Dimerization of Tryptophan Derivatives in Trifluoroacetic Acid[1]

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(Received February 23, 1981)

Side-products are formed when Ac–Trp–OMe, Ac–Gly–Trp–OMe, or Ac–Trp–Trp–OMe is stored in trifluoroacetic acid. Two products from each of Ac–Trp–OMe and Ac–Gly–Trp–OMe and one product from Ac–Trp–Trp–OMe are isolated by the column chromatography on silica gel and confirmed for homogeneity by the high-performance liquid chromatography. As confirmed by ^{1}H-NMR and mass spectral analyses, they have dimerized structures through the α-carbon to α′-carbon binding of two indole rings. One of the two products from each of Ac–Trp–OMe and Ac–Gly–Trp–OMe is the *trans*-isomer(s) and the other the *cis*-isomer(s). Only a *trans*-isomer(s) is obtained from Ac–Trp–Trp–OMe.

The acid-labile indole moiety in the tryptophan residue is responsible for several side-reactions under acidic conditions used for peptide synthesis. One type of reactions is an oxidation of the indole ring; Theodolopoulos and Fruton[2] reported a possible formation of "β-oxindolylalanine" in a treatment of *N*-(benzyloxycarbonyl)tryptophan peptide with hydrogen bromide in acetic acid, however this has not yet been proved. Another type of reactions is a substitution in the indole ring by a cation derived from a protecting group: Concerning this type, Alakhov *et al.*[3] first pointed out that when *N*-(*t*-butyloxycarbonyl)tryptophan peptide acids or *t*-butyl esters are treated with TFA, a *t*-butyl cation, released from the protecting peptides, is introduced onto the indole ring in the tryptophan residue. Subsequently, this was confirmed by Wünsch *et al.*,[4] who isolated a side product having been butylated at the NH group of the indole ring in the tryptophan residue. This substitution is reported also to occur at positions 2, 5, and 7 of the indole rings.[4,5] A third type of reactions is an intra- or intermolecular substitution of the indole ring; Uphaus *et al.*[6] isolated an α-carboline derivative formed through an intramolecular cyclization in a solution of acetyl-DL-tryptophan in TFA, but in low yield. Recently,[7] one of the authors and others isolated from a solution of Ac–Trp–OMe in TFA or HF a product having a dimerized structure in which the α-position of one indole ring binds with the α′-position of the other indole ring which is reduced simultaneously to an indoline, as shown in Fig. 1.

In this work, we isolated another product which is also formed when Ac–Trp–OMe is kept in TFA and determined its structure on the basis of ^{1}H-NMR and mass spectra. The spectral analysis suggests that the new product is a steric isomer of the compound previously isolated. We found also that when tryptophan-peptides (Ac–Gly–Trp–OMe and Ac–Trp–Trp–OMe) are stored in TFA under the same conditions as with Ac–Trp–OMe, side-products similar to those from Ac–Trp–OMe in structure are formed.

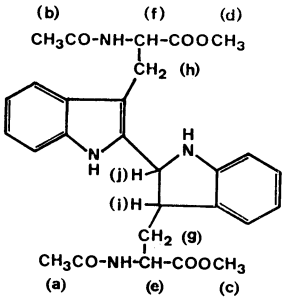

Fig. 1. Dimerized structure of Ac–Trp–OMe.

Results and Discussion

Isolation of Reaction Products. After keeping Ac–Trp–OMe in TFA, we detected two compounds with lower mobility than the starting compound on a TLC plate with a concentration zone (Fig. 2). These compounds gave yellow spots on being stained with the Ehrlich reagent. Previously,[7] these two compounds could not be separated from each other on a TLC plate with no concentration zone, and only one of them could be isolated on crystallization. In the present work we found that the previously isolated compound corresponds to the compound with the lower R_f value on the TLC plate with a concentration zone, which finding encouraged us to isolate the other compound. For isolation of this compound, a solution of Ac–Trp–OMe in TFA was subjected to a chromatography on a silica-gel

