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SYNTHESIS OF CARBOXAMIDE LINKED DIMERS, αT*αT AND αU^{Cl}*αT. - DUPLEX AND TRIPLEX STABILITIES OF THE CORRESPONDING α OLIGODEOXYNUCLEOTIDES

Abdel Aleem H. Abdel Aleem, Erik Larsen and Erik B. Pedersen*

Department of Chemistry, Odense University, DK-5230 Odense M, Denmark

Abstract: Two carboxamide linked dimers were synthesized by condensation of the appropiate α nucleosides with α thymidine-5'-carboxylic acid and incorporated in oligodeoxynucleotides. The oligodeoxynucleotides were investigated for hybridization to single and double stranded DNA.

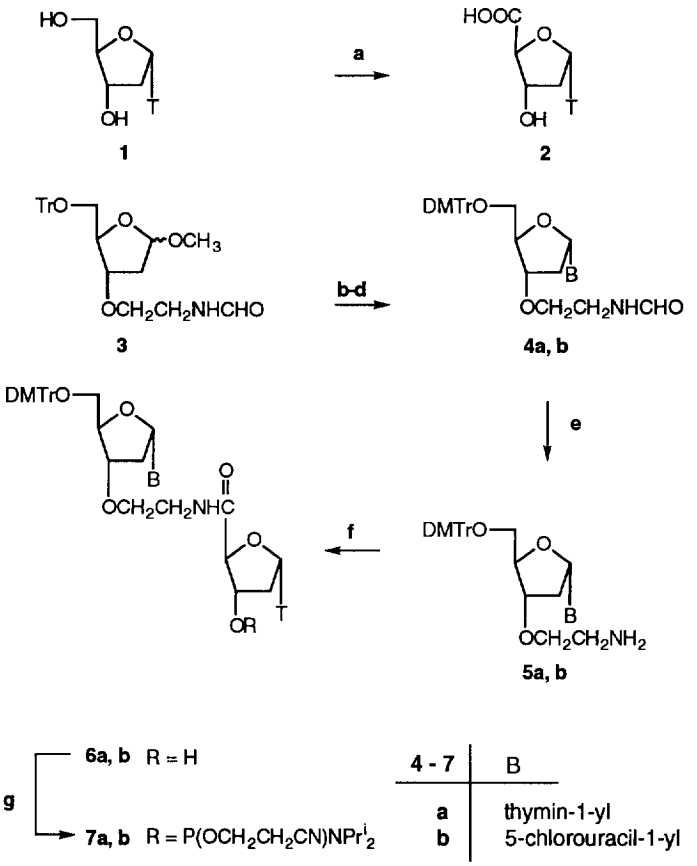
INTRODUCTION

α Oligonucleotides have shown good hybridization properties toward DNA or RNA with stability against nuclease degradation.[1-6] This makes them good candidates for antisense studies. Recently, we prepared a 5-atom carboxamide linked T*T dimer by alkylation of 5'-*O*-(4,4'-dimethoxytrityl)thymidine with 2-chloroethylamine and subsequent condensation with thymidine-5'-carboxylic acid.[7] Incorporation of the dimer into oligodeoxynucleotides showed good hybridization to natural DNA and high enzymatic stability. This promted us to device a more general synthetic method of the 3'-*O*-(2-aminoalkyl) nucleosides starting from 2-deoxy-D-ribose.[8] In order to investigate the dimer with other nucleobases and configurations we have used this method to prepare a αT*αT and a αU^{Cl}*αT dimer and incorporated it into oligodeoxynucleotides. This paper concerns the duplex and triplex properties of modified α oligodeoxynucleotides.

