Oligonucleotide analogues containing (2″*S*)- and (2″*R*)-2′-*O*,3′-*C*-((2″-*C*-hydroxymethyl)ethylene)-linked bicyclic nucleoside monomers:† Synthesis, RNA-selective binding, and diastereoselective formation of a very stable homocomplex based on T:T base pairing

Michael Raunkjær,[a] Carl E. Olsen[b] and Jesper Wengel*[a]

[a] *Center for Synthetic Bioorganic Chemistry, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*
[b] *Department of Chemistry, The Royal Danish Veterinary and Agricultural University, DK-1871 Frederiksberg, Denmark*

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The 2′-*O*,3′-*C*-[(2″*R*)-2″-*C*-(acetoxymethyl)ethylene]-linked and 2′-*O*,3′-*C*-[(2″*S*)-2″-*C*-(acetoxymethyl)ethylene]-linked bicyclic thymine nucleosides **12*R*** and **12*S*** have been synthesized and transformed into the phosphoramidite derivatives **14*R*** and **14*S***, respectively. On an automated DNA-synthesizer the novel 2′-*O*,3′-*C*-[(2″-*C*-hydroxymethyl)ethylene]-linked oligonucleotide analogues (2″*R*)-2″-hydroxymethyl-2′,3′-BcNA (***R***) and (2″*S*)-2″-hydroxymethyl-2′,3′-BcNA (***S***) have been prepared. The thermal stability of complexes involving these oligonucleotide analogues has been evaluated towards complementary single-stranded DNA and RNA and compared with the thermal stability of reference duplexes involving DNA and 2′-*O*,3′-*C*-ethylene-linked 2′,3′-BcNA (**B**). Oligonucleotide 5′-$\boldsymbol{S}_{13}$T exhibited RNA-selective binding with moderately enhanced thermal stability relative to the corresponding unmodified control. Remarkably strong intermolecular self-association was observed for 5′-$\boldsymbol{R}_{13}$T, but not for 5′-$\boldsymbol{S}_{13}$T.

Introduction

Conformational restriction has been successfully applied in recent years to the design of high-affinity oligonucleotides (ONs).[1,2] Several analogues containing bi- and tricyclic carbohydrate moieties have been synthesized and shown to display enhanced duplex stabilities[3–18] leading to increased thermal stability (melting temperature, T_m-value) of duplexes towards complementary single-stranded DNA and RNA. We have earlier introduced 2′-*O*,3′-*C*-ethylene-linked nucleoside and oligonucleotide analogues[8–10,19] and obtained RNA-selective binding with increased thermal stability for the analogue **B** (Fig. 1) termed 2′,3′-BcNA ($\Delta T_m = +12$ °C for the sequence 5′-$\mathbf{B}_{13}$T; thymine as nucleobase for monomer **B**; ΔT_m = overall increase in T_m compared with the value obtained for the unmodified reference ON). Combined molecular modelling and ^{1}H NMR studies on the parent 2′,3′-BcNA thymine nucleoside [(1*S*,5*R*,6*R*,8*R*)-5-hydroxy-6-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane] showed it to exist primarily in a C-1′-*exo* conformation.[10]

In this paper, synthesis and preliminary evaluation of 2″-*C*-hydroxymethyl-derivatized 2′,3′-BcNA are described, namely ONs containing the two diastereomerically pure monomers **R** [(2″*R*)-2″-hydroxymethyl-2′,3′-BcNA] and **S** [(2″*S*)-2″-hydroxymethyl-2′,3′-BcNA] shown in Fig. 1. This work was stimulated by (a) the possibility of enhancing the affinity towards complementary strands compared with 2′,3′-BcNA (additional hydrogen bonding or increased hydration), (b) our continued interest in branched ONs,[20,21] and (c) the possibility of obtaining conformationally restricted analogues of biologically active 3′-*C*-branched nucleosides.[22]

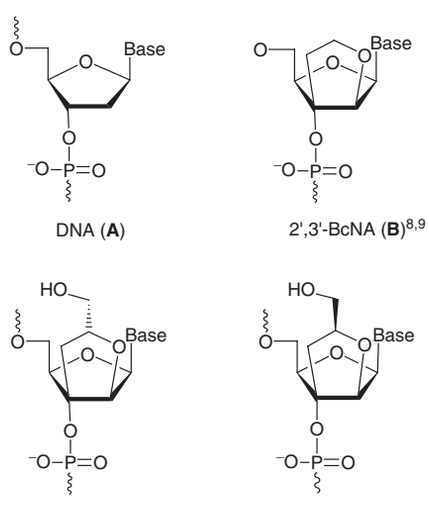


Fig. 1 The monomeric structures of DNA (**A**), 2′,3′-BcNA (**B**),[8,9] (2″*R*)-2″-hydroxymethyl-2′,3′-BcNA (***R***) and (2″*S*)-2″-hydroxymethyl-2′,3′-BcNA (***S***).

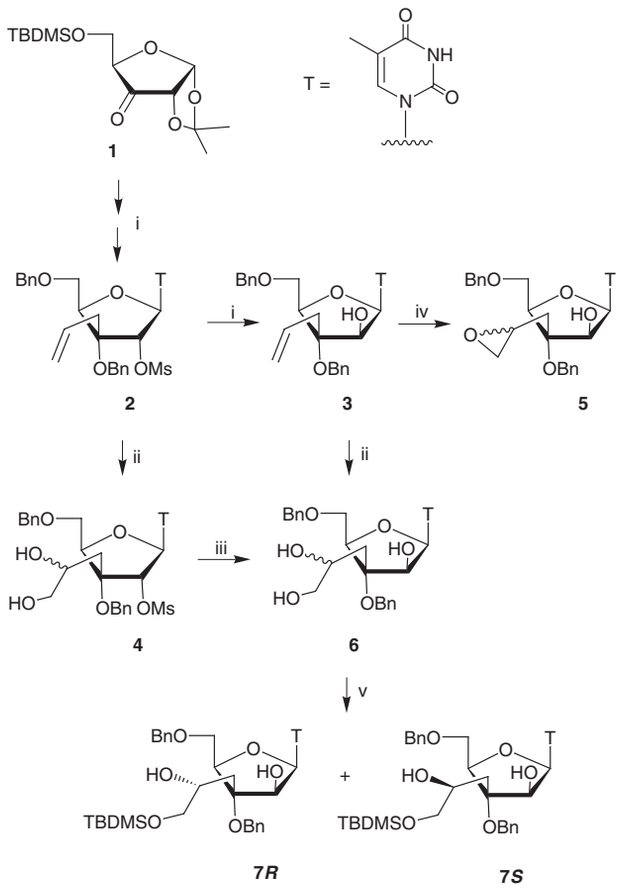
Results and discussion

The two diastereoisomeric phosphoramidite building blocks **14*R*** and **14*S*** were synthesized as shown in Schemes 1 and 2.

† The atom-numbering system used in this paper follows the conventional nucleoside style with the thymine base having the highest priority. The numbering used for the 3′-*C*-branched nucleosides **2**–**7** is continued for the bicyclic derivatives **8**–**14**. Thus, *e.g.*, the C-2″ carbon atom in the 3′-*C*-branch of **7** corresponds to the carbon atom carrying the hydroxymethyl group in nucleoside **9**. The names given in the Experimental section are of the von Baeyer type whereas the assignments of the signals in the NMR spectra follow the nucleoside style.

The 3′-*C*-allyl-branched nucleoside derivatives **2** and **3** were obtained using earlier published procedures starting from 3-ulose **1**.[9] Our initial synthetic strategy towards **14*R*** and **14*S*** involved a catalytic dihydroxylation of the 3′-*C*-allyl group of **2** or **3**. Thus, treatment with a catalytic amount of osmium tetraoxide in a mixture of THF and water in the presence of *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant afforded the diols **4** and **6** as inseparable diastereoisomeric pairs in yields of 90% and 88%, respectively. The dihydroxylations proceeded with moderate diastereoselectivity to give both **4** and **6** as a 2 : 1 stereoisomeric mixture. In order to selectively obtain the two C-2″ diastereoisomers the Sharpless asymmetric dihydroxylation method[23] should, theoretically, be useful. However, we have earlier experienced low yields and poor diastereoselectivity using this method on nucleoside alkenes,[24,25] and we therefore decided to proceed with the diastereoisomeric mixtures **4** and **6**. For synthesis of the bicyclic 2″-hydroxymethyl-2′,3′-BcNA nucleosides, *e.g.* **10*R***, **10*S***, **12*R*** and **12*S***, the arabino-configured derivative **6** appeared as an ideal intermediate which, *via* selective protection of the primary hydroxy group and subsequent selective activation of the C-2″-hydroxy group, should furnish the desired bicyclic nucleosides. The inversion of C-2′-stereochemistry could be obtained before[9] (**2** ⟶ **3** ⟶ **6**) or after (**2** ⟶ **4** ⟶ **6**) dihydroxylation. Thus, treatment of 2′-*O*-mesyl derivative **4** with excess of aq. sodium hydroxide in 96% ethanol furnished arabinofuranosyl nucleoside **6** in 98% yield. However, as an alternative synthetic key intermediate we also prepared epoxide **5** in 80% yield as a 2 : 1 diastereoisomeric mixture by oxidation of nucleoside **3** with 3-chloroperoxybenzoic acid (MCPBA). Cyclization of epoxide **5** (K_2CO_3, 18-crown-6, anhydrous DMF) yielded only a trace of nucleoside **9*R*** (~1%) in addition to several non-identified products after column chromatographic purification. We therefore focused our attention on selective protection of the primary hydroxy group of derivatives **6**, and silylation using *tert*-butyldimethylsilyl chloride (TBDMSCl) in anhydrous pyridine proved successful in affording the separable diastereoisomeric nucleosides **7*R*** and **7*S*** in yields of 31% and 53%, respectively, after column chromatographic purification. In addition, a mixture of **7*R*** and **7*S*** was isolated in 14% yield (Scheme 1). The stereochemistry of the two series of diastereoisomeric nucleosides **7**–**14** was assigned on the basis of nuclear Overhauser enhancement (NOE) experiments (*vide infra*).

