SYNTHESIS OF AMPHIKUEMIN AND ANALOGS: A SYNOMONE THAT MEDIATES PARTNER-RECOGNITION BETWEEN ANEMONEFISH AND SEA ANEMONE†

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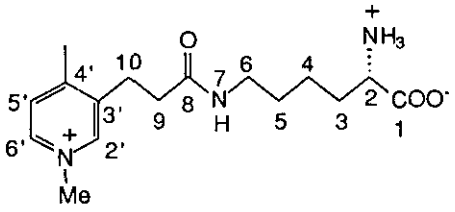
† Dedicated to the memory of Professor Tetsuji Kametani.

Abstract - Amphikuemin (**1**), a compound that is responsible for partner-recognition at the first encounter between anemonefish and sea anemones, has been synthesized to confirm its structure and to supply material for further biological studies. Synthesis of amphikuemin analogs (**11-15**) is also described.

The symbiosis between anemonefish and giant sea anemones is an intriguing phenomenon. Murata et al.[1] characterized the synomones, chemical substances that induce symbiosis, in two host-guest pairs; the results established[2] that an innate chemical recognition system is crucial for the species-specific association at the first encounter of the investigated symbionts. Several reports which described the existence of some locality-dependent variance in partnership,[3-6] suggested that chemical recognition toward the host anemone might be supplemented by pre- or post-hatching imprinting. An environment-adaptating process is necessary for the adult anemonefish when they spread their distribution to an area where the original partner anemone species are less populated. In such a locality, the anemonefish is forced to inhabit an alternative anemone species and lay eggs on the newly

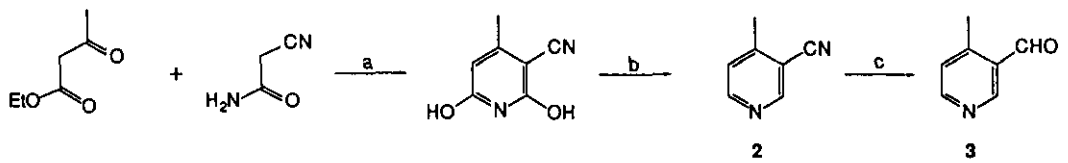
selected anemone; after hatching the fish enters a free-swimming planktonic larval period (ca. 10 days), which is followed by a juvenile period when the fish immediately return to inhabit the bonthos, their host. The imprinting system may serve to recognize the partner at the first encounter between the juvenile anemonefish and sea anemones. Since isolation from the host anemones begins at the planktonic period after hatching, influence by the host anemone via pre- or post-hatching imprinting must play an important role in recognition of the host by the juvenile fish upon the first encounter.

An understanding of the host recognition mechanism should be promoted by studies with amphikuemin **1**,[1] a synomone between Amphiprion perideraion Bleeker (fish) and Radianthus kuekenthali (=Heteractis crispa) Kwietniewski (sea anemone) living in the coastal region of Japan; 15 Kg of the anemone homogenate had yielded 48 μg of amphikuemin. Amphikuemin and its analogs **11-15** have been synthesized for structural confirmation and evaluation of biological properties.

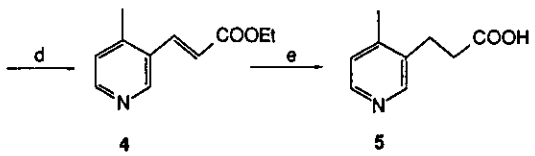


amphikuemin **1**

Procedure I [1]: 4-Methyl-3-pyridine carbonitrile **2** was prepared from 2-cyanoacetamide and ethyl acetoacetate. Diisobutylalminium hydride (DIBAL) reduction of **2** followed by acid hydrolysis gave the corresponding aldehyde (**3**), which then was subjected to the Wittig reaction to give the ester (**4**). Successive hydrogenation and hydrolysis of **4** afforded the pyridinecarboxylic acid (**5**). Spectral data of all new compounds were consistent with the assigned structures.



2 **3**



4 5

a. KOH / MeOH, 65°C, 8 h (95%)

b. (i) $POCl_3$, 180°C, 4 h (ii) H_2 / $PdCl_2$-AcONa, 3 atom, 40°C, 12 h (49%)

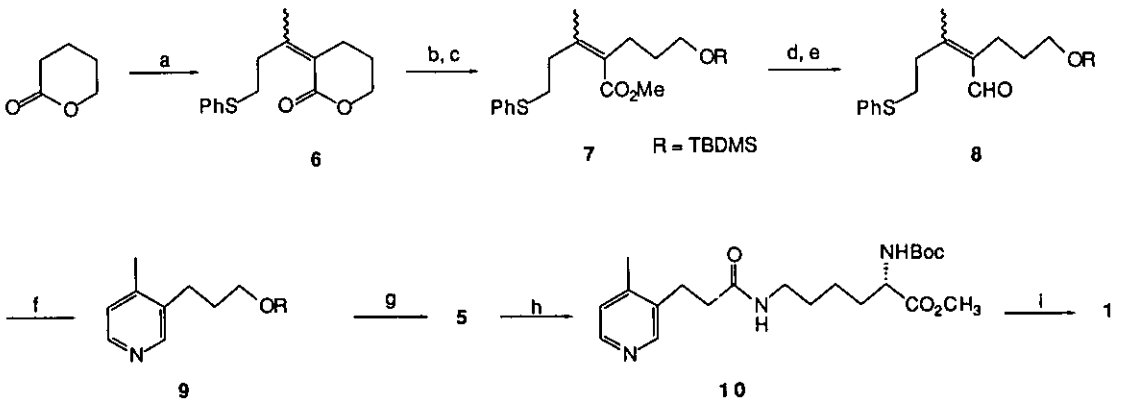
c. (i) DIBAL, CH_2Cl_2-hexane, -78°C → rt, 1 h (ii) 1N-HCl, H_2O, rt, 1 h (45%)

