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Optimized synthesis of LNA uracil nucleosides

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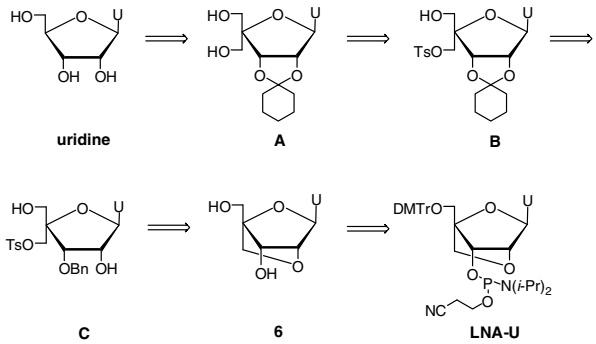
ABSTRACT

A short, very high yielding, and practical synthesis of LNA uracil diol **6** has been developed from the easily accessible glycosyl donor **1**. The concluding O3′-debenzylation of **5** resulted in significant reduction of the uracil moiety with many typical debenzylation conditions, while catalytic transfer hydrogenation using $Pd(OH)_2/C$ and formic acid largely suppressed this undesired side reaction. Facile access to **6** will allow full exploration of RNA-based LNA-technology applications, including polymerase-catalyzed synthesis of LNA-modified RNA-strands.

Oligonucleotides (ONs) continue to be extensively explored as gene regulatory agents within the antisense and siRNA regimes with the aim to develop powerful therapeutics.[1] Chemical modification of ONs for these applications is needed to provide adequate protection from enzymatic degradation by nucleases and to facilitate stronger binding to complementary nucleic acid targets. A very successful approach toward this end has been to incorporate conformationally restricted nucleoside building blocks into ONs.[2,3] Locked nucleic acid (LNA, β-D-*ribo* configuration, Scheme 1)[4,5] and analogs thereof[6–8] are particularly promising examples of this compound class. Incorporation of LNA monomers into ONs results in significant increases in thermal affinity against DNA/RNA complements of up to +10.0 °C per modification relative to unmodified ONs, and is accompanied by increased mismatch discrimination and improved stability against exo- and endonucleases.[9] These properties render the commercially available LNA building blocks of considerable therapeutic and diagnostic interest[10] and has stimulated their use in recent exploratory applications,[11] including enzymatic synthesis of LNA containing RNA-strands by transcription using LNA-ATP and T7 RNA Polymerase.[11d]



Scheme 1. Linear synthesis of LNA uracil diol **6**.[12]

The synthesis of LNA nucleosides with pyrimidine (U, T, C, 5-^{Me}C)[12] or adenine[13] moieties has been realized via linear approaches where natural nucleosides are used as starting materials (illustrated for uracil derivative[12] in Scheme 1). These linear strategies are intuitively appealing due to low number of synthetic steps and ready availability of inexpensive ribonucleoside starting materials, but pose a series of synthetic challenges resulting in low overall yields (Scheme 1).[2a] Briefly, these difficult challenges entail C4′-hydroxymethylation of ribonucleosides (uridine→→**A**), regioselective O5″-tosylations (**A**→**B**), and regioselective reduction of a O2′,O3′-benzylidene group to furnish O3′-benzylated nucleoside **C** as a suitable substrate for intramolecular closure which only occurs in moderate yield (Scheme 1).[12] LNA uracil diol **6** is, accordingly, only obtained in ~5% overall yield from uridine via this route.[12]

The original convergent strategy toward LNA nucleosides[4] utilizes commercially available diacetone-α-D-allose as the starting material, which is easily converted to C4-hydroxymethyl pentofuranose **D** in 75% yield (Scheme 2).[14]

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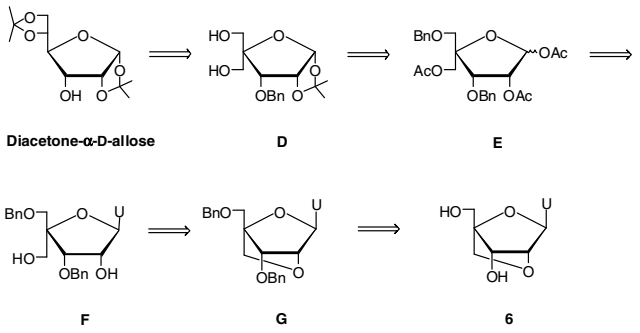
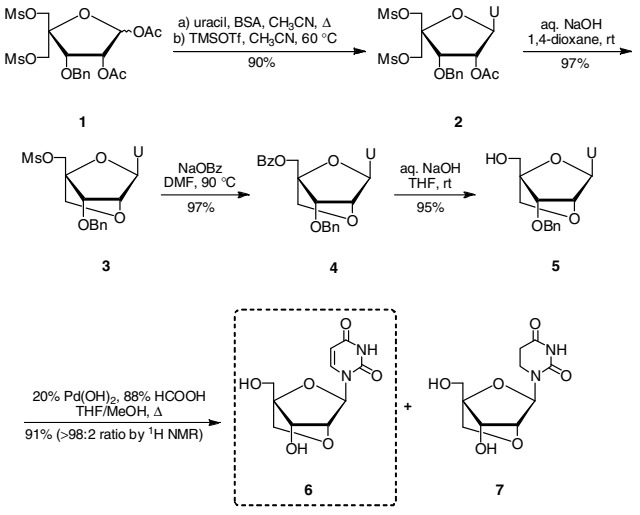


