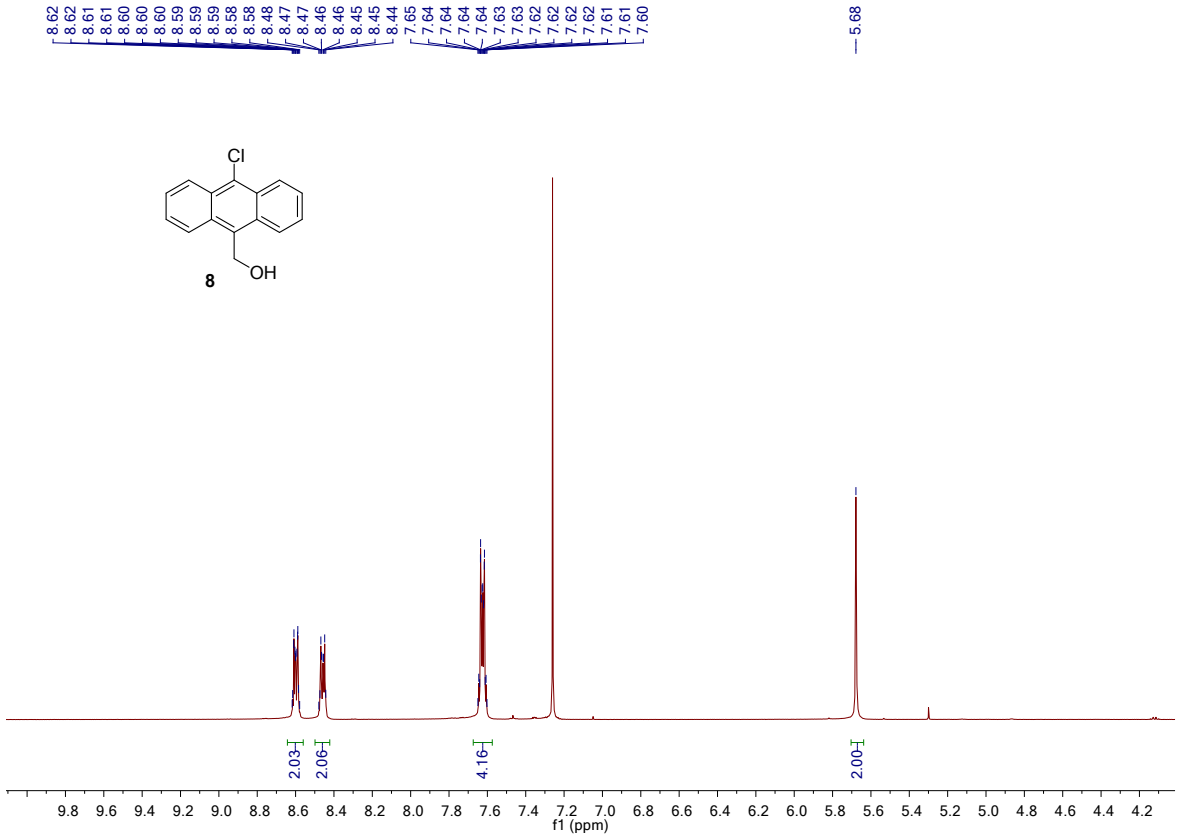
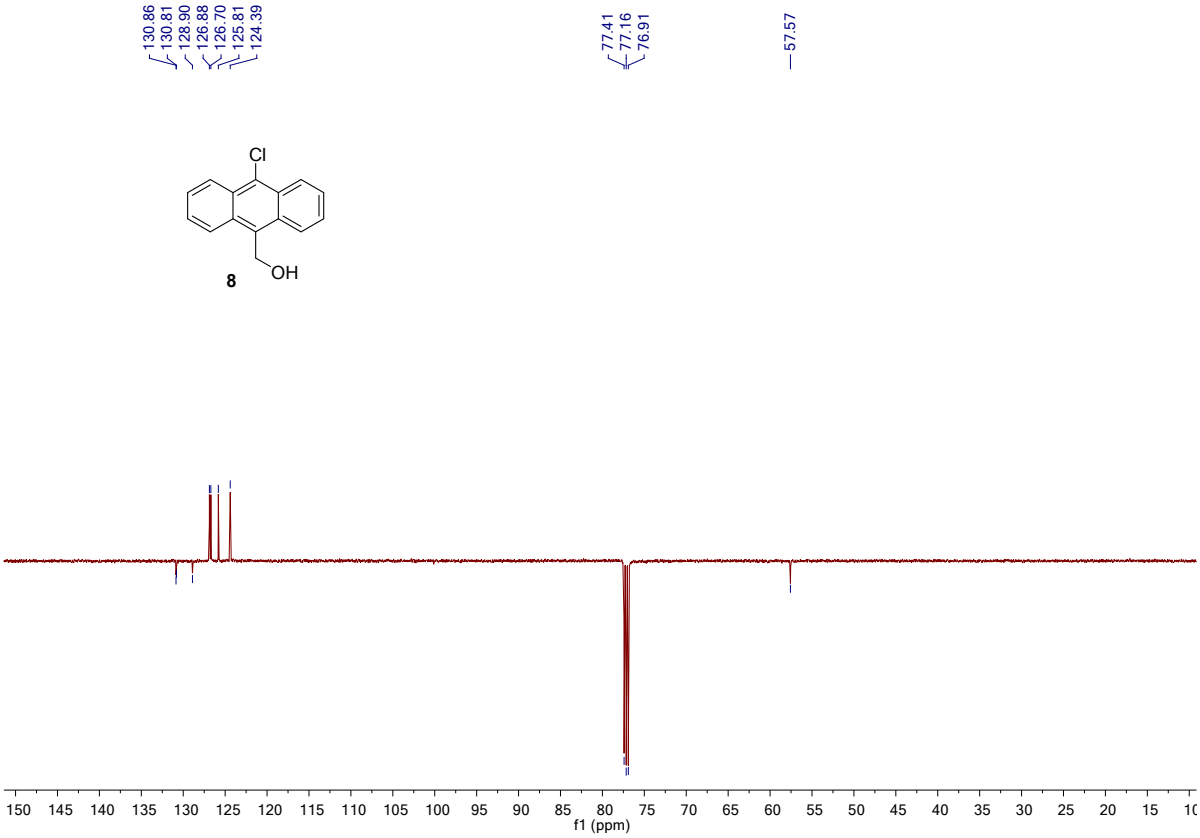
Supporting information for

DETERMINATION OF PROTONATION STATES OF IMINOSUGAR-ENZYME COMPLEXES USING PHOTOINDUCED ELECTRO TRANSFER

Bo Wang, Jacob Ingemar Olsen, Bo Wegge Laursen, Jens Christian Navarro Poulsen and Mikael Bols*

Contents:





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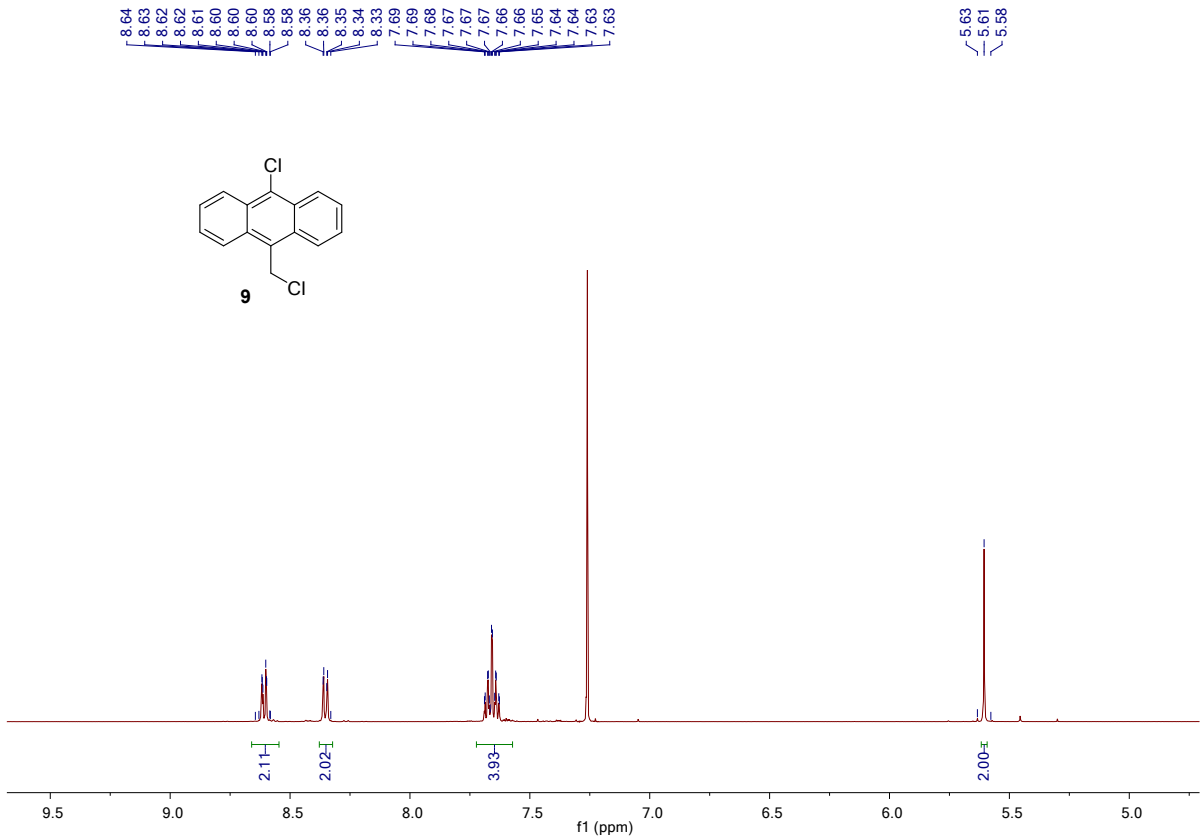

