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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All new compounds gave correct C, H, N values (± 0.3%) and appropriate IR and NMR spectral data.

Reactivity of the active esters

(a) Reaction with p-Chloroaniline: An active ester Z-Aib-n-OAt (17.8 mg. 0.05 mmol) was dissolved in 0.5 ml of $CDCl_3$ in an NMR sample tube and the spectrum scanned quickly. p-Chloroaniline (6.4 mg, 0.05 mmol) was added to the sample tube and the solution mixed by hand. The sample tube was quickly placed in the NMR probe with the halftime of the process being measured by the ratio of the peak integrals due to the gem-dimethyl groups of the active ester (60 MHz) at δ 1.84, 1.79, 1.78 and 1.77 in the case of the 7-, 6-, 5- and 4- isomers and those of the resulting amide (δ 1.58). The results are given in Table 1.

(b) Reaction with N-Methylbenzylamine: Z-Aib-n-OAt (25 mg, 0.07 mmol) was dissolved in 0.2 ml of DMSO-d_6 in a small vial. After 25 min (the time required for complete solubility), the solutions of the 7- and 4-ester were almost colorless, the 6-ester was brown and the 5-ester was dark brown. At that point to each solution, 1 eq (0.07 mmol) of N-methylbenzylamine in 0.4 ml of $CDCl_3$ was added from a stock solution prepared by dissolving 0.123 g of N-methylbenzylamine in 10 ml of $CDCl_3$. The halftime was determined from the ratio of peak integrals due to the gem-dimethyl groups of the active ester (60 MHz) at δ 1.84, 179, 1.78 and 1.77 in the case of the 7-, 6-, 5- and 4- isomers) and those of the resulting amide (δ 1.60). The results are given in Table 1.

(c) In the case of coupling to **benzylamine** under the same conditions in $CDCl_3$, all four active esters gave halftimes of less than 1 min.

Treatment of the O-Methyl Ethers of n-Aza-1-hydroxylbenzotriazole with Sodium Thiophenoxide

(a) To a stirred solution of 0.396 g (3.0 mmol) of sodium thiophenoxide in 5 ml of DMF/$CHCl_3$ (1:1) there was added 0.45 g (3.0 mmol, 1 eq) of 7-aza-1-methoxybenzotriazole (Me-7-OAt) at room temperature. A yellow precipitate separated within 5 min and stirring was continued at room temperature overnight, during which time the precipitate became thick and white. To the mixture was added 300 ml of $CHCl_3$ and the white solid filtered. The filtrate was washed with water (2 × 15 ml), dried over $MgSO_4$, and evaporated in vacuo to give a yellow liquid, which according to its ^{1}H-NMR spectrum, contained DMF. Chromatographic purification on silica gel 60 with elution by $CHCl_3$ gave 0.37 g (99.5%) of pure thioanisole, identified by IR and ^{1}H-NMR (60 MHz, $CDCl_3$) analysis [δ 2.46 (s, 3, CH_3), 7.24 (bs, 5, Ar)] and comparison with an authentic sample of thioanisole from Aldrich Chemical Co. The white solid was dissolved in about 5 ml of water and a few drops of conc. HCl were added causing the separation of 0.435 g of crude 7-HOAt as a white solid. Recrystallization from water twice gave 0.24 g (58.8%) of pure 7-HOAt as white needles: m.p. 216-217°C (lit.[13] 215-217°C), identified by comparison of IR and ^{1}H-NMR spectral data with that of an authentic sample.

(b) Determination of the Relative Demethylation Rates for the O-Methyl Ethers Derived from Isomeric Aza-1-hydroxybenzotriazoles by Treatment with Sodium Thiophenoxide. To a solution of 13.2 mg (0.1 mmol) of sodium thiophenoxide in 0.2 ml of DMSO-d_6 in a small vial was added 0.2 ml of $CDCl_3$ followed by 15 mg (0.1 mmol, 1 eq) of n-aza-1-methoxybenzotriazole (Me-n-OAt). The solution was mixed by hand and transferred to an NMR sample tube quickly. The halftime was determined by the ratio of the peak integrals due to the thiomethyl group of thioanisole at δ 2.45 (s, 3, CH_3) and the methoxy group of the four methoxybenzotriazoles (Me-n-OAt) near δ 4.99 (s, 3H, CH_3). Two solvent systems were examined . The results are given in Table 2.

