

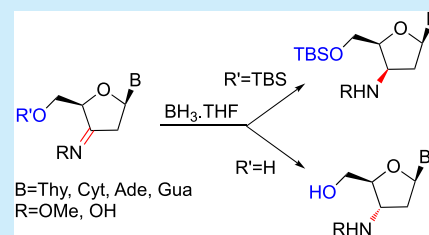
# Stereoselective Syntheses of 3'-Hydroxyamino- and 3'-Methoxyamino-2',3'-Dideoxynucleosides

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## Supporting Information

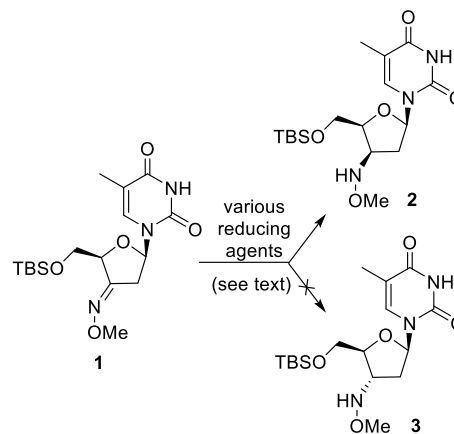
**ABSTRACT:** Aminonucleosides are used as key motifs in medicinal and bioconjugate chemistry; however, existing strategies toward 3'-hypernucleophilic amine systems do not readily deliver *deoxyribo*-configured products. We report diastereoselective syntheses of *deoxyribo*- and *deoxyxylo*-configured 3'-hydroxyamino- and 3'-methoxyamino-nucleosides 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<sub>3</sub> (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.<sup>1</sup> For example, the growth of L1210 cells was shown to be inhibited by 2'-deoxy-2'-(hydroxyamino) cytidine with an IC<sub>50</sub> of 1.84 μM; however, synthesis was achieved indirectly, via a uridine derivative.<sup>1b</sup> Tronchet et al.<sup>2</sup> 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 co-workers 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.<sup>3</sup> 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.<sup>4,2a</sup> Tronchet et al.<sup>2a</sup> reported the use of NaBH<sub>3</sub>CN to reduce **1**, albeit with low levels of conversion; thus, we explored the use of Bu<sub>3</sub>SnH/BF<sub>3</sub>·Et<sub>2</sub>O,<sup>5</sup> L-selectride,<sup>6</sup> and NaBH<sub>4</sub>;<sup>7</sup> 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



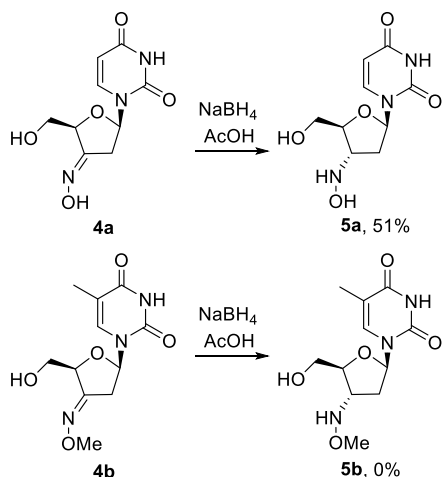
Sebesta et al.<sup>8</sup> and Matsuda and co-workers<sup>1b</sup> successfully synthesized 2'-(alkoxyamino)uridines via the intramolecular nucleophilic substitution upon 2,2'-*O*-anhydrouridine derivatives. Thus, we attempted nucleophilic substitution at the 3'-position of 2,3'-anhydrothymidine with 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.<sup>9</sup> Moreover, Matsuda and co-workers<sup>1b</sup> reported that 3'-(hydroxyamino) uridine with a *ribo*-configuration **5a** can be obtained from the corresponding 3'-hydroxyiminouridine **4a** by treatment with NaBH<sub>4</sub>/AcOH (Scheme 2). Thus, we

