# Synthesis and Triple-Helix-Stabilization Properties of Branched Oligonucleotides Carrying 8-Aminoadenine Moieties

by **Anna Aviño**, **Marta G. Grimau**, **Miriam Frieden**[1]), and **Ramon Eritja***

Department of Structural Biology, Instituto de Biología Molecular de Barcelona. C.S.I.C., Jordi Girona 18–26, E-08034 Barcelona
(phone: +34(93)4006145; fax: +34(93)2045904; e-mail: recgma@cid.csic.es)

The synthesis of several branched oligonucleotides, *i.e.*, of the parallel hairpins **5**–**8** and the Y-shaped **9** is described, together with their use in the formation of pyrimidine·pyrimidine·purine triple helices. Special attention was paid to the optimization of the assembly of the second strand from asymmetric branching molecules. The presence of 8-aminoadenine moieties in the *Watson–Crick* purine strand and 2′-*O*-methyl-RNA in the *Hoogsteen* pyrimidine strand produced strong stabilization of the triplex.

**Introduction.** – Oligonucleotides bind in a sequence-specific manner to homopurine·homopyrimidine sequences of duplex and single-stranded DNA and RNA to form triplexes [1]. Nucleic acid triplexes have potential applications in diagnostics, gene analysis, and therapy [1][2]. Recently, it has been reported that oligonucleotides carrying 8-aminopurine nucleosides form stable pyrimidine·pyrimidine·purine triplexes [3–6]. These triplex-stabilization properties of 8-aminopurines can be used to capture specific polypyrimidine sequences by using parallel-stranded hairpins with a polypyrimidine sequence linked to the complementary purine strand carrying 8-aminopurines [7][8]. The binding properties of parallel hairpins carrying 8-aminopurines stimulated the search for new types of hairpins with even better binding properties.

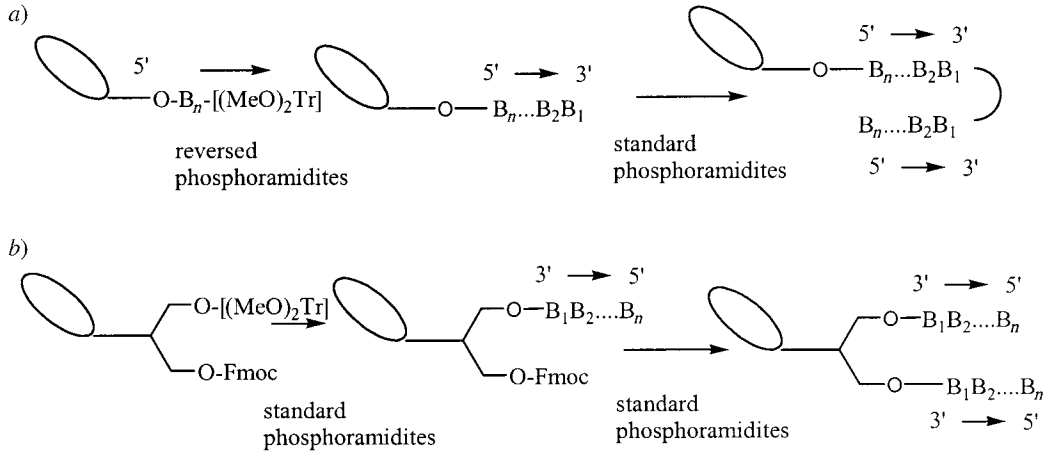
One interesting possibility is to use branched oligonucleotides (see [9] and ref. cit. therein). Most of the work on branched oligonucleotides was first focused on the study of these compounds as splicing intermediates of eukaryotic mRNAs [10–12]. Moreover, branched nucleic acids have been prepared by using nucleoside branching points other than the 2′- and 3′-positions of the regular ribonucleoside such as those provided by 4′-*C*-(hydroxymethyl)thymidine [13][14], *arabino*-uridine [15], or the nucleobase [16][17]. The complexity of the synthesis of the branching molecules and the low yields obtained triggered the search for non-nucleoside branching molecules, such as derivatives of hexane-1,2,6-triol [18], 1,3-diaminopropanol [19], and pentaerythritol [20][21]. These branching molecules can produce dendrimeric oligonucleotides that may be used as polylabelled DNA probes to increase sensitivity in hybridization experiments [17–20]. Moreover, oligonucleotides with three arms were found to be efficient templates for the directed synthesis of trimeric structures [22]. Finally, branched oligonucleotides have been used to produce V-shape oligonucleotides

[1]) Present address: *Cureon A/S*, Fruebjergvej 3, DK-2100 Copenhagen, Denmark.

with high affinity to single-stranded DNA or RNA by triplex formation [9][14][15][17][21][23][24] and to form alternate-strand triplexes [25][26].

Here we report the synthesis of parallel-stranded hairpins by using a non-nucleoside branching molecule. We also describe their use in the formation of pyrimidine·pyrimidine·purine triple helices. The previous method of synthesis [27][28] requires the use of reverse phosphoramidites to build half of the molecule (*Scheme 1,a*). In the present paper, the hairpins were synthesized from the middle of the molecule by the extension of one branch of an asymmetric branching molecule, followed by assembly of the next branch (*Scheme 1,b*). The combination of an acid-labile group (dimethoxytrityl, $(MeO)_2Tr$) and a base-labile group ((9*H*-fluoren-9-ylmethoxy)carbonyl, Fmoc) allowed assembly of the desired molecule without the need of reverse phosphoramidites. Finally, the three strands of a triplex were connected in a three-branch Y-shaped oligonucleotide by means of the asymmetric branching molecule. The binding properties of these molecules are discussed.

Scheme 1. *Two Possible Ways to Assemble 3′,3′-Linked DNA Hairpins with the Two Arms in a Parallel Orientation*[a])




[a]) All sequences $B_1B_2...B_n$ refer to 2'-deoxynucleotides.

*a*) *Standard method:* The synthesis starts at one end of the hairpin. The first half is assembled by using reversed phosphoramidites followed by the assembly of the second half by using standard phosphoramidites. *b*) *Method using branched molecules:* The hairpin is assembled from the loop by using a branched molecule that carries two OH groups protected by orthogonal groups: the acid-labile dimethoxytrityl ($(MeO)_2Tr$) and the base-labile (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc) group. This last method does not require the use of reversed phosphoramidites.

**Results.** – 1. *Structure of the Oligonucleotide Derivatives.* The oligonucleotide sequences of the prepared hairpins are shown in *Table 1*. The sequences of the hairpins are based on a triplex model sequence described previously [29][30]. Here, the *Hoogsteen* polypyrimidine strand was linked to the *Watson–Crick* polypurine strand.

