Oligonucleotides Composed of 2′-Deoxy-1′,5′-anhydro-D-mannitol Nucleosides with a Purine Base Moiety

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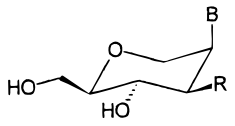
2′-Deoxy-D-mannitol nucleosides with a purine base moiety have been conveniently synthesized starting from 1,5-anhydro-4,6-*O*-benzylidene-D-glucitol. The 3-OH function of 1,5-anhydro-4,6-*O*-benzylidene-D-glucitol was selectively protected with *tert*-butyldimethylsilyl group, and the 2′-OH function was subsequently converted to the corresponding *O*-triflate derivative for the introduction of the nucleobase moieties. These nucleoside derivatives were transformed to 1,5-anhydro-4-*O*-(*P*-(2-cyanoethyl)-*P*-(*N*,*N*-diisopropylamino)phosphinyl)-2-deoxy-6-*O*-monomethoxytrityl-3-*O*-(*tert*-butyldimethylsilyl)-D-mannitol with either a 2-(N^6-benzoyladenin-9-yl) or a 2-(N^2-isobutyrylguanin-9-yl) substituent as the building blocks for oligonucleotide synthesis. The corresponding fully modified oligonucleotides afford considerably less stable duplexes with RNA as compared to the 3-deoxy hexitol nucleic acid analogues described previously. The reason for the lower stability was investigated using molecular modeling. MD simulations of single strand MNA(GCGTAGCG) and MNA(GCGTAGCG) complexed with RNA(CGCAUCGC) in aqueous solution were performed by use of AMBER 4.1 with the particle mesh Ewald (PME) method for the treatment of long-range electrostatic interactions. Frequent hydrogen bonds between the 3′-hydroxyl and the 6′-O of the phosphate backbone of the following base changed the conformation of the single strand as well as the MNA:RNA complex. The MNA:RNA backbone widens up and shows partial unwinding and disruption of base pair hydrogen bonds consistent with their low hybridization potential.

Introduction

An intriguing finding in the field of sugar-modified oligonucleotides is the observation that oligonucleotides composed of 1,5-anhydro-2,3-dideoxy-D-*arabino*-hexitol nucleosides[1] form very stable duplexes with natural nucleic acids.[1−4] The hexitol nucleic acid (HNA) obeys the same binding rules as found in nature and with even higher specificity.[4] This is due to the positioning of the base moiety in the 2′-position, instead of the anomeric position, allowing the oligomer to fold in a helix-like structure with the same geometry as found in the A-form of dsRNA.[5] The anhydrohexitol moiety of **1** (Figure 1) can be considered as a mimic of 2-deoxy-*ribo*-furanose frozen in its 2′-exo/3′-endo conformation. Studies of the duplex stability of HNA with DNA and RNA[4] reveal that the former duplexes (HNA−RNA) are more stable than the latter associations (HNA−DNA). This may be due to a better complementarity of HNA with RNA (in an A-form duplex) and/or to different degrees of hydration. To study further this structure−function relationship, we synthesized 2-deoxy-1,5-anhydro-D-mannitol nucleosides (**2a**,**b**) (Figure 1) and incorporated them into oligonucleotides (mannitol nucleic acids, MNA). These nucleoside analogues have a supplementary hydroxyl group in the 3′-β position, which we expect not to interfere with the conformational preference of the nucleoside. The synthesis of 2-deoxy-1,5-anhydro-D-mannitol nucleosides with a pyrimidine base moiety was described previously, starting from the D-altritol analogues.[6] The configuration in the 3′-position was inverted, making use of a pyrimidine O^2 neighboring group participation reaction. This reaction cannot be used for synthesis of the purine derivatives. Moreover, the synthetic strategy should take into account an easy way to selectively introduce a 3′-hydroxyl protecting group which would facilitate oligomerization of the new hexitol nucleoside. Therefore, we started synthesis from 1,5-anhydro-4,6-*O*-benzylidene-D-glucitol[7] and introduced the 3′-*O*-protecting group before attachment of the base moiety. The structure of the final compounds was proven by chemical and spectroscopical means before their conversion to phosphoramidites and their use for oligonucleotide synthesis. The influence of



1 R = H; B = adenin-9-yl, cytosin-1-yl, thymin-1-yl, guanin-9-yl
2a R = OH; B = adenin-9-yl
2b R = OH; B = guanin-9-yl

Figure 1.

(1) Herdewijn, P.; Dosboszewski, B.; De Winter, H.; De Ranter, C.; Verheggen, I.; Augustijns, K.; Hendrix, C.; Van Aerschot, A. *Am. Chem. Soc. Symp. Ser.* **1994**, *580*, 80−99.
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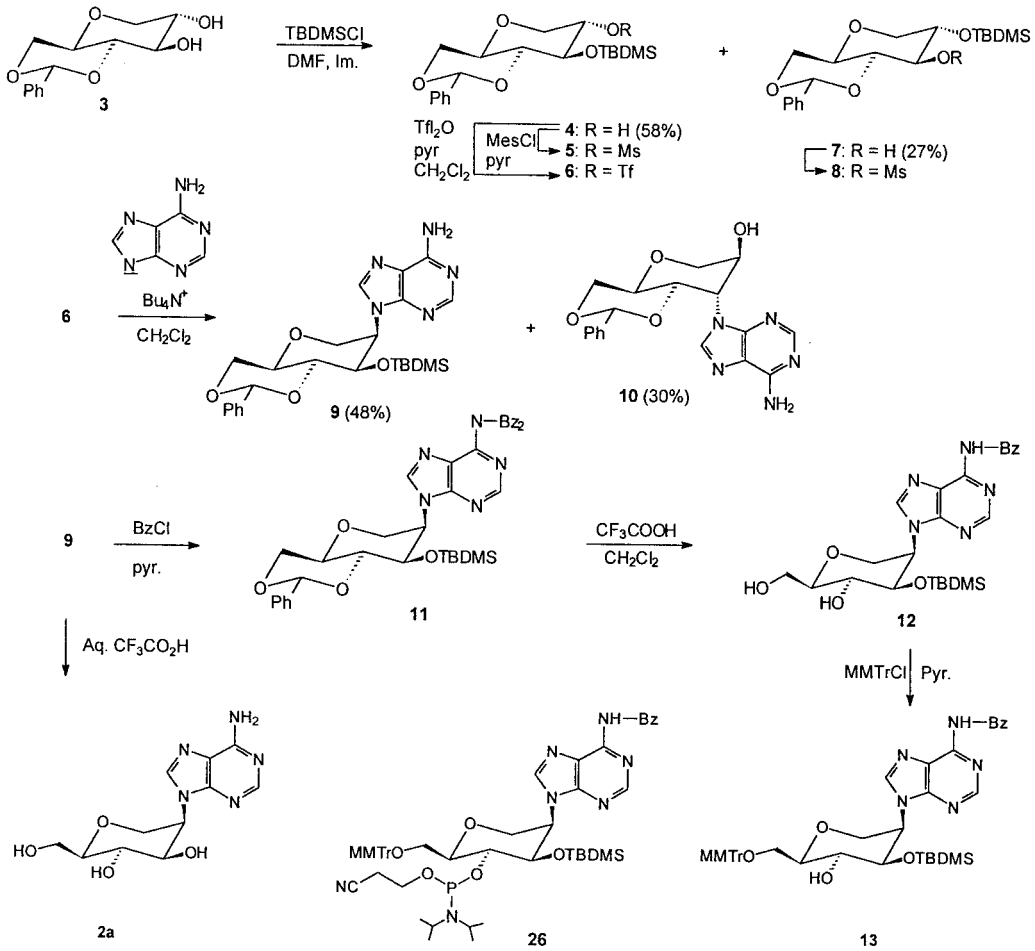
Scheme 1



the 3′-hydroxyl group on the conformation of the oligonucleotide was investigated through molecular modeling and free molecular dynamics simulations. The modeling of the complex of an octamer of mannitol nucleotides 6′-GCGTAGCG-4′ complexes with RNA (CGCAUCGC) was done by restraining the nucleobase positions on the positions of the nucleobases in an ideal A-DNA:RNA helix. Then free molecular dynamics simulations in aqueous solution of the duplex MNA:RNA in an A-RNA like conformation as well as of the single strand MNA, departing from the same conformation, were performed and the results compared with the conformation of natural A-RNA.

