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RACEMIZATION STUDIES DURING SOLID-PHASE PEPTIDE SYNTHESIS USING AZABENZOTRIAZOLE-BASED COUPLING REAGENTS[1,2]

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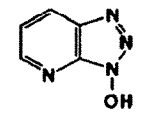
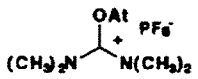
Key words: HATU, 1-hydroxy-7-azabenzotriazole, phosphonium salts, racemization, solid-phase peptide synthesis, segment coupling, uronium salts.

Abstract: *1-Hydroxy-7-azabenzotriazole (HOAt) and its corresponding uronium salts are shown to be more effective in avoiding racemization in a model solid-phase peptide segment coupling process than their benzotriazole analogs.*

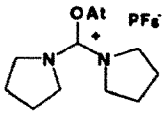
Recently, 1-hydroxy-7-azabenzotriazole (HOAt) in combination with carbodiimides, as well as the corresponding uronium and phosphonium analogs of *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) or benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) have been described as superior peptide coupling reagents for both solution and solid-phase syntheses.[3,4] These derivatives, which increase coupling yields in solution by about 6-32 times[3] and make possible the automated solid-phase synthesis of peptides containing hindered amino acids,[4] also reduce racemization in solution for segment coupling processes.[3,5] The present communication describes a model to study the racemization associated with the use of these derivatives when analogous couplings are effected by solid-phase techniques.



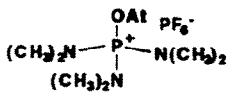
HOAt



HATU



HAPyU



AOP

The model studied involves the 2-hour coupling of Fmoc-Phe-Ser(*t*Bu)-OH onto H-Pro-PAL-PEG-PS-resin[6] under various conditions,[7] deblocking of Fmoc group with piperidine–DMF (2:8), cleavage of the tripeptide from the resin with TFA–H_2O (9:1), and separation of the crude diastereoisomers on a Delta Pak C_{18} column (5 μn, 100 A, 9 x 150 mm) eluted isocratically with 0.1% TFA in H_2O (r_t 6.0 and 6.8 min for H-L-Phe-L-Ser-L-Pro-NH_2 and H-L-Phe-D-Ser-L-Pro-NH_2, respectively).[8]

First, using HBTU as coupling reagent, the question of excess protected dipeptide was examined along with the effect of preactivation and reaction temperature. The results shown in Table I indicate that

preactivation should be totally avoided, that a greater excess of protected dipeptide leads to less racemization, and that temperature does not have a definite influence.

Table I. Effect of Excess Peptide Acid, Preactivation, and Temperature on HBTU Couplings

coupling method[a]	equiv	preact	temp (1st h)[b]	LDL-isomer
HBTU–DIEA, DMF	1.5	7 min	25 °C	40%
HBTU–DIEA, DMF	4.5	7 min	25 °C	40%
HBTU–DIEA, DMF	1.5	7 min	0 °C	40%
HBTU–DIEA, DMF	4.5	7 min	0 °C	39%
HBTU–DIEA, DMF	1.5	-	25 °C	21%
HBTU–DIEA, DMF	4.5	-	25 °C	12%
HBTU–DIEA, DMF	1.5	-	0 °C	17%
HBTU–DIEA, DMF	4.5	-	0 °C	15%

[a]The protected peptide, HBTU, and DIEA were stirred in DMF for 7 min at the corresponding temperature, and the mixture then added to the H-Pro-PAL-PEG-resin. Alternatively the mixture was added to the resin at once. [b]Total reaction time 2 h.

The data outlined in Table II, clearly show the effectiveness of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) in avoiding racemization, relative to HBTU, when 4.5 equiv of protected peptide is used, without preactivation. In the case of HATU coupling, racemization is slightly reduced when the temperature is kept at 0 °C during the 1st hour.

Table II. Effect of Temperature on HATU and HBTU Couplings

coupling method	equiv	preact	temp (1st h)	LDL-isomer
HATU–DIEA, DMF	4.5	-	0 °C	5%
HBTU–DIEA, DMF	4.5	-	0 °C	15%
HATU–DIEA, DMF	4.5	-	25 °C	6%
HBTU–DIEA, DMF	4.5	-	25 °C	12%

Next, the effect of adding an equivalent of HOAt or HOBt during the HATU or HBTU mediated coupling was evaluated. The results collected in Table III indicate that the presence of excess HOAt or HOBt during the process enhances racemization, in line with the fact that, as previously reported, under such conditions coupling yields are not improved.[4]

Table III. Effect of Excess HOAt or HOBt on HATU or HBTU Couplings

coupling method[a]	base	solvent	LDL-isomer
HATU	DIEA	DMF	5%
HATU–HOAt	DIEA	DMF	9%
HBTU	DIEA	DMF	12%
HBTU–HOBt	DIEA	DMF	20%

[a]Couplings were carried out with 4.5 equiv of dipeptide acid, at 0 °C for the 1st hour.

The results shown in Table IV indicate that different uronium and phosphonium salts derived from HOAt afford similar results, although the two dimethylamino derivatives (HATU and AOP) and the uronium salt (HAPyU) derived from pyrrolidine lead to slightly less racemization.[9]

Table IV. Effect of Uronium or Phosphonium Salt on Racemization

coupling method[a]	base	solvent	LDL-isomer
HATU	DIEA	DMF	5%
HAPyU	DIEA	DMF	5%
HAPipU	DIEA	DMF	7%
HAMTU	DIEA	DMF	8%
AOP	DIEA	DMF	5%
PyAOP	DIEA	DMF	7%

[a]Couplings were carried out with 4.5 equiv of dipeptide acid, at 0 °C for the 1st hour.

