[Chem. Pharm. Bull. 34(5)1961—1967(1986)]

Synthesis of 8,2′-Methanoguanosine and 9-(α-D-Arabinofuranosyl)-8,2′-methanoguanine (Nucleosides and Nucleotides. LXVII.[1])

HIROYUKI USUI, AKIRA MATSUDA, and TOHRU UEDA*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan

(Received September 25, 1985)

Guanosine fixed in the high-anti conformation by means of an 8,2′-methylene bridge was prepared. N^2-Acetyl-O^6-ethylguanosine (**2**) was converted to the 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl) derivative (**3**). Oxidation of **3** to the 2′-keto derivative (**4**) and successive coupling with methylenetriphenylphosphorane gave the 2′-methylidene derivative (**5**) and its α-anomer. The 2′-methylidene function of **5** was hydroxylated, and the 2′-hydroxymethyl group was modified to give the phenylthiomethyl derivative (**6**). Photocyclization of **6** followed by deprotection of the sugar and base protecting groups furnished 8,2′-methanoguanosine (**12**). The alpha anomer of **12** was likewise prepared. The circular dichroism spectra of **12**, its α-anomer, and related compounds were measured.

Keywords——8,2′-methanoguanosine; 9-(α-D-arabinofuranosyl)-8,2′-methanoguanine; *C*-cycloguanosine; Wittig reaction; photocyclization; nucleoside conformation; CD; NMR

In our earlier studies on the synthesis of carbon-bridged cycloguanosines, we have synthesized 5′-deoxy-8,5′-cycloguanosine[2] and its 2′, 3′-cyclic phosphate.[3] This nucleotide is useful for investigations on the mode of binding of guanylates to ribonuclease (RNase) T_1 and on the mechanism of cleavage by RNase T_1.[3,4] For further study of the stereochemistry of the enzyme–substrate binding of RNase T_1, it is expected that other *C*-cycloguanosines having different glycosyl torsion angles, such as 8,2′- or 8,3′-cycloguanosine derivatives, would also be useful. This paper deals with the synthesis of 8,2′-methanoguanosine in which the base portion is fixed in a high-anti conformation. Very recently, 2′-deoxy-8,2′-methanoguanosine was prepared by an intramolecular ionic cyclization reaction.[5]

We have recently reported the synthesis of carbon-bridged 8,2′-cycloadenosines by a photocyclization of 2′-phenylthiomethyladenosines.[6] This method was expected to be quite effective for the present purpose. As is well known, the solubility of guanine derivatives is very low and gel formation is quite general during attempts at crystallization, but protection of the base portion by O^6-alkylation is an effective means of increasing the solubility in organic solvents and reducing the reactivity of the N-1 position of the guanine moiety.[7] Therefore, in the present study we used the O^6-ethylated guanosine as a starting material. Treatment of N^2,2′,3′,5′-*O*-tetraacetylguanosine (**1**) with Mitsunobu reagents (triphenylphosphine and diethyl azodicarboxylate)[8] and ethanol afforded, after de-*O*-acetylation, N^2-acetyl-O^6-ethylguanosine[5] (**2**). The 3′- and 5′-hydroxyl groups of **2** were then protected with 1,1,3,3-tetraisopropyl-1,3-dichlorodisiloxane[9] ($TIPDSCl_2$) to give the 3′,5′-*O*-silyl derivative (**3**). Oxidation of **3** with dimethylsulfoxide and oxalyl chloride[10] afforded the O^6-ethyl-2′-ketoguanosine (**4**), which was used for the next step without purification. Treatment of crude **4** with methylenetriphenylphosphorane at 0 °C gave the expected 2′-methylidene derivative (**5**).

Although **5** was homogeneous as checked by chromatography on silica gel, the nuclear magnetic resonance (NMR) spectrum of **5** showed that it was an approximately equimolar

