Synthesis of Triple Helix Forming Oligonucleotides with a Phenanthroline Moiety into the 3′-3′ Inversion Site of Polarity

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A protocol for the synthesis of triplex forming oligonucleotides containing a chelating molecule in the 3′-3′ inversion site of polarity is reported. A number of 16-mers have been synthesized and UV spectroscopy employed to evaluate the stability of their triple helix complexes with the pertinent double-strand oligonucleotide targets.

Introduction

Antigene strategy, based on sequence-specific targeting of double-stranded DNA through triplex forming oligonucleotides (TFOs), is a fascinating topic in medicinal chemistry in view of its potential towards the rational design of new drugs capable of selectively inhibiting a gene expression.[1] A TFO may, indeed, interfere with the binding of proteins to the gene of interest and impede its transcription, specificity being insured by hydrogen bonding interactions occurring in the major groove of the double-stranded DNA between purine groups of Watson−Crick base pairs of the gene and the bases of the oligonucleotide.

Based on TFO sequence, triple helices can be generally classified as follows:

1) (TC) or pyrimidine motif, where TFO binds parallel to the purine strand of the duplex, forming canonical T·A*T and C·G*C^+ Hoogsteen base triplets;

2) (GA) or purine motif, with TFO binding to the purine strand of the duplex, through reverse Hoogsteen hydrogen bonds, giving rise to C·G*G and T·A*A base triplets;

3) (GT) or purine-pyrimidine motif, where TFO can bind either parallel or antiparallel to the purine strand of the duplex forming C·G*G and T·A*T base triplets.

These recognition models require homopurine stretches in double-stranded nucleic acids which, in addition, have to be at least 15−17 bases long to afford a stable triple helical complex with TFO. The need for these specifications in the target DNA severely limits the possible applications of antigene strategy. In order to overcome this restriction, TFOs containing a 3′-3′ inversion site of polarity have been devised which allow to extend the recognition to (purine)$_m$(pyrimidine)$_n$ sequences.[2−9] Such modified TFOs, in fact, have been shown to hybridize the adjacent purine blocks on alternate strands of a target double-stranded DNA by switching strand at the junction between the oligopurine and the oligopyrimidine domains.

From a chemical point of view, the 3′-3′ inversion site is represented by a suitable linker capable of crossing the major groove; furthermore, its structural features may, in principle, supply the TFO with useful properties, as well. In this frame, we have recently described a fully automated method useful to prepare TFOs containing a chelating agent in the 3′-3′ inversion site, namely a bipyridine moiety.[2] This molecule has been shown to form complexes with transition metals, such as Cu, Co, Rh and Ru, whose redox properties and DNA affinity have been exploited to develop chemical nucleases.[10−36] The incorporation of the ligand in the 3′-3′ inversion site was achieved by anchoring the ligand to the polymeric support through a trifunctionalized molecule (2-amino-1,3-propanediol). However, the presence of a labile amide bond between the linker and the bipyridine moiety required the use of *N*^{4}-*tert*-butylphenoxyacetyl-5′-*O*-DMT-2′-deoxycytidine phosphoramidite (expedite phosphoramidite) (DMT = dimethoxytrityl) to obtain an efficient deprotection of 2′-deoxycytidine residues by ammonia treatment at room temperature. Furthermore, the elongation of the oligonucleotide (ON) chains started from the two prochiral centers of 2-amino-1,3-propanediol, thus affording diastereomeric mixtures of TFOs.

This paper deals with a new procedure which does not suffer from the above drawbacks and permits the synthesis of two types of TFOs (A and B, Figure 1) with symmetrical sequences. These are characterized by a 3′-3′ inversion site containing a phenanthroline moiety as a chelating group and differ from each other for the position of the ligand on the respect of the oligonucleotide chains. Phenanthroline has been preferred to the previously utilized bipyridine be-

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cause in front of comparable chelating properties,[10] chemical reactivity of the former allows an easier preparation of a suitable functionalized derivative[28,32,36–42] for its incorporation into the polymeric support starting from commercially available compounds.

