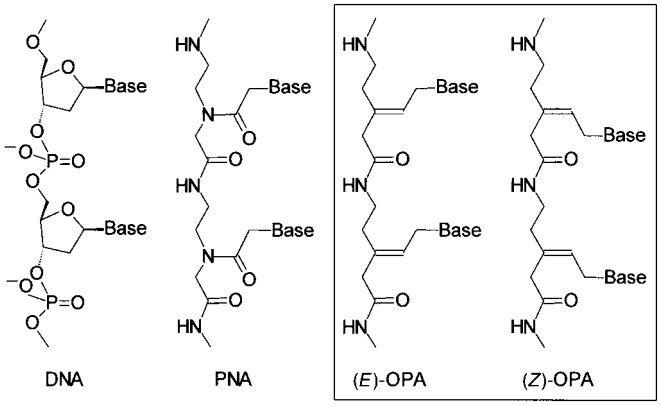
Olefinic Peptide Nucleic Acids (OPAs): New Aspects of the Molecular Recognition of DNA by PNA**

Rolf Schütz, Michel Cantin, Christopher Roberts, Beate Greiner, Eugen Uhlmann, and Christian Leumann*

Polyamide or peptide nucleic acids (PNAs), first described in 1991,[1] are DNA analogues based entirely on an achiral polyamide backbone (Scheme 1). The PNAs undergo sequence-specific and efficient Watson – Crick base pairing with



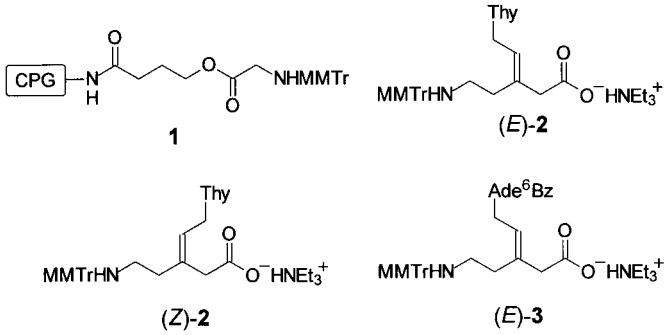
Scheme 1. Sections of the chemical structures of deoxyribonucleic acids (DNAs), polyamide or peptide nucleic acids (PNAs), and (*E*)- and (*Z*)-olefinic polyamide nucleic acids ((*E*)- and (*Z*)-OPAs).

complementary DNA and RNA.[2] Owing to their structural simplicity and their remarkable functional properties they have not only found application as tools in molecular biology,[3] but also have a large impact on antisense research[4] and prebiotic chemistry.[5] The ample literature describing the chemical and biological properties of PNAs has recently been reviewed.[6]

A key structural feature of PNAs is the central amide linker between the base and the backbone. This tertiary amide functionality is conformationally labile and occurs in both the *E*- and *Z*-rotameric forms in uncomplexed PNA.[7] However, it is uniformly oriented in the *Z*-rotameric form in PNA/DNA,[8, 9] PNA/RNA,[7] and PNA/PNA complexes,[10] as revealed by NMR and X-ray analysis. Early work on molecular modeling[11, 12] suggested that intra- or interresidue hydrogen bonds between the backbone and this tertiary amide group in the PNA strand might be important secondary structure-stabilizing elements—a statement that was not supported by the structural analyses.

To eliminate ambiguity regarding the conformation and to elucidate the structural and electrostatic role of this amide group on DNA and RNA binding, we designed olefinic polyamide nucleic acids (OPAs), in which this central amide functionality is replaced by an isostructural, configurationally stable C–C double bond in either the *Z* or *E* configuration (see Scheme 1). While we have already reported on the synthesis of the monomeric (*E*)- and (*Z*)-OPA building blocks containing the base thymine,[13, 14] we describe here the synthesis of (*E*)- and (*Z*)-OPA oligomers as well as preliminary studies on pairing with complementary DNA.

The synthesis of chimaeric PNA – oligomers containing OPA units was performed using the building blocks (*E*)/(*Z*)-**2** and (*E*)-**3** and followed the MMTr/acyl (MMTr = monomethoxytrityl) protecting group strategy developed earlier for the synthesis of PNAs.[15] This methodology was chosen because of its mild deprotection chemistry and its compatibility with DNA synthesis.



