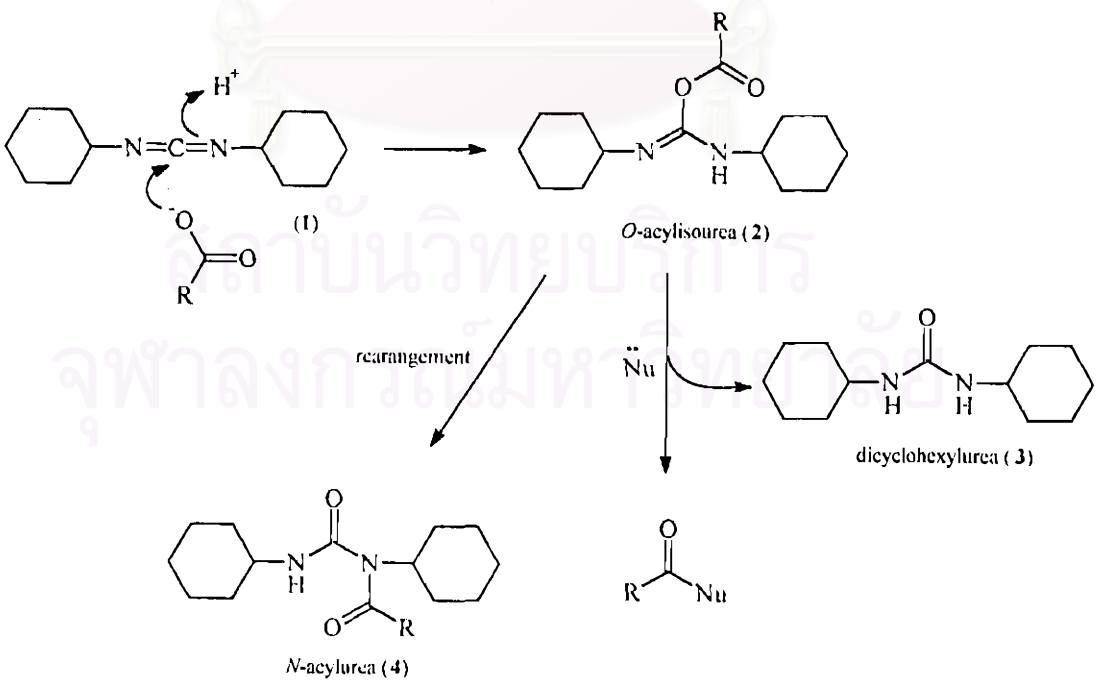
CHAPTER I

INTRODUCTION

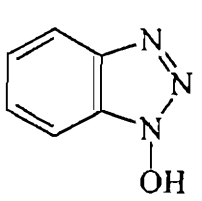
The most important stage in peptide synthesis is the coupling of one amino acid or peptide to another. The most common role of coupling reagents in the synthesis of peptides and esters are related to the activation of carboxylic acids by converting the hydroxy groups to good leaving groups. For example, *N,N*-dicyclohexylcarbodiimide (DCC)[1,2] (**1**) has long been the single most important reagent for activating carboxyl groups in peptide synthesis since Sheehan and Hess[3] reported their result in 1955. DCC may be used to generate activated carboxy derivatives such as symmetrical anhydride and active esters or as a direct coupling reagent. In all cases, the primary activating event is addition of the carboxy group to the carbodiimide functionality to give an *O*-acylisourea (**2**) which is a potent acylating agent as shown Scheme 1.1.[4]



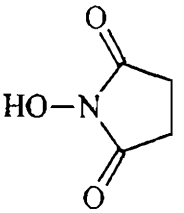
Scheme 1.1: Mechanism of carboxyl group activation by DCC

Direct peptide coupling with DCC is in principle very simple, involving mixing of the amino and carboxy components with DCC in equimolar amounts in an organic solvent, at ambient temperature or a little below.

