*Tetrahedron* Vol. 41, No. 23, pp. 5503 to 5506, 1985
Printed in Great Britain.

0040-4020/85 $3.00+.00


# PARTIAL PROTECTION OF CARBOHYDRATE DERIVATIVES. PART 18. SIMPLE, PREPARATIVE PROCEDURE FOR 5'-O-ACYLRIBONUCLEOSIDES; HIGHLY REGIOSELECTIVE O-DEACYLATION AT 2' AND 3' POSITIONS OF FULLY ACYLATED PURINE AND PYRIMIDINE RIBONUCLEOSIDE THROUGH SODIUM METHOXIDE - THF SYSTEM

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(*Received in Japan* 22 *July* 1985)

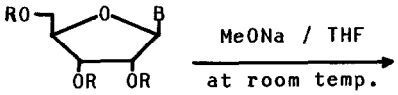
Abstract - A treatment of fully acylated purine and pyrimidine ribonucleosides with a small excess amount of sodium methoxide in THF at room temperature gave the corresponding 5'-acylates in excellent yields; N-acyl groups on the nucleic acid base moieties of adenosine and cytidine in addition to guanosine derivatives satisfactorily survived under the conditions used.

In a previous paper[1], we have reported utility of the sodium methoxide - THF system in regioselective 1-O-deacetylation of fully acetylated sugar derivatives bearing either furanose or pyranose ring structure; O-acetyl groups at the anomeric center was assumed to be the most active towards a nucleophile such as an alkoxide ion among the ester functions involved therein. On the other hand, the 2'-O-acyl groups of fully acylated ribonucleosides have also been demonstrated to be the most active towards a nucleophile such as hydrazine hydrate in 1:4 acetic acid - pyridine[2] and hydroxyaminium acetate in pyridine[3] among the O-acyl groups involved in the D-ribofuranosyl moieties. In these cases, the corresponding 5'-acylates were obtained in excellent yields on using an excess amount of a nucleophile towards the ribonucleoside acylates, although the procedure is still something tedious to perform so far as the work-up after the deacylation reaction. It has also been a point of important drawback of the procedure that N-acyl groups of the nucleic acid base moieties were undesirably removed by the nucleophiles preponderant over their O-acyl groups on the D-ribofuranosyl moieties. We were further interested in setting out to develop a more practical procedure for preparing the 5'-acylates from their peracylates in terms of the sodium methoxide - THF system[1], assuming the very simple procedure for regioselective 1-O-deacetylation of sugar peracylates; we now report the results thus obtained herein.

Ribonucleoside 5'-acylates have often been used as an important intermediate for the synthesis of ribonucleotide oligomers involving the 3'-5' phosphodiester linkage, e.g., Fromageot et al.[4] successfully prepared those by the use of the 2'-O-tetrahydropyran-2-yl derivatives of ribonucleosides which were derived from the 5'-acylates through the sequence of treatments, i.e., introduction of 2',3'-cyclic orthoester function, partial hydrolysis, fractional crystallization, tetrahydropyran-2-ylation, and then O-deacylation; Tener et al.[5] obtained a mixture of ribonucleoside 2'- and 3'-mono-

phosphates by the reaction with cyanoethyl phosphate - dicyclohexylcarbodiimide (DCC), followed by 5'-O-deacylation, in which phosphorylation was also occurred on their heterocyclic moiety. On the other hand, chemical modification of the 2' and 3' positions has also been continued from a viewpoint of developing new antiviral agents etc., e.g, Anzai et al.[6] prepared 2',3'-unsaturated adenosine derivatives by way of the corresponding 2',3'-dimesylate starting from the 5'-acylate.

As a model experiment, 2',3',5'-tri-O-benzoyluridine (**1a**)(0.5 mmol) was treated in a suspension of pulverized sodium methoxide (2.5 mol. equiv.) in tatrahydrofuran (THF, 6 mL) at room temperature for 1 h, after which the resulting mixture was quenched with Dowex-50W ($SO_3H$ type) and worked up to give 5'-O-benzoyluridine (**2a**)(83% yield; m.p. 168.8 - 169.5°C); the present procedure revealed the excellence in its simple work-up in addition to the high yield of **2a**. Other than THF, we attempted to use 1,4-dioxane, pyridine, acetonitrile, benzene, and methylene chloride as solvent, and only 1,4-dioxane was found useful for this purpose; THF was, however, preferred to use due to its wieldiness in purification and higher volatility. Quenching through the ion-exchange resin in the present procedure is more simple to perform compared with that through acetic acid used in regioselective 1-O-deacetylation of sugar peracetylates[1]. The procedure was thus extended




