1 General introduction

1.1 Peptides and peptidomimetics

Numerous small and large peptides, which are sequence and length-specific polymers composed of amino acids, represent compounds with significant therapeutic applications.[1] Peptides and their higher relatives proteins play a crucial role in almost all processes of the living cell. Representative examples include somatostatin, substance P, cholecystokinin, endorphin, enkephalin, angiotensin II and endothelin. As neurotransmitters, neuromodulators and hormones peptides are responsible for the regulation of biochemical processes in complex organisms such as cell-cell communication and control of vital functions like metabolism, immune response, digestion, respiration, sensitivity to pain, reproduction, behaviour and electrolyte levels. Since so many of peptides possess potent pharmacological properties, they are of enormous medicinal interest.

Unfortunately, many peptides composed of natural amino acids are readily degraded by proteases.[2] To design novel highly active, selective and metabolically stabile drugs, conformationally fixed or restricted peptidomimetics are often synthesized.[3] A peptidomimetic is a compound that imitates or blocks the biological effect of a peptide at the receptor level and typically constitutes a rigid template with the ability to exhibit conformational influences on the peptide backbone. As the ligand of an enzyme it can serve as substrate or as inhibitor. In addition, peptidomimetic oligomers may have reduced immunogenicity, increased selectivity and thereby fewer side effects, as well as improved bioavailability relative to peptide analogues.

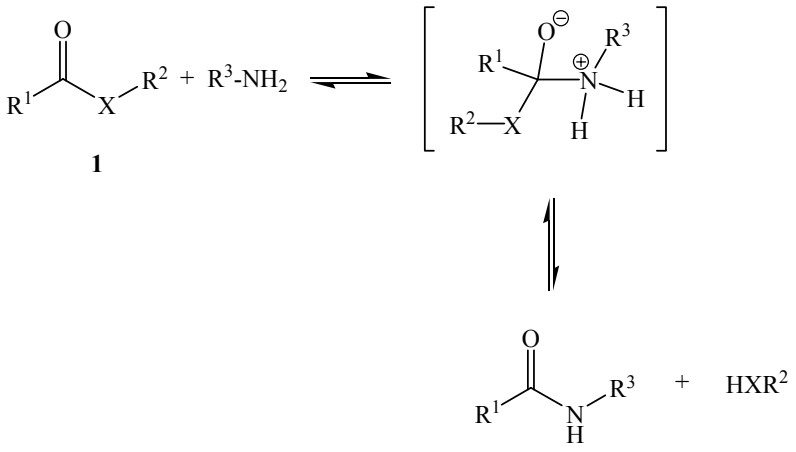
It has been shown that in most cases only four to eight amino acid side chains of the peptide are responsible for the recognition of the ligand by the receptor.[4] The rest of the molecular framework than serves to fix the pharmacophores in a specific spatial arrangement.

Incorporating unnatural functionality in amino acid-derived biomolecules has proven to be a successful strategy in the design of peptides with more desirable properties. The primary structure of a peptide refers to its amino acid sequence. Secondary structure is described by two characteristic: torsion angles and hydrogen bonding pattern. The major hydrogen bonding patterns in peptides which consist of natural amino acids are α-helix and β-sheets, as well as β-turns and loops. The pioneering studies on some unnatural peptidomimetic folding

oligomers discovered a unique type of secondary structure, which is referred as "mixed helix".[5, 6] In order to adopt a stable conformation in solution peptides composed of naturally occurring α-amino acids usually must have a length of at least 12 units, while oligopeptides constructed from unnatural amino acids assume surprisingly stable secondary structures even in short oligomers of several units.[7] These oligomers composed entirely of unnatural monomers that form characteristic secondary structures have attracted considerable attention in the last years.

1.1.1 Synthesis of peptides

Peptide bond formation is a nucleophilic substitution reaction of an amino group (nucleophile) at a carboxyl group *via* a tetrahedral intermediate. The carboxyl component **1** must be activated (its electrophilicity increased) prior to peptide bond formation by the introduction of electron-accepting moieties, because carboxylic acids normally react at room temperature with ammonia or amines to give an ammonium salt instead of carboxamide. The leaving group capacity (nucleofugicity) is another factor which influences the reaction rate; variation of the leaving group thus provides a broad spectrum of methods for peptide bond formation.