The conversion of nucleosides **7*S*** and **7*R*** into phosphoramidite building blocks **14*R*** and **14*S***, respectively, was conducted by the synthetic route depicted in Scheme 2. Following our expectation based on the proximity of the C-2′-hydroxy group to the nucleobase, the C-2″-hydroxy groups could be selectively activated for nucleophilic attack by reaction with even a large excess of toluene-*p*-sulfonyl chloride (TsCl) in anhydrous pyridine. Without isolation, the tosylated intermediates were cyclized with inversion of configuration (K_2CO_3, 18-crown-6, anhydrous DMF) to afford the 2,7-dioxabicyclo[3.3.0]octane nucleosides **8*R*** (from **7*S***) and **8*S*** (from **7*R***) in yields of 45% and 52%, respectively. Our attempts at selectively removing the *O*-benzyl protecting groups of derivatives **8** failed and we therefore decided to exchange the *O*-silyl protecting group with an *O*-acetyl group. The *O*-silyl group is known to be compatible with automated ON synthesis using the phosphoramidite approach[26–28] but use of the *O*-acetyl group alleviates the need for an additional desilylation step after completion of the desired sequence, cleavage from the solid support, and deprotection. Desilylation of **8*R*** and **8*S*** was accomplished using tetrabutylammonium fluoride (TBAF) in THF to give **9*R*** and **9*S*** in yields of 80% and 94%, respectively. Subsequent acetylation (Ac_2O, anhydrous pyridine) afforded the fully protected derivatives **11*R*** and **11*S*** in yields of 96% and 92%, respectively. In order to obtain the parent, completely deprotected, 2″-*C*-hydroxymethyl-derivatized 2′-*O*,3′-*C*-ethylene-linked bicyclic nucleosides **10*R*** and **10*S***, debenzylation of derivatives **9*R*** and



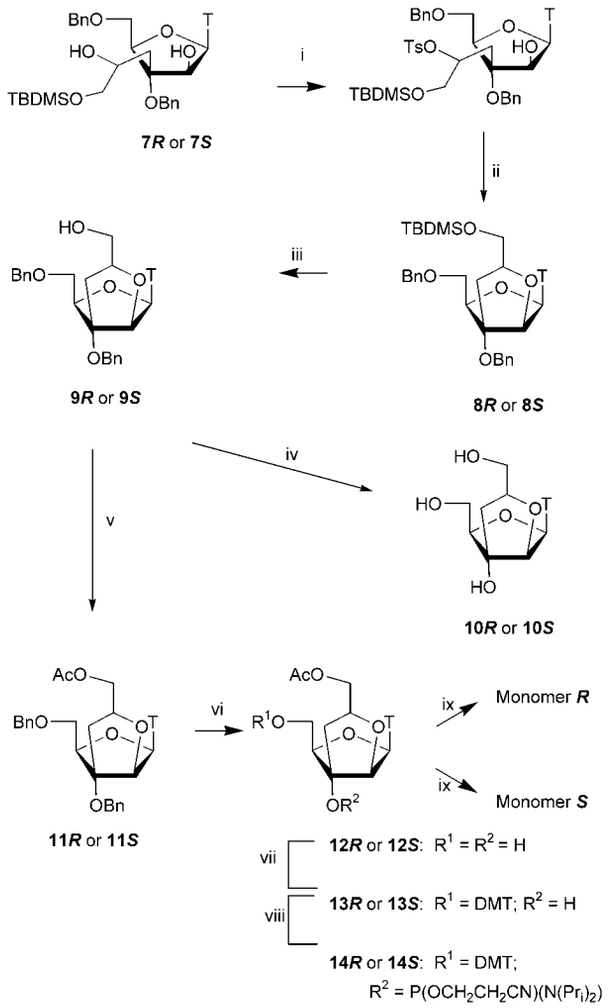
Scheme 1 *Reagents and conditions*: i) Ref. 9; ii) OsO_4, NMO, aq. THF (**4**: 90%; **6**: 88%); iii) NaOH, aq. EtOH (98%); iv) MCPBA, $NaHCO_3$, CH_2Cl_2 (80%); v) TBDMSCl, anhydrous pyridine (**7*R***: 31%; **7*S***: 53%).

9*S* was accomplished by hydrogenation over 20% $Pd(OH)_2$/C in 91% and 93% yield, respectively. Following the same procedure as for the preparation of nucleosides **10**, the monoacetylated diols **12*R*** (94% yield) and **12*S*** (95% yield) were obtained from **11*R*** and **11*S***, respectively. Transformation of diols **12*R*** and **12*S*** into the phosphoramidite derivatives **14*R*** and **14*S*** was accomplished *via* intermediates **13*R*** and **13*S*** by standard procedures, namely selective 5′-*O*-DMT protection by reaction with 4,4′-dimethoxytrityl chloride (DMTCl) in anhydrous pyridine (yields 92% for **13*R*** and 96% for **13*S***) followed by 3′-*O*-phosphitylation (yields 64% for **14*R*** and 50% for **14*S***).

To assign the stereochemistry of nucleosides **7**–**14** and monomers ***R*** and ***S***, NOE experiments were conducted on compounds **7**, **9** and **11**. In part due to overlapping signals for spectra recorded in different solvents, conclusive evidence could be obtained only in the case of nucleosides **9**. Thus, mutual NOEs were observed between the C-2″-C*H*$_2$O*H* protons and the *H*-6 and C-5-C*H*$_3$ protons in the thymine moiety (<1% to 3%) for **9*R***, but not for **9*S***, establishing the compounds **8*R***–**14*R*** and monomer ***R*** as having the *R*-configuration at C-2″.

The oligomers shown in Table 1 were synthesized on an automated DNA-synthesizer by use of the phosphoramidite approach.[28] The stepwise-coupling yields determined spectrophotometrically by the release of the 4,4′-dimethoxytrityl group after each coupling step were >98% for amidites **14*S*** and **14*R*** (couplings for 20 min) as well as for unmodified amidites (couplings for 1 min). The purity of all ONs synthesized was shown to be >90% by capillary gel electrophoresis and the compositions were verified by MALDI-MS analysis (see Experimental section for further details).

The stability of duplexes formed between the 2″-hydroxymethyl-2′,3′-BcNAs and complementary single-stranded DNA and RNA was evaluated by thermal denaturation studies as described earlier.[12] The results are shown in Table 1 as T_m-



Scheme 2 *Reagents and conditions*: i) TsCl, anhydrous pyridine; ii) K_2CO_3, 18-crown-6, anhydrous DMF (**8*R***: 45% from **7*S***; **8*S***: 52% from **7*R***); iii) TBAF, THF (**9*R***: 80%; **9*S***: 94%); iv) H_2, 20% $Pd(OH)_2$/C, EtOH (**10*R***: 91%; **10*S***: 93%); v) Ac_2O, anhydrous pyridine (**11*R***: 96%; **11*S***: 92%); vi) H_2, 20% $Pd(OH)_2$/C, EtOH (**12*R***: 94%; **12*S***: 95%); vii) DMTCl, pyridine (**13*R***: 92%; **13*S***: 96%); viii) 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite, *N*,*N*-diisopropylethylamine, dichloromethane (**14*R***: 64%; **14*S***: 50%); ix) DNA-synthesizer. T = thymin-1-yl.

values (melting temperatures) and ΔT_m values (total change in T_m compared with the corresponding reference duplex). The (2″*S*)-2″-hydroxymethyl-2′,3′-BcNAs (***S***, Fig. 1), for which the 2″-*C*-hydroxymethyl group is oriented away from C-5′ and the thymine moiety, displays hybridization properties closely resembling those obtained earlier for the parent 2′,3′-BcNA[8,9] (**B**, Fig. 1). Thus, consecutively modified (2″*S*)-2″-hydroxymethyl-2′,3′-BcNAs displayed pronounced RNA-selectivity with increased thermal affinity ($\Delta T_m = +1$ °C for 5′-T_5***S***$_4T_5$ and +14 °C for 5′-S_{13}T *towards RNA*; $\Delta T_m = -11$ °C for 5′-T_5***S***$_4T_5$ and <-21 °C for 5′-S_{13}T *towards DNA*), whereas (2″*S*)-2″-hydroxymethyl-2′,3′-BcNAs containing a single modification or three or four modifications alternating with unmodified monomers displayed unchanged or moderately decreased thermal stability compared with the unmodified reference duplexes. In comparison with the (2″*S*)-2″-hydroxymethyl-2′,3′-BcNAs, the corresponding (2″*R*)-2″-hydroxymethyl-2′,3′-BcNAs (***R***, Fig. 1) displayed an even lower affinity towards DNA and a similar, but less pronounced, tendency towards RNA-selective hybridization. This diastereoselectivity in duplex formation observed for the two stereoisomeric 2″-hydroxymethyl-2′,3′-BcNAs is most strongly expressed in the behavior of the two almost fully modified ONs, 5′-R_{13}T and 5′-S_{13}T. As mentioned above, 5′-S_{13}T displayed RNA-selective binding and

