Electronic Supplementary Information

Investigating the generation of hydrogen sulfide from the slow-release phosphonamidodithioate donor GYY4137

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Literature reference numbers and compound numbers correspond to those in the original article.

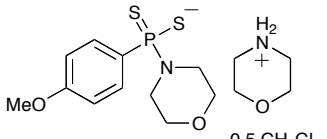
(i) Experimental procedures and characterisation data for the hydrolysis pathway and compounds prepared

General information

For general information regarding synthetic and analytical chemistry methods, see: M. E. Wood, S. Bissiriou, C. Lowe, A. M. Norrish, K. Sénéchal, K. M. Windeatt, S. J. Coles and M. B. Hursthouse, *Org. Biomol. Chem.* 2010, **8**, 4653.

In addition, ^{31}P NMR spectra were recorded at 121.5 MHz using a Bruker ACF300 spectrometer with external referencing.

Morpholinium 4-methoxyphenyl(morpholino)phosphinodithioate (GYY4137) 3[15]



3

In a typical preparation:

A solution of morpholine (1.75 ml, 20 mmol) in dichloromethane (6 ml) was added dropwise, to a stirred solution/suspension of Lawesson's reagent **4** (1.62 g, 4 mmol) in dichloromethane (6 ml) at 0 °C (ice-salt cooling bath). On completion of addition, the stirred reaction mixture was allowed to attain room temperature, with stirring being continued for 4 h. The precipitate formed was filtered off and washed with dichloromethane (3 x 2 ml) before drying *in vacuo* to give the *title compound* (1.87 g, 45% - corrected for CH_2Cl_2 content) as a white, crystalline solid as a 2 : 1 complex of **3** : CH_2Cl_2. Although a previously characterised literature compound,[15] NMR spectra were re-recorded, as a ^{1}H NMR signal originally reported as "2.04 to 2.09 (m, 4H, CH)," actually corresponds to d_5-acetone in the deuterated solvent and not a signal for GYY 4137 **3** itself.

δ_H (300.1 MHz; $(CD_3)_2CO$) 2.89 (4H, broad apparent q, J = 6.0 Hz, N(CH_2)$_2$ for morpholine attached to P), 3.36 (4H, m, morpholinium (CH_2)$_2$N), 3.54 (4H, broad t, J = 6.0 Hz, O(CH_2)$_2$ for morpholine attached to P), 3.82 (3H, s, CH_3O), 3.92 (4H, m, morpholinium (CH_2)$_2$O), 5.63 ("1H", s, 0.5 CH_2Cl_2), 6.89 (2H, dd, J = 3.0 and 9.0 Hz, arylC-*H o*-to OCH_3) and 8.06 (2H, dd, J = 6.0 and 9.0 Hz, arylC-*H o*-to P). Morpholinium $^+NH_2$ signal position unclear through line broadening.

δ_C (75.5 MHz; $(CD_3)_2CO$) 44.5 (morpholinium (CH_2)$_2$N), 46.7 (d, J = 2.3 Hz, N(CH_2)$_2$ for morpholine attached to P), 54.9 (CH_2Cl_2), 56.4 (CH_3O), 65.6 (morpholinium O(CH_2)$_2$), 68.6 (d, J = 11.3 Hz, O(CH_2)$_2$ for morpholine attached to P), 113.9 (d, J = 13.6 Hz, aryl*C*-H *o*-to OCH_3), 134.5 (d, J = 12.1 Hz, aryl*C*-H *o*-to C-P) and 162.5 (aryl*C*-OCH_3). Aryl*C*-P signal not visible.

δ_P (121.5 MHz; $(CD_3)_2CO$) 90.4.

m/z (ES$^-$) 290.0257 (10%), 289.0315 (12) and 288.0283 ([M-morpholinium]$^-$, 100) and 218.9708 (3). ($C_{11}H_{15}NO_2PS_2^-$ requires 288.0287.)

Crystal structure determination for GYY4137 **3**:

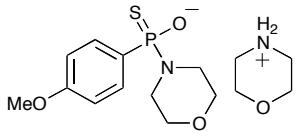
Suitable, dichloromethane-free crystals were prepared by slow diffusion of petroleum ether (b.p. 40-60 °C) into a dilute solution of GYY4137 dichloromethane complex **3** (above) in chloroform.

Table S1. Crystal data and structure refinement details for GYY4137 **3** (CCDC 1053548).

Empirical formula	$C_{15}H_{25}N_2O_3PS_2$	
Formula weight	376.46	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	*C*2/*c*	
Unit cell dimensions	a = 24.2051(3) Å	α = 90°
	b = 8.9799(2) Å	β = 101.4120(10)°
	c = 17.2946(3) Å	γ = 90°
Volume	3684.82(11) Å^3	
Z	8	
Density (calculated)	1.357 Mg / m^3	
Absorption coefficient	0.391 mm^{-1}	
F(000)	1600	
Crystal	Block; colourless	
Crystal size	0.20 × 0.12 × 0.08 mm^3	
θ range for data collection	3.22 – 27.47°	
Index ranges	$-31 \le h \le 31, -11 \le k \le 11, -22 \le l \le 22$	
Reflections collected	26236	
Independent reflections	4230 [R_{int} = 0.0416]	
Completeness to θ = 27.47°	99.7 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	0.9694 and 0.9260	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4230 / 0 / 217	
Goodness-of-fit on F^2	1.065	
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0329, $wR2$ = 0.0761	
R indices (all data)	$R1$ = 0.0386, $wR2$ = 0.0804	
Largest diff. peak and hole	0.301 and –0.274 e Å^{-3}	

Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit* sphere). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: *SADABS* (Sheldrick, G. M. (2007). SADABS. Version 2007/2. Bruker AXS Inc., Madison, Wisconsin, USA.). **Structure solution**: *SHELXS97* (Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.) **Structure refinement**: *SHELXL97* (G Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.). **Graphics:** *OLEX2* (Dolomanov, O. V., Bourhis, L. J., Gildea, R. J., Howard, J. A. K. & Puschmann, H. (2009). J. Appl. Cryst. 42, 339-341.)

Morpholinium 4-methoxyphenyl(morpholino)phosphinothioate 5



5

GYY4137 **3** (22.9 mg, 0.06 mmol) was dissolved in deuteriochloroform (0.6 ml), which had been stored in moist air and the solution was transferred into and sealed in a 5 mm internal diameter NMR tube, in which the sample depth was 5 cm. The sample was stored at room temperature and ^{31}P and ^{1}H NMR spectra were recorded at daily intervals, with additional deuteriochloroform being added at regular intervals in order to maintain the same sample depth. After 71 days, the chloroform was evaporated *in vacuo* to give the *title compound* as a straw-coloured syrup.

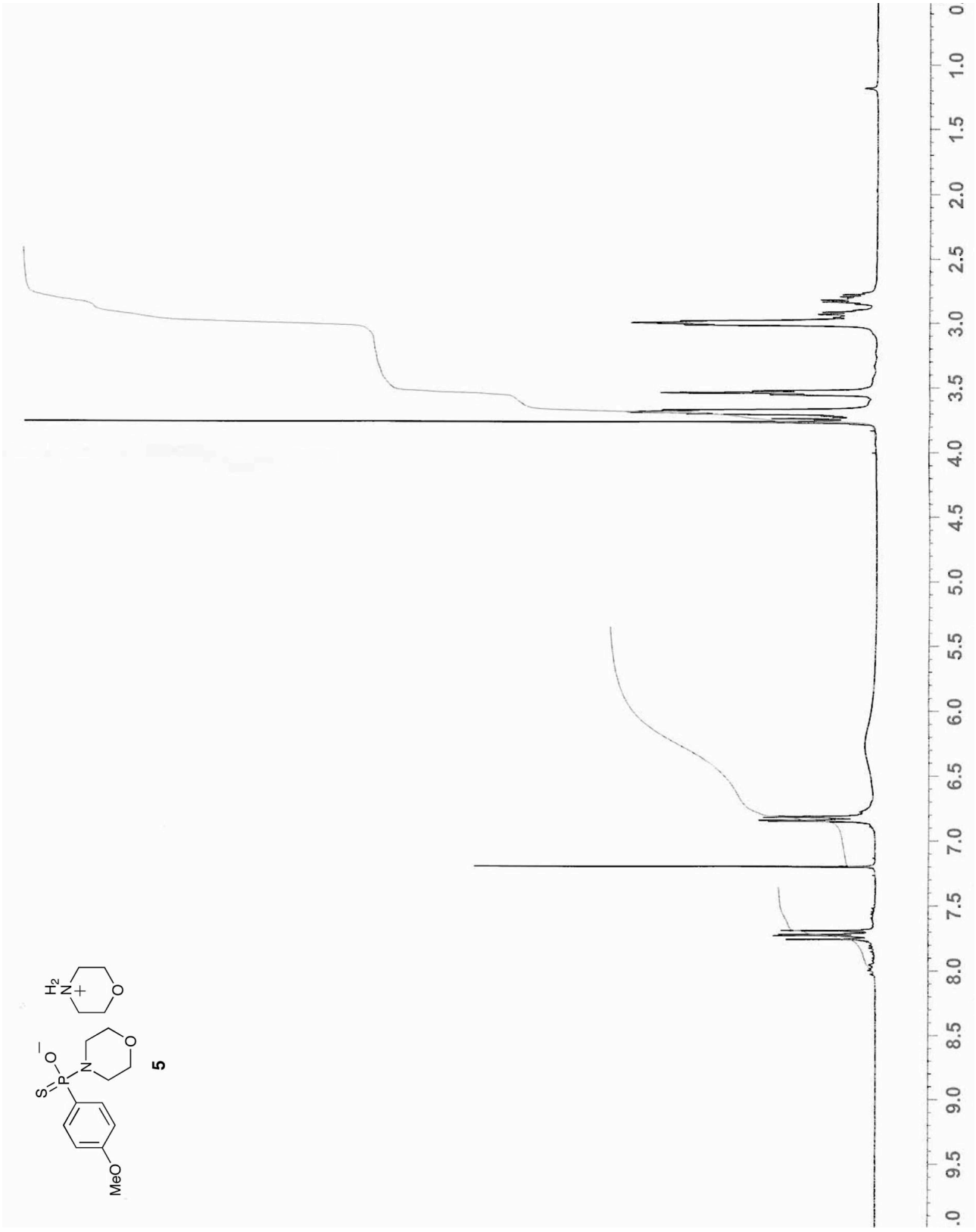
δ_H (300.1 MHz; $CDCl_3$) 2.69-2.78 and 2.83-2.92 (2 x 2H, 2 x m, $N(CH_2)_2$ for morpholine attached to P), 2.93 (4H, broad t, J = 4.5 Hz, morpholinium $(CH_2)_2N$), 3.47 (4H, t, J = 4.5 Hz, $O(CH_2)_2$ for morpholine attached to P), 3.62 (4H, broad t, J = 4.5 Hz, morpholinium $(CH_2)_2O$), 3.70 (3H, s, CH_3O), 6.08 (2H, broad s, morpholinium $^{+}NH_2$), 6.73 (2H, dd, J = 3.0 and 6.0 Hz, arylC-*H o*-to OCH_3) and 7.65 (2h, dd, J = 6.0 and 7.5 Hz, arylC-*H o*-to P).

δ_C (75.5 MHz; $CDCl_3$) 43.5 (morpholinium $(CH_2)_2N$), 45.2 ($N(CH_2)_2$ for morpholine attached to P), 55.3 (CH_3O), 64.4 (morpholinium $O(CH_2)_2$), 67.2 (d, J = 9.1 Hz, $O(CH_2)_2$ for morpholine attached to P), 113.2 (d, J = 13.6 Hz, aryl*C*-H *o*-to OCH_3), 132.5 (d, J = 11.3 Hz, aryl*C*-H *o*-to C-P) and 161.2 (aryl*C*-OCH_3). Aryl*C*-P signal not visible.

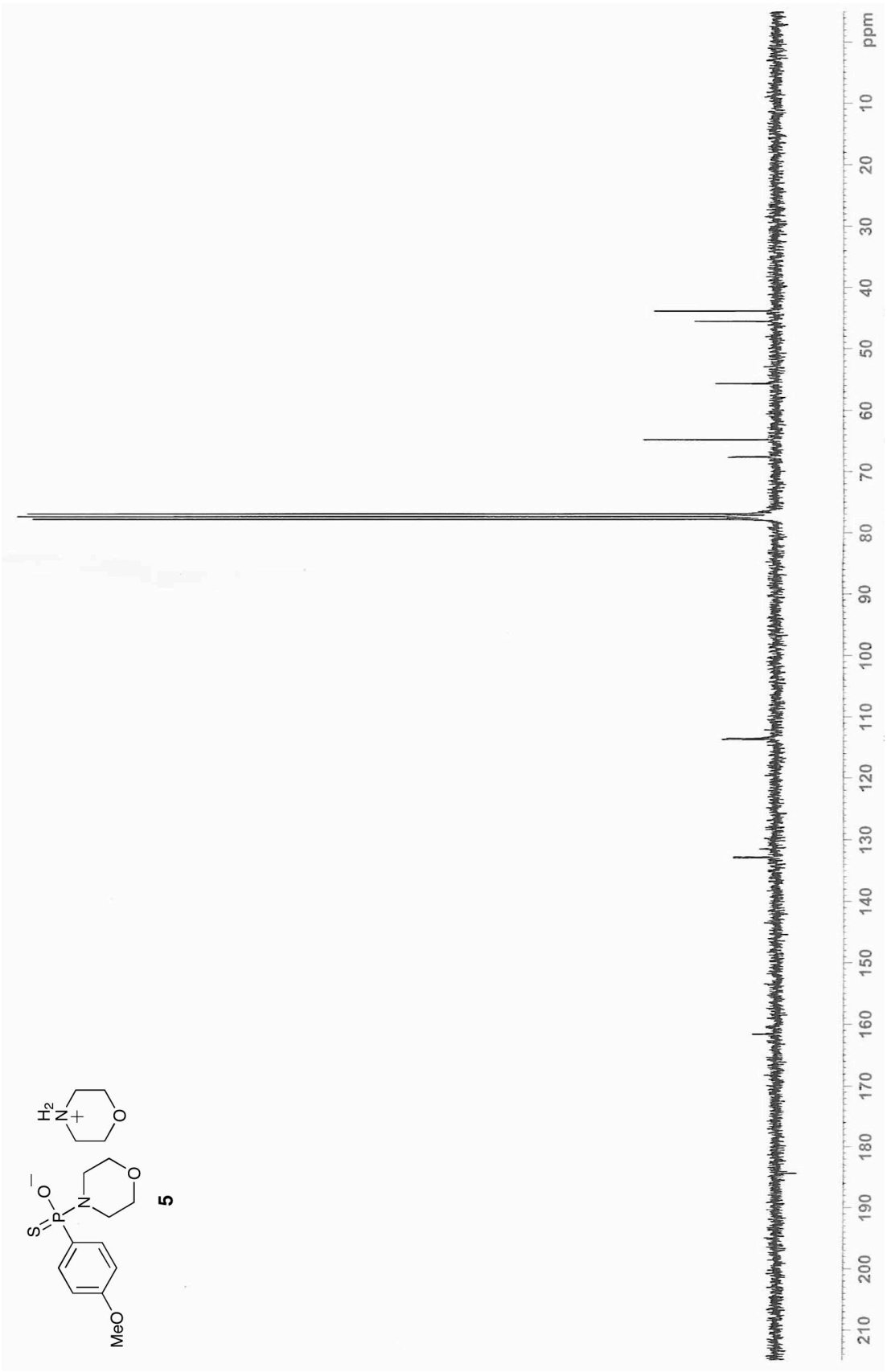
δ_P (121.5 MHz; $CDCl_3$) 64.9.

m/z (ES^-) 545.1081 ($[2M-2morpholinium+H]^-$, 20%) $C_{22}H_{31}N_2O_6P_2S_2^-$ requires 545.1110) and 272.0509 ($[M-morpholinium]^-$, 100). ($C_{22}H_{31}N_2O_6P_2S_2^-$ requires 545.1110 and $C_{11}H_{15}NO_3PS^-$ requires 272.0516.)

