

# MICROARRAYS, NANOTECHNOLOGY AND BEYOND

## 1. Aminolink at 21: maturity at last?

It may have been a (relatively) small step for Jerry Ruth 21 years ago, but it sure looks like a huge leap for oligokind (the world of oligonucleotide users) now: in 1984 the very first aminolink prototype was hatched<sup>[1]</sup>. Glen Research is celebrating this anniversary by offering its customers an advanced version of the aminolink phosphoramidite.

### 1.1. Aminolink and microarrays/DNA chips

Amino-modified oligos are mostly used for microarray manufacturing since DNA chip technology has become an indispensable tool for life sciences<sup>[2,3]</sup>. Fabrication is based on *in situ* synthesis on silicon chips or the more accessible approach of post-synthetic immobilization of oligos onto activated surfaces, predominantly glass slides. Large oligo synthesis houses, such as Biosearch Technologies, Dharmacon, Eurogentec, Fidelity Systems, IDT, Illumina, Invitrogen, Metabion, Operon, Sigma-Genosys-Proligo, TriLink Biotech, etc., use high-throughput processing to prepare libraries of tens of thousands of amino-modified oligos which are then spotted on surfaces to yield microarrays. The spotting process uses either piezo-based dispensers (GeSIM, Arrayjet) or the more popular pin-based spotters (Genomic Solutions, Genetix, Biorad, Telechem). The yield of the amino-modified oligos is vitally important since re-synthesis on this scale is time-consuming and expensive. Clearly, for this kind of high-volume throughput, purification of all these oligos using conventional methods like HPLC or PAGE is out of question.

The process has been optimized mainly in terms of immobilization chemistries, with the most popular methods relying on the reaction of amino-modified oligos with surfaces derivatized with moieties such as isothiocyanate or epoxy<sup>[4]</sup> groups.

### 1.2. Cartridge purification

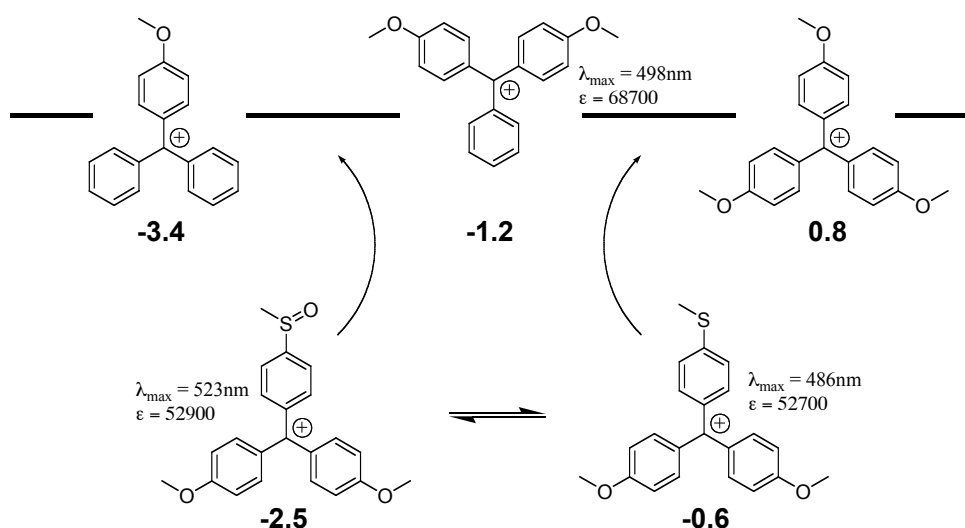
The most popular amino-modifier used today contains an amine protected by a TFA group. This modifier has the advantages of being inexpensive and reliable. However, it has a drawback that it does not allow the use of trityl-on purification techniques or even an estimate of the coupling yield by the trityl cation measurement. Moreover, since the removal of a TFA protecting group happens simultaneously with the deprotection of an oligo, side reactions such as Michael addition of acrylonitrile (formed from elimination of cyanoethyl protecting groups) take place, reducing the yield of the aminated product.

A reagent more suitable for preparation of thousands of amino-modified oligos contains an amine protected with a trityl group. Triphenylmethyl groups (trityls) are a popular family of protecting groups, used in oligonucleotide chemistry for hydroxyl (DMTr) and amino (MMTr) protection, and removable by mild acidic treatment. Conveniently, trityl cations have large extinction coefficients allowing stepwise coupling yields to be measured easily. Alternatively, due to the hydrophobicity of trityls, separation of the full-length product still bearing the trityl from capped failure sequences can be carried out, with subsequent acidic removal of the DMTr or MMTr protection as the final step.

