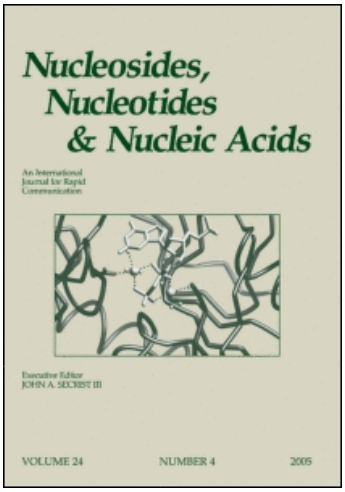

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SYNTHESIS OF A BRANCHED LOCKED NUCLEIC ACID (LNA) ANALOGUE

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□ *A 3′-C-branched LNA-type bicyclic nucleoside, containing a furanose ring locked in an N-type conformation, was synthesized from a known 3-C-vinyl allofuranose derivative using a strategy relying on the condensation with the nucleobase after the introduction of the branching hydroxymethyl chain by our recently developed RuO_4 based protocol. This branched LNA nucleoside has a potential as a monomer for the functionalization of LNA.*

Keywords Locked nucleic acid; N-type conformation; RuO_4 oxidation

In nucleic acid chemistry, conformationally restricted nucleoside building blocks are powerful tools for the design of oligodeoxynucleotides (ODNs) with selective and high-affinity recognition of complementary nucleic acids. Furthermore, the structural control embedded by these building blocks opens for the design of functional nucleic acid architectures.[1] Locked nucleic acid (LNA) represents the most absorbing example in which the conformation of the furanose moiety is locked in an N-type (C3′-*endo*) conformation (Figure 1).[2] The incorporation of one or more LNA monomers into an ODN strongly increases the thermal stability of the duplex formed with single-stranded DNA or RNA complements.[2] LNA has

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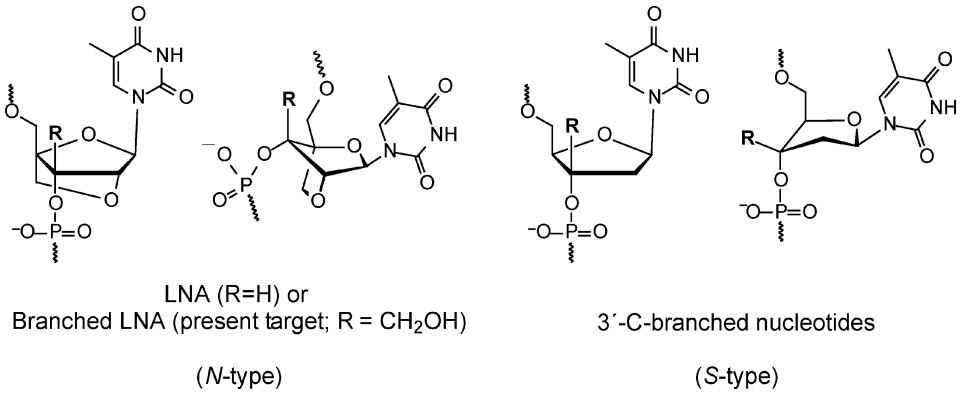
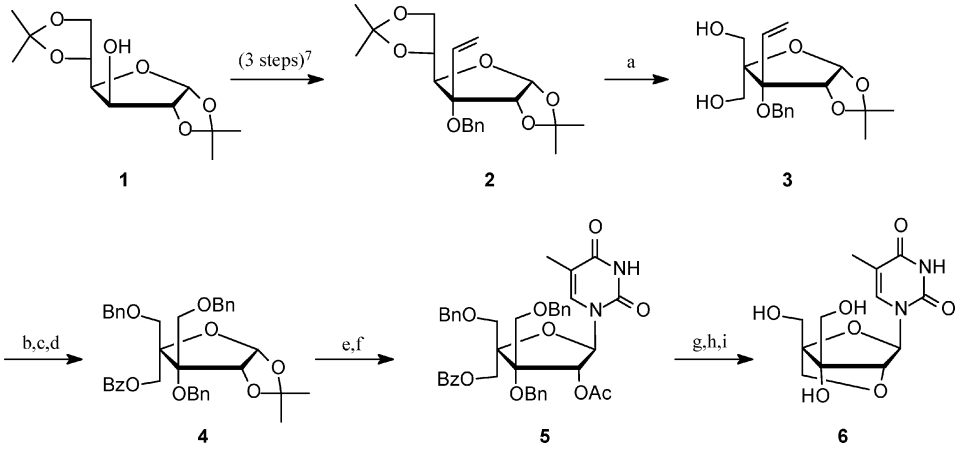


