Electronic Supporting Information

# Stereocontrolled Synthesis of the PPAR–γ Agonist 10-Nitrolinoleic Acid

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**Contents:** **Page No.**

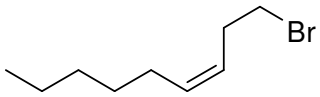
**Experimental directions:**

**General**

Reagents were obtained from commercial suppliers and were used without further purification. Lipozyme TL 100L was obtained from Novozymes. Dry THF and diethyl ether were distilled from sodium benzophenone ketyl radical and dry $CH_2Cl_2$ distilled from calcium hydride, under nitrogen. Low reaction temperatures were obtained with ice or an acetone/solid $CO_2$ bath. Thin-layer chromatography was performed on silica coated aluminium sheets (60 $F_{254}$) supplied by Merck. Flash column chromatography was performed using flash silica 60 Å (230-400 mesh) 9385 supplied by Merck. $^{1}H$ and $^{13}C$ NMR spectra were recorded using 300 MHz and 400 MHz instruments as indicated. Deuterochloroform was used as the solvent and chemical shifts are given in ppm relative to the standard reference TMS or residual chloroform.

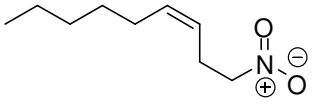
**Experimental:**

(*Z*)-1-Bromonon-3-ene, **SI_1**

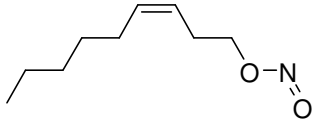



(*Z*)-3-Nonen-1-ol **5** (1.80 ml, 10.7 mmol, 1 equiv) was stirred with carbon tetrabromide (4.44 g, 13.4 mmol, 1.25 equiv) in $CH_2Cl_2$ (16 ml). Once all solids were dissolved, triphenylphosphine (4.22 g, 16.1 mmol, 1.5 equiv) was added portionwise at 0 $^{o}$C. This was stirred for 1.5 h before the solvent was removed *in vacuo*. The mixture was then poured into stirring pentane (35 ml) and filtered. The solvent was removed *in vacuo* and the filtration step was repeated three times to yield the title compound (1.15 g, 53%) as a clear oil.[1] $R_f$ = 0.9 (pentane). IR (film) 3013, 2959, 2928, 2856, 1620, 1438, 1028, 721 $cm^{-1}$. $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 8 Hz, 3H), 1.24-1.41 (m, 6H), 2.03 (q, *J* = 8 Hz, 2H), 2.62 (q, *J* = 8 Hz, 2H), 3.36 (t, J = 8 Hz, 2H), 5.32-5.39 (m, 1H), 5.50-5.58 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.1, 22.5, 27.4, 29.2, 30.9, 31.5, 32.6, 125.7, 133.2. HRMS $C_9H_{17}Br$ calcd. 204.0514; found 204.0514 ($^{79}Br$), 206.0503 ($^{81}Br$).

*Synthesis of nitro compound **4**:*




(*Z*)-1-Nitronon-3-ene, **4**




(*Z*)-Non-3-enyl nitrite, **6**

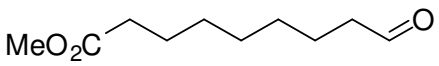
*Method A: Sodium nitrite reaction:*

(*Z*)-1-Bromonon-3-ene (1.22 g, 6.0 mmol, 1 equiv) was added dropwise to a solution of sodium nitrite (0.74 g, 10.8 mmol, 1.8 equiv) in anhydrous DMF (13 ml). This was left to stir overnight at room temperature under nitrogen. The solution was diluted with cold water (10 ml), washed with diethyl ether (3 × 15 ml), dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography (pentane) yielded (*Z*)-1-nitronon-3-ene **4** (0.38 g, 37 %) as a yellow oil and (*Z*)-non-3-enyl nitrite **6** (0.02 g, 1 %) as a clear oil. Starting material was also recovered (0.37 g, 30%). Data for (*Z*)-1-nitronon-3-ene, **4**: $R_f$ = 0.1 (pentane). IR (film) 3000, 2927, 2860, 1647, 1553, 1434, 1379, 1027 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.87-0.91 (3H, m), 1.21-1.39 (6H, m), 2.05 (2H, q, *J* = 8 Hz), 2.75 (2H, q, *J* = 8 Hz), 4.37 (2H, t, *J* = 8 Hz), 5.26-5.33 (1H, m), 5.56-5.62 (1H, m) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 25.5, 27.2, 29.1, 31.4, 75.1, 122.0, 134.9 ppm. HRMS $C_9H_{18}NO_2$ calcd. 172.1338; found 172.1330. Data for (*Z*)-non-3-enyl nitrite, **6**: $R_f$ = 0.4 (pentane). IR (film) 3000, 2927, 2858, 1633, 1455, 1280, 1022, 859 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.88-0.91 (m, 3H), 1.22-1.40 (m, 6H), 2.03 (q, *J* = 8 Hz, 2H), 2.48 (q, *J* = 8 Hz, 2H), 4.43 (t, *J* = 8 Hz, 2H), 5.30-5.37 (m, 1H), 5.54-5.61 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 25.1, 27.2, 29.1, 31.4, 72.3, 122.3, 134.4.

*Method B: Silver Nitrite reaction:*

(*Z*)-1-Bromonon-3-ene (0.55 g, 2.7 mmol, 1 equiv) was added to a flask containing silver nitrite (0.62 g, 4.0 mmol, 1.5 equiv) stirring in diethyl ether (6 ml) under nitrogen. The flask was covered in aluminium foil and left to stir at room temperature for seven days. The resulting suspension was filtered through silica and celite, washed with diethyl ether (3 × 20 ml), and the solvent was removed *in vacuo*. Purification by silica gel chromatography (pentane) yielded (*Z/E*)-1-nitronon-3-ene **4** (0.18 g, 40%) as a yellow oil and (*Z/E*)- non-3-enyl nitrite **6** (0.27 g, 58 %) as a clear oil with data as reported above.

*Synthesis of aldehyde* ***3****:*



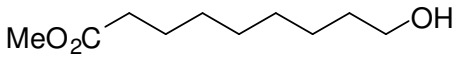

*Method A: Borane reduction followed by PCC oxidation:*

At -18°C and under nitrogen, *mono*-methyl azelate **7** (0.50 ml, 2.6 mmol, 1 equiv) was stirred in tetrahydrofuran (1 ml). To this $BH_3$·THF complex (2.50 ml, 2.5 mmol, 1.0 M solution in THF) was added dropwise over 20 min. The solution was stirred at -18°C for 10 min then left to stir at room temperature for 4 h. At 0°C, water (10 ml) was added, followed by $K_2CO_3$ (0.61 g, 4.4 mmol, 1.7 equiv) and diethyl ether (20 ml). The aqueous phase was separated and washed with diethyl ether (3

× 10 ml) and the combined organic layers were washed with brine (10 ml), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a yellow oil which was purified by column chromatography (pentane:diethyl ether; 1:1). The product was added dropwise to a solution of PCC (0.84 g, 3.9 mmol, 1.5 equiv) and celite (0.84 g) in $CH_2Cl_2$ (5 ml), under nitrogen. The solution was left to stir for 4 h at room temperature. Diethyl ether (10 ml) was added and the mixture filtered through silica. The solvent was removed *in vacuo* to yield aldehyde **3** (0.38 g, 82%) as a yellow oil.[2] $R_f$ = 0.9 (pentane:diethyl ether; 1:1). IR (film) 2932, 2856, 2719, 1738, 1437, 1249, 1171 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.26-1.36 (m, 6H), 1.58-1.66 (m, 4H), 2.30, (t, *J* = 8 Hz, 2H), 2.42 (dd, *J* = 4, 8 Hz, 2H), 3.67 (s, 3H), 9.76 (t, *J* = 4 Hz, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 22.0, 24.8, 28.90, 28.95, 29.0, 34.0, 43.9, 51.5, 174.2, 202.8.

*Method B: Three-step reduction/esterification and then oxidation:*

Methyl 9-hydroxynonanoate, **SI_2**



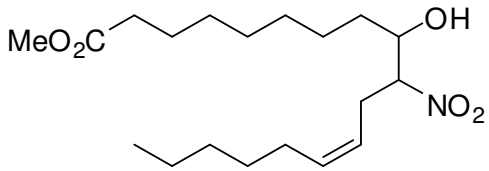

*Mono*-methyl azelate **7** (1.00 ml, 5.2 mmol, 1 equiv) was stirred in a 1:1 mixture of water (25 ml) and dioxane (25 ml). $NaBH_4$ (1.37 g, 36.2 mmol, 7 equiv) was added portion wise and the mixture left to stir at room temperature overnight. At 0°C, 1 M HCl (~20 ml) was added dropwise, followed by ethyl acetate (25 ml). The water layer was washed with ethyl acetate (3 × 20 ml). The organic layer was separated and dried with $MgSO_4$, filtered and solvent removed *in vacuo* to yield a white solid which was used without further purification. The crude hydroxy acid was heated to reflux in methanol (13 ml) with sulphuric acid (~1 ml) overnight. Diethyl ether (20 ml) and a saturated aqueous solution of $NaHCO_3$ (20 ml) were added. The aqueous phase was separated and washed with diethyl ether (2 × 15 ml). The combined organic layers were washed with water (15 ml), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a yellow oil. Purification by silica gel column chromatography (pentane:diethyl ether; 9:1) yielded methyl 9-hydroxynonanoate (0.24 g, 26%).[2] $R_f$ = 0.1 (pentane:diethyl ether; 9:1). IR (film) 3400, 2998, 2882, 2350, 1673, 1589, 1482, 1382 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.24-1.37 (m, 8H), 1.53-1.64 (m, 4H), 2.30 (t, *J* = 8 Hz, 2H), 3.64 (t, *J* = 8 Hz, 2H), 3.67 (s, 3H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 24.9, 25.6, 28.9, 29.0, 29.2, 32.7, 34.1, 51.4, 63.0, 174.3.

Oxidation: Under nitrogen, PCC (65 mg, 0.3 mmol, 1.5 equiv) and celite (65 mg) were stirred in $CH_2Cl_2$ (1 ml). To this, methyl 9-hydroxynonanoate (45 mg, 0.2 mmol, 1 equiv) was added dropwise and the solution was left to stir for 4 h. Diethyl ether (10 ml) was added and the mixture

filtered through silica. The solvent was removed *in vacuo* to yield **3** (27 mg, 60%) as a yellow oil. Data as above.

