225. Chemical Synthesis and Biological Activity of the Dogfish *(Squalus acanthias)* α-Melanotropins I and II, and of Related Peptides[1])

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In memoriam *Horst Hanson*, Halle

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Summary

The purpose of this investigation is to provide synthetic proof for the structure of dogfish *(Squalus acanthias)* α-MSH and to investigate the consequences of the presence of methionine in position 13 and the lack of the *N*-terminal acetyl group in this hormone. Because of the facile oxidation of methionine (13) during handling or storage, a number of specifically oxidized hormones (Met^4 and Met^{13}) as well as tripeptides belonging to the *C*-terminal second message sequence were investigated. All the products were prepared by classical methods in homogeneous solution; intermediate and end products were extensively purified and characterized *(Tables 3 and 4)*. The assays for melanotropic activity were performed *in vitro* with the modified reflectometric assay using the skin of *Rana pipiens*. It is concluded that the structures assigned to dogfish α-MSH I and II are correct and that the isolated samples contain slight quantities of [13-methionine(*S*-oxide)]dogfish α-MSH and [4,13-bis-methionine(*S*-oxide)]dogfish α-MSH. The peptides with methionine in position 13 are as active in this assay as those containing valine. This also holds for the $-NH_2/-OH$ interchange distinguishing dogfish α-MSH I from dogfish α-MSH II. However, the lack of the *N*-terminal acetyl group strongly reduces the biological activity. Its introduction into dogfish α-MSH I results in a product that is equipotent with mammalian α-MSH. These and other conclusions are discussed in detail.

[1]) Nomenclature and abbreviations [1]. Additional abbreviations are: ACTH = corticotropin, CLIP = corticotropin-like intermediate lobe peptide, LPH = lipotropin, MSH = melanotropin; Met(O) = L-methionine(R,S-*S*-oxide), $Met(O_2)$ = L-methionine(*S*-dioxide); DMF = *N*-dimethylformamide, DCCI = *N*,*N'*-dicyclohexylcarbodiimide, DCU = dicyclohexylurea, HOBT = 1-hydroxybenzotriazole; Ac = acetyl, BOC = *t*-butoxycarbonyl, MSOC = 2-(methylsulfonyl)-ethoxycarbonyl, NP = 4-nitrophenyl; RT. = (ambient) room temperature; i.V. = *in vacuo*. All chiral amino-acids are in the L-configuration except where indicated.

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Introduction. – α-Melanotropin (α-MSH) is presently the object of renewed, intense investigation, mainly because interesting, probably physiological functions in the brain and during the (human) embryonic state are being discovered [2]. Recent synthetic studies on structure/function relations have provided new insights into the one-dimensional organization of hormonal information in the mammalian-type α-MSH molecule [3]. This tridecapeptide hormone *(Table 1)* proved to be a typical example of a sychnologically organized molecule with *internally coded pleiotropic action* [4], much the same as ACTH. This means that different, sometimes overlapping amino-acid sequences within the peptide chain are responsible for causing different biological responses (triggering different receptors) in different target organs and cells [5]. Contrary to all previous experience with polypeptide hormones, α-MSH contains two independent message sequences, each of which is able to trigger the melanotropic response in amphibian skin [3a] [6]. One is the well-known 'classical' message, -His-Phe-Arg-Trp-(-H-F-R-W-), found as a common tetrapeptide sequence in ACTH, α-MSH, β-MSH, and β-LPH of all the species studied so far. The other is the tripeptide sequence, -Lys-Pro-Val- (-K-P-V-), found in ACTH and at the *C*-terminus of α-MSH. It consists of a basic (Lys), a conformation-restricting (Pro), and a hydrophobic (Val) amino-acid. The latter is present as an amide in all mammalian species.

The only known species-differences were found rather recently by *Lowry* and his colleagues in α-MSH from the elasmobranch, *Squalus acanthias* (dogfish): they consist in the loss of the *N*-terminal acetyl group (replacement by hydrogen) and in the exchange of the *C*-terminal valine amide against methionine amide (dogfish α-MSH I) or methionine with a free α-carboxy group (dogfish α-MSH II) [7].[3])

In this report, we describe the syntheses of *Squalus acanthias* (dogfish) α-MSH I (**14a**) and II (**15**), and of related peptides. The latter were chosen for the purpose of comparing biological activities and correlating them with structural changes, especially the presence or lack of acetyl and amide groups, the replacement of valine by methionine, and the oxidation of Met[4] and Met[13]. Thus, the compounds of *Table 2* were prepared and investigated biologically.

Table 1. *Structure of the α-melanotropins. (The amino-acid residues common to all known forms of α-MSH, β-MSH, ACTH, and LPH are shown in italics)*

1 2 3 4 5 6 7 8 9 10 11 12 13	
Ac · Ser-*Tyr*-Ser-Met-Glu-*His-Phe-Arg-Trp*-Gly-Lys-*Pro*-Val · NH_2	
α-MSH of mammalian origin: porcine [10], bovine [11], equine [12], simian [13], ovine [14], and camel [15]	
H · Ser-*Tyr*-Ser-Met-Glu-*His-Phe-Arg-Trp*-Gly-Lys-*Pro*-Met · NH_2	Dogfish α-MSH I (**14a**)
H · Ser-*Tyr*-Ser-Met-Glu-*His-Phe-Arg-Trp*-Gly-Lys-*Pro*-Met · OH	Dogfish α-MSH II (**15**)
α-MSH of elasmobranch origin: Squalus acanthias [7] [8]	

[3]) Originally [8], *Squalus acanthias* α-MSH I and II were believed to be the corresponding undecapeptides, lacking the dipeptide sequence H · Ser-Tyr- at the *N*-terminus. The undecapeptide amide was synthesized by *Watanabe et al.* [9], but it was impossible to remove all the Zn^{2+} introduced during the cleavage of the protecting groups. The biological activity *in vivo*, 4×10^8 U/g, was comparable to that of the isolated compound.

General aspects of the syntheses. – The syntheses were carried out in homogeneous solution, and the intermediates were, whenever possible or desirable, isolated, purified, and characterized. The strategies and tactics outlined in an early synthesis of mammalian α-MSH [16] were followed; however instead of the phthaloyl group, the more convenient 2-(methylsulfonyl)-ethoxycarbonyl (MSOC) group was used, as described in more recent work on α-MSH peptides [1].

Besides the two dogfish α-melanotropins a number of analogues with oxidized methionine residues were prepared (see *Table 2*). The synthetic dogfish α-MSH was more easily oxidized to sulfoxides, than synthetic mammalian α-MSH. Therefore [13-methionine(oxide)] dogfish α-MSH I (**14b**), [4, 13-bis-methionine(oxide)]dogfish α-MSH I (**17**), N^{α}-acetyl-[4, 13-bis-methionine(oxide)]dogfish α-MSH I (**21**), desacetyl-[4-methionine(oxide)]α-MSH (**18**), and [4-methionine(oxide)]α-MSH (**22**) were prepared. With the help of these compounds it was shown that an aged sample of dogfish α-MSH I[4]) contained some **14b** and less **17**, an indication that Met^{13} is very susceptible to oxidation. In order to exclude the possibility of an oxide exchange from Met^{13} to Met^{4}, [13-methionine(dioxide)] dogfish α-MSH I (**14c**) was prepared: its chemical and biological properties were closer to those of [13-methionine(oxide)]dogfish α-MSH I (**14b**) than to those of desacetyl-[4-methionine(oxide)]-α-MSH (**18**). Although not proof, this observation strengthens our argument that **14b** is not [4-methionine(oxide)]dogfish α-MSH I obtained through sulfoxide exchange $Met^{13} \rightarrow Met^{4}$. Therefore [4-methionine(oxide)]dogfish α-MSH I was not synthesized.

