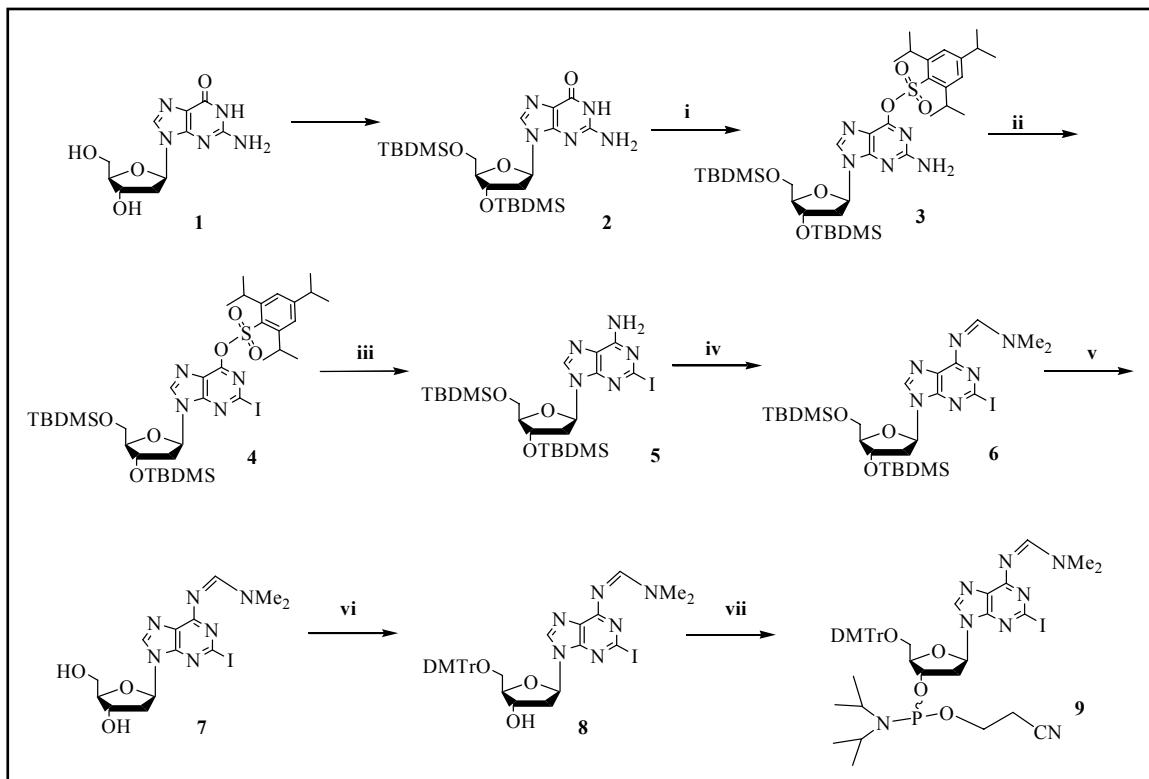


RNA-DNA Hybrid structure determined by EPR, CD and RNase H1

Supporting Information

1. Synthesis of 2-Iodo-deoxy-adenosine phosphoramidite

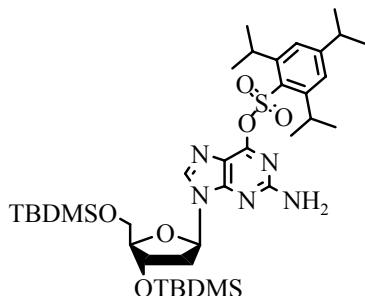


Scheme 1: synthesis of 2-Iodo-2'-deoxy-adenosine phosphoramidite

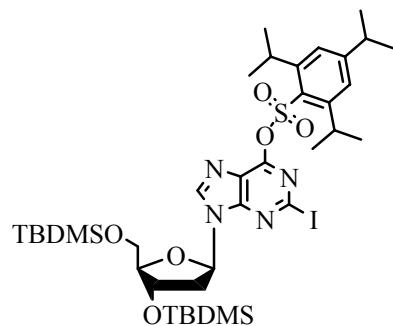
i) TiPBS-Cl, TEA, DMAP, 80%;¹ **ii)** I₂, CuI, CH₂I₂, isopentyl nitrite, 84%; **iii)** EtOH/NH₃, 78%; **iv)** DMF-DMA, 80%; **v)** TEA-3HF, 86%; **vi)** DMTr-Cl, pyridine, 75%; **vii)** 2-cyanoethoxy-bis(diisopropylamino)-phosphine, DCI, 82%

The reactions were monitored by thin-layer chromatography (TLC) analysis on silica gel aluminium plates (silica gel 60 *F*₂₅₄, 0.2 mm, Merck, Columbus, OH, USA). Flash column chromatography was performed on silica gel (*40-63 μm, 230-400 mesh, Merck*). Technical solvents were used after distillation for chromatography; absolute solvents, dried over molecular sieve, were purchased from FLUKA. ¹H, ¹³C and ³¹P-NMR spectra were recorded with Bruker AMX250/DPX 250 at 250 MHz, AMX300 at 300 MHz or AMX400 at 400 MHz as indicated. Electron Spray Ionization (ESI) masses were collected on a VG Platform II (Fisons Instruments, San Carlos, CA, USA). Elemental analyses were performed on CHN-O-

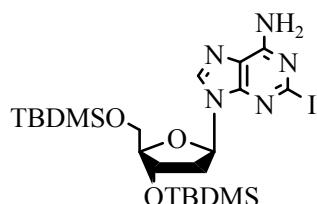
Rapid from Foss-Heraeus, Hanau, Germany. The definition of the carbons was assign by ¹H-¹³C-HSQC and -HMBC.



