New catalysts and procedures for the dimethoxytritylation and selective silylation of ribonucleosides[1]

GHOLAM H. HAKIMELAHI, ZBIGNIEW A. PROBA, AND KELVIN K. OGILVIE[2]

Department of Chemistry, McGill University, Montreal, P.Q., Canada H3A 2K6

Received December 2, 1981

GHOLAM H. HAKIMELAHI, ZBIGNIEW A. PROBA, and KELVIN K. OGILVIE. Can. J. Chem. **60**, 1106 (1982).

Procedures have been developed for the selective formation of (a) 2′,5′-silylated ribonucleosides and (b) 3′,5′-silylated ribonucleosides. These procedures also permit the selective silylation at either the 2′- or 3′-position of dimethoxytritylated ribonucleosides. The procedures involve nitrate or perchlorate ion catalysis for selective reaction at 2′-positions and a combination of silver ion and DABCO or 4-nitropyridine *N*-oxide for selective reaction at the 3′-position. During the course of this work a general and rapid procedure was developed for the preparation and isolation of the 5′-dimethoxytrityl derivatives of the four common ribonucleosides. Silver ion was found to have a marked effect on dimethoxytritylation reactions.

GHOLAM H. HAKIMELAHI, ZBIGNIEW A. PROBA et KELVIN K. OGILVIE. Can. J. Chem. **60**, 1106 (1982).

On a mis au point des méthodes de formation sélective des ribonucléosides silylés en positions 2′ et 5′ et des ribonucléosides silylés en positions 3′ et 5′. Ces méthodes permettent également la silylation sélective en positions 2′ ou 3′ des ribonucléosides diméthoxytritylés. Les méthodes impliquent une catalyse par l'ion nitrate ou l'ion perchlorate pour la réaction sélective en position 2′ et une combinaison de l'ion argent et du DABCO ou du *N*-oxyde de nitro-4 pyridine pour la réaction sélective en position 3′. On a développé, au cours de ce travail, une méthode rapide générale de préparation et de séparation des dérivés diméthoxytrityl-5′ des quatres ribonucléosides ordinaires. On a trouvé que l'ion argent a un effet marqué sur les réactions de diméthoxytritylation.

[Traduit par le journal]

Introduction

Progress in the chemical synthesis of oligodeoxynucleotides has culminated in the development of automated procedures for the synthesis of gene fragments (1). This success in the deoxy area is due principally to the early establishment of adequate and versatile protecting groups for the amino, hydroxyl, and phosphate functions of the growing chains (2). With the protecting group problem out of the way, successive developments in condensation procedures (3–6) have led to automation of the synthesis using a solid-support procedure.

Ribonucleotides have posed a more difficult challenge in terms of protecting groups. The presence of the 2′-hydroxyl group in ribonucleosides not only requires protection but places serious limitations on the nature of the protecting groups used. During the last few years we have been refining a new approach to the total synthesis of ribonucleotides (7). The approach is based on our development of the alkylsilyl protecting groups for the 2′-position of ribonucleosides (8). During this developmental stage we have used monomethoxytrityl protection of 5′-hydroxyl groups and trichloroethyl protection of phosphates. Amino groups have either been protected with benzoyl groups or left unprotected. Condensations have been effected using the "phosphite–triester" approach. We have recently adapted these developments to a solid-support procedure (*7a*) for ribonucleotide synthesis.

The procedures developed for the synthesis of ribonucleotides possessing the 3′–5′ phosphate bridge have been extended to 2′–5′ linked nucleotides (9). These molecules are of considerable interest because of the implication of 2′–5′ linked oligoadenylates in the interferon process (10). Thus the ready availability of 3′,5′-diprotected ribonucleosides is of importance.

At this stage in the development of a total procedure for ribonucleotide synthesis it became essential to reduce to a minimum the time involved in protection–deprotection of nucleosides and nucleotides. In terms of deprotection of nucleotides we wished to take advantage of the 10-fold reduction in time required to remove a dimethoxytrityl group compared to a monomethoxytrityl group. We also sought to speed up the rate of tritylation and silylation of ribonucleosides and to increase the selectivity of 2′ vs. 3′-silylation. In this report we describe: (1) procedures for the rapid and efficient dimethoxytritylation of ribonucleosides; (2) procedures for the rapid and highly selective 2′-silylation of ribonucleosides, and (3) procedures for the rapid and highly selective 3′-silylation of ribonucleosides.

Discussion

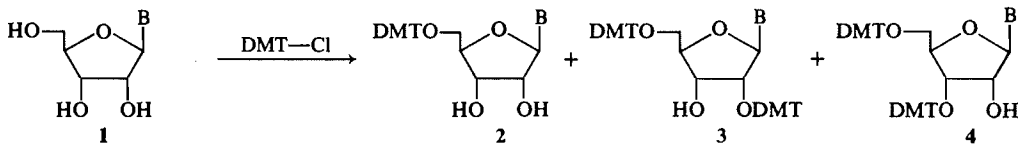
Dimethoxytritylation

The general procedures for dimethoxytritylation of ribonucleosides (Scheme 1) are based on those originally developed by the Khorana group in the

[1]This manuscript constitutes part XIX in a series on silyl protecting groups in nucleoside and nucleotide chemistry. Part XVIII is ref. 20.

[2]Author to whom correspondence may be addressed.

