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Application of coupling reagents in Amide bond formation

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A B S T R A C T

Amide bond is essential to sustain life, making up the peptide bonds in proteins, such as enzymes, and it is also one of the most prolific moieties in pharmaceutical molecules, agrochemicals and natural products. A key step in peptide production is the formation of the peptide bond, which involves amide bond formation. This requires the activation of a carboxylic acid, which is usually carried out by using peptide coupling reagent. The synthetic strategy adopted was the coupling of appropriately protected amino acids with carboxylic acid derivatives. Structures of newly synthesized compounds were confirmed by ^{1}H NMR, LCMS and IR spectral analysis.

1. Introduction

Peptides and protein play a wide variety of roles in living organisms and display a range of properties, from the potent hormonal activity of some small peptides to the structural support and protection for the organisms shown by insoluble proteins. A key step is the formation of the peptide bond, which involves amide bond formation.[1] Amide bonds are generally synthesized from the reaction of carboxylic acids and amines, but the reaction of these two functional groups does not occur spontaneously at ambient temperature, with the necessary elimination of water molecule only taking place at high temperatures (e.g.>200 ^{0}C),[2] conditions typically detrimental to the integrity of the substrates. For this reason, it is usually necessary to first activate the carboxylic acid, a process that usually takes place by converting the –OH of the acid into a good leaving group prior to treatment with the amine (Scheme 1).

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The process usually requires activation of a carboxylic acid which is usually carried out in the presence of coupling reagents. Activation consists of either the replacement of the hydroxyl group of the carboxylic acid with a leaving group as the acid or form salts with the amine. The reaction of the activated intermediate and the amine is known as the coupling reaction and the activators are coupling reagents.[3] Currently, the most common way to make an amide bond is used for carboxyl-protected amino acids[4] by using coupling reagents.

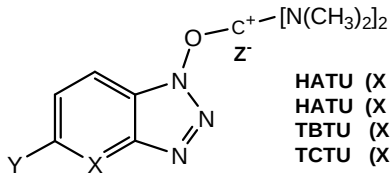
2. Coupling reagents:

There are many coupling reagents known in literature, some of the general ones are as given below:

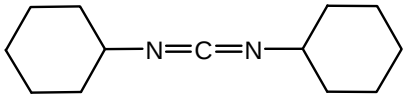
A. Carbodiimide derivatives[5]-

a) N, N'-dicyclohexylcarbodiimide (DCC)

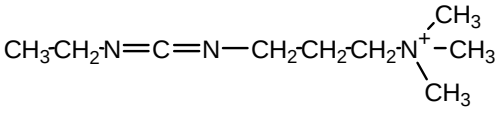
b)1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)



Aminium salts



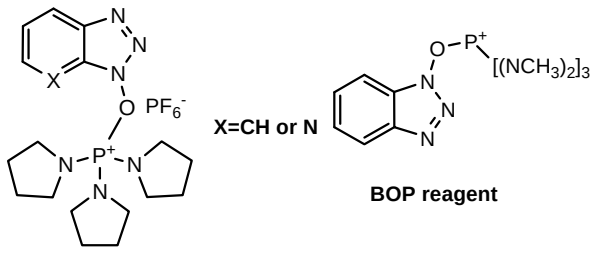
DCC



EDCI.HCl HCl

B. Phosphonium salts of Benzotriazoles derivatives-

Phosphonium salts of Benzotriazole derivatives-7-azabenzotriazol-1-yl-*N*-oxy-tris (pyrrolidino) phosphoniumhexaflurophosohate (PyAOP)



BOP reagent

PyBOP X = CH
PyAOP X = N

Benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)

phosphonium hexaflurophosohate (PyBOP)

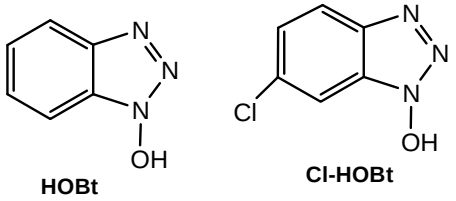
C. Ammonium salts of Benzotriazoles derivatives-

N-[(dimethylamino-1H-1,2,3-triazolo[4,5-b]pyridine-1-ylmethylene]-N-ethylmethanaminium hexafluorophosphate (HATU)

N-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium hexafluoro phosphate N-oxide (HBTU)

N-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium tetrafluoro borate N-oxide (TBTU)

N-[(1H-6-chlorobenzotriazol-1-yl)(dimethylamino) methylene]-N-methylmethanaminium tetrafluoroborate N-oxide (TCTU)

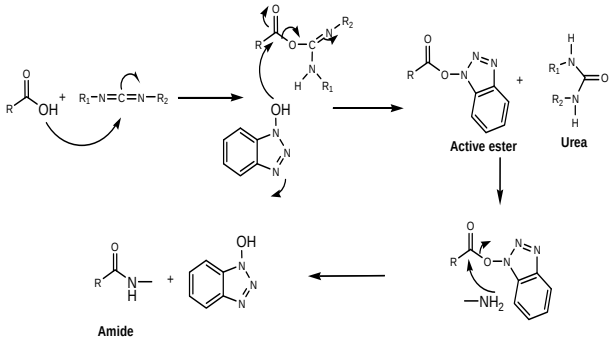


HOBt Cl-HOBt

These reagents can be used with or without the use of additives. Benzotriazole (HOBt) and chloro benzotriazole (Cl-HOBt) are commonly used as additives in coupling reaction.

