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Synthesis of Bombesin

We report the preparation of a peptide of the formula Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 according to the scheme, which involves the stepwise synthesis of the fragments II, X and XXII and their subsequent condensation. This synthetic peptide was found to be identical with natural bombesin[1,2].

Boc-His-NH-NH_2[3], treated in DMF at $-10°$ with HCl/THF and t.BuONO[4], yielded Boc-His-N_3 which was condensed with Leu-Met-NH_2[5] to give Boc-His-Leu-Met-NH_2 (I) (57% yield; mp 163–164°, AcOEt; $[\alpha]_D^{24}$ $-29.9°$; $E_{1\cdot2}$ = 0.6 Glu. Anal. Calcd. for $C_{22}H_{38}N_6O_5S$: C 53.0; H 7.7; N 16.9. Found: C 52.9; H 7.7; N 16.6) which by treatment with HCl/AcOH, followed by a passage on a IR-45 (OH^- cycle) column, afforded His-Leu-Met-NH_2 (II) (99% yield; mp 65–70°; $[\alpha]_D^{24}$ $-39.6°$; $E_{1\cdot2}$ = 1.25 Glu. Anal. Calcd. for $C_{17}H_{30}N_6O_3S$: C 51.2; H 7.6; N 21.1. Found: C 51.0; H 7.8; N 20.7).

Boc-Val-Gly-OEt[6] was treated with HCl/AcOH to give $^+H_2$-Val-Gly-OEt·Cl^- (armorphous; $E_{1\cdot2}$ = 1.15 Glu) which was condensed in THF with Boc-Ala[7], via the mixed anhydride (EtOCOCl) in the presence of MM, to give Boc-Ala-Val-Gly-OEt (III) (80% yield; mp 160°, AcOEt; $[\alpha]_D^{24}$ $-23.5°$. Anal. Calcd. for $C_{17}H_{31}N_3O_6$: C 54.7; H 8.4; N 11.3. Found: C 54.9; H 8.3; N 11.5) which by treatment with HCl/AcOH afforded $^+H_2$-Ala-Val-Gly-OEt·Cl^- (IV) (99% yield; mp 190°, MeOH-Et_2O; $[\alpha]_D^{24}$ $+5.5°$; $E_{1\cdot2}$ = 0.9 Glu. Anal. Calcd. for $C_{12}H_{23}N_3O_4$·HCl: C 46.5; H 7.8; N 13.6. Found: C 46.5; H 7.7; N 13.2). Z-Trp[8] in THF/DMF was condensed, via the mixed anhydride (i. BuOCOCl) in the presence of MM, with IV to give Z-Trp-Ala-Val-Gly-OEt (V) (81% yield; mp 213°, MeOH; $[\alpha]_D^{24}$ $-23.7°$. Anal. Calcd. for $C_{31}H_{39}N_5O_7$: C 62.7; H 6.6; N 11.8. Found: C 62.7; H 6.6; N 11.8) which by hydrogenation in the presence of Pd/C 10% in DMF yielded Trp-Ala-Val-Gly-OEt (VI) (94% yield; mp 205°, MeOH-Et_2O; $[\alpha]_D^{24}$ $-26.2°$; $E_{1\cdot2}$ = 0.6 Glu. Anal Calcd. for $C_{23}H_{33}N_5O_5$: C 60.1; H 7.2. Found: C 59.8; H 7.2) which was condensed in THF/DMF with Boc-Gln[7], via the mixed anhydride (i. BuOCOCl) in the presence of MM, to give Boc-Gln-Trp-Ala-Val-Gly-OEt (VII) (70% yield; mp 220°, MeOH; $[\alpha]_D^{24}$ $-11.6°$. Anal. Calcd. for $C_{33}H_{49}N_7O_9$: C 57.6; H 7.2; N 14.3. Found: C 57.5; H 7.4; N 14.3) which by treatment with TFA at 0° for 30 min, afforded $^+H_2$-Gln-Trp-Ala-Val-Gly-OEt·CF_3COO^- (VIII) (96% yield; mp 183°; $E_{1\cdot2}$ = 0.5 Glu)[9].