In a first set of experiments the PNA oligomers **5** – **9** (Table 1)—which contained single t^E ((*E*)-**2**) or t^Z mutations ((*Z*)-**2**) at various positions in the sequence, a hydroxyhexylamide C terminus, and a natural thymidine unit at the N terminus—were prepared according to published procedures,[16] and their structural integrity was verified by electrospray (ESI) mass spectrometry. The N-terminal thymidine

Table 1. Mass spectrometry data and T_m values [°C] (UV-melting curves, 260 nm) of DNAs, PNAs, and PNA sequences containing (*E*)-**2** or (*Z*)-**2** units with antiparallel complementary DNA ($c = 4\,\mu M$ in 100 mM NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0). Capital letters: deoxynucleotides; lowercase letters: PNA units; t^Z = (*Z*)-**2** unit, t^E = (*E*)-**2** unit, hex = *n*-hydroxyhexylamide; *g* = hydroxyethylglycyl variant of the PNA-guanine monomer ((guanin-9-yl)acetyl-*N*-2-hydroxyethylglycine).

		m/z calcd	*m/z* found (ESI⁺-MS)	T_m (antiparallel DNA)[a]
4	Tgtagatcact-hex	3132.0	3132.7	46.4
5	Tg**t**^{Z}agatcact-hex	3115.0	3115.0	43.4
6	Tgtaga**t**^{Z}cact-hex	3115.0	3114.6	32.2
7	Tgtaga**t**^{E}cact-hex	3115.0	3115.1	39.9
8	Tg**t**^{E}agatcact-hex	3115.0	3115.2	43.3
9	Tgtagatcac**t**^{E}-hex	3115.0	3115.0	47.7
10	d(GTAGATCACT)	–	–	33.5

[a] d(AGTGATCTAC).

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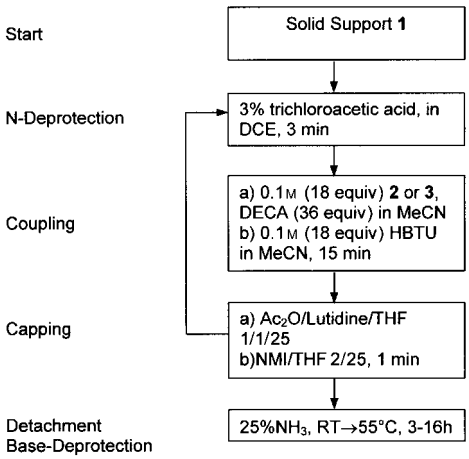
unit was introduced in order to facilitate purification of the oligomers (gel electrophoresis) and to increase their solubility in water.

The syntheses of the fully modified (*E*)- and (*Z*)-OPA decapeptides **13**–**15**, **17**, and **18** (Table 2) started with the glycine-containing solid support **1** and were performed on a 1-μmol scale using a Pharmacia Gene Assembler DNA synthesizer that was reprogrammed accordingly (Scheme 2).



Scheme 2. Cycle and conditions for the solid-phase synthesis of OPA oligomers. DCE = dichloroethane, DECA = diethylcylohexylamine, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NMI = *N*-methylimidazole.

The coupling yields per step, as measured by MMTr assay, were in the range of 88–95% for **2** and **3**, and thus slightly lower than for the synthesis of the PNA control sequence **12** (>98% per step). Whereas oligomers **12**–**14** were prepared with free N termini, sequences **15**–**18** were treated in the last cycle with a thymidine or deoxycytidine phosphoramidite DNA building block under typical DNA synthesis conditions[17] to produce a phosphonamidate cap. This step was again included in order to introduce a negative charge, which facilitates purification and enhances the solubility of the oligomer in water. Deprotection and detachment from the solid support was effected by treatment with concentrated NH_3, (room temperature (RT) or 55 °C, 3 or 16 h) and produced oligomers with C-terminal glycine amide residues. The oligomers **12**–**14** were purified by HPLC, and **15**–**18** by polyacrylamide gel electrophoresis. Again, ESI or matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry confirmed the strucutral integrity of the oligomers (Table 2).

Initially there was concern that migration of the C–C double bond (β,γ-unsaturated amide) could occur under the conditions of the synthesis and deprotection. The ^{1}H NMR spectrum of the crude trimer $t^Zt^Zt^Z$-Gly-NH_2, which was prepared manually on a 10-μmol scale to test this possiblity, showed no signals for products arising from double-bond tautomerism (<1%).

The T_m analysis (UV-melting curves) of the chimaeric PNA/OPA oligomers **5**–**9** paired to their antiparallel DNA complements indicated a general decrease in affinity, relative to the unmodified PNA oligomer **4**, for those cases (**5**–**8**) where the modifications are not at the end of the sequence (Table 1). A dramatic decrease was observed for **6** and **7**, which display modification in the middle of the sequence. Oligomer **7**, which contains t^E-OPA and represents the geometry of the base-linker unit observed for PNA in complexed form, is less destabilized ($\Delta T_m = 6.5$ K) than **6**, which contains the geometrically mismatched monomeric unit t^Z ($\Delta T_m = 14.2$ K).

