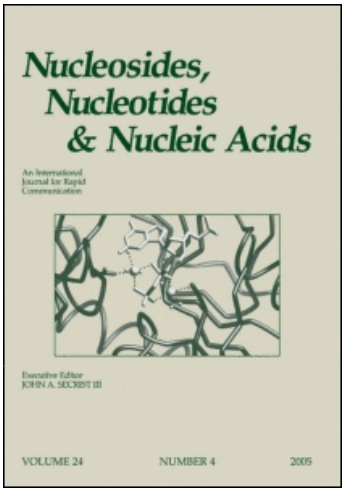

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Metal Carbonate-Mediated Complete Deacylation of Polyacyl Protected Nucleosides

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METAL CARBONATE-MEDIATED COMPLETE DEACYLATION OF POLYACYL PROTECTED NUCLEOSIDES

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Abstract

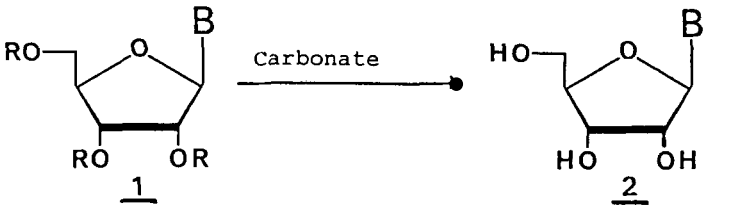
Treatment of poly-acetyl or -benzoyl protected ribonucleosides (1a-i) and 2'-deoxyribonucleosides (3a-d) with metal carbonates such as $NaHCO_3$ or Na_2CO_3 in MeOH gave the corresponding deacylated free ribonucleosides (2a-d and 4a-b) in excellent high yields.

Protection or deprotection of hydroxyl and amino groups is one of the most important procedures in the field of sugar, nucleoside, and nucleotide chemistry. In general, acetylation and benzoylation are most often used for protecting hydroxyl groups or amino groups in mono- and oligo-saccharides syntheses, as well as in nucleoside and nucleotide syntheses. Acyl groups of protected esters have generally been removed by treatment with alcoholic ammonia or strong bases such as metal hydroxides or alkoxides. However, when strong bases are used, the protecting ester groups can migrate, or other protecting groups can be untimely removed.[1] The selective or regioselective partial deacylation of protecting groups

in carbohydrate compounds has been reported and reviewed.[2] Recently, cyanide-catalyzed removal of acetate (and benzoate) groups of polyacylated sugars and acylated sugar moieties of nucleosides has been done under neutral conditions.[3]

We now wish to report the complete deacylation reaction of poly-acetyl or -benzoyl protected nucleosides (1a-i and 3a-d) in the presence of metal carbonates in MeOH. Deblocking of base-sensitive nucleoside triacetates by $NaHCO_3$ in MeOH has been reported earlier by Bambury and Wemple et al.[4]

The deacylation reaction was first examined by the use of 2',3',5'-tri-O-acetyluridine (1a) and N^4,2',3',5'-tetra-acetylcytidine (1b) under several reaction conditions. Treatment of 1a or 1b with 1.0 or 2.0 mol equiv.



1

2

1a R=Ac, B=U
b R=Ac, B=C^{NHAc}
c R=Ac, B=A
d R=Ac, B=A^{NHAc}
e R=Ac, B=G^{NHAc}
f R=Bz, B=U
g R=Bz, B=C^{NHBz}
h R=Bz, B=A^{NHBz}
i R=Bz, B=G^{NHBz}

2a B=U
b B=C
c B=A
d B=G

of $NaHCO_3$ or 1.0 mol equiv. of Na_2CO_3 in MeOH at ambient temperature or 50 °C (in an oil bath) gave the completely deacylated product (uridine 2a and cytidine 2b) in excellent high yields. These results are summarized in Table I.

