Pergamon

Tetrahedron Letters 40 (1999) 8301–8304

TETRAHEDRON LETTERS

A novel thiazolium type peptide coupling reagent for hindered amino acids

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Received 18 June 1999; accepted 14 September 1999

Abstract

A highly efficient coupling reagent, 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate (BEMT), was designed, synthesized and successfully applied to the synthesis of oligopeptides containing *N*-alkyl or α-*C*-dialkyl amino acids. Its efficiency was evaluated by HPLC and ^{1}H NMR methods, and demonstrated by synthesis of a number of *N*-methyl-rich peptide segments with good yields and negligible racemization. The mechanism of coupling was studied by HPLC, ^{1}H NMR and IR monitoring; it is proposed that labile (acyloxy)thiazolium salts and *N*-protected amino acid bromides were the major active intermediates with concomitant formation of *N*-ethyl-4-methyl thiazolidones and a small amount of oxazolones and *N*-protected amino acid anhydrides.

N-Methyl amino acids are present in many naturally occurring peptides exhibiting important biological properties, such as cyclosporines[1] and pseudopeptides of marine origin including didemins[2] and dolastatins.[3] It has also been established that the metabolic stability of biologically active peptides can be altered or enhanced by the introduction of *N*-methylated amino acid residues which has become a standard modification in peptide chemistry.

Unfortunately, the incorporation of hindered *N*-methyl amino acids into peptides results in difficult condensations using the usual DCC/HOBt or HOBt-derived coupling reagents. The problem also exists in the synthesis of peptides containing α-aminoisobutyric acids. Recently, this kind of condensation was improved by using HOAt-derived coupling reagents[4] and the halogenuronium or halogenophonium derivatives PyClU, PyCloP and PyBroP.[5] We have now designed and synthesized a novel, even more efficient coupling reagent, 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate (BEMT†), for the coupling of hindered amino acids based on our previous studies.[6]

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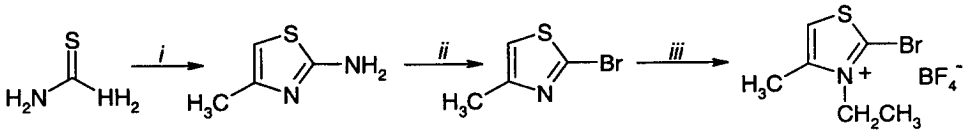
† Abbreviations: Aib, α-aminoisobutyric acid; BEMT, 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate; BTFFH, bis(tetramethylene) fluoroformamidinium hexafluorophosphate; CMBI, 2-chloro-1,3-dimethyl 1*H*-benzimidazolium hexafluorophosphate; DIEA, diisopropylethylamine; HOAt, 1-hydroxy-7-azabenzotriazole; OFm, 9-fluorenylmethyloxy; OBzl, benzyloxy; OBu^t, *tert*-butyloxy; PyBroP, bromotripyrrolidinophosphonium hexafluorophosphate; PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; PyClU, 1,1,3,3-bis(tetramethylene) chlorouronium hexafluoro-phosphate.

0040-4039/99/$ - see front matter
PII: S0040-4039(99)01763-3

BEMT was readily prepared from inexpensive and non-toxic starting materials (Scheme 1). The intermediate, 2-bromo-4-methylthiazole, which can be easily purified by stream distillation, was treated with triethyloxonium tetrafluoroborate to give the desired compound as a shelf-stable crystalline solid.[7]



Scheme 1. Synthesis of coupling reagent BEMT[a]. [a]Reagents: (i) (a) CH_3COCH_3, I_2, reflux, 4 h; (b) NaOH, rt; (ii) $NaNO_2$, NaBr, $CuSO_4$, H_2SO_4/H_3PO_4, 0°C; (iii) Et_3OBF_4, $ClCH_2CH_2Cl$, 60°C, 1 h

The efficiency of BEMT in the synthesis of oligopeptides containing hindered amino acids, the estimation of racemization and the influence of several reaction parameters such as solvent, base, as well as temperature were studied by HPLC (model reactions: Z-Aib-OH+Aib-OMe·HCl→Z-Aib-Aib-OMe and Z-Gly-Phe-OH+Val-OCH_3·HCl→Z-Gly-Phe-Val-OCH_3[8]) and by ^1H NMR methods (model reaction: Z-Me-Val-OH+Me-Val-OMe·HCl→Z-Me-Val-Me-Val-OMe).[9,10] It was observed that CH_2Cl_2 was the favored solvent with respect to low racemization, fast reaction rate and good yield among CH_3CN, DMF THF and CH_2Cl_2. The hindered strong base DIEA was more suitable than NEt_3 and 2,6-lutidine leading to less racemization and higher yield. The high reactivity of BEMT allowed the coupling reaction to be carried out at lower temperatures that will be a benefit to the reduction of epimerization.

