Process Development of an N-Benzylated Chloropurine at the Kilogram Scale

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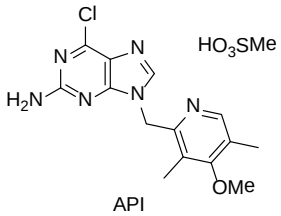
Supporting Information

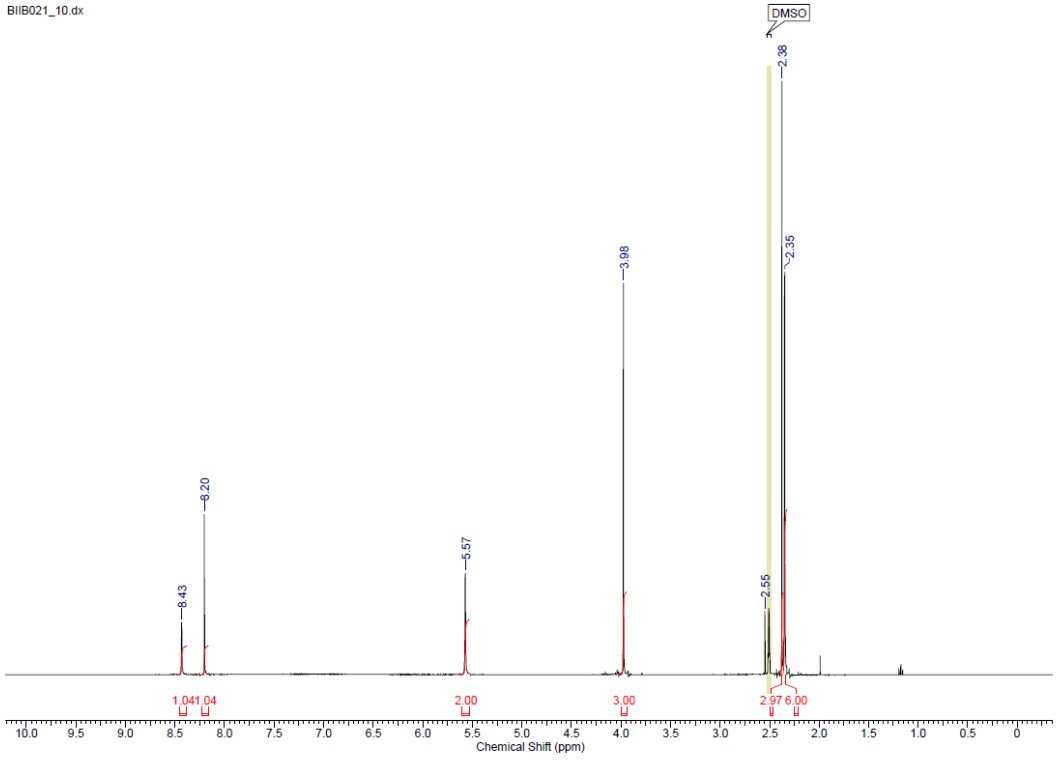
Contents

NMR spectra of the API (methanesulfonic acid salt)

