^{1}H NMR spectra of the ylides **1b** and **2b** show that both methyls are equivalent, suggesting that the π(CN) bond order is small enough to allow rotation of the NMe_2 group. The same is observed in the spectra of the parent $Ph_3P{=}CHC(O)NMe_2$ species.[13b]

The crystal structure of **1a**[19] was solved at −95 °C and is shown in Figure 1. The coplanarity of the atoms P, C(41), C(1), C(2), O(1), O(2) (within 0.04 Å) and the rotation of the C_6F_4CN ring by 59° out of this plane show that a delocalization of electron density affects mainly the O–C–C–P bonding arrangement. This effect is clearly shown by the P—C(1) bond distance [1.722 (3) Å], which is, as expected, somewhat shorter than the standard C_{sp^2}(Ar)—PR_3 bond distance [1.793 Å[20]], but longer than in non-carbonyl ylides such as $Ph_3P{=}CH_2$ [1.697 (8), 1.688 (3) Å].[21] However, only marginal shortening of the C(1)—C(2) bond [1.441 (4) Å vs mean value of 1.460 Å for a C=C—C(O)R bond[20]] and lengthening of the C(2)—O(2) bond [1.222 (3) Å vs mean value of 1.199 Å for a C=C—CO_2R[20]] are evident.

This study proves that para-C—F bond cleavage in C_6F_4X (X = CN, NO_2) is easily achieved by the weak nucleophiles $Ph_3P{=}CHC(O)R$ (R = OEt, NMe_2).

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Supplementary Material Available: Experimental information and data on compounds **1–3** and structure determination summary, data collection, solution and refinement and tables of atomic coordinates, bond lengths and angles, anisotropic displacement coefficients, and H-atom coordinates for **1a** (10 pages); listing of observed and calculated structure factors for **1a** (16 pages). Ordering information is given on any current masthead page.

(19) Crystal data for **1b**: pale yellow prism, 0.65 × 0.35 × 0.25 mm, monoclinic, space group $P2_1/c$, T = −95 °C, a = 15.217 (4) Å, b = 9.306 (2) Å, c = 18.465 (4) Å, β = 111.27 (2)°, V = 2436.8 (10) Å^3, Z = 4. The structure was solved by direct methods and refined to R = 0.043, wR = 0.047 for 2895 reflections > $4\sigma(F)$ to $2\sigma_{max}$ 50° (Mo Kα, λ = 0.710 69 Å).

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A DNA Hairpin as a Target for Antisense Oligonucleotides

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Artificial regulation of gene expression can be achieved by antisense oligonucleotides complementary to part of a messenger RNA.[1] Although RNAs can be written as single strands, self-pairing between adjacent or remote sequences gives rise to double-stranded regions. RNA hairpins will weaken or prevent the binding of an antisense oligomer if its target is sequestered in such a structure.[2] We propose here a strategy to bind an oligonucleotide to a hairpin without disrupting the structure, via the formation of base triplets.[3-8] Our suggestion is to form a "double hairpin" complex (Figure 1a). The antisense oligomer has two domains: the first one is complementary to the single-stranded sequence at the bottom of the hairpin, and the second one is designed to form a triplex with both the hybridized first domain and the stem of the hairpin.

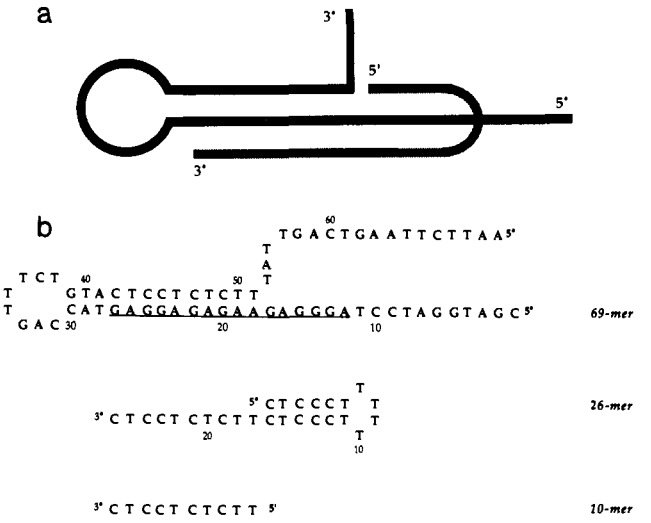

Figure 1. (a) Schematic representation of the double hairpin structure. (b) Sequence of the 69-mer showing the hairpin structure. The 16-purine target sequence is underlined. The sequences of the oligopyrimidine 26-mer and 10-mer are shown at the bottom. The antisense 26-mer is folded to evidence its association with the target 69-mer. Oligodeoxynucleotides were synthesized on an Applied Biosystem synthesizer using phosphoramidite chemistry and were purified by electrophoresis on polyacrylamide gels containing 7 M urea.

