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# DNA Conjugated Phenoxyaniline Intercalators: Synthesis of Diethanolaminoacetamide-type Linkers

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Two non-nucleosidic monomers conjugated to a phenoxyaniline intercalator have been synthesized and inserted into ODNs. The conjugated monomers were prepared from 2-chloro-*N*-(4-phenoxyphenyl)acetamide (**1**) either by reaction with diethanolamine and 4,4′-dimethoxytritylation, or by reaction with ethanolamine by subsequent peptide coupling type reaction with 4,4′-dimethoxytrityl protected glycolic acid. The modified ODNs moderately stabilize DNA three-way junctions when the intercalator was introduced at the branch point. For targeting RNA the results were more ambiguous.

The antisense method for controlling gene expression has great potential use in antiviral chemotherapy. Chemically synthesized oligodeoxynucleotides (ODNs) will, when bound to their target (mRNA) in a sequence-specific manner, induce RNase H digestion. RNase H is known to digest the RNA strand of DNA–RNA duplexes, and in this way, the synthesis of harmful proteins can be inhibited.[1] RNAs normally adopt many tertiary structures,[2] where a typical feature is a hairpin consisting of a double-stranded stem region bound to single-stranded regions at the foot of the stem. It is therefore attractive to investigate the use of structured DNAs and RNAs as targets for antisense oligonucleotides. By hybridizing an antisense ODN to single-stranded regions at the foot of a DNA or RNA hairpin a 'three way junction' (TWJ) is adopted which in several works[3–9] have been shown to be stabilized by bulged nucleotides at the branch point of the TWJ. In this way the TWJ is reformatted from a Y-shaped structure to a more folded structure where the stem will be in a coaxial stacking with one of the other arms of the TWJ.[4,5]

To increase the penetration of the antisense ODN into the cells and to enhance the stability of the duplex between the antisense and the target, there have been several approaches to conjugate intercalating derivatives.[10–12] Often the linkers are between the nucleobase and the intercalating agent, or the intercalator is directly linked to the sugar part. There are only few examples in the literature of intercalating systems which are linked to neither a sugar part nor a nucleobase.[13,14] The problem of the minor cellular uptake of antisense ODN should be overcome by introducing neutral 'backbones', e.g. amide linkers instead of the normal phosphate backbone in DNA.[15]

Diphenyl ether has recently been introduced[16] as an effective intercalator in TWJs. The diphenyl ether was here covalently linked to $N^4$ of 5-methylcytidine. In this investigation two new intercalating systems are introduced which consist of a diphenyl ether conjugated to two different acyclic linkers. The synthesized intercalating systems were incorporated into oligodeoxynucleotides and hybridized to both DNA and a RNA hairpins to study whether a TWJ is stabilized by the presence of more flexible linkers between the backbone and the intercalating conjugates than those used hitherto.

## Results and discussion

*Synthesis of intercalating monomers.* The synthesis of the two monomeric building blocks started from 2-chloro-*N*-(4-phenoxyphenyl)acetamide (**1**).[17] The intercalating phosphoramidate **4** is produced by a nucleophilic substitution on **1** with diethanolamine at reflux in a mixture of triethylamine and ethanol to give the *N*-alkylated derivative **2** in 47% yield after purification by column chromatography. Treatment of **2** with 4,4′-dimethoxytrityl chloride (DMTCl) in dry pyridine gave the corresponding mono *O*-DMT protected derivative **3** in 21% yield. The phosphitylation of **3** was accomplished with 2-cyanoethyl diisopropylchlorophosphoramidite in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in anhydrous methylene chloride under argon to give **4** in 51% yield.