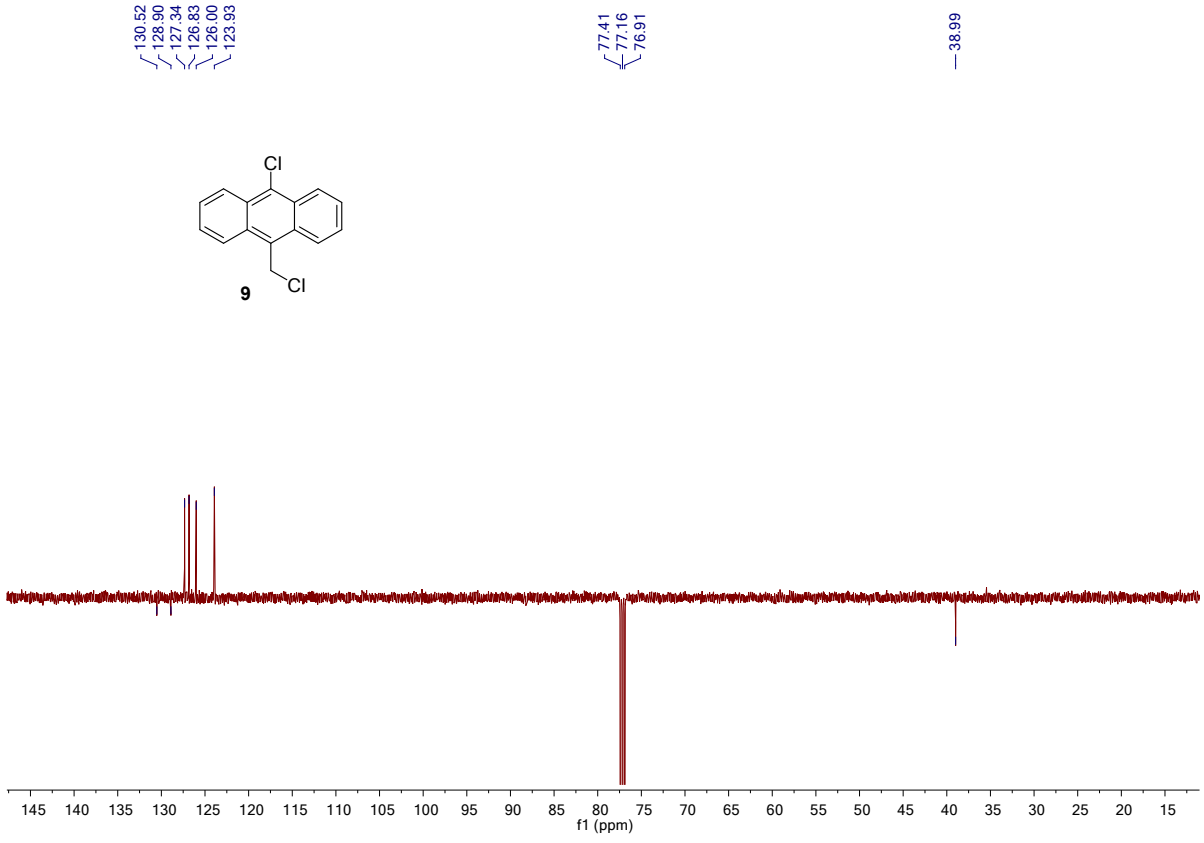

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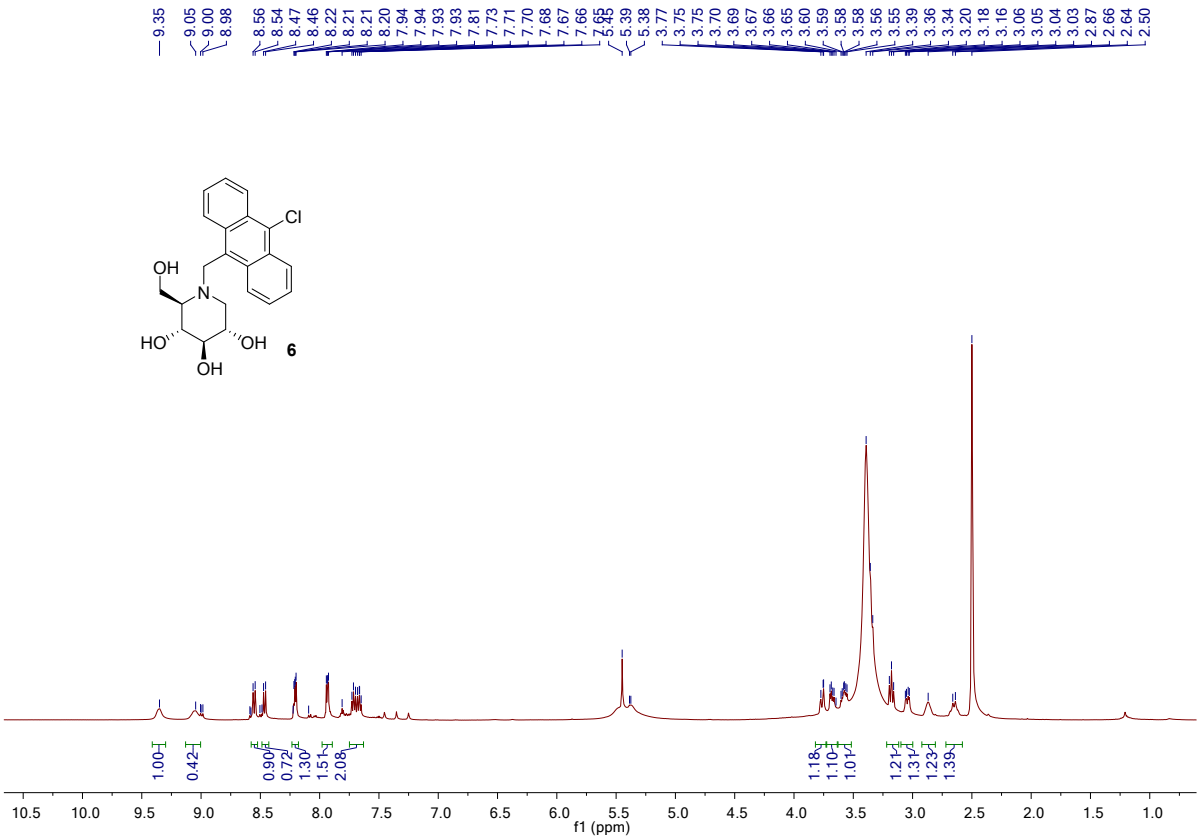

