Electronic Supplementary Information for:

Synthesis of Thiophosphoramidates: Click Chemistry for Phosphates

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Contents

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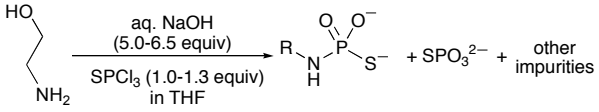
General procedures.

Reagents were purchased from Aldrich, Acros, Fisher, Apollo Scientific Ltd, Goss Scientific Instruments and Amersham Biosciences, and were used as supplied, except thiophophorylchloride, which was distilled under an atmosphere of nitrogen. Dry dichloromethane and tetrahydrofuran were produced by the solvent drying service within the Chemistry Department, Durham University (Pure Solv MD Solvent Purification System 400, Inovative technology). Measurements of pH were performed using a pre-calibrated (4.00 ± 0.01, 7.02 ± 0.01, 10.04 ± 0.01) HANNA 210 Microprocessor pH meter at 22 °C. For larger sample volumes, a Hanna combination electrode was used, whereas an Orion micro PerpHecT ROSS combination electrode was used for smaller volumes. Lyophilisation was carried out on a Jouan high vacuum system. Mass spectra were acquired on Thermo Electron LTQ and Micromass LCT spectrometers.

Optimization of ethanolamine thiophosphorylation (Manuscript Table 1, entries 1-4)

In order to optimize thiophosphorylation of ethanolamine, several experiments were performed where the number of equivalents of thiophosphoryl chloride was varied.



Ethanolamine was vigorously stirred with an aqueous solution of 1 M sodium hydroxide (see table 1 for quantities) in a 100 mL round bottomed flask with indentations that aim to ensure turbulent mixing, and the mixture was placed in an ice bath. Thiophosphoryl chloride (ESI Table 1) was dissolved in dry THF (7 mL) was added dropwise to the stirred aqueous mixture over the course of 10 minutes. The mixture was stirred vigorously for 1 h followed by

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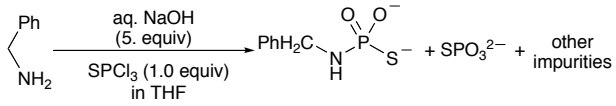
removal of THF under reduced pressure. The residual aqueous solution was then lyophilised to afford a white powder of crude ethanolamine thiophosphoroamidate. Products were then assessed using integrated ^{1}H and ^{31}P NMR spectra that are presented below.

ESI Table 1. Quantities of reagents used during ethanolamine thiophosphorylation experiments

Main paper Table 1 entry number	1 M NaOH			$PSCl_3$			ethanolamine	
	mL	mmol	Eq	mL	mmol	Eq	mL	mmol
1	11.5	11.5	5	0.232	2.3	1	0.135	2.3
2	12.6	12.6	5.5	0.255	2.53	1.1	0.135	2.3
3	13.8	13.8	6	0.278	2.76	1.2	0.135	2.3
4	14.95	14.95	6.5	0.301	2.99	1.3	0.135	2.3

The other impurities in the samples were inorganic phosphate and unidentified structures, containing phosphoryl groups. Summary of spectroscopic data (^{1}H and ^{31}P NMR spectra are available on p. 13-20): ^{1}H NMR (400 MHz; D_2O) δ 3.66 (2 H, t, J 5.7, OHCH_2), 2.97-2.91 (2 H, m, CH_2NH); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 44.7 (t, J 9.3, NHPS); ^{13}C NMR (101 MHz; D_2O) δ 62.6 (d, $^{3}J_{C\text{-}P}$ 8.1, OHCH$_2$), 44.6 (CH$_2$NH); HRMS (ES$^-$, *m/z*): calcd for $C_2H_7NO_3PS^-$: 155.9889; found: 155.9888.

