via distortions, also periodic, from regular helix geometries.

Can a small geometric distortion of a helical secondary structure quantitatively explain the experimentally observed fairly large sequence-related variability of helix shifts, and also the different behavior of amide and H_α protons? The magnitude of helix shifts of backbone protons in short distorted helices is unknown, but can be computed using a recent protein chemical shift model.[12] On the basis of average crystal geometries of protein helices,[18] we computed the helix shifts of one regular straight and two distorted peptide helices. On the whole, the computed and the experimental helix shifts of model helices (Figure 2) are in close agreement and coincide in features such as the two helix periods (2 and 4), the relative magnitudes of the amide and H_α signals within each helix, and the magnitudes of helix shifts in the two distorted helices. Other less obvious features also appear in both computed and experimental data; e.g., the amide helix shifts of the first helical turn differ in size from those of the other two turns. This is probably because the first turn lacks the preceding peptide CO groups whose magnetic anisotropy strongly influences the δ values of the subsequent residues.[8,11,12] It is therefore a terminal rather than a distortion effect, and it appears also in the δ values computed for the regular Arnott's helix[18] (Figure 2a) that, interestingly, do not show other sequence dependent amide helix shift variations.

Despite having a hydrophobic moment $\mu = 0.03$, the alternate model helix shows sequence-related variability of its H_α and amide helix shifts in both the experimental and computed shift vs sequence profiles (Figure 2b,c). The experimental amide helix shifts of the second and third helical turns in both model helices are clustered more strongly in the positive range than in the computed ones (Figure 2c,e). These more positive shifts probably reflect the existence of intrahelix hydrogen bonds in the second and third turns that are shorter than the hydrogen bonds between first-turn residues and solvent molecules.

The excellent agreement between observed and computed helix shifts in Figure 2d,e supports that natural amphipathic helices in solution are bent so that the hydrophobic side has shorter hydrogen bonds, as observed in protein crystals.[17-19] Bending seems to be a property of such helices in polar solvents rather than a packing requirement of the tertiary structure, since many isolated amphipathic[20] protein helices show δ periodicity[3,4,15,16] and therefore curvature.

The differences involved in the geometries of distorted helices are extremely small (typically 0.1-0.2 Å) but still detectable by helix shifts. The main reason is the aromatic-like behavior of the peptide CO groups[11,12] whose magnetic anisotropy strongly and very selectively influences the δ values of nearby protons. Also amide δ values are extremely sensitive to hydrogen bond lengths.[2,8,12,21] Although agreement between experimental and computed amide δ values can be obtained[26] using a simple hydrogen bond length model of chemical shifts,[2] hydrogen bonds alone cannot explain the now well established sequence variability of helix shifts of the H_α and H_β protons[16] nor those of amide protons not intramolecularly hydrogen bonded. Such minor geometric differences would be very difficult to detect by conventional NOE-based structure refinements.

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Supplementary Material Available: One table and two figures showing the NMR chemical shifts, NOE summary, and NOESY spectrum of the Ac(Leu-Lys-Leu-Lys)$_3$-NH_2 model peptide in mixed TFE/H_2O solvent, details on chemical shift computation, experimental procedures, and additional references (6 pages). Ordering information is given on any current masthead page.

(26) Zhou, N. E.; Zhu, B. Y.; Sykes, B. D.; Hodges, R. S. *J. Am. Chem. Soc.* **1992**, *114*, 4320.

Recognition of Guanine and Adenine in DNA by Cytosine and Thymine Containing Peptide Nucleic Acids (PNA)[1,2]

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Reagents that recognize specific sequences in RNA or double-stranded DNA may be developed into drugs which can modulate gene expression[3] or they may be used as diagnostic or molecular biological probes. We have recently found that nucleic acid analogues, in which the entire phosphate-sugar backbone has been replaced with a backbone consisting of (2-aminoethyl)glycine units to which thymines are attached through methylenecarbonyl bridges (PNA, **1**, Figure 1), bind very efficiently to complementary DNA.[4] The PNA[5] was found to bind to the complementary oligodeoxynucleotides with a 2:1 stoichiometry, as expected for analogues with neutral backbones.[6] It was also observed that the binding of PNA reagents containing 10 T ligands to a $(dA/dT)_{10}$ target in a 248 base pair double-stranded DNA fragment took place with strand displacement, i.e., binding to the A-strand with displacement of the opposite T-strand.[7]

Obviously, it would be most exciting to extend this recognition to other bases, and we now report the incorporation of a second base, cytosine, which is shown to recognize its complementary base, guanine, with a 2:1 stoichiometry presumably involving both Watson-Crick and Hoogsteen base pairing.