In the intercalating monomer **9**, an amide group was

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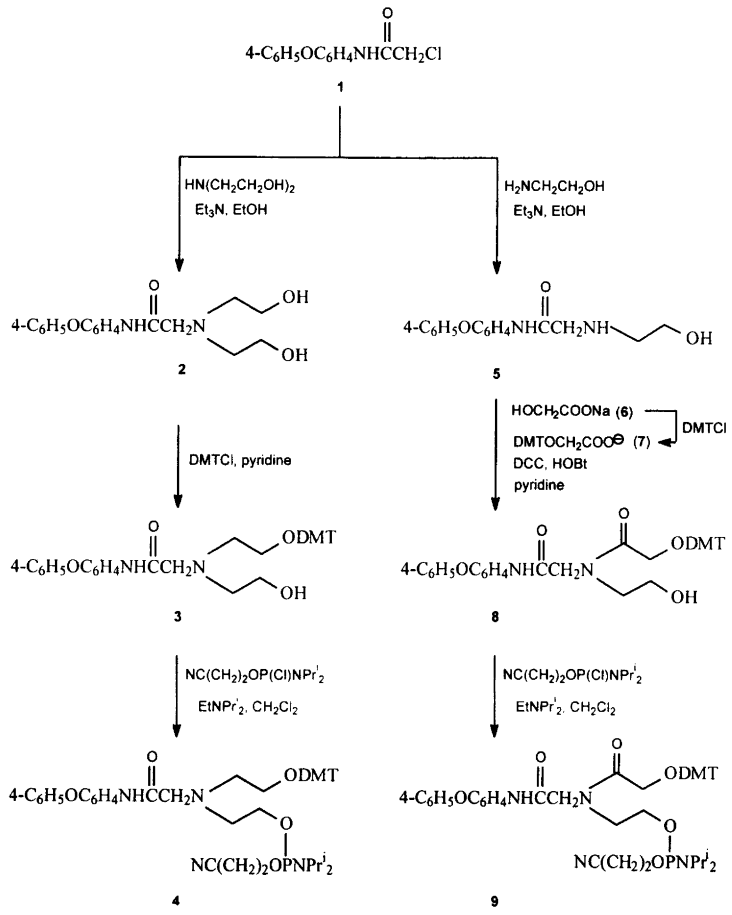


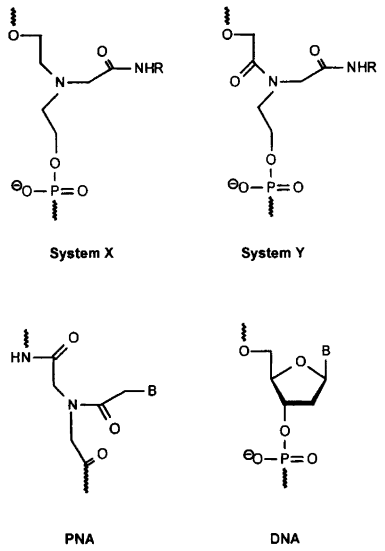

*Scheme 1.* Pathway for the synthesis of phosphoramidite derivatives **4** and **9**: DMTCl, 4,4′-dimethoxytrityl chloride; DCC, *N,N′*-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole.

introduced to make the linker more rigid. The first step is an *N*-alkylation of **1** with ethanolamine. The procedure followed the synthesis carried out for **2**, except that **5** was obtained by precipitation from dry ether followed by extraction with distilled chloroform in 81% yield. The purity of **5** was verified by microanalysis. In the next step a DMT-protected *N*-glycolyl group was introduced in a carbodiimide-mediated one-pot reaction. Thus selective *O*-DMT protection was achieved which otherwise might have been a problem later on because of bis-protection and low selectivity between the two primary hydroxy groups. Hovinen *et al.*[18,19] have shown that the sodium salt of glycolic acid (**6**) can be obtained as the DMT-protected derivative **7** after purification on a cation column and **7** was used for making amide linkers for solid-support synthesis of oligodeoxynucleotides. We decided to make the synthesis of **8** in a one-pot reaction in a similar manner. The sodium salt of glycolic acid (**6**) was reacted with DMTCl in dry pyridine for 7 h at room temperature, which according to analytical TLC gave the DMT-protected derivative **7** in quantitative yield. To the reaction mixture were added the amine **5**, 1-hydroxybenzotriazole (HOBt) and *N,N′*-dicyclohexylcarbodiimide (DCC) and the reaction was stirred overnight to give **8** in 51% yield after purification by column chromatography. The use of *N,N′*-diisopropylcarbodiimide and *N*-hydroxysuccinimide as a coupling reagent system produces **8** contaminated with *N,N′*-diisopropylurea. By using DCC this problem was avoided because of the insolubility of *N,N′*-dicyclohexylurea in organic solvents.[20,21] The phosphitylation of **8** to give **9** was accomplished as described for **4** in 46% yield.