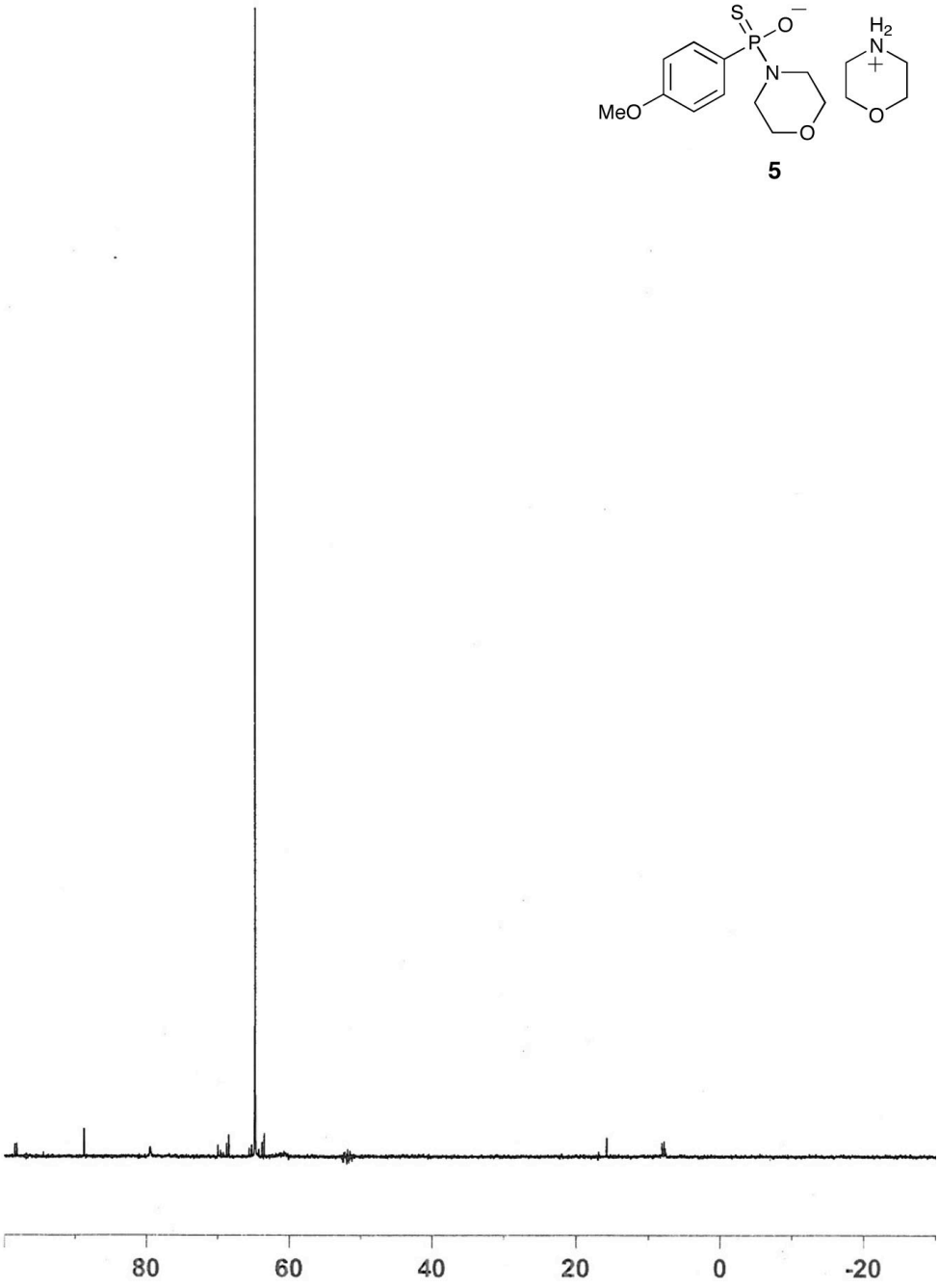
Morpholinium 4-methoxyphenyl(morpholino)phosphinothioate **5** ^{1}H NMR Spectrum (300.1 MHz; $CDCl_3$)



Morpholinium 4-methoxyphenyl(morpholino)phosphinothioate **5** ^{13}C NMR (75.5 MHz; $CDCl_3$)

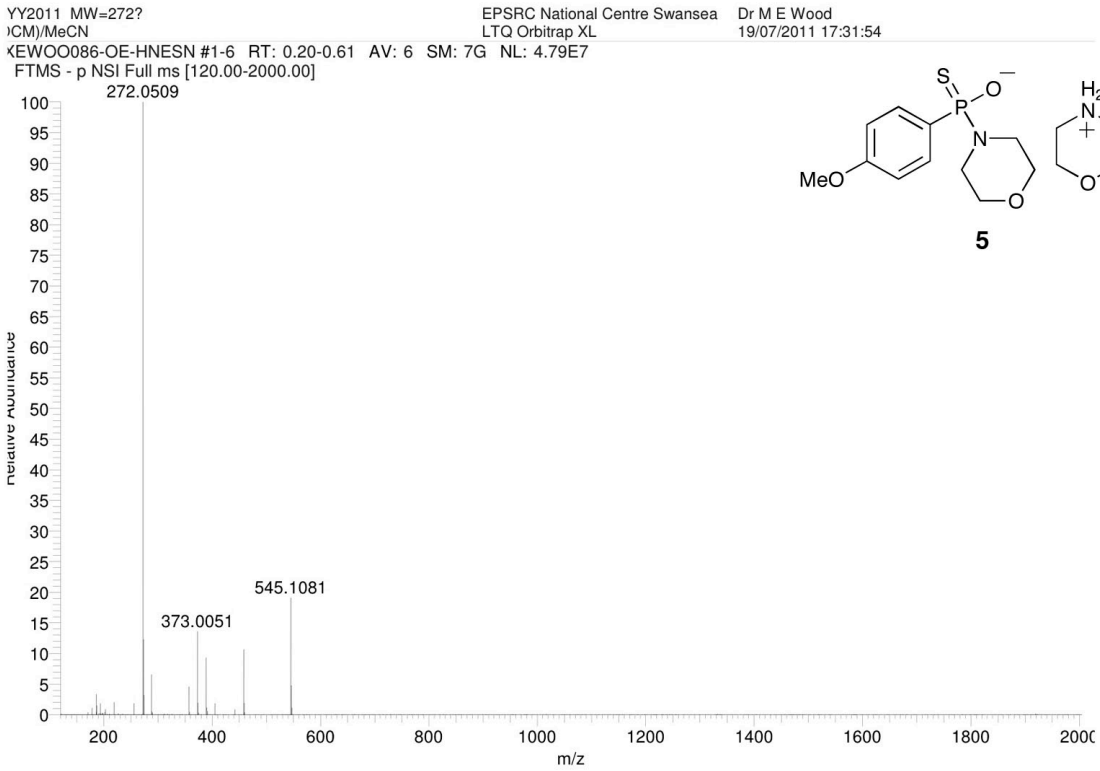


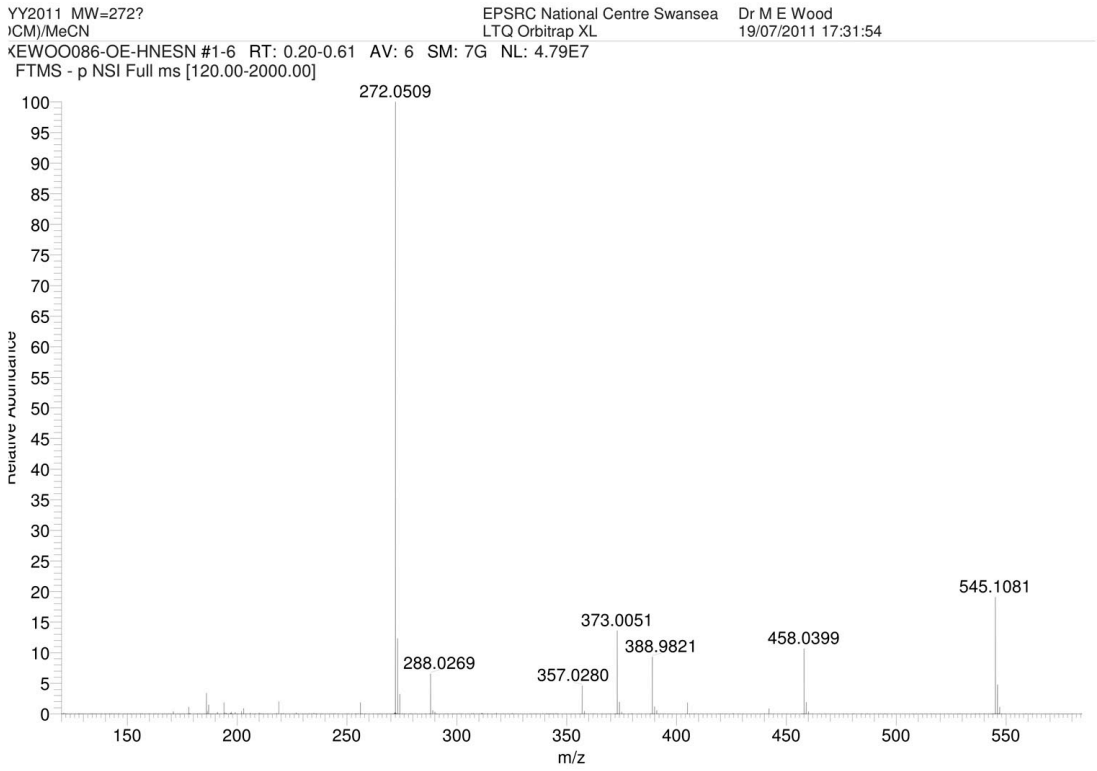
Morpholinium 4-methoxyphenyl(morpholino)phosphinothioate **5** ^{31}P NMR Spectrum (121.5 MHz; $CDCl_3$)

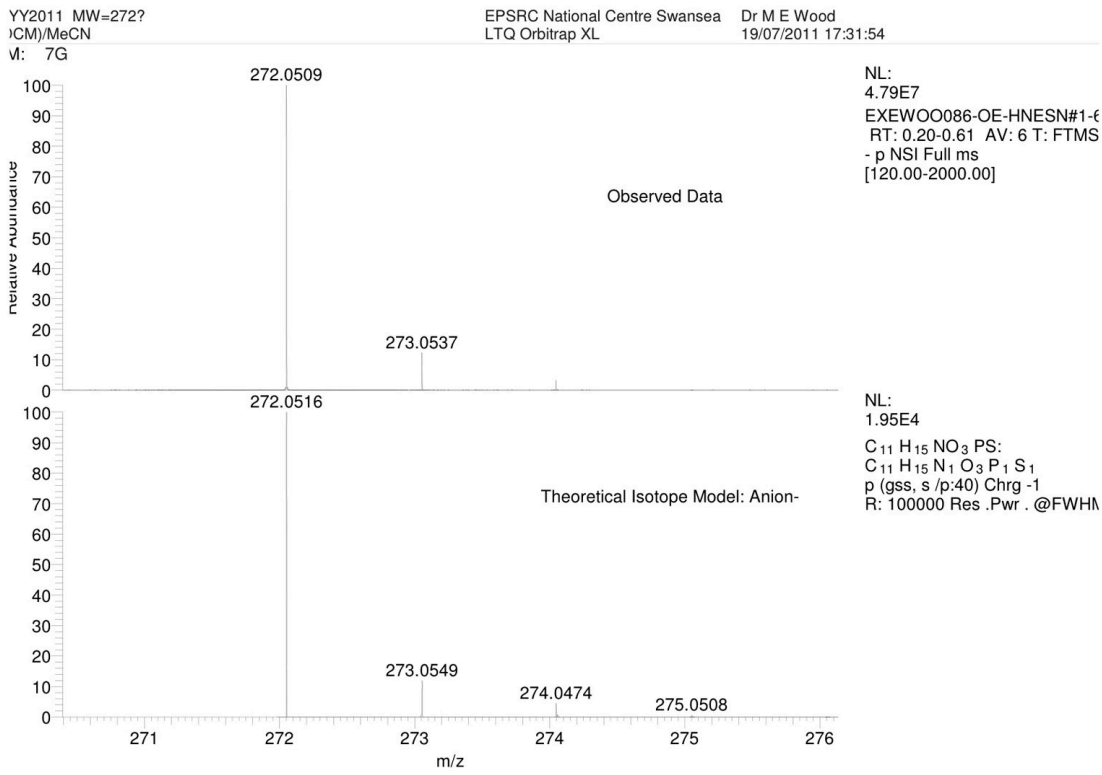


5

Morpholinium 4-methoxyphenyl(morpholino)phosphinothioate **5** HRMS (ES^-)



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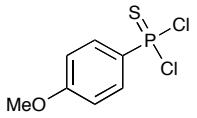
)CM)/MeCN
M: 7G
EPSRC National Centre Swansea Dr M E Wood
LTQ Orbitrap XL 19/07/2011 17:31:54
272.0509
273.0537
Observed Data
NL:
4.79E7
EXEWOO086-OE-HNESN#1-6
RT: 0.20-0.61 AV: 6 T: FTMS
- p NSI Full ms
[120.00-2000.00]
272.0516
273.0549
274.0474
275.0508
Theoretical Isotope Model: Anion-
NL:
1.95E4
C 11 H 15 NO 3 PS:
C 11 H 15 N 1 O 3 P 1 S 1
p (gss, s /p:40) Chrg -1
R: 100000 Res .Pwr . @FWHM
Relative Abundance
m/z

4-Methoxyphenylphosphonothioic dichloride 7[31]

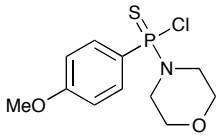


7

Prepared by an adaptation of the method described by Lecher *et al*.[31]

Sulfuryl chloride (1.65 ml, 20.4 mmol) was added dropwise to a stirred and cooled (0 °C, ice-water bath) solution/suspension of Lawesson's reagent **4** (2.50 g, 6.2 mmol) in carbon tetrachloride (12 ml). After completion of addition and further stirring at 0 °C for 1 h, the reaction mixture was stirred for a further 1 h at room temperature, before removal of the solvent and excess sulfuryl chloride by conventional distillation (atmospheric pressure, oil bath at 100 °C). After co-distilling the remaining volatile material from the residue with petroleum ether (b.p. 40-60 °C, 5 ml), the remaining crude material was purified by Kugelrohr distillation (25 mmHg, oven temperature 260 °C) to give the *title compound* as a slightly impure, pale yellow syrup (2.16 g, 72%), which was used without further purification/characterisation.

4-Methoxyphenyl(morpholino)phosphinothioic chloride 8



8

A solution of morpholine (0.55 ml, 6.4 mmol) and triethylamine (0.90 ml, 6.5 mmol) in dichloromethane (5 ml) was added dropwise to a stirred solution of crude 4-methoxyphenylphosphonothioic dichloride **7** (1.78 g, 7.4 mmol) in dichloromethane (10 ml) at room temperature. After stirring for 1 h, the solution was washed with water (2 x 10 ml) and brine (saturated, 10 ml) and dried (magnesium sulfate), filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluting with dichloromethane), to give the *title compound* (1.546 g, 83% based on morpholine) as a white, crystalline solid.

m.p. 74 °C.

ν_{max}/cm^{-1} (thin film) 2965 (w), 2912 (w), 2853 (w), 1595 (s), 1569 (w), 1500 (s), 1451 (w), 1296 (m), 1258 (s), 1183 (m), 1111 (s), 1086 (m), 1025 (w), 965 (s), 918 (w), 831 (m), 803 (w), 736 (s) and 693 (m).

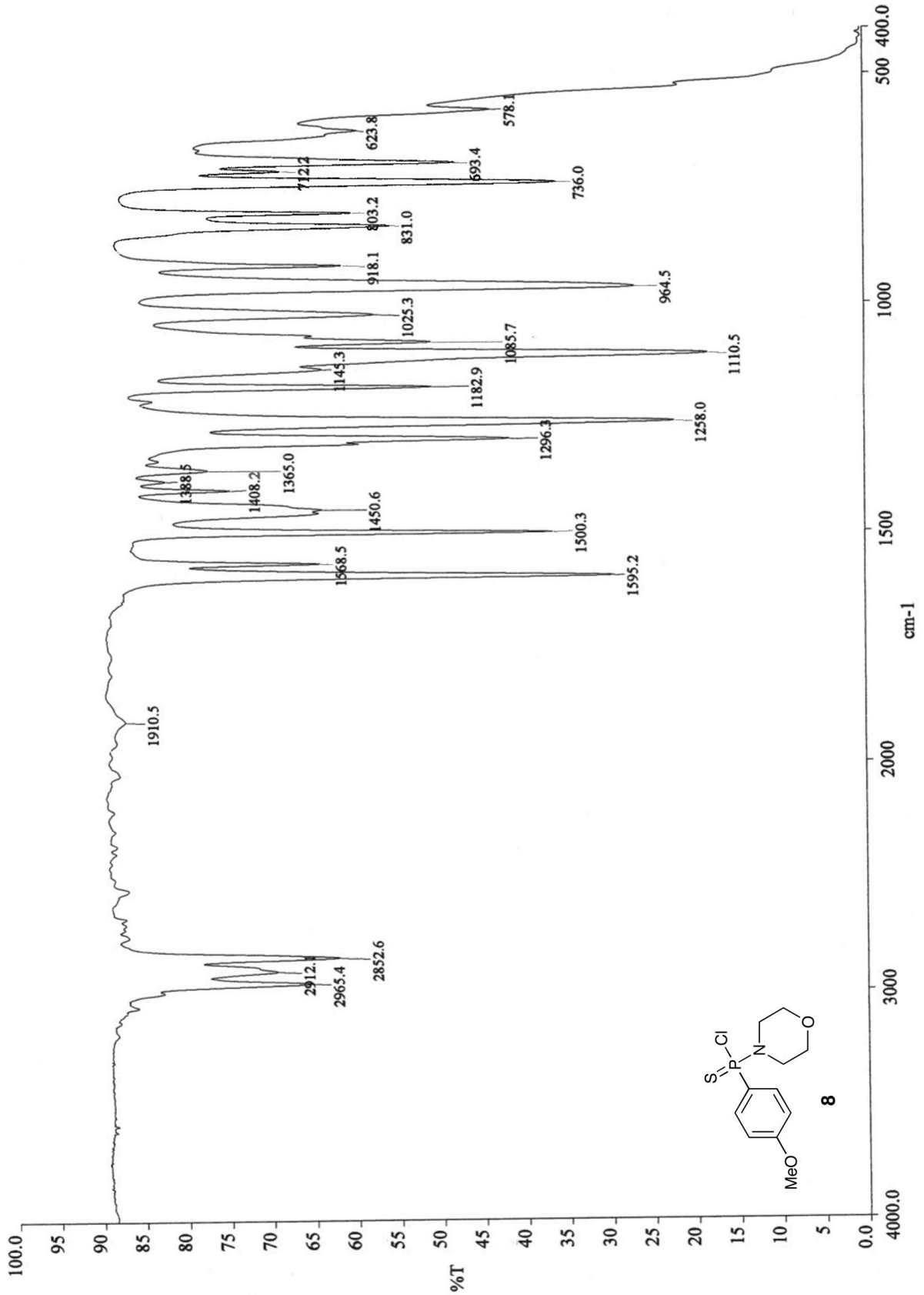
δ_H (300.1 MHz; $CDCl_3$) 3.10 and 3.47 (2 x 2H, 2 x octet, J = 4.5 Hz, N(CH_2)$_2$), 3.75 (4H, dt, J = 1.2 and 4.5 Hz, O(CH_2)$_2$), 3.92 (3H, s, CH_3O), 7.05 (2H, dd, J = 3.9 and 8.9 Hz, arylC-*H* *o*-to OCH_3) and 7.92 (2H, dd, J = 8.9 and 14.9 Hz, arylC-*H* *o*-to P).

