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Development and Characterization of Antibody-Oligonucleotides Conjugate Manufacturing Processes

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Disclaimer

The content provided herein includes information on investigational product(s) and/or investigational use(s) for which efficacy and safety have not been established. There is no guarantee that the compound(s) and AOC platform will become commercially available for use(s) under investigation. This is for your background and educational purposes only and may not be altered or further disseminated in any fashion.

Outline

- AOC overview
- AOC and oligonucleotides development, characterization, and manufacturing
- AOC control strategy
- PMO manufacturing process and control strategy

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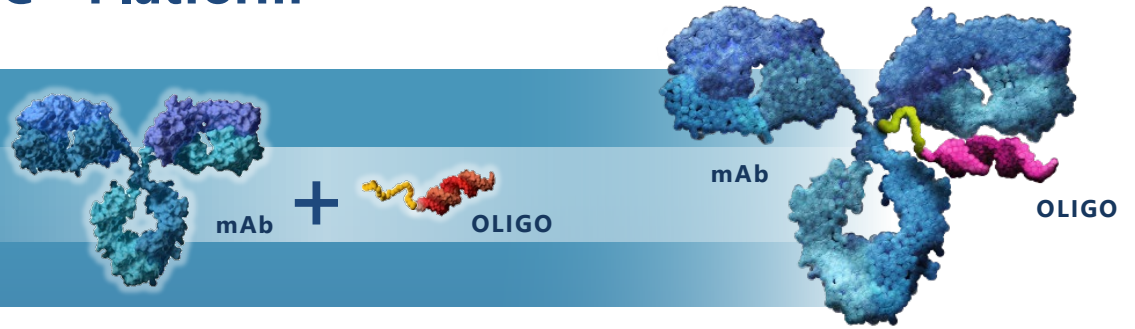
AOC, antibody oligonucleotide conjugate; PMO, phosphorodiamidate morpholino oligomer.

Introduction to Avidity/Novartis AOCs

The Avidity/Novartis AOC™ Platform

Combining the specificity of mAbs with the precision of oligonucleotide therapies[1,2]



MONOCLONAL ANTIBODIES

OLIGONUCLEOTIDE THERAPIES

ANTIBODY OLIGONUCLEOTIDE CONJUGATE (AOC)

AOC platform is designed to:

- ✓ Innovative approach to deliver a range of investigational oligonucleotides (e.g., siRNAs, PMOs)[1]
- ✓ Goal to target tissue and cell types beyond the liver[1]
- ✓ Be readily reproducible and scalable[1]

AOC platform is investigational. Safety and efficacy have not been established.

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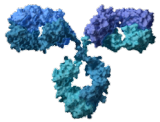
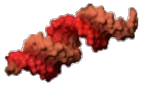
AOC, antibody oligonucleotide conjugate; mAb, monoclonal antibody; PMO, phosphorodiamidate morpholino oligomer; siRNA, small interfering ribonucleic acid.

1. Levin AA, et al. *N Engl J Med*. 2019; 380: 57–70. 2. Avidity BioSciences. https://www.aviditybiosciences.com/about/about-us/ (Accessed 12 march 2026).

