

## An orthogonal photolabile linker for the complete “on-support” synthesis/deprotection/hybridization of RNA

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### Supplementary Material

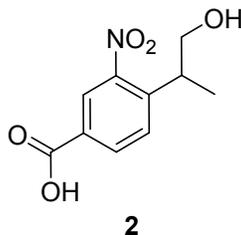
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## General experimental section

Thin layer chromatography was performed on EM Science Kieselgel 60 F- 254 (1mm) plates. Silicycle 40-63  $\mu\text{m}$  (230-400 mesh) silica gel was used for flash chromatography. Pyridine was distilled from  $\text{CaH}_2$  after refluxing for several hours. Acetonitrile, dichloromethane, THF, and toluene were dried using and Mbraun Inc. SPS 800 solvent dryer. All other anhydrous solvents were purchased from Sigma-Aldrich. Chemicals and reagents were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded on a Varian 300, 400 or 500 MHz spectrophotometer with chemical shift values reported in ppm.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were referenced to residual solvent.  $^{31}\text{P-NMR}$  were recorded at 80 MHz with a Varian 200MHz spectrophotometer and were measured from 85%  $\text{H}_3\text{PO}_4$  as an external standard. Preparations of the photoreactive solid supports were carried out in flame-dried solid-phase reaction vessels or in dry DNA synthesizer columns, all covered with aluminium foil. The solid phase syntheses of oligonucleotides were conducted on an ABI 3400 synthesizer. Standard cyanoethyl phosphoramidites and methoxyphosphoramidic chloride phosphitylation reagents were purchased from ChemGenes Inc. Underivatized NittoPhase<sup>®</sup> (420  $\mu\text{mol.g}^{-1}$  of free hydroxyl groups) was purchased from Kinovate Life Sciences Inc., 501 Via del Monte, Oceanside, CA. Microwave-assisted reactions were performed in a Biotage<sup>®</sup> Initiator using the supplied 0.5-2 ml vial and stir bar. Reactions were set at 60  $^\circ\text{C}$  for 10 min (12 W) without pre-stirring. UV spectra for oligonucleotide quantitation (absorbance measurements) were measured at 260 nm on a Varian Cary I or 300 UV-Vis dual beam spectrophotometer. Photolysis was performed in a Luzchem LZC photoreactor using their LZC-UVA lamps, which have a range of 316-400 nm.

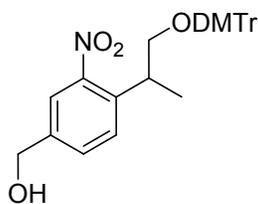
## Synthesis of NPPOC linker 3



### 4-(1-hydroxypropan-2-yl)-3-nitrobenzoic acid

Compound **1** (8.02 g, 28.5mmol), which was prepared according to published procedures,<sup>1</sup> was dissolved in 60 mL of freshly prepared solution of 25% TFA in DCM at room temperature. This was followed directly with 2.5 eq of triethylsilane (8.29 g, 11.38 mL, 71.3 mmol). This mixture was allowed to stir at room temperature for 3 h, at which point the mixture was concentrated under reduced pressure, resulting in a thick yellow oil. Compound **2** was purified by column chromatography using a gradient of hexanes/ethyl acetate 100:0 →60:40 with 1% acetic acid. Carboxylic acid **2** was obtained in 94% yield (6.05 g).

**Data for 2:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 1.46 (d, *J* = 7.03 Hz, 3 H) 3.87 (sxt, *J* = 6.64 Hz, 1 H) 4.48 - 4.64 (m, 2 H) 7.63 (d, *J* = 8.60 Hz, 1 H) 8.30 (dd, *J* = 8.21, 1.56 Hz, 1 H) 8.52 (d, *J* = 1.95 Hz, 1 H) 12.07 (br. s., 1 H) <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ ppm 17.31 (s, 1 C) 20.85 (s, 1 C) 33.37 (s, 1 C) 70.77 (s, 1 C) 126.26 (s, 1 C) 128.99 (s, 1 C) 129.56 (s, 1 C) 133.82 (s, 1 C) 141.45 (s, 1 C) 150.09 (s, 1 C) 169.79 (s, 1 C). Low resolution ESI-MS calculated: 225.20, found: 224.1[M-H]<sup>-</sup>



**3**

**(4-(1-(bis(4',4'-dimethoxytrityl)propan-2-yl)-3-nitrophenyl)methanol**

Compound **2** was dissolved in 100 mL of anhydrous pyridine and solid dimethoxytrityl chloride was added directly to the solution. This mixture was allowed to stir at room temperature overnight. The mixture was concentrated to near dryness and extracted in ethyl acetate with 10% w/v ammonium chloride x3 and once with brine. The organic layer was dried with magnesium sulfate and concentrated to dryness. This crude mixture oil was passed over a short silica column to remove the excess DMT-Cl. The remainder of the mixture was washed off the column with 10% methanol in DCM. The collected fractions were treated with triethylamine before being concentrated to dryness. This crude mixture was dissolved in 90 mL (0.2M solution) of THF and cooled to 0°C in an ice bath. This solution was then treated with 40 mL of a 1M solution of BH<sub>3</sub>-THF (39.5 mmol) added dropwise. This mixture was allowed to stir for 4 hours and allowed to slowly warm to room temperature, after which all starting material had been consumed. The excess BH<sub>3</sub> was quenched by the slow addition of methanol at 0°C. The crude mixture was concentrated to dryness and purified by column chromatography yielding pure compound **3**. Hexanes: ethyl acetate 100:0 → 70:30. Yield: 65% (6.43 g).