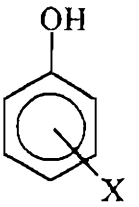
O-acylisourea formation is rapid, leading to peptide by immediate aminolysis with the concomitant formation of dicyclohexylurea (DCU) (**3**). Since DCU is slightly soluble in most solvents, it always contaminate the desired products and, in certain cases, could not be removed completely from the products, especially in small-scale reactions. Furthermore, the *O*-acylisourea intermediate shown in Scheme 1.1 are highly active and side-reaction can intervene, especially if the concentration of the amino component is low. Extensive racemization takes place with susceptible carboxy components *via* the formation of oxazolone intermediate[4] (see Scheme 3.8, p. 68). The collapse of the *O*-acylisourea by intramolecular acyl transfer sometimes competes significantly with the desired attack by external nucleophiles. When this happens, the much less reactive *N*-acylurea (**4**) is formed. *N*-acylurea formation not only reduces the yield, but may give rise to purification problems. Theoretically, this side-reaction can be prevented by adding acidic additives. Numerous possible additives have been investigated : *N*-hydroxysuccinimide[5] was the first, but 1-hydroxybenzotriazole (HOBt)[6,7] has been the most regularly used and electron deficient phenols like nitro- or polyhalogeno-phenols[8] were also used (Figure 1.1). These additives are able to react very rapidly with the *O*-acylisourea initially formed to give acyl derivatives of auxiliary nucleophiles such as *N*-hydroxysuccinimidyl ester or hydroxybenzotriazolyl ester before other side-reactions can take place (Scheme 1.2). However, anions of these auxiliary nucleophiles are also good leaving groups, so the acyl derivatives formed can react with a variety nucleophiles including another amino acid derivative carrying free -NH_2 group to give the coupling product. These additives can therefore reduce racemization, prevent the formation of *N*-acylurea and provide products in excellent yield and high purity.



1-hydroxybenzotriazole (HOBt)

