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The 5,6- and 4,5-Benzo Derivatives of 1-Hydroxy-7-azabenzotriazole†

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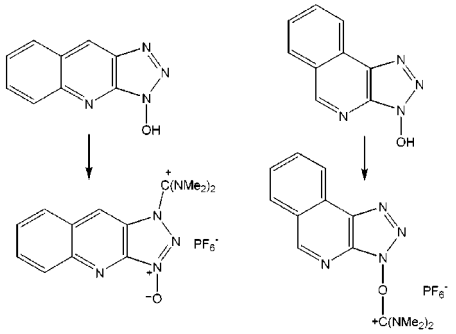
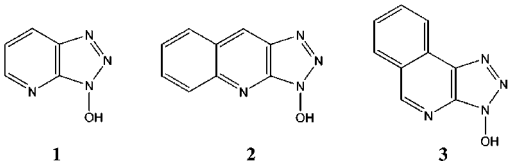
ABSTRACT



Syntheses of the two benzo derivatives of HOAt are described. Conversion of the two isomers to the corresponding onium-style coupling reagents gave in one case a guandinium species 14 and in the other, presumably as a result of steric factors, a uronium species 15. The two systems are compared as to their effectiveness in peptide coupling processes.

1-Hydroxy-7-azabenzotriazole[1] (HOAt) **1** is generally superior to 1-hydroxybenzotriazole (HOBt)[2] as an additive in peptide coupling. It has been postulated that at least part of the reason is that the intermediate active esters presumably involved in the coupling process are subject to a seven-ring anchimeric effect[1] involving the pyridine N-atom, which may be more effective in promoting reactivity than the analogous six-ring effect[3] thought to be important in the case of HOBt.



1 **2** **3**

This Letter concerns the synthesis of the two isomeric benzo derivatives of HOAt, which result from fusion of a benzo substituent across the 5,6- and 4,5-positions of the pyridine ring to give 5,6-B(HOAt) **2** and 4,5-B(HOAt) **3**, respectively. The former is a derivative of quinoline, the latter of isoquinoline. Since isoquinoline (pK_a 5.36) is slightly more basic than quinoline (pK_a 4.89)[4] it might be expected[5] that anchimeric effects at the stage of the active esters might be greater for the isoquinoline derivative.

A second key difference between the two benzo analogues is that steric effects for the quinoline derivative **2** would be expected to be little different from those characteristic of

† Abbreviations not cited in the text: ACP, acyl carrier protein decapeptide (65−74); Aib, α-aminoisobutyric acid; B, benzo; 5,6-B(HATU), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]quinolinium hexafluorophosphate 3-oxide; 4,5-B(HATU), *N*-[(dimethylamino)(3*H*-1,2,3-triazolo[4,5-*c*]isoquinolin-3-yloxy)-*N*-methylmethanaminium hexafluorophosphate; DIEA, diisopropylethylamine; HATU, 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3,-triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide; 5,6-B(HOAt), 3-hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]quinoline; 4,5-B(HOAt), 3-hydroxy-3*H*-1,2,3-triazolo[4,5-*c*]isoquinoline; TEA, triethylamine; TMP, 2,4,6-trimethylpyridine.

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(5) pK_a values in water solution appear to correlate directly with pK_{HB} values for closely related series of bases (pK_{HB} quinoline 1.85; isoquinoline 1.93). See: Taft, R. W.; Gurka, D.; Joris, L.; von R. Schleyer, P.; Rakshys, J. W. *J. Am. Chem. Soc.* **1969**, *91*, 4801. However, as pointed out by these authors, such data may not apply to cases involving intramolecular hydrogen bonding.

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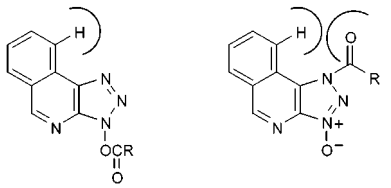
HOAt, whereas for the isoquinoline derivative **3** one arm of the benzo function occupies the 4-position, which could lead to destabilization of the *N*-acyl form of the mixed *O*,*N*-acyl species **4**, **5** believed to be involved in the acylation process.

