Synthesis and Properties of O^6-Substituted Guanosine Derivatives

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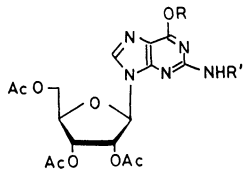
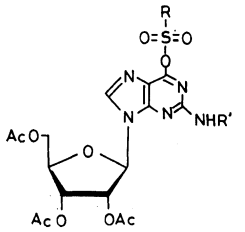
Reactions of 2′,3′,5′-tri-*O*-acetyl-N^2-protected or unprotected guanosine derivatives with phosphoryl, phosphinothioyl, arylsulfonyl, and silyl halides in dichloromethane gave the corresponding O^6-substituted guanosine derivatives in good yields. In these reactions, 4-(dimethylamino)pyridine (DMAP) was found to be very effective catalyst. The physical data of these products and their stabilities under acidic and basic conditions were described in detail. Selective detritylation of O^6-arylsulfonyl-N^2-tritylguanosine derivatives was accomplished by treatment with 80% acetic acid. It was found that O^6-dibutylphosphinothioyl derivatives were relatively stable under conditions where acetyl group was predominantly removed.

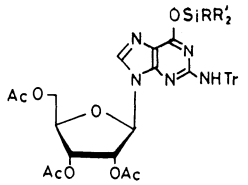
In recent years, introduction of new methodologies into nucleotide chemistry has facilitated dramatically the synthesis of oligonucleotides.[1] The modern phase of chemical synthesis of oligonucleotides rests mainly on the development of new types of condensing agents[2] and protecting groups[3] and the application of rapid purification and separation procedures.[4] For example, several (arylsulfonyl)azoles[2] have proved to be the most promising condensing agents since the reaction time for the condensation is being outstandingly shortened and the yields of oligomers are usually high. Narang[5] and Itakura[6] have recently showed rapid and practical methods for the synthesis of oligodeoxyribonucleotides demonstrating its successful application to the chemical synthesis of the fragments of the double stranded bioactive genes of lactose operator,[5a] human hormones,[6a,b] and insulin.[5b,c,6c,d] Ohtsuka and Ikehara[7] have also reported the utility of arenesulfonyl azoles in "unit-type" synthesis involving the total synthesis of $tRNA^{fMet}$. These new synthetic devices seem to be accepted satisfactorily. However, it appears that the preparation of guanosine-containing oligomers by the improved phosphotriester approach involves still synthetic problems, especially, on the aspect of the coupling yields. This problem is more severe in the case of the ribo-series. Several years ago, Reese[8–10] reported that guanosine derivatives were sulfonylated at the O^6-position of guanine moiety with arenesulfonyl chlorides in pyridine. It suggests strongly that during the elongation of oligonucleotide chains such unavoidable side reactions might occur at the O^6-position of guanosine. Indeed, we have met similar troubles owing to these side reactions in the synthesis of a guanosine monomer (as a unit nucleotide) and guanosine-rich oligomers. In connection with our project in oligoribonucleotide synthesis we have needed a highly lipophilic guanosine derivative capable for elaboration of a phosphate group at the 5′-ribose hydroxyl. Consequently, the coupling reaction between N^2-tritylguanosine and cyclohexylammonium *S*,*S*-bis(4-methoxyphenyl) phosphorodithioate (MPT) was performed by use of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (TPS–MPT reaction) according to the established procedure.[11]



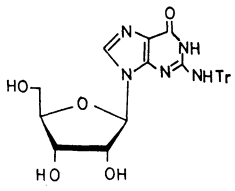
1: R = H, R′ = Tr
2: R = H, R′ = Bz
3: R = R′ = H



8a: R = 4-Me C_6H_4, R′ = Tr
b: R = 2,4,6-$(Me)_3C_6H_2$, R′ = Tr
c: R = 2,4,6-$(i\text{-}Pr)_3C_6H_2$, R′ = Tr
9: R = 2,4,6-$(i\text{-}Pr)_3C_6H_2$, R′ = Bz
10a: R = 4-MeC_6H_4, R′ = H
b: R = 2,4,6-$(Me)_3C_6H_2$, R′ = H
c: R = 2,4,6-$(i\text{-}Pr)_3C_6H_2$, R′ = H



7a: R = R′ = Me
b: R = t-Bu, R′ = Me
c: R = t-Bu, R′ = C_6H_5



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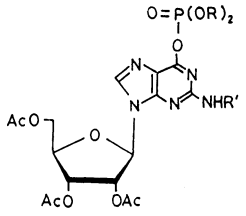
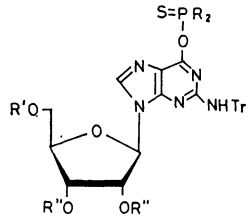


4a: R = Et, R′ = Tr
b: R = i-Pr, R′ = Tr
c: R = C_6H_5, R′ = Tr
d: R = 4-Cl-C_6H_4, R′ = Tr
5: R = C_6H_5, R′ = Bz



6a: R = Bu, R′ = R″ = Ac
b: R = Ph, R′ = R″ = Ac
12: R = Bu, R′ = R″ = H
13: R = Bu, R′ = Ac, R″ = H

However, the desired 5′-*O*-phosphorylated product was not obtained and some unidentified by-products were formed. Careful investigation of the TPS–MPT reaction on more simplified (*i.e.*, fully protected) guanosine derivatives such as 2′,3′,5′-tri-*O*-acetyl-N^2-trityl-guanosine (**1**) and 2′,3′,5′-tri-*O*-acetyl-N^2-benzoylguanosine (**2**) has revealed that O^6-substitution reactions of the above mentioned compounds occured easily under similar conditions. This led us to study extensively the protection of the O^6-position of guanosine residue, which would give some insight on the oligonucleotide synthesis.

