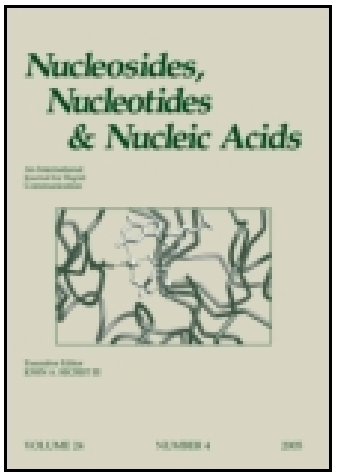

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NITROGEN MUSTARD DERIVATIVES OF PURINE BASES

Benjamin Boëns,[1] Mounir Azouz,[2] Tan-Sothea Ouk,[1] and Rachida Zerrouki[1]
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□ *This paper deals with the synthesis of nitrogen mustard analogs, derivatives of purine bases. Alkylation in position N-9 and diethanolamine fixation on position 6 were managed by microwave irradiations. Chlorination of these dihydroxylated intermediates led to a cyclization, giving tricyclic purine base analogs bearing a chloroethyl chain. Finally, MTT assays on obtained compounds do not show cytotoxicity on four different cancer cell lines.*

Keywords Alkylating agents; nitrogen mustards; purine bases; microwave activation

INTRODUCTION

The nitrogen mustards are still among the most useful clinical agents for the treatment of a number of cancers. All of them are bifunctional alkylating molecules which can react with two guanines in the DNA molecule. If these guanines belong to different strands, the result is a cross-link which prevents uncoiling of the double helix.[1] If the two guanines are on the same DNA strand, the result is called "limpet attachment." Monoalkylating agents can react with only one guanine. Limpet attachment and monoalkylation do not prevent the opening of the double helix but do prevent vital DNA processing enzymes.[2]

All these alkylation reactions result in an inhibition of cell division or cell death. Since cancer cells generally divide more rapidly than healthy cells, they are more sensitive to DNA damage by alkylating agents.

The pyrimidine and purine derivatives have elicited great interest for many years owing to their diversified biological activities. In particular, many authors performed the structural modification of pyrimidine or purine bases as well as the introduction of different functional groups into these bases

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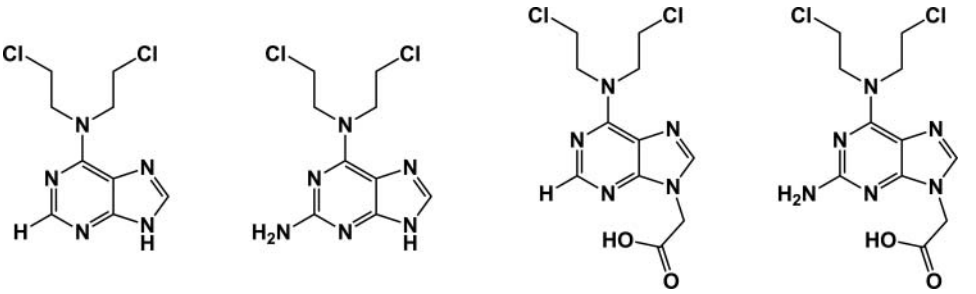


FIGURE 1 Target structures from purine bases.

in the aim of searching potential activity.[3–5] Although it was discovered in 1963,[6] bendamustine has recently known a rebirth, as this drug is more efficient than other canonical nitrogen mustards. It is even efficient in the cases of drug-resistant tumors. This molecule is composed of a benzimidazole cycle bearing the bis(2-chloroethyl)amine group. Thanks to this benzimidazole ring, bendamustine has structural similarities with purine analogs.

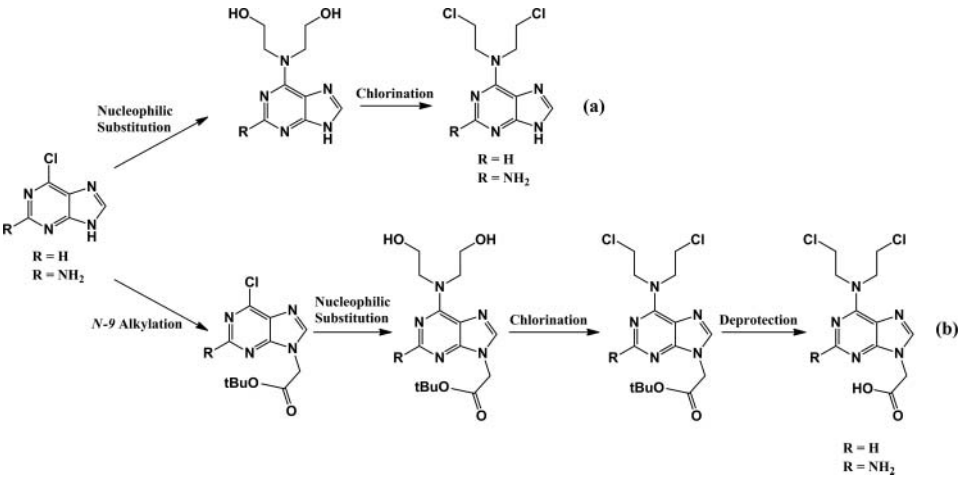
In connection with our research program on modified nucleosides and nucleic bases designed for cancer therapy,[7–9] we chose to synthetize four purine analogs, comprising a purine base bearing a bis(2-chloroethyl)amine moiety, as presented in Figure 1.

