NEIGHBORING GROUP PARTICIPATION IN PEPTIDE SYNTHESIS BY THE USE OF ARYLSULFONATES OF N-HYDROXYAZOLES AND RELATED COMPOUNDS —TAUTOMERIC EFFECT[1]

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The present paper deals with some attempt of the mechanistic elucidation on peptide bond formation using several arylsulfonates of N-hydroxy compounds and describes the important role of neighboring group involved in the N-hydroxy compounds in peptide synthesis.

Although much attention has recently been paid to the usefulness of arylsulfonate,[2] the phosphonium salt[3] and N,N-tetramethyluronium salt[4] of N-hydroxybenzotriazole (HOBt) as peptide coupling reagents, the mechanism of the peptide formation remains obscure.

The present paper describes the reaction mechanism in the following peptide formation reaction using p-chlorobenzenesulfonate of HOBt (Chart 1).

Dedicated to Prof. Dr. Adolf Butenandt on the occasion of his 75th birthday.

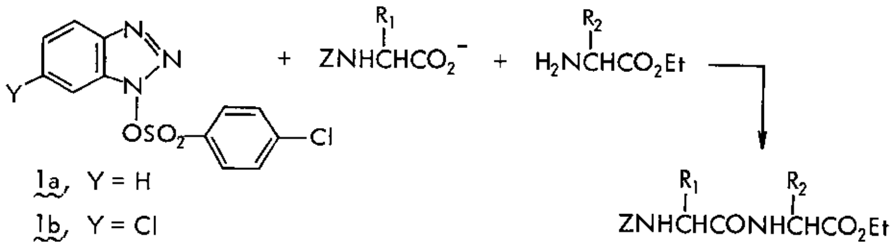
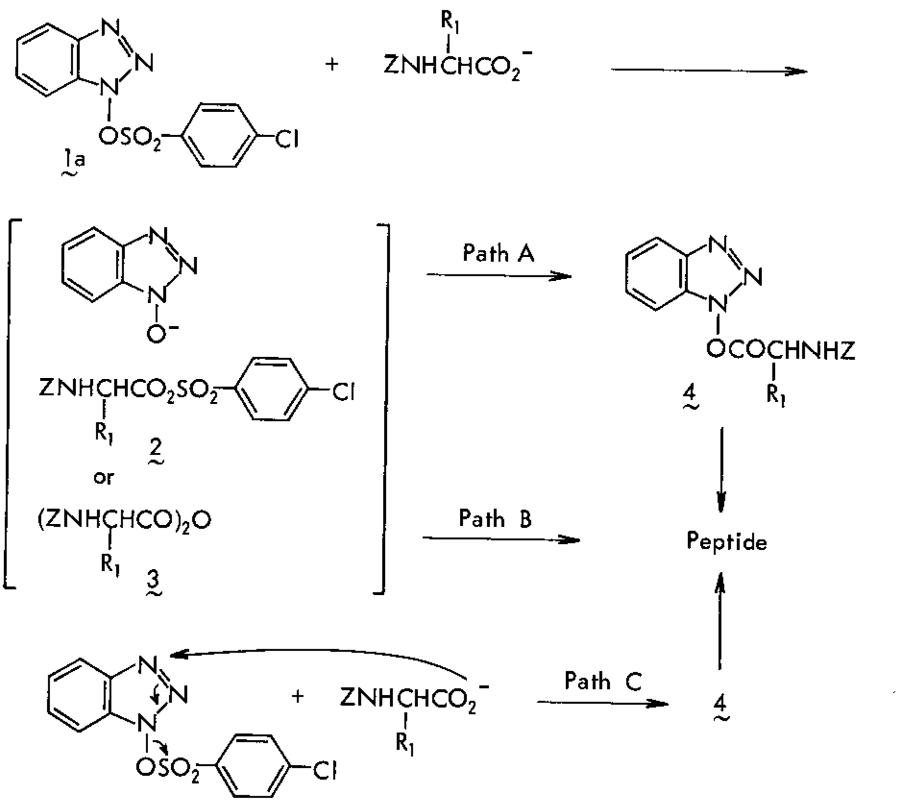