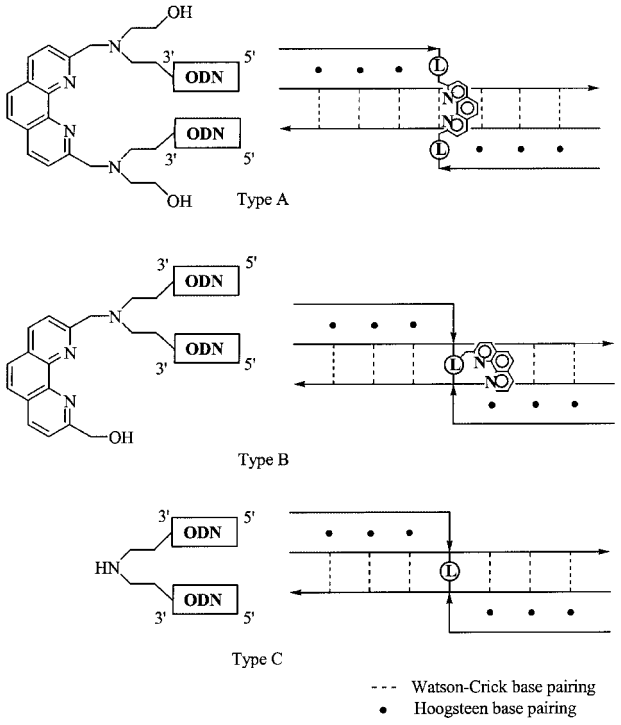


Figure 1. Types of TFOs synthesized

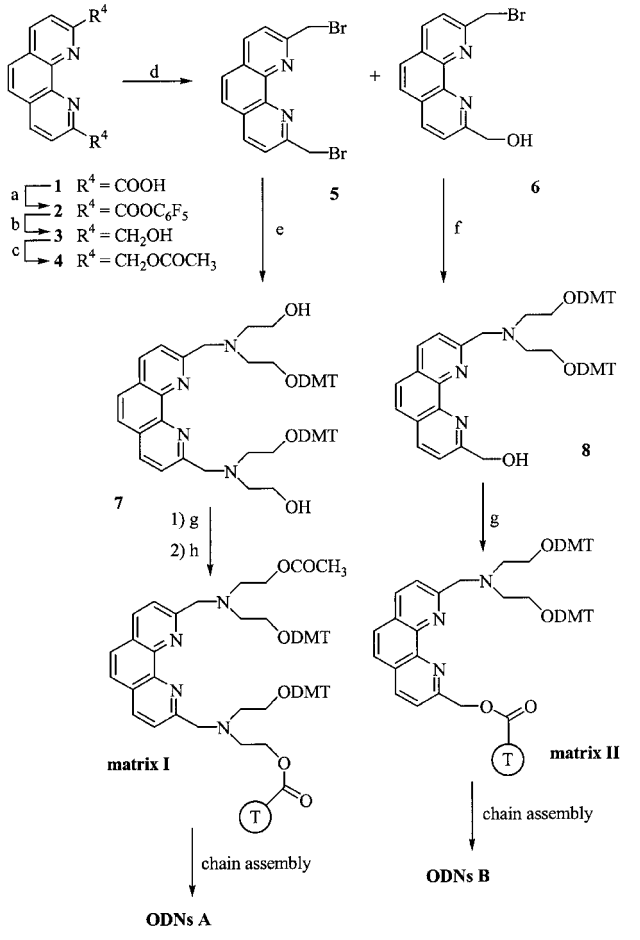
A comparison of the melting profiles of the triple helices formed by the two types (**A**, **B**, Figure 1) of TFOs and that containing only the diethanolamine linker in the 3′-3′ inversion site (type **C**, Figure 1) with a double-stranded ON is reported, and the effects of the presence and the relative mobility of the phenanthroline moiety is discussed.

Results and Discussion

Syntheses of TFOs of type **A** and **B** are performed on suitably functionalized polymeric supports, matrixes I and II (Scheme 1), obtained by reaction of TENTAGEL-COOH with phenanthroline derivatives **7** and **8**, respectively, both prepared starting from phenanthroline-2,9-dicarboxylic acid (**1**, Scheme 1). In order to selectively reduce the carboxylic functions without affecting the phenanthroline moiety, **1** is first converted into the active ester **2** by reaction with pentafluorophenyl trifluoroacetate in dry DMF and pyridine. After purification, derivative **2** is treated with $NaBH_4$ in dimethyl ether at room temperature for 4 h and the excess of reducing agent destroyed by addition of H_2O/MeOH. The resulting mixture containing derivative **3**, dried from the solvents, is submitted to reaction with Ac_2O, without any further purification, thus affording the derivative **4**, easily separated from boron salts thanks to its lipophilicity. Bromination of **4** by refluxing with 30% HBr in less than stoichiometric amounts, gives a mixture of **5** and **6** (54% and 37% yields, respectively based on the carboxylic derivative **1**).

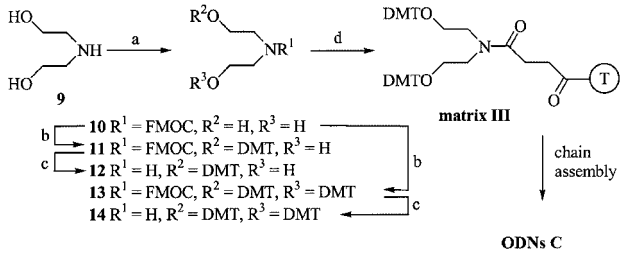
TFOs of Type A

Treatment of 2,9-bis(bromomethyl)phenanthroline (**5**) with the DMT derivative of diethanolamine (**12**) in dry CH_2Cl_2/Et_3N, gives the derivative **7** which is, in turn, anchored to the polymeric support (TENTAGEL-COOH) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (DMPEC) as a coupling agent in dry pyridine/DMF (50:50, v/v). DMT-diethanolamine **12** is prepared from diethanolamine (**9**) by reaction with 4,4′-dimethoxytrityl chloride in pyridine using 9-fluorenylmethoxycarbonyl (FMOC) as a transient protecting group for the amino function (Scheme 2).



