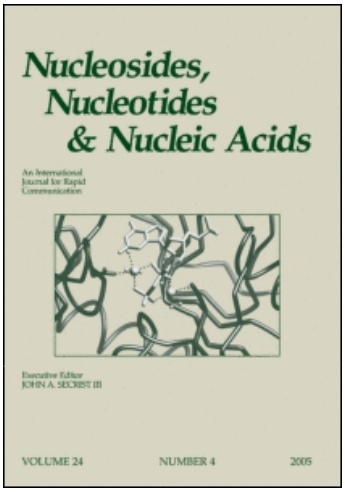


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of a Carboxamide Linked T*T Dimer with an Acyclic Nucleoside Unit and Its Incorporation in Oligodeoxynucleotides

Erik Larsen[a]; Krzysztof Danel[a]; Erik B. Pedersen[a]
[a] Department of Chemistry, Odense University, Odense M, Denmark

To cite this Article Larsen, Erik , Danel, Krzysztof and Pedersen, Erik B.(1995) 'Synthesis of a Carboxamide Linked T*T Dimer with an Acyclic Nucleoside Unit and Its Incorporation in Oligodeoxynucleotides', Nucleosides, Nucleotides and Nucleic Acids, 14: 9, 1905 — 1912
To link to this Article: DOI: 10.1080/15257779508010713
URL: http://dx.doi.org/10.1080/15257779508010713

NUCLEOSIDES & NUCLEOTIDES, 14(9&10), 1905-1912 (1995)

SYNTHESIS OF A CARBOXAMIDE LINKED T*T DIMER WITH AN ACYCLIC NUCLEOSIDE UNIT AND ITS INCORPORATION IN OLIGODEOXYNUCLEOTIDES

Erik Larsen, Krzysztof Danel and Erik B. Pedersen*

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

Abstract: A T*T dimer with * representing a 2'-$OCH_2CH_2NHC(O)$-4' linkage connecting two nucleoside units was prepared by condensation of (S)-1-[2-(2-aminoethoxy)-3-(4,4'-dimethoxytrityloxy)propyl]thymine with 1,2-dideoxy-1-thyminyl-β-D-*erythro*-pentofuranuronic acid. The T*T dimer was incorporated in oligodeoxynucleotides and investigated for hybridization to DNA.

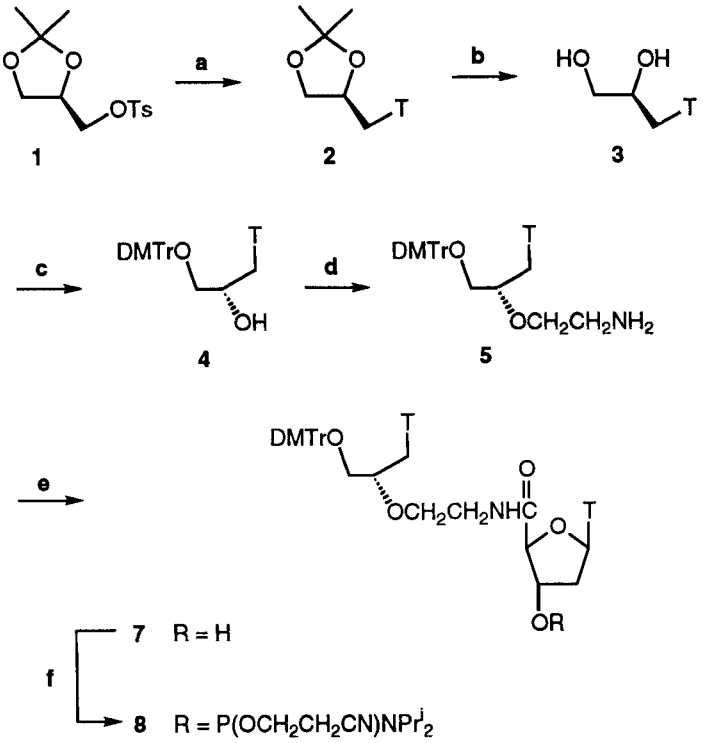
Modified oligonucleotide analogs have demonstrated their potential usefulness as therapeutical agents *in vitro* and *in vivo*.[1,2] The first oligonucleotide analogues to be prepared were modified at the phosphate moiety, e.g. the phosphorothioates[3,4] and the methylphosphonates,[5] because of their ease of synthesis and resistance to nuclease degradation but with some drawbacks such as introduction of chirality at phosphorus. Only a few acyclic oligonucleotide analogues have been prepared: Incorporation of 1-2 glyceronucleosides into a oligonucleotide sequence showed significant lowered duplex stability towards a complementary DNA string[6-9] but better hybridizations was observed with oligonucleotides incorporating acyclic nucleosides with 3,4-dihydroxybutyl-[10] , 3,5-dihydroxypentyl-[11] and 1-(2-hydroxyethoxy)-3-hydroxypropyl[12] sugar moieties, especially as end-modified. In search of better acyclic nucleoside analogues we have synthesized a T*T dimer with * representing a 2'-$OCH_2CH_2NHC(O)$-4' linkage connecting an acyclic and a cyclic thymidine analogue.

