[7]

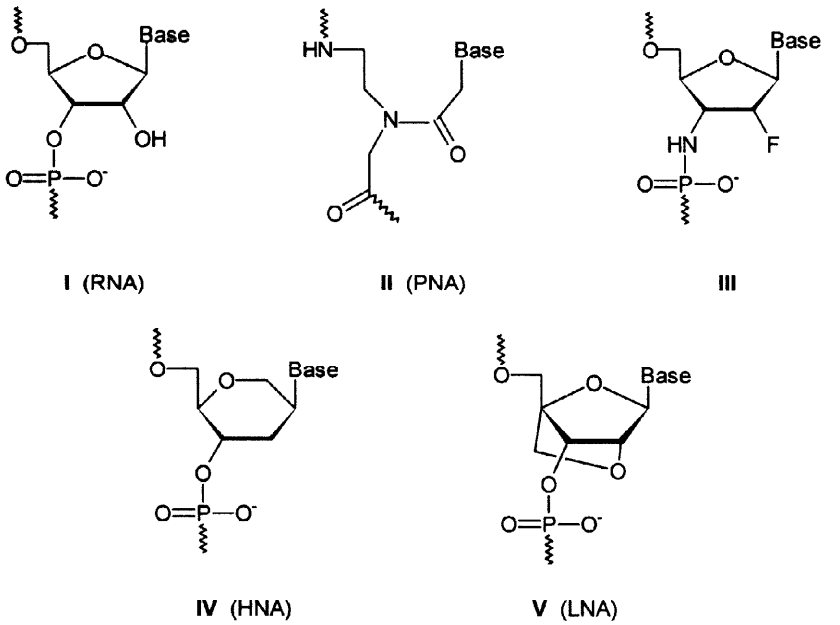


Figure 1. Structures of RNA and selected high-affinity nucleic acid analogues. RNA (**I**, in DNA the 2'-OH group is substituted with a 2'-H); PNA (**II**, ΔT_m ~ +1 to +2 °C for DNA and RNA complements)[5,6]; 2'-Fluoro N3'-P5'-phosphoramidates (**III**, ΔT_m ~ +3 to +5 °C for DNA and RNA complements)[7]; HNA (**IV**, ΔT_m ~ +1 to +3 °C for DNA complements, ΔT_m ~ +3 to +5 °C for RNA complements[8,9]. LNA (**V**). ΔT_m = change in melting temperature per modification. Base = nucleobase.

Based on the results and shortcomings described above for even successfully designed DNA mimics, we have attempted design and synthesis of a nucleic acid analogue containing a minimum of structural changes compared to parent DNA and RNA, but with superior nucleic acid recognition properties. As a possible candidate, we decided to synthesize the novel bicyclic oligonucleotide analogue, LNA (Locked Nucleic Acids, **V**, Figure 1). Promising properties of ONs containing bicyclic monomers have been reported,[3, 11-15] and molecular modelling and simple model building suggested to us that bicyclic LNA nucleoside monomers should be favourably preorganized in an N-type conformation (Figure 2) thus leading to the possible formation of entropically favoured duplexes with complementary DNA and RNA. Independently, another group, apparently stimulated by analogous structural considerations, has shown interest in LNA (termed so by us), and very recently synthesis of the uracil and cytosine nucleosides from uridine has been published.[16] As another attractive point for us, the structural change going from DNA (or RNA) to LNA is rather limited from a chemical perspective, namely the introduction of an additional 2'-*C*,4'-*C*-oxymethylene linker. This ether group is expected

to be of low reactivity, and the physical and chemical properties of LNA compared to the corresponding DNA should not be changed significantly by its introduction.

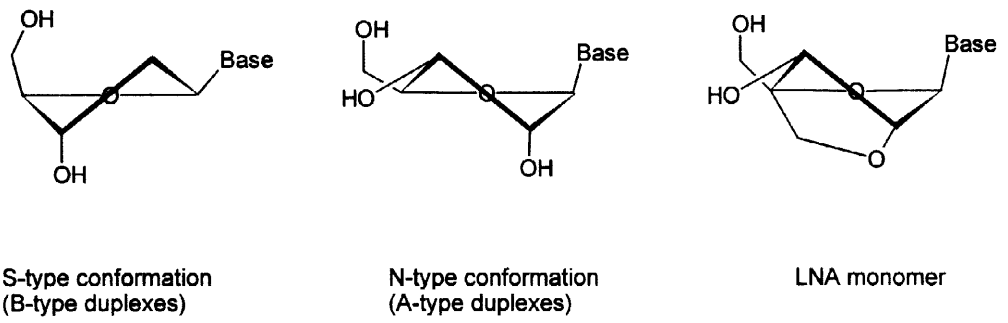
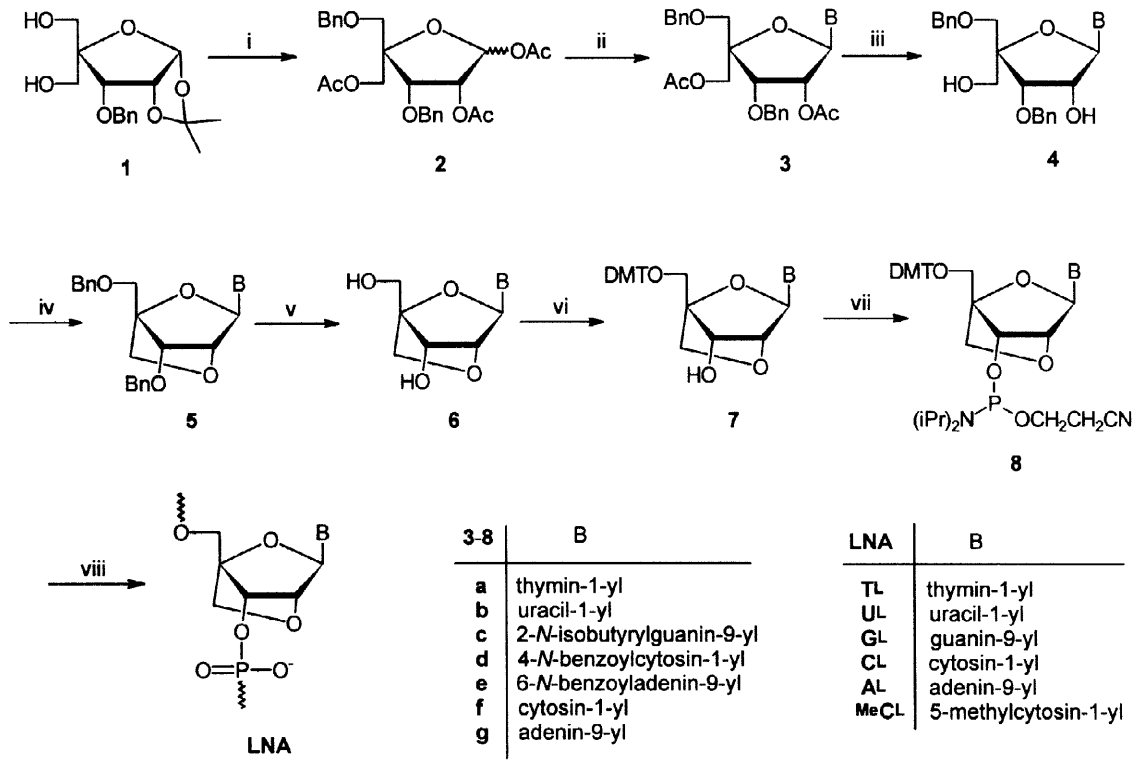


Figure 2. Conformations of pentofuranose nucleoside monomers in nucleic acid duplexes and the expected conformation of LNA monomers.

Synthesis of LNA (the term LNA is used for ONs containing one or more LNA monomer(s)) containing six different nucleobases, namely adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil is described in full detail for the first time. We have in a preliminary way communicated[17] synthesis of a limited number of guanine and thymine containing LNAs together with thermal stability studies of partly modified oligothymidylates and a few partly modified 9-mer mixed sequences (Table 1, entries 1 and (in part) 4; Table 2, entries 1 and 5). In this report, the very appealing features of LNA-mediated nucleic acid recognition are revealed for six different LNA-monomers and for fully modified LNA.

RESULTS AND DISCUSSION

Synthesis of a number of 4'-*C*-hydroxymethyl nucleosides has been reported earlier.[18-22]. For synthesis of the LNA monomers we chose a strategy starting from 4'-*C*-hydroxymethyl pentofuranose derivative **1**[18] (Scheme 1). Regioselective 5-*O*-benzylation, acetylation, and acetolysis followed by another acetylation, afforded in 55% yield the furanose **2**, a key intermediate for coupling reactions with a variety of silylated nucleobases. Stereoselective reaction with silylated thymine[23,24] afforded in 76% yield nucleoside **3a** which was deacetylated in 97% yield to give nucleoside diol **4a**. Tosylation followed by base-induced ring closure afforded the 2'-*O*,4'-*C*-linked bicyclonucleoside derivative **5a** in 42% yield. Debenzylation yielded efficiently the (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane thymine nucleoside analogue **6a**. The assigned structure of **6a** was verified by NMR, including NOE experiments (NOE contacts were observed between 1"-H_b (oxymethylene linker) and 1'-H, 6-H and 3'-H, 5'-OH and 5'-H, 5'-OH and 3'-H, and 5'-OH and 6-H). The absence of a coupling constant between 1'-H and 2'-H (known to be indicative of 1'-H,1'-C:2'-C,2'-H dihedral angles close to 90°),[25,26] and the unusual and strong mutual NOE effects (9%/8%) between 3'-H and 6-H (thymine base) indicate structural preorganization of the pentofuranose ring of **6a** into an N-type conformation. Similar results were obtained by X-ray crystallographic analysis of the corresponding uracil nucleoside **6b**.[16] Nucleoside **6a** was converted into the 5'-*O*-4,4'-dimethoxytrityl (5'-*O*-DMT) protected analogue **7a** in 93% yield and subsequently into the phosphoramidite derivative **8a** in 70% yield thus affording the desired monomeric building block for automated LNA synthesis.



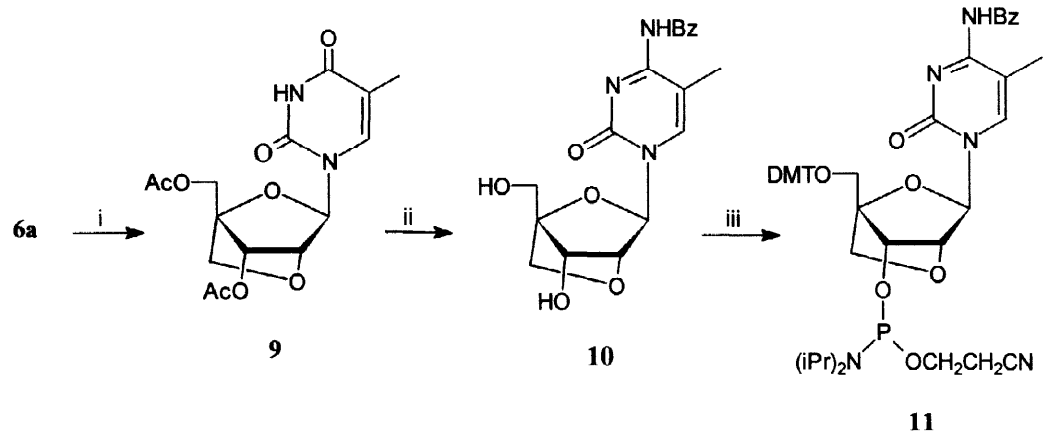
3-8	B
a	thymin-1-yl
b	uracil-1-yl
c	2-*N*-isobutyrylguanin-9-yl
d	4-*N*-benzoylcytosin-1-yl
e	6-*N*-benzoyladenin-9-yl
f	cytosin-1-yl
g	adenin-9-yl

LNA	B
TL	thymin-1-yl
UL	uracil-1-yl
GL	guanin-9-yl
CL	cytosin-1-yl
AL	adenin-9-yl
MeCL	5-methylcytosin-1-yl

Scheme 1. i) a) NaH, BnBr, DMF, b) acetic anhydride, pyridine, c) 80% AcOH,d) acetic anhydride, pyridine; ii) nucleobase, *N,O*-bis(trimethylsilyl)acetamide, TMS-triflate, acetonitrile (or dichloroethane); iii) $NaOCH_3$, methanol; iv) a) *p*-toluenesulphonyl chloride, pyridine, b) NaH, DMF; v) $Pd(OH)_2/C$, ethanol, H_2 (or 1,4-cyclohexadiene, 10% $Pd(OH)_2/C$; methanol, or BCl_3, dichloromethane, hexane); vi) DMTCl, pyridine; vii) *N,N*-diisopropylethylamine, 2-cyanoethyl *N,N*-diisoproylphosphoramidochloridite, dichloromethane; viii) DNA-synthesizer. See text and experimental section for further details.