δ_C (75.5 MHz; $CDCl_3$) 45.2 (d, J = 2.9 Hz, N(CH_2)$_2$), 55.5 (CH_3O), 66.4 (d, J = 10.2 Hz, O(CH_2)$_2$), 114.3 (d, J = 17.1 Hz, aryl*C*-H *o*-to OCH_3), 125.4 (d, J = 141.6 Hz, aryl*C*-P), 132.8 (d, J = 13.9 Hz, aryl*C*-H *o*-to C-P) and 162.9 (d, J = 3.7 Hz, aryl*C*-O).

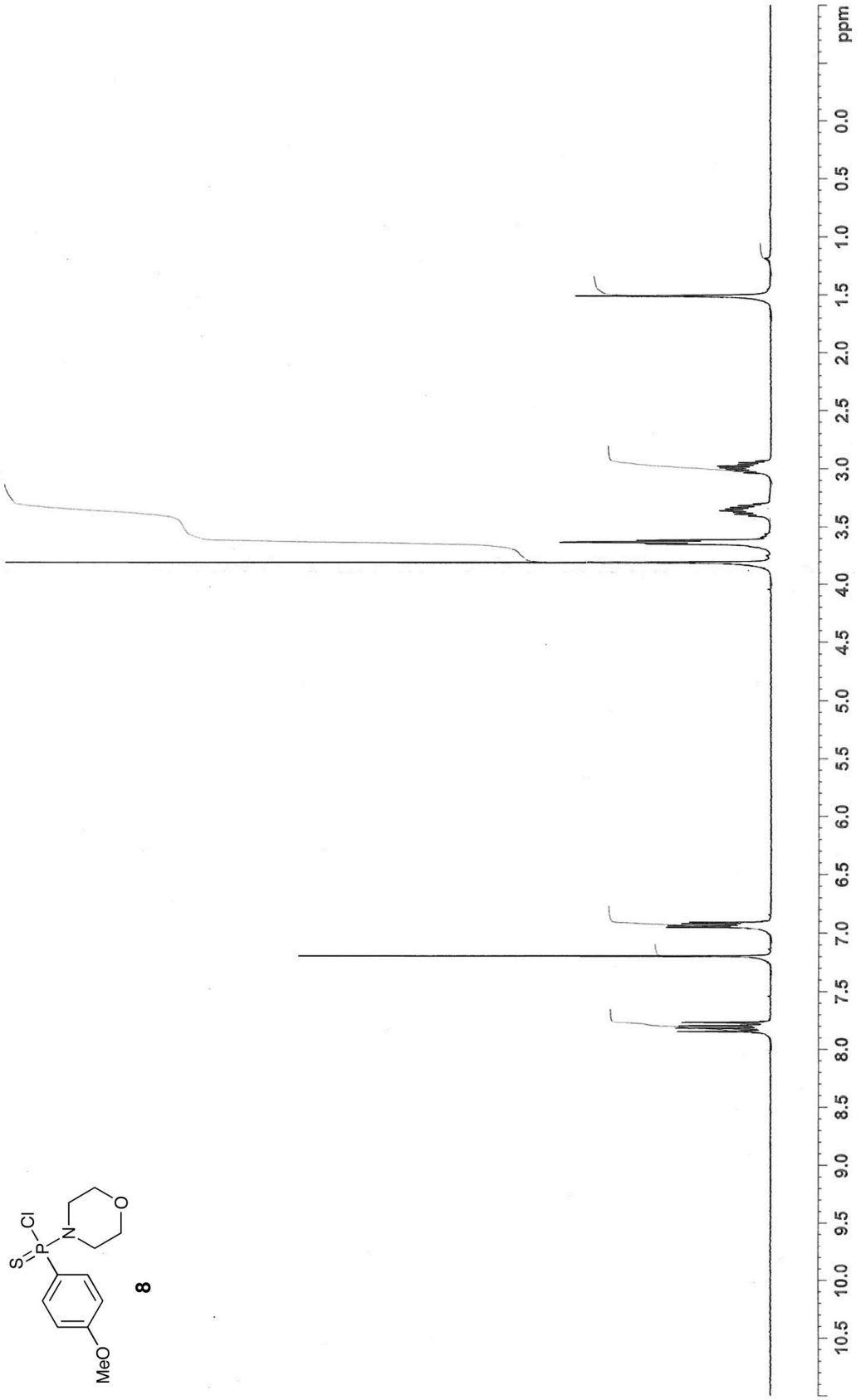
δ_P (121.5 MHz, $CDCl_3$) 86.9.

m/*z* (ES$^+$) 295.0326 (4%), 294.0295 ([M+H]$^+$ ^{37}Cl, 38), 293.0359 (12), 292.0329 ([M+H]$^+$ ^{35}Cl, 100) and 256.0561 (58). ($C_{11}H_{16}{}^{37}ClNO_2PS$ requires 294.0291 and $C_{11}H_{16}{}^{35}ClNO_2PS$ requires 292.0322.)

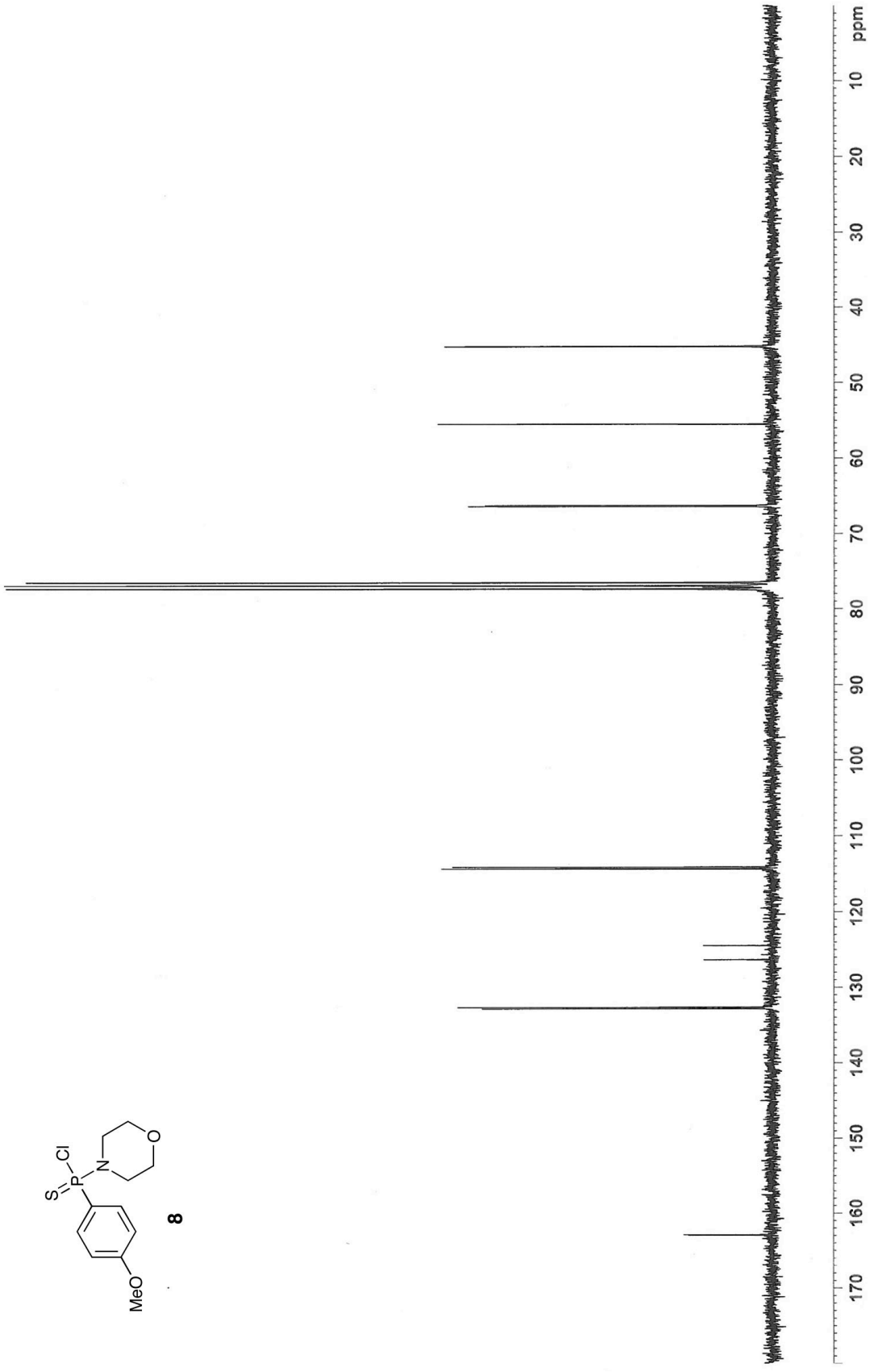
4-Methoxyphenyl(morpholino)phosphinothioic chloride **8** IR Spectrum (thin film)



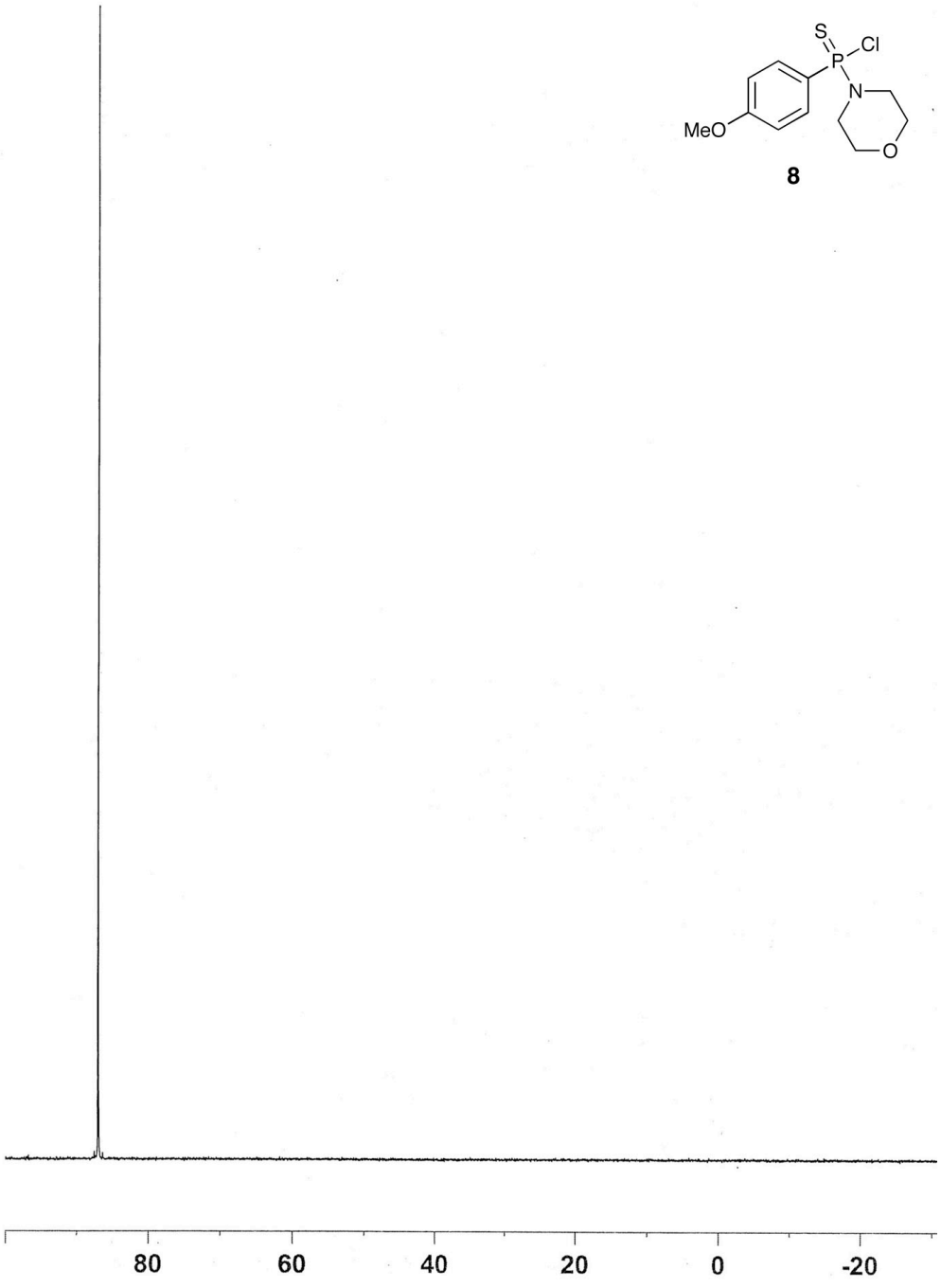
4-Methoxyphenyl(morpholino)phosphinothioic chloride **8** ^{1}H NMR Spectrum (300.1 MHz; $CDCl_3$)



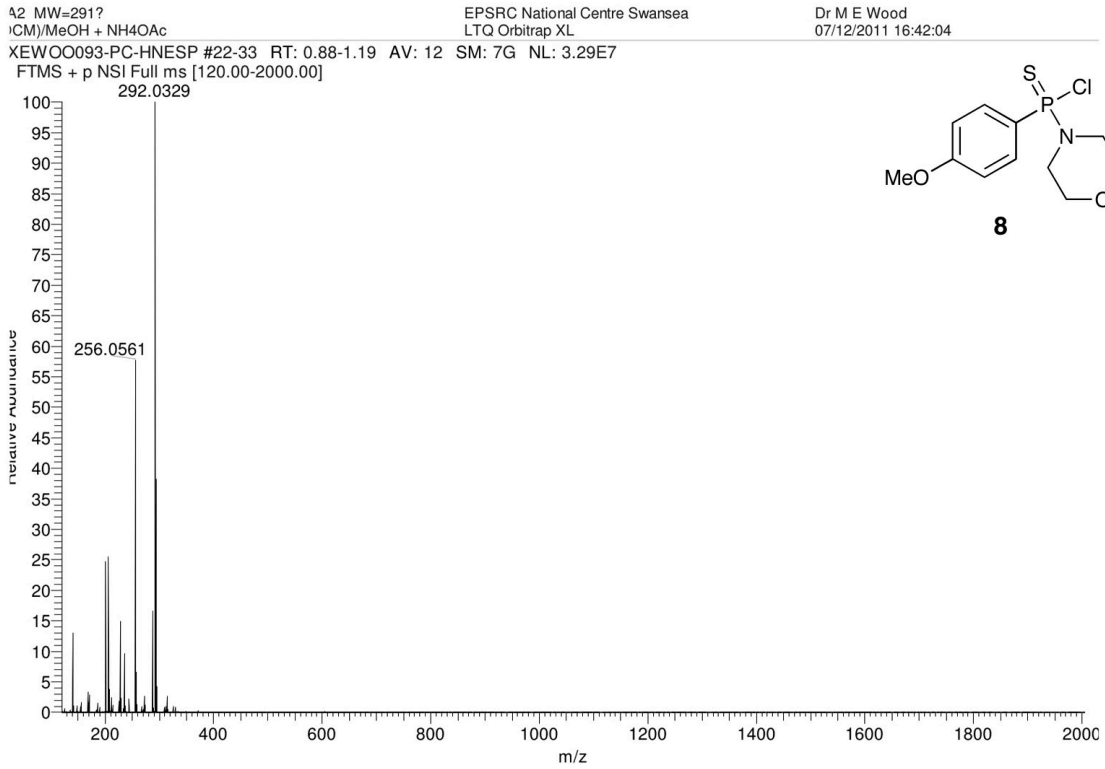
4-Methoxyphenyl(morpholino)phosphinothioic chloride **8** ^{13}C NMR Spectrum (75.5 MHz; $CDCl_3$)

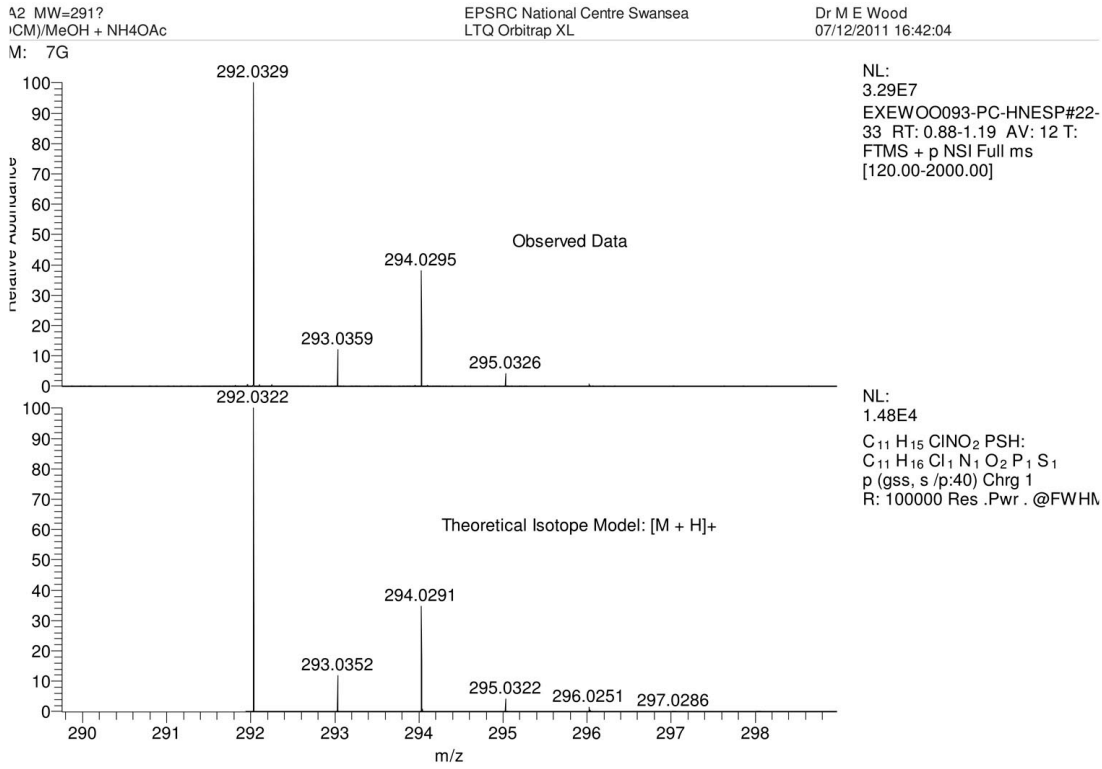


4-Methoxyphenyl(morpholino)phosphinothioic chloride **8** ^{31}P NMR Spectrum (121.5 MHz; $CDCl_3$)

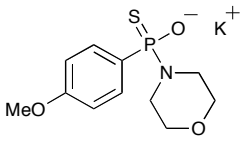


4-Methoxyphenyl(morpholino)phosphinothioic chloride **8** HRMS (ES^+)





Potassium 4-methoxyphenyl(morpholino)phosphinothioate 6



6

Potassium trimethylsilanolate (500 mg, 3.90 mmol) was added to a stirred solution of 4-methoxyphenyl(morpholino)phosphinothioic chloride **8** (400 mg, 1.37 mmol) in diethyl ether (12 ml) at room temperature. After stirring for 7 days, the resulting precipitate was filtered off to give the *title compound* (402 mg, 94%) as an off-white, crystalline solid.

m.p. 107 °C (dec.)

