Protecting groups for RNA synthesis: an increasing need for selective preparative methods

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RNA can be chemically synthesized by automated DNA/RNA synthesizers, using protected ribonucleosides activated as phosphoramidites. The efficiency of the synthesis depends greatly on the protecting groups used, especially the protecting group on the 2′-hydroxyl functionality. The strategies employed to place the protecting groups on the desired functionality are quite inefficient, requiring additional modifications of the substrate, or leading to mixtures of protected compounds. In this *tutorial review*, the methods available for the selective protection of ribonucleosides are commented on, introducing the reader to the synthetic challenges involved.

Introduction

DNA oligonucleotides are routinely obtained on a solid support from their corresponding monomers by using an automated synthesizer. This process is very efficient, and allows for control of the sequence, length and the incorporation of modified nucleosides, which has led to the development of interesting applications in different areas, such as nanotechnology[1] and biomedicine.[2,3]

On the other hand, the preparation of RNA oligonucleotides has been more problematic. The presence of the 2′-hydroxyl group on the sugar moiety of ribonucleosides has required the development of additional orthogonal protecting groups. Additionally, the synthesis of the monomers used to prepare the oligonucleotides is less efficient.

During the last few years, the interest in RNA research has increased significantly, since RNA is a key component of biological systems and performs many functions in the regulation of genetic information. Moreover, RNA is a versatile tool in molecular biology,[4] and has been used for catalysis (ribozymes),[5] specific protein binding (aptamers)[6] and in the control of gene expression (riboswitches[7] and small RNAs[8]).

The potential applications based on RNA have attracted interest from the scientific community. Unfortunately, the inefficient preparation of the monomers and their corresponding oligonucleotides can hinder the development of new RNA-based technologies.

In this tutorial review, the automated synthesis of oligonucleotides, particularly of RNA, and the protecting groups required are commented on. The methods available for the selective incorporation of these protecting groups are reviewed, pointing out the need for better approaches.

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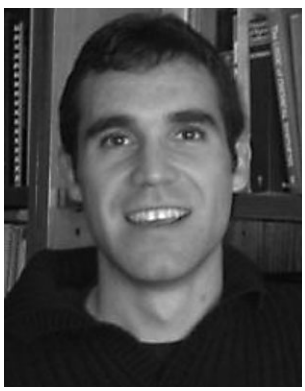

Alvaro Somoza

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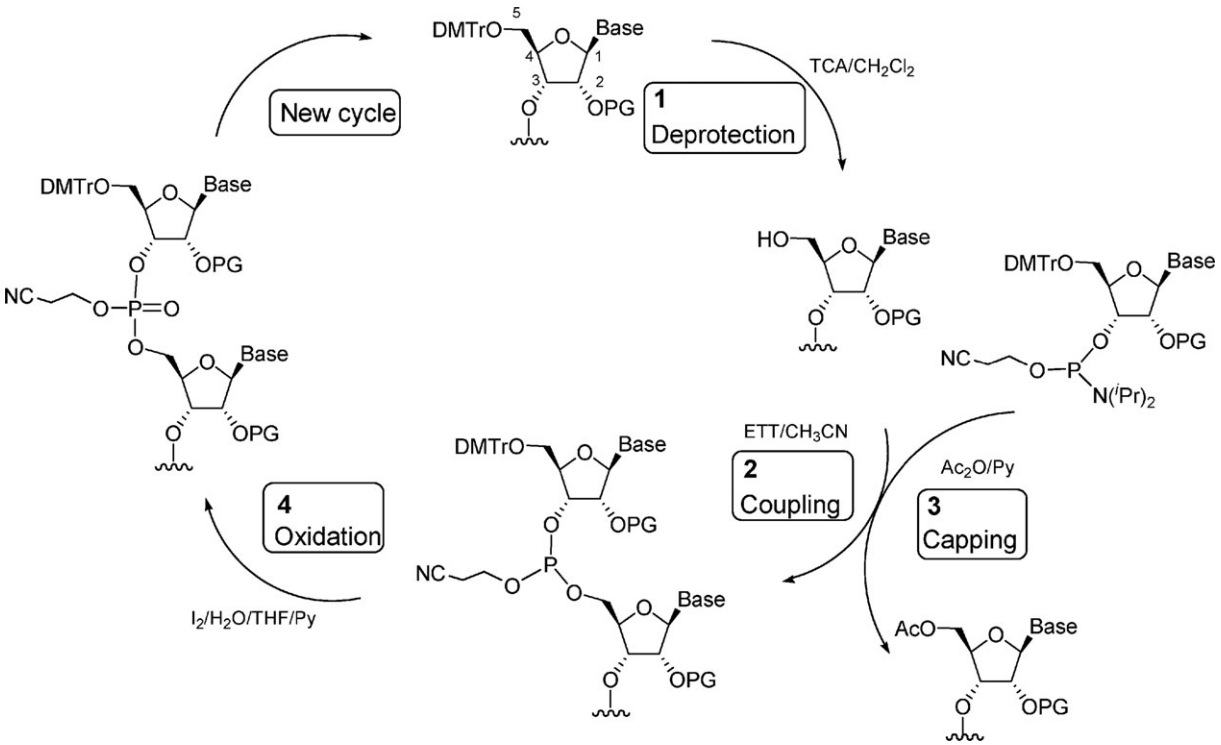
Synthesis of oligonucleotides

The most commonly used method for the chemical synthesis of oligonucleotides has been the phosphoramidite four-step process.[9,10] This method consists of the reaction of activated nucleoside phosphoramidites with solid-phase tethered nucleosides, and can be carried out by an automated synthesizer. The processes that take place during RNA synthesis are represented in Scheme 1. The synthesis starts with removal of the dimethoxytrityl (DMTr) protecting group at the 5′-hydroxyl functionality of the nucleoside attached to the solid support by treatment with a mild acid, such as trichloroacetic acid (TCA) or dichloroacetic acid (DCA) (step 1). The subsequent addition of an activated nucleoside gives rise to the formation of a phosphite triester internucleotide bond (step 2). The unreacted nucleosides on the solid support are capped by derivatization of the 5′-hydroxyl groups to the corresponding acetyl esters (step 3).

