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BOMI — A Novel Peptide Coupling Reagent

Peng Li and Jie Cheng Xu*

Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

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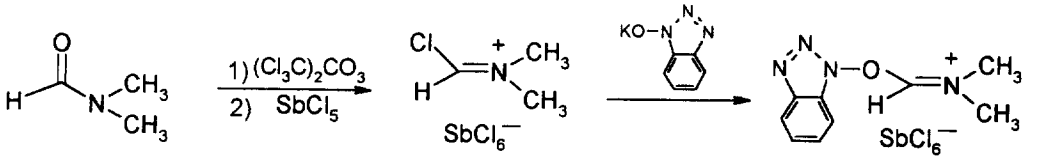
Abstract: A novel coupling reagent, benzotriazol-1-yloxy-*N,N*-dimethylmethaniminium hexachloroantimonate (BOMI), was synthesized and successfully applied to the synthesis of oligopeptides, the racemization and the influence of several reaction parameters such as solvent, base, as well as temperature were evaluated.

In recent years, many new coupling reagents have been suggested for replacement of the conventional methods for peptide synthesis to enhance the coupling efficiencies and eliminate racemization. We have developed several new highly reactive coupling reagents which are mainly based on uronium salts or phosphinates.[1a~d] In continuation of our studies towards this objective, we report here the synthesis of the immonium salt, benzotriazol-1-yloxy-*N,N*-dimethylmethaniminium hexachloroantimonate (BOMI) and evaluation of its efficiency in peptide coupling by comparison with other popular reagents.

Condensation of DMF with BTC [bis(trichloromethyl)carbonate] yielded an immonium chloride, which was stabilized with $SbCl_5$ and subsequently reacted with 1-hydroxyl benzotriazole to afford the desired compound, BOMI, which was a crystalline, pale yellow, solid, stable at room temperature.[2]



Using BOMI as a coupling reagent, several oligopeptides were prepared to assess effectiveness of the reagent (Table 1). The reactions were carried out similarly to coupling with HBPyU. In a typical experimental procedure, 2,6-lutidine (3 equiv.) was added to a cold mixture (-10 °C) of the N-protected amino acid

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(1 equiv.), the amino acid ester hydrochloride or trifluoroacetate(1.1 equiv.), and BOMI (1.1equiv.) in THF or CH_3CN (1mL/mmol). The reaction was monitored by TLC which showed that complete conversion was reached within approximately 1 hour. The reaction mixture was filtered and purified by flash chromatography on silica gel column to afford the desired product.

Table 1. Preparation of peptides using BOMI

		Found		Literature	
Entry	Peptide (yield %)[a]	m.p. (°C)	$[\alpha]_D$(conc.,solv.)	m.p.(°C)	$[\alpha]_D^{20}$(conc.,solv.)
1	Boc-Phe-Gly-OEt[3](91)	98~100	-5.3 (2, EtOH)	88~89.5	-4.2 (2, EtOH)
2	Boc-Phe-Phe-OMe[4](85)	116~117	-14 (1,EtOH)	114~115	-14 (1, EtOH)
3	Z-Ser-Gly-OEt[5](88)	107~108	-6.4 (1, AcOEt)	106~107	-5.9 (1, AcOEt)
4	Boc-Ile-Val-OMe[6](86)	165~166	-15.5 (1, AcOEt)	166~167	-15 (1, AcOEt)
5	Bz-Leu-Gly-OEt[7](84)	154~155	-31 (3.1, EtOH)	156.5~157	-34.0 (3.1, EtOH)

a) isolated yield based on N-protected amino acid

In order to optimize the reaction conditions, the influence of solvent, base and temperature were studied by an HPLC method using the model reaction: Z-Gly-Phe-OH+Val-OMe·HCl→Z-Gly-Phe-Val-OMe.[8, 9] It was observed that THF seemed to be the most suitable solvent for peptide synthesis with BOMI with respect to the yield and reaction rate but, in most cases, we ran the model couplings in CH_3CN because of its excellent solubility for substrates and BOMI. In the case of DMF, only a medium yield of product and relatively high racemization was obtained.

The influence of the nature of the tertiary amine such as DIEA, NEt_3 and *N*-methylmorpholine, which are often used for peptide synthesis, was checked. They all drive the reaction so fast that the reactions are complete within 5 minute, even at -70 °C. On the other hand the strong basicity of these bases causes the decomposition of the BOMI and side reactions and results in lower yield. Thus, a less basic tertiary amine, 2,6-lutidine, was adopted for general use.

Using the HPLC method (coupling of Z-Gly-Phe-OH and Val-OCH_3) and Young's test[7] (coupling of Bz-Leu-OH and Gly-OEt·HCl), the extent of the racemization was examined. In comparison with other coupling reagents, the racemization with BOMI was observed to be the very low as shown in Table 2. The reduced racemization used by BOMI may be due to the mild reaction conditions and the weak basicity of the tertiary amine 2, 6-lutidine.

