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Synthesis and Pairing Properties of α-Tricyclo-DNA

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Abstract: We describe the synthesis of the phosphoramidite building blocks of α-tricyclo-DNA (α-tc-DNA) covering all four natural bases, starting from the already known corresponding α-tc-nucleosides. These building blocks were used for the preparation of three α-tc-oligonucleotide 10-mers representing a homopurine, a homopyrimidine, and a mixed purine/pyrimidine base sequence. The base-pairing properties with complementary parallel and antiparallel oriented DNA and RNA were studied by UV-melting analysis and CD spectroscopy. We found that α-tc-DNA binds preferentially to parallel nucleic acid complements through Watson–Crick duplex formation, with a preference for RNA over DNA. In comparison with natural DNA, α-tc-DNA shows equal to enhanced affinity to RNA and also pairs to antiparallel DNA or RNA complements, although with much lower affinity. In the mixed-base sequence these anatiparallel duplexes are of the reversed Watson–Crick type, while in the homopurine/homopyrimidine sequences Hoogsteen and/or reversed Hoogsteen pairing is observed. Antiparallel duplex formation of two α-tc-oligonucleotides was also observed, although the thermal stability of this duplex was surprisingly low. The base-pairing properties of α-tc-DNA are discussed in the context of α-DNA, α-RNA, and α-LNA.

Keywords: DNA recognition · DNA · oligonucleotides · tricyclo-DNA

Introduction

Oligonucleotide-based gene silencing by antisense[1] and antigene[2] agents or by small interfering RNAs and microRNAs[3] is of prime interest[4] in human therapy and in DNA-based diagnostics. Chemically modified oligonucleotides have proven very useful in this context, providing solutions to the relatively low biostabilities and target affinities of natural oligonucleotides,[5] which are in many cases incapable of eliciting biological responses. One of the first oligonucleotide analogues investigated in detail was α-DNA, made up of α-anomeric 2′-deoxyribonucleosides (Figure 1). In early modeling[6] and structural[7,8] investigations it was found that α-DNA forms parallel oriented Watson–Crick duplexes with natural DNA, exhibiting thermal and thermodynamic stabilities similar to those of the corresponding natural duplexes.[9] In its own series, α-DNA forms antiparallel, Watson–Crick paired duplexes.[10] α-Oligodeoxynucleotides are substantially more stable than natural oligonucleotides towards degradation by nucleases[11] and, furthermore, hybrid α-DNA/β-RNA duplexes are not substrates for the RNA-cleaving enzyme RNase H.[12] In a similar vein, α-anomeric oligoribonucleotides have also been synthesized and shown to bind to the corresponding natural DNA in parallel orientation, although with reduced thermal stability.[13]

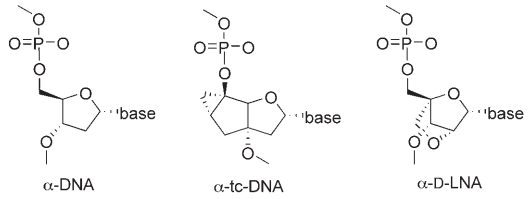

Figure 1. Chemical structures of α-DNA and analogues.

The search for novel carbohydrate-modified oligonucleotides has given rise to a class of conformationally constrained analogues that display high RNA and DNA affinity due to reductions in entropic cost upon duplex formation. Members of this class include, among others, the locked nucleic acids (LNAs),[14,15] the hexitol nucleic acids (HNAs),[16] and tricyclo-DNA (tc-DNA).[17,18] These third-generation analogues have in the past proven to be very useful antisense agents with generally superior efficacy in biological assays in rela-

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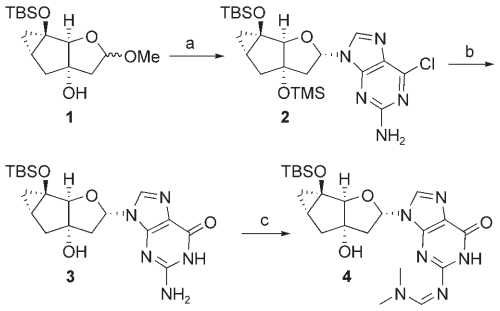
tion to their first- (phosphorothioates) or second-generation (2′-alkyl-RNA) analogues.

α-Anomeric forms of many of these carbohydrate-modified oligonucleotide analogues also exist, but data on the pairing properties of these are limited. Typically, though, binding properties inferior to those of the corresponding β-series are observed. In the case of α-D-LNA (Figure 1), however, it had previously been shown that pyrimidine sequences bind to complementary RNA in a parallel fashion and with high affinity, whilst, interestingly, no binding to DNA was observed.[19–21] In this article we report on the synthesis and base-pairing properties of α-tc-DNA with complementary RNA, DNA, and with itself.

Results

Synthesis of the phosphoramidite building blocks: For α-tc-guanosine (Scheme 1), a more practical synthesis of the

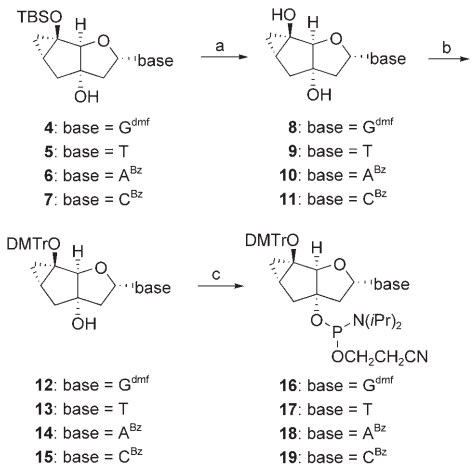


Scheme 1. a) 2-Amino-6-chloropurine, BSA, TMSOTf, 55 °C, 4 h, 48 %. b) 3-Hydroxypropionitrile, NaH, THF, 0 °C, 3 h, then CH_3OH/NH_3 3:2, 55 °C, 16 h, 91 %. c) $HC(OCH_3)_2N(CH_3)_2$, DMF, 55 °C, 3 h, 94 %.

N,*N*-dimethylformamidino-protected nucleoside tc-G^{dmf} (**4**) was developed, starting from the sugar building block **1** and the base 2-amino-6-chloropurine in analogy to a procedure in the LNA series.[22] This synthesis proved to be more efficient than that for the previously reported isobutyryl-protected tc-G^{ibu}.[17] The synthesis of the other α-tc-nucleoside phosphoramidites with the bases A^{Bz}, T, and C^{Bz} started from the known α-anomeric, base-protected tc-nucleosides **5**–**7** (Scheme 2).[17,23]

Nucleosidation of building block **1** with 2-amino-6-chloropurine in dichloroethane with TMSOTf as Lewis acid yielded nucleoside **2** in 48 % yield, together with its β-isomer (40 %). Subsequent conversion into tc-guanosine **3** with 2-cyanoethanol/NaH, followed by installation of the dimethylformamidine group by treatment with DMF dimethylacetal yielded the base-protected nucleoside **4** in 85 % yield over two steps.

The 5′-protected α-tc-nucleosides **4**–**7** were then desilylated in HF/pyridine to give the dihydroxynucleosides **8**–**11**. Subsequent tritylation with DMT-OTf in pyridine[24] afforded the 5′-protected nucleosides **12**–**15** in yields in the 90 % range, and phosphitylation under standard reaction conditions finally yielded the phosphoramidites **16**–**19** in reasonable yields. While desilylation and tritylation posed no problems, phosphitylation required a slight excess of the chlorophosphite reagent, which resulted after quenching with H_2O in an H-phosphonate by-product that was difficult to remove by extraction or column chromatography. A viable solution to this problem was found in quenching of the reaction with glycerol, to a large extent allowing extraction of excess reagent into the aqueous phase.



Scheme 2. a) HF·pyridine, pyridine, RT, 2–5 h, 60–100 %. b) DMT-OTf, pyridine, RT, 1–6 h, 91–99 %. c) $ClP[(OCH_2CH_2CN)N(iPr)_2]$, $EtN(iPr_2)_2$, CH_3CN, RT, 2 h, 58–83 %.