**Data for 3:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 1.30 (d, *J* = 6.64 Hz, 3 H) 2.36 (s, 1 H) 3.16 - 3.30 (m, 2 H) 3.56 - 3.69 (m, 1 H) 3.78 (s, 7 H) 4.72 (d, *J* = 4.69 Hz, 2 H) 6.78 (dd, *J* = 8.79, 1.76 Hz, 5 H) 7.13 - 7.34 (m, 15 H) 7.46 (d, *J* = 8.21 Hz, 1 H) 7.73 (s, 1 H) <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ ppm 18.32 (s, 1 C) 34.23 (s, 1 C) 55.20 (s, 1 C) 63.81 (s, 1 C) 67.96 (s, 1 C) 85.85 (s, 1 C) 112.95 (s, 1 C) 121.92 (s, 1 C) 126.63 (s, 1 C) 127.70 (s, 1 C) 128.09 (s, 1 C) 128.78 (s, 1 C) 129.04 (s, 1 C) 130.00 (s, 1 C) 130.33 (s, 1 C) 136.07 (s, 1 C) 138.00 (s, 1 C) 140.10 (s, 1 C) 144.86 (s, 1 C) 158.30 (s, 1 C). Low resolution ESI<sup>+</sup>-MS calculated: 513.59, found: 536.7 [M+Na]<sup>+</sup>

## Synthesis of photolabile solid support 5

Dry, underivatized polystyrene solid support (1g) (NittoPhase®) was first phosphitylated using a small peptide synthesis vessel. Significant excess of (*N,N*-diisopropylamino)methoxychlorophosphine (500mg, 2.5mmol) was used, with 5 mL of a solution of 10% triethylamine in THF solution. This mixture was allowed to gently shake at room temperature for 6h. The solid support was then rinsed with 30 mL of dry ACN affording the phosphitylated solid support (4). In order to ensure dry conditions for the subsequent coupling, the solid support 4 was washed with dry ACN (3 times) over an argon atmosphere. To this rinsed solid support (0.5 g), a 0.25M solution of dicyanoimidazole (DCI) and compound 3 (0.8 g, 1.8mmol) were added directly and very gently agitated for one hour. Anhydrous ethanol was then added to the reaction mixture to quench any remaining phosphoramidite on the solid support. The mixture was agitated for an additional 10 min. Excess reagents were then filtered through the reaction vessel. Unreacted hydroxyl groups on support from the initial phosphitylation were capped with an acetyl group using a mixture of Ac<sub>2</sub>O and *N*-methylimidazole, and phosphite linkages were oxidized with *tert*-butyl hydroperoxide. The resulting solid support 5 was then thoroughly washed with ACN and dried on high vac.

## Synthesis of photolabile solid support 6

Solid support 5 (13 mg) was weighed in a dry automated DNA synthesizer column and washed with ACN (20 ml) and CH<sub>2</sub>Cl<sub>2</sub> (20 ml). It was then first detritylated by passing a solution (10 ml) of 3% trichloroacetic acid in CH<sub>2</sub>Cl<sub>2</sub>. The solid support was thoroughly washed with CH<sub>2</sub>Cl<sub>2</sub> (20 ml), ACN (20 ml) then dried under vacuum. DMTr-protected dT phosphorodiamidite 7 (17 mg; 0.023 mmol; 1 eq.) and 3'-O-TBDMS protected dT 8 (8 mg; 0.023 mmol; 1 eq.) were mixed in a separate over-dried 1 Dram vial and dissolved in 150 µl ACN. DCI (7 mg; 0.059 mmol; 2.7 eq.) in 300 µl ACN was then added and after 20 min at r.t., the resulting mixture was transferred to the detritylated solid support. The coupling reaction was conducted at r.t. for 20 min with regular, manual percolations through the column, after which the column was flushed and the support washed with dry ACN (2 x 2 ml). The oxidation of the phosphite triester was then performed by adding an iodine solution (0.1 M I<sub>2</sub> in pyridine-water-THF; 1 ml) to the support and percolating for 2 min. The suspension was filtered, washed with ACN (2 x 2 ml) and the remaining unreacted hydroxyl groups were capped with the addition of a 2 ml 1:1 mixture of Cap A/Cap B. After a 2

min manual percolation at r.t., the reaction vessel was flushed, the support washed thoroughly with ACN (2 x 5 ml) and dried under vacuum.

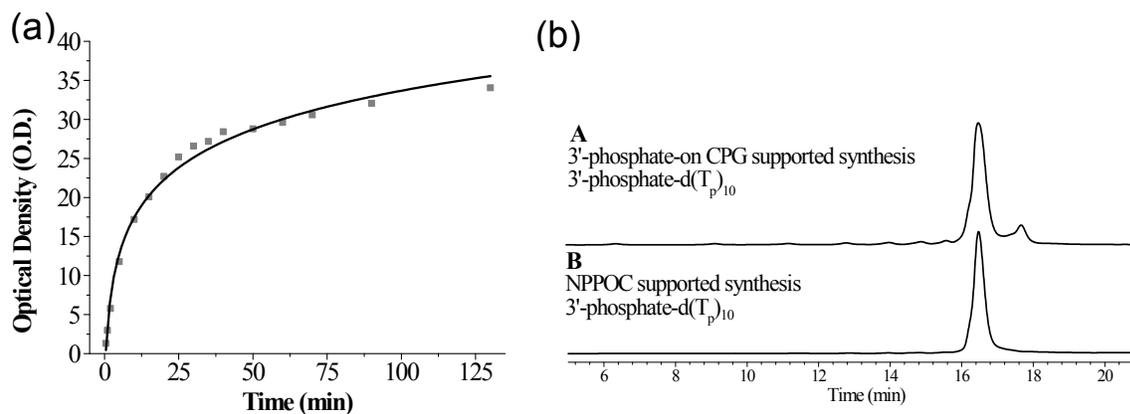
### Synthesis of DNA and RNA oligonucleotides