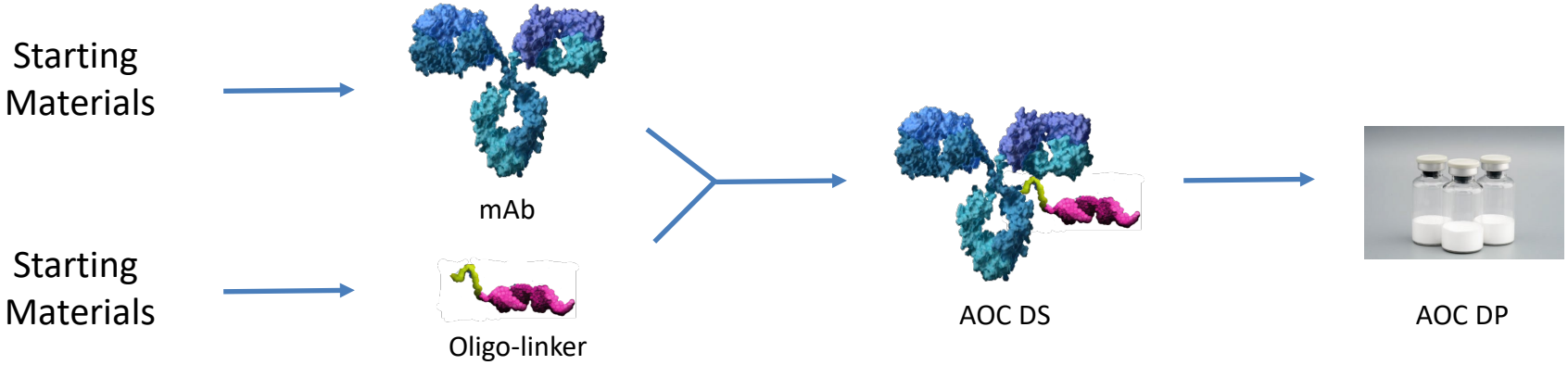
Each Component Engineered to Deliver AOC for Target*

AOC COMPONENTS	MOA COMPONENT CHARACTERISTICS
Monoclonal antibody 	mAbs profile: • High specificity and affinity • Long half-lives
Linker 	• Applicable to multiple oligo modalities
siRNA 	siRNA profile: • Nanomolar potency • Optimized DAR
PMO 	PMO profile: • Nanomolar potency • Optimized DAR

*Based on in vitro/in vivo data. Clinical significance is unknown.
AOC, antibody oligonucleotide conjugate; DAR, drug to antibody ratio; mAb, monoclonal antibody; MOA, mechanism of action PMO, phosphorodiamidate morpholino oligomer; siRNA, small interfering ribonucleic acid.
1. Malekova B, et al. *Nucleic Acids Research* 2023;51(12):5901–5910. 2. Cochran M, et al. *J Med Chem* 2024;67:14852–14867.

AOC and Oligonucleotide Development, Characterization and Manufacture

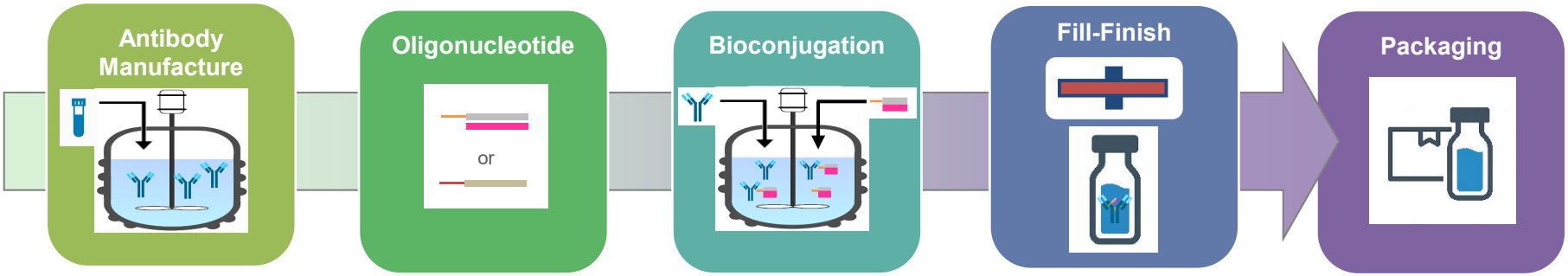
AOC Process Development and Characterization



- From the development and characterization perspectives, AOCs embed multiple programs in one
  - ❑ Manufacture of oligonucleotide
  - ❑ Manufacture of mAb
  - ❑ Manufacture of AOC drug substance
  - ❑ AOC Formulation and Drug Product Manufacture

AOC, antibody oligonucleotide conjugate; DP, drug product; DS, drug substance; mAb, monoclonal antibody.