In this paper, we wish to report the facile phosphorylation, sulfonylation, silylation of the O^6-position of guanosine derivatives and the properties of the O^6-substituted guanosine derivatives in detail.[12] During this work, we dealed mainly with N^2-tritylguanosine derivatives since the trityl group has proved to be useful and promising protecting group of the 2-amino group of guanine moiety because of its stability and great lipophilicity.

Results and Discussion

O^6-Substitution Reaction of Guanosine Derivatives. At first, pyridine was chosen as a solvent for the above mentioned O^6-substitution reaction, since it was the common solvent used for the TPS–MPT reaction. By use of pyridine, however, unsatisfactory results were obtained for O^6-sulfonylation and O^6-phosphorylation of N^2-substituted 2′,3′,5′-tri-*O*-acetylguanosines. Another disadvantage was difficult work-up procedure which allowed isolation of the O^6-substituted derivatives only in low yields. On the other hand, excellent results were obtained, when the reaction was carried out in the presence of a catalytic amount of 4-(dimethylamino)-pyridine (DMAP)[13,14] in dichloromethane. It can be

Table. 1 N^2-Substituted or unsubstituted 2′,3′,5′-tri-*O*-acetyl-O^6-(arylsulfonyl) guanosines

Compound	R^1	Sulfonylating agent[a]	Time h	Yield %	R_f value[b]	UV spectra, λ/nm $\lambda_{max}^{dioxane}$ ($\varepsilon \times 10^{-3}$)	UV spectra, λ/nm $\lambda_{min}^{dioxane}$
8a	Ph_3C	TsCl	3	75	0.58	303 (6.2) 258 (12.4)	278 248
8b	Ph_3C	MS	3	83	0.61	300 (6.2) 290 (6.2) 254 (12.3)	291 277 250
8c	Ph_3C	TPS	3	87	0.67	300 (7.7) 290 (7.7) 255 (14.9)	291 277 250
9	PhC(=O)	TPS	12	78	0.66	276 (7.3) 233 (12.0)	250 221
10a	H	TsCl	24	73	0.36	298 (8.1) 245 (12.4)	267 243
10b	H	MS	24	78	0.59	298 (6.2) 288 (6.2) 238 (12.4)	265 235
10c	H	TPS	24	80	0.43	299 (7.8) 289 (7.8) 240 (12.7)	265 238

a) TsCl=tosyl chloride. MS=mesitylenesulfonyl chloride. TPS=2,4,6-triisopropylbenzenesulfonyl chloride. b) Benzene–ethyl acetate (4 : 1, v/v).

Table 2. O^6-Phosphorylated 2′,3′,5′-tri-*O*-acetyl-N^2-tritylguanosines (**4**—**6**)

Compound	R	Phosphorylating agent	Yield %	R_f value[a]	UV spectra, λ/nm $\lambda_{max}^{dioxane}$ ($\varepsilon \times 10^{-3}$)	UV spectra, λ/nm $\lambda_{min}^{dioxane}$
4a	Ph_3C	$(EtO)_2P(O)Cl$	b)	0.23	b)	b)
4b	Ph_3C	$(i\text{-}PrO)_2P(O)Br$	35—40	0.31	291 (8.3) 257 (13.9)	277 244
4c	Ph_3C	$(PhO)_2P(O)Cl$	30—40	0.51	291 (7.5) 261 (13.3)	277 245
4d	Ph_3C	$(p\text{-}ClC_6H_4O)_2P(O)Cl$	30—40	0.65	292 (6.3) 257 (12.1)	286 250
5	PhC(=O)	$(PhO)_2P(O)Cl$	*ca.* 30	0.49	c)	c)
6a	Ph_3C	$Bu_2P(S)Br$	90—98	0.67	300 (9.25) 258 (12.9)	277 246
6b	Ph_3C	$Ph_2P(S)Cl$	50—60	0.66	299 (8.0) 256 (15.3)	279 247

a) Benzene–ethyl acetate (4 : 1, v/v). b) Compound **4a** was too unstable to be isolated in pure form. c) Compound **5** was not purified by chromatography because of partial decomposition.