Results and Discussions

1,5-Anhydro-4,6-*O*-benzylidene-D-glucitol (**3**) was prepared from commercially available bromo acetyl-α-D-glucose, in three steps.[7] Treatment of **3** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in pyridine at room temperature gave **4** (31%) and **7** (40%) (Scheme 1). The TBDMS group preferentially approaches from the α-face leading to **7** as a major product. However, as we need compound **4** to be the major product, other reaction circumstances were investigated. When **3** was treated with TBDMSCl in the presence of imidazole in DMF[8] at room temperature, **4** (58%) was formed as a major product along with **7** (27%) in a combined yield of 85%. To introduce the nucleobase, **4** was converted to the corresponding mesyl derivative (**5**) upon treatment with mesyl chloride in pyridine. When **5** was treated with the sodium salt of adenine in DMF at 110 °C, compounds **9** (20%) and **10** (30%) were obtained. This reaction was found to be not very reproducible (yields range from 10 to 20% for **9** and 30−50% for **10**). Therefore, we moved to the use of the triflate leaving group for the preparation of **9**.[9,10] In this approach, **4** was converted to the corresponding triflate derivative **6** upon treatment with trifluoromethanesulfonic anhydride in the presence of pyridine in CH_2Cl_2 at −5 °C. After standard workup **6** was reacted with tetrabutylammonium salt of adenine in CH_2Cl_2 to give **9** in 48% yield. Treatment of **9** with benzoyl chloride in pyridine at room temperature afforded **11**, which was treated with CF_3COOH in dry CH_2Cl_2 at room temperature to give **12** (85%). Compound **12** was treated with MMTrCl in pyridine to give **13** (74%). Complete deprotection of **9** with aqueous CF_3COOH gave **2a** in 52% yield.

Likewise, the triflate approach was used for the synthesis of the guanine analogue (Scheme 2). This approach gave reproducible yields both for **9** and **14**. Compound **6** was treated with tetrabutylammonium salt of 6-iodo-2-aminopurine[9,10] in CH_2Cl_2 to give **14** (70%).

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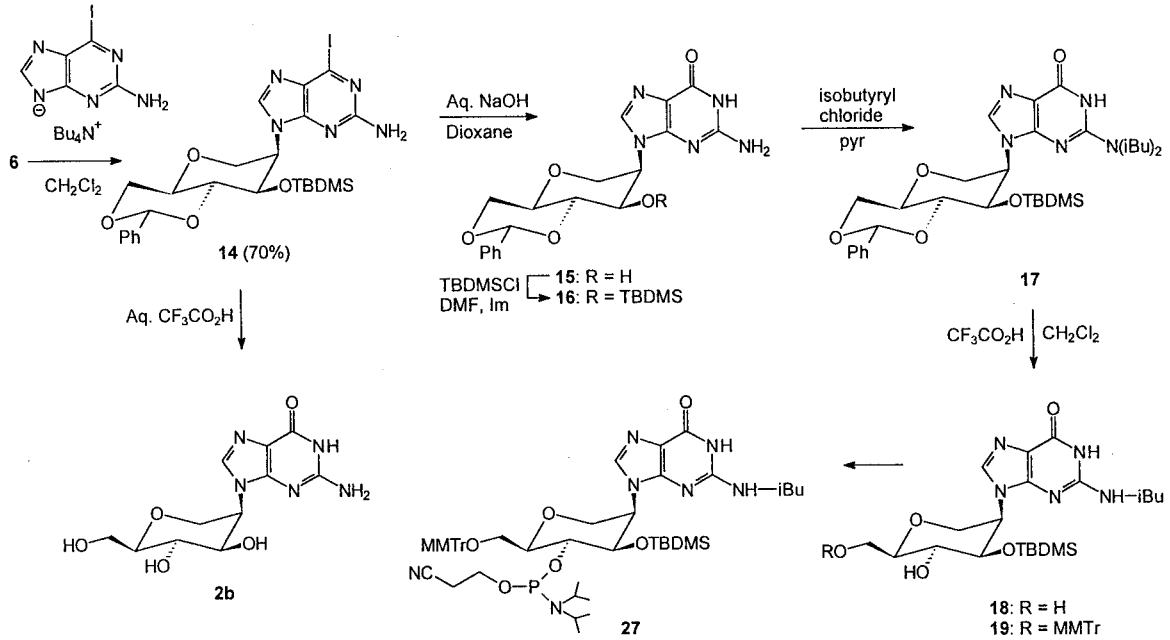
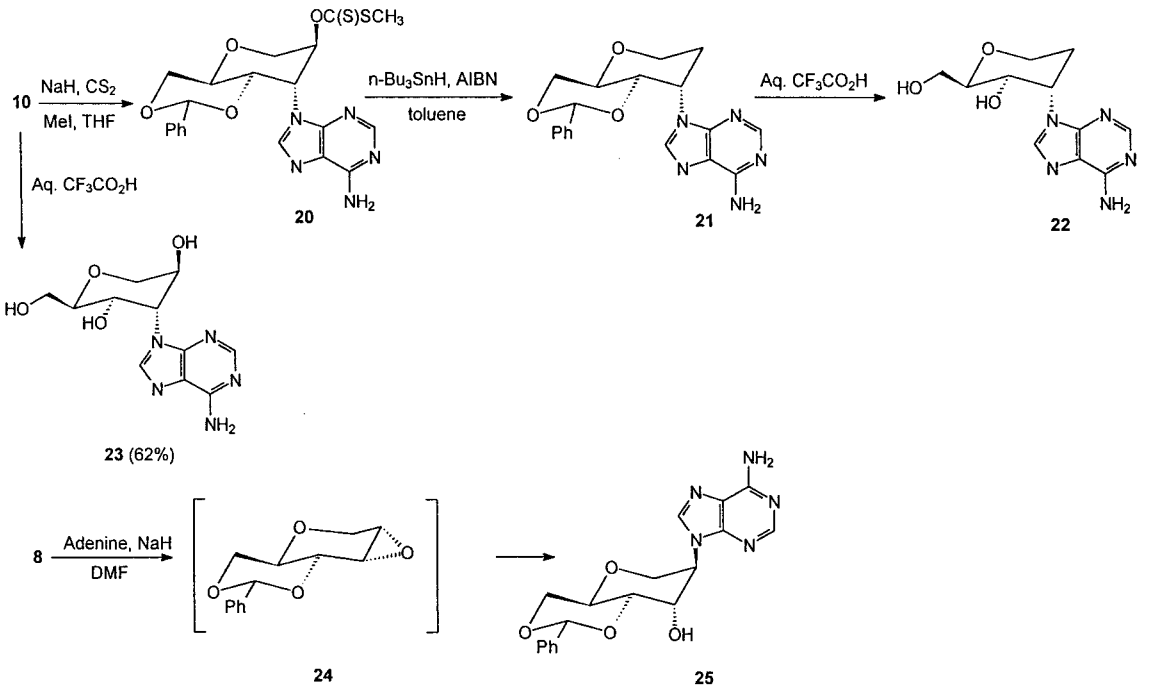
Scheme 2



Scheme 3



Compound **14** was treated with aqueous NaOH in dioxane at 55 °C to give **15** (96%) which can easily be precipitated out from the reaction mixture. These alkaline reaction conditions also removed the 3′-*O*-TBDMS group. Therefore, it was necessary to reprotect the 3′-OH group by treating **15** with TBDMSCl in the presence of imidazole in DMF. Compound **16** (70%) was obtained by simple filtration of the reaction mixture. Treatment of **16** with isobutyryl chloride in pyridine followed by removal of the benzylidene protecting group using CF_3COOH in CH_2Cl_2 gave **18** in 80% yield. Compound **18**, likewise, was easily crystallized from $MeOH/CH_2Cl_2$ in pure form. Finally, **18** was treated with MMTrCl in pyridine to give **19** (75%) after crystallization. It is worthwhile to mention that purification of guanine intermediates is very easy because all intermediates could be precipitated out or crystallized in pure form. The fully deprotected nucleoside, **2b**, was obtained from **14** in 94% yield using aqueous CF_3COOH. To assign the configuration of **4** and **7** together with the silylation site in an unambiguous chemical way, we further transformed **10** to **22** and **23** and also synthesized **25** from **8** (Scheme 3). Thus, **10** was treated with CS_2 and MeI in the presence of NaH in THF to give intermediate **20** which was converted to **21** upon treatment with n-Bu_3-SnH in the presence of AIBN in toluene. Finally, **21** was treated with aqueous CF_3COOH at room temperature to give **22**. The benzylidene protecting group in **10** was removed upon treatment with aqueous CF_3COOH to give **23**. The introduction of the *tert*-butyldimethylsilyl protecting group in the 2-position of **7** and the 3-position of **4** was proven by chemical means.