From these experiments, two conclusions can be drawn: 1) A geometric mismatch of the base-linker unit is associated with an energetic penalty on duplex formation; 2) stereochemical constriction of the base-linker unit into the pairing-competent geometry of PNA does not increase but rather decreases affinity. Assuming that replacement of the amide functionality by the double bond has a minor influence on the conformational preferences of the rest of the PNA unit, there exists a considerable electrostatic contribution of the amide functionality to pairing.

The pure (*Z*)-OPA and (*E*)-OPA decamers **13** and **14** also form complexes of comparable stability with the DNA complement $d(A)_{10}$, as inferred from UV-melting curves (Figure 1a). The T_m values are on the order of that of the DNA control duplex, but distinctly lower than that of the corresponding PNA_2/DNA triplex (Table 2).

Interestingly, neither the (*Z*)-OPA nor the (*E*)-OPA homodecamers **13**–**15** seem to form triplexes with $d(A)_{10}$. This was demonstrated in the case of **15** by a gel shift experiment (Figure 2). For a stoichiometric ratio of 2:1 for **15**:$d(A)_{10}$, a new band attributable to a complex arises on the gel together with a band for uncomplexed **15** (lane 3). At the same time, all the $d(A)_{10}$ was consumed. From this distribution of products we conclude that only a duplex had formed

Table 2. Mass spectrometry data and T_m values [°C] of DNAs, PNAs, and fully modified (*E*)- and (*Z*)-OPA sequences with antiparallel and parallel complementary DNA ($c = 4$ μM in 100 mM NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0). a^E = (*E*)-**3** unit; see Table 1 for further abbreviations.

		m/*z* calcd	*m*/*z* found	T_m	
				antiparallel DNA	parallel DNA
11	$d(T)_{10}$	2980.0	2977.2[a]	20.7	
12	$(t)_{10}$-Gly-NH_2	2736.7	2736.6[a]	66.9	
13	$(\mathbf{t^Z})_{10}$-Gly-NH_2	2566.8	2568.5[a]	23.0	
14	$(\mathbf{t^E})_{10}$-Gly-NH_2	2566.8	2566.6[a]	23.5	
15	$dT(\mathbf{t^E})_{10}$-Gly-NH_2	2869.9	2890.3[b]	n.d.[c]	n.d.[c]
16	dC(ttttaatata)-Gly-NH_2	3060.9	3060.9[b]	29.4	13.1
17	$dC(\mathbf{t^Et^Et^Et^Ea^Ea^Et^Ea^Et^Ea^E})$-Gly-$NH_2$	2891.0	2891.3[b]	<0	16.0
18	$dC(\mathbf{t^Et^Et^Et^Ea^Ea^Et^Za^Et^Ea^E})$-Gly-$NH_2$	2891.0	2891.0[b]	<0	13.5

[a] MALDI-TOF-MS (positive-ion mode). [b] ESI^+-MS. [c] n.d. = not determined.