The synthesis of α-tc-oligonucleotides by the established phosphoramidite synthesis method on a DNA synthesizer (1 μmol scale) was then examined, but it soon became clear that the synthesis was more difficult than expected and required considerable optimization. In initial attempts using standard α-tc-amidite solutions (0.1 mM in CH_3CN) we found coupling efficiencies in the 50 % range (trityl assay) even with extended coupling times (12 min). Increasing the amidite concentration twofold (0.2 mM) and using a 0.4 M solution of 5-(ethylthio)-1*H*-tetrazole (ETT) as activator finally provided coupling yields of 95 % per step. A second problem encountered was the oxidation step, which proved to be incomplete with the use of standard iodine oxidation procedures. This issue was solved by employment of a concentration of 20 mM and an oxidation time extended by 20 s. The third problem arose from the universal solid support used, which rapidly released a linker–oligonucleotide conjugate into solution during deprotection, whilst use of an extended deprotection time (60 h, 55 °C) was necessary to remove the linker unit from the oligomer. As in the case of β-tc-DNA synthesis, the 5′-end had to be phosphorylated to prevent loss of the 5′-terminal nucleotide unit initiated by thermal cyclopropanol ring-opening, followed by β-elimination of the 5′-keto nucleoside formed in situ.[25] This was done by addition of one further nucleotide unit ($n+1$) during synthesis, resulting after deprotection in the 5′-phosphorylated *n*-mer oligonucleotide. Using this procedure we prepared the three α-tc-DNA decamers **20**–**22** listed in

Table 1. Analysis by ESI⁻ mass spectrometry confirmed the expected masses and in addition revealed the presence of minor amounts (10–25%) of the 3′-phosphorylated oligonucleotides, arising from partial P–O bond hydrolysis between the 3′-terminal phosphate and the universal support linker. In our hands these mixtures were inseparable by standard RP-HPLC.

Table 1. Sequences of α-tc-oligonucleotides **20**–**22** and their ESI⁻-MS data.

	Sequence	*m/z* calcd	*m/z* found
20	α-tc(pGAGAAGGAAA)	3595.6	3594.9
21	α-tc(pTTTCCTTCTC)	3380.4	3379.6
22	α-tc(pGCACTGTCAA)	3472.5	3472.1

UV-melting experiments: With the three α-tc-oligonucleotides **20**–**22**—representing a pyrimidine, a purine, and a mixed purine/pyrimidine sequence—to hand we investigated their pairing properties with parallel and antiparallel complementary DNA and RNA by UV-melting. Table 2 shows a summary of the corresponding T_m data. Relevant melting curves are reproduced in Figures 2–5. None of the α-tc-DNA single strands **20**–**22** displayed any cooperative transition, ruling out self-complex formation (data not shown).

Table 2. Summary of T_m data (260 nm). c_t = 5.1 μM in 0.15 M NaCl, 10 mM NaH_2PO_4, at pH 7.0.

	Complementary DNA[a]		Complementary RNA[a]	
	parallel	antiparallel	parallel	antiparallel
20 α-tc(pGAGAAGGAAA)	33.0 (32.0[b])	14.0 (31.5)	44.8	18.3 (28.0)
21 α-tc(pTTTCCTTCTC)	16.5	<5	32.8	14.7
22 α-tc(pGCACTGTCAA)	34.7	17.8 (18.1)	45.1	27.0 (28.0)
23 d(GAGAAGGAAA)	<5	25.9	<10	12
24 r(GAGAAGGAAA)	19.1	41.5	23.2	47.8
25 d(AACTGTCACG)	n.m.[c]	44.4	n.m.[c]	42.5
26 r(AACUGUCACG)	n.m.[c]	40.1	n.m.[c]	52.2

[a] T_m values in parenthesis were determined at pH 6.0. [b] c_t = 2.6 μM. [c] n.m. = not measured.

The homopurine oligonucleotide **20** forms stable duplexes with parallel oriented DNA (pDNA) and RNA (pRNA), with a distinctly higher T_m for the RNA complement (Figure 2, Table 2). The hyperchromicities found are in the same range, indicating similar extents of base stacking in the corresponding duplexes. In both cases the T_m values are considerably higher than those for the corresponding antiparallel (ap) DNA or RNA duplexes with the sequence-identical deoxyoligonucleotide **23** and slightly reduced in relation to those with the sequence-identical oligoribonucleotide **24**. Interestingly, the pure ap α-tc-DNA duplex **20**/**21** is significantly less stable than any of the mixed backbone duplexes and showed much less hyperchromicity, which is in accord with reduced base stacking in the duplex. Evidence of duplex formation in the latter case was provided by a comparison of a plot of the sum of the melting curves of the individual single strands **20** + **21** with that of the **20**/**21** mixture (Figure 2), which shows significant deviation with a sigmoidal melting curve in the case of the mixture.

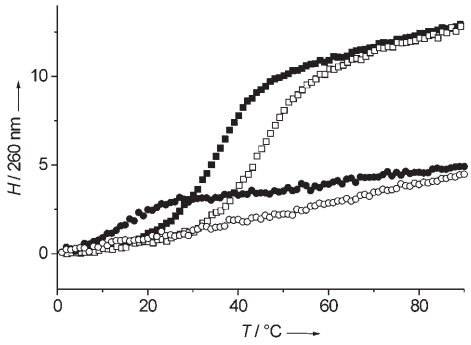


Figure 2. UV-melting curves (260 nm) of duplexes indicated. For experimental conditions see Table 2. ■: **20**/pDNA; □: **20**/pRNA; ●: **20**/**21**; ○: **20**+**21**.

In analogy to parallel α-DNA/DNA(RNA) duplexes we conclude that parallel α-tc-DNA/DNA(RNA) duplexes are also of the Watson–Crick type (Figure 5). This is supported by the T_m of **20** with its parallel DNA complement at pH 6.0 (Table 2), which is identical (within the error limits) to that at pH 7, thus excluding duplexes of the Hoogsteen or reversed-Hoogsteen type, which would require cytosine protonation.

The homopurine sequence **20** also undergoes duplex formation with apDNA and apRNA, although with distinctly lower T_m values than seen with the parallel complements at neutral pH. In these cases duplex formation is strongly pH-dependent, with higher T_m values at lower pH. Figure 3 illustrates this with the example of the duplex of **20** with apDNA. The pH dependence is a safe indication that protonated cytosines are involved, pointing towards Hoogsteen and/or reversed-Hoogsteen base pairing (Figure 5). Support for this interpretation is also provided by the known fact that α-DNA can bind as a third strand in parallel and antiparallel orientations to the purine strand of the underlying duplex through reversed-Hoogsteen and Hoogsteen base-pair formation.[26,27]

The homo-pyrimidine α-tc-oligonucleotide **21** also strongly favors binding to pDNA or pRNA, although with reduced T_m values relative to the purine sequence. Again, the duplex with pRNA is more stable than that with pDNA. For comparison, the oligodeoxyribonucleotide of identical sequence to **21** binds to apRNA and apDNA with slightly enhanced thermal stability, whilst the sequence-identical oligoribonu-

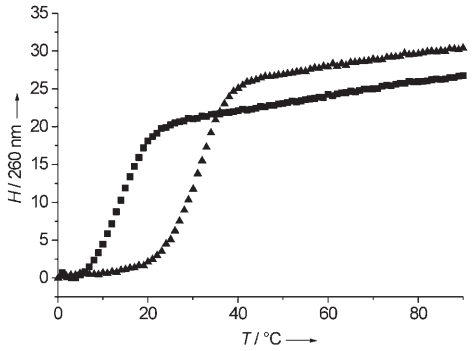


Figure 3. UV-melting curves (260 nm) of duplexes indicated. For experimental conditions see Table 2. ■: **20**/apDNA, pH 7.0; ▲: **20**/apDNA, pH 6.0.

cleotide shows weaker binding to DNA but stronger binding to RNA.

To address a more general sequence context we prepared the α-tc-oligonucleotide **22**, containing all four bases, and investigated its pairing properties with parallel and antiparallel DNA and RNA (Table 2, Figure 4). Pairing to complementary pRNA and pDNA is also preferred here, but some promiscuity exists in that duplexes with the antiparallel oriented complements were also observed. All duplexes show similar hyperchromicities, indicating similar extents of base pairing and base stacking. While Watson–Crick-paired structures are expected with parallel complements, the matter of the pairing mode in the antiparallel duplex was open; pH variation did not result in T_m differences, ruling out Hoogsteen and reversed-Hoogsteen structures, so the most likely interaction of the bases is the reversed-Watson–Crick mode (Figure 5), as is also observed in parallel β-DNA duplexes[28,29] and in antiparallel β-DNA/α-DNA duplexes exhibiting noncanonical G–A base pairs.[30] In this mixed sequence context, α-tc-DNA/RNA duplex stability is approximately the same as DNA/DNA duplex stability, lying between DNA/RNA and RNA/RNA duplex stability (Table 2).

