Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 975–981

BIOORGANIC & MEDICINAL CHEMISTRY

The Retro-Mannich Cleavage of δ_1,δ_1'-Tryptophan Dimers

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Received 19 November 1997; accepted 3 February 1998

Abstract—Under acidic conditions tryptophan sidechains crosslink to form δ_1, δ_1'-tryptophan dimers through a Mannich-type mechanism. Tryptophan dimers are readily cleaved at high temperatures under acidic conditions making it impossible to isolate tryptophan dimers under standard conditions of acidic protein hydrolysis. In a prescriptive sense this cleavage can be used to recover peptides that have undergone tryptophan crosslinking, although the yields drop with increasing peptide length due to competitive cleavage of the amide bonds. The best conditions for cleavage involve heating the dimeric peptides in dilute ethanolic HCl at 150 °C in the presence of ten equivalents of ethanedithiol.

Introduction

Tryptophan is notorious among the amino acids for its ability to cause problems during peptide synthesis.[1] The nucleophilicity of unprotected tryptophan leads to alkylation by the cationic species which are abundant under typical acidic peptide deprotection conditions. To overcome problems of alkylation, special deprotection cocktails have been formulated to include a variety of nucleophiles that can scavenge benzylic and *t*-butyl cations. These nucleophiles commonly include water, dimethylsulfide, anisole, thiocresol, and ethanedithiol.[1–3] Even in the absence of external electrophiles, tryptophan can undergo 'self alkylation' by acid-promoted Mannich dimerization.[4–6] We have found that tryptophan dimers, once formed, can be cleaved under acidic conditions at elevated temperatures. Amide bond hydrolysis competes with tryptophan dimer cleavage, so dilute acid gives the best yields.

The potential for crosslinking of tryptophan sidechains under acidic conditions was first proposed by Elliott in 1952[7]–long before the true structure of 3-alkylindole dimers was known.[8–10] Tryptophan dimerization was demonstrated over 20 years later by Shimonishi and coworkers using Ac-Trp-OMe, Ac-Gly-Trp-OMe, and Ac-Trp-Trp-OMe.[6] While this reaction produces diastereomeric tryptophan dimers, they may be convergently oxidized to afford ditryptophans, a new class of symmetrical homodimeric peptide crosslinks.[11]

Steam distillation of skatole dimer from a solution of dilute hydrochloric acid has been shown to produce skatole, presumably by the reverse of the Mannich-type dimerization shown in Scheme 1. We sought to develop thermodynamic conditions that could facilitate tryptophan dimer cleavage (without removal of the product by distillation).[9] Additionally we hoped to minimize amide bond hydrolysis and thus provide a means of salvaging intact peptides from product mixtures derived from uncontrolled crosslinking of tryptophan sidechains.

Results and Discussion

Preparation of substrates

Substrates were prepared by stirring tryptophan-containing peptides in neat trifluoroacetic acid at room temperature. This reaction leads to the formation of diastereomeric tryptophan dimers, which are typically isolable in about 25–75% yield after silica gel chromatography or reverse-phase HPLC. For example, stirring *N*-acetyltryptophan ethyl ester in anhydrous TFA under nitrogen for 12 h leads to formation of dimers **2a** and **2b** in 33% and 41% isolated yields, respectively [eq. (1)]. These dimers have the same relative indoline stereochemistry, and this stereochemistry is *trans*,[12] but we have not yet determined which structure corresponds to