3',5'-O-tert-butyl-dimethylsilyl-O'-(2,4,6-triisopropyl-benzene)sulfonic acid-deoxyguanosine (3).² The reaction has to be carried out under dry conditions and argon atmosphere. 3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxy-guanosine (3.13 g, 6.32 mmol) was solved in 70 ml dry CH₂Cl₂ and cooled down at 0°C. Afterwards 1.8 ml triethylamine (2 eq), 188 mg DMAP (0.24 eq) and 2,4,6-triisopropyl-benzenesulfonic-chloride (4.019 g, 2.1 eq) were added. The mixture was brought to room temperature and stirred for 20 hours. After the reaction was finished the reaction mixture was washed with saturated NaHCO₃-solution and water, dried over MgSO₄ and evaporated. The crude product was purified on silica gel (*n*-Hex/AcOEt (3/1, v/v); R_f 0.52). Pure product was obtained as white foam (3.852 g, 80%). Product was identified via ¹H-NMR (250MHz, DMSO-d₆): δ[ppm] 8.22 (s, 1H, H(8)), 7.33 (s, 2H, Ar-H), 6.64 (bs, 2H, NH₂), 6.22-6.17 (t, 1H, H(1'), J=6.5), 4.53-4.50 (m, 1H, H(3')), 4.16-4.06 (ph, 2H, *i*Pr in ortho, J=6.8), 3.84-3.80 (m, 1H, H(4')), 3.70-3.68 (dd, 1H, H(5'), J=5.5,11.1), 3.65-3.61 (dd, 1H, H(5'), J=4.4, 11.1), 2.30-2.28 (q, 1H, *i*Pr in para, J=6.8), 2.80-2.72 (pq, 1H, H(2')), 2.31-2.25 (ddd, 1H, H(2'), J=3.5, 6.4, 13.1), 1.29-1.19 (dd, 18H, (CH₃)₂, J=6.8, 9.0), 0.88 and 0.83 (2s, 18H, SiC(CH₃)₃), 0.10 (s, 6H, SiCH₃), 0.01 and -0.1 (2s, 6H, SiCH₃); ¹³C-NMR (400 MHz, DMSO-d₆): δ[ppm] 158.38 (C(6)), 147.5 (C(8)), 146.85 (Ar-*i*Pr)), 141.27 (Ar-C), 135.20 (C(2)), 134.20 (C-O), 121.24 (Ar-C), 119.22 (C(5)), 98.04 (C-S), 88.21 (C(4')), 85.26 (C(1')), 71.55 (C(3')), 60.90 (C(5')), 40.00 (C(2')), 33.23, and 28.07 (*i*Pr-C), 25.74 (*i*Pr), 24.74 (*t*Bu), 23.75 (*i*Pr), 17.71 (Si-C), -3.29 and -4.90 (SiCH₃); EA: calculated C(58.31), H(8.33), N(9.19); found C(58.54), H(8.36), N(9.10) and ESI(+/-): calculated 762.2; found 762.2 [M] and 763.6 [M+H⁺].

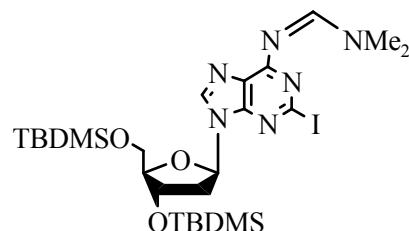


2-Iodo-3',5'-O-tert-butyl-dimethylsilyl-O'-(2,4,6-triisopropyl-benzene)sulfonic acid-deoxy-guanosine (4). The reaction needs inert atmosphere and dry conditions. 1.0 g 2,4,6-triisopropyl-benzene-sulfonic-acid 3',5'-O-tert-butyl-dimethylsilyl-O'-(2,4,6-triiso-propyl-benzene) sulfonic acid-deoxy-guanosine (1.31 mmol) was dissolved in dry THF (40 ml) and 333 mg iodine (1 eq) and 262 mg CuI (1.05 eq) were added. After further addition of 1.06 ml CH₂I₂ (10 eq) and 0.9 ml isopentyl nitrite (6.7 eq) the reaction was refluxed over 15 hours. The dried residue was dissolved in CH₂Cl₂ and the remaining iodo-compound was reduced with sat. Na₂S₂O₄. The organic layer was dried over MgSO₄, evaporated and purified by column chromatography (*n*-Hex/AcOEt: 10/0 to 9/1, v/v). Yield: 0.96 g (84%). R_f 0.52; ¹H-NMR (250 MHz, DMSO-d₆): δ [ppm] 8.67 (s, 1H, H(8)), 7.36 (s, 2H, Ar), 6.35-6.30 (dd, 1H, H(1'), J=5.3, 6.8), 4.76-4.68 (bm, 1H, H(3')), 4.10-4.04 (m, 2H, *i*Pr in ortho), 3.85-3.59 (m, 3H, H(4') and H(5')), 3.04-2.88 (m, 1H, H(2') and 1H, *i*Pr in para,), 2.42-2.31 (pq, 1H, H(2')), 1.25-1.19 (pt, 18H, *i*Pr), 0.89 and 0.74 (2s, 18H, SiC(CH₃)₃), 0.12 (s, 6H, SiCH₃), 0.06 and -0.14 (2s, 6H, SiCH₃); ¹³C-NMR (250 MHz, DMSO-d₆): δ [ppm] 155.89 (C(6)), 149.24 (C(2)), 147.08 and 146.67 (C(Phenyl-*i*Pr)), 141.77 (Ar-C), 139.72 (C(8)), 121.24 (C-S), 119.22 (C(5)), 117.66 (C(4)), 86.98 (C(4')), 83.53 (C(1')), 71.81 (C(3')), 62.44 (C(5')), 38.16 (C(2')), 33.17 and 27.91 (*i*Pr-C), 25.71 (*t*Bu), 24.72 and 23.75 (*i*Pr), 17.69 (Si-C), -3.29 (SiCH₃); EA: calculated C(50.90), H(7.04), N(6.42); found C(51.60), H(7.17), N(6.31) and ESI(+-) calculated 873.1; found 873.5 [M], 874.6 [M+H⁺] and 895.5 [M +Na⁺].

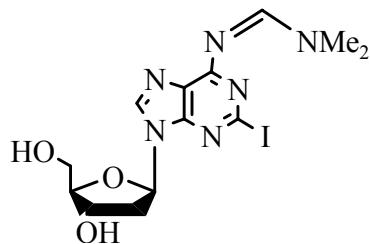


2-Iodo-3',5'-O-tert-butyl-dimethylsilyl-deoxyadenosine (5).³ Compound 4 (2.625 g, 3 mmol) was dissolved in a few millilitres of dry CH₂Cl₂ and transferred to a high-pressure tube. Following this the mixture was diluted with 350 ml ammonia saturated ethanol and cooled down to 0 °C. After three hours the reaction was brought to room temperature and left

