Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Photodegradable Antimicrobial Agents - Synthesis, Photodegradation, and Biological Evaluation

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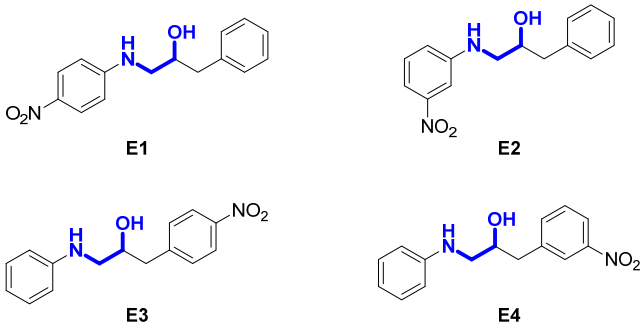
Supplementary Figures and Tables

a)



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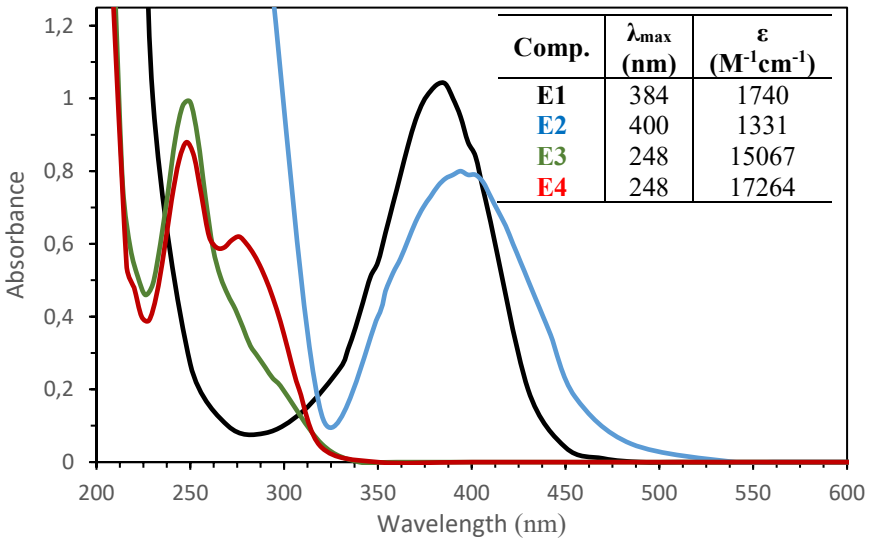


Comp.	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)
E1	384	1740
E2	400	1331
E3	248	15067
E4	248	17264

Figure S1. a) Compounds **E1-E4** were used as model compounds to study the photodecomposition strategy. After 2 hours of irradiation with a medium-pressure mercury-vapor lamp emitting light mainly at 254, 370, and 410 nm at pH 13, decomposition conversions were as follows: E1 – 100%, E2 – 17%, E3 – 40%, E4 – 56%. b) UV-Vis spectra of compounds **E1-E4**.

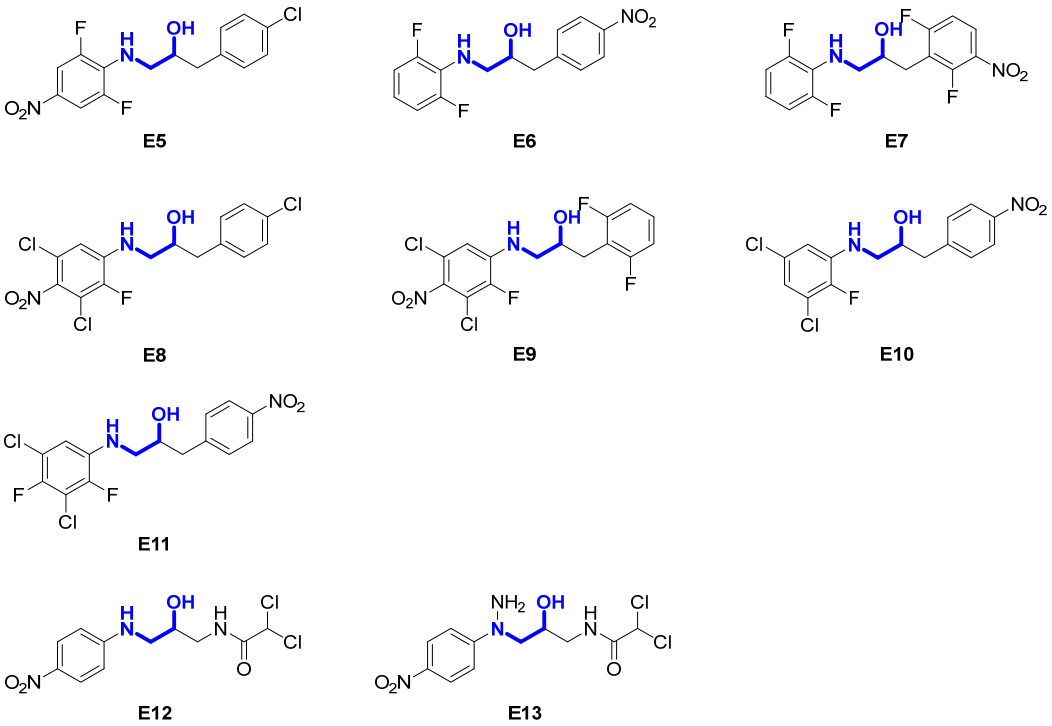
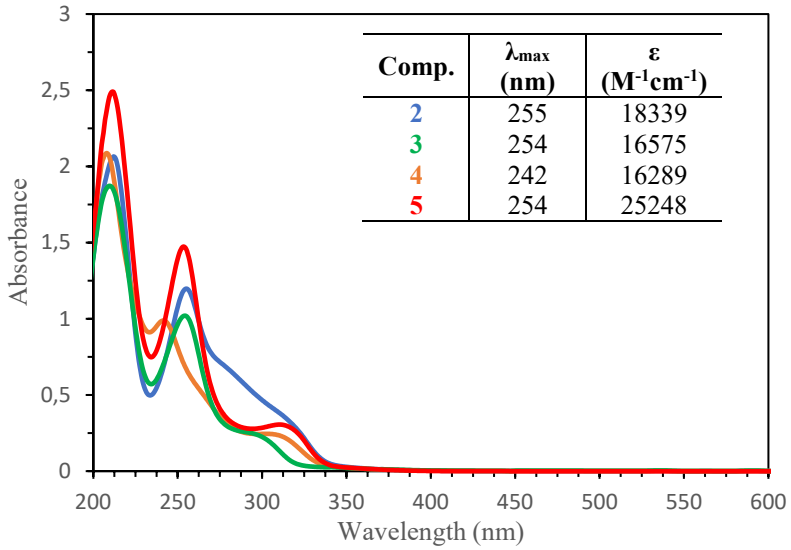


Figure S2. Additional compounds prepared and tested for antimicrobial activity. These compounds were inactive at 100 μM.



Comp.	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)
2	255	18339
3	254	16575
4	242	16289
5	254	25248

Figure S3. UV-visible spectra of compound **2-5** at 0.06 mM with corresponding λ_{max} and ε, determined as the slope of standard curves from concentrations 0.02, 0.04, 0.06, and 0.08 mM for each compound ($R^2 \geq 0.999$).

Table S1. Conversion of compounds **2**-**5** during irradiation for 24 hours at pH 13 and 8.

Compound	Conversion at pH 13	Conversion at pH 8
2	100%	25%
3	100%	100%
4	100%	19%
5	56%	100%

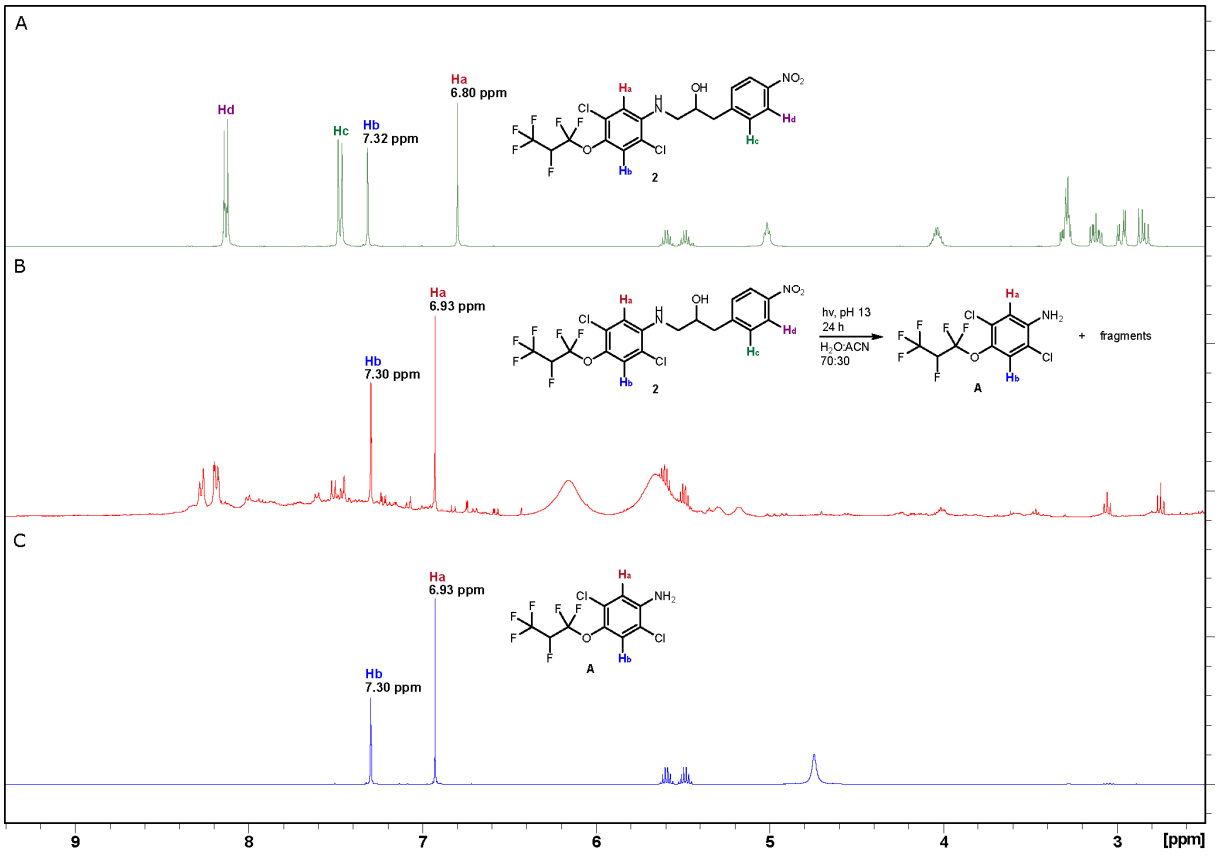


Figure S4. ^{1}H NMR spectra of compound **2** (0.7 mM) before irradiation (A); the reaction mixture after photodegradation of compound **2** (B); the decomposition product **A** from compound **2** (C). The NMR spectra were recorded in CD_3CN at 400 MHz.

