A Convenient Method for the Synthesis of N^2,N^2-Dimethylguanosine by Reductive C–S Bond Cleavage with Tributyltin Hydride

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A new method for the N-methylation of guanosine is described. The 1,3-benzodithiol-2-yl group was introduced into the 2-amino group of 2′,3′,5′-tri-*O*-acetylguanosine (3) and then converted into a methyl group by reductive C–S bond cleavage with tributyltin hydride. This method was also applied to the synthesis of N^2,N^2-dimethylguanosine. A similar reductive conversion of a (*p*-tolylthio)methyl group into a methyl group was studied.

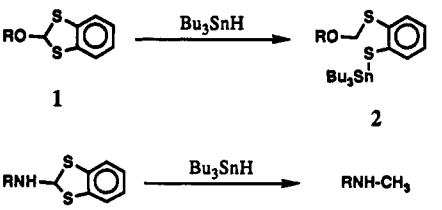
Several types of small nuclear RNAs (snRNAs) have been discovered in nuclei of eukaryotic cells and characterized.[1,2] Recent studies have revealed that a U series of these snRNAs plays an important role in the splicing of eukaryotic pre-mRNAs.[3] Their 5′-termini are modified with a so-called cap structure. The cap structure of U1, U2, U4, and U5 snRNAs involves a unique permethylated ribonucleoside of N^2,N^2,N^7-trimethylguanosine (TMG).[1] A TMG-capped mRNA has recently been prepared by transcription of β-globin DNA gene using chemically synthesized $m_3^{2,2,7}G^5'pppG^5$ as a primer to study the efficiency of the splicing reaction.[4] N^2,N^2-Dimethylguanosine (DMG) would be a useful starting material for the synthesis of snRNAs or mRNAs containing the TMG cap structure. This compound has been synthesized by several methods.[6-10]

In this paper, we report a new method for the synthesis of DMG and related compounds by reductive C–S bond cleavage of the 1,3-benzodithiol-2-yl (BDT) or the (*p*-tolylthio)methyl group.

Results and Discussion

We have reported[11-13] that the Nakayama reagent, 1,3-benzodithiolylium tetrafluoroborate[14] (BDTF) reacts with alcohols in pyridine to give 1,3-benzodithiol-2-yl ethers 1 in high yields. We also found that 1 could be quantitatively converted into ring-opened products 2 by treatment with tributyltin hydride (TBTH). Methylation of 2 with methyl iodide afforded the corresponding [[2-(methylthio)phenyl]thio]methyl ethers (MPTM ethers).[15] We recently demonstrated the utility of the MPTM group as a protecting group for the 2′-hydroxy group in oligoribonucleotide synthesis.[16]

Scheme I



In the present study, we have found that the BDT group attached to the 2-amino group of guanosine can be converted directly into a methyl group by treatment with tributyltin hydride. This result contrasts with that of a BDT group attached to a hydroxy group, where the reaction stops at the first stage of the ring-opening reaction (Scheme I).

Introduction of the BDT Group onto the 2-Amino Group of Guanosine Derivatives. 2′,3′,5′-Tri-*O*-acetylguanosine (3)[17] was treated with 1.5 equiv of BDTF in pyridine to prepare 2′,3′,5′-tri-*O*-acetyl-N^2-(1,3-benzodithiol-2-yl)guanosine (4). This reaction proceeded sluggishly, however, and did not reach completion even when excess BDTF was used. It is reported that the indirect conversion of N–H to N–Me can be effected by a two-step process involving alkylation with chloromethyl methyl sulfide followed by reduction of the C–S bond with Raney Ni.[18] However, this method is limited to N-methylation of amides and requires strong acids such as methanesulfonic acid for the first alkylation. Such drastic conditions are not applicable to the preparation of N-methylated guanosines. We reasoned that silylation of the O^6-position of 3 would increase the nucleophilicity of the exo amino group by fixation of the guanine ring into a pyrimidine structure, and that the exo amino group would then behave as a more reactive "adenosine-like" amino group.[19] When

