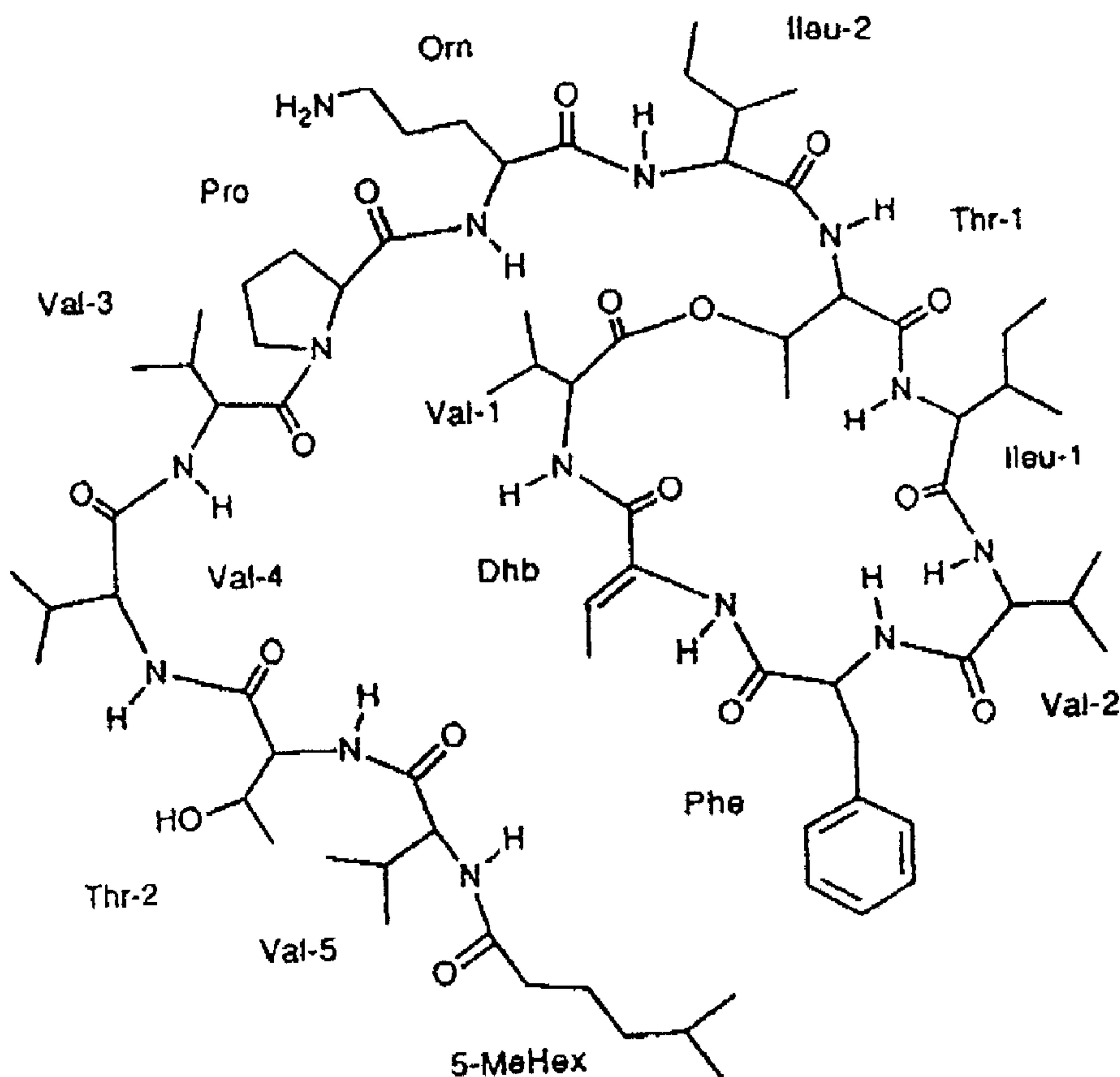




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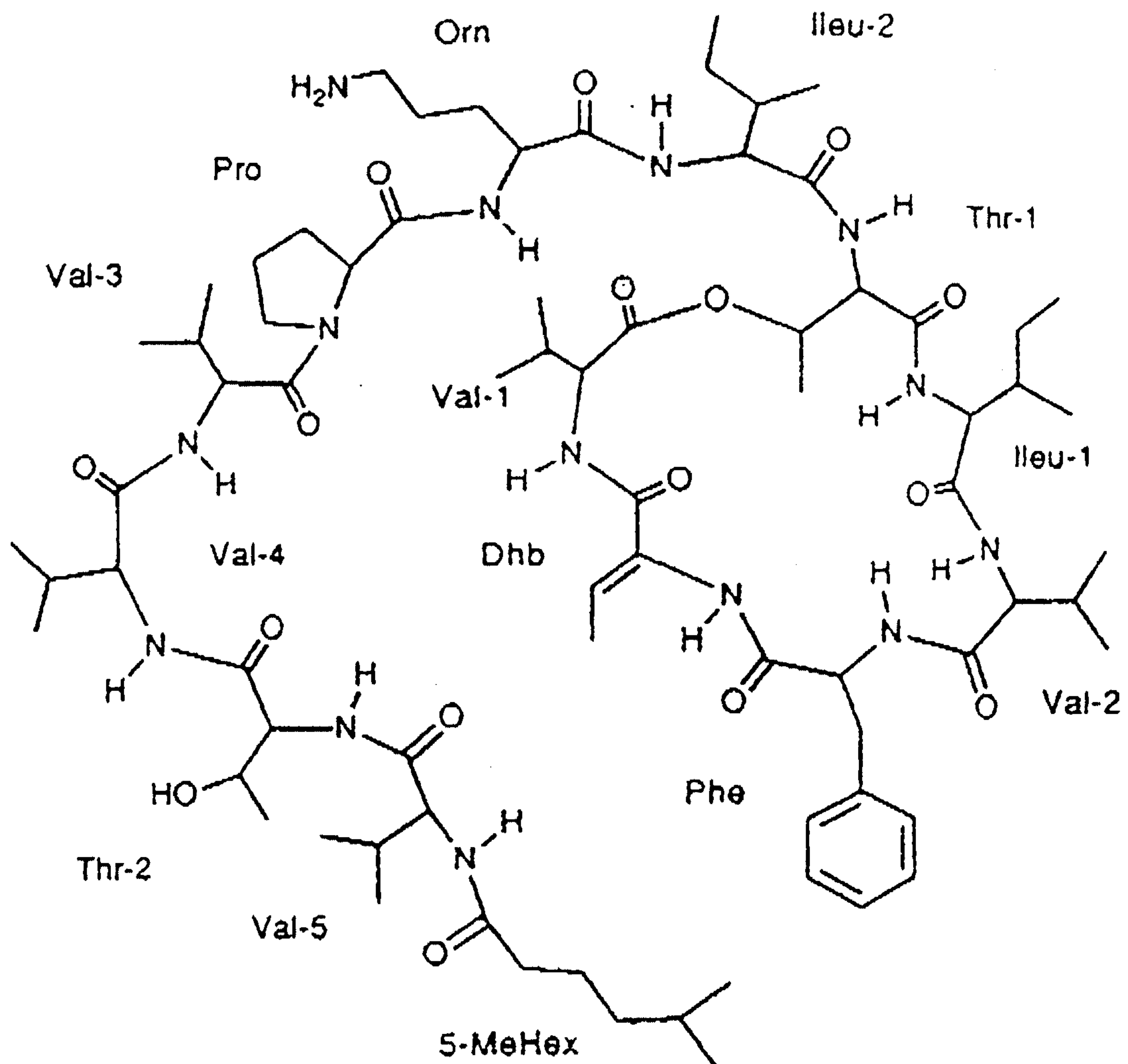
(57) Abrégé/Abstract:

Kalahide F, of formula I below, may be isolated from a sacoglossan. The compound may be used in the manufacture of pharmaceutical compositions or in the treatment of tumors or viral conditions. (see above formula)

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ABSTRACT

Kalahide F, of formula I below, may be isolated from a sacoglossan. The compound may be used in the manufacture of pharmaceutical compositions or in the treatment of tumors or viral conditions.

