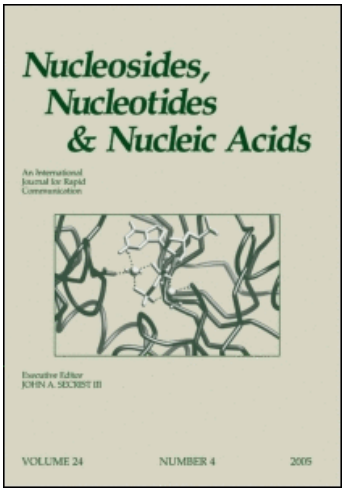

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Aldehydic Oligonucleotide: A Key Intermediate for the Preparation of Oligonucleotide Conjugates Through Oxime Bond Formation

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ALDEHYDIC OLIGONUCLEOTIDE: A KEY INTERMEDIATE FOR THE PREPARATION OF OLIGONUCLEOTIDE CONJUGATES THROUGH OXIME BOND FORMATION

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□ *Oligonucleotides functionalized with an aldehyde group are the key intermediates used for the preparation of peptide-oligonucleotide conjugates through the formation of an oxime linkage. Herein, we describe a brief overview of various synthetic protocols developed in our laboratory for the preparation of aldehyde containing oligonucleotides and their subsequent conjugation with peptides.*

Keywords Conjugation; Oxime; Oligonucleotide; Peptide

The synthesis of peptide-oligonucleotide conjugates (POC)[1] has attracted significant research interest. This is due to the fact that POCs have been shown to improve the cell specific targeting, cellular uptake efficiency and intracellular stability of natural oligonucleotides (ODN). Besides, increased binding strength with the target sequence has also been observed in some cases. The major approach being employed for the synthesis of POCs involves the separate assembly of peptide and oligonucleotide fragments followed by their solution-phase coupling.[2] This is accomplished by incorporating mutually reactive groups into each fragment leading usually to the formation of chemical linkages such as amide,[3] thioether,[4] disulfide,[5] and oxime.[6] Oxime linkage formed by the reaction between an aldehyde and an aminooxy group has become preferred method for ODN conjugation. In fact, the formation of oxime bond is chemoselective, can be carried out in aqueous medium without the use of activators and give high coupling efficiency. Furthermore, the oxime bond is stable over a wide pH range. ODN conjugation through oxime bond formation utilizes ODNs modified with an aldehydic functionality as key intermediate molecule. Our research efforts over the past few years have focused toward the development of facile and convenient synthetic procedures for ODN functionalization with aldehyde group and their subsequent use in the preparation of POCs. The main

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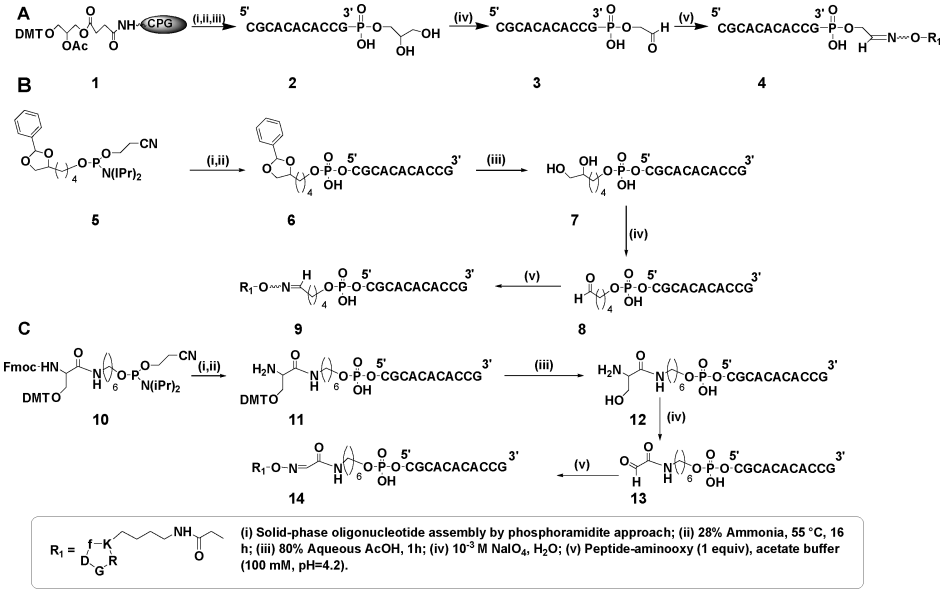
strategy for incorporating an aldehyde group on oligonucleotides consists in the oxidation of a 1,2-diol. We describe herein a brief overview of work accomplished in our laboratory using this strategy.

