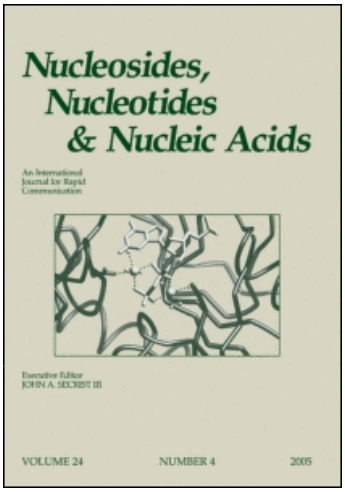

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Aleem, Abdel Aleem H. Abdel , Larsen, Erik and Pedersen, Erik B.(1995) 'Synthesis of a Carboxamide Linked $U^{Br*}U^{Br}$ Dimer-Duplex and Triplex Stabilities of the Corresponding Oligodeoxynucleotides', Nucleosides, Nucleotides and Nucleic Acids, 14: 9, 2027 — 2038
To link to this Article: DOI: 10.1080/15257779508010721
URL: http://dx.doi.org/10.1080/15257779508010721

NUCLEOSIDES & NUCLEOTIDES, 14(9&10), 2027-2038 (1995)

SYNTHESIS OF A CARBOXAMIDE LINKED $U^{Br}*U^{Br}$ DIMER — DUPLEX AND TRIPLEX STABILITIES OF THE CORRESPONDING OLIGODEOXYNUCLEOTIDES

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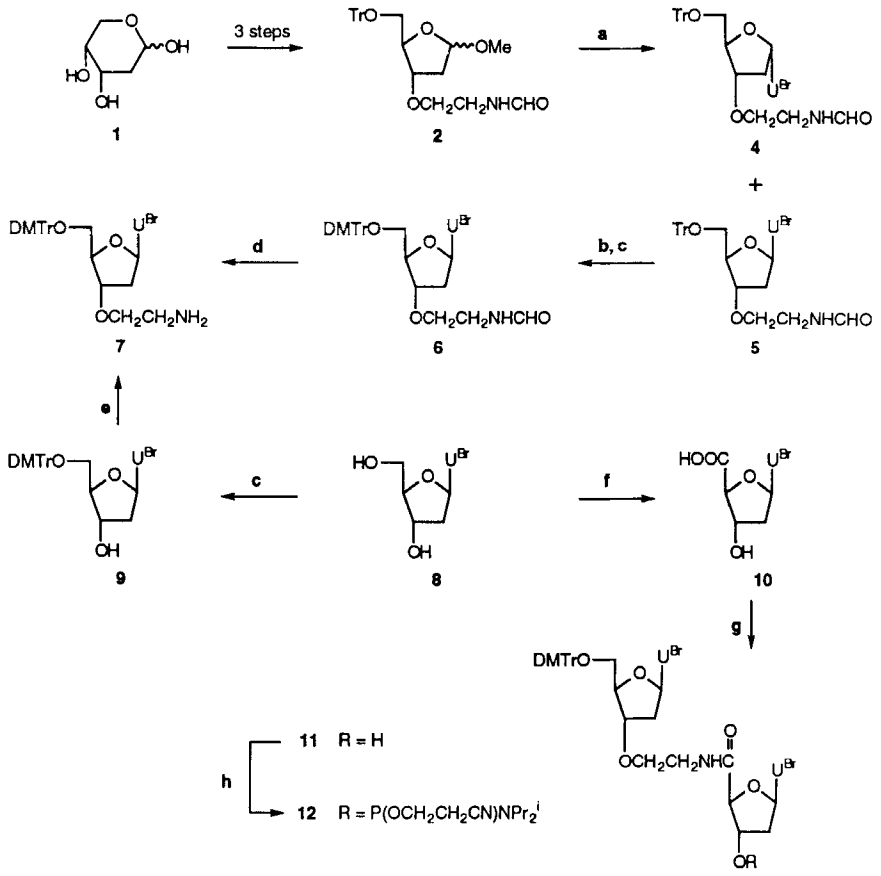
Abstract: A $U^{Br}*U^{Br}$ dimer was synthesized by connecting the appropiate nucleoside monomers through a 5-atom carboxamide linkage. The dimer was incorporated in oligodeoxynucleotides and investigated for hybridization properties toward single and double stranded DNA.