The first group of sequences, **1**–**4**, are parallel-stranded, and the polypurine strand is connected with the *Hoogsteen* polypyrimidine strand either by their 3′-ends (see **1**

Table 1. *Sequences of the Synthesized Parallel Hairpins* **1**–**8** *and of the Three-Branch Oligonucleotide* **9**

| | Sequence[a]) |
|---|---|
| **1** | 5′-GAAGGAGGAGA-heg-TCTCCTCCTTC-5′ |
| **2** | 5′-GAAGGNGGNGA-heg-TCTCCTCCTTC-5′ |
| **3** | 3′-AGAGGAGGAAG-heg-CTTCCTCCTCT-3′ |
| **4** | 3′-AGNGGNGGAAG-heg-CTTCCTCCTCT-3′ |
| **5** | 5′-GAAGGAGGAGA-TT-bpa-TT-TCTCCTCCTTC-5′ |
| **6** | 5′-GAAGGAGGAGA-TT-bppd-TT-TCTCCTCCTTC-5′ |
| **7** | 5′-GAAGGNGGNGA-TT-bppd-*UU-UCUCCUCCUUC*-5′ |
| **8** | 5′-GAAGGNGGNGA-TT-bppd-TT-TMTMMTMMTTM-5′ |
| **9** | 5′-GAAGGAGGAGA-heg-bpp-(heg-CTTCCTCCTCT-5′)-heg-TCTCCTCCTTC-5′ |

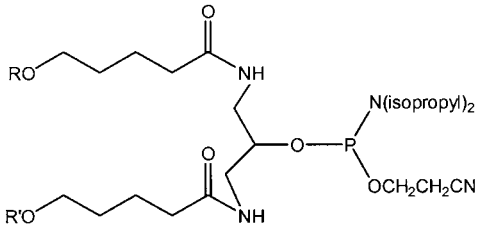
[a]) All sequences refer to 2′-deoxynucleotides, except for the branch of **7** printed in italics. heg = hexaethylene glycol; bpa = [−$PO_3$-O$(CH_2)_3$-CONH-$CH_2$]$_2$-CHOH; bppd = [−$PO_3$-O$(CH_2)_4$−CONH−$CH_2$]$_2$-CHO$PO_2$-O$CH_2CH_2CH_2$OH; bpp = [−$PO_3$-O$(CH_2)_4$-CONH−$CH_2$]$_2$-CHO$PO_3$-; N = 8-amino-2′-deoxyadenosine; M = 2′-deoxy-5-methylcytidine; *U*,*C* = 2-*O*′-methyl-RNA.

and **2**) or by their 5′-ends (see **3** and **4**) through a hexaethylene glycol (= 3,6,9,12,15-pentaoxaheptadecane-1,17-diol) ($(EG)_6$). In hairpins **2** and **4**, two adenines bases were replaced with two 8-aminoadenine bases. These hairpins were prepared by the standard method by using a combination of standard and reversed phosphoramidites (see *Scheme 1*,*a* [27][28]). The binding properties of these hairpins have been described previously [7][8], and they are used for comparison purposes.

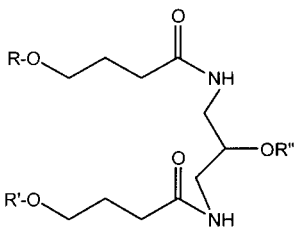
The second group of sequences, **5** and **6**, are 3′,3′-linked parallel hairpins like **1**. The hexaethylene glycol loop of **1** is replaced by a 4-hydroxybutanoic and 5-hydroxypentanoic derivative of 1,3-diaminopropan-2-ol in **5** and **6**, respectively, and four thymidine units (*Table 1*). These hairpins were prepared from the middle of the loop by using an asymmetric branching molecule as outlined in *Scheme 1*,*b* (for the solid support carrying a propan-1,3-diol molecule, see [31]). The difference between **5** and **6** is a 3-hydroxypropyl phosphate in the loop. The syntheses of **5** and **6** were used to find the optimal conditions for the assembly of the sequences by using asymmetric doublers. In this method, the combination of an acid-labile group ($(MeO)_2$Tr) and a base-labile group (Fmoc) allows the assembly of the hairpin from the middle of the loop. After the assembly of the first strand, the Fmoc group is removed, and the second strand is then synthesized.

The third group of sequences, **7** and **8**, are derivatives of **6** carrying two different types of substitutions. First, in both hairpins **7** and **8**, two adenine bases were replaced by two 8-aminoadenine bases. In addition, the whole *Hoogsteen* polypyrimidine strand of **6** was replaced with the 2′-*O*-methyl-RNA-backbone in hairpin **7**, and the six $C_d$ of **6** were replaced with 5-methyl-$C_d$ in hairpin **8**. The introduction of RNA and 2′-*O*-methyl-RNA [32–36] as well as 5-methyl-$C_d$ [29][37][38] in the *Hoogsteen* polypyrimidine strand have been reported to stabilize triplexes. We were interested in analyzing whether these stabilizing properties are additive to the triplex-stabilizing properties of 8-aminoadenine found in parallel hairpins. These hairpins were synthesized under the optimal conditions developed for the synthesis of **6**. These optimal conditions were also used for the synthesis of the three-branch oligonucleotide **9** having the three strands of a triplex (see *Table 1*).

2. *Synthesis of Parallel Hairpins.* The asymmetric branching phosphoramidite **10** (*Fig. 1*) used for the syntheses of **6**–**9** was obtained from commercial sources. For the preparation of the solid support **15** carrying the asymmetric branching molecule (see *Scheme 1,b*), 1,3-diaminopropan-2-ol was treated with $\gamma$-butyrolactone to yield the desired triol **11** [19] (*Fig. 1*). Next we tried the introduction of the $(MeO)_2Tr$ group at one position by limiting the amount of dimethoxytrityl chloride. The desired mono[$(MeO)_2Tr$] product was formed but was isolated from the mixture with the bis[$(MeO)_2Tr$] product in very low yields. Reaction of the mono[$(MeO)_2Tr$] derivative (isolated compound or as a mixture with the bis[$(MeO)_2Tr$] product) with 9*H*-fluoren-9-ylmethyl carbonochloridate gave the desired product, but, again, the recovery after column chromatography (CC; silica gel) was low. The introduction of the Fmoc group prior to that of the $(MeO)_2Tr$ group also yielded little product after CC purification (silica gel), although the Fmoc group was more-stable in the presence of silica gel than the $(MeO)_2Tr$ group. Finally, we selected the more stable monomethoxytrityl (MeOTr) group for the protection of the alcohol function and the desired monofunctionalized derivative **12** was isolated in good yield. The introduction of the Fmoc group also proceeded in moderate but better yields than before ($\rightarrow$ **13**). Compound **13** was further derivatized to give the solid support **15** *via* **14** according to standard protocols.




**10** R=$(MeO)_2Tr$, R'=Fmoc




**11** R=R'=R"=H
**12** R=MeOTr, R'=R"=H
**13** R=MeOTr, R'=Fmoc, R"=H
**14** R=MeOTr, R'=Fmoc, R"=$COCH_2CH_2COOH$
**15** R=MeOTr, R'=Fmoc, R"=$COCH_2CH_2CONH$-LCAA-CPG

Fig. 1. *Asymmetric branching phosphoramidite* **10** *and support* **15** *carrying the asymmetric branching molecule* (obtained *via* **11**–**14**)

Sequence **5** was used as model compound to study the synthesis of oligodeoxynucleotides having two different branches (*Scheme 2*). The first branch was assembled on a 1-μmol scale by using the standard synthesis cycle. The resulting support was acetylated, and the Fmoc group was removed by treatment with 0.5M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) solution in MeCN (10 min). Aliquots of 0.2 μmol of support were subjected to six different synthesis cycles as described in the *Exper. Part.* The changes introduced were the increased reaction times, especially coupling times, double-coupling protocols, introduction of an extra wash with *N*,*N*-dimethylpyridin-4-amine (DMAP)/1*H*-tetrazole (TET) solution, and nonaqueous oxidation conditions.

