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Reaction of O6-methylguanosine with nitrite in the presence of carboxylic acid: synthesis of the purin-2-yl carboxylate

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Abstract—O6-Methylguanosine derivative was treated with sodium nitrite or isoamylnitrite in the presence of carboxylic acid to give the purin-2-yl carboxylate, an unusual product bearing a carboxylic group at the 2-position of the purine moiety.

It has been considered that alkylating agents are the causative chemicals of cancer. Particularly important is the alkylation of guanosine. In 1986, monoclonal antibody-based immunoanalytical methods for detection of carcinogen-modified DNA components was developed and O6-alkyl-2′-deoxyguanosine in DNA was detected in cancer cells.[1] Repair of nucleosides alkylated at the O6-guanine is mediated by an O6-alkylguanine–DNA transferase (AGT) which removes only the alkyl group and inhibition of enzymatic repair of O6-methyl-2′-deoxyguanosine enhances mutagenesis in rat liver epithelial cells.[2] Recently, Ziegel et al. reported that endogenous 5-methylcytosine protects neighboring guanines from N7 and O6-methylation and O6-pyridyloxobutylation by the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.[3] When oligodeoxynucleotides modified site-specifically with O6-methyl-2′-deoxyguanosine (O6medG) were used as templates for DNA synthesis in primer-extension reactions dTMP, accompanied by small amounts of dCMP, was incorporated.[4] These miscodings during DNA synthesis predict the mutagenic potential of O6-alkylation of 2′-deoxyguanosine. Important alkylation agents such as *N*-alkyl-*N*-nitrosourea and *N*-alkyl-*N*-nitrosoamine are formed endogeneously from amine or urea by the action of the nitrite ion.[5] Since nitrates and nitrites have been used as additives for food preservation, the effect of the nitrites on tumors has long been discussed.[6] Also nitric oxide overproduction has been implicated in the pathogenesis of many disorders, including artherosclerosis, neurodegenerative diseases, inflammatory and auto-immune diseases as well as cancers.[7] However, no report has described the reaction of O6-alkylguanosine with nitrites.

We directed our attention to the aromaticity of the O6-alkylguanosines. It is anticipated that O6-alkylation of guanosine increases aromaticity of the purine moiety since the 6-oxo form was abolished. The diazonium ion prepared from O6-alkylguanosine could be stabilized by resonance and receive substitution by nucleophiles leaving N_2. Nair et al. have reported that the reaction of 2-aminopurine nucleosides with the nitrite ester in the presence of di-iodomethane gave 2-iodopurine nucleosides.[8] A nonaqueous diazotization–dediazoniation method which introduces halogen at the 2 position of purine has been further revised by Robins and co-workers.[9] When 2′-deoxyguanosine was treated with HNO_2 in the presence of NaCl, however, 2′-deoxyoxanosine and 2′-deoxyxanthosine were obtained in 13.4% and 68.9% yield, respectively, and the 2-halogenated product was obtained only in 3.3% yield.[10] It is also reported that the diazonium ion from an aromatic compound reacts with carboxylic acid to give an acyloxylated product.[11] This background prompted us to explore the reaction of O6-alkylguanosines with nitrite in the presence of carboxylic acid. In this letter, we describe the synthesis of purin-2-yl carboxylate, a new type of nucleoside derivative.

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O6-Methylguanosine was prepared from 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**1a**)[12] as follows. Compound **1a** was deacylated with ammonia in MeOH to give 2-amino-6-chloropurine riboside (**1b**). However, when **1a** was treated with 1 M NaOH in MeOH, O6-methylguanosine (**2a**) was obtained. Before treatment with the nitrite ion, the sugar-OHs of **2a** were protected to avoid undesired reaction. A trial to introduce an acetyl group using a conventional method gave the tetra-acetate (**3**) as a major product and the desired tri-*O*-acetate (**2b**) was obtained in only 15% yield. It is supposed that the basicity of the 2-NH_2 group of **2a** is increased compared with that of guanosine. This makes it difficult to introduce an acetyl group selectively at the sugar-OH of **2a**. Therefore, **1b** was reacted with *tert*-butyldimethylsilyl (TBS) chloride to afford tri-*O*-TBS derivative **1c** in good yield. Then, **1c** was subjected to the reaction with 1 M NaOH in MeOH under mild conditions to give the O6-methylguanosine derivative (**2c**) (Chart 1).[13]