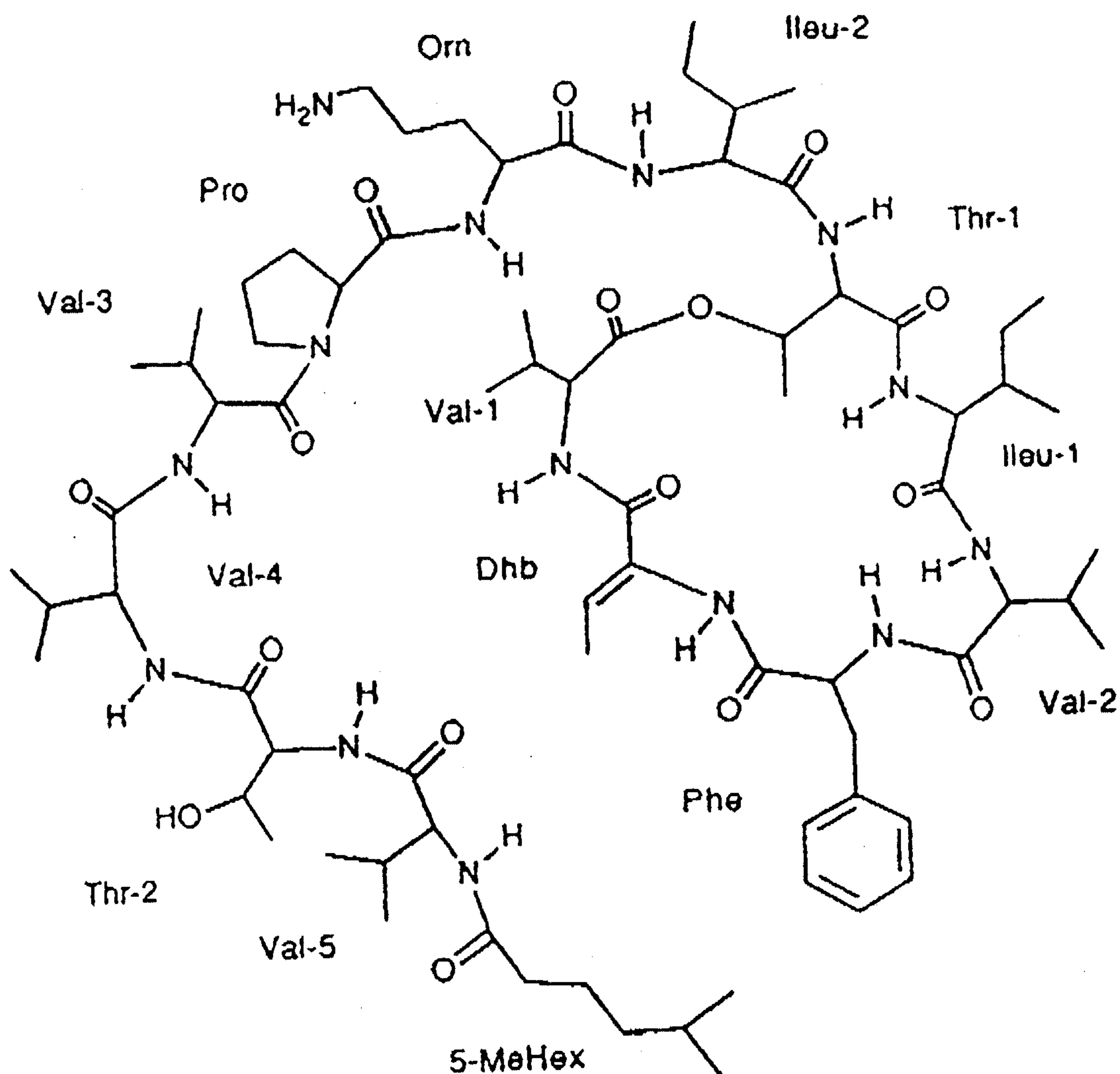


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CYTOTOXIC AND ANTIVIRAL COMPOUND

This invention is concerned with a cytotoxic and antiviral compound isolated from the sacoglossan, *Elysia rafescens*.

According to the invention there is provided, a new compound, the peptide, Kalahide F, of the formula:



The antitumor activities of this compound has been determined "in vitro" in cell cultures of human lung carcinoma A-549 and human colon

carcinoma HT-29. The procedure was carried out using the methodology described by Raymond J. Bergeron et al. *Biochem. Bioph. Res. Comm.* 1984, 121(3), 848-854 and by Alan C. Schroeder et al. *J. Med. Chem.* 1981, 24 1078-1083.

The antiviral activities of this compound have also been determined "in vitro" against HSV (Herpes simplex virus) and VSV (Vesicular stomatitis virus). The methodology used to carry out this determination is described by Raymond J. Bergeron et al. *Biochem. Bioph. Res. Comm.* 1984, 121(3), 848-854 and by Alan C. Schroeder et al. *J. Med. Chem.* 1981, 24 1078-1083.

Therefore, the present invention also provides a method of treating any mammal affected by a malignant tumor sensitive to compounds above described, which comprises administering to the affected individual a therapeutically effective amount of these compounds or a pharmaceutically composition thereof; and a method of treating viral infections in mammals, comprising administering to a patient in need of such treatment, an antiviral effective amount of the compounds described in the present invention.

