

## Supplementary Material

### **<sup>13</sup>C Metabolomics: NMR and IROA for Unknown Identification**

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## SUPPLEMENTARY METHODS

### Liquid Chromatography High Resolution Mass Spectrometry

Samples were analyzed using a mass range of 70-1000 in positive and negative ionization modes, externally calibrated, using a Thermo Scientific Q-Exactive Orbitrap mass spectrometer equipped with a Dionex UltiMate 3000 RS autosampler and pump. The Q-Exactive was equipped with a Heated Electrospray Ionization (HESI) source which operated at a spray temperature of 350 °C, a spray voltage of 3.5 kV, and sheath and auxiliary gas flow rates of 50 and 10 arbitrary units, respectively. 20 µL of each sample were injected onto an Waters Acquity UPLC HSS T3 (2.1x100 mm, 1.8 micron) column using a column temperature of 35 °C and a flow rate of 300 µL/min with solvent A (0.1% formic acid in water (Fisher LC-MS Optima)) held at 100% A for 1 min, followed by linear increase to 80% B (0.1% formic acid in acetonitrile) over 10 min, kept constant for 2 min, followed by a return to initial conditions over 0.5 min and equilibration for 3 min. The capillary temperature was held at 325 °C, and the S-lens RF Level was set to 40%. The FR resolution was set to 70,000 at  $m/z$  200. The accuracy achieved was routinely less than 1.5 ppm. In addition to full MS, data-dependent MS/MS was performed on the 10 most abundance peaks with an exclusion period of 10 seconds (40 normalized collision energy).

### Fractionation of IROA-labeled Material

Fractionation was performed using a Dionex UltiMate 3000 fraction collector by triggering every 30 seconds. Samples were dried under N<sub>2</sub> for 1 hour, lyophilized overnight, and resuspended in 50 µL of 99.98% D<sub>2</sub>O (Cambridge Isotope Laboratory). Forty µL of the resuspended material from each fraction was added to a 1.5-mm NMR tube (Norell).

### NMR Experiments

All NMR spectra were collected on an Agilent VNMRS-600 spectrometer using a custom 1.5-mm <sup>13</sup>C high temperature superconducting (HTS) probe [1]. <sup>13</sup>C spectra were collected for a total duration of a half hour with an observe frequency of 150.799, a 60° pulse, 2 sec relaxation delay and a 2 sec acquisition time, a 212 ppm (32051.3 Hz) spectral window. Two-dimensional HSQC and HSQC-TOCSY were collected with an observe frequency of 599.662, a 90° pulse, 1.5 sec relaxation delay, a 0.14 sec acquisition time. Indirect and direct dimensions were 205.5 ppm (30983.7Hz) and 12 ppm (7183.9 Hz) respectively. HSQC was collected with 32 scans and 512 increments. HSQC-TOCSY was collected with 96 scans and 512 increments using a 20 ms mixing time and a MLEV spin lock sequence. All NMR spectra were zero-filled 2x, Fourier transformed, phased and baseline corrected using NMRPipe [2].

### Chemical Shift Calculations

All molecules were optimized at the B3LYP/6-31+G\* level [3]. Next, NMR chemical shieldings were calculated using gauge-including atomic orbitals (GIAO) [4] at the B3LYP/6-311++G\*\* level, which has been shown to produce accurate NMR chemical shifts [5-6]. To account for solvent effects, we applied implicit solvent model IEFPCM [7] developed by Tomasi and co-workers. All calculations were carried out by using the Gaussian 09 package [8]. This approach has been demonstrated to identify the correct stereochemistry of a natural product from a walkingstick [9].

**Fig. (S1).** **A)** MS spectrum of the isolated fraction from 4.0 - 4.5 min (red chromatographic trace) with highlighted peaks from the known compounds Phenylalanine (F) and Inosine (I). **B)**  $^{13}\text{C}$  NMR spectrum of this fraction from five repeated LC-MS injections contained F and I resonances that were using database matching.

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**Fig. (S2).** MS/MS spectra of 3'-O-phospho- $\beta$ -D-glucopyranosyl-anthranilate. The mass spectra for the compound in **(A)** positive and **(D)** negative mode are shown with the entirely  $^{12}\text{C}$  isotopic peak ( $m/z$  380.0742) and entirely  $^{13}\text{C}$  isotopic peak ( $m/z$  393.1178) highlighted in blue and red, respectively. The MS/MS spectrum for the entirely  $^{12}\text{C}$  isotopic peak is shown in positive **(B)** and negative **(E)** ion modes, and for the entirely  $^{13}\text{C}$  isotopic peak in positive **(D)** and negative **(F)** ionization modes.

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**Fig. (S3).** **A)**  $^{13}\text{C}$  NMR spectra of fraction 10 with tryptophan peaks 2 and 3 highlighted in purple and green respectively. **B)** Tryptophan carbon #3 displays  $^{13}\text{C}$ - $^{13}\text{C}$  J couplings with adjacent carbons 2 (33.1 Hz) and 4 (46.6 Hz). **C)** Carbon #2 displays  $^{13}\text{C}$ - $^{13}\text{C}$  J couplings with adjacent carbons 1 (54.1 Hz) and 3 (33.1 Hz).

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**Fig. (S4).** HSQC of unknown compound. **A)** HSQC of aromatic region. **B)** HSQC of the aliphatic region shows consistency with a  $\beta$ -glucose.

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