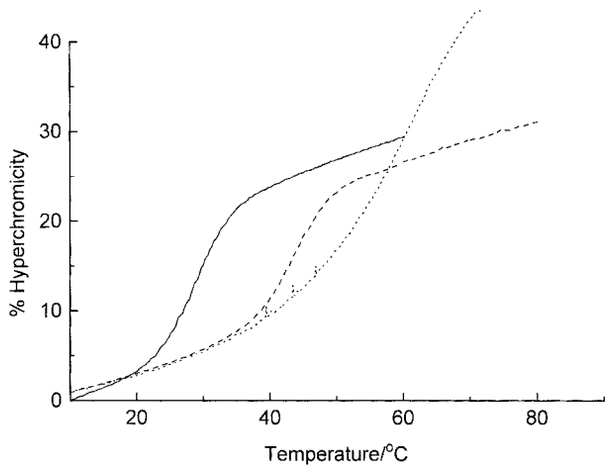


Fig. 2 Thermal denaturation curves for selected complexes. Conditions as described in the caption under Table 1 and in ref. 12 (——), T_{14}:rA_{14}; (– – –), 5′-S_{13}T:rA_{14}; (----), 5′-R_{13}T.

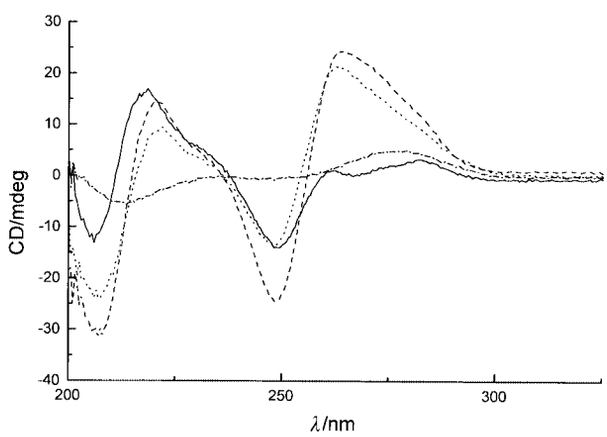


Fig. 3 CD curves for selected complexes recorded at 20 °C. Conditions otherwise as described in the caption below Table 1 and in ref. 12 (——), T_{14}:dA_{14}; (– – –), T_{14}:rA_{14}; (----), 5′-S_{13}T:rA_{14}; (–·–·–), 5′-R_{13}T.

a measured T_m-value towards the rA_{14} complement of 43 °C. In thermal denaturation experiments with 5′-R_{13}T mixed with dA_{14} or rA_{14}, T_m-values of 60 and 61 °C were determined. However, as an identical T_m-value (60 °C) and thermal denaturation curve (A_{260} *vs.* temperature) were obtained for 5′-R_{13}T in experiments conducted *without* the addition of a complementary strand, we ascribe the T_m-value of ≈60 °C to self-complexation for 5′-R_{13}T based on T:T base pairing. Our experiments showed no indication of the formation of a similar complex for the corresponding (2″*S*)-diastereoisomer. Thermal denaturation curves for selected complexes are shown above (Fig. 2). The large hyperchromicity change observed during denaturation for the 5′-R_{13}T homocomplex possibly reflects a very limited tendency towards base-stacking for the denatured non-base-paired state.

To study the nature of the complexes formed, CD spectra of selected complexes were recorded (Fig. 3). The similarity between the spectra for the T_{14}:rA_{14} duplex and the 5′-S_{13}T:rA_{14} complex is striking. This establishes 5′-S_{13}T as a close mimic of T_{14} and that the 5′-S_{13}T:rA_{14} complex is a regular duplex and not a triplex. The CD spectrum of the homocomplex formed by 5′-R_{13}T does not display normal A- or B-form duplex characteristics, but strongly reduced ellipticity values.

To further investigate the homocomplex, the T_m-value for 5′-R_{13}T without complement was measured at concentrations of 3.6 μM and 7.3 μM in addition to the 1.5 μM used earlier in the series of experiments shown in Table 1. An increase in the T_m-value with increasing concentration of 5′-R_{13}T (1.5 μM; T_m = 60 °C; 3.6 μM; T_m = 61 °C; 7.3 μM; T_m = 62 °C; measured

Table 1 ONs synthesized and thermal denaturation studies[a]

Sequence	X	Complementary dA_{14}		Complementary rA_{14}	
		T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
5′-T_{14}		31	reference	29	reference
5′-T_7**X**T_6	***R***	27	−4	25	−4
	S	30	−1	27	−2
5′-T_3(T**X**)$_4T_3$	***R***	17	−14	12	−17
	S	25	−6	19	−10
5′-T_5**X**$_4T_5$	***R***	10	−21	25	−4
	S	20	−11	30	+1
5′-**X**$_{13}$T	***R***	60	[b]	61	[b]
	S	<10	<−21	43	+14
5′-GTGATATGC		27	reference	25	reference
5′-GTGA**X**ATGC	***R***	28	+1	26	+1
	S	28	+1	26	+1
5′-G**X**GA**X**A**X**GC	***R***	18	−9	24	−1
	S	24	−3	25	0

[a] A = 2′-deoxyadenosine monomer, C = 2′-deoxycytidine monomer, G = 2′-deoxyguanosine monomer, T = thymidine monomer, ***R*** = (2″*R*)-2″-hydroxymethyl-2′,3′-BcNA thymine monomer, ***S*** = (2″*R*)-2″-hydroxymethyl-2′,3′-BcNA thymine monomer, **X** = monomer ***R*** or ***S*** as indicated in the second column. The melting temperatures (T_m-values) were determined in 3 cm^3 cuvettes as described earlier,[12] assuming identical extinction coefficients for the 2″-hydroxymethyl-2′,3′-BcNA-type ONs and the corresponding unmodified ONs. A medium salt buffer (10 mM sodium phosphate, pH 7.0, 100 mM sodium chloride, 0.1 mM EDTA) was used. ΔT_m-values are the calculated total change in T_m-value compared with the corresponding unmodified reference duplex. [b] A T_m-value of 60 °C was obtained in an analogous thermal denaturation experiment without the addition of a complementary strand.

in 1 cm^3 cuvettes whereas 3 cm^3 cuvettes were used in the experiments shown in Table 1) was detected. Although no definite conclusion can be drawn based on these minor changes, the observed tendency towards an increase in T_m with increasing concentration of 5′-***R***$_{13}$T *suggests* the homocomplex formed as being of intermolecular nature, probably a duplex. Similar strong self-pairing (*via* the formation of reverse-Hoogsteen base-pairs) has been reported for oligo-(2′,3′-dideoxy-β-D-glucopyranosyl)-adenine and -guanine (but not thymine),[29] and it is known that poly-U (but not poly-T) forms a poly-U : poly-U duplex of low stability.[30] In addition, only oligo-α-thymidylate derivatives[31] and 1′,5′-anhydrohexitol-thymine oligomers[32] have been shown to form thymine : thymine base-pairs. The observed self-complexation with a T_m-value of 60 °C/61 °C observed for 5′-***R***$_{13}$T is thus quite remarkable and we attribute its existence to the presence of the 2″-*C*-hydroxymethyl group pointing towards the thymine moiety. Molecular modelling suggests the nucleoside **10*R*** to exist in a C-1′-*endo* furanose conformation ‡ and the formation of an intramolecular hydrogen bond between the (2″*R*)-2″-*C*-hydroxymethyl group and the C-2-carbonyl group of the thymine moiety seems realistic. This could preorganize the thymine moiety in a *syn*-conformation allowing the formation of a stable homoduplex with intermolecular hydrogen bonds between two thymine moieties. It should be mentioned that no conclusions on the furanose conformation adopted by monomer **R** present in an oligomer can be drawn based on the modelling performed on nucleoside **10*R***. Likewise it is noteworthy that the formation of an intramolecular hydrogen bond between the (2″*R*)-2″-*C*-hydroxymethyl group and the C-2-carbonyl group in the thymine moiety appears likely also for several other possible furanose conformations of monomer ***R***, *e.g.* the C-1′-*exo* furanose conformation earlier shown[10] to be adopted by the bicyclic nucleoside leading to 2′,3′-BcNA (**B**, Fig. 1). In addition, the formation of this intramolecular hydrogen bond, and/or sterically unfavorable interactions involving the 2″-*C*-hydroxymethyl group of monomer ***R***, and/or a changed furanose conformation could explain the diastereoselective hybridization observed for the two isomers of 2″-hydroxymethyl-2′,3′-BcNA containing one, three or four modifications.

‡ Molecular modelling experiments were performed using Sybil™ Rel. 6.3 for Silicon Graphics using Tripos Force Field with MOPAC-derived charges for geometry optimizations and molecular dynamics studies.