0008-4042/82/091106-08$01.00/0



a B = uracil
b B = adenine
c B = *N*-benzoyladenine
d B = *N*-benzoylcytidine
e B = guanine
f B = *N*-benzoylguanine

DMT = dimethoxytrityl

SCHEME 1

deoxy area (11, 12). Two of the desired compounds **2***a* (13) and **2***d* (14) have been described in the literature. By this standard procedure the nucleoside and dimethoxytrityl chloride (DMT-Cl) are dissolved in pyridine and, after about 12 h, products are isolated by chromatography. We found that by using this procedure a mixture of desired product, ditritylated (2′,5′ and 3′,5′) material, *N*-tritylated material, and unreacted starting material was always obtained and that separation of these components was time-consuming.

We observed that a simple change in the procedure led to a much cleaner product mixture which could easily be separated on short column chromatography. Thus the nucleoside was completely dissolved in pyridine or pyridine–DMF (1:1) and dimethoxytrityl chloride was added in eight equal portions (1 per hour) to the solution maintained at 0°C. At the end of the reaction period (9 h) the solvent was concentrated at reduced pressure and mixed with an aqueous sodium bicarbonate solution. If this bicarbonate treatment was eliminated a significant amount of detritylation occurred. The product was then extracted into methylene chloride. Thin-layer chromatography at this point indicated that only a very little by-product was present. Consequently the solution was concentrated and applied to a short silica gel column from which the desired product was rapidly eluted. Yields ranged from 69% for the guanosine derivative **2***f* to 85% for the cytidine derivative **2***d*. The elimination of by-products was particularly evident in the preparation of the adenosine derivative **2***b* in 80% yield. *N*-Tritylation was not a problem in this case. However, *N*-tritylation is a serious problem using the original procedure (8*a*). The only drawback to this modified procedure was the time involved (8–10 h). However, we found that the addition of silver nitrate gave a marked improvement in the rate of product formation.

The use of silver nitrate was based on our initial use of this reagent in the selective silylation of nucleosides to be described below. The results summarized in Table 1 show that the use of silver nitrate caused complete reaction within 1 h leading to yields of **2** ranging from 70% for **2***d* to 95% for **2***f*. The results also show that while ditritylation could be rapidly achieved using silver nitrate catalysis, selective ditritylation occurred only for the uridine case (**3***a* (80%), **4***a* (15%)). It appears that *N*-protection is necessary when using silver nitrate since in a test reaction on adenosine (**1***b*) a significant amount of *N*-tritylation was observed.

Silver ion is the effective catalyst in these reactions. As expected, silver trifluoroacetate gave the same results as silver nitrate, while tetrabutylammonium nitrate was ineffective.

Silylations

A. *Selective 2′-silylations*

We have previously described procedures for the silylation of ribonucleosides and their 5′-methoxytrityl derivatives (8). These procedures always lead to a mixture of 2′,5′ and 3′,5′ derivatives in nearly equal amounts. While these isomers can be separated by silica gel chromatography, the yield of the 2′,5′-diprotected compound was lower than desired. We have investigated numerous procedures in an effort to obtain higher yields of the 2′,5′-protected compounds. Most of these procedures, including slow addition of silylating agent at lowered temperature, gave no significant improvement in selectivity. However, the addition of nitrates and perchlorates gave a remarkable improvement in the selectivity of silylation.

The reactions are outlined in Scheme 2 and the results are summarized in Table 2. The general procedure can be illustrated with the silylation of 5′-dimethoxytrityl-*N*-benzoylcytidine (**2***d*). The nucleoside is dissolved in THF after which pyridine (3.7 mmol/mmol $AgNO_3$) and silver nitrate (1.2 mmol/mmol nucleoside) are added and the solution

TABLE 1. Silver nitrate catalysis of dimethoxytritylation reactions*

Nucleoside	DMT-Cl (mmol)	$AgNO_3$ (mmol)	Pyridine (mmol)	Yield (%) 5′ (**2**)	2′,5′ (**3**)	3′,5′ (**4**)
U, **1***a*	1	1	5	90	—	—
U, **1***a*	2	2	10		80	15
A^{Bz}, **1***c*	1	1	5	80	—	—
A^{Bz}, **1***c*	2.5	2.5	10	10	40	40
C^{Bz}, **1***d*	1	1	5	70+	10	10
C^{Bz}, **1***d*	2	2	10		45	45
G^{Bz}, **1***f*	1	1	5	70	—	—
G^{Bz}, **1***f*	2	2	10	95	—	—
G^{Bz}, **1***f*	5	5	25	10	40	40

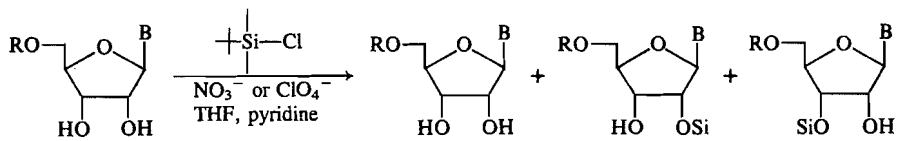
*Solvent was THF except for + where THF–DMF (1:1) was used and DMT–Cl was added portionwise in this case only. The time was 1 h in all cases except the last entry for **1***f* where 1.5 h were used. For the general procedure see the Experimental.

is stirred until the silver nitrate is completely dissolved (~ 5 min). At this point *tert*-butyldimethylsilyl chloride (TBDMS-Cl, 1.3 mmol/mmol **2***d*) is added all at once and the resulting mixture is stirred at room temperature for 1.5 h. The mixture is then filtered and the clear solution is mixed with 5% aqueous $NaHCO_3$ to prevent detritylation. The product is extracted into methylene chloride. The products are separated on short column chromatography to yield 5′-DMT-2′-TBDMS-C^{Bz} (**6***d*) in 68% and the 3′-isomer, **8***d*, in 21% yield. These results show very high conversion yields with remarkable selectivity in all cases except for *N*-benzoylguanosine derivatives which will be discussed later.

