

Supporting Information

Evaluation of synthetic substituted 1,2-dioxanes as novel agents against human leishmaniasis.

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[§] *These authors equally contributed to this work.*

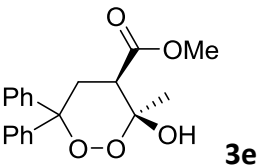
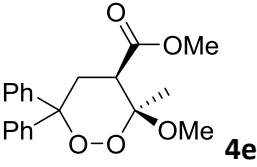
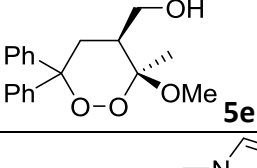
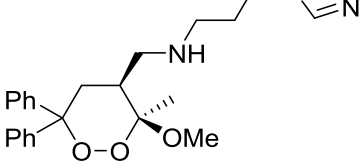
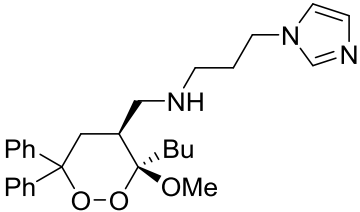
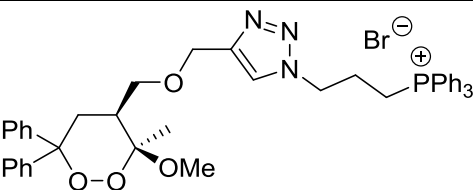
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A. Comparison Assays.

As carried out for C6-butyl endoperoxides (Table 1 in the manuscript), the C6-phenyl-substituted synthetic intermediates 3,4-*cis* **3e**, 3,4-*cis* **4e**, and 3,4-*cis* **5e** were tested on promastigotes of *L. donovani*. This study was aimed to confirm the crucial role played by the C4-side chain also in the presence of phenyl groups on C6. As already demonstrated for C6-butyl endoperoxides (Table 1 in the manuscript), the C6-phenyl derivatives resulted inactive (at 40 μM) when substituted on C4 with a methyl ester (**3e** and **4e**, Table S1) or with a hydroxymethyl group (**5e**, Table S1).

Table S1.

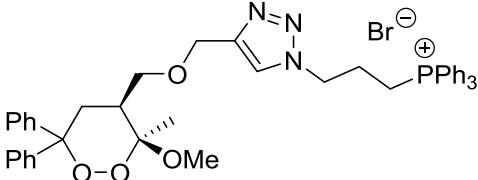
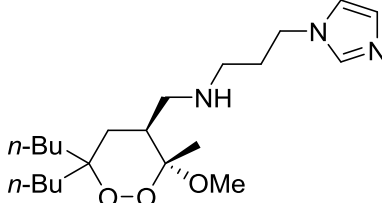
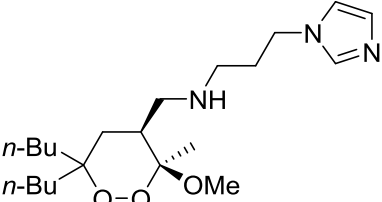
| Compound ^a | <i>L. donovani</i> promastigotes IC ₅₀ (μM) ^b | Vero CC ₅₀ (μM) ^c | SI ^d |
|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------|-----------------|
|  3e | >40 | nd | nd |
|  4e | >40 | nd | nd |
|  5e | >40 | nd | nd |
|  8g | 7.5 | 50.0 | 6.7 |
|  8h | 4.2 | 9.5 | 2.3 |
|  8p | 11.5 | 280.0 | 24.3 |

^a Compounds tested as racemates. ^b IC₅₀ represents the concentration of a compound that causes 50% growth inhibition. The experimental error was in the range 1 – 2.5 μM . See Section B (pag. S3) for details. ^c CC₅₀ represents 50% cytotoxic concentration. ^d Selectivity index (SI) = CC₅₀/IC₅₀. nd = not determined due to the low antileishmanial potency.

B. Experimental Error Evaluation.

To evaluate the experimental error associated to IC₅₀ results, we carried out three independent experiments for each selected compound for both *L. donovani* promastigotes (Table S2) and *L. donovani* amastigotes (Table S3). The experimental error associated to the bioassay protocol was in the range 1 – 2.5 μM. The evaluated experimental error allowed us to usefully compare the IC₅₀ values obtained for the endoperoxides library that resulted significantly different.

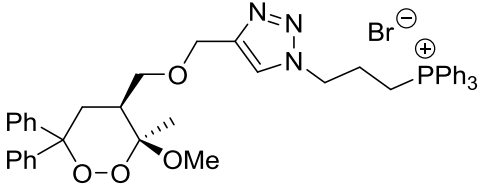
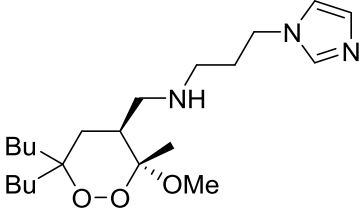
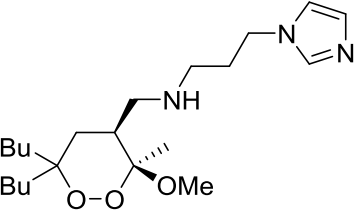
Table S2.

| Compound ^a | <i>L. donovani</i> promastigotes | | | SD ^c | IC ₅₀ (mean) ± SD (μM) |
|-----------------------------------------------------------------------------------------------|------------------------------------|------|------|-----------------|-----------------------------------|
| | IC ₅₀ (μM) ^b | | | | |
|  8p | 11.5 | 16.0 | 14.0 | 2.25 | 13.8 ± 2.3 |
|  8l | 4.0 | 4.7 | 6.8 | 1.46 | 5.2 ± 1.5 |
|  8b | 5.5 | 8.7 | 8.2 | 1.72 | 7.5 ± 1.7 |

^a Compounds tested as racemates. ^b IC₅₀ represents the concentration of a compound that causes 50 % growth inhibition.

^c SD = standard deviation.

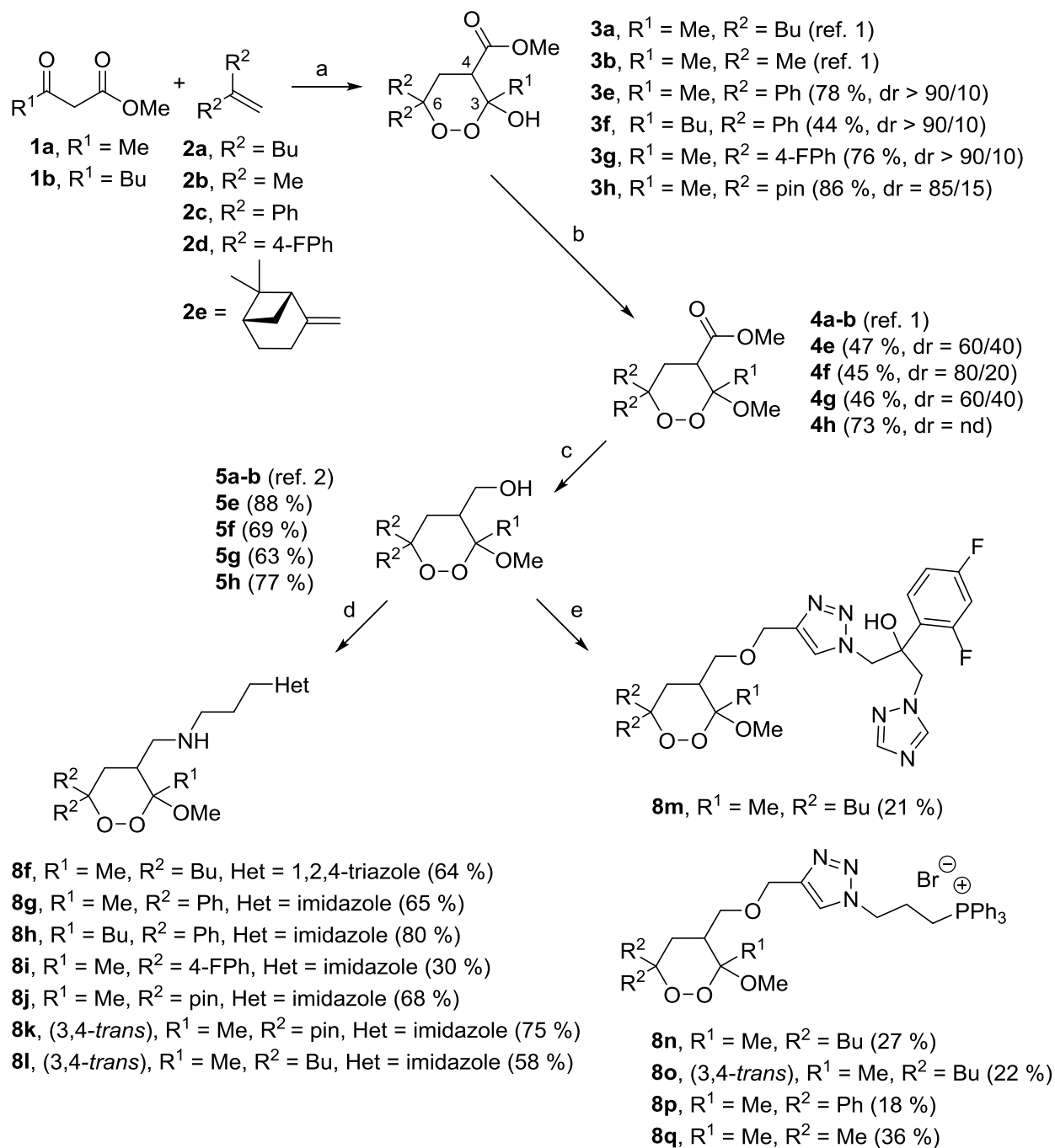
Table S3.

| Compound ^a | <i>L. donovani</i> amastigotes IC ₅₀ (μM) ^b | | | SD ^c | IC ₅₀ (mean) ± SD (μM) |
|-------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|------|------|-----------------|--------------------------------------|
| | | | | | |
|  <p style="text-align: right;">8p</p> | 15.0 | 18.0 | 16.5 | 1.50 | 16.5 ± 1.5 |
|  <p style="text-align: right;">8l</p> | 10.3 | 12.0 | 8.0 | 2.01 | 10.1 ± 2.0 |
|  <p style="text-align: right;">8b</p> | 12.0 | 13.5 | 11.0 | 1.26 | 12.2 ± 1.3 |

^a Compounds tested as racemates. ^b IC₅₀ represents the concentration of a compound that causes 50 % growth inhibition.

^c SD = standard deviation.

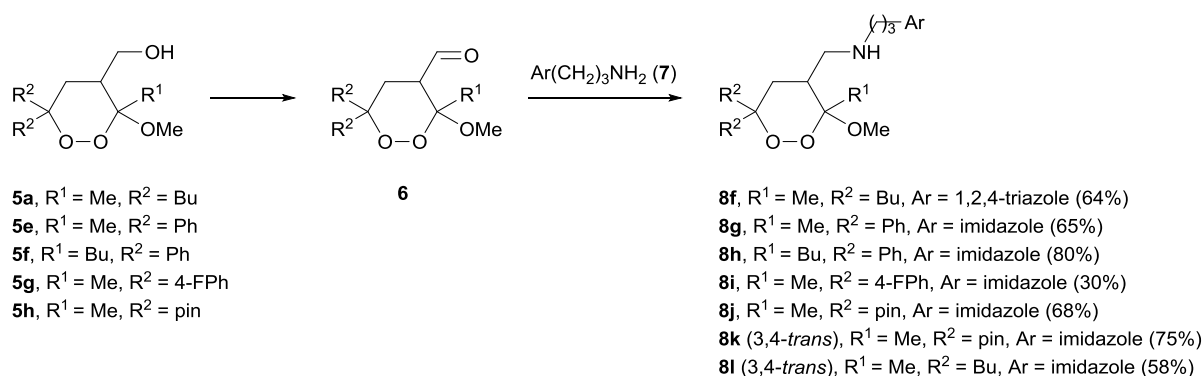
C. Synthetic Procedures.



Scheme S1.

- 1) M. Persico, A. Quintavalla, F. Rondinelli, C. Trombini, M. Lombardo, C. Fattorusso, V. Azzarito, D. Taramelli, S. Parapini, Y. Corbett, G. Chianese, E. Fattorusso, O. Tagliatela-Scafati, A New Class of Antimalarial Dioxanes Obtained through a Simple Two-Step Synthetic Approach: Rational Design and Structure–Activity Relationship Studies, *J. Med. Chem.* 54 (2011) 8526–8540. <http://dx.doi.org/10.1021/jm201056j>.
- 2) M. Persico, S. Parapini, G. Chianese, C. Fattorusso, M. Lombardo, L. Petrizza, A. Quintavalla, F. Rondinelli, N. Basilico, D. Taramelli, C. Trombini, E. Fattorusso, O. Tagliatela-Scafati, Further optimization of plakortin pharmacophore: Structurally simple 4-oxymethyl-1,2-dioxanes with promising antimalarial activity, *Eur. J. Med. Chem.* 70 (2013) 875-886. <http://dx.doi.org/10.1016/j.ejmech.2013.10.050>.

The general procedures for the synthesis of 3-hydroxy-1,2-dioxanes **3** (step a, Scheme S1), 3-methoxy-1,2-dioxanes **4** (step b, Scheme S1), 4-hydroxymethyl-1,2-dioxanes **5** (step c, Scheme S1), 4-aminopropyl derivatives **8f-l** (step d, Scheme S1), 4-triazolyl ether derivatives **8m-q** (step e, Scheme S1) were described in the experimental section of the manuscript. Herein, further details concerning the synthesis of intermediate 4-carbaldehyde-1,2-dioxanes **6** (Scheme S2), amines **7** (Schemes S2-S3), 4-propargyl ethers **9** (Scheme S4) and azides **10** (Schemes S4-S6) were provided.



Scheme S2.

cis-**6a**, 89% yield.

trans-**6a**, 83% yield.

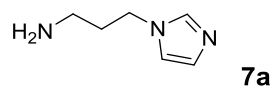
6e, 83% yield.

6f, 93% yield.

6g, 75% yield.

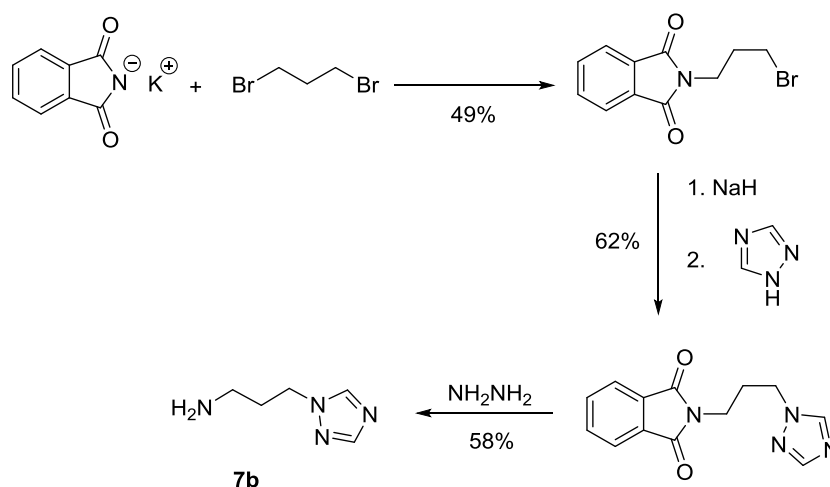
cis-**6h**, 89% yield.

trans-**6h**, 83% yield.



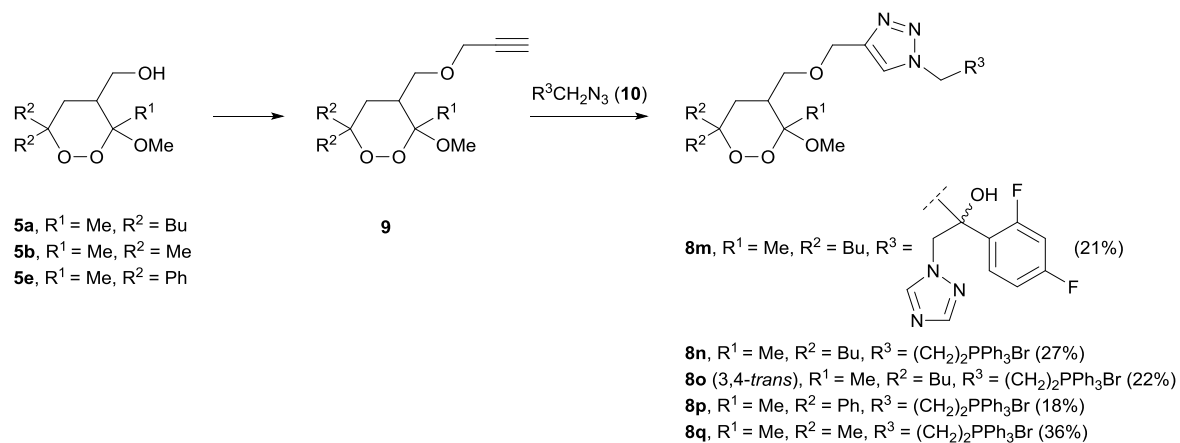
Aminopropyl imidazole **7a** was commercially available.

Aminopropyl 1,2,4-triazole **7b** was synthesized following a literature procedure³ (Scheme S3) and the spectroscopic data and physical properties of the obtained product were identical to the reported ones.⁴



Scheme S3.

- 3) V. Gauchot, M. Branca, A. Schmitzer, Encapsulation of a Catalytic Imidazolium Salt into Avidin: Towards the Development of a Biohybrid Catalyst Active in Ionic Liquids, *Chem. Eur. J.* 20 (2014) 1530-1538. <http://dx.doi.org/10.1002/chem.201303865>.
- 4) P. Gaillard, J.-P. Gotteland, I. Jeanclaude-Etter, M. Schwarz, R. J. Thomas, Azole methyldene cyanide derivatives and their use as protein kinase modulators, WO03/106455A1 (2003).



Scheme S4.