1 a~f 2 a~f

**a** : R = Bz, B = Ur
**b** : R = Bz, B = $Ad^{Bz}$
**c** : R = Bz, B = $Cy^{Bz}$
**d** : R = Tol, B = $Cy^{Tol}$
**e** : R = Bz, B = $Gu^{Bz}$
**f** : R = iBu, B = $Gu^{iBu}$

Table 1. Regioselective O-Deacylation of Fully Acylated Ribonucleosides Through Sodium Methoxide - THF System.[a]

| Entry | Ribonucleoside acylates | Sodium methoxide (mol. equiv.) | Reaction time ( h ) | Yield (%) of 5'-acylates | |
|---|---|---|---|---|---|
| 1 | **1a** | 2.5 | 1.0 | **2a** | 83 |
| 2 | **1b** | 4.0 | 0.3 | **2b** | 84 |
| 3 | **1c** | 3.0 | 1.0 | **2c** | 82 |
| 4 | **1d** | 4.5 | 0.5 | **2d** | 85 |
| 5 | **1e** | 4.0 | 3.0 | **2e** | 81 |
| 6 | **1f** | 6.0 | 4.5 | **2f** | 82 |

[a] See Experimental as for the details of reaction conditions.

to apply to other ribonucleoside peracylates, i.e., $N^6$,2',3',5'-tetrabenzoyladenosine (**1b**), $N^4$,2',3',5'-tetrabenzoylcytidine (**1c**), $N^4$,2',3',5'-tetra-o-toluoylcytidine (**1d**), $N^2$,2',3',5'-tetrabenzoylguanosine (**1e**), and $N^2$,2',3',5'-tetraisobutyrylguanosine (**1f**); conditions used and the results thus obtained are summarized in Table 1. $N^6$,5'-Dibenzoyladenosine (**2b**), $N^4$,5'-dibenzoylcytidine (**2c**), $N^4$,5'-di-o-toluoylcytidne (**2d**), $N^2$,5'-dibenzoylguanosine (**2e**), and $N^2$,5'-diisobutyrylguanosine (**2f**) were all obtained crystalline in 81 - 85% isolated yields, respectively.

Incidentally, the traditional procedures for preparation of ribonucleoside 5'-acylates are tedious to perform compared with the present procedure, i.e., 2',3'-O-isopropylidenation of ribonucleosides[7], followed by 5'-O-acylation and unmasking at 2' and 3' positions. 2',3'-O-Deiso-

propylidenation has considerably improved in terms of 90% aqueous trifluoroacetic acid[8], but still involves some drawback in the yield of the resulting 5'-acylates depending on the structure of their heterocyclic moieties, e.g., 5'-O-acetyl-8-bromo-2',3'-O-isopropylideneadenosine gave the 5'-acetate in a 51% yield[8]. This isopropylidene-method has usually been used for uridine derivatives[4,9].

On the whole, the present procedure is regioselective and practical enough for unmasking 2' and 3' position of fully acylated ribonucleosides without regard to the structure of nucleic acid base moieties, and, in addition, without harming the N-acyl groups of adenosine and cytidine derivatives.

## Experimental

Melting points were determined by a Yanagimoto Micro-Melting-Point apparatus, and are uncorrected. Proton NMR spectra were recorded on a Varian T-60 using tetramethylsilane as the internal standard, in chloroform-d, dimethyl sulfoxide-$d_6$, or a mixture with methanol-$d_4$ whose amount varied depending on each sample, unless otherwise noticed; the addition of methanol-$d_4$ conspicuously improved the spectra by sharpening each signal and made us possible to assign the structure of the nucleoside derivatives unambiguously and to confirm purity of the derivatives in terms of their anomeric proton signal sharpened. UV spectra were recorded on a Hitachi 220A model. TLC was conducted on Merck silica gel $60F_{254}$ by the use of chloroform - methanol (9:1, v/v); the development was repeatedly performed after drying when the separation of spots was unsatisfactory. Column chromatography was performed on silica gel of Wakogel C-300 (Wako Pure Chemical Co., Ltd.) by the use of chloroform - methanol system. Elemental analyses were performed by a Perkin-Elmer 240-002 apparatus. Sodium methoxide was purchased from Yoneyama Yakuhin Kogyo Co. Ltd. Dowex 50W resin was washed with acetone prior to use in order to remove moisture as far as possible.

