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TECHNICAL BULLETIN - 5'-AMINOOXY C11 MODIFIER C11

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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

Introduction

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

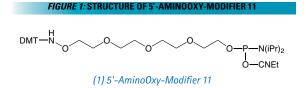
Deprotection

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 gelfiltration 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

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

- References
- 1. B. Cebon, et al., Australian Journal of Chemistry, 2000, 53, 333-339.
- H. Salo, et al., Bioconjugate Chemistry, 1999, **10**, 815-823.
 O.P. Edupuganti, E. Defrancq, and P. Dumy, J Org Chem, 2003, **68**, 8708-8710.
- 4. T.S. Zatsepin, D.A. Stetsenko, M.J. Gait, and T.S. Oretskaya, Bioconjugate Chemistry, 2005, 16, 471-489.
- 5. T.S. Zatsepin, D.A. Stetsenko, M.J. Gait, and T.S. Oretskaya, Tetrahedron Lett, 2005, 46, 3191-3195.