

Synthesis of the C19 Methyl Ether of Aspercyclide A *via* Germyl-Stille Macrocyclisation and ELISA Evaluation of Both Enantiomers Following Optical Resolution

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SUPPORTING INFORMATION

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General Directions

Solvents and Reagents: Solvents were dried as follows: MeCN and CH₂Cl₂ were distilled over CaH₂. Alternatively MeCN and CH₂Cl₂ were dried and deoxygenated with Grubbs-type solvent purification system. The moisture content of the solvents was monitored by Karl Fischer coulometric titration. Reagents were used as commercially supplied unless otherwise stated and handled in accordance with COSHH regulations.

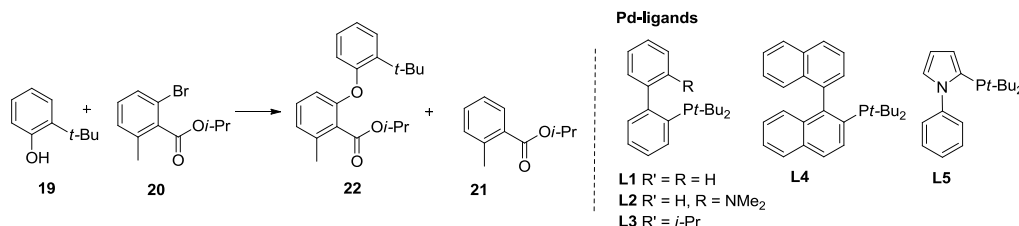
¹ **Microwave Irradiation:** These were performed on a Biotage Initiator microwave reactor with the temperature determined by IR. **Chromatography:** Flash chromatography was performed on silica gel (60 F₂₅₄, 230-400 mesh) according to the method of W.C. Still.² Pre-packed fluorous solid-phase extraction (F-SPE) cartridges were purchased from Fluorous Technologies Inc. and pre-conditioned with 50:50 MeCN-H₂O. Crude reaction mixtures were loaded on the cartridge using CH₂Cl₂ and eluted with MeCN-H₂O (1:1) as the fluorophobic eluant. Thin layer chromatography (TLC) was performed on aluminium plates pre-coated with silica (60 F₂₅₄, 0.2 mm) which were developed using standard visualising agents: ultra violet fluorescence (254 nm), KMnO₄/Δ or vanillin/Δ. **¹H NMR Spectra:** These were recorded at 500, 400 or 300 MHz. Chemical shifts (δ_H) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak, with the abbreviations s, d, t, and m denoting singlet, doublet, triplet and multiplet respectively. **¹³C NMR Spectra:** These were recorded at 125, 100 or 75 MHz. Chemical shifts (δ_C) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak, with the abbreviations s, d, t, and q denoting C, CH, CH₂ and CH₃ respectively. No attempt was made to resolve the chemical shifts of carbons on the fluorous-tag by applying fluorine-decoupling at δ -125.1 ppm during acquisition; these carbons are not reported. **¹⁹F NMR spectra:** These were recorded at 367 MHz. Chemical shifts (δ_F) are given in parts per million (ppm) as referenced to CFCl₃. **Infra Red Spectra:** These were recorded as thin films or as solids. Only selected absorbencies (ν_{max}) are reported. **Mass Spectra:** Molecular ions and major peaks only are reported for low resolution spectra. Intensities are given as percentages of the base peak. HRMS values are valid to 5 ppm. **Optical Rotations:** These were recorded on a Perkin-Elmer 241 polarimeter at 589 nm (Na D-line) with a path length of 1 dm. Concentrations (c.) are quoted in g/100 mL and specific rotations, [α]_D^T, are quoted in units of 10⁻¹ deg cm² g⁻¹ at the specified temperature, T. **CD Spectra:** These were recorded in MeOH with a Jasco J-715 using 10 mm quartz cuvettes. **UV Spectra:** These were extracted from the corresponding HPLC UV diode

¹ <http://www.hse.gov.uk/coshh/index.htm>.

² Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, 43, 2923.

array detector (DAD) data. **Melting Points:** Analyses were carried out using a hot-stage and are uncorrected.

General Procedure for Screening of Conditions for Palladium-Mediated Biaryl Ether Formation (Table 1).



Entry	Pd Source (mol%)	Base (eq.)	Ligand (mol%)	T (°C)	Outcome ^a (20:22:21)
1 ³	Pd(OAc) ₂ (2)	NaH (1.4)	L1 (3)	100	1:0:0
2 ³	Pd(OAc) ₂ (2)	K ₃ PO ₄ (2.0)	L1 (3)	100	5.7:0:1 (15% conv.)
3 ³	Pd(OAc) ₂ (2)	Na salt 19 (1.2)	L1 (3)	110	1:0:0
4 ³	Pd ₂ (dba) ₃ (1.2)	Na salt 19 (1.2)	L1 (3.75)	110	1:0:0
5 ³	Pd(OAc) ₂ (4)	NaH (2.2)	L1 (6)	115	1:0:1.4 (58% conv.)
6 ³	Pd(OAc) ₂ (2)	NaH (1.4)	L2 (3)	100	1:0:0
7 ³	Pd(OAc) ₂ (2)	K ₃ PO ₄ (2.0)	L2 (3)	100	9:0:1 (10% conv.)
8 ³	Pd(OAc) ₂ (2)	Na salt 19 (1.2)	L2 (3)	110	1:0:0
9 ³	Pd ₂ (dba) ₃ (1.2)	Na salt 19 (1.2)	L2 (3)	110	5.7:0:1 (15% conv.)
10 ³	Pd(OAc) ₂ (4)	NaH (2.2)	L2 (6)	115	1:0:2.5 (71% conv.)
11 ⁴	Pd(OAc) ₂ (2)	K ₃ PO ₄ (2.0)	L5 (4)	120	1.5:0:1 (40% conv.)
12 ⁵	Pd(OAc) ₂ (3)	K ₃ PO ₄ (2.0)	L4 (5)	110	1:0:1.5 (60% conv.)
13 ⁵	Pd(OAc) ₂ (3)	K ₃ PO ₄ (2.0)	L3 (5)	110	1:0:2.5 (71% conv.)

Table 1: Investigation of Pd-catalysed biaryl ether formation. *Reagents and conditions:* as above, 60 h, N₂ atmosphere, toluene, 0.25 mmol. ^a Determined by ¹H NMR analysis of the unpurified reaction mixture.

An oven-dried reaction vial was charged with a stirrer bar, aryl bromide **20**, base, phenol **19**, palladium catalyst and ligand. The vial was equipped with a Suba-seal, then respectively evacuated and purged with nitrogen (× 5) before addition of toluene.

The Suba-seal was replaced by a screw cap under a flow of nitrogen, and the reaction mixture was heated at the appropriate temperature for the desired time. After this time the reaction was allowed to cool to r.t., filtered through a pad of Celite[®], washed with acetone and concentrated *in vacuo*. Integration of key peaks in the ¹H NMR spectra of the unpurified reaction mixtures was used to determine the product distribution.

³ Aranyos, A.; Old, D. W.; Kiyomori, A.; Wolfe, J. P.; Sadighi, J. P.; Buchwald, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 4369-4378.

⁴ Harkal, S.; Kumar, K.; Michalik, D.; Zapf, A.; Jackstell, R.; Rataboul, F.; Riermeier, T.; Monsees, A.; Beller, M. *Tetrahedron Lett.* **2005**, *46*, 3237-3240.

⁵ Burgos, C. H.; Barder, T. E.; Huang, X.; Buchwald, S. L. *Angew. Chem. Int. Ed.* **2006**, *45*, 4321-4326.

General Procedure for Screening of Conditions for Copper-Mediated Biaryl Ether Formation (Table 2)

Entry	19 eq.	Cu Source (mol%)	Base (eq.)	Solvent	Additive (mol%)	T (°C)	Outcome ^a 20:22:21	% Conversion (% 22) ^a
1 ⁶	1.2	CuI (10)	K ₂ CO ₃ (2.0)	1,4-dioxane	Ni-Al alloy (30)	110	2:5:1	75 (63)
2 ⁷	1.4	(CuOTf) ₂ ·PhH (2.5)	Cs ₂ CO ₃ (1.4)	toluene	EtOAc (5)	110	0:1:0	>98 (>98) 72% isolated yield ^a
3 ⁸	1.5	Cu ₂ O (10)	Cs ₂ CO ₃ (2.0)	MeCN	L1 (20)	110	0:>20:1	>98 (98) 70% isolated yield ^a
4 ⁹	1.5	CuI (10)	Cs ₂ CO ₃ (2.0)	1,4-dioxane	L2 (30)	90	3:10:1	79 (71)
5 ¹⁰	2.0	CuCl (50)	Cs ₂ CO ₃ (2.0)	NMP	L3 (10)	120	0:18:1	100 (95)
6 ¹¹	2.0	CuCl (50)	Cs ₂ CO ₃ (2.0)	NMP	L4 (10)	120	0:>20:1	100 (98)

Table 2: Ullmann-type copper catalysed biaryl ether formation. *Reagents and conditions:* as above, 60 h, N₂ atmosphere, 0.25 mmol. ^aDetermined by ¹H NMR analysis of the unpurified reaction mixture.

An oven-dried reaction vial was charged with a stirrer bar, aryl bromide **20**, base, phenol **19**, copper salt and additive/ligand. The vial was equipped with a Suba-seal, then repeatedly evacuated and purged with nitrogen (× 5) before addition of the solvent. The Suba-seal was then replaced by a screw cap under a flow of nitrogen, and the reaction mixture was heated at the appropriate temperature for the desired time. After this time the reaction was allowed to cool to r.t., filtered through a pad of Celite[®], washed with acetone and concentrated *in vacuo*. In the case of reactions carried out in DMA as solvent, the crude reaction mixture was flushed through a silica plug with EtOAc:petrol (1:5) and concentrated *in vacuo*. Integration of key peaks in the ¹H NMR spectra of the unpurified reaction mixtures was used to determine the product distribution.

⁶ Xu, L.-W.; Xia, C.-G.; Li, J.-W.; Hu, X.-X. *Synlett* **2003**, 13, 2071-2073.

⁷ Marcoux, J.-F.; Doye, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1997**, 119, 10539-10540.

⁸ Cristau, H.-J.; Cellier, P. P.; Spindler, J.-F.; Taillefer, M. *Org. Lett.* **2004**, 6, 913-916.

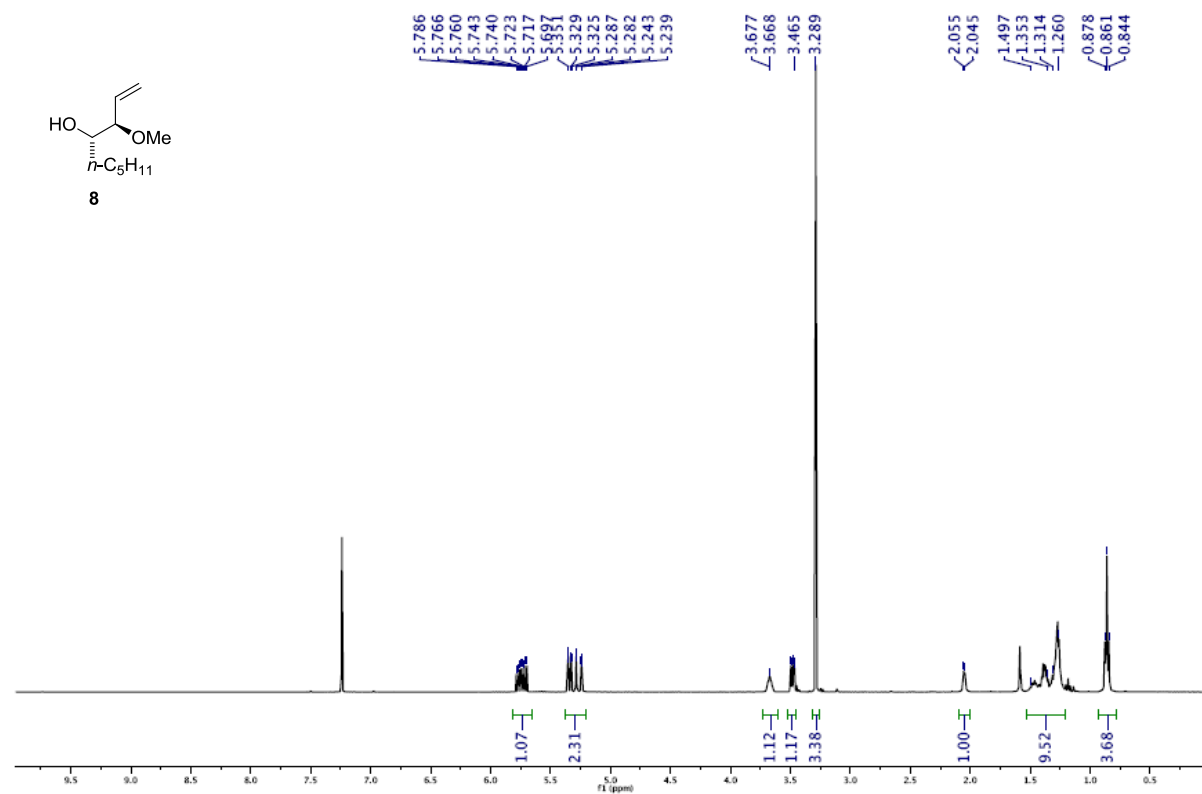
⁹ Ma, D.; Cai, Q. *Org. Lett.* **2003**, 5, 3799-3802.

¹⁰ Buck, E.; Song, Z. J.; Tschaen, D.; Dormer, P. G.; Volante, R. P.; Reider, P. J. *Org. Lett.* **2002**, 4, 1623-1626.

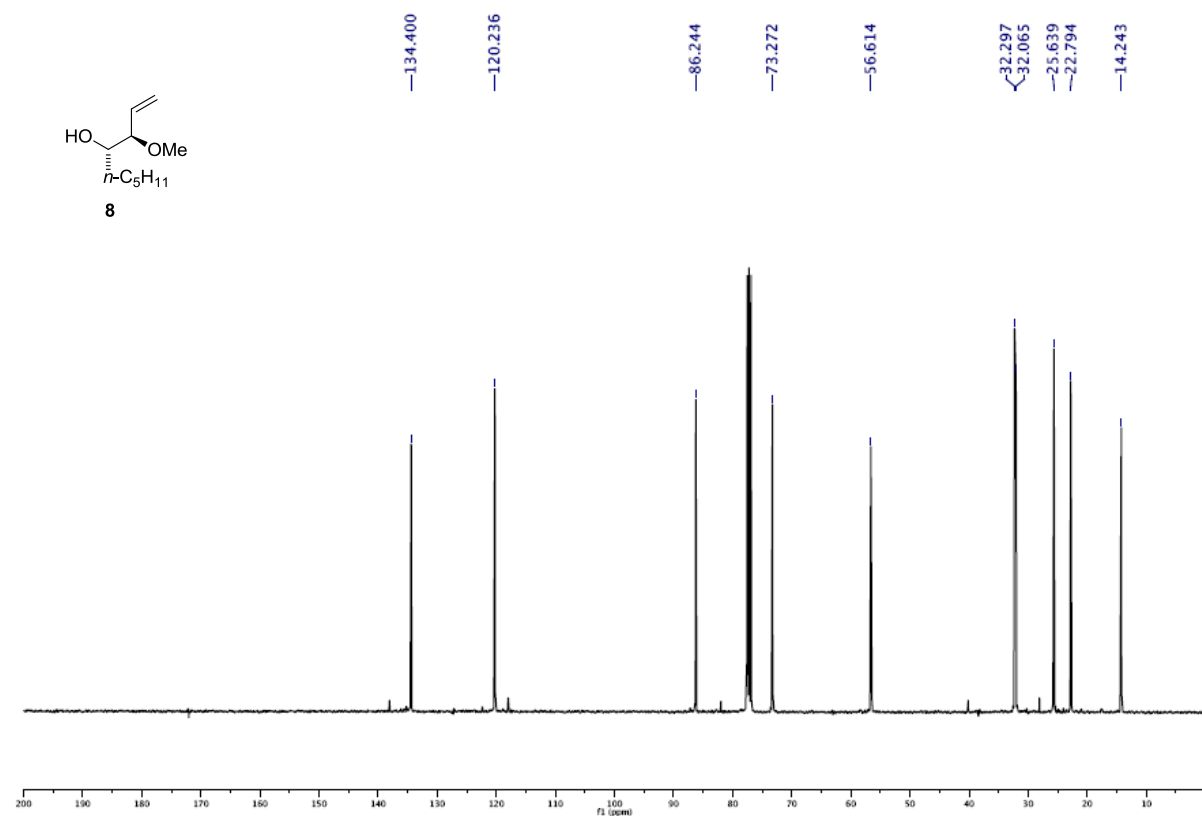
¹¹ Gujadhur, R. K.; Bates, C. G.; Venkataraman, D. *Org. Lett.* **2001**, 3, 4315-4317.

