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Optimized synthesis of [3-^{15}N]-labeled uridine phosphoramidites

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ABSTRACT

A short and high-yielding synthetic route to [3-^{15}N]-labeled uridine phosphoramidite **1** (26% overall yield from uridine) has been developed. This will enable automated synthesis of isotopically labeled RNA strands and facilitate their use in structural studies.

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Structural elucidation of specific segments in RNA by nuclear magnetic resonance (NMR) or infrared (IR) spectroscopy is often complicated by spectral congestion, arising due to the presence of numerous equivalent nucleotide units. Chemical or enzymatic incorporation of nucleotide analogs at specific sites in RNAs can provide valuable information about the structure and function of biological RNA.[1] However, this is a potentially invasive approach that may perturb the structure and function of native RNA. Incorporation of isotopically labeled ribonucleotides[2] is an elegant solution to this challenge. These minimal structural changes often result in sufficient shifts of, e.g., NMR/IR signals to enable structural elucidation and monitoring of dynamic events. [3-^{15}N]-Labeled pyrimidine nucleotides have been particularly useful to study the dynamic changes in Watson–Crick base-pairing patterns and protein–nucleic acid interactions.[2f–h]

We recently required access to [3-^{15}N]-labeled uridine phosphoramidites for the synthesis of isotopically labeled RNA strands to study RNA folding kinetics by time-resolved IR spectroscopy.[3] We were surprised only to find one synthetic route to [3-^{15}N]-labeled uridine phosphoramidites in the literature (Scheme 1).[2f] The key features of this approach include (a) the use of the 2'-*O*-[(triisopropylsilyl)oxy]methyl (TOM)-protecting group,[4] which is compatible with automated RNA synthesis protocols,[5] and (b) introduction of the ^{15}N-label by N-nitration of the N3-position and subsequent treatment with $^{15}NH_3$.[2a,6] The poor regioselectivity during TOM-protection in the initial phase of this route compromises the overall yield of the desired [3-^{15}N]-labeled uridine phosphoramidite (14% from uridine).[2f] More efficient routes to [3-^{15}N]-labeled uridine phosphoramidites for automated incorporation into RNA strands are therefore desirable. Herein, we describe short and efficient synthetic routes to [3-^{15}N]-labeled uridine phosphoramidite **1**.

We designed synthetic routes toward [3-^{15}N]-labeled uridine phosphoramidite **1**, which utilize a 2′-*O*-TBDMS rather than a 2′-*O*-TOM-protecting group approach (Schemes 2 and 3). Several advantages were anticipated from this approach including: (a) *improved overall yield* as efficient synthetic methodology for regioselective O2′-TBDMS protection of pyrimidine ribonucleosides is well-established,[7] (b) *reduced cost* since *tert*-butyldimethylsilyl chloride is less expensive and consumed in smaller quantities than the corresponding TOM–Cl reagent, and (c) *seamless incorporation of* **1** *into RNA strands* as the 2′-*O*-TBDMS protocol is the most widely established approach for automated RNA synthesis.[5]

Our original route to [3-^{15}N]-labeled uridine phosphoramidite **1** initiated from fully protected N3-nitrated nucleoside **2**.[2a] This starting material was obtained in 98% yield by triacylation of uridine[8] followed by treatment of the resulting crude product with nitronium trifluoroacetate generated in situ[2a] upon mixing ammonium nitrate and trifluoroacetic anhydride. While bubbling hydrogen chloride gas through a methanolic solution of **2** to facilitate deacylation as previously described,[2a] we observed pronounced cleavage of the N3-nitro group. In contrast, mild acidic deacylation of **2** by in situ formation of HCl in MeOH[9] more controllably furnished triol **3**[2a] in excellent yield (95%). Subsequent O5′-(4,4′-dimethoxy)tritylation of **3** using standard conditions (i.e., DMTr-

