Tetrahedron Letters No. 22, pp 1897 - 1900, 1977. Pergamon Press. Printed in Great Britain.

BEHAVIOR OF ACYLATED 1-HYDROXYBENZOTRIAZOLE[1]

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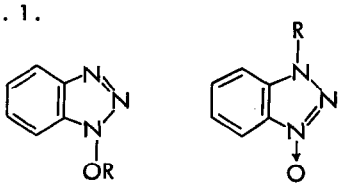
(Received in Japan 24 March 1977; received in UK for publication 19 April 1977)

König and Geiger[2] established that 1-hydroxybenzotriazole (HOBt) is an efficient catalyst of peptide bond formation with either dicyclohexylcarbodiimide or the active ester method. They also observed that some active HOBt esters of N-protected amino acids, except that of threonine, exist in the N-acyl form (2c) in the crystalline state in contrast to the O-acyl form (1c) in dioxane.

We successfully isolated both isomeric acetates (1a and 2a)[3] and phenyl acetates (1b and 2b) as crystals. As a preliminary experiment on the kinetics of the hydrolysis reaction, each isomer (1a and 2a) was allowed to stand in aqueous acetone with various amounts of water at room temperature to obtain an equilibrium mixture accompanying the simultaneous hydrolysis shown in Table 1.

Fig. 1.



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a: R = CH_3CO-
b: R = ΦCH_2CO-
c: R = N-protected amino acid
d: R = N-protected dipeptide

Table 1. Equilibrium using each acetate (1a and 2a) in aqueous acetone

Acetone, % (v/v)[a]	Ratio (1a/2a)[b] Using 1a (min.)[c]	Using 2a (min.)[c]
20	0.41 (10)	0.39 (15)
50	1.17 (40)	1.09 (60)
80	2.37 (210)	2.40 (180)

[a] To the sample in a 25-ml volumetric flask, an appropriate volume of acetone was added and the flask was filled to the mark with water. [b] Determined by integration of each methyl signal in the nmr in $CDCl_3$.[4] [c] The values in parentheses are the approximate time needed for equilibration.

Note that the isomeric ratio and equilibrium rates are influenced by the nature of solvent polarity, which causes the polar N-acetyl derivative (2a) to increase as it increases (Table 1). This behavior is similar to that found by Boyle and Jones[5] who investigated the solvent effects on the tautomerism of HOBt itself in aqueous and organic solutions. Although it is not known whether this acetyl migration takes place via an intramolecular or intermolecular pathway, the former seems likely based on the results of the alternative synthesis of the acylated HOBt derivative reported by Huisgen and Weberndörfer.[6] The dependence of equilibrium rates on solvent polarity for the acyl migration suggests an ionic process.

The structural differences of HOBt esters of N-protected amino acids in either solid or solution state prompted us to prepare several other esters in addition to those reported by König and Geiger, as shown in Table 2; the structures were readily confirmed by ir spectra.

Table 2. Preparation of HOBt esters

Substituent, R (structure)[a]	Mp, °C	Yield, %	IR, cm^{-1}
CH_3CO (1a)	104-106[c]		1825[b]
CH_3CO (2a)	104-105		1736[b]
ϕCH_2CO (1b)	101-101.5		1840, 1820[b]
ϕCH_2CO (2b)	124.5-127		1735[b]
Z-Gly (2c)	134.5-135.5[d]	94.5	1727[b]
^{t}Boc-Gly (2c)	126-128	69.7	1750, 1720[b]
Z-Phe (2c)	120-122[e]	66.4	1736
Z-Thr (1c)	136-138[f]	65.8-72	1825
Z-D.L.-Abu (2c)	116.5-119.5	85	1735
Z-β-Ala (2c)	113-115	98.5	1732
^{t}Boc-Thr(BZL) (1c)	119.5-120	70.3	1818
Z-Gly-Gly (1d)	162-163	89.6	1814
Z-β-Ala-Gly (2d)	149-151	75.5	1750

[a] All compounds were prepared by the procedure of König and Geiger.[2] Satisfactory elemental analyses were obtained. Abbreviations used: Z for Benzyloxycarbonyl, ^{t}Boc for ^{t}Butoxycarbonyl, Gly for glycine, Phe for phenylalanine, Thr for threonine, β-Ala for β-alanine and Abu for α-aminobutyric acid.