NMR Spectra for compounds 8, 12, 13, 14, 17a, 22, 28, 4b, 5, 30 and 31.

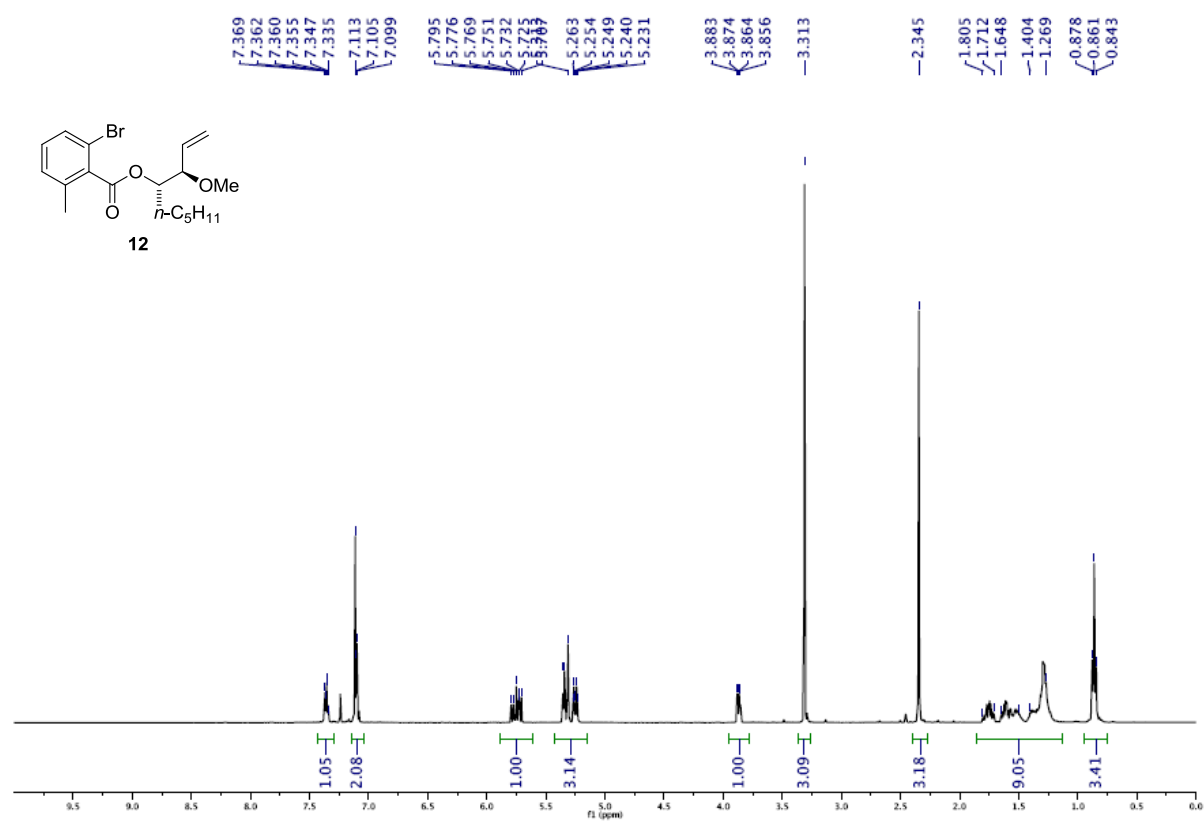
^1H NMR, 400 MHz, CDCl_3



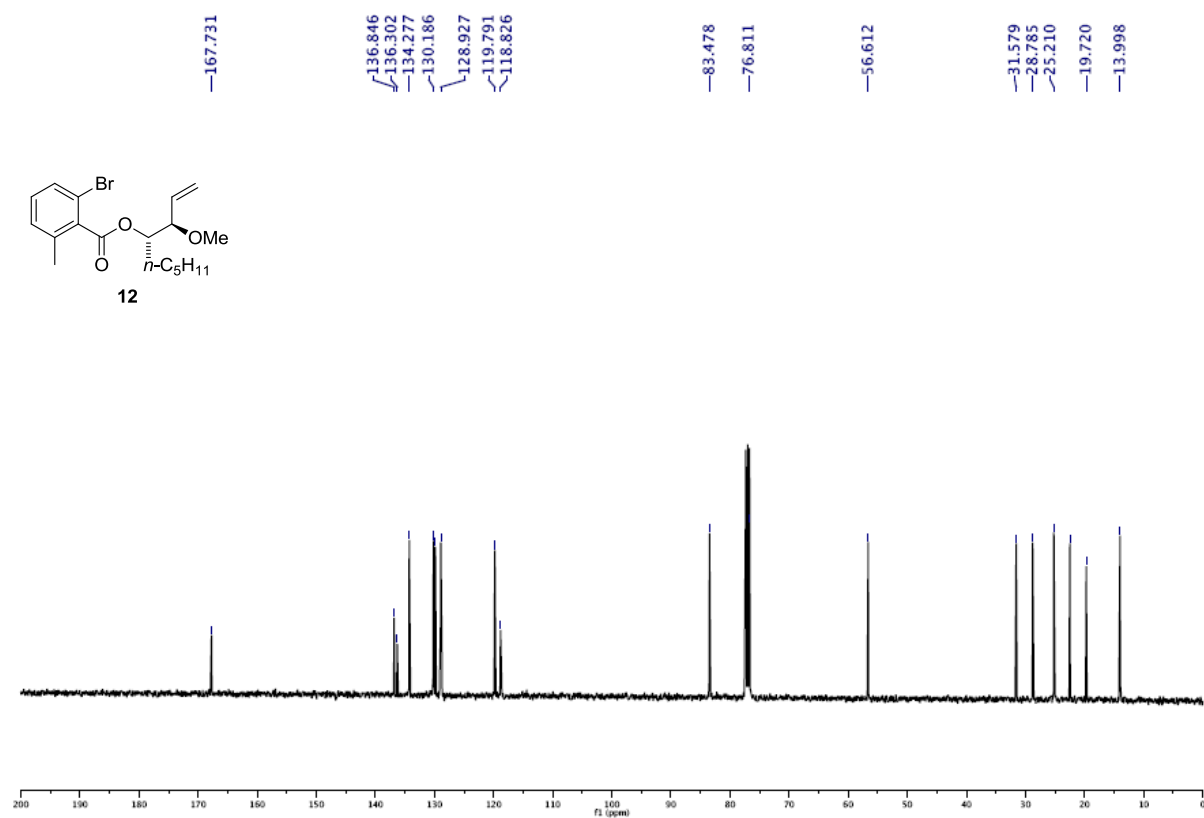
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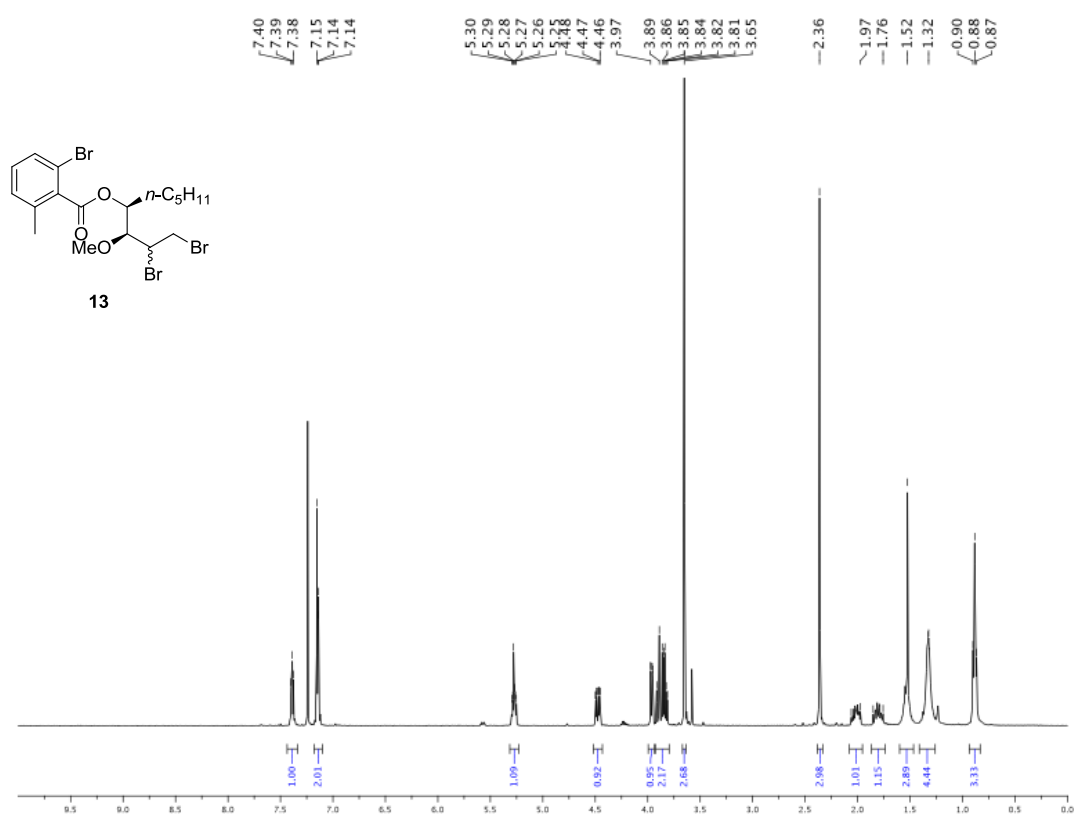
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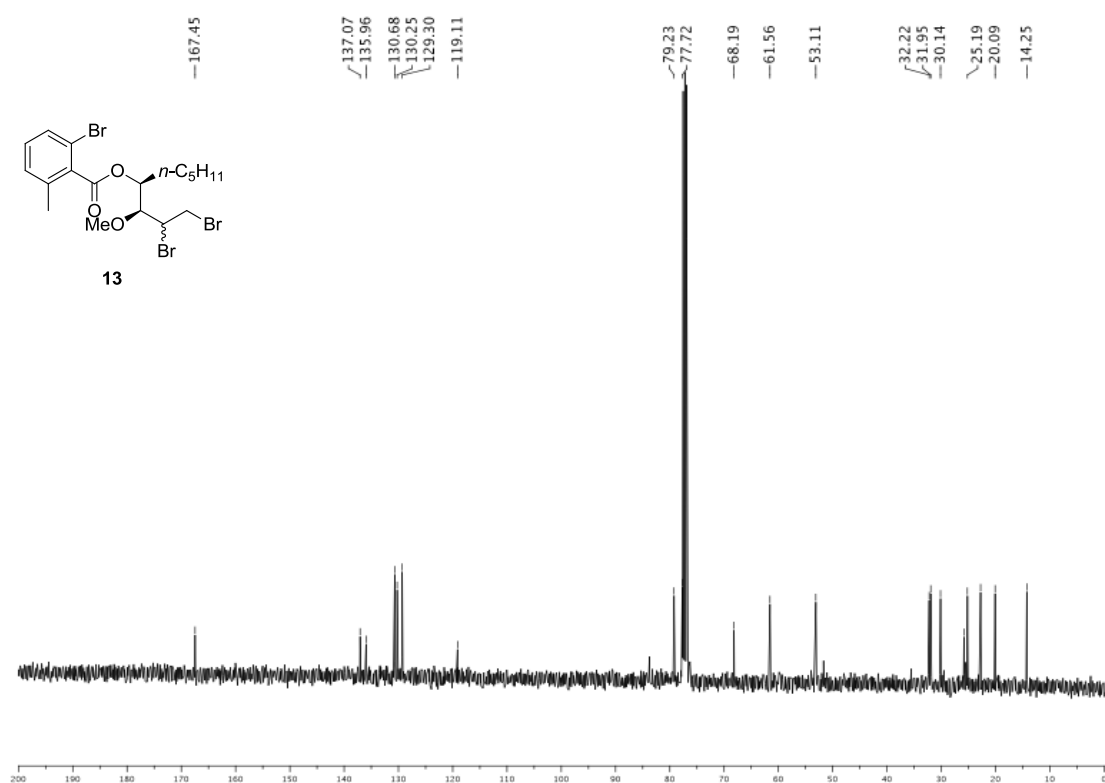
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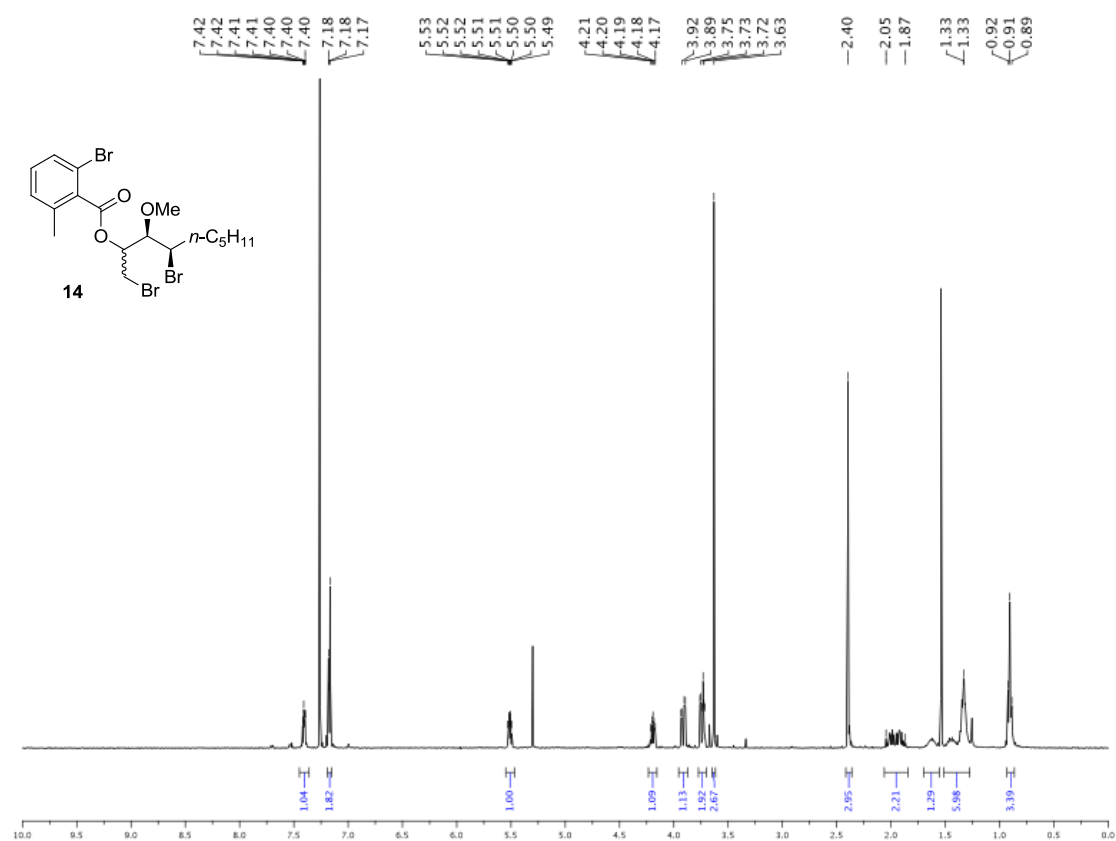
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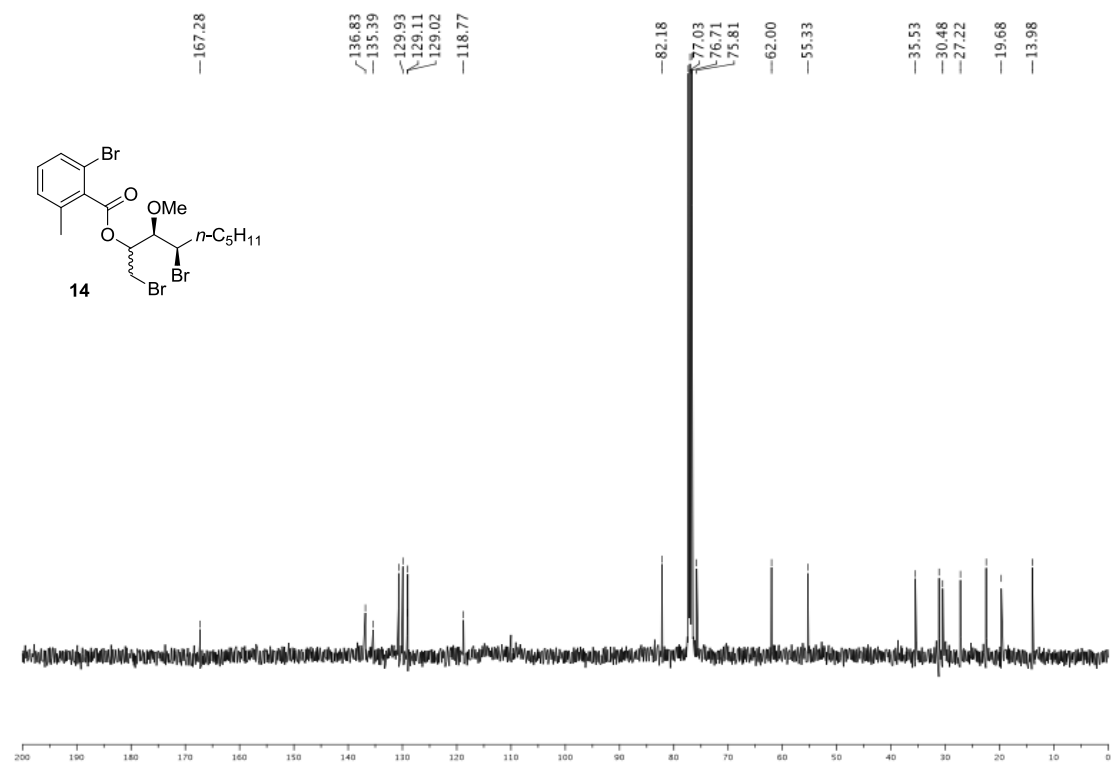
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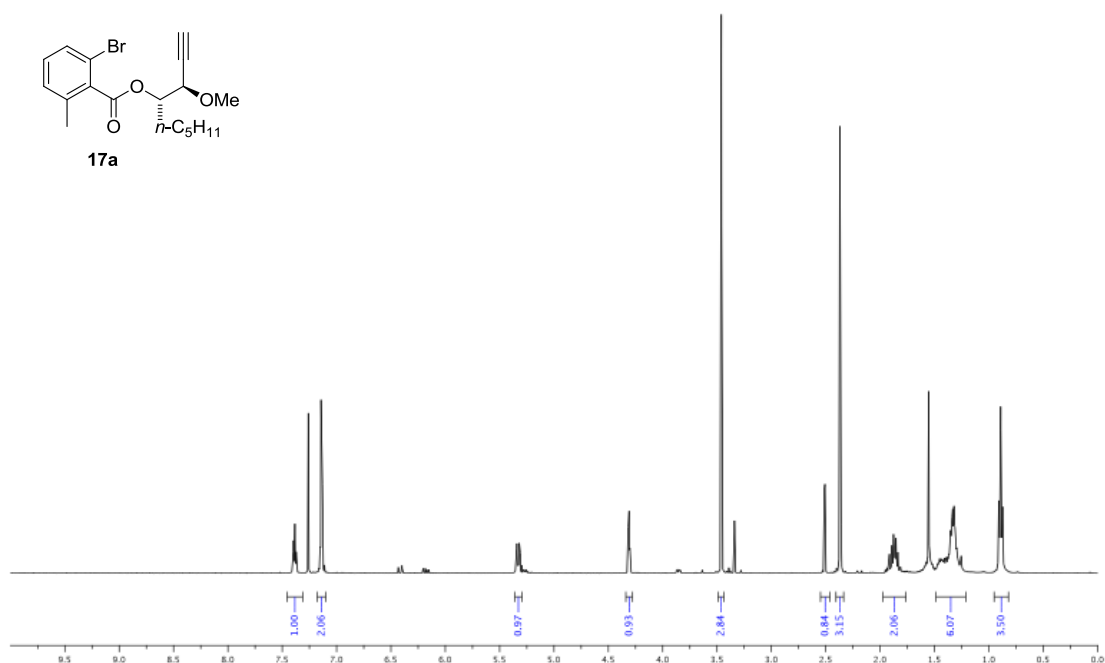
