Account

Highly efficient synthesis of peptides by rational utilization of novel coupling reagents

LI, Peng*(李鹏) XU, Jie-Cheng(徐杰诚)
Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

The efficiency of different peptide coupling reagents, including carbodiimides, HOBt or HOAt-derived uronium, phosphonium and immonium salts, halouronium, halophosphonium, 2-halopyridinium and 2-halothiazoliuim salts, was evaluated. The synthetic strategy for coded peptides andnonribosomal peptides was discussed with an emphasis on the rational selection of peptide coupling reagents.

Keywords Peptide, coupling reagent, racemization, reactivity, nonribosomal amino acid

Introduction

The chemical synthesis of peptide is necessary and effective means to study structure-activity relationship (SAR), elucidate structures and acquire ample quantities of compounds unavailable from natural sources or fermentation, especially for the peptide-based medicines and pharmaceuticals in reducing their toxicities or enhancing their activities, selectivities or bioavailabilities by syntheticmanipulation. To meet the needs for peptide synthesis, many chemical methods for the peptide synthesis have been developed, which in most cases involve activation of the carboxylic acid components in the peptide coupling reactions by the formation of anhydrides, acid azides, acid halides or active esters in advance, or by the *in situ* activation of the carboxylic acids by coupling reagents. Because the coupling reagents are convenient to use and often show high efficiency, they have developed very rapidly in the past decades. In this account, the exploited peptide coupling reagents are reviewed and the rational selection of the coupling reagents, as well as the mechanisms for the coupling reactions is discussed.

Coupling reagents used for the synthesis of peptide containing only coded amino acid residues

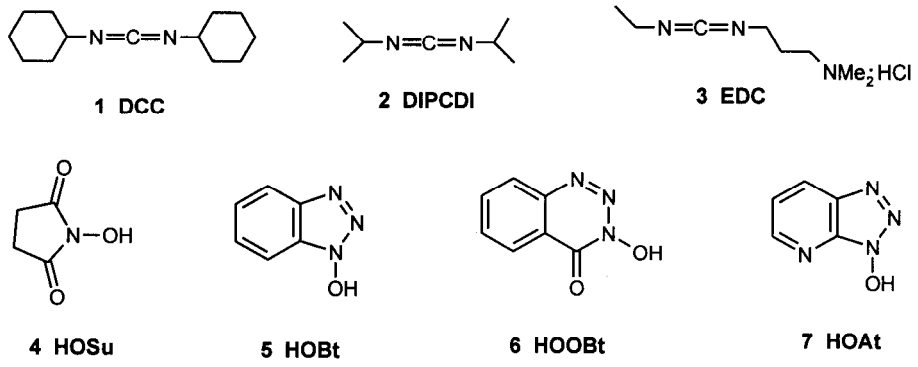
Synthesis of coded small or medium peptides using conventional coupling reagents has become routine. The most commonly used coupling reagents are the carbodiimides **1**—**3** shown in Scheme 1. These carbodiimides promote the formation of the amide bond by forming the corresponding intermediates of the *O*-acylisourea and symmetric anhydride, which subsequently undergo aminolysis with the amino component to give product. However, the coupling of protected peptide segments using the carbodiimides alone proceeds with extensive racemization of the *C*-terminal amino acid of the segment besides other well-known side reactions. Such racemization can be dramatically reduced by the addition of the additives **4**—**7**, which intercept the above two intermediates to form the corresponding active esters. The esters react rapidly with amino components to afford amides.

Among the additives, HOSu was the first used in peptide synthesis. However it was gradually replaced by HOBt due to the better racemization-suppressing ability and much higher reactivity of the latter. HOOBt can suppress the racemization more efficiently than the HOBt does. Nevertheless it has not been used as broadly as HOBt because it produces an azide by-product during coupling. HOAt is the most efficient additive up to now, which can effectively promote the formation of sterically hindered amide bonds and dramatically suppress the

* Received December 23, 1999; accepted March 21, 2000.
Project supported by the National Natural Science Foundation of China.

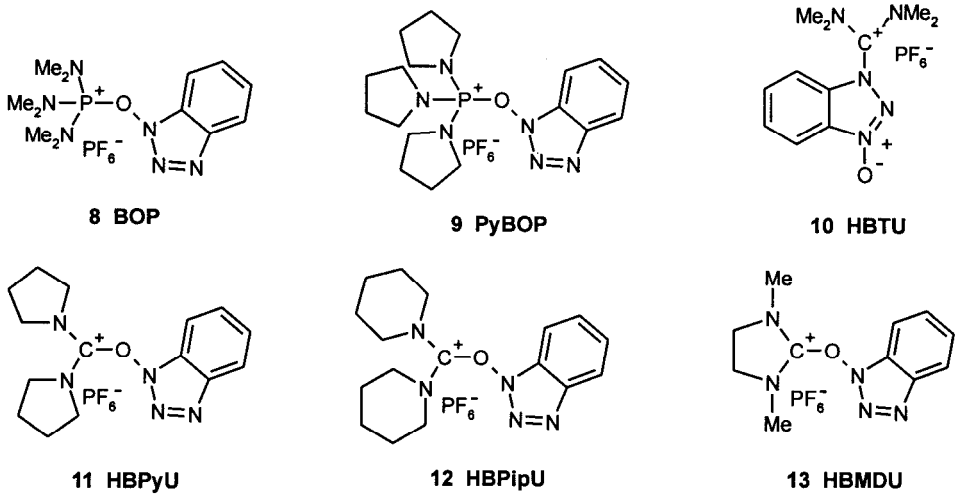
racemization because of the presence of an anchimeric assistance effect.