Scheme 1. Reagents: a) $CF_3COC_6F_5$/DMF; b) $NaBH_4$/dimethoxyethane; c) Ac_2O/Py; d) HBr 30%; e) **12** (Scheme 2)/DCM/Et_3N; f) **14** (Scheme 2)/DCM/Et_3N; g) TENTAGEL-COOH/DMPEC/Py/DMF; h) Ac_2O/1-methylimidazole/THF

After capping the unchanged hydroxy functions on the solid support (matrix I) by acetylation, automated ODN synthesis may proceed through the standard 2-cyanoethyl-



Scheme 2. Reagents: a) Fmoc-OSu/DMF; b) DMTCl/Py; c) piperidine/DMF; d) TENTAGEL-COOH/Py

phosphoramidite chemistry, with a symmetrical elongation of the two chains. Two 16-mers, $^{5'}(TC)_4{}^{3'}$-Phe-$^{3'}(CT)_4{}^{5'}$ (**A1**) and $^{5'}(CT)_4{}^{3'}$-Phe-$^{3'}(TC)_4{}^{5'}$ (**A2**), were prepared in satisfactory yields. After deprotection and removal from the polymeric matrix, the products were purified by HPLC on an ionic exchange column and analyzed by MALDI-TOF mass spectrometry which confirmed their identities.

TFOs of Type B

The polymeric matrix II is prepared by leaving it in contact with the derivative **8**, obtained by reaction of 2-(bromomethyl)-9-(hydroxymethyl)phenanthroline (**6**) with the di-DMT derivative of diethanolamine **14**, and TENTAGEL-COOH in pyridine/DMF, in the presence of DMPEC as a coupling agent. Compound **14** is obtained by treating the *N*-FMOC derivative of diethanolamine (**10**) with excess DMT-Cl and successive removal of the NH protecting group by treatment with piperidine in DMF.

Polymeric matrix II has been, finally, used to synthesize the sequences $^{5'}(TC)_4{}^{3'}$-Phe-$^{3'}(CT)_4{}^{5'}$ (**B1**) and $^{5'}(CT)_4{}^{3'}$-Phe-$^{3'}(TC)_4{}^{5'}$ (**B2**). Also in this case, the efficiency of the syntheses was confirmed, after purification of ODNs as described for TFOs of type **A**, by MALDI TOF mass spectrometry.

TFO of Type C

The polymeric matrix III, obtained by reaction of the diethanolamine derivative **14** with TENTAGEL-COOH in dry pyridine and DCCI, has been used to prepare the following TFOs: $^{5'}(TC)_4{}^{3'}$-L-$^{3'}(CT)_4{}^{5'}$ (**C1**), $^{5'}(CT)_4{}^{3'}$-L-$^{3'}(TC)_4{}^{5'}$ (**C2**), where L indicates diethanolamine linker. UV spectroscopy has been employed to assess the formation of the complex of each TFO with the pertinent double-stranded oligonucleotide target and to appraise the stability of the resulting triple helix, by monitoring hyperchromicity at 260 nm.

Representative UV melting curves for triple helix complexes between TFOs (**A1**, **B1**, **C1**) and the target duplex $(AGAGAGAGCTCTCTCT)_2$ are shown in Figure 2. Each thermal denaturation profile shows a biphasic pattern, the former transition being due to the dissociation of the third strand, the latter to the melting of the duplex into single strands.

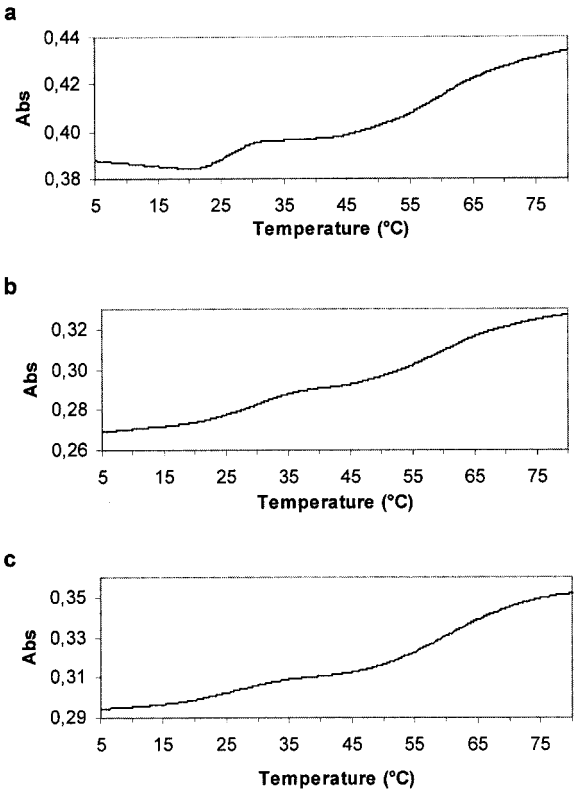


Figure 2. Thermal denaturation profile (pH = 6.2) of complexes of the duplex $d(AGAGAGAGCTCTCTCT)_2$ with TFOs **A1** (a), **B1** (b), **C1** (c)

