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CHEMICAL REACTIVITY OF ALKYLGUANINES. I. METHYLATION OF O^6-METHYLGUANINE DERIVATIVES

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Abstract: Reaction of O^6-methylguanine (**1**) with CH_3I gave O^6,3-dimethylguanine, 3,7-dimethylguanine and 3-methylguanine. In the presence of K_2CO_3, O^6,9-dimethylguanine and imidazole ring-opened products of O^6,7,9-trimethylguanine were produced. Methylations of O^6,9-dimethylguanine and O^6-methyguanosine with CH_3I gave the corresponding 7-methylated derivatives. Reaction of **1** with $(CH_3)_4N^+OH^-$ gave 1,7-, 1,9-, 3,7- and O^6,9-dimethylguanines.

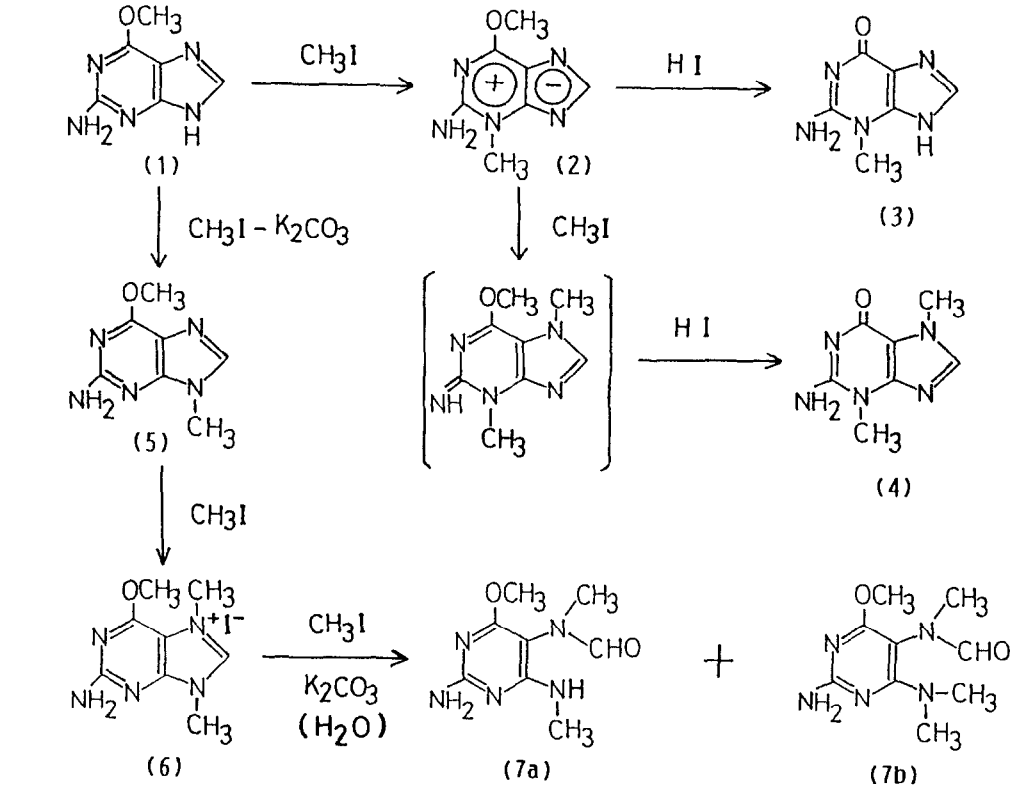
Alkylation of nucleic acid bases has long been a fundamental subject since 1950s, not only in nucleic acid chemistry but also in chemical carcinogenesis and mutagenesis by alkylating agents. The present study brought to light further methylation of O^6-methylguanine (**1**) and its 9-substituted derivatives with iodomethane and tetramethylammonium hydroxide (TMAH). The results revealed that **1** could be a key intermediate for the synthesis of some N-3 and N-9 substituted guanines that could not be prepared by direct alkylation of guanine. Alkylation of alkylated guanines has not been thoroughly studied in contrast to that of alkylated adenines.[1)]

