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Comparison of the Effects of 5- and 6-HOAt on Model Peptide Coupling Reactions Relative to the Cases for the 4- and 7-Isomers†,‡

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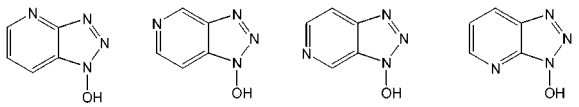
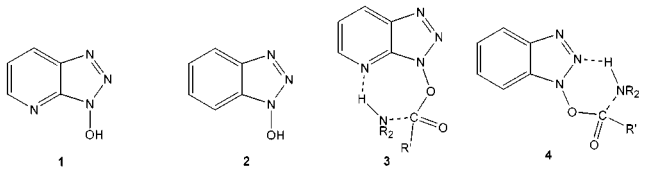
ABSTRACT



Synthesis of 5- and 6-HOAt has completed the full set of the four HOAt isomers derived from HOBt by insertion of a single nitrogen atom in the benzenoid nucleus. Comparison of the reactivity of all four isomers in model peptide coupling reactions has confirmed the unique character of the 7-isomer in promoting selectivity and maintaining configuration at the reactive carboxylic acid residue.

Upon discovery of the fact that HOAt **1** was more efficient than HOBt **2** as a peptide coupling additive, it was suggested[1] that a possible explanation was that the internal base-catalyzed process implied by the 7-ring transition state depicted in **3** could be more effective than the corresponding 6-ring process previously suggested[2] for **2** (structure **4**).[3] Alternatively, the special properties of **1** might simply be related to the presence of the pyridine-N atom, which with its electron-withdrawing properties[4] could provide for a better leaving group and thus increased reactivity for the derived *O*-acyl ester.



While the latter effect can explain the increase in reactivity observed for 7-HOAt over HOBt, it cannot explain the lesser

† Abbreviations used: Aib = α-aminoisobutyric acid; CM = cyclomonomer; EDC = 1-ethyl-3-(3′-(dimethylamino)propyl)carbodiimide; EDC·HCl = the corresponding hydrochloride; HAPyU = 1-(1-pyrrolidinyl)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate *N*-oxide; HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt = 7-HOAt = 7-aza-1-hydroxybenzotriazole (other isomers identified by a preceding 4-, 5-, or 6-); HOBt = 1-hydroxybenzotriazole; HOXt = n-HOAt or HOBt (generalized structure); PCA = *p*-chloroaniline; TMP = collidine = 2,4,6-trimethylpyridine; Z = benzyloxycarbonyl.

‡ Molecular structures for all four HOAt isomers and all of the methyl ethers have been confirmed by X-ray crystallography (BMF and MJV). Details of the structural data will be provided in the full paper.

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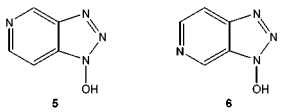
loss of configuration observed for 7-HOAt relative to both HOBt and 4-HOAt. The pK_a values for HOBt, 4-HOAt, and 7-HOAt are 4.6, 3.14, and 3.47, respectively.[5] Substitution of a nitro group into the HOBt molecule enhances the acidity but also leads to increased loss of configuration when used as an additive for segment coupling reactions. Thus, in the presence of 6-nitro-HOBt, the [2 + 1] coupling of Z-Phe-Val-OH with H-Pro-NH_2 leads to 16.3% of the LDL-isomer, whereas with HOBt and 7-HOAt the loss is 8.6% and 2.1%, respectively,[6] under the same conditions.

