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ISOMERIZATION OF *tert*-BUTYLDIMETHYLSILYL PROTECTING GROUPS IN RIBONUCLEOSIDES*

KELVIN K. OGILVIE AND DOUGLAS W. ENTWISTLE
Department of Chemistry, McGill University, Montreal, Quebec H3A 2K6 (Canada)
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ABSTRACT

The *tert*-butyldimethylsilyl group undergoes isomerization between O-2′ and O-3′ in ribonucleosides in solution. Isomerization is most rapid in protic solvents and extremely slow in such solvents as dry dimethyl sulfoxide, pyridine, oxolane, chloroform, or *N*,*N*-dimethylformamide. Isomerization is much faster for uridine and adenosine derivatives than for cytidine and guanosine derivatives. With *N*-benzoyl-nucleosides, an unusual debenzoylation of 2′- and/or 5′-silylated nucleosides occurs in methanol; it is fastest in *N*-benzoylcytidine and *N*-benzoyladenosine and much slower in *N*-benzoylguanosine. The isomerization and debenzoylation studies were monitored by l.c., and separation of the isomeric, silylated nucleosides by l.c. is described.

INTRODUCTION

We have been describing in detail the progressive development of an efficient synthesis of oligoribonucleotides[1–4]. A key feature was the introduction of alkylsilyl protecting groups[5–7], specifically the *tert*-butyldimethylsilyl (TBDMS) group, for protection of nucleosides. During this work we observed that, in ribonucleosides, isomerization of the TBDMS group between O-2′ and O-3′ occurred on silica gel surfaces and in wet solvents (*N*,*N*-dimethylformamide, dimethyl sulfoxide) or basic, protic solvents (pyridine–water)[2]. For alcohol solvents, isomerization was found to be much more rapid in methanol than in ethanol[3]. With the alcoholic solvents, we also observed a very unusual methanolysis of *N*-benzoyl groups from *N*-benzoylated nucleosides possessing a 2′-*O* and/or 5′-*O*-TBDMS group. The recent appearance of an article describing the isomerization of the TBDMS group in a single derivative of adenosine[8], and another describing the interconversion of one of the isomers of cytidine[9], has now prompted us to report our detailed finding.

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EXPERIMENTAL

Instrumentation. — A Spectra-Physics liquid chromatograph (model SP-8000 with microprocessor) equipped with a 254-nm, fixed wavelength detector (SP model 8210) was used for analytical separations of the isomeric, protected ribonucleosides. The isomers were separated by using a 10-μm, RP-8, reversed-phase column (Spectra-Physics RP-8) of 4.6 mm (i.d.) × 25 cm. The instrument was operated in the constant-flow mode at 2 mL/min with a column temperature of 30°. Samples were injected via a loop-type sample injector (10-μL loop), and concentrations of sample were ~1 mg/mL of methanol.

Solvents. — Three solvent-systems were used: *A*, 7:3 methanol–water; *B*, 4:9:3 acetonitrile–methanol–water; and *C*, a gradient system consisting of the following program:

time (min)	*MeOH(%)*	*CH_3CN(%)*	*H_2O(%)*
0.0	40	45	15
5.0	40	45	15
6.0	40	60	0
11.0	40	60	0
15.0	40	45	15

System *A* was used for the monosilylated nucleosides, because of their polarity and low retention on the column. System *C* was used for the fully protected nucleosides, which are the most strongly retained by the column. System *B* was used for all other compounds. The retention times and k' values (capacity factor) are recorded in Table I for all nucleoside derivatives.

Reaction studies. — Isomerization reactions were monitored by l.c. Generally, 1.5 mg of the compound in methanol was incubated at 30°. Samples were withdrawn at intervals as 10-μL aliquots and injected into the liquid chromatograph. The resulting chromatogram was used to determine product ratios (integrated automatically by the microprocessor). Reactions were usually performed in pairs (namely, in one case, the pure 2′ isomer and at the same time the pure 3′ isomer in another tube), and aliquots were withdrawn until identical isomer ratios (equilibrium) or decomposition of sample was observed. The rate constant for isomerization of the TBDMS group in any given compound was calculated from the following equation:

