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Synthesis of 3-deaza-3-nitro-2′-deoxyadenosine

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Abstract—Photoactivable deoxyadenosine mimic, 3-deaza-3-nitro-2′-deoxyadenosine (**2**), was prepared using two different synthetic routes. The first route involved base catalyzed glycosylation of 3-deaza-3-nitroadenine, which was prepared by regioselective nitration of 3-deazaadenine. In the second route, the convertible nucleoside 6-*O*-(2,4,6-trimethylphenyl)-3-deaza-2′-deoxyadenosine (**28**) was used to introduce 6-NH_2 group in the last step.

1. Introduction

Our current interest focuses on nitro nucleosides as a class of photoactivable nucleosides. Previously, we synthesized 7-nitroindole nucleoside **1**[1–5] and 3-deaza-3-nitro-2′-deoxyadenosine **2**[6], and incorporated them in oligonucleotides (Scheme 1). The structures of these nitro nucleosides[2,6] were shown to be constrained in an *anti*-like conformation due to steric hindrance of the nitro group, with the consequence that one of the oxygen atoms of the nitro group is in close proximity to H-1′. Upon photochemical irradiation, the excited nitro group abstracts the neighboring anomeric H-1′ atom to produce the C-1′ radical that subsequently generates in excellent yield 2-deoxyribonolactone **3** with departure of the heterocyclic moiety as nitroso derivative. 2-Deoxyribonolactone **3** is an important DNA lesion produced via the same C-1′ radical intermediate under the action of a variety of damaging agents including the neocarzinostatin chromophore[7] and porphyrin-manganese chemical nucleases.[8] Since 2-deoxyribonolactone **3** in a DNA strand is highly alkali- and thermo-labile,[4,9,10] the above nitro nucleosides when inserted in an oligonucleotide can be considered both as a 'caged' deoxyribonolactone site[11,12] and as a 'caged' photocleavable site.[13–17]

In a previous communication, we described the incorporation of 3-deaza-3-nitro-2′-deoxyadenosine **2** in oligonucleotides and reported on photochemical, photophysical, and cleavage properties of the resulting DNA strands.[6] Hybridization experiments showed notably that **2** behaves very much like deoxyadenosine as it possesses the same hydrogen-bonding pattern. In the present paper, we report full account of the synthesis of **2** including two different routes.

2. Results

2.1. Synthesis of 2 via glycosylation of 3-deaza-3-nitroadenine (5)

The first route to 3-deaza-3-nitro-2′-deoxyadenosine **2** involved base catalyzed glycosylation of 3-deaza-3-nitroadenine **5** by chloro sugar **6**[18] (Scheme 2). The former nitro compound **5** was prepared by regioselective nitration of 3-deazaadenine **4**, for which we developed an improved synthesis involving regioselective amination of 2,4-dichloro-3-nitropyridine **8** (Scheme 3).

2,4-Dichloro-3-nitropyridine **8** was prepared from 2,4-dihydroxy-3-nitropyridine **7**.[19] Direct treatment of **8** by ammonia gave a mixture of the two isomeric monosubstituted amino derivatives. However upon treatment of **8** with one equivalent of *p*-methoxybenzylamine, highly regioselective amination occurred on the 4-position affording compound **9** with 93% yield. Hydrogenation of **9** over Raney nickel to diaminopyridine **10** followed by classical cyclization in the presence of triethylorthoformate[20] to **11** and acidic deprotection gave readily the chloroimidazopyridine **12**.[21,22] Conversion of **12** to 3-deazaadenine **4** was achieved by hydrazine treatment followed by Raney nickel hydrogenolysis. Direct nitration of deazaadenine **4** in a number of experimental

