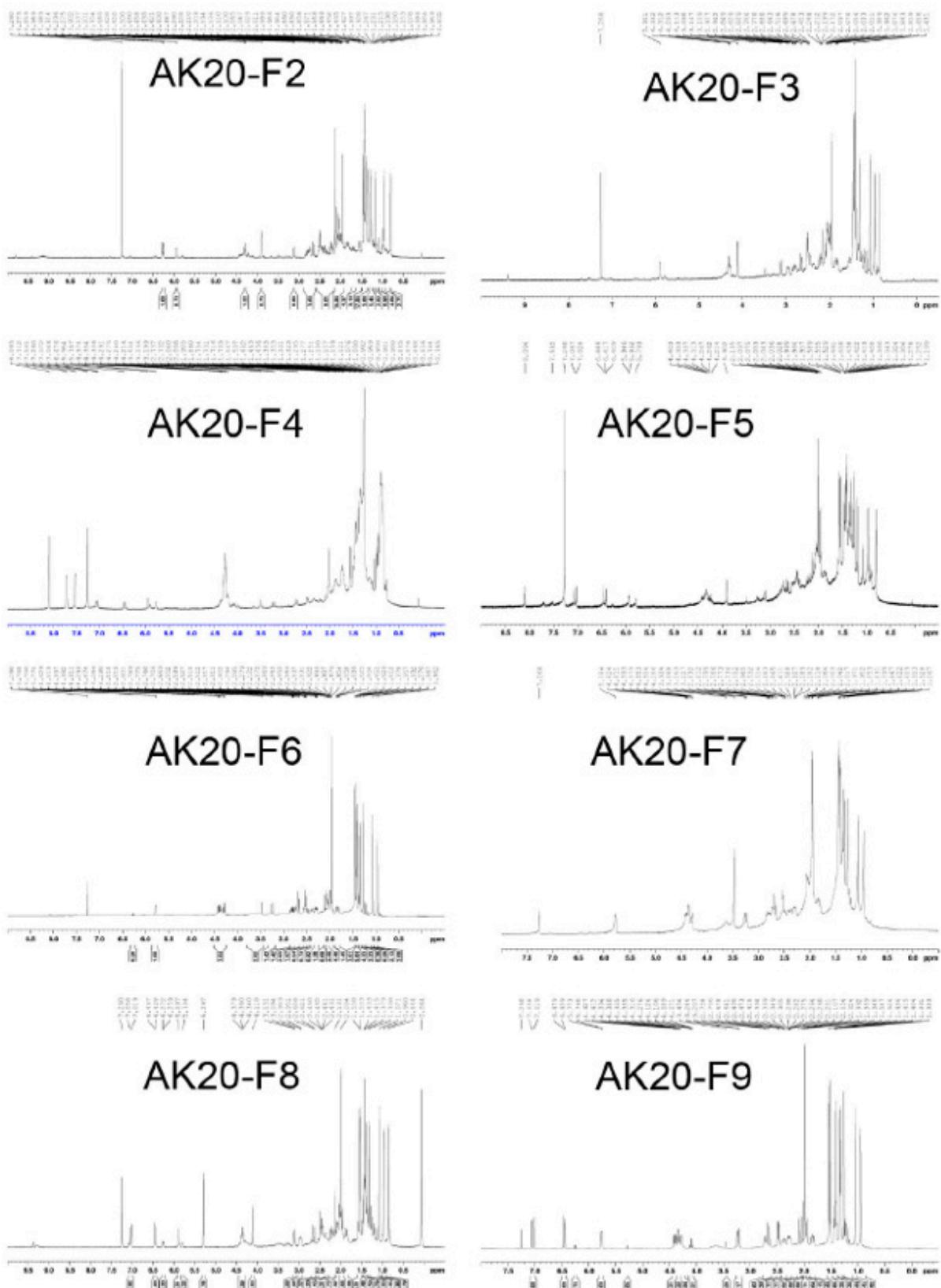


Supplemental Figure S1. NMR graphs of AK20 fractions.



Supplementary Figure S2. Chemical characterizations of AK20-F9.

The molecular formula of AK20-F9 was determined to be C₃₂H₄₆O₈.