Chart 1



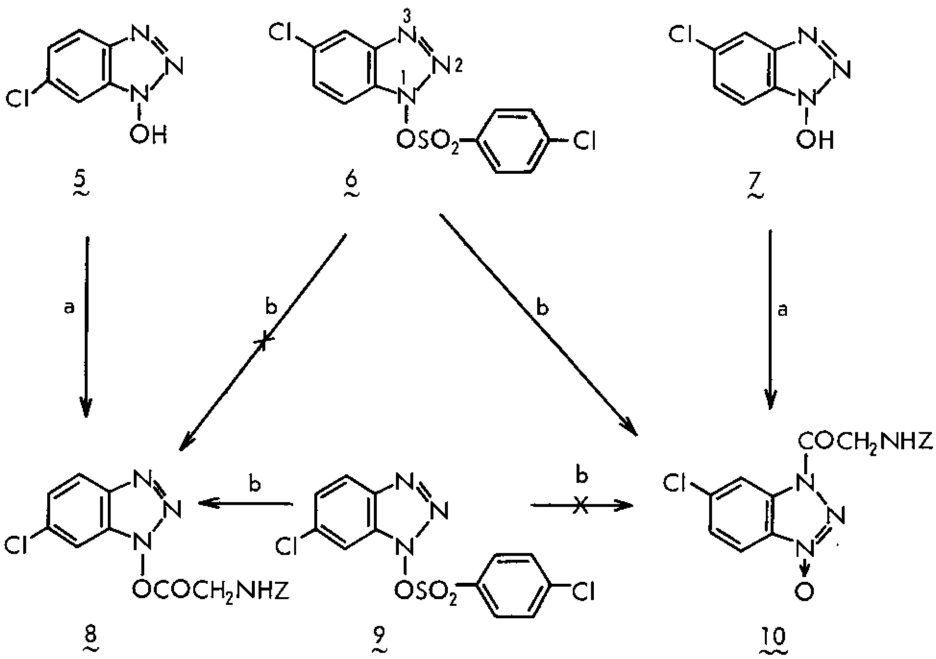
Abbreviation used:

Z = Carbobenzyloxy

Chart 2

The mechanism proposed by Galpin et al.[5] is that nucleophilic attack of the carboxylate anion on tetra-coordinated sulfur takes place to form mixed anhydride (2), which is subsequently attacked by oxy anion of HOBt to form the active ester (4) followed by aminolysis to afford peptides (Path A in Chart 2). Although another possibility existed of direct aminolysis of the mixed anhydride (2) or symmetrical anhydride (3) (Path B) where complete racemization occurred,[6] Anderson's test[7] for racemization detection showed partial racemization took place in the present synthesis.[8] This suggests Path B might be excluded.

In addition, Path C could be also readily ruled out by the following experiment (Chart 3). Treatment of 6-chloro-HOBt (5) and Z-glycine with DCC afforded the corresponding acyl derivative (8), which was also obtained by the reaction of p-chlorobenzenesulfonate of 6-chloro-HOBt (9) with Z-glycine in the presence of an equivalent triethylamine. The reaction of p-chlorobenzenesulfonate of 5-chloro-HOBt (6) with Z-glycine in the presence of triethylamine afforded the compound (10), which was obtained by an independent synthesis. These results suggest that the nucleophilic carboxylate ion did not attack the nitrogen at position 3 indicating that N-O bond fission did not take place. This could be reasonably understood by the following consideration. In the well-known S_N2' reaction, leaving group and entering group should be cis to each other as shown in Fig. 1A.[9] Therefore, if the carboxylate attacks the nitrogen at 3 perpendicularly to the plane of HOBt, which is energetically favorable due to the maximum overlap between π-orbital and the nucleophile, the leaving group (X) can not be expelled on the basis of stereoelectronic view-point (see Fig. 1B).



Reactions:

a: Reaction with Z-glycine with dicyclohexylcarbodiimide (DCC).

b: Reaction with Z-glycine in the presence of an equivalent triethylamine.

