

Toward hypoxia-selective DNA-alkylating agents built by grafting nitrogen mustards onto the bioreductively-activated, hypoxia-selective DNA-oxidizing agent 3-amino-1,2,4-benzotriazine 1,4-dioxide (tirapazamine)

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Figure S1

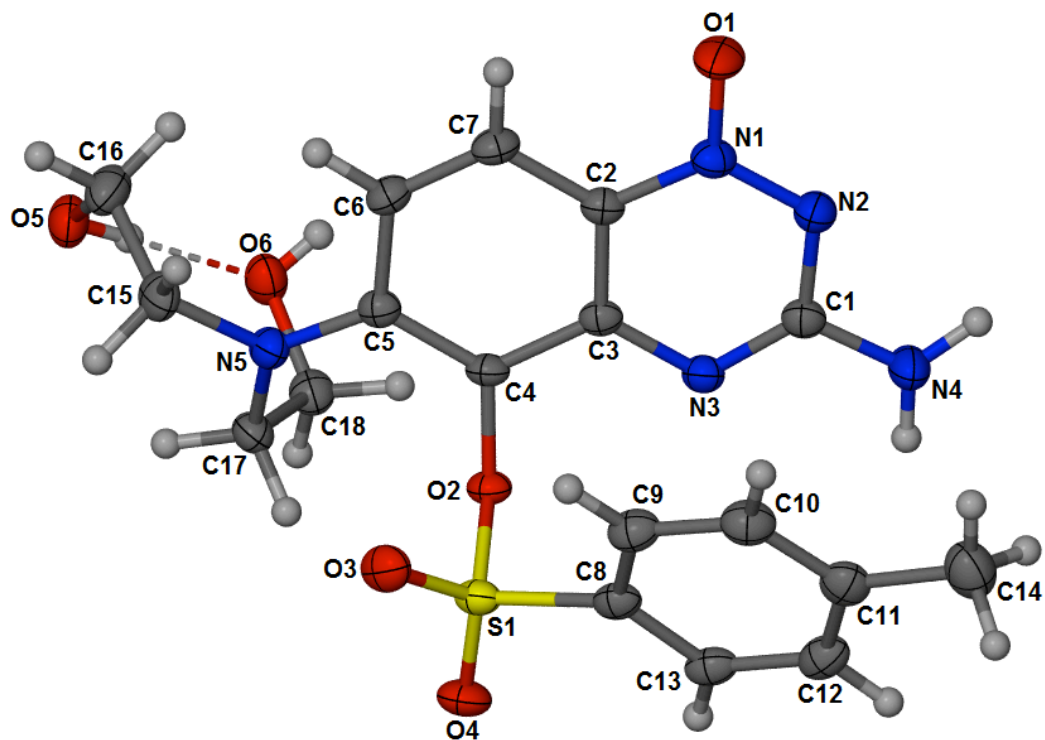


Figure S1. Crystal structure of **19** obtained from the reaction of tosyl chloride with **15**.

Table S1

| | |
|--|--------------------------------|
| | |
| Empirical formula | C18 H21 N5 O6 S |
| Formula weight | 435.46 |
| Temperature, (K) | 173(2) |
| W. length, (Å) | 0.71073 |
| Crystal system | Monoclinic |
| Space group | P 21/c |
| a, (Å) | 11.8137(17) |
| b, (Å) | 7.0470(10) |
| c, (Å) | 24.199(4) |
| α , (deg) | 90 |
| β , (deg) | 101.033 |
| γ , (deg) | 90 |
| Volume, (Å ³) | 1977.4(5) |
| Z/calculated density (Mg/m ³) | 4/1.463 |
| Absorption coefficient (mm ⁻¹) | 0.211 |
| Crystal size (mm) | 0.50 x 0.35 x 0.10 |
| Reflections collected/unique | 22323 / 4574 [R(int) = 0.0261] |
| Data/restraints/parameters | 4574 / 0 / 276 |
| GOF | 1.047 |
| R indices (all data) | R1 = 0.0413, wR2 = 0.0972 |

Table S1. Crystal and data collection parameters for the crystal structure of **19**.

Figure S2

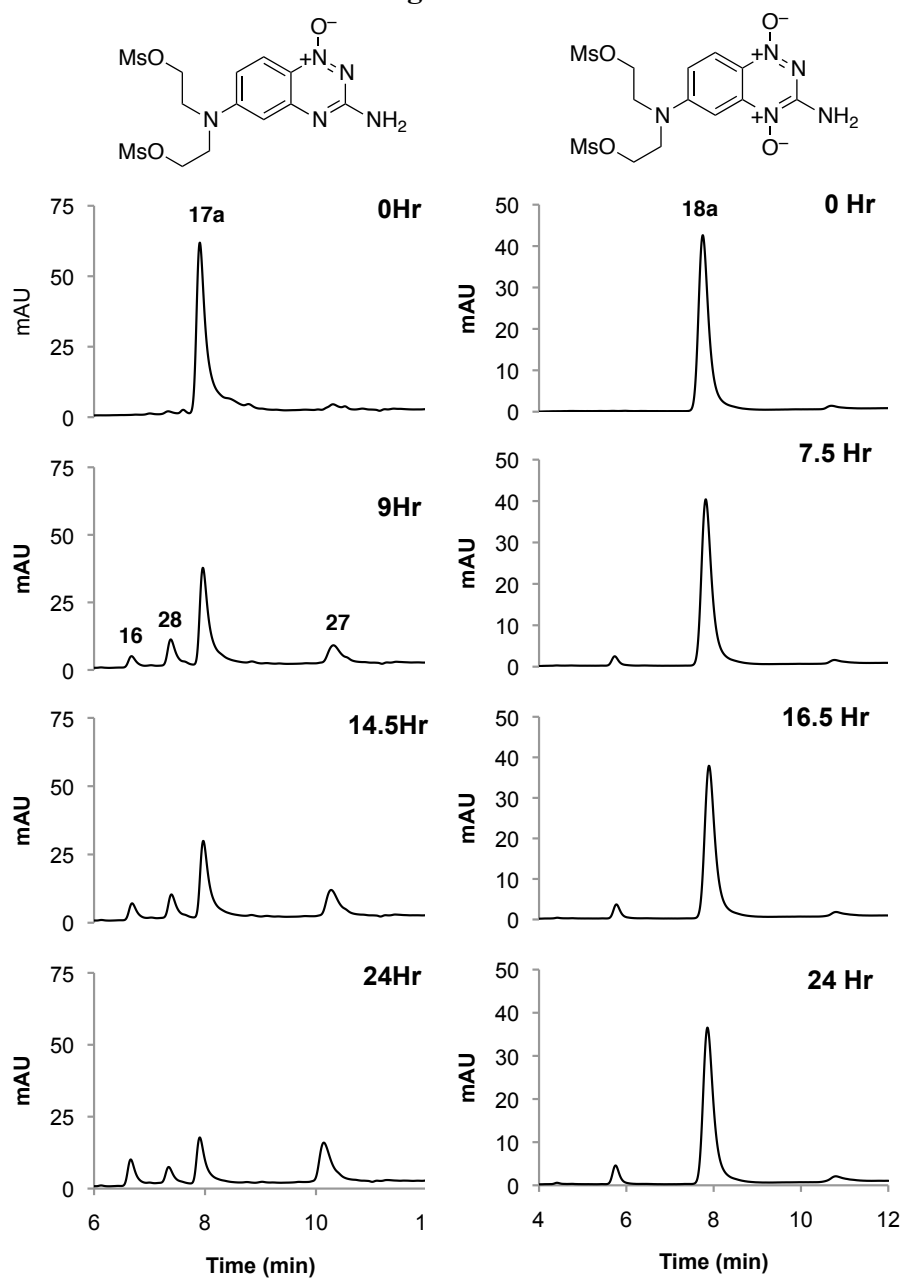


Figure S2. Representative HPLC chromatograms showing the hydrolysis of compounds **17a** (left) and **18a** (right) (250 μ M) in sodium phosphate buffer (25 mM, pH 7) containing DMF (2.5% v/v) at 50 $^{\circ}$ C. Compounds were detected by their absorbance at 280 nm. The disappearance of compound **17a** and appearance of hydrolysis products **16** and **27** over a 24 h time period is shown here (left). Detailed conditions are provided in the Experimental Section of the paper.

Figure S3

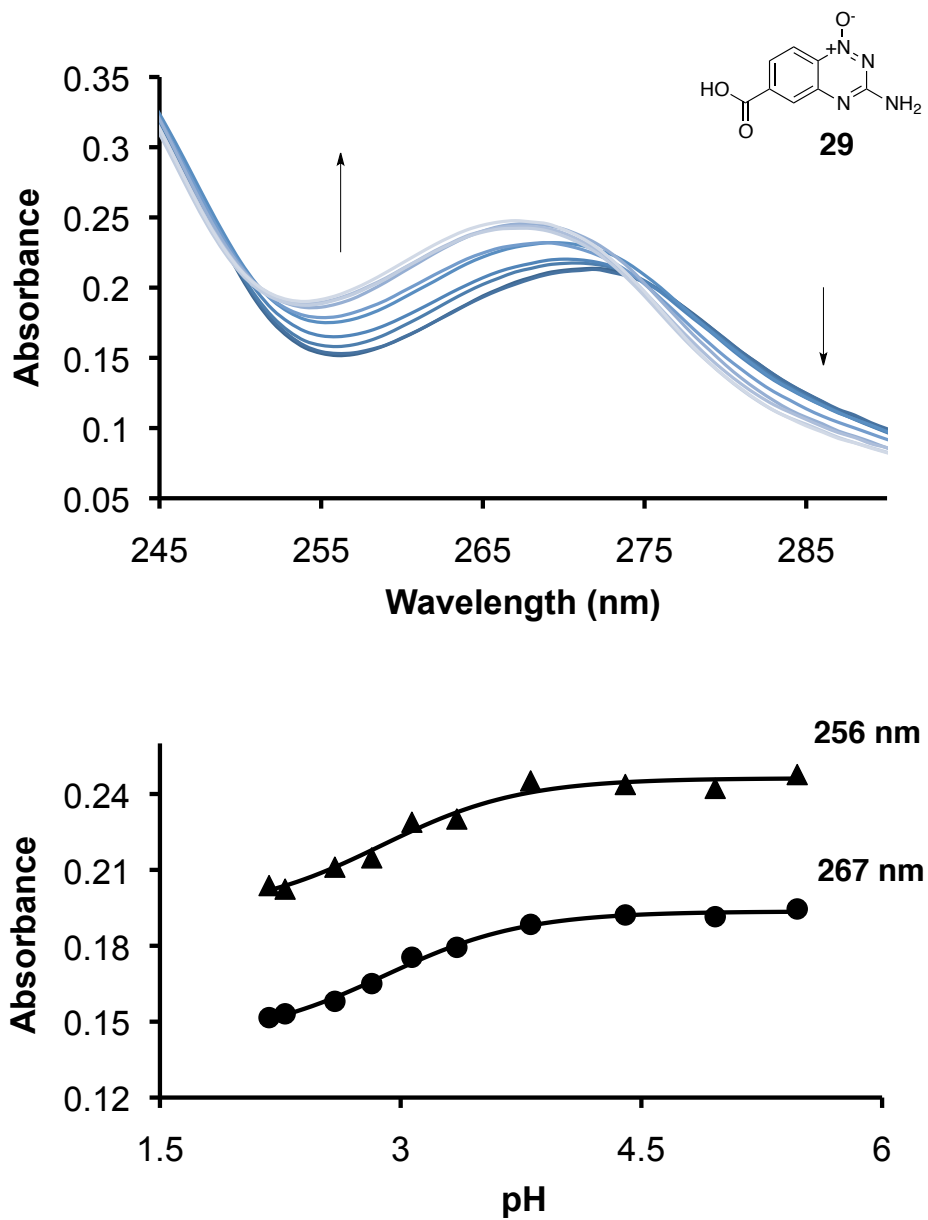


Figure S3. pK_a determination for **29**. Absorbance spectra (top) and titration curves (bottom) with least squares fitting for compound **29** from pH 2.18-5.47.

Figure S4

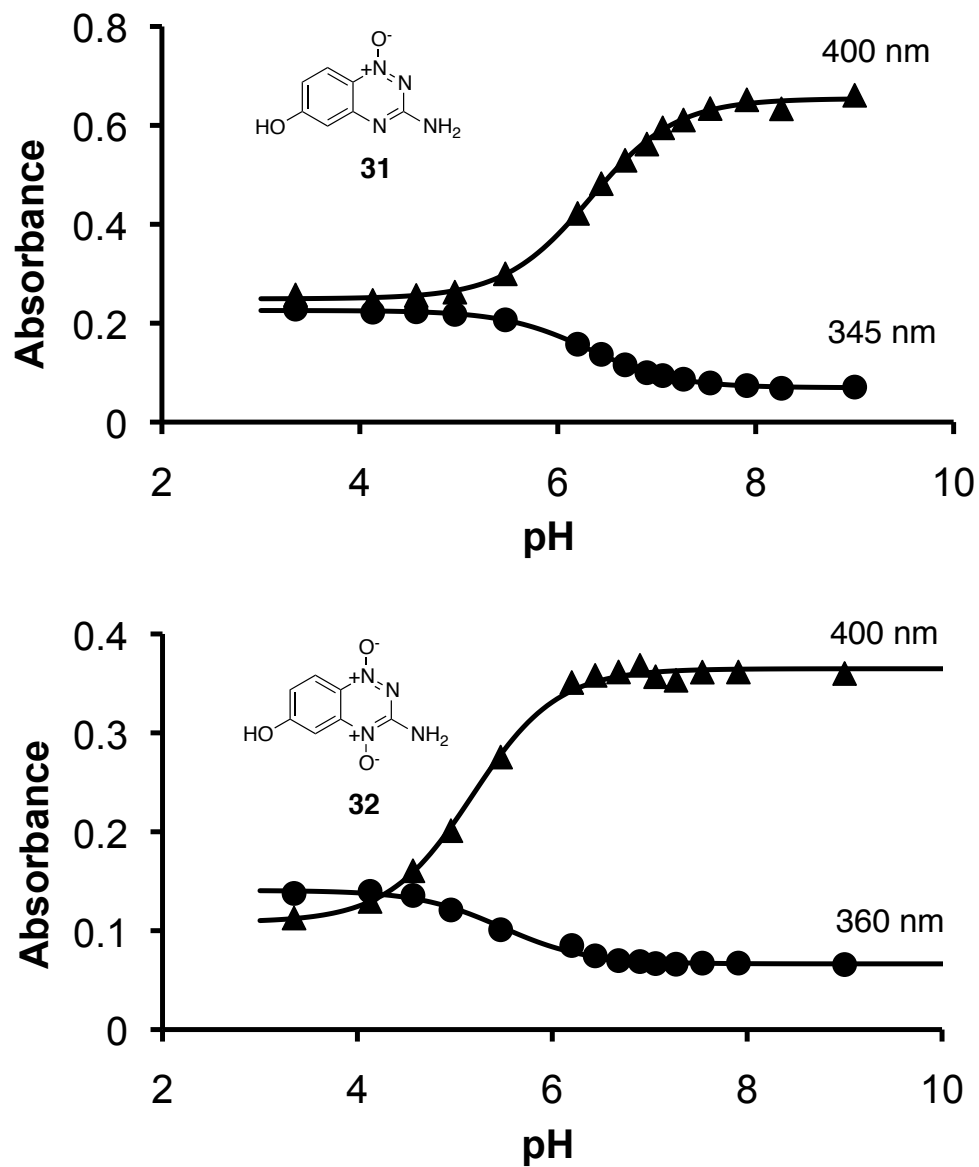


Figure S4. pK_a determination for compounds **31** and **32**. Titration curves with least squares fitting of compounds **31** (top) and **32** (bottom) from pH 3.35-9.00. The absorbance spectra of these compounds as a function of pH are shown in the manuscript.

Figure S5

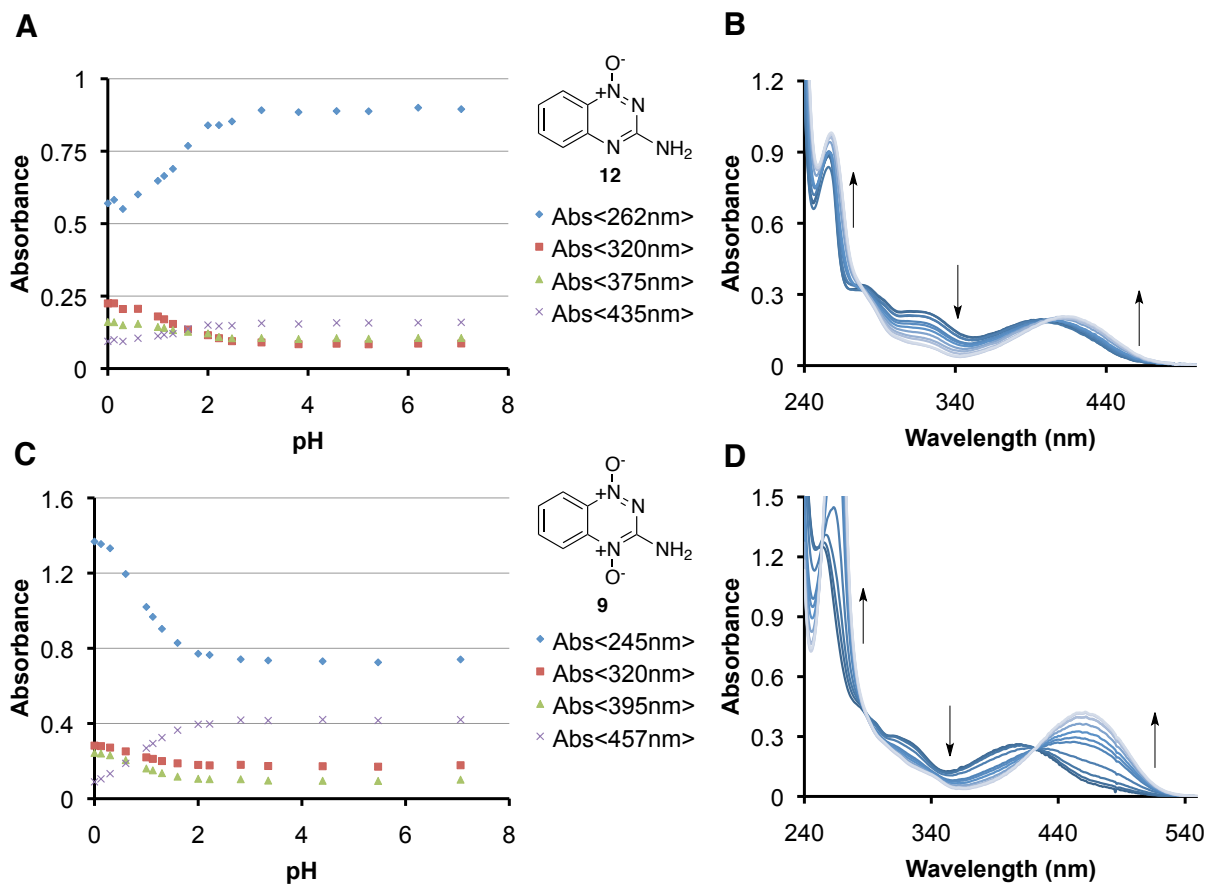


Figure S5. Control experiments showing the pH-dependent changes in the absorbance spectra of compounds **12** and **9**. There are not significant changes in the absorbance spectra for these molecules in the pH 3-7 region. This provides evidence that the pH-dependent changes in the absorbance spectra for **29**, **31**, and **32** in the pH 3-7 range are likely associated with protonation and deprotonation of the carboxylate and phenol groups of these analogs.