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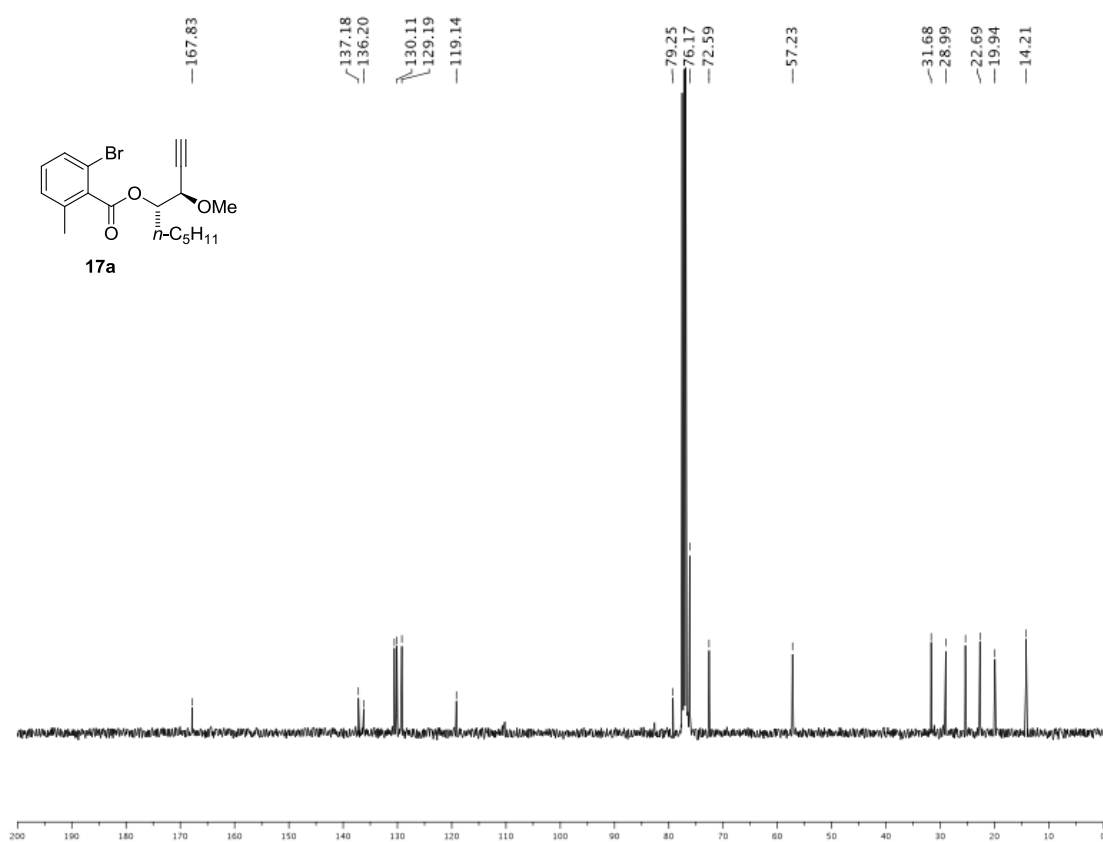
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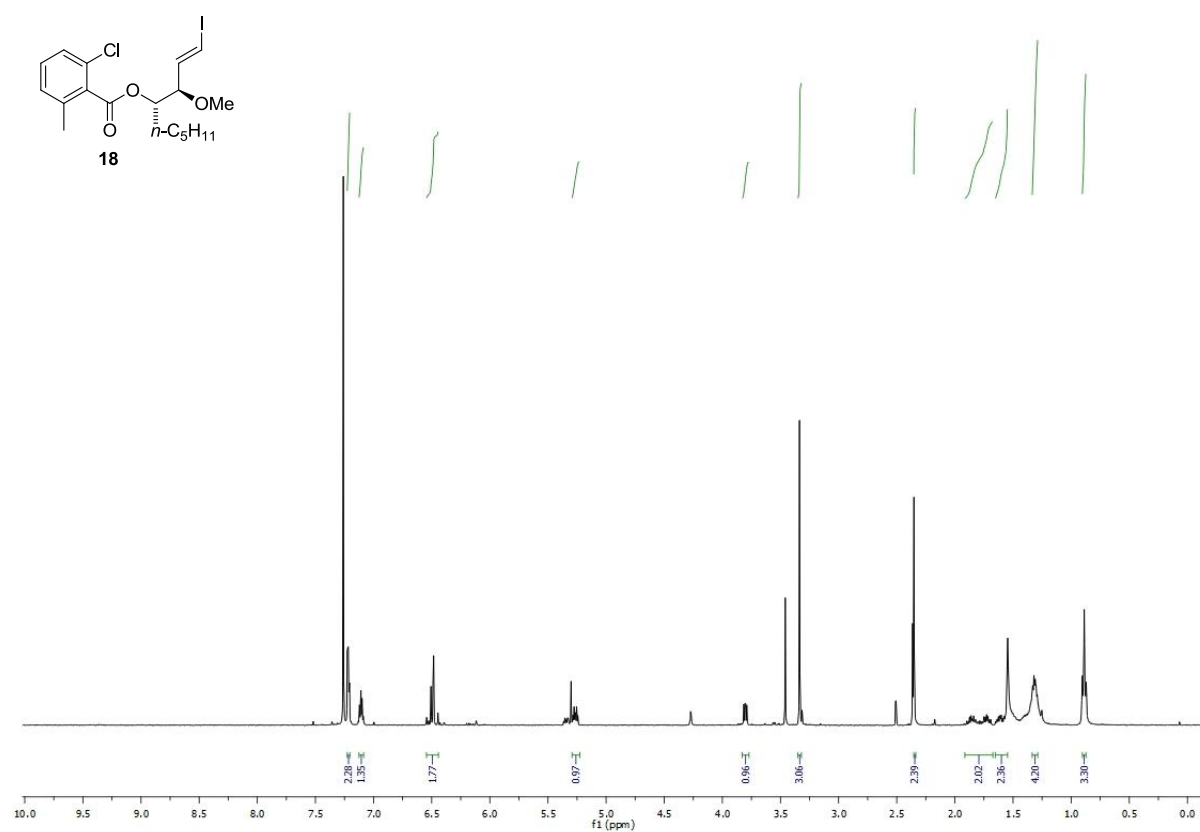
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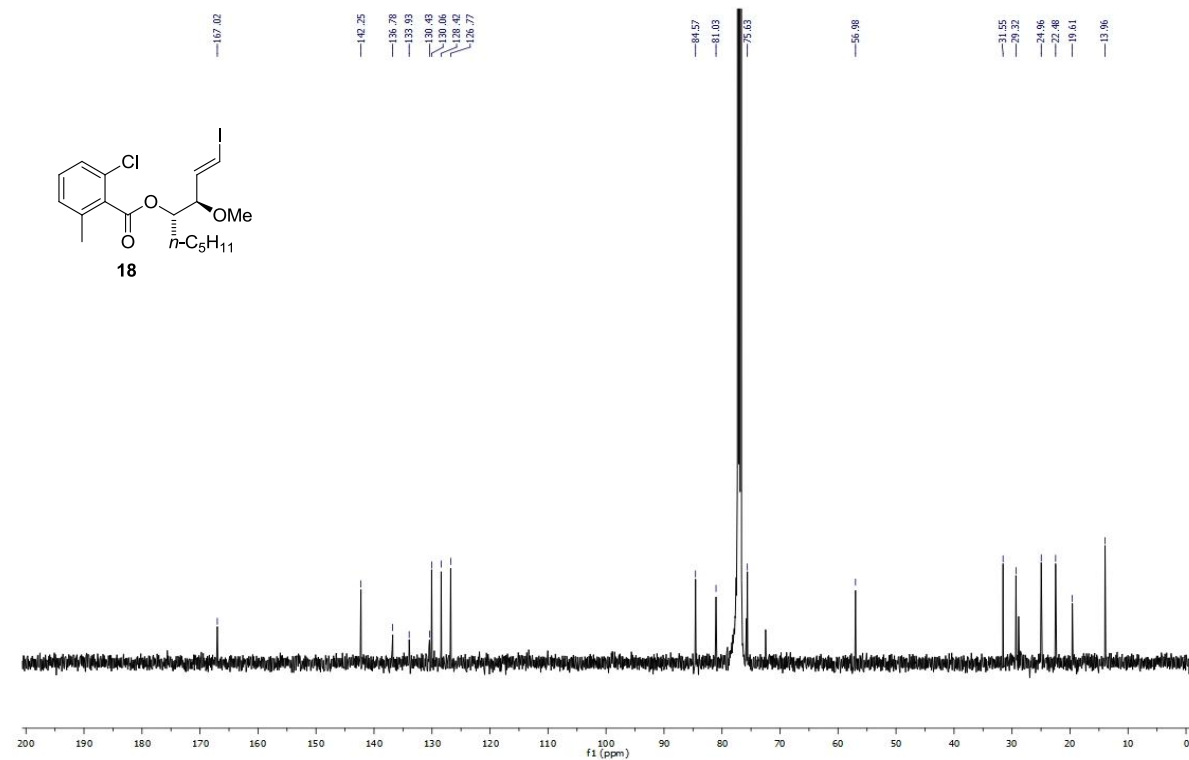
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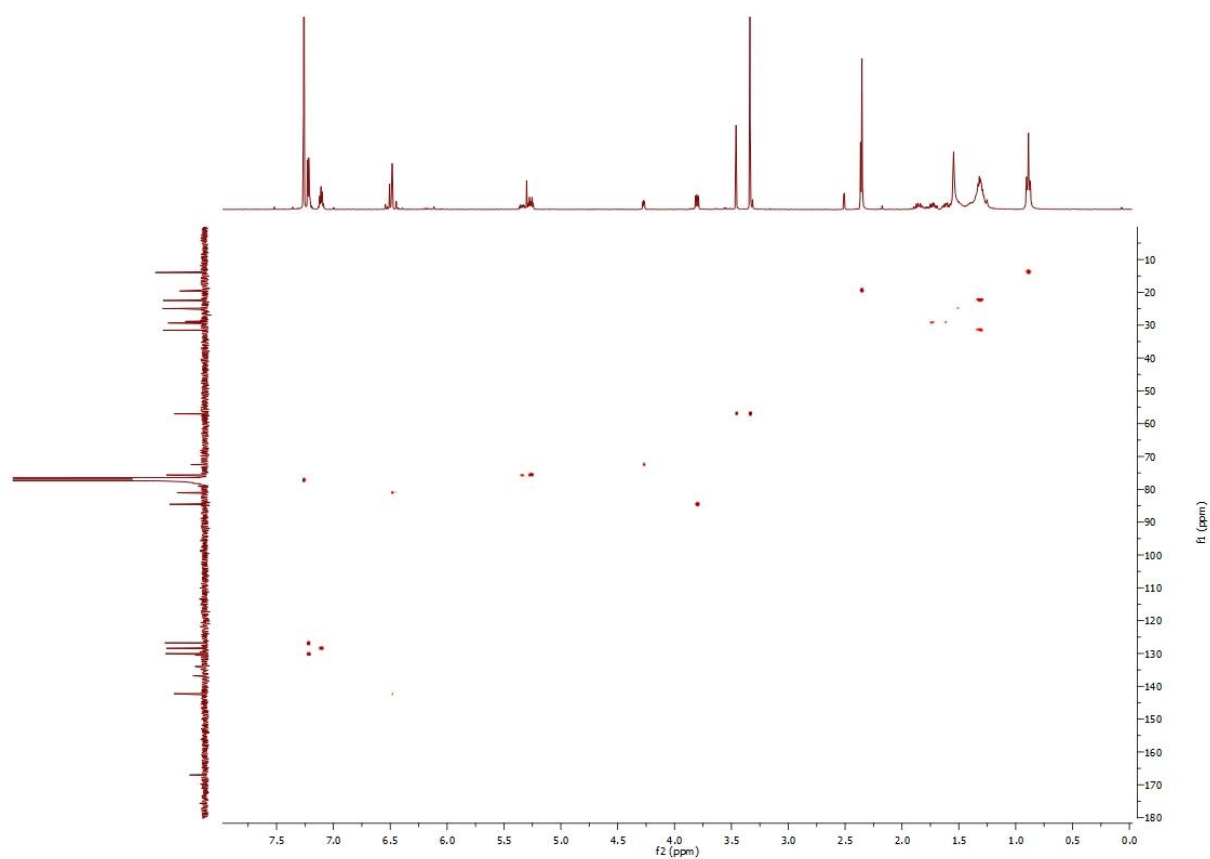
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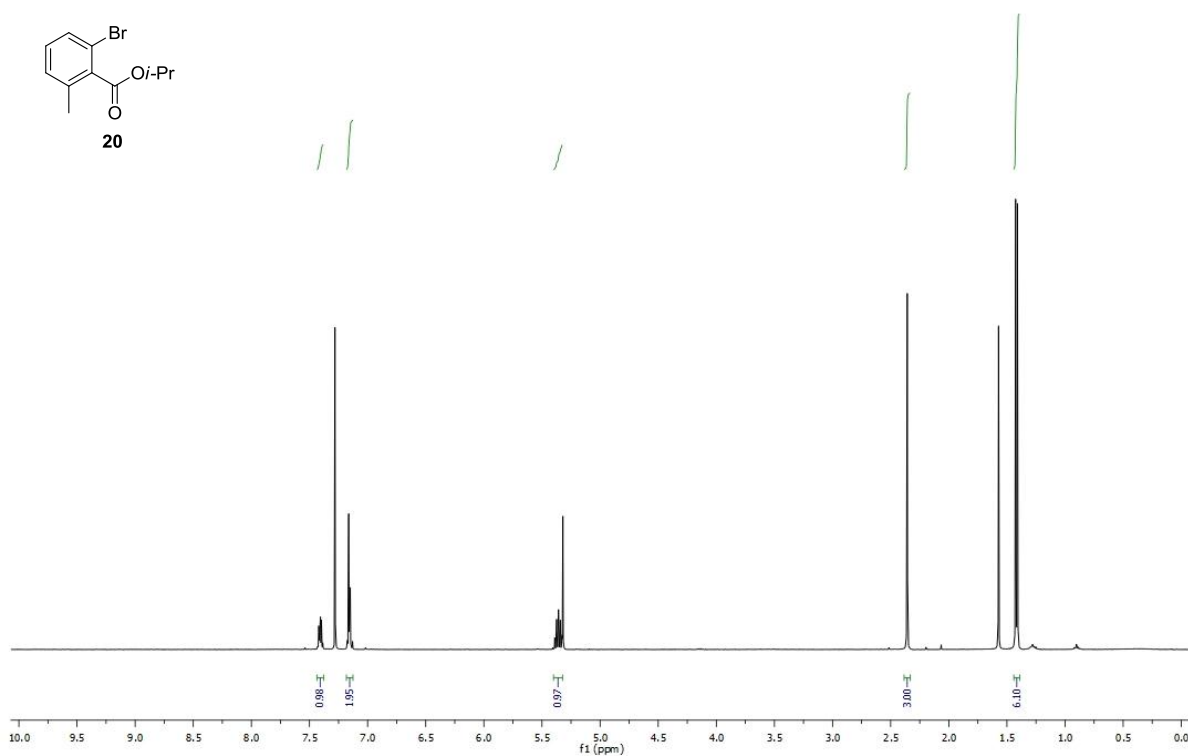
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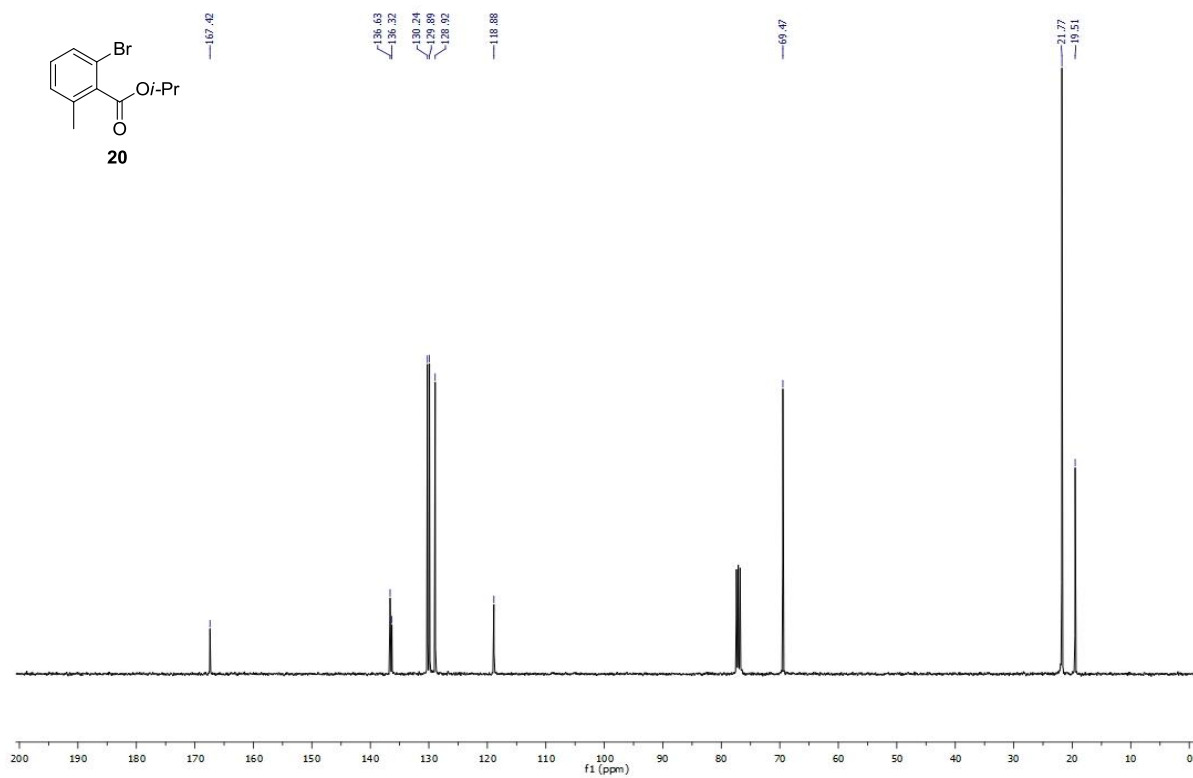
HSQC NMR, CDCl₃