AOC Manufacturing Steps



- Upstream processing
- Downstream processing

- SPOS or LPOS
- Purification
- Annealing
- Linker modification

- Bioconjugation of mAb and oligonucleotide-linker
- Purification
- DS Formulation

- Sterile fill-finish
- Lyophilization

- Global distribution

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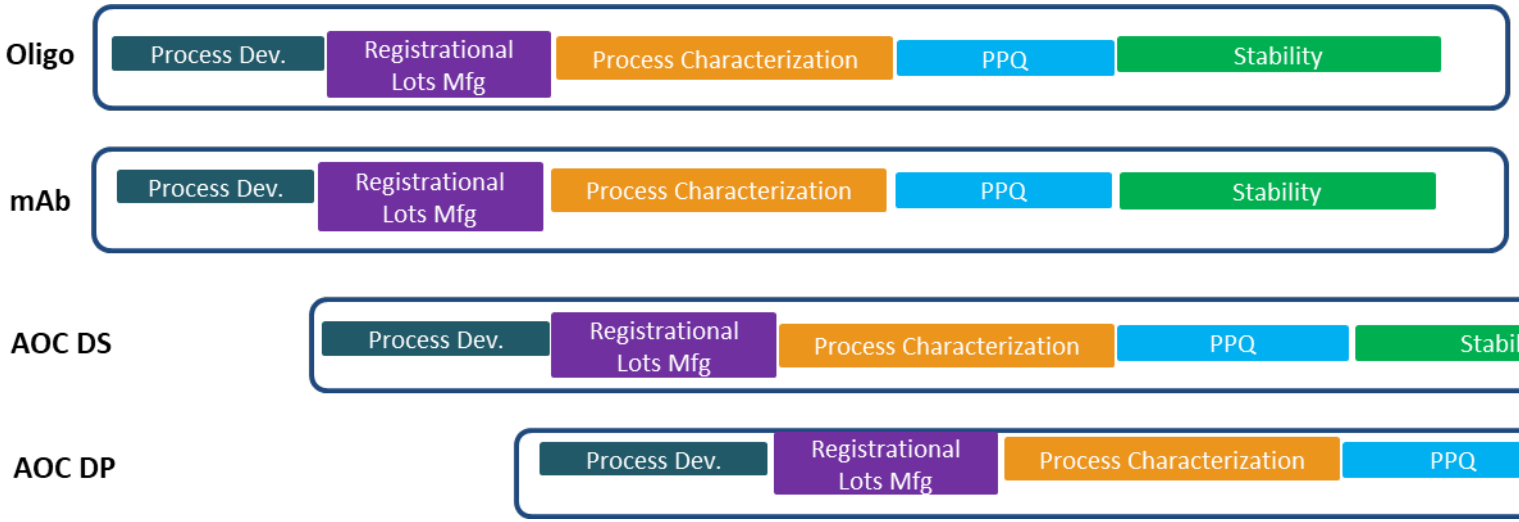
AOC, antibody oligonucleotide conjugate; DS, drug substance; LPOS, liquid phase oligonucleotide synthesis; mAb, monoclonal antibody; SPOS, solid phase oligonucleotide synthesis.

