Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1983–1992

BIOORGANIC & MEDICINAL CHEMISTRY

A 'Retro–Inverso' PNA: Structural Implications for DNA and RNA Binding

Achim H. Krotz,[a,*] Sine Larsen,[c] Ole Buchardt,[a,†] Magdalena Eriksson[b] and Peter E. Nielsen[b,‡]

[a]*Center for Biomolecular Recognition, Chemical Laboratory II, The H. C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark*

[b]*Center for Biomolecular Recognition, IMBG, Department. of Biochemistry B, The Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N*

[c]*Centre for Crystallographic Studies, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark*

Received 5 March 1998; accepted 21 May 1998

Abstract—'Retro–inverso' peptide nucleic acid (PNA) monomers of thymine (T*: *N*-(amidomethyl)-*N*-(N^1-thyminylacetyl)-β-alanyl) (and adenine) have been prepared and introduced in PNA oligomers. A homo 'retro–inverso' T^*_8 PNA was found not to hybridize to a complementary DNA or RNA oligonucleotide, whereas introduction of one retro–inverso thymine unit into the middle of a normal PNA 15-mer resulted in a ca. 8 °C destabilization of the complex of this oligomer with a complementary DNA or RNA oligomer. In an effort to compensate for the structural nucleobase 'phase-shift' caused by the T* monomer by also introducing a β-alanine monomer it is concluded that the effect of the T* backbone is −7 °C when hybridizing to DNA and −4.5 °C when hybridizing to RNA. Nonetheless, the T* unit shows good sequence discrimination comparable to that of normal PNA. Molecular dynamics simulations indicate an unfavourable conformation of the backbone amide carbonyl group resulting in reduced interaction with the aqueous medium and an 'electrostatic clash' with the carbonyl of the nucleobase linker. These results show that a simple inversion of an amide bond in the PNA backbone has a dramatic, and hardly predictable, effect on the DNA mimicking properties of the oligomer.

Introduction

Molecules that bind mRNA or double-stranded DNA with high affinity and sequence specificity represent promising drug candidates in the antisense and antigene therapy, and may also provide new tools in molecular biology and diagnostics.[1–4] In this context we recently described the remarkable hybridization properties of peptide nucleic acids (PNA) **A** (Scheme 1), a novel class of nucleic acid binding DNA analogues with an achiral and non-charged pseudo-peptide backbone consisting of *N*-(2-aminoethyl) glycinyl units to which the nucleobases are attached via methylenecarbonyl linkers.[5–11] PNA oligomers containing the four natural nucleobases are conveniently synthesized from monomeric building units by Merrifield solid-phase methodology.[10–12] Mixed purine–pyrimidine PNAs hybridize to complementary DNA oligomers in a sequence-specific manner by formation of double-helices obeying the Watson–Crick base-pairing rules,[9] whereas homopyrimidine PNAs bind to complementary homopurine DNA sequences with a 2:1 stoichiometry through $(PNA)_2$/DNA triplex formation by both Watson–Crick and Hoogsteen base-pairing.[13,14] Furthermore, such homopyrimidine PNAs bind to double stranded DNA by duplex invasion.[5,6]

Key words: Nucleic acid; hybridization; antisense; peptide nucleic acid.

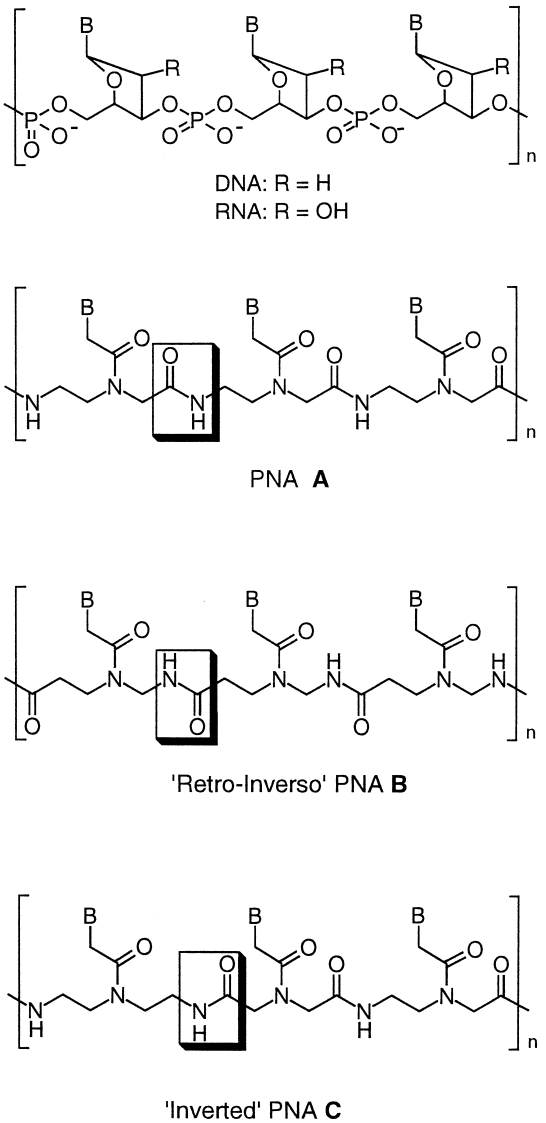
*Present address: Isis Pharmaceuticals, 2292 Faraday Av., Carlsbad, CA 92008, USA.

†The late.

‡Corresponding author. Tel.: 45 35 32 77 62; fax: +45-35396042; e-mail: pen@imbg.ku.dk

Abbreviations used: T: *N*-(amidoethyl)-*N*-(N^1-thyminylacetyl) glycinyl (n = 2, m = 1) β-ala-T: *N*-(amidoethyl)-*N*-(N^1-thyminylacetyl)-β-alanyl (n = 2, m = 2), T*: *N*-(amidomethyl)-*N*-(N^1-thyminylacetyl)-β-alanyl (n = 1, m = 2)

0968-0896/98/$ - see front matter
PII: S0968-0896(98)00152-7



Scheme 1. Upper, peptide nucleic acid (PNA **A**) with the original *N*-(2-aminoethyl) glycine backbone; middle, 'retro–inverso' PNA **B** with the *N*-(aminomethyl) β-alanine backbone; and lower, 'inverted' PNA **C**. Note the different positioning of polar groups on the backbone, while the number of bonds along the backbone between the nucleobases is kept constant. B = nucleobase.

In vitro studies have demonstrated both good antisense and antigene activity of PNAs.[15–21]

In order to understand the structural requirements for high affinity binding of nucleic acid analogs with pseudo-peptide backbones more fully, we have engaged in an investigation of the factors which may contribute to the stability of PNA–DNA hybrids. Significantly lower thermostability (T_m) of hybrids of DNA with PNA oligomers with extended backbones (e.g. *N*-(amidoethyl) β-alaninyl) suggested that the interbase distance (six bonds along the backbone) in PNA **A** is best suited for binding to a DNA target.[22] The interchanging of the positions of backbone methylene and side chain carbonyl group has been found to abolish any interactions with DNA,[23] and reduction of the linker amide likewise was found to be detrimental to the hybridization properties.[24] However, interchanging the position of one carbonyl group of the backbone with a methylene group from the backbone ('inverted amide', PNA C) did not affect the T_m of PNA–DNA hybrids significantly.[25]

As a logical extension of the work toward delineating the structural elements which are responsible for high binding affinity of nucleic acid analogs, we have recently designed a 'retro–inverso' structural isomer (PNA **B**) of the original aminoethyl-glycine (PNA **A**) (Scheme 1).[26,27] In this PNA isomer two of the presumed key elements present in the original aminoethyl glycine PNA backbone are retained; interbackbone distances (6 bonds along the backbone and 3 bonds from the backbone to the nucleobase) as well as restrained flexibility imposed by the two 'planar' amide groups.

