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REGIOSELECTIVE PROTECTION OF CARBOHYDRATE DERIVATIVES. PART. 20[1]. SIMPLE, EFFICIENT 2'-O-DEACYLATION OF FULLY ACYLATED PURINE AND PYRIMIDINE RIBONUCLEOSIDES THROUGH tert-BUTOXIDE

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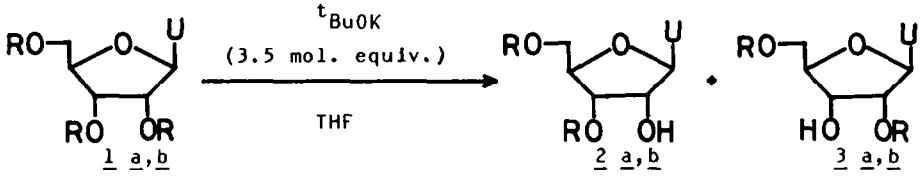
Abstract - A simple treatment of fully aroylated purine and pyrimidine ribonucleosides with pulverized potassium tert-butoxide in tetrahydrofuran (THF) or dichloromethane under a controlled condition gave a mixture of the corresponding di-O-aroyl derivatives in which 2'-OH derivatives are preponderant over 3'-OH derivatives; 3',5'-di-O-benzoyluridine, N^4,3',5'-tribenzoylcytidine, N^4,3',5'-tri-O-toluoylcytidine, N^2,3',5'-tribenzoylguanosine, and N^2-isobutyryl-3',5'-di-O-benzoylguanosine were obtained crystalline in 80%, 78%, 72%, 67%, and 65% yields, respectively.

One of the current strategies in RNA-type oligonucleotide synthesis involves the simultaneous protection of both of the 3' and 5' hydroxyl groups of ribonucleosides through 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl group[2]. The blocking group is used only for temporary protection of the hydroxyl groups, i.e., it was removed after the introduction of tetrahydrofuran-2-yl[3] and/or tetrahydropyran-2-yl group[4] to 2'-position in order to introduce 4,4'-dimethoxytriphenylmethyl group at the 5'-position and phosphor function at 3'-position in turn. It is inevitably required to introduce a protecting group toward an amino group on the nucleic acid base moiety prior to the protection of 3' and 5' positions in the cases of adenosine, guanosine, and cytidine. In a previous paper[5], on the other hand, we have reported a simple, preparative procedure for 5'-O-acylribonucleosides involving highly regioselective O-deacylation at 2' and 3' positions of fully acylated ribonucleosides through sodium methoxide - THF system, which has never been accompanied by the undesirable N-deacylation on the nucleic acid base moieties of adenosine and cytidine acylates different from the deacylation procedures involving hydrazinolysis[6] and hydroxyaminolysis[7]. We were, therefore, interested in exploring potential procedure for highly regioselective 2'-O-deacylation through the sodium methoxide - THF system; this was, however, unsuccessful to prepare the corresponding 2'-OH derivatives. Consequently, we applied tert-butoxide to the 2'-O-deacylation based on an assumption that the bulkier the substituent of an alkoxide, the higher the regioselectivity in 2'-O-deacylation; this was efficiently induced under a controlled condition as we expected with respect to every fully acylated purine and pyrimidine ribonucleoside. The results thus obtained will be described herein.

The effect of reaction temperature and solvent was first examined by the use of 2',3',5'-tri-O-benzoyluridine (**1a**); the results thus obtained and the conditions used are summarized in Table 1. A treatment of **1a** with potassium tert-butoxide in THF at room temperature gave a 2:1 mixture of 3',5'- (**2a**) and 2',5'-di-O-benzoyluridine (**3a**) in a low yield of 67%, which was separated by chromatography on a column of silica gel; the reaction was too fast to follow by TLC, so that it was impossible to fix an appropriate timing for getting maximized proportion of the mixture to **1a** and the corresponding 5'-benzoate. Lowering the temperature, the reaction was conspicuously improved; that performed at -20°C (ice-salt bath) gave a 4:1 **2a** - **3a** mixture in 74% yield (Entry 2), and that at -56°C (dry-ice - acetonitrile) gave a 5:1 mixture in 94% yield (Entry 3). The reaction was not induced in acetonitrile, and was rather slowed down in dichloromethane, which might be useful for the reaction of a nucleoside acylate extremely susceptible to O-deacylation reaction. To confirm

Table 1. Regioselective O-Dearoylation of 2',3',5'-Tri-O-aroyluridine with Potassium tert-Butoxide[a]



Entry	Uridine aroate R	Reaction temperature (°C)	Reaction time (min)	Total yield (%) of 2 and 3	Proportion of 2 and 3
1	**1a** Bz	room temp.	1	67	2 : 1
2	1a Bz	-20	2	74	4 : 1
3	**1a** Bz	-56	26	94 (75[b])	5 : 1
4[c]	1a Bz	-50	7	80	—
5	**1b** Tol[d]	-20	20	93	6 : 1

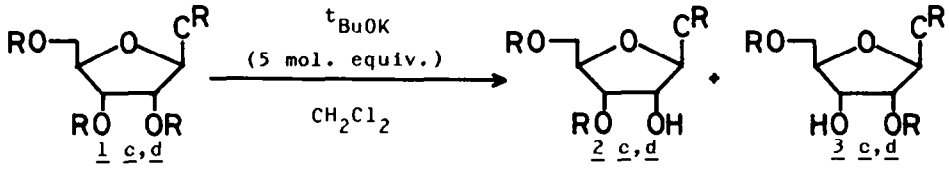
[a] All of the reactions were performed by the use of **1a** (0.3 mmol) and potassium tert-butoxide (3.5 mol. equiv.) in THF (8 mL). [b] Yield of **2a** obtained by crystallizing the resulting mixture from ethanol. [c] The reaction was performed by the use of **1a** (0.6 mmol) and potassium tert-butoxide (3.5 mol. equiv.) in THF (10 mL); **2a** was isolated by crystallization from ethanol - methanol, without column chromatography. [d] Tol stands for o-toluoyl group.