Synthetic dogfish α-MSH I was indistinguishable by TLC. from the main (unoxidized) component of the natural product[4]).

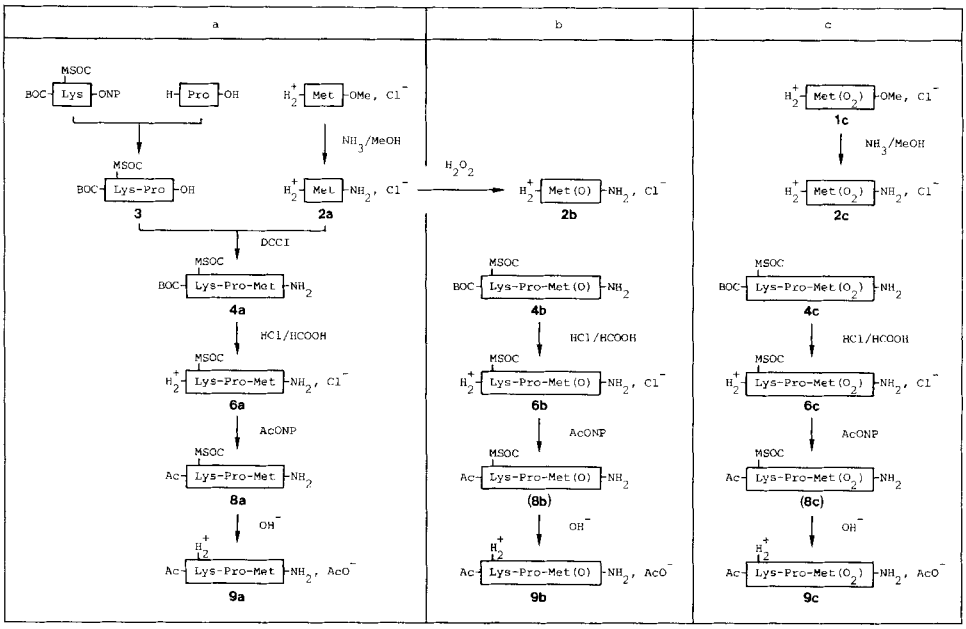
In order to facilitate comparisons of biological activities, all the tridecapeptides were also prepared in the N^{α}-acetylated form: **19a**, **19b**, **19c**, **21**, and **22** (see *Table 2*).

Finally, the acetylated tripeptides **9a**, **9b**, and **9c** were synthesized in order to evaluate the influence of the exchange of valine amide in the *C*-terminal message sequence, -Lys-Pro-Val · NH_2, against -Met · NH_2, -Met(O) · NH_2, and -Met(O_2) · NH_2. Even with these replacements, the tripeptides are still able to elicit the melanotropic response of the frog skins; this means that the second message sequence [6] is functional in unoxidized and oxidized dogfish α-MSH I.

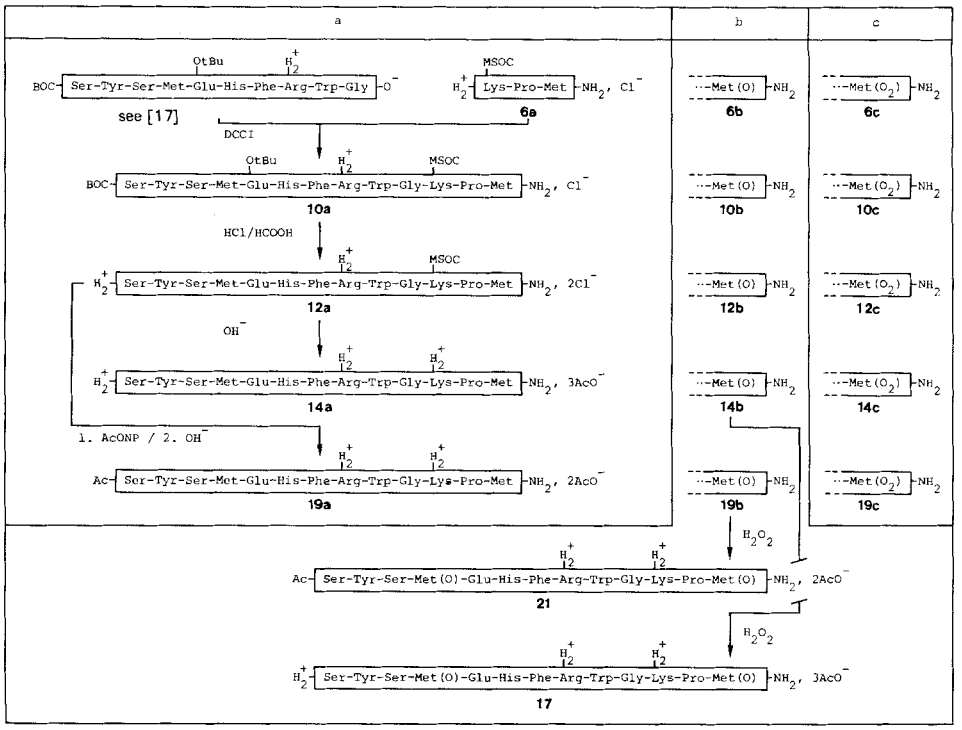
Special aspects of the syntheses. – The main difference from similar syntheses of mammalian-type α-MSH resides in the preparation of the *C*-terminal tripeptide sequences, **6a**, **6b**, and **6c** *(Scheme 1)*. Condensation with 4-nitrophenyl acetate and removal of the MSOC group with $Ba(OH)_2$ leads to the acetylated end products, **9a–c** *(Scheme 1)*, or to the tridecapeptides by condensation with the protected decapeptide, BOC · Ser-Tyr-Ser-Met-Glu(O*t*Bu)-His-Phe-Arg-Trp-Gly · OH [17], as shown in *Schemes 2 and 3*. Finally, mammalian-type α-MSH and desacetyl-α-MSH (described by *Guttmann & Boissonnas*[18])were freshly synthesized by a route shown in *Scheme 4* (similar to that of [1]). Methionine amide was prepared according to [19], its oxide by oxidation with H_2O_2. Separation of the diastereoisomers was not attempted, because acidic cleavage of the protecting groups would have racemized

[4]) Obtained through the courtesy of Dr. *P.J. Lowry*, Dept. of Chemical Pathology, St. Bartholomew's Hospital, London, England.