Figure S6

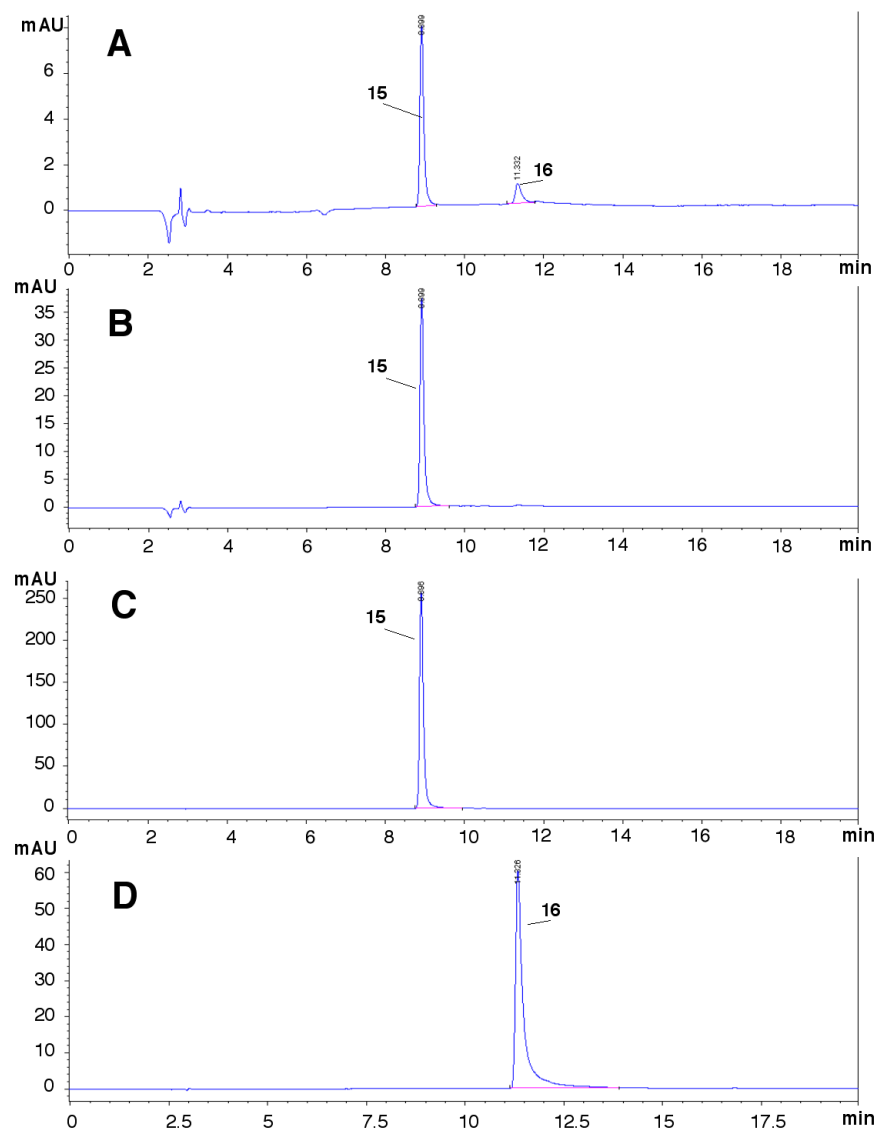


Figure S6. HPLC chromatograms showing the in vitro metabolic conversion of **15** to **16** by NADPH:cytochrome P450 reductase and associated controls: (A) reduction of **15** under anaerobic conditions, (B) same except under aerobic conditions, (C) authentic standard of **15**, and (D) authentic standard of **16**. Detailed conditions are provided in the Experimental Section of the paper.

Figure S7

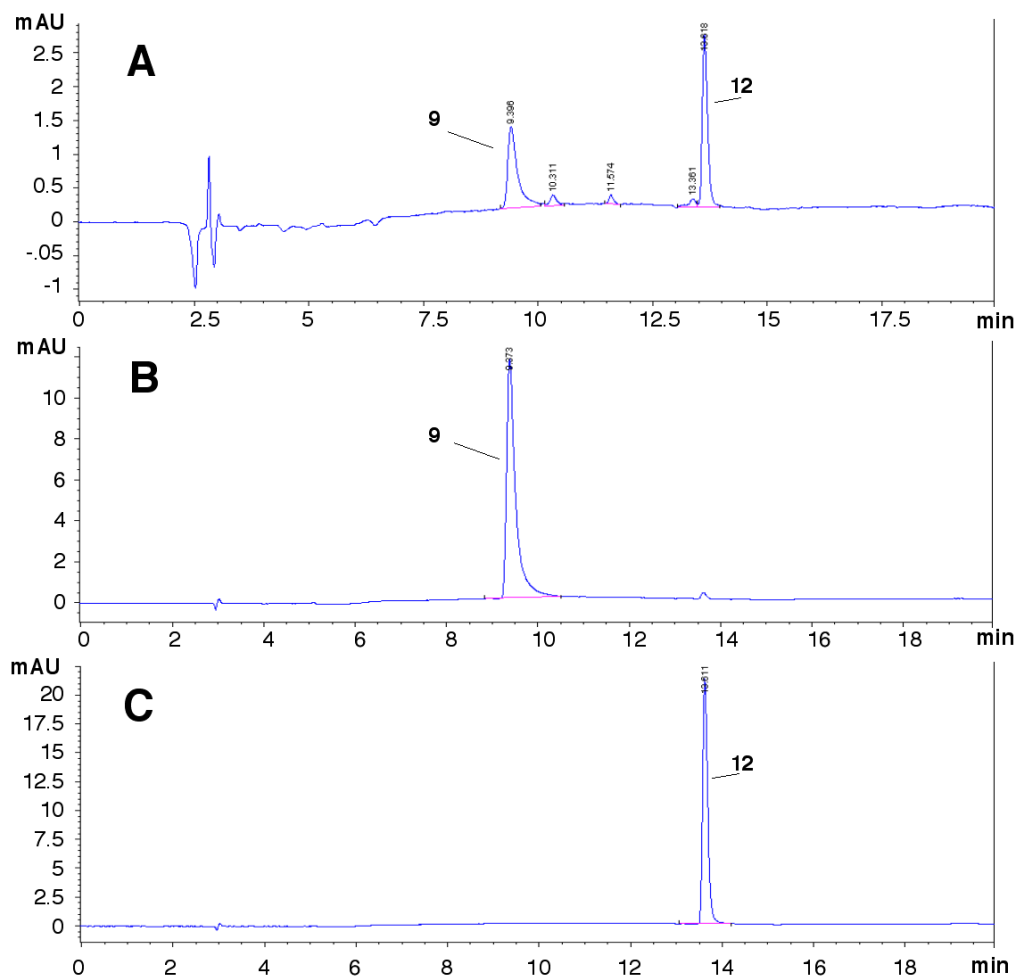
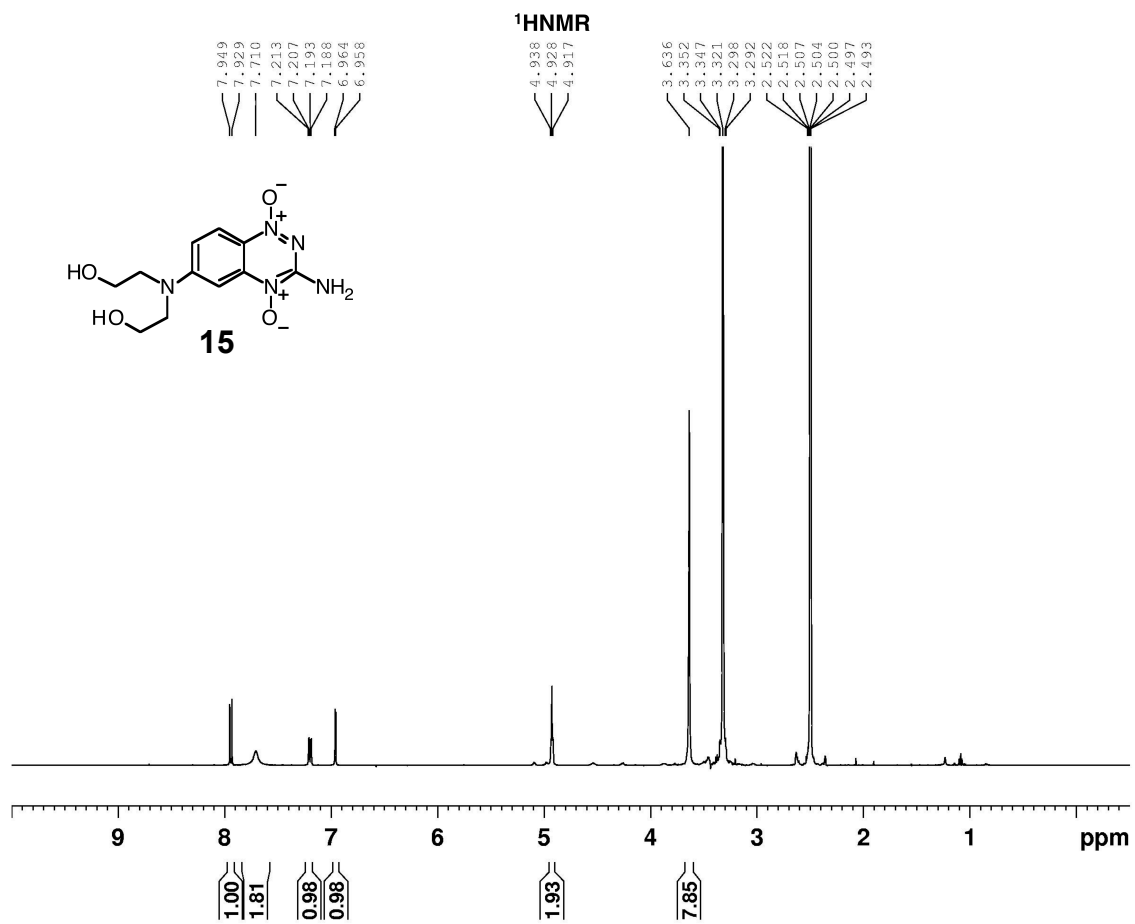
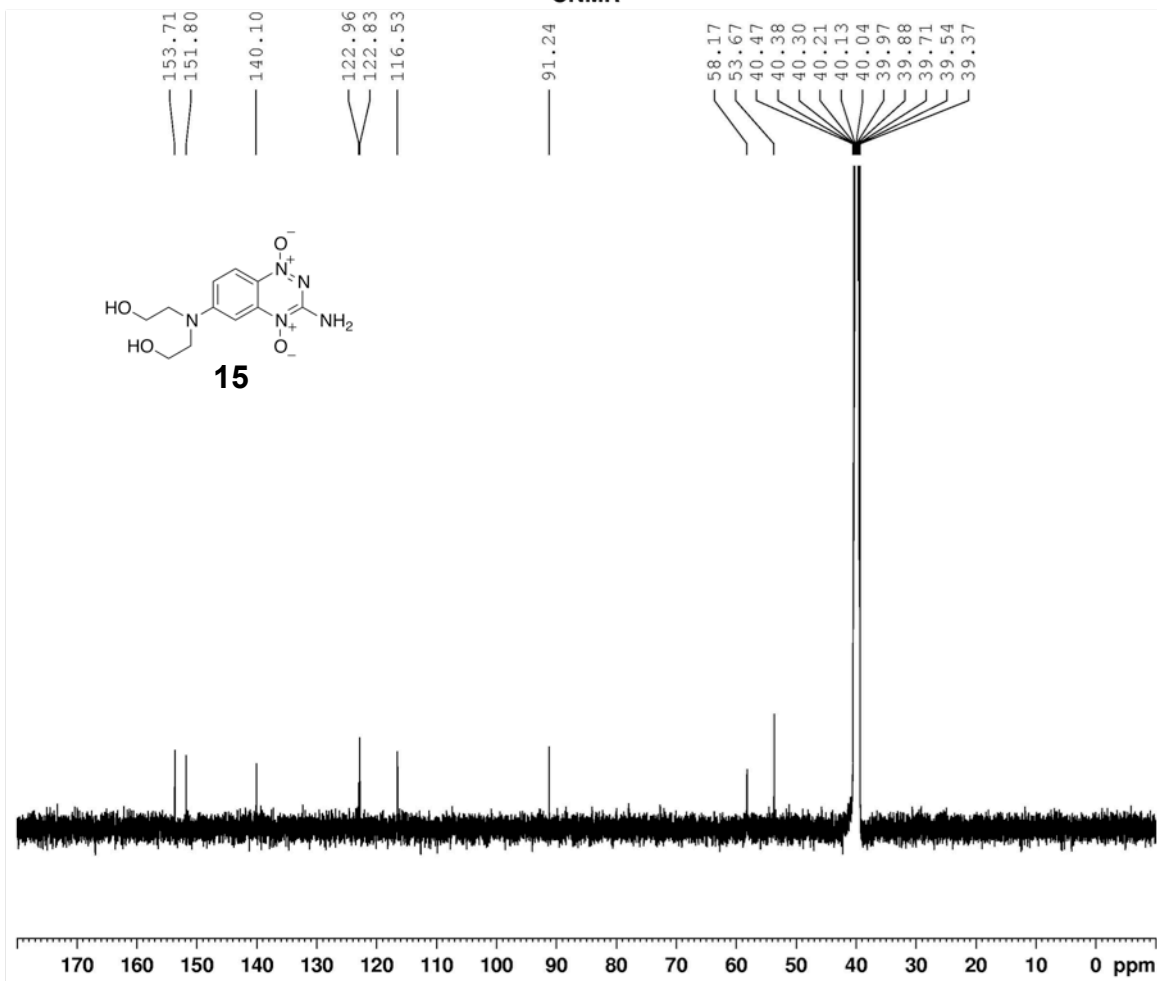


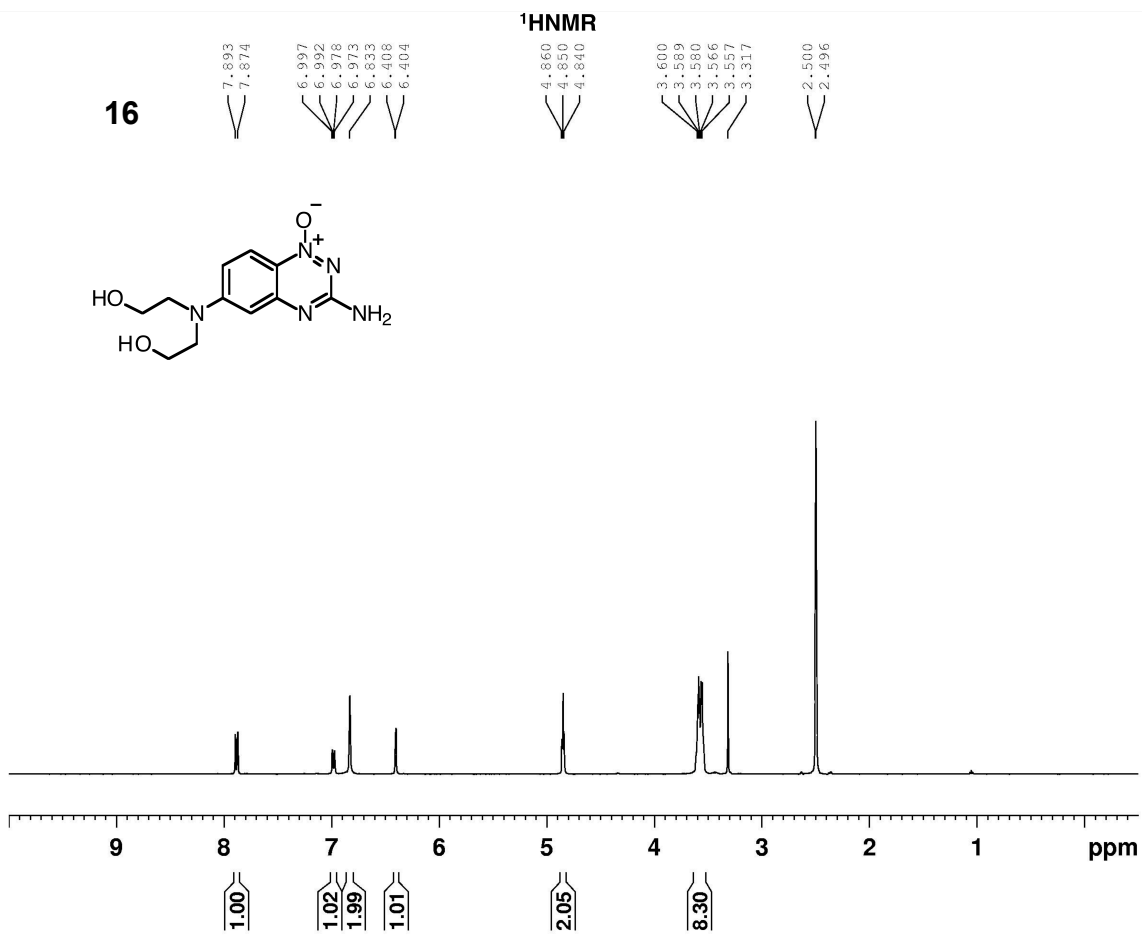
Figure S7. HPLC chromatograms showing the in vitro metabolic reduction of **9** to **12** by NADPH:cytochrome P450 reductase using the same conditions employed for the reduction of **15** to **16** (see: Experimental Section for details). Panel (A) Reduction of **9** under anaerobic conditions, (B) HPLC standard chromatogram of **9**, and (C) HPLC standard chromatogram of **12**.