Table 1. ^{1}H NMR Chemical Shifts (δ) and Coupling Constants (J) of 2a and 2b

		2a		2b	
proton	coupled to proton	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
1′ax (dd)		4.164		4.118	
	1′eq		−13.2		−13.2
	2′		2.4		2.4
1′eq (dd)		4.315		4.282	
	2′		1.0		1.5
2′ (m)		5.093		4.923	
	3′		5.1		5.3
3′ (dd)		4.187		4.130	
	4′		9.8		9.8
4′ (t)		3.694		3.690	
	5′		9.8		9.8
5′ (dddd)		3.588		3.548	
	6′A		5.4		5.4
	6′B		2.0		2.4
6′A (dd)		3.920		3.906	
	6′B		−12.2		−12.7
6′B (dd)		4.018		4.005	
A2 (s)		8.534		–	
A8 (s)		8.243		–	
G8 (s)		–		8.208	

Compound **4** was converted to the corresponding mesyl derivative **5** which was treated with the sodium salt of adenine in DMF. This reaction yields a mixture of **9** and **10** (compound **10** cannot be formed from the 2-*O*-silylated compound **8**). Compound **10** must be formed from the (β)2,3-epoxide intermediate. Deoxygenation of the 2-hydroxyl group of **10**, followed by removal of the benzylidene protecting group gave compound **22**. This compound shows a clearly distinct NMR spectrum from the previously synthesized α- and β-analogues of 2-substituted 1,5-anhydro-2,3-dideoxy-D-*erythro*-hexitol nucleosides[7,11] which demonstrate that opening of the β-epoxide occurred by nucleophilic attack at the 3-position of the carbohydrate moiety. Compound **7** was converted to the corresponding mesyl derivative **8** which was treated with adenine in the presence of NaH in DMF to give **25** via the epoxide **24**. Compound **25** is a known product.[12] These chemical transformations unambiguously prove the location of the silyl protecting group of the starting materials **4** and **7**.

The conformation of the final compounds **2a** and **2b** were deduced from ^{1}H NMR analysis. Table 1 shows the chemical shifts and coupling constant for all protons. The large coupling constant obtained for $J_{3',4'}$ and $J_{4',5'}$ together with the lower values obtained for $J_{2',3'}$ and $J_{1'eq,2'}$, $J_{1'ax,2'}$ may be indicative for the axial position of the purine base moiety as was also observed for the 1,5-anhydro-2,3-dideoxy-D-*arabino* hexitol nucleosides.[7,13]

The monomethoxytritylated precursors **13** and **19** were phosphitylated under standard conditions[14] to afford the phosphoramidites **26** and **27** in 86% and 91% yield, respectively. ^{13}C NMR analysis displayed the signals characteristic for cyanoethyl phosphoramidites, but complete assignment proved difficult. MS confirmed the expected structure.

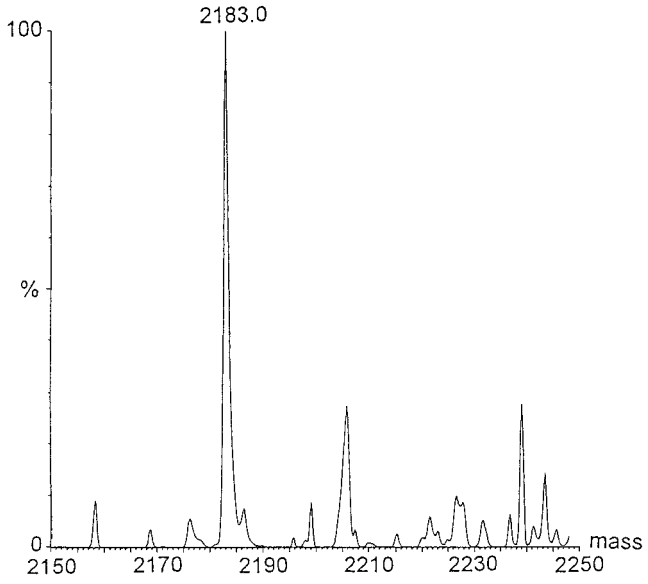


Figure 2. Deconvoluted electrospray mass spectrum of the mannitol nucleic acid 6′-AGGAGA-4′-propanediol.

The obtained building blocks were used for oligonucleotide assembly at a 1 μmol scale following the standard protocol (ABI 392) except for a prolonged coupling time of 10 min to ensure adequate coupling yields (95% according to trityl analysis) and a prolonged acid treatment (80 s) to fully deprotect the monomethoxytrityl group. Two hexamers and a dodecamer were assembled on a 1,3-propanediol functionalized support to avoid synthesis of the modified supports.[1−3] Following deprotection with ammonia and lyophylization, the residue was treated with a 1 M TBAF solution for 22 h at rt as for standard RNA synthesis procedures. Purification by ion exchange chromatography yielded the desired hexamers in moderate yield. Both displayed only a single sharp peak on RP-HPLC analysis (chromatogram not shown). Further treatment of the isolated peak with 1 M TBAF, however, yielded a pattern of breakdown products. Electrospray analysis of the obtained hexamers after conversion to their triethylammonium salts[15] went sluggishly, but the correct mass was determined after smoothing and deconvolution using the maximum entropy algorithm (Figure 2).

Recently it was claimed that deprotection of tBDMSi groups of oligonucleotides was improved using triethylamine trihydrofluoride, maximizing the yield of synthetic RNA.[16] Therefore, the dodecamer after ammonia deprotection was divided in two parts and treated with a 1 M TBAF solution or TEA, 3HF, respectively. The latter method, however, only yielded fully degraded product, while TBAF deprotection afforded a product mixture displaying a very broad peak on ion exchange chromatography at the expected position. However, isolation and RP-HPLC analysis of the obtained material displayed several peaks suggesting incomplete deprotection of the oligomer. The present methodology therefore did not allow isolation of pure dodecamers. Only oligonucleotides of short length (i.e. hexamers) could be obtained.

The obtained hexamers (mannitol nucleic acids, MNA) were analyzed for their hybridization properties via

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(14) *Methods in Molecular Biology*; Agrawal, S., Ed.; Humana Press: New Jersey, 1993; Vol. 20.
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(16) Westman, E.; Strömberg, R. *Nucleic Acid Res.* **1994**, *22*, 2430−2431.

Table 2: $T_{m\ Values}$[a] of Hexameric Mannitol Oligonucleotide Analogues (MNA) with Either DNA, RNA, or HNA Complementary Sequences in Comparison with the Hybridization Properties of Arabino Hexitol Oligonucleotides (HNA)

MNA	0.1 M NaCl	1 M NaCl
1. 6′-AGGAGA−4′/RNA	–	14
2. 6′-AGGAGA−4′/DNA	–	*b*
3. 6′-AGGAGA−4′/HNA	18	23
4. 6′-GAGAGA−4′/RNA	–	14
5. 6′-GAGAGA−4′/DNA	–	*b*
6. 6′-GAGAGA−4′/HNA	15	20
HNA		
7. 6′-AGGAGA−4′/RNA	45[c]	55
8. 6′-AGGAGA−4′/DNA	*b*	31[c]
9. 6′-AGGAGA−4′/HNA	55	69
10. 6′-GAGAGA−4′/RNA	43[c]	53
11. 6′-GAGAGA−4′/DNA	*b*	15[c]
12. 6′-GAGAGA−4′/HNA	54	69

[a] T_m (°C) were determined in a buffer containing 0.02 M KH_2PO_4, pH 7.5, 0.1 mM EDTA with 0.1 or 1 M NaCl and 4 μM of each oligonucleotide (only 2 μM for line 1). [b] No hypochromicity detectable. [c] Data from ref 3.

melting experiments, which proved disappointing. Only very weak hybridization was noticed for the mannitol oligonucleotide analogues with complementary RNA as well as with HNA sequences (Table 2, lines 1−6). No hypochromicity was detected with DNA as the complementary partner while the hexitol oligonucleotides (HNA) afford very good hybridization with RNA and display exceptionally strong self-pairing with a complementary HNA strand (lines 7−12). The very large discrepancy in stability for duplexes containing the mannitol hexamers versus the duplexes with arabinohexitol oligonucleotides (HNA) is shown in Figure 3 (with a RNA sequence and with a HNA sequence as the complementary partners). The very broad transitions for duplexes with MNA as a partner do not allow accurate T_m determination and suggest multiple transitions during melting. The presence of the extra hydroxyl group at the 3′β-position in the MNA analogues has a deleterious effect on their hybridization potential.

