**TECHNICAL BULLETIN - 5’-AMINOXY C11 MODIFIER C11**

**Introduction**

The use of nucleophilic aminooxy modifiers for DNA conjugations were first described in the mid 1990’s and several reviews/papers/authors have found applications for these alkyloxyamines, such as cyclizing DNA, fluorescent labeling, and peptide conjugations1-5. Aminooxy modifiers can be used in chemoselective conjugation reactions with aldehydes and ketones to form stable oximes. The oxime formed from the reaction of these alkyloxyamines creates a stable covalent bond that is compatible with standard oligonucleotide deprotection conditions. Oxime covalent bonds are a more stable than the imine formed by the conjugation of amines with aldehydes, which require subsequent reduction to secondary amines. The 5’-AminoOxy-Modifier 11 (1) contains the tetraethylene glycol linkage for improved solubility and minimizes the effects on hybridization of the oligo.

**Synthesis**

We recommend using 1H-Tetrazole with a 3 minute coupling time. No other changes to the synthesis cycle are required.

**Deprotection**

We recommend AMA deprotection, 10 minutes at 65°C. Compatible with:
- 28% ammonium hydroxide in water, 55°C, 17 hours;
- 28% ammonium hydroxide in water, room temperature, 4 hours;
- 0.05M Potassium Carbonate in Methanol;
- 0.4M Sodium Hydroxide in Methanol:Water (4:1).

**Solution Phase Conjugation**

Solution-phase with direct-conjugation to an aldehyde or ketone provides the best results. Synthesize the oligo DMT-ON and complete the deprotection as described above. After drying, complete the conjugation in 80% acetic acid to simultaneously remove the DMT group and catalyze the oxime formation. Subsequent gel-filtration using a Glen Gel-Pak removes the acetic acid and unreacted aldehydes or ketones.

**Solid Phase Conjugation**

The aminooxy conjugation can also be performed on the column after removal of the 5’-DMT group, provided the label is stable to the subsequent deprotection conditions. If the oligo will not be conjugated immediately, retain the DMT protecting group and remove with 3% TCA in DCM just prior to the conjugation.

**Solution Phase DMT-ON Protocol**

1. Synthesize the 5’-AminoOxy modifier, DMT-ON.
2. Cleave and deprotect with AMA for 10 minutes at 65°C.
3. Dry oligo.
4. Dissolve oligo in 0.2mL water.
5. Add 10 equivalents of aldehyde in a suitable solvent, 100μL.
6. Add 0.8mL acetic acid and mix well.
7. React for 60 minutes at room temperature.
8. Quench with 0.1M TEAA, 1mL.
9. Desalt on Glen Gel Pak or equivalent.

**Solution Phase DMT-Off Protocol**

1. Synthesize the 5’-AminoOxy modifier, DMT-ON.
2. Cleave and deprotect with AMA for 10 minutes at 65°C.
3. Dry oligo.
4. Dissolve oligo in 1mL 80% acetic acid.
5. Let stand for 30 minutes at room temperature.
6. Purify oligo by Glen Pak using desalting procedure.
7. Reconstitute in water.
8. Conjugate with 10 equivalents aldehyde at room temperature, overnight.
9. Desalt oligo using Glen Gel Pak or equivalent.
Solid Phase Conjugation

1. Synthesize the oligo-DMT-Off.
2. Immediately conjugate the oligo with 10 equivalents of aldehyde label in a suitable solvent.
3. Rinse support.
4. Deprotect and cleave the oligo using a method compatible with the label.
5. Purify oligo.

References