[b] Ir spectra were recorded in chloroform and others as Nujol mull.

[c] Lit.[3] mp 104-105°C. [d] Lit.[2] mp 147°C. [e] Lit.[2] mp 120-122°C. [f] Lit.[2] mp 150-153°C.

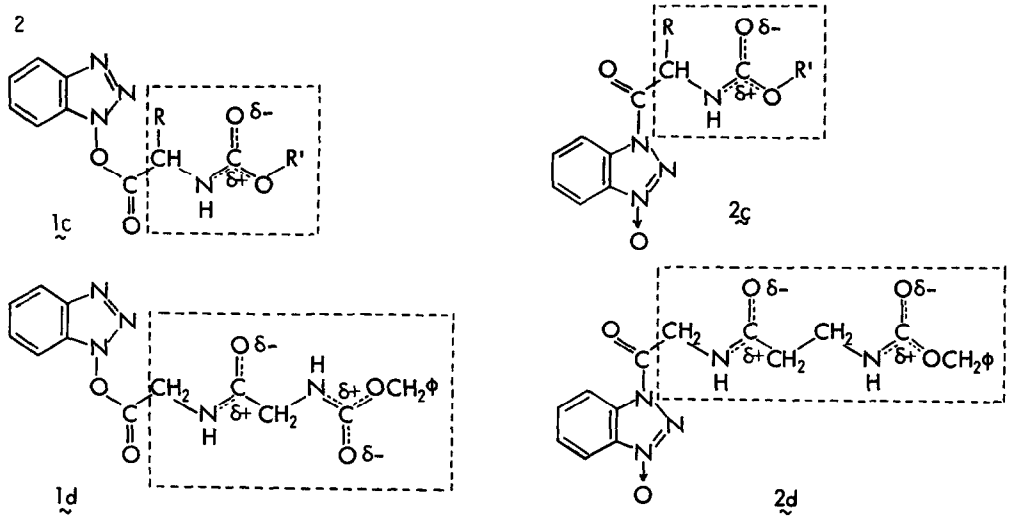
The nmr of Z-Gly-HOBt ester in dioxane-d_8 indicated that it exists in an equilibrium mixture of almost equal amounts of both isomers (1c and 2c) when the solution was heated on a water bath for a short period.[7] The nmr in chloroform showed that it exists as 2c in solid state. This observation is in contrast to that of König and Geiger[2] who reported that the ester exists in the O-acyl form (1c) in dioxane.

Preparation of acetyl HOBt (DCC with acetic acid or acetic anhydride) gave a mixture of both isomers (1a and 2a), which indicates that the acetyl carbonyl group has no effect on the distribution of products. On the other hand, the fact that one of the isomers was exclusively obtained in the case of HOBt esters of N-protected amino acids suggests that the protective group in the side chain might largely affect the structure in a crystalline state.

In order to interpret the cause of the structural differences among HOBt esters of N-protected amino acids in the solid state, the following generally accepted assumptions[8] were made: the most favorable conformation of the N-protected (Z- and ^{t}Boc-) amino acid chain is a β-sheet form (zig-zag conformation), and the urethane (Z- and ^{t}Boc-) protective group of the amino acid and the amide linkage are both polarized as shown in Fig. 2.

Therefore, we could focus our attention on the parts within the dotted lines which

Fig. 2



correspond to the alkoxycarbonylaminomethyl moiety because the other carbonyl parts outside the circle are the same for both the acetates and HOBt esters of N-protected amino acid.