Scheme 1



One of the most significant developments in peptide coupling reagents is the successful exploitation of HOBt-based phosphonium and uronium salts. Subsequent to the first phosphonium salt BOP, designed by Castro,[1] a series of HOBt-derived onium type coupling reagents, such as PyBOP, HBTU, HBPyU, HBPipU and HBMDU (Scheme 2), have been developed and applied to the peptide synthesis both in solution and solid phase. The previously predominant carbodiimide reagents are gradually replaced by the more efficient onium salts. Among these onium salts, HOBt-derived uronium type reagents are shown to be somewhat more efficient than the corresponding phosphonium analogues. Furthermore, HBPyU was proved to be the most efficient among HOBt-derived uronium salts, of course more efficient than phosphonium salts based upon HOBt.[2]

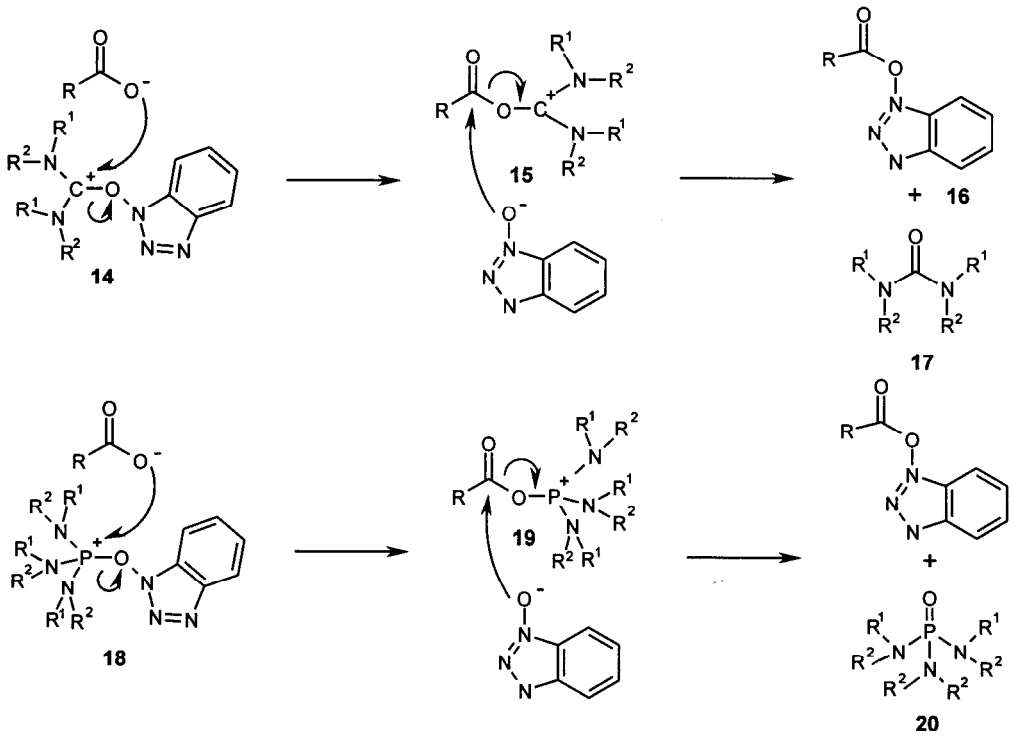
Scheme 2



The mechanism of these phosphonium and uronium salts mediated coupling reactions is proposed as shown in Scheme 3. The carboxylate ion of the carboxyl component attacks the onium salt **14** or **18** to form the corresponding unstable acyloxyuronium salt **15** or acyloxyphosphonium salt **19**, which is subsequently attacked by the oxyanion of HOBt to afford the benzotriazolyl ester **16**, releasing the by-product **17** or **20**. It is also proposed that all the intermediates, the acyloxyonium salt **15** or **19**, the active ester **16**, the amino acid anhydride and 5(4*H*)-oxazolone may react with amino component to give the final product. However, the benzotriazolyl

ester **16** is widely regarded as the main active intermediate. The racemization of products is mainly caused by the intramolecular deprotonation of the α-CH of the active ester **16** and the tautomerization or enolization of 5-(4*H*)-oxazolone under basic reaction conditions.

Scheme 3



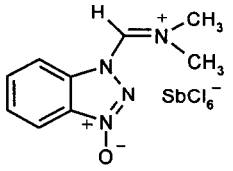
Although HOBt-derived phosphonium and uronium salts demonstrate high efficiency in peptide synthesis, the racemization is still a problem. For example, Young's tests show that the racemization of products were 25.4% and 39.8%, respectively, using HBTU or BOP as a coupling reagent.[2] To further suppress the racemization and enhance the coupling efficiency, a series of HOBt-based immonium salts, such as BOMI, BDMP and BPMP shown in Scheme 4, have been developed. Similar to the uronium/aminium salt HBTU, reagent BOMI crystallized in the *N*-substituted form **21**, rather than the isomeric *O*-substituted form **22**,[3] as shown by the X-ray analysis. The X-ray data for those analogous BDMP and BPMP as well as the uronium salts HBMDU and HBPyU have not been available yet. Therefore the structural representations are arbitrarily drawn as the *O*-substituted forms. Similar isomerization was also observed in the active ester **16**, and could be explained by molecular orbital calculation using semi-empirical method PM3.[4]

These immonium type reagents show extremely high reactivity, very low racemization and good yields for the couplings shown in Table 1 and Fig. 1. It is obvious that, both in terms of reactivity and racemization, the HOBt-based immonium salts are superior to the uronium salts HBPyU and HAPyU, which are widely regarded as the most effective reagents so far developed. Using BOMI as coupling reagent and NMM as base, the model coupling reaction can be accomplished within 5 min even at −70℃ (Entry 13).[5] The half-reaction time of these HOBt-based immonium salts is less than 20 min even at −10℃ in THF with sterically hindered 2,6-lutidine as base, while the half time of reaction ($t^{1/2}$) of the carbodiimides, HOBt-derived uronium and phosphonium salts are more than 120 min under the same reaction conditions.[3]