Scheme 2. *Outline of the Synthesis of Parallel Hairpin* **5**[a])


[a]) All sequences refer to 2'-deoxynucleotides.

In some experiments, we introduced a wash in DMAP/TET between detritylation and coupling. During the removal of the Fmoc group, a strong base was used (DBU), and, under these conditions, the 2-cyanoethyl group that protects the phosphate groups was also removed. The presence of unprotected phosphate moieties during the assembly of the oligonucleotides may explain the low yields obtained during the assembly of the second branch. In a similar situation, the use of DMAP/TET was recommended [39].

The progress of the assembly of the second strand was monitored by the absorption of the $(MeO)_2Tr$ group released after each coupling step. The first and second coupling after branching were lower than the subsequent couplings. The standard cycle (*A*) gave a 60% overall yield. Increasing the coupling time (cycle *B*) and double coupling (coupling *C*) were the best conditions, giving a 90% overall yield. The use of nonaqueous oxidation conditions (cycle *F*) produced no significant differences. The use of DMAP/TET gave lower yields than the standard cycle (30% overall yield). The results obtained by the measurement of the trityl released during the acid treatments

were confirmed by gel electrophoresis (*Fig. 2*). Moreover, the truncated sequences were observed mainly near the branching point. The resulting oligonucleotide **5** was purified by the trityl-on and trityl-off protocols, and the purified compound was characterized by mass spectrometry.

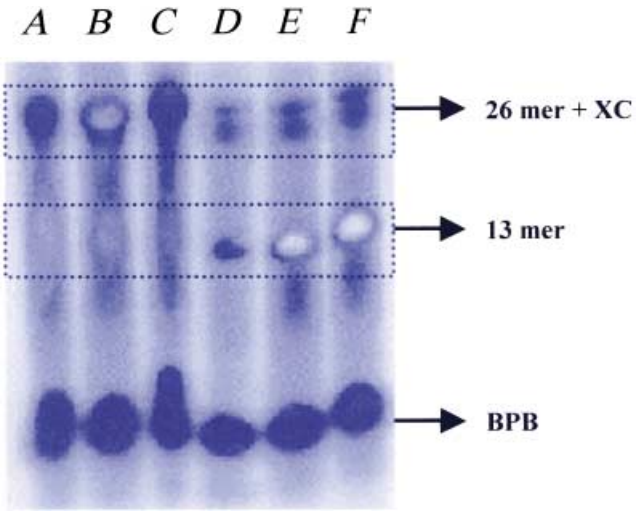



Fig. 2. *Analysis by gel electrophoresis* (20% acrylamide, 8M urea) *of the products obtained after the assembly of the parallel hairpin* **5** *obtained by the standard cycle* (*A*), *an increased coupling time* (*B*), *double coupling* (*C*), *the standard cycle* A *but adding a wash with DMAP/TET* (*D*), *the cycle* B *but adding a wash with DMAP/TET* (*E*), *and the cycle* C *but adding a wash with DMAP/TET* (*F*). BPB = bromophenol blue dye, 26 mer = **5**, XC = xylene cyanol dye.

In addition, we built the first strand with phosphoramidites carrying MeO groups instead of the 2-cyanoethyl group. Previous reports on the synthesis of branched oligonucleotides describe the use of methyl phosphoramidites [10–13]. In our model compound, the use of methyl phosphoramidites was not satisfactory. The coupling efficiency of the second strand was slightly worse than by using 2-cyanoethyl phosphoramidites, and the crude product clearly contained more shorter sequences as by-products near the branching point (data not shown).

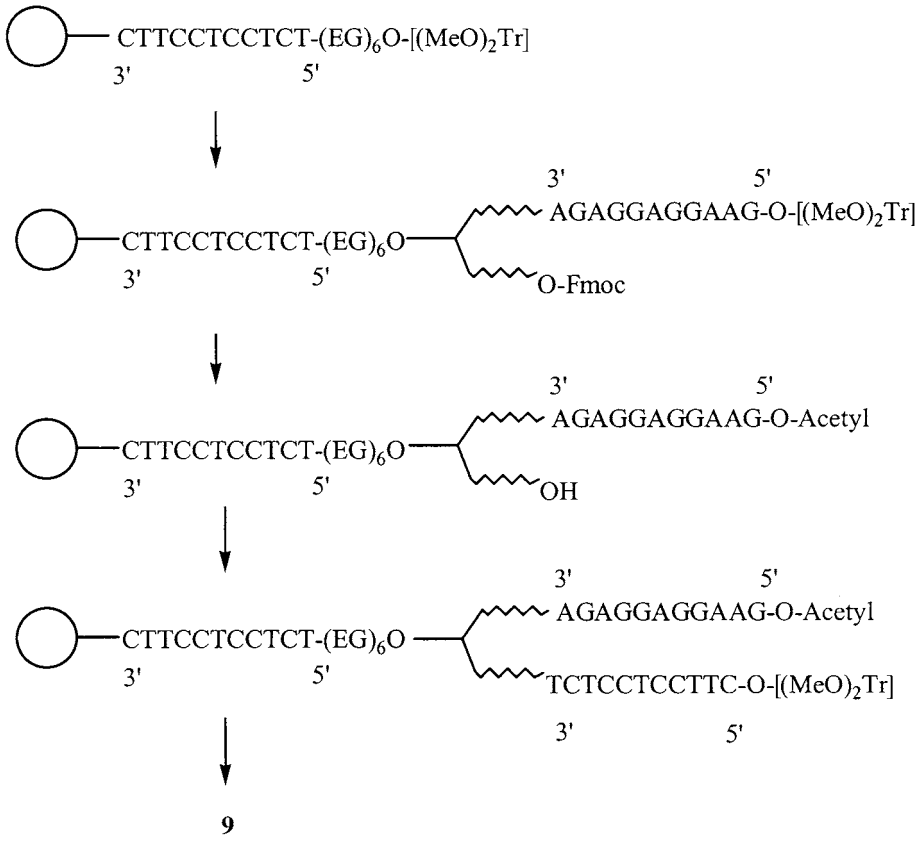
Next, the assembly of sequence **6** was performed. In this sequence, the phosphoramidite of the branching molecule is added to a solid support carrying a propane-1,3-diol molecule [31]. We assayed various changes in the cycle (described in the *Exper. Part*). As observed before, yields of the first and second coupling steps were low, but in this case even lower than before. With the standard cycle, the overall yield was only 20%. The use of *tert*-butyl hydroperoxide instead of $I_2$ (cycle *G*) was slightly better. Increasing the coupling time increased the overall yield to 60%. Increasing the time and the concentration of the phosphoramidite or using a double coupling protocol increased the overall yield to 80–90%. The presence of the phosphate near the branching point reduced the coupling yields of the second branch, but this effect was overcome by increasing time and the concentration of the phosphoramidite.