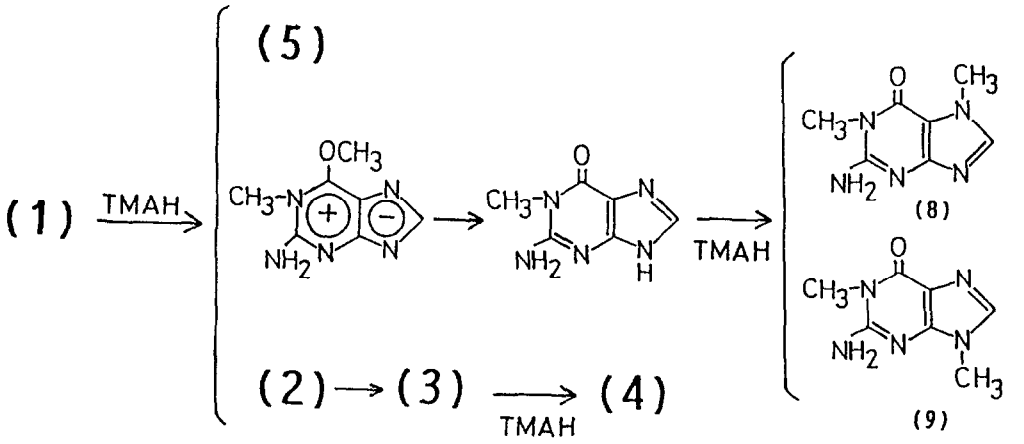
Biologically, O^6-methylguanine residue formed in cellular DNA has been proven to be a key DNA-damage component responsible for the induction of cancer and mutation by methylating agents.[2)] The present results may be extended to the dynamic electronic structure of O^6-methylguanine moiety, which may enable us to find a clue to the molecular mechanism operating in the enzymatic repair of O^6-methylguanine residues in cellular DNA.[3)]

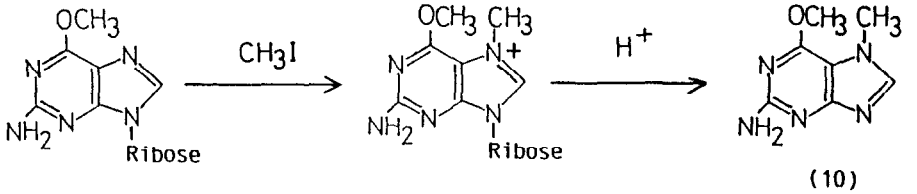
Methylation of O^6-methylguanine

Reaction of O^6-methylguanine (**1**) (82.5 mg, 0.5 mmol) with 2 eq. mol of CH_3I in 15 ml of DMF at 50°C for 17 hr resulted in the formation of O^6,3-dimethylguanine (**2**), 3-methylguanine (**3**) and 3,7-dimethylguanine (**4**) at a ratio of 1 : 1.3 : 1.3, respectively,[4)] in 91% overall yield. The structures of **2**, **3**, and **4** were identified by comparing their NMR, Mass and UV spectra with those of authentic specimens[5)] and by elemental analyses. Treatment of **2** with CH_3I under the same reaction condition as described for **1** gave **4** as the single product, while treatment of **3** with CH_3I did not give **4**. **2** was converted to **3** by acid hydrolysis. The probable reaction pathway is as follows: The attack of CH_3I takes place at the N-3 position of **1** to give **2**. Then, the HI thereby produced reacts with a part of **2** to result in protonation on the O-6 atom, followed by nucleophilic attack of I^- on the O-methyl group to form **3**. Another part of **2** is further methylated to form unstable O^6,3,7-trimethylguanine[6)] which is subsequently converted to stable **4** by the release of a methyl group from the O-6 atom.