AOC Process Development, Characterization, and Manufacturing Framework

Process Lock

Commercially Representative:
- Primary Stability
- Use in Registrational trial

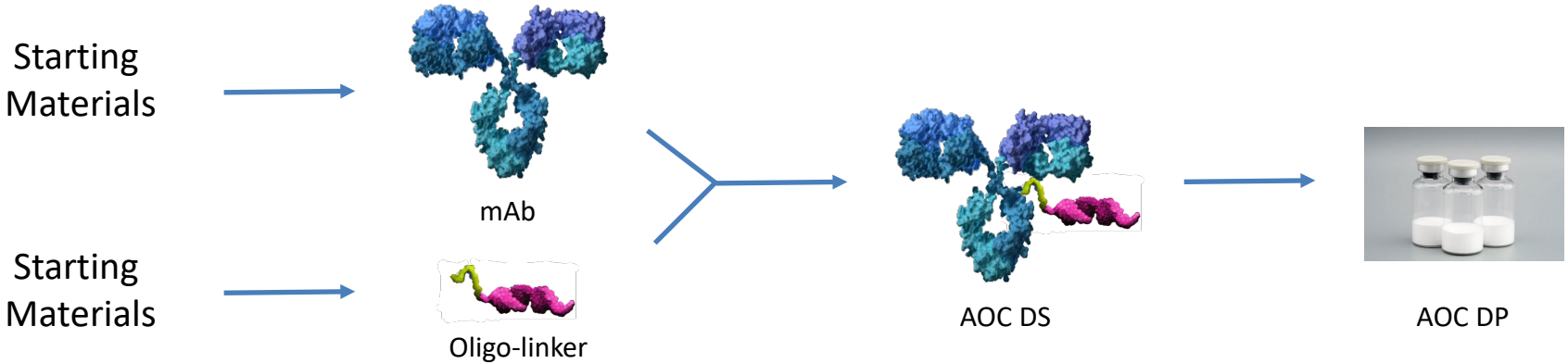


VALIDATED

➢ **Interlocked Execution of Development, Characterization, and Manufacturing Framework**

AOC, antibody oligonucleotide conjugate; DP, drug product; DS, drug substance; mAb, monoclonal antibody; PPQ, process performance qualification.

Potential Challenges of AOC Supply and Comparability

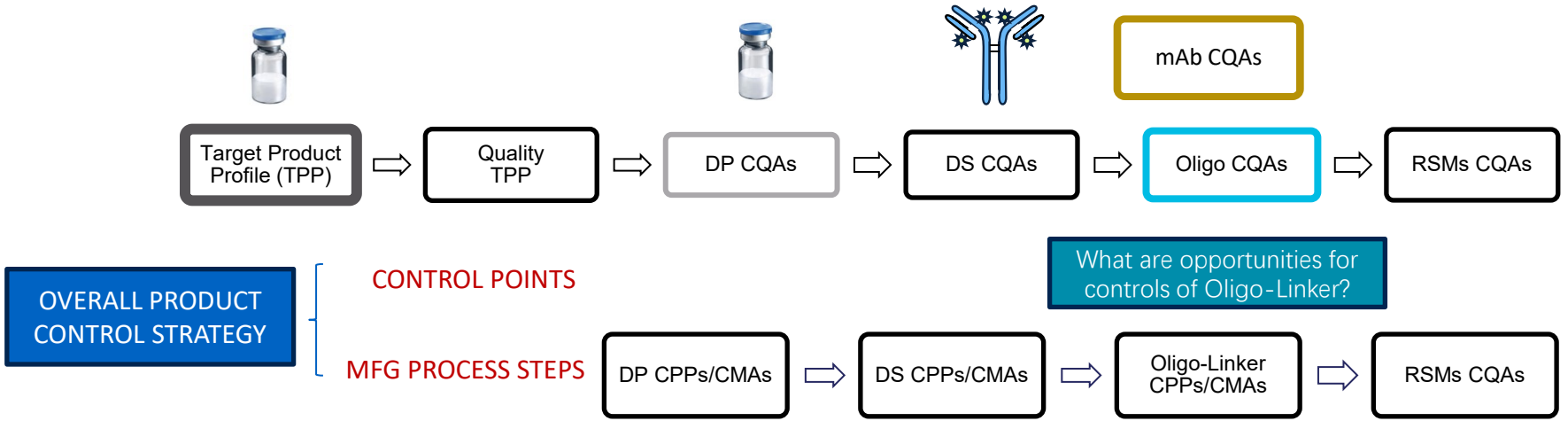


- **Comparability:**
  - **Generate sufficient representative comparability from registrational clinical and process validation lots**
- **Manufacture and Supply:**
  - **Non-sequential PPQ strategy may be utilized for marketing application approval**
  - **Strategy for manufacture of saleable goods from process validated across all unit operations**

AOC, antibody oligonucleotide conjugate; DP, drug product; DS, drug substance; mAb, monoclonal antibody; PPQ, process performance qualification.

