5-(1*H*-Benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-Pyrrolium Hexachloroantimonate (BDMP®): A Highly Efficient Immonium Type Peptide Coupling Reagent

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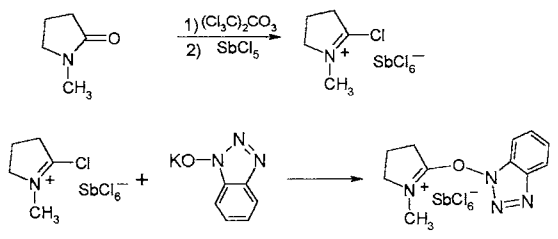
HOBt-based immonium type coupling reagent, 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (BDMP) has been designed, synthesized and utilized to synthesize oligopeptides and biologically active peptides both in solution and solid phase with good yield, low racemization and fast reaction rate.

With the development of peptide chemistry, many new peptide coupling reagents have been designed and synthesized to meet the needs of peptide synthesis. In the past decades, one of the significant development in this field was the exploitation of HOBt-based onium type reagents, such as BOP,[1] HBTU,[2] PyBOP,[3] HBPyU,[4] HBPipU,[5] HBMDU[6] *et al*, which were widely used in peptides synthesis both in solution and solid phase. Subsequently those reagents have been modified by replacing HOBt with HOAt to obtain HOAt-based onium reagents, such as HATU, HAPyU, AOP and PyAOP.[7] These reagents were more efficient than corresponding HOBt-based reagents due to the anchimeric assistance effect of HOAt.[8] Despite the above mentioned studies, another pathway, enhancing the efficiency and decreasing the racemization during coupling, is still assumable by replacing one of the substituted amino groups of central carbon atom of uronium reagents with hydrogen, alkyl or aryl. Thus the electron density of central carbon of immonium salts was much lower than that of corresponding uronium salts and the resulted immonium molecules were adequately activated due to obvious electronic effect. Based upon this consideration we designed and synthesized a series of immonium type coupling reagents. In previous studies,[9] we proved HOBt- based immonium salts BOMI to be very effective, Herein we will report its more efficient analogue 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate, BDMP.

The preparation of BDMP is outlined in Scheme 1. Condensation of *N*-methyl pyrrolidone with BTC [bis-(trichloromethyl)carbonate] yielded the immonium chloride which was stabilized subsequently with the $SbCl_5$ to give the intermediate 5-chloro-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate, which was subsequently reacted with the potassium salt of 1-hydroxyl benzotriazole to afford the desired compound, BDMP as a crystalline and shelf-stable solid.[10]



Scheme 1. Synthesis of coupling reagent BDMP.

In order to evaluate the effectiveness of BDMP, we examined the reactivity and the extent of racemization using the HPLC method[11] (model coupling of Z-Gly-Phe-OH and Val-OCH_3) and Young's test[12] (model coupling of Bz-Leu-OH and Gly-OEt·HCl). In comparison with other conventional coupling reagents, the racemization with BDMP was observed to be the lowest, even lower than HAPyU and its analogous BOMI as shown in Table 1. It is likely that the less racemization of BDMP can be attributed to the mild reaction condition and the less basic and hindered heterocyclic base, 2,6-lutidine.[13] On the contrary, products with considerable racemization and relatively slow reaction rate was observed during the above reaction condition was used for the coupling with other HOBt-derived phosphonium and uronium type coupling reagents.

Table 1. Comparison of racemization of BDMP with other coupling reagents [a]

Coupling Reagent	Racemization/DL% [a]	
	HPLC method	Young's test
BOP	9.6	39.6
HBPyU	7.9	18.0
HAPyU	5.7 (3.3 [c])	13.9
BOMI	6.4 (3.1 [c])	8.8 [c]
BDMP	4.6 (2.2 [c])	5.3 [c]

[a]All reactions were carried out in the same conditions which were commonly used for onium reagents[4]. [b]DL% equal to D-isomer% multiplied by two. [c]Reaction conditions were the same as those used in Table 2 which were suitable for immonium type coupling reagents.

