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New and Highly Efficient Immonium Type Peptide Coupling Reagents: Synthesis, Mechanism and Application

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Abstract—A series of novel immonium type coupling reagents BOMI, BDMP, BPMP, DOMP, SOMP, FOMP and AOMP were designed and synthesized. It was shown that most of these reagents were more efficient in peptide synthesis than conventional methods due to the advantages of high reactivity, low racemization and good yields. The reaction mechanism was proposed and studied by ^{1}H NMR, IR, UV and HPLC.

Introduction

In recent years a large number of the HOBt-based uronium and phosphonium type coupling reagents, such as PyBOP,[1] HBTU,[2] HBPyU,[3] HBPipU[4] and HBMDU,[5] have been developed and widely employed in peptide synthesis both in solution and solid phase since the first HOBt-derived phosphonium coupling reagent BOP was designed by Castro et al.[6] (Fig. 1). Although the predominance of carbodiimides and active ester methods are being gradually replaced with these onium salts based upon 1-hydroxybenzotriazole (HOBt),[7] the problem of racemization is still a challenge during the use of the above reagents.[3a,8] To suppress the level of racemization and enhance the coupling efficiency, the HOAt-derived phosphonium and uronium reagents, such as HATU, HAPyU, AOP, PyAOP,[9] were suggested with good virtues because of the anchimeric assistance effect of HOAt.[10] Here we report an alternative pathway to enhance the coupling efficiency by modifying the carbon skeleton of uronium salts by replacing one of the substituted amino groups with a hydrogen, alkyl or aryl group. Thus the novel immonium type coupling reagents were exploited and we observed that the reactivity of these reagents was much higher than that of the corresponding uronium salts. Using these immonium reagents to synthesize peptides not only enhanced the coupling efficiency but also substantially suppressed the extent of racemization under relatively mild reaction conditions.

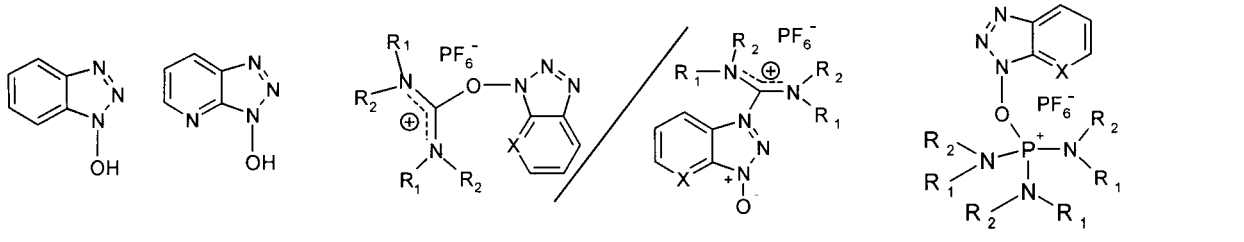

Figure 1. General structures of HOBt- and HOAt-based uronium/aminium and phosphonium type coupling reagents.

Keywords: peptide coupling reagents; reaction mechanism; immonium salts.

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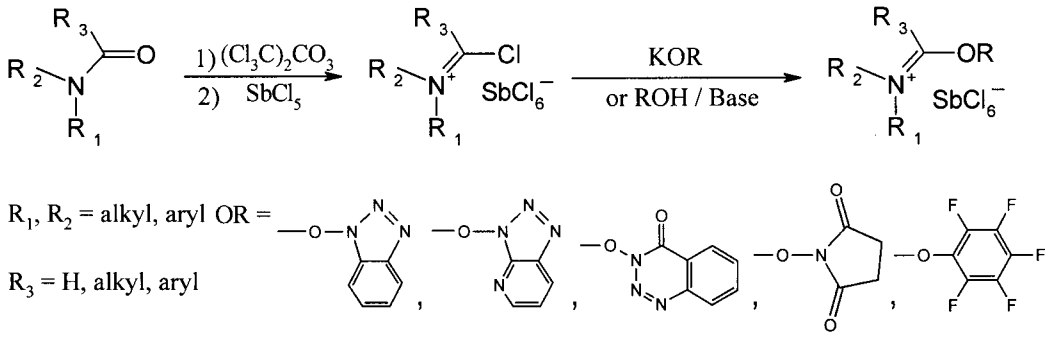

Scheme 1. Synthesis of immonium type coupling reagents.

Results and Discussion

HOBt- or HOAt-based onium salt-mediated amide formation reaction generally involves two steps, activation and coupling.[11] The activation step, in which coupling reagent reacts with *N*-protected amino acid to form the HOBt or HOAt active ester of the carboxylic acid, was mainly influenced by the nature of the carbon skeleton structure besides the influence of the carboxylic component. The coupling step, in which the active ester of the *N*-protected amino acid reacts with the amino component to form the peptide, was mainly determined by the nature of the active ester moiety of the coupling reagent and, of course, amino component. Therefore, one effective way to enhance the coupling efficiency is to modify the active ester moiety of the molecules, which has been proved by the successful design and application of HOAt-based onium salts to replace the HOBt, HOPfp, HOSu or HODhbt-derived onium salts. While our approach to enhance the coupling efficiency is based on the modification of the structures of the carbocation skeleton moiety of uronium/aminium type reagents which display relatively higher reactivity and lower racemization than the corresponding phosphonium analogues.[12]

Considering the structural feature of uronium salts shown in Fig. 1, it is obvious that the two substituted amino groups of these molecules provide two equal resonance structures to stabilize these uronium salts, the lone electron pairs of the two nitrogen atom are delocalized within the bonds N–C–N, which causes the carbocation to share a relatively high electron density; consequently the nucleophilic attack of the carboxylate anion is impeded during the activation step. To overcome this drawback, a series of novel immonium salts were designed and synthesized by replacing one of the substituted amino groups of the uronium reagents with hydrogen, alkyl or aryl. Thus the electron density of the central carbon of immonium salts was much lower than that of corresponding uronium salts, and resulted in improvement of the reactivity of these immonium type reagents due to the electronic effect.

These immonium type reagents can be readily prepared by treating *N*-disubstituted amides with bis(trichloromethyl) carbonate to yield the corresponding immonium chlorides, which were stabilized with $SbCl_5$ and subsequently reacted with the potassium salts of active hydroxyl compounds or hydroxyl compounds themselves in the presence of tertiary amine, such as NEt_3, to afford the desired compounds as crystalline and shelf-stable solids (Scheme 1).

