Supporting Information for

Rapid Phytotransformation of Benzotriazole Generates Synthetic Tryptophan and Auxin Analogs in *Arabidopsis*

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Total Pages (including this page)**: 25**

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Contains:

Method Details

1. Seed Sterilization Procedure. All procedures for seed sterilization were conducted over a flame and the bench and gloves were sterilized with a 70% ethanol spray solution to create a sterile working environment. 50 µl of seeds and 1 ml of seed sterilization solution were added to a 1.5 ml autoclaved tube. The seed sterilization solution consisted of 0.8 ml autoclaved water, 0.2 ml Bleach (8.25% sodium hypochlorite, Clorox brand) and 10 µL Tween 20 (Polyoxyethylene sorbitan monolaurate, BioRad Laboratories Inc.). The tube was vortexed briefly and slowly inverted for 5 minutes. The supernatant was removed using an autoclaved pipet. 1 mL of sterile water was added to wash the seeds from the sterilization solution and again the supernatant was removed. The washing step needed to be repeated for a total of four times. The seeds were then stored at 4°C overnight to stratify.

2. Spike-Recovery of Benzotriazole into Plant Tissue.

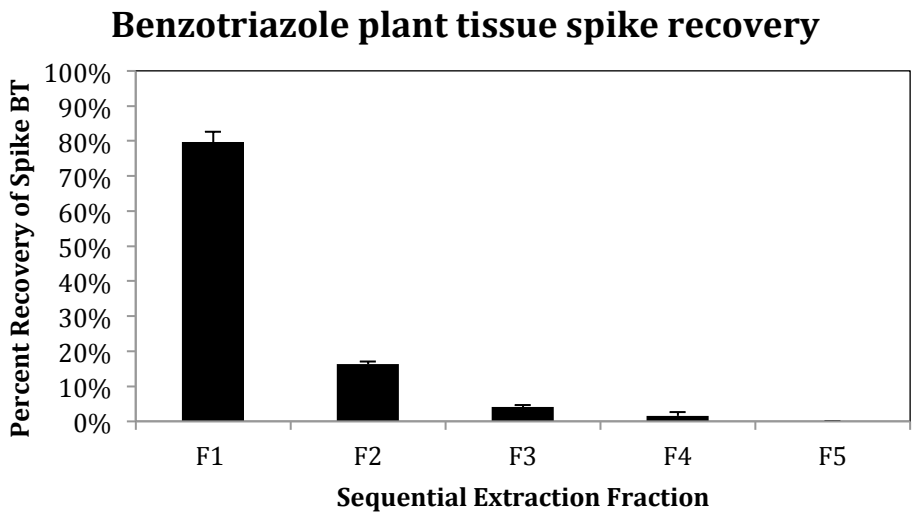


Figure S.1: Spike-recovery of benzotriazole into plant tissue with recovery from each sequential extraction shown. The mean value for each of the proportion of the total recovery measured in each sequential extractions fraction (e.g., F1, F2). Error bars are the standard deviation (n=2 spikes). The purpose of the spike-recovery test was to determine how much mass could be expected in each of the sequential extraction when using the procedure described in the Methods section of the manuscript body (i.e., freeze-thaw, mixer-mill, vortex, sonication, centrifugation) and to determine how many sequential extractions would be required to extract the majority of the mass for the experimental plants. In the approach used for the experiments (as described in the Methods), a total of three extractions were conducted, yielding a recovery of 100.5±4.0%. When conducting the benzotriazole extractions from the plant tissues for the experiment, deuterated benzotriazole was added as a surrogate to account for any losses during extraction. BT was quantified using a six-point internal standard normalized external calibration curve to account for surrogate recovery and matrix effects during ionization.

3. LC/MS/MS MRM Parameters.

Table S.1 LC-MS/MS parameters for benzotriazole and benzotriazole metabolites.

MRM Mass Transition	Q1 Mass (Da)	Q3 Mass (Da)	Declustering Potential (V)	Focusing Potential (V)	Entrance Potential (V)	Collision Energy (V)	Collision Cell Exit Potential (V)
Benzotriazole-1	119.951	65.110	51.000	240.000	10.000	32.000	4.000
Benzotriazole- 2	119.951	92.127	51.000	240.000	10.000	25.000	6.000
5-Methyl-Benzotriazole-1	134.000	77.000	56.000	195.000	10.000	40.000	4.000
5-Methyl-Benzotriazole-2	134.000	79.000	56.000	195.000	10.000	25.000	4.000
d4-Benzotriazole-1	123.972	69.100	41.000	170.000	10.000	35.000	4.000
d4-Benzotriazole-2	123.972	96.000	41.000	170.000	10.000	27.000	6.000
M282-1	281.900	119.900	23.000	200.000	10.000	25.000	10.000
M282-2	281.900	85.000	23.000	200.000	10.000	35.000	10.000
M207-1	207.088	120.000	36.000	170.000	10.000	22.000	8.000
M207-2	207.088	179.100	36.000	170.000	10.000	13.000	12.000
M247-1	247.080	118.000	-36.000	-150.000	-10.000	-24.000	-9.000
M247-2	247.080	157.900	-36.000	-150.000	-10.000	-24.000	-11.000

4. BT Uptake Ratio Calculation. *Note*: This value is reported as an approximation determined gravimetrically (hydroponic media specific gravity assumed = 1) and corresponds to the mass balance displayed in Figure 1 of the manuscript. It is meant only to estimate the approximate ratio of the observed pollutant mass uptake to that of the passive uptake (i.e., accumulation in the plant due to uptake with water through transpiration) during the same time period (i.e., one spike of benzotriazole). Exact measurements of transpiration were outside the scope of this work. The passive uptake of media was assumed to be represented by the volume of hydroponic media measured in the plant tissue plus the total lost due to evapotranspiration less the passive abiotic evaporation (as measured by a no plant control box).

(1) Mass of hydroponic media in plant at time of harvest=(Fresh weight – Dry weight)$_{final}$-(Fresh weight – Dry weight)$_{initial}$

(2) Total Evapotranspiration (for planted treatments)=(Mass Media)$_{final}$ – (Mass media)$_{initial}$

(3) Abiotic Transpiration (for no plant controls)=(Mass Media)$_{final}$ – (Mass media)$_{initial}$

(4) Total media uptake into / through the plant=Evapotranspiration+Media in plant at Harvest-evaporation

(5) Total Passive BT Uptake rate = Media Uptake (4) ×Media Conc. At t_o

(6) Measured BT uptake rate from media= Mass$BT_{initial}$ - MassBT_{final}

(7) "Active" uptake rate from media= Observed mass uptake rate / passive uptake rate

$$Passive\ Uptake = (Vol_{ET} + Vol_{InPlant})(\frac{Vol_i \times C_i}{Vol_i - Vol_{EVAP}})$$

$$Observed\ Uptake = (C_i Vol_i - C_f Vol_f)$$

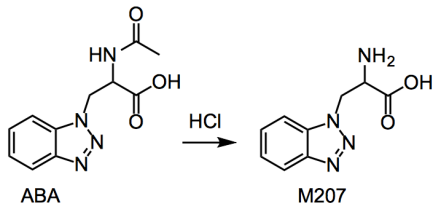
		Spike 1 (t=0 to t=3)	**Spike 2 (t=3 to t=8)**
Approximate Passive BT Uptake (ng)	Vol in Plant tissue (mL)	0.387	0.815
	Total E/T (mL)	1.36	3.05
	Abiotic Evaporation (mL)	0.89	0.54
	BT initial mass (C_iV_i) (ng)	72.4	46.8
	Initial Media Vol (mL)	25.13	25.86
	Total Passive Uptake	**5.22**	**7.14**
Observed BT Uptake (ng)	$Mi=C_iV_i$	72.4	46.8
	$Mf=C_fV_f$	0.95	0.61
	Total Observed Uptake	**71.5**	**46.2**
Ratio Observed BT mass uptake / Passive Uptake:		**13.69**	**6.46**

5. Synthesis of Benzotriazole Plant Metabolites M207 and M247.

General materials and methods. Flash column chromatography was performed using Silicycle P60 silica: 230-400 mesh (40-63 μm) silica. Reactions were monitored using thin layer chromatography (TLC, glass backed silica gel, 60 F_{254}, part number HX003787). TLC plates were visualized by UV fluorescence (254 nm) and followed by ceric ammonium molybdate stain.
Preparative HPLC purification was performed using an Agilent 1260 Infinity preparative-scale HPLC system with an Agilent 1100 diode array detector and a TARGA C18 10 µm 250 × 20 mm column (Higgins Analytical). Water with 0.1% TFA (A) and acetonitrile with 0.1% TFA (B) were used as the mobile phase components at a flow rate of 36 mL/min with the following method: 0-5 min, 3-25% B; 5-20 min, 25-40% B; 20-22 min, 40% B; 22-23 min, 40-97% B; 23-25 min, 97% B; 25-26 min, 97-3% B.