This step prevents the addition of subsequent nucleosides to this group, thereby minimizing the formation of undesired sequences. The last step is the oxidation of the phosphite triester group to the corresponding phospho triester (step 4),



Scheme 1 The synthetic cycle carried out by an automated synthesizer.

which can be achieved using iodine or *tert*-butylhydroperoxide. Multiple cycles of this process generate the oligoribonucleotide of desired length and sequence, after its cleavage from the solid support.

Protecting groups

The efficiency of oligonucleotide synthesis depends greatly on the protecting groups used on the nucleotide phosphoramidites, which have to be carefully chosen and selectively incorporated at different positions, such as the exocyclic amino group on the nucleobase and the hydroxyl groups at the 5′- and 2′-positions (Fig. 1).

The selective protection of these groups can be difficult due to their similar reactivity, giving rise to derivatives that are protected at undesired positions. The selection of appropriate reagents and conditions can afford protected deoxyribonucleosides at the nucleobase and the 5′-hydroxyl group without major difficulties. Unfortunately, in the case of ribonucleosides, the selective protection of the 2′-hydroxyl group is more complicated due to the presence of another secondary hydroxyl group at the 3′-position.

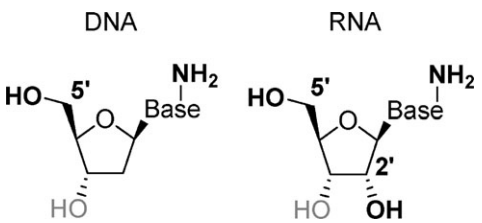


Fig. 1 The positions to be protected on nucleosides for DNA or RNA synthesis.

Due to the similarities between DNA and RNA monomers (Fig. 1), most of the protecting groups used in RNA synthesis have been adapted from work previously done on DNA. This is the case for the protecting groups used on the 5′-hydroxyl functionality and the nucleobases. However, in the case of the 2′-hydroxyl functionality, new protecting groups orthogonal to the nucleobase and the 5′-hydroxyl protecting groups had to be developed for RNA synthesis.

Nucleobase protecting groups

During the synthesis of oligoribonucleotides, the nucleophilic nitrogen present on the nucleobases has to be conveniently protected in order to prevent side reactions. The protecting groups used have to be stable during synthesis of the oligonucleotide, and are usually removed during cleavage of the strand from the solid support. The most common protecting groups used on the nucleobases are the acyl-type ones, such as acetyl (Ac), benzoyl (Bz) or isobutyryl (Ib) groups (Fig. 2).[11]

The main drawback of this kind of protecting group is the deprotection conditions, typically involving heating for long

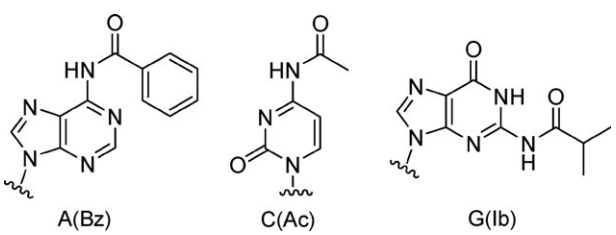


Fig. 2 Protected nucleobases.

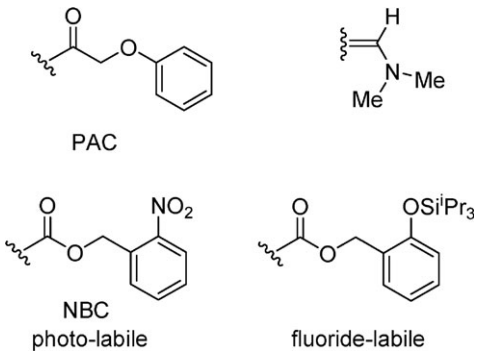


Fig. 3 Labile nucleobase protecting groups.

periods of time (NH_4OH 28%, 12 h, 55 °C), which can lead to partial degradation of the oligoribonucleotide.

When room temperature or short reaction times are needed, more labile protecting groups, such as phenoxyacetyl (PAC)[12] or dimethylformamidine,[13] can be used instead (Fig. 3).

In addition, photo-[14] and fluoride-[15]labile protecting groups have been developed, which can be removed using very mild conditions and are orthogonal to the protecting groups previously mentioned.

The methods employed for the incorporation of these groups onto the exocyclic amino moiety are quite selective, and it is even possible to directly monobenzoylate cytidine with a high selectivity.[16] On the other hand, transient protection of the hydroxyl group is necessary to selectively protect all of the other nucleobases. The methods reported by Rammler and Khorana,[17] and Jones *et al.*[18] allow the isolation of the monoprotected derivatives in excellent yields, with the strategy developed by Jones and co-workers being the method of choice for the protection of the exocyclic amino group on ribonucleosides. This strategy consists of the transient protection of the hydroxyl groups as trimethylsilyl ethers, leaving the exocyclic amino group available to react with the benzoyl chloride reagent. After removal of the trimethylsilyl (TMS) groups with ammonium hydroxide, the *N*-protected nucleosides are obtained in very good yields (Scheme 2).

