Supporting Information

Non-Peptide Macrocyclic Histone Deacetylase Inhibitors Derived from Tricyclic Ketolide Skeleton

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Contents

Experimentals:

Tricyclic Ketolide TE-802 (10)

Synthesis of TE-802 is according to protocol in ref.**25**. Purification by silica gel column chromatography (12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) afforded 62% of compound **10**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, *J* = 7.2 Hz), 1.04 (3H, d, *J* = 6. 8 Hz), 1.10-1.41 (12H, m), 1.49 (6H, s), 1.53-2.00 (8H, m), 2.26 (6H, s), 2.40-2. 49 (2H, m), 2.72 (3H, s) 2.84-3.25 (3H, m), 3.48-3.85 (4H, m), 3. 99-4.02 (1H, m), 4.20 (1H, d, *J* = 8.4 Hz), 4.29 (1H, d, *J* = 7.6 Hz), 4.95 (1H, d, *J* = 10.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) 10.4, 10.8, 12.8, 14.4, 16.4, 19.1, 19.6, 21.1, 22.0, 28.2, 36.3, 38.5, 40.2, 42.3, 42.7, 48.1, 49.1, 49.5, 51.2, 53.4, 59.9, 65.8, 69.5, 70.3, 76.4, 78.5, 79.1, 81.5, 103.8, 156.0, 169.5, 181.3, 204.2; HRMS (ESI) calc for $[C_{33}H_{55}N_3O_9 + H]^+$ 638.3973, found 638.4011

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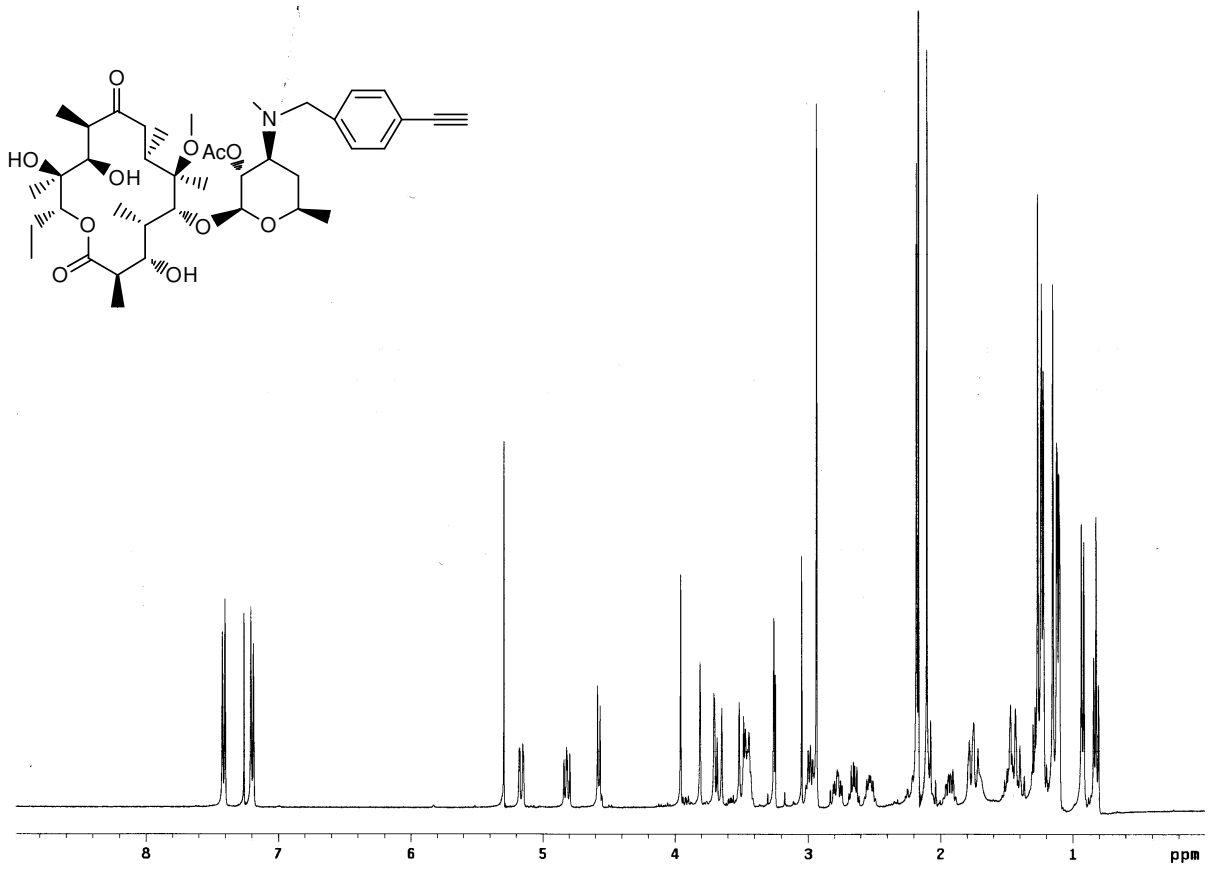
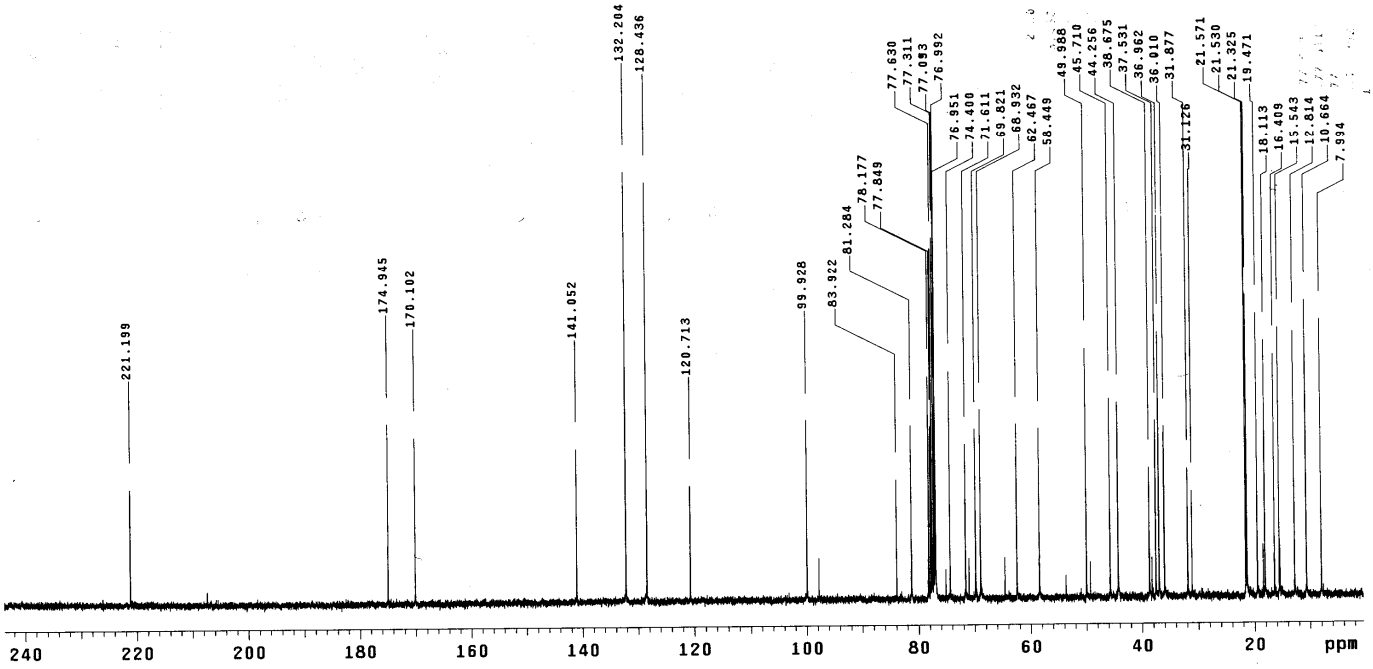
4-Desmethyltricyclic Ketolide (11)

To a solution of **tricyclic ketolide 10** (3.10 g, 4.88 mmol) in 60 ml of acetone, was added diethyl azodicarboxylate (0.94 g, 5.37 mmol) dropwise. The reaction was stirred at room temperature for 24 h and evaporated to dryness. The yellow residue was diluted in 10 ml of MeOH and 10 ml of saturated NH_4Cl and refluxed for 1 h. After evaporation to dryness, the yellow residue was taken up with 40 ml of water and the pH was adjusted to 8 with aqueous ammonium hydroxide. Extraction with ethyl acetate (3 x 100 ml), drying over Na_2SO_4, and evaporation of the solvent afforded the crude product which was purified on silica column chromatography (80:10:3 ethyl acetate/MeOH/Et_3N) to give 1.86 g (62 %) of **11** as a creamish foam. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, *J* = 7.2 Hz), 1.05 (3H, d, *J* = 6. 8 Hz), 1.10-1.45 (12H, m), 1.49 (6H, s), 1.53-2.02 (8H, m), 2.43 (3H, s), 2.47-2.54 (2H, m), 2.73 (3H, s) 2.91-3.15 (3H, m), 3.57-3.84 (4H, m), 3. 97-4.01 (1H, m), 4.23 (1H, d, *J* = 8.0 Hz), 4.29 (1H, d, *J* = 7.6 Hz), 4.94 (1H, d, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) 10.4, 10.8, 12.8, 14.4, 16.3, 19.1, 19.5, 20.9, 22.0, 32.8, 36.3, 37.0, 38.5, 42.3, 42.6, 47.8, 49.0, 49.4, 51.1, 59.8, 60.3, 60.6, 69.1, 73.8, 76.5, 78.5, 81.5, 103.0, 156.0, 169.5, 181.1, 204.0; HRMS (ESI) calc for $[C_{32}H_{53}N_3O_9 + H]^+$ 624.3796, found 624.3854

^{1}H NMR of **6**



^{13}C NMR of **6**



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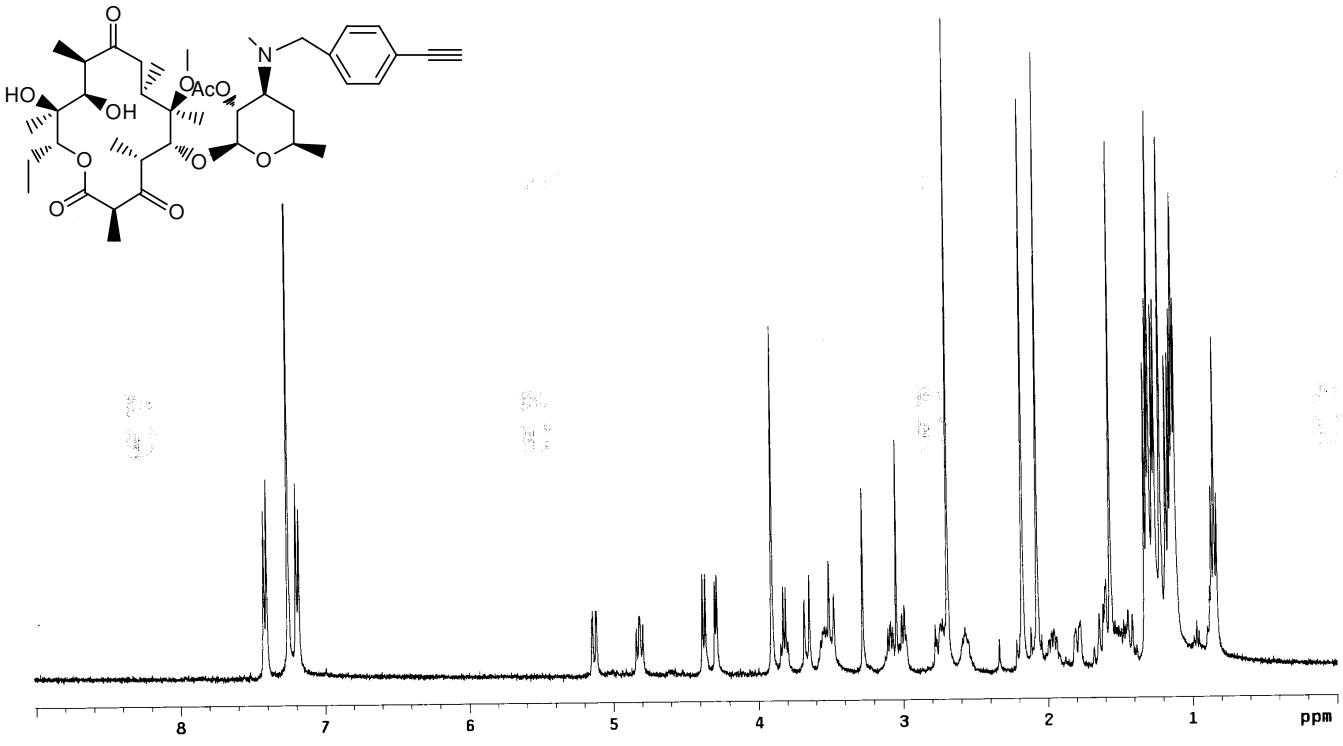

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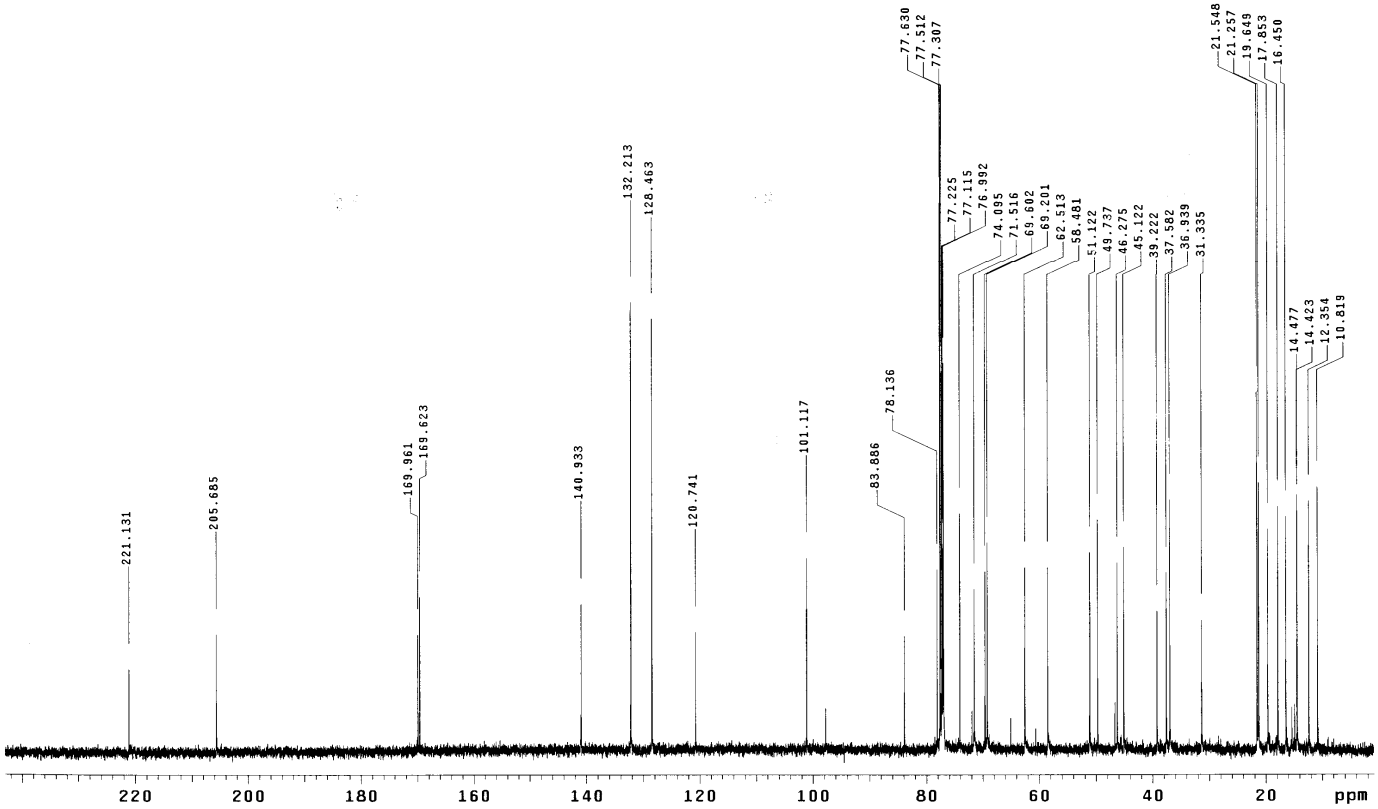