NMR solvents were purchased from Cambridge Isotope Laboratories. ^{1}H NMR (and ^{13}C-NMR) spectra were acquired at room temperature on a Varian 400 (100) MHz spectrometer. Chemical shifts (∂) are reported in parts per million (ppm) downfield from tetramethylsilane and referenced to the residual solvent peak(s) rounded to the nearest 0.01 for proton and 0.1 for carbon. Coupling constants (J) are reported in Hz to the nearest 0.1 Hz. Peaks multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Attribution of peaks was done using the multiplicities and integrals of the peaks.

Synthesis of M207: (adapted from Dilbeck et al. *J. Org. Chem.* **1978**, *43,* 4593)



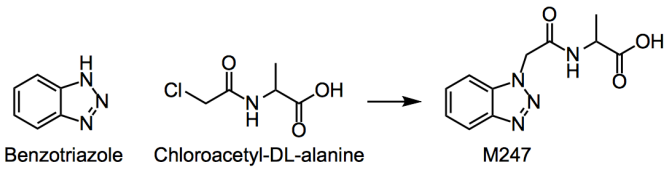
ABA M207

1.2 M HCl (1 mL) was added to 10 mg of *N*-acetyl-3-(1H-1,2,3-benzotriazol-1-yl)alanine (ABA; Sigma Aldrich # CCA001845, 40 μmole) in a borosilicate glass vial and sealed. The mixture was subjected to sonication to ensure complete dissolution. The reaction was heated to 100 °C for 8 h in a sand bath. The crude product was concentrated *in vacuo*, re-dissolved in 1 mL MiliQ water, frozen, and lyophilized to yield 7.1 mg (85 %, unoptimized) M207 as a layer of thin film. ^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (dt, J = 8.4, 0.9 Hz, 1H), 7.84 (dt, J = 8.5, 1.0 Hz, 1H), 7.63 (ddd, J = 8.2, 6.9, 1.0 Hz, 1H), 7.49 (ddd, J = 8.1, 7.0, 1.0 Hz, 1H), 5.37 (dd, J = 15.5, 5.7 Hz, 1H), 5.27 (dd, J = 15.5, 4.1 Hz, 1H), 4.79 (dd, J = 5.7, 4.1 Hz, 1H).

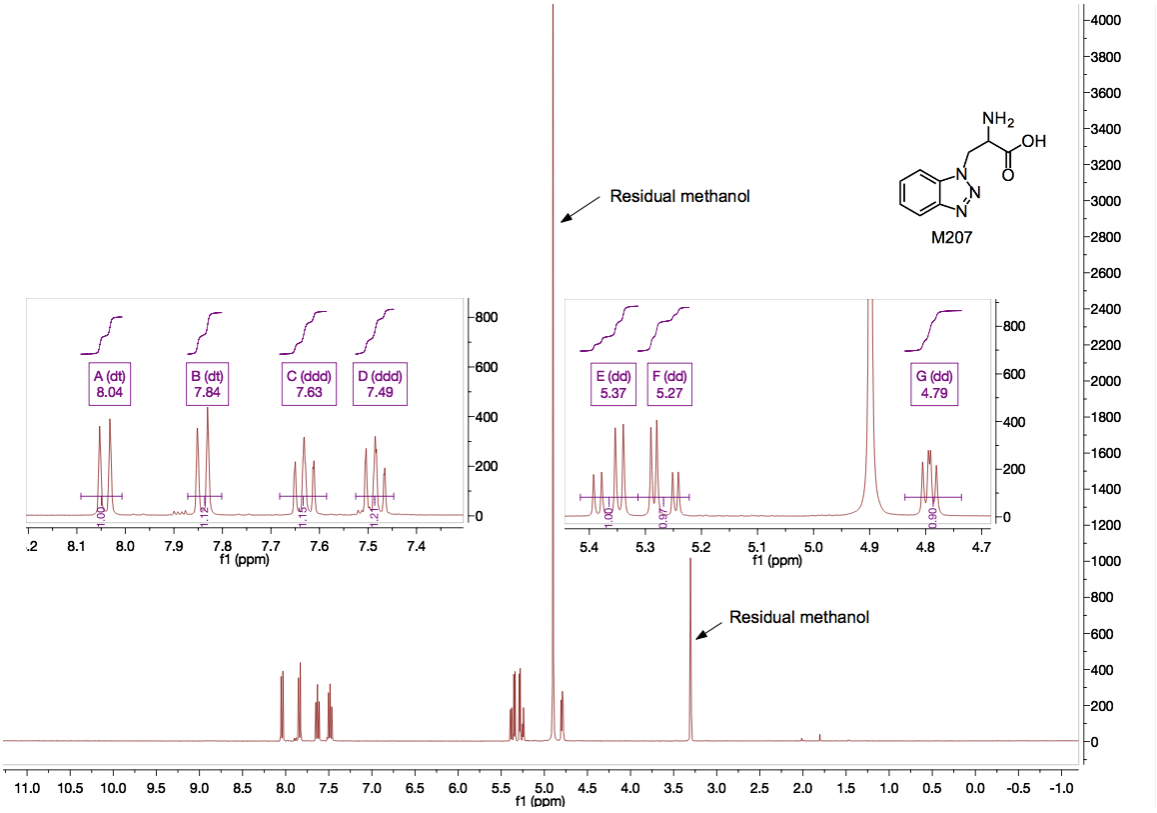
^{13}C-NMR (101 MHz, CD3OD) δ= 167.7, 145.6, 133.7, 128.3, 124.9, 119.1, 110.0, 52.3, 46.8.

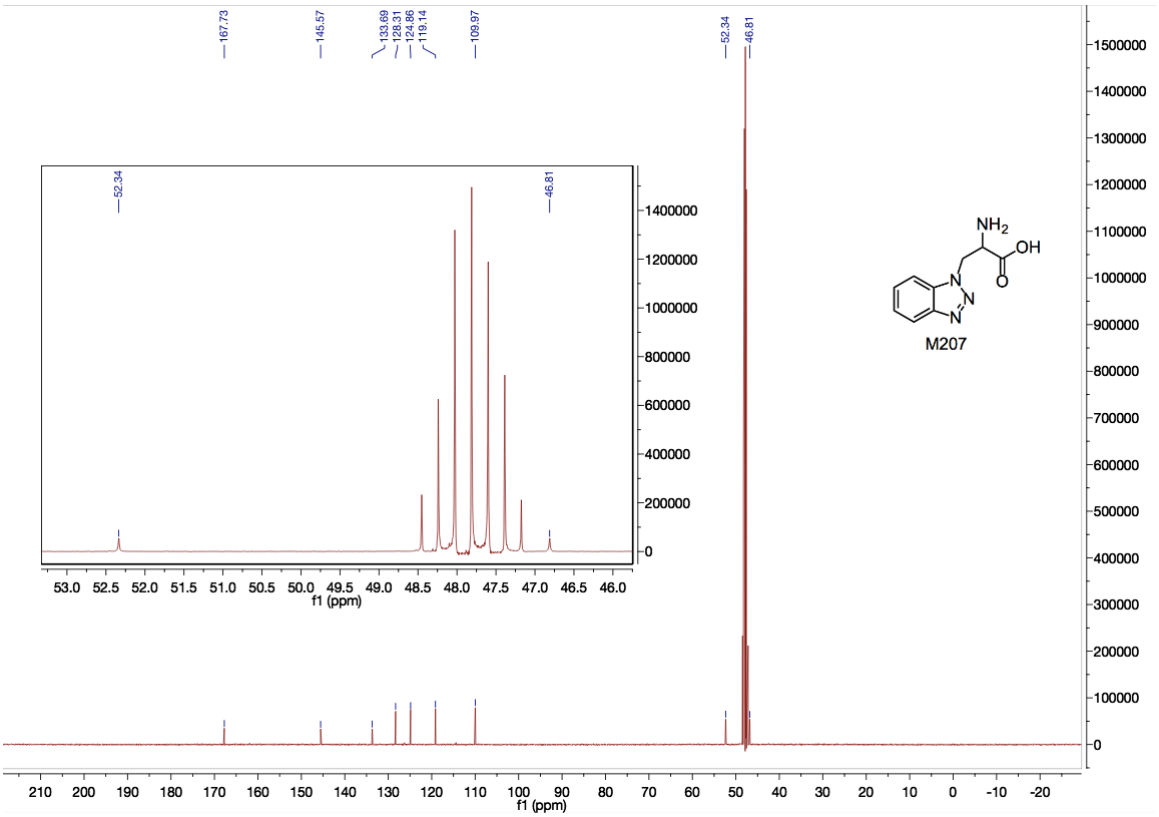
The ^{1}H NMR of the starting material (ABA) and the ^{1}H and ^{13}C NMR of the synthesized M207 and shown here in the SI.