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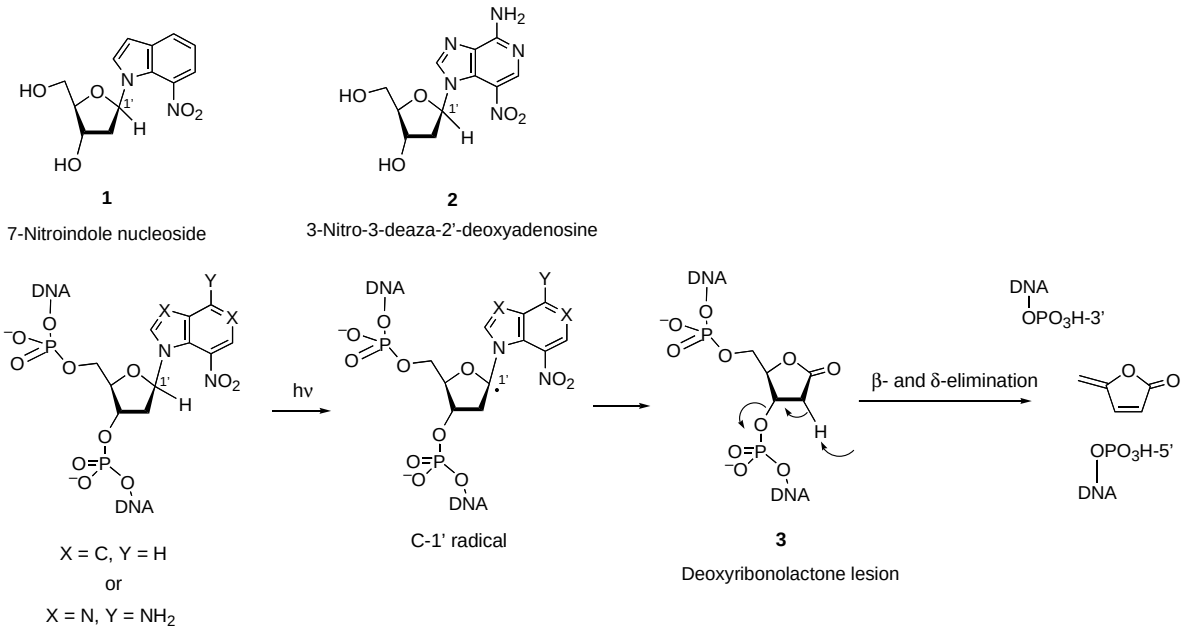
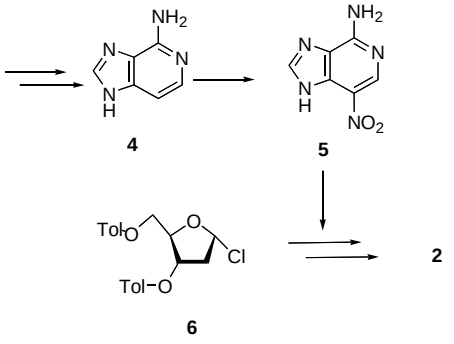
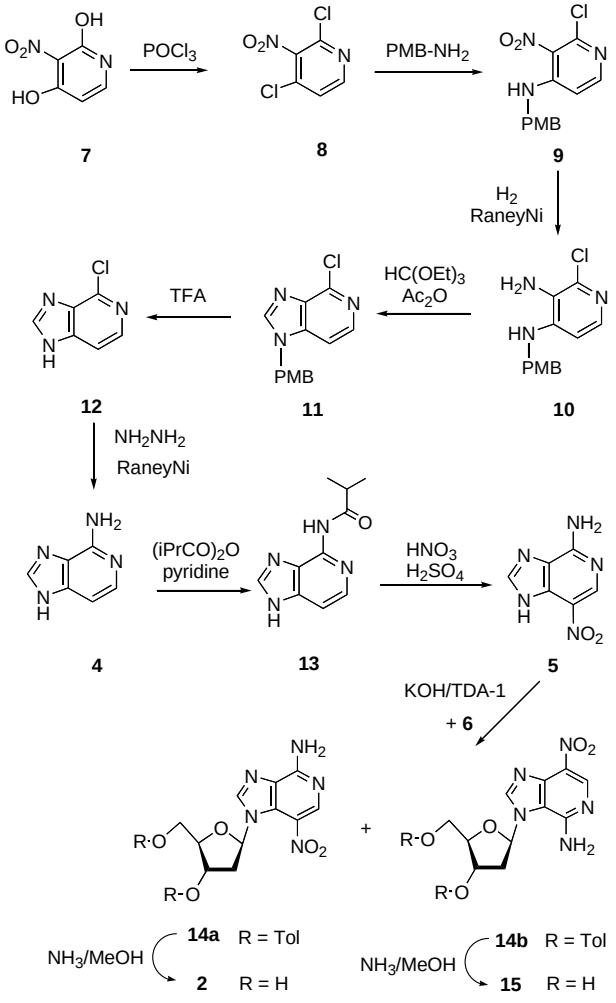


Scheme 1. Photoactivable nitro nucleosides and photochemical reaction of oligonucleotides containing these nucleosides leading to alkali- and thermo-labile deoxyribonolactone site.

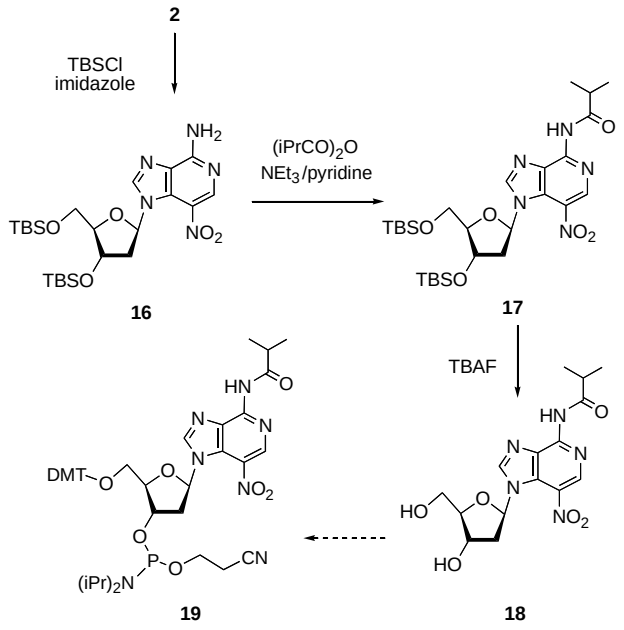


Scheme 2. Synthetic strategy of 3-deaza-3-nitro-2′-deoxyadenosine **2** (Tol = *p*-toluoyl).