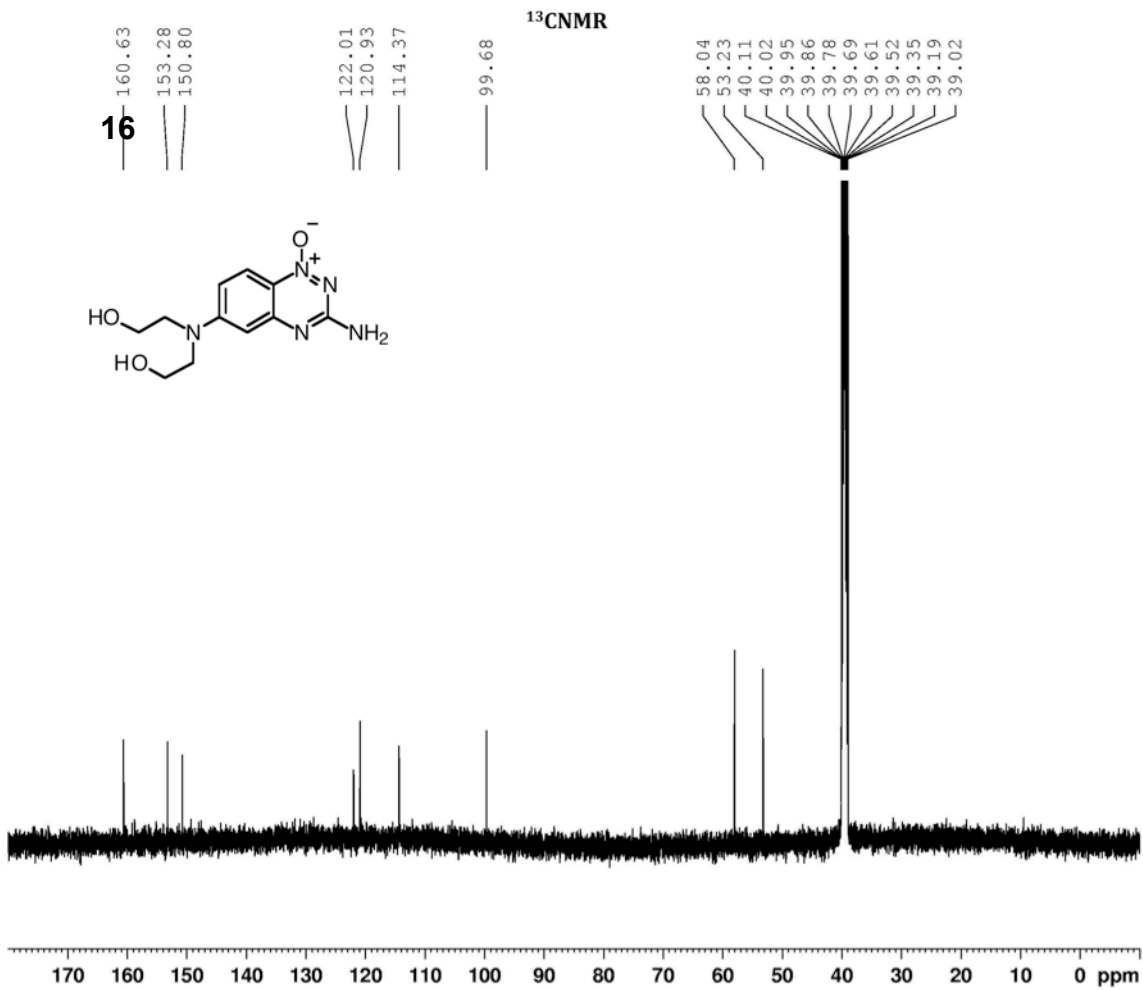
¹H-NMR and ¹³C-NMR Spectra

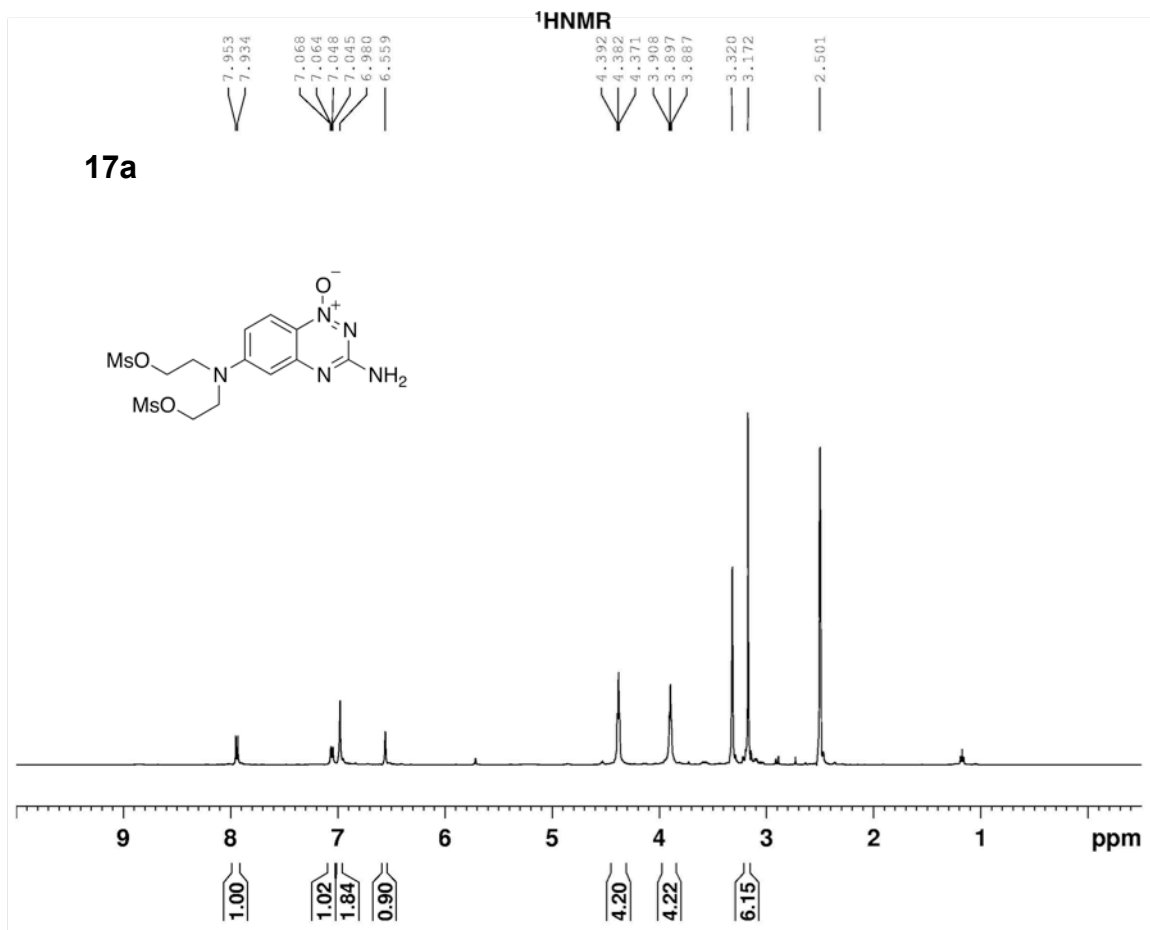


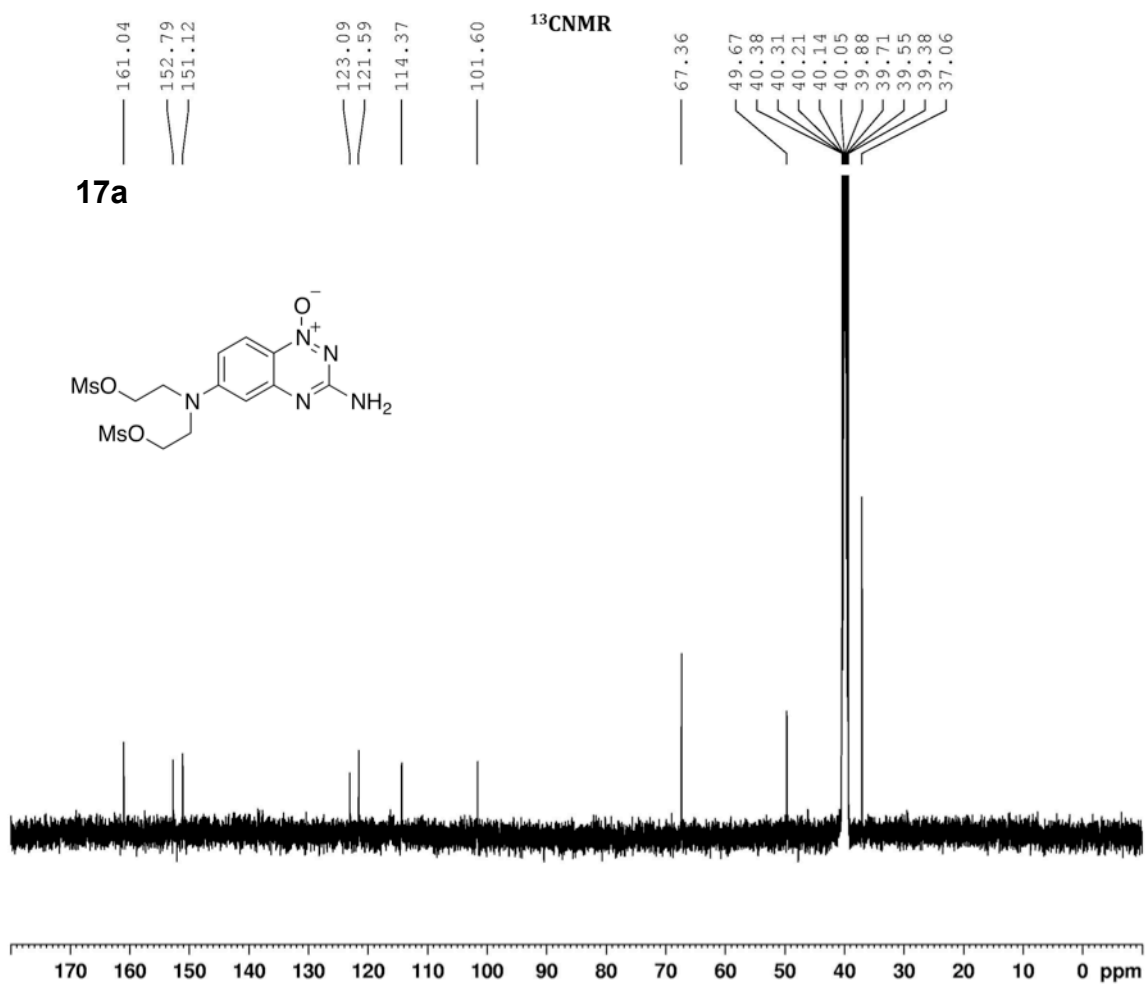
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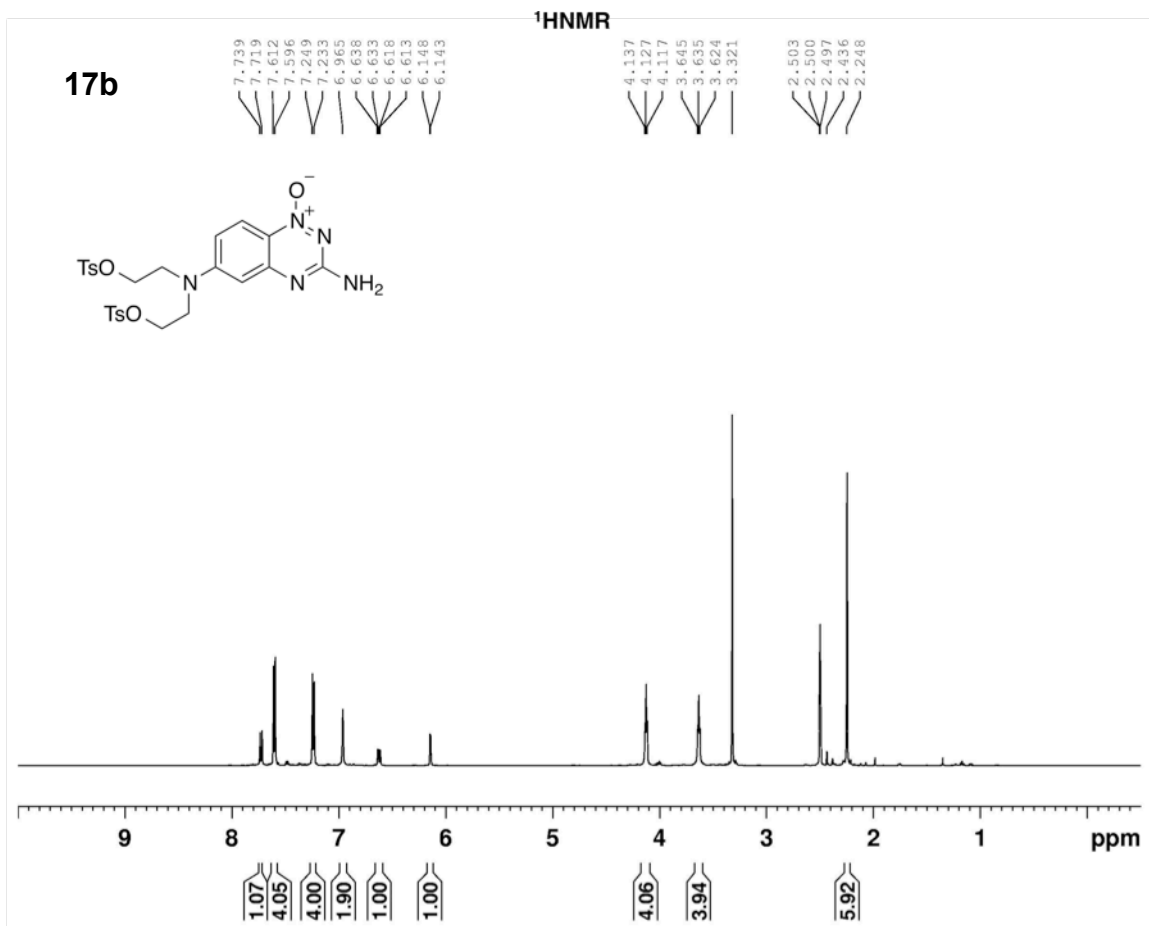