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(7) Gerster, J. F.; Robins, R. K. *J. Org. Chem.* **1966**, *31*, 3258.
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(12) Sekine, M.; Hata, T. *J. Org. Chem.* **1983**, *48*, 3112.
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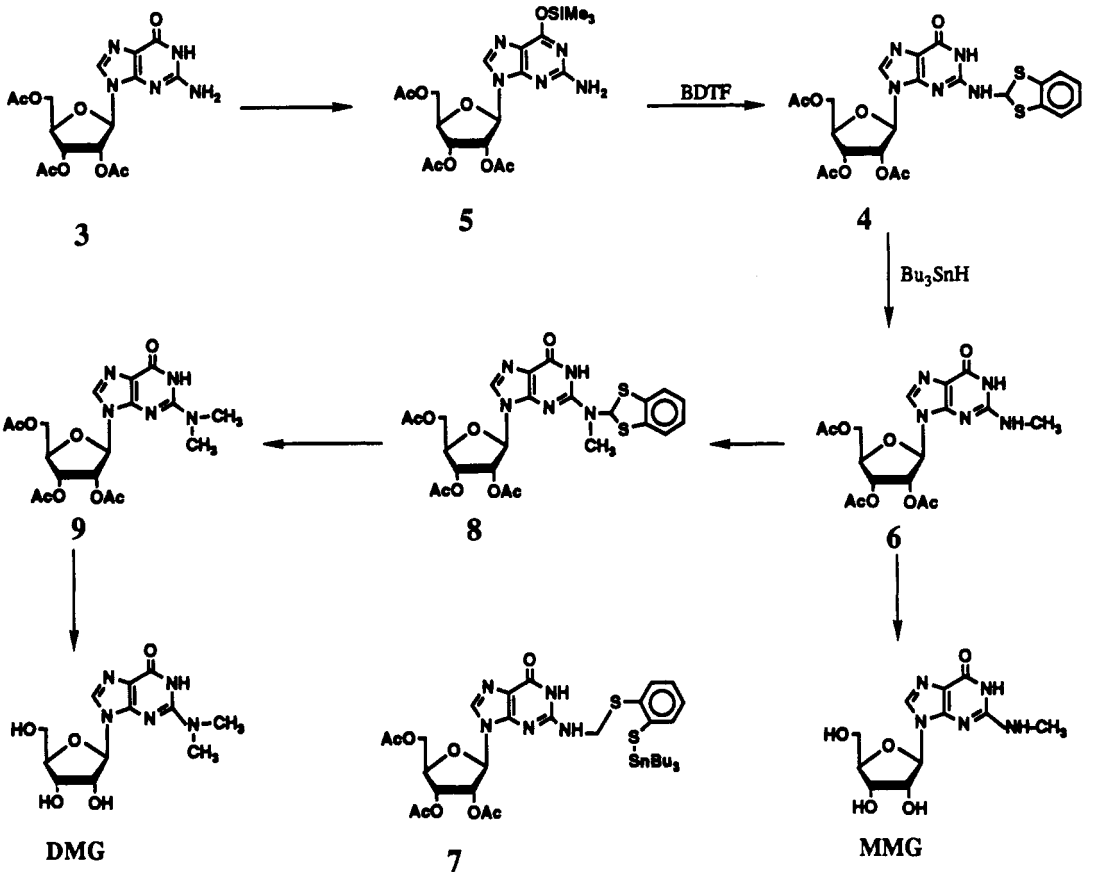
0022-3263/91/1956-1224$02.50/0

Scheme II



BDTF was added to the silylated guanosine intermediate **5**, smooth alkylation on the N^2-nitrogen occurred to give exclusively the monoalkylated product **4** in 94% yield. No appreciable dialkylation occurred even when a large excess of BDTF was employed in the reaction.

Reductive Cleavage of the C–S Bond of BDT Ether Derivatives. Reduction of the C–S bond in **4** with Raney Ni afforded the N^2-methylguanosine derivative **6**, but the yields were <19%. Several attempts to improve the yield of **6** were unsuccessful. We found, however, that reduction of **4** with 4 equiv of tributyltin hydride gave **6** as the sole product in 88% yield. A combination reagent of bis(tributyltin) oxide and polymethylhydrosiloxane[20] was also effective and afforded **6** in 75% yield. Oshima has reported the use of triethylborane as a radical initiator for several radical-induced reactions, such as the reduction of alkyl halides[21] and the deoxygenation of secondary alcohols via phenoxythiocarbonate esters.[22] This catalyst allows such radical reactions to be conducted at moderate temperatures, and it is compatible with solvents other than benzene and toluene. We therefore studied the reaction of **4** with tributyltin hydride in the presence of tributylborane and found that the reaction could be initiated at room temperature but was not complete in 24 h. The ring-opening intermediate **7** was formed as the predominant product at the initial stage. This controlled reaction is interesting since the AIBN mediated C–S bond cleavage causes competitive formation of **6** and **7** from the beginning.