Analogous synthetic procedures were applied to synthesize the derivatives **3b-3e** by coupling of furanose **2** with uracil, 2-*N*-isobutyrylguanine, 4-*N*-benzoylcytosine and 6-*N*-benzoyladenine (see experimental part for details). The transformation of nucleoside **3b** into phosphoramidite **8b** was accomplished as described for **3a**. Glycosylation using 2-*N*-isobutyrylguanine was performed under thermodynamic control applying conditions described for other glycosyl donors to give mainly the desired N-9 regioisomer.[27] We obtained a mixture of three products which was directly reacted and not separated before ring closure was accomplished to give nucleoside **5c**. The assigned structure of **5c** was further substantiated by the successful recognition of complementary cytosine residues (*vide infra*). Nucleoside **5c** was transformed into phosphoramidite **8c** essentially following the procedure described for **5a**. After cyclization of the 4-*N*-benzoylcytosine and 6-*N*-benzoyladenine nucleosides to give derivatives **5d** and **5e**, respectively, *O*-selective or complete debenzylation proved impossible in our hands. However, by debenzylation of **5d** using 1,4-cyclohexadiene and 10% $Pd(OH)_2/C$ and of **5e** using BCl_3, the fully deprotected bicyclonucleosides **6f** and **6g** were obtained and subsequently converted to the desired phosphoramidite derivatives **8d** and **8e** by transient protection (silylation) of the hydroxy groups, benzoylation of the nucleobases, desilylation, DMT-protection and phosphitylation (see experimental part for details). It was thus possible, using a general glycosylation strategy, to synthesize LNA monomers containing all five natural nucleobases. This approach is different from the one published for the uracil nucleoside, for which a linear strategy was applied.[16]

To obtain the corresponding 5-methylcytosine LNA-monomer, the thymine nucleoside **6a** was converted in 38% yield through the di-*O*-acetate **9** into the 4-*N*-benzoyl-5-methylcytosine nucleoside diol **10** by a general procedure known from the literature[28] (Scheme 2). By subsequent 5'-*O*-DMT-protection and phosphitylation, synthesis of the desired 4-*N*-benzoyl-5-methylcytosine building block **11** was achieved.



Scheme 2. i) Acetic anhydride, pyridine, DMAP; ii) a) 1,2,4-triazole, $POCl_3$, acetonitrile, triethylamine, b) 32% aqueous NH_3, c) benzoyl chloride, pyridine, d) NaOH, H_2O, methanol, pyridine; iii) a) DMTCl, pyridine, b) *N,N*-diisopropylethylamine, 2-cyanoethyl *N,N*-diisoproylphosphoramidochloridite, dichloromethane.

LNAs (Tables 1-4) were effectively synthesized using the phosphoramidite approach.[29] The stepwise coupling efficiencies of the LNA phosphoramidites **8a**, **8b**, **8c**, **8e** and **11** and for unmodified deoxynucleoside phosphoramidites were approximately 99% as determined spectrophotometrically by the release of the DMT-cation after each coupling step. The coupling efficiency of the cytosine phosphoramidite **8d** was slightly lower (approximately 95%) which can be explained by the presence of an impurity. After standard deprotection using 32% aqueous ammonia for 5-10 h at 55 °C, 5'-*O*-DMT-*ON* LNAs were purified by reversed phase chromatography followed by detritylation, whereas 5'-*O*-DMT-*OFF* LNAs were desalted. The purity (>90%) of all LNAs (Tables 1-4) were confirmed by reversed phase HPLC and/or capillary gel electrophoresis and the composition of all synthesized LNAs were confirmed by MALDI-MS (see experimental part for representative data). These results show that LNA phosphoramidites allow very efficient incorporation of LNA monomers into oligonucleotide strands, and by using a commercially available universal solid support, a fully modified LNA was efficiently synthesized.

The thermal stabilities of duplexes involving LNA oligonucleotides were determined towards DNA and RNA complements and compared to their unmodified references (Tables 1-4). Generally, sharp monophasic transitions were obtained with hyperchromicities of 1.2-1.4, and no indication of biphasic transitions was detected. Thermal affinity studies of oligothymidylate LNAs indicated the remarkable nucleic acid recognition potential of LNA as increases in the melting temperatures per LNA monomer of +4 to +6 °C were obtained.[17] More relevant for most applications, however, are the properties of LNAs involving mixed sequences. We chose a 9-mer sequence for our studies, and the results towards matched and singly mis-matched DNA complements are depicted in Table 1 for **LNA 1-LNA 6** containing, in turn, the six different LNA monomers ($\mathbf{X^L}$) flanked by unmodified deoxynucleoside monomers and two $\mathbf{T^L}$ monomers.

5'-d(GT^{L}GAX^{L}AT^{L}GC)-3'
3'-d(CACTYTACG)-5'

Entry	Y / X^{L}	Melting temperature (T_m / °C) A	C	T	G
12	**LNA 1: T^{L}**	**44**	23	27	30
	DNA-reference	**28**	11	12	19
3	**LNA 2: U^{L}**	**44**	24	25	32
45	**LNA 3: G^{L}**	26	**49**	33	28
	DNA-reference	17	**33**	16	15
6	**LNA 4: C^{L}**	32	n.d.	n.d.	**52**
7	**LNA 5: $^{Me}C^{L}$**	32	32	27	**53**
8	**LNA 6: A^{L}**	26	25	**45**	23

Table 1. Melting temperatures towards matched and singly mis-matched complementary DNA for 9-mer LNAs containing three LNA-monomers X^{L} (T^{L}, U^{L}, G^{L}, C^{L}, $^{Me}C^{L}$, A^{L}). A = adenosine monomer, C = cytidine monomer, G = guanosine monomer, T = thymidine monomer. Oligodeoxynucleotide sequences are depicted as d(sequence). n.d. = not determined. T_m values for matched sequences are shown in bold.

The results reveal that the DNA-hybridizing selectivity of mixed sequence LNAs is, if anything, improved compared to the corresponding DNAs. This can be extracted from entries 1, 3, 4 and 6-8 compared to entries 2 and 5 where mis-matched nucleotides in turn were introduced in the position opposite to the middle X^{L} monomers. The decreases in thermal stabilities obtained with these mis-matched ONs were slightly more pronounced for the LNAs than for the reference strands. For all the fully matched LNAs of Table 1, very convincing stabilizing effects were observed (ΔT_m = +5.3 °C for entries 1 and 4, and comparable T_m values were obtained for the LNAs of entries 3 and 6-8; ΔT_m = change in T_m per LNA monomer incorporated).

Results towards complementary RNA are shown in Table 2. Unprecedented increases in the thermal stability (ΔT_m = +7.3 and +8.3 °C, entries 1 and 5 compared to entries 2 and 6, respectively) were observed. From the results of entries 3 and 4 (single central T/C mis-match) it is indicated that the hybridization of LNA with an RNA-complement is associated with satisfactory selectivity.

Entry	Duplex	T_m (°C)
1	5'-d(GT^{L}GAT^{L}AT^{L}GC)-3' / 3'-CACUAUACG-5'	50
2	5'-d(GTGATATGC)-3' / 3'-CACUAUACG-5'	28
3	5'-d(GT^{L}GAT^{L}AT^{L}GC)-3' / 3'-CACUCUACG-5'	33
4	5'-d(GTGATATGC)-3' / 3'-CACUCUACG-5'	10
5	5'-d(GT^{L}GAG^{L}AT^{L}GC)-3' / 3'-CACUCUACG-5'	58
6	5'-d(GTGAGATGC)-3' / 3'-CACUCUACG-5'	33

Table 2. Melting temperatures towards matched and singly mis-matched complementary RNA for 9-mer LNAs containing three LNA-monomers (T^{L}, G^{L}). A = adenosine monomer, C = cytidine monomer, G = guanosine monomer, T = thymidine monomer, U = uridine monomer. Oligodeoxynucleotide sequences are depicted as d(sequence).

The additive effect of LNA-monomers can be extended to a fully modified LNA (Table 3, ΔT_m = +4.0 °C (entry 1) for the DNA complement, ΔT_m = +5.1 °C (entry 3) for the RNA complement). Despite the very high thermal affinities of these duplexes, introduction of a single T/G (entry 2) and T/C (entry 4) mismatch lead to significant depressions in the T_m values.

Entry	Duplex	T_m (°C)
1	5'-$G^LT^LG^LA^LT^LA^LT^LG^L\,^{Me}C^L$-3' / 3'-d(CACTATACG)-5'	64
2	5'- $G^LT^LG^LA^LT^LA^LT^LG^L\,^{Me}C^L$-3' / 3'-d(CACTGTACG)-5'	52
3	5'-$G^LT^LG^LA^LT^LA^LT^LG^L\,^{Me}C^L$-3' / 3'-CACUAUACG-5'	74
4	5'-$G^LT^LG^LA^LT^LA^LT^LG^L\,^{Me}C^L$-3' / 3'-CACUCUACG-5'	60

Table 3. Melting temperatures towards matched and singly mis-matched complementary DNA and RNA for a 9-mer fully modified LNA containing T^L, A^L, G^L and C^L. A = adenosine monomer, C = cytidine monomer, G = guanosine monomer, T = thymidine monomer, U = uridine monomer. Oligodeoxynucleotide sequences are depicted as d(sequence). See Table 1 for reference T_m values.

The results depicted in Table 3 can be extended to 13-mer sequences containing four and twelve LNA monomers (Table 4). Under medium salt conditions, ΔT_m values of +3 °C (towards DNA) and +4.5 °C (towards RNA) were obtained. As the melting temperature of the LNA:RNA duplex of entry 6 was above 92 °C in the standard medium salt buffer, these duplexes were in addition evaluated in a low salt buffer. As expected, significant lowered T_m values resulted both for the duplexes involving LNA and for the corresponding reference duplexes, but the relative increases in T_m values induced by the introduction of LNA monomers were matching those obtained under medium salt conditions.

Entry	Duplex	T_m (°C) medium salt	T_m (°C) low salt
1	5'-d(GGTGGTTTGTTTG)-3'/**ODN1**	47	31
2	5'-d(GGTGG$T^LT^LT^LG^L$TTTG)-3'/**ODN1**	57	40
3	5'- $G^LG^LT^LG^LG^LT^LT^LT^LG^LT^LT^LT^L$dG -3'/**ODN1**	83	67
4	5'-d(GGTGGTTTGTTTG)-3'/**ON2**	52	32
5	5'-d(GGTGG$T^LT^LT^LG^L$TTTG)-3'/**ON2**	70	50
6	5'- $G^LG\,^LT^LG^LG^LT^LT^LT^LG^LT^LT^LT^L$dG -3'/**ON2**	> 92	85

Table 4. Melting temperatures towards complementary DNA and RNA for 13-mer LNAs containing four and twelve LNA-monomers (T^L, G^L). G = guanosine monomer, dG = 2'-deoxyguanosine monomer, T = thymidine monomer. Oligodeoxynucleotide sequences are depicted as d(sequence). **ODN1** and **ON2** are the complementary DNA and RNA 13-mers, respectively. Medium salt: 10 mM Na_2HPO_4 pH 7.0, 100 mM NaCl, 0.1 mM EDTA; Low salt: 10 mM Na_2HPO_4 pH 7.0, 0.1 mM EDTA.

CONCLUSION

In this report it has been illustrated that LNA display unprecedented nucleic acid recognition properties (ΔT_m values +3 to +5 °C towards DNA and +4 to +8 °C towards RNA) which we ascribe to preorganization[3] of LNA monomers and LNA. However, definite conclusions concerning this await further studies. Improved properties were obtained for all six nucleobase analogues synthesized, and apparently pyrimidines and purines have comparable effects. It has been demonstrated that by the subtle structural change going from DNA to LNA, only involving the addition of a oxymethylene biradical to the 2-deoxyribose unit, profound improvements regarding affinity can be obtained without compromising hybridizing selectivity. It can be concluded that LNA obeys the standard Watson-Crick pairing rules and that the positive effect on the thermal affinity of consecutive LNA monomer substitutions in an oligomer are additive. Importantly, incorporation of LNA monomers into an ON is effective following the standard phosphoramidite chemistry thus allowing chemical insertion of an LNA monomer in any position in an (unmodified) ON. These features, proving the viability of the strategy of minimal structural perturbation in a DNA mimic, combined with the reported stability against 3'-exonucleolytic degradation[17] and excellent aqueous solubility, establish LNA as a very attracting novel nucleic acid analogue.

