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HOLISTIC CONTROL STRATEGY OF OLIGONUCLEOTIDES STARTING MATERIALS

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Location | Bubendorf
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A LEADING SPECIALIST FOR DRUG SUBSTANCES

- Contract development and manufacturing organization (CDMO)
- Broad capabilities in Peptides and Oligonucleotides (TIDES) as active pharmaceutical ingredients (API)
- Long-term partnerships with pharmaceutical and biotech companies
- Focused on chemical synthesis, committed to innovation
- Annual sales of CHF 577.3 million in 2023 and over 2,000 colleagues globally
- Reliable supply of APIs for WHO essential medicines benefitting patients worldwide

High quality GMP manufacturing

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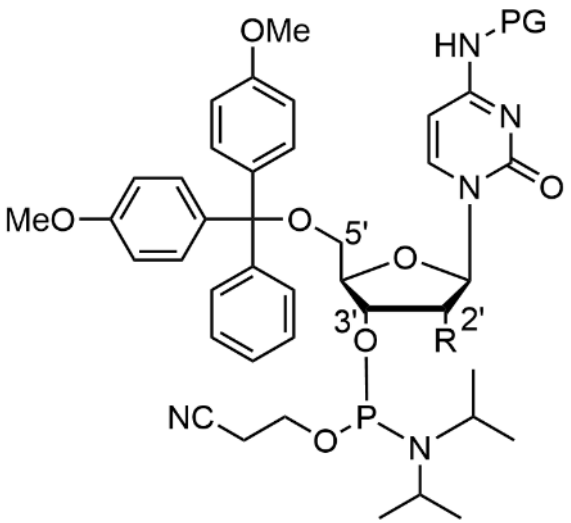
AGENDA

01 Oligonucleotide starting materials: structure and impurities

02 Method development and validation

03 Sourcing and target specifications

PHOSPHORAMIDITES: STRUCTURE



- DMT-2'Fluoro-dA(Bz) phosphoramidite
- DMT-2'Fluoro-dC(Ac) phosphoramidite
- DMT-2'Fluoro-dG(Ib) phosphoramidite
- DMT-2'Fluoro-dU phosphoramidite

- DMT-dA(Bz) phosphoramidite
- DMT-dC(Bz) phosphoramidite
- DMT-dG(Ib) phosphoramidite
- DMT-dT phosphoramidite

20 «standard» phosphoramidites

- DMT-2'O-Methyl-rA(Bz) phosphoramidite
- DMT-2'O-Methyl-rC(Ac) phosphoramidite
- DMT-2'O-Methyl-rG(Ib) phosphoramidite
- DMT-2'O-Methyl-rU phosphoramidite

- DMT-2'O-TBDMS-rA(Bz) phosphoramidite
- DMT-2'O-TBDMS-rC(Ac) phosphoramidite
- DMT-2'O-TBDMS-rG(Ib) phosphoramidite
- DMT-2'O-TBDMS-rU phosphoramidite

- DMT-2'O-MOE-rA(Bz) phosphoramidite
- DMT-2'O-MOE-rMeC(Bz) phosphoramidite
- DMT-2'O-MOE-rG(Ib) phosphoramidite
- DMT-2'O-MOE-rMeU phosphoramidite