Scheme 1



Scheme 2



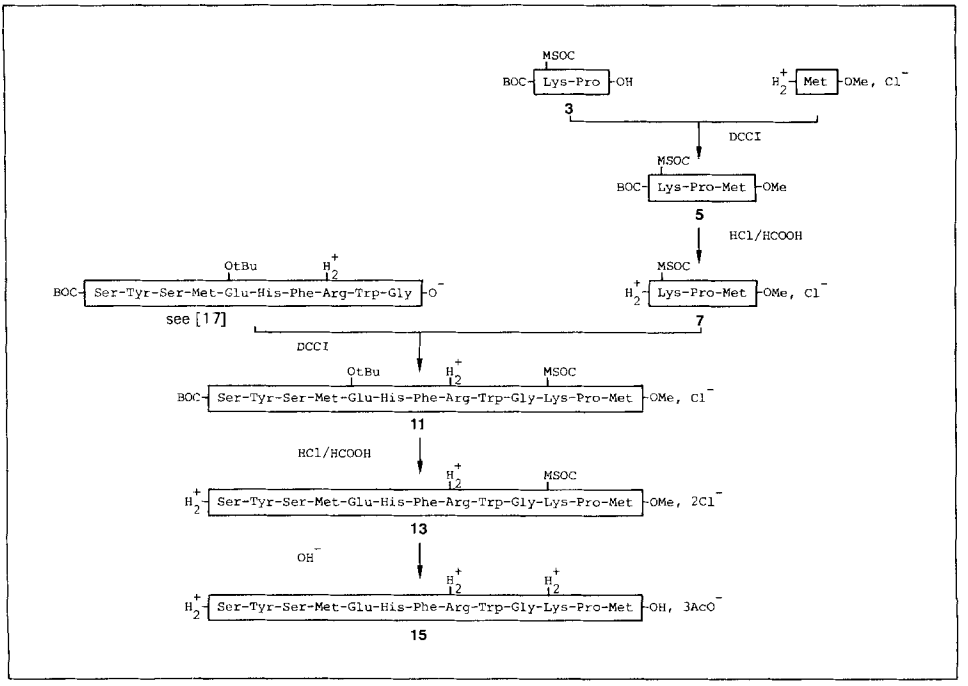
the chiral center on the sulfur as demonstrated by *Iselin* [20]. Methionine-*S*-dioxide was used as a starting material for the preparation of H · Met(O_2) · NH_2. The formation of side-products during tripeptide synthesis according to *Scheme 1* made it necessary to purify each stage very carefully; the acetylated tripeptides **9a**, **9b**, and **9c** were obtained absolutely pure after chromatography on Sephadex LH-20. However, they did not crystallize.

The syntheses of dogfish α-MSH I (**14a**) and its Met(O)[13] (**14b**) and Met(O_2)[13] (**14c**) analogues (series **a**, **b**, and **c**) are shown in *Scheme 2*. Acetylation of the selectively protected tridecapeptides **12a**, **b**, and **c** led to the corresponding N^{α}-acetyl-dogfish α-MSH I (**19a**) (differing only by the Val→Met exchange from mammalian α-MSH); oxidation of **19b** gave N^{α}-acetyl-[4, 13-bis-methionine (oxide)]dogfish α-MSH I (**21**), and oxidation of **14b**, [13-methionine (oxide)]dogfish α-MSH I, gave the bis-sulfoxide **17**.

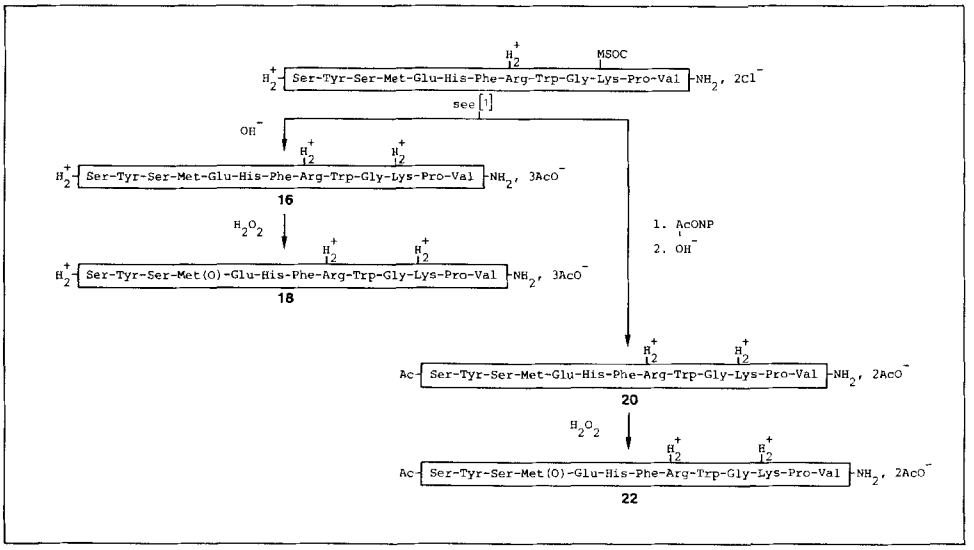
Dogfish α-MSH II (**15**) was prepared according to *Scheme 3* in a manner very similar to the synthesis of dogfish α-MSH I. The alkaline deprotection step **13**→**15** involved not only the removal of the MSOC group by β-elimination, but also the hydrolysis of the *C*-terminal methyl ester: however, the reaction was quantitative after 18 min with $Ba(OH)_2$ at RT. After purification the product **15** was indistinguishable from **14a** by TLC. in the solvent systems used routinely *(Table 4)* but exhibited a distinctly different behaviour on thin-layer electrophoresis (gain of 1 negative charge due to the carboxy group).

The reference compounds with the mammalian α-MSH sequence: desacetyl-α-

Scheme 3



Scheme 4



MSH (**16**) and its Met(O)[4] analogue (**18**), α-MSH (**20**) and its Met(O)[4] analogue (**22**) were prepared according to procedures described earlier [1] and here *(Scheme 4)*. We purified α-MSH with better yields than before [1] by repeated Sephadex LH-20 chromatography, thus avoiding the deleterious ion-exchange chromatography step (see experimental part).

Biological activity. – *Table 2* shows the melanotropic activities in the frog skin of 16 synthetic compounds and of natural dogfish α-MSH I and II.

Dogfish α-MSH I (**14a**), dogfish α-MSH II (**15**), and desacetyl mammalian α-MSH (**16**) have the same potency. This indicates that (i) the hydrophobic amino-acids valine and methionine are equivalent from the point of view of the *Rana pipiens* α-MSH receptors, and (ii) – rather unexpectedly – the replacement of the *C*-terminal, neutral amide by the charged carboxy group does not influence the activity. The synthetic materials were somewhat more active than previously reported [22] and than the natural material. (Probably due to its age, the latter was somewhat oxidized – *vide infra* – which could explain the slightly reduced potency.)