Currently, there is a great interest in the chemical synthesis of oligonucleotides bearing modification at the phosphodiester internucleotide linkages. These changes in the backbone of DNA fragments often lead to an increased stability towards enzymatic hydrolysis by nucleases and a greater overall lipophilicity without influencing too much the hybridization toward complementary single stranded DNA or RNA sequences. Therefore, such backbone-modified DNA fragments seem to be ideal tools for being applied in the control of gene expression on the RNA level known as the antisense approach.[1] This could finally lead to agents capable of stopping the growth of viruses or malignant cells.[2] Among the many types of oligonucleotide analogs,[3,4] methylphosphonates,[5-9] phosphorothioates,[10-13] phosphorodithioates[14,15] and phosphate-triesters,[16] have been intensively studied but often result in highly heterogeneous oligomers because of the introduction of chirality at phosphorous. Our research has focused on the development of modified nucleotides, replacing only the 3'-5'-phosphate linkage with a 5-atom



Scheme. a) *O,O'*-Bis(trimethylsilyl)-5-bromouracil (**3**), TMS triflate, MeCN, - 20 °C, b) 80 % aqueous AcOH, reflux, c) DMTrCl, pyridine, r.t., d) NaOMe, MeOH, reflux, e) $ClCH_2CH_2NH_2 \cdot HCl$, OH^- (excess), benzene/dioxane, 70 °C, f) Pt, O_2, H_2O, 80 °C, g) **7**, DPPA, Et_3N, DMF, h) $NCCH_2CH_2OP(Cl)NPr^i_2$, CH_2Cl_2, $EtNPr^i_2$. U^{Br} = 5-bromouracil-1-yl.

carboxamide.[17] This type of internucleoside linkage has been reported on several occassions since 1974.[18] The non-chiral carboxamide moiety having the advantage of being nonionic and much less polar than phosphate, has also been reported as a 4-atom internucleoside linkage.[19]

Methyl 2-deoxy-3-*O*-(2-formylaminoethyl)-5-*O*-trityl-α,β-*erythro*-pentofuranoside (**2**) was prepared from 2-deoxy-D-ribose (**1**) as described by Abdel Aleem *et al.*[20] Condensation of compound **2** with *O,O'*-bis(trimethylsilyl)-5-bromouracil (**3**) using the trimethylsilyl

trifluoromethanesulfonate (TMS triflate) method of Vorbrüggen *et al*[21] gave an anomeric mixture of the protected nucleosides which by chromatographic separation gave moderate yields of the α nucleoside **4** (37 %) and the corresponding β anomer **5** (10 %). To obtain 5-bromo-1-(2-deoxy-3-*O*-[2-(formylaminoethyl)]-5-*O*-[4,4'-dimethoxytrityl]-β-D-*erythro*-pentofuranosyl)uracil (**6**) compound **5** was detritylated with aqueous acetic acid at reflux temperature for 10 min, and protected again with 4,4'-dimethoxytrityl chloride in pyridine at room temperature. The product was purified by silica gel chromatography in 50 % overall yield. Deformylation of compound **6** by refluxing with MeONa/MeOH overnight gave 1-[3-*O*-(2-aminoethyl)-5-bromo-2-deoxy-*O*-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]uracil (**7**) in 85 % yield. Compound **7** could be synthesized by a simpler route by treatment of 5-bromo-2'-deoxyuridine **8** with 4,4'-dimethoxytrityl chloride in dry pyridine to give 5-bromo-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyuridine **9** in 76 % yield which on refluxing with 2-chloroethylamine and KOH in benzene/dioxane gave **7** in 20 % yield. Using the method of Moss *et al*[22] 5-bromo-2'-deoxyuridine was oxidized to 1-(5-bromouracil-1-yl)-1,2-dideoxy-β-D-*erythro*-pentofuranuronic acid (**10**) which was sufficiently pure after crystallization from water. The dimer (U^{Br}*U^{Br}, **11**) was prepared by condensation of **7** with **10** using diphenyl phosphorazidate ($(PhO)_2P(O)N_3$, DPPA)[23,24] as the condensing agent, and the product was obtained in 76 % yield after silica gel purification. Reaction of **11** with 2-cyanoethyl *N,N*-diisopropylphosphoroamido chloridite ($NCCH_2CH_2OP(Cl)NPr^i_2$) as described by Sinha *et al*[25] gave the corresponding phosphoramidite **12**.