We investigated this possibility with a 69-mer oligodeoxynucleotide, able to fold into a hairpin, the stem of which comprised 13 base pairs. The 5′ strand contained a sequence of 10 purines, and consequently, the 3′ strand contained 10 pyrimidines. In addition, a stretch of six purines, 5′AGGGAG, was immediately upstream of the stem, leading to a 16-purine-long sequence that we selected as a target for an antisense 26-mer oligopyrimidine (Figure 1b). We also synthesized a 10-mer complementary to the stem of the hairpin.

Electrophoretic analysis, on a nondenaturing polyacrylamide gel, showed that the 26-mer induced a mobility shift of the 69-mer indicating the association of the two oligomers (not shown). We then probed the double hairpin complex by chemical footprinting. Diethyl pyrocarbonate (DEPC) clearly mapped the stem region,[5,9] in the 69-mer from G(20) to A(29) (Figure 2a). The addition of the 10-mer did not reveal any significant change; but when the modification of the 69-mer was carried out in the presence of the 26-mer, the reactivity of purines from G(13) to A(19) was reduced, indicating the protection of this sequence by the antisense oligonucleotide (Figure 2a). Surprisingly, the reactivity of A(32) (in the loop) and of the purines on the 3′ side of the stem (from A(53) to A(62)) was increased, suggesting an overall conformation change of the 69-mer upon association of the 26-mer.

These conclusions were strengthened by the modification of the 69-mer by dimethyl sulfate (DMS). The addition of the 10-mer decreased the sensitivity to DMS from G(20) to G(27), indicating the formation of the expected 69-mer/10-mer triplex (Figure 2b). In the 69-mer + 26-mer mixture, the reactivity of G(13) to G(27) was reduced almost to 0, demonstrating that both the double-stranded region of the hairpin and the upstream sequence were involved in the binding of the antisense 26-mer. An unexpected hyperreactivity of G(27) was induced by the formation of the double-hairpin structure (Figure 2b). This might reveal the triplex–duplex junction, but such an increased sensitivity was not

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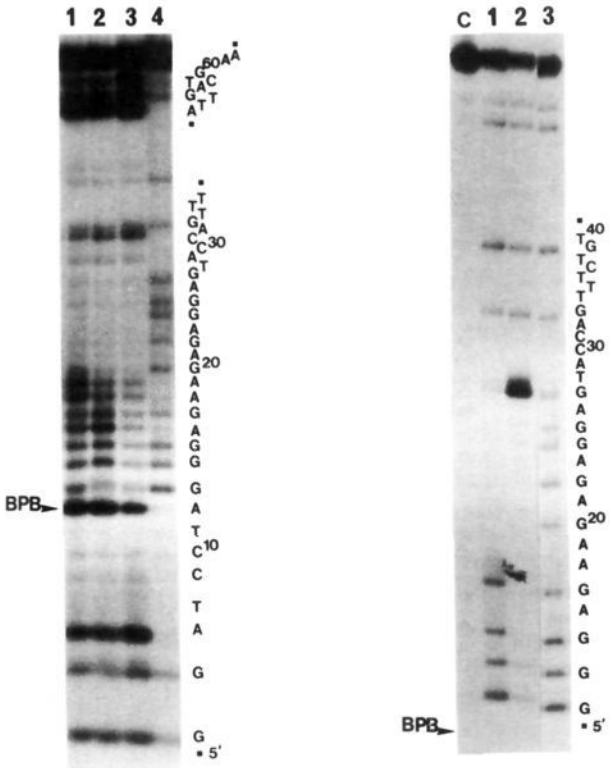