Thiophosphorylation of benzylamine (Manuscript Table 1, entry 5)



Benzylamine (1 Eq, 0.5 mL, 4.6 mmol) was vigorously stirred with an aqueous solution of 1 M sodium hydroxide (22.8 mL) and water (2.96 mL) in a 100 mL round bottomed flask with indentations that aim to ensure turbulent mixing, placed in an ice bath. Thiophosphoryl chloride (1 Eq, 0.464 mL, 4.6 mmol) was dissolved in dry THF (14 mL) and added dropwise to the stirred aqueous mixture over the course of 10 minutes. The mixture was stirred vigorously for 1 h followed by removal of THF under reduced pressure. The residual aqueous solution was then lyophilised, to afford a white powder of crude benzyl thiophosphoroamidate. Summary of spectroscopic data (^{1}H and ^{31}P NMR spectra are available on p. 21-22): ^{1}H NMR (400 MHz; D_2O) δ 7.39-7.25 (5 H, m, C_6H_5), 3.92 (2 H, d, J 6.8, CH_2NH); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 43.0 (t, J 6.8, NHPS); ^{13}C NMR (101 MHz; D_2O) δ 141.3 (d, $^{3}J_{C\text{-}P}$ 12.4, *i*-C_6H_5), 128.8 (*o*-C_6H_5), 128.2 (*m*-C_6H_5), 127.1 (*p*-C_6H_5), 47.1 (CH$_2$NH); HRMS (ES$^-$, *m/z*): calcd for $C_7H_9NO_2PS^-$: 202.0097 found 202.0099.

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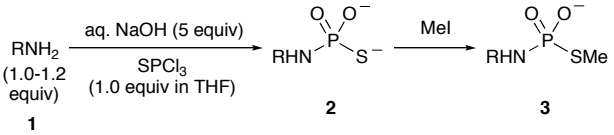
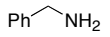
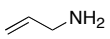
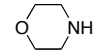
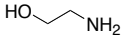
N-Thiophosphorylation of amines and *S*-methylation of *N*-thiophosphoramidates



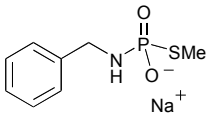
ESI Table 2. *N*-Thiophosphorylation of amines and *S*-methylation of *N*-thiophosphoramidates

entry	RNH_2	Eq	RNH_2 (μL)	mmol	*N*-thio phosphorylation (h)	*S*-methyl-ation (h)	conversion to *N*-alkyl *S*-methylthio phosphoramidate **8**, %
1		1.2	300	2.76	0.25	0.25	98[a],96[b]
2		1.2	206	2.76	0.25	0.3	98[a], 91 [b]
3		1.2	241	2.76	1	1.5	98[a], 92[b]
4		1	138	2.3	1	4	92[a], 89[b]

[a] Determined by ^{31}P NMR. [b] Determined by ^{1}H NMR.

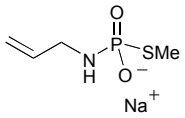
Amine RNH_2 (ESI Table 2) was mixed with sodium hydroxide (5 Eq, 1 M aqueous solution, 11.5 mL, 11.5 mmol) and water (1.48 mL) in a 50 mL round-bottomed flask with indentations in the flask walls that aimed to cause turbulent mixing. Thiophosphoryl chloride (1 Eq, 232 μL, 2.3 mmol) dissolved in THF (7 mL) was added dropwise to the aqueous mixture over the course of 10 minutes. After vigorous mixing for a prescribed period (ESI Table 2), methyl iodide was added (2 Eq, 286 μL, 4.6 mmol) and vigorous mixing was continued for a prescribed period (ESI Table 2). Then, ether (ESI and Manuscript Table 2, entries 1 and 4) or chloroform (ESI and Manuscript Table 2, entries 2 and 3) extraction (3 × 30 mL) was performed and the aqueous layer was lyophilised, to afford a white powder. This material was then subjected to NMR and MS analyses that are summarised below (^{1}H and ^{31}P NMR spectra are available p. 23-30).

Table 2, entry 1:



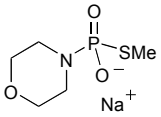
^{1}H NMR (400 MHz; D_2O) δ 7.38–7.23 (5 H, m, C_6H_5), 3.88 (2 H, d, J 10.4, CH_2NH), 1.93 (3 H, d, J 12.8, SCH_3); ^{31}P NMR [^{1}H] (283 MHz; D_2O) δ 26.2-25.8 (m, NH*P*S); ^{13}C NMR (176 MHz; D_2O) δ 141.1 (d, $^{3}J_{C\text{-}P}$ 8.7, *i*-C_6H_5), 129.0 (*m*-C_6H_5), 128.1 (*o*-C_6H_5), 127.5 (*p*-C_6H_5), 45.7 (CH_2NH), 12.2 (SCH_3); HRMS (ES^-, *m/z*): calcd for $C_8H_{11}NO_2PS^-$: 216.0253 found 216.0252.

