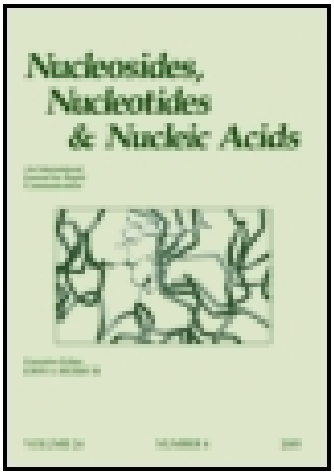

Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Synthesis and Evaluation of Novel Oligodeoxynucleotides Containing 3′-C-(3-Benzoyloxypropyl)thymidine and Bicyclo Nucleoside Derivatives

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Published online: 16 Aug 2006.

To cite this article: Henrik M. Pfundheller , Poul Nielsen & Jesper Wengel (1997) Synthesis and Evaluation of Novel Oligodeoxynucleotides Containing 3′-C-(3-Benzoyloxypropyl)thymidine and Bicyclo Nucleoside Derivatives, Nucleosides and Nucleotides, 16:7-9, 1439-1442, DOI: 10.1080/07328319708006201

To link to this article: http://dx.doi.org/10.1080/07328319708006201

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1439-1442 (1997)

SYNTHESIS AND EVALUATION OF NOVEL OLIGODEOXYNUCLEOTIDES CONTAINING 3'-*C*-(3-BENZOYLOXYPROPYL)THYMIDINE AND BICYCLO NUCLEOSIDE DERIVATIVES

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ABSTRACT: The stereoselective synthesis of 3'-*C*-Allyluridine derivative **2** has been accomplished. This nucleoside was used as a key synthon for the synthesis of oligodeoxynucleotides containing 3'-*C*-(3-benzoyloxypropyl)thymidine (**X**) or bicyclo nucleoside (**Y+Z**) monomers. Preliminary thermal experiments are reported.

The synthesis and incorporation of 3'-*C*-hydroxymethyl-,[1] 4'-*C*-hydroxymethyl-[2] and 5'-*C*-hydroxymethylthymidine[3] into oligodeoxynucleotides (ODNs) have recently been accomplished. DNA-DNA and RNA-DNA[2,3] duplexes involving these analogues exhibit promising thermal stabilities and increased resistance towards nucleolytic digestion. These results and our continued interest in *C*-branched ODN-analogues have prompted the synthesis and incorporation into ODNs of the modified nucleoside **4** containing a 3'-*C*-(3-benzoyloxypropyl) group.

DNA-DNA and DNA-RNA duplexes containing bicyclo nucleosides have shown promising results regarding thermal stability.[4,5] The bicyclic analogues (**5** and **6**), having the propyloxy chain fixed to the ribose on the 2'-position, were synthesized and incorporated into ODNs for comparison with the ODNs containing 3'-*C*-(3-benzoyloxypropyl)thymidine.

Synthesis of 3'-*C*-Allylthymidine derivatives using a divergent strategy gives unfortunately the desired *erythro* isomer in only minor yields.[6] We have therefore turned our attention to a convergent strategy and preliminary results are reported herein. Besides the obvious advantage of having the possibility of incorporating different nucleobases, the convergent strategy stereoselectively affords the key synthon **2,** useful not only for the synthesis of the desired 3'-C-(3-benzoyloxypropyl) nucleoside **4**, but also for the synthesis of the bicyclo nucleoside analogues **5** and **6**.

The key synthon **2** was synthesized in six steps from the silylprotected ulose **1**[7] in an overall yield of 40% (Fig.1). Stereoselective Grignard addition using allylmagnesium bromide was fol-

