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REVIEW ARTICLE

Recent development in peptide coupling reagents

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Organophosphorus reagents;
Triazine coupling reagents

Abstract Two decades of domination of benzotriazole-based chemistry stimulated the progress in peptide synthesis to a high level of effectiveness. However, the growing need for new and more complex peptide structures, particularly for biomedical studies and, very recently, for the large-scale production of peptides as drugs, required manufacturing peptide products by efficient synthetic strategies, at reasonably low prices. Therefore, the search for new, more versatile and low-cost reagents becomes a great challenge. Several comprehensive review articles summarized the great effort undertaken, but up to now, no versatile coupling reagent useful for both amide and ester bond formation, as well as for solution and solid-phase peptide synthesis has been yet developed. The most-widely used coupling reagents are carbodiimides on one hand and phosphonium and aminium salts on the other. Herein in this review article, we summarized the recent development in peptide coupling reagents during the last two decades.

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Contents

1. Introduction . . . 98
2. Chemistry . . . 98
2.1. Peptide bond formation . . . 98
2.2. Coupling reagents and activation . . . 100
2.2.1. Carbodiimides (Rich and Singh, 1979) . . . 100
2.2.2. Phosphonium and aminium salts . . . 103
2.2.3. Fluoroformamidinium coupling reagents . . . 106
2.2.4. Organophosphorus reagents . . . 108
2.2.5. Triazine coupling reagents . . . 109
References . . . 113

1. Introduction

The synthesis of proteins has challenged chemists for over a century. Among many methodologies used or currently being used, chemical synthesis offers attractive advantages over the molecular biology and genetic engineering approaches. Chemical synthesis confirms the covalent structure and provides material both for three-dimensional structure determination by NMR or X-ray crystallography and for biological evaluation. The synthesis of analogues allows the relation between molecular structure and pharmacological activity to be determined, and in favorable cases, compounds with activity, selectivity, or biostability superior to the original natural product could be produced on an industrial scale if desired (Lloyd-Williams et al., 1997). Since the difference between peptides and proteins is essentially one of size or of the length of the amino acid backbone, the problems involved in the chemical synthesis of proteins are basically those of the synthesis of peptides.

Since the first simple peptides were synthesized by Theodor Curtius and Emil Fisher a century ago, research in the methodology of peptide synthesis has undergone dramatic development. However, it is only over the last few decades, especially after Bruce Merrifield's invention of solid-phase peptide synthesis (SPPS) (Merrifield, 1963) that reliable methods for the preparation of the peptide have been achieved. Modern synthetic methods, whether manual or machine-assisted, using solid supports or in solution, allow many peptides to be synthesized without undue difficulty. However, long peptides and proteins, or those peptides having a high incidence of the more sensitive amino acids, still present difficulties. Side reactions can always occur even in quite simple sequences, but for larger molecules much more serious complications can manifest themselves. In solid-phase synthesis, incomplete deprotections and coupling reactions tend to become more pronounced as the length of the peptide chain increases, the protocols routinely used on an automatic multiple peptide solid-phase synthesizer take advantage of potent coupling reagents and a large excess of the acylating mixture. On the other hand, it is well-known that over activation may lead to undesired side reactions. Moreover, the high cost of some protected amino acids (particularly the un-natural ones) and coupling reagents suggests maintaining their consumption to a reasonably low level, compatibly with the success of the synthesis. In automatic multiple solid-phase synthesis, used to obtain simultaneously peptides not only of different sequences but also of different length, the coupling reagents should be efficient in terms of yield and preservation of optical purity of the final products. In particular, the coupling reagents should be applied repetitively under standard conditions to a wide range of substrates including less reactive and sterically hindered amino acids. In addition, using an automatic multiple peptide synthesizer, coupling reagents, preferably commercially available and easy-to-use, should have the following characteristics: fast reactions at room temperature, good solubility in the common solvents, and stability of their solutions for several days.

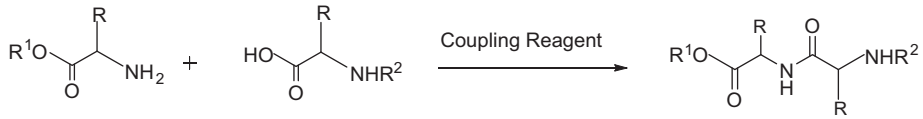
Synthesis in solution is only rarely a practical alternative for large peptides, since it is slow, labor-intensive, and dogged by the problems of racemization and poor solubility of the synthetic intermediates.

2. Chemistry

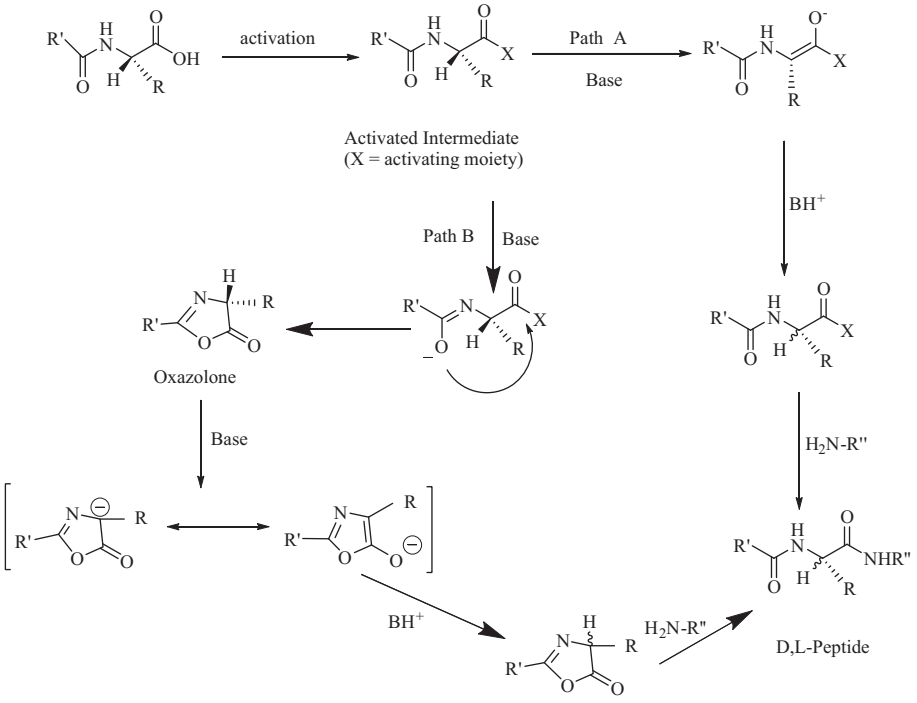
2.1. Peptide bond formation

Success in the chemical synthesis of peptides, as well as peptide libraries, relies on an efficient combination of protecting groups and coupling reagents (Scheme 1).

The formation of a peptide bond between two amino acids involves two steps. The first step is the activation of the



Scheme 1 Peptide bond formation.



Scheme 2 Mechanism of racemization.

carboxyl group of one residue; this step accounts for a key step in the synthesis of a large number of bioorganic molecules, in particular during peptide synthesis (Sheehan and Hess, 1955). If the activation of carboxylic acid is slow, the coupling reagents will be degraded and will no longer be able to activate the carboxyl function. This is crucial for cyclization steps or in convergent strategies during the fragment coupling steps because yields tend to be lower than those of other couplings (El-Faham and Albericio, 2008).

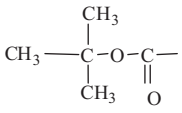
The second step is the nucleophilic attack of the amino group of the other amino acid derivative at the active carboxylic group (Albericio and Carpino, 1997; Albericio et al., 1998, 2001; Albericio and Kates, 2000; Han and Kim, 2004; Benoiton, 2006; El-Faham et al., 2006). This process is an energy-requiring reaction (Bodanszky, 1993), therefore, one of the carboxylic groups must be activated before the reaction can occur. Unfortunately, this activation step, along with the next coupling reaction, brings up a loss of configuration at the carboxyl residue undergoing the activation process.

Peptides are, therefore, assembled by amide bond formation between optically active monomers. This being so, the possibility of loss of chiral integrity must be considered, and understanding the mechanisms of racemization is surely necessary for its prevention. Two major pathways for the loss of configuration, both base-catalyzed, have been recognized: (a) direct enolization, and (b) 5(4*H*)-oxazolone formation (Scheme 2) (Antonovics and Young, 1967; Carpino, 1988; Bodanszky, 1993; Lloyd-Williams et al., 1997).

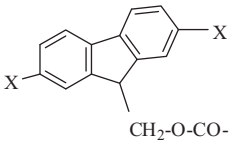
As shown in Scheme 2, the controlled formation of a peptide bond (the so-called ''coupling'' reaction) between two amino acids requires activation of the carboxyl group of one for facile reaction with the amino group of the other. The process of activation is that aspect of peptide synthesis, which has been most extensively developed, in recent years (Albericio and Carpino, 1997; Humphrey and Chamberlin, 1997; Lloyd-Williams et al., 1997; Albericio and Kates, 2000; Albericio et al., 2001; Han and Kim, 2004; Benoiton, 2006).

An essential feature of all coupling methods is that in addition to giving peptide bonds in good yield, the configurational integrity of the carboxylic component must be maintained. This duality, good yield and absence of racemization is often difficult to achieve, since usually the best methods involve conversion of the acid to a derivative bearing a good leaving group. Such leaving groups tend to increase the acidity of the α-proton and favor formation of an oxazolone, as shown in Scheme 2. Loss of configuration is especially prominent if oxazolone formation occurs, but also can occur at stages of the activated carboxyl derivatives.

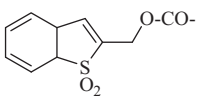
In stepwise solid-phase peptide synthesis (Merrifield, 1985) the problem of racemization is less dramatic than that for other strategies. Several parameters have been used to deal with such side reactions during peptide-coupling reactions. A key issue is N^{α}-protecting group of the amino acid to be coupled (the one with the carboxylic function activated) is normally a urethane function such as, *t*-butoxycarbonyl (Boc, **1**) (McKay and Albertson, 1957), 9-fluorenylmethyloxycarbonyl (Fmoc, **2**) (Carpino, 1987) or the recent type of base-sensitive amino protecting groups 1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxy-carbonyl (Bsmoc, **3**) (Carpino et al., 1997, 1999), 2-(*tert*-butylsulfonyl)-2-propyloxycarbonyl (Bspoc, **4**) (Carpino and Philbin, 1999), 2-methylsulfonyl-3-phenyl-1-prop-2-enyloxycarbonyl (Mspoc, **5**) (Carpino and Mansour, 1999), 2,7-di-*tert*-butyl-9-fluorenylmethyloxycarbonyl (Dtb-Fmoc, **6**) (Stigers et al., 2000), and 2,7-bis(trimethylsilyl)-9-fluorenylmethyloxycarbonyl (Bts-Fmoc, **7**) (Carpino and Wu, 2000).



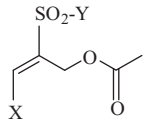
1 (Boc)



2 (Fmoc); X = H
6 (Dtb-Fmoc); X = -CMe_3
7 (Bts-Fmoc); X = -$SiMe_3$



3 (Bsmoc)



4 (Bspoc); X = H, Y = -CMe_3
5 (Mspoc); X = Ph, Y = -CMe_3

The presence of an electron withdrawing group (R′ = O-alkyl, Scheme 2) in these carbamates reduces the tendency to give the oxazolone (Romoff and Goodman, 1997). Furthermore, Fmoc (**2**), Bsmoc (**3**), Bspoc (**4**), and Mspoc (**5**) can be deblocked with a lower concentration of piperidine or weaker bases (e.g. morpholine), thus minimizing base-catalyzed side reactions (Carpino et al., 1997, 1999).

