Not everything likes water. Our new modifiers love it.

Hydrophilic terminal modifiers for rapid oligo labelling in aqueous solution

Until now, post-synthetic reaction of amino- or thiol- modified oligonucleotides in aqueous solution (e.g. in dye labelling) often required the use of a separate hydrophilic spacer phosphoramidite.

We have designed a new range of ethylene glycol-based terminal modifiers with hydrophilicity built-in. This greatly increases efficiency and lowers the overall cost of oligo production.

www.linktech.co.uk

link technologies
tools for molecular biology

Overview

The use of modified linkers to incorporate labels such as dyes, haptens, enzymes and antibodies into oligonucleotides is widely known. Most commonly these are thiol[1,2] or amino[2,3] modified linkers. Although there are 3'-modified linkers, it is more common that these are added to the 5' end on an oligonucleotide using phosphoramidite chemistry.

With the exception of MMTr-protected amino linkers, where on-column labelling is possible, the use of these modifiers requires the attachment of the desired label to be carried out post-synthetically and in aqueous solution. On the whole, solution-phase coupling with oligonucleotides modified with these linkers is sufficient. However, longer sequences, and those with significant secondary structure, often prove problematic and poor coupling of the label is often observed. To counteract this an amino modifier with a longer (C12) linker[4] was developed which has been shown to improve the post labelling reaction but in many cases, especially when coupling an enzyme or antibody to an oligonucleotide, there is a need to add a hydrophilic spacer such as spacer 18 to optimise the coupling reaction.

Although the use of the spacer improves the coupling reaction, it takes the label further away from the oligonucleotide. This can be problematic in some cases, e.g. molecular beacons where it is important that the quencher and the fluorophore are close enough in order to interact. In this case it is advisable to use a spacer at both the 5' and 3' end of the oligonucleotide. This clearly adds to the manufacturing cost of the oligonucleotide.

It is therefore desirable to have available a range of modifiers than can introduce a reactive functionality to the oligonucleotide which has inherent compatibility with aqueous chemistry.

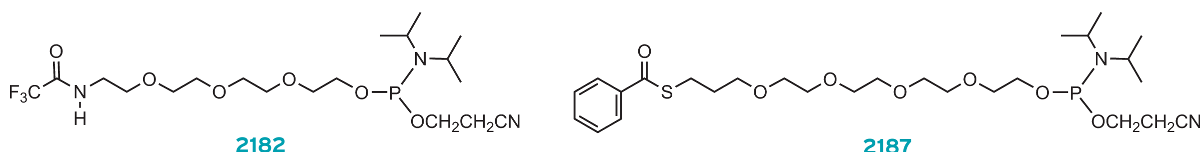
To this aim, we are developing a series of hydrophilic modifiers, the first two of which are an amino linker (**2182**) and the analogous thiol linker (**2187**). Both of these alleviate the need for the use of the hydrophilic spacer.

1 **Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specific probes**. B.A.Connolly and P.Rider, *Nucleic Acids Research*, **13**, 4485-4502, 1985.

2 **The preparation and application of functionalised synthetic oligonucleotides: III. Use of H-phosphonate derivatives of protected amino-hexanol and mercapto-propanol or -hexanol**, N.D. Sinha and R.M. Cook, *Nucleic Acids Research*, **16**, 2659-2670, 1988.

3 **The synthesis of oligonucleotides containing a primary amino group at the 5'-terminus**, B.A. Connolly, *Nucleic Acids Research*, **15**, 3131-3139, 1987.

4 R.I. Hogrefe, H. Mackie, and M.L. Powell, *Amer. Biotechnologies News Ed.*, **6**, 47, 1988.



Product Details

Both products are based on ethylene glycol spacer units. **2182** uses the common TFA protection on the amino group, whilst in **2187** the benzoyl protecting group was selected such that the free thiol is formed during standard oligonucleotide deprotection. Hence there is no need to use the silver salts required to remove the trityl group when using a S-Tr thiol modifier. The lack of the disulphide bridge, as is present in common S-S thiol modifiers, results in **2187** proving to be more stable; it is stable on the synthesiser for at least 48 hours.

Both modifiers are compatible with the standard cycles in oligo synthesis however a wash with 10-20% DEA/MeCN is required prior to cleavage and deprotection. This is true for all amino modifiers. Similarly, this wash prevents acrylamide capping on the free thiol generated during deprotection.

A comparison of existing amino and thiol linkers with the new hydrophilic linkers has been carried out. Oligo syntheses show that the automated coupling efficiencies are comparable to the current commercially available amino and thiol linkers. Post-synthetic labelling in aqueous solution has been carried out, conjugating oligos modified with **2182** to thioctic acid NHS ester (**2166**) and **2187**-modified oligos to Horse Radish Peroxidase (HRP) and 6-iodoacetamido fluorescein (6-IAF). In addition to the simplified method, in many cases we have found the coupling better to the hydrophilic linkers than when using the corresponding C6 linker plus spacer 18 (or HEG).

Ordering Hydrophilic Modifiers

Product	Pack Size	Cat. No.
Hydrophilic Amino-Modifier-11-CE Phosphoramidite	100µmol 250mg	2182-F100 2182-B250
Hydrophilic S-Bz TEG-CE Phosphoramidite	100µmol 250mg	2187-F100 2187-B250

Find out more...

For further information on hydrophilic modifiers, and our other products, please visit **www.linktech.co.uk**, e-mail us at sales@linktech.co.uk, or call +44(0)1698 849911. A full technical information sheet providing further data and protocols will be available soon.