The T*T dimer **7** was prepared using (R)-2,2-dimethyl-4-(tosyloxymethyl)dioxolane (**1**) as starting material. Reaction of thymine with **1**, deprotection of the hydroxyl groups with acetic acid and protection of the primary hydroxyl group with 4,4'-dimethoxytrityl chloride (DMTrCl) and 4-dimethylaminopyridine (DMAP) in pyridine gave (S)-1-[3-(4,4'-dimethoxytrityloxy)-2-hydroxypropyl]thymine (**4**) in 18 % overall yield. Compound **4** was alkylated with 2-chloroethylamine using the method of Chur *et al*[13] to give (S)-1-[2-(2-aminoethoxy)-3-(4,4'-dimethoxytrityloxy)propyl]thymine (**5**) in 34 % yield. Diphenyl phosphorazidate ($(PhO)_2P(O)N_3$, DPPA) was used to connect compound **5** through a carboxamide bond with 1,2-dideoxy-1-(thymin-1-yl)-β-D-*erythro*-pentofuranuronic acid (**6**) to give the T*T dimer **7** in 70 % yield after silica chromatography. Addition of pyridine to the eluent (1 %) is important to avoid very low yields (2-3 %) due to deprotection of the 4,4'-dimethoxytrityl group on the silica gel.

Using a standard procedure,[14] the T*T dimer **7** was reacted with 2-cyanoethyl *N,N*-diisopropyl phosphoramidochloridite in the presence of *N,N*-diisopropylethylamine to give the phosphoramidite **8** in 85 % yield after precipitation in petroleum ether (scheme).

In our laboratory we are currently investigating different approaches towards anti-HIV agents so we choose a oligonucleotide sequence (5'-GGGGAAAGAAAAAA-3') found in HIV-1[15] as the test substrate for the duplex melting point experiments. The oligodeoxynucleotides **9-16** were synthesized by standard phosphoramidite methodology on an automated DNA-synthesizer using commercial β-cyanoethylphosphoramidites and compound **8**. The coupling efficiencies for compound **8** 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 which also removed the protecting groups on the nucleobases and phosphorous. The hybridization properties of the oligodeoxynucleotides were examined by mixing each oligonucleotide with the complementary DNA strand and determining the melting points of the DNA-DNA-hybrids by UV measurements.[16] In the table the melting temperatures (T_m) and the differences between the modified and the unmodified oligodeoxynucleotides as the decrease in T_m per modification (ΔT_m) are showed.

