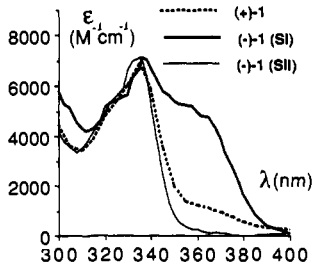

Figure 1.

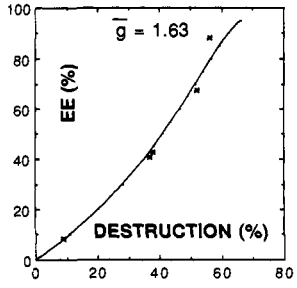

Figure 2.

protein bovine serum albumin (BSA). The optical enrichment is controlled by the difference in light absorption at the irradiation wavelength, and the extent of **1** phototransformation, analogous to the CPL photolysis.

Thus, when crystalline (−)-**1** is dissolved in an aqueous solution of BSA, it reversibly binds in two sites of BSA with binding constants higher than 10^8 M.[3] The properties of (−)-**1** in these binding sites strongly differ, as absorption, circular dichroism, and fluorescence spectra manifest. In particular, (−)-**1** in the higher affinity site, SI, shows a new absorption band in the 355–420-nm spectral region. Here it does not absorb when bonded to the second site, SII, nor when dissolved in CH_3CN, CH_3OH, and C_6H_{14}. When (+)-**1** is dissolved in aqueous BSA, the absorption in the 355–420-nm region is far less intense than that of (−)-**1** in SI[3] (Figure 1).

Finally, when (±)-**1** is dissolved in aqueous BSA, a (±)-**1**/BSA complex is formed.[4] The absorption spectrum of this complex is exactly the arithmetic average of the (+)-**1** and (−)-**1**/BSA complexes having half the concentration in the same BSA solution. When the number of (±)-**1** molecules on each BSA is, on the average, ≤1, practically every (−)-**1** is in SI, as happens when ≤0.5 (−)-**1** molecule is bound to BSA in the (−)-**1**/BSA complex. Thus, at 365 nm, the light absorption of (−)-**1** is $\epsilon^-(SI)/\epsilon^+ = 4850/970 = 5.0$ times greater than the (+)-**1**/BSA absorption. Hence, irradiation of the (±)-**1** complex with 365-nm light would preferentially both excite and, conceivably, photoconvert (−)-**1** so as to cause enrichment in the (+)-**1** antipode.

The ees obtained in a series of five irradiations on the (±)-**1**/BSA complex, 0.000 121/0.000 173 M, respectively, versus the photoconversion degree are reported in Figure 2.[5] The curve shows the ee expected (vide infra).

Here, contrary to classical CPL photolyses, high ees are reached with tolerable photoconversions (e.g., 89% ee with 57% destruction of **1**). The experimental data can be fitted according to the simple kinetic scheme

$$\frac{dc_{+1}}{dt} = -K\phi^+ I^+ = -KI\phi^+ \frac{\epsilon^+ c_{+1}}{\epsilon^- c_{-1} + \epsilon^+ c_{+1} + R} \qquad (1)$$

where ϕ^+ is the quantum yield of phototransformation, c_{+1} the concentration, I^+ the intensity of light absorbed, ϵ^+ the extinction coefficient, at the irradiation wavelength, of (+)-**1** bound to BSA. K and R depend on the volume of the solution and on the presence of other light-absorbing molecules, respectively. Integration of the differential equation obtained by dividing eq 1 by the corresponding equation for (−)-**1** and simple mathematics leads to

$$\bar{g} = \frac{\Delta\bar{\epsilon}}{\bar{\epsilon}} = \left| \frac{\ln [(1 + ee)/(1 - ee)]}{\ln (1 - x) + \frac{1}{2} \ln [1 - (ee)^2]} \right|$$

similar to a valid expression for CPL irradiation.[2]

In this expression, $\bar{\epsilon} = 0.5(\phi^+\epsilon^+ + \phi^-\epsilon^-)$, $\Delta\bar{\epsilon} = \phi^-\epsilon^- - \phi^+\epsilon^+$, and ee is the enantiomeric excess obtained when the fraction x of **1** has been transformed. In the reported experiments, the factor $\bar{g}$ has the value 1.63 ± 0.04, i.e., *3 orders of magnitude* higher than Kuhn's dissymmetry factor for **1**. With the above $\bar{g}$ value and with measured ϵ^+ and ϵ^-, at 365 nm, we calculate the ratio $\phi^+/\phi^- = 0.50$. Thus, BSA–substrate interactions also differently change the excited-state reactivity of the antipodes, doubling that of (−)-**1** with respect to that of (+)-**1**.