RESULTS AND DISCUSSION

The strategy envisioned for the synthesis of these modified purine bases is displayed in Scheme 1. In continuation of our research program, microwave activation was used whenever possible in order to study its influence on the investigated transformations.

Nitrogen mustard analogs were synthesized from commercial 6-chloropurine and 2-amino-6-chloropurine, in two ways. In the first way (a), the chloropurines are directly modified on position 6, to afford the bis(2-hydroxyethyl)amino intermediates. In the second way (b), chloropurines are alkylated on position 9 with *tert*butyl bromoacetate. The bis(2-hydroxyethyl)amino intermediates are obtained by using the same conditions as previously. These dihydroxylated intermediates are then chlorinated to afford the corresponding nitrogen mustards.

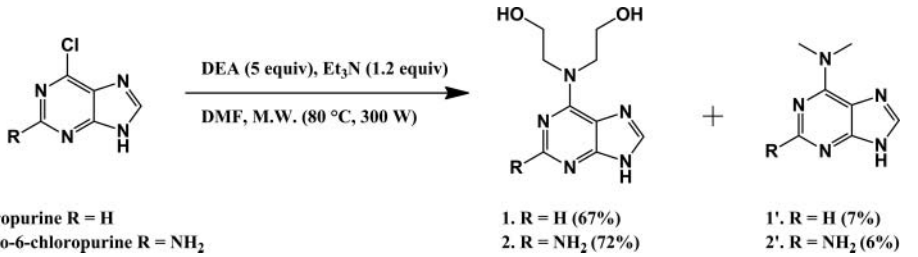
In the first way, the introduction of diethanolamino group onto 6-chloropurines was achieved in dimethylformamide, with an excess of diethanolamine (5 equiv) in the presence of triethylamine (1.2 equiv),[10] using microwave activation (80°C, 300 W). The best yields are obtained after 50 minutes of irradiation. The reaction gave a mixture of two products. Structural elucidation of these compounds indicates that one of them is the expected product (70% yield) and the second one results from the fixation



SCHEME 1 Strategy of synthesis.

of dimethylamine in position 6 (7%). A longer activation time leads to the increase of the yield of the dimethylamino by-product and complicates the purification.

The promotion of the dimethylamination of activated aromatic halides by diethanolamine has been reported by Park and Cho.[11] The in situ reaction of DMF with $HN(CH_2CH_2OH)_2$ led to the formation of dimethylamine and 2-((2-hydroxyethyl)amino)ethyl formate (Scheme 2). Then, the produced dimethylamine reacted with 6-chloropurines.



SCHEME 2 Synthesis of 6-bis(2-hydroxyethyl)aminopurine.

Chlorination of dihydroxyethylamino intermediates was conducted in thionyl chloride. After a short reaction time (10 minutes), TLC monitoring showed the disappearance of the starting material and the formation of two highly polar products. Extending the reaction time to 4 hours led to the disappearance of one of them.

NMR spectra (Table 1) of the compound **3** showed an important deshielding effect of the purine cycle protons, H-5 (8.78 ppm) and H-2 (8.58 ppm). Two deshielded triplets (4.79 ppm and 4.19 ppm) and an abnormally high coupling constant (9.5 Hz) were observed in comparison of

TABLE 1 NMR data of compound **3** in DMSO-d_6 (400 MHz for ^{1}H, 100 MHz for ^{13}C)

Position	δ_C	δ_H (mult; J in Hz)	HMBC
2	143.9	8.58 (*s*)	C-3a; C-9b
3a	115.4	—	—
5	144.6	8.78 (*s*)	C-3a; C-7; C-9a; C-9b
7	47.8	4.79 (*t*; 9.5)	sC-5; C-8; C-9a
8	48.3	4.19 (*t*; 9.5)	C-7; C-9a
9b	151.1	—	—
9a	149.7	—	—
α	48.0	4.42 (*t*; 5.9)	C-β; C-9a
β	41.1	4.07 (*t*; 5.9)	Cα

two other triplets (4.42 ppm and 4.07 ppm, J = 5.9 Hz). Moreover, HMBC experiences revealed a connection between H-7 protons (4.78 ppm) and C-5 (144.6 ppm) of purine cycle (Figure 2).

