Electronic Supplementary Information

for

Halogenated anilines as novel natural products from a marine biofilm forming microalga

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Materials and Methods

Chemicals

Ethyl acetate (GC grade), ethanol (99.7%) and methanol (UPLC grade) were purchased from VWR International GmbH (Darmstadt, Germany). Chloroform (GC grade) was purchased from Merck (Darmstadt, Germany), KBr from Roth (Karlsruhe, Germany), BSTFA from Thermo Scientific (Bremen, Germany), K_2HPO_4 from Fluka (Deisenhofen, Germany), 2,4,6-tribromoaniline (98%), KCl, and KH_2PO_4 were purchased from Alfa Aesar (Ward Hill, MA), aniline from Sigma (Deisenhofen, Germany), and 2,4,6-trichloroaniline (>99%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The starting material for the synthesis of 2,4,6-tribromo-3,5-*d*2-aniline (TBA-d_2) was benzene-*d*6 from the former Institute of Nuclear Research (IBJ, now NCBJ, Otwock-Świerk, Poland, 1976). Solvents and chemicals used in the synthesis were reaction grade only. Stable isotope labeled $NaH^{13}CO_3$ (98 atom % ^{13}C) and $Na^{15}NO_3$ (98 atom % ^{15}N) were purchased from Sigma Aldrich (Taufkirchen, Germany).

Microalgal cell cultures

The marine diatom *Nitzschia* cf. *pellucida* (strain DCG 0303) was obtained from the Belgian Coordinated Collection of Microorganisms. Cells were grown in polystyrene cell culture flasks for adherent cells (Sarstedt, Nümbrecht, Germany) in artificial seawater (ASW) which was prepared according to Maier *et al*.[1] Cells were grown under illumnination with daylight fluorescent tubes (40-50 µmol $m^{-2}s^{-1}$) in a 14:10 light:dark cycle coupled to a thermoregulated cycle (16 °C/12 °C day/night). The first microalgal extract that led to the discovery of the halogenated anilines was prepared from cells grown in a 12:12 light:dark cycle at 18 °C under daylight fluorescent tubes (50-60 µmol $m^{-2}s^{-1}$). Cell counts were obtained by counting cells with a Fuchs-Rosenthal hemocytometer (Laboroptik, Lancing, United Kingdom) using an inverted Leica DM IL LED light microscope (Heerbrugg, Switzerland).

Stable isotope labeling

ASW media were prepared as described elsewhere[1], but using stable isotope labeled $NaH^{13}CO_3$ (2.4 mM) or $Na^{15}NO_3$ (621 µM) instead of the unlabeled salts. Cultures in the late exponential growth phase (150,000 - 200,000 cells mL^{-1}) were centrifuged (4,000 ×*g*, 15 min) and the cells were washed twice with fresh labeled medium. The labeled medium was inoculated with washed cells (2,000 - 5,000 cells mL^{-1}). When the cultures reached late exponential growth phase (150,000 – 200,000 cells mL-1) two thirds of the $Na^{15}NO_3$–enriched medium was substituted with fresh labeled medium to enhance the degree of labeling. The culture flasks with $NaH^{13}CO$-enriched medium were stored upstanding to reduce the headspace and by that the uptake of $^{12}CO_2$ as a carbon source. The extractions of intracellular and extracellular metabolites were performed in triplicates as described in the next section, using *N.* cf. *pellucida* cultures in 40 mL medium (^{15}N-labeling) or 80 mL medium (^{13}C-labeling) in the stationary growth phase (150,000 -250,000 cells mL^{-1}).

Intracellular and extracellular extraction

Cells were carefully detached from the surface of the culture flask using a cell scraper (Sarstedt, Nümbrecht, Germany) and resuspended by slow shaking. An aliquot of 1 mL was obtained for the determination of the cell density. Cells were collected on a 25 mm GF/C glass microfiber filter (Whatman, Maidstone, United Kingdom) using a vacuum-coupled filter unit. The spent filtered medium was collected in a round bottom flask. The microfiber filters with cells were placed in a 2 mL tube and 1.8 mL ethyl acetate (for quantification) or a mixture of methanol:ethanol:chloroform (*v*:*v*:*v*, 2:6:2) (for screening) were added for extraction. Samples were sonicated for 5 min in an ultrasonic bath, and subsequently centrifuged (16,100 ×*g*, 15 min, room temperature). 1.4 mL of the supernatant were transferred into a new tube and centrifuged (16,100 ×*g*, 15 min, room temperature). Particle-free supernatants were transferred into fresh tubes, and solvent was evaporated to dryness under a constant nitrogen flow. Dried samples were stored at −20 °C.

The filtered spent medium was extracted by solid phase extraction using 60 mg Oasis HLB cartridges (Waters, Milford, MA, USA). Cartridges were stepwise treated with 2 mL ethyl acetate, 2 mL methanol, and 2 mL purified water. Filtered spent medium was loaded on the cartridges under application of vacuum, and 10 mL purified water were added for desalting. Elution was performed with 2 mL ethyl acetate. Extracts were dried under a constant nitrogen flow and samples were stored at −20 °C.

To determine the intracellular concentrations of TBA in *N.* cf. *pellucida*, an average volume of an individual cell was calculated according to Hillebrand *et al*. 1999.[2] It was assumed that the shape of a cell resembles a prism on an elliptic base.

$$cell\ volume = \frac{\pi}{4} \times length \times width \times depth\ \mu m^3$$

Cell dimensions were measured with a Leica DM IL inverted microscope (Table S3).

GC/HRMS method

Gas chromatography coupled to high-resolution mass spectrometry was performed on a GC TRACE™ 1310 coupled to a QExactive GC Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) operated in electron ionization (EI) mode. Samples (1 µL) were injected *via* a TriPlus™ RSH autosampler (Thermo Fisher Scientific, Bremen, Germany) into the S/SL injector, operated at 310 °C, split 10, and a He 5.0 carrier gas flow rate of 1 mL/min. Chromatography was performed on a ZB-SemiVolatiles column (30 m length, 10 m pre-column, 0.25 mm inner diameter, 0.25 µm film thickness, Phenomenex Aschaffenburg, Germany). Initial oven temperature was set to 60 °C that was held for 2 min, then increased to 120 °C at a rate of 20 °C min^{-1} and held for 1 min. The temperature was then further increased to 250 °C at a rate of 5 °C min^{-1}, and finally increased to a temperature of 320 °C at a rate of 10 °C min^{-1} which was held for 2 min. Mass spectrometric measurement started at 5.5 min probe run time in full scan mode using a scan range of *m*/*z* 50-600 at a resolution of 120,000 in positive EI mode (70 eV). Transfer line temperatures were set to 280 °C and ion source was operated at 300 °C. Database search was performed with the NIST library browser (Version 2.2), using the NIST14 library.

Monitoring the abundance of halogenated anilines in cultures

Cell cultures (80 mL) were prepared in five biological replicates, plus one blank for each time point, respectively. Time points were 8, 15, 22, and 29 days after inoculation of fresh medium. At each time point, cultures were extracted as described above. For absolute and relative quantification, tribromoaniline-3,5-d_2 was used as the internal standard. Intracellular extraction was performed with 1.8 mL ethyl acetate that contained 0.019 µg mL^{-1} tribromoaniline-3,5-d_2 (56.49 nmol L^{-1}, 0.1 nmol total amount per culture). The collected spent medium flow through was collected and 50 µL ethyl acetate containing 0.743 µg/mL tribromoaniline-3,5-d_2 (2.23 µmol mL^{-1}, 0.1 nmol total amount per culture) were added before solid phase extraction. Mass-to-charge ratios and retention times used for integration of peak areas in Thermo Xcalibur Quan Browser (version 3.0.63) can be found in Table S2. Integration of peak areas was performed, using a mass deviation of 3 ppm. All mass-to-charge-ratios used were calculated with the Xcalibur Qual Browser software (version 3.0.63). Simulation of peaks was also performed in Xcalibur Qual Browser. Since both dibromochloroanilines and both bromodichloroanilines were not unambiguously assigned, their peak areas were combined, respectively.

