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THE SYNTHESIS OF *ANTI*-FIXED 3-METHYL-3-DEAZA-2'-DEOXYADENOSINE AND OTHER 3H-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-CH₃ 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 *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.

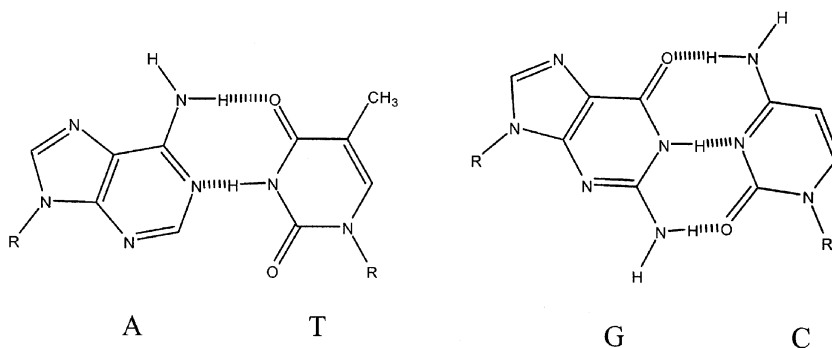


Figure 1. Watson-Crick base pairs showing *anti* conformation in base pairing.

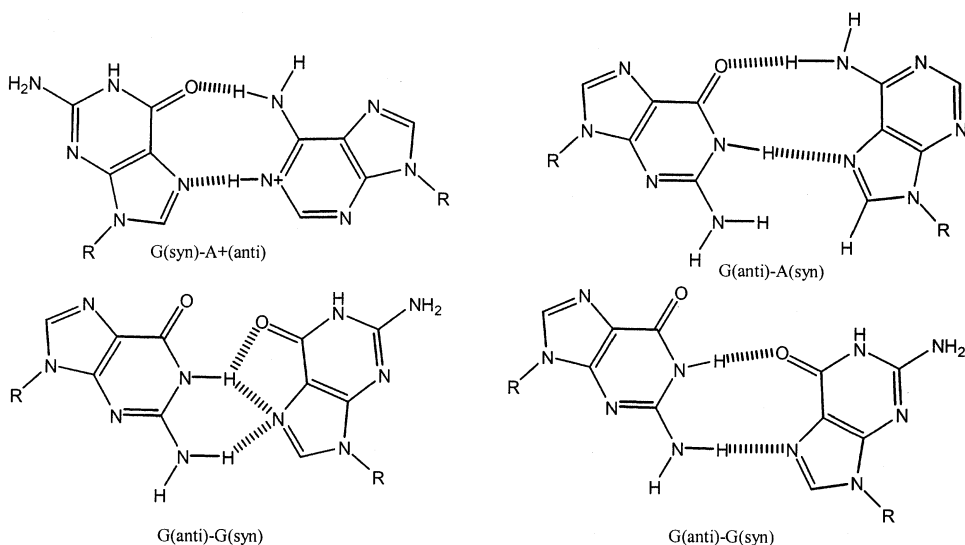


Figure 2. Purine-purine mismatches showing *syn* conformation in base pairing.