RESULTS AND DISCUSSION

α Thymidine **1** was oxidized at the 5'-position using Pt/O_2[9] to 1,2-dideoxy-1-thyminyl-α-D-*erythro*-pentofuranuronic acid (**2**). Methyl 2-deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-trityl-α,β-*erythro*-pentofuranoside (**3**) was obtained from 2-deoxy-D-ribose in three steps as described by Abdel Aleem *et al.*[8] Coupling of **3** with silylated uracil derivatives[10] using trimethylsilyl trifluoromethanesulfonate (TMS triflate) as Lewis acid catalyst

as described by Vorbrüggen *et al.*[11] gave an anomeric mixture of the protected nucleosides in moderate yield (44-60%) (scheme). The 1-(2-deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-[4,4'-dimethoxytrityl]-α-*erythro*-pentofuranosyl)uracil derivatives **4a,b** were obtained in 68% and 78% yields, respectively, by detritylation of the anomeric mixture with aqueous acetic acid at reflux temperature, separation of the anomers followed by protection of the α anomer with 4,4'-dimethoxytrityl chloride in dry pyridine. The α anomeric configuration was confirmed by NOE. The 2'α proton appeared as a large doublet in the ^{1}H-NMR spectrum due to geminal coupling to the 2'β proton. No couplings were observed from H2'α to H1' and H3'. This also confirmed the α anomeric configuration. Deformylation of compounds **4a,b** with MeONa in MeOH overnight gave



Scheme. a) Pt, H_2O, O_2, 80 °C, b) *O,O'*-bis(trimethylsilyl)uracil derivative, TMS triflate, CH_3CN, -20 °C, c) 80 % aqueous AcOH, reflux, d) DMTrCl, pyridine, r.t., e) NaOMe, MeOH, reflux, f) **2**, DPPA, Et_3N, DMF, g) $NCCH_2CH_2OP(Cl)NPr^i_2$, CH_2Cl_2, $EtNPr^i_2$.

compounds **5a,b** in 66% and 80% yield, respectively. Condensation of α thymidine-5'-carboxylic acid **2** with the 1-[3-*O*-(2-aminoethyl)-2-deoxy-5-*O*-(4,4'-dimet-hoxytrityl)-α-*erythro*-pentofuranosyl]uracil derivatives, **5a,b** using diphenyl phosphorazidate ($(PhO)_2P(O)N_3$, DPPA)[12,13] as the condensing agent, gave the dimers αT*αT **6a** and αU^{Cl}*αT **6b** in 81% and 82% yield, respectively. The dimers were further converted to their corresponding phosphoramidites **7a,b** using 2-cyanoethyl *N,N,*-diisopropylphosphor-amidochloridite ($NCCH_2CH_2OP(Cl)NPr^i_2$) as described by Sinha *et al.*[14]

α oligodeoxynucleotides base pair with a complementary β strand to form a parallel-stranded duplex.[15] The duplex hybridization properties were examined by synthesizing homo-pyrimidine α oligodeoxynucleotides, mixing each oligodeoxynucleotide with its complementary β DNA strand (5'-GGGGAAAGAAAAAA-3') and determining the melting points by UV-measurements.[16,17] The α oligodeoxynucleotides **9-15** were synthesized by standard phosphoramidite methodology on an automated DNA-synthesizer using the phosphoramidites of α 2'-deoxycytidine[15,18,19] and α thymidine,[15,19] compounds **7a,b** and silica derivatized with α thymidine.[19] The coupling efficiencies for compound **7a,b** were app. 95 % compared to 99 % for the other α amidites as monitored by the release of 4,4'-dimethoxytrityl cation after each coupling step. The oligodeoxynucleotides were deblocked and removed from the solid support by concentrated aqueous ammonia at 55 °C. In table 1 the melting temperatures (T_m) and the differences between oligodeoxynucleotide **9** and the modified oligomers **10-15** as the decrease in T_m per modification (ΔT_m) are shown.