Standard phosphoramidite solid-phase synthesis conditions were used for the synthesis of all oligonucleotides. Each synthesis was done at approximately 1  $\mu$ mole scale using either controlled pore glass (500  $\text{\AA}$  CPG) with a 5'-*O*-DMTr-thymidine derivatized monomer attached by a succinyl linker or Unylinker<sup>®</sup> solid support or photolabile solid supports **5** and **9**. All phosphoramidites were prepared as 0.15 M solutions in acetonitrile (ACN) for RNA and 0.1 M for DNA. For syntheses on photolabile supports **5** and **9**, *N*<sup>4</sup>-acetyl-protected dC and rC phosphoramidites were used in order to avoid transamination reactions when using 40% MeNH<sub>2</sub> in H<sub>2</sub>O as a nucleobase deprotection reagent. 5-(Ethylthio)-1*H*-tetrazole (ETT, 0.25 M in ACN) was used to activate phosphoramidites for coupling. Detritylations were accomplished with 3% trichloroacetic acid in CH<sub>2</sub>Cl<sub>2</sub> (110 s). Capping of failure sequences was achieved with a 16% *N*-methylimidazole in THF (CAP A) and acetic anhydride:pyridine:THF, (1:2:2, v/v/v) (CAP B) for (30 s). Oxidation was done using 0.1 M I<sub>2</sub> in pyridine-water-THF (1:2:10, v/v/v).

## On-support deprotection of DNA using photolabile solid support 5

*Deprotection.* The solid support was treated with either ammonium hydroxide for 48 h at room temperature or 40% methylamine in water for 30 min at 60°C. The excess reagents were simply removed by filtration. To cleave the oligonucleotides from solid supports, the polystyrene beads were placed in a 1 Dram vial that has been pre-treated with Sigmacote® followed by a small stir bar and 1 mL of 100 mM triethylammonium acetate (TEAA) pH 7.5, the starting buffer used with reverse phase HPLC column, for ease of subsequent analysis. This vial was then placed in a photoreactor equipped with UVA bulbs (315-400 nm), and the sample was exposed to the UVA with stirring for 1 hour. A small portion of the solution phase was aliquoted to quantitate the amount of sample obtained, and then subjected to reverse phase HPLC analysis.

*Rate of photocleavage and HPLC analysis of a model  $T_{10}$  oligonucleotide*

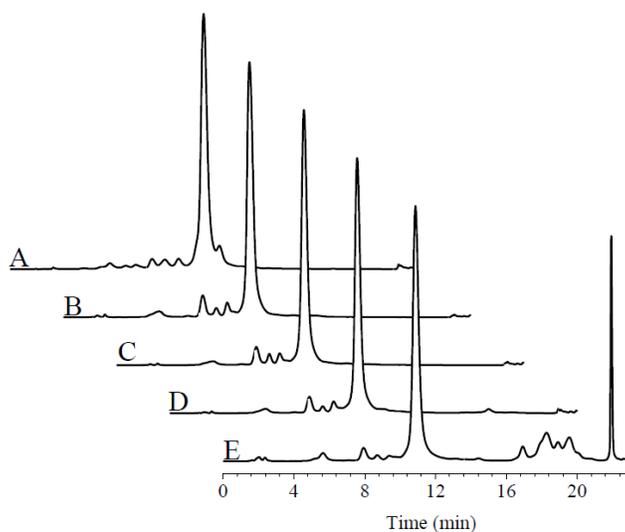


**Figure S1.** (a) Time-dependent release of a  $d(T_p)_{10}$  oligonucleotide from the NPPOC-linked solid support under UV irradiation. (b) reverse-phase (RP) HPLC chromatograms of a  $d(T_p)_{10}$  synthesized on (top) standard LCAA-CPG solid support and (bottom) on photolabile NPPOC-linked PS solid support.

## Determination of the optimal deprotection conditions for mixed sequence RNA oligonucleotides

A 12mer RNA sequence was synthesized on solid support **5** under the conditions described in “Synthesis of DNA and RNA oligonucleotides”, using 2'-TBDMS rG<sup>iBu</sup>, rA<sup>Bz</sup> and rC<sup>Ac</sup> phosphoramidites. The sequence was (5' to 3'): UGAUUCACGACU. After synthesis, the solid-supported RNA was deprotected first in 40% MeNH<sub>2</sub> in H<sub>2</sub>O for 30 min at 60 °C, filtered then washed with dry ACN. The solid support was then suspended in TREAT–HF and the desilylation reaction was performed under microwave irradiation at 80 °C for 5 min (trace C) or at 60 °C for 10 min (trace D). The desilylation was also performed at 65 °C for 10 min in the absence of microwave irradiation (trace E). A sample of RNA 12mer synthesized on **5** was first treated with NH<sub>3</sub>/EtOH instead of methylamine and then desilylated under microwave irradiation (trace B). Finally, for the purpose of comparison, the same sequence was prepared and deprotected according to conventional methods (trace A).

