

## Seminari Prof. Eray ÇALIŞKAN

### Type of Course the Teaching Activity

Graduate and upper-level undergraduate courses in Organic Chemistry, with a focus on Peptide Synthesis, Bioorganic Chemistry, and Functional Materials.

Focus: Advanced Organic Chemistry & Peptide Chemistry

Format: Lectures, interactive seminars, lab components (where applicable)

Tutti i seminari si svolgeranno in **Aula VII (CU032), 12:00 – 14:00**

### Week 1 — Fundamentals of Amino Acids + Introduction to Peptides (Lunedì 02/03)

#### Topics

- Definition and general structure of amino acids:  $\alpha$ -amino acid framework, chirality, zwitterionic forms
- Physicochemical properties: pKa values, isoelectric point (pI), buffering behavior, solubility, hydrogen bonding
- Classification: polar/nonpolar, aromatic, acidic/basic, sulfur-containing, hydroxyl-containing, etc.
- Amino acid chemistry: side-chain reactivity ( $-\text{NH}_2$ ,  $-\text{COOH}$ ,  $-\text{SH}$ ,  $-\text{OH}$ , aromatic ring) and common derivatization reactions
- Introduction to peptides: N-terminus/C-terminus, directionality, and general chemical structure
- Peptide stereochemistry: L/D configuration, the concept of epimerization, and preliminary conformational concepts
- Peptide bond formation: general approach, condensation logic, and elimination of water

#### Key learning outcomes

- Establish structure–property relationships of amino acids using pKa/pI concepts.
- Understand the reactivity of side-chain functionalities and the rationale for protection.
- Explain peptide directionality, stereochemistry, and the fundamental logic of peptide bond formation.

#### Review questions

### Week 2 — Peptide Types + Conditions for Peptide Bond Formation + Classification of Coupling Reagents (Introduction) (Lunedì 09/03)

#### Topics

- Peptide types: isopeptides (amide bond formed via a side chain), depsipeptides (ester bond in place of an amide), and peptidomimetics
- Differences from natural peptides: stability, protease resistance, and functional diversity
- Chemical and thermodynamic emphasis for peptide bond formation: equilibrium limitations in condensation and the concept of water removal
- Need for activation: conversion of the carboxyl group into a more reactive intermediate with a better leaving group
- Classification of peptide coupling reagents (framework): carbodiimides, active-ester strategies, uronium/phosphonium salts, and acid chloride/anhydride approaches
- Role of additives (e.g., HOBt-type, Oxyma-type) and bases: selectivity, rate enhancement, and control of side reactions

*Key learning outcomes*

- Differentiate isopeptides and depsipeptides based on bond type and connectivity.
- Explain why peptide bond formation is fundamentally constrained by equilibrium and activation requirements.
- Place major reagent families into logical classes and justify why coupling reagents are needed.

*Review questions*

**Week 3 — Peptide Coupling Reagents: Advantages/Limitations + General Mechanistic Frameworks (Lunedì 16/03)**

**Topics**

- Carbodiimides (DCC/DIC/EDC family): advantages, limitations, by-product management, epimerization risk, and core mechanism
- Active-ester strategies
- Uronium/phosphonium salts (HATU/HBTU/TBTU, etc.): rate/yield, performance in difficult couplings, cost/safety considerations, and mechanistic outline
- Acid chloride/anhydride approaches: high reactivity, selectivity issues, and compatibility with sensitive functional groups
- Side reactions and control parameters: epimerization, diketopiperazine formation, oligomerization; effects of solvent, temperature, base, and reagent choice

*Key learning outcomes*

- Compare reagent families along the reactivity–selectivity–epimerization axis.
- Recognize common reactive intermediates and connect conditions to outcomes.
- Justify coupling reagent selection based on target amino acids and sequence difficulty.