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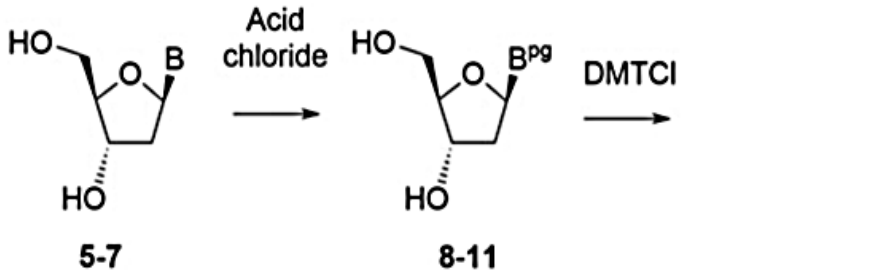
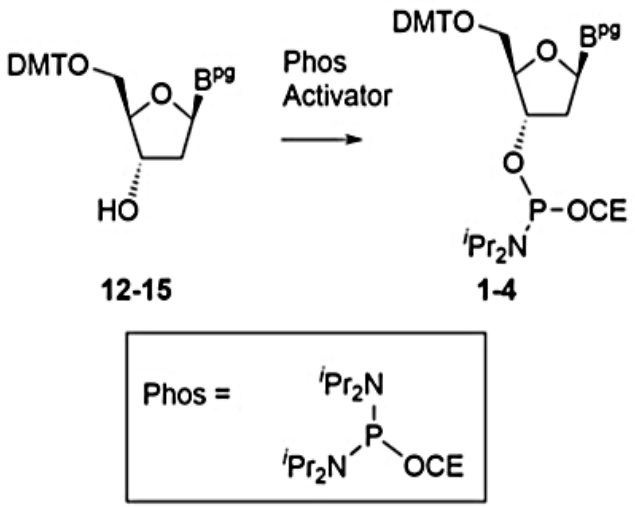
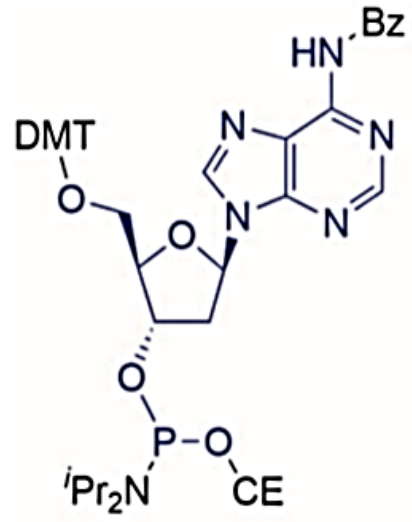
PHOSPHORAMIDITES: SYNTHESIS







General Synthesis Strategy published (Kiesmann, et al.; 2021)

Impurity profile depending on route of synthesis

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PHOSPHORAMIDITES: SUPPLIER SPECIFICATION

Test	Method	
Appearance	Appearance of solid Appearance of 0.1M solution	White to off white powder Report color and clarity of 0.1M solution in acetonitrile
Identity	Molecular Weight by MS ^{1}H NMR	Theoretical Mass +/- 2 Da Conform to structure
Purity	^{31}P NMR Trivalent Phosphorus (P(III)) impurities	≥ 98% ≤ 0.5%
Purity	HPLC (area%)	Purity ≥ 98% Total impurities ≤ 2.0% Any single impurity ≤ 0.7%
Water content	KF coul.	≤ 0.2%
Residual organic solvents	GC	Determine and report

- **Does the specification reflect the commercially available quality?**
- **Does it guarantee final API quality?**

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PHOSPHORAMIDITES: CONTROL STRATEGY