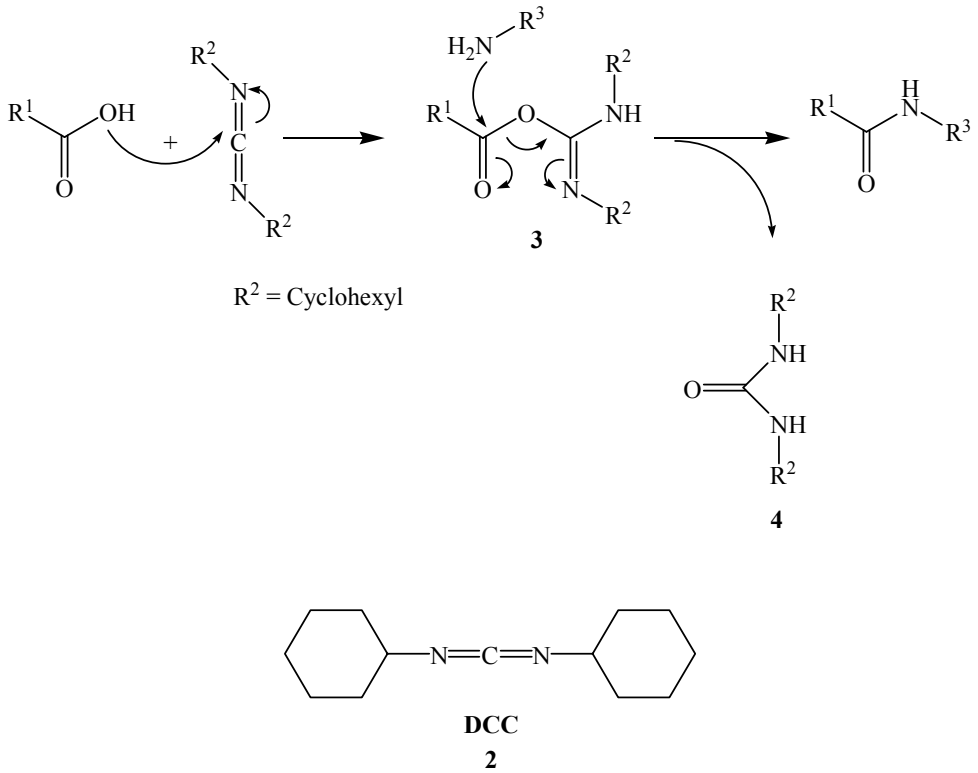
Scheme 1. Formation of a peptide bond

In the recent past, numerous activating reagents have appeared including those based on phosphonium salts, uronium (iminium) salts, preformed anhydrides, active esters and acid chlorides and fluorides. The need for these reagents arises from the synthetic requirements of

complex peptides, peptidomimetics and even protein structures. These new reagents must be effective for amide bond formation under conditions of high steric hindrance, and must lead to optically pure peptide molecules.

1.1.2 Coupling reactions with DCC

The use of carbodiimides such as dicyclohexylcarbodiimide (DCC) (**2**) for the activation of a carboxylic acid together with the addition of an amine is a frequently used procedure for the peptide bond formation in spite of troublesome formation and removal of urea by-products. N,N'-Dicyclohexyl carbodiimide (DCC) is probably the most utilized carbodiimide reagent that is relatively cheap and soluble in the solvents used in peptides synthesis. The reaction mechanism for carbodiimide-mediated peptide couplings is depicted in Scheme 2.[8]