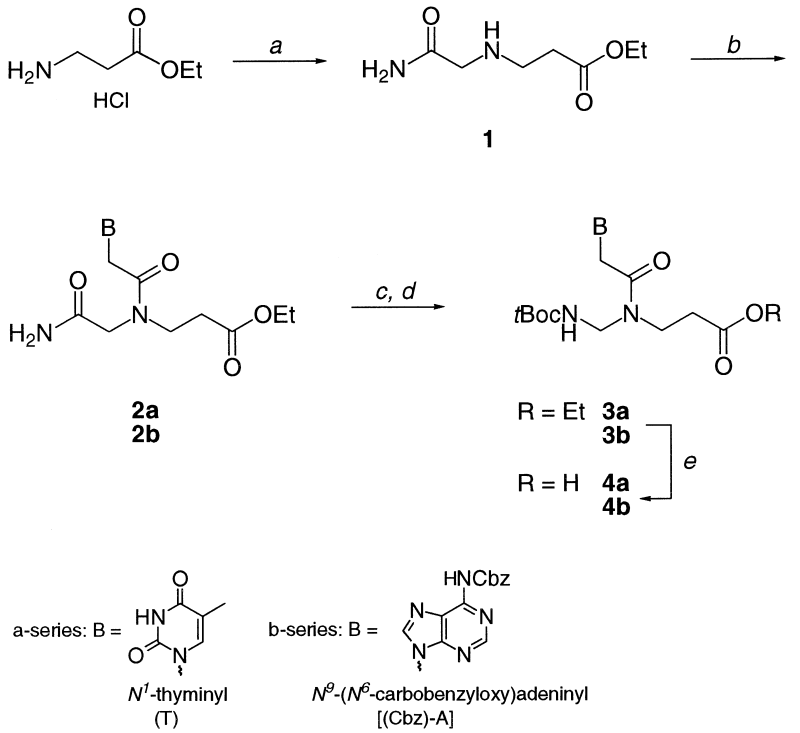
We describe in this report in detail the convenient synthesis of both a thymine and a N^6-Cbz adenine monomer suitable for the synthesis of 'retro–inverso' PNA **B**. Results from the first X-ray crystallographic study of a PNA monomer are also presented. Furthermore, the hybridization properties of different oligomers containing the new backbone modification with DNA, RNA and PNA oligomers are reported.

Results and Discussion

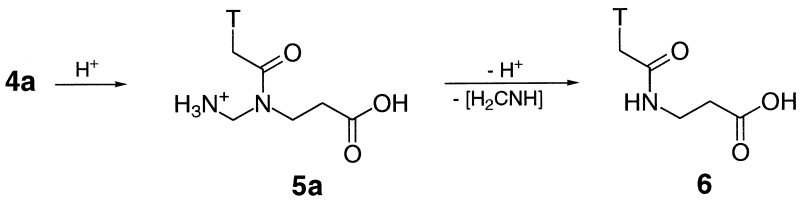
The four monomers of PNA **A** containing the natural nucleobases are prepared in a straightforward fashion from commercially available building blocks.[11–13] The retro-synthetic analysis of PNA **B** reveals a more challenging preparative task. The key structural feature of monomers **4a** and **4b** (Scheme 2) is the inherently labile *N*-(aminomethyl) amide moiety at the N terminus (Scheme 3).[27] As precursor we chose to prepare a primary amide, as the amide-to-amine conversion via Hofmann rearrangement using Loudon's variant[28,29] requires only very mild conditions. The synthetic route to **4a** and **4b** is depicted in Scheme 2 as previously reported.[26,27]

X-ray crystallography

The crystal structure analysis revealed the presence of two molecules in the asymmetric unit. As shown in



Scheme 2. Synthesis of the N-protected monomers **5a** and **5b**. *a*. 2-chloroacetamide, K_2CO_3, DMF, 90 °C, 24 h, 50%; *b*. BCH_2COOH, DCC/DhbtOH or HBTU, DMF/CH_2Cl_2 1:1, 0 °C to r.t., 1 h (58–61%); *c*. *I,I*-[bis(trifluoroacetoxy)-iodo]benzene, CH_3CN/H_2O, r.t., 20 h; *d*. di-*t*butyl dicarbonate, K_2CO_3, dioxane, r.t., 1 h (*c*, *d*: 64–75%). *e*. LiOH, THF/H_2O 1:1, r.t., 1 h (92%).



Scheme 3. Hydrolytic degradation of retro–inverso PNA.

Figure 1 they are almost related by non-crystallographic inversion symmetry through the hydrogen bonds that connect the two thymine rings. The almost planar conformation of the central amide group is also apparent. The X-ray crystal structure analysis of **4a** revealed a conformation of the tertiary amide group as shown as the major isomer (Scheme 4) found in solution.[26]

For the initial investigations of the hybridization properties of PNA with the new backbone modification we chose three types of poly-T oligomers: *N*-(aminoethyl) glycine PNA with one modification T* (**II**), *N*-(aminoethyl) glycine PNA with one T* and one β-ala-T monomer at the N terminus of T* (**IV**) and a polyT* PNA (**VI**) (Scheme 5).[27]

Hybridization Properties

The thermal stability of the PNA_2–DNA triplexes formed by T_{10}PNA oligomers and the complementary oligodeoxynucleotide (5′-dA_{10}-3′, 5′-dCGC-A_{10}-CGC-3′) were determined (Table 1). The incorporation of a single T* in position 5 of an oligomer **A** (**II**: H-T_4-T*-T_5-$LysNH_2$) resulted in a substantial decrease (48 °C) of the T_m ($T_m = 25$ °C) compared to the 10mer H-T_{10}-$LysNH_2$ ($T_m = 73$ °C). This large decrease in T_m is not totally surprising since the important interbase distance between the thymine of T* and the neighboring bases is reduced to 5 bonds along the backbone to the N terminal nucleobase and extended to 7 bonds to the C terminal nucleobase, respectively, compared to 6 bonds