1 **Common specification**

2 **Impurity investigation**

Theoretical investigation based on manufacturing process

Definition of critical impurities based on impact and occurrence

LC-MS investigation of available batches

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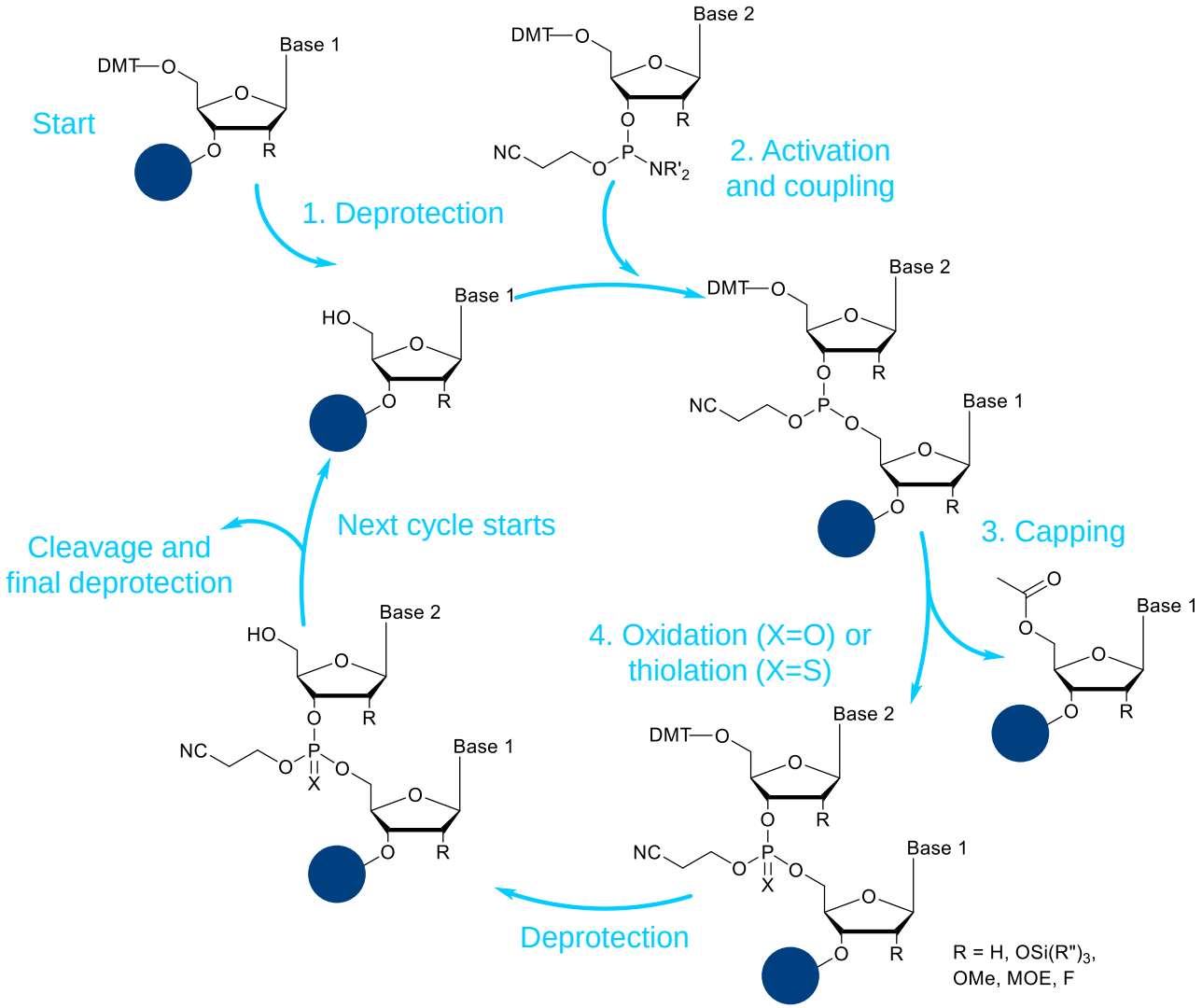
OLIGONUCLEOTIDE SYNTHESIS

Solid support is used for synthesis (preloaded resin)

- Step 1: The DMT group is removed with a solution of an acid (usually DCA dichloroacetic acid)
- Step 2: Coupling of the desired nucleotide (nucleoside phosphoramidite)
- Step 3: In the capping step unreacted solid phase bound 5'-OH groups are permanently blocked from further chain elongation to prevent the formation of oligonucleotides with an internal base deletion commonly referred to as (n-1) shortmers
- Step 4: Oxidation by iodine or thiolation of the tri-coordinated phosphite ester into a phosphate ester.



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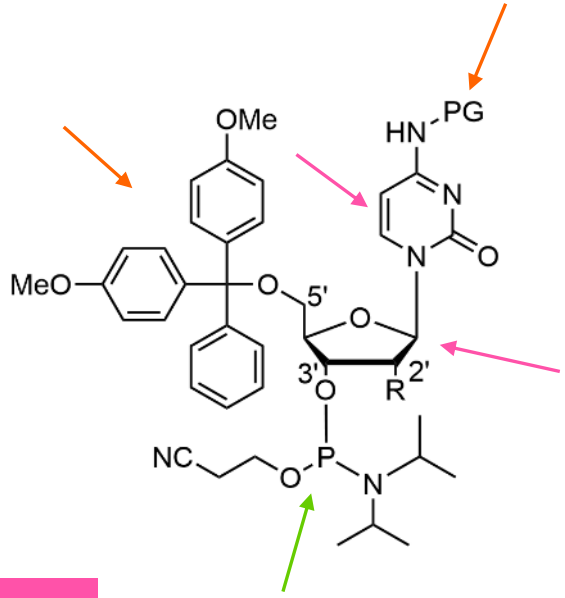
PHOSPHORAMIDITES: IMPURITY INVESTIGATION



