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Synthesis of α,β-Dehydrotryptophan by Reaction of Indole with the β-(*N*-Methylamino)dehydroalanine Derivative

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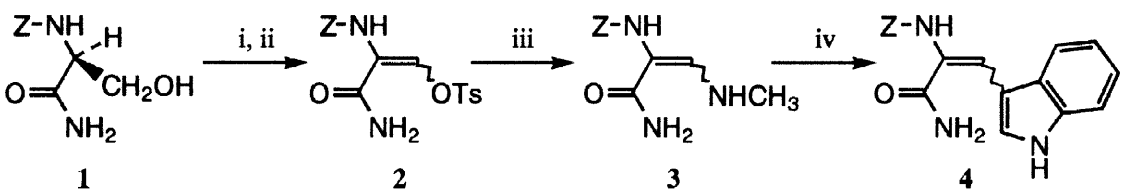
Abstract: α,β-Dehydrotryptophan **4** is synthesized by a sequence of reactions starting with the corresponding serine derivative **1**. This procedure includes substitution reaction between indole and the methylamino group of the *β-(N*-methylamino)dehydroalanine derivative **3** in acetic acid. The key intermediate **3** is obtained by oxidation of **1** with dimethylsulfoxide activated by *p*-toluenesulfonyl chloride, followed by reaction of the resulting *β-O*-tosyldehydroserine **2** with methylamine.

α,β-Dehydrotryptophan **4** is known to be involved in telomycin as a modified form of one of the tryptophan residues in this naturally occurring peptide antibiotics.[1] Many bacteria catabolite amino acids with oxidases and dehydrogenases; tryptophan side-chain oxidase[2] and L-tryptophan 2',3'-oxidase[3] specifically catalyze the transformation of a series of indolic substrates into the corresponding derivatives of **4**. Usually, these enzymes are capable of acting even on tryptophan residues in relatively small peptides, thus allowing to speculate about the biological process involved for the production of **4** in a peptide like telomycin. Along with the biochemical significance, researchers noted the interesting fluorescence properties of **4**.[2] Although these enzymes proved to be highly specific for tryptophan as well as its derivatives, the enzymes are not commercially available and there may be the intrinsic difficulty in adapting them to preparative purposes. Therefore, an efficient synthetic approach needs to be devised to bypass the disadvantages associated with enzymatic procedures. We have elaborated a novel synthetic route to yield **4** starting from the serine derivative **1** *via* a sequence of reactions (Scheme 1).



1 **2** **3** **4**

i: *p*-Toluenesulfonyl chloride (3 equiv.), Me_2SO/DMF (1:2), −5°C, 10 min.; ii: Et_3N, 1 hr.; iii: CH_3NH_2/MeOH; iv: Indole (1 equiv.)/AcOH, 40°C, 3 days. Z = Benzyloxycarbonyl.

Scheme 1

We reported the details of the initial steps (i)-(iii) in a previous paper.[4] We then found that either the tosyl group of **2** or the methylamino group of **3** could readily be replaced with a variety of nucleophiles to form other

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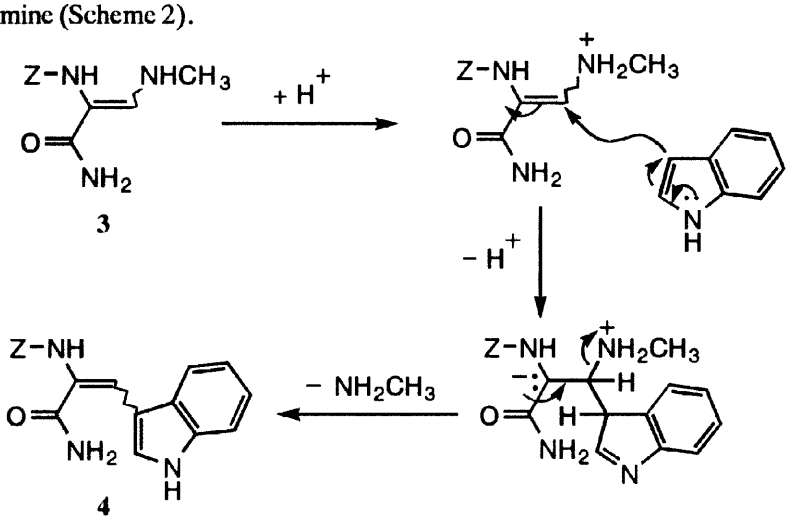
β-substituted dehydroamino acid derivatives; when indole was employed as a nucleophile and reacted with **3**, the corresponding derivative of **4** was obtained (step iv).