*Synthesis of oligodeoxynucleotides.* Using the phosphoramidite methodology[22] the two phosphoramidites **4** and **9** were used for the ODN synthesis on a Pharmacia Gene Assembler Special DNA-synthesizer. The coupling efficiencies for the modified phosphoramidite **4** (system X) were 68–85% (2 × 12 min couplings) and it was therefore necessary to purify the ODNs by HPLC (reversed-phase). The coupling efficiencies for the phosphoamidite derivative **9** (system Y) and commercial ones were 84–94% (2 × 12 min couplings) and 99% (2 min couplings), respectively. As representative examples, the composition of ODNs $B_X$, $B_Y$, $D_Y$, $E_Y$, $G_Y$ and $F_Y$ was confirmed by matrix-assisted laser desorption mass spectrometry (MALDI); ($B_X$: calc. 5427.5 Da, found 5426.9 Da; $B_Y$: calc. 5441.7 Da, found 5440.7 Da; $D_Y$: calc. 5745.9 Da, found 5744.8 Da; $E_Y$: calc. 5745.9 Da, found 5745.1 Da; $F_Y$: calc. 5848.0 Da, found 5848.1 Da;

$G_Y$: calc. 5848.0 Da, found 5846.4 Da). A short 10mer ODN was also synthesized for the introduction of X in the last step at the 5′ end of the ODN in order to hybridize one of the flanks in the TWJ. Unfortunately, MALDI-MS revealed that the diphenyl ether moiety was lost during the deprotection step with ammonia, most likely due to anchimeric assistance of the free *N*-hydroxyethyl group.

The structures of system X and Y can be seen as analogues of normal DNA and PNA[23] (see Scheme 2). Compared with DNA and PNA, the internucleosidic linker is two atoms longer.




*Scheme 2.* The structure resemblance between system X, system Y, PNA and DNA: R, 4-phenoxyaniline; B, nucleobase.

*DNA and RNA three-way junctions.* To investigate the effect of a conjugated intercalator at the TWJ branch point we selected a 36mer DNA-hairpin as a target which has been shown to form a TWJ when hybridized with a 17mer at the flanks of the hairpin.[24] Earlier studies have revealed that introduction of 2′-deoxy-5-methyl-$N^4$-(4-phenoxyphenyl)cytidine at the branch point results in a stabilization of 9 °C.[16]

Table 1 gives the melting points for the DNA TWJ for both systems X and Y. The best stabilization for system X is observed when introduced just in the centre of the junction region by hybridization with the ODN $B_X$. The thermal stabilization is moderate and is about 3 °C when compared with a wild-type strand and 0.8 °C when compared with a strand with an inserted C. The flexibility in X can reduce the ability of the ODNs to form duplexes, because the concomitant loss of entropy is much larger for flexible structures. The system Y is more rigid than X because of the amide functionality introduced into the linking chain between two nucleotides. As expected a slight increase in the values of $T_m$ is observed. Especially interesting is the increase in the thermal melting temperature of 5.2 °C (Table 1) when two modified monomers ($G_Y$) were introduced at the branch point.

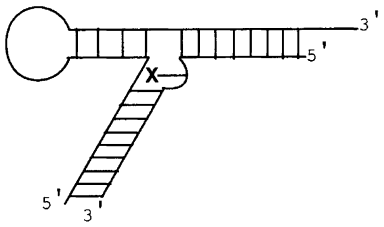
*Table 1.* Targeting DNA hairpin.

```
                T  T
             T     T
             G     C
             C     G        DNA
             G     C
             C     G
5′-TGGAAAGAGA      AGAAAAAATACAGT-3′
   3′-CCTTTCTC-TT-CTTTTTT-5′
```

| ODN | T, X and Y in junction | $T_m$/ °C | $T_m$[C]/ °C[a] | $\Delta T$/ °C[b] | $T_m - T_m$[C]/ °C[c] |
|---|---|---|---|---|---|
| — | -TT- | 27.6 | — | — | — |
| $A_X$ | -XTT- | 28.0 | 28.0 | +0.4 | 0.0 |
| $B_X$ | -TXT- | 30.8 | 30.0 | +3.2 | +0.8 |
| $C_X$ | -TTX- | 27.2 | 29.6 | −0.4 | −2.4 |
| $A_Y$ | -YTT- | 29.6 | 28.0 | +2.0 | +1.6 |
| $B_Y$ | -TYT- | 30.4 | 30.0 | +2.8 | +0.4 |
| $C_Y$ | -TTY- | 28.8 | 29.6 | +1.2 | −0.8 |
| $D_Y$ | -TYTT- | 31.2 | 30.4 | +3.6 | +0.8 |
| $E_Y$ | -TTYT- | 30.8 | 30.4 | +3.2 | +0.4 |
| $F_Y$ | -YYTT- | 30.0 | 26.4 | +2.4 | +3.6 |
| $G_Y$ | -TYYT- | 32.8 | 29.6 | +5.2 | +3.2 |
| $H_Y$ | -TTYY- | 29.6 | 28.4 | +2.0 | +1.2 |

[a] $T_m$[C], melting temperature upon insertion of C instead of X or Y. [b] $\Delta T$, the stabilizing effect in comparison with wild type (wt). [c] $T_m - T_m$[C], the stabilizing effect when comparing inserted C ODNs with inserted X or Y ODNs.