5'-O-Benzoyluridine (**2a**) from 2',3',5'-Tri-O-benzoyluridine (**1a**): To a suspension of sodium methoxide (68 mg, 1.25 mmol) in THF (6 mL), was added **1a** (274 mg, 0.5 mmol), and the mixture was stirred at room temperature for 1 h. The resulting mixture was neutralized by quenching with Dowex 50W, monitoring with a pH test paper (pH 1 - 11; Toyo Roshi Co., Ltd.), and the resin was filtered off; the resin was washed with ethanol (ca. 20 mL). The filtrate and washings were combined and evaporated to dryness. The residue was chromatographed on a column (d: 3 cm) of the silica gel (25 - 30 mL). The fractions containing **2a** was combined and evaporated to give a half-crystalline mass, which, on crystallization, gave **2a** (144 mg, 83% yield), m.p. 168.8 - 169.5°C (from ethanol - water) [lit.[10] 169 - 170°C(from ethanol or chloroform)], $^1$H-n.m.r. ($CDCl_3$-$CD_3OD$-TMS): δ 7.7 (2H, dd, J 2 Hz and 7.5 Hz, ortho protons of Bz group , 7.35 (1H, d, $J_{5,6}$ 8 Hz, H-6), 7.47 - 7.03 (3H, m, meta and para protons of Bz group), 5.66 (1H, d, $J_{1'2'}$ 2 Hz, H-1'), and 5.34 (1H, d, H-5).

$N^6$-Benzoyl-5'-O-benzoyladenosine (**2b**) from $N^6$-benzoyl-2',3',5'-tri-O-benzoyladenosine[11] (**1b**): A similar treatment of **1b** (342 mg, 0.5 mmol) as above described gave **2b** [216 mg, 91% yield as a glass, $^1$H NMR (JEOL JNM FX-200, $CDCl_3$-$CD_3OD$-TMS): δ 8.67, 8.23 (2H, 2 x s, H-2 and 8), 8.06, 8.02 (4H, 2 x d, ortho protons of two Bz groups), 7.6 - 7.3 (6H, m, meta and para protons of two Bz groups), 6.09 (1H, d, $J_{1',2'}$ 3.9 Hz, H-1'), 4.08 (1H, dd, $J_{2',3'}$ 5 Hz, H-2'), 4.75 (1H, dd, $J_{4',5"}$ 4.5 Hz, H-5"), 4.57 (1H, t, $J_{3',4'}$ 5 Hz, H-3'), and 4.43 (1H, ddd, H-4'), and ($CDCl_3$-TMS): 9.53 (1H, bs, NH), and 2.6 (2H, bs, 2 x OH). Anal. Calcd for $C_{24}H_{21}O_6N_5$: C, 60.62; H, 4.45, N, 14.73. Found: C, 60.34; H, 4.77; N, 14.49. $^1$H NMR spectrum of crystals of **2b** (199 mg, 84% yield)[m.p. 160 - 161°C (from methanol) and Anal. Found: C, 60.49; H, 4.52; N, 14.70] was superposable with that of the above glass and UV (EtOH): $\lambda_{max}$ 280 nm (ε 26000) and $\lambda_{min}$ 251 nm (ε 14000).

$N^4$-Benzoyl-5'-O-benzoylcytidine (**2c**) from $N^4$-bezoyl-2',3',5'-tri-O-benzoylcytidine[12] (**1c**): A similar treatment of **1c** (322 mg, 0.5 mmol), followed by a similar work-up, gave **2c** (185 mg, 82% yield), m.p.182 - 182.5°C (from ethanol-chloroform)[lit.[13] m.p. 185 - 186°C (from ethanol)], $^1$H NMR (DMSO-$d_6$-$CD_3OD$-TMS): δ 8.17 (1H, d, $J_{5,6}$ 8 Hz, H-6), 8.1 - 7.4 (10H, m, 2 x Bz), 7.34 (1H, d, H-5), 5.87 (1H, d, $J_{1',2'}$ 1.5 Hz, H-1'), 4.74 - 4.58 (2H, m, H-2' and 3'), and 4.42 - 4.16 (3H, m, H-4', 5', and 5"). An attempt at isolating **2c** through partial evaporation of neutralized solution brought about its crystallization to give **2c** (81 mg, 64% yield) starting from **1c**. Anal. Calcd for $C_{23}H_{21}O_7N_3$: C, 61.19; H, 4.69; N, 9.31. Found: C, 61.02; H, 4.75; N, 9.53.

