Stabilisation of RNA Bulges by Oligonucleotide Complements Containing an Adenosine Analogue

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Incorporation of 2′-deoxy-2′-β-(1-naphthylmethyl)tubercidin into an oligodeoxyribonucleotide mostly has little or a slightly negative effect on the T_m values of complexes with DNA complements. With the same naphthylmethyl-substituted nucleoside at the 3′-end of a 2′-O-methyloligoribonucleotide, however, a stabilisation of 1 – 2 °C in the corresponding complexes with both DNA and RNA is observed. When the target sequence is an RNA fragment forming a two- or three-nucleotide bulge, complexes with (naphthylmethyl)-tubercidin-modified oligodeoxyribonucleotides, as well as with the corresponding 2′-O-methyloligoribonucleotides, give stabilisations of 1 – 2 °C for the three-nucleotide bulge and of almost 4 °C for the two-nucleotide bulge. This stabilisation is specific to RNA, since the corresponding complexes with the DNA fragments do not display this effect. Thus, the (naphthylmethyl)tubercidin-containing oligonucleotides are the first reported oligonucleotide modifications that specifically stabilise bulged RNA.

Introduction

Largely thanks to the development of antisense therapy, numerous oligonucleotide modifications for the improvement of the stability of duplexes formed between modified oligonucleotides and their RNA target have been developed.[1–3] The occurrence of one or several bulged out nucleotides in an otherwise complementary duplex usually results in substantial destabilisation of the complex.[4] There are, however, potential gains imaginable if the antisense oligonucleotide induces and stabilises a bulge in the target RNA sequence. One may obtain interactions that give specificities other than those of Watson – Crick base-pairing (possibly also more discriminating). A potential site for binding of low molecular weight drugs is created, and may stabilise the oligonucleotide-target complex substantially.[5]

Bulged RNA is also known to be more susceptible to cleavage than RNA in a helical structure and is therefore particularly interesting as a target for artificial nucleases.[6] We are currently pursuing a programme devoted towards the development of oligonucleotide-based artificial nucleases (OBANs), with emphasis on the cleavage of induced bulges in a target RNA. However, since structures with bulges are inherently less stable than fully complementary duplexes,[4] an initially important issue is the introduction of modifications that can stabilise an RNA bulge, and thus the OBAN-target RNA complex. In addition, stabilisation of the bulged complex would also be expected to rigidify the structure, which—with correct positioning of the catalytic part of the OBAN—could give rise to higher cleavage rates.

Binding of a bisbenzimidazole derivative affects the stability of a structure containing an RNA bulge[5a] but to our knowledge no oligonucleotide modifications for stabilisation of RNA bulges have been designed. A bulge in the human immunodeficiency virus type 1 (HIV-1) Rev response element binding site for Rev protein, for which a crystal structure has been reported,[7] is one of the model bulges in our development of OBANs. Molecular modelling in this system suggested that the presence of a 1-naphthylmethyl group in the 2′-position (with *arabino* configuration) would be worth exploring as a first step in increasing affinity and specificity for bulged out RNA target sequences.

Results and Discussion

Synthesis of the modified building block and incorporation into oligonucleotides

Because of depurination problems observed in initial efforts on the derivatisation of adenosine, the naphthylmethyl moiety was introduced into the 7-deaza analogue of adenosine, known as tubercidin.[8]

The synthesis of the modified building block (Scheme 1) began with 6-deamino-6-chloro-tubercidin (**1**),[9] which was silylated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[10] ($TIPDSCl_2$, Scheme 1). Oxidation of **2** with CrO_3, followed by treatment with (1-naphthylmethyl)triphenylphosphonium chlor-

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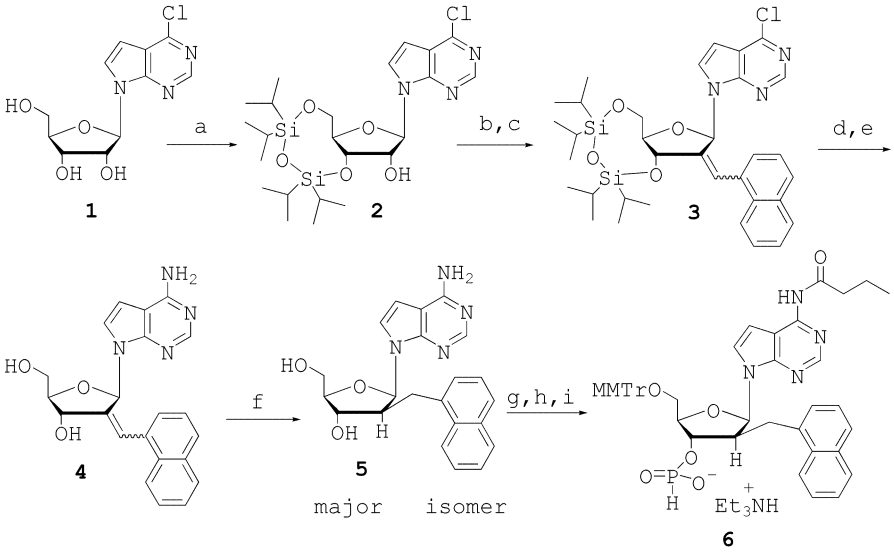


Scheme 1. *Synthesis of the 2′-(1-naphthylmethyl)-substituted H-phosphonate building block: a) $TIPDSCl_2$ (1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane), pyridine, RT, 12 h, 95%; b) CrO_3, acetic anhydride, pyridine, CH_2Cl_2, 86%; c) (naphthylmethyl)triphenylphosphonium chloride, nBuLi (1.75 equiv.), THF, RT, 73%; d) NH_3 (l), dioxane, 4 days, 80 °C; e) $Bu_4N^+F^-$, THF, 80% over two steps; f) H_2, Pd/C, CH_3OH, 5 h, 82%; g) 1) $(CH_3)_3SiCl$, pyridine, 2) butyric anhydride, 3) H_2O, 60%; h) $MMT^+BF_4^-$ (monomethoxytrityl tetrafluoroborate), Li_2CO_3, 2,6-lutidine, 0 °C to RT, 93%; i) 1) imidazole, PCl_3, Et_3N, CH_2Cl_2, −20 °C to −78 °C, 80% 2) H_2O.*

Further transformations were carried out to provide a building block suitable for oligonucleotide synthesis (Scheme 1). Base protection was accomplished with butyric anhydride, after which the 5′-OH function was protected by use of monomethoxytrityl tetrafluoroborate in the presence of lithium carbonate and 2,6-lutidine.[11] Subsequent phosphonylation with the PCl_3/imidazole reagent[12] yielded the H-phosphonate building block **6** in 80% yield.