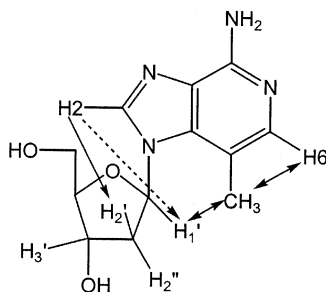


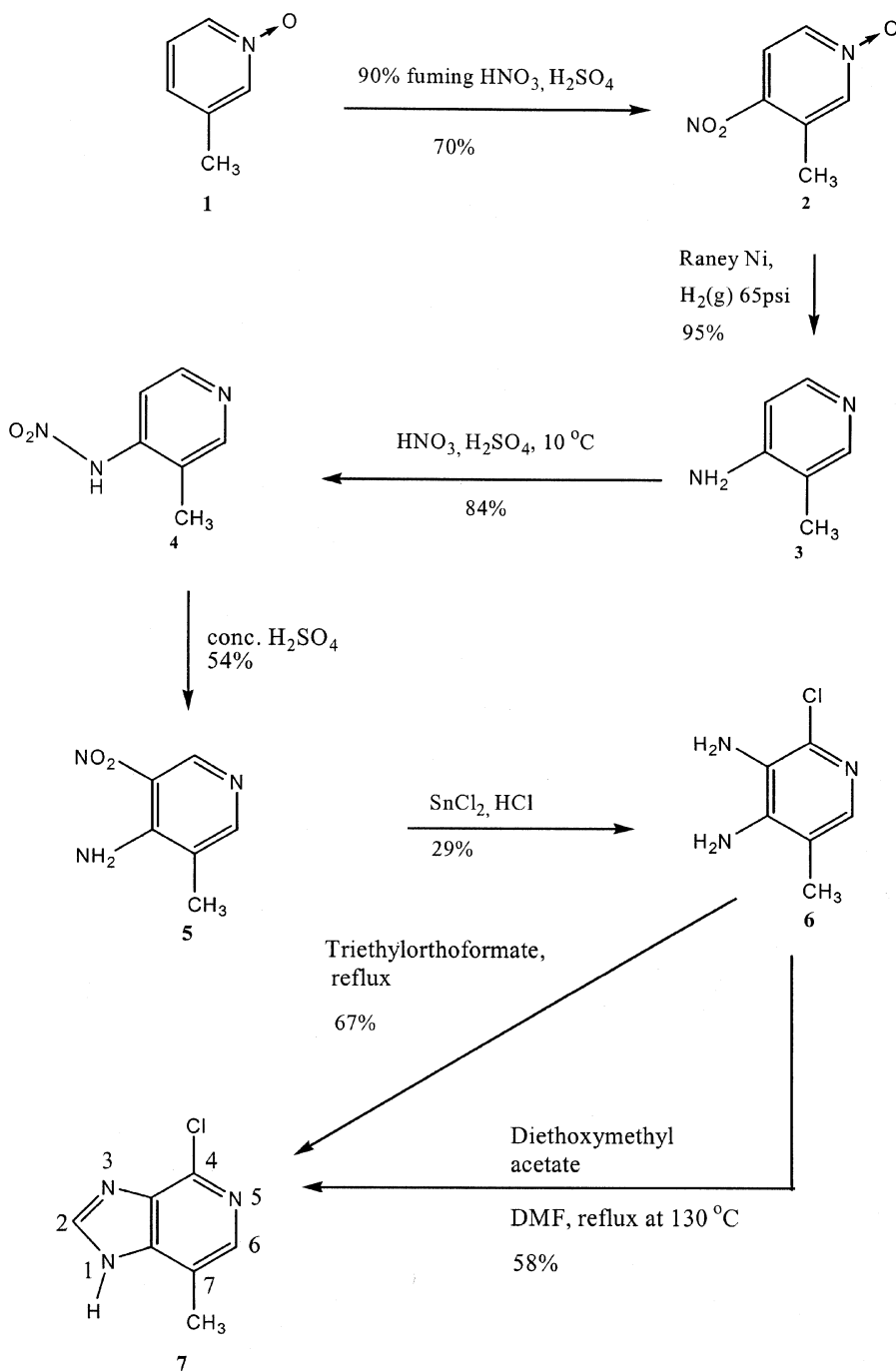
Figure 3. Schematic representation of important 1D NOE interactions for compound **11**. Solid arrows indicate strong NOEs and dashed arrows indicate weak NOEs.