the utility of this procedure, crystallization of **2a** from the resulting mixture obtained by the reaction of **1a** (0.6 mmol) with 3.5 mol. equiv. potassium tert-butoxide in THF (10 mL) at -50°C, after quenching with Dowex 50W without the chromatographic separation, was performed to give **2a** in 80% isolated yield (Entry 4). For a strategy of preparing **2a**, it is advantageous to minimize the formation of the corresponding 5'-benzoate and recycle the unchanged **1a**. A treatment of **1a** (2.783 g, 5 mmol) with 3.5 mol. equiv. of the alkoxide in THF (30 mL) at -50°C for 10 min, followed by quenching

with the ion-exchange resin, evaporation, crystallization from ethanol, was thus performed to give **2a** in 64% yield (82% yield assuming the recovery of **1a** 22% yield). Subsequently, 2',3',5'-tri-*O*-*o*-toluoyluridine (**1b**) was subjected to the reaction with potassium *tert*-butoxide. In this case, a treatment of **1b** under the same condition as was used in Entry 3 was unsuccessful and left a considerable amount of **1b** unchanged even after 45 min, and increment of the amount of the butoxide from 3.5 to 5.0 mol. equiv. was also unfruitful. A treatment of **1b** with 3.5 mol. equiv. of the butoxide at -20°C gave a 6:1 **2b** - **3b** mixture in 93% yield (Entry 5); at the moment, there has not been found any appropriate solvent for crystallization of **2b**, but regioselectivity is superior to that of **2a** and **3a**. *o*-Toluoyl group is not susceptible to acyl migration between 2' and 3' position[8], so that further investigation is in progress to use this mixture for a nucleoside chemistry, *e.g.*, deoxygenation at either 2' or 3' position.

The reaction was secondly examined by the use of N^4,2',3',5'-tetrabenzoyl- (**1c**) and -*o*-toluoylcytidine (**1d**) (Table 2). The reaction of **1c** performed in THF at room temperature was too fast to obtain a mixture of N^4,3',5'- (**2c**) and N^4,2',5'-tribenzoylcytidine (**3c**) effectively. The reaction of **1c** at a lower temperature was, however, impossible to perform due to its precipitation at the temperature. Therefore, the reaction was performed in dichloromethane, which was found to slow down the *O*-debenzoylation of **1a**; the reactions at -20°C gave a 5:1 **2c** - **3c** mixture in 89% (Entry 2) and 91% yields (**2c**, 78% yield obtained by crystallization) in contrast with that at room temperature,

Table 2. Regioselective *O*-Dearoylation of N^4,2',3',5'-Tetraaroylcytidines with Potassium *tert*-Butoxide[a]



Entry	Cytidine aroyl derivative	R	CH_2Cl_2 (mL)	Reaction temperature (°C)	Reaction time (min)	Total yield (%) of 2 and 3	Proportion of 2 and 3
1	**1c**	Bz	8	room temp.	25	65	—
2	**1c**	Bz	5	-20	60	89	5 : 1
3	**1c**	Bz	2.5	-20	25	91 (78[b])	5 : 1
4	**1d**	Tol[c]	7	-20	60	83 (72[b])	>12 : 1

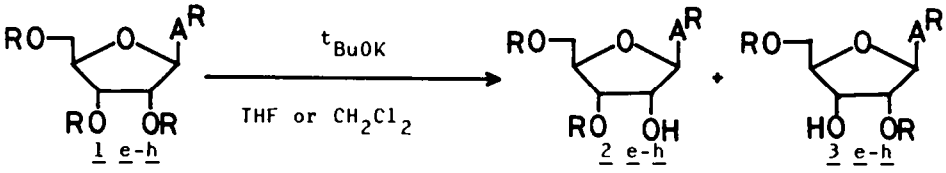
[a] The reactions in Entries 1 - 3 were performed by the use of **1c** (0.2 mmol) and potassium *tert*-butoxide (5.0 mol. equiv.), and that in Entry 4 by the use of **1d** (0.5 mmol) and potassium *tert*-butoxide (5.0 mol. equiv.) in dichloromethane. [b] Yield of 2'-OH derivatives obtained by crystallization of the resulting mixture. [c] Tol stands for *o*-toluoyl group.

which gave the mixture in 65% yield (Entry 1). Comparison of Entries 2 and 3 shows that higher

concentration shorten the reaction time from 60 to 25 min. Incidentally, **2c** is very easy to crystallize on evaporation of the eluate from the chromatography. Compound **3c** is quite soluble in chloroform, so that isolation of **2c** was easily attained by crystallizing from methanol containing chloroform, and that of **3c** was by crystallizing the residue obtained by evaporation of the filtrate, from methanol. The reaction of **1d** also proceeded as we expected to give a >12:1 **2d** - **3d** mixture in 83% yield (**2d**, 72% yield obtained by crystallization from ethanol)(Entry 4); isolation of **3d** was attained by crystallization of the residue which was obtained by evaporation of the filtrate.

The reaction was examined thirdly by the use of N^6,2',3',5'-tetra- (**1e**), N^6,N^6,2',3',5'-penta-benzoyl- (**1f**), N^6, 2',3',5'-tetra- (**1g**), and N^6,N^6,2',3',5'-penta-*o*-toluoyladenosine (**1h**)(Table 3). The reaction of **1e** in THF at room temperature was over in 1 min to give a 5:1 N^6,3',5'- (**2e**) - N^6,2',5'-tribenzoyladenosine (**3e**) mixture in 96% yield (Entry 1), in contrast with that in dichloromethane (Entry 2) giving the mixture in 46% yield in addition to the recovery of **1e** (47% yield) in spite of a longer reaction time. The reaction in acetonitrile was, however, unsuccessful to afford a complex mixture giving so many spots on monitoring by TLC; this aspect was not improved by performing the reaction at -20°C. In addition, the reaction of **1f** gave a complex mixture which