Despite the high yields achieved through this approach, methods selective enough to skip the transient protection step of the hydroxyl groups are desirable. In this context, Tripathi and co-workers have reported a direct approach in which the key step is the activation of acid derivatives of the protecting groups to the corresponding *p*-nitrophenoxy esters by DCC on a solid support.[19]

5′-Hydroxyl protecting groups

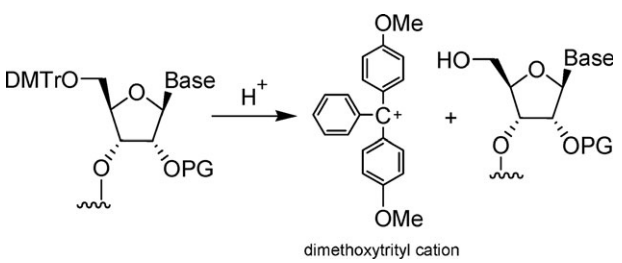
In contrast to the protecting groups on the nucleobase and the 2′-hydroxyl functionality, the protecting group at the 5′-hydroxyl position has to be cleaved during the oligonucleotide synthesis to allow coupling with a phosphoramidite. The conditions employed to remove it have to be completely compatible with the other protecting groups present on the molecule, and to give rise to the free 5′-hydroxyl group quantitatively and in a very short period of time. These conditions have been achieved using the DMTr group, which can be easily removed under mild acidic conditions. This group also allows the monitoring of the yield after every cycle by absorbance or conductivity measurements of the DMTr cation obtained after deprotection (Scheme 3).

The DMTr and other groups designed for protection of the 5′-hydroxyl group were initially established for DNA synthesis,[20] and only some of them have been developed during RNA synthesis research.[21,22] The most significant are the fluoride-labile groups described by Scaringe and co-workers[21] during the development of a new method for RNA synthesis, which is described below. A variety of silyl derivatives were tested as protecting groups, with the bis(trimethylsiloxyl)-cyclooctyloxylsilyl group being the best for 5′-hydroxyl protection. However, this group cannot be used to monitor the yield after every cycle; for this reason, some derivatives have been modified with the DMTr group to overcome this drawback (Fig. 4).

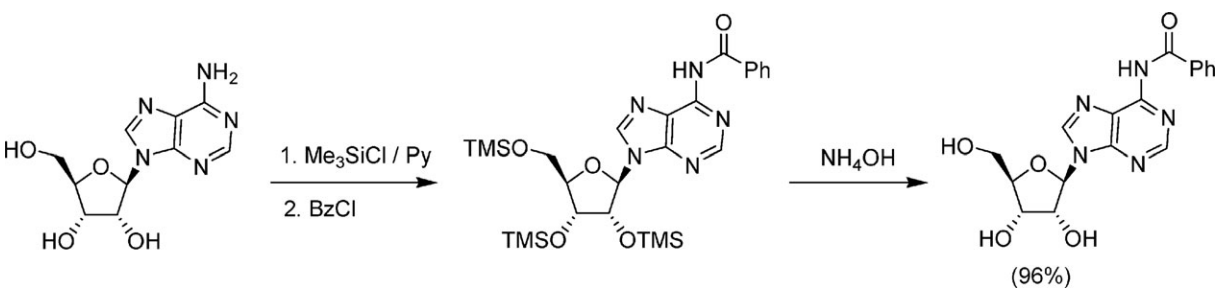
Protection of the 5′-hydroxyl group with DMTrCl is highly selective and special conditions are not required for this transformation. This is consequence of the large size of the protecting group, which reacts faster with the primary hydroxyl group than with the other reactive positions.

2′-Hydroxyl protecting groups

As has been mentioned previously, the protecting groups employed for the nucleobase and 5′-hydroxyl functionality



Scheme 3 The acid deprotection of DMTr.



Scheme 2 The selective benzoyl protection of adenine.

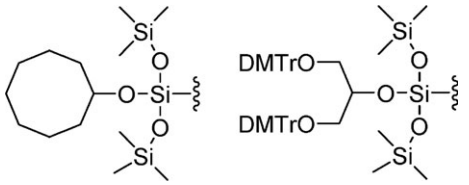
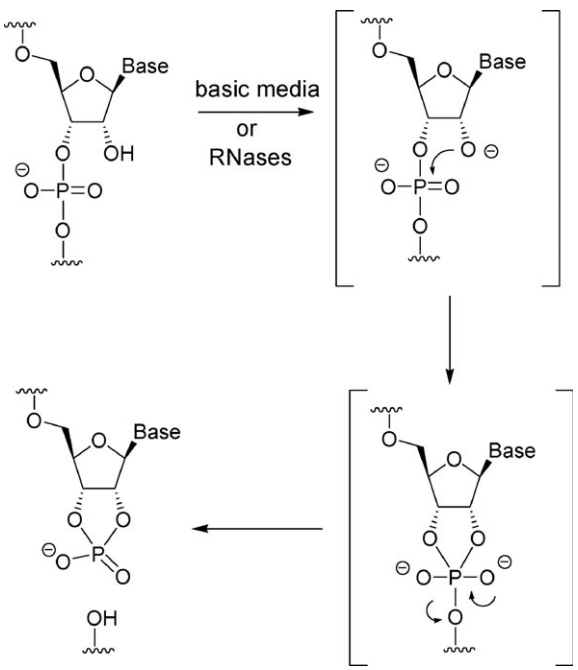


Fig. 4 Fluoride-labile protecting groups for the 5′-hydroxyl functionality

in RNA synthesis have been adapted from DNA synthesis, and standard protecting groups are commonly used. In contrast, there is not a consensus protecting group for the 2′-hydroxyl functionality due to the many requirements it has to fulfil. This protecting group must be stable under the conditions employed during the synthesis, such as the acidic media needed to remove the DMTr group after every cycle, or the basic treatment required to unblock the base residues and to cleave the RNA strand from the solid support.

The 2′-hydroxyl group is key to the stability of oligoribonucleotides because RNA degradation can take place in basic media, or in the presence of RNases through deprotonation of this group. Then, the generated alkoxy intermediate attacks the phosphorus atom, leading to the cleavage of the phosphodiester internucleotide bond (Scheme 4).[23] For this reason, removal of the protecting group at the 2′-position has to be performed under mild conditions to prevent RNA degradation.

It is also desirable that the 2′-protecting group does not interfere in the coupling step, which can be difficult to achieve, since it is very close to the reaction site where the formation of the internucleotide bond takes place (Scheme 1). Consequently, sterically demanding protecting groups should be avoided.