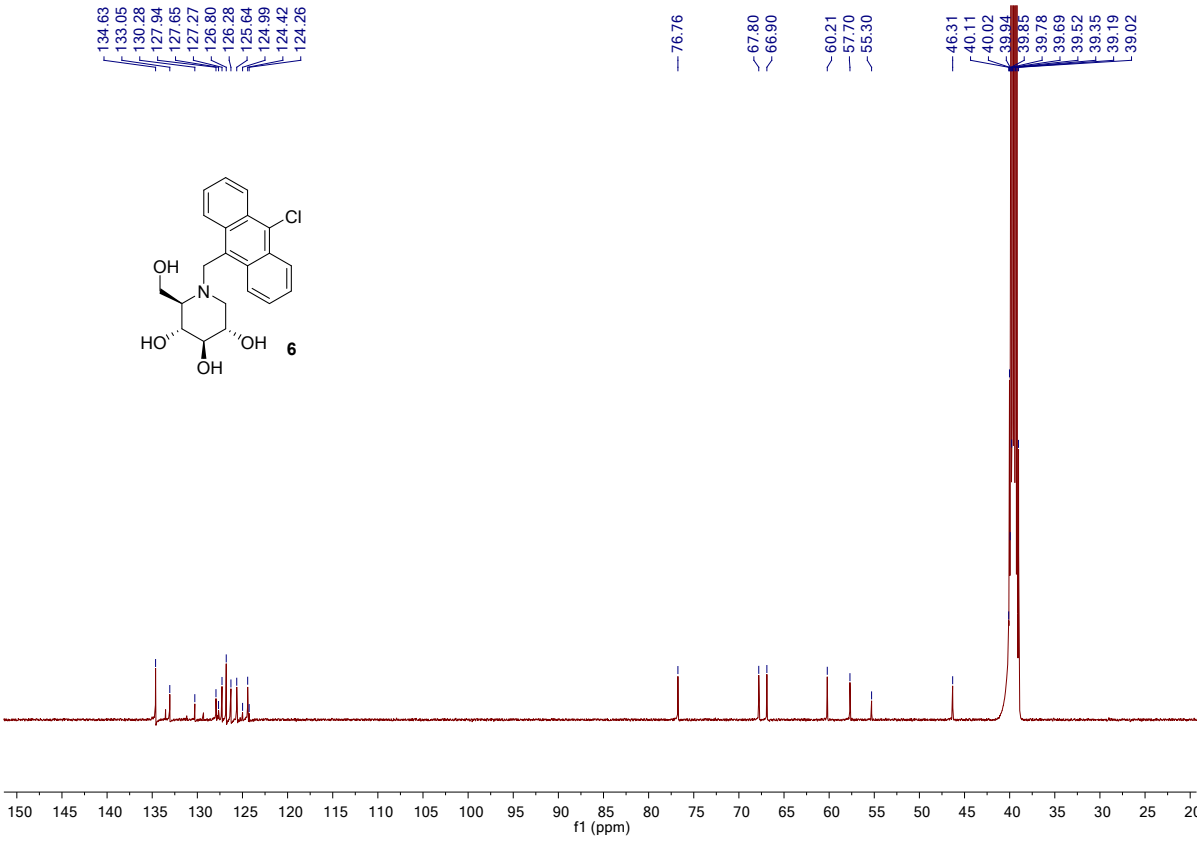

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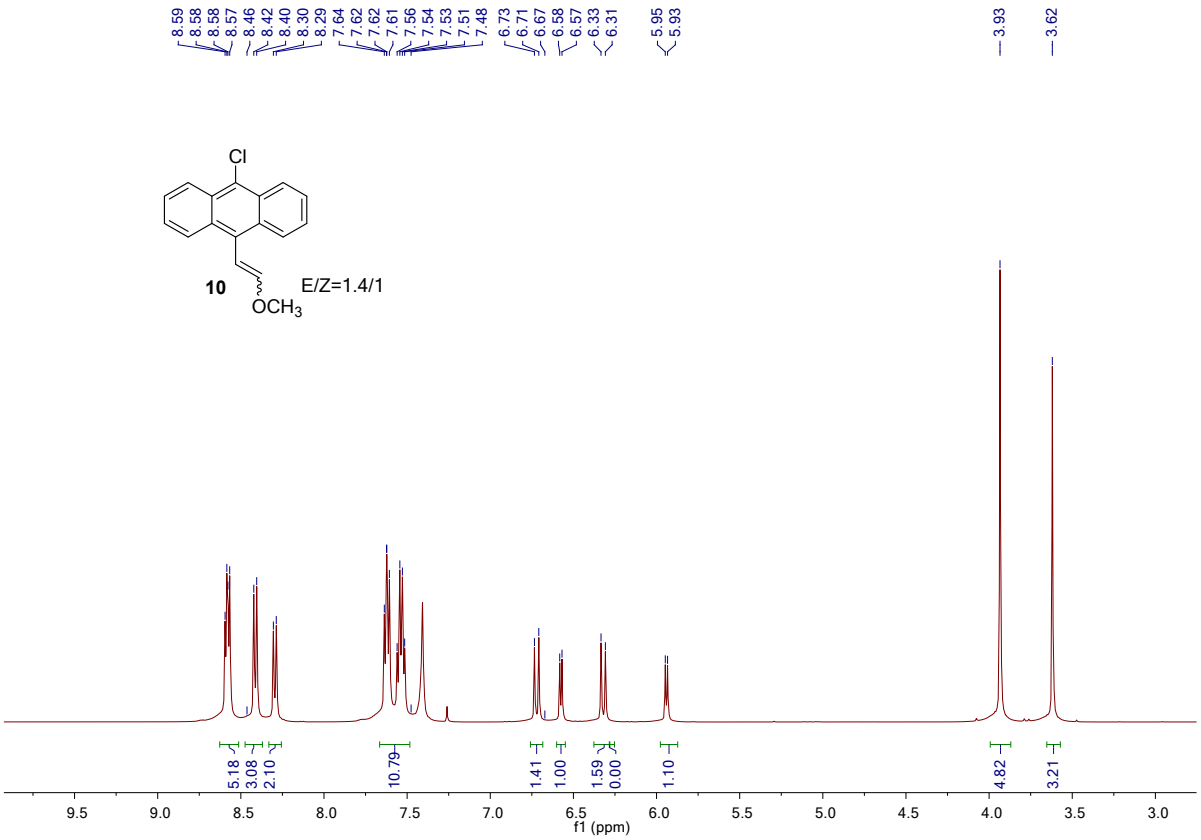

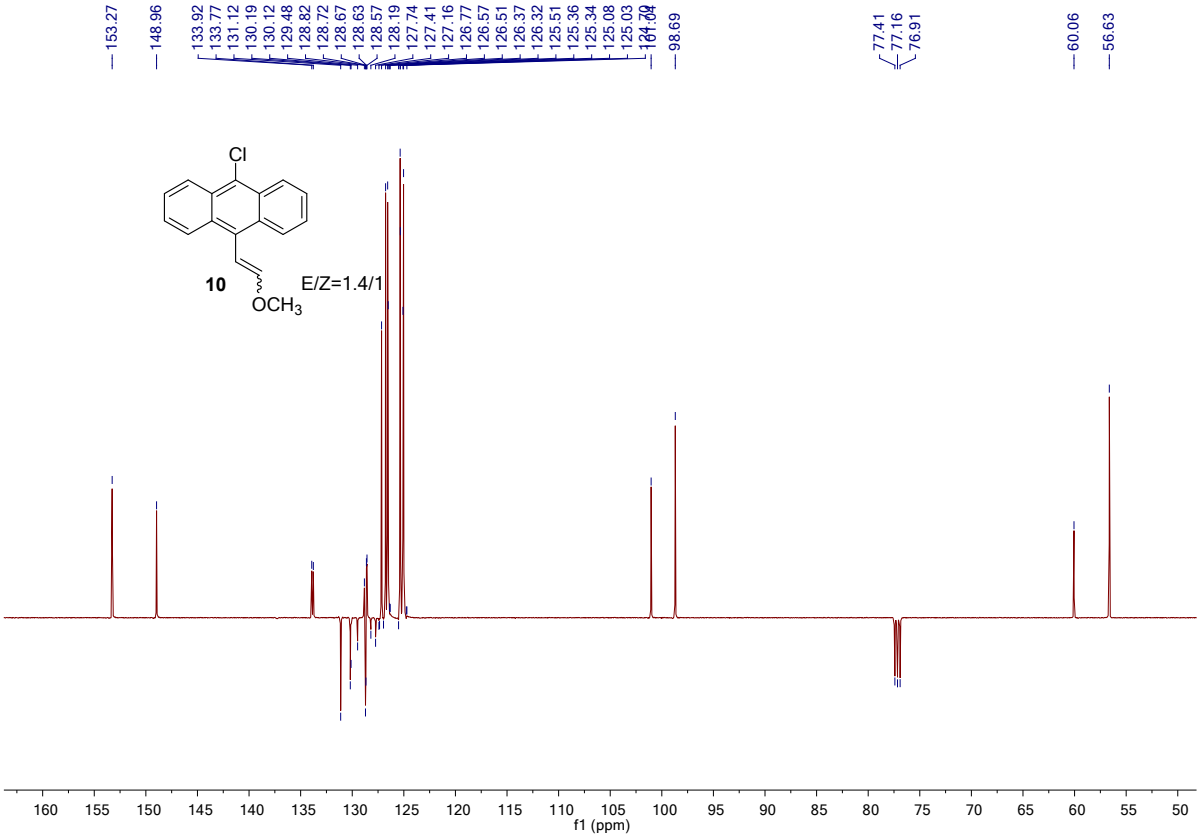

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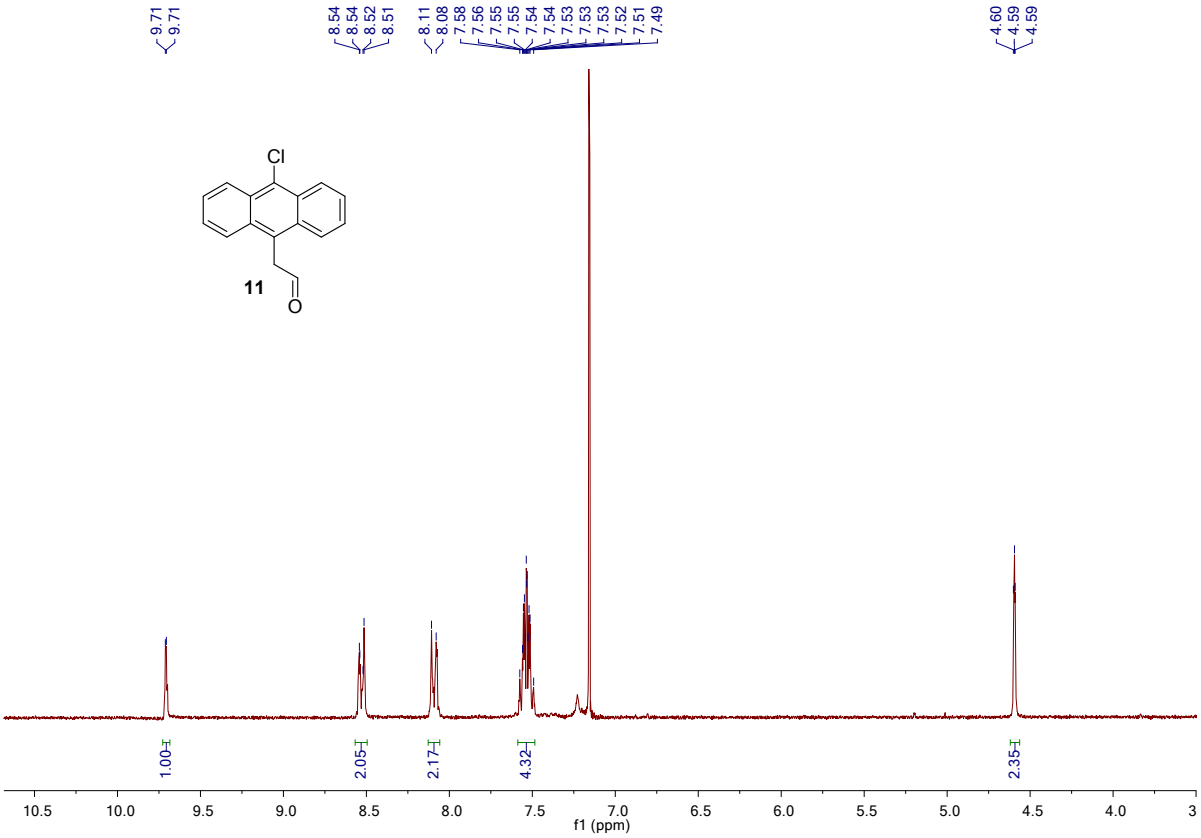

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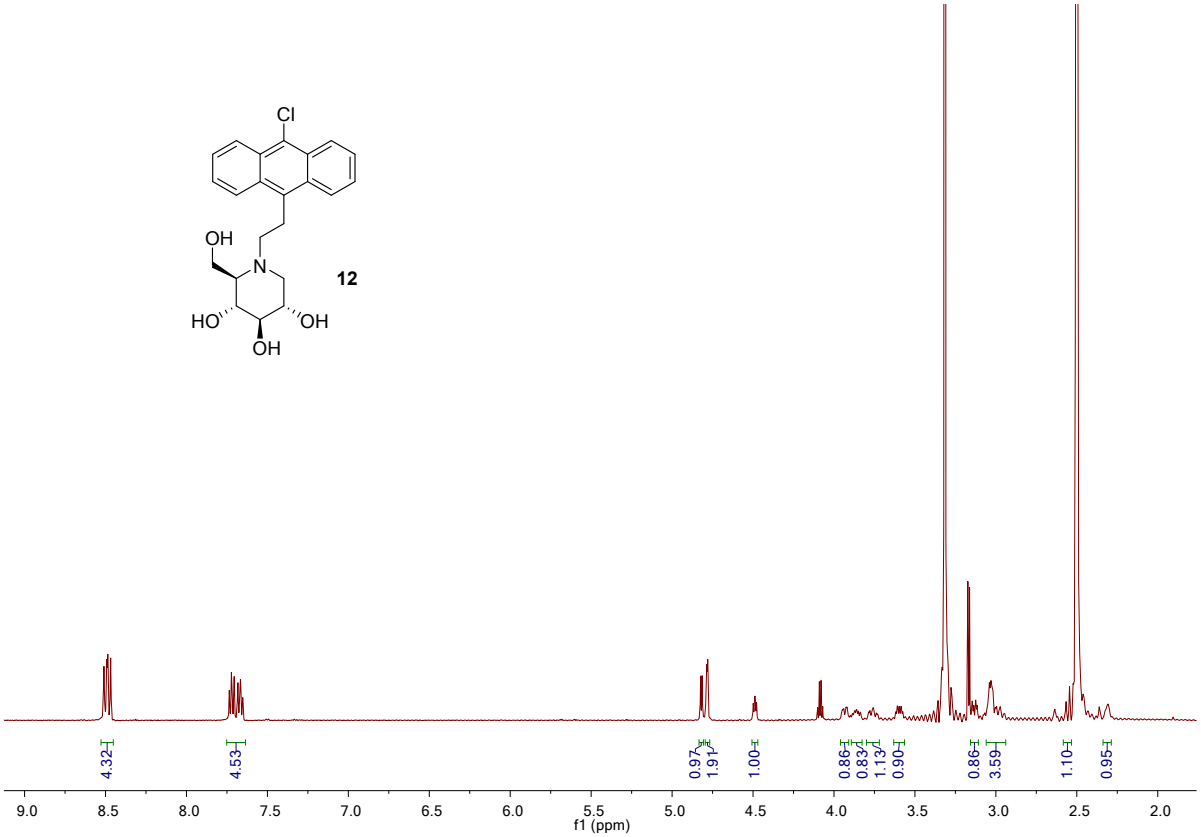