Compound **6** was deacetylated by treatment with NaOH to give crystalline MMG[23-27] in 73% yield. When aqueous ammonia in methanol[10] was employed for the deacetylation, crystallization of MMG was difficult, probably because of the presence of acetamide, and an amorphous gel was obtained.

A similar alkylation of **6** with BDTF led to the formation of the N-substituted product **8** in 84% yield. Reduction of **8** with tributyltin hydride gave the N^2,N^2-dimethylguanosine derivative **9** in 94% yield. Alkaline hydrolysis of the acetyl groups of **9** afforded crystalline DMG in 80% yield. The overall yield of DMG from guanosine by our six-step method was 47%, which is a considerable improvement over previous methods.[6-10]

The tributyltin hydride mediated C–S bond cleavage of **8** results in exclusive formation of **9** in high yield without the undesired C–N bond fission. Reese has reported that Raney Ni reduction of a similar compound, 2′,3′,5′-tri-*O*-acetyl-N^2-methyl-N^2-[(*p*-tolylthio)methyl]guanosine (**11**), gives **9** in 30% yield along with the undesired byproduct **6** (34%).[10]

(20) Grady, L. G.; Kuivila, H. G. *J. Org. Chem.* **1969**, *34*, 2014. Prisbe, E. J.; Martin, J. C. *Synth. Commun.* **1985**, *15*, 401.
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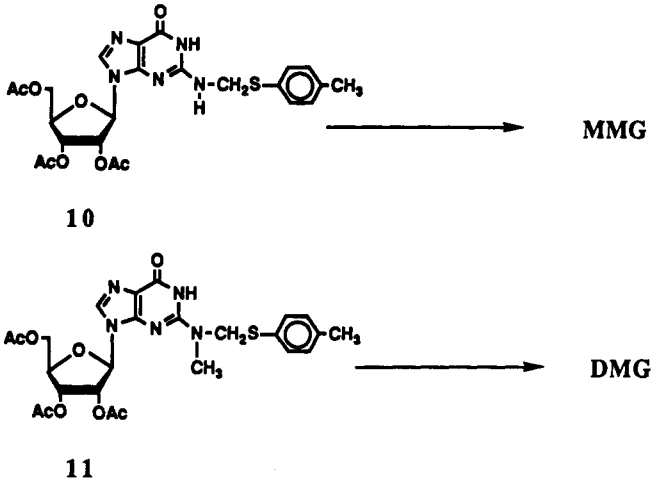
Scheme III



A recent improvement in the preparation of the Nakayama reagent makes it accessible on a large scale in an overall yield of 70% from anthranic acid in two steps.[28] This reagent is now commercially available.[29]

Our success in reducing the two C–S bonds in **4** at the same time with tributyltin hydride probably reflects the α-nitrogen stabilization of the primary carbon radical $NCH_2^{\bullet}$ formed by C–S bond fission, in contrast to the *O*-BDT derivatives where the oxygen is not as effective in stabilizing the $OCH_2^{\bullet}$ radical. This hypothesis is supported by the fact that the homolytic bond dissociation energies of the H–C bonds of $H–CH_2NHCH_3$ and $H–CH_2OCH_3$ are 87[30] and 93[31] kcal mol^{-1}, respectively.

The successful one-step cleavage of the BDT group to the methyl group stimulated us to study the reduction of **10**[10] with tributyltin hydride. Reduction of **10** with 2–4 equiv of tributyltin hydride in benzene or toluene under reflux resulted in incomplete C–S bond cleavage, giving the desired product **6** in poor yields. This result was due to the extremely poor solubility of **10** in refluxing benzene or toluene. To overcome this problem, we resorted to O^6-silylation of **10** to convert it into a more lipophilic species. When a mixture of hexamethyldisilazane, tributyltin hydride, and AIBN in benzene was heated under reflux, evolution of ammonia was observed, and **10** gradually went into solution, so that the reduction was achieved in a homogeneous medium. As a result, **6** was obtained in 83% yield.

A similar reduction of the N^2-methyl-N^2-[(*p*-tolylthio)methyl]guanosine derivative **11** was also investigated. In contrast to **10**, **11** was soluble in hot benzene, and its reduction with tributyltin hydride gave **9** in 95% yield.

