thylsilyl)acetamide (366 mg, 1.80 mmol), dimethyl malonate (238 mg, 1.80 mmol), and KOAc (3.0 mg, 0.03 mmol) were added successively. The reaction mixture was stirred for 14 h and was worked up in the usual manner to afford the desired product **11** as a colorless oil (174 mg, 90 % yield) after purification by flash chromatography (pentane/ethyl acetate 6/1). The enantiomeric excess (73 % *ee*) was determined by HPLC (Daicel Chiralcel OD column, 0.4 mL min^{-1}, heptane/2-propanol 99/1, UV detection at 254 nm).

Typical procedure for the enantioselective deprotonation: Preparation of **17a**: A Schlenk-flask was charged with a solution of **14** (236 mg, 0.47 mmol) in dry THF (10 mL) under argon. The solution was cooled to $-78\,^{\circ}$C and *n*BuLi (1.60 M in hexane, 0.58 mL, 0.94 mmol) was added dropwise. The reaction mixture was allowed to warm to $0\,^{\circ}$C over 15 min, and was stirred for another 15 min at this temperature. The reaction mixture was rapidly cooled to $-100\,^{\circ}$C and chlorotrimethylsilane (0.24 mL, 1.89 mmol) was added dropwise. After the reaction mixture had stirred for 2 min at this temperature a solution of 4-*tert*-butylcyclohexanone (58 mg, 0.38 mmol) in dry THF (0.8 mL) was added dropwise over 5 min. After 50 min triethylamine (2 mL) was added, followed by a saturated solution of $NaHCO_3$ (2.5 mL). The reaction mixture was warmed to room temperature, extracted with diethyl ether, and the organic phase washed with water (3×15 mL). The combined aqueous phase was extracted with diethyl ether (20 mL), and the organic phase was dried over Na_2SO_4. The crude reaction mixture obtained after evaporation of the solvents was subjected to Kugelrohr distillation ($150\,^{\circ}$C, 10^{-3} bar). This afforded the desired product **17a** (90 mg, 85 %) in 87 % *ee* (GC analysis: Chirasil-DEX CB (Chrompak), carrier gas H_2 (100 kPa), $80\,^{\circ}$C (1 min) $\rightarrow 120\,^{\circ}$C, *T* gradient: $2\,^{\circ}$C min^{-1}: t_R = 20.5 (*S*), 20.8 (*R*) min). Any urea **14** (205 mg, 87 %) recovered by distillation was reused in further reactions.

Typical procedure for the enantioselective alkylation: Preparation of **20**: A Schlenk flask was charged with a solution of **14** (505 mg, 1.00 mmol) in dry THF (7 mL) under argon. The solution was cooled to $-78\,^{\circ}$C and *n*BuLi (1.50 M in hexane, 0.67 mL, 1.00 mmol) was added dropwise. The reaction mixture was allowed to warm to $0\,^{\circ}$C over 15 min, and was stirred for another 15 min at this temperature. The reaction mixture was allowed to cool to $-40\,^{\circ}$C, and a solution of α-tetralone (120 μL, 0.90 mmol) in dry THF (2 mL) was added dropwise over 2 min. The reaction mixture was warmed to room temperature over 20 min and stirred for another 30 min. The reaction mixture was then cooled to $-78\,^{\circ}$C, and a solution of benzyl bromide (1.20 mL, 10.1 mmol) in dry THF (2 mL) was added dropwise over 15 min. The reaction mixture was allowed to warm to $-20\,^{\circ}$C and stirred for 28 h at this temperature. The reaction mixture was quenched with aqueous hydrochloric acid (1 M, 5 mL), and after warming to room temperature the reaction mixture was extracted with diethyl ether (80 mL) and the organic phase was washed successively with water (2×10 mL) and brine (10 mL). The combined aqueous phase was extracted with diethyl ether (10 mL) and the organic phase was dried (Na_2SO_4). Purification by flash chromatography (pentane/diethyl ether 3.5/1) afforded the desired product **20** as a colorless crystalline solid (180 mg, 83 %, m.p. $54\,^{\circ}$C). The enantiomeric excess was determined by HPLC as 81 % *ee* (Daicel Chiralcel OD column, 0.6 mL min^{-1}, heptane/2-propanol 99.5/0.5, UV detection at 254 nm).