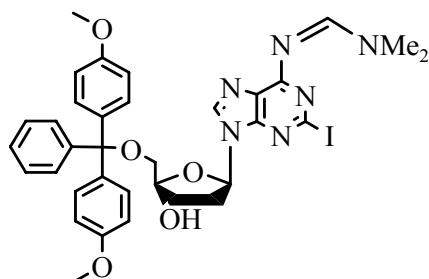
stirred over night at this temperature. Afterwards the solvent was removed in vacuo. The flash column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$: 98/2, v/v) yielded 1.42 g (78%) of 4. R_f 0.2; ^1H -NMR (250 MHz, DMSO-d₆): δ [ppm] 8.20 (s, 1H, H(8)), 7.67 (bs, 2H, NH₂), 6.25-6.21 (t, 1H, H(1'), J=6.5), 4.67-4.63 (bm, 1H, H(3')), 3.83-3.70 (m, 2H, H(4') and 1H of H(5')), 3.67-3.63 (dd, 1H, H(5'), J=4.3, 10.6), 2.91-2.84 (pq, 1H, H(2'), J=6.2, 6.5), 2.33-2.27 (m, 1H, H(2')), 0.90 and 0.84 (2s, 18H, tBu), 0.13 (s, 6H, SiCH₃), 0.04 and -0.01 (s, je 3H, SiCH₃); ^{13}C -NMR (250 MHz, DMSO-d₆): δ [ppm] 155.89 (C(6)), 149.24 (C(2)), 139.72 (C(8)), 119.22 (C(5)), 117.66 (C(4)), 86.98 (C(4')), 83.53 (C(1')), 71.81 (C(3')), 62.44 (C(5')), 38.16 (C(2')), 25.68 (tBu), 17.93 and 17.69 (Si-C), -4.74, -4.96 and -5.52 (SiCH₃); EA: calculated C(43.63), H(6.66), N(11.56); found C(43.82), H(6.71), N(11.41) and ESI(+/-): calculated 605.7; found 606.3 [M], 607.4 [M+H⁺] and 628.3 [M+Na⁺].



2-Iodo-3',5'-O-tert-butyl-dimethylsilyl-N,N-dimethyl-N'-formamidine-deoxyadenosine (6). Under argon atmosphere the mixture of 1.17 g of TBDMS-protected 2-Iodo-deoxyadenosine (1.93 mmol) and *N,N*-dimethylformamide-dimethylacetal (1.03 ml, 4 eq) in dry DMF (20 ml) was heated at 65 °C for 6 hours and afterwards stirred for 15 h at room temperature. After evaporation of the solvent, the oily residue was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$: 98/2; v/v, R_f : 0.26), yield 1.02 g (80%). ^1H -NMR (250 MHz, DMSO-d₆): δ [ppm]; 8.79 (s, 1H, CH), 8.30 (s, 1H, H(8)), 6.30-6.24 (t, 1H, H(1'), J=6.5), 4.66 (bs, 1H, H(3')), 3.34-3.63 (m, 3H, H(4'+5')), 3.21 (s, 3H, N(CH₃)), 3.12 (s, 3H, N(CH₃)), 2.98-2.85 (m, 1H, H(2')), 2.35-2.25 (m, 1H, H(2')), 0.89 and 0.83 (s, 18H, tBu), 0.12 (s, 6H, Si(CH₃)), 0.01 and -0.2 (2s, 6H, Si(CH₃)); ^{13}C -NMR (250 MHz, DMSO-d₆): δ [ppm]; 159.29 (CH), 158.14 (C(2)), 151.34 (C(6)), 141.42 (C(8)), 125.77 (C(5)), 119.96 (C(4)), 86.96 (C(4')), 83.57 (C(1')), 71.84 (C(3')), 62.42 (C(5')), 40.77 (N(CH₃)), 38.00 (C(2')), 34.67 (N(CH₃)), 25.58 (tBu), 17.83 and 17.56 (Si-C), -4.84, -5.04 and -5.94 (SiCH₃); EA: calculated C(45.44), H(6.86), N(12.72); found C(45.34), H(6.73), N(12.57); ESI(+/-): calculated: 659.7; found 660.8 [M+H⁺] und 675.3 [M+Na⁺]

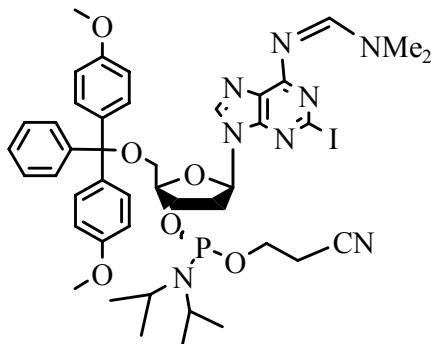


2-Iodo-N,N-dimethyl-N'-formamidin-deoxy-adenosine (7). 869 mg (1.32 mmol) of compound **6** were dissolved in 35 ml dry THF and cooled down to 0 °C. Keeping this temperature 3.5 eq (2.0 ml) of a TEA*3HF solution was dropwise added and the reaction was stirred for 1 h. After 20 h stirring at room temperature the reaction was quenched using 10 ml water, extracted with CH₂Cl₂ and dried over Na₂SO₄. The solid residue was purified by column chromatography (CH₂Cl₂/MeOH: (9/1), v/v, R_f 0.40) and yielded 489 mg (86%). ¹H-NMR (250 MHz, DMSO-d₆): δ [ppm]; 8.81 (s, 1H, CH), 8.38 (s, 1H, H(8)), 6.32-6.27 (t, 1H, H(1'), J=6.75), 5.33-5.31 (d, 1H, 3'-OH, J=4.25), 4.95-4.91 (t, 1H, 5'-OH, J=5.25), 4.66 (m, 1H, H(3')), 3.87-3.75 (m, 1H, H(4')), 3.64-3.45 (m, 2H, H(5')), 3.22 (s, 3H, N(CH₃)), 3.13 (s, 3H, N(CH₃)), 2.72-2.60 (m, 1H, H(2')), 2.33-2.24 (ddd, 1H, H(2'), J=3.25, 6.0, 13.25); ¹³C-NMR (250 MHz, DMSO-d₆): δ [ppm]; 159.29 (CH), 158.26 (C(2)), 151.54 (C(6)), 140.77 (C(8)), 125.45 (C(5)), 120.12 (C(4)), 87.84 (C(4')), 83.30 (C(1')), 70.69 (C(3')), 61.51 (C(5')), 40.80 (N(CH₃)), 39.15 (C(2')), 34.68 (N(CH₃)); EA: calculated C(36.13), H(3.96), N(19.44); found C(36.41), H(4.19), N(19.17); ESI(+/-): calculated 432.2; found 433.0 [M+H⁺].