Complete conversion was observed after 24 hours. This was substantiated by the disappearance of the two singlets at 6.80 and 7.32 ppm from compound **2** (due to H_a and H_b, respectively) and the appearance of the two singlets at 6.93 and 7.30 ppm due to aniline **A** (Figure S4). The ^{1}H NMR spectrum of a pure sample of compound **A**. is shown in Figure S4C. Additionally, the signals for the two protons denoted H_c and H_d in the 4-nitrophenyl group (doublets at 7.47 and 8.13 ppm in **2**, respectively) had shifted and overlapped with other signals in a complicated multiplet after degradation. Furthermore, the aliphatic protons around 3 ppm are no longer visible.

Table S2. Minimum inhibitory concentration (MIC) in μM for compounds **2-5** against Gram-positive and Gram-negative bacteria and toxicity tests against MRC5 and HepG2 cell lines. The activity was screened using the following concentrations 100, 75, 50, 25, 12.5, 6.3, 3.1, and 1.6 μM.[a]

Compound	MIC					Tox	
	S. epidermidis	***S. aureus***	***S. agalactiae***	***E. faecalis***	***E. coli***	**MRC5**	**HepG2**
2	I	I	6.3	I	I	50	75
3	50	50	50	I	I	50	75
4	I	50	I	I	I	50	50
5	I	I	6.3	I	I	25	50
2d	I	I	I	I	I	I	I
3d	I	I	I	I	I	I	I
4d	I	I	I	I	I	I	I
5d	I	I	I	I	I	I	I

[a]I = inactive at the tested concentrations.

Materials and Methods

Photodegradation reactions at ~0.7 mM for NMR analysis

All reactions were carried out in a 75 mL gas inlet flask (~0.25-0.35 mM) under a constant stream of nitrogen with a quartz well water-cooled cold finger for 24 hours. The appropriate aminol (~ 0.02 mmol) was dissolved in acetonitrile (23 mL), diluted with distilled water adjusted to pH 8 with sodium hydroxide (52 mL), and photolyzed using a 254 nm low-pressure mercury lamp. The aqueous solution was extracted with DCM (3 x 20 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to yield a black residue, which was analysed by ^1H NMR spectroscopy.

Compound	m (mg)	n (mmol)	C (mM)
2	10.2	0.0201	0.268
3	6.7	0.0170	0.226
4	9.8	0.0237	0.316
5	14.0	0.0258	0.344

Testing of antimicrobial activity and cytotoxicity

Growth inhibition assay

To determine and quantify antimicrobial activity, a bacteria growth inhibition assay in liquid media was executed. Compounds **2-5**, **2d-5d**, and **S5-S13** were tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 259233), *Enterococcus faecialis* (ATCC 29122), *Pseudomonas aeruginosa* (ATCC 27853) and *Streptococcus agalactiae* (ATCC 12386); all strains from LGC Standards (Teddington, UK). *S. aureus*, *E. coli*, and *P. aeruginosa* were grown in Muller Hinton broth (275730, Becton). *E. faecalis* and *S. agalactiae* were cultured in brain hearth infusion broth (53286, Sigma). Fresh bacterial colonies were transferred in the respective medium and incubated at 37 °C overnight. The bacterial cultures were diluted to a culture density representing the log phase and µL/well were pipetted into a 96-well microtiter plate (734-2097, NunclonTM, Thermo Scientific, Waltham, MA, USA). The final cell density was 1500-15.000 colony forming units/well. The compound was diluted in 2% (v/v) DMSO in ddH_2O, providing a final assay concentration of 50% of the prepared sample, since 50 µL of sample in DMSO/water were added to 50 µL bacterial culture. After adding the samples to the plates, they were incubated overnight at 37 °C and the growth was determined by measuring the optical density at λ = 600 nm (OD600) with a 1420 Multilabel Counter VICTOR3TM (Perkin Elmer, Waltham, MA, USA). A water sample was used as a reference control, growth medium without bacteria was used as a negative control and dilution series of gentamycin (A2712, Merck) from 32 to 0.01 µg/mL was used as positive control and visually inspected for bacterial growth. The positive control was used as a system suitability test and the results of the antimicrobial assay were only considered valid when positive control was passed. The final concentration of DMSO in the assays was ≤ 2% (v/v) known to have no effect in the tested bacteria. The data was processed using GraphPad Prism 8.

Cytotoxicity assay

Cytotoxicity was studied using HepG2 (human liver carcinoma, ATCC HB-8065) and MRC-5 cells (normal human lung fibroblasts, ATCC CCL-171) for 24 hr (HepG2) and 72 h (MRC5). For the 24 hr study, 20,000 HepG2 cells were seeded per well*. For the 72 hr study, 4000 MRC-5 cells were used**. HepG2 and MRC5 were grown over night. Fresh growth media was added together with 10 µL test compound at various concentrations, 100, 75, 50, 25, 12.5, 6.3, 3.1, and 1.6 µM, dissolved in 2% DMSO (v/v). After incubation, 10 µL of CellTiter 96 AQueous One Solution Reagent (Promega, Madison, WI, USA, G3581) was added and plates were then incubated further for 1 hr. Absorbance was measured at 485 nm in a DTX 880 Multimode Detector. Results were calculated as % survival compared to negative (assay media) and positive (10% DMSO; Sigma-Aldrich) control

* Growth medium: MEM Earl's (Biochrom, F0325) added Glutamine stable (Biowest, X0551-100), Nonessential amino acids (Biochrom, K0293), sodium pyruvate (Biochrom, L0473), gentamycin (Biochrom A2712), FBS (Biowest, S1810)