Table 1. Sequences and duplex melting experiments of synthesized oligodeoxynucleotides

Sequence	No	T_m[a]/°C	ΔT_m[b]/°C
5'-β(TTTTTTCTTTCCCC)-3'	**8**	43.6	-
5'-α(CCCCTTTCTTTTTT)-3'	**9**	47.2	-
5'-α(CCCCTU^{Cl}*TCTTTTTT)-3'	**10**	45.8	-1.4
5'-α(CCCCTT*TCTTTTTT)-3'	**11**	45.4	-1.8
5'-α(CCCCTTTCU^{Cl}*TTTTT)-3'	**12**	45.4	-1.8
5'-α(CCCCTTTCT*TTTTT)-3'	**13**	45.4	-1.8
5'-α(CCCCTT*TCT*TTTTT)-3'	**14**	43.8	-1.7
5'-α(CCCCTTTCT*TT*TTT)-3'	**15**	43.4	-1.9

[a] T_m estimated to be +/- 0.4 °C; [b] ΔT_m = change in T_m per modification.

From the table it can be seen that the unmodified α oligodeoxynucleotide hybridize better than the complementary β strand with T_m app. 3.6 °C higher for this 14-mer. Incorporation of the dimers in the α strand results in a slight destabilization of app. 1.4-1.9 °C per modification which seems to be independent of the

position of the modification. Its seems to be of no importance for the hybridization properties whether 5-chlorouracil or thymine is used as the nucleobase, in agreement with previous reported results.[20]

α Oligodeoxynucleotides with the nucleobases cytosine and thymine bind to the major groove of a DNA duplex in a antiparallel orientation.[21] The triplex hybridization properties of the oligodeoxynucleotides **8-15** were examined almost similarly as above[22] but with each oligonucleotide mixed with the complementary DNA duplex. In table 2 the melting temperatures (T_m) and the difference between the unmodified and the modified oligomers as the change in T_m per modification (ΔT_m) are shown.

Table 2. Triplex melting experiments of synthesized oligodeoxynucleotides

Sequence no.	T_m[a]/°C	ΔT_m[b]/°C
8	44.0	-
9	35.2	-
10	40.0	4.8
11	40.0	4.8
12	37.2	2.0
13	37.6	2.4
14	42.4	3.6
15	38.0	1.8

[a] T_m estimated to be +/- 0.4 °C; [b] ΔT_m = change in T_m per modification.

From table 2 it can be seen that the ability to form triplex is much lower (ΔT_m app. -8.8 °C) for the unmodified α oligodeoxynucleotide **9** compared to the complementary β strand **8**. Incorporation of the dimers resulted in a stabilization of the triplex; e.g. ΔT_m is app. 4.8 °C for middle modifications (sequence **10** and **11**) and 2.0-2.4 °C for modifications closer to the 3'-end (sequence **12** and **13**). No significant differences in hybridization properties between the two dimers was observed, indicating the same ability of 5-chlorouracil and thymine to do Hoogsteen base pairing. Incorporation of two dimers (**7a**) gave an increase in the melting points which can be considered as a sum of each contribution. Sequence **14** increases the melting point with 7.2 °C (4.8 °C + 2.4 °C) compared to the unmodified α sequence **9** and sequence **15** increases the melting point with 3.6 °C. Incorporation of dimers with longer linkages, especially in the middle of an α oligonucleotide, seems to be promising modifications in order to improve the triplex forming properties.

EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-250 FT NMR spectrometer at 250 MHz for ^{1}H NMR and 62.9 MHz for ^{13}C NMR with TMS as an internal standard. ^{31}P-NMR spectra were recorded on the same spectrometer with 85% H_3PO_4 as internal reference. FAB mass spectra were recorded on a Kratos MS-50 TS spectrometer. Silica gel (0.04-0.063 mm) and analytical silica gel TLC plates 60 F_{254} were purchased from Merck.