NII-Electronic Library Service

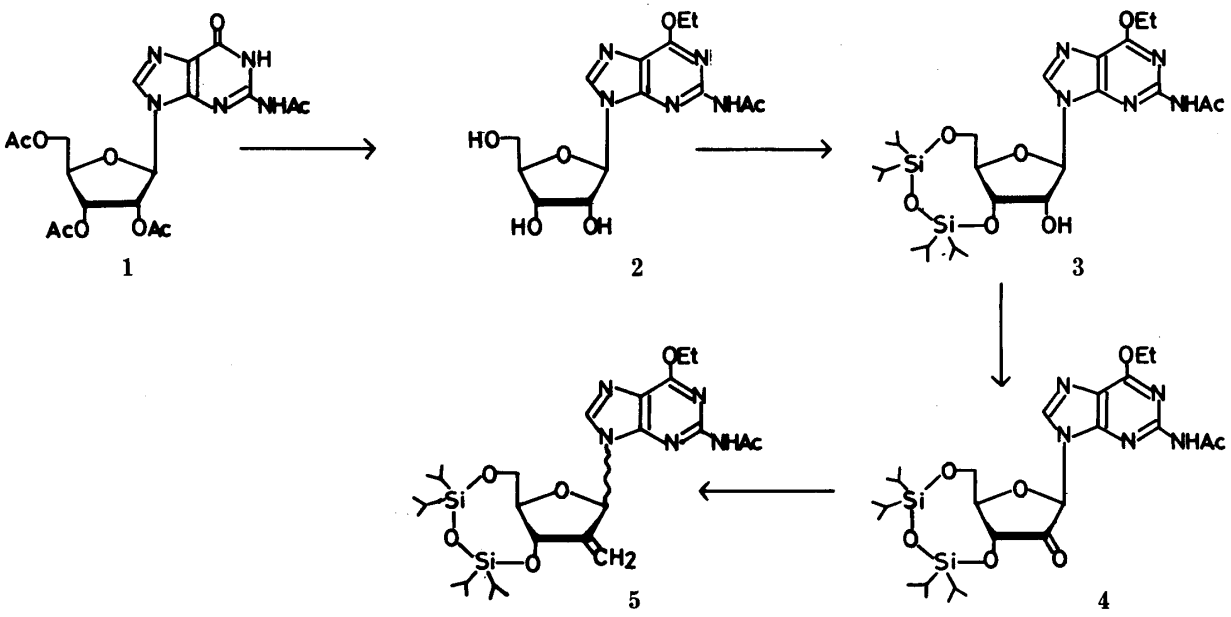

Chart 1

mixture of anomers. Therefore, anomerization of the 2′-ketoguanosine (**4**) under the coupling conditions must have occurred, although such anomerization had not been observed in the Wittig reaction of the 2′-ketoadenosine derivative.[6] Compound **5** was hydroxylated by treatment with osmium tetroxide and *N*-methylmorpholine *N*-oxide, then the 2′-hydroxymethyl compounds were converted to the β-(**6**) and α-anomer (**7**) of 2′-phenylthiomethylguanosine by mesylation and successive substitution with thiophenoxide. Compounds **6** and **7** were separated by silica gel column chromatography in yields of 23 and 21%, respectively. The configurations of the 2′-position of **6** and **7** were assigned as *R* and *S*, respectively, assuming that the hydroxylation occurred from the less hindered side[11] (*trans* to the base orientation) in each case.

Photoirradiation of **6** in acetonitrile in the presence of trimethyl phosphite[1,6] gave the 8,2′-cyclo products (**8** and its *N*-deacetylated derivative, **9**) in 67% yield. The mixture was deacetylated, desilylated, and then re-acetylated to give the di-*O*-acetate (**10**). Treatment of **10** with iodotrimethylsilane resulted in de-*O*-ethylation to give **11**, which was deacetylated by treatment with triethylamine in methanol to furnish 8,2′-methanoguanosine (**12**) in a cystalline form. The NMR spectrum and other instrumental analyses confirmed the structure. In paper electrophoresis in a borate buffer, **12** migrated similarly to guanosine, which supports the presence of a 2′,3′-*cis* diol system in **12**.

The α-anomer **7** was likewise photoirradiated to give a mixture of 8,2′-methano-cyclo derivatives (**13**, R=H or Ac), which was deacetylated, desilylated, and re-acetylated to furnish the O^6-ethyl cycloguanosine (**14**). Deprotection of **14** by treatment with iodotrimethylsilane followed by methanolic triethylamine gave crystalline 9-α-D-arabinofuranosyl-8,2′-methanoguanine (**15**). The structure of **15** was confirmed by comparison of its NMR spectrum with those of the β-anomer **12** and 8,2′-methanoadenosine.[6] The low field shift of one of the bridge-methylene signals and reversal of the chemical shifts of H-3′, and H-4′ are noteworthy in the α-anomer (**15**) as compared to the β-anomers of cycloadenosine and -guanosine (**12**). The slower migration of **15** on paper electrophoresis in borate buffer is also consistent with the *trans* configuration of the 2′,3′-diol system.

The circular dichroism (CD) spectra of **10** and **14** are also informative regarding the anomer relations. Compounds **10** and **14** showed inverted CD spectra with respect to each