An experiment sheds light on the origin of the above-described astonishing capability of the protein to discriminate. In fact, when (−)-**1** is dissolved in aqueous CH_3CN, at pH > 9, we measure UV and CD spectra similar to that of (−)-**1** in SI, i.e., with an intense band in the 355–420-nm range. Accordingly, it is reasonable to deduce that the acidic naphtholic moieties of (−)-**1** in the SI site are in a basic environment not accessible to the (+)-**1** antipode and manifest spectral features typical of anionic forms.

If the above simple interpretation is correct, we expect novel applications, also without intervention of light, of chiral recognition by biological macromolecules, particularly by serum albumins, when acidic or basic substrates, for which strong binding sites are known to be active, are involved.

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Registry No. (+)-1,1′-bi-2-naphthol, 18531-94-7; (−)-1,1′-bi-2-naphthol, 18531-99-2.

(3) Zandomeneghi, M., to be published.
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(5) In each experiment, 5 mL of (±)-**1**/BSA solution, at 10 °C under N_2, was irradiated with the 365-nm emission of a filtered, medium-pressure Hg lamp. Diethyl ether extraction from the irradiated mixtures and HPLC (RP18 column, CH_3CN/H_2O, 70/30 v/v) purification gave (+)-**1** of the reported ee. The optical purity (OP)/ee of samples was measured both with CD/UV, in comparison with samples (OP > 99%) from Aldrich, and with chiral HPLC analysis (TC-DNBPG column), with coincident results. Recovered BSA, after dialysis, was indistinguishable from original BSA as indicated by UV spectra, CD spectra, and new complexation experiments with **1**.

Single-Stranded DNA as a Target for Triple-Helix Formation

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Two strategies have been developed to selectively control gene expression by way of oligonucleotides targeted to nucleic acids. In the "antisense" strategy the oligonucleotide binds to a complementary sequence on messenger RNAs thereby inhibiting translation. In the "antigene" strategy the oligonucleotide recognizes a sequence of double-stranded DNA and forms a triple helix which is expected to block transcription and/or replication (for review, see ref 1). Here we demonstrate that an oligonucleotide can form a stable *triple helix* on a *single-stranded DNA* sequence by forming both Watson–Crick and Hoogsteen hydrogen bonds with the target sequence. In addition, covalent attachment of an intercalating agent to the 5′-end of the oligonucleotide

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(1) Hélène, C.; Toulmé, J. J. *Biochim. Biophys. Acta* **1990**, *1049*, 99–125.

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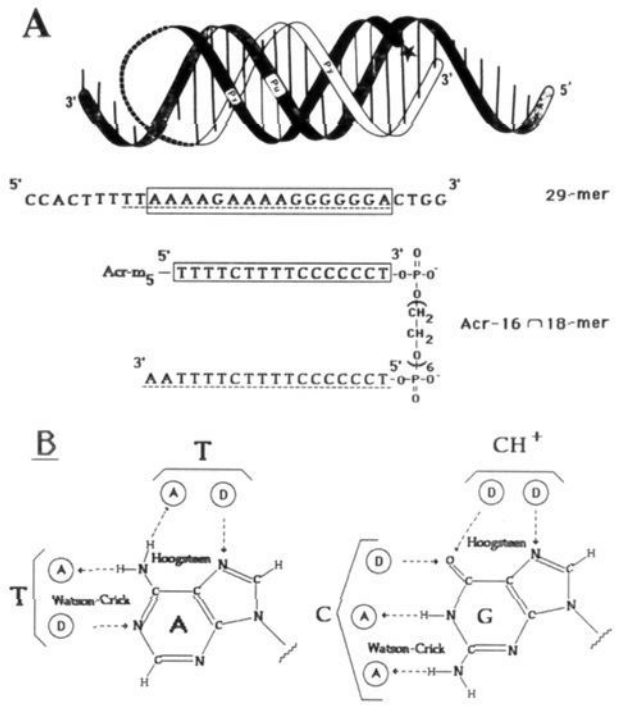

