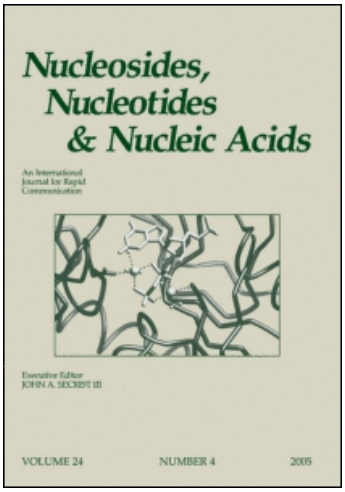

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THE SYNTHESIS OF *ANTI*-FIXED 3-METHYL-3- DEAZA-2'-DEOXYADENOSINE AND OTHER 3*H*-IMIDAZO[4,5-c]PYRIDINE ANALOGS

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THE SYNTHESIS OF *ANTI*-FIXED 3-METHYL-3-DEAZA-2′-DEOXYADENOSINE AND OTHER 3*H*-IMIDAZO[4,5-c]PYRIDINE ANALOGS

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ABSTRACT

Rotation of a heterocyclic base around a glycosidic bond allows the formation of *syn* and *anti* conformations in nucleosides. The *syn* conformation has been observed primarily in purine-purine mismatches in DNA duplexes. Such mismatches give rise to false positive oligonucleotide hybridization in DNA-based diagnostics. Here we describe the synthesis of an analog of 2′-deoxyadenosine that retains its Watson-Crick functional groups, but cannot form the *syn* conformation. In this analog, the N3 atom of 2′-deoxyadenosine is replaced by a C-CH_3 group to give 7-methyl-1-β-D-deoxyribofuranosyl-1H-imidazo[4,5-c]pyridin-4-ylamine or 3-methyl-3-deaza-2′-deoxyadenosine (3mddA). This modification sterically prevents the *syn* conformation and 3mddA becomes an *anti*-fixed nucleoside analog of 2′-deoxyadenosine. The synthesis and conformational analysis of 3mddA and several analogs with an 3H-imidazo[4,5-c]pyridine skeleton are described, as well as their potential applications.

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INTRODUCTION

Natural DNA nucleosides can adopt either the *syn* or the *anti* conformation about the glycosidic dihedral angle. Several nucleosides have been synthesized where the nucleoside is locked in either the *syn* or the *anti* conformation. 8-bromoadenosine and 8-bromoguanosine adopt exclusively the *syn* conformation,[1] while 3-deaza-3-methylinosine adopts exclusively the *anti* conformation.[2] X-ray studies on 3-deaza-3-methylinosine indicated that steric hindrance from the extra methyl group was adequate to hinder the rotation of the purine analog around the glycosidic bond.[3] Nuclear Overhauser effect (NOE) experiments showed that 3-deaza-3-halo (iodo, bromo, chloro) derivatives of guanosine, adenosine and inosine nucleosides also adopt an *anti*-fixed conformation.[4]

In Watson-Crick base pairs (Fig. 1) the nucleosides are in an *anti* conformation, but for purine-purine mismatches (Fig. 2), a variety of base pairing arrangements are possible. For single purine-purine mismatches, it has been observed that one of the nucleosides is often in the *anti* conformation and the other is in the *syn* conformation.[5–9] To destabilize the single purine-purine mismatch, we synthesized 3-methyl-3-deaza-2′-deoxyadenosine (3mddA; Fig. 3), which is an *anti*-fixed analog of 2′-deoxyadenosine. This nucleoside analog may have several applications: 1. in DNA oligonucleotide arrays, it could be used to reduce the formation false positive results caused by purine-purine mismatches,[10] 2. to improve the specificity and binding constant of target hybridization by DNA primers and probes in PCR and other hybridization-based assays, 3. to act as a probe to study protein-nucleic acid interactions in the minor groove, 4. the nucleoside 3mddA as well as the other adenosine analogs presented here are also candidates for antiviral activity, and 5. 3mddA is a stable uncharged analog of 3-methyl-2′-deoxyadenosine, which is a common alkylation product[11–15] and may be useful for mechanistic and X-ray structural studies in DNA mismatch repair of alkylated bases.

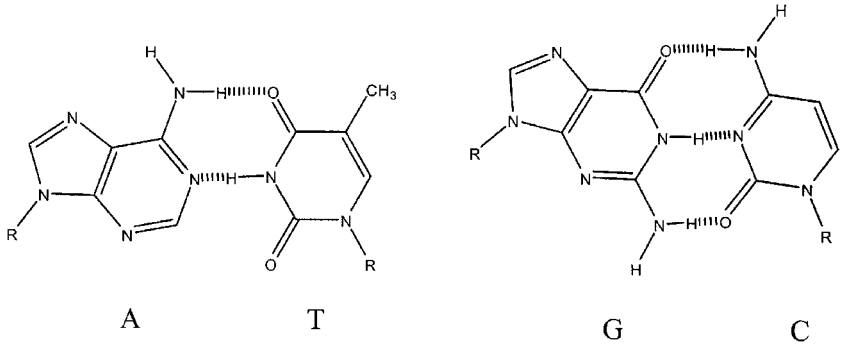


Figure 1. Watson-Crick base pairs showing *anti* conformation in base pairing.