(A) In the ¹H NMR spectrum, nine methyl signals at δ_H 0.96 (3H, s, H-18), 1.03 (3H, s, H-19), 1.42 (3H, s, H-21), 1.53 (3H, s, H-26), 1.55 (3H, s, H-27), 1.33 (3H, s, H-28), 1.27 (3H, s, H-29), 1.34 (3H, s, H-30), and 1.95 (3H, s, OCOCH₃).

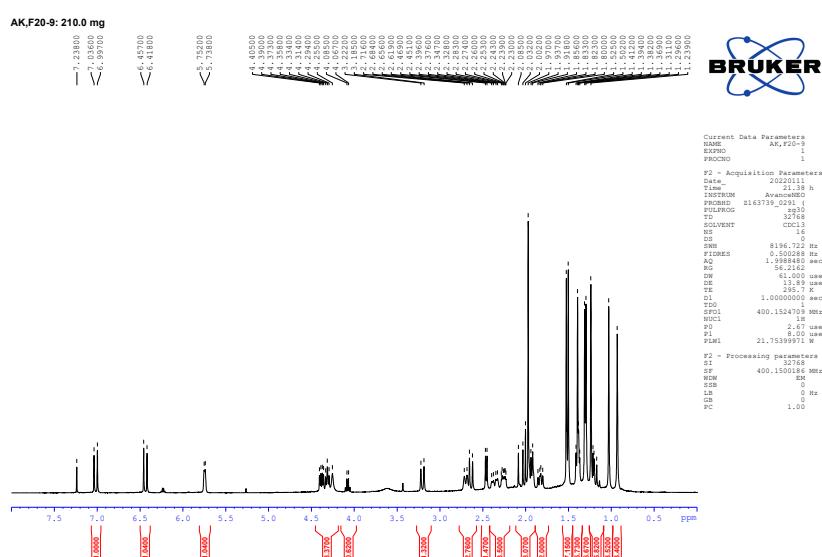
(B) Along with the 32 signals in the ¹³C NMR spectrum, AK20-F9 is found to be a cucurbitacin-type triterpene. The NMR signals further indicate 9 methyl, 4 methylene, 8 methane, and 11 quaternary carbons, as determined by the DEPT spectroscopy.

(C-E) Detailed analyses using HSQC, HMBC, and NOESY spectra assisted the complete assignment of its ¹H and ¹³C NMR data which were in according with those of cucurbitacin B. (ref: Natural Product Research, 2003, Vol. 17, No. 4, 229–233)

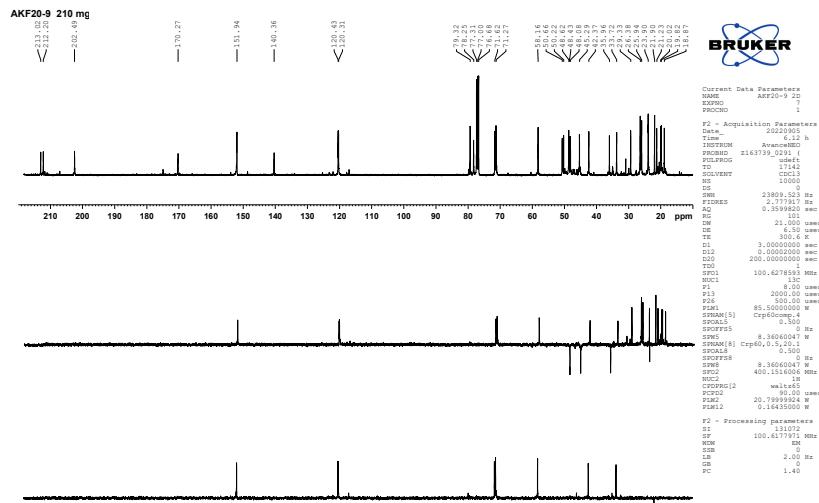
(F) The IR spectrum showed the characteristic absorptions of hydroxyl group (3470 cm⁻¹) and conjugated carbonyl group (1690 cm⁻¹).