Then, compound **2c** was subjected to diazotization–substitution in the presence of acetic acid or an amino acid. At first, an aqueous solution of sodium nitrite was added to a solution of **2c** in acetic acid and stirred at room temperature for 30 min. After work-up of the solution, the residue was chromatographed over a column of silica gel to give **4** in 79% yield as a colorless oil. ^{1}H NMR of compound **4** showed a signal attributable to the 2-acetate (2.33 ppm) instead of the 2-NH_2 group. The structure of **4**, combined with data of FAB-MS, UV, and IR spectra, was determined as 6-methoxy-9-[2,3,5-tris-*O*-[(1,1-dimethyl-ethyl)dimethylsilyl]-β-D-ribofuranosyl]purin-2-yl acetate.[14] Since **4** was a new type of nucleoside, the structure of **4** was further confirmed as follows. A solution of **4** in MeOH was treated with aqueous ammonia at room temperature for 30 min to give the O6-methylxanthosine derivative **5**.[15] UV spectra of **5** showed absorption maximum at 285 nm in alkaline solution, almost 20 nm longer compared with that in neutral condition. This result strongly indicates that N1–H of **5** was dissociated in alkaline condition and the enolate ion was formed by resonance. In conclusion, the structure of **4** is further enforced by the formation of **5**. Next, we extended this reaction to benzoic acid. Therefore, **2c** was treated with isoamylnitrite (2.4 equiv) in the presence of benzoic acid (2.1 equiv) in CH_2Cl_2 at room temperature for 1 h. TLC of the solution showed the presence of four compounds. After work-up of the solution and purification by silica gel column chromatography using hexane–AcOEt as eluants, the purin-2-yl benzoate (**6**) was obtained in 38% yield.[16] Also, 6-methoxy-9-[2,3,5-tris-*O*-(1,1-dimethyl-ethyl)dimethylsilyl]-β-D-ribofuranosyl]purine (**7a**)[17] and its 2-chloro congener (**7b**)[18] were obtained in 5% and 24% yields, respectively. Another major by-product was isolated from the last fraction in 32% yield and identified as O6-methylxanthosine **5**. These results prompted us to explore the reaction of **2c** with an amino acid derivative. Thus, compound **2c** was treated with *N*-*t*-Boc-L-valine in a similar manner to that of **6** to afford 2-*tert*-butoxycarbonyl-amino-3-methylbutyrate (**8**) in 49% yield.[19] Undesired products, **5** and **7b** were also obtained in 22% and 19%, respectively. These results indicate that acyloxy group could be introduced onto 2-position of O6-methylguanine nucleosides.

A reaction mechanism to provide purin-2yl carboxylates **4**, **6**, and **8** was considered as follows. To receive substitution similar to that of the Sandmyer reaction, a diazonium ion should be stable. In the case of the diazonium ion (**I**) derived from **2c**, the positive charge delocalizes by resonance as shown in Chart 2, in which structure **II** contributes to stabilizing this ion. The intermediary ion **I** receives nucleophilic attack of carboxylic acid to afford the purin-2-yl derivatives. In the case of **4**, a large excess of nucleophile, and acetic acid, favors the formation of S_N2 product **4**. However, compounds **6** and **8** were prepared by reaction of **I** with only 2.1 equiv of benzoic acid or *N*-*t*-Boc-L-valine in CH_2Cl_2. Under the conditions, two undesired products **7a**,**b** were obtained by radical-substitution of the **I**.[8,9] Formation of another