Figure 1. (A) Top: Schematic representation of the triple helix formed by a single-stranded nucleic acid containing a homopurine stretch with a dimeric oligonucleotide whose two sequences are linked by a polyethyleneglycol linker (see sequences below). The star indicates the site of attachment of the intercalating agent (5′-end of the Hoogsteen-bonded strand) Bottom: Sequence of the 29-mer single-stranded DNA fragment used as a substrate for binding of the oligonucleotides shown below. The underlined bases form Watson–Crick base pairs while the boxed sequences are engaged in Hoogsteen hydrogen bonding interactions. Acr indicates 2-methoxy-6-chloro-9-aminoacridine attached to the 5′-phosphate via a pentamethylene linker (m_5) as described in ref 2. The synthesis of 16∩18-mers was achieved on a Pharmacia automatic synthesizer using phosphoramidite chemistry as described in ref 6. (B) Hydrogen-bonding sites on adenines and guanines of the homopurine stretch engaged in interactions with bases of the dimeric oligonucleotide. The encircled A and D represent hydrogen-bond-acceptor and -donor groups, respectively. Adenine interacts with two thymines (C(4)=O and N(3)—H). Guanine interacts with a cytosine (three Watson–Crick hydrogen bonds involving C(2)=O, N(3), NH_2(6)) and a protonated cytosine (two Hoogsteen hydrogen bonds involving NH(3) and NH_2(6)).

allowed us to obtain a completely "locked" system.

The target chosen in these experiments is a 29-nucleotide-long single-stranded DNA fragment containing a stretch of 16 purines (Figure 1). We synthesized an oligonucleotide consisting of two parts, 18 and 16 nucleotides in length, connected by a hexaethyleneglycol bridge linking the 3′-phosphate of the 16-mer to the 5′-phosphate of the 18-mer (16∩18-mer, Figure 1). The sequences were chosen in such a way that the 18-mer forms Watson–Crick base pairs with the 29-mer and the 16-mer could form Hoogsteen hydrogen bonds with 16 of the 18 base pairs (see Figure 1). Two oligonucleotides made of 18 and 16 nucleotides, respectively, were used as controls. An acridine derivative (2-methoxy-6-chloro-9-aminoacridine) was covalently linked to the 5′-end of the 16-mer and 16∩18-mer oligonucleotides as described.[2] We previously showed that a triple helix was strongly stabilized when the third-strand oligonucleotide carried an intercalating agent attached to its 5′-end.[3] The 18-mer sequence in 16∩18-mer was chosen in such a way that an intercalation site was created in the Watson–Crick double helix for the acridine derivative attached to the 5′-end of the 16-mer third strand.

Complex formation between the 29-mer and the oligonucleotides described in Figure 1 was followed by the temperature dependence

