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Stereoselective Syntheses of 3′-Hydroxyamino- and 3′-Methoxyamino-2′,3′-Dideoxynucleosides

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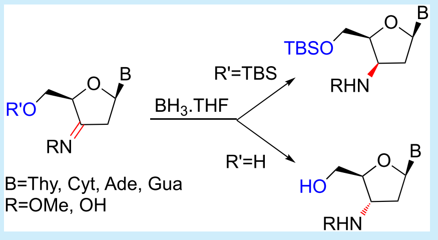
Supporting Information

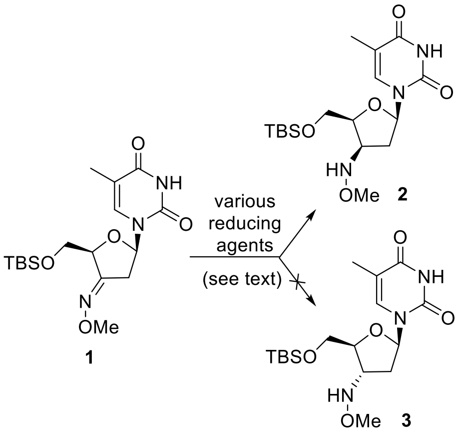
ABSTRACT: Aminonucleosides are used as key motifs in medicinal and bioconjugate chemistry; however, existing strategies toward 3′-hypernucleophilic amine systems do not readily deliver *deoxyribo*-configured products. We report diastereoselective syntheses of *deoxyribo*- and *deoxyxylo*-configured 3′-hydroxyamino- and 3′-methoxyamino-nucelosides from 3′-imine intermediates. The presence or absence of the 5′-hydroxyl-group protection dictates facial selectivity via inter- or intramolecular delivery of hydride from BH_3 (borane). Protecting group screening gave one access to previously unknown 3′-methoxyamino-deoxyguanosine derivatives.



Amino-functionalized nucleosides are key fragments for the development of antiviral agents, nucleic acids technologies, and bioconjugates. While the introduction of *aza*-functionalities at the 5′-position is relatively straightforward because of the limited effect of steric hindrance, 3′-functionalization is more challenging. Modified *ribo*- and *deoxyribo*-nucleosides with hydroxyamino and methoxyamino groups at their 3′-positions possess antiviral, anti-leukemic, and anti-HIV activities.[1] For example, the growth of L1210 cells was shown to be inhibited by 2′-deoxy-2′-(hydroxyamino) cytidine with an IC_{50} of 1.84 μM; however, synthesis was achieved indirectly, via a uridine derivative.[1b] Tronchet et al.[2] explored the synthesis of 3′-methoxyamino- and 3′-hydroxyamino-derivatives by stereoselective reduction of 3′-imines. They readily obtained *deoxyxylo*-configured systems as major or exclusive products across a range of reduction conditions. The *deoxyribo*-isomers, on the other hand, were usually minor products or absent, where syntheses have only been achieved via indirect, multistep methods. Richert, Szostak, and their coworkers have also exploited the nucleophilicity of amines for chemical primer extension studies; however, they have not taken advantage of the enhanced nucleophilicities of hypernucleophilic amines.[3] Thus, we sought to develop a stereoselective reduction strategy to access *deoxyribo*-configured 3′-hydroxyamino- and 3′-methoxyamino-nucleoside systems directly from 3′-imine intermediates.

Our initial investigations centered on thymidine systems because they do not require nucleobase protection and show reasonable solubility properties. We chose 5′-*O*-TBDMS-2,3-dideoxy-3-*N*-methoxyimino-thymidine **1** as our starting material, and it was prepared according to reported procedures.[4,2a] Tronchet et al.[2a] reported the use of $NaBH_3CN$ to reduce **1**, albeit with low levels of conversion; thus, we explored the use of $Bu_3SnH/BF_3{\cdot}Et_2O$,[5] L-selectride,[6] and $NaBH_4$;[7] however, in all cases, we were unable to obtain the desired *ribo*-configured compound **3** (Scheme 1), and the *xylo*-product was formed instead.