The scope of the reaction was investigated using the unprotected nucleoside. Treatment of **1** with 2.2 equivalents each of TBDMS-Cl and $AgNO_3$ in THF led to exclusive formation of the 5′-TBDMS derivatives **5** in ~ 95% isolated yields (Table 2) within 3 h. No disilylation was observed in doubling the amounts of reagents (entry 6). However, if the solvent were changed to DMF, a mixture of mono, di, and trisilylated products was obtained. Repeating the original THF reaction but adding 5 equivalents of pyridine gave a rapid and highly selective disilylation. Yields of the 2′,5′-disilyl derivatives **7** were 82–90%.

An interesting contrast exists between guanosine (**1***e*) and *N*-benzoylguanosine (**1***f*). In the former case the reaction showed good selectivity, while in the latter there was very little selectivity. We believe this is due to steric hindrance created by the N^2-benzoyl group in the vicinity of the 2′-hydroxyl group. In this regard it may be of interest to note our observations on separating isomeric-protected guanosine derivatives. These isomers have always given us considerable difficulty. However, we have found that in acetone – petroleum ether – triethyl amine (6:5.5:0.5) the 2′,5′-protected compound **6***f* has an R_f of 0.59 while the 3′,5′-isomer **8***f* has an R_f of 0.21. If the triethylamine is eliminated from this solvent, both isomers have an R_f of 0.41. In chloroform–acetone–triethylamine (9:0.5:0.5) compound **6***f* had an R_f of 0.30 while **8***f* was at 0.05. In the absence of triethylamine both had an R_f value of 0.28. This suggests that there may be hydrogen bonding between the 2′-hydroxyl in **8***f* and the 2-amide group. This H-bonding would be destroyed in the presence of triethylamine leading to a more polar structure for **8***f* and a resultant decreased R_f value (stronger absorption). As expected, we were not able to observe a similar effect with the other nucleosides.

Nitrates which are soluble in the reaction medium all give good results. For example, tetrabutylammonium nitrate (entries 33 and 34) gives the same rate and selectivity as silver nitrate. In



1, R = H
2, R = DMT
5, R = Si
6, R = DMT
7, R = Si
8, R = DMT
9, R = Si

a–f as in Scheme 1

Si = *tert*-butyldimethylsilyl

SCHEME 2

TABLE 2. Nitrate ion catalysis of silylation reactions*

Entry	Nucleoside	TBDMS-Cl (mmol)	$AgNO_3$ (mmol)	Base (mmol)	Time (h)	Product (%) 5′	2′,5′	3′,5′
1	DMT-U (**2***a*)	1.3	1.2	py (3.7)	1.5	—	70	15
2	DMT-A (**2***b*)	1.3	1.2	py (3.7)	5	—	65	21
3	DMT-C^{Bz} (**2***d*)	1.3	1.2	py (3.7)	1.5	—	68	20
4	DMT-G^{Bz} (**2***f*)	1.7	1.5	py (5)	5	—	45	40
5	U (**1***a*)	2.2	2.2	—	3	98	—	—
6	U (**1***a*)	4	4	—	3	98	—	—
7	U (**1***a*)	2.2	2.2	(DMF)	3	20	30	10
8	U (**1***a*)	2.2	2.2	py (5)	1	—	90	5
9	U (**1***a*)	2.2	2.2	col (5)	3	97	—	—
10	U (**1***a*)	2.2	—	py (5)	5	<10	—	—
11	U (**1***a*)	2.2	2.2	im (4)	1	—	70	15
12	A (**1***b*)	2	2	py (5)	1	—	68	22
13	A (**1***b*)	2.2	2.2	im (4)	1	—	60	20
14	A (**1***b*)	2.2	—	im (4)	30	25	13	12
15	A (**1***b*)	2.2	2.2	—	3	95	—	—
16	C^{Bz} (**1***d*)	2.2	2.2	py (5)	1	—	82	13
17	C^{Bz} (**1***d*)	2.2	2.2	—	3	95	—	—
18	C^{Bz} (**1***d*)	2.2	2.2	im (4)	1	—	65	15
19	G (**1***e*)	2.2	2.2	—	3	95	—	—
20	G (**1***e*)	2.2	2.2	py (5)	4	—	33	17
21	G (**1***e*)	2.2	2.2	py (5)	2	—	60	35
22	G^{Bz} (**1***f*)	2.2	2.2	—	4	97	—	—
23	G^{Bz} (**1***f*)	2.2	2.2	py (5)	2	—	48	40
24	G^{Bz} (**1***f*)	2.2	2.2	im (4)	3	—	50	40
25	U (**1***a*)	2.2	2.2	DMAP (5)	3	—	80	15
26	U (**1***a*)	2.2	2.2	4-Acpy (5)	1	98	—	—
27	U (**1***a*)	2.2	2.2	4-Acpy (5)	5	68	30	—
28	U (**1***a*)	2.2	2.2	4-Acpy (5)	10	35	60	—
29	U (**1***a*)	2.2	2.2	4-Acpy (5)	15	—	94	1
30	U (**1***a*)	2	KH_2PO_4 (2)	py (5)	2	—	—	—
31	U (**1***a*)	2	$(n\text{Bu})_4NI$ (2)	py (5)	2	—	—	—
32	U (**1***a*)	2	$(n\text{Bu})_4NSO_3H$ (2)	py (5)	2	90	—	—
33	U (**1***a*)	2.2	$(n\text{Bu})_4NNO_3$ (2.2)	py (5)	1	—	87	6
34	A (**1***b*)	2	$(n\text{Bu})_4NNO_3$ (2)	py (5)	1	—	67	23

*All reactions were carried out in THF except for No. 7 which was in DMF and Nos. 21, 23, and 24 which were carried out in THF–DMF (1:1). Abbreviations used are: py = pyridine, col = collidine, im = imidazole, DMAP = 4-dimethylaminopyridine, and 4-Acpy = 4-acetylpyridine.

another experiment in which adenosine was dissolved in THF–DMF (1:1) along with 2 equivalents each of TBDMS-Cl and potassium nitrate and 5 equivalents of pyridine, compounds **7***b* and **9***b* were produced in a 1.5:1 ratio.