Concerning the stability, activity, simplicity and easy availability of the amides, *N*,*N*-dimethyl formamide (DMF), *N*-methyl pyrrolidone (NMP) and *N*-benzoyl pyrrolidine were selected as substrates, thus a series of immonium type coupling reagents were synthesized. Some representative immonium salts, such as BOMI, BDMP, BPMP,

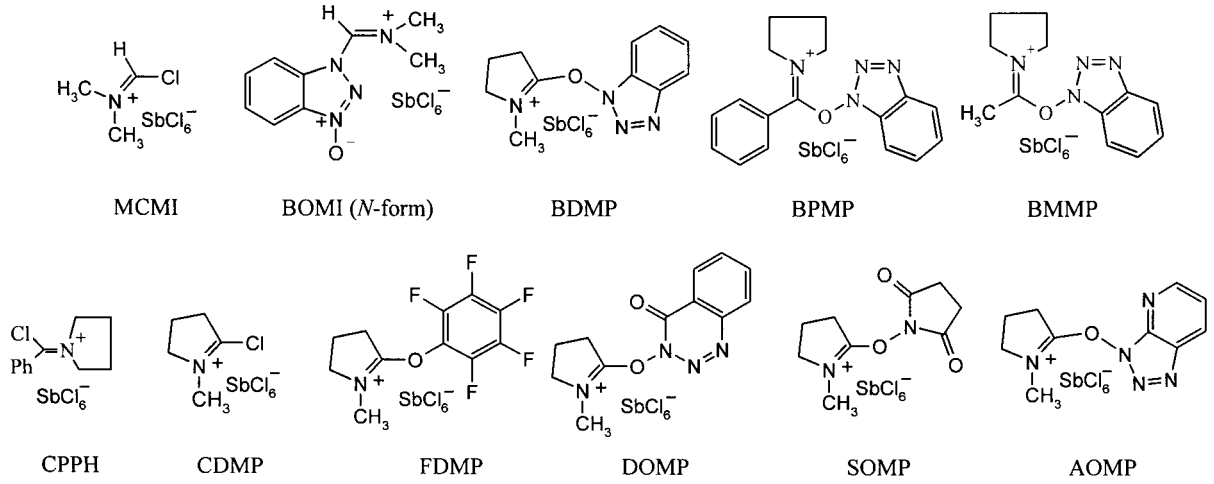

Figure 2. Structures of novel immonium type coupling reagents and their intermediates.

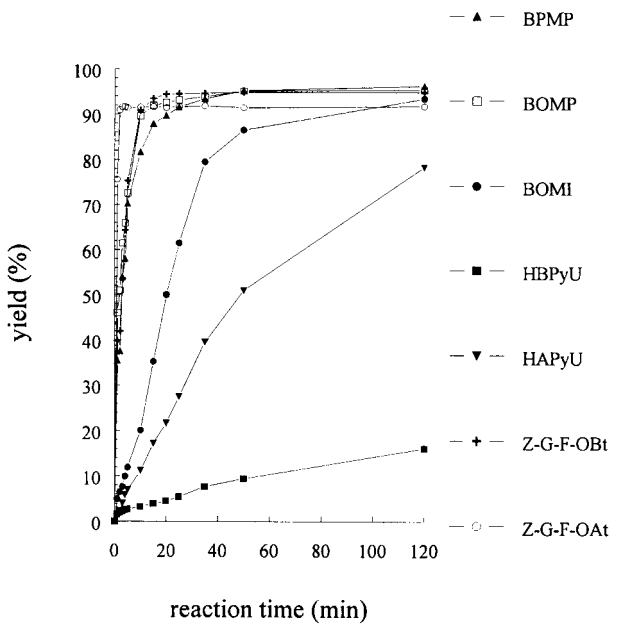

Figure 3. Comparison of reactivity of HOBt-based immonium salts vs HBPyU and HAPyU (reaction conditions: *T*, 10°C; base, 2,6-lutidine; solvent, THF. Substrate ratio: *N*-protected amino acid/amino acid ester hydrochloride/coupling reagent/base=1:1.1:1.1:3 (mol)).

DOMP, FOMP, SOMP, AOMP and their intermediates are listed in Fig. 2. X-Ray analysis indicated that the reagent BOMI presents as the *N*-substituted form[13] shown in Fig. 2, rather than as the isomeric *O*-substituted form.[14,15] This kind of isomerization was also observed in the HOBt-based esters,[16] and can be explained by molecular orbital calculations using the semi-empirical method PM3.[17]

The efficiency of these immonium reagents was evaluated by HPLC monitoring the segment coupling reaction: Z-Gly-Phe-OH+Val-OCH_3·HCl→Z-Gly-Phe-Val-OCH_3, which was first adopted by Van der Auwera et al.[18] From the results shown in Fig. 3 and Table 1, it is obviously that, both in reactivity and in racemization, HOBt-based immonium salts are superior to the uronium salt HBPyU which was very reactive among HOBt-based phosphonium and uronium salts,[3a,12] even better than HOAt-derived uronium salt HAPyU, one of the most efficient reagents up to now.[11,19] For example, the yield is up to 20.1% for BOMI, 89.6% for BDMP, 81.7% for BPMP, meantime only 3.3% for HBPyU and 11.2% for HAPyU after 10 min reaction at −10°C with 2,6-lutidine as base in THF (Table 1, entries 3, 7, 9, 13, 14). The extremely high reactivity of these reagents can also be seen by the result of entry 2. Adopting strong base DIEA or NMM, the coupling reaction was completed within 5 min using BOMI, even at −70°C. The efficiency of HOBt-base immonium salts, such as BOMI, can also be further enhanced by the addition of HOAt (entry 6).