EXPERIMENTAL

All reagents were obtained from commercial suppliers and were used without further purification. After drying any organic phase using Na_2SO_4, filtration was performed. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck. NMR spectra were recorded at 400 MHz or 250 MHz for ^1H NMR and 62.9 MHz for ^{13}C NMR and at 202.33 MHz for ^{31}P NMR. Assignments of NMR peaks are given according to standard nucleoside nomenclature. δ-Values are in ppm relative to tetramethylsilane as internal standard for ^1H NMR and ^{13}C NMR, and relative to 85% H_3PO_4 as external standard for ^{31}P NMR.

3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose.[30] To a solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**1**, 20.1 g, 0.064 mol)[18] in anhydrous DMF (100 cm^3) at -5 °C was added a suspension of NaH (60% in mineral oil (w/w), four portions during 1 h 30 min, total 2.85 g, 0.075 mol). Benzyl bromide (8.9 cm^3, 0.075 mol) was added dropwise and stirring at room temperature was continued for 3 h whereupon ice-cold water (50 cm^3) was added. The mixture was extracted with EtOAc (4 x 100 cm^3) and the combined organic phase was dried (Na_2SO_4). After evaporation, the residue was purified by silica gel column chromatography eluting with 5% EtOAc in petroleum ether (v/v) to yield the product (18.5 g, 71%). δ_C ($CDCl_3$) 138.0, 137.4, 128.5, 128.3, 128.0, 127.8, 127.6 (Bn), 113.5 ($C(CH_3)_2$), 104.4 (C-1), 86.5 (C-4), 78.8, 78.6 (Bn), 73.6, 72.6, 71.6 (C-2, C-3, C-5), 63.2, (C-1'), 26.7, 26.1 (CH_3). Found: C, 68.8; H, 7.1; $C_{23}H_{28}O_6$ requires C, 69.0; H, 7.1%.

4-*C*-Acetoxymethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose. To a solution of 3,5-di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (913 mg, 2.28 mmol) in anhydrous pyridine

(4.5 cm^3) was dropwise added acetic anhydride (1.08 cm^3, 11.4 mmol) and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of ice-cold water (50 cm^3) and extraction was performed with dichloromethane (3 x 50 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (2 x 50 cm^3), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane as eluent to give the product as a clear oil (911 mg, 90%). δ_H ($CDCl_3$) 7.34-7.25 (10 H, m, Bn), 5.77 (1 H, d, *J* 3.6, 1-H), 4.78-4.27 (8 H, m, Bn, H-5_a, H-5_b, H-3, H-2), 3.58 (1 H, d, *J* 10.3, H-$1'_a$), 3.48 (1 H, d, *J* 10.5, H-$1'_b$), 2.04 (3 H, s, $COCH_3$), 1.64 (3 H, s, CH_3), 1.34 (3 H, s, CH_3). δ_C ($CDCl_3$) 171.1 (C=O), 138.2, 137.9, 128.6, 128.1, 128.0, 128.0, 127,8 (Bn), 114.0 ($C(CH_3)_2$), 104.5 (C-1), 85.4 (C-4), 79.3, 78.6 (C-2, C-3), 73.7, 72.7, 71.2 (Bn, C-5), 64.9 (C-1'), 26.7, 26.3 ($C(CH_3)_2$), 21.0 ($COCH_3$). FAB-MS *m/z* 443 $[M+H]^+$. Found: C, 67.0; H, 6.5; $C_{25}H_{30}O_7$, $1/4H_2O$ requires C, 67.2; H, 6.9%.

4-*C*-Acetoxymethyl-1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-*D*-ribofuranose (2). A solution of 4-*C*-acetoxymethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose (830 mg, 1.88 mmol) in 80% acetic acid (10 cm^3) was stirred at 90 °C for 4 h. The solvent was removed under reduced pressure and the residue was coevaporated with ethanol (3 x 5 cm^3), toluene (3 x 5 cm^3) and anhydrous pyridine (3 x 5 cm^3), and was redissolved in anhydrous pyridine (3.7 cm^3). Acetic anhydride (2.85 cm^3) was added and the solution was stirred for 72 h at room temperature. The solution was poured into ice-cold water (20 cm^3) and the mixture was extracted with dichloromethane (2 x 20 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (2 x 20 cm^3), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane as eluent to give furanose **2** (β:α ~ 1:3) as an clear oil (789 mg, 86%). δ_C ($CDCl_3$) 171.0, 170.3, 170.0, 169.3 (C=O), 138.1, 137.6, 136.3, 128.9, 128.6, 128.2, 128.0, 128.0, 127.9, 127.7, 124.0 (Bn), 97.8, 97.8 (C-1), 87.0, 85.0, 78.9, 74.5, 74.4, 73.8, 73.6, 72.0, 71.8, 71.0, 70.9, 64.6, 64.4 (C-2, C-3, C-4, Bn, C-5, C-1'), 21.0, 20.8, 20.6 ($COCH_3$). FAB-MS *m/z* 509 $[M+Na]^+$. Found: C, 64.2; H, 6.3; $C_{26}H_{30}O_9$ requires C, 64.2; H, 6.2%.

1-(4-*C*-Acetoxymethyl-2-*O*-acetyl-3,5-di-*O*-benzyl-β-*D*-ribofuranosyl)thymine (3a). To a stirred solution of the anomeric mixture **2** (736 mg, 1.51 mmol) and thymine (381 mg, 3.03 mmol) in anhydrous acetonitrile (14.5 cm^3) was added *N,O*-bis(trimethylsilyl)acetamide (2.61 cm^3, 10.6 mmol). The reaction mixture was stirred at reflux for 1 h, then cooled to 0 °C. Trimethylsilyl triflate (0.47 cm^3, 2.56 mmol) was added dropwise under stirring and the solution was stirred at 65 °C for 2 h. The reaction was quenched with a cold saturated aqueous solution of sodium hydrogen carbonate (15 cm^3) and extraction was performed with dichloromethane (3 x 10 cm^3). The combined organic phase was washed with saturated aqueous solutions of sodium hydrogencarbonate (2 x 10 cm^3) and brine (2 x 10 cm^3), and was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give nucleoside **3a** as a white solid material (639 mg, 76%). δ_H ($CDCl_3$) 8.98 (1 H, br s, NH), 7.39-7.26 (11 H, m, Bn, 6-H), 6.22 (1 H, d, *J* 5.3, 1'-H), 5.42 (1 H, t, *J* 5.4, 2'-H), 4.63-4.43 (5H, m, 3'-H, Bn), 4.41 (1 H, d, *J* 12.2, 5'-H_a), 4.17 (1 H, d, *J* 12.1, 5'-H_b), 3.76 (1 H, d, *J* 10.2, 1"-H_a), 3.51 (1 H, d, *J* 10.4, 1"-H_b), 2.09 (3 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$), 1.53 (3 H, d, *J* 0.9, CH_3). δ_C ($CDCl_3$) 170.8, 170.4 (C=O), 163.9 (C-4), 150.6 (C-2), 137.4 (C-6) 137.4, 136.1, 128.9, 128.8, 128.4, 128.2, 127,9 (Bn), 111.7 (C-5), 87.2, 87.2, 86.1 (C-1', C-3', C-4'), 77.6 (C-2'), 74.8, 73.9, 71.1, 63.8 (Bn, C-1", C-5'), 20.9, 20.8 ($COCH_3$), 12.0 (CH_3). FAB-MS *m/z* 553 $[M+H]^+$. Found: C, 62.7; H, 5.9; N, 4.7; $C_{29}H_{32}N_2O_9$ requires C, 63.0; H, 5.8; N, 5.1%.

1-(3,5-Di-*O*-benzyl-4-*C*-(hydroxymethyl)-β-*D*-ribofuranosyl)thymine (4a). To a stirred solution of nucleoside **3a** (553 mg, 1.05 mmol) in methanol (5.5 cm^3) was added sodium methoxide (287 mg, 5.25 mmol). The reaction mixture was stirred at room temperature for 10 min, then neutralized with dilute hydrochloric acid. The solvent was partly evaporated and extraction was performed with dichloromethane (2 x 20 cm^3). The combined organic phase was washed with saturated aqueous sodium hydrogencarbonate (3 x 20 cm^3) and was dried (Na_2SO_4). The solvent was removed under reduced pressure to give nucleoside **4a** as a white solid material (476 mg, 97%). δ_H ($CDCl_3$) 7.47 (1 H, d, *J* 1.0 6-H), 7.36-7.22 (10 H, m, Bn), 6.07 (1 H, d, *J* 3.8, 1'-H), 4.87 (1 H, d, *J* 11.7, Bn), 4.55 (1 H, d, *J* 11.7, Bn), 4.50-4.32 (4 H, m, Bn, 2'-H, 3'-H), 3.84-3.53 (4 H, m, 5'-H_a, 5'-H_b, 1"-H_a, 1"-H_b), 1.50 (3 H, d, *J* 1.1, CH_3). δ_C ($CDCl_3$) 164.3 (C-4), 151.3 (C-2), 137.6 (C-6) 136.4, 136.3, 128.8, 128.6, 128.4, 128.3, 127,9 (Bn), 111.1 (C-5), 91.1, 91.0, 88.1 (C-1', C-3', C-4'), 77.4 (C-2'), 74.8, 73.8, 71.4, 63,2 (Bn, C-5', C-1"), 12.0 (CH_3). FAB-MS *m/z* 491 $[M+Na]^+$. Found: C, 63.4; H, 6.0; N, 5.5; $C_{25}H_{28}N_2O_7$, 1/4H_2O requires C, 63.5; H, 6.1; N, 5.9%.

(*1S,3R,4R,7S*)-7-Benzyloxy-1-benzyloxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (5a). A solution of nucleoside **4a** (225 mg, 0.48 mmol) in anhydrous pyridine (1.3 cm^3) was stirred and *p*-toluenesulphonyl chloride (118 mg, 0.62 mmol) was added in small portions at 0 °C. The solution was stirred at room temperature for 16 h and additional *p*-toluenesulphonyl chloride (36 mg, 0.19 mmol) was added. After stirring for another 4 h and addition of ice-cold water (15 cm^3), extraction was performed with dichloromethane (2 x 15 cm^3). The combined organic phase was washed with saturated aqueous sodium hydrogencarbonate (3 x 15 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give an intermediate (140 mg). This intermediate (102.2 mg) was dissolved in anhydrous DMF (0.8 cm^3). The solution was added dropwise to a stirred suspension of 60% sodium hydride in mineral oil (w/w, 32 mg, 0.80 mmol) in anhydrous DMF (0.8 cm^3) at 0 °C. The mixture was stirred for 72 h and then concentrated under reduced pressure. The residue was dissolved in dichloromethane (10 cm^3), washed with saturated aqueous sodium hydrogencarbonate (3 x 5 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give the bicyclic nucleoside **5a** as a white solid material (65.7 mg, 42% from **4a**). δ_H ($CDCl_3$) 9.24 (1 H, br s, NH), 7.49 (1 H, s, 6-H), 7.37-7.26 (10 H, m, Bn), 5.65 (1 H, s, 1'-H), 4.70-4.71 (5 H, m, Bn, 2'-H), 4.02-3.79 (5 H, m, 3'-H, 5'-H_a, 5'-H_b, 1"-H_a, 1"-H_b), 1.63 (3 H, s, CH_3). δ_C ($CDCl_3$) 164.3 (C-4), 150.1 (C-2), 137.7, 137.1 (Bn), 135.0 (C-6), 128.8, 128.7, 128.4, 128.0, 127.9 (Bn), 110.4 (C-5), 87.5, 87.3 (C-1', C-3'), 76.7, 75.8, 73.9, 72.3, 72.1 (Bn, C-5', C-2', C-4'), 64.5 (C-1"), 12.3 (CH_3). FAB-MS *m/z* 451 $[M+H]^+$. Found: C, 65.6; H, 5.8; N, 6.2; $C_{25}H_{26}N_2O_6$, 1/2 H_2O requires C, 65.3; H, 5.9; N, 6.1.