Experimental Section

Melting points were obtained on a Mitamura Melt-temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 60 MHz on a Hitachi 24B spectrometer (unless otherwise noted), at 270 MHz on a JEOL-GX 270 spectrometer with Me_4Si (for $CDCl_3$) or DSS (for D_2O) as the internal standard. TLC was performed on precoated TLC plates of silica gel 60 F-254 (Merck). Column chromatography was performed with silica gel C-200 (Waco Co. Ltd.), and a minipump for a goldfish bowl was used to attain sufficient pressure for rapid chromatographic separation. TBTH and TEB were purchased from Kanto Chemical Co. Ltd.

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(29) BDTF is available from Tokyo Kasei Co. Ltd.
(30) Griller, D.; Lossing, F. P. *J. Am. Chem. Soc.* **1981**, *103*, 1586. See also: McMillen, D. F.; Golden, D. M. Ann. *Rev. Phys. Chem.* **1982**, *33*, 493.
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Compounds **10** and **11** were prepared according to the literature procedure.[10]

2′,3′,5′-Tri-*O*-acetyl-N^2-(1,3-benzodithiol-2-yl)guanosine (4). Compound **3**[17] (0.819 g, 2 mmol) was rendered anhydrous by repeated evaporation with dry pyridine and finally suspended in dry pyridine (20 mL). To the suspension was added trimethylsilyl chloride (0.38 mL, 3 mmol) under an argon atmosphere. After being stirred at room temperature for 15 min, the mixture was treated with BDTF (0.72 g, 3 mmol). The resulting solution was stirred for 30 min and then quenched by addition of water (4 mL). The mixture was extracted with CH_2Cl_2. The extract was washed three times with water (saturated NaCl if necessary), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and chromatographed on a column of silica gel with CH_2Cl_2–MeOH (100:0–97:3, v/v) to give **4** (1.06 g, 94%): ^{1}H NMR ($CDCl_3$) δ 2.00 (3 H, s, Ac), 2.11 (6 H, s, Ac), 4.27 (3 H, m, 4′,5′-H), 5.59 (3 H, m, 1′,2′,3′-H), 6.10 (1 H, s, SCHS), 7.03 (4 H, m, ArH).

Anal. Calcd for $C_{23}H_{23}O_8N_5S_2 \cdot {}^1/_3H_2O$: C, 48.67; H, 4.20; N, 12.34. Found: C, 48.72; H, 4.17; N, 12.35.

2′,3′,5′-Tri-*O*-acetyl-N^2-methylguanosine (6). Method A. To a suspension of **4** (1.69 g, 3 mmol) in benzene (30 mL) were added AIBN (197 mg, 1.2 mmol) and TBTH (3.23 mL, 12 mmol). The resulting mixture was refluxed under an argon atmosphere for 1 h. Then the solvent was removed by evaporation under reduced pressure and the oily residue was poured into hexane (600 mL) with vigorous stirring. The precipitate was collected, washed with hexane, and crystallized two times from ethanol to give **6** (1.06 g). The mother liquor was evaporated, and the residue was chromatographed on a silica gel column to give additional crops of **6** (68 mg). The total yield was 1.13 g (88%): mp 250 °C dec; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.06 (3 H, s, Ac), 2.11 (3 H, s, Ac), 2.12 (3 H, s, Ac), 3.04 (3 H, d, J = 1.8 Hz, N^2-CH_3), 4.30 (1 H, m, 5′-Ha), 4.38 (1 H, m, 4′-H), 4.49 (1 H, m, 5′-Hb), 5.86 (1 H, t, J = 6.0 Hz, 3′-H), 5.97 (1 H, d, J = 3.7 Hz, 1′-H), 6.08 (1 H, t, J = 5.2 Hz, 2′-H), 7.68 (1 H, br, NH), 7.72 (1 H, s, 8-H).

Anal. Calcd for $C_{17}H_{21}O_8N_5 \cdot {}^1/_4H_2O$: C, 47.72; H, 5.07; N, 16.37. Found: C, 47.88; H, 5.06; N, 16.48.

Method B. A mixture of **4** (168 mg, 0.3 mmol), bis(tributyltin) oxide (0.76 mL, 1.5 mmol), polymethylhydrosiloxane (0.76 mL), and AIBN (25 mg, 0.15 mmol) was dissolved in benzene (3 mL). The mixture was refluxed under argon for 1 h. After the same workup as described above, chromatography gave **6** (95 mg, 75%).

Method C. To a suspension of Raney Ni (2 g) in dioxane (10 mL) was added **4** (281 mg, 0.5 mmol). The mixture was refluxed for 20 min. The Raney Ni was removed by filtration using hyflosupercell and washed with hot ethanol–water (9:1, v/v, 200 mL). The filtrate and washing were combined, evaporated under reduced pressure, and chromatographed in the above manner to give **6** (40 mg, 19%).

