HETEROCYCLES, Vol. 65, No. 8, 2005, pp. 1967 - 1973
Received, 15th April, 2005, Accepted, 27th May, 2005, Published online, 27th May, 2005

A PRACTICAL SYNTHESIS OF 8-HYDROXYACYCLOVIR AND 9-(CARBOXYMETHOXYMETHYL)GUANINE, METABOLITES OF ACYCLOVIR

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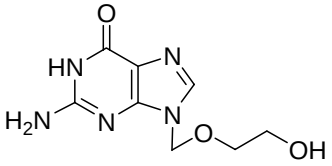
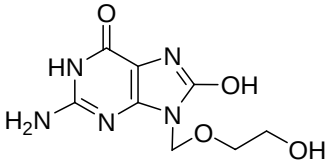
Abstract – Improved methods for the synthesis of 8-hydroxyacyclovir and 9-(carboxymethoxymethyl)guanine, metabolites of acyclovir, were examined. The methods were found to be useful for practical preparation of 8-hydoxyacyclovir and 9-(caboxymethoxymethyl)guanine of high purity. Careful spectroscopic analysis of 8-hydroxyacyclovir in DMSO-d_6 suggested that it may be exist in an 8-oxo tautomer rather than an 8-hydroxy tautomer under the solution conditions.

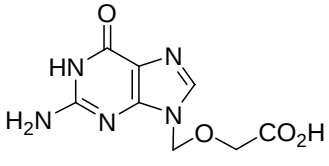
9-(2-Hydroxyethoxymethyl)guanine (**1**) (acyclovir), the acyclic nucleoside analogue of guanosine, is a selective inhibitor of the replication of herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus.[1] Many synthetic and metabolic studies of acyclovir have been reported in the last decade.[2] The metabolic disposition of acyclovir has been investigated in humans and several experimental species including mice, rats and dogs. In these metabolic studies, 8-hydroxy-9-(2-hydroxyethoxymethyl)guanine (8-hydroxyacyclovir) (**2**) and 9-(caboxymethoxymethyl)guanine (**CMMG**) (**3**) have been identified as major metabolites for humans and animals.[3]



acyclovir (1)



8-hydroxy acyclovir (2)



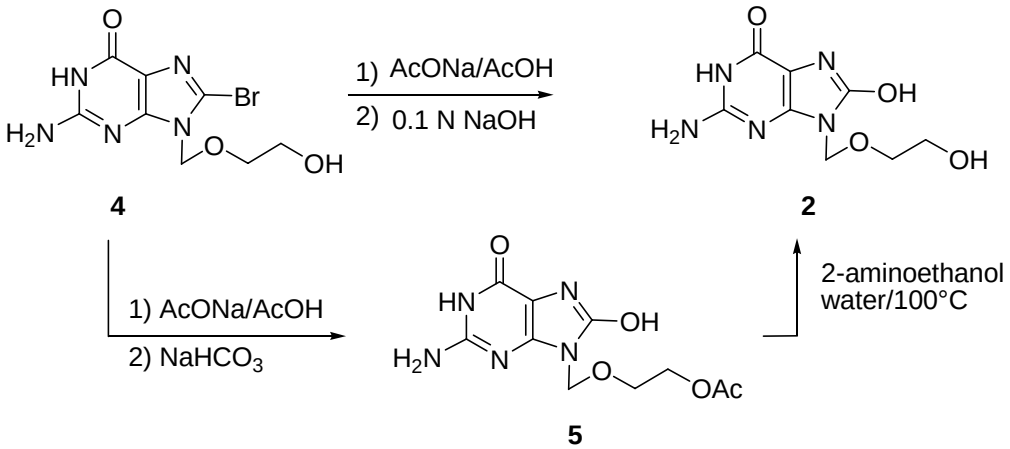
CMMG (3)

In the course of our investigation directed toward developing novel prodrugs of acyclovir, we required a large quantity of 8-hydroxyacyclovir and **CMMG** of high purity for the metabolic studies. Although a method for the synthesis of 8-hydroxyacyclovir (**2**) has been reported in the literature,[4] in our experience, the method could not be applied to large-scale synthesis. The structural analysis of **2** was not studied in detail. Moreover, synthetic methods for **CMMG** were not disclosed in the literature. In this paper we wish to describe experimental details for a practical synthesis of 8-hydroxyacyclovir and **CMMG** with their full physical constants.