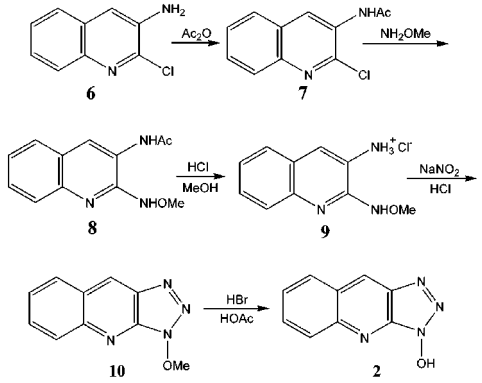


4 **5**

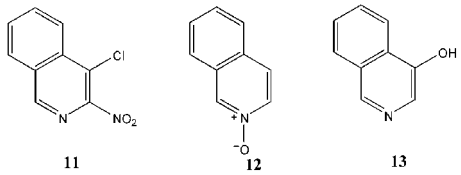
Since it has been shown that the *O*-acyl form is more reactive than the *N*-acyl form,[6] it is possible that this could be a second reason to expect increased effectiveness of **3** over **2** in coupling processes. With the promise of obtaining a better coupling additive than HOAt, these appeared to be cogent reasons for attempting the synthesis of these two compounds and comparing their reactivity with each other and with HOAt.

Possible intermediates that could be used for the synthesis of **2** via the now standard approach as applied to HOAt[1] are 2-nitro-3-chloro- or 2-nitro-3-methoxyquinoline. Neither of these compounds appears to have been reported. A possible convenient intermediate in their synthesis, 3-hydroxyquinoline[7] is not commercially available but can be obtained by diazotization of 3-aminoquinoline, available commercially but unfortunately quite expensive.[8]

Because of these difficulties in approaching the quinoline derivative **2** via the standard methodology, an alternative scheme was therefore examined based on a diazotization technique used in the synthesis of an *N*-amino analogue.[9] Thus, 2-chloro-3-aminoquinoline **6** was available by a sequence of steps that started with the Knoevenagel reaction between *o*-nitrobenzaldehyde and malonic acid.[10] Reductive cyclization followed by treatment with phosphorus oxychloride and ammonium hydroxide gave 2-chloroquinoline-3-carboxamide,[11,12] which was subjected to the Hofmann rearrangement to give **6**.[13] Since the free amino group of **6**, by its electron-donating effect, reduced the reactivity of the ring toward displacement of chloride ion, the amino function was first acetylated. The resulting acetamide **7** was treated with methoxyamine to give **8**, which was hydrolyzed, and the resulting amine salt was diazotized to give methoxyamine **10**. Deblocking of **10** gave the desired HOAt derivative **2** in an overall yield of 34.1% without the isolation of any intermediates.

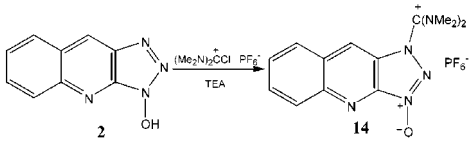


For the isoquinoline analogue **3** the standard HOAt methodology could be applied since the appropriate intermediate **11**[14] can be obtained via the readily available isoquinoline-*N*-oxide **12**.[15] Rearrangement of **12** on treatment with *p*-toluenesulfonyl chloride gave 4-hydroxyisoquinoline **13**,[16] which upon nitration and subsequent reaction with phosphorus oxychloride gave **11**. Upon treatment of chloro nitro compound **11** with hydrazine in ethanol in the standard manner,[1] cyclization occurred to give **3**.

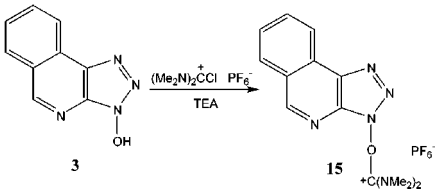


11 **12** **13**

Both **2** and **3** showed properties typical of an HOAt derivative. Both were tested as additives for peptide coupling in the presence of diisopropylcarbodiimide (DIC). Conversion to the corresponding uronium/guanidinium coupling reagents by reaction with tetramethylchloroformamidiunium hexafluorophosphate[1,17] gave, as for HOAt itself, the normal guanidinium species[18] 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] quinolinium hexafluorophosphate 3-oxide [5,6-B(HATU)] **14** in the case of **2**, whereas with **3** the uronium form *N*-[(dimethylamino)(3*H*-1,2,3-triazolo-[4,5-*c*]isoquinolin-3-yloxy)-*N*-methylmethanaminium hexafluorophosphate [4,5-B(HATU)] **15** resulted. Formation of