ν_{max}/cm^{-1} (KBr disc) 2957 (w), 2843 (w), 1632 (m), 1596 (s), 1499 (m), 1444 (w), 1403 (w), 1291 (m), 1253 (m), 1181 (w), 1118 (5), 1098 (s), 1071 (m), 1026 (m), 945 (5), 830 (w), 802 (m), 714 (m), 669 (s), 616 (m), 572 (m), 534 (m), 503 (m) and 455 (m).

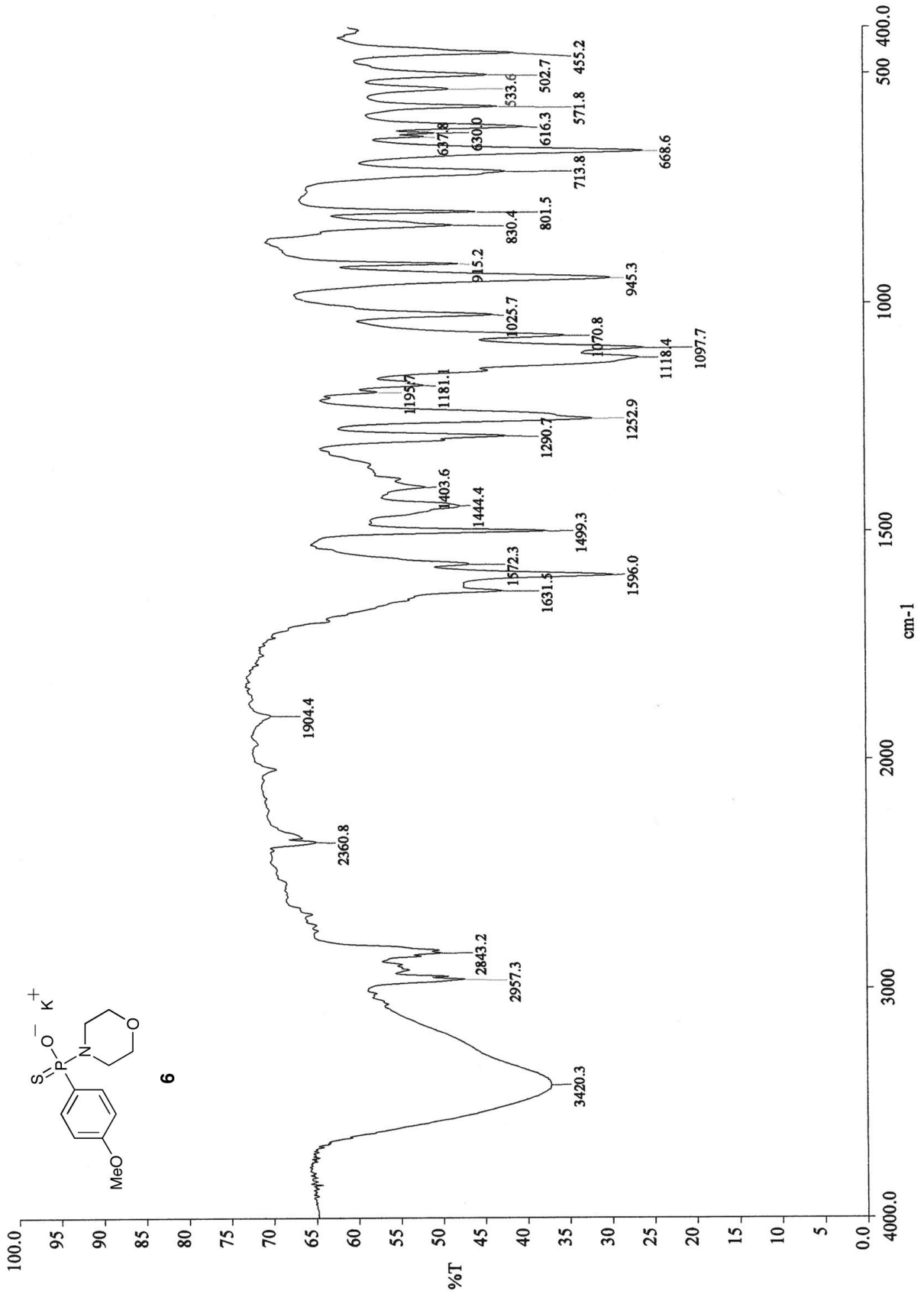
δ_H (300.1 MHz; D_2O) 2.71 (4H, m, N(CH_2)$_2$), 3.46 (4H, t, J = 4.3 Hz, O(CH_2)$_2$), 3.65 (3H, s, CH_3O), 6.84 (2H, dd, J = 2.2 and 8.7 Hz, arylC-*H* *o*-to OCH_3) and 7.57 (2H, dd, J = 8.7 and 12.2 Hz, arylC-*H* *o*-to P).

δ_C (75.5 MHz; D_2O) 44.1 (N(*C*H_2)$_2$), 54.9 (*C*H_3O), 66.3 (d, J = 9.5 Hz, O(*C*H_2)$_2$), 113.1 (d, J = 14.4 Hz, aryl*C*-H *o*-to OCH_3), 129.2 (d, J = 134.6 Hz, aryl*C*-P), 132.2 (d, J = 11.9 Hz, aryl*C*-H *o*-to C-P) and 160.5 (d, J = 3.1 Hz, aryl*C*-O).

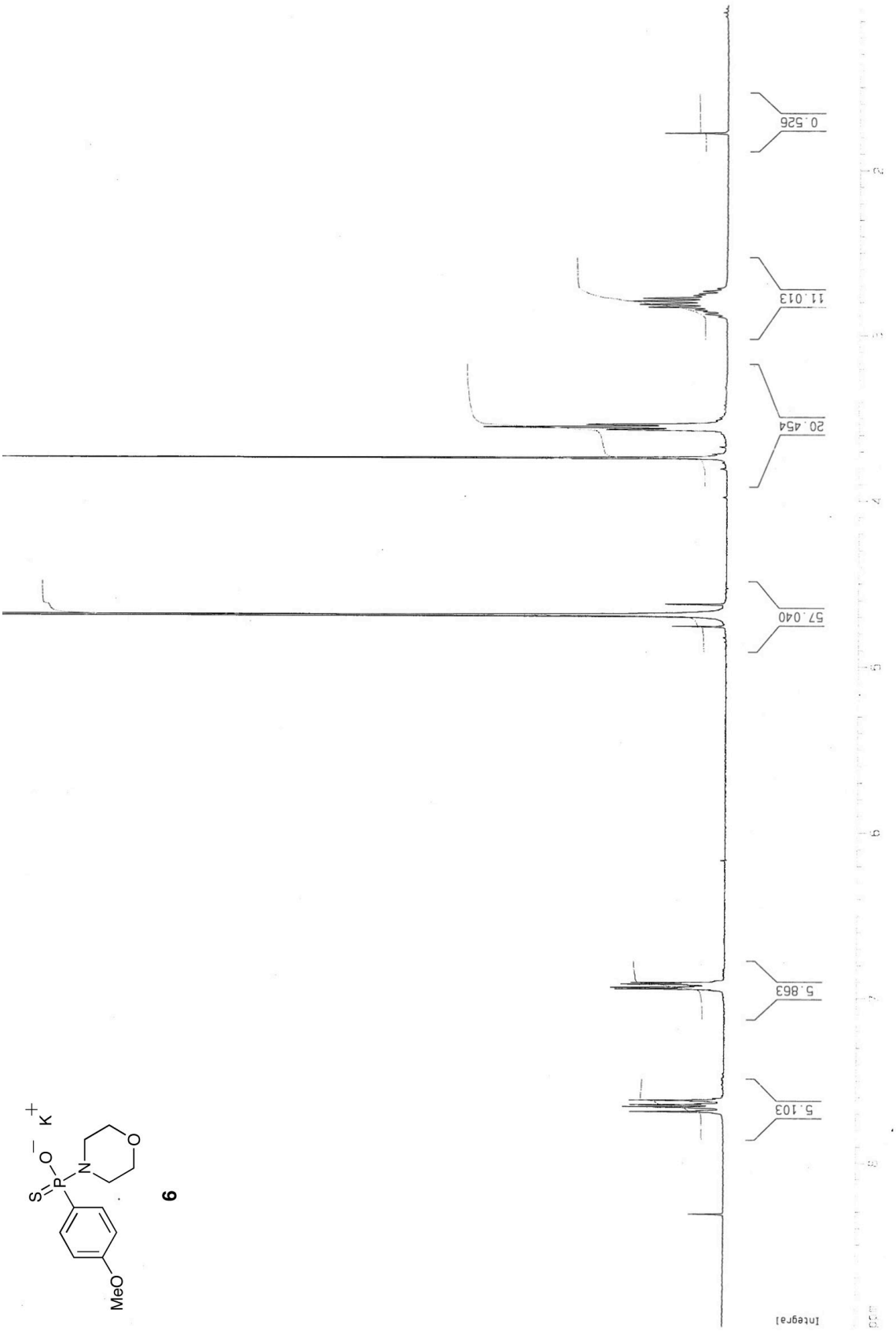
δ_P (121.5 MHz, D_2O) 64.5.

m/z (ES$^-$) 274.0476 (4%), 273.0551 (12) and 272.0524 ([M-K]$^-$, 100). ($C_{11}H_{15}NO_3PS^-$ requires 272.0516.)

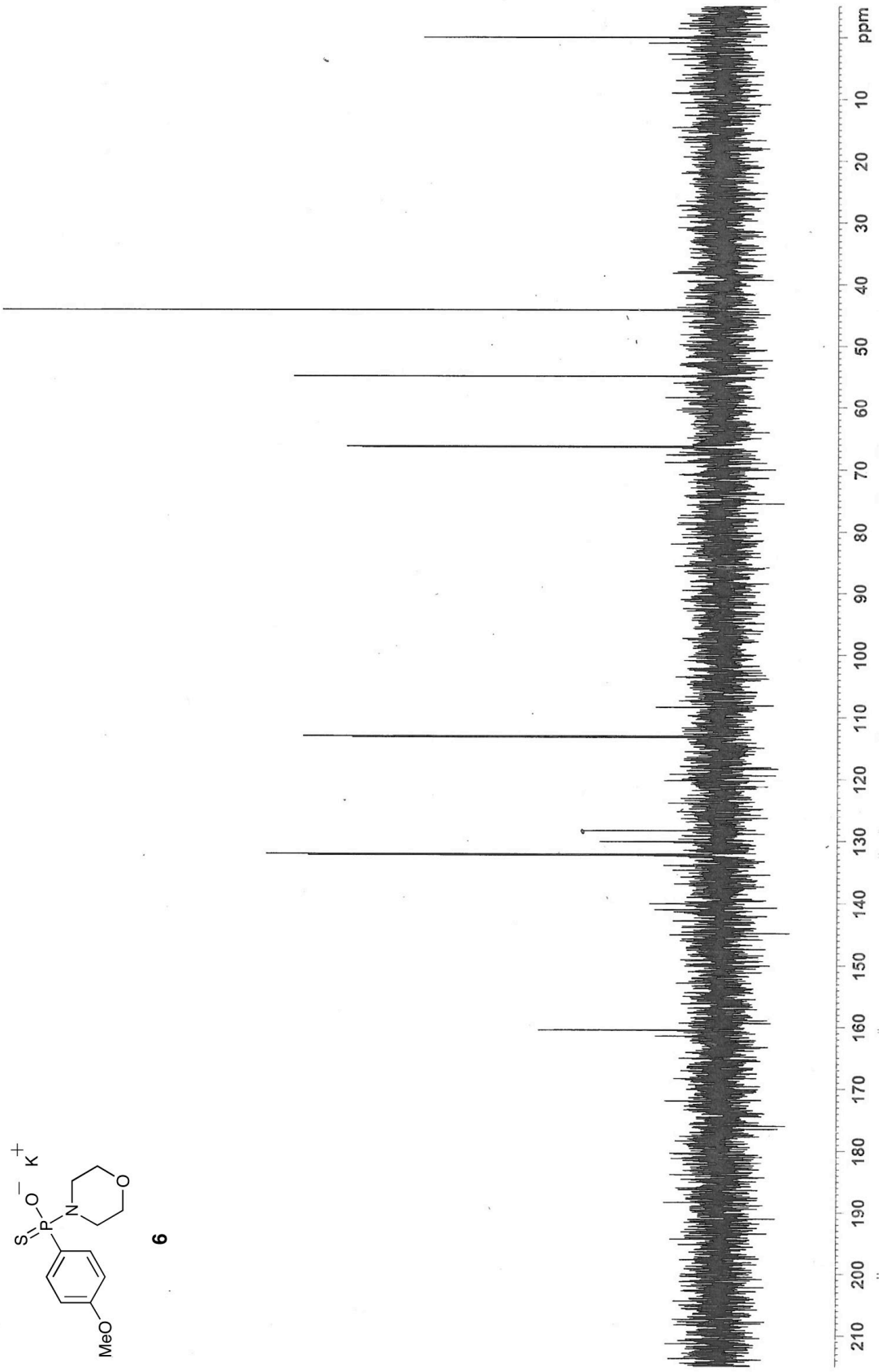
Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** IR Spectrum (KBr disc)



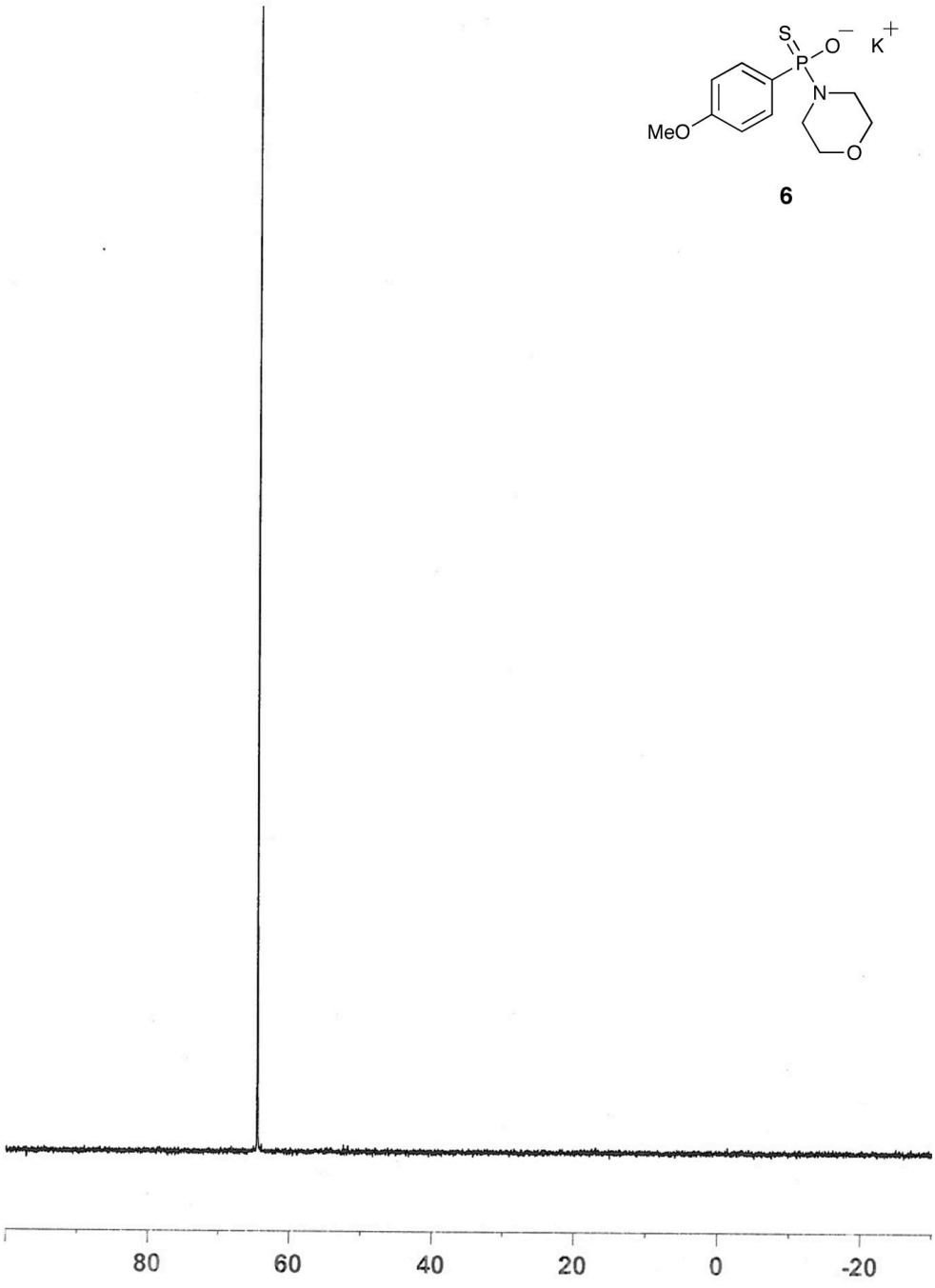
Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** ^{1}H NMR Spectrum (300.1 MHz; D_2O)



Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** ^{13}C NMR Spectrum (75.5 MHz; D_2O)

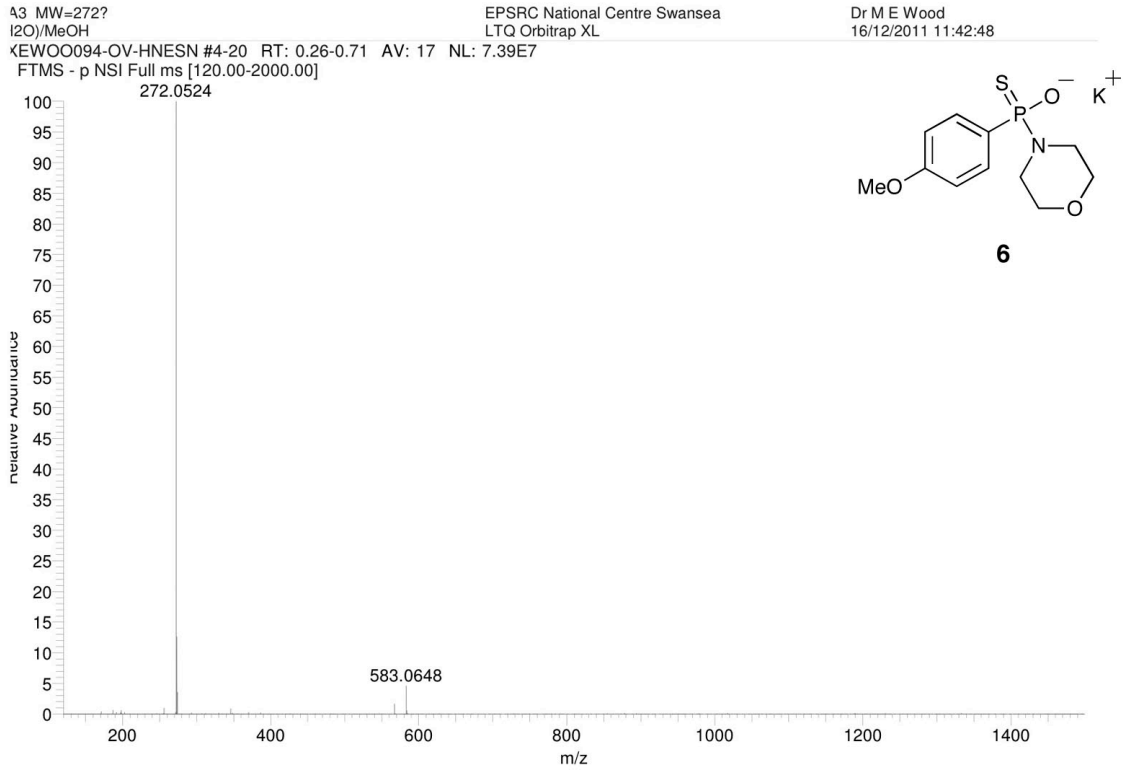


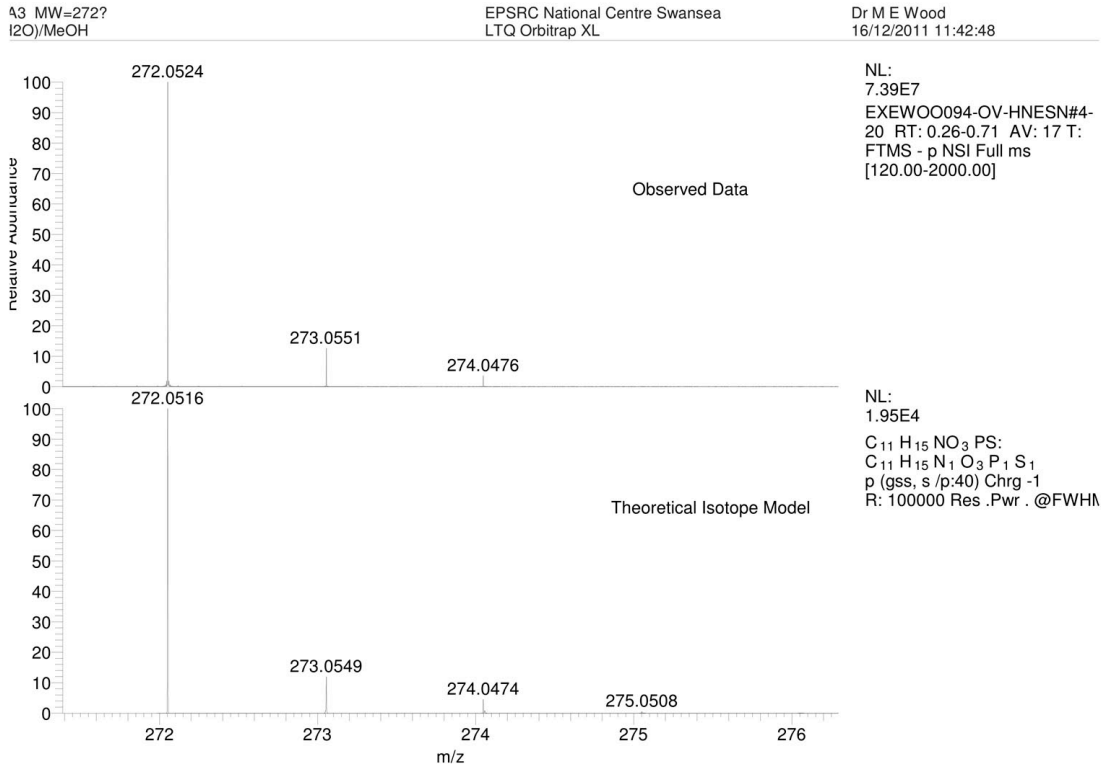
Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** ^{31}P NMR Spectrum (121.5 MHz; D_2O)



6

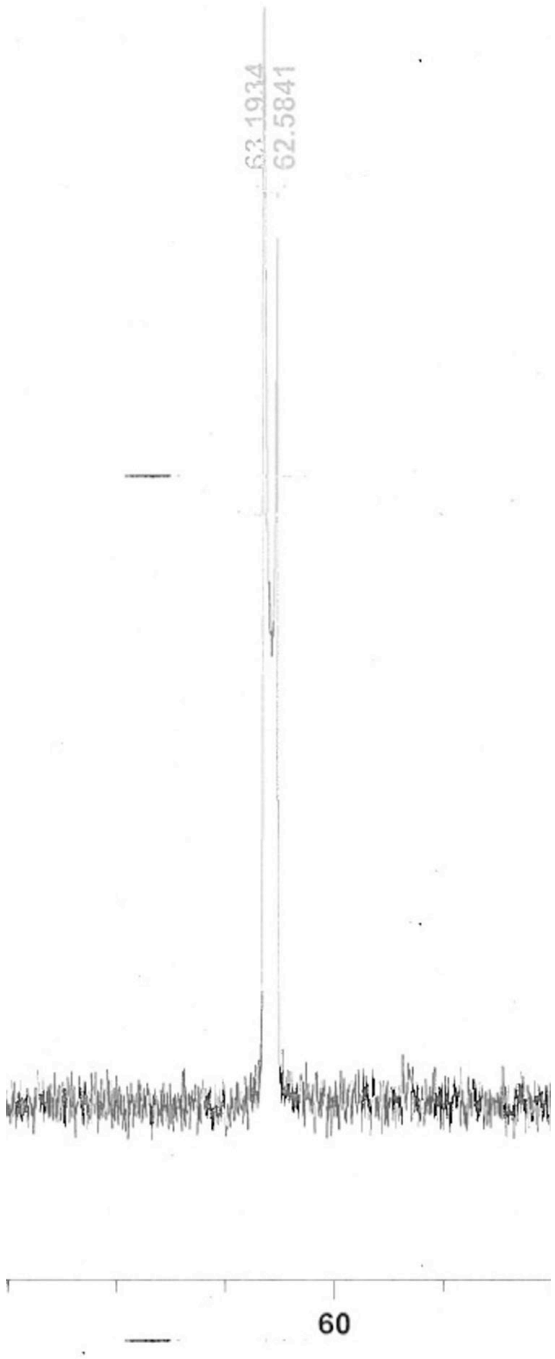
Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** HRMS (ES$^-$)



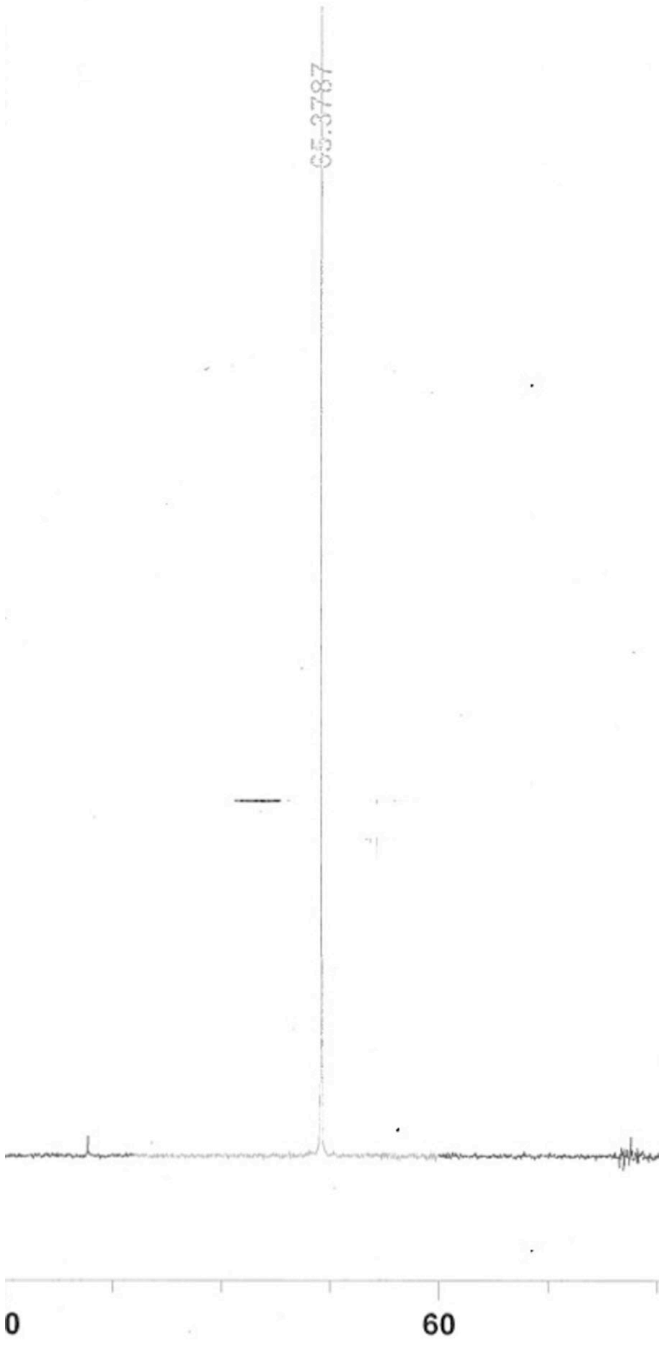


Morpholinium 4-methoxyphenyl(morpholino)phosphinothioate 5 and potassium 4-methoxyphenyl(morpholino)phosphinothioate 6 ^{31}P NMR mixing experiment

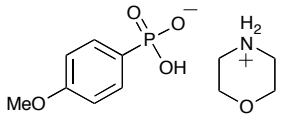
Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** in D_2O overlayered with morpholinium 4-methoxyphenyl(morpholino)phosphinothioate **5** in $(CD_3)_2CO$ ^{31}P NMR Spectrum (121.5 MHz)



Potassium 4-methoxyphenyl(morpholino)phosphinothioate **6** in D_2O fully mixed with morpholinium 4-methoxyphenyl(morpholino)phosphinothioate **5** in $(CD_3)_2CO$ ^{31}P NMR Spectrum (121.5 MHz)



Morpholinium 4-methoxyphenylphosphonate 9

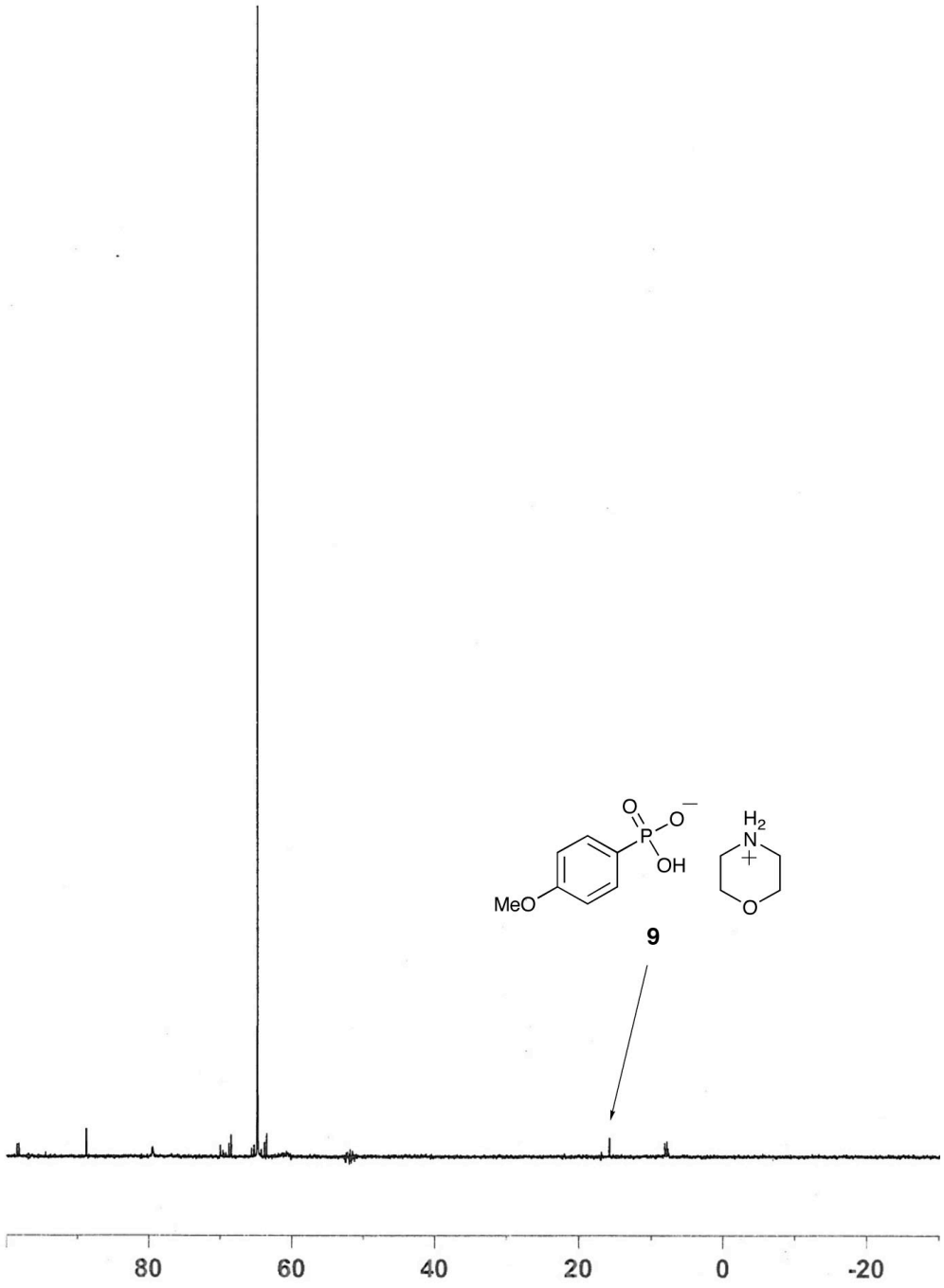


9

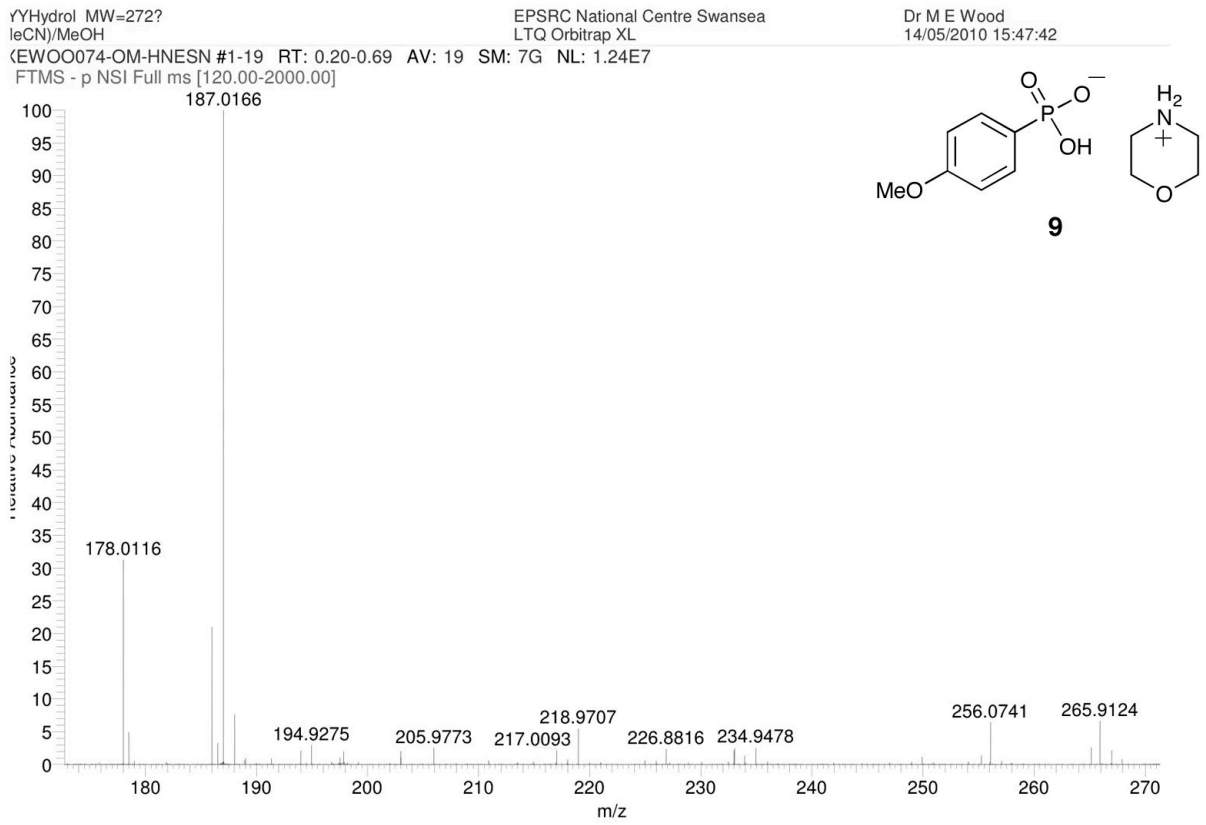
The ^{31}P NMR spectrum of the hydrolysis reaction product **5** above, also showed a signal of very low intensity at $\delta_P = 15.8$, corresponding to the *title compound*.