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A New Strategy for the Destabilization of Double-Stranded Nucleic Acids by Phenylalkylamine Derivatives**

Anmar Ali, Martin Gasiorek, and Hans-Jörg Schneider*

The unwinding of double-stranded (ds) nucleic acids is an important process for the biological functions of DNA and RNA. Until now, most of the organic ligands studied stabilize the double-stranded form of nucleic acids,[1] which is characterized by an increase in the thermal denaturation temperature T_m. Only with high concentrations of organic solvents, copper salts,[2] and with some steroidal amines[3] has a decrease in the melting point been reported; stronger destabilization effects are accompanied by DNA precipitation.[4] Kimura et al. found that zinc complexes could desta-

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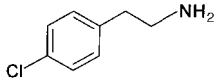
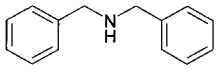
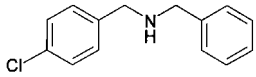
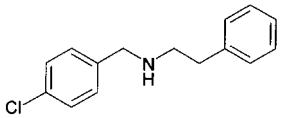
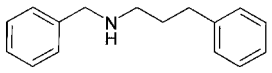
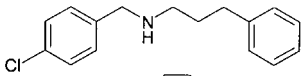
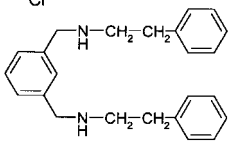
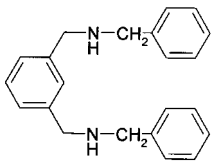
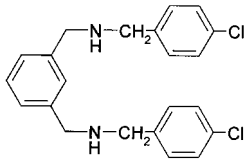
bilize ds poly(A:U), but could stabilize poly(dG:dC).[5] Less attention has been focused until now on the design of agents that can destabilize and unwind nucleic acid helices, although unpaired bases, particularly in RNA, are becoming an increasingly important target for new diagnostic and therapeutic strategies.[6] We describe here a new way to destabilize folded nucleic acid conformations by stabilizing unduplexed parts of the polymer, or single-stranded (ss) forms, which lead to destabilization effects of hitherto unknown magnitude with concentrations as low as 50 μM. The ligands chosen contain aromatic units that are not able to intercalate into ds nucleic acids, but are well suited for base stacking with ss nucleic acids. The new structural principles may interfere with replication and transcription and, therefore, may provide new lead compounds of medicinal interest.

Synthetic ligands that are designed to bind more strongly to ss than to ds nucleic acids should avoid large numbers of positive charges, as these bind and stabilize the duplex form. Moreover, they should contain aromatic units that can insert only between the flexible ss nucleic acid bases.[7] Since NMR spectroscopic measurements[8] have established that, in contrast to earlier assumptions,[9] the corresponding phenylalkylamines do not show the unwanted intercalation into ds forms, we have prepared ligands in which several phenyl rings are separated by spacers that allow bisintercalation-type binding into ss forms. Such compounds resemble building blocks of some proteins that bind to single-stranded nucleic acids.[10]

All compounds prepared with the strategy of having properly spaced phenyl rings and little positive charge indeed do cause destabilization of nucleic acid duplexes (Table 1), with a strong dependence on the spacers as well as on the substituents of the phenyl rings (Figure 1). The stacking interaction with a single phenyl ring is not sufficient to cause a significant reduction in the melting temperature, as seen with ligand **1**. The destabilization of the double strand becomes significant only if two phenyl rings are present, and only if

Table 1. Melting point changes ΔT_m [°C] of CT DNA complexed with various ligands.[a]

