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Synthesis and Incorporation into PNA of Fluorinated Olefinic PNA (F-OPA) Monomers

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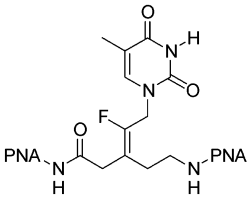
ABSTRACT



A fluorinated OPA monomer containing the base thymine ((*Z*)-t-F-OPA) was synthesized in 12 steps, featuring a highly selective allylic over homoallylic Mitsunobu substitution for the introduction of the nucleobase. F-OPA modified PNA decamers were prepared by the MMTr/acyl protection strategy. The thermal stability of duplexes of PNA decamers containing (*Z*)-t-F-OPA units with antiparallel complementary DNA was measured. We found a strong dependence of stability from the sequential position of the (*Z*)-t-F-OPA units, ranging from ΔT_m of +2.4 to −8.1 °C/modification relative to unmodified PNA.

The peptide nucleic acid (PNA) is a DNA analogue entirely based on an achiral polyamide backbone.[1] PNA readily undergoes sequence-specific Watson−Crick base-pairing with complementary DNA and RNA.[2] The relatively high binding affinity of PNA toward natural oligonucleotides is attributed to the lack of electrostatic repulsion between the uncharged PNA backbone and the negatively charged sugar−phosphate backbone of DNA and RNA.[3]

An interesting structural feature of PNA is the central amide linker, connecting the nucleobases with the amide backbone (Figure 1). The carbonyl oxygen of these units uniformly points toward the carboxy termini in PNA/DNA,[4,5] PNA/RNA,[6] and PNA/PNA[7] complexes, whereas both rotameric forms coexist in the free monomer. Furthermore, in the crystal structures of a 50% N-methylated PNA hexamer[8] and of a hexameric PNA containing the 1,8-naphthyridin-2(1*H*)-one base,[9] the same situation was encountered, revealing that structural alterations in the base and in the inter-residue amide bond have no influence on the orientation of the linker-carbonyl unit.

To structurally constrain the base-linker unit in PNA and to explore its electrostatic contribution to complementary DNA binding, we recently designed and investigated the olefinic peptide nucleic acids (OPA), built from units of **2** and **3**[10,11] (Figure 1).

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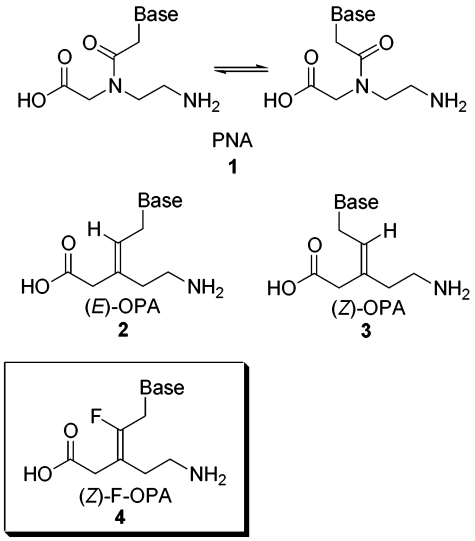


Figure 1. Two rotameric forms of the PNA monomers (top) and chemical structures of the (*E*)- and (*Z*)-OPA monomers (center) and of the F-OPA monomer (bottom).

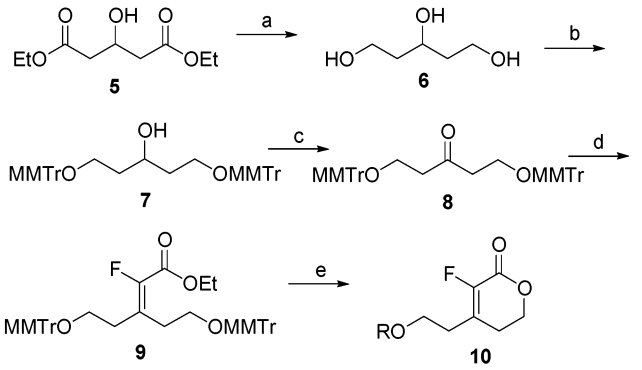
So far, we have investigated fully modified OPA-oligoamides containing the bases adenine and thymine. We found that (*E*)-OPA forms preferentially parallel duplexes with DNA of markedly lower affinity compared to unmodified PNA. Furthermore, oligothymine-OPA was unable to form triplexes with DNA. Thus, it became clear that the amide function in the base-linker unit in PNA significantly determines affinity and strand orientation in PNA/DNA duplexes. We reasoned that the differences in the recognition properties of OPA are mainly related not to conformational differences relative to PNA but rather to changes in the H-bonding capacity, electrostatic properties, or solvation.