Next, sequences **7** and **8** carrying 8-aminoadenine bases were prepared. First, the polypurine sequence was assembled on a solid support carrying the asymmetric

branching molecule coupled to propane-1,3-diol. Upon completion of the polypurine sequence, the support was removed from the synthesizer, and acetylation followed by the removal of the Fmoc group was performed in a syringe. Finally, the polypyrimidine strand was assembled in a synthesis cycle with the double-coupling protocol. The assembly of the second strand proceeded with an overall yield of 80%. The resulting oligonucleotides were purified by the trityl-on and trityl-off protocols, and the homogeneity was checked by gel electrophoresis.

Finally, the three-branch oligonucleotide **9** was prepared on a polystyrene support (*LV200*, *Applied Biosystems*). First the *Watson–Crick* pyrimidine strand was assembled, followed by the addition of hexaethylene glycol and the asymmetric branching molecule. The synthesis was continued by the addition of hexaethylene glycol and the assembly of the *Watson–Crick* purine strand (*Scheme 3*). At this point, the $(MeO)_2Tr$ support was detritylated and acetylated followed by the removal of the Fmoc group as described above. A third hexaethylene glycol molecule was then added, followed by the assembly of the *Hoogsteen* pyrimidine strand. Standard phosphoramidites were used.

Scheme 3. *Outline of the Synthesis of Oligonucleotide* **9**[a])




[a]) All sequences refer to 2'-deoxynucleotides.

The three strands were assembled by using the synthesis cycle recommended for low-volume columns. A longer coupling time (480 s instead of 40 s) was used only during the assembly of the third strand. The assembly of the third strand proceeded with an overall yield of 80%. The resulting oligonucleotide **9** was purified by the trityl-on and trityl-off protocols, and the homogeneity was checked by gel electrophoresis.

3. *Thermal Denaturation Experiments.* The relative stability of triple helices formed by parallel hairpins and the polypyrimidine target sequences was measured spectrophotometrically. In all cases, a single transition is observed and is assigned to the transition from triple helix to random coil [8]. *Table 2* shows the melting temperatures of triplexes made by mixing parallel hairpins **5**–**8** with the natural polypyrimidine target sequence (S11WC), the 2′-*O*-methyl-RNA target sequence (S11RNA) and the 5-methyl-$C_d$-modified target sequence (S11MeC). For comparison, melting temperatures of hairpins **1**–**4** are also shown. When hairpins were heated alone without the target sequence, a transition from parallel duplex to random coil is also observed. In all cases, except for **8**, this transition has a lower melting temperature and a lower hyperchromicity. Hairpin **5** has a melting temperature similar to that of hairpins **1** and **3**, but that of hairpin **6** is 6–7° lower. Hairpins having two 8-amino-$A_d$ residues have higher melting temperatures ($\Delta T_m$ 13° for **4** and $\Delta T_m$ 18° for **2**). The presence of two 8-amino-$A_d$ and six 5-methyl-$C_d$ produces a stabilization of the parallel duplex of 33° (see **8**, pH 6.0). The highest stabilization of the parallel duplex is obtained in the presence of two 8-amino-$A_d$ and when the backbone of the *Hoogsteen* strand is replaced by 2′-*O*-methyl-RNA ($\Delta T_m$ 40° for **7**).

Table 2. *Melting Temperatures* $T_m$ *of Parallel Hairpins* **1**–**8** (see *Table 1*) *and Triplexes in 0.1*M *Sodium Phosphate and Citric Acid pH 6.0 and pH 7.0* (last entries), *and 1*M *NaCl.* S11 = 5′-d(CTTCCTCCTCT)-3′; S11RNA = 5′-*CUUCCUCCUCU*-3′; S11MeC = 5′-d(MTTMMTMMTMT)-3′; *C*,*U* = 2′-*O*-methyl-RNA, $M_d$ = 2′-deoxy-5-methylcytidine.

| | $T_m$ [°] | | | |
|---|---|---|---|---|
| | no target | target S11WC | target S11RNA | target S11MeC |
| **3** | 25 | 45 | 38 | 55 |
| **1** | 25 | 47 | 42 | 58 |
| **6** | 18 | 40 | 35 | 52 |
| **5** | 24 | 43 | 36 | 53 |
| **2** | 43 | 56 | 55 | – |
| **4** | 38 | 51 | 52 | 62 |
| **7** | 58 | 66 | 71 | – |
| **8**, pH 6.0 | 51 | 51 | – | 63 |
| **8**, pH 7.0 | 29 | 32 | – | – |

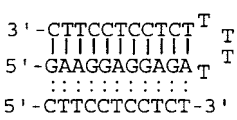
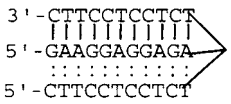
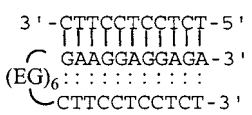
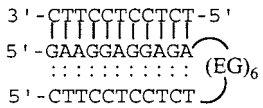
A similar but less-pronounced trend is observed in the melting temperatures of the triplexes. Melting temperatures of triplexes formed by **1**, **3**, **5**, and **6** with their natural DNA target (S11WC) are between 40–47°. The presence of two 8-amino-$A_d$ increase the $T_m$ values to 51–56° (**2** and **4** + S11WC), and the presence of two 8-amino-$A_d$ together with the replacement of the backbone of the *Hoogsteen* strand by 2′-*O*-methyl-RNA increases the $T_m$ values to 66° (**7** + S11WC). The presence of two 8-amino-$A_d$ and six 5-methyl-$C_d$ gives a $T_m$ value of only 51°, the same $T_m$ as that of the hairpin alone (see **8** + S11WC, pH 6.0). A similar result is observed at pH 7.0.

When the target sequence has the 2′-*O*-methyl-RNA backbone (S11RNA), melting temperatures of triplexes are slightly lower than those of triplexes formed with the DNA target ($T_m$ values of triplexes formed by **1**, **3, 5** and **6** 35–42°; $T_m$ values of triplexes formed by **2** and **4** 52–55°), except for the melting temperature of the triplex formed by the hairpin **7**, which is 71° (the highest). This result is especially important because it indicates that hairpins with mixed backbones bind more strongly to RNA targets.

When the target sequence has 5-methyl-$C_d$ instead of $C_d$ (S11MeC), melting temperatures of triplexes are between 10–12° higher than triplexes formed with the natural target sequence. This is 1.7–2° per substitution, consistent with the stability provided by the presence of the Me group at position 5 of the $C_d$ in the *Watson–Crick* position [40].