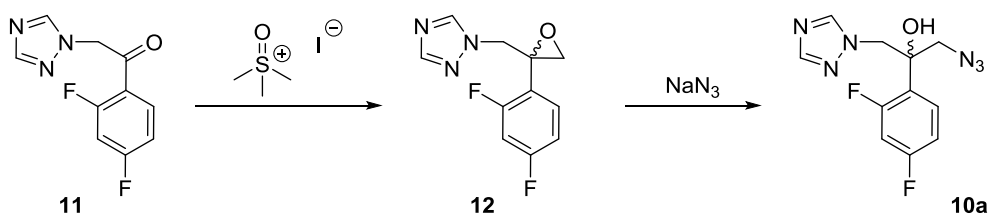
cis-**9a**, 47% yield.

trans-**9a**, 50% yield (DMF used as solvent).

9b, 52% yield.

9e, 30% yield.

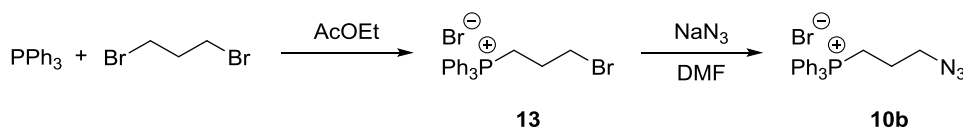
Azide **10a** was synthesized as follows (Scheme S5):



Scheme S5.

Commercially available ketone **11** was first converted into epoxide **12**,⁵ which was then transformed in the azidoalcohol **10a**⁶ following literature procedures and the spectroscopic data and physical properties of the obtained products were identical to the reported ones.

Azide **10b** was synthesized as follows (Scheme S6):



Scheme S6.

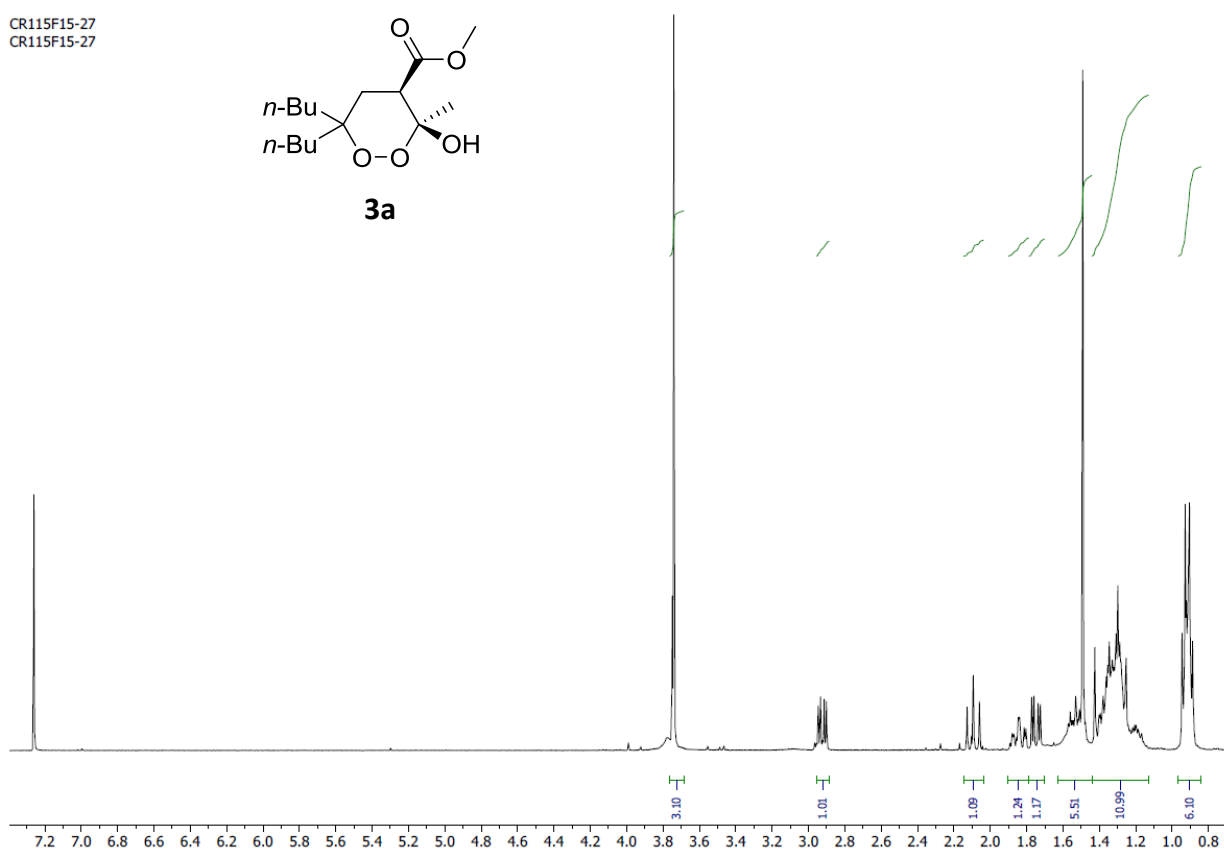
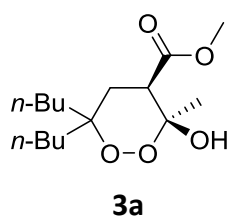
Triphenylphosphine (10 mmol) was dissolved in AcOEt (10 mL) and dibromopropane (4 equiv.) was added. The mixture was stirred overnight at room temperature and a white solid was observed. The mixture was refluxed for 8 h and, then, quenched by adding AcOEt with the aim to favor the phosphonium salt precipitation. The desired product **13** was isolated by filtration (75 % yield).

The obtained phosphonium salt **13** (2.5 mmol) was dissolved in DMF (5 mL) and NaN_3 (1.1 equiv.) was added. The mixture was stirred overnight at room temperature. Then, CH_2Cl_2 (10 mL) was added and the organic phase was repeatedly washed with water (5 x 7 mL) to eliminate DMF . Lastly, Et_2O was added to the organic phase to favor the product precipitation. The desired product **10b** was isolated by filtration (85 % yield). The spectroscopic data and physical properties of the obtained product were identical to the reported ones.⁷

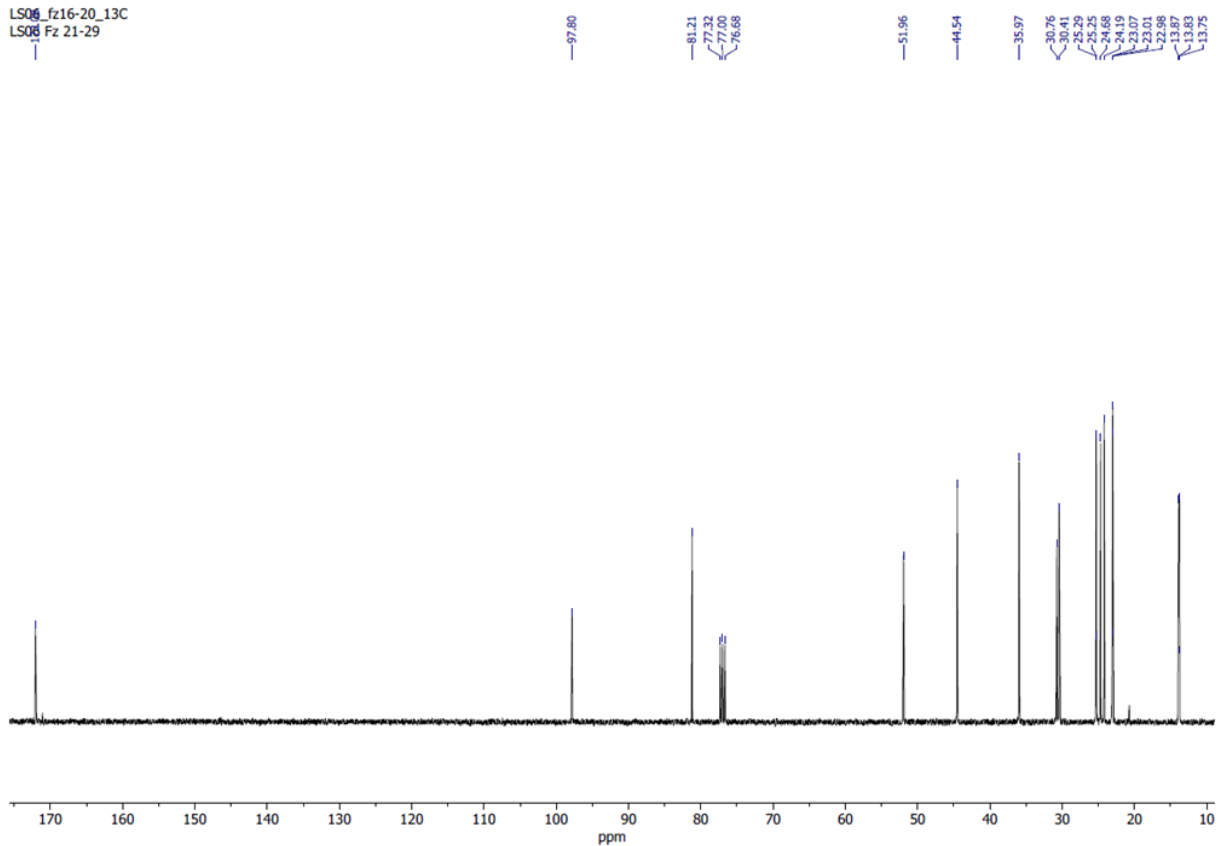
- 5) R. S. Upadhayaya, S. Jain, N. Sinha, N. Kishore, R. Chandra, S. K. Arora, Synthesis of novel substituted tetrazoles having antifungal activity, *Eur. J. Med. Chem.* 39 (2004) 579–592. <http://dx.doi.org/10.1016/j.ejmech.2004.03.004>.
- 6) T. Konosu, Y. Tajima, N. Takeda, T. Miyaoka, M. Kasahara, H. Yasuda, S. Oida, Triazole antifungals. II. Synthesis and antifungal activities of 3-acyl-4-methyloxazolidine derivatives, *Chem. Pharm. Bull.* 38 (1990) 2476–2486.
- 7) J. D. Knight, S. J. Sauer, D. M. Coltart, Asymmetric Total Synthesis of the Antimalarial Drug (+)-Mefloquine Hydrochloride via Chiral *N*-Amino Cyclic Carbamate Hydrazones, *Org. Lett.* 13 (2011) 3118–3121. <http://dx.doi.org/10.1021/ol2010193>.

D. NMR Spectra.

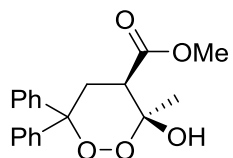
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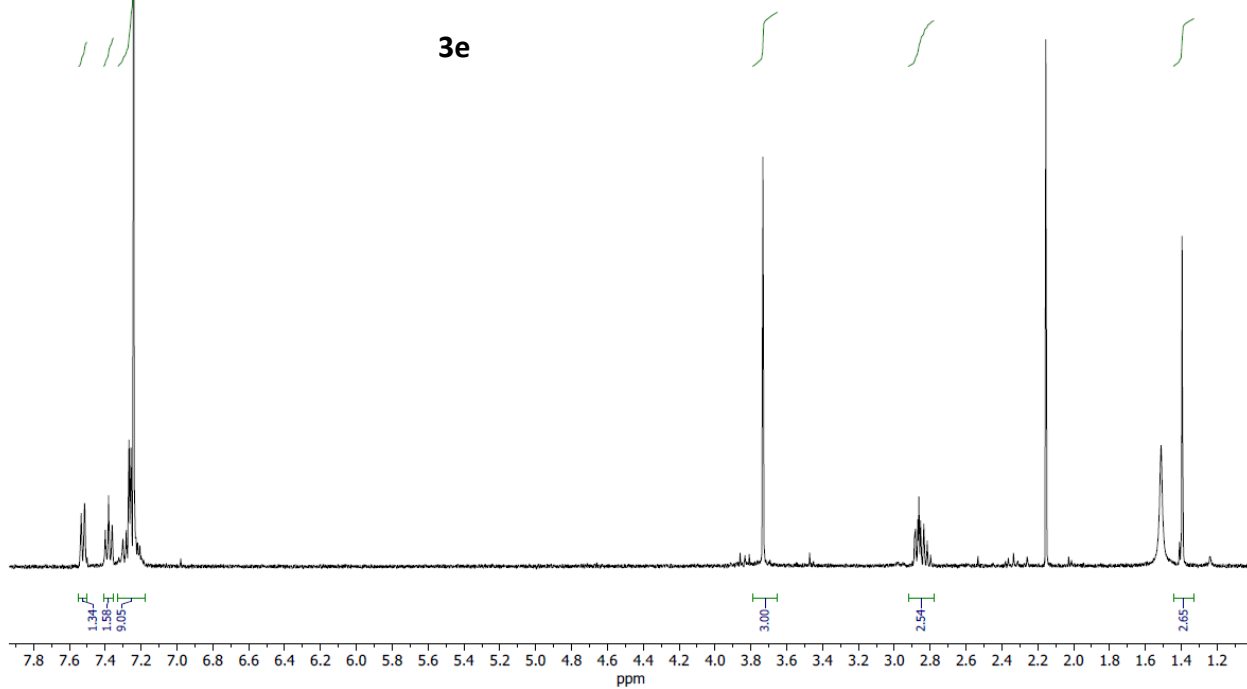
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LS08 Fz 21-29



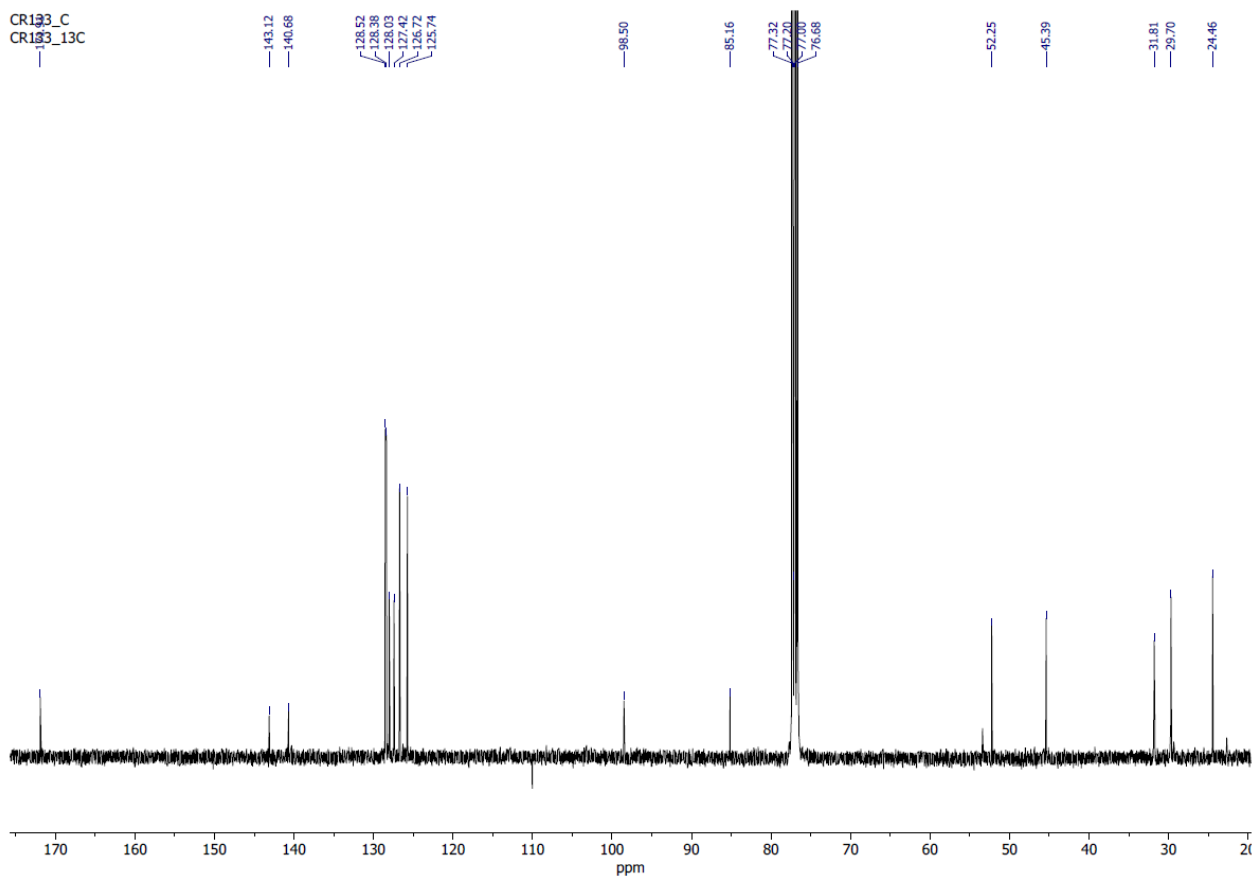
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cr133gr_solido