$$k_1 = \frac{x_e}{(a_o + x_o)t} \ln \frac{Ex_e - x_oE}{Ex_e - xE} \qquad (1)$$

for

$$A \underset{k-1}{\overset{k_1}{\rightleftharpoons}} B \qquad (2),$$

TABLE I

LIQUID-CHROMATOGRAPHIC RETENTION-TIMES[a] FOR SILYLATED NUCLEOSIDES

Isomer[b]	*Retention time (sec), k′ values*						
	Uridine	*Cytidine*	*Adenosine*	*Guanosine*	N^{Bz}-*Cytidine*	N^{Bz}-*Adenosine*	N^{Bz}-*Guanosine*
2′-Sil (**3**)	162,1.5[c]	154,1.4[c]	99,0.5	136,1.1[c]	340,4.2[c]		269,3.1[c]
3′-Sil (**6**)	193,2.0[c]	181,1.8[c]	111,0.7	193,2.0[c]	340,4.2[c]		344,4.3[c]
5′-MMT-2′-Sil (**4**)	211,2.2	194,2.0		341,4.2	341,4.2	291,3.5	230,2.5
5′-MMT-3′-Sil (**7**)	255,2.9	241,2.7	247,2.8		390,5.0	376,4.8	315,3.8
5′-Sil-2′-Sil (**5**)	205,2.2	199,2.1	203,2.2	167,1.6	366,4.6	328,4.0	229,2.5
5′-Sil-3′-Sil (**8**)	262,3.0	267,3.1	284,3.4	240,2.4	465,6.2	496,6.5	344,4.3
2′-Sil-3′-Sil (**9**)	230,2.5	239,2.7	259,3.0	226,2.5			310,3.8
2′,3′,5′-Tri-Sil (**11**)		673,9.3	909,13.0	903,12.9	395,5.1		1,603,21.7
5′-MMT-2′,3′-di-Sil (**10**)	703,9.8	691,9.6	703,9.8		791,11.2		1,430,21

[a]Solvents and column conditions are described in the experimental section. Solvent *B* was used if a superscript notation is given. [b]Sil = *tert*-butyldimethylsilyl; MMT = 4-methoxyphenyldiphenylmethyl. [c]System *A*. [d]System *C*.

where a_0 = initial concentration of A, x = actual concentration of B, x_e = equilibrium concentration of B, and x_0 = initial concentration of B. In some cases, initial samples of A are contaminated by trace quantities of B, which are detected and measured by l.c. Values of k_1 were obtained by plotting

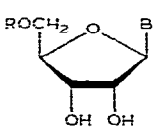
$$\frac{x_e}{a_0 + x_0} \ln \frac{x_e - x_0}{x_e - x}$$

vs time. For those samples where $x_0 = 0$, equation (*1*) reduces to the equation given by Laidler[10] for first-order, reversible reactions.

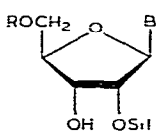
$$k_1 = \frac{x_e}{a_0 t} \ln \frac{x_e}{x_e - x} \qquad (3)$$

RESULTS AND DISCUSSION

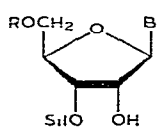
The l.c. characteristics of the isomeric, silylated ribonucleosides were first determined so as to permit the use of this technique for monitoring isomerization and debenzoylation reactions. Compounds used in this study are shown in Chart I. For the monosilyl derivatives **3** and **6**, the 2′ isomer (**3**) was always eluted before the 3′ isomer (**6**). In the disilyl isomers, the 2′,5′ isomer (**5**) was always eluted ahead of the 3′,5′ isomer (**8**). The same result was true for the 5′-*O*-methoxytritylated isomers **4** and **7**. The data are collected in Table I.



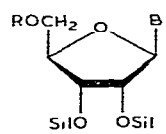
1 R = MMT
2 R = Sil



3 R = H
4 R = MMT
5 R = Sil



6 R = H
7 R = MMT
8 R = Sil



9 R = H
10 R = MMT
11 R = Sil

a B = U = Uracil
b B = C = Cytosine
c B = A = Adenine
d B = G = Guanine
e B = C^{Bz} = *N*-benzoylcytosine
f B = A^{Bz} = *N*-benzoyladenine
g B = G^{Bz} = *N*-benzoylguanine

MMT = 4-methoxyphenyldiphenylmethyl
Sil = *tert*-Butyldimethylsilyl

Chart I

TABLE II

RATE CONSTANTS[a] (IN UNITS OF 10^{-5} SEC^{-1}) AND HALF TIMES (H) FOR ISOMERIZATION OF SILYLATED NUCLEOSIDES IN METHANOL AT 30°