(*1S,3R,4R,7S*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6a). Nucleoside **5a** (97 mg, 0.215 mmol) was dissolved in ethanol (1.5 cm^3) and the mixture was stirred at room temperature and 20% palladium hydroxide over carbon (50 mg) was added. The mixture was degassed several times with argon and placed under a hydrogen atmosphere. After stirring for 4 h, the mixture was purified by silica gel column chromatography using dichloromethane-methanol (97:3, v/v) as eluent to give nucleoside **6a** as a white solid material (57 mg, 98%). δ_H ($(CD_3)_2SO$) 11.33 (1H, br s, NH), 7.62 (1H, d, *J* 1.1 Hz, 6-H), 5.65 (1H, d, *J* 4.4 Hz, 3'-OH), 5.41 (1H, s, 1'-H), 5.19 (1H, t, *J* 5.6 Hz, 5'-OH), 4.11 (1H, s, 2'-H), 3.91 (1H, d, *J* 4.2 Hz, 3'-H), 3.82 (1H, d, *J* 7.7 Hz, 1"-H_a), 3.73 (1H, s, H'-5_a), 3.76 (1H, s, 5'-H_b), 3.63 (1H, d, *J* 7.7 Hz, 1"-H_b), 1.78 (3H, d, *J* 0.7 Hz, CH_3). δ_C ($CDCl_3$) 166.7 (C-4), 152.1 (C-2), 137.0 (C-6), 110.9 (C-5), 90.5, 88.4 (C-1',

C-4'), 80.9, 72.5, 70.4 (C-2', C-3', C-5'), 57.7 (C-1"), 12.6 (CH_3). EI-MS *m/z* 270 $[M]^+$. Found: C, 48.9; H, 5.1; N, 10.1; $C_{11}H_{14}N_2O_6$ requires C, 48.9; H, 5.2; N, 10.3.

(*1R,3R,4R,7S*)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-3-thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (7a). To a solution of nucleoside **6a** (1.2 g, 4.44 mmol) in anhydrous pyridine (5 cm^3) was added 4,4'-dimethoxytrityl chloride (2.37 g, 7.0 mmol) at 0 °C. The solution was stirred at room temperature for 2 h whereupon the reaction was quenched with ice-cold water (10 cm^3) and extracted with dichloromethane (3 x 15 cm^3). The combined organic phase was washed with saturated aqueous solutions of sodium hydrogen carbonate (3 x 10 cm^3), brine (2 x 10 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give nucleoside **7a** as a white solid material (2.35 g, 93%). δ_H ($CDCl_3$) 9.89 (1H, br s, NH), 7.64 (1H, s, 6-H), 7.47-7.13 (9H, m, DMT), 6.96-6.80 (4H, m, DMT), 5.56 (1H, s, 1'-H), 4.53 (1H, br s, 2'-H), 4.31 (1H, m, 3'-H), 4.04-3.75 (9H, m, 1"-H_a, 1"-H_b, 3'-OH, OCH_3), 3.50 (2H, br s, 5'-H_a, 5'-H_b), 1.65 (3H, s, CH_3). δ_C($CDCl_3$) 164.47 (C-4), 158.66 (DMT), 150.13 (C-2), 144.56, 135.46, 135.35, 134.78, 130.10, 129.14, 128.03, 127.79, 127.05 (C-6, DMT), 113.32, 113.14 (DMT), 110.36 (C-5), 89.17, 88.16, 87.05 (C-1', C-4', DMT), 79.36, 71.81, 70.25, 58.38 (C-2', C-3', C-5', C-1"), 55.22 (OCH_3), 12.57 (CH_3). FAB-MS *m/z* 595 $[M+Na]^+$, 573 $[M+H]^+$.

(*1R,3R,4R,7S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (8a). To a solution of nucleoside **7a** (2.21 g, 3.86 mmol) in anhydrous dichloromethane (6 cm^3) were added *N,N*-diisopropylethylamine (4 cm^3) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (1 cm^3, 4.48 mmol) and stirring was continued for 1 h. MeOH (2 cm^3) was added, and the mixture was diluted with ethyl acetate (10 cm^3) and washed successively with saturated aqueous solutions of sodium hydrogencarbonate (3 x 5 cm^3) and brine (3 x 5 cm^3) and was dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the residue was purified by basic alumina column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give a white foam after evaporation under reduced pressure. This residue was dissolved in dichloromethane (2 cm^3) and the product was precipitated from petroleum ether (100 cm^3, cooled to -30 °C) under vigorous stirring. The precipitate was collected by filtration, and was dried to give nucleoside **8a** as a white solid material (2.1 g, 70%). δ_P ($CDCl_3$) 149.06, 148.74. FAB-MS *m/z* 795 $[M+Na]^+$, 773 $[M+H]^+$.

1-(4-*C*-Acetoxymethyl-2-*O*-acetyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)uracil (3b). To a stirred solution of the anomeric mixture **2** (3.0 g, 6.17 mmol) and uracil (1.04 g, 9.26 mmol) in anhydrous acetonitrile (65 cm^3) was added *N,O*-bis(trimethylsilyl)acetamide (9.16 cm^3, 37.0 mmol). The reaction mixture was stirred for 1 h at room temperature and cooled to 0 °C. Trimethylsilyl triflate (1.8 cm^3, 10.0 mmol) was added dropwise and the solution was stirred at 60 °C for 2 h. The reaction was quenched by addition of a saturated aqueous solution of sodium hydrogencarbonate (10 cm^3) at 0 °C and extraction was performed with dichloromethane (3 x 20 cm^3). The combined organic phase was washed with brine (2 x 20 cm^3) and was dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give nucleoside **3b** as a white solid material (2.5 g, 75%). δ_H ($CDCl_3$) 9.57 (1H, br s, NH), 7.63 (1H, d, *J* 8.2, 6-H), 7.40-7.24 (10H, m, Bn), 6.18 (1H, d, *J* 4.5, 1'-H), 5.39-5.32 (2H, m, 2'-H, 5-H), 4.61 (1H, d, *J* 11.6, Bn), 4.49-4.40 (5H, m, 3'-H, Bn, 1"-H_a), 4.37 (1H, d, *J* 12.3, 1"-H_b), 3.76 (1H, d, *J* 10.1, 5'-H_a), 3.49 (1H, d, *J* 10.1, 5'-H_b), 2.09 (s, 3H, $COCH_3$), 2.04 (3H, s, $COCH_3$). δ_C ($CDCl_3$)

170.47, 169.94 (C=O), 163.32 (C-4), 150.30 (C-2), 140.24 (C-6), 137.15, 136.95, 128.65, 128.52, 128.32, 128.19, 128.02, 127.77 (Bn), 102.57 (C-5), 87.41, 86.14 (C-1', C-4'), 77.09, 74.84, 74.51, 73.75, 70.60, 63.73 (C-2', C-3', C-5', C-1", Bn), 20.79, 20.68 ($COCH_3$). FAB-MS *m/z* 539 $[M+H]^+$.

1-(3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-β-D-ribofuranosyl)uracil (4b). To a stirred solution of nucleoside **3b** (2.0 g, 3.7 mmol) in methanol (25 cm^3) was added sodium methoxide (0.864 g, 95%, 16.0 mmol). The reaction mixture was stirred at room temperature for 10 min and neutralized with 20% aqueous hydrochloric acid. The solvent was partly evaporated and the residue was extracted with ethyl acetate (3 x 50 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 20 cm^3) and was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98.5:1.5, v/v) as eluent to give **4b** as a white solid material (1.58 g, 95%). δ_H ($CDCl_3$) 9.95 (1H, br s, NH), 7.69 (d, *J* 8.1, 6-H), 7.35-7.17 (10H, m, Bn), 6.02 (1H, d, *J* 2.3, 1'-H), 5.26 (1H, d, *J* 8.1, 5-H), 4.80 (1H, d, *J* 11.7, Bn), 4.47 (1H, d, *J* 11.7, Bn), 4.45-4.24 (4H, m, Bn, 2'-H, 3'-H), 3.81 (1H, d, *J* 11.9, 1"-H_a), 3.69 (2H, br s, 2'-OH, 1"-OH), 3.67 (2H, m, 5'-H_a, 1"-H_b), 3.48 (1H, d, *J* 10.3, 5'-H_b). δ_C ($CDCl_3$) 163.78 (C-4), 150.94 (C-2), 140.61 (C-6), 137.33, 137.22, 128.59, 128.18, 128.01 (Bn), 102.16 (C-5), 91.46, 88.36 (C-1', C-4'), 76.73, 74.66, 73.71, 73.29, 70.81, 62.81 (C-2', C-3', C-5', C-1", Bn). FAB-MS *m/z* 455 $[M+H]^+$.

(*1S,3R,4R,7S*)-7-Benzyloxy-1-benzyloxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (5b). A solution of nucleoside **4b** (1.38 g, 3.0 mmol), anhydrous pyridine (2 cm^3) and anhydrous dichloromethane (6 cm^3) was stirred at -10 °C and *p*-toluenesulfonyl chloride (0.648 g, 3.4 mmol) was added in small portions during 1 h. The solution was stirred at -10 °C for 3 h. The reaction was quenched by addition of ice-cold water (10 cm^3) and the mixture was extracted with dichloromethane (3 x 50 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 20 cm^3) and was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give an intermediate (0.9 g). This intermediate (0.7 g) was dissolved in anhydrous DMF (3 cm^3) and a 60% suspension of sodium hydride (w/w, 0.096 g, 24 mmol) was added in four portions during 10 min at 0 °C, and the reaction mixture was stirred for 12 h. The reaction was quenched with methanol (10 cm^3), and the solvents were removed under reduced pressure. The residue was dissolved in dichloromethane (20 cm^3), washed with saturated aqueous sodium hydrogencarbonate (3 x 6 cm^3) and was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/ethanol (99:1, v/v) as eluent to give nucleoside **5b** (0.30 g, 30% from **4b**). δ_H ($CDCl_3$) 9.21 (1H, br s, NH), 7.70 (1H, d, *J* 8.2, 6-H), 7.37-7.24 (10H, m, Bn), 5.65 (1H, s, 1'-H), 5.52 (1H, d, *J* 8.2, 5-H), 4.68-4.45 (5H, m, 2'-H, Bn), 4.02-3.55 (5H, m, 3'-H, 5'-H_a, 1"-H_a, 5'-H_b, 1"-H_b). δ_C ($CDCl_3$) 163.33 (C-4), 149.73 (C-2), 139.18 (C-6), 137.46, 136.81, 128.58, 128.54, 128.21, 128.10, 127.79, 127.53 (Bn), 101.66 (C-5), 87.49, 87.33 (C-1', C-4'), 76.53, 75.71, 73.77, 72.33, 72.00, 64.36 (C-2', C-3', C-5', C-1", Bn). FAB-MS *m/z* 459 $[M+Na]^+$.

(*1S,3R,4R,7S*)-7-Hydroxy-1-hydroxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6b). To a solution of compound **5b** (0.35 g, 0.8 mmol) in absolute ethanol (2 cm^3) was added 20% palladium hydroxide over carbon (0.37 g) and the mixture was degassed several times with hydrogen and stirred under an atmosphere of hydrogen for 4h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (9:1, v/v) as eluent to give nucleoside **6b** as a white

solid material (0.16 g, 78%). δ_H (CD_3OD) 7.88 (1H, d, *J* 8.1, 6-H), 5.69 (1H, d, *J* 8.1, 5-H), 5.55 (1H, s, 1'-H), 4.28 (1H, s, 2'-H), 4.04 (1H, s, 3'-H), 3.96 (1H, d, *J* 7.9, 1"-H_a), 3.91 (2H, s, 5'-H), 3.76 (1H, d, *J* 7.9, 1"-H_b). δ_C (CD_3OD) 172.95 (C-4), 151.82 (C-2), 141.17 (C-6), 101.97 (C-5), 90.52, 88.50 (C-1', C-4'), 80.88, 72.51, 70.50, 57.77 (C-2', C-3', C-5', C-1"). FAB-MS *m/z* 257 $[M+H]^+$. ^{1}H NMR data are in agreement with published data.[16]

(*1R,3R,4R,7S*)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (7b). To a solution of compound **6b** (0.08 g, 0.31 mmol) in anhydrous pyridine (0.5 cm^3) was added 4,4'-dimethoxytrityl chloride (0.203 g, 0.6 mmol) at 0°C and the mixture was stirred at room temperature for 2 h. The reaction was quenched with ice-cold water (10 cm^3) and extracted with dichloromethane (3 x 4 cm^3). The combined organic phase was washed with saturated aqueous solutions of sodium hydrogencarbonate (3 x 3 cm^3) and brine (2 x 3 cm^3) and was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give nucleoside **7b** as a white solid material (0.12 g, 69%). δ_H ($CDCl_3$) 9.25 (1H, br s, NH), 7.93 (1H, d, *J* 7.2, 6-H), 7.50-7.15 (9H, m, DMT), 6.88-6.78 (4H, m, DMT), 5.63 (1H, s, 1'-H), 5.59 (1H, d, *J* 8.0, 5-H), 4.48 (1H, s, 2'-H), 4.26 (1H, s, 3'-H), 3.88 (1H, d, *J* 8.1, 1"-H_a), 3.85-3.55 (7H, m, 1"-H_b, OCH_3), 3.58-3.40 (2H, m, 5'-H_a, 5'-H_b). δ_C ($CDCl_3$) 164.10 (C-4), 158.60 (DMT), 150.45 (C-2), 147.53 (DMT), 144.51 (C-6), 139.72, 135.49, 135.37, 130.20, 129.28, 128.09, 127.85, 127.07 (DMT), 113.39, 113.17 (DMT), 101.79 (C-5), 88.20, 87.10, 86.87 (C-1', C-4', DMT), 79.25, 71.79, 69.70, 58.13 (C-2', C-3', C-5', C-1"), 55.33 (OCH_3). FAB-MS *m/z* 559 $[M+H]^+$.