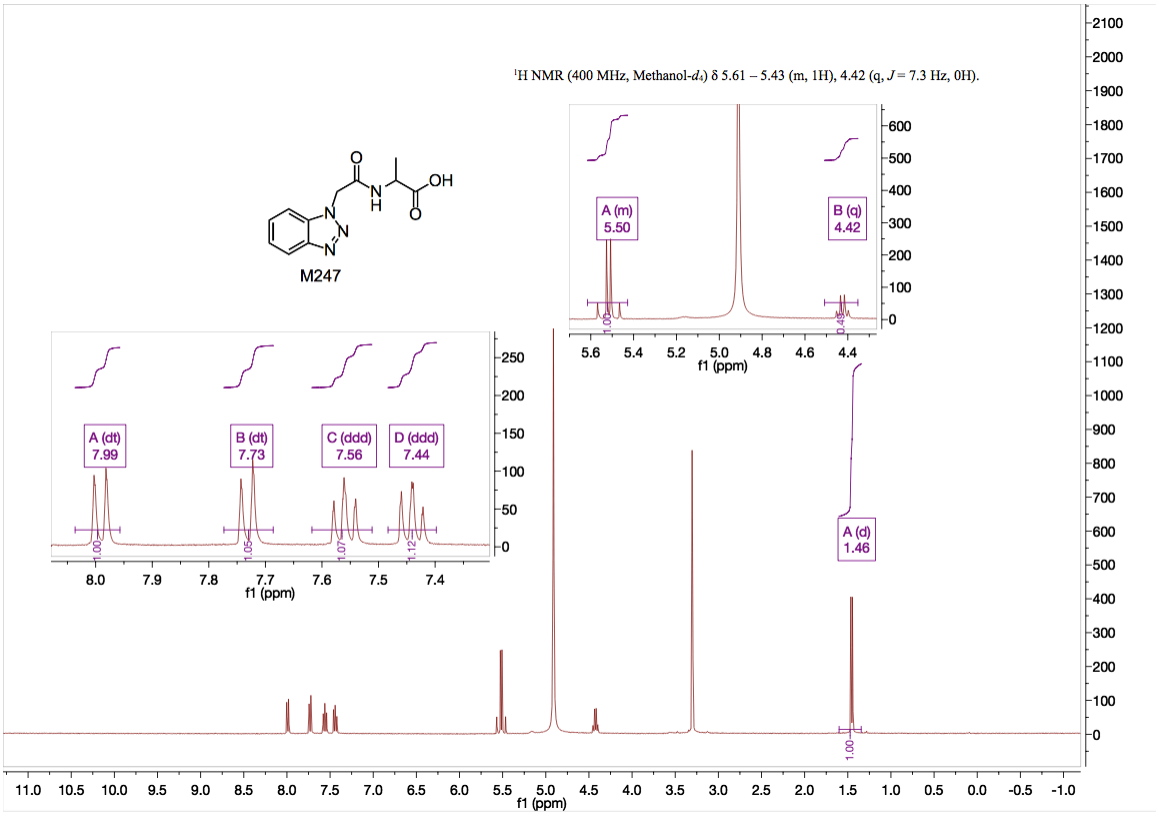
Synthesis of M247: (adapted from Chen et al. *J. Heterocyclic Chem*. **1996,** *3*, 1107)

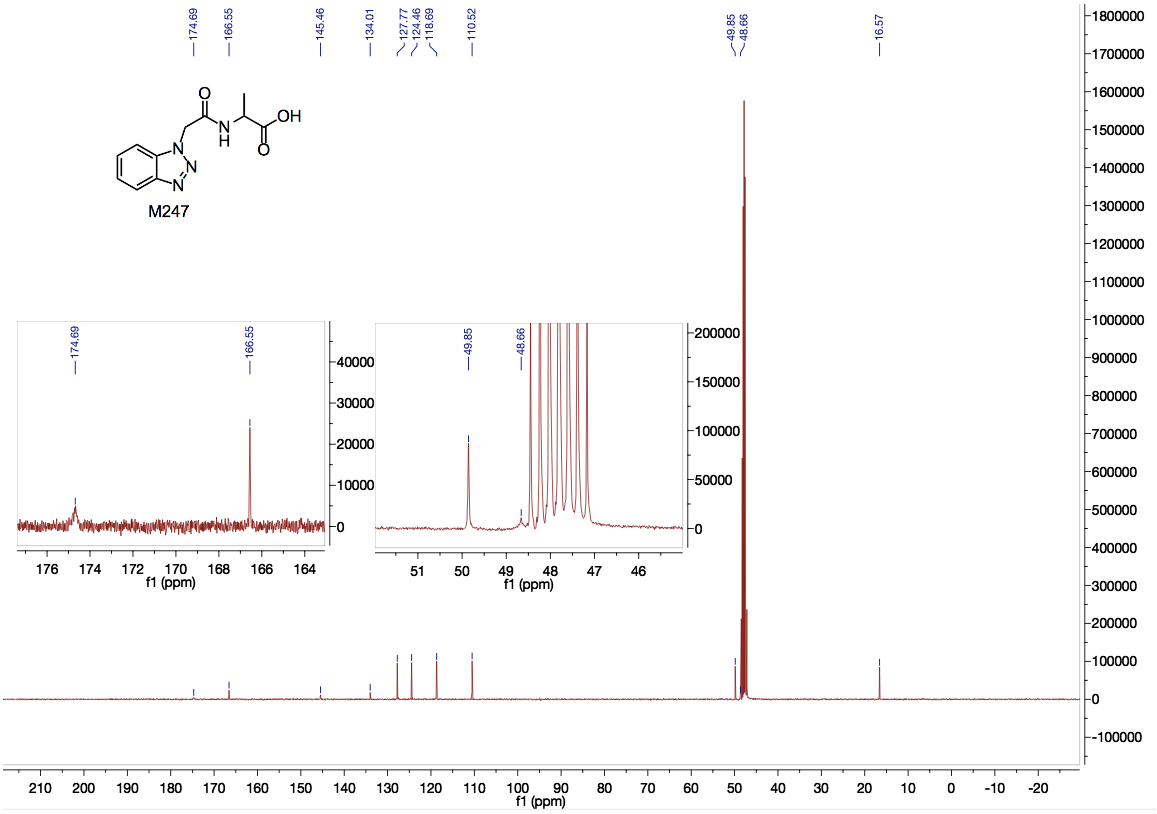


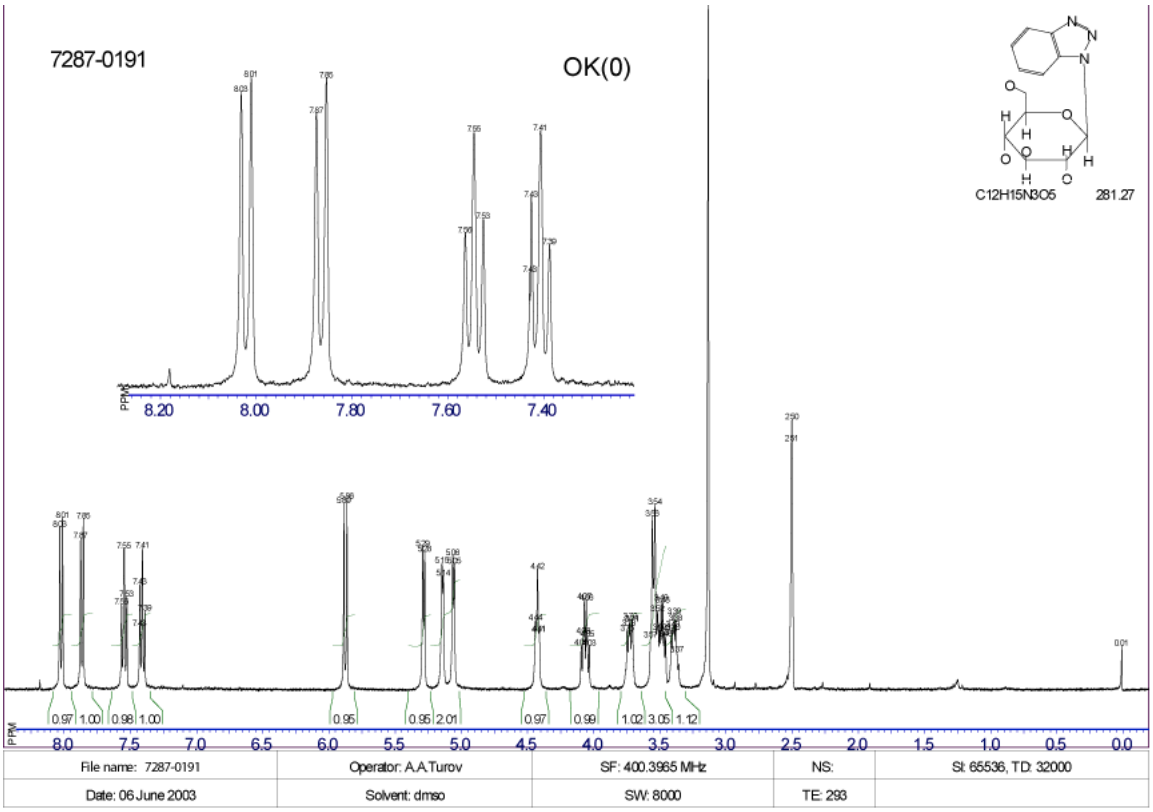
To a mixture of 4.8 mg of 1-H-benzotriazole (40 μmole, 1.0 eq) and 9.9 mg *N*-Chloroacetyl-DL-alanine (CAS: 1190-32-5; TCI America # C0097, 60 μmole, 1.5 eq) was added 0.5 mL of anhydrous toluene (Arcos organics) in a borosilicate glass vial and sealed. The mixture was subjected to sonication to ensure complete dissolution. The reaction was heated to 105° C for 15 hours. The solution was dried under a gentle stream of nitrogen to afford crystalline crude product, which was then subjected to preparative HPLC to remove unreacted *N*-Chloroacetyl-DL-alanine. The partially purified product was further purified via silica gel column chromatography (0.5 vol% formic acid and 5 vol% methanol in ethyl acetate) to afford 5.0 mg (50%, unoptimized) of M247. ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (dt, J = 8.4, 1.0 Hz, 1H), 7.73 (dt, J = 8.4, 1.0 Hz, 1H), 7.56 (ddd, J = 8.3, 6.9, 1.0 Hz, 1H), 7.44 (ddd, J = 8.1, 6.9, 1.0 Hz, 1H) , 5.61 – 5.43 (m, 1H), 4.42 (q, J = 7.3 Hz, 0H), 1.46 (d, J = 7.3 Hz, 1H). ^{13}C-NMR (101 MHz, CD3OD) δ= 174.7, 166.6, 145.5, 134.0, 127.8, 124.5, 118.7, 110.5, 49.9, 48.7, 16.6.