Chart 3

The pKa of HOBt is reported to be 7.88,[10] which is very close to that of p-nitrophenol (7.2), but the arylsulfonate of the latter did not afford peptides. Thus, our interest was focused on the reaction mechanism due to the ready reaction of HOBt sulfonate (1) and we synthesized several other arylsulfonates of N-hydroxy compounds to elucidate

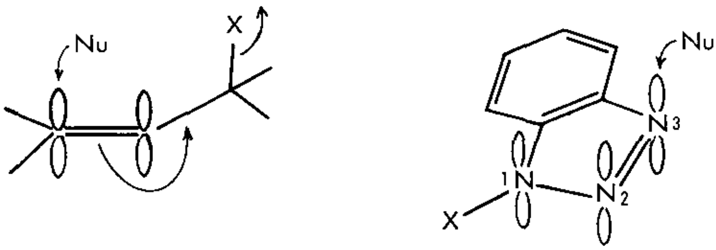
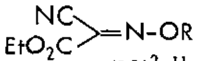
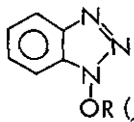
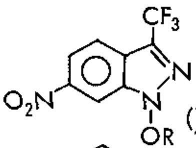
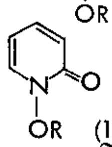
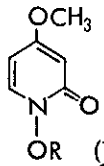
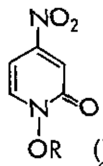
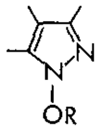
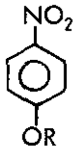
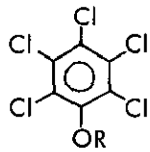
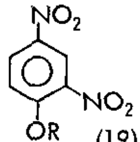
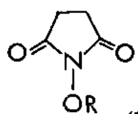


Fig. 1. Favorable S_N2' reaction in allylic system and difficult situation for Path C.

what plays major roles in the synthesis. p-Chlorobenzenesulfonates of N-hydroxybenzotriazole, substituted 1-hydroxy-2-pyridone, N-hydroxypyrazole, and the N-hydroxyindazole derivative including those of substituted phenols were synthesized and their melting points and pKa values are listed in Table 1.

A coupling reaction of Z-valine and ethyl glycinate using these arylsufonates was chosen as a model experiment and the yields of Z-valyl-glycyl ethyl ester are also included in Table 1. These results showed that substituted phenyl arylsulfonates which have pKa values for the leaving group similar to those of 1-hydroxy-2-pyridones did not afford the dipeptide. For example, arylsulfonates of pentachlorophenol (18) and p-nitrophenol (17) which had a pKa value for the leaving group comparable to that of 1-hydroxy-2-pyridone (13) and 4-methoxy-1-hydroxy-2-pyridone (14), respectively, did not afford the dipeptide while

Table 1. Reaction of Z-valine and ethyl glycinate with p-chlorobenzenesulfonate[a]

Compound	mp[b] (°C)	Yield, % (pKa)
 (11)[2,11]	103-104.5	65[c] (4.6)
 (1a)	94-95	66[c] (7.88)[e]
 (12)[12]	164-165	73-81[c] (4.57)
 (13)	111-112	55[d] (5.9)[f]
 (14)	152-154	24[d] (6.59)[f]
 (15)	171-173	62[d] (4.16)[f]
 (16)[13]	69-70	---[d] (6.11)
 (17)	109-112	---[d] (7.2)
 (18)	142-143.5	---[d] (5.3)
 (19)	116-117	40[g] (4.1)
 (20)	166-167.5	---[d] (6.1)

a) All reactions were carried out at room temperature by the reported procedure.[2] b) R = Cl-⟨O⟩-SO_2- ; Satisfactory elemental analyses were obtained for all compounds. c) Run overnight. d) Allowed to react for 6-10 days. e) After completion of this work, pKa of HOBt (in 10% EtOH) was found to be 4.2. f) Dr. E. Hirai of this laboratory kindly informed us of pKa values (in H_2O) of 1-hydroxy-2-pyridone derivatives. g) See text.

arylsulfonates of the latter did. This also shows that Path A seems unlikely to occur.