2.2. Coupling reagents and activation

Two decades of domination of benzotriazole-based chemistry stimulated the progress in peptide synthesis to a high level of effectiveness. However, the growing need for new and more complex peptide structures, particularly for biomedical studies and, very recently, for the large-scale production of peptides as drugs, required manufacturing peptide products by efficient synthetic strategies, at reasonably low prices. Therefore, the search for new, more versatile and low-cost reagents becomes a great challenge. Several comprehensive review articles (Albericio et al., 2001; Mizhiritskii and Shpernat, 2002; Najera, 2002; Patchornik, 2002; Basso and Wrubl, 2003; Bray, 2003; Eggen, 2003; Keller and Schauwecker, 2003; Kent, 2003; Marder and Albericio, 2003; Pittman, 2003; Stachelhaus, 2003; Zhang, 2003; Han and Kim, 2004) summarized the great effort undertaken, but up to now, no versatile coupling reagent useful for both amide and ester bond formation, as well as for solution and solid-phase peptide synthesis has been yet developed (Jastrząbek et al., 2007).

An important feature of the solid-phase approaches is the use of large excesses of reagents. The coupling reactions are generally faster in SPPS than in solution, thus minimizing loss of configuration. Although it is widely accepted that for stepwise SPPS the risk of racemization is practically nil, this is not completely true and the possibility of loss of configuration should always be kept in mind, especially for the amino acids which are sensitive, such as cysteine or histidine.

Among the vast number of coupling methods, there are two main classes of reagents. These involve (a) those that require *in situ* activation of the carboxylic acid, and (b) those that depend on the activated species that have previously been prepared, isolated, and characterized (Albericio and Carpino, 1997; Humphrey and Chamberlin, 1997; Kamiński, 2000; Albericio et al., 2001; Han and Kim, 2004; Montalbetti and Falque, 2005).

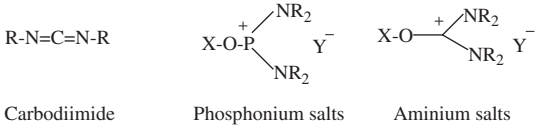


Figure 1 Coupling reagents.

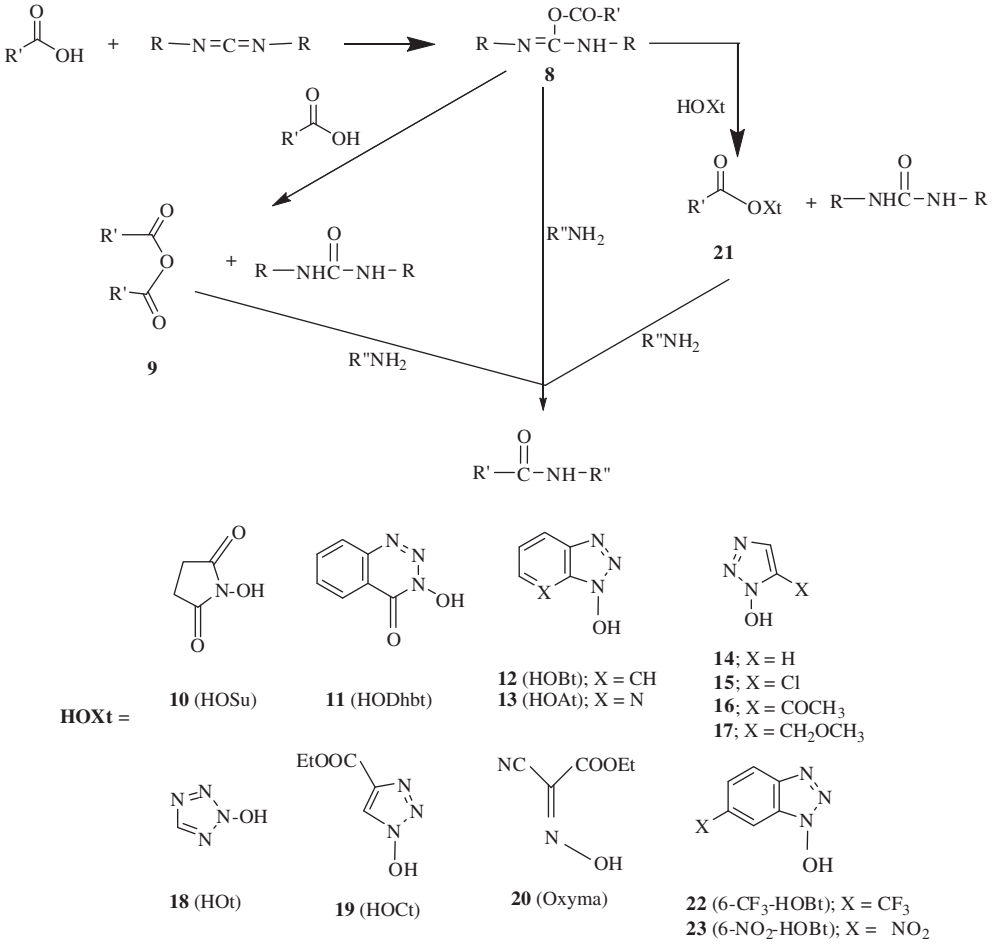
The most-widely used coupling reagents are carbodiimides (Fig. 1) on one hand, and phosphonium and aminium salts on the other (Sheehan and Hess, 1955; Castro et al., 1975; Coste et al., 1990; Li and Xu, 2001; Carpino et al., 2002). Phosphonium and aminium salts, which has a general structure as shown in Fig. 1 (Williams and Ibrahim, 1981).

2.2.1. Carbodiimides (Rich and Singh, 1979)

Reaction of a protected carboxylic acid with a carbodiimide is believed to involve a labile *O*-acylisourea (**8**), which reacts with the amino component to give the corresponding amide (Scheme 3). If 2 equiv. of carboxylic acid are used, the intermediate *O*-acylisourea reacts with the second equivalent of carboxylic acid to give the corresponding symmetric anhydride (**9**). If the activated process is carried out in the presence of a hydroxylamine derivative {HOXt; *N*-hydroxysuccinimide (HOSu, **10**) (Knorr et al., 1989), 1-oxo-2-hydroxydihydrobenzotriazine (HODhbt, **11**) (König and Geiger, 1970a), 1-hydroxybenzotriazole (HOBt, **12**) (König and Geiger, 1970b), 7-aza-1-hydroxybenzotriazole (HOAt, **13**) (Anderson, 1970; Carpino, 1993; Gibson et al., 1994), or 1-hydroxy-1,2,3-triazole derivatives (**14**, **15**, **16**, and **17**), *N*-hydroxytetrazole (HOt, **18**) (Majestich et al., 1998; Spetzler et al., 1998), or ethyl 1-hydroxy-1*H*-1,2,3-triazole-4-carboxylate (HOCt, **19**) (Jiang et al., 1998; Robertson et al., 1999)}, and the most recent additive ethyl-2-cyano-2-(hydroxyimino)acetate (Oxyma, **20**) (Subirós-Funosas et al., 2009), an active ester **21** is obtained, as shown in Scheme 3. Anyone of the three active species, *O*-acylisourea (**8**), symmetric anhydride (**9**), or active ester **21** is an excellent acylating reagent.

The notion of replacement of the benzene ring in typical active esters by the aromatic heterocyclic ring has inspired the designing of new reagents useful for the formation of the peptide bond. Esters of 2-hydroxypyridine (Effenberger and Brodt, 1985), pyrimidine (Kim and Kim, 1985), pyrazoline (Hudson, 1990), 8-hydroxyquinoline (Jakubke et al., 1967), hydroxybenzotriazole, 1,2,3-triazolopyrydin-3-ol (Carpino and El-Faham, 1994), and many others (Janoschek, 1993; Griehl et al., 1998; Bailen et al., 1999; Zacharie et al., 1999) have been accepted standards, are still in use, for the preparation of peptides. Many other heterocyclic compounds are currently being developed (Kamiński, 2000).

The effects of electron-withdrawing may decrease electron density at the carbon atom of the ester group directly attached



Scheme 3 Mechanism of peptide bond formation through carbodiimide.

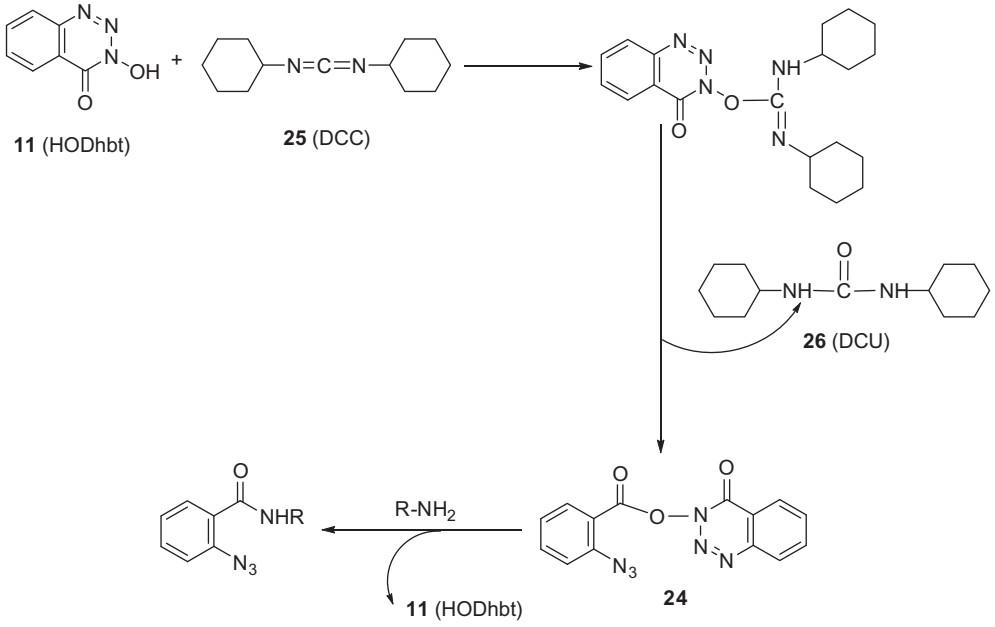
to the ring, thereby increasing its reactivity towards nucleophiles and thus improving active esters was based on increasing the number of electronegative heteroatoms in the ring (Kamiński, 2000). Some oximes reported as alcoholic components of active esters seem to be good nucleophiles, but the reactivity of these esters is somewhat lower than those of other types of active esters. It was expected, therefore, that strongly acidic and nucleophilic oximes, which possess electron-withdrawing groups in the molecule, might be suitable as additives (Bittner et al., 1965; Handford et al., 1965; Losse et al., 1965; Fujino and Nishimura, 1969).

The main advantage of using *N*-hydroxy compounds as additives is to reduce loss of configuration at the carboxylic acid residue (Carpino et al, 1995).

The HODhbt (**11**) reaction is accompanied by formation of a by-product namely 3-(2-azidobenzoyloxy)-4-oxo-3,4-dihydro-1,2,3-benzotriazine (**24**), which itself can react with the amino group to terminate chain growth (Scheme 4) (König and Geiger, 1970a; Albericio et al., 1998). 7-Aza-1-hydroxybenzotriazole (HOAt, **13**) has been described as being superior to HOBt (**12**) and its derivatives (Carpino et al., 1994a,b, 2000; Wijkmans et al., 1995; Kehler et al., 1996; Quibell et al., 1996; Carpino and El-Faham, 1999; El-Faham et al., 2006) 6-CF_3-HOBt (**22**) or 6-NO_2-HOBt (**23**) as an additive for both solution and solid-phase synthesis.