The free molecular dynamics simulations of the MNA:RNA duplex as well as the MNA single strand showed a strong influence of the axial 3′-hydroxyl group on the conformation, which led to a considerable amount of instability in both simulations. Both simulations exhibited large conformational changes which let them drift away from their A-RNA like starting conformations (Figure 4). The single strand MNA partially unwound during 300 ps of MD forming a kink at the position of MNA (T4A5) in the center of the strand. The structure is stabilized by frequent hydrogen bonds between the 3′ hydroxy and the 6′O in the phosphate backbone of the following nucleotide (Figure 4). Under the same conditions, single strand RNA totally unwinds, without any remaining intramolecular hydrogen bonds or base stacking interactions. During 1 ns of molecular dynamics simulation the complex of MNA:RNA showed a similar behavior as the single strand MNA (Figure 4). The frequent formation of 3′ hydroxy−6′O hydrogen bonds is the driving cause of the conformational change. The conformational transition consists of a widening of the nucleotide backbone with an accompanied partial unwinding of the MNA:RNA strands. The widening is most pronounced in the last three 4′-terminal base pairs, where the distance between the P atoms of the two

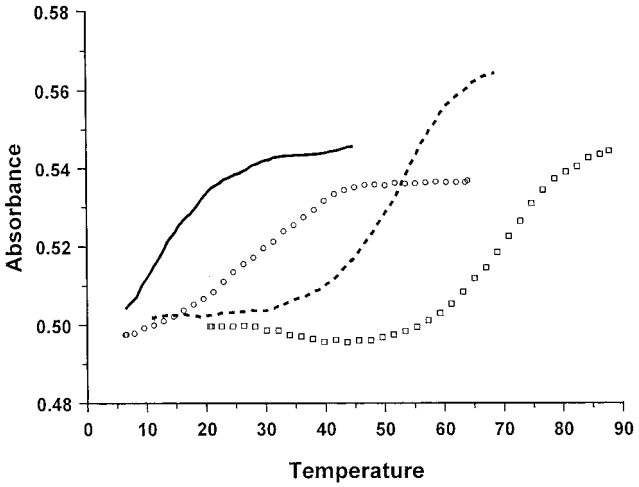

Figure 3. Melting profiles for the hexamer sequence 6′-AGGAGA (NMA and HNA) with the RNA complement 5′-(UCUCCU) and with the HNA sequence 6′-TCTCCT as the complement, at 4 μM in 1 M NaCl buffer (all normalized) (MNA−RNA (—); HNA−RNA (- - -); MNA−HNA (○); HNA−HNA (□)).

strands increased from 17.7 to 18.5 Å. The base pair hydrogen bonds are partially broken during MD simulation, as well as the base stacking interactions are weakened, again most pronounced in the last three 4′-terminal base pairs. The minor groove becomes wider (from 11.0 to 13.8 Å) and less deep as in natural A-RNA (Figure 4). These findings suggest that the MNA is conformational restricted and the more flexible RNA strand has to fit to the preformed MNA conformation, inducing conformational stress. This picture of instability and unwinding and widening of the MNA:RNA strands is consistent with the experimentally determined low hybridization potential of MNA with RNA as well as with MD simulations of natural RNA,[17] HNA:RNA, HNA:DNA[18] and Altritol Nucleic Acids[19] which, also consistent with their experimentally determined hybridization potentials, remain stable during MD simulation. These findings suggest that MD simulation by use of PME may be a useful tool for determining the stability of complexes of modified nucleic acids with RNA or DNA. The lower stability of the MNA:RNA duplex when compared with the HNA:RNA duplex may primarily be due to the presence of an intramolecular hydrogen bond and, consequently, unfavorable entropy and enthalpy for binding.

Experimental Section

Analytical instruments and general methods were described previously.[11] ^{1}H NMR spectra for **2a** and **2b** were recorded at 33 °C in D_2O on a Varian Unity-500 spectrometer (500 MHz for ^{1}H). The instrument was equipped with a 5-mm i.d. PFG probe. The FIDs were acquired in 32 K data points, with a spectral width of 8000 Hz. Data processing involved resolution enhancement by a Gaussian-to-Lorentzian window multiplication and zero filling to 64 K, resulting in a digital resolution of ~0.12 Hz/pt. Chemical shifts are listen in ppm, relative to internal DSS (measured against the residual HDO signal as secondary reference, δ 4.691 ppm at 33 °C). Coupling constant

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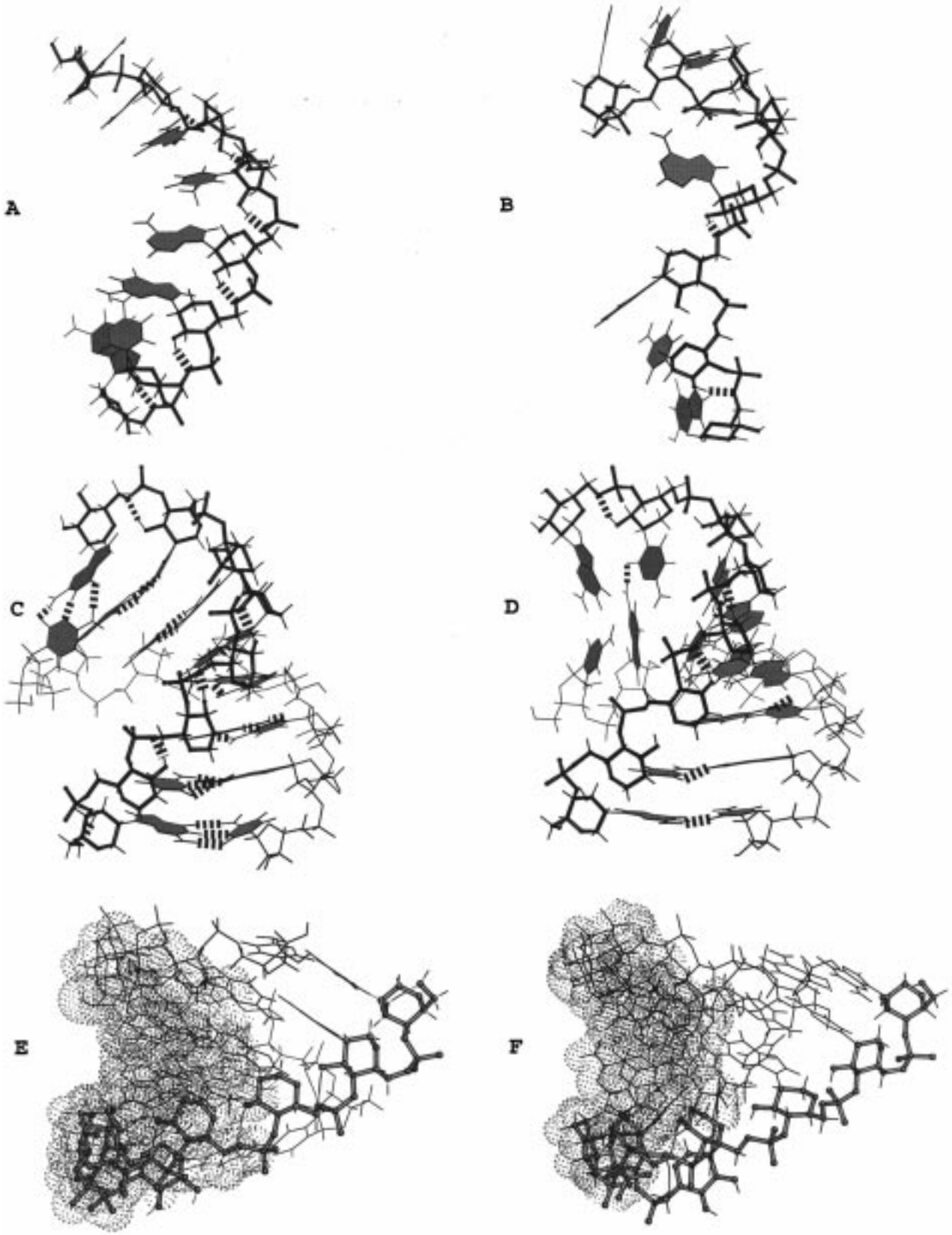