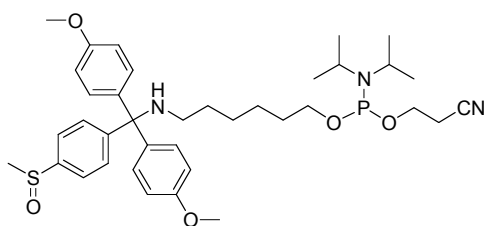
Manufacturing of large numbers of oligonucleotides requires cheap and fast purification techniques, ruling out HPLC and PAGE as too expensive and time-consuming. The popular reverse-phase (RP) cartridge purification method (e.g., PolyPak, Glen Research) does not allow detritylation of the monomethoxytrityl (MMTr) group from protected amino-modified oligonucleotides in high yield on the cartridge. The reason for this situation is that acidic cleavage of the N-trityl bond is an equilibrium reaction. On a reverse phase cartridge, where the trityl cation is not physically separated from the amine, the process results in substantial (up to 50%) reattachment of the MMTr-group back onto the amine. Subsequent elution of the amino-modified oligonucleotide results in a product that is up to 50% inactive. Interestingly, in addition to this reattachment problem, MMTr-amino-modified oligos were shown to be quite unstable when stored in aqueous ammonia, gradually losing the MMTr group over time.

### **1.3. The new aminolink phosphoramidite**

One way around these problems is to use a modified trityl with controlled  $pK_{R^+}$ , where  $pK_{R^+}$  is defined by the following formulae:



4,4'-Dimethoxy-4''-thiomethoxytrityl (DMS(O)MTr; sulfoxy-form) cation is more stabilized than  $MMTr^+$ , and so the DMS(O)MTr-protected amino group is easier to deprotect compared to the  $MMTr^+$ -protected one. The sulfoxy derivative survives conditions of oligonucleotide synthesis and can either be cleaved with standard deblock solution, or left intact for an HPLC purification. At the same time, the DMS(O)MTr group is fully compatible with cartridge purification: when detritylation on cartridge is carried out, the  $DMS(O)MTr^+$ , which is more stable than  $MMTr^+$ , does not reattach itself to an amine. The new aminolink phosphoramidite reagent utilizing this new trityl based protecting is shown below. The reagent is stable in acetonitrile at room temperature for at least two weeks. UV quantification for release of the new protecting group is possible. Extinction coefficients (L/(mol x cm)) shown in the Scheme above were measured in 2% TFA/DCM. In PolyPak detritylation experiments followed by HPLC measurements, the new reagent gave more than 20% improvement in deprotection yields compared to an  $MMTr^+$ -protected amino group labeled oligo (4% TFA, 5 min exposure time).



## 2. Treblers: Interfacing Oligo Synthesis and Nanotechnology

Several new applications for dendrimers in the oligonucleotide field have emerged since Glen Research commercialized branching reagents in 1999 (*The Glen Report*, 1999, 12, #1, p1-4). Examples include multiple fluorescent labeling<sup>[5]</sup>, controlled delivery of antisense oligos<sup>[6]</sup>, more efficient conjugation of oligos with nano-gold<sup>[7]</sup>, and more efficient quenching for molecular beacon applications<sup>[8]</sup>. New treblers described below, now available from Glen Research, possess features that will assist in some of these applications and thus further expand the arsenal of Glen Research's nano-Lego building blocks.

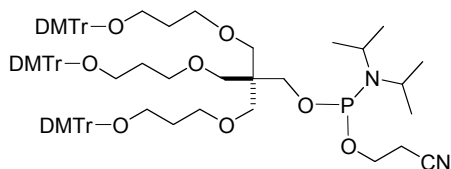
### 2.1. 'Long' Trebler Phosphoramidite

The **Trebler Phosphoramidite** currently available from Glen Research contains a phosphoramidite 'arm' that is somewhat crowded by the three adjacent DMTr-bearing branches. This leads to increased coupling times (recommended coupling time: 10-15 min).

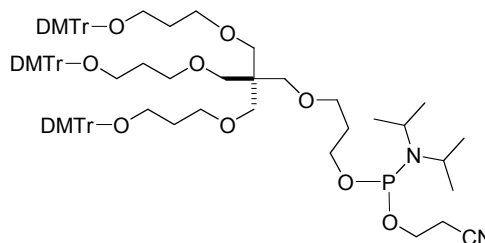
The Trebler of next generation (**'Long' Trebler Phosphoramidite**) contains an extended phosphoramidite arm, thus markedly reducing the problem of steric hindrance. The new reagent gives higher coupling yields and requires shorter coupling times. Large pore size CPG supports (1000Å and 2000Å) should be used when employing this phosphoramidite.

Nevertheless, the original version of Trebler is more compact and should still be employed when the size of a final assembly is an issue and manufacturing time is not.