In our laboratory we are currently investigating different approaches toward anti-HIV agents so we choose a HIV-1 oligonucleotide sequence (5'-GGGGAAAGAAAAAA-3')[26] as the test substrat for the duplex melting point experiments. Furthermore this homo-purine oligodeoxynucleotide sequence hybridized with its complementary DNA-strand should be a good test substrate for triplex melting point experiments. As the triple helix formation between an homo-pyrimidine strand and a DNA-duplex is paralleled orientated with respect to the homo-purine strand we choose, for convinience, the 3'-GGGGAAAGAAAAAA-5'/5'-CCCCTTTCTTTTTT-3' duplex as the test substrate. The homo-pyrimidine oligodeoxynucleotides **13-18** were synthesized by standard phosphoramidite methodology on an automated DNA-synthesizer using commercial 2-cyanoethyl phosphoramidites and compound **12**. The coupling efficiencies for compound

Table 1. Sequences and duplex melting experiments of synthesized oligodeoxynucleotides

Sequence[a]	No	T_m[b]/°C	ΔT_m[c]/°C
5'-CCCCTTTCTTTTTT-3'	**13**	47.2	
5'-$C^{Me}C^{Me}C^{Me}C^{Me}U^{Br}U^{Br}U^{Br}C^{Me}U^{Br}U^{Br}U^{Br}U^{Br}T$-3'	**14**	57.8	-
5'-$C^{Me}C^{Me}C^{Me}C^{Me}U^{Br}U^{Br}*U^{Br}C^{Me}U^{Br}U^{Br}U^{Br}U^{Br}T$-3'	**15**	54.0	-3.8
5'-$C^{Me}C^{Me}C^{Me}C^{Me}U^{Br}U^{Br}U^{Br}C^{Me}U^{Br}*U^{Br}U^{Br}U^{Br}T$-3'	**16**	53.8	-4.0
5'-$C^{Me}C^{Me}C^{Me}C^{Me}U^{Br}U^{Br}*U^{Br}C^{Me}U^{Br}*U^{Br}U^{Br}U^{Br}T$-3'	**17**	52.8	-2.5
5'-$C^{Me}C^{Me}C^{Me}C^{Me}U^{Br}U^{Br}U^{Br}C^{Me}U^{Br}*U^{Br}U^{Br}*U^{Br}U^{Br}T$-3'	**18**	53.2	-2.3

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

12 was app. 95 % compared to 99 % for the commercial 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.

The duplex hybridization properties of the oligonucleotides were examined by mixing each oligonucleotide with the complementary DNA-strand (3'-GGGGAAAGAAAAAA-5') and determining the melting points of the DNA-DNA-hybrids by UV measurements.[27,28]

In table 1 the melting temperatures (T_m) and the differences between oligodeoxynucleotide **14** and the other oligomers as the decrease in T_m per modification (ΔT_m) are shown. It can be seen that exchanging the nucleobases thymine and cytosine with 5-bromouracil and 5-methylcytosine gives a stabilization of the DNA duplex by 10.6 °C, which is in accordance with previously reported results.[29] Incorporation of the dimer gives a destabilization of the DNA duplex of 2-4 °C per modification. The decrease of the T_m per modification is higher when only one dimer is incorporated but more or less independant on its position on the DNA strand. The results are slightly higher compared to what has been reported for the corresponding T*T dimer (ΔT_m app. -1.5 - -2.5 °C).[17]

Table 2. Triplex melting experiments of synthesized oligodeoxynucleotides

Sequence no.	T_m[a]/°C	ΔT_m[b]/°C
14	32.4	-
15	28.8	-3.6
16	34.0	1.6
17	31.2	-0.6
18	34.4	1.0

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

The triplex hybridization properties of the oligodeoxynucleotides **14-18** were examined almost similarly as above[30] but with each oligonucleotide mixed with the complementary DNA duplex (5'-GGGGAAAGAAAAAA-3'/3'-CCCCTTTCTTTTTT-5'). The UV measurements were performed both at 260 nm and 284 nm, which gave allmost identical melting curves. 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.