TABLE 3. O^6-SILYLATED 2′,3′,5′-TRI-*O*-ACETYL-N^2-TRITYLGUANOSINES (**7**)

Compound	Silylating agent	R_f value[a]	Yield %	UV spectra, λ/nm $\lambda_{max}^{dioxane}$ ($\varepsilon \times 10^{-3}$)	$\lambda_{min}^{dioxane}$
7a	Me_3SiCl	b)	b)	b)	b)
7b	*t*-$BuMe_2SiCl$	0.64[c]	d)	c)	c)
7c	*t*-$BuPh_2SiCl$	0.66	98	290 (8.04) 271 (sh) 265 (sh) 261 (11.4) 254 (sh)	276 243

Other physical data (^{1}H NMR and elemental analysis) are given as follows: **7c**: ^{1}H NMR ($CDCl_3$): δ 1.18 (9H, s, $(CH_3)_3C$), 1.93 (3H, s, 5′-acetyl protons), 2.06 (6H, br. s, 2′- and 3′-acetyl protons), 5.06—5.53 (3H, m, ribose protons), 5.63 (3H, br. s, ribose protons), 7.10 (15 H, br. s, ArH), 7.17—7.83 (11H, Ph_2Si and H-8); Found: C, 69.07; H, 5.80; N, 7.47%. Calcd for $C_{51}H_{51}N_5O_8Si$: C, 68.82; H, 5.78; N, 7.87%. a) Benzene–ethyl acetate (4 : 1, v/v). b) Compound **7a** was too unstable to characterize its properties (see the text). c) It was not possible, according to the techniques developed in this report, to isolate **7b** in pure form. Therefore, no elemental analysis, precise, UV, ^{1}H NMR data are given here. R_f value for **7b** should be accepted with some reservation due to partial decomposition of **7b** during TLC. d) The yield of **7b** was *ca.* 90—95% (determined by the amount of triethylammonium hydrochloride and ^{1}H NMR appearance of the partially purified product; see General Procedure B.

emphasized that O^6-substitution reactions took place by use of appropriate sulfonyl and phosphoryl halides in the presence of DMAP and the reactions were very clean and the results were also satisfactory. The reaction conditions and the results are summarized in Tables 1—3.

Concerning the reaction rate of O^6-substitution reaction, it depended on the lipophilicity of the guanosine derivatives. For instance, compound **1** containing trityl group was more reactive than 2′,3′,5′-tri-*O*-acetylguanosine (**3**) having no trityl group, while **1** entered in O^6-substitutions a little faster than **2**. As one could easily see from Tables 1—3, electronic and steric effects in the O^6-substitution reactions have apparently a little influence on the reaction rates.

The successful isolation of this type of guanosine derivatives (**4** and **5**) is of great interest since O^6-phosphorylated species have been discussed as by-products in the synthesis of oligonucleotides involving guanosines but they have never been characterized.

On the other hand, two kinds of phosphinothioyl halides, *i.e.*, dibutylphosphinothioyl bromide and diphenylphosphinothioyl chloride, were employed in place of the phosphoryl halides in order to introduce phosphinothioyl groups into the O^6-position of guanosine derivatives. As a consequence, the corresponding O^6-phosphinothioylated guanosine derivatives (**6a** and **6b**) were successfully obtained in 98 and 60% yields, respectively.

Furthermore, this DMAP/Et_3N/CH_2Cl_2 system could be also extended to the O^6-silylation. We examined the DMAP-catalyzed silylation of **1** using trimethylsilyl chloride, *t*-butyldimethylsilyl chloride, and *t*-butyldiphenylsilyl chloride. The superiority of DMAP/Et_3N/CH_2Cl_2 system was also confirmed in this case by comparison with the conventional system (imidazole/*N*,*N*-dimethylformamide/silyl chloride[15]). Thus, the *t*-butyldiphenylsilyl derivative (**7c**) was successfully isolated in an excellent yield of 98%. However, in the case of the trimethylsilylation, it was impossible to isolate the silylated product (**7a**) and even to detect it on the TLC plate. Very rapid decomposition of **7a**

TABLE 4. ELEMENTAL ANALYSIS AND ^{1}H NMR SPECTRA OF **4** AND **6**[a]

Compound formula	Elemental analysis (%) Calcd / Found	C	H	N	[S] or (Cl)	^{1}H NMR ($CDCl_3$), δ
4b		60.36	5.68	8.58	—	1.38 (6H, d, J=6 Hz, $(CH_3)_2C$), 1.96 (3H, s, 5′-acetyl), 2.08 (6H, s, 2′,3′-acetyls), 4.16 (3H, br. s, ribose protons), 4.92 (2H, m, $(CH_3)_2CH$), 5.20—5.63 (3H, m, ribose protons), 6.56 (1H, s, NHTr), 7.23 (15H, br. s, ArH), 7.70 (1H, s, H-8)
$C_{41}H_{46}N_5O_{11}P$		60.29	5.69	8.53	—	
4c		63.86	4.80	7.92		1.93 (3H, s, 5′-acetyl), 2.08 (6H, s, 2′,3′-acetyls), 4.13 (3H, br. s, ribose protons), 5.13—5.55 (3H, m, ribose protons), 6.08 (1H, br. s, NHTr), 7.18 (25H, br. s, ArH), 7.70 (1H, s, H-8)
$C_{47}H_{42}N_5O_{11}P$		64.06	4.78	7.61		
4d		55.02	3.90	6.83	(6.94)	1.97 (3H, s, 5′-acetyl), 2.10 (6H, s, 2′,3′-acetyls), 4.17 (3H, br. s, ribose protons), 5.17—5.63 (3H, m, ribose protons), 6.27 (1H, br. s, NHTr), 7.20 (1H, s, H-8)
$C_{47}H_{40}N_5Cl_2O_{11}P \cdot 4H_2O$		55.04	3.98	6.46	(6.48)	
6a		61.00	5.91	8.27	[3.78]	0.67—2.43 (18H, m, C_4H_9), 2.00 (3H, s, 5′-acetyl), 2.06 (6H, s, 2′,3′-acetyls), 4.23 (3H, br. s, ribose protons), 5.22—5.80 (3H, m, ribose protons), 6.43 (1H, br, s, NHTr), 7.23 (15H, br. s, ArH), 7.73 (1H, s, H-8)
$C_{43}H_{50}N_5O_8PS \cdot H_2O$		61.39	6.06	8.03	[3.62]	
6b		64.34	4.82	7.98	[3.65]	1.93 (3H, s, 5′-acetyl), 2.04 (6H, s, 2′,3′-acetyls), 4.13 (3H, br. s, ribose protons), 5.17—5.63 (3H, m, ribose protons), 6.13(1H, s, NHTr), 7.08—8.10 (26H, br. s, H-8 and ArH)
$C_{47}H_{42}N_5O_8PS \cdot 1/2H_2O$		64.42	4.90	7.75	[3.45]	