(*1R,3R,4R,7S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (8b). To a solution of compound **7b** (0.07 g, 0.125 mmol) in anhydrous dichloromethane (2 cm^3) was added *N,N*-diisopropylethylamine (0.1 cm^3) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.07 cm^3, 0.32 mmol). After stirring for 1 h, the reaction was quenched with MeOH (2 cm^3), and the resulting mixture was diluted with ethyl acetate (5 cm^3) and washed successively with saturated aqueous solutions of sodium hydrogencarbonate (3 x 2 cm^3) and brine (3 x 2 cm^3), and was dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give a white foam. This foam was dissolved in dichloromethane (2 cm^3) and the product was precipitated from petroleum ether (10 cm^3, cooled to -30°C) under vigorous stirring. The precipitate was collected by filtration and was dried to give compound **8b** as a white solid material (0.055 g, 58%). δ_p ($CDCl_3$) 149.18, 149.02.

9-(4-*C*-Acetoxymethyl-2-*O*-acetyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine (3c). To a stirred suspension of the anomeric mixture **2** (1.8 g, 3.7 mmol) and 2-*N*-isobutyrylguanine (1.28 g, 5.6 mmol) in anhydrous dichloroethane (60 cm^3) was added *N,O*-bis(trimethylsilyl)acetamide (4 cm^3, 16.2 mmol). The reaction mixture was stirred at reflux for 1 h. Trimethylsilyl triflate (1.5 cm^3, 8.28 mmol) was added dropwise and the solution was stirred at reflux for 2 h. The reaction mixture was allowed to cool to room temperature during 1.5 h. After dilution to 250 cm^3 by addition of dichloromethane, the mixture was washed with a saturated aqueous solution of sodium hydrogencarbonate (200 cm^3) and water (250 cm^3). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using 1.25% (200 cm^3) and 1.5% (750 cm^3) methanol in dichloromethane (v/v) as eluents to give 2.10 g (87%) of a white solid that according to ^{1}H-NMR analysis consisted of three isomers (ratio: 12.5:2.5:1). The individual isomers

were not isolated and the mixture was used in the next step. For the main product assigned as **3c**: δ_H ($CDCl_3$) 12.25 (br s, NHCO), 9.25 (br s, NH), 7.91 (s, 8-H) 7.39-7.26 (m, Bn), 6.07 (d, *J* 4.6, 1'-H), 5.80 (dd, *J* 5.8, *J* 4.7, 2'-H), 4.72 (d, *J* 5.9, 3'-H), 4.59-4.43 (m, Bn, 1''-H_a), 4.16 (d, *J* 12.1, 1''-H_b), 3.70 (d, *J* 10.1, 5'-H_a), 3.58 (d, *J* 10.1, 5'-H_b), 2.65 (m, CHCO), 2.05 (s, $COCH_3$), 2.01 (s, $COCH_3$), 1.22 (d, *J* 6.7, CH_3CH), 1.20 (d, *J* 7.0, CH_3CH). δ_C ($CDCl_3$) 178.3 (COCH), 170.6, 169.8 ($COCH_3$), 155.8, 148.2, 147.6 (guanine), 137.6, 137.2 (guanine, Bn), 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7 (Bn), 121.2 (guanine), 86.2, 86.0 (C-1', C-4'), 77.8 (C-3'), 74.9, 74.5, 73.7, 70.4 (Bn, C-2', C-5'), 63.5 (C-1''), 36.3 (COCH), 20.8, 20.6 ($COCH_3$), 19.0 (CH_3CH). For the mixture: FAB-MS *m/z* 648 $[M+H]^+$, 670 $[M+Na]^+$. Found: C, 60.8; H, 6.0; N, 10.4; $C_{33}H_{36}N_5O_9$ requires C, 61.3; H, 5.6; N, 10.8%.

9-(3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-β-D-ribofuranosyl)-2-*N*-isobutyrylguanine (4c). A solution of the mixture containing compound **3c** (2.10 g, 3.25 mmol) in THF/Pyridine/methanol (2:3:4, v/v/v) (40 cm^3) was cooled to -10 °C and sodium methoxide (320 mg, 5.93 mmol) was added to the stirred solution. The reaction mixture was stirred at 10 °C for 30 min and neutralized with acetic acid (2 cm^3). The solvent was evaporated under reduced pressure and the residue was twice extracted in a system of dichloromethane/water (2 x 100 cm^3). The organic fractions were combine and evaporated under reduced pressure. The residue was purified by silica gel column chromatography eluting with a gradient (2-7%) of methanol in dichloromethane (v/v) to give a white solid material (1.62 g, 89%). According to ^{1}H-NMR it consisted of three isomers (ratio: 13.5:1.5:1). The individual isomers were not isolated and the mixture was used in the next step. For the main product assigned as **4c**: δ_H (CD_3OD) 8.07 (s, 8-H) 7.36-7.20 (m, Bn), 6.05 (d, *J* 3.9, 1'-H), 4.81 (d, *J* 11.5, Bn), 4.75 (m, 2'-H), 4.56 (d, *J* 11.5, Bn), 4.51-4.43 (m, Bn, 3'-H), 3.83 (d, *J* 11.7, 1''-H_a), 3.65 (d, *J* 11.7, 1''-H_b), 3.64 (d, *J* 10.6, 5'-H_a), 3.57 (d, *J* 10.3, 5'-H_b), 2.69 (m, CHCO), 1.20 (6 H, d, *J* 6.8, CH_3CH). δ_C (CD_3OD) 181.6 (COCH), 157.3, 150.2, 149.5 (guanine), 139.4, 139.3, 139.0 (guanine, Bn), 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8 (Bn), 121.2 (guanine), 90.7, 89.6 (C-1', C-4'), 79.2 (C-3'), 75.8, 74.5, 74.3, 72.2 (Bn, C-2', C-5'), 63.1 (C-1''), 36.9 (COCH), 19.4 (CH_3CH), 19.3 (CH_3CH). For the mixture: FAB-MS *m/z* 564 $[M+H]^+$. Found:C, 60.4; H, 6.0; N, 11.8; $C_{29}H_{32}N_5O_7$, H_2O requires C, 60.0; H, 5.9; N, 12.1.

(*1S,3R,4R,7S*)-7-Benzyloxy-1-benzyloxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (5c). A solution of the mixture containing **4c** (1.6 g, 2.85 mmol) in anhydrous pyridine (6 cm^3) was stirred at -20 °C and *p*-toluenesulphonyl chloride (0.81 g, 4.27 mmol) was added. The solution was stirred at -20 °C for 1 h and at -25 °C for 2 h. Then the mixture was diluted by addition of dichloromethane (to 100 cm^3) and immediately washed with water (2 x 100 cm^3). The organic phase was separated and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol as eluent (1-2%, v/v) to give an intermediate (980 mg). After elution of this intermediate from the column, the starting mixture containing **4c** (510 mg, 32%) was eluted using 8% methanol in dichloromethane (v/v) as eluent. This material was concentrated, dried under reduced pressure and treated in the same manner as described above to give additionally 252 mg of the intermediate. The intermediate (1.23 g) was purified by silica gel HPLC (PrepPak Cartridge, Porasil 15-20 μm 125 Å, flow rate 60 cm^3/min, eluent 0-4% of methanol in dichloromethane (v/v), 120 min). Fractions containing the intermediate were pooled and concentrated to give a white solid (1.04 g, 51%). According to ^{1}H-NMR it consisted of two main products, namely isomers of 1''-*O* and 2'-*O* monotosylated derivatives in a ratio of ~ 2:1. FAB-MS *m/z* 718 $[M+H]^+$. Found C, 60.4; H, 5.8; N, 9.3; $C_{36}H_{39}N_5O_9S$ requires C, 60.2; H, 5.5; N, 9.8%. To a solution of the intermediate (940 mg) in anhydrous THF

(20 cm^3) was added a 60% suspension of sodium hydride (w/w, 130 mg, 3.25 mmol) and the mixture was stirred for 1h at room temperature. Acetic acid (0.25 mL) was added and the mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane (100 cm^3) and was washed with water (2 x 100 cm^3). The organic phase was separated and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using methanol/dichloromethane (1-1.5%, v/v) as eluent to give nucleoside **5c** as a white solid material (451 mg, 29% from **4c**). δ_H ($CDCl_3$) 12.25 (1H, br s, NHCO), 10.12 (1H, br s, NH), 7.84 (1H, s, 8-H), 7.31-7.15 (10H, m, Bn), 5.72 (1H, s, 1'-H), 4.60-4.46 (5H, m, Bn, 2'-H), 4.14 (1H, s, 3'-H), 4.02 (1H, d, *J* 7.9, 1''-H_a), 3.85 (1H, d, *J* 7.9, 1''-H_b), 3.78 (2H, s, 5'-H), 2.81 (1H, m, CHCO), 1.24 (3H, d, *J* 6.8, CH_3CH), 1.22 (3H, d, *J* 6.4, CH_3CH). δ_C ($CDCl_3$) 179.5 (COCH), 155.6, 148.1, 147.3 (guanine), 137.3, 136.9, 136.0 (guanine, Bn), 128.4, 128.3, 127.9, 127.8, 127.5, 127.4 (Bn), 121.2 (guanine), 87.1, 86.2 (C-1', C-4'), 77.0 (C-3'), 73.6, 72.5, 72.3 (Bn, C-2', C-5'), 64.9 (C-1''), 36.1 (COCH), 19.0 (CH_3CH), 18.9 (CH_3CH). FAB-MS *m/z* 546 $[M+H]^+$. Found: C, 63.3; H, 5.9; N, 12.6; $C_{29}H_{30}N_5O_6$, 1/2 H_2O requires C, 62.9; H, 5.6; N, 12.7.

(*1S,3R,4R,7S*)-7-Hydroxy-1-hydroxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (6c). A mixture of nucleoside **5c** (717 mg, 1.31 mmol) and 10% palladium over carbon (500 mg) was suspended in methanol (8 cm^3). The mixture was degassed several times under reduced pressure and placed under a hydrogen atmosphere. After stirring for 24 h the mixture was purified by silica gel column chromatography using methanol/dichloromethane (8-20%, v/v) as eluent to give nucleoside **6c** as a glass (440 mg, 92%). δ_H (CD_3OD) 8.12 (1H, br s, 8-H), 5.89 (1H, s, 1'-H), 4.50 (1H, s, 2'-H), 4.30 (1H, s, 3'-H), 4.05 (1H, d, *J* 8.0, 1''-H_a), 3.95 (2H, s, 5'-H), 3.87 (1H, d, *J* 7.9, 1''-H_b), 2.74 (1H, m, CHCO), 1.23 (6H, d, *J* 6.9, CH_3CH). δ_C (CD_3OD, signals from the carbohydrate part) 90.2, 87.6 (C-1', C-4'), 81.1 (C-3'), 72.9, 71.3 (C-2', C-5'), 58.2 (C-1''), 37.1 (COCH), 19.5 (CH_3CH). FAB-MS *m/z* 366 $[M+H]^+$.