(G) The ESI-MS data exhibited a quasi-molecular ion peak at *m/z* 581.3073 [M+Na]⁺, confirming the molecular weight of AK20-F9 to be 558 g/mol.

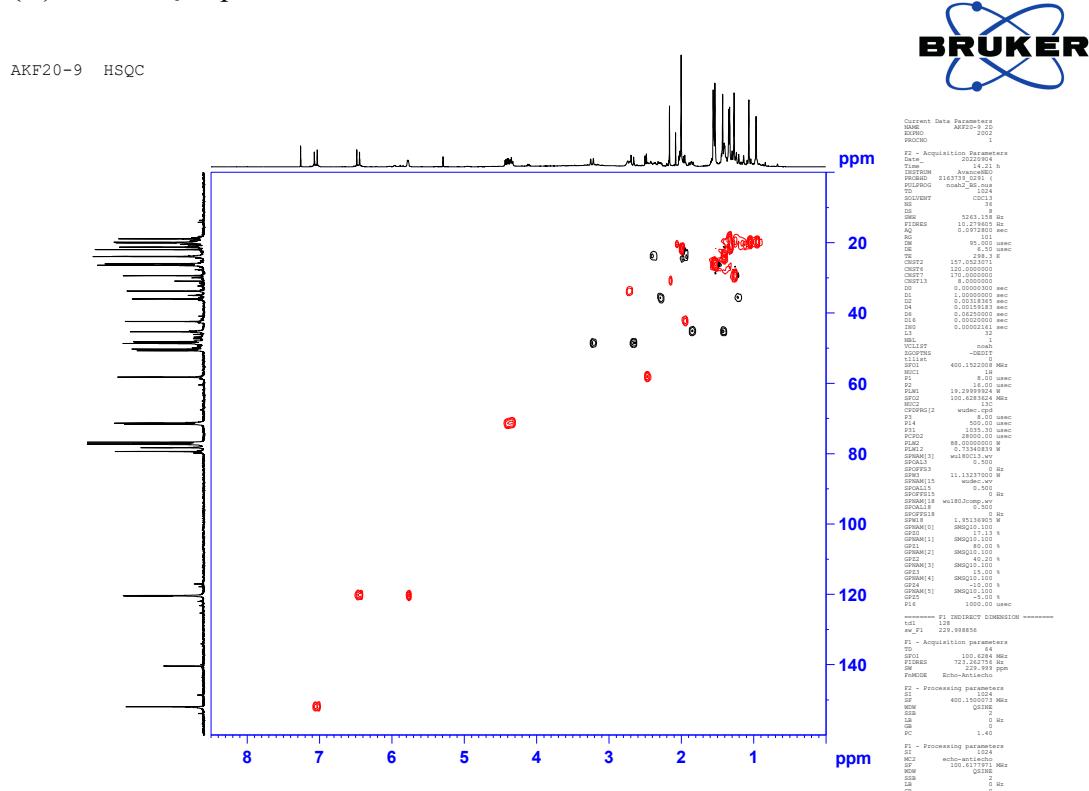
(A) ¹H-NMR (400 MHz, CD₃CL) spectrum of AK20-F9