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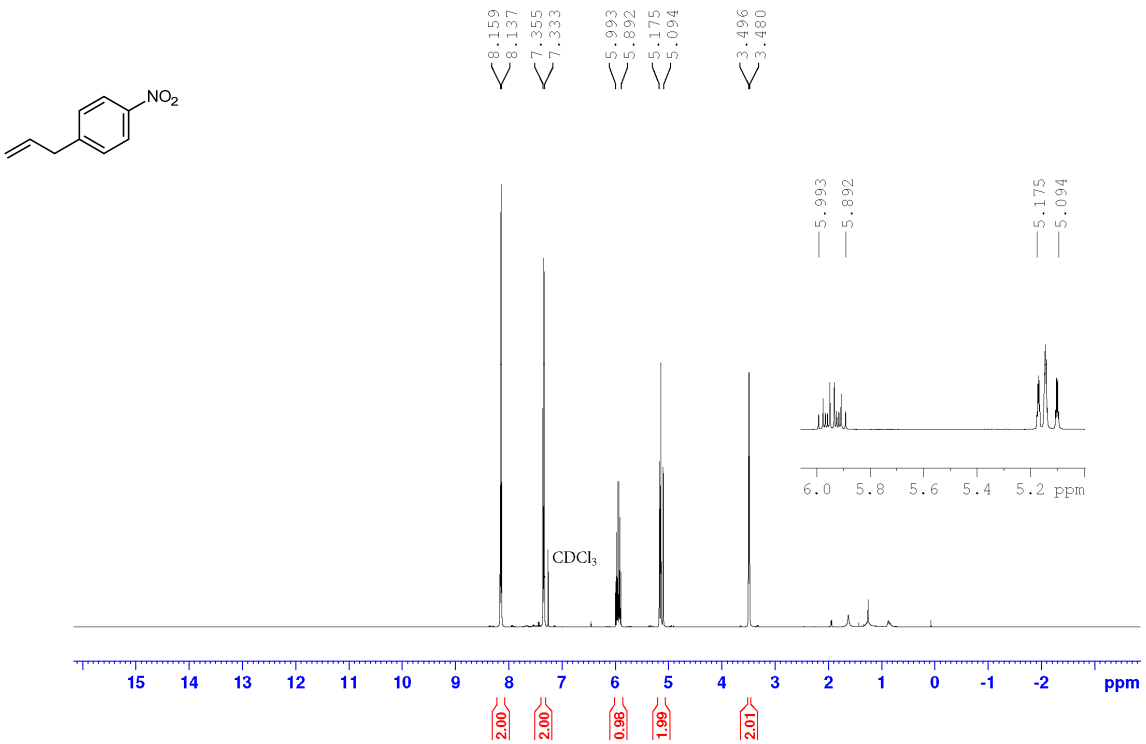
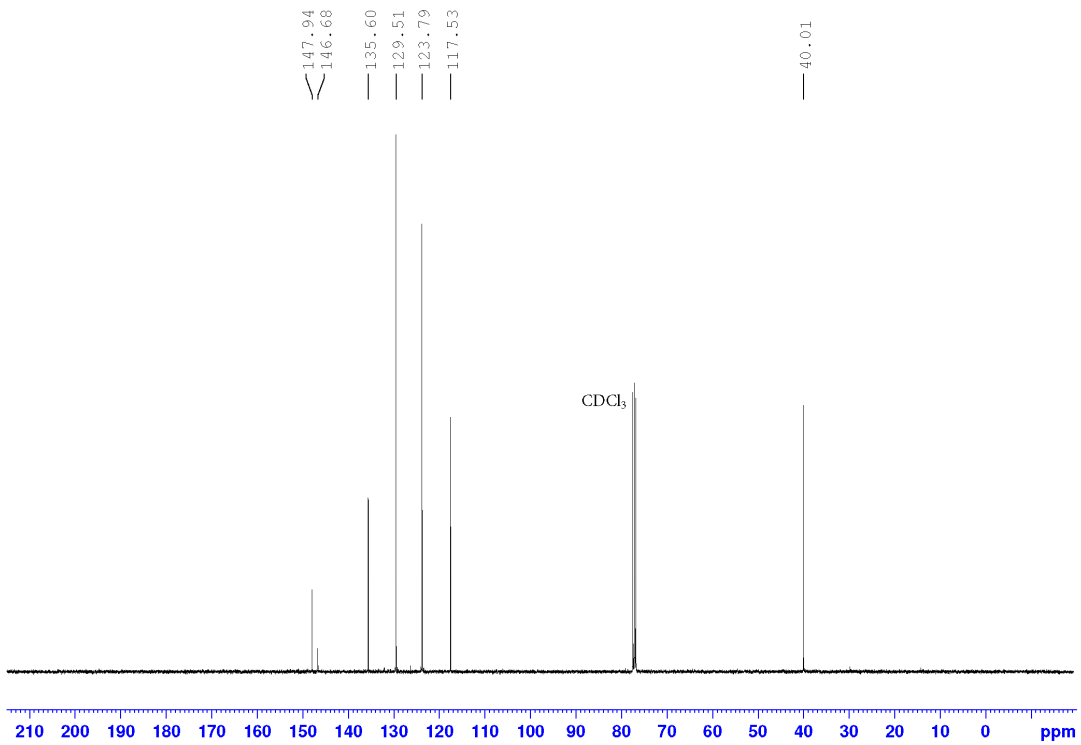
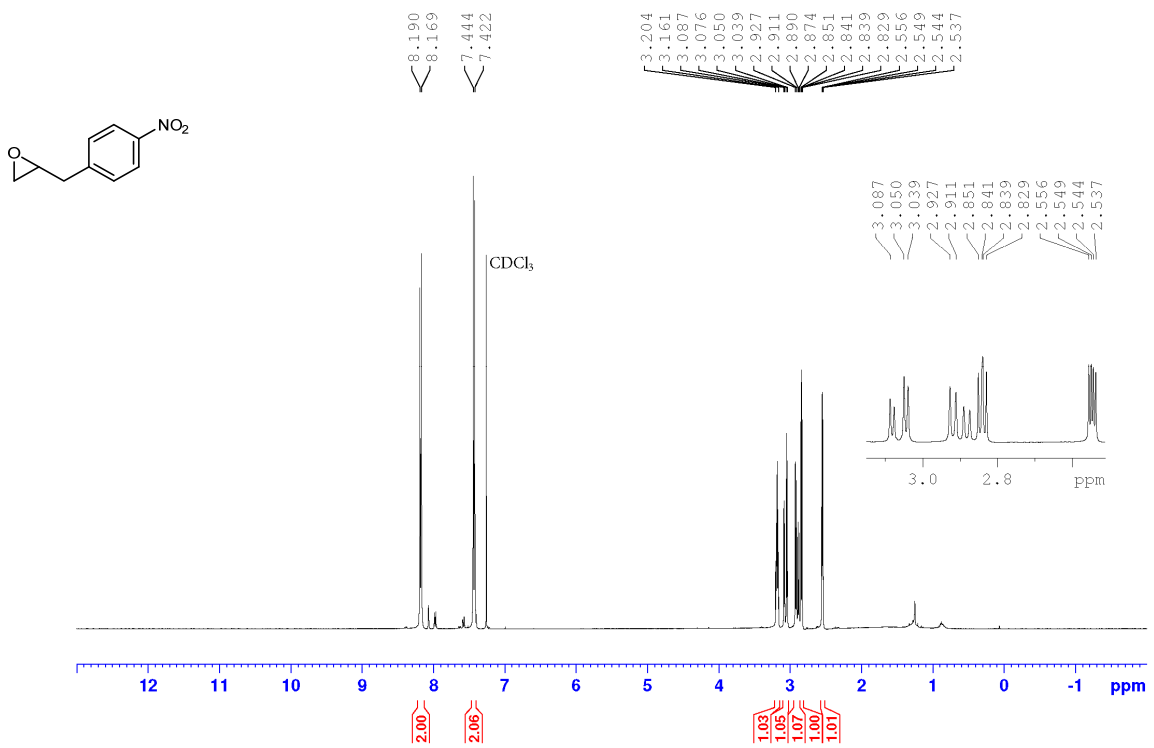
1-Allyl-4-nitrobenzene: ^{1}H NMR 400.13 MHz, $CDCl_3$



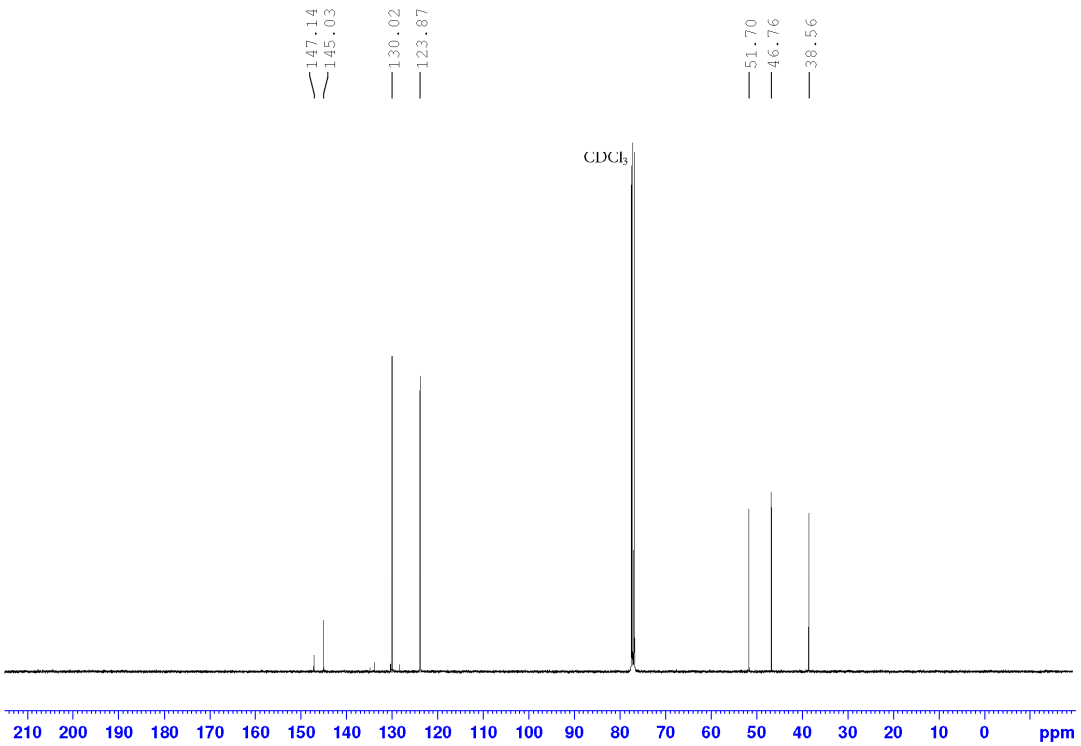
^{13}C NMR 100.61 MHz, $CDCl_3$



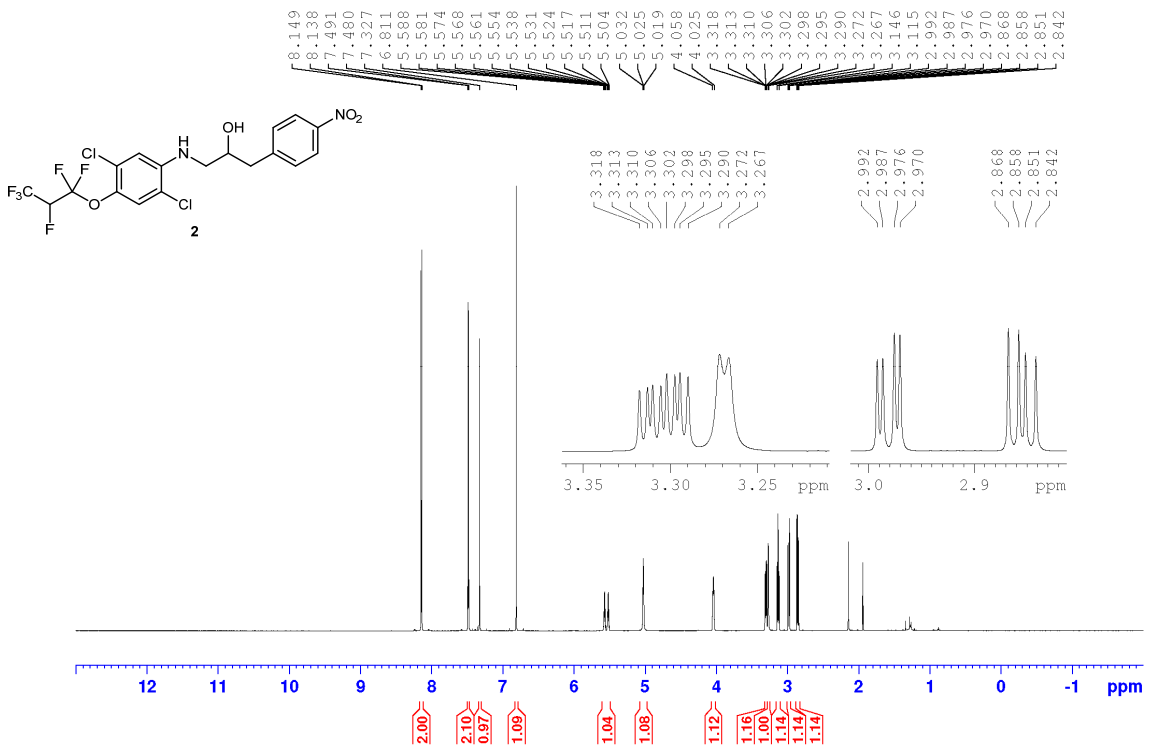
2-(4-Nitrobenzyl)oxirane: ^{1}H NMR 400.13 MHz, $CDCl_3$



^{13}C NMR 100.61 MHz, $CDCl_3$



1-((2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)amino)-3-(4-nitrophenyl)propan-2-ol (2): ^{1}H NMR 850.13 MHz, CD_3CN