Chemical ionization mass spectrometry analysis allowed identification of the molecular peak (M^+) at m/z 224 and the isotopic peak at m/z 226, confirming the formation of a cationic compound and the presence of only one chlorine in this molecule. It seems that the reaction of chlorination led to the

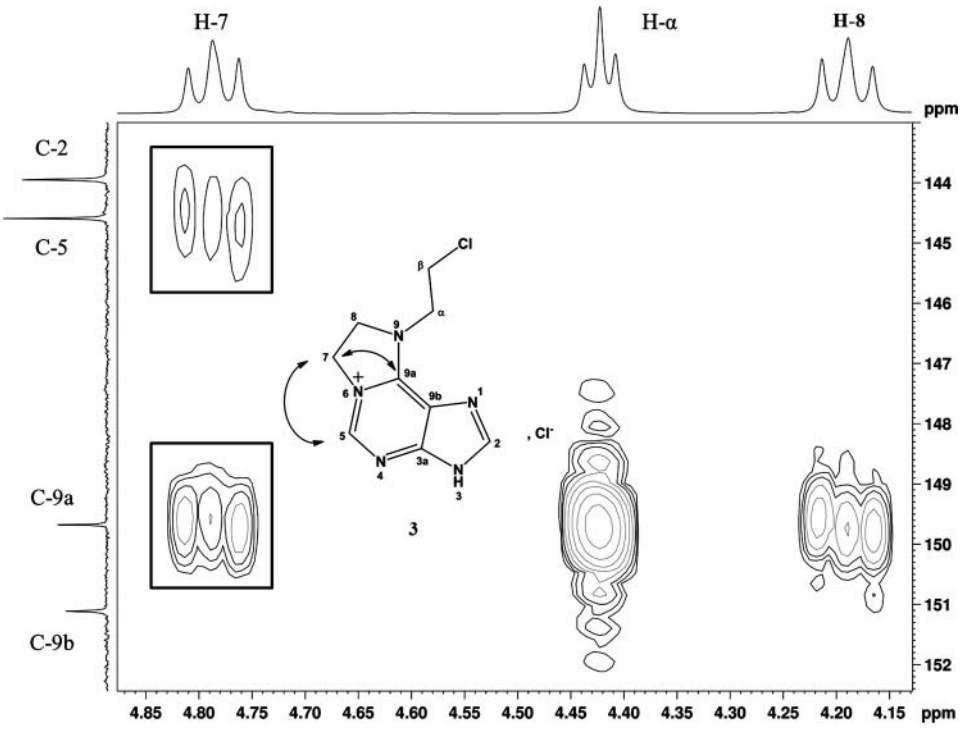


FIGURE 2 H-7 proton and C-5 / C-9a HMBC connections.

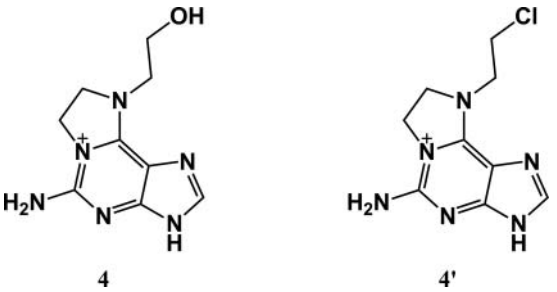
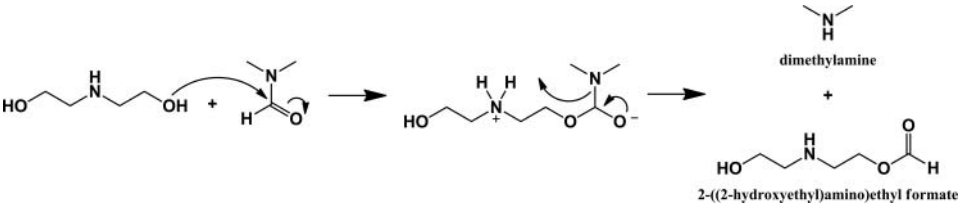


FIGURE 3 Two-compound mixture for compound **2** chlorination.

rapid formation of a five-membered cycle, derived from the intramolecular reaction of *N-1* with an ethyl chain.