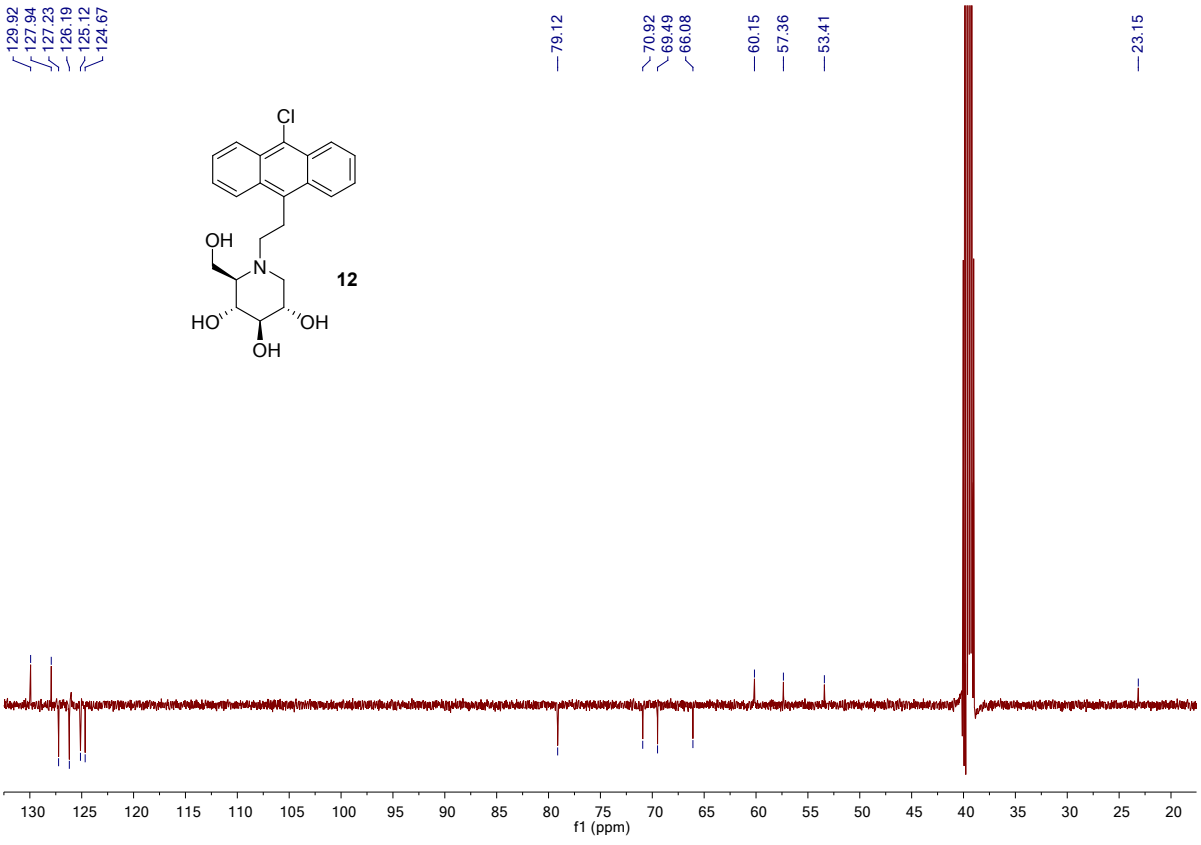

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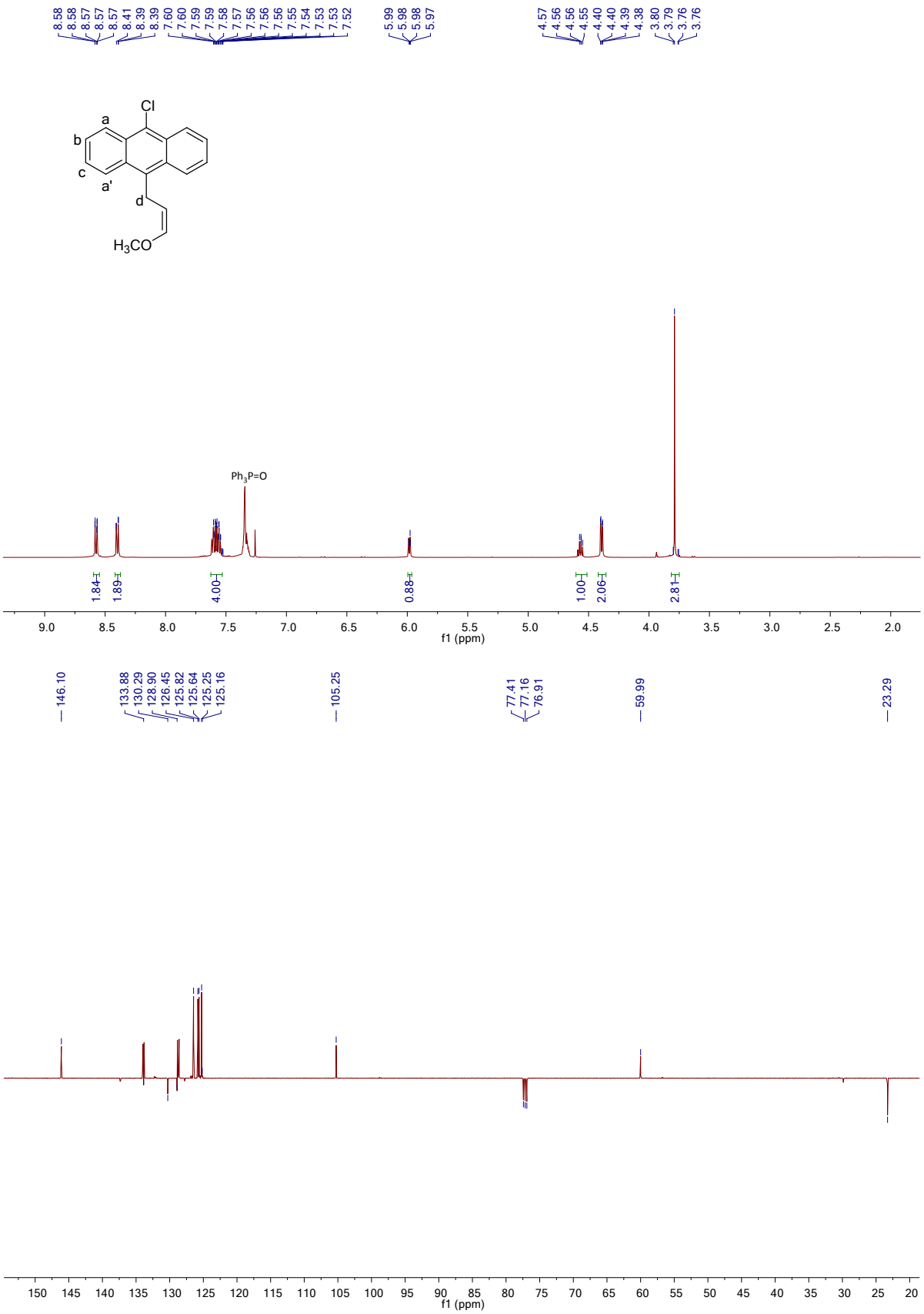

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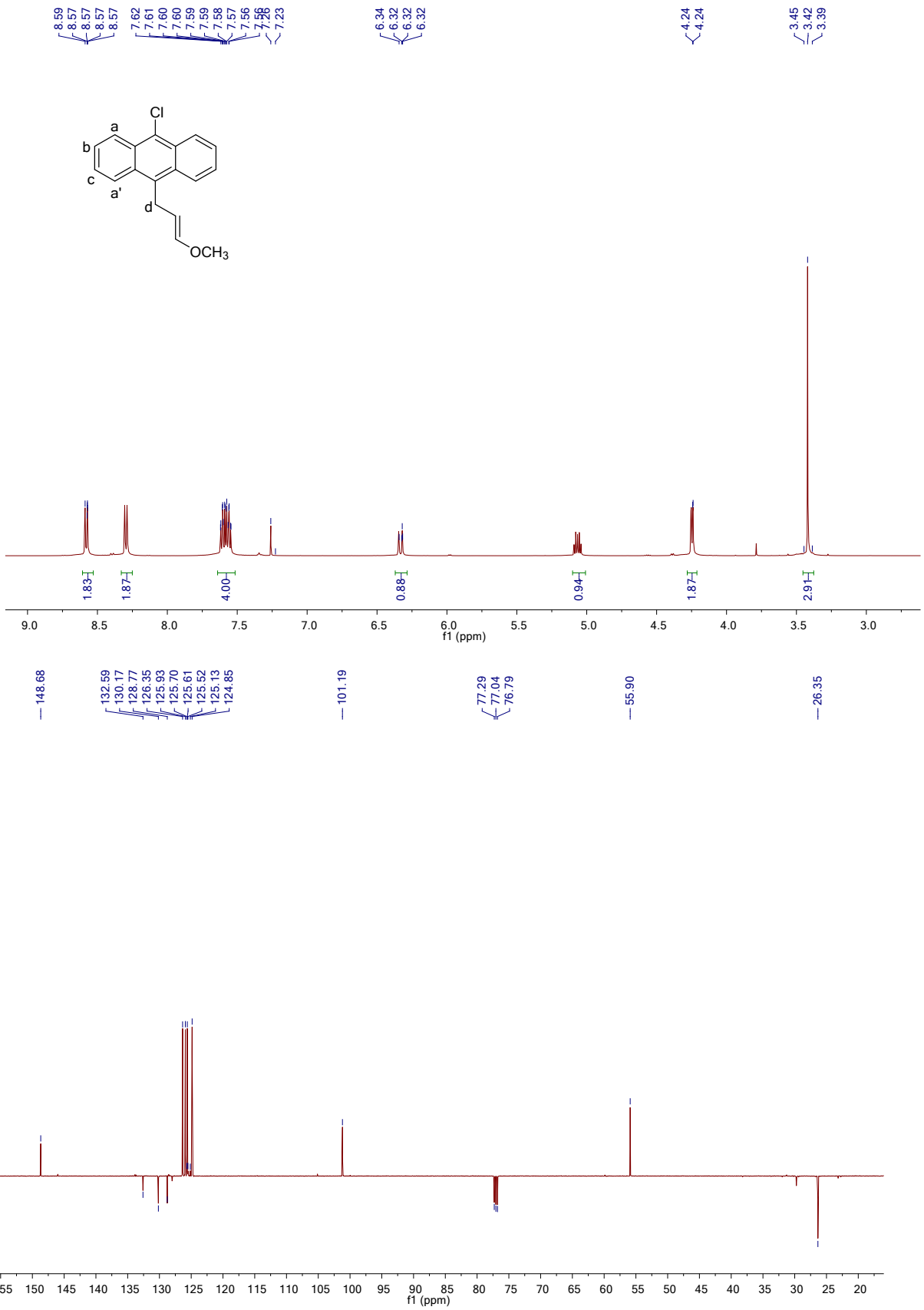