PNA Synthesis. The cytosine monomer (Boc-C[Z]aeg-OH, **3**, Scheme I), was prepared in a manner analogous to the preparation of the thymine monomer, with the exception that it was equipped with a benzyloxycarbonyl (Z) protecting group. The fully protected Boc-T_4C[Z]T_5-Lys(Cl-Z)-benzhydrylamine resin was assembled by stepwise Merrifield synthesis[8,9] utilizing an improved solid-phase procedure, i.e., in situ DCC coupling (0.15 M in 50% DMF/CH_2Cl_2) instead of the previously reported pentafluorophenyl ester activation. All of the coupling steps proceeded with an efficiency of 98-100%. Deprotection and release of the free PNA, H-T_4CT_5-Lys-NH_2, from the resin were accomplished with anhydrous HF under standard conditions. The purified product was homogeneous by analytical HPLC and showed the expected molecular weight by fast atom bombardment mass spectrometry (found (calcd), 2792.21 (2792.14)). The positively charged lysine amide at the C-terminus was originally

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(1) Dedicated to John D. Rockefeller Jr. Professor Bruce Merrifield on the occasion of his 70th birthday.
(2) Standard oligopeptide nomenclature is used; see ref 4.
(3) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544-584.
(4) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895-1897.
(5) We have chosen to call this type of structure peptide nucleic acids (PNA), since the structure is a chimera of a peptide and a nucleic acid.
(6) Letsinger, R. L.; Bach, S. A.; Eadie, J. S. *Nucleic Acids Res.* **1986**, *14*, 3487-3499.
(7) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.
(8) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.
(9) Merrifield, B. *Science* **1986**, *232*, 341-347. (Nobel Lecture.)

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Table I

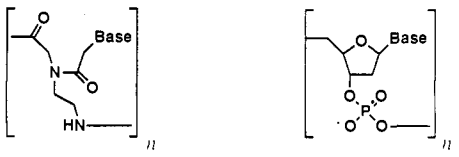
row no.	PNA oligomer	target	T_m,[a] °C	stoichiometry
1	H-T_{10}-Lys-NH2	5′-$(dA)_{10}$	73	2:1
2	H-T_{10}-Lys-NH_2	5′-$(dA)_4(dG)(dA)_5$	59	
3	H-T_4CT_5-Lys-NH_2	5′-$(dA)_4(dG)(dA)_5$	74	2:1
4	H-T_4CT_5-Lys-NH_2	5′-$(dA)_{10}$	54	
5	H-T_4CT_5-Lys-NH_2	5′-$(dA)_4(dT)(dA)_5$	43	
6	H-T_4CT_5-Lys-NH_2	5′-$(dA)_4(dC)(dA)_5$	46	
7	H-T_4CT_5-Lys-NH_2	5′-$(dA)_5(dG)(dA)_4$	75	2:1

[a] The melting temperatures of the hybrids were determined on a Gilford Response apparatus. The following extinction coefficients were used: A, 10.8; T, 8.8; c, 7.3; G, 11.7 mL/μmol-cm for both oligodeoxynucleotides and PNA. The solutions were 10 mM in phosphate, 140 mM in NaCl, and pH 7.2. Approximately 0.3 OD_{260}/mL of the PNA oligomer was hybridized with 1 equiv of the other strand (using the extinction coefficients above for both PNA and DNA) by heating to 90 °C for 5 min, cooling to room temperature, and storing for 30 min followed by storage at 5 °C for at least 30 min. The melting curves were recorded in steps of 0.5 °C/ca. 0.5 min. The T_m values were determined from the maximum of the first derivative of the plot of A_{260} versus temperature.