Scheme 1. Several Hydride-Transfer Agents Were Explored and Each Delivered Deoxyxylo-Configured Product 2 Exclusively



Sebesta et al.[8] and Matsuda and co-workers[1b] successfully synthesized 2′-(alkoxyamino)uridines via the intramolecular nucleophilic substitution upon 2,2′-*O*-anhydrouridine derivatives. Thus, we attempted nucleophilic substitution at the 3′-position of 2,3′-anhydrothymidine with methoxylamine under a range of reaction conditions; however, surprisingly, we only observed a hydrolytic opening of the anhydro-linkage.

Stereoselective reduction of 3′-keto nucleosides to ribonucleosides via intramolecular delivery of hydride, tethered through a free 5′-hydroxyl group, has been reported.[9] Moreover, Matsuda and co-workers[1b] reported that 3′-(hydroxyamino) uridine with a *ribo*-configuration **5a** can be obtained from the corresponding 3′-hydroxyiminouridine **4a** by treatment with $NaBH_4$/AcOH (Scheme 2). Thus, we

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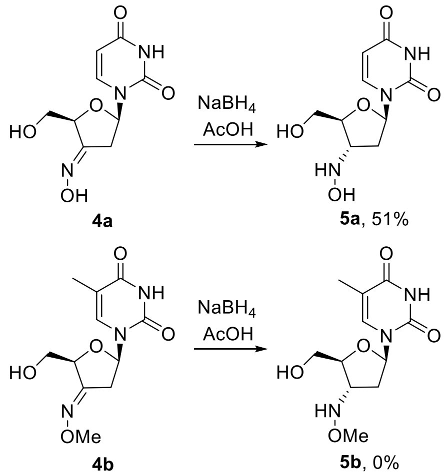
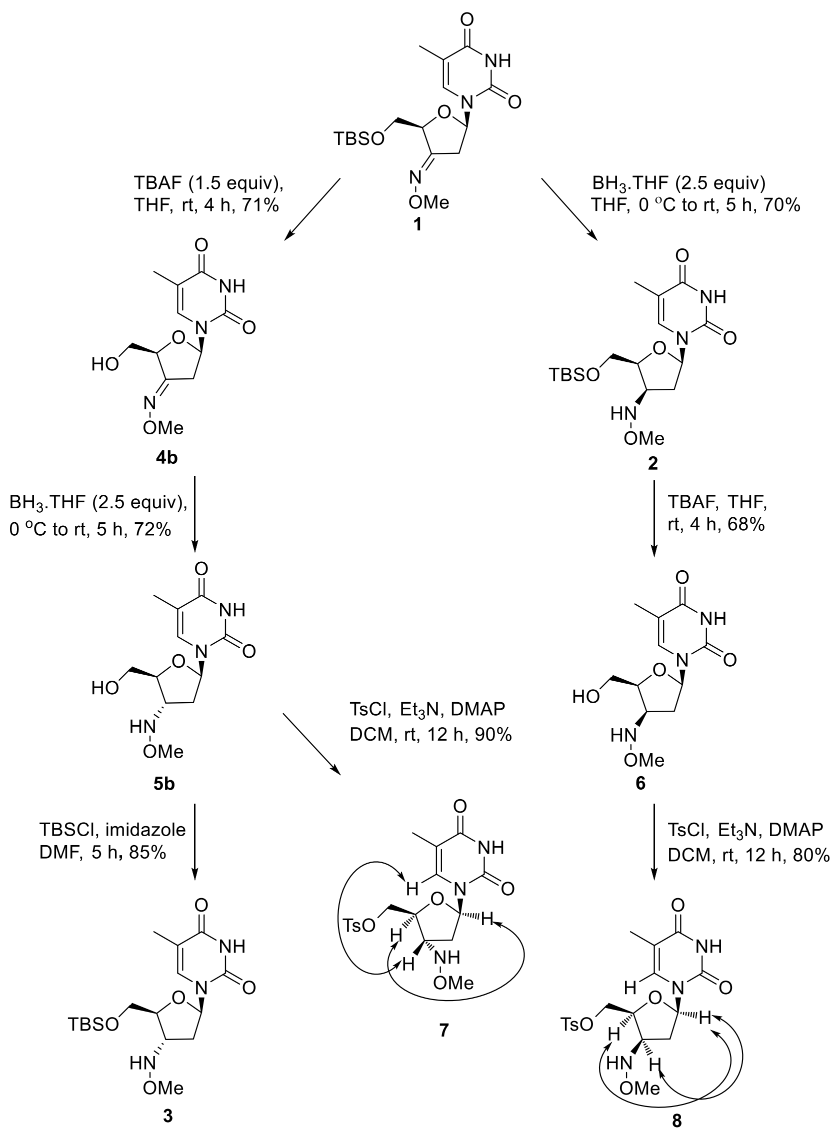
Scheme 2. Stereoselective Reduction of Uridine-Based Oxime 4a[1b] Is Observed but Not for the Thymidine Analog 4b