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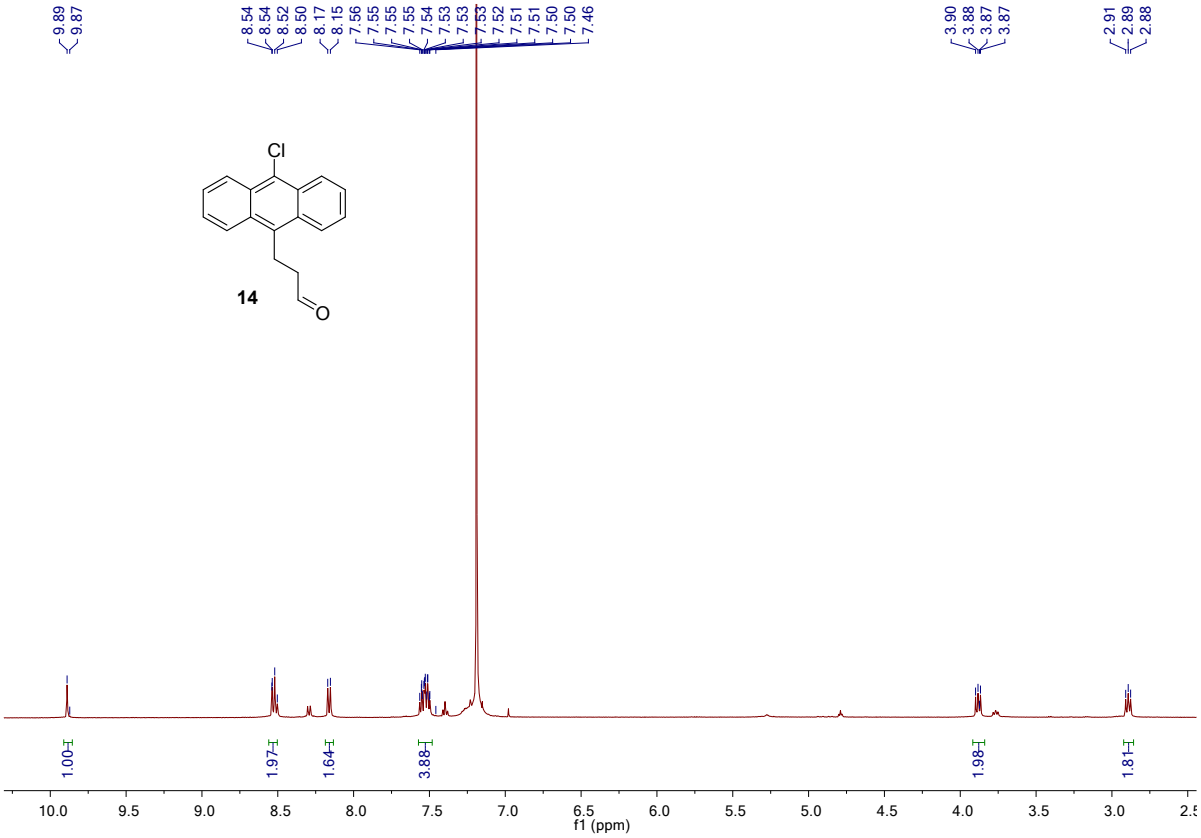

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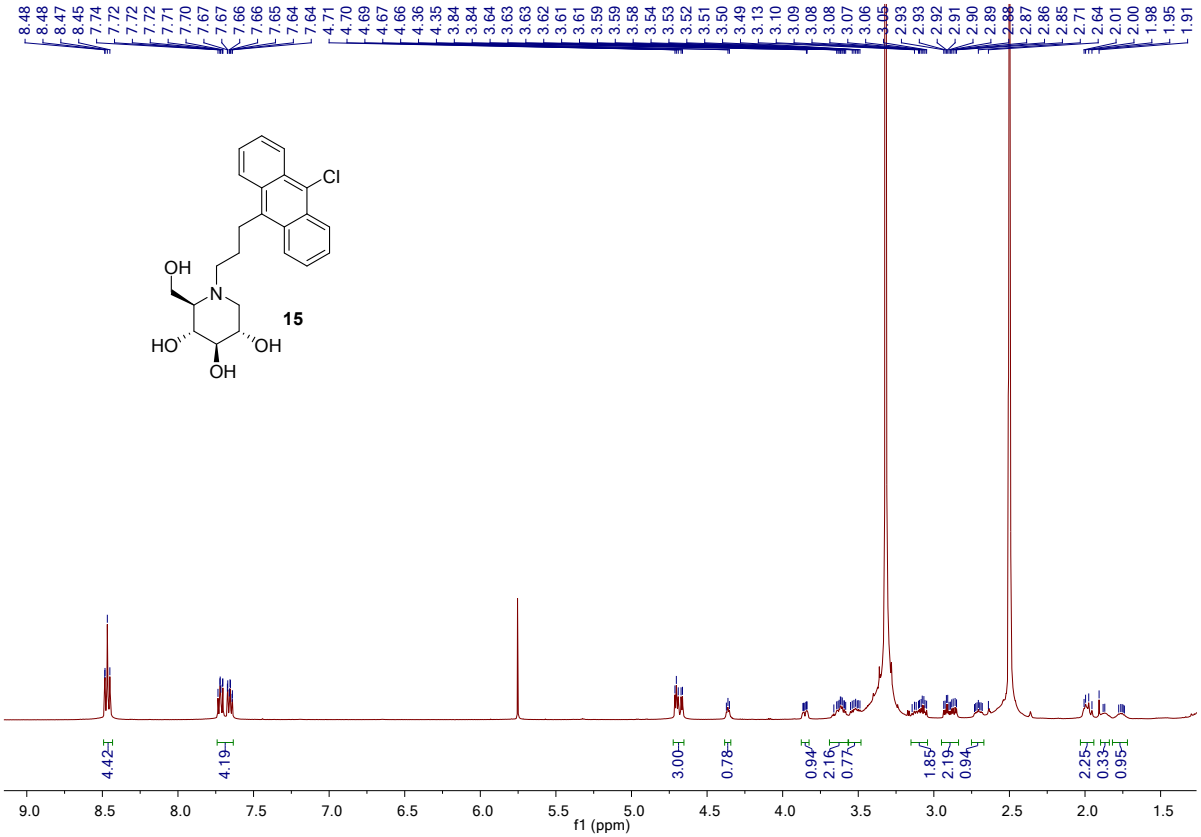

