Dimethylpyridin-4-ylamine-Catalysed Alcoholysis of 2-Amino-*N*,*N*,*N*-trimethyl-9*H*-purine-6-ylammonium Chloride: An Effective Route to O^6-Substituted Guanine Derivatives from Alcohols with Poor Nucleophilicity

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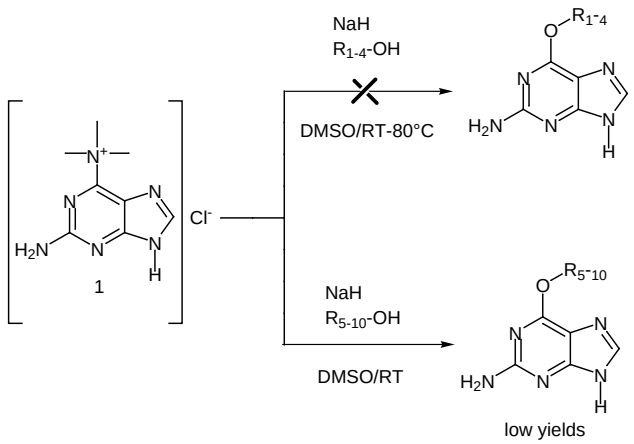
Abstract: Dimethylpyridin-4-ylamine (DMAP)-catalysed reactions of 2-amino-*N*,*N*,*N*-trimethyl-9*H*-purine-6-ylammonium chloride with fluoropyridine methoxides and various other alkoxides in DMSO at 60 °C gave the corresponding coupling products in moderate to good yields between 20–87%. Under these reaction conditions, fluorinated O^6-substituted Guanine derivatives have been synthesized which could not be obtained via known analogous literature procedures. The respective yields of known O^6-substituted guanine derivatives could be significantly improved by using this method. The efficient use of DMAP as an excellent nucleophilic catalyst in the syntheses of O^6-substituted Guanine derivatives has thus been demonstrated.

Key words: catalysis, coupling, fluorine, nucleophilic aromatic substitution, pyridines

The development of new efficient methods for the synthesis of O^6-substituted guanines continues to receive much attention, in part due to the fact that O^6-substituted guanines containing a benzyl or hetarylmethyl moiety are of great medical interest. O^6-Substituted guanines were shown to be important synthetic targets as they effectively inactivate O^6-alkylguanine-DNA alkyltransferase (MGMT).[1]

There are some synthetic methods described in the literature to obtain these compounds. Displacing the halogen with alkoxides from 2-amino-6-chloropurine at 130 °C with the alcohol as a solvent was introduced by Bowels et al.[2] for the synthesis of O^6-benzyl guanine. Annulation of an imidazole ring to 6-(benzyloxy)-2,4,5-triaminopyrimidine at 180 °C was applied by Robins et al.[3] The O^6-substituted guanines so far described[4] have usually been synthesized rather inefficiently by the former method since the preparation of 2,4,5-triaminopyrimidines is very unattractive. However, the method of Bowels et al.[2] is not useful in multistep syntheses if the starting alcohol is either expensive or difficult to prepare. Linn et al. applied DABCO as a catalyst for the alcoholysis reaction of 2-amino-9-benzyl-6-chloro-9*H*-purine mainly with primary and secondary alcohols.[5] The reaction of 2-amino-*N*,*N*,*N*-trimethyl-1*H*-purine-6-ammonium chloride (**1**) with the respective benzyl- and hetarylmethyl alcohols was proved to be the most suitable method by McElhinney et al.[6]

We applied this method to the reaction of several fluoropyridine methanols, which have a decreased nucleophilicity due to their electron-withdrawing N and F atoms. Different hetaryl methanols were synthesized as shown in Table 1. The coupling of these fluorine bearing alcohols with the appropriate guanine precursor **1** did not give any product even under modified conditions like increased temperature and extended reaction time. In addition, some other alkoxides gave only low yields, even when the method of McElhinney et al. was applied (Scheme 1).[6] Therefore, we modified this procedure and investigated DMAP as a nucleophilic catalyst in DMSO at 60 °C.



Scheme 1 McElhinney's method of preparing O^6-substituted guanine derivatives

DMAP is known for its excellent qualities as a catalyst in acylation and methylation reactions[7] and has never been applied before to the syntheses of O^6-substituted Guanine derivatives. There are some indications of pyridinium salts of Guanine and Purine derivatives reported in the literature for the syntheses of valuable intermediates in nucleoside chemistry[8] but not for the syntheses of O^6-(hetarylmethyl) guanines.