Method D. A mixture of **19** (273 mg, 0.5 mmol), hexamethyldisilazane (1.05 mL, 5 mmol), trimethylsilyl chloride (6.3 μL, 0.05 mmol), TBTH (0.34 mL, 1.25 mmol), and AIBN (16 mg, 0.1 mmol) in benzene (3 mL) was refluxed for 1.5 h. Precipitation of the mixture into hexane followed by chromatography gave **6** (176 mg, 83%).

2′,3′,5′-Tri-*O*-acetyl-N^2-(1,3-benzodithiol-2-yl)-N^2-methylguanosine (8). Compound **6** (2.26 g, 5.3 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (30 mL). Trimethylsilyl chloride (2.03 mL, 16 mmol) was added to the solution, and the mixture was stirred at room temperature for 30 min. The BDTF (1.92 g, 8 mmol) was added, and stirring was continued for 1.5 h. Water (5 mL) was added, and the mixture was extracted with CH_2Cl_2. The extract was washed three times with water (if necessary use saturated NaCl), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Chromatography gave **8** (2.56 g, 84%): ^{1}H NMR ($CDCl_3$) δ 2.03 (3 H, s, Ac), 2.06 (3 H, s, Ac), 3.01 (3 H, s, N^2-CH_3), 4.27 (3 H, m, 4′,5′-H), 5.38 and 5.60–5.95 (3 H, m, 1′,2′,3′-H), 6.71–7.33 (4 H, m, ArH), 7.53 (1 H, s, 8-H), 7.76 (1 H, s, SCHS).

Anal. Calcd for $C_{24}H_{25}O_8N_5S_2$: C, 50.08; H, 4.38; N, 12.17; S, 11.14. Found: C, 49.95; H, 4.30; N, 12.05; S, 11.03.

2′,3′,5′-Tri-*O*-acetyl-N^2,N^2-dimethylguanosine (9). Method A. A mixture of **8** (278 mg, 0.48 mmol), TBTH (0.77 mL, 2.88 mmol), and AIBN (47.6 mg, 0.29 mmol) in benzene (5 mL) was

refluxed under argon for 1 h. A workup similar to that described in the synthesis of **6** followed by chromatography gave **9** (198 mg, 94%) as a foam: ^{1}H NMR ($CDCl_3$) δ 2.03 (3 H, s, Ac), 2.06 (6 H, s, Ac), 3.20 (6 H, s, N^2-CH_3), 4.27 (3 H, m, 4',5'-H), 5.35–6.10 (3 H, m, 1',2',3'-H), 7.43 (1 H, s, 8-H).

Anal. Calcd for $C_{18}H_{23}O_8N_5 \cdot {}^1/_3H_2O$: C, 48.76; H, 5.38; N, 15.79. Found: C, 48.50; H, 5.10; N, 15.69.

Method B. A mixture of **11** (112 mg, 0.2 mmol), TBTH (0.25 mL, 0.9 mmol), and AIBN (8 mg, 0.05 mmol) in benzene (2 mL) was refluxed under argon for 3 h. The usual workup followed by chromatography gave **9** (83.2 mg, 95%) as foam.

Methylation of Guanosine by Trimethyl Phosphate in the Presence of Tetrabutylammonium Fluoride. A mixture of guanosine (283.2 mg, 1 mmol) and trimethyl phosphate (2 mL, 17.1 mmol) was dissolved in a 1 M THF solution of tetrabutylammonium fluoride (10 mL, 10 mmol). The resulting mixture was stirred at room temperature for 18 h and then diluted with dioxane to a volume of 20 mL. One-fifth of this solution was applied to Watman 3MM papers developed with 2-propanol–concentrated ammonia–H_2O (7:1:2, v/v/v). The strongest UV absorbing band of R_f 0.61 was eluted with water to give N^1-methylguanosine (2022 $A_{255.4}$, 64% calculated by using $\epsilon = 15.9 \times 10^3$). The aqueous solution was freeze-dried and analyzed by 270 MHz NMR: ^{1}H NMR (270 MHz, D_2O/DSS) δ 3.37 (3 H, s, N^1-CH_3), 3.83 (1 H, dd, $J_{5'a-5'b}$ = 12.6 Hz, $J_{5'a-4'}$ = 4.2 Hz, 5'-Ha), 3.92 (1 H, $J_{5'a-5'b}$ = 12.6 Hz, $J_{5'b-4'}$ = 3.1 Hz, 5'-Hb), 4.23 (1 H, dd, $J_{3'-4'}$ = 7.5 Hz, $J_{2'-3'}$ = 3.9 Hz, 3'-H), 4.42 (1 H, dd, $J_{2'-3'}$ = 3.9 Hz, $J_{1'-2'}$ = 5.5 Hz, 2'-H), 5.85 (1 H, d, $J_{1'-2'}$ = 5.5 Hz, 1'-H), 7.93 (1 H, s, 8-H); UV (H_2O) λ_{max} 255.4 nm, λ_{min} 227 nm, sh 268 nm. The positions of λ_{max} and sh in the UV spectra of this material did not change essentially over the pH range 7–12.