*Review questions*

**Week 4 — Solid-Phase Peptide Synthesis (SPPS): Principles, Strengths/Limitations, and Literature Examples (Lunedì 23/03)**

**Topics**

- Core principle of SPPS: resin attachment and iterative cycles of “deprotection + coupling + washing”
- Protection strategies: operational logic of Fmoc/tBu and Boc/Bn approaches
- Key strengths of SPPS: speed, reduced need for intermediate purification, and compatibility with automation
- Limitations/challenges: resin swelling and solvent compatibility, difficult sequences, aggregation, side reactions, and environmental burden (high solvent use)
- Representative literature concepts: historical Merrifield framework; modern solutions for difficult sequences such as double coupling, pseudoproline motifs, and backbone protection (conceptual overview)

*Key learning outcomes*

- Describe the SPPS cycle and protection chemistry step-by-step.
- Explain practical consequences of Fmoc vs Boc strategies (compatibility, side reactions).
- Identify difficult-sequence problems and select feasible classes of solutions.

## *Review questions*

### **Week 5 — Peptide Bioconjugation: Rationale, Applications, and Representative Conjugate Types (Lunedì 30/03)**

#### **Topics**

- Why bioconjugation is performed: improved stability, targeting, pharmacokinetics, and imaging/monitoring
- Major conjugation handles: N-terminus/C-terminus, Lys  $\epsilon$ -NH<sub>2</sub>, Cys –SH, Tyr phenolic –OH
- Conjugation strategies (framework): amide formation (NHS-ester logic), thiol-based approaches, and click-type reactions (conceptual overview)
- Selectivity considerations: single-site vs multi-site conjugation; heterogeneity and product distribution
- Application areas: drug delivery, targeted therapeutics, biosensors, and surface immobilization
- Representative examples: effects of PEGylation (half-life extension), peptide–fluorophore conjugates, and peptide–drug conjugates (targeting)

#### *Key learning outcomes*

- Link bioconjugation to design objectives and articulate the rationale.
- Evaluate functional-group selectivity and heterogeneity risk across strategies.
- Justify an appropriate conjugation chemistry for a given application scenario.

## *Review questions*

### **Week 6 — Protecting Groups and Orthogonal Protection Design (Lunedì 13/04)**

#### **Topics**

- Side-chain protection logic for common residues (e.g., Lys, Asp/Glu, Cys, Ser/Thr, Tyr, His, Arg)
- Orthogonal protection concepts: selective deprotection followed by selective functionalization
- Typical side reactions: aspartimide formation, Cys oxidation, side-chain alkylation, and mitigation strategies

#### *Key learning outcomes*

- Design and justify a protection strategy tailored to a target sequence.
- Relate orthogonal protection to branched peptide synthesis and bioconjugation objectives.

## *Review questions*

### **Week 7 — Cyclic Peptides and Synthetic Approaches (20/04)**

#### **Topics**

- Cyclization strategies and design principles: head-to-tail (N→C) macrocyclization, side-chain-to-side-chain (e.g., Lys–Asp/Glu), side-chain-to-terminus cyclization, and the concept of stapled peptides; effects of ring size, preorganization, and conformational constraint on activity/stability
- Practical solution-phase routes for macrocyclization: design of linear precursors (protecting groups, orthogonality), high-dilution conditions, pseudo-dilution techniques, minimization of dimerization/oligomerization, and approaches to improve ring-closure yield
- Bond-forming chemistries (lactam/lactone/disulfide and click-based rings): lactam (amide) bridges, lactone (ester) bridges, disulfide bridges with redox control; triazole rings via CuAAC/SPAAC, thiol–ene reactions, and stapling via olefin metathesis (RCM); selectivity and side reactions for each method

### *Key learning outcomes*

- Explain, with scientific rationale, why cyclic peptides are often preferred (conformational constraint, proteolytic stability, increased affinity/selectivity, and potential improvements in cell permeability).
- Classify major cyclization types: head-to-tail, side-chain↔side-chain, side-chain↔terminus, disulfide bridging, and stapled approaches.

### *Review questions*

## **Week 8 — Peptide Conformation and Folding: Structure–Function Relationships (Lunedì 27/04)**

### **Topics**

- The Ramachandran framework; secondary structure motifs:  $\alpha$ -helix,  $\beta$ -sheet
- Effects of proline and glycine on conformational behavior; *cis/trans* peptide bond isomerism (particularly X–Pro motifs)
- Influence of solvent, pH, and ionic strength; aggregation tendencies and strategies for their control

### *Learning Outcomes*

- Derives conformational tendencies from primary sequence and provides structural justification.
- Explains post-synthetic solubility and aggregation issues based on conformational principles.

