Strategies for Peptide Conjugations and Application to Peptide-carbohydrate and Peptide-lipid Conjugates
Customised Service Packages organized under Technology Platforms spanning the entire Pharmaceutical Manufacturing Process

Specialised Technologies for the Production of Oral, Sterile & Highly Potent Pharmaceutical Drug Products and their APIs

More than 40 years of Experience in European- and USA-based Pharmaceutical Manufacturing

CordenPharma Facilities are Audited and Certified by all Relevant Pharmaceutical Compliance and Approval Authorities
CordenPharma - Value Proposition

- Serving Global Pharma & Biotech Customers
- Organized under 6 Distinctive Technology Platforms
- Broad Range of Expertise:
  - API’s: Small Molecules, Peptides, Lipids, Carbohydrates, Highly Potents, Cytotoxics, Conjugates
  - Drug Products: Oral, Liquids, Injectables, Highly Potents, Anti-infectives / Antibiotics
- Global Coverage Allowing for Flexibility
- Your Full-Service Provider from Clinical Development to Full-Scale Commercial Supply of APIs & Drug Products
Solid-Phase Peptide Synthesis at any Scale

Solid-Phase Synthesis:

- Full range of synthesizer covering any scale at any stage of development
- Batch automation
- Large-volume solvent and waste-handling logistics
- Precipitation / isolation of fragments and final APIs
- Enfuvirtide fragment scale up to 920 kg

100-500ml  12 L  50 L  75 L  100 L  500 L  10,000 L
Peptide Purification Capabilities

- **Full range of HPLC, LPLC Scale**
  - Orthogonal chromatography capability
  - Continuous acetonitrile recovery
  - Diagnosis & control of gel formation

- **Precipitation, Isolation & Drying**
  - AP 300 BHS Autopress
  - PSL filter / dryer
Conjugation Targets

- Drug-Conjugates
  - Targeted drug delivery
  - Improving drug release properties: Plasma half-life, controlled release…
  - Improving pharmacological profile
  - Intracellular drug delivery

- Polymer-Conjugates
  - Engineered micro/nano-particles
    - Targeted drug release
    - Improving drug release properties: Plasma half-life, controlled release…
    - Cell-internalization by endocytosis
  - Large molecule conjugates (ex. PEG-conjugates)
    - Bioavailability
    - Improve pharmacology, decrease immunogenicity
Nanoparticle Conjugates

- Increase of plasma half-life
- Drug cargo
- Delivery to the target

Polymer Conjugates

- Synthetic Polymers: PEG, Polyethyleneimine (PEI), Polyvinylalkohol (PVA), Polyvinylpyrrolidone (PVP)...
- Natural Polymers: Dextran, Chitosan, Hyaluronic acid, Mannan, Proteins...
- Semi-Synthetic Polymers: Poly-Amino acids, PLG...
CordenPharma: Ex. Of Cationic Lipid Custom Manufacturing

Core Structure

Variable Lipophilic Tail(s)

(Functionalized) Head Group

Cationic 1,2-glycerol carbamate
CordenPharma:
Ex. Of Cationic Lipid Custom Manufacturing

- **Core Structure**
- **Head Group**
- **Lipophilic Tail(s)**
  - Cationic 1,2-glycerol carbamate
  - Cationic 1,3-glycerol carbamate
  - Cationic 3-amino-1,2-propanediol
  - Cationic 2,3-diacylmannosyl
  - Cationic threosamine

Ex. of Cationic Lipid Custom Manufacturing
Synthetic Carbohydrates: Targeting and Delivery Strategies

Modular Approach to Optimized Constructs

Nucleic acid

Liposome

Carbohydrate Targeting Agent

CPP-Conjugates

RNA-Conjugates

Mifamurtide API, osteosarcoma therapy

Experts taking care.
Peptide-Lipid nanoparticle conjugation

Cys-Cys-Gly-Asn-Lys-Arg-Thr-Arg-Gly-Cys-OH

Polymer-Protein conjugates

Polymer conjugation shielding effect:
- Increase bioavailability
- Decrease immunogenicity by silencing immunogenic sites
- Pharmacokinetic and intracellular trafficking

Important features:
- Conjugate/polymer size
- Hydrophobic/lipophylic balance
- Biodegradability
- Release properties
Control of Protein Glycosylation Pattern Can Improve Efficacy

Improved glycogen clearance in Pompe mice

Endocystosis—Inducing Conjugation

DC-SIGN

Dectin

ASGP-R

Man-6-P-R

High Mannose

β-Glucan

Gal/GalNAc Presenting

Man-6-P Presenting
Demonstrated Total Synthesis of Complex Carbohydrates

Mammalian Carbohydrate Structures:

- N-/O-linked structures (biotherapeutics)
- Highly branched structures (biotherapeutics)
- Human milk oligosaccharides, Lewis structures
- Heparan sulfates (coagulation, targeting)
Conjugated Synthetic Carbohydrates for Vaccines

Pathogen Carbohydrate Structures:

- Meningitis B
- Burkhoderia
- Leishmania
- Moraxella
- Group A Strep
- Malaria toxin
- Candida

Experts taking care.
Example: Phosphorylation

Increase of water solubility, improved bioavailability…

→ Negative charges → decrease of cell uptake

Esterification of the phosphate moiety:
- neutralization of the negative charges
- increased membrane permeation (diester > monoester)
- increased stability against enzymatic degradation
Anchimeric-assisted release

Control of the drug release by a diester hydrolysis sequence: Double pro-drug hydrolysis

Good permeation properties of the acyloxyalkyl phosphate diester
Esterification

Isopeptides = Switch peptides

Spontaneous O to N acyltransfer. Also triggered by esterase activity.