167.73
145.57
133.69
128.31
124.86
119.14
109.97
52.34
46.81
M207
f1 (ppm)


¹H NMR (400 MHz, Methanol-d_4) δ 5.61 – 5.43 (m, 1H), 4.42 (q, J = 7.3 Hz, 0H).
M247
A (m) 5.50
B (q) 4.42
A (dt) 7.99
B (dt) 7.73
C (ddd) 7.56
D (ddd) 7.44
A (d) 1.46
f1 (ppm)


174.69
166.55
145.46
134.01
127.77
124.46
118.69
110.52
49.85
48.66
16.57
M247
f1 (ppm)
f1 (ppm)
f1 (ppm)



Glycosylated-Benzotriazole (M282) NMR Specra (from Chem Div).

6. Harvesting of Exudates.

Exudates from *Arabidopsis* plants were harvested in a manner loosely based on LeFevre et al. [*Environ. Sci. Technol.*, **2013**, *47* (20), pp 11545–11553]. The purpose of this experiment was to determine if anything that is relegated to the plant but outside of it could be facilitating transformation of benzotriazole, for example, enzymes released by the plant in exudates or indirect photolysis due to organic matter release by plants. In short, dissolved exudates that may be released by the plant and present in the hydroponic medium were examined for possible contribution to BT transformation; no significant losses were observed (p=0.1670) in systems containing filter-sterilized root exudates.

For this exudate harvesting specific experiment, *Arabidopsis* plants were grown in MS hydroponic medium in the same manner described in Methods of manuscript (n=6 boxes of seedlings for 14 d). Plant tissues were removed leaving behind any dissolved exudates. The hydroponic medium from all boxes was combined to form a master mix. This master mix was filtered through a 0.22 µm PTFE filter and spiked with BT to a nominal concentration of 3 µg/L (the time=0 sample was measured exactly). The BT-spiked exudate solution was placed into new clean autoclaved Magenta boxes and placed into the plant growth chamber. Samples were harvested daily by removing the entire boxes (i.e., biological replicates) to determine if BT concentration had changed in the system.

8. Metabolomics Method Details: XCMS and R Code.

Agilent MassHunter data files were converted to mzXML format using Trapper (Seattle Proteome Center). mzXML files were analyzed by XCMS; procedural details are provided in the relevant references (Smith et al. 2006; DeVos et al. 2007). Further data processing of the resultant comparisons was described in the manuscript body in the Methods section. A sample R code for a comparison between treatment and control groups is as follows:

```
library(xcms)
xset<-xcmsSet()
xset<-group(xset)
xset2<-retcor(xset,family="s",plottype="m")
xset2<-group(xset2)
xset3<-retcor(xset2,family="s",plottype="m")
xset3<-group(xset3)
xset4<-retcor(xset3,family="s",plottype="m")
xset4<-group(xset4,bw=10)
xset5<-fillPeaks(xset4)
reporttab<-diffreport(xset5,"dBT","Control","dBT_vs_Control",500)
```

References cited:

1. Smith, C. A.; Want, E. J.; O'Maille, G.; Abagyan, R.; Siuzdak, G. XCMS: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching, and Identification. *Anal. Chem.* **2006**, *78*, 779-787.

2. De Vos, R.,C.H.; Moco, S.; Lommen, A.; Keurentjes, J. J. B.; Bino, R. J.; Hall, R. D. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nat. Protocols* **2007**, *2*, 778-791.

Supplementary Results

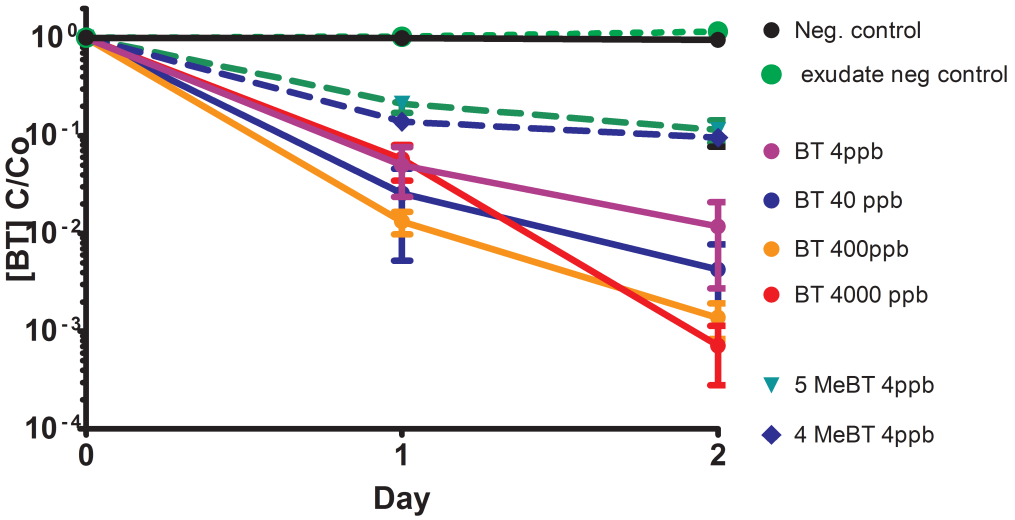


Figure S.2: Measurement of benzotriazole concentration in hydroponic media through time in planted systems. The negative control contained no plants and was exposed to the same lighting and temperature conditions to test for photodegradation and the BT plus filter-sterilized exudates tested for indirect photolysis or exudate catalyzed losses. No significant losses occurred in plant-free negative controls (p=0.4476) or controls containing filter-sterilized root exudates (p=0.1670), indicating that abiotic losses (including photolysis or extracellular plant enzyme processes (Gerhardt et al. 2009) were not relevant.

Initial BT concentration was varied between 4 and 4,000 ppb to examine the effect of initial conditions on uptake, specifically if any observable effects were evident. No significant difference in BT removal rate from the hydroponic medium was observed in experiments where the initial concentration was varied between 4 and 4000 µg/L (p=0.3794); however, more detailed, high frequency measurements would be required to examine uptake kinetics and is beyond the scope of this work. No negative effects (e.g., color, plant death) was evident at the tested exposure levels.

Two different methyl-BT isomers were also tested for plant uptake from media. The removal rates of 4- and 5-MeBT were not different from each other (p=0.2990), but by day 2 the removal of BT was significantly greater than either MeBT isomer (p=0.0019) at the same initial concentration (Fig 1). There is evidence of differential microbial recalcitrance (Weiss et al. 2006; Liu et al. 2013; McKeill and Cancilla 2009; Drummer 2014) among benzotriazoles (microbial recalcitrance: 4-MeBT>BT>5-MeBT), but the plant uptake rate is greater for BT than either MeBT isomer. Comprehensive metabolomics studies for MeBT were not conducted, but predicted analogous metabolites in treated plants and absent from controls were observed via an LC-QTOF-MS targeted formula search (S.8, Table S.4). Thus, the same transformation pathways observed for BT are presumed to occur for MeBT but at different rates as described above. The purpose in investigating MeBT in this preliminary experiment was to determine if the same uptake occurred for MeBT as for BT; the differences in uptake kinetics was not investigated for this study. Chemicals used included: 5-Methyl-1H-Benzotriazole (5-MeBT; Aldrich, CAS: 136-85-6), 4-Methyl-1H-Benzotriazole (4-MeBT; Fluka, CAS: 29878-31-7),

1. Dummer, N. 4(5)-Methylbenzotriazole: a review of the life-cycle of an emerging contaminant. Reviews in Environmental Science and BioTechnology 2014, 13, 53-61.

2. Gerhardt, K. E.; Huang, X.; Glick, B. R.; Greenberg, B. M. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Science 2009, 176, 20-30.

3. Liu, Y.; Ying, G.; Shareef, A.; Kookana, R. S. Biodegradation of three selected benzotriazoles in aquifer materials under aerobic and anaerobic conditions. J. Contam. Hydrol. 2013, 151, 131-139.

4. McNeill, K. S.; Cancilla, D. A. Detection of Triazole Deicing Additives in Soil Samples from Airports with Low, Mid, and Large Volume Aircraft Deicing Activities. Bull. Environ. Contam. Toxicol. 2009, 82, 265-269.

