250. α-Melanotropin Labelled at its Tyrosine[2] Residue: Synthesis and Biological Activities of 3′-Iodotyrosine[2]-, 3′-^{125}Iodotyrosine[2]-, 3′,5′-Diiodotyrosine[2]-, and (3′,5′-$^{3}H_2$)tyrosine[2]-α-Melanotropin, and of Related Peptides[1])

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Summary

α-MSH was labelled at its tyrosine[2] residue with tritium and iodine. Several synthetic routes were investigated by preparing 13 precursor or model compounds and 4 different labelled products (*via* about 40 intermediates). Their melanotropic activity was determined with an *in vitro* frog skin assay and, for some of the compounds, with a tyrosinase assay. The tritiation was performed on [Tyr(I_2)[2]]α-MSH by catalytic halogen/tritium exchange, yielding α-MSH of high specific radioactivity (34 Ci/mmol) and full biological activity. Iodination was studied in detail using five different techniques. An equimolar chloramine T procedure proved to be the most convenient and reproducible method, resulting in monoiodinated α-MSH containing 99% of the label in position 2. The biological activity was 50% that of α-MSH; the specific radioactivity, determined in a competitive binding assay with a highly specific α-MSH antiserum and [Tyr(I)[2]]α-MSH as competitor, was 1530 Ci/mmol. The labelling techniques and the biological results are discussed.

Introduction. – Recent findings of a possibly decisive involvement of α-melanotropin (α-MSH) in neural functioning and foetal development are the reason for renewed intense investigation of this tridecapeptide [3]. From synthetic studies on structure/function relations it appeared that different target cell receptors may recognize different portions of the α-MSH molecule [4]. In order to study such hormone-receptor interactions in more detail, α-MSH derivatives are synthesized which

[1]) Parts of this report have appeared in a preliminary form [1]. Nomenclature and abbreviations [2]. Additional abbreviations are: ACTH = corticotropin, MSH = melanotropin; Tyr(I) = 3′-iodotyrosine, Tyr(I_2) = 3′,5′-diiodotyrosine, Hpp = 3-(4-hydroxyphenyl)-propionic acid; DCC = *N,N′*-dicyclohexylcarbodiimide, DCU = dicyclohexylurea, DMF = *N,N*-dimethylformamide, DMSO = dimethylsulfoxide, HOBT = 1-hydroxybenzotriazole, HOSu = N-hydroxysuccinimide, Ac = acetyl, BOC = *t*-butoxycarbonyl, O*t*Bu = *t*-butoxy, Z = benzoxycarbonyl, BZL = benzyl, MsOC = 2-(methylsulfonyl)-ethoxycarbonyl, NP = 4-nitrophenyl; TLC. = thin layer chromatography, TLE. = thin layer electrophoresis, RT. = (ambient) room temperature, *i.V.* = *in vacuo*. All chiral amino acids are in the L-configuration except where indicated (D-configuration with small letters in the 3-letter-symbolism).

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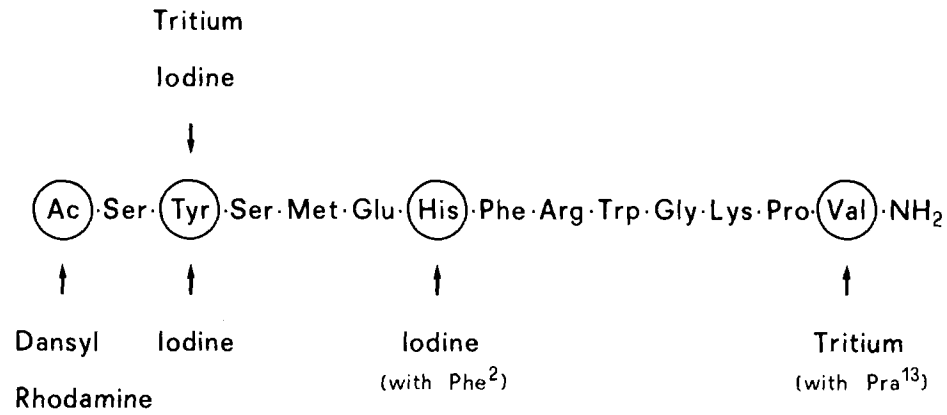


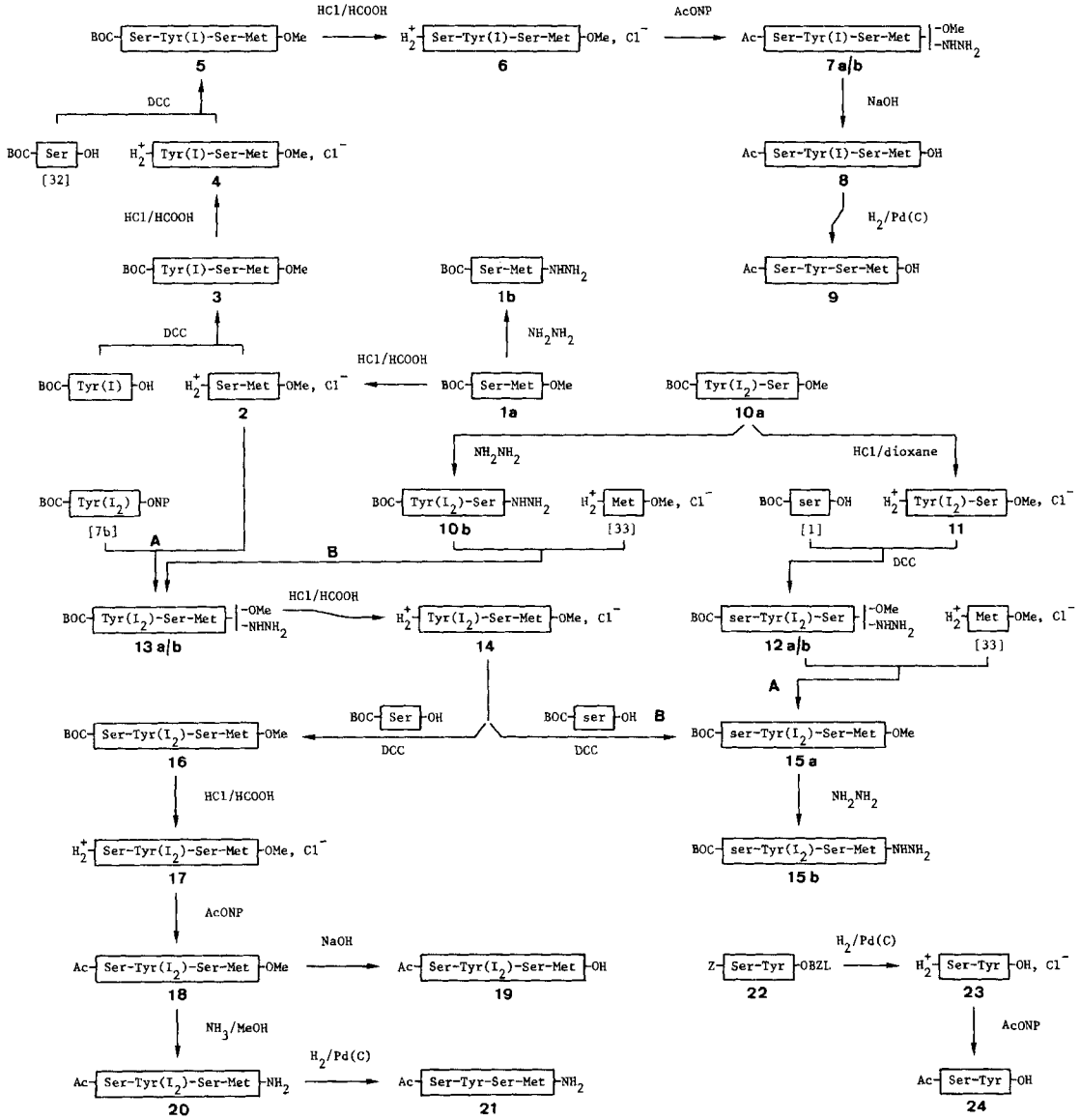
Fig. 1. *Structure of α-melanotropin and sites for specific labelling*

are labelled specifically at defined sites. This should enable us to gain insight into the degradation process of the hormone in plasma and tissue, as well as into the 'fate' of the hormone during and after the stimulation of cells. To perform such investigations at physiological concentrations (10^{-7} to 10^{-11} M), the derivatives must have both high specific radioactivity and unaltered biological activity. Therefore, tritiated and/or radio-iodinated α-MSH analogues have been synthesized carrying the label at position 2 or 13 (^{3}H), and 2 or 6 (^{125}I), respectively. In addition, derivatives with fluorescence marker groups instead of the *N*-terminal acetyl group are being prepared for microscopic studies *(Fig. 1)*.

In this, the first of three communications, we describe the preparation of α-MSH derivatives labelled at the tyrosine^2 residue. The syntheses of the intermediates and precursor molecules for the tritiated compounds were studied in great detail. Special attention was also paid to the problem of iodinating α-MSH because of reports claiming that iodination causes a considerable loss of activity in peptides like α-MSH, β-MSH, and ACTH [5]. Five different approaches for the iodination of α-MSH were compared, and a non-radioactive reference compound was synthesized in order to allow an exact determination of the specific radioactivity of the tracer. Altogether 58 intermediates and α-MSH derivatives are described.

General aspects of the syntheses. – The most convenient way of introducing tritium into the tyrosine residue of a peptide is by catalytic halogen-tritium exchange of a suitable precursor [6]. We employed this technique in the synthesis of α-MSH analogues carrying affinity and photoaffinity marker groups [7], where peptide derivatives containing 3′,5′-diiodotyrosine or 4′-amino-3′,5′-diiodophenylalanine were used for tritiation. *Nikolics et al.* [8] prepared tritiated α-MSH *via* a 3′,5′-dibromotyrosine^2 analogue, yielding a considerably lower specific radioactivity. In the present study, [Tyr(I_2)^2]α-MSH (**28**) and its D-serine^1 analogue (**32**) were synthesized as precursors for [Tyr($^2H_2$)^2]α-MSH (**29**) of high specific radioactivity. By inserting a residue with D-configuration into position 1, we expected to obtain a derivative of higher resistance to enzymic degradation. On simi-

Scheme 1. *Synthesis of the* N*-terminal peptide fragments*



lar synthetic routes, [Tyr(I)^2]α-MSH (**26**), [Tyr(I_2)^2]α-MSH(2–13) (**40**), and α-MSH(2–13) (**44**) were prepared for the analysis of [Tyr(^{125}I)^2]α-MSH (**48**) and of [Tyr(^{125}I)^2]α-MSH(2–13) (**51**), respectively. The partially protected α-MSH(3–13)-undecapeptide **38** was used as the *C*-terminal fragment for preparing iodinated α-MSH by the *Bolton-Hunter* approach (**51**, **52**) and by the fragment iodination procedure (**48** B). As methionine may easily be oxidized during iodination with

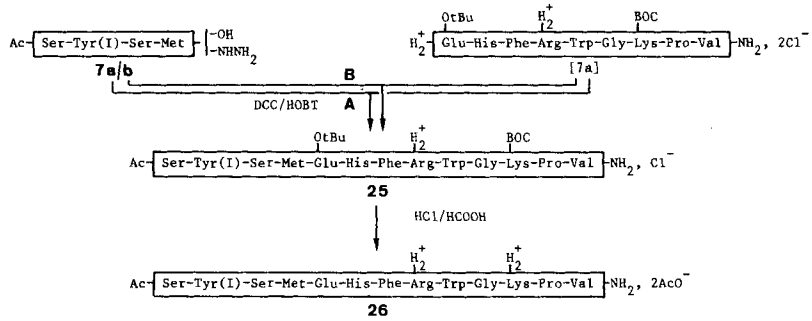
strong oxidants, two models containing norvaline instead of methionine[4] (**46**, **47**) were prepared to study the influence of such an alteration on the biological activity.

