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Addition of HOAt Dramatically Improves the Effectiveness of Pentafluorophenyl-Based Coupling Reagents

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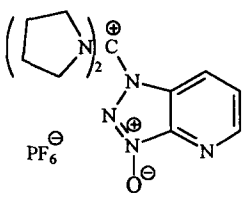
Abstract:
In the course of comparing the effectiveness of several HOAt- and HOPfp-derived coupling reagents by cyclization and segment condensation of model sequences, using HPLC to follow the time course and to determine the outcome in terms of oligomerization and epimerization, we found a striking improvement of the less effective HOPfp-based coupling reagent (HPyOPfp), both with regard to reaction rate and extent of epimerization, when HOAt was added.

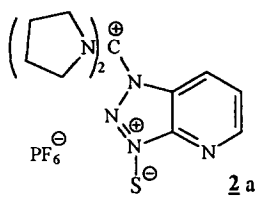
Due to their restricted conformational flexibility, cyclic peptides are of great interest in connection with structure-activity relationships, especially the elucidation of bioactive conformations.

In the special, and sometimes problematic case of head-to-tail cyclizations of all-L penta- and hexapeptides the search for efficient coupling methods is of great relevance, particularly in the design and synthesis of cyclic peptide libraries[1,2].

Phosphonium-, uronium- and guanidinium-based coupling reagents derived from 1-hydroxy-7-azabenzotriazole (HOAt), such as HATU, PyAOP and HAPyU[3], promote rapid peptide cyclizations with a minimum of epimerization[4-7]. Recently, the structures of these coupling reagents have been modified both in the uronium/guanidinium moiety[8] and by introducing alternative leaving groups, e.g. the pentafluorophenyl residue (OPfp)[9].

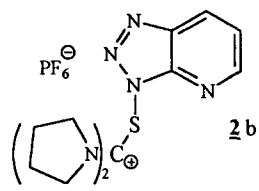


1, HAPyU



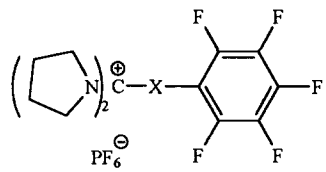
2 a

or



2 b

2, HAPyTU



3 a, X = O HPyOPfp
3 b, X = S HPySPfp

Figure 1. Examples of Coupling Reagents Used for Cyclization [10]

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Comparison of the effectiveness of these reagents[11] by cyclization of the sequence AANMeAAA and [3+3] segment condensation, Z-GGV + AGG-PAL-PEG-PS, following standard protocols[6,12], shows a clear superiority for the HOAt-derived coupling reagents compared to those derived from HOPfp both in terms of reaction rates and epimerization (Table 1, see also Ref. 13).

Table 1.

Coupling Reagent	AANMeAAA-Cyclization[a] (after 60 min reaction)		Z-GGV+AGG-PAL-PEG-PS Segment Condensation[b]	
	% all-L-c.m.[c]	%AANMeAAa c.m.	Yield [%]	%DL
HATU	53	2.3	97.7	2.0
HATTU			93.5	4.8
HAPyU	55	1.6	97.9	1.9
HAPyU / HOAt			95.6	0.8
HAPyTU	50	3.8	94.9	4.0
PyAOP[d]	54		97,1	2.9
PyAOP / HOAt			97.9	2.0
HPyOPfp	10[e]	39[e]	92.3	27.3
HPySPfp	11[e]	14[e]	91.2	33.8
HPyOPfp / HOAt	56	2.0	91.2	2.5

[a] 10^{-3} M in DMF, 3 eq. of DIEA, 1.1 eq. of coupling reagent; [b] 3 eq. each of acid, coupling reagent and TMP were preactivated for 30 sec and the mixture added to the resin; [c] c.m.: cyclic monomer; [d] 3 eq. of coupling reagent used for cyclization; [e] still 40-55% linear peptide

The effectiveness of these coupling reagents appears to be more strongly influenced by the nature of the 'active ester' and less by that of the uronium- /guanidinium- /phosphonium component. Moreover, the results indicate different mechanisms for HOAt- and HOPfp-derived coupling reagents and, supported by IR experiments, it has been concluded that reactions of the HOAt-derived coupling reagents involve a highly reactive intermediate, probably the OAt ester, whereas HOPfp-derived coupling reagent-promoted reactions involve the intermediacy of oxazolone and the relatively less reactive OPfp ester.

Remarkably high epimerization levels are observed for the OPfp-derived coupling reagents and it is in these cases where the IR data show extensive oxazolone formation. It has been shown previously that for other coupling reagents known to generate oxazolones, the addition of HOAt effects a shift to the safer, more reactive OAt ester[14]. The influence of HOAt on HPyOPfp-promoted coupling reactions is shown in Table 2 for [2+1] segment condensation giving the model tripeptide Z-Phe-Val-Pro-NH_2 under both solution and solid phase conditions and in Table 3 for cyclization.

The various protocols used for the investigation of HOAt-addition were as follows: (i) the acidic component was preactivated in the presence of coupling reagent (HPyOPfp) and base, and the amino component and HOAt were then added (experiments 5 and 6); (ii) the acidic component was preactivated in the presence of coupling reagent, HOAt and base, and the amino component was added (experiments 7 and 8); (iii) acidic- and amino component were allowed to react for a definite time in the presence of coupling reagent and base, and then HOAt was added (experiments 13 and 14) and (iv) all reactants were present in the reaction system at the start of reaction (experiments 9 and 12).