Incorporation of one or two dimers in the middle of the oligodeoxynucleotides resulted in a decrease of 11.4-14.6 °C per modification while incorporation in AT base pairs region gave a decrease of 6.6 °C. As stability of duplexes is mainly due to GC base



Scheme. a) NaH, thymine, DMF, 100 °C, b) 80 % aqueous AcOH, reflux, c) DMTrCl, DMAP, pyridine, d) $ClCH_2CH_2NH_2 \cdot HCl$, OH^- (excess), benzene/dioxane, 70 °C, e) 1,2-dideoxy-1-thyminyl-β-D-*erythro*-pentofuranuronic acid (**6**), DPPA, Et_3N, DMF, f) $NCCH_2CH_2OP(Cl)NPr^i_2$, CH_2Cl_2, $EtNPr^i_2$. T = thymin-1-yl.

pairs it is not surprising that introduction of modified dimer in the AT base pair region at the 3' end induced lower destabilization than when the modified dimer was introduced near GC base pairs region in the central part of the oligos. The decreases are in the same order previously reported for oligodeoxynucleotides modified with acyclic nucleosides.[12] The results show that combination of an acyclic and a cyclic nucleoside unit into a dimer offers no advantage over the usual acyclic nucleosides although better results might be obtained using other combinations of acyclic and cyclic units.

EXPERIMENTAL

NMR-spectra were recorded at 250 MHz for ^1H NMR and 62.9 MHz for ^{13}C NMR on a Bruker AC-250-FT NMR spectrometer and at 202.33 MHz for ^{31}P NMR on a Varian

Table. Sequences and melting experiments of synthesized oligodeoxynucleotides

Sequence	No	T_m[a]/°C	ΔT_m[b]/°C
5'-CCCCTTTCTTTTTT-3'	**9**	46.4	-
5'-CCCCT*TTCTTTTTT-3'	**10**	33.6	12.8
5'-CCCCTT*TCTTTTTT-3'	**11**	31.8	14.6
5'-CCCCTTTCTTTT*TT-3'	**12**	39.8	6.6
5'-CCCCTTTCT*TTTTT-3'	**13**	35.0	11.4
5'-CCCCTT*TCT*TTTTT-3'	**14**	20.2	13.1
5'-CCCCTTTCT*TT*TTT-3'	**15**	29.6	8.4
5'-CCCCTTTCTT*TT*TT-3'	**16**	33.0	6.7

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

Unity 500 spectrometer using tetramethylsilane (TMS) as an internal standard. Silica gel (0.040-0.063 mm) and analytical silica gel TLC plates 60 F_{254} were purchased from Merck.

(S)-2,2-Dimethyl-4-(thymin-1-ylmethyl)-1,3-dioxolane (2). Compound **2** was prepared from (R)-2,2-dimethyl-4-(tosyloxymethyl)-1,3-dioxolane (**1**) using a slightly modified procedure of Holý.[17] To a stirred dispersion of thymine (12.6 g, 0.10 mol) in anhydrous DMF (100 ml) was added NaH (4.0 g of a 60 % dispersion in mineral oil, 0.10 mol) and after allmost complete evolving of hydrogen the mixture was heated to 100 °C for 1 h. Then compound **1** (25 g, 0.087 mol) was added, the reaction mixture stirred for an additional 8 h at 100 °C, cooled to r.t. and filtered through Celite. The mixture was evaporated to dryness at reduced pressure, coevaporated with toluene (3x20 ml) and purified with silica gel chromatography using $CHCl_3$ as eluent. Yield 8.46 g (40 %). ^{1}H-NMR ($CDCl_3$/TMS): δ = 1.35 (3H, s, CH_3a), 1.43 (3H, s, CH_3b), 1.92 (3H, d, J = 0.9 Hz, CH_3), 3.70 (2H, m, H1'a, H3'a), 4.02 (1H, dd, J = 2.9, 14.2 Hz, H1'b), 4.10 (1H, m, H3'b), 4.38-4.41 (1H, m, H2'), 7.16 (1H, q, J = 1.0 Hz, H6), 10.10 (1H, s, NH).