Table 2, entry 2:



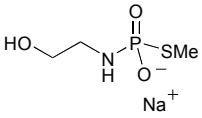
^{1}H NMR (400 MHz; D_2O) δ 6.03-5.91 (1 H, m CH_2=C*H'*), 5.26 (1 H, dq, *J* 17.2 and 1.6, CH*H*=CH' where *H* and H' are in a *trans*-relationship), 5.12 (1 H, app dq, *J* 10.2 and 1.6 C*H*H=CH' where *H* and H' are in a *cis*-relationship) 3.47 (2 H, app ddt, *J* 10.9, 5.6 and 1.6, CH_2NH), 2.13 (3 H, d, *J* 13.0, SCH_3); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 27.9-27.4 (m, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 137.4 (d, $^3J_{C\text{-}P}$ 7.3, CH_2=*C*H), 115.6 (*C*H_2=CH), 44.4 (*C*H_2NH), 12.3 (S*C*H_3); HRMS (ES^-, *m/z*): calcd for $C_4H_9NO_2PS^-$: 166.0097 found 166.0097.

Table 2, entry 3:



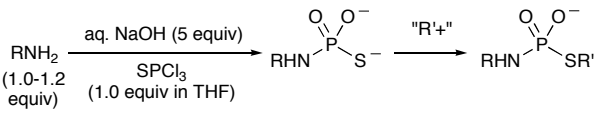
^{1}H NMR (400 MHz; D_2O) δ 3.78-3.74 (4 H, m, O(CH_2)$_2$), 3.17-3.12 (4 H, m, (CH_2)$_2$N), 2.23 (3 H, d, *J* 12.8, SCH_3); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 25.5-25.0 (m, N*P*S); ^{13}C NMR (101 MHz; D_2O) δ 67.4 (d, $^3J_{C\text{-}P}$ 7.3, O(*C*H_2)$_2$), 45.3 ((*C*H_2)$_2$N), 12.0 (d, $^2J_{C\text{-}P}$ 2.9, S*C*H_3); HRMS (ES^-, *m/z*): calcd for $C_5H_{11}NO_3PS^-$: 196.0203 found 196.0202.

Table 2, entry 4:



^{1}H NMR (400 MHz; D_2O) δ 3.57 (2 H, t, *J* 5.6, OHCH_2), 2.94-2.87 (2 H, m, CH_2NH), 2.05 (3 H, d, *J* 12.4, SCH_3); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 28.3-27.8 (m, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 62.3 (d, $^3J_{C\text{-}P}$ 8.1, OH*C*H_2), 43.5 (*C*H_2NH), 12.1 (S*C*H_3); HRMS (ES^-, *m/z*): calcd for $C_3H_9NO_3PS^-$: 170.0046 found 170.0046.

S-alkylation of *N*-thiophosphoramidates



Amine (ESI table 3, RNH_2) was mixed with sodium hydroxide (5 Eq, 1 M aqueous solution, 11.5 mL, 11.5 mmol) and water (1.48 mL) in a 50 mL round-bottomed flask with indentations that aim to ensure turbulent mixing. Thiophosphoryl chloride (1 Eq, 0.232 mL, 2.3 mmol) dissolved in THF (7 mL) was added dropwise to the aqueous mixture over the course of 10 minutes. After vigorous mixing for 15 minutes to allow *N*-

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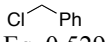
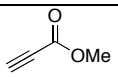
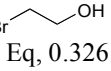
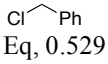
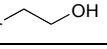
thiophosphorylation to take place, an alkylating agent was added (ESI Table 3, alkylating agent) along with additional sodium hydroxide solution (ESI table 3, additional 1 M NaOH) and vigorous mixing was continued for a prescribed period (ESI table 3, *S*-alkylation time). Then, ether (3 × 30 mL, ESI Table 2, entries 1-11) or chloroform (3 × 30 mL, entries 12-16) extraction was performed and the aqueous layer was lyophilised. The crude material was then subjected to ^{1}H and ^{31}P analyses to assess conversion levels (ESI table 3, conversion to *N*-alkyl *S*-alkyl thiophosphoramidate), and these spectra are available below (p. 31-46 and 51-66). Entry 8 was treated in a different manner, described in the table legend, and associated spectra are also available (p. 47-50).

