1-Hydroxy-7-azabenzotriazole. An Efficient Peptide Coupling Additive†

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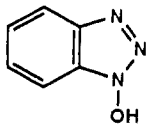
The most common peptide coupling additive is 1-hydroxybenzotriazole (HOBt, **1**),[1] used either in combination with a carbodiimide or another coupling agent or built into a stand-alone reagent such as 1-benzotriazolyloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP)[2] or an analogous uronium salt.[3] Such additives generally inhibit side reactions and reduce racemization. In this communication, 1-hydroxy-



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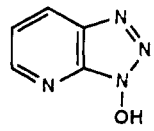

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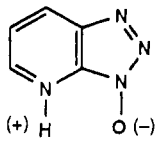
7-azabenzotriazole (HOAt) (**2**) is described as a more efficient additive which speeds up coupling processes, reduces the loss of chiral integrity, and provides a visual indication[4] (yellow-to-colorless) of the reaction endpoint.

While not new, HOAt[6-8] appears never to have been tested in any coupling reaction. Initial examination of **2** was inspired by the consideration that it incorporates within a single molecule both key elements of the 1:1 mixture of HOBt and a tertiary amine which is of greater catalytic effect than HOBt itself in couplings involving active esters.[10] In fact, pronounced reactivity enhancement was observed upon substitution of HOAt for HOBt in a variety of circumstances, although **2**, being insufficiently basic to form stable salts in aqueous media, is not likely to adopt the zwitterionic structure **3**. If analogy to the reactions of HOBt



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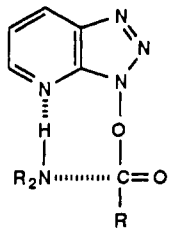

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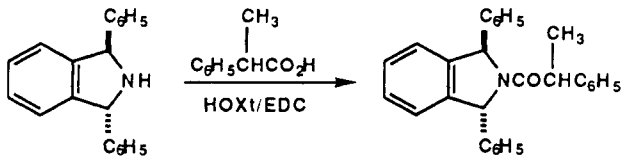
is taken as a guide, increased efficiency of **2** relative to **1** as a coupling additive could be governed by the formation or reactivity of an active ester intermediate. Although precise mechanistic details need yet to be established, reactions of HOAt esters appear to be enhanced relative to those of the HOBt analogs, and it is tempting to postulate the importance of the neighboring group effect depicted in **4** as an important factor.[11,12] As an example, the highly hindered secondary amine **5**[14] undergoes acylation by 2-phenylpropanoic acid within about 22 h in the presence of HOAt under conditions which with HOBt provide only a trace of the mixed diastereomeric amides. With the *cis*-isomer of **5**, acety-



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lation via acetic acid in the presence of HOAt and EDC gives within 30–50 min the corresponding amide, mp 162–164 °C, in 87% yield, whereas the analogous reaction with HOBt is still

† Abbreviations used: PG = protecting group; AA_1, AA_2 = amino acid or dipeptide fragment; PS = Proton Sponge (1,8-bis(*N*,*N*-dimethylamino)-naphthalene); EDC = *N*-ethyl-*N'*-(3-(dimethylamino)propyl)carbodiimide hydrochloride; PCA = *p*-chloroaniline; DIEA = diisopropylethylamine; NMM = *N*-methylmorpholine; DCC = dicyclohexylcarbodiimide; DCM = dichloromethane; DMF = dimethylformamide; Z = (benzyloxy)carbonyl; Bz = benzoyl; BOC = (*tert*-butyloxy)carbonyl; Aib = α-aminoisobutyric acid; HOBt = *N*-hydroxybenzotriazole; HOAt = 7-HOAt = 1-hydroxy-7-azabenzotriazole; 4-HOAt = 1-hydroxy-4-azabenzotriazole; HODhbt = 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine; BOP = 1-benzotriazolyloxytris(dimethylamino)-phosphonium hexafluorophosphate; HBTU = *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; HBPyU = *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate; HAPyU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate.

(1) (a) König, W.; Geiger, R. *Ber. Dtsch. Chem. Ges.* **1970**, *103*, 788. (b) König, W.; Geiger, R. *Ber. Dtsch. Chem. Ges.* **1970**, *103*, 2024, 2034.

(2) Review: Le Nguyen, D.; Castro, B. *Peptide Chemistry 1987*, *Proc. Jpn. Symp. Pept. Chem.* **1988**, 231.