Prevent aggregation, improve peptide solvation and solubility because of the free amine. Thermodynamically more stable.
Lipid Conjugation

- Hydrophobicity
- Lipid solubility
- Circulation half-life

Example of Lipid-Peptide linkages
Lipid Conjugation

Ex. GLP-1 conjugated peptides

- Improved Half-life
- Chemical properties

Cell uptake up to 5.5 fold compared to non-conjugated GLP-1
Half-Life up to 9 fold higher

**PEG-reagents**

Different linkages with or without a spacer (linker) for cleavage/drug release:
PEG-Controlled cleavage

Drug-controlled release by control of the hydrolysis kinetics
Ex. double pro-drug approach

Greenwald et al. *Bioconj. Chem.* 2003; 14; 395-403
PEG-Controlled cleavage

Bicin (bis 2-hydroxyethylglycineamide) system of drug controlled release from PEG chains

Delivery into the cell: pH-sensitive linkers

2 Main Considerations:

- Membrane translocation
  - Receptor mediated endocytosis
  - Conjugations with vectors/carriers
  - Fusion with cell membrane using liposomes, micelles & particles

- Escape from the endosomes, membrane disruption
  - Intracellular environment and peptide conformation properties
Poly-AA for Oligonucleotide (ON) Delivery

Poly-Lysine (PLL)
- Complex formation with the Oligonucleotide
- Enhance cellular uptake and nuclear delivery of the cargo
- Receptor mediated endocytosis by conjugating PLL to folic acid, Transferrin, steroids, growth factors

Poly-His (PLH)
Membrane translocation properties
- protonation of imidazole groups in the cytosol
  ➔ endosomal escape
- Partially « histidinylated » oligo-Lysines: 10-fold enhancement of the oligonucleotide uptake compared

NCA polymerization
MW average controlled by reaction conditions and initiator

Experts taking care.
Drug Conjugation Strategy

VECTOR
- Easy to conjugate
- Cost effective
- Non-toxic
- Metabolism

LINKER
- Easy to cleave
- Easy to conjugate
- Non-toxic
- Intrinsic activity
- Activity after cleavage
- Easy conjugation

DRUG
- Cell environment sensitive

Polymer (PEG, PLG, Peptide polymers…)
Peptide (CPPs)
Receptor substrate (targeted delivery)
others

API Manufacture
- Cost of API synthesis

Loading of active
- Cost of loading/engineering efficiency

Duration of activity
- Cost of low bioavailability
pH-Sensitive Linkers

- Drug release following endocytosis through: acidic pH in the endosomes and lysosomes (usually peptide linkers)
- Drug release to tumor cells. N-cis-aconityl linker for Doxorubicin-polysaccharide conjugates
Cell Penetrating Peptides (CPPs)

Endosome escape/Cell-membrane disruption:

- pH-sensitive peptides (CPPs) or polymers
  - coupling CPP to a polycationic polymer leads to endosome rupture by protonation of the polymer

- Conformation changes that trigger membrane disruption (fusogenic peptides)
  Ex.: AP6 from adenovirus VI, HGP from HIV-1 gp41 protein, Cathepsin sequence GKPIILFF. Retro sequence FFLIPKG more efficient internalization

- Treatment with external agents: chloroquine, transfectamine…
CPP-Lipid Conjugation for Oligo Delivery

- Ex. Arg-rich peptide:
  \((RxR)4\) where \(x = \alpha\)-aminohexanoic acid

Stearic-(RxR)4 increases cell uptake and endosome disruption

Nanoparticles were formed and protected DNA from degradation for >24h.

Naked DNA was completely digested after 24h.

Delivery of noncovalently bound DNA

CPPs for ON delivery: Ex. of Linkers

Covalent Linkages: Different linking possibilities

VECTOR → LINKER → DRUG

CPP Nucleic Acid
CPP Nucleic Acid
CPP Nucleic Acid
CPP Nucleic Acid
CPP Nucleic Acid
pH mediated delivery/cleavage

Ex. of pH sensitive linkers
- Hydrazones
- Vinyl ethers
- Acetals
- Ortho esters

Extracellular
Cytoplasm
Peptide-Orthoester-PEG model

5- and 6-membered orthoesters

Hydrolysis (Release profiles)

Hydrolysis at 37°C, pH 4.5 and 6.5

Hydrolysis (Release profiles) at pH 5.5

Conclusion

- Drug conjugation remains a powerful delivery strategy despite a long effort for lead-optimization.

- Understanding the drug conjugate chemical properties is key for:
  - Targeted delivery
  - Controlled drug release and site drug release
  - Drug stability, metabolism

- Importance of considering evaluating the conjugation early enough in the drug development process:
  - Anticipate potential drawbacks
  - Shorten development efforts and time to market
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