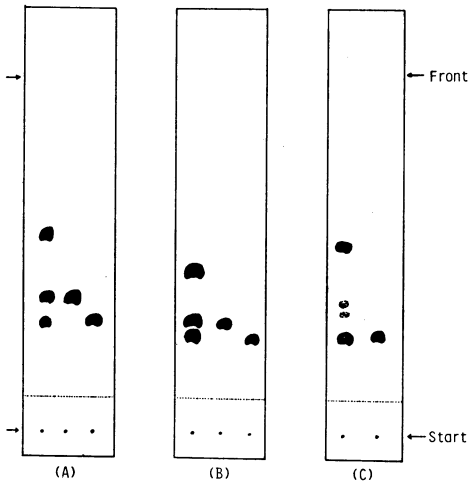

Fig. 2. TLC of products in TFA and isolated materials on silica gel 60 with a concentration zone (Merck Art. 11845). (A) Ac–Trp–OMe (from left, reaction mixture, compounds **1B** and **1A**), (B) Ac–Gly–Trp–OMe (from left, reaction mixture, compounds **2B** and **2A**) and (C) Ac–Trp–Trp–OMe (from left, reaction mixture and compound **3**). Solvent system: (A) and (C), CH_3CN–$CHCl_3$ (2 : 1), (B) CH_3CN–$CHCl_3$–MeOH (60 : 30 : 5). Staining with Ehrlich reagent.

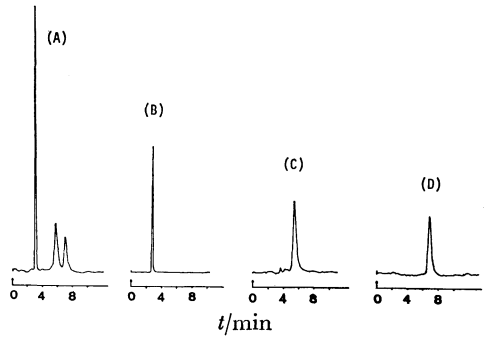

Fig. 3. Elution profiles at 280 nm of reaction mixture of Ac–Trp–OMe in TFA and isolated materials by HPLC on a μ-porasil column (0.39 cm × 30 cm). Solvent: CH_3CN–$CHCl_3$ (2 : 1). Flow rate: 1 ml/min. (A) reaction mixture, (B) starting material (Ac–Trp–OMe), (C) compound **1B**, and (D) compound **1A**.

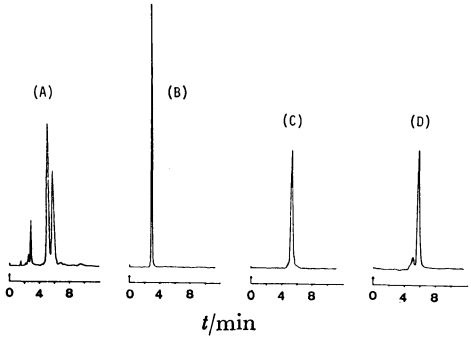

Fig. 4. Elution profiles at 280 nm of reaction mixture of Ac–Gly–Trp–OMe in TFA and isolated materials by HPLC on a μ-porasil column (0.39 cm × 30 cm). Solvent: CH_3CN–$CHCl_3$–MeOH (60 : 30 : 5). Flow rate: 1 ml/min. (A) reaction mixture ,(B) starting material (Ac–Gly–Trp–OMe), (C) compound **2B**, and (D) compound **2A**.