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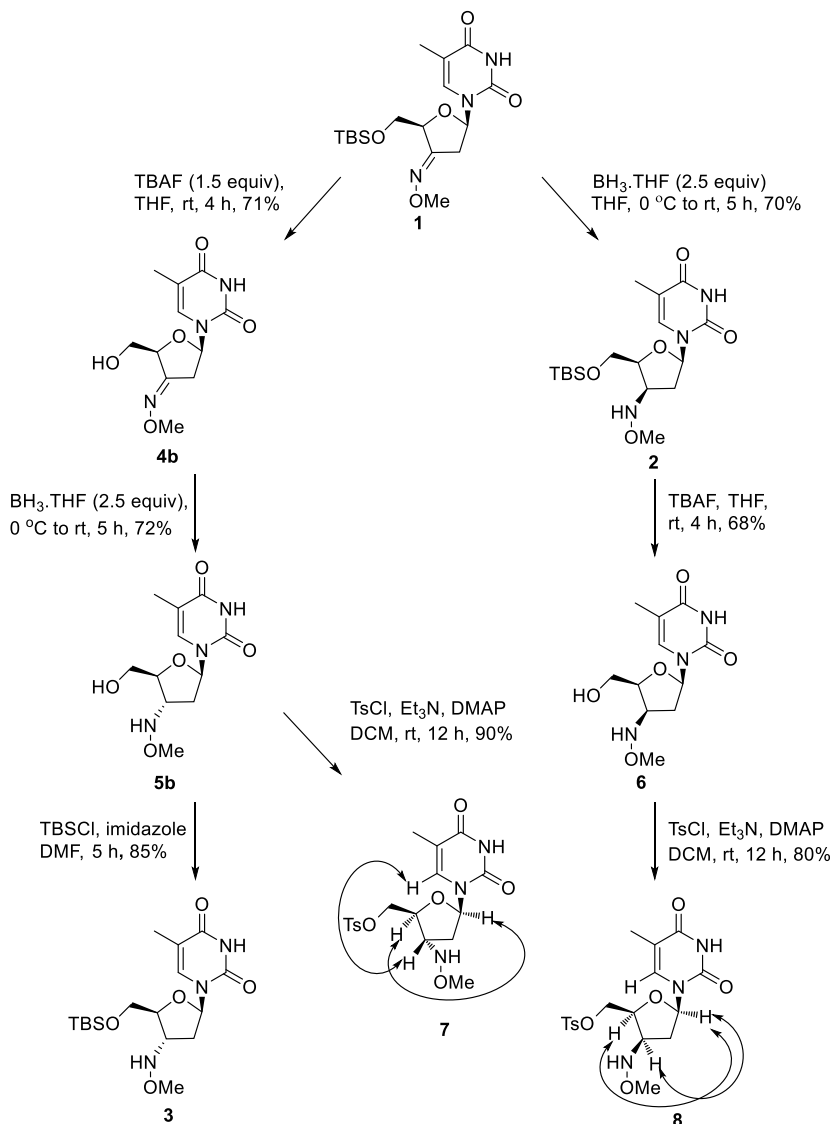
**Scheme 2. Stereoselective Reduction of Uridine-Based Oxime 4a<sup>1b</sup> Is Observed but Not for the Thymidine Analog 4b**



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.,<sup>2</sup> who used  $\text{NaBH}_3\text{CN}$  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  $\text{BH}_3\cdot\text{THF}$  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<sub>2</sub>OTBS) protons were overlapping in the <sup>1</sup>H NMR spectra, thus preventing clear assignments by NOESY correlations. We also attempted

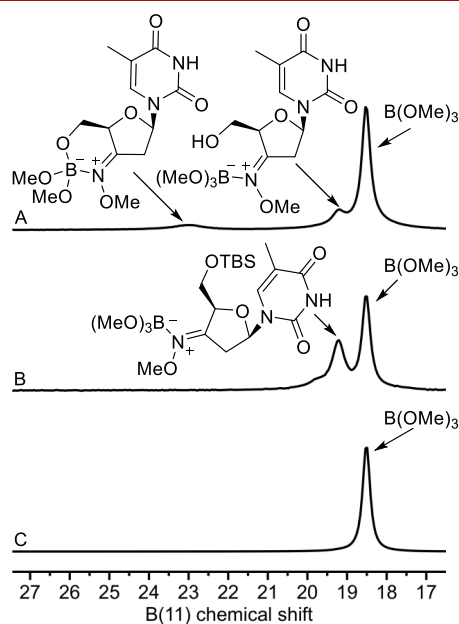
**Scheme 3. Stereoselective Syntheses of *Deoxyribo*- and *Deoxyxylo*-Configured 3'-Methoxyamino-Thymidines<sup>a</sup>**



<sup>a</sup>Arrows on structures **7** and **8** indicate observed NOESY correlations.

