Novel Three-Atom 2′-5′ Linkages in Antisense Nucleotides: Synthesis and Pairing Properties of Dinucleotides with a Carboxylic Ester Linkage

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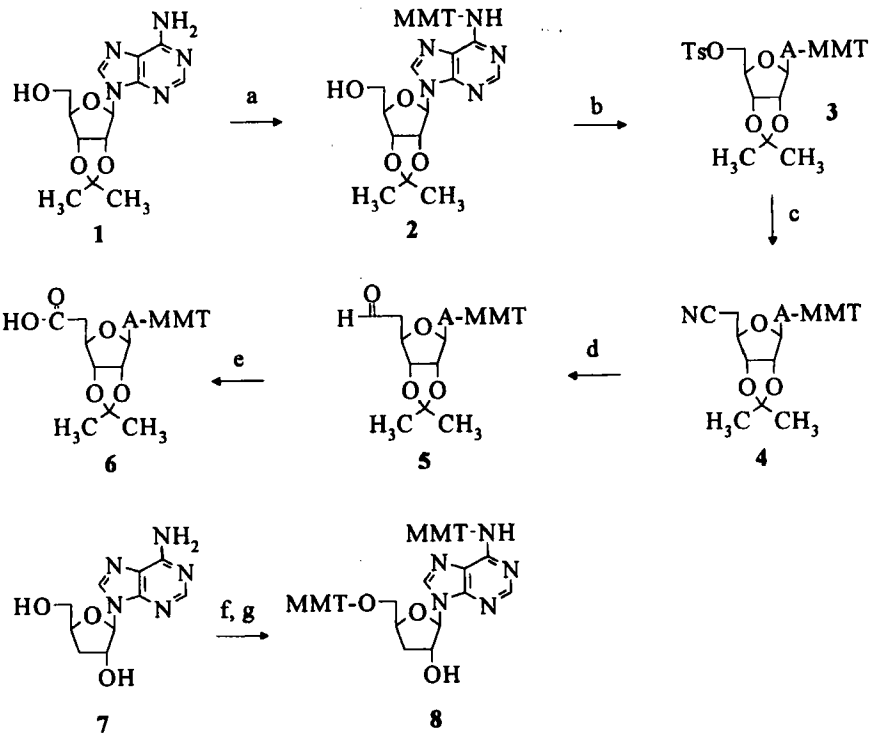
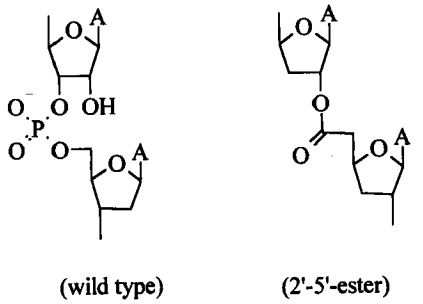
Inhibition of gene expression by the use of short oligodeoxynucleotides (ODNs) known as antisense oligonucleotides has recently become a promising field of biological research[1]. Ability of antisense ODNs to interfere specifically with mRNA targets provides a powerful tool for control of cellular and viral gene expression. However, insufficient cell penetration due to their charge and degradation by nucleases may render unmodified oligonucleotides unsuited for therapeutic application. Therefore, chemical modifications of ODNs are a prerequisite for their effective use as potential antisense drugs. Apart from modifications of the phosphate moiety, a number of oligonucleotides with 3′-5′ achiral neutral dephospho linkages (e.g. silyl[2], sulfonamide[3], carbamate[4], formacetal and thioformacetal[5]) has been reported. We describe a new class of backbone modified nucleotides, in which the nucleosides are 2′-5′-linked by a three atom ester moiety. Although in these compounds the number of exocyclic backbone atoms is reduced from four to three, the number of atoms per chain unit (six) remains the same as in naturally occurring 3′-5′-phosphodiester linked oligonucleotides, because – in addition to ring carbons C′-4 and C′-3 – C′-2 also is a member of this chain.