The UV data confirm the previous finding that TFOs containing dC nucleotides right before and after the 3′-3′ inversion site, provide triple helices more stable than those formed by TFOs of the same length and base composition, containing thymidine residues adjacent to the 3′-3′ inversion site.[4] Furthermore, a comparison of T_m values, indicates no significant difference in the thermal stability between triple helices formed by TFOs of type **A**−**C** with identical sequence, whereas an increase of T_m values (5 °C) is observed for triple helices involving the corresponding TFOs of type **B** (see Table 1).

Table 1. Estimated T_m values [°C] for triple helix complexes between the target duplex and the TFOs (n.t. = not tested; X = phenanthroline linker for types **A** and **B**; amino linker for type **C**)

Sequence	TFO type		
	A	**B**	**C**
$(TC)_4$-X-$(CT)_4$ (1)	26.4	31.4	26.0
$(CT)_4$-X-$(TC)_4$ (2)	n.t.	27.4	22.0

Conclusions

In this paper we report the synthesis of two types of TFOs with a 3′-3′ inversion of polarity containing a chelat-

ing agent, namely a phenanthroline moiety, which is known to bind to a variety of metal ions giving rise to tetrahedral as well as octahedral complexes. In one case (TFOs of type **A**), the ligand, as an integral part of the inversion site, is stiffly placed within the ODN chain. In the other case (TFOs of type **B**) a phenanthroline molecule is anchored, like a pendant, to the central part of the ODN, and possesses a certain freedom of independent orientation. The key step of the synthetic strategy here described is represented by the preparation of the suitable polymeric supports (matrixes I and II), whose functionality is achieved through the intermediates **5** and **6**, deriving from a common precursor. Matrixes I and II allow a fully automated chain assembly of ODNs, using commercially available 3′-phosphoramidite nucleosides. A third kind of polymeric support has been prepared (matrix III) to assemble TFOs just containing diethanolamine into the 3′-3′ inversion site, to be used as terms of comparison to evaluate the effects of the ligand in the stability of triple helices. UV thermal analyses suggest that all the TFOs tested are able to give intermolecular triplexes by cooperative, alternate strand, recognition of adjacent homopurine stretches with the pertinent target duplexes. Furthermore, T_m values demonstrate that the presence of the ligand does not affect negatively the triplex formation and, in the case of TFOs of type **B**, produces a stabilizing effect on the complex. This stabilization can be tentatively ascribed to the higher mobility of the phenanthroline moiety which is, in principle, able to establish positive interactions with groups present in the major groove of the target duplex and/or to intercalate between the base pairs as proposed by other authors for double stranded DNA.[23,43]

Preliminary experiments of site-specific cleavage activity performed on TFOs of type A and B in the presence of $CuCl_2$ and β-mercaptopropionic acid in a HEPES buffer,[23,28,44] are particularly promising for ODNs of the latter class. Further studies aimed at evaluating the coordination properties of the phenanthroline containing TFOs towards copper as well as other cations in view of their possible usage as chemical nucleases are currently in progress in our laboratory and will be published in due course.

Experimental Section

General: Resin (TENTAGEL©) and 1,10-phenanthroline-2,9-dicarboxylic acid (**1**) were purchased from Novabiochem and Avocado, respectively. Functionalization of solid supports was carried out in a short column (5 cm length, 1 cm i.d.) equipped with a sintered glass filter, a stopcock and a cap. The ODNs were assembled with a Millipore Cyclone plus DNA synthesizer using standard phosphoramidite chemistry. HPLC analyses were carried out with a Waters 515 Pump equipped with a UV detector and a computerized controller. Thermal denaturation experiments were run with a Jasco V 530 spectrophotometer, equipped with a thermo-controller. NMR spectra were recorded with a Bruker AMX500 spectrometer. All chemical shifts are expressed as δ values (ppm) in comparison with the residual solvent signal ($CDCl_3$). NMR signals were assigned to the pertinent nuclei through two-dimensional ^1H-^1H and ^1H-^{13}C COSY experiments.

