COUPLING METHODS FOR *Fmoc-His(Trt)-OH* RESULTING IN MINIMAL RACEMIZATION

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INTRODUCTION

The derivative Fmoc-His(Trt)-OH represents the most widely used building block for introduction of His by Fmoc-chemistry due to its commercial availability and the adequate acid lability. Unfortunately, upon activation, the free π-nitrogen of the imidazole moiety contributes to substantial racemization [1, 2]. Since π-protected derivatives, compatible with the Fmoc/tBu strategy, are not readily available, we have started to optimize the coupling reaction for Fmoc-His(Trt)-OH aiming at a minimal racemization.

RESULTS AND DISCUSSION

The recently developed model peptide Z-Ala-L-His-Pro-OH [1] and, for reasons of comparison, also its D-His epimer were synthesized to assess the degree of racemization during activation and coupling of Fmoc-His(Trt)-OH. For the syntheses of the L-epimer, the new coupling reagents DEPBT [3] and TOTT [4] (see Figure 1) were included in a list of activators used for the coupling of Fmoc-His(Trt)-OH (see Table 1). The percentages of D-His epimer, as determined by RP-HPLC of the crude products, are given in Table 2. Approximately 0.3% of Z-Ala-D-His-Pro-OH contaminating the all-L-epimer could reliably be detected.

In agreement with previous studies [2], the application of the commonly used reagent TBTU led to approximately 4% of the D-His peptide (see Figure 2B). The results obtained after synthesis of the D-His epimer, using TBTU/DIPEA activation for the coupling of Fmoc-D-His(Trt)-OH, nicely correlate with the findings reported in Table 2. In this case, the crude product contained 3.9% of the L-epimer.

The recently described DEPBT [3] in combination with diisopropylethylamine (DIPEA) turned out to represent an excellent activation reagent (see Figure 2C). However, the replacement of the base with collidine resulted in very slow coupling rates. TPTU [5] also proved to be a superior coupling reagent, preferably in combination with collidine. Another new activation reagent, TOTT [4], performed less satisfactory, especially in combination with collidine. Unexpectedly, TFFH [6] showed a disappointing result (see Figure 2A) and, according to this study, this derivative should not be employed for the incorporation of Fmoc-His(Trt)-OH. Activation with TATU [7] in combination with DIPEA distinctly promoted racemization, though a remarkable decrease of the D-epimer was observed when the base collidine was applied. The beneficial influence of collidine was also demonstrated in case of the reagent PyBOP [8]. A moderate degree of racemization resulted when activation of Fmoc-His(Trt)-OH was carried out in the presence of DCC/HOBT [9].

Figure 1

Structures of the activation reagents DEPBT, TOTT, and TPTU.

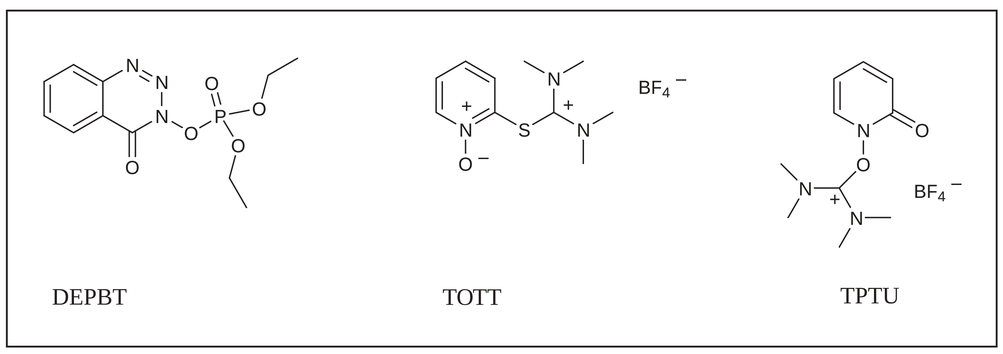


Table 1

Coupling reagents applied in this study.