Finally, the melting behavior of the triplex formed by the three-branch oligonucleotide **9** was compared with the melting behavior of the same triplex in which the strands are connected in different ways (*Table 3*). The triplex formed by three independent oligonucleotides (PurWC, S11WC, and S11) has two transitions at pH 6.0. The lower melting transition (20°) corresponds to the dissociation of the *Hoogsteen*

Table 3. *Melting Temperatures* $T_m$ *of Triplexes Having Different Constraints in 0.1*M *Sodium Phosphate and Citric Acid, and 1*M *NaCl.* H26 = 5′-d(GAAGGAGGAGA-$T_4$-TCTCCTCCTTC)-3′, S11 = 5′-d(CTTCCTCCTCT)-3′, S11WC = 5′-d(TCTCCTCCTTC)-3′; PurWC = 5′-d(GAAGGAGGAGA)-3′; for **1**, **3**, and **9**, see *Table 1.*

| | Triplex[a]) | $T_m$ [°] | | | |
|---|---|---|---|---|---|
| | | pH 5.5 | pH 6.0 | pH 6.5 | pH 7.0 |
| H26 + S11 |  | 40, 82 | 20, 82 | –, 82 | –, 82 |
| **9** |  | 54, 80 | 38, 80 | 27, 82 | 15, 80 |
| PurWC + S11WC + S11 |  | 44[b]) | 20, 50 | –, 50 | –, 50 |
| **3** + S11WC |  | 54[b]) | 45[b]) | 33[b]) | 20[b]) |
| **1** + S11WC |  | 56[b]) | 47[b]) | 36[b]) | 32[b]) |

[a]) All sequences refer to 2′-deoxynucleosides. [b]) Triplex-to-random-coil transition.

strand (S11), and the higher melting transition (50°) is due to the transition from a *Watson–Crick* duplex to a random coil. At higher pH, only the duplex-to-random-coil transition is observed. At pH 5.5, the triplex-to-duplex transition is stabilized by protonation of the $C_d$ residues, and the transition occurs at the same time as the duplex-to-random-coil transition.

The connection of the *Watson–Crick* strands produces a stabilization of the *Watson–Crick*-duplex structure without changing the stability of the triplex-to-duplex transition. The melting temperature of the duplex-to-random-coil transition of the triplex formed by H26 and S11 is 82° instead of 50°, and the melting temperatures of the triplex-to-duplex transitions are 40° and 20° at pH 5.5 and pH 6.0 (*Table 3*).

The connection of the *Hoogsteen* strand with the *Watson–Crick* purine strand produces the disappearance of the duplex-to-random-coil transition. Only one pH-dependent transition is observed at pH 4.5–7.0, and it is assigned to the triplex-to-random-coil transition. This is expected at pH 5.5–6.0 because of the high stabilization of the *Hoogsteen* pairing by linking the *Hoogsteen* strand with the *Watson–Crick* purine. Two transitions are expected at pH 7.0, one being the duplex-to-random-coil at around 50°. But instead, a single transition at 20° (**3** + S11WC) and 32° (**1** + S11WC) is observed (*Table 3*). We believe this melting temperature is due to a triplex-to-random-coil transition.

The connection of the three strands repeats the same situation found with the three independent strands, but now with strong stabilization of both transitions. Oligonucleotide **9** has two transitions. The higher melting temperature (duplex-to-random-coil transition) has the same value as the $T_m$ observed in the triplex H26 + S11, and it is pH-independent. The lower melting temperature (triplex-to-duplex transition) shows a similar trend to the transitions observed in the triplexes **3** + S11WC and **1** + S11WC, and it is pH-dependent. This behavior indicates that oligonucleotide **9** has the expected triplex structure.

**Discussion.** – The triplex-stabilizing properties of 8-aminopurines has triggered interest in the preparation of parallel hairpins for triplex formation [8]. In this paper, we described the use of Fmoc- and trityl-protected asymmetric branching molecules for the preparation of parallel hairpins and three-armed oligonucleotides. This synthetic approach allows the preparation of parallel hairpins without the need of the reversed phosphoramidites, thus facilitating the preparation of parallel hairpins carrying modified bases and backbones. In most of the previous work in this area, nucleosides were used as branching units [13][15][17][23][24], as the conformational rigidity imparted by the sugar could be exploited to preorganize and induce triplex formation. We preferred the use of the non-nucleoside branching unit **11** because it is easy to prepare and it is free of possible side reactions described for the synthesis of branched RNA such as phosphoryl migration and/or chain cleavage [10–12][24].

All asymmetric branching molecules prepared carry two OH groups protected by orthogonal groups: one acid-labile and one base-labile. The acid-labile group is always a trityl (MeOTr or $(MeO)_2Tr$) derivative, while the base labile group is frequently a levulinoyl moiety [10–21] and in some cases the (*tert*-butyl)dimethylsilyl group [24]. We believe that the extensive use of the levulinoyl moiety is due to the optimal use in the synthesis of branched RNA to avoid phosphoryl migration. In our case, this side

reaction could not happen, and we selected the Fmoc group as base-labile group. Although the conditions for the removal of the Fmoc group are very mild, this approach implies the removal of the Fmoc group in the middle of the synthesis to allow the second strand to grow. In this step, in addition to the Fmoc group, the 2-cyanoethyl group of the phosphate moieties of the assembled DNA half is also removed. Little data was available on the possible consequences of the premature removal of the 2-cyanoethyl groups of phosphates, although the general feeling was positive due to the fact that during the synthesis of branched RNA, the branching nucleoside should have an unprotected phosphodiester function to prevent phosphoryl migration [24]. We found that coupling reactions are slower after branching; these difficulties lasted in coupling of a few bases after branching, but after three or four bases, coupling became easier (as observed by the lack of the corresponding truncated sequences). This is consistent with results found during the synthesis of branched RNA [10–24], and it indicates that steric hindrance and electron-withdrawing effects of the groups at the branching site slow the coupling reactions more than the presence of unprotected phosphate groups. For these reasons, difficulties in coupling at the branching point are easily overcome by increasing the coupling time or by increasing the phosphoramidite concentration. On the other hand, changing the 2-cyanoethyl group to a Me group, changing the oxidation conditions, and addition of an extra washing step with DMAP/TET does not significantly improve coupling at the branching point.

We also measured the relative stability of purine · pyrimidine · pyrimidine triplexes formed by the parallel hairpins prepared in this work. RNA/DNA Hybrid hairpins carrying 8-aminopurines and 2′-*O*-methyl-RNA at the *Hoogsteen* strand were found to have the best affinity for both DNA and 2′-*O*-methyl-RNA polypyrimidine targets. On the other hand, substitution of $C_d$ by 5-methyl-$C_d$ at the *Hoogsteen* position induced a high stabilization of the parallel structure but not of the triplex. During the preparation of this manuscript, the synthesis of 3′,3′-linked oligonucleotides branched by a pentaerythritol linker was described [21]. These authors also showed a high stabilization of triplexes formed by single-stranded DNA or RNA sequences with branched oligonucleotides composed of 2′-deoxyribonucleotides and 2′-*O*-methylribonucleotides. Thus, our results are in agreement with these authors and indicate that parallel hairpins carrying 2′-*O*-methyl-RNA and 8-aminoadenine bases exhibit enhanced binding interactions with single-stranded polypyrimidine sequences by triplex formation.

