# Synthesis of 3′−3′-Linked Pyrimidine Oligonucleotides Containing an Acridine Moiety for Alternate Strand Triple Helix Formation

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Oligonucleotides with a 3′–3′ inversion of polarity, containing an acridine moiety attached to the nucleotide base flanking the 3′–3′ phosphodiester bond, have been synthesised, characterised and used to form alternate-strand triple helix complexes. These have been investigated by UV melting studies and CD experiments.


## Introduction

Oligodeoxynucleotides (ODNs) can bind the major groove of double-stranded DNA oligopurine tracts to form local triple helices by sequence-specific hydrogen bonding.[1] Pyrimidine-rich, triplex-forming oligonucleotides (TFOs) target, in a parallel orientation, homopurine strands in a duplex DNA by Hoogsteen-type triplets (T·AT and $C^+$·GC), while purine-rich TFOs can bind, in an antiparallel orientation, the purine strand of a duplex DNA by reverse Hoogsteen-type base triplets (G·GC and A·AT).

To extend the range of applicability of this gene-control strategy,[2] which is otherwise confined to the rare presence of a long — at least 16−17 bases — homopurine sequence on the target gene, it is possible to form triplex structures in which the duplex is composed of two adjacent and alternating oligopurine-oligopyrimidine tracts. In this approach, called "alternate strand triple helix formation" ODNs having a 3′−3′ or 5′−5′ internucleoside junction simultaneously hybridise the adjacent purine tracts by switching strand at the junction between the oligopurine and the oligopyrimidine domains of the duplex.[3] Several 3′−3′ and 5′−5′ internucleoside junctions have been proposed as linkers for alternate strand TFOs,[4−11] with the latter imparting a minor cooperativity in the binding of the two domains of the TFO with the target duplex.[5]

In the last few years we have focused our attention on the synthesis of ODNs containing a 3′−3′ inversion polarity motif, which is able to hybridize the target duplex, in parallel mode, by formation of Hoogsteen triplets.[12−15] Our results on the stability of these triplex structures pointed to the phosphodiester bond as a suitable 3′−3′ junction into TFOs.[12−14,16]

Nevertheless, molecular-mechanics calculations indicated that a distortion of the sugar-phosphate backbone around the 3′−3′ phosphodiester junction occurs, thus preventing the correct Hoogsteen-hydrogen-bond formation for the nucleotide bases flanking the site of inversion polarity.[17] In order to balance the destabilizing effects occurring in alternate strand triplexes, several research groups have introduced an intercalating agent near the 3′−3′ switching region of the TFO.[18−20] Among the possible DNA intercalating agents, acridine is particularly advantageous considering that: i) molecules containing an acridine moiety have been extensively studied as duplex and triplex DNA stabilizing agents; ii) acridine-linked ODNs have been proposed as antisense agents and as efficient TFOs;[21−23] iii) ODNs linking acridine derivatives can induce a DNA cleavage under photoirradiation when combined with metal ions;[24,25] and iv) a large number of acridine derivatives are commercially available.

We report here an easy and convenient method for the synthesis of TFOs carrying a chloro-methoxyacridine residue attached to the N-3 atom of a thymidine flanking the 3′−3′ phosphodiester bond, through an alkylamino linker. We chose the N-3 position of thymidine on the basis of its inherent reactivity towards alkylation and the reported stability of duplex and triplex DNA complexes containing intercalators, or other groups, attached to the N-3 position of a thymidine residue.[26,27]

## Results and Discussion

Our approach is based on a commercially available difunctionalised CPG support carrying a 2-methoxy-6-chlo-