In vitro bromination of aniline by a cell free algal extract

A phosphate buffer was prepared with K_2HPO_4 (27.8 mM) and KH_2PO_4 (72.2 mM) and purified water. The pH was adjusted to 6.4 with NaOH (1 M) and the buffer was subsequently sterilized in an autoclave. Cells from of a 2 month old culture in late stationary phase (60 mL) were collected on a 25 mm GF/C glass microfiber filter (Whatman, Maidstone, United Kingdom) using a vacuum-coupled filter unit. The cells were washed off the filter into 20 mL phosphate buffer and the cell suspension was subsequently sonicated for 5 min in an ultrasonic bath (VWR ultrasonic cleaner, Darmstadt, Germany). The cell lysate was sterile filtered through a Filtropur S 0.2 syringe filter (Sarstedt, Nümbrecht, Germany). The haloperoxidase activity of the resulting cell-free enzyme extract (EE) was initially confirmed by conversion of phenol red (phenolsulfonphthalein, λ_{max} 433 nm) to bromophenol blue (3′,3″,5′,5″-tetrabromophenolsulfonphthalein, λ_{max} 592 nm), modified after Hill and Manley (2009).[3] The conversion of phenol red was monitored with a Thermo Scientific™ GENESYS™ 10S UV/VIS spectrophotometer. The reaction conditions for the phenol red control are listed below (Table S1). The enzyme assays were performed with cell free extracts prepared as described above by addition of anilin instead of phenol red (Table S1). All reaction mixtures were incubated at room temperature for 15 min. Products from the enzyme assays with aniline were concentrated by solid phase extraction, using 30 mg Oasis HLB cartridges (Waters, Milford, MA, USA). Before extraction, the cartridges were rinsed with 2 mL ethyl acetate, 2 mL MeOH, and 2 mL purified water. Samples (1 mL) were loaded onto the cartridges which were then desalted with 2 mL purified water. For elution 2 mL ethyl acetate were used. The samples were dried under a gentle stream of N_2. The dried samples were dissolved in 250 µL pyridine and aliquots of 20 µL were combined with 20 µL *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) to react for 60 min at 60 °C. Samples were then submitted to GC-MS analysis. The cell-free enzyme extract was stored at −20 °C.

Table S1 Composition of enzyme assay and controls.

	Phenol red	Control Phenol red	Aniline KBr	Aniline KBr KCl	Control Aniline no H_2O_2	Control Aniline no H_2O_2 no KBr	Control Aniline no EE
Phenol red (5 mM in buffer) [µL]	10	10	-	-	-	-	-
Aniline (5 mM in DMSO) [µL]	-	-	10	10	10	10	10
KBr (1 M in buffer) [µL]	100	100	100	50	100	-	100
KCl (1 M in buffer) [µL]	-	-	-	50	-	-	-
H_2O_2 (0.116 M in buffer) [µL]	17	17	17	17	-	-	17
Enzyme extract (EE) [µL]	873	-	873	873	873	873	-
Buffer (0.1 M) [µL]	-	873	-		17	117	873

GC/MS method (for enzyme assay)

Gas chromatography coupled to mass spectrometry was performed on a GC TRACE™ 1300 coupled to a TSQ triple quadrupole mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) operated in electron ionization (EI, 70 eV) mode. Samples (1 µL) were injected *via* a TriPlus™ RSH autosampler (Thermo Fisher Scientific, Bremen, Germany) into the S/SL injector, operated at 300 °C, split 5, and He 5.0 carrier gas flow rate of 1 mL/min. Chromatography was performed on a ZB-SemiVolatiles column (30 m length, 10 m pre-column, 0.25 mm inner diameter, 0.25 µm film thickness, Phenomenex Aschaffenburg, Germany). Initial oven temperature was set to 80 °C that was held for 2 min, then increased to 120 °C at a rate of 20 °C min^{-1} and held for 1 min. The temperature was then further increased to 250 °C at a rate of 5 °C min^{-1}, and finally increased to a temperature of 320 °C at a rate of 10 °C min^{-1} which was held for 2 min. Mass spectrometric measurement started at 5.5 min probe run time in full scan mode using a scan range of *m*/*z* 50-600 in positive EI mode (70 eV). Transfer line temperatures were set to 280 °C and ion source was operated at 300 °C.

DeltaMS survey to detect polybrominated compounds

Putative identification of polybrominated compounds was performed with the DeltaMS package[4] (version 1.1.0, obtained from https://github.com/Pohnert-Lab/DeltaMS) in RStudio (version 1.1.463). The Thermo raw file of a

methanol:ethanol:chloroform (*v*:*v*:*v*, 2:6:2) cell extract of an unlabeled *N*. cf. *pellucida* culture was converted into the mzXML format, using MSConvert.exe.[5] The "Isotope signature" workflow was selected, searching for single positively-charged isotopologues containing $^{79/81}$Br with its natural occurring isotope abundance. An intensity deviation of 10% was allowed. Further parameters of the performed analysis in DeltaMS are listed below:

Peakdetection

centWave options

Parameter	Value	Parameter	Value
ppm	2.5	peakwidth (min / max)	5 / 20
prefilter (peaks / intensity)	3 / 5000	mzdiff	-0.001
snthresh	6	noise	0
polarity	positive	mzCenterFun	wMean
integrate	Use filtered data		

DeltaMS Settings

DeltaMS options

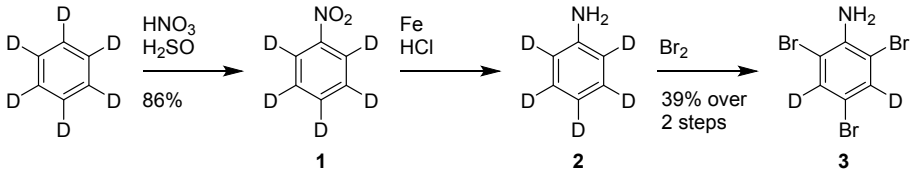
Parameter	Value	Parameter	Value
Rtwindow	10	ppmw	5
noiseCutoff	10	monoTol	FALSE
enriTol	0.1	intChoice	into
varEQ	FALSE	alpha	0.05
numAtom	9	dpeak	12
maxLT	4	compareOnlyDistros	FALSE

Determination of degree of labeling

To determine the degree of labeling (^{13}C and ^{15}N), it was assumed that the abundance of the measured isotopologues follows a Bernoulli distribution.[6] Peak areas of all $^{12}C_n{}^{13}C_m$ (n+m = 6) isotopologues of 2,4,6-tribromoaniline (TBA), dibromochloroaniline (DBCA), and putative tribromophenol, and peak areas of ^{14}N and ^{15}N isotopologues of TBA and DBCA were obtained (see Table S2). Measured areas were compared with simulated isotopologue distributions in an iterative manner in Microsoft Excel 2010. A minimized squared coefficient of variation between the measured and simulated distributions yielded the degree of ^{13}C labeling. The degree of ^{15}N labeling was determined by:

$$Degree\ of\ {}^{15}N\ labeling = \frac{area\ ({}^{15}N\ compound)}{(area\ ({}^{14}N\ compound) + \ area\ ({}^{15}N\ compound))}$$

Synthesis of the internal standard 2,4,6-tribromobenzen-3,5-*d2*-amine (TBA-d_2)



Scheme S1 Three step synthesis of 2,4,6-tribromobenzen-3,5-*d2*-amine (TBA-d_2).

1-Nitrobenzene-2,3,4,5,6-*d5* (1).[7]

Concentrated H_2SO_4 (14 mL) was added slowly to 13 mL of concentrated HNO_3 at 0 °C. After complete addition, this solution was added dropwise to 8.6 g of ice-cooled benzene-*d*6 (0.10 mol, 1 eq) at such a rate that the reaction temperature stayed below 10 °C. Stirring was continued for 3 h at 25 °C and the solution was poured onto 300 g of ice and stirred for 15 min. The organic phase was separated, the aqueous phase extracted with petrol ether (100 mL) and the combined organic phases were washed with 50 mL of saturated $NaHCO_3$ solution (50 mL) until neutral. After drying with $CaCl_2$ the product was distilled at ambient pressure to yield 11.2 g (0.087 mol, 86%) of nitrobenzene-*d*5 as an orange liquid. **^{13}C NMR** (75 MHz; $CDCl_3$): δ = 148.0 (C1), 134.0 (t, *J* = 25.0 Hz, C4), 128.7 (t, *J* = 25.1 Hz, C3),123.0 (t, *J* = 25.7 Hz, C2) ppm. **IR**: $\tilde{\nu}$ = 2851, 2303, 1512, 1358, 1339, 844 cm^{-1}. **GC**: 5.79 min. **MS** (EI): *m*/*z* = 128.06 $[M]^{\bullet+}$ (calc. for $C_6D_5NO_2$: 128.06), 112.06 $[M - O]^+$, 98.08 $[M - NO]^+$, 82.07 $[M - NO_2]^+$, 70.07 $[C_5D_5]^+$, 54.04 $[C_4D_3]^+$.