Oxidation of the methionine sulfur to the sulfoxide and sulfone stages invariably reduces the potency. Whether Met[13] is present as the R,S-*S*-oxide or as the *S*-dioxide makes no difference: the activity is reduced to 0.3 of the value for unoxidized dogfish α-MSH I (**14a**) in **14b** and **14c**, and to 0.25 of the value for unoxidized N^{α}-acetyl dogfish α-MSH I (or [Met[13]]α-MSH, **19a**) in the acetylated species **19b** and **19c**. Potency is even more susceptible to oxidation of Met[4]: desacetyl-[Met(O)[4]]α-MSH (**18**) and [Met(O)[4]]α-MSH (**22**) are only 0.13 and 0.05 times as potent as desacetyl-α-MSH (**16**) and α-MSH (**20**), respectively. The bis-(methionine-*S*-oxide)-analogues are the least active tridecapeptides: in **17** and **21** the potency is reduced to 0.067 and 0.023 times that of the unoxidized compounds, dogfish α-MSH I (**14a**) and [Met[13]]α-MSH (**19a**) respectively. This means that although

Table 2. *Melanotropic activities of* Squalus acanthias *(dogfish) α-MSH and related compounds determined with the modified* [6a] in vitro *reflectometric assay* [21] *using the skin of* Rana pipiens

No.	Amino-acid sequence[a])		MSH Units/mmol
14a	h · SYSMEHFRWGKPM · nh	dogfish α-MSH I	$3 \cdot 10^9$ ($2 \cdot 10^9$)[b])
14b	h · SYSMEHFRWGKPM̊ · nh		$9 \cdot 10^8$
14c	h · SYSMEHFRWGKPM̊̊ · nh		$9 \cdot 10^8$
15	h · SYSMEHFRWGKPM · oh	dogfish α-MSH II	$3 \cdot 10^9$ ($2 \cdot 10^9$)[b])
16	h · SYSMEHFRWGKPV · nh	desacetyl α-MSH	$3 \cdot 10^9$
17	h · SYSM̊EHFRWGKPM̊ · nh		$2 \cdot 10^8$
18	h · SYSM̊EHFRWGKPV · nh		$4 \cdot 10^8$
19a	ac · SYSMEHFRWGKPM · nh	[Met[13]]α-MSH	$4 \cdot 10^{10}$
19b	ac · SYSMEHFRWGKPM̊ · nh		$1 \cdot 10^{10}$
19c	ac · SYSMEHFRWGKPM̊̊ · nh		$1 \cdot 10^{10}$
20	ac · SYSMEHFRWGKPV · nh	α-MSH	$4 \cdot 10^{10}$
21	ac · SYSM̊EHFRWGKPM̊ · nh		$9 \cdot 10^8$
22	ac · SYSM̊EHFRWGKPV · nh		$2 \cdot 10^9$
9a	ac · KPM · nh		$5 \cdot 10^4$
9b	ac · KPM̊ · nh		$2 \cdot 10^4$
9c	ac · KPM̊̊ · nh		$2 \cdot 10^4$

[a]) h, oh, ac, and nh = H–, –OH, Ac–, and $-NH_2$, respectively, as used with the three-letter symbolism; M = Met(O), M̊ = Met(O_2).

[b]) Isolated, natural material kindly provided by Dr. *P.J. Lowry.*

Met[13] is part of the second message sequence, its oxidation alters the receptor interaction less than the oxidation of Met[4] in the *N*-terminal potentiating sequence (which has no direct capacity for triggering the receptor). This may reflect on one hand the relative insensitivity of the *C*-terminus towards a change of polarity (*vide supra:* **14a** *vs.* **15**), on the other the dependence of the potentiation effect on a good balance of hydrophobicity/hydrophilicity/hydrogen-bonding characteristics at the *N*-terminus [3a] [6].

In connection with the lower potency of the Met(O)[4] analogues the equivalence of the biological activities of the Met(O)[13] and the Met(O_2)[13] analogues means that most probably practically no oxide shift 13 → 4 has occurred.

In order to investigate the situation with respect to triggering *vs.* potentiation somewhat more closely, oxidized analogues of the *C*-terminal tripeptide message sequence of dogfish α-MSH I were prepared. Ac · Lys-Pro-Met · NH_2 (**9a**) is almost as active as the corresponding sequence from mammalian α-MSH, Ac · Lys-Pro-Val · NH_2, that was found to have $8 \cdot 10^4$ U/mmol in earlier experiments [6a]. Thus, the replacement of valine by methionine leaves intact the capacity of the tripeptide to act as a stimulus on *Rana pipiens* skin α-MSH receptors. Oxidation again reduces activity to 0.4 for both the oxide (**9b**) and the dioxide (**9a**). This reduction is practically the same as that observed with the corresponding tridecapeptides **14a**, **14b**, and **14c** *(vide supra).*

A *N*-terminal acetylation invariably enhances the melanotropic potency of peptides related to α-MSH [23]. This was first observed by *Guttmann & Boissonnas* [18] in the case of desacetyl-α-MSH (15-fold), by *Schwyzer* [23] for corticotropin-(1-24)-tetracosapeptide, and by *Eberle* [3a] [6] for numerous synthetic α-MSH fragments. Benzoylation of desacetyl-α-MSH enhanced its potency less than acetyla-

tion [23]. According to *Table 2*, the enhancement by acetylation is exactly the same for both dogfish α-MSH I (**14a**)→[Met^{13}]α-MSH (**19a**) and for desacetyl-α-MSH (**16**)→α-MSH (**20**), namely 13-fold. It is almost as strong in the case of the analogues with oxidized Met^{13} (11-fold for the oxides, **14b**→**19b**, and for the dioxides, **14c**→**19c**), but much less pronounced for the analogues with Met(O) in position 4 (4.5-fold for **17**→**21**, and 5-fold for **18**→**22**). These results again may reflect the special dependence of the *N*-terminal potentiator sequence, Ac · Ser-Tyr-Ser-Met-Glu-, as a whole on a correct balance of hydrophobic, hydrophilic, and hydrogen-bonding properties.

Conclusion. – From these studies we conclude that α-MSH peptides with *C*-terminal Met or Val are equipotent in the skin of the leopard frog, *Rana pipiens*. The lower potency in this assay of dogfish α-MSH as compared to mammalian-type α-MSH is quantitatively explained by one factor alone, the missing *N*-terminal acetyl group.

Whether the α-MSH receptors of *Squalus acanthias* as opposed to those of *Rana pipiens* recognize the desacetyl series better than the acetyl series (*e.g.* through an evolutionary optimization of their structure) is an open question; to answer it, an assay with elasmobranch skin instead of frog skin would have to be developed. The physiological reason for using unacetylated molecules as hormones might – as *Lowry & Scott* [24] have suggested – reside in differences of fine control over the α-MSH effects between elasmobranchs and mammals. Such a control might be facilitated with unacetylated hormones, because they would be expected to be more rapidly destroyed by enzymatic degradation than the acetylated ones.

The comparison of synthetic and natural dogfish α-MSH by TLC. indicates chemical identity. However the isolated material contains some oxidized compounds indistinguishable on TLC. from **14b** and **17**. The latter is present in a smaller amount than the former, indicating a more rapid oxidation of Met^{13}. The presence of these materials would suffice to explain the reduced potency.

In the light of the fact that Met peptides are as potent as Val peptides, one cannot be sure whether or not dogfish α-MSH I and II are the only melanotropic peptides of *Squalus acanthias* (besides, of course, ACTH). The undecapeptides, H · Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · X (X = –OH or $-NH_2$), postulated earlier [8], might well be present, because the activity of the isolated sample corresponded well not only to that of a synthetic product[3]) but also to that of mammalian-type α-MSH undecapeptides [3a] [18].