For a further evaluation of the efficiency of BEMT, a comparison between BEMT and PyClU, a commonly used coupling reagent, was also made by HPLC using the model reaction Z-Gly-Phe-OH+Val-OCH_3·HCl→Z-Gly-Phe-Val-OCH_3. It was observed that a result in favor of BEMT was obtained. The yield was up to 46% after 2 mins and racemization did not exceed 2.7% with BEMT; while the yield was no more than 12% and the racemization was 25% for PyClU. ^1H NMR confirmed that the epimerization during coupling using BEMT was lower than with PyClU. Similar results were also obtained on comparison with other halogenated reagents, such as BTFFH and CBMI.

The usefulness of the thiazolium type coupling reagent BEMT was supported by the successful synthesis of a series of peptides containing hindered amino acids (Table 1).

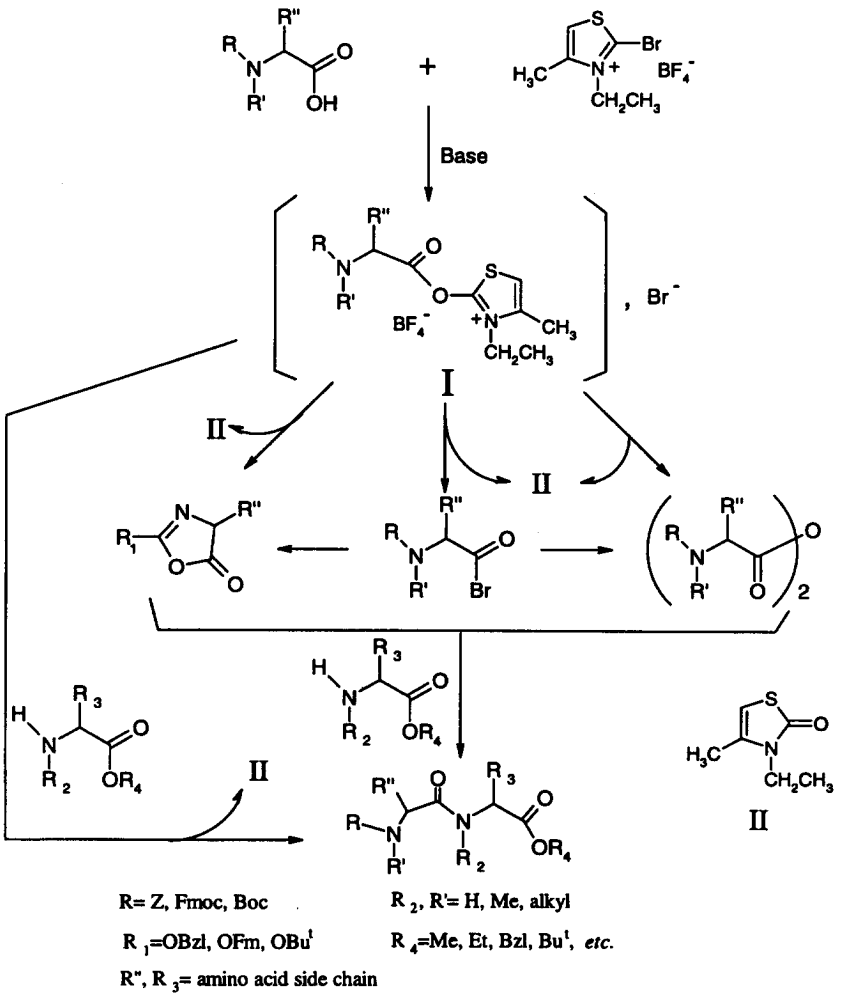
The reaction mechanism was studied by carrying out the coupling reaction in $CDCl_3$ and monitoring by ^1H NMR and IR. It is speculated that the first step is carboxylic acid activation by BEMT involving