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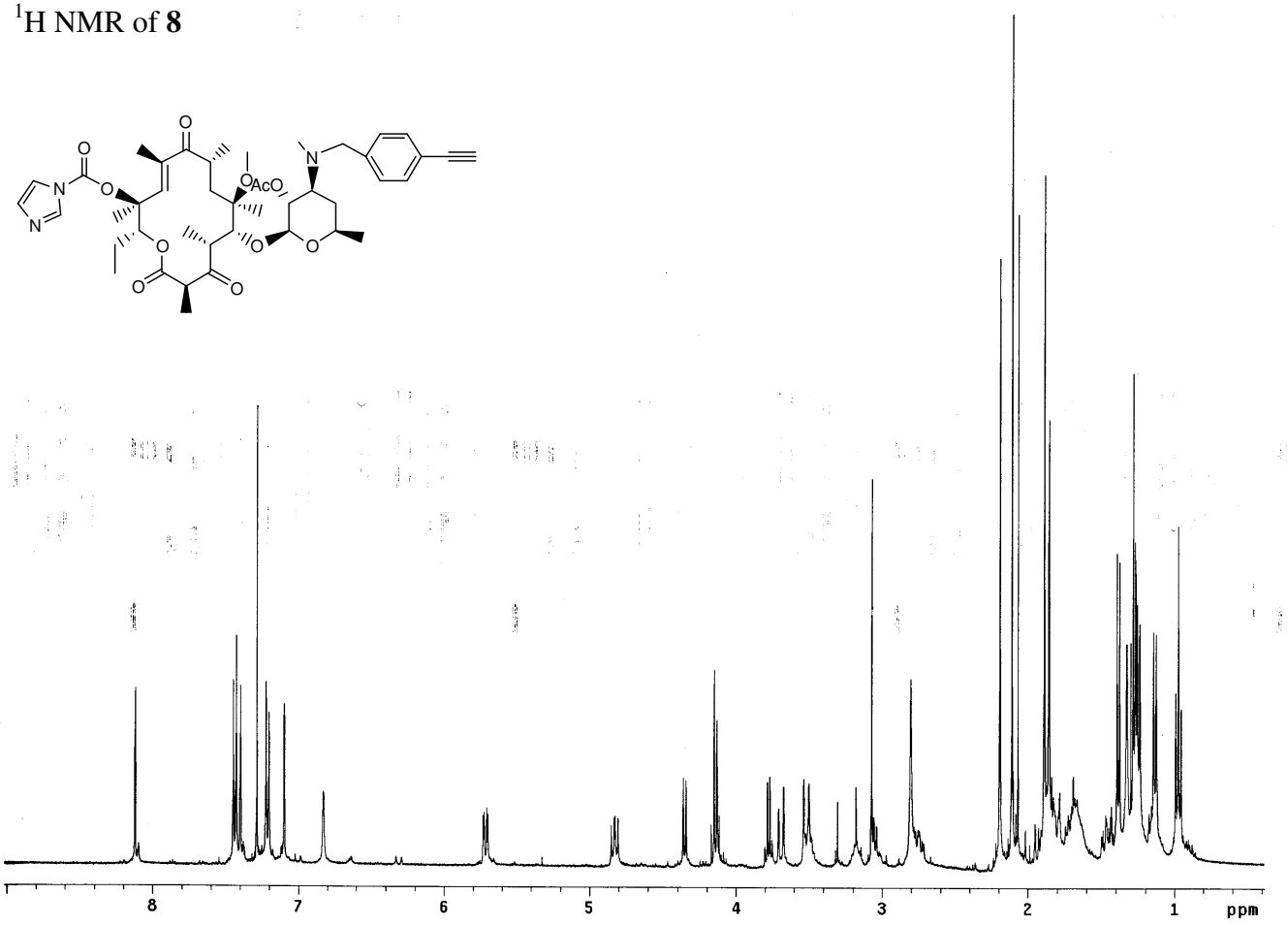

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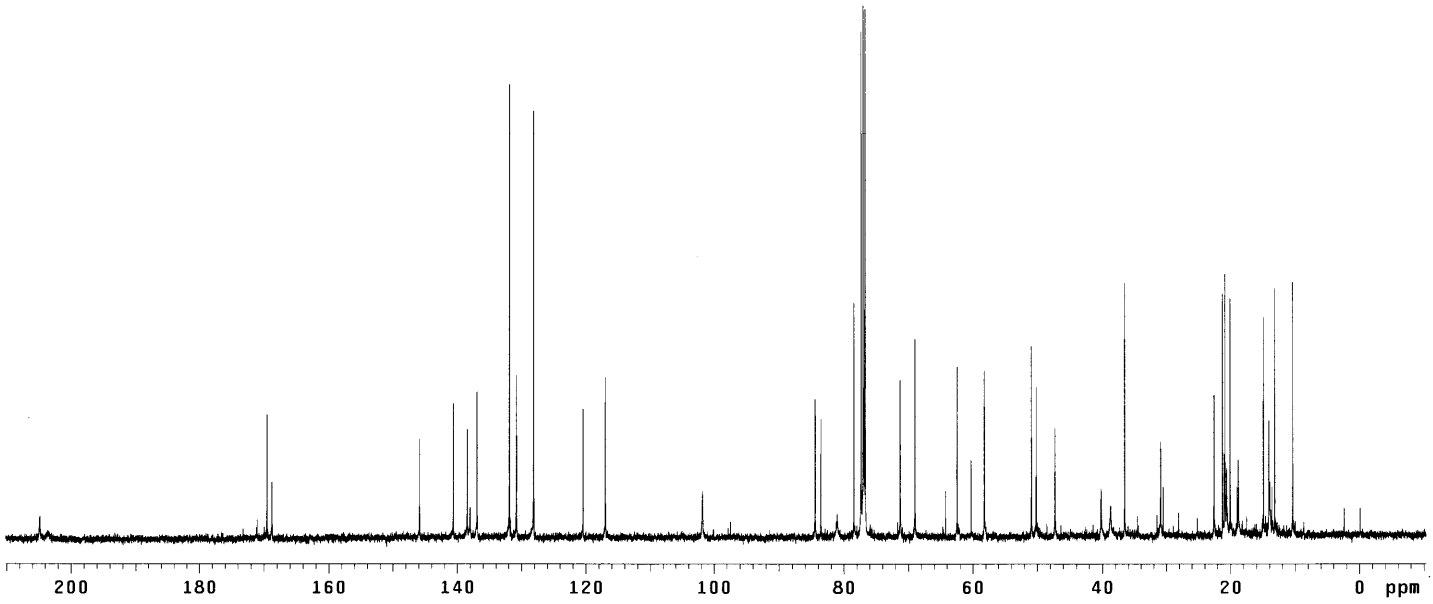

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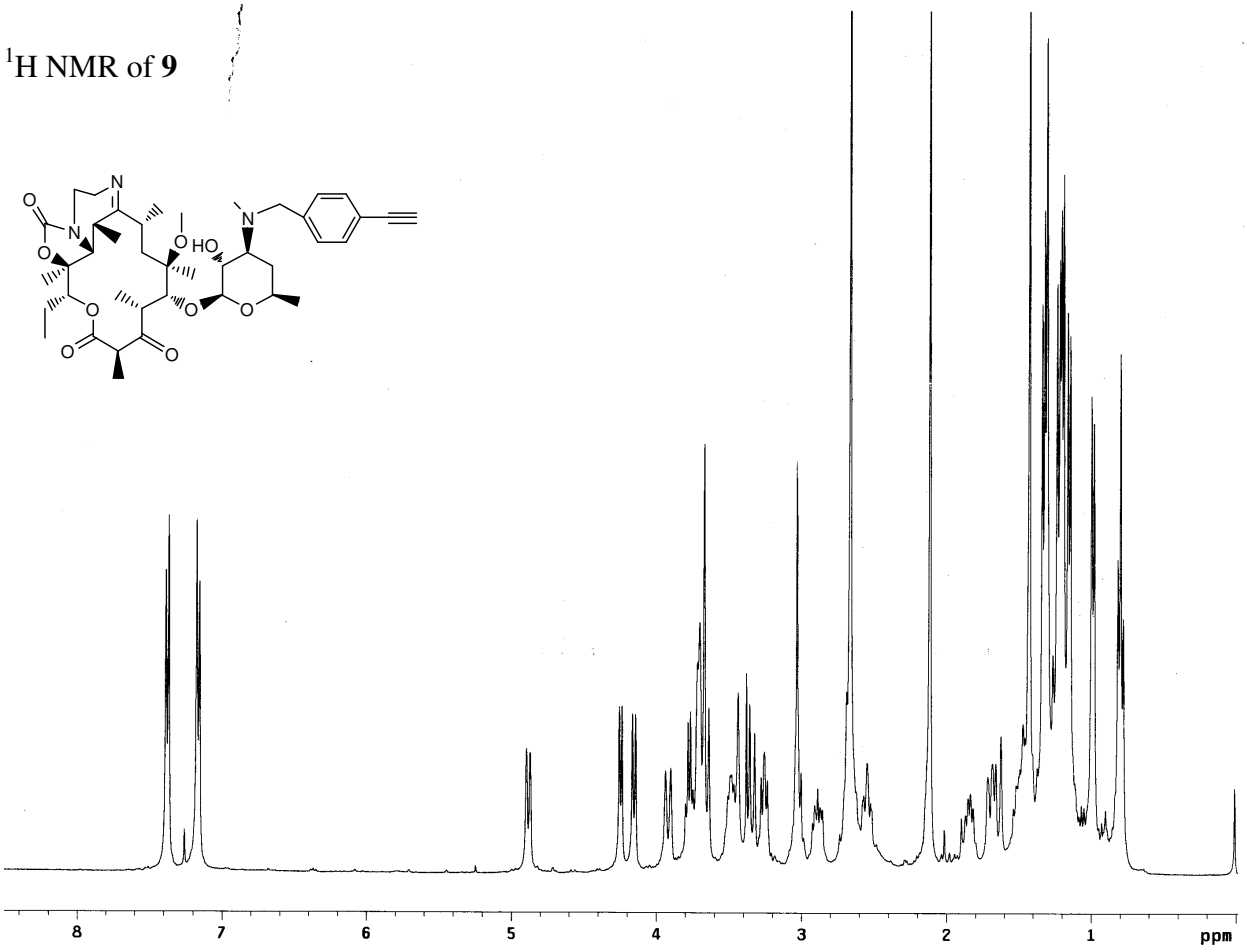

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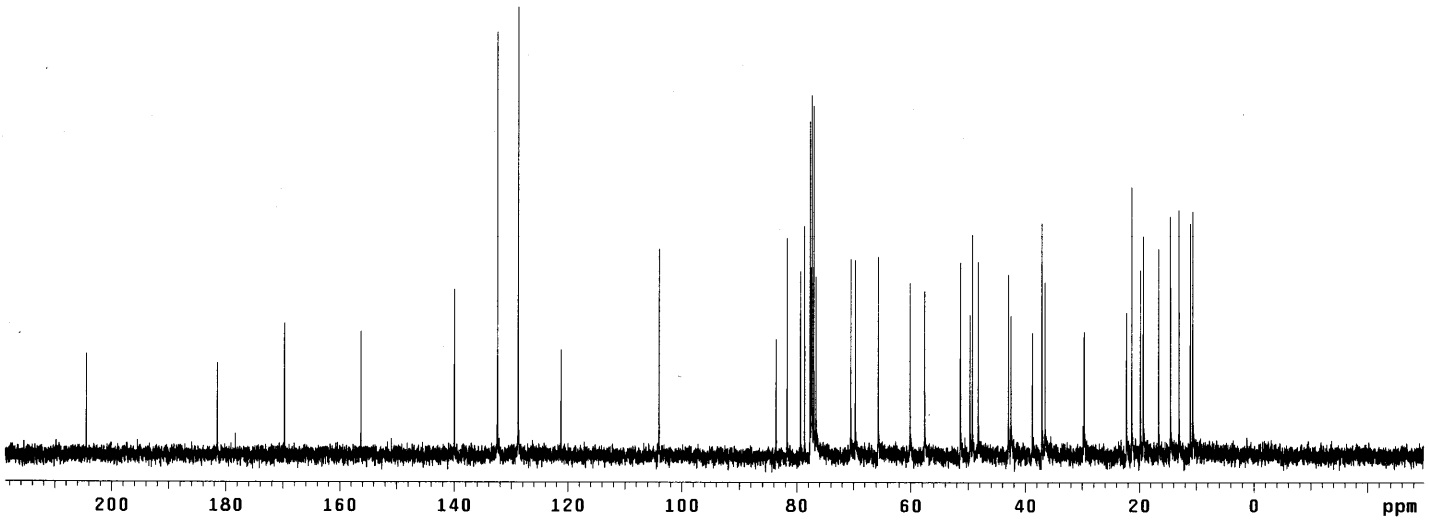
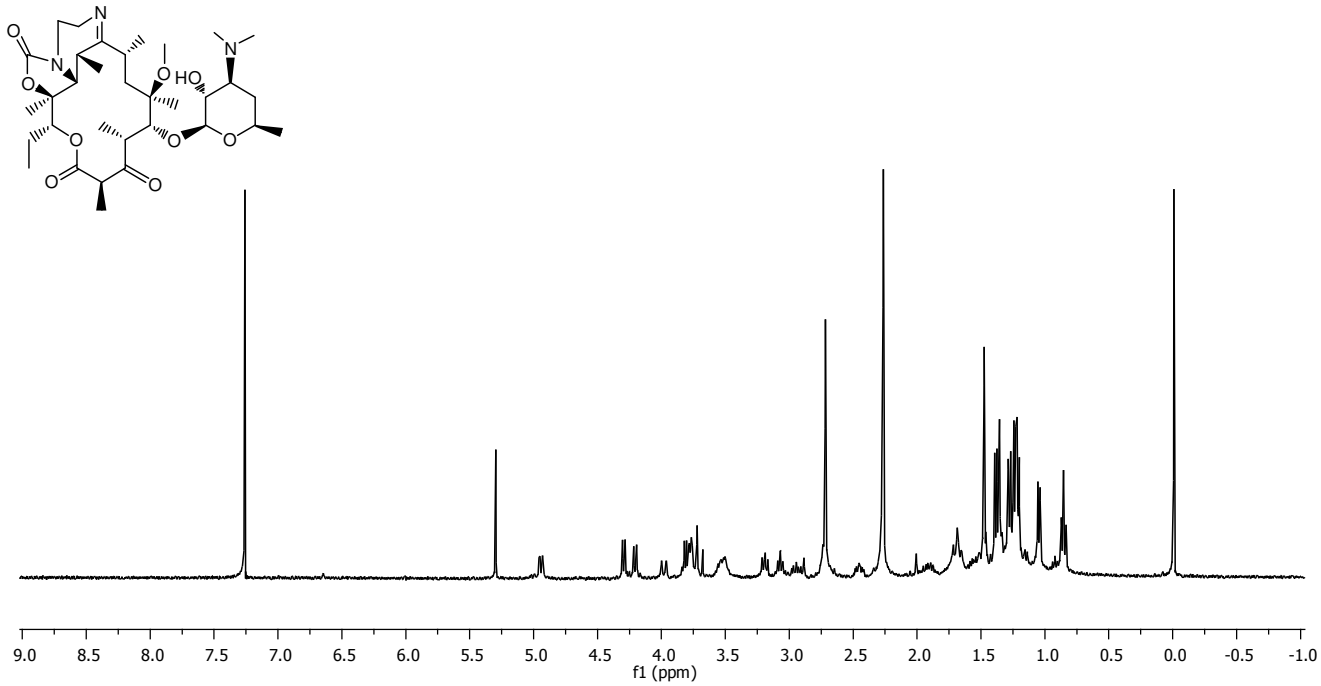


