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Synthesis of Bicyclo-DNA Nucleosides with Additional Functionalization in the Carbocyclic Ring

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§SCS Poster Prize Winner

Abstract: Two novel bicyclo nucleoside isomers carrying the base thymine in the furanose ring and an ester substituent in the carbocyclic ring were synthesized from a common bicyclic sugar precursor *via* a cyclopropanation/fragmentation pathway in nine steps. The relative configuration of the ester substituent in both isomers as well as the anomeric configuration in one nucleoside was determined by ^{1}H-NMR difference NOE spectroscopy.

Keywords: Antisense oligonucleotides · Bicyclo-DNA · Nucleosides · Thymine · Tricyclo-DNA

Introduction

The concept of conformational restriction[1] has been successfully applied in the past in nucleic acid chemistry and has produced analogues such as the family of the locked nucleic acids (LNA)[2] or tricyclo-DNA (tc-DNA)[3] (Fig. 1) which show strongly increased affinity to complementary RNA without compromising base-recognition selectivity. These analogues are currently considered as advanced generation antisense agents and are expected to replace the phosphorothioate DNA and some of the simpler 2'-O-alkyl-RNA analogues in therapy.[4] Besides this, some of these analogues have also proven to increase siRNA efficacy.[5] While chemistry has provided solutions to increase duplex stability with target RNA and to enhance resistance towards nuclease induced degradation, there are still a series of largely unsolved problems on the way to effective oligonucleotide drugs, the most prominent ones being cellular uptake and distribution,[6] as well as, depending on the mechanism of action, off target effects.[7]

The molecular scaffold of bicyclo- and tricyclo-DNA is ideally suited to accommodate further functional groups. Such groups can for example prove useful in cellular targeting and cellular uptake when modified with appropriate molecular entities. With this background we became interested in the bicyclo-DNA derivatives containing an additional carboxyl substituent, such as **1** and **2** (bc^{alk}-DNA, Fig. 1). From model building

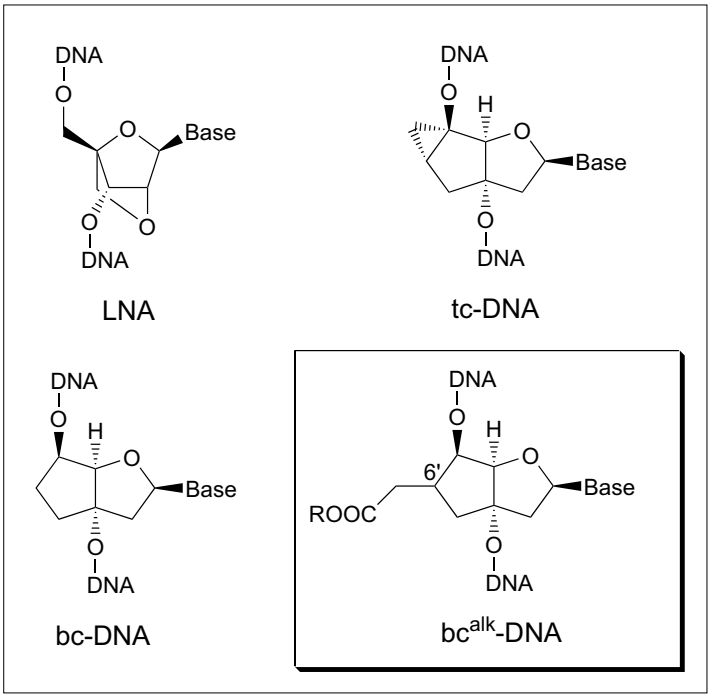

Fig. 1. Structures of selected examples of conformationally restricted DNA analogues as well as of bc^{alk}-DNA described in this paper