^{13}C-NMR ($CDCl_3$/TMS): δ 12.04 (CH_3), 24.91, 26.42 (2xCH_3), 49.69 (C1'), 66.17 (C3'), 73.95 (C2'), 109.68 (CMe_2), 110.00 (C5), 141.49 (C6), 151.43 (C2), 164.47 (C4).

(S)-1-(2,3-Dihydroxypropyl)thymine (3). Compound **3** was prepared according to ref. 17 by refluxing compound **2** in 80 % aqueous AcOH for 0.5 h Yield 4.49 g (67 %). ^{1}H-NMR (DMSO-d_6/TMS): δ 1.75 (3H, s, CH_3), 3.28-3.42 (3H, m, H1'a, H3'a, H3'b), 3.69 (1H, m, H2'), 3.88 (1H, dd, J = 3.5, 13.5 Hz, H1'b), 4.65 (1H, t, J = 5.6 Hz, OH3'), 4.93 (1H, d, J = 5.1 Hz, OH2'), 7.39 (1H, q, J = 0.7 Hz, H6), 11.03 (1H, br. s., NH). ^{13}C-NMR (DMSO-d_6/TMS): δ 11.83 (CH_3), 50.77 (C1'), 63.58 (C3'), 69.08 (C2'), 107.37 (C5), 142.73 (C6), 151.00 (C2), 164.30 (C4).

(S)-1-[3-(4,4'-Dimethoxytrityloxy)-2-hydroxypropyl]thymine (4). 4,4'-Dimethoxytrityl chloride (8.10 g; 24 mmol) was added to a stirred suspension of compound **3** (4.40 g; 22 mmol) and DMAP (122 mg, 1 mmol) in pyridine (250 ml) and the reaction mixture was stirred at r.t. for 16 h. After addition of MeOH (5 ml) the mixture was evaporated *in vacuo* and the residue partitioned between $CHCl_3$ (500 ml) and H_2O (200 ml). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. Silica gel (200 g) chromatography using 2 % MeOH in CH_2Cl_2 as eluent afforded compound **4**. Yield 7.61 g (67%). ^{1}H-NMR ($CDCl_3$/TMS): δ = 1.80 (3H, s, CH_3), 3.18 (2H, d, J = 5.1 Hz, H1'a, H1'b), 3.61-3.67 (1H, m, H3'a), 3.76 (6H, s, OCH_3), 4.01-4.07 (2H, m, H2', H3'b), 6.81 (4H, d, J = 8.8 Hz, H_{Arom}), 7.03 (1H, s, H6), 7.14-7.43 (9H, m, H_{Arom}), 9.67 (1H, br. s, NH). ^{13}C-NMR ($CDCl_3$/TMS): δ 12.11 (CH_3), 51.68 (C1'), 55.13 (OCH_3), 64.59 (C3'), 69.17 (C2'), 86.37 (CAr_3), 109.87 (C5), 113.18, 126.87, 127.84, 127.97, 129.90, 135.57 (C_{Arom}), 141.92 (C6), 144.53 (C_{Arom}), 151.77 (C2), 158.62 (C_{Arom}), 164.38 (C4).

(S)-1-[2-(2-Aminoethoxy)-3-(4,4'-dimethoxytrityloxy)propyl]thymine (5). Compound **4** (7.60 g, 15 mmol) was dissolved at 60°C in a mixture of benzene (60 ml) and 1,4-dioxane (30 ml). Powdered KOH (27 g) was added followed by 2-chloroethylamine (13.62 g, 0.171 mol) and the reaction mixture was vigorously stirred at 70°C for 8 h. After cooling the reaction mixture to r.t., H_2O (100 ml) was added followed by neutralization with 4 M acetic acid to pH 7.5. The benzene layer was separated, washed with H_2O (2 x 100 ml), dried over Na_2SO_4 and evaporated *in vacuo*. Silica gel (300 g) chromatogra-