Conclusions

The 2′-*O*,3′-*C*-[(2″*R*)-2″-*C*-(acetoxymethyl)ethylene]-linked and the 2′-*O*,3′-*C*-[(2″*S*)-2″-*C*-(acetoxymethyl)ethylene]-linked bicyclic arabinofuranosyl thymine nucleosides **12*R*** and **12*S*** and the corresponding phosphoramidite building blocks **14*R*** and **14*S*** have been synthesized, generally in good stepwise yields, starting from the C-2″-diastereoisomeric mixture **6**. Arabino-configured nucleosides **6** were obtained by catalytic dihydroxylation of 3′-*C*-allyl-branched nucleosides preceded or followed by inversion of stereochemistry at C-2′. Selective silylation of the primary hydroxy group of nucleosides **6** furnished the separable diastereoisomeric nucleosides **7*S*** and **7*R***. Key steps towards their conversion into phosphoramidites **14*R*** and **14*S*** were selective tosylation of the 2″-hydroxy groups followed by base-mediated ring-closure to give the desired 2,7-dioxabicyclo[3.3.0]octane skeleton. The amidites **14*R*** and **14*S*** were successfully elaborated on an automated DNA-synthesizer together with unmodified phosphoramidites to afford the novel bicyclic oligonucleotide analogues (2″*R*)-2″-hydroxymethyl-2′,3′-BcNA (***R***) and (2″*S*)-2″-hydroxymethyl-2′,3′-BcNA (***S***). The thermal stability of complexes involving these oligomers was evaluated towards complementary single-stranded DNA and RNA, and compared with the thermal stability of reference duplexes involving DNA and 2′-*O*,3′-*C*-ethylene-linked 2′,3′-BcNA (**B**). Oligomer 5′-***S***$_{13}$T, as observed for 2′,3′-BcNA, exhibited RNA-selective binding with moderately enhanced thermal stability compared with the corresponding unmodified reference. Remarkably strong intermolecular self-pairing was observed for oligomer 5′-***R***$_{13}$T, but not for the diastereoisomeric 5′-***S***$_{13}$T. We suggest that the formation of a unique intramolecular hydrogen bond between the (2″*R*)-2″-*C*-hydroxymethyl group and the C-2-carbonyl group in the thymine moiety leads to preorganization of monomer ***R*** (with the thymine moiety adopting a *syn*-conformation) allowing the formation of a very stable homocomplex based on T : T base pairing.

Experimental

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out on glass columns using Silica gel 60 (0.040–

0.063 mm). After drying of organic phases using Na_2SO_4, filtration was performed. 'Petroleum ether' of distillation range 60–80 °C was used. NMR spectra were recorded on a Varian Unity 400 or a Bruker AM 250 spectrometer. Chemical-shift-values δ are in ppm relative to tetramethylsilane as internal reference (^{1}H and ^{13}C NMR) and relative to 85% H_3PO_4 as external reference (^{31}P NMR). Assignments of signals from ^{1}H and ^{13}C NMR spectra are based on 2D NMR techniques when given. *J*-Values are given in Hz. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen. Matrix-assisted laser-desorption ionization–mass spectrometry (MALDI-MS) was performed on a Micromass Tof Spec E mass spectrometer.

1-{3,5-Di-*O*-benzyl-3-*C*-[(2*RS*)-2,3-dihydroxypropyl]-2-*O*-methylsulfonyl-β-D-ribofuranosyl}thymine 4

Nucleoside **2**[9] (8.89 g, 15.99 mmol) was dissolved in a mixture of THF (80 cm^3) and water (80 cm^3) followed by the addition of NMO (4.60 g, 39.40 mmol). A solution of OsO_4 in 2-methylpropan-2-ol [2.5% (w/w); 3.0 cm^3, 0.24 mmol] was added dropwise and the reaction mixture was stirred at room temperature for 7 h. The mixture was evaporated to dryness under reduced pressure and CH_2Cl_2 (300 cm^3) was added. Washing was performed successively using half-saturated aq. $NaHSO_4$ (3 × 100 cm^3), saturated aq. $NaHCO_3$ (100 cm^3) and water (100 cm^3). The organic phase was dried, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography [0→2% (v/v) MeOH in CH_2Cl_2 as eluent] to give nucleoside **4** as a 2:1 mixture of diastereoisomers as a white solid material (8.54 g, 90%), $\delta_C(CDCl_3)$ 163.8, 151.1, 137.7, 137.3, 136.4, 136.2, 135.7, 128.6, 128.4, 128.2, 128.2, 127.7, 127.6, 127.4, 127.0, 111.5, 85.2, 83.6, 83.3, 83.1, 82.8, 82.0, 81.5, 81.2, 73.5, 73.4, 69.5, 68.9, 67.8, 67.5, 66.7, 66.4, 66.3, 65.3, 38.8, 38.5, 33.6, 31.5, 11.8, 11.8; FAB-MS *m*/*z* 590.9 $[M + H]^+$ ($C_{28}H_{34}N_2O_{10}S\cdot0.5H_2O$ requires C, 56.1; H, 5.9; N, 4.7. Found: C, 55.8; H, 5.7; N, 4.4%).

1-{3,5-Di-*O*-benzyl-3-*C*-[(2*RS*)-2,3-epoxypropyl]-β-D-arabinofuranosyl}thymine 5

A suspension of MCPBA (60%; 650 mg, 2.27 mmol) and $NaHCO_3$ (47 mg, 0.55 mmol) was added to a solution of nucleoside **3**[9] (540 mg, 1.13 mmol) in CH_2Cl_2 (10 cm^3). The reaction mixture was stirred at 30 °C for 20 h and then washed successively with saturated aq. $NaHCO_3$ (2 × 5 cm^3) and water (1 × 5 cm^3). The organic phase was dried, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography [0→2% (v/v) MeOH in CH_2Cl_2] to give epoxide **5** as a 2:1 mixture of diastereoisomers as a white solid material (450 mg, 80%); $\delta_C(CD_3)_2SO$ 163.8, 163.7, 150.2, 150.1, 138.8, 138.4, 138.3, 138.1, 138.1, 137.3, 128.4, 128.3, 128.3, 128.0, 127.7, 127.6, 127.6, 127.5, 127.5, 108.0, 107.5, 93.8, 93.3, 84.2, 83.8, 83.7, 82.8, 82.7, 82.1, 80.6, 78.1, 72.5, 72.4, 68.9, 68.5, 66.9, 66.5, 62.7, 61.9, 33.9, 33.5, 12.3, 12.1; FAB-MS *m*/*z* 495.3 $[M + H]^+$.

1-{3,5-Di-*O*-benzyl-3-*C*-[(2*RS*)-2,3-dihydroxypropyl]-β-D-arabinofuranosyl}thymine 6

Nucleoside **3**[9] (19.01 g, 39.77 mmol) and NMO (9.99 g, 85.38 mmol) were dissolved in a mixture of THF (180 cm^3) and water (180 cm^3). A solution of OsO_4 in 2-methylpropan-2-ol [2.5% (w/w); 5 cm^3, 0.40 mmol] was added dropwise to the reaction mixture. After stirring at room temperature for 15 h the mixture was evaporated to dryness under reduced pressure. To the residue was added CH_2Cl_2 (300 cm^3) and washing was performed successively with half-saturated aq. $NaHSO_4$ (3 × 100 cm^3), saturated aq. $NaHCO_3$ (100 cm^3) and water (100 cm^3). The separated organic phase was dried, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography [0→2% (v/v) MeOH in CH_2Cl_2] to give nucleoside **6** as a 2:1 mixture of diastereoisomers as a white solid material. Yield 18.02 g (88%).

An alternative method for the preparation of 6. To nucleoside **4** (430 mg, 0.73 mmol) dissolved in a mixture of 96% EtOH (10 cm^3) and water (10 cm^3) was added 1.0 M aq. NaOH (3.0 cm^3, 3.0 mmol). The reaction mixture was stirred under reflux for 15 h and then cooled to room temperature. During cooling and stirring, 2.0 M aq. HCl (2 cm^3, 4.0 mmol) was added to the reaction mixture, which was subsequently evaporated to dryness under reduced pressure. To the residue was added CH_2Cl_2 (50 cm^3) and washing was performed successively using saturated aq. $NaHCO_3$ (2 × 20 cm^3) and water (15 cm^3). The separated organic phase was dried, and evaporated to dryness under reduced pressure to give a residue, which was purified by silica gel column chromatography [0→2% (v/v) MeOH in CH_2Cl_2] to give nucleoside **6** as a 2:1 mixture of diastereoisomers as a white solid material (366 mg, 98%); $\delta_C(CD_3)_2SO$ 164.0, 163.9, 150.4, 150.3, 138.7, 138.6, 138.4, 138.3, 137.4, 137.4, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.2, 107.0, 87.7, 87.4, 87.2, 87.0, 83.0, 80.6, 74.8, 73.3, 72.4, 72.3, 69.9, 69.8, 67.2, 67.1, 66.6, 66.0, 64.1, 63.7, 30.5, 29.5, 12.5; FAB-MS *m*/*z* 513.2 $[M + H]^+$ ($C_{27}H_{32}N_2O_8\cdot0.5H_2O$ requires C, 62.2; H, 6.4; N, 5.4. Found: C, 62.2; H, 6.1; N, 5.3%).