The reaction of **1** (82.5mg, 0.5 mmol) with CH_3I (1-5 eq. mol) in the presence of excess


OCH3
NH2
CH3I
HI
(1)
(2)
(3)
CH3I - K2CO3
CH3I
(5)
(4)
CH3I
(6)
K2CO3
(H2O)
(7a)
(7b)
CHO
NH
N-CH3


(1)
TMAH
(5)
(2) → (3) → (4)
TMAH
(8)
(9)


OCH3
CH3I
H+
Ribose
Ribose
(10)

K_2CO_3 (proton accepter) in 15 ml DMF at 50°C for 17 hr was examined. The products were O^6,9-dimethylguanine (**5**), imidazole ring-opened products (**7a,b**) and an unidentified product. The yields of these products varied depending on the concentration of CH_3I; with 3 eq. mol of CH_3I, the imidazole ring-opened products were dominant and **5** was not found. An optimal amount of CH_3I to yield **5** was 1.5 eq. mol, where the yield of **5** was around 60 %. The structure of **5** was supported by NMR, Mass and UV spectroscopies and elemental analysis[7] and further confirmed by leading **5** to 9-methylguanine[8] on heating in 1 N HCl. **7a** and **7b** were presumed to be an alkali-catalyzed decomposition product of O^6,7,9-trimethylguaninium ion (**6**) once produced, and a further methylated product of **7a**, respectively.[9]

The reaction of **1** (0.5 mmol) with TMAH (0.6 mmol) under reduced pressure at 200°C without solvent was examined[10] and four sublimed main products, 1,7-dimethylguanine (**8**),[11] 1,9-dimethylguanine (**9**),[11] 3,7-dimethylguanine (**4**) and 6,9-dimethylguanine (**5**), were obtained in similar ratio. When 1-methylguanine was treated with TMAH under the same condition as described for **1**, **8** and **9** were obtained in the ratio of 3 : 2. Further, when **3** was treated with TMAH, **4** was obtained selectively in around 50 % yield. From these results, the possible pathway of the reaction of **1** with TMAH is speculated as follows: The first methylation proceeds at N-1, N-3 or N-9 position of **1**. O^6,1-Dimethylguanine, once formed, decomposes to 1-methylguanine which is subsequently reacted with TMAH to form **8** and **9**. Similarly, **2**, once formed, decomposes to **3**, and then converted to **4** by further reaction with TMAH.

Methylation of 9-substituted O^6-methylguanines

The reaction of **5** with 1.5 eq. mol of CH_3I in DMF gave **6**, the structure of which was supported by NMR and UV spectroscopies[12] and confirmed by deriving it to 7,9-dimethylguanine by acid-hydrolysis. Treatment of **6** with CH_3I in DMF containing excess K_2CO_3 gave a mixture of **7a** and **7b**.

O^6-Methylguanosine (5 mg) was reacted with 2 eq. mol of CH_3I in 2 ml of DMF at 60°C for 17 hr. The products were separated by HPLC.[13] The main product was collected and heated in 0.1 N HCl for 1 hr. After the chromatographic purification, the product was isolated and proved to be O^6,7-dimethyguanine (**10**) by NMR, UV and Mass spectroscopies.[13]

Discussion

The first methylation by CH_3I took place at the N-3 position of **1** to give **2**, although the N-7 position is most reactive in guanine toward alkylating agents. **2** was further methylated at the N-7 position to form **4** via O^6,3,7-trimethylguanine. The reactivity of the purine-ring of **1** toward CH_3I is the same as that of adenine. The methylated site of 9-substituted O^6-methylguanine derivatives was the N-7 position, which is the same as that of 9-substituted guanines. The basicity of O^6-methylguanosine at N-7 was slightly higher (pKa=2.35) than that of guanosine (1.9). In the presence of a proton accepter, the reaction site of **1** toward CH_3I was the N-9 position, indicating that CH_3I attacks on the N-9 position, but not the sterically hindered N-7 position of the anionic form of **1**.

Methylations of adenine and guanine with TMAH are reported to yield corresonding 9-methyl derivatives.[8] It is worth noting, however, that methylation of **1** with TMAH took place at the N-1, N-3 and N-9 positions. Chemoselectivity might be lost at a high reaction temperature of 200°C.

It is worth emphasizing that **1** would be a key intermediate for synthesis of N-3 and N-9

substituted guanines since the O^6-methyl group of alkylated **1**'s is readily removable by acid hydrolysis (100°C for 1 hr in 1 N HCl). A paper discussing the preparation of some 9-substituted antiviral guanines was recently published.[14] Synthetic trials are in progress in our laboratory to prepare several N-3 or N-9 substituted guanines.