SYNTHESIS OF 4-HYDROXYACYCLOVIR

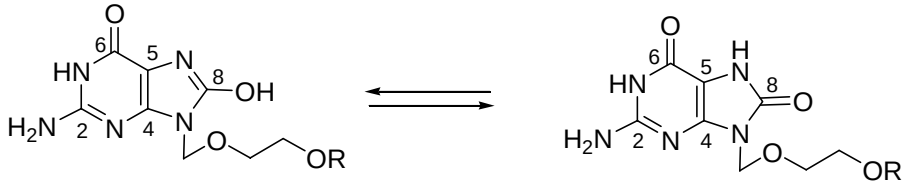
Robins *et al*. reported that 8-hydroxyacyclovir (**2**) could be prepared from 8-bromoacyclovir (**4**) by treatment with sodium acetate in glacial acetic acid, followed by neutralization with 0.1N NaOH.[4] However, our attempted application of the reported procedure for the synthesis in large scale was not successful, due to production of a mixture of **2** and the corresponding acetates in varying ratios, which were not readily separated. Then, we examined a modified procedure for obtaining **2** in large scale (Scheme 1).

Treatment of 8-bromoacyclovir (**4**) with sodium acetate in glacial acetic acid at reflux for 6 h, followed by neutralization with powdered $NaHCO_3$ gave the acetate (**5**) in 75% yield. The results suggest that the initial-formed 8-acetoxyacyclovir is transformed to **5** *via* concomitant migration of the acetyl group to the primary hydroxyl group under the conditions. In this procedure, we readily obtained multi-gram quantities of **5** of high purity after single recrystallization of the crude materials. When **5** was treated with 2-aminoethanol in boiling water for 5 h,[6] hydrolysis of the acetate group cleanly occurred to give the requisite 8-hydroxyacyclovir (**2**), which could be isolated in 96% yield after single recrystallization of the crude materials from 25% aqueous acetic acid. By using the step-wise method *via* the acetate (**5**), we obtained several grams of extremely pure 8-hydroxyacyclovir (**2**) in 72% yield for two steps.



Scheme 1

Although compound (**2**) was believed for a long time albeit without substantiating evidence to be in the 8-hydroxy tautomer, theoretically, **2** would exist in an equilibrium between the 8-hydroxy tautomer and 8-oxo tautomer (***oxo*-2**) (Scheme 2). Structural analysis of 8-hydroxy-2′-deoxyguanosine (**8-OH-dG**) has been reported by Culp and co-worker.[7] They found that chemical shifts of 5-C for **8-OH-dG** in solution of pH 6.0–14 are markedly dependent upon the pH value. The 5-C resonance exhibited a significant downfield chemical shift of *ca.* 10 ppm from 101 ppm with increasing pH, while the remaining base carbons exhibit minimal effects in this pH range. These marked shift in 5-C indicates that increasing basicity of the solution increases the 8-enolate form of **8-OH-dG** in an equilibrium. Then we carefully analyzed the ^{13}C NMR spectrum of **2** to elucidate the tautomeric structures in comparison with that of acyclovir and **CMMG** (Table 1). In the ^{13}C NMR spectrum (DMSO-d_6) of **2**, 5-C and 6-C resonated at δ 98.6 and 153.7, respectively. The corresponding carbons for acyclovir (**1**) resonated at δ 116.5 and 156.9, respectively. Although no significant differences in the chemical shifts for 6-C were observed between **2** and acyclovir, the signal due to 5-C of **2** is significantly shifted upfield by *ca.* 18 ppm as compared with that of acyclovir. The 5-C of **2** resonates at approximately the same chemical shift to that of **8-OH-dG** in a solution of pH 6.0.[7] While precise tautomeric ratios for **2** and ***oxo*-2** remain unclear at this stage, the results delineated in this section strongly suggest that 8-oxo tautomer (***oxo*-2**) would be a presumed major tautomer for compound (**2**) under the solution conditions. In a similar manner, compound (**5**) was also estimated to exist as 8-oxo tautomer (***oxo*-5**). A clear understanding of the pH-dependent tautomeric equilibrium for compounds (**2** and **5**) must await further experimentation.[7]