The mass spectrum of a sample of GYY4137 **3**, hydrolysed in wet $(CD_3)_2CO$ for 90 days, also showed a peak at $m/z = 187.0166$ corresponding to **9**. ($C_7H_8O_4P^-$ requires 187.0166.)

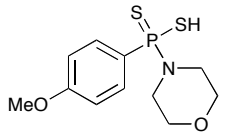
Morpholinium 4-methoxyphenylphosphonate **9** ^{31}P NMR Spectrum (121.5 MHz; $CDCl_3$)



Morpholinium 4-methoxyphenylphosphonate **9** HRMS (ES^-)



4-Methoxyphenyl(morpholino)phosphinodithioic acid 10



10

Glacial acetic acid (3.30 ml, 57.7 mmol) was added dropwise, to a cooled (0-5 °C), stirred solution of GYY4137 **3** (2 : 1 with CH_2Cl_2, 2.00 g, 4.83 mmol) in water (50 ml). After stirring at 0-5 °C for a further 25 min, the precipitate was filtered off, washed with water and dried to constant mass *in vacuo* (*ca* 15 mmHg). This gave the *title compound* (1.17 g, 84%) as a white, powdery solid.

m.p. 157-163 °C

ν_{max}/cm^{-1} (KBr disc) 3405 (w), 3054-2526 (broad, m), 1593 (s), 1497 (s), 1456 (m), 1290 (s), 1253 (s), 1177 (m), 1100 (s), 1021 (s), 870 (s), 830 (m), 800 (m), 718 (m), 652 (s) and 544 (m).

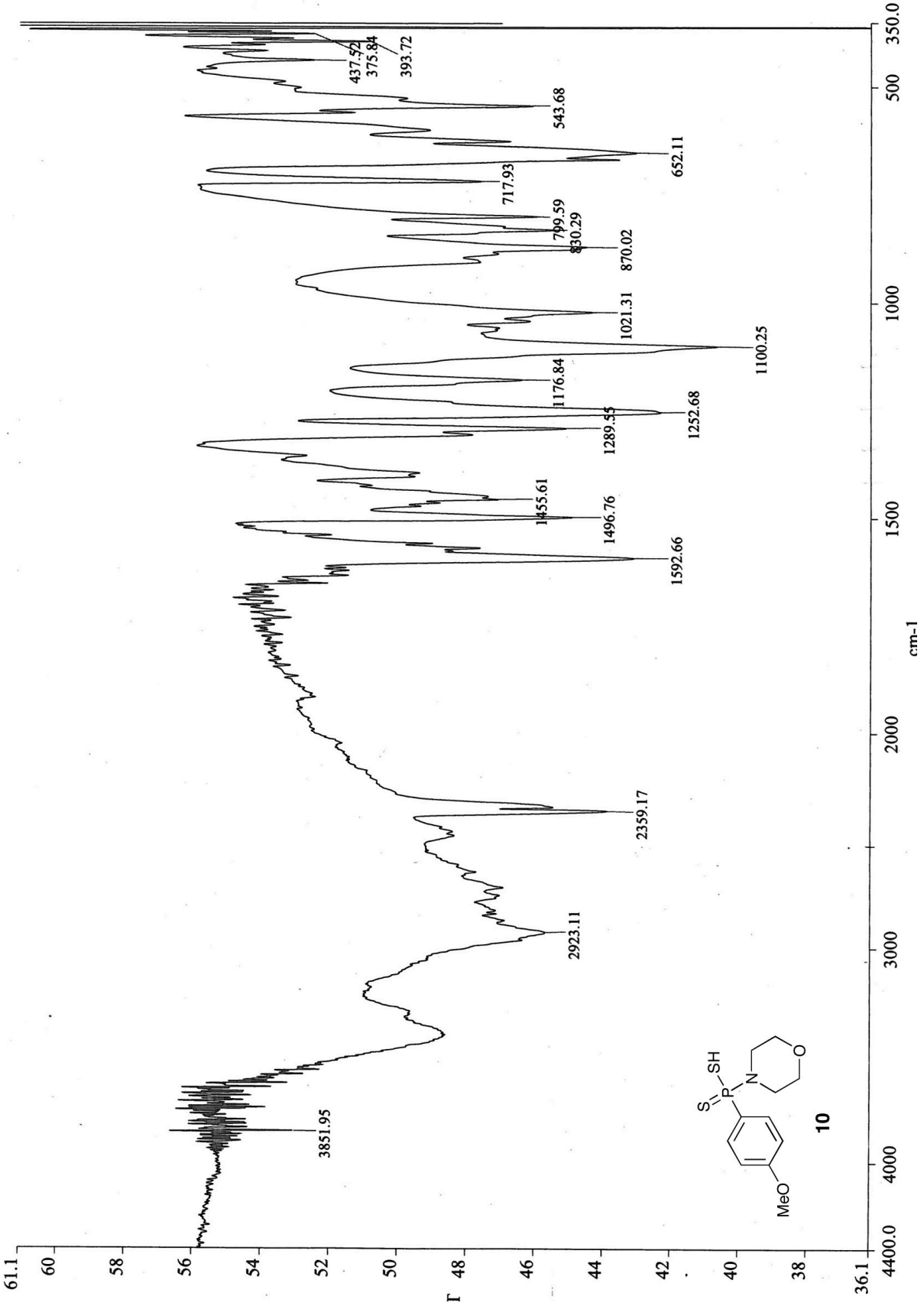
δ_H (300.1 MHz; $CDCl_3$) 3.19-3.43 (4H, broad m, N(C*H*$_2$)$_2$), 3.76-3.98 (4H, broad m, O(C*H*$_2$)$_2$), 3.90 (3H, s, C*H*$_3$O), 7.00 (2H, dd, *J* = 2.8 and 8.4 Hz, arylC-*H* *o*-to OCH_3), 8.06 (2H, dd, *J* = 8.4 and 13.9 Hz, arylC-*H* *o*-to P) and 9.52 (1H, broad s, S*H*).

δ_C (75.5 MHz; $CHCl_3$) 43.4 ((*C*H$_2$)$_2$N), 55.4 (*C*H$_3$O), 63.8 ((*C*H$_2$)$_2$O), 113.4 (d, *J* = 15.9 Hz, aryl*C*-H *o*-to OCH_3), 131.2 (d, *J* = 14.4 Hz, arylC-H *o*-to P),135.6 (d, *J* = 117.8 Hz, aryl*C*-P) and 161.4 (aryl*C*-OCH_3).

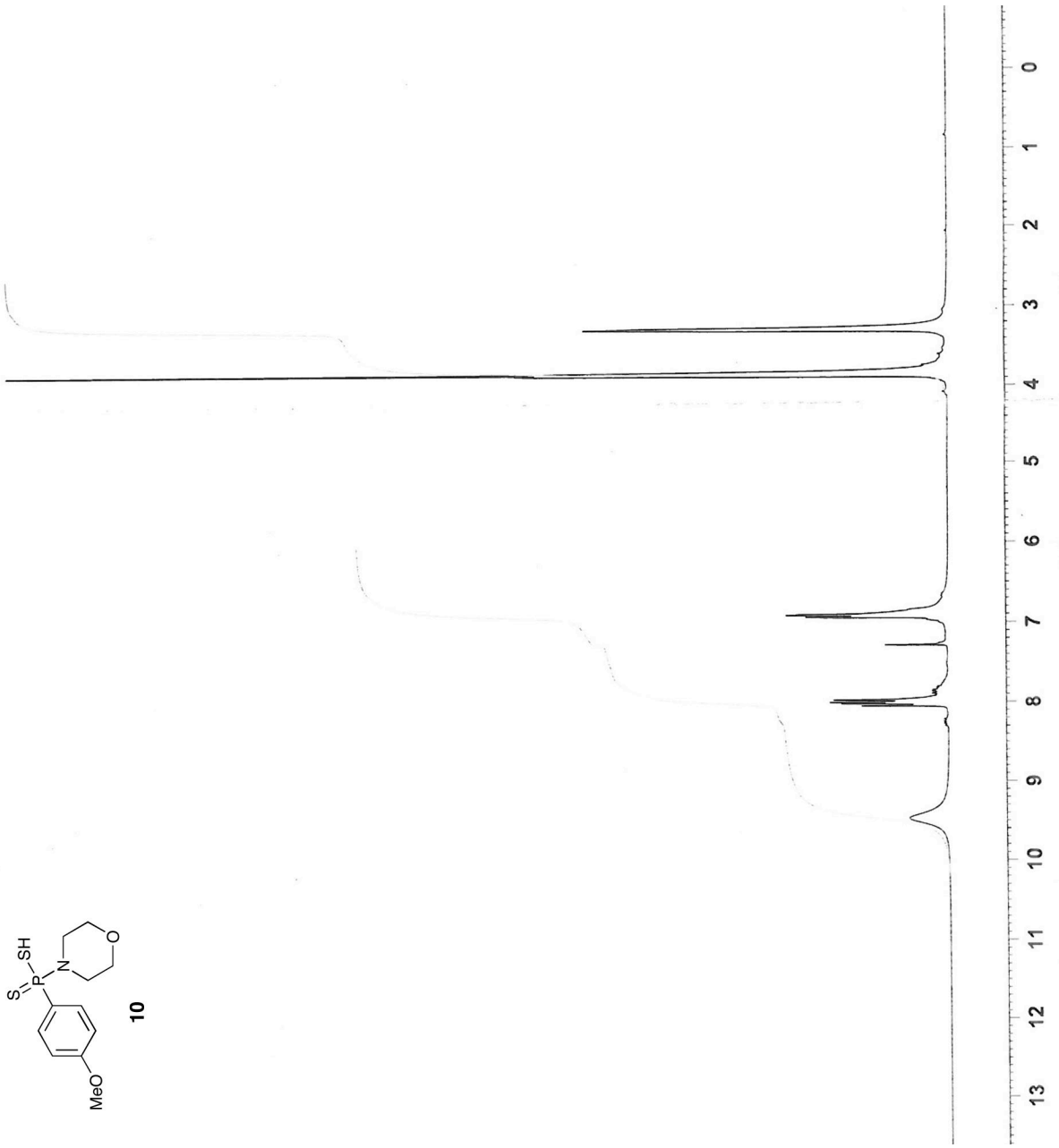
δ_P (121.5 MHz; $CDCl_3$) 82.1.

m/*z* (ES$^+$) Although ^{1}H and ^{13}C NMR spectra suggested a high level of purity, the mass spectrum revealed a number of peaks, which could not be assigned with certainty, at higher molecular weight than expected for **10**. These were found at *m*/*z* 510.0204 (21%), 538.9476 (24), 377.1118 (77) and 308.0540 (33). Peaks assignable to the molecular ion corresponding to structure **10** gave an excellent match to the theoretical isotope model at *m*/*z* 292.0393 (9%), 291.0468 (11) and 290.0435 (100). ($C_{11}H_{16}NO_2PS_2$ requires 290.0433.) Lower molecular weight peaks were also observed at *m*/*z* 220.9855 (31%) and 205.0083 (12).

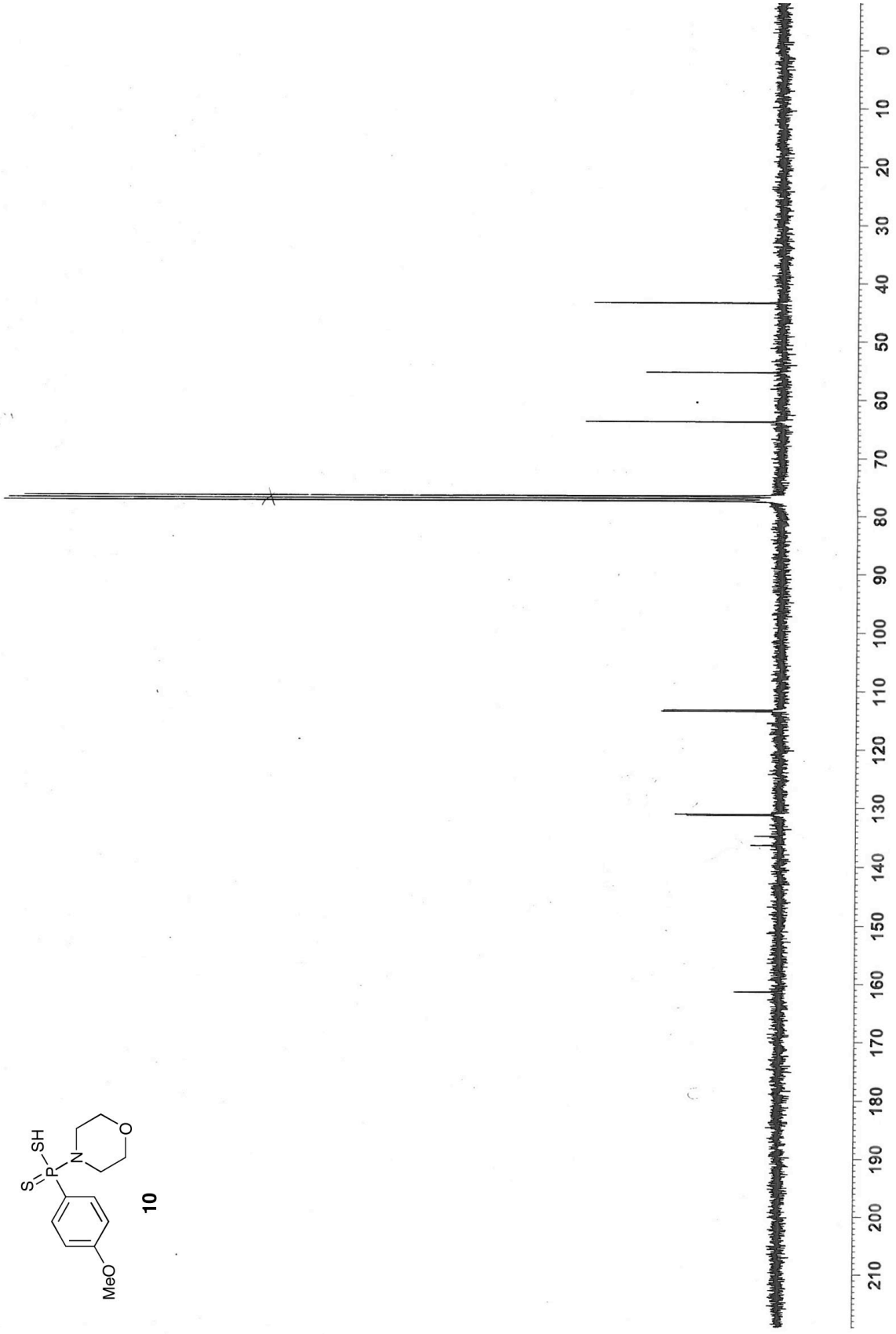
4-Methoxyphenyl(morpholino)phosphinodithioic acid **10** IR Spectrum (KBr disc)