The modified oligodeoxyribonucleotides and 2′-*O*-methyloligoribonucleotides were prepared by the H-phosphonate approach,[13] with building block **6** for incorporation of internal modifications (aimed at base-pairing to a uridine or thymidine adjacent to the bulged out region at the 3′-side in the target sequence). The modification was also incorporated at the 3′-end of oligonucleotides by use of the 5′-*O*-(4-methoxytrityl)-2′-deoxy-2′-(1-naphthylmethyl)tubercidin 3′-succinate attached to the solid support. The synthesized sequences are shown in Figure 1.

ide, gave **3**, which was further treated with liquid ammonia and then with tetrabutylammonium fluoride (TBAF) in THF, affording **4** as an isomeric *E*/*Z* mixture in 80% yield. Hydrogenation of **4** gave a ≈1/5 mixture of the α and β 2′-naphthylmethyl isomers. The mixture of 2′-isomers could be separated by column chromatography (see Experimental Section for more details). NMR studies were then performed to assign the 2′-configurations of the major and the minor isomers. 2D NMR experiments (COSY, HMQC) were carried out for both isomers to assign all the proton signals in the spectra unambiguously, and for evidence of a 2′-β (*arabino*) configuration could be found in the 2D NOESY and ROESY spectra. A strong interaction was observed between the benzylic protons ($\delta = 3.35$ and 2.71) and H3′ ($\delta = 4.53$); furthermore, an additional cross-peak between H2′ and H4′ confirms the 2′-β (*arabino*) configuration for this major isomer **5**. These effects were absent in the corresponding spectra for the minor isomer. In these spectra, however, a cross-peak was observed between H2′ ($\delta = 3.41$) and H8 ($\delta = 6.99$), confirming the 2′-α (*ribo*) naphthyl configuration for the minor isomer.

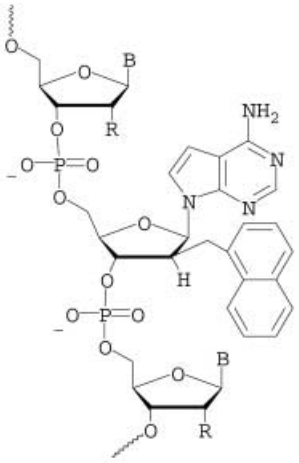
One feature of this novel nucleoside was reflected in a strong NOESY interaction between H3′ and H8 observed in the 2D ^{1}H NMR spectrum of **5**. This observation points towards a strongly preferred North (C3′-*endo*) conformation. This is further supported by the ^{1}H,^{1}H coupling constants (i.e., $J_{H3',H4'} = 8.0$ and $J_{H1',H2'} = 7.7$ Hz; whereas for the minor *ribo* isomer the $J_{H3',H4'}$ value of 0 Hz indicates a predominant South ($C_{2'}$-*endo*) conformation). The preference for the N-conformation of **5** may well be a result of a favourable stacking interaction between the naphthyl moiety and the heterocyclic base.

Studies on the effects on the stability of complexes with RNA or DNA complements

The binding of the 2′-deoxy-2′-(1-naphthylmethyl)tubercidin-modified oligonucleotides to DNA and RNA sequences was investigated by their UV absorbance melting behaviour in an aqueous neutral buffer solution. Hybridisation experiments were performed with fully complementary sequences and different complements that form varying bulge sizes (see Table 1 and Table 2). The melting profiles for duplexes containing a modification exhibited, in most cases, typical two-state transitions similar to those for the corresponding unmodified duplexes.

Incorporation of the (naphthylmethyl)tubercidin into an oligodeoxyribonucleotide mostly has little or a slightly negative effect on the T_m values of the complexes with the DNA complements (Table 1). This is also the case for internal naphthylmethyl modification in 2′-OMeRNA fragments (Table 2). When, however, the modification is present at the 3′-end of the 2′-*O*-methyloligribonucleotide a stabilisation of the complexes with DNA fragments is observed (ΔT_m from 0.4 up to 2.2 °C). As DNA/2′-OMeRNA duplexes are expected to be of the A-type, this stabilisation may be due to the high North conformation preference of the 3′-end naphthylmethyl nucleoside.

As would be expected from the steric demands of the naphthylmethyl group, all fully complementary duplexes with internal modifications display a substantial decrease in melting temperature. When, however, the target sequence is an RNA fragment forming a two- or three-nucleotide bulge, the complexes with the internally (naphthylmethyl)tubercidin-modi-



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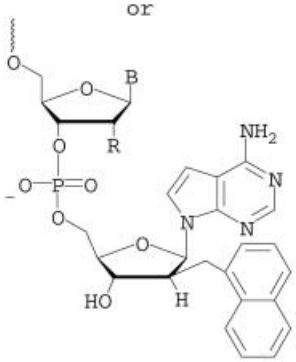

R = H
7 5′-d(GAGTACACAGA)= ref
8 5′-d(GAGTAC$_{7d}$A^{N}CAGA)
9 5′-d(GAGTACACAG$_{7d}$A^{N})

R = OCH_3
10 5′-2′OMe(GAGUACACAGA)= ref
11 5′-2′OMe(GAGUAC$_{7d}$A^{N}CAGA)
12 5′-2′OMe(GAGUACACAG$_{7d}$A^{N})
13 5′-2′OMe(GAGU$_{7d}$A^{N}CACAG$_{7d}$A^{N})

$_{7d}$A^{N}=2′-deoxy-2′-*C*-naphthylmethyl-7-deazaadenosine

Molecular weights of modified oligonucleotides as determined by ESI-TOF mass spectra.