conditions was not successful affording significant amounts of secondary product tentatively attributed to NH_2-nitrated product (data not shown).[23] However, by protecting the NH_2 group by an isobutyryl, group successful regioselective nitration could be achieved. The isobutyryl derivative **13** was thus treated by a mixture of nitric and sulfuric acids in which conditions both nitration and isobutyryl deprotection occurred to give compound **5** in 78% yield. Glycosylation of **5** with chloro sugar **6** was carried out in the presence of powdered KOH and tris[2-(2-methoxy)ethyl]amine[24] to afford two regioisomeric nucleosides **14a** and **14b** (60:40) in 45% yield, which were separated and characterized. ^{1}H-NOE experiments unambiguously proved the β-anomeric nature of the two isomers **14a** and **14b**. Hydrolysis of the toluoyl protections by ammonia afforded the two corresponding nucleosides **2** and **15**. X-ray analysis of **2** proved the structure as indicated with an *anti*-like conformation as observed previously for the 7-nitroindole nucleoside **1** and a distance between H-1′ and one of the oxygen atoms of the nitro group as 2.1 Å.[6]



Scheme 3. Synthesis of 3-deaza-3-nitro-2′-deoxyadenosine **2** via glycosylation of **5** (PMB = *p*-methoxybenzyl; TFA = trifluoroacetic acid; TDA-1 = tris[2-(2-methoxyethoxy)ethyl]amine; Tol = *p*-toluoyl).

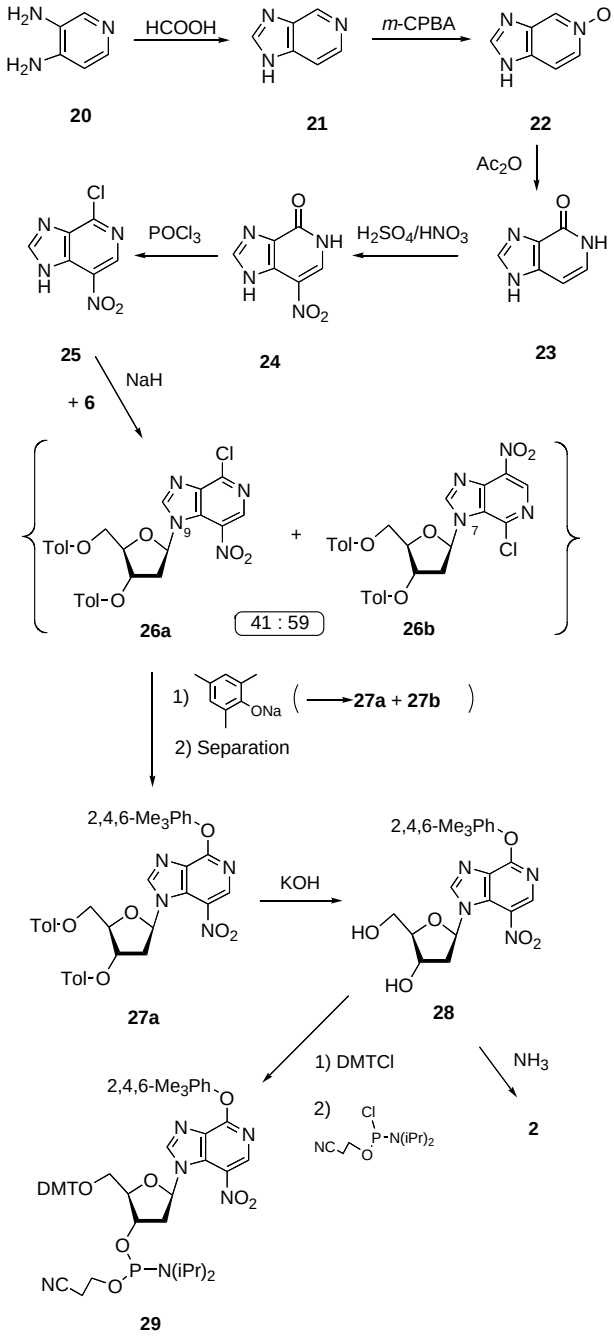


Scheme 4. Approach to phosphoramidite **18** (TBS = *t*-butyldimethylsilyl; TBAF = tetrabutylammonium fluoride; DMT = 4,4′-dimethoxytrityl).

It is interesting to note that preparation of the corresponding phosphoramidite **19** necessary for insertion of **2** in a DNA fragment could not be achieved satisfactorily, probably due to the high electron-withdrawing character of the nitro group (Scheme 4). Conventional preparation of the intermediate *N*-isobutyryl derivative **18** involving acylation of the NH_2 amino group followed by hydrolysis of the toluoyl ester groups failed as mild hydrolysis treatment of the intermediate amido diester derivative led to cleavage of the amide bond while the ester groups remained unchanged. Successful isobutyryl protection of **2** giving **18** was realized in three steps using *O*-silyl protection. However, attempts to tritylate **18** in standard conditions (DMTCl/pyridine/rt) could not be achieved satisfactorily leading to an extensive *N*-glycoside bond cleavage.