Soluble perchlorates give equally good results in selective silylations (Table 3). Silver perchlorate and $(n\text{Bu})_4NClO_4$ give excellent yields of 2′-silylated derivatives. The use of nitrates and perchlorates as catalysts in silylations is not new (15, 16) but the selectivity described here is unprecedented. Other soluble silver salts such as silver carbonate, silver trifluoroacetate, and silver cyanate have no effect on silylation under these conditions.

Pyridine is required for disilylation to occur. In the absence of pyridine, reaction stops at the 5′-monosilyl stage. Replacing pyridine with the hindered base 2,4,6-trimethylpyridine gives only monosilylation even after 20 h. These results represent an excellent procedure for the highly selective monosilylation of nucleosides.

In a separate experiment one equivalent each of TBDMS-Cl and $AgNO_3$ were mixed together in THF. The mixture was stirred at room temperature and after 30 min the solution was decanted and distilled. The presumed *tert*-butyldimethylsilyl nitrate distilled at 140–142°C (1 atm) and was collected as a pale yellow liquid. This material, TBDMS-NO_2, silylated uridine in the presence or absence of pyridine with the same results as described for TBDMS-Cl and $AgNO_3$.

B. *Selective 3′-silylation*

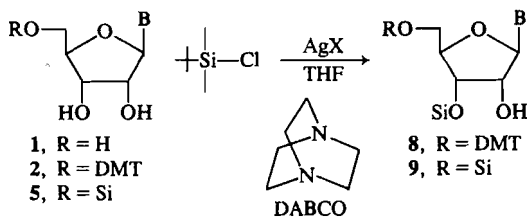
The selective protection of the 3′-position in ribonucleosides cannot be achieved with most common protecting groups. Only acylation has been reported (17) to give high yields of 3′-derivatization. This occurs through migration from the 2′-position and selective crystalization of the 3′-

TABLE 3. Perchlorate ion catalysis of silylation reactions*

Entry	Nucleoside	TBDMS-Cl (mmol)	$AgClO_4$ (mmol)	Base (mmol)	Time (h)	Product (%) 5′	2′,5′	3′,5′
1	U (1*a*)	2.2	2.2	py (5)	2	—	90	7
2	DMT-U (2*a*)	1.3	1.2	py (5)	2	—	75	15
3	Si-U (5*a*)	1.3	1.2	py (5)	2	—	90	6
4	U (1*a*)	2.2	2.2	—	2	90	2	2
5	A (1*b*)	2.2	2.2	py (5)	2	—	87	8
6	DMT-A (2*b*)	1.3	1.2	py (5)	4	—	70	20
7	Si-A (5*b*)	1.3	1.2	py (3)	2	—	80	10
8	C^{Bz} (1*d*)	2.2	2.2	py (5)	2	—	90	5
9	DMT-C^{Bz} (2*d*)	1.3	1.2	py (5)	2	—	70	20
10	Si-C^{Bz} (5*d*)	1.3	1.2	py (3)	2	—	92	5
11	G^{Bz} (1*f*)	2.5	2.5	py (6)	5	—	55	40
12	G^{Bz} (1*f*)	3	3	—	2	95	—	—
13	DMT-G^{Bz} (2*f*)	1.5	1.5	py (5)	5	—	55	42
14	Si-G^{Bz} (5*f*)	1.4	1.3	py (4)	5	—	60	35
15	U (1*a*)	2.2	$(n\text{Bu})_4NClO_4$ (2.2)	py (5)	2	—	90	5
16	A (1*b*)	2.2	$(n\text{Bu})_4NClO_4$ (2.2)	py (5)	2	—	85	5
17	C^{Bz} (1*d*)	2.2	$(n\text{Bu})_4NClO_4$ (2.2)	py (5)	2	—	93	4

*Reaction conditions are the same as for Table 2 and are described in the Experimental.

isomer from solution. Markiewicz (18) has described a novel bifunctional silylating agent, 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane which reacts with both the 5′- and 3′-positions. To date there has not been a report of the direct and selective protection of the 3′-position of ribonucleosides using a monofunctional silylating agent. We have developed procedures which accomplish this objective (Scheme 3). A preliminary account of some of these results has appeared (19, 20).

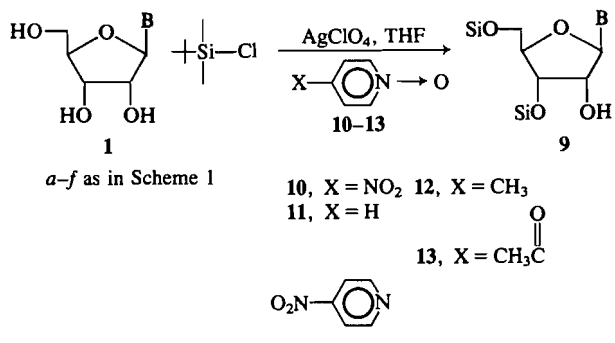


SCHEME 3

Two procedures have been developed. The first involves the silylation of ribonucleosides in the presence of DABCO (1,4-diazabicyclo[2.2.2]-octane) and silver salts. The second procedure employs silver salts with 4-nitropyridine *N*-oxide.