Among these immonium salts, HOBt-based immonium salts were shown to be the most promising. The rationality of the molecular design based upon the modification of HOBt-based uronium salts was also confirmed by the high reactivity of the HOBt-immonium salts. Although HOAt-derived immonium salt AOMP demonstrated high reactivity and low racemization, the coupling yield was not satisfactory yet. It was most likely that the extremely high reactivity of AOMP led to decomposition before activation of the *N*-protected amino acid was achieved in the presence of base, such as 2,6-lutidine. As for the HODhbt-derived reagent DOMP, it also showed low racemization, but the yield was relatively low. HOPfp- and HOSu-derived immonium salts give poor results due to the low reactivity of the pentafluorophenyl esters or succinimidyl esters formed in situ during coupling. It has been reported that benzotriazolyl esters undergo aminolysis up to 10^3 times faster than succinimidyl and related esters.[20] Among the developed HOBt-based immonium salts, the reactivity of BDMP was the highest which was probably due to the tension of the intra-annular imide bond of the molecule. The angles of C^{sp2}–N–C_2^{sp3} and N–C^{sp2}–C_4^{sp3} in the molecule of BDMP are equal to 105.7 and 113.7°, respectively, by calculating with PCMODEL software.[21] This tension or distortion may

Table 1. Comparison of reactivity and racemization of immonium type reagents with uronium type reagents and active esters using HPLC (model reaction: Z-Gly-Phe-OH+Val-OCH_3·HCl+coupling reagent+base→Z-Gly-Phe-Val-OCH_3)

Entry	Coupling reagent	Reaction conditions			Yield (%)			Reactivity $t^{1/2}$ (min)	Racemization DL%[a]
		Base	Temp. (°C)	Solvent	2 min	10 min	120 min		
1	BOMI	NMM	−30	CH_3CN	89.9	90.3	90.4	<1	3.1
2	BOMI	NMM	−70	CH_2Cl_2	87.6	89.1	89.3	<1	3.0
3	BOMI	2,6-Lutidine	−10	THF	6.43	20.1	93.4	20	3.1
4	BOMI[b]	DIEA	−10	CH_3CN	12.7	49.2	72.7	10.1	4.2
5	BOMI[b]	2,6-Lutidine	−10	THF	69.1	80.4	80.5	<1	3.3
6	BOMI+HOAt	2,6-Lutidine	−10	THF	41.1	73.5	86.0	4.5	1.7
7	BDMP	2,6-Lutidine	−10	THF	51.0	89.6	95.3	1.9	2.2
8	AOMP	2,6-Lutidine	−10	THF	64.1	64.2	64.2	<1	1.6
9	BPMP	2,6-Lutidine	−10	THF	37.6	81.7	96.2	2.8	2.3
10	BPMP	2,6-Lutidine	−10	THF	49.6	67.9	69.1	2.0	2.3
11	DOMP	2,6-Lutidine	−10	THF	–	–	5.57	>120	19.1
12	FOMP	2,6-Lutidine	−10	THF	9.2	13.8	54.3	110	12.2
13	HBPyU	2,6-Lutidine	−10	THF	2.0	3.29	16.1	>120	14.6
14	HAPyU	2,6-Lutidine	−10	THF	2.4	11.2	78.3	49	3.3
15	Z-G-F-OBt	2,6-Lutidine	−10	THF	42.0	90.8	94.8	2.7	4.4
16	Z-G-F-OAt	2,6-Lutidine	−10	THF	90.9	91.5	91.7	<1	

[a] DL% equal to D-isomer% multiplied by two.
[b] Preactivated 10 min before the amino component Val-OCH_3·HCl was added.

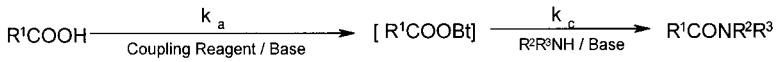

Figure 4. The two steps of the HOBt-based reagents mediated coupling reactions.

disturb the delocation of the lone electron pair of the nitrogen atom over the C=N$^+$ bond, consequently, lead the carbonation to possess relatively low electron density, which will be in favor of the nucleophilic attack of carboxylate anion.

To explain the high efficiency of HOBt-based immonium salts, the mechanism of the immonium salts mediated coupling reaction was proposed and studied. Since the amino component of the adopted model reaction was a primary amine without obvious hindrance and the benzotriazolyl esters elicited very high reactivity towards aminolysis in the presence of base, the rate of the amide formation reaction was substantially determined by the activation step, the formation of the benzotriazolyl esters, which determined the net overall rate $k \approx k_a$ (Fig. 4). Thus the reaction speed probably depends on the nature of the carbon skeleton of the coupling reagents when the amino component was an unhindered coded amino acid. This was confirmed by treating the preformed active ester intermediate Z-G-F-OBt with the amino component Val-OCH_3·HCl at −10°C in the presence of 2,6-lutidine to give over 90% yield after only 10 min (Table 1, entry 15). This indicated that the relatively higher reactivity of HOBt-based immonium salts than uronium salts HBPyU and HAPyU was mainly because the immonium carbon skeleton was more sensitive towards the attack of carboxylate anion than the uronium/aminium carbon skeleton, that is, $k_1 > k'_1$ (Fig. 5). The acyloxyimmonium salt intermediate **I** was also more reactive than the corresponding acyloxyuronium salt intermediate **I′**, that is, $k_2 > k'_2$, but this may not be the rate-determining step and not influence the overall reaction speed due to their extremely high reactivity.

Unexpectedly the reaction speed was slow and the racemization was increased during the model reaction carried out by the 10 min preactivation of the carboxylic component via BOMI mediation using DIEA as base (Table 1, entry 4). However the reaction speed was remarkably increased by the use of the weak base 2,6-lutidine (entry 5). This