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  $^{11}\text{B}$  NMR experiments.<sup>10</sup> The 5'-TBS protected thymidine imine **1** and deprotected 3'-methoxyimino thymidine **4b** were treated with  $\text{B}(\text{OMe})_3$  in  $\text{THF-}d_8$ . Starting with the addition of 0.5 equiv of  $\text{B}(\text{OMe})_3$ ,  $^{11}\text{B}$  NMR spectra were recorded for multiple additions of 0.5 equiv of  $\text{B}(\text{OMe})_3$  up to 2.5 equiv. **Figure 1** gives evidence for B–N complexation via the imine

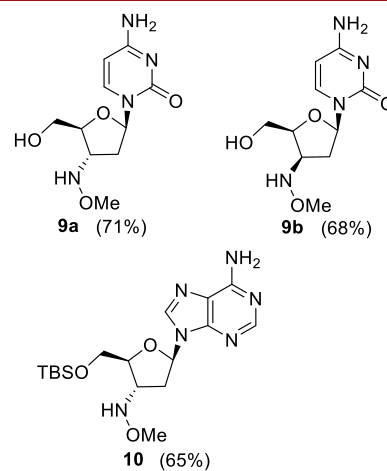


**Figure 1.**  $^{11}\text{B}$  NMR studies in  $\text{THF-}d_8$ . (A) 5'-OH imine **4b** (1.0 equiv) mixed with  $\text{B}(\text{OMe})_3$  (1.5 equiv). (B) 5'-OTBS imine **1** (1.0 equiv) mixed with  $\text{B}(\text{OMe})_3$  (1.5 equiv). (C)  $\text{B}(\text{OMe})_3$  alone.

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  $\text{B}(\text{OMe})_3$ . 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**).<sup>11</sup> 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  $\text{BH}_3\cdot\text{THF}$  was successfully performed on 5'-OH- and 5'-OTBS-3'-methoxyimino-2',3'-dideoxycytidine systems<sup>12</sup> 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-

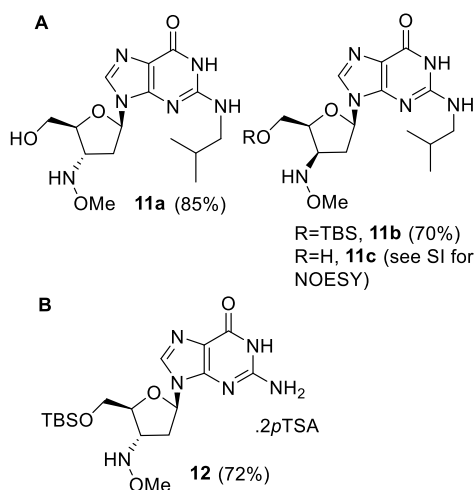


**Figure 2.** Product scope for deoxycytidine and deoxyadenosine systems.

yadenosine system<sup>12</sup> 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**).<sup>14,2b</sup>

We then moved on to explore the application of our  $\text{BH}_3\cdot\text{THF}$  reduction strategies toward guanosine systems. Guanosine systems present significant synthetic challenges because of their poor solubility properties.<sup>13</sup> With this in mind, we attempted reductions on the 5'-OTBS-*N*-isobutyryl-protected methoxyimino-derivative of deoxyguanosine and the analogous 5'-OH system<sup>12</sup> using  $\text{BH}_3\cdot\text{THF}$ . 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 isobutyryl group was also reduced. Thus, we moved to a *N*-DMT-protected substrate, which tolerated  $\text{BH}_3\cdot\text{THF}$  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  $\text{BH}_3\cdot\text{THF}$  reductions of 3'-hydroxyimino systems. The unprotected 3'-hydroxyimino-thymidine derivative<sup>4</sup> **13a** was reduced by  $\text{BH}_3\cdot\text{THF}$  stereoselectively to give *deoxyribo*-configured **14a**<sup>15</sup> as the major product alongside the *deoxyxylo*-derivative **14b**<sup>1c</sup> 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**<sup>2b</sup> afforded the *deoxyxylo*-product **15**<sup>2b</sup> exclusively. The NMR spectra of the TBS-protected *deoxyribo*-derivative **16** and *deoxyxylo*-isomer **15** matched NMR data reported by Tronchet et al.<sup>2b</sup> (**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<sup>14</sup>