(3) Knorr, R.; Trzeciak, A.; Bannworth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, 1927.

(4) The additive HODhbt also yields a yellow-colored anion.[5] More effective in reducing racemization than HOBt, this additive has had limited use due to the possible occurrence of side reactions involving attack at the carbonyl function. Comparisons with HOAt show that HODhbt is less effective in both reactivity enhancement and inhibition of racemization. According to Azev and co-workers,[6a] the pK_a values of HOBt, 4-HOAt, and 7-HOAt are 4.60, 3.02, and 3.28, respectively. These figures could account for some but not all of the special properties of HOAt and its derivatives.

(5) Atherton, E.; Cameron, L.; Meldal, M.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1986**, 1736.

(6) (a) Azev, Y.; Mokrushina, G. A.; Postovoskii, I. Ya.; Sheinker, Yu. N.; Anisimova, O. S. *Chem. Heterocycl. Compds.* **1976**, 1172. (b) Mokrushina, G. A.; Azev, Y.; Postovoskii, I. Ya. *Chem. Heterocycl. Compds.* **1975**, 880. (c) Azev, Y.; Mokrushina, G. A.; Postovoskii, I, Ya. *Chem. Heterocycl. Compds.* **1974**, 687. (d) Sacher, R. M.; Alt, G. H.; Darlington, W. A. *J. Agric. Food Chem.* **1973**, *21*, 132.

(7) Previously obtained from 3-fluoro-2-nitropyridine by reaction with hydrazine, the synthesis of **2** has been simplified by methylation [Yutilov, Y. M.; Ignatenko, A. G. *Khim. Prom-st., Ser. Reakt. Osobo Chist. Veshchestva* **1981**, 27 (*Chem. Abstr.* **1982**, *96*, 68773s)] of 2-nitro-3-pyridinol followed by treatment of the methyl ether with excess hydrazine according to the method described by Azev et al.[6] for the corresponding fluoride. Both HOAt and HATU are available from Millipore Corporation, Bedford, MA 01730.

(8) Assignment of structure (C/N/O skeleton) to HOAt and its 4-isomer follows from the synthetic methods adopted and analogy to HOBt syntheses. The active ester intermediates generated during acylation reactions involving these additives may participate in the same kinds of equilibria previously observed for the analogous esters derived from HOBt.[9]

(9) Cf.: Barlos, K.; Papaioannou, D.; Voliotis, S.; Prewo, R.; Bieri, J. H. *J. Org. Chem.* **1985**, *50*, 696 and references cited therein.

(10) See: Carpino, L. A.; Chao, H. G.; Beyermann, M.; Bienert, M. *J. Org. Chem.* **1991**, *56*, 2635 and references cited therein.

(11) Similar interactions have been proposed to account for the reactivity of esters of 8-hydroxyquinoline relative to that of the 3- and 6-isomers.[12,13] There is no evidence to date that the isomeric 1-hydroxy-4-azabenzotriazole[6a-c] shares all of the unique properties of the 7-aza derivative **2**. Thus, for the 4-aza compound color monitoring is possible and coupling rates are enhanced although racemization is not substantially reduced relative to the case of HOBt. In the reaction of the highly hindered α,α-disubstituted amino acid Z–Aib–OH with the weakly-basic aromatic amine *p*-chloroaniline (PCA) in DMF-d_7, rough NMR measurements show that about half of the PCA disappears into the corresponding amide within 40–45 min for 7-HOAt and 65–70 min for 4-HOAt, although reaching this stage requires over 24 h in the case of HOBt. About 260 min are required for comparable reactivity in the presence of the well-known additive HODhbt (see also footnote 4).

(12) (a) Jakubke, H.-D.; Voigt, A. *Chem. Ber.* **1966**, *99*, 2419. (b) Jakubke, H.-D.; Voigt, A.; Burkhardt, S. *Chem. Ber.* **1967**, *100*, 2367.

(13) For a deliberate although unsuccessful attempt to design an HOBt derivative for which intramolecular base catalysis by a neighboring sulfonate function might enhance reactivity and suppress racemization during peptide coupling, see: Savrda, J.; Ziane, N.; Guilhem, J.; Wakselman, M. *J. Chem. Res. (S)* **1991**, 36.

(14) Carpino, L. A. *J. Chem. Soc., Chem. Commun.* **1966**, 858.