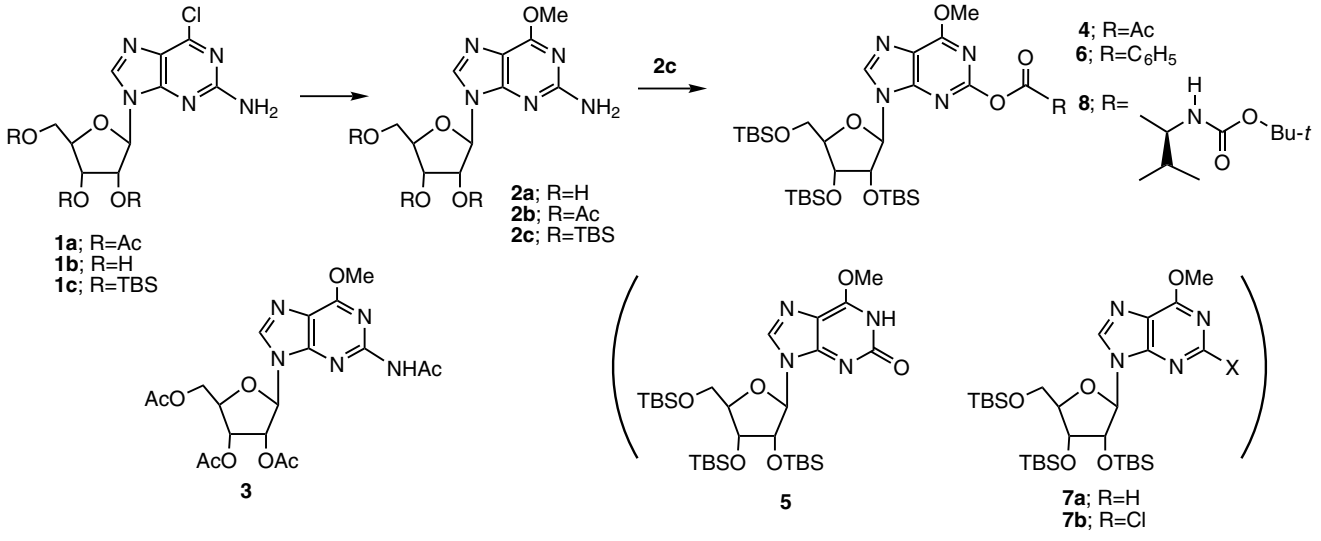

Chart 1.