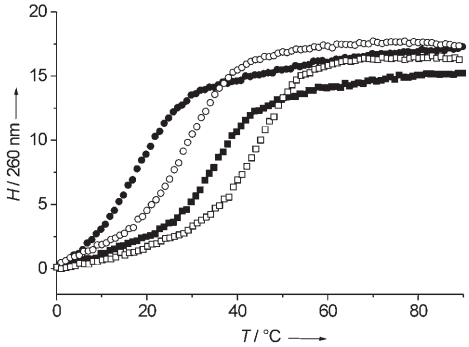


Figure 4. UV-melting curves (260 nm) of duplexes indicated. For experimental conditions see Table 2. ●: **22**/apDNA; ■: **22**/pDNA; ○: **22**/apRNA; **22**/pRNA.

We also determined the thermodynamic data for duplex formation by curve fitting to the experimentally derived melting curves in those cases in which the lower baseline was well defined.[31] These data are summarized in Table 3.

The order of the free enthalpies of duplex formation (ΔG) in all cases parallels the order of the corresponding

Table 3. Thermodynamic data for duplex formation between α-tc-oligonucleotides and parallel complementary DNA and RNA determined by curve fitting.

	ΔH [kcal mol^{-1}]	ΔS [cal K^{-1} mol^{-1}]	$\Delta G^{25\,°C}$ [kcal mol^{-1}]
α-tc(pGAGAAGGAAA) β-d(CTCTTCCTTT)	−60.5	−170.0	−9.8
α-tc(pGAGAAGGAAA) β-r(CUCUUCCUUU)	−64.8	−177.3	−12.0
α-tc(pTTTCCTTCTC) β-r(AAAGGAAGAG)	−63.0	−177.8	−10.0
α-tc(pGCACTGTCAA) β-d(GCATGTCAA)	−64.1	−180.2	−10.4
α-tc(pGCACTGTCAA) β-r(GCAUGUCAA)	−64.9	−177.0	−12.1
β-r(GAGAAGGAAA) β-d(TTTCCTTCTC)	−81.0	−229.0	−12.6
β-r(GAGAAGGAAA) β-r(UUUCCUUCUC)	−89.7	−250.9	−14.8

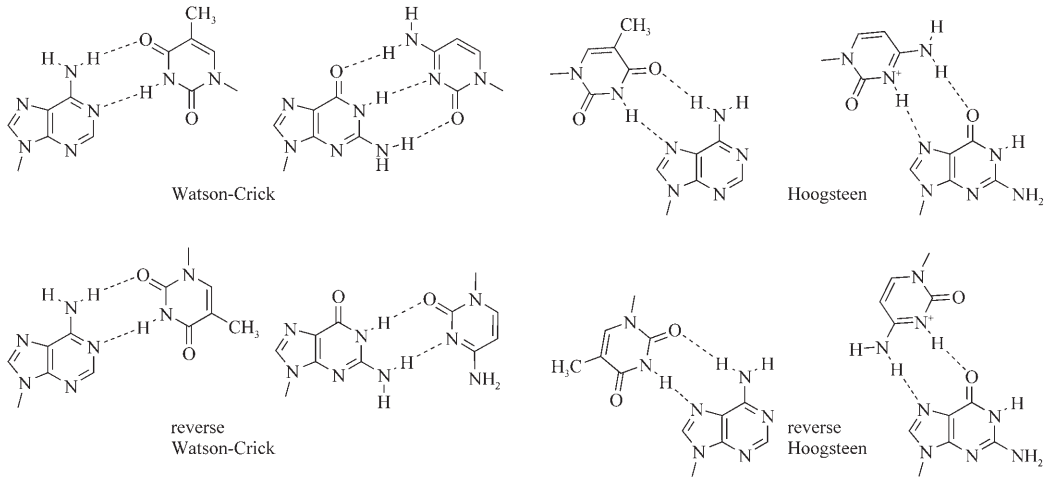


Figure 5. Chemical formulae of the four possible bi- or tridentate A–T(U) and G–C base pairs.

T_m. The enthalpies (ΔH) are also very similar for all α-tc-duplexes but distinctly smaller (less negative) than in the antiparallel DNA/RNA and RNA/RNA control duplexes. This is an indication that base stacking in the unnatural duplexes is less efficient than in the natural series, an obstacle that is compensated for by a smaller entropic penalty upon duplex formation (ΔS), most probably due to the higher preorganization of the α-tc-DNA backbone.

CD spectroscopy: We recorded CD spectra of **20** as an example of a homopurine sequence and of **22** as an example of a mixed-base sequence with their respective parallel and antiparallel DNA and RNA complements at different temperatures. Relevant spectra are reproduced in Figure 6. The temperature dependence of the CD spectra reflect the cooperative structural transitions from duplex to single strands observed in the UV-melting curves in all cases. Unfortunately, CD spectra do not provide detailed structural information on a duplex, but since CD signals arise from transition dipoles of adjacent base pairs they are very well suited to detection of changes in the base pair geometry within the stack in a comparative sense.

The CD traces of the mixed sequence **22** with its parallel and antiparallel RNA complements are very similar, indicating close base-pair geometries in both cases. This is not unusual and is also observed for parallel reversed-Watson–Crick and antiparallel Watson–Crick paired DNA.[28] In the cases of pDNA or apDNA as the complement, the two positive ellipticity maxima at 220 and 275 nm are inverted in their relative amplitude. As can be seen from the curves at high temperatures, at which the CD reflects the sum of the CD traces of the single strands, this inversion of maxima is to a large extent due to the spectral properties of the single strands and so is not a specific property of the ordered structure. From these observations we assume that Watson–Crick and reversed-Watson–Crick base pairs are also formed in the parallel and antiparallel duplexes in these cases.

The situation is somewhat different in the antiparallel duplexes of **20** (α-tc-homopurine sequence) with antiparallel DNA and RNA. In the DNA hybrid a relatively small but significant positive CD band is found around 290 nm in the duplex, together with a stronger positive band at 260 nm. Both fade away upon duplex melting and result in one large band around 270 nm in the single strands. This behavior is not observed in the α-tc-hybrid with RNA as a complement. As found before, the T_m values of both duplexes are pH-sensitive, indicating Hoogsteen or reversed-Hoogsteen base pairing. Given the differences in the CD spectra it is likely that one hybrid prefers the Hoogsteen and the other the reversed-Hoogsteen pairing mode. Promiscuity in Hoogsteen versus reversed-Hoogsteen base pairing with α-DNA has been observed in DNA triplexes, where it was shown that α-DNA homopyrimidine third strands can bind, in a sequence-dependent manner, to the major groove of a DNA duplex in either the parallel or the antiparallel modes.[26,27]