The reason why an intercalator at the junction is expected to stabilize the TWJ is based on the assumption of coaxial stacking of two arms and a lid effect when the intercalator is placed on the top of the third flexing arm. This lid effect has previously been described for self-complementary duplexes.[25]




*Scheme 3.* The coaxial stacked structure of the DNA hairpin after introduction of an intercalator (X) at the branch point of the TWJ.

One could also imagine a large bulging loop formed instead of the hairpin. In order to confirm the TWJ for the hairpin targeting, a new DNA stem-structure was synthesized. The foot-region in this target has an identical sequence with the flanking sites in the hairpin. The sequence for the stem was chosen so that this duplex would itself give a high melting point. A biphasic melting curve is, in this way, obtained where the first melting point ($T_m$), referring to the thermal stabilization of the TWJ, and the latter melting point (at ≈64 °C) represents the stabilization of the stem (see Fig. 1).

Results of the thermal stabilization of the TWJ brought about by hybridization of ODNs with inserted Y with

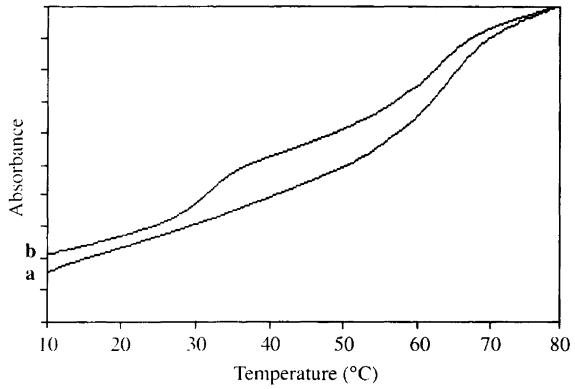


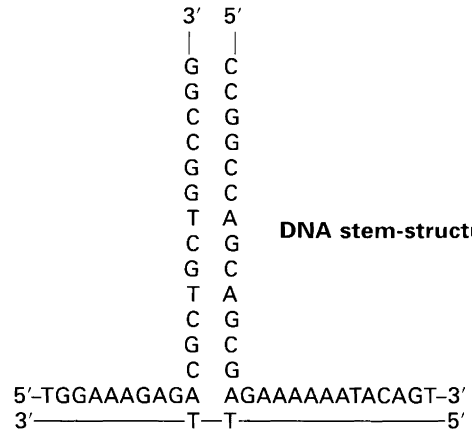

*Fig. 1.* Melting curves measured at 260 nm at pH 7.0 at 3 μM in each strand for a, the DNA stem-structure alone; b, the TWJ formed when ODN $G_Y$ is hybridized with the DNA stem-structure.

the DNA stem-structure are given in Table 2. The melting temperatures are of the same order of magnitude as the values given for the TWJ of the DNA hairpin. Again the best stabilization ($T_m$ 32 °C) is achieved by hybridizing the ODN $G_Y$. Also when compared with insertion of C instead of Y the variations of the thermal meltings are nearly the same for the two DNA targets, indicating the same type of TWJ in both cases.

System Y was chosen for measurement against an RNA hairpin[26] with the flanking sites identical with the DNA hairpin. As seen from Table 3 no stabilization was observed of the RNA–DNA TWJ. This is surprising when compared with ODNs with C inserted at the same position as Y performed a greater stabilization of the TWJ. Breaking of the lower CG base pair in the stem could allow base-pairing with the guanines from the stem with the extra cytidine and reveals a pronounced stabilization. In particular, insertion of two extra Cs in the DNA–RNA TWJ in the case of $G_C$ with an increase of 9.2 °C in $T_m$ demonstrates the difference between the DNA TWJ and the DNA–RNA TWJ, and the need for further research in this direction.

Compounds **2**, **3**, **5** and **8** did not show any significant activity against HIV-1 and HSV-1 when tested in MT-4 cells and Vero cells, respectively.[27] However, considering the aim of the present work, we found it interesting that cell toxicity in these cell lines was found for neither the monomer **2** nor the protected monomer **8**.