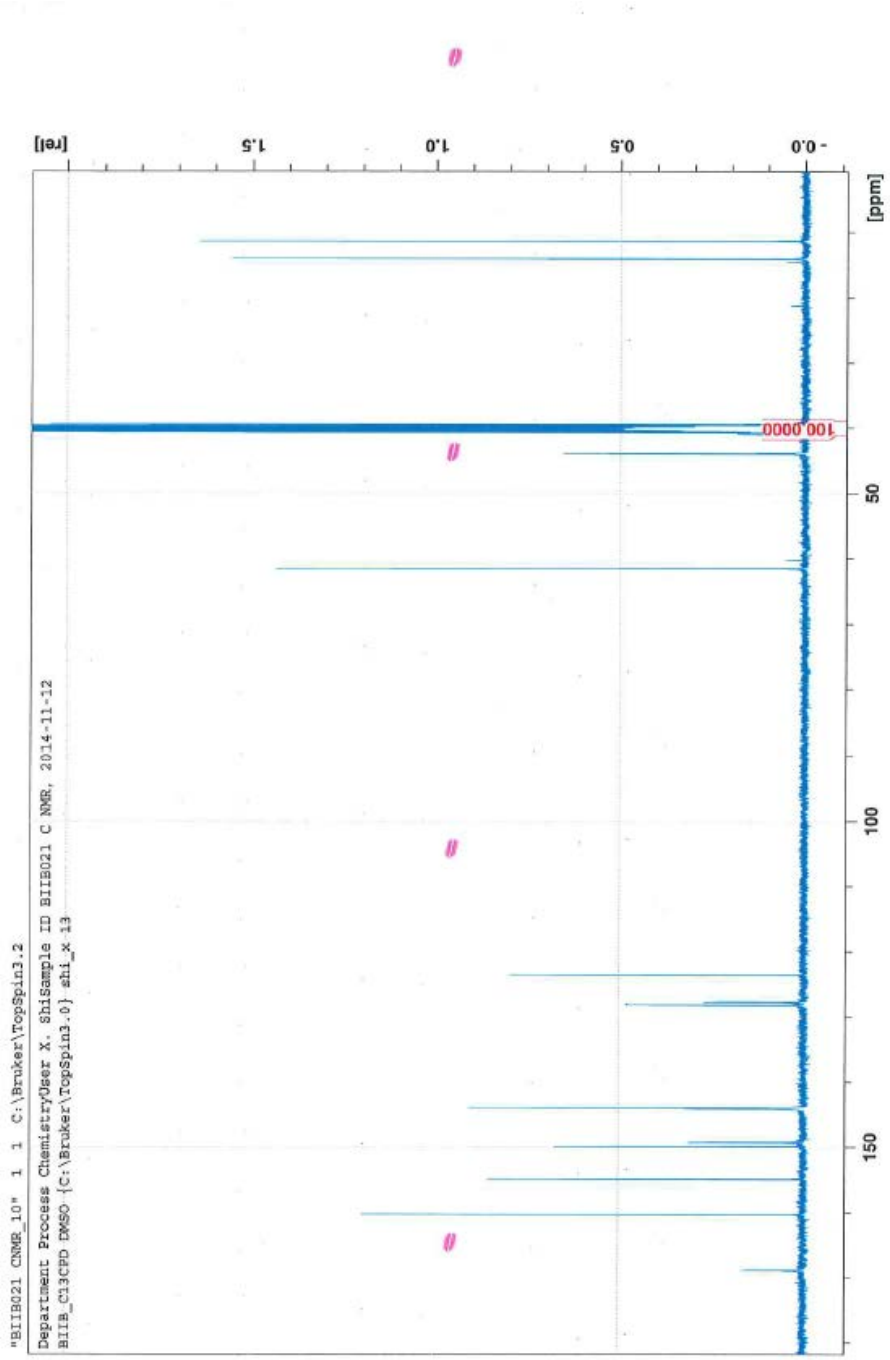


API

1H NMR (400 MHz, DMSO-d6)



^{13}C NMR Spectrum of the API (mesylate salt)



API Recrystallization Data Utilizing Revised Recrystallization Conditions

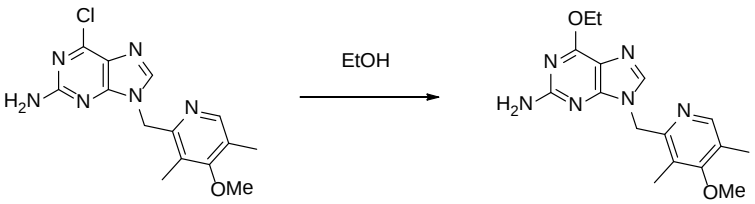
Table 1. Recrystallization Data from Last Pilot Campaign[a]

Product obtained (kg)	Purity (%)	Impurity **10** (%)
44.2	>99.90	< 0.05
51.9	>99.90	< 0.05
46.5	>99.90	< 0.05
5.1[b]	>99.90	< 0.05

[a] average yield 79%. [b] From filter heel.

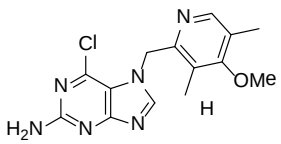
Preparation and Characterization of the Process Related Impurities

This section provides preparation, isolation, and characterization data of the impurities. Some of the impurities were initially identified and then confirmed by comparison with the samples synthesized using the following procedures. Some of them were isolated from the reaction mixtures. NMR spectra were recorded for ^{1}H NMR at 400 MHz, for ^{13}C NMR at 100 MHz, and data were processed using ACDLABSv12 software. Chemical shifts are expressed as δ (ppm) values using the residual signals of the solvents as the internal standard.



API free base Impurity **5**

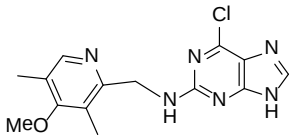
Impurity 5 [6-ethoxy-9-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-9H-purin-2-amine]. A mixture of the API free base (7.5 g, 23.5 mmol), potassium *tert*-butoxide (10.3 g, 91.8 mmol), and ethanol (25 mL) was stirred at rt for 2 days and additional potassium *tert*-butoxide (5.3 g, 47 mmol) was added. The mixture was stirred at rt for 3 h and filtered. The solid was washed with EtOH (150 mL) and dried to give product **5** (5.9 g, 77% yield). ^{1}H-NMR (400 MHz, DMSO-d6) δ 8.05 (s, 1H), 7.76 (s, 1H), 6.29 (s, 2H), 5.28 (s, 2H), 4.43 (t, *J* = 7.2 Hz, 2H), 3.72 (s, 3H), 2.26 (s, 3H), 2.11 (s, 3H), 1.35 (q, J = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, DMSO-d6) δ 163.9, 160.8, 160.3, 155.2, 153.9, 1449.3, 140.9, 125.6, 123.9, 114.1, 62.0, 60.4, 40.6, 15.2, 13.4, 10.9. LC-MS for $C_{16}H_{21}ClN_6O_2$ $(M+1)^+$: 329.