Benzen-*d*5-amine (2). Modified after Ueberschaar *et al*. 2013.[8]

To a solution of **1** (5.55 g, 43.5 mmol, 1 eq) and 14.6 g of Fe (261 mmol, 6 eq) in 250 ml MeOH under Ar at 70 °C was added dropwise 6 N hydrochloric acid (178 mL). After complete addition, the solution was stirred for 45 min at that temperature. After cooling to room temperature, the solution was diluted with 950 mL of H_2O and carefully neutralized with $NaHCO_3$. To the resulting green-brown solution EDTA was added, then it was extracted four times with $CHCl_3$ (300 mL). After solvent removal under reduced pressure, the resulting pale yellow liquid was used without further purification. **^{1}H NMR** (250 MHz; $CDCl_3$): δ = 3.63 (2H, br s, $–NH_2$) ppm. **^{13}C NMR** (63 MHz; $CDCl_3$): δ = 146.2 (C1), 128.7 (t, *J* = 24.3 Hz, C3), 118.0 (m, C4), 114.7 (t, *J* = 24.1 Hz, C2) ppm. **IR**: $\tilde{\nu}$ = 3428, 3352, 3219, 2274, 1616, 1568, 1398, 1302, 1198, 627 cm^{-1}. **GC**: 4.32 min. **MS** (EI): *m*/*z* = 98.09 $[M]^{\bullet+}$ (calc. for $C_6D_5NH_2$: 98.09), 97.08, 71.08.

2,4,6-Tribromobenzen-3,5-*d*2-amine (3). Modified after Coleman *et al*. 1933.[9]

The crude product (**2**, 5.70 g) was dissolved in H_2O (50 mL) and concentrated hydrochloric acid (10 mL) and then diluted with H_2O (235 mL). Br_2 (9.0 mL, 174 mmol, 3 eq) was slowly passed through the refluxing solution in 2 h using a gentle stream of N_2. After complete addition the solution was cooled to 0 °C and stirred for 1.5 h. The slurry was filtered and the solid washed three times with H_2O (50 mL). Drying *in vacuo* yielded 5.61 g (16.9 mmol, 39% over 2 steps) of 2,4,6-tribromobenzen-3,5-*d*2-amine as a pale brown solid. **^{1}H NMR** (300 MHz; $CDCl_3$): δ = 4.24 (2H, br s, $–NH_2$) ppm. **^{13}C NMR** (75 MHz; $CDCl_3$): δ = 141.3 (C1), 133.5 (t, *J* = 26.0 Hz, C3), 108.7 (C2), 108.6 (C4) ppm. **IR**: $\tilde{\nu}$ = 3414, 3298, 1609, 1412, 1344, 1088, 673 cm^{-1}. **GC**:

11.58 min. **MS** (EI): *m*/*z* = 328.80 $[M]^{\bullet+}$ (calc. for $C_6D_2Br_3NH_2$: 328.80), 249.89 $[M - Br]^+$, 169.96 $[M - 2Br]^+$, 142.95, 92.05, 64.03. **Isotopic purity** 92.9 atom % ^2H (refer to Table S4).

Table S2 Mass-to-charge-ratios of selected for integration of peak areas (3 ppm window)

Analyte	Sum formula	*m*/*z* for integration of peak areas (3 ppm window)
2,4,6-Tribromoaniline (TBA)	$C_6H_4NBr_3^{\bullet+}$	326.7888, 328.7868, 330.7847, 332.7827
	$^{13}C^{12}C_5H_4NBr_3^{\bullet+}$	327.7922, 329.7901, 331.7881, 333.7861
	$^{13}C_2{}^{12}C_4H_4NBr_3^{\bullet+}$	328.7955, 330.7935, 332.7915, 334.7894
	$^{13}C_3{}^{12}C_3H_4NBr_3^{\bullet+}$	329.7989, 331.7969, 333.7948, 335.7928
	$^{13}C_4{}^{12}C_2H_4NBr_3^{\bullet+}$	330.8023, 332.8002, 334.7982, 336.7961
	$^{13}C_5{}^{12}CH_4NBr_3^{\bullet+}$	331.8056, 333.8036, 335.8015, 337.7995
	$^{13}C_6H_4NBr_3^{\bullet+}$	332.8090, 334.8069, 336.8049, 338.8028
2,6-Dibromo-4-chloroaniline 2,4-Dibromo-6-chloroaniline (DBCA)	$C_6H_4NBr_2Cl^{\bullet+}$	282.8394, 284.8373, 286.8353, 288.8323
	$^{13}C^{12}C_5H_4NBr_2Cl^{\bullet+}$	283.8427, 285.8407, 287.8386, 289.8357
	$^{13}C_2{}^{12}C_4H_4NBr_2Cl^{\bullet+}$	284.8461, 286.8440, 288.8420, 290.8390
	$^{13}C_3{}^{12}C_3H_4NBr_2Cl^{\bullet+}$	285.8494, 287.8474, 289.8453, 291.8424
	$^{13}C_4{}^{12}C_2H_4NBr_2Cl^{\bullet+}$	286.8528, 288.8507, 290.8487, 292.8457
	$^{13}C_5{}^{12}CH_4NBr_2Cl^{\bullet+}$	287.8561, 289.8541, 291.8520, 293.8491
	$^{13}C_6H_4NBr_2Cl^{\bullet+}$	288.8595, 290.8574, 292.8554, 294.8524
4-Bromo-2,6-dichloroaniline 2-Bromo-4,6-	$C_6H_4NBrCl_2^{\bullet+}$	238.8899, 240.8878, 242.8849, 244.8819

dichloroaniline (BDCA)		
2,4,6-Trichloroaniline (TCA)	$C_6H_4NCl_3^{\bullet+}$	194.9404, 196.9374, 198.9345, 200.9315
Putative tribromophenol	$C_6H_3OBr_3^{\bullet+}$	327.7729, 329.7708, 331.7688, 333.7667
	$^{13}C^{12}C_5H_3OBr_3^{\bullet+}$	328.7762, 330.7742, 332.7721, 334.7701
	$^{13}C_2{}^{12}C_4H_3OBr_3^{\bullet+}$	329.7796, 331.7775, 333.7755, 335.7734
	$^{13}C_3{}^{12}C_3H_3OBr_3^{\bullet+}$	330.7829, 332.7809, 334.7788, 336.7768
	$^{13}C_4{}^{12}C_2H_3OBr_3^{\bullet+}$	331.7863, 333.7842, 335.7822, 337.7801
	$^{13}C_5{}^{12}CH_3OBr_3^{\bullet+}$	332.7896, 334.7876, 336.7855, 338.7835
	$^{13}C_6H_3OBr_3^{\bullet+}$	333.7930, 335.7909, 337.7889, 339.7868

Table S3 Cell dimensions of *N.* cf. *pellucida* measured with the inverted light microscope.

	Length [µm]	**Width [µm]**	**Depth [µm]**	**Volume [µm^3]**
Mean	16.36	7.35	7.60	717.91
Standard deviation	0.93	1.03	0.65	

Table S4 Isotopologue composition of 2,4,6-tribromobenzen-3,5-*d2*-amine standard (7 µg mL^{-1}).

Isotopologue	**Sum formula**	***m*/*z* for integration of peak areas (3 ppm window)**	**Area [a. u.]**	**Percentage [%]**
2,4,6-tribromobenzen-3,5-*d2*-amine	$C_6H_2D_2NBr_3^{\bullet+}$	328.8014, 330.7993, 332.7973, 334.7953	4.06×10^8	92.93
2,4,6-tribromobenzen-3-*d1*-amine	$C_6H_3DNBr_3^{\bullet+}$	327.7951, 329.7931, 331.7910, 333.7890	3.05×10^7	6.98
2,4,6-tribromoaniline	$C_6H_4NBr_3^{\bullet+}$	326.7888, 328.7868, 330.7847, 332.7827	4.08×10^5	0.09

Table S5 Mass-to-charge ratios found in the spectrum of 2,4,6-tribromoaniline (retention time 17.8 min) and calculated mass-to-charge ratios and mass deviations.