Boc-Asn-ONp[10] (1.4 equiv) was condensed, in DMF and in the presence of MM, with VIII to give Boc-Asn-Gln-Trp-Ala-Val-Gly-OEt (IX) (93% yield; mp 254° d, DMF-AcOEt; $[\alpha]_D^{24}$ $-36.4°$. Anal. Calcd. for $C_{37}H_{55}N_9O_{11}$: C 55.4; H 6.9; N 15.7. Found: C 55.4; H 7.0; N 15.5) which was hydrolyzed at room temperature with NaOH in EtOH–H_2O to yield Boc-Asn-Gln-Trp-Ala-Val-Gly (X) (95% yield; mp 212° d; DMF-AcOEt; $[\alpha]_D^{24}$ $-34°$. Anal. Calcd. for $C_{35}H_{51}N_9O_{11}\cdot0.5H_2O$: C 53.7; H 6.7; N 16.1. Found: C 53.6; H 6.8; N 15.8) which was treated in DMF with 1 equiv N-hydroxysuccinimide and 1 equiv DCCI (2 h at 0° and 12 h at room temp.). The solution, containing the activated ester, was filtered and 1 equiv of II was added to the filtrate. After 12 h the solution was worked up to give Boc-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (XI) (66% yield; mp 233° d, DMF-EtOH; $[\alpha]_D^{24}$ -25.7, c 1, HMPT; $E_{1\cdot2}$ = 0.35 Glu. Anal. Calcd. for $C_{52}H_{79}N_{15}O_{13}S$: C 54.1; H 6.9; N 18.2. Found: C 53.8; H 6.9; N 17.8) which by treatment with HCl/AcOH yielded $^+H_2$-Asn-Gln-Trp-Ala-Val-Gly-His($^+$H)-Leu-Met-NH_2·2 Cl^- (XII) (98% yield; mp 200° d, MeOH-i.PrOH; $[\alpha]_D^{24}$ $-24,4°$, c 1, HMPT; $E_{1\cdot2}$ = 0.65 Glu. Anal. Calcd. for $C_{47}H_{71}N_{15}O_{11}S\cdot2$ HCl: C 50.1; H 6.5. Found: C 50.3; H 6.8).

Boc-Gly[7] in THF was condensed with Z-NH-NH_2, via the mixed anhydride (EtOCOCl) in the presence of MM, to give Boc-Gly-NH-NH-Z (XIII) (96% yield; amorphous. Anal. Calcd. for $C_{15}H_{21}N_3O_5$: C 55.7; H 6.6; N 13.0. Found: C 55.7; H 6.6; N 13.0) which by treatment with HCl/AcOH gave $^+H_2$-Gly-NH-NH-Z·Cl^- (XIV) (90% yield; mp 185° d, EtOH; $E_{1\cdot2}$ = 1.15 Glu. Anal. Calcd. for $C_{10}H_{13}N_3O_3$·HCl: C 46.2; H 5.4; N 16.2. Found: C 46.0; H 5.5; N 16.0) which

[1] A. Anastasi, V. Erspamer and M. Bucci, Experientia, *27*, 166 (1971).

[2] All amino acids have the L-configuration. The following abbreviations are used throughout this paper: Pyr, pyroglutamic acid; Z, benzyloxycarbonyl; Boc, t.butyloxycarbonyl; TEA, triethylamine; MM, N-methylmorpholine; THF, tetrahydrofuran; DMF, dimethylformamide; HMPT, hexamethylfosfotriamide; DCCI, dicyclohexylcarbodiimide; ONp, *p*-nitrophenylester. Unless otherwise stated, the optical rotations were performed in DMF (c = 1), and Boc-deblocking was achieved in HCl/AcOH 1.3 *N* for 30 min at room temperature.

[3] E. Schröder and H. Gibian, Justus Liebigs Annln Chem. *656*, 190 (1962).

[4] J. Honzl and J. Rudinger, Colln. Czech. chem. Commun. *26*, 2333 (1961).

[5] L. Bernardi, G. Bosisio, R. de Castiglione, O. Goffredo and F. Chillemi, Gazz. chim. ital. *94*, 853 (1964).

[6] R. de Castiglione, Farmaco, Ed. Sci. *24*, 664 (1969).

[7] E. Schnabel, Justus Liebigs Annln Chem. *702*, 188 (1967).