^{1}H NMR of **10**



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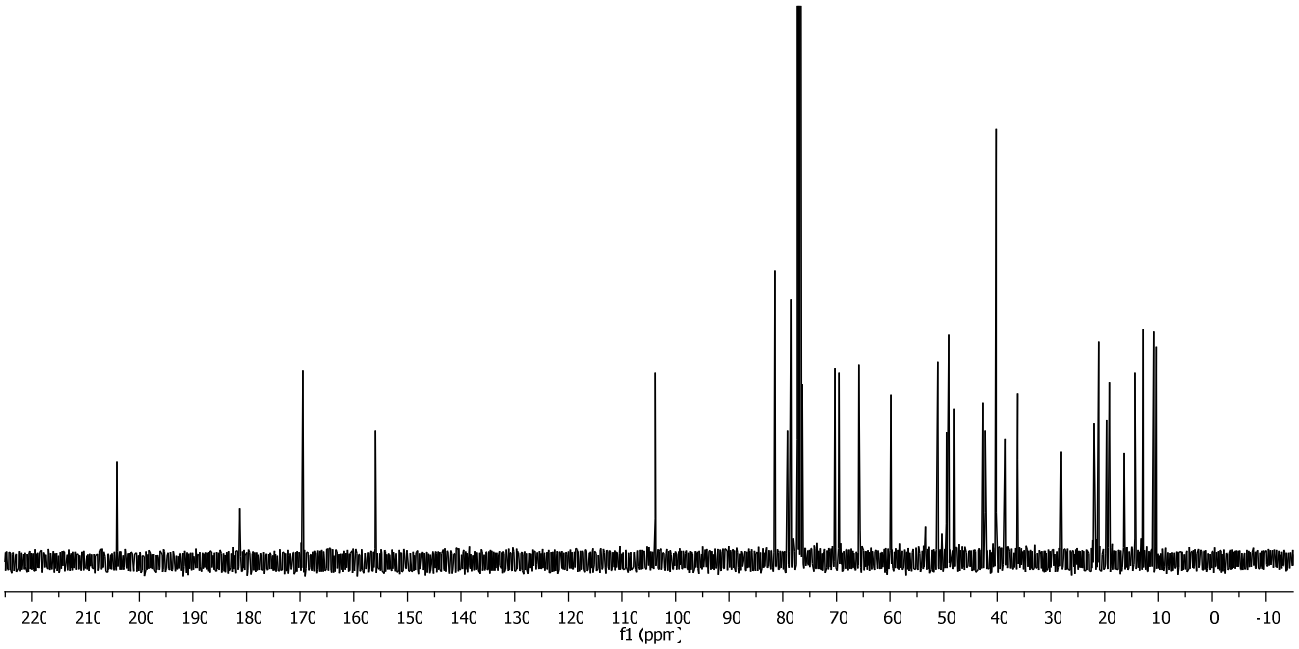

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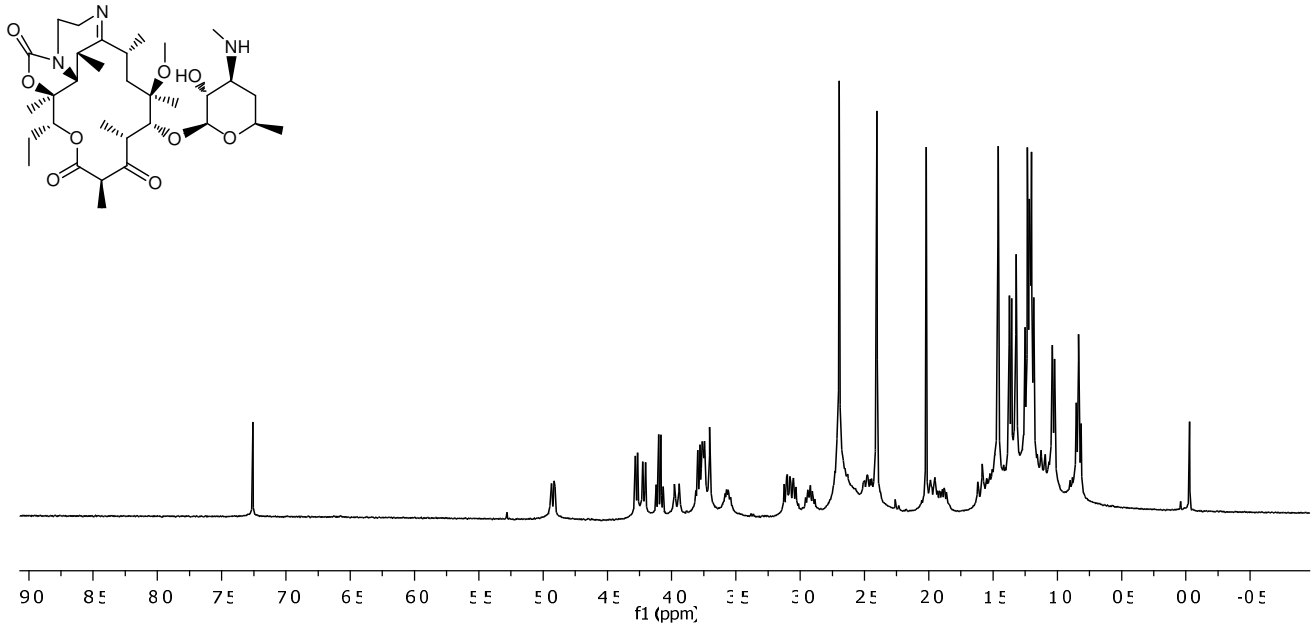

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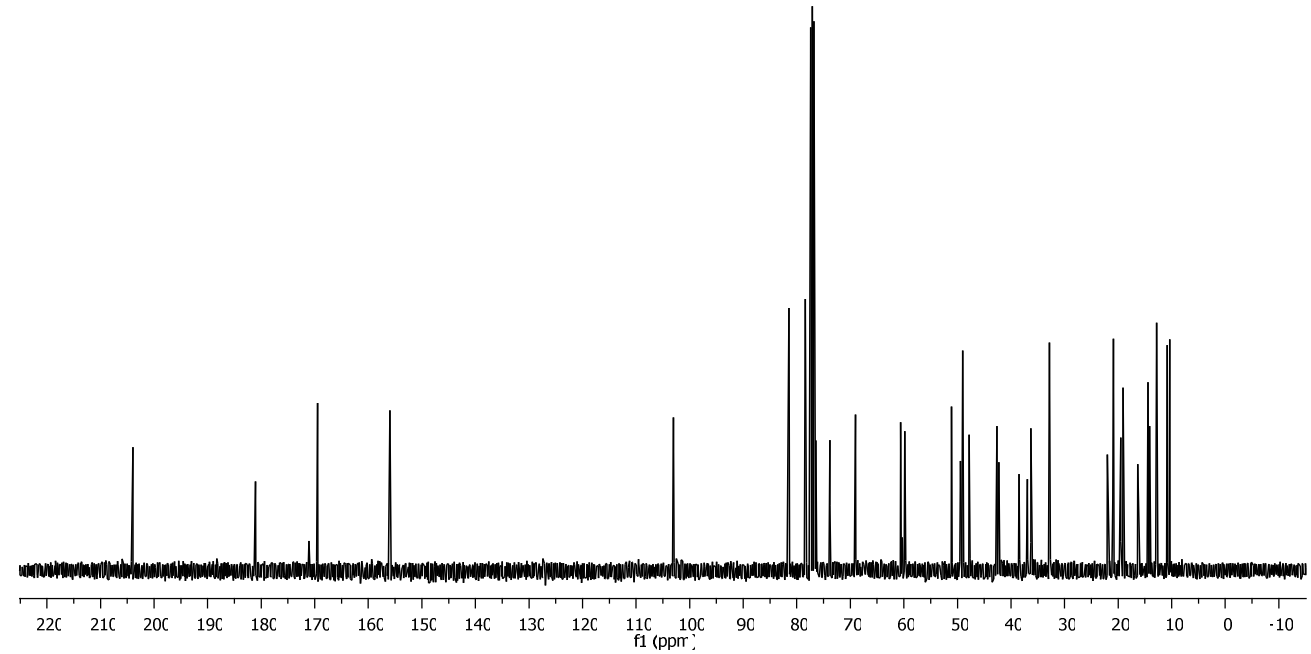

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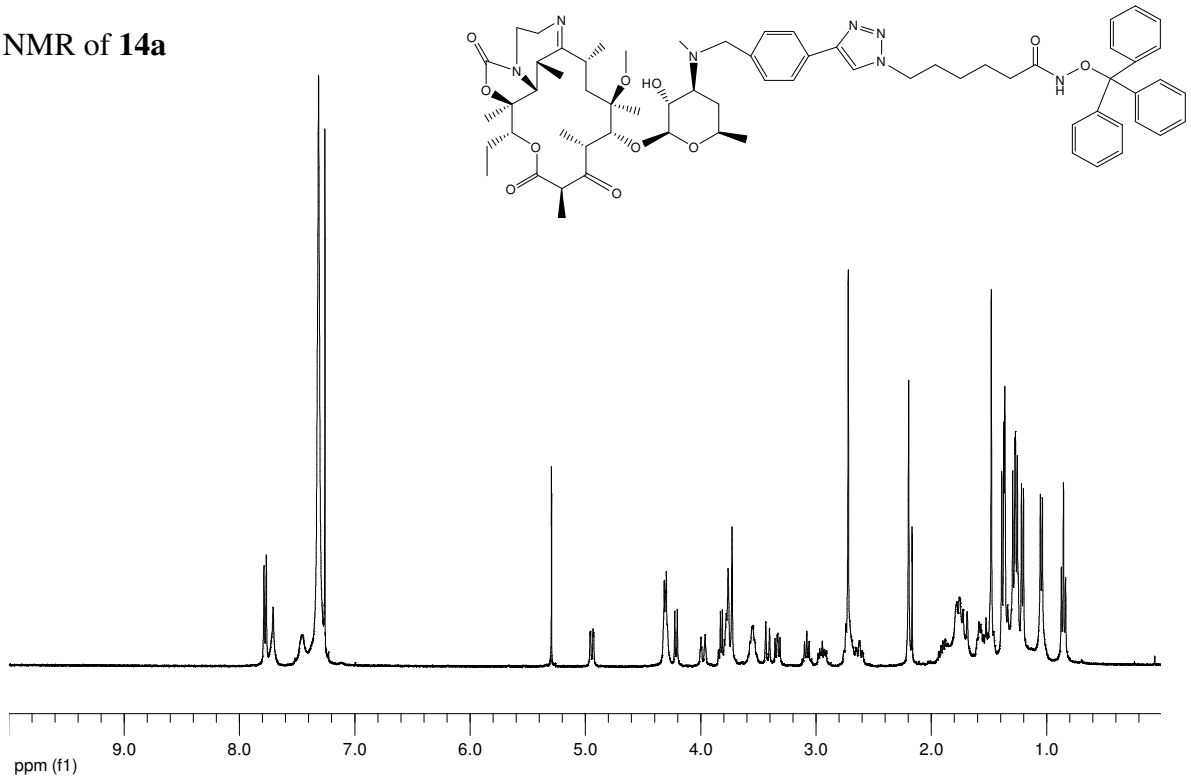

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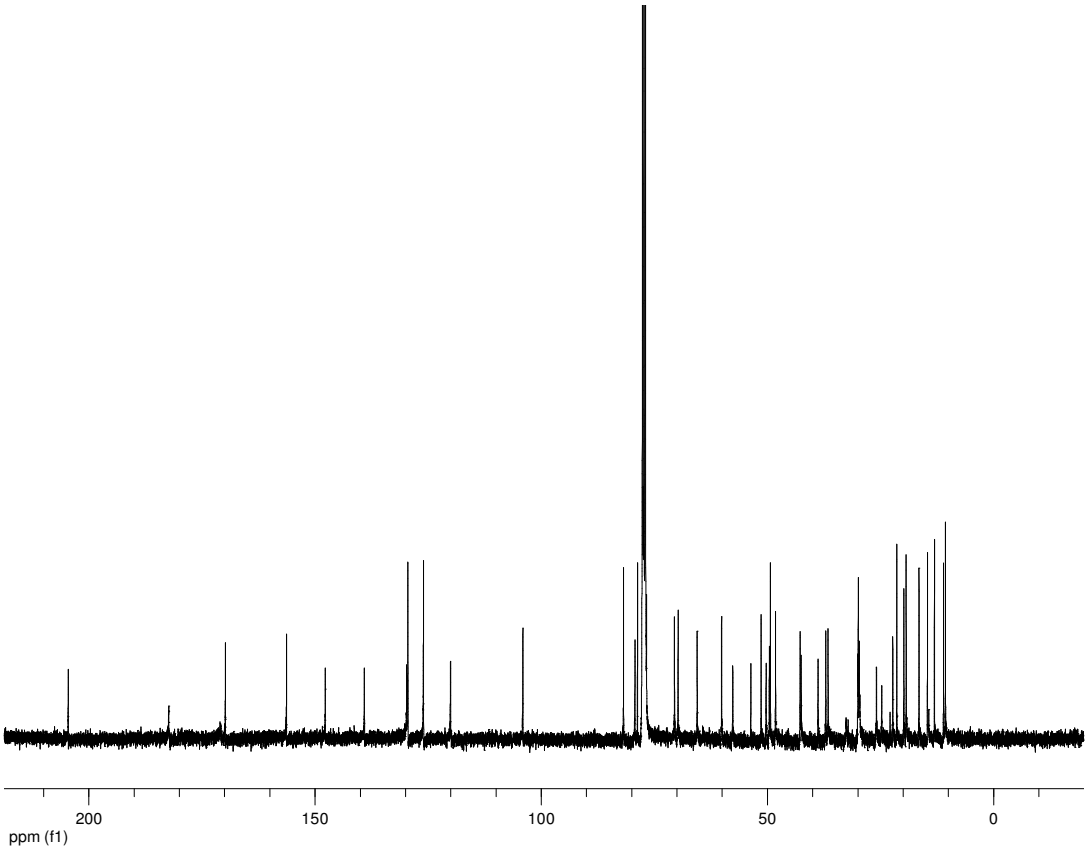