All compounds were synthesized by a classical approach in homogeneous solution, and the intermediates were, whenever possible or desirable, isolated, purified and characterized. Strategy and tactics for the syntheses were chosen so as to minimize the number of intermediates despite the rather large variety of modifications within the *N*-terminal tetrapeptide sequence. As a result, two derivatives of the non-modified *C*-terminal (5-13)-nonapeptide, containing BOC- or MsOC-groups to protect the lysine[11] side chain, were prepared and then condensed with the 8 different *N*-terminal di-, tri-, and tetrapeptide fragments. With the exception of glutamic acid[5], no other side chain function was protected ('minimal side chain protection' tactics). The following two main routes were used for the preparation of the tridecapeptides: (1) N^a-acetylation of the *N*-terminal tetrapeptide and condensation with the *C*-terminal nonapeptide containing BOC-protected lysine[11], followed by acidolysis of BOC/O*t*Bu, or (2) condensation of a BOC-protected *N*-terminal peptide fragment with the MsOC-containing *C*-terminal nonapeptide, followed by acidolytic deprotection, N^a-acetylation, and β-elimination. Similar approaches were outlined in earlier syntheses of α-MSH [7] [9] [10].

Special aspects of the syntheses. – For the preparation of the *N*-terminal peptide fragments, several synthetic routes were studied in order to test different methods for their applicability with peptides containing 3′-iodotyrosine or 3′,5′-diiodotyrosine *(Scheme 1)*. The two tetrapeptides **8** and **19** were prepared in a similar way by stepwise synthesis starting from the *C*-terminal protected dipeptide, BOC·Ser-Met·OMe (**1a**), using either activated esters or DCC for condensation, and usually, HCl/HCOOH for acidolytic deprotection steps. The overall yields were 23% for **8** and 41% for **19**. This difference probably resides in the fact that the two iodine atoms of the 3′,5′-diiodotyrosine residue protect its hydroxyl function almost completely, thus preventing *O*-acylation. As a result, such peptides need less purification than those with 3′-iodotyrosine. However, the solubility of certain derivatives is markedly impaired by the two iodine atoms; *e.g.*, **20** was only soluble in DMSO or aqueous ammonia. Furthermore, because of limited solubility of **18**, its hydrazide was only formed in trace amounts; therefore, **18** had to be saponified to its corresponding free acid **19**. In contrast, the analogous BOC-protected tetrapeptide derivative **15a** was easily transformed into the hydrazide **15b** with a yield of 80%. The synthesis of **15a** was performed both *via* the *N*-terminal tripeptide, BOC·Ser-Tyr(I_2)-Ser·OMe (**12a**), and *via* the *C*-terminal tripeptide, BOC·Tyr(I_2)-Ser-Met·OMe (**13a**). The former route corresponds to an approach used earlier for the synthesis of ACTH and α-MSH with non-iodinated tyrosine [11]; in the present case it resulted in an overall yield of only 17%, compared to 48% with the second route. The difference may reside in the less complicated purification steps of the latter.

The tridecapeptide derivative **25** was prepared by condensing the nonapeptide, H·Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(BOC)-Pro-Val·NH_2, 2 HCl [7a], with **7a** using the procedure of *König & Geiger* [12], or with **7b** *via* its azide as intermediate *(Scheme 2)*. Both methods gave identical products, the former with slightly better

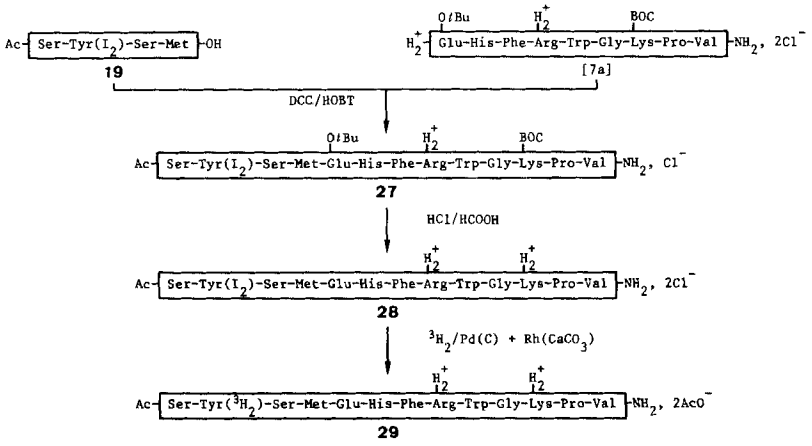
Scheme 2. *Synthesis of 3'-iodotyrosine²-α-melanotropin*



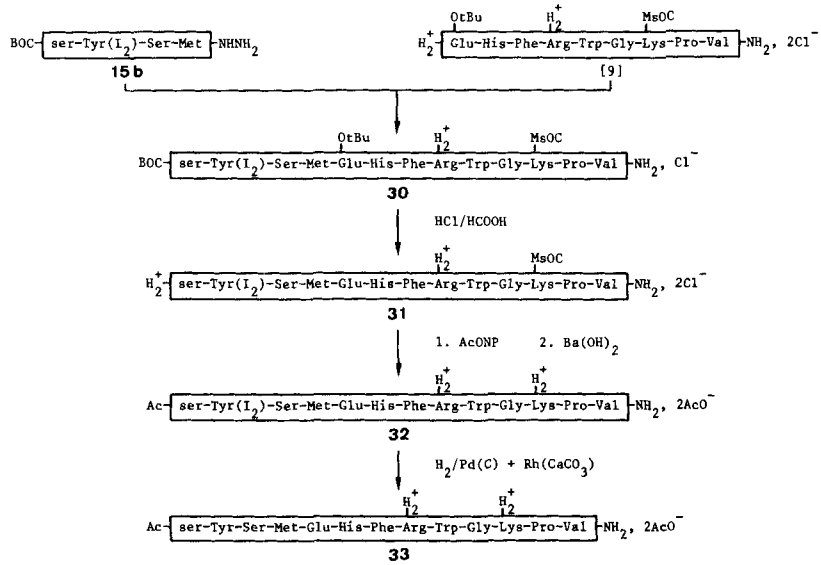
yield (81% *vs.* 68%). Acidolytic cleavage of the BOC/O*t*Bu groups was performed in 0.12N HCl in HCOOH containing 5% ethane-1,2-dithiol (or 10% mercaptoethanol), as used in our earlier syntheses. This procedure forms fewer side products than any other method (in particular, no butylation of tryptophan is observed [13]). When the concentration of HCl does not exceed 1.2 to 1.5 mol-equivalents per acid-labile group (plus 1 mol-equivalent per histidine), and when the reaction time is kept below 15 min, no formylation of the indole is observed.

The peptide **28** was synthesized in a similar way to **26** *(Scheme 3)*. Both peptides were purified by repetitive chromatography over *Sephadex* LH-20 in dilute acetic acid and were isolated in completely homogeneous form. The catalytic dehalogenation of **28** was performed with a mixture of 5% Pd/C and 5% $Rh/CaCO_3$ [14] in a new type of apparatus at a tritium pressure of 1030 mbar. These conditions resulted in a complete dehalogenation after 30 min (TLC.) despite the presence of the methionine residue. However, the incorporation of tritium was less than 100% because of a partial hydrogen/tritium exchange with the solvent during the experiment. (As the solubility of **28** was very poor in DMF, a mixture of DMF/H_2O 10:1 was

Scheme 3. *Synthesis of (3',5'-³H₂)tyrosine²-α-melanotropin*



Scheme 4. *Synthesis of* D-*serine*[1]*-α-melanotropin via its 3′,5′-diiodotyrosine*[2] *analogue*

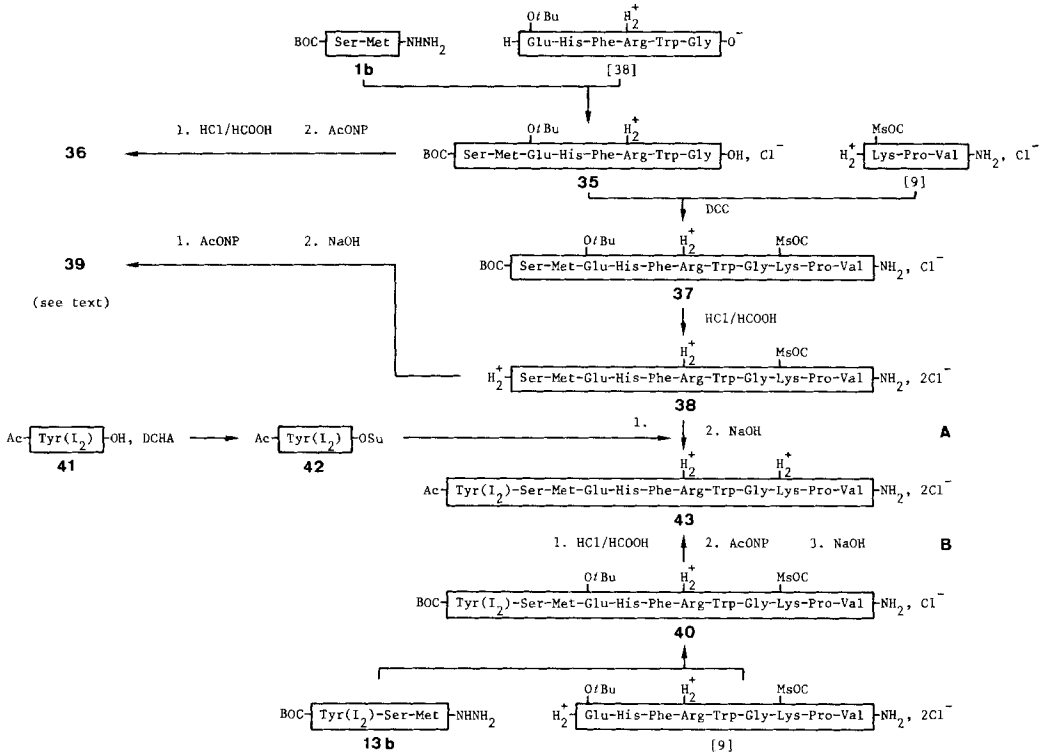


used for the tritiation). Chromatography of the tritiated product over *Sephadex* LH-20 (twice) eliminated the methionine[4]-*S*-oxide analogue (~ 10%) formed during the tritiation, and yielded homogeneous **29** with a specific activity of 34 Ci/mmol. The product was identical with α-MSH (**34**). In order to localize the radioactivity within the peptide, a sample of **29** was hydrolyzed with chymotrypsin, and the fragments formed were separated by TLC. and TLE. *(Fig. 3)*. Determination of the radioactivity of the different spots revealed that 92% of the tritium was incorporated into the *N*-terminal dipeptide (Tyr^2), 5% into the fragment (3–7) (mainly His^6; see [15]), 2% into Arg-Trp, and 1% into the fragment (10–13) *(Table 1)*. This clearly shows that only limited specificity can be attained upon tritiations of whole peptide sequences like α-MSH. To overcome this disadvantage, a short fragment may be tritiated with very good specificity and then be condensed with the rest of the molecule [16].