(*1R,3R,4R,7S*)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (7c). A mixture of compound **6c** (440 mg, 1.21 mmol) and 4,4'-dimethoxytrityl chloride (573 mg, 1.69 mmol) was dissolved in anhydrous pyridine (7 cm^3) and was stirred at room temperature for 4 h. The mixture was evaporated under reduced pressure to give an oil. Extraction was performed in a system of dichloromethane/water (1:1, v/v, 40 cm^3). The organic phase was separated and concentrated and redissolved in a minimal volume of dichloromethane containing 0.5% pyridine (v/v) which was applied to a silica gel column equilibrated by the same solvent. The product was eluted in a gradient of methanol (0.6-2%, v/v) in dichloromethane containing 0.5% pyridine (v/v) to give compound **7c** as a white solid material (695 mg, 86%). δ_H ($CDCl_3$) 12.17 (1H, br s, NHCO), 10.09 (1H, br s, NH), 7.87 (1H, s, 8-H), 7.42-6.72 (13H, m, DMT), 5.69 (1H, s, 1'-H), 4.59 (1H, s, 2'-H), 4.50 (1H, s, 3'-H), 3.98 (1H, d, *J* 8.1, 1''-H_a), 3.69-3.39 (9H, m, DMT, 5'-H, 1''-H_b), 2.72 (1H, m, CHCO), 1.17 (6H, d, *J* 6.8, CH_3CH). δ_C ($CDCl_3$) 179.8 (COCH), 158.8, 144.5, 135.6, 135.5, 130.1, 128.1, 127.7, 126.9, 113.2 (DMT), 155.8, 147.9, 147.5, 137.0, 120.8 (guanine), 87.6, 86.4, 86.1 (C-1', C-4', DMT), 79.7 (C-3'), 72.6, 71.4 (C-2', C-5'), 59.8 (C-1''), 55.2 (DMT), 36.1 (COCH), 19.1, 18.8 (CH_3CH). FAB-MS *m/z* 668 $[M+H]^+$.

(*1R,3R,4R,7S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (8c). Compound **7c** (670 mg, 1.0 mmol) was dissolved in anhydrous dichloromethane (5 cm^3) containing *N,N*-diisopropylethylamine (0.38 cm^3, 4 mmol). 2-Cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.36 cm^3, 2.0 mmol) was added dropwise under stirring.

After 5 h, methanol (2 cm^3) was added and the mixture was diluted to 100 cm^3 by addition of dichloromethane and washed with a saturated aqueous solution of sodium hydrogencarbonate (50 cm^3). The organic phase was separated and evaporated under reduced pressure. The residue was dissolved in the minimun amount of dichloromethane/petroleum ether (1:1, v/v) containing 0.5% pyridine (v/v) and was applied to a column packed with silica gel equilibrated by the same solvent mixture. The column was washed by dichloromethane/petroleum ether/pyridine (75:25:0.5, v/v/v, 250 cm^3) and the product was eluted using a gradient of methanol in dichloromethane (0-1%, v/v) containing 0.5% pyridine (v/v). The fractions containing the main product were evaporated and co-evaporated with toluene. The residue was dissolved in anhydrous dichloromethane (5 cm^3) and precipitated in petroleum ether (100 cm^3) to give compound **8c** as a white solid material (558 mg, 64%). δ_P ($CDCl_3$) 148.17, 146.07. FAB-MS *m/z* 868 $[M+H]^+$.

1-(4-*C*-Acetoxymethyl-2-*O*-acetyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)-4-*N*-Benzoylcytosine (3d). To a stirred solution of the anomeric mixture **2** (4.0 g, 8.22 mmol) and 4-*N*-benzoylcytosine (2.79 g, 13.0 mmol) in anhydrous acetonitrile (80 cm^3) was added *N,O*-bis(trimethylsilyl)acetamide (8.16 ml, 33.0 mmol). The reaction mixture was stirred for 1 h at room temperature and cooled to 0 °C. Trimethylsilyl triflate (3.0 cm^3, 16.2 mmol) was added dropwise and the mixture was stirred at 60 °C for 2 h. Dichloromethane (200 cm^3) was added and the mixture was washed with saturated aqueous solutions of sodium hydrogencarbonate (3 x 20 cm^3) and brine (2 x 20 cm^3), and the separated organic phase was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give compound **3d** as a white solid material (3.9 g, 74%). δ_H ($CDCl_3$), 8.28 (1H, d, *J* 7.5, 6-H), 7.94-7.90 (2H, m, Bz), 7.65-7.25 (13H, m, Bn, Bz), 7.16 (1H, d, *J* 7.1, 5-H), 6.22 (1H, d, *J* 2.8, 1'-H), 5.51 (1H, dd, *J* 2.8, 5.8, 2'-H), 4.62 (1H, d, *J* 11.6, Bn), 4.51 (1H, d, *J* 12.3, 1"-H_a), 4.49-4.34 (4H, m, 3'-H, Bn), 4.21 (1H, d, *J* 12.3, 1"-H_b), 3.85 (1H, d, *J* 10.3, 5'-H_a), 3.47 (1H, d, *J* 10.3, 5'-H_b), 2.13 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$). δ_C ($CDCl_3$) 170.52, 169.61 (C=O), 166.83, 162.27 (C-4, C=O), 154.26 (C-2), 145.26 (C-6), 137.25, 136.93, 133.18, 129.0, 128.75, 128.51, 128,45, 128.18, 128.10, 127.89, 127.71 (Bn, Bz), 96.58 (C-5), 89.42, 86.52 (C-1', C-4'), 76.21, 75.10, 74.17, 73.70, 69.70, 63.97 (C-2', C-3', Bn, C-5', C-1"), 20.82 ($COCH_3$). FAB-MS *m/z* 664 $[M+Na]^+$, 642 $[M+H]^+$. Found: C, 65.0; H, 5.7, N, 6.5; $C_{35}H_{35}N_3O_9$ requires C, 65.5; H, 5.5; N, 6.5%.

1-(3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-β-D-ribofuranosyl)-4-*N*-benzoylcytosine (4d). To a stirred solution of nucleoside **3d** (3.4 g, 5.3 mmol) in methanol (20 cm^3) was added sodium methoxide (0.663 g, 11.66 mmol). The reaction mixture was stirred at room temperature for 10 min and then neutralized with 20% aqueous HCl. The mixture was extracted with dichloromethane (3 x 50 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 20 cm^3) and was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98.5:1.5, v/v) as eluent to give compound **4d** as a white solid material (1.6 g, 54%). δ_H ($CDCl_3$) 9.95 (1H, br s, NH), 8.33 (1H, d, *J* 7.4, 6-H), 7.98 (2H, m, Bz), 7.60-7.12 (14H, m, Bn, Bz, 5-H), 6.17 (1H, d, *J* 1.6, 1'-H), 4.78 (1H, d, *J* 11.8, Bn), 4.48-4.27 (5H, m, Bn, 2'-H, 3'-H), 3.85 (1H, d, *J* 11.8, 5'-H_a), 3.66-3.61 (2H, m, 5'-H_b, 1"-H_a), 3.47 (1H, d, *J* 10.4, 1"-H_b). δ_C ($CDCl_3$) 167.5, 162.31 (C-4, C=O), 155.36 (C-2), 145.34 (C-6), 137.49, 137.08, 133.09, 133.01, 128.94, 128.67, 128.48, 128.30, 128.01, 127.90, 127.80 (Bn, Bz), 96.53 (C-5), 93.97, 89.35 (C-1', C-4'), 76.06, 75.28, 73.70, 72.76, 70.26, 62.44 (C-2', C-3', Bn, C-5', C-1"). FAB-MS *m/z* 558 $[M+H]^+$. Found: C, 65.7; H, 5.9; N, 7.4; $C_{11}H_{31}N_3O_7$, 1/2 H_2O requires C, 65.7; H, 5.7; N, 7.4.

(*1S,3R,4R,7S*)-3-(4-*N*-Benzoylcytosin-1-yl)-7-benzyloxy-1-benzyloxymethyl-2,5-dioxabicyclo[2.2.1]heptane (5d). A solution of nucleoside **4d** (2.2 g, 3.94 mmol) in anhydrous tetrahydrofuran (60 cm^3) was stirred at -20 °C and a suspension of 60% sodium hydride in mineral oil (w/w, 0.252 g, 6.30 mmol) was added in seven portions during 45 min. The mixture was stirred for 15 min at -20 °C followed by addition of *p*-toluenesulphonyl chloride (0.901 g, 4.73 mmol) in small portions. The solution was stirred for 4 h at -20 °C. Additional sodium hydride (0.252 g, 6.30 mmol) and *p*-toluenesulfonyl chloride (0.751 g, 3.93 mmol) was added. The reaction mixture was kept at -20 °C for 48 h. The reaction was quenched by addition of ice-cold water (50 ml) whereupon extraction was performed with dichloromethane (3 x 60 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 20 cm^3) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give an intermediate (1.80 g). This intermediate (1.80 g) was dissolved in anhydrous DMF (30.0 cm^3) and a 60% suspension of sodium hydride in mineral oil (w/w, 0.16 g, 3.9 mmol) was added in five portions during 30 min at 0 °C. The reaction mixture was stirred for 36 h at room temperature. The reaction was quenched by addition of ice-cold water (70 cm^3) and the resulting mixture was extracted with dichloromethane (3 x 50 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 30 cm^3) and dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99.5:0.5, v/v) as eluent to give compound **5d** as a white solid material (1.08 g, 51%). δ_H ($CDCl_3$) 8.95 (1H, br s, NH), 8.20 (1H, d, *J* 7.5, 6-H), 7.95-7.92 (2H, m, Bz), 7.66-7.22 (14H, m, Bn, Bz, 5-H), 5.78 (1H, s, 1'-H), 4.70-4.65 (3H, m, 2'-H, Bn), 4.60 (1H, d, *J* 11.6, Bn), 4.47 (1H, d, *J* 11.6, Bn), 4.05-3.78 (5H, m, 3'-H, 5'-H_a, 1''-H_a, 5'-H_b, 1''-H_b). δ_C ($CDCl_3$) 167.0, 162.36 (C-4, C=O), 154.5 (C-2), 144.58 (C-6), 137.46, 136.93, 133.35, 132.93, 129.11, 128.67, 128.50, 128.16, 128.11, 127.68, 127.60 (Bn), 96.35 (C-5), 88.38, 87.67 (C-1', C-4'), 76.14, 75.70, 73.79, 72.27, 72.09, 64.34 (Bn, C-5', C-1'', C-2', C-3'). FAB-MS *m/z* 540 $[M+H]^+$. Found: C, 68.4; H, 5.5; N, 7.5; $C_{31}H_{29}N_3O_6$, 1/4 H_2O requires C, 68.4; H, 5.5; N, 7.7.

(*1S,3R,4R,7S*)-3-(Cytosin-1-yl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (6f). To a solution of nucleoside **5d** (0.3 g, 0.55 mmol) in anhydrous methanol (22 cm^3) were added 1,4-cyclohexadiene (5.0 cm^3) and 10% palladium on carbon (0.314 g). The mixture was stirred under reflux for 18 h. Additional 10% palladium on carbon (0.380 g) and 1,4-cyclohexadiene (5.5 cm^3) were added and the mixture was refluxed for 54 h. The reaction mixture was filtered through a pad of silica gel which was subsequently washed with methanol (1500 cm^3). The combined filtrate was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (92.5:7.5, v/v) as eluent to give compound **6f** as a white solid material (0.051 g, 36%) which was used in the next step without further purification. δ_H ($(CD_3)_2SO$) 7.73 (1H, d, *J* 7.7, 6-H), 7.12-7.20 (2H, br s, NH_2), 5.74 (1H, d, *J* 7.7, 5-H), 5.61 (1H, br s, 3'-OH), 5.39 (1H, s, 1' -H), 5.12 (1H, m, 5'-OH), 4.08 (1H, s, 2'-H), 3.80 (1H, d, *J* 7.7, 1''-H_a), 3.81 (1H, s, 3'-H), 3.74 (2H, m, 5'-H_a, 5'-H_b), 3.63 (1H, d, *J* 7.7, 1''-H_b). δ_C ($(CD_3)_2SO$) 165.67 (C-4), 154.58 (C-2), 139.68 (C-6), 93.20 (C-5), 88.42, 86.74 (C-1', C-4'), 78.87, 70.85, 68.32, 56.05 (C-2', C-1'', C-3', C-5'). FAB-MS *m/z* 256 $[M+H]^+$.