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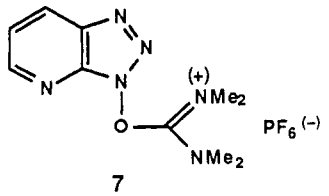
Table I. Loss of Chirality During Coupling of PG–AA_1–OH with H–AA_2–OMe·HCl or H–AA_2–OMe in DMF in the Presence of HOBt and HOAt[a]

run	PG	AA_1	AA_2	conditions	DL-isomer (%)
1	Z	Phg	Val	HOAt, PS (1 equiv), EDC, 9 h[b]	<1–2
2	Z	Phg	Val	HOBt, PS (1 equiv), EDC, 9 h[b]	3.7
3	Z	D-Phg	Val	HOAt, PS (1 equiv), EDC, 6 h	<1–2
4	Z	Phg	Val	HATU, PS (2 equivs), 7 h	<1–2
5	Z	Phg	Val	HBTU, PS (2 equivs), 7 h	4.0
6	Z	Phe-Val	Ala	HOAt, NMM (1 equiv), EDC, 1.25 h	<1–2
7	Z	Phe-Val	Ala	HOBt, NMM (1 equiv), EDC, 2.25 h	4.1
8	Z	Phe-Val	Ala	HATU, DIEA (2 equivs), 3.5 h	<1–2
9	Z	Phe-Val	Ala	HBTU, DIEA (2 equivs), 4 h	4.1
10	Bz	Val	Val	HOAt, NMM (1 equiv), EDC, 20 h	28.1
11	Bz	Val	Val	HOBt, NMM (1 equiv), EDC, 20 h	45.4
12	Bz	Val	Val	DCC, 24 h[c]	61.5[d]
13	Bz	Val	Val	HOAt, DCC, 24 h[c]	14.4
14	Bz	Val	Val	HOBt, DCC, 24 h[c]	41.9
15	Bz	Val	Val	HOAt, DCC, DCM solvent, 24 h[c]	<1–2
16	Bz	Val	Val	HATU, NMM (2 equivs), 3 h	28.3
17	Bz	Val	Val	HBTU, NMM (2 equivs), 3.5 h	46.8

[a] Test couplings were carried out by preparing a solution of 0.37 mmol of a protected amino or dipeptide acid, 0.33 mmol of HOAt or HOBt, and 0.33 mmol of an amino acid ester (or its hydrochloride plus an equivalent amount of a tertiary amine) in 1 mL of DMF. The mixture was cooled in an ice bath and treated with 0.37 mmol of DCC or EDC. For reactions involving uronium salts, 0.74 mmol of a tertiary amine was substituted for the HOAt or HOBt. For HOAt reactions in the absence of excess amino component or tertiary base, disappearance of the yellow color signaled completion of the coupling process, and workup proceeded soon thereafter. All reactions were carried out in DMF, except in the case of run 15 where DCM was used. Generally stirring was continued in the ice bath for 1.5–2 h and then at room temperature for the times indicated. Dilution with 15 mL of CH_2Cl_2 and 200 mL of water was followed by extraction with 4–5 10-mL portions of CH_2Cl_2 and washing in order with 10-mL portions of 10% HCl (twice), H_2O (once), and 0.5 M $NaHCO_3$ (twice). Drying and removal of solvent gave the crude peptide (generally in yields of 70–90%), which was examined by 200- or 300-MHz ^1H NMR for the presence of diastereomeric contamination (OMe and/or MeCH). In a few cases, the ^1H NMR analyses were confirmed by HPLC analysis. Thus for runs 6 and 7 HPLC analysis showed 0.3% and 4.7% of the LDL-isomer, respectively. Conditions for the separation of this set of diastereomers have been described by Miyazawa and co-workers.[19] [b] Under the same conditions, 4-HOAt and HODhbt led to 6.1% and 4.1% of the DL-form, respectively. [c] In these cases (runs 12–15), H–Val–OMe·HCl was converted to the free base by treatment with concentrated K_2CO_3 and extraction into CH_2Cl_2 prior to addition to the reaction mixture. [d] This figure is close to that reported as arising from the racemic oxazolone derived from *N*-benzoylvaline (65% DL), thus demonstrating the importance of asymmetric induction in the case of this particular coupling.[20]

incomplete after 7.5 h. The coupling of adjacent Aib residues is known to be difficult.[15] When BOC–Aib–OH is coupled to H–Aib–OMe in the presence of HOBt, reaction is incomplete after 24 h (ca. 35% of the HOBt ester remains unreacted), whereas with HOAt a theoretical yield of the dipeptide is obtained.