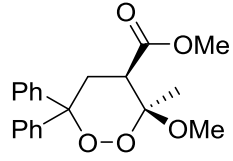
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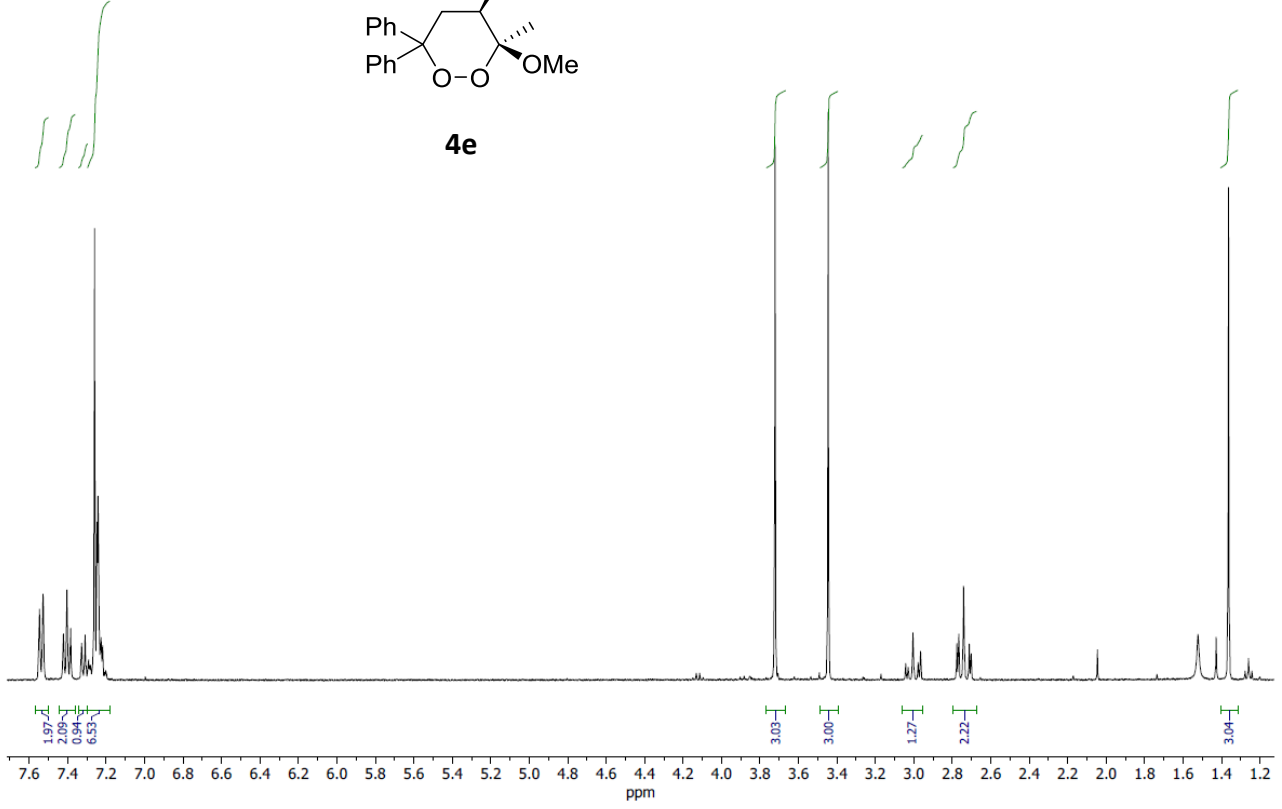
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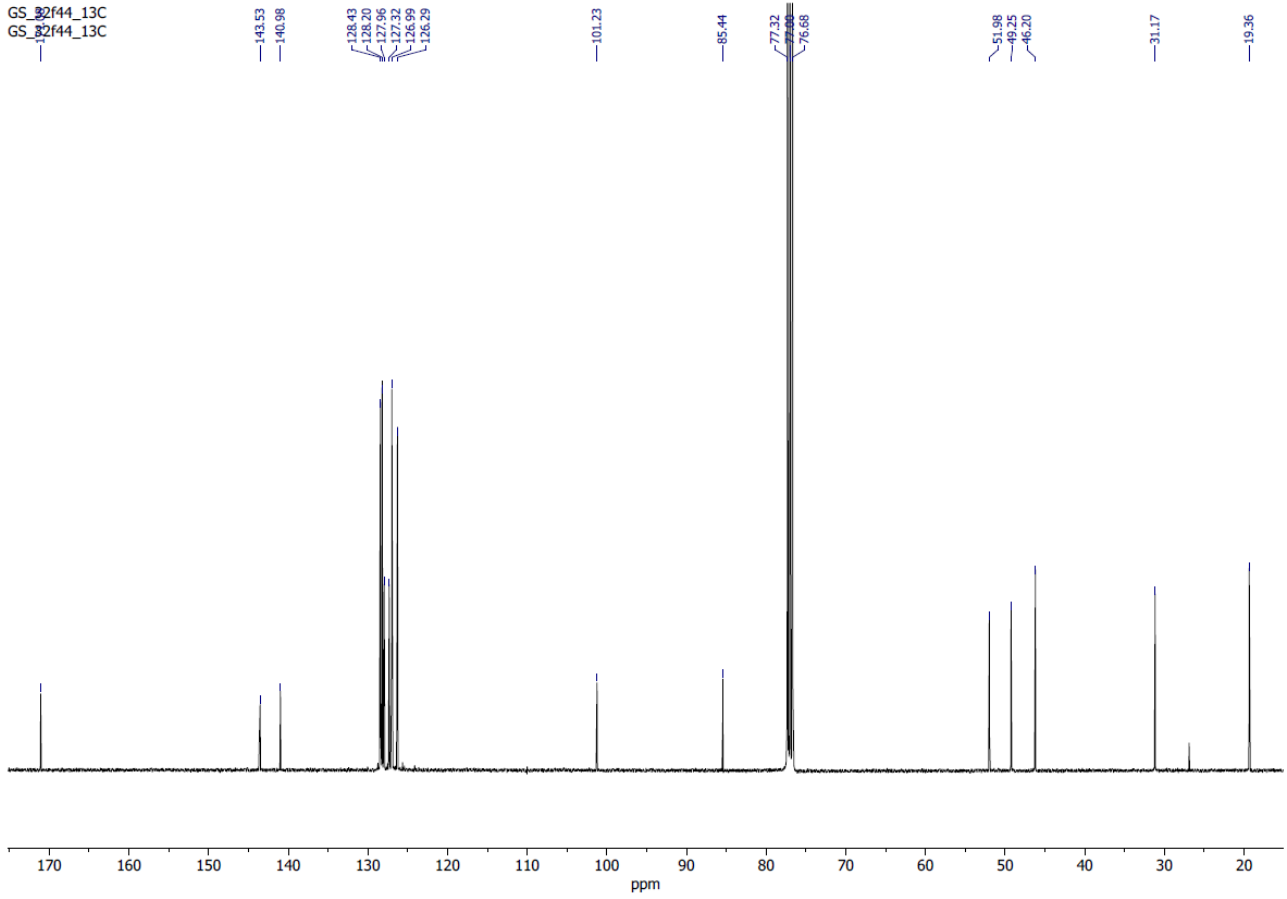
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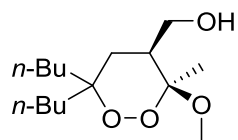
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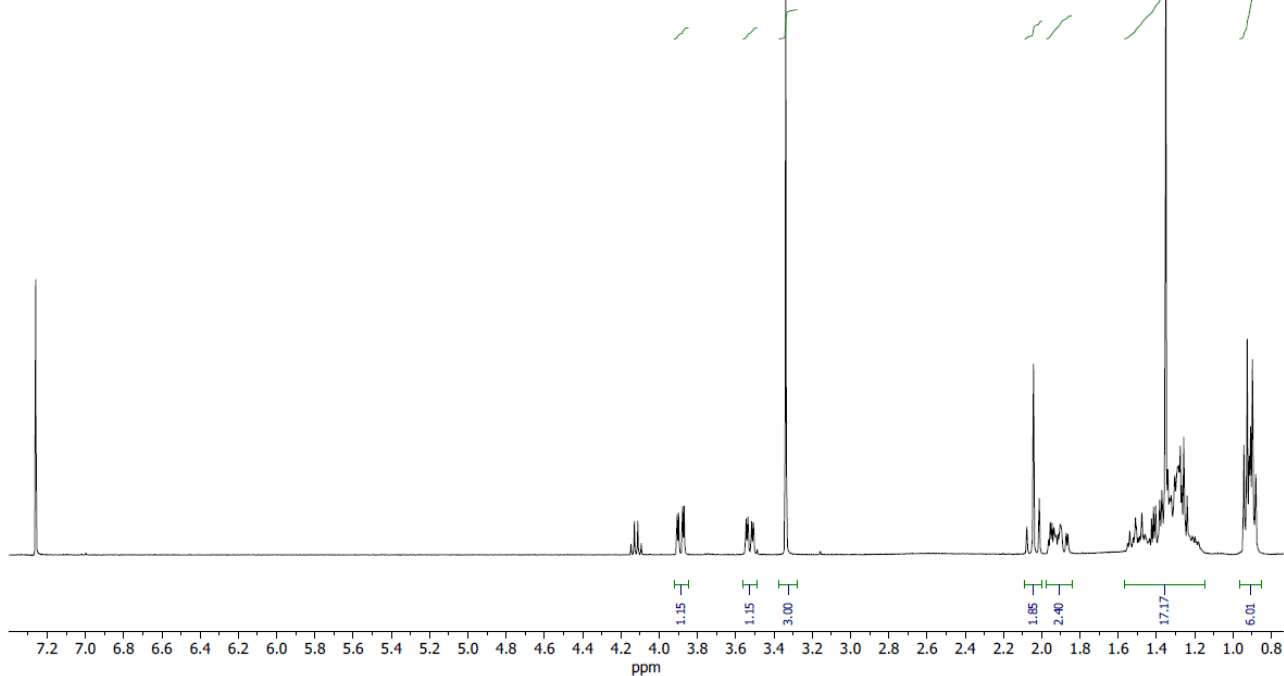
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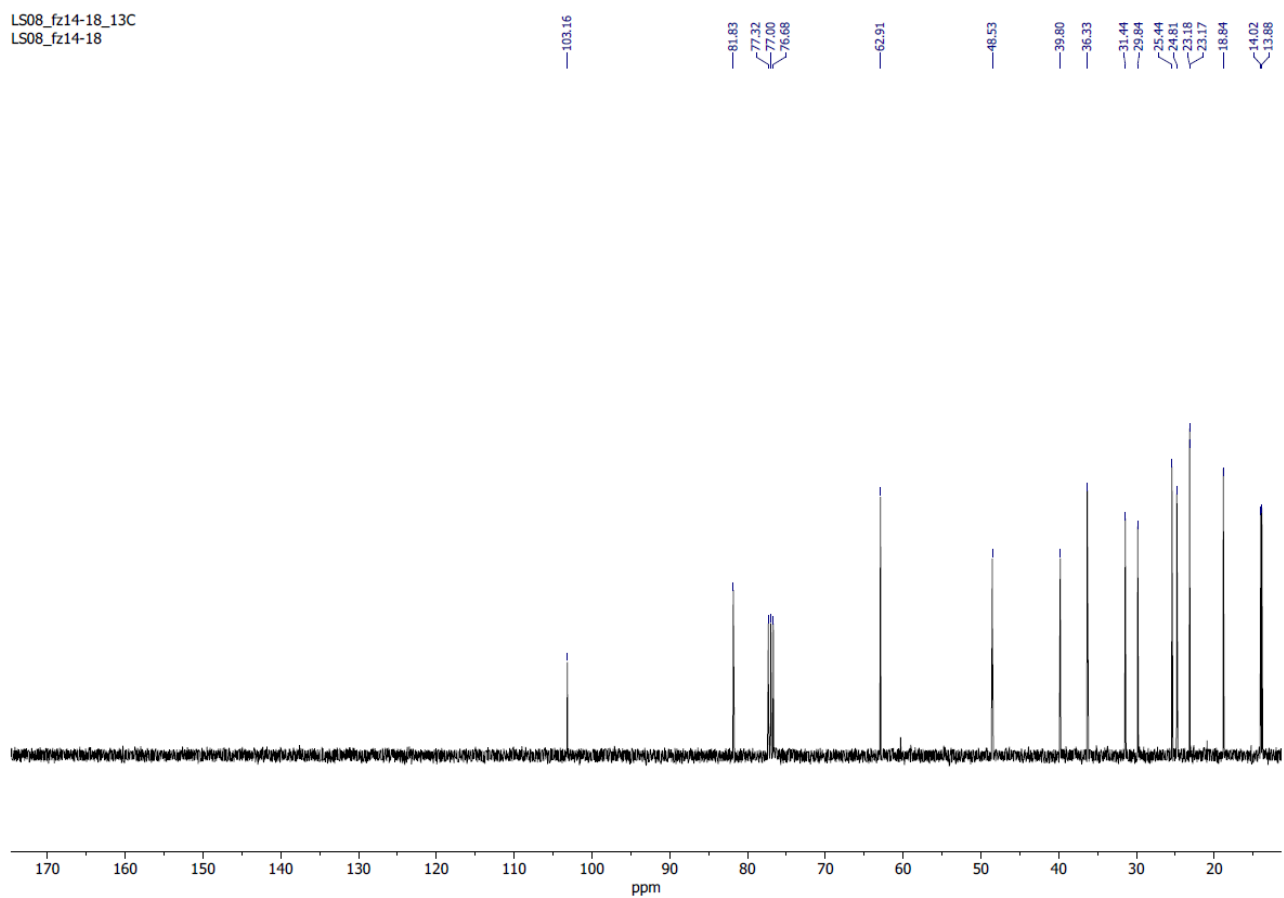
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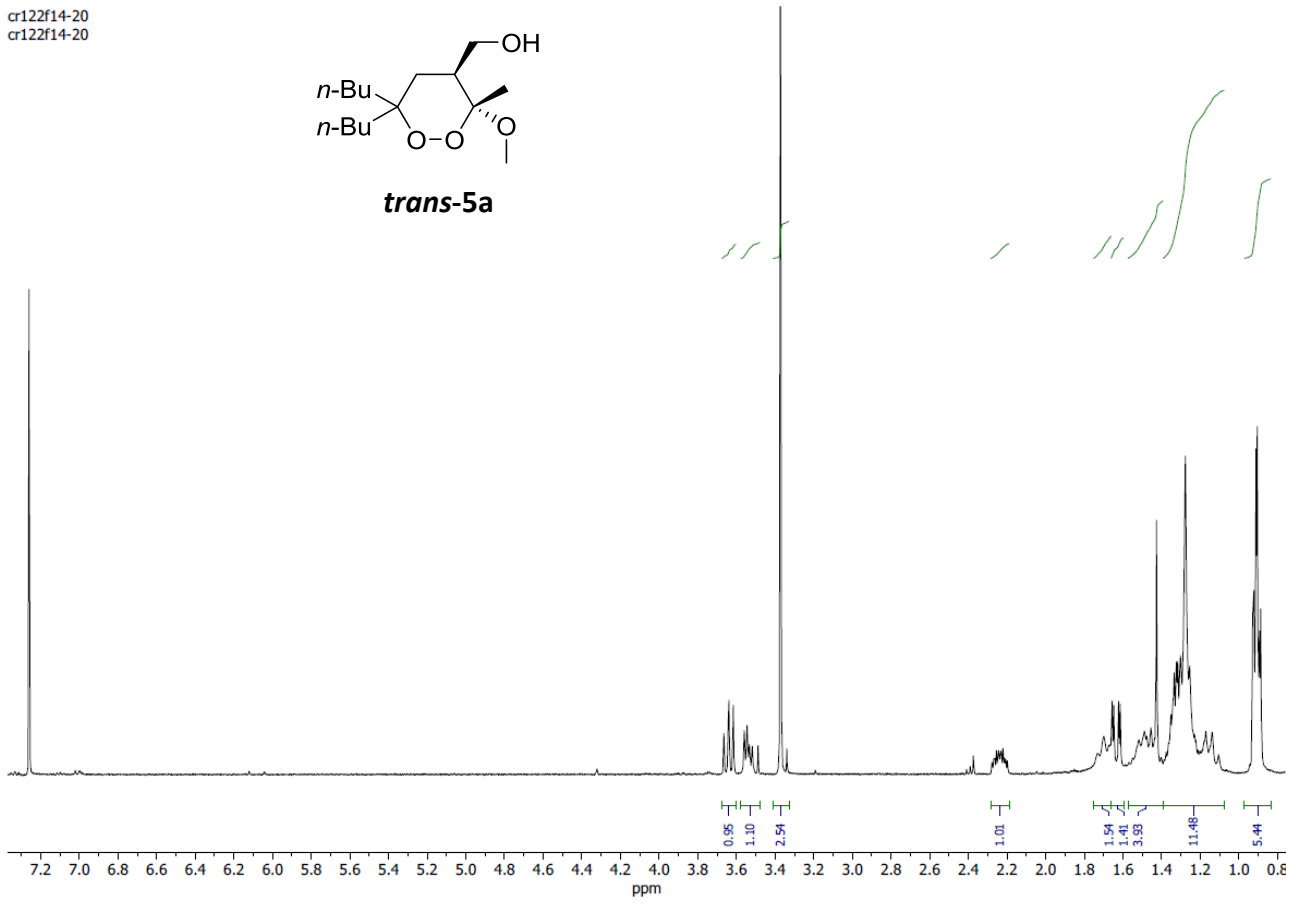
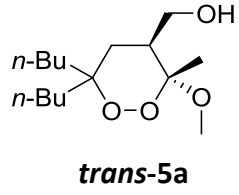
***cis*-5a**