*Nitro-aldol reaction:*

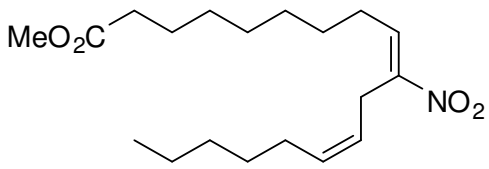
(*Z*)-Methyl 9-hydroxy-10-nitrooctadec-12-enoate, **8**




Under nitrogen, potassium *tert*-butoxide (0.02 g, 0.2 mmol, 0.1 equiv) was added to a solution of methyl 9-oxononanoate **3** (0.38 g, 2.0 mmol, 1 equiv), (*Z*)-1-nitronon-3-ene **4** (0.34 g, 2.0 mmol, 1 equiv) in THF (1 ml) and *tert*-butanol (1 ml) at 0°C. The mixture was stirred for 15 min at 0°C and then left to stir for 24 h at room temperature. Water (10 ml) and diethyl ether (10 ml) were added and the solution was transferred to a separating funnel where it was washed with a saturated aqueous solution of $NaHCO_3$ (10 ml), brine (10 ml) and diethyl ether (2 × 10 ml). The combined organic layers were dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography (pentane:diethyl ether; 19:1 to 9:1) yielded the product **8** (0.45 g, 62%).* Nitro compound **4** (0.11 g, 30%) was also recovered. $R_f$ = 0.03 (pentane:diethyl ether; 9:1). IR (film) 3450, 3100, 2929, 2857, 1739, 1551, 1438, 1377, 1201, 1169, 1107, 1030, 877 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 8 Hz, 3H), 1.29-1.63 (m, 18H), 2.03 (broad q, *J* = 8 Hz, 2H), 2.28 (t, *J* = 8 Hz, 2H), 2.54-2.61 (m, 1H), 2.78-2.92 (m, 1H), 3.67 (s, 3H), 3.85-3.94 (m, 0.6H), 4.03-4.09 (m, 0.4H), 4.41-4.47 (m, 1H), 5.25-5.33 (m, 1H), 5.54-5.60 (m, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 24.8, 25.3, 26.3, 27.2, 27.25, 28.6, 28.95, 29.0, 29.05, 29.10, 29.15, 31.45, 31.50, 33.2, 33.6, 34.0, 51.5, 71.6, 72.1, 91.9, 92.2, 121.5, 122.1, 135.0, 135.3, 174.3. HRMS $C_{19}H_{36}NO_5$ calcd. 358.2593; found 358.2579. *Mixture of diastereoisomers

*Dehydration:*

(9*E*,12*Z*)-Methyl 10-nitrooctadeca-9,12-dienoate, **9**

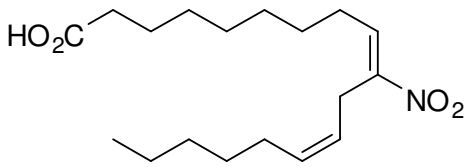



Under nitrogen, a solution of (*Z*)-methyl 9-hydroxy-10-nitrooctadec-12-enoate **8** (169 mg, 0.5 mmol, 1 equiv), DMAP (6 mg, 0.05 mmol, 0.1 equiv) and acetic anhydride (0.06 ml, 0.6 mmol,

1.25 equiv) was stirred in diethyl ether (1 ml) for 6 h and then concentrated. DMAP (73 mg, 0.6 mmol, 1.2 equiv) was added to a solution of the crude diastereomeric nitroacetates in $CH_2Cl_2$ (2 ml) and the solution was stirred at room temperature for 24 h. The solution was diluted with $CH_2Cl_2$ (10 ml) followed by washing with water (10 ml), 0.1 M HCl (10 ml), brine (10 ml) and $CH_2Cl_2$ (2 × 10 ml). The combined organic layers were dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography (pentane:diethyl ether; 15:1) yielded the product **9** (72 mg, 45%) as an oil. Further elution yielded the nitroacetates (42 mg, 26%) [HRMS $C_{21}H_{38}NO_6$ calcd. 400.2699; found 400.2679]. $R_f$ = 0.5 (pentane:diethyl ether; 9:1). IR (film) 3050, 2928, 2857, 1742, 1643, 1556, 1436, 1371, 1227, 1162, 1026 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J* = 8 Hz, 3H), 1.26–1.66 (m, 16H), 2.12 (q, *J* = 8 Hz, 2H), 2.24 (q, *J* = 8 Hz, 2H), 2.31 (t, *J* = 8 Hz, 2H), 3.34 (d, *J* = 8 Hz, 2H), 3.67 (s, 3H), 5.21-5.29 (m, 1H), 5.46-5.54 (m, 1H), 7.08 (t, *J* = 8 Hz, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 24.82, 24.83, 27.4, 27.9, 28.4, 28.93, 28.95, 29.0, 29.1, 31.5, 34.0, 51.4, 123.2, 133.0, 136.4, 150.6, 174.1. HRMS $C_{19}H_{34}NO_4$ calcd. 340.2488; found 340.2471. HPLC analysis (Agilent XDB-C18), gradient elution, MeCN, $H_2O$ (1% TFA), 1 ml/min, 40 °C, $t_r$ = 21.27 min.

*Hydrolysis:*

(9*E*,12*Z*)-10-Nitrooctadeca-9,12-dienoic acid, **2a**




Lipozyme TL 100L (400 mg) was added to a solution of nitrated linoleic acid methyl ester **9** (16 mg, 0.05 mmol) in acetone (0.6 ml) and aqueous phosphate buffer (6 ml, pH 7.4). The solution was vigorously stirred at room temperature for 24 h before acidification with 1 M HCl solution (pH 3) and extraction with diethyl ether (5 × 10 ml). The combined organic layers were dried with $MgSO_4$, filtered and the solvent removed *in vacuo*. Purification by silica gel column chromatography (pentane:diethyl ether; 5:1) yielded the title compound **2a** (11 mg, 73%) as a yellow oil.[3] Methyl ester **9** (4 mg, 25%) was also recovered. $R_f$ = 0.07 (pentane:diethyl ether; 5:1). IR (film) 3278, 3017, 2918, 2850, 1710, 1522, 1336, 911 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.83-0.95 (m, 3H), 1.18-1.67 (m, 16H), 2.13 (q, *J* = 8 Hz, 2H), 2.24 (q, *J* = 8 Hz, 2H), 2.36 (t, *J* = 8 Hz, 2H), 3.34 (d, *J* = 8 Hz, 2H), 5.21-5.30 (m, 1H), 5.46-5.54 (m, 1H), 7.08 (t, *J* = 8 Hz, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 24.6, 24.9, 27.4, 27.9, 28.4, 28.9, 28.95, 29.0, 29.1, 31.6, 33.8, 123.2, 133.0, 136.4, 150.7, 178.9. HRMS $C_{18}H_{32}NO_4$ calcd. 326.2331; found 326.2318. HPLC analysis (Agilent XDB-C18), gradient elution, MeCN, $H_2O$ (1% TFA), 1 ml/min, 40 °C, $t_r$ = 18.75 min.

