

## INVESTIGATIONS ON THE EFFECT OF BASE AND SOLVENTS ON THE ELIMINATION OF p-TOLUENE SULPHONYL GROUP IN THE SYNTHESIS OF DEHYDRO ALANINE

p-TOLUEN SÜLFONİL GRUBU ELİMİNASYONU İLE DEHİDROALANİN  
SENTEZİNDE BAZ VE SOLVANLARIN ETKİLERİNİN ARAŞTIRILMASI

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### ABSTRACT

*Dehydroamino acids are constituents of certain peptide antibiotics and represent an important class of compound. Considerable attention has been given recently to the preparation of dehydroaminoacids, particularly of the dehydroalanine unit, which is generally derived from serine derivatives. In this study the conversion of a serine to a dehydroalanine unit using different base and solvents was investigated using N-benzyloxycarbonyl-O-tosyl derivative of serine.*

**Key words:** dehydroamino acid, beta-elimination, serine, p-toluenesulfonyl chloride

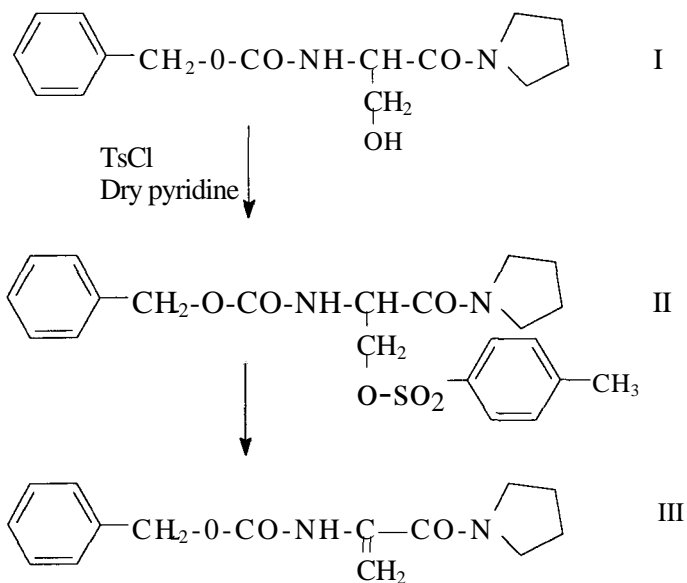
### ÖZET

*Dehidroamino asitler belli peptid antibiyotiklerinin yapısını oluşturur ve önemli bir sınıf maddeyi temsil ederler. Yakın zamanda özellikle serin amino asitinden türeyen dehidroalanin olmak üzere, dehidroamino asitlerin hazırlanışlarına dikkate değer bir ilgi vardır. Bu çalışmada serin türevi bir bileşiğin dehidroalanine dönüşümünde farklı çözücü ve bazların etkileri N-benziloksikarbonil-O-tosilserin kullanılarak araştırılmıştır.*

**Anahtar kelimeler:** dehidroaminoasit, beta eliminasyon, serin, p-toluensülfonil klorür

## INTRODUCTION

Dehydroalanine is in a variety of peptide antibiotics of bacterial origin, including the "lantibiotics" (1,2) and more highly modified peptides. Novel active antibiotics (3) have been synthesised by substituting dehydroalanine for other residues. It has been postulated that the dehydroamino acid plays an important role in giving the definite peptide conformation that is required for exhibition of biological activities (4). Recently structural and conformational properties of dehydroalanines take attention due to its importance in the peptide synthesis (5) and the role of N-methydehydroalanine to develop antibodies against toxic microcystins (6). In order to evaluate the synthesis of dehydroamino acids, series of experiments have been performed by researchers (7).



**Figure 1.** Synthesis of N-benzyloxycarbonyldehydroalanine pyrrolidine amid

Several approaches to the synthesis of  $\alpha,\beta$ -dehydro unit have been developed (8,9), but the most widely used method is based on  $\beta$ -elimination of O-tosylserine (or other  $\beta$ -hydroxyamino acids residues) using alkali or amines (4).