Compound		r[b] 0.26	0.52	0.78	1.3	2.6	3.9	6.5	13	26	39
1		–	–	–	–	–	–	–	+1	+1.3	+2
2		–	–	–	–	–	–	–	+1	+1	−0.3
3		–	–	–	–	–	–	–	−0.3	−0.3	−0.3
4		–	–	–	–	–	–	–	−0.4	−0.2	−21
5		–	–	–	–	–	–	–	−0.2	−0.8	−0.4
6		–	–	–	–	–	–	–	−0.5	−26	–[c]
7		–	–	–	+1	−17	−22	−28	−35		
8		–	–	–	–	–	–	−10	−17	−22	−24
9		+3	−18/+3	−28							

[a] Measurements with CT-DNA in MES buffer (MES = 2-(*N*-morpholino)ethanesulfonic acid) at pH 6.25; DNA base concentration 7.7×10^{-5} M. Absorption changes were followed at 260 nm as a function of temperature with a BioCary-1 spectrometer. The amines are fully protonated at the given pH. An average error of ΔT_m was determined as ±0.5 °C (duplicate runs). Values at other ligand concentrations could not be measured accurately as a result of the complex melting at too low a temperature or because of solubility problems. [b] Molar ratio of ligand to nucleic acid phosphate. [c] No deflection in the curve was detectable.

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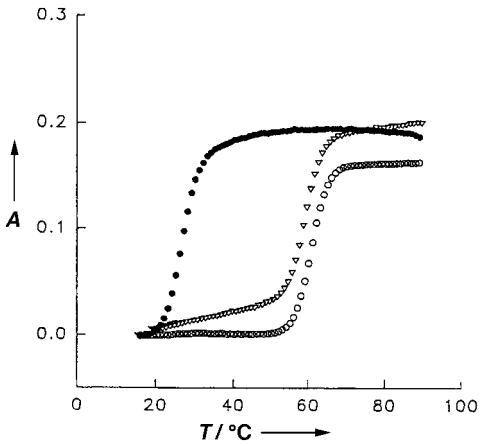

Figure 1. DNA melting curves of DNA alone (○), with **5** (▽), and with **6** (●); the molar ratio of ligand to nucleic acid phosphate is 1:26. The conditions are given in the footnote to Table 1.

they are arranged at a suitable distance by suitable spacers (Table 1). Higher ligand concentrations lead to complete denaturation of the nucleic acid duplex. The melting experiments suggest that the structure of the spacer between the phenyl rings must allow as far as possible strain-free insertion between neighboring nucleobases of single strands, or—in metastable, partially unfolded polymers—simultaneous insertion between two stacking base pairs. This will lead to substantial energies of complexation and, therefore, stabilization of unfolded nucleic acids. In line with this, short spacers as in structure **2** or **3** show less, or no melting point decrease. Remarkably, compound **4** with four atoms between the phenyl rings has a relatively large effect in contrast to the homologue **5** with a five atom spacer. However, introduction of chloro substituents has an even more dramatic influence on the destabilization of ds nucleic acids, as seen with ligand **6.** To enhance the affinity towards ss nucleic acids ligands **7** to **9** were designed with the help of computer-aided molecular modeling. These clamplike ligands provide a more convergent orientation of two phenyl rings toward a nucleobase of the single strand. Figure 2 demonstrates that compounds such as **9** indeed allow the stacking of a nucleobase without significant steric distortions. This ligand has by far a much stronger destabilizing effect than any other compound reported until now; a comparison of the results obtained with ligands **8** with **9** shows that this destabilization is clearly the result of the chlorine substituent.