2 R=H
5 R=Ac

***oxo*-2** R=H
***oxo*-5** R=Ac

Scheme 2

Table 1. Selected ^{13}C NMR spectral data of acyclovir derivatives[a]

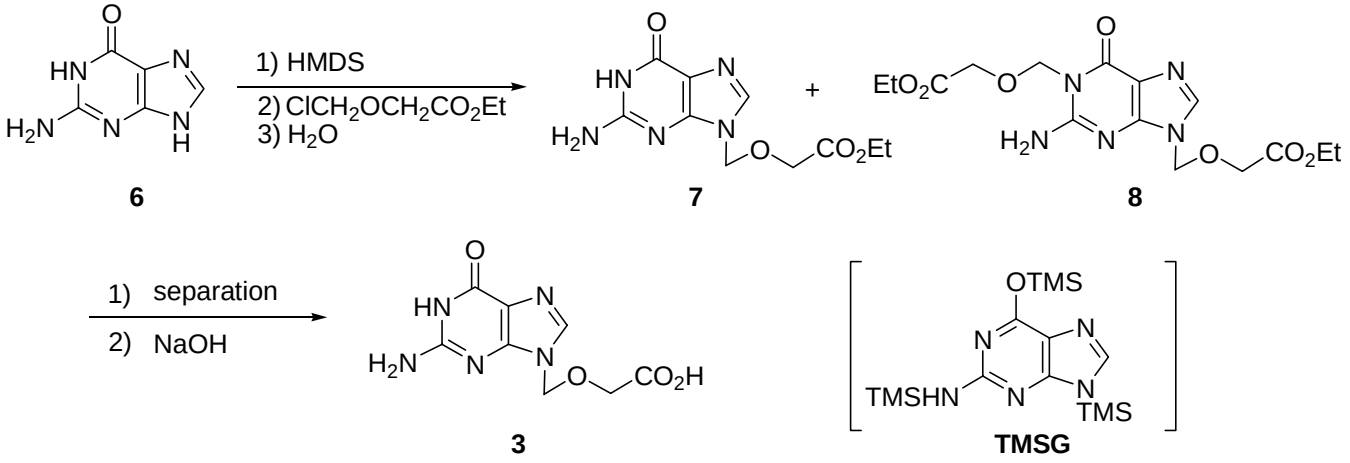
compound	chemical shifts, ppm				
	2-C	4-C	5-C	6-C	8-C
acyclovir (**1**)	153.9	151.5	116.5	156.9	137.9
CMMG	151.1	148.6	113.7	153.9	134.9
8-OH-acyclovir (**2**)	152.4	147.8	98.6	153.7	151.3
8-OH-acyclovir acetate (**5**)	151.0	147.4	98.5	152.1	153.4

[a] All spectra were obtained in DMSO-d_6

SYNTHESIS OF 9-(CABOXYMETHOXYMETHYL)GUANINE (CMMG)

Although **CMMG** was proved to be a major metabolite of acyclovir by Schaeffer *et al*.,[5] Wellcome Research Laboratories, to the best of our knowledge, details of synthetic methods for **CMMG** are not yet disclosed in scientific literature. We, therefore, examined a synthetic route for **CMMG** *via* *N*-glycosylation of guanine with ethyl (chloromethoxy)acetate (Scheme 3). The *N*-glycosylation of silylated guanine (**TMSG**) with ethyl (chloromethoxy)acetate has been reported by Russian chemists to give ethyl ester (**7**) of **CMMG**.[8] However, in our experimentation, the reported procedure was very difficult to isolate **7** of high purity owing to production of a large quantity of inseparable gelatinous products. Then we examined modified conditions for *N*-glycosylation of **TMSG** with ethyl (chloromethoxy)acetate.