In previously published papers (10,11) problems encountered during the synthesis of dehydroalanine derivatives were investigated and the most reasonable method that was the elimination of tosyl group from hydroxy amino acid derivatives was pointed. In this study

elimination of this bulky group from a serin derivative investigated and the effects of base and solvents on the reaction is given. The synthesis of N-benzyloxycarbonyldehydroalanine pyrrolidine amid from O-tosylated serine derivative is given in figure 1.

## MATERIALS AND METHODS

### Experimental

All chemicals were purchased from Aldrich Chemical Co.  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (62.9 MHz) NMR spectra were recorded on a Bruker AC 400 spectrometer. Tetramethylsilane was used as a reference. The deuteriated solvent was  $\text{CDCl}_3$  and all the chemical shifts were measured from TMS. Column chromatography was carried out with silica gel 60 (230-400 Mesh). All evaporations were made under reduced pressure. The abbreviations used are as follows: DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), Tosyl chloride ( $\text{TsCl}$ , p-toluenesulfonyl chloride), DCC (1,3-dicyclohexylcarbodiimide), DiCD (1,3-diisopropylcarbodiimide), DEAD (diethyl azodicarboxylate), WSC (water soluble carbodiimid),  $\text{Ph}_3\text{P}$  (triphenylphosphine), Z (benzyloxycarbonyl). All experiments were carried out under anhydrous conditions.

Synthesis of N-benzyloxycarbonyl-DL-serine pyrrolidine amide (I) (yield 59%, white crystals, m.p. 117-120 °C) and N-benzyloxycarbonyl-O-tosyl-DL-serine pyrrolidine amide (II) (yield 59 %, pale yellow solid, m.p. 89-91<sup>0</sup>C) has been stated in (11) in the previous paper. Dehydration attempts of the N-Z-DL-serine pyrrolidine amide (I) was unsuccessful using  $\text{Ph}_3\text{P}$ -DEAD (9), DCC, DiCD (12) or WSC. Then synthesis of N-benzyloxycarbonyldehydroalanine pyrrolidine amide (III) via tosyl elimination was planned and investigated.

Three different bases,  $\text{CH}_3\text{ONa}$ ,  $\text{Et}_3\text{N}$  (13), and DBU (14) were used to promote elimination of p-toluene sulphonic acid from compound (II) to give the dehydro derivative (III).

### Elimination of tosyl chloride using $\text{CH}_3\text{ONa}$ (15)

An oven-dried, round-bottomed flask sealed with a rubber septum and purged with  $\text{N}_2$ , was charged with N-Z-O-tosyl-DL-serine pyrrolidine amide (11) (II) (38 mg) in freshly dried  $\text{CH}_2\text{Cl}_2$  (1.5ml). Na (2mg) in dry MeOH (0.5ml) was added to the solution via a syringe under  $\text{N}_2$ . The reaction was almost complete after 1.5 h. stirring at room temperature. TLC analyses ( $\text{EtOAc}$ -petroleum spirit, 4:1 and 2:1) showed some starting material as a very pale spot

however because the longer reaction time caused many side products that made the purification impossible, the reaction was stopped after 1.5h. The reaction solution was then washed with water (2x10ml) to remove the salt. The organic layer was dried, filtered and the solvent removed under reduced pressure. This yielded the crude product (20mg) as a white solid that was purified by a column chromatography using EtOAc -petroleum-spirit to give the clear oily compound (**III**) (10mg, 42%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>,ppm) : 1.89 (4H, m, N(CH<sub>2</sub>-CH<sub>2</sub>)<sub>2</sub>), 3.52 and 3.65 (4H, dd, N(CH<sub>2</sub>-CH<sub>2</sub>)<sub>2</sub>), 5.03 (1H, s, C=CH), 5.14 (2H, s, Ph-CH<sub>2</sub>), 6.05 (1H, s, OCH), 7.34 (6H, m, Ph+NH). <sup>13</sup>C-NMR : 24.00 and 26.56 (N(CH<sub>2</sub>-CH<sub>2</sub>)<sub>2</sub>), 47.02 and 50.13 (N(CH<sub>2</sub>-CH<sub>2</sub>)<sub>2</sub>), 66.83 (Ph-CH<sub>2</sub>). 101.84 (=CH<sub>2</sub>), 128.12, 128.24 and 128.55 (ArC), 135.15 (C=), 136.01 (quaternary C), 153.37 (O=C-N), 164.80 (O=C-O)