FIGURE 1 An LNA nucleotide, a 3′-*C*-branched nucleotide and the target 3′-*C*-branched LNA nucleotide.

been shown to be very useful for antisense applications,[3] and amino-LNA has been used for the preparation of functionalized nucleic acids.[4]

3′-*C*-Branched nucleoside building blocks have been made to improve either enzymatic stability of the ODN or the binding affinity toward complementary targets (Figure 1).[5] In general, ODNs with 3′-*C*-branched nucleotides exhibit slightly decreased hybridization properties and improved stability against 3′-exonucleases. Furthermore, they contain branching points which can be used as conjugation sites for a variety of functional moieties such as peptides or other nucleotides.[5] A 3′-substituent is expected to be oriented in a pseudoequatorial position, driving the sugar pucker towards a C-2′-*endo* conformation, thus explaining why the binding affinity toward RNA is somewhat impaired.[5] On the other hand, the 3′-*C*-alkyl substituents (including the 3′-*C*-hydroxymethyl substituent) point into the major groove of DNA:DNA and DNA:RNA duplexes and are reasonably well tolerated in the duplex structure.[5] Therefore, it was appealing to study this structural feature in the context of an LNA monomer, in which the locked N-type conformation forces the 3′-hydroxymethyl group into an axial position. Longer alkyl groups in the same position have been studied before leading to some decrease in thermal stability.[6] However, we envisioned the smaller 3′-hydroxymethyl group to be better accommodated into duplexes.

For the introduction of the hydroxymethyl branch at the β-face of C3′ of a nucleoside, a convergent synthesis strategy starting from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **1** was chosen, and the known 3-*C*-vinyl derivative **2**[7] was prepared in three steps. In our first strategy, this compound was efficiently converted into a 3′-hydroxymethyl derivative by our recently developed ruthenium protocol for oxidative cleavage.[8] However, all attempt of making a successful preparation of the target nucleoside from this strategy failed due to problems with the 3′-hydroxymethyl moiety in the



SCHEME 1 Key: a) i) H_5IO_6, EtOAc; ii) HCHO, NaOH, $NaBH_4$, 94%; b) NaH, BnBr, DMF, 60%; c) i) BzCl, Pyridine; ii) $RuCl_3 \times H_2O$, $NaIO_4$, H_2O, EtOAc, CH_3CN; iii) $NaBH_4$, H_2O, THF; iv) $NaIO_4$; v) $NaBH_4$, 71%; d) NaH, BnBr, DMF, 75%; e) i) 80% Aq. AcOH; ii) Ac_2O, Pyridine, 91%; f) Thymine, BSA, CH_3CN, TMS-triflate, 93%; g) $NaOCH_3$, MeOH, 86%; h) i) MsCl, CH_2Cl_2, Pyridine; ii) NaH, Dioxane, 72%; i) H_2, $Pd(OH)_2/C$, EtOH, quantitative.

subsequent steps. Therefore, we decided to apply a second strategy in which the vinyl group is cleaved later, and **2** was eventually converted to **3** through oxidative cleavage of the C5–C6 bond and an aldol condensation. Selective protection followed by the ruthenium based oxidative cleavage[8] and a new benzylation afforded **4**. Adapted standard conversions gave the nucleoside **5**. A deprotection, a selective mesylation, ring-closure and a debenzylation afforded the target nucleoside **6**. Efforts towards the incorporation of **6** into functionalised ODNs are in progress.

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