Chlorination of the compound **2** using thionyl chloride gave after 4 hours a mixture of two compounds in the ratio 40/60, even after a long reaction time (more than 7 days), the mixture is still composed of two products but in the ratio 70/30 (Figure 3). NMR spectra and mass spectrometry analysis (Table 2) showed the same cyclization and an important deshielded effect of NH_2 group (7.78 ppm) and H-2 proton (8.08 ppm). In this case, chlorination of the second hydroxyethyl chain seems to be slow and completion of this reaction cannot be reached, even after several weeks of reaction and a large excess of thionyl chloride (Figure 3).

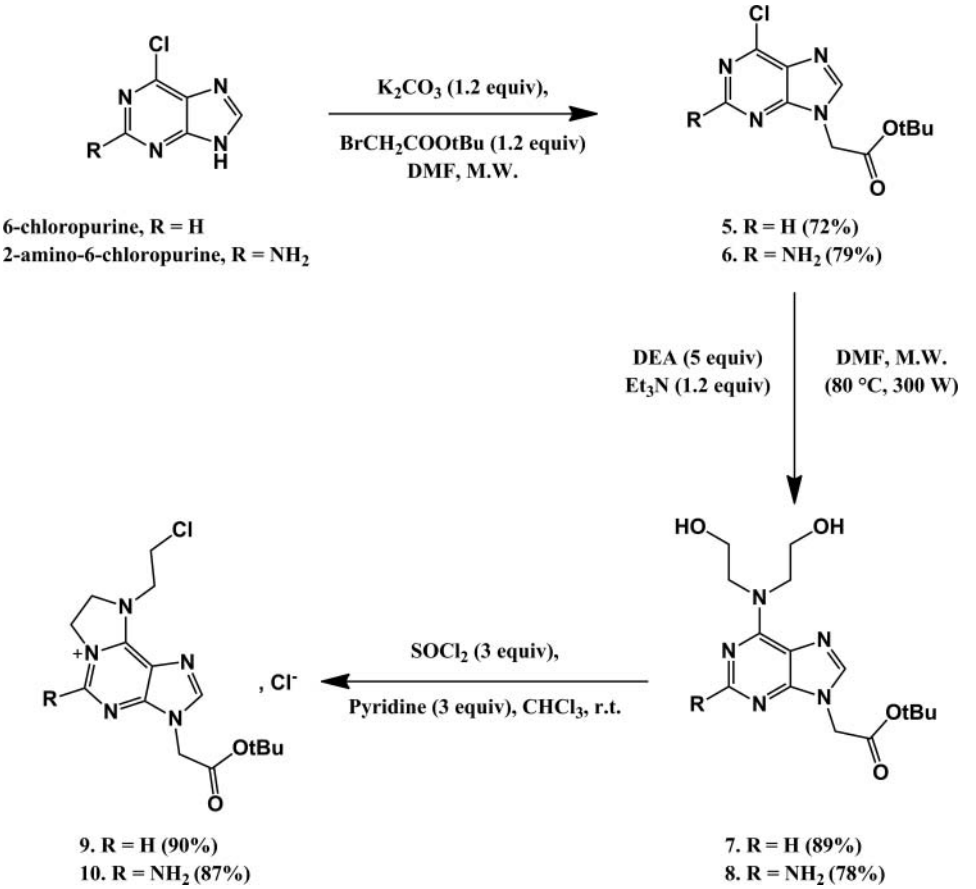
In the second way, the alkylation of purines was realized according to the method using *tert*-butylbromoacetate in the presence of potassium carbonate in *N,N*-dimethylformamide,[12,13] under microwave irradiations. This method gave, after 15 minutes, compounds **5** and **6** in 72% and 79% yield, respectively (Scheme 3). Only *N*-9 alkylation took place as *N*-7 no regioisomer was formed.



SCHEME 3 In situ formation of dimethylamine.

The reaction between *N-9* alkylated purines and diethanolamine was carried out as previously (Scheme 4) and led to the compounds **7** and **8** in 89% and 78%, respectively. In these cases, no dimethylamino by-products were observed.