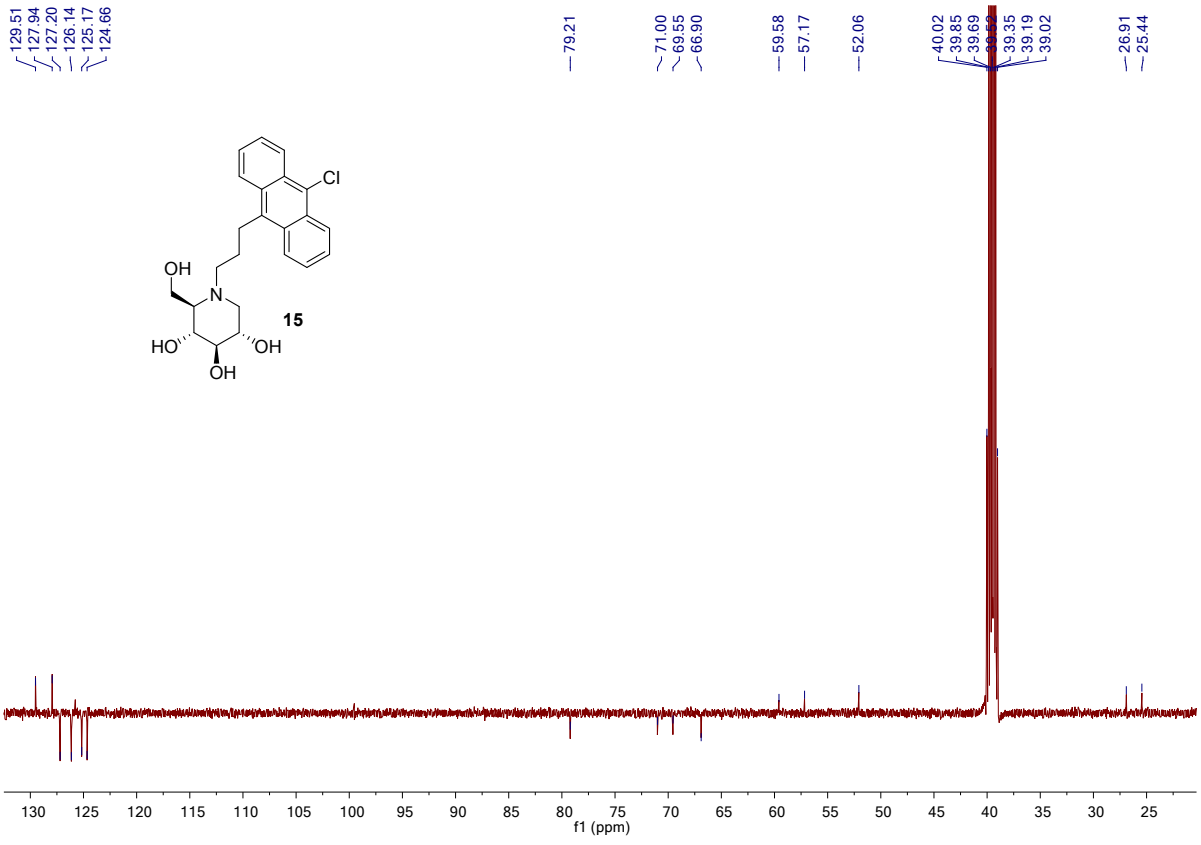

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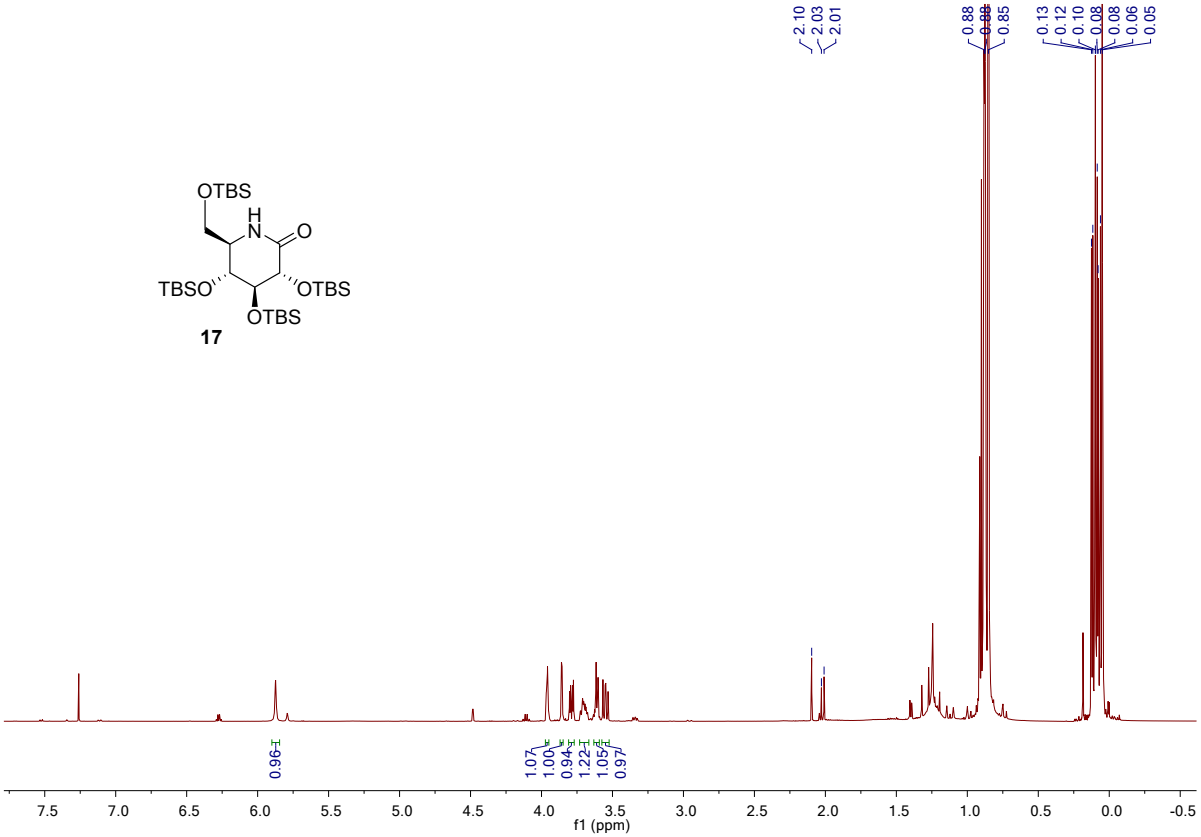

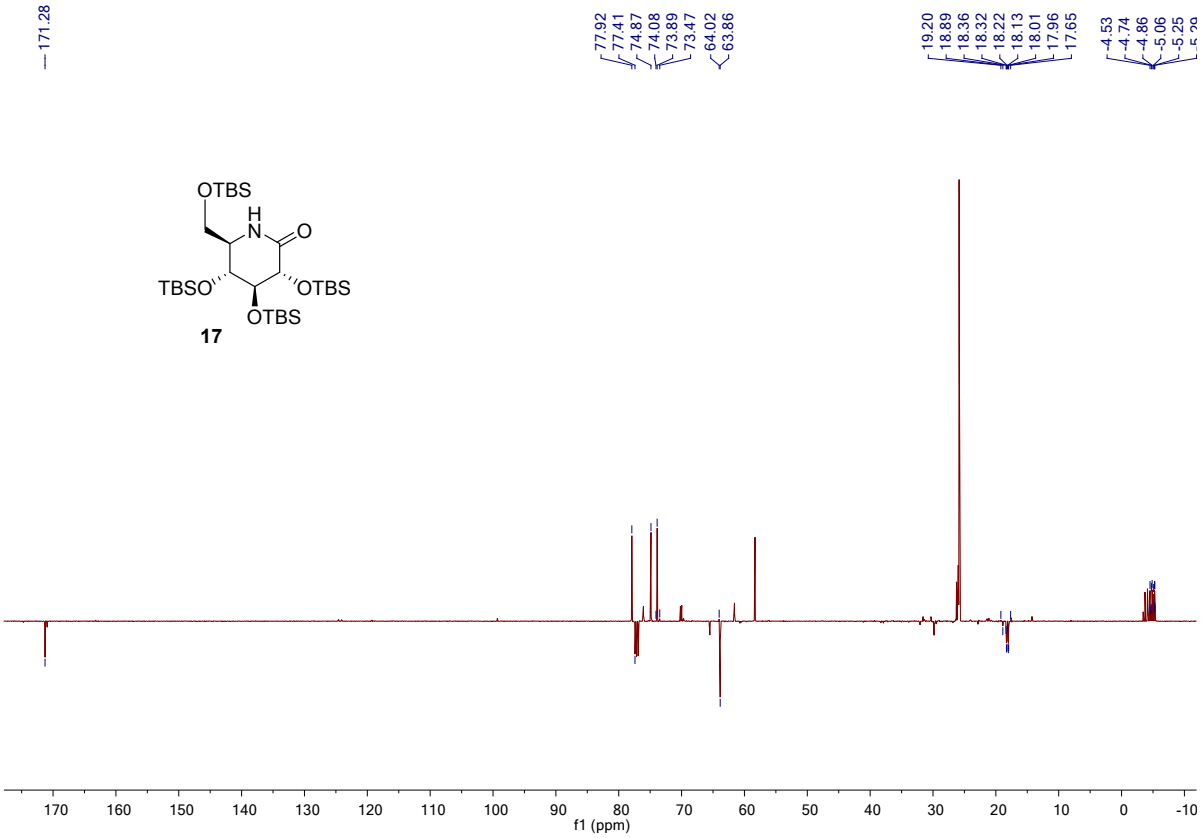

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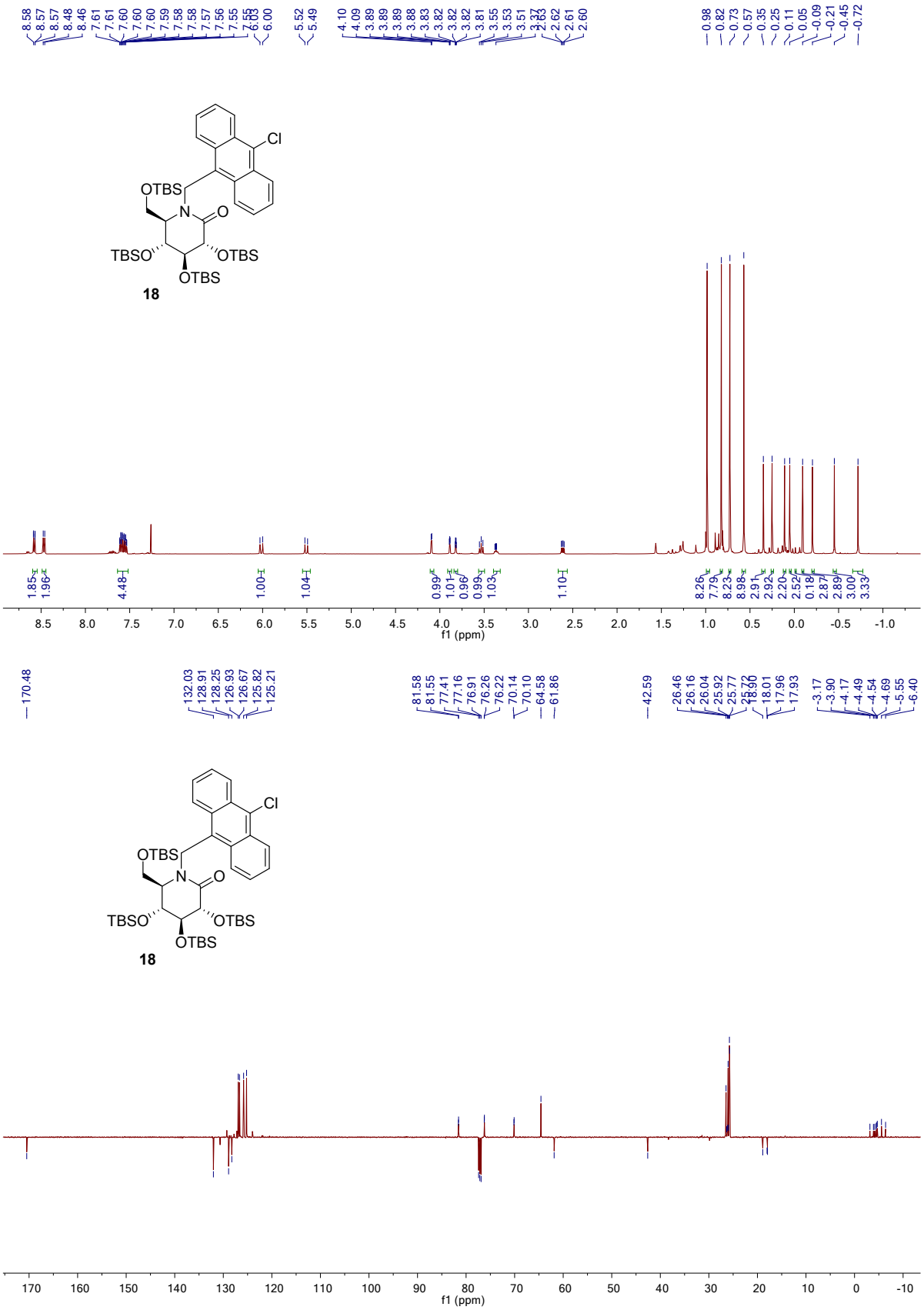