2.2. Synthesis of 2 via trimethylphenylether intermediate (28)

For the above reason another synthetic scheme was developed that allowed both preparation of nucleoside **2** and convenient insertion in oligonucleotides using the key 6-*O*-trimethylphenyl intermediate derivative **28** (Scheme 5). Synthesis involved the four-step preparation of 3-deaza-3-nitrohypoxanthine **24** from 3,4-diaminopyridine **20** using published procedures with introduction of some experimental improvements. We found notably that oxidation of imidazopyridine **21** was best accomplished using *m*-chloroperbenzoic acid giving the *N*-oxide **22** in 70% yield which rearranged in refluxing acetic anhydride to 3-deazahypoxanthine **23**. Nitration followed by $POCl_3$ treatment gave the chloronitroderivative **25**, which was glycosylated by chloro sugar **6** to the mixture of nucleoside regioisomers **26a** and **26b**. These were not separated at this stage for high scale prepara-



Scheme 5. Synthesis of 3-deaza-3-nitro-2′-deoxyadenosine **2** via 6-*O*-trimethylphenyl precursor **28**. Incorporation of **2** in oligonucleotides was done using phosphoramidite **29**. (Tol = *p*-toluoyl; DMT = 4,4′-dimethoxytrityl).

tions. Substitution of the chloro substituent by trimethylphenol treatment of the mixture yielded the two isomeric trimethylphenyl derivatives **27a** and **27b** (40:60) that were separated. Mild hydrolysis of **27a** with methanolic KOH gave the key trimethylphenyl nitro nucleoside **28** in which the trimethylphenyl ether was easily substituted by NH_3 to afford quantitatively the nitronucleoside **2**.[25,26]

Key intermediate **28** also proved quite convenient for oligonucleotide synthesis as it was readily transformed

by the standard procedure into phosphoramidite **29** that was used in solid-phase DNA synthesis. Final deprotection by ammonia in standard conditions gave modified oligonucleotide containing nitro nucleoside **2**.

3. Conclusion

We developed the synthesis of deoxyadenosine mimicking nitronucleoside **2**. The main problem in this synthesis is poor selectivity of N-9-glycosylation versus N-7-glycosylation. Incorporation of **2** in oligonucleotides was readily achieved using 6-*O*-trimethylphenyl precursor nucleoside **28**. Investigation in regard to enzymatic incorporation of **2** in oligonucleotides is in progress.

4. Experimental

4.1. General remarks

All commercially available chemical reagents were used without purification unless otherwise indicated. TLC: Merck Kieselgel 60 F_{254}, layer thickness 0.25 mm. Visualization by UV light (254 nm) or by phosphomolybdic acid solution. Preparative column chromatographies: Macherey–Nagel Kieselgel, 230–400 mesh. Mp: Reichert Thermovar (uncorrected). IR: Nicolet Impact 400. UV/vis: Varian Cary 400 scan. NMR: Bruker AC 200 Avance 300 and Varian U+500 spectrometers. NMR spectra were referenced to the residual solvent peak, chemical shifts δ in ppm, apparent scalar coupling constants J in Hz. MS: Esquire 3000+ (ES-MS) and Thermofinnigan Polaris Q (DCI and EI). Elemental analyses were performed by 'Service central de microanalyse du CNRS.'

4.2. 2,4-Dichloro-3-nitropyridine (8)

2,4-Dihydro-3-nitropyridine (24.4 g, 156 mmol) was dissolved in 150 mL $POCl_3$ and heated overnight at 90–100 °C. The excess $POCl_3$ was distilled and 110 g of ice was added. Aqueous solution was neutralized (pH 7) with concd NH_3 aqueous solution (110 mL). The precipitate was filtered and purified by flash chromatography (elution with CH_2Cl_2/cyclohexane 70:30) to give **8** (23.3 g, 77%). R_f (CH_2Cl_2): 0.58. Mp: 60 °C (litt.[21] 61.5–62.0 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.44 (d, 1H, J = 5.1 Hz, H-6), 7.47 (d, 1H, J = 5.1 Hz, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.7 (C-6), 145.2 (C-2), 143.2 (C-3), 137.5 (C-4), 125.0 (C-5). IR (KBr): 3069, 2922, 1561, 1546, 1436, 1346, 1219, 1180, 1114, 853, 824, 716, 596, 549, 483, 443 cm^{-1}.

4.3. 2-Chloro-4-(*p*-methoxybenzylamino)-3-nitropyridine (9)