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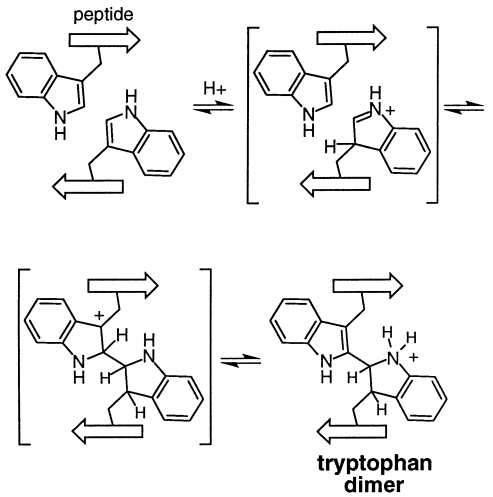
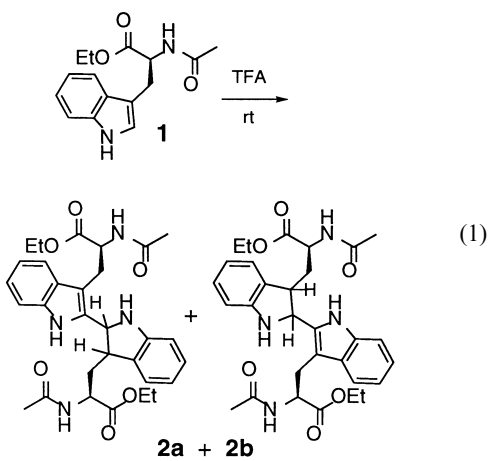
0968-0896/98/$19.00
PII: S0968-0896(98)00071-6



Scheme 1.

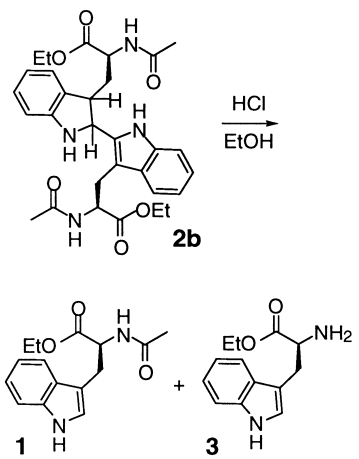
the low R_f isomer **2a** and which structure corresponds to the high R_f isomer **2b** (ethyl acetate/SiO_2). The low R_f isomer **2a** was used to optimize conditions for the retro-Mannich cleavage of tryptophan dimers without concomitant amide bond hydrolysis. Tryptophan dimers are not symmetrical so intramolecular crosslinking (as with Ac-Trp-Trp-OEt) is expected to lead to regioisomers as well as diastereomers. TFA cyclization of Ac-Trp-Trp-OMe and tosylation of the indoline nitrogen allowed the major regioisomer to be assigned to that which results from attack of Trp_1H+ on Trp_2.[6] For all of the tryptophan dimers in this work, convergent oxidation to the symmetrical ditryptophans using DDQ provided confirmation of the tryptophan dimer structure.[11]



(1)

Optimization of retro-Mannich cleavage conditions

In contrast to the initial report of Shimonishi, the dimerization of tryptophan derivative **1** in anhydrous trifluoroacetic acid was found to be reversible leading to a 1.0:4.2:1.7 ratio of **1**, **2a**, and **2b**, respectively. Tryptophan dimers are unstable to typical conditions[13,14] used for hydrolysis of proteins: 6 N aq HCl, 110 °C, degassed.[15] Under these conditions, tryptophan dimer **2a** is efficiently cleaved and hydrolyzed within 17 h to afford monomeric tryptophan in over 90% yield. Even at room temperature, about one third of the dimer is cleaved by 6 N aq HCl within 10 h. The important implication of these results is that tryptophan dimers can not be detected by acidic hydrolysis of proteins; their rapid cleavage makes them indistinguishable from unmodified tryptophan.



(2)

Harnessing the retro-Mannich cleavage of tryptophan dimers required that we develop conditions that would retain the integrity of the amide bonds. Ethanol was chosen as solvent for optimization of tryptophan dimer cleavage to facilitate solubility and ease of substrate handling [eq. (2), Table 1]. Also, on the assumption that monomer and dimer might be in equilibrium, reactions were run at low concentration (0.02 M) to favor monomer over dimer. Tryptophan dimers are stable in anhydrous 1 M ethanolic HCl at room temperature (entry 1), but at 78 °C they undergo retro-Mannich cleavage (entry 2). Unfortunately, under these strongly acidic conditions the mass balance was poor and monomer **3** was formed as the major product due to ethanolysis of the amide bond. Since acidic ethanolysis of amide bonds is first order in $[EtOH_2^+]$, we hoped to reduce this side reaction by reducing the concentration of acid.[16] The combined yield of monomers **1** and **3** was highest with 0.6 M HCl, but the mass balance was highest at 0.2 M HCl (entries 3 and 4). There was no reaction in dilute acid at 78 °C (entry 5). Since thiols are known to increase recovery of tryptophan during protein hydrolysis[17] we explored their effect on tryptophan dimer cleavage. It was found that addition of 1,2-ethanedithiol (EDT) led to enhanced mass balance (entries 6–8).