HOAt (**13**) enhances coupling rates and reduces the risk of racemization (Carpino et al., 1994a,b, 2000; Quibell et al., 1996; Carpino and El-Faham, 1999), possibly because it incorporates into the HOBt (**12**) structure a nitrogen atom strategically placed at position-7 of the aromatic system. Incorporation of a nitrogen atom in the benzene ring has two consequences. First, the electron withdrawing influence of a nitrogen atom (regardless of its position) effects stabilization of the leaving group leading to greater reactivity. Second, placement specifically at the 7-position makes feasible a classic neighboring group effect (Fig. 2) (Dourtoglou et al., 1984; Knorr et al., 1989; Kiso et al., 1992; Carpino, 1993; Abdelmoty et al., 1994; Carpino et al., 1994b, 1995, 2001; Bofill and Albericio, 1996; Albericio and Carpino, 1997; Albericio and Kates, 2000; Albericio et al., 2001; Han and Kim, 2004; Benoiton, 2006), which can both speed up the reactivity and reduce the loss of configuration. The corresponding 4-HOAt (**27**), 5-HOAt (**28**), and 6-HOAt (**29**) lacking the ability to take part in such a neighboring group effect have no influence on extent of stereomutation during segment coupling reaction relative to HOBt (**12**).

Subirós-Funosas et al. (2009) recently reported safe and highly efficient additive Oxyma (**20**) to be used mainly in the carbodiimide approach for forming the peptide bond. Oxyma (**20**) displays a remarkable capacity to suppress racemization



Scheme 4 HODhbt (**11**) side reaction.

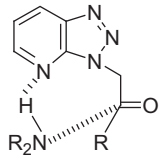
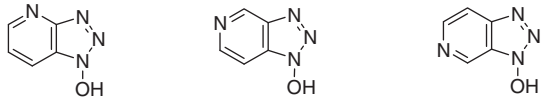
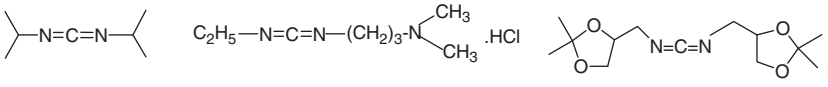


Figure 2 Neighboring group effect for HOAt (**13**).



nated from the reaction vessel due to its solubility during removal of the Boc (**1**) group with trifluoroacetic acid (TFA). The high solubility in *N*,*N*-dimethylformamide (DMF) of the urea derived from *N*,*N*′-diisopropylcarbodiimide (DIC, **30**) makes it the carbodiimide of choice when the Fmoc/*t*-Bu strategy is used. In fact, use of DCC (**25**) is not possible in this case, since (DCU, **26**) would plug the frit of either the reaction vessel or the column in the case of batch or continuous-flow synthesis, respectively. Finally, 1-ethyl-3-(3′-dimethyl-aminopropyl)carbodiimide hydrochloride (EDC.HCl, **31**), the urea by-product, which is soluble in aqueous solvent mixtures, is mainly used for synthesis carried out in solution. The newer carbodiimide 1,3-bis(2,2-dimethyl-1,3-dioxolan-4-ylmethyl)carbodiimide (BDDC, **32**) is also used for solution-phase peptide couplings with a maximum of 1.3% of epimerization (Gibson et al., 1994).



and an impressive coupling efficiency in both automated and manual synthesis.

For carrying out SPPS by Boc/benzyl (Bzl) strategy, among carbodiimides, the *N*,*N*′-dicyclohexyl carbodiimide derivative (DCC, **25**) is the most widely used. In the DCC method using additives, Anderson (1970) has concluded that a major factor for racemization suppression was not neutralization of basicity of DCC (**25**) by an additive, but its nucleophilic reactivity, the by-products *N*,*N*′-dicyclohexyl urea (DCU, **26**) can be elimi-

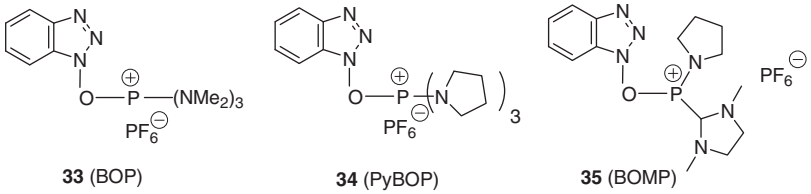
Carbodiimide-mediated coupling reagents are usually carried out with the preactivation of protected amino acid, at either 25 °C or 4 °C. If desired, after filtration of DCU (**26**) and evaporation of dichloromethane (DCM), DMF may be used as the coupling medium (Merrifield et al., 1982). In DMF where the activation process is slow, hindered base such as 2,4,6-trimethylpyridine (collidine; TMP) could be used to enhance the step involving preactivation of the carboxylic acid residue in contrast to the normal situation, in which bases such

as diisopropylethylamine (DIEA), *N*-methylmorpholine (NMM) or non hindered pyridine bases inhibit this step (Carpino and El-Faham, 1999). Comparison of coupling yields obtained in the presence of pyridine, triethylamine (TEA), NMM, and *N*,*N*-dimethylaniline (DMA) in solvents such as, tetrahydrofuran (THF), acetonitrile, DMF, *N*,*N*-dimethylacetamide and *N*-methylpyrrolidone reveal again that the best yield is obtained when the activation proceeds under standard conditions (Kamiński, 1985).

For large-scale synthesis (ca. 10 mmol of peptide), it is mandatory to preactivate at 4 °C, because the exothermic nature of the reaction increases the risk of racemization, even when urethane-type protecting groups are used.

2.2.2. *Phosphonium and aminium salts*

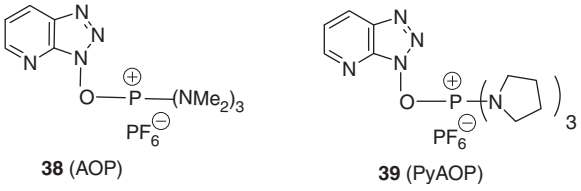
2.2.2.1. Phosphonium salts. Gawne et al. (1969) were the first to describe the use of phosphonium salts as coupling reagents, these species have only been widely adopted after the extensive studies of Castro and Dormoy (1973), Castro et al. (1975,1977) and Coste et al. (1990), who described the applicability of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, **33**) and (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP, **34**).

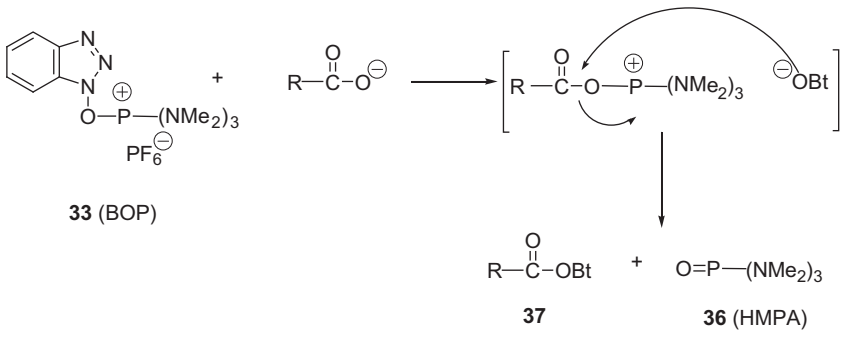


The BOP reagent (**33**) has been used in many syntheses since it is easy to use and promotes rapid coupling. However, it produces hexamethylphosphorotriamide (HMPA, **36**) as a side by-product, which has been classified as a potential human carcinogenic (Dykstra, 1995). PyBOP (**34**) is a good substitute that presents all of the advantages of BOP reagent (**33**) and yields less noxious by-products. Later, 2-(benzotriazol-1-yloxy)-1,3-dimethyl-2-pyrrolidin-1-yl-1,3-diazaphospholidinium hexafluorophosphate (BOMP, **35**) was used as an effective condensing reagent for rapid internucleotidic bond formation (Wada et al., 1997). It may also represent a new, useful reagent for peptide coupling. The main mechanistic pathway involved in coupling reactions mediated via BOP (**33**) and related reagents, is probably that as shown in Scheme 5.

Formation of OBt ester (**37**) is achieved in the presence of 1 equiv. of a tertiary base, such as diisopropylethylamine (DIEA), *N*-methylmorpholine (NMM) (Kim and Patel, 1994; Campagne et al., 1995; Coste and Campagne, 1995), or collidine (TMP) (Carpino and El-Faham, 1994; Carpino et al., 1996a). The presence of an extra equivalent of HOBt (**12**) can accelerate the coupling process as well as reduce the loss of configuration (Hudson, 1988). Some controversy has arisen regarding the possible intermediacy of an acyloxyphosphonium salt and its life time. Kim and Patel (1994) reported that such intermediates could exist at −20 °C in the absence of excess of HOBt (**12**). On the other hand, Coste and co-workers (Campagne et al., 1995; Coste and Campagne, 1995) suggested that this species is very unstable and even at low temperature undergoes conversion to the active ester.

Phosphonium salts derived from HOAt (**13**), such as (7-azabenzotriazol-1-yloxy)tris(di-methylamino)phosphonium hexafluorophosphate (AOP, **38**) and (7-azabenzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP, **39**) have also been prepared and are generally more efficient than BOP (**33**) and PyBOP (**34**) as coupling reagents (Carpino et al., 1994b; Ehrlich et al., 1996; Kates et al., 1996; Albericio et al., 1997; Han et al., 1997; Jou et al., 1997). The pyrrolidino derivative PyAOP (**39**) is slightly more reactive than the dimethylamino derivatives AOP (**38**), and in the activation step does not liberate HMPA (**36**).

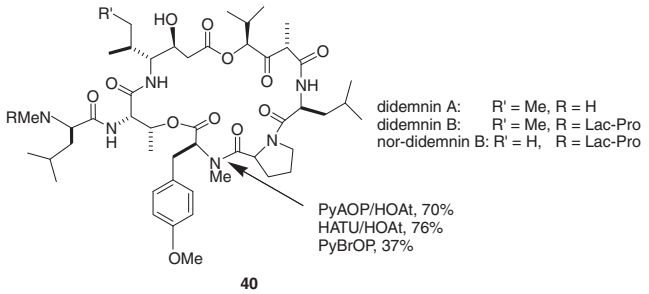




Scheme 5 Mechanism of BOP-mediated coupling reagent.

Intramolecular peptide forming reaction is the key step in the synthesis of constrained head-to-tail cyclopeptides due to the high tendency of the corresponding linear peptides to oligomerize. Classical approaches to the synthesis of a cyclic peptide generally involve preparation of the partially protected linear precursor (by solution or solid-phase approaches), followed by cyclization in solution under high dilution conditions. However, solution-phase methodologies, even in high dilution conditions, suffer from several drawbacks, such as cyclodimerization and cyclooligomerization side reactions. If the peptide remains anchored on a solid support, the cyclization takes advantage of the pseudodilution phenomenon, which favors intramolecular resin-bound reactions and thus minimizing interchain interactions (Rovero, 2000). It is already well-known that the cyclization with aminium (uronium) salts should be generally performed without excess of coupling reagent (1 equiv. of coupling reagent in the presence of 2 equiv. of tertiary base), to avoid the formation of guanidinium side products (Fernso et al., 2000). The same conditions (stoichiometric amounts of reagent) were applied, to compare the efficacy of all reagents under the same conditions. In all experiments, the cyclization yield (%) was determined as a ratio between the concentration of the cyclopeptide and the corresponding linear peptide isolated after the cleavage from the resin (Kamiński et al., 2005).