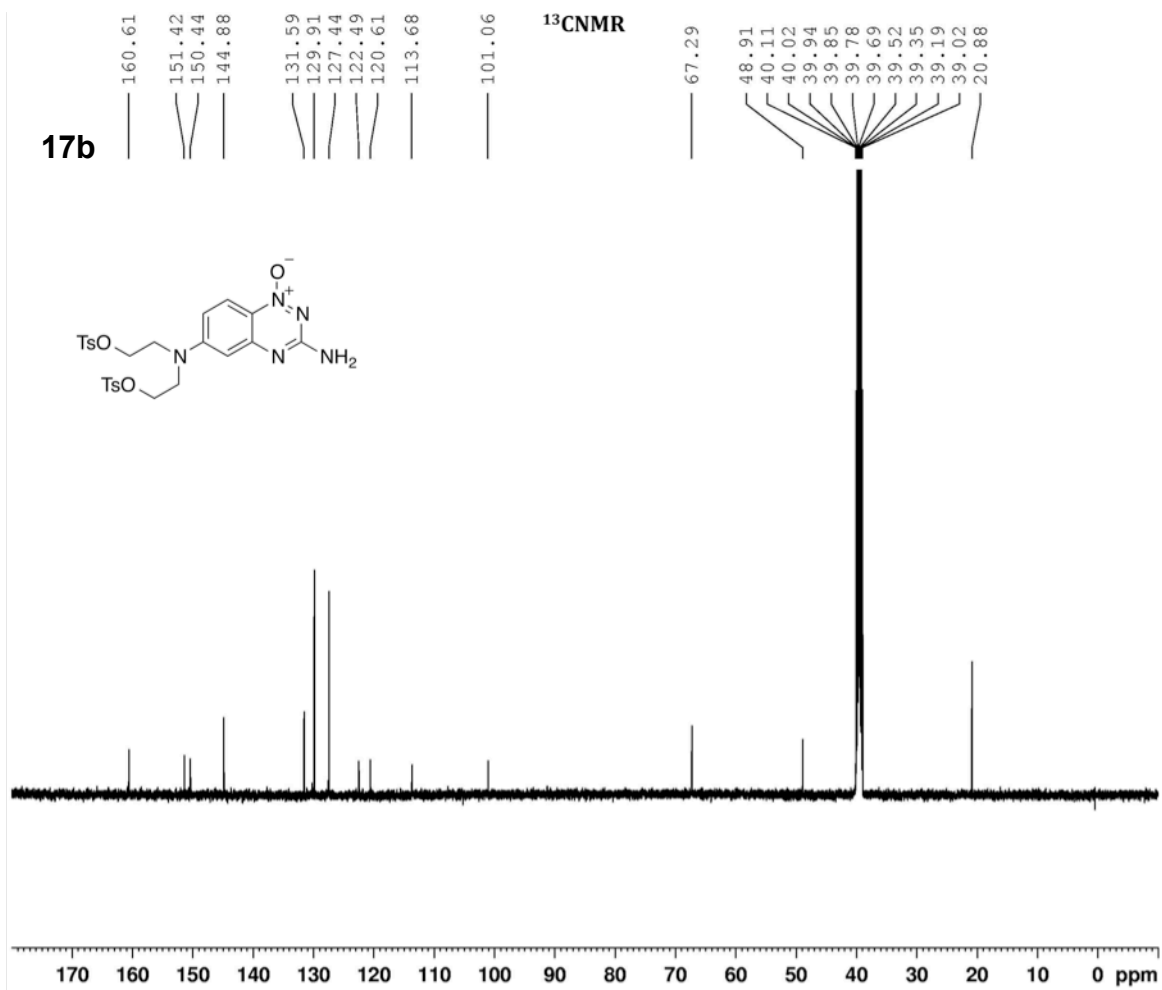




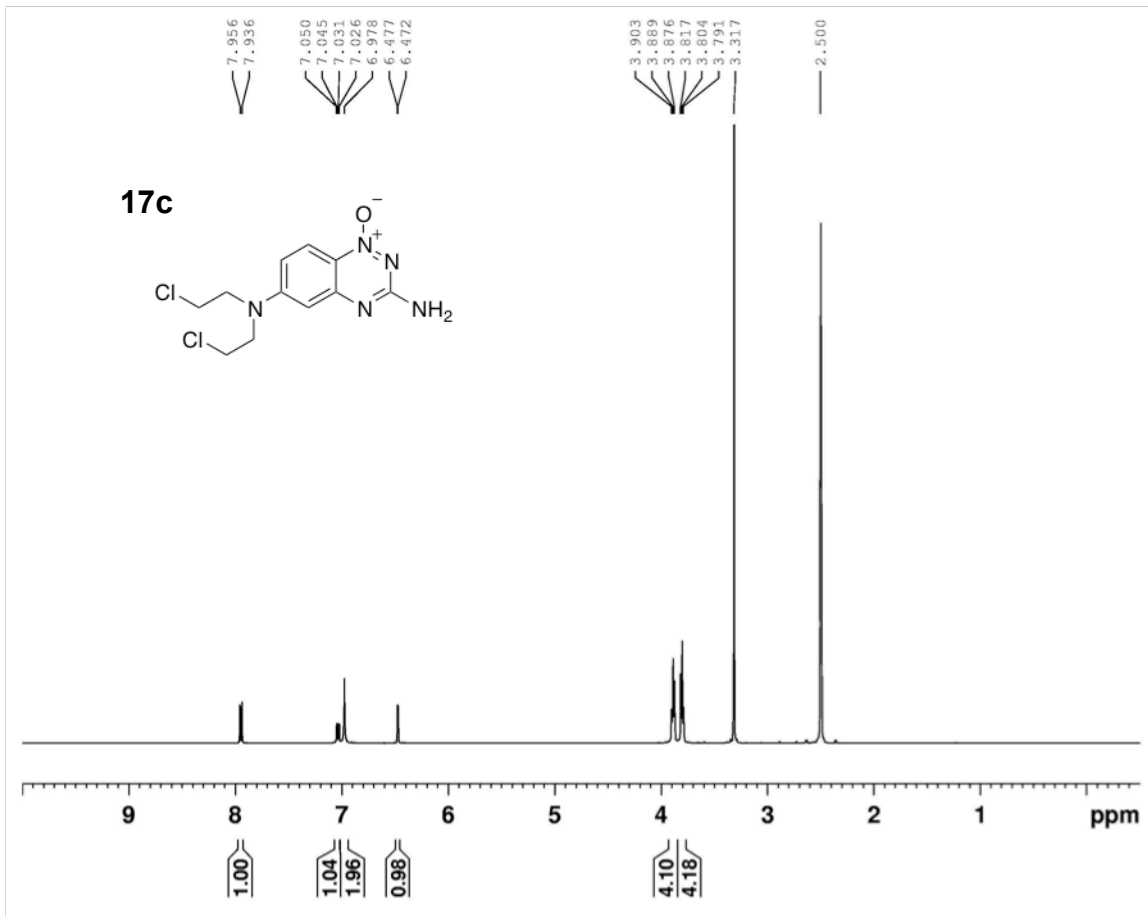


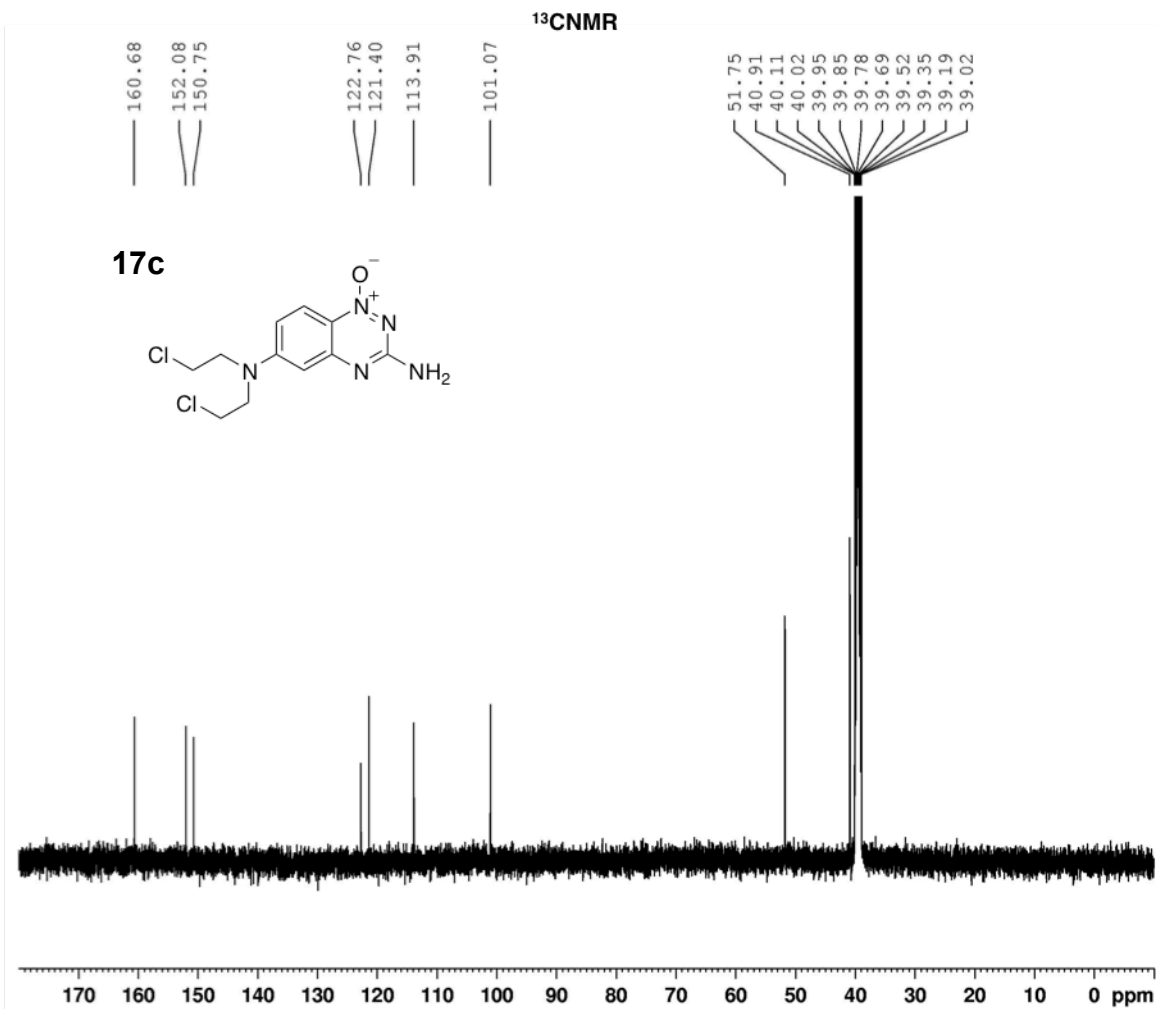


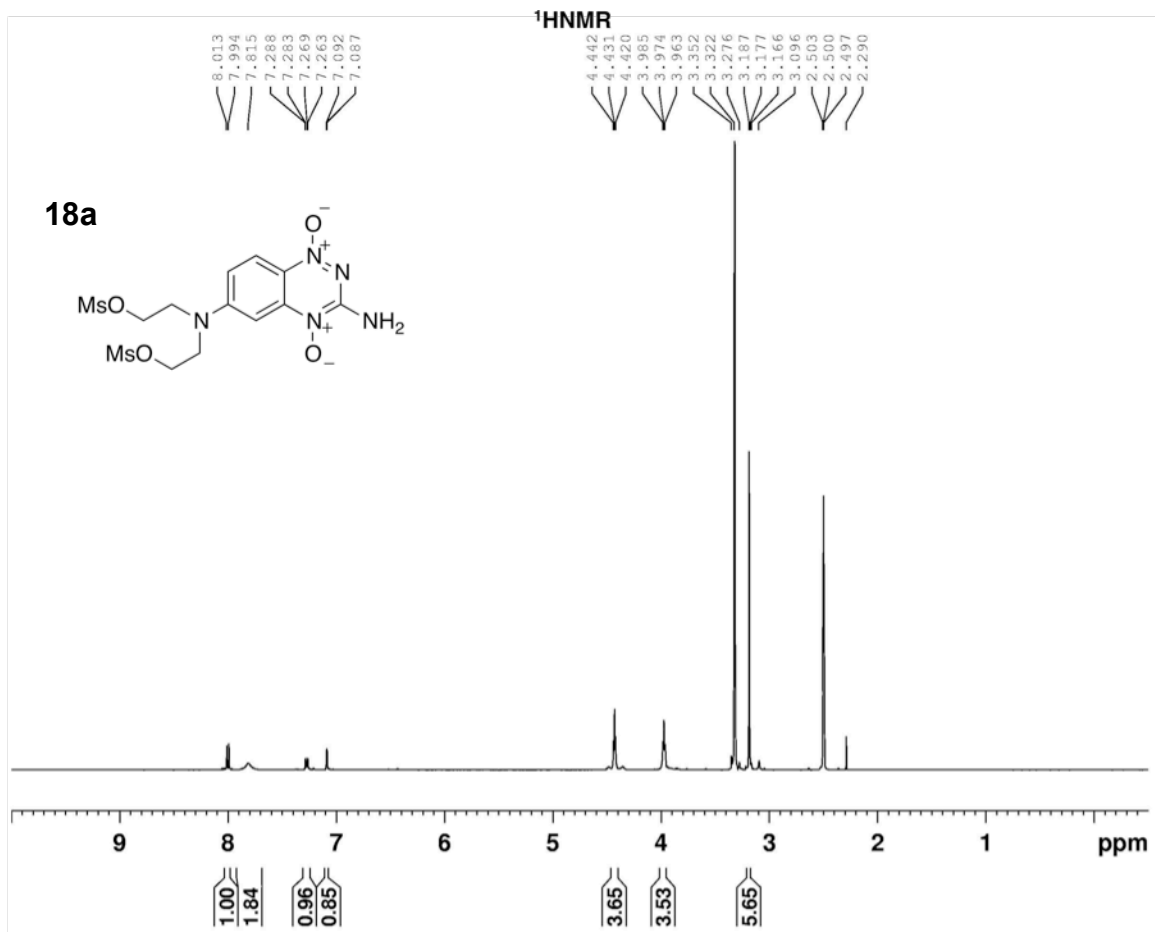


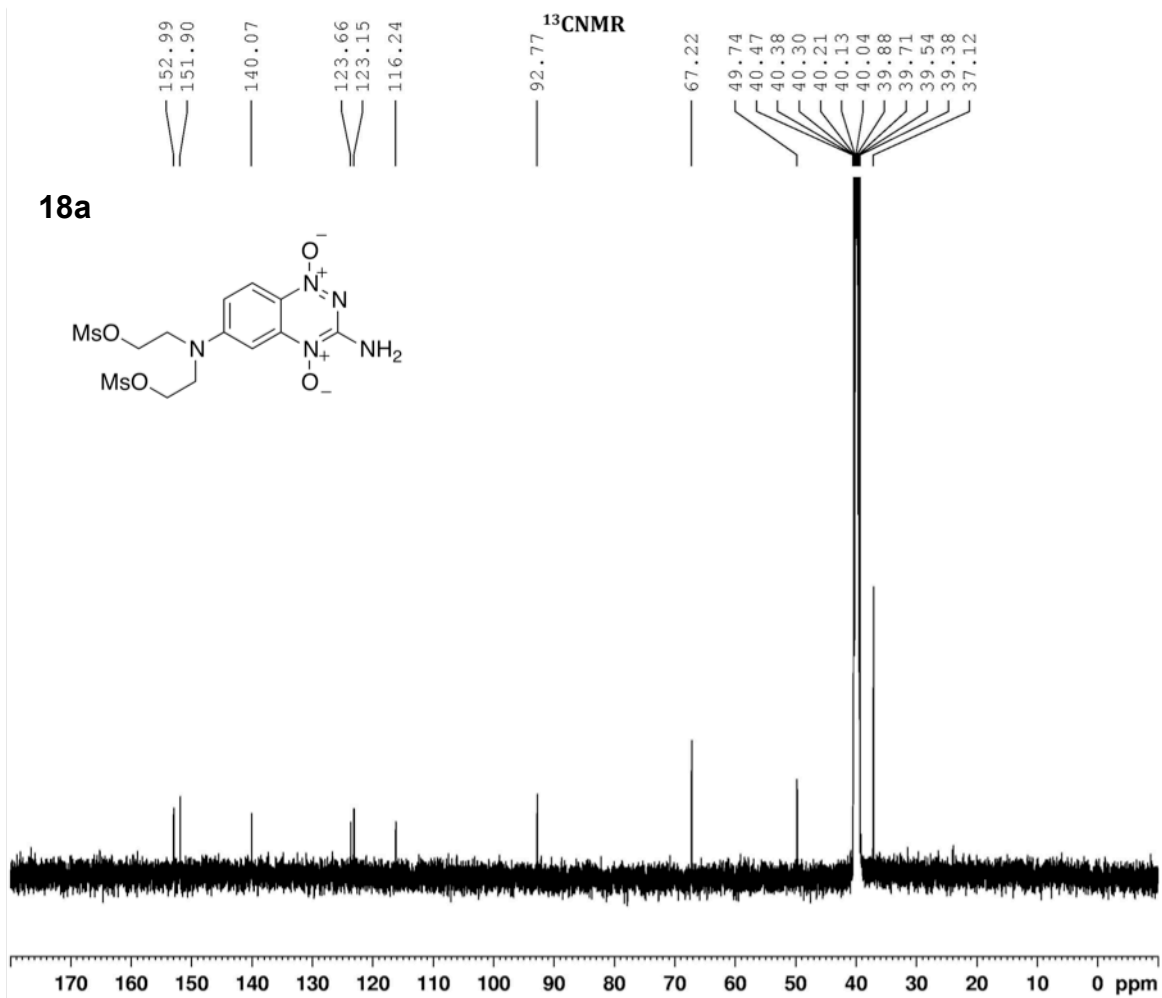


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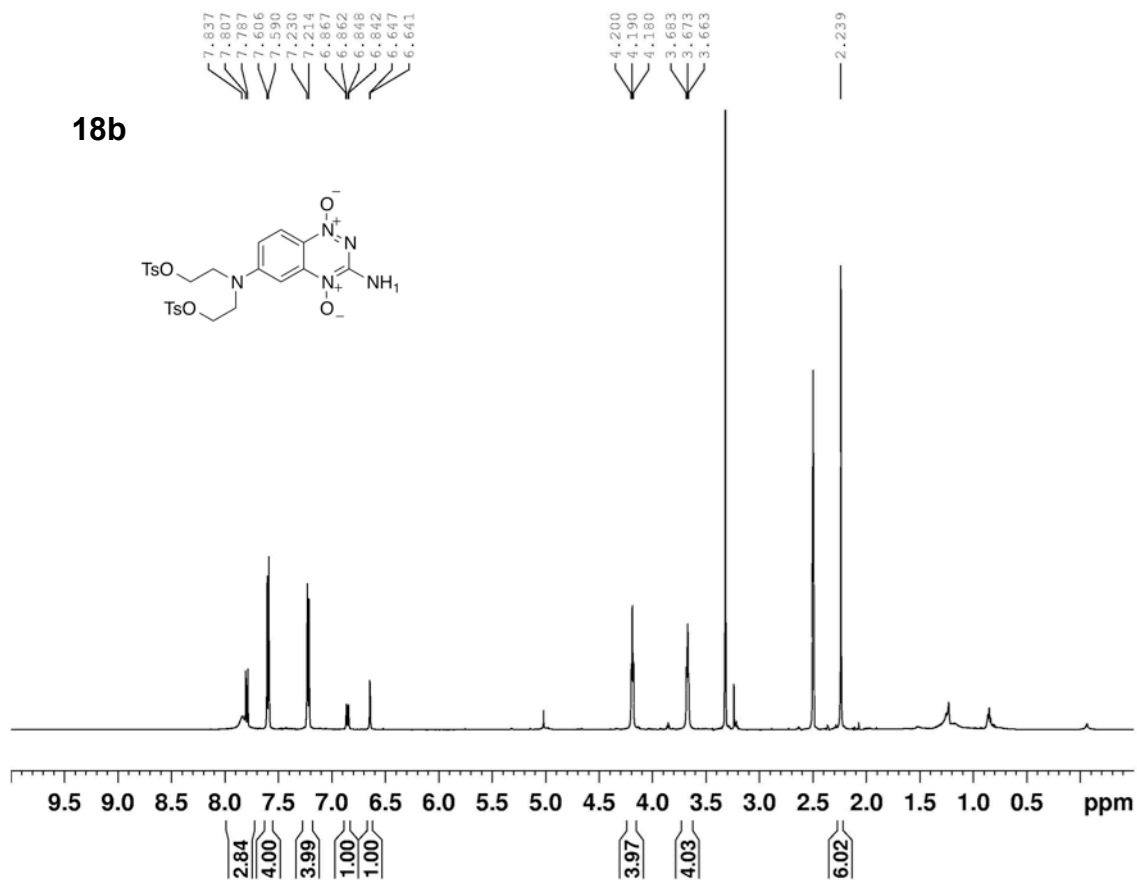
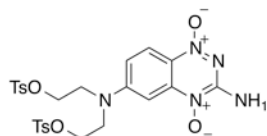




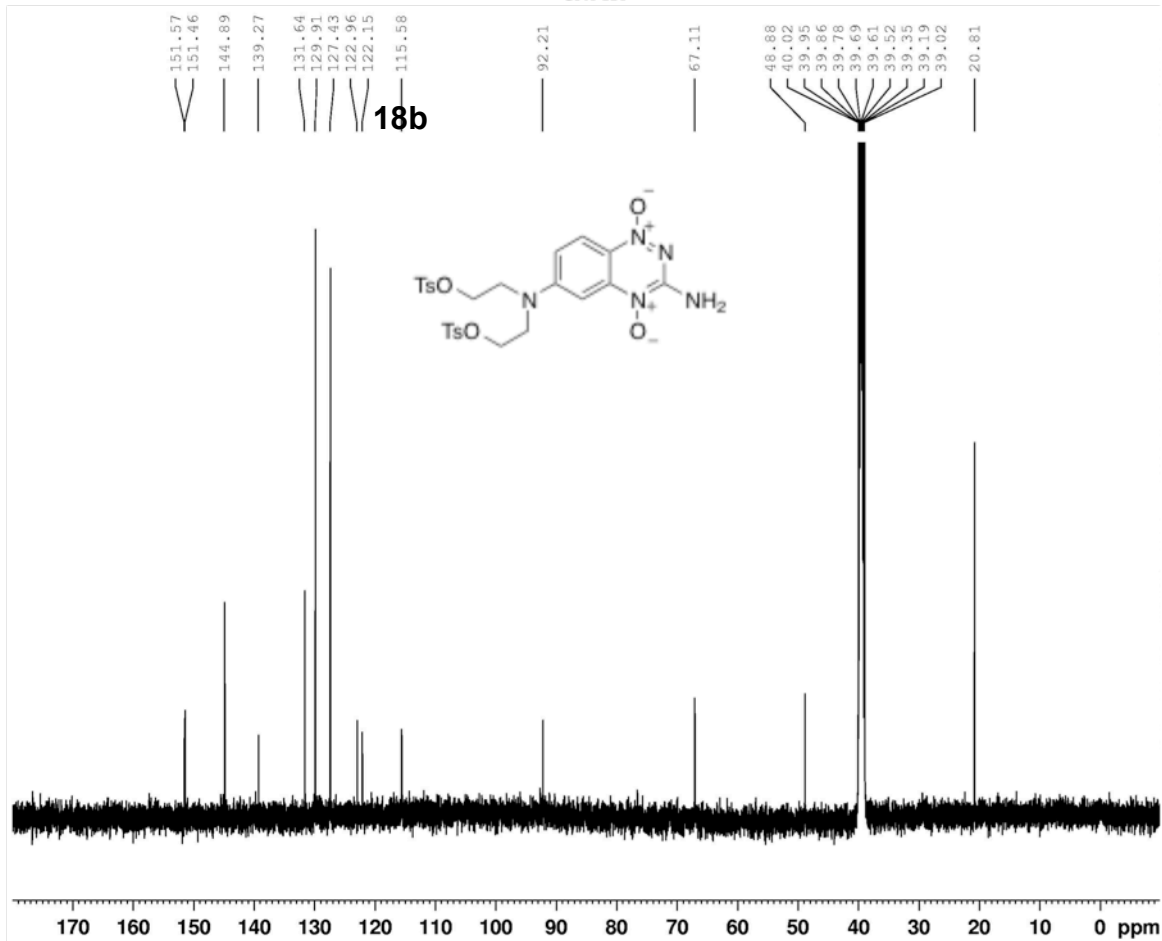


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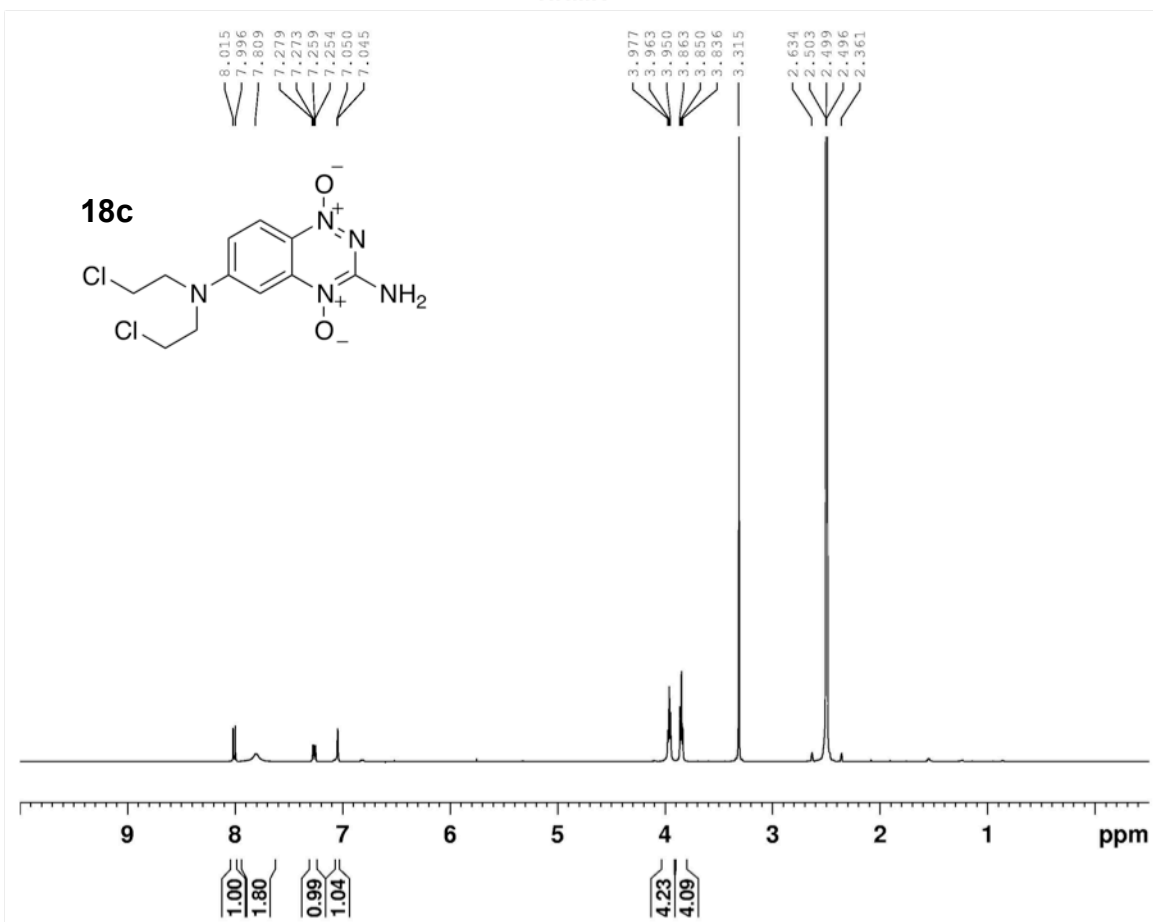
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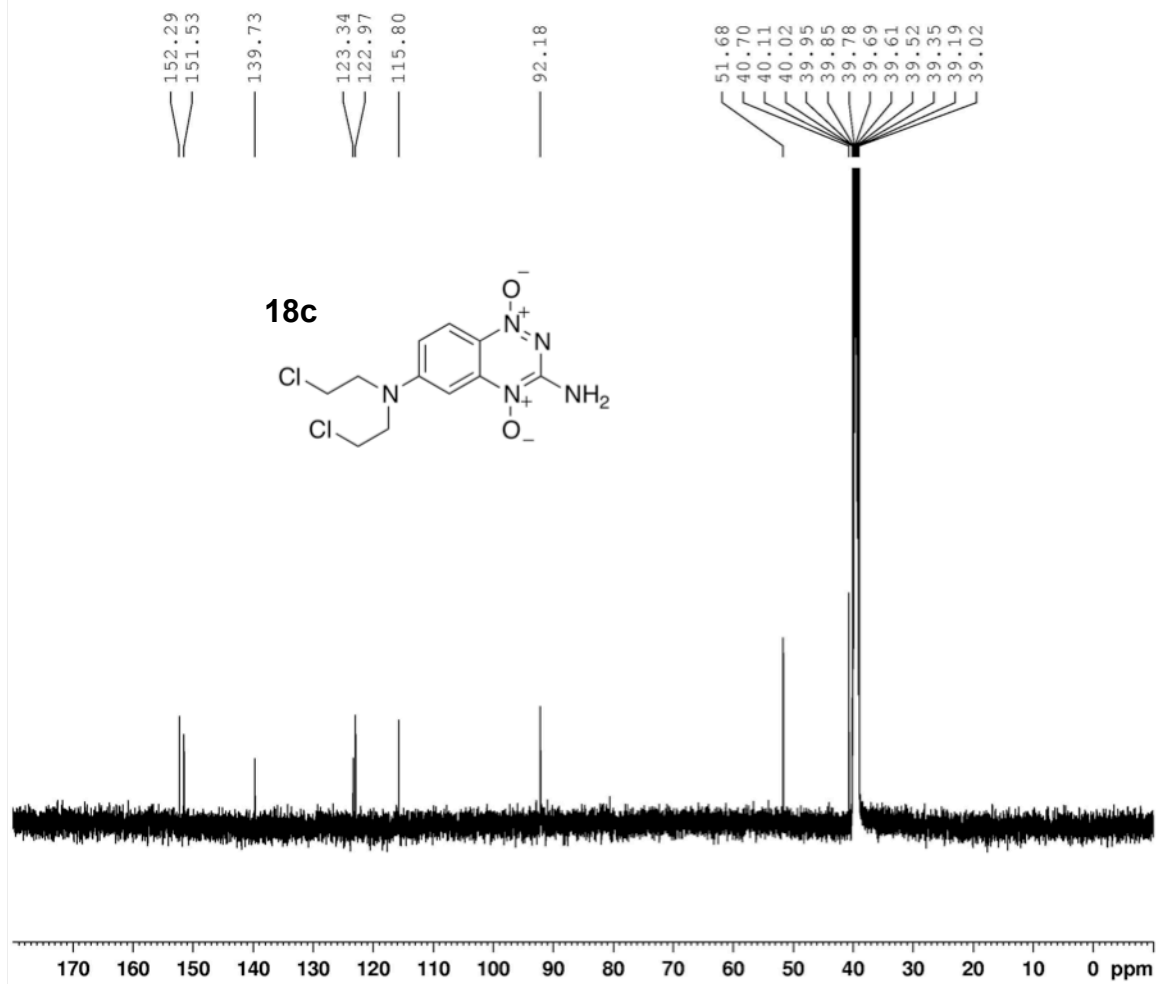
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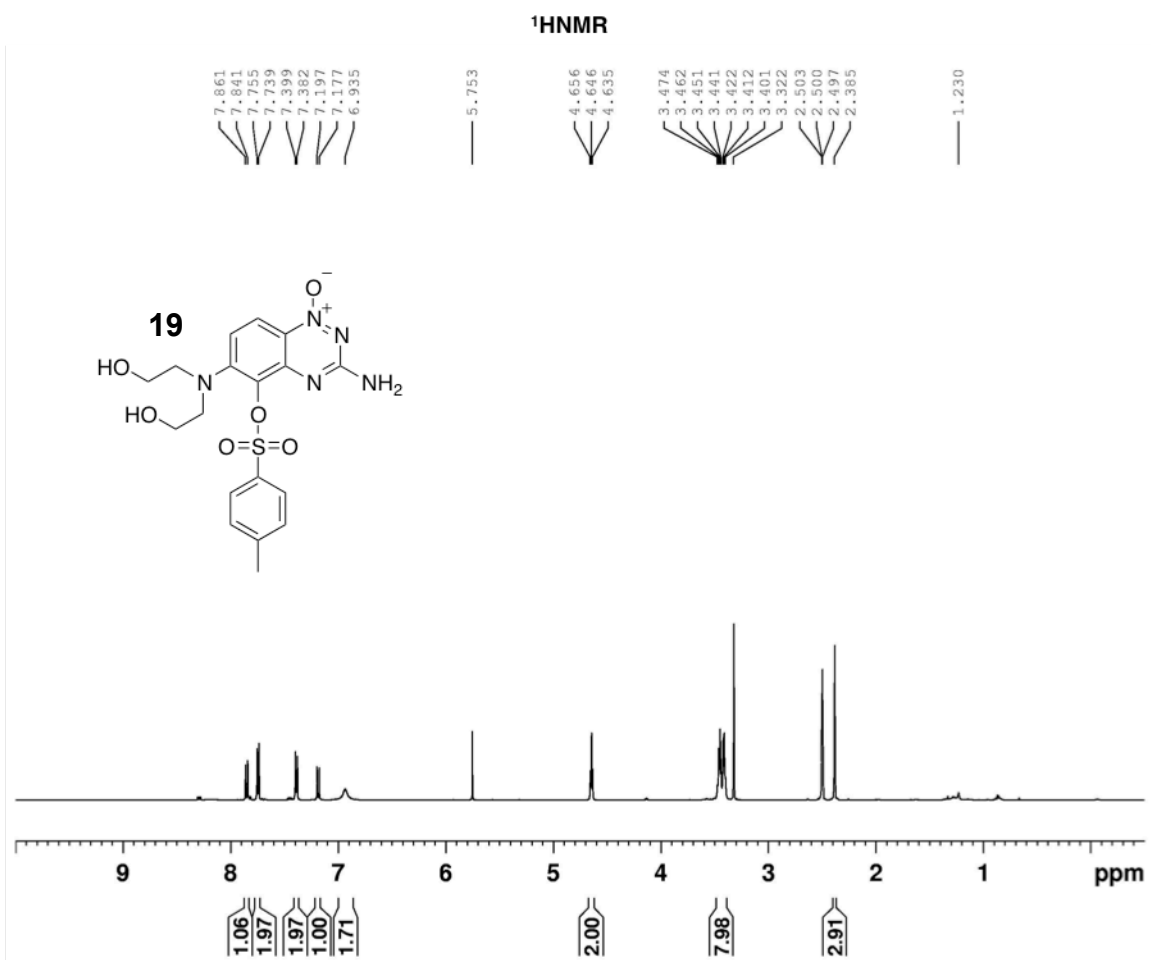