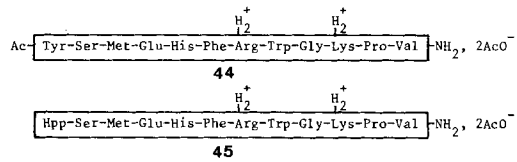
The synthesis of the D-serine[1] analogues of **28** and α-MSH, **32** and **33**, respectively, was performed according to *Scheme 4*, thereby partially following the procedure for the preparation of α-MSH [9]. The advantage of this route resides in the possibility of introducing an affinity label into the *N*-terminal end of the molecule at a late stage of the synthesis.

Iodination of α-MSH. – Radio-iodination of α-MSH, β-MSH, or ACTH with oxidants like chloramine T or lactoperoxidase/H_2O_2 may produce biologically poorly active or even inactive tracer molecules [5], mainly because of oxidation of methionine[4] to the corresponding methionine-*S*-oxide, which considerably impairs the biological activity [17]. Although we have described a monoiodinated α-MSH tracer of good quality using the lactoperoxidase method [1] [18], we tested several procedures in order to select the most convenient and reproducible method for

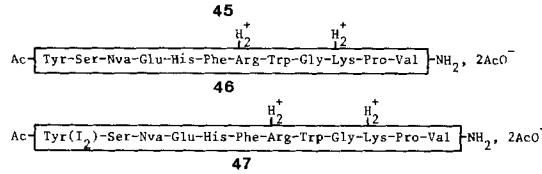
Scheme 5. *Synthesis of precursor and model compounds for the fragment iodination procedure*



In the same way, on route **A**:



In the same way, on route **B**:

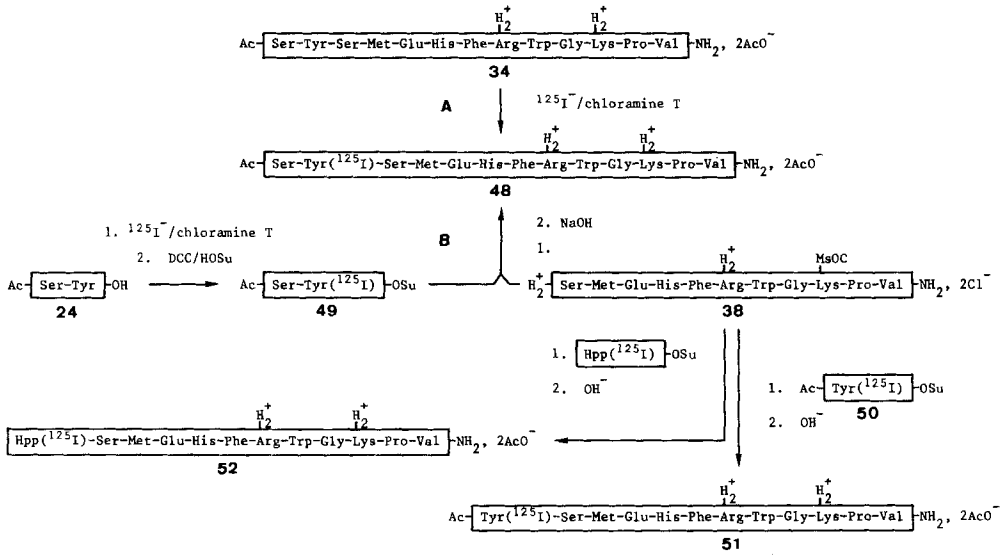


iodinating α-MSH specifically at position 2. The following procedures were examined: (1) fragment iodination, (2) *Bolton-Hunter* reagent method [19] and a modification thereof, (3) equimolar chloramine T method, (4) lactoperoxidase [20], and (5) electrolytical iodination [21].

From theoretical considerations, methods (1) and (2) are most attractive. They are both based on the idea of iodinating a small, non-oxidizable portion of the

molecule, isolating and purifying it and then condensing it with the partially protected remaining portion of the peptide molecule. Such an approach requires suitable intermediates, which were synthesized according to *Scheme 5*. α-MSH was divided into the *N*-terminal dipeptide Ac·Ser-Tyr·OH (**24**, *Scheme 1*), which has to be iodinated, and the (3–13)-undecapeptide **38**[2]) (with MsOC-protection for lysine[11]). The latter was prepared *via* the protected octapeptide **35**, condensed with the *C*-terminal tripeptide, H·Lys(MsOC)-Pro-Val·NH_2, HCl [9]. At this stage, the suitability of attaching iodinated **24**, *Bolton-Hunter* reagent [19], or iodinated N^{α}-acetyltyrosine[3]) to **38** was studied with non-radioactive Ac·Tyr(I_2)·OSu (**42**) as a model. The dodecapeptide **43** A resulting from condensation of **38** with **42** and β-elimination of MsOC was identical with **43** B obtained by a different route, *via* **40**. Similarly, **24**, Ac · Tyr · OSu [1], and Hpp were condensed with **38** *via* their HOSu esters, yielding, upon cleavage of MsOC, **34**, **44**, and **45**, respectively. This proves the suitability of the proposed pathway, at least when milligram amounts are used. Although iodination and isolation of the peptide fragment **24** in the microgram range was generally successful[4]), the problem of condensing such low quantities with good reproducibility could not be solved completely because of rather dilute reaction mixtures. Only about 40–50% of the iodination experi-

Scheme 6. *Iodination of α-melanotropin by the chloramine T and the fragment iodination procedure*



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2) The unprotected, N^{α}-acetylated analogues of **35** and **38**, the (3–10)-octapeptide **36** and the (3–13)-undecapeptide **39** have been partially described [22]. As their synthesis was not reported in [9], a short description is included here.

3) The reason for modifying the *Bolton-Hunter* reagent for iodinating **38** resides in the lack of the biologically important *N*-terminal acetylamide group in Hpp (see melanotropic activities of Hpp compounds in *Table 2*).

4) However, Ac·Tyr·OSu may easily be hydrolyzed during iodination if the isolation step is not performed as rapidly as possible.

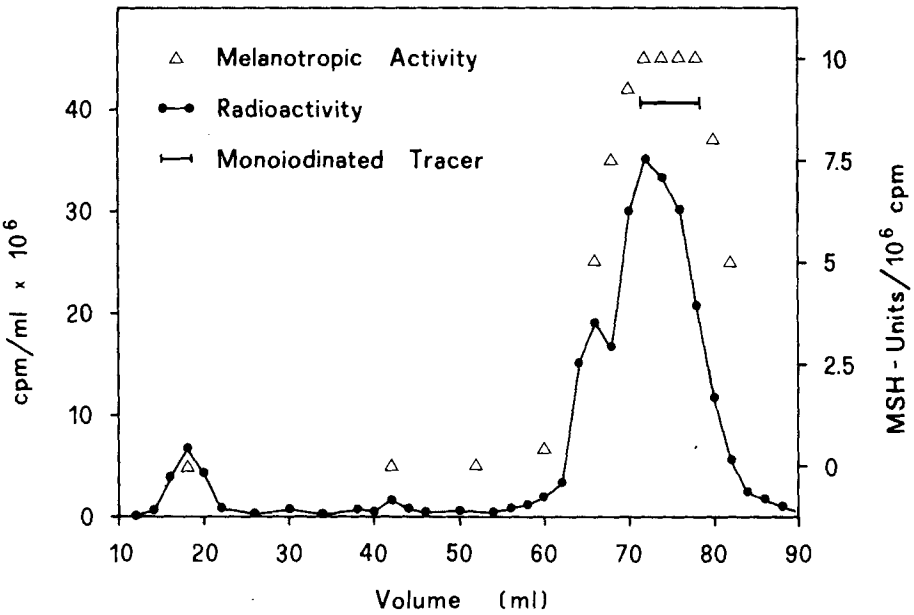

Fig. 2. *Gel filtration of iodinated α-melanotropin* (**48**) *on* Sephadex *LH-20 with an albumin-phosphate buffer* (●——●), *and melanotropic activity (frog skin) of various fractions* (△)

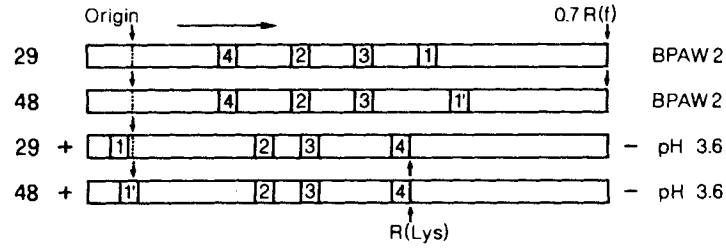

Fig. 3. *Separation of a chymotryptic hydrolysate of tritiated* (**29**) *and iodinated* (**48**) *α-melanotropin by TLC.* (BPEW 2) *and TLE.* (pH 3.6). The fragments were identified with synthetic Ac·Ser-Tyr·OH (1), H·Arg-Trp·OH (3), and H·Gly-Lys-Pro-Val·NH_2 (4).

ments of **48** B, **51**, or **52** *(Scheme 6)* resulted in a biologically active tracer of high specific radioactivity, even when commercial *Bolton-Hunter* reagent was used. Thus, the reproducibility of both procedures (1) and (2) is too low for routine application on α-MSH.

Best results for the preparation of monoiodinated, biologically active α-MSH were obtained with an equimolar chloramine T method. The original procedure of *Hunter* [23] was modified as follows: (1) $Na^{125}I$ and the hormone were present in equimolar amounts; (2) chloramine T was added in 3 equimolar portions at intervals of 30 s; (3) the reaction was stopped with an albumin buffer instead of $Na_2S_2O_5$. Purification of the tracer over *Sephadex* LH-20 *(Fig. 2)* yielded monoiodinated fractions exhibiting chemical and biological properties identical to **26**.

Table 1. *Determination of the radioactivity found in the chymotryptic fragments of tritiated* (**29**) *and iodinated* (**48**) *α-melanotropin*

Compound No.	Fragment No.	Structure of the fragment	Radioactivity	
			BPAW 2	pH 3.6
29	1	Ac·Ser-Tyr·OH	92%	92%
	2	H·Ser-Met-Glu-His-Phe·OH	5%	5%
	3	H·Arg-Trp·OH	2%	2%
	4	H·Gly-Lys-Pro-Val·NH_2	1%	1%
48	1′	Ac·Ser-Tyr(I)·OH	99.5%	100%
	2	H·Ser-Met-Glu-His-Phe·OH	0.5%	0
	3	H·Arg-Trp·OH	0	0
	4	H·Gly-Lys-Pro-Val·NH_2	0	0

This procedure proved to be highly reproducible, and the results were slightly superior to these obtained with lactoperoxidase [18].

For further characterization, **48** was hydrolyzed with chymotrypsin and the fragments were analyzed as described for **29**. The results are displayed in *Figure 3* and *Table 1*. It is shown that 99% of the iodine is localized in the tyrosine[2] residue.

The specific radioactivity of **48** was determined with a competitive binding experiment, using a highly specific α-MSH antiserum [18b] and **26** as competitor.