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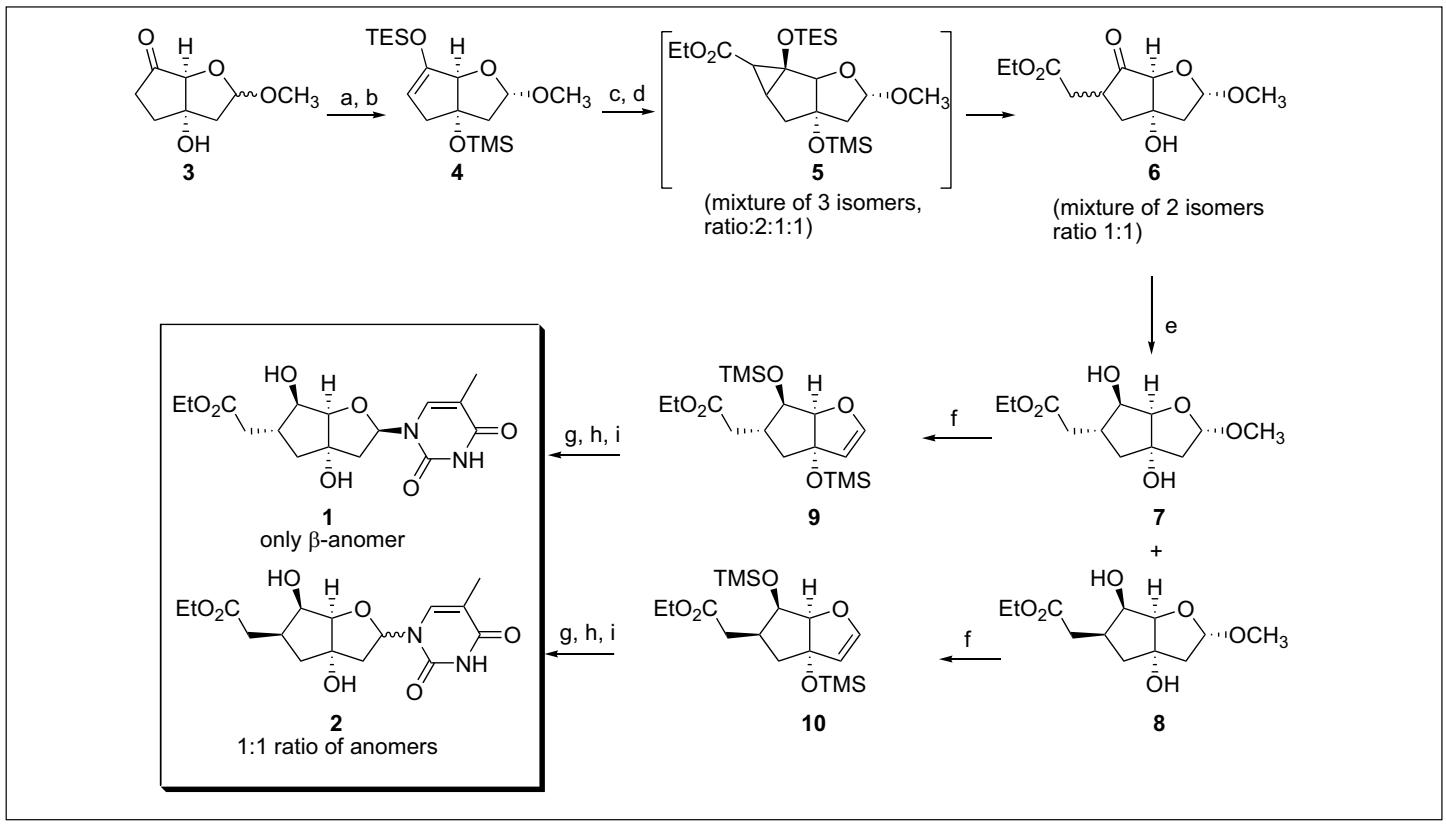

Scheme. Reagents and conditions: a) Li-DIPA, Et_3SiCl, THF, –78 °C, 2 h, 54%; b) BSA, pyridine, 17 h, rt, 86%; c) $N_2CHCOOEt$, $ClCH_2CH_2Cl$, $Cu(acac)_2$, 90 °C, 9 h, 80%; d) HF-pyridine, pyridine, r.t. 1h, 81%; e) $CeCl_3$, $NaBH_4$, 15 min, 0 °C, 57%; f), 2,6-lutidine, TMSOTf, CH_2Cl_2, 1h, rt, quant.; g) thymine, BSA, NIS, rt, 3h, h) AIBN, Bu_3SnH, toluene, reflux, 2h; i) HF.pyridine, pyridine, rt, 4h, 20–30% over the last three steps.

it appears that these functional groups are located on the rim of the backbone pointing away from the helical axis. Thus only little or no interference with duplex formation as a consequence of a molecular entity attached in this position can be expected. In this preliminary communication we describe a convenient synthetic access to the corresponding nucleosides carrying the base thymine.