*Table 2.* Hybridization results with the DNA stem-structure.




| ODN | Position for Y | $T_m$[Y]/ °C | $T_m$[C]/ °C[a] | $\Delta T$[Y]/ °C[b] | $T_m$[Y] − $T_m$[C]/ °C[c] |
|---|---|---|---|---|---|
| — | -TT- | 28.8 | — | — | — |
| $A_Y$ | -YTT- | 29.6 | 27.2 | +0.8 | +2.4 |
| $B_Y$ | -TYT- | 30.4 | 28.8 | +1.6 | +1.6 |
| $C_Y$ | -TTY- | 28.8 | 30.0 | 0.0 | −1.2 |
| $D_Y$ | -TYTT- | 31.6 | 30.8 | +2.8 | +0.8 |
| $E_Y$ | -TTYT- | 30.0 | 30.4 | +1.2 | −0.4 |
| $F_Y$ | -YYTT- | 30.4 | 26.4 | +1.6 | +4.0 |
| $G_Y$ | -TYYT- | 32.0 | 27.2 | +3.2 | +4.8 |
| $H_Y$ | -TTYY- | 28.8 | 26.4 | 0.0 | +2.4 |

[a] $T_m$[C], melting temperature upon insertion of C instead of X or Y. [b] $\Delta T$[Y], the stabilizing effect in comparison with wild type (wt). [c] $T_m$[Y] − $T_m$[C], the stabilizing effect when comparing inserted C ODNs with inserted Y ODNs.

## Conclusions

The intercalating systems presented here contain neither the normal sugar part nor nucleobase. Instead the intercalator is conjugated through an acetamide moiety to an acyclic 3-azapentane linker between two nucleotides. Only moderate stabilization of DNA TWJs was observed. The stabilization was improved as the linker became more rigid by introducing an amide functionality. For targeting RNA no stabilization was observed. Instead a rather dramatic stabilization of the DNA–RNA TWJ was observed when two extra cytidines were inserted to the junction region.

*Table 3.* Targeting RNA hairpin.




| ODN | Position for Y | $T_m$[Y]/ °C[a] | $T_m$[C]/ °C[b] | $\Delta T$[Y]/ °C | $\Delta T_m$[C]/ °C |
|---|---|---|---|---|---|
| — | -TT- | 36.4 | — | — | — |
| $A_Y$ | -YTT- | 36.8 | 41.2 | +0.4 | +4.8 |
| $B_Y$ | -TYT- | 34.4 | 41.6 | −2.0 | +5.2 |
| $C_Y$ | -TTY- | 33.2 | 38.0 | −3.2 | +1.6 |
| $D_Y$ | -TYTT- | 36.8 | 41.2 | +0.4 | +4.8 |
| $E_Y$ | -TTYT- | 35.2 | 41.6 | −1.2 | +5.2 |
| $F_Y$ | -YYTT- | 36.0 | 40.8 | −0.4 | +4.4 |
| $G_Y$ | -TYYT- | 36.0 | 45.6 | −0.4 | +9.2 |
| $H_Y$ | -TTYY- | 33.6 | 37.2 | −2.8 | +0.8 |

[a] $\Delta T$[Y], the stabilizing effect in comparison with wild type (wt). [b] $\Delta T_m$[C], the stabilizing effect for ODN with inserted C in comparison with wt.

## Experimental

NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz for $^1H$ NMR, 75 MHz for $^{13}C$ NMR and 101.3 MHz for $^{31}P$ NMR on a Bruker AC-250FT spectrometer; δ values are in ppm relative to tetramethylsilane as an internal standard ($^1H$ NMR and $^{13}C$ NMR), and relative to 85% $H_3PO_4$ as an external standard in $^{31}P$ NMR spectra. EI mass spectra were recorded on a Varian MAT 311A spectrometer. FAB mass spectra were recorded on a Kratos 50 TC spectrometer. Analytical silica gel TLC was performed on Merck precoated 60 $F_{254}$ plates. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Microanalyses were performed by the Atlantic Microlab, Inc., USA.