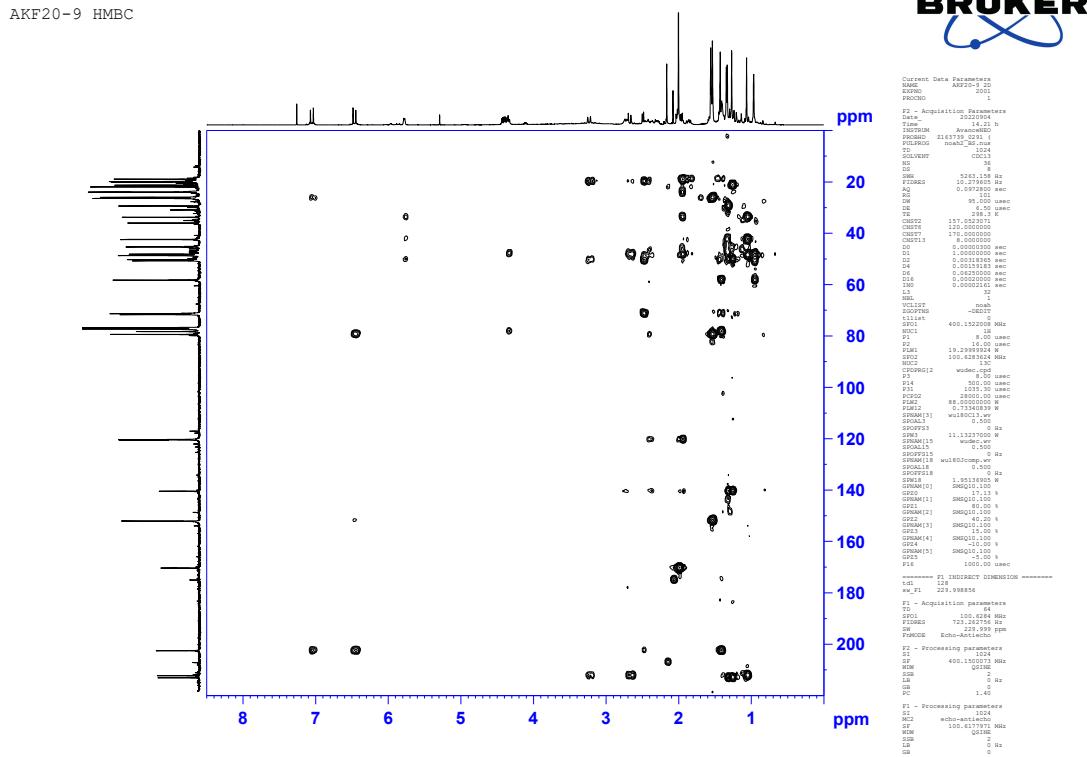
(B) ^{13}C -NMR (100 MHz, CD₃OD) and DEPT spectrum of AK20-F9