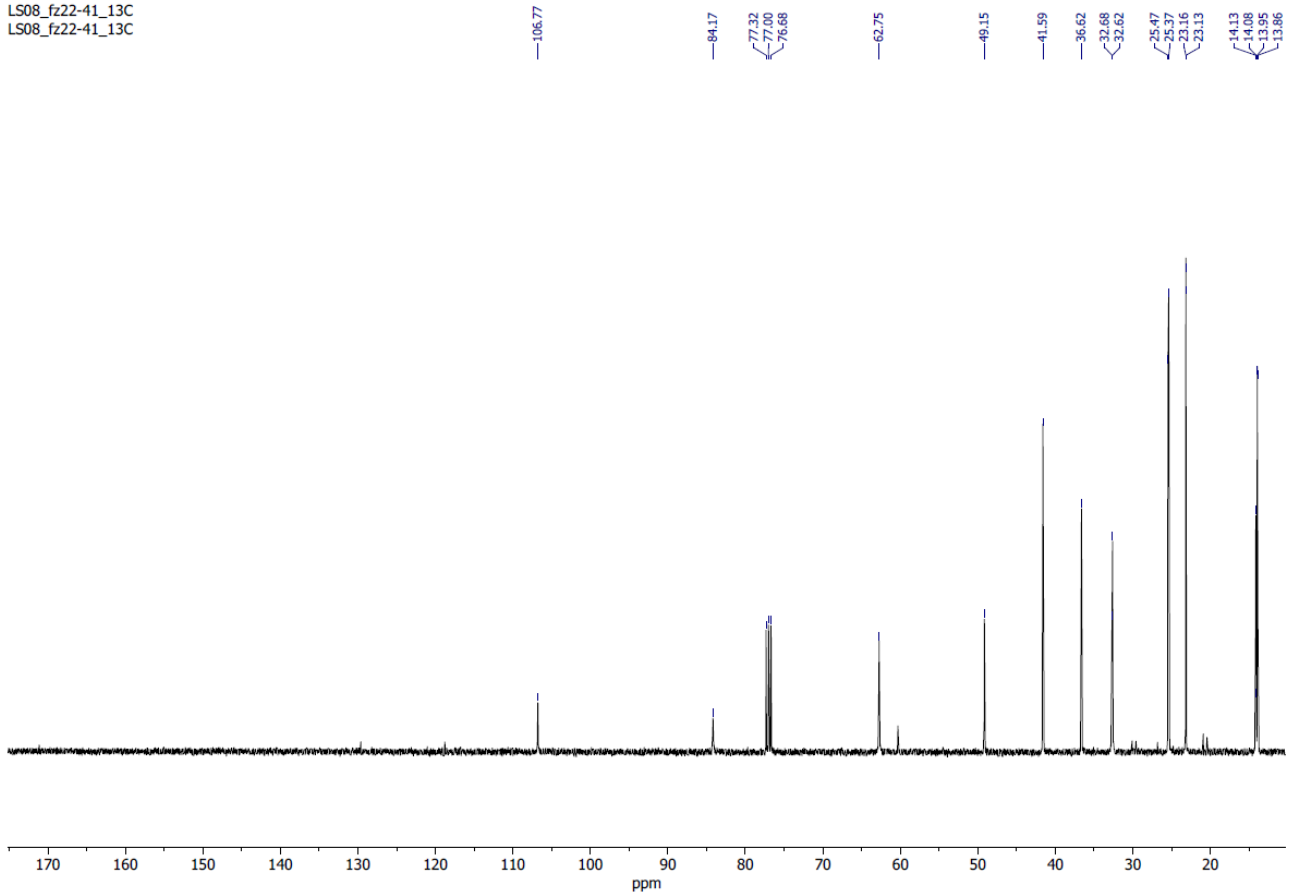
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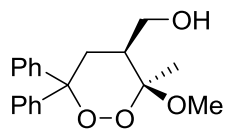
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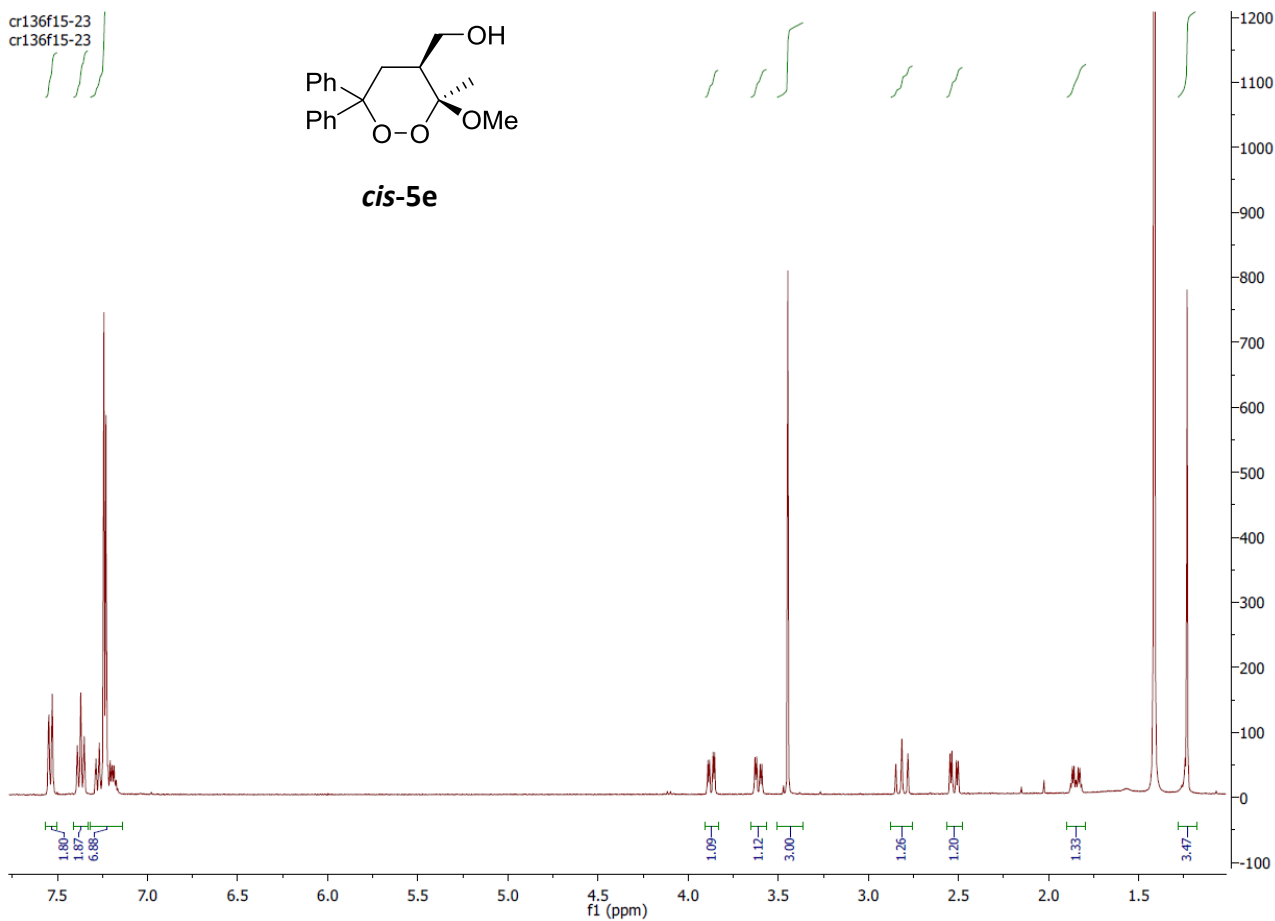
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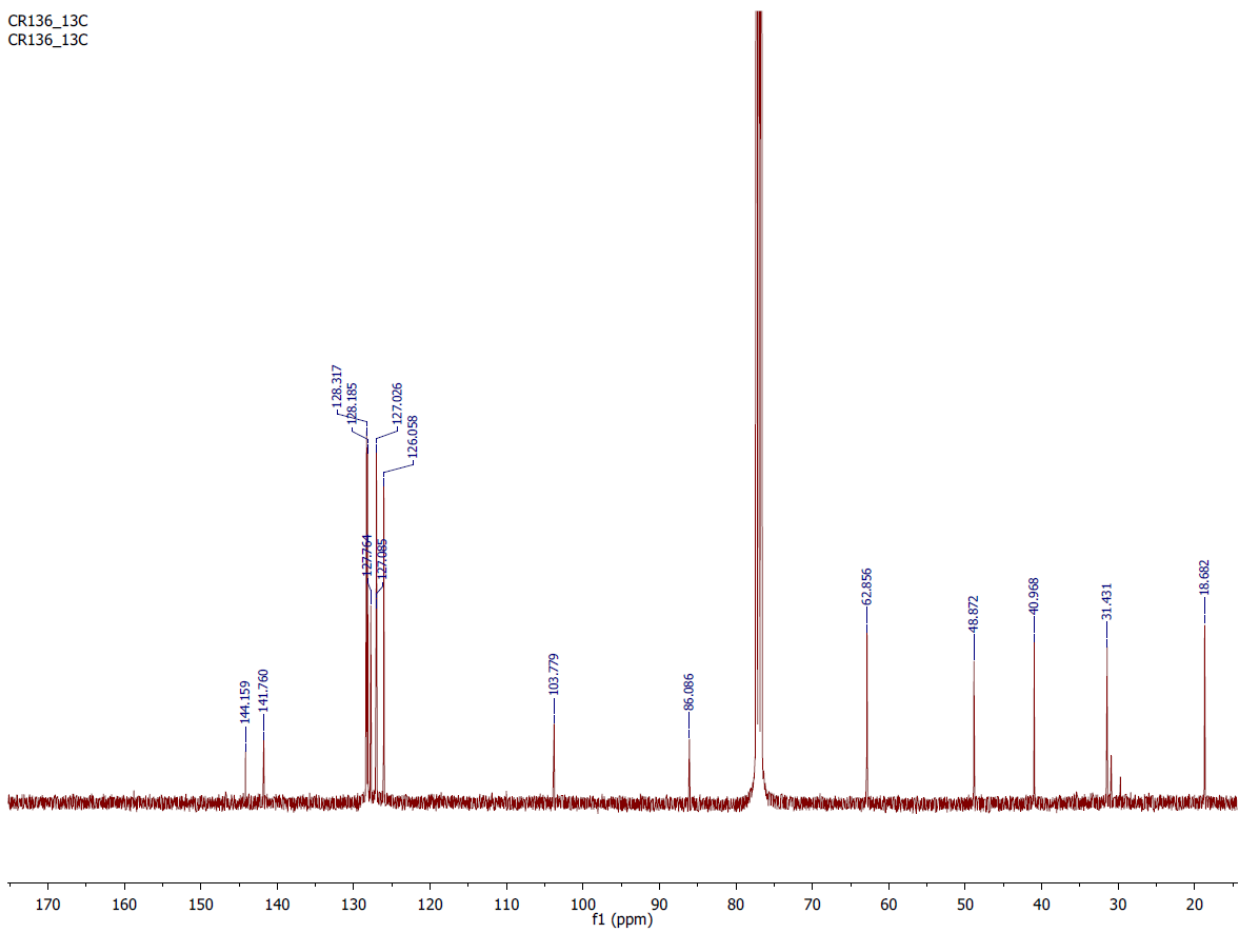
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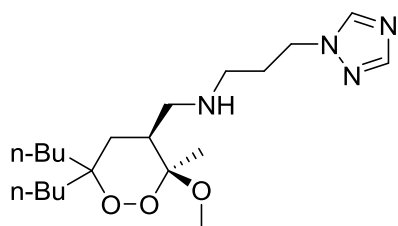
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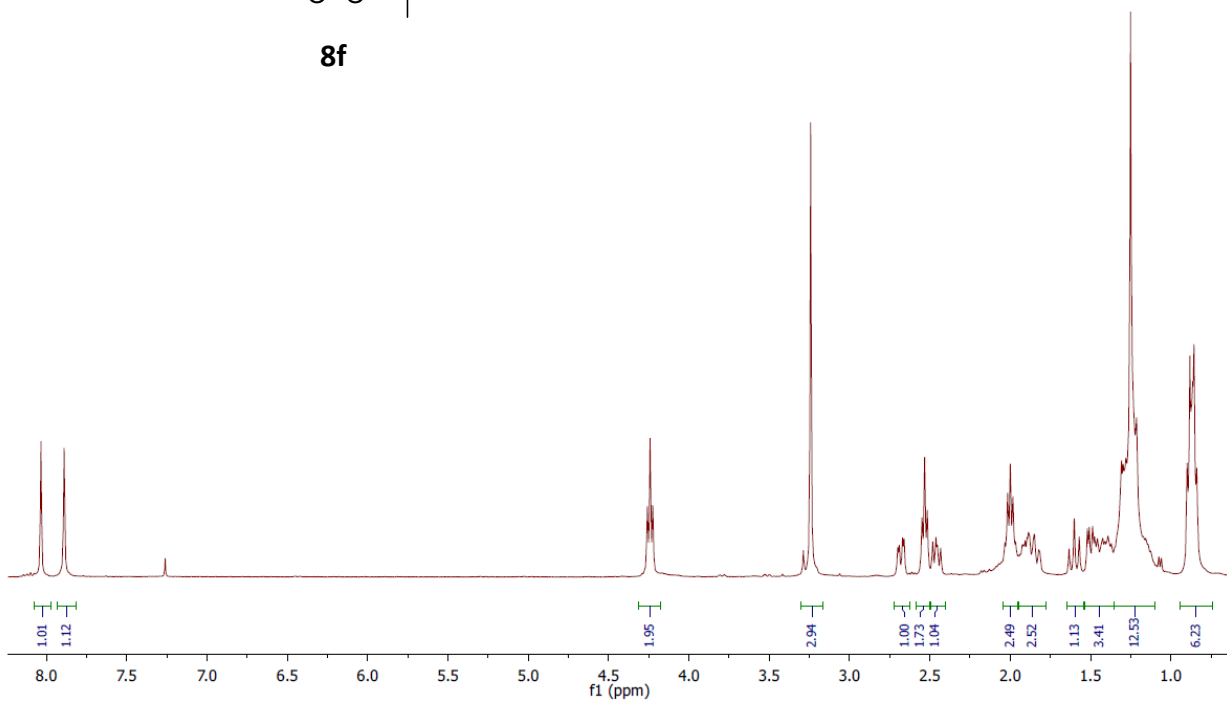
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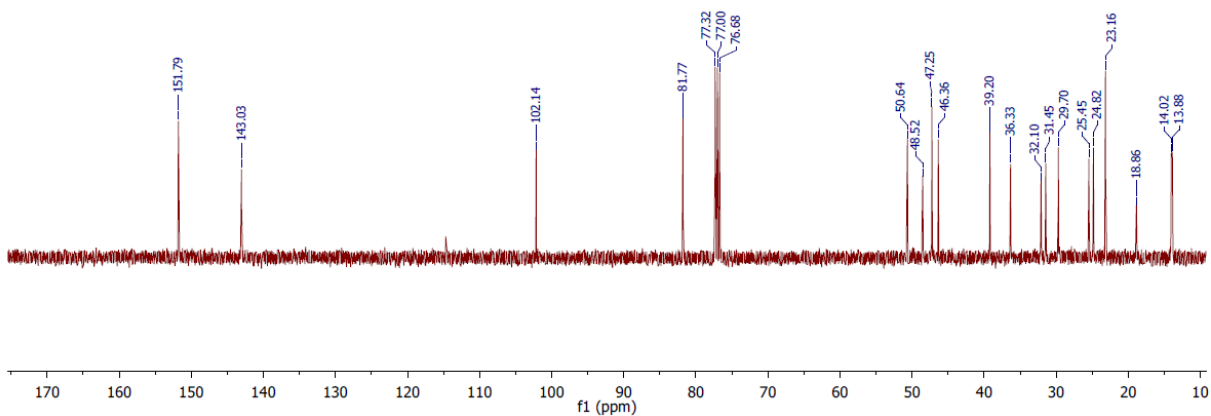
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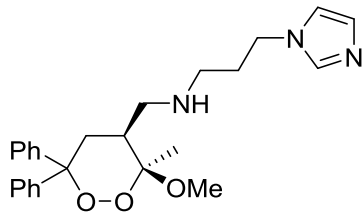
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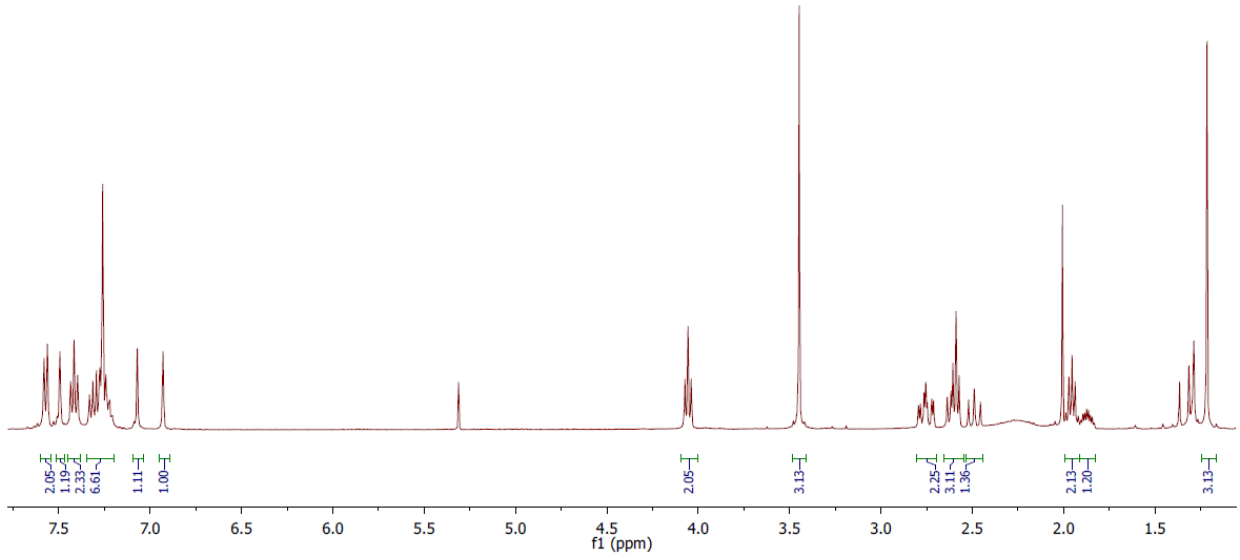
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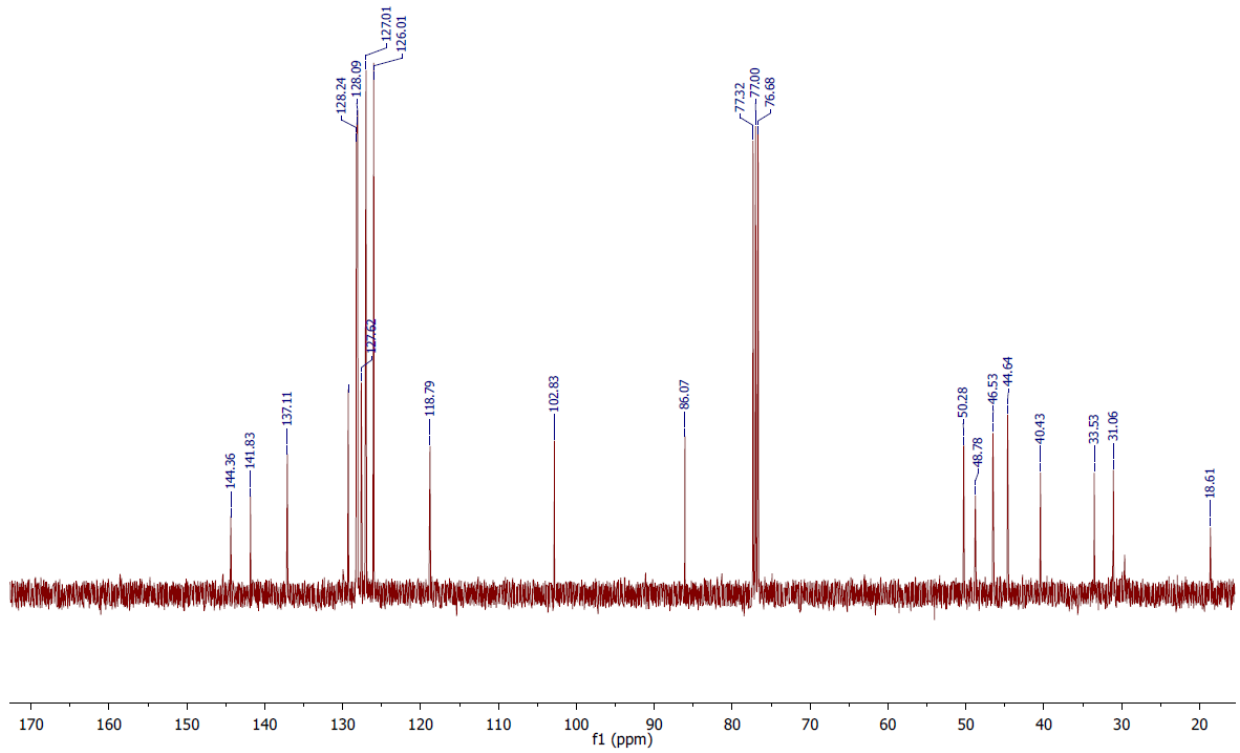
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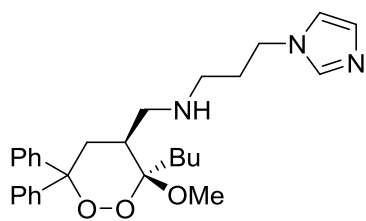
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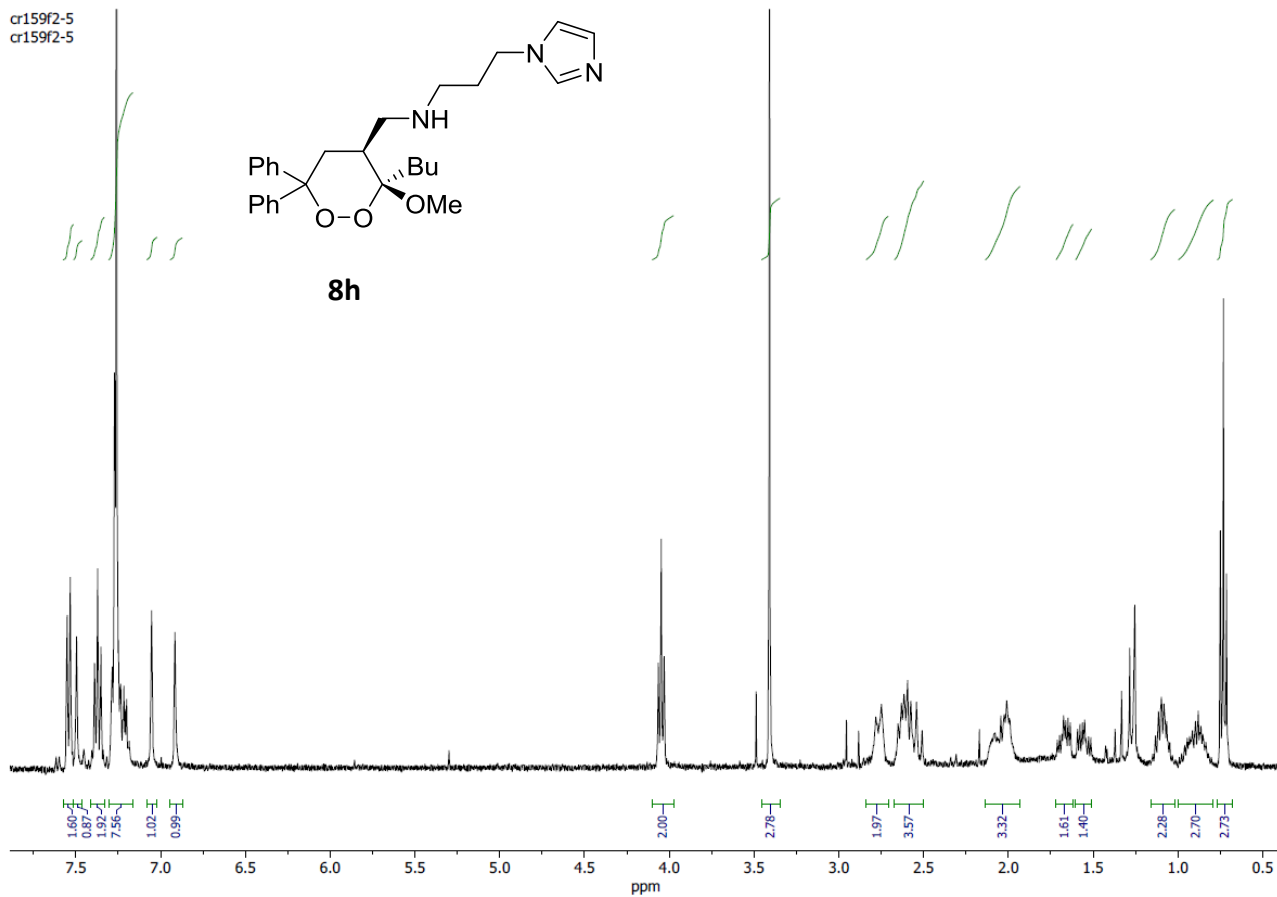
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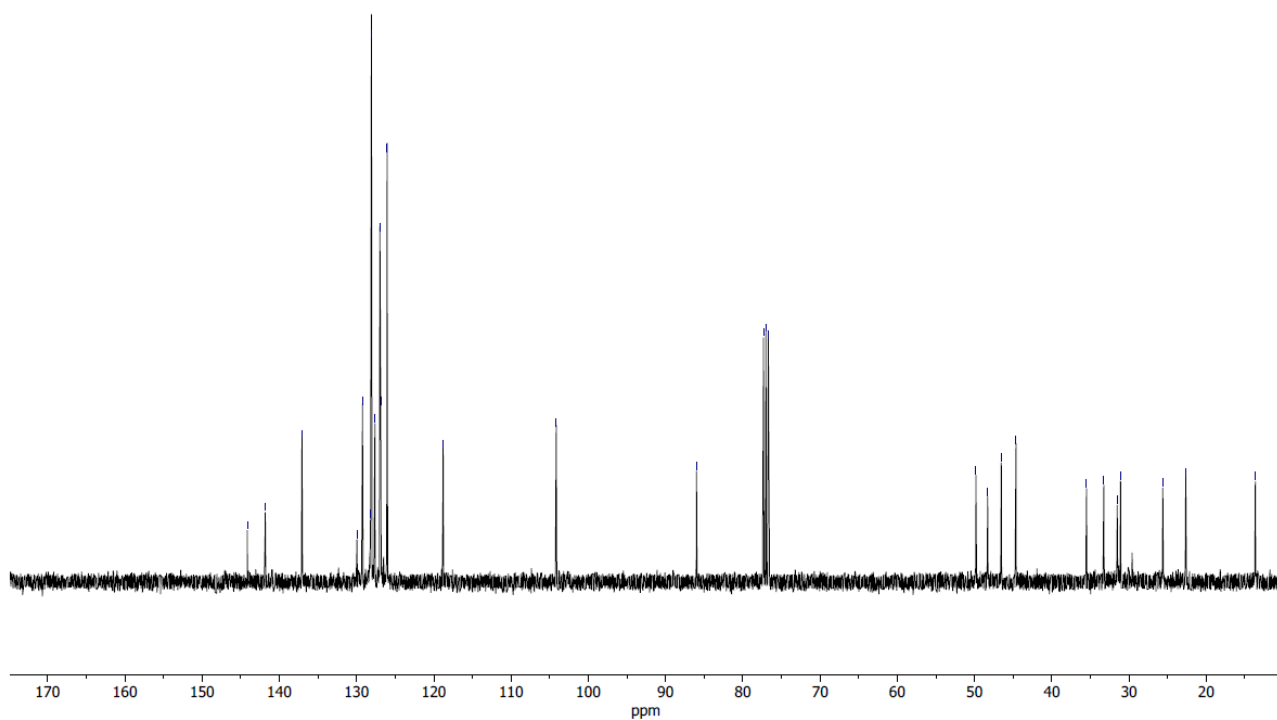