1,2-Dideoxy-1-thyminyl-α-D-*erythro*-pentofuranuronic acid (2). α Thymidine **1** (0.785 g, 3.2 mmol) was dissolved in an aqueous buffer (120 ml, 0.30 g $NaHCO_3$ and adjusted with Na_2CO_3 to pH 9) and reduced Adams catalyst[23] (0.5 g) was added. The reaction mixture was stirred for 4 h at 80 °C under an oxygen atmosphere. The catalyst was removed by decanting and Amberlite IR-120 (H^+ form, 12 ml) was added followed by stirring for 10 min. The mixture was filtered and the filtrate evaporated under reduced pressure. Compound **2** crystallized from water (10 ml) by standing at 5 °C overnight, was removed by filtration, washed with ice-cold water (5 ml) and finally dried *in vacuo*. Yield 0.45 g (55 %), colourless solid, mp 229-231°C. ^{1}H NMR (DMSO-d_6/TMS): δ 1.78 (3H, s, CH_3), 1.91 (1H, d, J = 14.7 Hz, H2'α), 2.45-2.57 (1H, m, H2'β), 4.46 (1H, d, J = 5.4 Hz, H3'), 4.63 (1H, s, H4'), 5.88 (1H, br. s, OH), 6.36 (1H, dd, J = 2.7, 8.3 Hz, H1'), 7.82 (1H, d, J = 0.8 Hz, H6), 11.24 (1H, s, NH), 13.01 (1H, br. s, COOH). ^{13}C NMR (DMSO-d_6/TMS): δ 38.60 (C2'), 72.29 (C3'), 85.02 (C1'), 85.61 (C4'), 109.18 (C5), 137.00 (C6), 150.52 (C2), 163.71 (C4), 171.77 (C5').

1-(2-Deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-[4,4'-dimethoxytrityl]-α-D-*erythro*-pentofuranosyl)uracil derivatives 4:

To a stirred solution of methyl 2-deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-trityl-α,β-pentofuranoside **(3)**[8] (6.8 mmol) and *O,O'*-bis(trimethylsilyl)uracil derivative[10] (11.2 mmol) in dry MeCN (100 ml) was added TMS triflate (1.3 ml, 6.8 mmol) at −30 °C. After complete addition, the reaction mixture was stirred at room temperature overnight, diluted with CH_2Cl_2 (300 ml) and extracted with cold saturated aqueous $NaHCO_3$ (150 ml). The aqueous solution was extracted with CH_2Cl_2 (2 × 150 ml). The combined organic layers were washed with cold H_2O, dried over Na_2SO_4 and evaporated under reduced pressure to give anomeric mixtures in moderate yields (48-60%) after purification using silica chromatography with CH_2Cl_2/MeOH (98:2) as eluent. The anomeric mixture was detritylated in 80 % aqueous acetic acid (5 ml) at reflux temperature for 10 min. After cooling, triphenylmethanol was filtered off and the filtrate was poured into ice-water (20 ml) and evaporated under reduced pressure. The residue was chromatographed on silica gel (100 g) with the gradient 5-10 % MeOH in CH_2Cl_2 to give the deprotected α anomers. The α anomers (10 mmol) were dissolved in dry pyridine (5 ml) and 4,4'-dimethoxytrityl chloride (3.50 g, 10.35 mmol) was added. After stirring for 5 h at room temperature the reaction mixture was quenched by addition of MeOH (1 ml). The mixture was evaporated under

reduced pressure and the residue was chromatographed on silica gel (75 g) with CH_2Cl_2/MeOH (98:2) as eluent to give **4a,b**.

1-(2-Deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-[4,4'-dimethoxytrityl]-α-D-*erythro*-pento-furanosyl)thymine (4a): Yield 1.71 g (41% from **3**). ^{1}H NMR ($CDCl_3$/TMS): δ 1.92 (3H, s, CH_3), 2.24 (1H, d, J = 14.6 Hz, H2'α), 2.63-2.70 (1H, m, H2'β), 3.14-3.23 (2H, m, H5'), 3.37-3.54 (4H, m, CH_2CH_2), 3.79 (6H, s, 2xOCH_3), 4.01 (1H, d, J = 5.7 Hz, H3'), 4.47 (1H, t, J = 4.1 Hz, H4'), 6.28 (1H, m, H1'), 6.85 (4H, d, J = 8.9 Hz, H_{Arom}), 7.19-7.41 (9H, m, H_{Arom}), 8.12 (1H, s, H6), 9.21 (1H, s, CHO). ^{13}C NMR ($CDCl_3$/TMS): δ 12.55 (CH_3), 37.72 (CH_2N), 38.18 (C2'), 55.10 (OCH_3), 63.85 (C5'), 67.76 (OCH_2), 80.47 (C3'), 85.65 (C1'), 86.56 (C4'), 86.84 (CAr_3), 109.53 (C5), 113.05, 126.88, 127.69, 127.86, 129.82 (C_{Arom}), 135.34 (C6), 144.26 (C_{Arom}), 150.27 (C2), 158.50 (C_{Arom}), 161.05 (CHO), 163.97 (C4).