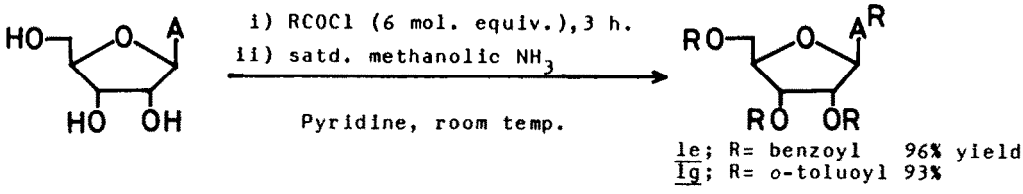
Table 3. Regioselective *O*-Dearoylation of N^6,2',3',5'-Tetraaroyladenosines with Potassium *tert*-Butoxide[a]



Entry	Adenosine aroyl derivative	R	^{t}BuOK (mol. equiv.)	Solvent (mL)	Reaction temperature (°C)	Reaction time (min)	Total yield (%) of 2 and 3	Proportion of 2 and 3
1	**1e**	Bz	3.0	THF (8)	room temp.	1	96	5 : 1
2	**1e**	Bz	3.0	CH_2Cl_2(8)	room temp.	40	46[b]	—
3	**1g**	Tol	4.0	CH_2Cl_2(2)	room temp.	5	85	6.5 : 1
4	**1g**	Tol	4.0	CH_2Cl_2(3)	room temp.	20	86	6.7 : 1
5	**1g**	Tol	6.0	CH_2Cl_2(3)	-20	2 h	82	8.0 : 1
6	**1h**[c]	Tol_5	5.0	THF (5)	-50	40	66[d]	6.8 : 1
7	**1h**[c]	Tol_5	5.5	CH_2Cl_2(2)	-20	4 h	83[d]	6 : 1

[a] The reactions in Entries 1 and 2 were performed by the use of **1e** (0.3 mmol), those in Entries 3, 4, and 5 were by the use of **1g** (0.3 mmol), and those in Entries 6 and 7 by the use of **1h** (0.2 mmol), respectively. [b] Recovery of **1e** was 47% yield. [c] Penta-*o*-toluoate was used as the substrate. [d] N^6-*o*-toluoyl derivatives (**2g** and **3g**) were obtained.

afforded a thin layer chromatogram involving many spots absorbing ultraviolet light strongly[9]. Successful preparation of the **2e** - **3e** mixture (Entry 1) prompted us to establish more effective way for the preparation of **1e**, which has been prepared by way of **1f** through fusing with 2 mol. equiv. of 2,4-dinitrophenol in 93% yield[10]. A series of attempts at minimizing $\underline{N}^6$,$\underline{N}^6$-debenzoylation through controlling the amount of benzoyl chloride and the reaction temperature for the benzoylation reaction were all unsuccessful. However, as shown in Scheme 1, quenching the resulting mixture from benzoylation of adenosine containing **1f** with ammoniacal methanol (saturated at 0°C) through one-pot manner afforded **1e** in 96% yield. A similar treatment of the resulting mixture from $\underline{o}$-toluoylation of adenosine also gave **1g** in 93% yield. These treatments are easy to perform in a



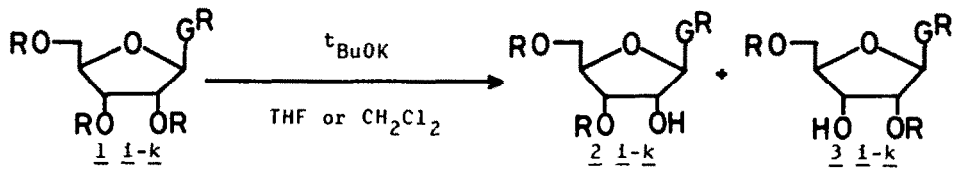
Scheme 1

one-pot manner in addition to an excellent yield of tetraaroyladenosines, which should be practically useful from the stand-point of nucleoside chemistry. Compound **1g** thus obtained was also subjected to the reaction with the $\underline{tert}$-butoxide; the reaction in THF gave a regioselectivity in the proportion of $\underline{N}^6$,3',5'- (**2g**) and $\underline{N}^6$,2',5'-tri-$\underline{o}$-toluoyladenosine (**3g**) lower than 5:1 even performing at -20°C, but the reaction in dichloromethane improved the selectivity up to 6.5:1 (Entry 3) - 6.7:1 (Entry 4) even at room temperature, and 8:1 (Entry 5) at -20°C in addition to high yields over 80%. A similar trend was observed in reaction time between Entries 3 and 4 arising from substrate concentration. The complex mixture-formation in the reaction of **1f** prompted us to perform the reaction of **1h** with the $\underline{tert}$-butoxide, which gave the mixture of **2g** and **3g** under both conditions in THF at -50°C and in dichloromethane at -20°C (Entry 7) in 66% and 83% yields, respectively, different from the reaction of **1f**. These reactions gave some TLC spots which absorbed ultraviolet light strongly but were not discolored on heating after spraying dilute methanolic sulfuric acid in addition to some small spots of other by-products.

In the last place, the reaction was examined by the use of $\underline{N}^2$,2',3',5'-tetrabenzoyl- (**1i**), -tetraisobutyryl- (**1j**), and $\underline{N}^2$-isobutyryl-2',3',5'-$\underline{O}$-benzoylguanosine (**1k**)(Table 4). As for the reaction of **1i** in THF, the solution obtained by heating up to 50°C was treated with the alkoxide when the inside temperature was lowered down to $\underline{ca.}$ 30°C on leaving the flask at room temperature; a 4.5:1 $\underline{N}^2$,3',5'- (**2i**) - $\underline{N}^2$,2',5'-tribenzoylguanosine (**3i**) mixture was obtained in 74% yield. The reaction in dichloromethane was, on the other hand, easy to perform due to its high solubility in the solvent; the yield was improved by lowering the temperature from room temperature (Entry 2) down to -20°C (Entry 3) to give **2i** (78% yield; **2i**, 67% yield obtained by crystallization from conc.

acetone solution by the addition of ethanol) as a sole product. Incidentally, isobutyryl group has been widely used as the protecting group for the 2-amino group of guanosine in the synthesis of

Table 4. Regioselective O-Dearoylation of N^2,2',3',5'-Tetraacylguanosines with Potassium tert-Butoxide[a]