From table 2 it can be seen that incorporation of the dimer either stabilize or destabilize the triplex dependant on the position of the dimer in the oligodeoxynucleotide. Incorporation of the dimer in the middle (sequence **15**) gives a destabilization of the triplex of app. 3.6 °C, incorporation in the "U^{Br} region" gives a stabilization of app. 1.0 - 1.6 °C (sequence **16** and **18**) and the combination a slight destabilization of app. 0.6 °C.

Incorporation of the corresponding T*T dimer has previously been shown to give protection against nuclease degradation, and similar results are to be expected with the $U^{Br}*U^{Br}$ dimer. Modification of oligonucleotides at the nucleobases in order to extend the triplex formation to physiological pH and with this carboxamide linkage at selected positions in order to prevent nuclease degradation, makes this a promissing tool in antigene theraphy.

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 tetramethylsilane as an internal standard. ^{31}P-NMR spectra were recorded on the same spectrometer with 85% H_3PO_4 as internal reference. Mass spectra (MS) were recorded using electron ionization (EI) on a varian Mat 311A spectrometer and fast atom bombardment (FAB) on a Kratos MS 50 spectrometer. Silica gel (0.040-0.063 mm) and analytical silica gel TLC plates 60 F_{254} were purchased from Merck.

5-Bromo-1-(2-deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-trityl-α-D-*erythro*-pentofuranosyl)uracil (4) and its β anomer 5. To a stirred solution of compound **2**[20] (4.0 g, 8.5 mmol) and *O,O'*-bis(trimethylsilyl)-5-bromouracil[31] (**3**) (3.75 g, 11.2 mmol) in anhydrous acetonitrile (100 ml) was added TMS triflate (1.67 ml, 8.6 mmol) dropwise at -30 °C. After complete addition the reaction mixture was stirred at room temperature overnight. The mixture was then diluted with CH_2Cl_2 (300 ml) and washed with a cold saturated aqueous solution of $NaHCO_3$ (150 ml). The aqueous solution was extracted with CH_2Cl_2 (2 x 150 ml). The combined organic layers was washed with cold H_2O, dried over Na_2SO_4 and evaporated under reduced pressure to give an anomeric mixture which was separated on silica gel (200 g) with CH_2Cl_2 and MeOH (98:2, v/v) to give the anomers **4** and **5**.

Compound 4: Yield 1.36 g (37 %). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 620 (M + H$^+$). ^{1}H NMR ($CDCl_3$): δ 2.23 (1H, d, J = 15.0 Hz, H2'a), 2.57-2.65 (1H, m, H2'b), 3.17-3.21 (m, 2H, H5'), 3.39-3.49 (4H, m, CH_2CH_2), 3.98 (1 H, d, J = 5.4 Hz, H3'), 4.52 (1H, t, J = 4.4 Hz, H4'), 5.27 (1H, s, NH), 6.26 (1H, d, J = 6.3 Hz, H1'), 7.21-7.44 (15H, m, H_{Arom}), 7.93 (1H, s, H6), 8.16 (1H, d, J = 1.3 Hz, CHO), 9.60 (1H, s, NH). ^{13}C NMR ($CDCl_3$): δ 37.99 (CH_2N), 38.31 (C2'), 63.95 (C5'), 67.76 (OCH_2), 80.30 (C-3'), 86.19 (C1'), 87.25 (CPh_3), 87.70 (C4'), 95.24 (C5), 127.24, 127.91, 128.42 (C_{Arom}), 140.48 (C6), 143.23 (C_{Arom}), 149.59 (C2), 159.23 (C4), 161.45 (CHO).

