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SYNTHESIS OF NELARABINE WITH PURE β-ANOMER THROUGH LATE-STAGE C-H NITRATION/NITRO-REDUCTION

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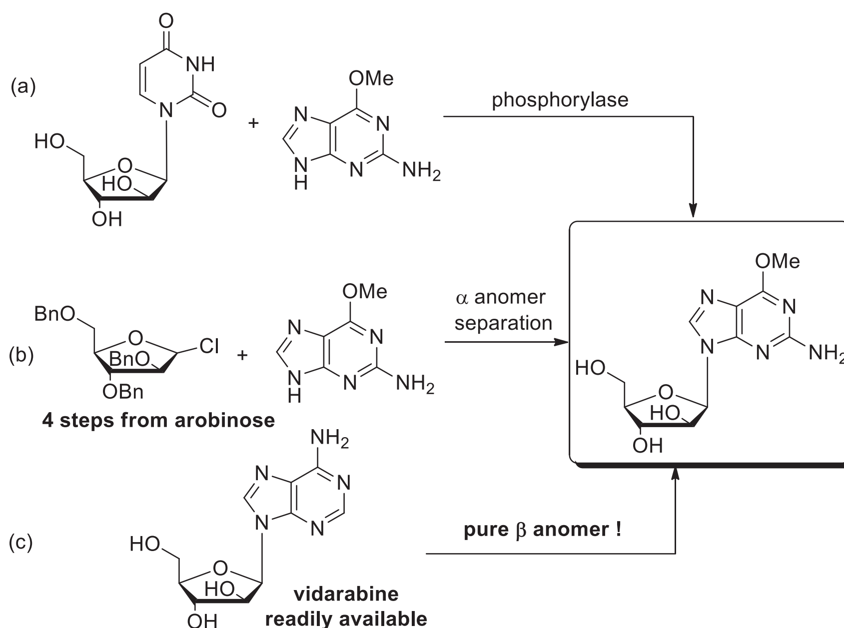
Abstract – An efficient and pure β-anomer synthesis of the clinical drug nelarabine from the readily available vidarabine has been achieved for the first time. The C6 amino group of vidarabine was transformed to methoxy group by diazotization/chlorination followed by methoxylation using Na_2CO_3/MeOH system. The formation of C(2)-N bond was achieved *via* the highly selective C-H bond functionalization by reacting with 2,2,2-trifluoroacetic anhydride (TFAA) and tetrabutylammonium nitrate. The final product was obtained in total yield of 58.6% by 5 steps-synthesis from vidarabine after the reduction of nitro group to amino group. Moreover, the drug nelarabine could be obtained in 100 grams scale successfully and no chromatography was needed, which made this route more attractive for industrial application.

The C-H bond functionalization has attracted much attention from both academia and industry in the past decade,[1] and various methods have been developed and applied in the synthesis of complex molecules.[2] However, synthesizing the clinical drugs *via* C-H bond functionalization remains a challenge. Among them, the synthesis of nucleosides *via* C-H bond functionalization is particularly important because the nucleosides are ubiquitous in RNA and DNA, which have broad applications in biological and pharmaceutical chemistry.[3]

Nelarabine (**1**) was the clinical nucleoside drug and approved by FDA (the US food and drug administration) in October 2005 for the treatment of T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) that has not responded to or has relapsed after treatment with at least two chemotherapy regimens.[4] To date, only two major classes of synthetic methods lead to nelarabine. The first class comprises the phosphorylase catalyzed *trans*-glycosylation between