Sequence	MW (calcd)	MW (found)
8	3514	3514
9	3514	3513
11	3799	3799
12	3799	3799
13	3909	3908

Figure 1. *DNA and 2′-OMe RNA fragments with an internal or 3′-end 2′-(1-naphthylmethyl)-tubercidin modification.*

Table 1. *Melting temperatures (T_m) for complexes of 2′-(1-naphthylmethyl)-tubercidin-modified oligodeoxyribonucleotides with DNA and RNA complements resulting in different bulge sizes.*[a]

Complement	T_m [°C][b] Complex with **7**	Complex with **8**	Complex with **9**
DNA-complements			
5′-TCTGTGTACTC-3′[c]	41.2	34.0	39.7
5′-TCTG^{A}TGTACTC-3′[c]	25.9	25.4	25.1
5′-TCTG^{AA}TGTACT C-3′[d]	19.8	19.0	19.1
5′-TCTG^{AAA}TGTACT C-3′[d]	14.0	15.8	13.6
RNA-complements			
5′-UCUGUGUACUC-3′[c]	37.5	28.5	38.3
5′-UCUG^{A}UGUACUC-3′[c]	21.8	19.7	20.8
5′-UCUG^{AA}UGUACUC-3′[d]	14.8	18.6	14.1
5′-UCUG^{AAA}UGUACUC-3′[d]	12.1	14.2	11.9

[a] Determined at 260 nm. Measured in 0.1 mM NaCl, 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA with 4 μM single strand concentration. [b] Deviations of ≤ 0.3 °C were obtained in triplicate experiments unless otherwise specified. [c] The T_m values were obtained by fitting of the melting profile to a two-state transition model. [d] T_m values were obtained as the maxima of the first derivatives of the absorbance versus temperature curves.

fied oligonucleotides are stabilised. There is clearly a preference in the stabilisation for the complexes with both the internally modified oligodeoxyribonucleotide (Table 1) and 2′-*O*-methylo-ligoribonucleotide fragments (Table 2), which give a stabilisation of 1–2 °C with the three-nucleotide (3 nt) bulge complex and of almost 4 °C with the two-nucleotide bulge complex. There is also a dependence on the bulge size, the 2 nt bulge being clearly preferred both to the larger 3 nt bulge and to the smaller 1 nt bulge. That this effect is only present with an RNA complement suggests a possible conformational dependence (in the target sequence) of the stabilising interaction.

The presence of a naphthylmethyl-modified nucleoside at the 3′-end of an oligonucleotide can give stabilisation of fully complementary duplexes. For 2′-OMeRNA/RNA and 2′-OMeRNA/DNA this is also observed with bulge-containing complements. Even more interesting, however, is that incorporation of the naphthylmethyl-modified nucleoside opposite to the bulge stabilises the complexes with RNA containing three and—in particular—two bulged-out nucleotides. This stabilisation is specific to RNA, as the corresponding complexes with DNA fragments do not display this effect. The stabilisation is relatively modest and it is possible that the result might be different with other sequences/bulges. Although this specific modification may not stabilise bulges with other sequences (which is not desirable anyway, if one wishes to achieve selectivity), we have proven the concept of a modification that can stabilise an RNA bulge. We report that (naphthylmethyl)tubercidin-containing oligonucleotides are capable of bulge stabilisation, and to the best of our knowledge this is the first reported oligonucleotide modification that specifically stabilises bulged RNA.

RNA bulge stabilisation could promote action by complementary modified OBANs. Stabilisation of the bulge should lead to a less flexible structure. We have recently shown the importance of linker and bulge size in several OBAN systems.[14] For further development, one possibility is that, if the catalytic group can be positioned close enough to the phosphodiester to be cleaved, a more rigid target bulge would provide a higher effective molarity and thus a higher rate of cleavage. It is likely that greater bulge stabilisation would be needed to obtain a substantial effect on oligonucleotide-based artificial nuclease cleavage. Further developments of bulge-stabilising modifications are currently being pursued not only to achieve greater stabilisation of the antisense-bulged RNA complex, but also since significant advantages due to specific selectivity patterns could be obtained.

Experimental Section

Materials and methods: All chemicals were purchased from Aldrich, Sigma or Fluka. Solvents were p. a grade. Pyridine, dichloromethane, acetonitrile and dimethylformamide were dried over 3 Å molecular sieves. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a Bruker AVANCE DRX 400 spectrometer operating at proton reso-

Table 2. *Melting temperatures (T_m) for complexes of 2′-(1-naphthylmethyl)tubercidin-modified 2′-O-methyloligoribonucleotides with DNA and RNA complements resulting in different bulge sizes.*[a]

	T_m [°C][b]			
Complement	Complex with **10**	Complex with **11**	Complex with **12**	Complex with **13**
DNA-complements				
5′-TCTGTGTACTC-3′	39.8[c]	32.2	41.1	33.1[c]
5′-TCTG^{A}TGTACTC-3′	29.6	26.6	31.7	27.9
5′-TCTG^{AA}TGTACT C-3′	23.9	24.5	26.1	25.6
5′-TCTG^{AAA}TGTACT C-3′	23.5[c]	20.6	23.9	21.8[c]
RNA-complements				
5′-UCUGUGUACUC-3′	55.3	42.9	57.2	43.0
5′-UCUG^{A}UGUACUC-3′	42.7	40.5	43.9	40.0
5′-UCUG^{AA}UGUACUC-3′	35.9	39.8	37.0	38.9
5′-UCUG^{AAA}UGUACUC-3′	33.7	34.9	35.2	34.8

[a] Determined at 260 nm. Measured in 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA with 4 μM single strand concentration. [b] The T_m values were obtained by fitting of the melting profile to a two-state transition model. Deviations of ≤ 0.3 °C were obtained in triplicate experiments unless otherwise specified. [c] Uncertainty of $\pm 0.5-1$ °C. The curves display a slight tendency towards biphasic behaviour.

nance frequencies of 400.23 MHz (100.62 MHz for ^{13}C, 161.98 MHz for ^{31}P). Chemical shifts are in ppm relative to TMS. *J* values are given in Hz. Thin layer chromatography was performed on Merck silica gel 60 F_{254} precoated plates, and column flash chromatography on silica gel 60 (Merck). HPLC analysis and purification was performed on Jasco HPLC systems. Highly crosslinked aminopolystyrene support was obtained from Applied Biosystems. Non-modified oligonucleotides used for the melting experiments were obtained from Dharmacon Research (Boulder, CO, USA). They were purified by reversed-phase HPLC and freeze-dried three times before use.