a) Compounds **4a** and **5** could not be purified because of their instability during work-up procedure. Therefore no elemental analysis and ^{1}H NMR spectral data are given here.

Table 5. Elemental analysis and ^{1}H NMR spectra of **8**—**10**

Compound formula	Elemental analysis (%) Calcd / Found C	H	N	S	^{1}H NMR ($CDCl_3$), δ
8a	62.60	4.88	8.69	3.98	1.95 (3H, s, 5′-acetyl), 2.06 (6H, s, 2′,3′-acetyls), 2.40 (3H, s, para-methyl), 4.15 (3H, br. s, ribose protons), 5.10—5.63 (3H, m, ribose protons), 6.42 (1H, br. s, NHTr), 7.18 (17H, s, ArH), 7.7 (1H, s, H-8), 7.78 (2H, d, J=8 Hz)
$C_{42}H_{39}N_5O_{10}S$	63.07	5.09	8.41	3.78	
8b	63.37	5.20	8.40	3.84	1.93 (3H, s, 5′-acetyl), 2.09 (6H, s, 2′,3′-acetyls), 2.26 (3H, s, p-CH_3), 2.7 (6H, s, o-CH_3), 4.15 (3H, br. s, ribose protons), 5.13—5.53 (3H, m, ribose protons), 6.17 (1H, br. s, NHTr), 6.83 (2H, s, m-ArH), 7.17 (15H, br. s, ArH), 7.70 (1H, s, H-8)
$C_{44}H_{43}N_5O_{10}S$	63.40	5.21	7.89	3.76	
8c	65.41	6.04	7.63	3.49	1.20 (18H, d, J=6 Hz, $(CH_3)_2C$), 1.86 (3H, s, 5′-acetyl), 2.03 (6H, s, 2′,3′-acetyls), 3.05 (1H, m, p-$(CH_3)_2CH$), 4.06 (3H, br. s, ribose protons), 4.10 (2H, m, o-$(CH_3)_2CH$), 5.00—5.46 (3H, m, ribose protons), 6.08 (1H, br. s, NHTr), 7.13 (15H, br. s, ArH), 7.62 (1H, s, H-8)
$C_{50}H_{55}N_5O_{10}S$	65.06	6.08	7.36	3.40	
9	58.53	5.82	8.98	4.11	1.26(12H, d, J=6 Hz, o-$(CH_3)_2C$), 1.23 (6H, d=6 Hz, p-$(CH_3)_2$-C), 2.00—2.23 (9H, br. d, acetyl), 2.92 (1H, m, p-$(CH_3)_2CH$), 4.20 (2H, m, o-$(CH_3)_2CH$), 4.50 (3H, m, ribose protons), 5.85—6.18 (3H, m, ribose protons), 7.1—8.00 (8H, m, ArH)
$C_{38}H_{45}N_5O_{11}S$	58.76	5.77	8.62	3.90	
10a	49.02	4.47	12.43	5.69	2.03—2.20 (9H, three singlets, acetyls), 2.42 (3H, s, p-CH_3), 4.33 (3H, br. s, ribose protons), 4.98 (2H, br. s, NH_2), 5.57—5.97 (3H, m, ribose protons), 7.23 (2H, d, J=8 Hz, ArH), 7.72 (1H, s, H-8) 7.92 (2H, d, J=8 Hz, ArH)
$C_{23}H_{25}N_5O_{10}S$	49.07	4.59	12.22	5.53	
10b	51.76	4.94	11.84	5.42	2.00—2.20 (9H, br. s, acetyls), 2.30 (3H, s, p-CH_3), 2.74 (6H, s, o-CH_3), 4.38 (3H, br. s, ribose protons), 5.08 (2H, br. s, NH_2), 5.60—6.00 (3H, m, ribose protons), 6.93 (2H, s, ArH), 7.80 (1H, s, H-8)
$C_{25}H_{29}N_5O_{10}S$	51.68	4.99	11.44	5.30	
10c	55.10	6.12	10.36	4.74	1.28 (18H, d, J=6 Hz, $(CH_3)_2C$), 2.00—2.13 (9H, three singlets, acetyls), 3.05 (1H, m, p-$(CH_3)_2CH$), 3.70—4.23 (2H, m, o-$(CH_3)_2CH$), 4.37 (3H, m, ribose protons), 4.97 (2H, br. s, NH_2), 5.60—6.00 (3H, m, ribose protons), 7.13 (2H, s, ArH), 7.68 (1H, s, H-8)
$C_{31}H_{41}N_5O_{10}S$	54.79	6.12	9.99	4.63	

to the starting material was observed. However, indirect evidence of the O^6-silylation was supported by the fact that a stoichiometric amount of triethylamine hydrochloride was obtained from the reaction mixture. When t-butyldimethylsilyl chloride was used for the silylation, it was possible to detect the corresponding silylated product (**7b**) by TLC and to separate it as a material which was contaminated with DMAP and traces of triethylammonium chloride (see Procedure B in Experimental Section).