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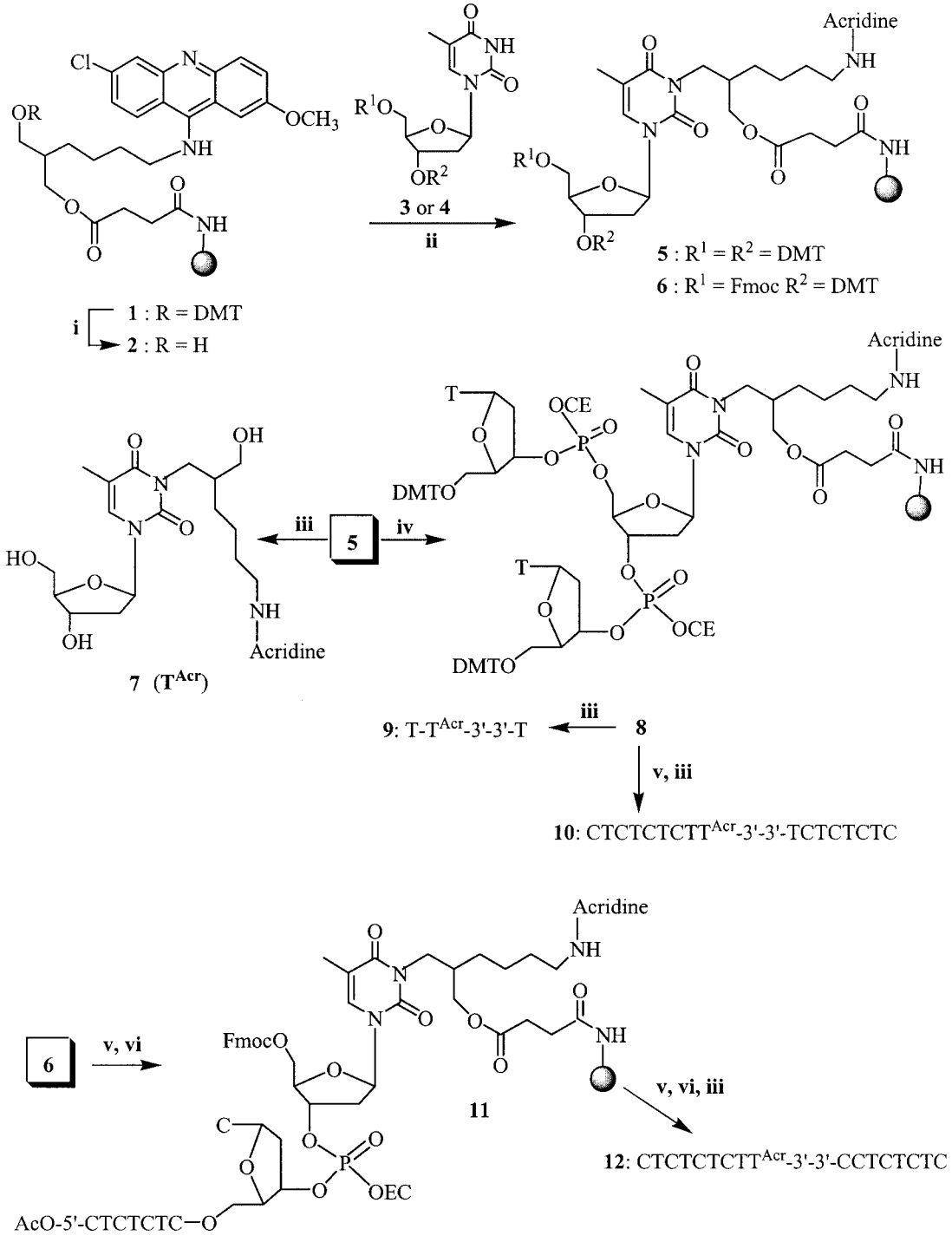


Scheme 1. Reagents and conditions: i) TCA 3% in DCM; ii) TPP, DEAD, THF, DCM; iii) NaOH 0.1 M in $CH_3OH/H_2O$ (4:1 v/v); iv) coupling with thymidine; v) pyridine/$NEt_3$ (95:5 v/v, 1.5 h); vi) chain elongation with 3′-phosphoramidite deoxynucleoside building blocks

roacridine moiety and a primary alcoholic hydroxy function protected with the acid-labile DMT group (**1**, Scheme 1).

A suitably 3′,5′-protected thymidine **3** or **4** was linked to support **2**, obtained by removal of the DMT protecting group, through the N-3 atom of the base under Mitsunobu condensation conditions,[28,29] thus affording supports **5** and **6**, respectively (70% yield by DMT spectrophotometric test). Supports **5** and **6** were found to be stable under the chemical treatments required by the DNA chain assembly[30] and cleavable, at the succinate function, in the final alkaline treatment for detachment and deprotection procedure. The cleavage from the support releases a thymidine base connected to the acridine residue through a hexylamino chain. Support **5**, after capping of the unchanged hydroxy functions and successive removal of the 3′,5′-DMT groups, reacted almost quantitatively with the 5′-DMT-thy-