ALDEHYDIC OLIGONUCLEOTIDE FOR 3′-CONJUGATION (SCHEME 1A)

The method utilizes a commercially available modified solid support (3-[(4,4′-dimethoxytrityl]-glyceryl-1-succinyl) long chain alkylamino controlled pore glass (1) to incorporate the protected diol group at 3′-terminus of ODN.[7] Briefly, automated DNA synthesis is carried out on this support by using the standard protocol. Subsequent treatment with ammonia cleaves the free 3′-diol containing ODNs (2) from support and releases it into the solution. Periodate oxidation then affords quantitatively the 3′-aldehyde containing ODNs (3). Further reaction with aminooxy containing peptide in aqueous buffer at slightly acidic pH leads to the formation of ODN-3′-conjugates (4).

ALDEHYDIC OLIGONUCLEOTIDE FOR 5′-CONJUGATION (SCHEME 1B AND C)

An elaborate preparation of modified phosphoramidite synthons is required to incorporate the masked reactive moieties at the 5′-terminus of



SCHEME 1 Synthesis of 3′-POCs through oxime bond formation.

ODNs. Two modified phosphoramidite derivatives have been successfully developed in our laboratory that are capable of introducing the aliphatic aldehyde and glyoxylic aldehyde precursors at the 5′-end.

5′-Aliphatic Aldehyde Oligonucleotide (Scheme IB)

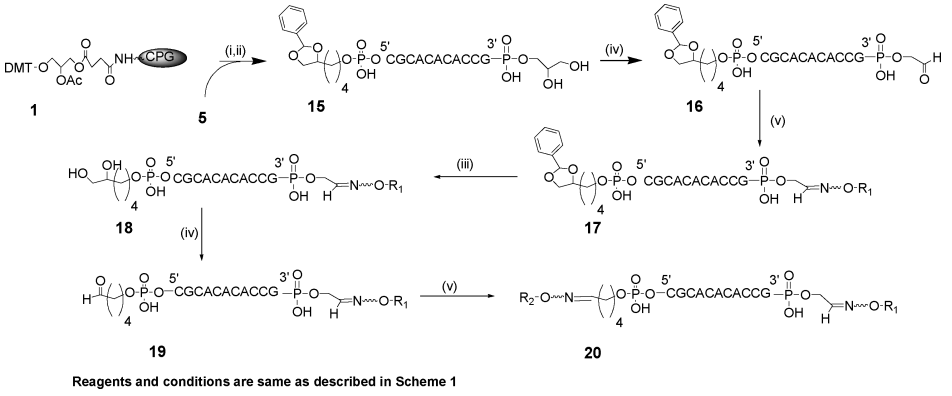
The phosphoramidite derivative (5) was designed for incorporating the benzylidene-protected diol at 5′-terminus of ODN.[8] This derivative is introduced into the growing support bound ODN chain during the last coupling step. It would be relevant to mention that this benzylidene protected phosphoramidite derivative similar to DMT group aids in the HPLC purification of oligonucleotide (6) on account of its hydrophobic character. The benzylidene protection is removed by treatment with 80% aqueous acetic acid thereby generating free diol at 5′-terminus (7). The ODNs with 5′-aldehyde (8) are prepared by periodate oxidation of 5′-diol. Subsequent conjugation with aminooxy containing peptide leads to the formation of 5′-conjugates with aldo-oxime bonds (9).

5′-Glyoxylic Aldehyde Oligonucleotide(Scheme IC)

Glyoxylic oxime linkage has been shown to be more stable than aldo-oxime. The glyoxylic aldehyde functionality is routinely prepared by periodate oxidation of a serine moiety. Consequently, a new phosphoramidite synthon (10) was prepared from serine.[9] This was added to the growing ODN chain during the last coupling step to incorporate serine modified phosphoramidite at the 5′-terminus. Further treatment with ammonia and acetic acid removes the Fmoc- and DMT- protection respectively present on the serine moiety. The ODNs with 5′-glyoxylic aldehyde (13) are prepared by periodate oxidation of oligonucleotide containing the modified serine linker. This is further reacted with aminooxy containing peptide to obtain 5′-conjugates with glyoxylic oxime bond (14). The stability of ODN conjugates containing glyoxylic oxime bond was compared to similar conjugate containing aldo-oxime bond. The glyoxylic oxime bonds were found to show higher stability in acidic to neutral media.