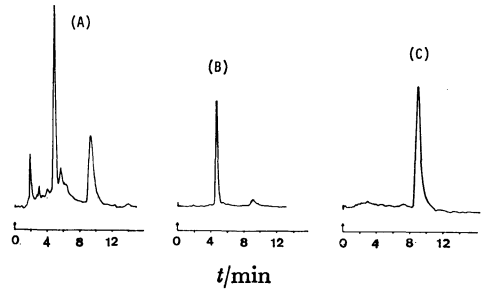

Fig. 5. Elution profiles at 280 nm of a reaction mixture of Ac–Trp–Trp–OMe in TFA and the isolated product by HPLC on a μ-porasil column (0.39 cm × 30 cm) in a solvent mixture of CH_3CN and $CHCl_3$ (2 : 1). Flow rate: 1 ml/min. (A) reaction mixture, (B) starting material (Ac–Trp–Trp–OMe), and (C) compound **3**.

column with a mixture of CH_3CN and $CHCl_3$ (v/v, 2/1). The first compound eluted was the starting compound, and the last compound eluted was identified as the compound previously isolated (denoted as compound **1A**). A compound (denoted as **1B**) eluted between the starting compound and compound **1A** was isolated; the purity of **1B** had been examined by a high-performance liquid chromatography (HPLC) using the same solvent

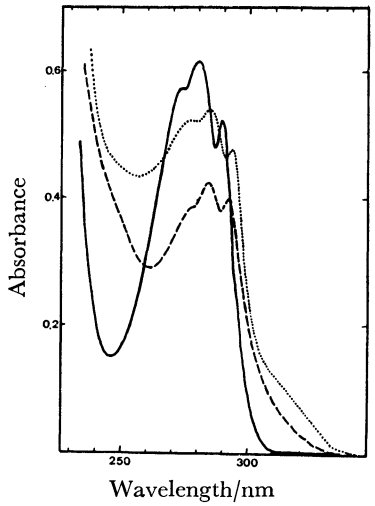

Fig. 6. UV absorption spectra of Ac–Trp–OMe (——), compounds **1A** and **1B** isolated from a solution of Ac–Trp–OMe in TFA (——), and compound **3** isolated from a solution of Ac–Trp–Trp–OMe in TFA (……) in EtOH.

system as for the preparative chromatography on silica gel to be found homogeneous on HPLC (Fig. 3).

After storage of solutions of tryptophan-peptides (Ac–Gly–Trp–OMe and Ac–Trp–Trp–OMe) in TFA under the same conditions as for Ac–Trp–OMe, TLC indicated presence of new compounds forming yellow spots when stained with the Ehrlich reagent, in addition to the starting compound, as illustrated in Fig. 2. The products from Ac–Gly–Trp–OMe were isolated by chromatography on a silica-gel column using a mixture of CH_3CN, $CHCl_3$, and MeOH (60/30/5) as eluting solvent. Two compounds (denoted as **2A** and **2B**) were eluted separately after the starting compound, and their purities were examined by HPLC (Fig. 4). From a solution of Ac–Trp–Trp–OMe in TFA, we could isolate only one product which was eluted more slowly than the starting peptide from a silica-gel column with a mixture of CH_3CN and $CHCl_3$ (v/v, 2/1) as solvent. Profiles of the reaction mixture and the isolated compound (denoted as compound **3**) on HPLC are shown in Fig. 5.

Structural Determination of Side-products. An elemental analysis showed that compound **1B** has the same composition as the starting compound and also as compound **1A** previously isolated. The mass spectrum of this compound showed a molecular ion at m/z=520, which is twice the m/z value of the starting compound but the same as that of compound **1A**. The UV absorption spectrum also was the same as that of compound **1A**, as shown in Fig. 6. The ^{1}H-NMR spectra of compound **1B** in DMSO-d_6 with and without D_2O suggest the presence of both one indole ring and one indoline ring in the molecule (Fig. 7), as in compound **1A**,[7] although the chemical shifts are slightly different from those of compound **1A**. The chemical shifts were assigned by the decoupling technique described in Ref. 7. These results suggest that compounds **1A** and **1B** are steric isomers associated with the asymmetric α- and β-carbons on the newly formed indoline rings