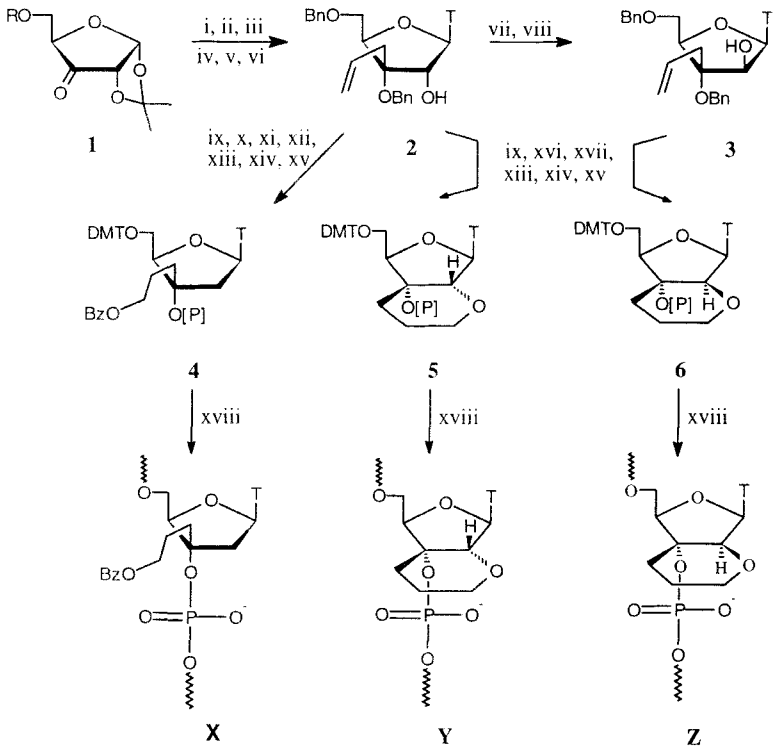

FIG. 1 : i) AllylMgBr, ether, THF; ii) TBAF, THF, 63% (2 steps); iii) BnBr, NaH, DMF, 87%; iv) 1) 80% AcOH (aq), 2) Ac_2O, pyridine, 90%; v) Thymine, BSA, CH_3CN, TMS-triflate, 82%; vi) NaOMe, MeOH, 99%; vii) MsCl, pyridine, 92%; viii) NaOH, H_2O, EtOH, 76%; ix) 1) BH_3:oxathiane, THF, 2) NaOH, H_2O_2, 54% (from **2**) and 58% (from **3**); x) BzCl, DCM, 2,6-lutidine, 62%; xi) $C_6F_5OC(S)Cl$, DMAP, pyridine, DCM, 54%; xii) Bu_3SnH, AIBN, benzene, 53%; xiii) H_2, $Pd(OH)_2$-C, EtOH, 65-80%; xiv) DMT-Cl, pyridine, 61-95%; xv) (*i*-$(Pr)_2NP(Cl)O(CH_2)_2CN$, DIPEA, DCM, 73-92%; xvi) TsCl, pyridine; xvii) NaH, DMF, 30-39% (2 steps); xviii) DNA-synthesizer; R = *t*$BuMe_2Si$- ; [P] = (i-$Pr)_2NPO(CH_2)_2CN$.

lowed by desilylation and standard benzylation. Cleavage of the acetal followed by diacetylation gave an anomeric mixture which was coupled with thymine by the Vorbrüggen method and deacetylated to give the key compound **2**. The *arabino*-isomer of this nucleoside **3** was synthesized using anhydro chemistry in 70% yield (2 steps).

For the synthesis of the 3'-*C*-(3-benzoyloxypropyl) nucleoside derivative **4**, compound **2** was subjected to a hydroboration followed by an oxidation (54% yield). After benzoylation of the primary hydroxy group, the pentafluorophenylthionocarbonate derivative was synthesized in 33% yield (2 steps). Barton deoxygenation afforded the 3',5'-di-*O*-benzylated compound, which

TABLE 1

	Complimentary DNA			Complimentary RNA		
ΔT_m/mod.	T = X	T = Y	T = Z	T = X	T = Y	T= Z
5´-T_7-$\mathbf{T}_{mod}$-T_6-3´	-3	-11	-9	-6	-6	-10
5´-T_6-$\mathbf{T}_{mod}$-$\mathbf{T}_{mod}$-T_6-3´	nd	-9	-9	nd	-7	-6
5´-T_6-$\mathbf{T}_{mod}$-T-$\mathbf{T}_{mod}$-T_5-3´	nd	-11	-9	nd	-8	-9

nd = not determined

was debenzylated in 34% yield (2 steps). DMT protection followed by phosphitylation afforded the phosphoramidite **4** in 63% yield (2 steps).

The bicyclic analogue **5** was synthesized from **2** and its isomer **6** was synthesized from **3** after hydroboration and oxidation. The cyclisations were accomplished by selective tosylations of the primary hydroxy groups followed by treatment with sodium hydride in yields of 30% (for **5**) and 39% (for **6**). The bicyclonucleosides were debenzylated, and the configurations of the deprotected bicyclic nucleosides were verified from ^{1}H-NMR NOE-experiments. DMT protection followed by phosphitylation afforded the phosphoramidites **5** and **6** in 86% and 61% yield, respectively (2 steps).

The phosphoramidites **4**, **5** and **6** were incorporated one or two times into ODNs (see Table 1) using standard solid phase methodology followed by deprotection and purification and hybridized with complimentary DNA and RNA. The composition of the ODNs was verified by matrix assisted laser desorption mass spectrometry.

Incorporation of 3'-*C*-(3-benzoyloxypropyl)thymidine (**X**) into an ODN results in a change in T_m (ΔT_m) of -3 °C of the resulting DNA-DNA duplex. A larger destabilisation was observed when the modified ODN was hybridized with RNA (Table 1). From earlier results with 3'-*C*-hydroxymethylthymidine, we expect the 3'-*C*-(3-benzoyloxypropyl)thymidine to prefer a C-2'-*endo*-conformation. Since DNA-RNA duplexes usually are in A-DNA form with the sugar ring in C-3'-*endo*-conformation, this could be an explanation of the larger destabilisation observed for the DNA-RNA duplex.

The thermal stabilities of oligodeoxynucleotides with 3'-*C*-(3-benzoyloxypropyl)thymidine incorporated exceed the stabilities of oligodeoxynucleotides with either of the two bicyclic analogues incorporated (Table 1). This is as expected from molecular modelling as the flexible benzoyloxypropyl group is able to find the empty space in the major group while the bicyclo nu-

cleotides through sterical hindrance partially distort the double helix. The *arabino*-isomer (**Z**) has as expected a minor effect compared to the *ribo*-isomer (**Y**) as the α- face of a nucleoside is more sterically hindered than the β-face in both A-DNA and B-DNA. The effects are for both isomers more pronounced for DNA-DNA (expected B-form) compared to DNA-RNA (expected A-form).

A conformational analysis[8] of the debenzylated *ribo*-bicyclo nucleoside indicated the additional pyran ring of the nucleoside to prefer a chair conformation and the sugar ring to prefer a C-2'-*endo*-conformation. As this conformation is normal in B-form DNA, the large destabilisation observed for the DNA-DNA duplex must be due to mainly sterical effects of the additional ring system. Conformational analyses of the *arabino*-isomer as well as further investigations on the 3'-*C*-(3-benzoyloxypropyl)nucleosides and oligodeoxynucleotides are in progress.

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