**References:**

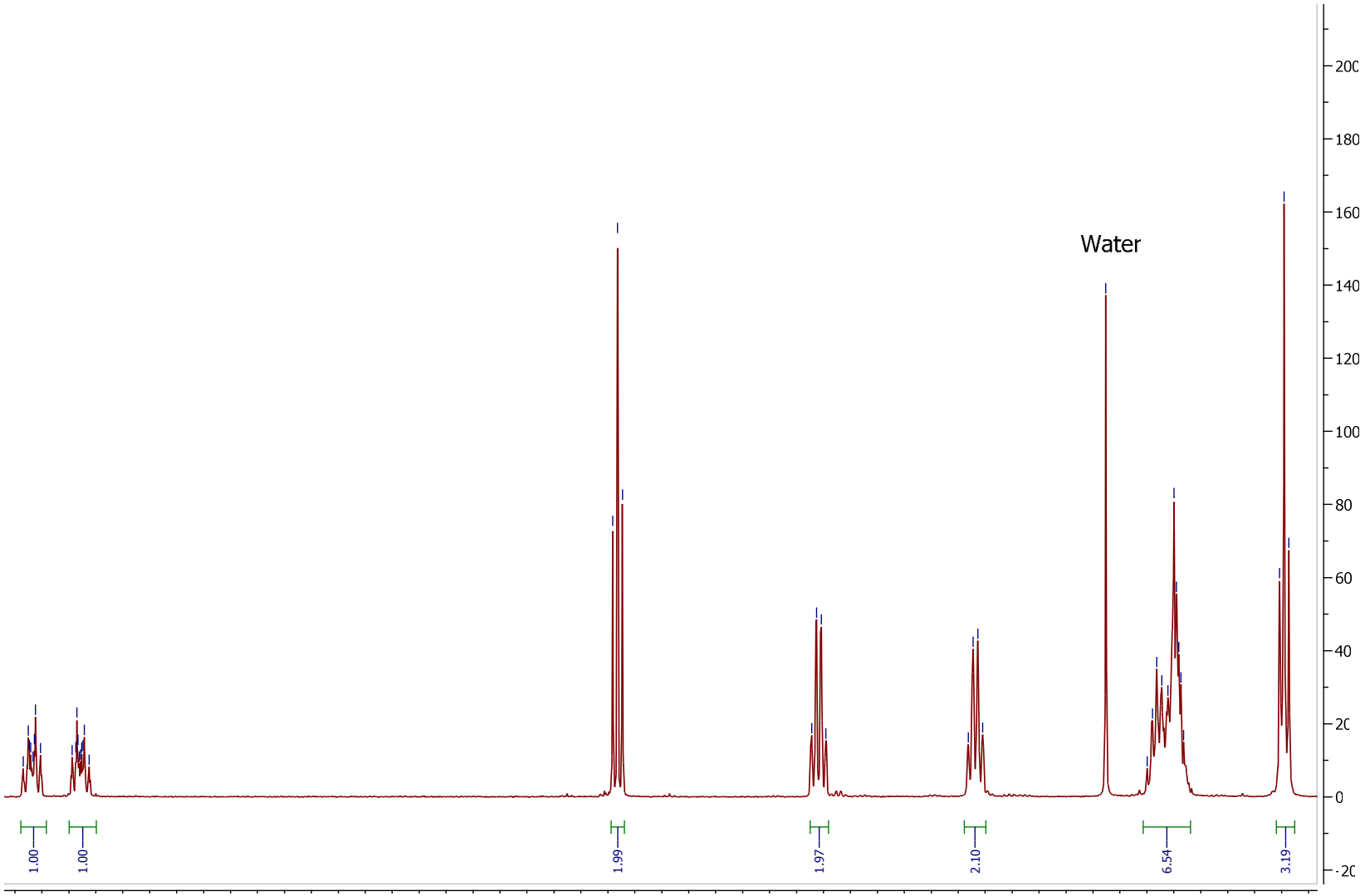

1. Pommier, A.; Pons, J. M.; Kocieński, P. J. *J. Org. Chem*. **1995**, *60*, 7334.

2. Gorczynski, M. J.; Huang, J.; King, S. B. *Org. Lett*. **2006**, *8*, 2305.

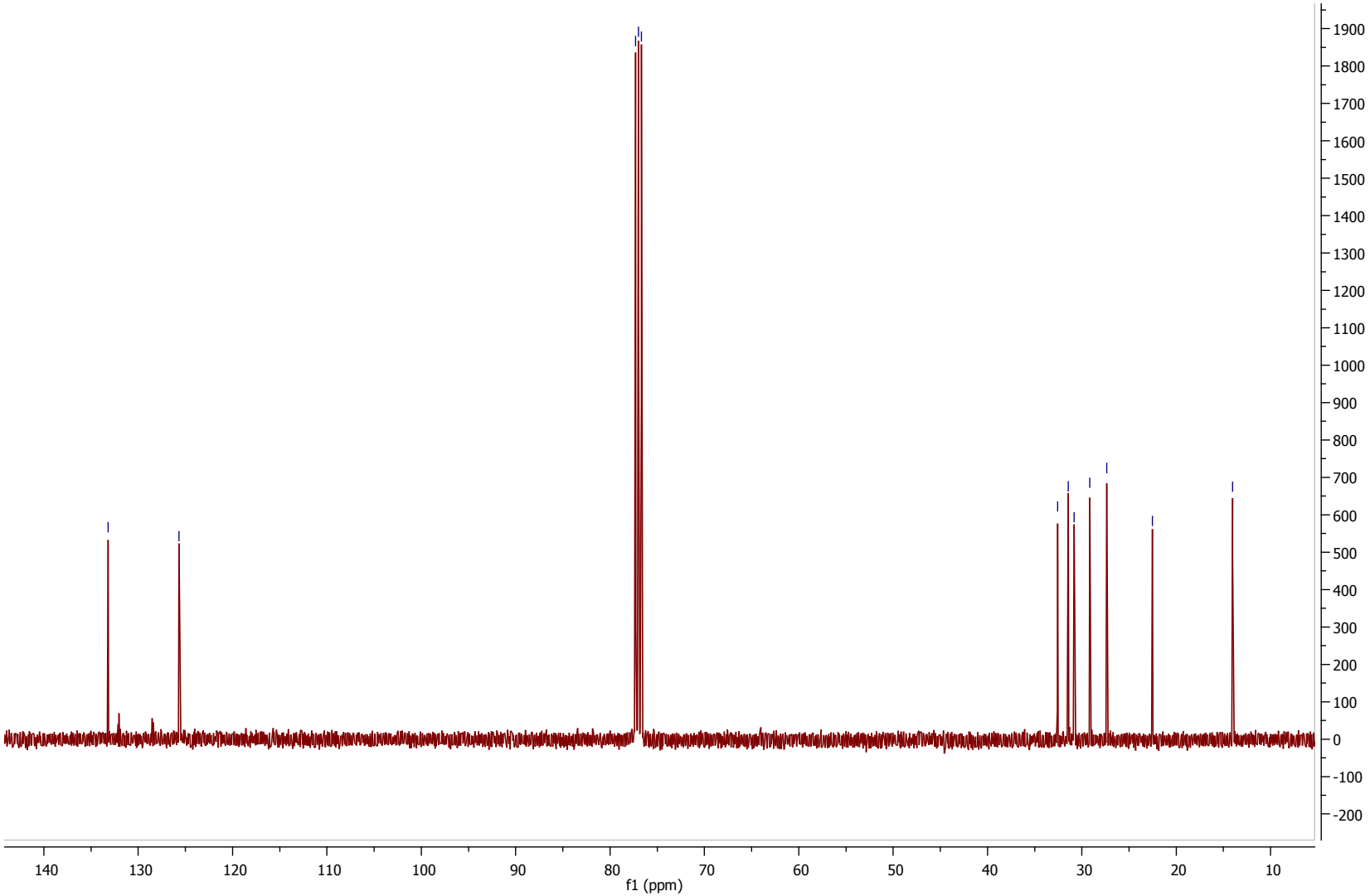
3. Manini, P.; Capelli, L.; Reale, S.; Arzillo, M.; Crescenzi, O.; Napolitano, A.; Barone, V.; d'Ischia, M. *J. Org. Chem*. **2008**, *73*, 7517

**Proton and carbon NMR spectra:**

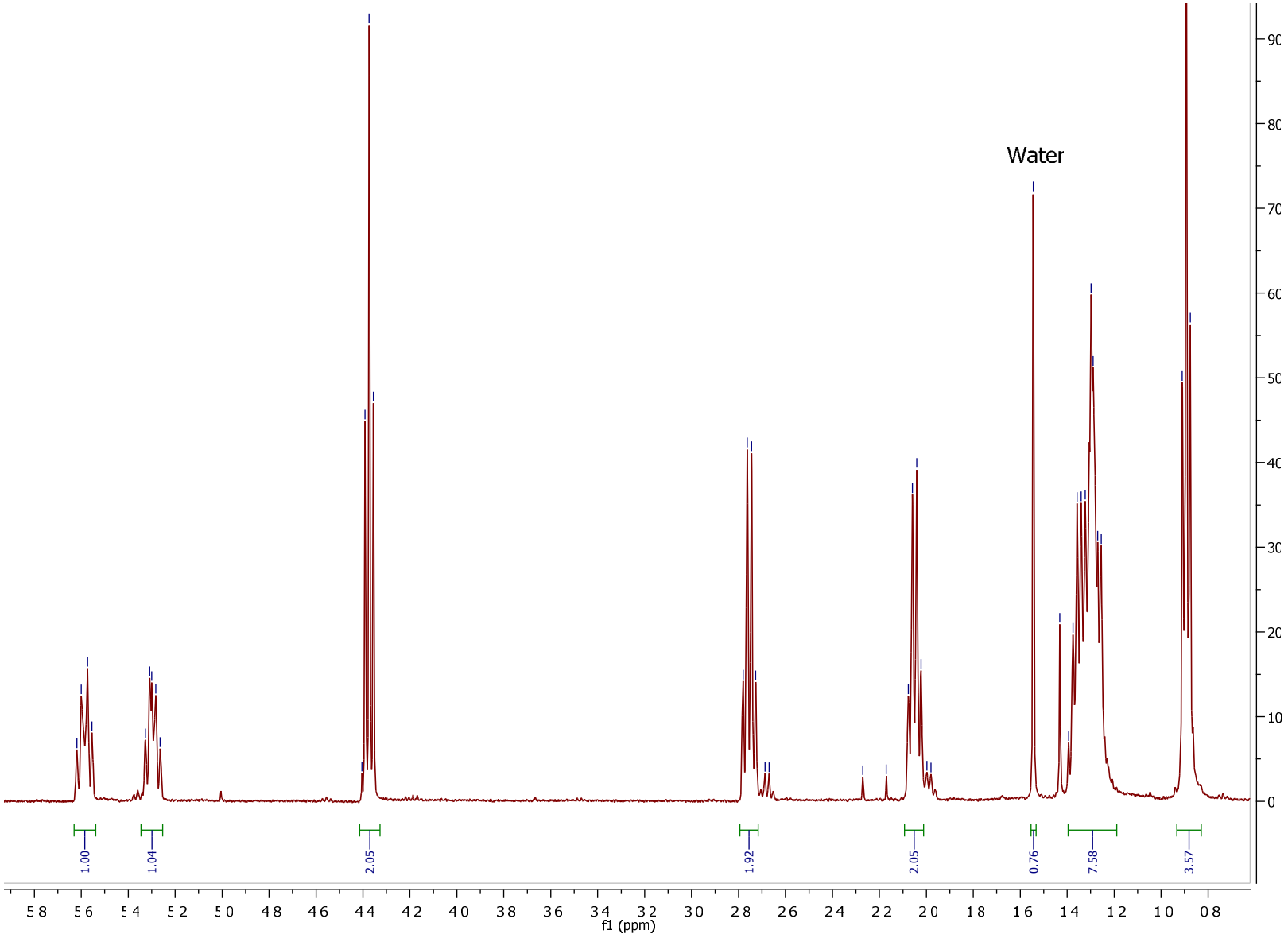
(*Z*)-1-Bromonon-3-ene, **SI_1** (400 MHz, $CDCl_3$):



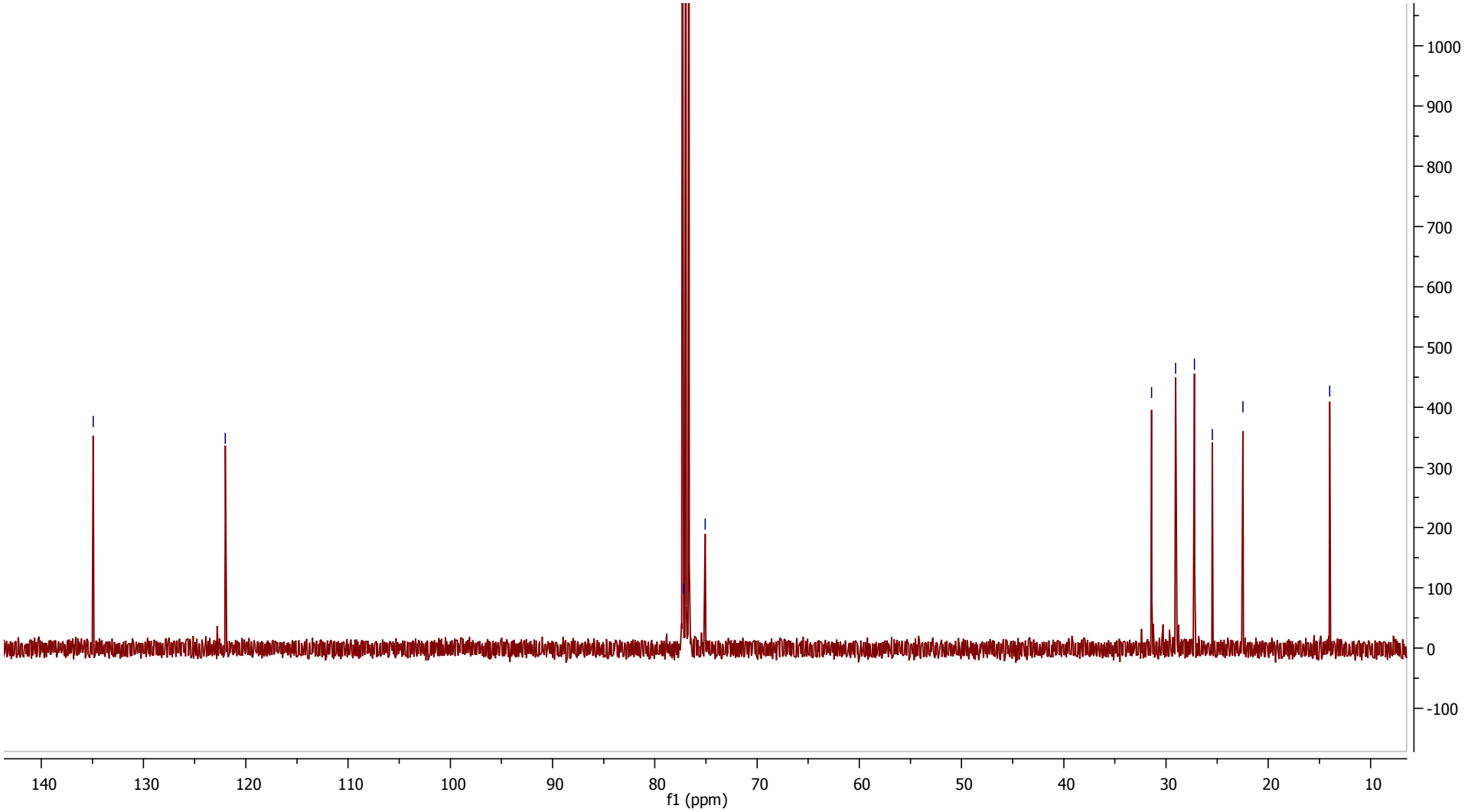
(*Z*)-1-Bromonon-3-ene, **SI_1** (100 MHz, $CDCl_3$):