Although large rate differences were observed, most of the compounds which possess a nitrogen or oxygen near the reacting position did afford the dipeptide, except the sulfonates of pyrazole derivative (16) and N-hydroxysuccinimide (HOSu) (20), which suggests the neighboring group might play an important role in the transition state. Although there are a number of arguments[14] concerning the formation of penta-coordinated sulfur, we would like to propose the following neighboring-group participation mechanism involving penta-coordinated sulfur, taking the pKa values of leaving groups into consideration (Chart 4).

In the three cases shown in Chart 4, the neighboring-group participation enhances the rate by facilitating the S-O bond fission caused by a sudden increase of the leaving ability which then results in the formation of polarized but neutral acyl derivatives such as 21, 22, and 24, thus lowering the activation energy in step 2 as depicted in the Fig. 2B. The rate differences observed by using arylsulfonates of N-hydroxyazoles and 1-hydroxy-2-pyridones would be attributed to the different nucleophilic characters of nitrogen and oxygen.

Some evidence was found for the existence of 21 and 22 as transient intermediates by alternative syntheses of 4 by Huisgen[15] and 23 by Sarantakis et al.[16] In the case of oxime (11), the nitrone derivative (24) might be formed as suggested by Smith.[17] We found that the reaction of Z-valine and ethyl glycinate with the sulfonate (19) afforded ethyl ester of N-(2,4-dinitrophenyl)glycine as a major product (36%) together with a small amount of Z-valyl-glycyl ethyl ester (0.5%). This result indicates that amine nitrogen is softer than carboxylate anion from the