The present invention also relates to pharmaceutical preparations which contain as active ingredient these compounds, or a pharmaceutical acceptable acid addition salt thereof, as well as the process for its preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) suitable composition for oral, topical or parenteral administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

The correct dosage of a pharmaceutical composition of these compounds will vary according to the particular formulation, the mode of application and particular situs, host and tumor being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of disease shall be taken in account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

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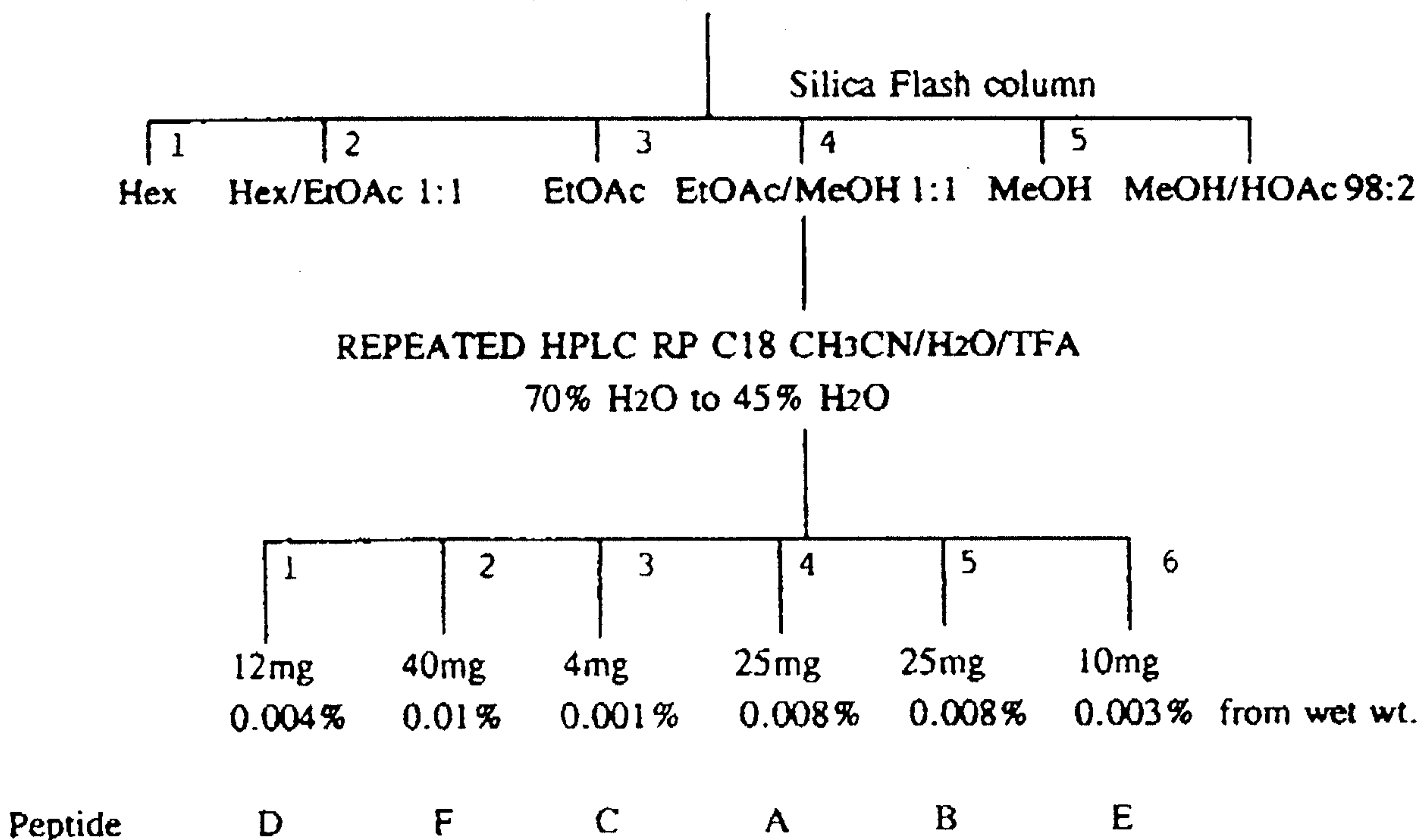
Kahalalide F was isolated from the sacoglossan, *Elysia rufescens* (family Plakobranchidae, order Sacoglossa), collected near Black point, Oahu. This animal varies in size between 1 and 4 cm; it is dark red-brown in color with light-colored spots. There is orange fringing of the parapodia, which have very small dark green spots from sequestered chloroplasts. *Elysia rufescens* feeds on the delicate, feather-like green alga *Bryopsis sp.*¹ Kahalalide F can also be isolated from this alga. Two hundred animals were collected over the period of several weeks during spring, 1991 and extracted with EtOH. The extracts were then chromatographed by silica gel flash chromatography (hexane, hexane/EtOAc (1:1), EtOAc, EtOAc (1:1), MeOH and MeOH/HOAc (98:2)). The peptides were eluted with EtOAc/MeOH (1:1). Final purification was accomplished by repeated HPLC (RP C18) using MeCN/H₂O with 0.1% TFA (70-45% H₂O) (Figure 1).