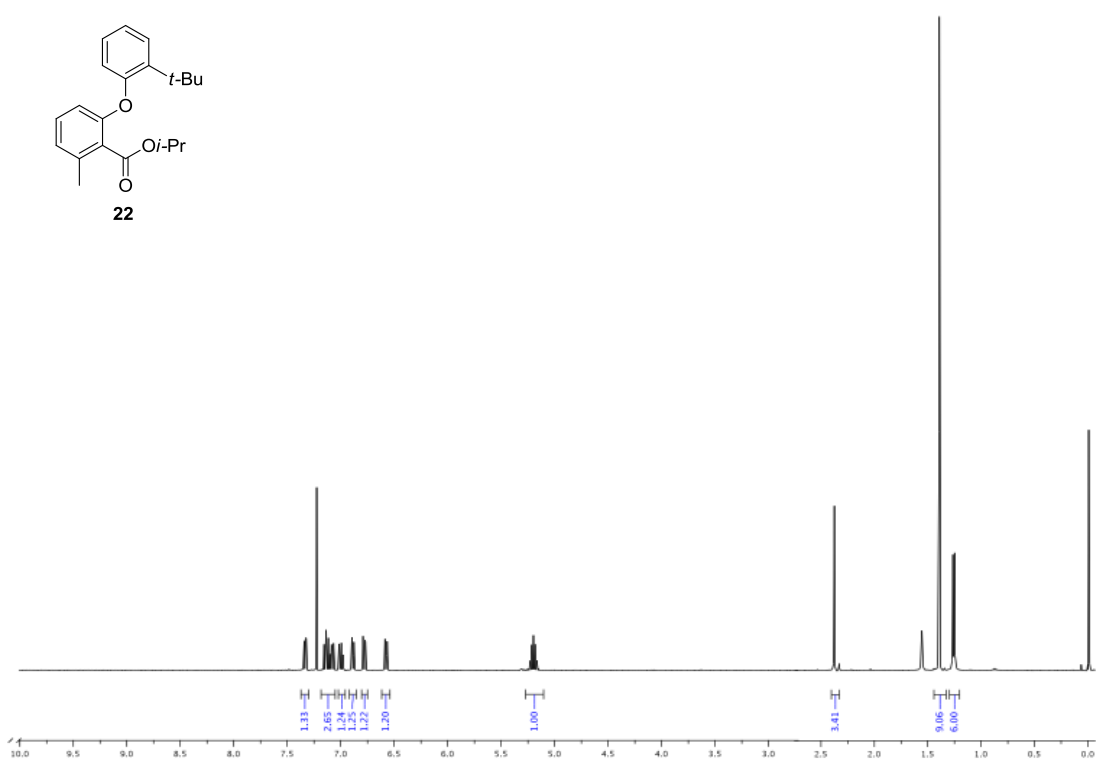
^1H NMR, 400 MHz, CDCl_3



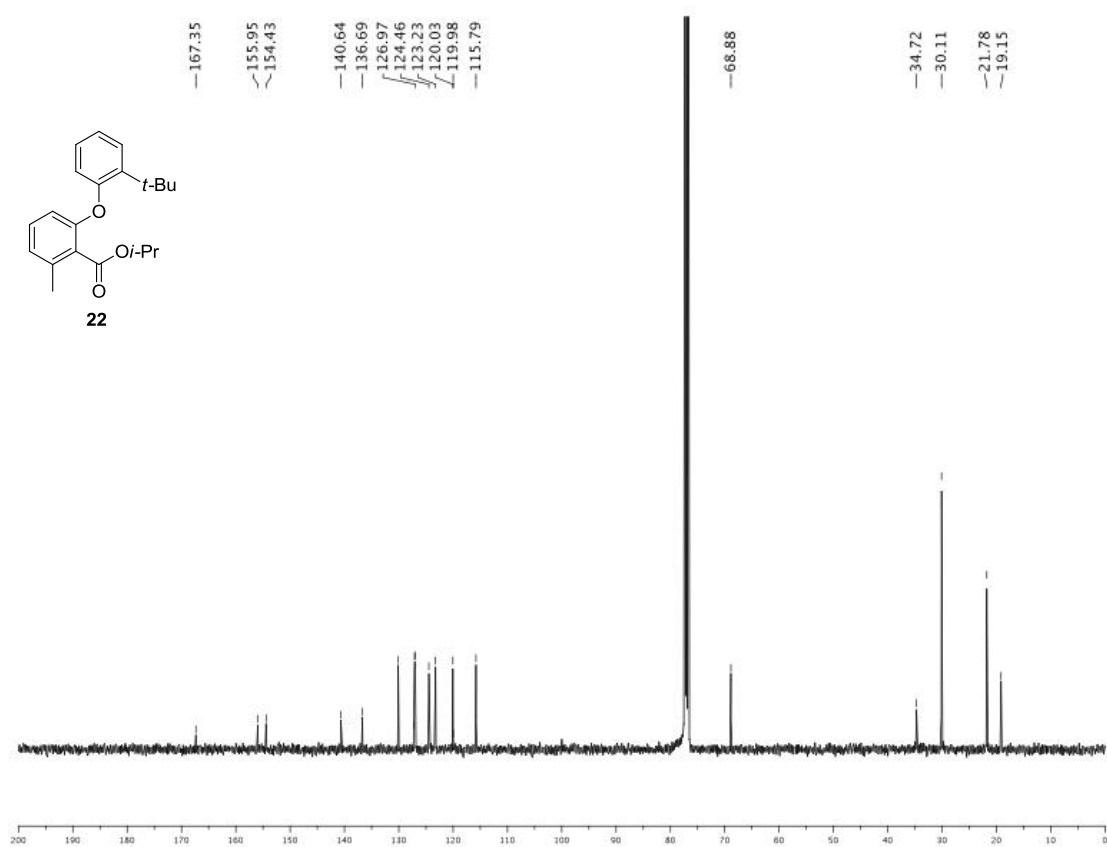
^{13}C NMR, 100 MHz, CDCl_3



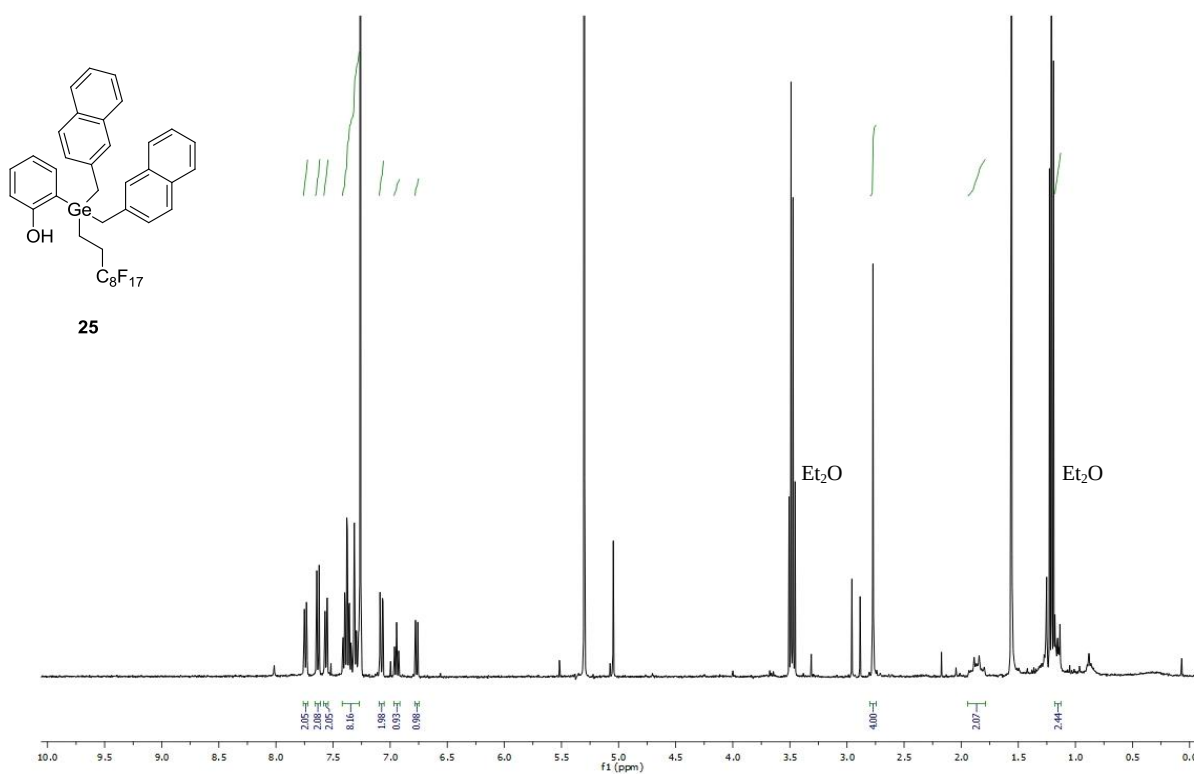
^1H NMR, 400 MHz, CDCl_3



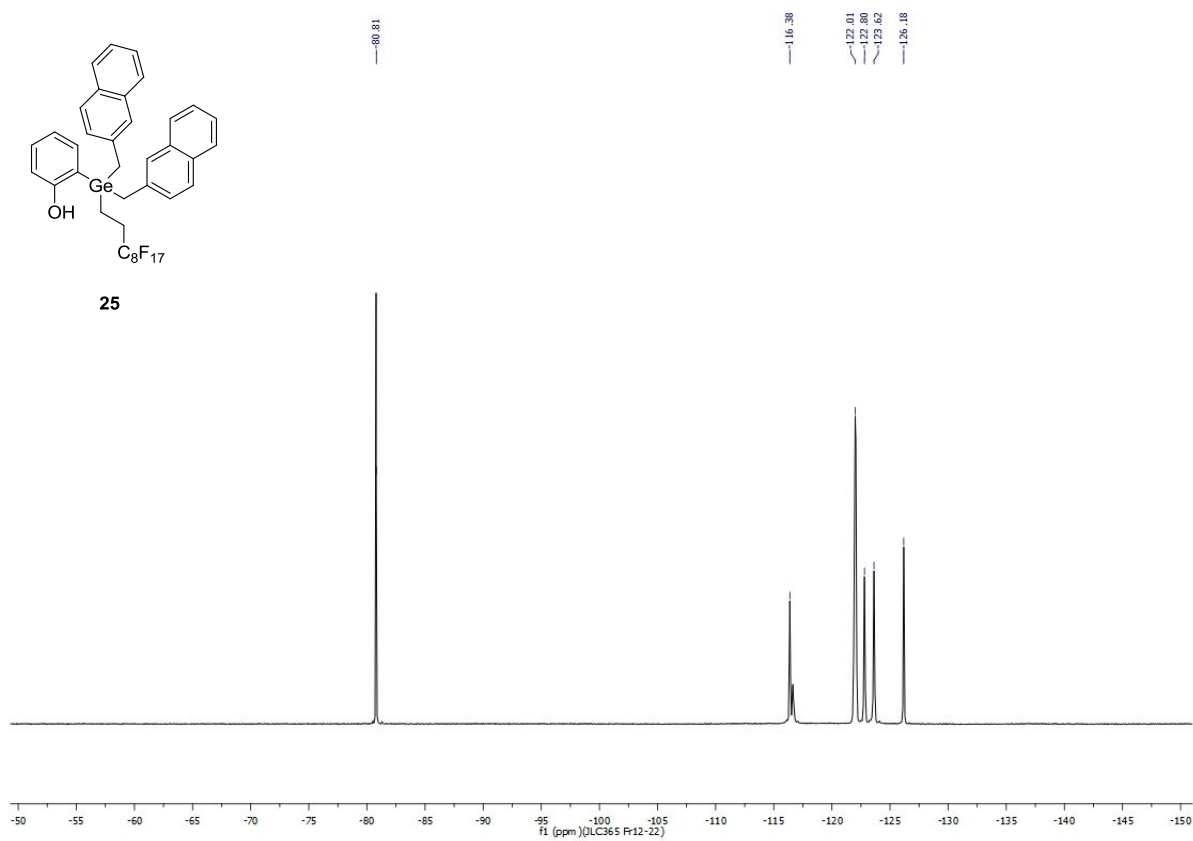
^{13}C NMR, 125 MHz, CDCl_3



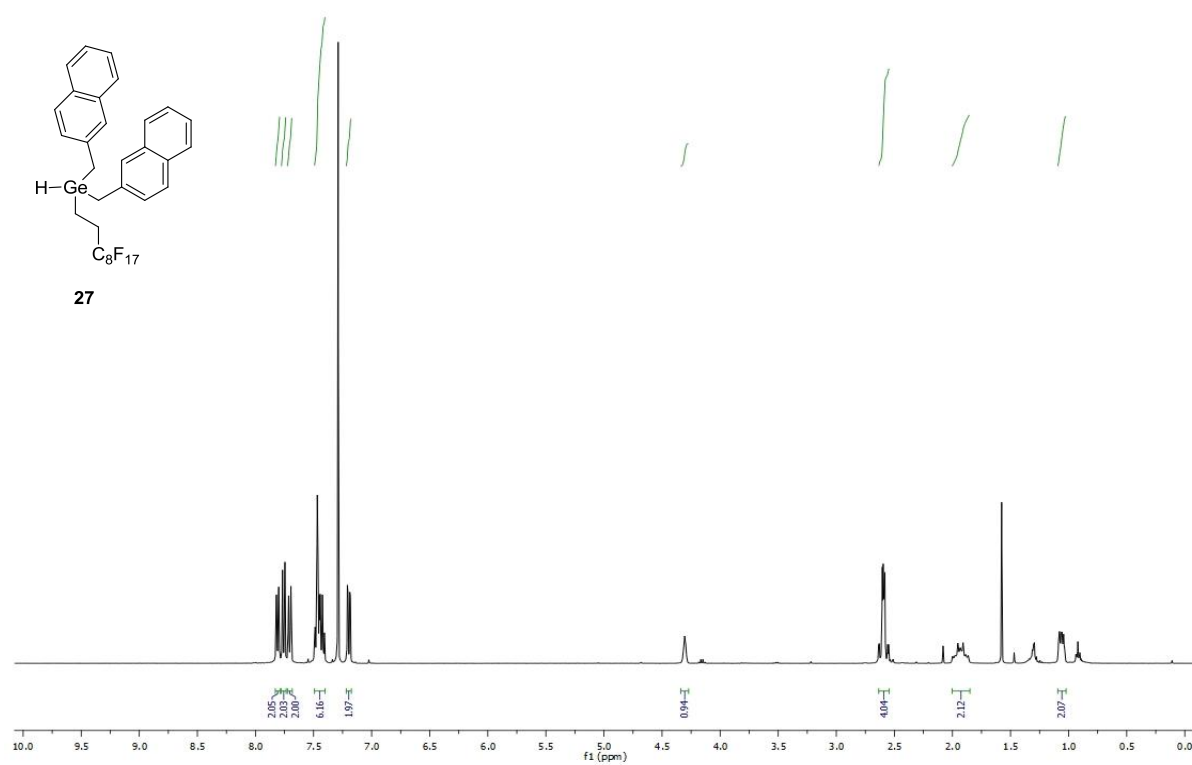
^1H NMR, 400 MHz, CDCl_3



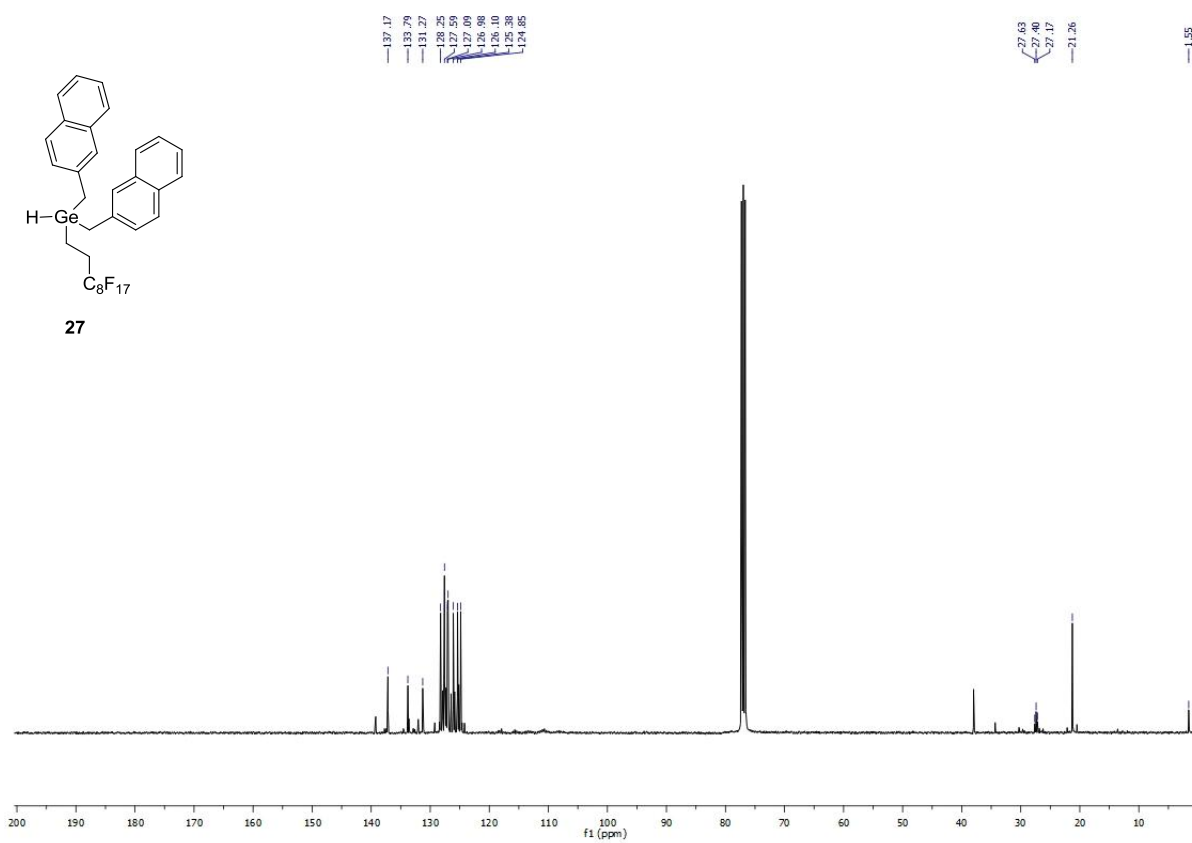
^{19}F NMR, 376 MHz, CDCl_3



^1H NMR, 400 MHz, CDCl_3



^{13}C NMR, 100 MHz, CDCl_3