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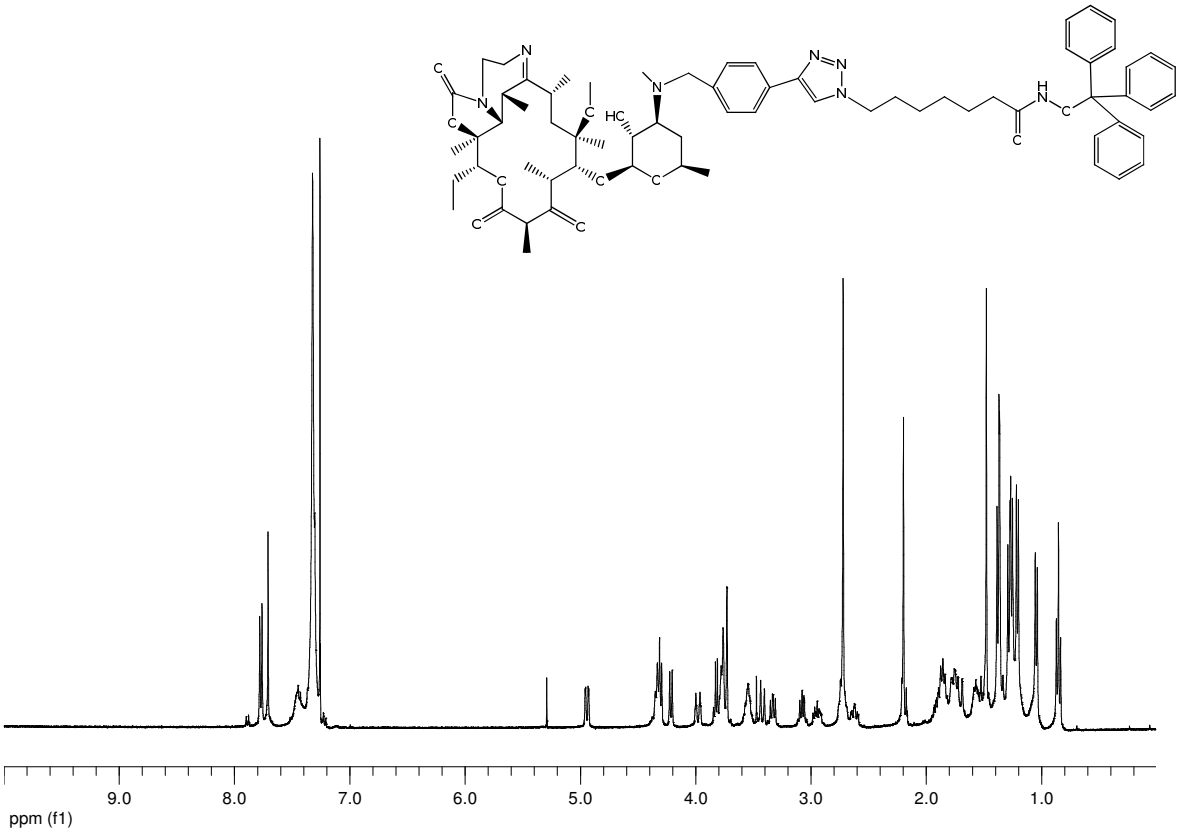

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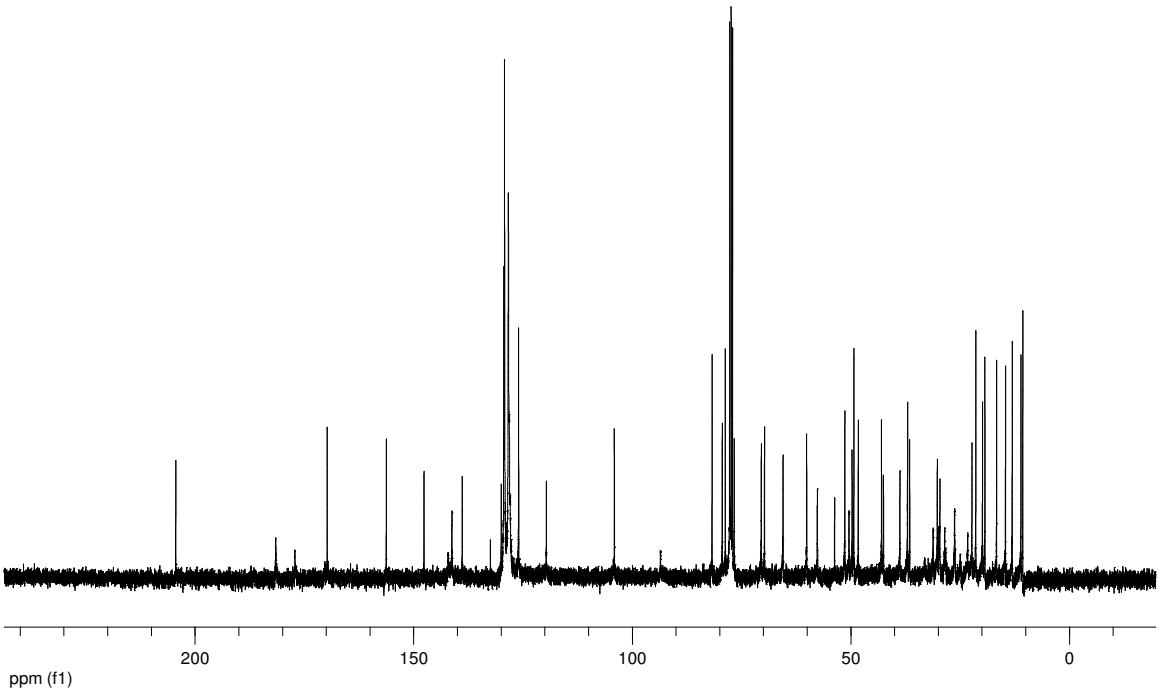

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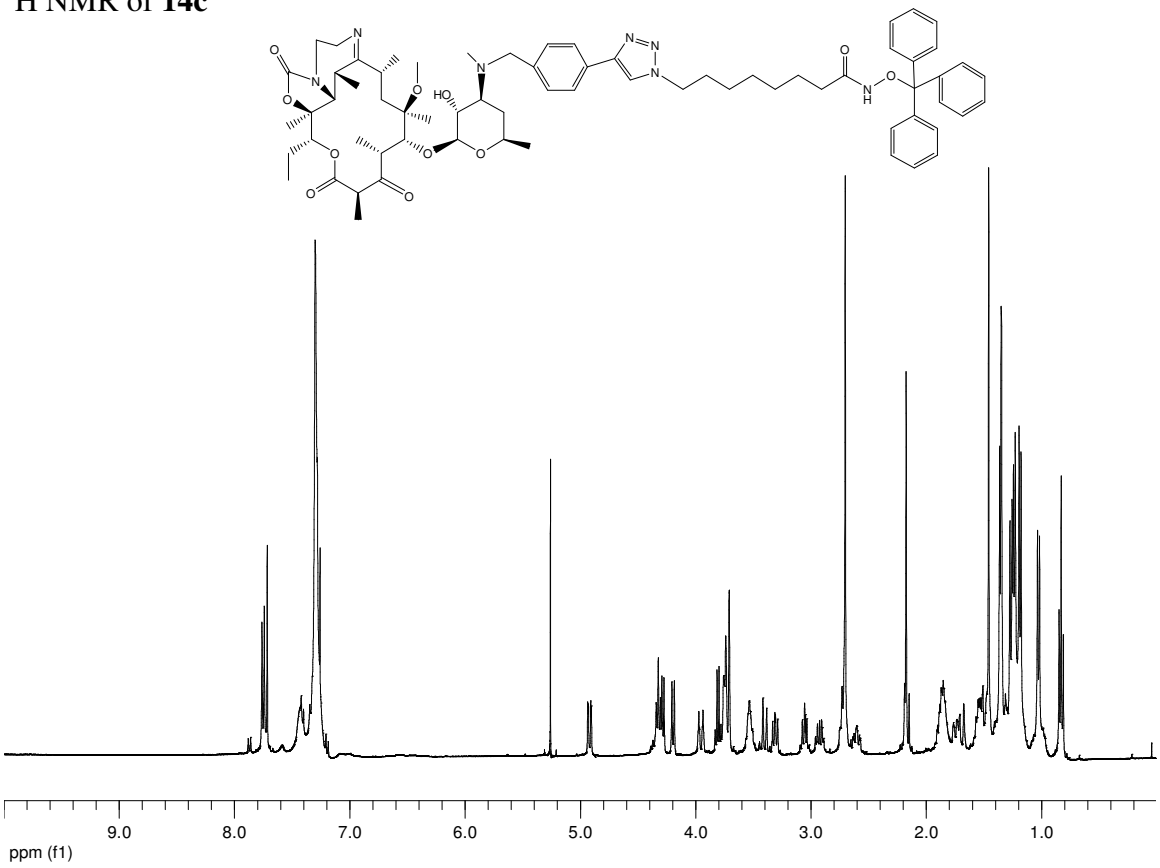

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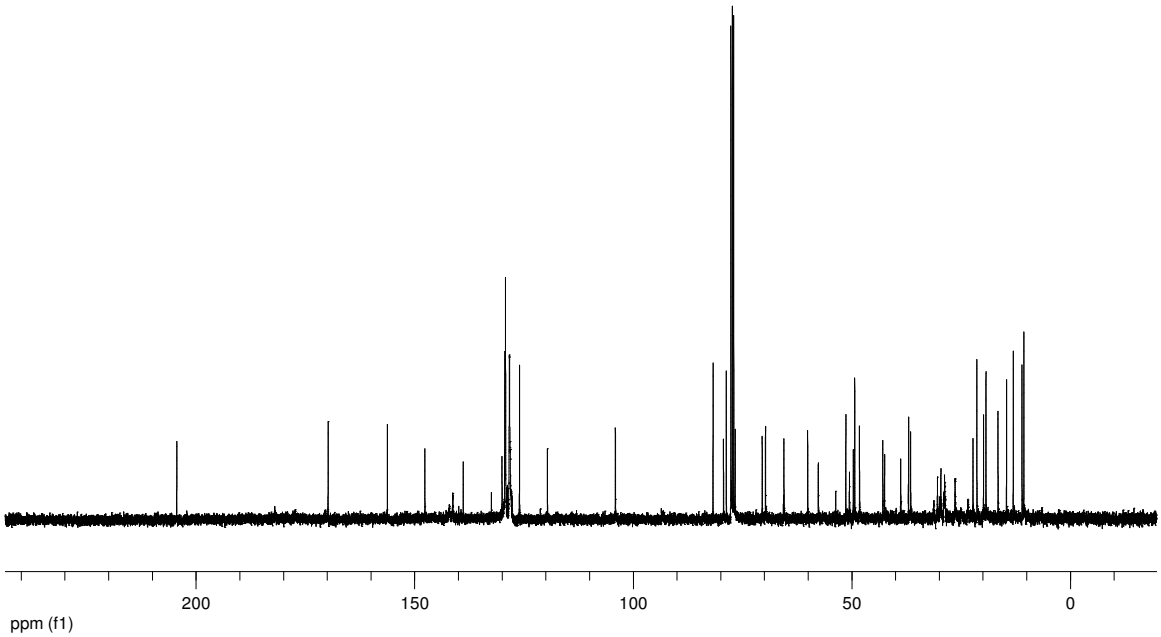

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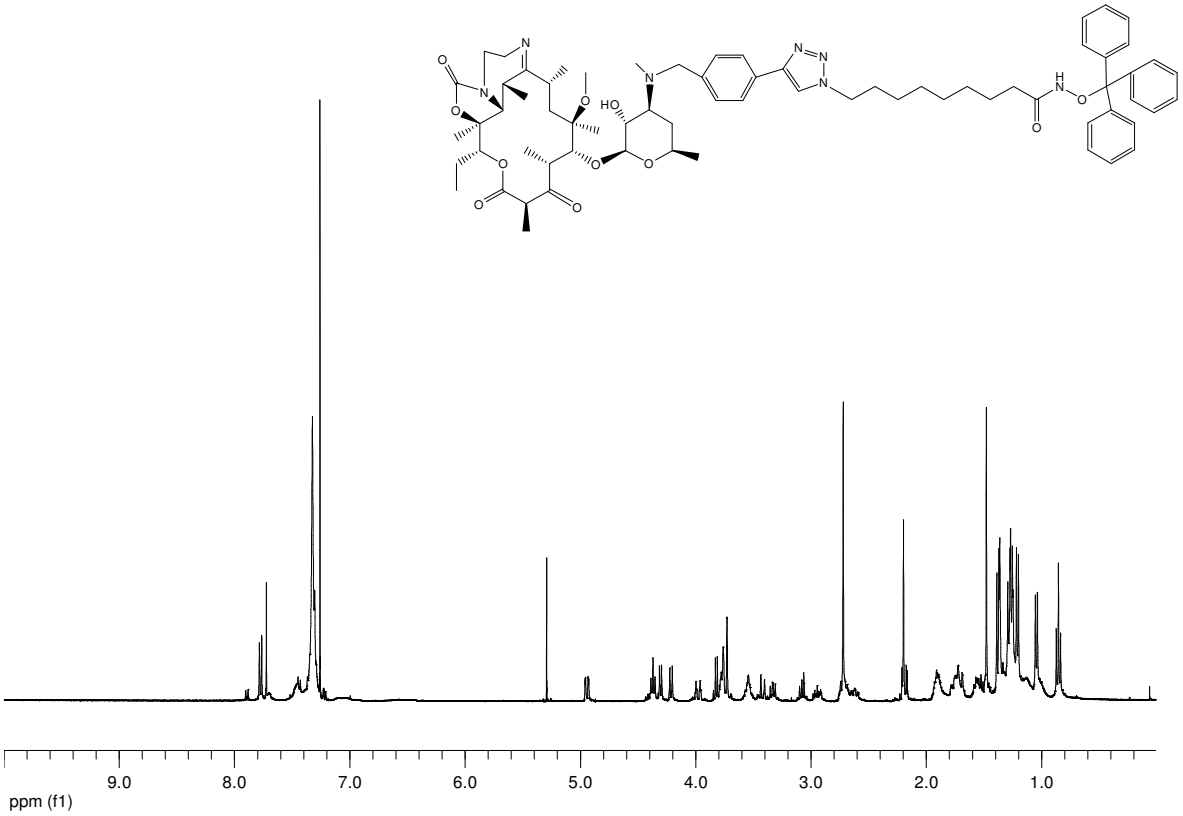

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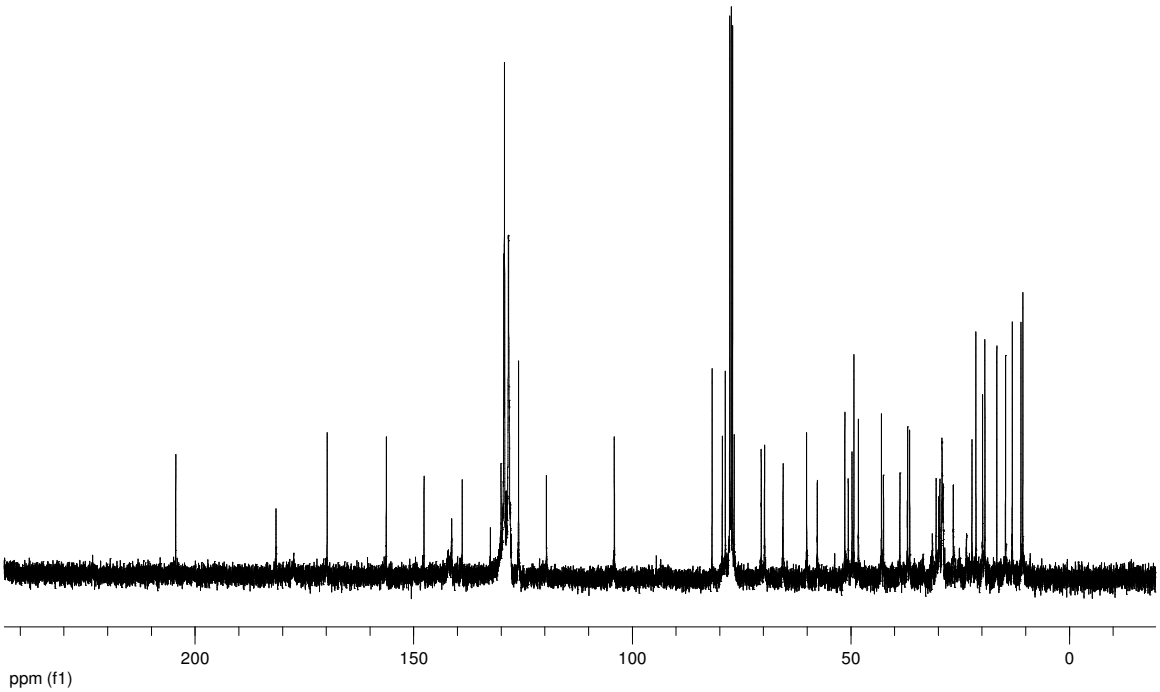