attempted the reduction of imine **4b** under similar conditions; however, poor conversion to **5b** was observed (Scheme 2). This result aligns with the findings of Tronchet et al.,[2] who used $NaBH_3CN$ upon **1** under acidic conditions to obtain low levels of the deoxyribomethoxyamino-product **5b** as part of a complex mixture that prevented the isolation of pure material.

We then explored the application of the borane–tetrahydrofuran complex for the reduction of **4b**, which we expected to show higher reactivity and higher levels of conversion. To our delight, we obtained 3′-methoxyamino-thymidine **5b** with the desired *deoxyribo*-configuration exclusively in 72% yield (Scheme 3). We were also able to reduce protected imine **1** with BH_3·THF to give *deoxyxylo*-configured product **2** in a yield of 70%. We sought to confirm the absolute configurations of the deprotected 3′-methoxyamino-products **5b** and **6** by 2D NMR spectroscopy. Unfortunately, the signals arising from the 3′-H [NCH(OMe)], 4′-H (OCH), and the 5′-H (OCH_2OTBS) protons were overlapping in the ^1H NMR spectra, thus preventing clear assignments by NOESY correlations. We also attempted

Scheme 3. Stereoselective Syntheses of *Deoxyribo*- and *Deoxyxylo*-Configured 3′-Methoxyamino-Thymidines[a]



[a]Arrows on structures **7** and **8** indicate observed NOESY correlations.

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similar analyses using the 5′-TBS-protected systems **2** and **3**; however, we encountered the same signal overlap problems. Thus, in order to increase the chemical shifts of the 5′-H signals and, to a lesser extent, 4′-H signals, we prepared 5′-tosyl derivatives **7** and **8**. This strategy allowed us to distinguish and assign each of the proton signals around the sugar rings. The *deoxyribo*-isomer **7** did not show NOESY correlation between the 3′- and the 1′-protons, whereas correlations were clearly observed for the *deoxyxylo*-isomer **8**. Additionally, in the case of *deoxyribo*-isomer **7**, NOESY signals were observed between the 3′-proton and thymine nucleobase, along with the expected NOESY correlation between the 4′- and the 1′-protons. The *xylo*-isomer **8** also showed the expected 4′−1′ NOESY correlations.

In order to gain mechanistic insights into the proposed intramolecular hydride delivery via complexation of the boron to the free hydroxyl group at the 5′-position, we carried out ^{11}B NMR experiments.[10] The 5′-TBS protected thymidine imine **1** and deprotected 3′-methoxyimino thymidine **4b** were treated with $B(OMe)_3$ in THF-d_8. Starting with the addition of 0.5 equiv of $B(OMe)_3$, ^{11}B NMR spectra were recorded for multiple additions of 0.5 equiv of $B(OMe)_3$ up to 2.5 equiv. Figure 1 gives evidence for B−N complexation via the imine