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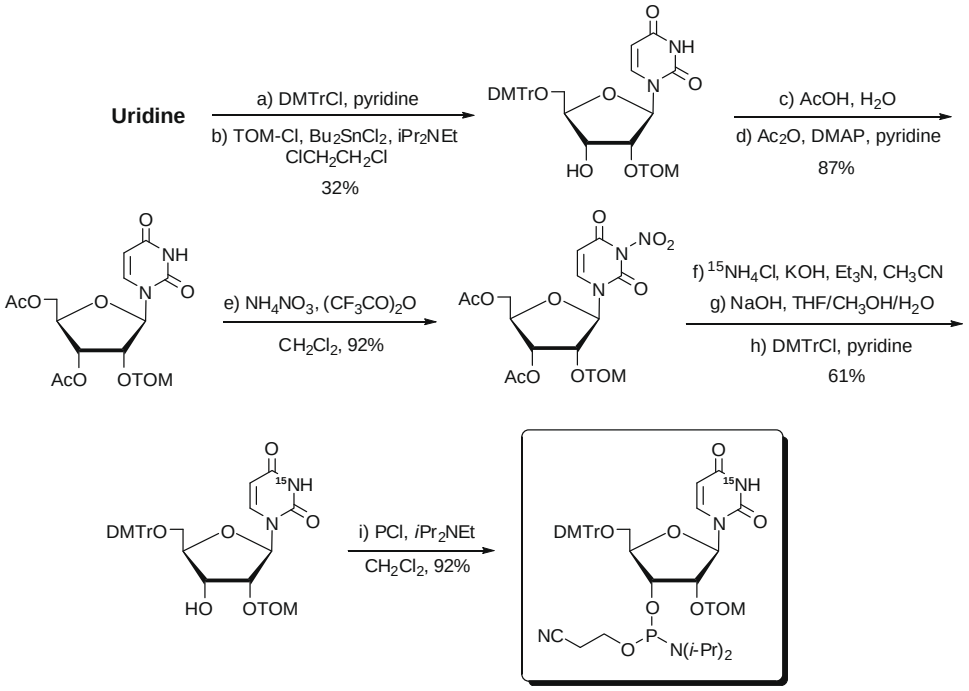
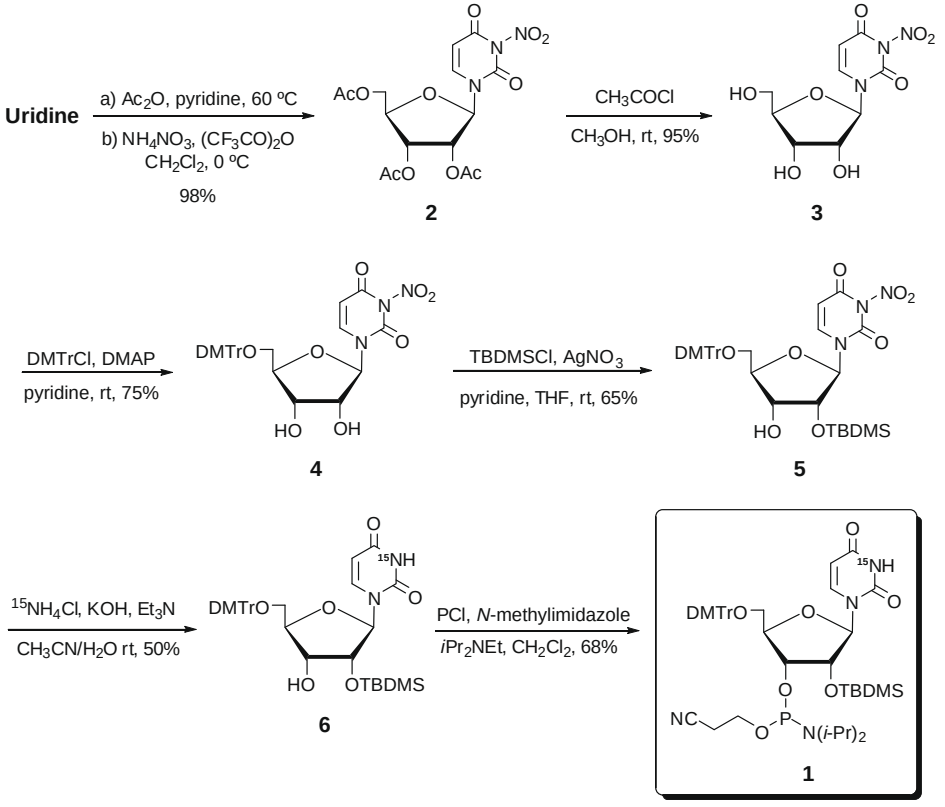
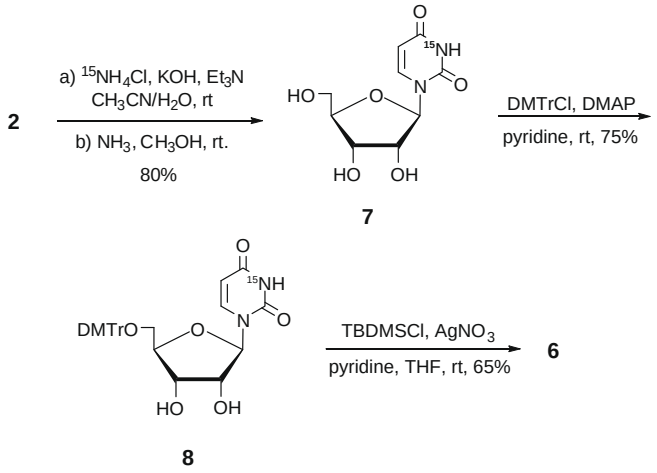