Isomer[b]	*Uridine*	*Cytidine*	*Adenosine*	*Guanosine*	N^{Bz}-*Guanosine*
2′-Sil (3)	5.1(1.6)		4.1(2)		0.35(26)
3′-Sil (6)	8.2(1.3)		7.1(1.5)		0.38(26)
2′,5′-Di-Sil (5)			4.3(1.9)	0.29(28)	0.36(18)
3′,5′-Di-Sil (8)			7.4(1.4)	0.94(12)	0.69(18)
5′-MMT-2′-Sil (4)	5.0(1.7)	0.27(35)	1.4(6.7)		1.3(6)
5′-MMT-3′-Sil (7)	6.9(1.6)	1.0(9.8)	3.3(2.9)		1.7(6)

[a]Determined over a 7-h period in each instance. In many examples, kinetic plots ceased to be linear at some point beyond 7 h. This may explain differences in K_{eq} values calculated from Tables II and III. [b]See footnote *b*, Table I

TABLE III

ISOMER RATIOS AT EQUILIBRIUM FOR SILYLATED NUCLEOSIDES

Isomer[a]	*Percent of mixture at equilibrium*				
	Uridine	*Cytidine*	*Adenosine*	*Guanosine*	N^{Bz}-*Guanosine*
2′-Sil (3)	57	60	57		52
3′-Sil (6)	43	40	43		48
2′,5′-Di-Sil (5)	64		56	58	66
3′,5′-Di-Sil (8)	36		44	42	34
5′-MMT-2′-Sil (4)	56	51	50		57
5′-MMT-3′-Sil (7)	44	49	50		43

[a]See footnote *b*, Table I.

Isomerization of 2′/3′-silylated nucleosides. — Several nucleosides silylated at either O-2′ or O-3′ were dissolved in methanol and maintained at 30°. The degree of isomerization was determined by l.c. as described in the experimental section. From equation (*1*) and the composition of the solutions at various time-intervals as determined by l.c., the first-order rate constants for isomerization were calculated and are collected in Table II.

In general, the uracil and adenine nucleosides isomerize much faster than the corresponding cytine and guanine isomers. In most cases, uridine and adenosine derivatives reach equilibrium in <24 h, and in some instances after only 12 h. Cytidine and guanosine derivatives required three days to reach equilibrium. With the exception of **3b** and **5a**, the 3′ isomer isomerized more rapidly than the 2′ isomer. This is also reflected in the equilibrium values shown in Table III, in which the 2′ isomer generally preponderates. Data were not available for the disilylated cytidines.

TABLE IV

STABILITY OF 2′,5′-Di-*O*-TBDMSURIDINE (5a) IN SELECTED SOLVENTS

Solvent	*Percent of* 5a *remaining after 24 h*
CH_3OH	64
EtOH	73.3
Me_2SO	95.6
Pyridine	96.9
Oxolane	99.3
CH_3CN	99.6
$CHCl_3$	100
$HCONMe_2$	100
$HCONMe_2$ — imidazole[a]	80

[a]2 equiv. of imidazole/1 equiv. 5a.

These compounds isomerized so slowly (30% after 7 days) that samples were beginning to decompose before equilibrium was reached.

Interestingly, changing the solvent from methanol to ethanol markedly decreased the rate of isomerization. Thus, for **7b**, the rate constant in ethanol was only 7.18×10^{-7} sec^{-1}, whereas in methanol it was 1.04×10^{-5} sec^{-1}.

The silylated derivatives are very stable in non-protic solvents. The data collected in Table IV are from studies in pure, dry solvents. The data in dimethyl sulfoxide differ from our previous report simply because of moisture in the reagent-grade dimethyl sulfoxide used in preparative experiments. Another interesting observation is that no isomerization was detected in pure *N*,*N*-dimethylformamide after 24 h. However, the addition of imidazole (2 eq/eq **5a**) caused 20% of isomerization during the following 24 h.

We have previously shown[7] that *O*-triisopropylsilyl derivatives of nucleosides are much more resistant to acid hydrolysis than the TBDMS derivatives. The same trend is true for isomerization in methanol. 2′,5′-Di-*O*-(triisopropylsilyl)uridine and 3′,5′-bis-*O*-(triisopropylsilyl)uridine had rate-constants of 2.65×10^{-6} sec^{-1} and 6.89×10^{-6}, which are lower than the values of 4.73×10^{-5} sec^{-1} and 1.92×10^{-5} sec^{-1} for **5a** and **8a**, respectively.

