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A Novel PNA-Monomer for Recognition of Thymine in Triple-Helix Structures

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ABSTRACT

To expand the triplex recognition repertoire of Nucleic Acids, novel nucleobases that recognize thymine in a T-A base pair are still required. A novel conformationally constrained PNA-monomer (II) capable of binding T in a triplex motif was designed and synthesized in 7 steps starting from commercially available dimethyl 2-oxoglutarate.

Triplex targeting of double-stranded DNA is essentially limited to homopurine stretches. Therefore novel nucleobases that recognise C(-G) and especially T (-A) are required. The known E-base PNA-monomer (\mathbf{I})^[1] synthesized some years ago is able to recognise T when incorporated into the Hoogsteen strand of a bis-PNA. But binding affinity is low which might be due to excessive flexibility and/or steric clash with the 5-methyl group of thymine. E-base PNAs bind somewhat stronger to uracil than thymine containing targets.^[1] The new analogue (**II**) contains a double bond, thereby introducing restricted flexibility in the linker. The hope is to preorganise the mono-

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Figure 1. Structures of the known E-base PNA-monomer^[1] (I) and the new analogue (II).

mer in a conformation more favourable for binding to thymine. The binding motif is the same for both monomers. The NH hydrogen functions as a donor and binds the 4-oxo group of thymine.

RESULTS AND DISCUSSION

In order to prepare the conformationally constrained PNA-monomer (8) commercially available dimethyl 2-oxoglutarate (1) was chosen as starting material. The first step was a ring closure to the known pyridazinone $(2)^{[2]}$ using hydrazine and acid catalysis in methanol. To improve hydrophobicity compound 2 was reacted with *p*-methoxybenzyl chloride and sodium hydride in DMF to obtain the PMB protected product (3). The ester functionality of 3 was subsequently reduced to the primary alcohol (4) using sodium borohydride in a refluxing mixture of THF and methanol.^[3] Some cleavage of the ester was also observed due to the alkaline conditions, but to a much lesser extent than when pure hydroxylic solvent was used. Treatment of compound 4 with activated manganese(IV) oxide in refluxing toluene afforded a tandem reaction where the primary alcohol was first oxidised to the aldehyde and next an oxidative aromatisation took place to give compound 5. The reduction-oxidation strategy to obtain the aldehyde was chosen since an earlier attempt with direct Dibal-H reduction of the ester was unsuccessful. The key step was a Wittig reaction between aldehyde (5) and 2-carboxyethyltriphenylphosphonium bromide.^[4] The reaction took place in a 1:1 mixture of THF and DMSO using sodium hydride as base and afforded only the desired E-isomer (6) as expected.^[5] Compound 6 was then condensed with methyl N-(2-Boc-aminoethyl)glycinate^[6] using DCC and DHbtOH as coupling reagents. Basic hydrolysis of the resulting ester (7) gave the monomer 8. Deprotection of p-methoxybenzyl is expected to take place under the acidic conditions required for cleavage of the other protecting groups when incorporated in PNA.



Scheme 1. Reagents, conditions and yields: a) NH₂NH₂, ACOH, MeOH, reflux (92%); b) *p*-methoxybenzylchloride, NaH, DMF, 0°C->RT (84%); c) NaBH₄, THF, MeOH, reflux (58%); d) activated MnO₂, toluene, reflux (30%); e) [P(Ph)₃(CH₂)₂COOH]⁺Br⁻, NaH, THF/DMSO, 0°C->RT (42%); f) methyl *N*-(2-Boc-aminoethyl)glycinate, DhbtOH, DCC, DMF, 0°C->RT (50%); g) 2M NaOH, MeOH, 0°C (46%).

CONCLUSION

The synthesis of a novel PNA-monomer has been accomplished. The triplex recognition properties of this E-base analogue is currently under investigations.

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