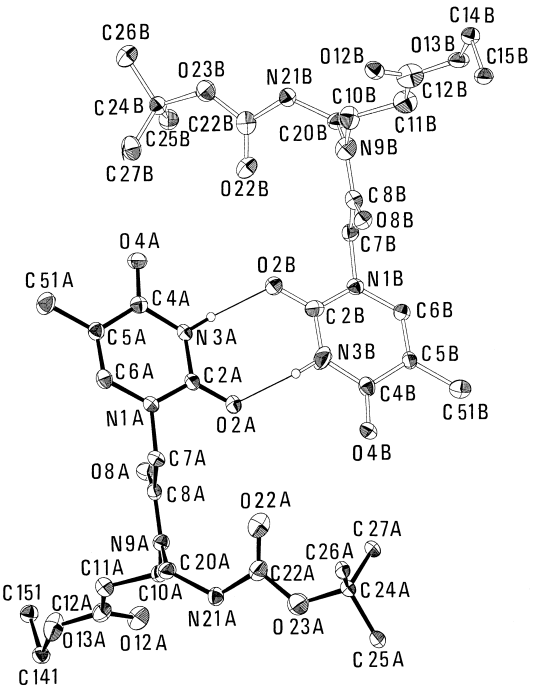
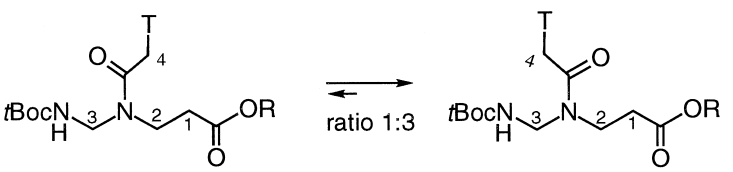


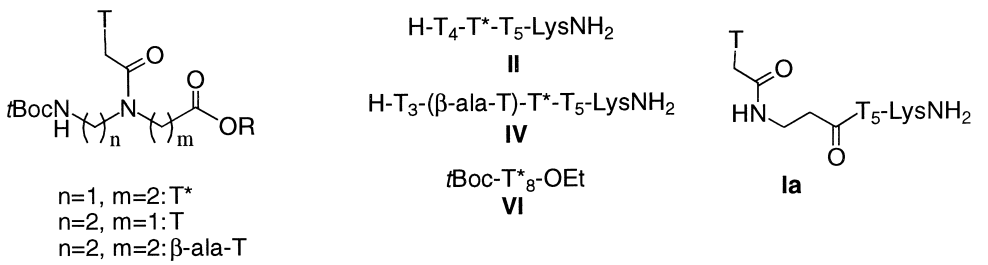
Figure 1. X-ray crystal structure of the 'retro–inverso' monomer. Drawing of molecule B and the more populated conformer (0.68) of molecule A. The two molecules are virtually mirror images of each other and differ only slightly in a few torsion angles: C6-N1-C7-C8 (A = 100°.0(2) B = −99°.7(2), N1-C7-C8-N9 (A = 176°.8(2) B = −175°.3(2)), C7-C8-N9-C10 (A = 179°.6(2) B = −179°.6(2)), C7-C8-N9-C20 (A = 4°.1(3), B = −3°.2(3)), N9-C20-N21-C22 (A = 82°.4(3) B = −88°.2(3)).

in PNA **A** (Scheme 6). The melting temperature of **II**/dA_{10} compares well with data obtained from PNA **A** H-T_5-LysNH_2 (T_m = 24 °C). Thus the incorporation of one backbone modified monomer into an oligomer **A** leads to a drastic distortion of the PNA **A** structure that prevents cooperative binding of the PNA oligomer to its complementary DNA. In an attempt to compensate partly for the different intraunit distances of the T* monomer compared to the original *N*-(aminoethyl) glycine backbone, we introduced an aminoethyl β-alanine unit[30,31] at the amino side of the T* unit. This resulted in a 23 °C increase in T_m (T_m = 48 °C), but the thermal stability was still lower than that of the unmodified PNA by 23 °C, and also lower by 11 °C than that of the PNA containing only the aminoethyl β-alanine unit (T_m = 59 °C).[31] However, from these experiments it is not clear whether this decreased stability is to be ascribed to the Watson–Crick or to the Hoogsteen strand of the triplex, or both.

Consequently, we incorporated the PNA T* unit into the middle of a 15-mer PNA (H-TGTACGT*CA-CAACTA-NH_2) that we have previously characterized for an aminoethyl glycine PNA,[9] and measured the thermal stability of this PNA complexed with complementary DNA, RNA or PNA oligomers. The results (Table 2) showed a dramatic decrease (ΔTm∼8 °C for the DNA and RNA complexes) in thermal stability upon introducing the T* unit. However, this unit also causes the thymine to be 'phase-shifted' −1 carbon on the amino-side and +1 carbon on the carboxyl-side of the nucleobase relative to the surrounding nucleobases



Scheme 4. Rotamer equilibrium of PNA **4a** (cf. ref[26])



Scheme 5. Retro–inverso monomers and oligomers.

Table 1. Thermal stabilities of PNA-oligoT-DNA complexes

PNA	T_m (°C)
H-T_{10}-LysNH_2 (**I**)	73
H-T_4-T*-T_5-LysNH_2 (**II**)	25
H-T_5-LysNH_2 (**III**)	24
H-T_3-βT-T*-T_5-LysNH_2 (**IV**)	48
H-T_4-βT-T_5-LysNH_2 (**V**)	59

(Scheme 6). This could be the cause of the reduced complex stability since it is known that introducing a β-alanine backbone unit into a PNA oligomer (e.g. PNA **XII**) (+1 carbon phase shift alone) results in a comparable reduction in complex stability[31] (Table 2, column 6).

Therefore we attempted to compensate for the −1 frame shift on the amino-side by introducing the β-alanine backbone guanine (PNA **IX**). This causes a further decrease (4 °C) in complex stability (column 3), and corresponds in terms of backbone structure to introducing one retro–inverso backbone (without phase shift) plus a β-alanine backbone on the thymine. Thus we should be able to indirectly deduce the effect of the retro–inverso backbone within the context of the original PNA glycine backbone by comparing PNA **IX** with PNA **X** (Table 2, columns 3 and 4). The results show ΔT_m of 6 °C and 4.5 °C for the DNA and RNA complexes and slightly less (3.5 °C) for the PNA complex. As a control, we also synthesized a PNA (PNA **XI**) in which the β-alanine unit would not be compensatory. As expected complexes with this PNA were less stable (column 5).

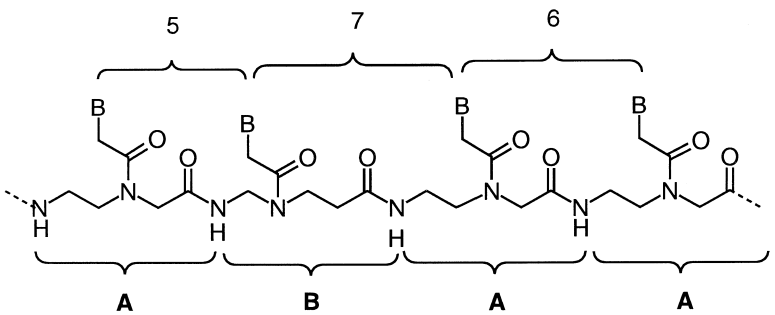
In order to analyze the integrity of the base pairs at and around the positions of the modified backbones, we studied the effects of base pair mismatches at three positions of the duplexes. All of the PNAs analyzed show very similar relative responses to mismatches in the complementary DNA or RNA oligonucleotide. Thus there is little doubt that all three nucleobases are engaged in conventional Watson–Crick base pairing despite possible structural constraints imposed by the changes in backbone structure.