Table 1. Tryptophan dimer cleavage versus amide hydrolysis in ethanol (eq. (2))

Entry	Time (h)	T (°C)	[HCl] (M)	EDT[a]	Recovered SM (%)	3 (%)	1 (%)
1	24	rt	1.00	(–)	100	–	–
2	5	78	1.00	(–)	0	28	4
3	5	78	0.60	(–)	0	41	4
4	5	78	0.20	(–)	29	27	7
5	40	78	0.01	(–)	100	–	–
6	5	78	0.20	10 equiv.	37	10	13
7	5	78	0.20	100 equiv.	35	9	14
8	16	78	0.20	10 equiv.	–	10	(71[b])
9	1.5	150	0.20	(–)	–	17	44
10	1.5	150	0.20	10 equiv.	–	–	88 (99[b])
11	1.5	150	0.20[c]	(–)	–	16	64

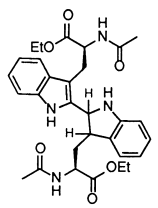
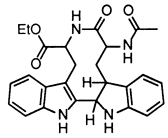
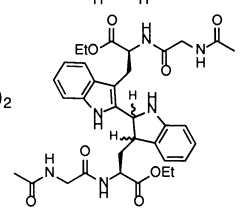
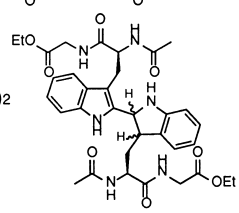
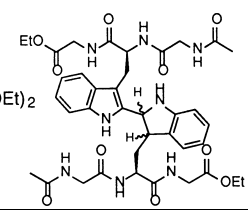
[a]EDT = ethanedithiol.
[b]HPLC yield (internal standard: *p*-nitrophenol).
[c]Degassed.

When the temperature was increased to 150 °C and 10 equiv. ethanedithiol was present, the retro-Mannich cleavage was complete in less than 2 h, and monomer **1** could be isolated before ethanolysis of the amide bond could occur (entries 9 and 10). Thiols are purported to improve yields of tryptophan recovery through an antioxidant effect.[17–20] Partial confirmation of this effect came through repeating the cleavage in a sealed tube with freeze-pump-thaw degassed solvent (entries 9 and 11). This led to an improved yield of **1**, but still did not match the effect of added thiol. In general, to cleave tryptophan dimers while minimizing amide bond hydrolysis one should heat at high temperatures for short times in dilute acid (ca. 0.2 M) with EDT as an additive.

Variation of peptide

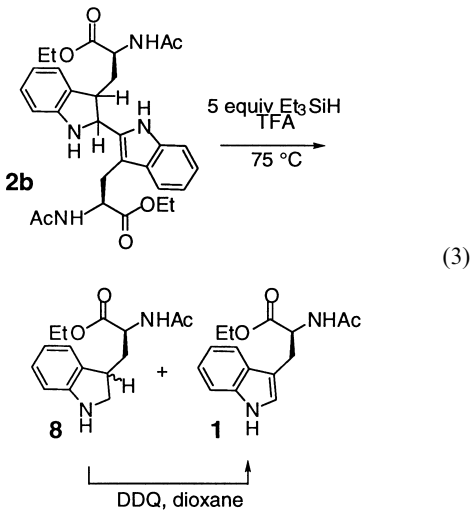
We next sought to investigate the generality of the optimized conditions from Table 1, entry 10: 0.02 M substrate, 0.2 M 1,2-ethanedithiol, 0.2 M ethanolic HCl, sealed tube, 150 °C, 1.5 h. The results are summarized in Table 2. Importantly, both diastereomers **2a** and **2b** are cleaved with equal efficacy using these conditions (Table 1, entry 10 and Table 2). Substrates with more amide bonds gave lower isolated yields, presumably due to competitive ethanolysis of the amide bonds. Interestingly, the cyclic tryptophan dimer **4**, which has two amide bonds, gave comparable yields to tryptophan dimers **5** and **6**, which have twice as many amide bonds. Yields are expected to decrease with increasing peptide length, but the peptide sequence is also likely to influence the cleavage yield just as it does in the formation of ditryptophans.[11] Since there were no other major products in these reaction mixtures, it is probable that non-selective ethanolysis of the amide bonds occurs both in the dimeric substrate and in the monomeric product.