Scheme 1. Known synthetic route to [3-^{15}N]-labeled uridine phosphoramidite suitable for incorporation into oligoribonucleotides.[2f] PCl: 2-cyanoethyl *N*,*N*-diisopropyl-phosphoramidochloridite.



Scheme 2. Synthetic route to [3-^{15}N]-labeled uridine phosphoramidite **1**.

Cl, cat. DMAP in pyridine) afforded diol **4** in 75% yield. Regioselective O2′-silylation of **4** following the Ogilvie protocol,[7b] i.e., TBDMS-Cl/$AgNO_3$/pyridine (1.3/1.2/4.0 equiv, respectively) in tetrahydrofuran cleanly furnished nucleoside **5** in acceptable yield (65%). Disappearance of the ^{1}H NMR signal from the exchangeable 3′-OH proton upon D_2O addition ascertained the O2′-silylated constitution of nucleoside **5** (for full NMR/MS-characterization of this and other reported compounds, see Supplementary data). The stability of the N3-nitro group during these conditions (**2**→→**5**) underlines its potential as a useful electron-withdrawing N3-pro-



Scheme 3. Alternative route to [3-^{15}N]-labeled key intermediate **6**.

tecting group.[10] Introduction of the isotopic label was realized upon treatment of **5** with $^{15}NH_3$, which was formed in situ[2a,6] by the addition of potassium hydroxide to $^{15}NH_4Cl$ in acetonitrile. Migration of the TBDMS-group from the O2′- to the O3′-position (<15%) lowered the yield of key intermediate **6** to 50%. The presence of the isotopic label was verified by the characteristic ^{15}N NMR signal at $\delta = -220$ ppm[2a] appearing as a doublet of doublets due to coupling between the [3-^{15}N] and the H3 and H5 protons. As expected, $^{1}H-^{15}N$ and $^{13}C-^{15}N$ couplings were also observed in the ^{1}H and ^{13}C NMR spectra of **6**. Subsequent O3′-phosphitylation of **6** using the 'PCl-reagent' (2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite) in DIPEA along with *N*-methylimidazole as a activator, afforded target phosphoramidite **1** in 68% yield. Thus, this route provides amidite **1** in 16% overall yield from uridine (Scheme 2), which is comparable to the synthetic route leading to the equivalent 2′-*O*-TOM building block (Scheme 1).

To further increase the yield of desired [3-^{15}N]-labeled uridine phosphoramidite **1**, a shortcut to key intermediate **6** was developed using N3-nitro nucleoside **2** as the starting material (Scheme 3). Introduction of the isotopic label using $^{15}NH_3$ generated in situ followed by completion of global deacylation using saturated methanolic ammonia, afforded [3-^{15}N]-uridine **7**[2a] in 80% yield. As before, the appearance of characteristic ^{15}N NMR signals verified the successful introduction of the isotopic label. O5′-DMTr-protection of **7** (75% yield) and subsequent regioselective O2′-TBDMS protection of **8** (65% yield) using similar protocols as described above, successfully afforded key intermediate **6** in high overall yield. Thus, the preferred route (i.e., uridine → **2** → **7** → **8** → **6** → **1**) affords the desired [3-^{15}N]-labeled uridine phosphoramidite **1** in 26% overall yield while only necessitating five purification steps. This constitutes a marked improvement in yield and feasibility compared to the 2′-*O*-TOM approach (Scheme 1).

In summary, a short and high-yielding route to [3-^{15}N]-labeled O2′-TBDMS-protected uridine phosphoramidite **1** has been developed. Synthesis of isotopically labeled RNA strands and their use in structural studies are ongoing and will be reported in due course.

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Supplementary data

Supplementary data (experimental protocols, physical data, and spectra of nucleosides **1**–**8**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.08.003.

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