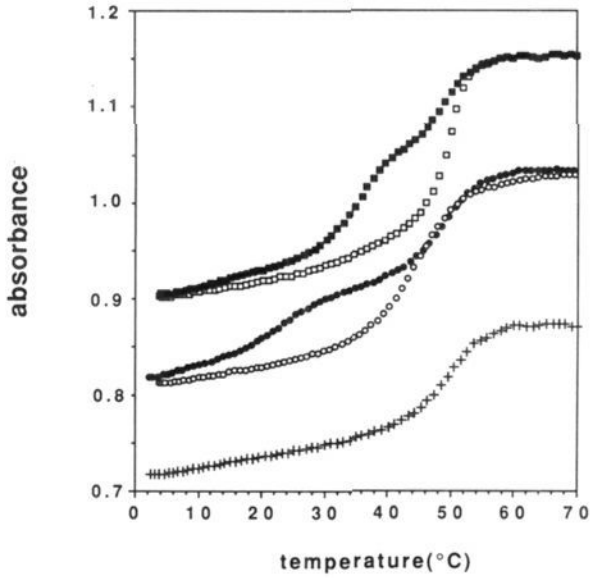

Figure 2. Transition curves obtained by measuring absorbance at 258 nm as a function of temperature. The rate of temperature variation was 0.15 °C/min. For the duplex, a slight excess of the 29-mer strand was used (1.9 μM) in order to ensure complete binding of the 18-mer (1.6 μM). A duplex solution at 1.6 μM concentration was then used for triplex formation with the 16-mer (1.3 μM) and Acr-16-mer (1.5 μM). Triplex formation by 16∩18-mer (1.3 μM) and Acr-16∩18-mer (1.5 μM) with the 29-mer (1.9 μM) was also followed at 258 nm. The buffer contained 11 mM sodium cacodylate at pH 6.0 and 100 mM NaCl. (+): 29-mer + 18-mer (abbreviated as D below) (T_m = 48 °C). ●: D + 16-mer (T_m = 20 °C and 48 °C). ■: D + Acr-16-mer (T_m = 34.5 °C and 48 °C). ○: 29-mer + 16∩18-mer (T_m = 42 °C). □: 29-mer + Acr-16∩18-mer (T_m = 49 °C). The first T_m value for D + 16-mer or D + Acr-16-mer corresponds to dissociation of the third strand from the duplex D; the second T_m value corresponds to melting of the duplex. The T_m values for 16∩18-mer and Acr-16∩18-mer refer to 50% hyperchromism of the single transition. Two curves (□ and ■) were translated in order to make the figure clearer.

of the ultraviolet absorption measured at 258 nm (Figure 2). 29-Mer and 18-mer formed a duplex containing 18 Watson–Crick base pairs. When this double-stranded DNA fragment was mixed with 16-mer or Acr-16-mer, two transitions were observed. The transition occurring in the upper temperature range (around 50 °C) was observed in the absence of any 16-mer oligonucleotide and was attributed to the thermal dissociation of the 18 Watson–Crick base pairs of the duplex. The transition in the lower temperature range was not observed in the absence of the 16-mer and was ascribed to the thermal dissociation of a triple-helical complex formed by the 16-mer oligopyrimidylate with the double-stranded DNA target.

Triple-helix formation was then analyzed when the single-stranded 29-mer target was mixed with 16∩18-mer. Figure 2 shows the corresponding melting curves. *A single transition* was observed, the amplitude of which was approximately the sum of the amplitudes for dissociation of 16-mer from the (29-mer + 18-mer) duplex and that attributed to the breakage of the Watson–Crick base pairs formed by the association of 29-mer with 18-mer. This result proved that both Watson–Crick and Hoogsteen hydrogen bonds formed between the 29-mer and 16∩18-mer dissociated in the same temperature range whereas two separate transitions were observed when the 16-nt and 18-nt oligomers were not covalently linked to each other. Attachment of the acridine derivative to the 5′-end of 16-mer and 16∩18-mer stabilized both triple helices, the one formed by three separate strands (29-mer + 18-mer + Acr-16-mer) and the other one formed by two single-stranded oligomers (29-mer + Acr-16∩18-mer). This result is in agreement with triple-helix stabilization previously observed when an oligonucleotide–intercalator conjugate was bound to the major groove of double-stranded DNA.[3]

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The influence of various parameters on triple-helix formation was examined. Addition of polyamines, such as spermine, at constant NaCl concentration, stabilized the triple helix as previously reported.[3,4] An increase in sodium chloride concentration, at constant spermine concentration, disfavored complex formation as shown in previous reports.[3,5] A pH decrease favored triplex formation as expected due to the requirement for cytosine protonation to form two Hoogsteen hydrogen bonds with a G·C Watson–Crick base pair (results not shown).

The present study shows that it is possible to form a stable complex on a single-stranded nucleic acid by an oligonucleotide capable of forming both Watson-Crick and Hoogsteen hydrogen bonds with a homopurine sequence. Most of the hydrogen-bonding sites on the purine bases of the target sequence are involved in complex formation: four hydrogen bonds are formed with A and five with G (Figure 1B). In addition we have shown that an intercalating agent attached to the 5′-end of the dimeric oligonucleotide strongly stabilizes the complex when the oligonucleotide is chosen such that the Watson–Crick sequence extends over a few base pairs (two in our study) outside the triple-helix-forming region. Formation of a triple-stranded structure on a single-stranded nucleic acid such as messenger RNA or viral RNA or DNA might prove more efficient to arrest translation, reverse transcription, or replication than double-helix formation.