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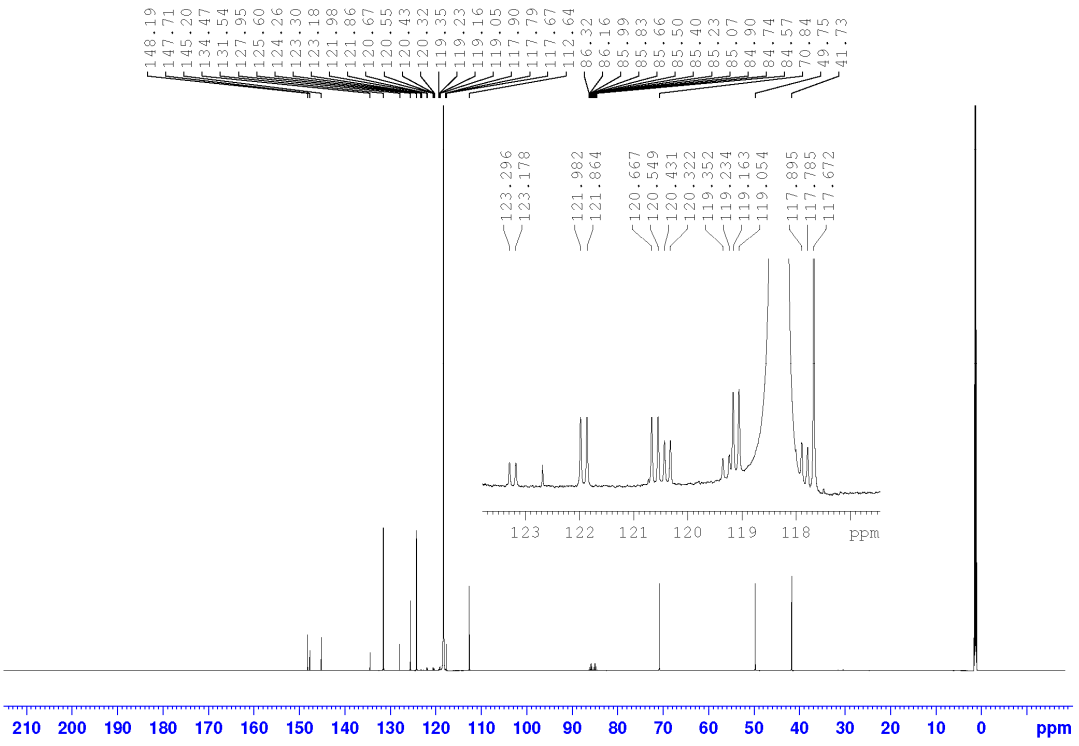
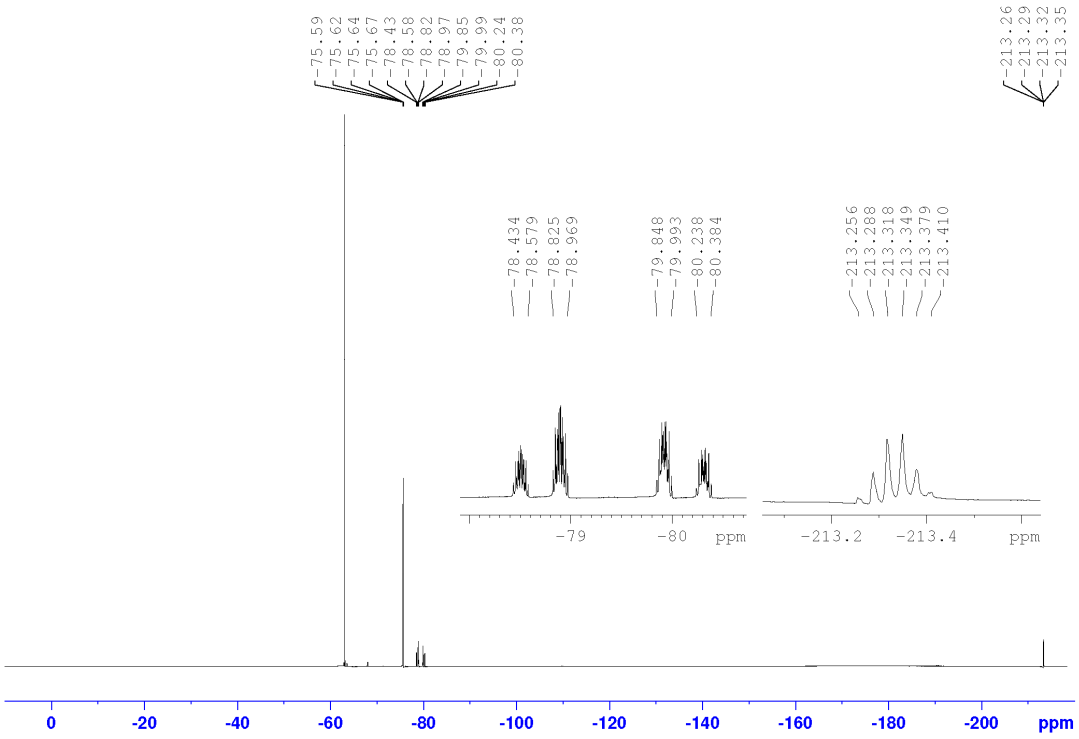


^{19}F NMR 376.46 MHz, CD_3CN



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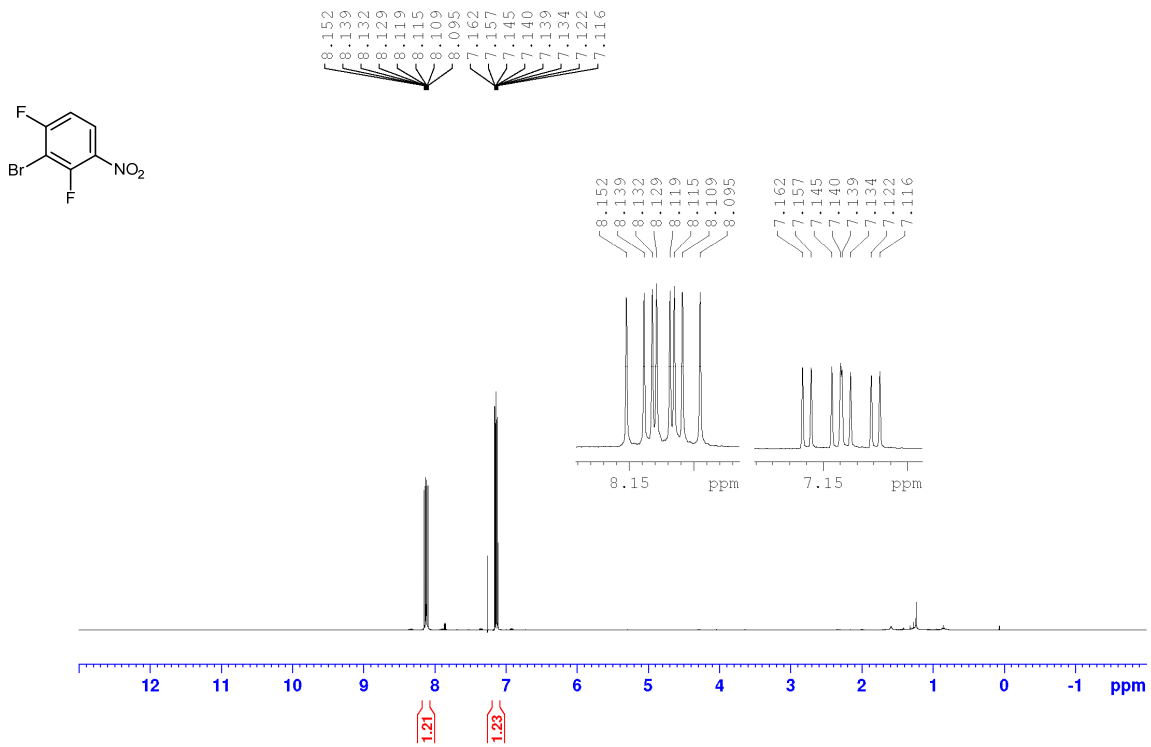

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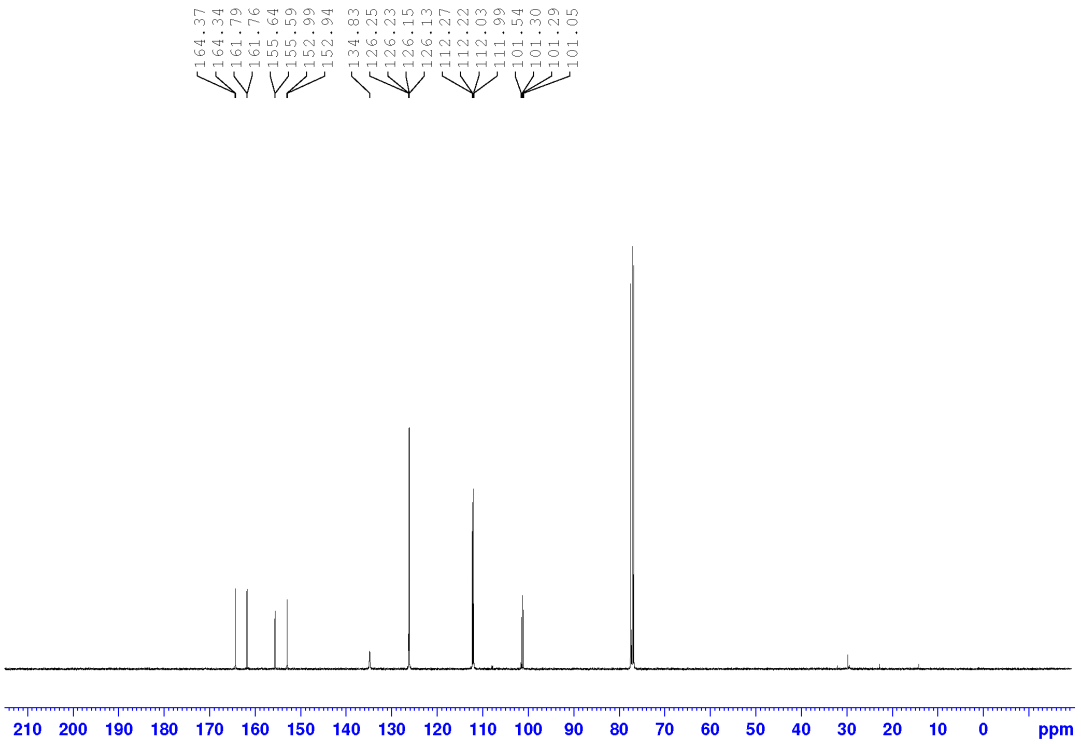