Dichloropyridine **8** (23.3 g, 121 mmol) was dissolved in DMF (200 mL). *p*-methoxybenzylamine (16.0 mL, 124 mmol) and triethylamine (15 mL, 107 mmol) were added and the solution was stirred at rt for 2 h. The forming salts were filtered and 400 mL of water was added to the filtrate in order to make precipitate the aminopyridine **9**, which was filtered and washed with water (33 g). Small amount of analytical samples was obtained by purification by flash chromatography (elution with CH_2Cl_2). R_f (CH_2Cl_2): 0.14. Mp: 106 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, 1H, J = 6.0 Hz, H-6), 7.24 (d, 2 H, J = 8.6 Hz, C*H*), 6.93 (d, 2H, J = 8.7 Hz, C*H*), 6.85 (s, 1H, N*H*), 6.67 (d, 1H, J = 6.0 Hz, H-5), 4.44 (d, 2H, J = 5.2 Hz, CH_2), 3.83 (s, 3H, O-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.5 (*C*-4′), 149.7 (C-6), 148.7 (C-4), 145.6 (C-2), 131.1 (C-3), 128,5 (C-2′), 127.4 (*C*-1′), 114.5 (C-3′), 107.5 (C-5), 55.3 (CH_3-O), 46.9 (CH_2). IR (KBr): 3240, 3110, 3004, 2914, 1593, 1528, 1510, 1456, 1365, 1336, 1242, 1176, 1060, 1028, 922, 853, 821, 774, 530, 447 cm^{-1}. MS (EI): 293 [M]$^+$. Anal. Calcd. for $C_{13}H_{12}ClN_3O_3$: C 53.16, H 4.12, N 14.31. Found C 53.35, H 4.13, N 14.38.

4.4. 3-Amino-2-chloro-4-(*p*-methoxybenzylamino)pyridine (10)

Compound **9** (30.0 g, 112 mmol) was dissolved in methanol (300 mL) and 25.0 g of Raney nickel was added. After 3.5 h stirring at rt under H_2, 80 mL of dichloromethane was added and the resulting solution was filtered through a Celite pad. The filtrate was evaporated to dryness to give **10** (28.6 g). R_f (CH_2Cl_2/MeOH 90:10): 0.55. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.66 (d, 1H, J = 4.4 Hz, H-6), 7.23 (d, 2H, J = 8.9 Hz, C*H*), 6.88 (d, 2H, J = 6.9 Hz, C*H*), 6.42 (d, 1H, J = 5.5 Hz, H-5), 4.67 (s, 1H, N*H*), 4.28 (d, 2H, J = 4.8 Hz, CH_2), 3.79 (s, 3H, O-CH_3). MS (DCI NH_3/isobutane): 264 [M]$^+$.

4.5. 4-Chloro-1-(*p*-methoxybenzyl)-1H-imidazo[4,5*c*]pyridine (11)

Compound **10** (28.6 g, 108.0 mmol) was dissolved in triethylorthoformate/acetic anhydride (490 mL, 1:1). The solution was refluxed for 1 h 30 min and then evaporated to dryness. Residue was purified by flash chromatography (elution with CH_2Cl_2/acetone 80:20) to afford **11** (20.1 g, 73.0 mmol) with 65% yield in three steps from **8**. R_f (CH_2Cl_2/acetone 80:20): 0.33. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.13 (d, 1H, J = 5.8 Hz, H-6), 7.99 (s, 1H, H-2), 7.17 (d, 1H, J = 5.6 Hz, H-7), 7.12 (d, 2H, J = 8.9 Hz, CH), 6.87 (d, 2H, J = 8.6 Hz, CH), 5.29 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.0 (*C*-OCH_3), 144.4 (C-2), 143.1 (C-4), 141.2 (C-6), 140.0, 138.1, 130.0 (C-*o*-benz), 130.9 (*C*-CH_2), 115.0 (C-*m*-benz), 106.2 (C-7), 57.5 (*C*H_3), 51.2 (CH_2). IR (KBr): 3102, 3012, 2955, 2832, 1600, 1561, 1510, 1484, 1365, 1339, 1303, 1256, 1161, 1024, 948, 919, 810, 759, 701, 643, 581, 545, 527 cm^{-1}. MS (EI): 273 [M]$^+$. Anal. Calcd. for $C_{14}H_{12}ClN_3O$: C 61.43, H 4.42, Cl 12.95, N 15.35. Found C 61.69, H 4.40, Cl 12.29, N 15.15.

4.6. 4-Chloro-1H-imidazo[4,5*c*]pyridine (12)

4-Chloro-1-(*p*-methoxybenzyl)-1H-imidazo[4,5*c*]pyridine **11** (20.0 g, 73.1 mmol) was dissolved in TFA (120 mL) and stirred for 2 h at 80 °C. Then the solution was evaporated and the residual oil was triturated with ether to obtain 4-chloro-1H-imidazo[4,5*c*]pyridine **12** as a powder (15.6 g). R_f (CH_2Cl_2/MeOH 90:10): 0.31. ^{1}H NMR

(300 MHz, DMSO-d_6): δ 8.44 (s, 1H, H-2), 8.01 (d, 1H, J = 5.6 Hz, H-6), 7.60 (d, 1H, J = 5.6 Hz, H-7). ^{13}C NMR (75 MHz, DMSO-d_6): δ 144.8 (C-2), 141.4 (C-1a), 141.0 (C-6), 139.9 (C-4), 135.8 (C-4a), 109.1 (C-7). MS (EI): 153 $[M]^+$.