Table 2. Cleavage of tryptophan dimers in 0.2 M HCl/EtOH at 150 °C

Substrate	Cleavage yield
(Ac-Trp-OEt)$_2$ **2a**	 **89%**
4	 **50%**
(Ac-Gly-Trp-OEt)$_2$ **5**	 **40%**
(Ac-Trp-Gly-OEt)$_2$ **6**	 **54%**
(Ac-Gly-Trp-Gly-OEt)$_2$ **7**	 **33%**

Reductive cleavage of tryptophan dimers

The forcing conditions required for the cleavage of tryptophan dimers led us to explore milder methods based on kinetic trapping of monomeric tryptophan. Since triethylsilane effectively reduces tryptophan in TFA we investigated the reductive removal of tryptophan from the monomer/dimer equilibrium.[2] Heating tryptophan dimer **2b** at 50 °C in TFA with 10 equiv. of triethylsilane established the monomer/dimer equilibrium, but reduction was sluggish at this temperature. By raising the temperature to 75 °C monomeric tryptophan was reduced slowly and selectively to dihydrotryptophan **8** over 12 h; no reduction of the tryptophan dimer was observed. The crude product was oxidized with DDQ in anhydrous dioxane to afford tryptophan monomer **1** in a 74% overall yield from dimer **2b**. While the two step reductive cleavage/oxidation sequence seems to work well, DDQ is unlikely to be compatible with peptides that contain oxidatively sensitive amino acids such as cysteine and methionine.



(3)

Conclusion

In conclusion, we have shown that the Mannich cross-linking of tryptophan sidechains, a side reaction in peptide synthesis, may be reversed by heating a dilute solution of peptide dimer in 0.2 M ethanolic HCl with 10 equiv. 1,2-ethanedithiol at 150 °C in a sealed tube. Ethanolysis also occurs under these conditions leading to a reduction in the isolated yield of monomeric peptide. Cleavage is also possible under reductive conditions leading to partial kinetic trapping of the tryptophan monomer. There have been no reports of tryptophan dimers in naturally occurring proteins or peptides. However, since tryptophan dimers are readily cleaved under typical conditions for protein hydrolysis (6 N HCl, 110 °C), and are not revealed by genetic sequence analysis, it is possible that tryptophan dimers play some role in the post-translational modification of proteins but have simply gone undetected.

Experimental

Peptide substrates were synthesized using published solution-phase BOC chemistry and DCC/HOBt couplings; BOC deprotections were carried out with ethanolic HCl.

General procedure I for the *N*α-acetylation of peptides

To a solution of the corresponding hydrochloride salt in pyridine (0.30 M) was added acetic anhydride (1.30 equiv.). The reaction was stirred at room temperature for 12 h and then concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with 1 N HCl. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by chromatography on silica gel.