Scheme 4 Internucleotide bond cleavage.

Nevertheless, a wide variety of protecting groups have been reported that fulfil these requirements, with varying degrees of success.[24] The most representative ones can be classified by the deprotection method used as either fluoride-, photo- or acid-labile.

Among the different 2′-protecting groups developed for RNA synthesis, the most extensively used has been the fluoride-labile *tert*-butyldimethylsilyl (TBDMS) group (Fig. 5).[25] TBDMS can be easily incorporated and removed, and is completely compatible with the DMTr chemistry, but has some significant drawbacks. The time required for the coupling step using the standard activator 1-*H*-tetrazole (Tet) is long (12 min), due to steric interactions with the bulky TBDMS group. However, this low reactivity can be overcome by using better activators that significantly reduce the coupling time, such as 5-ethylthio-1*H*-tetrazole (ETT)[26] or 5-(benzylmercapto)-1*H*-tetrazole (BTT) (Fig. 6).[27] These molecules activate the coupling through a two-step process. First, the activator protonates the

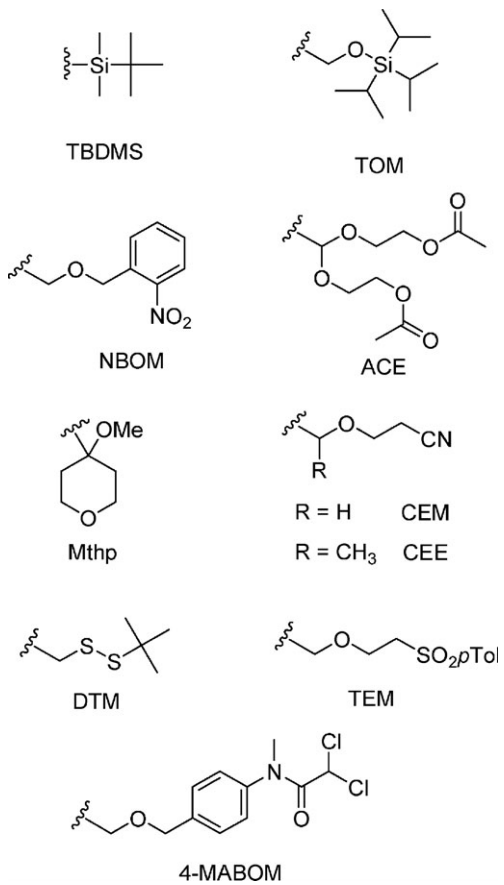


Fig. 5 Protecting groups for the 2′-hydroxyl functionality.

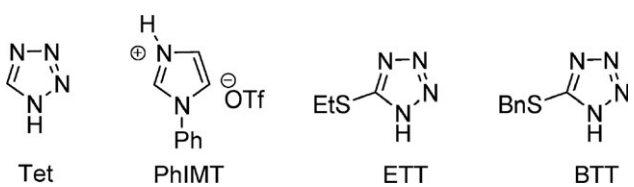
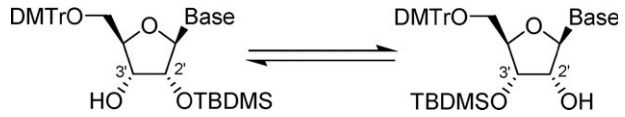


Fig. 6 Activators utilized in the coupling step.



Scheme 5 TBDMS migration in ribonucleosides.

diisopropylamino group of the phosphoramidite. Then, the deprotonated activator replaces the diisopropylamino group on the phosphorus atom, giving rise to a more reactive intermediate, which reacts with the 5′-hydroxyl group of the nucleoside attached to the solid support (Scheme 1). The use of activators can significantly improve the synthesis of RNA when bulky protecting groups such as TBDMS are employed at the 2′-hydroxyl functionality. In this context, a new family of azolium salts, such as *N*-(phenyl)imidazolium triflate (PhIMT), have shown excellent activation properties.[28]

Another issue related to the use of TBDMS is that it can migrate from the 2′- to the 3′-hydroxyl functionality during preparation of the protected ribonucleosides. Therefore, special precautions are needed to prevent the formation of undesired 3′-*O*-TBDMS ribonucleosides (Scheme 5).

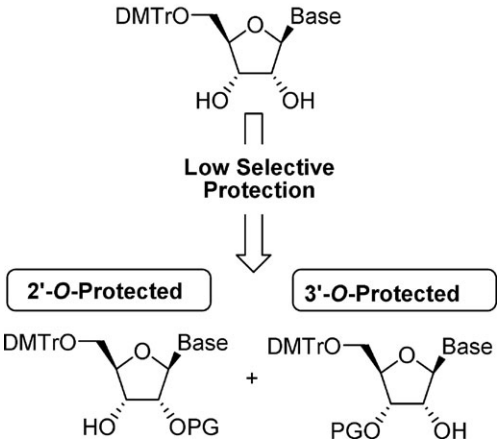
A great contribution to this field was made by Pitsch and co-workers, who reported the use of the [(triisopropylsilyl)oxy]-methyl group (TOM, Fig. 5) as a fluoride-labile protecting group.[29] TOM has shown several advantages compared to TBDMS; specifically, the TOM group does not migrate and also requires a reduced coupling time (6 min).

The interesting class of photo-labile protecting groups is stable under acidic and basic treatment, and can be cleaved under UV exposure. The coupling time required for this kind of protecting group, such as the [(2-nitrobenzyl)oxy]methyl group (NBOM, Fig. 5),[30] is short (2 min) and the yields obtained after every cycle are excellent. However, in long RNA strands, this protecting group cannot always be removed quantitatively due to the by-products generated during deprotection, which absorb the light required for removal of the remaining NBOM groups. Despite the improvements reported for deprotection, such as the continuous extraction to remove by-products,[31] this method has not reached the same popularity as previous ones.