It was found that when H_2O was used as a solvent, deacylation reaction did not proceed completely even for a

Table I. Deacylation of Pyrimidine Ribonucleoside Acetates[a]

Run	Compd.	Carbonate (mol. eq.)	Solvent	Temp. (°C)	Time (h)	Product 2 (yield %)[b]
1	1a	$NaHCO_3$ (1.0)	MeOH	r. t.	55	2a (97)
2	1a	$NaHCO_3$ (2.0)	MeOH	r. t.	45	2a (99)
3	1a	$NaHCO_3$ (2.0)	MeOH	50	7	2a (99)
4	1a	$NaHCO_3$ (2.0)	MeOH/H_2O (99:1)	50	7	2a (99)
5	1a	$NaHCO_3$ (2.0)	MeOH/H_2O (9:1)	50	8	2a (98)
6	1a	$NaHCO_3$ (2.0)	MeOH/H_2O (4:1)	50	8	2a (97)
7	1a	$NaHCO_3$ (2.0)	MeOH/H_2O (3:2)	50	10	2a (98)
8	1a	$NaHCO_3$ (2.0)	H_2O	r. t.	30	2a (–[c])
9	1a	$NaHCO_3$ (2.0)	H_2O	50	30	2a (–[c])
10	1a	Na_2CO_3 (1.0)	H_2O	r. t.	30	2a (–[c])
11	1a	Na_2CO_3 (1.0)	H_2O	50	30	2a (–[c])
12	1a	Na_2CO_3 (1.0)	MeOH	r. t.	7	2a (99)
13	1a	Na_2CO_3 (1.0)	MeOH	50	2	2a (99)
14	1a	$CaCO_3$ (1.0)	MeOH	r. t.	24	2a (0[d])
15	1a	$CaCO_3$ (1.0)	MeOH	50	48	2a (–[e])
16	1b	$NaHCO_3$ (2.0)	MeOH	r. t.	9	2b (99)
17	1b	$NaHCO_3$ (2.0)	MeOH	50	2	2b (99)
18	1b	Na_2CO_3 (1.0)	MeOH	r. t.	0.5	2b (99)
19	1b	$CaCO_3$ (1.0)	MeOH	r. t.	24	2b (0[f])
20	1b	$CaCO_3$ (1.0)	MeOH	50	24	2b (–[g])

[a] 1a–b (0.5 mmol), Solvent (15 mL). [b] Yields of purified products. [c] Starting compound remained mainly unchanged, small amount of 2a and several partially deacylated compounds were formed (by TLC analysis). [d] Starting compound was mainly unchanged and small amounts of several deacylated compounds were formed (by TLC analysis). [e] Trace amount of 1a remained; monoacetylated compound and 2a were present in the reaction mixture (about 1:1, by TLC analysis). [f] Small amount of 1b remained; several deacylated compounds were formed (by TLC analysis). [g] Monoacetylated compound and 2b were mainly present in the reaction mixture (about 2:3, by TLC analysis).

Table II. Deacylation of Purine Ribonucleoside Acetates[a]

Run	Compd.	Carbonate (mol. eq.)	Temp. (°C)	Time (h)	Product 2 (yield %)[b]
1	1c	$NaHCO_3$ (2.0)	r.t.	12	2c (98)
2	1c	$NaHCO_3$ (2.0)	50	2	2c (99)
3	1c	Na_2CO_3 (1.0)	r.t.	0.5	2c (99)
4	1d	$NaHCO_3$ (2.0)	r.t.	12	2c (99)
5	1d	$NaHCO_3$ (2.0)	50	2	2c (99)
6	1d	Na_2CO_3 (1.0)	r.t.	40 min.	2c (99)
7	1e	$NaHCO_3$ (2.0)	r.t.	120	2d (–[c])
8	1e	$NaHCO_3$ (2.0)	50	14	2d (97)
9	1e	Na_2CO_3 (1.0)	r.t.	7	2d (98)
10	1e	Na_2CO_3 (1.0)	50	2	2d (98)

[a] 1c–e (0.5 mmol), MeOH (15 mL). [b] Yields of purified products. [c] Product 2d was mainly formed; trace ammount of monoacetylated compound present in the reaction mixture (TLC analysis).

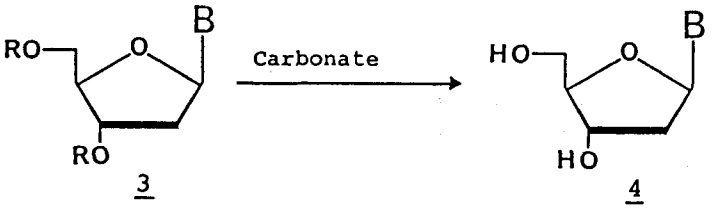
30 h reaction time at elevated temperature. Namely, starting nucleoside (1a) was predominant with a small amount of product (2a) and several partially deacylated compounds present in the reaction mixture as revealed by TLC (Table I, Run 8–11). When MeOH was used, elevation of the reaction temperature resulted in reaction rate acceleration. However, increasing the quantity of H_2O in the solvent (MeOH) resulted in a rate decrease (Table I, Run 4–7).