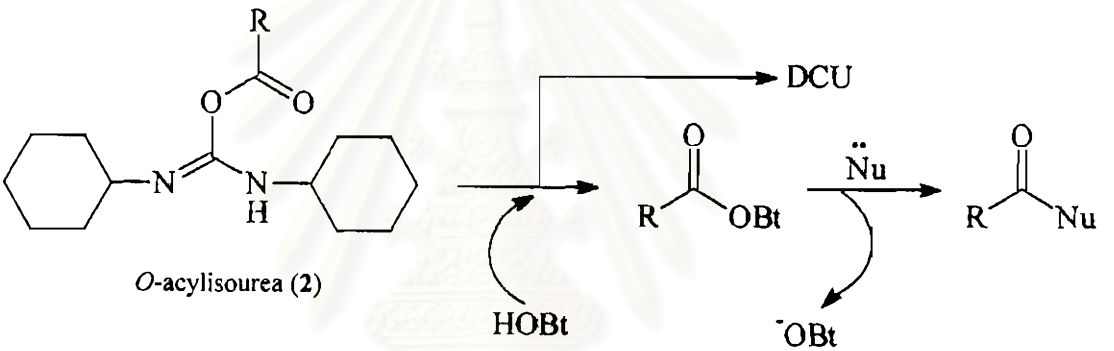


N-hydroxysuccinimide



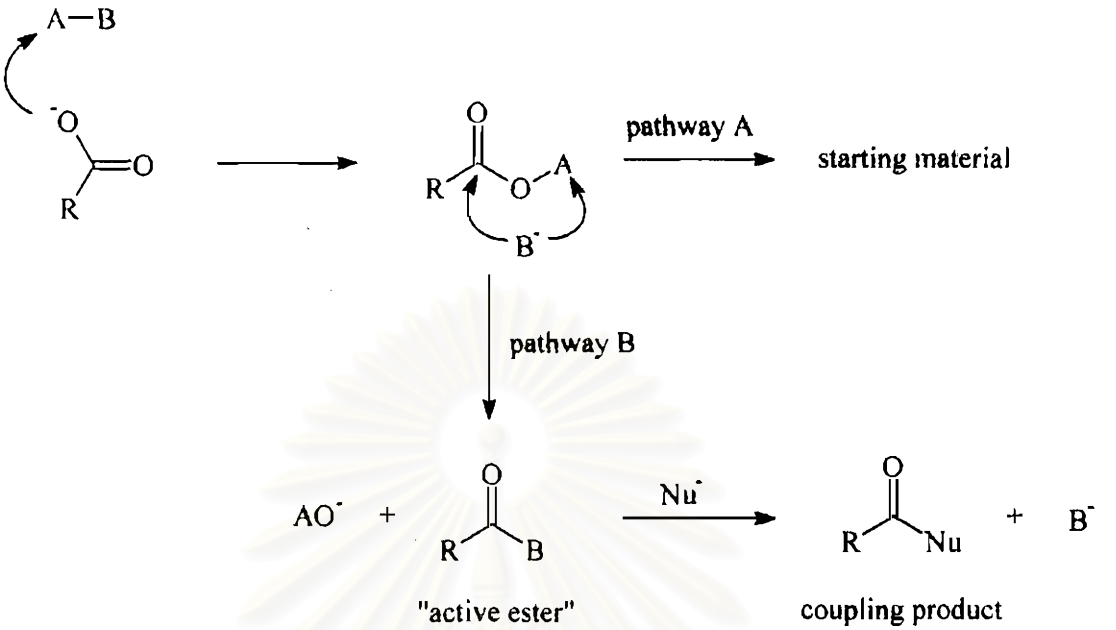
electron deficient phenols

Figure 1.1: Example of acidic additives generally used in peptide synthesis



Scheme 1.2: The role of HOBt in DCC-mediated coupling reaction

From the above-mentioned reasons, the combination of activating and auxiliary nucleophile property in the same molecule has been proposed in coupling reagents with a general formula A-B. A is an electrophilic group which upon attack by carboxylate ion gives an activated intermediate with concomitant release of anion of the auxiliary nucleophile B^-. Second nucleophilic attack of the acyl intermediate with anion of auxiliary nucleophile B generated from the first step affords an active ester which can react rapidly with a variety nucleophiles in system to give derivatives of carboxylic acids with liberation of the auxiliary nucleophile B as by-product as shown in Scheme 1.3.

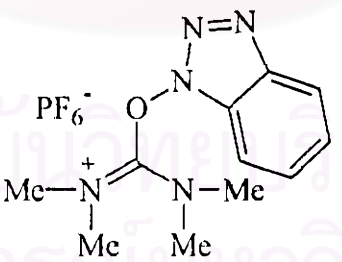


Scheme 1.3: Mechanism of carboxyl group activation by A-B reagent

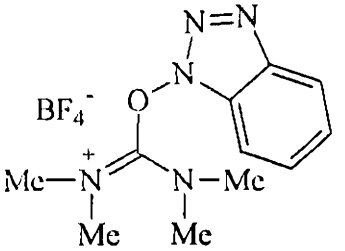
Therefore, reagents with the general formula A-B are potentially useful as coupling reagents for the synthesis of amides, esters and other carboxylic acid derivatives. Examples of these reagents have been reported as follows :

Uronium salts

HBTU[9,10] (*O*-Benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate)

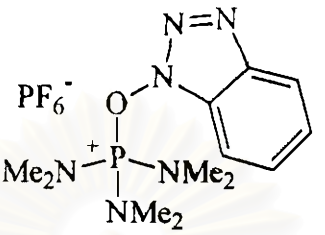


TBTU[10] (*O*-Benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate)

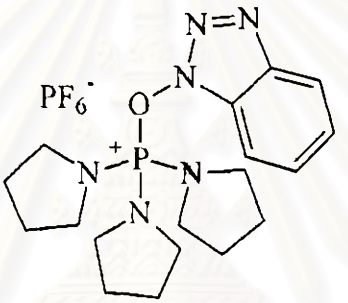


Phosphonium salt

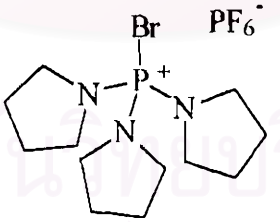
BOP[11,12] [Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate]



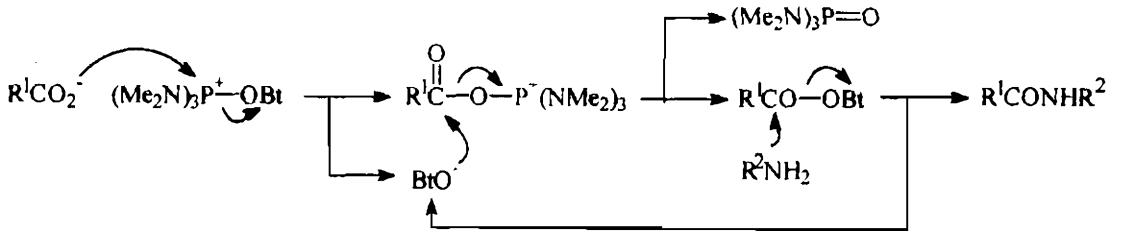
PyBOP[13] (Benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate)



PyBroP[14] (Bromotrispyrrolidinophosphonium hexafluorophosphate)