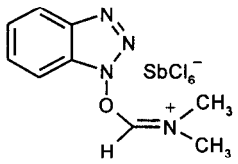
The relatively low reactivity of HBPyU and HAPyU is mainly due to the lower susceptivity of the carbocation in these uronium/aminium salts towards the attack of the carboxylate anion than that in the immonium salts. The two substituted amino groups of the uronium/aminium salts provide two equal resonance structures to stabilize

the central carbocation. The lone electron pairs of the two nitrogen atoms are delocalized over the bonds N – C – N, which makes the carbocations less susceptible to the nucleophilic attack of the carboxylate anion.[6] The higher reactivity of BDMP than its analogues BOMI is probably due to the tension of intra-annular imide bond. It has been shown by PCMODEL calculation that the angles of C^{sp2}—N—C_2^{sp3} and N—C^{sp2}—C_4^{sp3} in the molecule of BDMP are 105.7° and 113.7°, respectively, indicating that the five-membered ring of 2*H*-pyrrolium is constrained to some extent.[7]

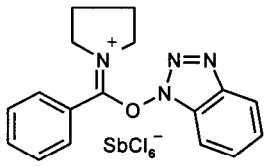
Scheme 4



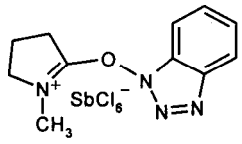
21 BOMI (*N*-from)
(ascertained by X-ray analysis)



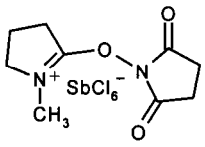
22 BOMI (*O*-from)



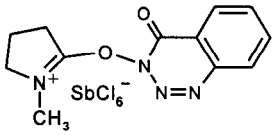
23 BPMP



24 BDMP



25 SOMP



26 DOMP

Table 1 Comparison of reactivity and racemization of various coupling reagents using HPLC[a] and Young's test[b]

Entry	Coupling reagent	Reaction conditions			Yield (%)			Reactivity $t^{1/2}$ (min)	Racemization (*DL*%)[c]	
		base	temp. (℃)	solvent	2 min	10 min	120 min		HPLC	Young's test
1	DCC + HOBt	2,6-lutidine[d]	– 10	THF	4.37	12.3	63.8	81	6.8	
2	DCC + HOBt	2,6-lutidine[d]	25	DMF	9.30	25.8	73.4	33	7.8	31.2
3	BOP	DIEA	25	DMF	80.1	91.2	98.9	< 1	9.6	39.6
4	HBTU	DIEA	25	DMF	95.5	99.3	99.3	< 1	9.8	24.3
5	HBPipU	DIEA	25	DMF	97.3	99.5	99.5	< 1	8.9	20.5
6	HBPyU	DIEA	25	DMF	98.9	99.6	99.6	< 1	7.9	18.0
7	HBPyU	2,6-lutidine	25	DMF	14.0	32.4	86.4	23	9.4	
8	HBPyU	2,6-lutidine	– 10	THF	2.0	3.29	16.1	> 120	14.6	
9	HAPyU	DIEA	25	DMF	99.1	99.6	99.6	< 1	5.7	13.9
10	HAPyU	2,6-lutidine	– 10	THF	2.4	11.2	78.3	49	3.3	
11	BOMI	2,6-lutidine	– 10	THF	6.43	20.1	93.4	20	3.1	8.8
12	BOMI + HOAt	2,6-lutidine	– 10	THF	41.1	73.5	86.0	4.5	1.7	
13	BOMI	NMM	– 70	CH_2Cl_2	87.6	89.1	89.3	< 1	3.0	
14	BDMP	2,6-lutidine	– 10	THF	51.0	89.6	95.3	1.9	2.2	5.3
15	BPMP	2,6-lutidine	– 10	THF	37.6	81.7	96.2	2.8	2.3	5.4
16	DOMP	2,6-lutidine	– 10	THF	49.6	67.9	69.1	2.0	2.3	5.0
17	AOMP	2,6-lutidine	– 10	THF	64.1	64.2	64.2	< 1	1.6	3.1
18	Z-G-F-OBt	2,6-lutidine	– 10	THF	42.0	90.8	94.8	2.7	4.4	
19	Z-G-F-OAt	2,6-lutidine	– 10	THF	90.9	91.5	91.7	< 1	2.0	

[a] Model reaction: Z-Gly-Phe-OH + Val-OCH_3·HCl→Z-Gly-Phe-Val-OCH_3.

[b] Model reaction: Bz-Leu-OH + Gly-OEt·HCl→Bz-Leu-Gly-OEt.

[c] *DL*% equal to *D*-isomer% multiplied by two.

[d] Base 2,6-lutidine (1 equiv.) was added to neutralize the amino component Val-OCH_3·HCl.

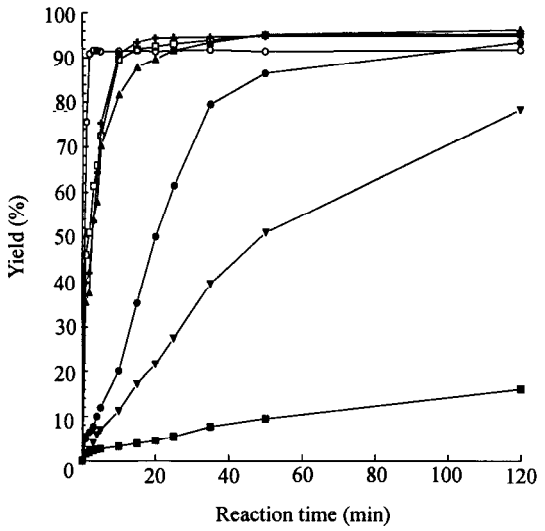


Fig. 1 Comparison of reactivity of immonium salts with uronium salts (▲ BPMP , □ BDMP , ● BOMI , HBPyU , ▼HAPyU, + Z-G-F-OBt, ○ Z-G-F-OAt, ■ HBPyU). Reaction conditions: T, − 10℃, base, 2,6-lutidine. Sol., THF. substrate ratio: N-protected amino acid: amino acid ester hydrochloride: coupling reagent: base = 1:1.1:1.1:3 (mol).