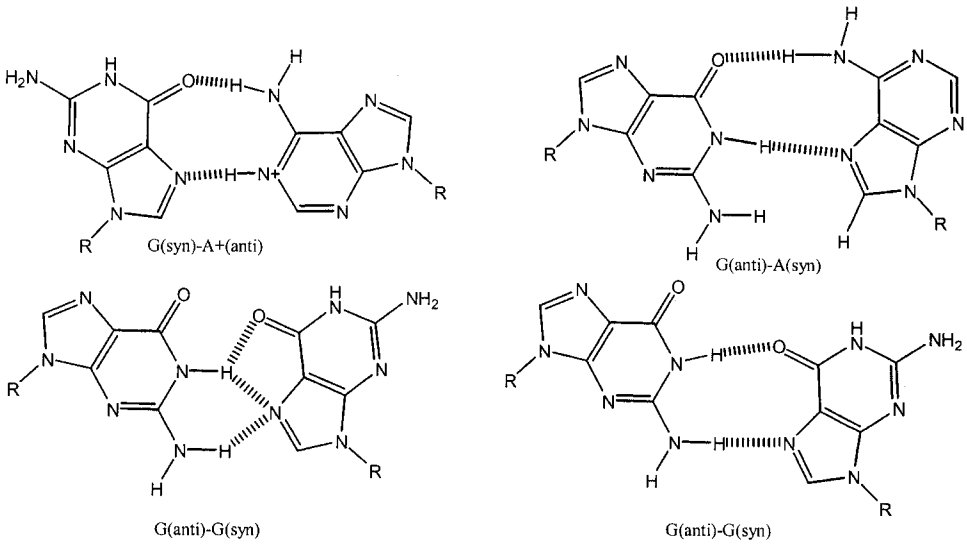


Figure 2. Purine-purine mismatches showing *syn* conformation in base pairing.

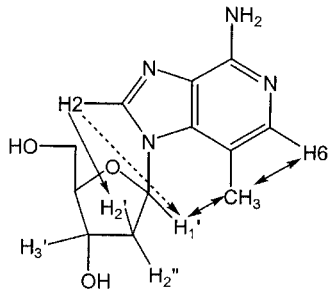


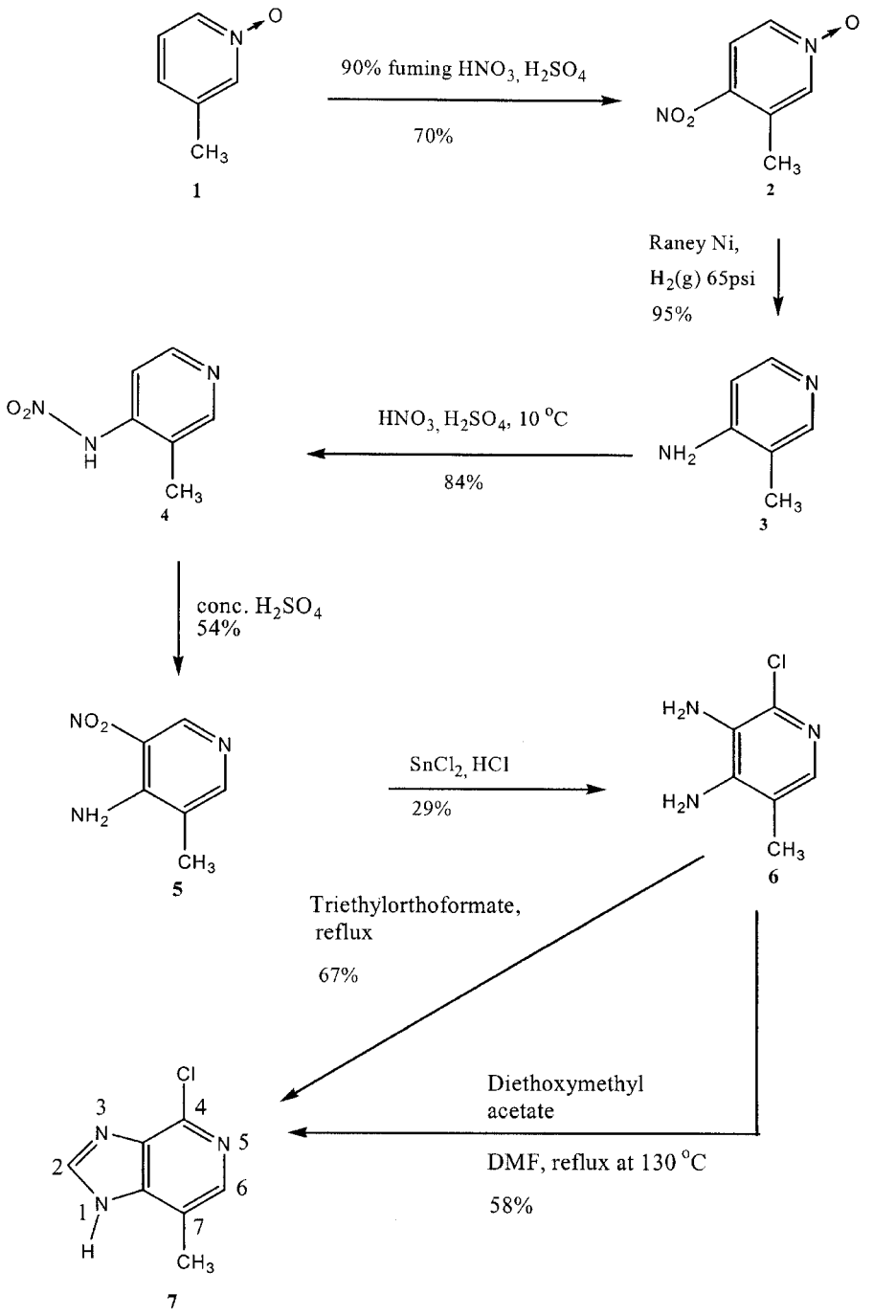
Figure 3. Schematic representation of important 1D NOE interactions for compound **11**. Solid arrows indicate strong NOEs and dashed arrows indicate weak NOEs.