The reaction between **1** and several alcohols and the mechanism of DMAP-catalysis suggested are shown in Scheme 2. Under DMAP-catalysis, the desired compounds could be obtained in yields ranging from 20–87%

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within 72 hours in DMSO at 60 °C. The trimethylammonium salt **1** was first synthesized by Kilburis and Lister.[9] The original synthetic route of **1** includes the low temperature condensation of gaseous trimethylamine (TMA) which is problematic owing to its high volatility and subsequent reaction with 2-amino-6-chloropurine. To avoid that critical step and to make the preparation more convenient, we established a synthesis starting from commercially available ethanolic TMA solution (4.2 N) and 2-amino-6-chloropurine.

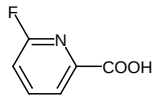
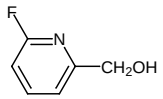
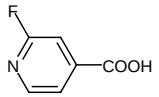
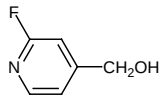
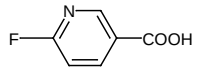
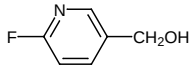
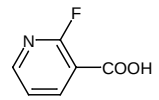
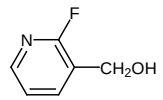
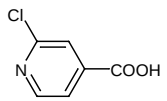
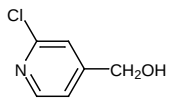
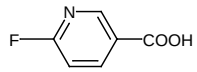
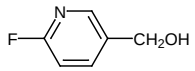
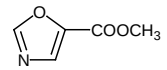
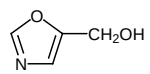
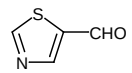
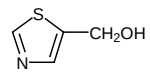
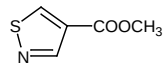
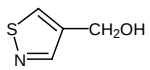
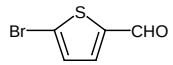
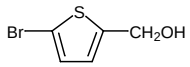
The DMAP-intermediate **2** could be prepared directly by the reaction of 2-amino-6-chloropurine with DMAP (2.5 equiv) in an anhydrous DMF/DMSO mixture at ambient temperature within 72 hours. The reaction was slow and the precipitated product could not be observed until after 48 hours of reaction time. Using the trimethylammonium salt **1** as starting material, however, traces of the unreacted precursor could not be removed in any way (Scheme 2).

Interestingly, the direct approach of the coupling between the isolated DMAP-intermediate **2** and the alcohols in DMSO within 72 hours gave lesser yields than under DMAP catalysis only, probably because of the low solubility of **2** in DMSO. Unfortunately, the purification method for the coupling products described by McElhinney et al.,[6] namely diluting the crude reaction mixture with diethyl ether and subsequent washing of the resulting precipitate with water to obtain the product was not suitable for the O^6-(fluoropyridinylmethyl)guanines because of their good solubility in diethyl ether. In the case of the other compounds, the DMAP-intermediate could not be removed by McElhinney's method either. Therefore, purification via column chromatography was necessary.

Table 2 summarizes the results of the coupling reaction between **1** and the hetaryl methoxides under DMAP-catalysis and by using **2** directly in comparison to known procedures which we carried out separately.

In summary we have demonstrated the catalytic effect of DMAP on the synthesis of some O^6-(hetarylmethyl) guanine derivatives in DMSO at a reaction temperature of 60 °C which could not be synthesized via the literature procedure[6] (in the case of fluorinated compounds) or only gave significantly lower yields. The intermediate **2** was synthesized directly and supports our suggested mechanism of the interaction of DMAP during the coupling reaction of alkoxides and **1**. The pyridinium compound **2** could be reacted with the deprotonated alcohols under the same reaction conditions but gave significantly lower yields of the alcoholysis product within the same reaction time probably due to its low solubility in DMSO. Nevertheless, compound **2** could become a useful tool for the syntheses of O^6-substituted Guanine derivatives. In particular, this reaction provides an improved synthetic pathway for the syntheses of O^6-substituted guanine derivatives especially when alcohols with decreased nucleophilicity are used.