5-Chloro-1-(2-deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-[4,4'-dimethoxytrityl]-α-D-*erythro*-pentofuranosyl)-uracil (4b). Yield: 1.60 g (37% from **3**). ^{1}H NMR ($CDCl_3$/TMS): δ 2.23 (1H, d, J = 14.5 Hz, H2'α), 2.60-2.86 (1H, m, H2'β), 3.16-3.18 (2H, m, H5'), 3.43-3.44 (4H, m, CH_2CH_2), 3.79 (6H, s, OCH_3), 3.98 (1H, m, H3'), 4.51 (1H, m, H4'), 6.26 (1H, d, J = 5.7 Hz, H1'), 6.77-6.85 (4H, m, H_{Arom}), 7.26-7.67 (9H, m, H_{Arom}), 7.85 (1H, d, J = 1.8 Hz, H6), 8.15 (1H, s, CHO), 8.62 (1H, s, NH).^{13}C NMR ($CDCl_3$/TMS): δ 37.95 (CH_2N), 38.34 (C2'), 55.13 (OCH_3), 63.83 (C5), 67.85 (OCH_2), 80.37 (C3'), 86.24 (C1'), 86.69 (C4'), 87.63 (CAr_3), 107.50 (C5), 113.20, 126.93, 127.88, 129.85, 135.32, 135.41 (C_{Arom}), 137.57 (C6), 144.22 (C_{Arom}), 149.55 (C2), 158.55 (C_{Arom}), 159.10 (C4), 161.31 (CHO).

1-[3-*O*-(2-Aminoethyl)-2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]uracil derivatives 5:

MeONa (0.49 g, 9.1 mmol) in MeOH (5 ml) was added dropwise to a stirred solution of the compound **4** (1.8 mmol) in MeOH (10 ml) at room temperature and the reaction mixture was refluxed overnight. After cooling, the solvent was evaporated and the crude product was purified by column chromatography on silica gel (50 g) with 5-10% MeOH in CH_2Cl_2 to give **5a,b**.

1-[3-*O*-(2-Aminoethyl)-2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]thymine (5a): Yield 0.70 g (66%). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 588 ($M+H^+$). ^{1}H NMR ($CDCl_3$/TMS): δ 1.91 (3H, s, CH_3), 2.19 (1H, d, J = 14.8 Hz, H2'α), 2.60-2.71 (1H, m, H2'β), 3.10-3.24 (2H, m, H5'), 3.44 (4H, t, J = 5.1 Hz, CH_2CH_2), 3.79 (6H, s, 2xOCH_3), 4.02 (1H, d, J = 5.6 Hz, H3'), 4.53 (1H, t, J = 4.3 Hz, H4'), 6.35 (1H, d, J = 6.3 Hz, H1'), 6.84 (4H, d, J = 9.0 Hz, H_{Arom}), 7.21-7.43 (9H, m, H_{Arom}), 7.51 (1H, s, H6). ^{13}C NMR ($CDCl_3$/TMS): δ 12.45 (CH_3), 38.11 (CH_2N), 41.49 (C2'), 55.07 (OCH_3), 63.97 (C5'), 70.94 (OCH_2), 80.30

(C3'), 85.77 (C1'), 86.46 (C4'), 86.55 (CAr_3), 109.66 (C5), 113.10, 126.80, 127.80, 127.90, 129.83 (C_{Arom}), 135.43 (C6), 144.32 (C_{Arom}), 150.44 (C2), 158.45 (C_{Arom}), 164.03 (C4).