The chlorination of alkylated compounds **7** and **8**, in thionyl chloride, under the same conditions as previously, led to degradation of the starting

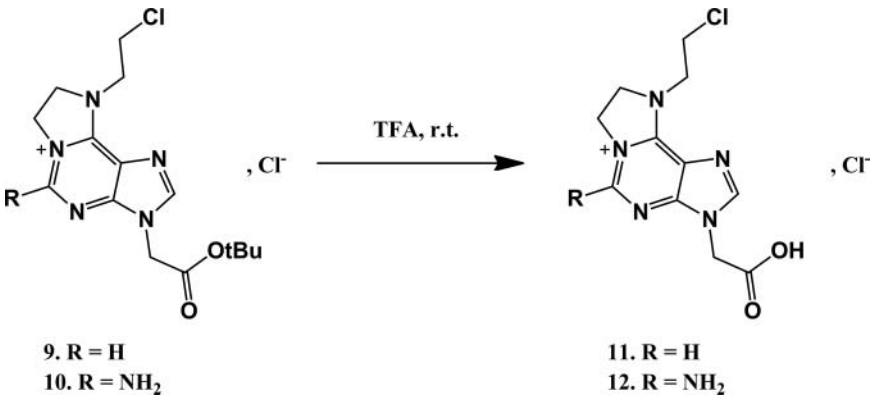


SCHEME 4 Preparation of alkylated compounds.

materials, due to the produced hydrochloric acid. By using pyridine to neutralize the reaction medium, chlorination conduced to the desired products without any loss of the *tert*butyl moiety. After purification, compounds **9** and **10** were obtained in 90% and 87% yield, respectively (Scheme 5).

TABLE 2 NMR data of compounds **4** and **4′** in DMSO-d_6 (400 MHz for ^{1}H)

	δ_H (mult; J in Hz)	
Position	**4**	**4′**
NH_2	7.86 (*s*)	7.78 (*s*)
2	8.12 (*s*)	8.08 (*s*)
7	4.33 (*t*; 8.7)	4.37 (*t*; 9.2)
8	4.12 (*t*; 8.7)	4.11 (*t*; 9.2)
α	4.02 (*t*; 5.2)	3.92 (*t*; 5.6)
β	3.73 (*t*; 5.2)	3.66 (*m*)



SCHEME 5 Deprotection of compounds **9** and **10**.

Carboxylic acid derivatives **11** and **12** were obtained in quantitative yields by solvolysis in trifluoroacetic acid (Scheme 5).

CONCLUSION

During this work, we achieved the synthesis of several purine analogs. Thanks to microwave irradiations, alkylation in position *N*-9 and diethanolamine fixation on position 6 were managed. Whatever the structure of the dihydroxylated intermediate is, the chlorination step led to the intramolecular reaction, conducing to the formation of a dihydroimidazolium cycle between *N*-1 and N^6 positions. Although these structures don't have bis(2-chloroethyl)amine, as expected for nitrogen mustards, the presence of one chloroethyl chain can lead to DNA alkylation. Moreover, the dihydroimidazolium cycle contains an electrophilic site at position C-7.

This structure is a potential alkylating agent as C-7 and C-β positions are electrophilic sites, available for alkylation of DNA. The cytotoxicity of these nitrogen mustards purine analogs was evaluated toward four cancer cell lines (JURKAT, K562, U266, and A431) using an MTT reduction assay.[14] For all of these cells, synthesized compounds didn't show any cytotoxicity, even at high concentration levels (2 mM). Further investigations on the cytotoxic activity of these molecules are in progress. Alkylation potency of these structures will also be investigated.

EXPERIMENTAL

General Methods

All the solvents and chemicals were commercially available and, unless otherwise stated, were used as received. Reactions were monitored by thin layer chromatography (TLC) on precoated 0.2 mm silica gel 60 F254 (Merck)

plates and visualized in several ways: with an ultraviolet light source at 254 nm or by spraying a solution of ninhydrin and acetic acid in butanol and heating to 200°C. Microwave irradiations were performed by the means of an Ethos 1600 MicroSynth reactor from Milestone. Temperature was measured with a fiber optic thermometer (ATC-FO)/Ethos. ^{1}H NMR spectra were recorded at 400.13 MHz and ^{13}C NMR spectra were recorded at 100.61 MHz with a Brüker DPX spectrometer (Germany). Chemical shifts (δ) are expressed in ppm with Me_4Si as an internal standard ($\delta = 0$). Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and br, broad), coupling constants (Hz) and assignment. Mass spectra were recorded with a R10-10 Nermag instrument.