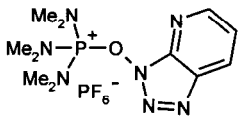
The efficiency of these immonium salts was further demonstrated by the successful synthesis of a series of oligopeptides and biologically active peptides, such as Leu—enkephalin, both in solution and solid phase. These immonium salts have also been employed in the synthesis of esters with excellent yields, especially the active esters such as benzotriazolyl, pentafluorophenyl, and succinimidyl esters, which are often used in the synthesis of lactones and lactams.[8]

Coupling reagents for nonribosomal peptide containing N-alkylated or C_α, C_α-disubstituted amino acid residues

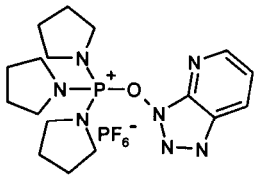
N-alkyl amino or imino acid residues in peptides, such as MeVal and Pro, exhibit reduced preference for *trans* conformation, which may lead to the biologically relevant β-turn structures. The abridgment of intra- and intermolecular hydrogen bonds in peptide containing N-alkyl amino acid residues further affect the secondary and tertiary structures of the peptides. As for C_α, C_α-disubstituted amino acids, such as Aib, they can rigidify the peptide backbone through the formation of helixes and β-turns, the organized structures responsible for the interesting biological activity of the peptaibols. The structurally unusual, nonribosomal peptide containing N-alkylated or C_α, C_α-disubstituted amino acid resi dues, which are mainly isolated from fungi, bacteria, marine sponges and other lower animal forms, usually exhibit a variety of activities including antitumor, antiviral antiinflammatory and immunosuppressive actions. These peptides or their analogues derived by SAR studies can be used as drugs or leading compounds in drug development. The chemical synthesis of these compounds is therefore necessary and valuable. Unfortunately, the incorporation of noncoded amino acids, especially sterically hindered N-methylated or C_α,C_α-dialkylated amino acids, into peptides could not be satisfactorily achieved using the conventional DCC/HOBt or HOBt-derived onium type coupling reagents. Although the reactivity of the N-methyl amino components should be higher than that of the primary amines in S_N2 reactions in view of the electronic effect, the steric hindrance of these secondary amines greatly outweighs their enhanced nucleophilicity and results in low coupling rates, leading to extend reaction time, undesired side reactions, and extensive racemization. Fortunately, this problem has been successfully circumvented with the exploitation of the new additive HOAt[9] and HOAt derived uronium, phosphonium and immonium type coupling reagents, such as HATU, HAPyU, HAPipU, HAMDU, AOP, PyAOP and AOMP (Scheme 5).[3,10] Just like HOBt-derived onium reagents, HOAt-based uronium salts are also more reactive than corresponding phosphonium analogues. HAPyU is shown to be the most efficient among these uronium salts.

Phosphonium type reagents are more suitable than the uronium salts in solid phase peptide synthesis because HOAt-derived uronium salts may react with amino groups to form undesired guanidino derivatives under the conditions commonly employed in peptide synthesis. Such side reactions partly terminate peptide chain elongation and may be a serious problem during the much slower activation of hindered amino acids, N_α-protected peptide segments, or carboxylic acids involved in cyclization steps.[10] The yields for coupling using immonium salt AOMP are usually poor because in the presence of a base AOMP decomposes before the activation of N-protected amino acid is achieved.

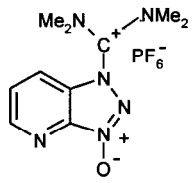
Scheme 5



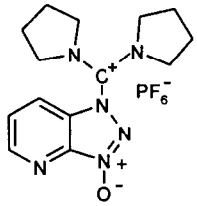
27 AOP



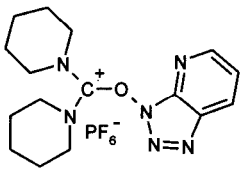
28 PyAOP



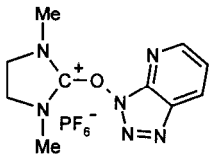
29 HATU



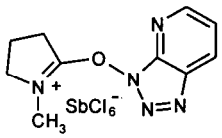
30 HAPyU



31 HAPipU



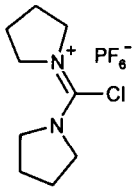
32 HAMDU



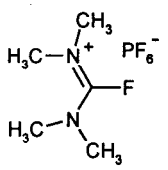
33 AOMP

Althaugh the HOAt-based coupling reagents mentioned above are shown to be very efficient for the construction of hindered peptides, they are very expensive and scale up is thus difficult. For these reasons another class of coupling reagents, halogenated uronium and phosphonium salts, such as PyClU, TFFH, BTFFH, PyBroP and PyCloP, become important in the synthesis of hindered peptides (Scheme 6). For instance, TFFH shows high efficiency during the synthesis of peptide containing Aib or *N*-methyl amino acid residues, and can be used both in solution and solid phase.[11] The main intermediate of the TFFH or BTFFH mediated coupling reactions is the N_α-protected amino acid fluoride. In the case of PyClU and PyCloP, the intermediate 5(4*H*)-oxazolone is also formed besides the corresponding N_α-protected amino acid chloride, resulting in more significant racemization than TFFH or BTFFH. PyBroP is also a powerful reagent for the formation of hindered amide bonds *via* corresponding acid bromide intermediate. The main disadvantage of using these halogenated onium salts is that the racemization of products is more serious than using HOAt-based onium reagents, especially for segment condensation. BOP-Cl demonstrated low racemization and high reactivity due to its intramolecular general base catalysis by the oxazolinone carbonyl of the mixed phosphorous anhydride active intermediates. However, as it reacts with primary amines under the commonly used reaction conditions, the acid components must be pre-activated. Consequently, the coupling reaction can not be carried out in a one-pot manner.[12]