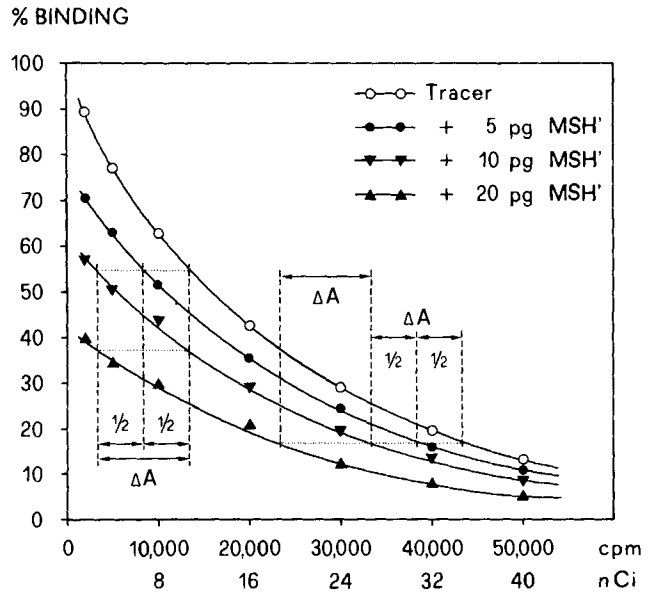

Fig. 4. *Determination of the specific radioactivity of monoiodinated α-melanotropin* (**48**) *with a competitive binding experiment.* Various amounts of **48** were incubated with a specific α-MSH antiserum, either alone or with 3 different concentrations of non-radioactive **26** (MSH') as competitor (see exper. part). The curves represent % binding of the tracer in relation to the total amount used. Each point is the mean of 4 different values. *ΔA* is the radioactivity displaced by 10 pg of **26** and corresponds to the specific radioactivity of 10 pg **48**.

The displacement of **48** (determined in nCi) by increasing amounts of **26** is directly proportional because both **48** and **26** exhibit exactly the same affinity to the antiserum. Thus, the radioactivity ΔA (in nCi) displaced by 10 pg of **26** corresponds to the same amount of **48** *(Fig. 4)*. With this, the specific radioactivity of **48** was calculated to 1530 Ci/mmol.

Biological activity. – *Table 2* shows the melanotropic activities in the frog skin assay of 14 synthetic compounds, as well as the tyrosinase-stimulating potency of 5 compounds, determined with *Cloudman* S-91 mouse melanoma cells in culture.

α-MSH (**34**), its D-serine[1] analogue **33**, and its tritiated analogue **29** display 100% activity in both assay systems. In the frog skin assay, monoiodination of tyrosine[2] causes a diminution of the melanotropic potency by a factor of 2 (**26**, **48**), whereas the introduction of a second iodine atom decreases the activity to 25% (**28**, **32**). The tyrosinase-stimulating potency is significantly more reduced, in particular upon diiodination of tyrosine[2]. This confirms our previous finding that a balanced hydrophilicity/hydrophobicity of the *N*-terminal tetrapeptide fragment is important for full biological activity of the hormone [4a]. (However, α-MSH-receptors appear to discriminate the tetrapeptide sequence to a lesser extent than ACTH-receptors; the steroidogenic activity of [Tyr(I_2)[2]]ACTH(1–24) is only 2.5% that of ACTH(1–24) [24]). In summary, insertion of only one iodine atom into position 2 of α-MSH results in a tracer which retains 50% (frog skin) or 35% (melanoma cells) of biological activity and is therefore suitable for biological studies.

Deletion of the *N*-terminal serine[1] causes a decrease of the melanotropic potency of about 50% (**44**; see also [22]). The same holds for iodinated α-MSH(2–13) derivatives **43**, **47**, **51**, **52**. Replacement of methionine[4] by norvaline (**46**) again

Table 2. *Melanotropic activities of α-melanotropin labelled at its tyrosine[2] residue.* Pigment-dispersing potencies, determined with the modified *in vitro* frog skin assay [22], are shown in Units/mmol. Tyrosinase-stimulating potencies, determined with *Cloudman* S-91 mous melanoma cells in culture, are expressed as relative molar potencies ($1/EC_{50} \cdot 100$; see [4b]).

Compound No.	Amino-acid sequence	Pigment-dispersion (Units/mmol)	Tyrosinase-stimulation ($1/EC_{50} \cdot 100$)
34	Ac·Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2 (α-MSH)	4×10^{10}	4×10^{10}
29	Ac·Ser-Tyr($^3H_2$)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	4×10^{10}	4×10^{10}
33	Ac·ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	4×10^{10}	4×10^{10}
26	Ac·Ser-Tyr(I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	2×10^{10}	1.4×10^{10}
48	Ac·Ser-Tyr(^{125}I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	2×10^{10}	–
28	Ac·Ser-Tyr(I_2)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	1×10^{10}	5×10^{9}
32	Ac·ser-Tyr(I_2)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	1×10^{10}	–
44	Ac·Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	2×10^{10}	–
51	Ac·Tyr(^{125}I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	1×10^{10}	–
43	Ac·Tyr(I_2)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	5×10^{9}	–
45	Hpp-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	4×10^{9}	–
52	Hpp(^{125}I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	1.5×10^{9}	–
46	Ac·Tyr-Ser-Nva-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	1.2×10^{10}	–
47	Ac·Tyr(I_2)-Ser-Nva-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2	4×10^{9}	–

proves that the biological activity is less susceptible to such a modification than to oxidation of methionine, as we have shown with similar compounds [17] [22]. In cases where hard conditions for iodinating α-MSH are to be used (iodination of histidine[6] [25]), analogues containing norvaline[4] or norleucine[4] may retain better biological activity than α-MSH itself. Therefore, such peptides are at present being prepared in our laboratory.

Discussion. – This report shows that monoiodinated α-MSH of high specific activity and good biological activity may be prepared using a modified chloramine T technique. In this method the label is inserted almost exclusively (99%) into position 2 of the molecule. Similar results were obtained with the fragment iodination procedure; however, this method (as well as the modified *Bolton-Hunter* approach) is too complicated and not reproducible enough for routine application. When the original chloramine T method [23] was used, the labelled α-MSH showes markedly impaired biological activity and affinity to the antiserum [1], compared to a tracer obtained with lactoperoxidase [18a]. The latter represents a suitable alternative to the equimolar chloramine T procedure; however, the reproducibility may depend on the quality of the enzyme preparation. The electrolytic iodination generally gave bad incorporation and did not prove to be suitable for α-MSH [1].

Incorporation of tritium into tyrosine[2] was less specific (92%) than iodination. This surprising finding stems from the fact that aromatic side chains (especially histidine) may undergo hydrogen/tritium exchange relatively easily during the tritiation experiment (*Wilzbach* effect [26]). Therefore, specific tritiations of α-MSH have to be performed on a rather small fragment, followed by completion of the whole synthesis (see [17]). Tritiated compounds have the advantage of being isosteric with the natural molecule, thus exhibiting identical chemical and biological properties (in particular identical hydrophilicity/lipophilicity). Whenever possible, they should be chosen before the iodinated compounds.

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Experimental Part

General. The compositions of solvent mixtures are indicated in volume parts. M.p. were determined in open capillaries and are uncorrected. Microanalyses were performed in the Laboratorium für Organische Chemie, ETHZ *(D. Manser)*. Amino acid analyses were carried out in this institute by Mrs. *Z. Zanivan* (Laboratory of Prof. Dr. *H. Zuber*) according to *Stein & Moore* with *Beckman* Mod. 120 B and 121 analyzers. The samples were hydrolyzed with 6N HCl containing 0.4% thioglycolic acid. UV. spectra (λ_{max} as nm) were recorded with a *Beckman* Acta V spectrometer. Radioactive samples were counted in *Nuclear Chicago* β- and γ-counters (Isocap and Mod. 4230, respectively). TLC. was carried out with *Merck* silica gel plates using the following solvent systems: AC = acetone/$CHCl_3$ 7:3, BAW 1 = 2-butanol/AcOH/water 72:7:21, BAW 2 = 1-butanol/AcOH/water 67:10:23, BPAW 1 = 1-butanol/pyridine/AcOH/water 50:12:12:25, BPAW 2 = 1-butanol/pyridine/AcOH/water 42:24:4:30, BPFW = 1-butanol/pyridine/HCOOH/water 40:20:10:30, BNH_3 = 2-butanol/3% ammonia 100:44, CM 1 = $CHCl_3$/methanol 1:1, CM 2 = $CHCl_3$/methanol 19:1, CMA = $CHCl_3$/methanol/AcOH 95:5:3, EBPAW = ethyl acetate/1-butanol/pyridine/AcOH/water 42:24:21:6:10. TLE. was performed

with *Merck* cellulose plates using an apparatus of *Camag* and the following buffers: pH 3.6 = AcOH/pyridine/water 100:10:890, and pH 6.4 = AcOH/pyridine/water 4:100:900. The compound spots on TLC. and TLE. plates were detected with I_2-vapour, ninhydrin, and *Reindel-Hoppe* [27] reagents, and (for special cases) *Sakaguchi* [28], *Pauly* [29], and *Ehrlich* [30] reagents. Radioactive spots were detected with an Actigraph III *(Nuclear Chicago)* TLC. scanner. Product characteristics are displayed in *Tables 3–5*.

Peptide synthesis. – The 6 main procedures used for the syntheses of the peptide derivatives **1** to **47** described below varied little between the individual steps and are therefore summarized in the following general form.

DCC/HOBT procedure (P.I) [12]. The carboxy and the (protonated) amino component were dissolved in DMF containing 1 equivalent of *N*-ethylmorpholine and treated at 0° with 2 mol-equiv. of HOBT and 1.1 mol-equiv. of DCC. After 2–3 h at 0° and 16 h at RT., a few drops of AcOH were added, the DCU was filtered off, the solvent evaporated at about 10^{-2} mbar, and the product isolated.

Azide procedure (P.II) [2]. The hydrazide component was dissolved in DMF (~5 ml/mmol peptide), cooled to −20° and treated with 2.5 mol-equiv. of HCl in dioxane (or ethyl acetate) and 1.2 mol-equiv. of *t*-butyl nitrite (freshly distilled). After 20 min at −20°, the amino component (dissolved in ~3 ml DMF/mmol) was precooled and slowly added together with 2.5 mol-equiv. of *N*-ethyldiisopropylamine (3.5 mol-equiv. if the amino component was in the protonated form). During the addition, the pH was checked with a moistened pH paper in order to avoid excess of base. All manipulations were performed under N_2 to exclude moisture. The temperature was kept at −15° for 1 h, then slowly raised and kept at 0° for 16 h. the solvent was evaporated and the product isolated.

HCl/HCOOH deprotection (P.III) [9] [31]. BOC- and O*t*Bu-protecting groups were removed by treating the peptide under N_2 with a solution of 0.12N HCl in HCOOH/mercaptoethanol 10:1 (10 ml/mmol) at RT. for 12 to 15 min. The reaction was stopped either by precipitating the product with peroxide-free ether, or by evaporating the solvent (and removing traces of the acid by repetitive solution of the residue in methanol and reevaporation).

Hydrazide formation (P.IV) [2]. The peptide methyl ester was dissolved in methanol (3–4 ml/mmol peptide) and treated with 3 mol-equiv. of hydrazine hydrate (155 µl/mmol peptide, *Fluka*) at RT. for 16 h. The crystallized hydrazide was filtered off, thoroughly washed with methanol/ether, recrystallized and dried in the desiccator over H_2SO_4.

N^a*-Acetylation* (P.V) [10]. The peptide (with protonated terminal amino group) was dissolved in pyridine/DMF 1:1 (or DMF) containing 1 mol-equiv. of *N*-ethylmorpholine. After addition of 1.5 mol-equiv. of 4-nitrophenyl acetate *(Merck)*, the solution was kept at RT. for 24 h. The solvent was evaporated and the product isolated.