  

midine-3′-phosphoramidite in a standard automated procedure, giving support **8**. The detritylation of supports **5** and **8**, followed by alkaline treatment with a 0.4 M solution of NaOH, furnished the expected acridine-nucleoside **7** and the acridine-trinucleotide **9**, respectively, the latter containing the 3′−3′ phosphodiester junction. The structures of both released products were confirmed by $^1$H NMR spectroscopy and mass spectrometry. Eight coupling cycles performed on support **5**, with appropriate 3′-phosphoramidite nucleotide units, furnished TFO **10**. Support **6**, in which the 5′- and 3′-OH functions are orthogonally protected with Fmoc and DMT groups, respectively, allowed the sequential synthesis of different ODN domains of the TFO. After the removal of the 3′-DMT group from support **6**, the elongation of the first ODN domain by the formation of the 3′−3′ phosphodiester junction was achieved, thus leading to **11**. Then, the 5′-Fmoc group was removed by pyridine/$NEt_3$ treatment and the second ODN domain was assembled, thus furnishing the TFO **12**. Between the two alternative 5- or 3′-OH Fmoc protections, the former was chosen on the basis of preliminary experiments performed on solid supports carrying 5′- or 3′-Fmoc-thymidine. These have shown that complete 5′-deprotection could be achieved by treatment with 5% triethylamine in pyridine in 1.5 hours, whereas a prolonged reaction time was needed to quantitatively deprotect the 3′-hydroxy function. TFOs **10** and **12** were synthesised on a 1 μM scale by a DNA synthesiser. The crude oligomers, detached from the support as described for trinucleotide **9**, were purified by HPLC on an anionic exchange column. The collected peaks were desalted, thus furnishing **10** and **12** with greater than 95% purity (by RP-HPLC) in 28 and 24 $OD_{260}$ units yields, respectively. The MALDI TOF-MS spectra of **10** and **12** were in agreement with their structures. Furthermore, the UV/Vis spectra of **10** and **12** confirmed the presence of the acridine moiety in the oligomers, showing a diagnostic absorption at $\lambda_{max}$ = 424 nm.

### UV and Circular Dichroism (CD) Studies

The triplexes **14** and **15** (Table 1) were formed by mixing equimolar amounts of TFO **10** and **12**, respectively, with the target duplex DNA **13**, and heating at 90 °C for 5 min. The solutions were then equilibrated for 15 h at room temperature before performing the analyses. The formation and stability of the triplexes **14** and **15**, compared with the acridine-devoid triplexes **16** and **17**, were studied by UV thermal denaturation experiments and by CD in 5 mM $NaH_2PO_4$, 140 mM KCl and 5 mM $MgCl_2$ buffer (at pH 5.5, 6.0 and 6.6). Figure 1 shows the melting profiles of the duplex **13** and triplexes **14**−**17**. The $T_m$ values are reported in Table 1.

Table 1. $T_m$ values (C°)/pH for duplex **13** and triplexes **14**−**17**

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5'-GAGAGAGAATCTCTCTC-3'
3'-CTCTCTCTTAGAGAGAG-5'
        Duplex 13

5'-CTCTCTCTX
5'-GAGAGAGAA TCTCTCTC-3'
3'-CTCTCTCTT AGAGAGAG-5'
            YCTCTCTC-5'
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**Triplex 14** : X = $T^{Acr}$; Y = T
**Triplex 15** : X = $T^{Acr}$; Y = C
**Triplex 16** : X = T; Y = T
**Triplex 17** : X = T; Y = C

| Complexes | pH 5.5 | pH 6.0 | pH 6.6 |
|---|---|---|---|
| Duplex **13** | 62.3 | 63.2 | 63.4 |
| Triplex **14** | 41.4 | 36.7 | 34.5 |
| Triplex **15** | 39.4 | 34.9 | 32.5 |
| Triplex **16** | 34.6 | 29.1 | 26.0 |
| Triplex **17** | 32.8 | 27.0 | 24.6 |

The melting curves for **14**−**17** show a typical biphasic behaviour, with the first sigmoid attributable to the triplex dissociation, while at higher temperatures the expected pH-insensitive duplex-to-single-strands transition can be observed. The UV melting data for the acridine-containing triplexes **14** and **15** indicate a stabilisation effect at all the pH values with respect to the corresponding acridine-devoid triplexes **16** and **17**. At pH 6.6 the presence of the acridine in the TFO led to a stabilisation, with $\Delta T_m$ values