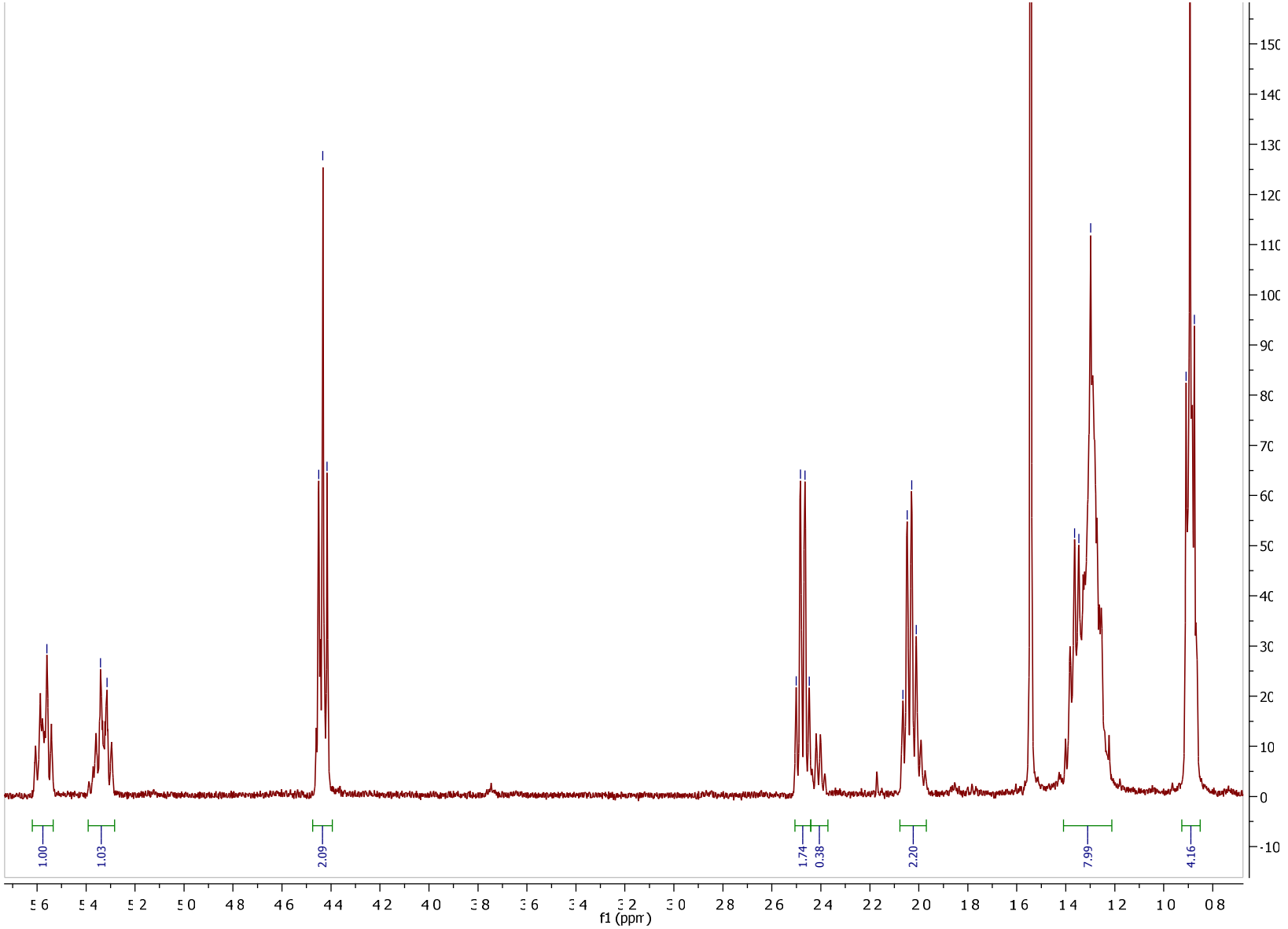
(*Z*)-1-Nitronon-3-ene, **4** (400 MHz, $CDCl_3$):



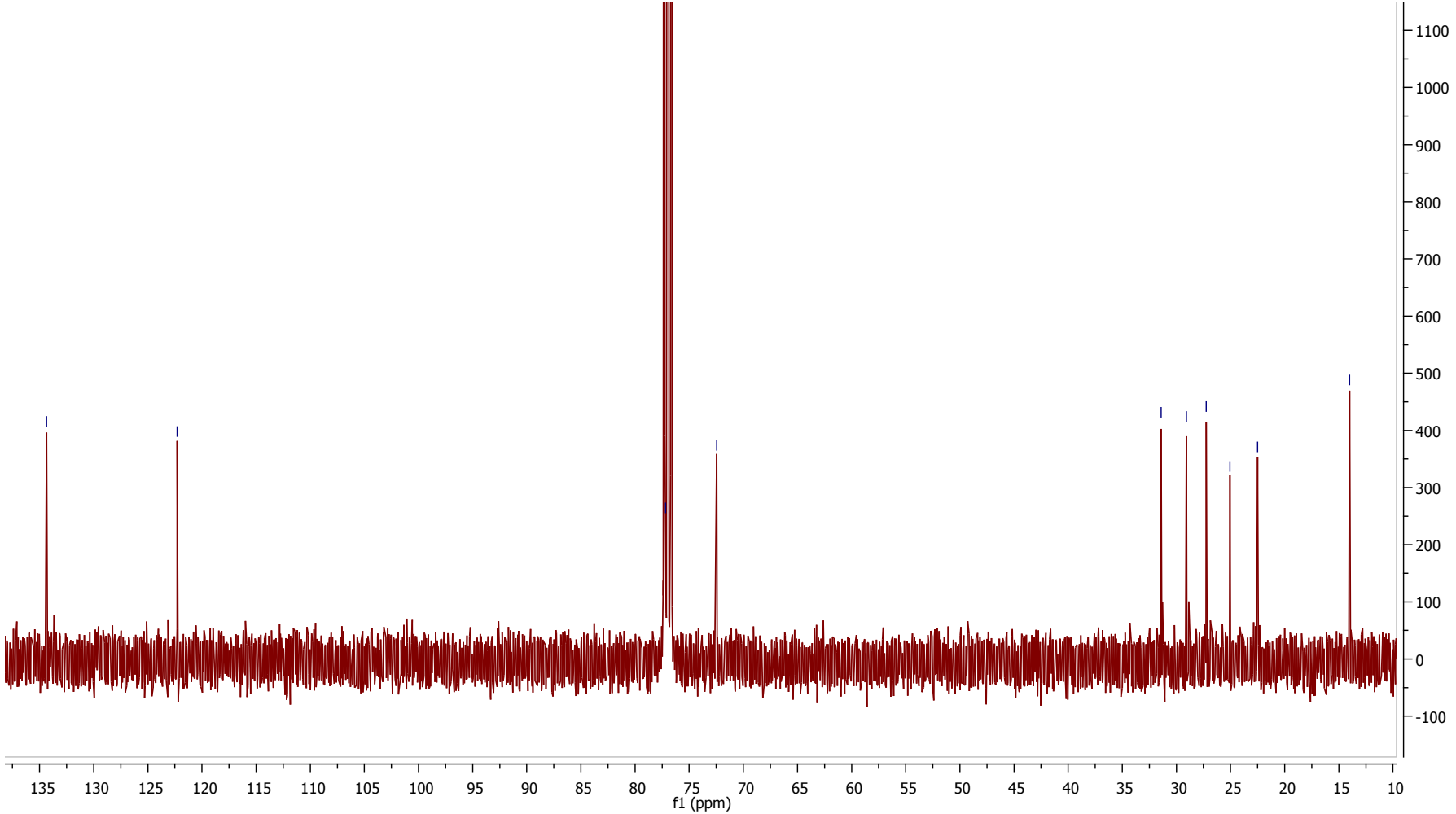
(*Z*)-1-Nitronon-3-ene, **4** (100 MHz, $CDCl_3$):



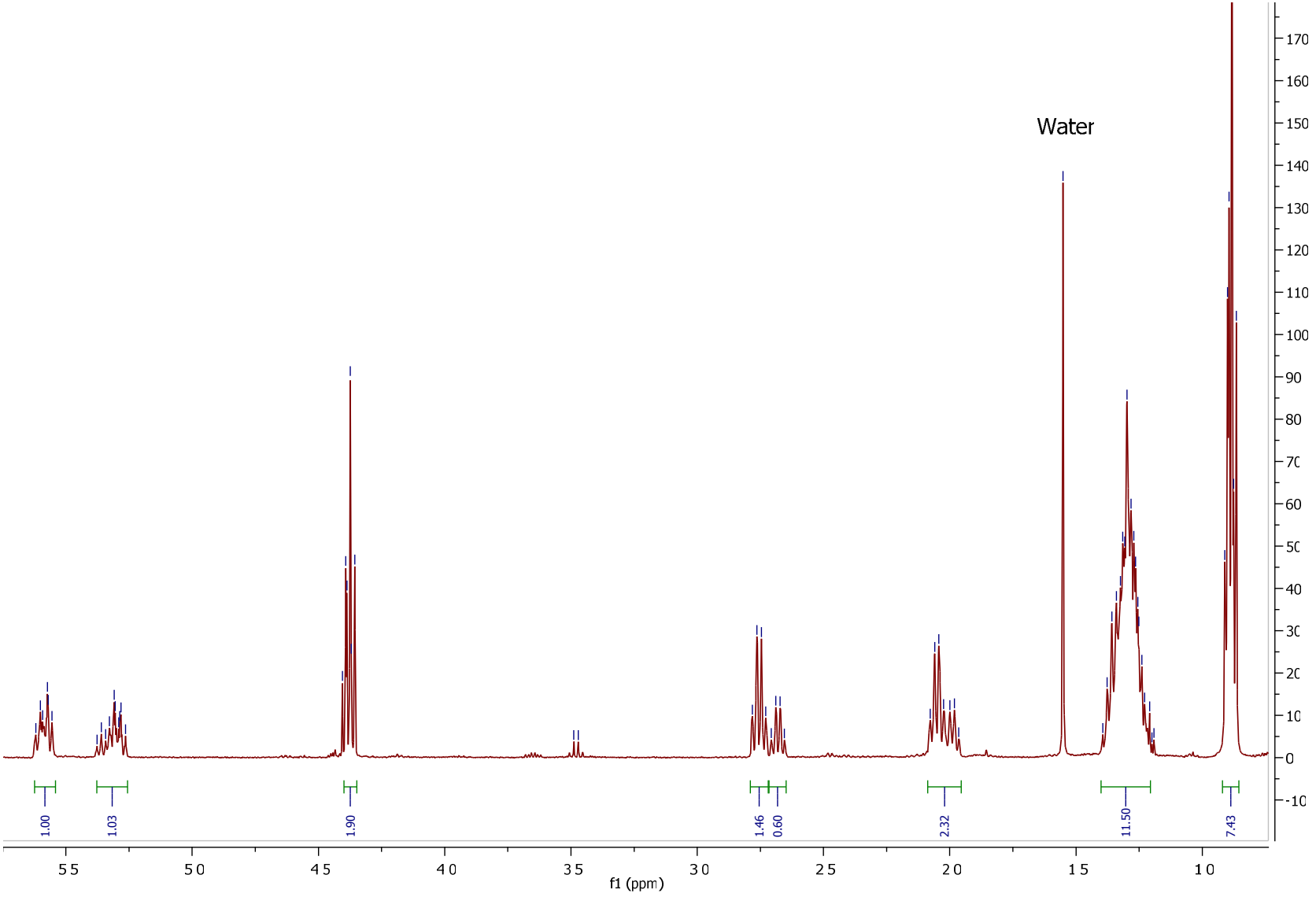
(*Z*)-Non-3-enyl nitrite, **6** (400 MHz, $CDCl_3$):