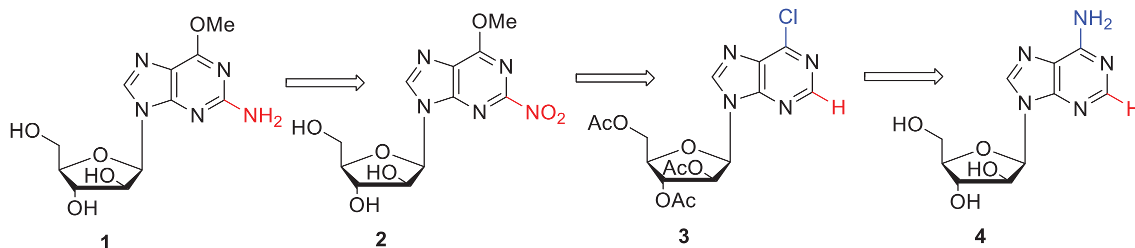
2-amino-6-methoxy-9*H*-purine and 1-β-D-arabinosyl-uracil (Scheme 1, route a).[5] These reactions are versatile, but the phosphorylase or modified arabinose was costly and commercially unavailable. A second class includes the coupling method between 2-amino-6-methoxy-9*H*-purine and modified arabinose (Scheme 1, route b).[6] In most cases, these reactions give α- and β-anomers which are difficult to separate and lead to low yields. Most of the biologically important nucleosides are required to be pure β-anomer for pharmaceutical applications. Therefore, searching for an efficient synthesis of nelarabine with pure β-anomer using available starting material is highly desirable.

In the context of ongoing projects on the synthesis of nucleosides *via* C-H bond functionalization,[7-9] herein we reported the synthesis of nelarabine with pure β-anomer employing readily available vidarabine (Scheme 1, route c).



Scheme 1. The selected routes to nelarabine

We aimed to develop a method for the late-stage functionalization of vidarabine that would avoid the formation of α-anomer. Our approach was inspired by the selective nitration reaction of purine nucleosides invented by Brændvang.[10] The retrosynthetic analysis for nelarabine is outlined in Scheme 2. It was our view that the C2 amino group of nelarabine could be transformed from nitro group (Scheme 2). The nitro-substituted nucleoside **2** could be synthesized *via* the functionalization of C(2)-H bond from nucleoside **3**, which could easily be prepared from commercially available vidarabine (**4**). At the outset of our studies, the hydroxy groups of **4** should be protected for the next transformations. Acetyl group was the best choice because the acetylating reagents were less costly.

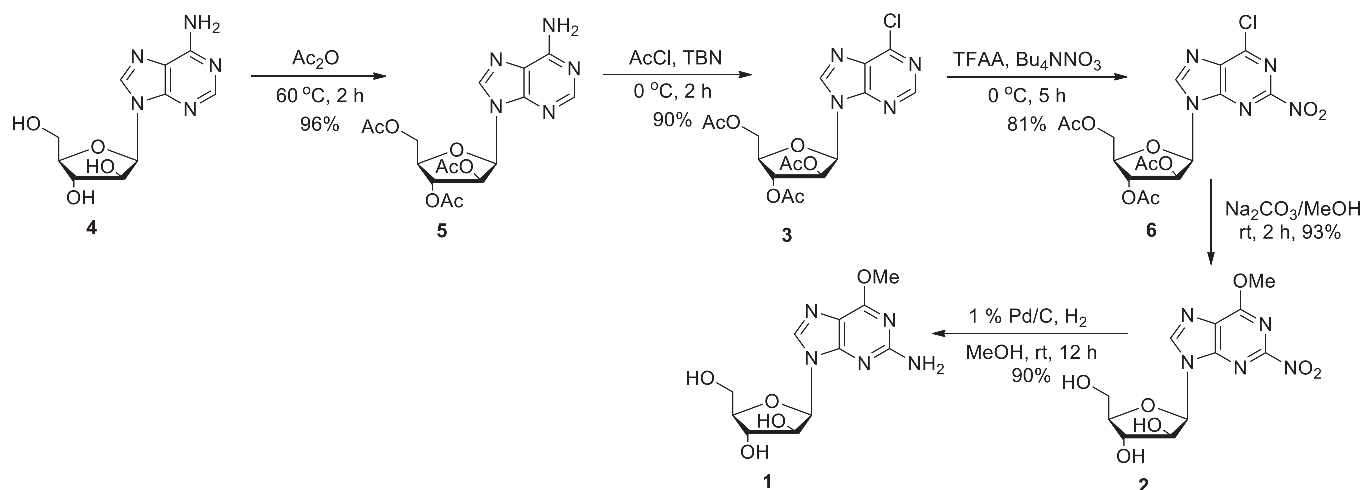


Scheme 2. The retrosynthetic analysis for nelarabine

Using acetic anhydride as acetylating reagent and solvent, the vidarabine reacted with acetic anhydride at 60 ºC for 2 h. On completion, the mixture was concentrated and coevapertaed with ethanol. The protected product **5** was obtained through recrystallization from ethanol in 96% yield. The acetylating step could be scaled up to 500 grams and the yield was maintained (Scheme 3).