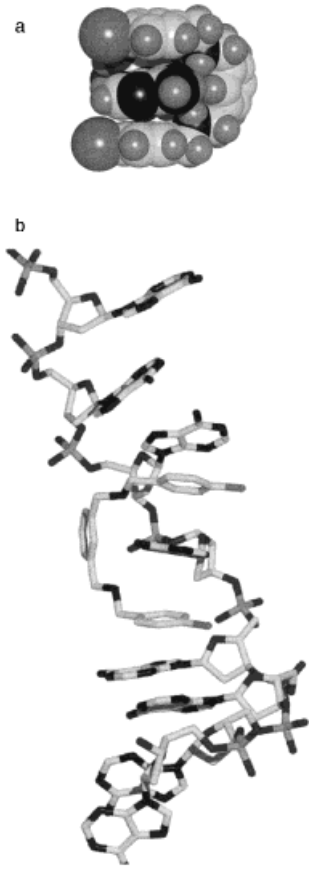

Figure 2. Computer-simulated (CHARMm energy minimization) stacked complex between ligand **9** and AMP (a) and of the intercalation of ligand **9** into a single-stranded poly(dA) strand (b).

Evidence for the mechanism of intercalation into single strands is provided not only by the dependence of T_m on the ligand structure. The NMR spectra of diphenylalkane compounds such as **6** with thermally denatured calf thymus DNA (CT-DNA) shows upfield shifts of the arene signals that are typical[8, 11] for exposure of the aryl protons to the anisotropy cone of the nucleobases (Figure 3). In contrast, the same

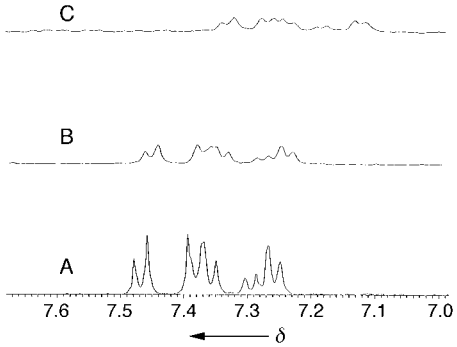

Figure 3. ^{1}H NMR spectra (400 MHz, 0.02 M sodium phosphate in D_2O, pD 7.4, sodium salt of trimethylsilylpropionic acid as an internal reference) of the aromatic region of ligand **6** (A), and its complexes with ds CT-DNA (B) and denatured CT-DNA (prepared by incubating CT-DNA at 80 °C for one hour followed by fast cooling) (C); the molar ratio of ligand to nucleic acid phosphate was 0.3:1.

derivative shows no appreciable shielding differences in a complex with ds DNA (Figure 3). This behavior differs considerably from the corresponding complexes with naphthylalkylamines, which are duplex intercalators and exhibit substantially larger line broadening.[9, 12] The shielding effects with ss DNA are, in comparison to those observed with duplexes, diminished by fast exchange of different protons. They are, however, noticeably larger for ligands that exhibit larger decreases in T_m, which again points to the insertion into single strands or unfolded parts of the nucleic acids as the driving force for the duplex destabilization.

Circular dichroism (CD) spectra of ds CT-DNA at 25 °C show a pronounced decrease in the intensity of the DNA band upon titration with compound **9** (Figure 4). Single stranded DNA is known to exhibit Cotton effects with ellipticities close to that of the duplex as a consequence of base stacking being preserved.[12] The observed decrease in the extinction coefficient $\Delta\varepsilon$ upon addition of the ligands indicates they bind to single strands with concommitant destruction of internucleobase stacking and loss of helicity. Similar observations were reported by Hélène et al for complexes of *p*-hydroxyphenylethylamine with ss DNA.[13] In accordance with their small effect on T_m ligands such as **3** have little influence on the CD spectra of ds DNA. However, a $\Delta\varepsilon$ reduction of approximately 50% is seen for **9** with both ds and ss DNA at concentrations of 5×10^{-5} M for the ligand and the DNA base pair. A corresponding affinity of $2 \times 10^4\,M^{-1}$ is in line also with the concentration of about 5×10^{-5} M of **9** that is needed to lower the melting point of ds DNA by approximately half of

