Pergamon

Tetrahedron Letters 41 (2000) 721–724

TETRAHEDRON LETTERS

HOBt and HOAt-derived immonium salts: new and highly efficient coupling reagents for peptide synthesis

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Received 29 September 1999; accepted 10 November 1999

Abstract

Novel HOBt- and HOAt-derived immonium type coupling reagents BDMP, BPMP and AOMP were shown to be very efficient for peptide synthesis with high reactivity, low racemization and good yields. X-Ray analysis indicates BOMI exists as the *N*-substituted form rather than as the *O*-substituted form assigned previously. A mechanism for amide bond formation mediated by these reagents was proposed and studied. © 2000 Elsevier Science Ltd. All rights reserved.

Subsequent to the first HOBt-derived phosphonium coupling reagent BOP, designed by Castro et al.,[1] many HOBt-based uronium and phosphonium type coupling reagents, such as PyBOP,[2] HBTU,[3] HBPyU,[4] HBPipU[5] and HBMDU,[6] have been developed and employed in peptide synthesis both in solution and solid phase. The predominance of carbodiimide and active ester techniques have been gradually replaced with onium salts based upon 1-hydroxybenzotriazole (HOBt),[7] but avoiding racemization is still a challenge even with these reagents.[4a,8] To suppress the level of epimerization and enhance the coupling efficiency, the HOAt-derived phosphonium and uronium reagents, such as HATU, HAPyU, AOP, PyAOP[9] were suggested and found to perform well because of the anchimeric assistance of HOAt.[10] Recently, we have synthesized a series of HOBt- and HOAt-derived immonium type coupling reagents, which were more reactive than the uronium reagents because the electron density of their central carbon is lower than that of the corresponding uronium salts. In previous studies,[11] we reported the synthesis of the HOBt-based immonium salt BOMI by replacing one of the dimethylamino groups of HBTU with hydrogen, and demonstrated its high efficiency in peptide synthesis. We report here the further design and synthesis of three immonium type coupling reagents BDMP, BPMP and AOMP based upon the modification of the structure of the uronium reagents HBMDU, HBPyU and HAMDU (Fig. 1).

These reagents can be readily prepared from condensation of the corresponding amides with bis(trichloromethyl) carbonate to yield the immonium chlorides, which were stabilized with $SbCl_5$ and subsequently reacted with the potassium salt of 1-hydroxybenzotriazole or 1-hydroxy-7-azabenzotriazole

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PII: S0040-4039(99)02125-5

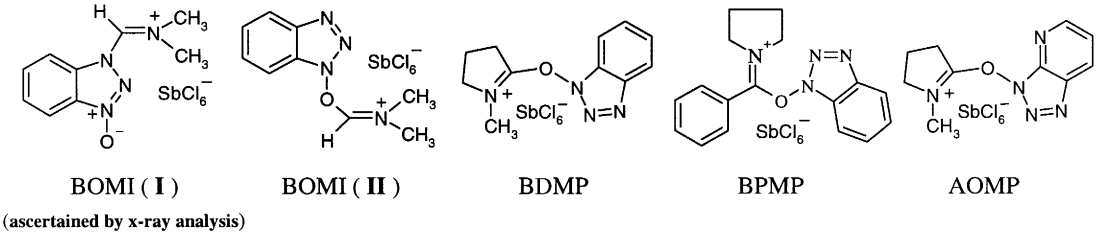
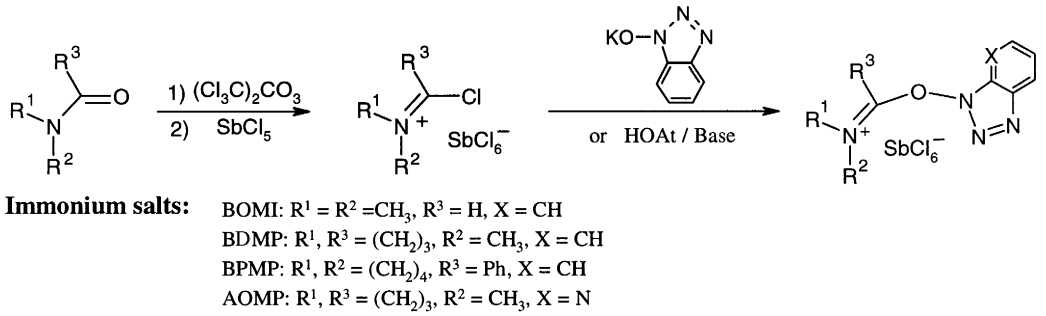


Fig. 1. Structures of HOBt- and HOAt-based immonium type coupling reagents

in the presence of a tertiary amine, such as NEt_3 to afford the desired compounds as crystalline and shelf-stable solids (Scheme 1).



Scheme 1. Synthesis of HOBt- and HOAt-derived immonium type coupling reagents

Recently, X-ray analysis indicated that BOMI is the *N*-substituted form **I**[12] shown in Fig. 1, rather than the isomeric *O*-substituted form **II**. Unfortunately, the X-ray data for the analogous BDMP, BPMP and AOMP have not yet been obtained, therefore the structural representations are arbitrarily assigned as the *O*-substituted forms in this article.