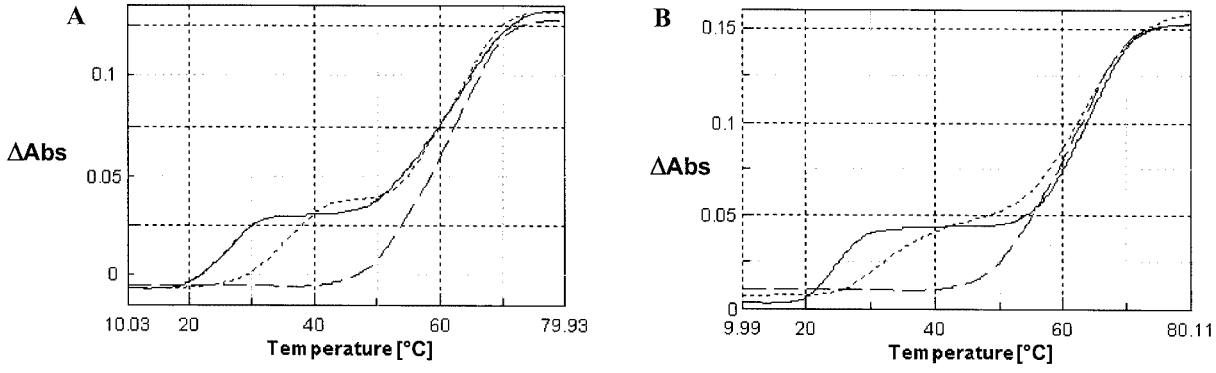


Figure 1. UV melting profiles (pH 6.6): A triplex **14** (····), triplex **16** (−) and duplex **13** (− −); B triplex **15** (····), triplex **17** (−) and duplex **13** (− −).

  

of 8.5 and 7.9 °C, respectively, for triplexes **14** and **15**, while at pH 5.5 a slightly smaller effect was observed ($\Delta T_m$ 6.8 and 6.6 °C, respectively). The $T_m$ values of the triplex **14** were higher than those of **15** at all the tested pH's and the same behaviour was observed for acridine-devoid triplexes **16** and **17**. These latter data indicate that the substitution of a thymine (in **14** and **16**) for a cytosine (in **15** and **17**) at the 3′−3′ junction in TFO induces, as expected, a destabilizing effect, probably due to the electrostatic repulsion between consecutive protonated cytosines.

The formation of a triplex structure for **14**−**17** was confirmed by circular dichroism measurements. The concentration of the triplex solution was in the range 1.9−2.3 × $10^{-5}$ M. The CD spectra of triplexes **14**−**17** (pH 6.6) are reported in Figure 2. Complexes **16** and **17**, as already reported for the same kind of complexes,[16,17,31] show a CD profile very similar to those observed for canonical Hoogsteen-type triplexes, characterised by a large positive band at 280 nm and a negative band centred around 213 nm.[12,15] The negative band, in particular, is indicative of the existence of a triplex structure.[32] Almost the same patterns were observed for acridine-containing triplexes **14** and **15**. The CD spectra of triplexes **14** and **15** were compared with the normalised summed spectra of the duplex **13** and appropriate single-strand TFOs (Figure 3). Significantly, for **14** and **15**, the diagnostic band at 213 nm is deeper than the corresponding band in the normalised sum spectra, and the 282 nm band decreases in intensity and shows a red shift. These data are clearly indicative of a triplex structures for complexes **14**−**17**.

## Conclusion

In this paper we report the easy synthesis of ODNs containing a 3′−3′-phosphodiester linkage and bearing an acridine residue on the thymidine base flanking the 3′−3′ junction. This synthesis was based on the preparation of a new kind of nucleoside-acridine solid support (**5** or **6**). In our opinion this synthetic strategy could be a useful entry to link an intercalating agent near to a 3′−3′ (or 5′−5′) phosphodiester linkage, thus furnishing a convenient method to stabilize alternate strand triple helices by minimizing the mismatching effect into the region between purine and pyrimidine domains. Both CD and UV melting data indicate that the acridine moiety, linked through a seven-atom spacer arm to the N-3 of a thymine, does not hamper the formation of a triplex structure. Furthermore, the stabilisation effect observed for triplexes **14** and **15** strongly suggests an intercalation of the acridine residue into the triplex structure.