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References and Notes

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2) A. Loveless, Nature, **223**, 206 (1969)

3) M. Olsson and T. Lindahl, J. Biol. Chem., **255**, 10569 (1980). Y. Nakabeppu, H. Kondo, S. Kawabata, S. Iwanaga and M. Sekiguchi, J. Biol. Chem., **260**, 7281 (1985)

4) The yield was determined by ^{1}H NMR spectroscopy (integral values of H-8 and CH_3). Rf values of **2**, **3** and **4** on a silicagel plate (MeOH/$CHCl_3$=2/5) were 0.53, 0.22 and 0.32, respectively. Products were separated by silicagel column chromatography.

5) L.B. Townsend and R.K. Robins, J. Am. Chem. Soc., **84**, 3008 (1962). I.M. Orcharova, L.A. Nikolaeva and E.S. Golovchinskaya, Khim. Farm. Zh., **2**, 18 (1968)

6) K. Yamauchi, T. Tanabe and M. Kinosita, J. Org. Chem., **41**, 3691 (1976). In our reaction condition, O^6,3,7-trimethylguanine was not isolated.

7) **5**; Crystallized from EtOH. mp 195-196°C. ^{1}H NMR (DMSO-d_6): δ 3.57 (s, 3H, NCH_3), 3.93 (s, 3H, OCH_3), 6.36 (broad s, 2H, NH_2), 7.76 (s, 1H, H-8); UV λ_{max}(nm): 242(sh) and 287 (pH 1), 246(sh) and 279 (H_2O and pH 12); MS: m/z=179. Anal. Calcd. for $C_7H_9N_5O$: C, 46.93; H, 5.03; N, 39.11. Found: C, 47.06; H, 5.03; N, 38.88.

8) T.C. Myers and L. Zeleznick, J. Org. Chem., **28**, 2087 (1963)

9) **7a**; ^{1}H NMR (DMSO-d_6): δ 2.74 (d, J=4.6Hz, 3H, $NHCH_3$), 2.83 (s, 3H, NCH_3), 3.73 (s, 3H, OCH_3), 6.23 (broad s, 2H, NH_2), 6.57 (broad q, 1H, NH), 7.74 (s, 1H, CHO). UV λ_{max}(nm): 240(sh) and 282 (pH 1), 240(sh) and 268 (H_2O and pH 12); MS: m/z=211. **7b**; ^{1}H NMR (DMSO-d_6): δ 2.77, 2.81 (s, 3H, $N(CH_3)_2$), 2.85 (s, 3H, NCH_3), 3.77 (s, 3H, OCH_3), 6.64 (broad s, 2H, NH_2), 7.77 (s, 1H, CHO). UV λ_{max}(nm): 230(sh), 240(sh) and 288 (pH 1), 240(sh) and 272 (H_2O and pH 12); MS: m/z=225.

10) The reaction condition reported (see Ref. 8) was employed.

11) W. Pfleiderer, Ann. Chem., **647**, 167 (1961)

12) **6**; Crystallized from EtOH containing 5% H_2O. mp 285-286°C. ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H, NCH_3), 4.00 (s, 3H, N^+CH_3), 4.09 (s, 3H, OCH_3), 7.36 (broad s, 2H, NH_2), 9.32 (s, 1H, H-8); UV λ_{max}(nm): 290 (pH 1 and H_2O), 267 (pH 12). Anal. Calcd. for $C_8H_{12}IN_5OH_2O$: C,28.32; H, 4.13; N, 20.65. Found: C, 28.50; H, 4.05; N, 20.63.

13) HPLC was carried out with a column (JASCO Fine Sil C_{18}, 4.6 x 200 mm) eluted with a gradient of phosphate buffer (33 mM, pH 7.4) - MeOH (10% to 35%). **10**; ^{1}H NMR (D_2O): δ 3.91 (s, 3H, CH_3), 4.07 (s, 3H, CH_3); UV λ_{max}(nm): 235(sh) and 287 (pH 1), 240(sh) and 288 (H_2O and pH 12); Ms: m/z=179. **10** was converted to 7-methylguanine by acid-hydrolysis.

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