Compound 5: Yield 361 mg (10 %). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 620 (M + H$^+$). ^{1}H NMR ($CDCl_3$): δ 2.13-2.16 (1H, m, H2'a), 2.51-2.54 (1H, m, H2'b), 3.37 (2H, m, H-5'), 3.46 (4H, m, CH_2CH_2), 4.10 (2H, m, H3', H4'), 5.29 (1H, s, NH), 6.21 (1H, m, H1'), 7.20-7.40 (15H, m, H_{Arom}), 8.03 (1H, s, H-6), 8.17 (1H, s, CHO), 9.29 (1H,

s, NH). ^{13}C NMR ($CDCl_3$): δ 37.74 (CH_2N), 38.24 (C2'), 63.79 (C5'), 67.83 (OCH_2), 80.08 (C3'), 84.07 (C1') 85.54 (CPh_3), 87.63 (C4'), 97.30 (C5), 127.32, 127.53, 128.43 (C_{Arom}), 138.93 (C6), 143.09 (C_{Arom}), 149.75 (C2), 159.00 (C4), 161.48 (CHO).

5-Bromo-1-(2-deoxy-3-*O*-[2-(formylamino)ethyl]-5-*O*-[4,4'-dimethoxytrityl]-β-D-*erythro*-pentofuranosyl)uracil (6). The nucleoside **5** (1.30 g, 2.1 mmol) was detritylated with 80 % aqueous acetic acid (5 ml) at reflux temperature for 10 min. The reaction mixture was left at room temperature for 3 h and precipitated triphenylmethanol was filtered off. The filtrate were poured into ice-water (20 ml). Water and acetic acid were evaporated under reduced pressure and the residue was chromatographed on silica gel (100 g) with the gradient 5-10% MeOH in CH_2Cl_2. The deprotected nucleoside was dissolved in dry pyridine (10 ml) and 4,4'-dimethoxytrityl chloride (0.85 g, 2.5 mmol) was added. The reaction mixture was stirred at room temperature for 5 h, quenched by addition of MeOH (1 ml) and evaporated under reduced pressure. The residue was chromatographed on silica gel (75 g) with CH_2Cl_2 and methanol (98:2, v/v) as eluent to give the nucleoside **6**. Yield 0.71 g (50 %). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 680 (M + H^+). ^1H NMR ($CDCl_3$): δ 2.16-2.19 (1H, m, H2'β), 2.48-2.56 (1H, m, H2'α), 3.31-3.42 (2H, m, H5'), 3.48 (4H, m, CH_2CH_2), 3.79 (6H, s, 2xOCH_3), 4.12 (2H, m, H3', H4'), 6.10 (1H, br. s, NHC=O), 6.24 (1H, dd, J = 5.5, 8.5 Hz, H1'), 6.86 (4H, d, J = 8.1 Hz, H_{Arom}), 7.22-7.40 (m, 9H, H_{Arom}), 8.05 (s, 1H, H6), 8.17 (s, 1H, CHO), 9.14 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 37.77 (CH_2N), 38.30 (C2'), 55.18 (OCH_3), 63.64 (C5'), 67.87 (OCH_2), 80.20 (C3'), 84.23 (C1'), 85.56 (C4'), 87.17 (CAr_3), 97.25 (C5), 113.31, 127.07, 127.69, 127.91, 127.99, 129.84, 129.93, 135.14, 135.24 (C_{Arom}), 139.01 (C6), 144.12 (C_{Arom}), 149.51 (C2), 158.70 (C4), 161.25 (CHO).

1-[3-*O*-(2-Aminoethyl)-5-bromo-2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]uracil (7). *Method A.* To a stirred solution of compound **6** (0.53 g, 0.78 mmol) in MeOH (10 ml) was dropwise added MeONa (0.21 g, 3.9 mmol) in methanol (5 ml) at room temperature followed by reflux temperature overnight. The mixture was evaporated to dryness under reduced pressure and the residue was chromatographed on silica gel (100 g) with the gradient 5-10% MeOH in CH_2Cl_2. Yield 0.43 g (85%). *Method B.* 5-Bromo-2'-deoxyuridine (**8**, 5.0 g, 16.3 mmol) was dissolved in dry pyridine (30 ml)