NII-Electronic Library Service

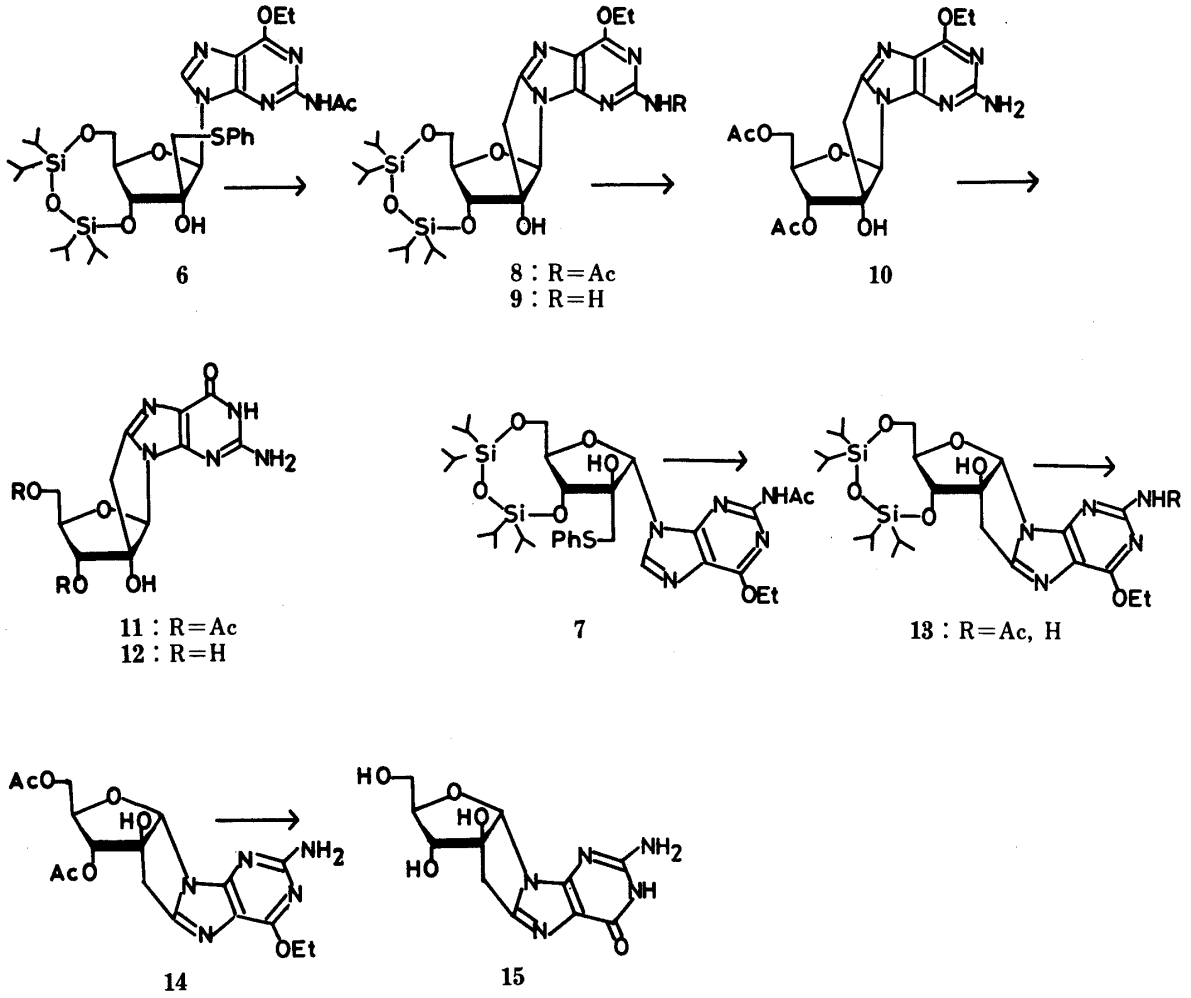

Chart 2

other in methanol (the two spectra are virtual mirror images), wherease the ultraviolet (UV) spectra are closely similar (Fig. 1). In contrast, the CD spectra of **12** and **15** are apparently more complex (Fig. 2). In the case of the β-anomer **12**, the CD band at the 280 nm region (the B_{2u} transition) is positive and that at the 250 nm region (B_{1u} transition) is negative. The CD bands of the α-anomer **15** exhibited positive bands both in the B_{1u} and B_{2u} transitions. On protonation at pH 1.0, the overall pattern of the CD spectrum of **12** remained essentially unchanged, though the molar ellipticity at the B_{1u} region was increased. Compound **15** also showed an increase at the B_{1u} region. In **15**, however, inversion of the sign at the longer wavelength (B_{2u}) region upon protonation was noted.

We have already reported the CD spectra of 5′-deoxy-8,5′-cycloguanosine 2′,3′-cyclic phosphate and 3′-phosphate,[3,4] and pointed out the inversion of the CD band (negative to positive) on N-7 protonation, as in the cases of naturally occurring guanosine and its phosphates. Therefore, it may be concluded that the inversion of the sign of the CD spectrum of guanosine by N-7 protonation is not a general feature of guanosines, but the phenomenon is rather a function of the glycosyl torsion angle as well as the nature of the transition. Therefore, it follows that guanosine having the glycosyl torsion angle fixed at the anti conformation (8,5′-cycloguanosines) shows positive CD bands on N-7 protonation and guanosine with high-anti conformation (**12**) shows a negative band on N-7 protonation. However, since guanosine derivatives generally show the partially separated UV spectra of B_{1u} and B_{2u} transitions, and these two transitions should both contribute to the CD

NII-Electronic Library Service

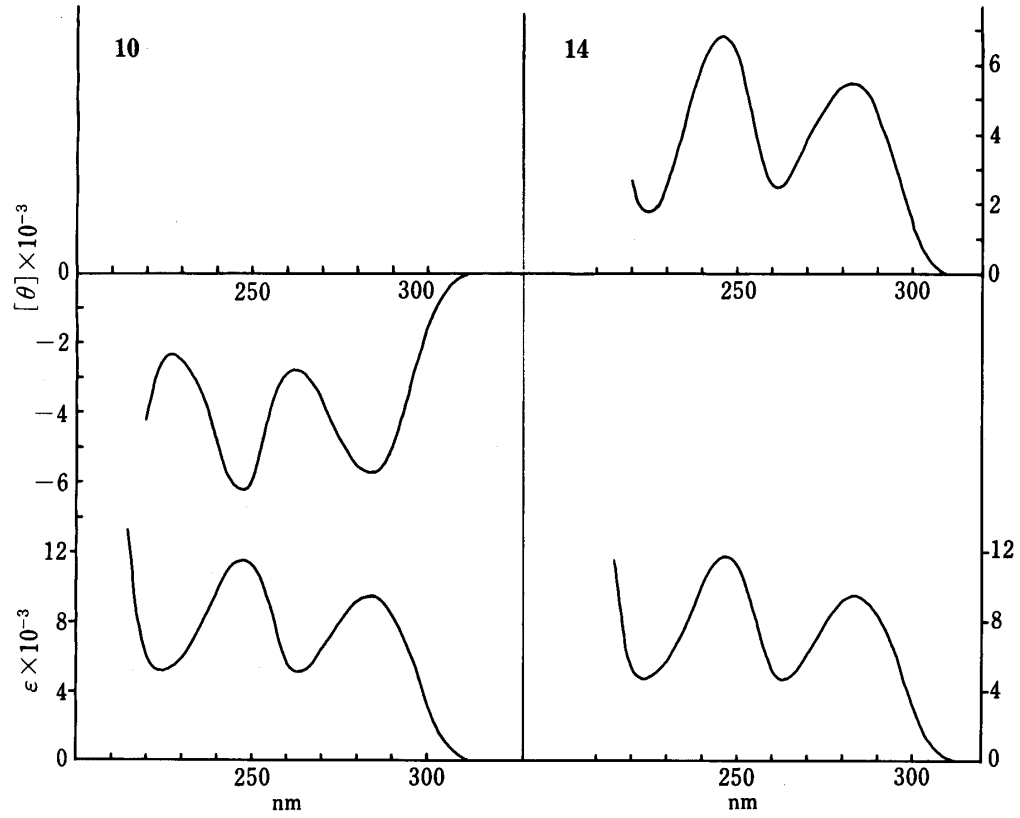

Fig. 1. CD Spectra of 3′,5′-Di-*O*-acetyl-O^6-ethyl-8,2′-methanoguanosine (**10**) and 2-Amino-6-ethoxy-9-(3,5-di-*O*-acetyl-α-D-arabinofuranosyl)purine (**14**) in Methanol