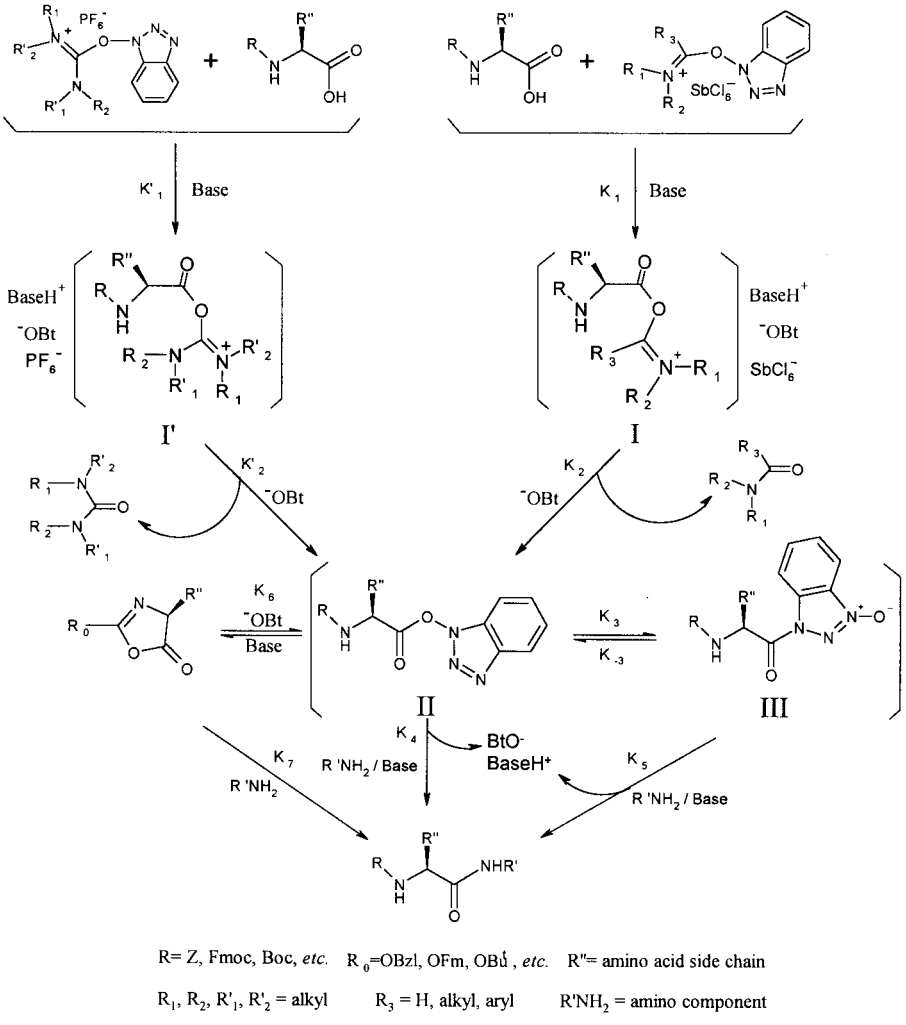

Figure 5. Proposed mechanism of the HOBt-derived immonium and uronium salts mediated coupling reaction.

Table 2. The spectra of the model active ester Z-G-F-OBt

Solvent	UV			IR		^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm)
	λ_{max} (nm)		Absorption (A)	ν(C=O) (cm^{-1})		
None (KBr)	–	–		**1808 (m)**	**O-form**	
				1722 (vs)	*N-form (overlapped)*	
CH_3CN				1660 (s)		7.05–8.38 (m, 14H, aryl)
	254		2.2114	**1821 (m)**	**O-form**	6.95, 6.76 (2d, J=7Hz,1H,NH-Phe)
	282	**O-form**	**1.0088**	1729 (vs)		5.74, 4.83 (2m, 1H, α-CH-Phe)
	328	N-form	0.8148	1715 (sh)	*N-form (partly overlapped)*	5.56, 5.61 (2br, 1H, NH-Gly)
	342		0.7819	1685 (m)		5.08–5.20 (m,2H,CH_2-CBZ)
				1635 (s)		3.76–4.09 (m, 2H, α-CH_2-Gly)
$CHCl_3$	256		0.5692	**1818 (m)**	**O-form**	3.18, 3.39 (2m, 2H, β-CH_2-Phe)
	286	**O-form**	**0.3337**	1732 (vs)	*N-form (partly overlapped)*	
	328	N-form	0.0517	1689 (vs)		
	342		0.0453			

phenomenon can be attributed to the fact that the preformed active intermediate benzotriazolyl ester of *N*-protected amino acid was gradually transformed into the less reactive and racemization prone species 5-(4*H*) oxazolone in the presence of base, especially the strong base DIEA.[22] ($k_7<k_4,k_5$).

As shown in Table 1, the rate of the coupling reagent BDMP mediated reaction is equal to, or even faster than, that of Z-G-F-OBt (Table 1, entries 7 and 15). It is suggested that the structural feature of BDMP considerably accelerates the speed of the activation step and leads to k_a nearly equal to k_c. This result may provide an insight into the mechanism of immonium salt-mediated coupling reactions and support the rationality of the molecular design of BDMP. The reason for the slightly higher reactivity of BDMP than the preformed active ester Z-G-F-OBt was most probably that the *O*-form isomer of the benzotriazolyl ester intermediate **II** is more reactive than the corresponding *N*-oxide isomer **III**[23] ($k_4>k_5$, Fig. 5) and the initially generated active ester from the BDMP mediation exists as the *O*-acylated isomer **II** under mild reaction conditions,[16b] and it reacts rapidly with the amino component to afford product ($k_4>k_3$); while a certain amount of the less reactive *N*-acylated isomer **III** seems to be involved in the case of the preformed ester Z-G-F-OBt. To verify the above speculation we studied and characterized the isomerization of the active ester by UV, IR and ^{1}H NMR. The UV spectra indicated that, besides the existence of the *O*-isomer characterized by the absorption at 1805–1825 cm^{-1}, Z-G-F-OBt did exist, to some extent, in the *N*-acylated form revealed by the characteristic absorption of *N*-oxide at 328 and 342 nm, and it appeared to a greater extent in CH_3CN than in $CHCl_3$ due to the higher polarity of CH_3CN stabilizing the *N*-oxide (Table 2). The ^{1}H NMR also revealed numerous signals corresponding to benzotriazole residues from the two forms of Z-G-F-OBt in the δ=7.1–8.4 ppm region. Similarly, during the preparation of Fmoc-MeVal-Sar-OBzl by treating Fmoc-MeVal-OH and Sar-OBzl-HCl with reagent BDMP in the presence of 2,6-lutidine at −10°C, the active intermediate Fmoc-MeVal-OBt was also isolated besides the dipeptide. This benzotriazolyl ester also exists in two forms, the less polar *O*-acylated isomer and the more polar *N*-acylated isomer, which can be separated by preparative thin layer chromatography. Either of the separated isomers gradually isomerises into two forms when dissolved in solvent.

As examined above, we evaluated the excellent performance of HOBt-based immonium type coupling reagents and discussed the mechanism and kinetics of coupling reactions mediated by these reagents using the model reaction: Z-Gly-Phe-OH+Val-OCH_3·HCl→Z-Gly-Phe-Val-OCH_3, in which the amino component is an unhindered primary amine. When an *N*-alkyl or C_α, C_α-disubstituted amino acid, such as Sar-OBzl·HCl or Aib-OCH_3·HCl was used as amino component, the coupling step becomes the key step of the peptide formation reaction ($k_a>k_c$). There is still a certain amount of active ester intermediate unreacted, and it

Table 3. Syntheses of amides and esters using HOBt-based immonium salts as coupling reagents (reaction conditions: base, 2,6-lutidine; temp., −10°C→r.t.; substrates ratio, carboxylic acid/amine/coupling reagent/base=1:1.1:1.1:3 (mol))