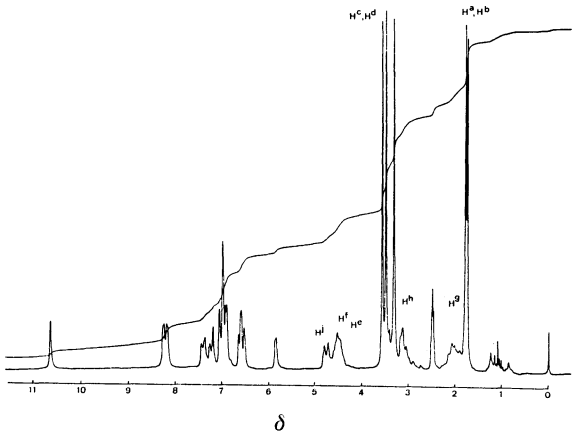

Fig. 7. ^{1}H-NMR spectrum of compound **1B** in DMSO-d_6 at 100 MHz.

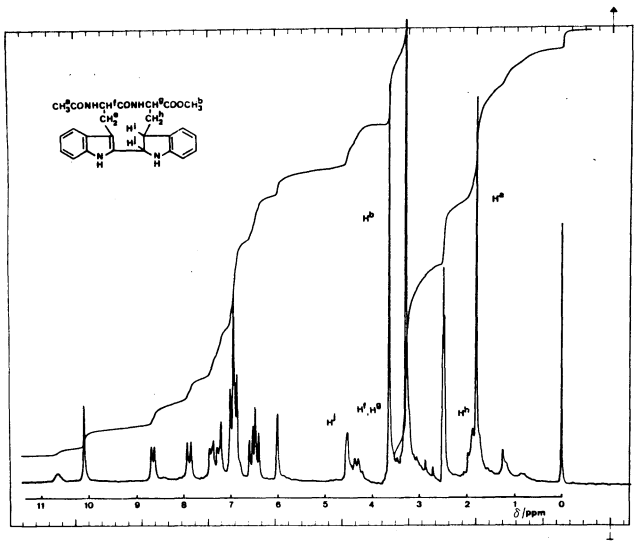

Fig. 8. ^{1}H-NMR spectrum of compound **3** in DMSO-d_6 at 100 MHz.

TABLE 1. COUPLING CONSTANTS (Hz) BETWEEN α-CH AND β-CH IN INDOLINE RINGS

Starting compound	Product	In DMSO-d_6 (+D_2O)	In DMSO-d_6 (+DCl)
Ac–Trp–OMe	**1A**	9.34	8.97
	1B	8.14	8.42
Ac–Gly–Trp–OMe	**2A**	9.33	0.08
	2B	8.79	8.80
Ac–Trp–Trp–OMe	**3**	3.28	3.03
Indoline[8]		J_{trans}=8.43	J_{trans}=6.63
		J_{cis}=8.43	J_{cis}=8.99

because the coupling constant between α-CH (i) and β-CH (j) of the indoline ring in compound **1B** is different from that of compound **1A**, as will be described later.

The two compounds **2A** and **2B** isolated from a solution of Ac–Gly–Trp–OMe in TFA gave analytical values similar to those of the starting peptide, but the molecular ions in their field-desorption mass spectra were at m/z=634, which is twice the value of that of the starting peptide. Their ^{1}H-NMR and UV absorption spectra are similar to those of compounds **1A** and **1B**, respectively, isolated from Ac–Trp–OMe. These results indicate that the compounds isolated from a solution of Ac–Gly–Trp–OMe in TFA have dimerized structures similar to those of the dimerized compounds from Ac–Trp–OMe, and further that they are steric isomers to each other because the coupling constants between α-CH and β-CH on the newly formedinidoline ring are different from each other, similarly to the above-mentioned case with compounds **1A** and **1B**.