***N*-Acetyl glycyltryptophan ethyl ester, Ac-Gly-Trp-OEt.** Glycyltryptophan ethyl ester, hydrochloride (5.88 g, 18.1 mmol) was acetylated according to general procedure **I** to afford Ac-Gly-Trp-OEt (5.84 g, 98%) as a white solid. Mp 148–150 °C ($CHCl_3$); R_f = 0.47 (10% MeOH/$CHCl_3$); IR (KBr) 3599, 3425, 3259, 3062, 2927, 1745, 1647 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.88 (s, 1H), 8.24 (d, J = 7.0 Hz, 1H), 8.05 (t, 5.3 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 2.0 Hz, 1H), 7.06 (t, J = 7.3 Hz, 1H), 6.98 (t, J = 7.0 Hz, 1H), 4.49 (q, J = 7.3 Hz, 1H), 3.99 (q, J = 7.0 Hz, 2H), 3.72 (dd, J = 16.8, 5.8 Hz, 1H), 3.67 (dd, J = 16.8, 5.8 Hz, 1H), 3.13 (dd, J = 14.0, 7.0 Hz, 1H), 3.05 (dd, J = 14.5, 8.0 Hz, 1H), 1.82 (s, 3H), 1.07 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 169.5, 169.0, 136.1, 127.1, 123.8, 121.0, 118.4, 117.9, 111.4, 109.2, 60.5, 53.1, 41.7, 27.1, 22.4, 13.9; LRMS, CI^+, m/e (%) 331 (MH^+, 125), 215 (52), 159 (15), 143 (20), 130 (100); HRMS (CI^+) calcd for $C_{17}H_{21}N_3O_4$, 331.1532; found 331.1533. Anal. calcd for $C_{17}H_{21}N_3O_4$: C, 61.60; H, 6.39; N, 12.69. Found: C, 61.65; H, 6.31; N, 12.65.

***N*-Acetyl tryptophanylglycine ethyl ester, Ac-Trp-Gly-OEt.** Tryptophanylglycine ethyl ester hydrochloride (4.99 g, 16.0 mmol) was acetylated according to general procedure **I** to afford Ac-Trp-Gly-OEt (4.96 g, 96%) as a white solid. mp 158–160 °C ($CHCl_3$); R_f = 0.54 (10% MeOH/$CHCl_3$); IR (KBr) 3388, 3292, 3070, 2927, 2850, 1730 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.46 (t, J = 5.8 Hz, 1H), 8.09 (d, J = 8.5 Hz, 1H),

7.60 (d, $J=8.0$ Hz, 1H), 7.32 (d, $J=8.5$ Hz, 1H), 7.14 (d, $J=1.5$ Hz, 1H), 7.06 (t, $J=7.5$ Hz, 1H), 6.98 (t, $J=7.0$ Hz, 1H), 4.57 (m, 1H), 4.09 (q, $J=7.0$ Hz, 2H), 3.81 (m, 2H), 3.17 (dd, $J=14.0$, 4.3 Hz, 1H), 2.89 (dd, $J=14.5$, 9.5 Hz, 1H), 1.77 (s, 3H), 1.19 (t, $J=6.8$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.9, 171.6, 170.7, 137.6, 129.0, 124.7, 123.7, 121.2, 120.2, 112.7, 112.0, 62.9, 55.2, 42.8, 29.6, 24.7, 15.5; LRMS, FAB$^+$, *m/e* (%) 332 (MH$^+$, 100), 273 (78), 230 (38), 202 (32), 129 (62); HRMS (FAB$^+$) calcd for $C_{17}H_{21}N_3O_4$ 331.1532; found 331.1533. Anal. calcd for $C_{17}H_{21}N_3O_4$: C, 61.60; H, 6.39; N, 12.69. Found C, 61.61; H, 6.45; N, 12.57.