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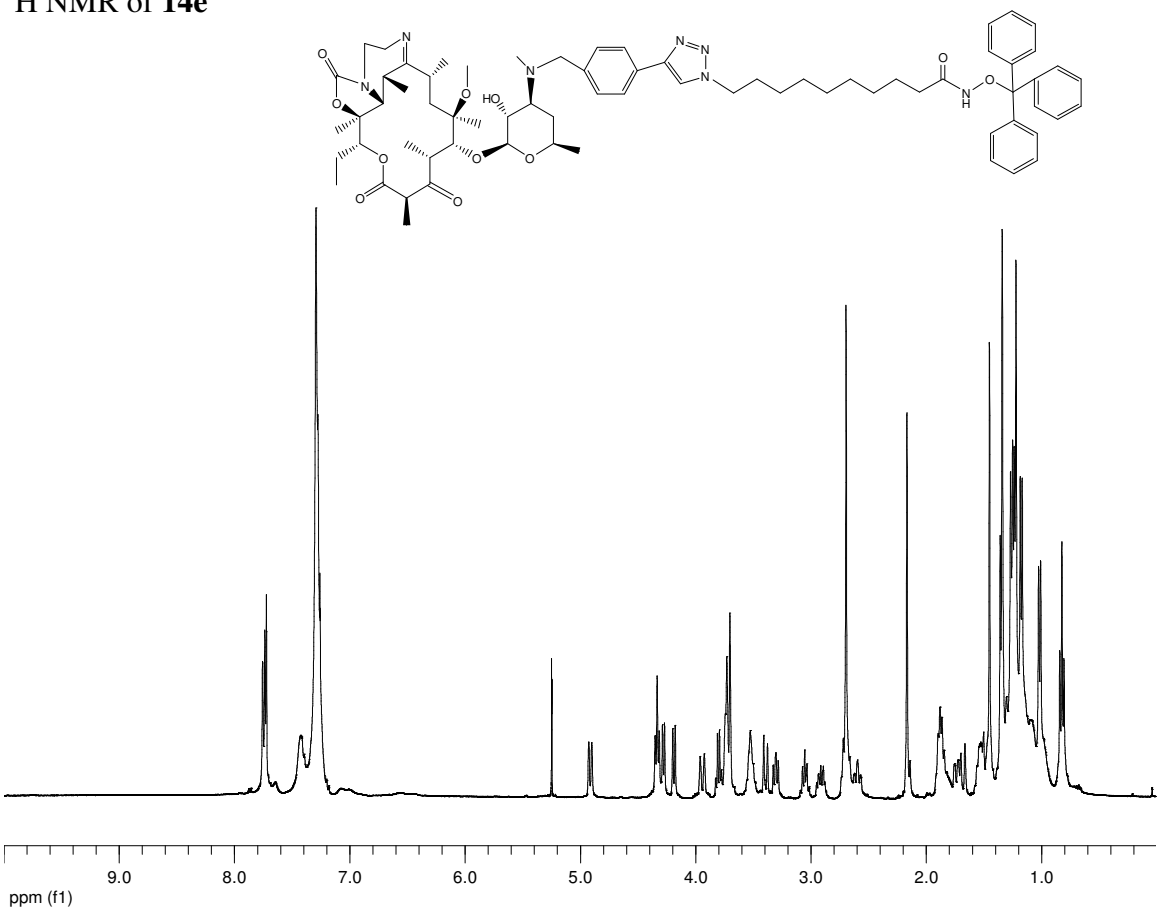

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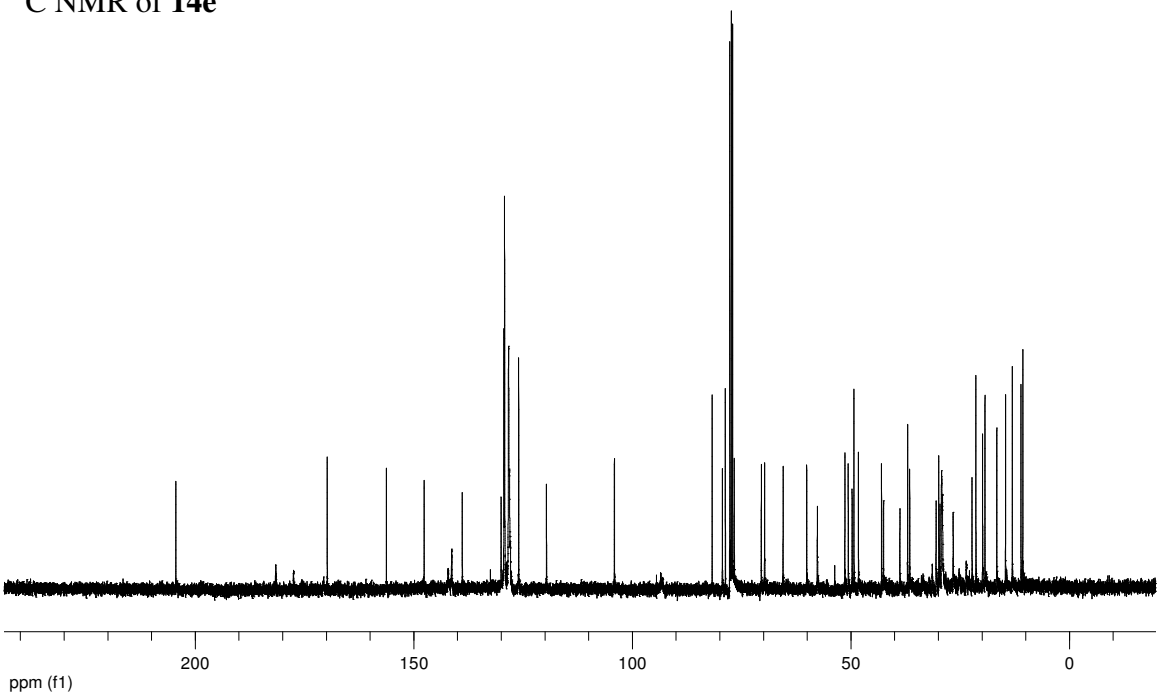

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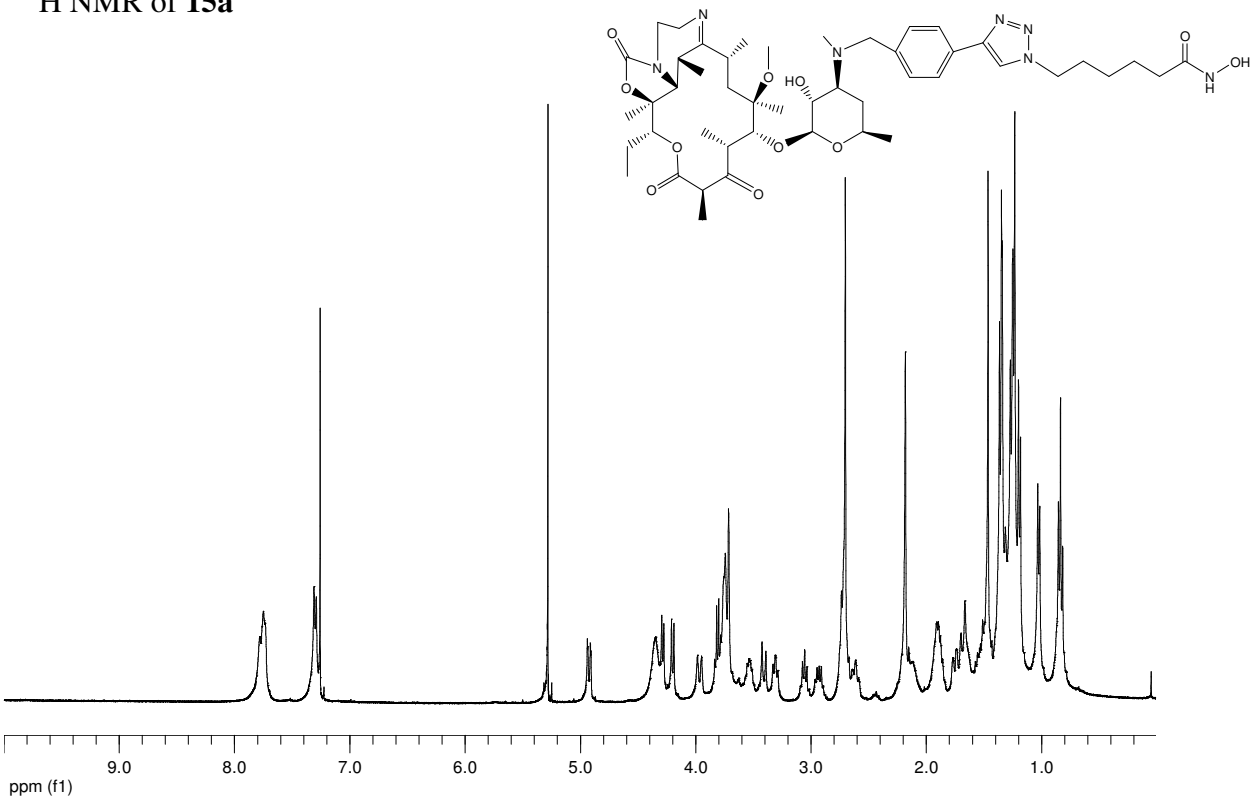

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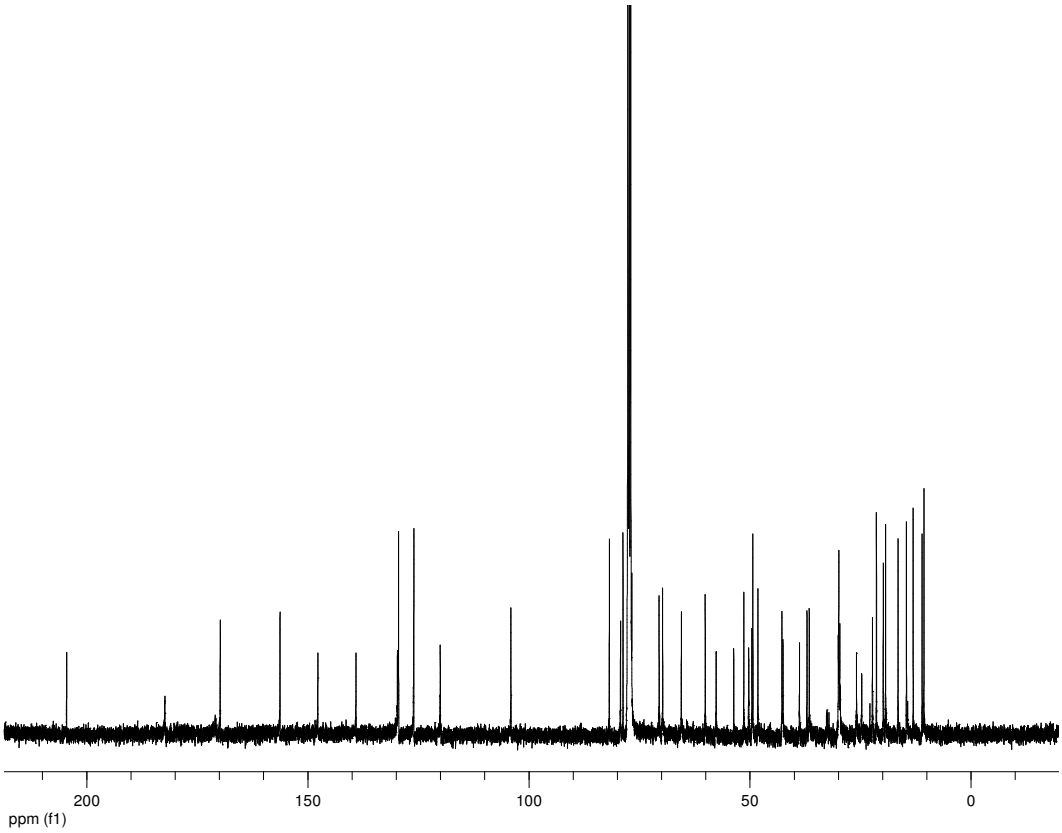

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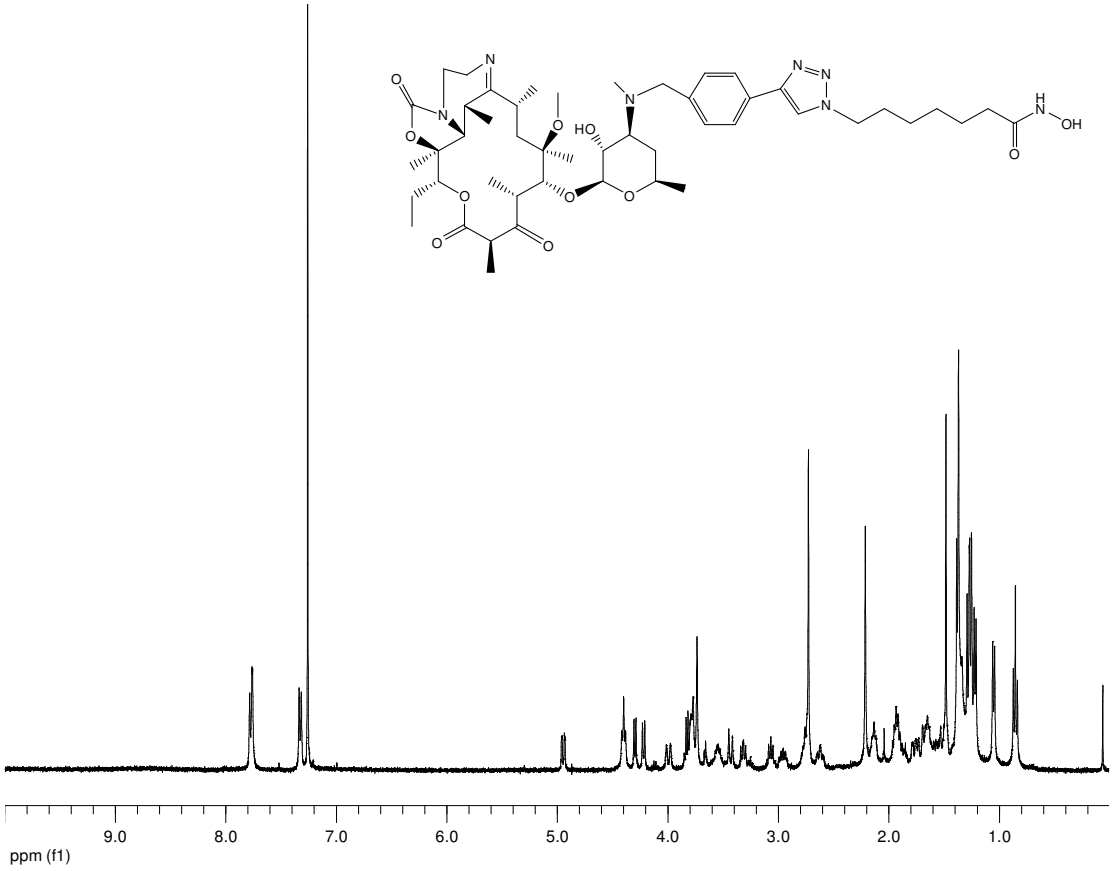

