Novel Achiral Building Blocks and Solid Supports for Preparation of Multi-Labeled Oligonucleotides

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Abstract

Novel achiral non-nucleosidic building blocks for the efficient preparation of single- or multi- labeled oligonucleotides have been developed. Incorporation of a wide variety of ligands including GalNAc cluster, fluorescent labels and quenchers, biotin, hydrophobic moieties, azido and alkyne groups suitable for the use in click-type conjugation has been demonstrated.

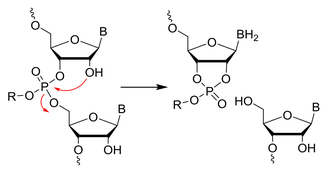
Introduction

i) Non-nucleosidic building blocks used in oligonucleotide chemistry for non-radioactive labeling and for attachment of various ligands should result in the attachment of a linker stable under the conditions of oligonucleotide chain assembly and deprotection by most common basic deprotection agents.

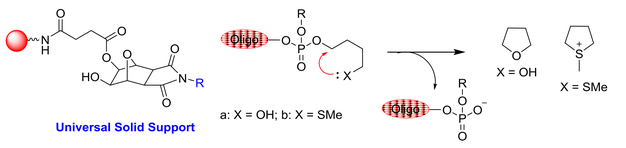
ii) The currently available building blocks derived from 1,2-diols suffer an unwanted loss of the linker due to cleavage of phosphodiester group.

iii) Generally the side chain of a building block is attached to the core structure via an amido function. When the amido group is placed in proximity to the nearest phosphodiester moiety, a minor loss of the entire linker via an intramolecular cyclization may occur and an oligonucleotide bearing a terminal phosphate group is formed.

iv) The attached linker should not generate new chiral centers in the oligonucleotide conjugate obtained. Ideally, the building block itself should not possess any chiral or prochiral carbon atoms.

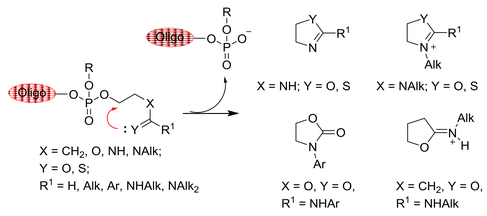


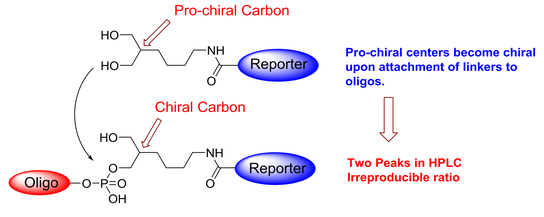
Base-catalyzed dephosphorylation is always present in
i) 1,2-diols,
ii) particularly rapid when the conformation is appropriate &
iii) particularly rapid when phosphate is protected



Universal Solid Support

Particularly rapid when the phosphate is protected. When X = SMe, cleavage is pH-independent.

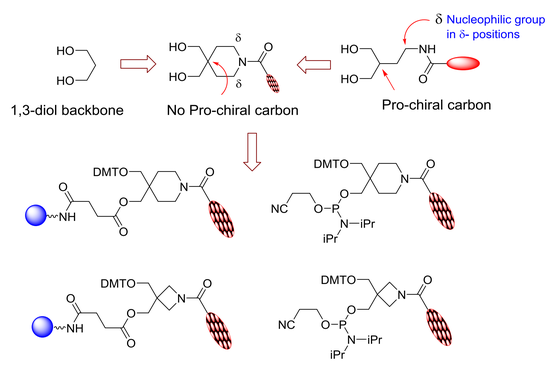




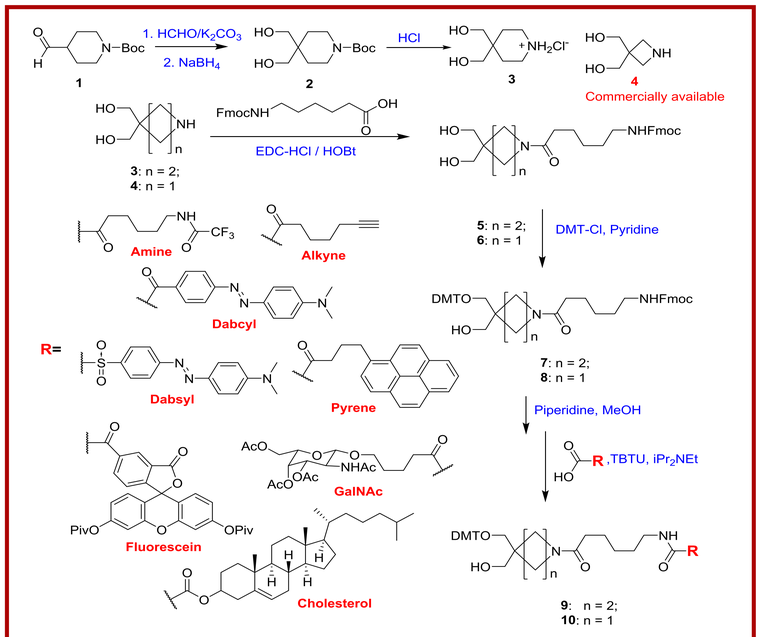
Pro-chiral centers become chiral upon attachment of linkers to oligos.