This study was supported by the *Dirección General de Investigación Científica* (BQU2000-0649 and CAL01-058-C2-2), the *Generalitat de Catalunya* (2000-SGR-0018, 2001-SGR-0049), and by *Cygene Inc.*

## Experimental Part

*General*. Phosphoramidites and ancillary reagents used during oligonucleotide synthesis were from *Applied Biosystems* (*PE Biosystems Hispania S.A.*, Spain), *Cruachem Ltd.* (Scotland), and *Glen Research Inc.* (USA). The rest of the chemicals were purchased from *Aldrich*, *Sigma*, or *Fluka* (*Sigma-Aldrich Química S.A.*, Spain). Long-chain-alkyl-amine controlled-pore glass (LCAA-CPG) was purchased from *CPG Inc.* (New Jersey, USA). Solvents were from *S.D.S.* (France). *N,N′*-(2-Hydroxypropane-1,3-diyl)bis[4-hydroxybutanamide] (**11**) was prepared as described in [19]. Oligonucleotide sequences were synthesized on a *Applied Biosystems 392* DNA synthesizer (*Applied Biosystems*, USA). UV Spectra and melting experiments: *Shimadzu UV-2101PC* spectrophotometer equipped with a temp. controller *SPR-8*. $^1$H- and $^{13}$C-NMR Spectra: *Varian Gemini-*

200 MHz spectrometer, $\delta$ in ppm. Matrix-assisted laser-desorption-ionization time-of-flight (MALDI-TOF) mass spectra were provided by the mass spectrometry service at the University of Barcelona.

*4-Hydroxy*-N-*{2-hydroxy-3-{[4-[(4-methoxytrityl)oxy]-1-oxobutyl}amino}propyl}butanamide* (**12**). Bis[4-hydroxybutanamide] **11** (5 g, 19 mmol) was dried after repeated evaporation of a soln. in anh. pyridine and dissolved in pyridine (20 ml). The soln. was cooled in an ice-bath and treated dropwise with a soln. of 4-methoxytrityl chloride (2.89 g, 9.3 mmol) in $CH_2Cl_2$ (20 ml) over 30 min. After the addition, the mixture was gradually warmed to r.t. and stirred for 90 min. The reaction was stopped by the addition of MeOH (0.5 ml), and the soln. was evaporated. The residue was dissolved in $CH_2Cl_2$ (200 ml), the org. soln. washed with 5% aq. $Na_2CO_3$ soln. (100 ml) and sat. NaCl soln. (100 ml), dried (anh. $Na_2SO_4$), and evaporated, and the residue purified by CC (silica gel, column packed with 0.5% pyridine/$CH_2Cl_2$, then eluted with $0 \rightarrow 5\%$ MeOH/$CH_2Cl_2$): 2.51 g (50%) of **12**. TLC (5% MeOH/$CH_2Cl_2$): $R_f$ 0.37. $^1$H-NMR ((D$_6$)DMSO): 7.4–6.4 (*m*, 16 H, MeO*Tr*, 2 NH); 4.6 (br. *s*, 1 H, CHO*H*); 3.71 (*s*, MeO); 3.6, 3.4, 3.2 (*m*, 8 H, OH, C*H*OH, C$H_2$OH, C$H_2$NH); 3.0 (*t*, 2 H, MeOTrOC$H_2$); 2.3 (*m*, 4 H, C$H_2$CO); 1.8 (*m*, 4 H, $CH_2CH_2CH_2$). $^{13}$C-NMR ($CDCl_3$): 174.1, 173.7 (2 CONH); 157.4 (MeO*Tr*); 143.6 (MeO*Tr*); 134.8 (MeO*Tr*); 129.2 (MeO*Tr*); 127.3 (MeO*Tr*); 126.8 (MeO*Tr*); 125.8 (MeO*Tr*); 112.0 (MeO*Tr*); 85.2 (quat. C, MeO*Tr*); 68.6 (CHOH); 60.8, 61.4 ($CH_2$OH, MeOTrOC$H_2$); 54.2 (MeO); 42.0 ($CH_2$NH); 32.4 (C$H_2$CONH); 27.2, 25.0 ($CH_2CH_2CH_2$). FAB-MS (pos.): 557.2 ($[M+Na]^+$, $C_{31}H_{38}N_2NaO_6^+$), 273.1 (MeOTr$^+$). Anal. calc. for $C_{31}H_{38}N_2O_6 \cdot 2\,H_2O$: C 65.25, H 7.42, N 4.91; found: C 65.84, H 7.84, N 4.92.

*4-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]oxy}*-N-*{2-hydroxy-3-{[4-[(4-methoxytrityl)oxy]-1-oxobutyl}amino}propyl}butanamide* (**13**). A soln. of **12** (0.63 g, 1.18 mmol) in pyridine (10 ml) was treated with 9*H*-fluoren-9-yl methyl carbonochloridate (0.31 g, 1.18 mmol). After 3.5 h stirring at r.t., the reaction was stopped by addition of MeOH (0.5 ml), and the soln. was evaporated. The residue was dissolved in $CH_2Cl_2$ (100 ml), the org. soln. washed with sat. NaCl soln. (100 ml), dried ($Na_2SO_4$), and evaporated, and the residue purified by CC (silica gel, column packed with 0.5% pyridine/$CH_2Cl_2$, then eluted with $0 \rightarrow 5\%$ MeOH/$CH_2Cl_2$): 0.2 g (23%) of **13**. TLC (5% MeOH/$CH_2Cl_2$): $R_f$ 0.78. FAB-MS (pos.): 779.5 ($[M+Na]^+$, $C_{46}H_{49}N_2NaO_8^+$), 273.2 (MeOTr$^+$). $^1$H-NMR ((D$_6$)DMSO): 7.9–6.8 (*m*, 24 H, MeO*Tr*, Fmoc, 2 NH); 4.9 (*d*, CHO*H*); 4.4 (*m*, $CH_2$ of Fmoc); 4.18 (*m*, CH of Fmoc); 3.75 (*s*, MeO); 3.5 (*m*, C*H*OH); 3.4–2.9 (*m*, 8 H, MeOTrOC$H_2$, FmocOC$H_2$, C$H_2$NH); 2.4–2.2 (*m*, 4 H, C$H_2$CO); 2.1–1.8 (*m*, 4 H, $CH_2CH_2CH_2$). $^{13}$C-NMR ((D$_6$)DMSO): 173.4, 172.5 (CONH); 157.4 (MeO*Tr*); 143.4 (MeO*Tr*); 142.6, 141.4, 140.2 (Fmoc); 129.5 (MeO*Tr*); 127.0 (MeO*Tr*, Fmoc); 126.6 (MeO*Tr*); 126.2, 125.9 (MeO*Tr*, Fmoc); 124.2 (Fmoc); 118.9 (Fmoc); 85.2 (quat. C, MeO*Tr*); 69.7, 68.9, 66.4, 61.5 (CHOH, MeOTrOC$H_2$, FmocOC$H_2$, $CH_2$ of Fmoc); 54.5 (MeO); 45.9 (CH of Fmoc); 41.8 ($CH_2$NH); 32.8, 31.1 (C$H_2$CO); 25.4, 23.8 ($CH_2CH_2CH_2$). Anal. calc. for $C_{46}H_{49}N_2O_8$: C 72.90, H 6.52, N 3.70; found: C 72.32, H 6.46, N 3.37.