***N*-Acetyl tryptophyltryptophan ethyl ester, Ac-Trp-Trp-OEt.** Tryptophanyltryptophan ethyl ester hydrochloride (7.72 g, 17.8 mmol) was acetylated according to general procedure **I** to afford Ac-Trp-Trp-OEt (7.66 g, 98%) as a white solid. mp 98–150 °C ($CHCl_3$); $R_f=0.48$ (10% MeOH/$CHCl_3$); IR (KBr) 3403, 3055, 2927, 1734, 1654 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.90 (s, 1H), 10.80 (s, 1H), 8.43 (d, $J=7.0$ Hz, 1H), 8.02 (d, $J=8.5$ Hz, 1H), 7.61 (d, $J=8.0$ Hz, 1H), 7.49 (d, $J=7.5$ Hz, 1H), 7.33 (t, $J=8.5$ Hz, 2H), 7.16 (d, $J=2.0$ Hz, 1H), 7.11 (d, $J=2.0$ Hz, 1H), 7.06 (q, $J=7.5$ Hz, 2H), 6.98 (q, $J=7.2$ Hz, 2H), 4.61 (m, 1H), 4.52 (q, $J=7.0$ Hz, 1H), 4.00 (q, $J=7.3$ Hz, 2H), 3.13 (m, 3H), 2.87 (m, 1H), 1.75 (s, 3H), 1.07 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.0, 171.7, 169.1, 136.1, 136.0, 127.3, 127.1, 123.7, 123.5, 121.0, 120.8, 118.5, 118.4, 118.2, 118.0, 111.4, 111.3, 110.2, 109.3, 60.4, 53.2, 53.0, 27.7, 26.9, 22.5, 13.9; LRMS, CI, *m/e* (%) 460 (MH$^+$, 100), 229 (32), 215 (70), 186 (12), 155 (13), 145 (18), 130 (100); HRMS (CI$^+$) calcd for $C_{26}H_{28}N_4O_4$, 460.2110; found 460.2111. Anal. calcd for $C_{26}H_{28}N_4O_4$: C, 67.79; H, 6.13; N, 12.17. Found C, 67.48; H, 6.06; N, 11.98.

General procedure II for the formation of tryptophan dimers. The corresponding tryptophan peptides were dissolved in trifluoroacetic acid (0.30 M) and stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo, taken up in ethyl acetate and washed with sat. aq. sodium bicarbonate. The aqueous phase was extracted with ethyl acetate and the combined organic layers were dried over $MgSO_4$. Solvent was removed in vacuo and the diastereomeric tryptophan dimers were separated from starting material by chromatography on silica gel or reverse phase HPLC (20–100% MeCN/H_2O over 30 min).

Formation of tryptophan dimers 2a and 2b. *N*-Acetyl tryptophan ethyl ester **1** (9.83 g, 35.8 mmol) was dimerized in trifluoroacetic acid according to general procedure **II**. The bulk of dimer **2a** was partially crystallized from the mixture of diastereomers (ethyl acetate) and the remaining mixture was separated by silica gel chromatography (33% acetone/chloroform) to afford dimer **2a** (3.25 g, 33%) and dimer **2b** (4.05 g, 41%). **2a**: mp 216–218 °C (EtOAc); $R_f=0.35$ (10% acetone/chloroform); IR (KBr) 3296, 3077, 2992, 1743, 1618, 1556 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H), 8.32 (d, $J=7.0$ Hz, 1H), 8.18 (d, $J=7.5$ Hz, 1H), 7.45 (d, $J=8.0$ Hz, 1H), 7.25 (d, $J=8.0$ Hz, 1H), 7.15 (d, $J=7.5$ Hz, 1H), 7.02 (ddd $J=16.5$, 7.1, 1.5 Hz, 2H), 6.94 (t, $J=7.3$ Hz, 1H), 6.67 (t, $J=7.5$ Hz, 1H), 6.61 (d, $J=8.0$ Hz, 1H), 6.00 (d, $J=3.0$ Hz, 1H), 5.97 (dd, $J=9.3$, 3.3 Hz, 1H), 4.53 (q, $J=6.5$ Hz, 1H), 4.26 (q, $J=6.5$ Hz, 1H), 3.96 (m, 4H), 3.46 (q, $J=6.5$ Hz, 1H), 3.17 (dd, $J=14.25$, 6.25 Hz, 1H), 3.08 (dd, $J=14.5$, 7.5 Hz, 1H), 2.09 (m, 1H), 1.98 (m, 1H), 1.80 (s, 3H), 1.60 (s, 3H), 1.05 (t, $J=3.75$ Hz, 3H), 1.03 (t, $J=3.5$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 171.9, 169.2, 169.2, 150.6, 136.9, 135.7, 130.5, 127.9, 127.7, 123.5, 120.9, 118.2, 118.0, 117.6, 111.1, 108.6, 107.1, 61.4, 60.5, 60.4, 53.5, 50.6, 45.5, 35.1, 26.3, 22.3, 21.9, 13.8, 13.8; LRMS, FAB$^+$, *m/e* (%) 549 (MH$^+$, 100), 520 (45), 257 (70), 169 (66), 130 (70); HRMS (FAB$^+$) calcd for $C_{30}H_{36}N_4O_6$, 548.2635; found 548.2652. Anal. calcd for $C_{30}H_{36}N_4O_6$: C, 65.66; H, 6.62; N, 10.22. Found: C, 65.40; H, 6.57; N, 10.16.