Synthesis of monomers

Synthesis of 3′,5′-TIPS-6-deamino-6-chloro-7-deazaadenosine (2): 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (4.80 g, 14.88 mmol) was added by syringe to a stirred solution of 6-chloro-7-deazaadenosine (3.54 g, 12.4 mmol) in dry pyridine (2 mL). The reaction mixture was stirred overnight at room temperature. After addition of methanol (1 mL) and dilution with dichloromethane, the mixture was extracted with sat. $NaHCO_3$ solution. After drying, filtration and concentration, the crude product was purified by column chromatography on silica gel with dichloromethane/methanol (20:1) as eluent, yielding the desired compound (6.25 g, 11.8 mmol, 95%). $R_f =$ 0.77 (6% methanol in dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.61$ (s, 1H), 7.46 (d, $J = 3.7$ Hz, 1H), 6.62 (d, $J = 3.7$ Hz, 1H), 6.14 (d, $J = 1.5$ Hz, 1H), 4.93 (dd, $J = 6.9$, 5.9 Hz, 1H), 4.47 (m, 1H), 4.03–4.15 (m, 3H), 1.00–1.15 (m, 28H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 150.8, 127.8, 118.6, 118.2, 100.4, 90.4, 82.0, 75.3, 70.7, 61.7, 17.6, 17.5, 17.5, 17.4, 17.2, 17.2, 17.1, 17.0, 13.5, 13.2, 13.0, 12.8 ppm; EIMS *m/z* (%): 527 ($[M]^+$, 1), 484 ($[M - i\mathrm{Pr}]^+$, 100).

Synthesis of 2′-(1-naphthylmethylene)-3′,5′-TIPS-6-chloro-7-deazaadenosine (3): CrO_3 (3.54 g, 35.4 mmol) was suspended in dry dichloromethane (71 mL) and the mixture was cooled to 0 °C. Subsequently, pyridine (5.72 mL, 70.8 mmol) was added, followed by acetic anhydride (3.35 mL, 35.4 mmol). The mixture was stirred at 0 °C under nitrogen atmosphere for 1 h. A solution of **2** (6.25 g, 11.8 mmol) in dichloromethane (45 mL) was added dropwise. The reaction mixture was stirred in an ice/water bath for 2 h, poured into cold ethyl acetate (400 mL) and filtered through a layer of silica gel. The product was isolated after chromatography on silica gel with dichloromethane/methanol (15:1), yielding 5.54 g (10.1 mmol, 86%) of the desired compound.

(1-Naphthylmethyl)triphenylphosphonium chloride was prepared by mixing (1-naphthylmethyl)chloride (3.53 g, 20.0 mmol) and triphenylphosphine (4.90 g, 18.7 mmol) and heating the mixture at 100 °C for 15 min. The salt precipitates and can be isolated in quantitative yield after cooling, powdering, thorough washing with diethyl ether and drying in vacuo.

n-Butyllithium (16.2 mL of a 1.6 M solution in hexane, 26 mmol, 2.6 equiv.) was slowly added by syringe, at room temperature, to a suspension of (1-naphthylmethyl)triphenylphosphonium chloride (12.5 g, 28.3 mmol, 2.8 equiv.) in THF (170 mL). The reaction mixture turned red. After the mixture had been stirred for 25 min, a solution of 2′-keto-3′,5′-TIPS-6-chloro-7-deaza-adenosine (5.54 g, 10.1 mmol) in THF (130 mL) was added dropwise. The reaction mixture was stirred for 3 h at 60 °C. Diethyl ether was then added, and the solid material was filtered off. The residue was poured into saturated $NaHCO_3$ solution, the aqueous phase was extracted three times with diethyl ether, and the combined organic phases were washed with brine. After drying over Na_2SO_4, the solvent was removed in vacuo. The crude material was purified by column chromatography on silica gel with dichloromethane/methanol (99:1) as eluent. The major isomer was isolated (4.79 g, 7.37 mmol, 73%). $R_f = 0.61$ (2% methanol/dichloromethane); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.47$ (s, 1H), 7.75 (d, $J = 7.8$ Hz, 1H), 7.69 (d, $J = 8.3$ Hz, 1H), 7.62 (d, $J = 8.3$ Hz, 1H), 7.30–7.46 (m, 2H), 7.08 (t, $J = 7.7$ Hz, 1H), 7.00 (d, $J = 3.7$ Hz, 1H), 6.75 (t, $J = 1.7$ Hz, 1H), 6.70 (d, $J = 7.1$ Hz, 1H), 6.38 (d, $J = 3.7$ Hz, 1H), 5.62 (dt, $J = 8.3$, 2.1 Hz, 1H), 4.14 (d, $J = 3.7$ Hz, 2H), 3.90 (m, 1H), 1.12–1.28 (m, 28H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 151.0$ (CH), 142.6 (C), 133.7 (C), 132.3 (C), 131.5 (C), 128.8 (CH), 128.6 (CH), 127.5 (CH), 126.5 (CH), 126.4 (C), 126.0 (CH), 125.8 (CH), 125.3 (CH), 124.2 (CH), 118.4 (C), 101.1 (CH), 82.9 (CH), 82.4 (CH), 73.4 (CH), 62.4 (CH_2), 17.9 (CH), 17.8 (CH), 17.7 (CH), 17.7 (CH), 17.5 (CH), 17.4 (CH), 14.1 (C), 13.5 (C), 13.3 (C), 13.2 (C) ppm; HRMS calcd. for $C_{34}H_{44}ClN_3O_4Si_2$ 650.2637, found: 650.2642.