Under microwave irradiation, fluoride-mediated desilylation is complete within 5 min at 80 °C and within 10 min at 60 °C (traces C and D, respectively). Aqueous MeNH<sub>2</sub> deprotection gave excellent results, requiring only 30 min treatment at 60°C compared to 2 days at r.t. for aqueous ammonia deprotection (trace C versus B). The benefits of microwave irradiation are appreciated by comparing the HPLC traces of an oligomer treated with TREAT–HF at 65°C for 10 min without microwave irradiation (trace E), to that of a sample treated under the same conditions with microwave irradiation (trace D). In addition to the deprotected product, significant amounts of silylated materials were observed in the sample not exposed to microwave irradiation (trace E)



**Figure S2.** RP- HPLC analysis of mixed-base RNA 12mer under various deprotection conditions.

A) Standard synthesis on 3' phosphate CPG deprotected by  $\text{NH}_3/\text{EtOH}$  (3:1, v/v), 48 h r.t. then TREAT-HF 48h r.t.  
 B)-E): synthesis on photolabile solid support **5**; deprotected by B)  $\text{NH}_3/\text{EtOH}$  (3:1) 48h r.t. then TREAT-HF, MW for 5 min at  $80^\circ\text{C}$ ; C) 40%  $\text{MeNH}_2$  in  $\text{H}_2\text{O}$  for 30 min at  $60^\circ\text{C}$  then TREAT-HF, MW for 5 min at  $80^\circ\text{C}$ ; D) 40%  $\text{MeNH}_2$  in  $\text{H}_2\text{O}$  for 30 min at  $60^\circ\text{C}$  then TREAT-HF, MW for 10 min at  $60^\circ\text{C}$ ; E) 40%  $\text{MeNH}_2$  in  $\text{H}_2\text{O}$  for 30 min at  $60^\circ\text{C}$  then TREAT-HF for 10 min at  $65^\circ\text{C}$  heat only (no MW). All sequences were characterized by ESI-MS  $m/z$  cal. 3836.3 found 3836.8.

## **On-support deprotection of RNA using photolabile solid supports 5 and 9**

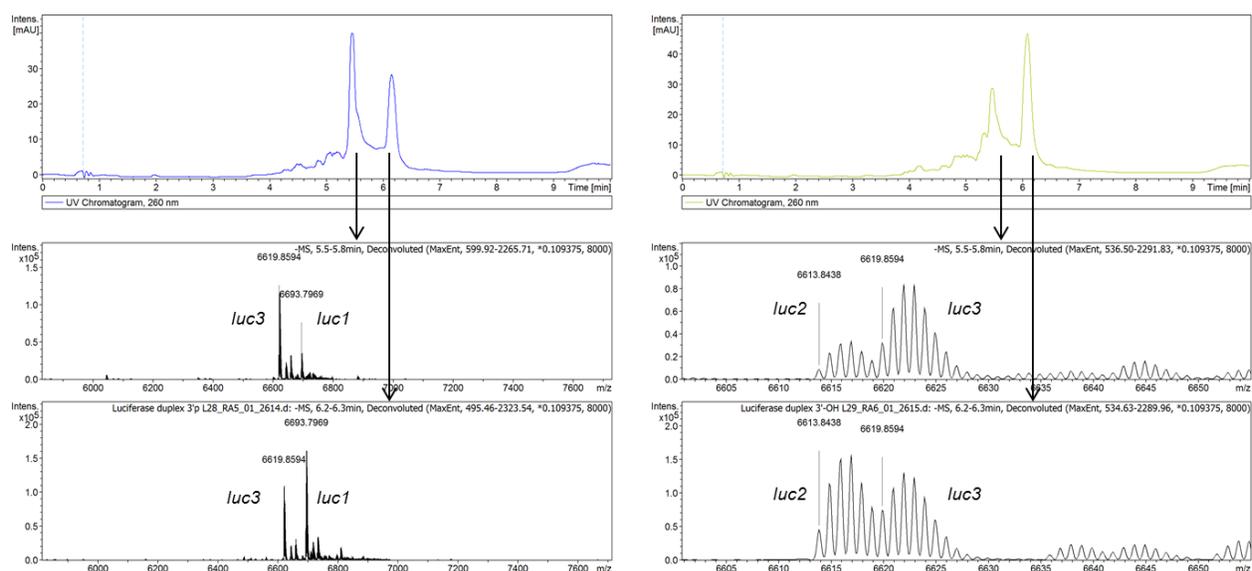
Post-synthesis the solid support was treated with either a 3:1 mixture of ammonium hydroxide/ethanol for 48 h at room temperature or 40% methylamine in water for 30 min at 60°C. The excess reagents were simply removed by filtration and the solid support was rinsed with ACN. Desilylation was achieved by suspending the solid support in 500 µL of TREAT-HF in a Biotage® Initiator reaction vial with the supplied stir bar, and subjected to microwave irradiation up to 60°C and held for 10 min. The excess reagents were then simply removed by filtration and the solid support was rinsed with ACN. To cleave the oligonucleotides from solid supports, the polystyrene beads were placed in a 1 Dram vial that has been pre-treated with Sigmacote® followed by a small stir bar and 1 mL of 100 mM triethylammonium acetate (TEAA) pH 7.5, the starting buffer used with reverse phase HPLC column, for ease of subsequent analysis. This vial was then placed in a photoreactor equipped with UVA bulbs (315-400 nm), and the sample was exposed to the UVA with stirring for 1 hour. A small portion of the solution phase was aliquoted to quantitate the amount of sample obtained, and then subjected to reverse phase HPLC analysis.