5. Weiss, S.; Jakobs, J.; Reemtsma, T. Discharge of Three Benzotriazole Corrosion Inhibitors with Municipal Wastewater and Improvements by Membrane Bioreactor Treatment and Ozonation,Ä. Environ. Sci. Technol. 2006, 40, 7193-7199.

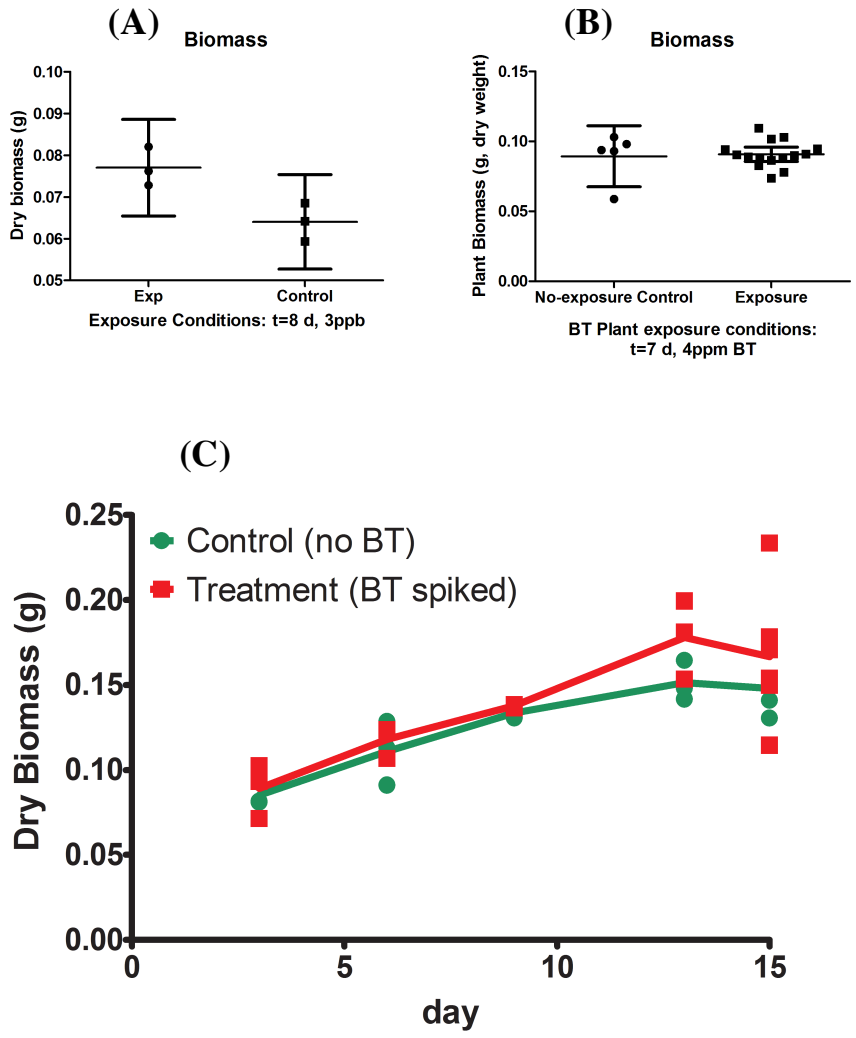


Figure S.3: Effect of benzotriazole on *Arabidopsis* overall plant biomass. Graph (A): The biomass in the treated experimental samples for an exposure of two spikes of 3 ppb after 8 d was compared to the positive controls (to test for seed viability) from the plants shown in Figure 1 BT uptake experiment of the manuscript (error bars show the 95% CI). There was no significant difference between the overall biomass in the samples (p=0.10), but the sample size was also small because the positive control was mainly included to ensure that plants were successfully grown. Graph (B): A biomass experiment comparing biomass after BT exposure at a much higher dose (4 ppm for 7 d) with no exposure plants grown simultaneously under the same conditions. No significant differences in overall biomass were observed (p=0.81). A higher concentration was investigated to attempt to induce an effect. Graph (C): A parallel harvest biomass study for treated (initial concentration in hydroponic media=600 µg/L) and untreated *Adrobadopsis* plants test was also conducted (at a somewhat higher than 3ppb to attempt to induce an effect, but much below 4 ppm) to provide a matched-pairs temporal comparison for the most statistical rigor because the treatment conditions can be compared at multiple time points. There was no significant difference between the treatment and control (p=0.06) after two weeks. The exact same growth conditions described for the experiment were used for the biomass production experiment. This separate more comprehensive biomass BT exposure experiment was conducted because the comparison of the biomass between the treatment and control for the BT exposure experiment (Figure 1 of the manuscript) was inconclusive (Graph (A)). A higher BT exposure level and more biological replicates were incorporated into the separate biomass. For this separate biomass effect experiment, samples (whole boxes planted with n=30 seeds and harvesting all plants from each biological replicate box) from treatment and controls were harvested started after initial BT exposure on days 3, 6, 9, 13, and 15. For the first four time points, three biological replicates each for the treatment and control were harvested and there were five replicate boxes harvested each for the treatment and control on the last time point (the reason for larger number of replicates at the last time point is because presumable any biomass differences would be more pronounced at a later time point). All biological replicates were used for statistical purposes to account for variability; specifically, each replicate for the treatment and control was used in a matched-pairs Student's t-test.

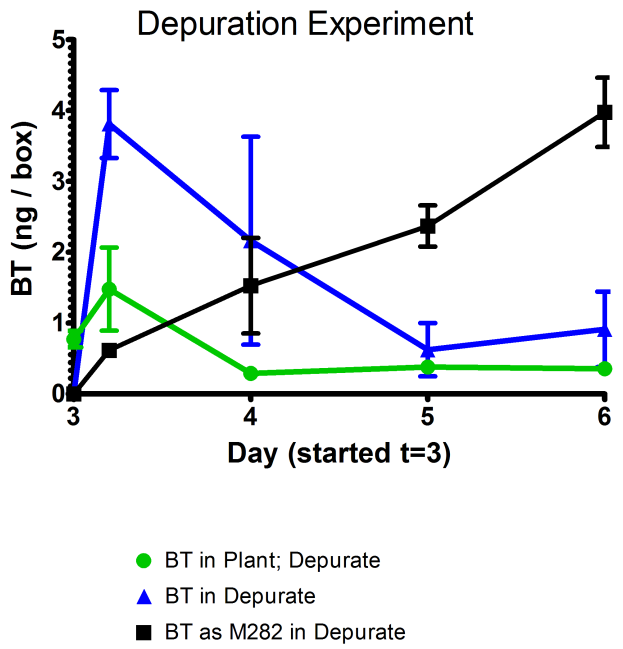


Figure S.4: Depuration experiment results in which plants previously exposed to benzotriazole were transferred to 'clean' (no benzotriazole) MS hydroponic media. BT parent compound did not significantly depurate from the plant tissues (p=0.94), but the BT metabolite M282 (expressed at BT molar mass equivalents) did release from the plant tissues. This depuration experiment was conducted using plant boxes (n=3 biological replicates harvesting all plants therein) from Figure 1 that were exposed to Spike 1 (indicated in Figure 1), thus the Depuration Experiment x-axis begins on day t=3 (three days since the initial BT exposure from Spike 1). During the depuration experiment, plants exposed to BT for 3 days were transferred to hydroponic medium that contained no BT ('clean medium'). There plants and medium were periodically harvested daily until day t=6. Samples during the first day were harvested immediately after exposure to clean hydroponic medium and then after two hours to observe any effects of reversible sorption/desorption of BT from the plant tissue. Please note the difference in scale on the y-axis in contrast to Figure 1 and that the y-axis is linear in contrast to the log-scale of Figure 1. The BT mass equivalents released during depuration was much less than the BT mass spiked into the system.

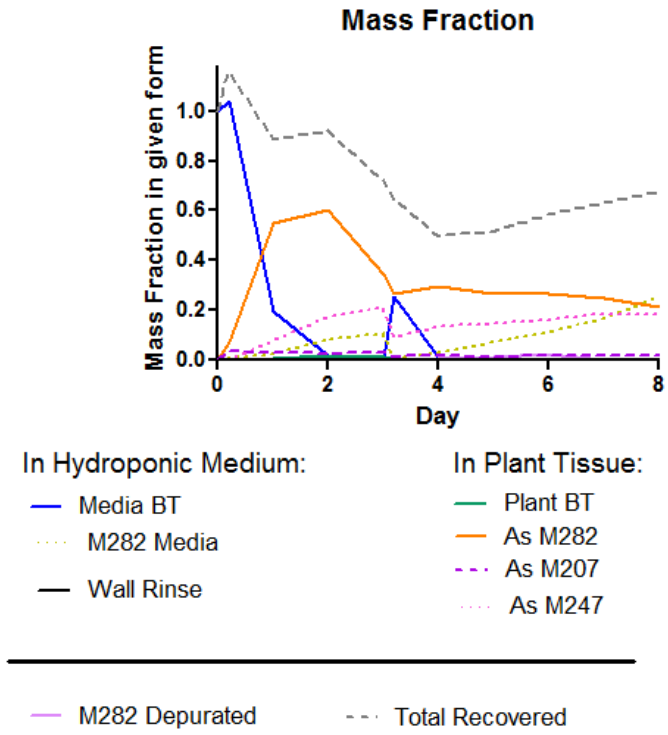


Figure S.5: Total mass fraction recovered of benzotriazole. Benzotriazole metabolites are represented in terms of benzotriazole molar equivalents. The BT rise in the media during day 3 occurs because the second BT spike occurred at that time (see Figure 1 in the text).