Figure 4. Initial structure (A) and average structure of the last 50 ps of single strand MNA (B) as well as initial structure (C, E) and average structure of the last 100 ps of the MNA:RNA duplex (D, F). The phosphate-sugar backbone of MNA is highlighted as sticks. The place of each base and the hydrogen bonds are indicated (A−D). A part of the solvent accessible surface forming the minor groove is shown with dots (E, F). Figures (A−D) were generated using BobScript,[26,27] and (E, F) by use of RasMol.[28]

values were derived by first-order spectral analysis. UV-melting experiments and mass spectrometric analysis of oligonucleotides were done as described.[15]

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-TBDMS-D-glucitol (4) and 1,5-Anhydro-4,6-*O*-benzylidene-2-*O*-TBDMS-D-glucitol (7). To a solution of **3** (7.0 g, 27.7 mmol) in DMF

(200 mL) was added imidazole (2.08 g, 30.5 mmol), followed by TBDMSCl (4.6 g, 30.6 mmol), and kept at room temperature for 24 h. The reaction mixture was concentrated. The residue was dissolved in CH_2Cl_2 (300 mL). After addition of saturated aqueous $NaHCO_3$ (50 mL), the two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layer was washed with H_2O (25 mL). The organic layer was dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (0−15% EtOAc in hexane) to give **4** (5.9 g, 58%) and **7** (2.8 g, 27%). When pyridine was used both as base and solvent the reactivity was altered, giving **7** as major product. **Compound 4**: ^{1}H NMR (DMSO-d_6) 7.49−7.29 (m, 5H); 5.58 (s, 1H); 5.10 (d, J = 5.8 Hz, 1H); 4.16 (dd, J = 4.3, 10.1 Hz, 1H); 3.78 (dd, J = 5.0, 10.8 Hz, 1H); 3.69−3.13 (m, 6H); 0.86 (s, 9H); 0.5 (s, 3H); 0.1 (s, 3H). ^{13}C NMR (DMSO-d_6) 137.8, 128.8, 128.0, 126.1, 100.6, 81.4, 76.3, 70.9, 70.6, 70.4, 68.0, 25.9, 18.2. HRMS calcd for $C_{19}H_{31}O_5Si_1$ $(M + H)^+$ 367.1940, found 367.1970. **Compound 7**: ^{1}H NMR (DMSO-d_6) 7.49−7.30 (m, 5H); 5.55 (s, 1H); 4.17 (dd, J = 3.4, 10.1 Hz, 1H), 3.77 (dd, J = 5.4, 10.6 Hz, 1H); 3.67−3.16 (m, 6H); 0.88 (s, 9H); 0.1 (s, 6H). ^{13}C NMR (DMSO-d_6) 137.9, 128.9, 128.1, 126.5, 100.8, 81.1, 74.4, 72.2, 71.0, 70.2, 68.0, 25.9, 18.0. HRMS calcd for $C_{19}H_{31}O_5Si_1$ $(M + H)^+$ 367.1940, found 367.1972.

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-TBDMS-2-*O*-mesyl-D-glucitol (5). To a cold (ice−water) solution of **4** (770 mg, 2.10 mmol) in CH_2Cl_2 (20 mL) was added Et_3N (465 μL, 3.34 mmol) followed by MsCl (243 μL, 3.14 mmol), and the reaction mixture was kept at 0 °C for 2 h. The reaction was quenched with ice−water and diluted to 100 mL by addition of CH_2Cl_2. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ (10 mL) and H_2O (5 mL), dried, filtered, and concentrated to give **5** (934 mg, 100%) which was used directly in the next step. ^{1}H NMR (DMSO-d_6) 7.50−7.30 (m, 5H); 5.61 (s, 1H); 4.49 (m, 1H); 4.17 (m, 2H); 3.96 (t, J = 8.5 Hz, 1H); 3.72−3.35 (m, 4H); 3.24 (s, 3H); 0.84 (s, 9H); 0.1 (s, 3H); −0.02 (s, 3H). ^{13}C NMR (DMSO-d_6) 137.4, 129.0, 128.0, 126.3, 101.0, 80.9, 78.5, 72.9, 70.5, 67.7, 66.9, 37.9, 25.7, 18.0. HRMS calcd for $C_{20}H_{33}O_7S_1Si_1$ $(M + H)^+$ 445.1716, found 445.1797.

1,5-Anhydro-2-(adenin-9-yl)-4,6-*O*-benzylidene-2-deoxy-3-*O*-TBDMS-D-mannitol (9) and 1,5-Anhydro-3-(adenin-9-yl)-4,6-*O*-benzylidene-3-deoxy-D-altritol (10). Method A. A mixture of adenine (564 mg, 4.17 mmol) and NaH (80%, 188 mg, 6.27 mmol) in DMF was heated at 100 °C for 30 min. Compound **5** (934 mg, 2.10 mmol) in DMF (2 mL) was added and the reaction mixture kept at 110 °C for 20 h. The solvent was removed. After addition of EtOAc (100 mL) and saturated aqueous $NaHCO_3$ (20 mL), two layers were separated. The aqueous layer was extracted with EtOAc (50 mL), and the combined organic layer was washed with H_2O (20 mL), dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (0−7% MeOH in CH_2Cl_2) to give **9** (200 mg, 20%) and **10** (300 mg, 30%) as a white solid. The yield of **9** varied from 10 to 20%.

Method B. To a cold (ice−NaCl) solution of **4** (1.03 g, 2.81 mmol) in CH_2Cl_2 (30 mL), pyridine (450 μL) was added, followed by slow addition of trifluoromethanesulfonic anhydride (705 μL in 515 μL CH_2Cl_2) and kept at −5 °C for 2 h. The reaction was quenched with ice−water, and the volume was increased to 60 mL by addition of CH_2Cl_2. Two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (30 mL). The combined organic layer was washed successively with prechilled (at −5 °C) aqueous saturated NaH_2PO_4 (3 × 10 mL) and H_2O (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated at 18 °C to give light yellow solid **6** (1.4 g, 100%). To a solution of tetrabutylammonium salt of adenine (1.37 g, 3.64 mmol) in CH_2Cl_2 (36 mL) at room temperature, **6** (1.4 g, 2.80 mmol) in CH_2Cl_2 (15 mL) was added and left at room temperature for 40 h. The reaction mixture was filtered, the precipitate washed with CH_2Cl_2, and the combined organic layer was concentrated. The residue was purified by silica gel column chromatography (0−3% MeOH in CH_2Cl_2) to give **9** (650 mg, 48%). **Compound 9**: ^{1}H NMR (DMSO-d_6) 8.38 (s, 1H); 8.15 (s, 1H); 7.49−7.30 (m, 5H); 7.20 (br s, 2H); 5.78 (s, 1H); 5.08 (br d, J = 5.4 Hz, 1H); 4.40 (dd, J = 5.6, 9.7 Hz, 1H); 4.29−3.94 (m, 5H); 3.58 (m, 1H); 1.00 (s, 9H); 0.00 (s, 3H); −0.1 (s, 3H). ^{13}C NMR (DMSO-d_6) 156.0, 152.3, 151.0, 139.9, 137.8, 128.9, 128.0, 126.1, 117.8, 100.9, 78.9, 72.1, 69.8, 69.6, 67.5, 54.6, 25.3, 17.8. HRMS calcd for $C_{24}H_{34}N_5O_4Si_1$ $(M + H)^+$ 484.2379, found 484.2374. Mp: 241−242 °C. **Compound 10**: ^{1}H NMR (DMSO-d_6) 8.51 (s, 1H); 8.18 (s, 1H); 7.33−7.16 (m, 5H); 5.88 (d, J = 3.7 Hz, 1H); 5.78 (s, 1H); 5.06 (br d, J = 3.9 Hz, 1H); 4.46 (dd, J = 5.3, 10.0 Hz, 1H); 4.25 (dd, J = 4.8, 9.8 Hz, 1H); 4.20−4.10 (m, 3H); 3.78 (m, 2H). ^{13}C NMR (DMSO-d_6) 156.2, 152.5, 150.6, 140.1, 137.6, 129.0, 128.1, 126.0, 118.2, 101.1, 74.3, 68.8, 68.1, 67.9, 67.0, 55.1. HRMS calcd for $C_{18}H_{20}N_5O_4$ $(M + H)^+$ 370.1515, found 370.1534.