*Butanedioic Acid Mono{2-{[4-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]oxy}-1-oxobutyl}amino}-1-{[{4-[(4-methoxytrityl)oxy]-1-oxobutyl}amino}methyl]ethyl} Ester* (**14**). Compound **13** (0.2 g, 0.26 mmol) was treated with succinic anhydride (31 mg, 0.31 mmol) in the presence of DMAP (38 mg, 0.31 mmol) in $CH_2Cl_2$ (25 ml). The mixture was stirred for 20 h at r.t., and the resulting soln. was diluted with $CH_2Cl_2$ (75 ml). The org. soln. was washed with 0.1M $NaH_2PO_4$ (pH 5; 75 ml) followed by sat. NaCl soln., dried ($Na_2SO_4$), and evaporated. The residue was dissolved with the minimum amount of $CH_2Cl_2$ and precipitated with hexane: 0.2 g (87%) of **14**, partially contaminated with DMAP. This white solid was used in the following step without further purification. $^1$H-NMR ((D$_6$)DMSO): similar to that of **13**, with the exception of a signal at 2.8 (*m*, C$H_2$COOH).

*4-(Controlled-pore Glass Long-chain-alkylamino)-4-oxobutanoic Acid 2-{[4-{[(9*H*-Fluoren-9-ylmethoxy)-carbonyl]oxy}-1-oxobutyl}amino}-1-{{[4-[(4-methoxytrityl)oxy]-1-oxobutyl}amino}methyl}ethyl Ester* (**15**). The support carrying the asymmetric doubler **13** was prepared by coupling of **14** to sarcosyl-LCAA-CPG [41] in the presence of $Ph_3P$, 2,2′-dithiobis[5-nitropyridine], and DMAP as condensing agents [42]. Coupling time was increased from 5 min to 45 min, which gave a functionalization of 34 μmol per g.

*Branched Oligonucleotides.* Phosphoramidites of the natural bases (bz$^6$A$_d$, bz$^4$C$_d$, ib$^2$G$_d$, and T; bz = benzoyl, ib = isobutyryl) were from commercial sources. The phosphoramidite of 8-amino-2-deoxyadenosine protected with the dimethylformamidine group was prepared as described elsewhere [3][4]. The *N*$^4$-benzoyl-2′-deoxy-5-methylcytidine (bz$^4$m$^5$C$_d$), 2′-*O*-methyl-U (U(Me)$^{2'}$), and *N*$^4$-benzoyl-2′-*O*-methylcytidine (bz$^4$C(Me)$^{2'}$), $(MeO)_2$Tr-protected hexaethylene glycol, and asymmetric doubler phosphoramidites were from commercial sources. The support carrying a $(MeO)_2$Tr-protected propane-1,3-diol moiety connected to succinyl-CPG was prepared as described elsewhere [31]. Oligonucleotides were synthesized on a 1- and 0.2-μmol scale with a DNA synthesizer. The supports carrying the first half of the parallel hairpin were synthesized on the 1-μmol scale. After the assembly of the first half of hairpins, the $(MeO)_2$Tr group at the 5′-end was removed, and the resulting supports were treated as follows: *a*) Acetylation of the 5′-end: treatment of the trityl-off supports

with 2 ml of a 1:1 mixture of the capping solns. from the DNA synthesizer ($A = Ac_2O$, 2,6-dimethylpyridine/tetrahydrofuran 1:1:8 and $B = 10\%$ 1-methyl-1*H*-imidazole in THF) for 10 min. *b*) Removal of the Fmoc group: treatment of the supports with 0.5M DBU in MeCN (2 ml) for 10 min and washings with 1% $Et_3N$/MeCN and MeCN to eliminate excess of DBU. These supports were divided in several aliquots to test the assembly of the second half of the hairpins by using several cycles of synthesis. The trials were performed on a 0.2-μmol scale. The following synthesis cycles were tried: *A*) Standard cycle: coupling time 30 s, capping time 15 s, oxidation time 30 s. *B*) Increased-coupling-time cycle: coupling time 5 min, capping time 75 s, oxidation time 75 s. *C*) Double coupling cycle: Two coupling reactions per base of 5 min, capping time 75 s, oxidation time 75 s. *D*) Neutralization cycle: standard cycle with an additional step of neutralization by DMAP/TET between the detritylation and coupling steps. *E*) Neutralization and increased-coupling-time cycle: increased-coupling-time cycle with an additional step of neutralization by DMAP/TET between the detritylation and coupling steps. *F*) Neutralization and double coupling cycle: double coupling cycle with an additional step of neutralization by DMAP/TET between the detritylation and coupling steps. *G*) Anhydrous oxidation cycle: standard cycle in which the $I_2$ soln. was replaced by a *tert*-butyl hydroperoxide soln. (20 ml of the commercially available soln. in decane (5–6M) was mixed with 40 ml of $CH_2Cl_2$), and the oxidation time was increased to 1 min. Finally the phosphoramidite concentration was 0.1M but in some cases a 0.2M soln. was also used.

The three-branch oligonucleotide **9** was prepared on a 0.2-μmol scale with polystyrene supports (*LV200* columns). The first two branches were assembled by the standard cycle recommended for these columns. After capping and removal of the Fmoc group as described above, the third branch was assembled by the same cycle but with increased coupling time (8 min).

After the assembly of the sequences, oligonucleotide supports were treated with 32% aq. ammonia at 55°. When *O*-methyl phosphoramidites were used, supports were first treated with 1 ml of thiophenol/$Et_3N$/dioxane 1:1:2 for 2 h at r.t., washed with MeCN, and dried. The resulting supports were then treated with conc. ammonia. The ammonia solns. were evaporated, and the products were purified by reversed-phase HPLC. Oligonucleotides were synthesized with the last $(MeO)_2Tr$ group at the 5′-end (trityl-on protocol) to facilitate reversed-phase purification. HPLC: Solvent $A = 5\%$ MeCN in 100 mM $(Et_3NH)OAc$ pH 6.5, solvent $B = 70\%$ MeCN in 100 mM $(Et_3NH)OAc$ pH 6.5; *PRP-1* columns (*Hamilton*), 250 × 10 mm; flow rate 3 ml/min, linear gradient 10 → 80% *B* (trityl on) for 30 min or linear gradient 0 → 50% *B* (trityl off) for 30 min. MALDI-TOF of **5**: 8358.9 ($[M + 4\,Na]^+$; calc. for $C_{265}H_{323}N_{93}O_{165}P_{26}$: 8275.4). Oligonucleotides were also checked by denaturing gel electrophoresis. In this case, a small aliquot of the $(MeO)_2Tr$-oligonucleotide was detritylated with 80% AcOH and dried. The resulting product was loaded on the gel by using glycerol loading buffer for the analysis of the purity.

Yields after purification: **5** (200 nmol), 8 *OD* units (cycle *B*); **6** (200 nmol), 7 *OD* units (cycle *C*); **7** (200 nmol), 3 *OD* units; **8** (200 nmol), 2 *OD* units; **9** (200 nmol), 4 *OD* units. Sequences **1**–**4** were prepared with polystyrene supports (200 nmol), and the amount obtained after the same HPLC purification were 5–10 *OD* units [8].