Bis(pentafluorophenyl) 1,10-Phenanthroline-2,9-dicarboxylate (2): Commercially available 1,10-phenanthroline-2,9-dicarboxylic acid (**1**, 2.0 g, 7.45 mmol) was treated with pentafluorophenyl trifluoroacetate (14.3 mL, 52.1 mmol) in anhydrous pyridine (16 mL) and DMF (24 mL). After 12 h at room temperature, TLC analysis (eluent $CHCl_3/CH_3OH$, 8:2, v/v) showed the complete conversion of the starting material into the ester. The solvent was then removed in vacuo and the residue dissolved in $CHCl_3$. The resulting solution was chromatographed on a silica gel column, eluted with $CHCl_3/CH_3OH$ (85:15, v/v). Fractions containing the product, taken to dryness, afforded ester **2** (4.3 g, 98% yield).^1H NMR ($CDCl_3$): δ = 8.91 (d, J = 10.3 Hz, 2 H, 4-H and 7-H), 8.32 (d, J = 10.3 Hz, 2 H, 3-H and 8-H), 7.81 (s, 2 H, 5-H and 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 188.5 (C=O), 158.4 (C-2 and C-9), 149.7 (C-2 and C-6 pentafluorophenyl group), 142.3 (C-4 pentafluorophenyl group), 140.4 (C-11 and C-12), 135.7 (C-4 and C-7), 138.7 (C-3 and C-4 pentafluorophenyl group), 128.1 (C-5 and C-6), 125.0 (C-13 and C-14), 120.3 (C-3 and C-8), 105.5 ppm (C-1 pentafluorophenyl group). ESI-MS: calcd. for $C_{26}H_6F_{10}N_2NaO_4$ 623.01; found 623 $[M + Na]^+$.

2,9-Bis(acetoxymethyl)-1,10-phenanthroline (4): Compound **2** (2.0 g, 3.3 mmol) was treated with $NaBH_4$ (17.0 g, 43.7 mmol) in anhydrous dimethoxyethane (50 mL), whilst stirring at room temperature. After 8 h, TLC analysis (eluent $CHCl_3/CH_3OH$, 85:15 v/v) showed the complete conversion of the ester into a single product. Excess $NaBH_4$ was then distroyed by addition of H_2O/CH_3OH whilst stirring for 2 h and the reaction mixture concentrated under reduced pressure and then co-evaporated with anhydrous pyridine (3 × 20 mL). The resulting residue was dissolved in anhydrous pyridine (15 mL) and acetic anhydride (2.5 mL) was added whilst stirring. After 8 h, TLC analysis (eluent $CHCl_3/CH_3OH$, 95:5, v/v) showed the complete conversion of the reduced intermediate into a less polar single product. The reaction mixture was concentrated and the residue, after dilution with H_2O, extracted with $CHCl_3$ (3 × 300 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to afford **4** (1.0 g, 95% yield). ^1H NMR ($CDCl_3$): δ = 8.50 (d, J = 9.8 Hz, 2 H, 4-H and 7-H), 7.95 (s, 2 H, 5-H and 6-H), 7.85 (d, J = 9.8 Hz, 2 H, 3-H and 8-H), 5.85 (CH_2OH phen.), 2.01 (CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 169.8 (C=O), 160.2 (C-2 and C-9), 140.2 (C-11 and C-12), 134.1 (C-4 and C-7), 127.2 (C-13 and C-14), 127.1 (C-5 and C-6), 119.1 (C-3 and C-8), 74.3 (CH_2O), 21.1 (CH_3) ppm. ESI-MS:calcd. for $C_{18}H_{16}N_2NaO_4$ 347.10; found 347 $[M + Na]^+$.

2,9-Bis(bromomethyl)-1,10-phenanthroline (5) and 2-(Bromomethyl)-9-(hydroxymethyl)-1,10-phenanthroline (6): A solution of **4** (1.0 g, 3.1 mmol) in 30% HBr (4 mL) was stirred under reflux for 2 h and, successively, for 20 min at 0 °C. Then, 20% aqueous Na_2CO_3 (150 mL) was slowly added and the resulting precipitate filtered and washed with H_2O. The solid was collected, dried under reduced pressure, dissolved in $CHCl_3$ and chromatographed on a silica gel column (eluent: increasing amounts of CH_3OH in $CHCl_3$). Fractions eluted with $CHCl_3/CH_3OH$ (98:2, v/v), after evaporation of the solvent, afforded **5** (736 mg) and **6** (418 mg), respectively (54% and 37% yields, based on 1,10-phenanthroline-2,9-dicarboxylic acid). ^1H and ^{13}C NMR spectra are in agreement with those reported in the literature.[36–40]

***N*-Fmoc-diethanolamine (10):** Diethanolamine (**9**) (2.0 g, 19.0 mmol) and FMOC-OSu (Su = Succinimide) (7.6 g, 2.9 mmol) in DMF/H_2O (98:2, v/v) (20 mL) were stirred at room temperature overnight. The resulting solution, diluted with H_2O, was extracted with $CHCl_3$ (3 × 200 mL). The organic layer was concentrated and purified by silica gel column chromatography, eluting with increas-

