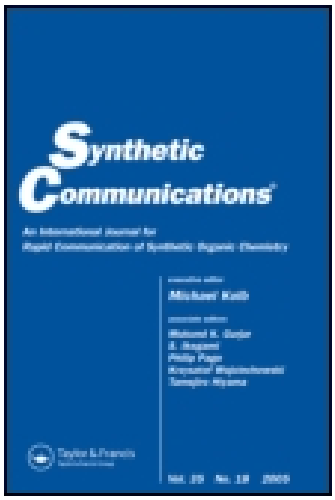

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DEHYDRATION OF THREONINE ESTERS DURING TOSYLATION

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ABSTRACT

Tosylation of *N*-α-protected threonine methyl-and benzyl esters was investigated. Depending on the protecting groups, the composition of the reaction mixtures showed different ratio between *O*-tosyl-and dehydrothreonine derivatives. The latter was formed exclusively in case of *N*-α-Fmoc-threonine benzyl ester.

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α,β-Dehydroamino acid units are frequently found in antibiotic and phytotoxic peptides.[1–3] Nisin, which contains both dehydroalanine- and β-methyl-dehydroalanine residues displays antimalarial action and release of lysosomal enzymes and lysis of erythrocytes.[4] Terminamycin A, a cyclo-dehydrodecapeptide with two dehydrodehydrodipeptide residues and seven dehydroamino acid moieties has been isolated from a culture of *Streptomyces bernensis*.[5]

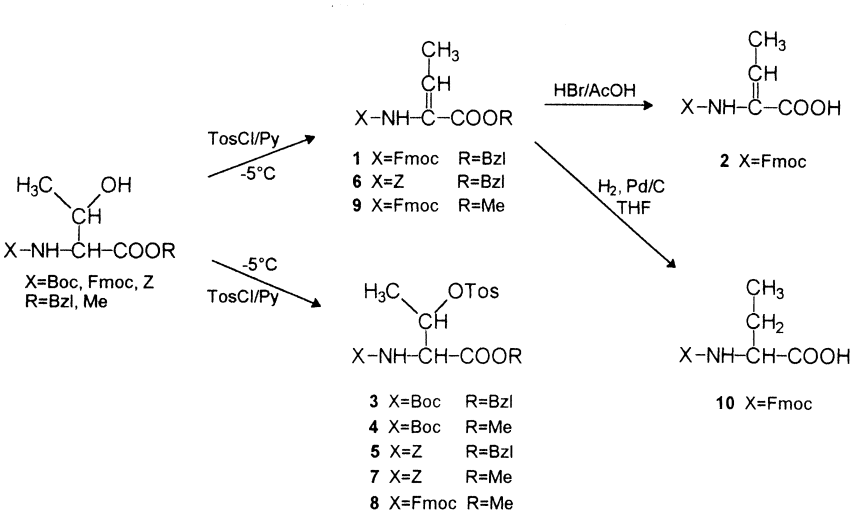
Dehydroalanine is one of the constituents of the toxin alternariolide, which responsible for necrotic brown spots of apple.[6] The partially recemized D-form of vinylglycine has been obtained from mushrooms[7] and it is an intermediate in the enzymatic conversions of homoserine to threonine and α-ketobutyrate. Various thiazole α-dehydroamino acids, including ΔGlu(OMe), ΔLeu, ΔPhe, ΔThr and ΔVal and their dehydrodi- and tripeptides were synthesized in 1992.[8] Different 2-(1-amino)alkenyl-thiazoline-4-carboxylates–synthesized by Shin and associates[9]– are also important partial skeleton of many thiostrepton antibiotics such as thiomycin A[10] and thiopeptin-Ba.[11] These examples show that much interest has been directed towards the synthesis and bioactivity of dehydropeptides containing α-dehydroamino acid residues.

