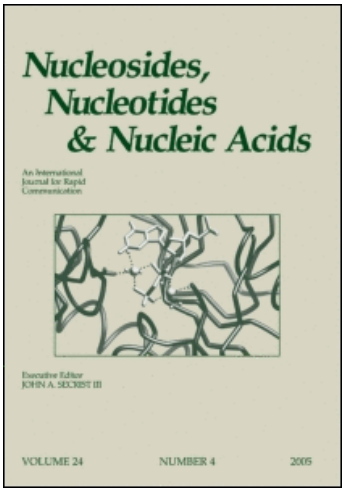

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PHENYLALKYL BACKBONE MODIFIED OLIGODEOXYNUCLEOTIDES, THEIR SYNTHESIS AND THE INFLUENCE OF THE ALKYL CHAIN LENGTH

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ABSTRACT

Phenylalkyl modified phosphoramidites (alkyl chain length $n = 1, 2, 3, 5$; Fig. 1) were synthesised and incorporated into a DNA hexamer (5′-d(GCC**p**-GCG); **p** = place of modification). The obtained diastereomeres were separated by RP-HPLC. After hybridisation with the complementary DNA strand T_m-value and thermodynamic data were measured. The stability of duplexes depends on the linker length and the absolute configuration of the backbone modified oligodeoxynucleotides (Rp, Sp).

INTRODUCTION

Backbone modified oligonucleotides have been well studied as antisense oligonucleotides (1). Benzyl modified oligonucleotides were investigated against Hepatitis C (2). Unfortunately most of the employed modifications decrease duplex stability to the complementary DNA/RNA strand. Therefore our aim is to design new modifications which could increase duplex stability by intramolecular intercalation. The potential intercalating agents were covalently linked via a methylene-linker to the oligonucleotide backbone. Further studies have shown the possible intercalation of intercalating agents at backbone modified oligonucleotides (3). Thereby oligothymidylates attached to an acridine dye through the 3′-phosphate

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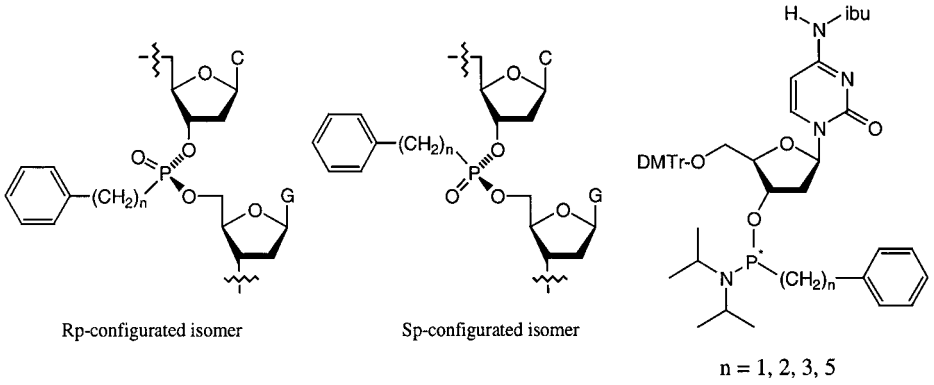

Figure 1. Structure of Rp- and Sp-configurated oligomers; synthesised phosphoramidites.

group specifically interact with the complementary sequence. We synthesised phenylalkyl modified phosphoramidites with different length of the alkyl chain, incorporated these into oligonucleotides (Fig. 1) and measured the T_m-value of the diastereomerically pure duplexes.

RESULTS AND DISCUSSION

1. Synthesis of phosphoramidites

For the synthesis of phenylalkyl modified phosphoramidites (Fig. 1) N^2-isobutyryl-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2′-deoxycytidine were reacted with phenylalkylchloro-*N*,*N*-diisopropylaminophosphane (n = 1, 2, 3, 5) (4,5). The phosphitylating agents were prepared by reaction of phenylalkylbromides with Mg, followed by transmetallation with $CdCl_2$ and reaction of these cadmium organic intermediates with phosporotrichloride at −80°C (argon atmosphere) to the corresponding phenylalkyldichlorphosphanes (5). The phosphanes obtained were subsequently substituted with two equivalents *N*,*N*-diisopropylamine to phenylalkyl-*N*,*N*-diisopropylaminochlorphosphanes (4).