Consequently, the retro–inverso backbone can be accommodated in a PNA–DNA (RNA or PNA) duplex, although at a considerable cost in duplex stability. Furthermore, our results indicate that this backbone is more easily accommodated in a PNA–RNA duplex than in a PNA–DNA duplex and most easily in a PNA–PNA duplex.

In agreement with the above results, we did not observe any indication of complex formation in the range of 5 to 90 °C between the fully 'retro–inverso' 8-mer PNA **B** (**VI**) and its complementary DNA oligomer; thereby supporting the conclusion that the PNA **B** structure has a low propensity of binding to DNA.

Considering only geometric aspects, the orientation of an amide group should have little impact on the hybridization properties of PNA. However, reversal of the amide groups in the backbone drastically reduces the binding affinity for complementary DNA sequences.

In order to better understand the results in structural terms, we performed molecular dynamics simulations on a (retro–inverso) PNA–DNA duplex. The solution structure of the duplex 5′dGACATAGC/H-GCTA TGTC-NH_2, previously determined by NMR methods,[32] was modified to contain only retro–inverso units in the PNA strand by slipping the -CONH region of the backbone on bond in the C-terminal direction, thereby switching place with one of the methylene groups. The modified structure was subjected to molecular dynamics (0.1 fs at 10 °C), keeping the DNA strand essentially fixed, followed by 10^4 steps of energy minimization. In the resulting structure the most notable feature is the direction of the carbonyl groups of the PNA backbone,



Scheme 6. 'Phase-shift' effect of introducing a 'retro–inverso' monomer (**B**) into a regular aminoethyl glycine (**A**) PNA oligomer. The internucleobase 'distances' are indicated above (5–7 bonds).

Table 2. Thermal stabilities (T_m, ΔT_m; °C)[a] of PNA–NA complexes PNA: H-TGT AC[**G TC**]A CAA CTA-NH_2 DNA/RNA: **3′**-ACA TG[**C AG**]T GTT GAT-5′

	PNA **VII** [GTC]	PNA **VIII**[c] [G(T*)C]	PNA **IX**[b] [(βG)(T*)C]	PNA **X** [G(βT)C]	PNA **XI** [G(T*)(βC]	PNA **XII** [(βG)TC]
T_m (ΔT_m)						
3′-[CAG]						
DNA	68,5 (0)	60,0 (8.5)	56,5 (12.0)	63,5 (5.0)	50,0 (18.5)	61,0 (7.5)
RNA	72,5 (0)	65,0 (7.5)	61,5 (11.5)	66,0 (6.5)	58,0 (14.5)	66,5 (6.0)
PNA[2]	83,0 (0)	78,5 (4.5)	75,5 (7.5)	79,0 (4.0)	73,5 (9.5)	79,5 (3.5)
			ΔT_m			
3′-[**C**TG]						
DNA	10.0	8.0	6.5	11.0	5.5	10.0
RNA	12.5	10.0	9.0	10.5	11.5	11.0
3′-[C**C**G]						
DNA	12.5	8.0	13.5	11.5	5.5	11.0
RNA	11.5	10.0	9.5	11.0	11.0	12.0
3′-[C**G**G]						
DNA	10.0	7.5	6.5	9.0	5.5	8.0
RNA	7.5	8.5	5.5	6.0	9.0	7.5
3′-[CA**C**]						
DNA	19.5	16.5	15.0	19.0	8.0	17.0
RNA	18.0	16.5	14.5	15.0	13.5	18.0
3′-[**A**AG]						
DNA	18.0	15.0	6.5	16.0	≥15	15.0
RNA	16.0	15.5	13.5	16.0	16.5	15.0

[a]Buffer: 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0.
[b]PNA: H-TAG TTG TGA CGT ACA-NH_2.
[c]T*: 'retro–inverso' thymine PNA unit.
[d]βG: β-alanine PNA unit.

which in most residues point into the helix, instead of being directed outwards into solution as in the regular PNA complexes.[14,32–34] This could have destabilizing effects on the (retro–inverso) PNA–DNA duplex for at least two reasons. First, the carbonyl group of the backbone is relatively close to the carbonyl group of the backbone–nucleobase linker of the same residue, which would be electrostatically unfavorable. Second, such relocation of the backbone carbonyl will result in reduced contact with the aqueous solvent which should likewise cause destabilization. On the other hand, one of the middle backbone units in the crystal structure of a PNA–PNA hexamer duplex indeed shows 50% 'carbonyl in' structure,[34] indicating that this conformation is not dramatically destabilized. Therefore, the modeling does not offer an obvious explanation for the poor hybridization properties of the 'retro–inverso' PNA backbone.

In conclusion we have shown that a simple inversion of the amide in the PNA backbone structure which in principle has conserved both the constrained flexibility in terms of two amide groups (one in the backbone itself and one in the linker to the nucleobase) as well as all interbackbone distances has a dramatic effect on the ability of the PNA to bind to complementary DNA or RNA oligonucleotides and thus to mimic DNA. The present results combined with those reported for numerous previously described 'peptide nucleic acids' clearly show that the structural window that allows for good DNA/RNA mimicking properties of a PNA is very narrow indeed.

Experimental

Synthesis

2-Chloroacetamide, β-alanine ethyl ester hydrochloride, 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (DhbtOH), 1,3-dicyclohexylcarbodiimide (DCC), [bis(trifluoroacetoxy)-iodo]benzene, di-*t*butyl dicarbonate and trifluoroacetic acid (TFA) were purchased from Aldrich. *O*-(l*H*-benzotriazolyl) *N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Nova. Buffers: pH 0: (HCl), pH 4: (56 mM citric acid, 68 mM NaOH, 44 mM NaCl, Fluka), pH 7 (26 mM KH_2PO_4, 41 mM

Na_2HPO_4, Fluka), pH 9 (13 mM sodium tetraborate, 4.6 mM HCl, Fluka). N^1-Thyminyl acetic acid and N^6-(benzyloxycarbonyl)-adenin-9-yl acetic acid were prepared as described.[2] Melting points (uncorrected) were determined in open capillaries on a Büchi melting point apparatus (acc. Dr Tottoli) and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker (250 MHz) or Varian spectrometer (400 MHz), respectively. Compounds **2**, **3** and **4** are mixtures of two rotamers. The resonances of the major isomer of **3a, 3b, 4a** and **4b** are labeled with an asterisk. T = Thymine, A = Adenine. High performance liquid chromatography (HPLC) was carried out with a Waters 600E System Controller, equipped with a Waters 486 Tunable Absorbance Detector (detector wavelength: 260 nm) and a Waters Data Module. We used a C_{18}-reversed phase column from Vydag. The chromatographic conditions were as follows: A: from CH_3CN:H_2O:TFA 1:99:0.1 to 10:90:0.1 in 20 min, flow rate = 1.5 mL min^{-1}, t_o = 2.3 min. B: from CH_3CN:H_2O:TFA 1:99:0.1 to 10:90:0.1 in 10 min, then to 70:30:0.1 in 30 min, flow rate = 1.5 mL min^{-1}. Fast-atom bombardment mass spectra (FAB-MS) were taken on a Masslab VG 12-250 quadrupole instrument fitted with a VG-FAB source and a JMS-Hx/Hx 110A High Performance Tandem Mass Spectrometer (JEOL). Peaks at higher m/z can be assigned to $[M+Li]^+$ or $[M+Na]^+$. Micro analyses were performed at the H. C. Ørsted Institute.