Debenzoylation. — We had initially observed that *N*-benzoylribonucleosides having a TBDMS group at O-5′ and/or O-2′ underwent debenzoylation in methanol[3] with the production of methyl benzoate. With *N*-benzoylcytidines, debenzoylation was much faster than isomerization, and these results are discussed later. The *N*-benzoyladenosines undergo isomerization more rapidly than debenzoylation. For example, when *N*-benzoyl-2′,5′-di-*O*-TBDMS-adenosine is dissolved in methanol at 30°, analysis shows that, after 7 h, the solution contains 79% of starting material, its 3′,5′ isomer (17%), 2′,5′-di-TBDMS-adenosine (3%), and 3′,5′-di-TBDMS-adenosine (1%). For the *N*-benzoylguanosines, debenzoylation was so slow that rates of isomerization of the silylated compounds could be determined.

TABLE V

DEBENZOYLATION OF *N*-BENZOYLCYTIDINES IN METHANOL AT 30°

Isomer[a]	*Percent of debenzoylation after 24 h*	*Time to complete loss of benzoyl group*
5′-Sil-C^{Bz} (**2e**)	49	~48 h
2′-Sil-C^{Bz} (**3e**)	34.5 (19.1 **3b**, 15.4 **6b**)	~72 h
3′-Sil-C^{Bz} (**6e**)	7 (3.7 **3b**, 3.3 **6b**)	23 days 50/50
3′,5′-Di-Sil-C^{Bz} (**5e**)	94.4 (85.7 **5b**, 8.7 **8b**)	~30 h
3′,5′-Di-Sil-C^{Bz} (**8e**)	99 (30.4 **5b**, 68.6 **8b**)	~24 h
5′-MMT-2′-Sil-C^{Bz} (**4e**)	98.4 (80.7 **4b**, 17.7 **7b**)	~48 h
5′-MMT-3′-Sil-C^{Bz} (**7e**)	2.3 (0.8 **4b**, 1.5 **7b**)	7 days 50/50

[a]See footnote *b*, Table I.

The results of the debenzoylation study of the cytidine derivatives are collected in Table V. For **2e**, **3e**, and **6e**, debenzoylation is quite rapid for the 5′ and 2′ isomers (**2c**, **3e**), but negligible for the 3′ isomer (**6e**). This trend is also observed for the 5′-"methoxytrityl" compounds **4e** and **7e**, where the 2′-*O*-silyl derivative **4e** is nearly completely debenzoylated after 24 h, whereas the 3′ isomer **7e** is virtually unaffected. With the disilylated isomers **5e** and **8e**, the 3′,5′-disilyl isomer **8e** is debenzoylated the more rapidly.

The nature of the alcohol solvent plays a major role in debenzoylation. Thus, whereas **4e** is completely debenzoylated in methanol within 24 h, no debenzoylation is observed in ethanol after 24 h. Indeed, only 5% of debenzoylation is observed in ethanol after 48 h.

This debenzoylation is of interest and probably involves silyl-group participation, either directly or by providing a unique, tertiary structure that traps a methanol molecule in just the right geometry for the transition state for methanolysis.

CONCLUSION

The TBDMS group constitutes a major breakthrough in the protection of hydroxyl groups in ribonucleosides. Our major goal has been the synthesis of oligoribonucleotides, and the TBDMS protecting-group, coupled with the phosphite condensation-procedure has allowed us to present a remarkably efficient synthesis of oligoribonucleotides[1-4]. A key to that success is the stability of the 2′-*O*-TBDMS group under the conditions employed during nucleotide condensation. We had previously shown that, in wet pyridine or dimethyl sulfoxide, isomerization of a 2′- (or 3′-) *O*-TBDMS group occurred to some extent, but that this could be prevented by the presence of sulfonic acids or sulfonyl chlorides[2]. In this study, we have detailed the results concerning a number of pure solvents. The results clearly show that in the key solvents (oxolane, pyridine, and *N*,*N*-dimethylformamide), the

TBDMS group is stable during a time-period compatible with any of the condensation procedures currently used in nucleotide synthesis. In particular, condensation times by the dichloridite procedure are only 30–120 min in oxolane, *N,N*-dimethylformamide, or pyridine solutions.

The debenzoylation of specifically silylated nucleosides is of interest. The clarification of this solvolysis will undoubtedly shed considerable light on the important question of nucleoside conformation and the effects of substituents in protected nucleosides.

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