Two Peaks in HPLC Irreproducible ratio

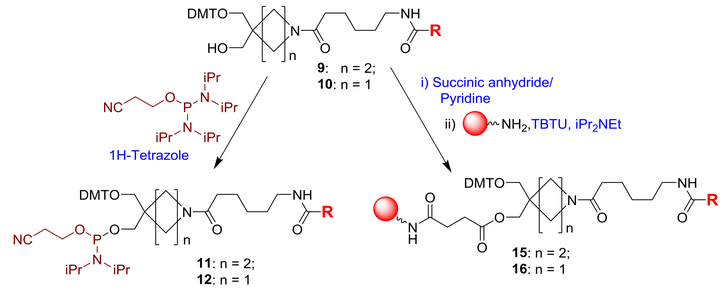
The Design We Want



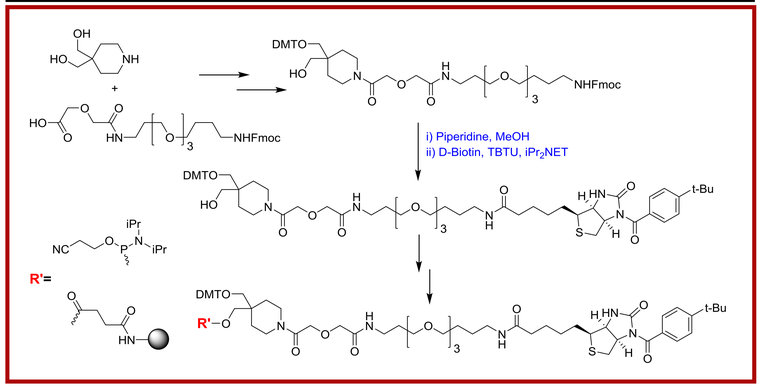
Synthesis of Azetidine- and Piperidine core structures and attachement of various ligands



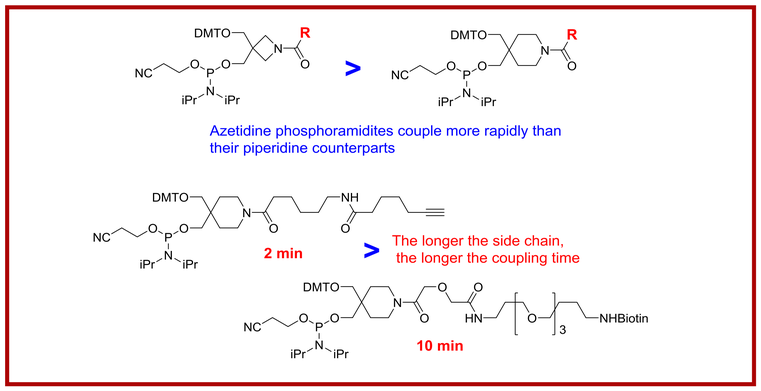
Synthesis of Solid Supports and Phosphoramidite Building Blocks



Synthesis of Biotinylated Phosphoramidite and Solid Support



Oligonucleotide Synthesis: Coupling Efficiency of the Non-Nucleosidic Phosphoramidites



Synthesis & deprotection of oligonucleotides

Sequence 1: 5'-TTT TTT TTT TTT TTT TTT TT-**Ligand** 3'
Sequence 2: 5'-TAG TGC TAG ATG CCT-**Ligand** 3'
Sequence 3: 5'-CCA CTA CCT GAG CAC CCA GTT-**Ligand** 3'
Sequence 4: 5'-CTG GGT GCT CAG GTA GTG GTT-**Ligand** 3'
Sequence 5: 5'-**Ligand**-CCA CTA CCT GAG CAC CCA GTT 3'
Sequence 6: 5'-**Ligand**-CCA CTA CCT GAG CAC CCA GTT-**Ligand** 3'
Sequence 7: 5'-CsCsAs CsTsAs CsCsTs GsAsGs CsAsCs CsCsAs GsTsT-**Ligand** 3'
Sequence 8: 5'-r(CCA CUA CCU GAG CAC CCA GUU)-**Ligand** 3'
Sequence 9: 5'-r(CsCsAs CsUsAs CsCsUs GsAsGs CsAsCs CsCsAs GsUsU)-**Ligand** 3'

Cleavage and deprotection of the oligonucleotides:

i) Aqueous NH_3 (28%), 60^{0}C, 8-12 hour.
ii) Acetonitrile-Diethylamine (5:1) treatment for 3 minutes, followed by treatment with aqueous NH_3 (28%), 60^{0}C, 8-12 hour.
iii) 1:1 AMA (Aqueous NH_3: Methylamine), 60^{0}C, 15 minutes
iv) Acetonitrile-Diethylamine (5:1) treatment for 3 minutes, followed by treatment with Toluene-Ethylenediamine (1:1) for 2 hours at room temperature.
v) 50 mM K_2CO_3 in Methanol at room temperature (45 ^{0}C for CF_3CO protected Amine oligonucleotides)
vi) Acetonitrile-Diethylamine (5:1) treatment for 3 minutes, followed by treatment with 50 mM K_2CO_3 in Methanol at room temperature (45 ^{0}C for CF_3CO protected Amine oligonucleotides)

Results:

All the Methods (i) to (vi) were suitable for oligonucleotides of Alkyne, Dabsyl, Dabcyl, Pyrene, Fluorescein, Cholesterol and GalNAc .
Method (ii) to (vi) were suitable for deprotection of Biotin labeled oligonucleotides.
Method (i), (ii) & (vi, 45 ^{0}C) lead to complete deprotection of the CF_3CO protecting group of the Amine labeled oligonucleotides.

HPLC profiles of Ligand-oligonucleotides conjugates