1,5-Anhydro-2-(N^6-benzoyladenin-9-yl)-2-deoxy-3-*O*-TBDMS-D-mannitol (12). To a cold (ice−water) solution of **9** (330 mg, 0.68 mmol) in pyridine (5 mL) was added benzoyl chloride (236 μL, 2.04 mmol) and kept at room-temperature overnight. The reaction mixture was cooled to 0 °C, and saturated aqueous $NaHCO_3$ (2 mL) was added and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with H_2O (10 mL), concentrated, and coevaporated with toluene. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 10 mL) and H_2O (10 mL). The organic layer was concentrated to give **11** which was directly treated with CF_3COOH (5 mL) in CH_2Cl_2 (5 mL) at room temperature for 22 h. The solvent was removed and coevaporated with MeOH and toluene. The residue was dissolved in MeOH (10 mL) and treated with NH_4OH (3 mL). The reaction mixture was concentrated and coevaporated with MeOH, and the residue was purified by silica gel column chromatography (0−4% MeOH in CH_2Cl_2) to give **12** (290 mg, 85%). ^{1}H NMR (DMSO-d_6) 11.08 (br s, 1H); 8.78 (s, 1H); 8.76 (s, 1H); 8.16−7.50 (m, 5H); 5.20 (br s, 1H); 5.10 (d, J = 5.1 Hz, 1H); 4.11 (m, 4H); 3.62 (m, 3H); 3.30 (m, 1H); 0.6 (s, 9H); 0.5 (s, 6H). ^{13}C NMR (DMSO-d_6) 165.7, 153.7, 151.1, 150.0, 144.0, 133.6, 132.4, 128.5, 124.4, 81.7, 73.3, 67.5, 66.7, 60.0, 54.9, 25.5, 17.7. HRMS calcd for $C_{24}H_{34}N_5O_5Si$ $(M + H)^+$ 500.2329, found 500.2317.

1,5-Anhydro-2-(N^6-benzoyladenin-9-yl)-2-deoxy-6-*O*-MMTr-3-*O*-TBDMS-D-mannitol (13). Compound **12** (290 mg, 0.58 mmol) was treated with MMTrCl (268 mg, 0.87 mmol) in pyridine (5 mL) at room-temperature overnight. The reaction mixture was cooled to 0 °C, and saturated aqueous $NaHCO_3$ (5 mL) was added and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with H_2O (5 mL), dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (0−1.5% MeOH in CH_2Cl_2) to give **13** (330 mg, 74%). ^{1}H NMR ($CDCl_3$) 9.26 (s, 1H); 8.86 (s, 1H); 8.70 (s, 1H); 8.08−6.89 (m, 19H); 5.18 (m, 1H); 4.22 (d, J = 12.8 Hz, 1H); 4.08−3.83 (m, 3H); 3.79 (s, 3H); 3.63 (m, 1H), 3.46 (m, 2H); 1.99 (d; J = 2.2 Hz, 1H); 0.61 (s, 9H); 0.1 (s, 6H). ^{13}C NMR ($CDCl_3$) 164.5, 158.6, 152.9, 152.4, 149.3, 144.1, 144.0, 143.3, 135.3, 134.0, 132.6, 130.3, 128.8, 128.4, 128.0, 127.8, 127.0, 121.6, 113.3, 86.9, 80.0, 73.7, 69.0, 68.8, 62.8, 55.2, 55.0, 25.4, 17.8. HRMS calcd for $C_{44}H_{49}N_5O_6SiNa$ $(M + Na)^+$ 794.3349, found 794.3361.

1,5-Anhydro-2-(adenin-9-yl)-2-deoxy-D-mannitol (2a). Compound **9** (200 mg, 0.41 mmol) was treated with 80% aqueous CF_3COOH (10 mL) at room-temperature overnight. The solvent was removed and coevaporated with MeOH and toluene. The residue was dissolved in MeOH (5 mL), treated with NH_4OH (3 mL), concentrated, coevaporated with MeOH and toluene, and crystallized from MeOH/CH_2Cl_2 to give **2a** (60 mg, 52%). ^{1}H NMR (D_2O, 33 °C at 500 MHz). 8.53 (s, 1H); 8.24 (s, 1H); 5.09 (m, 1H); 4.31 (dd, J = 1.0, 13.2 Hz, 1H); 4.18 (dd, J = 9.8, 5.1 Hz, 1H); 4.16 (dd, J = 2.4, 13.2 Hz, 1H); 4.01 (dd, J = 2.0, 12.2 Hz, 1H); 3.92 (dd, J = 5.4, 12.2 Hz, 1H); 3.69 (t, J = 9.8 Hz, 1H); 3.58 (ddd, J = 2.0, 5.4, 9.8 Hz, 1H). ^{13}C NMR (DMSO-d_6) 156.0, 152.2, 150.9, 140.4, 117.9, 81.7, 71.9, 68.0, 66.6, 60.2, 54.3. HRMS calcd for $C_{11}H_{16}N_5O_4$ $(M + H)^+$ 282.1202, found 282.1241. Anal. Calcd for $C_{11}H_{15}N_5O_4 \cdot 0.6CH_3OH$ C, 46.37, H, 5.84, N, 23.31. Found C, 46.33, H, 5.55, N, 22.92. Mp: 232−233 °C.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(N^6-iodo-2-aminopurine-9-yl)-3-*O*-TBDMS-D-mannitol (14). To a so-

lution of tetrabutylammonium salt of 6-iodo-2-aminopurine (3.6 g, 7.16 mmol) in CH_2Cl_2 (30 mL) was added **6** (2.59 g, 5.19 mmol) in CH_2Cl_2 (5 mL) and the reaction mixture kept at room temperature for 24 h. The precipitate was filtered off and washed with CH_2Cl_2 (10 mL). The combined filtrate was concentrated and purified by silica gel column chromatography (0−50% EtOAc in hexane) to give **14** (2.20 g, 70%). ^{1}H NMR (DMSO-d_6) 8.32 (s, 1H); 7.49−7.30 (m, 5H); 6.69 (br s, 2H); 5.78 (s, 1H); 4.85 (br d, J = 5.5 Hz, 1H); 4.37 (dd, J = 5.5, 9.5 Hz, 1H); 4.23 (dd, J = 4.8, 9.9 Hz, 1H); 4.17−3.86 (m, 4H); 3.55 (m, 1H); 0.55 (s, 9H); 0.00 (s, 3H); −0.01 (s, 3H). ^{13}C NMR (DMSO-d_6) 159.4, 151.5, 141.2, 137.7, 129.6, 128.9, 128.0, 126.0, 122.9, 100.9, 78.8, 72.0, 69.6, 69.1, 67.4, 54.7, 25.2, 17.8. HRMS calcd for $C_{24}H_{33}N_5O_4ISi$ (M + H)$^+$ 610.1348, found 610.1398. Mp: 129−131 °C.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(guanin-9-yl)-D-mannitol (15) and 1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(guanin-9-yl)-3-*O*-TBDMS-D-mannitol (16). Compound **14** (2.0 g, 3.28 mmol) was treated with aqueous NaOH (N) (50 mL) in dioxane (50 mL) at 55 °C for 60 h. The reaction mixture was cooled to 0 °C, and the pH was adjusted to 7.0 by addition of dilute aqueous HCl. The solvent was removed to give a white solid. After addition of H_2O (20 mL), the solid was crushed to powder with a spatula, filtered, washed with H_2O (10 mL), and dried to give **15** (1.21 g, 96%). ^{1}H NMR (DMSO-d_6) 7.94 (s, 1H); 7.53−7.30 (m, 5H); 6.50 (br s, 2H); 5.80 (s, 1H); 5.48 (d, J = 5.1 Hz, 1H); 4.78 (m, 1H); 4.23 (dd, J = 5.0, 10.2 Hz, 1H); 4.14−3.83 (m, 5H); 3.48 (m, 1H). ^{13}C NMR (DMSO-d_6) 157.0, 153.4, 152.6, 137.9, 136.7, 129.0, 128.1, 126.4, 115.7, 101.1, 79.0, 72.3, 69.7, 68.0, 67.6, 54.4. HRMS calcd for $C_{18}H_{20}N_5O_5$ (M + H)$^+$ 386.1464, found 386.1457. This compound was directly used in the next reaction without further purification.