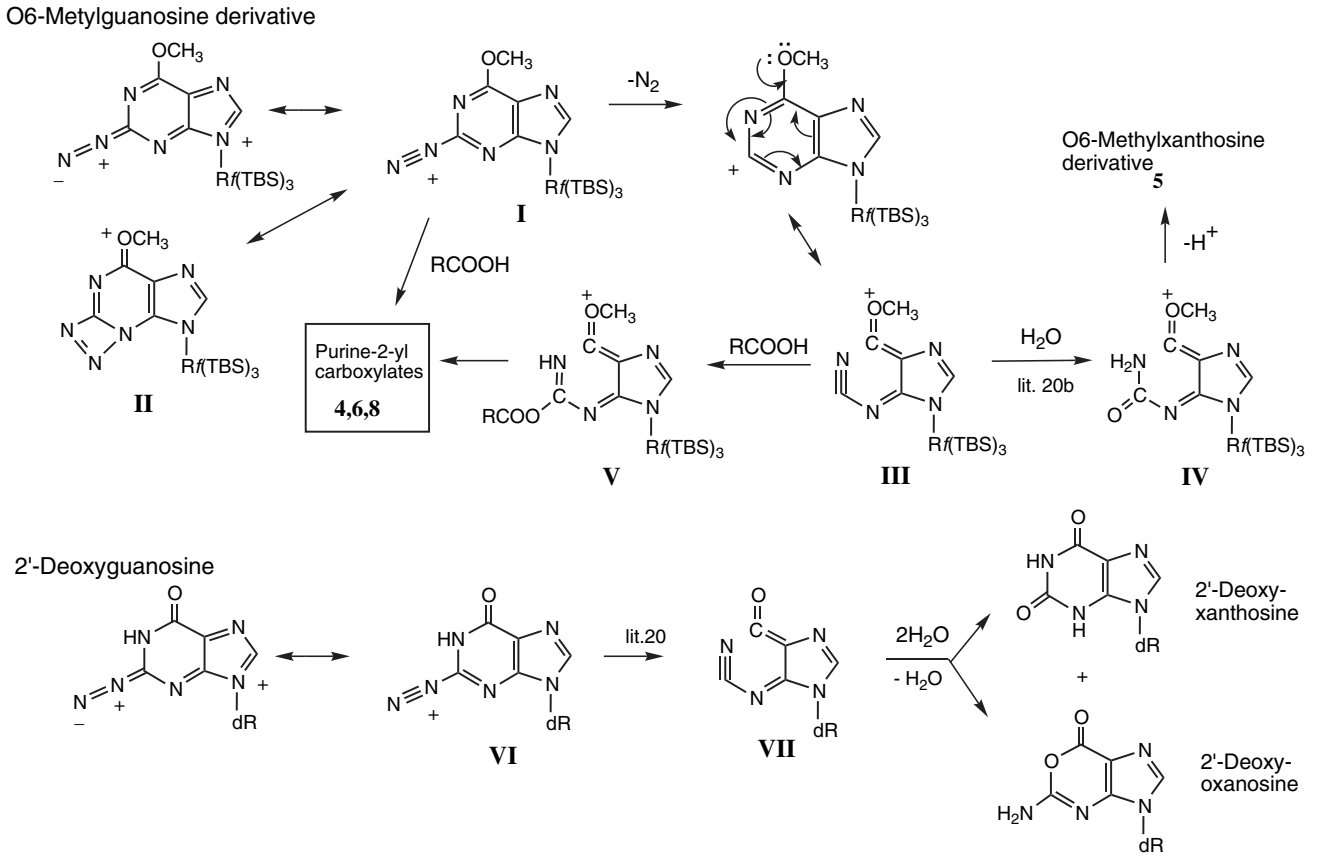

Chart 2.

undesired product **5** is explainable as follows. One possibility is hydrolysis of the purin-2-yl carboxylates **6** and **8**. Recently, it was shown that the deamination of 2′-deoxyguanosine gave 2′-deoxyoxanosine and 2′-deoxyxanthosine[10] and the cyanoimine **VII** has been proposed as the neutral key intermediate.[20] Therefore, it is possible that the cyanoimine intermediate **III** was formed from the diazonium ion **I**. When carboxylic acids were added to **III**, the additive **V** could be formed and subsequent ring closure affords purine-2-yl carboxylates **4**, **6** and **8**. On the other hand, attack of water to cyanoimine **III** affords undesired product **5** via **IV**. Like the reaction of 2′-deoxyguanosine with nitrous acid, however, the oxanosine derivative was not isolated by deamination of **2c** with nitrous acid or nitrite ester, suggesting 1-oxygen of 2′-deoxyoxanosine comes from O6 of 2′-deoxyguanosine.

We are looking forward to explore further reaction for the synthesis of the 6-methoxypurine ribosides bearing amino acids at the 2-position of purine moiety, a new type nucleoside derivative.