α,β-Unsaturated amino acid derivatives and dehydropeptides can be prepared by base catalyzed elimination of sulfonium derivatives[12] and sulfoxides,[13] furthermore by condensation reaction of 2-oxoalkanoic acid with benzyl carbamate or *p*-toluenesulfonamide.[14] The most frequently used method for synthesis of α,β-dehydroamino acids is β-elimination reaction of *O*-tosyl derivatives of 3-hydroxy amino acids described by I. Photaki.[15] *N*-acylcrotonates have been reported by R.K. Olsen and associates *via* β-chloro-and *O*-tosyl derivatives of the respective *N*-acyl-DL-threonine methyl esters using 1,4-diazabicyclo[2,2,2]octane (Dabco) as base in ethyl acetate.[16] On the other hand, *O*-tosyl threonine as well as peptides containing *O*-tosyl threonine residue have been easily converted into aziridine rather than α,β-dehydro compounds upon treatment with triethylamine.[12,17]

Direct tosylation of *N*-α-Fmoc-, *N*-α-Boc-and *N*-α-Z-protected threonine benzyl-and methyl esters is presented here. The original aim was to synthesize a new γ-lactam constrained dipeptide analogue to stabilize β-turn conformation in peptides.[18] Elongation of the side chain of threonine with one carbon atom by replacement the tosyl group by the nitrile group using tetraethylammonium cyanide in dichloromethane was the key step of the synthetic plan. For this purpose, *O*-tosyl derivative of *N*-protected threonine ester as starting material was needed.

First, *N*-α-Fmoc-threonine benzyl ester was treated with excess amount of *p*-toluenesulfonyl chloride in dry pyridine at $-5^{\circ}C$ for 2 h, then allowed to stand overnight as usual for tosylation reactions. NMR

REACTION SCHEME



measurements revealed that *N*-α-Fmoc-dehydrothreonine benzyl ester was obtained, unexpectedly almost quantitatively, without formation of the corresponding *O*-tosyl derivative. Direct β-elimination is known in the literature. In 1981 C. Shin and associates reported the synthesis of several dehydrodehydrodipeptides under mesylation by base catalyzed β-elimination.[19] In order to examine the solvent-dependence of the tosylation, the same reaction was repeated in other tertiary bases. Under the same reaction conditions, conversion of the starting material didn't result in main product in triethylamine and many spots have been observed on TLC. In 4-methylmorpholine a moderate amount of the appropriate dehydrothreonine was obtained, but the reaction mixture contained a portion of the starting material even after 2 days. The starting material in dry pyridine in the absence of *p*-toluenesulfonyl chloride at room temperature was stabile for after a week. Consequently, *O*-tosyl derivative as an intermediate has to be formed first, which serves the appropriate dehydrothreonine derivative by a fast β-elimination.

When *N*-α-Fmoc-threonine methyl ester was tosylated in dry pyridine, the desired *O*-tosyl compound and the dehydrothreonine derivative were isolated from the reaction mixture with 70% and 30% yield, respectively. The effect of replacement of the *N*-α-Fmoc group by the *N*-α-Boc-and the *N*-α-*Z* was also studied. Both the *N*-α-Boc and the *N*-α-*Z*-threonine

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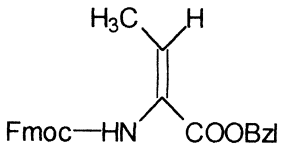
Table 1. Conversion of Protected Threonine Esters to *O*-Tosyl- and Dehydrothreonine Derivatives

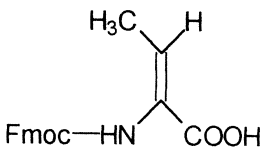
X	R	*O*-Tosyl Yield (%)	Dehydro Yield (%)
Fmoc	Bzl	0	100
Fmoc	Me	70	30
Z	Bzl	90	10
Z	Me	100	0
Boc	Bzl	100	0
Boc	Me	100	0

methyl esters gave exclusively *O*-tosyl products, therefore these protecting groups do not favour the formation of the α,β-unsaturated systems. Similarly, the *N*-α-Boc-threonine benzyl ester yielded only the *O*-tosyl derivative. On the other hand, both the *O*-tosyl- and the dehydrothreonine were obtained under the conversion of *N*-α-*Z*-threonine benzyl ester with 90% and 10% yields, respectively (Table 1).