2. Oligonucleotide synthesis

We incorporated the modified phosphoramidites (Fig. 1) on a DNA-synthesizer into a dC-dG hexamer (5′d(GCC**p**GCG); **p** = place of modification) using increased coupling times for modified amidites. In all cases purification and separation of diastereomers was achieved with the fully deprotected oligonucleotides (deprotection with EDA-mix for 3 hours (ethylenediamine/ethanol/acetonitrile/water 50:24:24:2) by RP-HPLC on a Lichrospher RP 18 column. The fast eluating isomer is, according to methyl-modified oligonucleotides Rp-configurated, the slow isomer is

Table 1. T_m-values and Thermodynamic Data of Non Self Complementry Modified and Unmodified 5′d(GCC**p**GCG) (p = place of Modification) Hexamers (Paired with 3′d(CGGCGC))[a)]

Duplexes	Isomer	T_m [°C]	ΔT_m [°C]	ΔH [kcal/mol]	ΔS [cal/mol]	ΔG [kcal/mol]
5′d(GCCGCG) 3′d(CGGCGC)	—	44.3	—	−53	−139	−11.6
5′d(GCC°GCG)	S_p	41.0	−3.3	−48	−126	−10.5
3′d(CGG CGC)	R_p	45.5	+1.2	−54	−142	−11.7
5′d(GCC×GCG)	S_p	39.8	−4.5	−50	−134	−10.1
3′d(CGG CGC)	R_p	37.8	−6.5	−49	−129	−10.6
5′d(GCC•GCG)	S_p	39.3	−5.0	−53	−142	−10.7
3′d(CGG CGC)	R_p	37.1	−7.2	−49	−130	−10.3
5′d(GCC∗ GCG)	S_p	38.9	−5.4	−47	−124	−10.0
3′d(CGG CGC)	R_p	36.2	−8.1	−48	−127	−10.1

[a)] measured in phosphate buffer (10 mmol phophate, 140 mmol Nacl, pH 7.0, oligomer concentration is 19 μmol/l). alkyl chain length: °n = 1; × n = 2; • n = 3; ∗ n = 5.

Sp-configurated (6). All oligonucleotides were characterised by MALDI-TOF-MS and were in good agreement with the calculated molecular weights.

3. Stability of the duplexes

The melting temperatures (recorded with a Varian Cary-UV/VIS spectrophotometer) and the thermodynamic properties of diastereomerically pure modified and unmodified duplexes are shown in Table 1.

The introduction of a benzyl group (n = 1) had been shown to decrease the T_m-value for the Sp-configurated isomer and a small stabilisation for the Rp-configurated modification compared to their unmodified analogues was observed. With the change of the alkyl length (n = 2, 2-phenylethyl) the T_m-value of duplexes decreased for both diastereomers. In this case we observed that the Sp-configurated isomer is more stable than the Rp-configurated isomer. An explanation of these findings are possible interactions like DNA-intercalation or other π-π contacts of the benzene ring (Sp-configurated isomer) into the DNA-duplex. Extension of the alkyl chain length to n = 3 (3-phenylpropyl) and n = 5 (5-phenylpentyl) caused only a small decrease respectively increase of duplex stability compared to the 2-phenylethyl modified duplexes (n = 2).

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REFERENCES

1. De Mesmacker, A.; Häner, R.; Martin, P.; Moser, H.E. *Acc. Chem. Res.* **1995**, *28*, 366–374.
2. a) Caselmann, W.H.; Eisenhardt, S.; Alt, M.; *Intervirology* **1997**, *40*, 394–399. b) Alt, M.; Eisenhardt, S.; Serve, M.; Renz, R.; Engels, J.W.; Caselmann, W.H. *Eur. J. Clin. Invest.* **1999**, *29(10)*, 868–876.
3. a) Asseline, U.; Delarue, M.; Lancelot, G.; Toulmé, F.; Thuong, N.T.; Montenary-Garestier, T.; Hélène, C. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3297–3301. b) Asseline, U.; Thuong, N.T.; Hélène, C. J. *Biol. Chem.* **1985**, 260, *15*, 8936–3941.
4. Amberg, S.; Engels, J.W. in preparation.
5. a) Samstag, W.; Engels, J.W. *Angew. Chemie, Int. Ed. Engl.* **1992**, *31*, 1386–1389. b) Collins, D.J.; Rowley, L.E.; Swan, J.M. *Aust. J. Chem.*, **1974**, *27*, 831–839.
6. a) Han, F.; Watt, W.; Duchamp, D.J.; Callahan, L.; Kézdy, F.J.; Agrawal, K. *Nucleic Acid Res.* **1990**, *18*, 2759–2767. b) Löschner, T.; Engels, J.W. *Nucleic Acid Res.* **1990**, *18*, 5083–5088.

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