Synthesis

1. General Procedure for N-9 *alkylation of 6-chloropurines*

To a solution of chloropurine (6.5 mmol) in DMF (100 mL), potassium carbonate (7.8 mmol, 1.08 g) and *tert*-butyl bromoacetate (7.8 mmol, 1.14 mL) were added. The resulting mixture was activated by microwave irradiation (15 min, 400 W, 100°C) and then evaporated in vacuo. The crude product was then purified by automated flash chromatography ($CHCl_3$/EP; 7/3; v/v to $CHCl_3$).

tert-*butyl (6-chloro-purin-9-yl)ethanoate (5):*

White powder (61%, 1.06 g); mp = 104°C; Rf 0.47 ($CHCl_3$/EtOH; 95/5; v/v); ^{1}H NMR (DMSO-d_6, δ): 8.81 (s, 1H, H-2), 8.68 (s, 1H, H-8), 5.16 (s, 2H, CH_2), 1.43 (s, 9H, $(CH_3)_3$), ^{13}C NMR (DMSO-d_6, δ): 166.2 (CO), 152.0 (C-4), 151.7 (C-2), 149.0 (C-6), 147.9 (C-8), 130.4 (C-5), ESIMS: m/z 269 and 270 (MH^+), 291 and 292 (MNa^+).

tert-*butyl (6-chloro-2-aminopurin-9-yl)ethanoate (6):*

White powder (62%, 1.14 g); mp = 165°C (decomposition); Rf 0.46 ($CHCl_3$/EtOH; 95/5; v/v); ^{1}H NMR (DMSO-d_6, δ): 8.09 (s, 1H, H-8), 6.96 (s, 2H, NH_2), 4.86 (s, 2H, CH_2), 1,42 (s, 9H, $(CH_3)_3$), ^{13}C NMR (DMSO-d_6, δ): 166.6 (CO), 159.8 (C-2), 154.2 (C-4), 149.3 (C-6), 143.5 (C-8), 122.8 (C-5), 82.2 (C-*tert*-butyl), 44.5 (CH_2), 27.6 ($(CH_3)_3$), ESIMS: m/z 284 and 286 (MH^+), 306 and 308 (MNa^+).

2. General Procedure for Diethanolamine Fixation

To a solution of chloropurine in 8 mL of DMF, 6 mmol (836 μL) of triethylamine and 25 mmol (2.4 mL) of diethanolamine were added. After activation by microwave irradiations (50 min, 300 W, 80°C), the mixture was dried under vacuum and purified by automated flash chromatography ($CHCl_3$ to $CHCl_3$ / EtOH; 7/3; v/v).

6-((N,N-di-2-hydroxyethyl)amino)purine (1):

White powder (84%, 937 mg, 4.2 mmol); mp = 215°C; Rf 0.53 ($CHCl_3$/EtOH; 1/1; v/v); ^{1}H NMR (DMSO-d_6, δ): 12.93 (s, 1H, NH), 8.17 (s, 1H, H-2), 8.09 (s, 1H, H-8), 4.80 (m, 2H, OH), 4.15 (m, 4H, N-CH_2), 3.68 (m, 4H, -CH_2-O), ^{13}C NMR (DMSO-d_6, δ): 153.6 (C-6), 151.6 (C-2), 151.1 (C-4), 130.0 (C-8), 118.5 (C-5), 59.6 (-CH_2-O), 51.7 (N-CH_2), ESIMS: m/z 224 (MH^+), 246 (MNa^+).

2-amino-6-((N,N-di-2-hydroxyethyl)amino)purine (2):

White powder (67%, 798 mg, 3.35 mmol); mp = 198°C; Rf 0.41 ($CHCl_3$/EtOH; 1/1; v/v); ^{1}H NMR (DMSO-d_6, δ): 7.66 (s, 1H, H-8), 5.63 (s, 2H, NH_2), 4.76 (s, 2H, OH), 3.96 (m, 4H, N-CH_2), 3.64 (m, 4H, -CH_2-O), ^{13}C NMR (DMSO-d_6, δ): 159.4 (C-6), 154.0 (C-4), 153.4 (C-2), 134.7 (C-8), 112.9 (C-5), 59.7 (-CH_2-O), 51.3 (N-CH_2), ESIMS: m/z 239 (MH^+), 261 (MNa^+).

tert-*butyl (6-((N,N-di-2-hydroxyethyl)amino)purin-9-yl)ethanoate (7):*

White powder (80%, 516 mg, 1.5 mmol); mp = 110°C; Rf 0.56 ($CHCl_3$/EtOH; 7/3; v/v); ^{1}H NMR (DMSO-d_6, δ): 8.20 (s, 1H, H-2); 8.12 (s, 1H, H-8); 4.95 (s, 2H, CH_2 acetate); 4.80 (t, 2H, J = 5.2 Hz, OH); 4.27 (m, 2H, N-CH_2); 3.83 (m, 2H, N-CH_2); 3.68 (m, 4H, -CH_2-O); 1.42 (s, 9H, $(CH_3)_3$); ^{13}C NMR (DMSO-d_6, δ): 166.9 (CO); 152.0 (C-4); 151.7 (C-2); 149.0 (C-6); 147.9 (C-8); 130.4 (C-5); 82.0 ($C(CH_3)_3$); 60.0 (-CH_2-O); 53.6 (N-CH_2); 45.5 (CH_2 acetate); 27.6 ($C(CH_3)_3$); ESIMS: m/z 338 (MH^+), 360 (MNa^+).