*2-[Bis(2-hydroxyethyl)amino]-N-(4-phenoxyphenyl)-acetamide* (**2**). Diethanolamine [2.64 g, 25 mmol in 16 ml $Et_3N$–EtOH (5:3)] was added dropwise at room temperature to a suspension of **1**[17] (6.66 g, 25 mmol) in 32 ml $Et_3N$–EtOH (15:1). The reaction mixture was refluxed for 2 h and diluted with 60 ml dry EtOH. The precipitated triethylammonium hydrochloride was filtered off and the mixture was evaporated to dryness. EtOH (100 ml) and $H_2O$ (100 ml) were added to the residual solid and extracted with $CH_2Cl_2$ (4 × 100 ml). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. The residual oil was purified on a silica gel column (MeOH–$CH_2Cl_2$, 0.5–50% v/v). Yield 3.90 g (47%); $R_f$ 0.64 (MeOH–EtOAc, 50% v/v). $^1H$ NMR ($CDCl_3$): δ 9.75 (br s, 1 H, NH), 6.90–7.57 (m, 9 H, $H_{arom.}$), 4.30 (br s, 2 H, OH), 3.63–3.66 (m, 4 H, $NCH_2CH_2OH$), 3.27 (s, 2 H, $COCH_2N$), 2.69–2.70 (m, 4 H, $NCH_2CH_2OH$). $^{13}C$ NMR ($CDCl_3$): δ 170.21 (C=O), 157.59, 153.39, 133.44, 129.72, 123.03, 121.56, 119.51, 118.29 ($C_{arom.}$), 59.59, 59.33 ($NCH_2CH_2OH + NCH_2CH_2OH$), 56.97 ($COCH_2N$). EI MS: *m/z* 330 ($M^+$). Anal. ($C_{18}H_{22}N_2O_4 \times 0.75\ H_2O$): C, H, N.

*2-{(2-Hydroxyethyl)[2-(4,4'-dimethoxytrityloxy)ethyl]-amino}-N-(4-phenoxyphenyl)acetamide* (**3**). To a stirred solution of **2** (1.50 g, 4.54 mmol) in dry pyridine (5 ml) was added 4,4'-dimethoxytrityl chloride (1.54 g, 4.54 mmol). The mixture was stirred for 90 min and then quenched with 0.5 ml MeOH and evaporated to dryness. The residual oil was diluted with $CH_2Cl_2$ (40 ml) and washed with satd. $NaHCO_3$ (3 × 40 ml). The organic layer was dried ($Na_2SO_4$) and evaporated *in vacuo*. The raw product was purified on a silica gel column (MeOH–$CH_2Cl_2$, 0–1% v/v with 0.5% v/v pyridine) and finally coevaporated with dry toluene (3 × 5 ml). Yield 0.612 g (21%); $R_f$ 0.30 (MeOH–EtOAc, 5% v/v). $^1H$ NMR ($CDCl_3$): δ 9.62 (s, 1 H, NH), 6.75–7.48 (m, 22 H, $H_{arom.}$), 3.69–3.73 (m, 8 H, $NCH_2CH_2OH + 2 \times OCH_3$), 3.28 (s, 2 H, $COCH_2N$), 3.21 (t, 2 H, $J$=5.0 Hz, $NCH_2CH_2ODMT$), 2.84 (t, 2 H, $J$=5.0 Hz, $NCH_2CH_2$), 2.74 (t, 2 H, $J$=5.1 Hz, $NCH_2CH_2$). $^{13}C$ NMR ($CDCl_3$): δ 169.80 (C=O), 157.59, 153.39, 158.60, 157.87, 152.98, 135.89, 133.72, 129.98, 129.69, 128.08, 127.95, 126.92, 125.32, 122.86, 121.20, 119.69, 118.16, 113.10 ($C_{arom.}$), 86.74 ($CAr_3$), 61.66 ($COCH_2N$), 59.78, 59.67 ($NCH_2CH_2O$), 55.06 ($2 \times OCH_3$), 57.40, 54.80 ($NCH_2CH_2O$). FAB MS (3-nitrobenzyl alcohol + $CH_2Cl_2$ + TFA): *m/z* 632 ($M^+$).