To mimic the serine residue in a peptide, we chose N^{α}-benzyloxycarbonyl-L-serine amide (**1**: Z-L-Ser-NH_2) as starting material for the synthesis of **4**. The oxidation using Me_2SO activated by *p*-toluenesulfonyl chloride afforded the *E* isomer of product **2** in 45% yield,[5] but the *Z* counterpart has not yet been isolated. Practically, enamine **3** could be obtained in more than 55% yield with respect to **1** when a crude mixture of the oxidation products was subjected to reaction with methylamine, indicating that the net yield of both isomers of **2** was at least 64% since the isolated pure *E* isomer of **2** gave enamine **3** in 85% yield.[4,6] The reaction of indole with **3** is carried out as follows: indole (120 mg, 1 mmol) was dissolved in acetic acid (4 ml) and then enamine **3** (250 mg, 1 mmol) was added. The solution was allowed to react for 3 days at 40°C. After evaporating the solvent, fine crystals of the *Z* isomer of N^{α}-benzyloxycarbonyl-α,β-dehydrotryptophan amide **4** (60 mg, 18%) were obtained from the AcOEt solution (about 2 ml) of the oily residue and were collected by filtration. To complete the separation of the isomers, the filtrate was chromatographed on silica-gel (toluene: AcOEt: AcOH; 9:9:2). The *E* isomer was isolated from the fraction eluted earlier, and obtained as pure material by recrystallization from methanol (15 mg, 4%).[7a] As the main product, the additional *Z* isomer eluted later than the *E* isomer and the crude materials obtained before and after the chromatography were combined and recrystallized from methanol. Total yield of the *Z* isomer was 158 mg (45%).[7b] The yields of these isomers were not appreciably improved when more than 2-fold excess of indole was applied in the reaction with **3**.

Enamine **3**, preferably in its protonated form, appears to undergo addition by indole, followed by elimination of methylamine (Scheme 2).



Scheme 2

This mechanism is analogous to that of the Mannich reaction in which the active immonium intermediate plays an important role. For example, the starting material **3** involves the enamine function that can readily undergo addition by indole to give a possible intermediate corresponding to the Mannich base, leading to **4** by the subsequent elimination of methylamine. Gramine, or 3-(*N*,*N*-dimethylaminomethyl)indole, has been known to be a Mannich base that is useful for synthesis of tryptophan derivatives,[8] but it differs from **3** in having the dimethylamino group to be displaced by the glycine moiety without being eliminated to give an unsaturated compound. There is a closer resemblance between **3** and methyl α-nitro-β-ethoxyacrylate; each compound bears

not only the potent electron-withdrawing group(s) inducing the positive charge on the C^{β} atom which is sufficiently reactive with the nucleophilic indole but also a leaving group appropriate to restore the C^{α}-C^{β} double bond in the product. Unfortunately, the reaction using the latter compound has not been pursued further by the authors because of various experimental difficulties.[9]

Although displacement of the tosyl group in **2** with indole occurred in acetic acid, the yield of **4** was only marginal (<10%) and the starting materials were recovered almost unreacted even after a prolonged reaction time (>1 week) at temperatures up to 70°C. Indole was found to react with neither **2** nor **3** in solutions where bases or nucleophiles could compete with indole for the electrophilic site. These facts suggest that protonation on the methylamino group of **3** in acetic acid effectively promotes the reaction without affecting the nucleophilicity of the indole. The yield of the substitution reaction could possibly be improved if a better leaving group could be introduced in place of the methylamino group of **3**. In this context, the dimethylamino analogue of **3** appeared promising as a substrate for the substitution, but its preparation was unsuccessful because reaction of **2** with dimethylamine led mostly to a glycine derivative.[4] The efficiency of the reaction can also be varied depending on the nature of a nucleophile. Therefore, only minor modifications of the present reaction conditions should suffice to obtain various ring-substituted tryptophan analogues using the related indole derivatives. Note that there are now at least two options open for the procedure in which either **2** or **3** may be applied for the reaction with a given nucleophile, thus making it possible to broaden the spectrum of dehydroamino acids to be synthesized.