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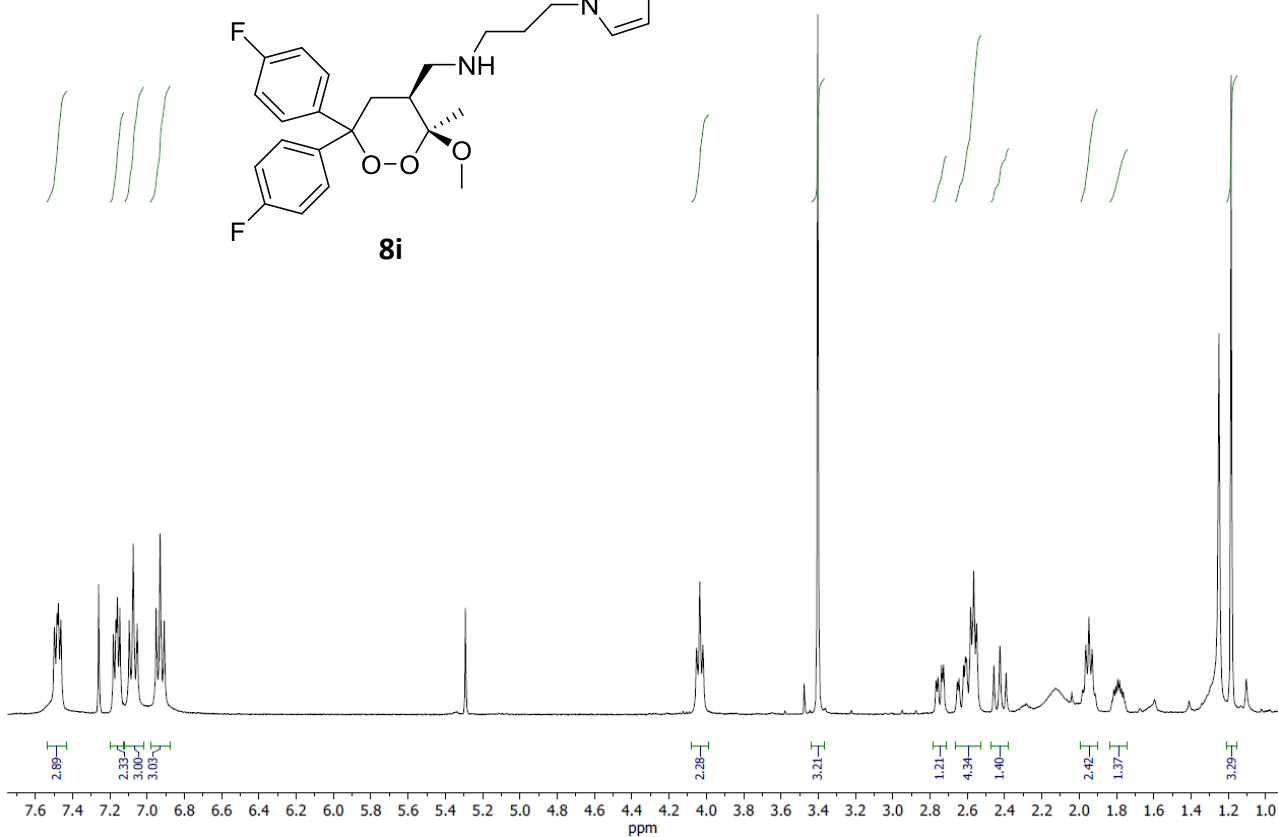
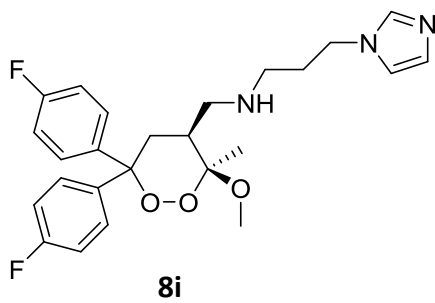