2-Iodo-5'-O-(4,4'-dimethoxytrityl-methyl)-N,N-dimethyl-N'-formamidine-deoxyadenosine (8) Compound **7** (475 mg, 1.1 mmol) was two times coevaporated with dry pyridine (5 ml) and dried on the vacuum line over night. For water free conditions the 4,4'-dimethoxy-trityl chloride (418 mg, 1.1 eq) was dried on the vacuum line for the same time. The starting material was dissolved in 5 ml dry pyridine and DMTr-Cl was added. After stirring at room temperature for 4 h the reaction was quenched by addition of methanol. The solid was evaporated and three times coevaporated with dry toluene. The pyridine free residue was dissolved in CH₂Cl₂ and washed with aqueous NaHCO₃. The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel by

using a gradient of CH₂Cl₂/MeOH 100/0 to 95/5, v/v. The product yielded in 605 mg (75%). R_f (CH₂Cl₂/MeOH 98/2, v/v) 0.25; ¹H-NMR (400 MHz, DMSO-d₆): δ [ppm]; 8.78 (s, 1H, N=CH), 8.29 (s, 1H, H(8)), 7.32-7.17 (m, 9H, DMTr), 6.78-6.70 (dd, 4H, J=9.2, 26), 6.36-6.32 (t, 1H, H(1'), J=6.0), 5.37-5.35 (d, 1H, J=4.8, 3'-OH), 4.50-4.40 (q, 1H, J=5.2Hz, H(3')), 3.98-3.93 (m, 1H, H(4')), 3.71 and 3.69 (2s, each 3H, OCH₃), 3.28-3.08 (m, overlapped by NCH₃, H(5')), 3.22 and 3.13 (2s, each 3H, NCH₃), 2.82-2.75 (m, 1H, H(2')), 2.39-2.32 (m, 1H, H(2')); ¹³C-NMR (400 MHz, DMSO-d₆): δ [ppm]; 159.31 (C(9)), 158.16 (C(2)), 157.95 (C(10)), 151.51 (C(6)), 144.89 (C(11)), 141.09 (C(8)), 135.54 and 135.48 (C(15)), 129.71 and 129.51 (C(12)), 127.62 (C(16)), 125.81 (C(5)), 120.19 (C(4)), 113.00 and 112.94 (C(13)), 86.09 (C(4')), 85.32 (C(14)), 83.46 (C(1')), 70.56 (C(3')), 64.09 (C(5')), 54.94 and 54.89 (C(OCH₃)), 40.85 (N(CH₃)), 39.15 (C(2'; overlapped by DMSO-d₆-signal)), 34.73 (N(CH₃)); EA: calculated C(55.59), H(4.80), N(11.44); found C(55.11), H(4.86), N(10.57); ESI(+/-): calculated 734.6; found 734.9 [M] and 735.6 [M+H⁺].



2-Iodo-5'-O-(4,4'-dimethoxytriphenyl-methyl)-3'-O-(2-cyanethoxydiisopropylphosphine)-N,N-dimethyl-N'-formamidine-deoxyadenosine (9) Compound **8** (300 mg, 40.8 mmol) under argon atmosphere was dissolved in 6 ml CH₂Cl₂. The solution was cooled down to 0°C and DCI (1.15 eq, 55.5 mg) and a solution of 2-cyanethoxy-di-(di-N,N-*iso*-propylamino) phosphine (1.2 eq) were added. After being stirred at room temperature for 3.5 h the reaction was stopped by adding NaHCO₃-solution and washed with brine. The organic layer was dried over Na₂SO₄ and evaporated in vacuo. The residue was purified by silica gel chromatography (*n*-Hex/Acetone, 55/45, v/v and 5% TEA) and yielded 315 mg (82%). R_f (*n*-Hex/Acetone, 55/45, v/v and 5% TEA) 0.25; ¹H-NMR (300 MHz, Acetone-d₆): δ [ppm]; 8.86 (s, 1H, N=CH), 8.15 (s, 1H, H(8)), 7.47-7.17 (m, 9H, DMTr), 6.84-6.73 (m, 4H), 6.36-6.32 (m, 1H, H(1'), diastereomeres), 4.94-4.86 (m, 1H, H(3')), 4.27-4.18 (m, 1H, H(4')), 3.94-3.85 (m, 1H, H(5')), 3.78-3.75 (m, 6H, OCH₃), 3.69-3.58 (m, 1H, H(5')), 3.30 and 3.21 (2s, each 3H, N(CH₃)), 3.12-3.02 (m, 1H, H(2')), 2.92-2.63 (m, 4H, Ethyl-H), 2.73-2.71 (m, 1H, H(2')).

1.28-1.11 (m, 12H, *i*Pr); ^{31}P -NMR (300 MHz, Acetone-d₆): δ [ppm]; 148.45 and 148.34; ESI(+/-): calculated 934.8; found 936.0 [M+H⁺] and 936.9 [M+2H⁺].

2. Oligonucleotide synthesis

The RNAs were synthesized on 0.2 μmol scale on a rebuilt ABI 392 synthesizer (Applied Biosystems, Foster City, CA, USA) with phosphoramidites purchased from Dharmacon (ACE chemistry)⁴. The ribo-oligonucleotides were synthesized following the procedure published in *Nature Protocols*.⁵ However, the synthesis cycles were performed without interruption after incorporating the iodinated compounds.

The DNA oligomers were synthesized on an Expedite D300+ synthesizer from Perseptive Biosystems by phosphoramidite chemistry on a 1 μmol scale. In this case the published procedure was also modified, the coupling time was the same for each phosphoramidite and the synthesis was done without interruption. The yields could be improved by this procedure.

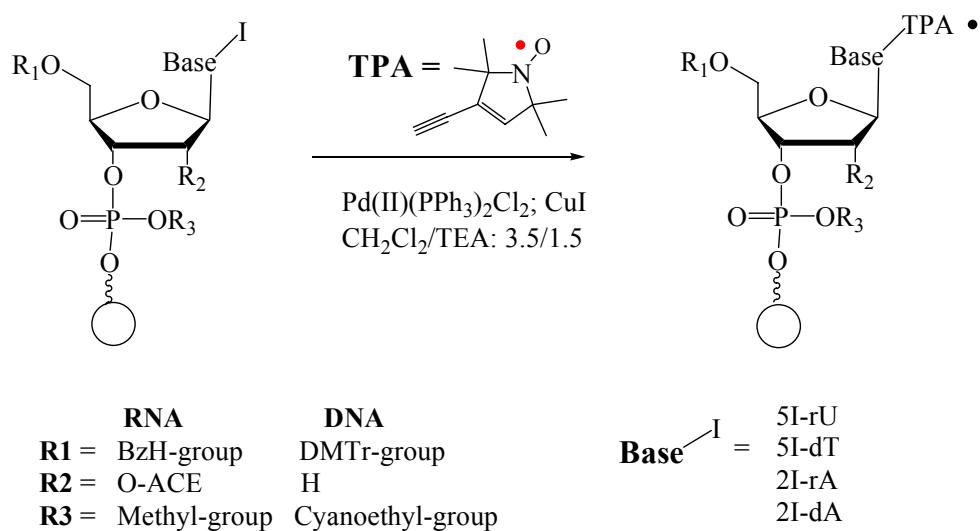
Table 1: Synthesized DNA- and RNA-strands