2 **14**



3 **15**

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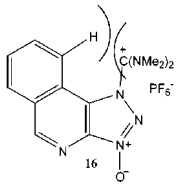
the uronium salt in this case is attributed to interference by the 4,5-benzo substituent with the possible guanidinium isomer **16**. A similar effect had previously been observed for 4-Me-HOAt.[19] Structural assignments were made on the basis of ^{1}H and ^{13}C NMR spectroscopy and comparisons with previously examined model compounds.[18,19]



As expected 4,5-B(HATU) effected conversion of a typical protected amino acid, e.g., *N*-tritylglycine, into the corresponding mixed *O*,*N*-acyl species[20] more readily than did 5,6-B(HATU) (30 vs 180 s, respectively). Similarly, for the more hindered amino acid derivative *Z*-Aib-OH, 4,5-B(HATU) showed a half-life of about 1 min for conversion to the activated species, whereas for HATU, which like 5,6-B(HATU) exists as a guanidinium species,, the half-life is about 6 min (Table 1).

Table 1. Approximate Half-Times for the Disappearance of Z-Aib-OH via Reaction with a Coupling Reagent in DMF in the Presence of TMP[a]

coupling reagent	$t_{1/2}$ (min)
4,5-B(HATU)	1
HATU	6

[a] Using 0.1 mmol each of coupling reagent, *Z*-Aib-OH, and base in 0.5 mL of DMF.

Since shorter activation times might be expected to lead to less loss of configuration during segment coupling, an examination of the assembly of several previously studied[21] segments was undertaken. The results are collected in Table 2 and show that while 4,5-B(HOAt) and 4,5-B(HATU) are more effective than the 5,6-analogues, the differences are rather small. Only in one of the cases examined, that of the nonsegment coupling to give *Z*-Phg-Pro-NH_2, was 4,5-B(HATU) slightly more effective than the parent system HATU. Perhaps because the effect was cumulative over the activation of several amino acids the effect of shorter activation times was greater in the case of peptide synthesis. For example, for assembly of the ACP decapeptide **17**

H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2
17

under so-called forcing conditions (1.5 mol equiv of amino acid and 1.5 min coupling)[22] the crude purity of the decapeptide was 81.8% for 4,5-B(HATU) and 73.6% for HATU (see Supporting Information for comparison HPLC traces).

Table 2. Effect of Coupling Reagent on Loss of Configuration during [2 + 1] Coupling Leading to *Z*-FVP-NH_2 and *Z*-GFP-NH_2, [3 + 3] Coupling to *Z*-GGVAGG-OMe, and [1 + 1] Coupling Leading to *Z*-Phg-Pro-NH_2 in DMF with or without 2 Equiv of TMP as Base[a]

coupling reagent/base	*Z*-FVP-NH_2[b]	*Z*-GFP-NH_2[c]	*Z*-GGVAGG-OMe[c]	*Z*-Phg-P-NH_2[c]
DIC/5,6-B(HOAt)	4.7 (65)			
DIC/4,5-B(HOAt)	3.3 (72)	0.6 (86)		
DIC/HOAt	2.4 (63)	0.2 (44)		
5,6-B(HATU)/TMP	5.0 (75)			
4,5-B(HATU)/TMP	4.0 (49)	0.8 (54)	2.5 (99)	2.4 (77)
HATU/TMP	3.8 (80)	0.5 (56)	1.6 (71)	3.0 (79)

[a] The figure in parentheses refers to the yield of di-, tri-, or hexapeptide obtained. [b] Percent LDL-form [c] Percent DL-form.

In summary, syntheses of the two isomeric benzo derivatives of HOAt are described. Reaction of each isomer with tetramethylchloroformamidinium hexafluorophosphate gave uniquely different results: in one case a guanidinium salt is formed, and in the other a uronium salt results. The structural differences of the salts led to differences that were observed during their use as coupling reagents. Differences in the extent of loss of configuration during segment coupling were modest, but differences in the speed with which the two salts effected the activation of protected amino acids were more pronounced.

Acknowledgment. We thank the National Institutes of Health (GM-09706), the National Science Foundation (NSF CHE-9003192), and the donors of the Petroleum Research Fund, administered by the American Chemical Society (PRF 26070-AC1), for support of this work. The National Science Foundation is also thanked for grants used to purchase the high-field NMR spectrometers used in this research.

Supporting Information Available: IR and ^{1}H NMR data for B(HOAt) and B(HATU) derivatives and comparison HPLC curves for the synthesis of the ACP 10-mer via 4,5-B(HATU) and HATU. This material is available free of charge via the Internet at http://pubs.acs.org.

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