Synthesis of 2′-(1-naphthylmethylene)-7-deazaadenosine (4): A suspension of **3** (0.958 g, 1.47 mmol) in dioxane (3 mL) was added to a cooled (−78 °C) pressure vessel containing liquid ammonia (60 mL). The vessel was sealed and heated at 80 °C for 4 days, then cooled down to −78 °C and opened. The ammonia was evaporated under a gentle N_2 stream, after which the residue was dissolved in methanol and concentrated. TLC analysis showed complete disappearance of starting material. THF (10 mL) was then added, followed by tetrabutylammonium fluoride (TBAF, 1.2 g, 4.61 mmol, 3 equiv.),

and the reaction mixture was stirred at RT for 1.5 h. After concentration, the crude reaction mixture was purified by column chromatography on silica gel (dichloromethane/methanol 88:12 →80:20), yielding the desired compound (459 mg, 1.18 mmol, 80%). $R_f = 0.33$ (10% methanol/dichloromethane); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.88$ (s, 1H), 7.87 – 7.90 (m, 1H), 7.72 (m, 1H), 7.53 (d, $J = 8.23$ Hz, 1H), 7.43 (s, 1H), 7.41 – 7.43 (m, 2H), 6.95 (m, 2H), 6.92 (d, $J = 3.79$ Hz, 1H), 6.75 (d, $J = 7.03$ Hz, 1H), 6.22 (d, $J = 3.73$ Hz, 1H), 5.10 (d, $J = 6.95$ Hz, 1H), 3.95 (m, 2H), 3.84 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 157.6$ (C), 150.9 (CH), 149.4 (C), 144.7 (C), 133.7 (C), 132.2 (C), 131.4 (C), 128.2 (CH), 127.9 (CH), 126.7 (CH), 126.0 (CH), 125.8 (CH), 125.6 (CH), 124.7 (CH), 124.3 (CH), 122.7 (CH), 103.1 (C), 100.1 (CH), 84.6 (CH), 82.2 (CH), 72.2 (CH), 61.8 (CH_2) ppm; TOF-MS-ES$^+$ *m/z* (%): 389.0 ($[M^+H]^+$, 100), 777.2 ($[2 \times M^+H]^+$, 22).

Synthesis of 2′-(1-naphthylmethyl)-7-deazaadenosine (5): Pd/C (100 mg) was suspended in methanol (3 mL) and the mixture was presaturated with hydrogen for 15 min. 2′-(1-Naphthylmethylene)-7-deazaadenosine (**4**, 459 mg, 1.18 mmol) was dissolved in methanol (8 mL) and the solution was added to the reaction mixture. After the mixture had been stirred for 5 h, TLC showed complete conversion of starting material. The reaction mixture was filtered through Celite, which was washed with methanol. At this stage it was difficult to separate the isomers by column chromatography. The solvent was removed in vacuo, yielding the desired compound (approximately 390 mg) as a ≈1:5.5 mixture of 2′-isomers (as estimated by ^{1}H NMR of the crude reaction mixture). The residue was then purified by column chromatography (CH_2Cl_2/methanol 92:8), yielding 47 mg of the first eluting minor isomer and 330 mg of an almost pure fraction of the major isomer. This last fraction was used as such in the subsequent reaction (base protection), after which the remaining traces of the minor isomer could be easily removed by chromatography.

A small amount of the last fraction was purified by repeated chromatography ($CHCl_3$/methanol 92:8) to provide the pure major isomer for spectral characterization.

First eluting (minor) isomer (2′-α): $R_f = 0.45$ (methanol/dichloromethane 8:92); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.96$ (s, 1H), 7.70 (d, $J = 8.1$ Hz, 1H), 7.53 (d, $J = 8.2$ Hz, 1H), 7.44 (d, $J = 8.4$ Hz, 1H), 7.32 (t, $J = 7.6$ Hz, 1H), 7.28 (d, $J = 6.7$ Hz, 1H), 7.15 (m, 2H), 6.99 (d, $J = 3.6$ Hz, 1H), 6.33 (d, $J = 3.6$ Hz, 1H), 6.18 (d, $J = 9.7$ Hz, 1H), 4.35 (d, $J = 5.0$ Hz, 1H), 4.14 (t, $J = 3.1$ Hz, 1H), 3.77 (dd, $J = 3.3$, 12.3 Hz, 1H), 3.70 (dd, $J = 3.0$, 12.3 Hz, 1H), 3.54 (m, 1H), 3.41 (m, 1H), 3.06 (dd, $J = 6.2$ Hz, 13.8 Hz, 1H) ppm; ^{13}C NMR (100 MHz, CD_3OD): $\delta = 152.0$ (CH), 136.8 (C), 135.7 (C), 130.0 (CH), 128.3 (CH), 128.2 (CH), 126.9 (CH), 126.7 (CH), 126.5 (CH), 125.0 (C), 124.6 (CH), 100.7 (CH), 91.9 (CH), 90.1 (CH), 74.7 (CH), 64.6 (CH_2), 37.3 (CH_2) ppm.

Last eluting (major) isomer (2′-β): $R_f = 0.41$ (methanol/dichloromethane 8:92); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.84$ (m, 1H), 7.84 (s, 1H), 7.78 (m, 1H), 7.60 (d, $J = 8.3$ Hz, 1H), 7.45 (d, $J = 3.6$ Hz, 1H), 7.41 (m, 1H), 7.02 (dd, $J = 7.2$, 8.0 Hz, 1H), 6.72 (d, $J = 3.6$ Hz, 1H), 6.46 (d, $J = 6.9$ Hz, 1H), 6.39 (d, $J = 7.7$ Hz, 1H), 4.53 (dd, $J = 8.0$, 9.2 Hz, 1H), 3.97 (dd, $J = 1.7$, 11.7 Hz, 1H), 3.85 (dd, $J = 3.9$, 11.7 Hz, 1H), 3.82 (m, 1H), 3.35 (m, 1H), 3.22 (m, 1H), 2.71 (dd, $J = 9.9$, 14.4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, CD_3OD): $\delta = 151.3$ (CH), 136.3 (C), 135.7 (C), 133.3 (C), 130.1 (CH), 128.4 (CH), 127.9 (CH), 127.1 (CH), 126.8 (CH), 126.5 (CH), 125.1 (CH), 124.8 (CH), 101.3 (CH), 86.3 (CH), 85.9 (CH), 75.2 (CH), 62.3 (CH_2), 53.1 (CH), 31.7 (CH_2) ppm; TOF-MS-ES$^+$ *m/z* (%) 391.0 ($[M^+H]^+$, 100); 781.2 ($[2 \times M^+H]^+$, 46); HRMS calcd. $C_{22}H_{22}N_4O_3$ 391.1770, found: 391.1778.