Oligomer	Sequence	Mass cal.	Mass found.
DNA(dT)	5'-CGC-TAC-ATA-GTG-AGC-3'	4577.0	4577.7
DNA(dA)	5'-GCT-CAC-TAT-GTA-GCG-3'	4568.0	4569.1
DNA(<u>dT</u>)	5'-CGC-TAC-ATA- <u>G</u> TG-AGC-3'	4725.2	4724.9
DNA(<u>dA</u>)	5'-GCT-CAC-TAT- <u>G</u> T <u>A</u> -GCG-3'	4730.2	4730.1
RNA(rU)	5'-CGC-UAC-AUA-GUG-AGC-3'	4775.0	4779.0
RNA(rA)	5'-GCU-CAC-UAU-GUA-GCG-3'	4751.9	4751.0
RNA(<u>rU</u>)	5'-CGC-UAC-AUA- <u>G</u> UG-AGC-3'	4937.1	4931.6
RNA(<u>rA</u>)	5'-GCU-CAC-UAU-GU <u>A</u> -GCG-3'	4914.2	4914.5

T, U und A: labelled with spin label TPA

General procedure for Sonogashira cross coupling reaction: The Sonogashira cross coupling for all oligonucleotides was done postsynthetically “on column” (on solid support). For that the column was removed from the synthesizer and maintained under argon atmosphere. 200

μl of a dried and deoxygenated $\text{CH}_2\text{Cl}_2/\text{TEA}$ solution (1.75 ml/0.75 ml) and 9.5 mg copper(I)iodide were added under argon atmosphere to a mixture of $\text{Pd}(\text{II})(\text{PPh}_3)_2\text{Cl}_2$ (2.1 mg) and TPA (2.0 mg). The yellow or orange solution was immediately given into the column and moved in it back and forth using two syringes.⁵ After the reaction time of 2.5 h in the dark the column was washed with 10 ml dry CH_2Cl_2 , dried for 10 min in vacuo and flushed with argon. To achieve better yields, the Sonogashira cross coupling was performed two times with the same amount of reagents.



Scheme 2: Sonogashira cross coupling reactions at different bases during solid phase synthesis

Finally, the DNA was cleaved from the controlled pore glass (CPG) support and completely deprotected with a mixture of 32%-ammonia/MeOH (3/1) at room temperature over 24 h. In the case of RNA, the cleavage of methyl-groups on the phosphate using a 0.4 M solution of disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate-trihydrate (S_2Na_2) in $\text{DMF}/\text{H}_2\text{O}$, 98/2 at room temperature in 30 min was done at first. Afterwards, in one step the oligonucleotide was cleaved from the solid-support (polystyrene) and the functional groups were deprotected with methylamine (40% in water), 16 h at +4°C.

3. Purification and Characterization

All oligonucleotids were purified by anionic exchange-HPLC (DNA Pack® PA-100, Semi-Prep, 9x 250 mm from Dionex) with 1 M LiCl. In the case of DNA a further purification by reversed phase-HPLC (Phenomenex, Jupiter 4 μ -Poter-90 A, gradient see Table 2) was necessary (data not shown).

The oligonucleotides were desalted with PD-10 Sephadex columns from Amersham Biosciences, Piscataway, NJ, USA.

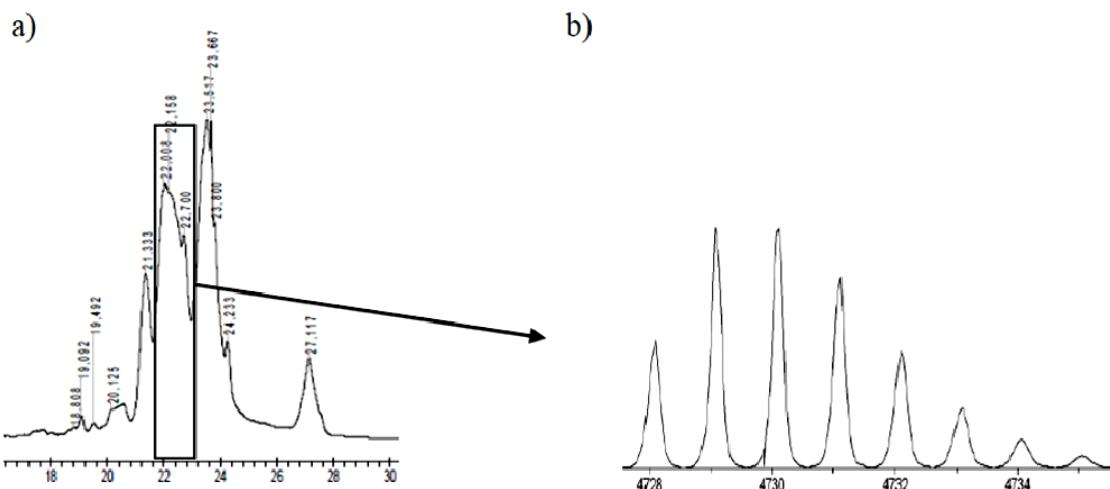


Figure 1: DNA(dA) a) anionic exchange-HPLC and b) ESI-mass spectrum

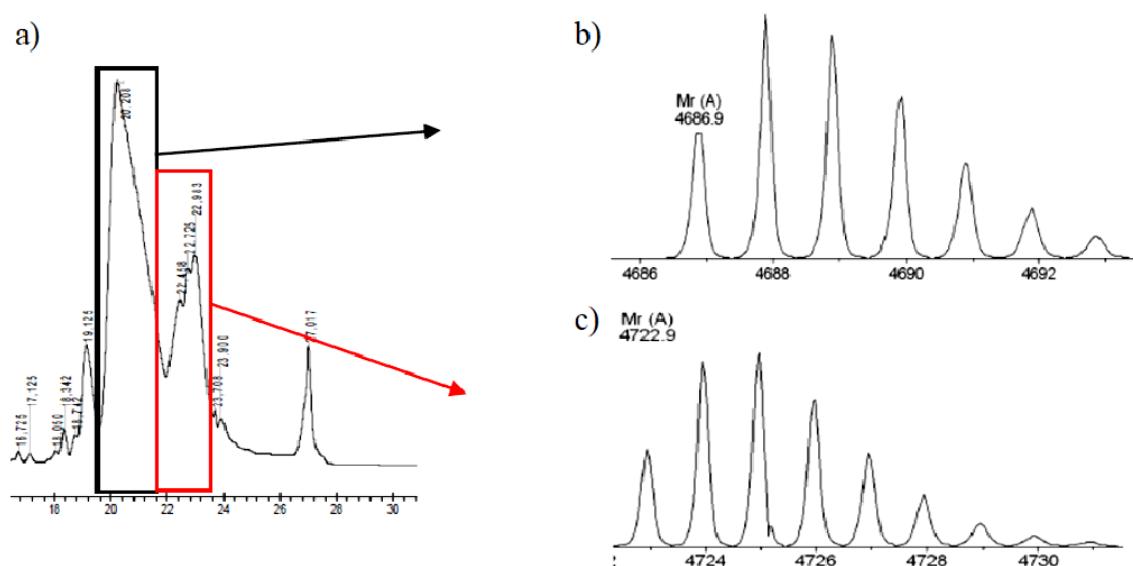


Figure 2: DNA(dT) a) anionic exchange-HPLC b) ESI-mass spectrum of iodinated DNA

c) ESI-mass spectrum of spin labelled DNA

It was possible to separate the iodinated from the spin labelled DNA (see Figure 2 b and c).