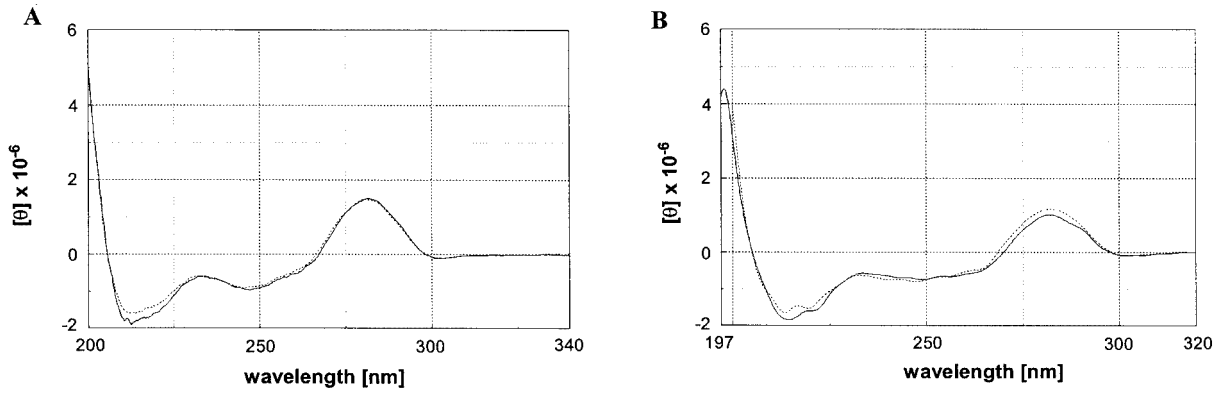


Figure 2. A: CD spectra at pH 6.6 of triplex **14** (−−) and triplex **16** (···); B: triplex **15** (−−) and triplex **17** (···)

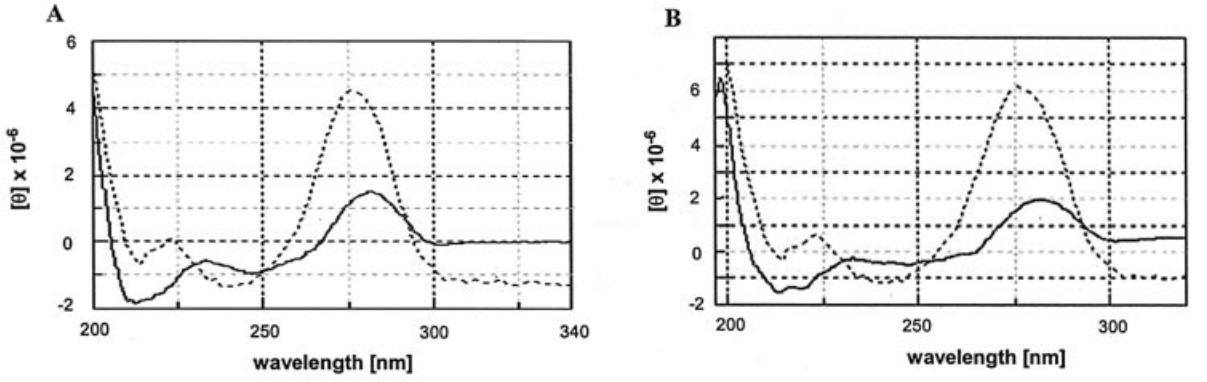


Figure 3. A: CD spectra at pH 6.6 of triplex **14** (−−) and normalised summed spectra (···) of duplex **13** and TFO **10**; B: CD spectra at pH 6.6 of triplex **15** (−−) and normalised summed spectra (···) of duplex **13** and TFO **12**

  

## Experimental Section

**General Remarks:** The following abbreviations are used throughout the paper: triplex forming oligonucleotide (TFO), fluorenylmethoxycarbonyl (Fmoc), 4,4′-dimethoxytrityl (DMT), trichloracetic acid (TCA), dichloromethane (DCM), triethylammonium hydrogen carbonate (TEAB), diethyl azadicarboxylate (DEAD), triphenylphosphane (TPP), tetrahydrofuran (THF).

The acridine CPG support was purchased from Glen Research. Functionalisation of solid supports was carried out in a short column (5 cm length, 1 cm i.d.) equipped with a sintered-glass filter, stopcock and a cap. The ODNs were assembled with a PerSeptive Biosystems Expedite DNA synthesiser using standard phosphoramidite chemistry. HPLC analyses and purification were carried out on a Waters 600E apparatus equipped with a UV detector. UV spectra and thermal denaturation experiments were run with a Jasco V 530 spectrophotometer, equipped with a Jasco 505T temperature controller unit. CD spectra were obtained with a Jasco 715 circular dichroism spectrophotometer. NMR spectra were recorded with a Bruker AMX500 spectrometer. ESI mass spectrometric analyses were performed on a API 2000 (Applied Biosystem) machine used in negative mode. MALDI TOF mass spectrometric analysis wad performed on a PerSeptive Biosystems voyager-De Pro MALDI mass spectrometer using picolinic/3-hydroxypicolinic acid mixtures as the matrix.