cr159BconcC
cr159B conc C

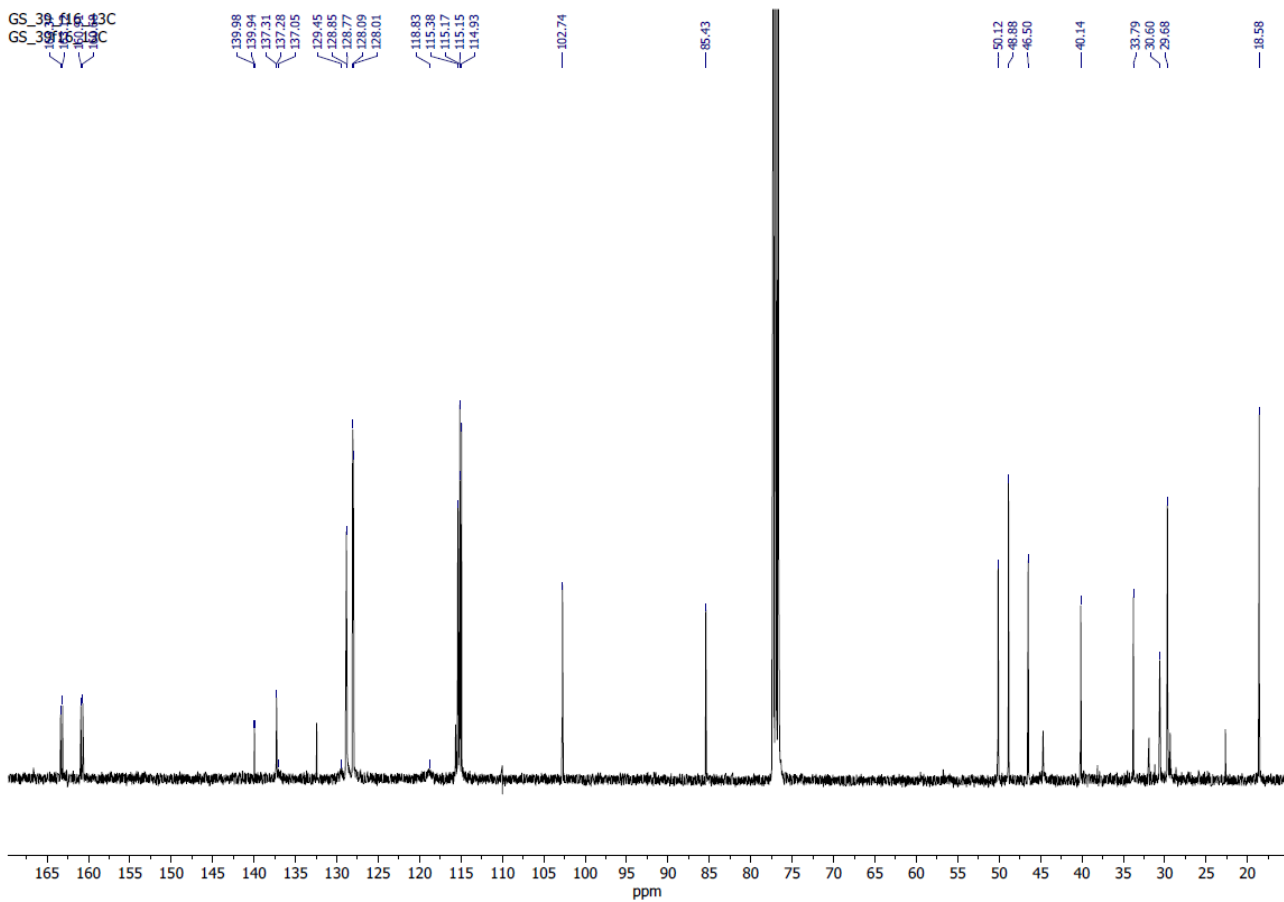
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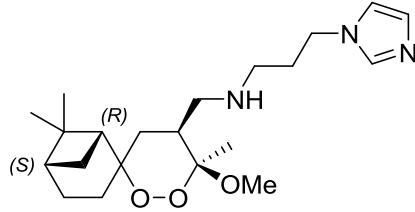
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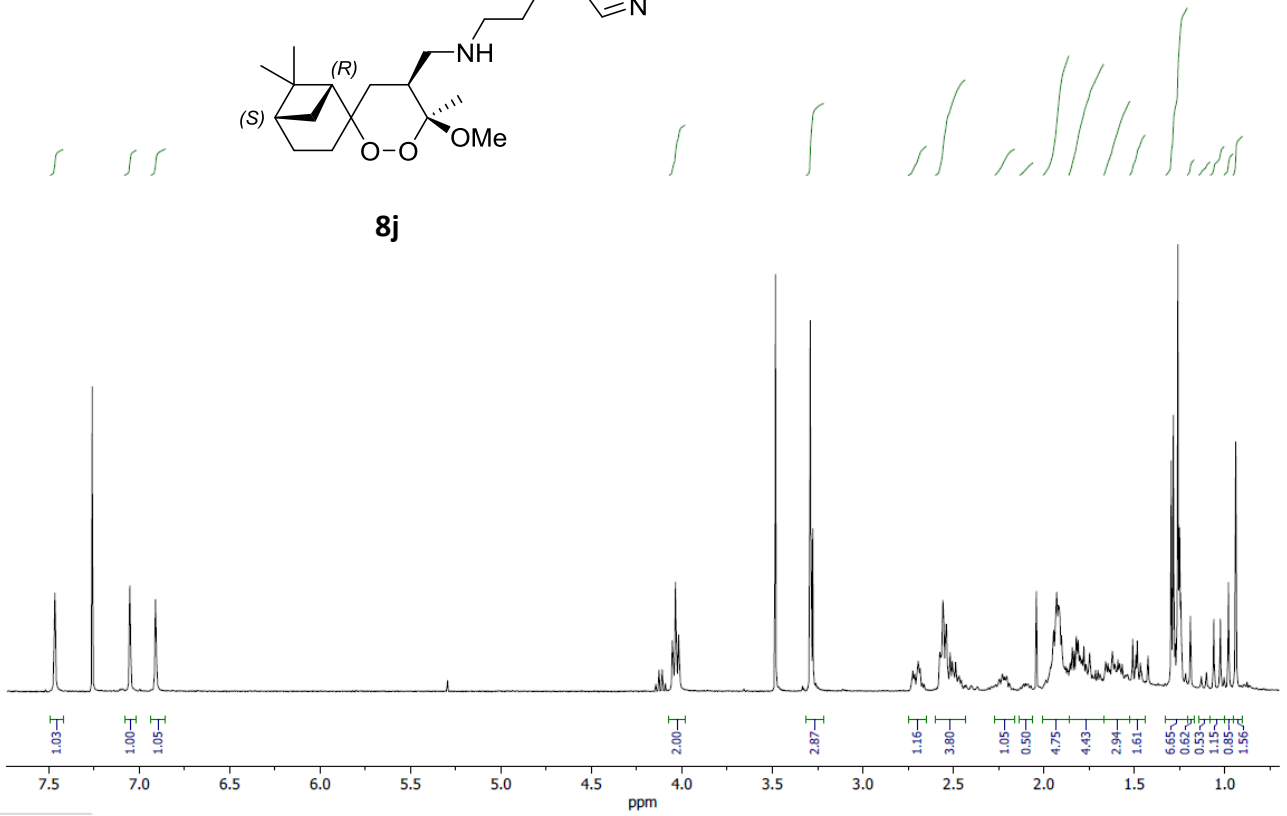
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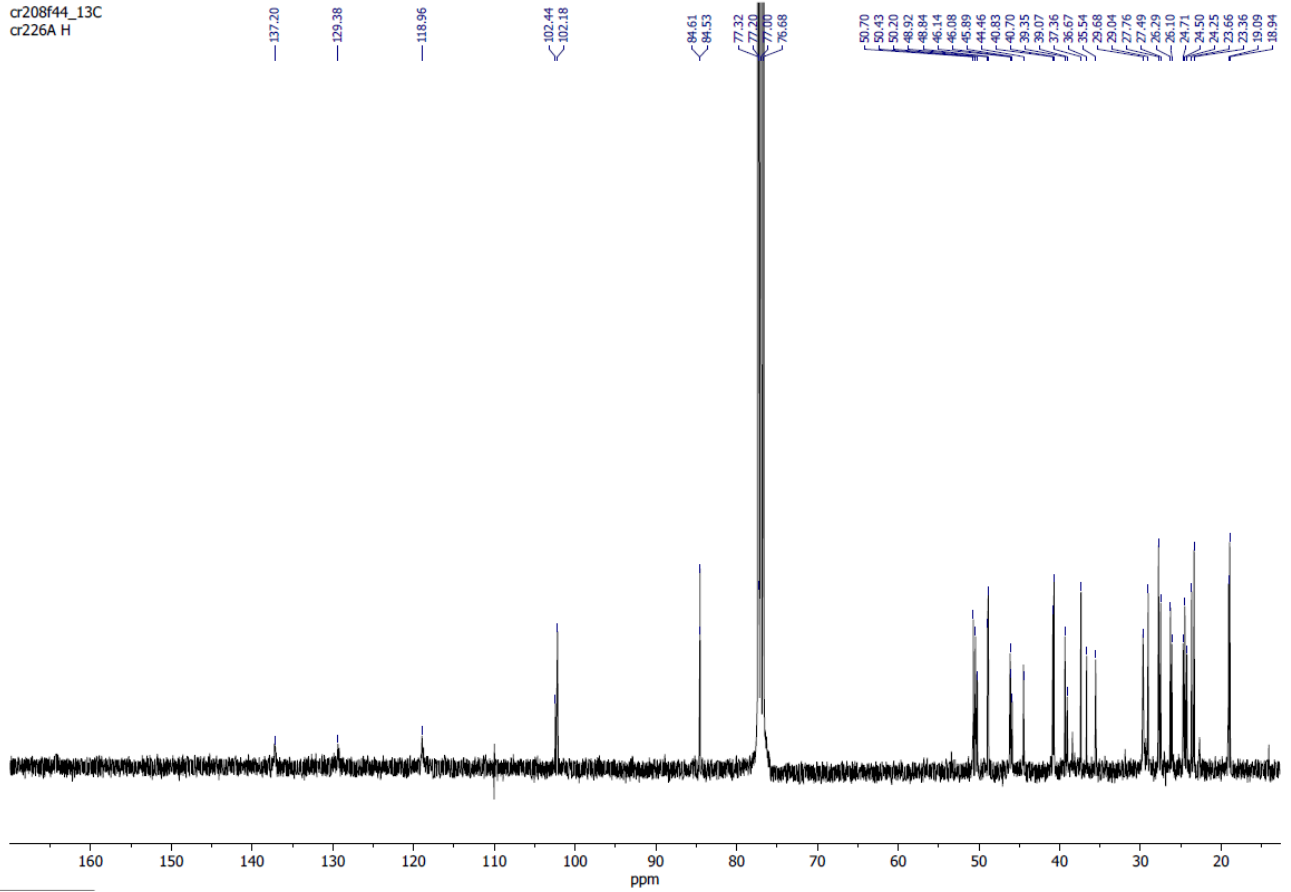
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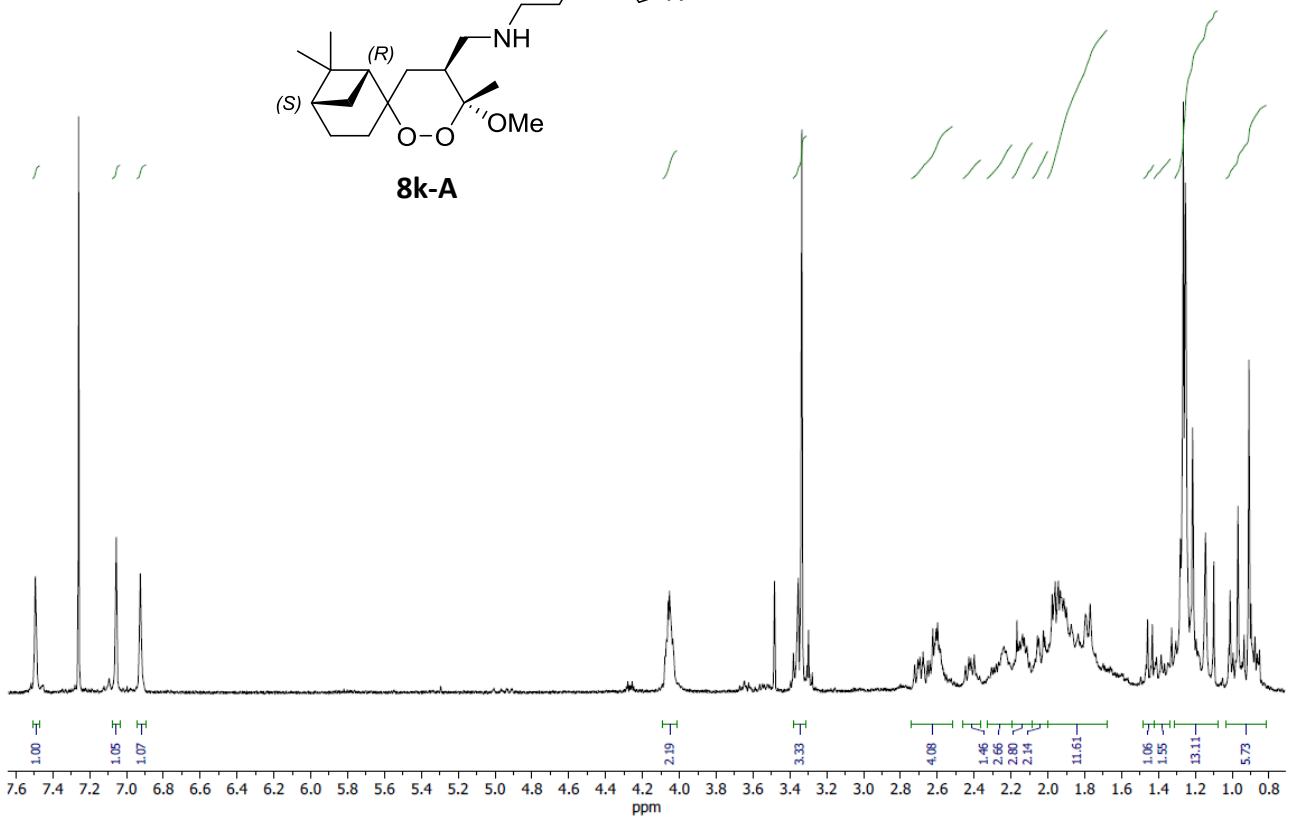
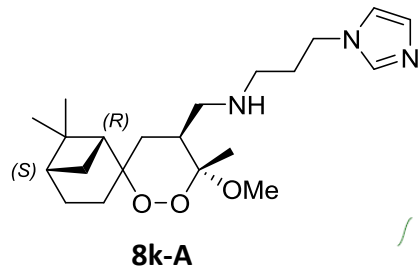
8j



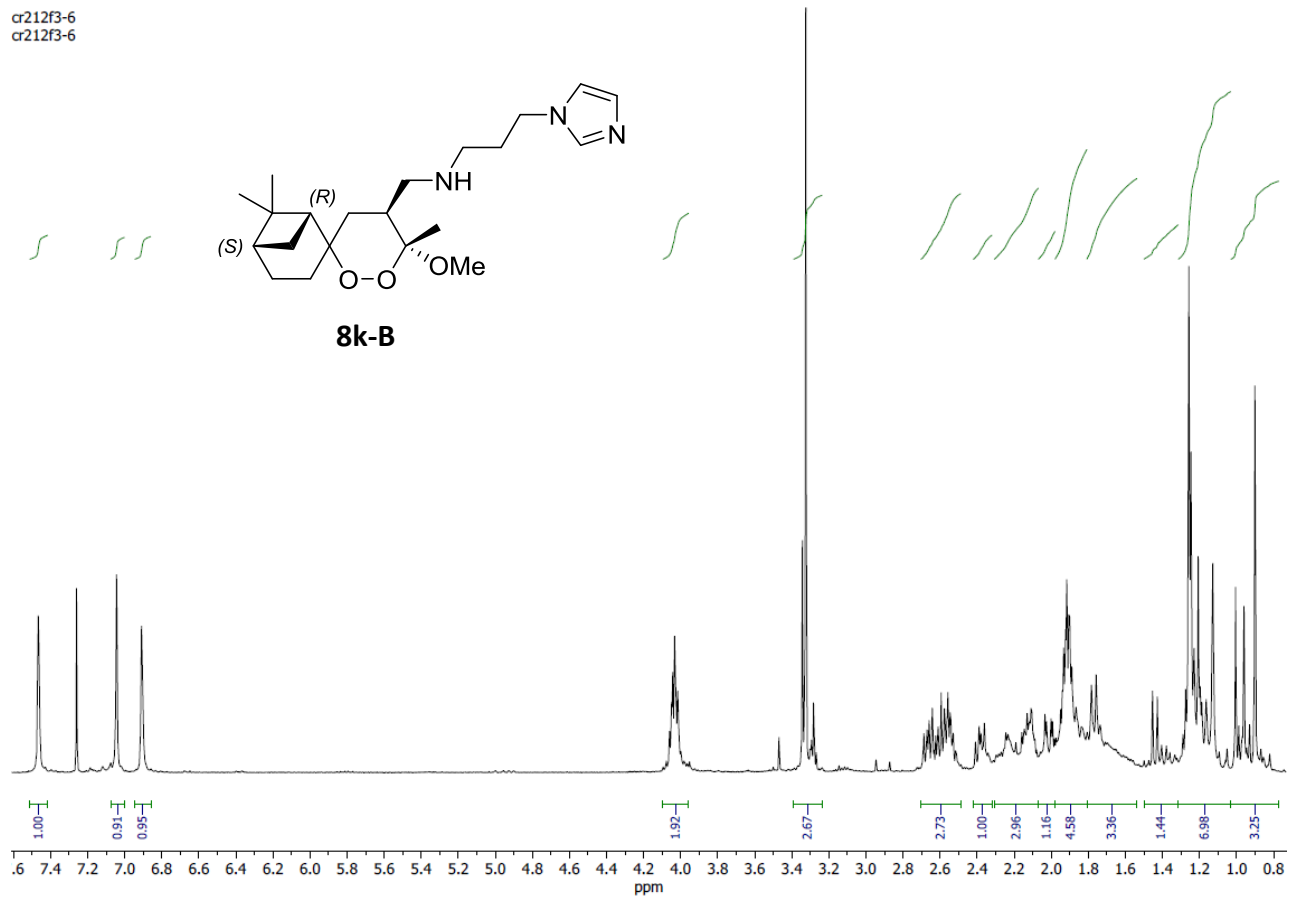
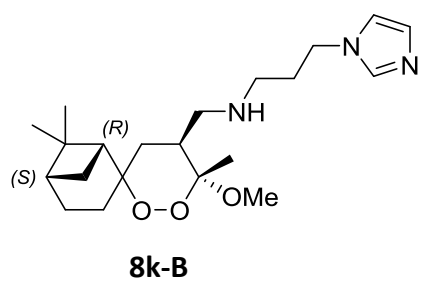
cr208f44_13C
cr226A H



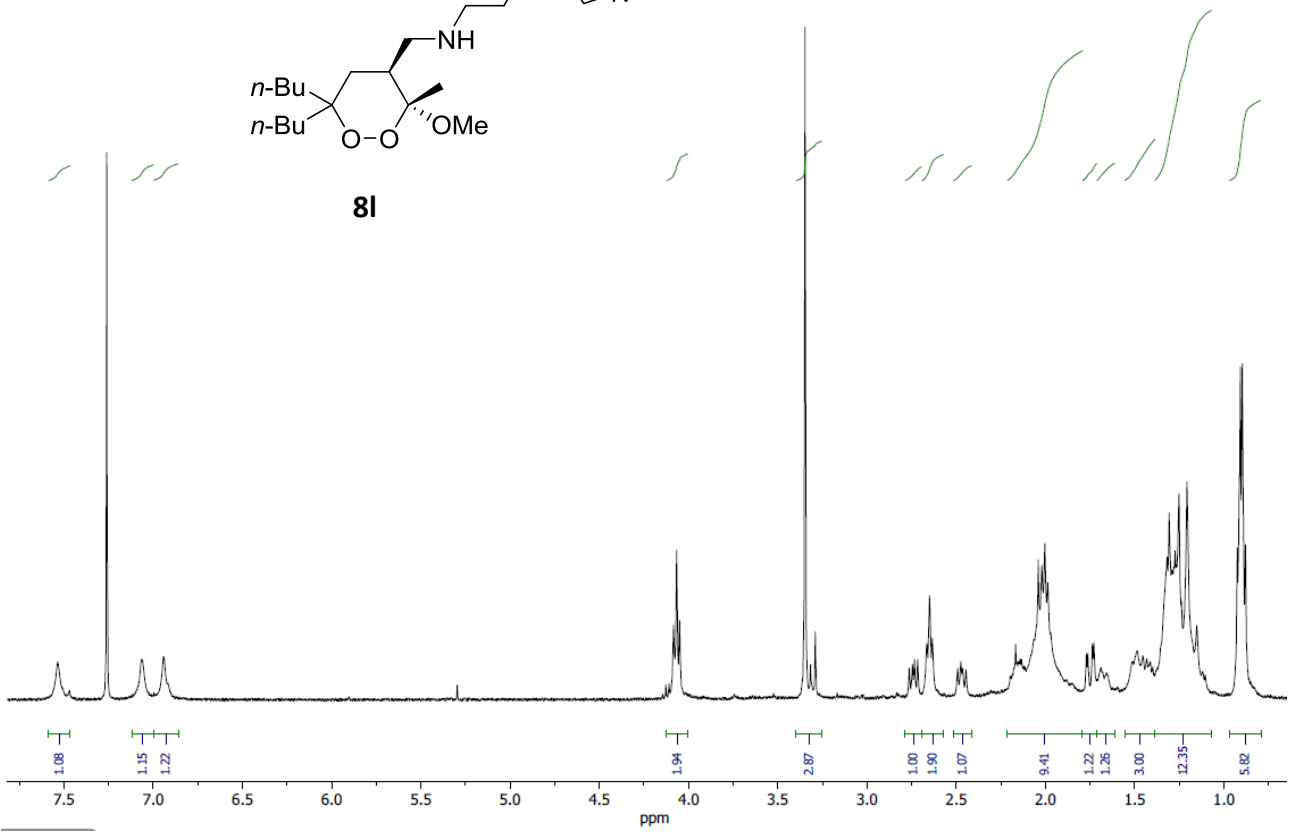
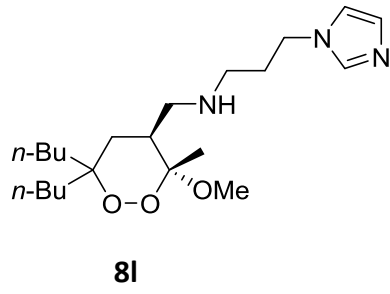
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cr212f2