Isolation (P.VI). The product is usually dissolved in $CHCl_3$ or ethyl acetate, then the organic solution is washed with dilute aqueous acid (5% $KHSO_4$-solution/5% K_2SO_4-solution 1:2), 5% aqueous $NaHCO_3$-solution, and saturated aqueous NaCl-solution, drying the organic phase (Na_2SO_4) and evaporating at about 15 mbar in a rotatory evaporator.

BOC·Ser-Met·OMe (**1a**). BOC·Ser·OH (9.5 g, 46 mmol) [32] and H·Met·OMe, HCl (9.2 g, 46 mmol) [33] were condensed in DMF (100 ml) (P.I) and isolated in $CHCl_3$ (P.VI). The residue was dissolved in ether, separated from a small insoluble portion, and crystallized from ether/pentane at 4°. Yield: 14.5 g (90%) of pure **1a**.

BOC·Ser-Met·NHNH$_2$ (**1b**) was prepared by treatment of **1a** (1.05 g, 3 mmol) with hydrazine hydrate (0.5 ml) in methanol (P.IV) and recrystallized from (hot) methanol/ether. Yield: 0.83 g (79%) **1b**.

H·Ser-Met·OMe, HCl (**2**). HCl/HCOOH deprotection (P.III) of **1a** (3.85 g, 11 mmol) gave **2** as colourless, resinous product. Yield: 3.1 g (98%) of pure (TLC.) **2**.

BOC·Tyr(I)-Ser-Met·OMe (**3**). BOC·Tyr(I)·OH[5]) (4.08 g, 10 mmol) was condensed with **2** (2.87 g, 10 mmol) (P.I), isolated in $CHCl_3$ (P.VI), and purified by dry column chromatography [34], with $CHCl_3$/methanol 19:1. Crystallization from 2-propanol/diisopropyl ether yielded 3.21 g (51%) **3**.

H·Tyr(I)-Ser-Met·OMe, HCl (**4**) was synthesized by treatment of **3** (0.87 g, 1.4 mmol) with HCl/HCOOH (P.III) for 10 min. Precipitation from methanol/ether yielded 0.69 g (88%) **4**.

BOC·Ser-Tyr(I)-Ser-Met·OMe (**5**). BOC·Ser·OH (0.45 g, 2.2 mmol) and **4** (1.27 g, 2.2 mmol) were condensed with DCC/HOBT (P.I) at RT. for 48 h. Isolation in ethyl acetate (P.VI) and precipitation from $CHCl_3$/petrol ether yielded 1.27 g (79%) **5**.

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[5]) DCHA salt: m.p. 130°; $[\alpha]_D^{25} = 28.6$ ($c = 1$, methanol).

Table 3. *Analytical data of the* N-*terminal peptide fragments* **1** *to* **24**

Compound No.	Structure (formula; M.W.)	m.p. °	$[\alpha]_D^{25}$ (*c*) solvent	UV. λ_{max} (ε) solvent (sh. = shoulder)		Elemental Analysis % Calc. % Found					Amino Acid Analysis[a])
						C	H	I	N	S	
1a	BOC·Ser-Met·OMe ($C_{14}H_{26}N_2O_6S$ 350.56)	68	−30.9 (1) MeOH			47.97 47.98	7.48 7.49		7.99 7.91	9.15 9.23	
1b	BOC·Ser-Met·$NHNH_2$ ($C_{13}H_{26}N_4O_5S$ 350.56)	190	−13.9 (1) DMF			44.55 44.64	7.48 7.47		15.99 15.79	9.15 9.26	
3	BOC·Tyr(I)-Ser-Met·OMe ($C_{23}H_{34}IN_3O_8S$ 638.49)	101– 104	−9.5 (1) MeOH			43.20 43.44	5.36 5.38		6.57 6.46	5.01 5.15	
4	H·Tyr(I)-Ser-Met·OMe, HCl ($C_{18}H_{27}ClIN_3O_6S$ 575.85)	173– 175	−3.7 (1) MeOH			37.54 37.64	4.73 4.83	22.04 22.18	7.30 7.38		
5	BOC·Ser-Tyr(I)-Ser-Met·OMe ($C_{26}H_{39}IN_4O_{10}S$ 726.59)	115– 117	−14.6 (1) MeOH			42.98 43.08	5.41 5.49		7.71 7.63		
6	H·Ser-Tyr(I)-Ser-Met·OMe, HCl ($C_{21}H_{32}ClIN_4O_8S$ 662.93)	199– 202 (dec.)	−2.0 (1) MeOH			38.05 38.08	4.87 4.98		8.45 8.30		
7a	Ac·Ser-Tyr(I)-Ser-Met·OMe ($C_{23}H_{34}IN_3O_8S$ 638.49)	186– 188	−23.2 (1) MeOH			41.32 41.25	4.98 4.99	18.98 18.72	8.38 8.19	6.69 6.78	
7b	Ac·Ser-Tyr(I)-Ser-Met·$NHNH_2$ ($C_{22}H_{33}IN_6O_8S$ 668.51)	190– 191	−11.5 (0.4) DMF			39.53 39.59	4.98 4.95	18.98 18.75	12.57 12.33		
8	Ac·Ser-Tyr(I)-Ser-Met·OH ($C_{22}H_{31}IN_4O_9S$ 654.48)	189– 190	−11.7 (0.75) 50% AcOH	283 (1965) 289 (1880) sh. 50% AcOH	305 (3310) 0.1N NaOH	40.38 40.16	4.77 4.56	19.39 19.09	8.56 8.32		S 1.87 *Y 1.00* M 0.95

Table 3 (continued)

Compound No.	Structure (formula; M.W.)	m.p. °	$[\alpha]_D^{25}$ (c) solvent	UV. λ_{max} (ε) solvent (sh. = shoulder)		Elemental Analysis % Calc. % Found C	H	I	N	S	Amino Acid Analysis[a]
9	Ac·Ser-Tyr-Ser-Met·OH ($C_{22}H_{32}N_4O_9S$ 528.59)	167–170	−34.6 (0.5) H_2O	275 (1290) 281 (1080) sh. 1N AcOH	293 (2220) 0.1N NaOH	49.99 50.18	6.10 6.23		10.60 10.48		S 1.85 *Y 1.00* M 0.98
10a	BOC·Tyr(I_2)-Ser·OMe ($C_{18}H_{24}I_2N_2O_7$ 634.21)	160–161	10.8 (1) MeOH			34.09 34.40	3.81 3.85	40.02 39.90	4.42 4.35		
10b	BOC·Tyr(I_2)-Ser·$NHNH_2$ ($C_{17}H_{24}I_2N_4O_6$ 634.21)	198–199	15.3 (1) DMF			32.20 32.40	3.81 3.92	40.02 40.10	8.83 8.82		
12a	BOC·ser-Tyr(I_2)-Ser·OMe ($C_{21}H_{29}I_2N_3O_9$ 721.28)	189–190 (dec.)	7.8 (1) MeOH			34.97 35.07	4.05 4.38	35.19 35.20	5.83 5.61		
12b	BOC·ser-Tyr(I_2)-Ser·$NHNH_2$ ($C_{20}H_{29}I_2N_5O_8$ 721.28)	172–174 (dec.)	3.4 (1) DMF			33.30 33.01	4.05 4.08	35.19 34.79	9.71 9.90		
13a	BOC·Tyr(I_2)-Ser-Met·OMe ($C_{23}H_{33}I_2N_2O_8S$ 765.40)	142–143	−4.9 (1) MeOH		287 (2850) 296 (2650) sh. EtOH	36.09 36.07	4.35 4.38	33.16 33.01	5.49 5.66	4.16 4.33	S 0.91 *Y 1.00* M 1.02
13b	BOC·Tyr(I_2)-Ser-Met·$NHNH_2$ ($C_{22}H_{33}I_2N_5O_7S$ 765.40)	180–182	15.1 (0.9) DMF			34.52 34.40	4.35 4.52	33.16 32.93	9.15 8.93		
14	H·Tyr(I_2)-Ser-Met·OMe, HCl ($C_{18}H_{26}ClI_2N_3O_6S$ 701.75)	180–181	2.7 (1) EtOH			30.81 30.66	3.73 3.69	36.17 35.91	5.99 5.71		
15a	BOC·ser-Tyr(I_2)-Ser-Met·OMe ($C_{26}H_{38}I_2N_4O_{10}S$ 852.48)	174–175	14.0 (0.5) AcOH		287 (2830) 296 (2740) EtOH	36.63 36.45	4.49 4.62	29.77 29.76	6.57 6.69	3.76 3.95	S 1.90 *Y 1.00* M 1.04

15b	BOC · ser-Tyr(I_2)-Ser-Met · $NHNH_2$ ($C_{25}H_{38}I_2N_6O_9S$ 852.48)	182	5.4 (1) DMF			35.22 35.52	4.49 4.61	29.77 29.48	9.86 9.59		
16	BOC · Ser-Tyr(I_2)-Ser-Met · OMe ($C_{26}H_{38}I_2N_4O_{10}S$ 852.48)	129–131	−12.5 (1) MeOH			36.63 36.51	4.49 4.65	29.77 29.59	6.57 6.39		
17	H · Ser-Tyr(I_2)-Ser-Met · OMe, HCl ($C_{21}H_{31}ClI_2N_4O_8S$ 788.84)	172–176	4.0 (1) MeOH			31.98 31.75	3.96 3.99	32.18 32.04	7.10 6.78		
18	Ac · Ser-Tyr(I_2)-Ser-Met · OMe ($C_{23}H_{32}I_2N_4O_9S$ 794.38)	210 (dec.)	−19.6 (1) MeOH			34.77 34.92	4.06 4.21		7.05 6.97		
19	Ac · Ser-Tyr(I_2)-Ser-Met · OH ($C_{22}H_{30}I_2N_4O_9S$ 780.38)	185–186	−8.0 (1) 50% AcOH	287 (2340) 295 (2310) 50% AcOH	310 (4370) 0.1N NaOH	33.86 33.66	3.88 3.97	32.52 32.30	7.18 6.69	S 1.82 *Y 1.00* M 1.02	
20	Ac · Ser-Tyr(I_2)-Ser-Met · NH_2 ($C_{22}H_{31}I_2N_5O_8S$ 779.39)	275–278 (dec.)	15.0 (0.5) DMSO					32.57 32.29		S 1.74 *Y 1.00* M 0.90	
21	Ac · Ser-Tyr-Ser-Met · NH_2 ($C_{22}H_{33}N_5O_8S$ 527.60)	199–200	−17.6 (0.5) MeOH					0 0.15		S 1.68 *Y 1.00* M 0.92	
24	Ac · Ser-Tyr · OH ($C_{14}H_{18}N_2O_6$ 310.31)	194–196	19.4 (0.5) H_2O			54.19 53.85	5.85 5.68		9.03 8.69	S 0.88 *Y 1.00*	
41	Ac · Tyr(I_2) · OH, DCHA ($C_{23}H_{34}I_2N_2O_4$ 656.34)	169–170	37.9 (1) MeOH			42.09 42.53	5.22 5.48	38.67 38.32	4.27 4.03		

[a]) Reference amino acid in italics.