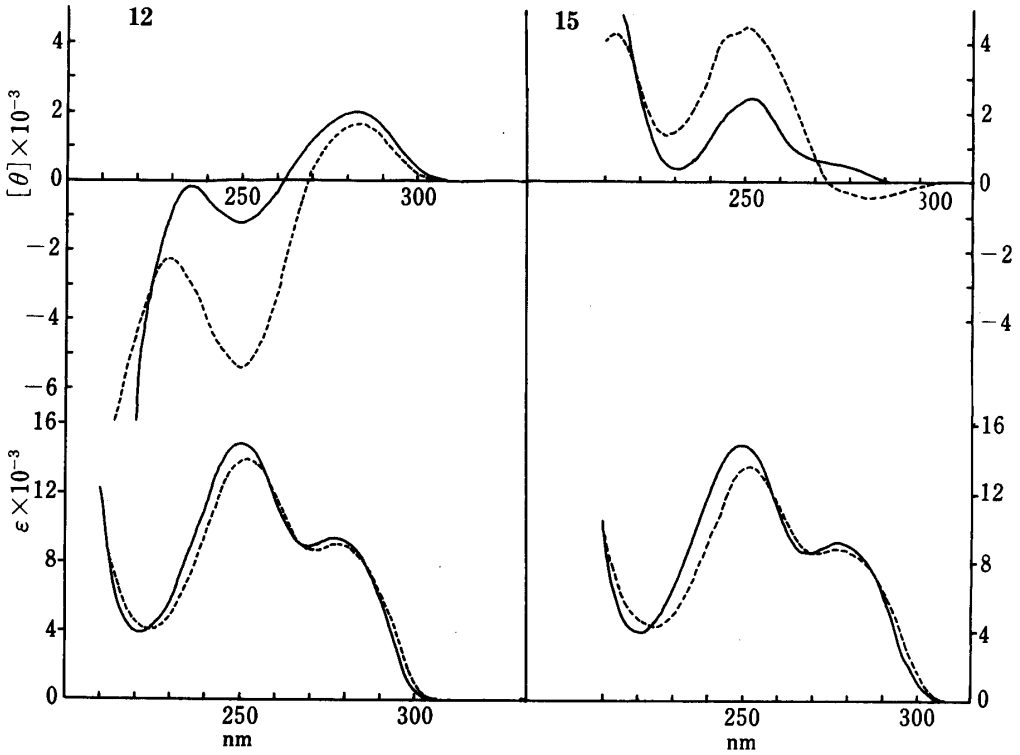

Fig. 2. CD Spectra of 8,2′-Methanoguanosine (**12**) and 9-(α-D-Arabinofuranosyl)-8,2′-methanoguanine (**15**) in Water

—— in H_2O; ------ in 0.1 N HCl.

NII-Electronic Library Service

spectra, further experiments on the synthesis of various *C*-cycloguanosines will be necessary to fully understand the CD spectra in terms of glycosyl torsion angles and transition moments. In fact, 2′-deoxy-8,2′-methanoguanosine was reported to exhibit somewhat different CD and UV spectra from those of **12** or other guanosine derivatives.[5]

Experimental

All melting points were determined on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The UV spectra were measured with a Shimadzu UV-260 spectrophotometer, and NMR spectra were taken on a JEOL JNM-FX 100FT or 200FT spectrometer with tetramethylsilane as an internal standard. Chemical shifts are repoted as δ (ppm) and the signal splitting is described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or b (broad). Mass spectra (MS) were taken on a JEOL D-300 spectrometer, and CD spectra were recorded on a JEOL J-500A spectropolarimeter at room temperature. The photoreaction was carried out in an apparatus fitted with an Eikosha PIL-60 60W low-pressure Hg vapor lamp (quartz filter) in an argon atmosphere. The starting nucleoside, guanosine, was from Yamasa Shoyu Co. Silica gel used for column chromatography or preparative thin layer chromatography (PTLC) was Wako gel C-200.

The abbreviation TIPDS is used for the tetraisopropyldisiloxane-1,3-diyl protecting group in the present experiment.

N^2-Acetyl-O^6-ethylguanosine (2)——This compound was prepared by a method essentially similar to that reported.[5] Triphenylphosphine (9.7 g, 1.2 eq), diethyl azodicarboxylate (5.8 ml, 1.2 eq) and EtOH (2.0 ml, 1.1 eq) were added to a solution of N^2-2′,3′,5′-*O*-tetraacetylguanosine (**1**, 13.9 g, 30.8 mmol) in dioxane (300 ml) and the whole was stirred for 15 min at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in a mixture of MeOH (200 ml) and conc. NH_4OH (200 ml). The mixture was stirred for 3 h at room temperature. The precipitated **2** was collected (3.66 g) by filtration and the filtrate was concentrated. The residue was triturated in $CHCl_3$ (200 ml) to give additional **2** (3.86 g). Further **2** (1.40 g) was obtained by silica gel column (200 g) chromatography of the mother liquor (eluted with 12% MeOH–$CHCl_3$). Total yield was 8.92 g (82%). An aliquot was recrystallized from H_2O to obtain a pure sample for analyses. mp 195—196 °C. UV $\lambda_{max}^{H_2O}$ nm (ε): 260 (16300), 218 (20600). MS *m/z*: 353 (M^+). NMR (DMSO-d_6 + D_2O) δ: 8.43 (1H, s, H-8), 5.89 (1H, d, H-1′, J=5.9 Hz), 4.67—4.46 (3H, m, H-2′, $CH_3C\underline{H}_2O$), 4.18 (1H, dd, H-3′, $J_{2',3'}$=5.1 Hz, $J_{3',4'}$=3.4 Hz), 3.93 (1H, m, H-4′) 3.59 (2H, m, H-5′ab), 2.22 (3H, s, Ac), 1.40 (3H, t, $C\underline{H}_3CH_2O$, J=7.1 Hz). *Anal.* Calcd for $C_{14}H_{19}N_5O_6$: C, 47.59; H, 5.42; N, 19.82. Found: C, 47.55; H, 5.35; N, 19.53.