In this paper, we discuss the syntheses and characterization of *anti*-fixed 3-methyl-3-deaza-2′-deoxyadenosine and several 3*H*-imidazo[4,5-c]pyridine analogs.

RESULTS AND DISCUSSION

Synthesis of 3-Methyl-3-deazaadenine

Scheme 1 shows the synthesis of the nucleobase from the starting material 3-picoline N-oxide (**1**). The IUPAC numbering system of imidazo[4,5-c]pyridines is different from that of purines and is shown in Sch. 1 compound **7**. Regioselective nitration of compound **1** with fuming nitric acid and concentrated sulfuric acid gave 4-nitro-3-picoline N-oxide (**2**), which on

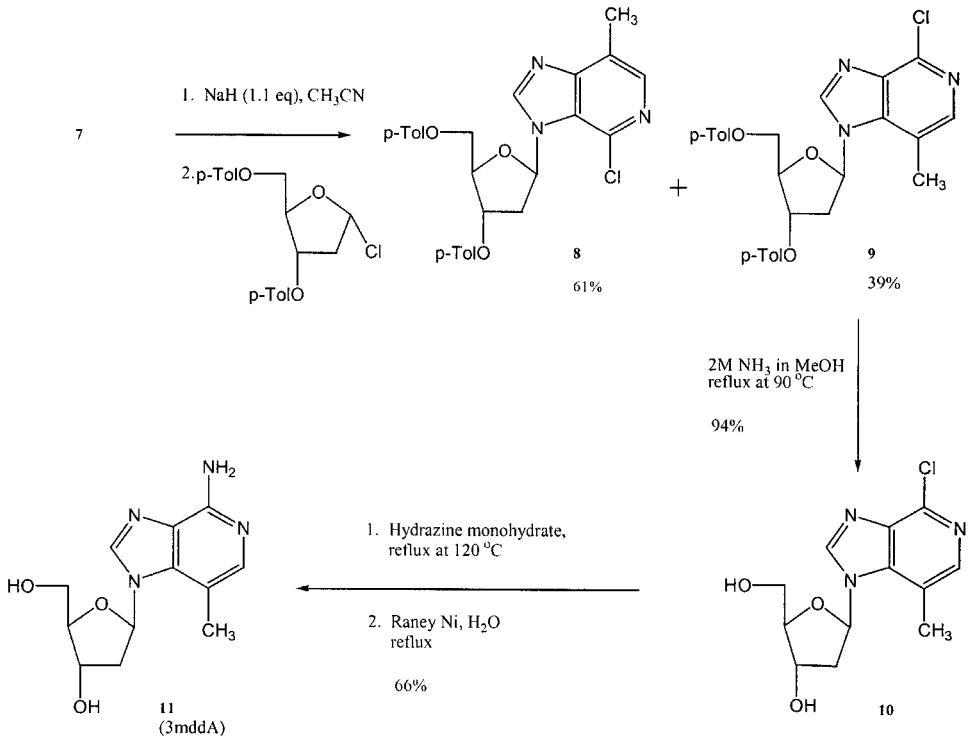


Scheme 1.

recrystallization from methanol was obtained in 70% yield, by adapting the literature procedure for other nitropyridines.[16,17] The next step was the reduction of compound **2** to 4-amino-3-methylpyridine (**3**), for which several different reaction conditions were tried. In the presence of tin (II) chloride and concentrated HCl, only the nitro group was reduced and the N-oxide was unaffected. On reduction with hydrogen catalyzed by palladium (10 wt. % on activated carbon) or platinum(IV) oxide, a mixture of partially reduced compounds along with compound **3** was generated. The same result was obtained when **2** was refluxed with iron powder in glacial acetic acid. The method of choice used for reduction of **2** to **3** was Raney nickel in methanol, under 65 psi of hydrogen gas in a Parr hydrogenator (95% yield). Nitration of **3** with concentrated nitric acid and concentrated sulfuric acid in an ice bath gave 4-nitramino-3-methylpyridine (**4**) in 84% yield, as described previously.[18,19] The nitro group migrated from the amine to the ring in the presence of concentrated sulfuric acid overnight at room temperature, to yield 5-nitro-4-amino-3-methylpyridine (**5**) with a 54% yield, as described previuosly.[18,19] Reduction of compound **5** in the presence of tin (II) chloride and HCl afforded 4,5-diamino-6-chloro-3-methylpyridine (**6**) with a yield of 29%, which is lower than the previously reported yield of 58%,[19,20] but sufficient for preparation of gram quantities for further steps. The tin (II) chloride reduction was a key step in the synthesis since it reduced the nitro group to an amine and regioselectively added a chloro group, which was used later in the synthesis to introduce the final amine into the nucleoside. Ring closure was accomplished by reflux of **6** with triethylorthoformate giving 4-chloro-7-methyl-1(*H*)-imidazo[4,5-c]pyridine (**7**) with a 67% yield. This is an improved method to **7** than the previously reported two-step procedure with formic acid ring closure and $POCl_3$ chlorination, which proceeded in 49% yield.[19] Compound **7** was also produced from **6** by reflux with diethoxymethyl acetate in dimethylformamide (DMF) at 130°C, though in lower yield (58%).