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

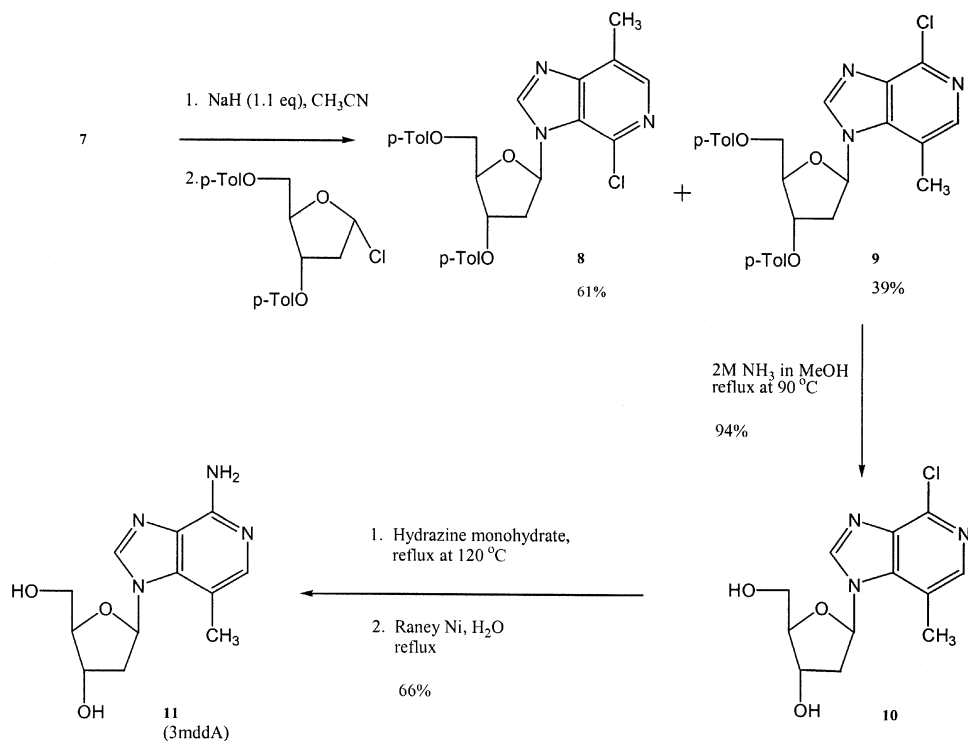


Scheme 1.

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 previously.^[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 POCl₃ 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 NH₃ 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

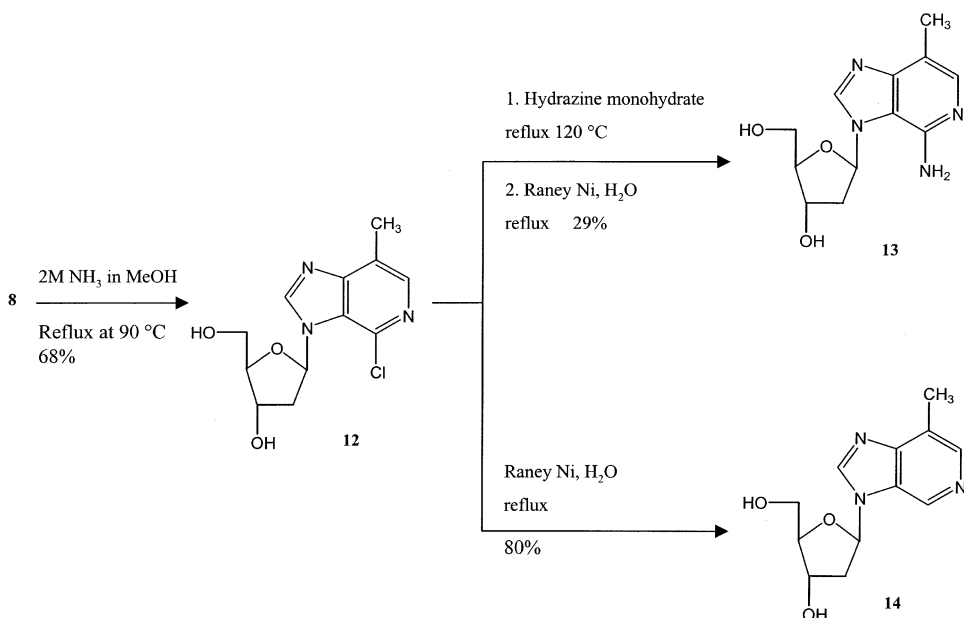


Scheme 2.