Impurity **6**

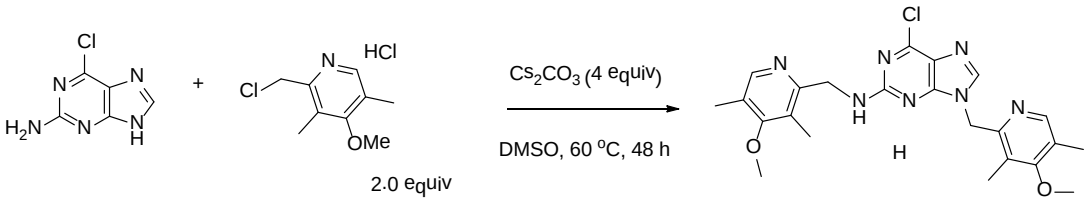
Impurity 6 [6-chloro-7-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-7H-purin-2-amine]. This impurity was isolated from the filter cake of the reaction mixture making the API free base by washing the filter cake with H_2O and DMF to remove inorganic salts and residual API free base. ^{1}H-NMR (400 MHz, DMSO-d6) δ 8.36 (s, 1H), 7.99 (s, 1H), 6.57 (s, 2H), 5.62 (s, 2H), 3.74 (s, 3H), 2.27 (s, 3H), 2.15 (s, 3H). This compound has

very low solubility and attempts to acquire a ^{13}C-NMR spectrum were not successful. HRMS (FT ICR) calcd. for $C_{14}H_{16}ClN_6O$ (M+1) 319.1074, found 319.1072.



Impurity **7**

Impurity 7 [6-chloro-N-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-9H-purin-2-amine]. This compound was isolated from the reaction mixture of the API free base formation using preparative TLC (SiO_2, EtOAc-EtOH (5:1, v/v). ^{1}H-NMR (400 MHz, DMF-d7) δ 8.30 (br, 2H), 8.18 (s, 1H), 7.97 (s, 1H), 5.95 (s, 2H), 3.99 (s, 3H) 2.57 (s, 3H), 2.37 (s, 3H). ^{13}C-NMR (100 MHz, DMF-d7) δ 164.0, 156.6, 156.5, 152.1, 151.1, 149.0, 148.6, 128.4, 125.8, 124.6, 59.9, 48.9, 12.5, 9.9. HRMS (FT ICR) calcd. for $C_{14}H_{16}ClN_6O$ (M+1) 319.1074, found 319.1069.

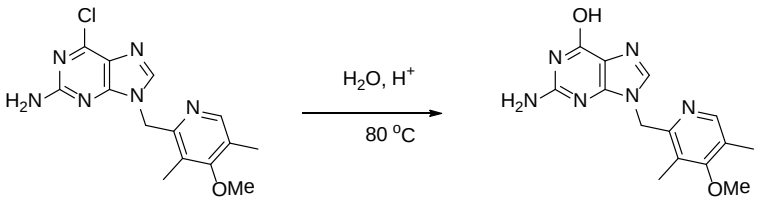


2.0 equiv

Impurity **8**

Impurity 8. [2-(((6-chloro-9-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-9H-purin-2-yl)amino)methyl)-3,5-dimethylpyridin-4-ol]. A mixture of 6-chloro-9H-purin-2-amine ((1.0 g, 3.1 mmol), 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (0.70 g, 3.1 mmol), Cs_2CO_3 (2.0 g, 6.3 mmol), and DMSO (8 mL) was