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13. Colorless oil (70%): ^{1}H NMR ($CDCl_3$): δ 7.98 (1H, s, 8-CH), 5.91–5.89 (1H, d, $J = 5.0$ Hz, 1′-CH), 4.78 (2H, s, 2-NH_2), 4.06 (3H, s, OMe); UV λ_{max} (MeOH) nm: 282, 249; MS *m*/*z*: 640.7 [M^++H].
14. Colorless oil (79%): ^{1}H NMR ($CDCl_3$): δ 4.15 (3H, s, OMe), 2.33 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 21.0 (2-Ac CH_3); FAB-MS *m*/*z* 683.4 (M^++H); UV λ_{max} (MeOH) nm: 262(sh), 253; FT-IR (cm^{-1}) 2930, 2858, 1780, 1606, 1474.
15. Colorless oil (93%); ^{1}H NMR ($CDCl_3$): δ 9.98 (1H, br s, 1-NH), 7.58 (1H, s, 8-CH), 4.15–4.10 (5H, m, 3′-CH, 4′-CH, OMe); UV λ_{max} (MeOH) nm: 267, 245, 238, λ_{max} (0.02 M NaOH) nm: 285, 248; ESI-MS *m*/*z*: 663.2667 [M^++Na]; FT-IR (cm^{-1}) 3226, 2955, 2931, 2860, 1667, 1630, 1473.
16. Colorless oil (38%): ^{1}H NMR ($CDCl_3$): δ 8.31 (1H, s, 8-CH), 8.23–8.21 (2H, d, $J = 9.6$Hz, Ph 2,6-CH), 7.67–7.63 (1H, t, $J = 8.0$Hz, Ph 4-CH), 7.53–7.50 (2H, t, $J = 7.8$ Hz, Ph 3,5-CH), 4.17 (3H, s, OMe); UV λ_{max} (MeOH) nm: 262(sh), 254(sh), 235; ESI-MS(+) *m*/*z*: 767.2801 (M^++Na); FT-IR (cm^{-1}) 956, 2931, 2859, 1747, 1608, 1473.
17. White crystals (5%); mp 97–98 °C; ^{1}H NMR ($CDCl_3$) δ : 8.52 (1H, s, 2-CH), 8.32 (1H, s, 8-CH), 4.18 (3H, s, OMe); FAB-MS *m*/*z*: 626.2 (M^++H); UV λ_{max} (MeOH) nm: 248. Anal. Calcd for $C_{29}H_{56}N_4O_5Si_3$: C, 55.73; H, 9.03; N, 8.96. Found: C, 55.51; H, 9.28; N, 9.02. These data were identical with that of the sample prepared from 6-chloropurine riboside by two steps.
18. Colorless oil (24%); ^{1}H NMR ($CDCl_3$): δ 8.30 (1H, s); FAB-MS *m*/*z*: 659, 661 (M^++H); UV λ_{max} (MeOH) nm: 257, 266(sh).
19. Colorless oil (49%): ^{1}H NMR ($CDCl_3$): δ 8.29 (1H, s, 8-CH), 5.92–5.91 (1H, d, $J = 4.2$ Hz, 1′-CH), 5.03–5.01 (1H, d, $J = 9.0$ Hz, NH), 4.52–4.51 (1H, m, valine 2-CH), 4.49–4.48 (1H, m, 2′-CH), 4.22–4.19 (1H, t, $J = 4.2$ Hz, 3′-CH), 4.07–4.03 (1H, m, OMe, 4′-CH), 3.95–3.90 (1H, dd, $J = 3.4$, 11.5 Hz, 5′-C*H*H), 3.72–3.68 (1H, dd, $J = 2.2$, 11.6 Hz, 5′-CH*H*), 2.34–2.26 (1H, m, valine 3-CH), 1.38 (9H, s, *t*-Bu), 1.04–0.97 (6H, m, valine 4-$CH_3 \times 2$), 0.87 (9H, s, 2′-TBS *t*-Bu), 0.83 (9H, s, 3′-TBS *t*-Bu), 0.72 (9H, s, 5′-TBS *t*-Bu), 0.06 (3H, s, 2′-TBS CH_3), 0.05 (3H, s, 2′-TBS CH_3), −0.01 (6H, s, 3′-TBS $CH_3 \times 2$), −0.09 (3H, s, 5′-TBS CH_3), −0.12(3H, s, 5′-TBS CH_3); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 170.2 (valine C=O), 162.1 (Boc C=O), 155.5 (4-C), 154.8 (2-C), 152.6 (6-C), 141.6 (8-CH), 120.5 (5-C), 88.5 (1′-CH), 85.0 (4′-CH), 79.9 (2′-CH), 76.2 (Boc C), 71.4 (3′-CH), 62.1 (5′-CH_2), 58.3 (valine CH), 54.7 (OMe), 31.7 (valine CH), 28.3 (Boc CH_3), 26.1, 25.8, 25.7 (*t*-Bu CH_3), 18.9 (valine CH_3), 18.5, 18.0, 17.8 (*t*-Bu C), 17.4 (valine CH_3), −4.39, −4.80, −4.91, −5.37, −5.43 (TBS CH_3); TOF-MS *m*/*z*: 862.4 (M^++Na), 878.5 (M^++K); UV λ_{max} (MeOH) nm: 262(sh), 253.
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