Coupling of the Base with Deoxyribose

Coupling of the nucleobase to the deoxyribose sugar was attempted by the enzymatic[21] and the sodium salt glycosylation[22] methods. The enzymatic method yielded no coupling product, however, indicating that purine nucleoside phosphorylase does not recognize the modified nucleobase as a substrate. As shown in Sch. 2, the sodium salt glycosylation method produced the desired compound **9**, in 39% yield and the undesired **8** in 61% yield. Compound **9** was deprotected by reflux at 90°C in 2 M NH_3 in methanol to produce compound **10** with a yield of 94%. The exchange of the chloride with an amino group at position 6 in purines is usually accomplished during the deprotection step.[23] Deazapurines, however, are

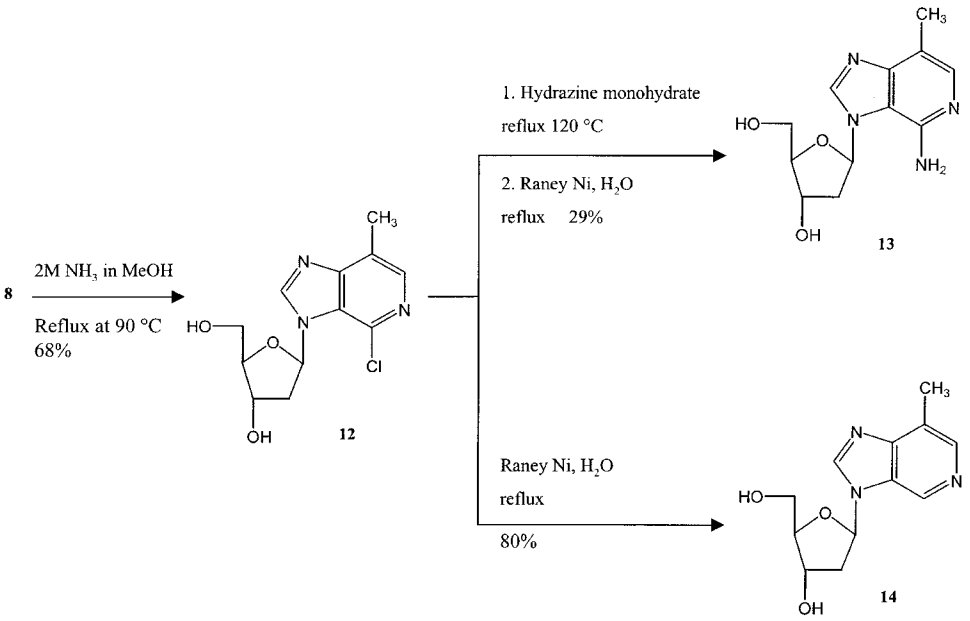


Scheme 2.

more electron-rich than purines and therefore hydrazine was used to affect the transformation.[24] 3mddA was obtained from compound **10** in 66% yield by reaction with hydrazine monohydrate at 120°C and subsequent reduction by reflux with Raney nickel in water.

Synthesis of Additional Nucleosides with A 3*H*-Imidazo[4,5-c]pyridine Skeleton

For the synthesis of a nucleoside with a 3*H*-imidazo[4,5-c]pyridine skeleton, compound **8** was used as the starting material. As shown in Sch. 3, compound **8** was deprotected using 2 M ammonia in methanol to give compound **12** in 68% yield. Compound **12** was converted to **13** in two steps. In step one, the chloride group was exchanged with hydrazine by reflux with hydrazine monohydrate at 120°C. This hydrazine intermediate was then reduced by reflux with Raney nickel in water to give compound **13** with a yield of 29%. In addition, compound **12** was also reduced by reflux with Raney nickel in water to give compound **14** in 80% yield.



Scheme 3.

Conformational Studies Using NMR Spectroscopy

The 1D NOE data for compound **11** are shown in Table 1 and Fig. 3. 1D NOE experiments are useful to qualitatively and semiquantitavely assess the *syn*/*anti* conformational equilibrium.[25] According to Rosemeyer et al.[25] for the *anti* conformation irradiation of H8 of purines (corresponding to H2 in 3mddA) exhibits a strong NOE to H2′ and a weak NOE to H1′. On the other hand, for the *syn* conformation irradiation of H8 of purines exhibits a strong NOE to H1′ and weaker NOEs to H2′ and H3′. The data in Table 1, indicate that upon irradiation of H2 of compound **11**, a strong NOE to H2′ (2.4%), and a weaker NOE to H1′ (2.0%) is observed as shown in Fig. 3.