The C6 amino group of **5** was converted into chloro group using acetyl chloride as chloride source and *tert*-butyl nitrite (TBN) as diazotization reagent.[11] The intermediate **3** was obtained in 90% yield after purification and this step could be enlarged to 100 grams scale.

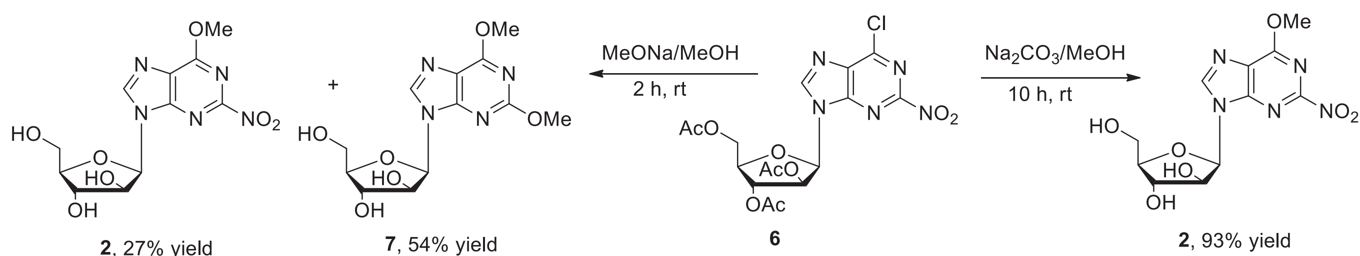
The intermediate **3** without activated hydrogen atoms was suitable for the nitration reaction *via* C-H bond functionalization which was realized by reacting with TFAA and tetrabutylammonium nitrate (Bu_4NNO_3) in CH_2Cl_2.[10] $F_3CCOO^-NO_2^+$ as an nitration reagent was produced *in situ* by Bu_4NNO_3 and TFAA, and NO_2^+ reacted at C2 of purine cycle selectively.[12] 6-Chloro-2-nitro-2′,3′,5′-*O*-triacetyl purine nucleoside (**6**) was synthesized in 81% yield at 0 ºC for 5 h and the reaction scale could be enlarged to 100 grams. The replacement of Bu_4NNO_3 with equivalent tetramethylammonium nitrate (Me_4NNO_3) or NH_4NO_3 decreased the yield to 52% and 31%, respectively. The main reason for lower yields was the poor solubility of other nitrates in CH_2Cl_2.



Scheme 3. The synthetic route

Next, we investigated the methoxylation of chloro group. MeONa/MeOH was the commonly used system to convert chloro group to methoxy group accompanied with removing acetyl groups in sugar cycle. When the reaction was carried out in MeONa/MeOH solution at room temperature for 2 h, the 2,6-dimethoxylated compound **7** was observed in 54% yield and the target product **2** was isolated in 27% yield. The formation of side product **7** was due to the substitution of nitro group by methoxy anion. Shortening the reaction time or reduce the amount of MeONa could not avoid the side product.

To assess the impact of bases on the yields of **2**, we performed a series of experiments with different bases (Scheme 4). To our delight, when the reaction was carried out in Na_2CO_3/MeOH solution at room temperature for 2 h, the chloro group was substituted by methoxy group accompanied with removing of acetyl groups. The product **2** was isolated in 93% yield and any formation of **7** was not observed. Moreover, the product **2** could be easily purified through recrystallization avoiding of chromatography.



Scheme 4. The base effects on methyoxylation

The most important method to obtain a 2-aminopurine derivative is based on the selective reduction of nitroarenes.[13] Therefore, we explored the reduction conditions to convert nitro group to amino group. The final product nelarabine was obtained in 90% yield using 1% Pd/C as catalyst in H_2 atmosphere. Finally, nelarabine was obtained in 58.6% yields and through 5 steps from vidarabine.

To evaluate the reproducibility and the stability of the reaction, then, the synthesis of nelarabine on a larger scale was performed. The synthesis of nelarabine on 100 grams scale was carried out and the reaction still worked well. More importantly, these 5 steps could be purified through washing or recrystallization and did not need chromatography or lengthy workup process which showed good potential for industrial applications.