The reaction involving ribonucleosides, TBDMS-Cl, DABCO, and silver salts leads to 3′,5′-di-TBDMS derivatives (**9**) in yields as high as 94% (Table 4). It is apparent from Table 4 that the silver ion is important in the reaction. All soluble silver salts tested ($AgNO_3$, $AgClO_4$, $AgOCOCF_3$) produce excellent selectivity while the salts $(n\text{Bu})_4NNO_3$ and $(n\text{Bu})_4NClO_4$ have no effect. In the absence of DABCO only 5′-silylation occurs. The procedure works equally well with dimethoxytritylated nucleosides (**2**) giving 3′-TBDMS-5′-DMT nucleosides (**8**) in yields of 70–93%.

The reaction with 4-nitropyridine *N*-oxide (Scheme 4) also leads to selective reaction at the 3′-position when silylation is carried out in THF with silver salts and TBDMS-Cl. We have only observed selectivity with the 4-nitropyridine *N*-oxide among those tested (Table 5). The one drawback with this procedure is that the reaction conditions rapidly remove dimethoxytrityl groups (e.g. from **2**). It is interesting to note that 4-nitropyridine (**14**) produces similar results to 4-nitropyridine *N*-oxide (**10**) but in a much slower reaction (10–20 h). This result contrasts with 4-acetylpyridine which gives a slow, but highly selective, silylation at the 2′-position (Table 2, entries 26–29).



SCHEME 4

TABLE 4*. Selective 3′-silylations with DABCO and Ag^+

Entry	Nucleoside	TBDMS-Cl (mmol)	Salt (mmol)	Time (h)	Products (%) 5′	3′,5′	2′,5′
1	U (**1***a*)	2.3	$AgClO_4$ (2.3)	3	—	(**9***a*) 90	(**7***a*) 5
2	U (**1***a*)	2.3	$AgNO_3$ (2.3)	3	—	(**9***a*) 91	(**7***a*) 3
3	U (**1***a*)	2.3	$AgOCOCF_3$ (2.2)	3	—	(**9***a*) 90	(**7***a*) 5
4	U (**1***a*)	2.3	$(n\text{Bu})_4NClO_4$ (2.2)	10	(**5***a*) 65	(**9***a*) 18	(**7***a*) 2
5	U (**1***a*)	2.3	$(n\text{Bu})_4NNO_3$ (2.2)	10	(**5***a*) 60	(**9***a*) 25	(**7***a*) 1
6	U (**1***a*)	2.3	—	10	(**5***a*) 85	(**9***a*) 2	—
7	DMT-U (**2***a*)	1.3	$AgClO_4$ (1.2)	2	—	(**8***a*) 91	(**6***a*) 5
8	Si-U (**5***a*)	1.3	$AgClO_4$ (1.2)	3	—	(**9***a*) 90	(**7***a*) 5
9	C^{Bz} (**1***d*)	2.4	$AgClO_4$ (2.3)	3	—	(**9***d*) 92	(**7***d*) 3
10	C^{Bz} (**1***d*)	2.4	$AgNO_3$ (2.3)	3	—	(**9***d*) 90	(**7***d*) 5
11	DMT-C^{Bz} (**2***d*)	1.3	$AgClO_4$ (1.2)	3	—	(**8***d*) 90	(**6***d*) 5
12	A (**1***b*)	2.4	$AgClO_4$ (2.3)	4	—	(**9***b*) 70	(**7***b*) 25
13	A (**1***b*)	2.4	$AgNO_3$ (2.3)	4	—	(**9***b*) 70	(**7***b*) 20
14	DMT-A (**2***b*)	1.3	$AgClO_4$ (1.2)	4	—	(**8***b*) 70	(**6***b*) 25
15	G^{Bz} (**1***f*)	2.6	$AgClO_4$ (2.5)	6	—	(**9***f*) 60	(**7***f*) 30
16	G^{Bz} (**1***f*)	2.6	$AgNO_3$ (2.5)	6	—	(**9***f*) 60	(**7***f*) 35
17	DMT-G^{Bz} (**2***f*)	1.5	$AgNO_3$ (1.5)	5	—	(**8***f*) 30	(**6***f*) 60

*Reactions were carried out in THF with 6 mmol DABCO/mmol of nucleoside, as described in the Experimental.

TABLE 5. Selective 3′-silylation using 4-nitropyridine *N*-oxide*

Entry	Nucleoside	Salt (mmol)	Catalyst* (mmol)	Time (h)	Products (%) 5′	3′,5′	2′,5′
1	U (**1***a*)	$AgClO_4$ (2.2)	**10** (2.3)	1.5	—	(**9***a*) 50	(**7***a*) 40
2	A (**1***b*)	$AgClO_4$ (2.2)	**10** (2.3)	2	—	(**9***b*) 89	(**7***b*) 5
3	C^{Bz} (**1***d*)	$AgClO_4$ (2.2)	**10** (2.3)	2	—	(**9***d*) 90	(**7***d*) 8
4	G^{Bz} (**1***f*)	$AgClO_4$ (2.5)	**10** (2.6)	2.5	—	(**9***f*) 98	—
5	C^{Bz} (**1***d*)	$AgClO_4$ (2.2)	**11** (2.3)	2	—	(**9***d*) 40	(**7***d*) 40
6	C^{Bz} (**1***d*)	$AgClO_4$ (2.2)	**12** (2.3)	2	—	(**9***d*) 40	(**7***d*) 40
7	C^{Bz} (**1***d*)	$AgClO_4$ (3.7)	**13** (3.5)	60	(**5***d*) 40	(**9***d*) 25	(**7***d*) 18
8	C^{Bz} (**1***d*)	$(n\text{Bu})_4NClO_4$ (2.2)	**10** (2.3)	5	—	—	—

*Reactions were carried out in THF (25 mL/mmol of nucleoside) using TBDMS-Cl (2.2 mmol/mmol of **1**) as described in the Experimental.