tert-*butyl (2-amino-6-((N,N-di-2-hydroxyethyl)amino)purin-9-yl) ethanoate (8):*

White powder (70%, 468 mg, 1.3 mmol); mp = 112°C; Rf 0.37 ($CHCl_3$/EtOH; 7/3; v/v); ^{1}H NMR (DMSO-d_6, δ): 7.69 (s, 1H, H-8), 5.81 (s, 2H, NH_2), 4.75 (t, 2H, J = 4.6 Hz, OH), 4.74 (s, 2H, CH_2 acetate), 4.02 (m, 4H, N-CH_2), 3.65 (m, 4H, -CH_2-O), 1.42 (s, 9H, $(CH_3)_3$), ^{13}C NMR (DMSO-d_6, δ): 167.3 (CO), 159.6 (C-6), 154.2 (C-4), 152.9 (C-2), 137.2 (C-8), 112.7 (C-5), 81.8 (C-tert-butyl), 59.6 (-CH_2-O), 51.2 (N-CH_2), 27.7 ($(CH_3)_3$), ESIMS: m/z 353 (MH^+), 375 (MNa^+).

9-(2-chloroethyl)-7,8-dihydroimidazo[2,1-i]purinium chloride (3):

Fifty milligrams (50 mg) of compound **2** (0.23 mmol) were introduced in 2 mL of thionyl chloride. After 6 hours, TLC monitoring showed the completion of the reaction. The white precipitate is filtered off and rinsed with diethyl ether. Product is obtained pure as a white solid (88%, 52 mg). NMR ^{1}H, (DMSO-d_6, δ): 8.78 (s, 1H, H-5), 8.58 (s, 1H, H-2), 6.00 (s, 1H, NH-3), 4.79 (t, 2H, J = 9.5 Hz, H-7), 4.42 (t, 2H, J = 5.9 Hz, H-α), 4.19 (t, 2H, J = 9.5 Hz, H-8), 4.07 (t, 2H, J = 5.9 Hz, H-β). NMR ^{13}C, (DMSO-d_6, δ): 151.1 (C-9b), 149. 7 (C-9a), 144.6 (C-5), 143.9 (C-2), 115.4 (C-3a), 48.3 (C-8), 48.0 (C-α), 47.8 (C-7), 41.1 (C-β). ESIMS: (M^+) *m/z* 224 et 226.

3. Chlorination of Compound 2:

Hundred milligrams (100) mg of compound **3** (0.42 mmol) were introduced in 2 mL of thionyl chloride. After 7 days, the white precipitate was filtered off and rinsed with diethyl ether. The two compounds **4** and **4′** were isolated by preparative TLC (eluent BuOH/AcOH/H_2O; 2/1/1; v/v/v).

5-amino-9-(2-chloroethyl)-7,8-dihydroimidazo[2,1-i]purinium chloride (4):

White powder (63%, 65 mg); mp = 273°C (decomposition); Rf 0.45 (BuOH/AcOH/H_2O; 2/1/1; v/v/v); NMR ^{1}H, (DMSO-d_6, δ): 8.12 (s, 1H, H-2), 7.86 (s, 1H, NH_2), 4,33 (t, 2H, J = 8.6 Hz, H-7), 4.12 (t, 2H, J = 8.6 Hz, H-8), 4.02 (t, 2H, J = 5.2 Hz, H-α), 3.73 (t, 2H, J = 5.2 Hz, H-β). HRMS: calcd for $C_9H_{12}N_6Cl$ (M^+): 239.0807, found: 239.0806.

5-amino-9-(2-hydroxyethyl)-7,8-dihydroimidazo[2,1-i]purinium chloride (4′):

White powder (27%, 26 mg); mp = 269°C; Rf 0.32 (BuOH/AcOH/H_2O; 2/1/1; v/v/v); NMR ^{1}H, (DMSO-d_6, δ): 8.08 (s, 1H, H-2), 7.78 (s, 1H, NH_2), 4.37 (t, 2H, J = 9.2 Hz, H-7), 4.11 (t, 2H, J = 9.2 Hz, H-8), 3.92 (t, 2H, J = 5.6 Hz, H-α), 3.66 (m, 2H, H-β). HRMS: calcd for $C_9H_{13}N_6O$ (M^+): 221.1145, found: 221.1144.