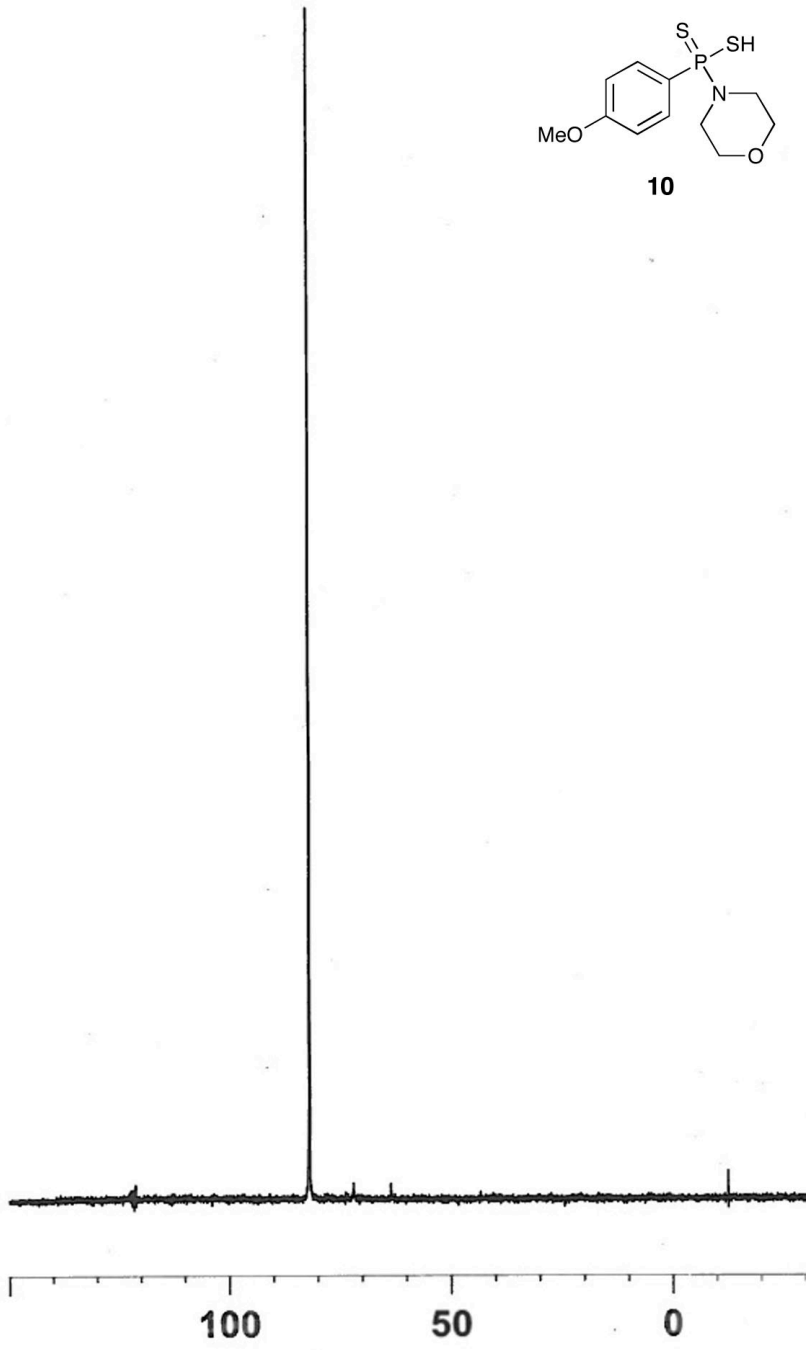
4-Methoxyphenyl(morpholino)phosphinodithioic acid **10** ^{1}H NMR Spectrum (300.1 MHz; $CDCl_3$)



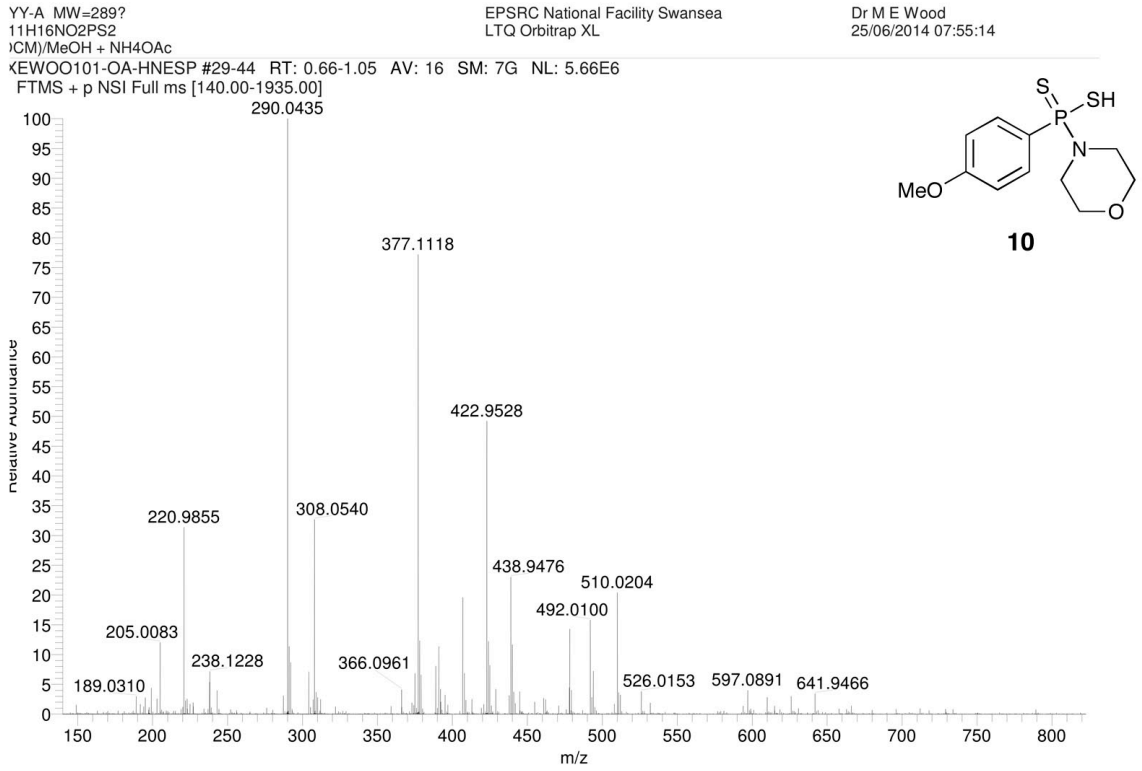
4-Methoxyphenyl(morpholino)phosphinodithioic acid **10** ^{13}C NMR Spectrum (75.5 MHz; $CDCl_3$)

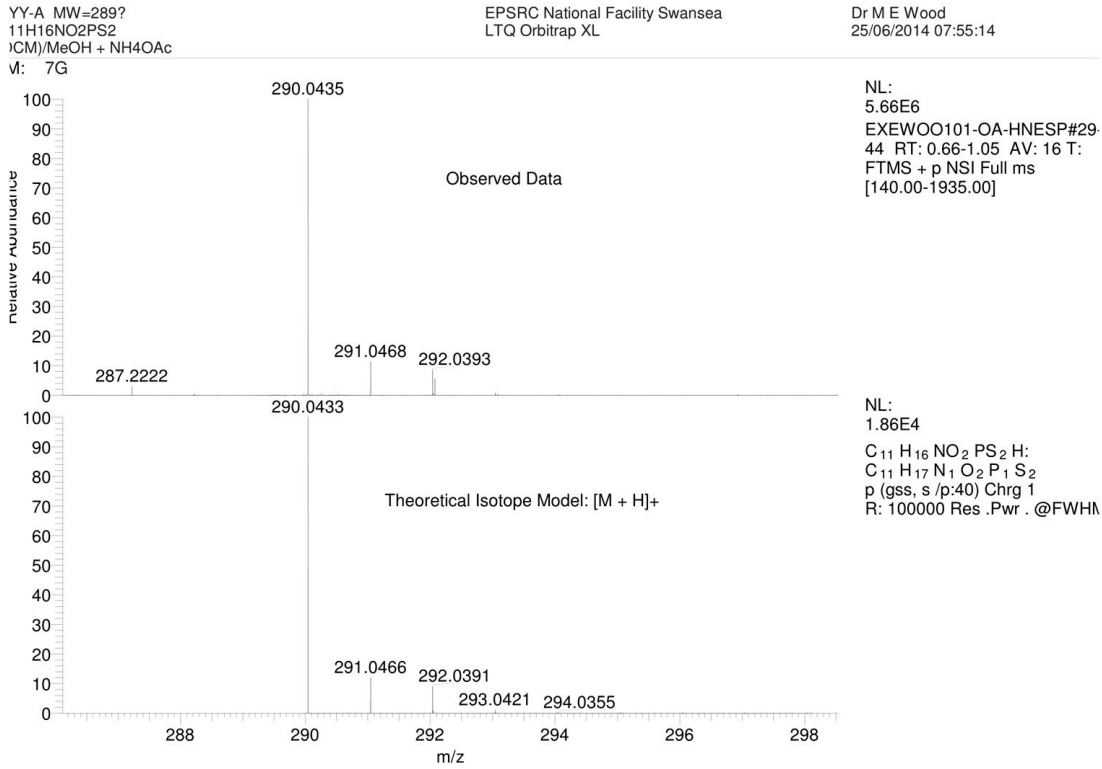


4-Methoxyphenyl(morpholino)phosphinodithioic acid **10** ^{31}P NMR Spectrum (121.5 MHz; $CDCl_3$)

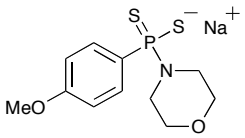


4-Methoxyphenyl(morpholino)phosphinodithioic acid **10** HRMS (ES^+)





Sodium 4-methoxyphenyl(morpholino)phosphinodithioate 11



11

Sodium hydride (60% dispersion in mineral oil, 140 mg, 3.5 mmol) was washed with petroleum ether (b.p. 40-60 °C, 3 x 5 ml) and dried in a gentle stream of dry nitrogen. After addition of diethyl ether (25 ml), 4-methoxyphenyl(morpholino)phosphinodithioic acid **10** (1.00 g, 3.5 mmol) was added portionwise to the stirred sodium hydride suspension over a period of 10 min, at 0 °C. After stirring at 0 °C for a further 30 min and at room temperature for 15 min, the reaction mixture was filtered and the resulting solid was washed with diethyl ether (3 x 25 ml) and dried *in vacuo* (*ca* 15 mmHg), to give the *title compound* (1.05 g, 98%) as a white, microcrystalline solid.

m.p. 220 °C (dec.)

ν_{max}/cm^{-1} (KBr disc) 2962-2833 (m), 1595 (s), 1568 (m), 1496 (s), 1453 (s), 1401 (m), 1386 (w), 1363 (w), 1301 (s), 1289 (s), 1252 (s), 1205 (w), 1183 (m), 1170 (m), 1145 (s), 1103 (s), 1073 (s), 1028 (s), 945 (s), 916 (s), 849 (w), 824 (s), 800 (s), 719 (m), 708 (s), 654 (s), 628 (s), 594 (m), 543 (s), 521 (m), 483 (m), 444 (m) and 430 (m).

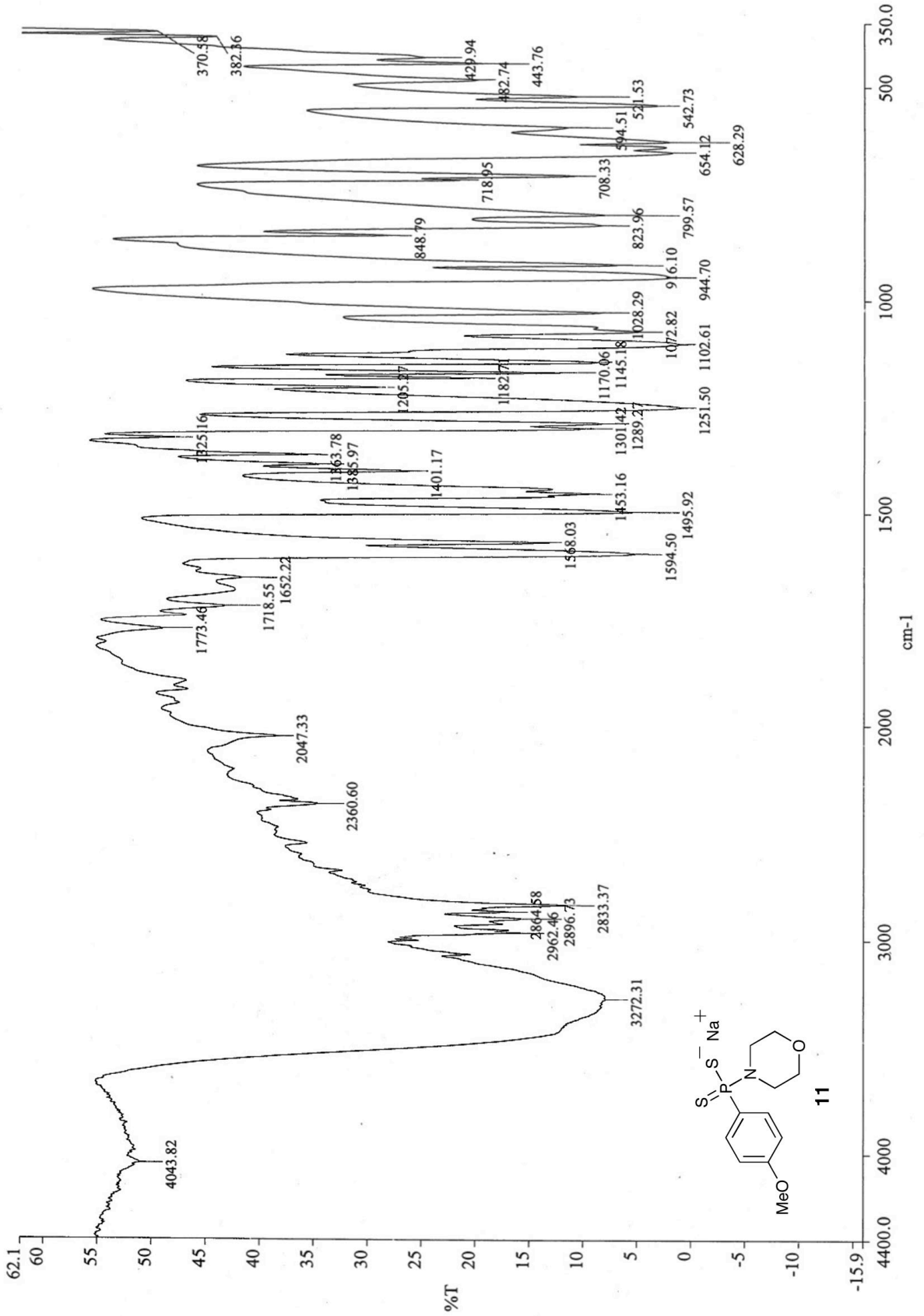
δ_H (300.1 MHz; D_2O) 2.87 (4H, broad apparent q, J = 5.4 Hz, N(CH_2)$_2$), 3.66 (4H, broad apparent t, J = 4.2 Hz, O(CH_2)$_2$), 3.81 (3H, s, CH_3O), 7.00 (2H, dd, J = 2.5 and 8.8 Hz, arylC-*H* *o*-to OCH_3) and 7.96 (2H, dd, J = 8.8 and 13.7 Hz, arylC-*H* *o*-to P).

δ_C (75.5 MHz; D_2O) 44.7 (d, J = 1.5 Hz, N(*C*H_2)$_2$), 55.5 (*C*H_3O), 66.7 (d, J = 11.3 Hz, O(*C*H_2)$_2$), 113.5 (d, J = 11.3 Hz, aryl*C*-H *o*-to OCH_3), 131.4 (d, J = 109.5 Hz, aryl*C*-P), 132.5 (d, J = 12.8 Hz, aryl*C*-H *o*-to C-P) and 161.0 (d, J = 3 Hz, aryl*C*-OCH_3).

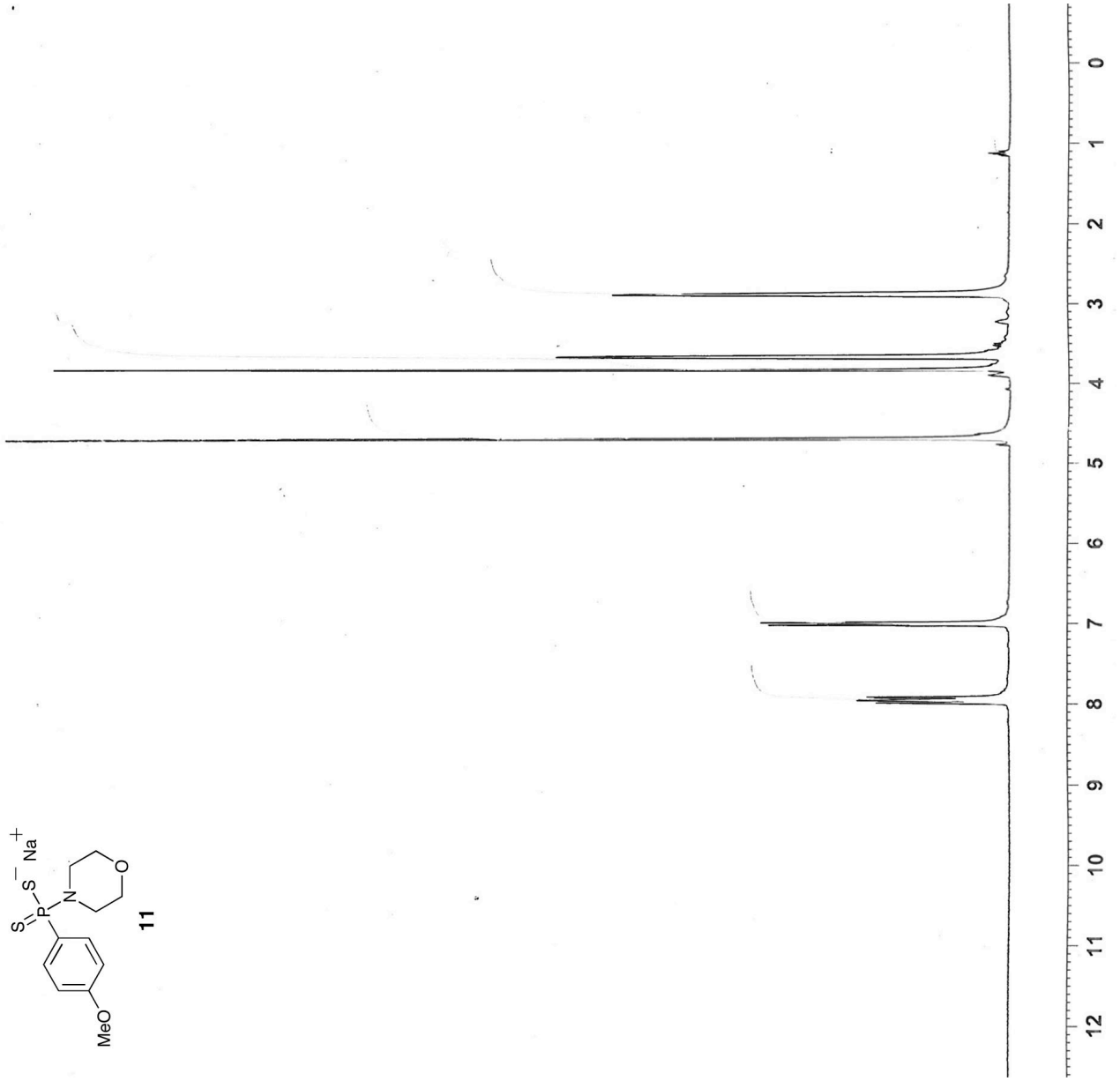
δ_P (121.5 MHz; D_2O) 89.0.

m/z (ES$^-$) 290.0235 (10%), 289.0311 (12) and 288.0281 ([M-Na]$^-$, 100). ($C_{11}H_{15}NO_2PS_2^-$ requires 272.0287.)