Consequently, the intriguing question arose as to what extent the dipole moment of the linker-carbonyl group in PNA influences its binding properties. The OPA scaffold is ideally suited to address this question. Since a C−F bond is structurally and electrostatically a decent mimic of a C═O bond without hydrogen bonding capacity[12] and was often used in that respect,[13] we decided to synthesize and investigate the pairing properties of the (*Z*)-F-OPA system **4** (please note that the (*Z*)-F-OPA system has the same geometric configuration as the (*E*)-OPA series). Introduction of the fluorine atom at the vinylic position thus mimicks the geometry and in part the dipole moment of the carbonyl group in bound PNA.

Herein, we report on a highly stereoselective synthesis of the novel fluorinated (*Z*)-F-OPA building block **17** containing the base thymine, its incorporation into PNA via the MMTr/acyl protecting group strategy, and first pairing experiments with complementary antiparallel DNA.

We started the synthesis of the tetrasubstituted olefin with the commercially available 3-hydroxy glutarate **5** and followed a Wittig strategy for the introduction of the vinylic fluorine. Desymmetrization of the double bond was controlled via lactone formation (Scheme 1).

Scheme 1[a]



[a] Reagents and conditions: (a) $LiAlH_4$, THF, rt, 4 h; (b) MMTrCl, DMAP, pyridine, rt, 8 h; (c) IBX, DMF, rt, 3 h; (d) *n*-BuLi, $(EtO)_2P(O)CHFCO_2Et$, THF, −78 °C, 1.5 h, then **8**, THF, −78 °C → rt, 5 h; (e) BCl_3, CH_2Cl_2, rt, 2 h.

Reduction of **5** with $LiAlH_4$ yielded triol **6** (65%), which was then bis-MMTr protected (82%). Oxidation of alcohol **7** under mild conditions afforded ketone **8** (79%) which was subsequently converted to the α-fluoroester **9** by means of a Wittig-Wadsworth-Emmons[14] reaction in 67% yield. *β*-Elimination of the MMTrO moieties as a side reaction during olefination was observed but could be controlled.

To chemically differentiate between the two alcohol moieties, ester **9** was converted to intermediate **10** by means of a deprotection−lactonization step mediated by BCl_3 in 77% yield.[15]

Lactone **10** was then TBDMS protected (99%) and reduced to diol **12** (94%) under Luche conditions with $NaBH_4/CeCl_3$ in methanol (Scheme 2).[16] In our plan, we had hoped to be able to chemically differentiate between the two primary hydroxyl functions in **12**, reasoning that the allylic nature of one of the two leads to an advantage in reactivity during substitution. Indeed, introduction of the N^3-benzoyl-protected thymine[17] under Mitsunobu conditions led to a rewarding 72% yield of **13**. No isomeric substitution product could be isolated (Scheme 2).

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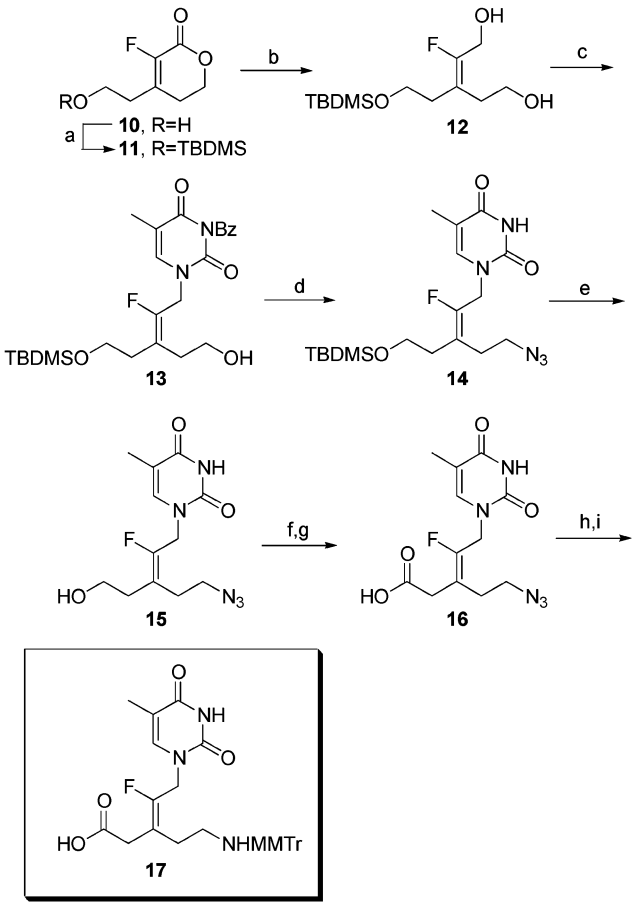
Scheme 2[a]