1 i-k 2 i-k 3 i-k

Entry	Guanosine acyl derivative	R	^{t}BuOK (mol. equiv.)	Solvent (mL)	Reaction temperature (°C)	Reaction time (min)	Total yield (%) of 2 and 3	Proportion of 2 and 3
1	**1i**	Bz	3.5	THF (6)	50 ⟶ room temp.	3	74	4.5 : 1
2	**1i**	Bz	3.5	CH_2Cl_2(4)	room temp.	1 h 50 min	56	**2i**[b]
3	**1i**	Bz	5.0	CH_2Cl_2(1)	-20	15	78 (67[c])	—
4	**1j**	iBu[d]	5.8	CH_2Cl_2(2)	-20	15	65	5 : 1
5	**1j**	iBu[d]	5.0	THF (2)	-20	10	64	3.7 : 1
6	**1j**	iBu[d]	5.0[e]	THF (2)	-50	50	85	4 : 1
7	**1k**[f]	—	5.0	CH_2Cl_2(3)	room temp.	35	81 (65[c])	4.4 : 1

[a] The reactions in Entries 1, 5, and 6 were performed by the use of 0.3 mmol, that in Entry 2 was by the use of 0.15 mmol, those in Entries 3 and 7 were by the use of 0.2 mmol, and that in Entry 4 was by the use of 0.35 mmol of guanosine acyl derivatives. [b] No 3i was contained according to the ^{1}H-NMR spectrum. [c] Yield of 2'-OH derivatives obtained by crystallization of the resulting mixture. [d] iBu stands for isobutyryl group. [e] The alkoxide was portionwise added similar to Entry 5, but taking a time of 35 min. [f] In this case, N^2-isobutyryl-2',3',5'-tri-O-benzoylguanosine was used as the substrate.

nucleotide oligomers. Thus, the reactions of **1j** and **1k** with the alkoxide were performed as shown in Table 4. The reactions of **1j** gave a mixture in 65% (**2j**:**3j** = 5:1)(Entry 4) and 64% yields (**2j**:**3j** = 3.7:1)(Entry 5), on carrying them out in dichloromethane and in THF at -20°C, respectively. The yield was improved by performing the reaction in THF at -50°C to give a 4:1 **2j** - **3j** mixture in 85% yield (Entry 6); **2j** was crystallized from benzene, and **3j** was obtained by crystallizing the residue which was obtained by evaporation of the filtrate, from diethyl ether - methanol or from acetone - water. The reaction of **1k** in dichloromethane at room temperature, on the other hand, gave a 4.4:1 **2k** - **3k** mixture in 81% yield (**2k**, 65% yield obtained by crystallization)(Entry 7). Incidentally, **1k** was prepared from **1j** by a sequence of treatments, O-deisobutyrylation through sodium methoxide in 1:2 THF - methanol and O-benzoylation with benzoyl chloride in pyridine (95% overall yield based on guanosine).

Present work disclosed the excellent utility of potassium tert-butoxide for regioselective O-

deacylation of fully acylated purine and pyrimidine ribonucleosides, which afforded crystalline **2a** (80% yield), **2c** (78% yield), **2d** (72% yield), **2i** (67% yield), and **2k** (65% yield), by a simple procedure. These derivatives thus obtained are something expecting as an appropriate key compound for RNA-type oligonucleotide synthesis without use of 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl protecting group[2]. In the case of adenosine derivatives, the resulting diaroates were all obtained as glass but preponderant in the proportion of 2'-OH derivatives over the corresponding 3'-OH derivatives; other glassy di-O-aroyl derivatives in addition to these are also useful for nucleoside as well as nucleotide chemistry, e.g., deoxygenation[11]. It should be stated that regioselectivity of O-deacylation depends on the reaction temperature, and that the reaction proceeds faster on enhancing the concentration of a nucleoside derivative.

EXPERIMENTAL

General methods. Melting points were determined by a Yanagimoto Micro-Melting-Point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX200 and a Varian T-60 apparatus with tetramethylsilane as the internal standard. Particularly, the addition of methanol-d_4 improved the spectra recording on a Varian T-60 apparatus[5]. TLC was conducted on Merck Silica Gel 60 F_{254} through the solvent system of 9:1 chloroform - methanol. Column chromatography was performed with Wakogel C-300 (Wako Pure Chemicals, Co. Ltd.) and Merck Kieselgel 60 (70 - 230 mesh ASTM) through the solvent system of chloroform - methanol. Each proportion of 2 and 3 was calculated in terms of area-ratio of their anomeric proton signals. Potassium tert-butoxide was purchased from Merck. Dowex 50W (SO_3H form) ion-exchange resin was used after removing moisture as far as possible by washing with acetone. Elemental analyses were performed by a Perkin-Elmer 240-002 apparatus.

3',5'-Di-O-benzoyluridine (2a). To a solution of **1a** (170 mg, 0.3 mmol) in THF (8 mL), chilled at -56°C in dry-ice - acetonitrile bath, was added potassium tert-butoxide (118 mg, 1.05 mmol, 3.5 mol. equiv.) with vigorous stirring. After stirring for 26 min, the resulting mixture was quenched by the addition of Dowex-50W to neutralize the alkoxide by monitoring with a Toyo Roshi pH test paper (pH 1 - 11), and the resin was filtered off. The resin was further washed with acetone, chloroform, and then with methanol. The filtrate and the washings were combined to evaporate to a syrup, which was then subjected to chromatographic purification on a column of silica gel (30 - 40 mL) to give a 5:1 **2a** - **3a** mixture (130 mg, 94% yield). Crystallization of the resulting syrup gave **2a** (103 mg, 75% yield), m.p. 188.2 - 188.5°C (from methanol)[lit.[12] m.p. 187 - 189°C (from methanol)], ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 5.90 (1H, d, $J_{1',2'}$ 4.5 Hz, H-1'), 5.49 (1H, d, $J_{5,6}$ 8 Hz, H-5), and 5.36 (1H, m, H-3')(Entry 3 in Table 1).

Anal. Calcd for $C_{23}H_{20}O_8N_2$: C, 61.06; H, 4.46; N, 6.19. Found: C, 61.10; H, 4.48, N, 5.91.