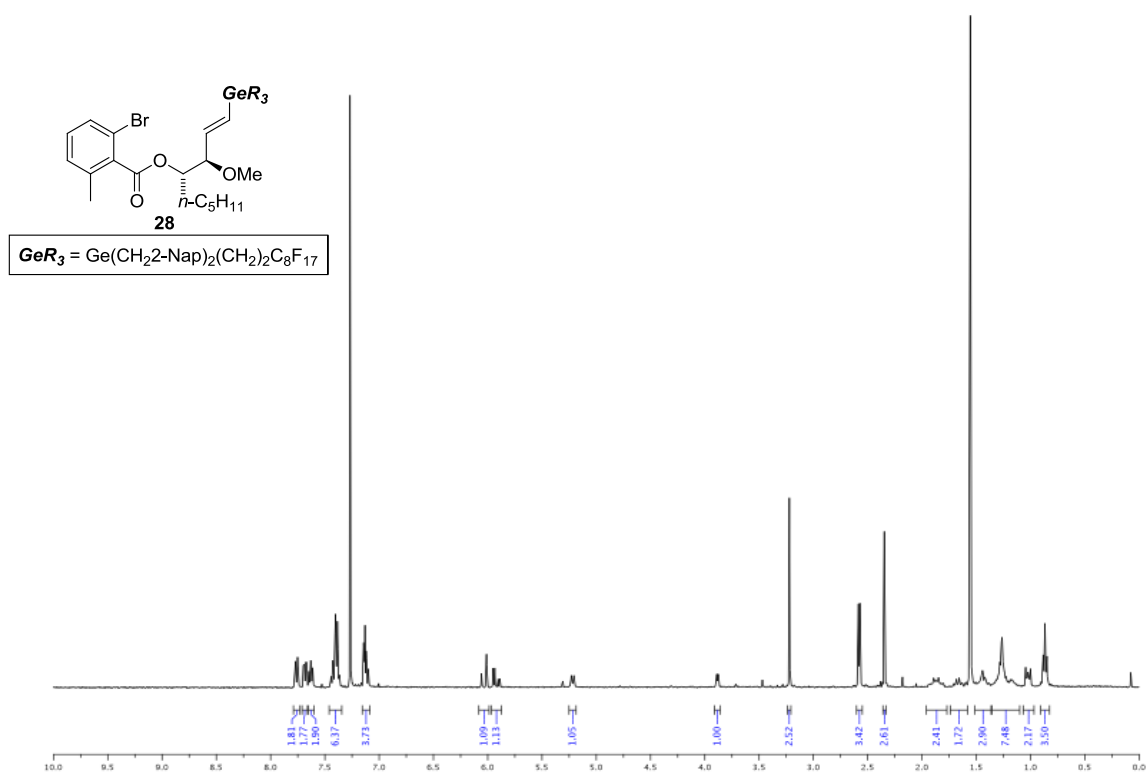
27

Chemical structure of compound **27** is shown. The structure features a germanium atom bonded to a hydrogen atom, a 1-phenylethyl group, a 1-(naphthalen-1-ylethyl) group, and a 2-(8-fluorooctyl)ethyl group.

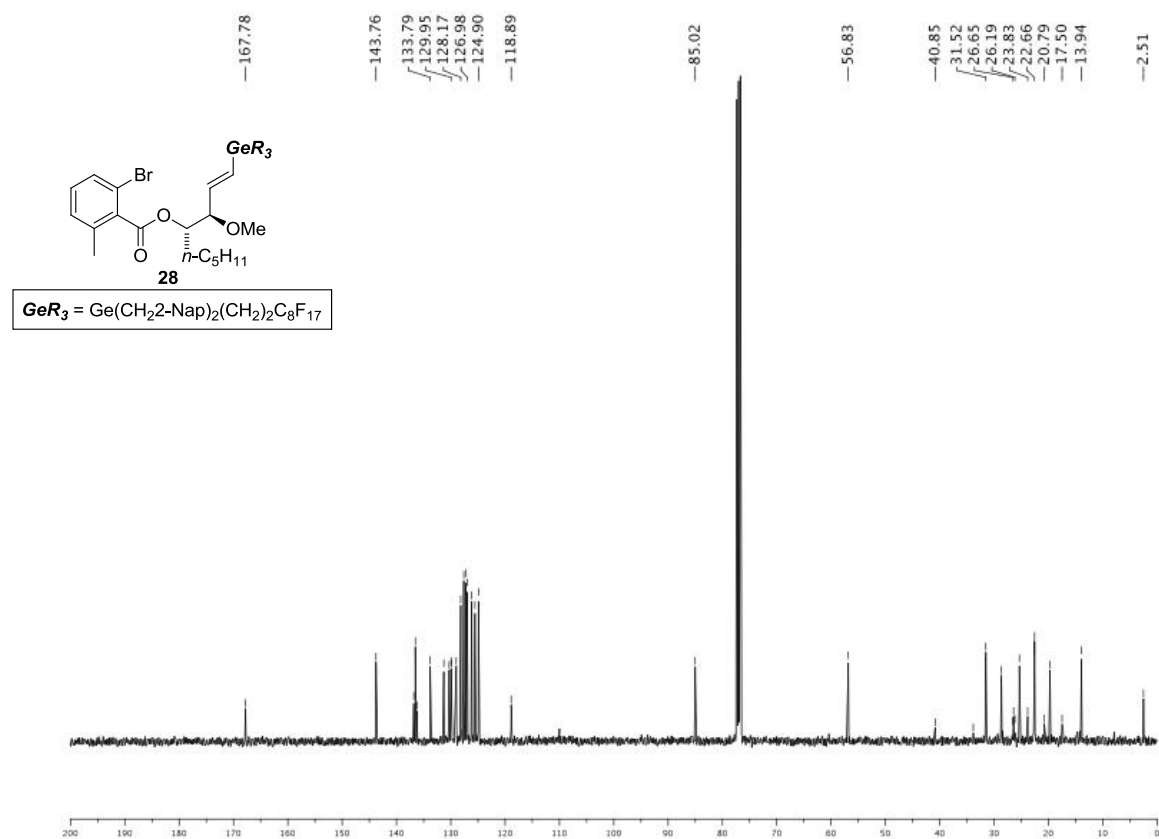
The ^1H NMR spectrum (CDCl₃) shows the following peaks (ppm):

- ~7.2 (broad, H-Ge)
- 7.0-7.5 (aromatic signals)
- ~4.2 (triplet, CH₂-Ge)
- 1.2-1.6 (alkyl chain signals)
- 1.26.13 (peak at ~1.2 ppm)