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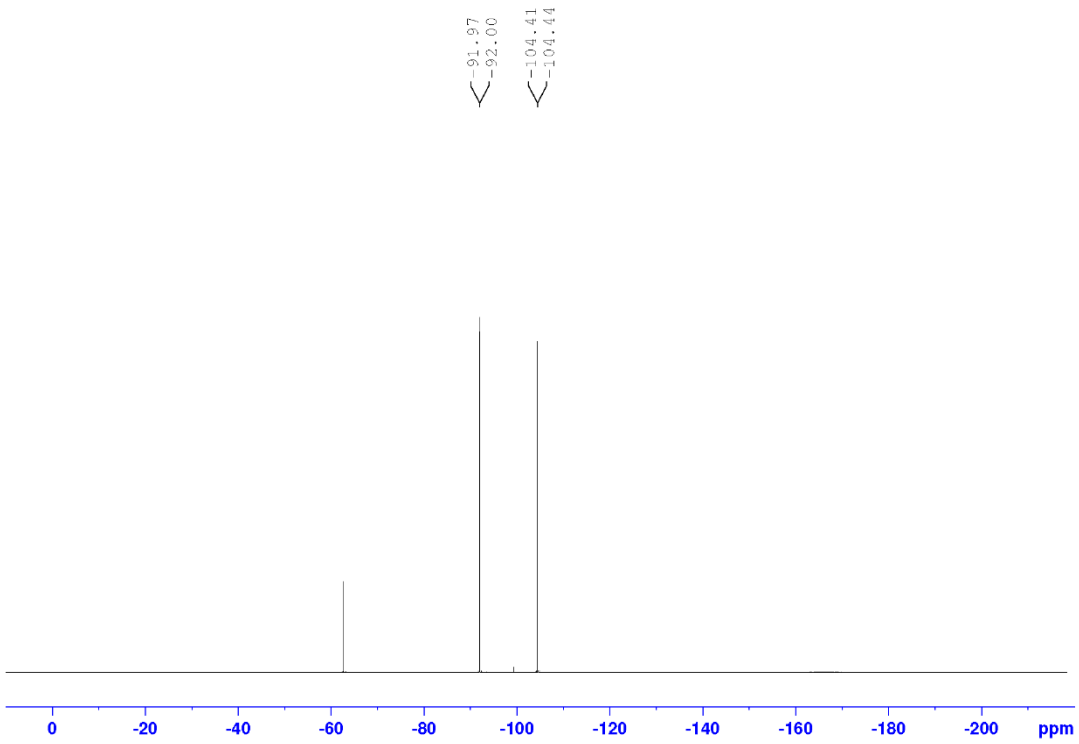
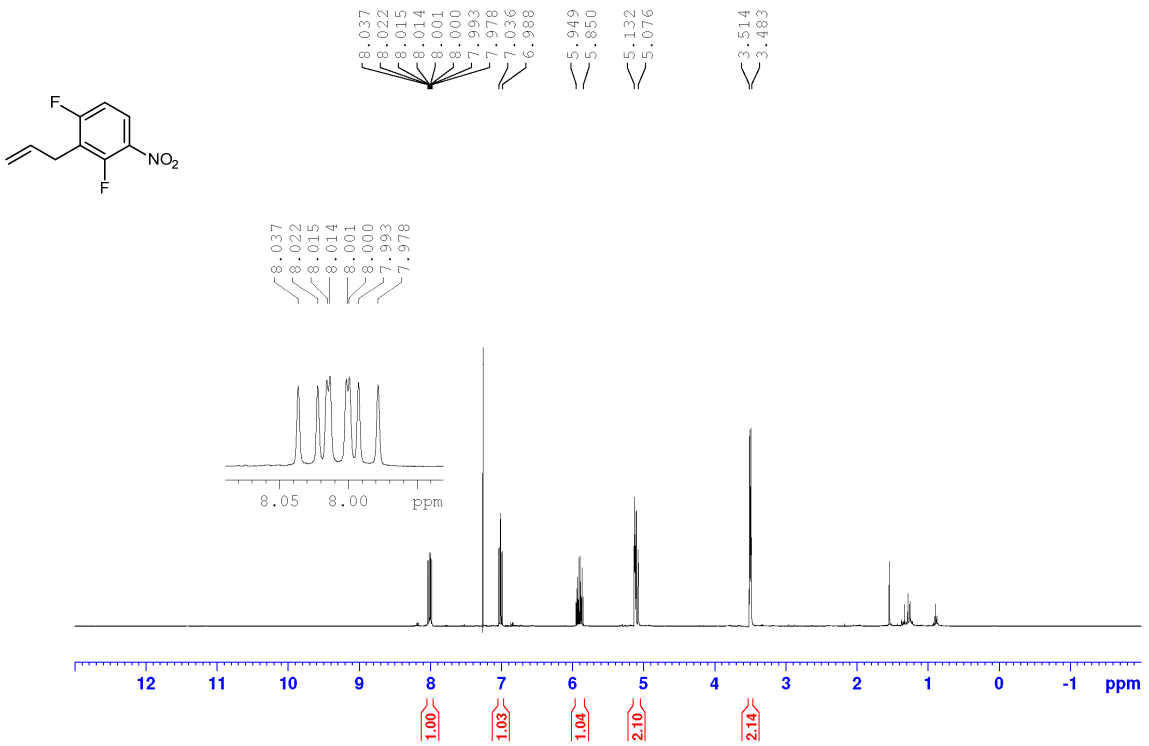


2-Allyl-1,3-difluoro-4-nitrobenzene: ^{1}H NMR 400.13 MHz, $CDCl_3$



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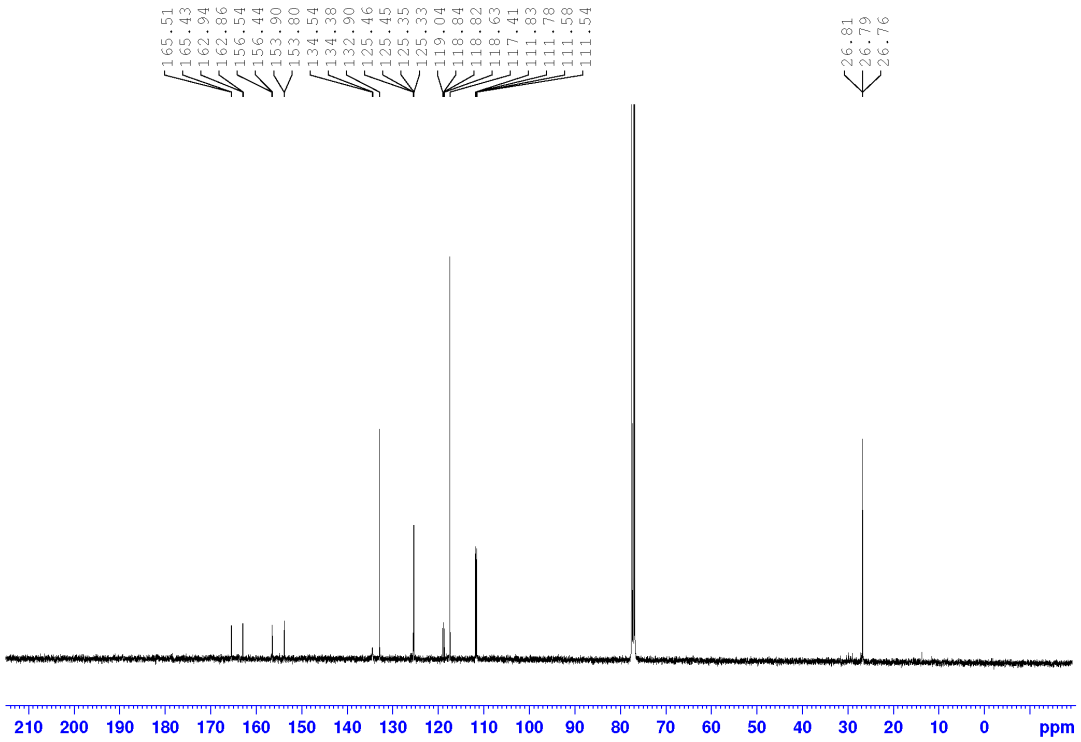

