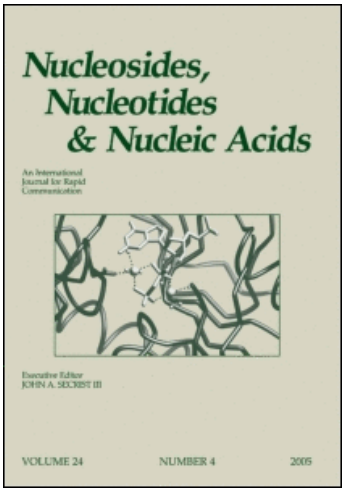

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Fluorinated Peptide Nucleic Acid

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ABSTRACT

The fluorinated olefinic peptide nucleic acid analogue (F-OPA) monomer containing the base thymine was synthesised in 13 steps. PNAs containing this unit were prepared and their pairing properties assessed by means of UV-melting experiments.

Polyamide or peptide nucleic acids **1**, first described in 1991, are DNA analogues entirely based on an achiral polyamide backbone.[1] The PNAs undergo sequence-specific and efficient Watson-Crick base pairing with complementary DNA and RNA.[2,3] One structural feature of PNA is the central amide linker connecting the base to the backbone. The carbonyl oxygens of this unit, uniformly point towards the carboxy termini in PNA/DNA,[4,5] PNA/RNA[6] and PNA/PNA[7] complexes, whereas both rotameric forms co-exist in the free monomer. In order to elucidate this structural ambiguity, the olefinic peptide nucleic acids (OPAs) have been synthesised and studied (Fig. 1).[3] Fully modified OPA oligoamides resulted in a marked decrease in affinity towards complementary DNA, compared to PNA. In order to investigate the effect of the dipole moment of the linker carboxy group while

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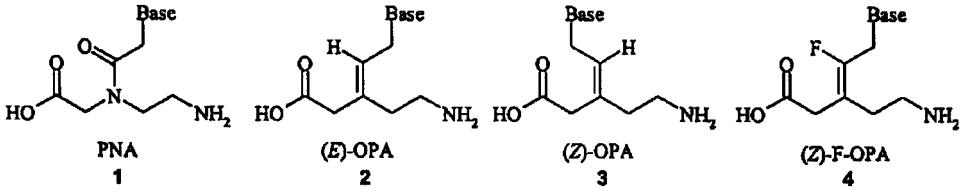
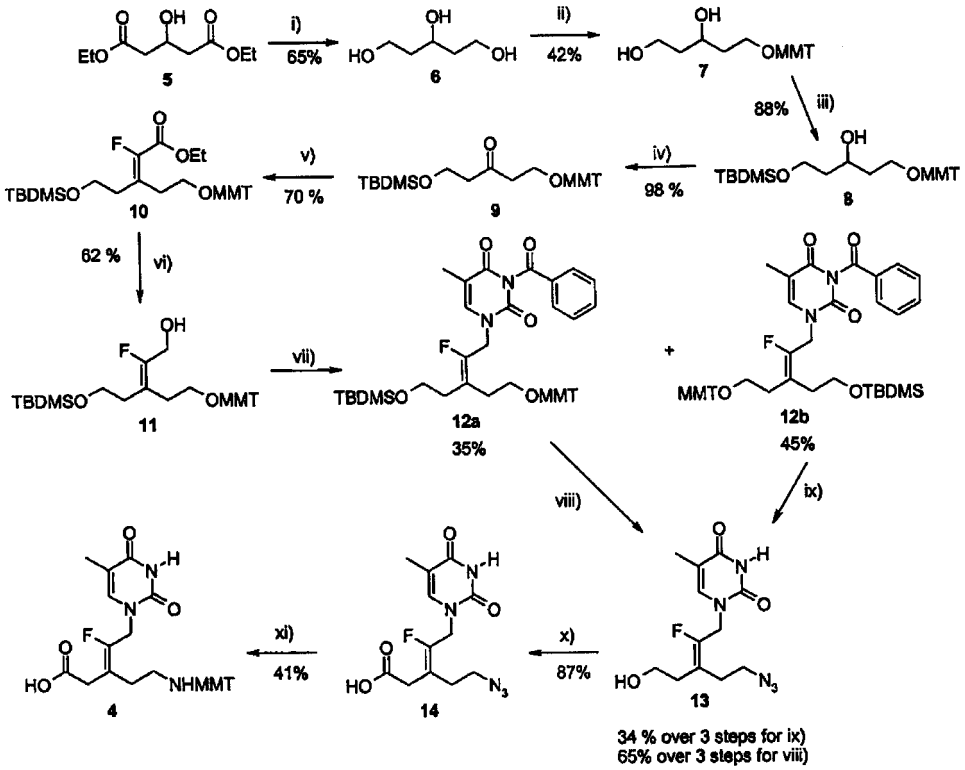


Figure 1. Chemical structure of the monomeric units of the different peptide nucleic acids.

maintaining the geometry of the C=C double bond, (*Z*) -t-F-OPA **4** was synthesised and incorporated into PNA.