followed by addition of 4,4'-dimethoxytrityl chloride (5.7 g, 16.8 mmol). After stirring for 3 h, the reaction mixture was quenched by addition of MeOH (1 ml), evaporated to dryness under reduced pressure and the resulting residue partioned between $CHCl_3$ (100 ml) and water (100 ml). The organic phase was dried over Na_2SO_4, evaporated and the residue chromatographed on silica gel with the gradient 5-10% MeOH in CH_2Cl_2. Yield 7.5 g (76 %) of 5-bromo-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyuridine (**9**). Compound **9** (4 g, 5.55 mmol) was dissolved in a mixture of benzene (40 ml) and 1,4-dioxane (20 ml). Powdered KOH (10.5 g) was added followed by 2-chloroethylamine hydrochloride (5.5 g, 65 mmol) and the reaction was refluxed for 3 h with vigorous stirring. The reaction mixture was cooled to room temperature and water (30 ml) was added followed by adjustment of pH to 7.5 with 4 M aqueous acetic acid. The benzene layer was separated, washed with water, dried over (Na_2SO_4), and the solvent evaporated. The product **7** was purified by silica gel chromatography using the gradient 5-10 % CH_3OH in CH_2Cl_2. Yield: 1.5 g (20 %). FAB MS ($CHCl_3$ + 3-nitrobenzylalcohol) *m/z* 652 ($M + H^+$). ^1H NMR ($CDCl_3$): δ 2.11-2.16 (1H, m, H2'a), 2.53-2.57 (1H, m, H2'b), 2.91 (2H, m, CH_2N), 3.30-3.52 (4H, m, OCH_2, H5'), 3.77 (6H, s, 2xOCH_3), 4.14 (1H, m, H3'), 4.32 (1H, m, H4'), 6.23 (1H, dd, J = 5.7, 7.8 Hz, H1'), 6.84 (1H, d, J = 8.5 Hz, H_{Arom}), 7.21-7.42 (9H, m, H_{Arom}), 8.03 (1H, s, H6). ^{13}C NMR ($CDCl_3$): δ 38.25 (CH_2N), 41.30 (C2'), 55.15 (OCH_3), 63.59 (C5'), 70.64 (C3'), 79.90 (OCH_2), 84.27 (C1'), 85.61 (C4'), 87.01 (CAr_3), 97.20 (C5), 113.28, 126.98, 127.94, 129.96, 135.28, 135.40 (C_{Arom}), 139.01 (C6), 144.23 (C_{Arom}), 149.87 (C2), 158.63 (C4).

1-(5-Bromouracil-1-yl)-1,2-dideoxy-β-D-*erythro*-pentofuranuronic acid (10). 5-Bromo-2'-deoxyuridine (**8**, 1.0 g, 3.2 mmol) was dissolved in an aqueous buffer (120 ml, 0.55 g $NaHCO_3$ and adjusted with Na_2CO_3 to pH 9) and reduced Adams catalyst[32] (0.5 g) was added. The reaction mixture was stirred for 8 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 to a volume of app. 20 ml. Compound **10** precipitated during standing at room temperature overnight and the crystals was removed by filtration, washed with water (10 ml) and dried *in vacuo*. Yield 0.87 g (85 %). ^1H NMR (DMSO-d_6): δ 1.93-2.04 (1H, m, H2'a), 2.19-2.26 (1H, m, H2'b), 4.38 (1H, s, H4'), 4.47 (1H, d,

J = 4.0 Hz, H3'), 5.80 (1H, br. s, OH), 6.29 (1H, dd, J = 5.4, 8.8 Hz, H-1'), 8.74 (1H, s, H6), 11.82 (1H, s, NH), 13.36 (1H, br. s, COOH). ^{13}C NMR (DMSO-d_6): δ 38.79 (C2'), 73.70 (C3'), 84.68 (C1'), 86.50 (C4'), 95.75 (C5), 140.42 (C6), 149.72 (C2), 159.01 (C4), 172.63 (C5').