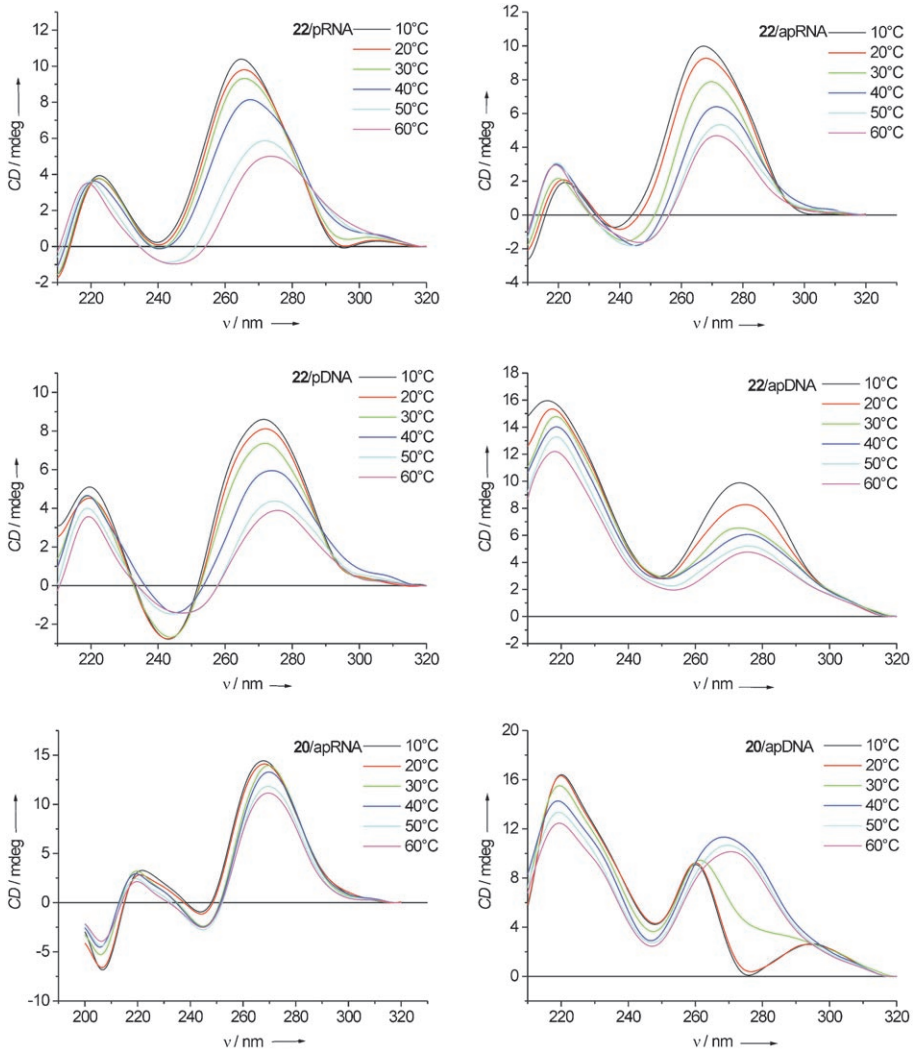


Figure 6. CD spectra of duplexes indicated at different pre- and post-melting temperatures. Experimental conditions were as indicated in Table 2.

Discussion

The synthesis of α-tc-DNA by phosphoramidite chemistry was more difficult than had been anticipated from previous experience with α-DNA and β-tc-DNA synthesis. In particular, the coupling step required higher α-tc-phosphoramidite

concentrations and a larger excess of monomeric unit per coupling step than is usual in natural DNA or β-tc-DNA synthesis. We ascribe this lack of reactivity during coupling to increased steric constraints imposed by the nucleobases at the activated P^{III} center. A further problem identified was the reduced release rate (in relation to oligodeoxynucleotides) from the universal solid support, which required prolonged treatment with conc. ammonia (60 h, 60 °C instead of 16 h, 60 °C) and produced not a uniformly free 3′-OH terminus but also 10–25 % 3′-phosphorylated termini arising from alternative P–O–linker bond scission. It has thus become clear that further improvement in α-tc-DNA synthesis would be necessary if larger quantities and a wider variety of α-tc-oligonucleotides were needed for, for example, structural or biological tests.

We compared affinities and preferred strand orientations of α-tc-DNA with those of α-DNA.[32] In mixed-base sequence contexts, both α-anomeric oligonucleotide types prefer parallel alignment over antiparallel in duplexes with DNA or RNA. Thermal stabilities with RNA complements are generally higher than those observed for DNA complements in the case of α-tc-DNA while no significant differences were found in the case of α-DNA. In a homopurine/homopyrimidine sequence context it has become clear that α-tc-DNA and α-DNA show inverted preferences. Tc-purine sequences form more stable duplexes with DNA and RNA than tc-pyrimidine sequences, whilst the opposite is observed for α-DNA. The relative thermal stabilities of α-DNA and α-tc-DNA with β-DNA are similar, implying that there is no major difference in thermal stabilities between the two backbone systems.

A direct comparison with β-DNA (Table 2) reveals that the affinity of α-tc-DNA to a parallel RNA complement is roughly of the same order as that of a β-DNA to an antiparallel RNA complement. Pure RNA duplexes of the same base sequence are generally slightly more stable than the mixed backbone duplexes. In summary, this analysis shows that, in mixed sequence contexts, α-tc-DNA performs similarly to DNA and α-DNA in terms of target affinity, whilst in homopurine sequence contexts α-tc-DNA outperforms the other two backbone systems.

An interesting difference between α-DNA and α-tc-DNA resides in the complementary base-pairing properties in their own series. While α-DNA forms relatively stable antiparallel duplexes with itself,[10,32] this is not the case for α-tc-DNA, as can be seen from the T_m values of **20**/**21**. This is a rather unusual feature, as for most backbone-modified oligonucleotide analogues—such as LNA[33] and β-tc-DNA[17]—the homo-backbone duplexes are more stable than hetero-backbone duplexes.

We also compared the pairing behavior of α-tc-DNA with that of α-bicyclo-(bc)-DNA, the structurally simpler version of tricyclo-DNA lacking the cyclopropane ring.[34] Because only α-bc-oligonucleotides with adenine and thymine as bases were investigated, only a very general comparison is possible. We find the same trend in that homo-backbone duplexes are less stable than hetero-backbone duplexes, but the differences are much more pronounced in the case of α-tc-DNA. Furthermore, the relative affinities to DNA and RNA, especially in purine/pyrimidine mixed sequences, are much higher in the case of α-tc-DNA, so the coherent picture of the superiority of natural nucleic acid recognition by the tc-DNA backbone relative to its bc-DNA counterpart also pertains in the α-anomeric series.

From NMR structures of α-DNA/DNA duplexes it is known that the deoxyribose units in the α-strand are predominantly in the 3′-*exo* conformation, giving rise to a right-handed duplex of the B conformational family.[35] X-ray analysis of the α-tc-C nucleoside showed the furanose unit to adopt a 3′-*endo* conformation.[17] An extrapolation of this structural feature to the α-tc backbone would result in a conformation similar to that of A-DNA. It is known that the ribose units of α-ribonucleosides also exist predominantly in N-type conformations (2′-*exo*).[36] α-RNA is known to form complementary parallel duplexes with both DNA and RNA complements, albeit with relatively low T_m values.[13] From these considerations it becomes clear that differences in ribose conformations in the three α-backbone systems alone do not account for the observed differences in affinity. It may well be that furanose conformation-dependent changes in the glycosidic torsion angles play an important role, which could also explain the rather peculiar situation encountered with α-D-LNA, in which the ribose units are locked in a 3′-*endo* conformation. In contrast with α-RNA, α-D-LNA recognizes complementary RNA with high affinity, but not DNA.[19] It may well be that the glycosidic torsion angle is also modulated here both by the sugar pucker and by the C-4′–O-2′-bridge. This is further supported by the UV-melting curves, which show weak hyperchromicities, and by the CD spectra, which are totally different from those of α-DNA and α-tc-DNA, pointing to duplexes with RNA of an as yet unknown structure.

Conclusion

α-tc-DNA has been prepared and its pairing properties with RNA and DNA have been analyzed. Like α-DNA, α-tc-DNA prefers Watson–Crick duplex formation with parallel-stranded RNA and DNA complements. The thermal stabilities of such duplexes are equal to or slightly enhanced in relation to corresponding DNA duplexes. Interestingly, antiparallel duplex formation within the α-tc-backbone system is weak. This special feature, together with the expected high biostability of α-tc-DNA, lends hope for successful applications as antisense agents or novel tools for biotechnology in general, and as DNA double-strand invaders in particular. Work in this direction is currently in progress.

Experimental Section

General: Reactions were performed under argon in distilled, anhydrous solvents. All chemicals were reagent grade from Fluka or Aldrich.

^{1}H NMR (300 MHz, 500 MHz) spectra were recorded on a Bruker AC 300 or a Bruker DRX 500 spectrometer; the chemical shifts δ in ppm were referenced to residual undeuterated solvent ($CHCl_3$: 7.27, CHD_2OD: 3.35), J in Hz. ^{13}C NMR (75 MHz) were recorded on a Bruker AC 300 instrument; the chemical shifts δ were referenced to residual undeuterated solvent ($CHCl_3$: 77.00, CHD_2OD: 49.3). ^{31}P NMR spectra (162 MHz) were recorded on a Bruker DRX 400 spectrometer; the chemical shift δ was referenced to 85% H_3PO_4 as external standard. ESI-MS mass spectra were recorded on a Fisons Instrument VG Platform. For TLC, pre-coated plates (SIL-G UV254, Macherey–Nagel) were used, these being viewed by UV and/or by dipping into a solution of $Ce(SO_4)_2$ (10.5 g), phosphomolybdic acid (21 g), H_2SO_4 (60 mL), and H_2O (900 mL). Flash chromatography (FC) was performed with silica gel 60 (230–400 mesh).