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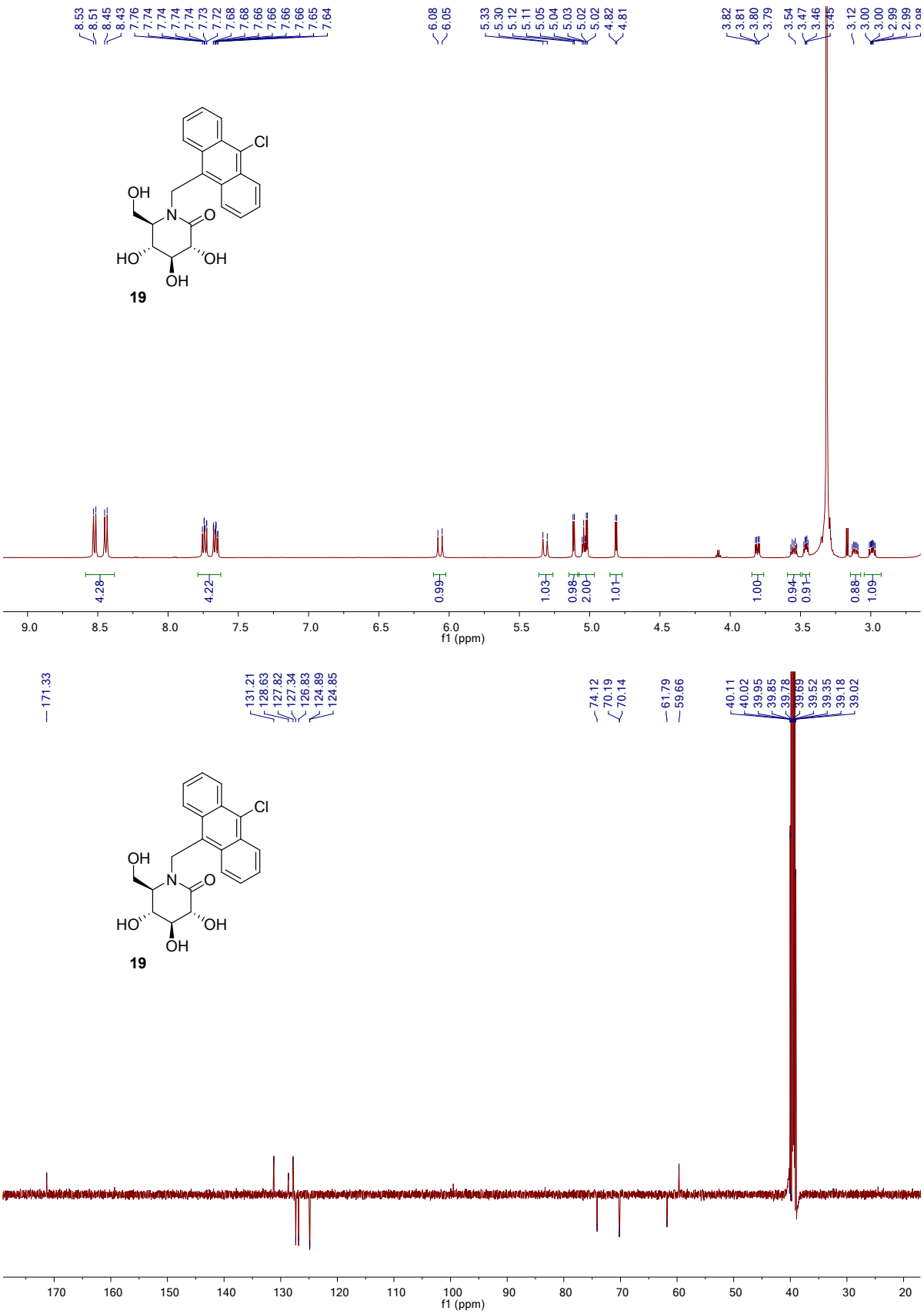
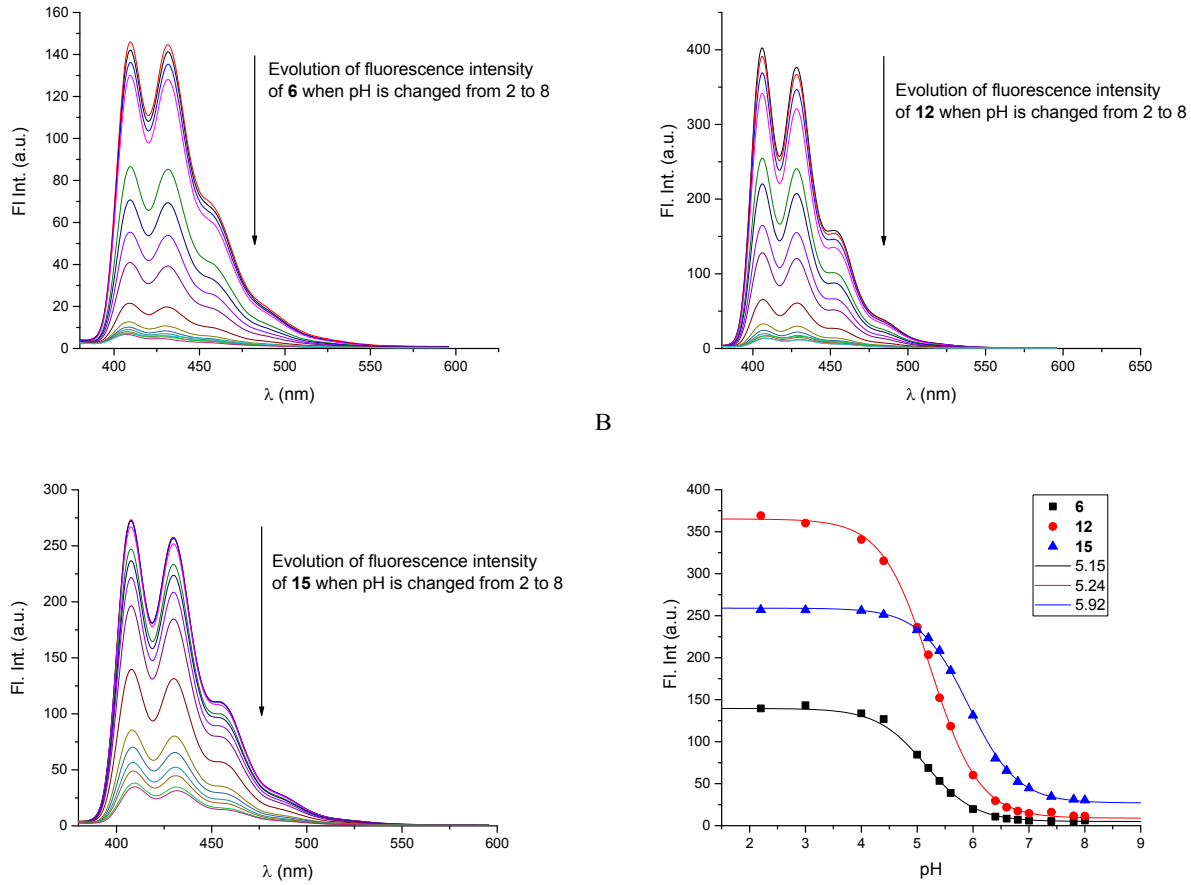


NMR spetra of Compound **19** in DMSO-d6

Determination of pK_a value of iminosugars 6, 12 and 15 by fluorescence titration.

Iminosugar **6**, **12** and **15** were dissolved respectively in DMSO to give 8 µmol/L stock solution. 0.05 mL was added to 0.45mL of phosphate buffers with different pH values (2.2, 3.0, 4.0, 4.4, 5.0, 5.2, 5.4, 5.6, 6.0, 6.4, 6.6, 6.8, 7.0, 7.4, 7.8, 8.0). Fluorescence spectra of each solution were recorded with excitation at 358 nm (Figure S1A-C).

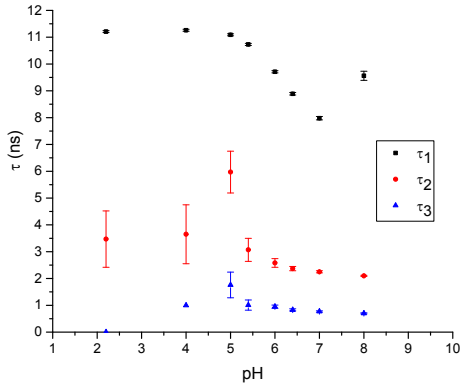
Figure S1



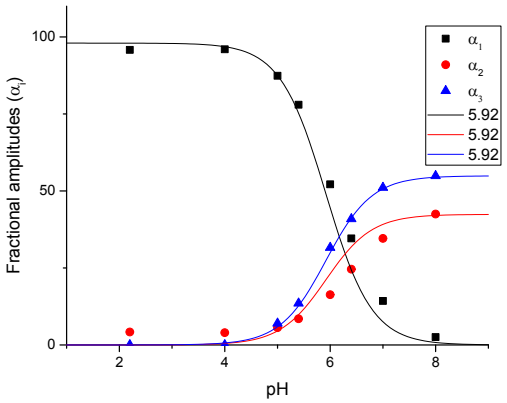
Determination of pK_a values for inhibitors: plotting the fluorescence intensity at 380 nm as function of pH gave for all three compounds clear sigmoidal curves, which show near perfect agreement with theoretical curves for monoprotic acids with pK_a values of 5.15, 5.24 and 5.92 for compounds **6**, **12**, and **15** respectively (Scheme S1D). For compounds **6** and **12** the residual fluorescence at pH = 8 and above (not shown) was found to be less than 2% of the signal at pH=2.2, thus showing practically full quenching of the deprotonated forms. For compound **15** the residual fluorescence was however found to be in the range of 10-12% of the maximal fluorescence measured at pH=2.2, clearly exceeded the values expected in case of full quenching.