Table 1
Preparation of peptides using BEMT as coupling reagent[a]

Entry	Peptide[b]	yield (%)	m.p. (°C)	$[\alpha]_D$(conc.,solv., temp)
1	Z-MeVal*-MeVal-OMe	88	oil	- 207 (1, MeOH, 19°C)
2	Z-Aib*-Aib-OCH_3	95	109 - 110	—
3	Fmoc-MeLeu*-MeVal-OBu^t	91	oil	- 102.7 (1,$CHCl_3$, 22°C)
4	Fmoc-MeLeu*-MeLeu-MeVal-OBu^t	87	n.d.[d]	- 152.1 (1, $CHCl_3$, 22°C)
5[c]	Fmoc-D-Ala*-MeLeu-MeLeu-MeVal-OBu^t	89	51 - 54	- 168 (0.1, $CHCl_3$, 22°C)
6	Fmoc-Nva-Sar*-MeLeu-Val-MeLeu-Ala-OBzl	86	80 - 81	- 114.6 (1, $CHCl_3$, 22°C)
7[c]	Fmoc-MeLeu*-Nva-Sar-MeLeu-Val-MeLeu-Ala-OBzl	92	48 - 49	- 114.8 (0.5, $CHCl_3$, 22°C)

[a] The reactions were carried out as for PyBroP. In a typical experimental procedure, DIEA (3 equiv.) was added to a cooled mixture (-10°C) of *N*-protected amino acid (1 equiv.), amino acid ester hydrochloride (1.1 equiv.), and BEMT (1.1equiv.) in CH_2Cl_2 (2-4mL/mmol), stirred for 1 minute cold and for an hour at room temperature; the reaction time should be properly prolonged for the coupling between *N*-methyl amino acids and can be monitored by TLC. [b]The CO-NH bond formed in the peptide is indicated by *. All products were confirmed by ^1H NMR, EIMS and other characterization. [c] Further confirmed by HMQC and ESI-MS analysis. [d] No distinct melting point.

the formation of an unstable acyloxythiazolium salt **I**, which in turn reacts directly with the amino component to give the product, or is competitively converted into the acid bromide which is subsequently converted into the dipeptide by aminolysis. A small amount of the corresponding acid anhydride and 5(4*H*)-oxazolone is also formed from the acyloxythiaozolium salt or acid bromide (Scheme 2). The main by-product of the reaction is *N*-ethyl-4-methyl thiazolidone **II** which can be isolated from the reaction mixture and characterized by elemental analysis, ^{1}H NMR, EI-MS and IR.



Scheme 2. The proposed reaction mechanism for the coupling reagent BEMT

In conclusion, the crystalline and shelf-stable thiazolium salt, BEMT, was shown to be a very efficient peptide coupling reagent for the acylation of *N*-methyl amino acids or α-aminoisobutyric acid with good yields, fast reaction rate and low racemizaton. Its efficiency may be due to the high reactivity of acyloxythiazolium salts and *N*-protected amino acid bromides intermediates.

Acknowledgements

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

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7. BEMT was easily synthesized from thiourea by three steps in 23% overall yield as a colorless crystals, mp 189–189.5°C. ^{1}H NMR (300 MHz, [D$_6$]acetone, 25°C, TMS): δ=8.15 (s, 1H, aryl), 4.72 (q, ^{3}J(H,H) =7.5 Hz, 2H, CH_2CH_2), 2.77 (s, 3H, CH_3), 1.55 (t, ^{3}J(H,H) =7.5 Hz, 3H, CH_2CH_3). IR (KBr): ν=3134, 1581, 1476, 1442, 1330, 1065, 958, 869, 763, 522 cm^{-1}. FAB-MS: 206, [M–BF_4^-] 208 [(M+2)–BF_4^-].
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10. The quantity of the D-L isomer can be calculated from the four sharp signals of OMe group. The two diastereoisomers: Z-L-Me-Val-L-Me-Val-OMe, Z-D-Me-Val-L-Me-Val-OMe and Z-D,L-Me-Val-L-Me-Val-OMe were synthesized, respectively, to determine the chemical shift and proportion of the OMe signal of the four conformers of each isomer.