Compound **3a** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.06 (1H, d, $J_{1',2'}$ 4.5 Hz, H-1'), and 5.48 (1H, d, $J_{5,6}$ 8 Hz, H-5).

Crystallization of the resulting mixture from the reaction of **1a** (0.6 mmol) prior to the chromatographic separation from ethanol - methanol, gave **2a** in 80% yield (Entry 4 in Table 1).

3',5'-Di-O-o-toluoyluridine (2b). A treatment of **1b** (120 mg, 0.2 mmol) under the conditions described in Entry 5 of Table 1, followed by a work-up, similar to those as above, gave a 6:1 **2b** - **3b** mixture (87 mg, 93% yield), whose crystallization gave **2b**, m.p. 81.5 - 82.5°C (from ethanol), ^{1}H-NMR($CDCl_3$ - CD_3OD): δ 8.1 - 7.7 (2H,m,phenyl protons ortho to the carbonyl group), 7.53 (1H, d, $J_{5,6}$ 8 Hz, H-6), 7.5 - 7.0 (6H, m, aromatic protons), 5.95 (1H, d, $J_{1',2'}$ 4 Hz, H-1'), 5.49 (1H, d, H-5), 5.5 - 5.2 (1H, m, H-3'), 4.8 - 4.4 (4H, m, H-2', 4', 5', and 5"), 2.55 (3H, s, CH_3), and 2.53 (3H, s, CH_3); ^{1}H-NMR ($CDCl_3$): δ 10.27 (1H, bs, NHCO).

Anal. Calcd for $C_{25}H_{24}O_8N_2$: C, 60.23; H, 5.25; N, 5.62. Found: C, 60.46; H, 5.43; N, 6.08.

Compound **3b** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.10 (1H, d, $J_{1',2'}$ 3.5 Hz, H-1').

N^4,3',5'-Tribenzoylcytidine (2c). A treatment of **1c** (130 mg, 0.2 mmol) under the conditions described in Entry 3 of Table 2, followed by a work-up, similar to that in the first experiment, gave a 5:1 **2c** - **3c** mixture (100 mg, 91% yield), whose crystallization gave **2c** (85 mg, 78% yield), m.p. 189.5 - 190.0°C (from chloroform - methanol)[lit.[13] m.p. 193 - 194°C (from ethanol)], ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.06 (1H, d, $J_{1',2'}$ 3 Hz, H-1') and 5.41 (1H, dd, $J_{2',3'} = J_{3',4'}$ 2 Hz, H-3').

Anal. Calcd for $C_{30}H_{25}O_8N_3$: C, 64.86; H, 4.54; N, 7.56. Found: C, 64.82; H, 4.56; N, 7.63.

Evaporation of the mother liquor of the above crystallization gave a residue, whose methanolic solution, on addition of a few drops of chloroform, afforded crystalline **3c**, m.p. 180.5 - 181.5°C [lit.[13] m.p. 177°C (from chloroform - petroleum ether)], ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.23 (1H, d, $J_{1',2'}$ 3 Hz, H-1') and 5.75 (1H, dd, $J_{2',3'}$ 4.5 Hz, H-2').

Anal. Calcd for $C_{30}H_{25}O_8N_3$: C, 64.86; H, 4.54; N, 7.56. Found: C, 65.16; H, 4.68; N, 7.42.

A treatment of **1c** (0.2 mmol) with potassium tert-butoxide (5 mol. equiv.) in dichloromethane (2.5 mL) at -20°C for 14 min, followed by a work-up without the chromatographic separation, similar to that above mentioned, and crystallization of the residue obtained by evaporation of the resul-

ting organic solution, from methanol gave 2c in 82% yield.

N^4,3',5'-Tri-o-toluoylcytidine (2d). A treatment of **1d** (358 mg, 0.5 mmol) under the conditions described in Entry 4 of Table 2, followed by a work-up, similar to that as above gave a >12:1 **2d** - **3d** mixture (248 mg, 83% yield), whose crystallization gave **2d** (215 mg, 72% yield), m.p. 122 - 123°C (from ethanol), ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 8.03 (1H, d, $J_{5,6}$ 7 Hz, H-6), 7.78 (3H, m, phenyl protons ortho to the carbonyl group), 5.87 (1H, d, $J_{1',2'}$ 2.5 Hz, H-1'), 5.23 (1H, dd, J 4.5 Hz and J 7 Hz, H-3'), 4.63 (4H, m, H-2', 4', 5', and 5"), and 2.50 (9H, m, CH_3 x 3).

Anal. Calcd for $C_{33}H_{31}O_8N_3$: C, 66.32; H, 5.23; N, 7.03. Found: C, 66.30; H, 5.27; N, 7.06.

Evaporation of mother liquor of the above crystallization, followed by crystallization of the residue from ethanol - chloroform, gave **3d**, m.p. 115 - 116°C, ^{1}H-NMR (CD_3OD): δ 8.01 (1H, d, $J_{5,6}$ 7.5 Hz, H-6), 7.79 (3H, m, phenyl protons ortho to the carbonyl group), 7.4 - 6.9 (6H, m, phenyl protons), 6.02 (1H, d, $J_{1',2'}$ 3 Hz, H-1'), 5.54 (1H, dd, $J_{2',3'}$ 4.5 Hz, H-2'), 2.52 (6H, 2 x s, CH_3 x 2), and 2.38 (3H, s, CH_3).

Anal. Calcd for $C_{33}H_{31}O_8N_3$: C, 66.32; H, 5.23; N, 7.03. Found: C, 66.30; H, 5.27; N, 7.06.

Alternative treatment of **1d** (358 mg, 0.5 mmol) in dichloromethane (4 mL) chilled down to -20°C with potassium tert-butoxide (327 mg, 2.9 mmol, 5.8 mol. equiv.) under vigorous stirring for 70 min, followed by quenching with acetic acid (0.2 mL), extraction with chloroform, drying over anhydrous sodium sulfate, evaporation to a syrup, and the column chromatographic separation, gave a syrup of **2d** almost pure judging from its ^{1}H-NMR spectrum (241 mg, 81% yield), whose crystallization gave crystalline **2d** (214 mg, 72% yield).