HOAt used in conjunction of *N,N'*-dicyclohexylcarbodimide (DCC) also lowered dramatically the extent of racemization when compared with HOBt or in absence of any additive (Table V).

Table V. Effect of HOAt or HOBt on DCC Coupling

coupling method[a]	solvent	LDL-isomer
DCC–HOAt	DMF	6%
DCC–HOBt	DMF	18%
DCC	DMF	22%

[a]Couplings were carried out with 4.5 equiv of dipeptide acid, at 0 °C for the 1st hour.

Because of its general utility in segment coupling processes, DMF was used as solvent in most cases. Dilution of DMF with a less polar solvent such as CH_2Cl_2 led to lowered racemization levels. On the other hand mixtures containing hexafluoroisopropanol (HFIP) and toluene gave results similar to those observed with DMF (Table VI).

Table VI. Solvent Effects on Racemization

coupling method[a]	solvent	LDL-isomer
HATU–DIEA	DMF	5%
HATU–DIEA	DMF–CH_2Cl_2 (1:2)	3%
HATU–DIEA	HFIP–CH_2Cl_2 (1:2)	5%
HATU–DIEA	DMF–toluene–CH_2Cl_2 (1:1:1)	5%
DCC–HOAt	DMF	6%
DCC–HOBt	DMF–CH_2Cl_2 (1:2)	3%

[a]Couplings were carried out with 4.5 equiv of dipeptide acid, at 0 °C for the 1st hour.

Finally, the effect of the base was studied (Table VII) and while *N*-methylmorpholine (NMM) gave results similar to those noted with DIEA, the use of collidine considerably reduced the level of racemization, in agreememt with results previously reported for coupling reactions carried out in solution.[5]

Table VII. Effect of Base on Racemization

coupling method[a]	base	solvent	LDL-isomer
HATU	DIEA	DMF	5%
HATU	NMM	DMF	5%
HATU	Collidine	DMF	3%
HATU	DIEA	DMF–CH_2Cl_2 (1:2)	3%
HATU	Collidine	DMF–CH_2Cl_2 (1:2)	2%

[a]Couplings were carried out with 4.5 equiv of peptide acid, at 0 °C for the 1st hour.

In order to confirm the effects observed for coupling reactions carried out in solution, reaction of the same dipeptide used for the solid-phase studies [Fmoc-Phe-Ser(*t*Bu)-OH] with H-Pro-NH_2 was examined under various conditions. The results (Table VIII) indicate that the combinations of collidine-HATU or collidine-HAPyU lead to near total suppression of racemization. In addition HATU and HAPyU again show their superiority relative to HBTU.

Table VIII. Effect of Base on Racemization for Couplings Carried out in Solution

coupling method[a]	base	solvent	LDL-isomer
HATU	DIEA	DMF	4%
HATU	Collidine	DMF	< 0.1%
HAPyU	DIEA	DMF	5%
HAPyU	Collidine	DMF	< 0.1%
HBTU	DIEA	DMF	17%
HBTU	Collidine	DMF	12%

[a]Equimolar amounts of dipeptide, prolylamide, and coupling reagent were mixed for 1h at 0 °C and 2 h at 25 °C in the presence of 2 equiv of base.

In conclusion, it is clear that coupling reagents derived from HOAt are markedly more effective than analogous reagents derived from HOBt in allowing solid-phase segment coupling with minimal racemization. Of major importance in the case of uronium salt couplings is the avoidance of any preactivation time. Additional improvements can be made by appropriate choice of the necessary, tertiary base and attention to solvent composition. Upon further definition of optimun conditions, including modifications designed to reduce racemization to levels observed in solution, this technique promises to be well adapted to the convergent solid-phase approach[10] to the synthesis of longer peptides.

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References and Notes

1) The term racemization is used in this paper to indicate epimerization at the *C*-terminal amino acid of the protected peptide.

2) Abbreviations used are as follows: AOP, 7-azabenzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; BOP, Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; *t*Bu, *tert.*-butyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HAMTU, *O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-trimethyleneuronium hexafluorophosphate; HAPipU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(pentamethylene)uronium hexafluorophosphate; HAPyU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HFIP, hexafluoroisopropanol; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; PAL, 5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid; PEG, polyethylene glycol; PS, polystyrene, PyAOP, 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; Amino acid symbols denote the L-configuration unless indicated otherwise.

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7) The dipeptide (1.5 or 4.5 equiv) and the coupling reagent (1.5 or 4.5 equiv) dissolved in the appropiate solvent were added to the prolyl resin (1 equiv), followed by the base used (3 or 9 equiv). All couplings took place with yields above 95%, as demonstrated by spectrophotometric determination of the Fmoc group.

8) Both diastereisomers were prepared independently using a Milllipore 9050 continuous-flow synthesizer following standard protocols; Fields, G.B.; Tian, Z.; Barany, G. In Grant, G.A. (Ed) Synthetic Peptides: A User's Guide, W.H. Freeman & CO., New York, 1992, pp. 77-183.

9) For the cyclization of linear peptides in solution, HAPyU leads to less racemization than HATU. See Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L.A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781-4784.

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