The efficiency of these immonium reagents in peptide synthesis was evaluated by HPLC monitoring of the racemization prone 2+1 peptide segment condensation during the formation of the model compound Z-Gly-Phe-Val-OCH_3 which was first adopted by Van der Auwera et al.[13] It was observed that the immonium reagents exhibited extremely high reactivity, very low racemization and good yields for couplings. From the results shown in Table 1, it is obvious that, both in terms of reactivity and racemization, the HOBt-based immonium salts are superior to the uronium salt HBPyU which is one of the most efficient among the HOBt-based phosphonium and uronium salts,[4a,14] even better than that of the HOAt-derived reagent HAPyU, which is widely regarded as the most effective reagent so far developed.[15] These data also indicate that BDMP is the most efficient reagent among these immonium salts with respect to the reaction speed, epimerization and coupling yield. However, the reagent AOMP is shown to be less efficient, the most likely reason being that the extremely high reactivity of AOMP causes its decomposition before achieving the activation of the *N*-protected amino acid in the presence of base, such as 2,6-lutidine.

Because the amino component of the adopted model reaction is a primary amine without obvious hindrance (R^2=H, Fig. 2), the reaction rate is probably determined by the activation step, the formation of the HOBt or HOAt active ester. To confirm this consideration, we synthesized the active ester of the model reaction Z-G-F-OBt, Z-G-F-OAt and subjected these active esters to the coupling reaction (Z-G-F-OBt/Z-G-F-OAt+Val-OCH_3·HCl+2,6-lutidine→Z-G-F-V-OCH_3). We found they coupled with the amine quickly with a yield of above 90% after 10 min under the same reaction conditions as used in Table 1. This indicated that the relatively low reactivity of HBPyU and HAPyU is mainly due to the

Table 1
Comparison of reactivity and racemization of immonium type reagents with uronium salts using HPLC[a,b] and Young's test[c]

Coupling Reagent	Reactivity[d] $t^{1/2}$ (min)	Yield (%) 2 min	10 min	120 min	Racemization (DL%)[e] HPLC	Young's test
BOMI	20	6.4	20.1	93.4	3.1	8.8
BDMP	1.9	51.0	89.6	95.3	2.2	5.3
BPMP	2.8	37.6	81.7	96.2	2.3	5.4
AOMP	< 1	64.1	64.2	64.2	1.6	3.1
HBPyU	> 120	2.0	3.3	16.1	14.6 (7.9[f])	18.0[f]
HAPyU	49	2.4	11.2	78.3	3.3 (5.7[f])	13.9[f]

[a] Model reaction: Z-Gly-Phe-OH + Val-OCH_3·HCl → Z-Gly-Phe-Val-OCH_3. [b] 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. [c] Model reaction: Bz-Leu-OH + Gly-OEt·HCl → Bz-Leu-Gly-OEt. [d] The reactions were monitored by HPLC, the reaction mixture was sampled at 1′, 2′, 3′, 4′, 5′, 10′, 15′, 20′, 25′, 35′, 50′, and 120′ respectively, thus half-reaction time $t^{1/2}$ was obtained from the time-yield curve drawn according to these original data. [e] DL% equal to D-isomer% multiplied by two. [f] The reactions were carried out under the conditions which were suitable for the uronium type coupling reagents.[4a]

lowered reactivity of the uronium/aminium carbon skeleton towards the attack of the carboxylate anion. The two substituted amino groups of the uronium/aminium salts provide two equal resonance structures to stabilize the molecule, and the lone electron pairs of the two nitrogen atoms are delocalized within the bonds N–C–N, which causes the carbocation to share a higher electron density than the immonium salts; consequently, the nucleophilic attack of the carboxylate anion was impeded. It is assumed that the higher reactivity of BDMP over other immonium salts is probably due to the intra-annular imide bond. The electronic effect also makes BDMP more reactive than its analogues due to the relatively low electron density of the central carbon of BDMP.

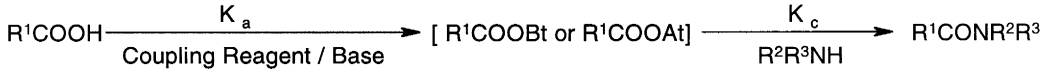


Fig. 2. The two steps of coupling reactions mediated by HOBt- and HOAt-based reagents

When the *C*-protected amino acid ester is an *N*-alkyl or an α,α-dialkyl amino acid ester, such as *N*-Me-Val-OCH_3·HCl and Aib-OCH_3·HCl, the coupling step becomes the rate-determining step in peptide bond formation ($k_a > k_c$). In this case, HOBt-based coupling reagents including these novel immonium salts prove to be inefficient, the major isolated products being the HOBt active esters when the amino component is an *N*-alkylated amino acid ester.[3b,11b] However, the HOAt-based aminium salt HAPyU displays very high efficiency for the construction of hindered amide bonds.[15b]

To verify the performance of these novel immonium salts, we synthesized a series of oligopeptides and bioactive peptides both in solution and solid phase with good yields and low racemization.[11b] We also synthesized the immunosuppressive cyclic undecapeptide Cyclosporin O using these reagents combined with other reagents.[16] These reagents can also be used for ester formation with satisfactory yields, especially for the preparation of active esters such as benzotriazolyl, pentafluorophenyl and succinimidyl esters which are useful for the synthesis of lactones and lactams.

In conclusion, several novel HOBt- and HOAt-derived immonium coupling reagents BOMI,[11] BDMP, BPMP and AOMP were designed and synthesized. Their utility for peptide synthesis was demonstrated by rapid reaction speed, low racemization and good yields. In the case of the coupling of normal coded

amino acids and peptide segments, HOBt-derived immonium salts, BOMI, BDMP and BPMP[17] seem to be more efficient than HOBt- or HOAt-derived uronium/aminium and phosphonium salts.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (9772045).

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