N^6,2',3',5'-Tetrabenzoyladenosine (1e) from Adenosine. To a solution of adenosine (267 mg, 1 mmol) in pyridine (6 mL), was dropwise added benzoyl chloride (0.79 mL, 6 mmol) at 0°C and the mixture was stirred at room temperature for 3 h, after which were added THF (4 mL), methanol (4 mL), and then ammoniacal methanol (saturated at 0°C)(3 mL). The resulting mixture was monitored by TLC, and further portion of the ammoniacal methanol (2 mL) was added after 10 min. After 20 min, TLC gave no spot of N^6,N^6,2',3',5'-pentabenzoyladenosine (**1f**), and the solution was evaporated by the use of aspirator. The residual solution was further evaporated through high vacuum rotary pump to remove pyridine. The residue was distributed between chloroform and 0.1 N hydrochloric acid, and the organic layer was successively washed with water, aqueous solution of sodium bicarbonate, and water. The organic solution was, after drying over anhydrous sodium sulfate as usual, evaporated to a syrup, which gave crystals of benzamide [m.p. 128.5 - 129.2°C] on dissolving in benzene. The filtrate was evaporated to a syrup and was subjected to the chromatographic purification on a column of silica gel to give **1e** (657 mg, 96% yield) as glass, ^{1}H-NMR ($CDCl_3$): δ 9.05 (1H, s, NHBz), 8.70, 8.18 (2H, 2 x s, H-2 and 8), 8.11, 8.02, 8.00, 7.93 (8H, 4 x d, J 7.3 Hz, J 7.6 Hz, J 6.6 Hz, and J 7.8 Hz, phenyl protons ortho to the carbonyl group), 7.6 - 7.3 (12H, m, phenyl protons), 6.50 (1H, d, $J_{1',2'}$ 5.1 Hz, H-1'), 6.43 (1H, dd, $J_{2',3'}$ 5.7 Hz, H-2'), 6.28 (1H, dd, $J_{3',4'}$ 4.6 Hz, H-3'), 4.94 (1H, dd, $J_{5',5"}$ 11.8 Hz, $J_{4',5'}$ 3.1 Hz, H-5'), 4.85 (1H, ddd, $J_{4',5"}$ 3.8 Hz, H-4'), and 4.71 (1H, dd, H-5"); the spectrum was superposable with that of an authentic specimen[10] and was also found to be chromatographically pure for the subsequent use.

N^6,3',5'-Tribenzoyladenosine (2e) from 1e. A treatment of **1e** (210 mg, 0.3 mmol) under the conditions described in Entry 1 of Table 3, followed by a work-up, similar to that as above gave a 5:1 **2e** - **3e** mixture (172 mg, 96% yield) as a glass, **2e** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.19 (1H, d, $J_{1',2'}$ 5.5 Hz, H-1'), 5.78 (1H, m, H-3'), 5.33 (1H, t, $J_{2',3'}$ 6 Hz, H-2'), and **3e** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.39 (1H, d, $J_{1',2'}$ 4 Hz, H-1') and 6.07 (1H, dd, $J_{2',3'}$ 5.5 Hz, H-2').

Anal. Calcd for $C_{31}H_{25}O_7N_5$: C, 64.24; H, 4.35; N, 12.09. Found: C, 63.92; H, 4.39; N, 11.92.

N^6,2',3',5'-Tetra-o-toluoyladenosine (1g) from Adenosine. Adenosine (1335 mg, 5 mmol) was, after azeotropic removal of moisture from pyridine, dissolved in pyridine (32 mL). To the resulting solution, was dropwise added o-toluoyl chloride (3.9 mL, 6 mol. equiv.) at 0°C, and the mixture was continued to stir at room temperature for 3 h although white crystalline materials precipitated out. Twenty mL of the ammoniacal methanol was added to the mixture, and further portions of 6 mL, after 50 min, and 6 mL, after 40 min, were added; the time-course of the conversion of N^6,N^6,2',3',5'-penta-o-toluoyladenosine (**1h**) to **1g** was monitored by TLC, and the reaction was over in 3 h. The resulting mixture was evaporated with aspirator to remove an excess ammonia, and then white mass of pyridinium chloride was removed by filtration. The filtrate was concentrated with a high vacuum rotary pump to a syrup, which was distributed between chloroform - saturated aqueous solution of sodium bicarbonate. White crystals (o-toluamide, m.p. 142°C) which are insoluble in chloroform were filtered off, and the filtrate was, after drying over anhydrous sodium sulfate, evaporated to a syrup, which was then subjected to the chromatographic purification to give **1g** (3435 mg, 93% yield) as a glass, ^{1}H-NMR ($CDCl_3$): δ 9.91 (1H, bs, NHTol), 8.63, 8.15 (2H, 2 x s, H-2 and 8), 8.02, 7.93, 7.86 (3 x 1H, 3 x d, J 7.3 Hz, J 7.8 Hz, J 8.1 Hz, phenyl protons ortho to the carbonyl group of O-o-toluoyl group), 7.60 (1H, d, J 7.3 Hz, ortho proton of N-o-toluoyl group), 7.4 - 7.1 (12H, m, phenyl protons), 6.9 (1H, d, $J_{1',2'}$ 5.4 Hz, H-1'), 6.34 (1H, t, $J_{2',3'}$ 5.4 Hz, H-2'), 6.20 (1H, dd, $J_{3',4'}$ 4.2 Hz, H-3'), 4.84 (1H, d, $J_{5',5"}$ 13.3 Hz, H-5'), 4.82 (1H, t, H-4'), 4.72 (1H, dd, $J_{4',5'}$ 5.6 Hz, H-5'), 2.60, 2.53, 2.52, and 2.47 (4 x 1H, 4 x s, CH_3 x 4).

Anal. Calcd for $C_{42}H_{37}O_8N_5$: C, 68.19; H, 5.04; N, 9.47. Found: C, 67.95; H, 5.01; N, 9.16.