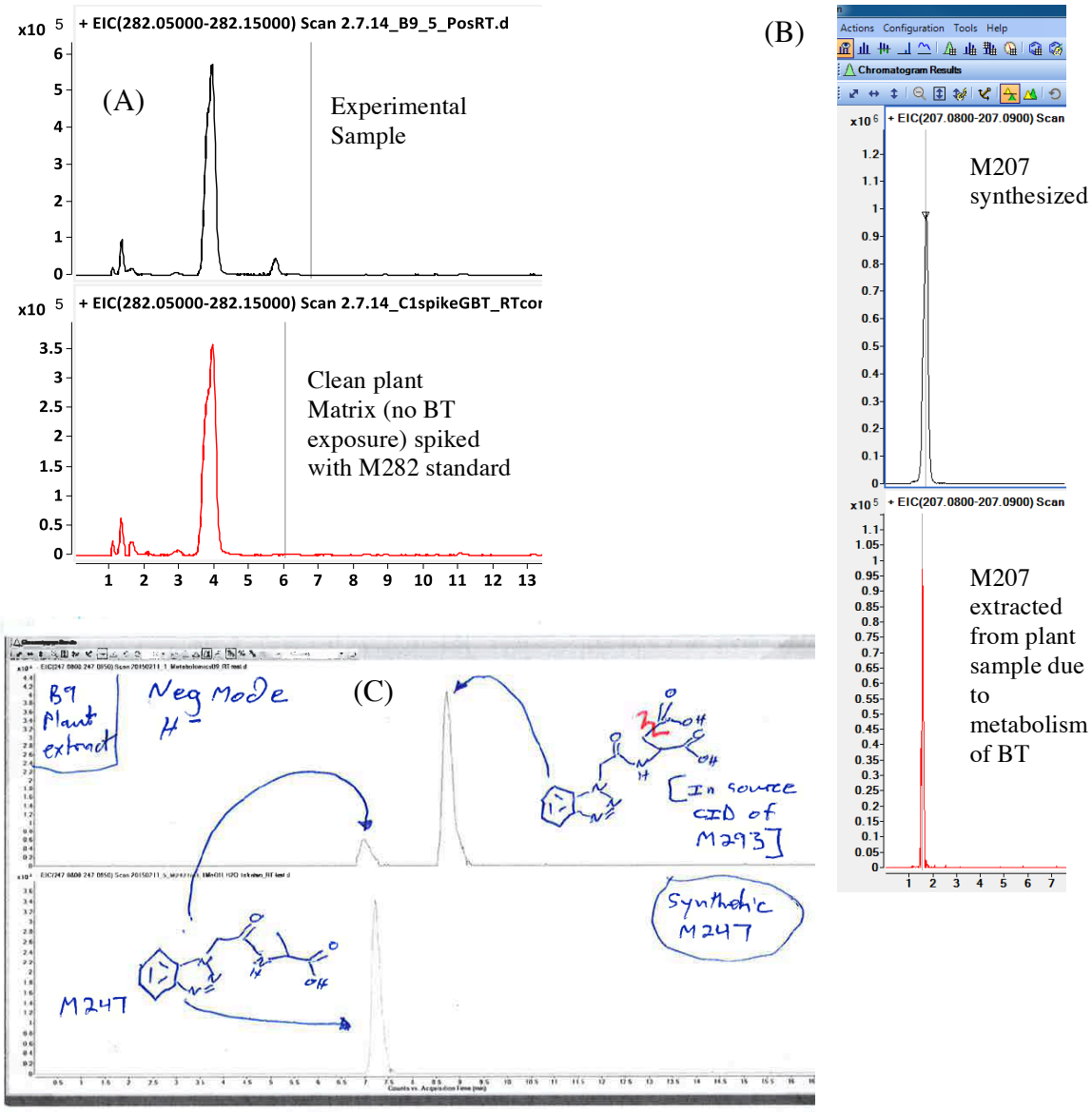


Figure S.6: Examples of retention time verification for metabolites. The retention time of the synthesized metabolite spiked into an unexposed plant matrix was compared to the retention time of the proposed metabolite from the exposed plant extract. The upper left chromatograms (A) are for M282, the right at for M207 (B), and the lower (C) are for M247. The targeted formula search for M247 in the plant extract reveals the same retention time for the synthetic as for the metabolomics experiments (see Table 1); in source collision induced dissociation (CID) of M293 also creates a peak with an m/z value of 247 but the differing retention time (see Table 1) demonstrates that this peak is a different compound and not an adduct.

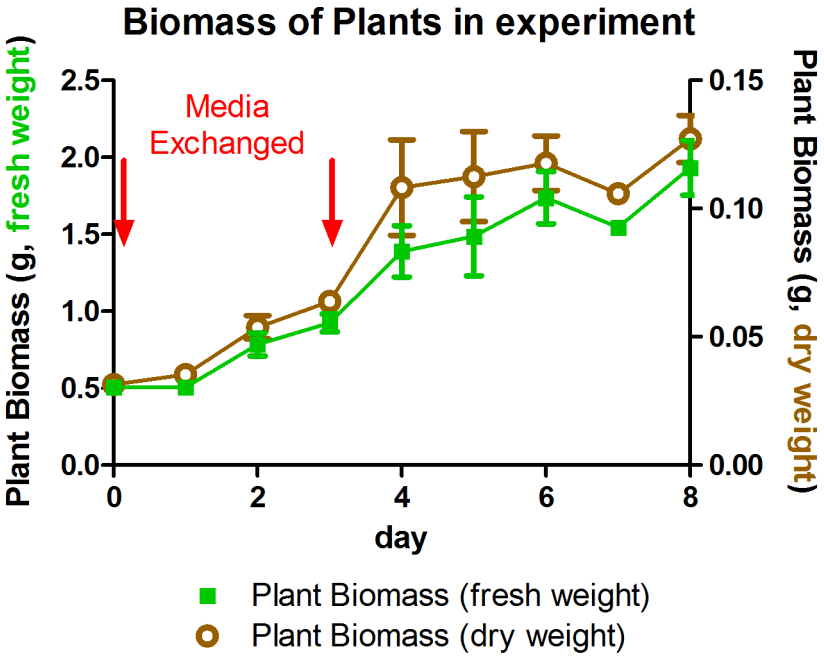


Figure S.7: Fresh weight and dry weight of the plant biomass of the plants harvested in the BT repeated spike exposure experiment (results in Figure 1 of the manuscript). Note that the y-axis for the fresh and dry weights are on the left and right sides, respectively, and are at different scales. The points at which the hydroponic medium was exchanged are shown in the red arrows; plants appeared to respond to the new influx of nutrients present in the fresh medium. Error bars are the standard error of the mean (n=3 biological replicates); some error bars are obscured by the data symbols.

Table S.2: Benzotriazole and metabolite data with full MS/MS fragment ion data. This table supplements Table 1 in the Manuscript Body.

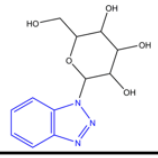
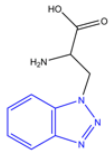
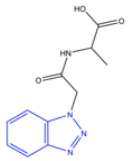
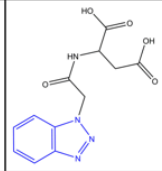
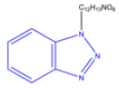
Benzotriazole Plant Metabolites											Fragment Ions			
Compound Name, Description			Proposed Structure	Proposed Formula	Confidence Level*	RT (sec)	ESI Mode (+/-)	Accurate Mass (m/z)	Accurate Mass Deviation (ppm)	d_4-Accurate Mass (m/z)	Fragment ions (nominal mass; m/z)	Accurate mass (m/z)	Accurate Mass Deviation (ppm)	Proposed Molecular Formula
BT Parent Compound	BT	Benzotriazole		$C_6H_5N_3$	Level 1; Standard confirmation, HR-MS, MS/MS, RT confirmed	398	+H	120.07226	-0.84	124.08004	NA	NA	NA	NA
Glucose Conjugates	M282	Glycosylated Benzotriazole		$C_{12}H_{15}N_3O_5$	Level 1; Standard confirmation, HR-MS, MS/MS, RT confirmed	94	+H	282.10690	5.49	286.13870	120	120.0553274	2.49	$C_6H_5N_3$
Amino Acid Conjugates	M207	Benzotriazole Alanine ("BT-tryptophan")		$C_9H_{10}N_4O_2$	Level 1; Standard synthesized (^1H, ^{13}C NMR), HR-MS, MS/MS, RT confirmed	95	+H	207.08746	0.92	211.11268	120	120.05533	2.49	$C_6H_5N_3$
											88	88.03934	-0.35	$C_3H_5NO_2$
											146	146.05905	-2.43	$C_7H_5N_4$
											164	164.06984	-3.52	$C_7H_7N_4O$
	M247	Benzotriazole Acetyl-Alanine		$C_{11}H_{12}N_4O_3$	Level 1; Standard synthesized (^1H, ^{13}C NMR), HR-MS, MS/MS, RT confirmed	471	-H	247.08246	-0.72	251.10784	118	118.04087	1.69	$C_6H_5N_3$
											160	160.03924	-1.15	$C_7H_5N_4O$
											132	132.0445	-2.66	$C_6H_5N_4$
											146	146.03593	0.39	$C_7H_5N_3O$
											104	104.0483	-3.87	$C_4H_9O_3$
	M293	Benzotriazole Acetyl-Aspartate		$C_{12}H_{12}N_4O_5$	Level 2b: HR-MS, MS/MS	494	+H	293.08803	0.06	297.11312	120	120.05533	2.49	$C_6H_5N_3$
											174	174.03928	2.41	$C_6H_7NO_5$
											128	128.03427	-0.42	$C_5H_5NO_3$
											156	156.02857	3.66	$C_6H_5NO_4$
Unknown	M387	Niacin Glycosylated Benzotriazole		$C_{18}H_{18}N_4O_6$	Level 3: HR-MS, MS/MS	633	+H	387.13045	-1.39	391.15477	120	120.0553274	2.49	$C_6H_5N_3$
											124	124.0384743	6.75	$C_6H_5NO_2$
											268	268.0808471	2.68	$C_{12}H_{13}NO_6$
											106	106.0289129	-1.64	C_6H_3NO
	M468	Unknown	Unknown	Ambiguous	Level 5; HR-MS, exact mass	509	-H	468.16095	-	472.18685	223	223.05937	-	N/A