1-{3,5-Di-*O*-benzyl-3-*C*-[(2*R*)-3-*tert*-butyldimethylsilyloxy-2-hydroxypropyl]-β-D-arabinofuranosyl}thymine 7*R* and 1-{3,5-di-*O*-benzyl-3-*C*-[(2*S*)-3-*tert*-butyldimethylsilyloxy-2-hydroxypropyl]-β-D-arabinofuranosyl}thymine 7*S*

Nucleoside **6** (18.00 g, 35.16 mmol) was dissolved in anhydrous pyridine (200 cm^3) and TBDMSCl (13.99 g, 92.82 mmol) was added. After stirring at room temperature for 12 h the mixture was evaporated to dryness under reduced pressure. To the residue was added CH_2Cl_2 (350 cm^3) and washing was performed successively with saturated aq. $NaHCO_3$ (3 × 100 cm^3) and water (100 cm^3). The separated organic phase was evaporated to dryness under reduced pressure to give a residue after coevaporation with toluene (4 × 100 cm^3), which was purified by silica gel column chromatography [0→2% (v/v) MeOH in CH_2Cl_2] to give the two separated diastereoisomers **7*R*** (7.02 g, 31%) and **7*S*** (11.70 g, 53%), and a mixture of **7*R*** and **7*S*** (3.10 g, 14%), all as white solid materials.

Data for 7*R*. $\delta_H(CD_3)_2SO$ 11.28 (1 H, s, NH), 7.40–7.27 (11 H, m, 6-H and Ar), 6.09 (1 H, d, *J* 3.5, 1′-H), 5.24 (1 H, d, *J* 5.0, 2″-OH), 5.20 (1 H, d, *J* 4.2, 2′-OH), 4.63–4.42 (5 H, m, 4′-H and 2 × C*H*$_2$Ph), 4.14 (1 H, m, 2′-H), 3.82–3.76 (2 H, m, 2″-H and 5′-H^a), 3.63–3.55 (2 H, m, 3″-H^a and 5′-H^b), 3.38 (1 H, dd, *J* 7.5 and 9.7, 3″-H^b), 2.23 (1 H, d, *J* 15.4, 1″-H^a), 1.71 (3 H, s, CH_3), 1.62 (1 H, dd, *J* 9.6 and 15.6, 1″-H^b), 0.86 (9 H, s, Bu^t), 0.04 (3 H, s, SiMe), 0.03 (3 H, s, SiMe); $\delta_C(CD_3)_2SO$ 163.9 (C-4), 150.3 (C-2), 138.4 and 138.2 (Ar), 137.4 (C-6), 128.3, 128.3, 128.2, 127.6, 127.5, 127.4 and 127.3 (Ar), 107.0 (C-5), 87.6 (C-3′), 86.9 (C-1′), 80.4 (C-4′), 74.7 (C-2′), 72.4 (*C*H$_2$Ph), 69.7 (C-5′), 67.1 (C-3″), 66.6 (C-2″), 63.6 (*C*H$_2$Ph), 29.1 (C-1″), 25.9 [C(*C*H$_3$)$_3$], 18.0 [*C*(CH$_3$)$_3$], 12.5 (CH$_3$), −5.4 and −5.4 [Si(CH$_3$)$_2$]; FAB-MS *m*/*z* 627.4 $[M + H]^+$ ($C_{33}H_{46}N_2O_8Si$ requires C, 63.2; H, 7.4; N, 4.5. Found: C, 62.8; H, 7.3; N, 4.5%).

Data for 7*S*. $\delta_H(CD_3)_2SO$ 11.27 (1 H, s, NH), 7.43 (1 H, s, 6-H), 7.37–7.26 (10 H, m, Ar), 6.05 (1 H, d, *J* 3.3, 1′-H), 5.76 (1 H, d, *J* 5.8, 2′-OH), 4.73 (1 H, d, *J* 4.7, 2″-OH), 4.64–4.50 (4 H, m, 2 × C*H*$_2$Ph), 4.44 (1 H, m, 4′-H), 4.14 (1 H, dd, *J* 3.4 and 5.6, 2′-H), 3.87–3.77 (3 H, m, 2″-H and 5′-H$_2$), 3.52–3.42 (2 H, m, 3″-H$_2$), 2.02 (1 H, m, 1″-H^a), 1.71 (1 H, m, 1″-H^b), 1.69 (3 H, s, CH_3), 0.85 (9 H, s, Bu^t), 0.03 (3 H, s, SiMe), 0.02 (3 H, s, SiMe); $\delta_C(CD_3)_2SO$ 164.1 (C-4), 150.5 (C-2), 138.7 and 138.4

(Ar), 137.4 (C-6), 128.4, 127.7, 127.6 and 127.5 (Ar), 107.1 (C-5), 87.4 (C-3′), 87.3 (C-1′), 83.4 (C-4′), 73.1 (C-2′), 72.3 (*C*H_2Ph), 69.9 (C-5′), 68.0 (C-3″), 67.0 (C-2″), 64.1 (*C*H_2Ph), 30.1 (C-1″), 26.0 [C(*C*H_3)$_3$], 18.1 [*C*(CH$_3$)$_3$], 12.6 (CH$_3$), −5.1 and −5.2 [Si(CH$_3$)$_2$]; FAB-MS *m*/*z* 627.4 $[M + H]^+$. Found: C, 63.1; H, 7.4; N, 4.4%).

(1*S*,3*R*,5*R*,6*R*,8*R*)-5-Benzyloxy-6-benzyloxymethyl-3-(*tert*-butyldimethylsilyloxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (8*R*)

Nucleoside **7*S*** (440 mg, 0.70 mmol) was dissolved in anhydrous pyridine (20 cm^3), a solution of TsCl (1.33 g, 7.00 mmol) in anhydrous pyridine (10 cm^3) was added dropwise and the mixture was stirred for 72 h at room temperature. After evaporation to dryness under reduced pressure the residue was dissolved in CH_2Cl_2 (30 cm^3) and washed successively with saturated aq. $NaHCO_3$ (3 × 10 cm^3) and brine (10 cm^3). The organic phase was dried, and evaporated to dryness under reduced pressure to give a residue, which was purified by filtration through a short silica gel column [1% (v/v) MeOH in CH_2Cl_2]. Evaporation of the filtrate under reduced pressure gave a white solid material, which was dissolved in anhydrous DMF (6 cm^3) followed by addition of K_2CO_3 (50 mg, 0.36 mmol) and 18-crown-6 (66 mg, 0.25 mmol). The reaction mixture was stirred at 70 °C for 24 h and subsequently evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 cm^3) and washing was performed with successively saturated aq. $NaHCO_3$ (3 × 5 cm^3) and water (5 cm^3). The separated organic phase was dried, and evaporated to dryness under reduced pressure to give a residue which was purified by silica gel column chromatography [0→1% (v/v) MeOH in CH_2Cl_2] to give the product nucleoside **8*R*** as a white solid material (195 mg, 45%). In addition, desilylated derivative **9*R*** (39 mg) was obtained as a white solid material.

Data for 8*R*. δ_H(CD$_3$)$_2$SO 11.40 (1 H, s, NH), 7.50 (1 H, d, *J* 1.1, 6-H), 7.38–7.29 (10 H, m, Ar), 5.97 (1 H, d, *J* 4.4, 1′-H), 4.63 (2 H, m, C*H*$_2$Ph), 4.57 (3 H, m, 2′-H, C*H*$_2$Ph), 4.26 (1 H, m, 4′-H), 4.18 (1 H, m, 2″-H), 3.82 (2 H, m, 5′-H$_2$), 3.71 (1 H, dd, *J* 8.2 and 11.2, 3″-H^a), 3.60 (1 H, dd, *J* 5.3 and 11.2, 3″-H^b), 2.17 (1 H, dd, *J* 4.6 and 13.6, 1″-H^a), 1.92 (1 H, dd, *J* 11.3 and 13.3, 1″-H^b), 1.82 (3 H, d, *J* 1.1, CH$_3$), 0.86 (9 H, s, Bu^t), 0.01 (3 H, s, SiMe), 0.00 (3 H, s, SiMe); δ_C(CD$_3$)$_2$SO 163.7 (C-4), 150.0 (C-2), 138.4 and 138.0 (Ar), 137.6 (C-6), 128.3, 128.2, 127.5, 127.4 and 127.4 (Ar), 107.7 (C-5), 93.3 (C-3′), 83.9 (C-2′), 82.5 and 82.5 (C-1′ and -2″), 78.5 (C-4′), 72.3 (*C*H_2Ph), 68.8 (C-5′), 66.4 (*C*H_2Ph), 64.6 (C-3″), 33.9 (C-1″), 25.8 [C(*C*H_3)$_3$], 18.1 [*C*(CH$_3$)$_3$], 12.2 (CH$_3$), −5.4 and −5.4 [Si(CH$_3$)$_2$]; FAB-MS *m*/*z* 609.4 $[M + H]^+$ ($C_{33}H_{44}N_2O_7Si\cdot 0.25H_2O$ requires C, 64.6; H, 7.3; N, 4.6. Found: C, 64.5; H, 7.2; N, 4.3%).