Although the Russian chemists used dichloroethane as a solvent for the *N*-glycosylation reaction, we examined several low-boiling solvents rather than dichloroethane to minimize formation of the gelatinous products. We found the gelatinous compounds were minimally formed when **TMSG**, prepared from guanine and hexamethyldisilazane (HMDS) in toluene, was treated with ethyl (chloromethoxy)acetate in toluene at 70ºC for 12 h. The crude mixture was chromatographed on silica gel to give the desired **7** in 14.2% yield. We also isolated 1,9-bis(ethoxycarbonylmethoxymethyl)guanine (**8**) in 12.4% yield as a gelatinous product. Although the yield of **7** was modest, the method was available for synthesis of several grams quantities of **7**. Finally, compound (**7**) was hydrolyzed with aqueous NaOH to give the requisite **CMMG** (**3**) in quantitative yield.



Scheme 3

EXPERIMENTAL

All melting points are uncorrected. The NMR spectra were measured using 300 or 400 MHz spectrometers with DMSO-d_6 as the solvent and $SiMe_4$ as the internal standard. The assignment of ^{13}C

carbon signals is based on DEPT data. IR spectra were recorded as a KBr pellet. Anhydrous toluene was purchased from Kanto Chemical Co., Ltd. and used without further purification. Mass spectra were recorded using electrospray ionization (ESI) techniques.

2-Amino-8-bromo-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (4)

To a stirred suspension of bromine (10.55 g, 66 mmol) in water (300 mL) was added a solution of acyclovir (13.52 g, 60 mmol) in water (2.3 L) at 40–45ºC. The mixture was stirred at rt for 30 min and cooled with ice-cold water. The precipitates were filtered by suction and recrystallized from water to give **4** (15.9 g, 87%) as pale-yellow crystals: mp>300ºC (lit.,[4] mp ~280ºC). ^{1}H NMR (300 MH, DMSO-d_6) δ 3.42-3.51 (m, 4H, 2xCH_2), 4.68 (t, *J*=5.1 Hz, 1H, O*H*), 5.29 (s, 2H, NCH_2O), 6.62 (s, 2H, NH_2), 10.73 (s, 1H, N*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.5 (6-C), 154.2 (2-C), 152.9 (4-C), 120.9 (8-C), 116.6 (5-C), 72.4 (N-*C*H_2-O), 70.8 (O*C*H_2CH$_2$OH), 59.9 (OCH$_2$*C*H_2OH). MS (ESI) *m/z* 304 (MH$^+$). HRMS (ESI) calcd for $C_8H_{11}N_5O_3Br$ (MH$^+$): 304.0045. Found: 304.0045.

2-[(2-Amino-6,8-dioxo-1,6,7,8-tetrahydro-9*H*-purin-9-yl)methoxy]ethyl acetate (5)

A mixture of **4** (12.16 g, 40 mmol) and AcONa (24.61 g, 300 mmol) in AcOH (650 mL) was stirred under reflux for 6 h. The volatile components of the mixture were removed *in vacuo*. The resulting white solids were dissolved in hot water and neutralized by powdered $NaHCO_3$. The solution was cooled by ice water and the resulting precipitates were collected and recrystallized from water to give **5** (7.53 g, 78%) as colorless crystals: mp 268-269ºC (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.96 (s, 3H, CH_3CO), 3.69 (t with small splits, *J*=4.7 Hz, 2H, CH_2), 4.06 (t with small splits, *J*=4.7 Hz, 2H, CH_2), 5.00 (s, 2H, NCH_2O), 6.50 (s, 2H, NH_2), 10.66 (s, 1H, N*H*), 10.69 (s, 1H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 170.1 (C=O), 153.4 (8-C), 152.1 (6-C), 151.0 (2-C), 147.4 (4-C), 98.5 (5-C), 68.6 (N*C*H$_2$O), 66.6 (O-*C*H_2CH$_2$), 62.9 (CH$_2$*C*H_2O), 20.6 (*C*H$_3$). IR (KBr) 1723, 1689, 1647, 1602 cm^{-1}. MS (ESI) *m/z* 284 (MH$^+$). Anal. Calcd for $C_{10}H_{13}N_5O_5$•H_2O: C, 39.86; H, 5.02; N, 23.25. Found: C, 39.88; H, 5.22; N, 23.47.