Using ACE chemistry in the case of RNA one further deprotection step is necessary prior to use. The deprotection of the 2'-hydroxy group was performed under sterile conditions with a TEMED/acetic acid buffer pH 3.8, 30 min at 60°C. After lyophilisation the probe is ready to use.

Table 2: Gradient for the reversed phase-HPLC

time [min] buffer [%]	0	25	27	30	32	35
1M TEAA ^[a]	10	10	0	0	10	10
H ₂ O	85	55	0	0	85	85
AcCN ^[b]	5	35	100	100	5	5

[a] triethyl-ammonium acetate [b] acetonitrile

Table 3: Calculated melting points and the parameters for the characteristic maxima of A- and B-helix types (phosphate buffer, pH 7)⁶

Oligomer	T _m [°C]	ΔT _m [°C]	λ ₁	
			x[nm]	y[mdeg]
DNA(dT)-DNA(dA)	48.9±0.1	0	276	2.62
DNA(d <u>T</u>)-DNA(d <u>A</u>)	48.6±0.1	-0.3	276	1.09
DNA(dT)-RNA(rA)	50.9±0.2	0	273	3.38
DNA(dA)-RNA(rU)	49.4±0.4	0	277	3.99
DNA(d <u>T</u>)-RNA(r <u>A</u>)	47.8±0.2	-3.1	270	2.41
DNA(d <u>A</u>)-RNA(r <u>U</u>)	45.7±0.2	-3.7	282	1.94
RNA(rU)-RNA(rA)	63.7±0.2	0	261	6.74
RNA(r <u>U</u>)-RNA(r <u>A</u>)	63.5±0.4	-0.2	266	2.46

The synthesized oligonucleotides were characterized besides mass spectroscopy by measuring T_m-values and CD-spectra. The masses were determined on an ESI microTOF QII mass

spectrometer from Bruker. UV melting curves (T_m) of the duplexes dissolved in phosphate buffer (10 mM Na₂HPO₄, 10 mM NaH₂PO₄, 140 mM NaCl, 0.01 mM duplex, pH 7) were recorded on a UV-/VIS-600 (Jasco) spectrophotometer equipped with a Peltier thermostat from Jasco. The detection was monitored at 260 nm, in the range from 5 to 95°C and with a heating rate of 0.5 °C/min. CD spectra were measured on a temperature-controlled J-710 spectropolarimeter from Jasco. The scans ranged from 200 to 350 nm and the temperature was 20°C. Typical A-helix curve was observed for RNA and a B-helix type for the DNA duplex.⁶ In case of hetero hybrids differentiation between labelled and unlabelled double helices is necessary. In contrast to the labelled the unmodified ones show A-like structure. Both modified hybrids exhibited two distinguishable forms, one A- and one B-like form depending on the spin label position. The comparison is shown in figure 3.

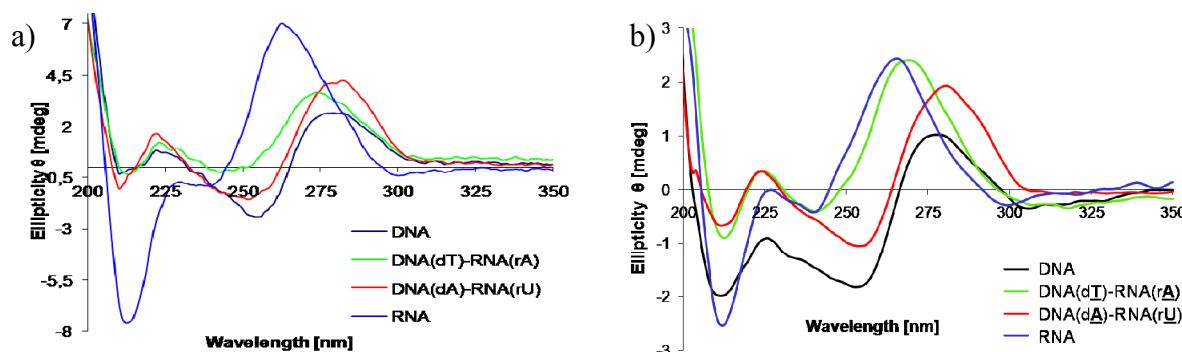


Figure 3: a) CD-spectra of unmodified b) double spin-labelled helices

4. PELDOR-experiments

The duplexes for distance measurements were dissolved in the same buffer with addition of 20%-ethylene glycol

The dead-time free 4-pulse PELDOR sequence was used for all experiments.⁷ A Bruker Elexsys E580 X-band spectrometer equipped with a PELDOR unit (E580-400U) was used. Microwave pulses were amplified by a 1 kW TWT amplifier (ASE 117x). Typical pulse lengths were 32 ns ($\pi/2$ and π) for the probe pulses and 12 ns for the pump inversion pulse. The time delay between first and second probe pulse was varied between 132 and 196 ns in 8 ns steps to reduce contributions from proton modulations. The pulse separation between the second and third probe pulses was 1.5 μ s. The frequency of the pump pulse was fixed to the central peak of the nitroxide powder spectrum to obtain maximum pumping efficiency. The probe frequency was chosen 70 MHz above this frequency. This frequency offset corresponds

to a large PELDOR effect and minimum of unwanted artefacts due to detection/pump pulse overlap. The temperature was set to 40 K for all experiments and controlled by an Oxford flow cryostat. Typically, the signal was averaged for approximately 6 h to obtain sufficient signal-to-noise ratio.