### **HPLC and LC-MS conditions**

HPLCs were performed on a Waters or an Agilent 1200 series machine. Reverse phase HPLCs were performed with a Hamilton PRP-1 (5 µm 100Å 2.1 x 150 mm, analytical scale) and (10 µm 10 x 250 mm, preparative scale) HPLC columns. Synthesized oligoribonucleotides were analyzed using a 0-15% gradient over 20 min (analytical scale) or 0-70% gradient over 60 min (preparative scale). Solvent A: 100 mM TEAA pH 7.0. Solvent B: 100% ACN. The purified oligonucleotides were analyzed by LC-MS using a Dionex Ultimate 3000 UHPLC coupled to a Bruker Maxis Impact QTOF mass spectrometer in negative ESI mode. Samples were run through an Acclaim RSLC 120 C<sub>18</sub> column (2.2 µm 120A 2.1 x 50 mm) using a gradient of 98% mobile phase A (100 mM HFIP and 5 mM TEA in H<sub>2</sub>O) and 2 % mobile phase B (MeOH) to 40 % mobile phase A and 60% mobile phase B in 8 minutes. The data was processed and deconvoluted using the Bruker DataAnalysis software version 4.1.

**Table S1.** LC-MS data for RNA oligonucleotides synthesized on photolabile solid supports **5** and **9**

Entry	Sequence (5' to 3')	M <sub>calcd</sub> (m/z)	M <sub>found</sub> (m/z)
1	UGAUUCACGACU	3836.3	3836.8
2	GCUUGAAGUCUUUAAUUAAtt (3'-phosphate)	6693.85	6693.78
3	GCUUGAAGUCUUUAAUUAAtt (3'-OH)	6613.75	6613.83



**Figure S3.** LC-MS of duplexes *luc1/luc3* and *luc2/luc3* made on solid supports **5** and **9**, respectively, and characterized after photocleavage in siRNA buffer under UV irradiation (315-400 nm) for 1h at r.t. Separate single strands usually elute ~5.3-5.5 min under the same conditions, which suggests that peaks at 6.2-6.3 min correspond to duplexes dissociating during ionization and thus being detected as single strands.

## **On-support hybridization experiments**

Representative experimental procedure: Supported luciferase sense strand (*luc2*) was suspended in 0.5 ml sterile MQ-H<sub>2</sub>O. A 10 µl aliquot containing a few beads was added to an aliquot of gel-purified antisense strand (*luc3*, 13 nmol) and dried down. The mixture was then resuspended in 20 µl of sodium citrate buffer (20X; 3 M NaCl and 300 mM citrate) and left to hybridize at 45°C for 3h. The supernatant was then removed, diluted and its absorbance measured to account for the excess of *luc3* that was collected (9 nmol). The beads were then washed with 20 µl SSC 20X at 45°C for 2 min, the supernatant removed and its absorbance measured (2.6 nmol). Finally, the beads were washed with 1) 20 µl SSC 10X at 45°C for 2 min then 2) 20 µl SSC 5X at 45°C for 2 min, releasing virtually no *luc3*. Beads were then filtered and washed with ACN. They were then transferred to a 1 Dram vial that has been pre-treated with Sigmacote® and with a small stir bar and 200 µl sterile MQ-H<sub>2</sub>O. This vial was then placed in a photoreactor equipped with UVA bulbs (315-400 nm), and the sample was exposed to the UVA with stirring for 1 hour. The supernatant was then recovered, dried down and then rediluted in 25 µl siRNA buffer (60 mM KCl, 30 mM HEPES, 0.2 mM MgCl<sub>2</sub>, pH 7.4).