This work further confirms and extends the discovery [6a] that the *C*-terminal tripeptide sequence of α-MSH is a message sequence capable of triggering the frog skin receptors.

Experimental Part

General remarks. 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 *(W. 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 6 N HCl containing 4% thioglycolic acid.

Table 3. *Analytical data of the C-terminal tripeptides* **1** *to* **9**

Compound No.	Structure (formula; M.W.)	m.p. °C	$[\alpha]_D^{25}$ (c) solvent	TLC. (Rf)					Elemental Analysis %calc. % found				Amino Acid Analysis (reference amino acid in italics)
				BAW	BNH_3	CM	BPAW1	BPAW2	C	H	N	S	
1c	H · Met(O_2) · OMe, HCl ($C_6H_{14}ClNO_4S$ 231.70)	164	23.2 (1) 0.1N HCl				0.48		31.10 31.18	6.09 6.05	6.05 6.03	13.84 13.82	
			14.5 (1) DMF										
2a	H · Met · NH_2, HCl ($C_5H_{13}ClN_2OS$ 184.69)	206–208	16.4 (1) 0.1N HCl	0.30	0.48		0.56		32.52 32.45	7.10 7.15	15.17 15.03	17.36 17.23	
			25.5 (1) DMF										
2b	H · Met(O) · NH_2, HCl ($C_5H_{13}ClN_2O_2S$ 200.69)	144–145	20.3 (1) 0.1N HCl		0.19		0.23		29.92 29.75	6.53 6.59	13.96 13.71	15.98 15.75	
			21.2 (1) DMF										
2c	H · Met(O_2) · NH_2, HCl ($C_5H_{13}ClN_2O_3S$ 216.69)	170–172	19.2 (1) 0.1N HCl	0.13	0.26		0.30		27.72 27.79	6.05 6.12	12.93 12.80	14.80 14.70	
			−9.5 (1) DMF										
3	BOC · Lys(MSOC)-Pro · OH ($C_{20}H_{35}N_3O_9S$ 493.58)	75–77	−38.2 (1) MeOH	0.46		0.48	0.65	0.63	48.67 48.72	7.15 7.13	8.51 8.38		
4a	BOC · Lys(MSOC)-Pro-Met · NH_2 ($C_{25}H_{45}N_5O_9S_2$ 623.69)	60–65	−51.0 (1) MeOH	0.51		0.75	0.81		48.13 48.02	7.27 7.36	11.23 11.14	10.28 10.20	*K 1.00/* P 1.05/ M 0.95

4b	BOC · Lys(MSOC)-Pro-Met(O) · NH_2 ($C_{25}H_{45}N_5O_{10}S_2$ 639.79)	84–85	−42.2 (0.5) MeOH	0.23		0.59		46.93 46.75	7.09 6.93	10.95 10.74	10.02 9.91	*K 1.00/* P 1.02/ M 0.96
4c	BOC · Lys(MSOC)-Pro-Met(O_2) · NH_2 ($C_{25}H_{45}N_5O_{11}S_2$ 655.79)	87–88	−36.6 (0.5) MeOH	0.32		0.61		45.79 45.67	6.92 6.95	10.68 10.55	9.78 9.70	*K 1.00/* P 1.05/ M′ 0.99
5	BOC · Lys(MSOC)-Pro-Met · OMe ($C_{26}H_{46}N_4O_{10}S_2$ 638.81)	–	−26.6 (1.5) MeOH			0.69	0.67					
6a	H · Lys(MSOC)-Pro-Met · NH_2, HCl ($C_{20}H_{38}ClN_5O_7S_2$ 560.14)	56–60	−29.4 (1) MeOH	0.19	0.20	0.41	0.53	42.89 42.65	6.84 6.71	12.50 12.30	11.45 11.31	
6b	H · Lys(MSOC)-Pro-Met(O) · NH_2, HCl ($C_{20}H_{38}ClN_5O_8S_2$ 576.14)	75–80	−14.4 (0.5) MeOH	0.05		0.31		41.70 41.81	6.65 6.71	12.16 12.01		
6c	H · Lys(MSOC)-Pro-Met(O_2) · NH_2, HCl ($C_{20}H_{38}ClN_5O_9S_2$ 592.14)	125–130	−14.0 (0.6) MeOH	0.07		0.36		40.57 40.53	6.47 6.40	11.83 11.69		
7	H · Lys(MSOC)-Pro-Met · OMe, HCl ($C_{21}H_{39}ClN_4O_8S_2$ 575.15)	122–124	−48.5 (1) MeOH	0.37		0.56	0.59	43.86 43.68	6.83 6.71	9.74 9.52		*K 1.00/* P 1.08/ M 0.93
8a	Ac · Lys(MSOC)-Pro-Met · NH_2 ($C_{22}H_{39}N_5O_8S_2$ 565.71)	–	−49.5 (1) MeOH			0.65	0.69	46.71 46.65	6.95 6.83	12.38 12.19	5.67 5.54	
9a	Ac · Lys-Pro-Met · NH_2, AcOH ($C_{20}H_{37}N_5O_6S$ 475.61)	–	−64.5 (1) 1% AcOH			0.41	0.45					*K 1.00/* P 1.06/ M 0.98
9b	Ac · Lys-Pro-Met(O) · NH_2, AcOH ($C_{20}H_{37}N_5O_7S$ 491.61)	–	−55.7 (1) 1% AcOH			0.22	0.22					*K 1.00/* P 1.02/ M 0.90
9c	Ac · Lys-Pro-Met(O_2) · NH_2, AcOH ($C_{20}H_{37}N_5O_8S$ 507.61)	–	−50.0 (1) 1% AcOH			0.29	0.32					*K 1.00/* P 1.07/ M′ 1.02