Since we observed that the equilibrium ratio of O- and N-acetates in aqueous acetone depends on the medium polarity (the N-acetyl isomer increases with increasing medium polarity), the structure of HOBt esters of N-protected amino acids in the solid state may be strongly affected by the polarized protecting group, assuming that this group plays a role similar to that of a polar solvent. HOBt esters of Z- or ^{t}Boc-protected amino acids would therefore take the polar N-acyl form (2c) due to the polarization effects of the protecting group (dipole-induced dipole), except when R in Fig. 2 contains a polar substituent, such as a hydroxyl or benzyloxy group in threonine. The effects of the protecting group may occur either intramolecularly or intermolecularly in the crystal lattice. The exception could be considered to occur when a polar hydroxyl group involved in the substituent R (as in the case of Z-Thr-HOBt ester) takes a conformation to compensate for the dipole of the polarized urethane group. To extend these considerations, we synthesized a dipeptide ester (Z-Gly-Gly-HOBt ester) in which the two dipoles in the protecting group and the amide bond face opposite directions, compensating each other, to give the nonpolar O-acyl form (1d) in Fig. 2. We also synthesized Z-β-Ala-Gly-HOBt ester which was expected to have both dipoles in the same direction and thus the N-acyl structure (2d). The results agreed with our hypothesis shown in Fig. 2 and Table 2. As already mentioned, Z-Gly-HOBt ester in dioxane, which is in an equilibrium mixture, is interpreted as being a result of a perturbation of the dipole in the protecting group due to solvation which may partially neutralize the polarized group. Even after Z-Gly-HOBt ester was dissolved in THF for equilibration,[9] evaporation of the solvent without heating gave only the original N-acyl isomer, indicating that acyl migration is quite rapid on crystallization, probably due to the polar Z group. On the other hand, the successful isolation of both isomeric acetates can be best explained by a slow isomerization due to lack of a polar group in the side chain.

In conclusion, the most important factor which governs acylated HOBt structure is whether an apparent dipole exists in the protected amino acids and dipeptides as discussed above.

Situations are complicated for 5- or 6-chloro-HOBt esters of Z-amino acids which involve another dipole of a chlorine atom on the benzene nucleus, in which the above hypothesis can not be simply applied.

Acknowledgment: The author expresses his gratitude to Professor M. Bodanszky for sending reprints concerning the conformational studies and to Professor G. A. Berchtold for helpful discussions.

REFERENCES AND NOTES

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3. Compound (1a) was already synthesized by N. J. Leonard and K. Golankiewicz, J. Org. Chem. 34, 359 (1969). The isolation of 2a was carried out by the following procedure: the crude acetylated HOBt was treated with aqueous acetone for 10 min at room temperature followed by extraction with benzene. The benzene extract was lyophilized to give a crystal, which was recrystallized from n-hexane-acetone mixture to give pure 2a.
4. After treatment of each pure 1a and 2a with appropriate aqueous acetone for appropriate periods as shown in Table 1 followed by extraction with benzene, the benzene extract was lyophilized to afford a crystalline residue. The nmr spectra were recorded on a Varian A-60 instrument using TMS as an internal standard. Acetyl methyl protons of 1a appeared at δ 2.48 and that of 2a at δ 2.78, respectively.
5. F. T. Boyle and R. A. Y. Jones, J. Chem. Soc. Perkin Trans. II 160 (1973). For the dipole directions of heterocyclic compounds, see Z. Simon, "Quantum Biochemistry and Specific Interactions," English Ed., Abacus Press, Kent (1976), p. 89.
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7. Two doublets of α-methylene protons of Z-Gly-HOBt ester (1c and 2c) appeared at δ 4.45 (J = 6 Hz) and 4.68 (J = 6 Hz), respectively.
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9. The equilibrium in THF solution was observed by ir spectrum.