Table 4. *TLC. of the* N-*terminal peptide fragments* **1** *to* **24**

Compound No.	AC	BAW 1	BAW 2	BPAW 1	BPAW 2	BPFW	BNH_3	CM 1	CM 2	CMA	EBPEW
1a			0.70							0.51	
1b		0.63		0.70							
2		0.45						0.63			
3				0.74					0.55		
4				0.62	0.58						
5		0.75		0.73							
6		0.37		0.60							
7a		0.51		0.67	0.69						
7b				0.63	0.64						
8				0.53	0.56		0.40				
9				0.38	0.43		0.30				
10a			0.76							0.44	0.70
10b		0.79				0.81					0.90
11		0.49									
12a		0.71								0.22	
12b			0.53	0.66							
13a	0.81	0.91		0.74						0.46	0.94
13b		0.65		0.73							
14		0.51	0.47	0.70							
15a		0.77		0.79							
15b		0.65		0.70							
16		0.81		0.77							
17		0.40		0.57	0.59						
18		0.65		0.70							
19		0.37		0.51	0.47						
20		0.58		0.64	0.64						
21				0.59	0.55						
22		0.77								0.43	
23				0.30							
24				0.41	0.55						
41		0.63	0.78		0.63						

H·Ser-Tyr(I)-Ser-Met·OMe, HCl (**6**). Deprotection of **4** (1.09 g, 1.5 mmol) (P.III, reaction time: 10 min) yielded 0.81 g (88%) **6** after crystallization of the residue from methanol/ether.

Ac·Ser-Tyr(I)-Ser-Met·OMe (**7a**) was obtained by acetylation of **6** (0.99 g, 1.5 mmol) with 4-nitrophenyl acetate (0.38 g) in pyridine/DMF 1:1 (10 ml) (P.V), followed by chromatography over *Sephadex* LH-20 (3×80 cm) in DMF/water 9:1. The pure fractions (TLC.) were combined and evaporated, whereupon the product was precipitated from 2-propanol/diisopropyl ether. Yield: 0.85 g (85%) **7a**.

Ac·Ser-Tyr(I)-Ser-Met·NHNH$_2$ (**7b**) was prepared from **7a** in 83% yield using P.IV. Recrystallization from DMF/ether.

Ac·Ser-Tyr(I)-Ser-Met·OH (**8**). A solution of **7a** (393 mg, 0.6 mmol) in methanol (12 ml) was treated with 0.1N NaOH (12 ml) at RT. for 10 min. The solution was neutralized with 1N HCl at 0° and evaporated. The residue was dissolved in ethanol, filtered from insoluble salts, and precipitated with ether. Recrystallization from (hot) ethanol/ether. Yield: 382 mg (97%).

Ac·Ser-Tyr-Ser-Met·OH (**9**). **8** (650 mg, 1 mmol) was hydrogenated in the presence of 5% Pd/C (200 mg; *Engelhard*) and 5% Rh/$CaCO_3$ (100 mg; *Engelhard*) in DMF (8 ml) at 1000 mbar and RT. for 1 h. Purification by chromatography over *Sephadex* LH-20 with methanol and precipitation from methanol/ether yielded 410 mg (78%) of **9** identical with the N^{α}-acetylated H·Ser-Tyr-Ser-Met·OH, HCl (prepared by saponification of H·Ser-Tyr-Ser-Met·OMe, HCl [11]).

BOC·Tyr(I_2)-Ser·OMe (**10a**). BOC·Tyr(I_2)·OH (10.66 g, 20 mmol) [7b] and H·Ser·OMe, HCl (2.30 g, 20 mmol) [35] reacted with DCC/HOBT (P.I) in DMF (60 ml). The product was isolated in ethyl acetate (500 ml; P.VI), chromatographed over silica gel (500 g; *Merck*) with ethyl acetate/methanol 49:1, and crystallized from ethyl acetate/methanol 4:1 by addition of ether. Yield: 8.2 g (71%)**10a**.

BOC·Tyr(I_2)-Ser·$NHNH_2$ (**10b**). Treatment of **10a** (5.0 g, 7.9 mmol) with hydrazine hydrate (0.8 ml; P.IV), and crystallization by adding the double volume of ether to the reaction mixture yielded 3.8 g (76%) **10b**.

H·Tyr(I_2)-Ser·OMe, HCl (**11**). A solution of **10a** (13.95 g, 22 mmol) in dioxane (125 ml) treated with 6N HCl in dioxane (25 ml), was kept at RT. for 1 h, whereupon **11** crystallized from the reaction mixture. Quantitative crystallization was obtained by adding ether. The product was filtered off under N_2, washed thoroughly with ether, and dried in the desiccator over KOH. Yield: 11.45 g (91%) of very hygroscopic, crystalline **11**.

BOC·ser-Tyr(I_2)-Ser·OMe (**12a**). The condensation product of **11** (11.4 g, 20 mmol) with BOC·D-Ser·OH (4.1 g, 20 mmol) [1] using DCC/HOBT in DMF (50 ml) (P.I), was isolated in $CHCl_3$ (500 ml; P.VI), and purified by dry column chromatography [34] over silica gel (4.5×40 cm; *Woelm*, 'for dry column chromatography') with $CHCl_3$/methanol/AcOH 95:5:3 (details for this procedure see [1]). Crystallization of the pure fractions from 2-propanol/petrol ether yielded 7.2 g (50%) **12a**.

BOC·ser-Tyr(I_2)-Ser·$NHNH_2$ (**12b**) was prepared from **12a** (P.IV) and crystallized from DMF as long, white needles in 77% yield.

BOC·Tyr(I_2)-Ser-Met·OMe (**13a**). A) A solution of **2** (2.87 g, 10 mmol) in DMF (40 ml) was treated with BOC·Tyr(I_2)·ONP (6.54 g, 10 mmol) [7b] and *N*-ethylmorpholine (1.15 g, 10 mmol) at RT. for 24 h. The product was isolated in ethyl acetate (500 ml; P.VI) and crystallized 3 times from $CHCl_3$/petrol ether and once from acetone/water. Yield: 6.2 g (81%) of pure **13a**. – B) According to P.II **10b** (2.53 g, 4 mmol) reacted with H·Met·OMe, HCl (0.80 g, 4 mmol). The product was purified by dry column chromatography with CMA; the homogeneous fractions were pooled, evaporated, and precipitated from $CHCl_3$/petrol ether: 2.10 g (69%) **13a** identical with the product obtained from A).

BOC·Tyr(I_2)-Ser-Met·$NHNH_2$ (**13b**) was prepared from **13a** (P.IV) in 77% yield. A minute contaminant was removed by chromatography over *Sephadex* LH-20 with DMF.

H·Tyr(I_2)-Ser-Met·OMe, HCl (**14**) was obtained from acid treatment of **13a** (P.III) and crystallization from methanol/ether in 92% yield.

BOC·ser-Tyr(I_2)-Ser-Met·OMe (**15a**). A) Condensation of **12b** with H·Met·OMe, HCl according to P.II yielded **15a** in pure form (70%). – B) Using a modified DCC/HOBT procedure (P.I), BOC·ser·OH (0.41 g, 2 mmol) [1] was preactivated with HOBT (0.54 g, 4 mmol) and DCC (0.46 g, 2.2 mmol) in DMF (10 ml) at 0° for 10 min. Separate solutions of **14** (1.40 g, 2 mmol) and *N*-ethylmorpholine (0.23 g, 2 mmol) in DMF (8 ml) were then added to the reaction mixture. The crude product was crystallized several times from methanol/water. Yield: 1.25 g (74%)**15a** identical with **15a** A.

BOC·ser-Tyr(I_2)-Ser-Met·$NHNH_2$ (**15b**). A solution of **15a** (852 mg, 1 mmol) in DMF was evaporated i.V. The residual oil was dissolved in methanol (8 ml) (**15a** in crystalline form is difficult to dissolve in methanol), and treated with hydrazine hydrate (P.IV). Crystallization was quantitative upon addition of ether (8 ml) to the reaction mixture: 680 mg (80%) **15b**.

BOC·Ser-Tyr(I_2)-Ser-Met·OMe (**16**). BOC·Ser·OH (0.41 g, 2 mmol) was condensed with **14** (1.40 g, 2 mmol) as described for **15a** B. Crystallization and recrystallization (2×) from methanol/water yielded 1.44 g (85%) of pure **16**.

H·Ser-Tyr(I_2)-Ser-Met·OMe, HCl (**17**). Deprotection of **16** (10.2 g, 12 mmol) with HCl/HCOOH (P.III) for 12 min, and precipitation from methanol/ether yielded 8.42 g (89%) **17**.

Ac·Ser-Tyr(I_2)-Ser-Met·OMe (**18**). A solution of **17** (3.95 g, 5 mmol) in pyridine (40 ml, freshly distilled) was acetylated (P.V). The product was crystallized from (hot) methanol/ether. Yield: 3,95 g (99%).

Ac·Ser-Tyr(I_2)-Ser-Met·OH (**19**). A solution of **18** (1.6 g, 2 mmol) in dioxane/methanol/0.2N NaOH 1:1:2 (40 ml) was kept at RT. for 10 min. After neutralization at 0° with 1N HCl, the solvent was evaporated, and the residue was dissolved in warm ethanol, separated from insoluble NaCl, and recrystallized twice from (hot) ethanol (for quantitative crystallization, a small amount of ether was added to the still warm solution). Yield: 1.3 g (83%) **19**.

Ac·Ser-Tyr(I_2)-Ser-Met·NH_2 (**20**). Treatment of **18** (0.4 g, 0.5 mmol) in methanol (4 ml) with ammonia (4 ml, dried over Na) for 3 days gave **20** crystallizing quantitatively from the reaction mix-