phy with the gradient 1-4 % MeOH in CH_2Cl_2 gave compound **5**. Yield 2.8 g (34%). ^{1}H-NMR ($CDCl_3$/TMS): δ 1.80 (3H, s, CH_3), 2.83 (2H, t, J = 5.2 Hz, CH_2N), 3.10-3.15 (1H, m, H1'a), 3.21-3.27 (1H, m, H1'b), 3.40-3.70 (4H, m, OCH_2, H3'a, H3'b), 3.76 (6H, s, 2xOCH_3), 4.10 (1H, m, H2'), 6.83 (4H, d, J = 8.9 Hz, H_{Arom}), 7.08 (1H, s, H6), 7.19-7.46 (9H, m, H_{Arom}). ^{13}C-NMR ($CDCl_3$/TMS): δ 11.96 (CH_3), 41.46 (CH_2N), 49.58 (C1'), 55.05 (OCH_3), 62.46 (C3'), 71.78 (C2'), 77.00 (OCH_2), 86.21 (CAr_3), 109.61 (C5), 113.09, 126.76, 127.74, 127.89, 129.76, 135.56, 135.63 (C_{Arom}), 141.81 (C6), 144.48 (C_{Arom}), 151.33 (C2), 158.46 (C_{Arom}), 164.63 (C4).

T*T Dimer 7. Compound **5** (0.47 g; 0.92 mmol) and 1,2-dihydroxy-1-thyminyl-β-D-*erythro*-pentofuranuronic acid[18] (**6**) (0.197 g; 0.77 mmol) was dissolved in dry DMF (14 ml) and cooled to -20°C. DPPA (0.256 g, 0.92 mmol; 0.20 ml) was added followed by addition of Et_3N (0.097 g, 0.92 mmol, 0.13 ml). The resulting solution was stirred at room temperature for 3 h and evaporated to dryness *in vacuo*. The product **7** was purified by silica gel (100 g) chromatography with the gradient 1-5 % MeOH in CH_2Cl_2/pyridine (99:1). Yield 0.53 g (85 %). ^{1}H-NMR (DMSO-d_6/TMS): δ 1.70 (3H, s, CH_3), 1.74 (3H, s, CH_3), 2.04-2.22 (2H, m, H2''), 2.96-3.13 (2H, m, H1'), 3.20-3.49 (4H, m, CH_2CH_2), 3.56-3.70 (2H, m, H3'), 3.78 (6H, s, 2xOCH_3), 3.80-3.91 (1H, m, H2'), 4.25 (1H, s, H4''), 4.93 (1H, m, H3''), 5.57 (1H, d, J = 3.8 Hz, OH), 6.34 (1H, dd, J = 5.8, 8.5 Hz, H1''), 6.89 (4H, d, J = 8.8 Hz, H_{Arom}), 7.11-7.42 (10H, m, H_{Arom}, H6), 8.08 (1H, q, J = 0.8 Hz, H6 in thymidine), 8.21 (1H, t, J = 5.2 Hz, NHCO), 11.18 (1H, s, NH), 11.29 (1H, s, NH). ^{13}C-NMR (DMSO-d_6/TMS): δ 11.68, 12.13 (2xCH_3), 38.19, 38.54 (C2'', CH_2N), 49.58 (C1'), 54.89 (OCH_3), 63.05 (C3'), 68.30 (C3''), 73.57 (OCH_2), 76.54 (C4''), 85.35, 85.55 (CAr_3, C1''), 107.87, 109.37 (2xC5), 113.07, 125.16, 127.53, 127.70, 128.05, 128.75, 129.51, 135.36, 135.38 (C_{Arom}), 136.79, 141.94 (2xC6), 144.66 (C_{Arom}), 150.54, 150.81 (2xC2), 158.00 (C_{Arom}), 163.57, 164.07 (2xC4), 170.28 (C5'').