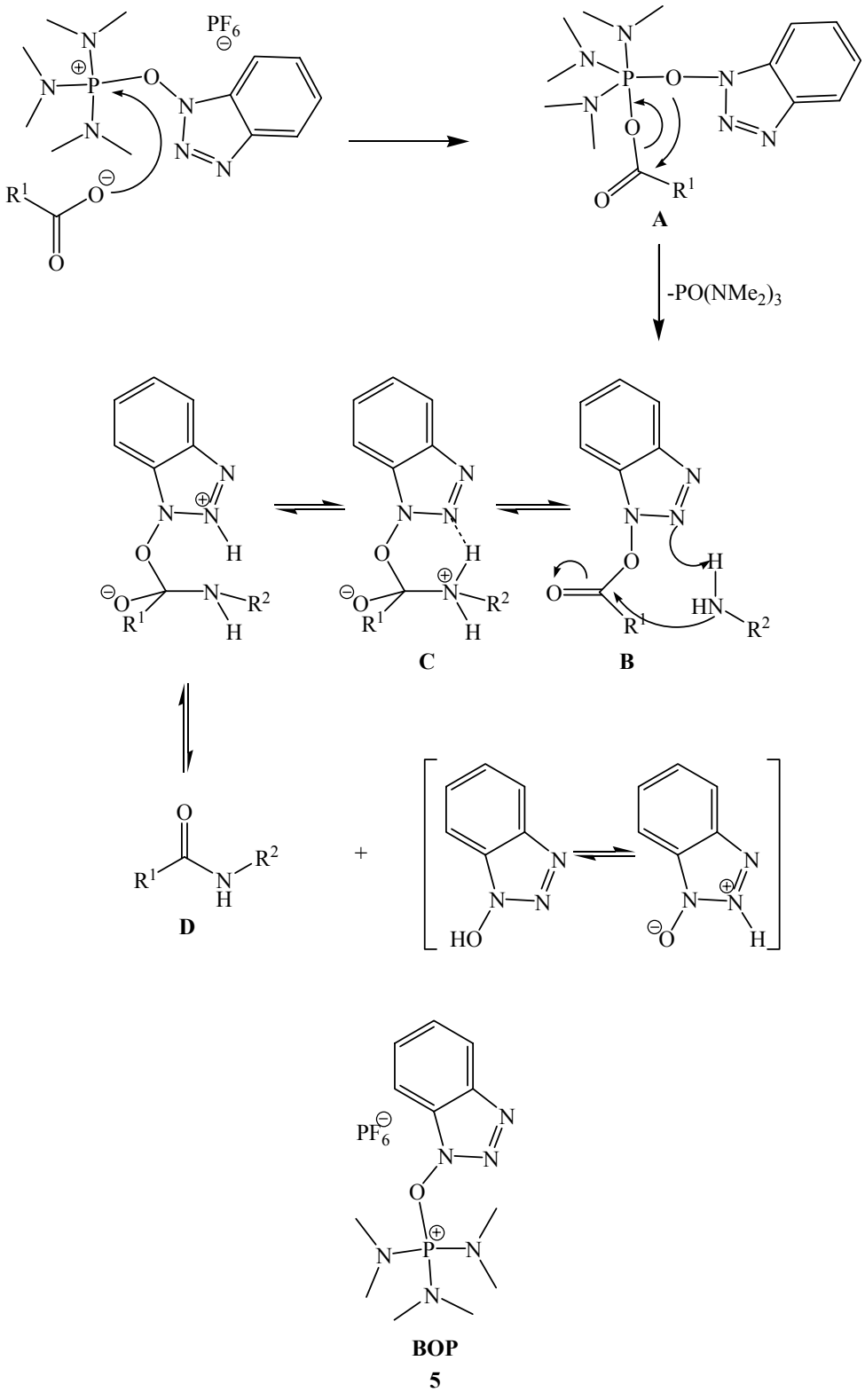
Scheme 2. Reaction mechanism for carbodiimide-mediated peptide couplings

The diimide moiety of DCC, which contains an electrondeficient central carbon atom, is attacked by the carboxylate and the highly reactive O-acylisourea **3** is formed. The addition of an amine results in the formation of a peptide bond. The driving force for the reaction is the

formation of the particularly stable dicyclohexylurea (**4**). The DCC method is a reliable variant for the stepwise assembly of peptides using urethane-protected amino acids (Z, Boc, Fmoc) in both solid-phase and solution-phase peptide synthesis, as well as for segment condensation.

1.1.3 Coupling reactions with BOP (*via* active esters)

Peptide bond formation by ester aminolysis is a reaction analogous to ester saponification reaction (X = O, S, Se). The rate of peptide bond formation correlates with the leaving group capacity of XR^2 (Scheme 1). Some active esters feature an additional proton-accepting group stabilizing a hydrogen-bonded transition state during aminolysis which leads to high activity. One of the coupling reagents allowing *in situ* generation of such active esters is benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (**5**) developed by Castro et al. BOP is a very efficient reagent which allows *in situ* formation of hydroxybenzotriazolyl esters. Its main disadvantage is that the highly toxic and carcinogenic HMPA is formed during the course of the reaction. The mechanism of coupling which involves BOP is presented on the Scheme 3.[8, 9] A tertiary amine is required to generate the carboxylate anion of the amino acid, which then attacks the phosphorus atom of BOP. The resulting intermediate **A** rearranges to form the hydroxybenzotriazolyl ester **B**, which is then attacked by the amine component to form the amide product **D** *via* the stabilized hydrogen-bonded intermediate **C**.