Scheme 2. a: 1. TMS-Cl, 2. MMT-Cl, 3. NH_4OH, pyridine, 88 %; b: *p*-tosyl chloride, pyridine, 81 %; c: tetraethylammonium cyanide, DMF, 57 %; d: DIBAL, –78 °C, toluene, 44 %; e: $KMnO_4$, CH_2Cl_2/DMF 4/1, 0°C, 42 %; f: 1. TMS-Cl, 2. MMT-Cl, 3. NH_4OH, pyridine, 92 %; g: MMT-Cl, pyridine, 87 %.



Scheme 1

Molecular mechanics and molecular dynamics calculations of these novel backbone modified oligonucleotides have been carried out to investigate the structural characteristics and pairing properties of this new backbone. Conformational analysis and molecular dynamics simulations of duplexes containing a modified and a natural RNA strand showed that they are capable of recognizing natural RNA. A detailed account of the computer simulations will be published elsewhere.

The synthesis of a building block **6** for 2′-5′-linked ester-type oligonucleotides, which was also used for synthesis of dinucleotides **10** and **12**[6], is outlined in Scheme 2. Starting from 2′,3′-O-isopropylidene adenosine **1** the 6-N position was protected using monomethoxytrityl chloride (MMT-Cl). The 5′-O-position was activated by tosylation in pyridine. Tosylate **3** was treated with tetraethylammonium cyanide in DMF at room temperature (RT) to yield nitrile **4** in 57 % yield.

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Reduction of the nitrile to the aldehyde **5** was carried out using DIBAL in toluene. The major side product (10 %) was the 4′-α epimer. The oxidation of aldehyde **5** to acid **6**[7] was achieved with $KMnO_4$ in $CHCl_2$/DMF 10/1 at 0 °C.

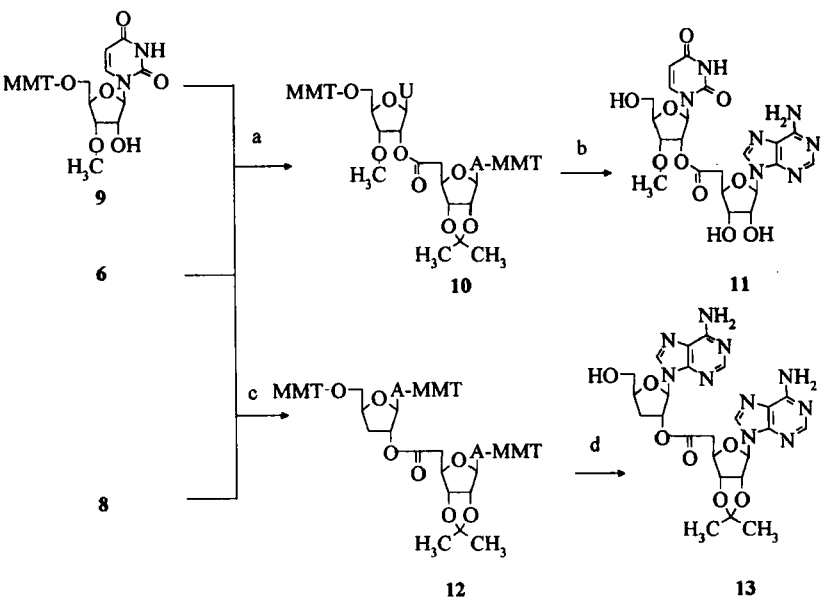
Stepwise monomethoxytritylation of cordycepin **7** furnished an adenine building block **8**, which was used to form the ester-type dinucleotides **12** and **13**. The preparation of the uridine building block **9** is described in ref.[8].

Coupling of acid **6** with alcohols **8** and **9**, respectively was achieved by the use of DCC and dimethylaminopyridine (DMAP) (Scheme 3). Deprotection of the dimer **10** and **12** gave the U-ester-A dimer **11**[9] and the A-ester-A dimer **13**[10].

To study base pairing properties of the ester-type dinucleotides we used the known fact that polyuridylic acid (poly U) can form complexes not only with adenosine oligonucleotides but even with adenosine dinucleotides[11]. Three-stranded helix sections formed by the system (ApA 2 poly U) were characterized by Ackermann et al.[12]. Thus two poly U strands are a prerequisite for formation of a thermodynamically stable cooperative unit with a complementary strand as short as an ApA dimer. The type of pairing per base residue was spectroscopically determined to be the same Watson-Crick/Hoogsteen type as exhibited in the (poly A + 2 poly U) triplex.