To a suspension of **15** (1.10 g, 2.85 mmol) in DMF (45 mL) was added imidazole (327 mg, 4.80 mmol), followed by TBDMSCl (683 mg, 4.53 mmol), and the reaction mixture kept at room temperature for 40 h. The solvent was removed, and H_2O (20 mL) was added, followed by EtOAc (50 mL), to give a white precipitate. The precipitate was filtered off, washed successively with H_2O and EtOAc, and dried to give **16** (1.0 g, 70%). ^{1}H NMR (DMSO-d_6) 10.59 (br s, 1H); 7.97 (s, 1H); 7.50−7.28 (m, 5H); 6.30 (br s, 2H); 5.77 (s, 1H); 4.78 (m, 1H); 4.38−3.88 (m, 6H); 3.52 (m, 1H); 0.66 (s, 9H); −0.1 (s, 6H). ^{13}C NMR (DMSO-d_6) 157.2, 153.2, 152.6, 137.8, 136.7, 128.8, 128.0, 126.0, 115.5, 100.8, 78.8, 72.0, 69.6, 69.5, 67.5, 54.2, 25.3, 17.8. HRMS calcd for $C_{24}H_{34}N_5O_5Si$ (M + H)$^+$ 500.2329, found 500.2284. Anal. Calcd for $C_{24}H_{33}N_5O_5Si\cdot 1.0H_2O$ C, 55.69, H, 6.81, N, 13.53. Found C, 55.62, H, 6.75, N, 13.84.

1,5-Anhydro-2-deoxy-2-(*N*^{2}-isobutyrylguanin-9-yl)-3-*O*-TBDMS-D-mannitol (18). To a cold (0 °C) suspension of **16** (660 mg, 1.32 mmol) in pyridine (20 mL) was slowly added isobutyryl chloride (414 μL, 3.96 mmol). The ice bath was removed and the reaction mixture kept at room temperature for 5 h. The reaction mixture was quenched with ice−water and extracted with EtOAc (3 × 25 mL). The combined organic layer was concentrated and coevaporated with toluene to remove the traces of pyridine. The residue was dissolved in EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ (4 × 10 mL) and H_2O (10 mL). The organic layer was concentrated to give **17** which was directly treated with CF_3-COOH (10 mL) in CH_2Cl_2 (10 mL) at room temperature for 22 h. The solvent was removed, coevaporated with MeOH/toluene. The residue was dissolved in MeOH (30 mL) and treated with NH_4OH (4 mL). The solvent was removed and product was crystallized from CH_2Cl_2 in MeOH to give **18** (510 mg, 80%). ^{1}H NMR (DMSO-d_6) 12.0 (s, 1H); 11.50 (s, 1H); 8.18 (s, 1H); 5.23 (d, J = 6.3 Hz, 1H); 4.88 (br s, 1H); 4.73 (br d, J = 5.4 Hz, 1H); 3.98 (m, 3H); 3.77−3.51 (m, 3H); 3.25 (m, 1H); 2.80 (m, 1H); 1.15 (d, J = 3.9 Hz, 3H); 1.07 (d, J = 3.9 Hz, 3H); 0.62 (s, 9H); 0.05 (s, 3H); 0.01 (s, 3H). ^{13}C NMR (DMSO-d_6) 180.1, 155.0, 149.9, 147.4, 139.5, 119.0; 81.7, 73.2, 67.1, 66.8, 60.0, 54.7, 34.7, 25.4, 19.3, 18.5, 17.6. HRMS calcd for $C_{21}H_{36}N_5O_6Si$ (M + H)$^+$ 482.2434, found 482.2432. Anal. Calcd for $C_{21}H_{35}N_5O_6Si\cdot 0.3CH_2Cl_2$ C, 50.45, H, 7.08, N, 13.81. Found C, 50.28, H, 6.98, N, 13.55.

1,5-Anhydro-2-deoxy-2-(*N*^{2}-isobutyrylguanin-9-yl)-6-*O*-MMTr-2-deoxy-3-*O*-TBDMS-D-mannitol (19). The reaction was performed using a reaction condition described for **13** using **18** (340 mg, 0.70 mmol) and MMTrCl (432 mg, 1.40 mmol) in pyridine (14 mL) to give **19** (400 mg, 75%). This product was crystallized from CH_3OH/CH_2Cl_2. ^{1}H NMR (DMSO-d_6) 12.05 (br s, 1H); 11.50 (br s, 1H); 8.18 (s, 1H); 7.55−7.20 (m, 12H); 6.86 (m, 2H); 5.18 (d, J = 7.0 Hz, 1H); 4.77 (m, 1H); 4.16−3.90 (m, 3H); 3.78 (s, 3H); 3.76−3.38 (m, 4H); 2.80 (m, 1H); 1.12 (d, J = 2.1 Hz, 3H); 1.10 (d, J = 2.3 Hz, 3H); 0.58 (s, 9H); 0.00 (s, 6H). ^{13}C NMR (DMSO-d_6) 180.1, 158.2, 155.0, 149.8, 147.6, 144.6, 139.0, 135.2, 130.2, 128.2, 127.9, 126.9, 119.0, 113.2, 85.8, 80.2, 73.3, 67.5, 67.1, 62.8, 55.1, 54.6, 34.7, 25.4, 19.3, 18.6, 17.6. HRMS calcd for $C_{41}H_{51}N_5O_7SiNa$ (M + H)$^+$ 776.3455, found 776.3402.

1,5-Anhydro-2-deoxy-2-(guanin-9-yl)-D-mannitol (2b). Compound **14** (305 mg, 0.50 mmol) was treated with 80% CF_3-COOH in H_2O (20 mL) at room temperature for 60 h. The solvent was removed and coevaporated with MeOH and toluene. The residue was dissolved in MeOH (10 mL) and treated with NH_4OH (2 mL). The reaction mixture was concentrated, and **2b** (140 mg, 94%) was crystallized from $MeOH/CH_2Cl_2$. ^{1}H NMR (D_2O, 33 °C at 500 MHz) 8.20 (s, 1H); 4.92 (m, 1H); 4.28 (dd, J = 1.5, 13.2 Hz, 1H); 4.13 (dd, J = 5.3, 9.8 Hz, 1H); 4.11 (dd, J = 2.4, 13.2 Hz, 1H); 4.00 (dd, J = 2.4, 12.7 Hz, 1H); 3.90 (dd, J = 5.4, 12.7 Hz, 1H); 3.69 (t, J = 9.8 Hz, 1H); 3.54 (ddd, J = 9.8, 2.4, 5.4 Hz, 1H). ^{13}C NMR (DMSO-d_6) 157.0, 153.2, 152.4, 137.2, 115.6, 81.6, 71.8, 67.9, 66.8, 60.2, 53.8. HRMS calcd for $C_{11}H_{16}N_5O_5$ (M + H)$^+$ 298.1151, found 298.1168. Anal. Calcd for $C_{11}H_{15}N_5O_5\cdot 2.5H_2O$ C, 38.60, H, 5.89, N, 20.46. Found C, 38.80, H, 5.82, N, 20.36.

1,5-Anhydro-3-(adenin-9-yl)-4,6-*O*-benzylidene-2,3-dideoxy-D-altritol (21). A mixture of **10** (350 mg, 0.95 mmol), imidazole (25 mg), and NaH (80%) (120 mg) in THF (10 mL) was stirred at 0 °C for 30 min and then at room temperature for 30 min. After addition of CS_2 (570 μL) and stirring at room temperature for 60 min, MeI (234 μL) was added and kept at room temperature for 3 h. After addition of H_2O (2 mL), the mixture was concentrated, diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ (3 × 10 mL), H_2O (10 mL), dried, filtered, and concentrated to give **20** which was directly treated with n-Bu_3SnH (505 μL, 1.88 mmol) and AIBN (38 mg, 0.23 mmol) in toluene (10 mL) at 110 °C overnight. The solvent was removed, and the residue was purified by silica gel column chromatography (0−4% MeOH in CH_2Cl_2) to give **21** (150 mg, 45%). ^{1}H NMR ($CDCl_3$) 8.50 (s, 1H); 8.38 (s, 1H); 7.48−7.27 (m, 5H); 6.12 (br s, 2H); 5.67 (s, 1H); 5.19 (m, 1H); 4.40 (dd, J = 4.6, 10.5 Hz, 1H); 4.22−3.68 (m, 5H); 2.93 (m, 1H); 2.39 (m, 1H). ^{13}C NMR($CDCl_3$) 155.6, 152.6, 150.9, 140.9, 136.6, 129.2, 128.3, 125.9, 119.3, 102.5, 78.3, 69.7, 67.7, 63.3, 51.4, 29.7. HRMS calcd for $C_{18}H_{20}N_5O_3$ (M + H)$^+$ 354.1566, found 354.1577.