Scheme 2. Convergent synthesis of LNA uracil diol **6**.[4]

Regioselective O5-benzylation of **D**, followed by a series of protecting group manipulations provides common glycosyl donor **E** in 55% yield from **D**.[4] Briefly, glycosylation of **E** with silylated nucleobases (U, T, C^{Bz}, A^{Bz}, and G^{Ibu}), selective deacylation to give **F** (illustrated for the uracil derivative), regioselective O5″-tosylation, and a base-induced intramolecular S_N2-reaction affords LNA intermediate **G**, which upon O3′-debenzylation provides unprotected LNA diol **6** (Scheme 2). Since regioselective O5-benzylation of **D** is difficult to obtain in a reliable and satisfying yield on a larger scale,[15,16] and the selective O5″-tosylation and ring closure steps generally only proceed in low to moderate yields, the overall yield of LNA uracil diol **6** from diacetone-α-D-allose via this route is below 7%.[4]

Although a significantly improved convergent strategy to LNA nucleosides has recently been described,[15] there still remains *a need for a concise and highly efficient synthesis of LNA uracil nucleosides* to fully explore novel RNA-based LNA-applications[11b,11d] including T7 RNA Polymerase-catalyzed incorporation of LNA monomers in RNA-strands.[11d] This shortcoming addressed herein as a convergent route toward LNA uracil diol **6** is described, initiating from glycosyl donor **1** (Scheme 3).[15] The identity of reported compounds was fully ascertained by NMR (^1H, ^{13}C, COSY, and/or HSQC) and FAB-HRMS, while purity was determined by 1D NMR.[17]

Glycosyl donor **1** is obtained on a multigram scale from commercially available diacetone-α-D-allose in 71% overall yield by a highly optimized procedure.[14] Following Koshkin's general route,[15] treatment of the anomeric mixture of glycosyl donor **1** with persilylated uracil and trimethylsilyl triflate as Lewis acid in refluxing acetonitrile initially afforded desired nucleoside **2** via anchimeric assistance in a moderate 65% yield. It was noticed that prolonged exposure (~16 h) to refluxing acetonitrile resulted in decreased yields of **2**. Satisfyingly, a decrease in reaction temperature to 60 °C improved the reaction yield to 90% after purification by column chromatography. Conducting this reaction at 60 °C in acetonitrile for extended periods (>36 h) did not lead to a reduction in yield of desired nucleoside **2**, which underlines the importance of temperature control in this conversion. Similar yields were obtained when the reaction was performed in 1,2-dichloroethane at 60 °C. Treatment of β-nucleoside **2** with aqueous sodium hydroxide in 1,4-dioxane resulted in efficient tandem O2′-deacylation and O2′,C4′-ring closure to afford LNA nucleoside **3** in 97% yield. Structural validation for the proposed LNA-skeleton of **3** was obtained by: (a) appearance of ^1H NMR signals from H1′, H2′, and H3′ as singlets or narrow doublets ($J < 0.5$ Hz), a known characteristic of LNA nucleosides,[4] and (b) comparison of NMR spectral data of known downstream products (vide infra). Subsequent treatment of **3** with sodium benzoate in DMF at 90 °C resulted in nucleophilic displacement of the O5′-mesylate group to furnish O5′-benzoate **4** (97%), which was hydrolyzed using sodium hydroxide in aqueous THF to furnish known alcohol **5**[12] in 95% yield. Purification of nucleosides **3**–**5** was very conveniently accomplished by *simple precipitation*, whereby resource-intensive column chromatography purification was avoided.



Scheme 3. Novel route toward LNA uracil diol **6**.