Table 1 Syntheses of Hetaryl Methanols R_{1-10} from Their Corresponding Precursors

Entry	Precursors (%)[a]	Lit.	Yield of Corresponding Alcohols R_{1-10}–OH[b]	Lit.
1		Ref.[10]		-
2		Ref.[11]		Ref.[11]
3		Ref.[12]		Ref.[14]
4		Ref.[11]		Ref.[11]
5		Ref.[13]		Ref.[15]
6		-[c]		Ref.[16]
7		Ref.[17]		Ref.[18]
8		Ref.[19]		Ref.[20]
9		Ref.[21]		-
10		-[c]		Ref.[22]

[a] Carboxylic acids were all synthesized via oxidation of the corresponding methylpyridines with potassium permanganate in water under reflux.
[b] Pyridinemethanols were synthesized according to a published general literature procedure[11] and all compounds gave satisfactory spectroscopic data (^{1}H, ^{13}C and ^{19}F NMR, FD MS).
[c] Commercially available.

Analytical TLC was performed using plates from Merck (Silica gel 60 F_{254}, thickness 0.25 mm). Column chromatography was performed with silica gel (Si-60, Merck). All solvents for column chromatography were p.a. grade. ^{1}H, ^{19}F and ^{13}C NMR spectra were recorded using a DRX 400 spectrometer (Bruker Analytik GmbH). Chemical shifts are quoted in δ (ppm) downfield from TMS as an internal standard. MS spectra were obtained on a MAT90 spectrometer (Finnigan). Elemental analyses were performed with an EL2 system (Elementar vario). Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected.

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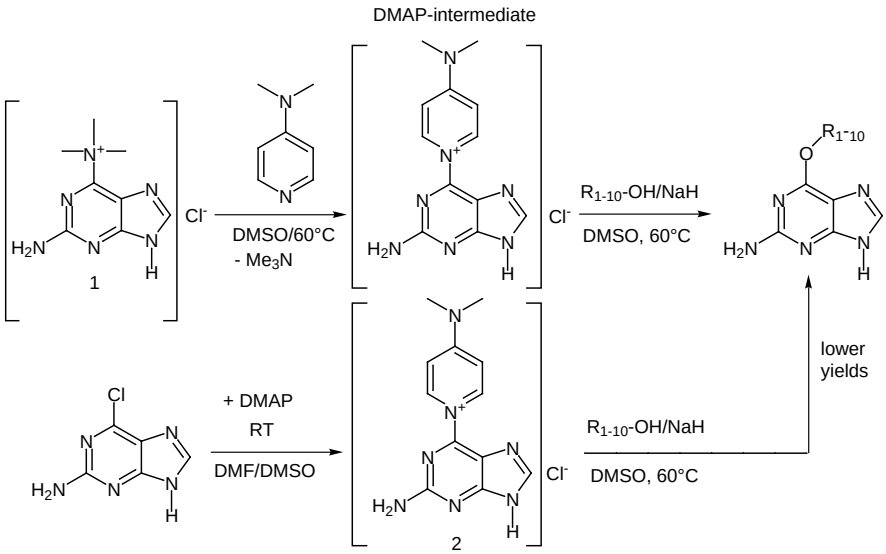

Scheme 2 DMAP (0.3 equiv)-catalyzed reaction of alcohols (5.6 equiv) and **1** (1 equiv) as well as the synthesis of **2** and its direct alcoholysis

2-Amino-*N,N,N*-trimethyl-9*H*-purine-6-ylammonium Chloride (1)

To a stirred solution of 2-amino-6-chloropurine (0.5 g, 2.95 mmol) in anhyd DMSO (10 mL) was added dropwise a 4.2 N ethanolic solution of TMA (0.75 mL, 2.95 mmol) and the solution was stirred at r.t. for 1 h. An additional quantity of TMA (0.75 mL, 2.95 mmol) was added to complete the reaction. The reaction mixture was allowed to stirr for 11 h. Addition of anhyd Et_2O (100 mL) gave a crude precipitate, which was filtered off, and washed with anhyd Et_2O to give the product **1** as a white solid which was dried under vacuum (1 mbar) (0.51 g, 76%); mp 192–194 °C (Lit.[9] mp 191–193 °C).

^{1}H NMR (D_2O): δ = 8.11 (s, 1 H), 3.63 (s, 9 H).

^{13}C NMR (D_2O): δ = 158.86, 157.96, 152.46, 143.36, 115.90, 54.39.