Stability of the O^6*-Substituted Guanosine Derivatives.* Since it was quite important for our synthetic purpose, we undertook a careful examination of the stability of the above mentioned compounds (**4**—**10**) either during work-up procedure, in solid state, or in several acidic and basic mediums. Consequently, their stabilities have varied in a quite wide range. The summary of the results is given below (for further details see Table 6 and Experimental Section). In connection with their stability in solid state, we have found that N^2-unsubstituted guanosine derivatives were unstable and after two months at room temperature a significant decomposition has been observed.

It was proved also that an introduction of the bulky substituents near O^6-oxygen of the O^6-substituted guanosines increases their stability both during work-up and in the solid state. Thus, in the O^6-sulfonylated derivatives of **3** the order of stability in the solid state was **10a**>**10b**>**10c**. On the other hand, in O^6-phosphorylation of **1**, it was found that the O^6-diethylphosphoryl derivative (**4a**) was not stable enoguh to be isolated in pure form, while the O^6-diisopropylphosphoryl compound (**4b**) could be isolated, although the yield was relatively low (*ca.* 30%).

Because of the circumstances under which this study was undertaken we have been in keen interest for lability of the substituent on O^6-guanosine oxygen, especially, in different basic mediums. Our principal goal is to obtain O^6- and N^2-disubstituted guanosines so that we can call them "fully protected" selectively in the guanine part. As a consequence, we tried to check the stability of O^6-substituted 2′,3′,5′-tri-O-acetyl-N^2-tritylguanosines in more detail. It was found that most of substituents of O^6-guanosine oxygen were too unstable in several alkaline mediums to serve as protecting groups for O^6-position of the compounds involved. The O^6-sulfonylated derivatives (**8**—**10**) were particularly susceptible to nucleophilic substitution at C^6 carbon. A similar reaction of 2′,3′,5′-tri-O-acetyl-O^6-mesitylsulfonylguanosine (**10b**) has been described by Reese.[9] Unfortunately, it was not possible to find a suitable reagent which would attack preferably ribose acetoxyl groups, but not the C^6-carbon in the guanine moiety.

In order to gain information on the possibility of using O^6-substituents as protecting groups some data on their stability in acidic mediums were also required. We have found that, as a rule, they were quite labile under acidic conditions (see Table 6). It was, however, possible to find removal conditions of trityl group selectively from 2′,3′,5′-tri-O-acetyl-O^6-sulfonyl-N^2-tritylguanosine (**8a**—**c**) where the sulfonyl groups remained intact to afford **10a**—**c** in almost quantitative yields. On

TABLE 6. REMOVAL OF THE PROTECTING GROUPS OF SOME O^6-SULFONYLATED PHOSPHORYLATED, OR SILYLATED COMPOUNDS IN ACIDIC OR BASIC MEDIUMS

Compound	Acidic or basic medium[a)]	Time/h	Product
8a	80% acetic acid in dioxane–water (4 : 1, v/v)	1.0	**10a**
8b		1.0	**10b**
8c		1.0	**10c**
8a	60% formic acid	0.5	**3**
8b		0.5	**3**
8c		0.5	**3**
8a	14% methanolic ammonia[c)]	2.0	b)
7c		24	**11**
6a	*t*-butylamine in methanol–dichloromethane[d)]	6.0	**12** and **13**[e)]
6a	butylamine in dichloromethane	48	no reaction
6a	*t*-butylamine in methanol	0.25	**12** and **13**[f)]

a) All operations were carried out at room temperature, if not otherwise stated. b) The TLC ($CHCl_3$– MeOH, 9 : 1, v/v) of the reaction mixture after 2 h showed that at least 4 products were formed (no starting material remained). No attempts were made to identify these compounds, but at least for one of them it could be judged (from R_f value) that it was N^2-tritylguanosine. c) In 5% methanolic ammonia a similar reaction took place. d) This was one of the best solvent systems which have been tested for aminolysis of **6a**. The reaction was sufficiently slow for isolation of monoacetyl- and deacetylated products of **6a**. e) See Experimental section. f) See the text.

the other hand, simultaneous removal of both trityl and arylsulfonyl groups could be accomplished by employing 60% formic acid (see Table 6).

The O^6-phosphorylated guanosine derivatives (**4** and **5**) were also quite labile in basic mediums, and the O^6–P bond was cleaved easily in either aqueous or anhydrous solvents to give rise to N^2-tritylguanosine (**11**) as a principal reaction product.