3′,5′-*O*-TIPDS-N^2-acetyl-O^6-ethylguanosine (3)——A solution of **2** (9.49 g, 26.86 mmol) and $TIPDSCl_2$ (9.3 ml, 1.1 eq) in pyridine (200 ml) was stirred for 3 h at room temperature. A small volume of H_2O was added, the solvent was removed *in vacuo* and the residue was partitioned between EtOAc (200 ml) and H_2O (100 ml). The organic layer was separated and passed through a Whatman 1PS filter paper, then the filtrate was concentrated *in vauco*. The residue was taken up in toluene and a trace of H_2O was removed by co-distillation. The residue was dissolved in $CHCl_3$, and applied to a column of silica gel (300 g). The eluate with 1—2% MeOH–$CHCl_3$ was concentrated to leave **3** (14.02 g, 88%) as a foam. UV λ_{max}^{MeOH} nm 268; λ_{max}^{pH2}: 272, 255. MS *m/z*: 595 (M^+), 552 (M − isoPr). NMR ($CDCl_3$) δ: 8.00 (1H, s, H-8), 7.86 (1H, br s, HN-2), 5.98 (1H, d, H-1′, J=1.7 Hz), 4.69—4.40 (4H, m, H-2′, 3′, $CH_3C\underline{H}_2O$), 4.15—4.10 (3H, m, H-4′, 5′ab), 3.16 (1H, d, HO-2′, J=2.0 Hz), 2.59 (3H, s, Ac), 1.10—0.98 (28H, m, isoPr).

3′,5′-*O*-TIPDS-N^2-acetyl-O^6-ethyl-2′-ketoguanosine (4)——DMSO (4.67 ml, 2.8 eq) in CH_2Cl_2 (20 ml) was added dropwise to a solution of $(COCl)_2$ (2.67 ml, 1.3 eq) in CH_2Cl_2 (100 ml) at −60——−70 °C. After 30 min, **3** (14.0 g, 23.5 mmol) in CH_2Cl_2 (70 ml) was added over a period of 30 min. Et_3N (16.4 ml, 5 eq) was added to the solution after 30 min of stirring, and the whole was then brought to room temperature. The mixture was partitioned between $CHCl_3$ (100 ml) and H_2O (200 ml), the aqueous layer being acidified to pH 4 by addition of 1 N HCl, and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (50 ml), and the combined organic layer was concentrated. The residue was dissolved in $CHCl_3$ and applied to a column of silica gel (300 g). The eluate with 10—20% EtOAc–$CHCl_3$ was concentrated to leave **4** (9.7 g, 69%) as a foam, which was dried over P_2O_5 overnight and used for the next step without further purification.

3′,5′-*O*-TIPDS-N^2-acetyl-O^6-ethyl-2′-deoxy-2′-methylideneguanosine (5)——Compound **4** (9.7 g, 16.3 mmol) in THF (35 ml) was added dropwise to a solution of methylenetriphenylphosphorane[6] (2 eq) in THF and DMSO at 0 °C, and the solution was stirred for 1 h at that temperature. Stirring was continued for a further 2.5 h at room temperature. After neutralization of the mixture by addition of AcOH, the solvent was removed *in vacuo* and the residue was partitioned between EtOAc and H_2O (200 ml each). The organic layer was passed through a Whatman 1PS filter paper and the solvent was evaporated off. The residue was applied to a column of silica gel (200 g). The eluate with 1% EtOH in $CHCl_3$ was concentrated and the residue was subjected to silica gel column chromatography several times. From the concentrate of the eluate, a small amount of **5** and a 1 : 1 mixture of **5** and its α-anomer (as judged from the NMR signal of H-1′) were obtained in a combined yield of 1.47 g (15%). MS *m/z*: 591 (M^+), 576 (M − Me), 548 (M − isoPr). NMR ($CDCl_3$) δ: 7.90 (1H, s, H-8), 7.85 (1H, br s, HN), 6.59 (1H, dd, H-1′), 5.48 (2H, m,

NII-Electronic Library Service

$=CH_2$ at 2′), 5.08 (1H, m, H-3′), 4.59 (2H, q, $CH_3C\underline{H}_2O$), 4.15—4.08 (2H, m, H-5′ab), 3.80 (1H, m, H-4′, $J_{3',4'}=8.8$ Hz), 2.61 (3H, s, Ac), 1.49 (3H, t, $C\underline{H}_3CH_2O$), 1.11—1.07 (28H, m, isoPr). The signal of H-1′ of the α-anomer appeared at 6.70 ppm as a broad triplet. Other signals appeared at the same positions as or overlapped with those of the β-anomer, and were not characterized, since the pure α-anomer could not be isolated.