The UV spectrum of the *Z* isomer of **4** in methanol exhibited characteristic absorption maxima at 275 nm and 332 nm in good agreement with that reported in literatures.[1,2] In contrast, the *E* isomer showed an UV spectrum with maxima at 282 nm and 295 nm (shoulder) which were very close to those of the parent indole chromophore (280 nm and 290 nm (shoulder)). Obviously, the appearance of these spectra is closely related to the extent of rotation about the C^{β}-C^{γ} bond as a measure of the strain due to the main-chain atoms in contact with the bulky indole ring that otherwise tends to be coplanar. The finding that the *Z* isomer is obtained in much higher yield compared to the *E* isomer is thus understandable considering the presumably less constrained configuration of the former isomer being more stable. These findings about the isomers of **4** agree well with the N^{α}-acetyl-α,β-dehydrotryptophan derivatives,[2,9] except for some spectroscopic properties arising from possible differences in their conformations reflecting the respective steric constraints.

Many dehydrotryptophan derivatives have been synthesized as intermediates leading to tryptophan.[8-10] The main features of the present reaction scheme are that it allows to convert a serine residue in a peptide chain into **4** by means of chemical modification techniques, and that it involves reasonably mild procedures like those generally adopted in peptide synthesis. Furthermore, there may be a variety of nucleophiles in place of indole to be incorporated in the side chain of a dehydroamino acid residue. In peptide chemistry, this can be especially advantageous for the structural and functional studies where a wide spectrum of analogues or specifically labeled species with appropriate probes are often required. As noted in this study, the remarkable UV spectral properties of **4** will also be of use to probe the environment around the indole chromophore with appreciable sensitivity.