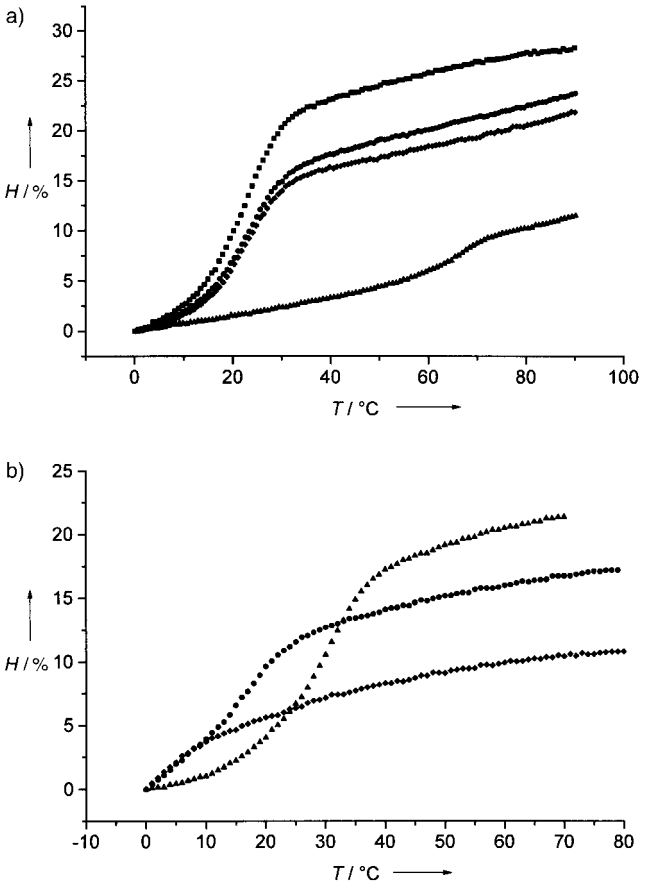


Figure 1. a) The UV-melting curves (H = relative hyperchromicity, λ = 260 nm) of a) ● **11**/dA_{10}, ▲ **12**/dA_{10}, ■ (**13**)/dA_{10}, and ◆ **14**/dA_{10} as well as b) ▲ **16**/antiparallel DNA, ● **17**/parallel DNA, and ◆ **17**/antiparallel DNA. Oligonucleotide concentrations and buffer conditions as in Table 2.

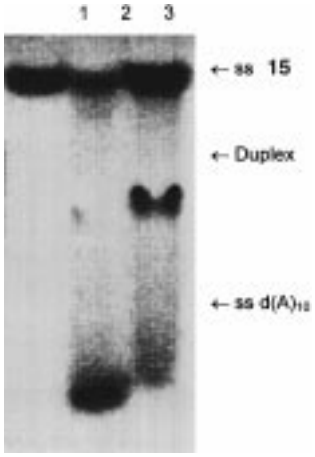


Figure 2. Binding of **15** to $d(A)_{10}$. UV shadowing of a 20% nondenaturing polyacrylamide gel. Buffer: 90 mM Tris · borate, pH 8.1, 10 mM $MgCl_2$. The samples were dissolved in 20 µL of loading buffer (8% sucrose, 10 mM $MgCl_2$, 90 mM Tris · borate) and incubated at 0 °C for 20 min. The gel was run at 120 V for 1 h, then at 90 V for 12 h (T = 5 °C). Lane 1: **15**; lane 2: **15**:$d(A)_{10}$ 1:1; lane 3: **15**:$d(A)_{10}$ 2:1.

under these conditions. The fact that no triplex had formed is also supported by the very different shapes of the melting curves of OPA/DNA and PNA/DNA hybrids (Figure 1 a) as well as by the large differences in T_m between the PNA_2/DNA triplex $(\mathbf{12})_2$:$d(A)_{10}$ and PNA/DNA duplex **16**:d(TATATTAAAA) compared to the relatively similar T_m values of the OPA/DNA duplexes of **13**, **14**, and **17** with the corresponding DNA complements (see Figure 1 and Table 2). Furthermore, in contrast to PNA, no hysteresis was observed in melting/renaturation cycles (rate = 0.5 K min^{-1}), which is again in accord with the fact that no triplex was formed. Thus the behavior of OPA seems to be distinctly different from that of PNA, in that it has lost its ability to form triplex structures with complementary homo-purine DNA sequences.

With oligomer **17**, having an asymmetric sequence distribution of thymine and adenine bases, we investigated the preferred strand orientation in OPA/DNA duplexes (Table 2). Surprisingly it was determined that **17**, in which the bases are preorganized in the same orientation as in PNA/nucleic acid complexes, only forms duplexes with the parallel DNA complement and thus deviates completely from the preferred pairing orientations in PNA/nucleic acid complexes.

Oligomer **18**, in which one t^E unit is replaced by a t^Z unit, was prepared in order to investigate the effect of one stereochemical mismatch in an OPA sequence. When paired to its parallel DNA complement, this structural mismatch results in a penalty of 2.5 K in T_m relative to **17** and highlights a nonnegligible preference for the stereochemically uniform arrangement of the base-linker units in OPA.

The key results obtained are: 1) Both (*E*)- and (*Z*)-OPA sequences bind to complementary DNA with similar affinities as DNA itself; 2) in contrast to PNA, there are no indications of OPA_2/DNA triplex formation with homobasic thymine-containing OPA sequences; 3) in contrast to PNA, OPA binds preferentially in the parallel mode to DNA; 4) se quences containing both (*E*)- and (*Z*)-OPA units in the same strand bind less efficiently than homoconfigured sequences.