ing amounts of CH_3OH in $CHCl_3$. Fractions eluted with $CHCl_3$/CH_3OH (98:2, v/v) afforded pure **10** (6.0 g, 98% yield). ^1H NMR ($CDCl_3$): δ = 7.85 (d, *J* = 7.4 Hz, 2 H, fluorenyl group), 7.70 (d, *J* = 7.4 Hz, 2 H, fluorenyl group), 7.12 (t, *J* = 7.4 Hz, 2 H, fluorenyl group), 6.89 (t, *J* = 7.4 Hz, 2 H, fluorenyl group), 4.39 (d, *J* = 6.8 Hz, 2 H, CH_2 fluorenyl group), 4.24 (t, *J* = 6.8 Hz, 1 H, CH fluorenyl group), 3.87 (t, *J* = 6.2 Hz, 4 H, HOCH_2CH_2N), 2.85 (t, *J* = 6.2 Hz, 4 H, HOCH$_2$CH_2N) ppm. ^{13}C NMR ($CDCl_3$): δ = 157.3, 143.4, 137.2, 129.4, 127.5, 126.3, 126.1, 71.4, 64.4, 51.3, 47.8 ppm. ESI-MS: calcd. for $C_{19}H_{21}NNaO_4$ 350.14; found 350 $[M + Na]^+$.

Compound 12: A mixture of compound **10** (3.0 g, 9.2 mmol), DMTCl (2.8 g, 8.3 mmol) and DMAP (44.0 mg, 0.04 mmol) in dry pyridine was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the residue chromatographed on a silica gel column, eluting with increasing amounts of ethyl acetate in *n*-hexane. Fractions eluted with *n*-hexane/ethyl acetate (1:1, v/v), after removal of the solvent, afforded the derivative **11** (2.6 g, 45% yield) which was, in turn, treated with piperidine/DMF (2:8, v/v) (10 mL) for 2.5 h. The solvent was then removed in vacuo and the residue purified by silica gel column chromatography, eluting with increasing amounts of CH_3OH in $CHCl_3$ containing 0.5% of pyridine. Fractions eluted with $CHCl_3$/CH_3OH (85:15, v/v), taken to dryness, afforded **12** (1.6 g, 43% overall yields). ^1H NMR ($CDCl_3$): δ = 6.9−7.3 (m, 13 H, DMT aromatic protons), 3.65 (s, 6 H, CH_3O), 3.55 (t, *J* = 5.9 Hz, 2 H, HOCH_2CH_2N), 3.45 (t, *J* = 6.2 Hz, 2 H, DMTOCH_2CH_2N), 2.82−2.78 (m, 4 H, HOCH$_2$CH_2N and DMTOCH$_2$CH_2N) ppm. ^{13}C NMR ($CDCl_3$): δ = 158.1, 142.1, 135.3, 131.4, 128.6, 128.4, 128.3, 111.5, 89.1, 65.9, 64.3, 58.8, 49.2, 48.4 ppm. ESI-MS: calcd. for $C_{25}H_{30}NO_4$ 408.22; found 408 $[MH]^+$.

Compound 14: Compound **10** (3.0 g, 9.2 mmol) was treated with DMTCl (7.5 g, 22.1 mmol) and DMAP (9.8 mg, 0.08 mmol), according to the same procedure described in the previous section, thus affording **13** (8.4 g, 98% yield), purified by silica gel column chromatography, eluting with *n*-hexane/ethyl-acetate (8:2, v/v). Transformation of **13** into **14** was performed under the same experimental conditions used to obtain **12** from **11**. Final purification of the desired product was achieved by silica gel column chromatography, eluting with increasing amounts of ethyl acetate in *n*-hexane containing 0.5% of pyridine. Fractions eluted with *n*-hexane/ethyl acetate (1:1) gave pure **14** (6.3 g, 97% yield). ^1H NMR ($CDCl_3$): δ = 6.90−7.40 (m, 26 H, DMT aromatic protons), 3.62 (s, 12 H, CH_3O), 3.60 (t, *J* = 6.1 Hz, 4 H, DMTOCH_2CH_2N), 2.65 (t, *J* = 6.1 Hz, 4 H, DMTOCH$_2$CH_2N) ppm. ^{13}C NMR ($CDCl_3$): δ = 159.1, 147.4, 135.6, 130.3, 129.1, 129.0, 128.7, 112.3, 89.8, 67.3, 58.8, 49.4 ppm. ESI-MS: calcd. for $C_{46}H_{47}NNaO_6$ 732.33; found 732 $[M + Na]^+$.

Functionalization of the Resin: A mixture of TENTAGEL-NH_2 (1 g, 0.24 mequiv./g of amino groups) and succinic anhydride (1.3 g, 12 mmol) in anhydrous pyridine (10 mL) was shaken for 20 h at room temperature. The resulting resin (TENTAGEL-COOH), after filtration, was washed with pyridine and dichloromethane and dried in vacuo.