Entry	Substrate		Main product	Reagent	Reaction time	Yield (%)[a]
	Carboxylic acid	Amine				
1	Z-Gly-Phe-OH	Val-OCH_3·HCl	Z-Gly-Phe-Val-OCH_3	BPMP	2 h	96.2
2	Z-Gly-Phe-OH·DCHA	Val-OCH_3·HCl	Z-Gly-Phe-Val-OCH_3	BOMI	2 h	64.5
3	PhCOOH	Not added	PhCOOBt	BOMI	2 h	87.5
4	PhCOOH	$PhNH_2$	PhCONHPh	BOMI	2 h	90.0
5	PhCOOH	CHA	$PhCONHC_6H_{11}$	BOMI	2 h	79.4
6	PhCOOH	DCHA	PhCOOBt	BOMI	2 h	75.8
7	Z-Aib-OH	Not added	Z-Aib-OBt	BDMP	1 h	88.7
8	Z-Aib-OH	Aib-OCH_3·HCl	Z-Aib-OBt	BOMI	1 h	36.2
9	Fmoc-MeVal-OH	Sar-OBzl-OCH_3·HCl	Fmoc-MeVal-OBt	BDMP	3 h	51.6

[a] Isolated yield based on carboxylic acid component except entry 1 which was the yield determined by HPLC.

can be isolated from the reaction mixture during reaction with hindered amines, such as Z-Aib-OBt and Fmoc-MeVal-OBt (Table 3, entries 8, 9). In the case of more hindered dicyclohexylamine (DCHA), the reaction stops at the HOBt ester stage and the corresponding amide was not observed (entry 6), it appears that the DCHA salt of carboxylic acid component can be used directly in peptide synthesis (entry 2). In the absence of amine components, the corresponding active esters are obtained as the final product (entries 3, 7). These results also give an insight into the mechanism discussed above.

To verify the applicability of these novel immonium salts, we synthesized a series of oligopeptides, biological peptides both in solution and solid phase with good yield, and low racemization.[14] We also synthesized an immunosuppressive cyclic undecapeptide cyclosporin O using these reagents combined with other reagents designed by us.[24] These reagents can also be used in ester formation with satisfactory yield, especially the preparation of active esters such as benzotriazolyl ester, pentafluorophenyl ester and succinimidyl ester which are widely used in the syntheses of lactones and lactams.

In conclusion, several novel immonium coupling reagents BOMI, BDMP, BPMP, SOMP, DOMP, FOMP and AOMP, which can be easily prepared from the corresponding amide, were designed and synthesized by modifying the structure of the uronium/aminium salts. HPLC monitoring of these immonium salts mediated coupling reaction demonstrated that these novel reagents, especially the HOBt and HOAt-derived immonium salts, displayed extremely high reactivity and low racemization. Among the HOBt-based immonium salts, BDMP was the most efficient in terms of high reactivity, low racemization and good yields. In the case of the coupling of normal coded amino acids and peptide segments, HOBt-derived immonium salts, such as BOMI, BDMP and BPMP were even more efficient than the HOAt-derived uronium/aminium reagent HAPyU, the high reactivity of these reagents may be attributed to their structural features. However, in the case of the coupling of *N*-methyl or C_α,C_α-disubstituted amino acids, the HOAt-based reagents are more efficient since the reaction rate is mainly determined by the reactivity of the active ester intermediates.

Experimental

Amino acid derivatives were obtained from commercial sources or synthesized according to the literature. Melting points were determined in capillary tubes and are uncorrected. IR spectra were measured with an IR-440 spectrometer. ^{1}H NMR spectra were recorded on Bruker AM 300 MHz and are reported as parts-per-million (ppm) downfield from a tetramethylsilane internal standard. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br). Mass spectra were taken with HP5890A, and VG QUATTRO mass spectrometers. Elemental analyses for carbon, hydrogen and nitrogen were determined on a MOD-1106 elemental analyzer. Optical rotations were determined using a Perkin–Elmer 241 MC polarimeter. HPLC were carried out on Varian-SY-5000 with Kromasil RP-18 (0.5×25 cm) column. Flash column chromatography was performed with 300–400 meshes silica gel, and analytical thin layer chromatography was performed on precoated silica gel plates (60F-254) with the systems (v/v) indicated. Solvents and reagents were purified by standard methods as necessary. Amino acids were L-configuration if not otherwise stated.

Preparation of immonium type coupling reagents

Dimethylchloromethaniminium hexachloroantimonate (MCMI, general procedure I). A solution of *N*,*N*-dimethyl formamide (0.77 mL, 10 mmol) in CH_2Cl_2 was added dropwise to a solution of bis(trichloromethyl)carbonate (0.989 g, 3.33 mmol) in CH_2Cl_2 (5 mL) at 0°C under nitrogen atmosphere. After being stirred approximately 1 h, when the evolution of carbon dioxide has ceased, a 0.887 M solution of $SbCl_5$ in $CHCl_3$ (10.7 mL) was added dropwise at −30°C under vigorous stirring. The reaction mixture was stirred at 0°C for 2 h, the resultant suspension was filtered under nitrogen atmosphere, the filter cake was washed with cold CH_2Cl_2 and dried in vacuo. Recrystallization from CH_3CN–CH_2Cl_2 gave pure product as colorless crystals (4.07 g, 86.9%), mp 220–222°C; [Found: C, 8.59; H, 1.72; N, 3.25. $C_3H_7Cl_7NSb$ requires C, 8.44; H, 1.65; N, 3.28%]; ν_{max} (KBr) 3066w, 1658s, 1436m, 1407sh, 1322m, 1152m, 1033m, 800w, 710w; ^{1}H NMR ([D$_6$]acetone): δ=8.25 (s, 1H, α-*H*), 3.20 (s, 3H, C*H*$_3$), 3.02 (s, 3H, C*H*$_3$). EI-MS *m*/*z*: 92 $[M-SbCl_6]^+$, 94 $[M-SbCl_6+2]^+$.

***N*-(1*H*-Benzotriazol-1-ylmethylene)-*N*-methylmethanaminium hexachloroantimonate *N*-oxide (BOMI, general procedure II).** KOBt (0.173 g, 1 mmol) was added to a solution of MCMI (0.427 g, 1 mmol) in dry CH_3CN (3 mL) at −30°C with stirring under nitrogen atmosphere. After the reaction mixture was stirred at room temperature for 2 h, it was filtered and the filtrate was concentrated under reduced pressure, the residue was recrystallized from CH_3CN–Et_2O to give the yellowish crystalline product BOMI (0.460 g, 87.4%), mp 152–153°C (dec.); [Found: C, 20.78; H, 2.12; N 10.63. $C_9H_{11}Cl_6N_4Osb$ requires C, 20.56, H, 2.11, N, 10.66%]; ν_{max} (KBr) 3050w, 1702s, 1496m, 1461sh, 1446m, 1388m, 1340m, 1043m, 755w, 732w; ^{1}H NMR ([D$_6$]acetone): δ=7.99 (1H, s, α-*H*), 7.95–7.45 (4H, m, aryl), 2.97 (3H, s, C*H*$_3$), 2.81 (3H, s, C*H*$_3$).