ESI Table 3. *S*-alkylation of *N*-thiophosphoramidates

Main paper Table 3 entry number	**RNH_2 (mL)**	**Alkylating agent (mL)**	**Additional 1 M NaOH (mL)**	***S*-alkylation time (h)[a]**	**conversion to *N*-alkyl *S*-alkyl thio-phosphoramidate (%)**
1	 1.2 Eq, 0.300	 2 Eq, 0.326	0	17	>99[b],>99[c]
2	 1.2 Eq, 0.300	 2 Eq, 0.529	0	22	>99[b],>99[c]
3	 1.2 Eq, 0.300	 2 Eq, 0.448	0	25	>99[b],>99[c]
4	 1.2 Eq, 0.300	 10 Eq, 0.229, (11Eq, 0.251)	11.5 (23)	96[d] (24)[e,f]	>92[b],>92[c] (>90[b],>99[c])
5	 1.2 Eq, 0.300	 2 Eq, 0.555	0	5[g]	96[b], 97[c]
6	 1.2 Eq, 0.300	 2 Eq, 0.526	0	19	90[b,i]
7	 1.2 Eq, 0.300	 2 Eq, 0.411	0	5	93[b], 96[c]
8	 1.0 Eq, 0.038	 2 Eq, 0.270 mg	1	~80[d,h]	82[b]
9	 1.2 Eq, 0.206	 2 Eq, 0.326	2	1.5	>99[b], 95[c]
10	 1.2 Eq, 0.206	 2 Eq, 0.529	0.9	23	>99[b], >99[c]
11	 1.2 Eq, 0.206	 2 Eq, 0.448	0	120	>99[b], 98[c]
12			1.8	1	92[b],87[c]

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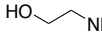
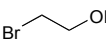
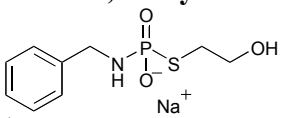
	1.2 Eq, 0.241	2 Eq, 0.326			
13	 1.2 Eq, 0.241	 2 Eq, 0.529	3.9	4[f]	93[b], 85[c]
14	 1.2 Eq, 0.241	 2 Eq, 0.448	0.9	7[f]	98[b], 99[c]
15	 1.2 Eq, 0.241	 10 Eq, 0.229	9.2	17[f,j]	94[b], 89[c]
16	 1.2 Eq, 0.138	 2 Eq, 0.326	0	20	91[b], 78[c]