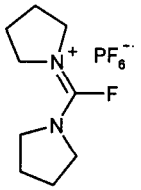
Scheme 6



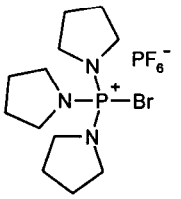
34 PyClU



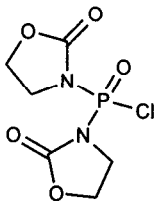
35 TFFH



36 BTFFH



37 PyBroP

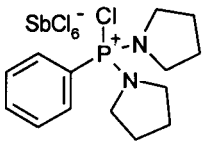


38 BOP-Cl

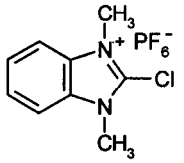
To tackle above problems, we have developed a series of halogenated reagents for peptide synthesis (Scheme 7). Table 2 and Fig. 2 show that 2-bromothiazolium salt[13] BEMT and 2-halopyridinium salts BEP, FEP, BEPH and FEPH are much more reactive than the commonly used halogenated reagents PyBroP, PyClU, BTFFH and BOP-Cl. The coupling could be accomplished within 2 min under the adopted reaction conditions. The coupling yield could be further improved if the base was added slowly. As shown in Table 2, the racemization of product using 2-halopyridinium or 2-halothiazolium salts as coupling reagents was much lower

than using other halogenated coupling reagents (except BOP-Cl). If HOAt was added as an additive, the racemization can be further suppressed due to its anchimeric assistance effect. Fig. 3 also demonstrates the high reactivity of thiazolium and pyridinium type coupling reagents in the coupling of sterically hindered *N*-methyl amino acids. Their high efficiency was further proved by the successful synthesis of the extensively *N*-methylated immunosuppressive cyclic undecapeptide Cyclosporin O (CsO) in 20—23% overall yield and the protected pentapeptide moiety of Dolastatin 15 in 57.7% overall yield.

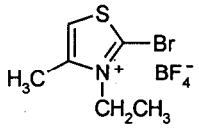
Scheme 7



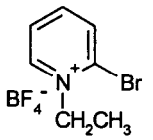
39 PyClOPP



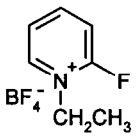
40 CMBI



41 BEMT

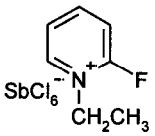


42 BEP

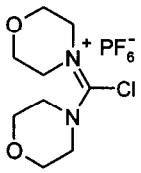


43 FEP

44 BEPH



45 FEPH



46 CMMM

Table 2 Comparison of reactivity and racemization of halogenated coupling reagents using HPLC[a]

Entry	Coupling reagent	Yield (%)[b]	$t^{1/2}$ (min)	*D*-isomer contant (%)
1	PyBroP	6.10	79	22.3
2	BOP-Cl	5.34	~ 90[d]	4.13
3	CMBI	19.7	92	16.8
4	PyClU	5.64	> 120	33.2
5	BTFFH	9.35	49	25.9
6	CMMM	2.31	> 120	31.4
7	BEMT	45.9	—[e]	2.72
8	BEMT[c]	84.3	< 2[e]	1.33
9	FEP	75.6	< 2[e]	2.33
10	FEPH	67.7	< 2[e]	2.08
11	BEP	50.8	< 2[e]	4.59
12	BEPH	52.3	< 2[e]	4.56
13	FEP[c]	86.7	< 2[e]	1.54
14	BEP[c]	75.9	< 2[e]	1.42

[a] Model reaction: Z-Gly-Phe-OH + Val-OCH_3·HCl→Z-Gly-Phe-Val-OCH_3. Reaction conditions: Sol. CH_2Cl_2(10 L/mol); temp. −10℃; base DIEA. [b] Reaction time t = 2 min. [c] HOAt (1 equiv.) was added as an additive. [d] The $t^{1/2}$ value of BOP-Cl cannot be evaluated accurately due to its poor solubility in CH_2Cl_2. [e] The coupling reactions were accomplished within 2 min.

The proposed mechanism of the coupling reactions mediated by the pyridinium type reagents is as follows: First the carboxylic acid is activated by the 2-halopyridinium salts due to the formation of an unstable acyloxypyridinium salt intermediate **47**, which in turn reacts with the amino component to give product and release the by-product *N*-ethyl-4-pyridone **48**. Alternatively, **47** may be converted, on a competitive basis, into the corresponding acid halide before being transformed into the dipeptide by aminolysis. A small amount of 5(4*H*)-oxa-

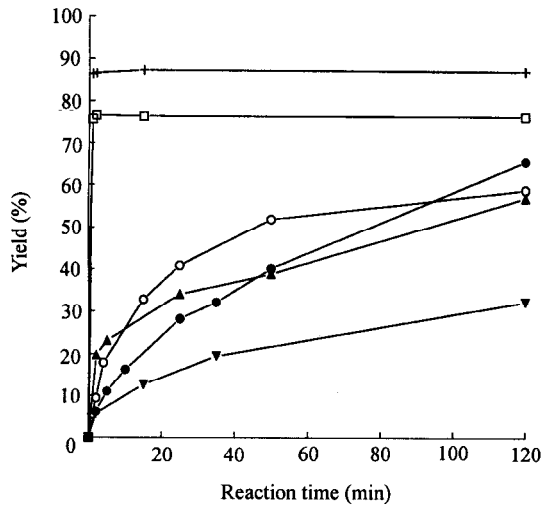


Fig. 2 Comparison of reactivity of various halogenated coupling reagents (+ FEP-HOAt, □ FEP, ○ BTFFH, ▼ PyClU, ▲ CMBI, ● PyBroP). The reaction model and conditions were the same as in Table 2.