For aminolysis of 2′,3′,5′-tri-*O*-acetyl-O^6-dibutylphosphinothioyl-N^2-tritylguanosine (**6a**), ammonia, *t*-butylamine, and butylamine in different solvent systems were tested. It was found that both ammonia (in methanol) and butylamine (in methanol–dichloromethane) had given unsatisfactory results. The aminolysis was too fast and O^6-dibutylphosphinothioyl group was cleaved considerably. On the contrary, *t*-butylamine [in methanol and especially in methanol–chloroform (or dichloromethane)] had given good results both in terms of aminolysis reaction and stability of the O^6-dibutylphosphinothioyl group. The aminolysis with *t*-butylamine appeared to be very sensitive to the solvent system. For instance, there was no reaction between **6a** and *t*-butylamine in solvents such as dichloromethane, 2-propanol, and chloroform, while the reaction was very fast in methanol (only 15 min was enough for disappearance of **6a**) and relatively slow in ethanol. Thus, it was shown that the dibutylphosphinothioyl group was relatively stable during the aminolysis of the corresponding guanosine derivatives with *t*-butylamine. On the other hand, an easy cleavage of the group was detected in acidic mediums (for example, 80% acetic acid) to give **3**. Therefore, it seems to us that the dibutylphosphinothioyl group (BPT) is useful for O^6-protection of guanosine derivatives, because it has the following useful features; 1) ribose acetyl groups undergo more rapid aminolysis by *t*-butylamine than the O^6-BPT group; 2) it is possible to cleave easily O^6-BPT groups in either acidic or appropriate basic mediums; 3) the corresponding O^6-phosphinothioyl compounds could be obtained conveniently in high yields; 4) compounds **6a**—**c** are stable in solid state at room temperature for a long period of time and in aqueous pyridine for one day and can survive TLC or other purification procedures; 5) O^6-dibutylphosphinothioyl derivatives of N^2-tritylguanosine can be detected easily on TLC plates because of their characteristic blue fluorescence at 254 nm; 6) compounds **6**, **12**, or **13** have very characteristic UV spectra (see the later section); 7) butyl groups are easily detectable by ^1H NMR and it might be useful in nucleotide synthesis, because no significant signals appeared in that region.

Diphenylphosphinothioyl group showed similar properties to the BPT group but the introduction of the former to the O^6-imidoyl group was performed only in moderate yields and it does not have blue fluorescence like BPT-containing compounds.

Thus, as a result of this investigation, it seems that the BPT group might be useful for protection of the O^6-oxygen of guanosine. It could be a valuable starting point for designing similar and even better groups. Further efforts toward this goal are now in progress in our laboratory.

Solubility of O^6*-Substituted Guanosine Derivatives.* It is well documented by Guschlbauer that guanosine forms rigidly stable hydrogen bonded complexes of cyclic structure.[16)] This greatly diminishes the solubility of the molecule in many organic solvents and makes it impossible to perform some interesting synthetic transformations. Generally, in the oligonucleotide synthesis, the longer the chain of an oligonucleotide is, the poorer its solubility is so that the R_f becomes low and the separation of the oligonucleotide from other polar by-products is difficult. Therefore, from the two points of the prevention of the side reactions at the O^6-position of guanine moiety and the enhance of the solubility of guanosine derivatives, introduction of a protecting group into the O^6-imidoyl group seems to be important. The solubility of the O^6-substituted guanosine derivatives obtained in the present works increases dramatically in several organic solvents. For example, the *t*-butyldiphenylsilylated derivative (**7c**) is freely soluble in ether and fairly even in hexane. The high solubility of the O^6-substituted guanosine derivatives is seen from the R_f values described in Tables 1—3 and 7.

UV and ^1H *NMR Spectra of* O^6*-Substituted Guanosine Derivatives.* All O^6-substituted guanosines have similar UV spectra which are quite different from the corresponding UV spectral data of O^6-unsubstituted derivatives. All O^6-substituted compounds have a

TABLE 7. SOME PHYSICAL DATA FOR O^6-DIBUTYLPHOSPHINOTHIOYL-N^2-TRITYLGUANOSINE (**12**) AND 5′-O-ACETYL-O^6-DIBUTYLPHOSPHINOTHIOYL-N^2-TRITYLGUANOSINE (**13**)

Compound	Elemental analysis (%) Formula		R_f value[a]	UV spectra λ/nm		^{1}H NMR ($CDCl_3$), δ
	Calcd	Found		$\lambda_{max}^{dioxane}$ ($\varepsilon \times 10^{-3}$)	$\lambda_{min}^{dioxane}$	
12	$C_{37}H_{44}N_5O_5PS$		0.14	300(8.75)	276	0.73—2.57 (18H, m, C_4H_9), 1.92 (3H, s, 5′-acetyl), 3.02 (1H, br. s, OH), 3.77—4.20 (5H, m, 2′,3′,4′, 5′-ribose protons), 5.38 (1H, d, $J_{1'-2'}$=1.1 Hz,H-1′), 6.47 (1H, s, NHTr), 7.23 (15H, br. s, ArH), 7.57 (1H, s, H-8)
	C, 63.32	63.42		258(10.6)	246	
	H, 6.32	6.30				
	N, 9.98	9.82				
13	$C_{39}H_{46}N_5O_6PS$		0.30	299(8.6)	277	0.67—2.33 (18H, m, C_4H_9), 3.33—4.57 (8H, m, 2′, 3′,4′,5′-ribose protons and three OH groups), 5.53 (1H, d, $J_{1'-2'}$=1.1 Hz, H-1′), 6.47 (1H, s, NHTr), 7.27 (15H, br. s, ArH), 7.68 (1H, s, H-8)
	C, 62.97	62.85		260(12.4)	298	
	H, 6.23	6.30				
	N, 9.41	9.03				

a) Benzene–ethyl acetate (4 : 1, v/v).

maximum at 290—305 nm (ε=6000—9000) which is not characteristic of guanosine-type derivatives. In the case of the O^6-sulfonylated compounds this maximum lies between 300—305 nm, while the corresponding O^6-silylated and phosphinothioylated derivatives have their higher wavelength maximum at 290 and 300 nm, respectively.