Scaringe and co-workers developed the acid-labile protecting group bis(acetoxyethoxy)methyl ether (ACE), which allows the synthesis of oligoribonucleotides in high yields and purity, with a coupling time of 1 min (Fig. 5).[21,32] However, the orthoester group is sensitive to acid and therefore is not compatible with the widely used DMTr group previously mentioned. As a result, a new family of fluoride-labile protecting groups orthogonal to ACE was developed for the 5′-hydroxyl functionality (Fig. 4). The main drawback of the ACE strategy is that the DNA/RNA synthesizer usually has to be modified to use the deprotection reagent (HF/TEA), thus limiting the spread of this method.

Selective 2′-hydroxyl protection methods

Despite the different protecting groups developed for this functionality, the synthetic strategies to incorporate them on the sugar moiety are not numerous. Moreover, the methods reported so far are not efficient, requiring additional manipulations or leading to a mixture of 2′- and 3′-protected ribonucleosides that are usually difficult to purify (Scheme 6).



Scheme 6 The mixture obtained during the protection of ribonucleosides.

This issue becomes a severe problem when non-natural nucleosides are needed for the preparation of modified oligoribonucleotides, since their synthesis can be tedious and expensive. The methods available up to now for the selective protection of the 2′-hydroxyl functionality are commented on below.

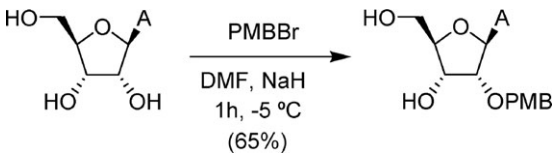
Direct protection

Direct protection of the 2′-hydroxyl group is difficult since there are other reactive positions on the nucleoside that can be protected as well. However, it can be achieved with some selectivity due to the difference in acidity between the 2′- and 3′-hydroxyl groups. The 2′-hydroxyl is slightly more acidic than the 3′-hydroxyl and, in some conditions, the 2′-protected derivative can be isolated in a moderate yield. In the example below, the addition of *p*-methoxybenzyl bromide to a solution of adenine and NaH in dimethylformamide (DMF) at −5 °C afforded the 2′-*O*-protected derivative in 65% yield (Scheme 7).[33]

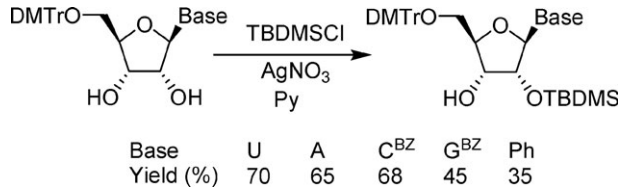
Unfortunately this approach does not work for every nucleoside or protecting group, and therefore additional strategies have been developed to afford 2′-protected ribonucleosides.

Additives for selective silyl protection

During the evaluation of TBDMS as a protecting group for ribonucleosides, Ogilvie and co-workers found that the use of silver nitrate in the reaction could increase the selectivity significantly.[34] The role of this additive is not completely clear, but it has been shown that the use of $TBDMSNO_3$ instead of a mixture of TBDMSCl and $AgNO_3$ gives rise to the same result, showing the key role of the nitrate anion in the selectivity. 2′-*O*-TBDMS ribonucleosides can be isolated in



Scheme 7 The direct protection of ribonucleosides.



Base	U	A	C^{BZ}	G^{BZ}	Ph
Yield (%)	70	65	68	45	35

Scheme 8 Selective TBDMS protection promoted by silver nitrate.

moderate to good yields using natural ribonucleosides, but the yield drops with modified ribonucleosides, as in the case shown in Scheme 8 where the base has been replaced by a benzene ring.[35]

The use of silver nitrate has also proven to be useful with the bulkier triisopropylsilyl (TIPS) group, allowing isolation of the 2′-*O*-TIPS derivatives in better yields.[36]

Another reagent that has been utilized for selective TBDMS protection is ammonium phosphonate, which was reported by Jones and co-workers.[37] This additive promotes the formation of a mixture of two phosphonate-*O*-TBDMS intermediates in equilibrium, which mainly afforded the 2′-*O*-TBDMS protected phosphonate. The final addition of pivaloyl (Piv) or adamantoyl chloride, and ethylene glycol or glycerol, completely removes the phosphonate on the 3′-hydroxyl group, leading to the final 2′-*O*-TBDMS ribonucleoside (Scheme 9). The selectivity observed in this procedure is very low in the case of uridine and cytidine, but purine nucleosides give high selectivities and overall yields of 60–70%.

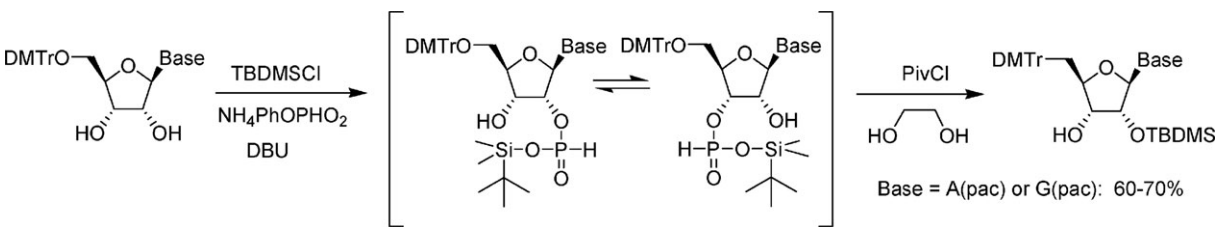
An interesting feature of these two methods is that they allow the direct protection of the 2′-hydroxyl group in a one-pot procedure. However, the variability of the results and the limitations of TBDMS as a protecting group make other approaches preferable.