FD-MS: *m/z* (%) = 326 (6), 208 (42), 193 (100), 179 (70).

1-(2-Amino-9*H*-purine-6-yl)-4-dimethylaminopyridinium Chloride (2)

A suspension of 2-amino-6-chloropurine (0.5 g, 2.95 mmol) in DMF (10 mL) was heated to 70 °C under stirring. DMSO was added dropwise (about 1 mL) until a clear solution was obtained. DMAP (0.7 g, 5.91 mmol) was added and the mixture was allowed to cool to r.t. The solution turned yellow and remained clear for 48 h before a yellow solid started to precpitate. After 72 h the crude product was filtered off, washed first with DMSO (10 mL), then with Et_2O (20 mL), and was dried under vacuum (1 mbar) for 24 h to yield **2** as a yellow solid (0.35 g). A second fraction was obtained via dilution of the remaining DMSO filtrate with Et_2O (5 mL). Isolation of the crude product by filtration and workup as described above gave an additional amount of product (0.12 g). The total yield was 49% (0.47 g); mp >350 °C (dec.).

^{1}H NMR (90 °C, D_2O): δ = 9.6 (d, 2 H), 8.7 (s, 1 H), 7.4 (d, 2 H), 3.9 (s, 6 H).

^{1}H NMR (100 °C, DMSO-d_6): δ = 9.6 (d, 2 H), 8.05 (s, 1H), 7.9 (s, 1H), 7.2 (d, 2 H), 6.4 (s, 2 H), 3.4 (s, 6 H).

^{1}H NMR spectra were not well enough resolved to give coupling constants.

^{13}C NMR (90 °C, D_2O): δ = 159.7, 159.2, 157.7, 146.4, 145.5, 138.1, 116.5, 107.8, 40.6.

FD-MS: *m/z* (%) = 256.7 (100), 255.7 (81), 169.5 (24.07), 122.6 (38).

Anal. Calcd for $C_{12}H_{14}ClN_7$ (291.7): C 49.40, H 4.84, N 33.61. Found C 49.21, H 4.62, N 33.72.

6-Fluoropyridin-2-ylmethanol; Typical Procedure[11]

To a suspension of 6-fluoropyridine-2-carboxylic acid[10] (2.1 g, 15 mmol) in benzene (80 mL) was added Et_3N (1.59 g, 15.8 mmol) at r.t. After the solution was clear, ethyl chloroformate (1.71 g, 15.8 mmol) was added. The mixture was stirred for 1 h at r.t. The precipitated triethylammonium chloride was filtered off and the filtrate was evaporated to dryness to give the mixed anhydride. THF (50 mL) was added to the residue and the mixture was added dropwise to a suspension of $LiAlH_4$ (0.6 g, 15.8 mmol) in THF (20 mL) at –78 °C. The resulting mixture was stirred for 30 min at the same temperature, passed into a sat. aq NH_4Cl solution (60 mL) and was extracted with Et_2O (150 mL). The organic phase was dried (Na_2SO_4), evaporated under vacuum, and purified via column chromatography on silica gel (EtOAc–*n*-hexane, 75:25, R_f 0.7). The product was obtained as a white slurry (0.9 g, 47%).

^{1}H NMR (DMSO-d_6): δ = 7.8 (dd, 1 H, *J* = 16.0, 8.0 Hz), 7.35 (m, 1 H), 6.9 (dd, 1 H, *J* = 8.0, 3.0 Hz), 5.4 (s, 1 H), 4.5 (s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 163.64, 161.55, 141.57, 117.92, 107.5, 63.68.

^{19}F NMR (DMSO-d_6): δ = –69.38 (d).

FD-MS: *m/z* (%) = 128.7 (100%).

Anal. Calcd for C_6H_6FNO (127.1): C 56.69, H 4.76, N 11.02. Found: C 56.64, H 4.70, N 11.12.