***N*-(Carbamoylmethyl)-β-alanine ethylester (1).** A suspension of 2-chloroacetamide (2.34 g, 25.0 mmol), β-alanine ethyl ester hydrochloride (15.36 g, 100 mmol) and K_2CO_3 (42.0 g) in dry DMF (200 mL) was heated at 90 °C for 24 h. After cooling to r.t. the reaction mixture was filtered and the solvent and unreacted β-alanine ethyl ester were removed by distillation under reduced pressure (50 °C, 1 Torr). A yellow oil (6.0 g) remained. Pure **1** (2.24 g, 50%) was obtained as colorless oil by flash chromatography (silica 20×5 cm, 1. ethyl acetate, 2. ethyl acetate:ethanol 2:1, 3. ethanol). TLC (methanol, det. ninhydrin), R_f(**1**) = 0.65. ^1H NMR ([D_6]DMSO): δ = 1.18 (t, J = 7.1 Hz, 3H, CH_3), 2.42, 2.70 (2t, J = 6.7 Hz, 4H, CH_2CH_2), 3.03 (s, 2H, CH_2), 4.06 (q, J = 7.1 Hz, 2H, OCH_2), 7.02, 7.24 (2s (br), 2H, NH_2) ppm. ^{13}C NMR ([D_6]DMSO): δ = 14.1 (CH_3), 34.5 (CH_2CO), 44.7 (NCH_2CH_2), 51.8 (NCH_2CN), 59.8 (OCH_2), 172.1 (C = O), 173.5 (C = O) ppm. FAB-MS: m/z = 175.1 $(M+1)^+$.

A solution of **1** (530 mg) in diethyl ether was treated with HCl (4.7M in diethyl ether). The precipitate **1**·HCl was filtered, rinsed with cold diethyl ether and dried in vacuo, white powder.

^1H NMR ([D_6]DMSO): δ = 1.21 (t, J = 7.1 Hz, 3H, CH_3), 2.80, 3.16 (2t, J = 7.5 Hz, 4H, CH_2CH_2), 3.69 (s, 2H, CH_2), 4.10 (q, J = 7.1 Hz, 2H, OCH_2), 7.53, 7.96 (2s, 2H, NH_2), 9.22 (s, 2H, NH_2^+) ppm. ^{13}C NMR ([D_6]DMSO): δ = 14.0 (CH_3), 30.2 ($CH_2C(O)$), 42.4 (NCH_2CH_2), 47.6 ($NCH_2C(O)$), 60.5 (OCH_2), 166.8 (amide C=O), 170.0 (ester C=O) ppm. $C_7H_{15}ClN_2O_3$ (210.66): calcd C 39.91, H 7.18, N 13.30; found C 39.45, H 7.10, N 13.22.

***N*-(Carbamoylmethyl)-*N*-(thymin-1-ylacetyl)-β-alanine ethylester (2a).** DhbtOH (9.37 g, 57.4 mmol), N^1-thyminyl acetic acid (10.57 g, 57.4 mmol) and CH_2Cl_2 (100 mL) were added to a solution of **1** (10.0 g, 57.4 mmol) in dry DMF (100 ml). The solution was cooled to 0 °C in an ice bath and a solution of DCC (1.90 g, 9.18 mmol) in DMF:CH_2Cl_2 (100 ml, 1:1, v/v) was added dropwise within 20 min. The ice bath was removed and after stirring for 1 h at r.t. the reaction mixture was filtered, the precipitate (DCU) was rinsed with CH_2Cl_2 (50 mL) and the organic solutions and CH_2Cl_2 (450 mL) were combined. A colorless precipitate formed overnight. Filtration, rinsing with CH_2Cl_2 and drying in vacuo afforded **2a** (12.0 g, yield 61%), mp 207–208 °C.

Due to restricted conformations at the tertiary amide group two sets of signals (ratio 1:1) are observed for most protons and carbons in the NMR spectra, the coalescence temperature is 85 °C. ^1H NMR ([D_6]DMSO, r.t.): δ = 1.16, 1.18 (2t, J = 7.1 Hz, 3H, CH_2CH_3), 1.73 (s, 3H, T-CH_3), 2.49, 2.70, 3.45, 3.58 (4t, J = 7 Hz, 4H, CH_2CH_2), 3.84, 4.04 (2s, 2H, CH_2), 4.03, 4.07 (2q, J = 7 Hz, 2H, OCH_2), 4.42, 4.65 (2s, 2H, T(N^1)-CH_2), 7.01, 7.22, 7.26, 7.54 (4s, 2H, NH_2), 7.28, 7.32 (2s, 1H, CH), 11.22, 11.24 (2s, T, NH) ppm. ^{13}C NMR ([D_6]DMSO): δ = 11.92, 11.95 (T, CH_3), 14.1 (CH_2CH_3), 32.1, 32.7 (CH_2CO), 43.4, 43.9 (NCH_2CH_2), 47.85, 47.91 ($NCCH_2N$), 48.5, 50.2 (T(N^1)-CH_2), 60.1, 60.2 (OCH_2), 108.0, 108.1 (CCH_3), 142.2, 142.3 (CH), 151.0, 151.1 (T, NC(O)N), 164.4 (T, C = O), 167.1, 167.7 (tert. amide C = O), 169.8, 170.0 (prim. amide C = O), 171.2 (ester C = O) ppm. FAB-MS: m/z = 341.1 $(M+1)^+$. $C_{14}H_{20}N_4O_6$ (340.34): calcd C 49.41, H 5.92, N 16.46; found C 48.76, H 5.76, N 16.34.

***N*-(Carbamoylmethyl)-*N*-(*N*^{6}-(benzyloxycarbonyl)adenin-9-ylacetyl)-β-alanine ethylester (2b).** According to the procedure for the preparation of **2b, 1** (2.00 g, 11.5 mmol) was reacted with N^9-(N^6-benzyloxycarbonyl)adeninyl acetic acid (3.76 g, 11.5 mmol), DhbtOH (1.88 g, 11.5 mmol) and DCC (2.37 g, 11.5 mmol). Flash chromatography on silica (twice, 25×5 cm, ethyl acetate: methanol 3:1) afforded **2b** (4.0 g, DCU content ca. 15%).

Using HBTU as the activating agent we obtained **2b** of higher purity. **1·HCl** (211 mg, 1.0 mmol) was dissolved in DMF (2.5 mL), pyridine (2.5 mL) and *N*-diisopropyl-

ethyl amine (0.64 mL). N^9-(N^6-benzyloxycarbonyl)-adeninyl acetic acid (327 mg, 1.0 mmol) and HBTU (380 mg, 1.0 mmol) were added subsequently. After stirring for 1 h at r.t. toluene (30 mL) was added. The precipitate was isolated by filtration. After flash chromatography (silica, 8×2 cm, eluent: methanol:ethyl acetate 1:1) **2b** (280 mg, yield 58%) was obtained as a white amorphous solid, mp 202–203 °C.