2-Amino-9-[(2-hydroxyethoxy)methyl]-7,9-dihydro-1*H*-purine-6,8-dione (8-hydroxyacyclovir) (2)

A suspension of **5** (5.5 g, 19.4 mmol) and 2-aminoethanol (2.33 g, 36.6 mmol) in water (85 mL) was heated under reflux for 5 h. The mixture was cooled. The resulting precipitates were collected and recrystallized from 25% aqueous acetic acid (16 mL) to give **2** (4.45 g, 95.5%) as colorless flakes: mp 289-290ºC (decomp) (lit.,[4] mp 260ºC (decomp)). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.43–3.50 (m, 4H, 2xCH_2), 4.61 (t, *J*=5.7 Hz, 1H, O*H*), 4.99 (s, 2H, NCH_2O), 6.49 (s, 2H, NH_2), 10.63 (s, 1H, N*H*), 10.68 (s, 1H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-d_6) 153.7 (6-C), 152.4 (2-C), 151.3 (8-C), 147.8 (4-C), 98.6 (5-C),

70.8 (N-*C*H_2-O), 68.9 (O-*C*H_2-CH_2), 60.1 (*C*H_2-OH). IR (KBr) 1727, 1704, 1645, 1598 cm^{-1}. MS (ESI) *m/z* 242 (MH^+). HRMS (ESI) calcd for $C_8H_{12}N_5O_4$ (MH^+): 242.0889. Found: 242.0901. Anal. Calcd for $C_8H_{11}N_5O_4$•3/4H_2O: C, 37.72: H, 4.95; N, 27.50. Found: C, 37.97; H, 5.10; N, 27.56.

Ethyl (chloromethoxy)acetate

To a stirred suspension of ethyl glycolate (26 g, 250 mmol) and paraformaldehyde (6.76 g, 325 mmol) in toluene (700 mL) was passed a stream of dry hydrogen chloride for 15 min at –10ºC. Then the mixture was treated with Na_2SO_4 (50 g) and stirred at the same temperature for 12 h. The temperature was elevated to 0ºC. After being stirred for an additional 12 h, the solids were filtered. The volatile components of the filtrates were removed *in vacuo* at below 30ºC to leave the title compound (23.5 g, 61.6%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, *J*=7.2 Hz, 3H, C*H*$_3$), 4.20 (q, *J*=7.2 Hz, 2H, OC*H*$_2$$CH_3$), 4.28 (s, 2H, OC*H*$_2$CO-), 5.54 (s, 2H, ClC*H*$_2$O). This liquid was used for the next reaction without further purification.

Ethyl (2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetate (7) and Ethyl ({2-amino-1-[(2-ethoxy-2-oxoethoxy)methyl]-6-oxo-1,6-dihydro-9*H*-purin-9-yl}methoxy)acetate (8).