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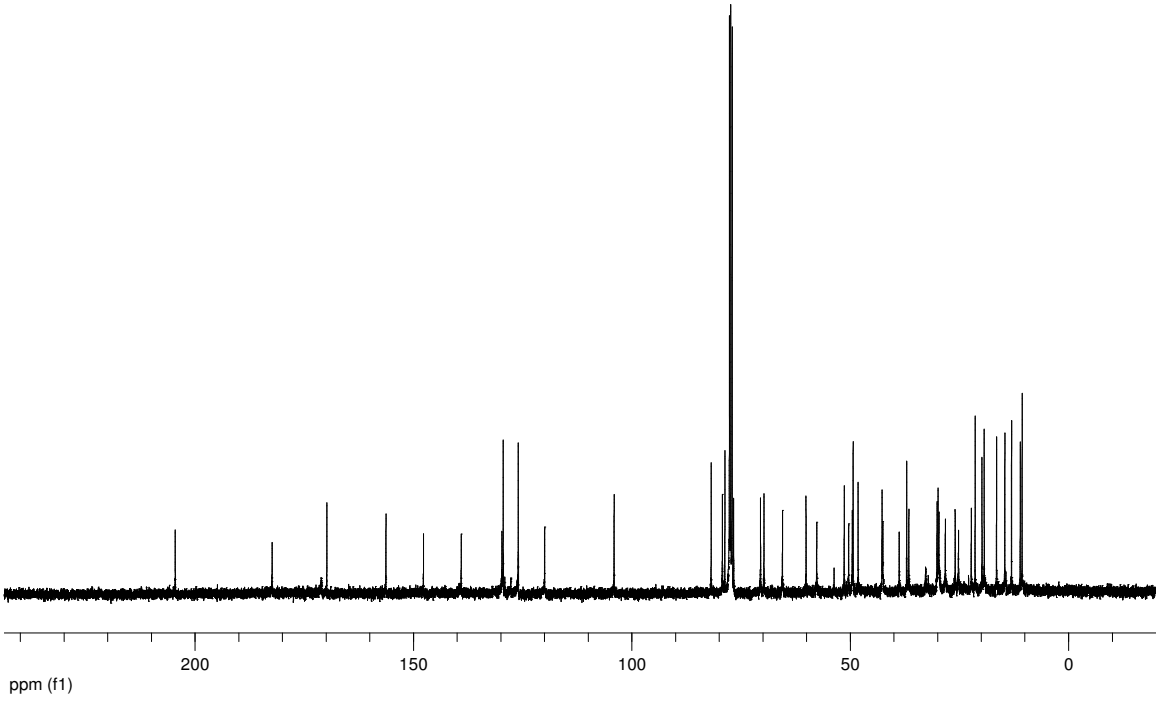

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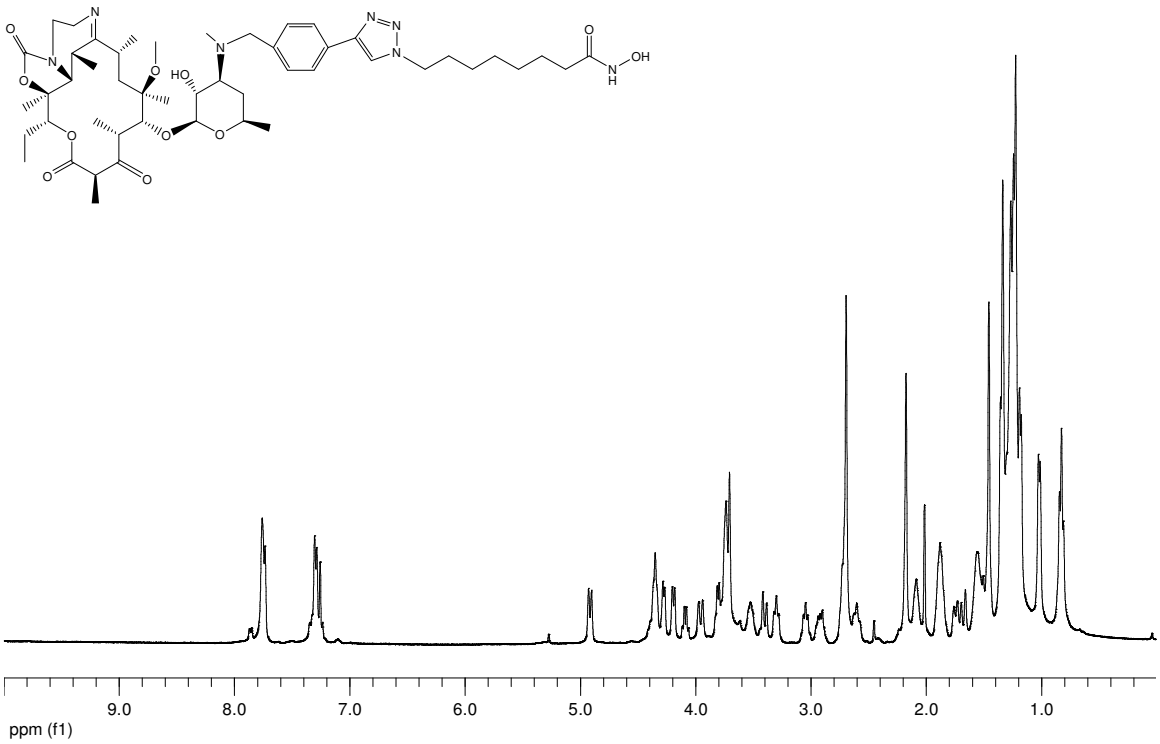

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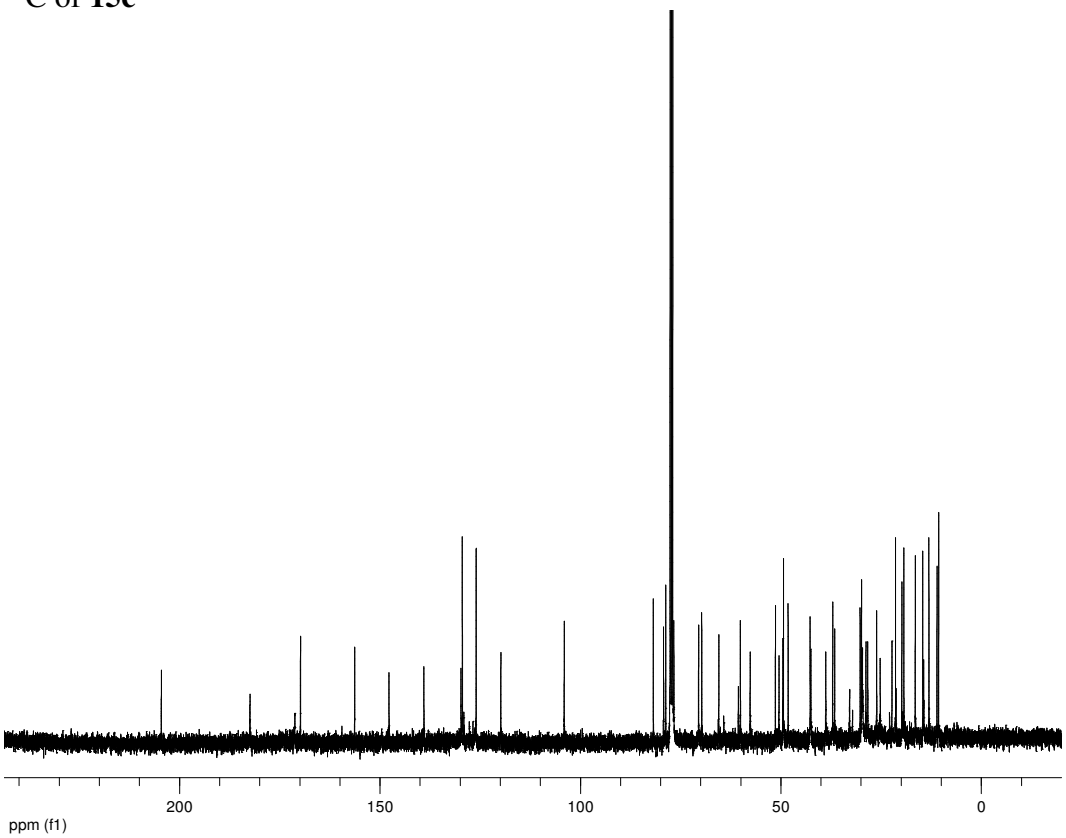

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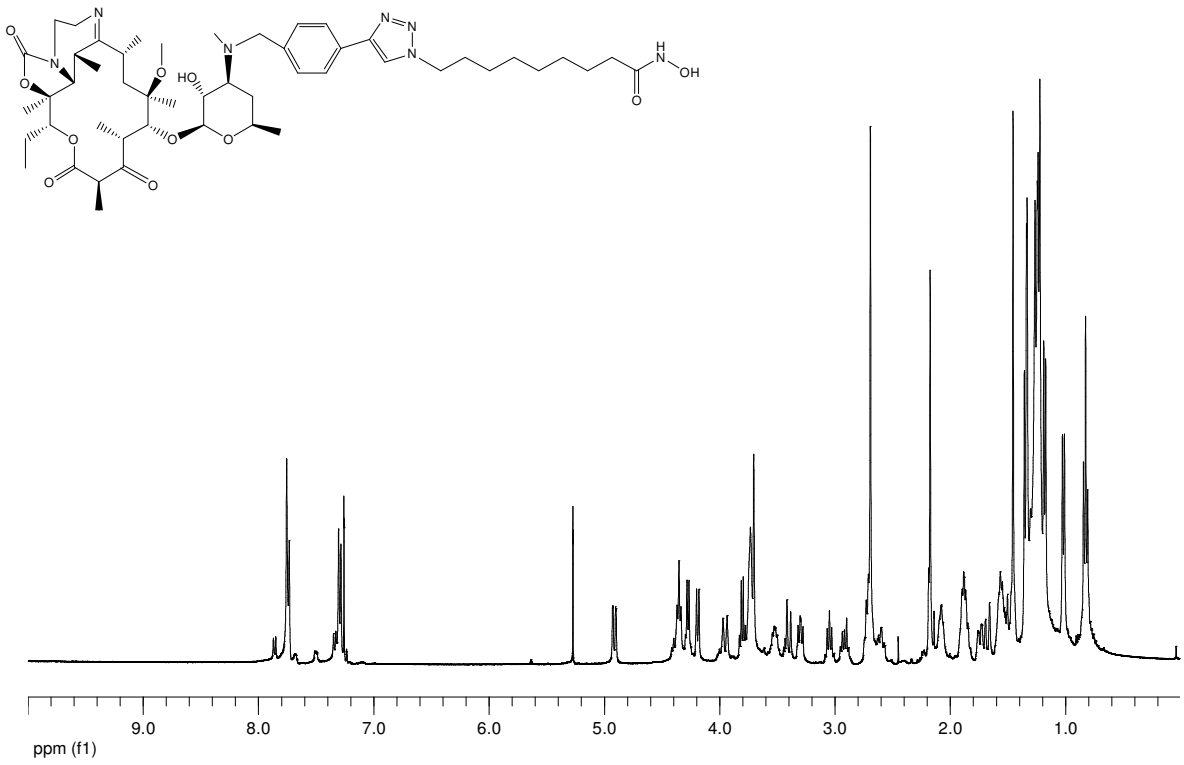

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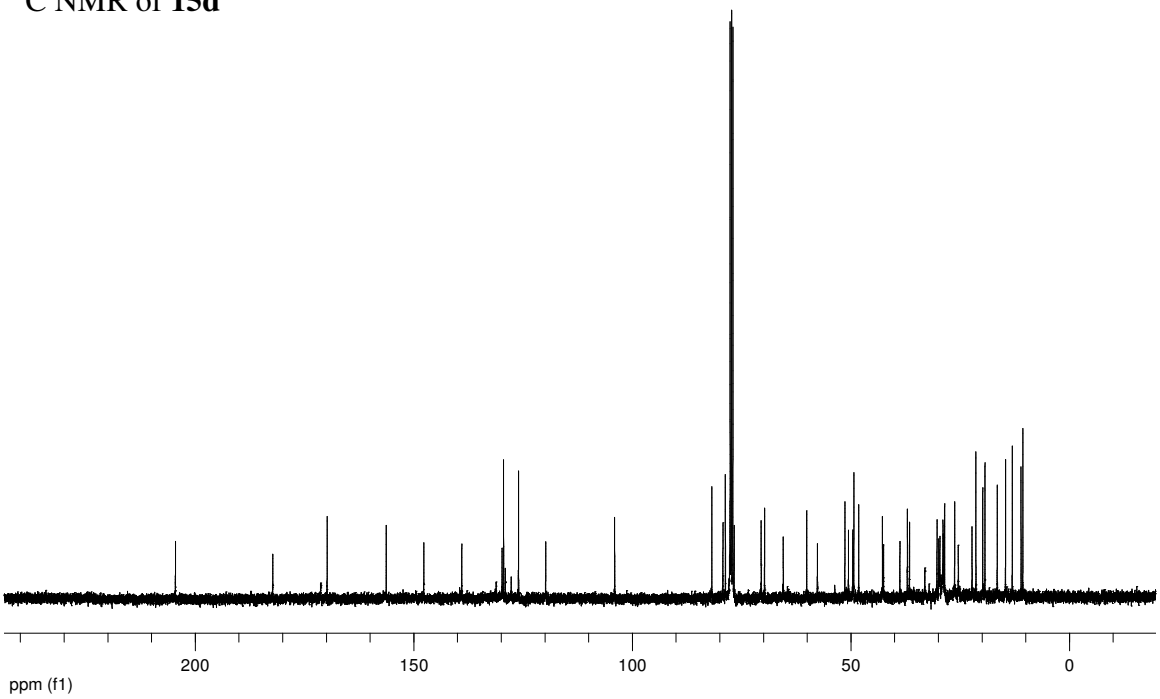