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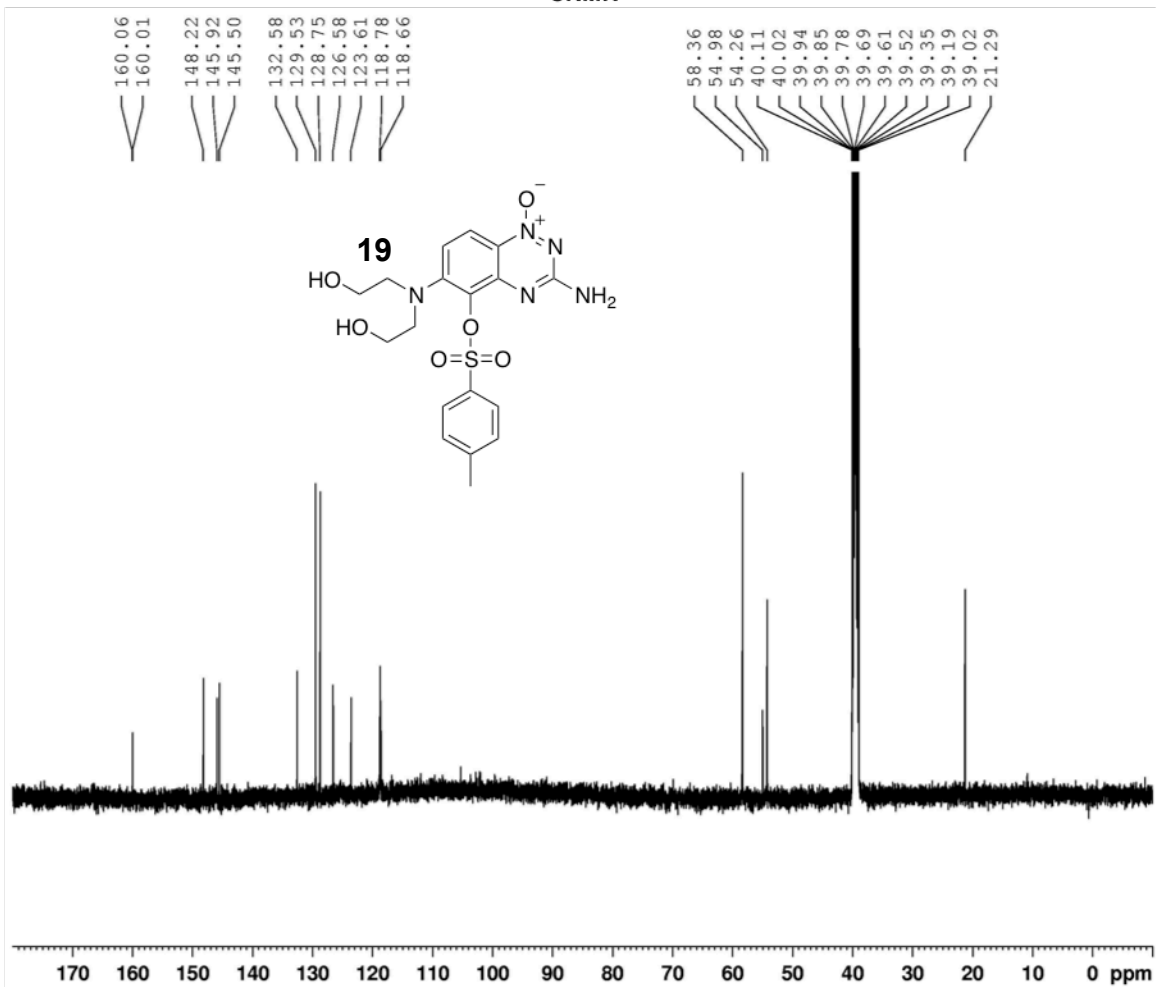


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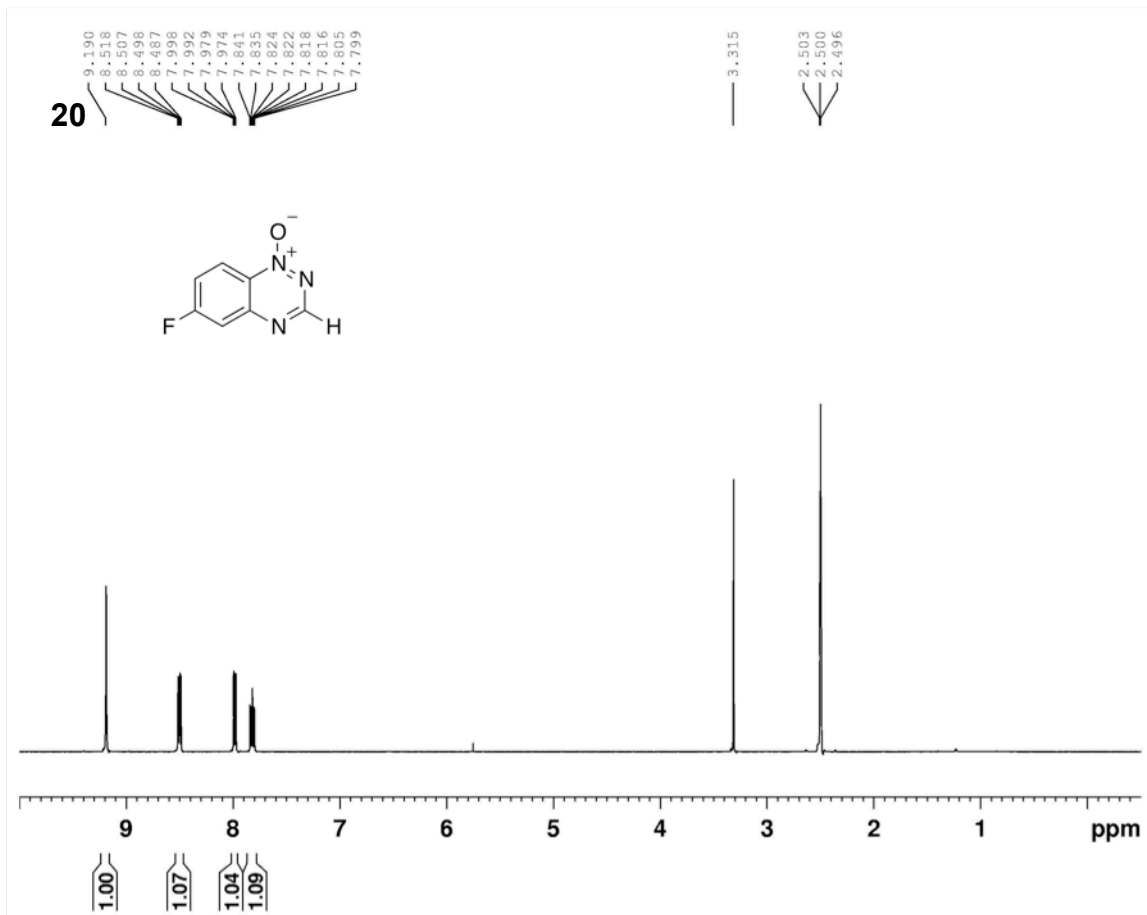


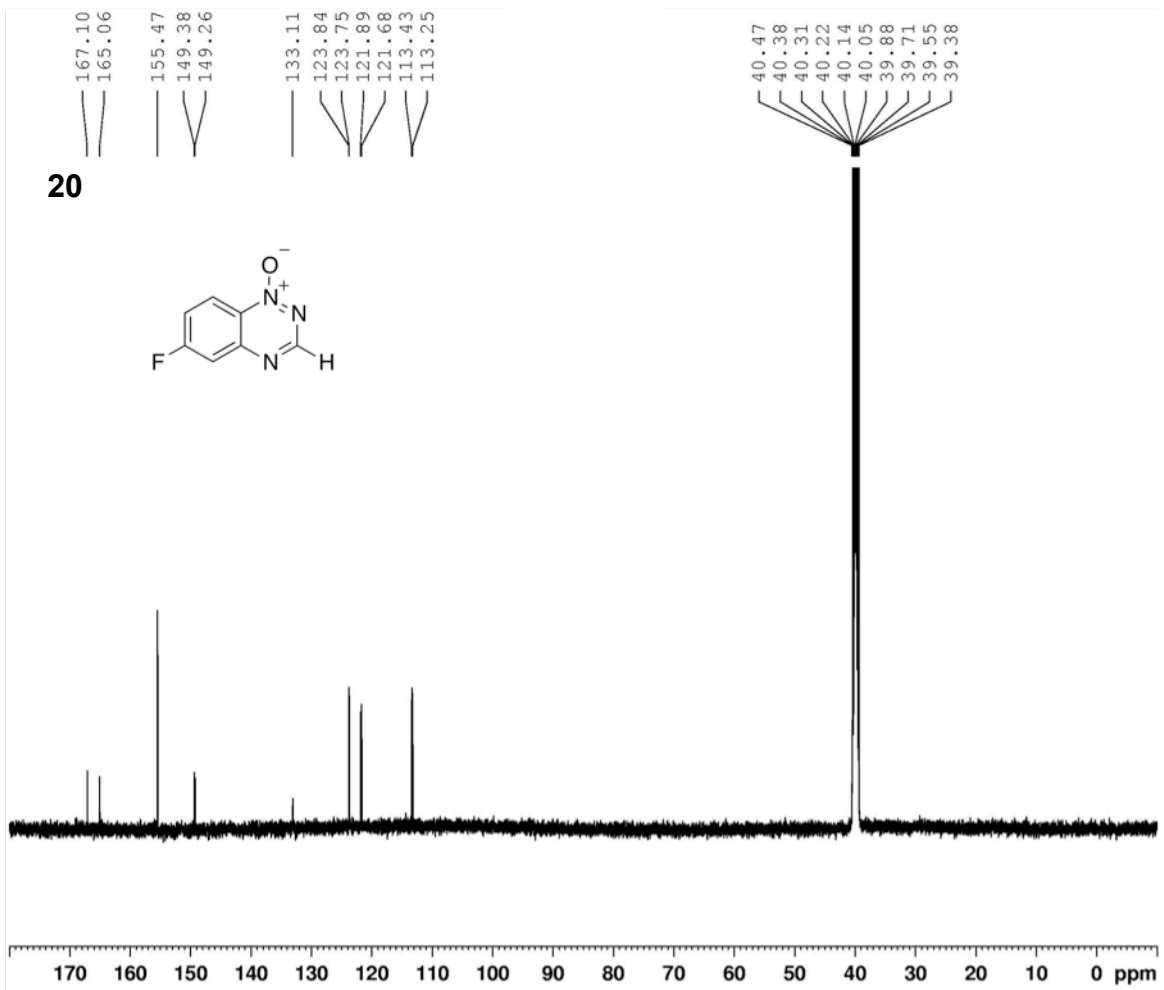


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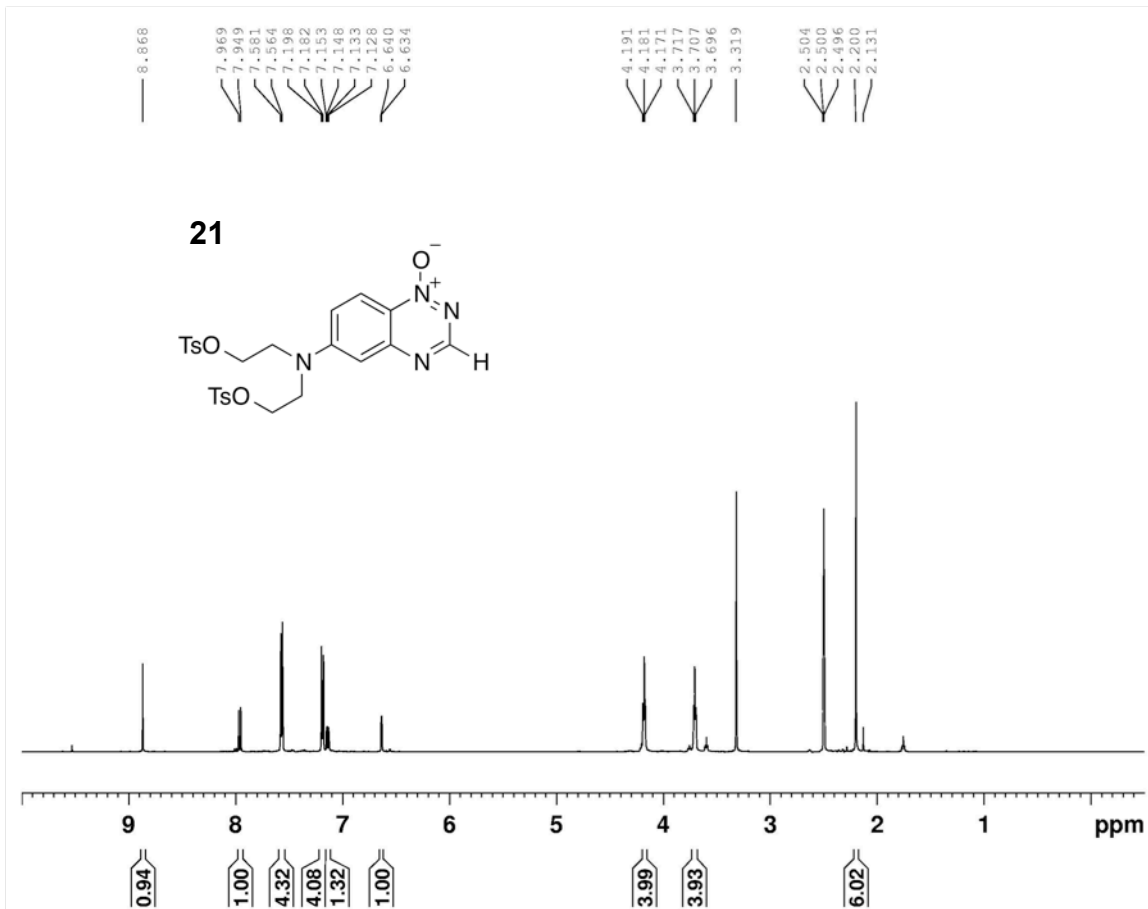


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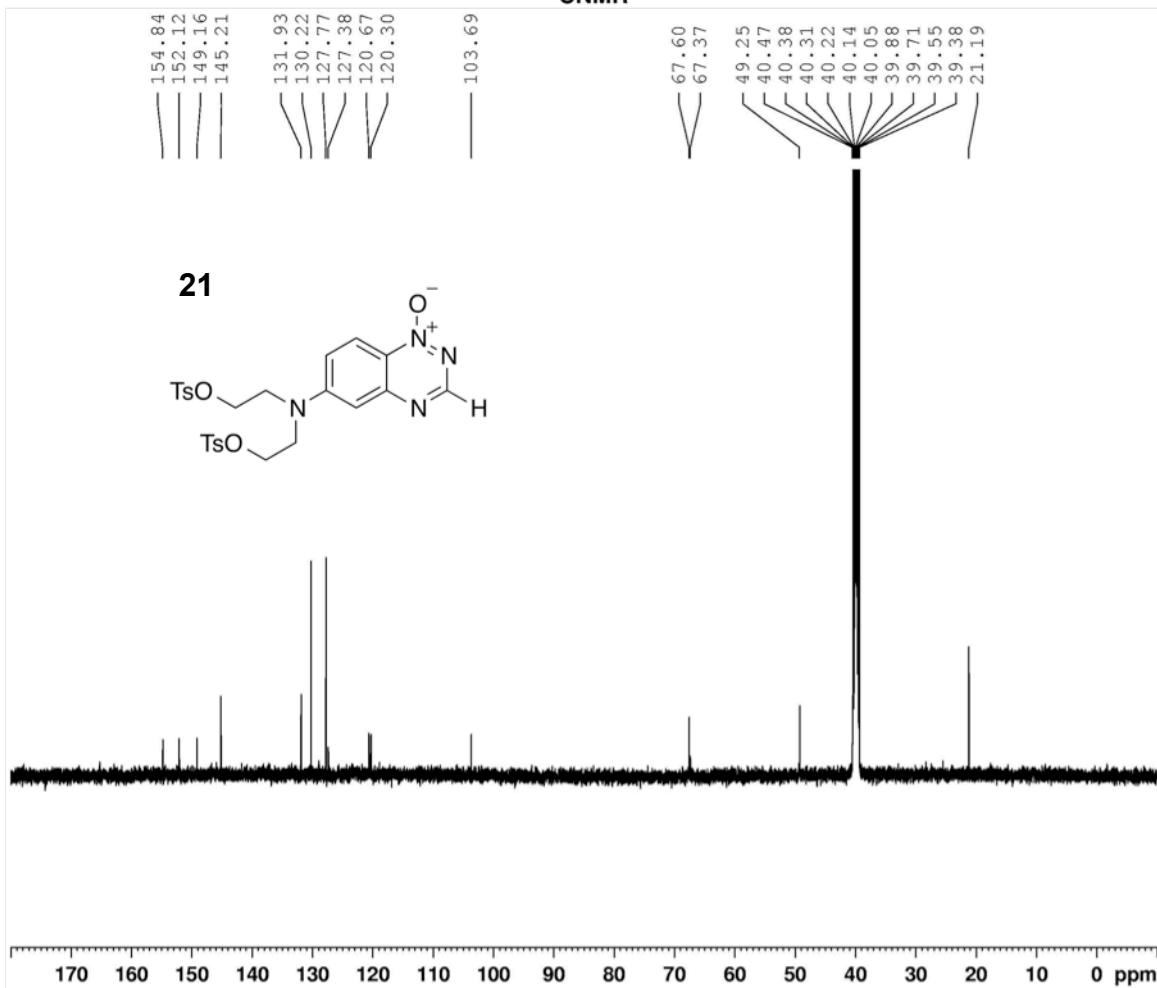