Abbreviation	Coupling Reagent	Reference
DCC/HOBT	Dicyclohexylcarbodiimide/1-hydroxybenzotriazole	[9]
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-(3H)-one	[3]
PyBOP	Benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate	[8]
TATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate	[7]
TBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate	[5]
TFFH	Tetramethylfluoroformamidinium hexafluorophosphate	[6]
TOTT	S-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethyluronium tetrafluoroborate	[4]
TPTU	O-(1,2-dihydro-2-oxo-1-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate	[5]

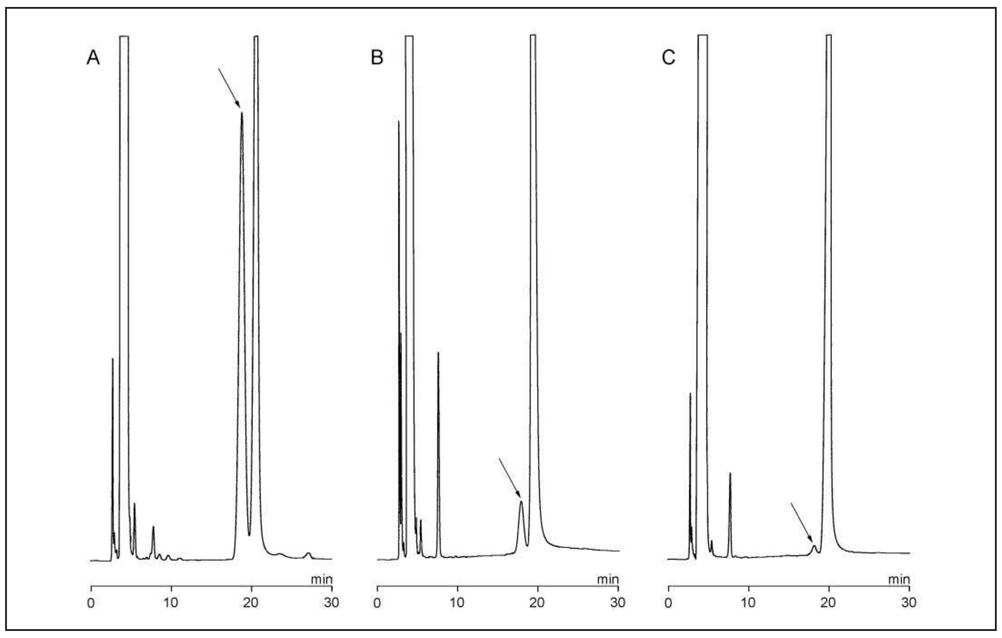
Table 2

Extent of epimerization of the peptide Z-Ala-His-Pro-OH during coupling of Fmoc-His(1-Trt)-OH as determined by HPLC.

coupling reagent	base	% D-His peptide
DCC/HOBT	---	2.8
DEPBT	DIPEA	0.8
DEPBT	collidine	0.8
PyBOP	DIPEA	12.7
PyBOP	collidine	4.4
TATU	DIPEA	13.9
TATU	collidine	2.8
TBTU	DIPEA	4.5
TBTU	collidine	4.1
TFFH	DIPEA	43.3
TFFH	collidine	29.3
TOTT	DIPEA	2.9
TOTT	collidine	5.5
TPTU	DIPEA	1.0
TPTU	collidine	0.6

Figure 2

HPLC-profiles of crude Z-Ala-His-Pro-OH obtained after syntheses following different coupling protocols for Fmoc-His(Trt)-OH.
***A**: coupling reagent, TFFH/DIPEA, **B**: coupling reagent, TBTU/DIPEA, **C**: coupling reagent, DEPBT/DIPEA (the arrow indicates the LDL-epimer).*
Conditions of analytical HPLC analyses: Nucleosil C_{18}, 50 Å (Macherey-Nagel) at 40°C; buffer system: 0.095 M H_3PO_4 and 0.09 M triethylamine in water (pH 2.3); buffer A: 10% acetonitrile, buffer B: 60% acetonitrile; gradient: 10 %B to 40 %B in 40 min; flow: 0.6 mL/min; detection at 220 nm.



CONCLUSION

In this study, we have demonstrated that the racemization of activated Fmoc-His(Trt)-OH can be markedly reduced by proper choice of the coupling reagent. In our hands, DEPBT/DIPEA and TPTU/collidine turned out to represent the optimal reagents for activation. Application of these coupling methods improved the quality of the crude product significantly and, thus, this contribution represents a further step towards an optimal strategy for the incorporation of His. In combination with a suitably π-protected His derivative (yet to be developed), these methods could help to overcome the problem of racemization for the His residue, a prerequisite for the synthesis of large peptides and proteins.

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