#### **Elimination of tosyl chloride using Et<sub>3</sub>N (13,16)**

The same procedure was carried out described above using N-Z-O-Tosyl-DL-serine pyrrolidine amide (**II**) (20mg) with dry Et<sub>3</sub>N (5mg). The reaction was followed by NMR and TLC. Analyses of the crude product showed the starting material only and no alkene. All the attempts with different parameters are listed in table 1.

#### **Elimination of tosyl chloride using DBU (14,17)**

To a solution of N-Z-O-tosyl-DL-serine pyrrolidine amide (**II**) (0.025g) in dry THF (1ml) in an oven dried round-bottomed flask sealed with a rubber septum, DBU (0.020g) was added via syringe under N<sub>2</sub>. The reaction mixture was stirred at 40°C and the reaction was followed by NMR and TLC (EtOAc-petroleum spirit). One spot (R<sub>f</sub>: 0.5, starting material R<sub>f</sub>: 0.6) was detected on the TLC plate. The reaction mixture was evaporated to dryness to give a sticky residue (0.050g) which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and washed with water (3x25ml). The organic layer was dried, filtered and evaporated to dryness. NMR analysis of the half solid crude product (0.030g) showed no starting material (**II**) and no alkene signals (**III**). The compound could not be identified. The mass spectrum was complicated and no M or M+1 ions were detected for the alkene or the starting material.

## RESULTS AND DISCUSSION

The synthesis and the solvent and base behaviour were investigated using the most popular solvents and base for the elimination of tosyl group. In view of the outstanding importance of proteins and peptides containing hydroxy amino acids (18,19) and the complex problems with their synthesis the difficulties previously encountered have involved mainly interference by the side chain. To eliminate this interference, this study performed on one derivative of serine in order to compare the results. Although the  $\beta$ -elimination is the most widely used method (4) to make dehydro compounds of serine derivatives, this study proved that it was not a straight forward reaction. There are some problems encountered with the base or the solvent systems or unwanted side reactions and neighbouring effects of the protecting groups are occurred.

The best result (see Table 1) was obtained when the  $\text{CH}_3\text{ONa}$  was used as a base giving a clear oily dehydro derivative (**III**) in 42% yield after column chromatography. The compound was analysed by  $^1\text{H-NMR}$  and the expected alkene signals were detected at  $\delta$  5.03 and 6.05. This preparation did not require heating or a long reaction time. In

**Table 1.** The reaction conditions and the parameters for each attempt

| Base                    | Solvent*                       | Conditions                 | NMR Analysis of crude product                |
|-------------------------|--------------------------------|----------------------------|--|
| $\text{CH}_3\text{ONa}$ | $\text{CH}_2\text{Cl}_2$ -MeOH | 1 day R.T + 4h50°          | No S.M. and alkene and unidentified products |
| $\text{CH}_3\text{ONa}$ | $\text{CH}_2\text{Cl}_2$ -MeOH | overnight R.T              | No S.M. but alkene and unidentified products |
| $\text{CH}_3\text{ONa}$ | THF                            | 1.5 days R.T               | S.M. was recovered                           |
| $\text{CH}_3\text{ONa}$ | MeOH                           | 30 min. R.T                | S.M. +alkene (1:1)                           |
| $\text{CH}_3\text{ONa}$ | MeOH                           | 1h R.T                     | S.M. +alkene (1:2)                           |
| $\text{CH}_3\text{ONa}$ | MeOH                           | 1.5h R.T                   | alkene + ignorable amount of S.M.            |
| $\text{Et}_3\text{N}$   | $\text{CH}_2\text{Cl}_2$       | 8 days R.T                 | S.M. was recovered                           |
| $\text{Et}_3\text{N}$   | $\text{CH}_2\text{Cl}_2$       | 1 day R.T + 1 day 30°      | S.M. was recovered                           |
| $\text{Et}_3\text{N}$   | EtOAc                          | 1 day 50° + ovnernight 70° | S.M. was recovered                           |
| $\text{Et}_3\text{N}$   | MeOH                           | 1 day 50° + ovnernight 70° | S.M. was recovered                           |
| DBU                     | THF                            | 3h 40° +                   | No S.M. or alkene but unidentified           |

