

Plant Derived Cholesterol Modifications

A Comparison of Commercially Available Cholesterol Phosphoramidites and Solid Supports for use in Oligonucleotide Synthesis of DNA and RNA (TC and TBDMS chemistries)

Sheena Aitken, Ulrich Ixkes, **Catherine McKeen** and Douglas Picken

Introduction

The use of cholesterol labelled oligonucleotides as a means of improving cell penetration is well known and with increasing frequency these are entering into various stages of clinical trials. As regulatory guidelines become more stringent, there is a need to have non-animal derived cholesterol building blocks. We have developed the latter in the form of 5'-cholesterol cyanoethyl phosphoramidites (**1**, **5**) and a 3'-cholesterol solid support (**2**) where the cholesterol is derived from plants.

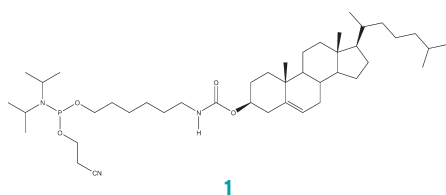
A comparison of commercially available cholesterol phosphoramidites (**1**, **3**, **5**) and solid supports (**2**, **4**) for use in oligonucleotide synthesis of DNA and RNA (TBDMS and TC chemistry) using varying deprotection conditions was carried out. Oligonucleotides modified with dR cholesterol (**6**) were also included.

In particular, degradation during deprotection was investigated where the cholesterol modification can potentially be lost *via* cleavage at the carbamate bond (**all**), the anomeric position (**2** or **6**) or elimination of the entire modification (**3** or **4**) during base deprotection as a result of the 1,2-diol arrangement where the terminal OH reacts with the neighbouring phosphate bond. The optimum deprotection conditions for each of these modifications were then determined

Summary Results & Discussion

5'-Cholesterol CEP (**1**)

1 is based on the standard 5'-C6 linker, the only possible degradation product being cleavage across the carbamate bond.

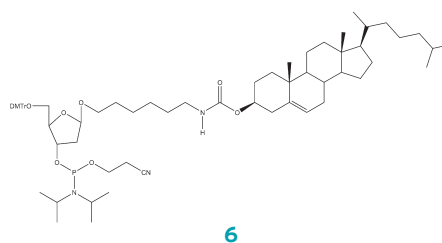
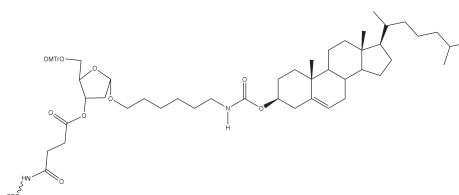


This was shown to be compatible with all DNA and RNA conditions used. The unlabelled oligonucleotide was a result of either failures in synthesising the oligonucleotide itself or incomplete coupling during the addition of the modification. In all cases MS indicated that there

was negligible cleavage across the carbamate bond.

3'-Cholesterol (**2**) and dR cholesterol (**6**)

These modifications are based on the deoxyribose backbone and the only possible degradation products are cleavage across the carbamate bond or at the anomeric position.



The oligonucleotides were synthesised both DMT-OFF and DMT-ON although, since the modification occurs at the 3'-end, no difference in the results was expected. This was not the case: a reproducible pattern was observed showing purer full-length product with DMT-ON oligonucleotides. However, the difference in the percentage of full-length product (~5%) is outweighed by the benefits of synthesising the oligonucleotide DMT-OFF (i.e. solubility, purification, no post-synthetic detritylation).

The results indicated that 3'-cholesterol (**2**) and dR cholesterol (**6**) are relatively stable to all deprotection conditions used and are compatible with both TC and TBDMS-RNA chemistries. There was no evidence of any cleavage at the anomeric position and negligible cleavage across the carbamate bond with the exception of the use of EDA/toluene with the DNA oligonucleotide where 14% cleavage was observed.

Cholesterol TEG (**3**)

This modification is based on a 1,2-diol linker, which is known to result in loss of the label during

deprotection. Additionally there is the possibility of cleavage across the carbamate bond. Of all the cholesterol modifications tested, **3** proved the least stable to most deprotection conditions.

As expected, when cholesterol TEG (**3**) modified oligonucleotides are detritylated prior to deprotection, the main product is either the unlabelled oligonucleotide or the oligonucleotide where cleavage has taken place across the carbamate bond. Even when the oligonucleotide is synthesised DMT-ON, if the deprotection process involved heating, significant cleavage occurs.