(1*S*,3*R*,5*R*,6*R*,8*R*)-5-Benzyloxy-6-benzyloxymethyl-3-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (9*R*)

A 1.0 M solution of TBAF in THF (3.2 cm^3, 3.2 mmol) was added to a solution of nucleoside **8*R*** (1.90 g, 3.13 mmol) in anhydrous THF (60 cm^3) and the resulting mixture was stirred for 3 h at room temperature. After evaporation to dryness under reduced pressure the residue was dissolved in a mixture of 'petroleum ether' and CH_2Cl_2 [200 cm^3; 1:1 (v/v)] and washing was performed using, successively, saturated aq. $NaHCO_3$ (3 × 70 cm^3) and brine (50 cm^3). The separated organic phase was evaporated to dryness under reduced pressure and coevaporated with toluene (3 × 100 cm^3). The residue was purified by silica gel column chromatography [10→30% (v/v) ethyl acetate in 'petroleum ether'] to give nucleoside **9*R*** as a white solid material (1.24 g, 80%); δ_H(CD$_3$)$_2$SO 11.36 (1 H, s, NH), 7.58 (1 H, d, *J* 1.3, 6-H), 7.37–7.27 (10 H, m, Ar), 6.00 (1 H, d, *J* 4.8, 1′-H), 4.81 (1 H, t, *J* 5.1, 3″-OH), 4.65 (2 H, m, C*H*$_2$Ph), 4.55 (3 H, m, 2′-H and C*H*$_2$Ph), 4.22 (1 H, t, *J* 5.5, 4′-H), 4.19 (1 H, m, 2″-H), 3.80 (2 H, d, *J* 5.5, 5′-H$_2$), 3.53–3.47 (2 H, m, 3″-H$_2$), 2.13 (1 H, dd, *J* 4.8 and 13.6, 1″-H^a), 2.02 (1 H, dd, *J* 11.2 and 13.5, 1″-H^b), 1.78 (3 H, d, *J* 1.3, CH$_3$); δ_C(CD$_3$)$_2$SO 163.8 (C-4), 150.2 (C-2), 138.8 (C-6), 138.4, 138.1, 128.3, 128.3, 127.6, 127.6 and 127.4 (Ar), 107.5 (C-5), 93.7 (C-3′), 83.6 (C-2′), 82.7 (C-2″), 82.0 (C-1′), 78.0 (C-4′), 72.3 (*C*H_2Ph), 68.8 (C-5′), 66.4 (*C*H_2Ph), 61.8 (C-3″), 33.9 (C-1″), 12.0 (CH$_3$); FAB-MS *m*/*z* 495.2 $[M + H]^+$ ($C_{27}H_{30}N_2O_7$ requires C, 65.6; H, 6.1; N, 5.7. Found: C, 65.3; H, 6.1; N, 5.6%).

(1*S*,3*R*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-5-benzyloxy-6-benzyloxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 11*R*

Nucleoside **9*R*** (1.30 g, 2.63 mmol) was dissolved in anhydrous pyridine (40 cm^3) under stirring and Ac_2O (0.40 cm^3, 4.24 mmol) was added dropwise. After stirring at room temperature for 15 h the mixture was evaporated to dryness under reduced pressure to give a residue, which was dissolved in a mixture of 'petroleum ether' and CH_2Cl_2 [150 cm^3; 1:1 (v/v)]. Washing was performed using, successively, saturated aq. $NaHCO_3$ (3 × 100 cm^3) and water (100 cm^3) and the separated organic phase was evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (3 × 100 cm^3) to give a white solid material, which was purified by silica gel column chromatography [10→40% (v/v) ethyl acetate in 'petroleum ether'] to give nucleoside **11*R*** as a white solid material (1.36 g, 96%); δ_H(CD$_3$)$_2$SO 11.41 (1 H, s, NH), 7.48 (1 H, d, *J* 1.0, 6-H), 7.39–7.27 (10 H, m, Ar), 6.00 (1 H, d, *J* 4.6, 1′-H), 4.64 (2 H, m, C*H*$_2$Ph), 4.58–4.51 (3 H, m, 2′-H and C*H*$_2$Ph), 4.31 (1 H, m, 2″-H), 4.25 (1 H, dd, *J* 3.3 and 6.8. 4′-H), 4.15–4.04 (2 H, m, 3″-H$_2$), 3.87–3.77 (2 H, m, 5′-H$_2$), 2.23 (1 H, dd, *J* 5.0 and 13.9, 1″-H^a), 2.02 (1 H, m, 1″-H^b), 2.00 (3 H, s, COCH$_3$), 1.79 (3 H, d, *J* 0.8, CH$_3$); δ_C(CD$_3$)$_2$SO 170.1 (*C*OCH$_3$), 163.7 (C-4), 150.1 (C-2), 138.3 and 138.1 (Ar), 137.9 (C-6), 128.3, 128.3, 127.6, 127.5 and 127. 5 (Ar), 107.7 (C-5), 93.4 (C-3′), 84.0 (C-2′), 82.2 (C-1′), 79.3 (C-2″), 78.3 (C-4′), 72.3 (*C*H_2Ph), 68.7 (C-5′), 66.5 (*C*H_2Ph), 64.6 (C-3″), 34.0 (C-1″), 20.6 (CO*C*H$_3$), 12.0 (CH$_3$); FAB-MS *m*/*z* 537.2 $[M + H]^+$ ($C_{29}H_{32}N_2O_8$ requires C, 64.9; H, 6.0; N, 5.2. Found: C, 64.9; H, 6.0; N, 5.1%).

(1*S*,3*R*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-5-hydroxy-6-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 12*R*

20% $Pd(OH)_2$/C was added to a solution of nucleoside **11*R*** (1.20 g, 2.24 mmol) in EtOH (20 cm^3). The mixture was degassed with hydrogen and stirred in an atmosphere of hydrogen for 96 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness under reduced pressure and coevaporated with a mixture of toluene and CH_2Cl_2 [3 × 50 cm^3; 1:1 (v/v)] to give diol **12*R*** as a white solid material (750 mg, 94%); δ_H(CD$_3$)$_2$SO 11.35 (1 H, s, NH), 7.43 (1 H, s, 6-H), 5.82 (1 H, d, *J* 4.4, 1′-H), 5.72 (1 H, s, 3′-OH), 4.89 (1 H, s, 5′-OH), 4.23 (1 H, m, 2″-H), 4.16 (1 H, d, *J* 4.6, 2′-H), 4.12–4.00 (2 H, m, 3″-H$_2$), 3.75–3.65 (3 H, m, 4′-H and 5′-H$_2$), 2.01 (3 H, s, COCH$_3$), 1.91 (1 H, m, 1″-H^a), 1.78 (4 H, m, 1″-H^b and CH$_3$); δ_C(CD$_3$)$_2$SO 170.2 (*C*OCH$_3$), 163.8 (C-4), 150.1 (C-2), 138.1 (C-6), 107.5 (C-5), 87.9 (C-2′), 87.1 (C-3′), 83.2 (C-4′), 82.0 (C-1′), 78.9 (C-2″), 64.8 (C-3″), 59.6 (C-5′), 37.0 (C-1″), 20.7 (CO*C*H$_3$), 12.0 (CH$_3$); FAB-MS *m*/*z* 357.1 $[M + H]^+$ ($C_{15}H_{20}N_2O_8$ requires C, 50.6; H, 5.7; N, 7.9. Found: C, 50.3; H, 5.7; N, 7.6%).

(1*S*,3*R*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-6-(4,4′-dimethoxytrityloxymethyl)-5-hydroxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]-octane 13*R*

DMTCl (1.40 g, 4.13 mmol) was added to a solution of nucleoside **12*R*** (740 mg, 2.08 mmol) in anhydrous pyridine (8 cm^3).

The solution was stirred for 15 h and subsequently poured into a mixture of 'petroleum ether', CH_2Cl_2 and water [60 cm^3; 1:1:1 (v/v/v)]. The separated organic phase was washed with saturated aq. $NaHCO_3$ (3 × 15 cm^3), dried, and evaporated to dryness under reduced pressure to give a yellowish solid material. This material was purified by silica gel column chromatography using dichloromethane–methanol–pyridine [0–2.0% methanol, 0.5% pyridine (v/v/v)] as eluent to give nucleoside **13*R*** as a white solid material (1.27 g, 92%); $\delta_H(CD_3)_2SO$ 11.46 (1H, s, NH), 7.50–7.27 (10H, m, 6-H and Ar), 6.95 (4H, m, Ar), 6.00 (1H, d, *J* 4.6, 1′-H), 5.84 (1H, s, 3′-OH), 4.25 (1H, m, 2″-H), 4.24 (1H, d, *J* 4.6, 2′-H), 4.12–3.99 (3H, m, 3″-H_2 and 4′-H), 3.80 (6H, s, OCH_3), 3.40 (1H, dd, *J* 2.6 and 7.7, 5′-H^a), 3.24 (1H, dd, *J* 2.7 and 10.1, 5′-H^b), 1.97 (3H, s, $COCH_3$), 1.85 (3H, d, *J* 1.1, CH_3), 1.77–1.69 (2H, m, 1″-H_2); $\delta_C(CD_3)_2SO$ 170.0 (*C*OCH$_3$), 163.7 (C-4), 158.2 (Ar), 150.1 (C-2), 144.9 (Ar), 137.6 (C-6), 135.6, 135.4, 129.8, 127.9, 127.8, 126.8 and 113.2 (Ar), 107.7 (C-5), 87.8 and 78.8 (C-2′ and 2″), 87.2 and 85.8 (C-3′ and Ar$_3$*C*), 82.2 (C-1′), 81.1 (C-4′), 64.5 (C-3″), 62.3 (C-5′), 55.1 (OCH_3), 37.1 (C-1″), 20.5 (CO*C*H$_3$), 12.0 (CH_3).