Sum formula	***m*/*z* found**	***m*/*z* calculated**	**Mass deviation [ppm]**
$C_6H_4NBr_3^{\bullet+}$	326.7888	326.7887	−0.33
$C_5H_2Br_3^+$	298.7700	298.7701	−0.42
$C_6H_4NBr_2^+$	247.8705	247.8705	−0.21
$C_6H_3NBr^+$	167.9442	167.9443	−0.59

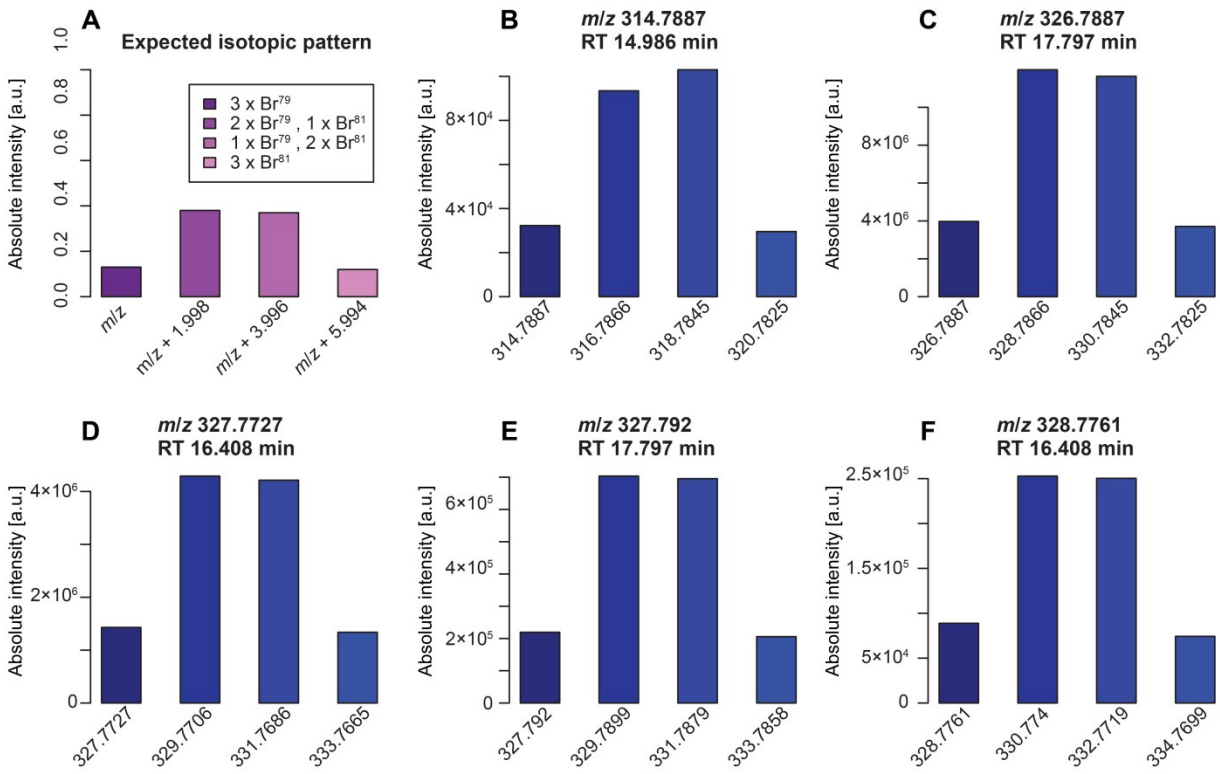


Figure S1 Absolute intensity plots obtained from the DeltaMS analysis of the methanol:ethanol:chloroform (*v*:*v*:*v*, 2:6:2) cell extract of an unlabeled *N.* cf. *pellucida* culture. Only plots that show potential candidate ions with three incorporated bromine are shown. **A** Expected isotope pattern of a candidate ion that contains three bromine. **B** Unknown compound, potential sum formula $C_5H_4NBr_3^{\bullet+}$ (calc. 314.7888, found 314.7887, −0.4 ppm). **C** and **E** belong to 2,4,6-tribromoaniline (TBA), whereby **C** shows ions of $^{12}C_6$-TBA and **E** shows ions of $^{13}C^{12}C_5$-TBA. **D** and **F** show ions of putatively identified tribromophenol ($C_6H_3OBr_3$), whereby **D** shows ions of putative $^{12}C_6$-tribromophenol and **F** shows ions of putative $^{13}C^{12}C_5$- tribromophenol.

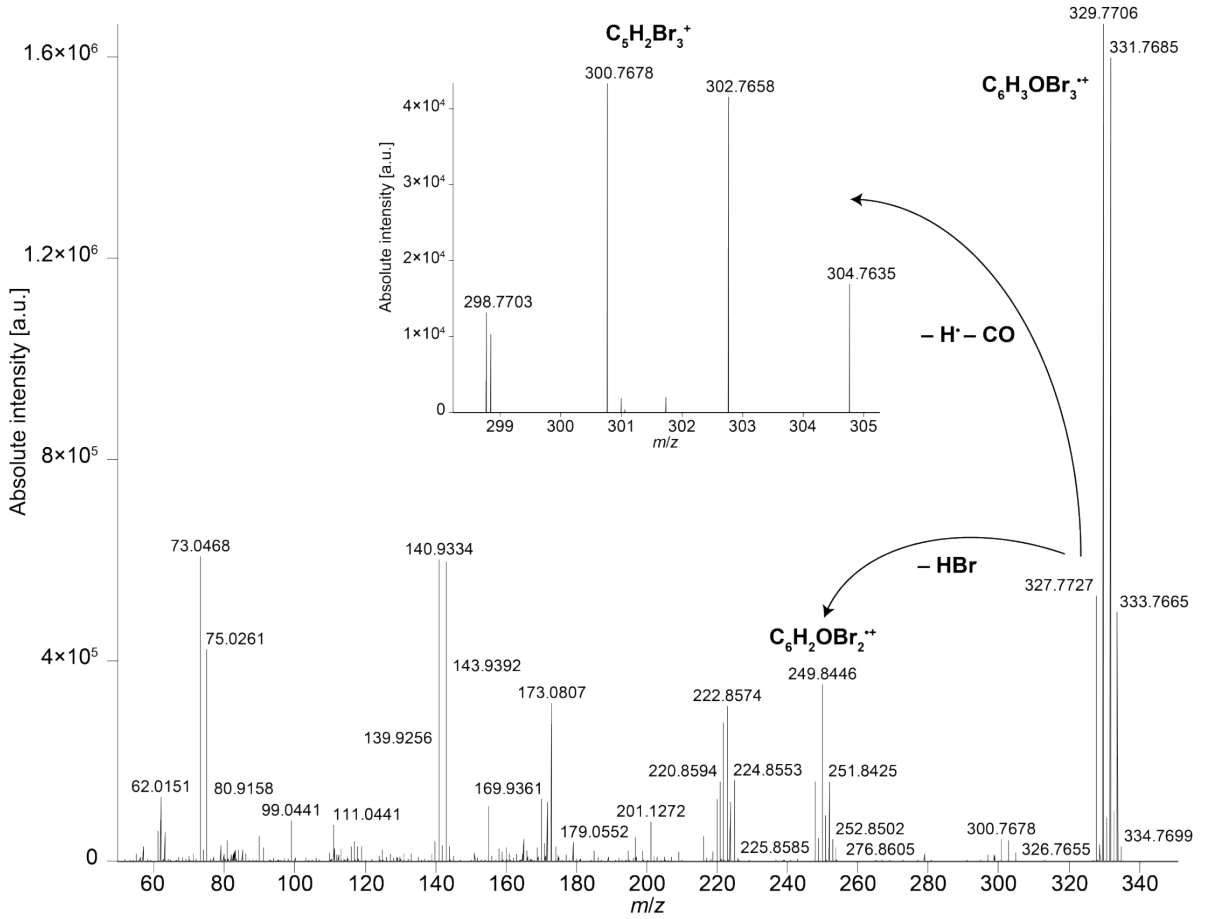


Figure S2 Spectrum of putatively identified tribromophenol (retention time 16.4 min, $C_6H_3OBr_3^{\bullet+}$, calc. 327.7729, found 327.7727, −0.4 ppm). The characteristic net loss of a formyl radical (M−H•−CO) of the phenol is highlighted ($C_5H_2Br_3^+$, calc. 298.7701, found 298.7703, 0.5 ppm). In addition, one HBr loss is shown ($C_6H_3OBr_2^+$, calc. 247.8467, found 247.8466, −0.2 ppm).