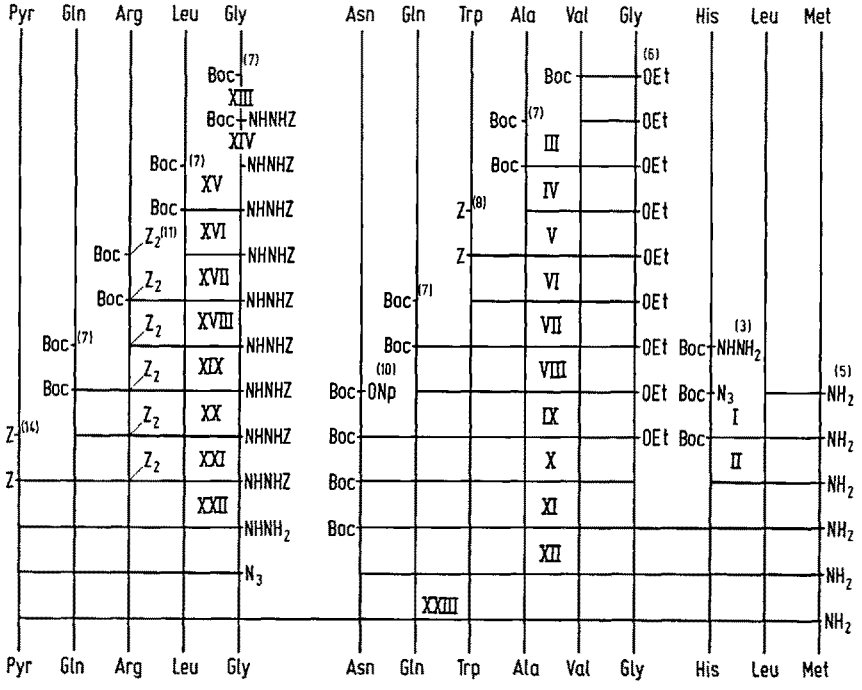
[8] E. L. Smith, J. biol. Chem. *175*, 39 (1948).

[9] A sample of VII, treated with 99% HCOOH (3 h) and then with HCl/AcOH, gave, by dilution with Et_2O, $^+H_2$-Gln-Trp-Ala-Val-Gly-OEt·Cl^-, mp 220°, MeOH; $[\alpha]_D^{24}$ —4.3. Anal. Calcd. for $C_{28}H_{41}N_7O_7$·HCl: C 53.9; H 6.8; N 15.7. Found: C 53.8; H 6.9; N 15.5.

[10] E. Schröder and E. Klieger, Justus Liebigs Annln Chem. *673*, 208 (1964).

was condensed with Boc-Leu[7], via the mixed anhydride (EtOCOCl) in the presence of MM, to yield Boc-Leu-Gly-NH-NH-Z (XV) (92% yield; mp 133°, Me_2CO-pet.ether; $[\alpha]_D^{24}$ −10°. Anal. Calcd. for $C_{21}H_{32}N_4O_6$: C 57.8; H 7.4; N 12.8. Found: C 57.9; H 7.4; N 13.0) which by treatment with HCl/AcOH afforded $^+H_2$-Leu-Gly-NH-NH-Z·Cl^- (XVI) (95% yield; mp 196°, AcOH/Et_2O; $[\alpha]_D^{22}$ +33.2°, c 0.6, AcOH 95%; $E_{1\cdot2}$ = 0.85 Glu. Anal. Calcd. for $C_{16}H_{24}N_4O_4$·HCl: C 51.5; H 6.8; N 15.0. Found: C 51.7; H 6.8; N 14.6). Boc-Arg(Z_2)[11] was condensed in THF-DMF with XVI, via the mixed anhydride (EtOCOCl) in the presence of MM, to afford Boc-Arg(Z_2)-Leu-Gly-NH-NH-Z (XVII) (89% yield, mp 146°, $CHCl_3$-AcOEt; $[\alpha]_D^{24}$ −12.4°. Anal. Calcd. for $C_{43}H_{56}N_9O_{11}$: C 60.0 H 6.6; N 13.0. Found: C 59.7; H 6.6; N 13.2) which was treated with TFA at 0° for 30 min[12]. By dilution with Et_2O, $^+H_2$-Arg(Z_2)-Leu-Gly-NH-NH-Z. CF_3COO^- (XVIII) was isolated (98% yield; mp 103° d; $[\alpha]_D^{24}$ +3.5; $E_{1\cdot2}$ = 0.85 Glu. Anal. Calcd. for $C_{38}H_{48}N_8O_9$. CF_3COOH; C 54.9; H 5.7. Found: C 54.5; H 5.8). Boc-Gln[7] was condensed in THF-DMF with XVIII, via the mixed anhydride in the presence of MM, to give Boc-Gln-Arg(Z_2)-Leu-Gly-NH-NH-Z (XIX) (68% yield; mp 185°, MeOH-AcOEt; $[\alpha]_D^{24}$ −11.3°. Anal. Calcd. for $C_{48}H_{64}N_{10}O_{13}$: C 58.3; H 6.5; N 14.2. Found: C 58.0; H 6.5; N 14.2) and treated with TFA at 0° for 20 min to yield, after dilution with Et_2O[13], $^+H_2$-Gln-Arg(Z_2)-Leu-Gly-NH-NH-Z. CF_3COO^- (XX) (98% yield; mp 108°; $[\alpha]_D^{24}$ −2.3°; $E_{1\cdot2}$ = 0.9 Glu) which was condensed in THF-DMF with Z-Pyr[14], via the mixed anhydride (EtOCOCl) in the presence of MM, to give Z-Pyr-Gln-Arg(Z_2)-Leu-Gly-NH-NH-Z (XXI) (77% yield; mp 194°, MeOH-i.PrOH; $[\alpha]_D^{24}$ −12.7°. Anal. Calcd. for $C_{56}H_{67}N_{11}O_{15}$: C 59.3; H 6.0; O 21.1. Found C 58.8; H 6.0; O 21.0).