Table 2. Comparison of racemization of BOMI with different coupling reagents[a]

Reagent	HPLC method[b] DL%[d]	Young's test[c] DL%[d]
DCC	19.7	72.1
BOP	9.6	39.6
HBTU	9.8	24.3
HBPyU	7.9	18.0
HBPipU	8.9	20.5
BOMI	6.4(3.1[e])	8.8[e]

[a]All reactions were carried out in the same conditions which were suitable for the HOBt-derived uronium reagents such as BOP, HBTU and HBPyU[1b]. [b]Coupling of Z-Gly-Phe-OH and Val-OCH_3[8, 9]. [c]Coupling of Bz-Leu-OH and Gly-OEt·HCl[7]. [d]DL% equal to D-isomer% multiplied by two. [e]Reaction conditions were the same as those used in Table 1 which were suitable for the HOBt-derived immonium coupling reagent— BOMI.

When we tried the utilization of BOMI in the synthesis of peptides containing hindered amino acids such as *N*-methyl valine or α-aminoisobutyric acid (Aib), similar results were obtained as using other HOBt-derived coupling reagent, the ester-formation step was fast and the peptide-formation step was slow.[10]

$$\text{Z-Aib-OH + Aib-OCH}_3 \xrightarrow[\text{fast}]{\text{BOMI}} \text{[Z-Aib-OBt]} \xrightarrow{\text{slow}} \text{Z-Aib-Aib-OCH}_3$$

To explore further the effectiveness of BOMI on peptide coupling, the synthesis of Leu-enkephalin[11] was accomplished in solution. The 42.4% overall yield of the protected Leu-enkephalin was obtained via seven coupling steps. The product of each step was confirmed by elemental analysis and other methods. Using HPLC and ESI-MS the final product was shown to be identical with an authentic sample from Sigma.

BOMI can also been used in solid phase peptide synthesis using Merrifield's resin. In order to optimize the reaction conditions and evaluate the efficiency of BOMI in SPPS the model reaction: Boc-Phe-OH + TFA·NH_2-Leu-resin → Boc-Phe-Leu-resin was adopted. It was observed that 2,6-lutidine was proved to be more suitable than DIEA in the coupling. The coupling efficiency was above 99% as judged by using the quantitative ninhydrin method;[12] moreover, TFA·NH_2-Leu-resin can be used directly in the coupling without pre-neutralization. Thus the optimized conditions used in the coupling were: (3 equiv. Boc-AA-OH/ CH_2Cl_2 + 3 equiv. BOMI reagent + 7 equiv. 2,6-lutidine)/ 1 equiv. TFA·NH_2-AA'-resin with the coupling time of 2 hr.

Based on these results, we successfully synthesized Leu-enkephalin according to the general SPPS principle using the optimized reaction conditions. The final product was purified and characterized by HPLC and

ESI-MS[13] and shown to be identical with the product obtained from the solution method and an authentic sample.

In conclusion, the immonium salt, BOMI, a new type of coupling reagent, was shown to be a very efficient peptide coupling reagent in both the solution and solid phase peptide synthesis in terms of yield, rate and low racemization.

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REFERENCES AND NOTES:

Abbreviations: AA, AA': amino acid; BOMI: (1H-Benzotriazol-1-yloxy)-*N,N*-dimethylmethaniminium hexachloroantimonate; BOP: (1H-benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluoro-phosphate; DCC: Dicyclohexylcarbodiimide; DIEA: diisopropylethylamine; HBPipU: O-(1H-benzotriazol-1-yl)-*N,N,N',N'*-bis(pentamethylene)uronium hexafluorophosphate; HBTU: O-(1H-benzotriazol-1-yl-tetramethyluronium hexafluorophosphate; HBPyU: O-(1H-benzotriazol-1-yl)-*N,N,N',N'*-bis-tetramethylene) uronium hexafluoro-phosphate; HOBt: 1-hydroxy benzotriazole; SPPS: Solid Phase Peptide Synthesis; Z: benzyloxycarbonyl.

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2. BOMI was obtained from DMF in 76% yield after recrystallization from CH_3CN/Et_2O in the form of pale yellow crystals, mp 152-153°C (dec.). ^{1}H-NMR (CD_3COCD_3): δ 7.99(1H, s α-H), 7.95-7.45(4H, m, aryl), 2.97(3H, s, CH_3), 2.81(3H, s, CH_3). Anal. Calcd. for $C_9H_{11}Cl_6N_4OSb$: C, 20.54; H, 2.09; N, 10.65. Found: C, 20.78, H, 2.12; N, 10.63.
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9. The two diastereometric tripeptides, Z-Gly-L-Phe-Val-OMe and Z-Gly-D-Phe-Val-OMe, can be easily separated by HPLC and monitored at 220 nm. The extent of racemization was determined by measuring the ratio of peak areas between Z-Gly-D-Phe-Val-OMe and Z-Gly-L-Phe-Val-OMe. The reaction speed and yield were also determined by measuring the ratio of peak areas between products and internal standard, Boc-Phe-Val-OMe, during different time periods of reaction, Z-Gly-DL-Phe-Val-OMe was used as reference compound to correct the molar ratio of LL and DL isomers.
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13. t_R = 19.08min, HPLC conditions: Column: Lichrosorb RP-18(0.5 × 30cm). Solvent A:0.1%TFA in water; Solvent B: 75% CH_3CN (0.1% TFA), Gradient: 20% solvent B to 60% solvent B in 20 min. Flow rate: 1.0 ml/min. Detection: 230nm (0.5 AUFS). ESI-MS: 278.3 [$(M+2H^+)/2$], 556.8 ($M+H^+$), 578.5 ($M+Na^+$), 1112.6 ($M+M+H^+$), 1134.0 ($M+M+Na^+$).