Removal of the O3′-benzyl group of **5** using previously established hydrogenolysis conditions (H_2 and 10% Pd/C in MeOH, entry 1, Table 1),[12] in our hands, repeatedly afforded mixtures (~30:70 ratio by ^1H NMR) of desired LNA uracil nucleoside **6**[12] and nucleoside **7** in which the C5–C6 double bond of the nucleobase is reduced, which were very difficult to separate. The tentative assignment of **7** was based on mass spectra (FAB-HRMS *m*/*z* 259.0945 ($[M+H]^+$, $C_{10}H_{14}N_2O_6{\cdot}H^+$, calcd 259.0925)) and on key signals in ^1H and COSY NMR spectra including an internally coupling multiplet at 3.26–3.51 ppm (integration of four) arising from H5/H6-protons and a broad exchangeable singlet at 10.26 ppm from the nucleobase imino proton.

Numerous debenzylation conditions, summarized in Table 1, were accordingly screened to prevent nucleobase reduction. Debenzylation under strongly acidic conditions such as 1 M boron trichloride in CH_2Cl_2 (entry 2) or methanesulfonic acid[18] (entry 3) was anticipated to render the C5–C6 olefin bond of the uracil moiety unharmed, but yet failed to afford desired diol **6**, most likely as a consequence of ether bond cleavage between O2′–C5″ and/or cleavage of the glycosidic bond as very polar products were observed (results not shown). Although a change of catalyst to Pearlman's catalyst (20% $Pd(OH)_2/C$) afforded a remarkably improved ratio in favor of desired diol **6** relative to the original conditions, unacceptable levels of reduced product **7** were still observed (entry 4). Catalyst poisoning with pyridine[19] did not improve yield and selectivity (entry 5), nor did the use of catalytic transfer hydrogenation conditions with ammonium formate[20] as hydrogen donor (entry 6). Gratifyingly, changing to 88% formic acid as a hydrogen

Table 1
Debenzylation conditions for conversion of **5** into desired LNA uracil diol **6**

Entry	Conditions	Ratio 6:7[a]
1	10% Pd/C, H_2, MeOH, rt	30:70
2	1 M BCl_3 (5 equiv), CH_2Cl_2, −78 °C for 3 h, then rt	—
3	$MeSO_3H$, $CHCl_3$, rt	—
4	20% $Pd(OH)_2/C$, H_2, THF/MeOH (9:1, v/v), rt	85:15
5	20% $Pd(OH)_2/C$, H_2, pyridine (0.5 equiv), THF/MeOH (9:1, v/v), rt	5:95
6	20% $Pd(OH)_2/C$, $HCOONH_4$ (3.5 equiv), MeOH, Δ	60:40
7	20% $Pd(OH)_2/C$, 88% HCOOH, THF/MeOH (9:1, v/v), Δ	>98:2

[a] Estimated from ^1H NMR of crude products.

donor[21] in concert with 20% $Pd(OH)_2/C$ as catalyst and THF/MeOH (9:1, v/v) as reaction solvent, in addition to desired diol **6**, *only resulted in traces* of the reduced nucleoside **7** in the crude. After separation of **7** during column chromatography, desired LNA uracil diol **6** was afforded in 91% yield, pure by ^1H NMR.[17] In fact, the uracil moiety of **6** proved highly stable to these hydrogenation conditions as excess formic acid (up to 13 equiv) and prolonged reaction times (up to 22 h) did never result in more than 2% formation of the reduced product. The problematic susceptibility of the C5–C6 double bond of pyrimidine nucleobases to undergo reduction during hydrogenation conditions has been noted.[8b,22] To our knowledge, the presented results describe the first use of catalytic transfer hydrogenation conditions with formic acid to avoid nucleobase reduction, and warrant further investigations of these conditions for applications in nucleoside chemistry.

To sum up, a short, high yielding and very practical synthesis of LNA uracil diol **6** has been developed from the easily accessible glycosyl donor **1**.[14,15] Diol **6** can ultimately be obtained from commercially available diacetone-α-D-allose in ~52% yield, and remarkably only necessitates two chromatographic purification steps. Thus, the developed route toward **6** represents a significant improvement in overall yield and convenience compared to existing routes[4,12] and works reliably on larger scale synthesis (~10 g). Facile access to **6** will allow full exploration of RNA-based LNA-technology applications[11b,d] as it: (1) is easily converted to the corresponding phosphoramidite building block **LNA-U** over two steps (Scheme 1)[4,12] for automated incorporation into ONs, and/or (2) could be converted to the corresponding 5′-triphosphates and used in enzyme-catalyzed synthesis of LNA-U-modified RNA-strands for generation of aptamers via SELEX.[23]

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Supplementary data

General experimental section, experimental description, and characterization data of compounds **2**–**6**, copies of ^1H, ^{13}C NMR, ^1H–^1H COSY, and/or ^1H–^{13}C HSQC spectra of **2**–**6**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.09.165.

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