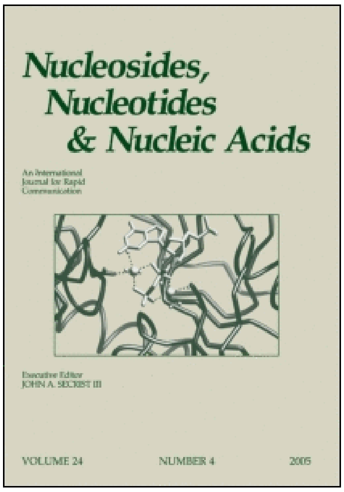

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SYNTHESIS OF NUCLEOSIDES WITH ADDITIONAL NUCLEOBASES

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□ *The syntheses of two nucleosides with additional nucleobases in the 2′-position are presented. The nucleosides have two- and one-carbon linkers to the additional nucleobase, respectively. The two nucleosides are synthesized from different strategies. The nucleoside with two carbons in the linker has been incorporated into oligonucleotides and showed stabilization of a tree-way junction.*

Keywords Double-headed nucleobase; RNA secondary structure; 2′-modification

The secondary structures of RNA are targets for modified oligonucleotides. In order to bind to RNA, forming other secondary structures than duplexes, oligonucleotides with additional nucleobases are suggested.[1,2] For this purpose, we have developed different strategies in synthesizing double headed nucleosides. These also can be useful in the design of double coding DNA.[3] A linear strategy with uridine as starting material resulted in the synthesis of 2′-deoxy-2′-*C*-(2-(thymine-1-yl)ethyl)uridine.[1] A convergent strategy starting from D-ribose resulted in 2′-deoxy-2′-*C*-(thymine-1-ylmethyl)uridine.

For the linear approach (Scheme 1), uridine was selectively protected at the 3′-*O*- and 5′-*O*—positions and the 2′-hydroxygroup was replaced by the allyl group in a radical deoxygenation reaction.[4] The olefin was converted to the alcohol by an oxidative cleavage followed by a reduction. To avoid intramolecular ring formation, the N3-position of uracil was protected by a benzoyl group with a selective and very efficient phase-tranfer procedure.[5] The additional nucleobase was introduced by a Mitsunobu reaction, and deprotection gave the desired compound **1**. The coupling constant $J_{H1',H2'}$ of **1** was 8.5 Hz indicating that the molecule primarily adopts an S-type conformation. Compound **1** was converted to the phosphoramidite and

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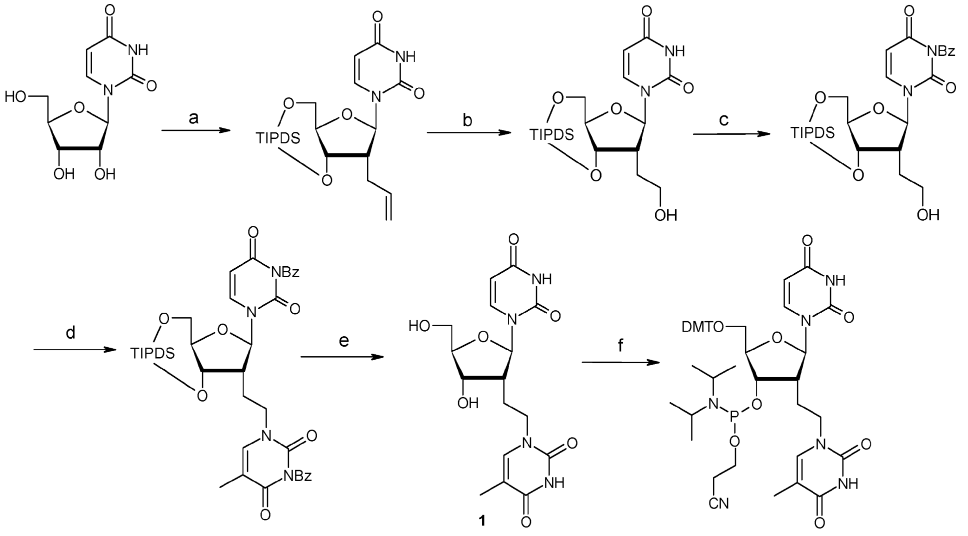
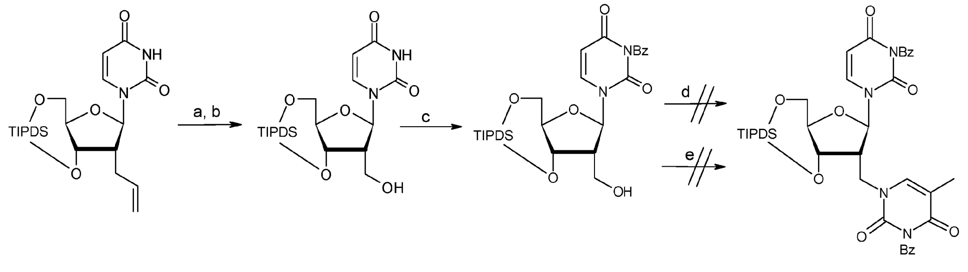