Figure 2. Autoradiograms of polyacrylamide gel electrophoresis of the double hairpin complex. The ^{32}P 5′ end-labeled 69-mer (5×10^{-8} M) was preincubated for 30 min at 15 °C in a 50 mM sodium acetate buffer, pH 6.0, containing 10 mM $MgCl_2$, in the absence or in the presence of the desired oligomer. Left (a): Following preincubation the samples were reacted for 90 min at 25 °C with diethyl pyrocarbonate (10% final concentration). After addition of 1 vol of 5 mM EDTA, the mixture was extracted with ethyl ether and the DNA was ethanol precipitated. The modified DNA was then treated (30 min at 90 °C) with 1 M piperidine, precipitated twice by ethanol, solubilized in 80% formamide containing marker dyes (bromophenol blue (BPB) and xylene cyanol), and analyzed on a 16% polyacrylamide gel containing 7 M urea in TBE buffer. Lane 1: 69-mer. Lane 2: 69-mer + 6×10^{-5} M 10-mer. Lane 3: 69-mer + 5×10^{-6} M 26-mer. Lane 4 shows a G reaction of the 69-mer whose sequence is written to the right. Right (b): Following preincubation the samples were incubated for 20 min at 25 °C in the presence of 0.5% dimethyl sulfate, treated with piperidine, and analyzed as described above. Lane 1: 69-mer + 6×10^{-5} M 10-mer. Lane 2: 69-mer + 2×10^{-5} M 26-mer. Lane 3: 69-mer. Lane C corresponds to the 69-mer treated with piperidine without any other previous treatment. The 69-mer sequence is given to the right of the panel.

detected with the 10-mer, suggesting a particular geometry of this region in the 26-mer/69-mer complex, due to the binding of the 5′ part of the 26-mer.

The formation of the complex depicted in Figure 1 was also monitored from the antisense standpoint. The reactivity of T's to potassium permanganate was determined for the 26-mer either in the presence or in the absence of the 69-mer target. A reduced sensitivity was observed from T(15) up to the 3′ end of the oligomer (not shown), in the oligonucleotide mixture compared to the 26-mer alone. No modification of the reactivity was observed either for T(6) or for T(1). As A(12) was not protected by the 26-mer from reaction with DEPC (Figure 2b), this suggested that this TA·T triplet is not formed in the 26-mer/69-mer hybrid.

From the above study we conclude that a stable complex can be formed, between a DNA hairpin structure and a complementary oligonucleotide, through the formation of both Watson–Crick and Hoogsteen hydrogen bonds with the homopurine target sequence. Preliminary data indicate that such complexes might be formed with RNA targets. These structures might be efficient at inhibiting either translation of mRNA or reverse transcription of viral RNA.

Molecular Recognition in Water: New Receptors for Adenine Derivatives

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Individual base pairs between nucleic acid components are generally not observed in water, since the entropic price of bimolecular association is generally not paid by the newly formed intermolecular hydrogen bonds. Accordingly, such interactions are studied in contexts where other forces are involved, and interpretation is difficult.[1,2] We have initiated a program to evaluate these forces in water and we report here our preliminary results.

We use the imides **1**, related to those used in organic solvents,[3] as the complement to adenine. These hydrophilic analogs of Kemp's triacid were prepared as shown in Scheme I. Alkylation of the trianion of triester **2** with chloromethyl benzyl ether gave the all-cis tribenzyloxy trimethyl ester **3**[4] after recrystallization from formic acid. Saponification yielded the triacid **4**, which provided the anhydride **5a** upon dehydration. Ammonolysis of **5a** yielded the imide acid **5b**, which was reacted with $SOCl_2$ to form the imide acid chloride **6**. Coupling of imide acid chloride **6** with aromatic amines gave the protected J-shaped receptors **7**. Debenzylation of these systems was quantitative using HBr(g) in formic acid, providing the water-soluble receptors **1**.

The solubility of these receptors ranged from 15 mM for the anilide **1a** (Table I) to 0.2 mM for the anthracyl derivative **1e** at 10 °C.[5] These systems were titrated with 9-ethyladenine (**8**) to obtain the association constants. In 9:1 H_2O/D_2O, the use of binomial solvent suppression[6] at 10 °C[7] permitted monitoring of the exchangeable protons. The imide peak moved consistently downfield from 10.6 ppm to a limiting value of ~13 ppm upon addition of 9-ethyladenine, behavior that confirms hydrogen-bonded base pairing as shown in Scheme II. The association constant was obtained from this movement using a nonlinear least-squares fit to the 1:1 binding isotherm, with allowance for guest dimerization.[8] Scheme II shows only the Watson–Crick mode of base pairing; not shown are the Hoogsteen, reverse Watson–Crick, and reverse Hoogsteen base pairs. The association constants reported in Table I represent the sum of all four binding modes.

Hydrophobic surface contacts are revealed to be the most significant contributors to binding. The phenyl-substituted receptor

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(8) The dimerization constant for 9-ethyladenine was measured as 13.8 M^{-1} under the titration conditions.

(9) Dilution studies of the receptors **1a–e** show that no host dimerization or aggregation occurs at the concentrations used for this study.

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