T*T-Deoxyribonucleosid-3'-yl 2-cyanoethyl *N,N*-diisopropylphosphoramidite (8). Compound **7** (188 mg, 0.25 mmol) was dried by coevaporation with anhydrous MeCN (2 ml) and dissolved in anhydrous CH_2Cl_2 (1.5 ml) under nitrogen. *N,N*-diisopropylethylamine (0.23 ml) was added followed by dropwise addition of 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.1 ml, 0.57 mmol). The reaction was quenched by

addition of MeOH (0.04 ml) after 1 h followed by dilution with ethyl acetate (6 ml). The mixture was washed with a saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was redissolved in anhydrous toluene (1.0 ml) and precipitated in ice-cold petroleum ether (200 ml) to give compound **8** after filtration and drying *in vacuo*; yield: 195 mg (82 %). ^{31}P NMR (DMSO-d_6/TMS): δ 153.39; 153.53.

Oligodeoxynucleotide 9-16. The synthesis of oligonucleotides **9-16** 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-cyanoethylphosphoramidites as well as compound **8**. The synthesis followed the regular protocol of the DNA-synthesizer for 2-cyanoethylphosphoramidites. The coupling efficiency of **8** was slightly lower (app. 95 %) than those of the unmodified 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.

REFERENCES AND NOTES

1) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.

2) Cook, P. D. *Anticancer Drug. Res.* **1991**, *6*, 585.

3) Matsukura, M.; Zon, G.; Shinozuka, K.; Robert-Guroff, M.; Shimada, T.; Stein, C. A.; Mitsuya, H.; Wong-Staal, F.; Cohen, J. S.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 4244.

4) Agrawal, S.; Temsamani, J.; Tang, J. Y. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 7595.

5) Miller, P. S.; *Biotechnology* **1991**, *9*, 358.

6) Usman, N.; Juby, C. D.; Ogilvie, K. K. *Tetrahedron Lett.* **1988**, *29*, 4831.

7) Schneider, K. C.; Benner, S. A. *J. Am. Chem. Soc.* **1990**, *112*, 453.

8) Azymah, M.; Chavis, C.; Lucas, M.; Morvan, F.; Imbach, J.-L. *Nucleosides Nucleotides* **1992**, *11*, 1241.

9) Ogilvie, K. K.; Nguyen-Ba, N.; Gillen, M. F.; Radatus, B. K.; Cheriyan, U. O.; Hanna, H. R.; Smith, K. O.; Galloway, K. S. *Can. J. Chem.* **1984**, *62*, 241.

10) Augustyns, K.; Van Aerschot; A.; Van Schepdael, A.; Urbanke, C.; Herdewijn, P. *Nucleic Acids Res.* **1991**, *19*, 2587.

11) Vandendriessche, F.; Augustyns, K.; Van Aerschot, A.; Busson, R.; Hoogmartens, J.; Herdewijn, P. *Tetrahedron* **1993**, *49*, 7223.

12) Nielsen, P.; Kirpekar, F.; Wengel, J. *Nucleic Acids Res.* **1994**, *22*, 703.

13) Chur, A.; Holst, B.; Dahl, O.; Valentin-Hansen, P.; Pedersen, E. B. *Nucleic Acids Res.* **1993**, *21*, 5179.

14) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843.

15) Froehler, B.; Krawczyk, S.; Matteucci, M. D.; Milligan, J. PCT Int. Appl. WO 9209705 (23 Nov.1992), Gilead Sciences, Inc.; *C.A.* **1993**, *118*, 143019.

16) The 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.

17) Holý, A. *Collect. Czech. Chem. Commun.* **1975**, *40*, 187.

18) Moss, G. P.; Reese, C. B.; Schofield, K.; Shapiro, R.; Lord Todd *J. Chem. Soc.* **1963**, *85*, 1149.

Received March 7, 1995
Accepted August 9, 1995