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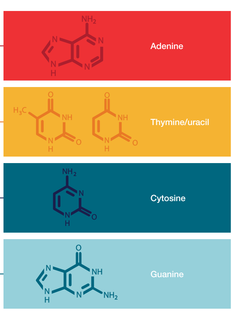
Building oligos with phosphoramidites

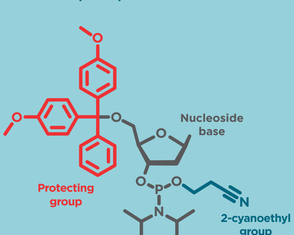
Oligonucleotides, also known as oligos, are short strands of DNA or RNA used in a variety of diagnostic kits and therapeutic applications. While oligos can be produced through biosynthetic methods, chemical synthesis remains the industry standard approach for therapeutic and diagnostic applications.

This infographic explores the role of phosphoramidites as essential building blocks in oligo manufacturing and the considerations for their usage in diagnostic and therapeutic development.

Oligos may be used as single or double strands and contain nucleic acid bases such as:



What are phosphoramidites?



Phosphoramidites, the chemical building blocks of oligo synthesis, consist of a protecting group (e.g., dimethoxytrityl, DMT) and a cyanoethyl reactive group.

Since phosphoramidites are chemically manufactured, they are easy to modify or label for desired characteristics (e.g., thermostability, low immunogenicity, and target specificity).

Attributes and applications

Phosphoramidites should be carefully selected for oligo synthesis since modification options and compliance requirements may differ depending on the intended use of the oligo. Below are some aspects of phosphoramidites that should be considered during product design and manufacturing.

Diagnostic applications

Purpose	Example	Commonly used in [1,2,5]:
Fluorescent detection of a target	Fluorescent dye–labeled phosphoramidite	Immunofluorescence assays, PCR, fluorescence *in situ* hybridization
Fast deprotection for removal of protecting groups under mild conditions without affecting labels	*N*-isopropyl phenoxyacetyl (iPr-PAC)	DNA sequencing, fluorescence *in situ* hybridization, PCR, SNP genotyping
Alteration of stability and melting point of oligo duplexes	N^6-methyl deoxyadenosine (N^6-Me-dA)	Methylation microarrays, PCR, DNA sequencing
Linker for tagging and labeling	5′-aminohexyl linker	Next-generation sequencing, DNA microarrays, PCR
Add space between a moiety and the hybridizing region of the oligo	Hexaethyleneglycol (HEG) (Spacer 18)	Nucleic acid hybridization, PCR, DNA microarrays

Therapeutic applications

Purpose	Example	Commonly used in [2,4]:
Thermal stability, nuclease resistance, and low immunogenicity	2′-O-methyl (2′-OMe) 2′-O-methoxy-ethoxy (2′-MOE) 2′-fluoro	Antisense oligos (ASOs), small-interfering RNAs (siRNAs), aptamer-based therapeutics
Targeted delivery	*N*-acetylgalactosamine (GalNAc)	ASOs, siRNAs
Linker for tagging and labeling	5′-aminohexyl linker	Attachment of a desired moiety (e.g., GalNAc) to ASOs, siRNAs, etc.

Other important attributes to consider while choosing phosphoramidites for oligo manufacturing:

Benefits	Diagnostic applications	Therapeutic applications
Scalability	✓	✓
Specificity	✓	✓
Reduction of reactive and critical impurities [6]		✓
Strict quality control to help achieve regulatory compliance		✓

Discover more about phosphoramidites at **thermofisher.com/phosphoramidites**

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References

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3. Senthilvelan S, Shanmugasundaram M, Kore AR (2022) Efficient and Improved Solution-Phase Synthesis of Modified RNA Dinucleotides: Versatile Synthons in Cap 1 mRNA Therapeutics. *Org. Process Res. Dev.*26(9):2771–2778.
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6. ThermoFisherScientific (2020) Classification and characterization of impurities in phosphoramidites used in making therapeutic oligonucleotides.