Sodium 4-methoxyphenyl(morpholino)phosphinodithioate **11** IR Spectrum (KBr disc)

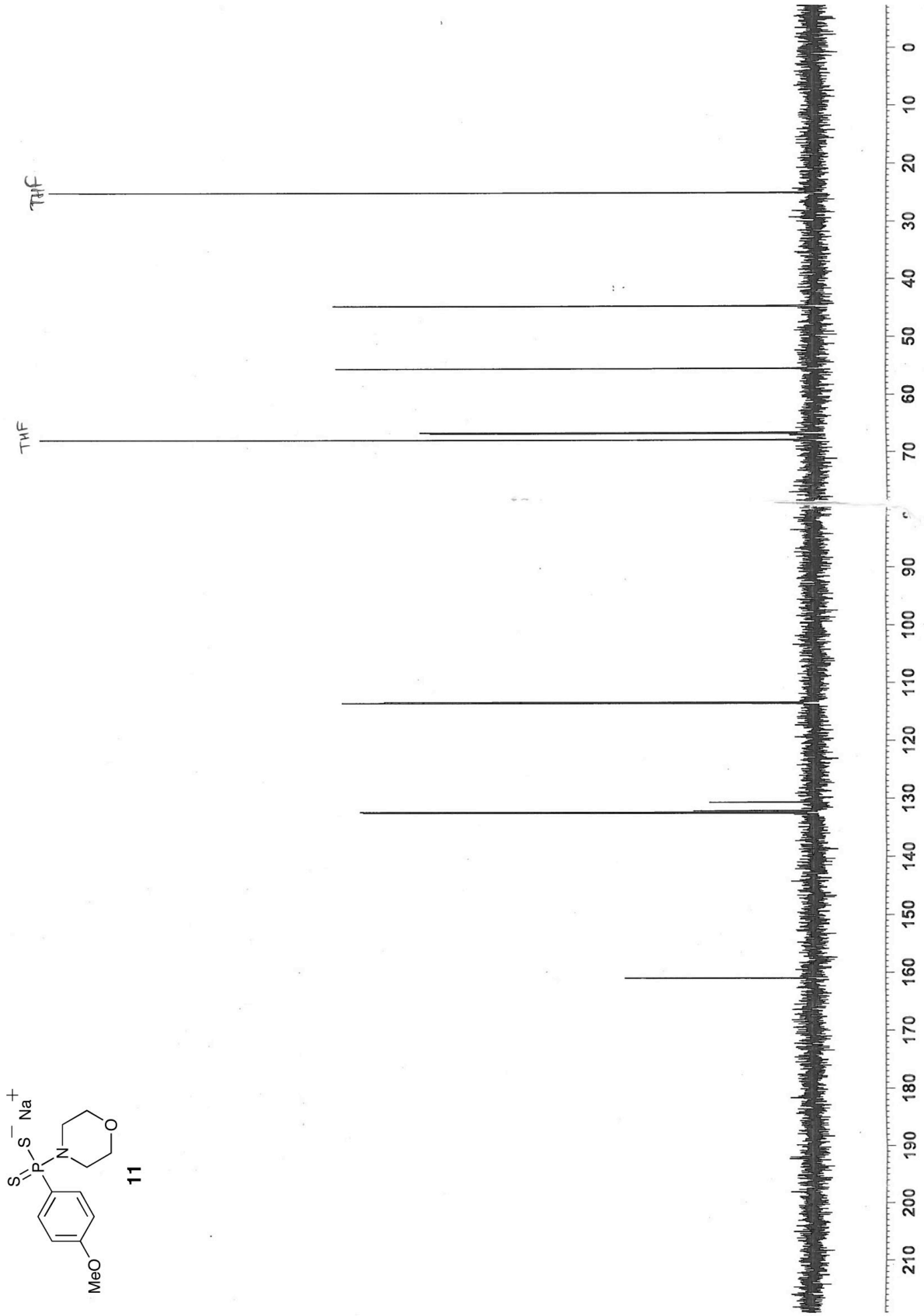


Sodium 4-methoxyphenyl(morpholino)phosphinodithioate **11** ^{1}H NMR Spectrum (300.1 MHz; D_2O)

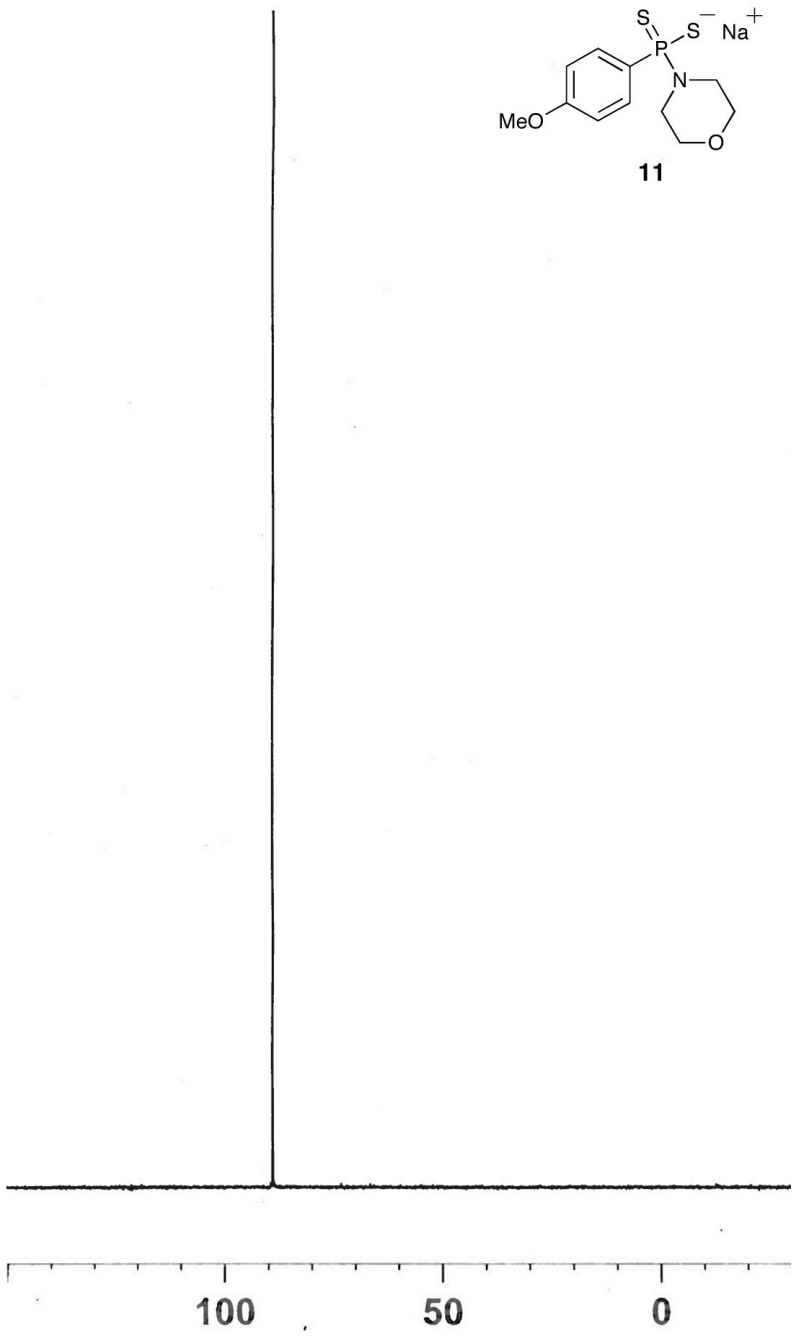


Sodium 4-methoxyphenyl(morpholino)phosphinodithioate **11** ^{13}C NMR Spectrum (75.5 MHz; D_2O)

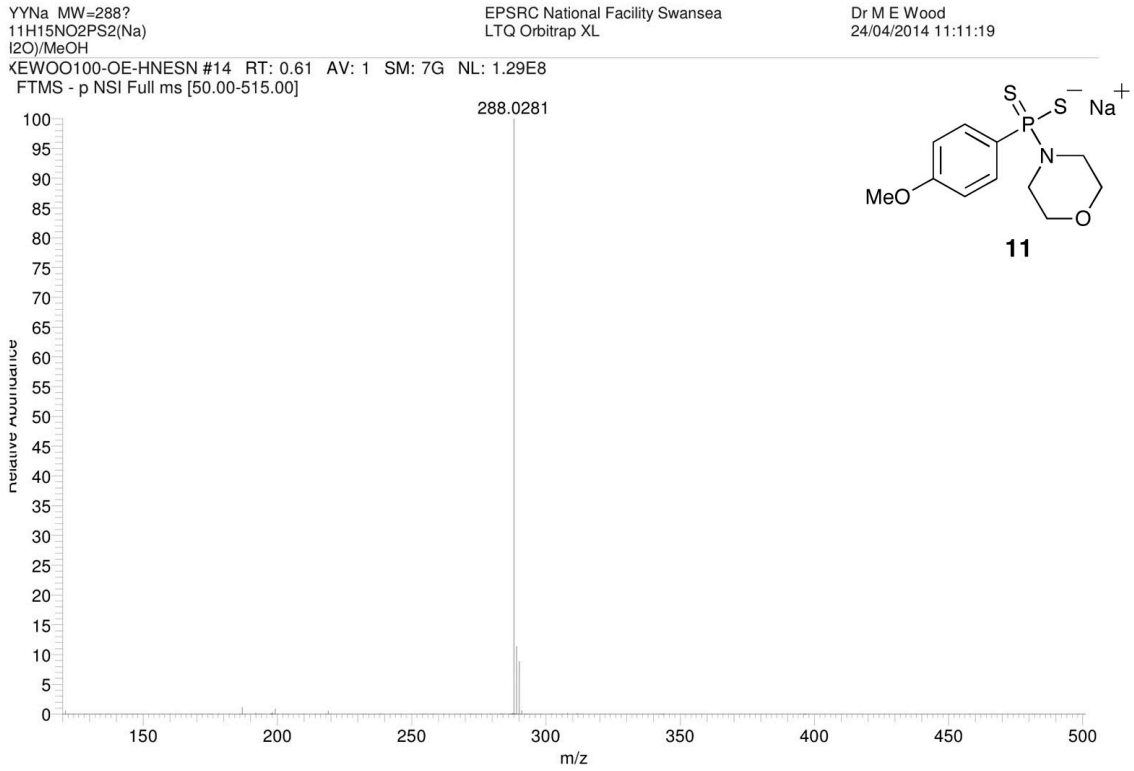
Internal THF used for referencing

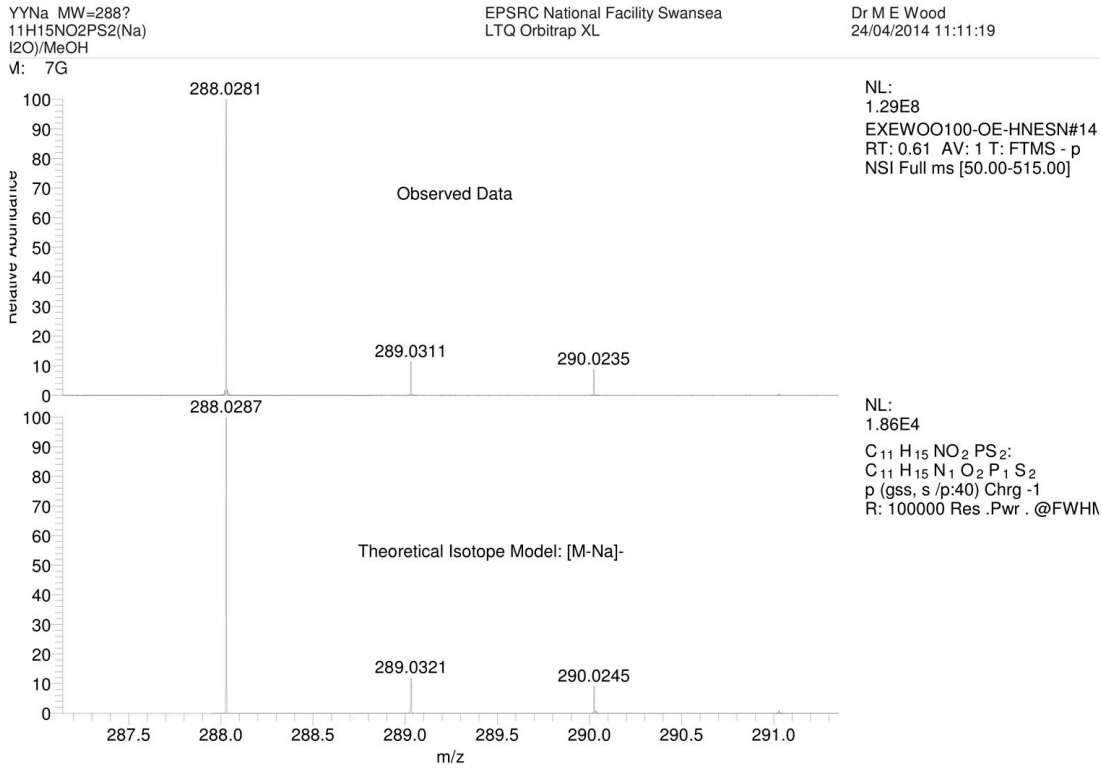


Sodium 4-methoxyphenyl(morpholino)phosphinodithioate **11** ^{31}P NMR Spectrum (121.5 MHz; D_2O)



Sodium 4-methoxyphenyl(morpholino)phosphinodithioate **11** HRMS (ES^-)





(ii) Experimental procedures for cell culture, H_2S generation, cytotoxicity assays and LPS treatment of RAW264.7 cells

Cell culture

Human Jurkat T-cells and murine RAW264.7 macrophages were purchased from the American Tissue Type Collection and cultured in RPMI and DMEM media respectively, containing 10% v/v fetal calf serum and 1% penicillin/streptomycin (Life Technologies) and cultured as described in references 34 and 14 respectively.

H_2S generation

Generation of H_2S from **3**, **6** and **11** in phosphate buffered saline (PBS) was determined using 5,5'-dithiobis-(2-nitrobenzoic acid) as described in reference 15. Intracellular determination of H_2S in human Jurkat cells was achieved using WSP-1 (Cayman, 11179-5).[19,36] In these experiments, cells were seeded at a density of 2 x 10^5 cells per ml in 24-well culture plates (Nalgen Nunc International) in RPMI, containing 10 μM WSP-1. After 30 min, **3**, **6**, or **11** were added for a further 60 min and fluorescence (Ex465nm, Em515nm) was determined using a Molecular Devices M2e microplate reader.

Cytotoxicity assays

To induce oxidative stress, Jurkat cells (2.5 x 10^5 cells per ml) were exposed to SIN-1 (100 μM)[37] or the lipid aldehyde product of lipid peroxidation in vivo, 4-hydroxynonenal (4-HNE; 20 μM) for 24 h in the presence or absence **3**, **6** and **11** (200 μM). After this time, cell viability, as an end point for cytotoxicity, was assessed using Trypan Blue (0.4% (w/v).[38] The concentration of 200 μM for **3**, **6**, and **11** was chosen based on reports in the literature, showing cytoprotection by GYY4137 **3** against oxidative stress in other cell types and this concentration was therefore, used for comparative purposes.[19,35]

LPS treatment of RAW264.7 cells

To induce an inflammatory response *in vitro*, murine RAW264.7 cells were used. Cells were seeded overnight on 24-well cell culture plates (Greiner) at a density of 2.5 x 10^5 cells per ml and allowed to attach overnight. Following attachment, cells were treated with **3**, **6** and **11** (200 μM) for 1 h,

prior to the addition of bacterial lipopolysaccharide (LPS; 1 μg per ml, Sigma) for 24 h. After this time, cell culture media was collected. Nitrite (NO_2^-) formation, as an index for NO synthesis, was assessed by Griess assay. PGE_2 levels in cell culture media were determined by commercial ELISA (Cayman). The concentration of 200 μM for **3**, **6**, and **11** was chosen based on reports in the literature showing inhibition of NO and PGE_2 synthesis by GYY4137 **3** in RAW264.7 and this concentration was therefore, used for comparative purposes.[14,28]

Statistical analysis

All graphs in the original article are plotted with mean +/- standard deviation. In all cases, the mean values were calculated from data taken from at least six separate experiments, performed on separate days using freshly prepared reagents. Where significance testing was performed, ANOVA with post-hoc t-test was used; *$p < 0.05$.

Additional references for cell culture experiments

36 C. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton and M. Xian, *Angew. Chem., Int. Ed.*, 2011, **50**, 10327.

37 M. K. Johnson and G. Loo, *Mutat. Res.*, 2000, **459**, 211.

38 S. Romeo, L. Zeni, M. Sarti, A. Sannino, M. R. Scarfi, T. Vernier and O. Zeni, *PLoS One*, 2011, **6**, e28419.