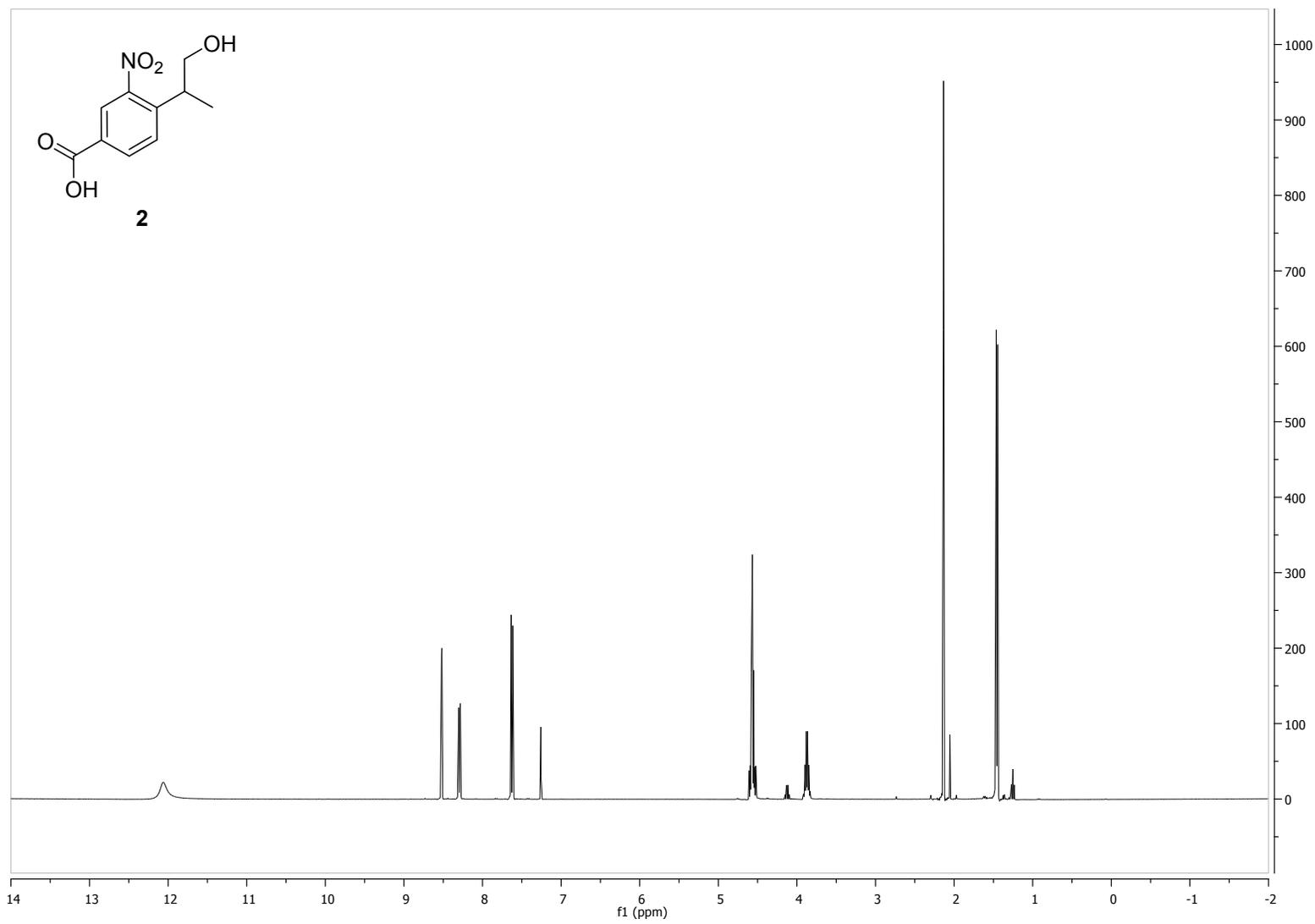
## **Non-denaturing polyacrylamide gel conditions**

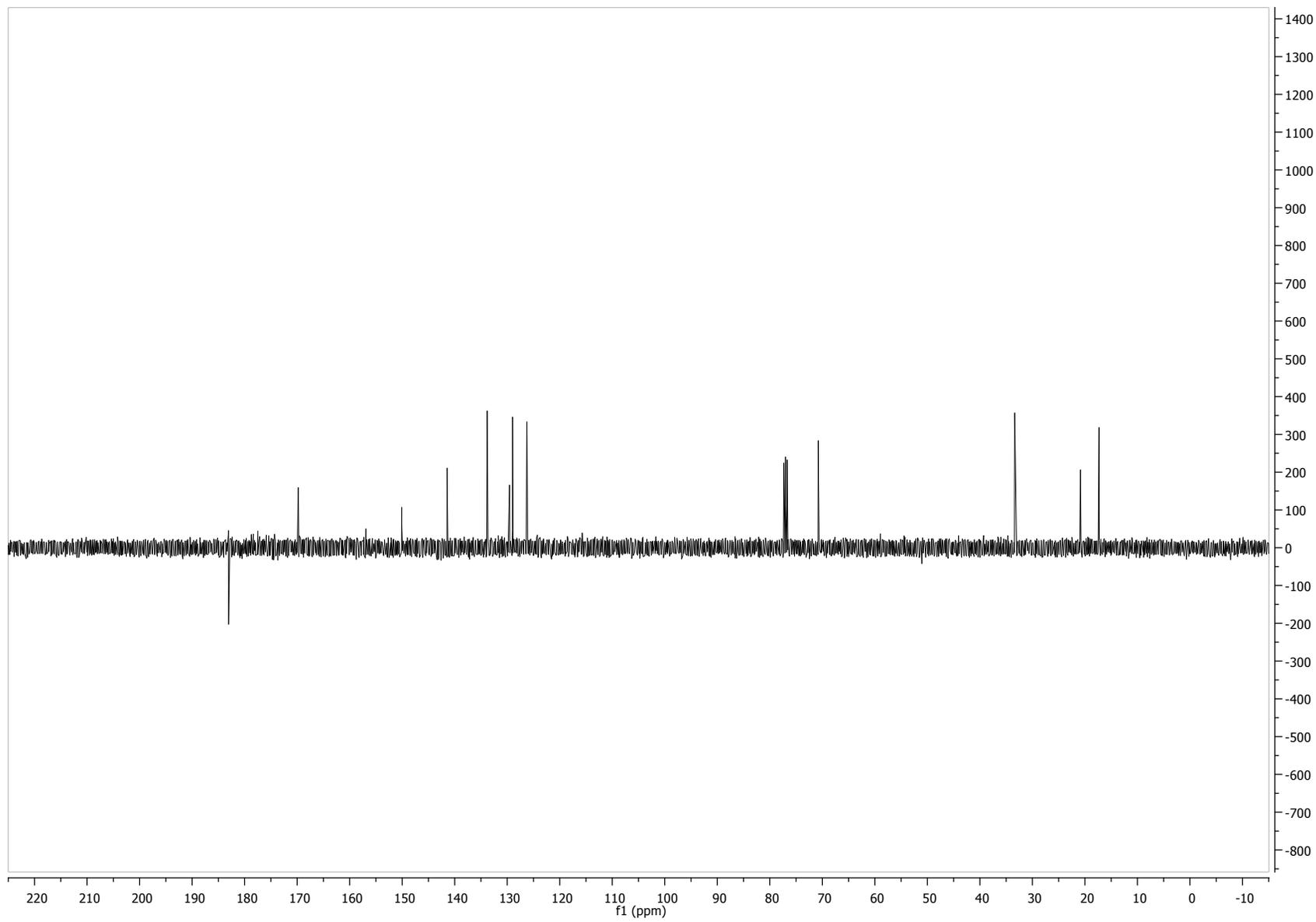
Duplexes made “on-support” or “in solution” (150 pmol per duplex) and their respective single strands (300 pmol per strand) were heated up to 90°C for 5 min and then slowly cooled down to r.t. overnight. They were loaded in 10% glycerol onto 24% non-denaturing polyacrylamide gel and electroeluted with 0.5X Tris/borate/EDTA buffer at 4°C for 20h at 210V. The gel was then visualized by dipping in Stains-All for 2h, removed from the staining chamber, washed then scanned.