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Stereochemical and Mechanistic Studies of CDP-D-glucose Oxidoreductase Isolated from *Yersinia pseudotuberculosis*

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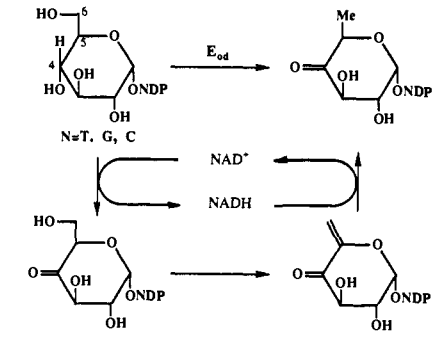
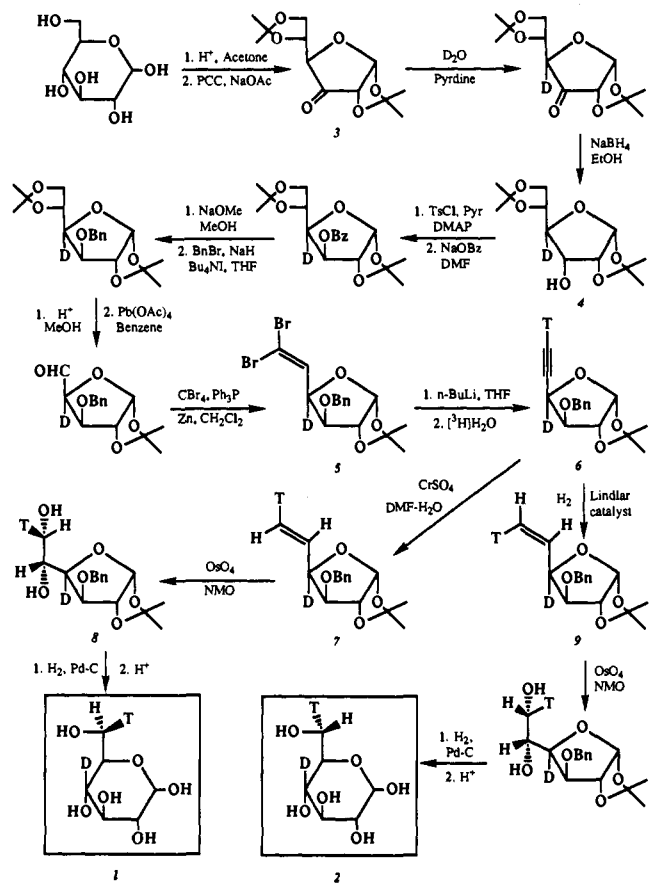
The great majority of deoxy sugars are 6-deoxyhexoses, which are commonly encountered as components of bacterial antigens and are also found widely in plant and animal tissues.[1] Studies of several naturally occurring deoxy sugars have shown that the first step unique to their formation involves the conversion of a nucleoside diphosphohexose to the corresponding 4-keto-6-deoxyhexose mediated by an oxidoreductase.[2] As depicted in Scheme I, studies of TDP-D-glucose[2] and GDP-D-mannose oxidoreductase[3] established that the reaction proceeds with an oxidation at C-4, followed by a dehydration between C-5 and C-6 and then a reduction at C-6.[4] An active-site-bound NAD^+ in these enzymes serves as a hydride carrier which mediates the internal hydrogen transfer from C-4 to C-6.[2] The stereochemistry of the displacement at C-6 of this intramolecular oxidation–reduction has been shown to occur with inversion.[5] However, study of the biosynthesis of 3,6-dideoxyhexoses in *Pasturella pseudotuberculosis*[6] led to the isolation of a CDP-D-glucose oxidoreductase which is readily resolved from its cofactor by simple purification steps.[6] The absolute requirement of NAD^+ for activity makes this enzyme fundamentally distinct from most of its counterparts[7] and thus throws into doubt whether its catalysis still follows the mechanism shown in Scheme I. As part of our efforts to study the biosynthesis of 3,6-dideoxyhexoses,[8] we have recently

Scheme I



Scheme II



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(4) Exemplified in Scheme I is the reaction mechanism of a generic nucleoside diphosphate-D-glucose oxidoreductase. NDP represents the nucleoside diphosphate group.
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(7) The other exception of this class enzyme is a TDP-D-glucose oxidoreductase isolated from *Saccharopolyspora erythraea*, which also requires added NAD^+ for activity (Vara, J. A.; Hutchinson, C. R. *J. Biol. Chem.* **1988**, *263*, 14992).

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