(*1R,3R,4R,7S*)-3-(4-*N*-Benzoylcytosine-1-yl)-1-(4,4'-dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (7d). To nucleoside **6f** (0.030 g, 0.11 mmol) suspended in anhydrous pyridine (2.0 cm^3) was added trimethylsilyl chloride (0.14 cm^3, 1.17 mmol) and stirring was continued for 1h at room temperature. Benzoyl chloride (0.07 cm^3, 0.58 mmol) was added at 0 °C and the mixture was stirred for 2 h at

room temperature. After cooling the reaction mixture to 0 °C, water (3.0 cm^3) was added, and after stirring for 5 min an aqueous solution of ammonia (1.5 cm^3, 32%, w/w) was added and stirring was continued for 30 min at room temperature. The mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (97.5:2.5, v/v) as eluent to give an intermediate (0.062 g). To a solution of this intermediate (0.042 g) in anhydrous pyridine (1.5 cm^3) was added 4,4'-dimethoxytrityl chloride (0.06 g, 0.17 mmol). The reaction mixture was stirred at room temperature for 3.5 h, cooled to 0 °C, and a saturated aqueous solution of sodium hydrogencarbonate (20 cm^3) was added. Extraction was performed using dichloromethane (3 x 10 cm^3), the combined organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol/pyridine (98.0:1.5:0.5, v/v/v) as eluent to give nucleoside **7d** as a white solid material (0.031 g, 63% from **6f**). δ_H (C_5D_5N) 12.32 (1H, br s, NHCO), 8.75-7.06 (20H, m, DMT, Bz, H-5, H-6), 6.24 (1H, s, 1'-H), 5.11 (1-H, s, 2'-H), 4.90 (1H, s, 3'-H), 4.38 (1H, d, *J* 7.6, 1"-H_a), 4.10 (1H, d, *J* 7.6, 1"-H_b), 4.02 (1H, d, *J* 10.6, 5'-H_a), 3.87 (1H, d, *J* 10.6, 5'-H_b), 3.77, 3.76 (2 x 3H, 2 x s, 2 x OCH_3). δ_C (C_5D_5N) 169.00 (NHCO), 164.24 (C-2), 159.39 (DMT), 155.50, 145.62, 144.31, 136.57, 136.30, 132.89, 130.82, 130.72, 129.09, 129.02, 128.90, 128.61, 127.61, 113.96 (DMT), 96.97 , 89.02, 87.18, 79.91, 72.57, 70.26 (C-5, C-1', C-4', C-2', C-1", C-3'), 59.51 (C-5'), 55.34 (OCH_3). FAB-MS *m/z* 662 $[M+H]^+$.

***(1R,3R,4R,7S)*-3-(4-*N*-Benzoylcytosine-1-yl)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (8d).** To a solution of nucleoside **7d** (0.025 g, 0.03 mmol) in anhydrous dichloromethane (1.5 cm^3) was added *N,N*-diisopropylethylamine (0.03 cm^3, 0.17 mmol) followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.02 cm^3, 0.09 mmol). After stirring for 5h at room temperature, the reaction mixture was cooled to 0 °C, dichloromethane/pyridine (10.0 cm^3, 99.5:0.5, v/v) was added, and washing was performed using a saturated aqueous solution of sodium hydrogencarbonate (3 x 8 cm^3). The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol/pyridine (99.0:0.5:0.5, v/v/v) as eluent to give amidite **8d** as a light yellow oil (0.038 g) which was used for automated LNA-synthesis without further purification. δ_P ($CDCl_3$) 147.93 (in addition a peak at 13.29 was present in the spectrum).

9-(4-*C*-Acetoxymethyl-2-*O*-acetyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)-6-*N*-benzoyladenine (3e). To a stirred suspension of the anomeric mixture **2** (5.0 g, 10.3 mmol) and 6-*N*-benzoyladenine (3.76 g, 15.7 mmol) in anhydrous dichloroethane (200 cm^3) was added *N,O*-bis(trimethylsilyl)acetamide (15.54 cm^3, 61.8 mmol). The reaction mixture was stirred at reflux for 1 h and then cooled to room temperature. Trimethylsilyl triflate (7.0 cm^3, 38.7 mmol) was added dropwise and the mixture was refluxed for 20 h. The reaction mixture was allowed to cool to room temperature and the volume of the mixture was reduced to 1/4 under reduced pressure. Dichloromethane (250 cm^3) was added, and the solution was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 50 cm^3) and water (50 cm^3). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (99.5:0.5, v/v) as eluent to give nucleoside **3e** as white solid material (3.65 g, 52%). δ_H ($CDCl_3$) 9.25 (1H, br s, NH), 8.71 (1H, s, 8-H), 8.24 (1H, s, 2-H), 8.0 (2H, d, *J* 7.5, Bz), 7.60-7.23 (13H, m, Bn, Bz), 6.35 (1H, d, *J* 4.6, 1'-H), 5.99 (1H, dd, *J* 4.9, 5.3, 2'-H), 4.78 (1H, d, *J* 5.6, 3'-H), 4.64-4.42 (5H, m, Bn, 1"-H_a), 4.25 (1H, d, *J* 12.1, 1"-H_b), 3.72 (1H, d, *J* 10.1, 5'-H_a), 3.56 (1H, d, *J* 10.1, 5'-H_b), 2.07 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$). δ_C ($CDCl_3$) 170.42, 169.72 ($COCH_3$), 164.60 (NHCO), 152.51 (C-6), 151.45 (C-2),

149.46 (C-4), 141.88 (C-8), 137.04, 137.00, 133.50, 132.60, 128.86, 128.66, 128.53, 128.41, 128.38, 128.18, 128.06, 127.91, 127.88, 127.79, 127.63, 123.26 (Bz, Bn, C-5), 86.38 (C-1'), 86.25 (C-4'), 77.74, 74.74, 74.44, 73.48 (C-2', C-3', 2 x Bn), 70.11 (C-1"), 63.42 (C-5'), 20.70, 20.54 ($COCH_3$). FAB-MS *m/z* 666 $[M+H]^+$. Found: C, 63.8; H, 5.4; N, 9.7; $C_{36}H_{35}N_5O_8$, H_2O requires C, 63.2; H, 5.4; N, 10.2.

9-(3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-β-*D*-ribofuranosyl)-6-*N*-benzoyladenine (4e). To a stirred solution of nucleoside **3e** (4.18 g, 6.28 mmol) in methanol (50 cm^3) was added sodium methoxide (0.75 g, 13.8 mmol) at 0 °C. The reaction mixture was stirred for 2 h, and ice-cold water (10 cm^3) was added. The mixture was neutralized using a 20% aqueous solution of HCl. Extraction was performed using dichloromethane (3 x 75 cm^3), the organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (98.5:1.5, v/v) as eluent to give nucleoside **4e** as a white solid material (2.68 g, 73%). δ_H ($CDCl_3$) 9.42 (1H, br s, NH), 8.58 (1H, s, H-8), 8.16 (1H, s, 2-H), 7.96 (2H, d, *J* 7.2, Bz), 7.52-7.08 (13H, m, Bn, Bz), 6.18 (1H, d, *J* 2.5, 1'-H), 4.85-4.38 (4H, m, Bn, 2'-H, 3'-H), 4.33 (2H, s, Bn) 3.90 (1H, d, *J* 11.9, 1"-H_a), 3.71 (1H, d, *J* 11.8, 1"-H_b), 3.50-3.39 (2H, m, 5-H). δ_C ($CDCl_3$) 164.98 (NHCO), 152.19 (C-6), 151.00 (C-2), 149.34 (C-4), 142.28 (C-8), 137.32, 137.25, 133.46, 132.70, 128.69, 128.49, 128.40, 128.11, 128.03, 127.94, 127.83, 127.62, (Bz, Bn), 122.92 (C-5), 90.94, 88.75 (C-1', C-4'), 77.65, 74.08, 73.44, 73.20, 71.12, 62.39 (C-1", C-5', C-2', C-3', 2 x Bn). FAB-MS *m/z* 582 $[M+H]^+$. Found: C, 65.6; H, 5.5; N, 11.7; $C_{32}H_{31}N_5O_6$ requires C, 66.1; H, 5.4; N, 12.0%.

(*1S,3R,4R,7S*)-3-(6-*N*-Benzoyladenin-9-yl)-7-benzyloxy-1-benzyloxymethyl-2,5-dioxabicyclo[2.2.1]heptane (5e). A solution of nucleoside **4e** (2.43 g, 4.18 mmol) in anhydrous tetrahydrofuran (25 cm^3) was stirred at -20 °C and a 60% suspension of sodium hydride in mineral oil (w/w, 0.28 g, 7.0 mmol) was added in four portions during 30 min. After stirring for 1 h, *p*-toluenesulphonyl chloride (1.34 g, 7.0 mmol) was added in small portions. The mixture was stirred at -10 °C for 15 h. Ice-cold water (50 cm^3) was added and extraction was performed with dichloromethane (3 x 50 cm^3). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (2 x 25 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give an intermediate (1.95 g). This intermediate (1.90 g) was dissolved in anhydrous DMF (20 cm^3) and a 60% suspension of sodium hydride in mineral oil (w/w, 0.16 g, 3.87 mmol) was added in small portions at 0 °C. The mixture was stirred for 10 h at room temperature and then evaporated under reduced pressure. The residue was dissolved in dichloromethane (75 cm^3), washed with a saturated aqueous solution of sodium hydrogencarbonate (2 x 25 cm^3), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give nucleoside **5e** as white solid material (1.0 g, 44% from **4e**). δ_H ($CDCl_3$) 8.71 (H, s, 8-H), 8.23 (1H, s, 2-H), 8.02 (2H, m, *J* 7.0, Bz), 7.99-7.19 (13H, m, Bn, Bz), 6.08 (1H, s, 1'-H), 4.78 (1H, s, 2'-H), 4.61-4.50 (4H, m, 2 x Bn), 4.24 (1H, s, 3'-H), 4.12 (1H, d, *J* 7.8, 1"-H_a), 4.00 (1H, d, *J* 7.9, 1"-H_b), 3.85-3.78 (2H, m, 5'-H_a, 5'-H_b). δ_C ($CDCl_3$) 164.62 (NHCO), 152.32 (C-6), 150.62 (C-2), 149.35 (C-4), 140.67 (C-8), 137.25, 136.76, 133.34, 132.67, 128.69, 128.40, 128.29, 127.95, 127.77, 127.52 (Bn, Bz), 123.44 (C-5), 87.15, 86.52 (C-1', C-4'), 77.22, 76.78, 73.57, 72.57, 72.28, 64.65 (C-2', C-3', C-1", 2 x Bn, C-5'). FAB-MS *m/z* 564 $[M+H]^+$.

(*1S,3R,4R,7S*)-3-(Adenin-9-yl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (6g). To a stirred solution of nucleoside **5e** (0.80 g, 1.42 mmol) in anhydrous dichloromethane (30 cm^3) at -78 °C was

dropwise during 30 min added BCl_3 (1 M solution in hexane; 11.36 cm^3, 11.36 mmol). The mixture was stirred for 4 h at -78 °C, additional BCl_3 (1M solution in hexane, 16.0 cm^3, 16.0 mmol) was added dropwise, and the mixture was stirred at -78 °C for 3 h. The temperature of the mixture was raised slowly to room temperature and stirring was continued for 30 min. Methanol (25.0 cm^3) was added at -78 °C, and the mixture was stirred at room temperature for 12 h. The mixture was co-evaporated with methanol (3 x 10 cm^3), and the residue was purified by silica gel column chromatography using dichloromethane/methanol (92:8, v/v) as eluent to give nucleoside **6g** as a white solid material (0.332 g, 84%). δ_H ($(CD_3)_2SO$) 8.22 (1H, s, 8-H), 8.15 (1H, s, 2-H), 7.33 (2H, s, NH_2), 5.89 (1H, s, 1'-H), 5.83 (1H, d, *J* 4.2, 3'-OH), 5.14 (1H, t, *J* 5.9, 5'-OH), 4.14 (1H, s, 2'-H), 4.25 (1H, d, *J* 4.2, 3'-H), 3.92 (1H, d, *J* 7.8, 1"-H_a), 3.81-3.41 (3H, m, 5'-H_a, 5'-H_b, 1"-H_b). δ_C ($(CD_3)_2SO$) 155.90 (C-6), 152.64 (C-2), 148.35 (C-4), 137.72 (C-8), 118.94 (C-5), 88.48, 85.17 (C-1', C-4'), 79.09, 71.34, 69.83, 56.51 (C-2', C-3', C-1", C-5'). FAB-MS *m/z* 280 $[M+H]^+$.