*Phosphoramidite derivative* (**4**). Compound **3** (250 mg, 0.4 mmol) was coevaporated with 3 × 5 ml anhydrous $CH_3CN$ and dried overnight *in vacuo*. It was then dissolved in anhydrous $CH_2Cl_2$ (1.2 ml) under Ar, after which *N,N*-diisopropylethylamine (0.4 ml, 2.3 mmol) and 2-cyanoethyl diisopropylchlorophosphoramidite (0.16 ml, 0.71 mmol) were added with stirring at room temperature. After 80 min the reaction was quenched with MeOH (1 ml), diluted with EtOAc (15 ml) and washed with satd. aq solution of $NaHCO_3$ (3 × 15 ml) and $H_2O$ (3 × 15 ml). The organic phase was dried ($Na_2SO_4$), filtered and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc–$CH_2Cl_2$–$Et_3N$, 45:45:10 v/v). The resulting gum was dissolved in 3 ml dry toluene and the solution was added dropwise to cold petroleum ether (b.p. 60–80 °C, 50 ml, cooled to 0 °C). A gum was formed, collected, redissolved in anhydrous $CH_3CN$ and evaporated *in vacuo* to give a colourless hard material. Yield 0.169 g (51%); $R_f$ 0.72 (EtOAc–$CH_2Cl_2$–$CH_3CN$, 45:45:10 v/v). $^{31}P$ NMR ($CDCl_3$): δ 149.20.

*2-(2-Hydroxyethylamino)-N-(4-phenoxyphenyl)acetamide* (**5**). At room temperature ethanolamine [7.6 g, 124 mmol in 62 ml $Et_3N$–EtOH (25:6)] was added dropwise to a suspension of **1** (6.51 g, 25 mmol) in 32 ml $Et_3N$–EtOH (15:1). The mixture was refluxed for 30 min and evaporated *in vacuo*. The residue was stirred vigorously in $Et_2O$ (150 ml) after which compound **5** could be filtered off and washed with $NaHCO_3$ (100 ml) by stirring for 5 min. After filtration, compound **5** was dissolved in distilled $CHCl_3$ (200 ml) and dried ($Na_2SO_4$). Evaporation to dryness afforded **5** as a white powder. Yield 5.76 g (81%); $R_f$ 0.46 (50% MeOH–EtOAc). M.p. 108–109 °C. $^1H$ NMR ($CDCl_3$): δ 9.95 (br s, 1 H, $NH_{arom.}$), 6.96–7.70 (m, 9 H, $H_{arom.}$), 4.69 (br s, 1 H, NH), 3.50 (t, 2 H, $J$=5.2 Hz, $NCH_2CH_2OH$), 3.31 (s, 2 H, $COCH_2N$), 2.64 (t, 2 H, $J$=5.5 Hz $NCH_2CH_2OH$). $^{13}C$ NMR ($CDCl_3$): δ 170.43 (C=O), 157.55, 151.81, 134.79, 130.01, 122.98, 120.91, 119.53, 117.86 ($C_{arom.}$), 60.38 ($COCH_2N$), 52.68 ($NCH_2CH_2OH$), 51.61 ($NCH_2CH_2OH$). EI MS: *m/z* 286 ($M^+$). Anal. $C_{16}H_{18}N_2O_3$: C, H, N.

*N-(2-Hydroxyethyl)-2-(4,4'-dimethoxytrityloxy)-N-(4-phenoxyanilinocarbonylmethyl)acetamide* (**8**). 4,4'-Dimethoxytrityl chloride (2.37 g, 7 mmol) was added at room temperature to a suspension of the sodium salt of glycolic acid (**6**) (0.686 g, 7 mmol) in dry pyridine (50 ml). The reaction was stirred for 7 h after which time analytical TLC showed disappearance of the starting

material **6**. To the reaction mixture were added the amine **5** (2.10 g, 7.35 mmol) and 1-hydroxybenzotriazole (0.950 g, 7 mmol) and this was followed by addition (after 10 min of stirring) of *N,N'*-dicyclohexylcarbodiimide (1.46 g, 7 mmol). The reaction mixture was stirred overnight at room temperature. The precipitated dicyclohexylurea was filtered off and the reaction mixture was evaporated to dryness. The residue was dissolved in $CH_2Cl_2$ (100 ml) and washed with satd. aq $NaHCO_3$ (2 × 50 ml) and $H_2O$ (50 ml). The organic phase was dried ($Na_2SO_4$), filtered and evaporated *in vacuo*. Compound **8** was obtained as a yellowish powder after purification on a silica gel column [EtOAc–petroleum ether (b.p. 60–80 °C), 50–90%]. Yield 2.30 g (51%); $R_f$ 0.65 (10% MeOH–EtOAc). M.p. 73–76 °C. $^1$H NMR ($CDCl_3$): δ 9.47 (br s, 1 H, $NH_{arom.}$), 6.75–7.51 (m, 22 H, $H_{arom.}$), 4.67 (br t, 1 H, OH), 4.09 (s, 2 H, $COCH_2N$), 3.99 (s, 2 H, $NCOCH_2ODMT$), 3.77 (s, 6 H, 2 × $OCH_3$), 3.61 (m, 2 H, $NCH_2CH_2OH$), 3.37 (m, 2 H, $NCH_2CH_2OH$). $^{13}$C NMR ($CDCl_3$): δ 171.32, 168.71 (C=O), 158.79, 157.62, 153.46, 144.22, 135.29, 133.33, 130.01, 129.73, 128.05, 127.12, 122.99, 121.70, 119.45, 119.37, 118.40, 113.33 ($C_{arom.}$), 87.28 ($CAr_3$), 63.23 ($COCH_2ODMT$), 59.63 ($NCH_2CH_2OH$), 55.12 ($OCH_3$), 52.19, 52.05 ($COCH_2NCO$ and $NCH_2CH_2OH$). FAB MS (3-nitrobenzyl alcohol): *m/z* 647 ($M+H^+$). Anal. ($C_{39}H_{38}N_2O_7 \times 0.25\ H_2O$): C, H, N.