|     |                                 | overnight 40°             | products                                    |
|-----|---------------------------------|---------------------------|---|
| DBU | MeOH                            | 3h 40° +<br>overnight 40° | No S.M. or alkene but unidentified products |
| DBU | CH <sub>2</sub> Cl <sub>2</sub> | 3h 40° +<br>overnight 40° | No S.M. or alkene but unidentified products |

\* Freshly dried solvents were used, S. M: starting material, R.T: room temperature

fact, heating at 50°C and a longer reaction time than an hour and a half gave no alkene product at the end of the reaction but some unknown products. The optimum reaction conditions involved stirring at room temperature for an hour and a half. Although a very small quantity of the starting material was still present, despite excess reagent, at the end of the 1.5 h reaction time, a longer reaction time was avoided to reduce the formation of the side-products that were detected on the TLC plate. These side-products could not be identified even after purification by chromatography or crystallisation, and they reduced the yield of the alkene. Best solvent system is CH<sub>2</sub>Cl<sub>2</sub>-MeOH.

Interestingly Et<sub>3</sub>N did not react at all with the starting material O-tosylated serine pyrrolidine amide using the same anhydrous reaction conditions as in the preceding experiment (see table 1). The starting material was recovered and characterised by <sup>1</sup>H-NMR (δ 2.43 3H, s, TS-CH<sub>3</sub> in CDCl<sub>3</sub>) at the end of each attempt. Basicity of Et<sub>3</sub>N is not enough to carry the reaction so it is not possible to talk about a solvent effect, there is a problem with base.

Attempts to form the dehydroalanine derivative via tosyl elimination from N-Z-O-Ts-DL-serine pyrrolidine amide (II) failed using DBU (14) in dry THF (see table 1). During the first 3 h of the reaction, only the starting material was detected by <sup>1</sup>H-NMR and TLC. After heating of the reaction mixture at 40°C, the starting material disappeared but the product was not the expected elimination product. The NMR analyses of the crude product showed neither the starting material nor the alkene signals but some impurities and DBU signals. This route was found to be unsuccessful and was not investigated further.

Although THF works perfectly for Ph<sub>3</sub>P/DEAD method to make dehydroalanines by elimination of water (9), it did not give the expected product with all the bases in this study. Also β-elimination was found to be effected by treatment with DBU in Fmoc-based

glycopeptide synthesis (14) but this was found to be unaffected in this study using with THF, MeOH and CH<sub>2</sub>Cl<sub>2</sub>.

The  $\beta$ -elimination reaction is particularly pronounced when the hydroxy groups are acylated with a tosyl group that generate a good leaving group. Under mild conditions and slightly excess base such as CH<sub>3</sub>ONa has to be chosen. In literature (20) some  $\beta$ -elimination reactions of N(Z)-O(Ts)-serine derivative give oxazolines. This was not observed by NMR on the analysis of the products in this study.

#### ACKNOWLEDGEMENT

The author is deeply grateful to Dr. J. M. Williams for his help during this study, the EPSRC Mass Spectrometry Unit (Swansea) for the mass spectra, to Mr M Nettle for NMR spectra, and the EPSCR for a grant for NMR equipment.