4.7. 4-Amino-1H-imidazo[4,5*c*]pyridine (3-deazaadenine) (4)

4-Chloro-1H-imidazo[4,5*c*]pyridine **12** (11.2 g, 153 mmol) was dissolved in a mixture of hydrazine 99% (200 mL) and propan-1-ol (140 mL). The solution was stirred at 120 °C overnight and then evaporated to dryness. Water (400 mL) and Raney nickel (20 g) were added. The mixture was refluxed for 1 h and filtered through a Celite pad. The filtrate was evaporated to afford 3-deazaadenine **4** (10.9 g). ^{1}H NMR (300 MHz, D_2O): δ 8.32 (s, 1H, H-2), 7.61 (d, 1H, J = 7.0 Hz, H-6), 7.17 (d, 1H, J = 7.0 Hz, H-3). MS (EI): 134 $[M]^+$.

4.8. 4-(Isobutyrylamino)-1H-imidazo[4,5*c*]pyridine (6-*N*-Isobutyryl-3-deazaadenine) (13)

To a suspension of 3-deazaadenine **4** (10.1 g, 73.0 mmol) in pyridine (140 mL) at 80 °C, isobutyric anhydride (24 mL, 146 mmol) was added dropwise. The mixture was stirred at 80 °C for 5.5 h, and then the solution was evaporated to dryness. Three hundred milliliters of dichloromethane and 300 mL of a Na_2CO_3 saturated aqueous solution were added. The mixture was stirred at rt for 2 h and then diluted with water (20 mL). Aqueous phase was extracted with dichloromethane (3 × 300 mL) and combined extracts were dried on $MgSO_4$. After the evaporation of the solvent, the residual oil was triturated with ether to obtain **13** as a white powder (4.6 g, 22.5 mmol, 31% after three steps from **11**). ^{1}H NMR (200 MHz, $CDCl_3$): δ 11.89 (s, 1H, N*H*), 8.98 (s, 1H, N*H*), 8.13 (s, 1H, H-2), 8.05 (d, 1H, J = 5.8 Hz. H-6), 7.54 (d, 1H, J = 5.5 Hz, H-7), 2.70 (m, 1H, C*H*), 1.27 (d, 6H, J = 6.9 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.8 (C=O), 151.5 (C-4), 142.7 (C-6), 139.2 (C-2), 121.5 (2C, C-4a, C-1a), 112.5 (C-7), 36.2, 19.7 (2C, CH_3). MS (DCI NH_3/isobutane): 205 $[M+1]^+$. Anal. Calcd. for $C_{10}H_{12}N_4O$: C 58.81, H 5.92, N 27.43. Found C 58.47, H 6.18, N 26.17.

4.9. 4-Amino-7-nitro-1H-imidazo[4,5*c*]pyridine (3-nitro-3-deazaadenine) (5)

Compound **13** (4.30 g, 21.1 mmol) was added in small portions to H_2SO_4 (43 mL, 92%) at 80 °C (oil bath temperature). Fuming nitric acid (36.6 mmol) was then added dropwise and the mixture was stirred at 80 °C for 45 min. After cooling by ice/acetone bath, the solution was neutralized (pH 9) with concd NH_3 aqueous solution. The precipitate was centrifuged and washed with water to give **5** (2.9 g, 78%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.72 (s, 1H, H-6), 8.19 (s, 1H, H-2), 7.91 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.3 (C-4), 142.5 (C-6), 141.6 (C-2), 131.2 (C-1a), 125.3 (C-4a), 124.3 (C-7). IR (KBr): 1669, 1613, 1595, 1397, 1319, 1277, 1186, 1112, 939, 621 cm^{-1}. MS (EI): 179 $[M]^+$, 133 $[M–NO_2]^+$. UV (100 μM in H_2O): 366 (9440).

4.10. 4-Amino-1-[2′-deoxy-3′,5′-di-*O*-(4-toluoyl)-β-D-*erythro*-pentofuranosyl]-7-nitro-1H-imidazo[4,5*c*]pyridine (14a) and 4-amino-3-[2′-deoxy-3′,5′-di-*O*-(4-toluoyl)-β-D-*erythro*-pentofuranosyl]-7-nitro-3H-imidazo-[4,5*c*]-pyridine (14b)

KOH (490 mg, 8.60 mmol) and TDA-1 (70 μL, 1.8 μmol) were suspended in dry acetonitrile (40 mL) and the mixture was stirred at rt for 10 min. To this suspension, compound **5** (700 mg, 3.92 mmol) was added and the mixture was stirred for 1 h at 80 °C under argon. When the temperature was gone down to 50 °C, α-chloro sugar **6** (2.27 g, 5.85 mmol) was added in small portions. After 20 min stirring at 80 °C, 40 mL of methanol was added and the resulting solution was filtered through a Celite pad. The filtrate was evaporated to dryness. The residue was purified by flash chromatography (elution with CH_2Cl_2/acetone 85:15 to 70:30). A mixture of two isomers (**14a**/**14b**, ratio = 60:40) was obtained (944 mg, 45%). The two isomers were separated by recrystallizations in acetonitrile. The isomer **14a** (30%) precipitated, whereas the isomer **14b** (33%) was soluble in the filtrate.