Table 1. Results of 1D NOE Experiments on the 3mddA Nucleoside

Proton Irradiated	NOE (%)
H2	H1′ (2.0), 5′-OH (0.5), H3′ (0.9), H2′ (2.4)
H6	CH_3-7 (2.1)
H1′	H2 (0.8), H4′ (0.5), H2′ (0.3), CH_3-7 (2.8), H2″(2.3)
H3′	H2 (0.6), 3′-OH (1.7), H5′/H5″ (0.8), H2′ (1.4), H2″ (0.7)
H2′	H2 (1.9), H1′ (1.1), H3′ (1.3), H2″ (3.0)
CH_3-7	H6 (1.4), H1′ (1.4)
H2″	H1′ (2.7), H3′ (0.6), H2′ (3.8)

These data indicate that **11** is in the *anti* conformation. Irradiation of H6 in compound **11**, resulted in a strong NOE (2.1%) to the methyl group and no NOEs to sugar protons, consistent with the *anti* conformation. Irradiation of the H1′ of compound **11** shows the strongest NOE (2.8%) to the methyl group indicating the proximity of the H1′ to the methyl group on the base, thus further confirming its *anti* conformation. The *anti* conformation is also consistent with the irradiation of the methyl group which only shows NOEs to the H6 (1.4%) and H1′ (1.4%).

CONCLUSIONS

The synthesis of 3mddA and the 3*H*-imidazo[4,5-c]pyridine analogs have further expanded the nucleoside alphabet. 3mddA is an *anti*-fixed nucleoside analog with potential applications in several fields including DNA microarrays, hybridization-based genotyping methods, and studies of DNA mismatch repair and DNA-protein interactions. In the future, we plan to study the thermodynamic and structural effects of 3mddA on single purine-purine mismatches in duplex DNA. At present, 3mddA and the 3*H*-imidazo[4,5-c]pyridine described here are being tested for their antiviral potential.

EXPERIMENTAL SECTION

General Methods

Physical data were measured as follows: Melting points are uncorrected. ^{1}H and ^{13}C NMR data were measured at 300, 400 or 500 MHz instruments in d_6-DMSO or d_6-acetone. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) using TMS or residual non-deuterated solvent as reference. Multiplicity is indicated using the following abbreviations: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) or br (broad). The NOE experiments were performed in d_6-DMSO. Each peak was irradiated, in an interleaved fashion to minimize errors due to spectrometer drift, for 250 μs yielding a saturation of $\leq$95%. The recycle delay was set at 3 s and the acquisition time was 1.9 s.

1-chloro-2-deoxy-3′,5′-di-O-p-toluoyl-α-D-erythro-pentofuranose was prepared from 2′-deoxyribose as described by Hoffer.[26] The starting material 3-picoline-N-oxide (compound **1**), thymidine, thymidine phosphorylase, and purine nucleoside phosphorylase were purchased from Sigma-Aldrich. Silica gel used for column chromatography was Merck, grade 9385, 230–400 mesh, 60 Å purchased from Sigma-Aldrich.

4-Nitro-3-picoline N-oxide (2). Compound **1** (20 g, 0.183 mol) was dissolved in 100 mL of concentrated H_2SO_4 and warmed to 60°C. While keeping the temperature below 80°C, 40 mL of 90% fuming HNO_3 was added dropwise. After 2 h, the reaction was cooled to room temperature and poured onto crushed ice. The cooled mixture was then titrated with concentrated NH_4OH until a yellow precipitate formed. This precipitate was filtered and dried in a vacuum oven. Upon recrystallization from methanol, yellow crystals of compound **2** were obtained with a 70% yield: mp 136–138°C, ^{1}H NMR (d_6-acetone) δ 8.29 (s, 1H, H2), 8.19 (m, 1H, H6), 8.11 (d, $J = 12$ Hz, 1H, H5), 2.59 (s, 3H, CH_3); EI-MS m/z 154 (M^+). Anal. Calcd. for $C_6H_6N_2O_3$: C, 46.74; H, 3.93; N, 18.18. Found: C, 45.85; H, 4.00; N, 17.98.

4-Amino-3-methylpyridine (3). Eight grams of wet Raney Ni was added to 100 mL of methanol and pre-reduced in a Parr hydrogenator at 60 psi for 5 min. To this mixture was added compound **2** (8 g, 0.052 mol) and reduced at 65 psi for 4 h with vigorous shaking. The mixture was then filtered through celite and the filtrate was immediately dried in vacuo. Note that compound **3** is easily oxidized and therefore it was dried and stored under argon. Compound **3** was obtained in 95% yield: mp 103–105°C,^{1}H NMR (d_6-acetone) δ 7.96 (s, 1H, H2), 7.94 (d, 1H, $J = 4.8$ Hz, H6), 6.55 (d, $J = 4.8$ Hz, H5), 5.24 (br s, 2H, NH_2), 2.08 (s, 3H, CH_3); EI-MS m/z 108 (M^+). Anal. Calcd. for $C_6H_8N_2$: C, 66.62; H, 7.42; N, 25.92. Found: C, 66.70; H, 7.47; N, 25.85.