$N^4$-o-Toluoyl-2',3',5'-tri-O-o-toluoylcytidine (**1d**) from cytidine: This preparation was performed according to the method for that of the correspoding benzoate[12] as follows. Cytidine (2 g, 8.2 mmol) was suspended in pyridine and co-evaporated; this operation was repeated two times more. The resulting syrup was dissolved in pyridine (40 mL) and chilled in an ice-bath, to which was dropwise added o-toluoyl chloride (5.6 mL, 10.7 mmol) and the mixture was stirred for 1.5 h. The resulting mixture was then quenched by the addition of methanol (2 mL), and, after 15 min, was evaporated; the residue was distributed between 0.2 M hydrochloric acid and chloroform, and the organic layer was evaporated and chromatographed on a column of silica gel. The fraction containing the objective **1d** was evaporated to a syrup, which was then crystallized to give **1d** (5.38 g, 92% yield), m.p. 85 - 86.5°C (from methanol-chloroform-n-hexane), $^1$H NMR ($CDCl_3$-$CD_3OD$-TMS): δ 7.92 (1H, d, $J_{5,6}$ 7.5 Hz, H-6), 7.8 - 6.9 (17H, m, 2 x o-toluoyl ring protons and H-5), 6.19 (1H, d, $J_{1',2'}$ 2 Hz, H-1'), 5.79 - 5.64 (2H, m, H-2' and 3'), 4.78 - 4.58 (3H, m, H-4', 5', and 5"), and 2.62 - 2.37 (12H,

4 x o-toluoyl $CH_3$). Anal. Calcd for $C_{41}H_{37}O_9N_3$: C, 68.80; H, 5.21; N, 5.87. Found: C, 68.50; H, 5.25; N, 6.12.

$N^4$-o-Toluoyl-5'-O-o-toluoylcytidine (2d) from $N^4$-o-toluoyl-2',3',5'-tri-O-o-toluoylcytidine (1d): A similar treatment of 1d (358 mg, 0.5 mmol) with MeONa (122 mg, 2.26 mmol; 4.5 mol. equiv.) in THF (5 mL), followed by a similar work-up as above, gave 2d (204 mg, 85% yield), m.p. 187.5 - 188.0°C (from ethanol), UV (EtOH): $\lambda_{max}$ 302 nm (ε 10600) and 256 nm (ε 22400), $\lambda_{min}$ 293 nm (ε 10000) and 245 nm (ε 19600), $^1$H NMR ($CDCl_3$-$CD_3OD$-TMS): δ 8.12 (1H, d, $J_{5,6}$ 7.5 Hz, H-6), 7.80 (1H, dd, J 3 Hz and 7 Hz, aromatic protons ortho to the carbonyl group), 7.5 - 7.1 (8H, m, aromatic protons and H-5), 5.82 (1H, d, $J_{1',2'}$ 1 Hz, H-1'), 4.8 - 4.1 (5H, m, H-2', 3', 4', 5', and 5"), 2.59 (3H, s, $CH_3$), and 2.47 (3H, s, $CH_3$). Anal. Calcd for $C_{25}H_{25}O_7N_3$: C, 62.62; H, 5.26; N, 8.77. Found: C, 62.71; H, 5.26; N, 8.62.

$N^2$-Benzoyl-5'-O-benzoylguanosine (2e) from $N^2$-benzoyl-2',3',5'-tri-O-benzoylguanosine (1e): A similar treatment of 1e (700 mg, 1.0 mmol), followed by a similar work-up, gave 2e (396 mg, 81% yield), m.p. 225 - 227°C (from ethanol - methanol)[lit.[2] m.p. 235 - 236°C (from methanol)], $^1$H NMR (DMSO-$d_6$-$D_2O$-TMS): δ 8.15 (1H, s, H-8), 5.83 (1H, d, $J_{1',2'}$ 5.0 Hz, H-1'), and 4.50 (1H, t, $J_{2',3'}$ 5.0 Hz, H-2').

$N^2$-Isobutyryl-5'-O-isobutyrylguanosine (2f) from $N^2$-isobutyryl-2',3',5'-tri-O-isobutyrylguanosine (1f): A similar treatment of 1f (564 mg, 1.0 mmol) in THF (10 mL) with NaOMe (324 mg, 6.0 mmol), which was portionwise added to the reaction mixture from time to time during 4 h, at room temperature with vigirous stirring, after which the resulting mixture was further stirred for 30 min, A similar work-up gave 2f (347 mg, 82% yield), m.p. 200 - 200.5°C (from benzene - methanol), $^1$H NMR ($CDCl_3$-$CD_3OD$-TMS): δ 7.81 (1H, s, H-8), 5.87 (1H, d, $J_{1',2'}$ 4 Hz, H-1'), 4.66 (1H, t, $J_{2',3'}$ 4 Hz, H-2'), 4.55 - 4.23 (4H, m, H-3', 4', 5', and 5"), 2.64 (2H, dq, J 7 Hz, 2 x $Me_2CH$), 1.28 (6H, d, $CH_3$). Anal. Calcd for $C_{18}H_{25}O_7N_5$: C, 51.05; H, 5.95; N, 16.54. Found: C, 51.25; H, 5.86; N, 16.62.

**ACKNOWLEDGEMENT**

The authors thank Mrs. Akiko Hasegawa, Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, for the elemental analyses, and Ministry of Education, Japanese Government, for the Scientific Research Grant-in-aid (No. 59430006).

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