2,9-Bis{[(2-hydroxyethyl){2-[bis(4-methoxyphenyl)(phenyl)methoxy]-ethyl}amino]methyl}-1,10-phenanthroline (7): A solution of **5** (300 mg, 0.82 mmol), **12** (835 mg, 1.66 mmol) and freshly distilled triethylamine (265 μL, 1.66 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature and the reaction monitored by TLC (eluent $CHCl_3$/CH_3OH, 97:3, v/v). After 4 h, the solvent was removed under reduced pressure and the residue purified on a silica gel column eluted with an increasing amount of CH_3OH in $CHCl_3$ containing 0.5% of pyridine. Fractions eluted with $CHCl_3$/CH_3OH (95:5, v/v), taken to dryness, afforded **7** (800 mg, 96% yield). ^1H NMR ($CDCl_3$): δ = 8.21 (d, *J* = 10.9 Hz, 2 H, 4-H and 7-H), 7.69 (s, 2 H, 5-H and 6-H), 7.22 (d, *J* = 10.9 Hz, 2 H, 3-H and 8-H), 4.12 (s, 4 H, CH_2N phen.), 3.84 (m, 4 H, NCH$_2$CH_2OH), 3.62 (s, 12 H, CH_3O), 3.59 (m, 4 H, NCH$_2$CH_2ODMT), 2.63 (m, 4 H, NCH_2CH$_2$OH), 2.58 (m, 4 H, NCH_2CH$_2$ODMT), 6.90−7.25 (m, 26 H, DMT aromatic protons) ppm. ^{13}C NMR ($CDCl_3$): δ = 160.2 (C-9 and C-2), 140.4 (C-11 and C-12), 138.4 (C-4 and C-7), 129.3 (C-13 and C-14), 128.6 (C-5 and C-6), 125.2 (C-3 and C-8), 160.4, 133.2, 120.1, 111.7 (DMT methoxyphenyl carbon atoms), 143.0, 128.8, 128.4, 124.6 (DMT phenyl carbon atoms), 90.3 (DMT aliphatic quaternary carbon atom), 66.3 (HO*C*H$_2$CH$_2$N), 64.5 (DMTO*C*H$_2$CH$_2$N), 62.4 (CH_2N phen.), 58.8 (CH_3O of DMT group), 57.1 (DMTOCH$_2$*C*H$_2$N), 54.3 (HOCH$_2$*C*H$_2$N) ppm. ESI-MS: calcd. for $C_{64}H_{66}N_4NaO_8$ 1041.48; found 1041 $[M + Na]^+$.

2-[(Bis{2-[bis(4-methoxyphenyl)(phenyl)methoxy]ethyl}-amino)methyl]-9-(hydroxymethyl)-1,10-phenanthroline (8): A mixture of the bromo derivative **6** (300 mg, 0.99 mmol), **14** (913 mg, 1.29 mmol) and freshly distilled triethylamine (190 μL, 1.23 mmol) in anhydrous CH_2Cl_2 (10 mL) was kept at room temperature whilst stirring. After 4 h, TLC analysis (eluent $CHCl_3$/CH_3OH, 9:1, v/v) showed the complete conversion of **6** into **8**. The solvent was then removed under reduced pressure and the residue purified on a silica gel column eluted with increasing amounts of CH_3OH in $CHCl_3$ containing 0.5% of pyridine. Fractions eluted with $CHCl_3$/CH_3OH (97:3, v/v), after removal of the solvent, afforded pure **8** (920 mg, 98% yields). ^1H NMR ($CDCl_3$): δ = 8.04 (d, *J* = 11.0 Hz,1 H, 4-H), 8.01 (d, *J* = 12.7 Hz, 1 H, 7-H), 7.70 (d, *J* = 8.0 Hz, 1 H, 5-H), 7.40 (d, *J* = 8.0 Hz, 1 H, 6-H), 7.34 (d, *J* = 12.7 Hz, 1 H, 8-H), 7.22 (d, *J* = 11.0 Hz, 1 H, 3-H), 6.90−7.20 (m, 26 H, DMT aromatic protons), 5.21 (s, 2 H, CH_2OH phen.), 4.42 (s, 2 H, CH_2N phen.), 4.32 (m, 4 H, DMTOCH_2CH_2N), 3.62 (s, 12 H, CH_3O), 3.01 (m, 4 H, DMTOCH$_2$CH_2N) ppm. ^{13}C NMR ($CDCl_3$): δ = 161.5 (C-9), 156.4 (C-2), 139.8 (C-12), 139.5 (C-7), 135.4 (C-4), 130.5 (C-11), 129.2 (C-14), 128.4 (C-13), 128.1 (C-5), 125.6 (C-6), 124.8 (C-8), 118.7 (C-3), 161.3, 133.1, 119.3, 111.2 (DMT methoxyphenyl carbon atoms), 143.0, 128.7, 128.1, 123.0 (DMT phenyl carbon atoms), 90.3 (DMT aliphatic quaternary carbon atom), 70.4 (CH_2OH phen.), 64.3 (DMTO*C*H$_2$CH$_2$N), 60.9 (CH_2N phen.), 58.4 (CH_3O), 52.6 (DMTOCH$_2$*C*H$_2$N) ppm. ESI-MS: calcd. for $C_{60}H_{57}N_3NaO_7$ 954.41; found 955 $[M + Na]^+$.