A mixture of guanine (**6**) (12.86 g, 85 mmol), hexamethyldisilazane (116.5 mL, 550 mmol), and $(NH_4)_2SO_4$ (1.0 g) in toluene (120 mL) was refluxed for 2 days. The mixture was cooled to rt. The resulting precipitates were filtered. The volatile components of filtrates were removed *in vacuo* to leave a yellow oil, which was treated with ethyl (chloromethoxy)acetate (13 g, 85 mmol) in toluene (120 mL) at 70ºC for 20 h. The mixture was neutralized by Et_3N (8.7 g, 86 mmol) and the solvent was removed *in vacuo*. The resulting yellow solid was heated in boiling water (60 mL) for 1 h. After being cooled to rt, the solid materials (21.3 g) were collected and treated with a mixture of EtOH (50 mL) and $CHCl_3$ (200 mL) at reflux. The solid materials were removed by centrifugal separation. The supernatant was concentrated and the residue was chromatographed on silica gel. Elution with $CHCl_3$/EtOH(4:1) gave **8** (4.03 g, 12.4%) as white crystals: mp 191–193ºC (from 70%EtOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.12–1.19 (m, 6H, 2xC*H*$_3$), 4.02–4.13 (m, 4H, 2xOC*H*$_2$$CH_3$), 4.26 (s, 2H, OC*H*$_2$CO), 4.28 (s, 2H, OC*H*$_2$CO), 5.54 (s, 2H, NC*H*$_2$O), 5.71 (s, 2H, NC*H*$_2$O), 7.15 (s, 2H, N*H*$_2$), 8.10 (s. 1H, 8-H). Anal. Calcd for $C_{15}H_{21}N_5O_7$: C, 46.99; H, 5.52; N, 18.27. Found: C, 46.83; H, 5.59; N, 17.70. Successive elution with $CHCl_3$/EtOH (7:3) gave white solids (3.92 g), which was recrystallized from water to give **7** (3.45 g, 14.2%) as colorless crystals: mp 223–224ºC (lit.,[8] mp 211–213ºC). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.14 (t, *J*=7.2 Hz, 3H, C*H*$_3$), 4.05 (q, *J*=7.2 Hz, 2H, OC*H*$_2$$CH_3$), 4.19 (s, 2H, OC*H*$_2$O), 5.39 (s, 2H, NC*H*$_2$O), 6.49 (s, 2H, N*H*$_2$), 7.80 (s, 1H, 8-*H*), 10.61 (s, 1H, N*H*$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4 (*C*OOEt), 156.8 (6-C), 153.9 (2-C), 151.5 (4-C), 137.7 (8-C), 116.5 (5-C), 71.9 (N-*C*H_2-O), 65.9

(O-*C*H_2CO), 60.4 (O*C*$H_2$$CH_3$), 13.9 (*C*$H_3$). IR (KBr) 1764, 1687, 1631, 1575 cm^{-1}. MS (ESI) *m/z* 268 (MH^+). HRMS (ESI) calcd for $C_{10}H_{14}N_5O_4$ (MH^+): 268.1022. Found: 268.1066. Anal. Calcd for $C_{10}H_{13}N_5O_4$•H_2O: C, 42.10; H, 5.30; N, 24.56. Found: C, 42.31; H, 5.49; N, 24.30.

[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]acetic acid (3).

Ethyl ester (**7**) (2.58 g, 9 mmol) was heated for 10 min in boiling water. After being cooled to rt, the mixture was treated with 10% NaOH (9 mL, 22.5 mmol) for 4 h under stirring. The mixture was neutralized with $MeSO_3H$ (2.28 g, 24 mmol). The resulting white solids were washed with water and filtered. The solid was recystallized from 25%AcOH to give **3** (2.13 g, 99%). mp>300ºC. R_f=0.56 ($CHCl_3$/MeOH=3:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.12 (s, 2H, OC*H*$_2$O), 5.38 (s, 2H, NC*H*$_2$O), 6.51 (s, 2H, N*H*$_2$), 7.82 (s, 1H, 8-*H*), 10.61 (s, 1H, N*H*), 12.75 (s, 1H, COO*H*). ^{13}C NMR (75.5 MHz) δ 168.1 (*C*O_2H), 153.9 (4-C), 151.1 (6-C), 148.6 (8-C), 134.9 (2-C), 113.7 (5-C), 69.1 (N*C*H_2O), 62.9 (O*C*H_2CO). IR (KBr) 1727, 1648, 1602 cm^{-1}. MS (ESI) *m/z* 240 (MH^+). HRMS (ESI) calcd for $C_8H_{10}N_5O_5$ (MH^+): 240.0733. Found: 240.0735. Anal. Calcd for $C_8H_9N_5O_4$: C, 40.17; H, 3.79; N, 29.29. Found: C, 40.08; H, 3.92; N, 28.97.

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