ALDEHYDIC OLIGONUCLEOTIDE FOR 3′, 5′-BIS-CONJUGATION (SCHEME 2)

The above-mentioned procedure described for the preparation of 3′- or 5′-conjugates can easily be applied in sequential fashion for the preparation of 3′,5′-bis-conjugates.[10] Briefly, solid-phase ODN synthesis is carried on modified solid support (1). The modified phosphoramidite derivative (5) is added to the growing ODN chain during the last coupling step. The support bound ODNs are treated with ammonia to achieve



SCHEME 2 Synthesis of 3′,5′-bis-conjugates.

base deprotection, cleavage and releases of ODNs (15) with free 3′-diol into the solution. This is easily converted to 3′-aldehyde ODN (16) by periodate oxidation. Reaction with appropriately functionalised peptide gives the 3′-conjugates (17). The benzylidene protection is removed next by treatment with 80% acetic acid and the 5′-aldehyde functionality (19) is generated by periodate oxidation. This is subsequently reacted to appropriately functionalised similar or different peptide to obtain the 3′-,5′ bis-conjugates (20).

In conclusion, we have designed and developed several convenient synthetic procedures to incorporate aldehyde functionality at 3′- and/or 5′- termini of ODNs. These ODNs containing aldehyde functionality are crucial intermediates required for the preparation of POCs through oxime bond formation. The efficiency of the procedure so developed has been demonstrated by utilizing these for the preparation of oligonucleotide 3′- and/or 5′- conjugates with peptides through aldo-oxime linkages. The ODNs containing 5′-glyoxylic aldehyde have been successfully prepared and we are now engaged in developing method to synthesize 3′-glyoxylic aldehyde containing ODNs. Moreover, the procedure described herein have been successfully employed by our group for the preparation of various other oligonucleotides conjugates also besides POCs.[11,12]

REFERENCES

1. Gait, M.J. Peptide-mediated cellular delivery of antisense oligonucleotides and their analogues. *Cell. Mol. Life Sci.* **2003**, 60, 844–853.
2. Stetsenko, D.A.; Malakhov, A.D.; Gait, M.J. Total stepwise solid-phase synthesis of oligonucleotide-(3′-*N*)-peptide conjugates. *Org. Lett.* **2002**, 4, 3259–3262.
3. McMinn, D.L.; Greenberg, M.M. Convergent solution-phase synthesis of a nucleopeptide using a protected oligonucleotide. *Bioorg. Med. Chem. Lett.* **1999**, 9, 547–550.
4. Harrison, J.G.; Balasubramanian, S. Synthesis and hybridization analysis of a small library of peptide-oligonucleotide conjugates. *Nucleic Acids Res.* **1998**, 26, 3136–3145.

5. Maurel, F.; Debart, F.; Cavelier, F.; Thierry, A.R.; Lebleu, B.; Vasseur, J.J.; Vives, E. Toward high yield synthesis of peptide-oligonucleotide chimera through a disulfide bridge: a simplified method for oligonucleotide activation. *Bioorg. Med. Chem. Lett.* **2005**, 15, 5084–5087.
6. Zatsepin, T.S.; Stetsenko, D.A.; Arzumanov, A.A.; Romanova, E.A.; Gait, M.J.; Oretskaya, T.S. Synthesis of peptide-oligonucleotide conjugates with single and multiple peptides attached to 2′-aldehydes through thiazolidine, oxime, and hydrazine linkages. *Bioconjugate Chem.* **2002**, 13, 822–830.
7. Villien, M.; Defrancq, E.; Dumy, P. Chemoselective oxime and thiazolidine bond formation: a versatile and efficient route to the preparation of 3′-peptide-oligonucleotide conjugates. *Nucleosides, Nucleotides, Nucleic Acids* **2004**, 23, 1657–1666.
8. Forget, D.; Boturyn, D.; Defrancq, E.; Lhomme, J.; Dumy, P. Highly efficient synthesis of peptide-oligonucleotide conjugates: chemoselective oxime and thiazolidine formation. *Chem. Eur. J.* **2001**, 7, 3976–3984.
9. Singh, Y.; Defrancq, E.; Dumy, P. New method to prepare peptide-oligonucleotide conjugates through glyoxylic oxime formation. *J. Org. Chem.* **2004**, 69, 8544–8546.
10. Edupuganti, O.P.; Singh, Y.; Defrancq, E.; Dumy, P. New strategy for the synthesis of 3′,5′-bifunctionalized oligonucleotide conjugates through sequential formation of chemoselective oxime bonds. *Chem. Eur. J.* **2004**, 10, 5988–5995.
11. Garanger, E.; Boturyn, D.; Renaudet, O.; Defrancq, E.; Dumy, P. Chemoselectively addressable template: a valuable tool for the engineering of molecular conjugates. *J. Org. Chem.* **2006**, 71, 2402–2410.