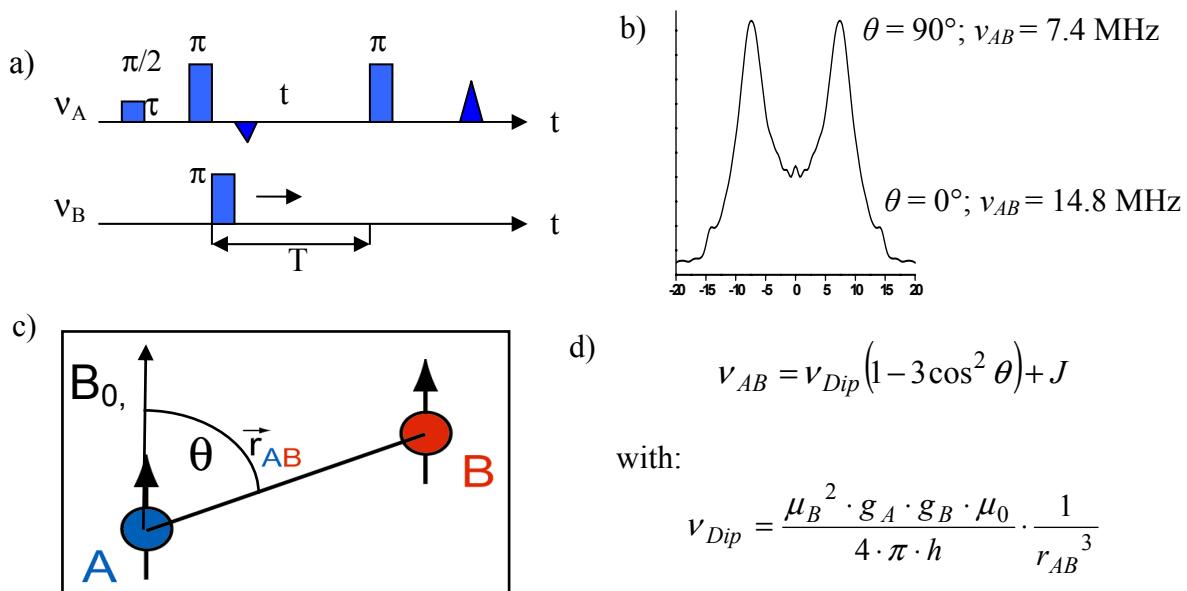


Figure 4: a) 4-Pulse ELDOR sequence b) Pake Power Pattern (Fourier Transform of PELDOR time domain signal c) definition of angle θ d) equation for distance calculation (secular approximation) J = exchange coupling constant

In figure 5 the background divided experimental traces (black) together with the Tikhonov fit (red) is shown in the right upper panel, the distance distribution resulting from the Tikhonov-regularisation⁸ is shown in the lower left panel and the final Pake Power Pattern resulting from a Fourier-transformation from the PELDOR time trace is represented in the lower right panel. The comparison of the oscillation depth and the oscillation time resulted in two types. The PELDOR time traces of the B-helix type samples (figure 5 a and c) show a long pronounced dipolar modulation resulting in a narrow distance distribution, whereas for the A-helix type samples this oscillation is much faster damped (figure 5 b and d). The modulation depth of the RNA probe is about 0.4 corresponding to a very high labelling efficiency. The other three spin labelled duplexes show somewhat reduced modulation depth (DNA: 0.3; DNA(dT)-RNA(rA): 0.25 and DNA(dA)-RNA(rU): 0.3).

The PELDOR-measurements resulted in only one main distance for each duplex. In the case of A-like helix the distance distribution is wider as in the B-like conformation.

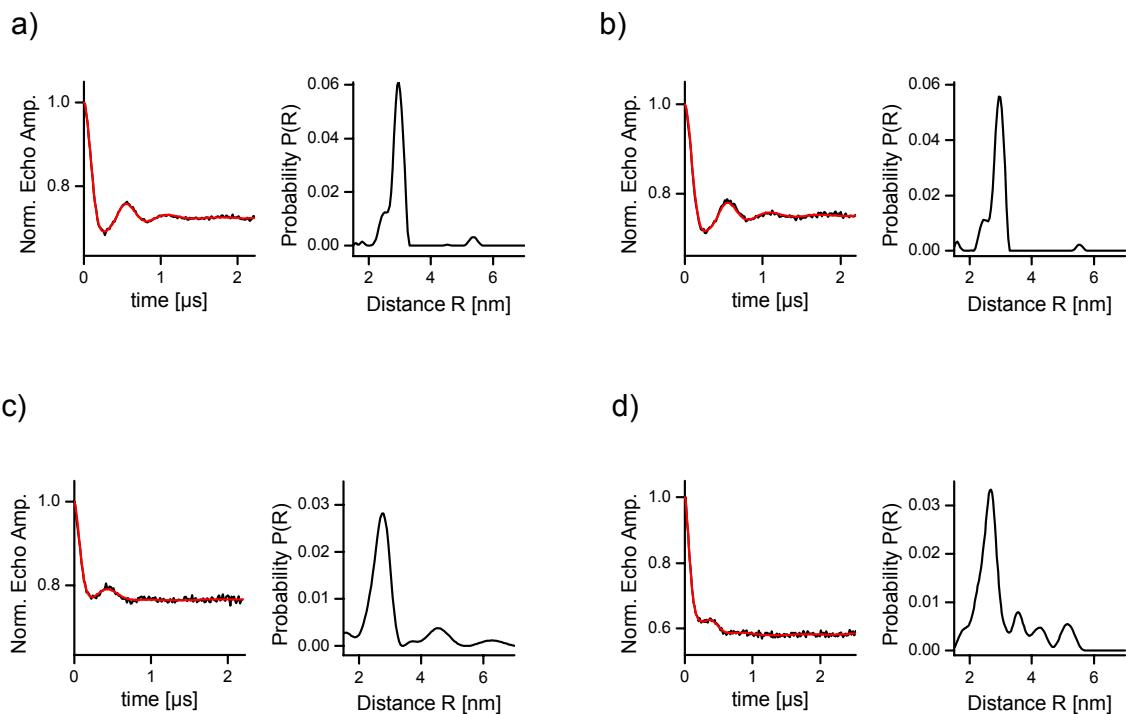


Figure 5: a) DNA-duplex b) DNA(dA)-RNA(rU) hybrid c) DNA(dT)-RNA(rA) hybrid d) RNA-duplex

5. RNase H kinetic experiments

For treatment with the enzyme RNase H (*Bacillus*) the double helices (6.5 nmol of each oligomer) were formed in sterile water (43 μL). After the injection of RNase H-buffer (5 μL of a 10x stock buffer; 50 mM Tris-Cl, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, pH 8.3) and the enzyme (2 μL) the probes were incubated at 37°C. After different time lapses 5 μL of the incubated solution was taken out and the enzymatic reaction was stopped by freezing to -20°C. The cleavage was followed by anionic exchange HPLC (Dionex DNAPac® PA-100, 9x 250mm; 1M LiCl, gradient 0 to 56% in 34 min).

The modified hetero hybrid with A-helix like conformation was cleaved faster than the rest of the tested duplexes. As control the pure DNA- and pure RNA-double helixes were also incorporated. Here, no cleavage was observed.