**3′-*O*-(4,4′-Dimethoxytriphenylmethyl)-5′-*O*-fluorenylmethoxycarbonylthymidine (4):** $^1$H NMR ([D$_6$]acetone): $\delta$ = 9.90 [br. s, 1 H, N(3)-H], 8.02 (s, 1 H, H-6), 7.90−6.80 (m, 21 H's, aromatic protons), 6.32 (m, 1 H, H-1′), 4.52 (m, 2 H, CH$_2$ Fmoc residue), 4.32 (m, 1 H, H-3′), 4.24 (t, 1 H, CH Fmoc residue), 3.98 (m, 3 H, H$_2$-5′ and H-4′), 3.81 (s, 6 H, OCH$_3$ DMT), 1.92 and 1.72 (m, 1 H each, H$_2$-2′), 1.65 (s, 3 H, CH$_3$-5) ppm.

**Preparation of Supports 5 and 6, and Product 7:** Commercially available support **1** (200 mg; 0.044 mequiv./g) was washed with DCM and then treated with 3 × 2.0 mL of a DCM solution of TCA (3%, w/v) for 2 min. This treatment was repeated three times and the resulting support **2** was exhaustively washed with DCM and then dried under reduced pressure.

DEAD (207 μL, 1.3 mmol) was added to a solution of triphenylphosphane (TPP; 340 mg, 1.3 mol) dissolved in 800 μL of THF at 0 °C. After 10 min this mixture was added to 200 mg of support **2** suspended with a mixture of 0.26 mmol of **5** (or **6**) in 700 μL of anhydrous DCM. After 5 hours at room temperature the support was exhaustively washed with DCM, pyridine and then treated with a solution of pyridine/acetic anhydride (2.0 mL, 9:1 v/v) for 30 min at room temperature. Finally, the support was washed with DCM and Et$_2$O, and dried under reduced pressure. Incorporation yields of nucleoside **3** and **4** onto **2** were in the range of 65−80% (0.029−0.035 mequiv./g), as determined by a quantitative DMT test performed on dried and weighed samples of the obtained supports **5** and **6**.

Treatment of 50 mg of support **6** with 1.5 mL of a 0.4 M solution of NaOH in methanol/water (4:1, v/v) for 17 h at room temperature, followed by neutralisation of the alkaline solution with a 2 M solution of triethylammonium acetate, furnished product **7** (0.5 mg, after RP HPLC purification).

**7:** $^1$H NMR (D$_2$O): $\delta$ = 8.02, 7.50, 7.48, 7.42, 7.26, 7.18, 7.06 (1 H each, acridine protons and H-6 thymine), 5.56 (m, 1 H, H-1′), 4.60 (m, 1 H, H-3′), 3.90 (s, 3 H, CH$_3$-O), 3.90−3.70 [m's, 5 H, overlapped signals, CH$_2$-NH, CH$_2$-N(1), H-4′], 3.60 (m, 2 H, CH$_2$OH), 3.48 (m, 2 H, H$_2$-5′), 2.25 (m, 2 H, H$_2$-2′), 1.61 (s, 3 H, CH$_3$-5), 1.85−1.40 (m's, 7 H, 3 CH$_2$ and CH) ppm. UV/Vis (CH$_3$OH): $\lambda_{max}$ = 265 and 422 nm. ESI-MS: *m*/*z* = 613 [MH$^+$].

**Oligonucleotide Synthesis. Products 9, 10 and 12. Symmetric Oligomer Elongation:** Support **5** (50 mg, 0.0016 mequiv.) was used for each synthesis in the automated DNA synthesiser following standard phosphoramidite chemistry, with a 45 mg/mL solution of 3′-phosphoroamidite building block in anhydrous CH$_3$CN. A commercially available *N*(4)-acetyl-protected cytidine 3′-phosphoramidite building block was used to avoid deamination when sodium hydroxide was used for final cleavage and deprotection. Three-mer **9** and ODN **10** were synthesised by performing one or eight coupling cycles, respectively, with the appropriate nucleotide unit. The coupling yields were always better than 98% (by DMT test). After completion of the desired ODN sequence and final DMT removal, the support was treated with 1.0 mL of a 0.4 M solution of NaOH in methanol/water (4:1, v/v) for 17 h at room temperature. The filtered solution and washings, neutralised with 5.0 mL of a 2 M solution of triethylammonium acetate, were concentrated under reduced pressure, redissolved in water and purified by HPLC.

Crude **9** was purified on an RP C$_{18}$ column (4.6 × 250 mm), eluting with a linear gradient of MeOH in TEAB buffer (pH 7.0, 0 to 10% in 50 min, flow 0.8 mL/min); retention time 35.5 min. The final product (45 units OD$_{260}$ from 50 mg of **5**) was lyophilized and characterised.