AOC Control Strategy

AOC PRODUCT QUALITY RISK ASSESSMENT



- **Control strategy:** A planned set of controls, derived from current product and process understanding, that assures process performance and product quality (ICH Q11)

- ***Establish overall AOC product control strategy from AOC DS, Oligo-Linker and mAb***
  - Identify DP CQAs impacted by CQAs of the AOC/DS and upstream intermediates (mAb, Oligo-Linker)
  - Identify points of controls in the manufacturing process steps (CPPs, CMAs)

AOC, antibody oligonucleotide conjugate; CMA, critical material attributes; CPP, critical process parameters; CQA, critical quality attributes; DP, drug product; DS, drug substance; mAb, monoclonal antibody; PPQ, process performance qualification; RSM, response surface methodology; TPP, target product profile.

AOC COMPARABILITY AND RELEASE ATTRIBUTES

Benchmark Attributes

	mAb	Oligo-Linker	AOC DS	AOC DP
Appearance	■	■	■	■
Osmolarity			■	■
Charge and Size Variants	■		■	■
Glycosylation	■			
pH	■	■	■	■
Potency and Effector Function	■		■	■
Unconjugated mAb			■	■
DAR Profile			■	■
Unconjugated Oligo-linker			■	
Conjugatable Impurities		■		
Non-Conjugatable Impurities		■		
Stereochemical Footprint		■		
Residual Solvents/Reagents		■	■	
Bioburden and Endotoxin	■	■	■	■
Mutagenic Impurities/Nitrosamine		■		
Leachable and Extractable	■	■	■	■

Comparability Assessment Variables

	mAb	Oligo-Linker	AOC DS	AOC DP
Change in Route of Synthesis		■	■	
Changes in Manufacturing Process Unit Operations/Procedures	■	■	■	■
Change in Batch Scale	■	■	■	■
Change in Contract Manufacturer	■	■	■	■
Change in Manufacturing Site/Equipment	■	■	■	■
Change in Impurity Profile	■	■	■	■
Change in Container Closure			■	■

- **Opportunities to streamline comparability and attributes requirements**
- **When is comparability of intermediate attribute sufficient?**

AOC, antibody oligonucleotide conjugate; DAR, drug antibody ratiol; DP, drug product; DS, drug substance; mAb, monoclonal antibody.

CONJUGATABLE VS NON-CONJUGATABLE OLIGO IMPURITIES IN DS MANUFACTURING PROCESS

Non-conjugatable

- "Mass dilution" between oligo-linker impurities and large molecule AOC DS

Worst-case assumption: no purging in purification[1]

$$\text{Impurity level in AOC} = \text{Impurity level in Oligo} \times \text{Oligo equiv. used} \times \frac{\text{Impurity MW}}{\text{AOC MW}}$$

2% impurity in Oligo-Linker $\rightarrow$ 0.2% impurity in AOC DS[2]

- Purge factor for OAC process consider UF/DF and chromatographic purification
- Q NMR can be used to determine non-conjugatable impurity content

Conjugatable

- Conjugation test with simple acetyl-cysteine to determine extent of conjugation by LCMS
- "Mass dilution " has also been proposed
- Consider structure of impurity for oligonucleotide impurities
- Consider tox coverage
- Qualification and identification levels set at 1.5% and 1.0%, respectively for oligonucleotides[2,3]

AOC, antibody oligonucleotide conjugate; DS, drug substance; LCMS, liquid chromatography mass spectrometry; mAb, monoclonal antibody; MW, molecular weight; PPQ, process performance qualification; Q NMR, qualitative nuclear magnetic resonance; UF/DF, ultrafiltration/diafiltration.