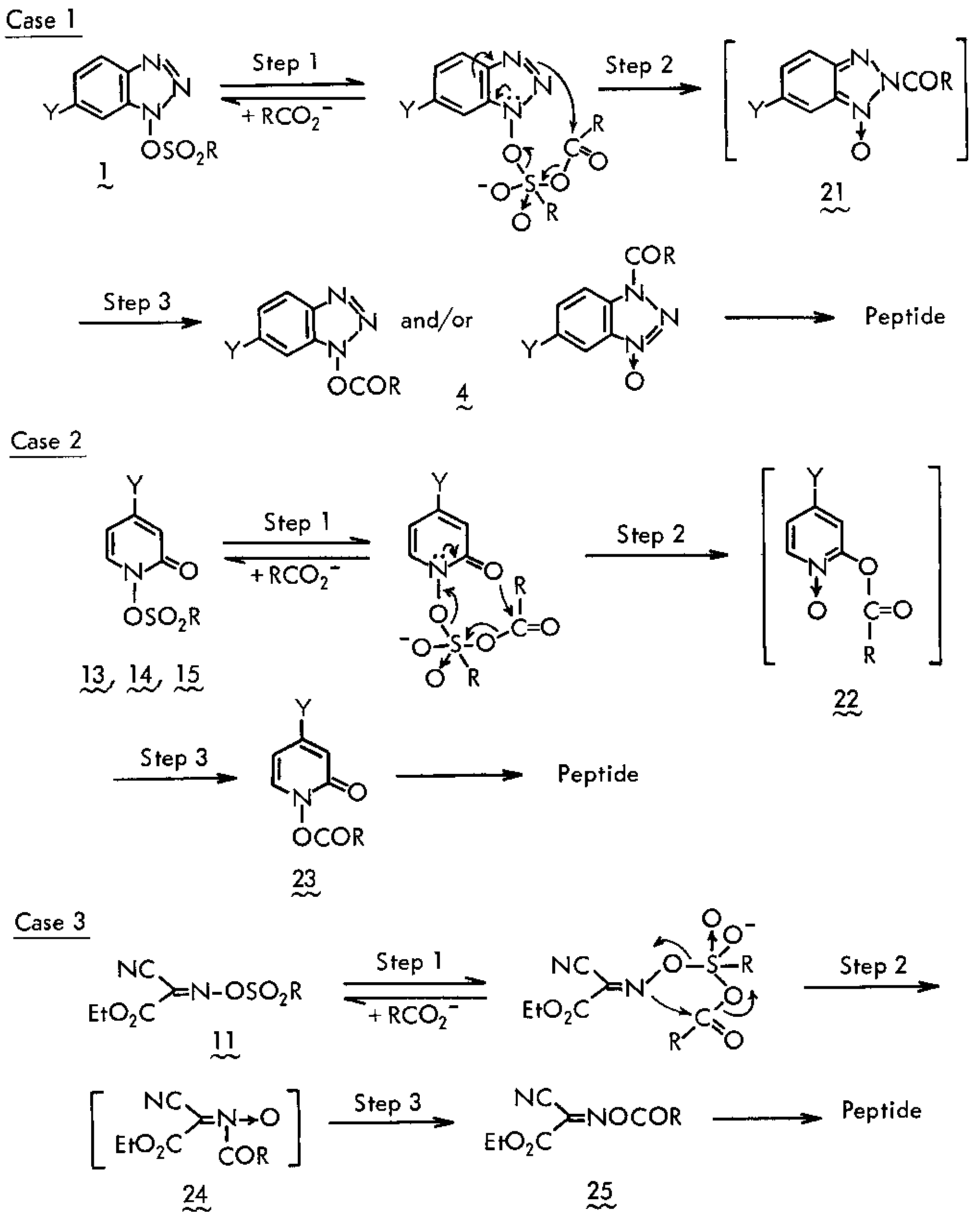

Case 1
Step 1
+ RCO2⁻
Step 2
21
Step 3
and/or
4
Peptide
Case 2
13, 14, 15
22
23
Case 3
11
24
25

Chart 4

view-point of HSAB principle.[18] Since sulfonyl sulfur which is in a very high oxidation state is assumed very hard, attack of hard carboxylate to the sulfur might be preferable to that of amine. Consequently, it is reasonably understood why no sulfonamide was detected in the reaction with p-chlorobenzenesulfonates of N-hydroxy compounds. Although the reaction was very slow, the reaction of Z-valine with 19 in the absence of amine component for 11 days gave 2,4-dinitrophenyl ester of Z-valine which was detected by tlc and subsequent treatment of the ester with ethyl glycinate afforded the dipeptide as shown in Table 1. Despite their having no neighboring group, compounds possessing leaving groups of pKa of ca. 4 or smaller than 4, such as in 2,4-dinitrophenylsulfonate as seen above and tosyl chloride,[6] afford dipeptides, because the leaving ability of the 2,4-dinitrophenoxide and chloride is similar to or better than that of the carboxylate[19] in the addition intermediate. These results and those involving neighboring-group participation are summarized in Table 2.

The question arises of why pyrazole and HOSu derivatives (16 and 20) did not yield the peptide. The reason is not clear at present but, in the case of the pyrazole derivative (16), this may be due to it having no prototropic (tautomeric) tendency.[20] In the case of HOSu derivative (20), generation of positive charge next to the carbonyl group requires large activation energy in Step 2 as shown in Chart 5.

However, Chapman and Freedman[21] recently carried out the reaction of trifluoromethanesulfonate (triflate) of HOSu with thallous acetate in DMF to obtain the corresponding N-hydroxysuccinimide-O-acetate, which is in sharp contrast with our result. They considered the double displacement mechanism similar to that of Path A. The large difference in the reactivity between these two sulfonates might be attributed to the degree

Table 2. Relationships between leaving group pKa and neighboring-group participation in peptide-bond formation

Leaving group pKa ≤ 4.1	? > Leaving group pKa > ca. 4.2 NGP present	NGP absent
Yes	Yes	No

NGP: Neighboring group participation.