O^6-(Hetarylmethyl) Guanines from 1 and Alcohols Using DMAP as a Catalyst; General Procedure

To a stirred solution of the heterocyclic alcohol (Tables 1, 14.5 mmol) in DMSO (3–5 mL) was carefully added NaH (0.2 g, 5.7 mmol, 60% w/w on mineral oil) and the stirring was continued for 45 min. 2-Amino-*N,N,N*-trimethyl-9*H*-purine-6-ylammonium chloride (**1**; 0.5 g, 2.6 mmol) and DMAP (104 mg, 0.85 mmol) were added and the mixture was heated to 60 °C for 72 h. HOAc (0.44

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mL) was added, the mixture was adsorbed on silica gel (3 g), dried under vacuum (1×10^{-3} bar), loaded on a silica gel column, and purified via flash chromatography (detailed TLC-conditions and chemical yields are shown in Table 2).

O^6-(Hetarylmethyl) Guanines from 2 and Alcohols; General Procedure

The synthesis was carried out as described above. Instead of **1**, compound **2** (0.66 g, 2.6 mmol) was used directly. Workup was the same as described above (detailed TLC-conditions and chemical yields are shown in Table 2).

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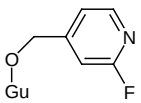
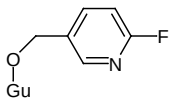
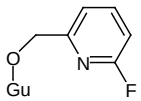
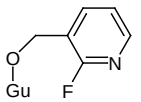
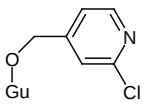
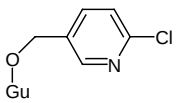
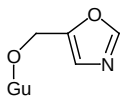
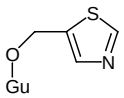
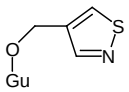
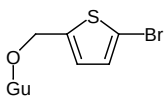
Table 2 Yields of O^6-(Hetarylmethyl) Guanine Derivatives **2** via Different Methods[a]

Entry[b]	Coupling Products Guanine (Gu)	Yield (%)[e]	Yield (%)[f]	Yield (%)[c]	R_f[d]	^{1}H NMR (DMSO-d_6), δ	^{13}C NMR (DMSO-d_6), δ	^{19}F NMR (DMSO-d_6)	FD-MS *m/z* (M^+, 100%)
1		80	40	0	0.31	12.5 (s, 1 H), 8.25 m, 1 H), 7.8 (s, 1 H), 7.4 (m, 1 H), 7.2 (s, 1 H), 6.3 (s, 2 H), 5.5 (s, 2 H)	164.7, 162.3, 159.8, 148.0, 141.0, 138.0, 120.6, 108.0, 107.6, 64.7	–69.18	260.7
2		66	34	0	0.35	12.5 (s, 1 H), 8.4 (m, 1 H), 8.1 (m, 1 H), 7.75 (s, 1 H), 7.1 (dd, 1 H), 6.25 (s, 2 H), 5.4 (s, 2 H)	164.7, 162.3, 159.8, 154.8, 153.7, 148.4, 143.3, 134.7,109.8, 109.4, 63.7	–69.5	260.7
3		60	15	0	0.39	12.5 (s, 1 H), 7.9 (m, 1 H), 7.8 (s, 1 H), 7.4 (d, 1 H), 7.1 (d, 1 H), 6.3 (s, 2 H), 5.4 (s, 2 H)	161.5, 159.8, 159.7, 154.8, 153.7, 143.2, 134.7, 113.6, 109.9, 108.6, 66.4	–68.5	260.7
4		20	10	0	0.33	12.5 (s, 1 H), 8.2 (d, 1 H), 8.17 (m, 1 H), 7.8 (s, 1 H), 7.4 (m, 1 H), 6.35 (s, 2 H), 5.45 (s, 2 H)	162.5, 159.8, 159.6, 155.65, 155.65, 147.5, 142.3, 122.5, 119.2, 118.9, 60.8	–69.3	260.7
5		85	60	10	0.41	-	-	-	-
6		85	62	58	0.40	-	-	-	-
7		72	42	32	0.30	-	-	-	-
8		83	40	40	0.30	-	-	-	-
9		68	35	28	0.34	-	-	-	-
10		87	39	14	0.39	-	-	-	-

[a] All alcoholysis reactions were carried out in DMSO at 60 °C with DMAP as catalyst.
[b] The spectroscopic data (^{1}H, ^{13}C NMR and FD-MS) of all the products listed under entries 5–10 were in accordance with the literature data.
[c] Yields refer to the reactions carried out according to the method of McElhinney et al.[6]
[d] Solvent system: benzene–MeOH (4:1).
[e] Yields via in situ generation of **2**.
[f] Yields via direct use of **2**.

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