2 **Impurity investigation**

Theoretical investigation based on manufacturing process

Definition of critical impurities based on impact and occurrence

Non-reactive and uncritical	Reactive and uncritical	Reactive and critical
Molecules without amidite moiety	No impact on final oligonucleotide quality	Amidite modifications
Removed during synthesis/purification	Base protection group modifications	Base modification
Not incorporated in final oligonucleotide	5’ Protecting group modification DMT	Stereo/regio isomers (Pos. 2’/3’/5’)
		Modifications at the sugar moiety

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PHOSPHORAMIDITES: IMPURITY INVESTIGATION

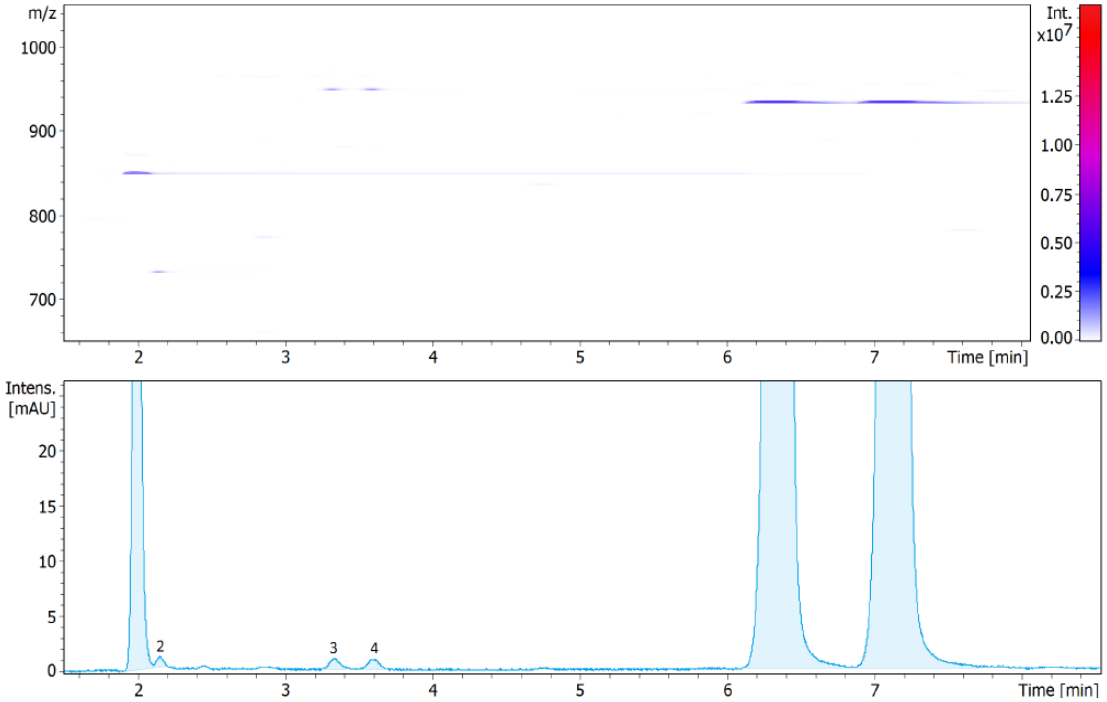
2

LC-MS impurity investigation

High number of batches from the 20 “standard” phosphoramidites analyzed and evaluated with current chromatographic method

Impurity identification approach:

- Mass difference
- Comparison with literature e.g Thermo technote
- High resolution MS-MS fragmentation



#	RT [min]	Max. m/z	Compound Label
1	2.00	849.2881	M-83.111 u; poss. DMT-3’-CE-H-phosphonate
2	2.15	732.2937	M-200.106 u; poss. DMT-2’(3’)-O-MOE-nucleoside
3	3.33	948.3940	M+15.995 u; poss. DMT-3’-amidate
4	3.61	948.3941	M+15.995 u; poss. DMT-3’-amidate
5	6.34	932.4172	Mmono = 931.4033 u; DMT-2'O-MOE-rA(bz) phosphoramidite
6	7.13	932.4172	Mmono= 931.4033 u; DMT-2'O-MOE-rA(bz) phosphoramidite