The compound isolated from the solution of Ac–Trp–Trp–OMe in TFA was subjected to a field-desorption mass spectrometric analysis and found to have a molecular weight corresponding to m/z=446, the same as that of the starting peptide; an elemental analysis on this compound gave analytical results similar to those on the starting peptide. However, its UV absorption spectrum is different from that of the starting peptide, but similar to those of compounds **1A** and **1B**, as illustrated in Fig. 6. Furthermore, only one NH proton in the indole ring and one NH proton of the indoline ring are observed on the ^{1}H-NMR spectrum, as shown in Fig. 8. These results suggest that in this compound the two indole rings are joined together not intermolecularly but intramolecularly, as shown in Fig. 8.

It is known that protonation of the indoline NH will cause the coupling constant between the α-CH and β-CH of indoline ring increase in the case of *cis*-conformation and to decrease in the case of *trans*-conformation.[8] The coupling constant between α-CH (i) and β-CH (j) in compound **1A** decreased by addition of DCl in DMSO-d_6, while that of compound **1B** increased, as shown in Table 1; such phenomena were observed also in compounds **2A** and **2B**. Also, an addition of DCl to a solution of compound **3** in DMSO-d_6 decreased the coupling constant between α-CH and β-CH of the indoline ring. These facts suggest that compounds **1A**, **2A**, and **3** obtained from Ac–Trp–OMe, Ac–Gly–Trp–OMe, and Ac–Trp–Trp–OMe, respectively, have the *trans*-conformation, while compounds **1B** and **2B** isolated from Ac–Trp–OMe and Ac–Gly–Trp–OMe, respectively, have the *cis*-conformation. These results are summarized in Table 1.

Change in Quantity of Side-products with Time. An HPLC elution profile of a reaction product from Ac–Trp–OMe in TFA is shown in Fig. 3. It is clear that compounds **1A** and **1B** are completely separated not only from the starting compound but also from each other, so that they can be used as a standard for their quantitative determination for a mixture on HPLC. When Ac–Trp–OMe was stored in TFA, besides compounds **1A** and **1B** some fluorescence material, which would not move on a thin layer chromatogram, was formed in too small an amount. Therefore, the amount of the products was calculated by taking the sum of the amounts of the unreacted compound, compounds **1A** and **1B** as 100%. When Ac–Trp–OMe was dissolved in TFA, immediately concentrated under reduced pressure, extracted with ethyl acetate, applied to a column of HPLC, the unreacted compound amounted to 91.4% of the starting reactant, and the percentages of compounds **1A** and **1B** were 2% and 6.6%, respectively, as measured by the method described above.

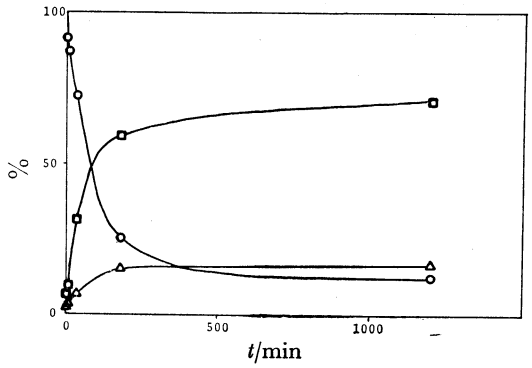

Fig. 9. Relative rate of decrease of Ac–Trp–OMe (—○—) and yields of compounds **1A** (—□—) and **1B** (—△—) in TFA. Values are averages for triplicate measurements.

As illustrated in Fig. 9, a 3-h storage in TFA at 20 °C caused the amount of the reactant to decrease rapidly to less than 25% of the starting reactant. On the other hand, the formation of compound **1A** surprisingly proceeded to more than 60%, but this compound could not be recovered preparatively in a similar yield; the formation of compound **1B** amounted to about 15%. These results suggest either that **1A** will be formed sterically with more advantage or that it is sterically more stable than **1B**. The isolated compounds **1A** and **1B** were stable at room temperature when stored in TFA. Therefore, it seems likely that the dimerized compounds are formed by an irreversible reaction from the starting monomer.