Results and Discussion

In our synthetic strategy we envisaged the introduction of the functional group into C(6') of the sugar unit *via* a cyclopropanation/fragmentation pathway on enol ether **4**. This pathway also provides an entry into functionalized tricyclo-DNA building blocks and was therefore considered to be more appropriate than direct alkylation strategies. The synthesis started with ketone **3** which is a known key intermediate in the synthesis of tricyclo-DNA.[8] (Scheme). Ketone **3** was converted into the silyl enol ether **4** by standard reactions. Cyclopropanation of **4** with ethyldiazoacetate in the presence of $Cu(acac)_2$ yielded **5** as a mixture of three isomers in a ratio of roughly 2:1:1 in a combined yield of 80%. Desilylation with HF·pyridine afforded under concomitant cleavage of the cyclopropane ring a mixture of ketoesters **6** (81%) in a 1:1 ratio that was inseparable by standard column chromatography. We assume that this isomeric mixture reflects thermodynamic equilibrium although we did not investigate this fact in detail. This mixture of ketones **6** was subsequently reduced with $NaBH_4/CeCl_3$ to the hydroxyesters **7** and **8** that could be isolated as pure isomers. The relative configuration of **7** and **8** was unambiguously assigned by ^{1}H-NMR NOE spectroscopy (Fig. 2).

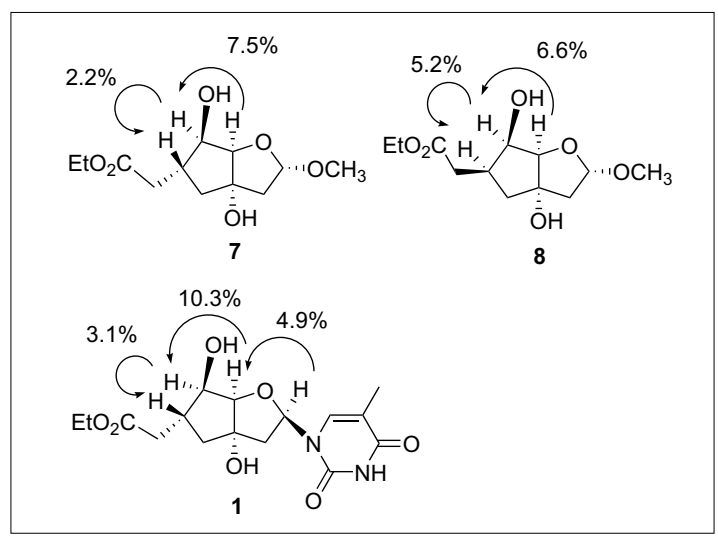

Fig. 2. Intensities of observed (mutual) ^{1}H-NMR difference NOE effects of the relevant protons used for assigning the *cis*- and *trans*-relationship of substituents in the carbocyclic ring in **7** and **8** as well as the β-anomeric configuration in **1**

In the light of previous results in the synthesis of tricyclo-nucleosides[9] we planned the introduction of the nucleobase in a two-step procedure *via* NIS induced nucleosidation[10] of enol ether **9** and **10**, followed by radical dehalogenation, as this promised to yield stereoselectively only the β-nucleosides and thus seemed superior to the standard Vorbrüggen procedure,[11] in which a mixture of anomers had to be expected. To this end the

isomers **7** and **8** were separately converted into enol ethers **9** and **10** by treatment with TMSOTf. Subsequent reaction with persilylated thymine and N-iodosuccinimide (NIS) followed by radical reduction with Bu_3SnH/AIBN gave the corresponding nucleoside **1** as a single β-isomer while nucleoside **2** was obtained as a 1:1 mixture of anomers. We rationalized the mixture of anomers in the NIS mediated addition to **9** as the consequence of the ester substituent at C(6'). In the concave-shaped intermediate after iodine addition, this substituent protects the β-face at the anomeric center from nucleophilic attack by the nucleobase, thus leading to partial *syn* addition. The relative configuration at the anomeric center in **1** was again assigned on the basis of ^{1}H-NMR NOE spectroscopy (Fig. 2).

Conclusions

We have successfully synthesized two novel bicyclo-nucleoside modifications with an additional ester function on the carbocyclic ring. These nucleosides will now be converted into the corresponding building blocks for automated DNA synthesis. The next set of experiments will then be devoted to the incorporation of these units into oligodeoxynucleotides and the study of the effect of the substituent at C(6') on the hybridization behavior towards complementary DNA and RNA.

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