Deprotection of the benzyl ester group to obtain *N*-α-Fmoc-dehydrothreonine (**2**) was achieved using 30% HBr in acetic acid solution. The geometry of the double bond was investigated by one-dimensional NOE experiments (Nuclear Overhauser Effect). The selective excitation of the methyl group at 1.71 ppm in **1** and at 1.68 ppm in **2** was used to identify the isomers in solution phase. The experiment provided enhanced signals on the NH protons at 8.87 ppm in **1** and at 8.64 ppm in **2**. These data revealed that a single stereoisomer of dehydrothreonine was formed which has a *Z* geometry in both cases. The existence of the *Z* isomer was proved further as the saturation of the benzyl CH_2 at 5.15 ppm in **1** in the NOE experiment did not yield any enhancements on the methyl doublet at 1.71 ppm. However, an enhanced signal was observed on the olefinic quartet at 6.60 ppm. Our observations are in a close correlation with formation of the more stable *Z* isomer of 2-acylaminocrotonates [16, 20].



1 (Z)



2 (Z)

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The catalytic hydrogenation of *N*-α-Fmoc-dehydrothreonine benzyl ester (**1**) with Pd/C simultaneously cleaved the benzyl ester and saturated the α,β-olefin bond yielding racemic mixture of *N*-α-Fmoc-norvaline (**10**) in the ratio of 1 : 1 (the product showed no optical rotation).

N-α-Fmoc-threonine benzyl ester can be converted into *N*-α-Fmoc-dehydrothreonine benzyl ester almost quantitatively in direct tosylation reaction in dry pyridine by β-elimination. After deprotection of the benzyl ester group, *N*-α-Fmoc-dehydrothreonine was obtained, which can be used as a building block for peptide synthesis and for tritium labelling of peptides.

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EXPERIMENTAL

All reagents and solvents were of reagent grade and purchased from commercial sources. Melting points were determined using a PAMK VEB apparatus and are uncorrected. TLC was performed on silica gel precoated glass plates 60 F_{254} (Merck). ^{1}H NMR spectra were obtained using a Bruker DRX 500 MHz spectrometer. Micoanalysis were made on a CHN Analyser (Prague).

General Procedure for Tosylation

The *N*-α-protected-threonine esters (10–15 mmol, 65 mmol for *N*-αFmoc-threonine benzyl ester) were dissolved in dry pyridine and cooled to -5°C, then *p*-toluenesulfonyl chloride (1.5 equiv.) was added in a small portions. The reaction mixtures were kept at -5°C, allowed to stand overnight in fridge, then poured onto ice-water, acidified with 10% $NaHSO_4$ solution. The precipitates were collected by filtration, washed with water to neutral pH and dried.

N-α-Fmoc-dehydrothreonine Benzyl Ester (1)

Mp.: 137–138°C from ethyl acetate/*n*-hexane; 95% yield; R_f: 0.48 (*n*-hexane/ethyl acetate, 3 : 2); ^{1}H NMR ($CDCl_3$), $\delta = 1.78$ ppm (d, 3H, CH_3),

4.20 ppm (t, 1H, CH), 4.41 ppm (d, 2H, CH_2), 5.20 ppm (s, 2H, CH_2), 6.83 ppm (q, 1H, CH), 7.20–7.80 ppm (m, $13H_{aromatic}$); $C_{26}H_{23}NO_4$ (413.45), calcd. C (75.53%), H (5.61%), N (3.39%), found C (75.35%), H (5.49%), N (3.20%).

N-α-Fmoc-dehydrothreonine (2)

N-α-Fmoc-dehydrothreonine benzyl ester (**1**, 1 g, 2.43 mmol) was dissolved in 30 wt.% HBr solution in acetic acid (10 ml) and stirred for 1 h at room temperature. The reaction mixture was concentrated *in vacuo* to dryness, the residue was suspended in distilled water, filtered, washed with water to neutral pH and dried, 600 mg (77%). The crude product was purified by column chromatography on silica gel (eluent CH_2Cl_2/MeOH, 95 : 5). The appropriate fractions were combined, evaporated *in vacuo* to dryness. The residue was triturated with diethyl ether to give the product as white crystalline powder, 300 mg (39%), mp.: 220–222°C, R_f: 0.30 (dichloromethane/methanol, 9 : 1); ^{1}H NMR ($CDCl_3$), $\delta = 1.69$ ppm (d, 3H, CH_3), 4.24–4.33 ppm (m, 3H, CH_2 and CH), 6.56 ppm (q, 1H, CH_β), 7.31–7.95 ppm (m, $8H_{aromatic}$); $C_{19}H_{17}NO_4$ (323.33), calcd. C (69.04%), H (5.30%), N (4.33%), found C (68.91%), H (5.13%), N (4.21%).