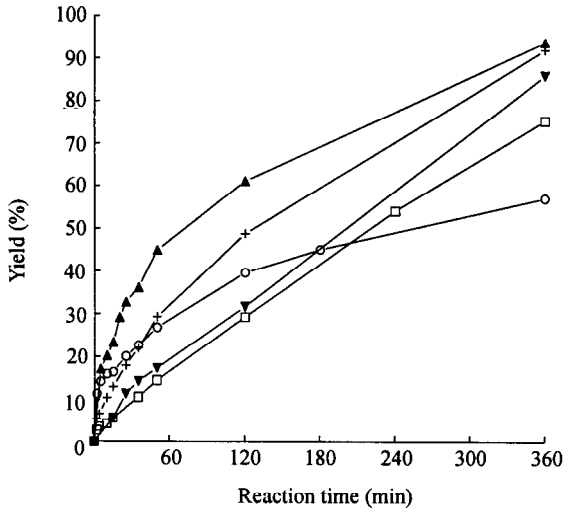
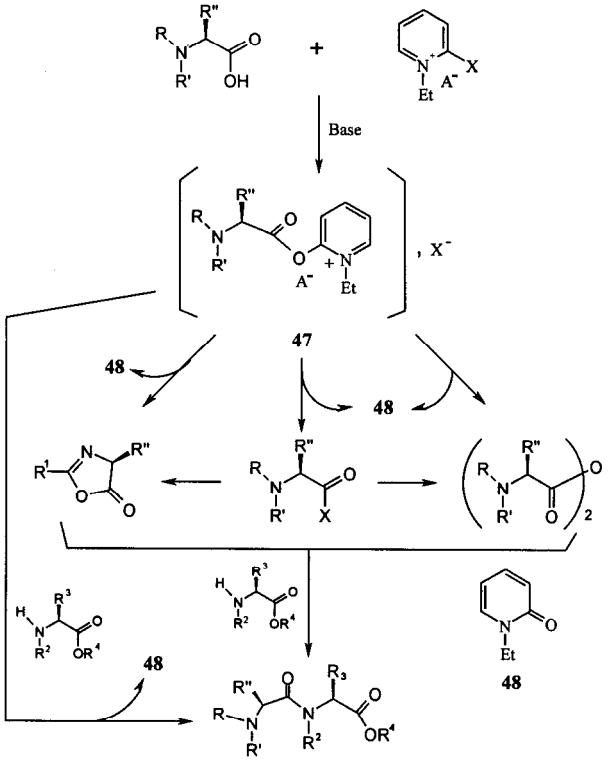


Fig. 3 Comparison of reactivity of BEP with other coupling reagents (+ BEP, □ PyBroP, ○ CMBI, ▼ BOP-Cl, ▲ BEMT). Model reaction: Z-MeVal-OH + MeVal-OCH_3 · HCl → Z-MeVal-MeVal-OCH_3. Reaction conditions: *T*: 25℃; Base: DIEA; Solvent: CH_2Cl_2 (10 L/mol); Substrate ratio: *N*-protected amino acid: amino acid ester hydrochloride: Coupling reagent: Base = 1:1.1:1.1:3.2.

zolone and symmetric anhydride of Boc-Val-OH are generated as minor active intermediates during coupling. The former was also very liable to racemization due to its tautomerization and enolization (Fig. 4). Similarly, the first step of carboxylic acid activation by thiazolium type reagent BEMT involves the formation of an unstable acyloxythiazolium salt **49**, which either reacts rapidly with the amino component to give the product, or is competitively converted into the acid bromide before reacting with the amino component to give the product. The corresponding acid anhydride and 5(4*H*)-oxazolone are also formed as minor intermediates during coupling. The main by-product of the reaction is *N*-ethyl-4-methyl thiazolidone **50**.[13]

Comparing to halouronium and halophosphonium type coupling reagents, the high reactivity of pyridinium and thiazolium type reagents may be attributed to the high susceptivity of 2-halopyridinium and 2-halothaizolium salts themselves, as well as the higher reactivity of the generated (acyloxy) pyridinium and (acyloxy) thiazolium intermediates than those of (acyloxy) uronium and (acyloxy) phosphonium intermediates due to the obvious electronic effect.



R= Z, Fmoc, Boc
R¹= OBzl, OFm, OBu^t
R", R^3 = amino acid side chain
R^2, R' = H, Me, alkyl
R^4 = Me, Et, Bzl, Bu^t, *et al.*

Fig. 4 Proposed mechanism for the 2-halopyridinium salts mediated coupling reactions.

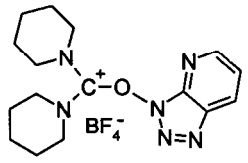
Coupling reagents for cyclic peptide synthesis

The structural features of cyclic peptides, *i.e.* the absence of polar *C*- and *N*-termini and a high proportion of *cis* amide bonds, confer greater stability to proteolytic enzymes, which can increase bioavailability and therapeutic potential. Furthermore, cyclic structures reduce the conformational freedom of the peptide and often result in high receptor binding affinities by reducing unfavorable entropic effects. For these reasons, the cyclic peptides often make promising leading compounds for drug discovery. Therefore, chemical syntheses of these cyclic peptide or peptidomimetic derived by SAR studies are very useful and significative.