1-[3-*O*-(2-Aminoethyl)-2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl]-5-chlorouracil(5b). Yield 0.87 g (80%). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 608 ($M+H^+$). ^1H NMR ($CDCl_3$/TMS): δ 2.27 (1H, d, J = 15.0 Hz, H2'α), 2.59-2.71 (1H, m, H2'β), 2.86 (2H, t, J = 5.1 Hz, CH_2N), 3.10-3.24 (2H, m, H5'), 3.42-3.51 (2H, m, OCH_2), 3.79 (6H, s, OCH_3), 4.01 (1H, d, J = 5.4 Hz, H3'), 4.59 (1H, t, J = 4.3 Hz, H4'), 6.30 (1H, d, J = 6.8 Hz, H1'), 6.84 (4H, d, J = 8.9 Hz, H_{Arom}), 7.18-7.41 (9H, m, H_{Arom}), 7.91 (1H, s, H6). ^{13}C NMR ($CDCl_3$/TMS): δ 38.20 (CH_2N), 41.26 (C2'), 55.11 (OCH_3), 63.95 (C5'), 70.69 (OCH_2), 80.22 (C3'), 86.39 (C1'), 86.53 (C4'), 87.57 (CAr_3), 107.76 (C5), 113.14, 126.86, 127.86, 129.84, 135.39 (C_{Arom}), 137.80 (C6), 144.30 (C_{Arom}), 149.69 (C2), 158.49 (C_{Arom}), 159.50 (C4').

Carboxamide Linked Deoxydinucleosides 6a,b. General Procedure:

Compound **5** (1.64 mmol) and compound **2** (1.37 mmol) were dissolved in dry DMF (20 ml) and cooled to −30°C. DPPA (1.62 mmol) was added followed by triethylamine (1.64 mmol). The resulting solution was stirred at room temperature for 3 h and evaporated to dryness under reduced pressure. The products were purified on silica gel using 10 % MeOH in CH_2Cl_2 as eluent to give **6a,b**.

αT*αT Carboxamide Linked Deoxydinucleoside 6a. Yield 1.01 g (81%). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 826 ($M+H^+$). ^1H NMR (DMSO-d_6/TMS): δ 1.71 and 1.82 (6H, 2xs, 2xCH_3), 2.03-2.08 (2H, m, H2''), 2.37-2.50 (2H, m, H2'), 3.08 (2H, s, CH_2N), 3.25 (2H, m, H5'), 3.45 (2H, s, OCH_2), 3.75 (6H, s, 2xOCH_3), 4.02 (1H, s, H4'), 4.40-4.47 (2H, m, H3', H4''), 4.62 (1H, s, H3''), 5.73 (1H, br. s, OH), 6.27 (1H, m, H1'), 6.37-6.39 (1H, m, H1''), 6.92 (4H, d, J = 7.5 Hz, H_{Arom}), 7.18-7.39 (9H, m, H_{Arom}), 7.79 (1H, s, H6'), 8.07 (1H, s, H6''), 8.56 (1H, s, NH linker), 11.25 (2H, s, 2xNH). ^{13}C NMR (DMSO-d_6/TMS): δ 12.12, 12.21 (2xCH_3), 37.17 (CH_2N), 38.10, 38.44 (C2', C2''), 54.91 (OCH_3), 63.73 (C5'), 66.88 (C3''), 72.61 (OCH_2), 79.45 (C3'), 84.04, 84.78 (C1', C1''), 85.77, (C4', C4''), 87.37 (CAr_3), 108.49, 109.03 (2xC5), 113.15, 126.62, 127.58, 127.77, 129.56 (C_{Arom}), 136.40, 136.82 (C6), 144.57 (C_{Arom}), 150.32, 150.43 (C2), 158.05 (C_{Arom}), 163.69 (C4), 169.41 (C5'').