Table S6 Top NIST14 library search results for spectra shown in Fig.1A and Fig. S2.

Spectrum	SI	RSI	Compound
Fig S2	830	859	2,4,6-tribromophenol
Fig 1	812	853	2,4,6-tribromoaniline

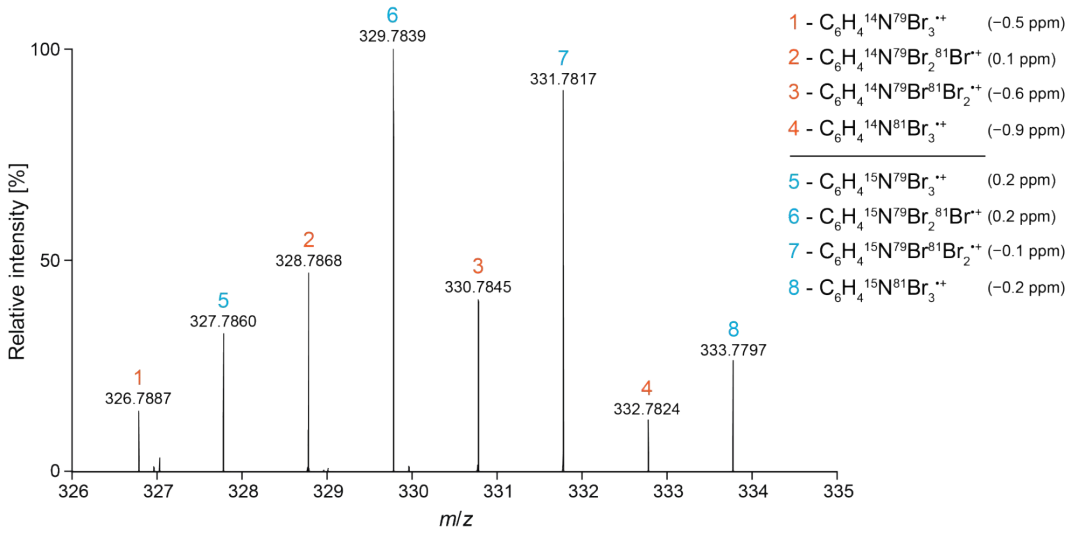


Figure S3 Mass spectrum of ^{15}N-labeled 2,4,6-tribromoaniline. Mass deviations to calculated *m*/*z* values are shown in brackets.

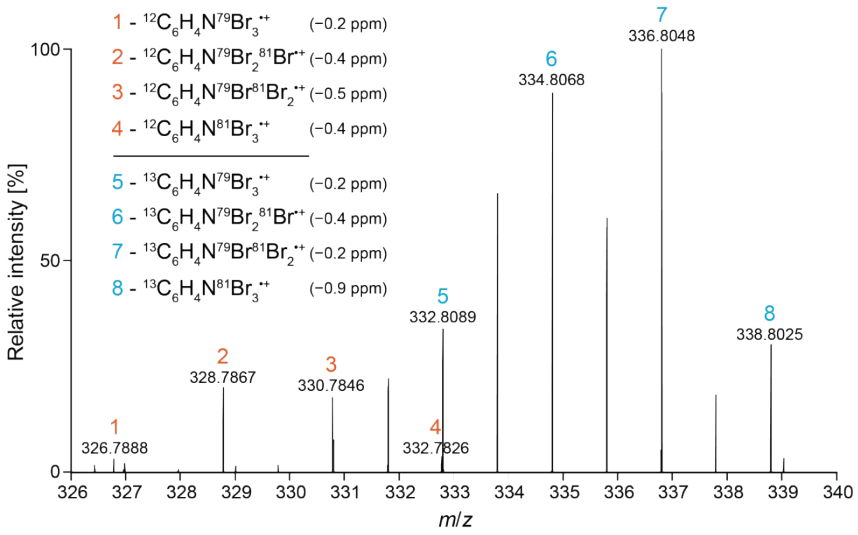


Figure S4 Mass spectrum of $^{13}C_6$-labeled 2,4,6-tribromoaniline. Mass deviations to calculated *m*/*z* values are shown in brackets.

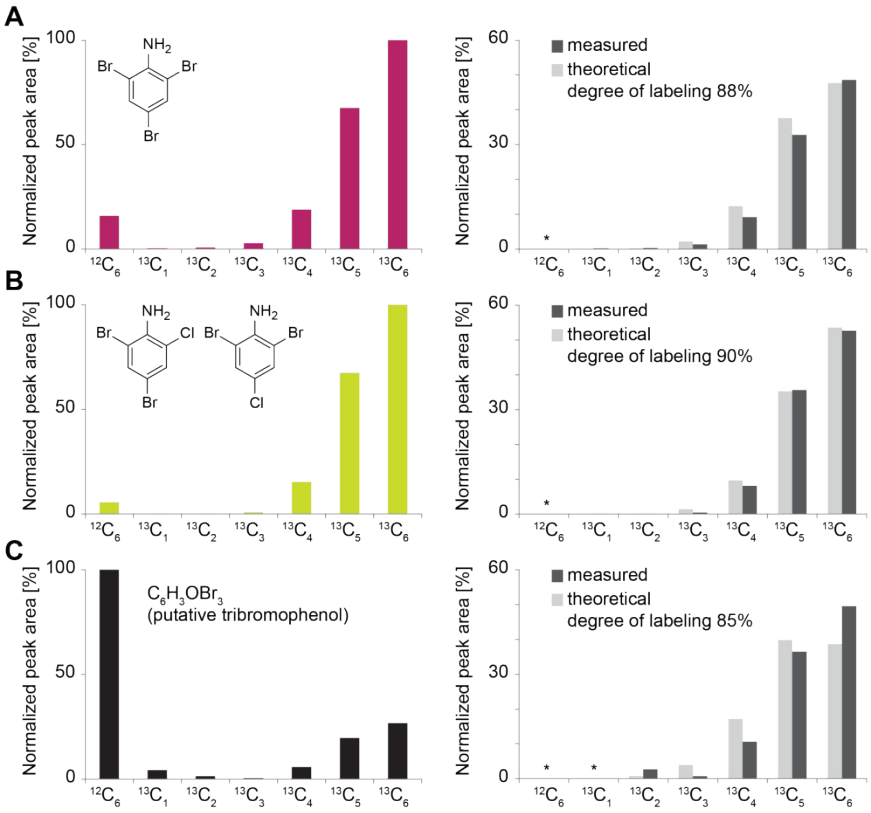


Figure S5 Determination of degree of ^{13}C labeling. Left bar plot shows measured normalized peak areas of ^{13}C-labeled compound, whereby areas were normalized to the isotopologue with the biggest area. Right bar plot shows sum normalized peak areas of a measured (dark grey) compound and theoretical C_6-compound (light grey), whereby the abundance of the theoretical isotopologues follow a Bernoulli distribution.[5] Leftover compounds from unlabeled culture conditions, indicated by a strong intensity of $^{12}C_6$ and $^{13}C_1$, relative to the heavier isotopologues, aggravate a precise calculation of the degree of labeling. Therefore, the $^{12}C_6$ and $^{13}C_1$ were removed if necessary. Asterisks indicate the removed isotopologues. **A** The degree of ^{13}C labeling for 2,4,6-tribromoaniline was determined to be around 88%. **B** Degree of ^{13}C labeling for the dibromochloroanilines was determined to be around 90%. **C** The degree of ^{13}C labeling for the putative tribromophenol ($C_6H_3OBr_3$) was determined to be around 85%.

Table S7 Degree of ^{15}N labeling of TBA and DBCA.

