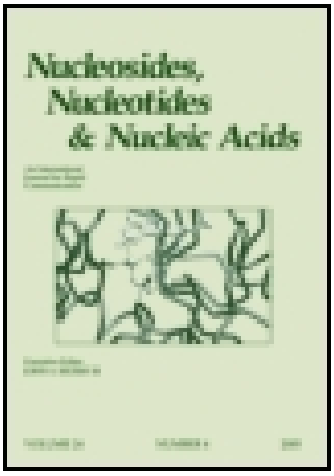

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Synthesis and Hybridization Properties of Modified Oligonucleotides with PNA-DNA Dimer Blocks

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SYNTHESIS AND HYBRIDIZATION PROPERTIES OF MODIFIED OLIGONUCLEOTIDES WITH PNA-DNA DIMER BLOCKS

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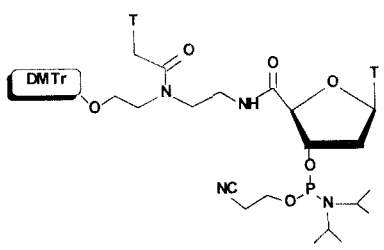
ABSTRACT: Different modified PNA-DNA dimer-analogous synthons (I and II) were synthesized as phosphoramidites. These dimer units were assembled by a 5'-modified deoxythymidine and a modified PNA monomer. These synthons were used in the routine coupling procedure for oligonucleotides. Therefore no PNA coupling chemistry is necessary to synthesize PNA-DNA chimeric oligonucleotides. Various deoxyoligonucleotides were synthesized introducing the dimer blocks I and II at different positions in the sequences. Melting temperatures of the modified oligonucleotides with their complementary DNA analogues were determined.

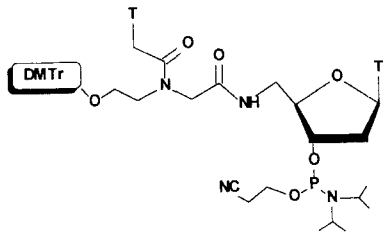
Backbone modifications of oligonucleotides are required in the antisense strategy for protection against endonucleolytic cleavage in biological environment. Peptide nucleic acids (PNA fragments) are known to be nuclease resistant analogues, which show stable and discriminating hybridization[1]. For this reason we prepared chimeric PNA-DNA oligomers by incorporation of two different modified PNA-DNA dimer blocks (Scheme A) into oligonucleotides. Melting temperatures of the modified oligonucleotides with their complementary DNA were determined.

Results and Discussion

The syntheses of the modified PNA backbone structures 1 and 2 is outlined in scheme B. Synthesis of modification 1 started with N-(2-hydroxyethyl)-ethylenediamine. We selectively protected the hydroxyl-group with tert.-butyldiphenylchlorosilane and the amino

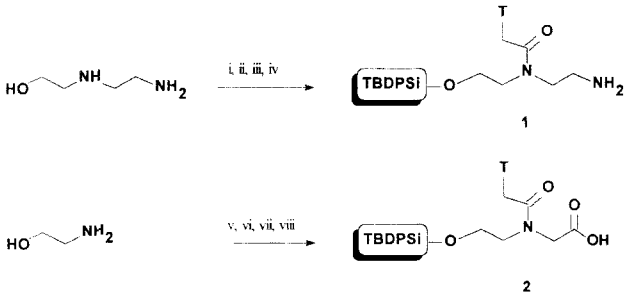


Synthon I



Synthon II

Scheme A



(i) TBDPSiCl in pyridine, 8h. (ii) FMOC-ONSu in dichlormethane, 4h. (iii) DCC, DhbtOH, TCH_2COOH in DMF 1h 0°C, 3h RT. (iv) 10% piperidine in dichlormethane 0°C, 0.5h. [DhbtOH = 3.4-dihydro-3-hydroxy-4-oxo-1.2.3-benzotriazine]

(v) TBDPSiCl in pyridine, 8h. (vi) $BrCH_2COOCH_3$, Et_3N in toluene and dioxane. (vii) DCC, DhbtOH, TCH_2COOH in DMF. (viii) 1M LiOH aq. in THF, 1h/ 1M HCLaq.