Secondly, the deacylation reaction was examined using 2',3',5'-tri-O-acetyladenosine (1c), N^6,2',3',5'-tetra-acetyladenosine (1d), and N^2,2',3',5'-tetra-acetyl-guanosine (1e). Deacylation of 1c–e was effected with $NaHCO_3$ or Na_2CO_3 in MeOH at ambient temperature or 50 °C. Corresponding free ribonucleosides (adenosine 2c and guanosine 2d) were obtained in excellent high yields (Table II). This complete deacylation of 1b–d was more rapid than deacylation of 1a or 1e. In contrast, deacy–

lation of 1a or 1b with 1.0 mol equiv. of $CaCO_3$ in MeOH at ambient temperature proceeded slowly in both and gave no free nucleosides (2a or 2b) within a 24 h reaction time (Table I, Run 14 and 19).

Thirdly, deacylation of benzoyl protected nucleosides was examined using 2',3',5'-tri-O-benzoyluridine (1f), N^4,2',3',5'-tetrabenzoylcytidine (1g), N^6,2',3',5'-tetrabenzoyladenosine (1h), and N^2,2',3',5'-tetrabenzoylguanosine (1i) (Table III). As in 1a-e deacetylation, complete debenzoylation of 1f-i was also effected with $NaHCO_3$ or Na_2CO_3 in MeOH at ambient temperature or 50 °C. Corresponding free nucleosides (2a-d) were obtained in excellent high yields. Using aqueous MeOH as a solvent resulted in a rate decrease (Table III, Run 3).

Finally, deacylation of some poly-acetyl and -benzoyl protected pyrimidine or purine 2'-deoxyribonucleosides was



3 4

3a R=Ac, B=C^{NHAc} c R=Ac, B=A^{NHAc} 4a B=C

b R=Bz, B=C^{NHBz} d R=Bz, B=A^{NHBz} b B=A

examined using N^4,3',5'-triacetyl-2'-deoxycytidine (3a), N^4,3',5'-tribenzoyl-2'-deoxycytidine (3b), N^6,3',5'-triacetyl-2'-deoxyadenosine (3c), and N^6,3',5'-tribenzoyl-2'-deoxyadenosine (3d) (Table IV).
Complete deacylation of 3a-d was also effected under same reaction conditions for 1a-i. Corresponding free nucleosides (2'-deoxycytidine 4a and 2'-deoxyadenosine (4b) were obtained in excellent high yields.

From a comparison of the results in Table I, II, III, and IV, the deacylation reactions with Na_2CO_3 proceeded more readily to completion than those with $NaHCO_3$. Also,

Table III. Deacylation of Ribonucleoside Benzoates[a]

Run	Compd.	Carbonate (mol. eq.)	Temp. (°C)	Time (h)	Product 2 (Yield %)[b]
1	1f	$NaHCO_3$ (2.0)	r. t.	120	2a (–[c])
2	1f	$NaHCO_3$ (2.0)	50	20	2a (97)
3[d]	1f	$NaHCO_3$ (2.0)	50	35	2a (99)
4	1f	Na_2CO_3 (1.0)	r. t.	20	2a (98)
5	1f	Na_2CO_3 (1.0)	50	6	2a (99)
6	1g	$NaHCO_3$ (2.0)	r. t.	84	2b (99)
7	1g	$NaHCO_3$ (2.0)	50	5	2b (99)
8	1g	Na_2CO_3 (1.0)	r. t.	6	2b (99)
9	1g	Na_2CO_3 (1.0)	50	1.5	2b (98)
10	1h	$NaHCO_3$ (2.0)	r. t.	45	2c (97)
11	1h	$NaHCO_3$ (2.0)	50	9	2c (97)
12	1h	Na_2CO_3 (1.0)	r. t.	12	2c (96)
13	1h	Na_2CO_3 (1.0)	50	3	2c (98)
14[e]	1i	$NaHCO_3$ (2.0)	r. t.	120	2d (–[f])
15[e]	1i	$NaHCO_3$ (2.0)	50	120	2d (93)
16[e]	1i	Na_2CO_3 (1.0)	r. t.	110	2d (95)
17[e]	1i	Na_2CO_3 (1.0)	50	24	2d (92)