3′,5′-*O*-TIPDS-N^2-acetyl-O^6-ethyl-2′(*R*)-phenylthiomethylguanosine (6)——A mixture of **5** and its α-anomer (1.14 g, 1.92 mmol) was dissolved in a mixture of THF (9 ml), *tert*-BuOH (9 ml), and H_2O (3 ml). *N*-Methylmorpholine-*N*-oxide (0.26 g, 1 eq) and OsO_4 (0.98 ml of 0.5% *tert*-BuOH solution, 0.01 eq) were added to the solution and the whole was stirred at 4 °C for 3 d. Further *N*-oxide (80 mg) and OsO_4 solution (0.5 ml) were added and the reaction mixture was stirred for an additional 3 d. The mixture was partitioned between EtOAc and 1 N $NaHSO_3$ (30 ml each), and the organic layer was separated, and passed through a Whatman 1 PS filter paper. The filtrate was concentrated. The residue was applied to a column of silica gel (50 g). The eluate with 1—2% MeOH in $CHCl_3$ was concentrated to leave the 2′-(*R* and *S*)-hydroxymethyl derivatives (0.925 g, 77%). The compound was dissolved in pyridine (10 ml) and MsCl (0.17 ml, 1.5 eq) was added to the solution. After 2 h, a small volume of H_2O was added, the solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and sat. $NaHCO_3$ (20 ml each). The orgaic layer was separated and the solvent was removed. The residue was dried by codistillation with toluene, and the residue was dissolved in DMF (10 ml). PhSH (0.152 ml) and KO*tert*-Bu (158 mg) were added and the solution was stirred for 1 h at room temperature. After neutralization of the solution by addition of 1 N HCl, the solvent was removed and the residue was partitioned between EtOAc and H_2O saturated with NaCl. The organic layer was separated, the solvent was removed, and the residue was subjected to silica gel column chromatography several times. Compound **6** (321 mg, 23%) was obtained as a foam from the slower-eluating fractions with 2—4% EtOAc in $CHCl_3$. UV λ_{max}^{MeOH} nm 257. MS *m/z*: 717 (M^+). NMR ($CDCl_3$) δ: 8.10 (1H, s, H-8), 7.81 (1H, br s, HN), 7.17—6.96 (5H, m, SPh), 6.14 (1H, s, H-1′), 4.76 (1H, d, H-3′), 4.53 (2H, q, $CH_3C\underline{H}_2O$), 4.31—4.00 (3H, m, H-4′, 5′ab), 3.40 (1H, s, HO-2′), 3.24 (1H, d, H-6′a, $J_{a,b}=13.2$ Hz), 2.95 (1H, d, H-6′b), 2.58 (3H, s, Ac), 1.14—1.05 (28H, m, isoPr).

2-Acetamido-6-ethoxy-9-[3,5-*O*-TIPDS-2(*S*)-phenylthiomethyl-α-D-arabionofuranosyl]purine (7)——Compound **7** (288 mg, 21%) was obtained by evaporation of the solvent from the above faster-eluting fractions. UV λ_{max}^{MeOH} nm 258. MS *m/z*: 717 (M^+). NMR ($CDCl_3$) δ: 8.21 (1H, s, H-8), 8.02 (1H, br s, HN), 7.15—7.03 (5H, m, SPh), 6.17 (1H, s, H-1′), 4.67—4.28 (5H, m, H-3′, 4′, HO-2′, $CH_3C\underline{H}_2O$), 4.07 (2H, m, H-5′ab), 3.35 (1H, d, H-6′a, $J_{a,b}=13.7$ Hz), 3.20 (1H, d, H-6′b), 2.50 (3H, s, Ac), 1.49 (3H, t, $C\underline{H}_3CH_2O$), 1.13—1.06 (28H, m, isoPr).

3′,5′-*O*-TIPDS-N^2-acetyl-O^6-ethyl-8,2′-methanoguanosine (8) and 3′,5′-*O*-TIPDS-O^6-ethyl-8,2′-methanoguanosine (9)——Compound **6** (380 mg, 0.53 mmol) and trimethyl phosphite (1 ml) were dissolved in acetonitrile (350 ml) and the solution was irradiated for 1.5 h at room temperature. The solvent was removed *in vacuo* and the residue was applied to a column of silica gel (30 g). The eluate with 1—2% EtOH in $CHCl_3$ was concentrated to give **8** and **9** as a 45 : 55 mixture (208 mg, 67%). A portion was separated by PTLC ($CHCl_3$–acetone, 1 : 1) to obtain **8** and **9**.

Physical constants of **8**. UV λ_{max}^{MeOH} nm: 268; λ_{max}^{pH2}: 270.5, 258.1. MS *m/z*: 607 (M^+), 564 (M−isoPr). NMR ($CDCl_3$) δ: 7.93 (1H, br s, HN), 5.94 (1H, s, H-1′), 4.58 (2H, q, $CH_3C\underline{H}_2O$), 4.18—3.68 (4H, m, H-3′, 4′, 5′ab), 3.32 (1H, d, H-6′a, $J=16.2$ Hz), 3.15 (1H, d, H-6′b), 2.58 (3H, s, Ac), 1.49 (3H, t, $C\underline{H}_3CH_2O$), 1.08—0.93 (28H, m, isoPr).

Physical constants of **9**. UV λ_{max}^{MeOH} nm: 283, 248; λ_{max}^{pH2}: 290, 245. MS *m/z*: 565 (M^+), 522 (M−isoPr). NMR ($CDCl_3$) δ: 5.89 (1H, s, H-1′), 4.96 (2H, br s, NH_2), 4.52 (2H, q, $CH_3C\underline{H}_2O$), 4.25—3.68 (4H, m, H-3′, 4′, 5′ab), 3.72 (1H, s, HO-2′), 3.30 (1H, d, H-6′a, $J=17.3$ Hz), 3.09 (1H, d, H-6′b), 1.44 (3H, t, $C\underline{H}_3CH_2O$), 1.08—0.94 (28H, m, isoPr).