**9:** $^1$H NMR (D$_2$O): $\delta$ = 7.95, 7.90, 7.85, 7.45, 7.40, 7.32, 7.20, 7.15, 7.02 (9 H, partially overlapped, acridine protons and 3H-6), 6.32−6.40 (m's, 3 H, 3 H-1′), 1.65, 1.68, 1.70 (s's, 3 H each, 3 CH$_3$-5) ppm. $^{31}$P NMR (D$_2$O): $\delta$ = 1.55, 1.38 ppm. UV/Vis (H$_2$O): $\lambda_{max}$ = 265, 422 nm. ESI-MS: *m*/*z* = 1221 [M + H]$^+$, 1243 [M + Na]$^+$.

Crude **10** was purified an a Nucleogel SAX column (Macherey-Nagel, 1000-8/46); buffer A: 20 mM aqueous KH$_2$PO$_4$ solution, pH 7.0, containing 20% (v/v) CH$_3$CN; buffer B: 1.0 M KCl, 20 mM aqueous KH$_2$PO$_4$ solution, pH 7.0, containing 20% (v/v) CH$_3$CN; using a linear gradient from 0 to 100% B in 20 min; flow rate 1.0 mL/min. The collected peak at retention time 18.8 min, desalted on a Sep-Pak column (C18), furnished 28 OD$_{260}$ units of pure **10**. Maldi TOF-MS (positive mode): calculated mass 5357.03; found 5358.83 [M + H], 5380.86 [M + Na].

**Asymmetric Oligomer Elongation:** Support **6** (50 mg, 0.0015 mequiv.) was used for the synthesis of oligomer **12** following standard phosphoramidite chemistry as described for product **10**. After the elongation of the first ODN tract, the final DMT group was removed and the resulting 5′-OH function capped by reaction with 1 mL of pyridine/Ac$_2$O solution (4:1, v/v) leading to **11**. Removal of the Fmoc protecting group was achieved by pyridine/NEt$_3$ treatment (1.5 h, 95:5, v/v). The resulting 5′-OH support was successively coupled with the required 3′-phosphoramidite derivatives to complete the desired ODN sequence, following a standard automated procedure. Crude **12** was detached from the support, deprotected and purified as described for **10**. The collected peak at retention time 20.5 min, after desalting, furnished 24 OD$_{260}$ units of pure **12**.

After HPLC purification 24 OD$_{260}$ units of **12** were obtained. Maldi TOF-MS (positive mode): calculated mass 5342.03; found 5343.65 [M + H], 5383.06 [M + K].

**UV Thermal Denaturation Experiments:** The concentrations of the synthesised oligomers were determined spectrophotometrically at $\lambda$ = 260 nm and 80 °C, using the molar-extinction coefficient calculated for unstacked oligonucleotides from the following extinction coefficients: 11700 (G); 8800 (T) cm$^{-1}$ M$^{-1}$. An aqueous solution of 5 mM NaH$_2$PO$_4$, 140 mM KCl and 5 mM MgCl$_2$ (at pH 5.5, 6.0

  

and 6.6) was used for the melting experiments. Melting curves were recorded using a concentration of approximately 1 μM of single strand in 1 mL of the tested solution in Teflon$^R$-sealed quartz cuvettes of 1-cm optical path-length. The resulting solutions were then heated at 90 °C for 5 min, then slowly cooled and kept at 20 °C for 15 h. After thermal equilibration at 20 °C the UV absorption at $\lambda = 260$ nm was monitored as function of the temperature, which was increased at a rate of 0.5 °C/min. The melting temperatures were determined as the maximum of the first derivative of the absorbance vs. temperature plots.

**Circular Dichroism:** CD spectra were registered in the same buffer as used for UV melting experiments at 25 °C in a 0.1 cm path-length cuvette. The wavelength was varied from 200 to 340 nm at 5 nm/min, and the spectra recorded with a response of 16 s, at 2.0 nm bandwidth and normalised by subtraction of the background scan with buffer. The temperature was kept constant at 25 °C with a thermoelectrically controlled cell holder (JASCO PTC-348).