*Melting Experiments*. For the melting experiments with triple helices, solns. of equimolar amounts of hairpins and the target *Watson–Crick* pyrimidine strand (11-mer) were mixed in 0.1M sodium phosphate/citric acid buffer of pH ranging from 4.5 to 7.0 and 1M NaCl. The DNA concentration was determined by UV absorbance measurements (260 nm) at 90°, by using for the DNA coil state the following extinction coefficients: 7500, 8500, 12500, 15000, and 15000 $M^{-1}\,cm^{-1}$ for $C_d$, $T_d$, $G_d$, $A_d$, and 8-amino-$A_d$, resp. The solns. were heated to 90°, allowed to cool slowly to r.t., and stored at 4° until the UV was measured. UV Absorption spectra and melting experiments (absorbance *vs.* temp.) were recorded in 1-cm-path-length cells with a spectrophotometer, a temp. controller, and a programmed temp.-increase rate of 0.5°/min. Melts were run on duplex concentration of 3–4 μM at 260 nm.

## REFERENCES

[1] N. T. Thuong, C. Hélène, *Angew. Chem., Int. Ed.* **1993**, *32*, 666.
[2] P. P. Chan, P. M. Glazer, *J. Mol. Med*. **1997**, *75*, 267.
[3] K. Kwai, I. Saito, H. Sugiyama, *Tetrahedron Lett*. **1998**, *39*, 5221.
[4] R. Güimil García, E. Ferrer, M. J. Macias, R. Eritja, M. Orozco, *Nucleic Acids Res.* **1999**, *27*, 1991.
[5] R. Soliva, R. Güimil García, J. R. Blas, R. Eritja, J. L. Asensio, C. González, F. J. Luque, M. Orozco, *Nucleic Acids Res.* **2000**, *28*, 4531.
[6] E. Cubero, R. Güimil García, F. J. Luque, R. Eritja, M. Orozco, *Nucleic Acids Res.* **2001**, *29*, 2522.

[7] A. Aviño, J. C. Morales, M. Frieden, B. G. de la Torre, R. Güimil García, E. Cubero, F. J. Luque, M. Orozco, F. Azorín, R. Eritja, *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1761.
[8] A. Aviño, M. Frieden, J. C. Morales, B. G. de la Torre, R. Güimil-García, F. Azorín, J. L. Gelpí, M. Orozco, C. González, R. Eritja, *Nucleic Acids Res.* **2002**, *30*, 2609.
[9] Y. Ueno, M. Takeba, M. Mikawa, A. Matsuda, *J. Org. Chem*. **1999**, *64*, 1211.
[10] M. J. Damha, K. Ganeshan, R. H. E. Hudson, V. Zabarylo, *Nucleic Acids Res.* **1992**, *20*, 6565.
[11] R. Kierzek, D. W. Kopp, M. Edmonds, M. H. Caruthers, *Nucleic Acids Res.* **1986**, *14*, 4751.
[12] M. Grotli, R. Eritja, B. Sproat, *Tetrahedron* **1997**, *53*, 11317.
[13] E. Azhayeva, A. Azhayev, A. Guzaev, H. Lönnberg, *Nucleic Acids Res.* **1995**, *23*, 4255.
[14] H. Thrane, J. Fensholdt, M. Regner, J. Wengel, *Tetrahedron* **1995**, *51*, 10389.
[15] M. Meldgaard, N. K. Nielsen, M. Bremmer, O. S. Pedersen, C. E. Olsen, J. Wengel, *J. Chem. Soc., Perkin Trans. 1* **1997**, 1951.
[16] T. Horn, C. A. Chang, M. S. Urdea, *Nucleic Acids Res.* **1997**, *25*, 4842.
[17] A. H. Uddin, M. A. Roman, J. R. Anderson, M. J. Damha, *J. Chem. Soc., Chem. Commun*. **1996**, 171.
[18] S. Teigelkamp, S. Ebel, D. W. Will, T. Brown, J. D. Beggs, *Nucleic Acids Res.* **1993**, *21*, 4651.
[19] V. A. Korshun, N. B. Pestov, E. V. Nozhevnikova, I. A. Prokhorenko, S. V. Gontarev, Y. A. Berlin, *Synth. Commun.* **1996**, *26*, 2531.
[20] M. S. Shchepinov, I. A. Udalova, A. J. Bridgman, E. M. Southern, *Nucleic Acids Res.* **1997**, *25*, 4447.
[21] Y. Ueno, A. Shibata, A. Matsuda, Y. Kitade, *Bioconjugate Chem*. **2003**, *14*, 684.
[22] L. H. Eckardt, K. Naumann, W. M. Pankau, M. Rein, M. Schweitzer, N. Winhab, G. von Kiedrowski, *Nature (London)* **2002**, *420*, 286.
[23] R. H. E. Hudson, A. H. Uddin, M. J. Damha, *J. Am. Chem. Soc*. **1995**, *117*, 12470.
[24] R. S. Braich, M. J. Damha, *Bioconjugate Chem*. **1997**, *8*, 370.
[25] S. Hoshida, Y. Uneo, A. Matsuda, *Bioconjugate Chem*. **2003**, *14*, 607.
[26] Y. Uneo, M. Mikawa, S. Hoshida, A. Matsuda, *Bioconjugate Chem*. **2001**, *12*, 635.
[27] J. H. Van de Sande, N. B. Ramsing, M. W. Germann, W. Elhorst, B. W. Kalisch, B. W. Kitzing, R. T. Pon, R. C. Clegg, T. M. Jovin, *Science (Washington, D.C.)* **1988**, *241*, 551.
[28] E. R. Kandimalla, S. Agrawal, *Biochemistry* **1996**, *35*, 15332.
[29] L. E. Xodo, G. Manzini, F. Quadrifolio, G. A. van der Marel, J. H. Boom, *Nucleic Acids Res.* **1991**, *19*, 5625.
[30] G. Manzini, L. E. Xodo, D. Gasparotto, *J. Mol. Biol*. **1990**, *213*, 833.
[31] R. Eritja, A. R. Díaz, E. Saison-Behmoaras, *Helv. Chim. Acta* **2000**, *83*, 1417.
[32] M. Shimizu, A. Konishi, Y. Shimada, H. Inoue, E. Ohtsuka, *FEBS Lett*. **1992**, *302*, 155.
[33] R. W. Roberts, D. M. Crothers, *Science (Washington, D.C.)* **1992**, *258*, 1463.
[34] C. Escudé, J. C. François, J. Sun, G. Ott, M. Sprinzl, T. Garestier, C. Hélène, *Nucleic Acids Res.* **1993**, *21*, 5547.
[35] S. Wang, E. T. Kool, *Nucleic Acids Res.* **1995**, *23*, 1157.
[36] F. Morvan, J. L. Imbach, B. Rayner, *Antisense Nucl. Acid Drug Develop*. **1997**, *7*, 327.
[37] T. J. Povsic, P. B. Dervan, *J. Am. Chem. Soc*. **1989**, *111*, 3059.
[38] E. Ferrer, C. Fàbrega, R. Güimil-García, F. Azorín, R. Eritja, *Nucleosides Nucleotides* **1996**, *15*, 907.
[39] A. P. Guzaev, M. Manoharan, *J. Org. Chem*. **2001**, *66*, 1798.
[40] S. Wang, E. T. Kool, *Biochemistry* **1995**, *34*, 4125.
[41] K. P. Stengele, W. Pfleiderer, *Tetrahedron Lett*. **1990**, *31*, 2549.
[42] K. C. Gupta, P. Kumar, D. Bhatia, A. K. Sharma, *Nucleosides Nucleotides* **1995**, *14*, 829.

*Received June 11, 2003*