Isotopologue	**Area [a.u.]**	**Degree of ^{15}N labeling**
$C_6H_4NBr_3^{\bullet+}$	2.42×10^5	68%
$C_6H_4{}^{15}NBr_3^{\bullet+}$	5.09×10^5	
$C_6H_4NBr_2Cl^{\bullet+}$	4.24×10^4	77%
$C_6H_4{}^{15}NBr_2Cl^{\bullet+}$	1.41×10^5	

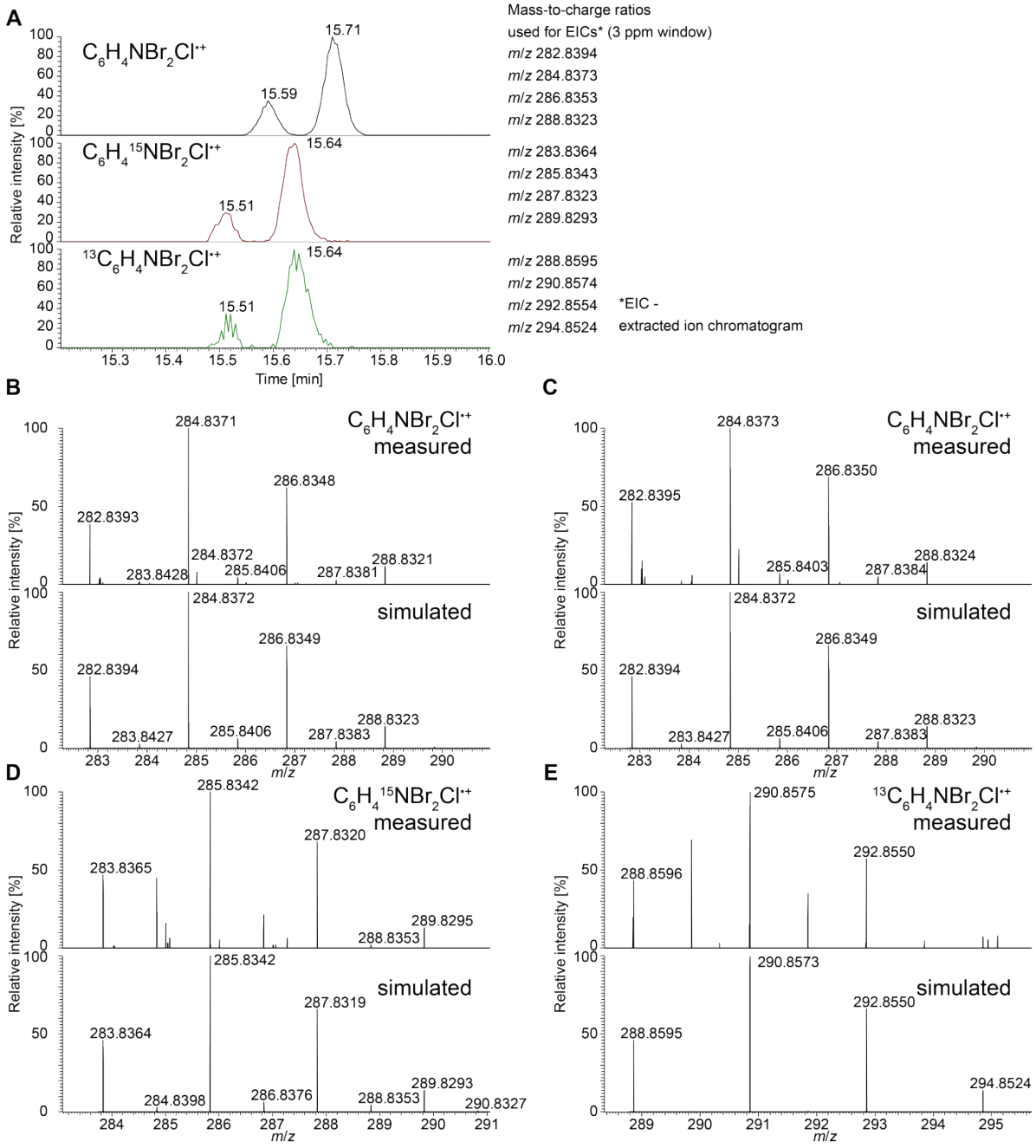


Figure S6 A Extracted ion chromatograms of unlabeled (top), ^{15}N-labeled (mid), and ^{13}C-labeled (lower) dibromochloroaniline ($C_6H_4NBr_2Cl$). Retention time drift is due to shortening of the GC column after measurements of unlabeled samples. Measured high-resolution mass spectra (top) and simulated mass spectra (lower, FWHM 120,000) of dibromochloroaniline: **B** Retention time 15.59 min (left peak). **C** Retention time 15.71 min (right peak). **D** Background subtracted spectrum of ^{15}N-labeled dibromochloroaniline at retention time 15.64 min. **E** ^{13}C-labeled dibromochloroaniline at retention time 15.64 min.

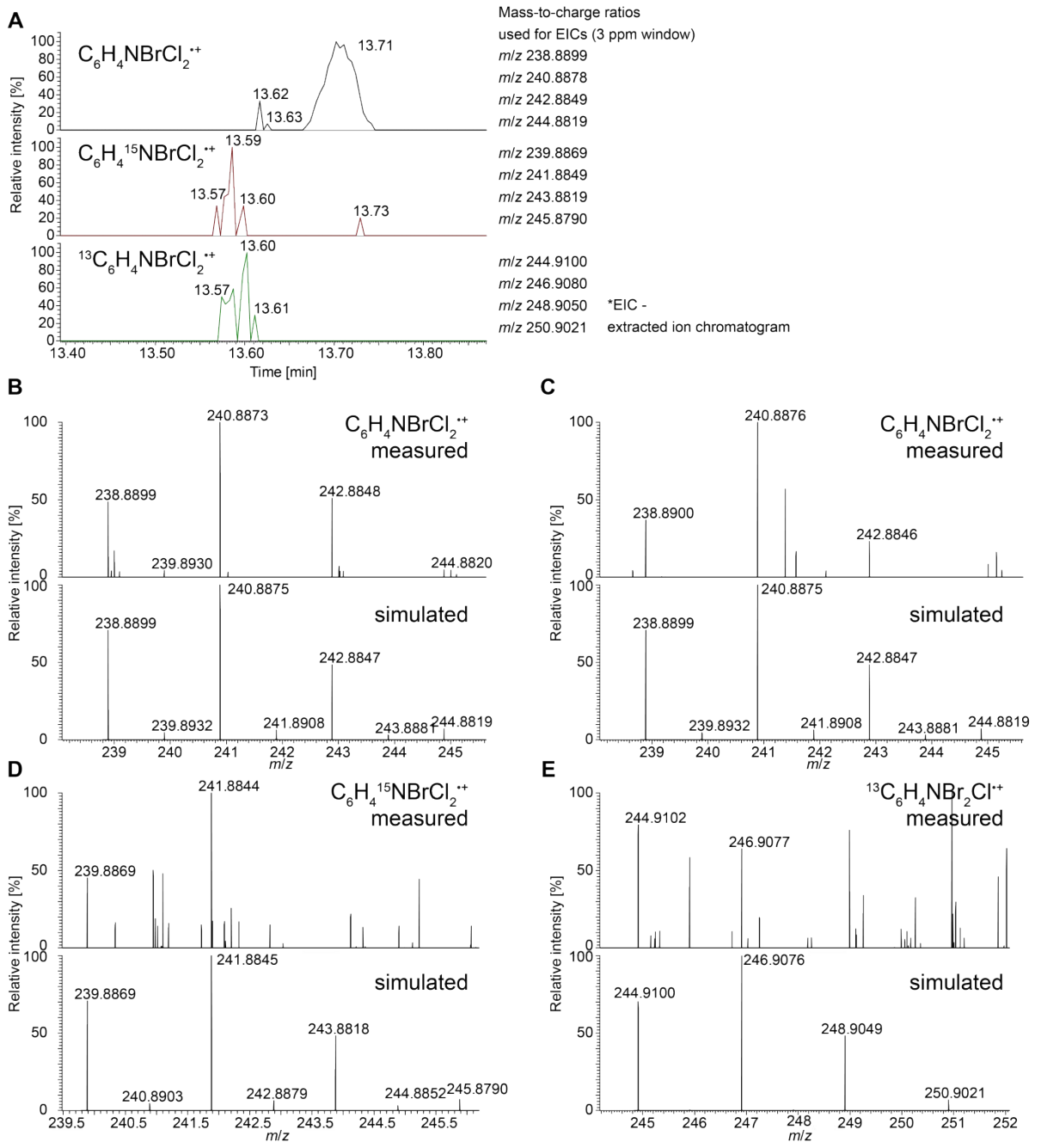


Figure S7 A Extracted ion chromatograms of unlabeled (top), ^{15}N-labeled (mid), and ^{13}C-labeled (lower) bromodichloroaniline ($C_6H_4NBrCl_2$). Retention time drift is due to shortening of the GC column after measurements of unlabeled samples. Measured high-resolution mass spectra (top) and simulated mass spectra (lower, FWHM 120,000) of bromodichloroaniline: **B** Background subtracted spectrum at retention time 13.71 min (right peak). **C** Background subtracted averaged spectrum (8 spectra) at retention time ~ 13.61 min (left peak). **D** Background subtracted averaged spectrum (14 spectra) of ^{15}N-labeled bromodichloroaniline at retention time ~ 13.59 min. **E** Background subtracted averaged spectrum (12 scans) of ^{13}C-labeled bromodichloroaniline at retention time ~ 13.59 min.