Table II. T_m Values as a Function of pH

row no.	PNA oligomer	target	pH	T_m,[a] °C	stoichiometry
1	H-T_{10}-Lys-NH2	5′-$(dA)_{10}$	5.0	71	
2	H-T_{10}-Lys-NH_2	5′-$(dA)_{10}$	7.2	73	2:1
3	H-T_{10}-Lys-NH_2	5′-$(dA)_{10}$	9.0	71	
4	H-T_4CT_5-Lys-NH_2	5′-$(dA)_4(dG)(dA)_5$	5.0	80	2:1
5	H-T_4CT_5-Lys-NH_2	5′-$(dA)_4(dG)(dA)_5$	7.2	74	2:1
6	H-T_4CT_5-Lys-NH_2	5′-$(dA)_4(dG)(dA)_5$	9.0	71	2:1

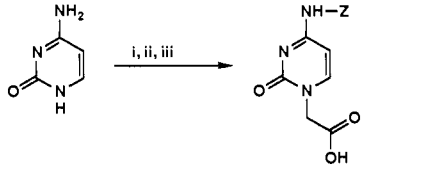
[a] See footnote to Table I.



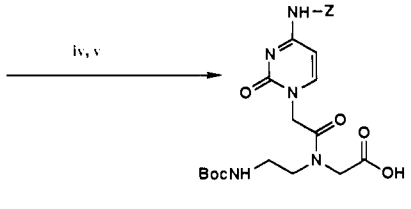
PNA (1) DNA (2)

Figure 1.

Scheme I[a]



4 5



3

[a] (i) Z-Cl/pyridine at 0 °C. (ii) $BrCH_2CO_2CH_3/K_2CO_3$ in DMF at room temperature. (iii) Aqueous NaOH. (iv) BocNH-$(CH_2)_2NHCH_2CO_2C_2H_5$/DCC/DhbtOH in DMF/CH_2Cl_2 at 0 °C. (v) LiOH in THF/water at 0 °C.

introduced in order to suppress the tendency for aggregation in thymine PNA oligomers. The inclusion of C appears to significantly diminish this problem. The lysine amide has, however, been retained in order to compare these experiments with the ones previously reported.

DNA Recognition. Melting curves of hybrids between H-T_4CT_5-Lys-NH_2 and complementary and noncomplementary oligodeoxynucleotides were determined. The hybrids showed well-defined single-phased melting profiles. The T_m values are listed in Table I (values for hybrids between H-T_{10}-Lys-NH_2 and oligodeoxynucleotides are listed for comparison).

Introduction of a guanine residue in the DNA strand (Table I, row 2) caused a lowering in the T_m compared to that of the H-T_{10}-Lys-NH_2/$(dA)_{10}$ hybrid of 13 °C, as expected when introducing a mismatch. Introduction of a cytosine at the complementary site in the PNA increases the T_m to 74 °C (Table I, row 3), whereas introduction of other mismatches opposite the cytosine moiety led to significant decreases in the T_m (Table I, rows 4–6).

The stoichiometry of the H-T_{10}-Lys-NH_2/$(dA)_{10}$ hybrid was found to be 2:1,[4] and since cytosine can participate in both Watson–Crick and Hoogsteen base pairing, the H-T_4CT_5-Lys-NH_2/5′-$(dA)_5(dG)(dA)_4$ hybrid was also expected to have 2:1 stoichiometry, as determined by UV–titration curves. Hoogsteen base pairing requires a protonated cytosine, and consequently the T_m of the hybrid should be sensitive to changes in pH if Hoogsteen base pairing is involved in the recognition. The data in Table II clearly demonstrate such a pH dependency, i.e., higher T_m at low pH (Table II, rows 4–6). UV–titrations showed that the stoichiometry was 2:1 at pH 5 and 7 and even at pH 9 where the cytosine is not expected to be protonated.[10] It is furthermore seen (Table I, rows 3 and 7) that the orientation does not seem to play a significant role in this case. From the present data it cannot be determined whether the two PNA strands are in a parallel or an antiparallel orientation in the 2:1 complex.

Conclusion. It has been demonstrated that it is possible to incorporate cytosine into a PNA oligomer. The results further demonstrate that cytosine in PNA specifically recognizes guanine in DNA. Experiments elucidating the specificity against double-stranded DNA of cytosine containing PNAs will be reported in due time. Work with more complex sequences is expected to reveal the relative stability of 2:1 and 1:1 complexes, as well as any directional preferences. Experiments are currently underway toward incorporating the purines as well as incorporating more cytosines in the same PNA oligomer.

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(10) Hanvey, J. C.; Shimizu, M.; Wells, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6292–6296.