The two strategies commonly utilized for the selective protection of the 2′-hydroxyl groups are: (1) the transient protection of the 3′- and 5′-hydroxyl groups by a disiloxane-based protecting group, and (2) the activation of the 2′- and 3′-hydroxyl groups *via* a dibutylstannylene intermediate. These two approaches have been applied to the recently reported DTM and TEM protecting groups, respectively, and are discussed below.

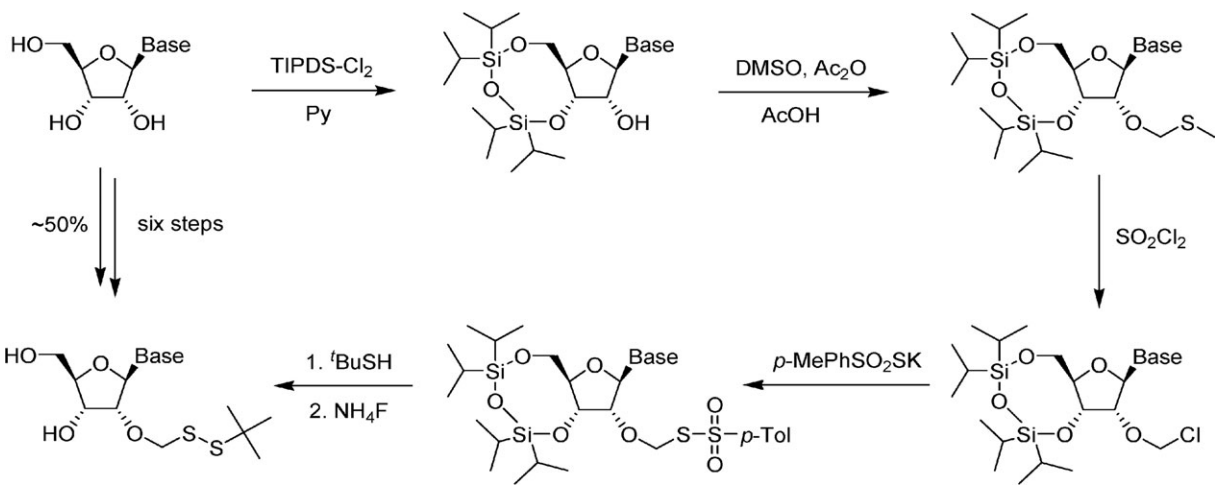
Transient 3′- and 5′-hydroxyl protection

The use of a disiloxane to protect the 5′- and 3′-hydroxyl functionalities on nucleosides with protected nucleobases leaves the 2′-hydroxyl group available to react with a second protecting group. The final removal of the disiloxane group gives rise to the desired 2′-protected ribonucleoside exclusively. This strategy has been employed by Kwiatkowski and co-workers for the selective incorporation of a new protecting group, *tert*-butyldithiomethyl (DTM),[38] on ribonucleosides.

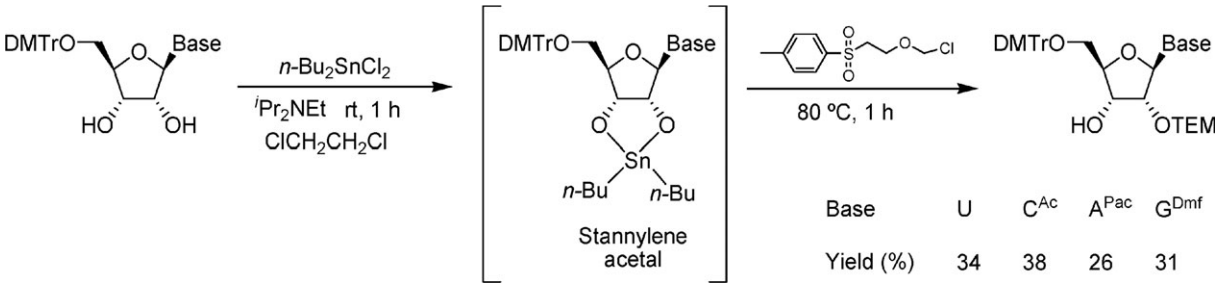
In this case, the protecting group is synthesized on the substrate through a multi-step sequence (Scheme 10). The synthesis of the DTM group starts by the addition of an electrophilic sulfonium species, generated *in situ* from DMSO and Ac_2O in acid media, leading to the corresponding methylthiomethyl ether derivative. The addition of sulfuryl chloride replaces the thiomethyl group with a chlorine, and the subsequent addition of potassium *p*-toluenethiosulfonate affords the reactive intermediate needed for the final step. The addition of *tert*-butyl mercaptan and deprotection with



Scheme 9 TBDMS protection by the phosphonate method.



Scheme 10 DTM protection of ribonucleosides.



Scheme 11 The TEM protection of ribonucleosides through a stannylene acetal.

ammonium fluoride gives rise to the corresponding 2′-*O*-DTM nucleosides in about 50% overall yield.

The main drawback of the disiloxane method is the two additional manipulations required to introduce and remove the transient protecting group, which reduce the efficiency of the synthesis. Moreover, in this report, multiple steps are required to introduce the DTM protecting group (six steps).

Regardless of the synthetic route, the new DTM group has shown interesting features, including deprotection in serum. This means that the complete DTM deprotection of RNA may not be necessary for *in vivo* experiments. Another attractive characteristic is the orthogonality found between silyl protecting groups and DTM. 2′-*O*-silyl protecting groups have been removed selectively in a strand with a DTM-protected nucleotide.[38]

The 3′,5′-disiloxane approach for the selective protection of the 2′-hydroxyl group has proven to be effective for a variety of protecting groups, such as tetrahydro-4-methoxy-2*H*-pyran-2-yl (Mthp),[39] 1-(2-cyanoethoxy)ethyl (CEE),[40] 2-cyanoethoxymethyl (CEM)[41] and 4-(*N*-dichloroacetyl-*N*-methylamino)benzyloxymethyl (4-MABON).[42] However, fluoride-labile groups should be avoided since the disiloxane protecting group is also removed by a fluoride reagent, although in some cases mild fluoride reagents can be used to selectively cleave the disiloxane in the presence of a fluoride-sensitive protecting group.[41] In this sense, the incorporation of the widely utilized TBDMS[43] and TOM[44] groups has been achieved through a related strategy, using the di-*tert*-butylsilanediyl protecting group for the 3′- and 5′-hydroxyl functionalities.