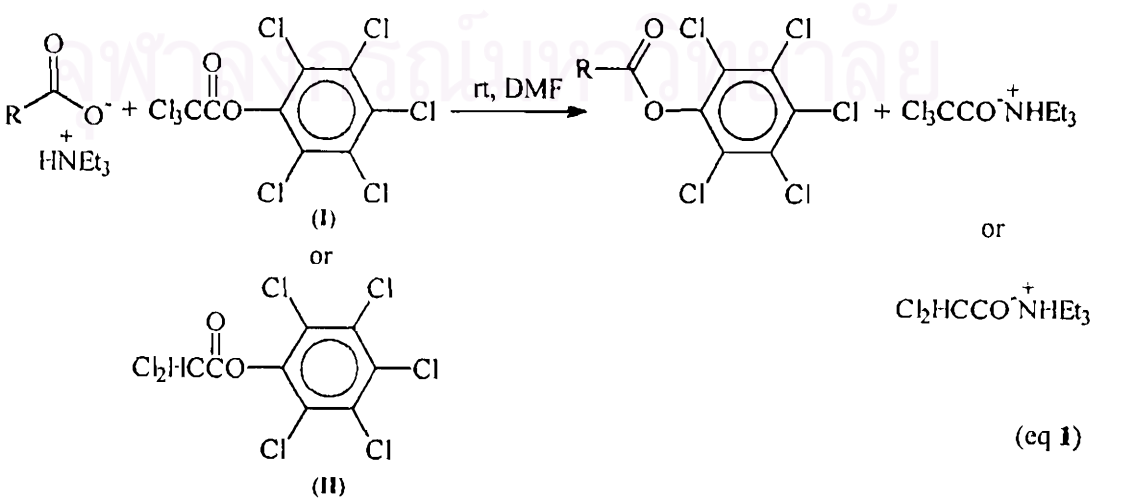
These reagents are becoming more popular over classical DCC method and are now commonly used in peptide synthesis.[4] It is assumed that the first step in carboxylic acid activation involves formation of an (acyloxy)uronium or phosphonium salts. This initially formed salt is attacked by the benzotriazolyloxy anion to form the benzotriazole ester (Bt ester) which is considered to be the same intermediate as in coupling reaction with DCC+HOBt which will be finally aminolysed. Mechanism of carboxyl group activation by BOP[4], for example, was shown in Scheme 1.4.



Scheme 1.4: Mechanism of BOP reagent in peptide coupling

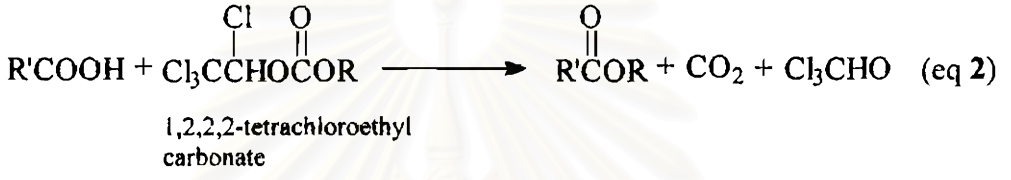
Active esters such as benzotriazole ester are interesting by themselves because they are quite stable activated derivatives which are generally used in peptide synthesis. The standard preparation of these activated esters has typically been achieved with a DCC-mediated coupling of a protected amino acid and the corresponding hydroxy compounds[15-18] but this method has an disadvantage because of the formation of the DCU as by-product which are difficult to remove. In order to avoid the need for DCC, alternative syntheses to DCC-mediated synthesis of active ester of amino acids have been developed.

In 1968 Masahiko Fujino and Chitoshi Hatanaka[19] reported that the pentachlorophenyl trichloroacetate (**I**) and pentachlorophenyl dichloroacetate (**II**), which were prepared from reaction of pentachlorophenol with trichloroacetylchloride and dichloroacetylchloride, were subjected to the ester-exchange reaction with trialkylammonium salts of acylamino acids. The corresponding aryl esters of acyl amino acids were obtained in good yield. As a result they are excellent reagents for preparation of the pentachlorophenyl active ester of acylamino acids (eq 1).