Experimental

All melting points were measured by the capillary method and are given as uncorrected values. TLC was performed on silica gel 60 (Merck Art. 11845) with a concentration zone using the following solvent systems (volume ratios): $CHCl_3$–acetone (2 : 1), $CHCl_3$–CH_3CN (4 : 1), and $CHCl_3$–MeOH–AcOH (95 : 5 : 5). HPLC was performed on a Toyosoda high speed liquid chromatograph HLC-803 and/or a Shimadzu HPLC LC-3A equipped with a data processor, Chromatopac C-R1A. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter. UV-absorption spectra were measured by using a Hitachi spectrometer type-124, equipped with a recording attachment. ^{1}H-NMR spectra were recorded on a JEOL PFT-100 pulse Fourier transform NMR spectrometer, locked on deuterium and equipped with an FT-1A pulse control system. Chemical shifts were measured relative to an internal reference, tetramethylsilane. Mass spectra were recorded with a second-order double focusing mass spectrometer[9] with a mono field-desorption ion source, equipped with a data processor (JEOL JMA-2000 mass data analysis system).

Ac–Trp–OMe. Ac–Trp–OH (17.4 g) was dissolved in MeOH (300 ml) and mixed with an ethereal solution of diazomethane in an ice–water bath under stirring. The solution was concentrated to dryness under reduced pressure. The residue was recrystallized from ethanol and hexane: wt 14.6 g (79.0%); mp 153—154 °C (lit,[10] 151 °C); $[\alpha]_D^{25}$ +13.2° (*c* 1.0, MeOH) (lit,[10] +13.2°); MS, *m/z*, 260 (M^+); ^{1}H-NMR [DMSO-d_6], CH_3CO δ=1.78 (3H, s), β-CH_2 ≈3.14 (2H, m), OCH_3 3.57 (3H, s), α-CH ≈4.59 (1H, t-d), aromatic CH ≈7.55 (5H, m), amide NH ≈8.32 (1H, d), and indole NH 10.82 (1H, s).

Found: C, 64.87; H, 6.16; N, 10.90%. Calcd for $C_{14}H_{16}O_3N_2$: C, 64.60; H, 6.20; N, 10.76%.

Ac–Gly–Trp–OMe. Z–Gly–Trp–OMe (3.39 g) was dissolved in MeOH (100 ml) and hydrogenated over a palladium–charcoal catalyst under atmospheric pressure. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to a syrupy residue, which was dissolved in DMF (50 ml). The solution was stirred with Ac–ONp (2.8 g) at room temperature for 1 d, and then concentrated to an oil under reduced pressure. The oil was purified on a column (3 cm × 60 cm) of silica gel using a mixture of ethyl acetate and benzene (v/v, 1/1) as elution solvent. Fractions containing the compound were collected and concentrated to a syrup under reduced pressure. The syrupy material was solidified in ethyl acetate and hexane: wt 0.69 g (27%); mp 178 °C; $[\alpha]_D^{24.5}$ +14.0° (*c* 1.0, MeOH); MS, *m/z*, 317 (M^+); ^{1}H-NMR [DMSO-d_6], CH_3CO δ=1.82 (3H, s), β-CH_2 (Trp) ≈3.13 (2H, m), OCH_3 3.55 (3H, s), α-CH_2(Gly) ≈3.70 (2H, d), α-CH(Trp) ≈4.53 (1H, m), aromatic CH ≈7.49 (5H, m), amide NH(Gly) ≈7.99 (1H, t), amide NH(Trp) ≈8.21 (1H, d), and indole NH 10.82 (1H, s).

Found: C, 60.67; H, 5.97; N, 13.37%. Calcd for $C_{16}H_{19}O_4N_3$: C, 60.55; H, 6.04; N, 13.24%.