Synthesis of 2′-(1-naphthylmethyl)-5′-monomethoxytrityl-6-*N*-butyryl-7-deazaadenosine: 2′-(1-Naphthylmethyl)-7-deazaadenosine (**5**, 136 mg, 0.35 mmol) was dried by evaporation of added dry pyridine (twice) under reduced pressure. Subsequent removal of solvent was carried out at low pressure for 3 h. After **5** had again been dissolved in dry pyridine (3 mL), trimethylsilylchloride (TMSCl, 0.220 mL, 1.74 mmol, 5 equiv.) was added and the reaction mixture was stirred at room temperature for 1 h. TLC analysis showed complete silylation. Butyric anhydride (0.057 mL, 0.35 mmol, 1 equiv.) was added, whereupon the mixture was stirred at room temperature for 5.5 h. Water (0.3 mL) was then added, and the resulting mixture was stirred overnight. After dilution with dichloromethane, the organic layer was washed with brine and dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel with dichloromethane/acetone (7:3). This yielded the butyryl-protected material (76 mg, 47%) as a pure isomer. In addition, some of the other, minor isomer (19 mg, 12%) was also isolated, together with some dibutyrylated material (23%). $R_f = 0.20$ (dichloromethane/acetone/methanol 80:18:2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.71$ (br d, $J = 9.0$ Hz, 1H; NH), 8.48 (s, 1H), 7.90 (d, $J = 7.7$ Hz, 1H), 7.82 (d, $J = 7.1$ Hz, 1H), 7.67 (d, $J = 8.2$ Hz, 1H), 7.44 – 7.51 (m, 2H), 7.30 (m, 1H), 7.19 (t, $J = 7.6$ Hz, 1H), 7.04 (d, $J = 3.5$ Hz, 1H), 6.79 (d, $J = 6.8$ Hz, 1H), 6.55 (d, $J = 7.4$ Hz, 1H), 4.88 (t, $J = 8.3$ Hz, 1H), 4.06 (d, $J = 10.8$ Hz, 1H), 3.90 (m, 2H), 3.23 (m, 1H), 2.95 (dd, $J = 14.1$, 7.7 Hz, 1H), 2.82 (dd, $J = 14.1$, 7.5 Hz, 1H), 2.52 (t, $J = 7.4$ Hz, 2H), 2.35 (m, 1H; O*H*), 1.80 (sextet, $J = 7.4$ Hz, 2H), 1.04 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 171.3$, 152.4, 150.3, 150.3, 134.3, 133.9, 131.3, 129.0, 127.7, 126.5, 126.4, 125.8, 125.3, 123.2, 104.3, 84.3, 73.2, 60.7, 51.3, 39.3, 30.7, 18.6, 13.7 ppm; TOF-MS-ES$^+$ *m/z* (%) 462.1 ($[M^+H]^+$, 92), 922.3 ($[2 \times M^+H]^+$, 100), 1381.4 ($[3 \times M^+H]^+$, 68), 1842.6 ($[4 \times M^+H]^+$, 15); HRMS calcd. for $C_{26}H_{28}N_4O_4$ 461.2189, found: 461.2187.

The 2′-(1-naphthylmethyl)-6-*N*-butyryl-7-deazaadenosine (72 mg) was then dried under high vacuum overnight. Monomethoxytrityl tetrafluoroborate (84 mg, 0.16 mmol) (prepared according to published procedures)[11] and Li_2CO_3 (32 mg, 0.43 mmol) were dried overnight in a drying pistol under high vacuum. The reagents were mixed in a reaction flask and kept under high vacuum for 2 h. 2,6-Lutidine (2.5 mL, cooled to 0 °C) was added to the cooled reaction mixture. The reaction mixture was stirred at 0 °C for 10 min and then for 2 h at room temperature. The orange colour of the trityl reagent disappeared within 5 min. After dilution with dichloromethane, the organic layer was extracted twice with sat. $NaHCO_3$ solution and dried over Na_2SO_4. After filtration and concentration, the residue was coevaporated twice with toluene to remove residual 2,6-lutidine. The crude product was purified by column chromatography with dichloromethane/methanol (98:2) containing 0.1% Et_3N. The desired product was isolated in 93% yield (109 mg, 0.15 mmol). $R_f = 0.30$ (acetone/dichloromethane 6:94); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.62$ (br, 1H), 8.44 (s, 1H), 7.92 (d, $J = 8.2$ Hz, 1H), 7.81 (d, $J = 7.6$ Hz, 1H), 7.67 (d, $J = 8.2$ Hz, 1H), 7.56 (d, $J = 3.5$ Hz, 1H), 7.41 – 7.52 (m, 6H), 7.40 (m, 4H), 7.28 (m, 2H), 7.20 (t, $J = 7.6$ Hz, 1H), 7.06 (d, $J = 3.7$ Hz, 1H), 6.83 – 6.88 (m, 4H), 4.53 (t, $J = 8.2$ Hz, 1H), 3.94 (m, 1H), 3.82 (s, 3H), 3.53 – 3.60 (m, 2H), 3.17 (quint, $J = 8.4$ Hz, 1H), 2.96 (dd, $J = 14.0$, 6.6 Hz, 1H), 2.75 (dd, $J = 13.8$, 9.0 Hz, 1H), 2.55 (t, $J = 7.4$ Hz, 2H), 1.84 (sextet, $J = 7.4$ Hz, 2H), 1.07 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.1$, 153.1, 150.7, 147.6, 144.5, 135.7, 134.8, 134.3, 131.8, 130.9, 129.3, 128.9, 128.4, 127.9, 127.5, 126.8, 126.7, 126.2, 125.6, 125.3, 123.7, 113.6, 104.8, 87.5, 85.3, 83.1, 75.5, 63.4, 55.7, 51.4, 39.7, 31.0, 19.1, 14.1 ppm; TOF-MS-ES$^+$ *m/z* (%) 733.34 ($[M^+H]^+$, 100); HRMS; calcd. for $C_{46}H_{44}N_4O_5$ 733.3390, found: 733.3398; elemental analysis calcd. (%) for $C_{46}H_{44}N_4O_5$: C 75.39, H 6.05, N 7.64; found C 75.20, H 6.12, N 7.71.