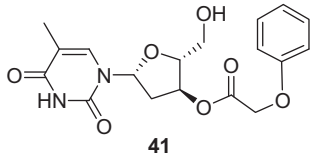
It has been found that PyAOP (**39**) is very effective in the solution phase cyclization of all L-pentapeptide, a very difficult case due to the restricted conformational flexibility of the linear precursor (Jou et al., 1997). For example, for the cyclization of H-Arg(NO_2)-Lys(*Z*)-Asp(OBn)-Val-Tyr-OH, PyAOP (**39**) gave 56% cyclomonomer with only 10.9% of the D-Tyr-isomer after 1 h, whereas PyBOP (**34**) led to 52% cyclomonomer with 13% D-isomer and BOP (**33**) to only 38% of the desired product with 20.2% C-terminal epimerization. In the course of the cyclization of H-Arg(H^+)-Lys(Ac)-Ala-Val-Tyr-OH, only 8.7% C-terminal epimerization was detected for PyAOP (**39**) whereas for PyBOP (**34**) and BOP (**33**), epimerization level reached 16.8% and 20.7%, respectively. By use of the same solution phase cyclization technique, the potent immunosuppressive and anticancer didemnins (**40**) were synthesized in excellent yields (70%) using PyAOP (**39**) in the presence of HOAt (**13**) (Albericio et al., 1997).



40

Further research has revealed that PyAOP/HOAt is also an effective reagent for cyclization of the highly hindered *N*-methylamino acids (Kates et al., 1996; Han et al., 1997).

In the course of the chemical synthesis of oligodeoxyribonucleotides, Wada et al. (1997) found that PyAOP (**39**) was a highly reactive internucleotidic bond coupling. For condensation of an H-phosphonate monomer with 2 equiv. of 3′-*O*-(phenoxyacetyl)thymidine (**41**) in CD_3CN-pyridine, the half-life time for the reaction with PyAOP (**39**) is only 4 min, in comparison with the corresponding figures for PyBOP (**34**) (12 min), BOP (**33**) (125 min) and PyDOP (**42**) (18 h).

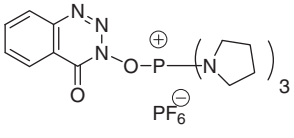


41

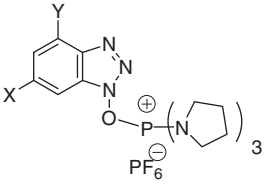
Several other HOBt (**12**) derivatives (Wijkmans et al., 1995; Kehler et al., 1996) bearing an electron-withdrawing group at the 6-position of the HOBt ring, such as 6-nitro-1-hydroxybenzotriazole (**43**), 6-trifluoromethyl-1-hydroxybenzotriazole (**44**), and 4-nitro-6-trifluoromethyl-1-hydroxybenzotriazole (**45**) were prepared and used in peptide coupling reactions.

While [(6-nitrobenzotriazol-1-yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate (PyNOP, **43**) (Høeg-Jensen et al., 1994, 1996; Wijkmans et al., 1995; Kehler et al., 1996), [[6-(trifluoromethyl)benzotriazol-1-yl]oxy]tris(pyrroli-dino)phosphonium hexafluorophosphate (PyFOP, **44**) (Høeg-Jensen et al., 1994, 1996; Kim and Patel, 1994; Campagne et al., 1995; Coste and Campagne, 1995), [[4-nitro-6-(trifluoromethyl)benzotriazol-1-yl]oxy]tris(pyrrolidino)phosphonium hexafluorophosphate (PyNFOP, **45**), and [(6-nitrobenzo-triazol-1-yl)oxy]tris(dimethyl-amino)phosphonium hexafluorophosphate (NOP, **46**) (Høeg-Jensen et al., 1994), were only used in peptide thioacylation and rapid internucleotidic bond formation, they should be applicable to peptide synthesis.

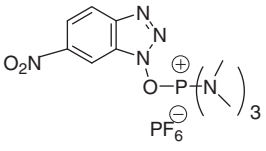
Meanwhile, as alternative to the BOP reagent (**33**), 1-β-naphthalenesulfonyloxy benzotriazole (NSBt, **47**) (Nakajima et al., 1988; Kundu et al., 1989, 1994) and 1-β-naphthalenesulfonyloxy-6-nitrobenzotriazole (*N*-NSBt, **48**) (Devadas et al., 1993; Kundu and Agarwal, 1996) have also been demonstrated to be efficient peptide coupling reagents in solid and solution phase synthesis.



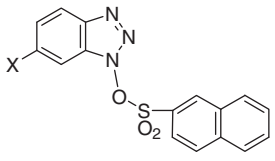
42 (PyDOP)



43 (PyNOP); X = NO_2, Y = H
44 (PyFOP); X = CF_3, Y = H
45 (PyNFOP); X = CF_3, Y = NO_2

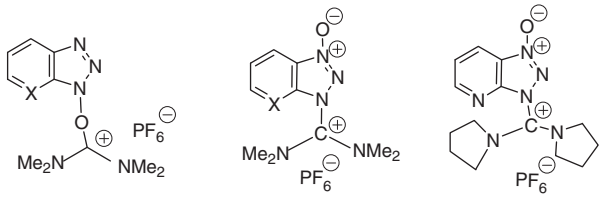


46 (NOP)



47 (NSBt); X = H
48 (*N*-NSBt); X = NO_2

2.2.2.2. Uronium/aminium salts. Aminium salt bearing a positive carbon atom in place of the phosphonium residue have also been reported (Dourtoglou et al., 1984; Knorr et al., 1989; Kiso et al., 1992; Carpino, 1993; Carpino and El-Faham, 1994, 1999; Carpino et al., 1994a,b, 1996a, 2000; Quibell et al., 1996; Carpino and El-Faham, 1999) and aminium salt initially assigned an uronium-type structure, HBTU (**49**) and HATU (**50**) presumably by analogy with the corresponding phosphonium salts. Later (Abdelmoty et al., 1994; Bofill and Albericio, 1996; Carpino et al., 2001), it has been determined by X-ray analysis that *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (*N*-HBTU, **51**), *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (*N*-HATU, **52**), and 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene)pyrrolidinmium hexafluorophosphate *N*-oxide (HAPyU, **53**) (Carpino and El-Faham, 1994; Carpino et al., 1996a; Fernso et al., 2000; Li and Xu, 2000) crystallize as aminium salts (guanidinium *N*-oxides) rather than the corresponding uronium salts (**49** and **50**). NMR studies in the case of HAPyU (**53**) show that the same structure is found in solution (Carpino et al., 2001).

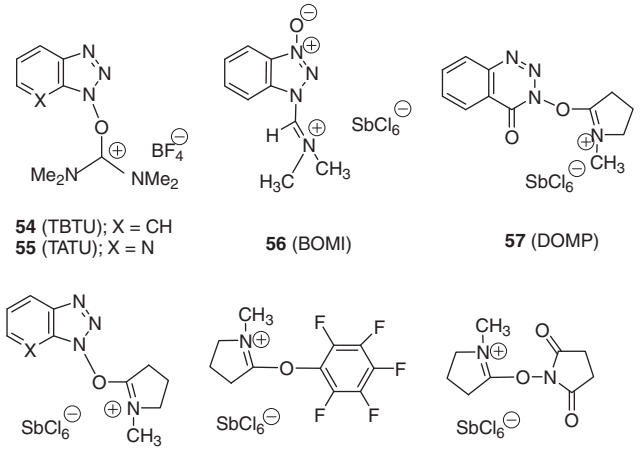


49 (HBTU); X = CH
50 (HATU); X = N

51 (*N*-HBTU); X = CH
52 (*N*-HATU); X = N

53 (HAPyU)

TBTU (**54**) and TATU (**55**), the tetrafluoroborates salts related to **49** and **50**, have also been prepared and used in SPPS. A series of novel aminium type coupling reagents such as {(BOMI, **56**), (DOMP, **57**), (BDMP, **58**), (AOMP, **59**), (FOMP, **60**) and (SOMP, **61**)}, were designed, synthesized and used in peptide synthesis (Devadas et al., 1993; Kundu and Agarwal, 1996; Rovero, 2000). It was shown that most of these reagents were more efficient in the form of HOAt-based coupling reagents than their HOBt analogous in terms of reactivity and racemization control in both solution and solid phase peptide synthesis (Fernso et al., 2000; Li and Xu, 2000).



54 (TBTU); X = CH
55 (TATU); X = N

56 (BOMI)

57 (DOMP)

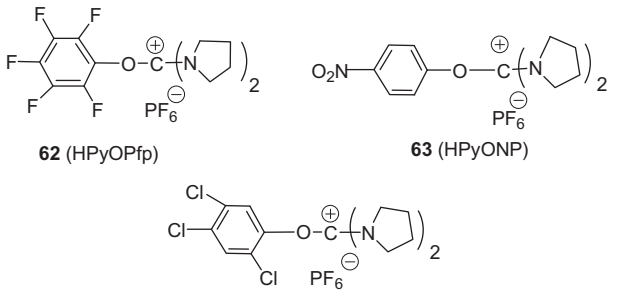
58 (BDMP); X = CH
59 (AOMP); X = N

60 (FOMP)

61 (SOMP)

HOAt-based uronium reagents are also very favorable for the coupling of hindered amino acids shown by excellent synthesis of peptide containing consecutive *N*-methylamino acids or 2-aminoisobutyric acid (Aib) or diethyl glycine (Deg) units (Frérot et al., 1992; Schnoelzer et al., 1992; Sapia et al., 1995; Humphrey and Chamberlin, 1997; Marsh et al., 1997).

Novel series of coupling reagents contain alternative leaving groups, e.g. pentafluorophenyl (HPyOPfp, **62**), P-nitrophenyl (HPyONp, **63**) and 2,4,5-trichlorophenyl (HPyOTcp, **64**) were synthesized and used in coupling reactions (Habermann and Kunz, 1998a; Klose et al., 1999; El-Faham, 2000).



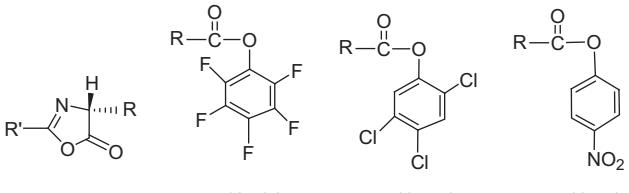
62 (HPyOPfp)

63 (HPyONP)

64 (HPyOTcp)

Comparison of the effectiveness of these reagents by cyclization of the sequence Ala-Ala-MeAla-Ala-Ala, (3 + 3) segment condensation, *Z*-Gly-Gly-Val-OH + Ala-Gly-Gly-PAL-PS (Klose et al., 1999) and (2 + 1) segment condensation, *Z*-Phe-Val-OH + Pro-PAL-PS (Klose et al., 1999; El-Faham, 2000), following the standard protocol (Ehrlich et al., 1993; Carpino et al., 1995), shows a clear superiority for the HOAt-derived coupling reagents HAPyU (**53**) and HATU (**50**) (in the presence or absence of HOAt (**13**)) relative to those derived phenol-based coupling reagents both in terms of reaction rates and epimerization.

The effectiveness of various coupling reagents (e.g. **39**, **50**, **53**, **62**) appears to be strongly influenced by the nature of the 'active ester' and less by that of the uronium/guanidinium/phosphonium component. Moreover, the results indicate different mechanism for HOAt (**13**) and phenol-derived coupling reagents and are supported by IR experiment (El-Faham, 2000), it has been concluded that reactions of the HOAt-derived coupling reagents involve a highly reactive intermediate, probably the OAt ester, whereas phenol-derived coupling reagents are promoted reactions that involve the intermediacy of oxazolone (**65**) and relatively less reactive OPfp (**66**), OTcp (**67**), ONp (**68**) esters (Fig. 3).