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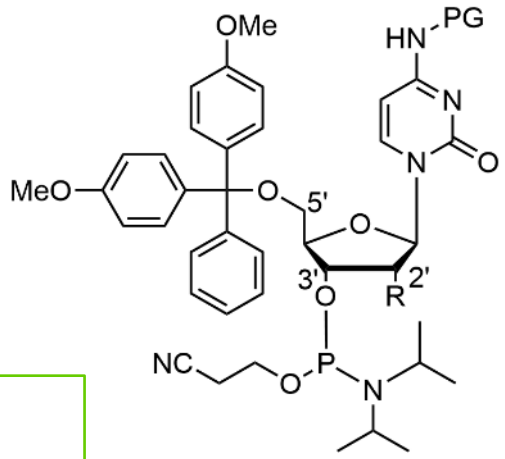
PHOSPHORAMIDITES: IMPURITY INVESTIGATION

2 LC-MS impurity investigation: regular occurring impurities



1. DMT-3’-CE-H-phosphonate (M-83.110 u)
2. DMT-3’-amidate (M+15.995 u)
3. DMT-2’(3’)-Fluoro/TBDMS/OMe-nucleoside (M-200.108 u) loss of phosphoramidite group
4. DMT-3’-H-phosphonoamidate (M-53.027 u)
5. 5’,3’-Bis-DMT-nucleoside (M+102.023 u)
6. 5’-chlorinated-trityl-3’-amidite (M+33.961 u)
7. DMT-3’-(N,N-amino-ethyl-isopropyl)-amidite (M-14.016 u)
8. Isomers (M+0 u) rarely

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PHOSPHORAMIDITES: CONTROL STRATEGY

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Method development

- Optimal parameter applicable for standard oligo startings (16 + 4)
- High sensitivity [LOQ ≤ 0.02%]
- Compatible with LC-MS
- No impurity below main peak [≥ 0.10%]
- Proof of concept with isomer spiking

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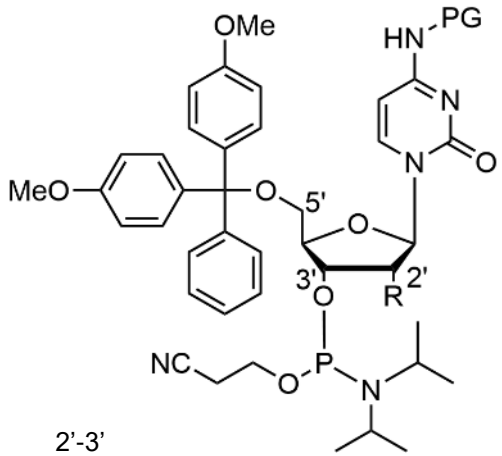
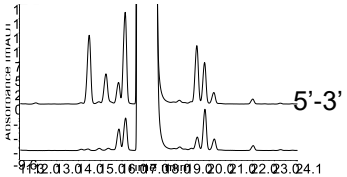
PHOSPHORAMIDITES: LC METHOD DEVELOPMENT

3 Method development: isomer spiking proof of concept



DMT-2'O-TBDMS-rG(Ib) phosphoramidite



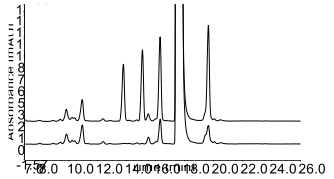
2'-3'

5'-3'

2'-3'

5'-3'

5'-DMT-2'O-TBDMS-rU phosphoramidite



2'-3'

5'-3'

2'-3'

5'-3'