Synthesis of 2′-(1-naphthylmethyl)-5′-monomethoxytrityl-6-*N*-butyryl-7-deaza-adenosine-3′-H-phosphonate (6): Imidazole (78 mg, 1.144 mmol, 10.75 equiv.) was dissolved in dry dichloromethane (3 mL) and the solution was cooled to −10 °C. PCl_3 (0.032 mL, 0.371 mmol, 3.5 equiv.) and Et_3N (0.166 mL, 1.193 mmol, 11.25 equiv.)

were added with vigorous stirring. The resulting mixture was stirred at $-10\,^{\circ}C$ for 30 min and was then cooled to $-78\,^{\circ}C$. A solution of 2′-(naphthylmethyl)-5′-monomethoxytrityl-6-*N*-butyryl-7-deazaadenosine (78 mg, 0.106 mmol) in dry dichloromethane (1.5 mL) was added over a period of 30 min, after which stirring was continued at $-78\,^{\circ}C$. After 1 h the reaction mixture was quenched at $-78\,^{\circ}C$ with 2 mL of 2.0 M triethylammonium bicarbonate (pH 7.5). The reaction mixture was extracted with dichloromethane. The organic phase was dried over Na_2SO_4. Purification by column chromatography with dichloromethane/methanol (95:5, 0.1 % Et_3N) yielded the H-phosphonate as a pure crystalline product (93 mg, 98 %). $R_f = 0.41$ (methanol/dichloromethane 12:88); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (s, 1 H), 7.78 (m, 1 H), 7.74 (d, $J = 8.3$ Hz, 1 H), 7.69 (d, $J = 7.9$ Hz, 1 H), 7.52 (s, 0.5 H), 7.50 (m, 1 H), 7.44 – 7.46 (m, 5 H), 7.32 (m, 3 H), 7.15 – 7.26 (m, 6 H), 7.05 (m, 2 H), 6.97 (d, $J = 3.7$ Hz, 1 H), 6.94 (t, $J = 7.6$ Hz, 1 H), 6.75 (d, $J = 8.7$ Hz, 2 H), 6.61 (d, $J = 7.2$ Hz, 1 H), 6.41 (d, $J = 6.9$ Hz, 1 H), 5.95 (s, 0.5 H), 5.06 (dd, $J = 7.9$, 18.1 Hz, 1 H), 4.19 (m, 1 H), 3.75 (s, 3 H), 3.60 (m, 1 H), 3.51 (m, 1 H), 3.48 (m, 1 H), 3.43 (m, 1 H), 3.03 (q, $J = 7.3$ Hz, 6 H), 2.66 (dd, $J = 9.5$, 14.3 Hz, 1 H), 2.55 (t, $J = 7.3$ Hz, 2 H), 1.80 (m, 2 H), 1.33 (t, $J = 7.3$ Hz, 9 H), 1.03 (t, $J = 7.3$ Hz, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.9$, 158.8, 153.0, 150.4, 150.2, 144.6, 144.5, 135.7, 134.6, 134.0, 131.8, 130.9, 129.0, 129.0, 128.1, 127.3, 126.8, 126.1, 125.6, 125.5, 125.4, 123.8, 113.4, 108.5, 105.0, 87.1, 84.6, 82,7, 82.69, 76.6, 63.4, 55.6, 50.5, 46.1, 39.6, 30.2, 19.1, 14.1, 9.0 ppm; ^{31}P NMR (161 MHz, decoupled, $CDCl_3$): $\delta = 3.15$(s) ppm; ^{31}P NMR (161 MHz, $CDCl_3$): $\delta = 3.77$ (d, $J = 630$ Hz) ppm; TOF-MS-ES$^+$ *m/z* (%): 797.1 ($[M^{\cdot} - Et_3N]^+$, 100), 898.26 ($[M^{\cdot}]^+$, 10), 999.36 ($[M^{\cdot} + Et_3N]^+$, 10); HRMS calcd. for $C_{46}H_{45}N_4O_7P$ 797.3104, found: 797.3098.

Preparation of modified solid support

Synthesis of succinic acid mono-[2′-(1-naphthylmethyl)-5′-monomethoxytrityl-6-*N*-butyryl-7-deaza-adenosin-3′-yl] ester: 2′-(1-Naphthylmethyl)-5′-monomethoxytrityl-6-*N*-butyryl-7-deazaadenosine (95 mg, 0.130 mmol) was dissolved in dry dichloromethane (1.5 mL) and the solution was cooled to $-78\,^{\circ}C$. Succinic anhydride (16 mg, 0.156 mmol, 1.2 equiv.) and *N*,*N*-dimethylaminopyridine (24 mg, 0.195 mmol, 1.5 equiv.) were added, and the reaction mixture was stirred at RT for 5 h. Dilution with dichloromethane was followed by extraction with cold 10 % citric acid (2 ×), water and brine. The aqueous layer was back-extracted with dichloromethane and the combined organic phases were dried over Na_2SO_4. Purification by column chromatography with dichloromethane/methanol (dichloromethane/methanol; 97:3 → 92:8; 0.1 % Et_3N) yielded the desired succinate in 88 % yield (95 mg, 0.1 mmol). $R_f = 0.18$ (methanol/dichloromethane 5:95); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 10.1 - 11$ (br, 1 H; COO*H*), 9.73 (br s, 1 H), 8.32 (s, 1 H), 7.76 (dd, $J = 12.4$, 8.3 Hz, 1 H), 7.64 (d, $J = 3.5$ Hz, 1 H), 7.58 (d, $J = 8.3$ Hz, 1 H), 7.49 – 7.50 (m, 4 H), 7.37 – 7.45 (m, 4 H), 7.27 – 7.31 (m, 4 H), 7.21 – 7.23 (m, 2 H), 7.12 (t, $J = 7.6$ Hz, 1 H), 7.03 (d, $J = 3.6$ Hz, 1 H), 6.82 (d, $J = 8.8$ Hz, 2 H), 6.78 (t, $J = 7.3$ Hz, 2 H), 5.83 (t, $J = 7.5$ Hz, 1 H), 4.07 (m, 1 H), 3.78 (s, 3 H), 3.51 (m, 2 H), 3.48 (m, 1 H), 1.69 (dd, $J = 14.5$, 6.8 Hz, 1 H), 2.81 (dd, $J = 14.5$, 8.7 Hz, 1 H), 2.51 (t, $J = 7.4$ Hz, 2 H), 2.42 (t, $J = 6,9$ Hz, 2 H), 2.25 – 2.32 (m, 1 H), 2.14 – 2.20 (m, 1 H), 1.79 (sextet, $J = 7.4$ Hz, 2 H), 1.03 (t, $J = 7.4$ Hz, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 177.6$, 172.4, 172.2, 159.0, 152.8, 150.5, 149.8, 144.5, 144.3, 135.6, 134.2, 134.1, 131.7, 130.9, 129.1, 129.0, 128.9, 128.2, 127.6, 127.4, 126.9, 126.5, 125.9, 125.5, 123.7, 113.5, 108.8, 105.3, 87.4, 85.3, 81.9, 75.5, 63.0, 55.6, 49.0, 39.4, 30.7, 30.4, 30.0, 19.1, 14.1 ppm; TOF-MS-ES$^+$ *m/z* (%): 834.06 ($[M^{\cdot} + H]^+$, 100); HRMS calcd. for $C_{50}H_{48}N_4O_8$ 833.3550, found 833.3567; elemental analysis calcd (%) for $C_{50}H_{48}N_4O_8$: C 72.10, H 5.81, N 6.73; found: C 71.89, H 5.73, N 6.63.