Table 4. *Analytical data of the tridecapeptides* **10** *to* **22**

Com-pound No.	Structure (formula; M.W.)	$[\alpha]_D^{25}$ (c) solvent	UV. λ_{max} (ε) solvent (sh = shoulder)	TLC. (Rf) BPAW1	BPAW2	TLE. (R Arg) pH 3.6	pH 5.6	Amino Acid Analysis (reference amino acid in italics)
10a	BOC · Ser-Tyr-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met · NH_2, HCl ($C_{88}H_{130}ClN_{21}O_{24}S_3$ 1997.79)	−28.5 (0.33) AcOH	279 (7000) 289 (5800) sh 10% AcOH	0.70	0.69			S 1.87/Y 1.00/M 1.81/E 1.04/ H 1.00/F 0.98/R 0.98/W 1.02/ *G 1.00*/K 0.90/P 1.06
10b	BOC · Ser-Tyr-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met(O) · NH_2, HCl ($C_{88}H_{130}ClN_{21}O_{25}S_3$ 2013.79)	−27.3 (0.45) AcOH	279 (6500) 289 (4800) sh 10% AcOH	0.56	0.55			S 1.78/Y 1.02/M 1.91/E 1.04/ H 1.02/F 1.00/R 1.04/W 0.91/ *G 1.00*/K 1.05/P 1.08
10c	BOC · Ser-Tyr-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met(O_2) · NH_2, HCl ($C_{88}H_{130}ClN_{21}O_{26}S_3$ 2029.79)	−26.4 (0.33) AcOH	279 (6525) 289 (4820) sh 10% AcOH	0.63	0.60			S 1.78/Y 0.99/M 1.01/E 1.07/ H 0.98/F 1.01/R 1.04/W 0.95/ *G1.00*/K 1.04/P 1.05/M′ 0.96
11	BOC · Ser-Tyr-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met · OMe, HCl ($C_{89}H_{131}ClN_{20}O_{25}S_3$ 2012.80)	−26.1 (0.33) AcOH	279 (6820) 289 (5630) sh 10% AcOH	0.73	0.68			S 1.75/Y 1.03/M 1.85/E 1.02/ H 0.95/F 0.98/R 1.06/W 0.85/ *G1.00*/K 1.06/P 1.08
12a	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met · NH_2, 2 HCl ($C_{79}H_{115}Cl_2N_{21}O_{22}S_3$ 1878.03)	−29.3 (0.27) 80% AcOH	280 (6110) 289 (4640) sh 10% AcOH	0.40	0.38			S 1.85/Y 0.96/M 1.71/E 1.10/ H 0.92/F 1.01/R 1.02/W 0.95/ *G1.00*/K 1.07/P 1.05
12b	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met(O) · NH_2, 2 HCl ($C_{79}H_{115}Cl_2N_{21}O_{23}S_3$ 1894.03)			0.34	0.32			S 1.81/Y 1.05/M 1.68/E 1.08/ H 1.02/F 1.03/R 1.05/W 0.88/ *G1.00*/K 1.06/P 1.01
12c	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met(O_2) · NH_2, 2 HCl ($C_{79}H_{115}Cl_2N_{21}O_{24}S_3$ 1910.03)			0.38	0.35			S 1.88/Y 0.96/M 0.95/E 1.02/ H 0.96/F 0.99/R 1.02/W 0.85/ *G 1.00*/K 1.01/P 0.97/M′ 0.92
13	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met · OMe, 2 HCl ($C_{80}H_{116}Cl_2N_{20}O_{23}S_3$ 1893.04)			0.45	0.42			S 1.75/Y 0.99/M 1.78/E 0.99/ H 0.95/F 0.95/R 1.02/W 0.81/ *G 1.00*/K 0.98/P 0.95
14a	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · NH_2, 3 AcOH ($C_{81}H_{119}N_{21}O_{24}S_2$ 1835.11)	−66.3 (0.33) 1% AcOH	281 (6815) 289 (6583) 0.1N NaOH	0.32	0.28	0.80	0.66	S 1.85/Y 1.01/M 1.79/E 1.09/ H 0.98/F 1.02/R 1.07/W 0.82/ *G 1.00*/K 1.04/P 1.06
14b	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O) · NH_2, 3 AcOH ($C_{81}H_{119}N_{21}O_{25}S_2$ 1851.11)	−59.6 (0.25) 1% AcOH	281 (6800) 289 (6545) 0.1N NaOH	0.28	0.23	0.64	0.63	S 1.80/Y 1.02/M 1.83/E 0.99/ H 0.95/F 0.98/R 1.01/W 0.87/ *G 1.00*/K 1.01/P 1.02

14c	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O_2) · NH_2, 3 AcOH ($C_{81}H_{119}N_{21}O_{26}S_2$ 1867.11)	−57.0 (0.33) 1% AcOH	281 (6685) 289 (6460) 0.1N NaOH	0.30	0.27	0.66	0.58	S 1.75/Y 1.01/M 0.89/E 1.01/ H 0.94/F 1.02/R 1.02/W 0.88/ *G 1.00*/K 1.02/P 1.03/M′ 1.03
15	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · OH, 3 AcOH ($C_{81}H_{118}N_{20}O_{25}S_2$ 1836.09)	−61.5 (0.33) 1% AcOH	281 (6945) 289 (6660) 0.1N NaOH	0.32	0.29	0.53	0.52	S 1.78/Y 1.02/M 1.92/E 1.05/ H 0.96/F 1.01/R 1.03/W 0.92/ *G 1.00*/K 1.02/P 1.07
16	H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val · NH_2, 3 AcOH ($C_{81}H_{119}N_{21}O_{24}S$ 1803.04)	−56.5 (0.2) 1% AcOH	281 (6880) 289 (6570) 0.1N NaOH	0.32	0.28	0.80	0.66	S 1.81/Y 0.98/M 0.95/E 1.04/ H 0.98/F 1.03/R 1.08/W 0.89/ G 1.02/K 1.03/P 1.03/*V 1.00*
17	H · Ser-Tyr-Ser-Met(O)-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O) · NH_2, 3 AcOH ($C_{81}H_{119}N_{21}O_{26}S_2$ 1867.11)	−54.0 (0.2) 1% AcOH	281 (6857) 289 (6570) 0.1N NaOH	0.22	0.17	0.64	0.61	S 1.80/Y 1.03/M 1.86/E 1.08/ H 1.02/F 1.04/R 1.01/W 0.90/ *G 1.00*/K 0.99/P 0.98
18	H · Ser-Tyr-Ser-Met (O)-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val · NH_2, 3 AcOH ($C_{81}H_{119}N_{21}O_{25}S$ 1819.04)	−55.0 (0.2) 1% AcOH	281 (6610) 289 (6405) 0.1N NaOH	0.25	0.18	0.77	0.63	S 1.72/Y 0.99/M 0.91/E 1.04/ H 0.92/F 1.02/R 1.08/W 0.86/ G 1.03/K 1.08/P 1.10/*V 1.00*
19a	Ac · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{23}S_2$ 1817.10)	−63.0 (0.27) 1% AcOH	281 (6940) 289 (6710) 0.1N NaOH	0.35	0.33	0.61	0.58	S 1.66/*Y 1.00*/M 1.89/E 1.16/ H 1.02/F 1.02/R 0.92/W 0.97/ G 1.16/K 0.92/P 1.13
19b	Ac · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O) · NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{24}S_2$ 1833.10)	−54.0 (0.25) 1% AcOH	281 (6730) 289 (6435) 0.1N NaOH	0.26	0.24	0.60	0.53	S 1.69/Y 0.98/M 1.81/E 1.12/ H 1.03/F 1.01/R 0.99/W 0.91/ *G 1.00*/K 0.99/P 1.08
19c	Ac · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O_2) · NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{25}S_2$ 1849.10)	−52.8 (0.33) 1% AcOH	281 (6666) 289 (6420) 0.1N NaOH	0.30	0.28	0.60	0.53	S 1.78/Y 1.03/M 0.96/E 1.09/ H 0.95/F 1.02/R 1.08/W 0.88/ *G 1.00*/K 1.07/P 1.08/M′ 0.96
20	Ac · Ser-Tyr-Ser-Met-Glu-His-Phe-Trp-Gly-Lys-Pro-Val · NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{23}S$ 1785.02)	−63.3 (0.33) 1% AcOH	281 (6860) 289 (6550) 0.1N NaOH	0.35	0.33	0.61	0.58	S 1.83/Y 1.02/M 0.91/E 1.04/ H 0.96/F 0.99/R 1.04/W 0.83/ G 1.02/K 1.05/P 1.15/*V 1.00*
21	Ac · Ser-Tyr-Ser-Met(O)-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O) · NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{25}S_2$ 1849.10)	−50.8 (0.25) 1% AcOH	281 (6562) 289 (6250) 0.1N NaOH	0.23	0.18	0.60	0.53	S 1.78/Y 0.99/M 1.80/E 1.10/ H 0.93/F 1.01/R 1.08/W 0.78/ *G 1.00*/K 1.05/P 1.11
22	Ac · Ser-Tyr-Ser-Met(O)-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val · NH_2, 2 AcOH ($C_{81}H_{117}N_{21}O_{24}S$ 1801.02)	−61.5 (0.33) 1% AcOH	281 (6820) 289 (6515) 0.1N NaOH	0.28	0.24	0.60	0.57	S 1.80/Y 1.03/M 0.92/E 1.14/ H 0.98/F 1.04/R 1.09/W 0.83/ G 1.04/K 1.02/P 1.13/*V 1.00*