(1*S*,3*R*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-5-[2-cyanoethoxy(diisopropylamino)phosphinoxy]-6-(4,4′-dimethoxytrityloxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 14*R*

2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.41 cm^3, 1.82 mmol) was added dropwise to a solution of nucleoside **13*R*** (592 mg, 0.90 mmol) in a mixture of anhydrous CH_2Cl_2 (2,5 cm^3) and *N*,*N*-diisopropylethylamine (0.8 cm^3). After stirring at room temperature for 18 h, the mixture was poured into a mixture of ethyl acetate and water [15 cm^3; 2:1 (v/v)]. The separated organic phase was washed with saturated aq. sodium hydrogen carbonate (3 × 10 cm^3), dried, and evaporated to dryness under reduced pressure to give a yellowish oil. This residue was purified twice by silica gel column chromatography using as eluent first CH_2Cl_2–methanol–pyridine [90:1:9 (v/v/v)] and then 'petroleum ether'–ethyl acetate–pyridine [70:29:1 (v/v/v)] to give a white solid material after evaporation of the solvents under reduced pressure. This residue was dissolved in CH_2Cl_2 (2 cm^3) and added dropwise with vigorous stirring to 'petroleum ether' (80 cm^3; −30 °C) to afford phosphoramidite **14*R*** (498 mg, 64%) as a white solid material after filtration and drying, $\delta_P(CD_3)_2SO$ 142.0, 141.8. This material was used on an automated synthesizer without further purification but ^{31}P NMR showed the presence of an impurity at δ_P 14.2.

(1*S*,3*S*,5*R*,6*R*,8*R*)-5-Benzyloxy-6-benzyloxymethyl-3-(*tert*-butyldimethylsilyloxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo-[3.3.0]octane 8*S*

The same procedure as the method described first for preparation of **8*R*** was used: Nucleoside **7*R*** (7.00 g, 11.18 mmol), TsCl (9.98 g, 52.53 mmol), anhydrous pyridine (150 cm^3), K_2CO_3 (651 mg, 4.72 mmol), 18-crown-6 (300 mg, 1.13 mmol) and anhydrous DMF (60 cm^3) afforded a product, which was purified by silica gel column chromatography [10→40% (v/v) ethyl acetate in 'petroleum ether'] to give nucleoside **8*S*** as a white solid material (3.55 g, 52%); $\delta_H(CD_3)_2SO$ 11.40 (1 H, s, NH), 7.39–7.28 (11 H, m, 6-H and Ar), 5.89 (1 H, d, *J* 3.5, 1′-H), 4.61–4.49 (4 H, m, 2 × CH_2Ph), 4.48 (1 H, d, *J* 3.5, 2′-H), 4.35 (1 H, t, *J* 5.7, 4′-H), 4.19–4.13 (1 H, m, 2″-H), 3.85 (2 H, d, *J* 6.0, 5′-H_2), 3.61 (1 H, dd, *J* 4.1 and 11.2, 3″-H^a), 3.55 (1 H, dd, *J* 5.7 and 11.2, 3″-H^b), 2.36 (1 H, dd, *J* 6.2 and 13.2, 1″-H^a), 2.10 (1 H, dd, *J* 8.6 and 13.0, 1″-H^b), 1.77 (3 H, d, *J* 1.1, CH_3), 0.84 (9 H, s, Bu^t), −0.02 (3 H, s, SiMe), −0.03 (3 H, s, SiMe); $\delta_C(CD_3)_2SO$ 163.6 (C-4), 150.0 (C-2), 138.2 and 138.1 (Ar), 137.0 (C-6), 128.3, 128.2, 127.6, 127.5 and 127.4 (Ar), 107.9 (C-5), 93.1 (C-3′), 84.2 (C-2′), 83.9 (C-1′), 82.2 (C-2″), 80.5 (C-4′), 72.4 (*C*H$_2$Ph), 68.4 (C-5′), 66.8 (*C*H$_2$Ph), 64.7 (C-3″), 33.3 (C-1″), 25.8 [C(*C*H$_3$)$_3$], 18.0 [*C*(CH$_3$)$_3$], 12.3 (CH_3), −5.3 [Si(CH$_3$)$_2$]; FAB-MS *m*/*z* 609.4 [M + H]$^+$ ($C_{33}H_{44}N_2O_7Si$ requires C, 65.1; H, 7.3; N, 4.6. Found: C, 64.9; H, 7.4; N, 4.5%).

(1*S*,3*S*,5*R*,6*R*,8*R*)-5-Benzyloxy-6-benzyloxymethyl-3-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 9*S*

The same procedure as for preparation of **9*R*** was used: Nucleoside **8*S*** (3.50 g, 5.76 mmol), a 1.0 M solution of TBAF in THF (8.75 cm^3, 8.75 mmol) and anhydrous THF (80 cm^3) gave a product, which was purified by silica gel column chromatography [0→2% (v/v) MeOH in CH_2Cl_2] to give nucleoside **9*S*** as a white solid material (2.68 g, 94%); $\delta_H(CD_3)_2SO$ 11.42 (1 H, s, NH), 7.80–7.28 (11 H, m, 6-H and Ar), 5.90 (1 H, d, *J* 3.6, 1′-H), 4.79 (1 H, t, *J* 5.7, 3″-OH), 4.65–4.49 (4 H, m, 2 × CH_2Ph), 4.46 (1 H, d, *J* 3.5, 2′-H), 4.33 (1 H, t, *J* 5.4, 4′-H), 4.25–4.12 (1 H, m, 2″-H), 3.85 (2 H, d, *J* 5.6, 5′-H_2), 3.48–3.34 (2 H, m, 3″-H_2), 2.32 (1 H, dd, *J* 6.2 and 13.1, 1″-H^a), 2.15 (1 H, dd, *J* 8.4 and 13.2, 1″-H^b), 1.78 (3 H, d, *J* 0.8, CH_3); $\delta_C(CD_3)_2SO$ 163.7 (C-4), 150.1 (C-2), 138.3 and 138.1 (Ar), 137.3 (C-6), 128.3, 127.6 and 127.5 (Ar), 108.0 (C-5), 93.2 (C-3′), 84.1 (C-2′), 83.7 (C-1′), 82.8 (C-2″), 80.5 (C-4′), 72.4 (*C*H$_2$Ph), 68.5 (C-5′), 66.8 (*C*H$_2$Ph), 62.6 (C-3″), 33.4 (C-1″), 12.2 (CH_3); FAB-MS *m*/*z* 495.2 [M + H]$^+$ ($C_{27}H_{30}N_2O_7$ requires C, 65.6; H, 6.1; N, 5.7. Found: C, 65.2; H, 6.2; N, 5.7%).

(1*S*,3*S*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-5-benzyloxy-6-benzyloxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 11*S*

The same procedure as for preparation of **11*R*** was used: Nucleoside **9*S*** (110 mg, 0.22 mmol), anhydrous pyridine (4 cm^3) and Ac_2O (0.1 cm^3, 1.06 mmol) gave a product, which was purified by silica gel column chromatography [0→1% (v/v) MeOH in CH_2Cl_2] to give nucleoside **11*S*** as a white solid material (110 mg, 92%); $\delta_H(CD_3)_2SO$ 11.43 (1 H, s, NH), 7.38–7.26 (11 H, m, H-6 and Ar), 5.94 (1 H, d, *J* 3.8, 1′-H), 4.60–4.52 (5 H, m, 2′-H and 2 × CH_2Ph), 4.35 (2 H, m, 2″- and 4′-H), 4.05 (2 H, m, 3″-H_2), 3.86 (2 H, d, *J* 5.5, 5′-H_2), 2.43 (1 H, dd, *J* 7.0 and 13.3, 1″-H^a), 2.09 (1 H, dd, *J* 7.6 and 13.2, 1″-H^b), 1.98 (3 H, s, $COCH_3$), 1.79 (3 H, d, *J* 0.7, CH_3); $\delta_C(CD_3)_2SO$ 170.2 (*C*OCH$_3$), 163.7 (C-4), 150.1 (C-2), 138.2 and 138.1 (Ar), 137.3 (C-6), 128.4, 127.7 and 127.5 (Ar), 108.1 (C-5), 93.1 (C-3′), 84.0 (C-2′), 83.4 (C-1′), 79.9 and 79.4 (C-2″ and -4′), 72.5 (*C*H$_2$Ph), 68.4 (C-5′), 66.8 (*C*H$_2$Ph), 65.1 (C-3″), 34.0 (C-1″), 20.6 (CO*C*H$_3$), 12.2 (CH_3); FAB-MS *m*/*z* 537.2 [M + H]$^+$ ($C_{29}H_{32}N_2O_8$ requires C, 64.9; H, 6.0; N, 5.2. Found: C, 64.6; H, 6.0; N, 5.1%).

(1*S*,3*S*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-5-hydroxy-6-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 12*S*

The same procedure as for preparation of **12*R*** was used: Nucleoside **11*S*** (1.50 g 2.80 mmol), 20% $Pd(OH)_2$/C (300 mg) and EtOH (25 cm^3) afforded a product, which was isolated as a white solid material (950 mg, 95%); $\delta_H(CD_3)_2SO$ 11.38 (1 H, s, NH), 7.37 (1 H, d, *J* 1.2, 6-H), 5.82 (1 H, d, *J* 3.9, 1′-H), 5.74 (1 H, s, 3′-OH), 4.92 (1 H, t, *J* 5.5, 5′-OH), 4.33–2.23 (1 H, m, 2″-H), 4.21 (1 H, d, *J* 4.0, 2′-H), 4.11–3.95 (2 H, m, 3″-H_2), 3.78–3.70 (3 H, m, 4′-H and 5′-H_2), 2.35 (1 H, dd, *J* 6.6 and 13.2, 1″-H^a), 1.98 (3 H, s, $COCH_3$), 1.79 (3 H, d, *J* 0.9, CH_3), 1.75 (1 H, m, 1″-H^b); $\delta_C(CD_3)_2SO$ 170.3 (*C*OCH$_3$), 163.8 (C-4), 150.1 (C-2), 137.6 (C-6), 107.8 (C-5), 86.9 (C-3′), 86.7 (C-2′), 84.3 (C-4′), 83.2 (C-1′), 79.3 (C-2″), 65.2 (C-3″), 59.5 (C-5′), 36.8 (C-1″), 20.7 (CO*C*H$_3$), 12.2 (CH_3); FAB-MS *m*/*z* 357.1 [M + H]$^+$.