N-α-Fmoc-norvaline (10)

N-α-Fmoc-dehydrothreonine benzyl ester (**1**, 2.07 g, 5 mmol) was dissolved in dry tetrahydrofuran (60 ml) and hydrogenated over 10% Pd/charcoal for 2 h. The catalyst was filtered and the filtrate was concentrated *in vacuo* to dryness. The crude product (1.4 g, 88%) was recrystallized from ethyl acetate and *n*-hexane solvent mixture, mp.: 161–163°C, R_f: 0.22 (dichloromethane/methanol, 9 : 1); ^{1}H NMR (DMSO), $\delta = 0.87$ ppm (t, 3H, CH_3), 1.63 and 1.75 ppm (m, 2H, CH_2), 3.89 ppm (m, 1H, CH_α), 4.21–4.29 ppm (m, 3H, CH_2 and CH), 7.11–7.89 ppm (m, $8H_{aromatic}$); $C_{19}H_{19}NO_4$ (325.35), calcd. C (70.16%), H (5.88%), N (4.30%), found C (69.85%), H (5.53%), N (4.19%).

N-α-Boc-*O*-tosyl-threonine Benzyl Ester (3)

Mp.: 128–130°C from methanol/water; 70% yield; R_f: 0.55 (*n*-hexane/ethyl acetate, 3 : 2); ^{1}H NMR ($CDCl_3$) $\delta = 1.33$ ppm (d, 3H, CH_3), 1.43 ppm (s, 9H, CH_3), 2.42 ppm (s, 3H, CH_3), 4.46 ppm (d, 1H, CH), 4.92 and

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5.12 ppm (d, 2H, $CH_{2,benzyl}$), 5.19 ppm (m, 1H, CH), 5.20 ppm (d, 1H, NH), 7.20–7.70 ppm (m, $9H_{aromatic}$); $C_{23}H_{29}NO_7S$ (463.50), calcd. C (59.60%), H (6.31%), N (3.02%), found C (59.37%), H (6.10%), N (2.85%).

N-α-Boc-*O*-tosyl-threonine Methyl Ester (4)

Oil; 80% yield; R_f: 0.45 (*n*-hexane/ethyl acetate, 3 : 2); ^1H NMR ($CDCl_3$), $\delta = 1.36$ ppm (d, 3H, CH_3), 1.44 ppm (s, 9H, CH_3), 2.44 ppm (s, 3H, CH_3), 3.57 ppm (s, 3H, OCH_3), 4.69 ppm (d, 1H, CH), 5.13 ppm (m, 1H, CH), 5.22 ppm (d, 1H, NH), 7.34–7.80 ppm (m, $4H_{aromatic}$); $C_{17}H_{25}NO_7S$ (387.50), calcd. C (52.69%), H (6.50%), N (3.62%), found C (52.40%), H (6.31%), N (3.49%).

N-α-*Z*-*O*-tosyl-threonine Methyl Ester (7)

Mp.: 70–72°C from methanol/water; 90% yield; R_f: 0.30 (*n*-hexane/ethyl acetate, 3 : 2); ^1H NMR ($CDCl_3$), $\delta = 1.35$ ppm (d, 3H, CH_3), 2.43 ppm (s, 3H, CH_3), 3.59 ppm (s, 3H, OCH_3), 4.48 ppm (d, 1H, CH), 5.11 ppm (s, 2H, CH_2), 5.16 ppm (m, 1H, CH), 5.46 ppm (d, 1H, NH), 7.30–7.70 ppm (m, $9H_{aromatic}$); $C_{20}H_{23}NO_7S$ (421.50), calcd. C (56.99%), H (5.50%), N (3.32%), found C (56.77%), H (5.45%), N (3.23%).

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