^{1}H NMR spectra of O^6-substituted guanosines also have some very characteristic features. For instance, all O^6-substituted N^2-tritylguanosines have a moderately broad singlet around δ 6.0—6.5 (due to N^2-proton)[9] which disappears after D_2O exchange. Also all O^6-substituted 2′,3′,5′-tri-O-acetyl-N^2-tritylguanosines have their ribose protons split in two three protons groups, one located as a characteristic multiplet around δ 5.0—5.6 and the other as a broad singlet around δ 4.2. Resonance positions of trityl, acetyl, and H-8 protons are also of great value, but usually their chemical shift on NMR scale does not depend so crucially on O^6-substitution pattern. In the case of O^6-substituted 2′,3′,5′-tri-O-acetyl-N^2-benzoyl (or unsubstituted) derivatives the same pattern of resonance for the ribose protons was observed.

In conclusion, preparative new findings on the O^6-substitution reactions of guanosine derivatives have been obtained by use of 4-(dimethylamino)pyridine as a catalyst. All O^6-substituted guanosine compounds are highly soluble in many nonpolar organic solvents. It might be due to the cleavage of hydrogen bond between N^1 and O^6 by the introduction of the O^6-substituent. The O^6-dibutylphosphinothioyl derivatives are promising synthetic intermediates of guanosine compounds in nucleotide chemistry, because of some useful characteristics of the BPT group.

From the synthetic point of view in oligonucleotide synthesis, it is highly likely that coupling agents such as arenesulfonyl chlorides or azoles and phosphorylating species activated by the coupling agents attack the O^6-imidoyl group of guanine residue during the desired internucleotidic bond formation. This seems to be the reason why yields of the coupling reactions of oligonucleotides containing guanosine residue are remarkable unsatisfactory.

Experimental

Proton nuclear magnetic resonance spectra (60 MHz) were taken on a Hitachi Model R-24 spectrometer and are reported in parts per million from internal tetramethylsilane on the δ scale (δ=0). Ultraviolet spectra were recorded on a Hitachi Model 124 spectrophotometer. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta. All solvents were distilled prior to use. Dichloromethane and pyridine were dried over 3A molecular sieves and calcium hydride, respectively. t-Butyldiphenylsilyl chloride,[17] diphenylphosphinothioyl chloride,[18] and dibutylphosphinothioyl bromide[19] were prepared according to the literature procedures.

General Procedure for O^6-*Sulfonylation, Silylation, or Phosphorylation of* N^2-*Substituted*[a] *or Unsubstituted 2′,3′,5′-Tri*-O-*acetylguanosines (Procedure A).* Two mmol of N^2-substituted or unsubstituted 2′,3′,5′-tri-O-acetylguanosine and 0.12 mmol of DMAP were dissolved[b] in 20 ml of dichloromethane and then to this solution were added successively 3.3 ml of triethylamine and 2.94 mmol of the corresponding sulfonyl, silyl, or phosphoryl halide.[c] After stirring of the homogeneous solution[d] for several hours,[e] the solvent was evaporated at 30 °C. The crystalline residue was redissolved[f] in 4 ml of dichloromethane and purified by column chromatography on silica gel. Elution was performed with benzene–ethyl acetate. Yields of the products are listed in Tables 1—3.

a) N^2-Substituent refers to trityl, benzoyl, or hydrogen. For a specific compound details are given in Tables 1—3.

b) In the case of 2′,3′,5′-tri-O-acetylguanosine, it was not possible to dissolve completely the guanosine derivative before addition of the corresponding sulfonylating, phosphorylating, or silylating reagent.

c) The corresponding silyl chloride (2.4 mmol) was used in the case of O^6-silylation reaction.

d) In the case of 2′,3′,5′-tri-O-acetylguanosine the reaction mixture became homogeneous after 30 min.

e) The reaction times for each individual compound are listed in Tables 1—3.

f) In some cases it was not possible to dissolve completely the white solid residue in 4 ml of dichloromethane. This did not, however, hamper the purification because it was possible to apply this suspension to the silica-gel column.

General Procedure for Partial Purification (Removal of Triethylammonium Chloride) of O^6-*Substituted* N^2-*Substituted or Unsubstituted 2′,3′,5′-Tri*-O-*acetylguanosines (Procedure B).* The general procedure A was followed until the solid residue was obtained after evaporation of the solvent. Then the solid was suspended

in 20 ml of dry ether, stirred for 10 min. and the white precipitate of triethylamine hydrochloride was filtered. The O^6-substituted-N^2-substituted or unsubstituted 2′,3′,5′-tri-*O*-acetylguanosine was in the filtrate together with DMAP and the excess of the corresponding sulfonylating, silylating, or phosphorylating agent. This procedure is especially useful for a quick confirmation that the reaction between guanosine derivative and the corresponding O^6-substitution reagent takes place. It is supported by the formation of triethylamine hydrochloride.