*Phosphoramidite derivative* (**9**). Compound **8** (0.400 g, 0.62 mmol) was coevaporated with anhydrous $CH_3CN$ (3 × 5 ml) and dried overnight *in vacuo*. It was then dissolved in anhydrous $CH_2Cl_2$ (1.5 ml) under Ar, and *N,N*-diisopropylethylamine (0.4 ml, 2.3 mmol) and 2-cyanoethyl diisopropylchlorophosphoramidite (0.25 ml, 1.12 mmol) were added with stirring at room temperature. After 30 min at room temperature analytical TLC showed the disappearance of the starting material **8**, and the reaction was quenched with MeOH (1 ml). The reaction mixture was diluted with EtOAc (15 ml) and washed with satd. aq $NaHCO_3$ (3 × 15 ml) followed by $H_2O$ (3 × 15 ml). The organic phase was dried ($Na_2SO_4$), filtered and evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc–$CH_2Cl_2$–$Et_3N$, 45:45:10 v/v) and the resulting thick oil was dissolved in dry toluene (5 ml) and the product precipitated from petroleum ether (b.p. 60–80 °C, 50 ml, cooled to −10 °C) as white crystals. Yield 0.239 g (46%); $R_f$ 0.73 (EtOAc–$CH_2Cl_2$–$CH_3CN$, 45:45:10 v/v). $^{31}$P NMR ($CDCl_3$): δ 149.46. FAB MS (3-nitrobenzyl alcohol): *m/z* 846 ($M^+$).

*Oligodeoxynucleotide synthesis and hybridization experiments.* All oligodeoxynucleotides were synthesised on a Pharmacia Gene Assembler Special® DNA-synthesizer. Solid supports on a 0.2 μmol scale were obtained from Cruachem. The amidite solution volume applied for all couplings was 75 μl. Commercial phosphoroamidites were used in 0.1 M concentration with 2 min coupling time. Synthesized phosphoroamidites **4** and **9** were used in 0.05–0.15 M concentrations and the coupling time was extended to 2 × 2 min. The DMT group of the nucleotide incorporated last was removed as the last step on the synthesizer. The oligodeoxynucleotides were deprotected and cleaved off the solid support by incubation in 25% aq ammonia at room temperature for 4 days. Desalting of all oligodeoxynucleotides was accomplished using disposable NAP-10 columns (Pharmacia). The oligodeoxynucleotides were obtained by evaporation to dryness (Heatovac VR1 centrifuge) and then redissolved in $H_2O$ (system 1) or autoclaved $H_2O$ (system 2). The UV extinction at 260 nm was then measured to determine the concentration. The extinction coefficient at 260 nm was determined for compound **2** to be 15 800 l mol$^{-1}$ and used for the modified nucleotides when calculating the extinction coefficient of the modified ODNs. Melting experiments were carried out on a Perkin–Elmer UV–VIS spectrometer Lambda fitted with a PTP-6 Peltier temperature programming element with 3 μM of each DNA strand in a buffer consisting of 1 mM EDTA, 10 mM $Na_2HPO_4$ and 140 mM NaCl at pH 7.0. Before each experiment, all samples were heated at 90 °C in a water bath for 5 min and then cooled slowly to 0 °C. The increase in the UV absorbance at 260 nm as a function of time was recorded while the temperature was raised gradually (1 °C min$^{-1}$) from 10–70 °C (for DNA hairpin) or 10–80 °C (for RNA hairpin) in a 1 cm cuvette.

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