Scheme B

function with [N-(9-fluorenylmethoxycarbonyl)-succinimide]. In a third step the thyminyl-acetic-acid (TCH_2COOH) was coupled via an amide bond to the secondary amino function. Under the deprotection conditions in step (iv) no acyl migration was observed. However we observed an acyl migration under different deprotection conditions (e.g. <20% piperidine in dichlormethane, 25°C, 1h)[2]. The total yield for the synthesis of PNA construct 1 was 25%. The synthesis of PNA modification 2 started, deviating from the synthesis of the ordinary PNA monomers with aminoethanol. The hydroxyl-group of aminoethanol was also selectively protected by TBDPSiCl. The further synthesis of construct 2 was the same as described for the ordinary PNA monomer[3] (Total yield 29%). PNA constructs 1 and 2 (Scheme B) were both coupled via an amide bond with modified

Tab. 1 T_m values of the modified oligonucleotides

Modified oligonucleotide no. (5'-3')	unmodified T_m	modified T_m
1 CACCAACT*TCTTCCACA	60.5 (60.0)	49.5 (48.5)
2 CACCAACT*TCT*TCCACA	60.5 (60.0)	37.6 (37.2)
3 TTAACTTCTTCACAT*TC	49.5 (48.2)	44.8 (43.3)
4 CACCAACT#TCTTCCACA	60.5 (60.0)	50.6 (49.7)
5 CACCAACT#TCT#TCCACA	60.5 (60.0)	38.5 (37.2)
6 TTAACTTCTTCACAT#TC	49.5 (48.2)	45.2 (43.2)
7 T#TAACTTCTTCACAT#TC	49.5 (48.2)	48.4 (47.3)

T*T = Synthon I, **T#T** = Synthon II, Buffer: 1xSSC (165mM Na^+), ramp: up, (down), ramp rate: 0.5°C/min.

deoxythymidine (1 with thymidine-5'-carboxylic acid and 2 with 5'-amino-5'-deoxy-thymidine). The hyroxyl-group was selectively deprotected by TBAF. After tritylation by DMTrCl and phosphorylation by β-cyanoethyl N,N-diisopropylchlorophosphoramidite we obtained synthon I and synthon II. Various oligonucleotides were prepared by introducing the two dimer blocks I and II at different positions in the sequences (Tab.1). The coupling efficiencies of the dimer blocks in a commercial DNA synthesizer were similar as for commercially available phosphoramidites (ca. 99%)

After purification of the oligonucleotides by a RP-HPLC, the melting temperatures of the modified oligonucleotides with their complementary DNA sequences were determined and compared to the corresponding T_m values of the natural DNA-DNA duplexes (Tab. 1). The introduction of a single PNA-DNA dimer block in the middle of the sequences lowered the T_m values in a range from -11.5°C (synthon I) to -9.9°C (synthon II) and ca.--4.5°C on positioning the dimer blocks at the end of the sequences (Tab. 1.). This very high decrease of T_m is approximately in the same range as for incorporation of unmodified PNA monomers in a natural DNA[4]. Incorporation of two dimer blocks leads to a nearly additive drop of T_m (ca. 22°C). Otherwise conjugates composed of a PNA and a DNA block show very stable and discriminating hybridization[5] and small decrease of T_m. Oligonucleotide no.7 (Tab.1) with the synthon II at both ends of the sequence shows a lower decrease of T_m (ca. 1°C, synthon II, Tab.1).

Experimental

Syntheses were controlled by ^1H, ^{13}C, ^{31}P, 2D H,H COSY and 2D H,C COSY 200MHz and 500MHz-NMR (Bruker AC 200, Bruker AM 500) and by MS (Varian MAT 711, Finnigan MRT, Finnigan TSU 7000). The synthesis of the oligonucleotides was done on

a DNA synthesizer (Pharmacia Gene Assembler 4 Primers). The oligonucleotides were purified by a RP-HPLC system (Applied Biosystem Modell 152 A, LiChroCART 125-4 Merck). Melting temperatures were determined with a Beckmann DU 7500 Spectrophotometer.

References and Footnotes

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