(1*S*,3*S*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-6-(4,4′-dimethoxytrityloxymethyl)-5-hydroxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]-octane 13*S*

The same procedure as for preparation of **13*R*** was used: Nucleoside **12*S*** (0.90 g, 2.58 mmol), DMTCl (1.70 g, 5.02

mmol) and anhydrous pyridine (10 cm^3) gave a product, which was purified by silica gel column chromatography [0.5% (v/v) pyridine, 0→2% (v/v) MeOH in CH_2Cl_2] to give nucleoside **13*S*** as a white solid material (1.60 g, 96%); $\delta_H(CD_3)_2SO$ 11.45 (1 H, s, NH), 7.46–7.22 (10 H, m, 6-H and Ar), 6.90 (4 H, m, Ar), 5.94 (1 H, d, *J* 4.0, 1′-H), 5.81 (1 H, s, 3′-OH), 4.27 (1 H, d, *J* 4.0, 2′-H), 4.16 (1 H, m, 2″-H), 4.08–4.01 (2 H, m, 3″-H^a and 4′-H), 3.91 (1 H, dd, *J* 3.5 and 11.7, 3″-H^b), 3.75 (6H, s, OCH_3), 3.38 (1 H, dd, *J* 7.5 and 10.4, 5′-H^a), 3.23 (1 H, dd, *J* 3.1 and 10.3, 5′-H^b), 2.08 (1 H, dd, *J* 7.0 and 13.2, 1″-H^a), 1.96 (3 H, s, $COCH_3$), 1.81 (3 H, d, *J* 0.9, CH_3), 1.66 (1 H, dd, *J* 6.0 and 13.3, 1″-H^b); $\delta_C(CD_3)_2SO$ 170.1 (*C*OCH_3), 163.7 (C-4), 150.1 (C-2), 137.1 (C-6), 158.2, 144.8, 135.5, 135.4, 129.8, 127.9, 127.8, 126.8 and 113.3 (Ar), 108.0 (C-5), 87.0 and 85.9 (C-3′ and Ar_3C), 86.5 (C-2′), 83.4 (C-1′), 82.2 (C-4′), 79.3 (C-2″), 65.1 (C-3″), 62.0 (C-5′), 55.1 (OCH_3), 36.9 (C-1″), 20.6 ($CO*C*H_3$), 12.3 (CH_3).

(1*S*,3*S*,5*R*,6*R*,8*R*)-3-Acetoxymethyl-5-[2-cyanoethoxy(diisopropylamino)phosphinoxy]-6-(4,4′-dimethoxytrityloxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 14*S*

The same procedure as for preparation of **14*R*** was used: Nucleoside **13*S*** (600 mg, 0.91 mmol), 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.50 cm^3, 2.22 mmol), CH_2Cl_2 (2.50 cm^3) and *N*,*N*-diisopropylethylamine (0.80 cm^3) afforded a product, which was purified by silica gel column chromatography [1% (v/v) pyridine, 30% (v/v) ethyl acetate in 'petroleum ether'] to give **14*S*** as a white solid material (395 mg, 50%), δ_P $(CD_3)_2SO$ 143.1 and 143.0.

(1*S*,3*R*,5*R*,6*R*,8*R*)-5-Hydroxy-3,6-bis(hydroxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 10*R*

The same procedure as for preparation of **12*R*** was used: Nucleoside **9*R*** (240 mg, 0.49 mmol), 20% $Pd(OH)_2$/C (50 mg) and EtOH (4.0 cm^3) afforded the product **10*R***, which was obtained as a white solid material (140 mg, 91%); $\delta_H(CD_3)_2SO$ 11.31 (1 H, s, NH), 7.50 (1 H, s, 6-H), 5.82 (1 H, d, *J* 4.8, 1′-H), 5.62 (1 H, br s, OH), 4.86 (1 H, br s, OH), 4.73 (1 H, br s, OH), 4.14 (1 H, d, *J* 4.8, 2′-H), 4.07 (1 H, m, 2″-H), 3.65 (3 H, m, 4′-H and 5′-H_2), 3.43 (2 H, m, 3″-H_2), 1.90 (1 H, m, 1″-H^a), 1.76 (3 H, s, CH_3), 1.72 (1 H, m, 1″-H^b); $\delta_C(CD_3)_2SO$ 163.9 (C-4), 150.3 (C-2), 139.0 (C-6), 107.4 (C-5), 87.6 (C-2′), 87.3 (C-3′), 83.0 (C-4′), 82.3 (C-2″), 81.8 (C-1′), 62.0 (C-3″), 59.7 (C-5′), 36.8 (C-1″), 12.0 (CH_3); FAB-MS *m*/*z* 314.9 $[M + H]^+$.

(1*S*,3*S*,5*R*,6*R*,8*R*)-5-Hydroxy-3,6-bis(hydroxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane 10*S*

The same procedure as for preparation of **12*R*** was used: Nucleoside **9*S*** (200 mg, 0.40 mmol), 20% $Pd(OH)_2$/C (50 mg) and EtOH (4.0 cm^3) gave a product, which was purified by silica gel column chromatography [0→15% (v/v) MeOH in CH_2Cl_2] to give nucleoside **10*S*** as a white solid material (119 mg, 93%); $\delta_H(CD_3)_2SO$ 11.36 (1 H, s, NH), 7.38 (1 H, d, *J* 1.1, 6-H), 5.81 (1 H, d, *J* 3.8, 1′-H), 5.67 (1 H, br s, OH), 4.88 (1 H, br s, OH), 4.78 (1 H, br s, OH), 4.17 (1 H, d, *J* 3.8, 2′-H), 4.07 (1 H, m, 2″-H), 3.85–3.54 (3 H, m, 4′-H and 5′-H_2), 3.48–3.37 (2 H, m, 3″-H_2), 2.27 (1 H, dd, *J* 6.6 and 13.1, 1-H^a), 1.78 (4 H, m, 1″-H^b and CH_3); $\delta_C(CD_3)_2SO$ 163.8 (C-4), 150.1 (C-2), 137.7 (C-6), 107.8 (C-5), 87.0 (C-3′), 86.7 (C-2′), 84.6 (C-4′), 83.6 (C-1′), 82.6 (C-2″), 63.1 (C-3″), 59.7 (C-5′), 36.8 (C-1″), 12.2 (CH_3).

Oligonucleotide synthesis

All ONs were prepared on a Biosearch 8700 DNA Synthesizer using the phosphoramidite approach.[28] Coupling of amidites **14*R*** and **14*S*** was performed by 'hand coupling' (premixing amidite and the activator 1*H*-tetrazole in acetonitrile in a syringe followed by flushing of the column reactor approximately twice every minute throughout the coupling time applied; CPG solid supports). The stepwise coupling yields for amidites **14*R*** and **14*S*** were >98% (couplings for 10–20 min; otherwise the standard RNA program of the synthesizer was used). The unmodified 2′-deoxynucleoside 2-cyanoethyl *N*,*N*-diisopropylphosphoramidites were coupled using the standard DNA program of the synthesizer. After completion of the sequences, deprotection, using conc. ammonia in methanol [32% (w/w); 55 °C; 12 h], of 5′-*O*-DMT-OFF ONs and ethanol precipitation yielded the final ON products, which by capillary gel electrophoresis were shown to be >90% pure. The composition of the modified ONs was verified by MALDI-MS analysis $[M - H]^-$: 5′-T_7***R***T_6 Calc.: 4267.9. Found: 4264.7; 5′-T_7***S***T_6 Calc.: 4267.9. Found: 4266.8; 5′-T_3(T***R***)$_4T_3$ Calc.: 4484.1. Found: 4484.4; 5′-T_3(T***S***)$_4T_3$ Calc.: 4484.1. Found: 4485.6; 5′-T_5***R***$_4T_5$ Calc.: 4484.1. Found: 4482.2; 5′-T_5***S***$_4T_5$ Calc.: 4484.1. Found: 4483.7; 5′-***R***$_{13}$T Calc.: 5132.6. Found: 5132.6; 5′-***S***$_{13}$T Calc.: 5132.6. Found: 5134.8; 5′-d(GTGA***R***ATGC) Calc.: 2824.9. Found: 2825.8; 5′-d(GTGA***S***ATGC) Calc.: 2824.9. Found: 2826.4; 5′-d(G***R***GA***R***A***R***GC) Calc.: 2969.1. Found: 2972.8; 5′-d(G***S***GA***S***A***S***GC) Calc.: 2969.1. Found: 2968.7.

Thermal stability studies

Melting temperatures (T_m-values) were determined as described earlier[12] assuming identical extinction coefficients for the 2″-hydroxymethyl-2′,3′-BcNA-type ONs and the corresponding unmodified ONs. The T_m-value of 5′-***R***$_{13}$T was measured without complement at three different ON concentrations: 1.5 μM: T_m = 60 °C; 3.6 μM: T_m = 61 °C; 7.3 μM: T_m = 62 °C.

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