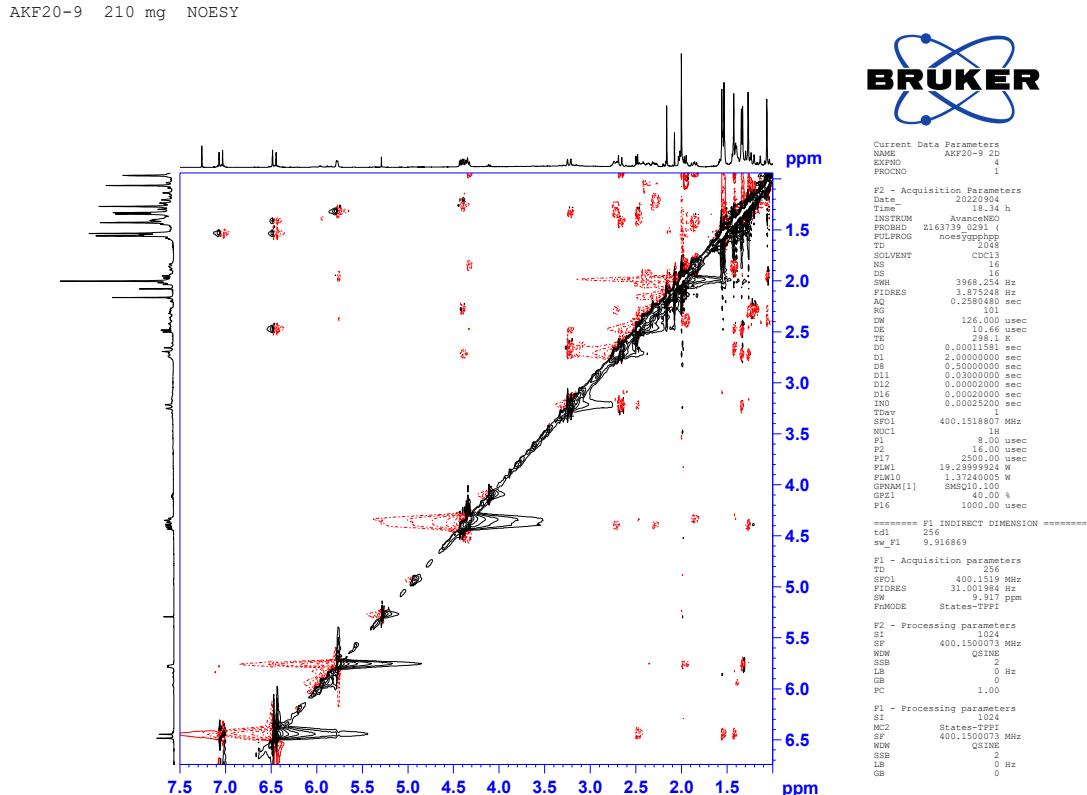
(C) The HSQC spectrum of AK20-F9



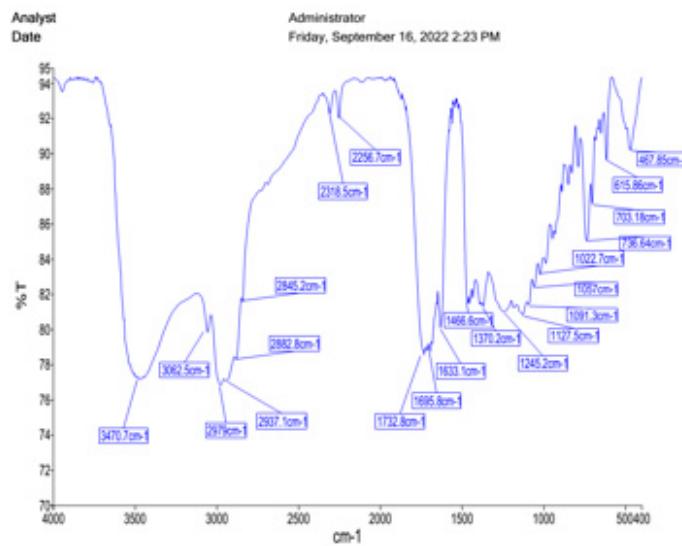
(D) The HMBC spectrum of AK20-F9



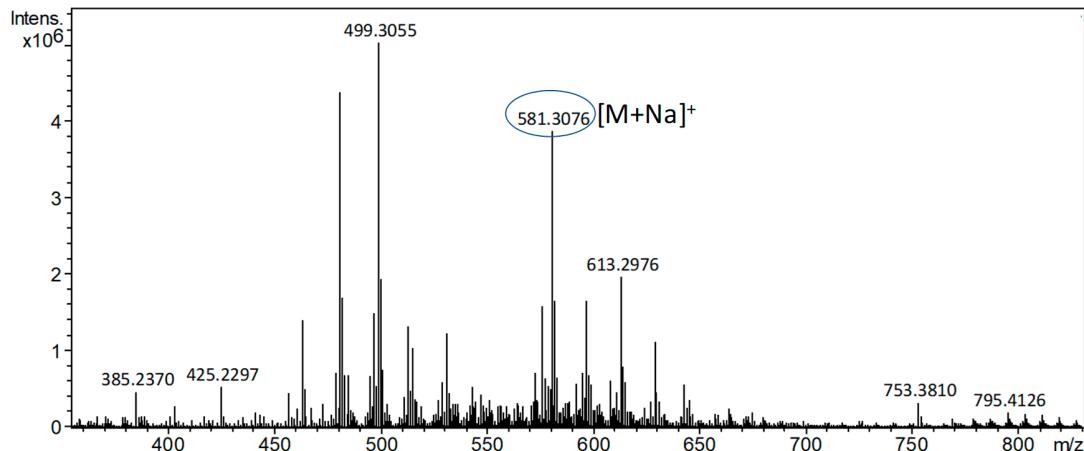
(E) The NOESY spectrum of AK20-F9



(F) The IR spectrum of AK20-F9



(G) The ESIMS spectrum of AK20-F9



Supplementary Figure S3. Surface sensory system in zebrafish embryo.

(A-B) Zebrafish embryos stained with the anti-acetylated tubulin showed no distinguishable difference of the surface neuronal network in the trunk between the (A) wild type and (B) AK20-F9 treated embryos.

(C) The anti-ZO-1 antibody labeled the bump in the head (arrow) of the AK20-F9 treated embryo.