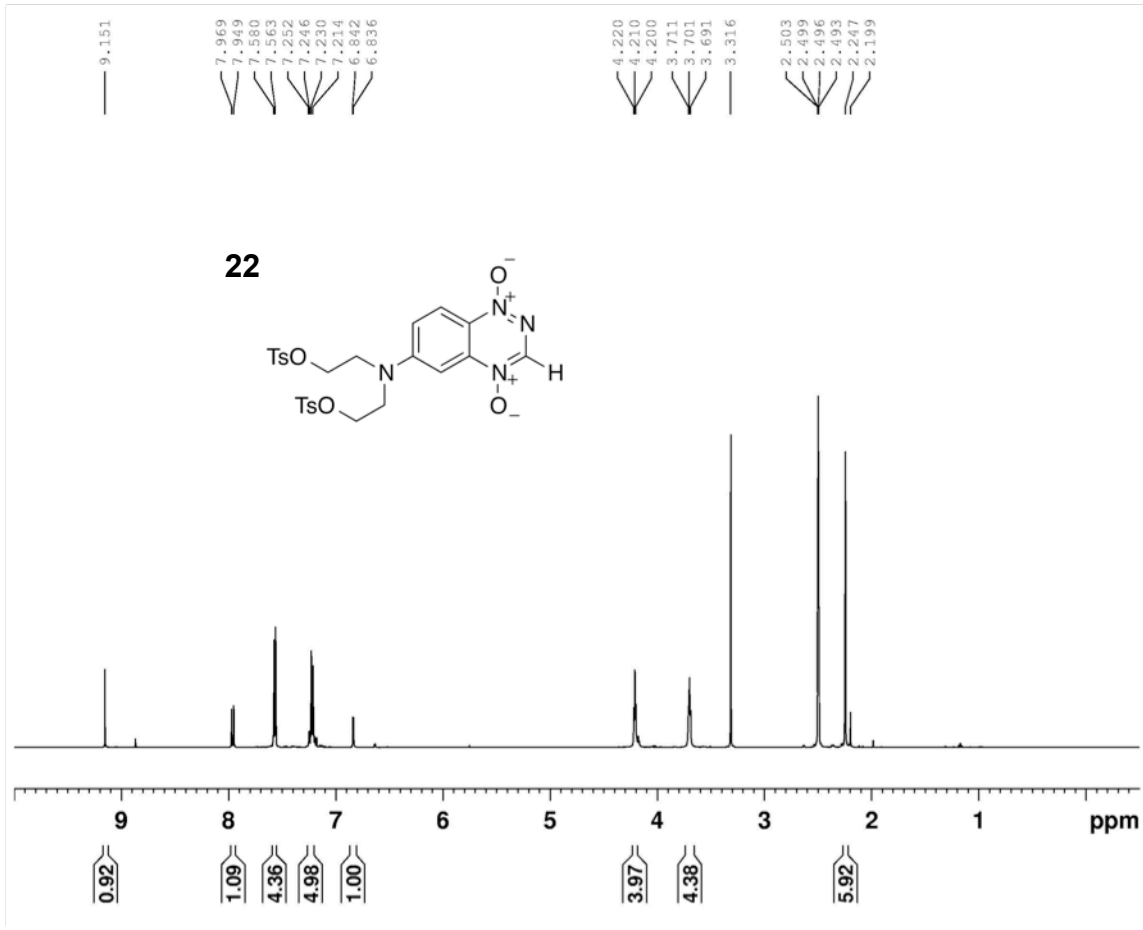
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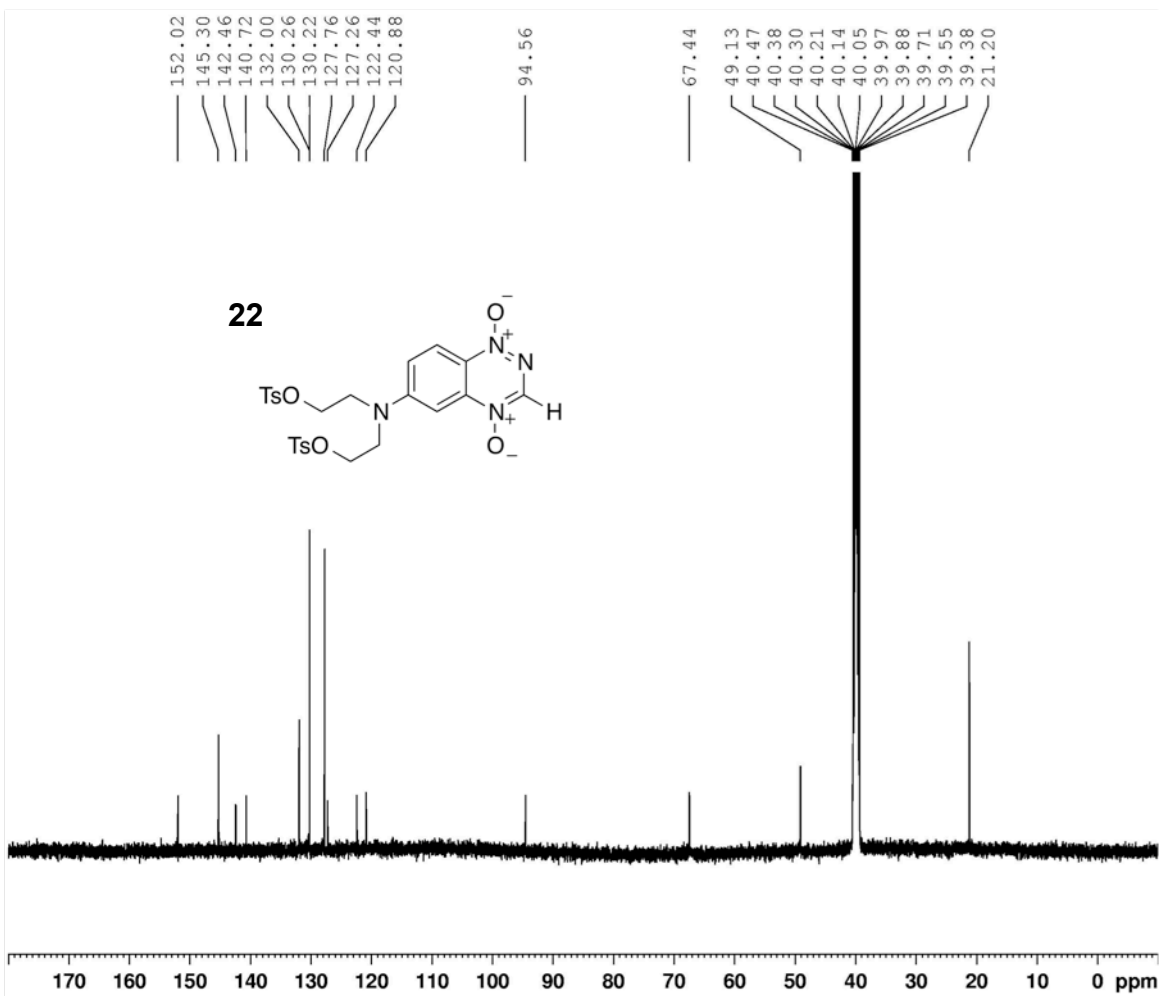
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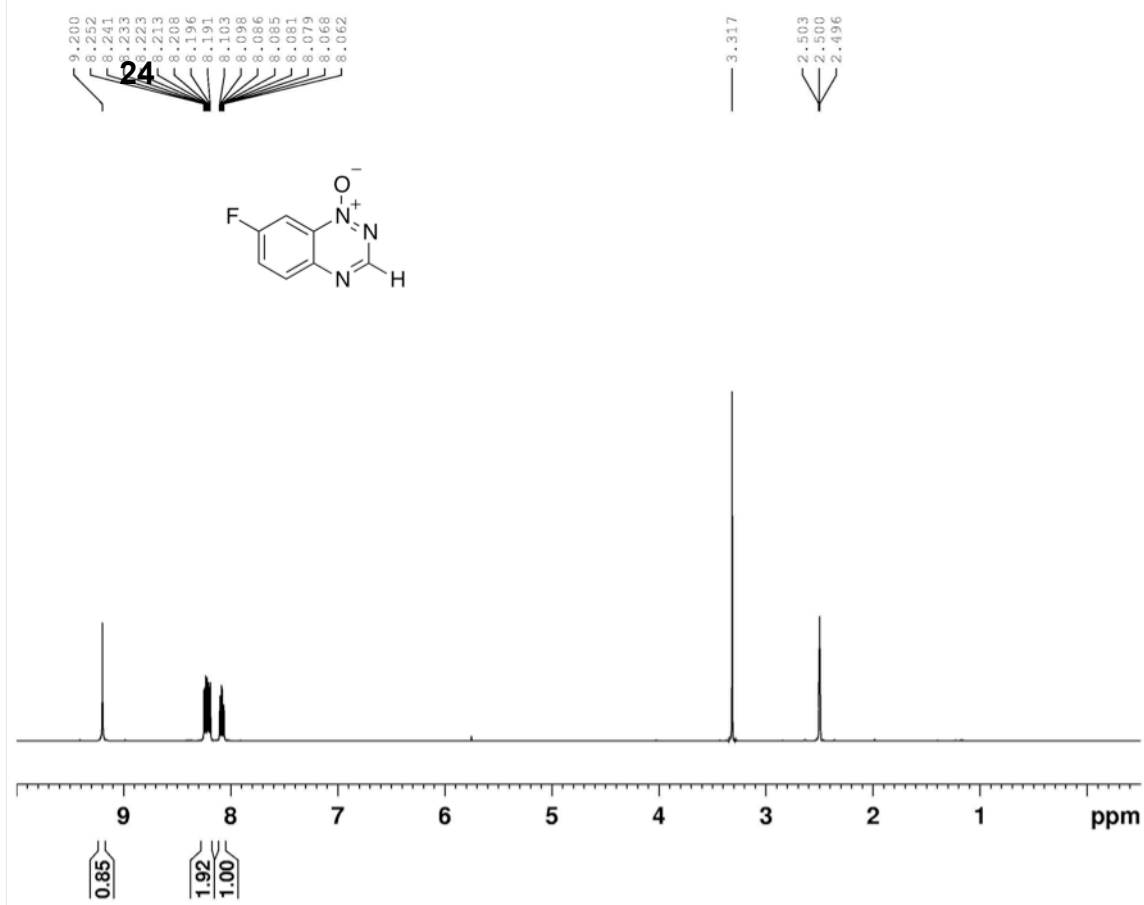
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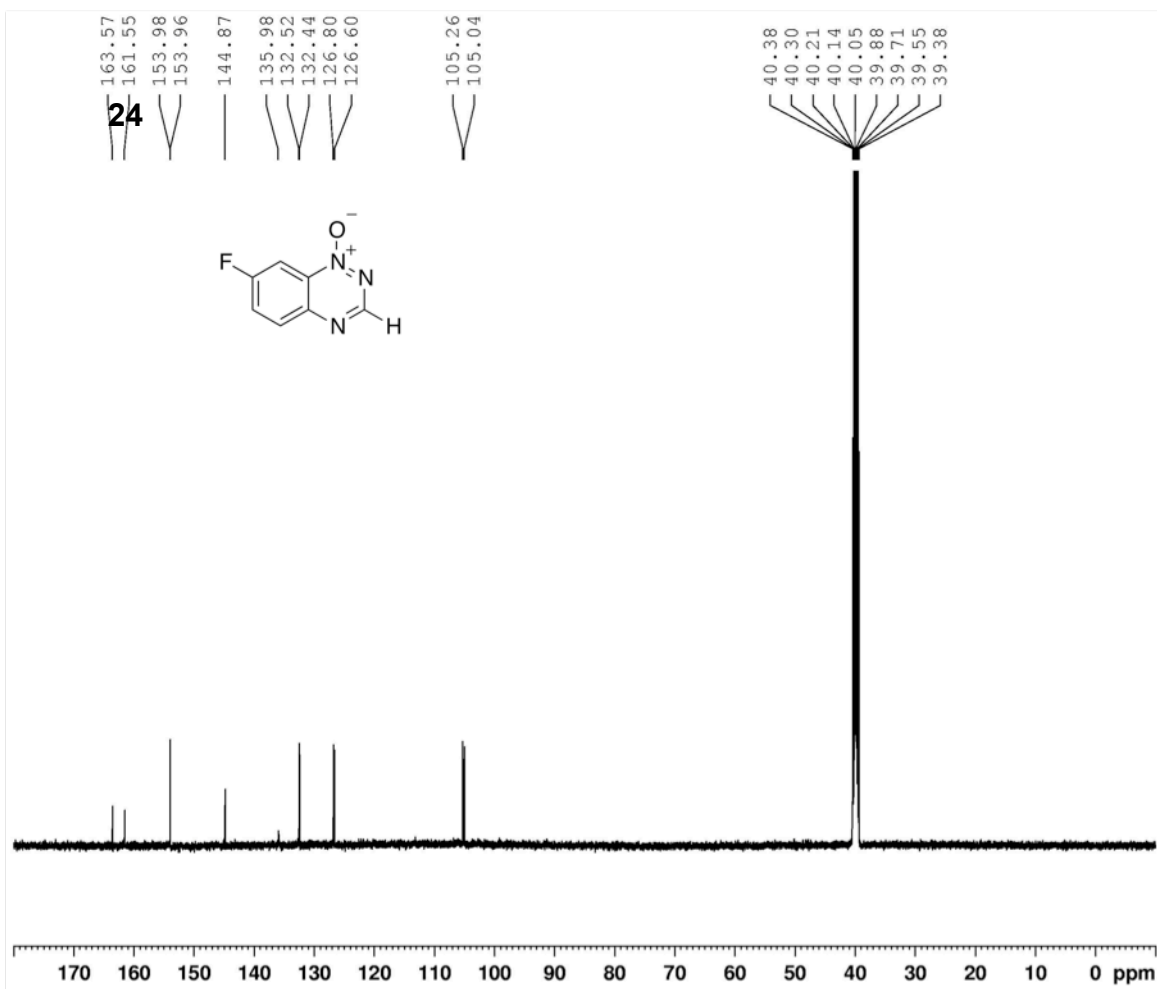


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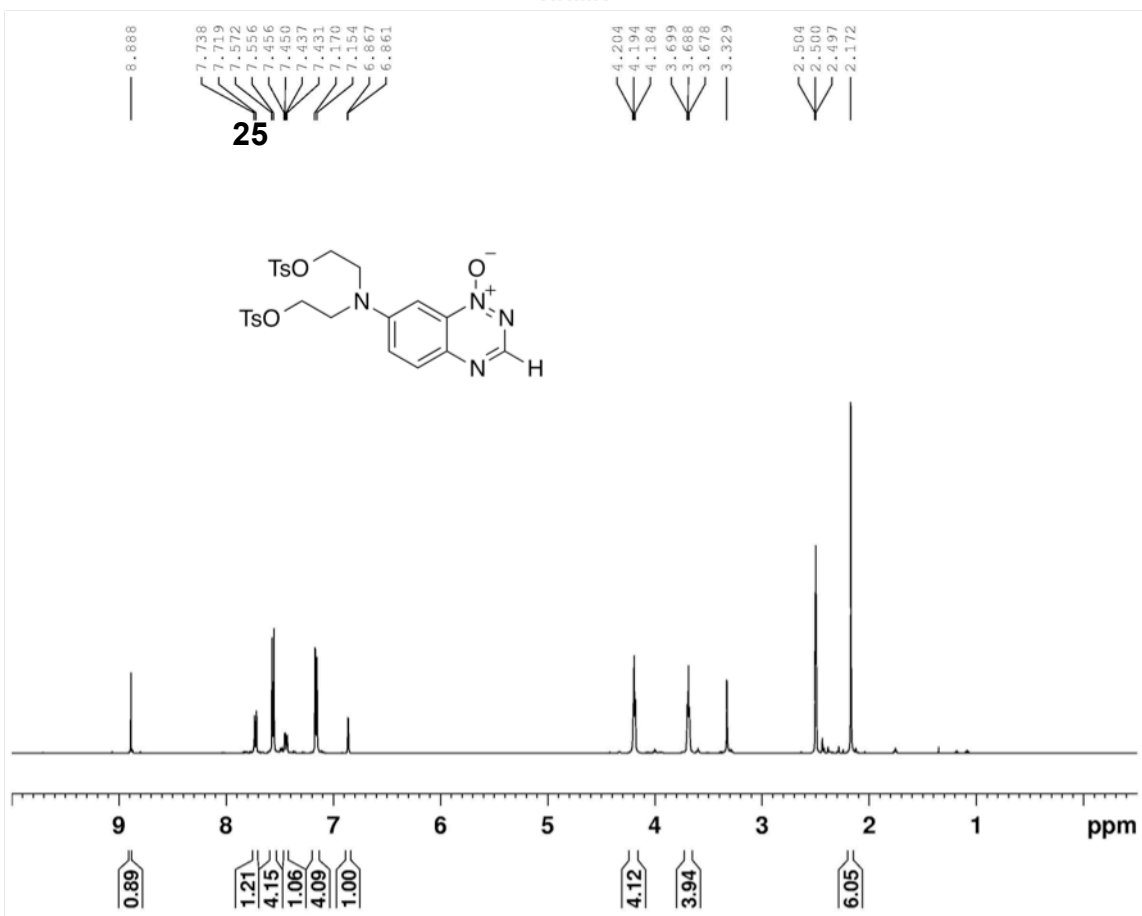


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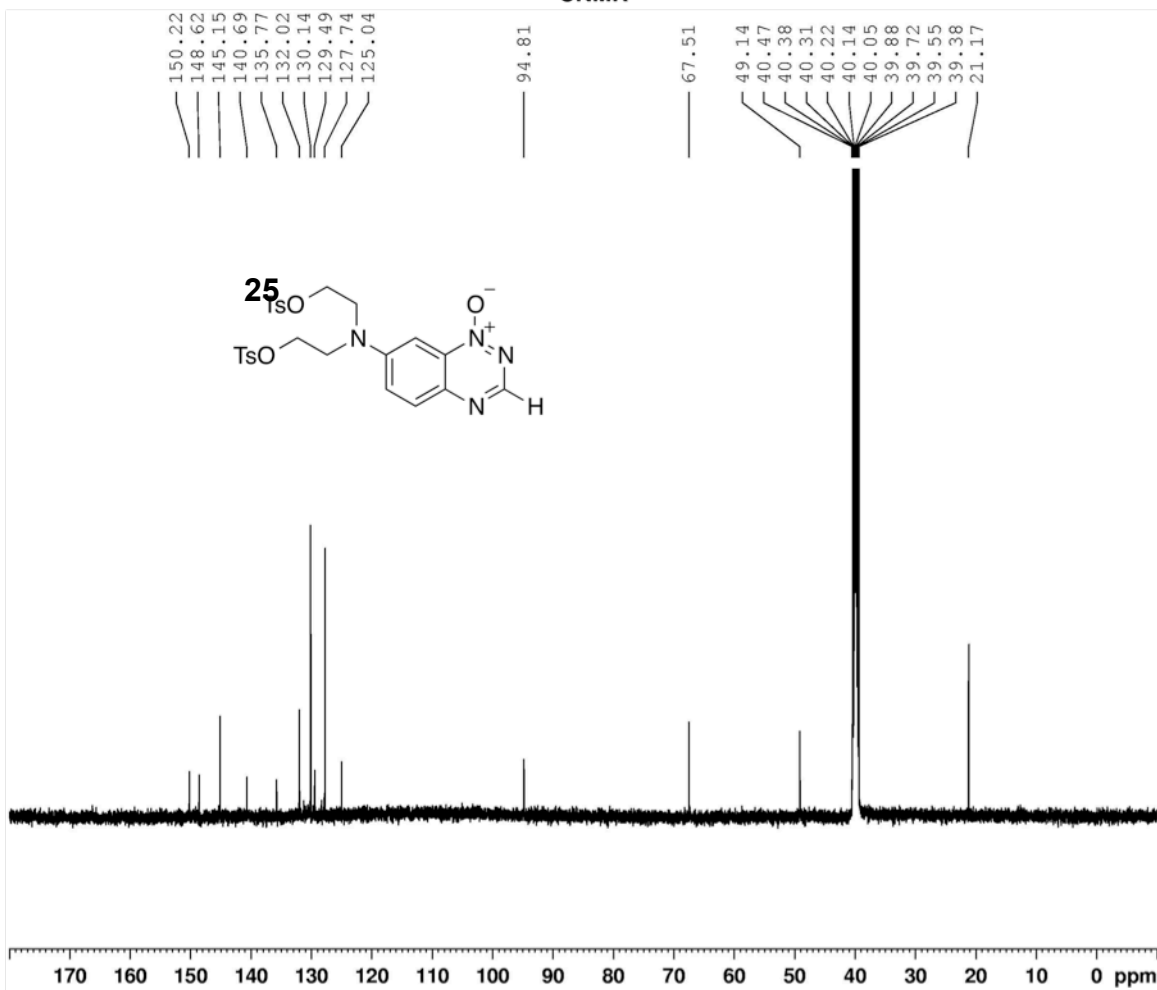