**Trebler Phosphoramidite**  
Glen Catalog # 10-1922-XX



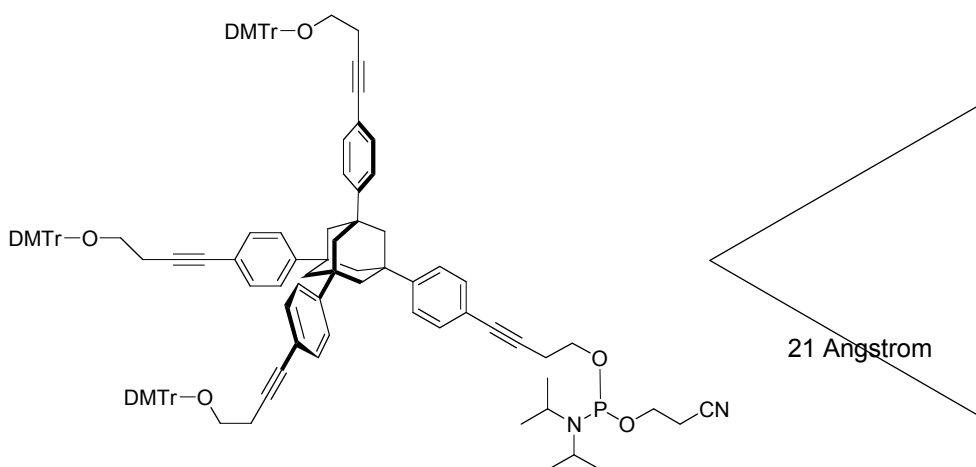
**'Long' Trebler Phosphoramidite**  
Glen Catalog # XX-XXXX-XX



### 2.2. 'Rigid' Trebler Phosphoramidite

The branches of treblers described above are flexible, permitting end-groups to occasionally come into a contact (brush against each other). This may be undesired when tags such as fluorophores are attached to these end-groups as it may lead to quenching<sup>[5]</sup>. Besides, the

'branch span'- the distance between end-groups when all three arms are fully stretched- is only around 15Å.

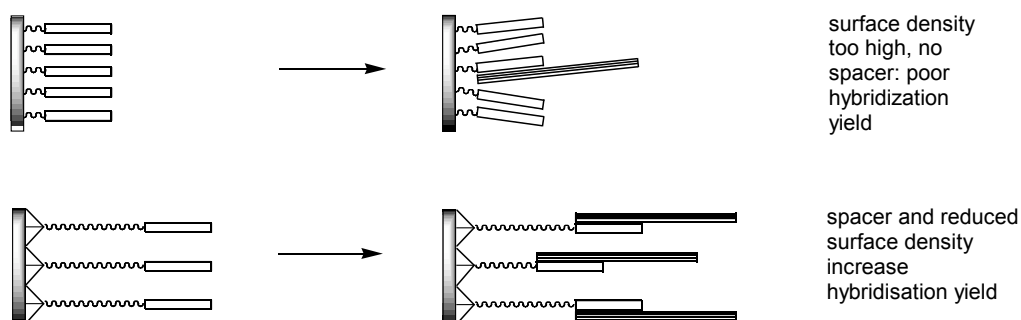


For applications when stiff (rigid) branches with larger branch spans are required, for example to build nano-structures or to increase fluorescent signal without self-quenching, the new **Rigid Trebler Phosphoramidite** has been designed. It has a branch-span of 21Å and requires 2000Å CPG support for optimal coupling yields.

### 3. Some Suggested Applications for Microarrays

#### 3.1. Improved surface and spatial density

Spatial factors greatly affect the quality of DNA chips. When the probes are too far apart, there would not be enough fluorescence signal to be detected. With probes positioned too close to one another on a surface, there would not be enough room for the target DNA to squeeze through and hybridize - again compromising the quality of the detection.



Reducing the surface loading chemically improves the hybridization yield and signal quality, and so does linking the oligos to a microarray surface through long spacers. Up to 150 times improvement in hybridization yield compared to oligos directly attached to a surface at high density can be achieved by optimizing spacing and surface density<sup>[9]</sup>.

A recently published approach involves a multistep procedure whereby an aminated surface is treated with two-sided bulky structures<sup>[10-12]</sup>. One side of such a structure non-covalently sticks to several reactive sites on the surface, whereas the opposite part of the structure has

just one reactive group of its own, thus effectively reducing the surface loading. The method relies on non-covalent binding and requires commercially available glass slides to be additionally modified.

The same result can be achieved in a simpler and more controllable way by employing new phosphoramidites available from Glen. Upon completion of an oligo synthesis on a large pore CPG (1000Å or 2000Å), the 5' end can be derivatized with spacer modifiers 9, C12 or C18 (Glen Research), followed by a trebler amidite and an amino-modifier amidite. Following standard deprotection, the resulting amino-modified probe will be spotted on to an activated glass surface. The probe will not only immobilize faster courtesy of multiple amino groups, but will also take up more surface area to generate increased spacing, thereby improving the yield in hybridization experiments.

**For further technical information on these products please contact:**

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