By comparing the reactivity of BDMP with other conventional coupling reagents by HPLC using the model reaction: Z-Gly-Phe-OH+Val-OMe·HCl→Z-Gly-Phe-Val-OMe, we found that the reactivity of BDMP was much higher than HOBt-based uronium salt HBPyU, even higher than HAPyU, which is widely regarded as the most efficient coupling regent[14] (Figure1). Because the two substituted amino groups of the central carbon atom of uronium salts provide two equal resonance structures to stabilize the molecules, but decrease the reactivity of these reagents. However BDMP averts this drawback by replacing one of the substituted amino group of the uronium reagents with alkyl. It is assumed that the higher reactivity of BDMP than its analogues BOMI was probably due to the tension of intra-annular imide bond. It was found 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°, indicating that the five number ring of 2*H*-pyrrolium was to some extent constraint calculated by PCMODEL software.[15] Electronic effect also makes BDMP more reactive than BOMI, which can be indirectly reflected by the results of calculation that the charge of central carbon of BDMP (Q = + 0.31) is higher than that of BOMI (Q = + 0.27).

The favorable coupling efficiency of BDMP was verified by the synthesis of oligopeptides with good yield, negligible

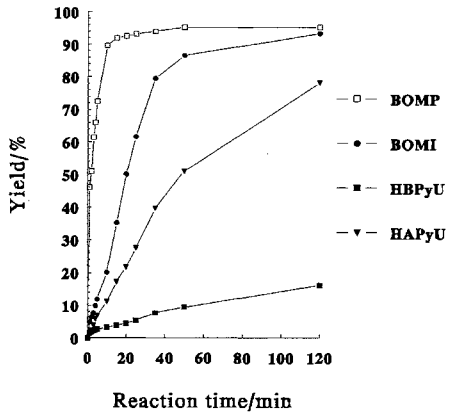


Figure 1. Comparison of reactivity of BDMP with other coupling reagents[a].

[a]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: 3.2.

Table 2. Synthesis of oligopeptides using BDMP in solution

Peptide [a]	Yield/% [b]	mp/°C [c]	$[\alpha]_D$ (conc., solv.) [c]
Boc-Phe$^{\Phi}$-Leu-OMe	91.1	102-103	-26.4° (1, MeOH)
Boc-Gly$^{\Phi}$-Phe-Leu-OMe	92.4	43-44	-22.9° (1, MeOH)
BOC-Tyr(Bzl)$^{\Phi}$-Gly-OEt	83.3	127-128	1.1° (1, MeOH)
Boc-Tyr(Bzl)-Gly-Gly$^{\Phi}$-Phe-Leu-OMe	84.1	147-148	-9.1° (1, MeOH)
Bz-Leu$^{\Phi}$-Gly-OEt	86.9	154-155	-32.2° (3.1, EtOH)
Fmoc-MeLeu$^{\Phi}$-Ala-OBzl	91.3	oil	-43.0° (0.9, MeOH)
Fmoc-Meleu$^{\Phi}$-Val-Meleu-Ala-OBzl	92.9	44-46	-91.8° (0.5, MeOH)

[a]The CO-NH bond formed in the peptides is indicated by Φ. [b]Isolated yields based on N-protected amino acid. [c]Melting points and $[\alpha]_D$ values are in accord with the reported values and all products are confirmed by elemental analysis, the deviations were no more than 0.3%.

racemization and simple experimental procedures (Table 2).

To further explore the effectiveness of BDMP on peptide coupling, the bioactive peptide Leu-enkephaline[16a] was synthesized in solution. The protected pentapeptide was obtained via seven steps in 51.4% overall yield, which was deprotected and purified to give Leu-enkephalin. The solid-phase synthesis of Leu-enkephalin on Merrifield's resin using BDMP was performed according to the general SPPS principle. The final product was purified and characterized by HPLC and ESI-MS and shown to be identical with the product obtained from the solution method and an authentic sample from Sigma.[16b] We also successfully synthesized immunosuppressive cyclic undecapeptide Cyclosporin O[17] using BDMP combined with other coupling reagents.

In conclusion, novel HOBt-based immonium salt BDMP is shown to be a very efficient peptide coupling reagent with extremely high reactivity, very low racemization and excellent yield during peptide synthesis. Its effectiveness is demonstrated by the successful synthesis of a series of oligopeptides and biologically active peptides both in solution and solid phase.

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