These selective silylations at the 3′-position apparently involve direct attack at the 3′-position since the 2′-silyl derivatives (e.g. **6** and **7**) appear to be stable under the reaction conditions (i.e., they do not rearrange to the 3′-position). The mechanistic implications of the combination of silver ion and DABCO or 4-nitropyridine *N*-oxide are being explored.

Conclusion

This report describes procedures for the facile preparation of ribonucleosides protected with two of the most versatile and useful blocking groups, the dimethoxytrityl group and the *tert*-butylmethylsilyl group. The procedures incorporate truly novel catalytic effects leading to highly selective protection at the 2′-position and the unprecedented highly selective silylation at the 3′-position.

Experimental

General methods

Thin-layer chromatographic data (R_f values) are recorded from Merck Kieselgel 60 F 254 analytical sheets. All uv spectra were recorded on a Cary 17 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian T-60A instrument. Infrared spectra were obtained on a Perkin Elmer 297 spectrophotometer. Melting points were determined on a Fisher–Johns melting point apparatus and are reported uncorrected.

Reagent grade pyridine was distilled first from *p*-toluenesulfonyl chloride, redistilled from calcium hydride, and stored over molecular sieves (type 4A). Tetrahydrofuran was refluxed with benzophenone and sodium for at least 3 h and was freshly distilled before use. *N*,*N*-Dimethylformamide (DMF) and 2,4,6-trimethylpyridine were distilled from calcium hydride and stored over molecular sieves (type 4A).

Short column chromatography

Short columns of Merck silica gel 60 (230–400 mesh) were packed in glass columns 5 cm in diameter using 10 g of silica gel per gram of crude mixture. Columns were washed first with a low polarity solvent to remove tritanol and other fast-moving impurities. The desired products were then eluted with a more polar solvent. Solvents used are indicated for each preparation described below. Fractions of 15 mL were collected every 5 min using a fraction collector. Contents of fractions were determined by analytical tlc.

General preparation of 5′-dimethoxytritylnucleosides

(a) *5′-Dimethoxytrityluridine (2a)*

Uridine (**1***a*, 24.4 g, 0.10 mol) was dissolved in pyridine (200

TABLE 6. Properties of protected nucleosides*

Compound	Melting point (°C)	(EtOH) λ_{max} (nm)	R_f (tlc)†
DMT-U **2***a*	111–112	233, 265	0.28[a]
DMT-A **2***b*	145–146	234, 258	0.16[a]
DMT-A^{Bz} **2***c*	113–115	234, 261, 284	0.14[b]
DMT-C^{Bz} **2***d*	109–111	234, 259, 305	0.30[a]
DMT-G^{Bz} **2***f*	169–171	234, 255(sh), 265, 291	0.26[a]
2′,5′-DiDMT-U **3***a*	144–146	231, 265	0.41[b]
2′,5′-DiDMT-A^{Bz} **3***c*	139–141	233, 262, 283	0.39[b]
2′,5′-DiDMT-C^{Bz} **3***d*	148–150	235, 261, 305	0.40[b]
2′,5′-DiDMT-G^{Bz} **3***f*	158–160	233(sh), 261(sh), 281, 295	0.79[a], 0.14[c], 0.72[b]
3′,5′-DiDMT-U **4***a*	136–138	231, 265	0.32[b]
3′,5′-DiDMT-A^{Bz} **4***c*	130–131	233, 262, 283	0.29[b]
3′,5′-DiDMT-C^{Bz} **4***d*	150–153	235, 261, 305	0.30[b]
3′,5′-DiDMT-G^{Bz} **4***f*	154–156	233(sh), 261(sh), 281, 295	0.71[b], 0.03[c]
DMT-U^{Si}_{OH} **6***a*	100–102	233, 265	0.22[d]
DMT-A^{Si}_{OH} **6***b*	102–103	235, 261	0.35[b], 0.57[e]
DMT-$^{Bz}_{C}$ $^{Si}_{OH}$ **6***d*	112–113	232, 261, 306	0.77[b], 0.75[e]
DMT-$^{Bz}_{G}$ $^{Si}_{OH}$ **6***f*	148–150	233, 256(sh), 264, 291	0.41[f], 0.59[g], 0.30[h], 0.29[i]
DMT-U^{OH}_{Si} **8***a*	109–110	233, 265	0.07[d]
DMT-A^{OH}_{Si} **8***b*	172–173	235, 261	0.28[b], 0.54[e]
DMT-$^{Bz}_{C}$ $^{OH}_{Si}$ **8***d*	110–111	232, 261, 306	0.48[b], 0.59[e]
DMT-$^{Bz}_{G}$ $^{OH}_{Si}$ **8***f*	140–142	233, 265(sh), 264, 291	0.41[f], 0.21[g], 0.05[h], 0.28[i]

*The nmr and ir spectra of all compounds agreed with the reported structures.
†Solvents employed were (a) chloroform–methanol (9:1); (b) chloroform–methanol (9.5:0.5); (c) chloroform–triethylamine (9.5:0.5); (d) hexane–ether (1:3); (e) chloroform–methanol–triethylamine (9.5: 0.5: 0.5); (e) acetone – petroleum ether (6:5.5); (g) acetone – petroleum ether – triethylamine (6:5.5:0.5); (h) chloroform–acetone–triethylamine (9:0.5:0.5); (i) chloroform–acetone (9:0.5).

mL) and kept at 0°C. Dimethoxytrityl chloride (41 g, 0.12 mol) was added in portions (5.1 g, 0.015 mol) during an 8 h period. After an additional 2 h methanol (50 mL) was added and the solution was concentrated at reduced pressure. A 5% $NaHCO_3$ solution (200 mL) was added and the resulting solution was extracted with methylene chloride (500 mL). The methylene chloride solution was washed once with water, then dried over magnesium sulfate and evaporated to leave the crude product.