^{1}H NMR ([D$_6$]DMSO): (two rotamers, ratio 1:1) δ = 1.25, 1.30 (2t, J = 7.1 Hz, 3H, CH_3), 2.60 (overl. with solvent), 2.89, 3.57, 3.81 (4t, J = 7.1 Hz, 4H, CH_2CH_2), 3.96, 4.29 (2s, 2H, CH_2), 4.12, 4.21 (2q, 2H, CH_2CH_3), 5.23, 5.48 (2s, 2H, CH_2), 5.30 (s, 2H, CH_2Ph), 7.12, 7.77 (2s, 1H, NH_2), 7.40–7.55 (m, 6H, aromatic H, NH_2), 8.41 (s, 1H, A-H), 8.67, 8.68 (2s, 1H, A-H), 10.71 (s, 1H, *H*NCbz) ppm. ^{13}C NMR ([D$_6$]DMSO): (two rotamers) δ = 14.08, 14.13 (CH_3), 32.08, 32.64 (CH_2CO), 43.51, 43.95, 44.12, 44.16 (CH_2NCH_2), 48.47, 50.29 (A(N^9)CH_2), 60.05, 60.23 (OCH_2CH_3), 66.27 (OCH_2Ph), 122.90, 122.96, 127.83, 127.96, 128.40, 136.40, 145.27, 145.32, 149.31, 151.43, 152.18, 152.40, 152.44, 166.44, 167.12 (tert. amide C=O), 169.78, 169.83 (prim. amide C=O), 171.22 (ester C=O) ppm. FAB-MS: m/z = 484.12 $(M+H^+)$. $C_{22}H_{25}N_7O_6$ (483.48) calcd C 54.65, H 5.21, N 20.28; found C 52.93, H 5.09, N 19.84.

***N*-(*N*-*tert*-Butyloxycarbonyl-aminomethyl)-*N*-(N^1-thyminylacetyl) β-alanine ethylester (3a).** A solution of *I,I*-[bis(trifluoroacetoxy)iodo]benzene (16.71 g, 38.8 mmol) in CH_3CN (55 mL) was added dropwise within 10 min to a stirred solution of **2a** (11.5 g, 33.8 mmol) in CH_3CN:H_2O (275 mL, 2:3 v/v). After 20 h at r.t. the volume was reduced to ca. 50 mL under reduced pressure (1 Torr, 30 °C) and the solution was extracted with diethyl ether (150 mL). The aqueous phase was evaporated to dryness in vacuo (1 Torr, 30 °C). The remaining solid was rinsed with diethyl ether (20 mL) and dried in vacuo. 1,4-Dioxane (150 mL), K_2CO_3 (12.2 g) and a solution of di-*tert*-butyl dicarbonate (8.4 g, 38.5 mmol) in 1,4-dioxane (50 mL) were added. After stirring for 1 h at r.t. the reaction mixture was filtered and the solvent was removed under reduced pressure. Purification by column chromatography (silica, 35×10 cm, hexane: acetone 1:1) and subsequent precipitation from MeOH (60 mL) by addition of hexane (300 mL) afforded **3a** (8.6 g, yield 64%), mp 174–175 °C. TLC (MeOH, det. UV, ninhydrin): R_f(**2a**) = 0.73, R_f(i) = 0.5, R_f(**3a**) = 0.85. ^{1}H NMR ([D$_6$]DMSO) δ = 1.17 (t, J = 7 Hz, 3H, CH_2CH_3), 1.38, 1.42* (2s, 9H, *t*Bu), 1.75 (s, 3H, T-CH_3), 2.50*, 2.72 (2t, J = 7.9 Hz, 2H, NCH_2CH_2), 3.31*, 3.50 (2t, 2H, NCH_2CH_2), 4.05*, 4.08 (2q, 2H, OCH_2), 4.58, 4.61* (2d, 2H, CH_2), 4.61 (overlap.), 4.74* (s, 2H, CH_2), 7.26*, 7.35 (2s, 1H, CH), 7.4, 7.8 (2m, 1H, NH), 11.25 (s, 1H, NH) ppm. ^{13}C NMR (major rotamer, [D$_6$]DMSO): δ = 11.9 (T, CH_3), 14.1 (CH_2CH_3), 28.0 (*t*Bu-CH_3), 32.2 (CH_2CO), 41.4 (NCH_2CH_2), 48.1 (T(N^1)CH_2), 53.1 (NCH_2N), 60.0 (OCH_2), 78.8 ($C(CH_3)_3$) 108.2 (T, CCH_3), 142.0 (CH), 151.1(T, NC(O)N), 155.7 (carbamate C=O), 164.4 (T, C=O), 167.0 (tert. amide C=O), 171.1 (ester C=O) ppm. FAB-MS: m/z = 413.20 $(M+H)^+$. $C_{18}H_{28}N_4O_7$ (412.44) calcd C 52.42, H 6.84, N 13.58; found C 52.37, H 6.68, N 13.57.

***N*-(*N*-*tert*-Butyloxycarbonyl-aminomethyl)-*N*-(N^9-benzyloxycarbonyl-adenin-9-ylacetyl) β-alanine ethylester (3b).** A solution of amid **2b** (2.60 g, 5.38 mmol) and *I,I*-[bis(trifluoroacetoxy)iodo]benzene (2.77 g. 6.45 mmol) in CH_3CN/H_2O (50 mL) was kept for 20 h at r.t.. The mixture was filtered and the solution was evaporated to dryness under reduced pressure. 1,4-Dioxane (50 mL) and Na_2CO_3 (5.0 g) were added, followed by addition of a solution of di-*tert*-butyl dicarbonate (1.65 g, 7.56 mmol) in 1,4-dioxane (10 mL). The mixture was stirred at r.t. for 24 h, filtered and **3b** (2.24 g, yield 75%) was obtained by flash chromatography (silica, 20×5 cm, ethyl acetate:methanol 97:3). TLC (MeOH:ethyl acetate 1:1, det. UV, ninhydrin): R_f(**2b**) = 0.65, R_f(i) = 0.47, R_f(**3b**) = 0.85. ^{1}H NMR ([D$_6$]DMSO): δ = 1.23*, 1.30 (2t, J = 7.0 Hz, 3H, CH_2CH_3), 1.46, 1.52* (2s, 9H, *t*Bu), 2.6* (overl. with solvent), 2.90 (2t, CH_2CO_2), 3.60*, 3.90 (2t, J = 7.3 Hz, 2H, NCH_2CH_2), 4.11*, 4.20 (2q, J = 7.1 Hz, 2H, CH_2CH_3), 4.65, 4.82* (2d, 2H, NCH_2N), 5.30 (s, 2H, CH_2Ph), 5.41, 5.53* (2s, 2H, CH_2-A), 7.40–7.60 (m, 5H, aromatic H), 8.02 (t, NH), 8.37, 8.40, 8.66 (3s, A-H), 10.72 (s, NH) ppm. FAB-MS: m/z = 556.4 $(M+1)^+$. $C_{26}H_{33}N_7O_7$ (555.59) calcd C 56.21, H 5.99, N 17.65; found C 55.65, H 5.89, N 17.16.