Table S.3: MS/MS fragment analysis (further details to Table S.2) from Agilent Mass Hunter.

Parent :	Fragment Name:	Measured m/z	Formula	Score	Mass	Mass Calc	m/z calc	Diff (ppm_	m/z	DBE*
M387	120	120.05533	C6 H5 N3	99.14	119.04805	119.04835	120.05562	2.49	120.05533	6
	124	124.03847	C6 H5 N O2	93.6	123.0312	123.03203	124.0393	6.75	124.03847	5
	268	268.08085	C12 H13 N O6	97.67	267.07357	267.07429	268.08156	2.68	268.08085	7
Fragment 268 (from M3287)	124	124.03847	C6 H5 N O2	93.6	123.0312	123.03203	124.0393	6.75	124.03847	5
	120	120.05533	C6 H5 N3	99.14	119.04805	119.04835	120.05562	2.49	120.05533	6
	106	106.02891	C6 H3 N O	99.68	105.02164	105.02146	106.02874	-1.64	106.02891	6
M293	120	120.05533	C6 H5 N3	99.14	119.04805	119.04835	120.05562	2.49	120.05533	6
	174	174.03928	C6 H7 N O5	98.76	173.03201	173.03242	174.0397	2.41	174.03928	4
	128	128.03427	C5 H5 N O3	99.97	127.027	127.02694	128.03422	-0.42	128.03427	4
	156	156.02857	C6 H5 N O4	97.49	155.02129	155.02186	156.02913	3.66	156.02857	5
M315	120	120.05533	C6 H5 N3	99.14	119.04805	119.04835	120.05562	2.49	120.05533	6
M207	120	120.05533	C6 H5 N3	99.14	119.04805	119.04835	120.05562	2.49	120.05533	6
	88	88.03934	C3 H5 N O2	99.99	87.03206	87.03203	88.0393	-0.35	88.03934	2
	146	146.05905	C7 H5 N4	98.96	145.05177	145.05142	146.0587	-2.43	146.05905	7.5
			C9 H7 N O	92.09	145.05177	145.05276	146.06004	6.83	146.05905	7
	164	164.06984	C7 H7 N4 O	97.53	163.06256	163.06199	164.06926	-3.52	164.06984	6.5
			C9 H9 N O2	95.62	163.06256	163.06333	164.0706	4.71	164.06984	6

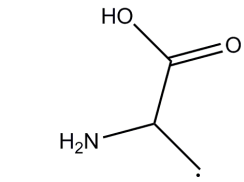
*Estimated Double Bond Equivalent

Table S.4: Metabolite exact mass defect data for benzotriazole and BT-plant metabolites measured by accurate mass LC-QTOF-MS. The exact m/z values for BT and the metabolites are shown as well as the observed mass defect. The ratio of the exact mass target compound and the most abundant isotope are provided for both the plants exposed to BT and BT-d4. The mass shift between the BT exposed and BT-d4 compounds are evident (i.e., four neutrons different). The calculated isotopic mass defect for the observed isotopes is provided and was *calculated using ChemCalc (Chemcalc.org). The measured relative abundance is based on the peak areas observed during metabolomics (average of n=9 samples per comparison). The measured relative abundance of the peak areas of the isotopes is comparable to the expected calculated values. For some metabolites, no isotopes were observed; however, all proposed metabolites were identified with a d4-shift.

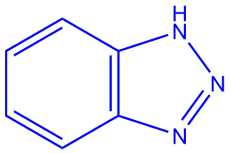
Name	Formula	Ionization Mode	Exact mass m/z	Observed mass defect	Measured Relative Abundance	d4 metabolite exact mass	d4-mass defect	Measured Relative Abundance	Calculated* Isotopic Relative Abundance
Benzotriazole	$C_6H_5N_3$	+H	120.07226	121.07619	7.8%	124.08004	125.08340	6.8%	6.5%
M282	$C_{12}H_{15}N_3O_5$	+H	282.10690	283.09807	14.2%	286.13870	287.14262	14.7%	13.0%
M293	$C_{12}H_{12}N_4O_5$	+H	293.08803	None observed	NA	297.11312	-	-	-
M387	$C_{18}H_{18}N_4O_6$	+H	387.13045	388.13327	21.9%	391.15477	392.16542	24.7%	19.5%
M207	$C_9H_{10}N_4O_2$	+H	207.08746	None observed	NA	211.11268	-	-	-
M247	$C_{11}H_{12}N_4O_3$	-H	247.08246	248.08539	14.1%	247.08246	248.08539	13.4%	11.9%
M468	Unknown	-H	430.13067	None observed	NA	434.15025	-	-	-

Figure S.8: Visual representations of MS/MS fragment analysis for M207, M282, M247, and M293.

M207:

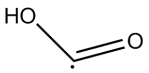


Chemical Formula: $C_6H_5N_3$
Exact Mass: 119.0483

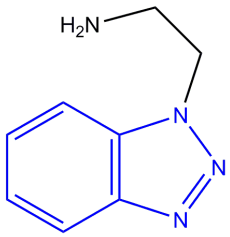


Chemical Formula: $C_3H_6NO_2^{\bullet}$
Exact Mass: 88.0399

M207: Benzotriazole propanoic acid
BT-"tryptophan"

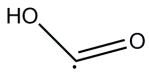


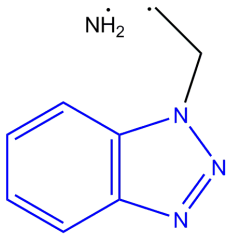
Chemical Formula: $CHO_2^{\bullet}$
Exact Mass: 44.9977



Chemical Formula: $C_8H_{10}N_4$
Exact Mass: 162.0905

M207: Benzotriazole propanoic acid
BT-"tryptophan"



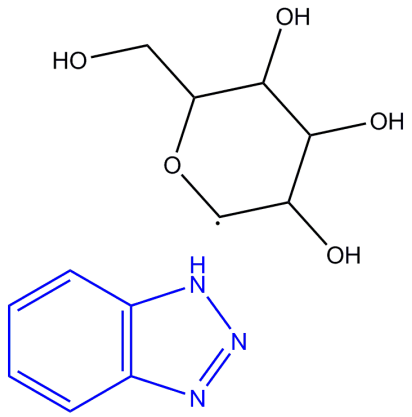


Chemical Formula: $C_8H_8N_3^{\bullet}$
Exact Mass: 146.0718

M207: Benzotriazole propanoic acid
BT-"tryptophan"

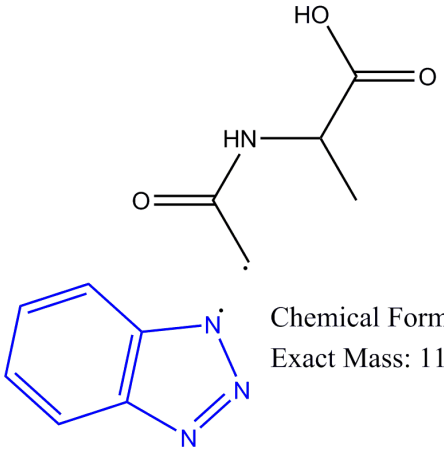
M282:

Chemical Formula: $C_6H_5N_3$
Exact Mass: 119.0483

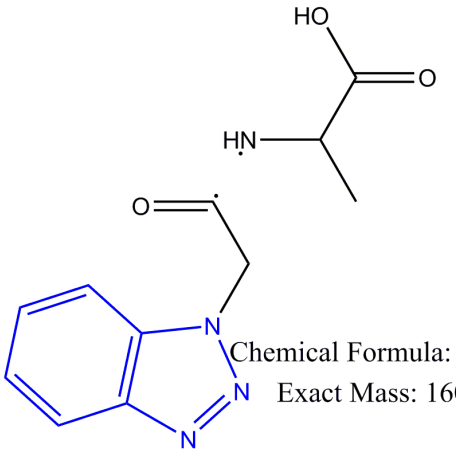


M282: Glycosylated Benzotriazole

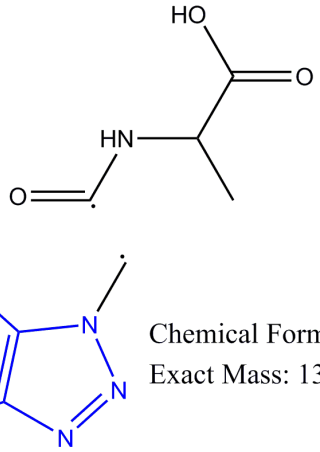
M247:



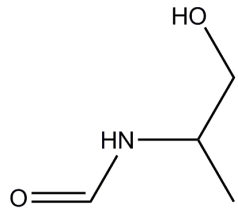
Chemical Formula: $C_6H_4N_3^{\bullet}$
Exact Mass: 118.0405



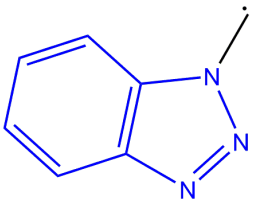
Chemical Formula: $C_8H_6N_3O^{\bullet}$
Exact Mass: 160.0511

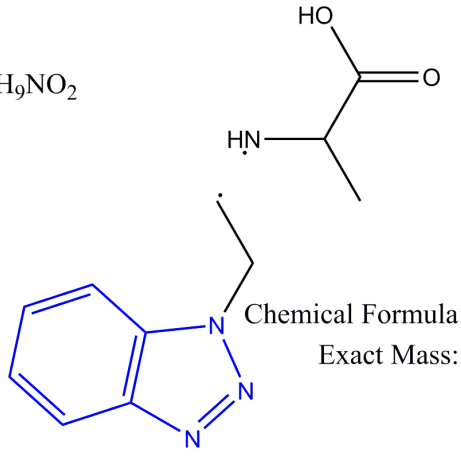


Chemical Formula: $C_7H_6N_3^{\bullet}$
Exact Mass: 132.0562



Chemical Formula: $C_4H_9NO_2$
Exact Mass: 103.0633

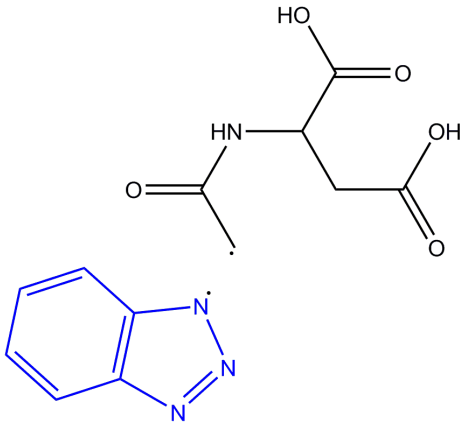




Chemical Formula: $C_8H_8N_3^{\bullet}$
Exact Mass: 146.0718

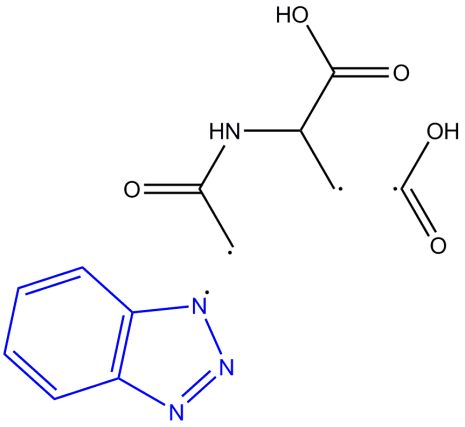
M293:

Chemical Formula: $C_6H_8NO_5^{\bullet}$
Exact Mass: 174.0402

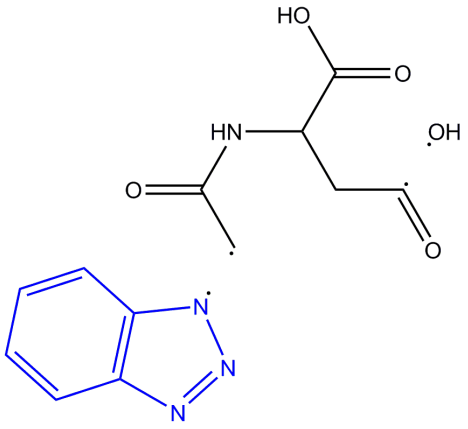


Chemical Formula: $C_6H_4N_3^{\bullet}$
Exact Mass: 118.0405

Chemical Formula: $C_5H_7NO_3^{2\bullet}$
Exact Mass: 129.0426



Chemical Formula: $C_6H_7NO_4^{2\bullet}$
Exact Mass: 157.0375



Targeted Formula Search for methylated BT metabolites

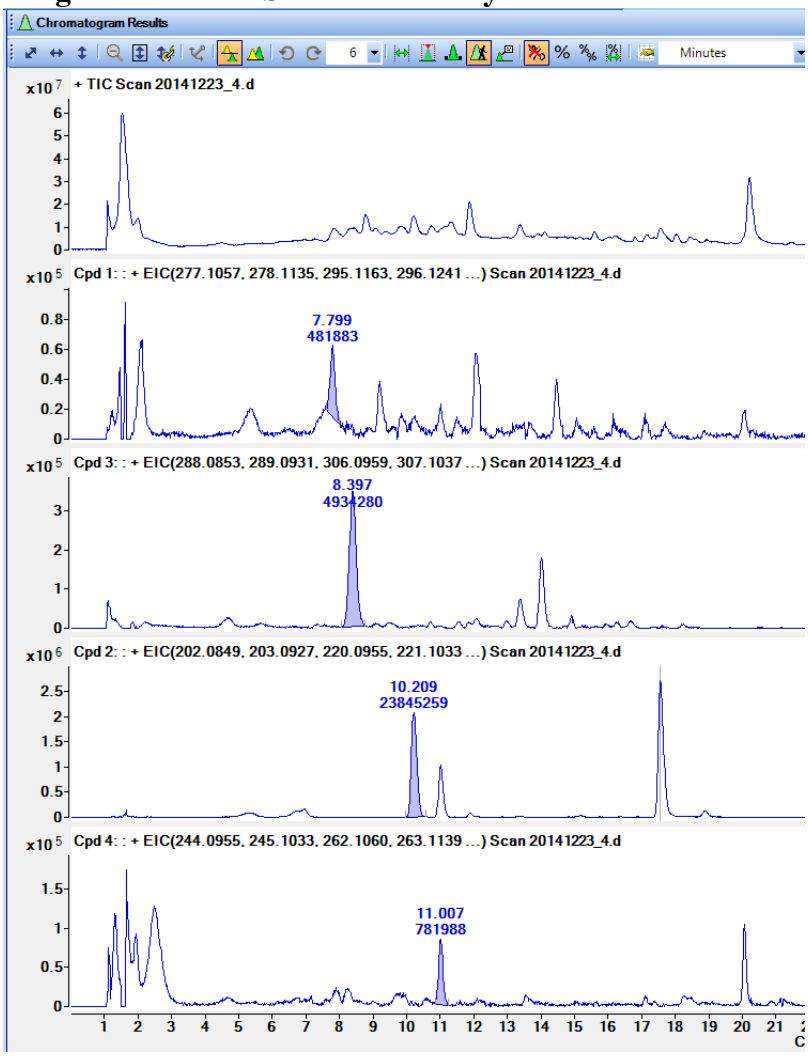


Figure S.9: Targeted formula search for predicted methylated benzotriazole metabolites in plant extract exposed to 5MeBT (samples from Fig. 1, initial concentration 4 µg/L). The formulae were generated assuming the same metabolites observed for BT also occurred for MeBT. Target formula search was conducted using Agilent Mass Hunter and results are presented below in Table S.4. All targeted metabolites were also searched in unexposed plant extracts and were absent.

Table S.5: Results of targeted formula search for proposed methylated BT metabolites using Agilent Mass Hunter, assuming same metabolites occur with except with addition of methyl group. Note that the exposure concentration was lower than for the untargeted metabolomics work for BT and differences in structure (i.e., extra methyl group) are likely responsible for different retention time. All targeted MeBT metabolites were absent from the negative control plant extracts measured.

Proposed metabolite	Formula	Score	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Diff (Tgt, mDa)	Polarity	RT	Algorithm
Methyl-M207	C10H12N4O2	88.62	220.09746	220.09603	6.53	1.44	Positive	10.209	Find By Formula
Methyl-247	C12H14N4O3	78.23	262.10794	262.10659	5.16	1.35	Positive	11.007	Find By Formula
Methyl-M293	C13H14N4O5	79.96	306.0983	306.09642	6.14	1.88	Positive	8.397	Find By Formula
Methyl-M282	C13H17N3O5	74.84	295.11552	295.11682	-4.41	-1.3	Positive	7.799	Find By Formula