Matrixes I and II: A mixture of the solid support (TENTAGEL-COOH) (200 mg) in DMF/pyridine (1:1, v/v, 4 mL) was shaken for 20 h at room temp in the presence of DMPEC (383 mg, 2.0 mmol) and derivative **7** (250 mg, 0.2 mmol) or **8** (200, 0.2 mmol). The resulting polymers were washed with pyridine, dichloromethane, diethyl ether and dried in vacuo. The loadings were estimated by spectroscopic measurement (λ = 498 nm; ε = 71700 cm^{-1} M^{-1}) of the DMT cation released upon acidic treatment (70% $HClO_4$/EtOH, 3:2, v/v) of a weighed amount of dried support and resulted to be 0.050−0.052 and 0.040−0.042 mequiv./g for matrix I and matrix II, respectively. For matrix I a capping step was performed before chain assembly to block the unchanged OH functions.

Matrix III: A solution of DCCI (115 mg, 0.56 mmol) in pyridine (500 μL) and a solution of **14** (100 mg, 0.14 mmol) in pyridine (500 μL) were added to the solid support (TENTAGEL-COOH) (200 mg). The mixture was shaken for 20 h at room temperature. The resulting matrix III was washed with pyridine, dichloromethane, diethyl ether and dried in vacuo and the loading (0.10−0.12 mequiv./g) measured as described above for matrixes I and II.

Chain Assembly: Syntheses of TFOs were typically carried out starting from the pertinent solid support (40 mg). After the initial removal of DMT groups, the assembly of the two domains was achieved according to the standard automated procedures, with coupling yields invariably in the range of 96−98% per cycle, as verified by DMT tests. Synthesis of the target duplex was carried out by the standard phosphoramidite method.

Deprotection, Purification and Characterization of Oligomers: The assembled oligomers were deprotected and detached from the matrix by treatment with concd. aq. ammonia at 50 °C overnight. The supernatant liquid was filtered and the support washed with water. The filtrate and washings were concentrated in vacuo, the residue was dissolved in water and purified by ion-exchange HPLC on a VA-50/4.6 Nucleogel SAX 1000-8 column eluted with a linear gradient from 0 to 100% in 30 min of buffer B in buffer A (flow rate 1 mL/min) (buffer A: aq. 20 mM KH_2PO_4, pH = 7, containing 20% v/v CH_3CN; buffer B: aq. 1 M KCl, 20 mM KH_2PO_4, pH = 7, containing 20% v/v CH_3CN). The resulting ODNs were desalted by Sep-Pak© RP-18 columns and their purity (> 98%, in any case) checked by HPLC on a Partisil RP-18 analytical column (125 × 4.0 mm, 5 μm), using a linear gradient (from 0 to 15% in 40 min) of CH_3CN in 0.1 M aq. triethylammonium acetate buffer at pH = 6.8 (flow rate 1 mL/min). MS analyses of all ODNs were performed after ion exchange on Dowex H^+ resin. MALDI TOF of ODN type **A**: $^{5'}(TC)_4{}^{3'}$-Phe-$^{3'}(CT)_4{}^{5'}$ *m/z* = 5160.37 [MH^+] and $^{5'}(CT)_4{}^{3'}$-Phe-$^{3'}(TC)_4{}^{5'}$ *m/z* = 5160.16 [MH^+]; ODN type **B**: $^{5'}(TC)_4{}^{3'}$-Phe-$^{3'}(CT)_4{}^{5'}$ *m/z* = 5072.90 [MH^+] and $^{5'}(CT)_4{}^{3'}$-L$^{3'}(TC)_4{}^{5'}$ *m/z* = 5073.07 [MH^+]; ODN type **C**: $^{5'}(TC)_4{}^{3'}$-L$^{3'}(CT)_4{}^{5'}$ *m/z* = 4744.74 [MH^+] and $^{5'}(CT)_4{}^{3'}$-Phe-$^{3'}(TC)_4{}^{5'}$ *m/z* = 4744.81 [MH^+].

Thermal Denaturation Experiments: The thermal denaturation experiments were carried out using 1 mL of a solution containing 140 mM KCl, 5 mM NaH_2PO_4, 5 mM $MgCl_2$ at pH = 6.2 and the oligomer of interest at a concentration of approximately 1 μM for each strand, determined spectrophotometrically at λ = 260 nm and at 85 °C using the following molar extinction coefficients for each base: 15400 (A), 11700 (G), 7300 (C), 8800 (T) $cm^{-1}\,M^{-1}$ and 4200 $cm^{-1}\,M^{-1}$ for the phenanthroline moiety. Each solution was heated at 80 °C for 15 min, then allowed to slowly cool and kept at 5 °C for 45 min. After thermal equilibration, UV absorption at λ = 260 nm was monitored as a function of the temperature (increasing at a rate of 0.5 °C/min within the range 5−80 °C). The melting temperatures were determined as the maximum of the first derivative of absorbance vs. temperature plots.

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