(3′*S*,5′*R*,6′*R*)-2-Amino-6-chloro-9-{5′-*O*-[(*tert*-butyl)-dimethylsilyl]-3′*O*-trimethylsilyl-2′-deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}purine (2): Tricyclo-sugar **1** (1.5 g, 5 mmol) was dissolved in 1,2-dichloroethane (19.5 mL), and 2-amino-6-chloropurine (1.3 g, 7.7 mmol) was added, followed by *N*,O-bis(trimethylsilyl)acetamide (3 mL, 12.3 mmol). The reaction mixture was heated to 40 °C until a clear solution appeared (30 min) and was then cooled to 0 °C. Trimethylsilyl triflate (1.3 mL, 7.2 mmol) was added over 15 min and the reaction mixture was heated to 55 °C for 4 h, allowed to cool to RT, and poured into sat. $NaHCO_3$ (40 mL). $CHCl_3$ (24 mL) was added and the mixture was stirred for 30 min. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (3×20 mL). The combined organic phases were washed with sat. $NaHCO_3$/brine (1:1, 40 mL), dried ($MgSO_4$), filtered, and concentrated to give a yellow oil. Purification by CC (hexane/EtOAc 2:1) gave title compound **2** (1.06 g, 48%), together with the corresponding β-anomer (890 mg, 40%). Data for the α-anomer: R_f = 0.29 (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.10, 0.13 (2×s, 15H; Me_3Si, Me_2Si), 0.85 (s, 9H; Me_3C), 0.95, 1.01 (2×m, 2H; H_2-C(8′)), 1.57 (m, 1H; H-C(6′)), 1.82 (d, J = 13.8 Hz, 1H; H-C(7′)), 2.31 (dd, J = 4.9, 13.8 Hz, 1H; H-C(7′)), 2.62 (dd, J = 6.2, 13.2 Hz, 1H; H-C(2′)), 2.76 (dd, J = 6.7, 13.1 Hz, 1H; H-C(2′)), 4.36 (s, 1H; H-C(4′)), 5.20 (s, 2H; NH_2), 6.25 (t, J = 6.4 Hz, 1H; H-C(1′)), 8.04 ppm (s, 1H; H-C(8)); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = −3.80, −3.76, 1.9, 17.9, 18.3, 23.9, 25.7, 40.2, 46.9, 64.9, 84.4, 88.0, 90.8, 140.6, 151.3, 153.1, 159.0 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{22}H_{36}ClN_5O_3Si_2Na$: 532.1943; found: 532.1927 $[M+Na]^+$.

(3′*S*,5′*R*,6′*R*)-9-{5′-*O*-[(*tert*-Butyl)-dimethylsilyl]-2′-deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}guanine (3): A solution of 3-hydroxypropionitrile (0.95 mL, 13.91 mmol) in anhydrous THF (20 mL) was cooled to 0 °C. After careful addition of NaH (60%, 673 mg, 16.97 mmol) the ice-bath was removed, the mixture was stirred for 30 min at RT, and compound **2** (1.57 g, 3.08 mmol) dissolved in THF (20 mL) was then added at 0 °C. The reaction was complete after 3 h at RT. A 1:1 mixture of brine and sat. $NaHCO_3$ (60 mL) was then slowly added, and the organic phase was separated and dried over $MgSO_4$. Evaporation of the solvent yielded a mixture of **3** and 3′-O-TMS-**3** in a ratio of 1:5. The crude solid was dissolved in CH_3OH (60 mL) and conc. NH_3 (40 mL) and the mixture was heated at 55 °C overnight. After evaporation of the solvent and CC (silica gel, CH_2Cl_2/CH_3OH 7:1) title compound **3** (1.17 g, 2.78 mmol, 91%) was isolated as a white foam. R_f = 0.21 (CH_2Cl_2/CH_3OH 7:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.00, 0.04 (2×s, 6H; Me_2Si), 0.76 (m, 10H; Me_3C, H-C(8′)), 0.96 (m, 1H; H-C(8′)), 1.48 (m, 1H; H-C(6′)), 1.67 (d, J = 13.6 Hz, 1H; H-C(7′)), 2.18 (dd, J = 6.0, 13.0 Hz, 1H; H-C(7′)), 2.48–2.59 (m, 2H; H_2C(2′)), 4.20 (s, 1H; H-C(4′)), 5.28 (s, 1H; OH), 6.06 (t, J = 7.1 Hz, 1H; H-C(1′)), 6.44 (s, 2H; NH_2), 7.89 (s, 1H; H-C(8)), 10.63 ppm (br, 1H; NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = −3.9, −3.6, 16.6, 17.7, 24.0, 25.8, 40.1, 46.4, 65.6, 82.4, 84.8, 88.3, 116.9, 135.4, 151.0, 153.8, 156.9 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{19}H_{29}N_5O_4Si$: 420.1989; found: 420.2089 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-N^2-{[*N*,*N*-Dimethylformamidino)-9-(5′-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}guanine (4): Compound **3** (830 mg, 1.98 mmol) was dissolved in DMF (13 mL), and *N*,*N*-dimethylformamide dimethylacetal (0.5 mL, 3.70 mmol) was added. The mixture was heated at 55 °C and stirred for 3 h. After removal of the solvent under reduced pressure the residue was purified by CC (silica gel, CH_2Cl_2/CH_3OH 8:1) to yield title compound **4** (882 mg, 1.86 mmol, 94%) as a yellow foam. R_f = 0.31 (CH_2Cl_2/CH_3OH 7:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.01, 0.05 (2×s, 6H; Me_2Si), 0.76 (m, 10H; Me_3C, H-C(8′)), 0.99 (m, 1H; H-C(8′)), 1.48 (m, 1H; H-C(6′)), 1.65 (d, J = 13.6 Hz, 1H; H-C(7′)), 2.24 (dd, J = 4.4 Hz, 1H; 13.6 Hz, H-C(7′)), 2.64 (d, J = 7.1 Hz, 2H; H_2C(2′)), 3.03, 3.13 (2×s, 6H; Me_2N), 4.25 (s, 1H; H-C(4′)), 5.37 (s, 1H; OH), 6.19 (t, J = 7.1 Hz, 1H; H-C(1′)), 8.00 (s, 1H; H-C(8)), 8.66 (s, 1H; N=C*H*NMe$_2$), 11.32 ppm (br, 1H; NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = −4.0, −3.6, 16.7, 17.7, 23.8, 25.8, 34.8, 39.7, 40.8, 45.9, 65.7, 82.6, 84.8, 88.4, 119.9, 136.7, 149.7, 157.4, 157.8, 158.4 ppm; MS (ESI-MS$^+$): m/z: calcd for: $C_{22}H_{34}N_6O_4Si$: 474.24; found: 475.27 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-N^2-(*N*,*N*-Dimethylformamidino)-9-(2′-deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl)guanine (8): HF·pyridine (70% HF, 3.1 mL) was added to a solution of **4** (1.11 g, 2.34 mmol) in pyridine (25 mL). After the system had been kept for 2 h at RT, silica gel (2 g) was added and the solvent was evaporated. Purification by CC (silica gel, CH_2Cl_2/CH_3OH 7:1) yielded fully deprotected **8** (506 mg, 1.40 mmol, 60%) as a white foam. R_f = 0.15 (CH_2Cl_2/CH_3OH 7:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.73 (m, 1H; H-C(8′)), 0.90 (t, J = 4.5 Hz, 1H; H-C(8′)), 1.36 (m, 1H; H-C(6′)), 1.65 (d, J = 13.6 Hz, 1H; H-C(7′)), 2.20 (dd, J = 4.3 Hz, 13.6 Hz, 1H; H-C(7′)), 2.65 (d, J = 7.0 Hz, 2H; H_2C(2′)), 3.03, 3.14 (2×s, 6H; Me_2N), 4.21 (s, 1H; H-C(4′)), 5.30, 5.51 (2×s, 2H; 2×OH), 6.21 (t, J = 7.0 Hz, 1H; H-C(1′)), 8.03 (s, 1H; H-C(8)), 8.68 (s, 1H; N=C*H*NMe$_2$), 11.33 ppm (br, 1H; NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = 16.45, 23.4, 40.8, 46.3, 48.8, 63.6, 82.6, 84.8, 88.5, 119.9, 124.1, 136.3, 137.0, 149.7, 149.8, 157.3, 157.8, 158.5 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{16}H_{21}N_6O_4$: 361.1546; found: 361.1624 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-1-(2′-Deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl)thymine (9): Compound **5** (320 mg, 0.81 mmol) was dissolved in pyridine (10.2 mL) and HF·pyridine (70% HF, 0.8 mL, 2.4 mmol) was added dropwise. After 3 h a second aliquot of HF·pyridine (0.3 mL, 0.9 mmol) was added, and after 2 h the reaction was quenched by the addition of silica gel (0.5 g), the solvent was evaporated, and the residue was purified by CC (silica, EtOAc/EtOH 9:1). Compound **9** (225 mg, 0.80 mmol, 99%) was readily isolated as a white foam. R_f = 20 (hexane/*i*PrOH 3:1); ^{1}H NMR (300 MHz, CH_3OD): δ = 0.93 (m, 1H; H-C(8′)), 1.00 (m, 1H; H-C(8′)), 1.57 (m, 1H; H-C(6′)), 1.75 (d, J = 14.0 Hz, 1H; H-C(7′)), 1.96 (d, J = 1.1 Hz, 3H; Me-C(5)), 2.25 (m, 2H; H-C(2′), H-C(7′)), 2.57 (dd, J = 6.1, 12.9 Hz, 1H; H-C(2′)), 4.32 (s, 1H; H-C(4′)), 6.22 (dd, J = 6.1, 7.8 Hz, 1H; H-C(1′)), 7.64 ppm (s, 1H; H-C(6)); ^{13}C NMR (75.5 MHz, CH_3OD): δ = 12.77, 17.72, 25.55, 25.65, 41.63, 65.40, 86.81, 86.87, 90.79, 112.15, 138.07, 152.60, 166.65 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{13}H_{17}N_2O_5$: 281.1138; found: 281.1137 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-N^6-Benzoyl-9-(2′-deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl)adenine (10): Compound **6** (817 mg, 1.41 mmol) was dissolved in pyridine (15 mL) and HF·pyridine (70% HF, 1.11 mL, 3.5 mmol) was added at RT. After 4 h the reaction was complete and was quenched by the addition of silica gel (2 g). Evaporation of the solvent and purification by CC (silica gel, CH_2Cl_2/CH_3OH 9:1) yielded the deprotected nucleoside **10** (450 mg, 1.15 mmol, 81%) as a white foam. R_f = 0.30 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 0.81 (m, 1H; H-C(8′)), 0.90 (m, 1H; H-C(8′)), 1.44 (m, 1H; H-C(6′)), 1.66 (d, J = 13.7 Hz, 1H; H-C(7′)), 2.26 (dd, J = 4.5, 13.4 Hz, 1H; H-C(7′)), 2.71 (dd, J = 6.7, 13.1 Hz, 1H; H-C(2′)), 2.82 (dd, J = 7.0, 13.2 Hz, 1H; H-C(2′)), 4.27 (s, 1H; H-C(4′)), 5.39, 5.58 (2×s, 2H; 2×OH), 6.46 (t, J = 6.8 Hz, 1H; H-C(1′)), 7.54 (m, 2H; arom. H), 7.64 (m, 1H; arom. H), 8.04 (d, J = 7.1 Hz, 2H; arom. H), 8.69, 8.77 (2×s, 2H; H-C(2), H-C(8)), 11.21 ppm (brs, 1H; HN-C(6)); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = 16.7, 23.7, 45.9, 63.5, 83.5, 85.0, 86.9, 89.0, 126.0, 128.6, 132.6, 133.6, 143.3, 150.6, 151.8 ppm.