N^6,3',5'-Tri-o-toluoyladenosine (2g) from 1g. A treatment of **1g** (222 mg, 0.3 mmol) under the conditions described in Entry 4 of Table 3, followed by a work-up, similar to that as above gave a 6.7:1 **2g** - **3g** mixture (160 mg, 86% yield), **2g** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 8.53, 8.20 (2H, 2 x s, H-2 and 8), 8.0 - 7.0 (12H, m, phenyl protons), 6.14 (1H, d, $J_{1',2'}$ 5 Hz, H-1'), 5.71 (1H, m, H-3'), 5.23 (1H, t, $J_{2',3'}$ 5 Hz, H-2'), 4.7 (3H, m, H-4', 5', and 5"), 2.64, 2.57, and 2.56 (9H, 3 x s, CH_3 x 3), **3d** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.34 (1H, d, $J_{1',2'}$ 4 Hz, H-1').

Anal. Calcd for $C_{34}H_{31}O_7N_5$: C, 65.69; H, 5.03; N, 11.27. Found: C, 65.47; H, 4.97; N, 10.99.

Compound 1h from Adenosine. To a solution of adenosine (267 mg, 1 mmol) in pyridine (6 mL), was dropwise added a solution of o-toluoyl chloride (0.91 mL, 7 mol. equiv.) in pyridine (2 mL) at 0°C taking a time of 5 min, and the mixture was stirred at room temperature for 4 h. The resulting mixture was quenched by the addition of water (1 mL), and stirred for 30 min, after which the mixture was evaporated to a syrup. The syrup was distributed between chloroform - dilute hydrochloric acid, and the organic layer was successively washed with water, saturated aqueous solution of sodium bicarbonate, and water. The organic solution was, after drying over anhydrous sodium sulfate, evaporated to a glass, which was then subjected to the chromatographic purification to give **1h** (845 mg, 98% yield) containing only a trace amount of **1g** according to TLC; ^{1}H-NMR ($CDCl_3$): δ 8.50, 8.07 (2 x 1H, 2 x s, H-2 and 8), 7.9 - 7.4, 7.3 - 6.8 (20H, m, o-toluoyl phenyl protons x 5), 6.43 - 6.0 (3H, m, H-1', 2', and 3'), 4.68 (3H, bs, H-4', 5', and 5"), and 2.6 - 2.4 (15H, m, CH_3 x 5). This material was used for the subsequent experiment.

Compound **2g** from **1h**. A treatment of **1h** (172 mg, 0.2 mmol) under the conditions described in Entry 7 of Table 3, followed by a work-up, similar to that as above gave a 6:1 **2g** - **3g** mixture (103 mg, 83% yield) as a glass, whose ^{1}H-NMR spectrum was superposable with that obtained above.

N^2,3',5'-Tribenzoylguanosine (**2i**) from N^2,2',3',5'-Tetrabenzoylguanosine (**1i**). A treatment of **1i**[12] (140 mg, 0.2 mmol) under the conditions described in Entry 3 of Table 4, followed by a work-up, similar to that as above gave a glass (93 mg, 78% yield), which was almost composed from **2i** based on the ^{1}H-NMR spectroscopy, and whose crystallization from hot acetone with partial evaporation of the solvent afforded **2i** (80 mg, 67% yield), m.p. 216.8 - 217°C, and 222.8 - 223.2°C (from acetone - ethanol)[lit[6]. m.p. 230 - 231.5°C (from methanol), as monohydrate], ^{1}H-NMR (DMSO-d_6 - CD_3OD): δ 6.11 (1H, d, $J_{1',2'}$ 6 Hz, H-1'), 5.78 (1H, m, H-3'), 5.15 (1H, t, $J_{2',3'}$ 6 Hz, H-2'), and ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.09 (1H, d, $J_{1',2'}$ 5 Hz, H-1') and 5.21 (1H, t, $J_{2',3'}$ 6 Hz, H-2').

Anal. Calcd for $C_{31}H_{25}O_8N_5$: C, 62.52; H, 4.23; N, 11.76. Found: C, 62.82; H, 4.50; N, 11.47.

The above mother liquor was evaporated to dryness, and dissolved in acetone without heating, followed by the addition of several drops of methanol, to give N^2,2',5'-tribenzoylguanosine (**3i**), m.p. 146.7 - 148.5°C, ^{1}H-NMR (DMSO-d_6 - CD_3OD): δ 6.34 (1H, d, $J_{1',2'}$ 4 Hz, H-1'), 6.00 (1H, dd, $J_{2',3'}$ 6 Hz, H-2'), and 5.09 (1H, t, H-3'), and ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.29 (1H, d, $J_{1',2'}$ 4 Hz, H-1').

Anal. Calcd for $C_{31}H_{25}O_8N_5 \cdot 1/2H_2O$: C, 61.58; H, 4.33; N, 11.59. Found: C, 61.48; H, 4.18; N, 11.68.

N^2,3',5'-Triisobutyrylguanosine (**2j**) from N^2,2',3',5'-Tetraisobutyrylguanosine (**1j**). A treatment of **1j**[4] (169 mg, 0.3 mmol) in THF (2 mL) at -50°C (chilled in a dry-ice - acetonitrile bath) with potassium tert-butoxide (168 mg, 1.5 mmol, 5.0 mol. equiv.) which was portionwise added taking a time of 35 min, followed by stirring for 25 min and similar work-up as above, gave a 4:1 **2j** - **3j** mixture (126 mg, 85% yield), a portion of which was crystallized to give **2j**, m.p. 123.5 - 124.7°C (from benzene), ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 7.98 (1H, s, H-8), 5.90 (1H, d, $J_{1',2'}$ 5.5 Hz, H-1'), 5.24 (1H, m, H-3'), 4.93 (1H, t, $J_{2',3'}$ 5.5 Hz, H-2'), 4.37 (3H, s, H-4', 5', and 5"), 2.70 (3H, dq, =CH), and 1.26 (18H, t, J 6 Hz, CH_3-C-CH_3 x 3).

Anal. Calcd for $C_{22}H_{31}O_8N_5 \cdot 1/2H_2O$: C, 52.59; H, 6.41; N, 13.94. Found: C, 52.49; H, 6.19; N, 13.69.