Table 5. *Analytical data of the peptide derivatives* **25** *to* **47**

Compound No.	Structure (formula; M.W.)	$[a]_D^{25}$ (*c*) solvent	UV. λ_{max} (ε) solvent	TLC. (Rf) BPAW 1	TLC. (Rf) BPAW 2	TLE. (R Arg) pH 3.6	TLE. (R Arg) pH 6.4	Amino Acid Analysis (reference amino acid in italics) (V′ = norvaline)
25	Ac·Ser-Tyr(I)-Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(BOC)-Pro-Val·NH_2, HCl ($C_{86}H_{125}ClIN_{21}O_{21}S$ 1983.51)	−31.6 (0.5) AcOH	281 (6450) 288 (5750) 10% AcOH	0.70	0.74			S 1.82/Y 0.98/M 0.95/E 1.08/ H 0.94/F 0.99/R 1.07/W 0.90/ G 1.03/K 1.02/P 1.11/*V 1.00*
26	Ac·Ser-Tyr(I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{81}H_{116}IN_{21}O_{23}S$ 1910.93)	−57.9 (0.33) 1N AcOH	281 (6595) 289 (6690) 0.1N NaOH	0.39	0.36	0.55	0.38	S 1.89/Y 0.97/M 0.95/E 1.06/ H 0.93/F 0.99/R 1.04/W 0.88/ G 0.96/K 1.08/P 1.12/*V 1.00*
27	Ac·Ser-Tyr(I_2)-Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(BOC)-Pro-Val·NH_2, HCl ($C_{86}H_{124}ClI_2N_{21}O_{21}S$ 2109.40)	−24.8 (0.25) AcOH		0.76	0.81			S 1.72/Y 0.96/M 0.92/E 1.12/ H 0.98/F 1.01/R 1.08/W 0.91 G 0.99/K 1.09/P 1.09/*V 1.00*
28	Ac·Ser-Tyr(I_2)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{81}H_{115}I_2N_{21}O_{23}S$ 2036.83)	−56.4 (0.33) 1N AcOH	281 (6315) 289 (6250) 310 (5092) 0.1N NaOH	0.45	0.40	0.49	0.28	S 1.83/Y 1.01/M 1.02/E 1.10/ H 0.95/F 1.00/R 1.07/W 0.92/ G 1.03/K 1.13/P 1.03/*V 1.00*
29	Ac·Ser-Tyr($^3H_2$)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{81}H_{115}{}^3H_2N_{21}O_{23}S$ 1789.05)	−62.7 (0.33) 1N AcOH	281 (6810) 289 (6510) 0.1N NaOH	0.35	0.33	0.61	0.46	S 1.69/Y 0.98/M 0.95/E 1.06/ H 0.98/F 1.01/R 1.10/W 0.89/ G 0.99/K 1.03/P 1.12/*V 1.00*
30	BOC·ser-Tyr(I_2)-Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH_2, HCl ($C_{88}H_{128}ClI_2N_{21}O_{24}S_2$ 2216.83)	−19.0 (0.4) AcOH	281 (8050) 1N AcOH	0.68	0.69			S 1.77/Y 0.97/M 0.90/E 1.08/ H 0.97/F 0.98/R 1.03/W 0.85/ *G 1.00*/K 1.04/P 1.02/V 1.01
32	Ac·ser-Tyr(I_2)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{81}H_{115}I_2N_{21}O_{23}S$ 2036.83)	−51.0 (0.33) 1N AcOH	281 (7305) 288 (6810) 1N AcOH	0.44	0.41	0.48		S 1.83/Y 1.02/M 0.91/E 1.10/ H 0.98/F 1.02/R 1.04/W 0.89/ *G 1.00*/K 0.99/P 0.99/V 0.98
33	Ac·ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{23}S$ 1785.02)	−57.5 (0.33) 1N AcOH	281 (6830) 289 (6525) 0.1N NaOH	0.34	0.33	0.60		S 1.80/Y 1.03/M 0.92/E 1.09/ H 0.96/F 1.03/R 0.99/W 0.84/ *G 1.00*/K 1.06/P 1.05/V 0.98
34	Ac·Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{23}S$ 1785.02)	−63.3 (0.33) 1N AcOH	281 (6860) 289 (6550) 0.1N NaOH	0.35	0.33	0.61	0.46	S 1.78/Y 1.02/M 0.91/E 1.05/ H 0.98/F 0.99/R 1.04/W 0.83/ *G 1.02*/K 1.05/P 1.15/V 1.00

35	BOC·Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly·OH, HCl ($C_{56}H_{81}ClN_{14}O_{14}S$ 1241.87)	−20.4 (0.33) MeOH	280 (5365) 1N AcOH	0.58	0.57		S 0.95/M 0.92/E 1.04/H 0.95/F 1.02/R 1.03/W 0.85/*G 1.00*
36	Ac·Ser-Met-Glu-His-Phe-Arg-Trp-Gly·OH, HCl ($C_{49}H_{67}ClN_{14}O_{13}S$ 1127.68)	−54.1 (0.33) 1N AcOH	280 (5500) 289 (5150) 0.1N NaOH	0.34	0.22	0.48	S 0.90/M 0.93/E 1.05/H 0.98/F 0.98/R 1.02/W 0.83/*G 1.00*
37	BOC·Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH_2, HCl ($C_{76}H_{116}ClN_{19}O_{20}S_2$ 1715.47)	−33.6 (0.33) AcOH	280 (5315) 1N AcOH	0.61	0.64		S 0.98/M 0.97/E 1.09/H 1.02/F 0.97/R 0.96/W 0.82/G 1.07/K 1.08/P 1.11/*V 1.00*
38	H·Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH_2, 2 HCl ($C_{67}H_{101}Cl_2N_{19}O_{18}S_2$ 1595.71)	−53.2 (0.33) 1N AcOH	280 (5320) 289 (4810) 1N AcOH	0.41	0.37	0.68	S 0.96/M 0.99/E 1.11/H 0.95/F 0.98/R 1.05/W 0.81/G 1.04/K 1.04/P 1.08/*V 1.00*
39	Ac·Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{68}H_{103}N_{19}O_{19}S$ 1522.76)	−54.2 (0.2) 5% AcOH	280 (5275) 289 (4530)[a] 0.1N NaOH	0.32	0.38	0.66	S 0.92/M 0.93/E 1.08/H 0.95/F 1.02/R 1.02/W 0.89/G 1.03/K 1.05/P 1.09/*V 1.00*
40	BOC·Tyr(I_2)-Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH_2, HCl ($C_{85}H_{123}ClI_2N_{20}O_{22}S_2$ 2130.44)	−26.3 (0.4) AcOH		0.75	0.68		S 0.91/Y 1.02/M 0.91/E 1.02/H 0.93/F 0.96/R 0.97/W 0.86/*G 1.00*/K 1.11/P 1.12/V 1.04
43	Ac·Tyr(I_2)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 HCl ($C_{74}H_{104}Cl_2I_2N_{20}O_{17}S$ 1902.56)	−57.1 (0.2) 1N AcOH	280 (7100) 1N AcOH	0.48	0.46	0.51	S 0.94/Y 1.08/M 0.95/E 1.08/H 1.01/F 0.99/R 1.02/W 0.82/G 1.03/K 1.08/P 1.12/*V 1.00*
44	Ac·Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{78}H_{112}N_{20}O_{21}S$ 1697.95)	−58.2 (0.2) 1N AcOH	280 (6825) 289 (6520)[a] 0.1N NaOH	0.38	0.35	0.61	S 0.94/Y 1.05/M 0.98/E 1.10/H 0.98/F 0.98/R 1.05/W 0.90/G 1.01/K 1.04/P 1.10/*V 1.00*
45	Hpp-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{76}H_{109}N_{19}O_{20}S$ 1640.90)	−55.0 (0.2) 1N AcOH	280 (6735) 1N AcOH	0.46	0.45	0.57	S 0.82/M 0.91/E 1.05/H 0.91/F 1.02/R 1.08/W 0.84/G 1.01/K 1.03/P 1.09/*V 1.00*
46	Ac·Tyr-Ser-Nva-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{78}H_{113}N_{20}O_{21}$ 1666.90)	−51.2 (0.33) 1N AcOH	280 (6870) 289 (6510) 0.1N NaOH	0.37	0.35	0.59	S 0.89/Y 1.02/V′ 0.98/E 1.03/H 1.01/F 0.99/R 1.03/W 0.93/G 1.02/K 1.02/P 0.97/*V 1.00*
47	Ac·Tyr(I_2)-Ser-Nva-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH ($C_{78}H_{111}I_2N_{20}O_{21}$ 1918.67)	−40.0 (0.33) 1N AcOH	280 (6630) 289 (6120) 0.1N NaOH	0.47	0.44	0.52	S 0.92/Y 1.02/V′1.01/E 1.09/H 0.93/F 1.01/R 1.04/W 0.89/G 1.01/K 0.99/P 0.98/*V 1.00*

[a] sh. = shoulder.

ture; it was filtered off, washed with methanol, and dried over P_2O_5. Yield: 0.34 g (87%) **20** which was only soluble in DMSO or ammonia.

Ac·Ser-Tyr-Ser-Met·NH$_2$ (**21**). A solution of **20** (100 mg, 0.12 mmol) in 5% aqueous ammonia was hydrogenated in the presence of 5% Rh/$CaCO_3$ and 5% Pd/C (50 mg of each; *Engelhard*) at RT. for 2 h. The catalyst was removed by centrifugation, the solvent was evaporated, and the product was precipitated from methanol/ether and dried over P_2O_5. Yield: 50 mg (76%).

Z·Ser-Tyr·OBZL (**22**). Z·Ser·ODNP (405 mg, 1 mmol) [36] was condensed with H·Tyr·OBZL, HCl (443 mg, 1 mmol) [2] in DMF (10 ml) in the presence of *N*-ethylmorpholine (0.13 ml, 1 mmol) at RT. overnight. Isolation in ethyl acetate (P.VI) and crystallization from benzene/petrol ether yielded 462 mg (93%).

Ac·Ser-Tyr·OH (**24**). A solution of **22** (330 mg, 0.67 mmol) in methanol/acetic acid 4:1 (12 ml) was hydrogenated in the presence of 10% Pd/C (40 mg; *Fluka*) under continuous flow for 90 min. The catalyst was filtered off, the solvent was evaporated, and the residue was treated with pyridinium hydrochloride (in methanol), yielding **23** as resinous solid. A solution of 150 mg (0.5 mmol) of **23** in DMF/H_2O 9:1 (5 ml) was acetylated (P.V). The product was purified by dry column chromatography with methanol/$CHCl_3$ 1:1. Crystallization from methanol/ether gave 80 mg (55%) of pure **24**. (A different approach for **24** is described in [37].)

Ac·Ser-Tyr(I)-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(BOC)-Pro-Val·NH$_2$, HCl (**25**). A) Condensation of **7a** (132 mg, 0.25 mmol) with H·Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(BOC)-Pro-Val·NH_2, 2 HCl (346 mg, 0.25 mmol) [8a] was effected in DMF (5 ml) (P.I) using HOBT (68 mg, 0.5 mmol) and DCC (62 mg, 0.3 mmol) in the presence of *N*-ethylmorpholine (29 mg, 0.25 mmol). After 2 h at 0° and 36 h at RT. the DCU was filtered off, and the product was chromatographed over *Sephadex* LH-20 (3.5 × 100 cm) with DMF/H_2O 9:1. Precipitation from DMF/ether yielded 401 mg (81%) **25**. – B) The azide form of **7b** (67 mg, 0.1 mmol) was coupled to the nonapeptide derivative (138 mg, 0.1 mmol) (P.II). Chromatography over *Sephadex* LH-20 yielded **25** in 68% yield, which was identical with **25A**.

Ac·Ser-Tyr(I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH$_2$, 2 AcOH (**26**). After treatment of **25** (396 mg, 0.2 mmol) with 0.12N HCl in HCOOH/1,2-ethanedithiol 19:1 (10 ml) at RT. for 15 min, the product was precipitated with ether, washed thoroughly with 2-propanol and dried over KOH. Chloride ions were exchanged against acetate on a weakly basic ion exchange resin (*Merck* No. II) with 1N AcOH. Purification by repetitive chromatography (3×) over *Sephadex* LH-20 (3.5 × 100 cm) with 1N AcOH yielded 280 mg (73%) of homogeneous **26**.

Ac·Ser-Tyr(I$_2$)-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(BOC)-Pro-Val·NH$_2$, HCl (**27**) was synthesized and purified as described for **25** A. Yield: 80%.

Ac·Ser-Tyr(I$_2$)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH$_2$, 2 AcOH (**28**). Deprotection of **27** (422 mg, 0.2 mmol) was performed as described above for **26**. Purification by chromatography over *Sephadex* LH-20 (2×) with 10% AcOH. Yield: 290 mg (73%).

The syntheses of **30, 31, 32** and **33** were accomplished in a similar way; a detailed description is in [1] (analytical data are displayed in *Table 5*).

BOC·Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly·OH, HCl (**35**). After condensation of **1b** (700 mg, 2 mmol) with H·GluO*t*Bu)-His-Phe-Arg-Trp-Gly·OH, HCl (1845 mg, 2 mmol) [38] (P.II), the product was purified by partition chromatography over *Sephadex* G-25 (4 × 120 cm) with 1% acetic acid/1-butanol. Pure fractions (TLC.) were pooled, evaporated, and **35** was precipitated from DMF/ether: 1.49 g (60%).