Using **3** with TC-RNA chemistry resulted predominately in the cleaved product regardless of whether the synthesis was DMT-ON or DMT-OFF. While TBDMS deprotection using $\text{NEt}_3/\text{Et}_3\text{N}:3\text{HF}/\text{NMP}$ results in the desired product, there is a significant drop (~27%) in the percentage full length product compared to the first deprotection with AMA.

3'-Cholesterol TEG (**4**)

4 is also based on a 1,2-diol linker, but is known to be more stable than **3**. The expected degradation products are complete cleavage of the label and cleavage of the cholesterol across the carbamate bond.

As with **2**, the oligonucleotides were synthesised both DMT-OFF and DMT-ON and the same pattern was observed in that the DMT-ON oligonucleotides gave slightly higher purity than DMT-OFF. This modification is compatible with both TC and TBDMS chemistries.

It is interesting to note that even although **4** has essentially the same structure as **3**, the former proved to be more stable to deprotection. This has been attributed to a combination of the difference in reactivity between the primary and secondary alcohol and the difference in spatial orientation

of the two modified oligonucleotides between the free OH and the nearest phosphate bond (4.5Å for **3** and 4.8Å for **4**).

5'-Cholesterol TEG (**5**)

5 is based on a TEG linker where the only expected degradation product is cleavage across the carbamate bond. For this modification a comparison was made of plant and animal derived materials. In terms of oligonucleotide synthesis, cleavage and deprotection, no difference in performance was observed.

This modification has proved to be more stable to deprotection than **3** but slightly less stable than **1**. This is compatible with both TC and TBDMS-RNA chemistries.

Conclusions

With the exception of **3**, all the cholesterol modifications tested were compatible with all deprotection conditions used. A summary of the results are shown in [Table 1](#) below.

While there is no major difference between the 3'-modifications (**2**, **4** and **6**), the order of stability for the 5'-modifications proved to be 5'-cholesterol (**1**) > 5'-cholesterol TEG (**5**) > cholesterol TEG (**3**).

Further Information

Any queries regarding this work should be directed to Dr Catherine McKeen, Product Manager, Link Technologies Ltd, by e-mail to catherine@linktech.co.uk or telephone on +44(0)1698 849911.

Copies of the full poster and a detailed technical information sheet will be made available *via* our web site at www.linktech.co.uk.

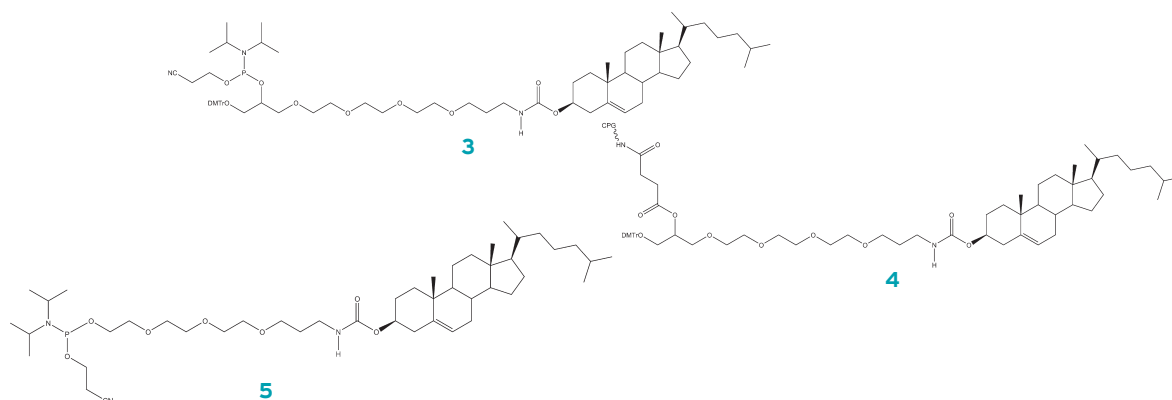


Table 1. Summary of results

Modification	Optimum Deprotection Conditions (DNA)	Compatibility with RNA Chemistry		Plant Derived
		TC	TBDMS	
5'-Cholesterol (1)	AMA, RT, 2h	Yes	Yes	Yes
3'-Cholesterol (2)	AMA, 65°C, 10min	Yes	Yes	Yes
3'-Cholesterol TEG (3)	AMA, RT, 2h	No	Yes	No
Cholesterol TEG (4)	AMA, 65°C, 10min	Yes	Yes	No
5'-Cholesterol TEG (5)	AMA, RT, 2h	Yes	Yes	Yes
dR Cholesterol (6)	AMA, RT, 2h	Yes	Yes	No