Activation of 2′- and 3′-hydroxyl groups

The activation of the vicinal hydroxyl groups by dialkyl tin reagents for the protection of ribonucleosides has been applied in the recently reported 2-(4-tolylsulfonyl)ethoxy methyl (TEM) protecting group (Scheme 11).[45] The protection of the 2′-hydroxyl group takes place in a one-pot, two step sequence. The addition of dibutyl tin chloride and a base to a solution of the ribonucleoside promotes the formation of the corresponding stannylene acetal, thus activating the 2′- and 3′-hydroxyl groups. The addition of the TEMCl reagent at 80 °C gives rise to a mixture of 2′ and 3′-*O*-protected ribonucleosides, in which the corresponding 2′-*O*-TEM derivatives can be isolated in about a 30% yield.

The new TEM protecting group reported has increased base stability compared to the related CEM protecting group. This feature prevents degradation of the RNA strand during the ammonia treatment needed for deprotection of the base residues and for cleavage of the strand from the solid support.

Besides the low yields usually obtained by this approach, another important disadvantage is the equimolar amount of the tin reagent that is required, which, in addition to its toxicity, can make the purification of the final compounds difficult.

Despite these disadvantages, this method has been employed with a variety of protecting groups, such as NBOM,[30] TOM[28] or CEM,[46] and is the preferred method for fluoride-labile protecting groups.

Conclusions

Despite the advances made in RNA synthesis, new protecting groups and selective protection methods are still needed to facilitate the development of new RNA technologies. The protection of the nucleobase and the 5′-hydroxyl group can be achieved without major difficulties; on the other hand, the 2′-hydroxyl group is more problematic, and the examples presented herein illustrate the inefficiency of the strategies employed for 2′-*O*-protection in ribonucleosides. It is remarkable that, despite the diverse number of protecting groups developed, there are still very few protection methods, and a direct and selective approach with a suitable protecting group is still lacking.

Methods that achieve this goal would be of immense value as they would facilitate the preparation of oligoribonucleotides. This issue is especially significant in the case of chemically modified ribonucleotides, since their preparation is costly and the inefficient protection of the 2′-hydroxyl group can limit their use.

An interesting approach to address this issue would be the use of a catalytic reaction to protect the 2′-hydroxyl group. In this way, the equimolar quantities of the activator or the additional protecting group could be avoided. Although the development of these kinds of catalysts is very challenging, the successful reports for the catalytic protection of 1,2-diols should encourage synthetic chemists to seek efficient catalysts for ribonucleoside protection.[47]

Abbreviations

Ac	Acetyl
ACE	Bis(acetoxyethoxy)methyl ether
Bz	Benzoyl
BTT	5-(Benzylmercapto)-1*H*-tetrazole

CEE	1-(2-Cyanoethoxy)ethyl
CEM	2-Cyanoethoxymethyl
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTr	Dimethoxytrityl
DTM	*Tert*-butyldithiomethyl
ETT	5-Ethylthio-1*H*-tetrazole
Ib	Isobutyryl
4-MABON	4-(*N*-Dichloroacetyl-*N*-methylamino)benzyloxymethyl
Mthp	Tetrahydro-4-methoxy-2*H*-pyran-2-yl
NBC	[(2-Nitrobenzyl)oxy]carbonyl
NBOM	[(2-Nitrobenzyl)oxy]methyl
PAC	Phenoxyacetyl
PG	Protecting group
PhIMT	*N*-(Phenyl)imidazolium triflate
Piv	Pivaloyl
PMB	*p*-Methoxybenzyl
Py	Pyridine
TBDMS	*Tert*-butyldimethylsilyl
TCA	Trichloroacetic acid
TEM	2-(4-Tolylsulfonyl)ethoxy methyl
Tet	Tetrazole
THF	Tetrahydrofuran
TIPDS	Tetraisopropyl disiloxane
TMS	Trimethylsilyl
TOM	[(Triisopropylsilyl)oxy]methyl