REFERENCES AND NOTES

1. Sheehan, J.C.; Mania, D.; Nakamura, S.; Stock, J.A.; Maeda, K. *J. Am. Chem. Soc.* **1968**, *90*, 462-470.

2. Noda, Y.; Takai, K., Tokuyama, T.; Narumiya, S.; Ushiro, H.; Hayaishi, O. *J. Biol. Chem.* **1977**, *252*, 4413-4415.; Ushiro, H.; Noda, Y.; Takai, K., Tokuyama, T.; Narumiya, S.; Hayaishi, O. *J. Biol. Chem.* **1978**, *253*, 4819-4822.; Takai, K.; Hayaishi, O. *Methods Enzymol.* **1987**, *142*, 195-217.
3. Genet, R.; Bénetti, P-H.; Hammadi, A.; Ménez, A. *J. Biol. Chem.* **1995**, *270*, 23540-23545.
4. Nakazawa, T.; Suzuki, T.; Ishii, M. *Tetrahedron Lett.* **1997**, *38*, 8951-8954.
5. N^{α}-Benzyloxycarbonyl-β-*O*-tosyldehydroserine amide (**2**: Z-Δ(Ts)Ser-NH_2): mp. 113-114°C, Anal. Calc. for $C_{18}H_{18}N_2O_6S$: C, 55.37; H, 4.65; N, 7.17; S, 8.21. Found: C, 55.28; H, 4.69; N, 7.14; S, 8.44; IR (KBr) 3479, 3354, 1721, 1662, 1507, 1180, 1085 cm^{-1}; ^1H NMR (270 MHz, Me_2SO-d_6) δ 2.43 (3H, s, C*H*$_3$-Ph), 5.05 (2H, s, C*H*$_2$-Ph), 7.12 (1H, br. s, *H*NHCO), 7.30-7.40 (6H, m, arom.(Z)-C*H*, and *H*NHCO), 7.50 (2H, d, J = 8.1 Hz, Ts-3,5-*H*), 7.80 (2H, d, J = 8.1 Hz, Ts-2,6-*H*), 9.63 (1H, s, $N^{\alpha}H$).
6. N^{α}-Benzyloxycarbonyl-β-(*N*-methylamino)dehydroalanine amide (**3**: Z-Δ(β-NHMe)Ala-NH_2): mp. 184-185°C. For other physical constants and analytical data, see our previous paper.[4]
7. (a) The ***E* isomer** of N^{α}-Benzyloxycarbonyl-α,β-dehydrotryptophan amide **4** (Z-(*E*)-ΔTrp-NH_2): mp. 175-178°C; Anal. Calc. for $C_{19}H_{17}N_3O_3$·(1/4)$C_6H_5CH_3$: C, 69.54; H, 5.34; N, 11.72. Found: C, 69.64; H, 5.52; N, 11.83; λ_{max}(MeOH)/nm 281.5 (ε/dm^3 mol^{-1} cm^{-1} 7 850), 295sh (6 880); ν_{max}(KBr)/cm^{-1} 3409, 1675, 1619, 1510, 1457, 1340, and 1248; ^1H NMR (270 MHz, Me_2SO-d_6) δ 5.06 (2H, s, C*H*$_2$-Ph), 6.96 (1H, s, *H*NHCO), 7.15-7.80 (12H, m, arom.*H*, $C^{\beta}H$, and *H*NHCO), 8.58 (1H, br. s, $N^{\alpha}H$), 11.57 (1H, s, indole $N^{\varepsilon 1}H$). Despite the close resemblance between the one-dimensional ^1H NMR spectra, long-range NOE correlations corresponding to those of the *Z*-isomer were not detected for this isomer. This accords with the implication of the UV spectrum (see text): a possible distortion of the molecule could separate the relevant protons beyond a detectable range of NOE interactions. About 1/4 equivalent of toluene (1.90 ppm, 0.7H, s, Ph-C*H*$_3$) could not be removed.
(b) The ***Z* isomer** of **4** (Z-(*Z*)-ΔTrp-NH_2): mp. 239-240°C (dec.); Anal. Calc. for $C_{19}H_{17}N_3O_3$: C, 68.04; H, 5.11; N, 12.53. Found: C, 68.14; H, 5.18; N, 12.47; λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 5 950), 331.5 (13100); ν_{max}(KBr)/cm^{-1} 3404, 3372, 1715, 1686, 1605, 1389, and 1339 cm^{-1}; ^1H NMR (270 MHz, Me_2SO-d_6) δ 5.10 (2H, s, C*H*$_2$-Ph), 6.98 (1H, s, *H*NHCO), 7.15-7.80 (12H, m, arom.*H*, $C^{\beta}H$, and *H*NHCO), 8.58 (1H, br. s, $N^{\alpha}H$), 11.56 (1H, s, indole $N^{\varepsilon 1}H$). All the resonances including the multiplets of aromatic protons were assigned using the phase-sensitive ^1H-^1H DQF-COSY and NOESY experiments in which the NOE cross peaks characteristic of the *Z*-configuration were observed clearly between signals of $N^{\alpha}H$ (8.58 ppm) and the indole-$C^{\delta 1}$ proton (7.68 ppm, d), and between one of CON*H*$_2$ (6.98 ppm) and the C^{β} proton (7.53 ppm, s) at a mixing time of 450 ms.
8. Hellmann, H. *Z. Physol. Chem.* **1949**, *284*, 163-167; Howe, E.E.; Zambito, A.J.; Snyder, H.R.; Tishler, M. *J. Am. Chem. Soc.* **1945**, *67*, 38-39.
9. Hengartner, U.; Valentine, Jr., D.; Johnson, K.K.; Larscheid, M.E.; Pigott, F.; Scheidl, F.; Scott, J.W.; Sun, R.C.; Townsend, J.M.; Williams, T.H. *J. Org. Chem.* **1979**, *44*, 3741-3747, and references cited therein.
10. For a review, see: Greenstein, J.P.; Winitz, M. *Chemistry of the Amino Acids*. Vol. 1; John Wiley & Sons, Inc., New York 1961; pp. 706-708.