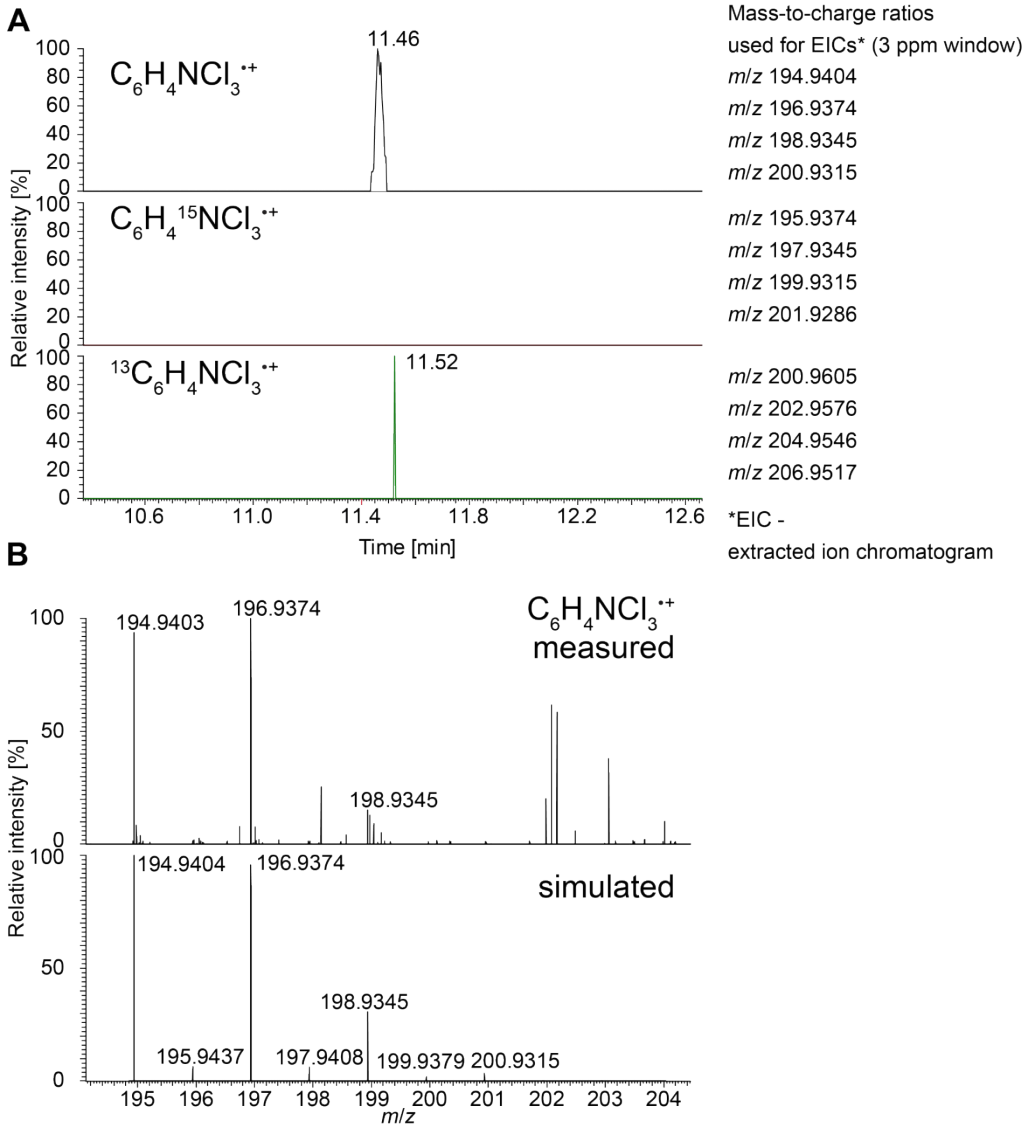


Figure S8 A Extracted ion chromatograms of unlabeled (top), ^{15}N-labeled (mid), and ^{13}C-labeled (lower) trichloroaniline ($C_6H_4NCl_3$). Measured high-resolution mass spectra (top) and simulated mass spectrum (lower, FWHM 120,000) of trichloroaniline: **B** Background subtracted averaged spectrum (15 scans) at retention time ~ 11.47 min.

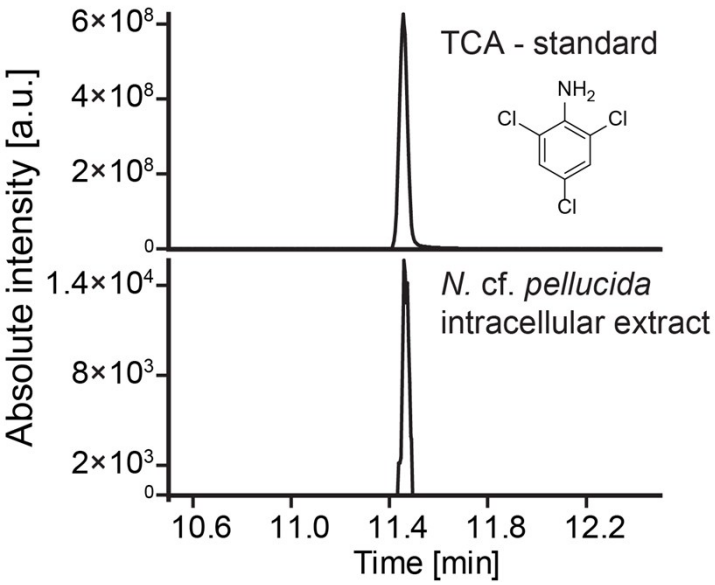


Figure S9 Comparison of retention times of a commercially available standard of 2,4,6-trichloroaniline and the ion trace of TCA ($C_6H_4NCl_3$). Masses used for production of the extracted ion chromatogram can be found in Figure S8.