4-Nitramino-3-methylpyridine (4). Compound **3** (6.80 g, 0.0630 mol) was dissolved in 51 mL of concentrated H_2SO_4 and the resulting mixture was cooled to 5°C in a dry ice/ethanol bath. Then 21 mL of concentrated HNO_3 was added slowly to maintain the reaction temperature below 10°C. After 1 h, the reaction mixture was poured onto 204 g of crushed ice. Concentrated NH_4OH was then added until a creamy precipitate of compound **4** was formed at pH 7. The mixture was filtered and the precipitate was dried in a vacuum oven. Crystals of compound **4** (8.11 g, 84% yield) were obtained by recrystallization from hot water: mp 204–206°C,^{1}H NMR (d_6-DMSO) δ 8.15 (d, 2H, Ar-H), 8.00 (d, 1H, $J = 6.6$ Hz, Ar-H), 2.08 (s, 1H, CH_3); EI-MS $m/z = 153$ (M^+). Anal. Calcd. for $C_6H_7N_3O_2$: C, 47.04; H, 4.61; N, 27.45. Found: C, 46.78; H, 4.55; N, 27.09.

4-Amino-3-methyl-5-nitropyridine (5). Compound **4** (2.00 g, 0.013 mol) was slowly added to 19 mL of concentrated H_2SO_4 to avoid increasing the reaction temperature. The mixture was stirred at room temperature overnight and the reaction was quenched by pouring over 200 g of crushed ice. This mixture was then slowly titrated with concentrated NH_4OH while cooling in an ethanol/dry ice bath. When the mixture reached pH 7, a yellow precipitate of compound **5** was formed. The precipitate was filtered and dried yielding

1.02 g (54%) of pure compound **5**: mp 194–196°C,[1]H NMR (d_6-DMSO) δ 8.87 (s, 1H, H6), 8.09 (s, 1H, H2), 7.66 (s br, 2H, NH_2), 2.12 (s, 3H, CH_3). EI-MS m/z 153 (M^+). Anal. Calcd. for $C_6H_7N_3O_2$: C, 47.04; H, 4.61; N, 27.45. Found: C, 47.29; H, 4.59; N, 27.40.

4,5-Diamino-6-chloro-3-methylpyridine (6). Six grams of $SnCl_2$ was added to 300 mL of concentrated HCl and refluxed at 120°C for 45 min. 3.0 g (0.019 mol) of compound **5** was then added and allowed to react for another 45 min. The heating was then turned off and the reaction allowed to cool to room temperature. The cooled mixture was poured over 700 g of crushed ice and 7 M NaOH was added until the solution turned basic. The basic solution was extracted with ethyl acetate (3 × 100 mL). The organic layer was dried *in vacuo* and the residue was silica gel column purified by elution with 7:3 chloroform:methanol. After purification 0.9 g (29%) of compound **6** was obtained: mp 150–152°C,[1]H NMR (d_6-DMSO) δ 7.20 (s, 1H, H2), 5.47 (s br, 2H, NH_2), 4.63 (s br, 2H, NH_2), 1.95 (s, 3H, CH_3). EI-MS m/z 157 (M^+). Anal. Calcd. for $C_6H_8N_3Cl$: C, 45.85; H, 5.13; N, 26.75; Cl, 22.27. Found: C, 45.65; H, 5.19; N, 26.70; Cl, 22.46.

4-Chloro-7-methyl-1(H)-imidazo[4,5-c]pyridine (7). Method A. Under argon atmosphere, 25 mL of anhydrous triethylorthoformate was added to 0.7 g (0.0045 mol) of compound **6** and refluxed at 160°C for 4 h. After 4 h the heating was turned off and the reaction cooled to room temperature. The cooled reaction mixture was then dried *in vacuo* and silica gel column purified by elution with 7:3 chloroform:methanol. After purification, 0.5 g (67%) of compound **7** was obtained: mp 258–260°C,[1]H NMR (d_6-DMSO) δ 8.45 (s, 1H, H2), 7.91 (s, 1H. H6), 2.44 (s, 3H, CH_3). EI-MS m/z 167 (M^+). Anal. Calcd. for $C_7H_6N_3Cl$: C, 50.29, H, 3.62, N, 25.15, Cl, 20.94. Found: C, 50.14, H, 3.77, N, 24.39, Cl, 21.59.

Method B. To 2.03 g (0.013 mol) of compound **6** was added 120 mL of dry N,N-dimethylformamide and then 20 mL of diethoxymethyl acetate was added under argon. The mixture was refluxed at 130°C overnight. After the reaction was cooled to room temperature, it was dried *in vacuo* and the residue purified by silica gel chromatography eluting with 7:3 chloroform:methanol. After chromatography 1.26 g (58%) of compound **7** was obtained.

3′,5′-O-p-toluoyl-7-chloro-4-methyl-1-β-D-deoxyribofuranosyl-3(H)-imidazo[4,5-c] pyridine (8) and 3′,5′-O-p-toluoyl-4-chloro-7-methyl-1-β-D-deoxyribofuranosyl-1(H)-imidazo[4,5-c] pyridine (9). Under argon atmosphere, compound **7** (1.00 g, 0.006 mol) was dissolved in 100 mL of dry acetonitrile and then 0.5 g (2.1 eq) of 60% sodium hydride was added to form the sodium salt of the heterocyclic base.[22] Evolution of H_2 gas was observed during the formation of the sodium salt. After 30 min 5.0 g (1.1 eq) of 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose was added and allowed to react for 15 h. The reaction was then filtered and dried *in vacuo*. The

residue was purified by silica gel column chromatography with 7:3 chloroform:methanol. The reaction yielded 1.9 g (61%) of compound **8** and 1.2 g (39%) of compound **9**.