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[1] N. T. Thuong, C. Hélène, *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 666−690.
[2] For a general review, see: B. P. Casey, P. M. Glazer, *Prog. Nucleic Acid Res.* **2001**, *67*, 163−192.
[3] D. A. Horne, P. B. Dervan, *J. Am. Chem. Soc.* **1990**, *112*, 2435−2437.
[4] A. Ono, C. N. Chen, L. Kan, *Biochemistry* **1991**, *30*, 9914−9921.
[5] S. Mc Curdy, C. Moulds, B. C. Froehler, *Nucleosides Nucleotides* **1991**, *10*, 287−290.
[6] B. C. Froehler, T. Terhorst, J. P. Shaw, S. N. Mc Curdy, *Biochemistry* **1992**, *31*, 1603−1609.
[7] M. Durand, S. Peloille, N. T. Thuong, J. C. Maurizot, *Biochemistry* **1992**, *31*, 9197−9204.
[8] P. A. Beal, P. B. Dervan, *J. Am. Chem. Soc.* **1992**, *114*, 4976−4982.
[9] S. D. Jayasena, B. H. Johnston, *Nucl. Acids Res.* **1992**, *20*, 5279−5288.
[10] U. Asseline, N. T. Thuong, *Tetrahedron Lett.* **1993**, *34*, 4173−4176.
[11] U. Asseline, N. T. Thuong, *Tetrahedron Lett.* **1994**, *35*, 5221−5224.
[12] L. De Napoli, A. Messere, D. Montesarchio, A. Pepe, G. Piccialli, M. Varra, *J. Org. Chem.* **1997**, *62*, 9024−9030.
[13] L. De Napoli, G. Di Fabio, A. Messere, D. Montesarchio, G. Piccialli, M. Varra, *Eur. J. Org. Chem.* **1998**, 2119−2125.
[14] L. De Napoli, G. Di Fabio, A. Messere, D. Montesarchio, D. Musumeci, G. Piccialli, *Tetrahedron* **1999**, *55*, 9899−9914.
[15] G. Barone, L. De Napoli, G. Di Fabio, C. Giancola, A. Messere, D. Montesarchio, L. Petraccone, G. Piccialli, *Bioorg. Med. Chem.* **2001**, *9*, 2895−2900.
[16] C. Giancola, A. Buono, G. Barone, L. De Napoli, D. Montesarchio, D. Palomba, G. Piccialli, *J. Therm. Anal. Cal.* **1999**, *56*, 1177−1184.
[17] C. Giancola, A. Buono, D. Montesarchio, G. Barone, *PhysChem ChemPhys.* **1999**, *1*, 5045−5049.
[18] Y. Ueno, M. Mikawa, S. Hoshika, A. Matsuda, *Bioconjugate Chem.* **2001**, *12*, 635−642.
[19] Y. Ueno, A. Shibata, A. Matsuda, Y. Kitade, *Bioconjugate Chem.* **2003**, *14*, 684−689.
[20] V. Esposito, A. Galeone, L. Mayol, G. Oliviero, A. Randazzo, M. Varra, *Eur. J. Org. Chem.* **2002**, 4228−4233.
[21] E. Washbrook, K. R. Fox, *Biochem. J.* **1994**, *301*, 569−575.
[22] G. C. Silver, C. H. Nguyen, A. S. Boutorine, E. Bisogni, T. Garestier, C. Hélène, *Bioconjugate Chem.* **1997**, *8*, 15−22.
[23] A. D. Stewart, X. Xu, S. D. Thomas, D. M. Miller, *Nucleic Acid Res.* **2002**, *30*, 2565−2573.
[24] A. Kuzuya, R. Mizoguchi, F. Morisawa, K. Machida, M. Komiyama, *J. Am. Chem. Soc.* **2002**, *124*, 6887−6894.
[25] M.-P. Teulade-Fichou, D. Perrin, A. Boutorine, D. Polverari, J.-P. Vigneron, J.-M. Lehn, J.-S. Sun, T. Garestier, C. Hélène, *J. Am. Chem. Soc.* **2001**, *123*, 9283−9292.
[26] M. Kubuota, K. Ono, *Nucl. Acids Res. Suppl.* **2002**, *2*, 33−34.
[27] K. Ono, Jpn. Kokai Tokkyo Koho 2003, 8 pp. CODEN: JKXXXAF JP 2003088374 A2 20030325 CAN 138:255457 AN 2003:227376 CAPLUS.
[28] O. Mitsunobu, *Synthesis* **1981**, 1−28.
[29] M. de Champdoré, L. De Napoli, G. Di Fabio, A. Messere, D. Montesarchio, G. Piccialli, *Chem. Commun.* **2001**, 2598−2599.
[30] *Oligonucleotides and Analogues: A Practical Approach* (Ed.: F. Eckestein), IRL Press, Oxford, UK, **1991**.
[31] L. M. Xodo, G. Manzini, F. Quadrifoglio, *Nucleic Acids Res.* **1990**, *18*, 3557−3564.
[32] V. P. Antao, D. M. Grey, R. L. Ratliff, *Nucleic Acids Res.* **1988**, *16*, 719−783.

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