cr212f3-6
cr212f3-6

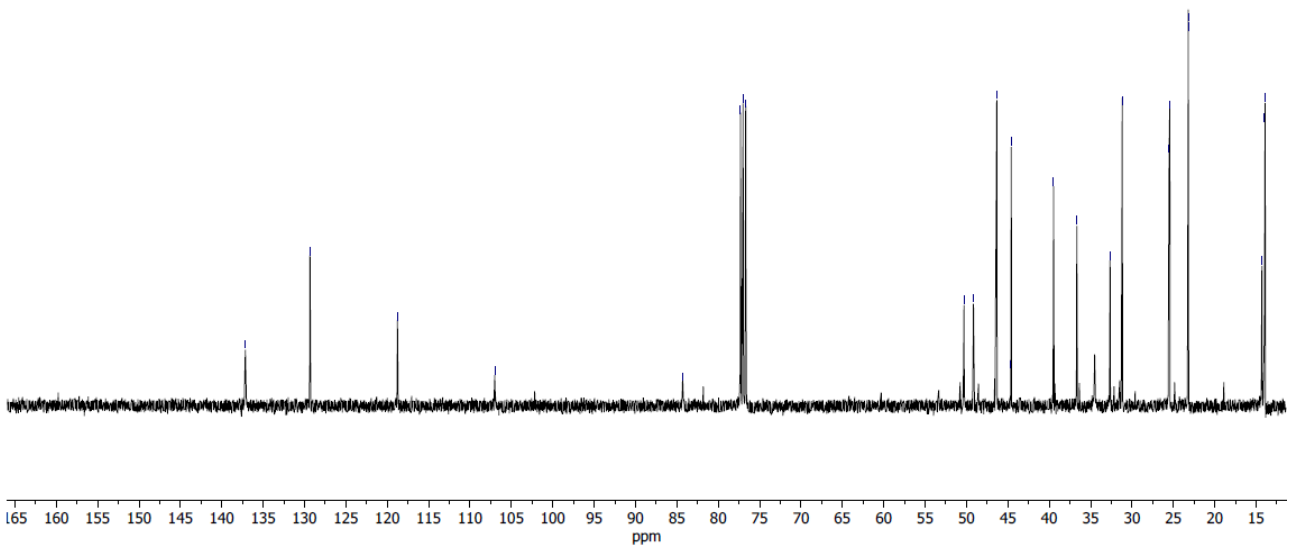


LS11b_1H
LC11_13C

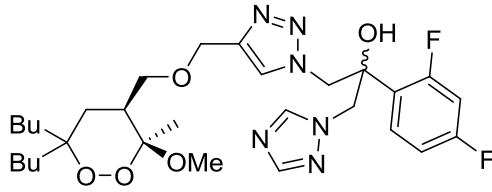


LS11b_13C
LS11b 13C

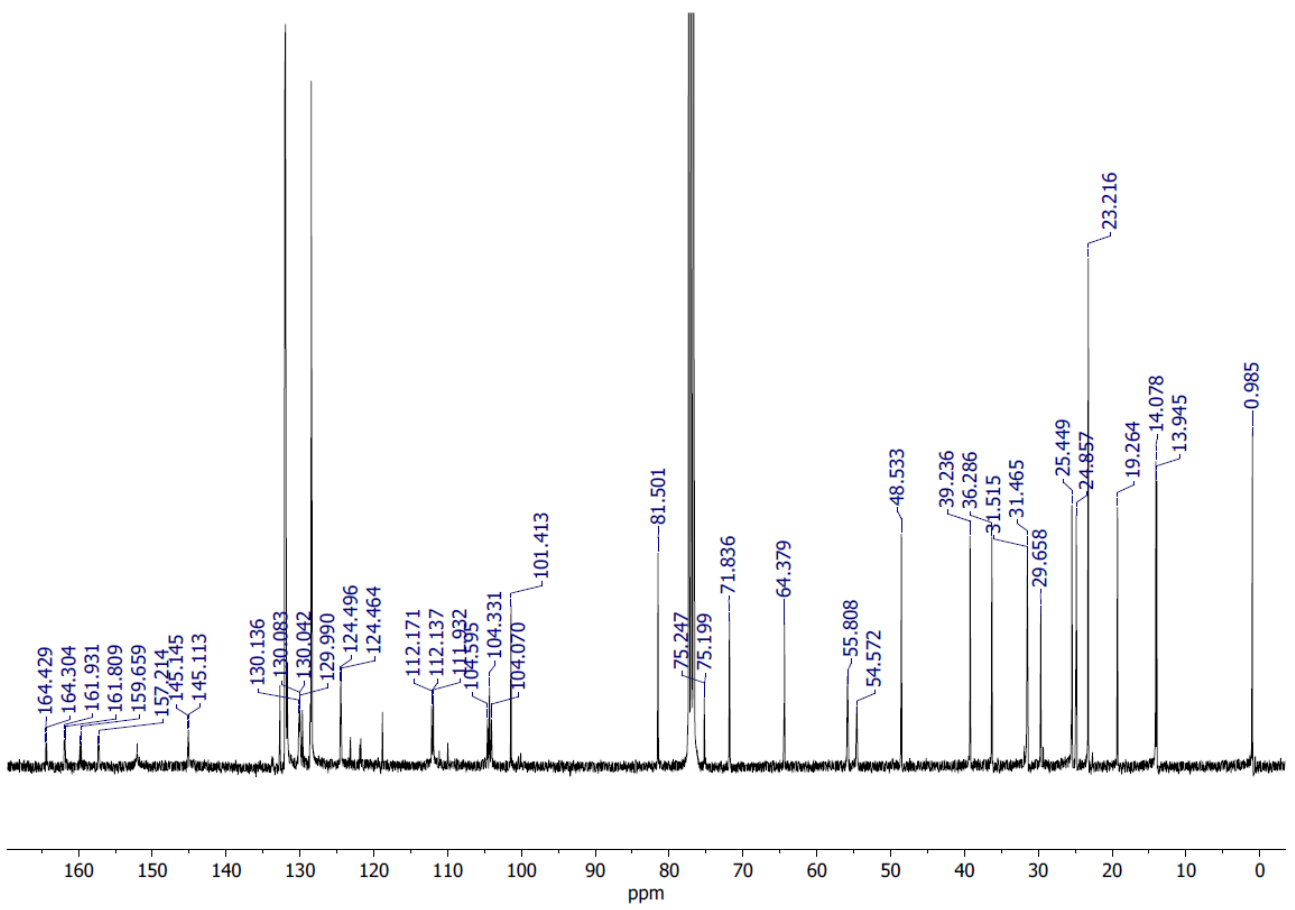
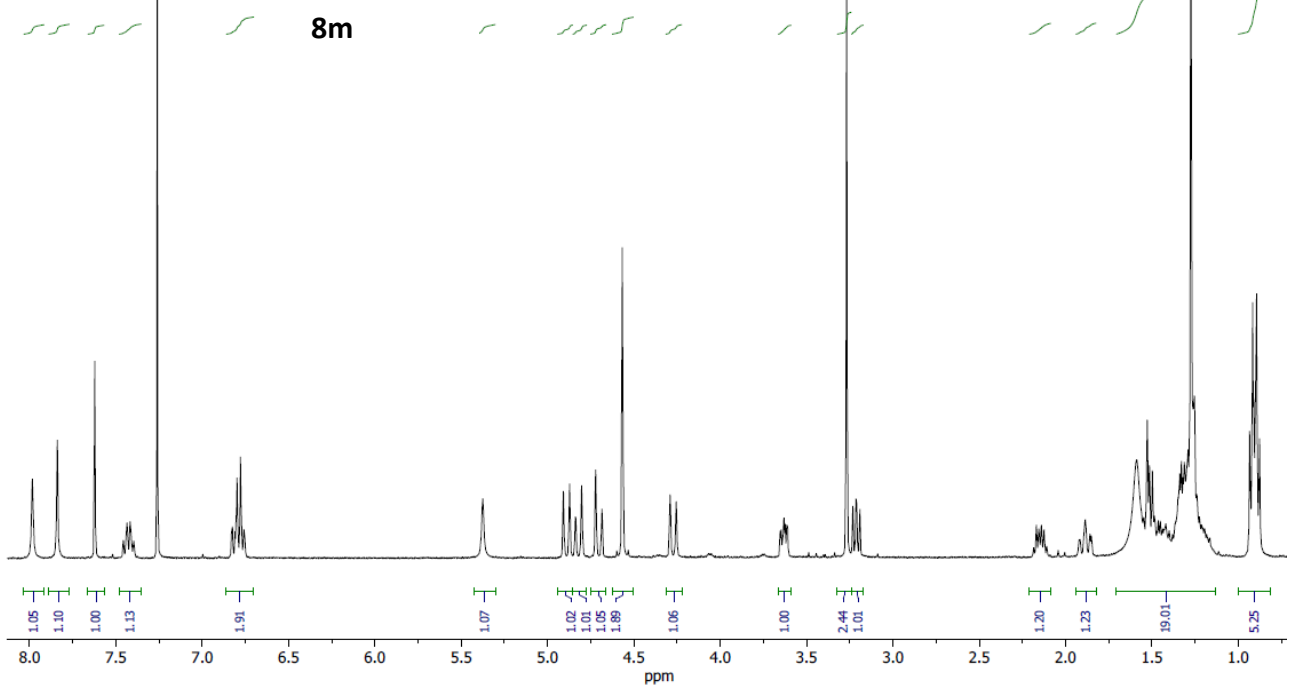
137.15 129.34 118.77 107.01 84.29 77.32 77.00 76.68 50.30 49.16 46.34 44.63 44.59 39.48 36.67 32.65 31.18 25.52 25.43 23.20 23.16 14.30 13.89 13.91



cr191b-f25
cr191b-f25

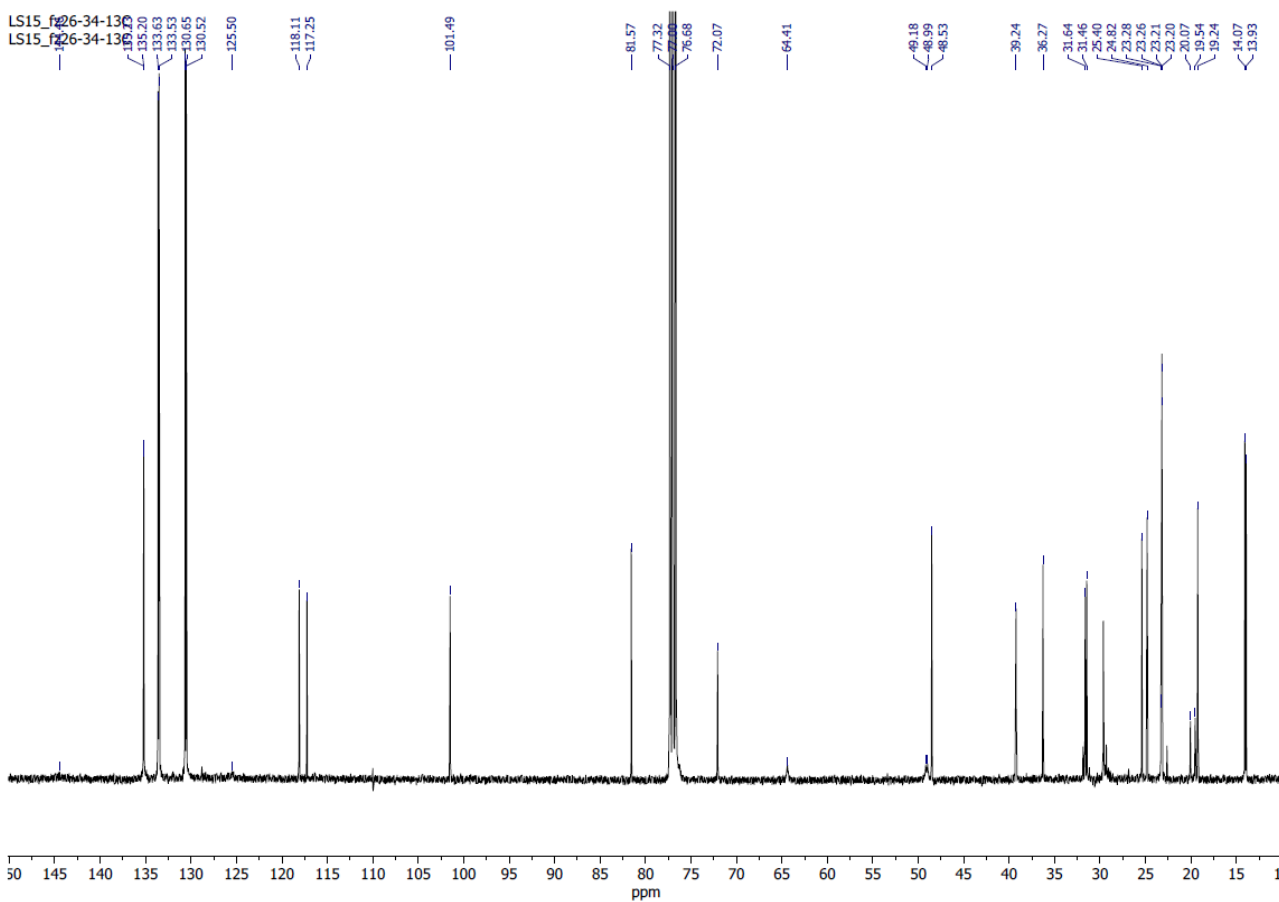
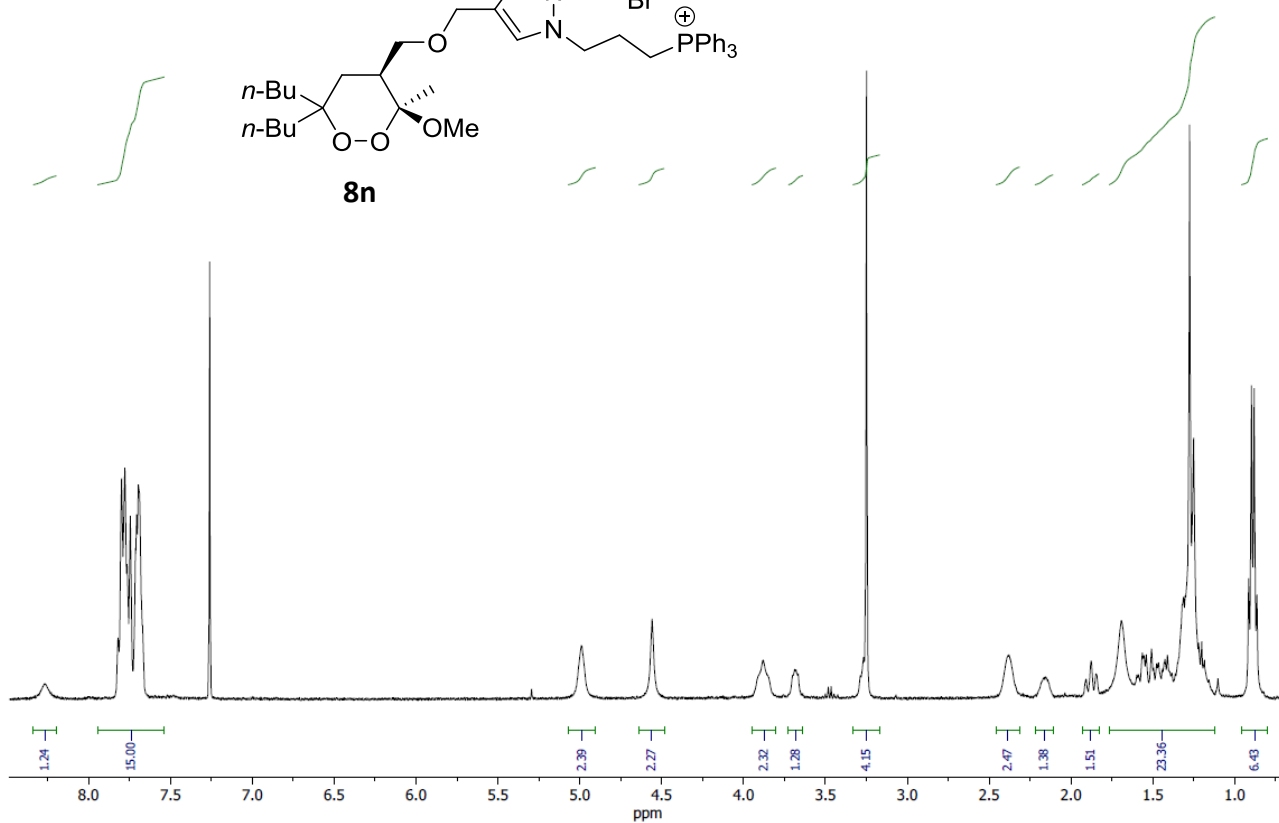
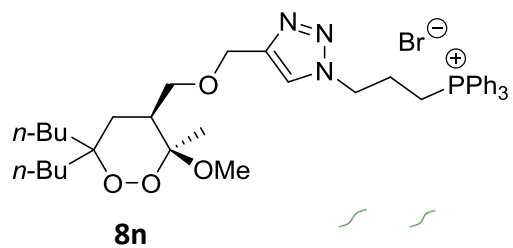