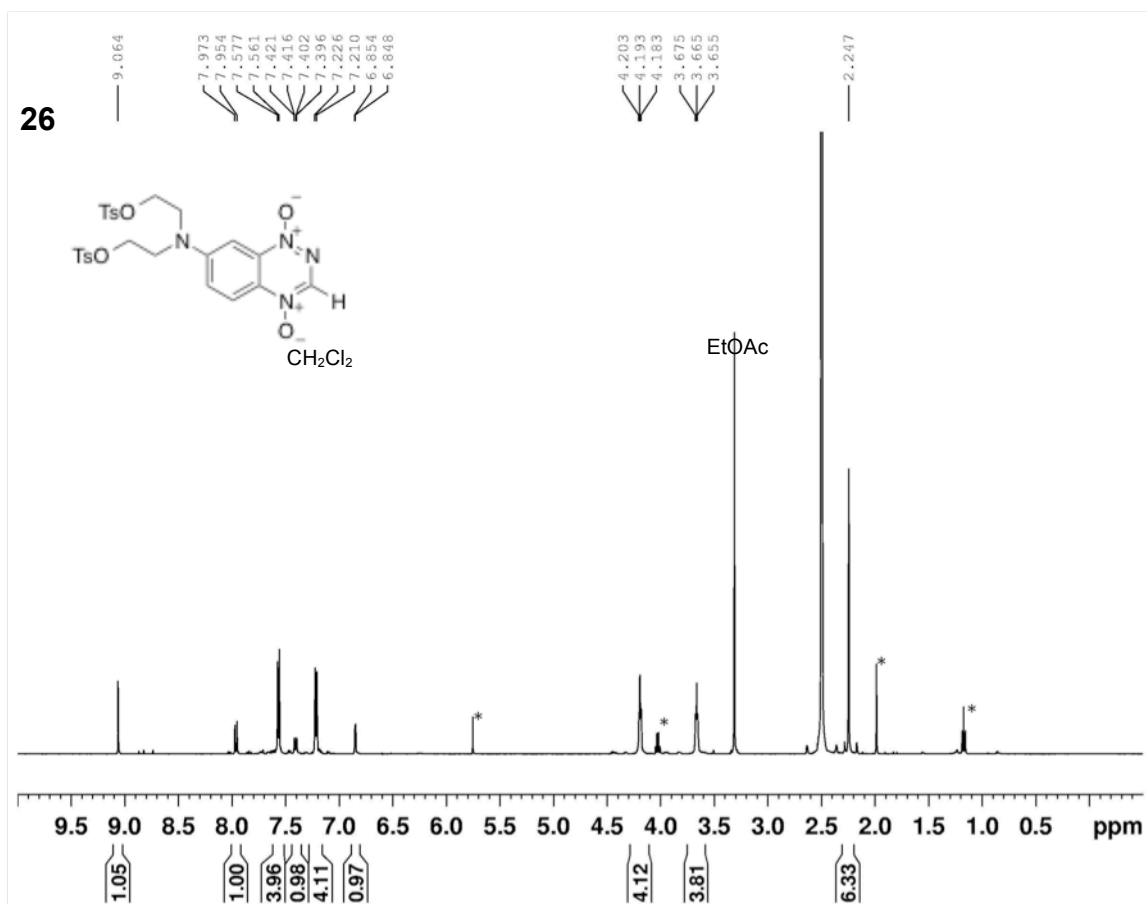
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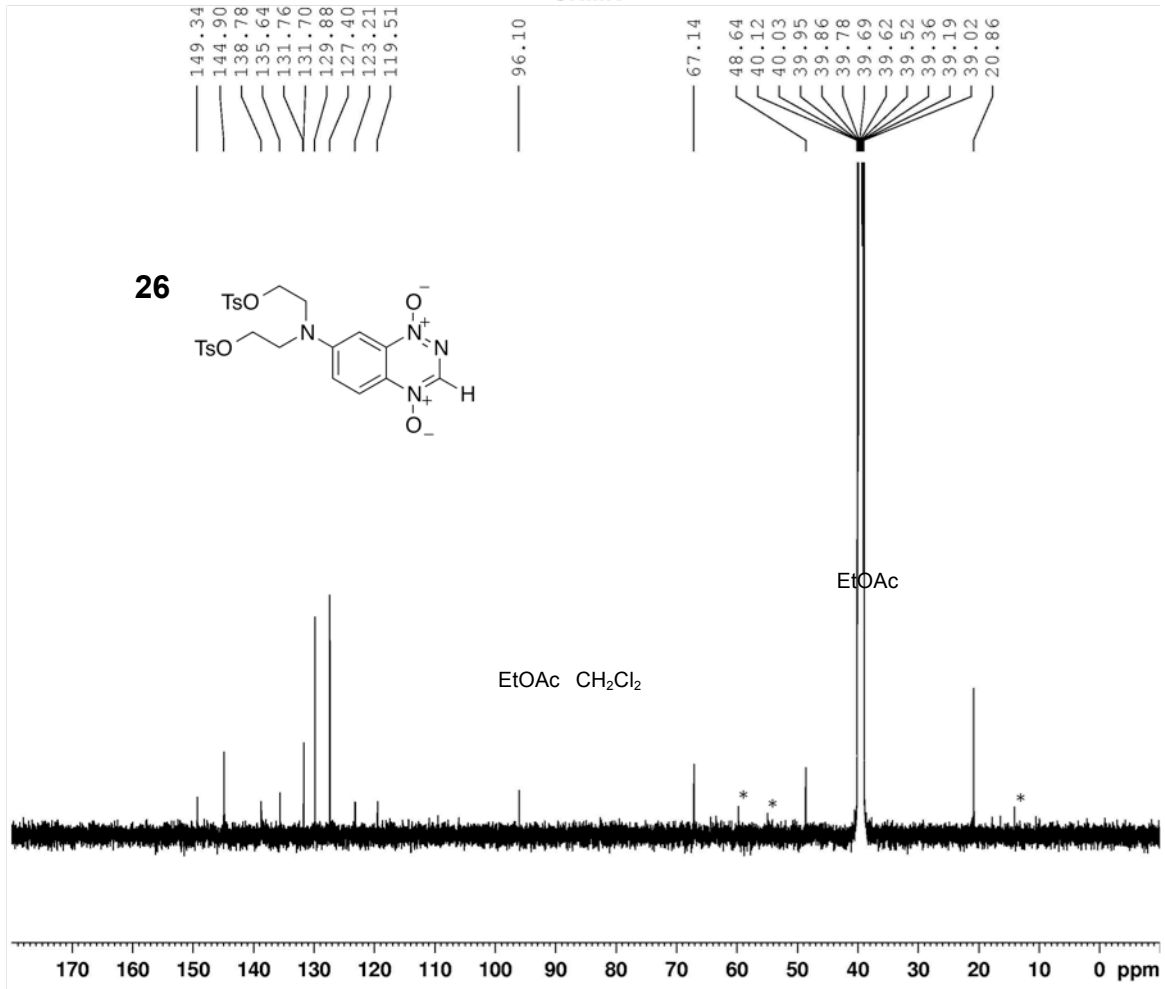
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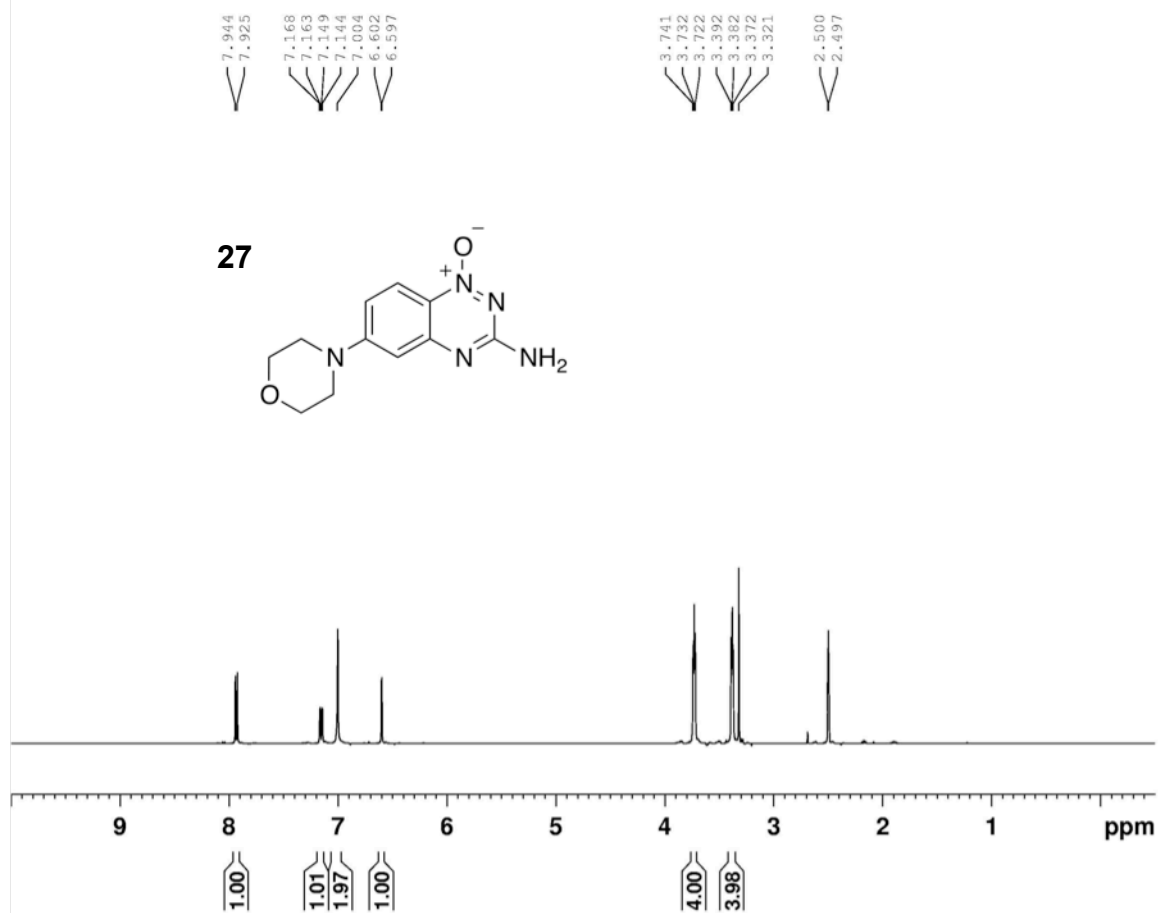
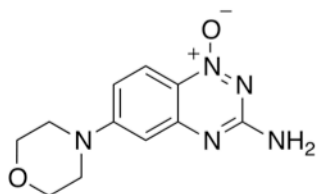


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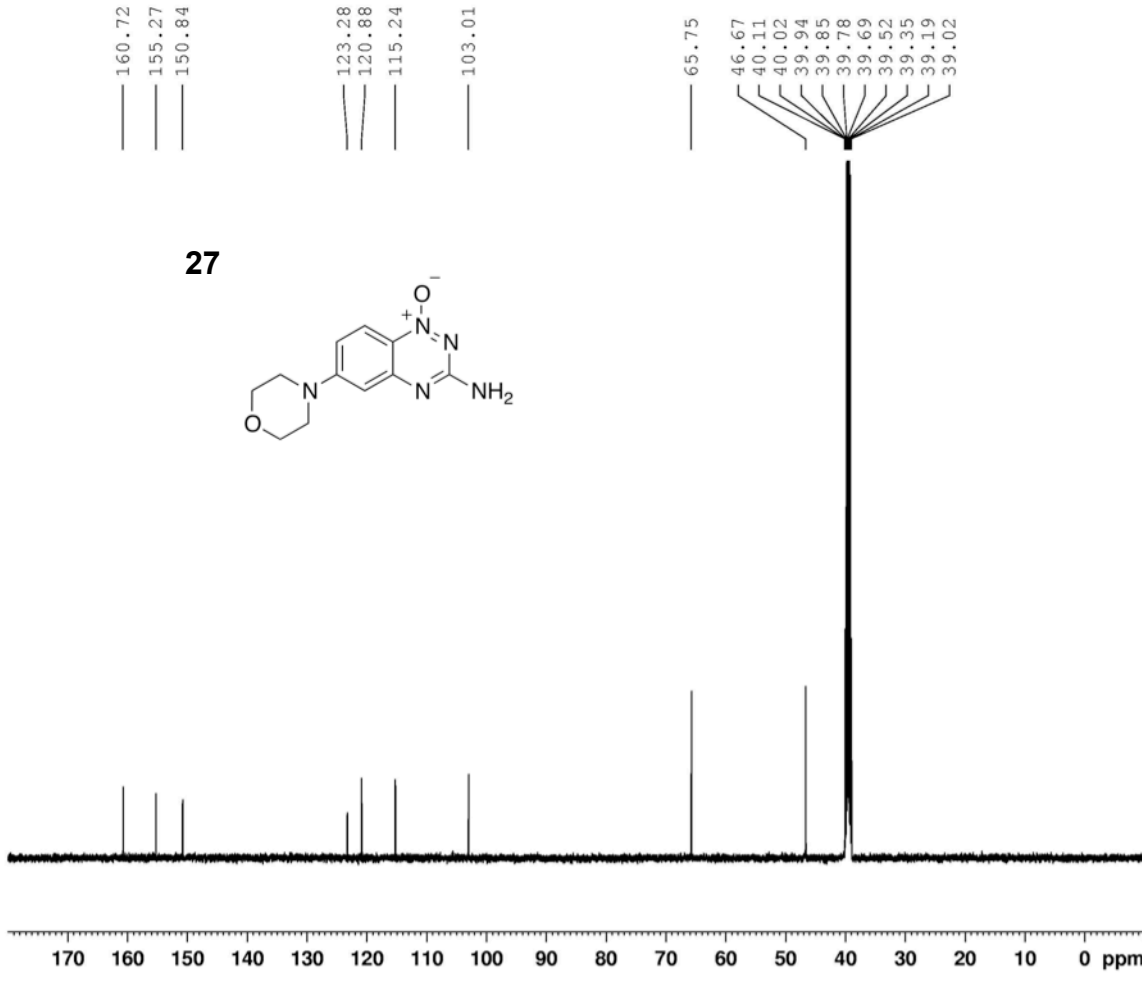


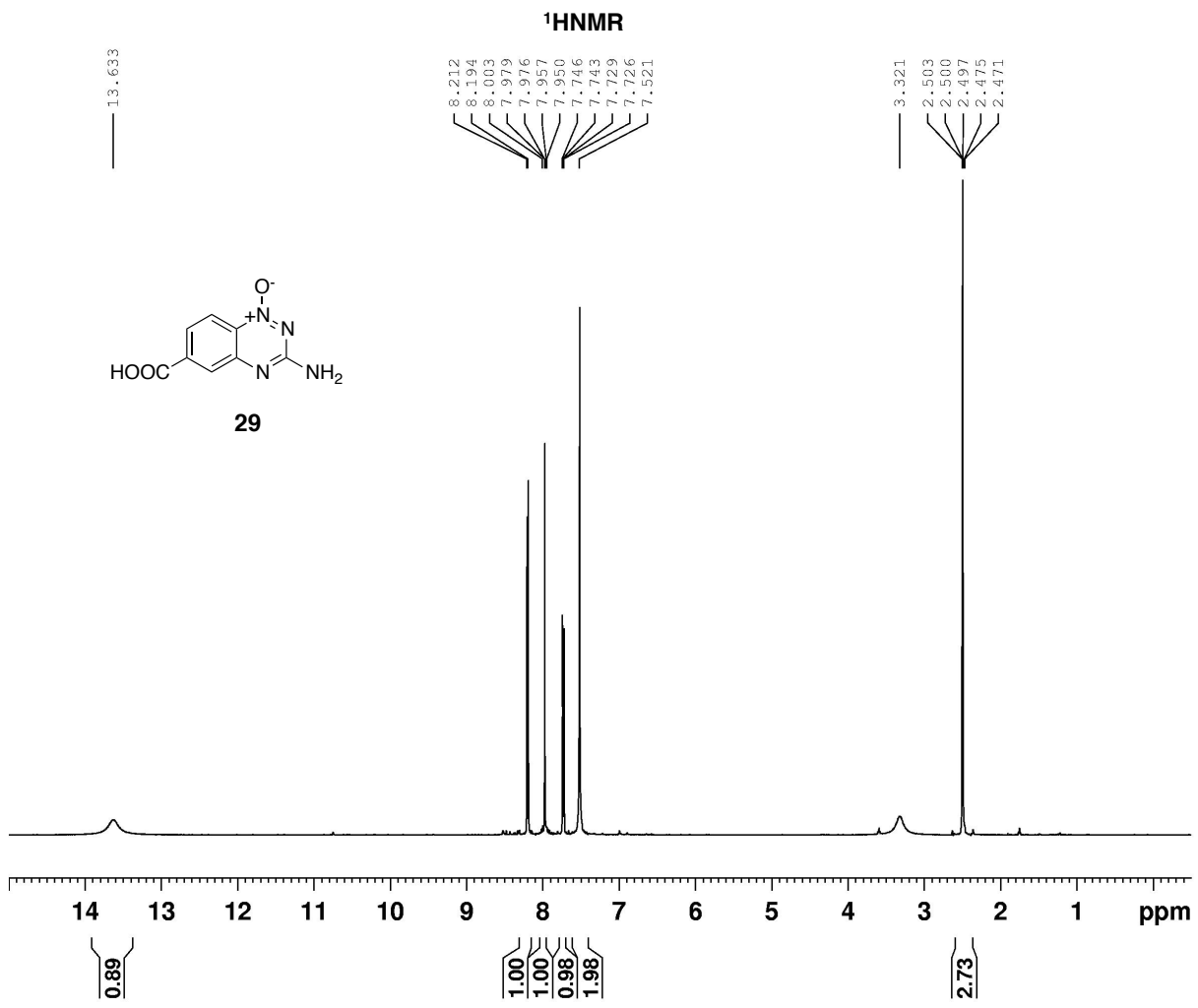
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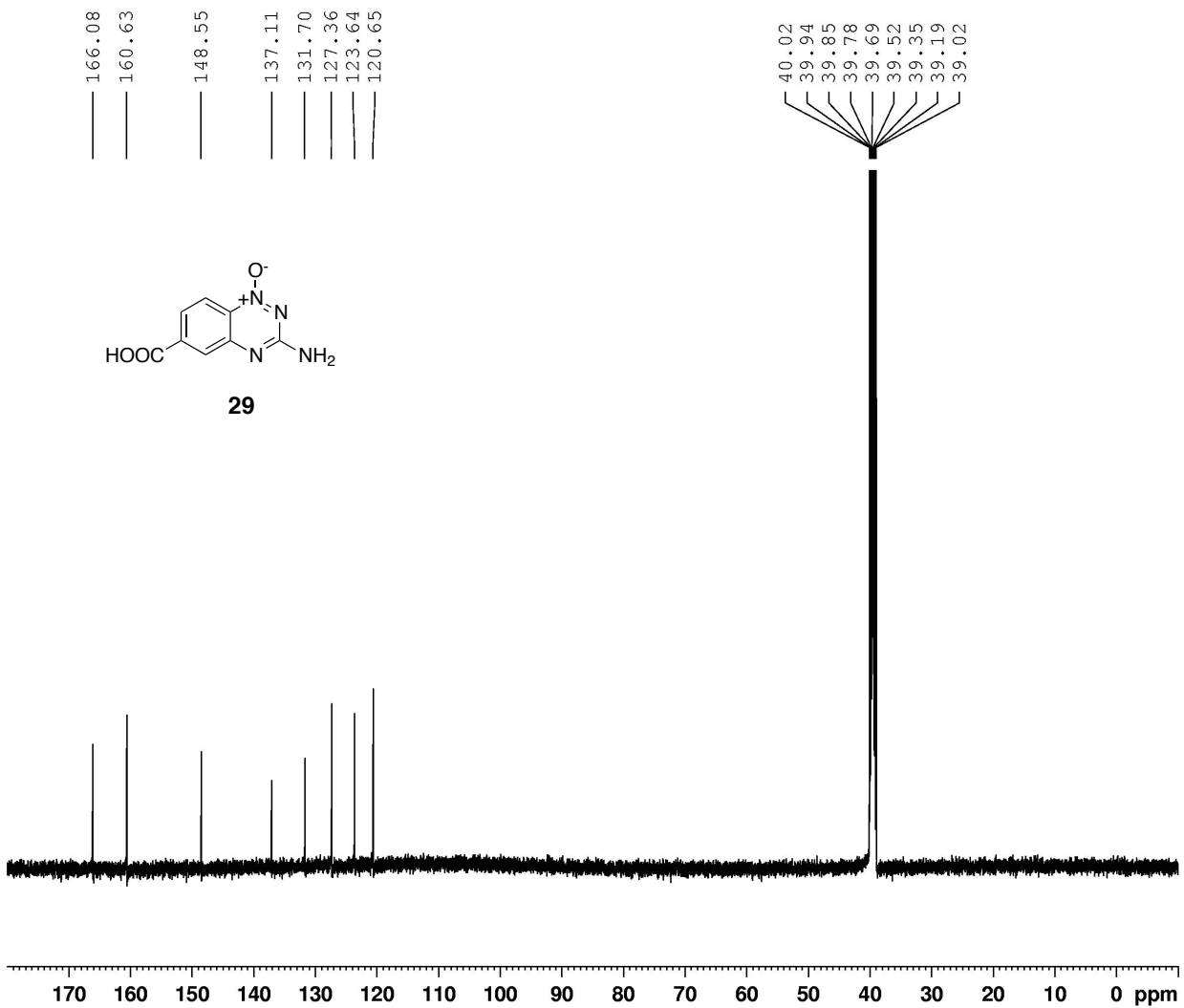
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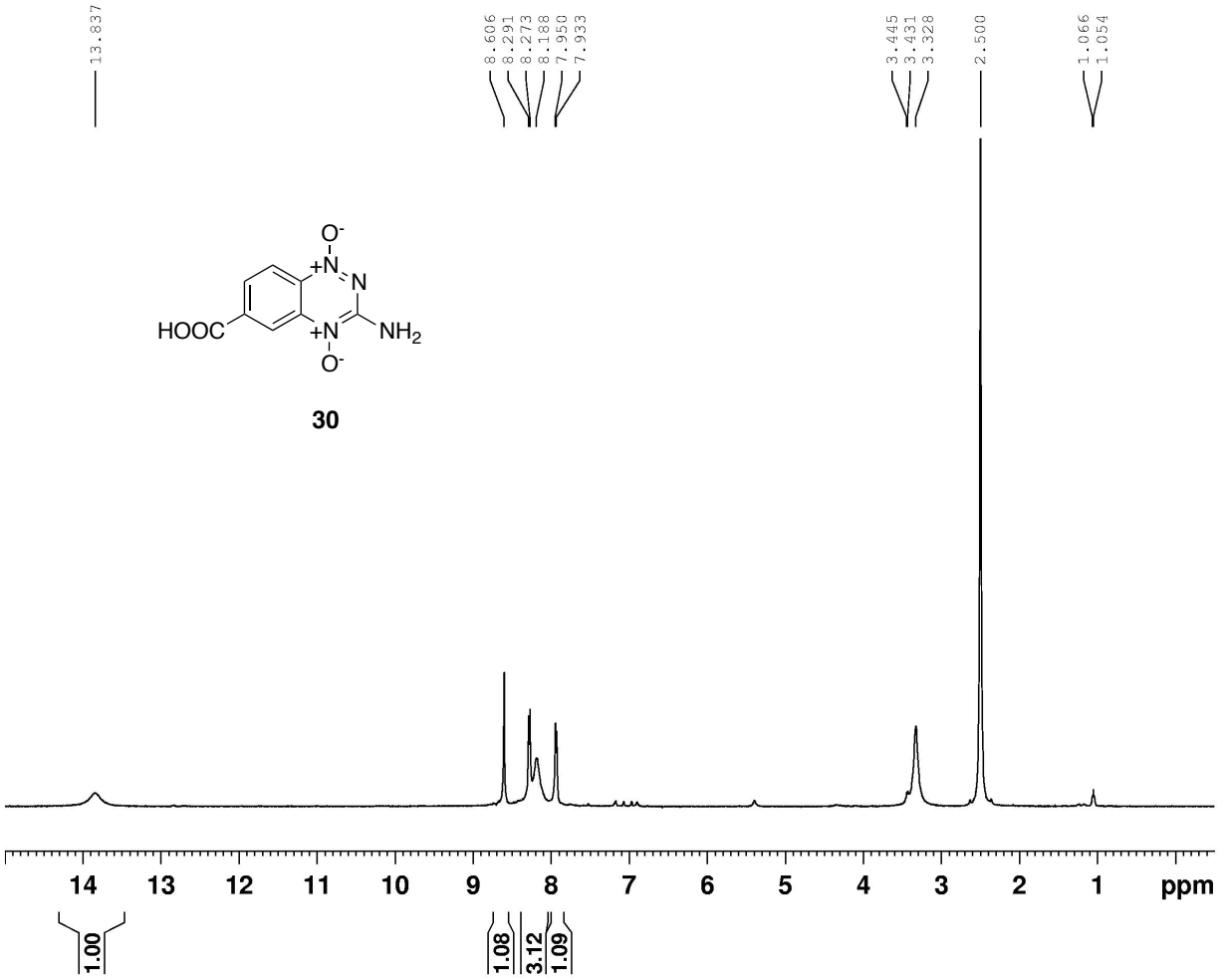
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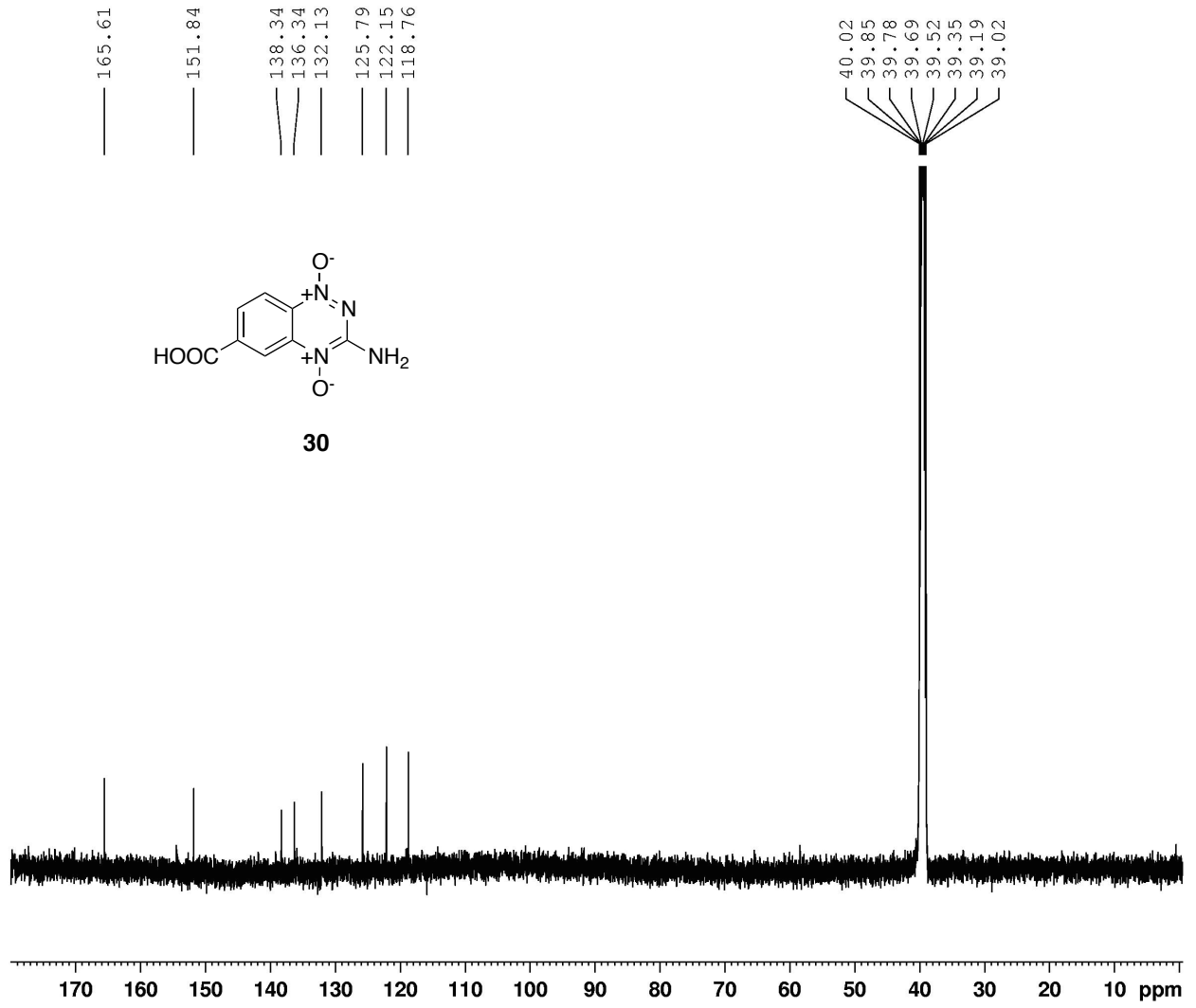