Similar enhanced reactivity is shown by uronium salt **7** (HATU)[16,17] relative to its HOBt analog HBTU.[3] Table I collects



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examples which illustrate the effectiveness of **2** in reducing racemization. One model system which is easy to follow via ^1H NMR techniques involved coupling of a urethane-protected derivative of the sensitive nonproteinogenic amino acid α-phenylglycine.[18] Upon treatment of the (benzyloxy)carbonyl derivative with valine methyl ester hydrochloride in the presence of HOBt and 1 equiv of PS or with HBTU and 2 equivs of PS, 3.7–4.0% of the DL-diastereomer was formed (runs 2 and 5). This was reduced to less than 1–2% by substitution of HOAt for HOBt in these reactions (runs 1 and 4).

A second example, relevant to the technique of segment coupling, involved reaction of *Z*-Phe–Val–OH with alanine methyl ester.[19] With this system, HOBt or HBTU coupling (runs 7 and 9) in the presence of NMM or DIEA gave 4.1% of the LDL-isomer. Again in this case the use of HOAt or HATU lowers the extent of racemization to less than 1–2% (runs 6 and 8). Finally, the highly sensitive coupling of benzoylvaline with valine methyl ester[20] shows that HOAt reduces racemization to about one-third or one-half the level found for comparable HOBt reactions (runs 10–17). For this system, even with HOAt, only in a nonpolar solvent such as dichloromethane was it possible to effect coupling without detectable racemization.

Additional applications of HOAt and reagents derived therefrom to both solution and solid-phase coupling reactions, ester formation, etc., as well as modification of the basic structural elements deemed responsible for the unique nature of these systems (neighboring group effects), are under active scrutiny.

Acknowledgment. We are indebted to the National Institutes of Health (GM-09706) and the National Science Foundation (CHE-9003192) for support of this work, which was carried out during the tenure of a University of Massachusetts Faculty Fellowship. Drs. Chuck Phoebe, Steven A. Kates, Michael Schümann, and Dieter Runald are thanked for some of the NMR and HPLC data.

(15) Coupling reactions involving protected Aib which are carried out in the presence of HOBt often lead to the isolation of stable OBt esters. See, for example: Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259.

(16) In honor of the originators of these reagents, suggested abbreviations for these new modifications will keep to the earlier style in spite of the fact that the word order in the French language reverses that of the English equivalents. Thus, HATU for **7**, HAPyU for its pyrrolidine analog, TATU for the tetrafluoroborate analog of **7**, etc. The ^1H-NMR spectra of these compounds exhibit the highly characteristic ABX/AMX pattern (three double doublets) of a pyridine nucleus disubstituted by electron-withdrawing groups at the 2,3-positions. Thus HOAt ($CDCl_3$–DMSO-d_6): δ 7.35 (dd, 1, β-H), 8.3 (dd, 1, γ-H), 8.66 (dd, 1, α-H); $J_{\alpha,\beta}$ = 4.2 Hz, $J_{\beta,\gamma}$ = 8.2 Hz, $J_{\alpha,\gamma}$ = 1.6 Hz. HATU (DMSO-d_6): δ 3.2 (d, 12, CH_3), 8.0 (dd, 1, β-H), 8.45 (dd, 1, γ-H), 8.9 (dd, 1, α-H), $J_{\alpha,\beta}$ = 4.4 Hz, $J_{\beta,\gamma}$ = 8.4 Hz, $J_{\alpha,\gamma}$ = 1.8 Hz.

(17) Prepared from tetramethylchloroformamidinium hexafluorophosphate by analogy to a published method [Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572].

(18) Carpino, L. A. *J. Org. Chem.* **1988**, *53*, 875.

(19) Yamada, T.; Kurokawa, S.; Dejima, K.; Watanabe, K.; Kotani, M.; Miyazawa, T.; Kuwata, S. *Mem. Konan Univ., Sci. Ser.* **1985**, *32*, 11.

(20) Miyazawa, T.; Otomatsu, T.; Yamada, T.; Kuwata, S. *Chem. Express* **1991**, *6*, 61.