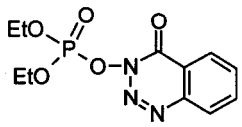
For the synthesis of macrocyclic peptide, the ring disconnection plays an important role towards the peptide macrocyclization reactions. Proper disconnection can increase cyclization rates and suppress such side-reactions as dimerization, oligomerization, and racemization of the *C*-terminal residue. The guidelines for ring disconnection have been well studied and reviewed,[14] and therefore are not covered in this account. Another important factor affecting peptide macrocyclizations is the rational selection of coupling methods. Although the mixed anhydride and active ester methods sometimes give good results, macrolactamization using coupling reagents is still of practical importance. Among the commonly used coupling reagents, HOAt-derived uronium/aminium salts, such as HAPyU, TAPipU and HTAU, have been shown to be very efficient in terms of reaction speed and low racemization extent. HOBt-based uronium, phosphonium and immonium salts also demonstrate good performance in peptide macrocyclization, but usually result in relatively high racemization than the HOAt-derived analogues, especially for the cyclization of the peptide that do not containing turn-inducing units such as Gly, Pro, or *N*-alkyl amino acid residues. The active intermediates of the macrocyclizations using these HOBt- or HOAt-based onium salts are the benzotriazolyl or 7-azabenzotriazolyl esters of the acid terminals. Using onium salts HAPyU or BDMP, we have achieved the macrocyclization of the undecapeptide H-D-Ala-MeLeu-MeLeu-MeVal-MeLeu-Nva-Sar-MeLeu-Val-MeLeu-Ala-OH to give immunosuppressive cyclic peptide Cyclosporin O in 84% and 68% yield, respectively. The cyclization of pentapeptide Leu-enkephalin was also realized by using HBPyU to give cyclo-Leu-enkephalin in nearly quantitative yield.[15]

Reagent DEPBT demonstrates high efficiency in peptide coupling and macrocyclization.[16] Elicited by the good results of pentafluorophenyl ester method for cyclic peptide synthesis, we designed and synthesized reagent FDPP[17] and FDP (Scheme 8).[18] Reagent FDPP not only demonstrated high efficiency in oligopeptide and bioactive peptide synthesis, but also gave better results in peptide macrolactamization than other reagents, such as DPPA and DEPC.[19] Reagent BOP-Cl was also proved to be effective for peptide cyclization, especially when the *N*-terminal was hindered amino acid residues.[20]

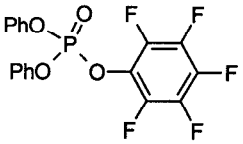
Scheme 8



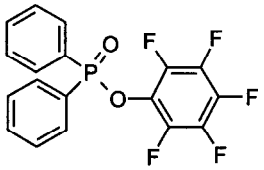
51 TAPipU



52 DEPBT



53 FDP



54 FDPP

Besides the two important factors affecting the results of peptide macrocyclization reactions, ring disconnection and selection of coupling method, other factors such as solution concentration, base, additives, ratio of substrates, temperature and reaction time, also affect these macrolactamization reactions. For complex target rings, model reactions must be done to help to optimize reaction procedure and conditions, and predict cyclization results.

Conclusion

The development of coupling reagents has significantly promoted the efficient synthesis of peptide. Recently developed HOBt-based immonium type reagents provide better choice for the synthesis of coded peptide

with low or negligible racemization. For sterically hindered nonribosomal peptide synthesis, 2-halopyridinium and 2-halothiazolium salts, such as BEP, FEP and BEMT, should be the most preferred candidates besides HOAt-based onium salts, especially for the preparation of peptide on large scale. As for the macrolactamization of peptide, HOAt-based onium salts, FDPP, DEPBT and BOP-Cl have been shown to be efficient to promote the macrocyclization reactions. These guidelines may vary for the synthesis of different peptide sequence because many factors influence peptide chain elongations or cyclizations. These general rules may provide useful references during the rational selection of coupling reagents for the efficient synthesis of peptide or peptidomimetics targets.

References and notes

Abbreviation: α-aminoisobutyric acid (Aib); 5-(1*H*-7-azabenzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (AOMP); (1*H*-7-azabenzotriazol-1-yloxy) tris (dimethylamino) phosphonium hexafluoro-phosphate (AOP); 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (BDMP); 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate (BEMT); 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP); 2-bromo-1-ethyl pyridinium hexachloroantimonate (BEPH); *N*-(1*H*-benzotriazol-1-ylmethylene)-*N*-methylmethanaminium hexachloroantimonate *N*-oxide (BOMI); (1*H*-benzotriazol-1-yloxy) tris (dimethylamino) phosphonium hexafluorophosphate (BOP); *N*, *N*′-bis (2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl); 1-(1*H*-benzotriazol-1-yloxy) phenylmethylene pyrrolidinium hexachloroantimonate (BPMP); 1, 1, 3, 3-bis (tetramethylene) florouronium hexafluorophosphate (BTFFH); 2-chloro-1, 3-dimethyl 1*H*-benzimidazolium hexafluorophosphate (CMBI); chloro (4-morphoino) methylene morpholinium hexafluorophosphate (CMMM); dicyclohexylcarbodiimide (DCC); 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4 (3*H*)-one (DEPBT); diethylphosphorocyanidate (DEPC); *N*, *N*′-diisopropylethylamine (DIEA); diisopropylcarbodiimide (DIPCDI); 5-(3′,4′-dihydro-4′-oxo-1′,2′,3′-benzotriazin-3′-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (DOMP); 1-ethyl-3-(3′-(dimethylamino) propyl) carbodiimide (EDC); 4-dimethylaminopyridine (DMAP); diphenylphosphoryl azide (DPPA); pentafluorophenyl diphenylphosphate (FDP); pentafluorophenyl diphenylphosphinate (FDPP); 2-fluoro-1-ethyl pyridinium tetrafluoroborate (FEP); 2-fluoro-1-ethyl pyridinium hexachloroantimonate (FEPH); *O*-(1*H*-7-azabenzotriazol-1-yl)-(*N*, *N*′-dimethyl-*N*, *N*′-dimethyleneuronium hexafluorophosphate (HAMDU); *O*-(1*H*-7-azabenzotriazol-1-yl)-*N*, *N*, *N*′, *N*′-bis (pentamethylene) uronium hexafluorophosphate (HAPipU); 1-(1-pyrrolidinyl-1*H*-1, 2, 3-triazolo [4, 5-*b*] pyridin-1-ylmethylene) pyrrolidinium hexafluorophosphate *N*-oxide (HAPyU); (*N*-(dimethylamino)-1*H*-1, 2, 3-triazolo [4, 5-*b*] pyridin-1-ylmethylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU)); O-(1*H*-benzotriazol-1-yl)-*N*, *N*′-dimethyl-*N*, *N*′-dimethyleneuronium hexafluorophosphate (HBMDU); *O*-(1*H*-benzotriazol-1-yl)-*N*, *N*, *N*′, *N*′-bis (pentamethylene) uronium hexafluorophosphate (HBPipU); *O*-(1*H*-benzotriazol-1-yl)-*N*, *N*, *N*′, *N*′-bis (tetramethylene) uronium hexafluorophosphate (HBPyU); *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino) methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU); 1-hydroxy-7-azabenzotriazole (HOAt); 1-hydroxybenzotriazole (HOBt); 3-hydroxy-3, 4-dihydro-4-oxo-1, 2, 3-benzotriazine (HOOBt); *N*-hydroxysuccinimide (HOSu); (1*H*-7-azabenzotriazol-1-yloxy) tris (pyrrolidino) phosphonium hexafluorophosphate (PyAOP); (1*H*-benzotriazol-1-yloxy) tris (pyrrolidino) phosphonium hexafluorophosphate (PyBOP); bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP); chlorotripyrrolidinophosphonium hexafluorophosphate (PyCloP); chlorobispyrrolidinophenyl phosphonium hexachloroantimonate (PyCloPP); 1, 1, 3, 3-bis (tetramethylene) chlorouronium hexafluorophosphate (PyClU); 5-(succinimiddyloxy)-3, 4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (SOMP); *O*-(1*H*-7-azabenzotriazol-1-yl)-*N*, *N*, *N*′, *N*′-bis (pentamethylene) uronium tetrafluoroborate (TAPipU); tetramethylfluoromamidinium hexafluorophosphate (TFFH).