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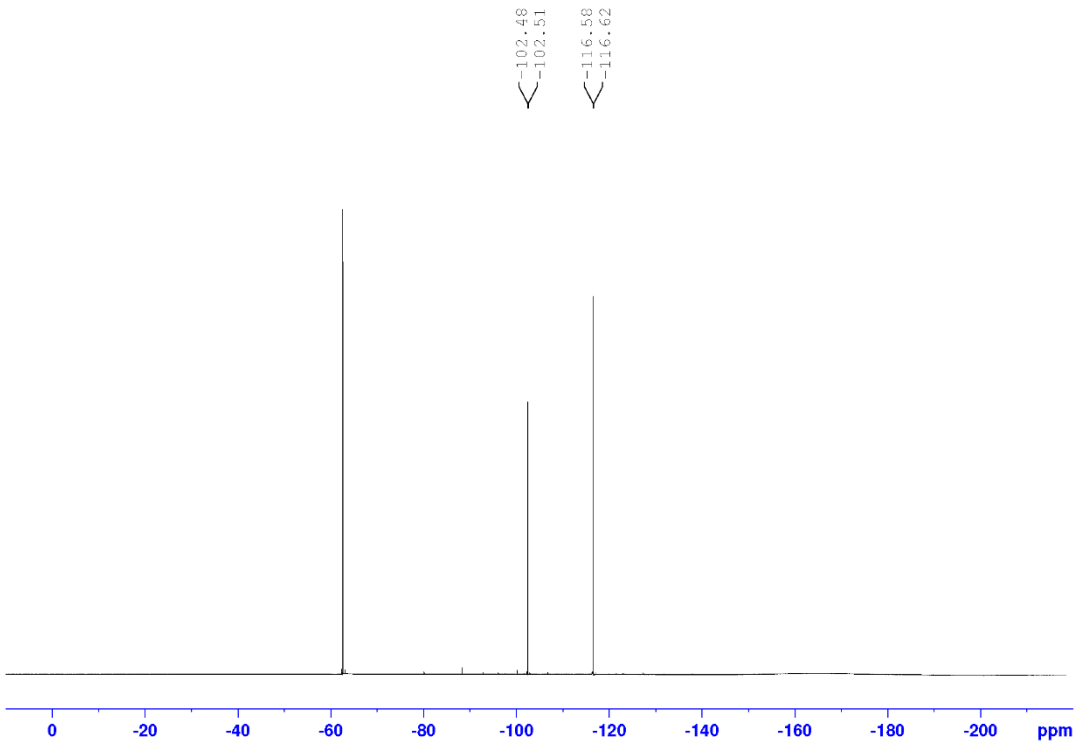
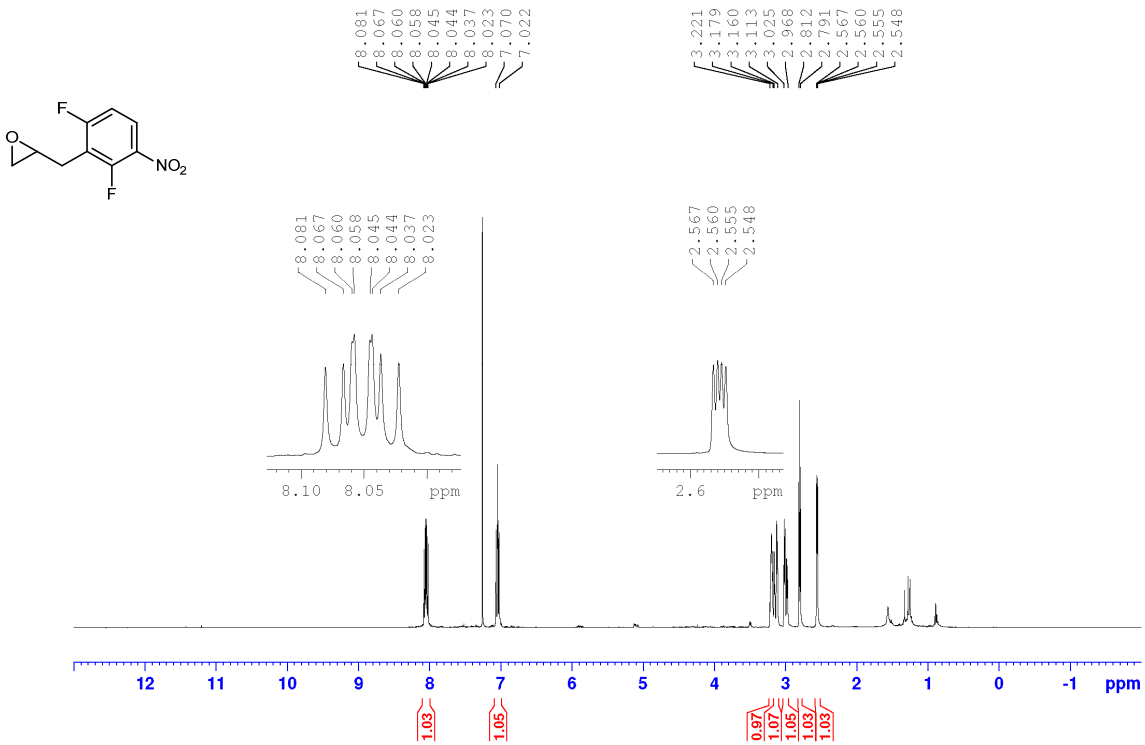


2-(2,6-Difluoro-3-nitrobenzyl)oxirane: ^{1}H NMR 400.13 MHz, $CDCl_3$



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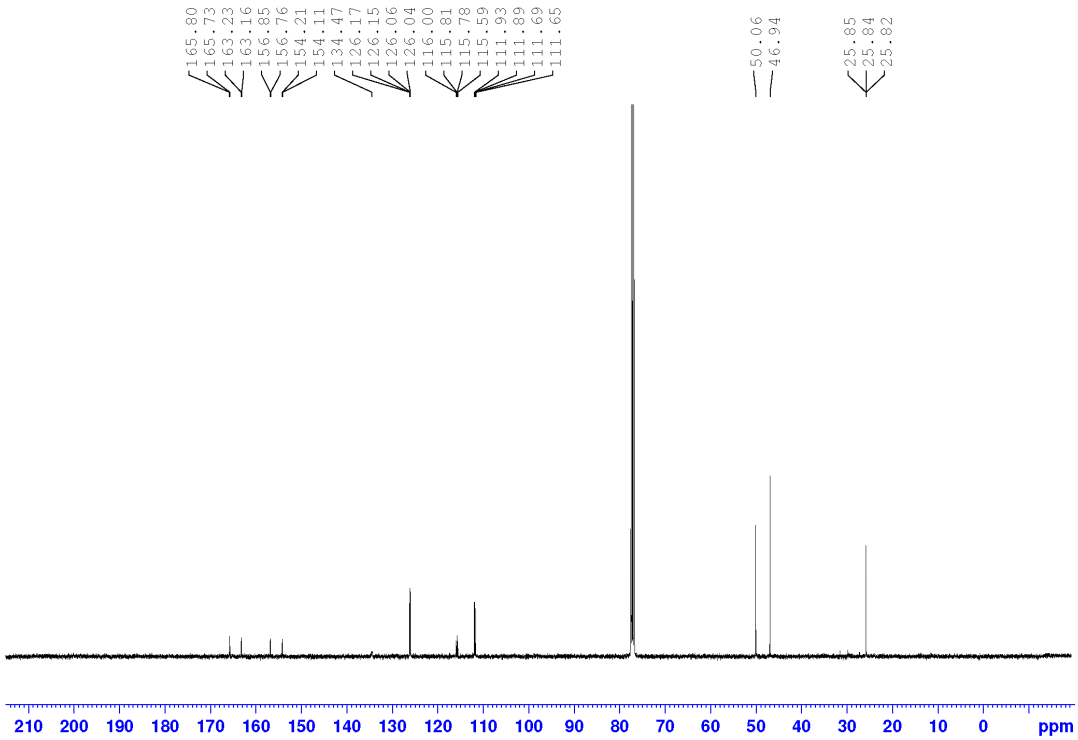

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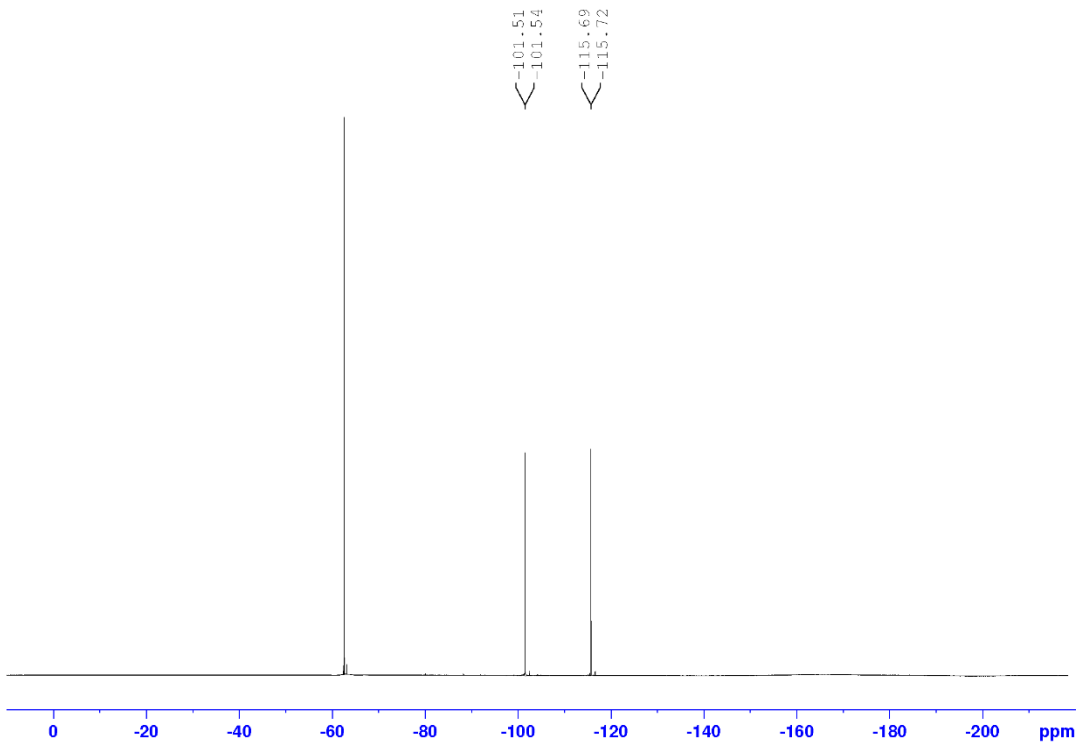

1-((3,5-Dichloro-2-fluorophenyl)amino)-3-(2,6-difluoro-3-nitrophenyl)propan-2-ol (3):