3′,5′-Di-*O*-acetyl-O^6-ethyl-8,2′-methanoguanosine (10)——A mixture of **8** and **9** (200 mg, 0.34 mmol) in MeOH saturated with NH_3 (25 ml) was heated at 100 °C for 3 d in a sealed tube. The solvent was removed and the residue was treated with Bu_4NF (0.3 ml of 1 M THF) in THF (5 ml) at room temperature for 1.5 h. The solvent was removed and the residue was dissolved in acetonitrile (5 ml) containing Ac_2O (71 μl), Et_3N (105 μl), and N^4-dimethylaminopyridine (3 mg). The solution was stirred for 15 min, then the solvent was removed and the residue was applied to a column of silica gel (20 g). The eluate with 2—4% MeOH in $CHCl_3$ was concentrated to leave **10** (69 mg, 69%). UV λ_{max}^{MeOH} nm (ε): 284 (9500), 246 (11500); λ_{min}: 264 (5040). CD (MeOH) [θ] (nm): −5760 (284), −2780 (262), −6250 (248), −2330 (227). MS *m/z*: 407 (M^+), 392 (M−Me). NMR ($CDCl_3$) δ: 5.89 (1H, s, H-1′), 5.08 (2H, br s, NH_2), 5.02 (1H, d, H-3′, $J_{3',4'}=6.4$ Hz), 4.60—4.32 (3H, m, H-4′, $CH_3C\underline{H}_2O$), 4.26 (1H, dd, H-5′a, $J_{4',5'a}=4$ Hz, $J_{5'a,b}=12$ Hz), 4.05 (1H, dd, H-5′b, $J_{4',5'b}=4.2$ Hz), 3.62 (1H, d, H-6′a, $J_{6'a,b}=18.1$ Hz), 3.19 (1H, d, H-6′b), 2.20, 1.91 (3H each, s, Ac), 1.44 (3H, t, $C\underline{H}_3CH_2O$).

3′,5′-Di-*O*-acetyl-8,2′-methanoguanosine (11)——Me_3SiCl (113 μl) was added to a solution of NaI (143 mg) in acetonitrile in the dark. After 5 min, a solution of **10** (71 mg) in acetonitrile (2 ml) was added and the whole was stirred at 50 °C for 6 h. H_2O (0.65 ml) and then Et_3N (133 μl) were added under cooling in an ice bath. After being stirred for 5 min, the solution was concentrated and the residue was subjected to PTLC ($CHCl_3$–MeOH, 5 : 1). The appropriate band was extracted with the same solvent to give **11** (41 mg, 62%). Crystallization from H_2O–EtOH gave a pure sample, mp 237 °C (dec). UV $\lambda_{max}^{MeOH-H_2O}$ nm: 277, 250; λ_{max}^{pH11}: 254. MS *m/z*: 379 (M^+). NMR ($CDCl_3$) δ: 10.66 (1H, br s, HN-1), 6.52 (2H, br s, H_2N), 6.40 (1H, s, H-1′), 5.63 (1H, s, HO-2′), 4.94 (1H, s, H-3′, $J_{3',4'}=6.8$ Hz),

NII-Electronic Library Service

4.48—4.11 (2H, m, H-4′, 5′a), 3.98 (1H, dd, H-5′b), 3.40 (1H, d, H-6′a, $J_{6'a,b}$=17 Hz), 2.85 (1H, d, H-6′b), 2.12, 1.89 (3H each, s, Ac).

8,2′-Methanoguanosine (12)——Compound **11** (18 mg) was added to 20% Et_3N–MeOH (3 ml) and the suspension was stirred overnight at 50 °C. The solvent was removed *in vacuo* and the resuidue was crystallized from H_2O–MeOH to give **12** (6.5 mg, 47%), mp >300 °C. UV $\lambda_{max}^{H_2O}$ nm (ε): 277 (9300), 250 (14800); λ_{max}^{pH1}: 278 (8900), 252 (13800); λ_{max}^{pH11}: 270 (11100), 255 (11600), 214 (18100). pK_a value determined from the UV spectra: 2.05. CD (H_2O) [θ] (nm): +2020 (282), 0, (262), −1200 (250). NMR (D_2O) δ: 5.72 (1H, s, H-1′), 4.04 (1H, ddd, H-4′), 3.82 (1H, d, H-3′, $J_{3',4'}$=8.3 Hz), 3.74 (1H, dd, H-5′a, $J_{4',5'a}$=2.7 Hz, $J_{5'a,b}$=12.7 Hz), 3.32 (1H, d, H-5′b, $J_{4',5'b}$=5.4 Hz), 3.19 (1H, d, H-6′a), 2.86 (1H, d, H-6′b, $J_{6',a,b}$=17.1 Hz). Relative migration with respect to guanosine on paper electrophoresis (0.2 M boric acid–sodium borate, pH 7.5, 700 V, 70 min): 1.14. *Anal.* Calcd for $C_{11}H_{13}N_5O_5 \cdot 1/4\ H_2O$: C, 44.08; H, 4.54; N, 23.36. Found: C, 44.08; H, 4.32; N, 23.08.

2-Acetamido-(and 2-amino)-6-ethoxy-8,2′-methano-9-[3,5-*O*-TIPDS-α-D-arabinofuranosyl]purine (13)——Compound **7** (340 mg, 0.474 mmol) and trimethyl phosphite (1 ml) in acetonitrile (300 ml) were irradiated for 1.5 h at room temperature. The solvent was removed *in vacuo* and the residue was applied to a column of silica gel (30 g). The eluate with 3—4% EtOH in $CHCl_3$ was concentrated to leave **13** (R=Ac and R=H, 46:54, 202 mg, 73%). A part of **13** was separated by PTLC as in the case of **8** and **9**. Physical constants of **13** (R=Ac). UV λ_{max}^{MeOH} nm: 273; λ_{max}^{pH2}: 270, 254. MS *m/z*: 607 (M^+), 564 (M−isoPr). NMR ($CDCl_3$) δ: 8.02 (1H, s, HN), 5.92 (1H, s, H-1′), 5.22 (1H, br s, HO-2′), 4.53 (3H, m, H-3′, $CH_3C\underline{H}_2O$), 3.98—3.79 (3H, m, H-5′ab, 6′a), 3.46 (1H, m, H-4′, $J_{3',4'}$=10 Hz), 2.87 (1H, d, H-6′b, $J_{6'a,b}$=19 Hz), 2.51 (3H, s, Ac), 1.49 (3H, t, $C\underline{H}_3CH_2O$), 1.16—1.04 (28H, m, isoPr). Physical constants of **13** (R=H). UV λ_{max}^{MeOH} nm: 284, 247; λ_{max}^{pH2}: 292, 245. MS *m/z*: 565 (M^+), 522 (M−isoPr). NMR ($CDCl_3$) δ: 5.79 (1H, s, H-1′), 5.52 (1H, br s, HO-2′), 5.02 (2H, br s, H_2N), 4.64—4.36 (3H, m, H-3′, $CH_3C\underline{H}_2O$), 3.95 (2H, m, H-5′ab), 3.80 (1H, d, H-6′a, $J_{6'a,b}$=19.0 Hz), 3.50 (1H, m, H-4′, $J_{3',4'}$=9.3 Hz), 2.78 (1H, d, H-6′b), 1.45 (3H, t, $C\underline{H}_3CH_2O$), 1.15—1.03 (28H, m, isoPr).