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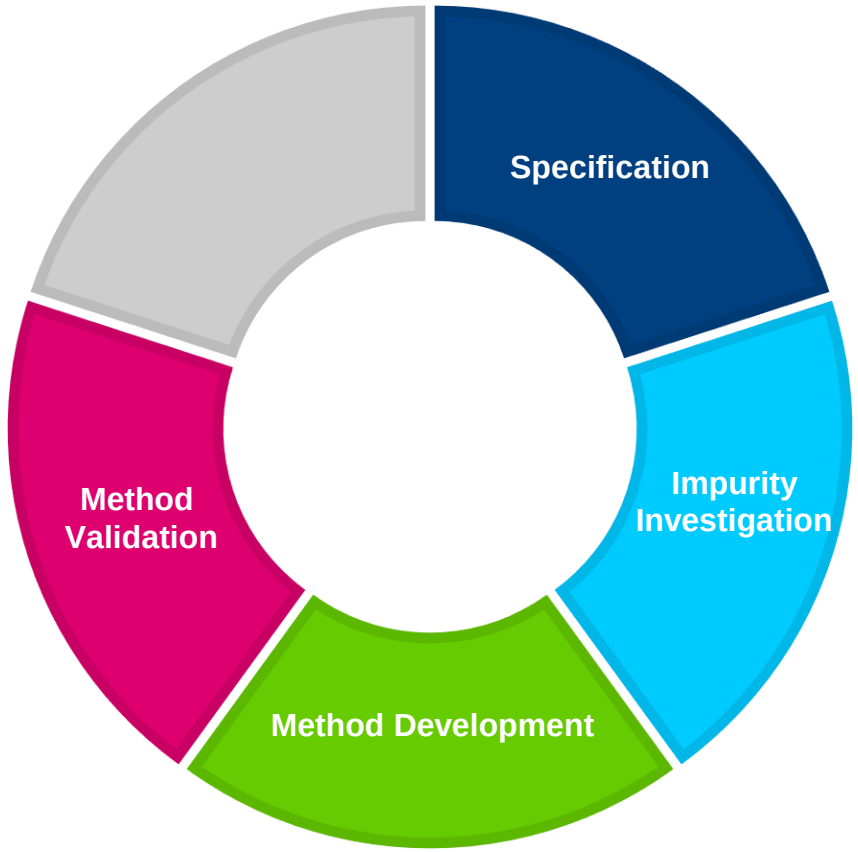
PHOSPHORAMIDITES: CONTROL STRATEGY



4

Method validation

- New concept according ICH Q2(R2) guidelines

  “Impurities or related substances are not available”
- Substance specific approach:

  specificity, LOQ, stability of solution
- With a model substance approach:

  linearity, accuracy by spiking, precision
- Identification of impurities by LC-MS and MS-MS
- Implementation of specific impurities into chromatographic data system

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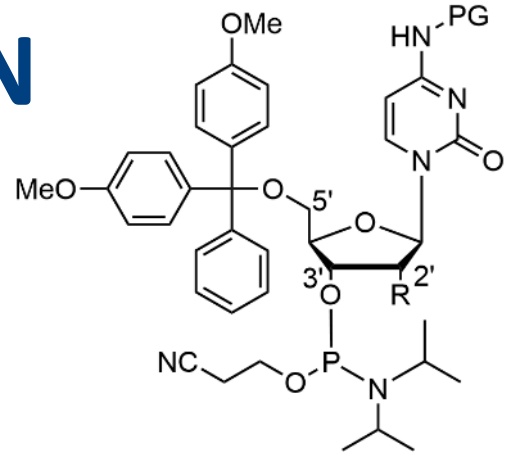
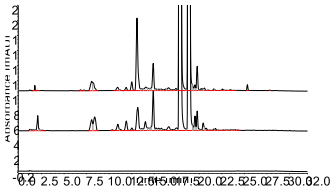
PHOSPHORAMIDITES: LC METHOD VALIDATION