This procedure reduced methionine sulfoxide, Met(O), to methionine, whereas methionine sulfone, Met(O_2), remained unchanged. IR. spectra ($\tilde{\nu}$ as cm^{-1}) and UV. spectra (λ_{max} as nm, ε values in parentheses) were recorded with *Beckman* IR 33 and Acta V spectrometers, respectively. Optical activity was determined with a *Perkin-Elmer* 141 polarimeter. Thin-layer chromatography (TLC.) was carried out with *Merck* silica gel plates using the following solvent systems: CM = chloroform/methanol 1:1, BAW = 2-butanol/acetic acid/water 72:7:21, BNH_3 = 2-butanol/3% ammonia 100:44, BPAW1 = 1-butanol/pyridine/acetic acid/water 50:12:12:25, BPAW2 = solvents of BPAW1, but 42:24:4:30. Thin-layer electrophoresis (TLE.) was performed with *Merck* cellulose plates using an apparatus of *Camag* and the following buffers: pH 3.6 = acetic acid/pyridine/water 100:10:890, and pH 5.6 = acetic acid/pyridine/water 6:23:970. The compound spots on TLC. and TLE. plates were detected with iodine vapor, ninhydrin, and *Reindel-Hoppe* reagents. The usual isolation procedure consisted in dissolving the products in an organic solvent poorly miscible with water, washing the solution consecutively 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 with anhydrous Na_2SO_4, and evaporating it at about 10 Torr in a rotatory evaporator. The analytical data are displayed in *Tables 3* (*C*-terminal tripeptides) and *4* (tridecapeptides). The [13-methionine-*S*-oxide]melanotropin analogues (series **b**) and the [13-methionine-*S*-dioxide]melanotropin analogues (series **c**) were synthesized in the same manner as the methionine-containing parent compounds (series **a**) with the exception of the BOC elimination that was carried out with HCl in formic acid without added thiol. The syntheses of **11** and **13** were practically identical with those of **10a** and **12a** and are therefore not recorded explicitly.

L-*Methionine*-S-*dioxide methyl ester hydrochloride* (**1c**). L-Methionine-*S*-dioxide (5 g, 27.6 mmol, *Fluka*) was esterified by the thionyl chloride procedure [25] (15 ml methanol, 23 ml thionyl chloride, heated under reflux for 4 h). After evaporation to dryness, the residue was crystallized from methanol/ether at 4°. Yield 5.65 g (88%) **1c** as colourless needles. – IR. (Nujol): 1110, 1250 ($-SO_2-$).

L-*Methionine amide hydrochloride* (**2a**) was prepared in a manner analogous to [19]. A methanolic solution of the crude product was filtered through Amberlyst A-21 (weakly basic) and evaporated to dryness. The residue was then dissolved in 1 eq. of pyridine hydrochloride in ethanol and, after evaporation of the solvent, crystallized from peroxide-free methanol/ethyl acetate. Yield 91% **2a**.

L-*Methionine*-S-*oxide amide hydrochloride* (**2b**), compare [20]. L-Methionine amide hydrochloride (**2a**, 460 mg) was dissolved in methanol (5 ml) and treated with 35% H_2O_2-solution (0.25 ml). After 2 h at RT. the mixture was evaporated to a small volume, diluted with 2-propanol, and crystallized at 4°. The very hygroscopic product was recrystallized from methanol/2-propanol. Yield 395 mg (79%) **2b**. – IR. (Nujol): 1000 ($-SO-$).

L-*Methionine*-S-*dioxide amide hydrochloride* (**2c**) was prepared from **1c** in a manner analogous to **2a**. Yield 98%. – IR. (Nujol): 1110, 1250 ($-SO_2-$).

BOC · Lys(MSOC)-Pro · OH (**3**). BOC · Lys(MSOC) · ONP (2.07 g, 4 mmol) [1] was dissolved in DMF (15 ml) and treated with a solution of L-proline (0.5 g, 4.4 mmol) and triethylamine (0.55 ml, 4.4 mmol) in water (1.2 ml). After 24 h at RT. the mixture was evaporated at 10^{-3} Torr, the residue dissolved in a small volume of chloroform/methanol 9:1 and chromatographed on a silica gel column (50 × 3 cm). The residue obtained after solvent evaporation was crystallized from 2-propanol/diisopropyl ether: 1.9 g (96%) **3**.

BOC · Lys(MSOC)-Pro-Met · NH_2 (**4a**). A solution on *N*-ethyl morpholine (0.4 ml, 3.2 mmol), **2a** (0.59 g, 3.2 mmol), and **3** (1.48 g, 3 mmol) in DMF (10 ml) was treated with HOBT (0.43 g, 3.2 mmol) and then DCCI (0.66 g, 3.2 mmol). After 36 h at RT., the crystalline DCU was removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in $CHCl_3$ and subjected to the usual isolation procedure. The product was chromatographed on silica gel (50 × 3 cm) with $CHCl_3$/methanol (9:1) and then crystallized from 2-propanol/diisopropyl ether. Yield 1.56 g (83%) **4a**.

BOC · Lys(MSOC)-Pro-Met · OMe (**5**). Preparation analogues to **4a**. The pure product was obtained as an oil in 94% yield.

H · Lys(MSOC)-Pro-Met · NH_2, HCl (**6a**). A solution of **4a** (1.2 g, 1.9 mmol) in a mixture of 0.12N HCl in formic acid/mercaptoethanol/anisole 10:1:1 was kept for 15 min at RT. and then evaporated to dryness. The residue was chromatographed on silica gel (70 × 2.5 cm) with $CHCl_3$/methanol 7:3. The pure product was dissolved in methanol and the solvent evaporated i.V.; the solution/evaporation procedure was repeated a few times. The final product crystallized from methanol/ether. Yield 1.0 g (93%) **6a**.

H · Lys(MSOC)-Pro-Met · OMe, HCl (**7**). Cleavage of the BOC group from **5** and purification analogous to **4a** → **6a**. Crystallization from 2-propanol/diisopropyl ether. Yield 70% **7**.