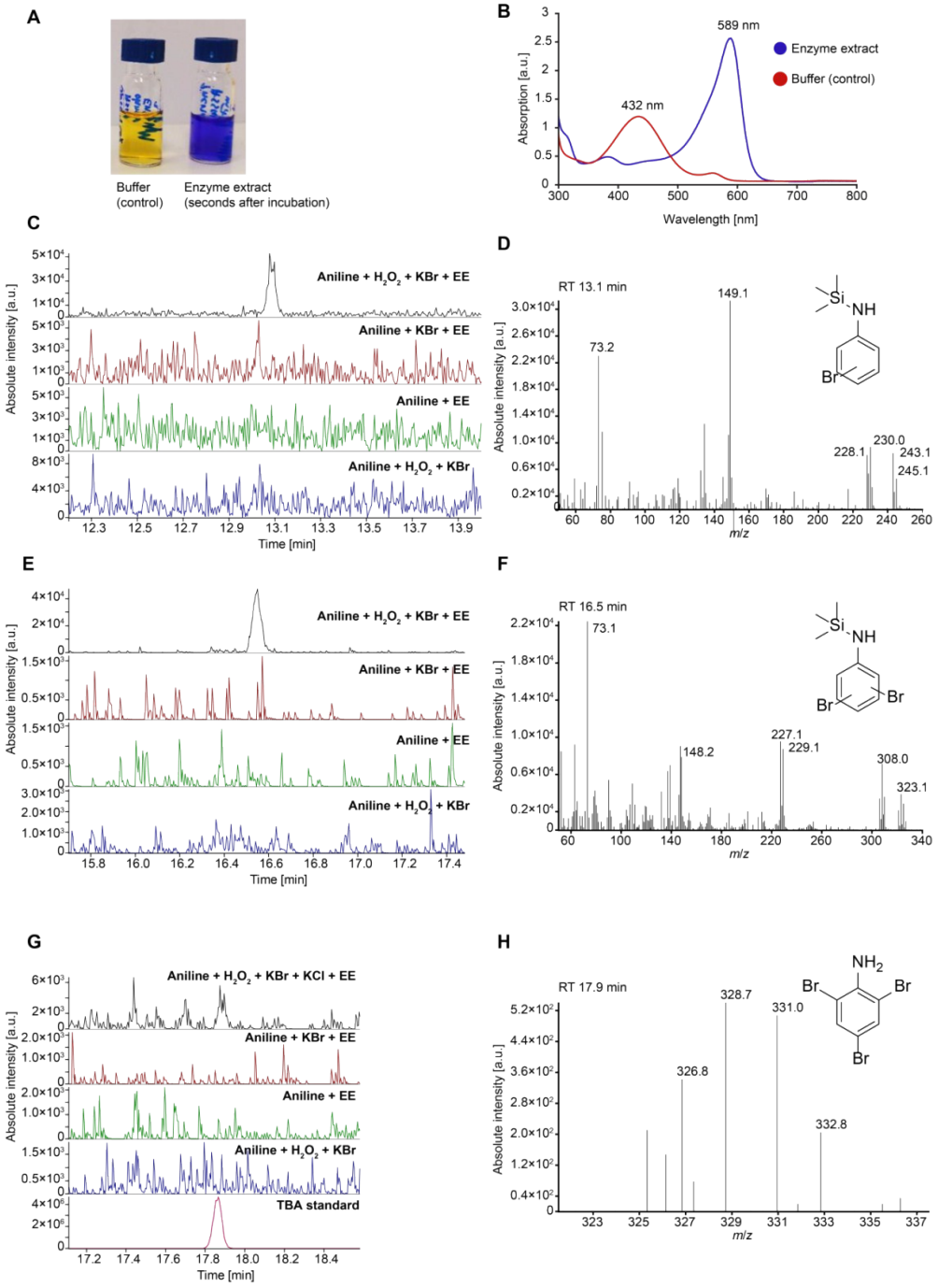
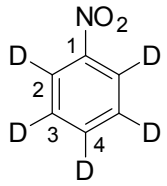


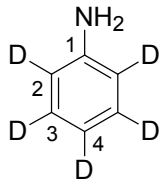
Figure S10 A Phenol red assay to confirm haloperoxidase activity of the cell-free enzyme extract (EE). Left vial shows control without EE. Right vial shows reaction mix with EE after few seconds at room temperature. **B** Absorption spectra of phenol red assay control (red line) and reaction mixture with EE (blue line). **C** Extracted ion chromatograms (EICs) of bromoaniline trimethylsilane (149 *m/z*, 243 *m/z*, 245 *m/z*). **D** Spectrum of bromoaniline trimethylsilane at RT 13.1 min. **E** Extracted ion chromatograms (EICs) of dibromoaniline trimethylsilane (227 *m/z*, 229 *m/z*, 321 *m/z*, 323 *m/z*, 325 *m/z*). **F** Spectrum of dibromoaniline trimethylsilane at RT 16.5 min. **G** Extracted ion chromatograms (EICs) of 2,4,6-tribromoaniline (248 *m/z*, 250 *m/z*, 252 *m/z*, 327 *m/z*, 329 *m/z*, 331 *m/z*, 333 *m/z*). TBA standard is shown (pink chromatogram). No derivatized TBA was found. **H** Spectrum of tribromoaniline at RT 17.9 min. Red, green and blue chromatograms show reaction controls, whereby the control in red lacks H_2O_2, green lacks H_2O_2 and KBr, and blue lacks EE.

^{13}C NMR (75 MHz, $CDCl_3$)



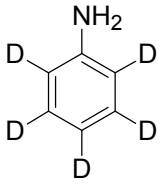
1

^{13}C NMR (63 MHz, $CDCl_3$)



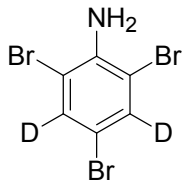
2

^{1H} NMR (250 MHz, $CDCl_3$)

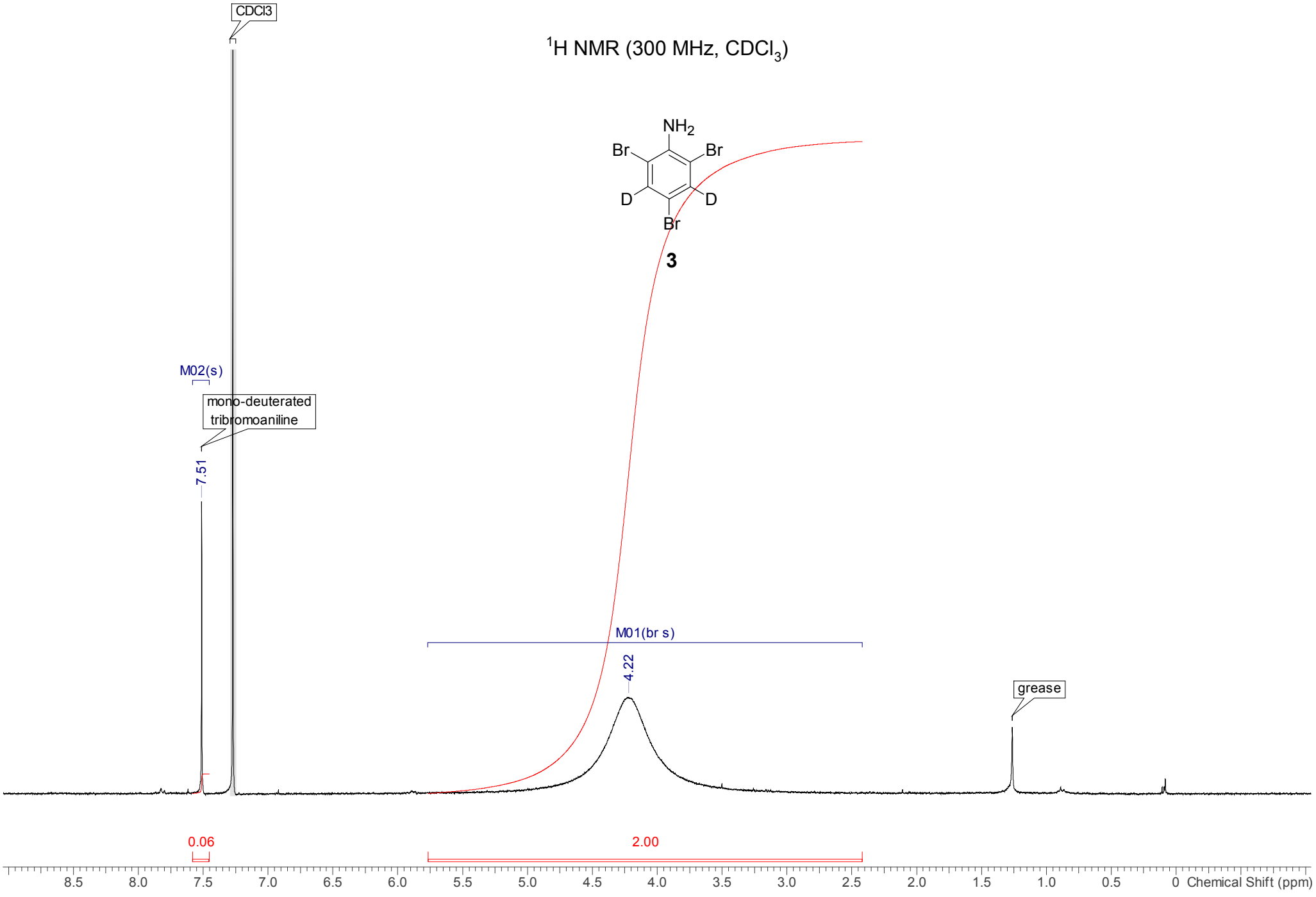


2

^{13}C NMR (75 MHz, $CDCl_3$)



3


^{1}H NMR (300 MHz, $CDCl_3$)
CDCl3
NH_2
Br
Br
D
D
Br
3
M02(s)
mono-deuterated
tribromoaniline
7.51
M01(br s)
4.22
grease
0.06
2.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

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