Time resolved fluorescence study inhibitor 15 as function of pH: in order to identify the origin of the residual fluorescence from **15** in basic solutions the fluorescence decay as function of pH was recorded on a FluoTime 300 system (PicoQuant, Berlin, Germany) using a pulsed 355 nm laser as excitation source (THG of 1064 nm, VisUV module). For all solutions the fluorescence decays was fitted to three exponentials yielding variable contributions from one long (11 ns to 8 ns) and two shorter lifetime components (~2.5 ns and ~1 ns) as summarized in the figure below (Figure S2A). As the radiative rate constant is assumed to be constant the fractional amplitudes (α) can be used as direct measurements of the relative abundance of various emitters (protonated and non-protonated inhibitor). By plotting the variation of the fractional amplitude of the long lifetime component (α_1) with pH a clear sigmoidal behavior matching the pKa of 5.92 is found (Figure S2B). The amplitudes of the two short components (α_2 and α_3) behave as expected for the corresponding base showing that both originates from the non-protonated inhibitor. We tentatively assign these two populations to different conformations of the C3 linker and thus different quenching rates (Figure S2B).

Figure S2



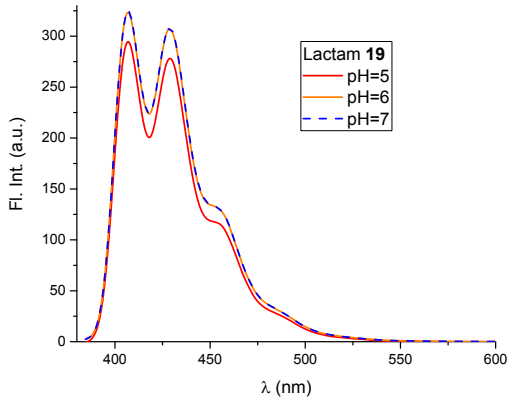
A



B

Fluorescence of lactame inhibitor (19):

Figure S3



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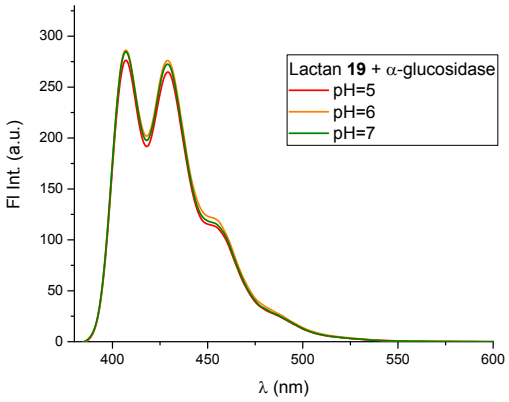
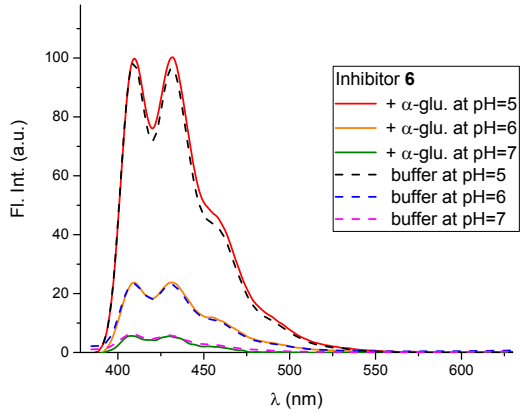


B

Fluorescence of lactame inhibitor **19** was practically independent of pH in the relevant range (Figure S3A). The pH independent fluorescence intensity was also demonstrated in the presence of excess (54 μM) α-glucosidase (Figure S3B).

Effect of glucosidase:

Figure S4



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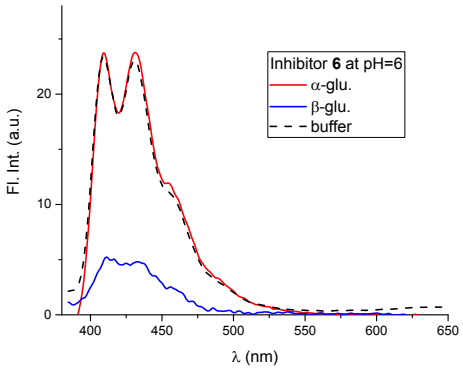

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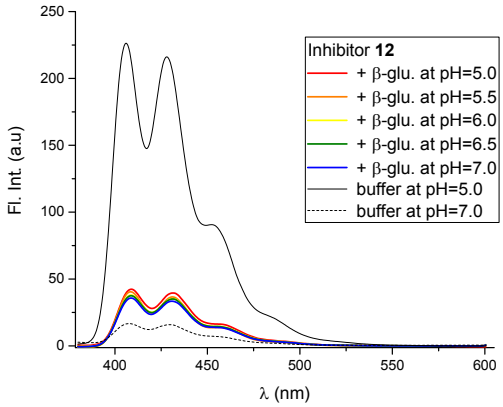
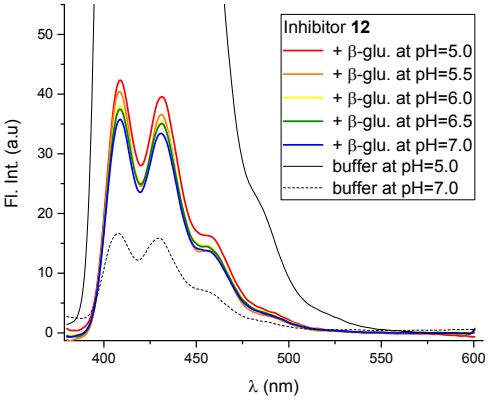
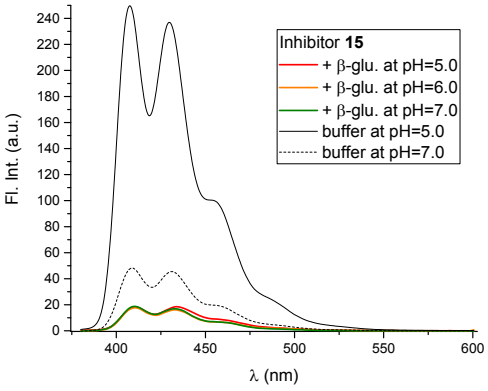


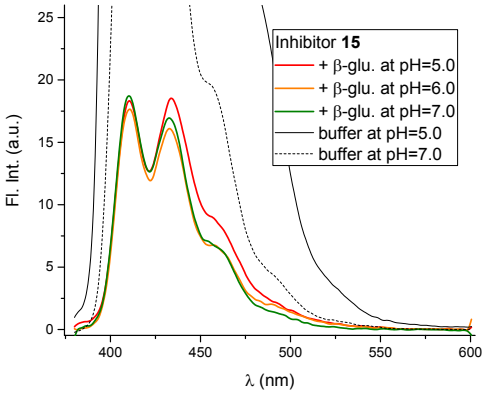
C



D



E



F

Procedure for fluorescence measurements. The inhibitor **6**, **12**, **15** or **19** was dissolved at 0.012 mg/mL in DMSO and diluted 4 times with DMSO to create a stock solution of approximately 8.0 μM. Normally fluorescence were measured on our types of solutions:

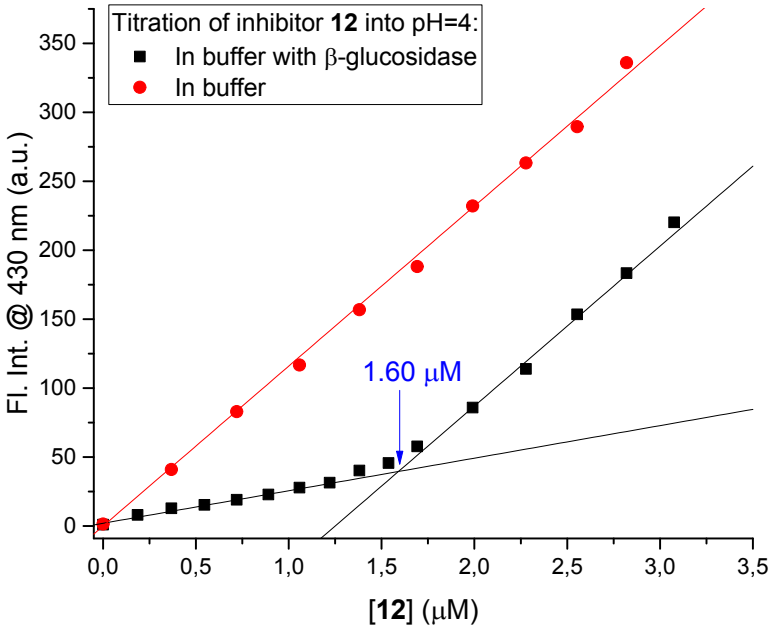
A. Blank (0.45 mL phosphat buffer and 0.05 mL DMSO)
B. Inhibitor (0.45 mL phosphat buffer, and 0.05 mL inhibitor stock solution, [I] = 0.8 μM)
C. Enzyme (0.1-0.2 mL enzyme stock solution in phosphate buffer, and 0.25-0.35 mL phosphate buffer and 0.05 mL DMSO. [E] = 5.8-72 μM)

D. Enzyme and inhibitor (0.1-0.2 mL enzyme stock solution in phosphate buffer, and 0.25-0.35 mL phosphate buffer and 0.05 mL inhibitor stock solution. [I] = 0.8 μM, [E] = 5.8-72 μM)

Fluorescence spectra was taken of samples A-D exciting at 358 nm on a Perkin Elmer LS50 instrument. The subtracted spectra (B-A), (D-C) gave the net effect of inhibitor fluorescence when free and bound.

Titration of β-glucosidase with inhibitor 12:

Figure S5



The increase in fluorescence intensity as function of [**12**] in buffer at pH=4 and at concentrations beyond the concentration of β-glucosidase are identical and the intensity of fluorescence from bound inhibitor is 20% of that of free inhibitor at pH=4, in agreement with the pH response curves of **12** and the pH independent fluorescence when bound. By this measurement the concentration of enzyme binding sites is determined to 1.60 micromolar.