Ac–Trp–Trp–OMe. Z–Trp–Trp–OMe[11] (4.11 g) was dissolved in MeOH (80 ml) and hydrogenated over a palladium–charcoal catalyst under reduced pressure. The catalyst was filtered off and the filtrate was concentrated to a syrup under reduced pressure. The syrup was dissolved with Ac–ONp (2.8 g) in DMF (50 ml), stirred for a day at room temperature and concentrated to an oil under reduced pressure. The oil was purified on a column (3 cm × 60 cm) of silica gel using a mixture of benzene and ethyl acetate (v/v, 1/1) as eluting solvent. Fractions containing the compound were pooled and concentrated under reduced pressure to a syrupy material, which was triturated in ethyl acetate and hexane: wt 1.21 g (35.5%); mp 78 °C; MS, *m/z*, 466 (M^+); ^{1}H-NMR [DMSO-d_6], CH_3CO δ=1.74 (3H, s), β-CH_2 ≈3.14 (2H × 2, m), OCH_3 3.54 (3H, s), α-CH ≈4.55 (1H × 2, m), aromatic CH ≈7.60 (5H × 2, m), amide NH ≈7.98 (1H, d) and ≈8.36 (1H, d), and indole NH 10.74 (1H, s) and 10.83 (1H, s).

Found: C. 68.01; H, 5.95; N, 12.31%. Calcd for $C_{25}H_{26}O_4N_4$: C, 67.25; H, 5.87; N, 12.55%.

*Compounds **1A** and **1B** from Ac–Trp–OMe.* Ac–Trp–OMe (1.00 g) was dissolved in TFA (10 ml). The solution was kept for 3 h at room temperature in a brown glass vessel to exclude light and then concentrated to dryness under reduced pressure. The residue was dissolved in ethyl acetate, washed with 5% aq $NaHCO_3$ and water and then dried over anhydrous Na_2SO_4. The dried solution was concentrated to a pale yellow solid under reduced pressure. **1A** was crystallized from EtOH and ether and collected to wt *ca.* 280 mg. The filtrate was concentrated to dryness under reduced pressure. The residue was subjected to chromatography in a brown glass column (3 cm × 60 cm) of silica gel 60 suspended in $CHCl_3$, in such a way that the sample was dissolved in $CHCl_3$, charged on the column, washed with a mixture of CH_3CN and $CHCl_3$ (v/v, 1/4) and then eluted with CH_3CN and $CHCl_3$ (v/v, 2/1). Fractions containing compound **1B** were pooled and concentrated to dryness under reduced pressure. The residue was recrystallized from ethyl acetate and hexane: wt *ca.* 50 mg.

*Compound **1A**:* Mp 218 °C; MS, *m/z*, 520 (M^+); ^{1}H-NMR [DMSO-d_6], CH_3CO δ=1.55 (3H, s), and 1.79 (3H, s) β-CH_2

(g) ≈2.16 (2H, m), β-CH_2(h) ≈3.18 (2H, q), OCH_3 3.49 (3H, s) and 3.53 (3H, s), indoline 3-CH(i) concealed under OCH_3, α-CH(e) ≈4.32 (1H, m), α-CH(f) ≈4.60 (1H, m), indoline 2-CH(j) ≈4.73 (1H, d-d), indoline NH 5.97 (1H, d), aromatic CH ≈7.50 (8H, m), amide NH ≈8.23 (1H, d) and ≈8.39 (1H, d), and indole NH 10.88 (1H, s).

Found: C, 64.37; H, 6.15; N, 10.99%. Calcd for $C_{28}H_{32}O_6N_4$: C, 64.60; H, 6.20; N, 10.76%.

Compound ***1B***: Mp 215 °C; MS, *m/z*, 520 (M^+); ^{1}H-NMR (Fig. 7).

Found: C, 64.23; H, 6.23; N, 10.73%. Calcd for $C_{28}H_{32}O_6N_4$: C, 64.60; H, 6.20; N, 10.76%.