Isomer **14a**: R_f (CH_2Cl_2/acetone 85:15): 0.21. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (s, 1H, H-2), 8.51 (s, 1H, H-8), 7.81 (d, 2H, J = 8.1 Hz, Harom.), 7.73 (d, 2H, J = 7.9 Hz; Harom.), 7.36 (d, 2H, J = 8.1 Hz, Harom.), 7.27 (d, 2H, J = 8.1 Hz, Harom.), 6.87 (t, 1H, J = 8.2, 6.3 Hz, H-1′), 5.61 (m, 1H, H-3′), 4.53 (m, 3H, H-4′, H-5′ and H-5″), 2.90 (m, 2H, H-2′ and H-2″), 2.39 (s, 3H, CH_3), 2.36 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.4 (C=O), 165.2 (C=O), 156.5 (C-6), 144.1 (2C, C-*p*-Tol.), 143.9 (C-2), 140.8 (C-8), 129.4 (4C, C-*m*-Tol.), 129.2 (4C, C-*o*-Tol.), 129.0 (C-4), 126.4 (C-5), 126.3 (2C, C-Tol.), 126.1 (C-3), 87.6 (C-1′), 81.8 (C-4′), 74.3 (C-3′), 63.8 (C-5′), 38.8 (C-2′), 21.1 (2C, CH_3). MS (EI): 532.2 $[M+1]^+$. UV (23 μM in CH_3CN/H_2O 1:1): 370 (9869). Anal. Calcd. for $C_{27}H_{25}N_5O_7$: C 61.01, H 4.74, N 13.18. Found: C 60.94, H 4.89, N 13.11.

Isomer **14b**: R_f (CH_2Cl_2/acetone 85:15): 0.25. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (s, 1H, H-2), 8.57 (s, 1H, H-8), 7.90 (m, 4H, NH_2 and Harom.), 7.35 (m, 6H, Harom.), 6.93 (m, 1H, H-1′), 5.55 (m, 1H, H-3′), 5.07 (m, 1H, H-4′), 4.50 (m, 2H, H-5′ and H-5″), 3.01 and 2.49 (m, 2H, H-2′ and H-2″), 2.39 (s, 3H, CH_3), 2.33 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.4 (C=O), 164.8 (C=O), 156.6 (C-6), 143.9 (C-2), 143.7 (2C, C-*p*-Tol.), 141.8 (C-8), 129.3 (4C, C-*o*-Tol.), 129.1 (4C, C-*m*-Tol.), 128.8 (C-4), 127.0 (C-5), 126.5 (C-Tol.), 126.2 (C-Tol.), 125.7 (C-3), 90.4 (C-1′), 84.5 (C-4′), 74.7 (C-3′), 64.0 (C-5′), 38.6 (-2′), 21.1 (2C, CH_3). UV (23 μM in CH_3CN/H_2O 1:1)]: 371 (9739).

4.11. 4-Amino-1-[2′-deoxy-β-D-*erythro*-pentofuranosyl]-7-nitro-1H-imidazo[4,5*c*]pyridine (3-deaza-3-nitro-2′-deoxyadenosine) (2)

Isomer **14a** (199 mg, 0.37 mmol) was suspended in THF/MeOH/NH_3 and the solution was heated at 60 °C overnight. The solvents were evaporated, the residual solid was washed with dichloromethane and recrystallized in MeOH/H_2O 90:10 to afford **2** (102 mg, 93%). ^{1}H

NMR (300 MHz, DMSO-d_6): δ 8.66 (s, 2H, H-2 and H-8), 7.83 (s, 2H, NH_2), 6.66 (t, 1H, J = 5.5 and 5.8 Hz, H-1′), 5.27 (m, 1H, 3′-OH), 5.05 (m, 1H, 5′-OH), 4.28 (m, 1H, H-3′), 3.83 (m, 1H, H-4′), 3.56 (m, 2H, H-5′ and H-5″), 2.41 (m, 2H, H-2′ and H-2″). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.5 (C-6), 143.7 (C-2), 141.5 (C-8), 129.2 (C-4), 126.5 (C-5), 125.9 (C-3), 87.8 (C-1′), 87.5 (C-3′), 69.1 (C-4′), 60.5 (C-5′), 42.3 (C-2′). MS (DCI NH_3/isobutane): 295 $[M+1]^+$. UV (100 μM in H_2O): 371 (8900). Anal. Calcd. for $C_{11}H_{15}N_5O_6$: C 42.17, H 4.,83, N 22.36. Found: C 41.86, H 4.51, N 22.25.

4.12. 4-Amino-3-[2′-deoxy-β-D-*erythro*-pentofuranosyl]-7-nitro-3H-imidazo[4,5*c*]pyridine (3-deaza-3-nitro-2′-deoxyadenosine) (15)

Isomer **14b** (200 mg, 0.38 mmol) was suspended in THF/MeOH/NH_3 and the solution was heated at 60 °C overnight. The solvents were evaporated, the residual solid was washed with dichloromethane and recrystallized in MeOH/H_2O 3:1 to afford **15** (108 mg, 96%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.68 (s, 1H, H-2), 8.35 (s, 1H, H-8), 7.79 (s, 2H, NH_2), 6.69 (m, 1H, H-1′), 5.01 (m, 1H, 3′-OH), 4.86 (m, 1H, 5′-OH), 4.25 (m, 2H, H-3′ and H-4′), 3.45 (m, 2H, H-5′ and H-5″), 2.49–2.10 (m, 2H, H-2′ and H-2″). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.5 (C-6), 143.6 (C-2), 142.2 (C-8), 128.8 (C-4), 126.8 (C-5), 125.9 (C-3), 90.0 (2C, C-1′ and C-3′), 70.7 (C-4′), 61.7 (C-5′), 42.5 (C-2′). MS (DCI NH_3/isobutane): 295 $[M+1]^+$.