***N*-(*N*-*tert*-Butyloxycarbonyl-aminomethyl)-*N*-(N^1-thyminylacetyl) β-alanine (4a).** A solution of **3a** (5.45 g, 13.2 mmol) in THF (80 ml) and aqu. LiOH (0.5 M, 80 mL) was stirred at r.t. for 1 h and extracted with ethyl acetate. The aqueous layer was acidified to pH 3.5 by dropwise addition of hydrochloric acid (4 M) and kept at 4 °C for 3 h. The colorless precipitate was filtered, rinsed with water and dried in vacuo to afford **4a** (4.40 g), mp 196 °C (dec.). The filtrate was extracted with ethyl acetate, the organic phase dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was crystallized from MeOH:ethyl acetate: hexane to afford additional **4a** (280 mg), total yield 92%. TLC ($CHCl_3$:MeOH:NEt_3 7:2:1, UV-detection): R_f(**3a**) = 0.94, R_f(**4a**) = 0.52). ^{1}H NMR ([D$_6$]DMSO): (two rotamers, ratio 3:1) δ = 1.46, 1.50* (2s, 9H, *t*Bu), 1.83 (s, 3H, T-CH_3), 2.52*, 2.73 (2t, J = 7.5 Hz, 2H, NCH_2CH_2), 3.54*, 3.64 (2t, J = 7.3 Hz, 2H, NCH_2CH_2), 4.64, 4.69 (2d, J = 6.4 Hz, J = 7.0 Hz. ^{13}C NMR (major rotamer, [D$_6$]DMSO): δ = 11.9 (T, CH_3), 28.1 (*t*Bu-CH_3), 32.2 (CH_2CO), 41.6 (NCH_2CH_2), 48.1 (T(N^1)CH_2), 53.2 (NCH_2N), 60.0 (OCH_2), 78.9 ($C(CH_3)_3$),

108.2 (T, CCH_3), 142.0 (CH), 151.1 (T, NC(O)N), 155.7 (carbamate C=O), 164.4 (T, C=O), 166.9 (tert. amide C=O), 172.7 (carboxylic acid C=O) ppm. FAB-MS: $m/z = 385.15$ $(M+H)^+$. $C_{16}H_{24}N_4O_7$ (384.39): calcd C 50.00, H 6.29, N 14.58; found C 49.95, H 6.40, N 14.23.

***N*-(*N*-*tert*-Butyloxycarbonyl-aminomethyl)-*N*-(*N*^{6}-benzyl-oxycarbonyladenin-9-ylacetyl) β-alanine (4b).** A mixture of ester **3b** (2.34 g, 4.21 mmol) in THF (25 ml) and aqu. LiOH (0.5 M, 25 mL) was stirred at r.t. for 1.5 h, when TLC indicated that hydrolysis was complete. Extraction with CH_2Cl_2 (extract discarded), acidification with HCl (2N) and extraction with CH_2Cl_2 afforded **4b** (2.1 g, 95%). ^{1}H NMR ([D$_6$]DMSO-d$_6$): δ = 1.47, 1.53* (2s, 9H, *t*Bu), 2.51*, 2.78 (2t, $J = 7.5$ Hz, 2H, NCH_2CH_2), 3.57*, 3.75 (2t, $J = 7.2$ Hz, 2H, NCH_2CH_2), 4.65, 4.83* (2d, NCH_2N), 5.29 (s, 2H, CH_2Ph), 5.44, 5.54* (2s, 2H, CH_2), 7.4–7.6 (5H, aromat.), 8.02 (NH), 8.39, 8.67 (2s, aromat.), 10.7 ppm. FAB-MS: $m/z = 528.1$ $(M+1)^+$. $C_{24}H_{29}N_7O_7$ (527.53): calcd C 54.64, H 5.54, N 18.59; found C 54.86, H 5.50, N 18.81.

***N*,*N*-(ammoniummethyl)-(*N*^{1}-thyminylacetyl)-β-alanine trifluoroacetate (5a).** At room temperature, **4a** (102 mg, 0.265 mmol) was dissolved in 95% TFA/cresol (1 mL). After 5 min the solution was evaporated in vacuo and the residue was dissolved in ethanol (0.5 mL). Upon addition of diethyl ether a precipitate formed (75 mg). FAB-MS: $m/z = 285.1$ (M^+).

***N*-(*N*^{1}-thyminylacetyl)-β-alanine (6).** B. A suspension of **4a** (100 mg, 0.26 mmol) and HCl/1,4-dioxane (0.5 M) was heated for 5 min at 100 °C. Evaporation and flash chromatography of the residue on silica afforded **4** in nearly quantitative yield. ^{1}H NMR ([D$_6$]DMSO): δ = 1.82 (s, 3H, CH_3), 2.47 (t, $J = 6.8$ Hz, 2H, NCH_2CH_2), 3.34 (m, 2H, $HNCH_2$), 4.33 (s, 2H, T-CH_2), 7.49 (s, 1H, CH), 8.27 (t, $J = 5.4$ Hz, 1H, HNC(O)), 11.3 (s, 1H, T-NH), 12.3 (s, (br), 1H, OH) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 11.9 (CH_3), 33.8, 34.9 (CH_2CH_2), 49.3 (T-CH2), 107.9 (CCH_3), 142.3 (CH), 151.0, 164.4, 166.9, 172.7 (4C=O) ppm. FAB-MS: $m/z = 256.1$ $(M+1)^+$.

Peptide coupling; general procedures. A. In a typical experiment, amino acid ester (0.1–1 mmol) was dissolved in trifluoroacetic acid:CH_2Cl_2 (0.2–2 ml, 1:1, v/v). After stirring for 20 min at room temperature the clear solution was evaporated to dryness in vacuo (0.5 Torr). Diethylether (2 mL) was added and the mixture was evaporated to dryness.

B. Equimolar amounts of *t*Boc-protected amino acid (0.1–1 mmol) and HBTU (0.1–1 mmol) were dissolved in a solution of DMF:pyridine (0.5–5 mL, 1:1, v/v) and diisopropylethyl amine (0.2–2 mL). This solution was then added to the solid residue obtained under A. After stirring for 2 h at room temperature the reaction mixture was purified by column chromatography on silica using a methanol/ethyl acetate gradient as eluent to give the oligomers.

Hydrolysis, general procedure. C. In a typical experiment, the oligomer-ester (0.1–1 mmol) was added to a mixture of THF (0.8–8 mL) and LiOH (0.5 M, 0.8–8 mL) and the mixture was stirred at room temperature until TLC indicated that hydrolysis was complete (ca. 2 h). After extraction with *n*-butanol, the aqueous phase was acidified with HCl (2N). A precipitate was isolated by filtration and/or the solution was extracted with *n*-butanol. The organic layer was separated and dried over Na_2SO_4, evaporated and the crude products were combined and purified by flash chromatography (silica) using a methanol/ethyl acetate gradient to give the oligomer-acid of high purity (>95%, acc. to HPLC). The identity was confirmed by mass spectroscopic analysis.