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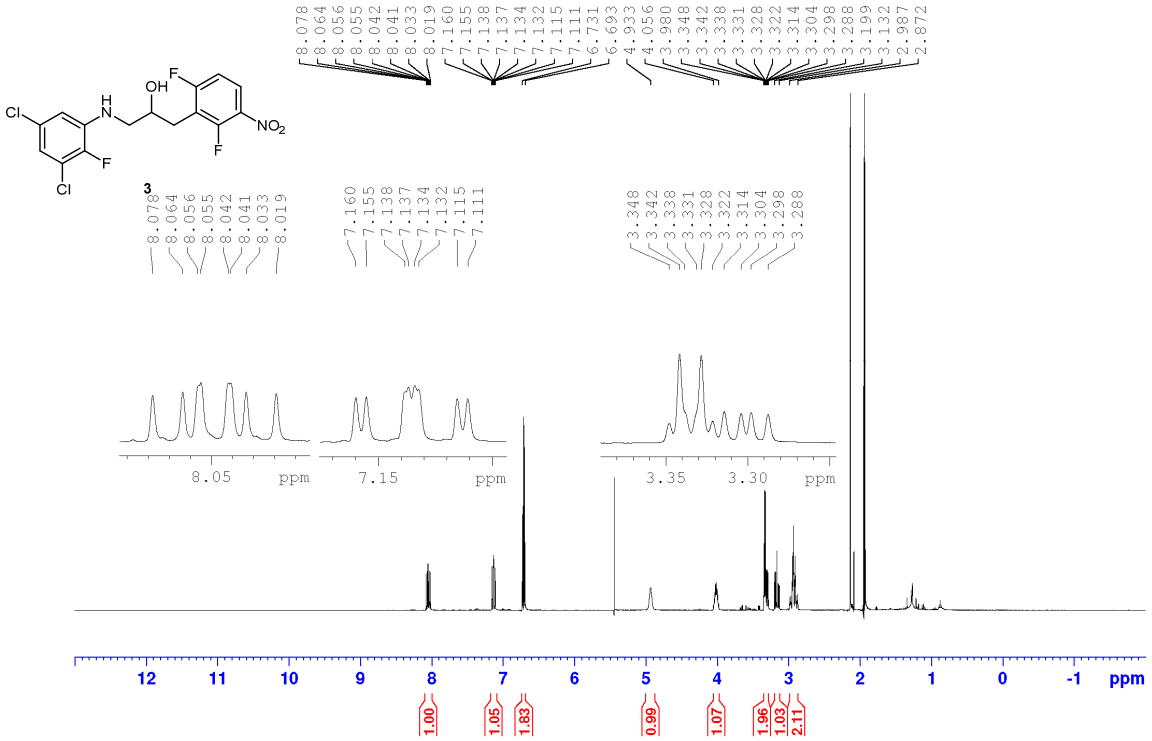

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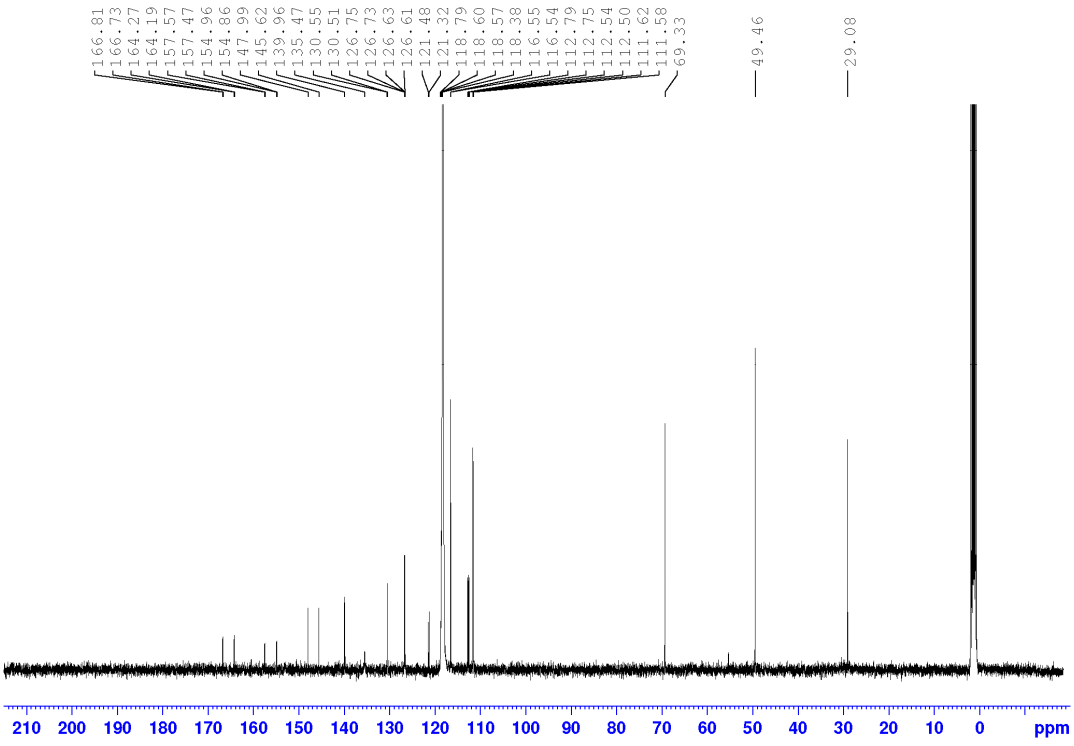

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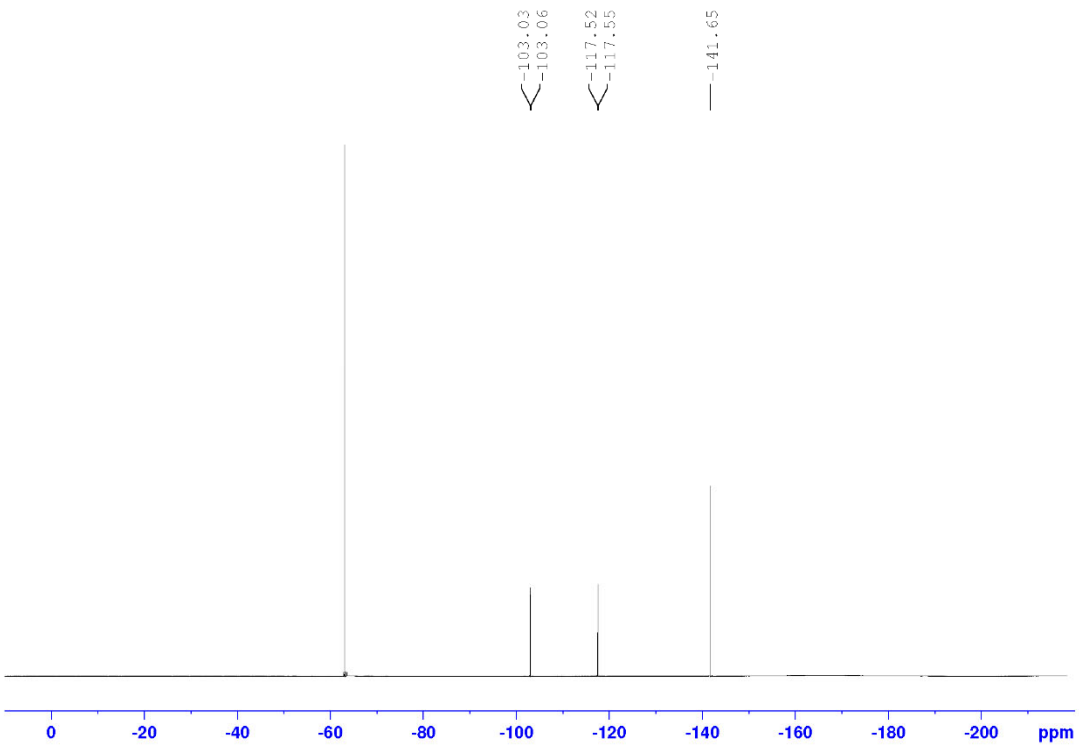
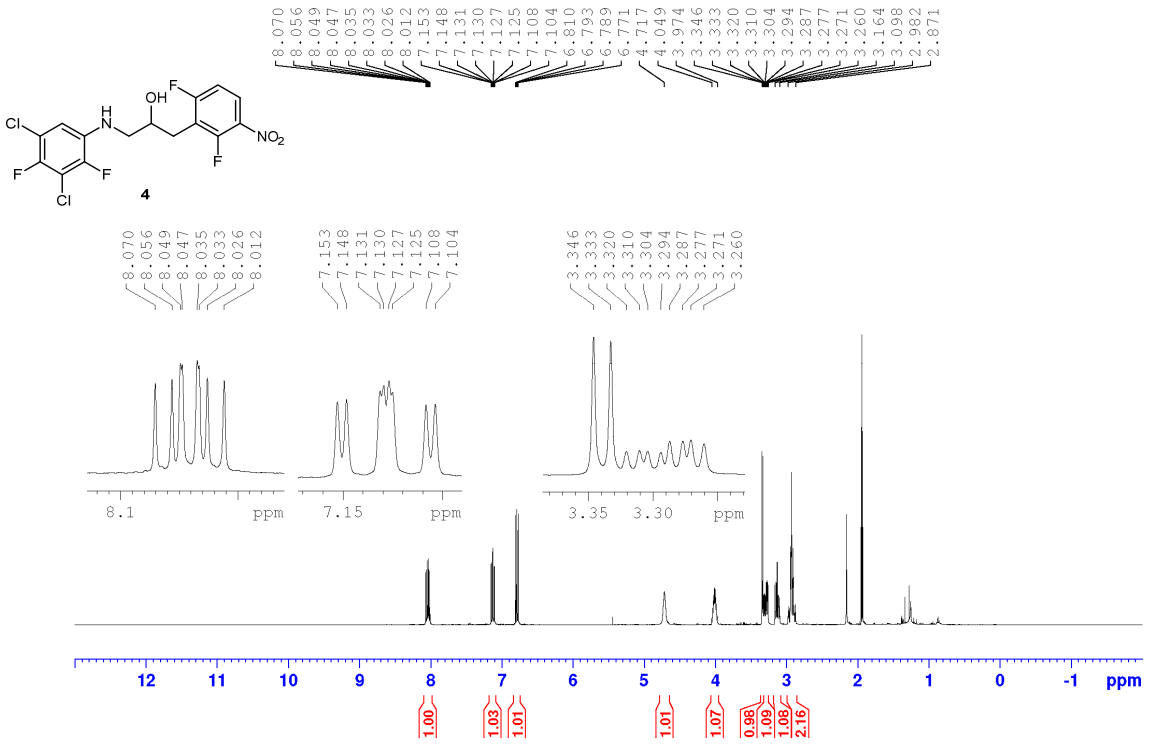


1-((3,5-Dichloro-2,4-difluorophenyl)amino)-3-(2,6-difluoro-3-nitrophenyl)propan-2-ol (4): ^{1}H NMR 400.13 MHz, CD_3CN