1,5-Anhydro-3-(adenin-9-yl)-2,3-dideoxy-D-altritol (22). Compound **21** (80 mg, 0.22 mmol) was treated with 80% CF_3-COOH in H_2O (5 mL) at room-temperature overnight. The solvent was removed and coevaporated with MeOH/toluene. The residue was dissolved in MeOH (10 mL), treated with NH_4-OH (2 mL), concentrated, and purified by silica gel column chromatography (0−12% MeOH in CH_2Cl_2) to give **22** (50 mg, 83%). ^{1}H NMR (DMSO-d_6) 8.60 (s, 1H), 8.52 (s, 1H); 7.60 (br s, 2H); 5.70 (br s, 1H); 5.29 (m, 2H); 4.40−4.08 (m, 6H); 2.82 (m, 1H); 2.19 (m, 1H). ^{13}C NMR (DMSO-d_6) 156.0, 152.3, 149.6, 140.5, 118.5, 79.7, 65.5, 61.2, 60.0, 51.3, 26.7. HRMS calcd for $C_{11}H_{16}N_5O_3$ (M + H)$^+$ 266.1253, found 266.1267.

1,5-Anhydro-3-(adenin-9-yl)-3-deoxy-D-altritol (23). Compound **10** (150 mg, 0.40 mmol) was treated with 80% CF_3-COOH in H_2O (5 mL) at room-temperature overnight. The solvent was removed and coevaporated with MeOH/toluene. The residue was dissolved in MeOH (5 mL) and treated with NH_4OH (2 mL), concentrated, and precipitated from MeOH/CH_2Cl_2 to give **23** (70 mg, 61%). ^{1}H NMR (DMSO-d_6) 8.15 (s, 1H); 8.14 (s, 1H); 7.25 (br s, 2H); 5.47 (br s, 1H); 5.17 (br d, J = 5.9 Hz, 1H); 4.92 (br s, 1H); 4.64 (dd, J = 2.9, 10.2 Hz, 1H); 4.34 (br s, 1H); 3.94−3.33 (m, 6H). ^{13}C NMR (DMSO-d_6) 155.8, 152.0, 150.0, 140.6, 118.4, 79.9, 67.0, 66.6, 63.6, 59.3, 57.8.

HRMS calcd for $C_{11}H_{16}N_5O_4$ $(M + H)^+$ 282.1202, found 282.1225. Anal. Calcd for $C_{11}H_{15}N_5O_4 \cdot 0.9CH_2Cl_2$ C, 39.96, H, 4.73, N, 19.58. Found C, 39.90, H, 4.87 and N, 19.54.

1,5-Anhydro-2-(adenin-9-yl)-4,6-*O*-benzylidene-2-deoxy-D-altritol (25). Compound **8** was prepared as described for **5**, using **7** (184 mg, 0.5 mmol), Et_3N (125 μL, (0.9 mmol), and MsCl (54 μL, (0.7 mmol) in CH_2Cl_2 (5 mL) to give **8** (223 mg, 100%) which was directly used for the next step. A mixture of adenine (135 mg, 1.0 mmol) and NaH (80%, 60 mg, 2.0 mmol) in DMF (5 mL) was heated at 95 °C for 60 min. Compound **8** (223 mg, 0.5 mmol) in DMF (2 mL) was added and the reaction mixture kept at 95 °C for 20 h. The solvent was removed, and EtOAc (50 mL) was added followed by saturated aqueous $NaHCO_3$ (15 mL). The two layers were separated. The aqueous layer was back extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with H_2O (10 mL), dried, filtered, concentrated, and purified by silica gel column chromatography (0−7% MeOH/CH_2Cl_2) to give **25** (100 mg, 51%). The spectroscopic properties of **25** are identical as reported previously.[12]

Phosphoramidite Synthesis. The phosphitylation reaction was carried out on 0.45 mmol of **13** or **19**, respectively, in 8 mL of dichloromethane using 3 equiv of *N*,*N*-diisopropylethylamine and 1.5 equiv of *N*,*N*-diisopropylchlorophosphoramidite. Reactions were complicated as only a slight change in R_f value was seen on TLC analysis. Therefore, the reaction was stirred for 2 h at room temperature and quenched by addition of 2 mL of ethanol and stirring for 10 min more. The mixture was partitioned between 30 mL of dichloromethane and 30 mL of saturated aqueous $NaHCO_3$. The organic phase was washed with brine (2 × 25 mL), dried on Na_2SO_4, and evaporated. The obtained residue was flash purified on 30 g of silica gel (hexane/acetone/triethylamine 50/48/2) and precipitated in cold hexane.

26: yield 86%, LSIMS (neg. mode, NBA) $[M - H]^-$ 970.

27: yield 91%, LSIMS (neg. mode, NBA) $[M - H]^-$ 952.

Oligonucleotide Synthesis and Purification. Standard operations for oligonucleotide assembly were as described.[15] Following assembly (trityl off) the support was treated with NH_4OH concentrated/EtOH 3:1 for 16 h at 50 °C, and the supernatants was lyophylized. The residue was taken up in 1 mL of TBAF/THF 1 M and left in the dark for 22 h at rt. After dilution with 1 mL of 0.03 M KH_2PO_4, the mixture was gel filtered in 2 portions over a NAP-25 column. Following concentration a second gel filtration was done eluting with buffer A (0.03 M KH_2PO_4 pH 6.7, 20% CH_3CN), and the eluate was purified by ion exchange chromatography on a Mono Q HR 10/10 column with a KH_2PO_4 gradient. Product containing fractions were desalted on Sephadex G50 and analyzed on a polystyrene reverse phase column (PLRP-S, Polymer Laboratories) with a CH_3CN gradient in aqueous 0.1 M TEAA.

For the dodecamer, the residue after ammonia deprotection was divided in two, one part treated as above, while the other part was dissolved in 1 mL of TEA, 3HF/DMF 3:1 and heated for 1 h 30 at 55 °C. After addition of 1 mL aqueous 0.1 M TEAA the mxiture was worked-up as above.

While analysis of the first part showed a broad peak on ion exchange chromatography at the expected position, the deprotection mixture by TEA, 3HF only displayed peaks with short retention times.

Molecular Modeling. The force field parameters and partial charges for RNA were taken from the AMBER 4.1 force field parameter database.[20] The force field parameters for MNA were taken from Amber 4.1 without modification. The partial charges of MNA were obtained by a 2 stage fitting procedure (RESP[21]) of the charges to the 6-31G*-derived electrostatic potential from a GAMESS calculation.[22] The modeling of the MNA:RNA complex in A-RNA geometry was done by modeling d(GCGTAGCG):r(CGCUACGC) in Arnott's canonical A-RNA geometry and subsequent docking of the p(GCGTAGCG) strand onto the DNA strand. The MNA strand was then minimized by restraining the base atoms onto the corresponding positions of the DNA strand by a force constant of 10 kcal/mol Å^2 per atom. After deletion of the DNA strand, the MNA:RNA complex was energy minimized to a rms gradient of 0.1 kcal/mol Å using restraints on the base hydrogen bonds (10 kcal/mol Å^2). The resulting duplex was put in a rectangular box of TIP3P water extending approximately 12 Å in each direction from the nucleic acid atoms. The 14 water molecules with the highest negative electrostatic potential were replaced by sodium ions which results in 3529 remaining waters and an initial box size of 50.9 × 43.7 × 45.9 Å. The single strand MNA was cut out of the duplex MNA:RNA, soaked in a water box and the 7 waters with highest electrostatic potential replaced by sodium ions, resulting in a rectangular box of 50.0 × 40.6 × 36.3 Å with 2347 water molecules. The subsequent molecular dynamics simulations were applied using constant temperature (300 K) with a separate but equal temperature coupling time constant of 0.4 ps^{-1} for solute and solvent atoms,[23] and constant pressure (1 bar) conditions with a compressibility of 44.6 × 10^{-6} bar^{-1} and a pressure relaxation time of 0.4 ps^{-1}. The integration time step was 2 fs and SHAKE[24] was applied on all bond lengths. A 9 Å cutoff for the Lennard−Jones interactions was used while updating the nonbonded pairlist every 10 steps. Long-range electrostatic interactions were treated by the particle mesh Ewald (PME) method[25] with a PME charge grid spacing of approximately 1 Å in each direction, an order of 4 of the βspline interpolarion and a direct sum tolerance of 10^{-4}. Equilibrium was achieved by energy minimization of the whole system until the gradient of the energy dropped below 0.1 kcal/mol Å. Then the systems were heated to 300 K over a period of 10 ps after which the production runs over 1 ns for the duplex MNA:RNA and 300 ps for the single strand MNA were initiated, saving the conformations every 0.2 ps. The energy minimizations and the MD simulations were performed with the SANDER module and the results analyzed with the CARNAL module of AMBER 4.1.[20]

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