X-ray crystallography. Crystals were obtained by slow evaporation from a [D$_6$]DMSO solution. Crystal size $0.15\times0.30\times0.60$ mm^3, monoclinic, space group $P2_1/n$, T = 122K, a = 9.9263(13), b = 19.098(5), c = 22.043(4) Å, β = 93.786(12)°, V = 4169.6(14) A^3, Z = 8, $d_{cal} = 1.311$ gcm^{-3}, CAD4 diffractometer, CuKα radiation, $2!\theta \leq 140°$.9698 measured reflections, 7893 unique, corrections for background, Lorentz, polarization and decay with DREAD programs.[35] Structure solved by direct methods SHELXS-86, full matrix least squares refinement on /F/^{2} SHELXL-93.[36] The ethyl ester group of one of the independent molecules is disordered, it can be described by two differently populated (0.68(4) and 0.32(3)) conformers. The disordered carbon atoms were given isotropic, all other non-hydrogen atoms anisotropic displacement parameters. Hydrogen atoms were located in the difference map apart from those attached to the disordered ethyl group. Their positional parameters were not refined, displacement parameters for CH_3 hydrogen atoms are $1.5\times U_{iso}$ of the atoms to which they are bonded, $1.2\times U_{iso}$ for all other hydrogen atoms. $wR_2 = 0.204$ $R_1 = 0.0668$ for reflections with $/F_o/ > 4\sigma$ $(/F_o/)$. Further details of the crystal structure investigation are available on request from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, GB-Cambridge CB2 1 EZ (UK) on quoting the full journal citation.

Acknowledgements

This work was supported by the Danish National Research Foundation and a DANVIS fellowship to AHK. We thank Inga Nordstrøm, Flemming Hansen

and Annette W. Jørgensen for skilful technical assistance.

References and Notes

1. Crooke, S. T. In *Burger's Medical Chemistry and Drug Discovery*; Wolff, M. E, Ed.; John Wiley & Sons, Inc.: 1995, pp 863–900.

2. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544–584.

3. De Mesmaeker, A.; Altmann, K. H.; Waldner, A.; Wendeborn, S. *Current Opinion in Structural Biology* **1996**, *5*, 343.

4. Matteucci, M.; Wagner, R. W. *Science* **1996**, *384(supp)* 20.

5. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.

6. Cherny, D. Y.; Belotserkovskii, B. P.; Frank-Kamenetskii, M. D.; Egholm, M. E.; Buchardt, O.; Berg, R. H.; Nielsen, P. E. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 1667.

7. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895.

8. Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H.; *J. Am. Chem. Soc.* **1992**, *114*, 9677–9678.

9. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566.

10. Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Org. Chem.* **1994**, *59*, 5767.

11. Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E.; Coull, J.; Berg, R. H. *J. Peptide Sci.* **1994**, *3*, 175.

12. Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, F. C.; Luzzio, M. J; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179.

13. Kim, S. K.; Nielsen, P. E.; Egholm, M.; Buchardt, O.; Berg, R. H.; Nordén, B. *J. Am. Chem. Soc.* **1993**, *115*, 6477.

14. Betts, L; Josey, J. A.; Veal, J. M.; Jordan, S. R. *Science* **1995**, *270*, 1838.

15. Nielsen, P. E.; Egholm, M.; Buchardt, O. *Gene*, **1994**, *149*, 139.

16. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Anti-Cancer Drug Design* **1993**, *8*, 53–63; *11*, 384.

17. Hanvey, J. C.; Peffer, N. J.; Bisi, J. E.; Thomson, S. A.; Cadilla, R.; Josey, J. A.; Ricca, D. J.; Hassman, C. F.; Bonham, M. A.; Au, K. G.; Carter, S. G.; Bruckenstein, D. A.; Boyd, A. L.; Noble, S. A.; Babiss, L. E. *Science* **1992**, *258*, 1481.

18. Knudsen, H.; Nielsen, P. E. *Nucleic Acids Res.*, **1996**, *24*, 494.

19. Vickers, T. A.; Griffith, M. C.; Ramasamy, K.; Risen, L. M.; Freier, S. M. *Nucleic Acids Res.*, **1995**, *23*, 3003.

20. Bonham, M. A.; Brown, S.; Boyd, A. L.; Brown, P. H.; Bruckenstein, D. A.; Hanvey, J. C.; Thomson, S. A.; Pipe, A.; Hassman, F.; Bisi, J. E.; Froehler, B. C.; Matteucci, M. D.; Wagner, R. W.; Noble, S. A.; Babiss, L. E. *Nucleic Acids Res.*, **1995**, *23*, 1197.

21. Good, L.; Nielsen, P. E. *Antisense and Nucleic Acid Drug Development* **1997**, *7*, 431.

22. Hyrup, B.; Egholm, M.; Rolland, M.; Nielsen, P. E.; Berg, R. H.; Buchardt, O. *J. Chem. Soc. Chem. Commun.* **1993**, 518.

23. Almarsson, O.; Bruice, T. C.; Kerr, J.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7518.

24. Hyrup, B.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083.

25. Lagriffoul, P.-H.; Egholm, M.; Nielsen, P. E.; Berg, R. H.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1081.

26. Krotz, A. H.; Buchardt, O.; Nielsen, P. E. *Tetrahedron Lett.* **1995**, *36*, 6937.

27. Krotz, A. H.; Buchardt, O.; Nielsen, P. E. *Tetrahedron Lett.* **1995**, *36*, 6941.

28. Loudon, G. M.; Radhakrishna, A. S.; Almond, M. R.; Blodgett, J. K.; Boutin, R. H. *J. Org. Chem.* **1984**, *49*, 4272.

29. Loudon, G. M.; Almond, M. R.; Jacob, J. N. *J. Am. Chem. Soc.* **1981**, *103*, 4508.

30. Hyrup, B.; Egholm, M.; Rolland, M.; Nielsen, P. E.; Berg, R. H.; Buchardt, O. *J. Chem. Soc. Chem. Commun.* **1993**, 518.

31. Hyrup, B.; Egholm, M.; Nielsen, P. E.; Wittung, P.; Nordén, B.; Buchardt, O. *J. Amer. Chem. Soc.* **1994**, *116*, 7964.

32. Eriksson, M.; Nielsen, P. E. *Nature Struct. Biol.* **1996**, *3*, 410–413.

33. Brown, S. C.; Thomson, S. A.; Veal, J. M.; Davis, D. G. *Science* **1994**, *265*, 777–780.

34. Rasmussen, H.; Kastrup, J. S.; Nielsen, J. N.; Nielsen, J. M.; Nielsen, P. E. *Nature Structural Biology* **1997**, *4*, 98.

35. Blessing, R. H. *Cryst. Rev.* **1987**, *1*, 3.

36. Sheldrick, G. M. Program for the refinement of crystal structures. University of Göttingen, Germany, 1993.