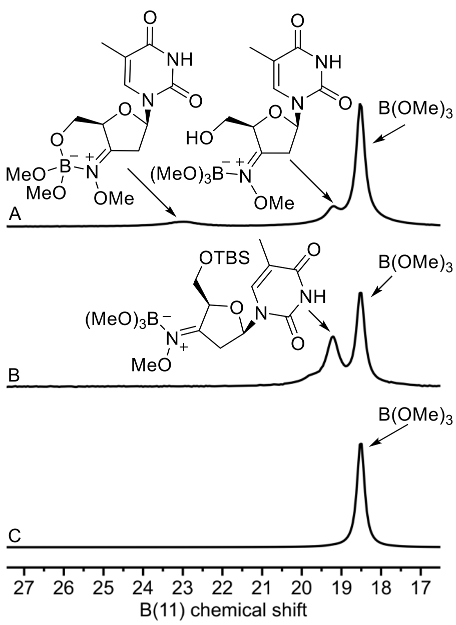

Figure 1. ^{11}B NMR studies in THF-d_8. (A) 5′-OH imine **4b** (1.0 equiv) mixed with $B(OMe)_3$ (1.5 equiv). (B) 5′-OTBS imine **1** (1.0 equiv) mixed with $B(OMe)_3$ (1.5 equiv). (C) $B(OMe)_3$ alone.

nitrogen of 5′-TBS-protected 3′-methoxyimino-thymidine **1** via a signal at 19.19 ppm, which persists even after overnight incubation with 2.5 equiv of $B(OMe)_3$. In the case of the 5′-hydroxy 3′-methoxyimino-thymidine **4b**, we observed two distinct signals at 22.98 ppm (RO−B−N) and 19.20 ppm that indicate the complexation of boron with the free hydroxyl group at the 5′-position and B−N complex, respectively (Figure 1).[11] Taken together, these simple experiments support the idea of a critical role for 5′-OH complexation in the reduction of **4b** to deliver the *deoxyribo*-configuration observed in **5b**.

On the basis of our promising results with the thymidine system, we applied the same strategies to the adenosine and cytidine systems. Reduction with BH_3·THF was successfully performed on 5′-OH- and 5′-OTBS-3′-methoxyimino-2′,3′-dideoxycytidine systems[12] to afford *deoxyribo*-product (**9a**) and *deoxyxylo*-product (**9b**), respectively, in 71% and 68% yields (Figure 2). The 5′-OH-3′-methoxyimino-2′,3′-dideox-

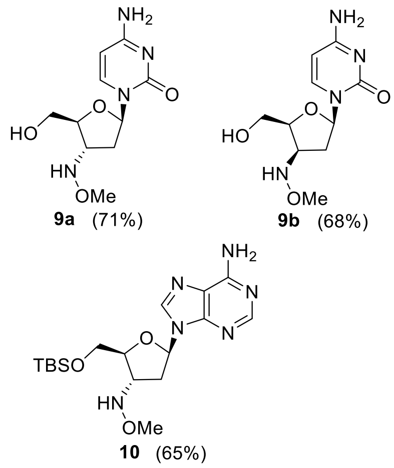

Figure 2. Product scope for deoxycytidine and deoxyadenosine systems.

yadenosine system[12] afforded the deoxyribomethoxylamine product **10** exclusively, which was derivatized at the 5′-position (Figure 2) to minimize conformational changes and, thus, confirm configuration (see the Supporting Information).[14,2b]

We then moved on to explore the application of our BH_3·THF reduction strategies toward guanosine systems. Guanosine systems present significant synthetic challenges because of their poor solubility properties.[13] With this in mind, we attempted reductions on the 5′-OTBS-*N*-isobutyroyl-protected methoxyimino-derivative of deoxyguanosine and the analogous 5′-OH system[12] using BH_3·THF. These reactions resulted in the reduction of the imines to the desired *deoxyxylo*-product (**11b**) and *deoxyribo*-product (**11a**) in 85% and 70% yield, respectively, but the isobutyroyl group was also reduced. Thus, we moved to a *N*-DMT-protected substrate, which tolerated BH_3·THF to yield the *deoxyribo*-product **12** after TBS protection, as its tosic acid salt in 80% yield upon deprotection of the DMT group (Figure 3). The configurations of the derivatives of all guanosine products were confirmed by NOESY analysis of the 5′-derivatives (see the Supporting Information).