N^2-Methylguanosine (MMG). Compound **6** (127 mg, 0.3 mmol) was dissolved in pyridine (9 mL), and 0.5 M sodium hydroxide (9 mL) was added. The solution was kept at room temperature for 20 min and then passed through a column of Dowex 50W X8 (pyridinium form, 13 mL). The column was washed successively with water (50 mL) and 10% aqueous pyridine (40 mL). The eluate and washings were combined and evaporated under reduced pressure. The residue was coevaporated several times with water to remove the last traces of pyridine. Crystallization of the residue from hot water (30 mL) gave MMG (69 mg, 73%): This material did not give a clear melting pattern as reported by Robins.[7] Softening began at ca. 179 °C and from then gradual decomposition was observed until ca. 230 °C: ^{1}H NMR ($CDCl_3$) δ 2.81 (3 H, s, N^2-CH_3), 3.68 (2 H, m, 5'-H), 3.98 (1, m, 4'-H), 4.55 (1 H, m, 2'-H), 5.67 (1 H, d, J = 6 Hz, 1'-H), 7.7 (1 H, s, 8-H).

Anal. $C_{11}H_{15}O_5N_5 \cdot {}^3/_4H_2O$: C, 42.51; H, 5.35; N, 22.53. Found: C, 42.21; H, 5.38; N, 22.60.

N^2,N^2-Dimethylguanosine (DMG). This compound was synthesized in a manner similar to that described for the synthesis of MMG and was recrystallized from water. DMG: mp 238–239 °C dec (lit.[6] mp 242 °C): ^{1}H NMR (d_6-DMSO–D_2O) δ 3.02 (6 H, s, N^2-CH_3), 3.79 (1 H, m, 4'-H), 4.07 (1 H, m, 3'-H), 4.44 (1 H, m, 2'-H), 5.61 (1 H, d, J = 5.4 Hz, 1'-H), 7.77 (1 H, s, 8-H).

Anal. Calcd for $C_{12}H_{17}O_5N_5$: C, 46.30; H, 5.50; N, 22.50. Found: C, 46.13; H, 5.47; N, 22.55.

Registry No. **3**, 6979-94-8; **4**, 130469-17-9; **6**, 4395-48-6; **8**, 130469-18-0; **9**, 73196-87-9; **10**, 130469-15-7; **11**, 130469-16-8; BDTF, 57842-27-0; DMG, 2140-67-2; MMG, 2140-77-4; guanosine, 118-00-3; N^1-methylguanosine, 2140-65-0.

New Diterpenoids from the Caribbean Gorgonian *Eunicea calyculata*. Photochemical Interconversion of the Cembrene and Cubitene Skeletons

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Seven new diterpenoids of the cubitane and cembrane classes (**1**–**7**) have been isolated from the Caribbean sea whip *Eunicea calyculata* (Ellis and Solander). The structures of these new compounds were assigned on the basis of spectral studies and an interconversion of the major metabolites via a photochemically induced 1,3-acyl migration. The first transformation of a cembrene to a cubitene diterpenoid is reported.

Marine octocorals of the order Gorgonacea, the sea whips and sea fans (phylum Cnidaria), are recognized as a rich source of biologically active and structurally unique secondary metabolites.[1] In the Caribbean Sea, sea whips of the genus *Eunicea* (Family Plexauridae) are particularly abundant and form a major component of the shallow water invertebrate fauna.[2] Early chemical investigations of *Eunicea* species, beginning in the early 1960s, showed these animals to be a chemically complex resource for cembrene lactones.[3] More recent studies of *Eunicea* species, based upon a chemotaxonomic approach, have shown that this gorgonian genus produces a wider diversity of secondary metabolites.[4-7]

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