U^{Br}*U^{Br} Carboxamide Linked Deoxydinucleoside 11. Compound **7** (1.07 g, 1.64 mmol) and compound **10** (0.44 g, 1.37 mmol) was dissolved in dry DMF (20 ml) and cooled to -30 °C. DPPA (0.45 g, 1.62 mmol) was added followed by triethylamine (165 mg, 1.64 mmol). The resulting solution was stirred at room temperature for 3 h and evaporated to dryness *in vacuo*. The mixture was separated on silica gel using the gradient 0-10 % MeOH in CH_2Cl_2/pyridine (99:1) to give compound **11**. Yield: 1.19 g (76 %). ^{1}H NMR (DMSO-d_6): δ 2.13 (2H, m, H2''), 2.31 (2H, m, H2'), 3.24-3.44 (6H, m, H5', CH_2CH_2), 3.72 (6H, s, 2xOCH3), 4.00 (1H, m, H4'), 4.12 (1H, m, H3'), 4.29-4.31 (2H, m, H4'', H3''), 5.74 (1H, s, OH), 6.04-6.08 (1H, m, H1'), 6.25-6.28 (1H, m, H1''), 6.89 (4H, d, J = 8.3 Hz, H_{Arom}), 7.22-7.37 (9H, m, H_{Arom}), 8.00 (1H, s, 1H, H6'), 8.55 (s, 1H, NH), 8.85 (1H, s, H6''), 11.7 (2H, s, NH). ^{13}C NMR (DMSO-d_6): δ 36.41 (CH_2N), 38.80, 39.44 (C2', C2''), 54.92 (OCH_3), 63.58 (C5'), 66.94 (C3''), 73.67 (C3'), 78.95 (OCH_2), 84.88, 85.35 (C1', C1''), 85.95 (C4', C4''), 86.02 (CAr_3), 95.83, 96.11 (C5), 113.16, 123.74, 126.47, 127.36, 127.51, 127.63, 127.79, 129.56, 135.20, 135.30 (C_{Arom}), 139.38, 140.92 (C6), 144.53 (C_{Arom}), 149.64, 149.85 (C2), 158.03, 159.03 (C4), 170.51 (C5'').

U^{Br}*U^{Br} 3'-*O*-(2-Cyanoethyl *N,N*-Diisopropylphosphoramidite) 12.

Compound **11** (239 mg, 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 (0.1 ml, 0.57 mmol). After 1.5 h when analytical TLC showed no more starting material the reaction was quenched with CH_3OH (0.04 ml) and diluted with AcOEt (5.5 ml). The mixture was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 ml) and NaCl (3 x 5 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The residual gum was redissolved in anhydrous toluene (1.0 ml) and precipitated in ice-cold petroleum ether (200 ml). The product was collected by filtration and dried under vacuum to give compound **12** as a colourless solid. Yield: 180 mg (75 %). ^{31}P-NMR ($CDCl_3$): δ 153.00, 153.35.

Oligodeoxynucleotide 13-18. The synthesis of oligonucleotides **13-18** 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 commercial 2-cyanoethyl phosphoramidites as well as compound **12**. The synthesis followed the regular protocol of the DNA-synthesizer for 2-cyanoethyl phosphoramidites. The coupling efficiency of **12** was slightly lower (app. 95 %) than those of the commercial amidites (app. 99 %). The oligonucleotides 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 nucleobases and the phosphorous. Purification of the oligodeoxynucleotides including 5'-*O* detritylation were performed on disposable reverse-phase chromatography cartridges.

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28) The duplex melting experiments were carried out in a medium salt buffer, 1 mM EDTA, 20 mM Na_2HPO_4, 140 mM NaCl, pH 7.2. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised lineary from 10-80 °C with a rate of 1 °C per minute.

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30) The triplex melting experiments were carried out in a 15 mM NaH_2PO_4, 5 mM Na_2HPO_4, 280 mM NaCl, pH 6.5. Although the triplex melting points could be measured at pH 7.0 and with 140 mM NaCl, it was much more difficult to measure the exact melting points. The increase in absorbance at 260 nm and 284 nm as a function of time was recorded while the temperature was raised lineary from 10-80 °C with a rate of 1 °C per minute.

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Received April 27, 1995
Accepted September 13, 1995