Physical data for compound **8**: mp 121–123°C,^{1}H NMR (d_6-acetone) δ 8.70 (s, 1H, H2), 8.00–7.90 (m, 4H, toluoyl), 7.98 (s, 1H, H5), 7.36–7.14 (m, 4H, toluoyl), 7.15 (t, 1H, $J = 7.5$ Hz and 5.5 Hz, H1′), 5.83 (m, 1H, H3′), 4.77 (m, 1H, H4′), 4.76–4.74 (m, 2H, H5′/H5″), 3.11–3.05 (m, 2H, H2′/H2″), 2.54 (s, 3H, CH_3-toluoyl), 2.42 (s, 3H, CH_3), 2.39 (s, 3H, CH_3-toluoyl). ESI-MS m/z 542 ($M + Na^+$). Anal. Calcd. for $C_{28}H_{26}N_3O_5Cl$: C, 64.72, H, 5.05, N, 8.09, Cl, 6.74. Found: C, 64.71, H, 5.02, N, 7.99, Cl, 6.71.

Physical data for compound **9**: mp 143–145°C,^{1}H NMR (d_6-acetone) δ 8.63 (s, 1H, H2), 8.00–7.98 (m, 4H, toluoyl), 7.94 (s, 1H, H6), 7.39–7.28 (m, 4H, toluoyl), 6.86 (t, 1H, $J = 7.5$ Hz and 5.5 Hz, H1′), 5.87 (m, 1H, H3′), 4.75 (m, 1H, H4′), 4.69–4.61 (m, 2H, H5′/H5″), 3.32–3.28 (m, 1H, H2′), 3.10–3.06 (m, 1H, H2″), 2.74 (s, 3H, CH_3), 2.43 (s, 3H, CH_3-toluoyl), 2.40 (s, 3H, CH_3-toluoyl). ESI-MS m/z 542 ($M + Na^+$). Anal. Calcd. for $C_{28}H_{26}N_3O_5Cl$. C, 64.72, H, 5.05, N 8.09, Cl, 6.74. Found: C, 64.66, H, 5.07, N, 7.99, Cl, 6.99.

4-Chloro-7-methyl-1-β-D-deoxyribofuranosyl-1*H*-imidazo[4,5-c] pyridine (10). One gram (0.0019 mol) of compound **9** was added to 20 mL of 2 M NH_3 in methanol. The reaction mixture was refluxed for 15 h at 90°C. Upon cooling to room temperature the mixture was dried *in vacuo* and the residue purified by silica gel column chromatography (8:2 chloroform:methanol). This yielded 0.5 g (94%) of compound **10** after purification: mp 146–148°C,^{1}H NMR (d_6-DMSO) δ 8.72 (s, 1H, H2), 7.92 (s, 1H, H6), 6.52 (dd, 1H, H1′, $J_{H2'\text{-}H1'} = 6.4$ Hz, $J_{H2''\text{-}H1'} = 5.6$ Hz), 5.37 (d, 1H, 3′-OH, $J_{H3'\text{-}3'\text{-}OH} = 4.8$ Hz), 4.92 (dd, 1H, 5′-OH, $J_{H5'\text{-}5'\text{-}OH} = 5.6$ Hz, $J_{H5''\text{-}5'\text{-}OH} = 4.8$ Hz), 4.39 (m, 1H, H3′), 3.87 (m, 1H, H4′), 3.48 (m, 2H, H5′/H5″), 2.71 (m, 1H, H2′), 2.61 (s, 3H, CH_3), 2.40 (m, 1H, H2″). ESI-MS m/z 284 ($M + H^+$). Anal. Calcd. for $C_{12}H_{14}N_3O_3Cl$: C, 50.87, H, 4.98, N, 14.84, Cl, 12.35. Found: C, 50.59, H, 4.95, N, 14.50, Cl, 11.97.

7-Methyl-1-β-D-deoxyribofuranosyl-1*H*-imidazo[4,5-c]pyridin-4-ylamine (3mddA, 11). To 0.5 g (0.0018 mol) of compound **10** was added 15 mL of hydrazine monohydrate and refluxed at 120°C for 10 h. The reaction was then cooled to room temperature, dried *in vacuo*, and the residue dissolved in 20 mL of H_2O. Raney Ni (3 g wet) was added to the aqueous mixture and refluxed for 6 h. The hot reaction mixture was then filtered through celite and the filtrate dried in vacuo. The residue was then dissolved in a minimal volume of 30% methanol/water and purified through a Dowex 1×2–400 (OH^- form) column. After chromatography, 0.31 g (66%) of compound **11** was obtained: mp 208–210°C,^{1}H NMR (d_6-DMSO) δ 8.38 (s, 1H, H2), 7.41 (s, 1H, H6), 6.42 (dd, 1H, H1′, $J_{H2'\text{-}H1'} = 7.6$ Hz, $J_{H2''\text{-}H2'} = 5.6$ Hz , 5.89

(s, 2H, NH_2), 5.32 (d, 1H, 3′-OH, $J_{H3'\text{-}3'\text{-}OH} = 4$ Hz), 4.90 (dd, 1H, 5′-OH, $J_{H5'\text{-}5'\text{-}OH} = 5.6$ Hz, $J_{H5''\text{-}5'\text{-}OH} = 4.8$ Hz), 4.34 (m, 1H, H3′), 3.83 (m, 1H, H4′), 3.47 (m, 2H, H5′/H5″), 2.61 (m, 1H, H2′), 2.40 (s, 3H, CH_3), 2.33 (m, 1H, H2″). EI-MS m/z 265 (M^+). Anal. Calcd. for $C_{12}H_{16}N_4O_3$: C, 54.52, H, 6.10, N, 21.21. Found: C, 54.43, H, 6.15, N, 20.63.