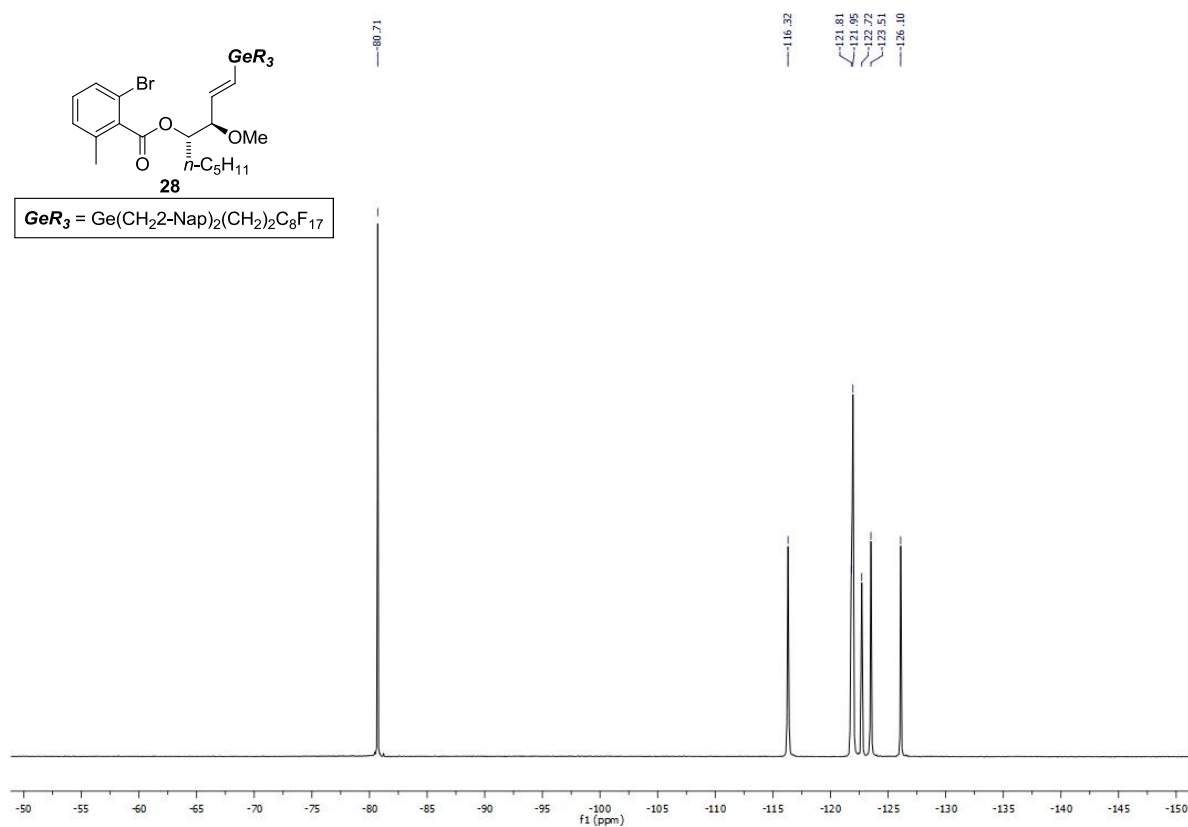
^1H NMR, 400 MHz, CDCl_3



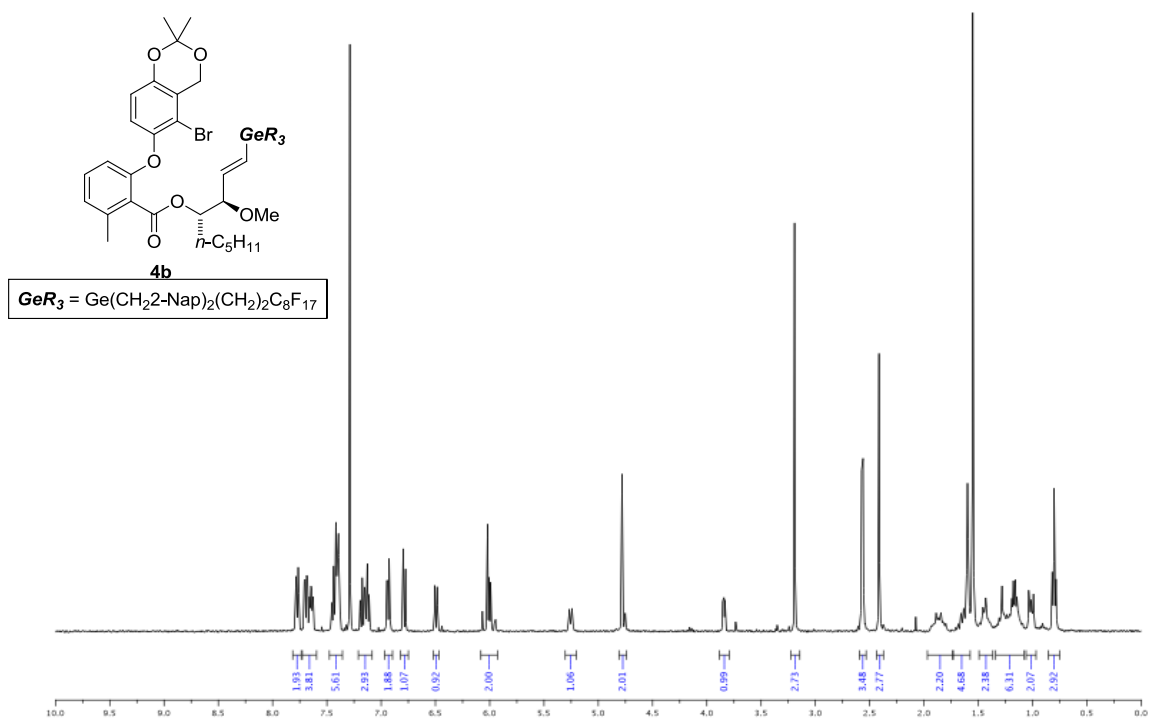
^{13}C NMR, 125 MHz, CDCl_3



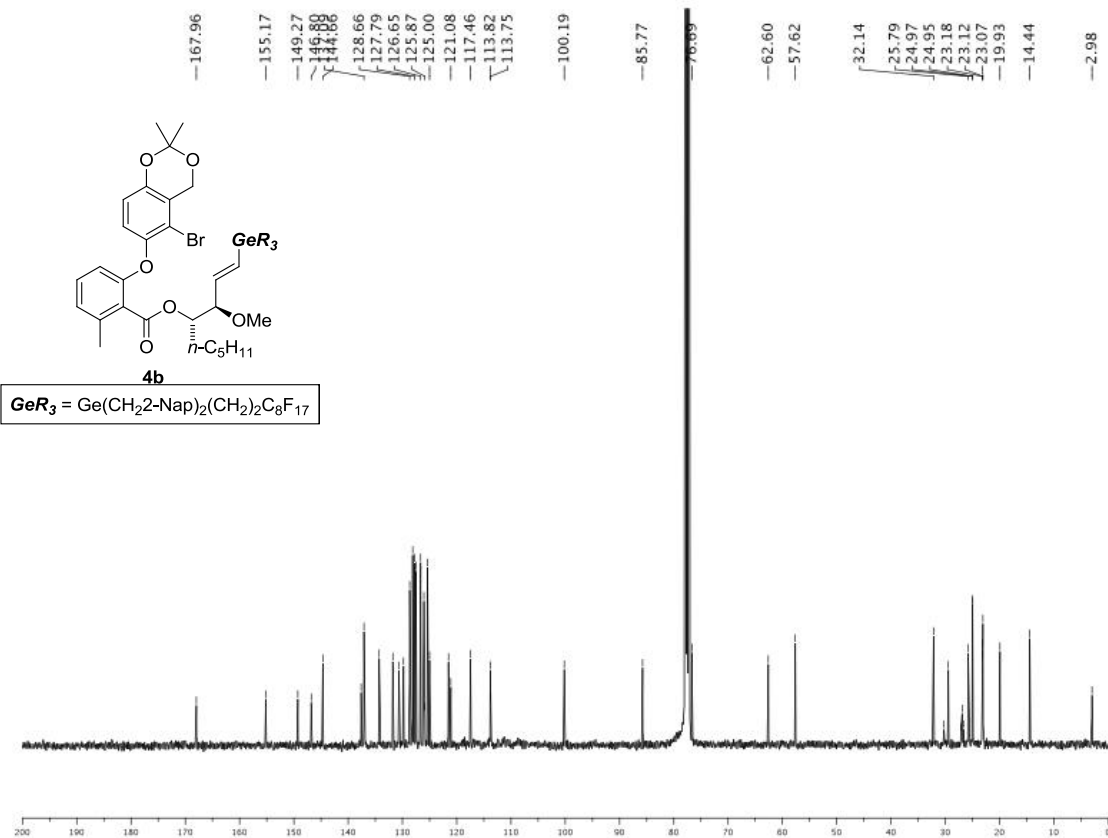
^{19}F NMR, 376 MHz, CDCl_3



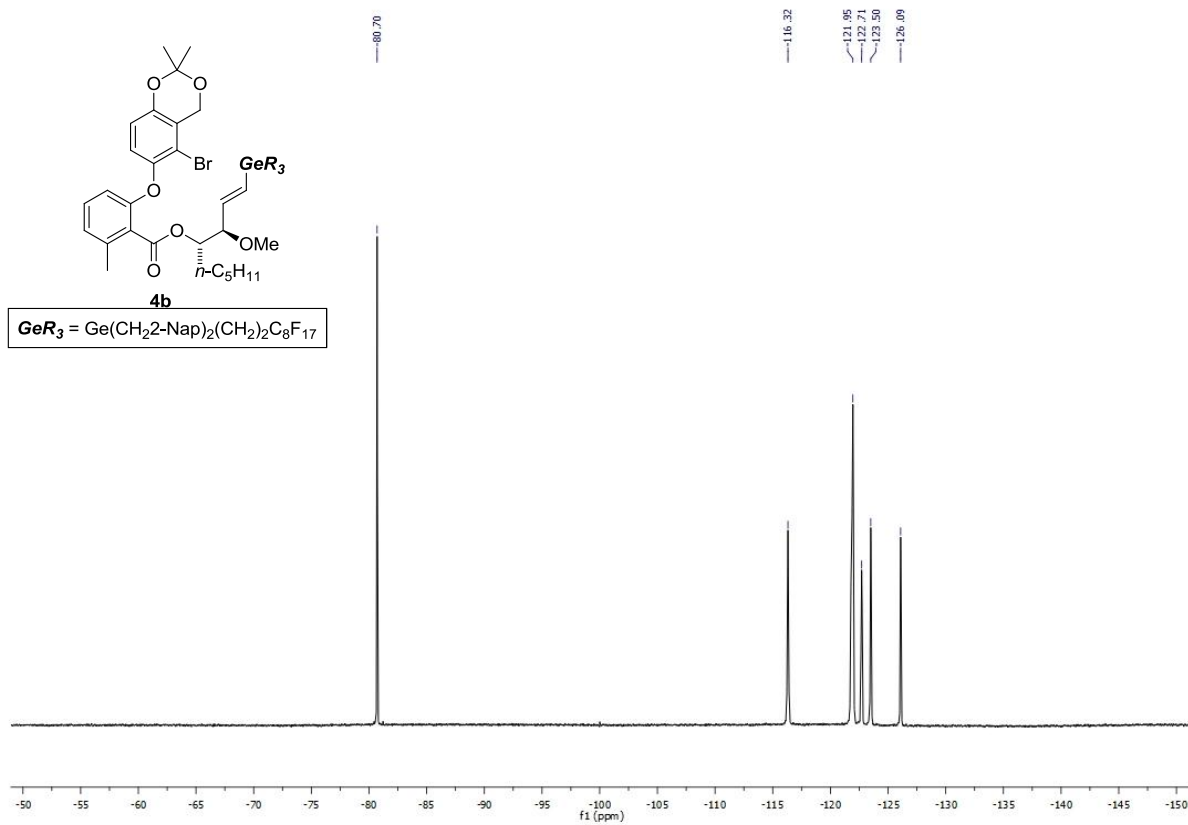
^1H NMR, 400 MHz, CDCl_3



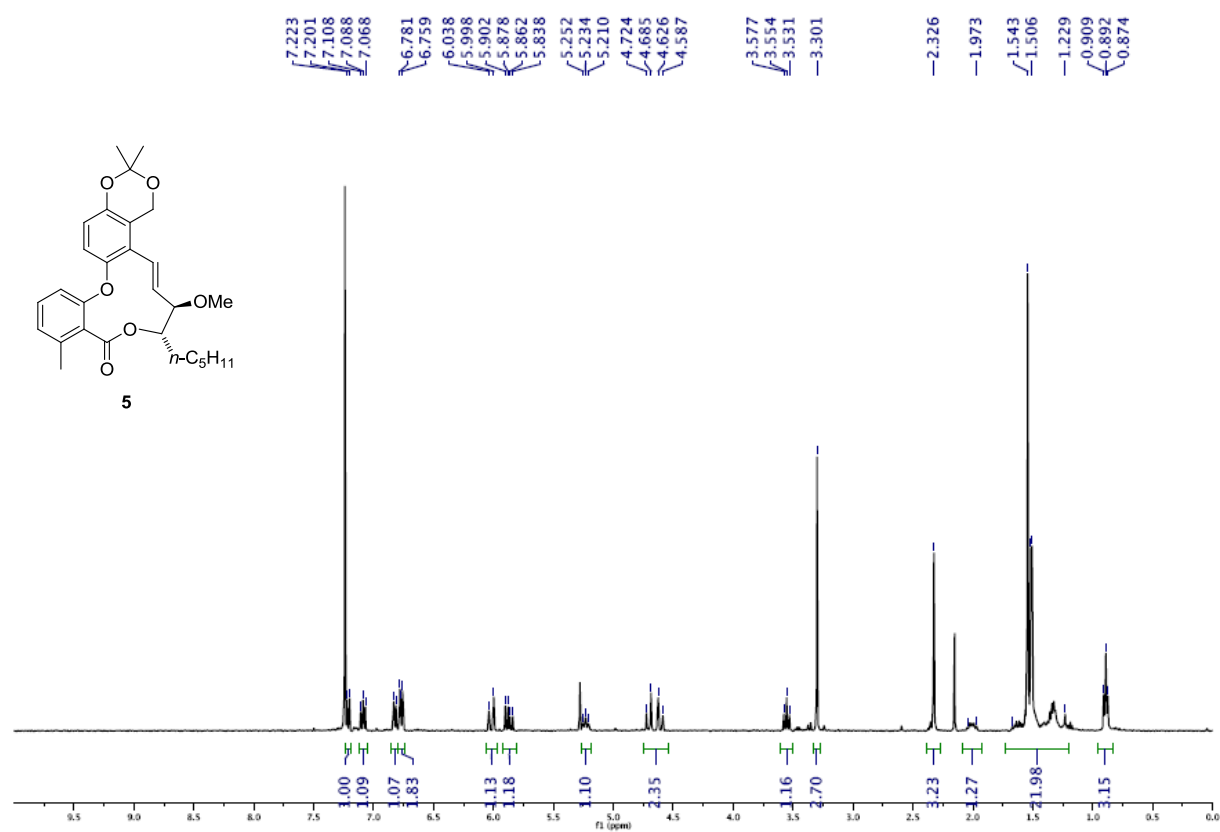
^{13}C NMR, 125 MHz, CDCl_3



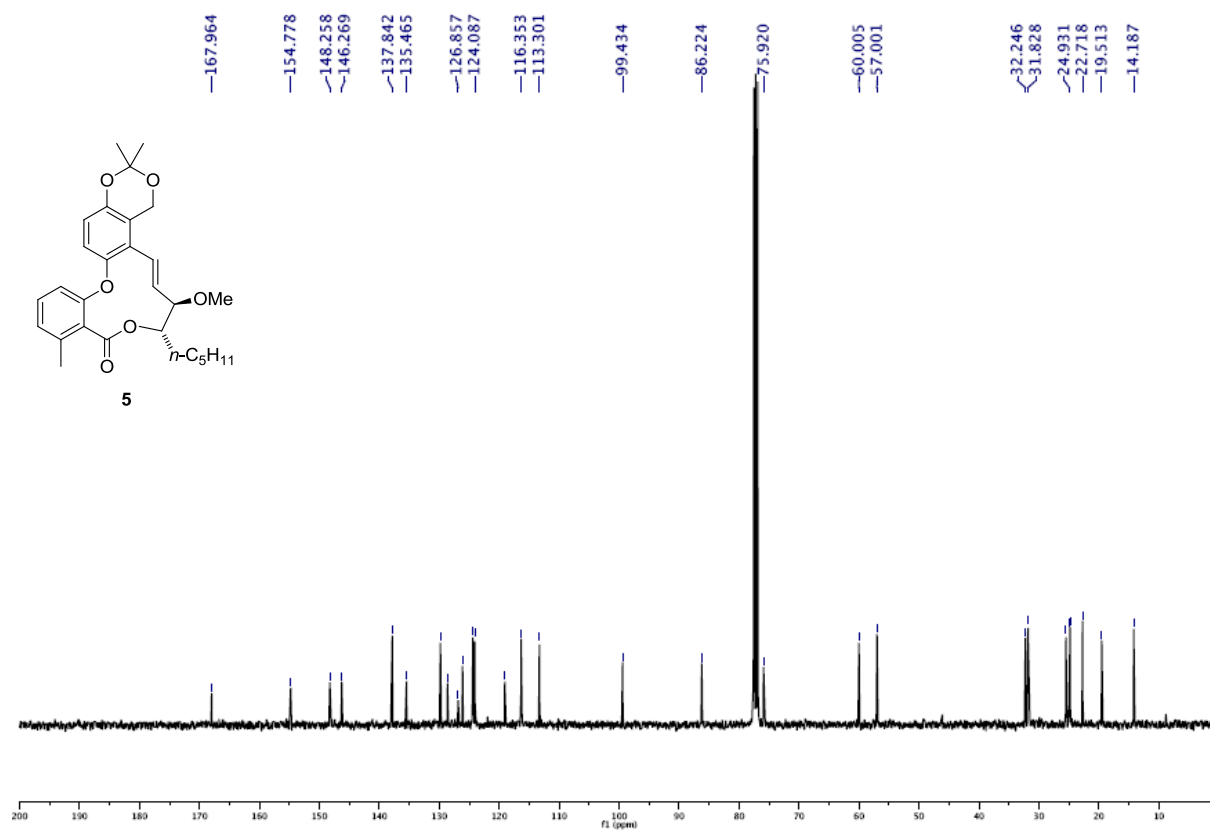
^{19}F NMR, 376 MHz, CDCl_3



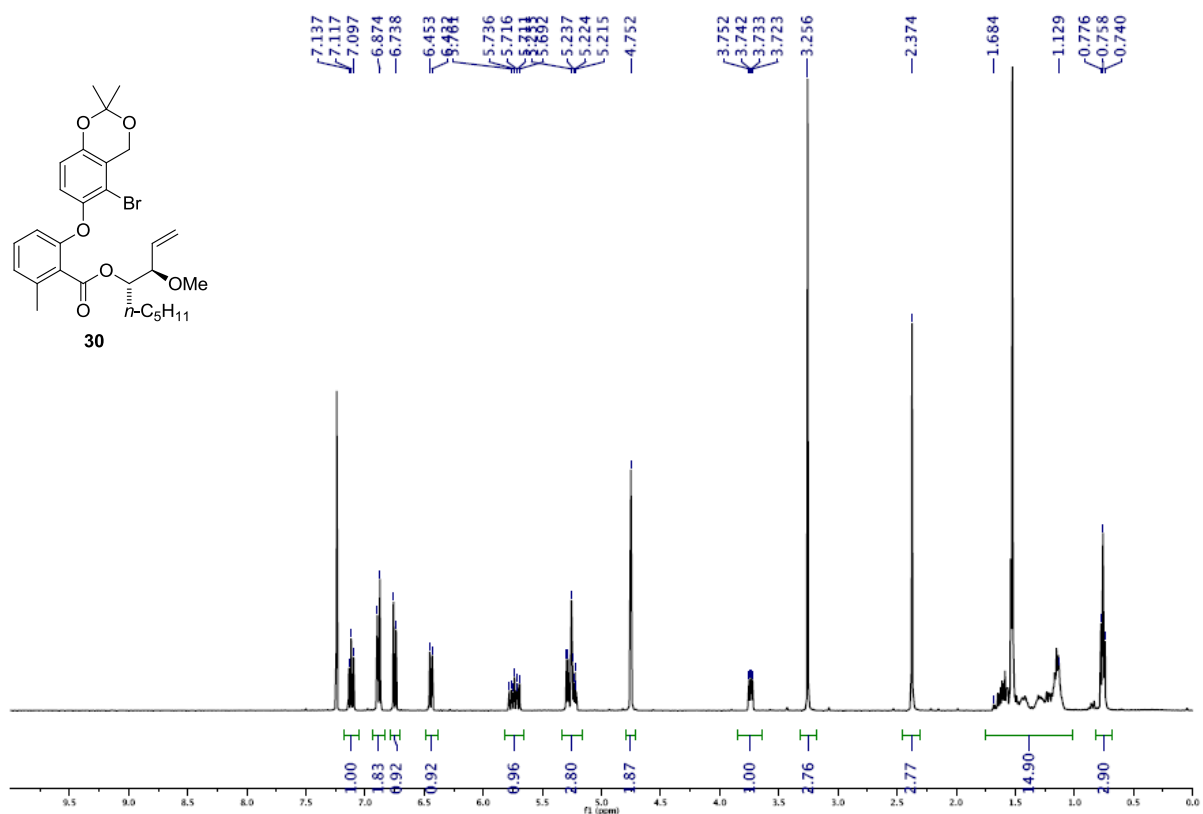