2-Amino-6-ethoxy-8,2′-methano-9-[3,5-di-*O*-acetyl-α-D-arabinofuranosyl]purine (14)——Compound **13** (173 mg) was converted to **14** (56 mg, 46%) by the method described for the preparation of **10**. UV λ_{max}^{MeOH} nm (ε): 284 (9500), 247 (11700); λmin: 263 (4640), 224 (4640). CD (MeOH) [θ] (nm): +5530 (284), +2460 (262), +6840 (246), +1760 (225). MS *m/z*: 407 (M^+), 392 (M−Me). NMR ($CDCl_3$) δ: 6.09 (1H, s, H-1′), 5.02 (2H, br s, H_2N), 4.95 (1H, d, H-3′), 4.52 (2H, q, $CH_3C\underline{H}_2O$), 4.36—3.95 (3H, m, H-4′, 5′ab), 3.40 (1H, d, H-6′a, $J_{6'a,b}$=18.3 Hz), 2.92 (1H, d, H-6′b), 2.23, 2.11 (3H each, s, Ac), 1.45 (3H, t, $C\underline{H}_3CH_2O$).

9-(α-D-Arabinorufanosyl)-8,2′-methanoguanine (15)——Compound **14** (50 mg) was treated with chlorotrimethylsilane (78 μl) and NaI (101 mg) in acetonitrile (4 ml) for 4 h, then H_2O (0.5 ml) and Et_3N (94 μl) were added to the mixture. The solvent was removed and the residue was subjected to PTLC (*n*-BuOH–H_2O, 86:14). The appropriate band was extracted with 30% MeOH–$CHCl_3$. The solvent was removed *in vacuo* to leave 18 mg of the de-ethyl derivative. This compound was taken up in 20% Et_3N–MeOH (5 ml) and the suspension was refluxed for 3 h. The solvent was removed and the residue was crystallized from aqueous EtOH to give **15** (5.3 mg, 15%), mp 300 °C. UV $\lambda_{max}^{H_2O}$ nm: 278, 249. CD (H_2O) [θ], assuming ε_{249} as 14800 (nm): +2440 (252). FD-MS *m/z*: 295 (M^+). NMR (D_2O) δ: 5.78 (1H, s, H-1′), 4.05 (1H, d, H-3′, $J_{3',4'}$=9.3 Hz), 3.70—3.49 (3H, m, H-4′, 5′ab), 3.52 (1H, d, H-6′a), 2.71 (1H, d, H-6′b, $J_{6'a,b}$=18.6 Hz). Relative migration with respect to guanosine on paper electrophoresis (0.2 M boric acid–sodium borate, pH 7.5, 700 V, 70 min): 0.34.

Acknowledgements We are grateful to the staff of the Center for Instrumental Analysis, Hokkaido University, for the instrumental analyses. This work was supported, in part, by a Grant-in-Aid for Scientific Research (No. 59470117) from the Ministry of Education, Science and Culture, Japan.

References

1) This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University in March 1986: Part LXVI: A. Matsuda and T. Ueda, *Chem. Pharm. Bull.*, **34**, 1573 (1986).
2) A. Matsuda, M. Tezuka, K. Niizuma, E. Sugiyama, and T. Ueda, *Tetrahedron*, **34**, 2633 (1978).
3) T. Ueda, A. Matsuda, T. Asano, and H. Inoue, "Chemistry and Biology of Nucleosides and Nucleotides," R. E. Harmon, R. K. Robins, and L. B. Townsend, Eds., Academic Press, New York, 1978, p. 397.
4) A. Matsuda and T. Ueda, *Nippon Kagaku Kaishi*, **1981**, 845.
5) A. Matsuda, K. Watanabe, T. Miyasaka, and T. Ueda, *Nucleic Acids Res. Symposium Series*, **15**, 57 (1984).
6) H. Usui and T. Ueda, *Chem. Pharm. Bull.*, **34**, 15 (1986).
7) F. Himmelsbach, B. F. Schulz, T. Trichtinger, R. Charubala, and W. Pfleiderer, *Tetrahedron*, **40**, 59 (1984).
8) O. Mitsunobu, *Synthesis*, **1981**, 1.
9) J. T. Markiewicz, *J. Chem. Res.* (*M*), **1979**, 181.
10) *a*) A. J. Mancuso, S.-L. Huang, and D. Swern, *J. Org. Chem.*, **43**, 2480 (1978); *b*) K. Omura and D. Swern, *Tetrahedron*, **34**, 1651 (1978).
11) T. Sano, S. Shuto, H. Inoue, and T. Ueda, *Chem. Pharm. Bull.*, **33**, 3617 (1985).

NII-Electronic Library Service