d. $Ph_3P=CHCO_2Et$ / PhH, C_6H_6, rt, 5 h (81%)

e. (i) H_2 / Pd-C, EtOH, 2 atm, 50°C, 3 h (ii) KOH, H_2O (74%)

Procedure II : Alternatively **5** was synthesized by the newly developed method for pyridine synthesis.[7] Condensation of δ-valerolactone with 4-thiophenyl-2-butanone, followed by dehydration gave a 3:1 geometrical mixture of sulfide **6**, which upon NaOMe/MeOH treatment and subsequent silyl protection produced the ester **7**. This was converted to the corresponding aldehyde **8** in two steps (i. DIBAL reduction, ii. MnO_2 oxidation). The pyridine ring **9** was formed in good yield from **8** through sequential reactions of (i) oxidation to the sulfoxide ($NaIO_4$-Na_2HPO_4), (ii) the Pummerer reaction (TFAA/Py), and (iii) cyclization (aq. NH_4OH). Simultaneous deprotection and oxidation of **9** with $KMnO_4$/HCl gave pyridinecarboxylic acid **5**. Condensation of acid **5** with N_α-^{t}Boc-L-lysine methyl ester using DPPA produced amide **10** in high yield. Successive treatment of **10** with NaOH, MeI and TFA finally gave amphikuemin **1**, which was identical to the natural product in uv, ^{1}H-nmr, CD spectra and tlc mobility. The synthetic amphikuemin elicited the fish up-stream swimming behavior[1] at the same concentration as that of the natural product. Thus the structure of amphikuemin is confirmed as being **1**.

Analogs **11-15** were synthesized by procedurs similar to those described above. Thus, deprotection of **10** with NaOH and TFA gave N-demethyl analog **11**, from which the pentamethyl compound **12** was obtained by treatment with MeI. 4-Demethyl compound **13** was prepared from nicotinaldehyde. Condensation of 3-pyridineacetic acid with N_α-^{t}Boc-L-lysine methyl ester led to the other analogs **14** and **15**.



a. (i) $PhS(CH_2)_2COCH_3$ / LDA, -78°C, 15 min (ii) $SOCl_2$ / DMAP, 0°C, 15 min (81%)

b. NaOMe / MeOH, rt, 16 h (67%)

c. $ClSi(Me)_2{}^tBu$ (Cl-TBDMS) / DMAP, rt, 2 h (87%)

d. DIBAL (diisobutylaluminum hydride), 0°C, 30 min (99%)

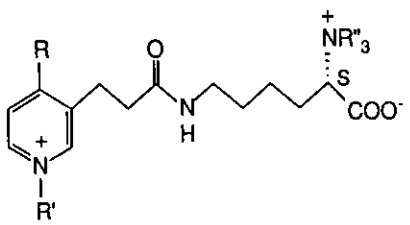
e. MnO_2, rt, 1 h (85%)

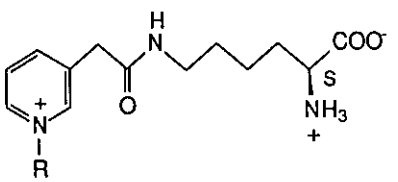
f. (i) $NaIO_4$-Na_2HPO_4 / MeOH-H_2O, rt, 16 h (ii) TFAA / Py, 0°C, 1 h (iii) aq. NH_4OH / MeOH, rt, 16 h (53%)

g. $KMnO_4$ / HCl rt, 16 h (60%)

h. N_α-^{t}Boc-L-lysine methyl ester, DPPA-Et_3N / DMF, rt, 16 h (83%)

i. (i) NaOH, rt, 1 h (ii) MeI / MeOH, 55°C, 1 h (iii) TFA, rt, 1 h (60%)





11 R = Me, R'= R"= H

12 R = R'= R"= Me

13 R = H, R'= Me, R"= H

14 R = H

15 R = Me

Olfactory responses[8a] exhibited the presence of a putative receptor at the nose membrane connected to the sensory system. The minimum effective dose of **1** against electrocephalographic study[8a] was found to be around 10^{-8} M, instead of the 10^{-10} M dose

found against fish behavior.[8b] Natural amphikuemin was more effective than several modified compounds; as reported earlier,[1] N-demethylamphikuemin **11** was totally devoid of behavioral activity. Details of biological studies will be reported elsewhere.[8a,b]

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