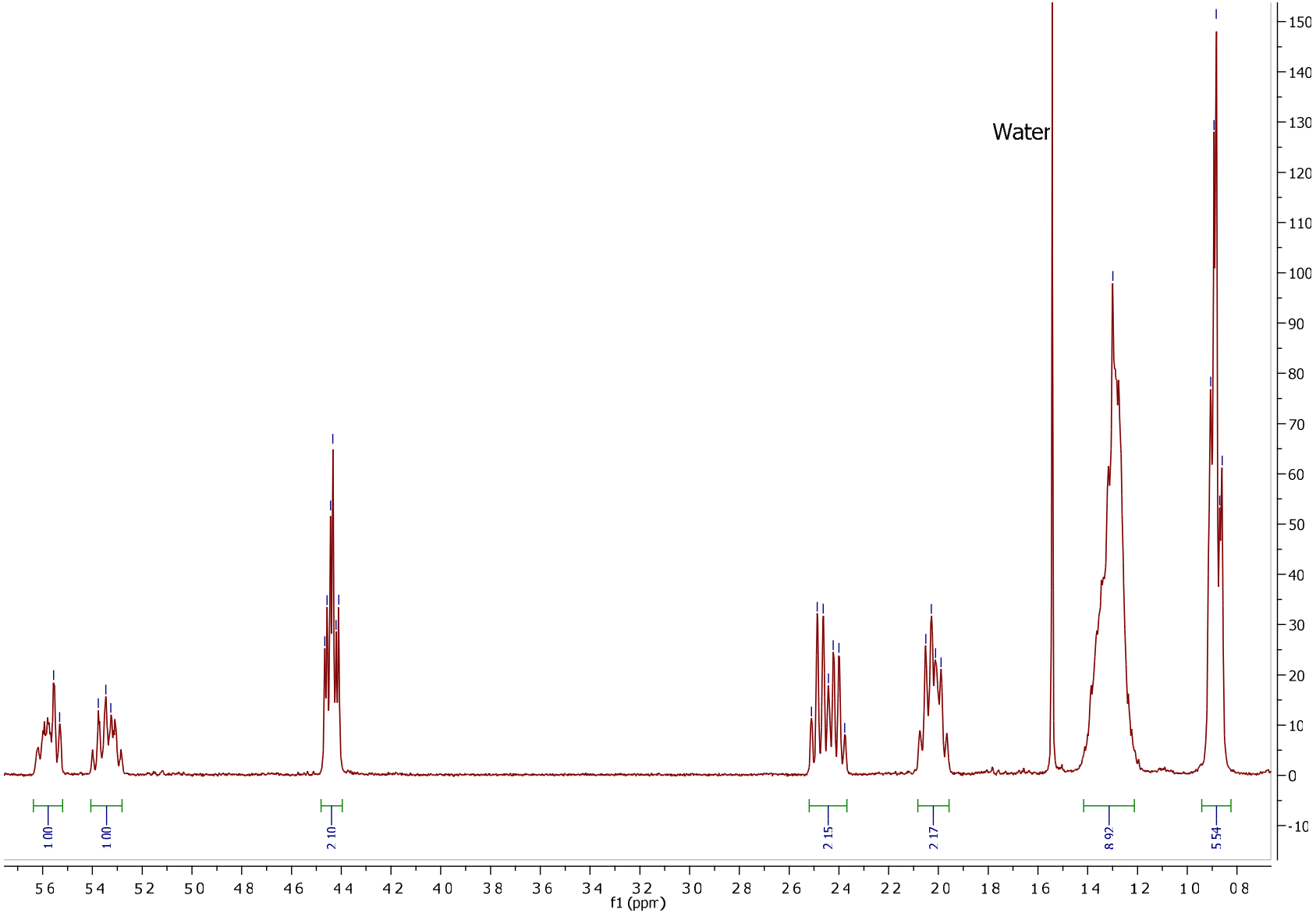
(*Z*)-Non-3-enyl nitrite, **6** (100 MHz, $CDCl_3$):



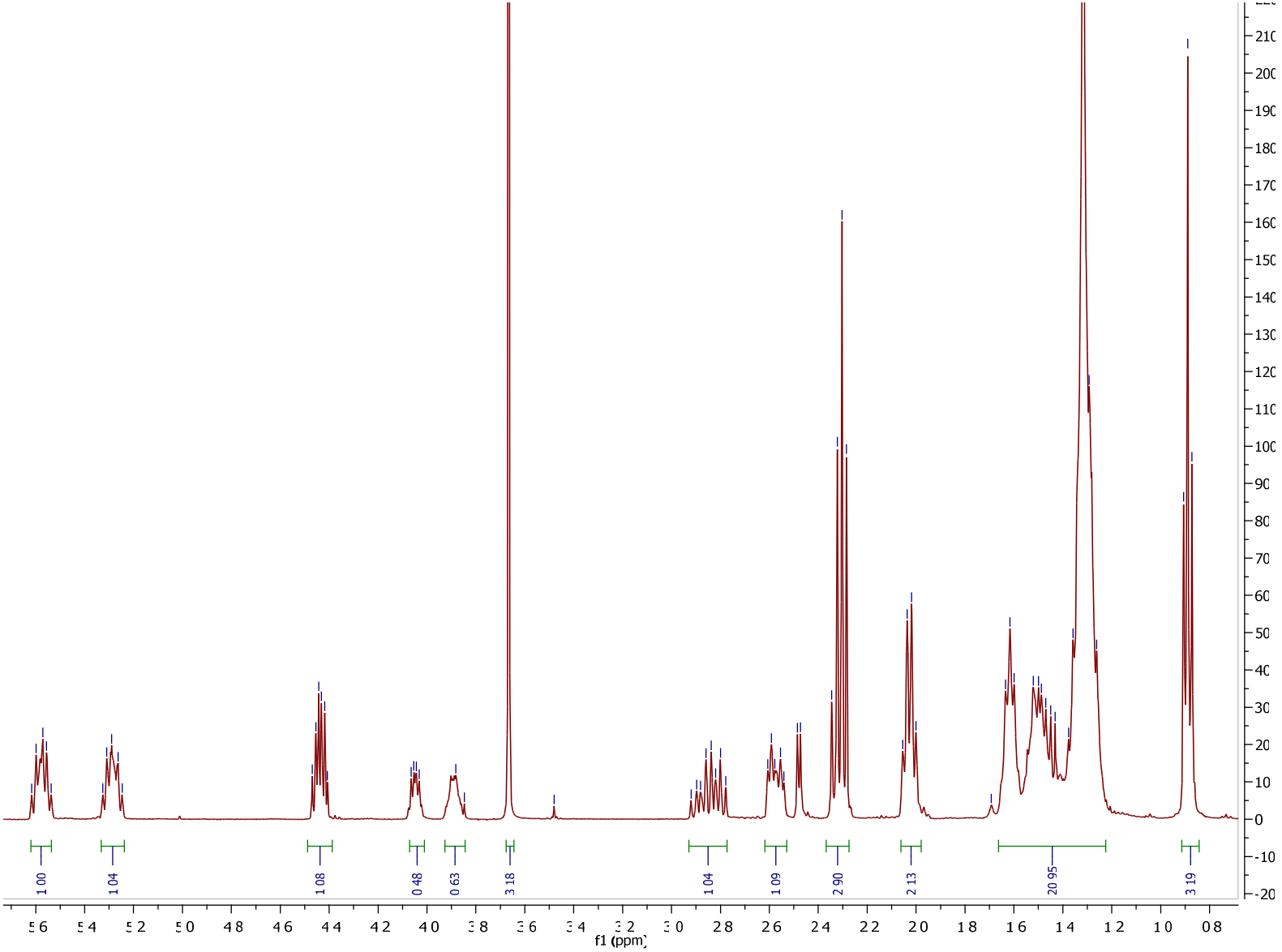
Mixture of (*E*) and (*Z*)-1-Nitronon-3-ene, **4** (300 MHz, $CDCl_3$):