4. General Procedure for Chlorination of Compounds 7 and 8:

Hundred milligrams (100) mg of compound are dissolved in 2 mL of chloroform. Then 0.9 mmol of pyridine (73 μL) and 0.9 mmol of thionyl chloride (66 μL) are injected. After 24 hours of reaction, compounds are obtained by recrystallization of ethanol.

3-(tert-butoxycarbonylmethyl)-9-(2-chloroethyl)-7,8-dihydroimidazo[2,1-i]purinium (9):

White powder (90%, 101 mg, 0,27 mmol); mp = 224°C; Rf 0.51 (BuOH/AcOH/H_2O; 2/1/1; v/v/v); NMR ^{1}H, (DMSO-d_6, δ): 8.88 (s, 1H, H-5), 8.65 (s, 1H, H-2), 5.19 (s, 2H, H-2'), 4.83 (t, 2H, J = 9.5 Hz, H-7), 4.43 (t, 2H, J = 5.8 Hz, H-α), 4.23 (t, 2H, J = 9.5 Hz, H-8), 4.09 (t, 2H, J = 5.8 Hz, H-β), 1.44 (s, 9H, CH_3 tBu). NMR ^{13}C, (DMSO-d_6, δ): 166.1 (C-1'), 150.3 (C-9b), 149.8 (C-9a), 145.9 (C-5), 141.8 (C-2), 115.4 (C-3a), 82.8 ($C_{quat.}$ tBu), 48.5 (C-8), 47.9 (C-α), 47.8 (C-7), 45.5 (C-2'), 41.1 (C-β), 27.6 (CH_3 tBu). HRMS: calcd for $C_{15}H_{21}N_5O_2Cl$ (M^+): 338.1378, found: 338.1379.

5-amino-3-(tert-butoxycarbonylmethyl)-9-(2-chloroethyl)-7,8-dihydroimidazo[2,1-i]purinium (10):

White powder (87%, 95 mg, 0,24 mmol); mp = 236°C; Rf = 0.51 (BuOH/AcOH/H_2O; 2/1/1; v/v/v); NMR ^{1}H, (DMSO-d_6, δ): 8.15 (s, 1H, H-2), 8,02 (s, 2H, NH_2), 4.86 (s, 2H, H-2'), 4.37 (t, 2H, J = 9.4 Hz, H-7), 4.32 (t, 2H, J = 5.6 Hz, H-α), 4.12 (t, 2H, J = 9.4 Hz, H-8), 4.01 (t, 2H, J = 5.6 Hz,

H-β), 1.43 (s, 9H). HRMS: calcd for $C_{15}H_{22}N_6O_2Cl$ (M^+): 353.1487 found: 353.1488.

5. General Procedure for Chlorination of Compounds 9 and 10:

Fifty (50) mg of *tert*butylated compound are dissolved in 1 mL of trifluoroacetic acid (TFA). After two hours, solvent is evaporated and the crude is rinsed with glacial ethanol.

3-(carboxymethyl)-9-(2-chloroethyl)-7,8-dihydroimidazo[2,1-i]purinium chloride (11):

White powder (100%, 41 mg); Rf = 0,36 (BuOH/AcOH/H_2O; 2/1/1; v/v/v); NMR ^1H, (DMSO-d_6, δ): 8.80 (s, 1H, H-5), 8.59 (s, 1H, H-2), 5.15 (s, 2H, H-2'), 4.78 (t, 2H, J = 9.5 Hz, H-7), 4.41 (t, 2H, J = 5,9 Hz, H-α), 4.19 (t, 2H, J = 9.5 Hz, H-8), 4.07 (t, 2H, J = 5.9 Hz, H-β). HRMS: calcd for $C_{11}H_{13}N_5O_2Cl$ (M^+): 282.0752, found: 282.0754.

5-amino-3-(carboxymethyl)-9-(2-chloroethyl)-7,8-dihydroimidazo[2,1-i]purinium chloride (12):

White powder (100%, 43 mg); Rf = 0.29 (BuOH/AcOH/H_2O; 2/1/1; v/v/v); NMR ^1H, (DMSO-d_6, δ): 8.13 (s, 1H, H-2), 7.94 (s, 2H, NH_2), 4.87 (s, 2H, H-2'), 4.33 (t, 2H, J = 8,9 Hz, H-7), 4.32 (t, 2H, J = 6.0 Hz, H-α), 4.12 (t, 2H, J = 8.9 Hz, H-8), 4.01 (t, 2H, J = 6.0 Hz, H-β). HRMS: calcd for $C_{11}H_{14}N_6O_2Cl$ (M^+): 297.0861, found: 297.0864.

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