The following conclusions can be drawn with respect to molecular recognition of DNA by PNA: The amide functionality in the base-linker unit in PNAs determines significantly the affinity and preferred strand orientation in PNA/DNA duplexes. Furthermore, it seems to be responsible for the propensity to form PNA_2/DNA triplexes. These properties do not depend on the conformational constraints that the amide functionality exerts on the base-linker unit,[18] but rather on its electrostatic properties. Whether solvation, solvent-mediated intrastrand hydrogen bonding, or dipole effects in the OPA strands are responsible for the observed behavior remains open at this point and has to be clarified by further experiments.

With OPA, new and rather unexpected structure – activity relationships in PNA/DNA recognition could be obtained. The results are of importance for the further modification of the PNA skeleton in order to enhance its chemical and biological properties. Furthermore, OPA offers the unique possibility, with respect to PNA, to introduce a fourth substituent on the double bond. This could be of interest, for example, for improving binding or solubility properties, or for the attachment of functional units that may act on bound complementary RNA or DNA.

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An Unexpected Coupling – Isomerization Sequence as an Entry to Novel Three-Component-Pyrazoline Syntheses**

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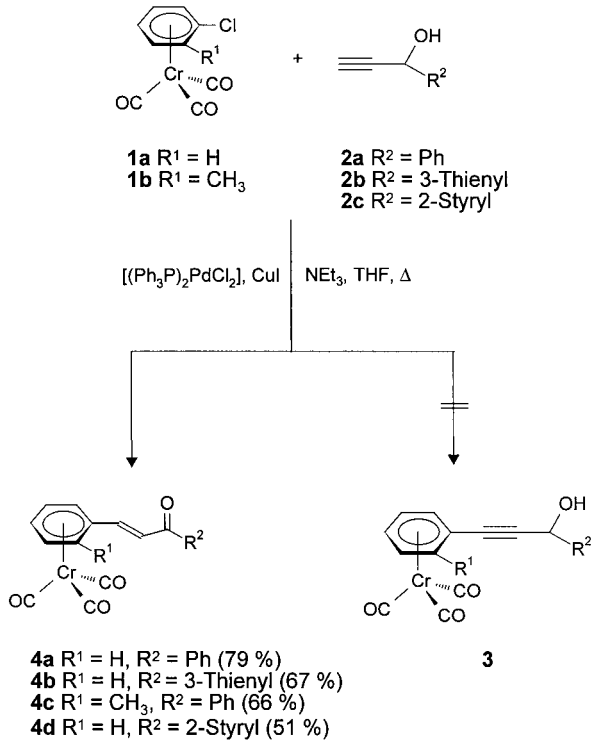
Consecutive reactions[1] have gained increasing importance and interest since efficient syntheses inherently claim to generate a maximum of structural complexity in few steps and in good yields from simple starting materials and with high chemo-, regio-, and stereoselectivity. In such reactions, the directly preceding step forms the functionality necessary for the following transformation. Particularly with regard to multicomponent reactions, the development of novel cascade reactions for the in-situ-generation of reactive functional groups is an intensively pursued goal. Ideally, all these processes occur in a consecutive fashion, occasionally by successive addition of reagents, without the isolation of intermediates and in the sense of a "one-pot synthesis". A model for these consecutive processes are multicomponent condensations[2] as well as palladium-catalyzed cascade reactions,[3] which have proved particularly useful in many cases owing to the mild reaction conditions and the pronounced tolerance for functional groups.

In the course of our work on the chemistry of (arene)Cr-$(CO)_3$ complexes with conjugated side chains,[4] we now have found that the usually reliable Sonogashira coupling[5] of chloroarene complexes **1** with 1-aryl prop-2-ynoles **2** does not furnish the expected alkyne coupling products, the propargyl alcohols **3**, but that the isomeric aryl complexed chalcones **4**[6] are formed in good yields (Scheme 1).



Scheme 1. Synthesis of chromium carbonyl complexed chalcones by means of a coupling – isomerization sequence.

The constitution of the complexed chalcone **4a** was elucidated unambiguously by a selectively ^{1}H-decoupled ^{13}C NMR experiment, and thus, a Meyer – Schuster rearrangement could be ruled out.[7] Remarkably, the chalcones are formed with excellent *trans* selectivity ($^3J = 16$ Hz), indicating a thermodynamically controlled double bond formation.

To the best of our knowledge this unusual reaction has so far only been observed for the coupling of 2-halogen-substituted pyrimidines.[8] In this case the authors explained the mechanism by a coordination of an intermediate during a hydropalladation – dehydropalladation catalytic cycle to the heterocyclic nitrogen atom.[8c] Additionally, some transition metal catalyzed redox isomerizations of propargyl alcohols to

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