In summary, we realized the efficient synthesis of the clinical drug nelarabine from the readily available vidarabine for the first time. The main steps were the late-stage C(2)-H nitration of vidarabine by reacting with TFAA and Bu_4NNO_3 followed by reduction of nitro group. The amino group was transformed to methoxy group by diazotization/chlorination followed by methoxylating using Na_2CO_3/MeOH system. The final product was obtained in total yield of 58.6% by 5 steps-synthesis from vidarabine. Compared to

known methods for the synthesis of nelarabine, our method affords pure β anomer and the separation of anomers was avoided. Moreover, the drug nelarabine was afforded successfully in 100 grams scale and no chromatography was needed, which showed good potential for industrial application. Further studies into the applications of this method for other nucleoside drugs are currently underway.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were determined on a Bruker AC 400 spectrometer (Bruker, Billerica, MA, USA) as DMSO-d_6 or $CDCl_3$ solution. Chemical shifts were expressed in parts permillion (δ) downfield from the internal standard tetramethylsilane and were reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets) and coupling constants J were given in hertz (Hz). High resolution mass spectra are taken using Q-TOF system, with Electrospray Ionization (ESI) as the ionization method used for the HRMS measurement. All reactions were monitored by thin layer chromatography (TLC). All reagents and solvents were purchased from commercial sources and purified commonly before used.

(2*R*,3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-5-(6-amino-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (5)
Vidarabine (0.53 g, 2.0 mmol), TEA (1.2 mL, 8.6 mmol), DMAP (0.04 g, 0.3 mmol) were stirred slowly in MeCN (20 mL), and then acetic anhydride (0.68 mL, 7.2 mmol) was added slowly. The mixture was heated to 60 °C and kept at this temperature for 2 h. TLC indicated vidarabine disappeared. The solvent was removed *in vacuo* and the residue was purified by recrystallization from EtOH to give the desire product **5** (0.75 g in 96% yield) as a white solid.

The experimental procedure of 500 grams scale: Vidarabine (500 g, 1.87 mol), TEA (1.03 L, 7.48 mol), and DMAP (11.4 g, 0.09 mol) were stirred for 30 min in MeCN (2 L) and cooled to 0-5 °C. The acetic anhydride (212 mL, 2.24 mol) was added slowly keeping the temperature below 5 °C. On completion, the mixture was heated to 60 °C and kept at this temperature for 4 h. TLC indicated vidarabine disappeared. The excess of acetic anhydride and the formed acetic acid were removed *in vacuo* and could be reused after separation in the future. The residue was coevaporated with EtOH (50 mL×2) and 500 mL EtOH was added, followed by heating to reflux to make the residue dissolve in EtOH. Then the solution was cooled and the product precipitated slowly. The solid product **5** was filtrated and the mother liquor was evaporated to dryness (625 g), and to be purified by recrystallization from EtOH to give the other batch of **5** (73 g). The combined yield was 95%.

White solid, mp 128-130 °C, literature[14] mp 128.5-129 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.00 (s, 1H), 6.58 (d, J = 4.4 Hz, 1H), 5.96 (brs, 2H), 5.51-5.50 (m, 1H), 5.44-5.43 (m, 1H), 4.47-4.45 (m, 2H), 4.28-4.24 (m, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3,

169.6, 169.4, 155.6, 153.0, 149.7, 138.9, 120.1, 86.3, 80.3, 73.2, 70.7, 63.1, 20.7, 20.5, 20.4; HRMS calcd for $C_{16}H_{19}N_5NaO_7$[M+Na]$^+$ 416.1177, found 416.1182.

(2*R*,3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-5-(6-chloro-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (3)

A solution of AcCl (0.20 mL, 2.8 mmol) in dried CH_2Cl_2 (10 mL) under N_2 was chilled in a cold bath (-5 to 0 °C) for 20 min. TBN (0.26 mL, 2.2 mmol) in dried CH_2Cl_2 (5 mL) was immediately added dropwise to the solution of AcCl/CH_2Cl_2. A solution of **5** (0.20 g, 0.5 mmol) in dried CH_2Cl_2 (5 mL) was added to the cold solution and stirring was continued for 2 h (TLC, 9:1 CH_2Cl_2/MeOH, showed complete conversion of **5** into a single spot). The reaction mixture was poured into a cold and vigorously stirred mixture of saturated aqueous $NaHCO_3$ and CH_2Cl_2 (20 mL). The layers were separated, and the organic phase was washed with H_2O (10 mL) and dried over anhydrous $MgSO_4$. The volatiles were evaporated *in vacuo* to give **3** (0.18 g in 90% yield) as an oil.