Next, we explored the BH_3·THF reductions of 3′-hydroxyimino systems. The unprotected 3′-hydroxyimino-thymidine derivative[4] **13a** was reduced by BH_3·THF stereoselectively to give *deoxyribo*-configured **14a**[15] as the major product alongside the *deoxyxylo*-derivative **14b**[1c] in a 4:1 ratio, where the mixture could be separated by column chromatography. On the other hand, the 5′-TBS-protected 3′-hydroxyimino-thymidine derivative **13b**[2b] afforded the *deoxyxylo*-product **15**[2b] exclusively. The NMR spectra of the TBS-protected *deoxyribo*-derivative **16** and *deoxyxylo*-isomer **15** matched NMR data reported by Tronchet et al.[2b] (Scheme 4). This strategy was also successfully applied to deoxycytidine and deoxyadenosine systems to afford mixtures of *deoxyribo*- and *deoxyxylo*-isomers, in ∼4:1 ratios, which could also be isolated by chromatography. The products were derivatized to **17a**, **17b**, and **18** to minimize conformational equilibration[14]

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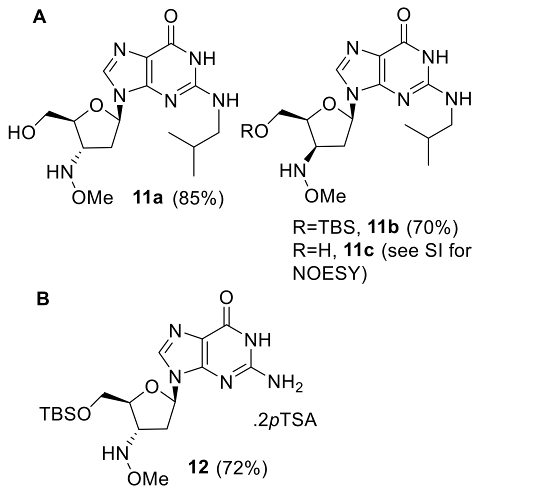


Figure 3. Deoxyguanosine systems. (A) The protecting groups of the isobutyroyl-protected imine substrates were also reduced. (B) DMT-protected imine substrate afforded the desired deoxyribo-configured methoxyamino-nucleoside upon DMT deprotection (*p*TSA = *para*-toluenesulfonate).

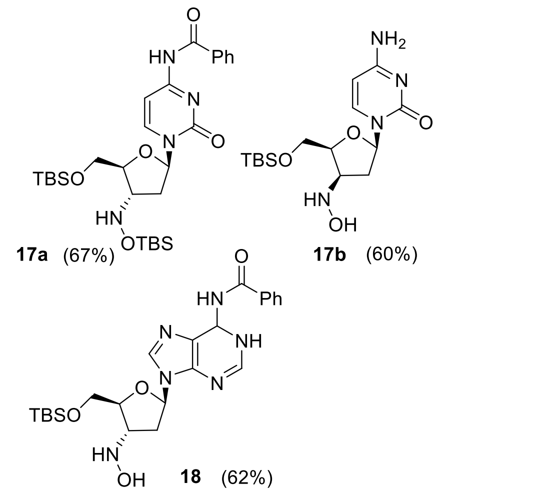


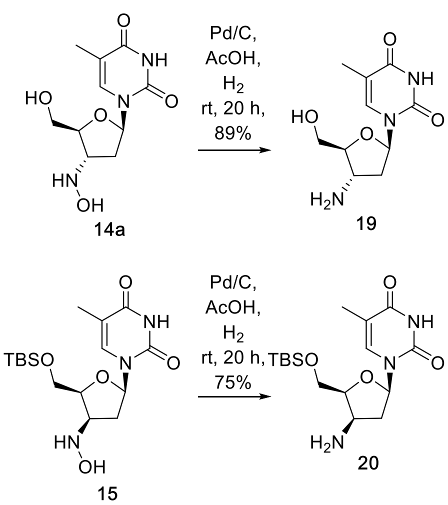
Figure 4. Product scope for deoxycytidine and deoxyadenosine systems.