### *Review Questions*

## **Week 9 — Unnatural Peptides I (Backbone and Bond Modifications) (Lunedì 04/05)**

### **Topics**

- Backbone Modifications: Peptides containing  $\beta$ - and  $\gamma$ -amino acids; peptoids (N-substituted glycines); azapeptides and N-methylated peptides.
- Bond Type Variations: Depsipeptides (ester linkages), thioamide-containing peptides, urea/carbamate/sulfonamide linkages, and amide isosteres.
- Electronic and Conformational Effects: Amide resonance, planarity, hydrogen-bond donor/acceptor properties; effects of bond length and torsional angle variations on secondary structure.
- Reactivity and Mechanistic Comparisons: Kinetic and thermodynamic evaluation of classical amide bond formation versus alternative bond-forming mechanisms.

### *Learning Outcomes*

- Analyzes modified peptide backbones in terms of bond character, resonance, and conformational consequences.
- Compares the electronic and steric effects of amide isosteres in the context of reactivity and stability.
- Explains alternative bond formation mechanisms at a mechanistic level.

### *Review Questions*

## **Week 10 — Unnatural Peptides II (Stereochemistry, Topology, and Design Strategies) (Lunedì 11/05)**

### **Topics**

- Stereochemical Modifications: Peptides incorporating D-amino acids; retro-inverso analogues; effects of chirality inversion on conformation and proteolytic stability.

- Topological Modifications: Macrocyclic unnatural peptides; stapled peptides; branched systems and artificial cross-linking strategies.
- Conformational Restriction and Stability: Entropic and enthalpic contributions; the concept of preorganization; influence of ring size and bond geometry on biomolecular interactions.
- Synthetic Planning: Solution-phase-focused strategies; orthogonal protecting group schemes; epimerization control and management of oligomerization risks.

#### *Learning Outcomes*

- Explains the effects of stereochemical and topological modifications on conformation and stability using chemical principles.
- Designs an appropriate synthetic strategy for an unnatural peptide with mechanistic justification.

#### *Review Questions*

### **Week 11 — Peptide Bioconjugation (Lunedì 18/05)**

#### **Topics**

- Chemical Principles of Bioconjugation: Concepts of chemoselectivity, regioselectivity, and orthogonality; evaluation of reactive functional groups in peptides (N-terminal amine, Lys  $\epsilon$ -amine, Cys thiol, Asp/Glu carboxyl groups) in terms of pKa and nucleophilicity.
- Amine- and Carboxyl-Based Conjugations: NHS ester activation for amide bond formation; carbodiimide (EDC)-mediated couplings; reductive amination; side reactions and hydrolysis kinetics.
- Thiol-Selective Reactions: Maleimide–thiol Michael addition (mechanism and retro-Michael considerations); haloacetyl derivatives; disulfide formation and redox-controlled conjugation.
- Bioorthogonal Approaches: CuAAC and SPAAC reactions; Staudinger ligation; reaction rates, selectivity, and issues related to metal residues in aqueous systems.

#### *Learning Outcomes*

- Analyzes peptide bioconjugation reactions in terms of mechanism, chemoselectivity, and reaction conditions.
- Selects appropriate conjugation strategies for different functional groups based on reactivity, stability, and side-reaction risk.

#### *Review Questions*

### **Week 12 — Final Lecture: Synthesis Planning + Data Interpretation (Lunedì 25/05)**

#### **Topics**

- Case study: design of reagent selection, protection strategy, SPPS cycle, and purification plan for a given target peptide
- Typical challenges: incomplete coupling, side-product formation, epimerization, aggregation; cause–solution mapping
- Decision-making based on analytical data: interpretation of representative NMR and LC–MS outputs (in-class application)

#### *Learning Outcomes*

- Develops a complete end-to-end synthetic strategy with integrated risk management.
- Demonstrates data-driven problem-solving and decision-making skills.