ISOLATION SCHEME

Elysia rufescens

EXTRACTION WITH ETOH

300g wet weight, 200 animals



The structures of the peptides were elucidated by 2D NMR experiments (HMQC, HMBC, TOCSY, COSY and ROESY).

Kahalalide F was isolated as a white amorphous powder in 0.02% yield. A molecular formula of $C_{75}H_{124}N_{14}O_{16}$ was deduced from detailed analyses of the ^{13}C and 1H NMR spectra and the high resolution FAB mass spectrum. The 14 substructures in this compound arise from five valines, two isoleucines, two threonines, ornithine, dehydroaminobutyric acid, proline, phenylalanine and 5-methylhexanoic acid (5-MeHex). Kahalalide F is the largest peptide in this series of compounds.

EXPERIMENTAL

General Considerations

Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Infrared spectra were recorded on a Nicolet MX-5 FTIR spectrometer. Gas chromatography was accomplished using a Hewlett-Packard Model 5890 instrument. Mass spectra were measured on a VG-70SE magnetic sector mass spectrometer. NMR spectra were measured on a General Electric QE-300 or a GN OMEGA 500 instrument. 1H NMR chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standards. ^{13}C NMR chemical shifts are reported in ppm by using the natural abundance ^{13}C of the solvent as an internal standard. Ultraviolet spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrophotometer. All solvents were distilled from glass before use.

Two hundred sacoglossans (*Elysia rufescens*, Fig. 33) were collected at Black Point, O'ahu during April and May 1992, and extracted 3 times with EtOH. Spring appears to be the time of year *Elysia rufescens* is in greatest abundance at Black Point. The combined extracts were then chromatographed using silica gel flash chromatography (hexane, hexane/EtOAc (1:1), EtOAc, EtOAc/MeOH (1:1), MeOH, MeOH/HOAc (98:2). The depsipeptides were found in the EtOAc/MeOH (1:1) fraction. Repeated HPLC RP18 MeCN/H₂O/TFA (55/45/1) - MeCN/H₂O/TFA ((30/70/1) yielded six new depsipeptides. For details see Fig. 1.