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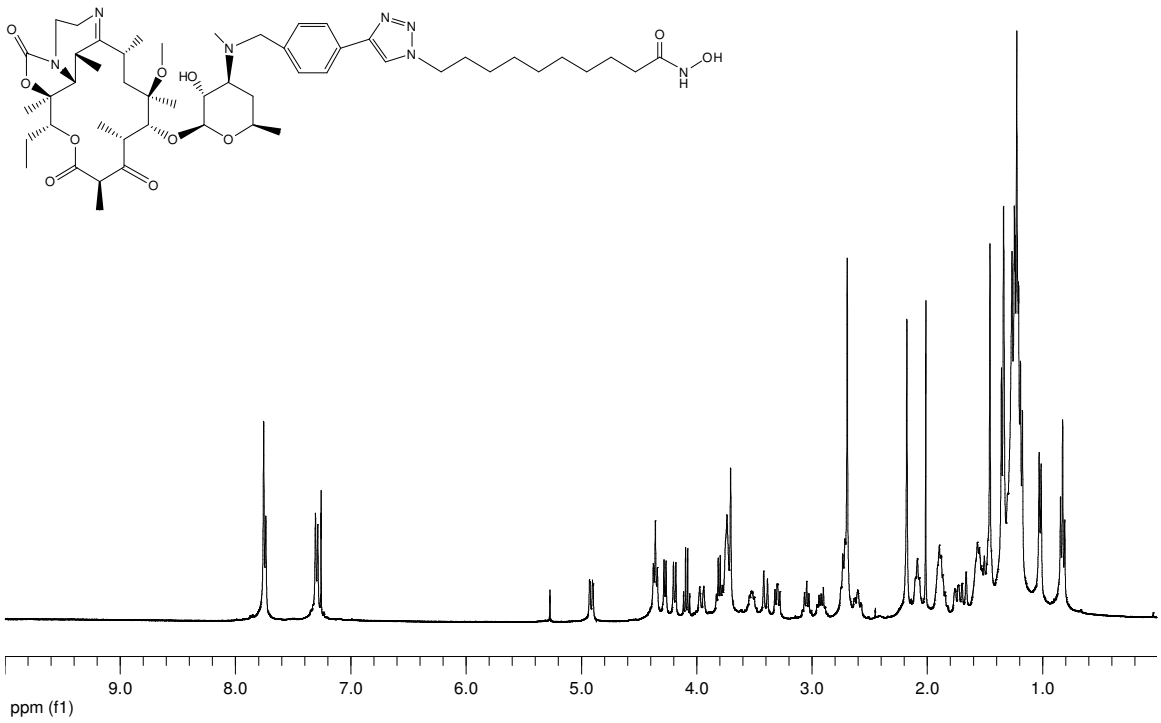
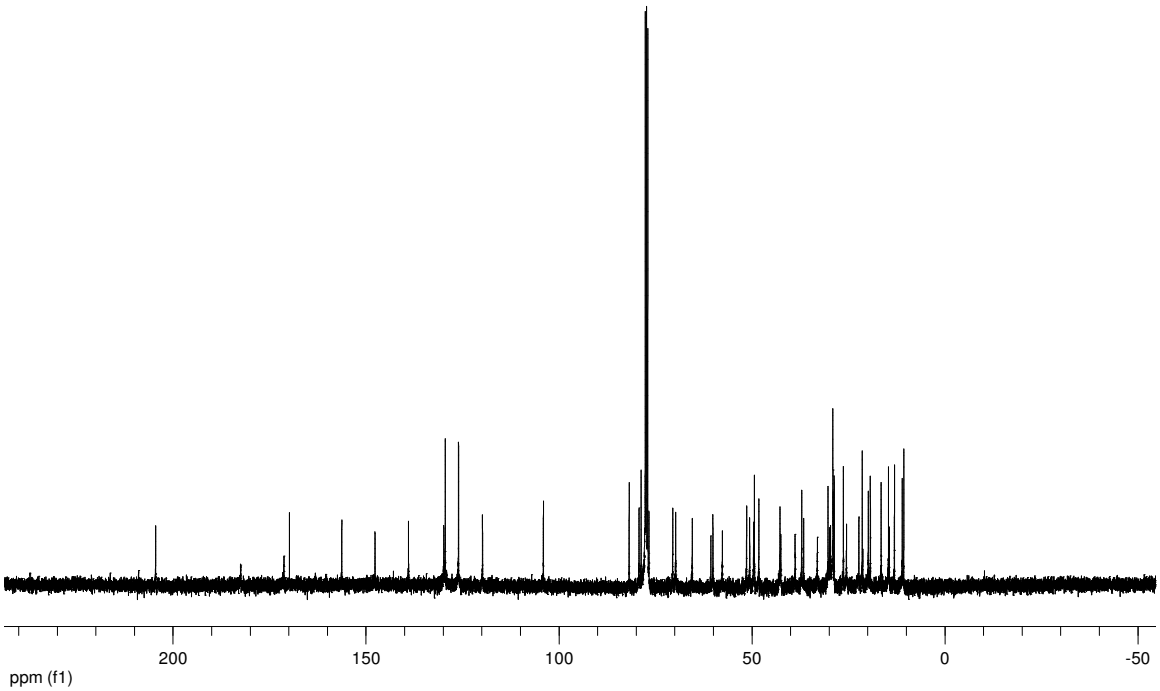
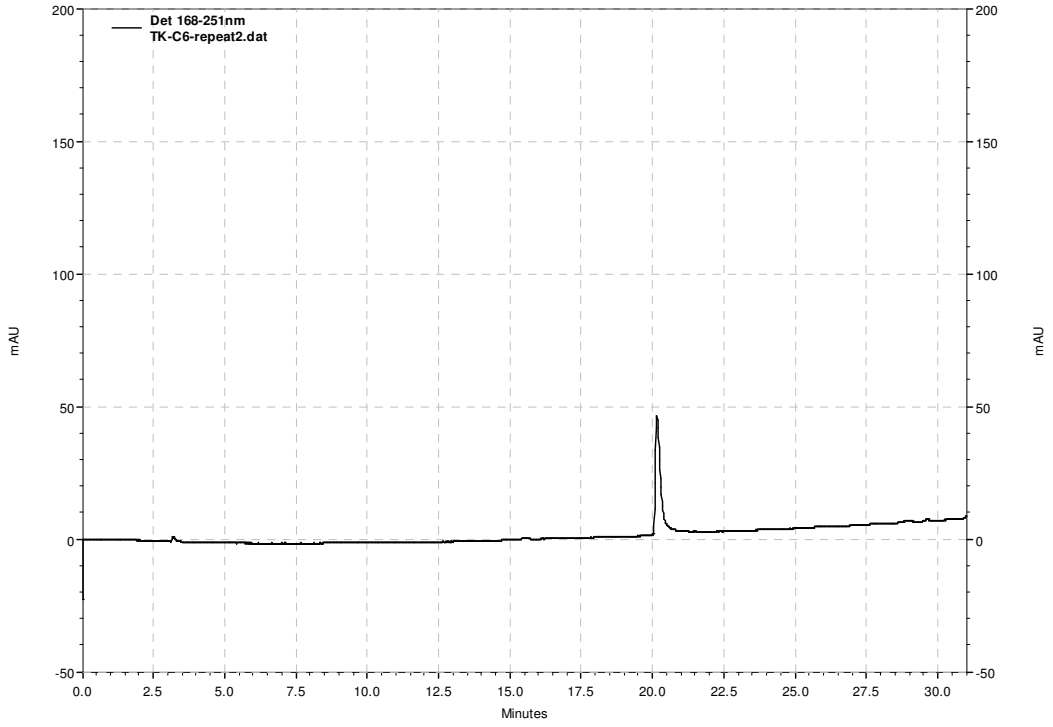


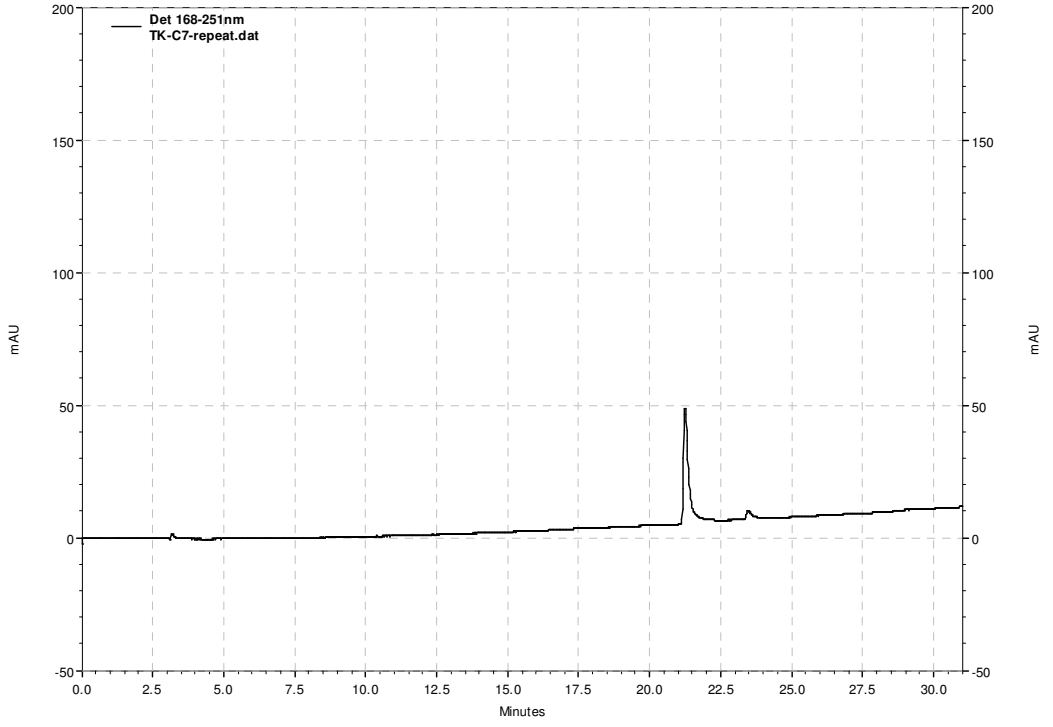
^{13}C NMR of **15e**



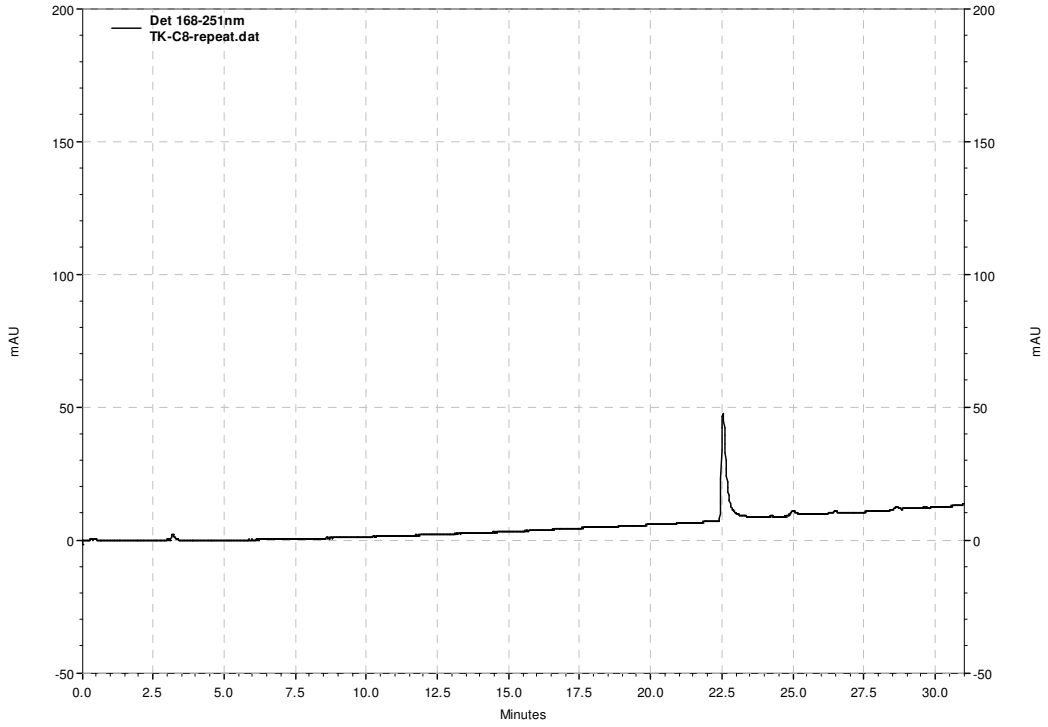
HPLC Trace of **15a**
Retention Time: 20.167 min



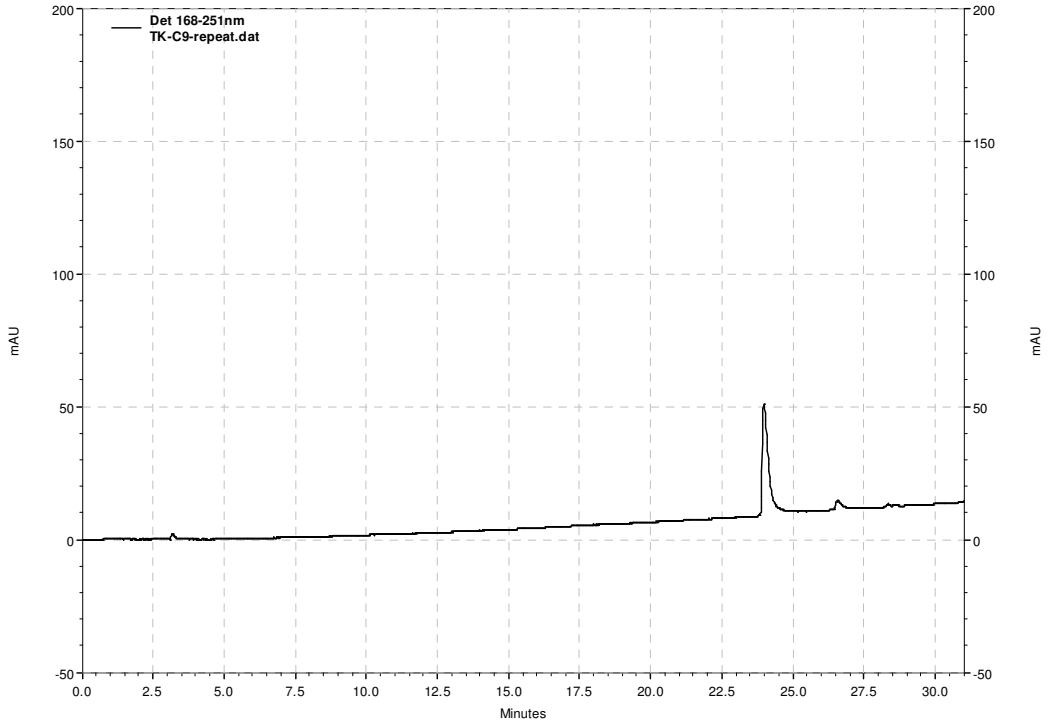
HPLC Trace of **15b**
Retention Time: 21.233 min



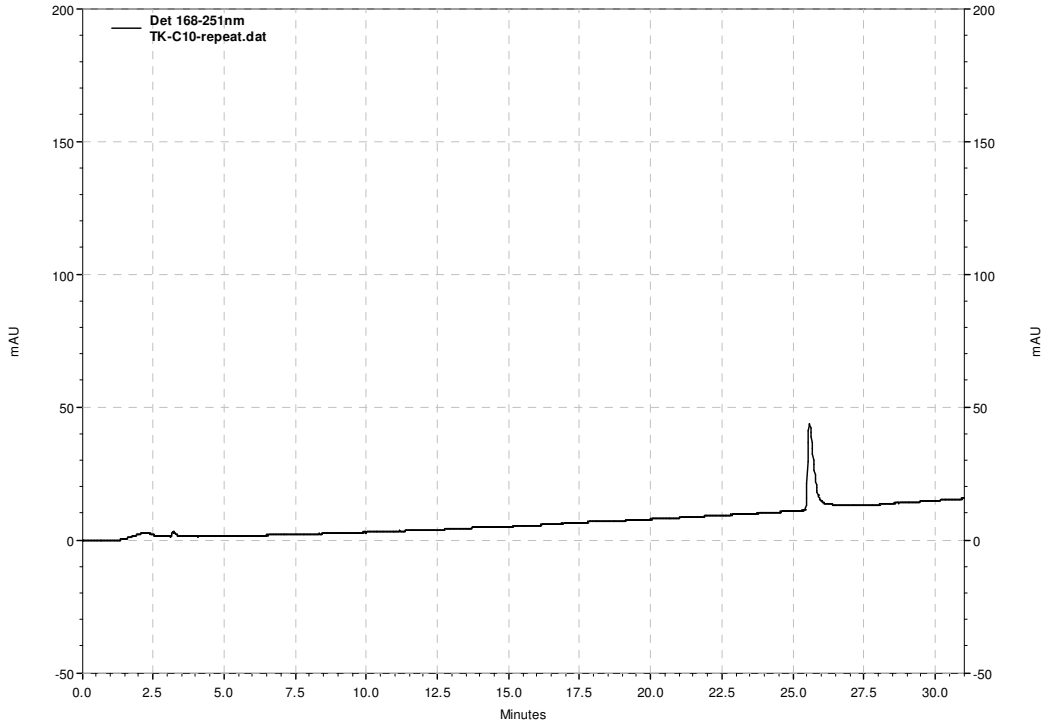
HPLC Trace of **15c**
Retention Time: 22.533 min