Compounds ***2A*** *and* ***2B*** *from Ac–Gly–Trp–OMe.* Ac–Gly–Trp–OMe (1.22 g) was dissolved in TFA (10 ml) and kept under the conditions used for Ac–Trp–OMe. The solution was concentrated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with 5% aq $NaHCO_3$ and water and then dried over anhydrous Na_2SO_4. The dried solution was concentrated to a solid under reduced pressure. The solid was dissolved in $CHCl_3$, applied to silica gel 60 in $CHCl_3$ in a brown glass column (3 cm × 60 cm) and washed with a mixture (50 ml) of $CHCl_3$ and CH_3CN (v/v, 2/1). Materials were eluted with a mixture of CH_3CN, $CHCl_3$, and MeOH (v/v/v, 60/30/5). The starting material was eluted first, followed by compounds **2B** and **2A**. Fractions containing compounds **2A** and **2B** were separately pooled and concentrated to dryness under reduced pressure. The residues were collected with ethyl acetate and hexane: wt **2A**, *ca.* 20 mg; **2B**, *ca.* 70 mg.

Compound ***2A***: Mp 152 °C; MS, *m/z*, 634 (M^+); ^{1}H-NMR [DMSO-d_6], CH_3CO δ=1.80 (3H×2, s), β-CH_2(Ind) ≈2.07 (2H, m), β-CH_2(Trp) ≈3.10 (2H, d), OCH_3 3.46 (3H, s) and 3.50 (3H, s), α-CH_2(Gly) ≈3.61 (2H, d) and ≈3.67 (2H, d), indoline 3-CH concealed under OCH_3, α-CH (Ind) ≈4.27 (1H, m), α-CH(Trp) ≈4.60 (1H, m), indoline 2-CH ≈4.77 (1H, d-d), indoline NH 5.91 (1H, s (broad)), aromatic CH ≈7.45 (8H, m), amide NH(Gly) ≈7.76 (1H, t) and ≈7.92 (1H, t), amide NH (Trp and Ind) 8.24 (1H, d) and 8.32 (1H, d), and indole NH 10.80 (1H, s).

Found: C, 60.74; H, 6.27; N, 12.83%. Calcd for $C_{32}H_{38}O_8N_6$: C, 60.55; H, 6.04; N, 13.24%.

Compound ***2B***: Mp 151 °C; MS, *m/z*, 634 (M^+); ^{1}H-NMR [DMSO-d_6], CH_3CO δ=1.80 (3H×2, s), β-CH_2(Ind) ≈2.18 (2H, m), β-CH_2(Trp) concealed under OCH_3, OCH_3 3.27 (3H×2, s), indoline 3-CH concealed under 3.40—3.50, α-CH_2 (Gly) ≈3.66 (2H×2, d), α-CH(Trp and Ind) ≈4.53 (1H×2, m), indoline 2-CH ≈4.86 (1H, d-d), indoline NH 5.92 (1H, s (broad)), aromatic CH ≈7.38 (8H, m), amide NH(Gly) ≈7.85 (1H, t) and ≈7.90 (1H, t), amide NH (Trp and Ind) ≈8.24 (1H, d) and ≈8.31 (1H, d), and indole NH 10.70 (1H, s).

Found: C, 60.41; H, 6.21; N, 12.85%. Calcd for $C_{32}H_{38}O_8N_6$: C, 60.55; H, 6.04; N, 13.24%.

Compound ***3*** *from Ac–Trp–Trp–OMe.* Ac–Trp–Trp–OMe (800 mg) was dissolved in TFA (10 ml) and treated under conditions similar to those for Ac–Trp–OMe. The extracted reaction mixture was subjected to chromatography on silica gel in $CHCl_3$ in a brown glass column (3 cm × 60 cm). Materials were eluted successively with CH_3CN and $CHCl_3$ (v/v, 1/2) and CH_3CN and $CHCl_3$ (v/v, 2/1). Fractions containing compound **3** were collected and concentrated under reduced pressure to an oil, which was triturated in ethyl acetate and hexane: wt 46 mg; mp 118 °C; MS, *m/z*, 446 (M^+); ^{1}H-NMR (Fig. 8).

Found: C, 66.92; H, 6.01; N, 12.18%. Calcd for $C_{22}H_{26}O_4N_4$: C, 67.25; H, 5.87; N, 12.55%.

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