The residue was washed with chloroform–hexane (1:20, 100 mL) to remove residual pyridine. The residue was then applied to a short column (5 cm diameter) packed with 450 g (10 g/g of crude product) of Merck silica gel 60 (230–400 mesh). The column was washed with ether (500 mL) to remove impurities, followed by ethyl acetate (800 mL). Fractions of 15 mL were collected (fraction collector) every 5 min. The product was recovered from the ethyl acetate fractions which, on evaporation, gave 43.7 g of **2***a* (80%). Compound **2***a* crystallizes from ether: mp 111–112°C. Properties of **2***a* are listed in Table 6.

(b) *5′-Dimethoxytrityladenosine (***2**b)

This procedure was very similar to that of (a) except that the solvent consisted of 100 mL each of pyridine and DMF. At the end of the reaction period (10 h) the solution was poured into 2 liters of cold water. A pale yellow precipitate was obtained which was collected by filtration, washed with water, and dried under air. The crude product was dissolved in hot xylene (2500 mL) and, on cooling, the product **2***b* crystallized as a white solid which was collected by filtration and washed with ether (yield 80%, mp 145–146°C). Properties of **2***b* are listed in Table 6.

(c) *5′-Dimethoxytrityl-*N*-benzoylcytidine (***2**d)

This procedure was virtually the same as that described for **2***a*, including isolation of the product on short silica gel column chromatography using first chloroform (400 mL) and then chloroform–methanol (9:1, 600 mL) as solvent. The yield of **2***d* was 85% (mp 109–111°C). Properties are listed in Table 6.

(d) *5′-Dimethoxytrityl-*N*-benzoylguanosine (***2**f)

This preparation was identical to that described above for **2***d* except that, during short column chromatography of the reaction products, 500 mL of chloroform was used to wash off impurities and the product was recovered in 800 mL of chloroform–methanol (9:1). The yield of **2***f* was 69% and its properties are listed in Table 6.

Preparation of dimethoxytritylated nucleosides using silver nitrate as catalyst

The general procedure is outlined for the synthesis of 5′-dimethoxytrityl-*N*-benzoyladenosine (**2***c*). *N*-Benzoyladenosine was dissolved in THF (30 mL/mmol of nucleoside). Pyridine (5 mmol/mmol of **1**), silver nitrate (1 mmol/mmol **1**), and dimethoxytrityl chloride (1 mmol/mmol **1**) were added successively and the mixture was stirred at room temperature for 1 h. The mixture was filtered and the solution added to a 5% sodium bicarbonate solution. The product was extracted into methylene chloride which was dried and evaporated to give **2***c* in an 80% yield.

When the above reaction was repeated except that the amounts of pyridine, silver nitrate, and dimethoxytrityl chloride were doubled, the 2′,5′ and 3′,5′-ditritylated products **3***c* and **4***c* were each obtained in 40% yields. The results for all of the nucleosides are listed in Table 1 and the properties of the products are listed in Table 6.

Silylation of dimethoxytritylated nucleosides **2** *using nitrate (or perchlorate) catalysts*

(a) The standardized procedure can be illustrated by the silylation of 5′-dimethoxytrityladenosine (**2***b*). Compound **2***b* (5.69 g, 10 mmol) was dissolved in THF (100 mL) followed by the addition of pyridine (3 g, 37 mmol) and silver nitrate (2 g, 12 mmol). The solution was stirred (5 min) until all the silver nitrate had dissolved and then *tert*-butyldimethylsilyl chloride (2 g, 13 mmol) was added and the mixture was stirred at room temperature for 5 h. The solution was then filtered into a 5% $NaHCO_3$ solution (100 mL). This solution was extracted with methylene chloride (300 mL) and the extracts were dried and evaporated.

Ether (100 mL) was added to the crude product (6.5 g). The solution was separated from undissolved **2***b* by filtration. The ether solution was concentrated, at reduced pressure without heating, to about 60 mL. During this period the 2′-silylated derivative precipitated and 3 g of **6***b* was collected by filtration. By repeated concentration it has been possible to collect most of the 2′-isomer. However, a more direct procedure is to collect the residue from the remaining ether solution and apply it directly to short column chromatography using chloroform–methanol (9.5:0.5) for elution. The yield of the 2′-isomer **6***b* was 65%, and that of the 3′-isomer **8***b* was 21%. Properties of these compounds are collected in Table 6.

(b) 5′-Dimethoxytrityluridine was silylated using the same procedure. Products **6***a* and **8***a* were obtained in 70% and 15% yields respectively from short column chromatography using ether–hexane (1:1). Properties are listed in Table 6.

(c) 5′-Dimethoxytrityl-*N*-benzoylcytidine was silylated using the standard procedure. Products **6***d* and **8***d* were obtained in 68% and 20% yields respectively after short column chromatography using ether–hexane (3:1). Properties of products are listed in Table 6.

(d) 5′-Dimethoxytrityl-*N*-benzoylguanosine was silylated using the standard procedure except that 1.5 equivalents of silver nitrate and 1.7 equivalents of TBDMS-Cl were used. The products were separated on short column chromatography using chloroform – methylene chloride – triethylamine (9:0.5:0.5). The collected fractions were neutralized by passing them through Dowex 50W × 8 (H^+ form) resin. The solvent was evaporated and the individual products were obtained as white solids on precipitation from chloroform with petroleum ether. The 2′-isomer **6***f* was obtained in 45% yield and the 3′-isomer **8***f* in 40% yield. Properties are summarized in Table 6.