[a] 1f–i (0.5 mmol), MeOH (15 mL). [b] Yields of purified products. [c] Free nucleoside was mainly formed with a trace amount of monobenzoylated compound present in the reaction mixture (TLC analysis). [d] MeOH/H_2O (3:2, 15 mL) was used. [e] MeOH of 25 mL was used. [f] Free nucleoside was mainly formed and small (or trace) amount of several partially debenzoylated compounds present in the reaction mixture (TLC analysis).

cleavage of acetates was more rapid than cleavage of corresponding benzoates. It seems that the deacylation rates between corresponding ribonucleosides (1b, d, g, and h) and 2'-deoxyribonucleosides (3a–d) derivatives were not so difference; except in the case of deacylation of benzoylcytidines (1g and 3b), and deacylation of benzoyl-adenosines (1h and 3d) by $NaHCO_3$ at ambient temperature.

Table IV. Deacylation of Polyacylated 2'-Deoxynucleosides[a]

Run	Compd.	Carbonate (mol. eq.)	Temp. (°C)	Time (h)	Product 4 (Yield %)[b]
1	3a	$NaHCO_3$ (2.0)	r.t.	10	4a (98)
2	3a	$NaHCO_3$ (2.0)	50	3	4a (99)
3	3a	Na_2CO_3 (1.0)	r.t.	40 min.	4a (99)
4	3b	$NaHCO_3$ (2.0)	r.t.	44	4a (97)
5	3b	$NaHCO_3$ (2.0)	50	5.5	4a (99)
6	3b	Na_2CO_3 (1.0)	r.t.	3.5	4a (98)
7	3b	Na_2CO_3 (1.0)	50	40 min.	4a (99)
8	3c	$NaHCO_3$ (2.0)	r.t.	13	4b (98)
9	3c	$NaHCO_3$ (2.0)	50	2	4b (98)
10	3c	Na_2CO_3 (1.0)	r.t.	1	4b (99)
11	3d	$NaHCO_3$ (2.0)	r.t.	62	4b (96)
12	3d	$NaHCO_3$ (2.0)	50	8	4b (98)
13	3d	Na_2CO_3 (1.0)	r.t.	15	4b (99)
14	3d	Na_2CO_3 (1.0)	50	3	4b (99)

[a] 3a–d (0.5 mmol), MeOH (15 mL). [b] Yields of purified products.

(Table III, Run 6, 8, and 9 : Table IV, Run 4, 6, and 7; Table III, Run 10 : Table IV, Run 11). Further, the reaction rate was greatly influenced by the particular nucleoside derivative in the order of cytidine) adenosine) uridine) guanosine.

In summary, this metal carbonate-mediated complete deacylation reaction of polyacyl protected nucleosides described herein has some positive features. Excellent high yields (almost quant.) of free nucleosides are produced. Inexpensive reagents are easily and safely handled. Reaction and workup procedures are simple and straightforward. However, this metal carbonate-mediated deacylation reaction could not control for regioselective deacylation. This procedure may be generally useful for deacylation of a variety of acyl protected organic compounds.

Experimental Section

General Procedure

Melting points were determined on a hot plate stage apparatus and are uncorrected. Proton NMR spectra were recorded with a Jeol GX-400 spectrometer at 400 MHz in Me_2SO-d_6 with Me_4Si as internal standard. Electron-impact high-resolution mass spectra were determined by the Mass Spectroscopy Laboratory, Kinki University, on Jeol HX-100 spectrometer. Elemental analyses were performed by the Analytical Center of Dainippon Pharmaceutical Co., Ltd.. UV spectra were obtained in MeOH with a Hitachi 323 spectrophotometer. MeOH for deacylation reaction was refluxed over and distilled from calcium hydride.
Starting acylated riboucleosides were prepared from corresponding free ribonucleosides by using Ac_2O/pyridine, Ac_2O/4-N, N-dimethylaminopyridine, or benzoyl chloride/pyridine system.[5] Chromatographic solvents used: A, EtOAc/*i*-PrOH/H_2O (4:1:2, upper phase); B, EtOAc/MeOH/H_2O (4:2:1).
Reaction was monitored by TLC with Merck silica gel 60 F-254 sheets. Merck silica gel 60 (230-400 mesh) was used for silica gel column chromatography.