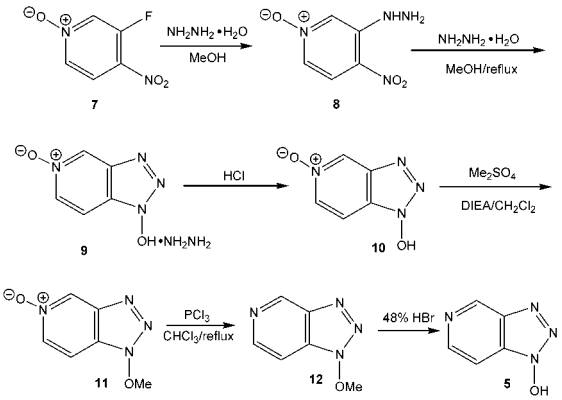
At the time the 4- and 7-HOAt isomers were examined, the 5- and 6-isomers were unknown. Recently we developed synthetic routes for these two isomers, **5** and **6**, and in the



present Letter describe a comparison of their properties with those of the known isomers.

The synthesis of 5-HOAt is outlined in Scheme 1. The synthesis began with the fluoronitropyridine *N*-oxide **7**,[7]

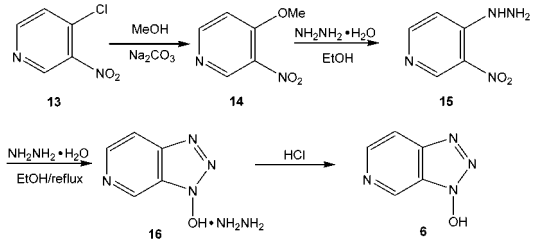
Scheme 1. Synthesis of 5-HOAt



which with hydrazine quickly gave the hydrazine intermediate **8**, which on extensive treatment with excess hydrazine underwent cyclization to give the hydroxybenzotriazole *N*-oxide, which could be deoxygenated by a standard method following prior methylation.

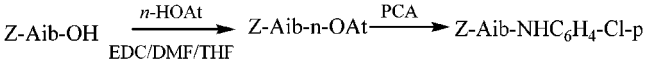
The 6-isomer was obtained similarly from 4-chloro-3-nitropyridine **13**[8] (Scheme 2) although in this case cyclization

Scheme 2. Synthesis of 6-HOAt



occurred without the need for prior formation of the pyridine *N*-oxide.

A first test involved the reactivity of the active esters derived from Z-Aib-OH (eq 1).[9] The esters **18** were



separately prepared via EDC coupling and then treated with *p*-chloroaniline, the relative reactivities being determined by ^{1}H NMR analysis by following the disappearance of the CMe_2 unit of the esters at δ 1.75−1.85 and appearance of the corresponding peak of the amide **19**, δ 1.58. Half-lives are collected in Table 1. The 7-ester was three times more

Table 1. Approximate Halftimes for the Reaction of Z-Aib-n-OAt with (A) *p*-Chloroaniline and (B) *N*-Methylbenzylamine in $CDCl_3$ and $CDCl_3$−DMSO-d_6, Respectively

	$t_{1/2}$ (min)	
ester	A	B
Z-Aib-7-OAt	7−8	2
Z-Aib-6-OAt	25	2
Z-Aib-5-OAt	95[a]	5
Z-Aib-4-OAt	95	7
Z-Aib-OBt	210	

[a] Z-Aib-5-OAt was not initially completely soluble in the reaction medium.

reactive than the 6-isomer and more than 10 times as reactive as the 5- and 4-isomers. Against the more reactive aliphatic amine, *N*-methylbenzylamine, the 7-isomer is again the most reactive, although not distinguished from the 6-isomer. The fact that the 6-isomer appears to be next in reactivity to the 7-isomer suggests that, of the 6-, 5-, and 4-isomers, it is this isomer for which the polarity of the C═N bond is such as to most enhance the acidity of the *N*-hydroxy compound.

(3) For a discussion of the favored ring size for a cyclic H-bonded transition state, see: Gandour, R. *Tetrahedron Lett.* **1974**, *15*, 295.

(4) The relationship between pyridine and nitrobenzene has often been remarked upon. See: Barnes, R. A. Properties and Reactions of Pyridine and its Hydrogenated Derivatives. In *The Chemistry of Heterocylic Compounds*; Klingsberg, E., Ed.; Interscience: New York, 1960; Vol. 14, Part 1, p 12.