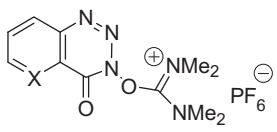


65 **66** (OPfp) **67** (OTcp) **68** (ONp)

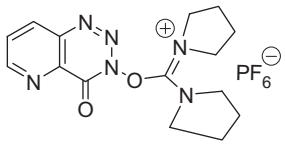
Figure 3 Oxazolone (**65**) and esters (**66**–**68**).

From HPLC analysis remarkably high epimerization levels are observed for the phenol-based coupling reagents due to the formation of oxazolone (El-Faham, 2000). It has been shown previously that for other coupling reagents known to generate oxazolones, the addition of HOAt (**13**) effects a shift to the safer, more reactive OAt ester (Akaji et al., 1994, 1996; Carpino et al., 1996a; El-Faham, 1998a). The addition of HOAt (**13**) strikingly improved the results for the HOPfp-derived coupling reagents compared to the analogous reactions carried out in absence of HOAt (**13**) (Klose et al., 1999; El-Faham, 2000).

Recently, Carpino et al. (2004a) reported the new coupling reagents (HDATU, **69**) and (HDAPyU, **70**). These were prepared by a method analogous to that used for the preparation of (HDTU, **71**) (Carpino et al., 1995).



69 (HDATU); X = N
71 (HDTU); X = CH



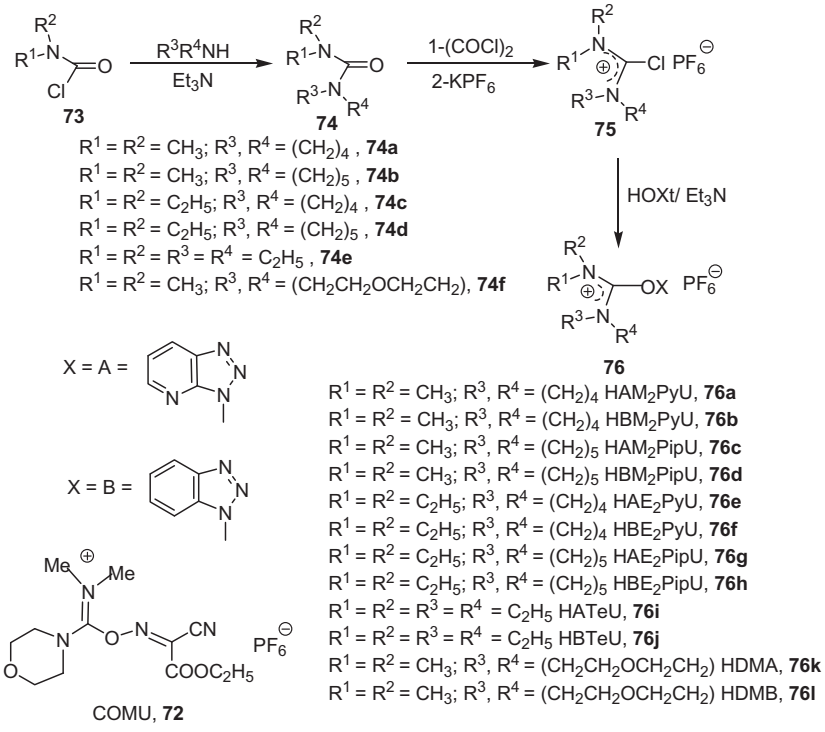
70 (HDAPyU)

El-Faham and Albericio described a new family of immonium-type coupling reagents based on the differences in the carbocation skeletons of coupling reagents which correlated with differences in stability and reactivity (El-Faham et al., 2006, 2009; El-Faham and Albericio, 2007, 2008). The dihydroimidazole derivatives are highly hygroscopic, while the salts derived from dimethyl morpholino are the most stable, and the pyrrolidino derivatives are of intermediate stability. Regarding both coupling yield and retention of configuration, derivatives of Oxyma (COMU, **72**) have been confirmed to show superior performance to those of HOBt (**12**) in all cases and the same performance as HOAt (**13**) or sometimes better. The recent uronium-type reagents can be readily prepared by treating *N*,*N*-dialkylcarbamoyl chloride **73** with secondary amines, such as diethylamine, pyrrolidine, piperidine or morpholine to give the corresponding urea derivatives **74** (Scheme 6). The urea derivatives then react with oxalyl chloride to yield the corresponding chlorosalts **75** (Scheme 6), which is stabilized by the formation of a PF_6 salt. Subsequent reaction with HOXt (X = A or B) in the presence of a tertiary amine such as Et_3N affords the desired compound **76** as crystalline and shelf-stable solids (Scheme 6).

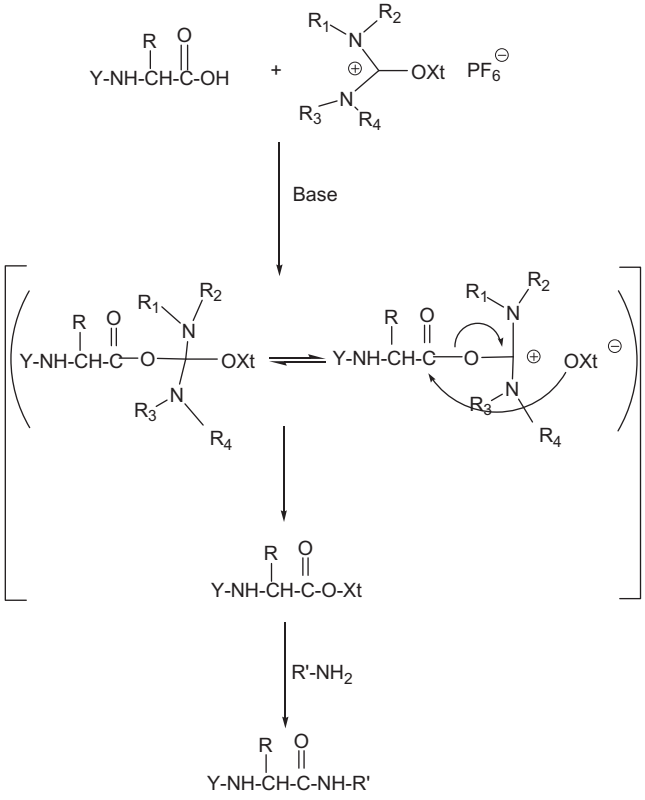
Mechanistically, aminium/uronium salts are thought to function in a similar manner to the phosphonium analogues (Scheme 7). Formation of carboxyl uronium salts, which generate an active ester is achieved in the presence of 1 equiv. of tertiary base such as, DIEA, NMM, or TMP (Carpino and El-Faham, 1994; Carpino et al., 1996a).

2.2.3. *Fluoroformamidinium coupling reagents*

Among the most reactive of the common coupling reagents are the preformed amino acid fluorides. A more convenient method of making use of these efficient reagents is to generate the acid fluorides *in situ* via the aminium reagents 1,1,3,3-tetramethylfluoroformamidinium hexafluorophosphate (TFFH, **77**) (Carpino and El-Faham, 1995; Carpino et al., 1996b), *bis*(tetramethylene)fluoroformamidinium hexafluorophosphate (BTFFH, **78**) (El-Faham, 1998b) and 1,3-dimethyl-2-fluoro-4,5-dihydro-1*H*-imidazolium hexafluorophosphate (DFIH, **79**) (El-Faham, 1998a) with these reagents even His and Arg, which are the only two proteinogenic amino acids that can not be converted to shelf-stable Fmoc-protected amino acid

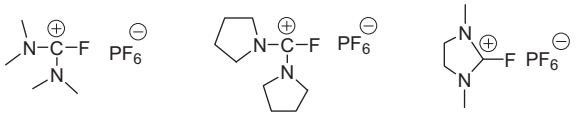


Scheme 6 Synthesis of non-symmetric uronium-type coupling reagents.



Scheme 7 Proposed mechanism for activation by uronium salt.

fluorides, can be routinely coupled in this form. In some cases for these two amino acids as well as for Asn, better results are obtained if 1 equiv. of HOAt (**13**) is present during the coupling process (Akaji et al., 1994; Carpino and El-Faham, 1995; Carpino et al., 1996b; Quibell et al., 1996; El-Faham and Albericio, 2008).



77 (TFFH) **78** (BTFFH) **79** (DFIH)

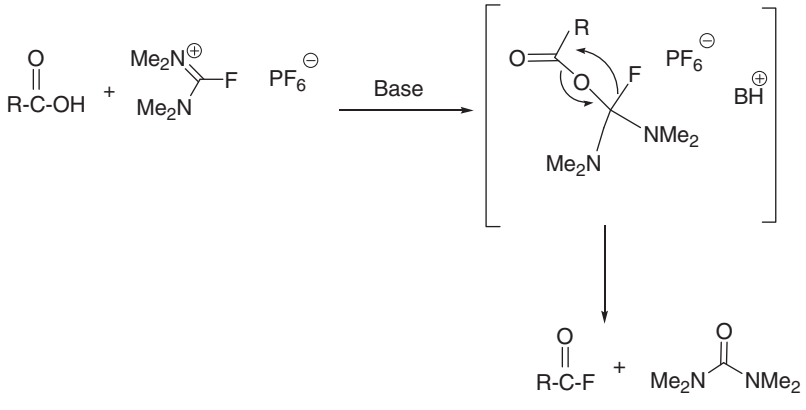
Infrared examination shows that in the presence of DIEA, Fmoc-Amino acids are converted to the acid fluorides by mean of (TFFH, **77**) or its analogous (Carpino and El-Faham, 1995; Carpino et al., 1996b; El-Faham, 1998a,b).

IR absorption (CH_2Cl_2 solution) characteristic of the carbonyl fluoride moiety (1842 cm^{-1}) appears after 3 min, with complete conversion to the acid fluoride occuring after 8–15 min. If desired, the acid fluorides can be isolated and purified (Carpino and El-Faham, 1995; Carpino et al., 1996b; El-Faham, 1998a,b) (Scheme 8).

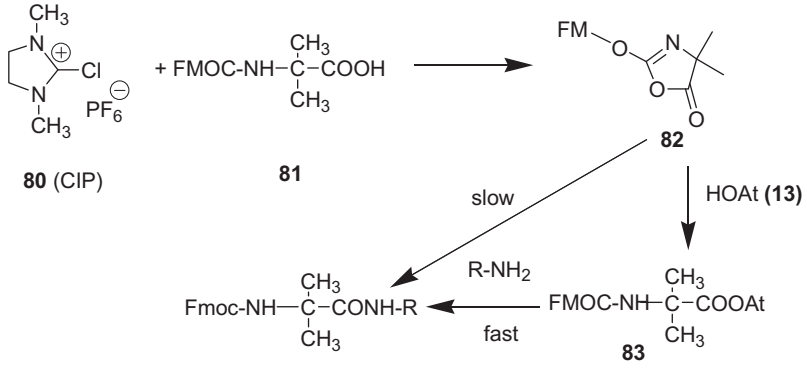
The related chloroformamidinium salt CIP (**80**) in presence of HOAt (**13**) as additive (Akaji et al., 1994, 1997; Kuriyama et al., 1997) mediates the solution phase 2-aminoisobutyric acid (Aib) coupling in excellent yield (82-90% for *Z*-Aib-Aib-OMe) (Akaji et al., 1994). Two peptaibols, Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Pheol (alamethicin F-30) and Ac-Aib-Asn-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (trichovirin I4-A) were successfully synthesized using CIP/HOAt coupling techniques (Akaji et al., 1997). For Fomc-Aib-OH (**81**) activation, the CIP-mediated reaction proceeds first through the oxazolone (**82**) and then through HOAt active ester (**83**) (Scheme 9) (Akaji et al., 1994, 1996; El-Faham, 1998a).