2b. Mp 103–105 °C ($CHCl_3$); $R_f=0.44$ (33% acetone/chloroform); IR (KBr) 3349, 3048, 2971, 1723, 1631, 1529 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.29 (m, 2H), 7.47 (d, $J=8.0$ Hz, 1H), 7.28 (d, $J=8.0$ Hz, 1H), 7.07 (d, $J=7.0$ Hz, 1H), 7.02 (m, 2H), 6.95 (t, $J=7.3$ Hz, 1H), 6.63 (t, $J=6.5$ Hz, 1H), 5.89 (s, 1H), 4.83 (d, $J=8.5$ Hz, 1H), 4.52 (m, 2H), 4.02 (m, 2H), 3.97 (m, 2H), 3.40 (q, $J=6.5$ Hz, 1H), 3.19 (dd, $J=14.1$, 4.8 Hz, 1H), 3.06 (dd, $J=14.1$, 9.2 Hz, 1H), 2.14 (m, 1H), 1.95 (m, 1H), 1.79 (d, $J=1.5$ Hz, 3H), 1.75 (d, $J=2.0$ Hz, 3H), 1.08 (dt, $J=14.0$, 7.0 Hz, 3H), 1.04 (dt, $J=14.5$, 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 172.2, 169.6, 169.5, 151.0, 137.5, 135.8, 129.7, 127.8, 127.7, 124.1, 120.9, 118.3, 117.9, 117.4, 111.3, 108.8, 107.3, 60.7, 60.5, 59.8, 53.6, 49.8, 45.3, 35.7, 26.3, 22.4, 22.2, 13.9, 13.9; LRMS, FAB$^+$, *m/e* (%) 549 (MH$^+$, 100), 404 (43), 259 (26), 154 (37), 130 (31); HRMS (FAB$^+$) calcd for $C_{30}H_{36}N_4O_6$, 548.2635; found 548.2627. Anal. calcd for $C_{30}H_{35}N_4O_6$: C, 65.66; H, 6.62; N, 10.22. Found: C, 65.43; H, 6.58; N, 10.17.

Formation of cyclic tryptophan dimer (4). Ac-Trp-Trp-OEt (0.20 g, 0.43 mmol) was cyclized with trifluoroacetic acid according to general procedure **II** to afford tryptophan dimer **4** as a mixture of diastereomers (0.060 g, 31%). $R_f=0.45$ (10% methanol/chloroform); LRMS, FAB$^+$, *m/e* (%) 461 (MH$^+$, 100), 442 (27), 271 (33), 259 (79), 245 (76), 130 (100); HRMS (FAB$^+$) calcd for $C_{26}H_{28}N_4O_4$, 460.2110; found 461.2196 (MH$^+$).

Formation of tryptophan dimer [Ac-Gly-Trp-OEt]$_2$ (5). Ac-Gly-Trp-OEt (0.50 g, 1.50 mmol) was dimerized with trifluoroacetic acid according to general procedure **II** to afford tryptophan dimer **5** as a mixture of diastereomers (white solid: 0.36 g, 72%). R_f = 0.10 (5% methanol/chloroform); LRMS, FAB$^+$, *m/e* (%) 663 (MH$^+$, 100), 596 (11), 549 (13), 523 (9), 509 (5); HRMS (FAB$^+$) calcd for $C_{34}H_{42}N_6O_8$, 662.3063; found 663.3142 (MH$^+$).

Formation of tryptophan dimer [Ac-Trp-Gly-OEt]$_2$ (6). Ac-Trp-Gly-OEt (0.20 g, 0.60 mmol) was dimerized with trifluoroacetic acid according to general procedure **II** to afford tryptophan dimer **6** as a mixture of diastereomers (white solid: 0.07 g, 33%). R_f = 0.11, 0.14 (5% methanol/chloroform); LRMS, FAB$^+$, *m/e* (%) 663 (MH+, 100); HRMS (FAB+) calcd for $C_{34}H_{42}N_6O_8$, 662.3063; found 663.3150 (MH$^+$).