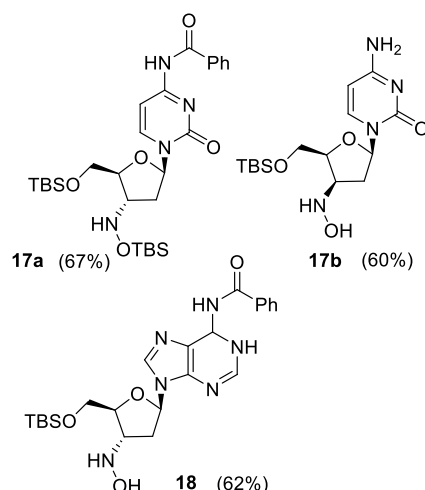


**Figure 3.** Deoxyguanosine systems. (A) The protecting groups of the isobutyryl-protected imine substrates were also reduced. (B) DMT-protected imine substrate afforded the desired deoxyribo-configured methoxyamino-nucleoside upon DMT deprotection (*p*TSA = *para*-toluenesulfonate).

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).

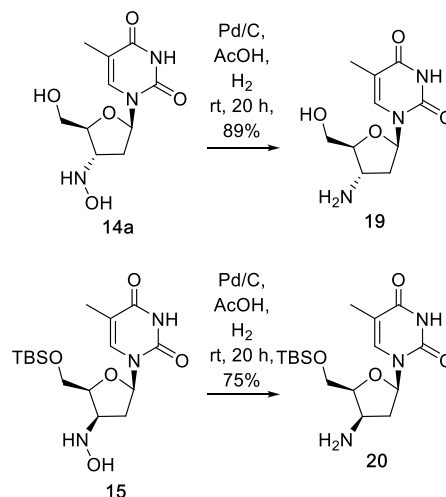
Kojima et al. demonstrated that 3'-hydroxylamine systems can be further reduced to 3'-amines by Pd/C and hydrogen to afford 3'-amino-ribonucleoside analogs.<sup>16</sup> We applied the same methodology to hydroxylamine-systems **14a** and **15**, and we were pleased to observe clean conversion to the 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

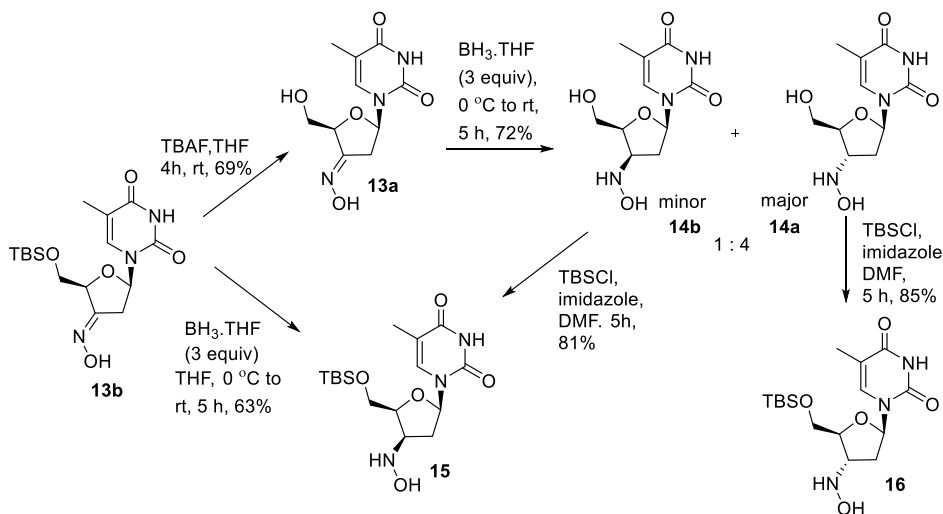


**Figure 4.** Product scope for deoxycytidine and deoxyadenosine systems.

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



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



intermediates, via stereoselective reductions of the corresponding 3'-imino deoxynucleosides using  $\text{BH}_3 \cdot \text{THF}$ . 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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