The use of these various onium salts requires careful attention to the tertiary base used and the preactivation time. Although, in the case of some authentic synthesizers, the preactivation time comes dictated by the instrument; in others and for manual synthesis it can be modulated. For onium salts incorporating HOAt (**13**), the activation of ordinary amino acids gives the corresponding OAt esters almost instantly. Thus, in such cases, the preactivation time should be kept to the minimum, since on standing alone the activated species can give rise to several side-reactions including racemization, formation of δ-lactam (Arg), cyno derivatives (Asn or Gln), or α-aminocrotonic acid (Thr). The same consideration applies to coupling reagents, which incorporate HOBt (**12**).

Regarding the use of a base during the coupling process, for those coupling reactions, which involve amino acids which are not likely to lose their configuration (all except Cys and His), the reactions are carried out in the presence of 1.5–2 equiv. of a tertiary amine, such as DIEA or NMM. For the coupling of protected peptides, where resistance to conversion to oxazolone does not apply, and for the coupling of Cys and His, the use of only 1 equiv. of a weaker or more hindered base is



Scheme 8 Synthesis of *N*-protected amino acid fluoride using TFFH (**77**).



Scheme 9 CIP-mediated reaction.

to be more recommended. For such systems, 2,4,6-trimethylpyridine (collidine, TMP) and the more basic 2,6-di-*tert*-butyl-4-(dimethylamino)pyridine (DB(DMAP)) are very promising (Carpino and El-Faham, 1994; Carpino et al., 1996a).

2.2.4. Organophosphorus reagents

Since Yamada introduced the mixed carboxylic-phosphoric anhydride method using DPPA (**84**) from diphenylphosphorochloridate and sodium azide to peptide chemistry in 1972 (Shioiri et al., 1972; Shioiri and Yamada, 1974), various organophosphorus compounds have been developed as peptide-coupling reagents (Fig. 4). This method usually gives higher regioselectivity towards nucleophilic attack by the amine component than a mixed carbonic anhydride method (Takeuchi and Yamada, 1974; Jackson et al., 1976).

Modification of DPPA (**84**) has led to the development of thiophosphinic-type coupling reagents such as MPTA (**88**) and MPTO (**89**) (Fig. 4) (Ueki et al., 1979; Ueki and Inazu, 1982; Katoh and Ueki, 1993).

These reagents are crystalline and stable for long-term storage. Since MPTA (**88**) generated a carbamoyl azide or urea derivative as the by-product, Ueki introduced MPTO (**89**), in which the azide group of MPTA (**88**) is replaced by a 2-oxazolone group. On the basis of the earlier development of organophosphorus reagents, a great amount of effort has been focused on developing various coupling reagents of a similar kind. For example, NDPP (**91**) (Kiso et al., 1980), Cpt-Cl (**92**) (Ramage et al., 1984; Poulos et al., 1992), BMP-Cl (**93**) (Miyake et al., 1985; Panse and Kamat, 1989), DEBP (**94**) (Kim et al., 1985), BDP (**95**) (Watanabe and Mukaiyama, 1981), bis(*o*-nitrophenyl)phenyl phosphonate (Mukaiyama et al., 1981), (5-nitro-pyridyl)diphenyl phosphinate (Kunieda et al., 1981), diphenyl 2-oxo-3-oxazolinyl phosphonate (Ueda and Oikawa, 1985), and 1,2-benzisoxazol-3-yl diphenyl phosphate (Fan et al., 1996) have been prepared by several research groups (Fig. 5).

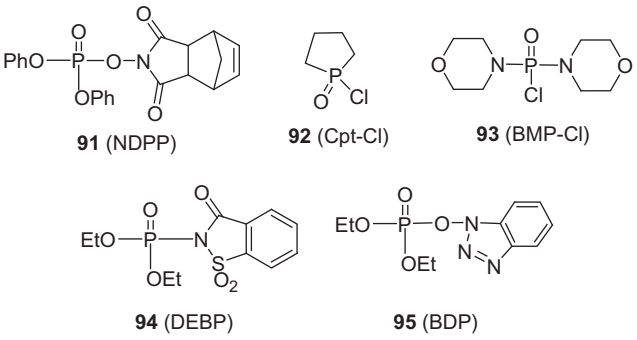


Figure 5 Structures of organophsphorus reagents.

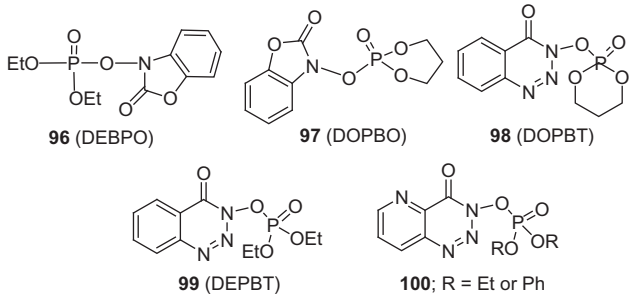


Figure 6 Structures of organophsphorus reagents.

More recently, Ye developed DEPBO (**96**), DOPBO (**97**), DOPBT (**98**), and DEPBT (**99**) (Fig. 6) (Li et al., 1999; Xie et al., 2000; Tang et al., 2002). DEPBT (**99**) derived from DEPC (**87**) and HODhbt (**11**) has been evaluated against other

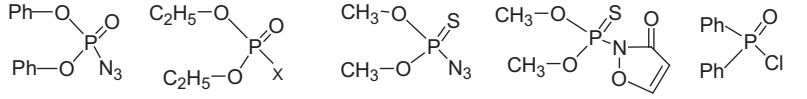
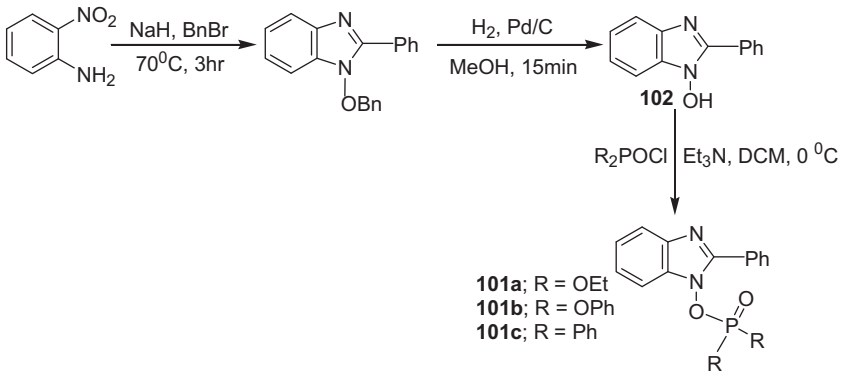


Figure 4 Structures of organophsphorus reagents.



Scheme 10 Synthesis of phosphorus reagents of 1-hydroxy 2-phenylbenimidazole (**102**).

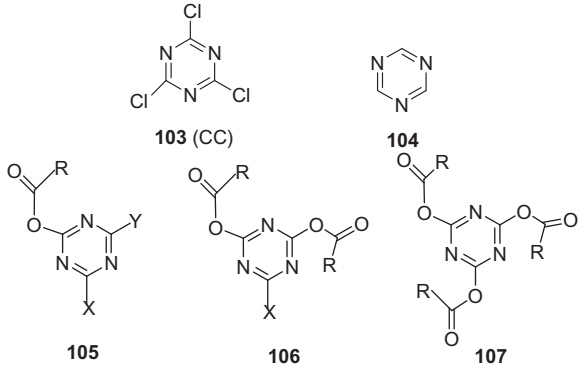
peptide-coupling reagents and gave good results in segment coupling reactions. Although the racemization-suppressing capacity of HODhbt (**11**) is greater than that of HOBt (**12**), its utility was limited due to side reactions.

Later Carpino et al. (2004b) introduced new organophosphorus reagents (**100**, Fig. 6). In this case the neighboring group effects believed to be relevant to the properties of HOAt (**13**) are superimposed on the effects that enhance the efficiency of the phosphorus moiety. On the basis of the results described, these effects are related to the greater speed with which protected amino acids are converted to their active esters by the phosphorus derivatives. Long-term storage, unfortunately, requires great care because of the hydrolytic sensitivity of these materials. Given that these new phosphate esters are clearly superior to the older uronium/guanidinium reagents for segment coupling and, under certain conditions, for solid-phase peptide assembly, it was considered essential to search for reagents with greater shelf stability.

Recently (Kokare et al., 2007), phosphoric acid diethyl ester 2-phenylbenzimidazol-1-yl ester (**101a**), diphenylphosphinic acid 2-phenylbenzimidazol-1-yl ester (**101b**) (Scheme 10) and phosphoric acid diphenyl ester 2-phenylbenzimidazol-1-yl ester (**101c**) have been reported as highly efficient coupling reagents. Their efficiency was evaluated through the synthesis of a range of amides and peptides, and the extent of racemization was found to be negligible. 1-Hydroxy-2-phenylbenzimidazole (**102**) was synthesized by coupling *ortho*-nitroaniline with benzyl bromide using sodium hydride as base, followed by benzyl deprotection (Scheme 10) (El-Faham and Albericio, 2009). The coupling reagents phosphoric acid diethyl ester 2-phenylbenzimidazol-1-yl ester (**101a**), diphenylphosphinic acid 2-phenylbenzimidazol-1-yl ester (**101b**) and phosphoric acid diphenyl ester 2-phenylbenzoimidazol-1-yl ester (**101c**) were synthesized by reaction of **102** with diethyl chlorophosphate, diphenylphosphorochloridate or diphenylphosphinic chloride, respectively, using TEA base in DCM.

2.2.5. Triazine coupling reagents

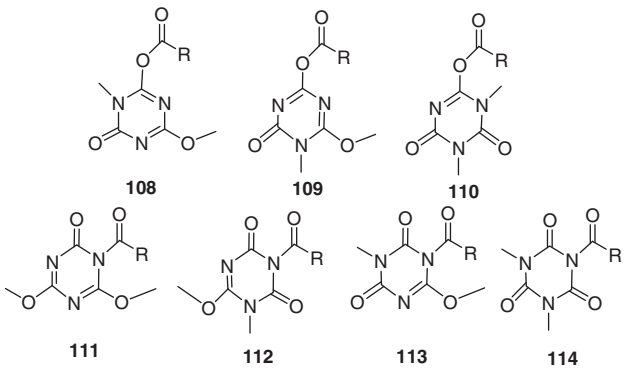
1,3,5-Triazines have also been used as coupling reagent. Thus the highly reactive 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride, **103**) produced by trimerization of chlorocyane is manufactured on the scale of several millions tons per year and is among the lowest price reagents. Derivatives of cyanuric chloride (CC, **103**) have found numerous applications as herbicides, in the dyestuff industry, polymer manufacturing, explosives, and many others. Their reactivity and properties have been studied most intensively. Considering any potential application of triazines as donors of an acyl group, the most symmetric 1,3,5-triazines (**104**) can be recognized as the most promising target (Kamiński, 1985). It is only in the case of symmetric 1,3,5-triazine (**104**) that each acyl group (derivatives **105**–**107**) can be transferred to the nucleophile via the intramolecular nucleophilic assistance of nitrogen atoms.