(3′*S*,5′*R*,6′*R*)-N^4-Benzoyl-1-(2′-deoxy-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl)cytosine (11): HF·Et_3N (70% HF, 1.2 mL, 4.65 mmol, 3 equiv) was added to a solution of nucleoside **7** (750 mg, 1.55 mmol) in pyridine (19.5 mL) and the mixture was stirred for 3 h, after which another aliquot of HF·Pyr (0.3 mL, 1.67 mmol) was added. After an additional 2 h the re-

action was quenched with silica gel (1 g), the solvent was evaporated, and the residue was purified by CC (silica gel, EtOAc/EtOH 9:1) to yield compound **11** as a white foam (100%). R_f = 0.62 (EtOAc/EtOH 9:1); ^{1}H NMR (300 MHz, CD_3OD): δ = 0.80 (m, 1H; H-C(8′)), 0.95 (m, 1H; H-C(8′)), 1.48 (m, 1H; H-C(6′)), 1.59 (d, J = 13.9 Hz, 1H; H-C(7′)), 2.10 (dd, J = 6.2, 13.4 Hz, 1H; H-C(7′)), 2.24 (dd, J = 5.1, 13.9 Hz, 1H; H-C(2′)), 2.71 (dd, J = 6.2, 13.4 Hz, 1H; H-C(2′)), 4.32 (s, 1H; H-C(4′)), 6.09 (t, J = 6.2 Hz, 1H; H-C(1′)), 7.45 (m, 2H; arom. H), 7.55 (m, 2H; arom. H), 7.99 (m, 2H; arom. H), 8.21 ppm (d, J = 7.5 Hz, 1H; H-C(6)); ^{13}C NMR (75 MHz, CD_3OD): δ = 18.4, 18.7, 41.5, 58.61, 87.3, 89.8, 91.7, 98.8, 129.5, 130.1, 134.4, 135.0, 146.3, 158.0, 165.1, 169.4 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{19}H_{20}N_3O_5$: 370.1400; found: 370.1402 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-N^2-(*N*,*N*-Dimethylformamidino)-9-{2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}guanine (12): DMT-OTf (1.2 g, 2.36 mmol) was added to a solution of **8** (460 mg, 1.27 mmol) in anhydrous pyridine (4.4 mL) and ethyldiisopropylamine (2.2 mL, 13.2 mmol). After 1 h at RT the reaction mixture was diluted with EtOAc (5 mL) and washed with sat. $NaHCO_3$ (5 mL). The organic phase was then dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. CC (silica gel, 5% CH_3OH in CH_2Cl_2) yielded **12** (816 mg, 1.23 mmol, 97%) as a yellow foam. R_f = 0.68 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.49 (m, 1H; H-C(8′)), 1.23 (m, 1H; H-C(8′)), 1.83 (d, J = 43.7 Hz, 1H; H-C(7′)), 1.87 (m, 1H; H-C(6′)), 2.29 (dd, J = 4.9, 13.7 Hz, 1H; H-C(7′)), 2.50 (s, 1H; H-C(4′)), 2.59 (dd, J = 2.3, 14.7 Hz, 1H; H-C(2′)), 2.78 (dd, J = 8.2, 14.6 Hz, 1H; H-C(2′)), 3.09, 3.13 (2×s, 6H; NMe_2), 3.49, 3.71 (2×s, 6H; 2×MeO), 5.13 (s, 1H; OH), 6.10 (dd, J = 2.3, 8.0 Hz, 1H; H-C(1′)), 6.63 (m, 4H; arom. H), 7.14 (m, 3H; arom. H), 7.31 (m, 4H; arom. H), 7.42 (m, 2H; arom. H), 7.71 (s, 1H; H-C(8)), 8.11 ppm (s, 1H; N=C*H*NMe_2); HRMS (ESI-MS$^+$): m/z: calcd for $C_{37}H_{39}N_6O_6$: 663.2853; found: 663.2918 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-1-{2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}thymine (13): DMT-OTf[24] (760 mg, 1.64 mmol, 2 equiv) was added to a solution of compound **9** (230 mg, 0.82 mmol) in pyridine (3.1 mL). The mixture was protected from light, stirred for 6 h at RT, and then diluted with CH_2Cl_2 (20 mL) and washed with sat. $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The resulting oil was purified by CC (silica, EtOAc/hexane 9:1 + 1% Et_3N) to yield the tritylated compound **13** (436 mg, 0.75 mmol, 91%) as a yellowish foam. R_f = 0.18 (EtOAc/hexane 9:1 + 1% Et_3N); ^{1}H NMR (300 MHz, CD_3OD): δ = 0.64 (t, J = 5.6 Hz, 1H; H-C(8′)), 1.21 (m, 1H; H-C(8′)), 1.62 (d, J = 14.0 Hz, 1H; H-C(7′)), 1.80 (m, 1H; H-C(6′)), 1.94 (d, J = 1.1 Hz, 3H; Me-C(5)), 2.28 (m, 2H; H-C(2′), H-C(7′)), 2.61 (m, 2H; H-C(2′), H-C(4′)), 3.82, 3.83 (2×s, 6H; 2×MeO), 6.19 (t, J = 6.6 Hz, 1H; H-C(1′)), 6.86 (m, 4H; arom. H), 7.23–7.33, 7.35–7.42 ppm (2×m, 7H; arom. H); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 56.0, 68.5, 87.6, 90.4, 114.0, 128.7, 130.3, 132.6, 132.7, 137.7, 138.7, 150.4, 178.7 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{34}H_{34}N_2O_7Na$: 605.2261; found: 605.2263 $[M+Na]^+$.