To investigate the binding affinity of our novel dinucleotide, the interaction between the A-ester-A dinucleotide **13** and poly U was studied under these triple helix conditions[13] using ultraviolet spectroscopy techniques as described earlier[14]. Heat induced UV melting profiles showed cooperative transition in the region of 6 to 30 °C with a hyperchromic effect of 36 %. The melting temperature (T_m = 15 °C) of this system was higher than for the corresponding ApA dinucleotide/2 poly U system (13.5 °C)[11]. These UV studies suggest binding affinity of the 2′-5′-ester dinucleotide **13** to RNA via formation of a stable poly U/dinucleotide triplex segment, thus indicating base pairing properties corresponding to those of adenosine dinucleotides and giving the first experimental evidence that a three atom 2′-5′ linkage is an acceptable modification of the natural nucleotide backbone.

Further studies on incorporating such dinucleotides into ODNs and synthesizing homo 2′-5′-oligonucleotides linked by a three atom ester moiety are ongoing.



Scheme 3. a: DCC, DMAP, in CH_2Cl_2, 65 %; b: CCl_3COOH, CH_2Cl_2, H_2O, 80 %; c: DCC, DMAP, in CH_2Cl_2, 23 %; d:. CCl_3COOH, CH_2Cl_2, 38 %.

References and Notes

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[7] Compound **6**: ^{1}H-NMR (d-DMSO) (300 MHz): δ (ppm) = 1.30 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.70 (m, 2H, 5′-CH_2), 3.68 (s, 3H, MMT OCH_3), 4.52 (m, 1H, 4′-H), 4.90 (m, 1H, 3′-H), 5.50 (m, 1H, 2′-H), 6.05 (d, 1H, 1′-H), 6.85 (d, 2H, MMT), 7.40–7.15 (m, 12H, MMT), 7.95 (s, 1H, 8-H), 8.48 (s, 1H, 2-H).

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[9] Compound **11**: ^{1}H-NMR (D_2O) (300 MHz): δ (ppm) = 3.0 (m, 2H, 5′-CH_2-A), 3.25 (s, 3H, OCH_3), 3.85 (m, 1H, 5′-H′U), 3.96 (m, 1H, 5′-H-″U), 4.1 (m, 2H, 3′ and 4′H-U), 4.35 (m, 1H, 3′-H-A), 4.5 (m, 1H, 4′-H-A), 4.85 (m, 1H, 2′-H-U), 5.45 (m, 1H, 2′-H-U), 5.71 (d, 1H, 5-H Uridine), 5.93 (d, 1H, 1′-H-U), 6.10 (d, 1H, 1′-H-A), 7.65 (d, 1H, 6-H Uridine).

[10] Compound **13**: ^{1}H-NMR (D_2O) (300 MHz): δ (ppm) = 1.44 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 2.10 (m, 1H, 3′-H″-A′), 2.40 (3′-H′-A′), 2.78 (m, 1H, 5′-H″-A″), 2.92 (m, 1H, 5′-H′-A″), 3.66 (m, 1H, 5′-H″-A′), 3.86 (m, 1H, 5′-H′-A′), 4.30 (m, 1H, 4′-H-A″), 4.68 (m, 1H, 4′-H-A″), 5.09 (m, 1H, 3′-H-A″), 5.57 (m, 1H, 2′-H-A″), 5.65 (m, 1H, 2′-H-A′), 6.12 (d, 1H, 1′-H-A′), 6.20 (d, 1H, 1′-H-A″), 8.35 (s, 1H, 8-H), 8.41 (s, 2H, 2-H, 8-H), 8.56 (s, 1H, 2-H).

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[13] Buffer: 0.04 M $MgSO_4$/10 mM TrisHCl pH 7.5; $Conc._{A\text{-ester-}A}$(Extinction$_{257}$ = 0.962 (25 °C)) = 0.064 mM nucleoside; $Conc._{Poly\ U}$ (Extinction$_{257}$ = 1.238 (25 °C)) = 0.13 mM nucleoside.

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