1,3,5-Triazines (**104**) bearing one acyl group can form several isomeric structures, resulting from partial or total O → N migration of substituents. Most of these have been synthesized and isolated in the pure form or at least identified as intermediates in reactions.

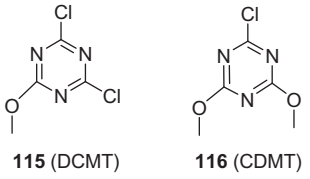
The anticipated higher reactivity of imine functionality, which is present in structures (**108**–**113**), and the increased risk of migration of substituents and double bonds in the non aromatic but partially unsaturated triazine ring, opens the possibility for many synthetic applications, but definitely has the important disadvantage considering the requirements of peptide preparation. However, acyl derivative (**114**), which bears a completely saturated ring, has to be recognized as a more promising acylating reagent than its other partially unsaturated counterparts (**108**–**113**).

Bearing in mind the synthetic potential of acyl derivatives of triazine and the convenient preparation of the whole family of 1,3,5-triazines (**104**) from the inexpensive cyanuric chloride (CC, **103**), it has been found valuable to develop these closely related compounds into the new group of triazine-based condensing reagents (TBCRs).



Cyanuric chloride (CC, **103**), 2,4-dichloro-6-methoxy-1,3,5-triazine (DCMT, **115**), and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, **116**) are the TBCRs commercially available from chemical reagent suppliers. In the laboratory, these TBCRs as well as numerous analogues can be readily prepared from (CC, **103**) by substitution of one or two chlorine atoms with the appropriate nucleophile. Usually substitution proceeds stepwise (Dudley et al., 1951; Kamiński, 2000).

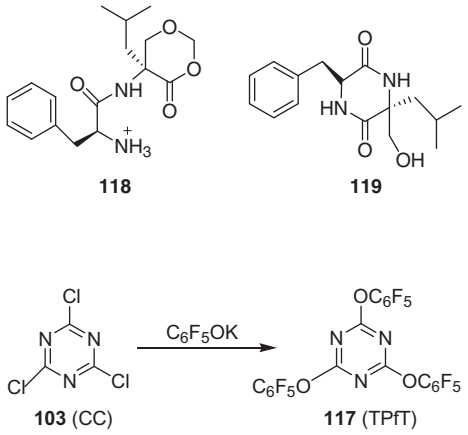
(CC, **103**), the parent compound of all TBCRs, has been successfully used as a condensing reagent in the preparation of amides (Baccolini et al., 1969; Rayle and Fellmath, 1999), dipeptides (Venkataraman and Wagle, 1979), macrocyclic lactones (Venkataraman and Wagle, 1980), formation of the β-lactam ring, etc. Cyanuric chloride (CC, **103**) activation of carboxylic acids followed by condensation with esters of alkyloiminocarboxylic acids (Schiff bases) afforded the β-lactam ring (Manhas et al., 1981). The excessive reactivity of (CC, **103**) creates serious problems when this reagent is used for the synthesis of multifunctional compounds. These impair the chance of its comprehensive application in the peptide synthesis, but certainly do not exclude its application to a limited extent (Kamiński, 2000).



The application of chiral coupling reagents for enantioselective incorporation of uncoded amino acids, often available in both configurations but in the racemic form only, is an interesting option in the screening of structure–activity relationships. The presence of three chlorine atoms makes the cyanuric chloride (CC, **103**) an interesting template for attachment of a chiral auxiliary and for preparing on this way a broad range of chiral TBCRs (Kamiński et al., 1998a, 1999).

Preliminary results have shown that the presence of a chiral center in TBCR may control activation of the carboxylic function as well as aminolysis. The symmetrically substituted 2,4,6-tris(pentafluorophenyloxy)-1,3,5-triazine (TPfT, **117**) has been prepared by reaction of cyanuric chloride (CC, **103**) with potassium pentafluorophenolate in acetonitrile in 67% yield (Scheme 11), it was shown to be less reactive than pentafluorophenol (Pfp) phosphonium and uronium derivatives (Habermann and Kunz, 1998b).

In the search for a fast and efficient coupling procedure preventing undesired side reaction of 4-oxo-1,3-dioxane (**118**) (Kamiński and Leplawy, 1986) to piperazine (**119**), (CDMT, **116**) in the presence of NMM has been successfully applied as a condensing reagent.

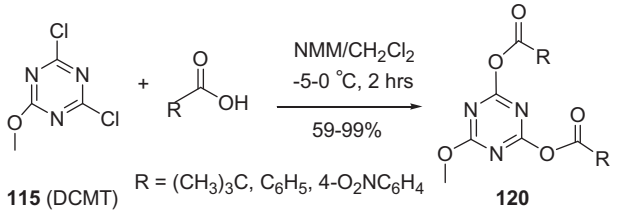


Scheme 11 Synthesis of 2,4,6-tris(penta fluorophenyloxy)-1,3,5-triazine (TPfT, **117**).

The most often used in peptide synthesis is the monofunctional coupling reagent CDMT (**116**). The first successful application of CDMT (**116**) for the preparation of peptides has remained relatively unknown because of the doubts concerning the mechanism of coupling (Kamiński et al., 1983).

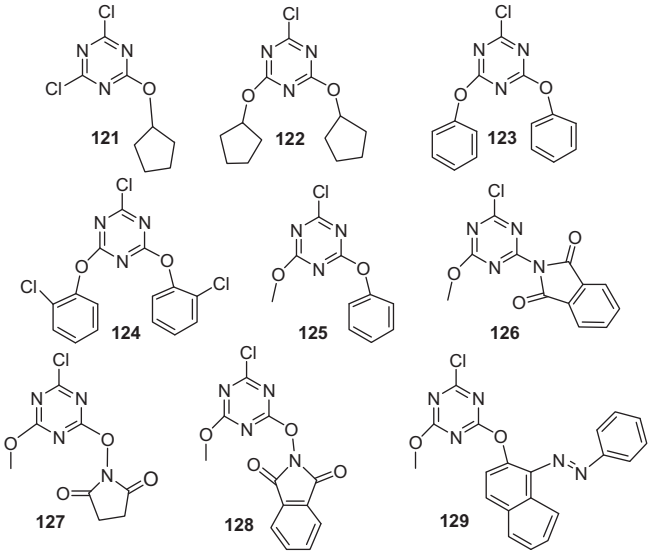
(DCMT, **115**) is stable, however, only when the reagent has been highly purified. The presence of impurities causes its decomposition (Siegel, 1978). Analogously, the high purity of the preparation is a necessary requirement for the successful storage of (CDMT, **116**). In the disadvantageous case, the formation of gaseous products may generate a rapid pressure increase in the container, and accordingly, precautions should be taken to avoid serious risk of blowout of toxic gases. In the pure state, however, (DCMT, **115**) as well as (CDMT, **116**) are stable almost indefinitely (20 years in the author's laboratory) without any traces of decomposition (Kamiński, 1996).

The first successful isolation of acyloxy-1,3,5-triazines by Kamiński has been performed in experiments involving chloro-1,3,5-triazines substituted with methoxyl groups CDMT (**116**) (Kamiński, 1990) and DCMT (**115**) (Kamiński, 1991). 2,4-diacyloxy-6-methoxy-1,3,5-triazines (**120**) were obtained in 55–99% yield by treating (DCMT, **115**) with 2 equiv. of carboxylic acid in the presence of *N*-methylmorpholine (NMM) (Scheme 12).



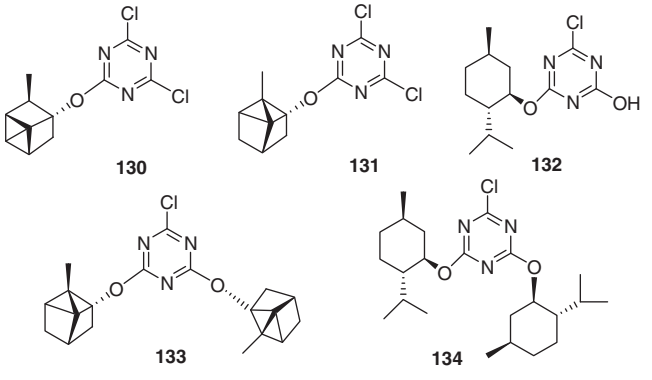
Scheme 12 Synthesis of 2,4-diacyloxy-6-methoxy-1,3,5-triazines (**120**).

Many analogues of (CDMT, **116**) (**121**–**129**) were obtained by varying the number and the nature of substituents in the triazine ring. Their utility in condensation reactions has been confirmed (Kamiński, 1996). In most cases, the alteration of the structure is based on inclusion of modified alkoxy or aryloxy groups into the triazine ring. All were prepared from cyanuric chloride (CC, **103**) by stepwise substitution of chlorine atoms. As a general rule, for the best results it is necessary to follow the optimal reaction conditions typical for stepwise substitution. The first substituent needs to be introduced at 0–30 °C followed by subsequent replacement of the second chlorine atom under more vigorous conditions, even in the case of symmetrically disubstituted triazines.



The reactivity of triazine in nucleophilic substitutions of halogen atoms has been studied (Rys et al., 1971; Ostrogovich et al., 1981; Hunter et al., 1995). The comparison of halogen susceptibility toward nucleophilic attack shows the order F > Br > Cl (Kamiński, 2000).

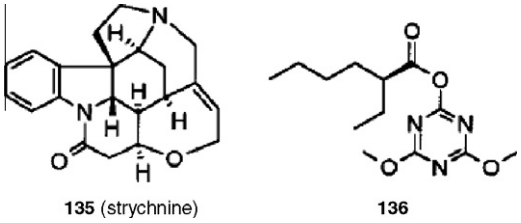
Several chiral monochloro- and dichloro-TBCRs (**130**–**134**) have been obtained and applied as enantiodifferentiating coupling reagents (Kamiński et al., 1999).



It is well known that the presence of nucleophilic heteroatoms in the substrate may severely deteriorate the course of coupling reactions. To overcome this barrier, it became necessary to introduce protecting groups in order to modify the reactivity of heteroatoms. Such a strategy is most common in the cases of nucleophilic amino, hydroxyl and thiol groups.

The fairly well-documented application of (CDMT, **116**) in the synthesis of compounds bearing heteroatoms illustrates two aspects of TBCRs. On the one hand, these powerful acylating species are efficient in reactions involving such troublesome substrates as *tert*-butylamine, *N*-alkylamino acids or $C^{\alpha,\alpha}$ disubstituted glycines. On the other hand, there are many examples demonstrating the surprising selectivity of TBCR in the synthesis involving substrates with unprotected $-NH_2$ and –OH groups.

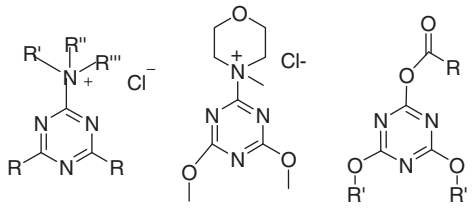
CDMT (**116**) is a cheap reagent which has been used mainly in SPPS. In the presence of *N*-methylmorpholine (NMM) as base, the reagent gives low levels of racemization (< 4.2%). It has also been used for the coupling of hindered amino acids (Kamiński, 1987, 1994). Treatment of 2-ethylhexanoic acid with (CDMT, **116**) in the presence of a chiral tertiary amine (strychnine, **135**) gave a weakly basic product, which was isolated and identified (Siegel, 1978; Kamiński, 1996) as the expected triazine ester **136**.