HPLC Trace of **15d**
Retention Time: 23.983 min



HPLC Trace of **15e**
Retention Time: 25.583 min



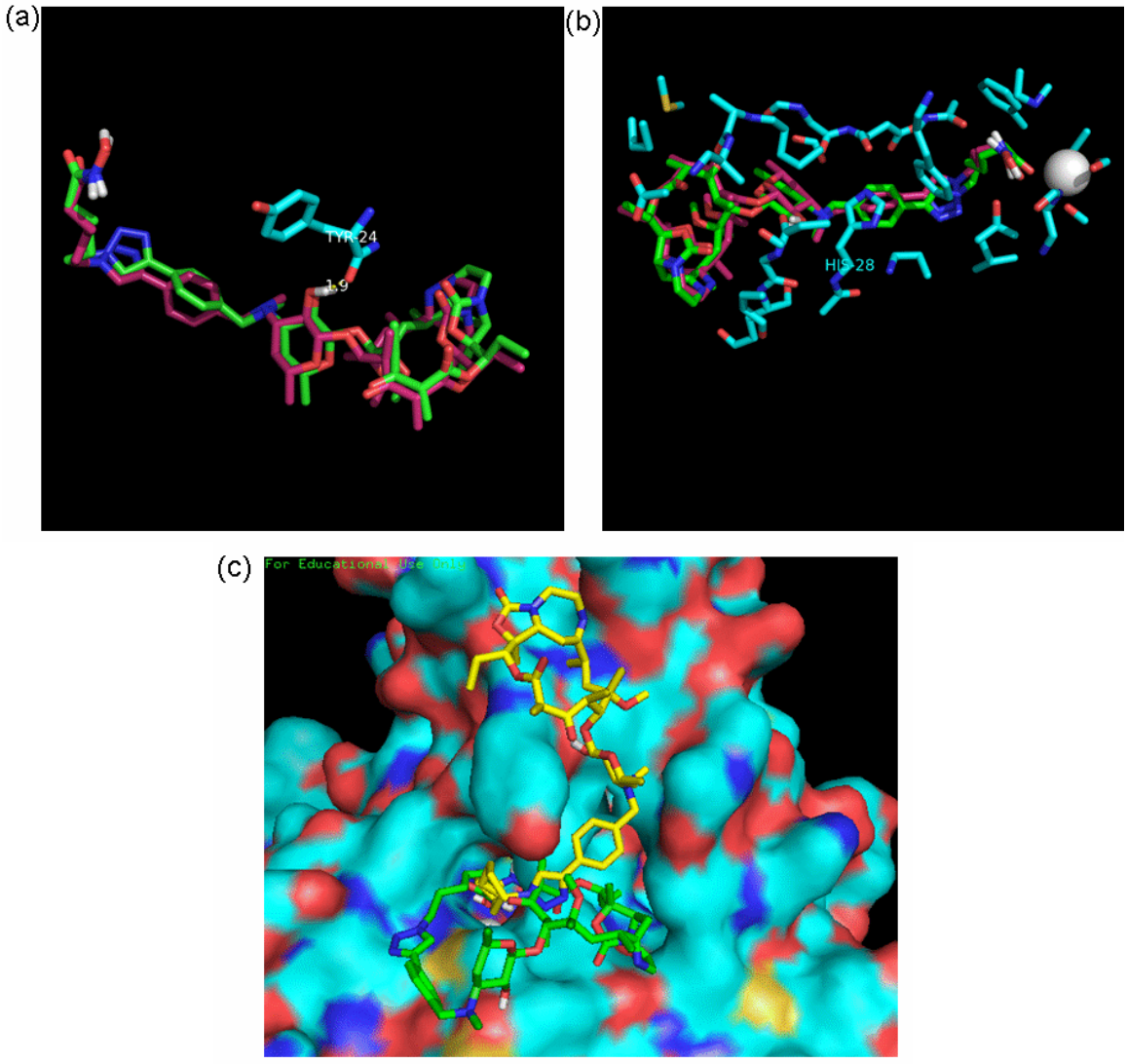


Figure S1: Docked structures of **15a-b** and **15e** at the outer rims of HDAC1 and HDAC8. (**a**) Compounds **15a** (pink) and **15b** (green) adopt docked orientations with their desosamine sugar 2'-OH group within 1.9 Å of the backbone carbonyl group of HDAC1 Tyr24 with which it could engage in a stabilizing H-bonding interaction. (**b**) A 5 Å cut of HDAC1 residues around **15a** (pink) and **15b** (green), note the placement of the His28 imidazole ring for a possible stacking interaction with the aryl moiety of **15b**. (**c**) Relative orientation of the macrocyclic rings of **15b** (green) and **15e** (yellow) within distinct hydrophobic pockets on HDAC8 outer rim.

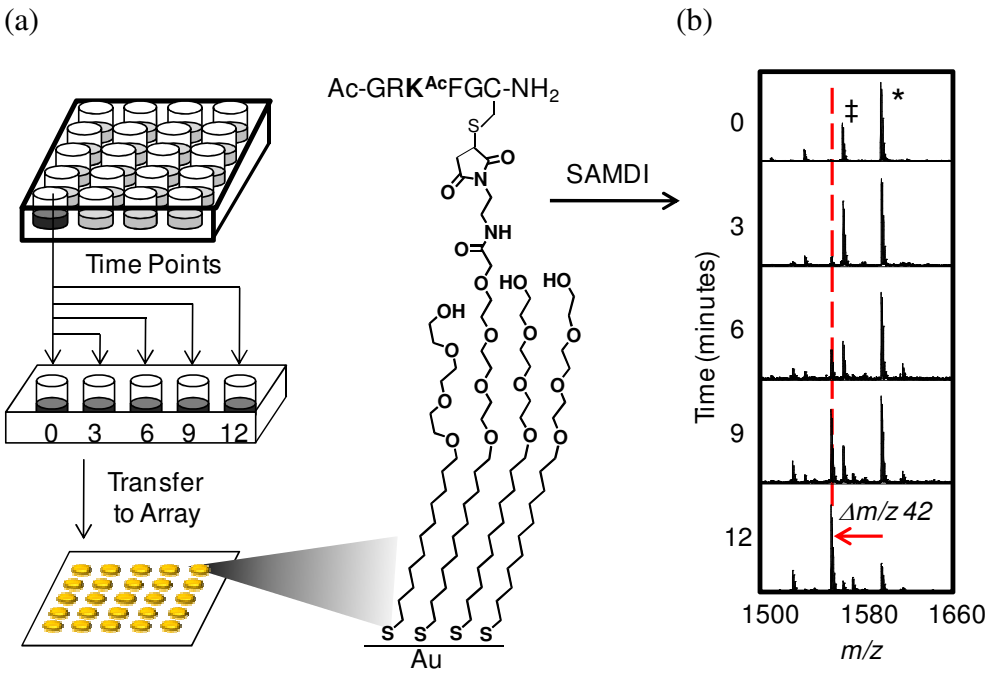
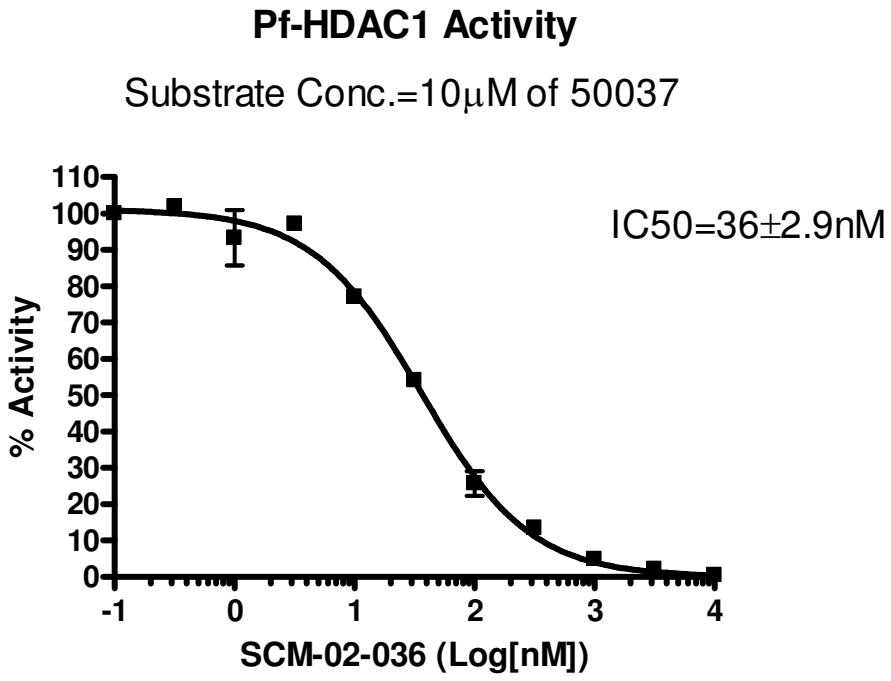


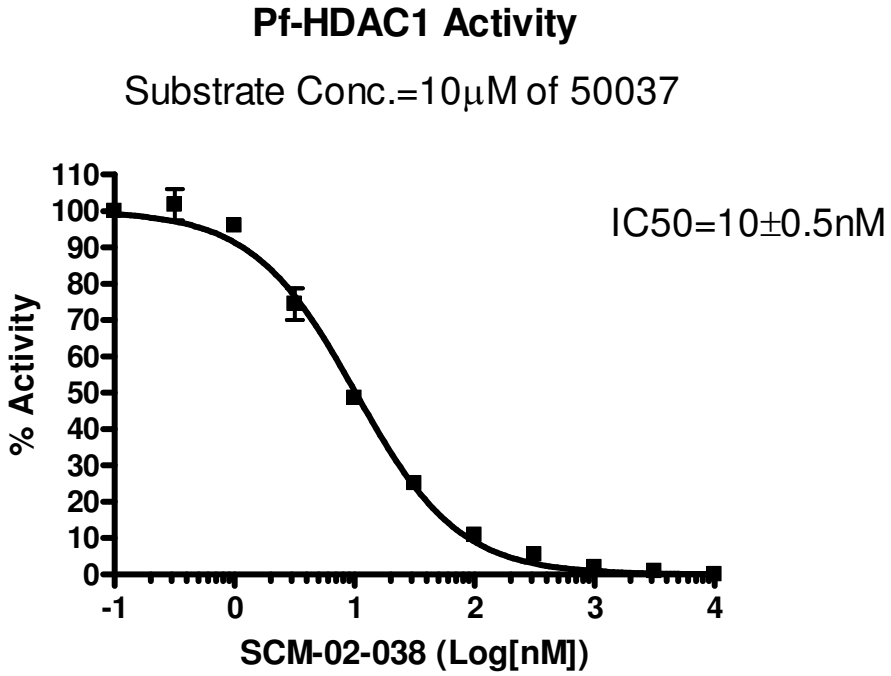
Figure S2: Schematic of the SAMDI Assay (Self-Assembled Monolayers for MALDI Mass Spectrometry). (a) Reactions were performed in a 96-well plate and reaction aliquots were removed at designated time points, terminated, and transferred to an array of gold circles presenting maleimide-terminated SAMs for substrate immobilization. (b) Representative SAMDI spectra after treatment with 250 nM **15b** where a shift of *m/z* 42 signifies deacetylation. (* indicates acetylated peptide; ‡ indicates a fragment from loss of sulfur; red line indicates deacetylated peptide)

Pf-HDAC1 Activity Dose-response Curves:

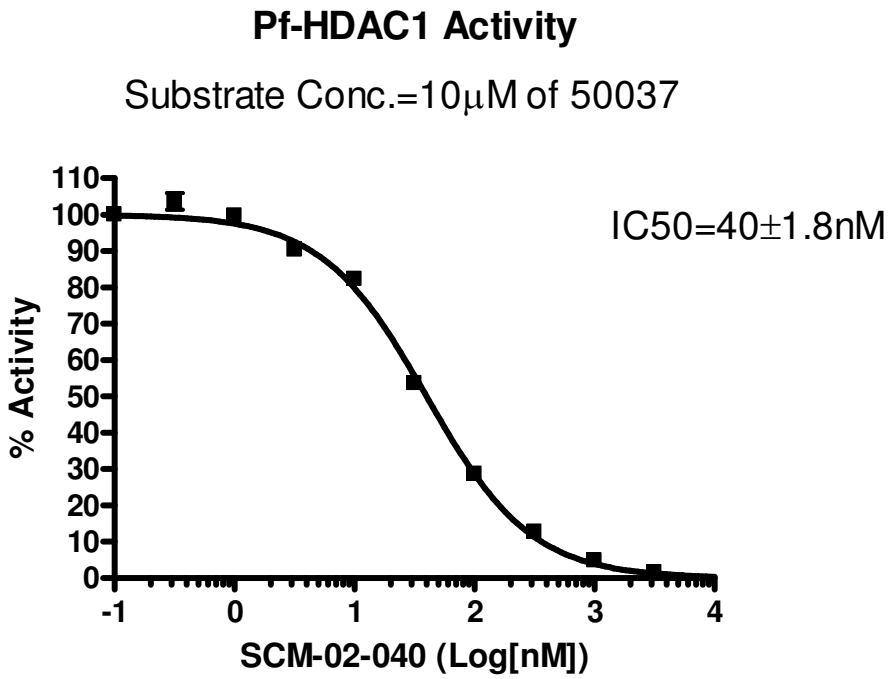
Compound 15a



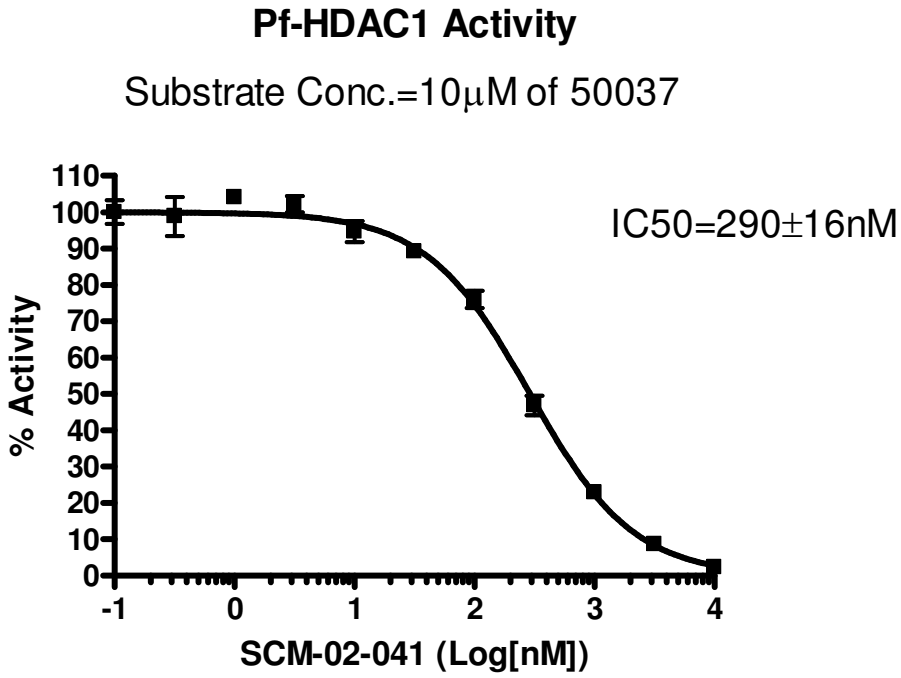
Compound 15b



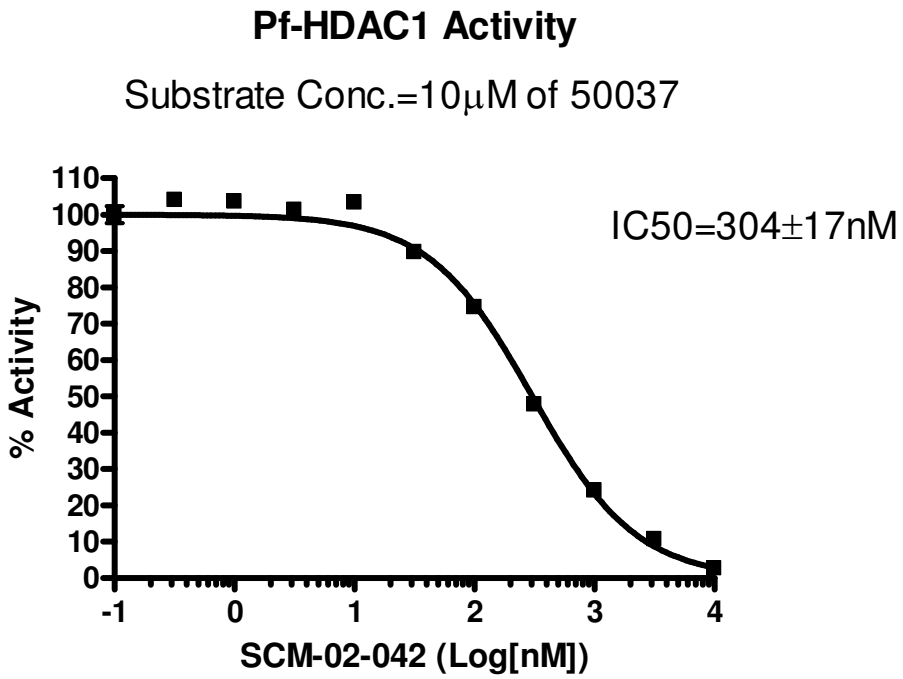
Compound 15c



Compound 15d



Compound 15e



SAHA