(3′*S*,5′*R*,6′*R*)-N^6-Benzoyl-9-{2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}adenine (14): DMT-OTf (1.0 g, 2.28 mmol, 2 equiv) was carefully added to a solution of **10** (450 mg, 1.14 mmol) in pyridine (4.6 mL) and the mixture was stirred at RT for 3 h. After addition of EtOAc (20 mL) the yellow solution was washed twice with sat. $NaHCO_3$ (30 mL), the organic phase was dried over $MgSO_4$ and evaporated, and the residue was then purified by CC (silica gel, EtOAc + 5% Et_3N). Tritylated compound **14** (730 mg, 1.05 mmol, 92%) was readily obtained as a yellow foam. R_f = 0.19 (EtOAc + 5% Et_3N); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.57 (t, J = 5.6 Hz, 1H; H-C(8′)), 1.15 (m, 1H; H-C(8′)), 1.89 (m, 1H; H-C(6′)), 1.97 (d, J = 4.9 Hz, 1H; H-C(7′)), 2.06 (s, 1H; H-C(4′)), 2.31 (dd, J = 5.0, 13.8 Hz, 1H; H-C(7′)), 2.86 (m, 2H; $H_2C(2')$), 3.66, 3.67 (2×s, 6H; 2×MeO), 5.79 (s, 1H; OH), 6.17 (m, 1H; H-C(1′)), 6.52 (m, 4H; arom. H), 7.01 (m, 1H; arom. H), 7.09 (m, 2H; arom. H), 7.27 (m, 4H; arom. H), 7.39 (d, J = 7.5 Hz, 2H; arom. H), 7.56 (m, 3H; arom. H), 8.03, 8.68 (2×s, 2H; H-C(2), H-C(8)), 8.09 (d, J = 7.0 Hz, 2H; arom. H), 9.22 ppm (brs, 1H; C(6)-NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.8, 16.4, 24.7, 34.4, 41.6, 55.2, 66.3, 86.8, 87.4, 89.6, 112.3, 112.3, 127.2, 127.9, 128.6, 130.7, 132.9, 133.7, 144.1, 146.2, 150.4, 158.4, 158.5 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{41}H_{38}N_5O_6$: 696.2787; found: 696.2822 $[M+H^+]$.

(3′*S*,5′*R*,6′*R*)-N^4-Benzoyl-1-{2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}cytosine (15): DMT-OTf (1.01 g, 2.20 mmol, 2.1 equiv) was added to a solution of compound **11** (392 mg, 1.06 mmol) in pyridine (4.2 mL) and the mixture was stirred for 4 h at RT. The reaction mixture was then diluted with EtOAc (10 mL) and washed with sat. $NaHCO_3$ (10 mL), the organic phase was dried over $MgSO_4$, the solvent was evaporated, and purification by CC (silica gel, EtOAc/EtOH 9:1 + 1% Et_3N) yielded compound **15** (706 mg, 1.05 mmol, 99%) as a yellow foam. R_f = 0.70 (EtOAc/EtOH 9:1 + 1% Et_3N); ^{1}H NMR (300 MHz, CD_3OD): δ = 0.70 (t, J = 5.7 Hz, 1H; 1H-C(8′)), 1.28 (t, J = 7.0 Hz, 1H; 1H-C(8′)), 1.63 (d, J = 13.7 Hz, 1H; H-C(7′)), 1.82 (m, 1H; H-C(6′)), 1.98 (dd, J = 5.0, 14.1 Hz, 1H; H-C(7′)), 2.33 (dd, J = 5.1, 13.7 Hz, 1H; H-C(2′)), 2.75 (s, 1H; H-C4′)), 2.88 (m, 1H; H-C(2′)), 3.84, 3.85 (2×s, 6H; 2×OMe), 6.11 (t, J = 5.8 Hz, 1H; H-C(1′)), 6.91 (m, 4H; arom. H), 7.15 (m, 1H; arom. H), 7.16 (m, 4H; arom. H), 7.41 (m, 4H; arom. H), 7.50 (m, 2H; arom. H), 7.62 (m, 4H; arom. H), 8.02 ppm (d, J = 7.2 Hz, 2H; arom. H); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 7.02, 25.76, 56.06, 68.26, 87.70, 90.14, 114.03, 114.09, 128.81, 129.45, 130.14, 130.25, 130.75, 132.63, 132.80, 138.57, 148.29, 152.28, 160.71, 160.81, 217.98, 234.12 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{40}H_{38}N_3O_7$: 672.2720; found: 672.2709 $[M+H]^+$.

(3′*S*,5′*R*,6′*R*)-N^2-(*N*,*N*-Dimethylformamidino-9-{3′-*O*-[2-cyanoethoxy)-(diisopropylamino)phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}guanine (16): Tritylated nucleoside **12** (389 mg, 0.59 mmol) was dissolved in anhydrous CH_3CN (5.4 mL) and ethyldiisopropylamine (0.4 mL, 2.32 mmol). Chloro-(2-cyanoethyl)-diisopropylaminophosphine (0.27 mL, 1.29 mmol) was then added and the solution was stirred for 2 h at RT. The reaction was quenched by the addition of anhydrous glycerol (0.1 mL, 1.33 mmol), and stirring was continued for 15 min. The mixture was diluted with EtOAc (10 mL) and washed with a H_2O/sat. $NaHCO_3$ (10:1) solution (10 mL). After drying of the organic phase over $MgSO_4$ and evaporation of the solvent, the residue was subjected to CC (silica gel, 3% CH_3OH in CH_2Cl_2) and phosphoramidite **16** (290 mg, 0.34 mmol, 58%) was obtained as a white foam. R_f = 0.59 (6% CH_3OH in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.65, 0.80 (2×t, J = 5.6 Hz, 1H; H-C(8′)), 1.10 (m, 12H; (Me_3-CH)$_2$N), 1.19 (m, 1H; H-C(8′)), 1.26 (m, 1H; H-C(7′)), 1.87 (m, 1H; H-C(6′)), 2.25 (m, 1H; H-C(7′)), 2.51 (m, 3H; H-C(4′), NC-CH_2), 2.61 (m, 1H; H-C(2′)), 2.73 (m, 1H; H-C(2′)), 3.12, 3.13 (2×s, 3H; *Me*N), 3.21 (s, 3H; *Me*N), 3.49 (m, 3H; CH_2O, C*H*Me_2), 3.77, 3.79 (2×s, 6H; 2×MeO), 6.35 (m, 1H; H-C(1′)), 6.71 (m, 4H; arom. H), 7.14 (m, 3H; arom. H), 7.28 (m, 4H; arom. H), 7.42 (m, 2H; arom. H), 8.62, 8.65 (2×s, 1H; H-C(8)), 9.06 ppm (br, 1H; N=C*H*NMe_2); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ = 142.13, 144.33 ppm; HRMS (ESI-MS$^+$): m/z: calcd for $C_{46}H_{56}N_8O_7P$: 863.4009; found: 863.4005 $[M+H]^+$.

(3′*S*,5′*R*,6′*S*)-1-{3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}thymine (17): Compound **13** (400 mg, 0.69 mmol) and ethyldiisopropylamine (0.69 mL, 3.97 mmol) were dissolved in dry CH_3CN (3.5 mL), chloro-(2-cyanoethyl)diisopropylaminophosphine (0.30 mL, 1.84 mmol) was added dropwise, and the clear solution was stirred for 2 h at RT. The reaction was quenched by the addition of glycerol (0.5 mL) and stirring was continued for an additional hour. After dilution with EtOAc (30 mL) and washing with sat. $NaHCO_3$ (30 mL), the organic phase was dried over $MgSO_4$ and the solvent was evaporated. CC (3 g silica conditioned with EtOAc + 1% Et_3N, elution with EtOAc) afforded phosphoramidite **17** (432 mg, 0.55 mmol, 80%) as a white foam. R_f = 0.39, 0.34 (2 diastereomers, hexane/EtOAc/Et_3N 10:20:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.64, (t, J = 5.6 Hz, 1H; H-C(8′)), 1.11 (m, 12H; 2×Me_3CH), 1.18 (m, 2H; H-(7′), H-(8′)), 1.81 (m, 1H; H-C(6′)), 1.95 (d, J = 1.0 Hz, 3H; MeC(5)), 2.20 (m, 2H; H-C(6′), H-C(7′)), 2.5–2.7 (2×m, 4H; $H_2C(2')$, H-C(4′), NC-CH_2), 3.41 (m, 2H; P-O-CH_2-C), 3.5–3.7 (2×m, 2H; 2×C*H*$(Me)_2$), 3.79, 3.80 (2×s, 6H; 2×MeO), 6.18 (m, 1H; H-C(1′)), 6.79 (m, 4H; arom. H), 6.91, 6.94 (2×s, 1H; H-C(6)), 7.23 (m, 3H; arom. H), 7.38 (m, 4H; arom. H), 7.49 (m, 2H; arom. H), 8.13 (s, 1H; H-N(3)) ppm; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ = 142.51,

144.28 ppm; HRMS (ESI-MS$^+$): *m*/*z*: calcd for $C_{43}H_{52}N_4O_8P$: 783.3489; found: 783.3522 [*M*+H$^+$].