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 2M 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 semiquantitatively 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)

1 These data indicate that **11** is in the *anti* conformation. Irradiation of H6 in
2 compound **11**, resulted in a strong NOE (2.1%) to the methyl group and no
3 NOEs to sugar protons, consistent with the *anti* conformation. Irradiation of
4 the H1' of compound **11** shows the strongest NOE (2.8%) to the methyl
5 group indicating the proximity of the H1' to the methyl group on the base,
6 thus further confirming its *anti* conformation. The *anti* conformation is also
7 consistent with the irradiation of the methyl group which only shows NOEs
8 to the H6 (1.4%) and H1' (1.4%).
9
10

11 CONCLUSIONS

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

25 General Methods

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27 Physical data were measured as follows: Melting points are uncorrected.
28 ¹H and ¹³C NMR data were measured at 300, 400 or 500 MHz
29 instruments in d₆-DMSO or d₆-acetone. Chemical shifts (δ) are reported in
30 parts per million (ppm) downfield from tetramethylsilane (TMS) using
31 TMS or residual non-deuterated solvent as reference. Multiplicity is indicated
32 using the following abbreviations: s (singlet), d (doublet), dd (doublet
33 of doublets), t (triplet), q (quartet), m (multiplet) or br (broad). The NOE
34 experiments were performed in d₆-DMSO. Each peak was irradiated, in an
35 interleaved fashion to minimize errors due to spectrometer drift, for 250 μs
36 yielding a saturation of ≤95%. The recycle delay was set at 3 s and the
37 acquisition time was 1.9 s.
38

39 1-chloro-2-deoxy-3',5'-di-O-p-toluoyl-α-D-erythro-pentofuranose was
40 prepared from 2'-deoxyribose as described by Hoffer.^[26] The starting material
41 3-picoline-N-oxide (compound **1**), thymidine, thymidine phosphorylase,
42 and purine nucleoside phosphorylase were purchased from Sigma-Aldrich.
43 Silica gel used for column chromatography was Merck, grade 9385, 230–400
44 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 H₂SO₄ and warmed to 60°C. While keeping the temperature below 80°C, 40 mL of 90% fuming HNO₃ 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 NH₄OH 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, ¹H NMR (d₆-acetone) δ 8.29 (s, 1H, H2), 8.19 (m, 1H, H6), 8.11 (d, *J* = 12 Hz, 1H, H5), 2.59 (s, 3H, CH₃); EI-MS *m/z* 154 (M⁺). Anal. Calcd. for C₆H₆N₂O₃: 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 mmol) 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, ¹H NMR (d₆-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, NH₂), 2.08 (s, 3H, CH₃); EI-MS *m/z* 108 (M⁺). Anal. Calcd. for C₆H₈N₂: 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 H₂SO₄ and the resulting mixture was cooled to 5°C in a dry ice/ethanol bath. Then 21 mL of concentrated HNO₃ 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 NH₄OH 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, ¹H NMR (d₆-DMSO) δ 8.15 (d, 2H, Ar-H), 8.00 (d, 1H, *J* = 6.6 Hz, Ar-H), 2.08 (s, 1H, CH₃); EI-MS *m/z* = 153 (M⁺). Anal. Calcd. for C₆H₇N₃O₂: 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 H₂SO₄ 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 NH₄OH 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 1.02 g (54%) of pure compound **5**: mp 194–196°C, ^1H NMR (d_6 -DMSO) δ
2 8.87 (s, 1H, H6), 8.09 (s, 1H, H2), 7.66 (s br, 2H, NH₂), 2.12 (s, 3H, CH₃).
3 EI-MS m/z 153 (M⁺). Anal. Calcd. for C₆H₇N₃O₂: C, 47.04; H, 4.61; N,
4 27.45. Found: C, 47.29; H, 4.59; N, 27.40.