1. IQ WG paper: Gong *et al. AAPS PharmSciTech* 2018;19:971. **2.** Assumptions: AOC MW 160,000-170,000, Impurities MW 7000-15000.
3. EPOC paper: Capaldi *et al. Nucleic Acid Therapeutics* 2017;27:309.

PMO Process Development and Control Strategy

PMO MANUFACTURING PROCESS

PMO Monomers Include specified impurities at levels exceeding paper-justification to meet EMA/EPOC ID threshold

Impurities can be generated as a function of protecting group selection (G), including pMIs

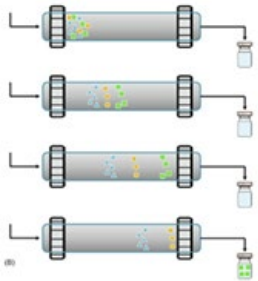
Limiting linker equivalents and minimizing temperature to control impurity formation

Starting Materials → **PMO Synthesis** → **PMO Cleave/Deprotection** → **PMO Purification** → **Linker Conjugation**

Lipophilic-Tag Assisted Liquid Phase Process:

- Monomer equivalents and coupling temperature increase during chain elongation
- pH modulation during trityl removal is necessary to limit backbone linkages hydrolysis

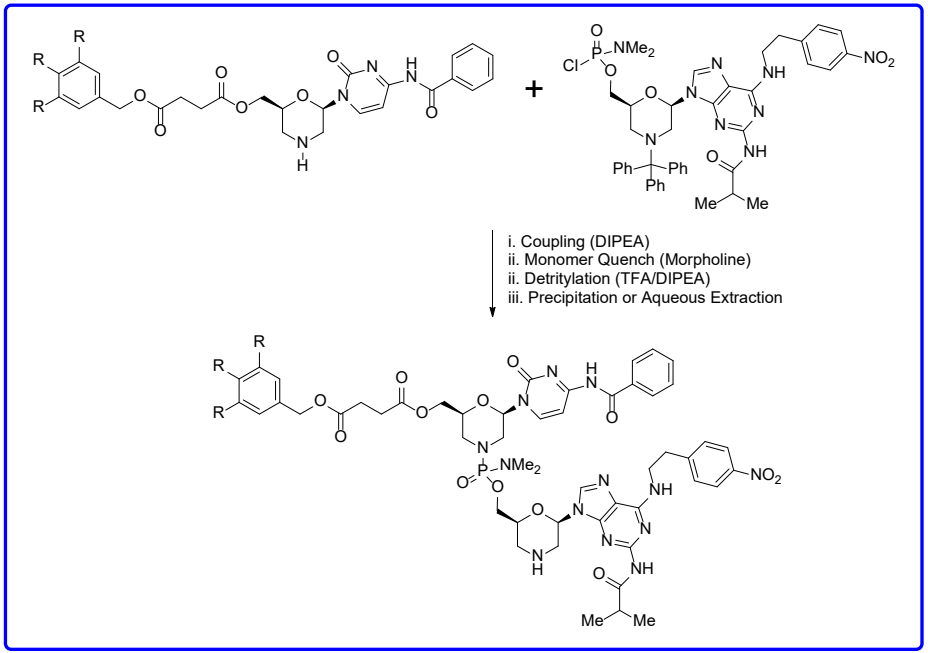
Sole purification opportunity prior to final chemical transformation



PMO, phosphorodiamidate morpholino oligomer.

LPOS PMO CHAIN ELONGATION CYCLE AND GLOBAL DEPROTECTION/CLEAVE PROCESS

- **Coupling**
  - For early couplings, monomer equiv. are near 1.0 but must be increased for longer chains
  - Coupling temperature is also increased throughout the cycle
  - Acceptable range finding during process characterization is thus performed by families of steps or target specific step
  - Process stereospecific at backbone phosphorus, however PMO monomer starting materials are near 1/1 mixture of diastereomers
- **Detritylation**
  - pH modulated using TFA and DIPEA to ensure stability of backbone linkages



DIPEA, N,N-diisopropylethylamine; LPOS, liquid phase oligonucleotide synthesis; PMO, phosphorodiamidate morpholino oligomer; TFA, trifluoroacetic acid.