The experimental procedure of 100 grams scale: **5** (100 g, 0.25 mol) in dried CH_2Cl_2 (800 mL) was chilled in a cold bath (-5 to 0 °C), then AcCl (100 mL, 1.4 mol) was added slowly for 20 min. TBN (130 mL, 1.1 mol) was added dropwise and the mixture was stirred for 5 h. The mixture is then carefully neutralized to about pH 7 with saturated aqueous $NaHCO_3$. Care being taken not to allow the temperature to rise above 0 °C. The layers were separated, and the aqueous solution is extracted with CH_2Cl_2 (100 mL). The combined organic phase was washed with H_2O (200 mL×2), decolored and dried over anhydrous $MgSO_4$. The volatiles were evaporated *in vacuo* to give **3** (95 g in 92% yield) as an oil.

Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.18 (s, 1H), 6.62 (d, *J* = 4.4 Hz, 1H), 5.48-5.40 (m, 2H), 4.45-4.38 (m, 2H), 4.25-4.21 (m, 1H), 2.12 (s, 3H), 2.07 (s, 3H), 1.84 (s, 3H); 13C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.7, 169.5, 156.3, 154.5, 150.7, 138.8, 118.6, 85.9, 80.5, 73.3, 70.7, 63.1, 20.8, 20.6, 20.5; HRMS calcd for $C_{16}H_{17}ClN_4NaO_7$[M+Na]$^+$ 435.0678, found 435.0678.

(2*R*,3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-5-(6-chloro-2-nitro-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (6)

A nitrating mixture was prepared by adding cold TFAA (4.6 mL, 33.0 mmol) over 2 min to a solution of Bu_4NNO_3 (10.1 g, 33.0 mmol) in dried CH_2Cl_2 (50 mL) at 0 °C. After 20 min the solution was added to **3** (8.3 g, 20 mmol) in dried CH_2Cl_2 (50 mL) at 0 °C. After 5 h at 0 °C, the reaction mixture was poured into a cold mixture of H_2O (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and CH_2Cl_2 (100 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were washed with brine (200 mL×2), dried over $MgSO_4$ and evaporated *in vacuo* to give **6** (7.4 g in 81% yield) as an oil. The crude product was pure enough for the next reaction.

The experimental procedure of 100 grams scale: TFAA (56 mL, 0.39 mol) was added to a solution of Bu_4NNO_3 (120 g, 0.39 mmol) in dried CH_2Cl_2 (200 mL) at 0 °C and stirred for 30 min. **3** (100 g, 0.24 mol) in dried CH_2Cl_2 (300 mL) was added dropwise to the solution, warmed to room temperature and

kept at room temperature for 5 h. The mixture is then carefully neutralized to about pH 7 with saturated aqueous $NaHCO_3$ and the organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 (50 mL×2). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (100 mL) followed by brine (100 mL), dried over $MgSO_4$ and evaporated *in vacuo* to about 100 mL. The MeOH was added slowly to the concentrated organic extracts and the solid **6** (93.4 g precipitated slowly in 85% yield).

White solid, mp 154-156 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 6.67 (d, J = 4.4 Hz, 1H), 5.49-5.47 (m, 1H), 5.40-5.38 (m, 1H), 4.44-4.36 (m, 2H), 4.28-4.24 (m, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 1.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.7, 169.6, 156.4, 154.4, 150.6, 138.8, 118.5, 85.8, 80.4, 73.2, 70.6, 63.1, 20.8, 20.6, 20.5; HRMS calcd for $C_{16}H_{16}ClN_5NaO_9$[M+Na]$^+$ 480.0529, found 480.0529.