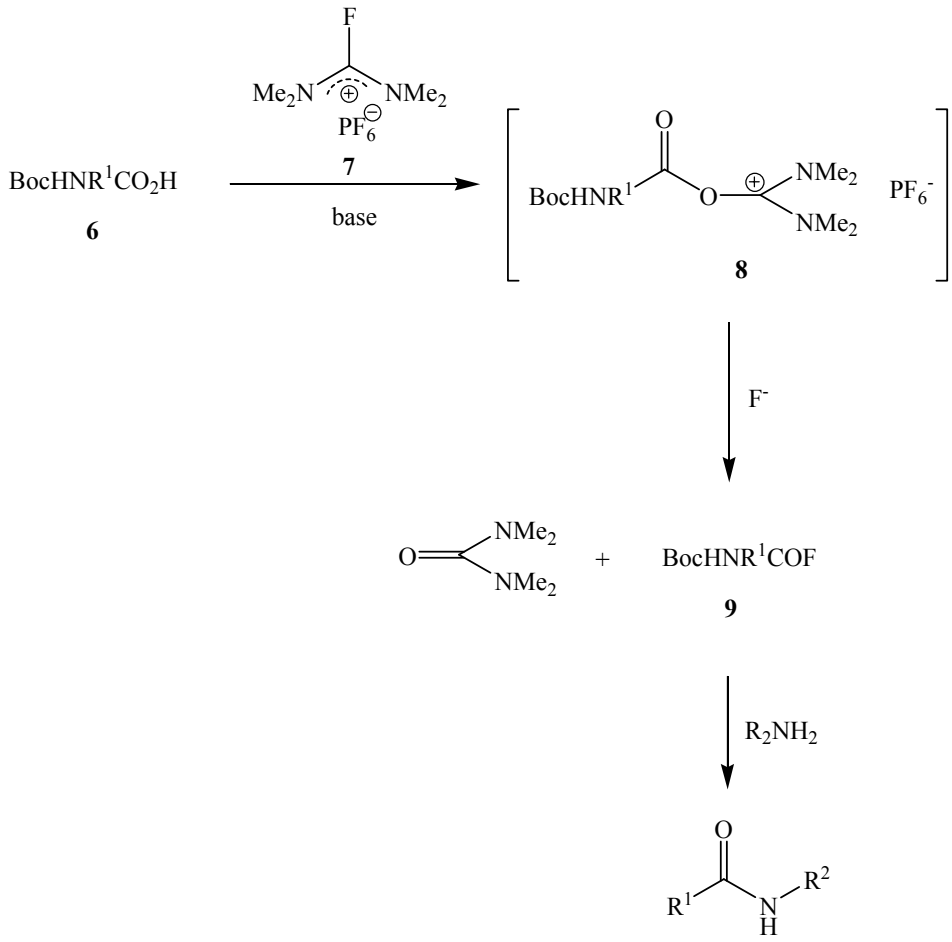


Scheme 3. Reaction mechanism for BOP-mediated peptide couplings

1.1.4 Coupling reactions with TFFH (*via* acid fluorides)

Amino acid fluorides have recently been shown to be rapidly acting species for peptide synthesis in solution or for the solid-phase synthesis of simple peptides. Amino acid chlorides have never been widely used except in special circumstances as they are reputed to be "overactivated" and prone to numerous side reactions, including racemization. Urethane-protected amino acids are not sufficiently stable under acidic conditions necessary to obtain acid chlorides. Most of the protecting groups stable enough to survive conversion to an acid chloride are generally difficult to deblock. The development of the Fmoc amino-protecting group enabled the use of amino acid chlorides in somewhat greater extent.
On the contrary, urethane-protected amino acid fluorides with protection of *tert*-butyl or trityl-type have been successfully synthesized and applied to peptide synthesis. The main reasons for their advantage in the synthesis of systems that incorporate sterically hindered amino acids compared to other coupling methods may be attributed to an efficient stabilization of the tetrahedral transition state by the highly polarized C-F dipole and the relatively small size of the fluoride leaving group.[8]
Tetramethylfluoroformamidinium hexafluorophosphate (TFFH) (**7**) is a non-hygroscopic salt that is suitable for generating the acyl fluoride *in situ* on treatment with ethyldiisopropylamine. It serves as a coupling reagent for solution- or solid-phase-syntheses and is especially suited for the coupling of sterically hindered amino acids, which are not easily handled by standard techniques.[10]

N-Boc protected amino acids **6** are converted *via* TFFH into acid fluorides **9**. The fluorination of amino acids most probably proceeds through the intermediate active ester **8**. If desired, the acid fluorides may be isolated and purified, or used directly in the coupling reaction without isolation (Scheme 4).[10] Typical procedure for couplings with TFFH includes addition of TFFH to a mixture of starting carboxylic acid, amine and DIEA in dichloromethane at 0°C. The reaction mixture is then allowed to warm to room temperature and after completion of reaction acidic workup is performed.



Scheme 4. Conversion of amino acids to acid fluorides with TFFH