1. Data on File. Avidity Biosciences, Inc. Number: VV-QUAL-004009 Version: 2.0.

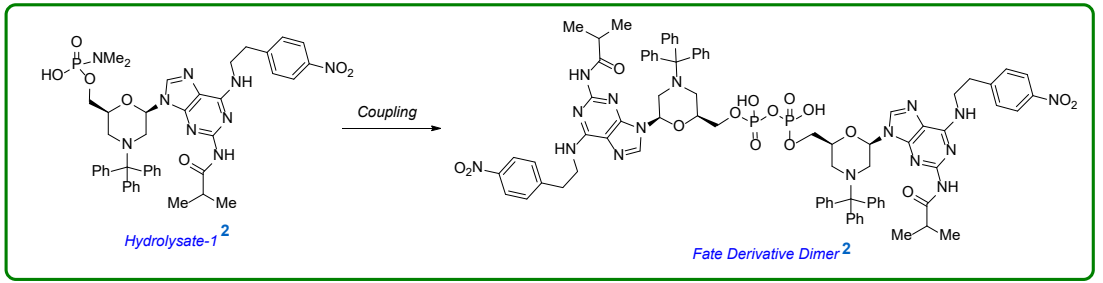
CONTROL OF STARTING MATERIAL IMPURITIES

➢ Non-Critical Impurities (Green boxes)

- ❑ Specified at 10X unspecified limit
- ❑ Do not get incorporated in PMO chain
- ❑ Rejected in extraction or purification, purge factors are measured from last point of introduction in process

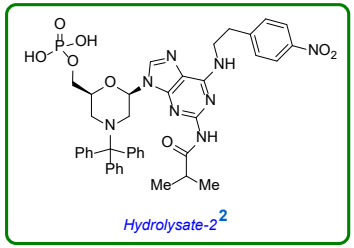
➢ Critical Impurities (Blue boxes)

- ❑ If present above unspecified impurity limit, fate derivative in full-length PMO is evaluated during chromatography and purge factor measured

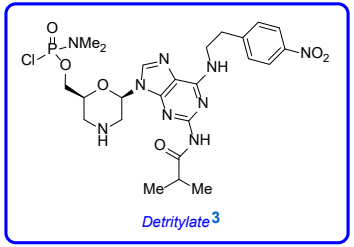


Hydrolysate-1 [2]

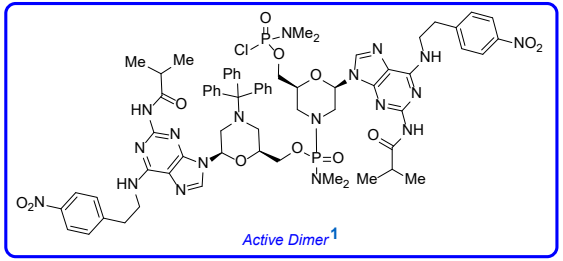
Fate Derivative Dimer [2]



Hydrolysate-2 [2]



Detritylate [3]



Active Dimer [1]

PMO, phosphorodiamidate morpholino oligomer.

