Synthesis of JA-Ile (and 13C JA-Ile): **Gustavo 12/07/07**

JA (30 mg [Sigma]) was dried under vacuum for ~3h to remove excess water. After drying, JA was dissolved in 2 mL of dry THF containing 27.5 µL (0.198 mmol) of triethylamine. Under stirring, 19 µL (0.197 mmol) of ethylchloroformate were added at 0°C (ice-water). After 3 min, 30 mg of Isoleucine –previously dissolved in 1.4 mL 0.3 N NaOH- were added ($^{13}C_6$-Ile [Cambridge Isotope Laboratories, 98% (CLM-2248-0.1)] is used for $^{13}C_6$-Ile-JA synthesis). After 5 min, the ice bath was removed and the mixture stirred (with a magnetic bar) for 1 h at room temperature. The reaction was then adjusted to pH 3-4 with 5 N HCl (~3 drops of a Pasteur pipette) and extracted 3 times with 3 mL of CH_2CL_2. The combined organic phases were first dried with Na_2SO_4 and then evaporated to dryness under a stream of N_2. Purification was achieved by silica gel chromatography: 3 g of silica 60 gel, preconditioned with CL_3CH/acetic acid 100/1 (v/v). The sample was reconstituted in 1-2 mL of CL_3CH/acetic acid 100/1 (v/v) and loaded onto the silica column. After the sample has entered the column, the column was washed two times with 5 mL of CL_3CH/acetic acid 100/1 (v/v) (discard flow-through). 5 mL of CL_3CH/ethyl-acetate/acetic acid 14/6/1 (v/v/v) were added and the flow-trough was collected (~5 mL). This step was repeated two more times collecting 5 mL fractions during each step.
The fractions were concentrated under a stream of N_2 and checked for purity by silica 60 TLC plates (run with CL_3CH/ethyl-acetate/acetic acid 14/6/1 (v/v/v) first half then full, stained with iodine vapor) and by LC-MS/MS for quantification and purity (run against a known amount of JA-Ile).
Note: two isoforms of JA-Ile are produced (seen as 2 bands on TLC or two peaks on LC). Depending on the LC conditions, these isoforms can be separated or not.