[a] Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, 12 h; (b) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, methanol, 0 °C→ rt, 4.5 h; (c) N^3-benzoyl thymine, PPh_3, DIAD, THF, rt, 12 h; (d) LiN_3, CBr_4, PPh_3, DMF, 0 °C→ rt, 12 h; (e) TBAF, THF, rt, 5 h; (f) Dess−Martin periodinane, CH_2Cl_2, rt, 2 h; (g) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH, rt, 12 h; (h) PPh_3, pyridine, concentrated NH_3, rt, 3 h; (i) MMTrCl, pyridine, rt, 12 h.

Azidation of **13**, again by Mitsunobu reaction,[18] provided **14** in excellent yield (92%). The N^3-benzoyl protecting group of the nucleobase neatly departed under these conditions. TBDMS deprotection to alcohol **15** (75%) was followed by a two-step oxidation of the hydroxy function. In the first step, **15** was oxidized to the corresponding aldehyde using the Dess−Martin periodinane and was subsequently further oxidized with sodium chlorite in *t*BuOH, with 2-methyl-2-butene as a Cl^+ scavenger,[10] to the acid **16** (68% for the two steps). Conversion of the azide to the amine under Staudinger conditions, followed by MMTr protection, afforded the monomer building block **17** in 35% yield (two steps).

PNA oligoamides containing the (*Z*)-t-F-OPA unit and the corresponding (*Z*)- or (*E*)-OPA units for comparison were synthesized according to the MMTr/acyl solid-phase peptide synthesis developed earlier.[19] A CPG support, carrying a glycine ester unit as the anchoring point was used as a solid phase,[11] and the N-termini of the oligoamides were equipped with lysine units in order to enhance water solubility and ease purification. The oligomers were isolated by reversed-phase HPLC using typical procedures for PNA purification. The composition of the purified oligomers was confirmed by ESI^+-TOF mass spectrometry as shown in Table 1. The coupling yields for the OPA and (*Z*)-t-F-OPA building blocks were in the range of 90−95%, as measured by the trityl assay.

Table 1. Mass Spectrometric and T_m Data from UV-Melting Curves of PNA Sequences Containing (*E*)-t-OPA, (*Z*)-t-OPA, or (*Z*)-t-F-OPA Units with Complementary DNA

	sequence[a]	m/z (calcd)	m/z (found)[b]	T_m (°C)[c]
18	Lys-TTTTAATATA-Gly-NH_2	2900.9	2900.1	33.2
19	Lys-TTTTAA**t^E**ATA-Gly-NH_2	2883.9	2883.3	36.7
20	Lys-TTTTAA**t^Z**ATA-Gly-NH_2	2883.9	2883.3	28.0
21	Lys-TTTTAA**t^F**ATA-Gly-NH_2	2901.9	2901.3	35.6
22	Lys-TTT**t^E**AATATA-Gly-NH_2	2883.9	2883.2	30.0
23	Lys-TTT**t^F**AATATA-Gly-NH_2	2901.9	2901.6	27.0
24	Lys-TT**t^E**TAATATA-Gly-NH_2	2883.9	2883.2	28.1
25	Lys-TT**t^F**TAATATA-Gly-NH_2	2901.9	2901.6	25.1

[a] t^Z = (*Z*)-t-OPA, t^E = (*E*)-t-OPA, and t^F = (*Z*)-t-F-OPA. [b] ESI^+-MS. [c] T_m data are from UV-melting curves (260 nm) at 4 μM duplex concentration in 100 mM NaCl, 10 mM Na_2HPO_4, pH 7.0; with antiparallel DNA complement d(TATATTAAAA).