(2*R*,3*S*,4*S*,5*R*)-2-(Hydroxymethyl)-5-(6-methoxy-2-nitro-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (2)

A solution of **6** (9.2 g, 20 mmol) and Na_2CO_3 (2.1 g, 20 mmol) in MeOH (100 mL) was stirred at room temperature for 2 h. The solution was filtrated and the filtrate was evaporated to dryness. The product was recrystallized from H_2O to give **2** (6.1 g in 93% yield) as a white solid.

The experimental procedure of 100 grams scale: **6** (100 g, 0.22 mol) and Na_2CO_3 (22.9 g, 0.22 mol) were added to MeOH (800 mL), and were stirred at room temperature for 6 h. The solution was filtrated and the filtrate was evaporated *in vacuo* to dryness. The product was recrystallized from H_2O to give **2** (67 g in 93% yield).

White solid, mp 234-236 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 5.88 (d, J = 4.4 Hz, 1H), 5.47-5.44 (m, 2H), 5.21-5.20 (m, 1H), 4.62-4.58 (m, 1H), 4.15-4.12 (m, 1H), 4.07 (s, 3H), 3.97-3.95 (m, 1H), 3.69-3.64 (m, 1H), 3.57-3.52 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.3, 152.9, 149.8, 140.8, 118.7, 84.5, 84.0, 76.1, 75.4, 61.3, 53.9; HRMS calcd for $C_{11}H_{14}N_5O_7$[M+H]$^+$ 328.0888, found 328.0886.

(2*R*,3*S*,4*S*,5*R*)-2-(2,6-Dimethoxy-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (7)

A solution of **6** (9.2 g, 20 mmol) and MeONa (2.7 g, 50 mmol) in MeOH (100 mL) was stirred at room temperature for 10 h. The solution was filtrated and the filtrate was evaporated to dryness. TLC showed two products were formed which were separated by chromatography (eluent: $CHCl_3$/MeOH = 7/3). One product was **7** in 54% yield (2.3 g) and the other was **2** in 27% yield (1.7 g).

White solid, m.p. 244-246 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 6.25 (d, J = 4.4 Hz, 1H), 5.61 (d, J = 4.4 Hz, 1H), 5.49 (d, J = 3.6 Hz, 1H), 5.04 (brs, 1H), 4.07-4.05 (m, 2H), 3.94 (s, 3H), 3.85 (s, 3H), 3.74-3.73 (m, 1H), 3.62-3.60 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.6, 152.9, 149.5, 140.4, 119.8, 88.4, 86.4, 73.9, 71.2, 62.2, 54.4, 54.0; HRMS calcd for $C_{12}H_{17}N_4O_6$[M+H]$^+$ 313.1143, found 313.1145.

Nelarabine (1)

A solution of compound **2** (1.6 g, 5 mmol) in MeOH (20 mL) was treated with Pd/C (1%, 16 mg) under H_2 (1.0 atm) and stirred for 12 h at room temperature. On completion, the reaction mixture was filtered through a Celite pad and washed with MeOH. The filtrate was evaporated *in vacuo*, and the residue was recrystallized from EtOH to give the desired product **1** (1.3 g, 90% yield).

The experimental procedure of 100 grams scale: The compound **2** (100 g, 0.31 mol) was dissolved in MeOH (800 mL), treated with Pd/C (1%, 1 g) under H_2 (1.0 atm) and stirred for 12 h at room temperature. On completion, the reaction mixture was filtered through a Celite pad and washed with MeOH (50 mL×2). The filtrate was evaporated *in vacuo*, and the residue was recrystallized from EtOH to give **1 (**73.6 g). The mother liquor was recycled to give the other batch of **1 (**10.1 g). The combined yield was 91% yield. White solid. mp 264-266 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.92 (s, 1H), 6.46 (brs, 2H), 6.12 (d, J = 4.0 Hz, 1H), 5.64 (d, J = 5.2 Hz, 1H), 5.56 (s, 1H), 5.08 (s, 1H), 4.07 (s, 2H), 3.96 (s, 3H) , 3.75 (d, J = 4.4 Hz, 1H), 3.63 (d, J = 4.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.0, 160.2, 154.4, 139.5, 113.6, 84.6, 83.8, 75.9, 75.7, 61.4, 53.6; HRMS calcd for $C_{11}H_{15}N_5NaO_5$ $[M+Na]^+$ 320.0965, found 320.0967.

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