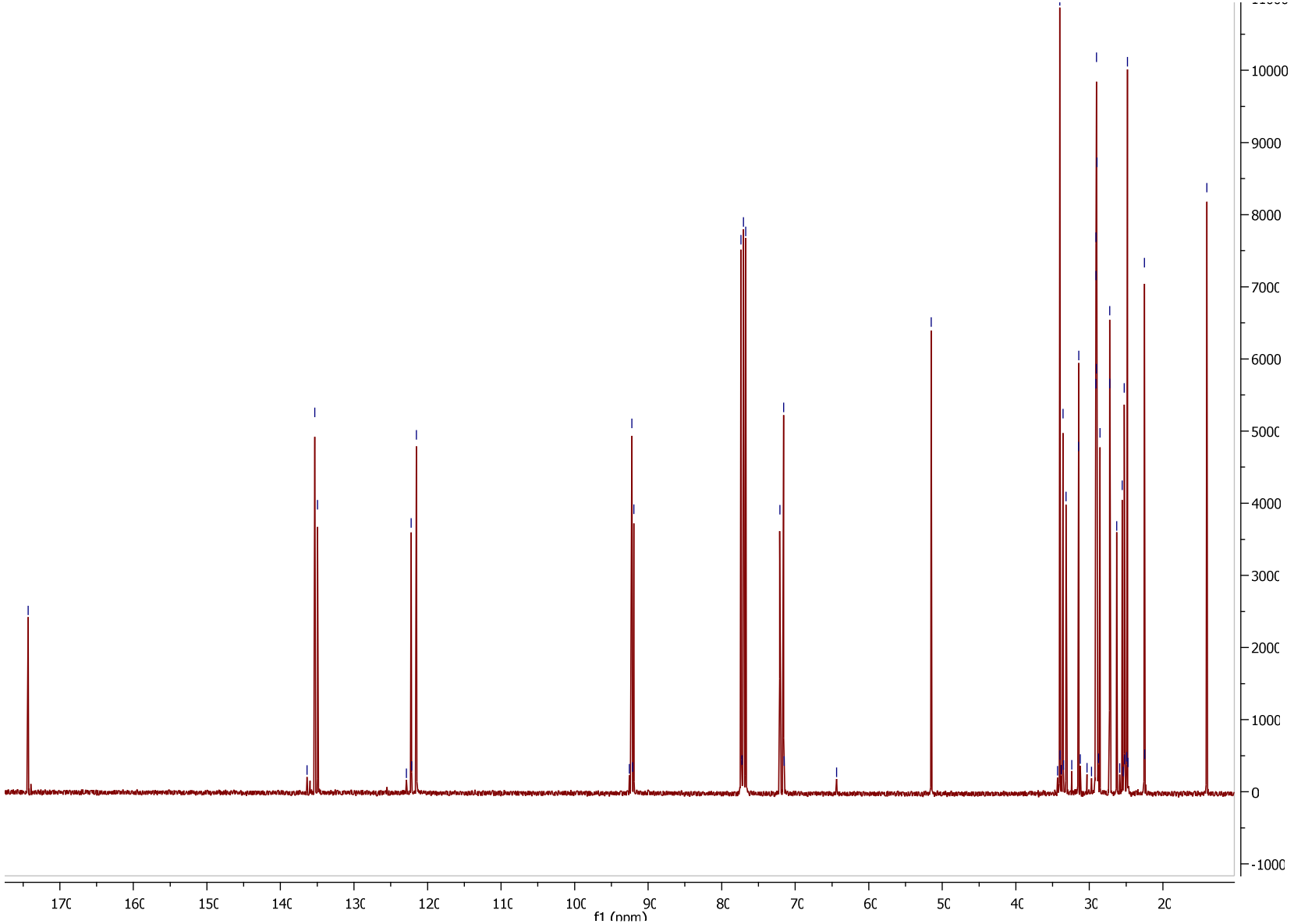
Mixture of (*E*) and (*Z*)-Non-3-enyl nitrite, **6** (300 MHz, $CDCl_3$):



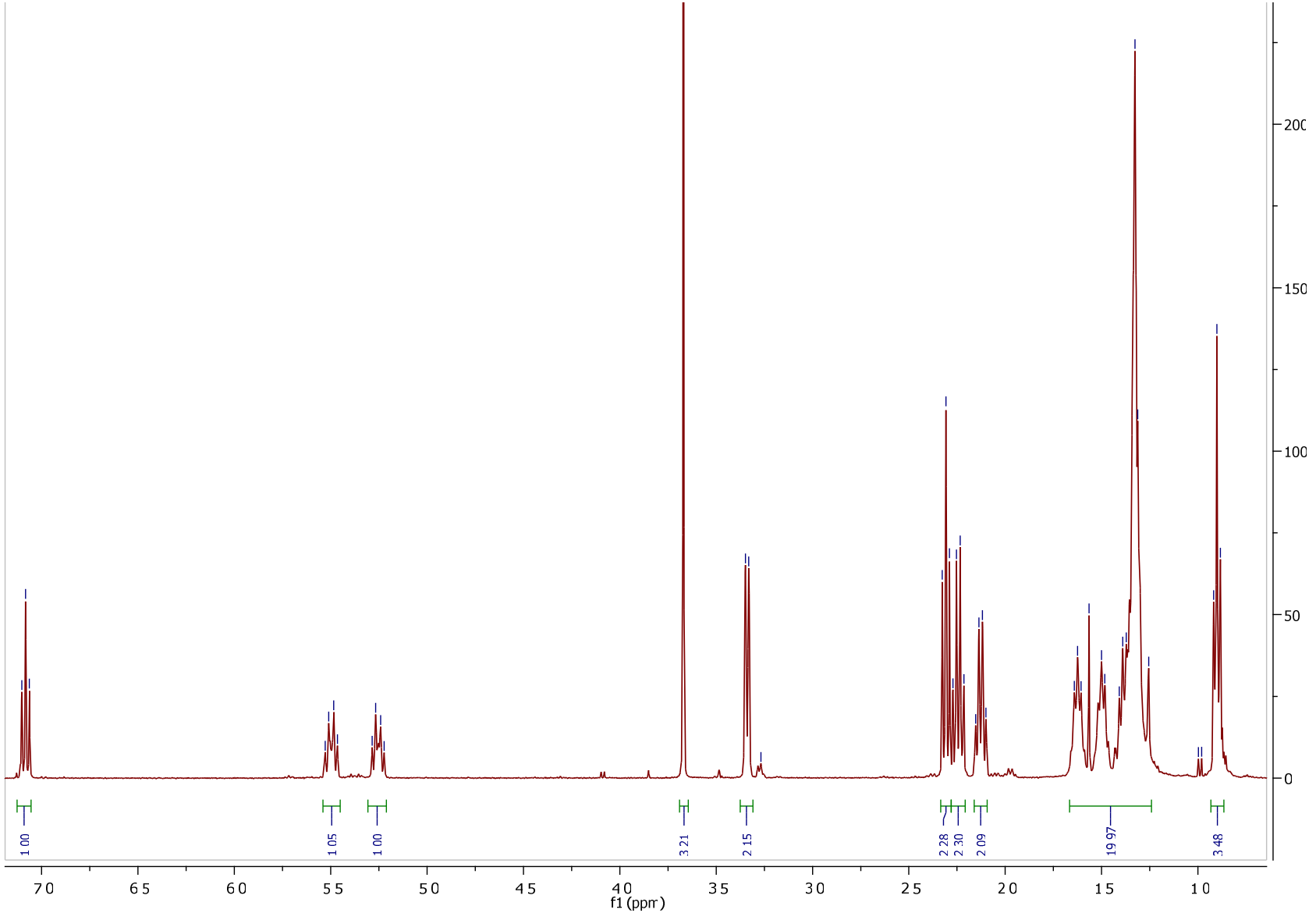
(*Z*)-Methyl 9-hydroxy-10-nitrooctadec-12-enoate, **8** (400 MHz, $CDCl_3$):



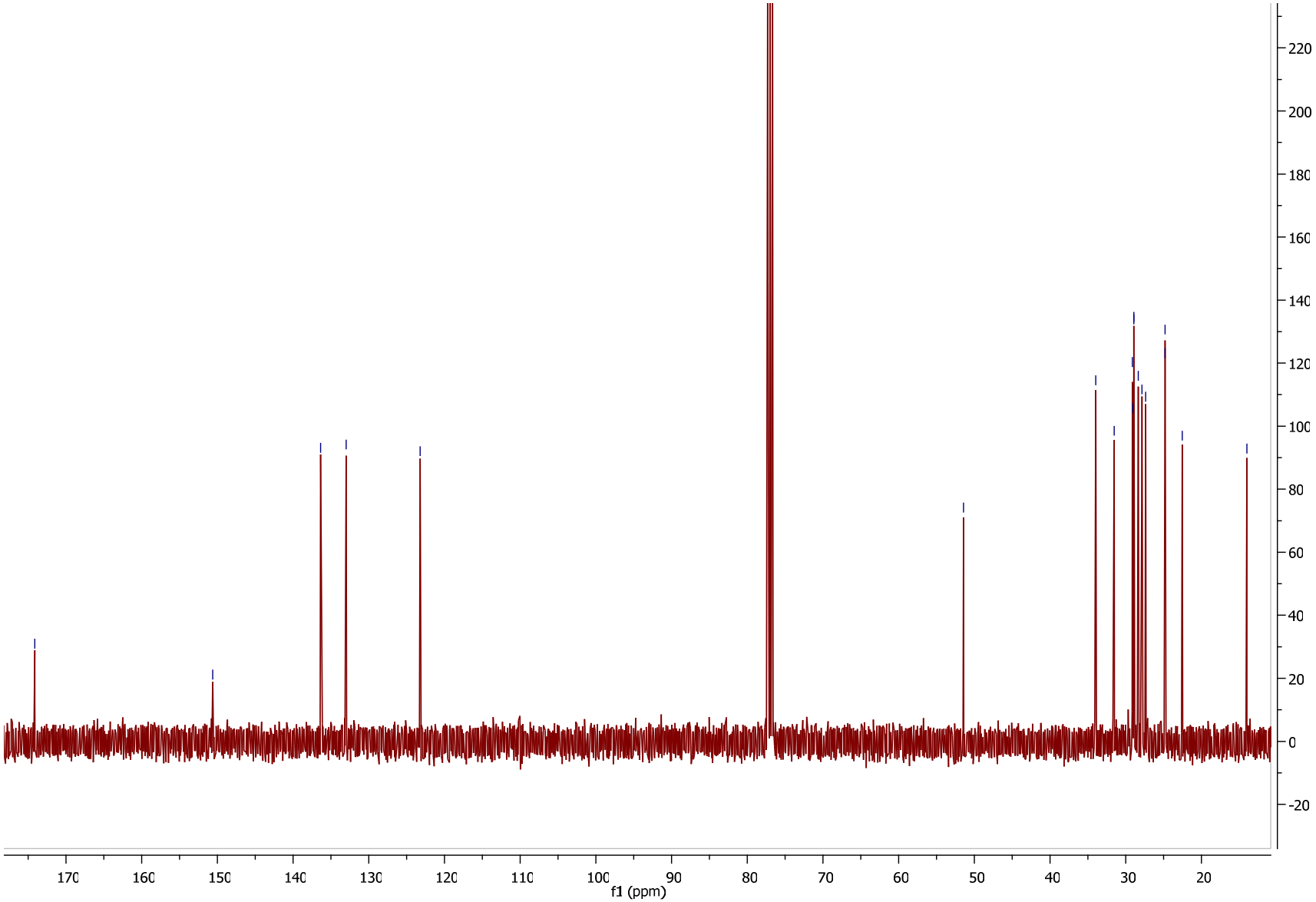
(*Z*)-Methyl 9-hydroxy-10-nitrooctadec-12-enoate, **8** (100 MHz, $CDCl_3$):