Preparation of succinic acid mono-[2′-(1-naphthylmethyl)-5′-monomethoxytrityl-6-*N*-butyryl-7-deaza-adenosine-3′-yl] ester modified polystyrene: Aminomethylpolystyrene (32.9 μmol g^{-1}, 150 mg) was washed with DMF and treated for 10 min with 2 mL DMF/piperidine (4:1). After washes with DMF (2 × 1 mL), dichloromethane (3 × 1 mL), acetonitrile (3 × 1 mL) and diethyl ether (3 × 1 mL), the resin was dried under high vacuum overnight. Succinate **14** (95 mg, 0.1 mmol) was coevaporated with dry pyridine (3 × 2 mL) and dissolved in 1 mL of dry dichloromethane. *N*,*N*′-dicyclohexylcarbodiimide (11 mg, 0.053 mmol) was added, and the solution was stirred for 30 min at RT and for 1 h at 4 °C. The white precipitate was removed by filtration through a syringe filter, and the filtrate was concentrated, redissolved in 0.5 mL DMF/pyridine (4:1) and placed on the support. The suspension was shaken at RT for 24 h. After filtration, the support was washed with DMF (5 × 5 mL), methanol (5 × 5 mL) and acetonitrile (5 × 5 mL). The resin was then capped by treatment with 1 mL of acetic anhydride/pyridine (1:1) containing 1 % of Et_3N, subsequently washed with pyridine (3 × 1 mL), acetonitrile (5 × 1 mL) and dry dichloromethane (2 × 1 mL) and dried under high vacuum. A small sample was treated with 1 % trifluoroacetic acid in dichloroethane and the loading was determined to be 31.6 μmol g^{-1} by UV quantification at 480 nm.

Synthesis and purification of modified oligonucleotides: The solid-phase synthesis of oligonucleotides including the use of labile *N*-protecting groups in the H-phosphonate approach has been reported previously.[13] Oligonucleotides were synthesized by use of H-phosphonate building blocks on a DNA synthesizer (Pharmacia Gene Assembler). The average coupling efficiency was 98.9 %, including for the modified building blocks. Synthesized oligonucleotides were cleaved from the support and deprotected with 32 % NH_4OH for 12 – 15 h at 55 °C. The ammonia solutions were lyophilised and the residue was dissolved in 30 % CH_3CN and filtered through a disposable syringe filter (Millex GV13, 0.22 μm). The oligonucleotides were analysed and purified on ion-exchange HPLC. Dionex NucleoPac PA-100 (4.250) was used for analytical runs and Dionex NucleoPac PA-100 (9.250) for preparative runs, with a linear gradient of 0 – 90 mM $LiClO_4$ in 20 mM sodium acetate (pH 6.5), 30 % CH_3CN. The collected fractions were lyophilised and then purified on reversed-phase HPLC Hypersil ODS (25 · 4.6 mm) for analytical runs and Hypersil ODS (25 · 10 mm) for preparative runs. A linear gradient of 0 – 25 % CH_3CN in 50 mM triethylammonium acetate (pH 6.5) was used. The oligonucleotides were collected, lyophilised, redissolved in water and lyophilised before quantification. The integrity of the purified oligonucleotides was verified by TOF-ES mass spectral analysis recording in negative mode.

Determination of duplex stability: Oligonucleotides (commercial and synthesized) were quantified by their calculated extinction coefficients, assuming only nearest-neighbour interactions among the bases in the sequence.[15] For the 2′-(1-naphthylmethyl)-tubercidin derivative building block the extinction coefficient was approximated by summation of the ε_{260} value for 7-deazaadenosine[16] and the ε_{260} value for naphthalene.[17] Duplex stability was determined by published procedures.[18] Absorbance versus temperature profiles were measured on a Cary 3E UV/VIS spectrophotometer (Varian, Australia) containing a Cary thermoelectrical temperature-controlled 6 × 6 sample holder and interfaced to an IBM-compatible PC computer. All solutions were prepared with a buffer containing 10 mM sodium phosphate, 100 mM NaCl and 0.1 mM EDTA, adjusted to pH 7.0. The mixtures (containing a 1:1 strand ratio of oligonucleotides, 4 μM total strand concentration) were heated to 90 °C and allowed to cool to 1 °C. After 5 min at that temperature, denaturation was monitored at 260 nm as a function of temperature, increasing from 1 to 80 °C at a ramp rate of 0.2 °C min^{-1}. Melting temperatures (T_ms) below 20 °C were obtained as the maxima of the first derivatives of the absorbance versus temperature functions and have errors of ± 0.3 °C (unless otherwise specified). T_m values above

20 °C were calculated by a two-state model using the hyperchromicity method from the CARY UV-Win Software; errors in these cases were in the range of ±0.3 °C (unless otherwise specified).

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