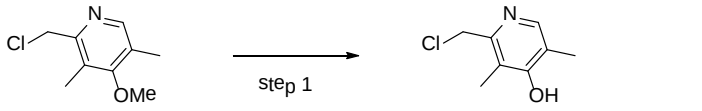
heated at 60 °C and stirred for 48 h. During the time, additional 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (0.70 g, 3.1 mmol) and Cs_2CO_3 (2.0 g, 6.3 mmol) were added. To the reaction mixture was added H_2O and the supernatant was decanted. The residual material was dissolved in MeOH and crystallized at rt to provide crude material, which was dissolved in hot MeOH and crystallized to afford pure **8**. This compound was also isolated from the reaction mixture of API free base formation using preparation TLC (SiO_2, EtOAc-EtOH (5:1, v/v). ^{1}H-NMR (400 MHz, DMF-d7) δ 8.40 (s, 1H), 8.39 (s, 1H), 7.55 (br, 1H), 5.67 (s, 2H), 4.75 (m, 2H), 3.98 (s, 3H), 3.97 (s, 3H), 2.60 (s, 3H), 2.43(s, 3H), 2.41 (s, 3H), 2.37 (s, 3H). ^{13}C-NMR (100 MHz, DMF-d7) δ 164.1, 163.9, 159.0, 155.1, 153.6, 149.4, 148.5, 125.8, 125.1, 123.9, 59.9, 59.8, 45.6, 44.7, 12.7, 9.8, 10.0. HRMS (FT ICR): calcd. for $C_{23}H_{27}ClN_7O_2$ (M+1), 467.1915, found 468.1912.



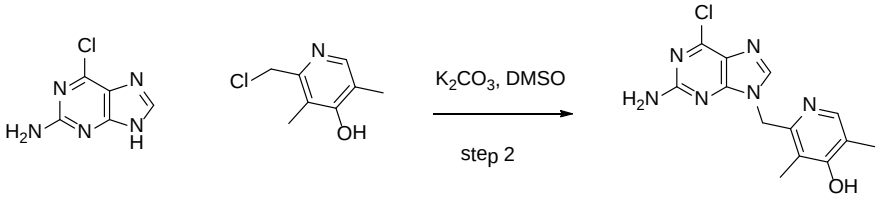
API free base Impurity **10**

Impurity 10 [2-amino-9-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-9H-purin-6-ol]. A mixture of API free base (7.5 g, 23.5 mmol) and hydrogen chloride in water (1.0 M, 300 mL) was heated at 80 °C for 5 h and then cooled and stirred at rt for 1 h. The mixture was neutralized with aqueous NaOH solution to pH ~ 10 resulting in the formation of a white precipitate. The suspension was washed with chloroform (the white precipitate remained suspended in the aqueous layer) and the pH was adjusted to 4 with a few drops of 1M HCl. The white solid was collected by vacuum filtration to yield a solid

(5.1 g), which was purified by dissolving in DMSO (150 mL) followed by precipitation with water. The solid obtained by filtration was triturated with DMSO-H_2O twice and then dried to give the product (3.1 g, 44%). ^{1}H-NMR (400 MHz, DMSO-d6) δ 10.45 (br, 1H), 8.04 (s, 1H), 7.53 (s, 1H), 6.29 (s, 2H), 5.17 (s, 2H), 3.69 (s, 3H), 2.22 (s, 3H), 2.12 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d6) δ 163.9, 157.5, 154.0, 153.9, 152.1, 149.4, 138.6, 125.6, 124.1, 116.8, 60.4, 40.6, 13.4, 10.9. LC-MS for $C_{14}H_{16}N_6O_2$ $(M+1)^+$: 301.



2-(chloromethyl)-3,5-dimethylpyridin-4-ol (**A**)



Impurity **11**

Impurity 11 [2-((2-amino-6-chloro-9H-purin-9-yl)methyl)-3,5-dimethylpyridin-4-ol].

Step 1. Preparation of intermediate 2-(chloromethyl)-3,5-dimethylpyridin-4-ol, **A**. A suspension of 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (20.0 g, 90.0 mmol) in toluene (150 mL) was heated to reflux for 25 h. Water (160 mL) and NH_4OH (20 mL) were added. The mixture was filtered and the solid was washed with water and CH_2Cl_2, dried to provide intermediate **A** (7.0 g, 45% yield).

Step 2. A mixture of 6-chloro-9H-purin-2-amine (5.5 g, 32.4 mmol), 2-(chloromethyl)-3,5-dimethylpyridin-4-ol, **A** (5.1 g, 32.2 mmol), potassium carbonate (7.7 g, 61.5 mmol), and DMSO (150 mL) was stirred at rt for 16 h. Water (600 mL) was added and the solid precipitated was filtered, washed with H_2O (600 mL), MeOH (200

mL) and dried to afford crude impurity **11**. The crude product was purified by dissolving in DMSO and precipitation with H_2O to give **11** as a solid (4.6 g, 48% yield). ^{1}H-NMR (400 MHz, DMSO-d6) δ 10.81 (br, 1 H), 8.07 (s, 1 H), 7.51 (s, 1 H), 6.87 (s, 2H), 5.29 (s, 2H), 2.02 (s, 3H), 1.89 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d6) δ 160.0, 154.9, 150.1, 143.6, 124.1, 121.9, 120.8, 40.6, 13.9, 10.7. LCMS for $C_{13}H_{13}ClN_6O$ $(M+1)^+$: 305.

^{1}H and ^{13}C NMR Spectra of the Impurities