In general, the addition of HOAt strikingly improved the results for the HOPfp-derived coupling reagents compared to the analogous reactions carried out in the absence of HOAt. In some cases the results were comparable to, or even better, than those observed for the HOAt-derived coupling reagents, both with regard to the time course of the reaction and the percentage of epimerized product.

Table 2.

Coupling Reagent	Z-Phe-Val-OH+H-Pro-NH_2 Segment Condensation[a] in Solution		on Solid Phase	
	Yield [%]	%LDL	Yield [%]	%LDL
1 HAPyU	86.5	3.3	98.6	8.9
1a HAPyU(30s)[b]	87.6	3.5	98.6	12.9
2 HAPyU(30s)/HOAt[c]	89.9	3.5	96.2	13.1
3 HAPyU(0)/HOAt[d]	89.1	2.3	98.2	9.0
4 HPyOPfp	85.6	33.7	90.0	39.5
4a HPyOPfp(30s)[b]	84.3	35.1	91.2	41.2
5 HPyOPfp(30s)/HOAt[c]	80.1	2.7	94.5	16.9
6 HPyOPfp(7m)/HOAt[c]	82.3	3.2		
7 HPyOPfp(30s)/HOAt[b]	81.1	2.6		
8 HPyOPfp(7m)/HOAt[b]	83.1	3.3		
9 HPyOPfp(0)/HOAt[d]	80.4	1.7	93.9	12.4

[a] Coupling in solution was carried out with 1 eq. each of acid, coupling reagent and base (TMP) whereas on solid phase the conditions of footnote (b) of Table 1 were used.
[b] Z-FV-OH was preactivated with 1 Eq. of HPyOPfp and 3 Eq. TMP 30 sec or 7 min in DMF, then H-Pro-NH_2 was added.
[c] Z-FV-OH was preactivated with 1 Eq. of HPyOPfp and 3 Eq. TMP 30 sec or 7 min in DMF, then 1 Eq. of HOAt and H-Pro-NH_2 were added.
[d] no preactivation
[e] Z-FV-OH was preactivated with 1 Eq. of HPyOPfp, 1 Eq. of HOAt and 3 Eq. TMP 30 sec or 7 min in DMF, then H-Pro-NH_2 was added.

Table 3.

Coupling Reagent	AANMeAAA-Cyclization (after 24 h reaction)	
	% all-L-c.m.[a]	%AANMeAAa c.m.
10 HAPyU	55	1.6
11 HPyOPfp	16[c]	60
12 HPyOPfp/HOAt(0)[b]	56	2.0
13 HPyOPfp/HOAt(30s)[c]	45	27
14 HPyOPfp/HOAt(7m)[d]	36	45

[a] c.m.: cyclic monomer
[b] 1.1 eq. each of HPyOPfp and HOAt were added to the linear peptide in DMF, then 3 eq. of DIEA were added.
[c] HOAt was added 30 sec after DIEA addition to peptide / HPyOPfp in DMF.
[d] HOAt was added 7 min after DIEA addition to peptide / HPyOPfp in DMF.

Although there was no notable difference in the outcome of segment condensations when the amino component was added to the 30-sec or 7-min-preactivated acidic component alone or along with HOAt, reactions performed according to protocol (iv) gave the best results for both cyclization and segment condensation. If the amino component is present in the reaction mixture along with the acidic component (e.g. cyclizations), the time of HOAt addition has a clear influence on the outcome: the later the HOAt is added the more is the product epimerized.

Based on the results shown and the accompanying IR experiments, one can summarize the data as follows:

(a) If no HOAt is present the reaction mixture contains oxazolone and OPfp ester in a ratio of about 1 : 1. The presence of a large amount of oxazolone can explain the extensive epimerization.

(b) If HOAt is present, the mixture contains OPfp ester and OAt ester in a ratio of about 1 : 1 after 1 min although later the ratio shifts to a greater amount of OPfp ester. The OAt ester reacts rapidly and the OPfp ester continues to shift to OAt ester. The later HOAt is added to the reaction system the more oxazolone formation occurs, leading to a higher degree of epimerization.

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2. Gordon, E.M.; Barett, R.W.; Dower, W.J.; Fodor, S.P.A., Gallop, M.A. *J. Med. Chem.*, **1994**, 27, 1385.
3. Abbreviations: DIEA: *N,N*-diisopropylethylamine; DMF: dimethylformamide; HAPyTU: S-(7-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)thiouronium hexafluorophosphate; HAPyU: 1-(1-pyrrolidinyl)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate N-oxide; HATTU: S-(7-azabenzotriazol-1-yl)-1,1:3,3-tetramethylthiouronium hexafluorophosphate; HATU: N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide; HOAt: 1-hydroxy-7-azabenzotriazole; HOPfp: pentafluorphenol; HPyOPfp: O-(pentafluorophenyl)-1,1:3,3-bis(tetramethylene)uronium hexafluorophosphate; HPySPfp: S-(pentafluorophenyl)-1,1:3,3-bis(tetramethylene)thiouronium hexafluorophosphate; PyAOP: (7-aza-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate; PyPfpOP: (pentafluorophenoxy)tris(pyrrolodino)phosphonium hexafluorophosphate; TMP: 2,4,6-trimethylpyridine.
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9. Habermann, J.; Kunz, H. *Tetrahedron Lett.*, **1998**, 39, 265.
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