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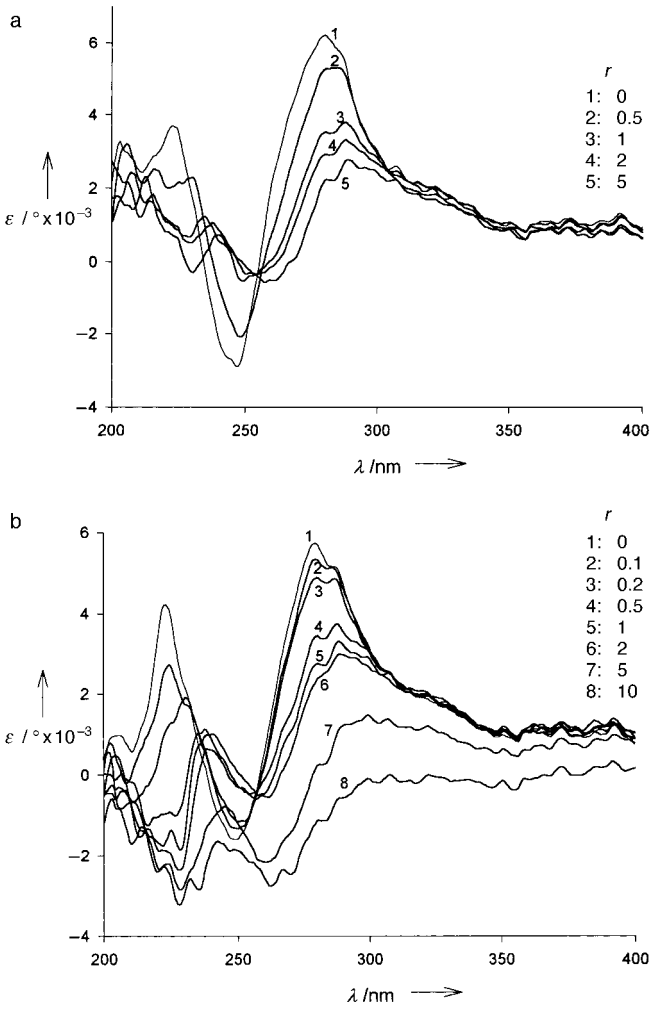

Figure 4. CD spectra of CT-DNA (see footnote to Table 1 for conditions); with ligand **9** at different ratios *r* to the nucleic acid phosphate (a) and thermaly denatured CT-DNA with ligand **9** at different ratios of ligand to nucleic acid phosphate (b). The CD spectra were obtained with a J-715 spectropolarimeter (JASCO) at 25 °C in cuvettes with a 1 cm path length. Curves presented are the average of three smothed scans. No Cotton effect was detected for the buffer.

the maximum value. An exact evaluation of the binding isotherms is difficult in view of the only approximately monophasic melting behavior and of solubility problems; with the latter also hampering NMR measurements.

The unpairing of double strands by base flipping is known to be involved in some protein–DNA interactions.[14] Only recently such a base flipping has also been achieved by a synthetic azoniacyclophane; on complexation with ds RNA the nucleobase becomes encapsulated in the cyclophane cavity.[15] In contrast to all these ligands the new phenylalkylamine derivatives are designed to interact preferentially with the ss form of nucleic acids and leave the duplex intact. The phenyl rings in the new ligands allow the introduction of a rich variety of substituents, which have a distinct influence on the observed duplex destabilization. Little is known about the dependence of the affinity towards single-stranded nucleic acids on the structure of the intercalating ligand. Studies with DNA duplexes do show appreciable variations in affinity upon substitution of the intercalating unit.[16, 17]

Agents that can unwind duplexes and bind selectively to unfolded nucleic acids can be the basis of potential antiviral and anticancer drugs. These compounds could disrupt RNA secondary structures such as hairpin stem–loop conformations, which are important recognition sites for gene regulatory proteins that control viral replication. Moreover, these compounds might be valuable in molecular biology methods such as sequencing or PCR (polymerase chain reaction), in which dissociation of folded nucleic acid parts is a necessary step.[18]

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[18] The synthesis of the new ligands will be reported elsewhere. All new compounds showed satisfactory analytical data.

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