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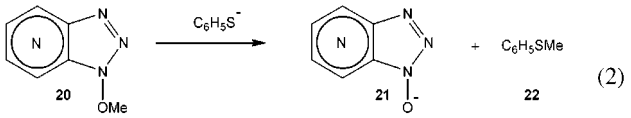
(9) A similar method was used previously but without isolation of the intermediate active esters. See: Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.

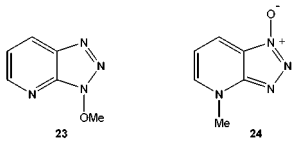
Unfortunately, reliable pK_a data could not be obtained for the 5- and 6-isomers since both appeared to undergo decomposition under the conditions of the determination.[10]

In contrast to the results of these two coupling reactions, it was of interest to compare reactivities for a system in which neighboring group effects cannot be involved. Such a reaction is that outlined in eq 2, namely the S_N2 demethylation of the methyl ethers of the HOAt derivatives by thiophenoxide ion.[11] Here the order should be determined only by the leaving group ability of **21**, which should follow the order of acidity of the HOAt derivatives.

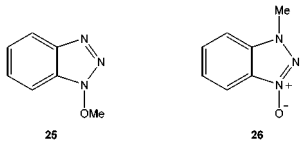


(2)

The ethers were easily synthesized by reaction with various methylating agents.[12] Previously methylation products derived from the 7- and 4-isomers have been described, with the 7-compound being assigned the normal *O*-ether structure **23** and the 4-compound the *N*-structure **24**.[13]



In earlier work, methylation of HOBt was shown to give a mixture of *O*- and *N*-forms **25** and **26**.[12b] Of the two *N*-hydroxy benzotriazole dervivatives, **25** shows in its ^{1}H NMR spectrum a singlet for the *O*-methyl ether at δ 4.39 whereas the *N*-methyl peak of **26** is found at δ 4.11. All four ethers derived from the four HOAt isomers show singlets at δ 4.43−4.49 indicative of the *O*-methyl structure.



The compound described by Azev and co-workers[13] as **24** had a mp 136−138 °C. Carrying out the methylation under comparable conditions gave in our hands a compound, mp 140−141 °C, that we assign as the *O*-methyl ether structure, based on the X-ray crystallographic determination, which confirms the ^{1}H NMR assignment. It is not certain whether these two compounds are the same. The various methylation products are listed in Table 5, Supporting Information.

Rough demethylation reactivities were easily determined by an NMR method using the thiophenoxide ion[11b] as nucleophile. The results are collected in Table 2. As expected for the systems for which pK_a data have been reported,[5] the speed of S_N2 demethylation is correlated to the acidity of the HOXt system, with the most acidic derivative providing for the highest reactivity. For the 4- and 7-HOAt isomers, these reactivities are the reverse of those seen during peptide coupling reactions.

Table 2. Approximate Halftimes for the Demethylation of the *O*-Methyl Ethers of Various 1-Hydroxybenzotriazoles by Means of Sodium Thiophenoxide

ether	pK_a of the appropriate *n*-HOAt or HOBt	$t_{1/2}$ (min) for DMSO-d_6/$CDCl_3$	$t_{1/2}$ (min) for DMF-d_7/$CDCl_3$
Me-7-OAt	3.47	19	26
Me-6-OAt		4	4
Me-5-OAt		6	8
Me-4-OAt	3.14	11	12
Me-OBt	4.70	50	

The high reactivity shown by the 5- and 6-isomers, especially the latter, is suggestive of a higher acidity for these isomers relative to the 4-isomer. Validation of this prediction must await a determination of the appropriate pK_a values.

In addition to enhancing the reactivity of active esters derived from 7-HOAt, the neighboring group effect has also been suggested as an explanation for the lowered loss of configuration observed generally for segment coupling processes involving 7-HOAt as an additive relative to cases involving either 4-HOAt or HOBt.[1] These tests have now been extended to include the 5- and 6-isomers.