## **Luciferase knockdown assay**

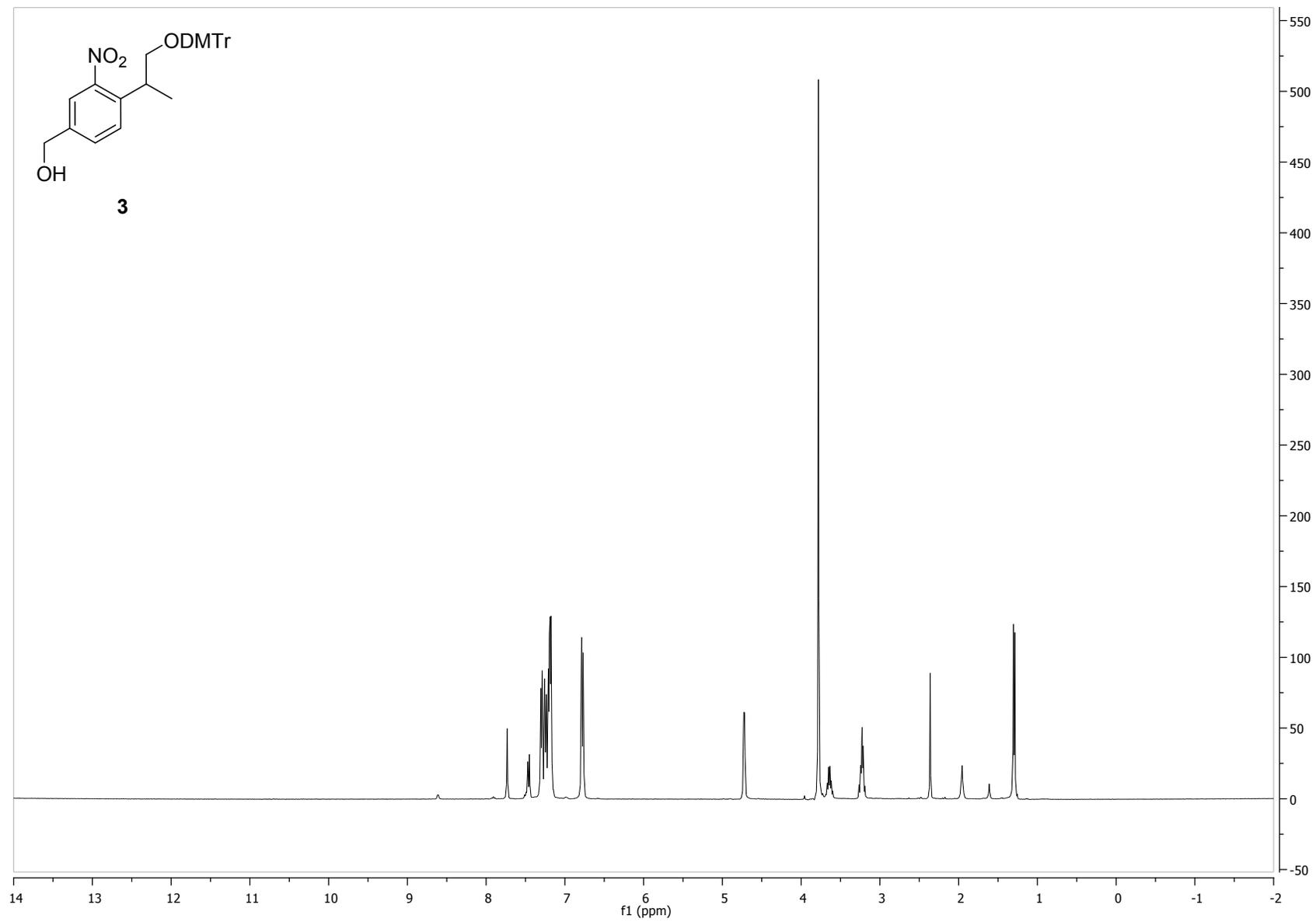
Luciferase knockdown assays were performed as described in Deleavey et al.<sup>2</sup> with a few modifications. Typically, HeLa cells were counted and seeded at a density of 10,000 cells/well in an 96-well plate. Cells were allowed to recover for 24 hours at 37°C with 5% CO<sub>2</sub>. Subsequently, cells were washed once with serum-free DMEM media and then 80 µl of serum-free DMEM media was added. siRNA and control nucleic acid preparations were diluted up to 20 µl with serum-free media and transfection reagent (Oligofectamine, Invitrogen) and added to the appropriate well (for a total of 100 µL) at increasing concentrations (0.16, 0.8, 4, and 20 nM). Cells were incubated for 4 hours, at that point each well was supplemented with 50 µL of serum-enriched DMEM media. Cells were further incubated overnight (for a total of 24 hours post-siRNA addition). Then 50 uL of ONE-Glo luciferase reagent (Promega, USA) was added to each well and luminescence was measured and normalized to protein levels using a Biotek Synergy HT plate reader. Data was acquired with the Gen5 software suite and data was manipulated and plotted using Graphpad Prism software suite.