It has been found that successful activation of carboxylic acids by means of 2-chloro-4,6-disubstituted-1,3,5-triazines requires the presence of a tertiary amine in the reacting medium. Generally, this reaction of amine with (CDMT, **116**) could be considered erratic because only a few of all tertiary amines respond to treatment with this reagent.

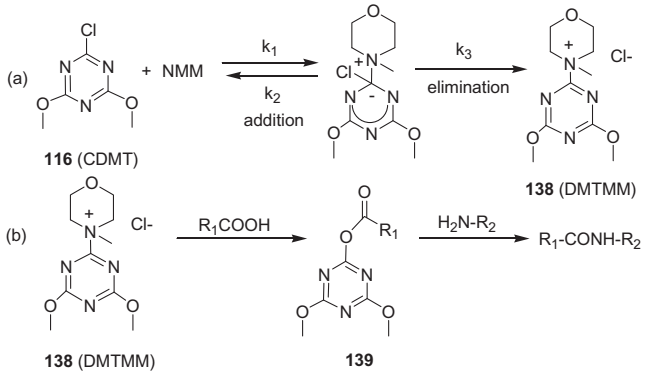
Moreover, the borderline between reactive amines does not correlate with the basicity of amines in polar solvents. This suggests participation of an intermediate, formed in the first step, involving an amine as the obligatory component. Further studies on the activation of carboxylic acids by means of (CDMT, **116**) confirmed a multistep process proceeding via triazinylammonium salts, such as **137** and (DMTMM, **138**) formed *in situ* in the presence of the appropriate amine (Kamiński et al., 1998a,b; Kamiński, 2000).

The rate of formation of triazinylammonium salts **137** and **138** strongly depends on the steric hindrance of *N*-substituents (amine). For more hindered amines, rapid loss of reactivity of **116** is observed. Only amines prone to the formation of salts such as, **137** and **138** when treated with **116** are useful in activation of the carboxylic function. Triethylamine (Et_3N), which does not form a quarternary ammonium salt at low temperature in the reaction with (CDMT, **116**) was entirely incapable of activating benzoic acid. Moreover, the addition of a tertiary amine, which otherwise is inactive toward (CDMT, **116**) has a negligible influence on the rate of activation of carboxylic acids. Thus, the addition of *N*,*N*-dimethylaniline to NMM does not accelerate the rate of activation of carboxylic acids by (CDMT, **116**) (Kamiński et al., 1998b).

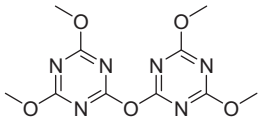


137 **138** (DMTMM) **139**

Undoubtly, the crucial stage in the activation of a carboxylic acid by (CDMT, **116**) comprises the two subsequent substitution reactions in the triazine ring (Fig. 5). The first one (a), which involves substitution of the chlorine atom by amine leading to quarternary ammonium salt **138** has been found to be extremely sensitive to steric hindrance of amine substituents. The second reaction (b), which is exceptionally tolerant to the steric hindrance of the activated carboxylic acid, involves substitution of an amine leaving group by the carboxylate ion, affording triazine ''superactive esters'' **139** (Kamiński, 2000).



Alcoholysis of acyloxy-1,3,5-triazines (**139**) proceeds much slower than aminolysis. Usually, activation of serine with an unprotected side-chain hydroxyl group and subsequent coupling with the amino component leads to efficient formation of peptide. In a large excess of primary or secondary alcohol, under more vigorous conditions, alcoholysis of **139** takes place after a few days giving the appropriate esters in 41–92% yield (Kamińska et al., 1999). In order to accelerate the alcoholysis of **139**, $MgBr_2$ or DMAP has been employed as a transesterification catalyst. Another approach to esterification of carboxylic acids has involved 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (**138**) used as coupling reagent in the presence of *N*-methylmorpholine (Kunishima et al., 1999b). Application of 2 equiv. of **138** gave esters in 34–99% yield in a relatively fast reaction without addition of any catalysts. This discrepancy in the efficacy of procedures postulating essentially the same reactive intermediates requires some comment. It seems most probable that exceedingly fast acylation reported in experiments involving an excess of amine and quarternary triazinium salt **138** (Kunishima et al., 1999b) versus experiments based on the standard (CDMT, **116**) procedure (Kamińska et al., 1999) results probably from a different mechanism operating in the former case. In the presence of a large excess of amine, triazine esters **139** disproportionate to corresponding anhydrides and *bis*(4,6-di-methoxy-1,3,5-triazin-2-yl)-ether (**140**), which remain unreactive under the reaction conditions described (Kamiński et al., 1998b). Moreover, this explains the need for the 2 equiv. of coupling reagent required for efficient coupling mediated by **138**, taking into account that only half of the acyl groups of anhydride are consumed in the acylation, but the activation of the other requires the second equivalent of activating reagent.



140

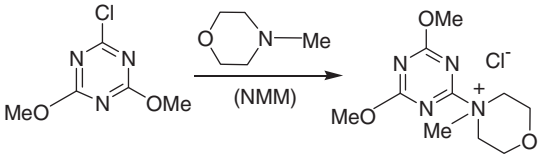
The versatility of (CDMT, **116**) has been documented by the successful synthesis of carotenoid–porphyrin–quinone triads used in the studies of photoinduced electron transfer processes (Kuciauskas et al., 1997).

Numerous oligopeptides of 3-heteroaryloamino-2,3-dehydroalanine have been obtained using (CDMT, **116**) as the coupling reagent. Opening of 2-phenyl-4-heteroaryloaminomethylene-5(4*H*)-oxazolones with a sodium salt of amino acid gave *N*-benzoyldipeptides, which were further extended in a stepwise procedure involving (CDMT, **116**) as coupling reagent.

The reported yield dramatically depends on the structure of the heterocyclic moiety (Svete et al., 1997). Unfortunately, no other concurrent coupling method was used to compare the efficacy of TBCR with the other reagents and the verification of results is not possible in this case.

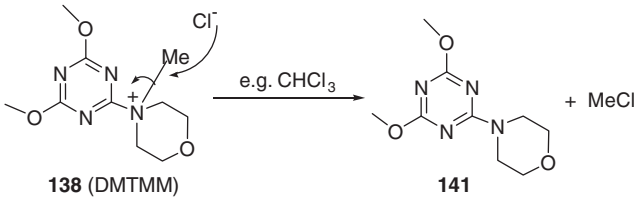
Relatively high rates of synthesis, diminished sensitivity to steric hindrance of substrates, along with convenient monitoring of every synthetic stage supplemented by purification of intermediates (if necessary), make the repetitive synthesis in solution a valuable tool for large-scale preparation of oligopeptides. Recently, the solution methodology has been intensively exploited as a valuable supplementation of the tools of combinatorial chemistry. This includes solution-phase preparation of sublibraries as well as its cooperative use in combination with solid-phase techniques. The hybrid approach, a combination of solution methodology and synthesis on the solid support, benefits from advantages of both, is, therefore, of promising utility when approaching new synthetic purposes (Felix et al., 1998; Flynn et al., 1998; Kim et al., 1998). One of the restrictions of solution synthesis is the absence of a general strategy that allows convenient manipulation with excess of reagents. Compared to the solid state approach, which assures filtration as a convenient and natural way of removing excess soluble components, developing repetitive methodology in solution always requires special care to avoid these limitations. Bearing in mind the weakly basic properties of all triazine derivatives, an advantage is offered of an acidic wash process for removing both excess of triazine condensing reagent, as well as any side triazine products. Accordingly, efforts have been made to elucidate the usefulness of TBCR in repetitive synthesis in solution.

The classic solution procedure, involving mixed anhydrides, named REMA (Repetitive Excess Mixed Anhydrides) (Tilak, 1970; van Zon and Bayerman, 1976) while assuring high rates



116 (CDMT) **138** (DMTMM)

Scheme 13 Synthesis of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, **138**).



138 (DMTMM) **141**

Scheme 14 Synthesis of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-morpholine (**141**).

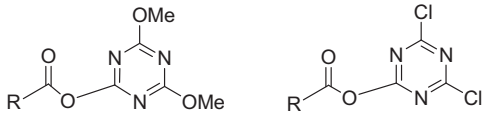
of coupling and convenient removal of excess of acylating reagent, inevitably leads to an ambiguous course of peptide bond formation due to the presence of two different carbonyl groups. The analogous procedure of removing excess of acylating reagent has been found efficient also in the case of peptide synthesis. In all coupling proceeding in THF or DMF, the final product has been isolated by filtration or extraction. Any further trace of the slightly basic triazine derivatives has been successfully removed by washing of the final product with diluted acids (Kamiński et al., 2005).

By a simple reaction of CDMT (**116**) with *N*-methyl-morpholine (NMM), the related triazine, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, **138**), has been prepared in 79% yield (Scheme 13) (Kamiński et al., 1998b) and used for the synthesis of amides. Reaction rates in THF are faster than with the CDMT/NMM system (Kunishima et al., 1999a).

In recent years, DMTMM (**138**) (Kamiński et al., 1998b; Kunishima et al., 1999a) has come to prominence as an effective coupling agent, finding applications in amidation (Kunishima et al., 1999c, 2001; Kjell et al., 2005), esterification (Kunishima et al., 1999c), glycosidation (Paoline et al., 2007; Tanaka et al., 2008) and phosphonylation (Wozniak et al., 2007) methodology. However, the utility of DMTMM (**138**) as a coupling agent is compromised, especially at large scale, by its instability in organic solution as it undergoes self-immolative degradation, yielding 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-morpholine (**141**) and chloromethane (Scheme 14). In chloroform at ambient temperature, this results in complete degradation in just 3 h (Kunishima et al., 1999c) (97% degradation is observed in 2 h) (Steven, 2009).

Recently a new generation of triazine-based coupling reagents (TBCRs) (Kamiński, 1985, 1987, 2000), designed according to the concept of "superactive esters" (Kamiński, 1994), was obtained by treatment of (DMTMM, **138**) with lithium or silver tetrafluoroborate. To demonstrate the efficacy of the modified triazine reagents, with improved stability due to the use of non-nucleophilic tetrafluoroborate counterion, in a very broad range of typical synthetic applications. The idea of acceleration of the coupling process in the case of "superactive esters" is based on facile departure of the leaving group by its rearrangement in energetically favored, consecutive process to a stable, chemically inert, and neutral side product. This general approach for designing coupling reagents can be considered as a valuable alternative, which eliminates the over activation of acylating intermediate. The new coupling reagents are highly versatile in ester and peptide synthesis in solution as well as in the solid phase. Their application in the synthesis gave very regular coupling results for the whole range of amino acids substrates derived from natural and un-natural sterically hindered amino acids and substantially reduced amount of side products under broad range of reaction conditions (Kamiński et al., 2005).

The 1,3,5-triazinylmethylmorpholinium chloride DMTMM (**138**) is an efficient condensing agent which leads to the formation of esters in the presence of NMM (Kunishima et al., 1999a,b) e.g. 2-acyloxy-1,3,5-triazines (**142**) (Farloni et al., 1999a) and **143** (Farloni et al., 1999b).



142 **143**

Previous studies proved the participation of 2-acyloxy-1,3,5-triazines (**142**) (Kamiński, 1990; Sochacki and Kamiński, 1994) as powerful acylating intermediates in condensations involving TBCRs. In fact, triazine esters **142** have already been found more reactive than any other acylating reagent, including *N*-hydroxybenzotriazole esters (Gówka et al., 1990). While esters derived from 1-oxo-2-hydroxydihydrobenzotriazine (HODhbt, **11**) gave better coupling rates than when used as additive. They have been employed in solid-phase peptide synthesis of the corresponding Fmoc-protected amino acids and they show a good resistance to racemization (Atherton et al., 1988).

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