and thus allow differentiation between the *deoxyribo*- and *deoxyxylo*-products through NOESY assignments. *Bis*-TBS-protected 3′-hydroxyamino-cytidine derivative **17a** exhibited NOESY correlations between the 3′-proton and the 6-(nucleobase)-proton, whereas the debenzoylated-*deoxyxylo*-derivative **17b** exhibited 1′-H to 3′-H NOESY correlation. Similarly, the TBS-protected-*deoxyribo*-3′-hydroxyamino-adenosine **18** exhibited NOESY correlations between the protons 3′- and 8-H of the nucleobase (Figure 4).

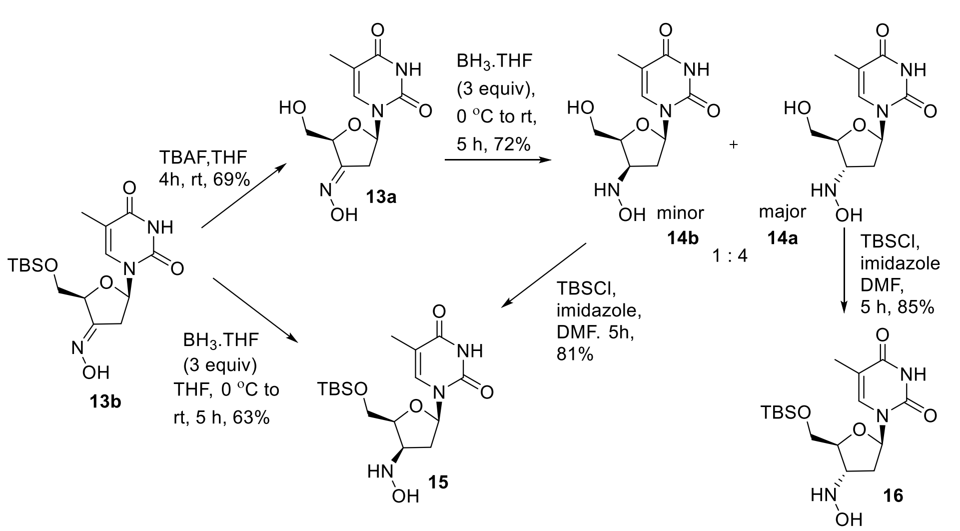
Kojima et al. demonstrated that 3′-hydroxylamine systems can be further reduced to 3′-amines by Pd/C and hydrogen to afford 3′-amino-ribonucleoside analogs.[16] We applied the same methodology to hydroxylamino-systems **14a** and **15**, and we were pleased to observe clean conversion to the corresponding amine systems **19** and **20** in 89% and 75% yield, respectively (Scheme 5).

In conclusion, we have developed efficient, direct strategies to obtain *deoxyribo*- and *deoxyxylo*-isomers of 3′-methoxyamino- and 3′-hydroxyamino-deoxynucleosides, from common

Scheme 5. Synthesis of 3′-Aminonucleoside Systems via Catalytic Reductions of Hydroxylamines



Scheme 4. Synthesis of *Deoxyribo*- and *Deoxyxylo*-Configured 3′-Hydroxyamino Thymidine Derivative



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intermediates, via stereoselective reductions of the corresponding 3′-imino deoxynucleosides using BH_3·THF. Our approach has delivered *ribo*-configured deoxynucleosides in good yields, which are otherwise difficult to obtain. To the best of our knowledge, the *ribo*-deoxycytidine derivative **9a**, deoxyadenosine derivative **10**, and *ribo*- and *xylo*-deoxyguanosine derivatives **11a–c** and **12** containing the 3′-methoxyamino-functionality are novel compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b03474.

Experimental procedures and characterizations (PDF)

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Notes

The authors declare no competing financial interest.

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