5-Chloro-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (CDMP). CDMP was synthesized from *N*-methyl pyrrolidone according to the general procedure I. Yield 90.7%. Mp 191–193°C (dec). [Found: C, 13.47; H, 2.06; N, 3.03; Cl, 54.99. $C_5H_9Cl_7NSb$ requires C, 13.25; H, 2.00; N, 3.09; Cl, 54.78.]; ν_{max} (KBr) 2922w, 1661vs, 1462w, 1446w, 1410m, 1320s, 1121m, 1095w. ^{1}H NMR ([D$_6$]acetone): δ=3.60 (t, *J*=7 Hz, 2H, α-C*H*$_2$), 2.91 (s, 3H, C*H*$_3$), 2.59 (t, *J*=8 Hz, 2H, γ-C*H*$_2$), 2.04–2.16 (m, 2H, β-C*H*$_2$). FABMS: 118 $[M-SbCl_6^-]^+$.

5-(1*H*-Benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (BDMP). BDMP was synthesized from CDMP and KOBt according to the general procedure II. Yield 88.2%. Mp 165–166°C (dec.); [Found:

C, 23.83; H, 2.13; N, 10.24. $C_{11}H_{13}Cl_6N_4Osb$ requires C, 23.95; H, 2.37; N, 10.15%]; ν_{max} (KBr) 3127w, 1655vs, 1496s, 1479m, 1445m, 1314m, 1165m, 1066m, 763m, 640w. ^{1}H NMR ([D$_6$]acetone): δ=7.34–7.95 (m, 4H, aryl), 3.48 (t, J=7 Hz, 2H, α-CH_2), 2.83 (s, 3H, CH_3), 2.39 (t, J=8 Hz, 2H, γ-CH_2), 1.98–2.09 (m, 2H, β-CH_2). FABMS: 217 [M−SbCl$_6^-$]$^+$.

5-(*N*-Succinimidyloxyl)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (SOMP). SOMP was synthesized from CDMP and KOSu according to the general procedure II. Yield 89.3%. Mp 166–168°C (dec.); [Found: C, 20.22; H, 2.45; N, 5.23.$C_9H_{13}Cl_6N_2O_3Sb$ requires C, 20.33; H, 2.46; N, 5.27%]; ν_{max} (KBr) 2978w, 1754vs, 1710vs, 1454s, 1420m, 1400m, 1335m, 1216s, 1162s, 1067m, 801m, 633w; ^{1}H NMR ([D$_6$]acetone): δ=3.70 (t, J=7.2 Hz, 2H, α-CH_2), 3.00 (s, 3H, *N*-CH_3), 2.71 (t, J=7.5 Hz, 2H, γ-CH_2), 2.68 (s, 4H, 2CH_2–OSu), 2.12–2.24 (m, 2H, β-CH_2). FABMS: 197 [M−SbCl$_6^-$]$^+$.

5-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3yloxy)-1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl pyrrolium hexachloroantimonate (DOMP). DOMP was synthesized from CDMP and KODhbt according to the general procedure II. Yield 83.6%. Mp 120–121°C (dec.); [Found: C, 24.71; H, 2.24; N, 9.72. $C_{12}H_{13}Cl_6N_4O_2Sb$ requires C, 24.86; H, 2.26; N, 9.66%]; ν_{max} (KBr) 1725vs, 1708sh, 1681sh, 1460m, 1395s, 1174w, 943s, 768m, 676m; ^{1}H NMR ([D$_6$]acetone): δ=8.29 (d, J=8.3 Hz, 1H, 8-C*H*-aryl), 8.18 (d, J=8.1 Hz, 1H, 5-*H*-aryl), 8.08 (m, 1H, 7-C*H*-aryl), 7.92 (m, 1H, 6-C*H*-aryl), 3.66 (t, J=7.4 Hz, 2H, α-CH_2), 2.96 (s, 3H, CH_3), 2.66 (t, J=7.4 Hz, 2H, γ-CH_2), 2.15 (m, 2H, β-CH_2).

5-Pentafluorophenyloxy-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (FOMP). FOMP was synthesized from CDMP and KOPfp according to the general procedure II. Yield 85.7%. Mp 182–183°C (dec.); [Found: C, 21.50; H, 1.34; N, 2.16. $C_{11}H_9Cl_6F_5NOSb$ requires C, 21.99; H, 1.50; N, 2.33%]; ν_{max}(KBr) 1705s, 1530sh, 1520s, 1398m, 1034w, 1001m, 954w cm^{-1}. ^{1}H NMR ([D$_6$]acetone): δ=3.46 (t, J=7 Hz, 2H, α-CH_2), 2.81 (s, 3H, CH_3), 2.35 (t, J=8 Hz, 2H, γ-CH_2), 2.02 (m, 2H, β-CH_2). ^{19}F NMR ([D$_6$]acetone, CF$_3$COOH) δ=−76.8 to −77.1 (m, 2F), −80.2 to 80.3 (m, 2F), −91.1 to −91.4 (m, 1F). FABMS: 266 [M−SbCl$_6^-$]$^+$.

Chlorophenylmethylene pyrrolidinium hexachloroantimonate (CPPH). CPPH was synthesized from *N*-benzoyl pyrrolidine according to the general procedure I. Yield 82.6%. Mp 176–177°C; ν_{max} (KBr) 1621vs, 1438s, 1343m, 1267m, 769m, 692s, 647w; ^{1}H NMR ([D$_6$]acetone): δ=1.98 (m, 4H, 2β-CH_2), 3.62 (m, 4H, 2α-CH_2), 7.34–7.63 (m, 5H, Ph). FABMS: 194 [M−SbCl$_6^-$]$^+$, 196 [M−SbCl$_6^-$+2]$^+$.