^1H NMR, 400 MHz, CDCl_3



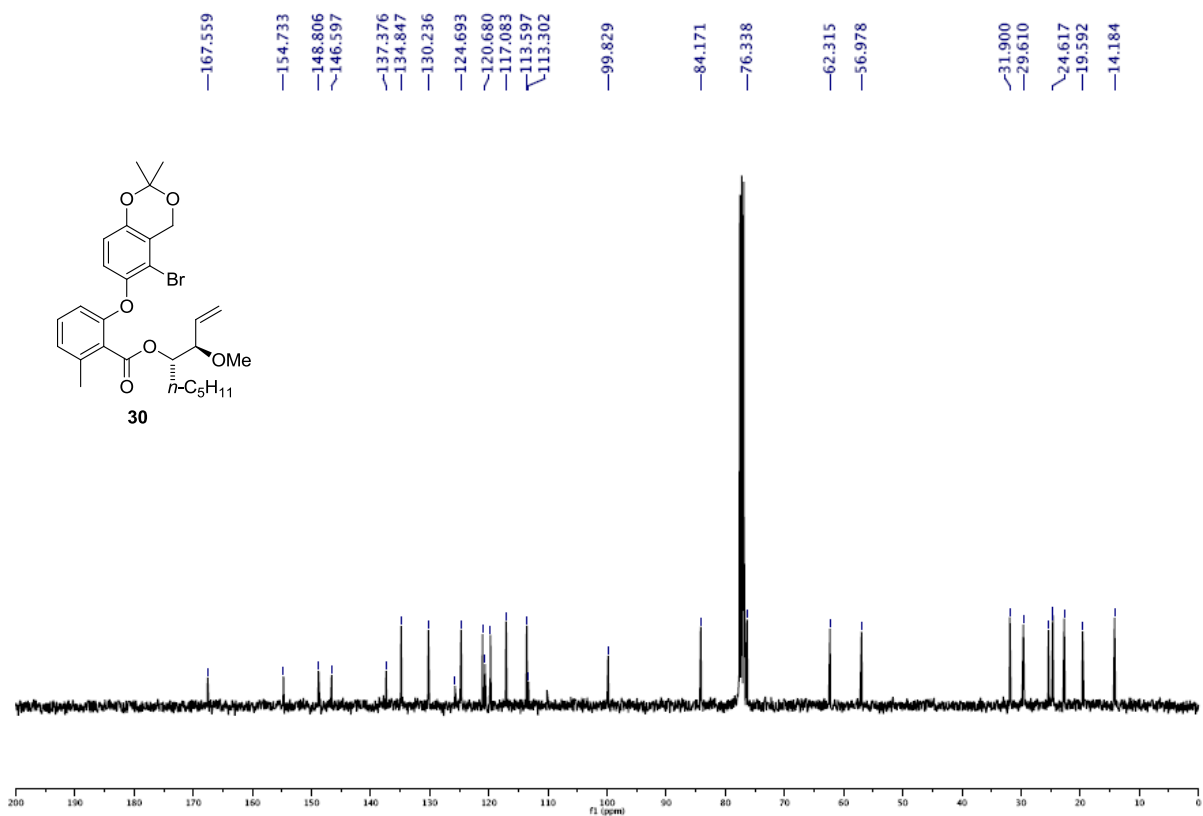
^{13}C NMR, 100 MHz, CDCl_3



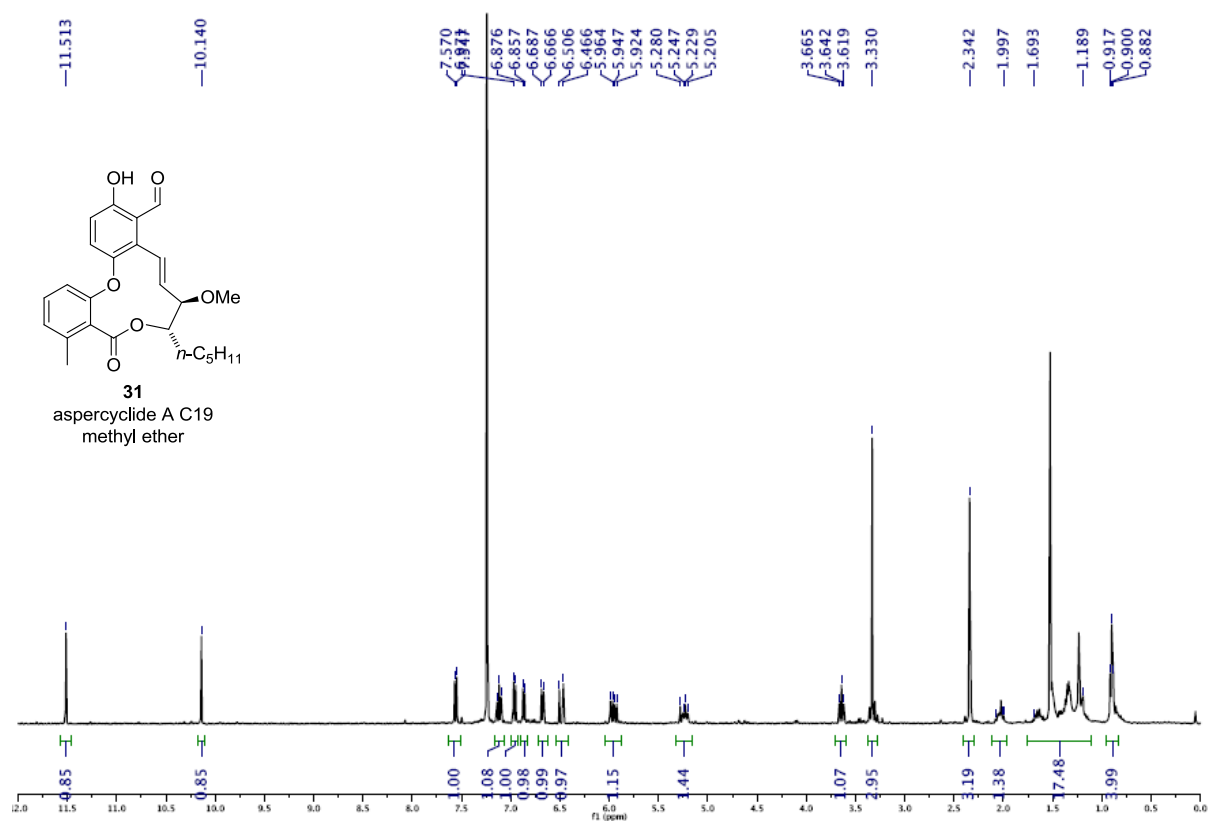
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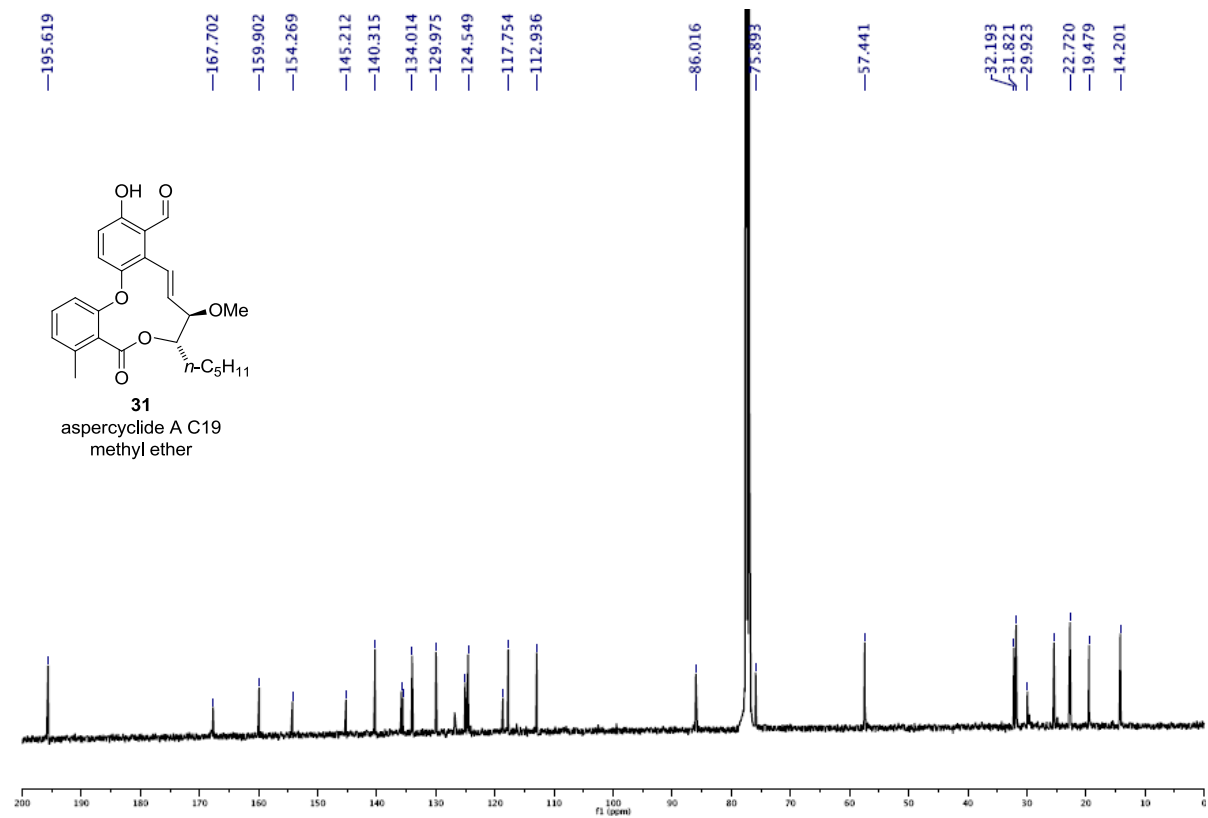
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^1H NMR, 400 MHz, CDCl_3



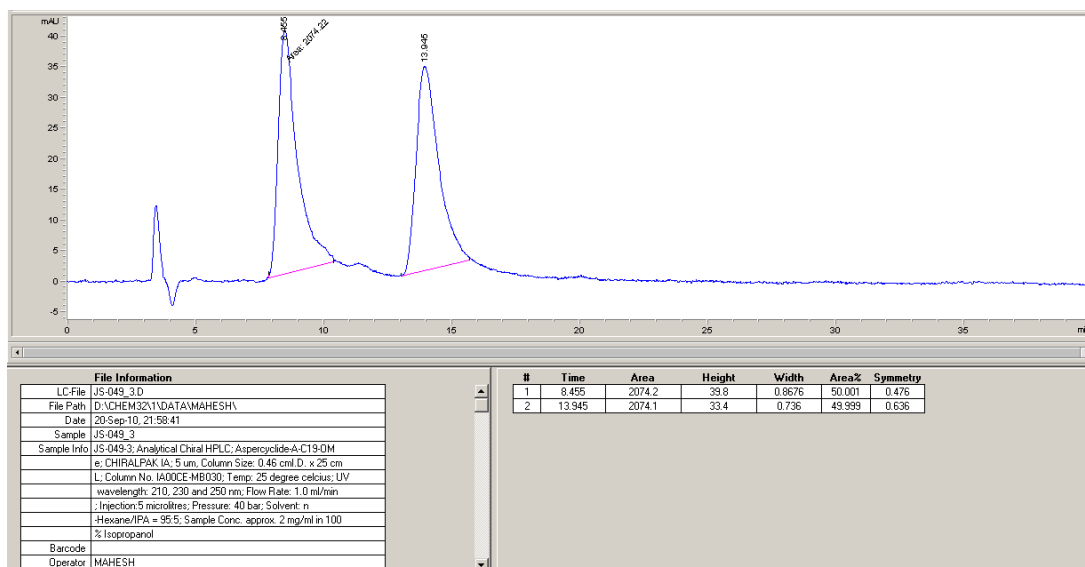
^{13}C NMR, 100 MHz, CDCl_3



HPLC Separation of enantiomers of aspercyclide A C19 methyl ether (31)