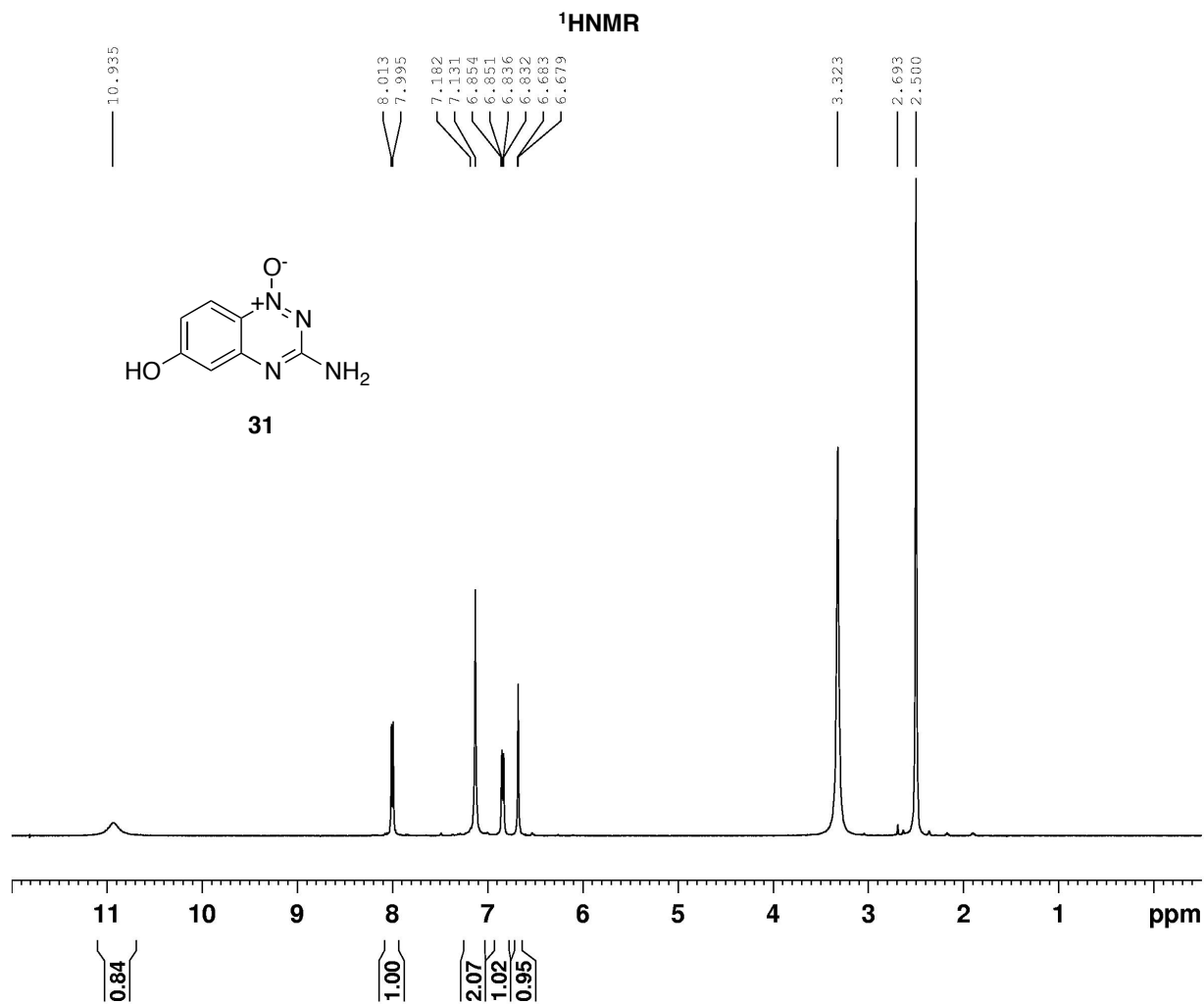


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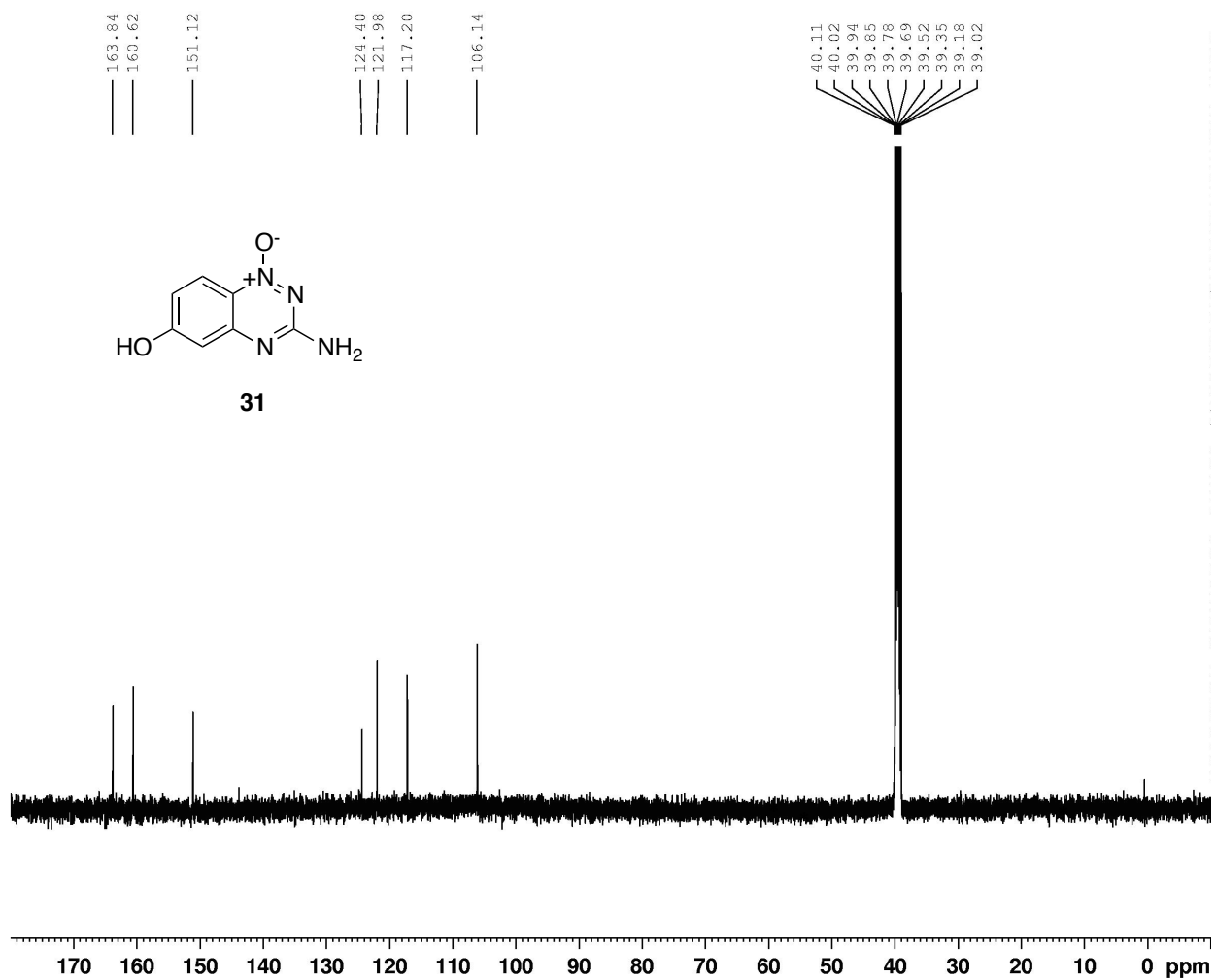


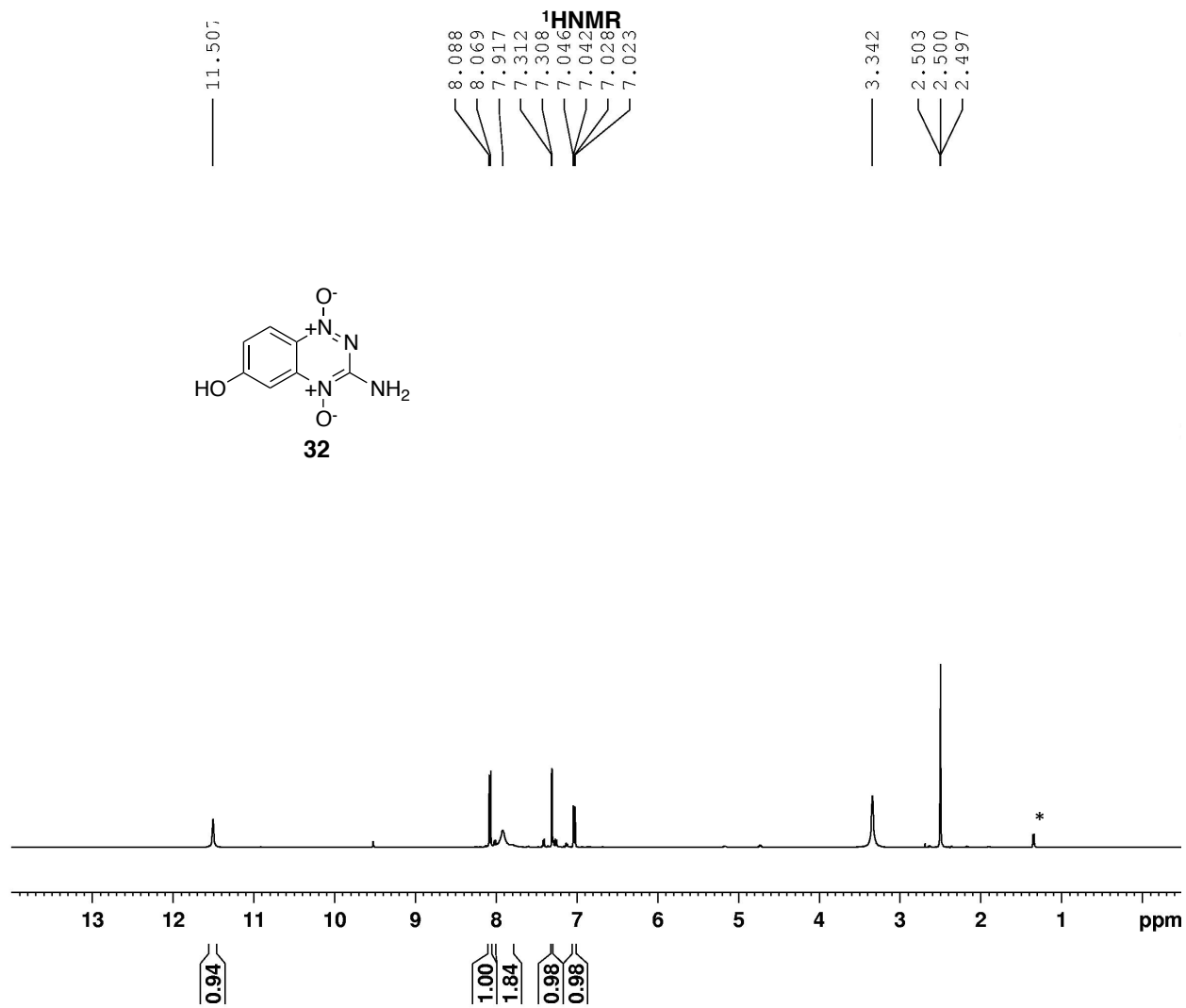
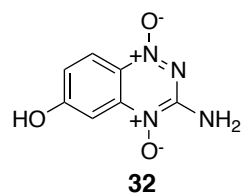
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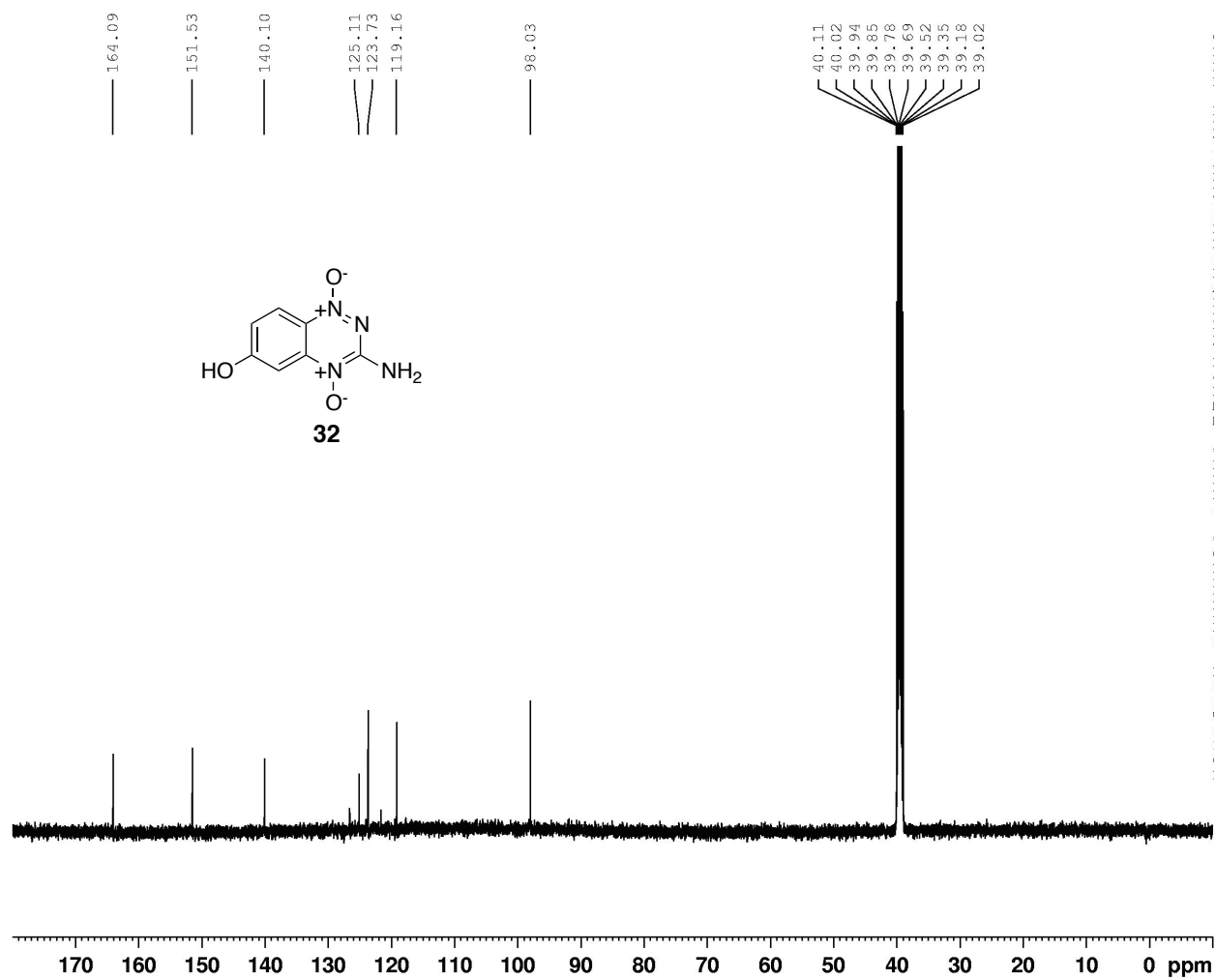


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¹³CNMR



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