(1*H*-Benzotriazol-1-yloxy)phenylmethylene pyrrolidinium hexachloroantimonate (BPMP). BPMP was synthesized from CPPH and KOBt according to the general procedure II. Yield 91.1%. Mp 93–94°C (dec.); [Found: C, 32.23; H, 2.61; N, 8.84.$C_{17}H_{17}Cl_6N_4Osb$ requires C, 32.52; H, 2.73; N, 8.92%]; ν_{max} (KBr) 3100w, 1616vs, 1494s, 1467s, 1417m, 1331s, 1151m, 1065m, 752s, 705m, 638w; ^{1}H NMR ([D$_6$]acetone): δ=1.95 (m, 4H, 2β-CH_2), 3.57 (m, 4H, 2α-CH_2), 7.34–7.97 (m, 9H, aryl).

5-(1*H*-7-Azabenzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (AOMP). The solution of HOAt (0.136 g, 1 mmol) and NEt$_3$ (139 μL, 1 mol) in CH$_2$Cl$_2$ (5 mL) was added dropwise to a suspension of CDMP (0.453 g, 1 mmol) in dry CH$_2$Cl$_2$ (6 mL) at 0°C with stirring under argon atmosphere. After the reaction mixture was stirred at room temperature for 2 h, it was filtered and the filter cake was washed with cold CH$_2$Cl$_2$ and dried in vacuo. Recrystallization from CH$_3$COCH$_3$–Et$_2$O gave 0.47 g pure product as yellowish crystalline solid. Yield 85.1%. Mp 109–110°C (dec.); [Found: C, 21.60; H, 2.11; N, 12.74. $C_{10}H_{12}Cl_6N_5Osb$ requires C, 21.73; H, 2.19; N, 12.67%]; ν_{max} (KBr) 3111w, 1667vs, 1496s, 1475s, 1459s, 1408m, 1314m, 1070s, 793m, 760w, 638w; ^{1}H NMR ([D$_6$]acetone): δ=8.76 (dd, ^3J=4.4 Hz, ^4J=1.4 Hz, 1H, α-*H*-aryl), 8.45 (dd, ^3J=8.4 Hz, ^4J=1.4 Hz, 1H, γ-*H*-aryl), 7.53 (dd, ^3J=4.4 Hz, ^3J=8.4 Hz, 1H, β-*H*-aryl), 3.62 (t, J=7.2 Hz, 2H, α-CH_2), 2.93 (s, 3H, *N*-CH_3), 2.59 (t, J=8.0 Hz, 2H, γ-CH$_2$), 2.13 (m, 2H, β-CH_2).

***N*-(Benzyloxycarbonyl)-glycyl-phenylalanine 1*H*-benzotriazolyl ester (Z-G-F-OBt, general procedure III).** To a solution of Z-Gly-Phe-OH (0.713 g, 2 mmol) and HOBt (0.270 g, 2 mmol) in THF (6 mL), DCC (0.413 g, 2 mmol) was added at room temperature. After the reaction mixture was stirred for 1 h, it was cooled to −30°C for 2 h to make DCU precipitate completely. The cold mixture was filtered, the filtrate was concentrated and dried in vacuo. Recrystallization from THF–AcOEt–Pe gave 0.766 g pure product as colorless crystalline solid. Yield 80.9%. Mp 124–125°C. FABMS: 474 [M+H]$^+$, 339 [M−OBt]$^+$, 91 [PhCH$_2$]$^+$. Other spectra are shown in Table 2.

***N*-(Benzyloxycarbonyl)-glycyl-phenylalanine 1*H*-7-azabenzotriazolyl ester (Z-G-F-OAt).** This compound was synthesized from Z-Gly-Phe-OH and HOAt according to the general procedure III. Yield 86.9%, mp 157–159°C. ν_{max} (KBr) 3069w, 2929w, 1815m, 1732vs, 1627vs, 1539s, 1393m, 1237s, 800m, 698m, 516w; UV (CHCl$_3$) λ_{max}=258, 288 nm; ^{1}H NMR ([D$_6$]-DMSO) δ=8.85–7.11 (m, 13H, aryl), 7.05 (br, 1H, N*H*-Phe), 5.62 (d, 1H, N*H*-Gly), 5.04 (s, 2H, CH_2^{CBZ}), 4.47 (m, 1H, α-C*H*-Phe), 3.75–3.52 (m, 2H, α-CH_2-Gly), 3.12–2.83 (m, 2H, β-CH_2-Phe).

Reaction speed and racemization test by HPLC using the model reaction: Z-Gly-Phe-OH+Val-OMe·HCl→Z-Gly-D/L-Phe-Val-OMe. Z-Gly-Phe-OH (50 mg, 0.14 mmol) and Val-OMe·HCl (26 mg, 0.154 mmol) were coupled with tested immonium type reagent (0.154 mmol) or the same equivalent of other coupling reagent. Boc-Phe-Val-OMe (66 mg, 0.175 mmol) was added as the internal reference. Test of reaction were performed on total 1.5 mL scale. Aliquots (10 μL) from the reaction mixture was quenched and dissolved in 100 μL buffer solution (CH$_3$OH/H$_2$O/TFA: 50/50/1). The resultant samples were analyzed by HPLC to give the following result: Z-Gly-Phe-OH (t_R=4.04 min); Z-Gly-L-Phe-Val-OMe (t_R= 9.24 min); Z-Gly-D-Phe-Val-OMe (t_R=10.28 min); Boc-Phe-Val-OMe (t_R=15.82 min). Peak areas were compared in order to obtain the chemical yields

(yield(%) = $[(LL/X_1 + DL/X_2)/a{\cdot}S] \times 100\%$). Percentage of epimers was calculated according to the equation: $D\% = [DL/X_2/(LL/X_1 + DL/X_2)] \times 100\%$; where *LL* refers to the area of Z-Gly-L-Phe-Val-OMe, *DL* refers to that of Z-Gly-D-Phe-Val-OMe, *S* refers to that of Boc-Phe-Val-OMe, a=0.778, which is the molar ratio between Z-Gly-OH and Boc-Phe-Val-OMe; X_1=1.269 and X_2=1.254 which are the determined correction factors for absorption difference (220 nm) between the references.

General procedure for peptide synthesis using immonium type coupling reagents

2,6-Lutidine (3 equiv.) was added to a cold mixture (−10°C) of N-protected amino acid (1 equiv.), amino acid ester hydrochloride (1.1 equiv.), and coupling reagent (1.1 equiv.) in THF (3–5 mL/mmol), stirred for 1 min cold and 1 h at room temperature. For large scale and coupling between hindered amino acids, the reaction time should be moderately prolonged. After the completion of reaction, the reaction mixture was diluted with THF, the resultant suspension was filtered and the filtered cake was washed with THF. The combined filtrate was concentrated under reduced pressure to give the crude product which was purified by flash chromatography on silica gel column to afford the desired product.