The synthesis of the monomeric unit **4** containing the base thymine is outlined in Sch. 1.



Scheme 1. i) $LiAlH_4$, THF, RT, 3 h. ii) MMTrCl, Pyridine, RT, overnight. iii) TBDMSCl, Pyridine, RT, overnight. iv) IBX, THF/DMSO 1/1, RT, 6 h. v) 1) n-BuLi, $(EtO)_2P(O)$-$CHFCO_2Et$, THF, −78°C, 2 h. 2) Ketone, −78°C to RT, 4 h. vi) $LiAlH_4$, Et_2O, RT, 2 h. vii) TBz, PPh_3, DIAD, THF, RT, overnight. viii) 1) BCl_3, CH_2Cl_2, −40°C, 30 min. 2) LiN_3, PPh_3, CBr_4, DMF, RT, overnight. 3) TBAF, THF, RT, overnight. ix) 1) TBAF, THF, RT, 5 h. 2) LiN_3, PPh_3, CBr_4, DMF, RT, overnight. 3) BCl_3, CH_2Cl_2, −40°C, 30 min. x) 1) Dess-Martin, CH_2Cl_2, RT. 2) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, t-BuOH. xi) 1) Lindlar catalyst, H_2, RT. 2) MMTrCl, Pyridine, RT.

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Table 1. Mass spectrometry data and *T*m values [°C] (UV-melting curves, 260 nm) of PNA sequences containing (*E*)-t-OPA, (*Z*)-t-OPA or (*Z*)-t-F-OPA units with parallel and antiparallel DNA (c = 4 μM in 100 mM NaCl, 10 mM Na_2HPO_4, pH 7.0). Lowercase letters: PNA units; t^Z = (*Z*)-t-OPA, t^E = (*E*)-t-OPA and t^F = (*Z*)-t-F-OPA.

		m/z calcd	m/z found (ESI^+-TOF)	T_m (antiparallel DNA)[a]	T_m (parallel DNA)[b]
15	Lys-ttttaatata-Gly-NH_2	2900.9	2900.10	33.2	< 0
16	Lys-ttttaa**t**$^{\mathbf{E}}$ata-Gly-NH_2	2883.9	2883.30	36.7	n.d.[c]
17	Lys-ttttaa**t**$^{\mathbf{Z}}$ata-Gly-NH_2	2883.9	2883.32	28.0	< 0
18	Lys-ttttaa**t**$^{\mathbf{F}}$ata-Gly-NH_2	2901.9	2901.26	35.6	n.d.
19	Lys-ttt**t**$^{\mathbf{E}}$aatata-Gly-NH_2	2883.9	2883.17	30.0	11.0, 34.0
20	Lys-tt**t**$^{\mathbf{E}}$taatata-Gly-NH_2	2883.9	2883.19	28.1	n.d.

[a]d(AAAATTATAT).
[b]d(TATATTAAAA).
[c]Not determined.

In order to study the pairing properties, oligomers **15**–**20** were prepared and the stability of the duplexes formed with anti-parallel and parallel DNA was assessed by means of UV-melting curves (Table 1). Introduction of the modified units leads to a marked difference in T_m as a function of the position of the modification in the sequence. Indeed, positioning of a (*E*)-t-OPA unit between 2 purine bases leads to a stabilisation of the duplex ($\Delta T_m = +3.5°C$), while introduction of this unit between 2 pyrimidine units leads to a marked destabilisation ($\Delta T_m = -5.1°C$). Positioning between one pyrimidine and one purine base leads, as expected, to an intermediate value ($\Delta T_m = -3.2°C$). The (*Z*)-t-F-OPA modification leads to a stabilisation comparable to the one observed for (*E*)-t-OPA ($\Delta T_m = +2.4°C$), whereas a substantial decrease of duplex stability is observed for the (*Z*)-t-OPA unit ($\Delta T_m = -5.2°C$).

The introduction of the fluorine atom at that location could alter the electrostatic properties and result in a reduced stacking ability. This could account for the lower T_m value obtained for oligomer **18** compared to the one for oligomer **16**. However, the effect on the dipole moment on the whole oligomer is yet unknown, and only a fully modified (*Z*)-t-F-OPA strand could provide with an answer.

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