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References

1 N. C. Seeman, *Mol. Biotechnol*., 2007, **37**, 246.
2 A. P. Silverman and E. T. Kool, *Chem. Rev*., 2006, **106**, 3775.
3 E. Uhlmann and A. Peyman, *Chem. Rev*., 1990, **90**, 543.
4 F. J. Isaacs, D. J. Dwyer and J. J. Collins, *Nat. Biotechnol*., 2006, **24**, 545.
5 P. C. Bevilacqua and R. Yajima, *Curr. Opin. Chem. Biol*., 2006, **10**, 455.
6 A. C. Yan, K. M. Bell, M. M. Breeden and A. D. Ellington, *Front. Biosci*., 2006, **11**, 89.
7 H. Schwalbe, J. Buck, B. Fürting, J. Noeske and J. Wöhnert, *Angew. Chem., Int. Ed*., 2007, **46**, 2.
8 D. H. Kim and J. J. Rossi, *Nat. Rev. Genet*., 2007, **8**, 173.
9 S. L. Beaucage and M. H. Caruthers, in *Current Protocols in Nucleic Acids Chemistry*, ed. S. L. Beaucage, D. E. Bergstrom, G. D. Glick and R. A. Jones, Wiley, New York, 2000, unit 3.3, pp. 1–20.
10 C. Reese, *Org. Biomol. Chem*., 2005, **3**, 3851.
11 R. P. Iyer, in *Current Protocols in Nucleic Acid Chemistry*, ed. S. L. Beaucage, D. E. Bergstrom, G. D. Glick and R. A. Jones, John Wiley & Sons, New York, 2000, unit 2.1, pp. 1–17.
12 J. H. Boal, A. Wilk, N. Harindranath, E. E. Max, T. Kempe and S. L. Beaucage, *Nucleic Acids Res*., 1996, **24**, 3115.
13 H. Vu, C. McCollum, K. Jacobson, P. Theisen, R. Vinayak, E. Spiess and A. Andrus, *Tetrahedron Lett*., 1990, **31**, 7269.
14 A. Stutz and S. Pitsch, *Synlett*, 1999, 930.
15 A. Stutz, C. Höbartner and S. Pitsch, *Helv. Chim. Acta*, 2000, **83**, 2477.
16 P. Nahar, *Tetrahedron Lett*., 1997, **38**, 7253.
17 D. H. Rammler and H. G. Khorana, *J. Am. Chem. Soc*., 1962, **84**, 3112.
18 G. S. Ti, B. L. Gaffney and R. A. Jones, *J. Am. Chem. Soc*., 1982, **104**, 1316.
19 S. Tripathi, K. Misra and Y. S. Sanghvi, *Bioorg. Med. Chem. Lett*., 2005, **15**, 5045.
20 H. Seliger, in *Current Protocols in Nucleic Acid Chemistry*, ed. S. L. Beaucage, D. E. Bergstrom, G. D. Glick and R. A. Jones, John Wiley & Sons, New York, 2000, unit 2.3, pp. 1–34.
21 S. A. Scaringe, F. E. Wincott and M. H. Caruthers, *J. Am. Chem. Soc*., 1998, **120**, 11820.
22 I. Hirao, M. Koizumi, Y. Ishido and A. Andrus, *Tetrahedron Lett*., 1998, **39**, 2989.
23 M. Oivanen, S. Kuusela and H. Lönnberg, *Chem. Rev*., 1998, **98**, 961.
24 C. B. Reese, in *Current Protocols in Nucleic Acid Chemistry*, ed. S. L. Beaucage, D. E. Bergstrom, G. D. Glick and R. A. Jones, John Wiley & Sons, New York, 2000, unit 2.2, pp. 1–24.
25 B. S. Sproat, in *Oligonucleotide Synthesis: Methods and Applications*, ed. P. Herdewijn, Humana Press, New Jersey, 2005, vol. **288**, pp. 17–31.
26 B. Sproat, F. Colonna, B. Mullah, D. Tsou, A. Andrus, A. Hampel and R. Vinayak, *Nucleosides Nucleotides*, 1995, **14**, 255.
27 R. Welz and S. Müller, *Tetrahedron Lett*., 2002, **43**, 795.
28 Y. Hayakawa, R. Kawai, A. Hirata, J.-I. Sugimoto, M. Kataoka, A. Sakakura, M. Hirose and R. Noyori, *J. Am. Chem. Soc*., 2001, **123**, 8165.
29 S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz and X. Wu, *Helv. Chim. Acta*, 2001, **84**, 3773.
30 M. E. Schwartz, R. R. Breaker, G. T. Asteriadis, J. S. deBear and G. R. Gough, *Bioorg. Med. Chem. Lett*., 1992, **2**, 1019.
31 S. Pitsch, *Helv. Chim. Acta*, 1997, **80**, 2286.
32 S. A. Hartsel, D. E. Kitchen, S. A. Scaringe and W. S. Marsal, in *Oligonucleotide Synthesis: Methods and Applications*, ed. P. Herdewijn, Humana Press, New Jersey, 2005, vol. **288**, pp. 33–49.
33 E. Ohtsuka, S. Tanaka and M. Ikehara, *Synthesis*, 1977, 453.
34 G. H. Hakimelahi, Z. A. Proba and K. K. Ogilvie, *Can. J. Chem*., 1982, **60**, 1106.
35 J. Matulic-Adamic, L. Beigelman, S. Portmann, M. Egli and N. Usman, *J. Org. Chem*., 1996, **61**, 3909.
36 M. D. Erlacher, K. Lang, B. Wotzel, R. Rieder, R. Micura and N. Polacek, *J. Am. Chem. Soc*., 2006, **128**, 4453.
37 Q. Song, W. Wang, A. Ficher, X. Zhang, B. L. Gaffney and R. A. Jones, *Tetrahedron Lett*., 1999, **40**, 4153.
38 A. Semenyuk, A. Földesi, T. Johansson, C. Estmer-Nilsson, P. Blomgren, M. Brännvall, L. A. Kirsebom and M. Kwiatkowski, *J. Am. Chem. Soc*., 2006, **128**, 12356.
39 J. M. Brown, C. Christodolulou, S. S. Jones, A. S. Modak, C. B. Reese, S. Sibanda and A. Ubasawa, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1735.
40 T. Umemoto and T. Wada, *Tetrahedron Lett*., 2004, **45**, 9529.
41 Y. Shiba, H. Masuda, N. Watanabe, T. Ego, K. Takagaki, K. Ishiyama, T. Ohgi and J. Yano, *Nucleic Acids Res*., 2007, **35**, 3287.
42 J. Cieślak, A. Grajkowski, J. S. Kauffman, R. J. Duff and S. L. Beaucage, *J. Org. Chem*., 2008, **73**, 2774.
43 Y. Saito, A. Nyilas and L. A. Agrofoglio, *Carbohydr. Res*., 2001, **331**, 83.
44 V. Serebryany and L. Beigelman, *Nucleosides, Nucleotides Nucleic Acids*, 2003, **22**, 1007.
45 C. Zhou, D. Honcharenko and J. Chattopadhyaya, *Org. Biomol. Chem*., 2007, **5**, 333.
46 T. Ohgi, Y. Masutomi, K. Ishiyama and H. Kitagawa, *Org. Lett*., 2005, **7**, 3477.
47 Y. Zhao, J. Rodrigo, A. H. Hoveyda and M. L. Snapper, *Nature*, 2006, **443**, 67.