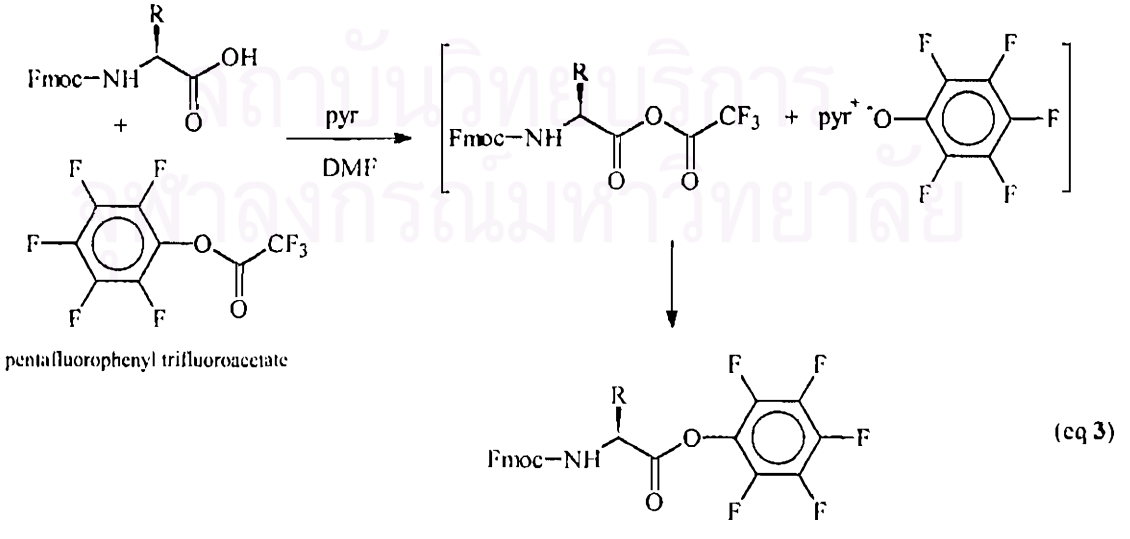
(eq **1**)

In 1987 Jean Martinez and co-workers[20] reported the preparation of active esters of *N*-protected amino acids using 1,2,2,2-tetrachloroethyl carbonates. The mixed aryl tetrachloroethyl carbonates were obtained from reaction of 1,2,2,2-tetrachloroethyl chloroformate with phenols. These carbonates readily reacted with protected amino acid derivatives to yield the corresponding active esters, carbon dioxide (CO_2) and chloral which was transformed to the water-soluble hydrate on hydrolysis (eq 2), therefore reaction work-up procedure were simple.

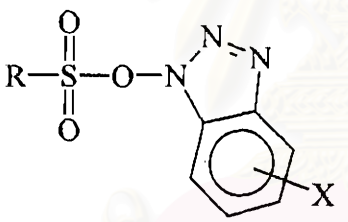


R = *N*-oxysuccimidyl, 2,4,5-trichlorophenyl, pentafluorophenyl

In 1990 Micheal Green and Judd Berman[21] reported that pentafluorophenyl trifluoroacetate, which was prepared easily from pentafluorophenol and trifluoroacetic anhydride, was a good reagent for preparing pentafluorophenyl esters of Fmoc-protected amino acids. The procedure made use of a base-catalyzed transesterification reaction of pentafluorophenyl trifluoroacetate (eq 3).



As for aryl sulfonates, they are stable crystalline compounds and are extensively used in synthetic chemistry involving palladium and nickle-catalyzed reactions.[22-24] Sulfonate esters of strongly acidic *N*-hydroxy compounds such as 1-hydroxybenzotriazole have been reported by Masumi Itoh and co-workers[25] in 1974 to be efficient coupling agents *via* formation of active esters.[25-28] These reagents were prepared by reaction of alkyl- or aryl-sulfonyl chlorides with the corresponding *N*-hydroxy compounds under condition of Schotten-Baumann reaction in aqueous or organic solvent. The mechanism was believed to result from nucleophilic substitution by the carboxylate ion at the sulfur atom to form a mixed carboxylic-sulfonic acid anhydride which was further attacked by the anion of the strongly acidic compound liberated from the previous step. More recent examples of coupling agents for peptide and oligonucleotide synthesis based on benzotriazol-1-yl sulfonates may be found in the literature.[26-29]



R = CH_3, C_4H_9, C_6H_5, *p*-C_6H_4Cl

X = H, Cl

Although these reagents are becoming more popular, many of them still posses undesirable properties such as availability and long-time stability and are somewhat limited by the relatively high cost of starting materials. Thus there are still need to develop new reagents which are superior in all of these aspects.

Goal of the research

The goal of this research is to synthesize the convenient and potential coupling reagents in the synthesis of amides and esters. Several kinds of activating part (A) and auxiliary nucleophiles (B) will be screened to compare their ability as a coupling reagent including the study of suitable reaction condition. In addition, these developed reagents will be evaluated by comparison with standard reagents such as DCC, DCC+HOBt, HBTU.