1. Castro, B.; Dromoy, J.R.; Evin, G.; Selve, C., *Tetrahedron Lett.*, 1219(1975).
2. Chen, S.Q.; Xu, J.C., *Tetrahedron Lett.*, **33**, 647 (1992).
3. Li, P.; Xu, J.C., *Tetrahedron Lett.*, **41**, 721(2000).
4. Anders, E.; Katritzky, A.R.; Malhotra, N.; Stevens, J., *J. Org. Chem.*, **57**, 3698(1992).
5. Li, P.; Xu, J.C., *Tetrahedron Lett.*, **40**, 3605(1999).
6. Li, P.; Xu, J.C., *J. Pept. Res.*, **55**, 110(2000).
7. Li, P.; Xu, J.C., *Chem. Lett.*, 1163(1999).
8. Li, P.; Xu, J.C., *Chin. J. Chem.*, **18**, 85(2000).
9. Carpino, L.A., *J. Am. Chem. Soc.*, **115**, 4397 (1993).
10. Albericio, F.; Bofill, J.M.; El-Faham, A.; Kates, S.A., *J. Org. Chem.*, **63**, 9678(1998).
11. Carpino, L.A.; El-Faham, A., *J. Am. Chem. Soc.*, **117**, 5401(1995).
12. Van der Auwera, C.; Anteunis, M.J.O., *Int. J. Pept. Protein Res.*, **29**, 574(1987).
13. Li, P.; Xu, J.C., *Tetrahedron Lett.*, **40**, 8301(1999).
14. Wenger, R.M., *Helv. Chim. Acta.*, **67**, 502(1984).
15. Chen, S.Q.; Xu, J.C., *Chin. Chem. Lett.*, **4**, 847 (1993).
16. Li, H.; Jiang, X.; Ye, Y.H.; Fan, C.X.; Romoff, T.; Goodman, M., *Organic Lett.*, **1**, 91(1999).

17. Chen, S.Q.; Xu, J.C., *Tetrahedron Lett.*, **32**, 6711 (1991).
18. Chen, S.Q.; Xu, J.C., *Chin. J. Chem.*, **13**, 175 (1995).
19. Deng, J.G.; Hamada, Y.; Shioiri, T., *Tetrahedron Lett.*, **37**, 2261(1996).
20. Ishiwata, H.; Sone, H.; Kigoshi, H.; Yamada, K., *J. Org. Chem.*, **59**, 4712(1994).

Peng LI was born in Hailin, China in 1972. He received his B.S. degree (1994) and M.S. (1997) at Dalian University of Technology under the direction of Prof. Zuwang Wu. As a Ph.D. candidate, He is currently studying at Shanghai Institute of Organic Chemistry (SIOC), Chinese Academy of Sciences under the supervision of Prof. Jiecheng XU. His research interests are the design and synthesis of novel peptide coupling reagents as well as the total synthesis of biologically active peptides and peptidomimics.
(E-mail: Lipeng@pub.sioc.ac.cn)

Prof. **Jiecheng XU** graduated from the department of Chemistry, Nanjing University in 1955 and achieved his graduate studies at the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences in 1962. He was appointed associate Professor in 1977, Professor in 1986. He was a visiting scholar at the University of Texas at Austin for the period of 1980—1982. He engaged in the total synthesis of Crystalline Bovine Insulin and was the co-recipient of the National Natural Science Award, China in 1982. He received the Cathay Award in 1996, which was sponsored by the H.H. Liu Education Foundation, and the QiuShi Scientific and Technological Award in 1997. His research interests focus broadly on peptide chemistry, synthesis of biologically active peptides, developing new peptide coupling reagents, as well as the design and synthesis of peptidomimics.
(E-mail: Xjcheng@pub.sioc.ac.cn)

(E9912181 SONG, J.P.; DONG, L.J.)