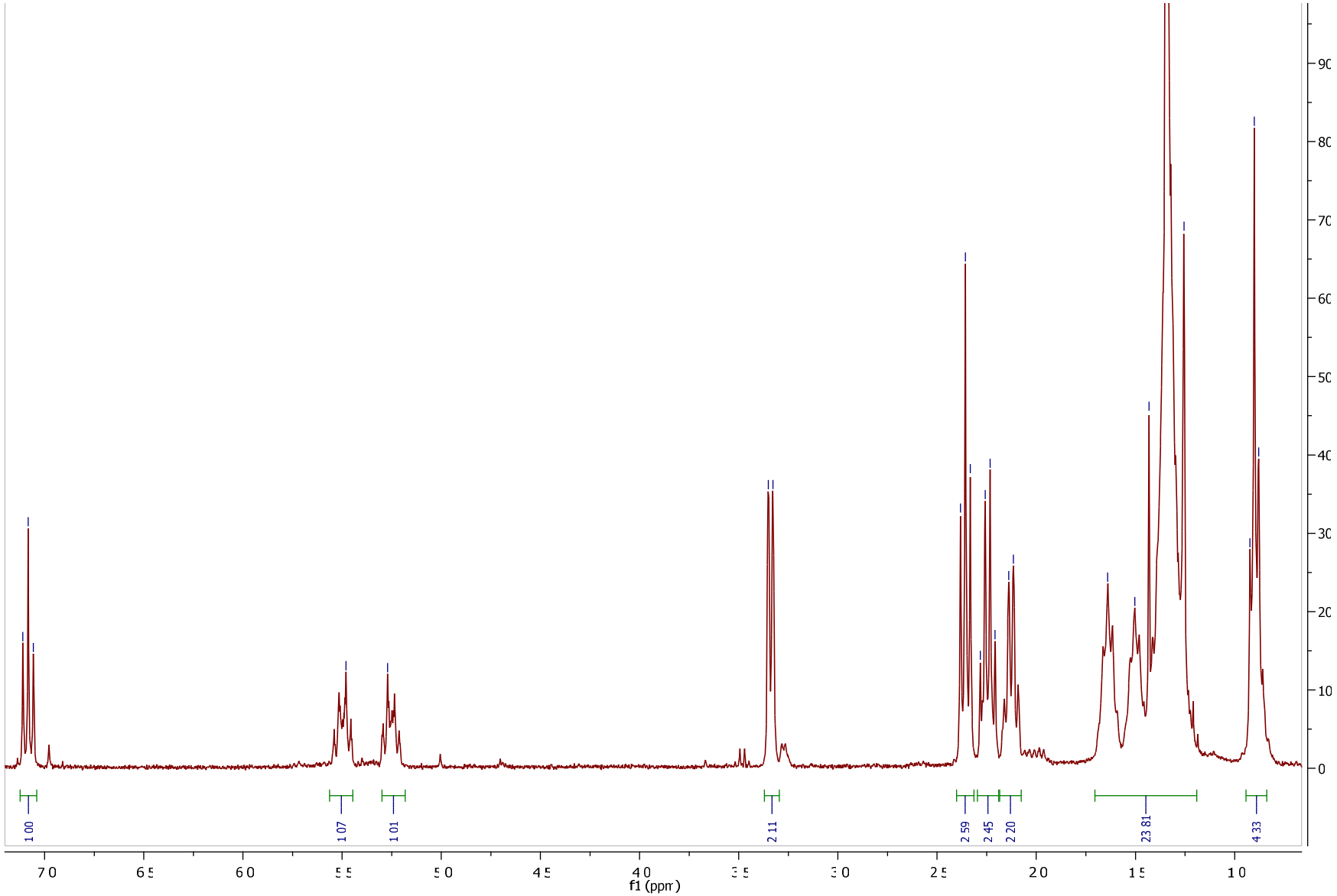
(9*E*,12*Z*)-Methyl 10-nitrooctadeca-9,12-dienoate, **9** (400 MHz, $CDCl_3$):



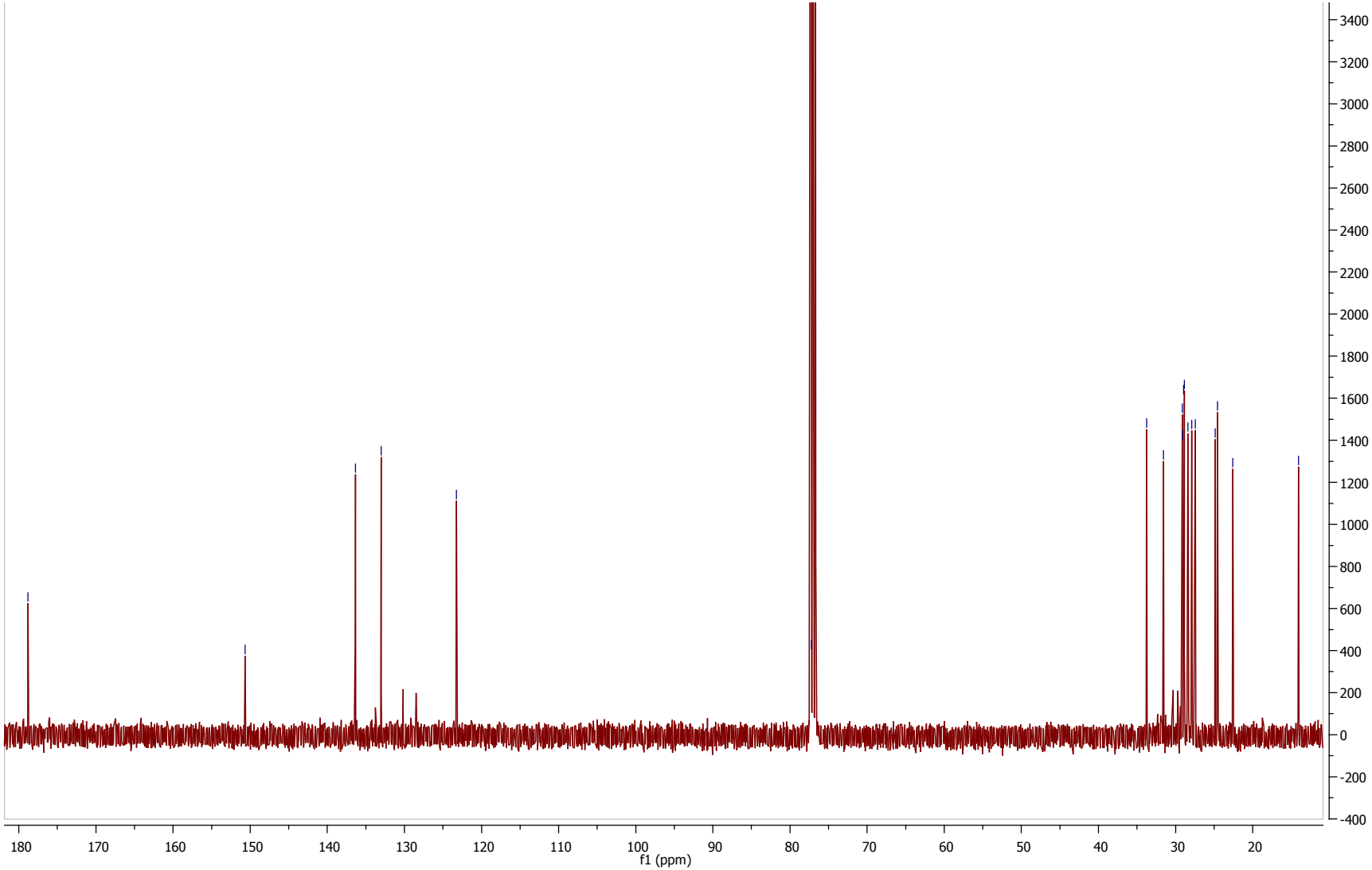
(9*E*,12*Z*)-Methyl 10-nitrooctadeca-9,12-dienoate, **9** (100 MHz, $CDCl_3$):



(9*E*,12*Z*)-10-Nitrooctadeca-9,12-dienoic acid, **2a** (400 MHz, $CDCl_3$):



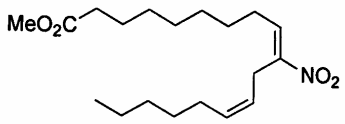
(9*E*,12*Z*)-10-Nitrooctadeca-9,12-dienoic acid, **2a** (100 MHz, $CDCl_3$):

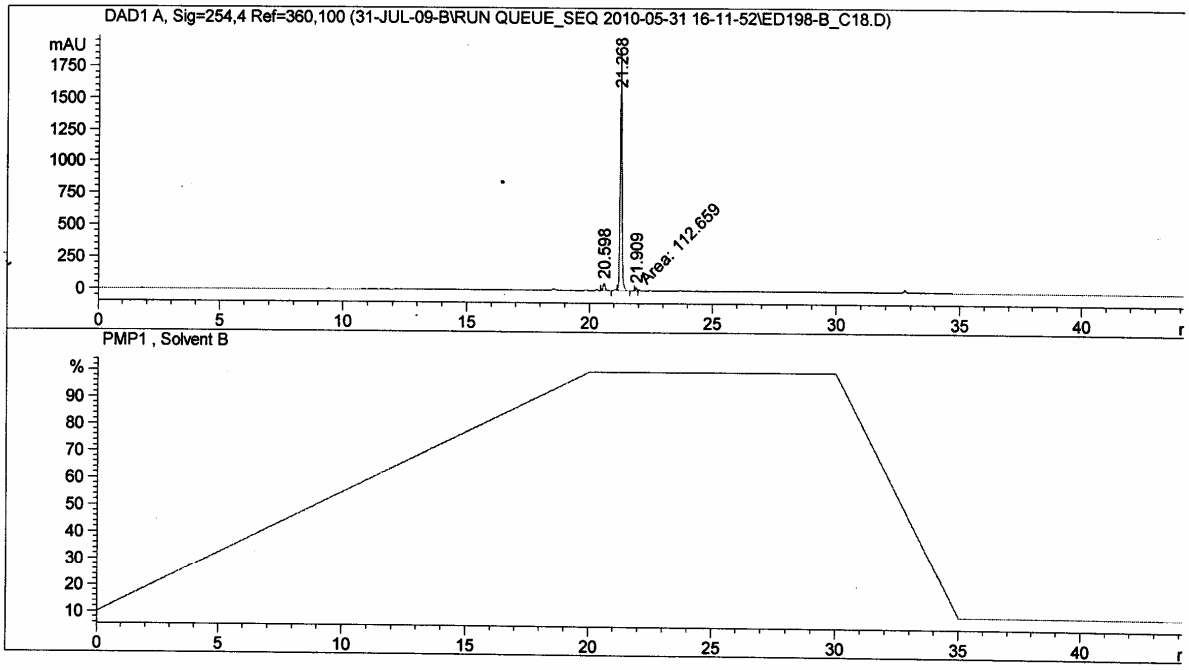


## HPLC traces:

(9*E*,12*Z*)-Methyl 10-nitrooctadeca-9,12-dienoate, **9**

```
==========================================================================
Acq. Operator   : Kevin conboy                    Seq. Line :   2
Acq. Instrument : HPLC-2                          Location : Vial 41
Injection Date  : 5/31/2010 4:58:34 PM                Inj :   1
                                                  Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\31-JUL-09-B\RUN QUEUE_SEQ 2010-05-31 16-11-52\
                  LINOLEICACIDMETHYLACID.M
Last changed    : 5/31/2010 12:23:43 PM by Kevin conboy
Analysis Method : C:\CHEM32\1\METHODS\04_PE GROUP\LINOLEICACIDMETHYLACID.M
Last changed    : 5/31/2010 12:23:43 PM by Kevin conboy
Method Info     : Analysis name:
                  Column: XDB-C18
                  Gradient run in water (A)/MeCN(B)
                  Run time: 45 min
                  Flow: 1 ml/min
                  Temperature: 40
```