Formation of tryptophan dimers [Ac-Gly-Trp-Gly-OEt]$_2$ (7). Ac-Gly-Trp-Gly-OEt (0.10 g, 0.26 mmol) was dimerized with trifluoroacetic acid according to general procedure **II** to afford tryptophan dimer **7** as a mixture of diastereomers (white solid: 0.08 g, 77%). R_f = 0.26, 0.32 (10% methanol/chloroform); LRMS, FAB$^+$, *m/e* (%) 777 (MH$^+$, 100), 577 (12), 549 (19), 535 (14), 518 (12), 509 (7); HRMS (FAB$^+$) calcd for $C_{38}H_{48}N_8O_{10}$, 776.3493; found 777.3566 (MH$^+$).

Reductive cleavage of tryptophan dimer 2b

To a solution of tryptophan dimer **2b** (0.50 g, 0.91 mmol) in trifluoroacetic acid (45 mL) was added triethylsilane (0.73 mL, 4.54 mmol) dropwise at 0 °C. The mixture was stirred at 0 °C for 0.5 h then at 70 °C for 12 h followed by concentration in vacuo. The crude product was oxidized without further purification.

The crude product mixture was dissolved in 1,4-dioxane (20 mL) and oxidized with DDQ (0.25 g, 1.10 mmol). After 4 h of stirring at room temperature, ethyl acetate was added (30 mL) and the mixture was washed with sat. aq. sodium bicarbonate (10×15 mL) until the washes were no longer colored. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Chromatography on silica gel (100% EtOAc) afforded **1** (0.18 g, 74% overall) as a white solid.

General procedure III for the nonreductive cleavage of tryptophan dimers

The corresponding tryptophan dimers (**2a**, **2b**, **4**, **5**, **6**, and **7**) were dissolved in ethanol (0.20 M) and treated with concentrated HCl (0.20 M) and ethanedithiol (10 equiv.). The reaction mixture was sealed and heated to 150 °C under nitrogen without degassing. After stirring at 150 °C for 1.5 h the mixture was allowed to cool to room temperature and concentrated in vacuo. The crude product was purified by chromatography on silica gel.

Cleavage of 2a. Tryptophan dimer **2a** (0.10 g, 0.18 mmol) was cleaved according to general procedure **III** to afford **1** (0.088 g, 88%) as a white solid.

Cleavage of 2b. Tryptophan dimer **2b** (0.10 g, 0.18 mmol) was cleaved according to general procedure **III** to afford **1** (0.089 g, 89%) as a white solid.

Cleavage of [Ac-Gly-Trp-OEt]$_2$ (5). Tryptophan dimer **5** (0.10 g, 0.15 mmol) was cleaved according to general procedure **III** to afford Ac-Gly-Trp-OEt (0.04 g, 40%).

Cleavage of [Ac-Trp-Gly-OEt]$_2$ (6). Tryptophan dimer **6** (0.10 g, 0.15 mmol) was cleaved according to general procedure **III** to afford Ac-Trp-Gly-OEt (0.054 g, 54%).

Cleavage of cyclic tryptophan dimer 4. Cyclic tryptophan dimer **4** (0.10 g, 0.22 mmol) in ethanol was cleaved with concentrated HCl and ethanedithiol according to general procedure **III** to afford Ac-Trp-Trp-OEt (0.05 g, 50%).

Cleavage of [Ac-Gly-Trp-Gly-OEt]$_2$ (7). Tryptophan dimer **7** (0.08 g, 0.10 mmol) in ethanol was cleaved with concentrated HCl and ethanedithiol to afford Ac-Gly-Trp-Gly-OEt (0.025 g, 33%).

Acknowledgements

This work is supported by the the Petroleum Research Fund, Camille and Henry Dreyfus Foundation, Glaxo-Wellcome, NSF (CHE-9623903) and the NIH (GM54523).

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