Hydrogenolysis of XXI in DMF, in the presence of Pd/C 10% and 2 equiv HCl afforded Pyr-Gln-Arg($^+H_2$)-Leu-Gly-NH-NH^+_3. $2Cl^-$ (XXII) (95% yield; amorphous; $[\alpha]_D^{24}$ −8.5°; $E_{1\cdot2}$ = 0.95 Glu. Anal. Calcd. for $C_{24}H_{43}N_{11}O_7$. 2HCl: C 43.0; H 6.8; Cl 10.6. Found: C 43.2; H 6.9; Cl 10.1) which was treated in DMF with 1 equiv n·BuONO[4] and 4 equiv HCl/THF at −25°. The solution was then neutralized with 6 equiv TEA and added to a DMF-HMPT solution of XII (0.75 equiv) containing 1.5 equiv TEA. After 4 days at −10°, the reaction mixture was filtered, concentrated in vacuo and diluted with i·PrOH. The separated tetradecapeptide was dissolved in MeOH and precipitated with EtOAc and finally was crystallized from 99% EtOH to give Pyr-Gln-Arg($^+H_2$)-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His(^+H)-Leu-Met-NH_2·2 Cl^- (XXIII) (50% yield; mp 185° d; $[\alpha]_D^{24}$ −20.6°, c 0.65, DMF-HMPT 8:2; $E_{1\cdot2}$ = 0.67 Glu. Anal. Calcd. for $C_{71}H_{110}N_{24}O_{18}S$·2HCl: C 50.4; H 6.7; N 19.9; Cl 4.2. Found: C 50.4; H 6.7; N 19.6; Cl 4.3)



which was found to be homogeneous and showed the same electrophoretic and chromatographic properties, the same reactions to trypsin and chymotrypsin and the same degradative pattern and biological properties[15] of natural bombesin, thus confirming the formula deduced from degradative experiments.

Riassunto. Viene riportata la sintesi della piroglutamil-glutaminil-arginil-leucil-glicil-asparaginil-glutaminil-triptofanil-alanil-valil-glicil-istidil-leucil-metioninamide, un peptide identico per proprietà fisiche, chimiche e biologiche alla bombesina.

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[11] C. GROS, M. PRIVAT DE GARILHE, A. COSTOPANAGIOTIS and R. SCHWYZER, Helv. chim. Acta *44*, 2042 (1961). We are indebted to Dr. G. BOSISIO for the preparation of this intermediate.

[12] At room temperature one of the Z groups of Arg is split off!

[13] It is necessary to avoid any heating during the work up, because the Gln residue very easily cyclizes to a Pyr residue.

[14] H. GIBIAN and E. KLIEGER, Justus Liebigs Annln Chem. *640*, 145 (1961).

[15] We are indebted to Dr. A. ANASTASI and to Prof. V. ERSPAMER for these assays.