Silylation of nucleosides **1**

The general procedure is illustrated for the silylation of uridine. Uridine (1 mmol) was dissolved in THF (30 mL). Pyridine (5 mmol) and silver nitrate (2.2 mmol) were added and stirring was continued for 5 min until all silver nitrate had dissolved. TDBMS-Cl (2.2 mmol) was added and the mixture was stirred for 1 h. The solution was collected by filtration and the solvents concentrated to a small volume and applied to 4 tlc plates which were developed in ether–hexane (4:1). The yields are shown in Table 2 for all compounds tested.

For monosilylation the above procedure is repeated exactly except that pyridine is omitted. Yields of the 5′-monosilyl derivatives are collected in Table 2. All silylated compounds were identified by comparison to authentic material (8).

The general procedure described above was employed for all nitrate and perchlorate salts tested. The results of all of these reactions are summarized in Tables 2 and 3. It is important to add the reagents in the order described above. All solvents used in the reactions should be purified by distillation.

Selective 3′-silylations

A. *General procedure using DABCO*

The general procedure is much the same as described above. DABCO (0.67 g, 6 mmol/mmol of nucleoside) was dissolved in dry THF (25 mL/mmol nucleoside). Silver nitrate (0.37 g, 2.2 mmol/mmol of nucleoside) was added and the solution was stirred for 5 min. At this point *tert*-butyldimethylsilyl chloride (0.35 g, 2.2 mmol/mmol of nucleoside) was added. After an additional 5 min the nucleoside (1 mmol) was added and stirring continued (for time of reaction, see Table 4). At the end of the reaction period the solution was collected by filtration, diluted with 50 mL of water, and extracted with chloroform (80 mL). The organic layer was dried and concentrated and the products were isolated by short column chromatography.

The results of individual experiments are collected in Table 4.

B. *General procedure using pyridine* N-*oxide*

This procedure is identical to that described in A above except that a pyridine *N*-oxide is used in place of DABCO. The ratio of reagents used and the results of individual experiments are collected in Table 5.

Acknowledgements

We gratefully acknowledge generous financial support from the Natural Sciences and Engineering Research Council of Canada (NSERCC Strategic Grant) and the Government of Quebec (FCAC).

1. G. Alvarado-Urbina, G. M. Sathe, W-C. Liu, M. F. Gillen, P. D. Duck, and K. K. Ogilvie. Science, **214**, 270 (1981).
2. H. Kossel and H. Seliger. Prog. Chem. Org. Nat. Prod. **32**, 298 (1975).
3. H. G. Khorana. Some recent developments in the chemistry of phosphate esters of biological interest. J. Wiley & Sons, New York. 1961. pp. 93–140.
4. R. L. Letsinger and K. K. Ogilvie. J. Am. Chem. Soc. **89**, 4801 (1967).
5. S. A. Narang, R. Brousseau, H. M. Hsiung, and J. J. Michniewicz. Methods Enzymol. **65**, 610 (1980).
6. R. L. Letsinger, J. L. Finnan, G. A. Heavner, and W. B. Lunsford. J. Am. Chem. Soc. **97**, 3278 (1975).
7. (*a*) K. K. Ogilvie and M. J. Nemer. Tetrahedron Lett. **21**, 4159 (1981); (*b*) K. K. Ogilvie and R. T. Pon. Nucleic Acids Res. **8**, 2105 (1980); (*c*) K. K. Ogilvie, N. Y. Theriault, J-M. Seifert, R. T. Pon, and M. J. Nemer. Can. J. Chem. **58**, 2686 (1980); (*d*) K. K. Ogilvie and M. J. Nemer. Can. J. Chem. **58**, 1389 (1980); (*e*) K. K. Ogilvie and N. Y. Theriault. Can. J. Chem. **57**, 3140 (1979).
8. (*a*) K. K. Ogilvie, A. L. Schifman, and C. L. Penney. Can. J. Chem. **57**, 2230 (1979); (*b*) K. K. Ogilvie, S. L. Beaucage, A. L. Schifman, N. Y. Theriault, and K. L. Sadana. Can. J. Chem. **56**, 2768 (1978).
9. K. K. Ogilvie and N. Y. Theriault. Tetrahedron Lett. 2111 (1979).
10. B. R. G. Williams and I. M. Kerr. Nature, **276**, 88 (1978).
11. K. L. Agarwal, A. Yamazaki, P. J. Cashion, and H. G. Khorana. Angew. Chem. Int. Ed. Engl. **11**, 451 (1972).
12. H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana. J. Am. Chem. Soc. **85**, 3821 (1963).
13. M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana. J. Am. Chem. Soc. **84**, 430 (1962).
14. D. H. Rammler and H. G. Khorana. J. Am. Chem. Soc. **84**, 3112 (1962).
15. D. J. Ager and I. Flemming. J. Chem. Research (S), 6 (1977).
16. T. J. Barton and C. R. Tully. J. Org. Chem. **43**, 3649 (1978).
17. C. B. Reese and D. R. Trentham. Tetrahedron Lett. 2467 (1965).
18. W. T. Markiewicz. J. Chem. Research (S), 24 (1979).
19. G. H. Hakimelahi, Z. A. Proba, and K. K. Ogilvie. Tetrahedron Lett. **22**, 4775 (1981).
20. G. H. Hakimelahi, Z. A. Proba, and K. K. Ogilvie. Tetrahedron Lett. **22**, 5243 (1981).