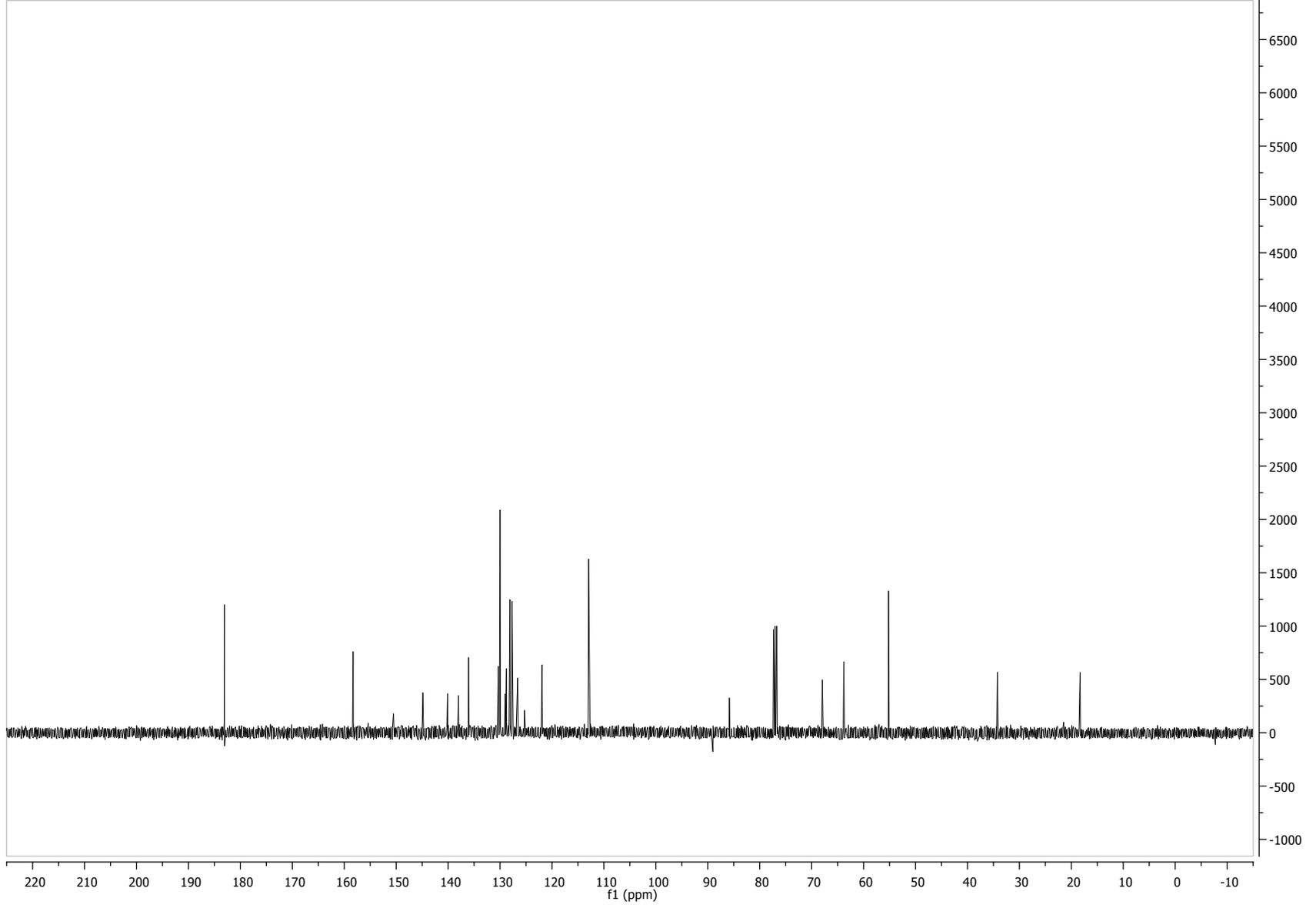
## NMR Spectra of compounds 2 and 3





S15





S17

## References

1. (a) Smirnova, J.; Wöll, D.; Pfeleiderer, W.; Steiner, U. E., *Helv. Chim. Acta* **2005**, *88*, 891-904; (b) Fahim, H. A.; Fleifel, A. M., *J. Chem. Soc.* **1952**, 4519-4521.
2. Deleavey, G. F.; Watts, J. K.; Alain, T.; Robert, F.; Kalota, A.; Aishwarya, V.; Pelletier, J.; Gewirtz, A. M.; Sonenberg, N.; Damha, M. J., *Nucleic Acids Res.* **2010**, *38*, 4547-4557.