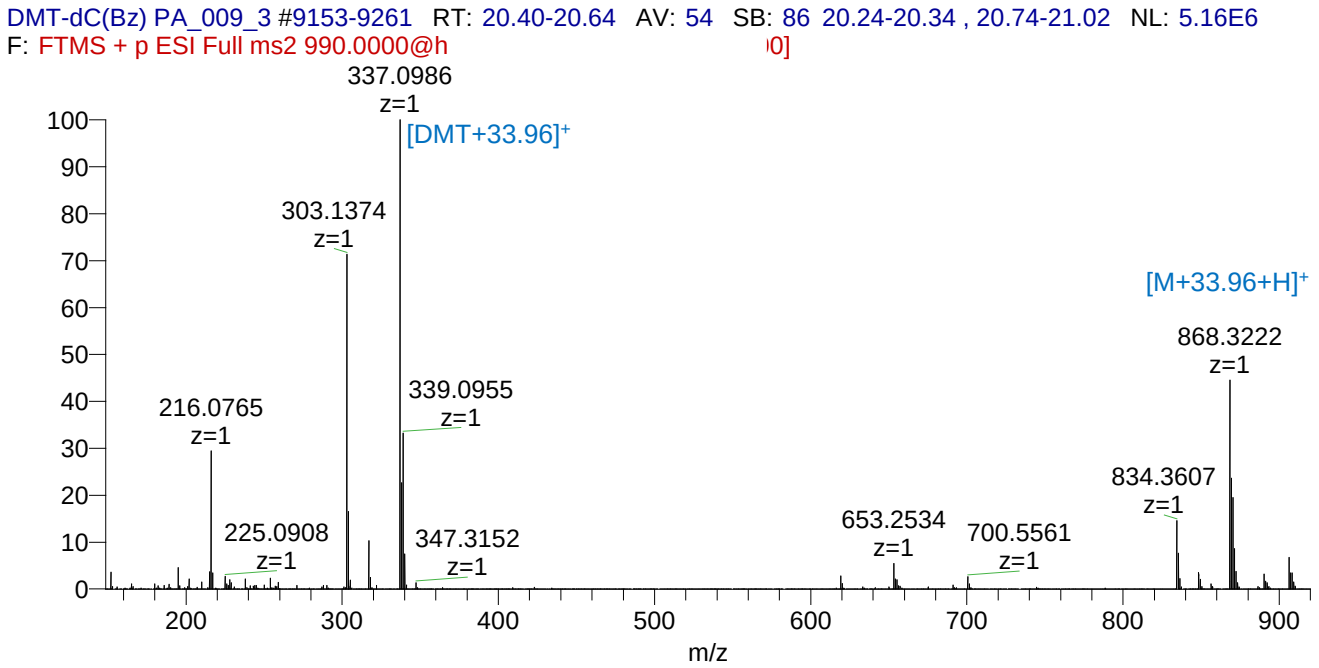
4 Impurity identification by MS and MS-MS approach

DMT-dC(Bz) phosphoramidite



Peak 13

Chlorinated DMT-dC(Bz) phosphoramidite



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PHOSPHORAMIDITES: CONTROL STRATEGY

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Sourcing – supplier quality agreement

- Quality system requirements
- Right to audit
- Change control
- Data reporting – maintenance record
- Deviation and investigations

Additional elements

- Key technical contacts
- Route of synthesis
- Material Specification
- Adequate measures in place to effectively control isomers impurities to max 0.15%

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PHOSPHORAMIDITES: TOWARDS TARGET SPECIFICATION

Test	
Purity and related impurities (HPLC)	Purity ≥ 98% Total impurities ≤ 2.0% Any single impurity ≤ 0.7%
Purity and related impurities (^{31}P NMR)	Purity ≥ 98% Total P(III) impurities ≤ 0.5%

Test	
Purity and related impurities (HPLC)	Purity ≥ 99.0% Any critical impurity ≤ 0.15% Total critical impurities ≤ 0.30% Total uncritical impurities ≤ 1.0% Any unspecified impurity ≤ 0.15%
Purity and related impurities (^{31}P NMR)	Purity ≥ 98.0% Total P(III) impurities ≤ 0.30%
Residual organic solvents (GC)	Primary alcohols ≤ 100 mg/kg (each)

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A HOLISTIC CONTROL STRATEGY REDUCES RISKS FOR PROJECTS AND CLIENTS

MITIGATION OF RISK

THROUGH

HIGH QUALITY

OF STARTINGS

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- **QC team:**

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NMR: Patrik Plattner, Mario Schleep, Larissa Casper, Emmanouil Veroutis

Compliance: Stefan Neimeier

- **Oligonucleotide manufacturing:**

Susanne Kruse, Adrian Sevenich, Henning Loui

- **External supply QA:**

Manuel Weber

QUALITY MATTERS

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THANK YOU

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