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KAHALALIDE F

Final purification was accomplished by HPLC on RP18 MeCN/H₂O/TFA (55/45/1). Physical data: $[\alpha]_D^{25}$ (c 4.32, MeOH); ¹H NMR (500 MHz, TFA/DMF): amino acid unit, δ (carbon position, mult, J): *Val-1* 4.16 (2, t, J=9.0 Hz), 7.11 (NH on 2, d, J=8.9 Hz), 1.77 (3, m), 0.95 (4, m), 0.95 (5, m); *Dhb* 9.20 (NH on 2, s), 6.48 (3, q, J=6.9 Hz), 1.43 (4, d, J=6.6 Hz); *Phe* 4.68 (2, q, J=6.6 Hz), 8.62 (NH on 2, d, J=6.6 Hz), 3.2 (3, dd, J=13.7, 7.2 Hz), 3.0 (3, dd, J=13.7, 9.0 Hz), 7.32 (5, d, J=7.2 Hz), 7.28 (6, t, J=7.5 Hz), 7.21 (7, t, J=7.2 Hz); *Val-2* 4.36 (2, m), 7.82 (NH on 2, d, J=6.6 Hz), 2.12 (3, m), 0.85 (4, m), 0.77 (5, d, J=6.6 Hz); *Ileu-1* 4.53 (2, m), 8.38 (NH on 2, d, J=9.6 Hz), 1.98 (3, m), 0.92 (4, d, J=6.6 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, t, J=7.2 Hz); *Thr-1* 4.63 (2, t, J=9.3 Hz), 8.12 (NH on 2, d, J=5.7), 5.07 (3, dq, 9.6, 6.0 Hz), 1.18 (4, d, J=6.3 Hz); *Ileu-2* 4.52 (2, m), 7.72 (NH on 2, d, J=8.4 Hz), 1.88 (3, m), 0.88 (4, d, J=6.3 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, d, J=7.2 Hz); *Orn* 4.48 (2, m), 7.92 (NH on 2, d, J=7.8 Hz), 1.76 (3, m), 1.83 (4, m), 3.10 (5, p, J=5.1 Hz); *Pro* 4.42 (2, m), 2.12 (3, m), 1.97 (3, m), 2.02 (4, m), 1.88 (4, m), 3.75 (5, m), 3.68 (5, m); *Val-3* 4.41 (2, m), 7.90 (NH on 2, d, J=7.2 Hz), 2.12 (3, m), 0.95 (4, m), 0.85 (5, m); *Val-4* 4.34 (2, m), 7.68 (NH on 2, d, J=8.1 Hz), 2.17 (3, m), 0.95 (4, m), 0.90 (5, m); *Thr-2* 4.46 (2, m), 7.77 (NH on 2, d, J=8.1), 4.21 (3, dq, 6.3, 3.6 Hz), 1.12 (4, d, J=6.6); *Val-5* 4.32 (2, m), 7.85 (NH on 2, d, J=8.1 Hz), 7.82 (NH on (second conformation), d, J=8.1 Hz), 2.14 (3, m), 0.95 (4, m), 0.90 (5, m); *5-MeHex* 2.26 (2, m), 1.60 (3, m), 1.20 (4, m), 1.55 (5, m), 0.87 (6, d, J=7.2 Hz), 0.87 (7, d, J=7.2 Hz); *5-MeHex* 2.29 (2, m), 1.65 (3, m), 1.40 (3, m), 1.13 (4, m), 1.35 (5, m), 0.90 (6, m), 0.90 (7, m); ¹³C NMR (125 MHz TFA/DMF): amino acid unit, δ (carbon position): *Val-1* 170.40 (1), 60.31 (2), 30.75 (3), 19.58 (4), 18.76 (5); *Dhb* 164.54 (1), 130.30 (2), 131.26 (3), 12.68 (4); *Phe* 171.31 (1), 56.27 (2), 36.79 (3), 138.23 (4), 129.86 (5), 128.77 (6), 126.98 (7); *Val-2* 172.94 (1), 58.57 (2), 32.38 (3), 18.92 (4), 17.60 (5); *Ileu-1* 171.87 (1), 57.48 (2), 38.78 (3), 14.56 (4), 26.78 (5), 11.67; *Thr-1* 169.68 (1), 57.37 (2), 71.05 (3), 17.34 (4); *Ileu-2* 171.92 (1), 57.29 (2), 38.01 (3), 14.78 (4), 26.55 (5), 11.63 (6); *Orn* 172.01 (1), 52.87 (2), 29.63 (3), 24.39 (4), 40.05 (5); *Pro* 172.55 (1), 60.23 (2), 29.58 (3), 25.38 (4), 48.03 (5); *Val-3* 171.28 (1), 57.57 (2), 30.54 (3), 19.61 (4), 18.80 (5); *Val-4* 171.83 (1), 59.10 (2), 31.26 (3), 19.45 (4), 18.08 (5); *Thr-2* 170.97 (1), 58.89 (2), 67.36 (3), 19.66 (4); *Val-5* 172.67 (1), 59.64 (2), 30.66 (3), 19.61 (4), 18.43 (5); *5-MeHex* 173.83 (1), 36.28 (2), 23.99 (3), 38.96 (4), 28.10 (5), 22.54 (6), 22.50 (7); *5-MeHex* (second conformation) 174.08 (1), 33.86 (2), 32.84 (3), 29.75 (4), 34.54 (5), 19.51 (6), 11.20 (7); IR neat (NaCl): 3287 (s, br), 2964 (s, br), 1646 (s), 1528 (s), 1465 (s), 1388 (m), 1228 (m), cm⁻¹; mass spectrum HRFAB *m/z* (fragment, %) 1477.9408 (M⁺ + 1, 85) (calcd for C₇₅H₁₂₅N₁₄O₁₆: 1477.9398); UV (MeOH): λ_{max} 204 (89,630) nm.

Amino acid analysis by GC-MS with a Chirasil-Val column indicates that Kahalalide F consists of D-Ileu, -Orn, L-Phe, D-Pro, L-Thr, D-Allo-Thr, 3 D-Val and 2 L-Val.