αU^{Cl}*αT Carboxamide Linked Deoxydinucleoside 6b. Yield: 1.13 g (82%). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 846 ($M+H^+$). ^1H NMR (DMSO-d_6/TMS): δ 1.84 (3H, s, CH_3), 2.20-2.26 (2H, m, H2''α), 2.43-2.56 (2H, m, H2'), 3.15 (2H, s, H5'), 3.29-3.51 (4H, m, CH_2CH_2), 3.81 (6H, s, 2xOCH_3), 4.10 (1H, s, H4'), 4.45 (1H, s, H4''), 4.63-4.67 (2H, m, H3', H3''), 5.80 (1H, br. s, OH), 6.27-6.29 (1H, m, H1'), 6.41-6.44 (1H, m, H1''), 6.98 (4H, d, J = 8.1 Hz, H_{Arom}), 7.24-7.43 (9H, m, H_{Arom}), 7.84 (1H, s, H6'), 8.00 (1H, s, H6''), 8.11 (1H, s, NH linker), 11.31 (1H, s, NH), 11.87 (1H, s, NH). ^{13}C NMR (DMSO-d_6/TMS): δ 12.21 (CH_3), 37.31

(CH_2N), 38.02, 38.43 (C2', C2''), 54.91 (OCH_3), 63.78 (C5'), 66.63 (C3''), 72.57 (OCH_2), 79.57 (C3'), 84.75, 85.80 (C1', C1''), 85.88, 86.07, (C4', C4''), 87.33 (CAr_3), 106.62, 108.48 (2xC5), 113.15, 126.65, 127.57, 128.04, 129.55 (C_{Arom}), 136.82, 138.08 (C6' and C6''), 144.54 (C_{Arom}) 149.46, 150.32 (2xC2), 158.05 (C_{Arom}), 158.92, 163.70 (2xC4), 169.41 (C5'').

Phosphoramidites 7. General Procedure:

Compound **6** (0.25 mmol) was dried by co-evaporation with anhydrous CH_3CN (2 ml) and dissolved under N_2 in anhydrous CH_2Cl_2 (1.5 ml). *N,N*-diisopropylethylamine (0.23 ml) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.10 ml, 0.57 mmol). After 1 h the reaction was quenched with CH_3OH (0.04 ml) and diluted with ethyl acetate (5.5 ml). The solution was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 ml) and a saturated aqueous solution of NaCl (3 × 5 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was redissolved in anhydrous toluene (1.0 ml) and precipitated in ice-cold petroleum ether (b.p. 60-80 °C, 200 ml). The products were collected by filtration and dried under reduced pressure to give compounds **7a,b** as colourless powders, respectively.

αT*αT 2-Cyanoethyl *N,N*-diisopropylphosphoramidite 7a.

Yield: 152 mg (63 %). ^{31}P NMR (DMSO-d_6): δ 154.21, 154.88

αU^{Cl}*αT 2-Cyanoethyl *N,N*-diisopropylphosphoramidite 7b.

Yield: 152 mg (58 %). ^{31}P NMR (DMSO-d_6): δ 154.21, 154.70

Oligodeoxynucleotide 8-15. The synthesis of oligonucleotides **8-15** were performed on a Pharmacia Gene Assembler Special^R DNA-synthesizer in 0.2 μmol-scale (5 μmol amidite per cycle, Pharmacia primer support^{TM}) using the 2-cyanoethyl phosphoramidites of 5'-*O*-(4,4'-dimethoxytrityl)-α-thymidine and 3-*N*-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-α-2'deoxycytidine as well as compounds **7a,b**. The synthesis followed the regular protocol of the DNA-synthesizer for 2-cyanoethylphosphoramidites. The coupling efficiencies of compounds **7a,b** was slightly lower (app. 95 %) than those of the unmodified α amidites (app. 99 %). The oligodeoxynucleotides were removed from the solid support by treatment with concentrated ammonia at 55 °C for 12 h which also removed the protecting groups on the nucleobase and the phosphorous. Purification of the oligodeoxynucleotides including 5'-*O* detritylation were performed on disposable reverse-phase chromatography cartridges.

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