8m

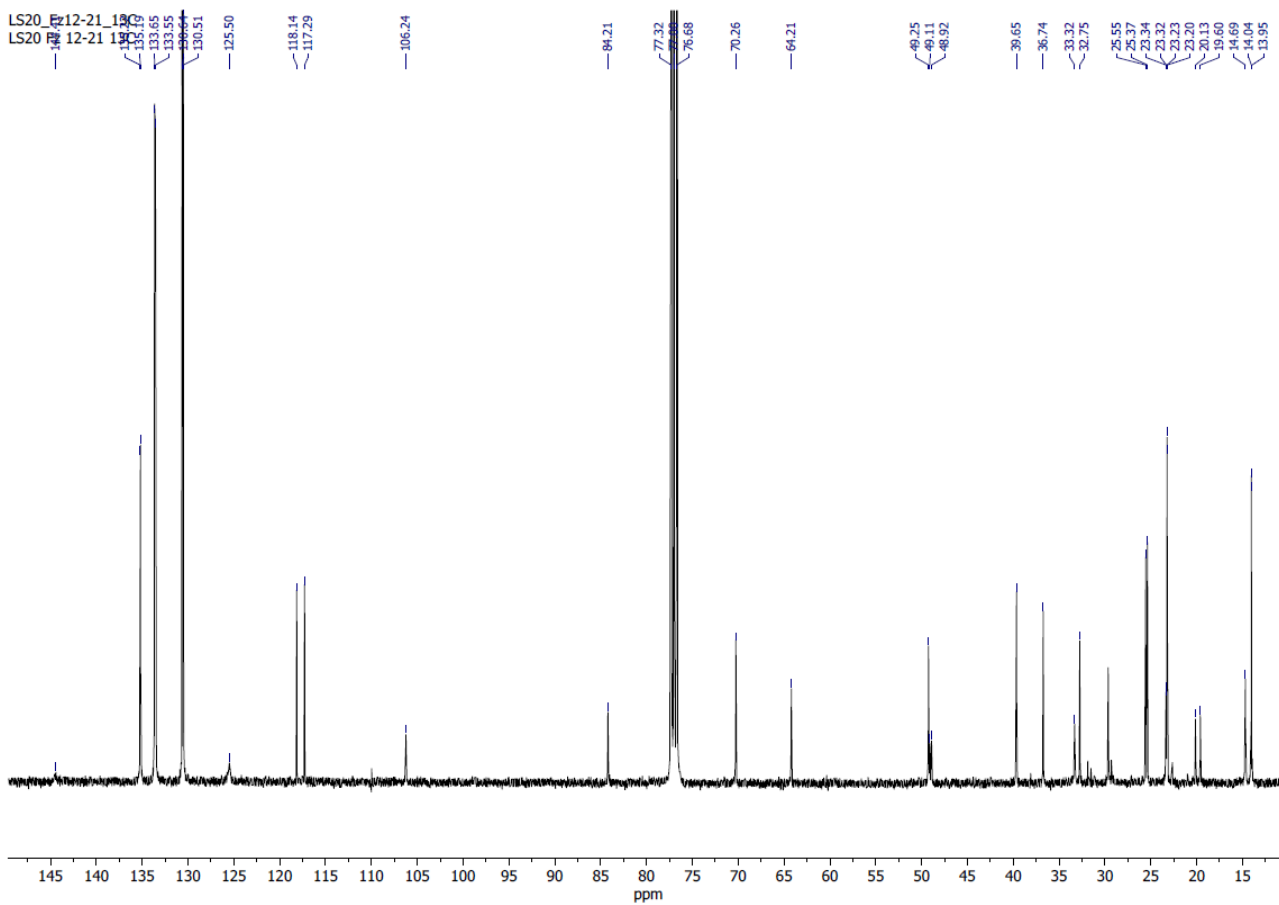
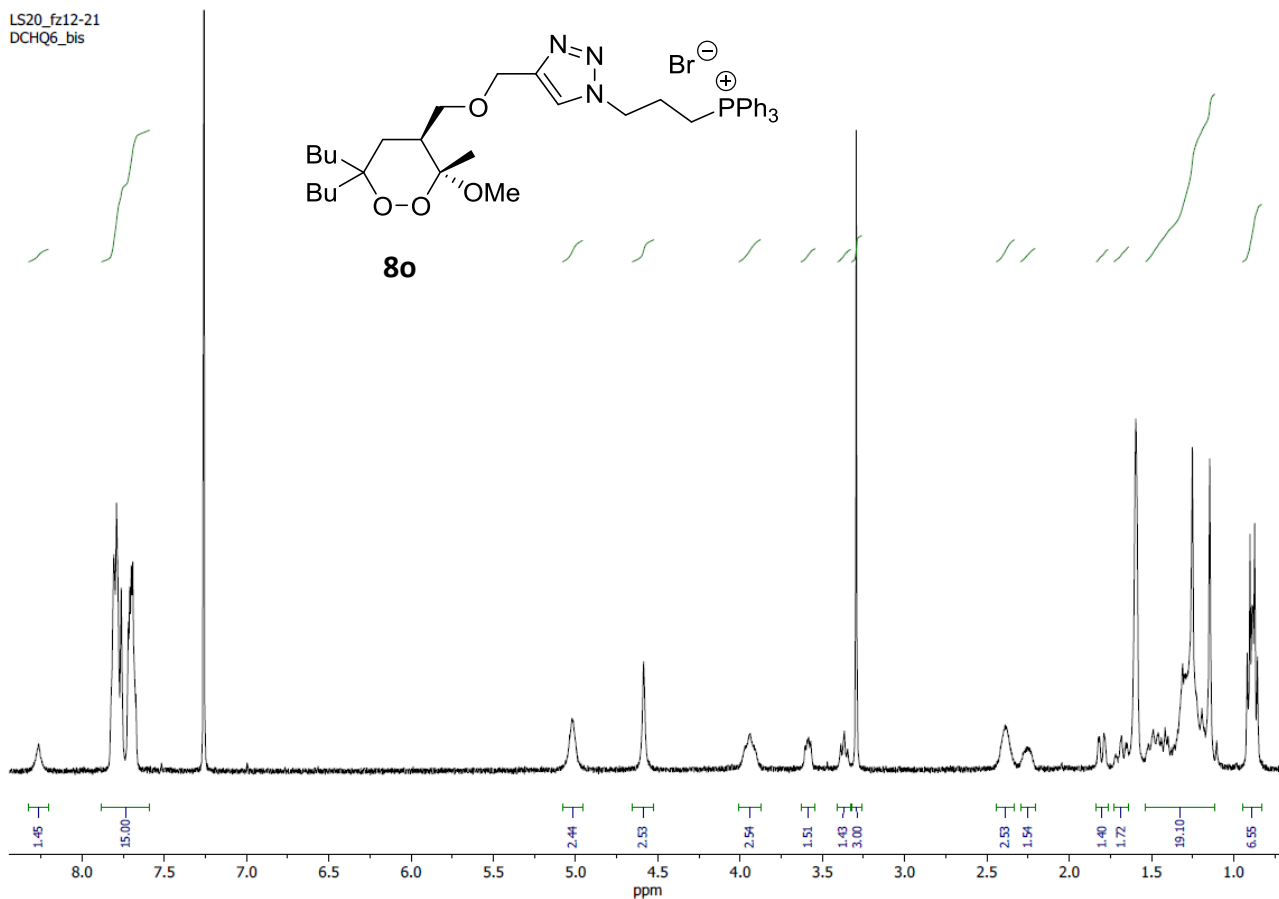
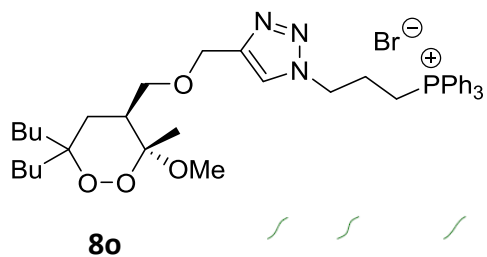


The purification of product **8m** from $\text{Ph}_3\text{P}=\text{O}$ was very difficult. We obtained a small amount of pure **8m** (see ^1H NMR spectrum) to be assayed against *Leishmania* spp.. However, to record the ^{13}C NMR spectrum in a reasonable time, we employed a sample of **8m** contaminated by $\text{Ph}_3\text{P}=\text{O}$ (see ^{13}C NMR spectrum).

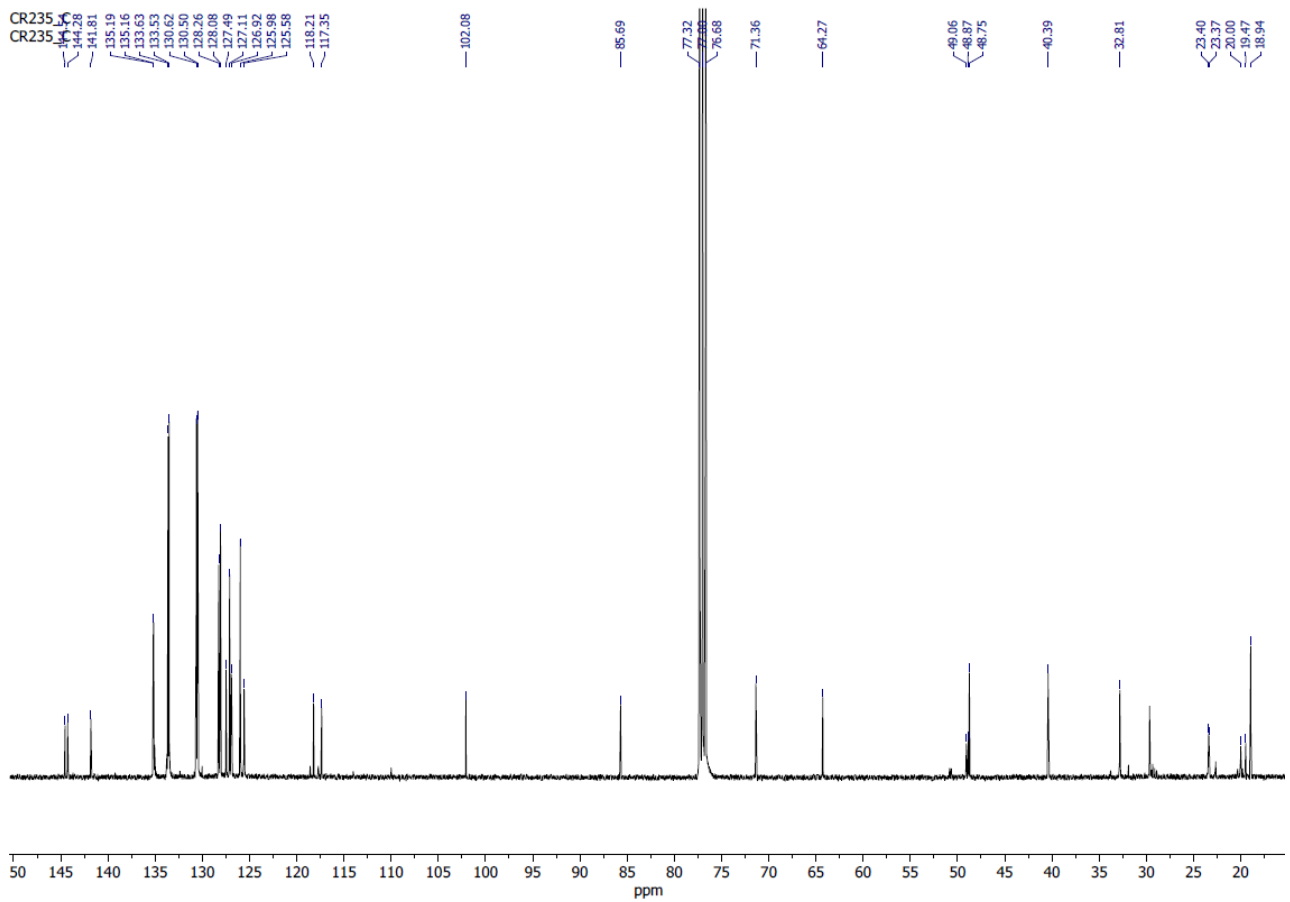
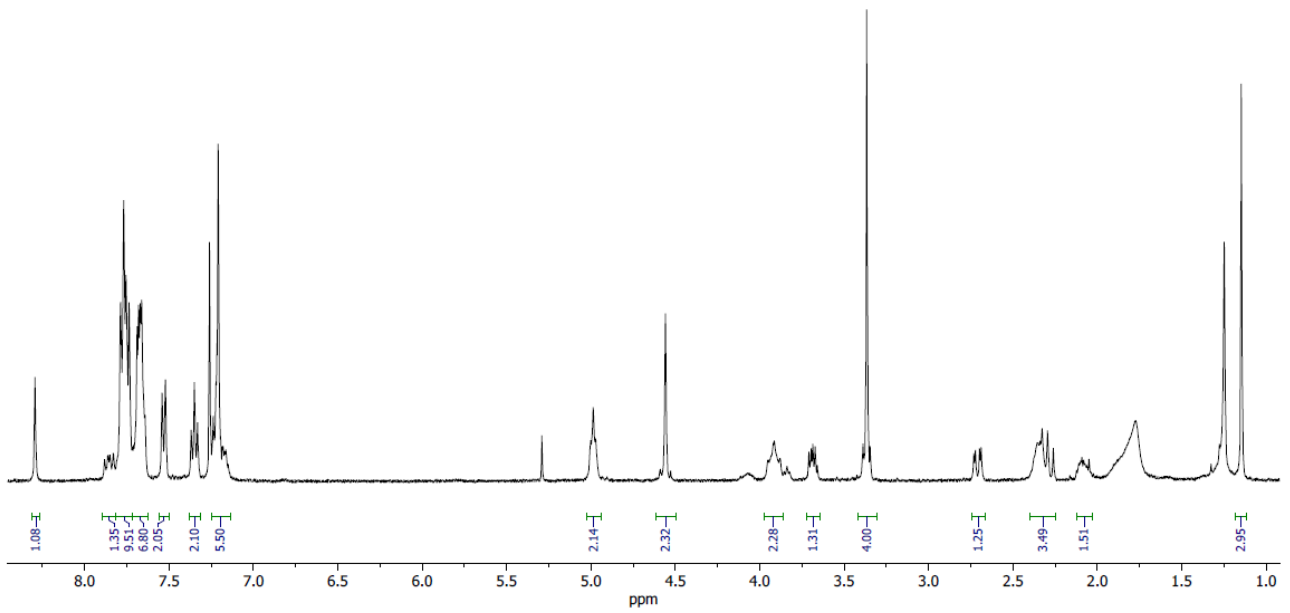
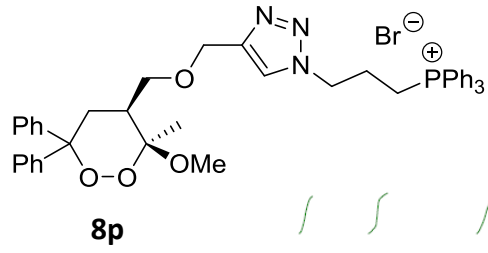
LS15b
AM98



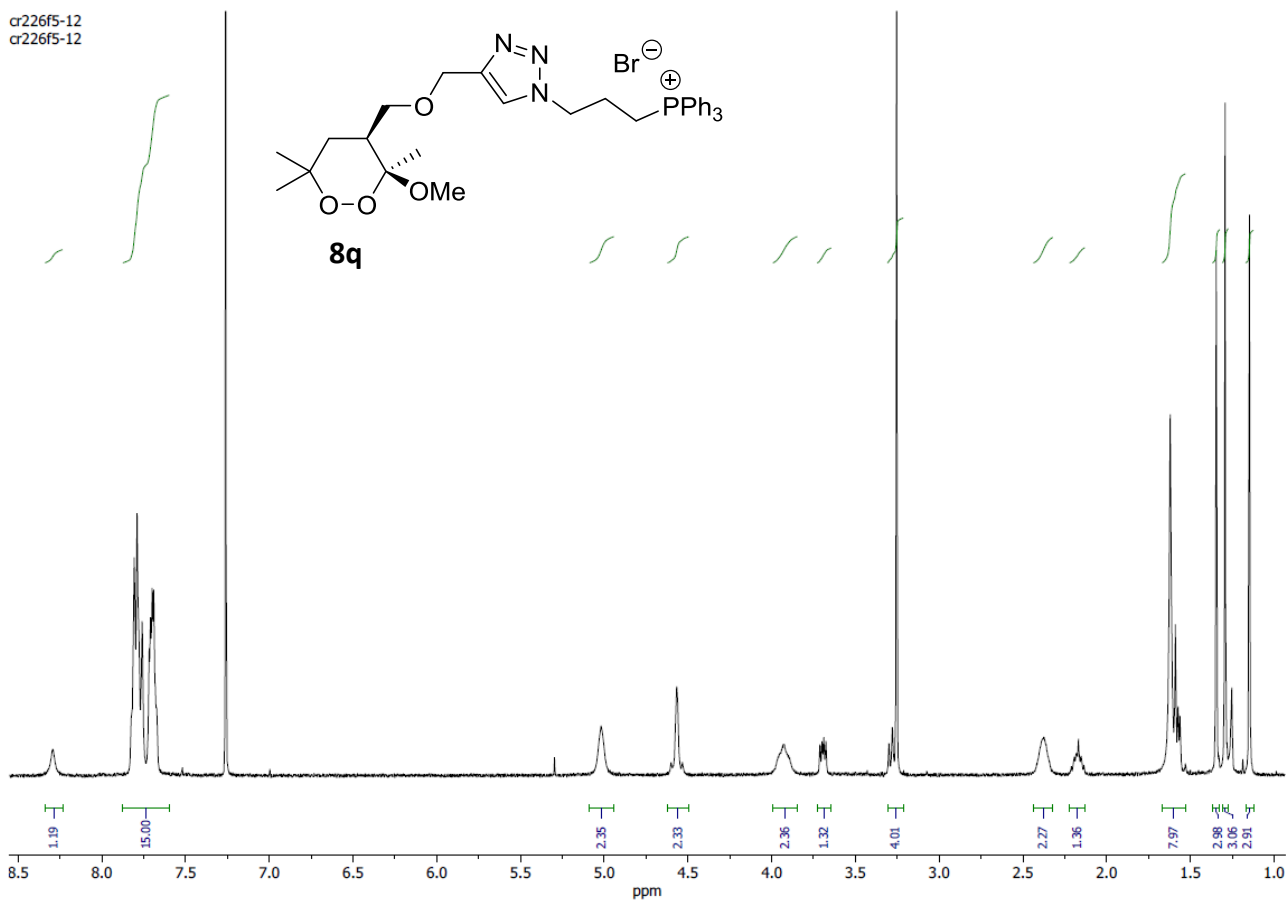
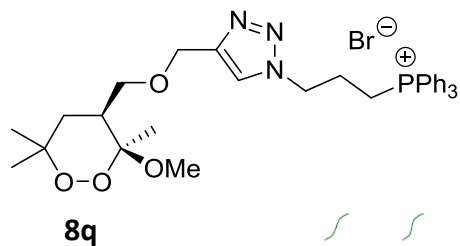
LS20_fz12-21
DCHQ6_bis



cr235dt
CR235dt



cr226f5-12
cr226f5-12



cr226-13C
cr226-13C