The oligomers were hybridized with the corresponding antiparallel DNA complement, and the thermal stability of the duplexes was examined by UV-melting curve analysis. Representative melting curves (260 nm) are reproduced in Figure 2. The corresponding T_m data are summarized in Table 1.

PNA oligomer **21**, containing the (*Z*)-t-F-OPA unit in a central position of the sequence, showed a stabilization of the duplex (ΔT_m = +2.4 °C) compared to the unmodified PNA oligomer **18**. Introduction of an (*E*)-OPA unit at the same location in the sequence (**19**) also led to a stabilization (ΔT_m = +3.5 °C), while a (*Z*)-OPA unit (**20**) strongly destabilized the duplex, due to the structural mismatch imposed by the (*Z*)-configuration of the double bond.

A marked sequence dependence that had already been observed previously,[11] but to a lesser extent, appeared upon introducing (*E*)-t-OPA units in various locations of the strand. Indeed, insertion of an (*E*)-t-OPA unit between two adenine PNA units, as in **19**, leads to stabilization, whereas positioning of a (*E*)-t-OPA between two thymine PNA units, as in **24**, leads to a significant decrease in the T_m (ΔT_m = −5.1 °C). Positioning between an adenine and a thymine PNA unit, as in **22**, leads to an intermediate T_m (ΔT_m = −3.2 °C compared to **18**). Substitution by (*Z*)-t-F-OPA, as in **21**, **23**, and **25**, essentially follows the same stability pattern as in

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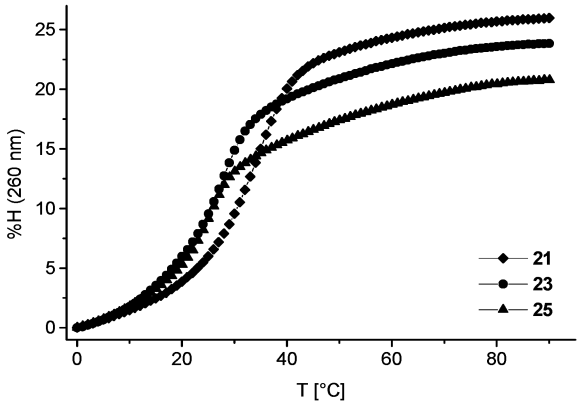

Figure 2. UV-melting curves (260 nm) of duplexes of oligoamides **21**, **23**, and **25** with the corresponding antiparallel oligodeoxynucleotide d(TATATTAAAA). Experimental conditions were the same as those in Table 1.

the case of the (*E*)-t-OPA units, but with more pronounced sequence-stability effects. Here the differences in T_m, relative to PNA, vary between +2.4 and −8.1 °C.

Various effects can be responsible for the observed sequence dependence in DNA affinity. It is certainly possible that OPA units intrinsically may prefer a base-linker conformation that is different from that in PNA. Assuming that this preferred conformation is independent of nearest neighbor influence, this would not, however, explain the sequence effects. Replacing a nonpolar H-atom at the double bond by a more polar F-atom does not change the sequence−affinity pattern of the OPA system, ruling out dipole differences in the base-linker units as being mainly responsible for this. Thus, there remain differential stacking interactions of the OPA−base-linker units and/or differential solvation patterns of this subunit as likely sources for the differential behavior relative to PNA.

In conclusion, we have synthesized a novel, fluorinated olefinic peptide nucleic acid monomer (*Z*)-t-F-OPA in 12 steps, the key steps being a lactonization as a stereodifferentiating step in the elaboration of the double bond and a highly selective Mitsunobu substitution of an allylic over a homoallylic hydroxy function by the thymine base. Incorporation of (*Z*)-t-F-OPA into PNA was achieved by the MMTr/acyl strategy. It was found that duplex stability strongly depends on the position of the modification within the strand. The reason for this is still unknown, but might likely be caused by the differential stacking interactions or solvation properties of the OPA units relative to those of PNA. Dipole effects of the base-linker unit could be excluded.

With the synthetic access to F-OPA accomplished, it will now certainly be of interest to investigate the pure F-OPA system. Differential analysis of the pairing properties with PNA and OPA will shed light on dipolar vs solvation or H-bonding effects of the respective structural elements on duplex stability and will thus advance our understanding of fundamental aspects of supramolecular folding and assembly.[20]

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Supporting Information Available: Experimental procedures and analytical data of compouds **7**−**17** as well as a general procedure for the synthesis and purification of oligomers **18**−**25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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