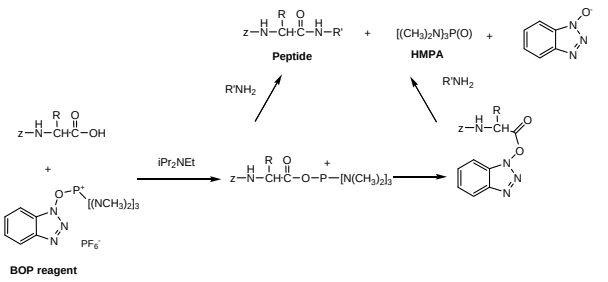
Carbodidiimide reagents along these additives are used very commonly for peptide synthesis.HOBt and Cl-HOBt are added to the carbodiimide[6] based coupling reagents leads to the formation of the benzotriazole active esters that are less reactive than the O-acylisourea, thereby reducing racemization[7]of the protected amino acid and avoiding the formation of other side products. Cl-HOBt performs at least as well as HOBt, but since it is more acidic (pka: 3.35 for Cl-HOBt and 4.60 for HOBt) it is better leaving group and its active esters are more reactive than OBt esters. Chlorine atom in Cl-HOBt stabilized the structure, making Cl-HOBt a less hazardous reagent. HOBt and Cl-HOBt are also added to the aminium salts mediated coupling reactions, with the purpose of favoring the active ester formation. These additives are capable of acting as proton acceptor aiding deprotection of ammonium ion intermediate and thereby greatly increasing reaction rate.

Among carbodiimide reagents EDC is more preferable as by product (urea, **Scheme-1**), which is water-soluble in this case and is a problem with DCC where dicyclohexylurea (DCU) is water insoluble and difficult to remove. For present work, EDC in combination with HOBt was used for amide formation of amino esters.



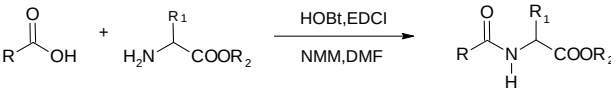
Scheme-1. Mechanism for formation of Amide.

The use of acylphosphinium as reactive intermediates for the formation of amide bond was first postulated by Kenner in 1969 for hindered amine and acids.[8] However, compounds that promote peptide coupling by the formation of acylphosphonium salts did not become widely used until the discovery of benzo triazol-1-yloxy tris(dimethylamino) Phosphonium hexafluorophosphate (BOP). Since then use of phosphonium salts in peptide chemistry has increased dramatically. The BOP reagent has been used in peptide synthesis since its introduction and it is easy to use and rapid coupling of hindered amines and acids. A drawback of its use is that it produces the toxic hexamethyl phosphoromide (HMPA). The proposed mechanism of BOP reagent is given below. The use of most reactive aminium salts (HATU, HBTU, TBTU, TCTU), is inconvenient because of price, [9]which makes it detrimental for industry. The mechanism for aminium salts is same as for phosphonium salts which are relatively economical.



Scheme-2 Mechanism of BOP reagent

For present work EDC in combination with HOBt was used for amide formation of amino esters (**Scheme-3**).



Scheme-3 Formation amide from amino esters

3. Materials and Methods

All reagents were obtained from commercial sources. Solvents were either commercially obtained as analytical grade or freshly distilled prior to use. Analytical thin layer chromatography was carried out on precoated silica gel $60F_{254}$ plates using either UV absorption or iodine staining for visualization. Column chromatography was carried out using 100–200 mesh silica gel. Melting points were determined on a Buchi instrument and are uncorrected. IR spectra were recorded on a Paragon 1000pc FT-IR spectrophotometer. ^{1}H NMR spectra were recorded at 400 MHz with a Bruker DRX spectrometer; chemical shift values are reported relative to tetramethylsilane as internal standard; peak positions are given in parts per million. LCMS spectra were obtained from a PE-SCIEX AP_1 3000 LC/MS/MS system using electron spray ionization at 80–250 ºC using 6 mM ammonium acetate buffer of pH 6.7 in a positive ion mode.

4. Experimental

Preparationof(S)-2-Amino-3-methyl-butyric acid methyl ester(1): L-Valine (5 g, 42.68 mmol) was dissolved in methanol (50 ml). Thionyl chloride (6.55 g, 3.97 ml, 55.48mmol) was added to the reaction mixture at 0^0C with stirring. The reaction was continued for 4h at room temperature. The reaction mixture was evaporated to give crude residue, dried and to obtain the title compound (**17**, 4.8 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.796(bs, 2H), 3.98(bs, 1H), 3.83(s, 3H), 2.496-2.452(m, 1H), 1.144(t, 6H); LCMS (m/e): 131.9 (M+1), 153.2 (M+23).