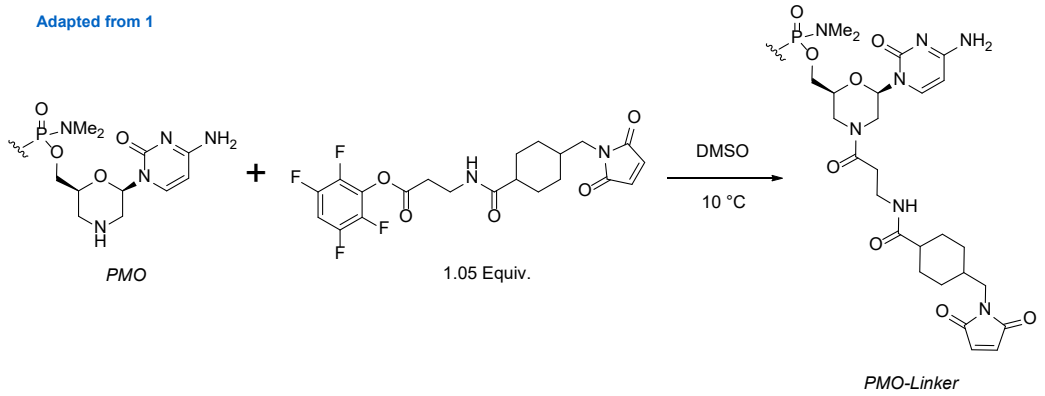
1. Data on File. Avidity Biosciences, Inc. Number: VV-QUAL-004837 Version: 1.0. **2.** Data on File. Avidity Biosciences, Inc. Number: VV-QUAL-004838 Version: 1.0.

3. Data on File. Avidity Biosciences, Inc. Number: VV-QUAL-004005 Version: 2.0

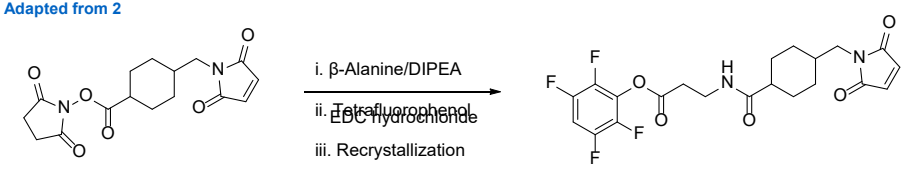
LINKER CONJUGATION PROCESS AND CONTROL

- **Linker Conjugation**
  - Optimization for Equiv. of Linker reagent and temperature
  - Enables control of impurities:
    - Starting Material PMO
    - Maleimide Hydrolysis
    - Attachment of 2nd linker on PMO
- **No purification of PMO-linker post amidation transformation**
- **2-Step GMP process to prepare linker reagent from starting materials SMCC and β-Alanine with no chromatography**

Adapted from 1



Adapted from 2



DIPEA, N,N-diisopropylethylamine; DMSO, dimethylsulfoxide; EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; GMP, good manufacturing practices; PMO, phosphorodiamidate morpholino oligomer; SMCC, Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate.

1. Data on File. Avidity Biosciences, Inc. Number: VV-QUAL-004009 Version: 2.0. **2.** Data on File. Avidity Biosciences, Inc. Number: VV-QUAL-004040 Version: 2.0.

PMO-LINKER CONTROL STRATEGY

AOC, antibody oligonucleotide conjugate; mAb, monoclonal antibody; PMO, phosphorodiamidate morpholino oligomer.

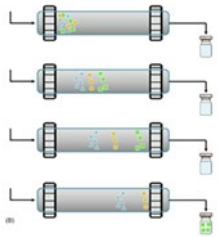
PMO monomer F&P studies in Synthesis and Purification to Meet EPOC Threshold

Crude target impurity limits based on pure PMO specifications and process purging capability (batch history and scale-down purification experiments)

PMO-Linker specified impurity limits based on AOC CQA control, batch history and tox coverage

Starting Materials → **PMO Synthesis** → **PMO Cleave/Deprotection** → **PMO Purification** → **Linker Conjugation**

i. Parameter acceptable ranges as coupling families
ii. Specific parameter acceptable range defined based on major sequenced impurity



Purified PMO specified impurity limits based on batch history and tox coverage

AOC, antibody oligonucleotide conjugate; CQA, critical quality attributes ; F&P, Fate and Purge; PMO, phosphorodiamidate morpholino oligomer.

Thank you for your Attention