4.13. 4-Amino-1-[2′-deoxy-3′,5′-bis-*O*-(*t*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]-7-nitro-1H-imidazo[4,5*c*]pyridine (16)

To a solution of **2** in DMF (2 mL) were added imidazole (172 mg, 2.5 mmol) and TBSCl (388 mg, 2.5 mmol). The solution was stirred at room temperature for 45 min, concentrated, and purified by flash chromatography (elution with cyclohexane/AcOEt 1:1). Compound **16** (222 mg, 85%) was obtained as a yellow powder. R_f (cyclohexane/AcOEt 1:1): 0.37. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (s, 1H, H-2), 8.64 (s, 1H, H-8), 6.80 (m, 1H, H-1′), 5.93 (s, 2H, NH_2), 4.53 (m, 1H, H-3′), 3.94 (m, 3H, H-4′, H-5′ and H-5″), 2.64 and 2.33 (m, 2H, H-2′ et H-2″), 0.95 (s, 9H, *t*-Bu), 0.89 (s, 9H, *t*-Bu), 0.15 (s, 3H, CH_3), 0.13 (s, 3H, CH_3), 0.06 (s, 6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.0 (C-6), 143.7 (C-2), 142.1 (C-8), 129.7 (C-4), 128.1 (C-5), 127.4 (C-3), 89.2 (C-1′), 87.7 (C-4′), 69.7 (C-3′), 61.8 (C-5′), 44.4 (C-2′), 25.8 (3C, CH_3), 25.6 (3C, CH_3),18.6 (Cquat), 18.1 (Cquat), –4.3, –4.7, –5.2, –5.3 (4C, CH_3). MS (DCI NH_3/isobutane): 524.2 $[M+1]^+$. Anal. Calcd. For C 52.74, H 7.89, N 13.17. Found: C 52.52, H 7.91, N 12.74.

4.14. 4-Isobutyrylamino-1-[2′-deoxy-3′,5′-di-*O*-(*t*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl]-7-nitro-1H-imidazo[4,5c]pyridine (17)

A solution of **16** (216 mg, 0.41 mmol) in CH_2Cl_2 (2 mL), pyridine (198 μl), and triethylamine (340 μL) was cooled to 0 °C. Isobutyryl chloride (214 μL, 2.05 mmol) were added and the mixture was stirred at 0 °C for 30 min. 2 mL of methanol and 2.5 of concd NH_3 aqueous solution was added. The solution was stirred at room temperature for 45 min, extracted with water (10 mL), and dried on $MgSO_4$. After the evaporation of the solvent, the residual oil was purified by flash chromatography (cyclohexane/AcOEt 75:25 to 70:30) to obtain **17** (216 mg, 89%). R_f (cyclohexane/AcOEt 2:1): 0.35. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.04 (s, 1H, H-2), 8.78 (s, 1H, H-8), 8.76 (s, 1H, NH), 6.76 (m, 1H, H-1′), 4.53 (m, 1H,H-3′), 3.86 (m, 3H, H-4′, H-5′ and H-5″), 3.32 (m, 1H, CH), 2.65 and 2.35 (m, 2H, H-2′ and H-2″), 1.31 (d, 6H, J = 6.8 Hz, CH_3), 0.95 (s, 9H, *t*-Bu), 0.89 (s, 9H, *t*-Bu), 0.15 (s, 3H, CH_3), 0.13 (s, 3H, CH_3), 0.06 (s, 6H, CH_3). MS (DCI NH_3/isobutane): 593.6 $[M]^+$, 594.2 $[M+1]^+$.

4.15. 4-Isobutyrylamino-1-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-7-nitro-1H-imidazo[4,5c]pyridine (18)

Compound **17** (367 mg, 0.62 mmol) was dissolved in THF (15 mL) and TBAF (1M/THF, 3.1 mL, 3.1 mmol) was added. The solution was stirred at room temperature for 15 min and directly purified by flash chromatography (cyclohexane/AcOEt 90:10 to 85:15) to afford the title compound (202 mg, 89%). R_f (cyclohexane/AcOEt 2/1): 0.35. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 8.93 (s, 1H, H-2), 8.87 (s, 1H, H-8), 6.72 (t, 1H, J = 5.5 and 5.6 Hz; H-1′), 4.85 (m, 1H, H-3′), 3.99 (m, 1H, H-4′), 3.71 (m, 2H, H-5′ and H-5″), 2.97 (m, 1H, CH), 2.60 (m, 2H, H-2′ and H-2″), 1.27 (d, 6H, J = 6.9 Hz, CH_3). MS (DCI NH_3/isobutane): 364.9 $[M]^+$.

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