Table II ¹H and ¹³C NMR Data for Kahalalide F (1) in DMF/TFA

Amino Acid	Carbon	¹³ C, ppm ^a	Mult.	¹ H, ppm ^b	Multiplicity
Valine-1	1	170.4	s	(NH) 7.11	d, J=8.9
	2	60.3	d	4.16	t, J=9.0
	3	30.8	d	1.77	m
	4	19.6	q	0.95	m
	5	18.8	q	0.95	m
Dehydroamino butyric acid	1	164.5	s	(NH) 9.20	s
	2	130.3	s		
	3	131.3	d	6.48	q, J=6.9
	4	12.7	q	1.43	d, J=6.6
Phenylalanine	1	171.3	s	(NH) 8.62	d, J=6.6
	2	56.3	d	4.68	q, J=6.6
	3	36.8	t	3.23	dd, J=13.7, 7.2
				3.00	dd, J=13.7, 9.0
	4	138.2	s		
	5, 5'	129.9	d	7.32	d, J=7.2
	6, 6'	128.8	d	7.28	t, J=7.5
Valine-2	7	127.0	d	7.21	t, J=7.2
	1	172.9	s	(NH) 7.82	d, J=6.6
	2	58.6	d	4.36	m
	3	32.4	d	2.12	m
	4	18.9	q	0.85	m
Isoleucine-1	5	17.6	q	0.77	d, J=6.6
	1	171.9	s	(NH) 8.38	d, J=9.6
	2	57.5	d	4.53	m
	3	38.8	d	1.98	m
	4	14.6	q	0.92	d, J=6.6
	5	26.8	t	1.40, 1.13	m, m
Threonine-1	6	11.7	q	0.88	t, J=7.2
	1	169.7	s	(NH) 8.12	d, J=5.7
	2	57.4	d	4.63	t, J=9.3
	3	71.1	d	5.07	dq, J=9.6, 6.0
Isoleucine-2	4	17.3	q	1.18	d, J=6.3
	1	171.9	s	(NH) 7.72	d, J=8.4
	2	57.3	d	4.52	m
	3	38.0	d	1.88	m
	4	14.8	q	0.88	d, J=6.3
	5	26.6	t	1.40, 1.13	m, m
Ornithine	6	11.6	q	0.88	t, J=7.2
	1	172.0	s	(NH) 7.92	d, J=7.8
	2	52.9	d	4.48	m
	3	29.6	t	1.76	m
	4	24.4	t	1.83	m
Proline	5	40.1	t	3.10	p, 5.1
	1	172.6	s		
	2	60.2	d	4.42	m
	3	29.6	t	2.12, 1.97	m, m
	4	25.4	t	2.02, 1.88	m, m
	5	48.0	t	3.75, 3.68	m, m

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Table II Continued

Valine-3	1	171.3	s	(NH) 7.90	d, $J=7.2$
	2	57.6	d	4.41	m
	3	30.5	d	2.12	m
	4	19.6	q	0.95	m
	5	18.8	q	0.85	m
Valine-4	1	171.8	s	(NH) 7.68	d, $J=8.1$
	2	59.1	d	4.34	m
	3	31.3	d	2.17	m
	4	19.5	q	0.95	m
	5	18.1	q	0.90	m
Threonine-2	1	171.0	s	(NH) 7.77	d, $J=8.1$
	2	58.9	d	4.46	m
	3	67.4	d	4.21	dq, $J=6.3, 3.6$
	4	19.7	q	1.12	d, $J=6.6$
Valine-5	1	172.7	s	(NH) 7.85,	d, $J=8.1$
		conf. #2		(NH) 7.82	d, $J=8.1$
	2	59.6	d	4.32	m
	3	30.7	d	2.14	m
	4	19.6	q	0.95	m
5-Methyl - Hexanoic acid	5	18.4	q	0.90	m
	1	173.8	s		
	2	36.3	t	2.26	m
	3	24.0	t	1.60	m
	4	39.0	t	1.20	m
	5	28.1	d	1.55	m
	6	22.5	q	0.87	d, $J=7.2$
7	22.5	q	0.87	d, $J=7.2$	
5-Methyl - Hexanoic acid (second conformation)	1	174.1	s		
	2	33.9	t	2.29	m
	3	32.8	t	1.65, 1.40	m
	4	29.8	t	1.13	m
	5	34.5	d	1.35	m
	6	19.5	q	0.90	m
	7	11.2	q	0.90	m

^a at 125 MHz, DMF signal at 35.2 ppm; ^b at 500 MHz, DMF signal at 2.91 ppm.

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Table I. *In vitro* Activity of Kahalalide F from *Elysia rufescens*
Assay (M.I.C. $\mu\text{g/mL}$)

Cytotoxicity $\mu\text{g/mL}$ (IC₅₀)