General Procedure for Aminolysis of Compounds 4—8 (Procedure C). O^6-Sulfonylated, O^6-phosphorylated, or O^6-silylated N^2-substituted 2′,3′,5′-tri-*O*-acetylguanosine (0.26 mmol) was dissolved in 1 ml of the corresponding organic solvent (dichloromethane, chloroform, methanol, ethanol, 2-propanol, or mixtures of two of them) and 2.8 mmol of the amine (ammonia, *t*-butylamine, or butylamine) in 2 mL of the above mentioned solvent was added at once. The course of the reaction was monitored by TLC (chloroform–methanol, 9 : 1, v/v). In the case of 2′,3′,5′-tri-*O*-acetyl-O^6-dibutylphosphinothioyl-N^2-tritylguanosine (**6a**: 1.59 mmol), *t*-butylamine (16.4 mmol) in methanol (12 ml) were stirred for 15 min at room temperature. The organic solvent was evaporated at 25 °C and the solid residue was chromatographed on silica gel with benzene–ethyl acetate to give **12** and **13** as white foams in 50 and 10% yields, respectively. In Table 7 the data are given for compounds **12** and **13**.

General Procedure for Acid Hydrolysis of O^6*-Sulfonylated 2′,3′,5′-Tri-*O*-acetyl-*N^2*-tritylguanosines to* O^6*-Sulfonylated 2′,3′,5′-Tri-*O*-acetylguanosines (Procedure D).* 2′,3′,5′-Tri-*O*-acethyl-O^6-arylsulfonyl-N^2-tritylguanosine[g] (0.1 mmol) was dissolved in 1 ml of 80% acetic acid in dioxane–water (4 : 1, v/v).[h] After 1 h water (4 ml) was added to the homogeneous reaction mixture and the resulting suspension was extracted with dichloromethane (3 × 2 ml). The combined dichloromethane solutions were washed with water (2 × 2 ml), dried over anhydrous sodium sulfate and evaporated at 25 °C. The remaining residue was redissolved in a small amount of dichloromethane and purified by column chromatography on silica gel (2 g). Benzene was used as the first eluent for removal of triphenylmethanol (about 10 ml of the eluate were collected) and the desired O^6-sulfonylated 2′,3′,5′-tri-*O*-acetylguanosine was eluted by ethyl acetate. The yield was nearly quantitative. The results are summarized in Table 6. All obtained 2′,3′,5′-tri-*O*-acetyl-O^6-(arylsulfonyl)guanosines (see Table 6) were proved to be identified (^{1}H NMR, R_f values, and UV spectra) with the corresponding 2′,3′,5′-tri-*O*-acetyl-O^6-(arylsulfonyl)guanosine obtained from direct O^6-sulfonylation of 2′,3′,5′-tri-*O*-acetylguanosine (see Table 1).

[g] Compounds **8a**—**c** were subjected to this reaction.

[h] No reaction took place in the absence of water.

*General Procedure for Conversion of 2′,3′,5′-Tri-*O*-acetyl-*O^6*-arylsulfonyl-*N*^{2}*-tritylguanosines to 2′,3′,5′-Tri-*O*-acetylguanosine (Procedure E).* 2′,3′,5′-Tri-*O*-acetyl-O^6-arylsulfonyl-N^2-tritylguanosine (0.1 mmol) was suspended in 70% aqueous formic acid (2 ml) and stirred at room temperature for 30 min. The white precipitate of tritylcarbinol was filtered off and the colorless filtrate was poured into 8 ml of water and extracted with chloroform (3 × 2 ml). After evaporation of the chloroform at 30 °C the remaining residue was chromatographed on silica gel (2 g) with chloroform–methanol to give the desired 2′,3′,5′-tri-*O*-acetylguanosine (**11**). The yield of **11** was nearly quantitative in each experiment.

*2′,3′,5′-Tri-*O*-acetyl-*N*^{2}*-tritylguanosine (1)* 2′,3′,5′-Tri-*O*-acetyguanosine (26.35 g, 64 mmol) was suspended in 400 ml of dry pyridine and heated at 100 °C and 53.9 g (0.193 mmol) of trityl chloride was added. After stirring at 100 °C for 6 h, the reaction mixture was poured into 1.5 l of water and extracted with dichloromethane (3 × 400 ml). The organic solvent was then removed by evaporation and the remaining oily residue was subjected to coevaporation with benzene (2 × 150 ml) for removal of the traces of pyridine. After that the dark brown oil (sometime it could crystallize) was suspended and stirred in 500 ml of ether for 1 h at room temperature. The resulting yellow precipitate was filtered off. After drying at 40 °C *in vacuo*, the crude 2′,3′,5′-tri-*O*-acetyl-N^2-tritylguanosine was dissolved in 90 ml of dichloromethane–methanol (9 : 1, v/v) and was purified by column chromatography on silica gel (60 g) by using dichloromethane–methanol as eluent. After evaporation of the eluate, the resulting light yellow solid was dissolved in 1.6 l of boiling methanol. The hot solution was filtered and the filtrate was concentrated by evaporation until 800 ml of methanol remained. External cooling (10—15 °C) was applied and after stirring of the suspension for 3 h the purified 2′,3′,5′-tri-*O*-acetyl-N^2-tritylguanosine was filtered and washed with a small amount of methanol. The yield was usually 70—80%.

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