Two systems were examined. One involved the [2 + 1] coupling of Z-Phe-Val-OH and H-Pro-NH_2 by a method

Table 3. Effect of Various Additives on Loss of Configuration at Valine during Formation by Segment Coupling in DMF of the Tripeptide Z-Phe-Val-Pro-NH_2 and the Hexapeptide Z-Gly-Gly-Val-Ala-Gly-Gly-OMe[a]

additive	Z-Phe-Val-Pro-NH_2, % LDL (yield, %)	Z-Gly-Gly-Val-Ala-Gly-Gly-OMe, % DL (yield, %)
7-HOAt	6.2 (95)	1.9 (89)
6-HOAt	11.0 (95)	4.7 (85)
5-HOAt	12.0 (95)	4.7 (84)
4-HOAt	13.2 (95)	3.6 (91)
HOBt	19.8	5.3 (98)

[a] Conditions: EDC·HCl/TMP (1)/*n*-HOAt (1). The results given are the averages for three separate runs.

(10) For the method used, see: Isaacs, N. S. *Experiments in Physical Organic Chemistry*; Macmillan: London, 1996; pp 8−14.

(11) The demethylation of aryl methyl ethers is a well-known model for this reaction. For examples of useful nucleophiles and selectivities seen based on the position of substitution by electron-withdrawing groups, see: (a) Cahn, R. *J. Chem. Soc.* **1931**, 1121. (b) Hansson, C.; Wickberg, B. *Synthesis* **1976**, 191. (c) Feutrill, G. I.; Mirrington, R. N. *Tetrahedron Lett.* **1970**, *12*, 1327. (d) Sheehan, J. C.; Daves, G. D., Jr. *J. Org. Chem.* **1964**, *29*, 2006.

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Table 4. Results for the Cyclization of AAMeAAA (10^{-3} M in DMF, 1.1 equiv of Coupling Reagent, 3 equiv of DIEA) via HAPyU and HATU Derivatives after 60 min, if Not Otherwise Indicated

coupling reagent	all L-linear peptide after 15 s	all L-linear peptide after 30 s	all L-CM (%)	epimeric CM (%)
4-HAPyU	17.6	10.8	67.1	< 0.3
5-HAPyU	9.0	7.3	55.2	1.9
6-HAPyU	11.7	9.1	59.0	3.0
7-HAPyU	1.9	-	56.7	< 0.3
4-HATU	49.0	37.8	50.9	2.7
5-HATU	6.9	2.7	57.6	< 0.3
6-HATU	10.3	5.5	62.1	0.9
7-HATU	6.7	2.3	41.1	< 0.3

previously described involving the presence of 1 equiv of an appropriate HOAt additive, 1 equiv of collidine, and 1 equiv of EDC·HCl.[14] The second system involved the analogous [3 + 3] coupling of Z-Gly-Gly-Val-OH with H-Ala-Gly-Gly-OMe, also described previously.[9] Results for both systems are collected in Table 3. These data are also consistent with the view that the 7-isomer of HOAt exerts a special influence on the coupling process.

When HAPyU- and HATU-type reagents derived from the four isomeric HOAt compounds were examined in the cyclization of the pentapeptide, H-Ala-Ala-MeAla-Ala-Ala-OH, it was shown that the reactivity sequence differed somewhat from that exhibited by the isolated active esters. However, for both HAPyU and HATU the 7-system was most reactive. There was also some scatter in terms of loss of configuration at the C-terminal amino acid but, again, the 7-system appeared to be more consistently protective (Table 4).

In conclusion, model peptide coupling tests extended to the 5- and 6-isomers of 7-HOAt confirm the unique character of the 7-isomer in boosting reactivity and minimizing loss of configuration at the reactive carboxylic acid residue. The results are consistent with the idea of a neighboring group effect being exerted by the 7-situated pyridine nitrogen atom.

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Supporting Information Available: Details of determining the relative reactivities of the active esters, Z-Aib-n-OAt, demethylation of the methyl ethers, Me-n-OAt (physical properties tabulated), and configurational loss upon segment condensation. This material is available free of charge via the Internet at http://pubs.acs.org.

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