Ac · Lys(MSOC)-Pro-Met · NH₂ (**8a**). 4-Nitrophenyl acetate (145 mg, 0.8 mmol; *Merck*), *N*-ethyl morpholine (58 mg, 0.5 mmol), and **6a** (280 mg, 0.5 mmol) reacted for 24 h at RT. in DMF (3 ml). After evaporation of the solvent at 10^{-3} Torr, the residue was precipitated and reprecipitated from methanol/ether as a very hygroscopic, colourless solid that was dried over P_2O_5. Yield 255 mg (90%) **8a**.

Ac · Lys-Pro-Met · NH₂, AcOH (**9a**). A solution of **8a** (266 mg, 0.4 mmol) in 1 ml methanol was treated with 0.1N $Ba(OH)_2$ (6 ml). After 5 min at RT. the mixture was neutralized with 0.2N H_2SO_4 (3 ml). The precipitated $BaSO_4$ was eliminated by centrifugation, the supernatant acidified to pH ~ 4 with dilute acetic acid and filtered through a small column of Dowex 3 with 1% acetic acid. The product was purified by chromatography over Sephadex LH 20 (80×3 cm) in 1% acetic acid as eluent. The fractions pure by TLC. were combined and lyophilized. Yield: 161 mg (84%) of colourless, resinous **9a**.

BOC · Ser-Tyr-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met · NH₂, HCl (**10a**). A solution of BOC · Ser-Tyr-Ser-Met-Glu(OtBu)-His-Phe-Arg-Trp-Gly · OH [17] (291 mg, 0.2 mmol) and **6a** (118 mg, 0.21 mmol) in DMF (4 ml) was prepared at 40° and then cooled to RT. and treated consecutively with HOBT (54 mg, 0.4 mmol), and DCCI (50 mg, 0.24 mmol). After 3 days at RT. one drop of glacial acetic acid was added, the mixture cooled, and the DCU filtered off. The filtrate was evaporated to dryness at 10^{-3} Torr; the residue was chromatographed over Sephadex LH 20 (100×3 cm) in DMF/water (9:1). The pure fractions (TLC.) were combined and evaporated to a small volume at 10^{-3} Torr whereupon the product was precipitated with ether as a colourless, amorphous solid. Yield: 360 mg (90%) **10a**.

H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Met · NH₂, 2 HCl (**12a**). A solution of **10a** (203 mg, 0.1 mmol) in 0.12N HCl in formic acid (4 ml, containing 10% each of anisole and mercaptoethanol) was kept at RT. for 15 min and then evaporated at low temperature. The residue was dissolved in methanol/water and isolated by evaporation of the solvent *in vacuo*. After several repetitions of the solution/evaporation process, the residue was triturated with ether. Yield: 185 mg (98%) of **12a** as a colourless powder that contains a minute contaminant with a larger Rf on TLC.

Dogfish α-MSH I, H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · NH₂, 3 AcOH (**14a**). A solution of **12a** (92 mg, 0.05 mmol) in 0.1N $Ba(OH)_2$ (2.5 ml) was kept at RT. for 5 min. Addition of 0.1N H_2SO_4 (2.5 ml) at 0° precipitated the barium as sulfate. After centrifugation at pH 4, the solution was applied to a column (20×1.5 cm) of CM-Sephadex C 25. The column was washed thoroughly with 0.1M trimethylammonium acetate buffer at pH 7, and the chromatogram then developed with a linear gradient, 0.1→1.0M, of trimethylammonium acetate buffer, pH 7. The pure fractions (TLC.) were combined and most of the buffer removed by evaporation and repeated dissolution in water and evaporation. The last traces of buffer were removed by chromatography of the product in 1% acetic acid over Sephadex LH 20 (100×3 cm). Yield 46 mg (51%) of lyophilized, pure **14a**. The ratio Tyr/Trp was found to be 1.01 (spectroscopic determination in NaOH).

Dogfish α-MSH II, H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · OH, 3 AcOH (**15**). A solution of **13** (95 mg, 0.05 mmol, *Scheme 3*) in 0.1N $Ba(OH)_2$ (5 ml) was kept for 18 min at RT. to remove the MSOC and OMe groups. The product was isolated and purified in the same manner as **14a**. Yield 45 mg (50%) of colourless, pure **15** after lyophilization. Tyr/Trp = 1.04 (spectroscopic determination in NaOH).

Des-acetyl α-MSH, H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val · NH₂, 3 AcOH (**16**). H · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys(MSOC)-Pro-Val · NH_2, 2 HCl [1] (92 mg, 0.05 mmol) was treated with 0.1N $Ba(OH)_2$ (2.5 ml) followed by H_2SO_4 as described for **14a**. The product was chromatographed twice over Sephadex LH 20 with 1% acetic acid. Yield: 80 mg (90%) of pure, lyophilized **16**. Tyr/Trp = 0.99 (spectroscopic determination in NaOH).

H · Ser-Tyr-Ser-Met(O)-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met(O) · NH₂, 3 AcOH (**17**). A solution of **14b** (18.5 mg, 10 μmol, *Scheme 2*) in glacial acetic acid (1 ml) was treated with 1M H_2O_2 (15 μl) and kept over night at RT. The mixture was chromatographed over Sephadex LH 20 (100×2 cm) with 1% acetic acid, and the pure **17** separated from about 10% of unchanged educt, **14b**. Yield: 15 mg (81%).

Similar procedures were used to oxidize **16**, **19b**, and **20** to their 4-methioninesulfoxide analogues, **18**, **21**, and **22** (*Schemes 2* and *4*).

Ac · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met · NH₂, 2AcOH (**19a**). A solution of **12a** (188 mg, 0.1 mmol) and *N*-ethyl morpholine (11.5 mg, 0.1 mmol) in DMF (2 ml) was treated for 36 h at RT. with 4-nitrophenyl acetate (30 mg, 0.17 mmol). The mixture was evaporated at 10^{-3} Torr to a small volume, and the product precipitated with diisopropyl ether: 170 mg (90%), Rf = 0.56 (BPAW 1), 0.54 (BPAW 2), single spots. To remove the MSOC group, the solution of this product in a very small amount

of DMF was treated for 6 min at RT. with 0.1N $Ba(OH)_2$ (3 ml) and then neutralized with 0.1N H_2SO_4. Isolation and purification as for **14a**. Yield: 81 mg (43%). Tyr/Trp = 1.02 (spectroscopic determination in NaOH).

α-MSH, Ac · Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val · NH_2, 2AcOH (**20**). The synthesis of α-MSH has been reported in detail [1], so that only a simplified purification procedure giving higher yields is reported here. Instead of using a CM-Sephadex C 25 ion exchange column, the crude product was chromatographed twice or thrice with 1% acetic acid over Sephadex LH 20 (100 × 3 cm). Yields: 80–90% of **20**, pure by all criteria. Tyr/Trp = 1.01 (spectroscopic determination in NaOH).

Bioassay. The melanotropic activities of **9a**, **9b**, **9c**, **14a**, **14b**, **14c**, **15**, **16**,**17**,**18**,**19a**, **19b**, **19c**, **20**, **21**, and **22** were determined with the modified [6a] reflectometric test system of *Schizume et al.* [21] using the skin of the leopard frog, *Rana pipiens.*

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