5
6 **4,5-Diamino-6-chloro-3-methylpyridine (6)**. Six grams of SnCl₂ was
7 added to 300 mL of concentrated HCl and refluxed at 120°C for 45 min. 3.0 g
8 (0.019 mol) of compound **5** was then added and allowed to react for another
9 45 min. The heating was then turned off and the reaction allowed to cool to
10 room temperature. The cooled mixture was poured over 700 g of crushed ice
11 and 7 M NaOH was added until the solution turned basic. The basic solution
12 was extracted with ethyl acetate (3 × 100 mL). The organic layer was dried
13 in vacuo and the residue was silica gel column purified by elution with 7:3
14 chloroform:methanol. After purification 0.9 g (29%) of compound **6** was
15 obtained: mp 150–152°C, ^1H NMR (d_6 -DMSO) δ 7.20 (s, 1H, H2), 5.47 (s br,
16 2H, NH₂), 4.63 (s br, 2H, NH₂), 1.95 (s, 3H, CH₃). EI-MS m/z 157 (M⁺).
17 Anal. Calcd. for C₆H₈N₃Cl: C, 45.85; H, 5.13; N, 26.75; Cl, 22.27. Found: C,
18 45.65; H, 5.19; N, 26.70; Cl, 22.46.

19
20 **4-Chloro-7-methyl-1(H)-imidazo[4,5-c]pyridine (7)**. Method A. Under
21 argon atmosphere, 25 mL of anhydrous triethylorthoformate was added to
22 0.7 g (0.0045 mol) of compound **6** and refluxed at 160°C for 4 h. After 4 h the
23 heating was turned off and the reaction cooled to room temperature. The
24 cooled reaction mixture was then dried in vacuo and silica gel column pur-
25 ified by elution with 7:3 chloroform:methanol. After purification, 0.5 g (67%)
26 of compound **7** was obtained: mp 258–260°C, ^1H NMR (d_6 -DMSO) δ 8.45 (s,
27 1H, H2), 7.91 (s, 1H, H6), 2.44 (s, 3H, CH₃). EI-MS m/z 167 (M⁺). Anal.
28 Calcd. for C₇H₆N₃Cl: C, 50.29, H, 3.62, N, 25.15, Cl, 20.94. Found: C, 50.14,
29 H, 3.77, N, 24.39, Cl, 21.59.