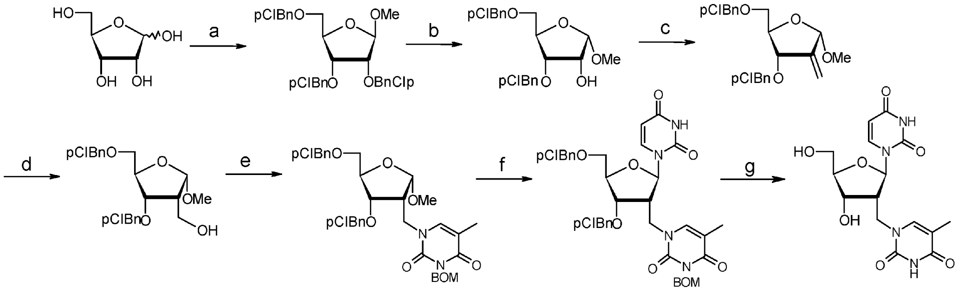
SCHEME 1 Ref. 1, a) i) TIPDSCl$_2$, pyridine; ii) PhOCSCl, DMAP, CH$_3$CN; iii) Bu$_3$SnAllyl, AIBN, toluene (36%) ref. 4; b) i) OsO$_4$, NMO, acetone, H$_2$O; ii) NaIO$_4$, dioxane, H$_2$O; iii) NaBH$_4$, methanol (64%); c) BzCl, Na$_2$CO$_3$(aq), Bu$_4$NBr, CH$_2$Cl$_2$ (93%); d) T^{Bz}, Ph$_3$P, DEAD, THF; e) i) TBAF, THF; ii) NH$_3$ aq. MeOH (67%); f) i) DMTCl, pyridine; ii) 4,5-dicyanoimidazol, NC(CH$_2$)$_2$OP(N(i-Pr)$_2$)$_2$, CH$_3$CN (33%).

incorporated successfully into oligonucleotides. The hybridization data shows slightly destabilization of dsDNA and DNA:RNA duplexes when **1** is incorporated in the middle of the sequences.[1] This shows, on the other hand, that the additional base is reasonably well accommodated in the duplexes. When the target is a tree-way junction, stabilization is observed when the additional nucleobase is positioned in the branching point.[1]

Compound **2**, 2′-deoxy-2′-*C*-(thymine-1-ylmethyl)uridine, was synthesized to investigate whether this stabilizing effect is dependent on the length and flexibility of the carbon linker. In the first attempt, the same starting material was used in a similar linear strategy. The double bond in the allyl group was rearranged in an *ene*-reaction, and the new double bond was cleaved to



SCHEME 2 a) Methyltriazoline, DCM (70%); b) i) O$_3$, DCM; ii) NaBH$_4$, MeOH, ref. 6 (74%); c) BzCl, Na$_2$CO$_3$ (aq), Bu$_4$NBr, DCM (63%); d) T^{Bz}, Ph$_3$P, DEAD, THF; e) i) MsCl, NEt$_3$, DCM; ii) Cs$_2$CO$_3$, T^{Bz}, DMF.



SCHEME 3 a) i) MeOH, H_2SO_4; ii) pClBnCl, NaH, DMSO (43%); b) $SnCl_4$ DCM, ref. 7 (74%); c) i) Dess-matrin periodinane, DCM; ii) Ph_3PCH_3Br, BuLi, THF (42%); d) i) 9-BBN, THF; ii) H_2O_2, NaOH (87%); e) T^{BOM}, Ph_3P, DEAD, THF (87%); f) Uracil, BSA, TMSOTf, CH_3CN (48%); g) H_2, $Pd(OH)_2/C$, MeOH.

give the alcohol by ozonolysis followed by a reduction.[6] The strategy was again to introduce the additional nucleobase by an S_N2 reaction, and to prevent an anhydro formation, N3 was protected by a benzoyl group. Nevertheless, Mitsunobu conditions as well as other attempts were not successful in introducing the additional nucleobase (Scheme 2).

An alternative strategy for the synthesis of **2** started from D-ribose following a convergent strategy. A published procedure for the preparation of a 5,3-diprotected methyl furanoside was used (Scheme 3).[7] The free hydroxyl group was oxidized, followed by a Wittig reaction and hydroboration to give the primary hydroxyl group at the 2-position. The nucleobase was successfully introduced by a Mitsunobu reaction, and a Vorbrüggen method resulted in the coupling of the uracil. Finally, a hydrogenation gave the desired nucleoside **2**. The $J_{H1',H2'}$ of **2** was 9.3 Hz indicating an S-type conformation. Incorporation of this nucleoside **2** into oligonucleotides is in progress.

In conclusion, nucleoside **1** was incorporated into oligonucleotides and hybridization studies showed increased stability of a tree-way junction when arranged with the additional nucleobase in the branching point. The shorter linkage in nucleoside **2** will lead to a less flexible organisation of the additional base. The consequences of this in nucleic acid recognition are currently studied.

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