Formation of Z-Gly-Gly-Val-Ala-Gly-Gly-OCH_3 by [3 + 3] coupling in DMF. To a stirred and ice-cooled solution of 0.0475 g (0.135 mmol) of Z-Gly-Gly-Val-OH 0.0317 g (0.125 mmol) of H-Ala-Gly-Gly-OMe•HCl and 0.0184 g (0.135 mmol) of the n-HOAt additive in 1 ml of DMF (dried over molecular sieves and treated with a stream of nitrogen for 2h), was added 34.4 μl (0.26 mmol) of TMP and 0.0259 g (0.135 mmol) of EDC•HCl. The mixture was stirred at 0°C for 1 h and at room temperature overnight. The solvent was evaporated in vacuo to give the crude hexapeptide methyl ester from which non-

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§Molecular structures for all 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.

peptide materials were removed by chromatography on silica gel 60 with elution by $CHCl_3$/MeOH/AcOH (7:3:0.1). Both LL- and DL-diastereomeric hexapeptides came off the column in the same fraction. The material from the column was directly analyzed for loss of configuration by HPLC analysis using a Waters C_{18} column (isocratic, 20% CH_3CN/80% H_2O/0.1% TFA). The results are given in Table 3.

Formation of Z-Phe-Val-Pro-NH_2 by [2 + 1] Coupling in DMF. To a stirred and ice-cooled solution prepared from 0.05 g (0.125 mmol) of Z-Phe-Val-OH, 0.0143 g (0.125 mmol) of H-Pro-NH_2 and the appropriate n-HOAt additive (0.017 g, 0.125 mmol) in 1 ml of DMF (dried over molecular sieves and treated with a stream of N_2 for 2 h) was added 16.5 µl (0.125 mmol) of TMP and 0.024 g (0.125 mmol) of EDC•HCl. The mixture was stirred at 0°C for 1 h and at room temperature overnight. The mixture was diluted with 25 ml of EtOAc and washed with 1 N HCl (2 × 5 ml), 1 N $NaHCO_3$ (2 × 5 ml) and saturated NaCl (2 × 5 ml) and finally dried over $MgSO_4$. The solvent was removed in vacuo to give a white residue, which was dissolved in CH_2Cl_2. Addition of hexane gave a white precipitate, which was filtered and directly analyzed for loss of configuration by HPLC analysis. For HPLC separation, a 4 µm C_{18} Waters Nova Pak column (3.9 × 150 mm, 60 Å) was used with a linear gradient system 25 to 50% CH_3CN in H_2O/0.1% TFA in 20 minutes. The results are given in Table 3.

Table 5. Methylation of *n*-HOAt.

HOXt	method[a]	yield (%)	mp ° C	^{1}H-NMR of methyl group, • (ppm)
7-HOAt	A	100	93-4 (lit.[13] 92-3)	4.49
7-HOAt	B	83	93-4	4.49
6-HOAt	A	67	112-114	4.49
6-HOAt	C	67	108-110	4.49
5-HOAt	C	67	113-115	4.43
4-HOAt	A	67	142-44	4.44
4-HOAt	C	66	141-43	4.44
4-HOAt	E	33	140-41	4.44
HOBt	F	14	87-89 (lit.[12b] 89)	4.39
		22	142-144.5 (lit.[12b] 145)	4.11

[a] A. Me_2SO_4/K_2CO_3 in Me_2CO[12a]; B. Me_2SO_4/DIEA in Me_2CO/MeOH (9:1); C. Me_3SiCHN_2/DIEA in CH_3CN/MeOH (9:1)[12c,d]; D. Me_2SO_4/DIEA in DCM; E. MeI/MeONa in MeOH[12b]; F. Me_2SO_4 in aq. NaOH.[12b]