(3′*S*,5′*R*,6′*S*)-*N*^{6}-Benzoyl-9-{3′-*O*-[(2-cyanoethoxy)(diisopropylamino)-phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}adenine (18): Ethyldiisopropylamine (0.93 mL, 5.35 mmol) and chloro-(2-cyanoethyl)diisopropylaminophosphine (0.49 mL, 3.01 mmol) were added to a stirred solution of compound **14** (540 mg, 0.78 mmol) in dry CH_3CN (4.5 mL). After 2 h the reaction was quenched with glycerol (0.5 mL) and after another hour the mixture was diluted with EtOAc (20 mL) and washed with $NaHCO_3$/sat. H_2O 1:10. The organic phase was then dried over $MgSO_4$ and evaporated. Purification of the residue by CC (10 g silica, conditioned with EtOAc + 1% Et_3N, eluent: EtOAc) afforded amidite **18** (528 mg, 0.59 mmol, 83%) as a yellow foam. R_f = 0.51 (EtOAc + 1% Et_3N); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.68, 0.82 (2×m, 1H; H-C(8′)), 1.00–1.15 (m, 13H; 2×C*Me*$_2$, H-C(8′)), 1.87 (m, 1H; H-C(7′)), 2.12 (m, 1H; H-C(6′)), 2.31 (m, 1H; H-C(7′)), 2.42, 2.47 (2×t, *J* = 6.2 Hz, 2H; NC-C*H*$_2$-), 2.55 (s, 1H; H-C(4′)), 2.77, 2.84 (2×m, 2H; H$_2$(C2′)), 3.50 (m, 4H; 2×C*H*CH$_3$, -OC*H*$_2$-), 3.78, 3.78, 3.79, 3.79 (4×s, 6H; 2×MeO), 6.45, 6.52 (2×m, 1H; H-C(1′)), 6.72 (m, 4H; arom. H), 7.20 (m, 3H; arom. H), 7.33 (m, 4H; arom. H), 7.44 (m, 2H; arom. H), 7.58 (m, 3H; arom. H), 7.99, 8.04, 8.77, 8.81 (4×s, 2H; H-C(2), H-C(8)), 8.05 (m, 2H; arom. H), 9.05 ppm (brs, 1H; NH); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ = 143.22, 144.35 ppm; HRMS (ESI-MS$^+$): *m*/*z*: calcd for $C_{50}H_{55}N_7O_7P$: 896.3908; found: 896.3908 [*M*+H]$^+$.

(3′*S*,5′*R*,6′*S*)-*N*^{4}-Benzoyl-1-{3′-*O*-[(2-cyanoethoxy)(diisopropylamino)-phosphino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′,5′-ethano-5′,6′-methano-α-D-ribofuranosyl}cytosine (19): Ethyldiisopropylamine (0.26 mL, 1.52 mmol) and chloro-(2-cyanoethyl)diisopropylaminophosphine (0.19 mL, 0.92 mmol, 3 equiv) were added drop by drop to a solution of the tritylated nucleoside **15** (204 mg, 0.30 mmol) in dry CH_3CN (1.9 mL). After 2 h at RT the reaction mixture was quenched by the addition of glycerol (0.3 mL) and allowed to stir for an additional hour. The mixture was then diluted with EtOAc (4 mL) and washed with sat. $NaHCO_3$ (6 mL), and the organic phase was dried over $MgSO_4$ and evaporated. Purification by CC (5 g silica, conditioned with EtOAc + 1% Et_3N, elution with EtOAc) afforded amidite **19** (186 mg, 0.21 mmol, 71%) as a white foam. R_f = 0.53, 0.45 (2 diastereomers, EtOAc + 1% Et_3N); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.60, 0.76 (2×m, 1H; H-C(8′)), 1.06 (m, 12H; 2×C*Me*$_2$), 1.13 (m, 1H; H-C(8′)), 1.84 (m, 1H; H-C(6′)), 2.05–2.35 (2×m, 2H; H$_2$C(7′)), 2.38 (m, 1H; H-C(2′)), 2.56 (m, 2H; NC-CH$_2$), 2.57, 2.77 (2×s, 1H; H-C(4′)), 2.86, 3.03 (2×m, 1H; H-C(2′)), 3.38 (m, 2H; OCH$_2$), 3.55, 3.66 (2×m, 2H; 2×C*H*Me$_2$), 3.81, 3.82 (2×s, 6H; 2×MeO), 6.12 (m, 1H; H-C(1′)), 6.83 (m, 4H; arom. H), 7.29 (m, 2H; arom. H), 7.37 (m, 4H; arom. H), 7.50 (m, 6H; arom. H), 7.61 (m, 2H; arom. H), 7.90 (m, 2H; arom. H), 8.66 ppm (brs, 1H; NH-C(4)); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ = 143.22, 144.35 ppm; HRMS (ESI-MS$^+$): *m*/*z*: calcd for $C_{49}H_{55}N_5O_8P$: 872.3822; found: 872.3788 [*M*+H]$^+$.

α-tc-Oligonucleotide synthesis: The syntheses of oligonucleotides **20**–**22** were performed by a modified phosphoramidite method on a 1.0 μmol scale in the trityl-off mode on an Applied Biosystems Expedite 8909 DNA synthesizer. The following modifications of the standard DNA synthesis cycle were made: i) the commonly used activator tetrazole was replaced by the more reactive 5-(ethylthio)-1*H*-tetrazole (0.4 M in MeCN), ii) 90 μL of 0.2 mM amidite solution in CH_3CN were used per coupling, iii) the coupling time was increased to a total of 15 min, and iv) a 20 mM I_2 oxidizing solution was used and the oxidation time was extended to 40 s. All α-tc-oligonucleotides were synthesized on a universal solid support (universal support UL1, CTGen, Milpitas, CA). 5′-End phosphorylation was effected by terminal coupling with an additional α-tc-C building block, which was lost upon deprotection, leaving behind a 5′-phosphate terminus.[25] After synthesis, the solid support was suspended in concentrated NH_3 solution (ca. 1 mL) and left for 60 h at 60–65 °C. The crude oligonucleotides were purified by RP-HPLC on a Source 15RPC ST 4.6/100 column (Amersham Pharmacia Biotech) with a CH_3CN gradient in Et_3N/AcOH buffer (0.1 M, pH 7.0, 60 °C). Purified oligonucleotides were desalted over SEP-PAK C-18 cartridges (Waters). All modified oligonucleotides were analyzed by ESI$^-$ mass spectrometry; the corresponding MS data are given in Table 1. Natural oligonucleotides were synthesized and purified by standard methodology, mass spectrometry also being used here as quality control.

UV melting curves: UV melting curves were determined at 260 nm on a Varian Cary 3E spectrophotometer fitted with a Peltier block with use of Varian WinUV software. Complementary oligodeoxynucleotides were mixed to 1:1 stoichiometry with a final total single-strand concentration of 5 μM. A heating-cooling-heating cycle (0–90 °C) was applied with a temperature gradient of 0.5–1.0 °C min^{-1}. Each T_m values was defined as the maximum of the first derivative of the melting curve with the aid of the Origin™ v 5.0 software package. Thermodynamic data for duplex formation were obtained as indicated in the text.

CD spectra: CD spectra were recorded on a Jasco J-715 spectropolarimeter with a Jasco PFO-350S temperature controller. The temperature was measured directly in the cell (path length 10 mm).

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