Deacylation of acetyluridine (1a)

A mixture of 1a (0.5 mmol), metal carbonate (1.0 or 2.0 mol equiv.) and solvent (15 mL) was stirred at ambient temperature or 50 °C (in an oil bath) (monitored by TLC solvent A). To the mixture 5 mL of H_2O was added followed by neutralization (pH ~ 6) with Dowex 50W-X8 (H^+) ion-exchange resin. The resin was filtered and washed with 50 % aqueous MeOH (15 mL). The combined filtrate and washings were evaporated and coevaporated with EtOH/EtOAc/toluene (1:1:2, 15 mL x 2) under reduced pressure to dryness to give TLC (solvent A) pure 2a as crystalline powder (see Table I for yields). Further, a small portion was crystallized from MeOH/Et_2O (diffusion) to yield an analytical sample. m. p. 2a, 168-169.5 °C.

Deacylation of acetylcytidines (1b and 3a)

Deacylation of 1b and 3a was carried out by the same procedure used for 1a (monitored by TLC, solvent B). After the addition of H_2O (5 mL) to the reaction mixture, the resulting solution was applied to a column (1.5 x 20 cm) of Dowex 1 (OH^-) ion-exchange resin (100-200 mesh). The product was eluted with 50 % aqueous MeOH, and appropriate fractions were pooled and evaporated. The residue was coevaporated with EtOH/EtOAc/toluene (1:1:2, 15 mL x 2) to give 2b or 4a (see Table I and IV for yields) as a homogeneous crystalline powder (TLC solvent B). A small portion was crystallized from EtOH (2b) or EtOH/Et_2O (diffusion, 4a) to yield an analytical sample.
m.p. 2b, 203-204 °C dec.; 4a, 193-197 °C dec..

Deacylation of acetyladenosines (1c, 1d, and 3c)

Deacylation of 1c, 1d, and 3c used the same reaction and workup procedures as for 1b and 3a. TLC (solbent A) homogeneous crystalline powder of 2c or 4b was obtained (see Table II and IV for yields), and an analytical sample was further crystallized from MeOH (2c) or MeOH/Et_2O (4b).
m.p. 2c, 225-227 °C dec.; 4b, 179-181 °C.

Deacylation of acetylguanosine (1e)

Deacylation of 1e was carried out by the same procedures used for 1a-d, 3a, or 3c (monitored by TLC, solvent B). To the mixture 15 mL of H_2O was added, and the solution was treated with Dowex 50W-X8 (pyridinium form)[6] ion exchange resin. The resin was filtered and washed with H_2O (20 mL). The combined filtrate and washings were evaporated, and coevaporated with EtOH/EtOAc/toluene (1:1:2, 15 mL x 2) to give TLC (solvent B) pure 2d (see Table II for yields) as crystalline powder. A small portion was crystallized from MeOH/H_2O to yield an analytical sample.
m.p. 2d, 227-234 °C dec..

Deacylation of benzoyl nucleosides (1f-i, 3b, and 3d)

Deacylation of 1f-i, 3b, and 3d employed the same procedures used for the corresponding acetylated nucleosides.

In the workup of 1f, the reaction mixture was evaporated and coevaporated with solvent A (15 mL x 2). The resulting residue was dissolved in a minimum volume of solvent A, and this solution was applied to a dry-packed silica gel column (1.5 x 25 cm). After elution with solvent A, appropriately pooled fractions were evaporated then coevaporated with EtOH/EtOAc/toluene (1:1:2) to give pure 2a as a dry crystalline powder. An analytical sample was crystallized from MeOH/Et_2O (diffusion). Workup of 1g-i, 3b, and 3d was carried out by the same procedures as corresponding nucleosides 1b-e, 3a, or 3c (see Table III, IV for yields).

Analytical data (^1H NMR, MS, UV-spectra, elemental analyses, and melting points) on these completely deacylated free ribonucleosides (2a-d and 4a-b) agreed with commercialy available authentic compounds.

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