A-549	2.5
HT-29	0.25-0.5

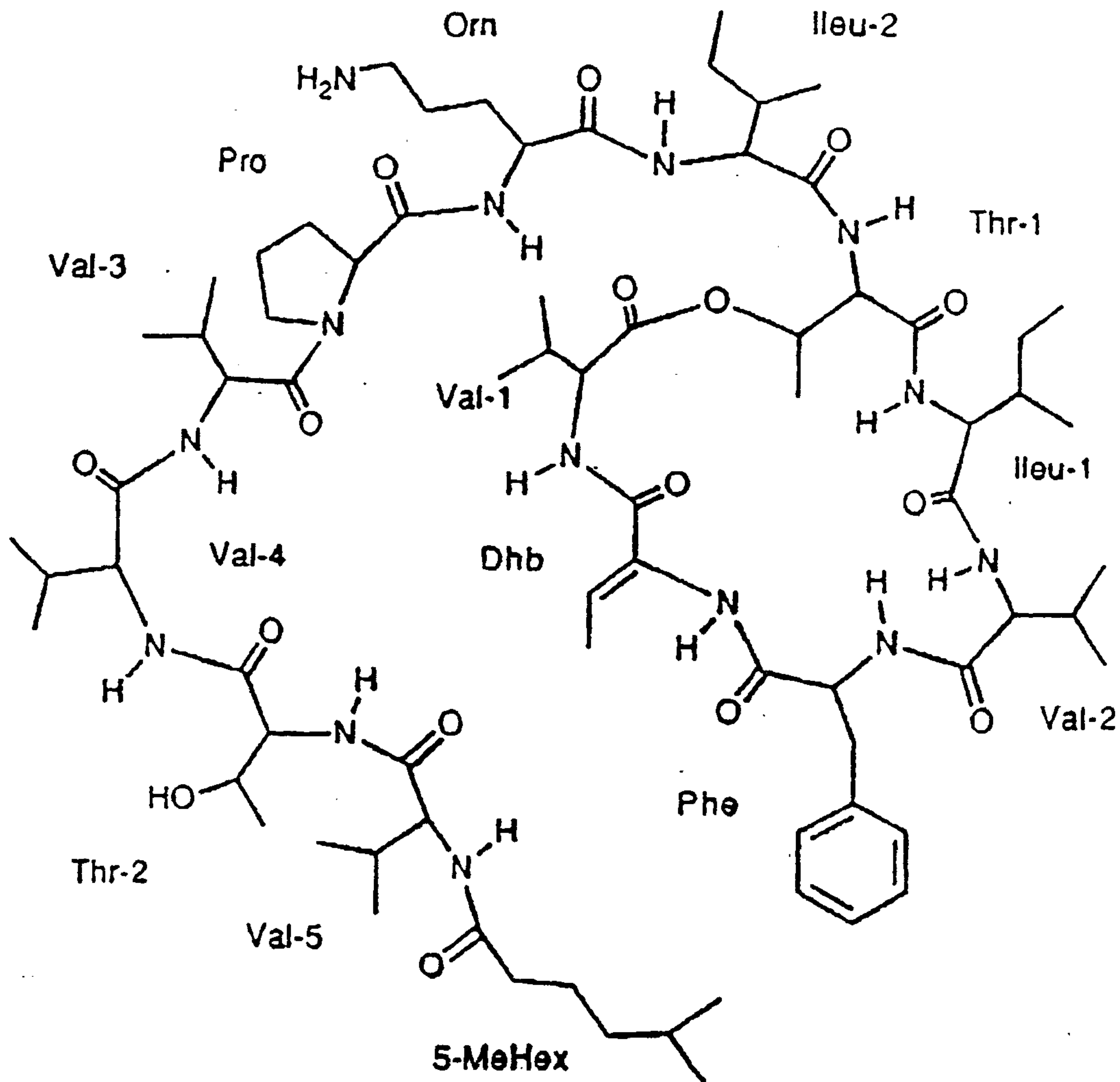
Antiviral $\mu\text{g/mL}$ (% reduction)

Mv 1 Lu/HSV II	0.5 (95%)
CV-1/HSV-1	> 8
BHK/VSV	> 8

Antifungal 6mm disk	50 $\mu\text{g/disk}$
<i>Aspergillus oryzae</i>	19 mm
<i>Penicillium notatum</i>	26 mm
<i>Tricophyton mentagrophy</i>	34 mm
<i>Saccharomyces cerevisiae</i>	neg
<i>Candida albicans</i>	16 mm

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A Kahalalide F compound of the formula:



2. A pharmaceutical composition comprising a Kahalalide F compound as defined in claim 1, in association with a pharmaceutical carrier or diluent.
3. Use of Kahalalide F as defined in claim 1, in the manufacture of an antitumor or antiviral pharmaceutical composition.

4. A process for the preparation of a Kahalalide F compound as defined in claim 1, by extraction from sacoglossan *Elysia rufescens*.

5. A process for preparing a pharmaceutical composition comprising admixing a Kahalalide F compound obtained by a process as defined in claim 4, and a pharmaceutical carrier or diluent.

6. Use of a pharmaceutical composition of claim 2, in the treatment of a mammal affected by a malignant tumor or in the treatment of a viral infection.