(*1R,3R,4R,7S*)-3-(6-*N*-Benzoyladenin-9-yl)-1-(4,4'-dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (7e). To a stirred solution of nucleoside **6g** (0.32 g, 1.15 mmol) in anhydrous pyridine (1 cm^3) was added trimethylsilyl chloride (0.73 cm^3, 5.73 mmol) and the mixture was stirred at room temperature for 20 min. Benzoyl chloride (0.67 cm^3, 5.73 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C and ice-cold water (15.0 cm^3) was added. After stirring for 5 min, a 32% (w/w) aqueous solution of ammonia (1.5 cm^3) was added and the mixture was stirred for 30 min. The mixture was evaporated to dryness and the residue was dissolved in water (25 cm^3). After evaporation of the mixture under reduced pressure, the residue was purified by silica gel chromatography using dichloromethane/methanol (97:3, v/v) as eluent to give an intermediate (0.55 g) (FAB-MS *m/z* 384). To a stirred solution of this intermediate (0.50 g) in anhydrous pyridine (20 cm^3) was added 4,4'-dimethoxytrityl chloride (0.71 g, 2.09 mmol) and DMAP (0.1 g). After stirring for 2 h at room temperature and for 1 h at 50 °C, the reaction mixture was cooled to 0 °C and a saturated aqueous solution of sodium hydrogencarbonate (100 cm^3) was added. After extraction using dichloromethane (3 x 50 cm^3), the combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography eluting with dichloromethane/methanol/pyridine (98.0:1.5:0.5) to give nucleoside **7e** as a white solid material (0.36 g, 50% from **6g**). δ_H (C_5D_5N) 12.52 (NHCO), 9.10 (2H, d, *J* 7.7, Bz), 8.88 (1H, s, 8-H), 8.50-7.11 (17H, m, DMT, Bz, 2-H), 6.65 (1H, s, H-1'), 5.25 (2H, s, H-2', H-3'), 4.71 (1H, d, *J* 7.8, 1"-H_a), 4.56 (1H, d, *J* 7.8, 1"-H_b), 4.20 (1H, d, *J* 10.8, 5'-H_a), 4.07 (1H, d, *J* 10.8, 5'-H_b), 3.82, 3.81 (2 x 3H, 2 x s, 2 x OCH_3). δ_C (C_5D_5N) 167.56 (NHCO), 159.24 (C-6), 152.50, 152.08, 151.81, 145.84, 141.45, 136.52, 136.28, 132.55, 130.76, 130.70, 129.32, 128.85, 128.76, 128.46, 127.38, 126.33 (DMT, Bz, C-2, C-4, C-8), 113.84 (C-5), 88.64, 87.20, 86.85, 80.52, 73.13, 72.16, 60.86 (C-1', C-4', DMT, C-2', C-3', C-1", C-5'), 55.24 (OCH_3). FAB-MS *m/z* 686 $[M+H]^+$. Found: C, 68.3; H, 5.0; N, 9.7; $C_{39}H_{35}N_5O_7$ requires C, 68.3; H, 5.1; N, 10.2%).

(*1R,3R,4R,7S*)-3-(6-*N*-Benzoyladenin-9-yl)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (8e). To a solution of compound **7e** (190 mg, 0.277 mmol) in anhydrous dichloromethane (1.5 cm^3) were added *N,N*-diisopropylethylamine (0.16 cm^3, 0.94 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.1 cm^3, 0.42 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 5h. The solution was diluted by dichloromethane (50 cm^3), washed by a saturated aqueous solution of sodium hydrogencarbonate (2 x 30 cm^3) and evaporated under reduced pressure. The products were isolated by silica gel HPLC (PrepPak cartridge, 25 x 100 mm, Prep Nova-

Pak® HR Silica 6 μm 60Å; gradient of solution B in solution A (from 0% to 15% during 25 min and from 15% to 100% during another 25 min, solution A: petroleum ether/dichloromethane/pyridine, 60/39.6/0.4, v/v/v, solution B: ethyl acetate). The fractions containig the two main products (retention times 30-40 min) were joined, evaporated under reduced pressure, co-evaporated with anhydrous toluene (2 x 40 cm^3) and dried. The residue was dissolved in anhydrous dichloromethane (4 cm^3) and precipitated by adding this solution into anhydrous petroleum ether (80 cm^3) under intensive stirring. The precipitate was collected by filtration, washed by petroleum ether (2 x 20 cm^3) and dried under reduced pressure to give compound **8e** (178 mg, 73%) as a white solid material. δ_P (CD_3CN) 148.42, 147.93.

(*1S,3R,4R,7S*)-7-Acetoxy-1-acetoxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (9). To a stirred solution of nucleoside **6a** (209.8 mg, 0.78 mmol) in anhydrous pyridine (2.5 cm^3) was added acetic anhydride (0.3 cm^3, 3.23 mmol) and a catalytical amount of DMAP (5 mg). After stirring for 2 h, ethanol was added (4 cm^3) and the mixture was evaporated under reduced pressure. The residue was redissolved in dichloromethane (10 cm^3) and washed with a saturated aqueous solution of sodium hydrogencarbonate (7 cm^3). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (97:3, v/v) as eluent affording nucleoside **9** as a white solid material (249 mg, 90%). δ_C ($CDCl_3$) 169.59, 163.20, 149.50, 133.55, 110.64, 87.05, 85.38, 77.84, 71.70, 71.02, 58.60, 20.62, 20.53, 12.78. FAB-MS *m/z* 355 $[M+H]^+$.

(*1S,3R,4R,7S*)-7-Hydroxy-1-hydroxymethyl-3-(5-methyl-4-*N*-benzoylcytosine-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (10). To a solution of nucleoside **9** (232.7 mg, 0.66 mmol) in anhydrous acetonitrile (3 cm^3) was added a solution of 1,2,4-triazole (420 mg, 6.1 mmol) and $POCl_3$ (0.12 cm^3, 1.3 mmol) in anhydrous acetonitrile (5 cm^3). The reaction mixture was cooled to 0 °C and anhydrous triethylamine (0.83 cm^3) was added, whereupon the mixture was keept for 90 min at room temperature. Triethylamine (0.54 cm^3) and water (0.14 cm^3) were added, and the reaction mixture was stirred for 10 min and evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with a saturated aqueous solution of sodium hydrogencarbonate (2 x 9 cm^3) and water (9 cm^3). The aqueous phase was extracted with dichloromethane (3 x 20 cm^3). The combined organic phase was evaporated under reduced pressure and the residue was redissolved in dioxane (4 cm^3) and 32% aqueous ammonia (0.7 cm^3) was added. After stirring for 3 h, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in anhydrous pyridine (3.6 cm^3) and benzoyl chloride (0.42 cm^3, 3.6 mmol) was added. After stirring for 2 h, the reaction was quenched with water (1 cm^3) and the reaction mixture was evaporated under reduced pressure. The residue was redissolved in ethyl acetate and washed with water (3 x 7 cm^3). The organic phase was evaporated under reduced pressure, and the residue was dissolved in pyridine/methanol/water (13:6:1, v/v/v, 14 cm^3) at 0 °C, and a 2M solution of NaOH in pyridine/methanol/water (13:6:1, v/v/v, 7 cm^3) was added. After stirring for 20 min, the reaction mixture was neutralized using a 2M solution of HCl in dioxane, and the reaction mixture was evaporated under reduced pressure. The residue was purified by silica column chromatography using dichloromethane/methanol (95:5, v/v) as eluent to give nucleoside **10** as a yellow foam (94.6 mg, 38%) which was used in the next step without further purification. δ_H (CD_3OD) 8.21 (1H, br, s), 8.02 (1H, m), 7.84-7.9 (1H, m), 7.41-7.58 (4H, m), 5.61 (1H, s), 4.36 (1H, s), 4.10 (1H, s), 3.98 (1H, d, *J* 8.0), 3.94 (2H, s), 3.78 (1H, d, *J* 7.9), 2.11 (3H, d, *J* 1.0). δ_C (CD_3OD, selected signals) 133.66, 132.90, 130.63, 129.50, 129.28, 128.65, 90.71, 88.86, 80.57, 72.47, 70.22, 57.53, 14.01. FAB-MS *m/z* 374 $[M+H]^+$.

(*1R,3R,4R,7S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityl-oxymethyl)-3-(5-methyl-4-*N*-benzoylcytosine-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (11). To a stirred solution of nucleoside **10** (82 mg, 0.22 mmol) in anhydrous pyridine (1.5 cm^3) was added 4,4'-dimethoxytrityl chloride (80 mg, 0.24 mmol) and stirring was continued at room temperature for 12 h. Additional 4,4'-dimethoxytrityl chloride (80 mg, 0.24 mmol) was added, and stirring was continued for another 12 h. Methanol (0.5 cm^3) was added and the reaction mixture was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography using dichloromethane/methanol/pyridine (98.5:1.0:0.5, v/v/v). The resulting intermediate (50 mg) (FAB-MS *m/z* 676) was dissolved in anhydrous dichloromethane (0.62 cm^3). *N,N*-Diisopropylethylamine was added (0.1 cm^3) followed by addition of 2-cyanoethyl *N,N*-diisopropyl-phosphoramidochloridite (0.3 cm^3, 0.11 mmol). After stirring for 3 h at room temperature, water (1 cm^3) was added and the resulting mixture was diluted with ethylacetate (10 cm^3), washed with saturated aqueous solutions of sodium hydrogencarbonate (3 × 6 cm^3) and brine (3 × 6 cm^3). The organic phase was separated, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column HPLC and precipitated as described for **8e** affording compound **11** as a white solid material (29.5 mg, 0.03 mmol, 14%). δ_P (CH_3CN) 148.46, 147.49.

LNA synthesis and analysis. Standard coupling conditions according to the protocol (0.2 μmol scale) of the DNA-synthesizers (Pharmacia Gene Assembler Special®, Biosearch 8750 DNA Synthesizer) were used except that the coupling time for LNA amidites **8** and **11** was increased from the standard two minutes to six or twelve minutes. The step-wise coupling yield for **8** (except for **8d**) and **11** as well as for unmodified deoxynucleoside phosphoramidites was approximately 99%, and for **8d** approximately 95%. Standard 2'-deoxynucleoside CPG or polystyren solid supports were generally used. For synthesis of the fully modified LNA (Table 3), a Universal CPG Support (BioGenex) was applied (coupling yields as for the standard supports were obtained; deprotection followed the manual of the manufacturer). After completion of the desired sequences, cleavage from the solid support and removal of protecting groups was accomplished using 32% aqueous ammonia (55 °C for 5 or 10 h). After filtration through Sephadex G-25 (NAP-10 columns, Pharmacia) for 5'-*O*-DMT-*OFF* LNAs and unmodified reference strands, capillary gel electrophoresis was used to verify the purity (>90%) of the synthesized oligonucleotides. LNAs synthesized in the 5'-*O*-DMT-*ON* mode were purified by reversed-phase HPLC (Delta Pak C-18, 300A, column dimensions 0.4 x 30 cm) in a concentration gradient of acetonitrile in 0.05 M triethylammonium acetate buffer pH 7.0 (flow rate 1.5 cm^3/min). Fractions containing 5'-*O*-DMT-*ON* LNAs (retention times of 30-35 minutes) were evaporated and detritylated in 80% acetic acid (1 cm^3, room temperature, 1 h). After evaporation, the LNAs were re-purified by reversed-phase HPLC (eluting as single peaks) as described above. Representative data from MALDI-MS analysis: **LNA3** $[M-H]^-$ 2862.2; calcd. 2861.9; **LNA4** $[M-H]^-$ 2822.6; calcd. 2822.8; **LNA5** $[M-H]^-$ 2835.8; calcd. 2836.8; **LNA6** $[M-H]^-$ 2846.1; calcd. 2846.8; 5'-$G^LT^LG^LA^LT^LA^LT^LG^L\,^{Me}C^L$ $[M-H]^-$ 3019.5; calcd. 3019.8.

Thermal affinity studies. The thermal stabilities of the duplexes were determined spectrophotometrically using a spectrophotometer equipped with a thermoregulated Peltier element. Hybridization mixtures of 1 cm^3 were prepared using a medium salt buffer solution (10 mM Na_2HPO_4 pH 7.0, 100 mM NaCl, 0.1 mM EDTA) and equimolar (1.0 or 1.5 μM) amounts of the oligonucleotides (see caption Table 4 for low salt buffer solution). The absorbance at 260 nm was recorded while the temperature was raised linearly from 10-90 °C (1 °C/min). The melting temperatures (T_m values) were obtained as the maxima (+/- 1 °C) of the first derivatives of the

melting curves. No transitions were observed when running LNAs without complements in control experiments.

ACKNOWLEDGEMENTS

We thank The Danish Natural Science Research Council, The Danish International Development Agency, and Exiqon A/S, Denmark for financial support. Ms Britta Dahl is gratefully thanked for performing LNA synthesis.

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