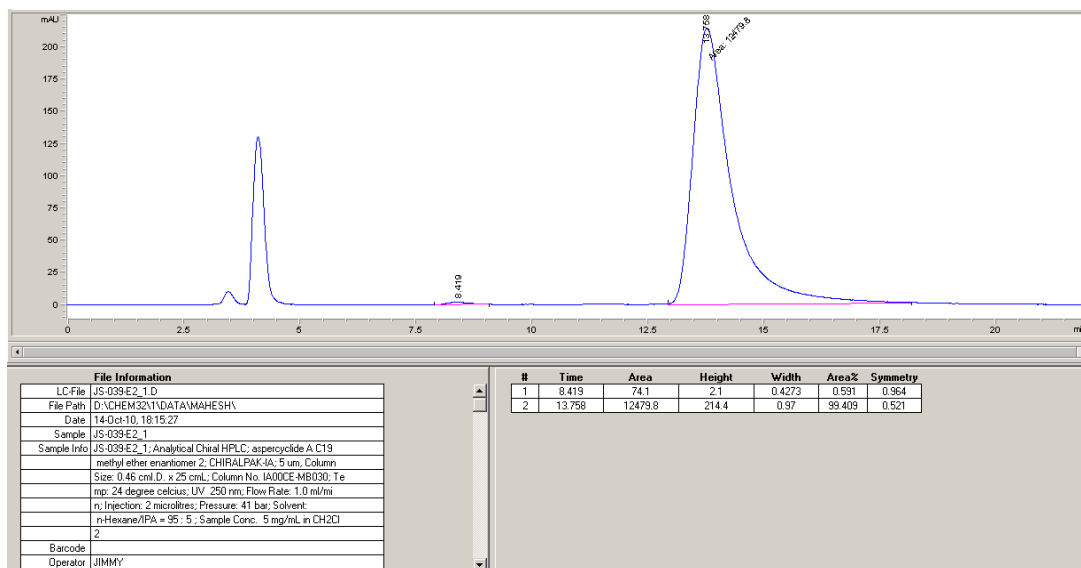
Synthetic (±)-aspercyclide A C19 methyl ether, (±)-(31)

HPLC (CHIRALPAK-IA column, sample conc. 2 mg/mL in CH₂Cl₂, injection volume 5 µL)

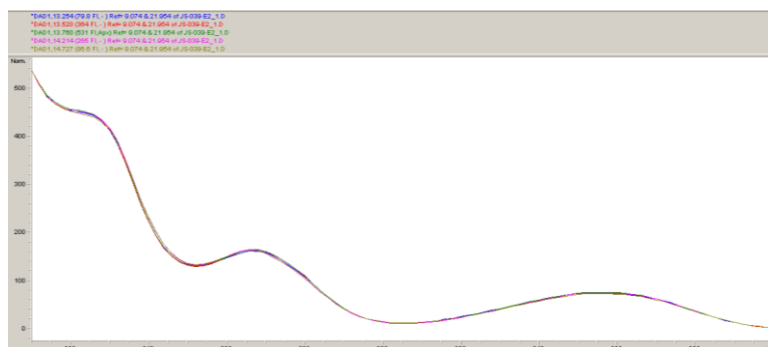


Synthetic (-)-aspercyclide A C19 methyl ether, (-)-(31)

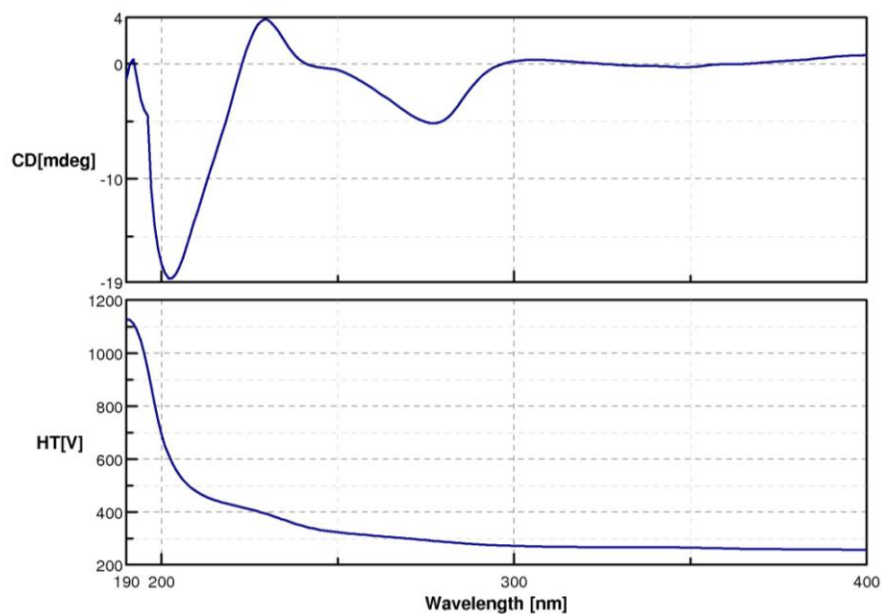
HPLC (CHIRALPAK-IA column, sample conc. 5 mg/mL in CH₂Cl₂, injection volume 2 µL)



UV (*n*-hexane/*i*-PrOH, 95:5)

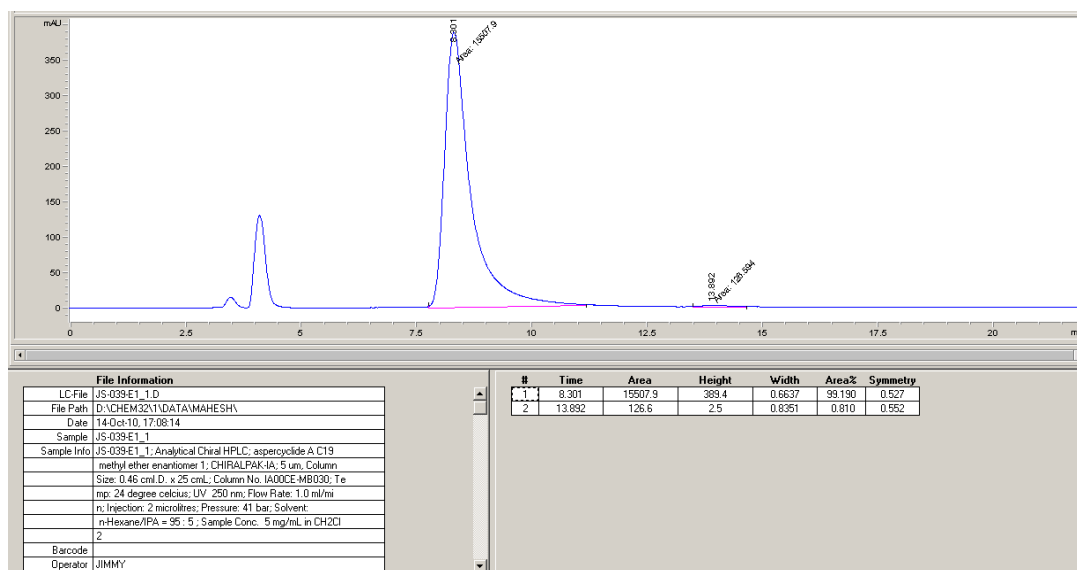


CD (MeOH, 0.02 mg/mL)

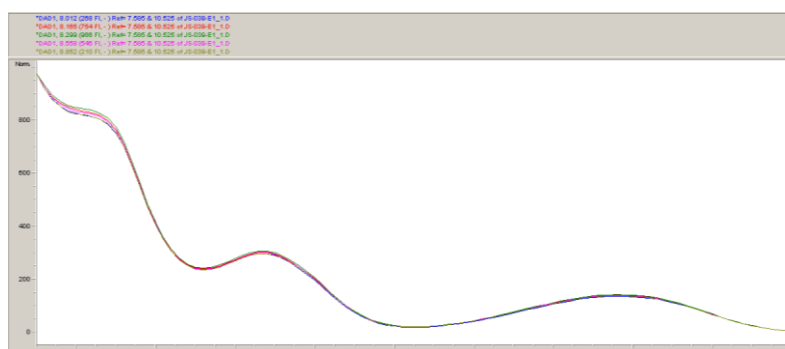


Synthetic (+)-aspercyclide A C19 methyl ether, (+)-(**31**)

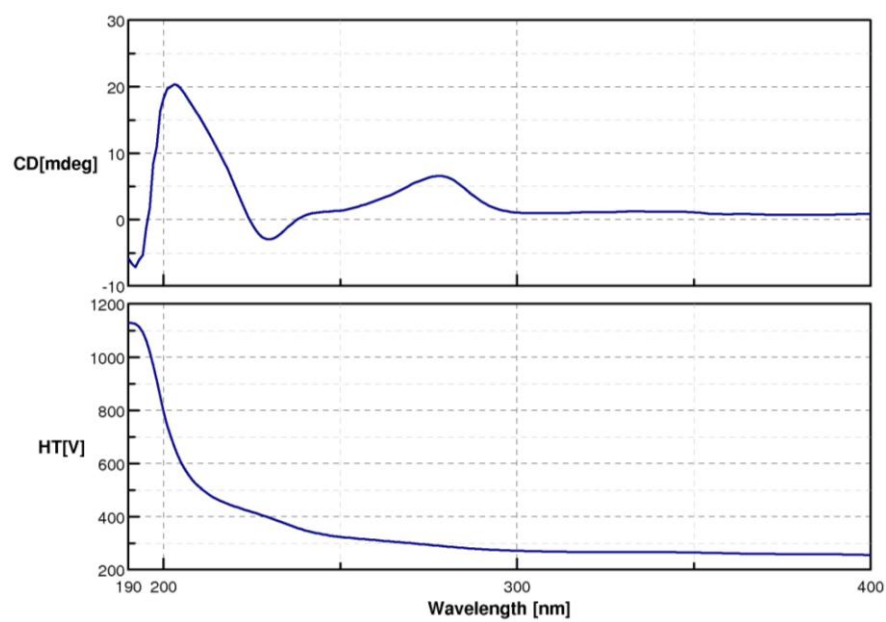
HPLC (CHIRALPAK-IA column, sample conc. 5 mg/mL in CH₂Cl₂, injection volume 2 μ L)



UV (*n*-hexane/*i*-PrOH, 95:5)

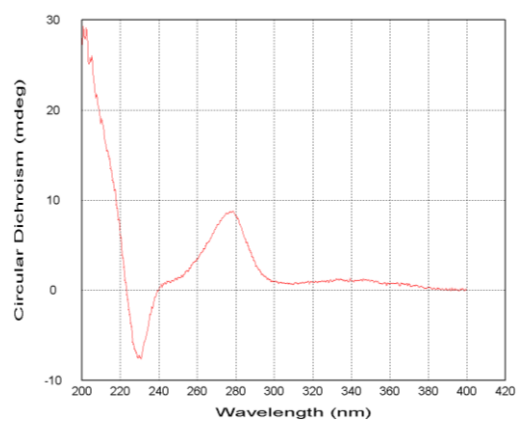


CD (MeOH, 0.02 mg/mL)



Natural (+)-aspercyclide A, (+)-**1**

CD (MeOH, 1.0 mg/mL)



IgE Receptor Binding Enzyme-linked Immunosorbent Assay (ELISA)

Compounds were evaluated in a receptor binding ELISA assay using IgE-Fc (Cε2-4) protein, a chimeric construct of the α-subunit of FcεRI fused to the Fc region of IgG4 [referred to below as AG (alphagamma) receptor protein], Biotinylated anti-IgE (Vector Laboratories), Streptavidin-Horse Radish Peroxidase conjugate (Biosource International, Inc.) and o-phenylenediamine dihydrochloride (OPD, Sigma-Aldrich) in P96 Maxisorp NUNC 439454 Immunoplates (Nalge Europe Ltd.) named ELISA plate.

The protocol was as follows: The ELISA plate was coated with 100 µL/well of an 2 µg mL⁻¹ AG receptor protein solution made up in aqueous carbonate buffer (0.045 M NaHCO₃ + 0.018 M Na₂CO₃, pH 9.8) then was incubated overnight at 4 °C. After 3 washing steps with 300 µL/well of PBS, 0.1% Tween 20 v/v for 2-3 min, to remove residual AG protein, the unbound sites were blocked by adding 300 µL/well of PBS, 2 % Bovine Serum Albumin (BSA) v/v. The plate was then incubated for 1 h at 37 °C. Controls and antagonists' solutions (*i.e.* compounds (+)-**31**, (-)-**31** and (±)-**31**) were prepared on a classic clear 96-wells plate (NUNC 262162) separately from the ELISA plate. Each well has a total volume of 110 µL and a final DMSO concentration of 5% v/v. The antagonists were tested in duplicate from 1 mM to 30.5 nM final concentrations. First, 180 µL of the 1 mM concentrations were prepared in PBS, 5 % DMSO from the 100 mM stock solutions in DMSO, then serial 1/2 dilutions were performed by taking 90 µL of each concentration in 90 µL of PBS 5 % DMSO. After the last dilution, 90 µL of the solution is removed in order to have 90 µL in all the wells. Six different controls named Fc, D, RB, AG, P and W were prepared in triplicate for each plate as indicated in the table below. Rose Bengal (Sigma-Aldrich) was used as a control antagonist as well as alphagamma. In all the wells except in the P and W controls, were added 20 µL of an IgE Fc solution (0.0137 ng/µL in PBS) in order to have a total volume of 110 µL. The plate was covered and left at room temperature for a 1 h pre-incubation step.

Controls for 1 well volumes in µL	IgE Fc 0.0137 ng/µL in PBS	DMSO	Rose Bengal 10 mM DMSO	Alphagamma 1.15 mg/mL in PBS	PBS	MilliQ water
Fc	20	-	-	-	90	-
RB	20	5.39	0.11	-	84.5	-
AG	20	5.5	-	1	83.5	-
D	20	5.5	-	-	84.5	-
P	-	5.5	-	-	104.5	-
W	-	5.5	-	-	-	104.5

The blocked ELISA plate was washed with PBS-Tween 20 (0.1% v/v, 3 × 300 µL/well, 2-3 min) then 100 µL/well were transferred from the pre-incubated plate to the ELISA plate keeping to the same template. The ELISA plate was then incubated for 1 h at 37 °C to allow the binding of IgE to its receptor. The plate was washed with PBS-Tween 20 (0.1% v/v, 3 × 300 µL/well) with a first washing step of 30 min, then 2-3 minutes for the 2 others washing steps in order to ensure the removal of the compounds. Bound IgE-Fc was detected by adding 100 µL/well of biotinylated anti-IgE (1/5000 dilution in PBS, 1% v/v BSA), then the plate was incubated for 1 h at 37 °C. After 3 washing steps with PBS-Tween 20 (0.1% v/v, 300 µL/well, 2-3 min), 100 µL/well of streptavidin-HRP (1/5000 dilution in PBS, 1% v/v BSA) were added. The plate was incubated 1 h at 37 °C, and then washed with PBS (3 × 300 µL/well, 2-3 min). The HRP substrate solution was prepared by dissolving 1 tablet of OPD in 9 mL of water and 1 mL of 10X peroxide buffer, then 50 µL/well were added to the plate. After 4 min incubation at room temperature in the dark, the enzyme reaction was stopped by addition of 50 µL/well of HCl 3 M. Absorbance detection of the plate was then performed at 492 nm using an ELISA plate reader (Titertek Multiskan).

All the titrations were performed twice in duplicate. All data were plotted and analyzed using Kaleidagraph® software (Synergy Software, Reading, PA). For each well, the percent of inhibition was calculated using the following equation (1):

$$\% \text{ inhibition} = 100 - ((100 * (OD_{492 \text{ nm}} - \mu c^-) / (\mu c^+ - \mu c^-)) \quad (1)$$

in which $OD_{492 \text{ nm}}$ is the absorbance measured at 492 nm; μc^- is the average of the negative control P (100% inhibition); μc^+ is the average of the positive control D (0% inhibition).

To obtain the inhibition curves, the logarithms of each compounds concentration were plotted *versus* the average inhibition percentages obtained for each concentration. The data were fitted using the sigmoidal equation (2):

$$Y = (100 * x)^b / (C^b + x^b) \quad (2)$$

where $x = \text{Log [concentration } \mu\text{M}]$, $b = \text{Hill number}$; $C = IC_{50}$.