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

36 **3',5'-O-p-toluoyl-7-chloro-4-methyl-1-β-D-deoxyribofuranosyl-3(H)-imi-**
37 **dazo[4,5-c] pyridine (8)** and **3',5'-O-p-toluoyl-4-chloro-7-methyl-1-β-D-deox-**
38 **yribofuranosyl-3(H)-imidazo[4,5-c] pyridine (9)**. Under argon atmosphere,
39 compound **7** (1.00 g, 0.006 mol) was dissolved in 100 mL of dry acetonitrile
40 and then 0.5 g (2.1 eq) of 60% sodium hydride was added to form the sodium
41 salt of the heterocyclic base.^[22] Evolution of H₂ gas was observed during the
42 formation of the sodium salt. After 30 min 5.0 g (1.1 eq) of 1-chloro-2-deoxy-
43 3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose was added and allowed
44 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, ^1H 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, CH₃-toluoyl), 2.42 (s, 3H, CH₃), 2.39 (s, 3H, CH₃-toluoyl). ESI-MS m/z 542 (M + Na⁺). Anal. Calcd. for C₂₈H₂₆N₃O₅Cl: 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, ^1H 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, CH₃), 2.43 (s, 3H, CH₃-toluoyl), 2.40 (s, 3H, CH₃-toluoyl). ESI-MS m/z 542 (M + Na⁺). Anal. Calcd. for C₂₈H₂₆N₃O₅Cl. 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-1H-imidazo[4,5-c] pyridine (10). One gram (0.0019 mol) of compound **9** was added to 20 mL of 2 M NH₃ 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, ^1H NMR (d_6 -DMSO) δ 8.72 (s, 1H, H2), 7.92 (s, 1H, H6), 6.52 (dd, 1H, H1', $J_{\text{H2}'\text{-H1}'} = 6.4$ Hz, $J_{\text{H2}''\text{-H1}'} = 5.6$ Hz), 5.37 (d, 1H, 3'-OH, $J_{\text{H3}'\text{-3'-OH}} = 4.8$ Hz), 4.92 (dd, 1H, 5'-OH, $J_{\text{H5}'\text{-5'-OH}} = 5.6$ Hz, $J_{\text{H5}''\text{-5'-OH}} = 4.8$ 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, CH₃), 2.40 (m, 1H, H2''). ESI-MS m/z 284 (M + H⁺). Anal. Calcd. for C₁₂H₁₄N₃O₃Cl: 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-1H-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 H₂O. 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 \times 2–400 (OH⁻ form) column. After chromatography, 0.31 g (66%) of compound **11** was obtained: mp 208–210°C, ^1H NMR (d_6 -DMSO) δ 8.38 (s, 1H, H2), 7.41 (s, 1H, H6), 6.42 (dd, 1H, H1', $J_{\text{H2}'\text{-H1}'} = 7.6$ Hz, $J_{\text{H2}''\text{-H2}'} = 5.6$ Hz), 5.89

(s, 2H, NH₂), 5.32 (d, 1H, 3'-OH, $J_{\text{H}3'-3'-\text{OH}} = 4$ Hz), 4.90 (dd, 1H, 5'-OH, $J_{\text{H}5'-5'-\text{OH}} = 5.6$ Hz, $J_{\text{H}5''-5'-\text{OH}} = 4.8$ 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, CH₃), 2.33 (m, 1H, H2''). EI-MS m/z 265 (M⁺). Anal. Calcd. for C₁₂H₁₆N₄O₃: 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-3H-imidazo[4,5-c]pyridine (12). Compound **8** (2.7 g, 0.0052 mol) was mixed with 20 mL of 2 M NH₃ 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, ¹H NMR (d₆-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, CH₃). ESI-MS m/z 306 (M + Na⁺). Anal. Calcd. for C₁₂H₁₄N₃O₃Cl: 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-3H-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, ¹H NMR (d₆-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, NH₂), 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, CH₃). ESI-MS m/z 265 (M + H⁺). Anal. Calcd. for C₁₂H₁₆N₄O₃: 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-3H-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 × 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, ¹H NMR (d₆-DMSO) δ 8.92 (s, 1H, H2), 8.61 (s, 1H, H7), 8.18 (s, 1H, H5), 6.42 (dd, 1H, H1', $J \sim 6$ Hz and $J \sim 6$ Hz), 5.39 (d, 1H, 3'-OH, $J = 3$ Hz), 5.02 (t, 1H, 5'-OH, $J = 4.5$ 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, CH₃), 2.34–2.29 (m, 1H, H2''). ESI-MS *m/z* 250 (M + H⁺). Anal. Calcd. for C₁₂H₁₅N₃O₃: C, 57.80, H, 6.07, N, 16.86. Found: C, 55.50, H, 5.99, N, 15.58.

ACKNOWLEDGMENTS

We thank Kathleen Fleming for her help in searching the chemical abstracts registry file. This work was funded by NIH grant number HG02020.

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Received March 27, 2002

Accepted August 5, 2002