Crystallization of the residue obtained by evaporation of the above mother liquor, gave **3j**, m.p. 143.5 - 145.0°C (from diethyl ether - methanol) and 112 - 113°C (from acetone - water)[lit.[7] 121 - 122°C (acetone - hexane)], ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 7.89 (1H, s, H-8), 6.02 (1H, d, $J_{1',2'}$ 4 Hz, H-1'), 5.58 (1H, dd, $J_{2',3'}$ 5.5 Hz, H-2'), 4.64 (1H, t, $J_{3',4'}$ 5 Hz, H-3'), and 4.35 (2H, bs, H-5' and 5").

Anal. Calcd for $C_{22}H_{31}O_8N_5$: C, 53.54; H, 6.33; N, 14.19. Found: C, 53.13; H, 6.16; N, 13.90.

N^2-Isobutyryl-2',3',5'-tri-O-benzoylguanosine (**1k**) from Guanosine. Guanosine (568 mg, 2 mmol) was, after azeotropic removal of moisture from pyridine as usual, suspended in a mixture of pyridine (2 mL) and chloroform (15 mL), to which was added a solution of isobutyryl chloride (1.4 mL, 13 mmol) at room temperature taking a time of 5 min, and the mixture was continued to stir for 1.5 h. The resulting mixture was distributed between 1 N hydrochloric acid in a separating funnel, and the aqueous layer was extracted with chloroform three times. The organic layers were combined and successively washed with water, saturated aqueous solution of sodium bicarbonate, and water. The organic solution was, after drying over anhydrous sodium sulfate, evaporated to a syrup. The syrup was dissolved in toluene and dried (3 times) by the azeotropic technique, again. After evaporation, the resulting glass was dissolved in a mixture of THF (4 mL) and methanol (8 mL), and was added sodium methoxide (432 mg, 8 mmol). After stirring for 30 min, the resulting solution was quenched by the addition of Dowex 50W. After filtering off the resin, the filtrate was evaporated to a syrup, which was, after azeotropic removal of moisture from pyridine, dissolved in pyridine (10 mL). To the solution, was slowly added benzoyl chloride (1.16 mL, 10 mmol) and the resulting solution was stirred overnight at room temperature, and was then treated with ammoniacal methanol (saturated at 0°C)(10 mL) with stirring. After 30 min at room temperature, the solution was evaporated to a syrup which was then dissolved in chloroform and insoluble substance was filtered off through filter cell. The filtrate was distributed between chloroform and dilute hydrochloric acid. The organic layer was successively washed with water, saturated aqueous solution of sodium bicarbonate, and water. The organic solution was, after drying over anhydrous sodium sulfate, evaporated to a syrup, which was then dissolved in hot benzene. Allowing cool at room temperature, crystals of benzamide were precipitated out, and were filtered off. The filtrate was evaporated to a syrup, which was then subjected to the chromatographic purification, to give **1k** (1260 mg, 95% yield) as glass, ^{1}H-NMR ($CDCl_3$): δ 9.30 (1H, s, NHCO), 8.0 - 7.3 (16H, m, H-8 and C_6H_5 x 5), 6.53 (1H, t, $J_{2',3'} = J_{3',4'}$ 5.3 Hz, H-3'), 6.37 (1H, dd, $J_{1',2'}$ 3.2 Hz, H-2'), 6.91 (1H, d, H-1'), 4.92 (1H, dd, $J_{5',5"}$ 10.0 Hz, $J_{4',5'}$ 3.6 Hz, H-5'), 4.84 (1H, ddd, $J_{4',5"}$ 4.2 Hz, H-4'), 4.76 (1H, dd, H-5"), 2.73 (1H, quintet, J 6.8 Hz, CH_3-C-CH_3), 1.32, 1.30 (3H x 2, 2

x d, CH_3 x 2); a portion of this glass was crystallized from ethanol - water to give **1k**, m.p. 117.4 - 118°C.

Anal. Calcd for $C_{35}H_{31}O_9N_5$: C, 63.15; H, 4.69; N, 10.52. Found: C, 62.85; H, 4.81; N, 10.26.

N^2-Isobutyryl-3',5'-di-O-benzoylguanosine (**2k**) from **1k**. A treatment of **1k** (133 mg, 0.2 mmol) under the conditions as described in Entry 7 of Table 4, followed by a work-up, similar to that as above to give a 4.4:1 **2k** -**3k** mixture (91 mg, 81% yield), whose crystallization gave **2k** (73 mg, 65% yield), m.p. 187 - 188°C (from methanol - chloroform), ^{1}H-NMR ($CDCl_3$ - D_2O): δ 8.12 (2H, d, J 8.3 Hz, phenyl protons ortho to the carbonyl group), 7.97 (2H, d, J 7.1 Hz, phenyl protons ortho to the carbonyl group), 7.54 (1H, s, H-8), 7.6 - 7.3 (6H, m, phenyl protons including para and meta positions), 5.91 (1H, d, $J_{1',2'}$ 6.8 Hz, H-1'), 5.76 (1H, dd, $J_{2',3'}$ 4.8 Hz, $J_{3',4'}$ ca. 1 Hz, H-3'), 5.57 (1H, dd, H-2'), 5.17 (1H, dd, $J_{4',5'}$ 7.4 Hz, $J_{4',5''}$ 11.2 Hz, H-4'), 4.77 (1H, dd, $J_{5',5''}$ 4.2 Hz, H-5'), 4.67 (1H, dd, H-5"), 2.85 (1H, quintet, J 6.8 Hz, =CH), 1.38, and 1.30 (2 x 3H, 2 x d, CH_3 x 2), ^{1}H-NMR ($CDCl_3$): δ 9.58 (1H, s, NHCO) and 6.66 (1H, bd, HO-2').

Anal. Calcd for $C_{28}H_{27}O_8N_5$: C, 59.88; H, 4.85; N, 12.47. Found: C, 59.75; H, 4.83; N, 12.18.

Compound **3k** had ^{1}H-NMR ($CDCl_3$ - CD_3OD): δ 6.17 (1H, d, $J_{1',2'}$ 3 Hz, H-1').

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