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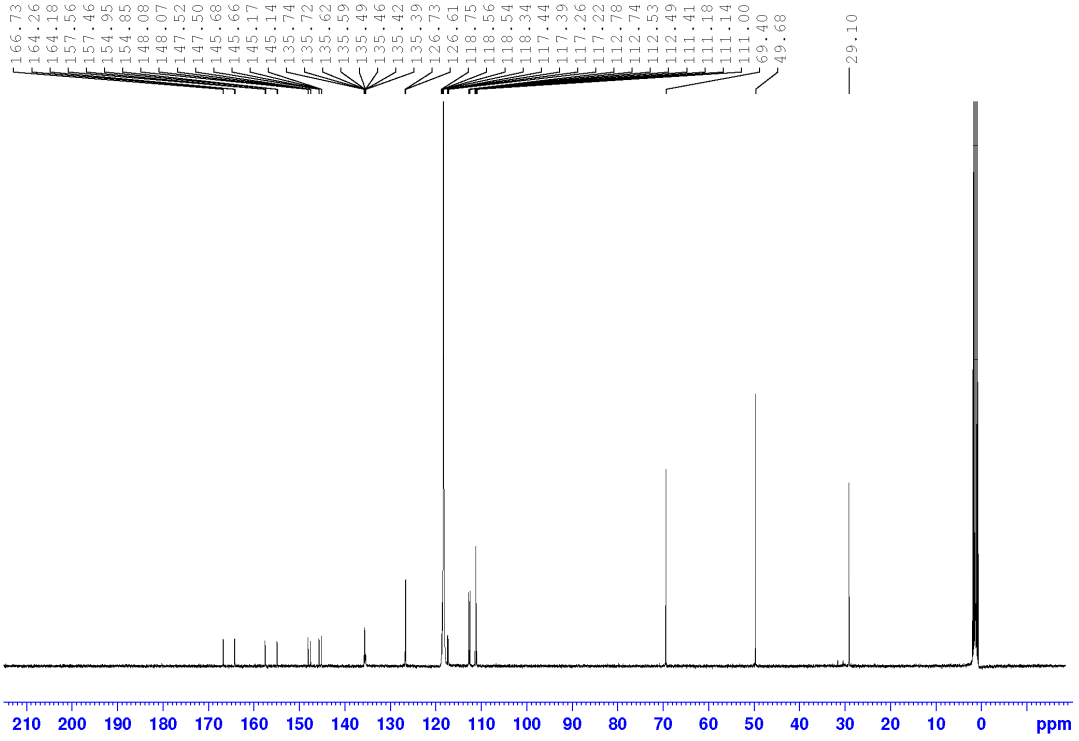

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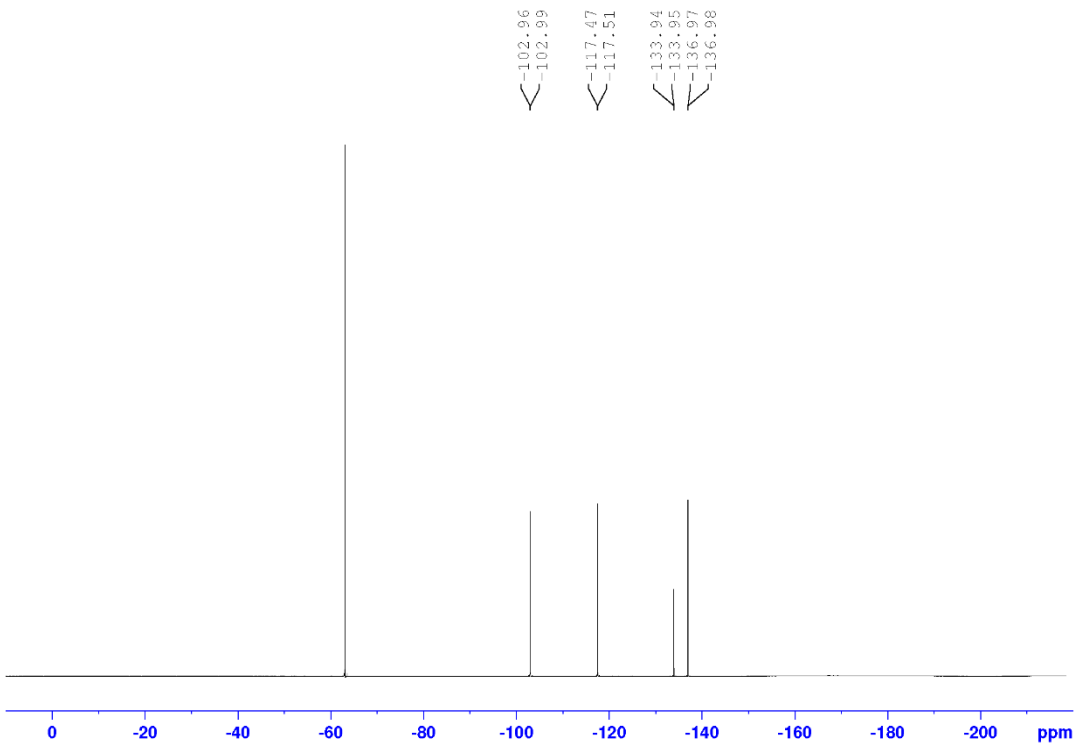

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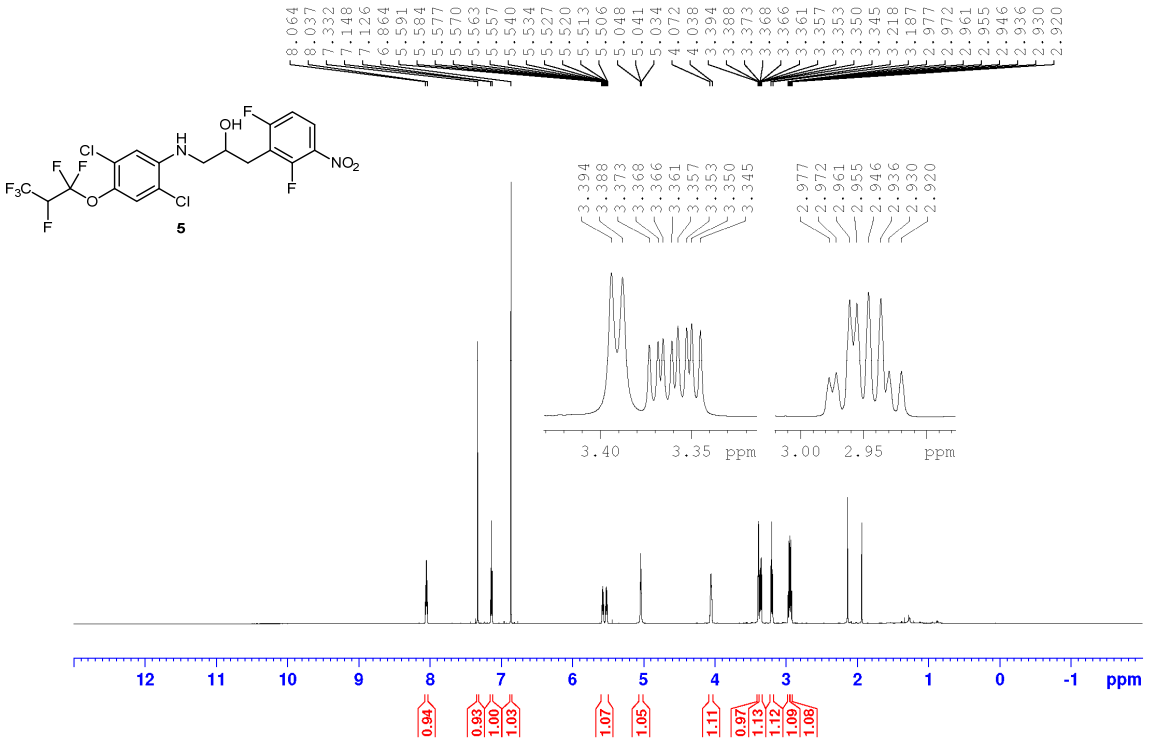

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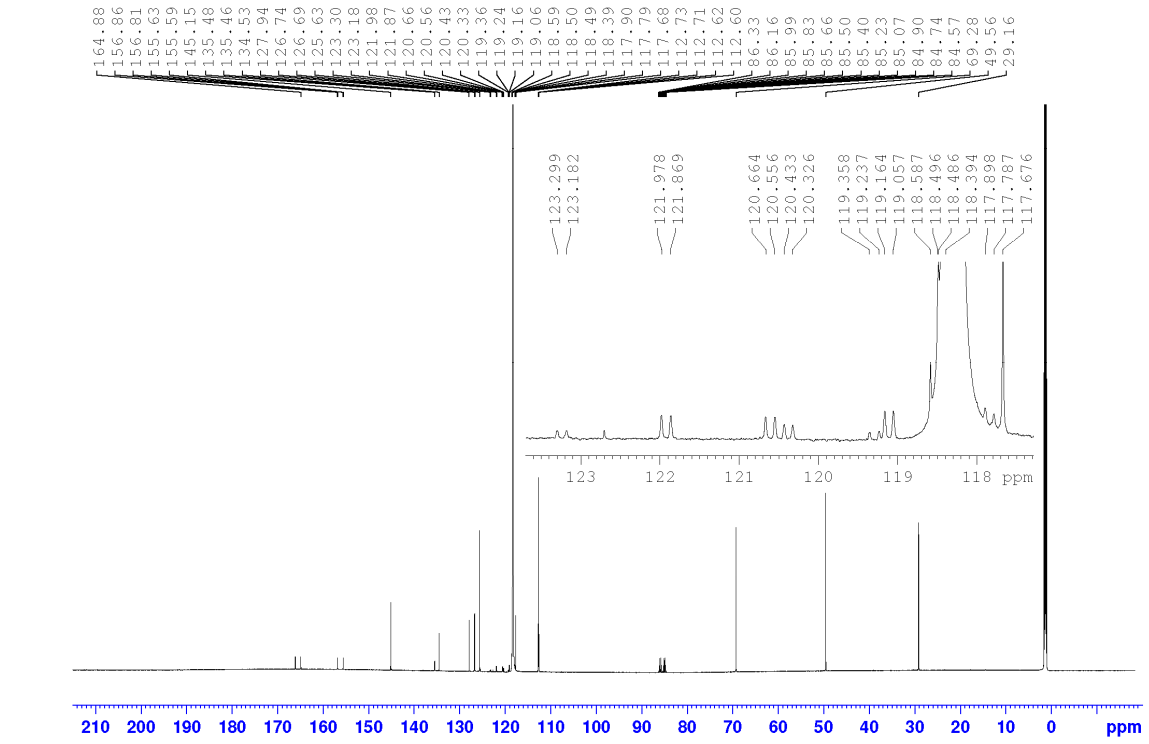

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