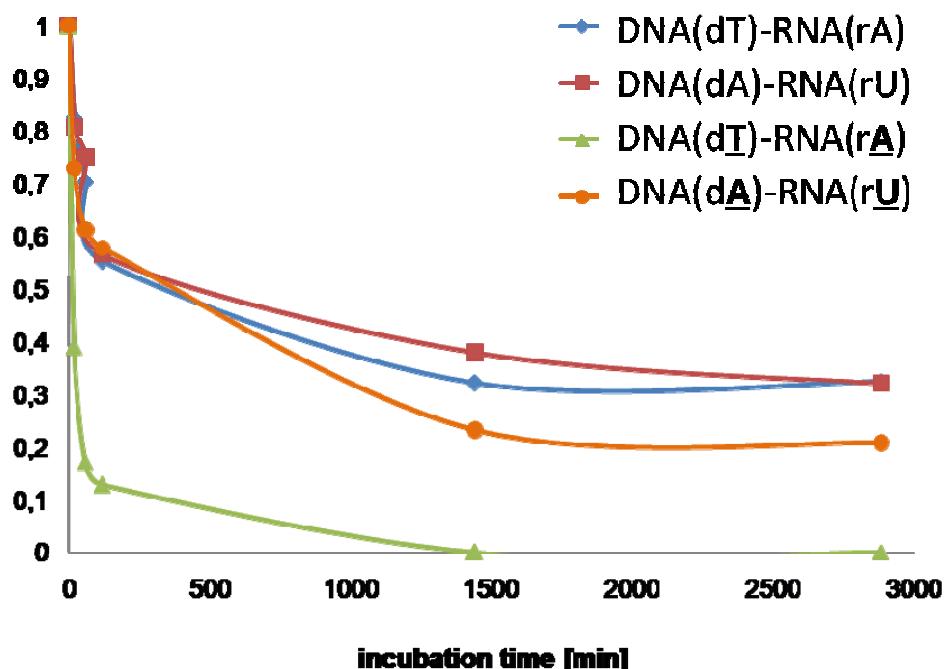


Figure 6: RNase H1-cleavage of the modified and unmodified hetero helices

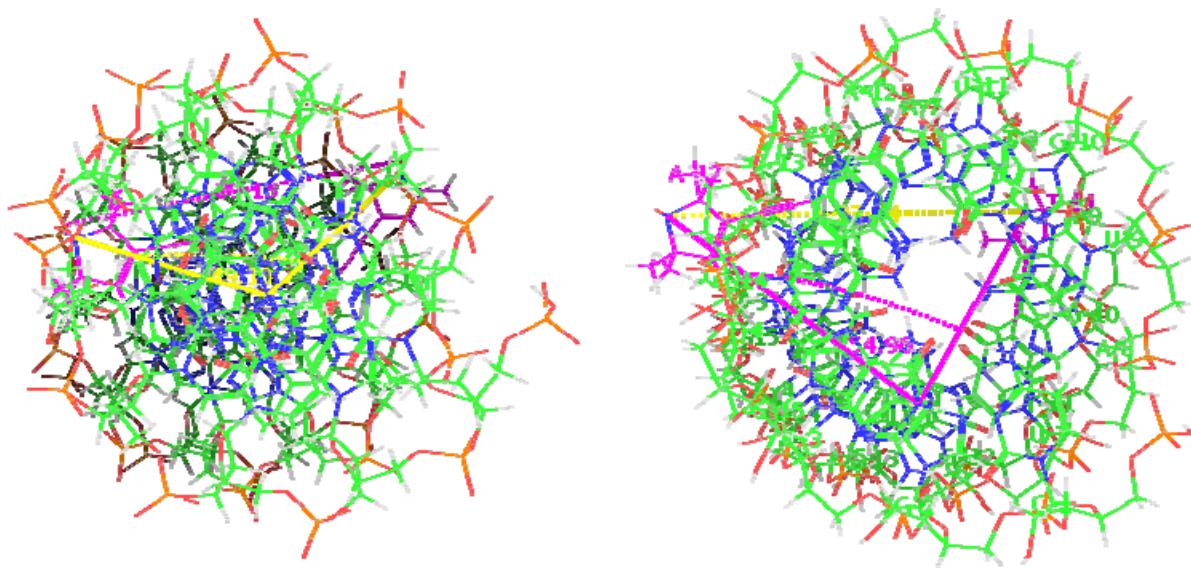


Figure 7: angel between both TPA spin labels a) DNA-duplex (B-helix) b) RNA-duplex (A-helix)

6. PyMol modelling⁹

To observe the sterical hindrance of the spin label TPA we modified the optimal B- and A-helix with TPA. The resulting pictures indicate the possible interactions between the incorporated label and the DNA- or RNA-construct (shown in fig. 7).

The major groove of the B-helix accommodates the paramagnetic spin label better in contrast to the minor groove. Here the methyl groups of the spin label touch the phosphate moiety (shown in fig. 8).

Incorporation of the nitroxide TPA at the minor groove of the A-form does not disturb the structure, because of the width of this groove. In the case of the major groove, the spin label is placed very close to the phosphate backbone.

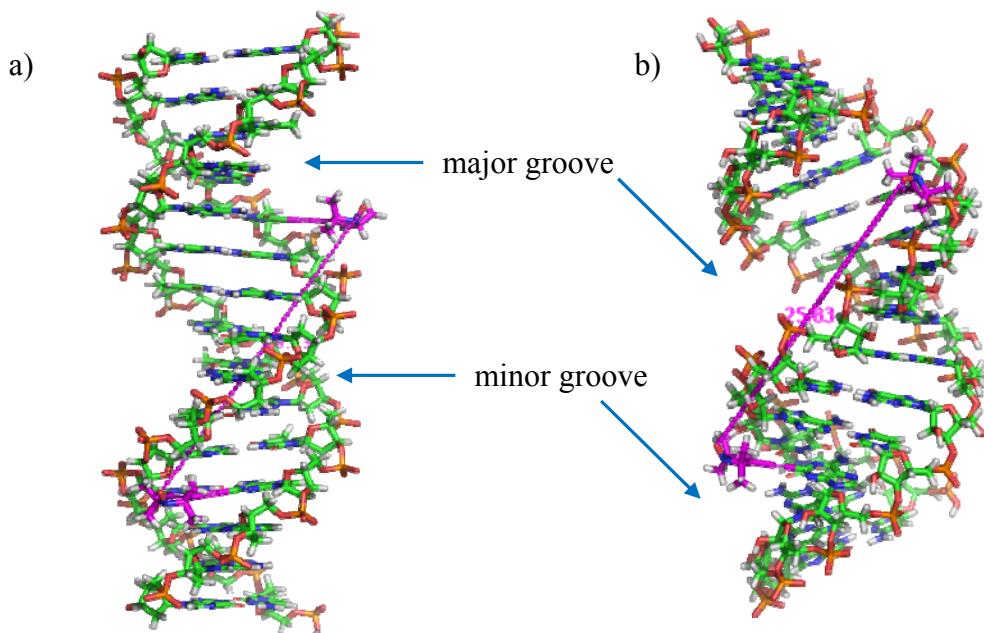


Figure 8: TPA labelled a) DNA-duplex (B-helix) b) RNA-duplex (A-helix)

7. Literature

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