7-Chloro-4-methyl-1-β-D-deoxyribofuranosyl-3*H*-imidazo[4,5-c]pyridine (12). Compound **8** (2.7 g, 0.0052 mol) was mixed with 20 mL of 2 M NH_3 in methanol. The reaction mixture was refluxed at 90°C for 12 h. Upon cooling to room temperature the mixture was dried *in vacuo* and the residue purified by silica gel chromatography (8:2 chloroform:methanol). This yielded 1.0 g (68%) of compound **12** after purification: mp 150–152°C,^{1}H NMR (d_6-DMSO) δ 8.84 (s, 1H, H2), 7.98 (s, 1H, H5), 6.98 (dd, 1H, H1′, $J = 5.6$ Hz and 6.4 Hz), 4.62 (m, 1H, H3′), 4.08 (m, 1H, H4′), 3.87–3.81 (m, 2H, H5′/H5″), 2.66–2.63 (m, 2H, H2′/H2″), 2.52 (s, 3H, CH_3). ESI-MS m/z 306 ($M + Na^+$). Anal. Calcd. for $C_{12}H_{14}N_3O_3Cl$: C, 50.87, H, 4.98, N, 14.84, Cl, 12.35. Found: C, 50.77, H, 4.97, N, 14.69, Cl, 12.67.

4-Methyl-1-β-D-deoxyribofuranosyl-3*H*-imidazo[4,5-c]pyridine-7-ylamine (13). Compound **12** (0.3 g, 0.0011 mol) was added to 10 mL of hydrazine monohydrate and refluxed at 120°C for 15 h. The reaction mixture was then dried *in vacuo*. The residue was then dissolved in 15 mL of water. To this mixture was added 2 g (wet) of Raney nickel and refluxed for 2 h. After 2 h, the hot reaction mixture was filtered through celite and the filtrate dried *in vacuo*. The residue was then purified by silica gel chromatography (7:3 chloroform:methanol). This yielded 1.0 g (29%) of compound **13** after purification: mp 170–172°C, ^{1}H NMR (d_6-DMSO) δ 8.40 (s, 1H, H2), 7.52 (s, 1H, H5), 6.38 (dd, 1H, H1′, $J = 6.4$ Hz and 7.6 Hz), 5.60 (br s, 2H, NH_2), 5.39 (d, 1H, 3′-OH, $J = 4$ Hz), 5.02 (br s, 1H, 5′-OH), 4.37 (m, 1H, H3′), 3.88 (m, 1H, H4′), 3.51 (m, 2H, H5′/H5″), 2.61 (m, 1H, H2′), 2.33 (m, 1H, H2″), 2.29 (s, 3H, CH_3). ESI-MS m/z 265 ($M + H^+$). Anal. Calcd. for $C_{12}H_{16}N_4O_3$: C, 54.52, H, 6.10, N, 21.21. Found: C, 53.92, H, 5.90, N, 20.13.

4-Methyl-1-β-D-deoxyribofuranosyl-3*H*-imidazo[4,5-c]pyridine (14). Compound **12** (0.1 g, 0.00035 mol) was added to 40 mL of water and 3 g (wet) Raney nickel was added to the mixture. This reaction mixture was refluxed for 2 h. After 2 h, the hot reaction mixture was filtered through celite and the filtrate dried ***in vacuo***. The residue was dissolved in a minimal volume of 30% methanol/water and purified through a Dowex $1 \times 2 - 400$ (OH^- form) column using 30% methanol/water as the eluent. After chromatography, 0.07 g (80%) of compound **14** was obtained: mp 166–168°C,^{1}H NMR (d_6-DMSO) δ 8.92 (s, 1H, H2), 8.61 (s, 1H, H7), 8.18 (s, 1H, H5), 6.42 (dd, 1H, H1′, $\boldsymbol{J} \sim 6$ Hz and $\boldsymbol{J} \sim 6$ Hz), 5.39 (d, 1H, 3′-OH, $\boldsymbol{J} = 3$ Hz), 5.02 (t, 1H, 5′-OH, $\boldsymbol{J} = 4.5$ Hz), 4.40 (m, 1H, H3′), 3.88 (m, 1H, H4′), 3.57 (m, 2H,

H5′/H5″), 2.62–2.57 (m, 1H, H2′), 2.49 (s, 3H, CH_3), 2.34–2.29 (m, 1H, H2″). ESI-MS m/z 250 ($M + H^+$). Anal. Calcd. for $C_{12}H_{15}N_3O_3$: C, 57.80, H, 6.07, N, 16.86. Found: C, 55.50, H, 5.99, N, 15.58.

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