(S)- 3-Methyl-2- (2-pyridin-4yl-acetylamino)-butyric acid methyl ester(2): L-Valine methyl ester (**17**, 1.5 g, 8.94 mmol) and 4-Pyridyl acetic acid (1.553 g, 8.94 mmol) was dissolved in dry dimethylformamide (20 ml) and 1-Hydroxybenzotriazole (1.32 g, 9.18 mmol) followed by N-methyl morpholine (4.51 g, 44.65 mmol) were added to reaction mixture at 0ºC. Reaction mixture was stirred for 30 mins and EDCI.HCl (1.8 g, 13.12 mmol) was added maintaining same temperature. Reaction mixture was stirred for 8h at room temperature and diluted with water (20 ml), which was then extracted with ethyl acetate (2 x 20 ml). Combined organic extracts were washed with water (2 x 15 ml), aqueous sodium bicarbonate solution (2 x 15 ml) and finally with brine solution (2 x 15 ml). Organic layer was then dried (anhydrous Na_2SO_4) and concentrated under reduced pressure to get crude residue, which was purified by column chromatography using eluent ethyl acetate: n-hexane (40:60) as eluent to obtain the title compound (**1**, 2 g, 95% yield).

FT-IR (DCM) ν_{max}: 3278.8, 2965, 1741.7, 1656.9, 1602.6, 1543.5, 1418.7, 1265.8, 1208, 1156, 804.3 cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ 8.582 (d, J= 5.2 Hz, 2H), 7.276-7.25(m, 2H), 4.578-4.545(m, 1H), 3.736(s, 3H),

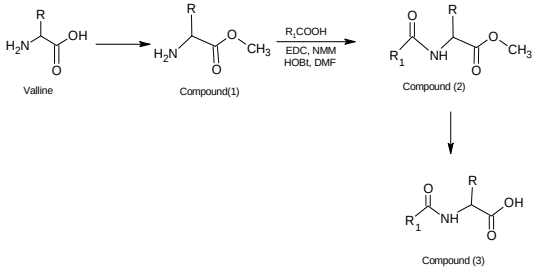
3.605(s, 2H), 2.170-2.089(m, 1H) 0.905-0.836(dd, J = 6.8Hz, 6H); LCMS (m/e): 251.2(M+1), 273.2 (M+23).

***(S)-3-Methyl-2-(2-pyridin-4yl-acetylamino)-butyricacid(3)*:**(S)-3-Methyl-2-(2-pyridin-4ylacetylamino)-butyric acid methyl ester (**21**, 2.3 g, 9.21 mmol) was taken with mixture of solvent system tetrahydrofuran: methanol: water (3:1:1 of total 20 ml). Lithium hydroxide mono hydrate (0.579 g, 13.8 mmol) was added to the reaction mixture with stirring and the reaction was continued for 1h at room temperature The reaction mixture was concentrated to 2\3rd of its volume, diluted with water (20 ml) and extracted with ethyl acetate (3 x 20 ml). Aqueous layer was then acidified with sodium hydrogen sulphate to pH-2 and extracted with ethyl acetate (2 x 20 ml). The combined organic extract was washed with brine solution (2 x 10ml) and dried over anhydrous sodium sulphate. The solvent was evaporated to obtain the title compound (**2**, 2 g, 92%yield).

^{1}H NMR (400MHz, $CDCl_3$): δ 8.467-8.452(m, 2H), 8.317(s, 1H), 7.283(s, 2H), 4.051-3.997(m, 2H), 1.985(d, J=8Hz, 2H), 0.845-0.8(m, 6H); LCMS (m/e): 236.2 (M+1), 259.2(M+23).

5. Results and Discussion

This methodology describes a method for selective carboxyl-protection of any amino acid as its ester, which is to be coupled as well as method for selective deprotection of an ester to its acid derivative. Thus, compound**(2)** was prepared via **Scheme-4**,wherein L-valine was first esterified by treating it with thionyl chloride in methanol to get L-valine methyl ester (**1)** as its hydrochloride salt in 86% yield.



Scheme-4: Formation of amide.

The identity of compound was proved by ^{1}H NMR wherein a singlet at 3.83 for OCH_3 was observed and by LCMS indicating a peak at 131.9 for (M+1). L-Valine methyl ester (**1**) so obtained was then coupled with appropriate acids in presence of EDC and HOBt to get respective amide (**2),** in 95% yield. These esters were then hydrolyzed under basic conditions using lithium hydroxide to get desired acids (3), in 92%yields. All compounds and intermediates were well characterized with ^{1}H NMR, LCMS and IR spectra.

6. Conclusion

The formation of amide bonds plays an important role in organic chemistry and biochemistry. The formation of amide bond together with chirality of the molecules plays a important role in the preparation of a broad range of organic compounds. It begins with the small molecules through the preparation of peptides to fully synthetic proteins. Advances in the area of protein structure and function are based on strategies developed from a simple chemical reaction — amide bond formation — and illustrate the power of chemical science in elucidating protein–ligand interactions. These developments will give a way to find out new therapeutic targets and suggest novel mechanism based therapeutic paradigms.

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