General procedure for esters synthesis using immonium type coupling reagents

2,6-Lutidine (2 equiv.) was added to a cold mixture (−10°C) of carboxylic acid (1 equiv.), alcohol (1.1 equiv.), and coupling reagent (1.1 equiv.) in THF (3–5 mL/mmol), stirred for 1 min cold, then raise to room temperature, the reaction was monitored by TLC. For the preparation of HOBt active ester, no alcohol need be added and the quantity of base was one equivalent. The work-up was the same as that of peptide synthesis.

Special examples for the mechanism studied of immonium salts mediated coupling reaction

PhCOOBt (Table 3, entry 6). To a solution of PhCOOH (61 mg, 0.5 mmol) in CH_3CN (2 mL), DCHA (101 μL) was added, then BOMI (0.289 g, 0.55 mmol), 2,6-lutidine (116 μL, 1 mmol) were added at −10°C. The reaction mixture was stirred 2 h, then it was filtered via a pad of silica gel, and washed with AcOEt/Pe=1:4 (200 mL), the filtrate was concentrated, dried and recrystallized from benzene–hexane to give 0.91 g PhCOOBt as main product. Yield 75.8%, mp 80–81°C; [Found: C, 65.30; H, 3.64; N, 17.92. $C_{13}H_9N_3O_2$ requires C, 65.27; H, 3.79; N, 17.56%]; ν_{max} (KBr) 3067w, 1808sh, 1779vs, 1597m, 1454m, 1230s, 1085m, 987s, 705s; ^{1}H NMR ([D_6]acetone): δ=7.48–8.46 (m, 9H, aryl). EI-MS *m/z*: 239 M^+, 105 $[M-BtO]^+$, 77 $[C_6H_5]^+$.

Z-Aib-OBt (Table 3, entry 8). 2,6-Lutidine (0.174 mL, 1.5 mmol) was added to a cold mixture (−10°C) of Z-Aib-OH (119 mg, 0.5 mmol), Aib-OCH_3.HCl (84 mg, 0.55 mmol), and BOMI (289 mg, 0.55 mmol) in THF (2 mL), stirred for 1 min cold and 1 h room temperature. The reaction mixture was treated according to the general procedure and purified by flash chromatography on silica gel column eluted with AcOEt/Petroleum ether (1:2) to give 64 mg of Z-Aib-OBt (36%), accompanied with the dipeptide Z-Aib-Aib-OCH_3 (76 mg, yield:45%). [Found: C, 61.26; H, 5.28; N, 15.53.$C_{18}H_{18}N_4O_4$ requires C, 61.01; H, 5.12; N, 15.81%]; R_f (AcOEt/Pe=1:2) 0.51; ^{1}H NMR ($CDCl_3$):δ=7.26–8.04 (m, 9H, aryl), 5.57 (s, 1H, N*H*), 5.21 (s, 2H, PHCH_2), 1.78 (s, 6H, 2CH_3); ν_{max} (Nujol)=3322, 1817, 1713, 1521, 1270, 1047 cm^{-1}. FABMS *m/z*: 355 $[M+H]^+$, 220 $[M-BtO]^+$, 91 $[PhCH_2]^+$.

Fmoc-MeVal-OBt (Table 3, entry 9). 2,6-Lutidine (0.37 mL, 3.2 mmol) was added to a cold mixture (−10°C) of Fmoc-MeVal-OH (353 mg, 1.0 mmol), Sar-OBzl·HCl (237 mg, 1.1 mmol), and BDMP (607 mg, 1.1 mmol), in THF (5 mL), stirred for 1 min cold and 3 h at room temperature. The reaction mixture was directly purified by flash chromatography on silica gel column eluted with AcOEt/Petroleum ether (1:4) to give Fmoc-MeVal-OBt besides the dipeptide Fmoc-MeVal-Sar-OBzl. Yield: 243 mg (51.6%), mp 43–44°C; [Found: C, 68.39; H, 5.57; N, 11.62. $C_{27}H_{26}N_4O_4{\cdot}0.25H_2O$ requires C, 68.27; H, 5.62; N, 11.79%]; R_{f1} (*O*-form, AcOEt/Pe=1:2) 0.67, R_{f2} (*N*-form, AcOEt/Pe=1:2) 0.40. ν_{max} (KBr) 3067w, 2966w, 1818m, 1702vs, 1464m, 1451sh, 1169m, 740s cm^{-1}; UV (CH_2Cl_2) λ_{max}=262, 289, 329, 344 nm; ^{1}H NMR ($CDCl_3$): δ=0.70, 0.85, 1.01, 1.23 (4d, J=6.4 Hz, 6H, 2ν-CH_3-Val), 2.16, 2.41 (2m, 1H, β-C*H*-Val), 2.92, 2.98, 3.02, 3.04 (4s, 3H, *N*-CH_3), 4.08–4.85 (m, 4H, CH_2-Fmoc, 9-C*H*-Fluorenyl, α-C*H*-Val), 7.08–8.12 (m, 12H, aryl).

Abbreviations: Aib, α-aminoisobutyric acid; AOMP, 5-(1*H*-7-azabenzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate; AOP, (1*H*-7-azabenzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BOMI, *N*-(1*H*-benzotriazol-1-ylmethylene)-*N*-methylmethanaminium hexachloroantimonate *N*-oxide; BDMP, 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate; BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BPMP, 1-(1*H*-benzotriazol-1-yloxy)phenylmethylene pyrrolidinium hexachloroantimonate; CHA, cyclohexyl amine; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexyl amine; DIEA, *N*,*N*′-diisopropylethylamine; DOMP; 5-(3′,4′-dihydro-4′-oxo-1′,2′,3′-benzotriazin-3′-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate; FOMP; 5-(pentafluorophenyloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate; HAPyU, 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate *N*-oxide; HBMDU, (1*H*-benzotriazol-1-yloxy)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate; HBPipU, (1*H*-benzo-triazol-1-yloxy)-*N*,*N*,*N*′,*N*′-bis(pentamethylene) uronium hexafluorophosphate; HBPyU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate; HBTU, *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; HODhbt, 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine; HOSu, *N*-hydroxysuccinimide; PyBOP, (1*H*-benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate;

SOMP, 5-(succinimiddyloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate. Nomenclature and symbols of amino acids and peptides generally follow the recommendations of the IUPAC–IUB Joint Commission of Biochemical Nomenclature in: *Pure Appl. Chem.* **1984**, *56*, 595–624.

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