Ac·Ser-Met-Glu-His-Phe-Arg-Trp-Gly·OH, HCl (**36**). Deprotection of **35** (0.62 g, 0.5 mmol) (P.III) and acetylation yielded 410 mg (73%) **7a** (P.V) after purification over *Sephadex* LH-20 with 1N AcOH.

BOC·Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH$_2$, HCl (**37**). After condensation of **35** (621 mg, 0.5 mmol) with H·Lys(MsOC)-Pro-Val·NH_2, HCl (285 mg, 0.52 mmol) [9] (P.I), pure **37** was obtained upon chromatography over *Sephadex* LH-20 with DMF/H_2O 9:1. Yield: 685 mg (80%).

H·Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH$_2$, 2 HCl (**38**). The BOC/O*t*Bu groups of **37** were removed (P.III), and **38** was precipitated from water/acetone as a pure, amorphous solid. Yield: 360 mg (90%)

Ac·Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH$_2$, 2 AcOH (**39**) was obtained from **38** upon N^a-acetylation (P.V) and β-elimination of the MsOC group with 0.1N $Ba(OH)_2$/MeOH 5:1 (5 min

at RT.). Filtration through *Dowex-3* ion exchange resin ($Cl^- \rightarrow AcO^-$) and chromatography over *Sephadex* LH-20 yielded pure **39** (87%).(An earlier synthesis of this compound has been described [39].)

BOC·Tyr(I_2)-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH_2, HCl (**40**). The azide of **13b** was condensed with H·Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly-Lys(MsOC)-Pro-Val·NH_2, 2 HCl [7b] (P.II). The product was purified over *Sephadex* LH-20. Yield: 57%.

Ac·Tyr(I_2)·OH, DCHA (**41**). 3′,5′-Diiodo-L-tyrosine, 2 H_2O (4.7 g, 10 mmol; *Fluka*) was suspended in water (70 ml) at 80° while Ac_2O (8 ml, 88 mmol) was slowly added over 40 min, and the reaction was continued at 80° for another hour. The solvent was evaporated, the residue was dissolved in ethyl acetate (100 ml) and treated with dicyclohexylamine (1.8 g, 10 mmol), and diisopropyl ether (100 ml) was added. Crystallization at 4° yielded **41** as an almost colourless solid: 4.2 g (64%). The NMR. spectrum in (D_6)DMSO showed only a sharp singulet at $\delta = 7.7$ ppm (no partial splitting of the iodine during acetylation).

Ac·Tyr(I_2)·OSu (**42**) was prepared by the mixed-anhydride method as described for Z·Glu(O*t*Bu)·OSu in [9]. It was freshly prepared before use and was stored at −20°. M.p. 90–95° (unstable in the TLC.).

Ac·Tyr(I_2)Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 HCl (**43**). A) A solution of **38** (135 mg, 75 μmol) and **42** (40 mg, 120 μmol) in DMF/pyridine/water 1:1:1 (1 ml) was kept at RT. for 6 h. The MsOC-group was eliminated *in situ* by treatment with $Ba(OH)_2$ (see **39**). Purification over *Sephadex* LH-20 yielded 114 mg (80%). – B) In a similar way, **43** was obtained after deprotection of **40** with HCl/HCOOH (P.III), N^{α}-acetylation (P.V), and elimination of the MsOC-group. Yield: 70%. A and B were identical (TLC.).

The dodecapeptides **44** and **45** (**46** and **47**) were synthesized in a similar way to **43** A (**43** B). Details are in [1] (analytical data see *Table 5*).

Tritiation of [Tyr(I_2)^2]α-MSH. – The tritiation experiments were performed in a new type of tritiation apparatus constructed by *A. Zeller* (*Radium-Chemie*, Teufen, Switzerland) to be described in a forthcoming publication. The synthesis depicted below was performed twice with similar results.

Ac·Ser-Tyr($^3H_2$)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH (**29**). A solution of **28** (50 mg, 25 μmol) in DMF/H_2O 10:1 (3 ml) at 40°, cooled to RT., was transferred into a 25 ml reaction flask containing 5% Pd/C and 5% Rh/$CaCO_3$ (50 mg of each; *Engelhard*). The flask was screwed on to the tritiation apparatus, cooled with liquid N_2, and evacuated ($p \leq 0.001$ mbar). The solution was degassed by repetitive thawing and freezing (the pressure was maintained at $p \leq 0.001$ mbar). The tritiation was carried out at RT. with $^3H_2$ of high purity (> 98%) at $p = 1030$ mbar. After 30 min, the $^3H_2$ was removed, the solvent was distilled off, and the dry residue was suspended in DMF/H_2O 1:1 in which the peptide was readily soluble. The catalyst was filtered off (over *Celite*). The filtrate was evaporated and redissolved in the same solvent; this procedure was repeated several times in order to exchange all 'loosely' bound ^{3}H-atoms from the peptide. The dry residue was dissolved in 1N AcOH, filtered through weakly basic ion exchange resin (*Merck* No.II; 1 × 20 cm), and chromatographed twice over *Sephadex* LH-20 (2 × 80 cm) with 1N AcOH. Yield: 17.8 mg (40%) of pure **29** which was identical with αMSH (**34**) *(Table 5)*. The product was dissolved in 100 ml of H_2O/EtOH 50:1 and stored in 1 ml – ampoules at −196°. Specific activity of **29**: 19.1 mCi/mg or 34 Ci/mmol ±5%.

Chymotryptic analysis of **29**. α-MSH (0.35 mg, 200 nmol) and **29** (0.4 μCi) were suspended in 0.1M ammonium acetate (100 μl, pH 8.5) and 5 μl of a solution containing 1 mg chymotrypsin *(Boehringer)* per ml of the same buffer were added. The probe was kept at 37° for 16 h (the long incubation period is necessary because of a very slow hydrolysis of the Trp-Gly bond, see [40]). The hydrolysate was analyzed by TLC. and TLE. *(Fig. 2)*, and the radioactivity of each spot was determined in the β-counter *(Table 1)*.

Radioiodination of α-MSH. – ^{125}I was used exclusively for all iodination experiments because of the long usability of α-MSH tracers prepared with this isotope (up to 8 weeks). The experiments were performed in a special isotope hood of a B-type laboratory giving full protection and preventing any incorporation of radioactivity.

Ac·Ser-Tyr(^{125}I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH_2, 2 AcOH (**48**). A) *Equimolar chloramine T method.* A small conic glass vial containing a solution of 2 mCi $Na^{125}I$ (5–10 μl; *NEN*; low pH) was equipped with a miniature magnetic Teflon stirrer. Consecutively, 5–10 μl of a 0.1M NaH_2PO_4 solution, 10 μl 0.25M sodium phosphate buffer (pH 7.4), and 1.5 μg α-MSH dissolved in

10 µl of the same buffer were added. The reaction was started immediately by adding 5 µl of a chloramine T solution (30 µg/ml sodium phosphate buffer, freshly prepared in concentrated form and diluted immediately before use) to the stirred reaction mixture. Addition of the same amount of chloramine T was repeated after 30 and 60 s. After 90 s the reaction was stopped by adding 100 µl of 1% AcOH containing 0.5% bovine serum albumin (BSA) and 0.05% NaN_3. The solution was filtered through weakly basic ion exchange resin (*Merck* No.II) in a *Pasteur* pipette with the same BSA-solution as eluent (elimination of free iodide). The eluted tracer (1–1.5 ml) was chromatographed over *Sephadex* LH-20 (1 × 80 cm) with 0.05M sodium phosphate buffer, pH 7.0, containing 0.5% BSA and 0.05% NaN_3. With a minute front peak iodinated BSA was removed. The biologically and immunologically most active fractions were found in the peak-maximum and shortly afterwards *(Fig. 3);* they were identical with **26** (mono-iodinated α-MSH). The tracer was stored at −25° at a maximal concentration of 10 µCi/ml. The chymotryptic analysis was performed as described above for **29** using **26** (0.38 mg, 200 nmol) and **48** A (~20,000 cpm). – B) *Fragment iodination method.* **24** (0.3 µg, 1 nmol) in 10 µl sodium phosphate buffer was iodinated as described above for α-MSH. Purification by paper-electrophoresis at pH 6.4. The peptide **49** was eluted with methanol/water 1:1 and the solution concentrated at the bottom of a small conic glass vial. A solution of DMF (10 µl, highest purity) containing HOSu (0.3 µg) and DCC (0.5 µg) was added; after 1 h of preactivation, a second portion of DMF (10 µl) with **38** (0.8 µg) and *N*-ethylmorpholine (0.05 µg) was added and stirred at RT. for 12 h. The reaction mixture was treated with 0.1N NaOH (20 µl) for 2 min and then neutralized with 0.1N HCl (20 µl). Filtration over ion exchange resin (*Merck* No.II) and chromatography over *Sephadex* LH-20 as depicted for A). Iodinated α-MSH appeared as major peak (identical to **48** A), and then a minor peak with unreacted **49** was eluted. The chymotryptic analysis of **48** B gave identical results with **48** A.

Ac·Tyr(¹²⁵I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH₂, 2 AcOH (**51**). The original procedure [1] for preparing **50** has been improved by speeding up the isolation of the active ester; however, this step remains the critical point of the whole method. In summary, Ac·Tyr·OSu [1] was iodinated as described for **49** (reaction time: 20 s). The iodinated active ester was isolated immediately afterwards by extraction with $CHCl_3$; it was concentrated in a small conic glass vial, condensed with **38** and purified as above. The specific activity ranged between 500 and 1000 Ci/mmol.

Hpp(¹²⁵I)-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val·NH₂, 2 AcOH (**52**). Reaction of 2 mCi of Hpp(¹²⁵I)·OSu (*Bolton-Hunter* reagent; *NEN*) with **38** occurred in the same way as **49** and **50**. The product was isolated after 6 h, the MsOC group was eliminated, and **52** was purified over *Sephadex* LH-20. Specific radioactivity: 1000 to 2000 Ci/mmol.

Determination of the specific radioactivity of iodinated α-MSH. A highly specific goat anti-α-MSH serum [18b] was diluted 1:100,000 with a 0.05M sodium phosphate buffer (pH 7.4) containing 0.1% gelatine (white; *Merck*), 0.9% NaCl, and 0.01% merthiolate (sodium ethylmercurithiosalicylate; *Fluka*). Diluted tracer solutions (50 µl) containing 2% normal goat serum and 2000, 5000, 10,000, 20,000, 30,000, 40,000, or 50,000 cpm of **48** and 50 µl buffer containing 0, 5, 10, or 20 pg **26** were pipetted to 500 µl of the antiserum in polystyrene tubes (4 tubes for each concentration). Incubation for 3 days at 4°; separation of bound from unbound tracer with charcoal (details see [41]). The probes were counted in a γ-counter with a counting efficiency of 56%. The results are displayed in *Figure 4. AA* = 10,000 cpm (8 pCi) for 10 pg **48**. The specific activity of **48** was 1530 Ci/mmol.

Bioassay. – The melanotropic activities of **26, 28, 29, 32–34, 43–48, 51,** and **52** were determined with the modified [22] reflectometric test system of *Shizume et al.* [42] using the skin of the leopard frog, *Rana pipiens*. The tyrosinase assay was performed with *Cloudman* S-91 mouse melanoma cells in culture [4b].

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