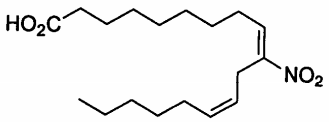



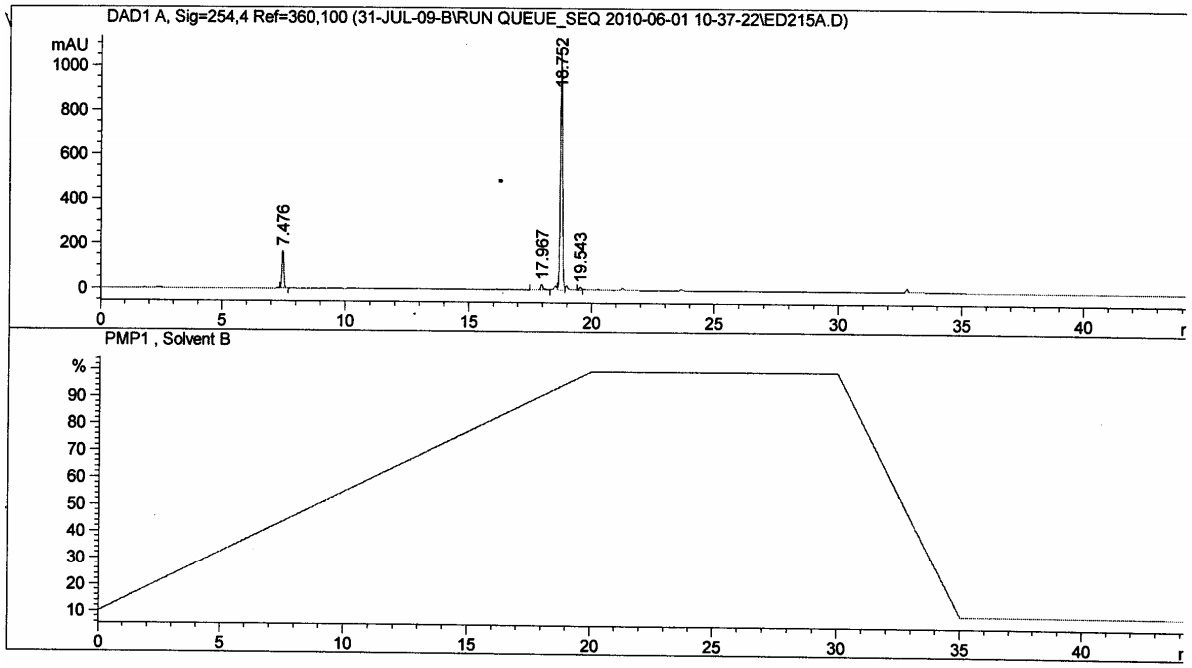

```
==========================================================================
                        Area Percent Report
==========================================================================
```

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 20.598 | VV | 0.0909 | 345.12186 | 56.81932 | 3.2359 |
| 2 | 21.268 | VB | 0.0850 | 1.02076e4 | 1889.87036 | 95.7078 |
| 3 | 21.909 | MM | 0.0824 | 112.65858 | 22.77433 | 1.0563 |
| Totals : | | | | 1.06654e4 | 1969.46401 | |

(9*E*,12*Z*)-10-Nitrooctadeca-9,12-dienoic acid, **2a**

```
================================================================================
Acq. Operator   : Kevin conboy                    Seq. Line :   3
Acq. Instrument : HPLC-2                          Location : Vial 44
Injection Date  : 6/1/2010 12:10:31 PM                 Inj :   1
                                                  Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\31-JUL-09-B\RUN QUEUE_SEQ 2010-06-01 10-37-22\
                  LINOLEICACIDMETHYLACID.M
Last changed    : 5/31/2010 12:23:43 PM by Kevin conboy
Analysis Method : C:\CHEM32\1\METHODS\04_PE GROUP\LINOLEICACIDMETHYLACID.M
Last changed    : 5/31/2010 12:23:43 PM by Kevin conboy
Method Info     : Analysis name:
                  Column: XDB-C18
                  Gradient run in water (A)/MeCN(B)
                  Run time: 45 min
                  Flow: 1 ml/min
                  Temperature: 40
```




```
================================================================================
                          Area Percent Report
================================================================================
```

| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 7.476 | VB | 0.0823 | 904.44275 | 169.30902 | 11.7579 |
| 2 | 17.967 | BV | 0.1102 | 183.17776 | 24.77938 | 2.3813 |
| 3 | 18.752 | VV | 0.0945 | 6522.71631 | 1078.65308 | 84.7965 |
| 4 | 19.543 | VV | 0.0924 | 81.86695 | 13.56055 | 1.0643 |
| Totals : | | | | 7692.20377 | 1286.30203 | |

## PPAR-γ ligand binding assay and $IC_{50}$ calculation:

Experimental conditions

| Assay | Origin | Ligand | Conc. | $K_d$ | Non-specific ligand | Incubation | Detection |
|---|---|---|---|---|---|---|---|
| PPAR-γ (*h*) (agonist radio-ligand) | Human recombinant (*E. coli*) | [$^3$H]rosi-glitazone | 5 nM | 5.7 nM | Rosiglitazone (10 μM) | 120 min, 4 °C | Scintillation counting |

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand.

The results are expressed as a percent of control specific binding [(measured specific binding/control specific binding) X 100] obtained in the presence of **2a** and **9**.

The $IC_{50}$ value (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficient (nH) were determined by non-linear regression analysis of the competition curve generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{nH})]$, where Y = specific binding, D = minimum specific binding, A = maximum specific binding, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.).

The inhibition constant ($K_i$) was calculated using the Cheng Prusoff equation ($K_i = IC_{50}/(1+(L/K_d))$, where L = concentration of radioligand in the assay, and $K_d$ = affinity of the radioligand for the receptor). A Scatchard plot is used to determine the Kd.

**Reference:** Ferry, G.; Bruneau, V.; Beauverger, P.; Goussard, M.; Rodgiguez, M.; Lamamy, V.; Dromaint, S.; Canet, E.; Galizzi, J. –P.; Boutin, J. A., *Eur. J. Pharmacol.* **2001**, *417*, 77.

| Compound 9 | | | |
|---|---|---|---|
| **Test concentration** | **% Control specific binding** | | |
| | 1st | 2nd | **Mean** |
| 5.0 X $10^{-9}$ M | 116.6 | 98. 6 | **107.6** |
| 10.0 X $10^{-9}$ M | 106.9 | 89.2 | **98.0** |
| 50.0 X $10^{-9}$ M | 79.7 | 77.0 | **78.4** |
| 0.1 X $10^{-6}$ M | 59.3 | 23.2* | **59.3** |
| 0.5 X $10^{-6}$ M | 12.9 | 6.6 | **9.7** |
| 1.0 X $10^{-6}$ M | 8.6 | 14.3 | **11.5** |
| 5.0 X $10^{-6}$ M | 2.6 | 1.9 | **2.2** |
| 10.0 X $10^{-6}$ M | 4.9 | -1.0 | **1.9** |

*Result omitted

| Compound 2a | | | |
|---|---|---|---|
| **Test concentration** | **% Control specific binding** | | |
| | 1st | 2nd | **Mean** |
| 5.0 X $10^{-9}$ M | 109.8 | 108.4 | **109.1** |
| 10.0 X $10^{-9}$ M | 111.4 | 107.9 | **109.6** |
| 50.0 X $10^{-9}$ M | 93.4 | 85.4 | **89.4** |
| 0.1 X $10^{-6}$ M | 85.6 | 65.6 | **75.6** |
| 0.5 X $10^{-6}$ M | 43.5 | 33.5 | **38.5** |
| 1.0 X $10^{-6}$ M | 26.8 | 23.0 | **24.9** |
| 5.0 X $10^{-6}$ M | 8.0 | 4.2 | **6.1** |
| 10.0 X $10^{-6}$ M | 0.5 | 2.0 | **1.2** |

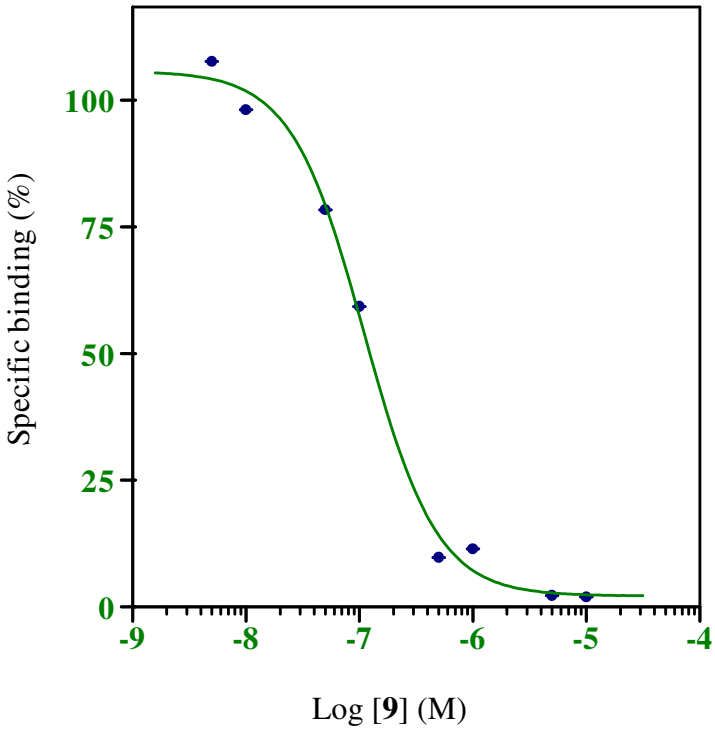
Summary and reference compound data:

| **Compound** | $IC_{50}$ | $K_i$ | nH |
|---|---|---|---|
| **9** | 1.1 X $10^{-7}$ M | 5.9 X 10-8 M | 1.3 |
| **2a** | 2.2 X $10^{-7}$ M | 1.2 X 10-7 M | 0.9 |

| | | | |
|---|---|---|---|
| Rosiglitazone | 1.1 X $10^{-8}$ M | 6.0 X $10^{-9}$ M | 0.9 |

Competition curve for **9**:

$IC_{50}$ = 1.1 X $10^{-7}$ M; nH = 1.3




Competition curve for **2a**:

$IC_{50}$ = 2.2 X $10^{-7}$ M; nH = 0.9