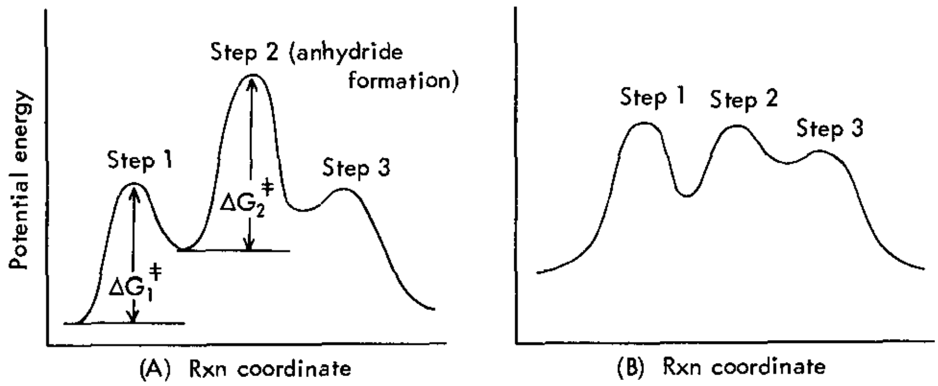
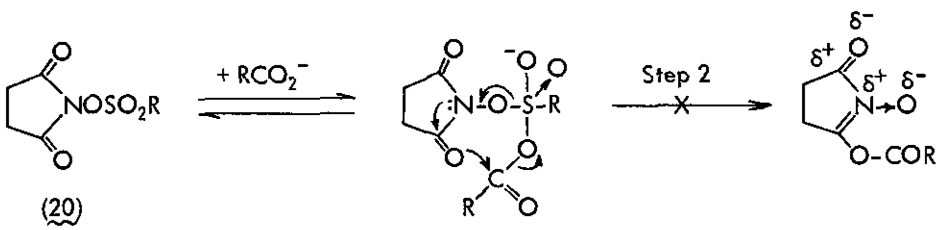


Fig. 2. Schematic energy profile.
A: NGP absent
B: NGP present



(20)

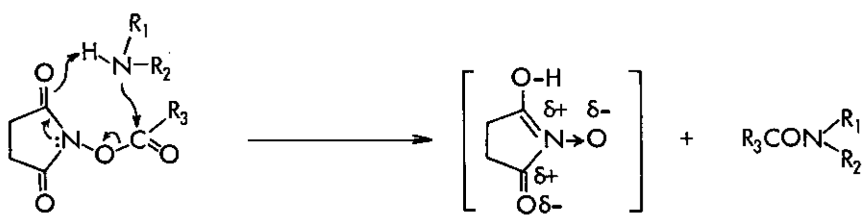
Chart 5

of polarization on the sulfonyl sulfur between the p-chlorobenzenesulfonate and the triflate of HOSu.

In this case, no nucleophilic carbonyl group participation could be anticipated by the above-mentioned reason. However, the triflate sulfur would be much positively polarized, which might bring about lowering the activation energy of step 1 ($\Delta G_1^{\ddagger}$) in Figure 2A, thus leading to decrease in the total activation energy ($\Delta G_1^{\ddagger} + \Delta G_2^{\ddagger}$). This might facilitate the formation of acetylated HOSu via Path A. A similar result was reported by Ciuffarin and Senatore[22] in the alkaline hydrolysis of benzenesulfonyl fluoride, where a very large rho value was obtained by the Hammett treatment indicating a large electronic influence on the reactivity.

In relation to this, we would like to discuss briefly on the mechanism of peptide bond formation by using O-acyl HOSu (26) which is widely used in peptide syntheses.

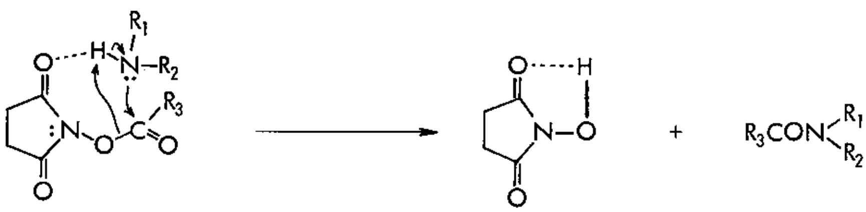
Goodman and Glaser[23] have proposed that the neighboring carbonyl group participation accompanied by the shift of the lone electron pair of the nitrogen in O-acyl HOSu (26) might be responsible for the efficient aminolysis as shown in Chart 6. However, this would lead to an energetically unfavorable N-oxide (27). Therefore, since such a carbonyl group participation is also unlikely in this case, we propose that the character of chelate formation[24] inherent in hydroxamic acid plays an important role for the efficient aminolysis. In this case the carbonyl group will merely act as a hydrogen acceptor to afford the stable



(26) (27)

Chart 6

and neutral HOSu, (28). Consequently the lone electron pair of the nitrogen is no longer needed to shift to the carbonyl group in the transition state (Chart 7).



(26) (28)

Chart 7

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References and Notes

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