#### REFERENCES

1. **Kellner, R., Jung, G., Horner, T., Zahner, H., Schnell, N., Entian, K.D., Gotz, F.D.,** "Gallidermin a new lanthionine containing polypeptide antibiotic" *Eur. J. Biochem.*, **177**, 53-59 (1988).
2. **Banerjee, S., Hansen, J.N.,** "Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic" *J. Biol. Chem.*, **263**, 9508-9514 (1988).
3. **Ando, S., Kato, T., Izumiya, N.,** "Studies of peptide antibiotics" *Int. J. Pept. Protein Res.*, **25**, 15-26 (1985).
4. **Pohataki, I.,** "Transformation of serine to cysteine" *J. Am. Chem.Soc.*, **85**, 1123-1126 (1963).
5. **Inai, Y., Oshikawa, t., Yamashita, M., Hirabayashi, T., Hirako, T.,** "Structural and conformational properties od (Z)-beta-(1-naphthyl)-dehydroalanine residue" *Biopolymers*, Jan;58(1), 9-19 (2001).
6. **Mikhailov,A., Harmala-Brasken, A., Meriluoto, J., Sorokina, Y., Dietrich, D., Eriksson, J.E.,** "Production and specificity of mono and polyclonal antibodies against

- microcystins conjugated through N-methyldehydroalanine" *Toxicol.*, Apr; 39(4), 477-483 (2001).
7. **Andersson, E., Holm, N.G.**, "The stability of some selected amino acids under attempted redox constrained hydrothermal conditions" *Orig. Life Evol. Biosph.*, 30, 9-23 (2000).
  8. **Breitholle, E.G., Stammer, C.H.**, "Synthesis of some dehydropolyalanine peptides" *J. Org. Chem.*, 41, 1344-1349 (1976).
  9. **Wojciechowska, H., Pawłowicz, R., Andruszkiewicz, R., Gryzbowska, J.**, "Conversion of protected serine and threonine to corresponding dehydroamino acids under mild conditions" *Tet. Lett.*, 42,4063-4064 (1978).
  10. **Süzen, S., Williams, J.M.**, "Behaviour of dehydroalanine derivatives under hydrazinolysis conditions. Possible relevance to glycoprotein hydrazinolysis" *J Pept Sci*, 5:6, 283-286 (1999).
  11. **Süzen, S., Williams, J.M.**, "Investigation of the synthesis of some dehydroalanine derivatives" *Turkish J. Chem.* (In press) (2000)
  12. **Miller, M.J.**, "Iso-urea mediated preparation of dehydro amino acids" *J. Org. Chem.*, 45, 3131-3132(1980).
  13. **Rich, D.H., Tam, J.P.**, "Synthesis of tentoxin and related dehydrocyclic tetrapeptides" *J. Org. Chem.*, 42, 3815-3820 (1977).
  14. **Meldal, M., Bielfeldt, T., Peters, S., Jensen, K J., Paulsen, H., Bock, K.**, "Susceptibility of glycans to beta-elimination in Fmoc-based O-glycopeptide synthesis" *Int. J. Peptide Protein Res.*, 43, 529-536 (1994).
  15. **Ginsburg, S., Wilson, I.B.**, "Factors affecting the competitive formation of oxazolines and dehydroalanines from serine derivatives" *J. Am. Chem. Soc.*, 86,4716-4720 (1964).
  16. **Nakagawa, Y., Tsuno, T., Nakajima, K.**, "Studies on hydroxy amino acids" *Bull. Chem. Soc. Jap.*, 45, 1162-1167 (1972).
  17. **Srinivasan, A., Stephenson, R.W., Olsen, R.K.**, "Conversion of threonine derivatives to dehydroaminoacids by elimination of beta-chloro and O-tosyl derivatives" *J. Org. Chem.*, 42,2256-2260(1977).
  18. **Süzen, S.**, "The role of dehydroalanines in enzyme and peptide chemistry" *J. Fac. Pharm. Ank. Univ.*, 28, 129-141 (1999)



19. Süzen, S., " The characterisation of the carbohydrate moieties of glycoproteins and the role of hydrazinolysis" *FABAD J. Pharm. Sci.*, 24,233-239 (1999)
20. Sheehan, J. C, Goodman, M., Hess, G.P.," Peptide derivatives containing hydroxyamino acids" *J.Am. Chem. Soc.*, 78,1367-1369 (1956).

**Başvuru Tarihi : 22.11.2000**

**Kabul Tarihi : 1.2.2001**