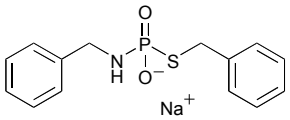
[a] All alkylations were performed at room temperature except where stated. [b] Determined by ^{31}P NMR. [c] Determined by ^{1}H NMR. [d] Alkylation reaction heated at 50 °C. [e] Alkylating agent was added in three portions (2×0.114 and 0.023 ml) followed by additional 1 M NaOH in two portions (ESI Table 3, 2×11.5 mL) after the first and second additions of alkylating agent. [f] Alkylation reaction heated at 80 °C. [g] Reaction mixture was adjusted to pH 8 with HCl (0.05 M, 46 mL, 2.3 mmol) prior to solvent removal. [h] Benzylamine (1 Eq, 0.038 mL, 0.34 mol) was mixed with sodium hydroxide solution (5 Eq, 1 M aqueous solution, 1.7 mL, 1.7 mmol) and water (0.218 mL) in a round-bottomed flask with indentations that aimed to ensure turbulent mixing. Thiophosphoryl chloride (1 Eq, 0.034 mL, 0.34 mmol), dissolved in THF (1 mL) was added dropwise to the aqueous mixture over the course of 10 minutes. 5′-deoxy-5′-iodoguanosine (ESI Table 3, alkylating agent) was added in two equal portions along with the additional sodium hydroxide solution (ESI Table 3, additional 1 M NaOH) just after the first portion. The reaction mixture was vigorously mixed at 50 °C. The second portion of 5′-deoxy-5′-iodoguanosine) was added after 48 h. After 80 h total reaction time, ether extraction (3 × 10 mL) was performed and the resulting aqueous solution was lyophilised. The crude lyophilised product was purified via anion exchange chromatography (DEAE Sepharose® Fast Flow chromatography media, 50 mL bed volume, 10 × 3 cm column dimensions, 3 mL/min flow rate) using a gradient of 50-500 mM triethylammonium bicarbonate (TEAB), pH 7.6 buffer. Cation exchange chromatography was carried out on a column packed with Dowex® 50W×2, 200-400 (50 mL, 30 × 2 cm, 3 mL/min) that had been converted to Na^+ form by washing with two column volumes of aqueous sodium hydroxide solution (0.1 M) and then water. Elution was performed using water. [i] A mixture of isomers (63:26) was obtained, where attack at the less hindered end was favoured. [j] Alkylation agent was added over two portions (2×0.114 ml) along with the additional 1 M NaOH (2×11.5 ml). The second portion of alkylating agent and sodium hydroxide solution were added after 1 h of reaction time. The reaction was then allowed to proceed for an additional 16 h.

Table 3, entry 1:



^{1}H NMR (400 MHz; D_2O) δ 7.37-7.19 (5 H, m, C_6H_5), 3.91 (2 H, d, J 10.8, CH_2NH), 3.59-3.55 (2 H, m, CH_2OH), 2.68-2.61 (2 H, m, SCH_2); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 25.6 (app qn, J 11.5, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 140.7 (d, $^{3}J_{C\text{-}P}$ 8.1, *i*-C_6H_5), 129.1 (*m*-C_6H_5), 128.1 (*o*-C_6H_5), 127.5 (*p*-C_6H_5), 61.7 (d, $^{3}J_{C\text{-}P}$ 5.1, CH_2OH), 45.4 (CH_2NH), 32.1 (SCH_2); HRMS (ES^-, *m/z*): calcd for $C_9H_{13}NO_3PS^-$: 246.0359, found 246.0356.

Table 3, entry 2:



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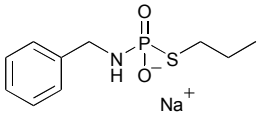
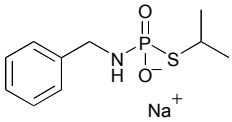
^{1}H NMR (400 MHz; D_2O) δ 7.31-7.18 (10 H, m, 2 × C_6H_5), 3.75 (4 H, 2 × d, both J 10.4, PhCH_2NH and PhCH_2S); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 24.5 (qn, J 10.4, NHPS); ^{13}C NMR (101 MHz; D_2O) δ 140.5 (d, $^{3}J_{C\text{-}P}$ 8.1, i-$C_6H_5CH_2NH$), 139.2 (d, $^{3}J_{C\text{-}P}$ 5.1, i-$C_6H_5CH_2S$), 128.8 (m-$C_6H_5CH_2NH$), 128.7 (m-$C_6H_5SCH_2$), 128.6 (o-$C_6H_5CH_2NH$), 127.7 (o-$C_6H_5SCH_2$), 127.3(p-$C_6H_5CH_2NH$), 127.2 (p-$C_6H_5SCH_2$), 45.4 (CH$_2$NH), 34.5 (SCH$_2$); HRMS (ES$^-$, *m/z*): calcd for $C_{14}H_{15}NO_2PS^-$: 292.0566, found 292.0564.

Table 3, entry 3:



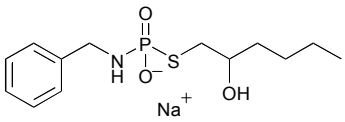
^{1}H NMR (400 MHz; D_2O) δ 7.28-7.18 (5 H, m, C_6H_5), 3.90 (2 H, d, J 10.4, CH_2NH), 2.47 (2 H, dt, J 10.8 and 7.3, SCH_2), 1.44 (2 H, app sx, J 7.3, $CH_2CH_2CH_3$), 0.78 (3 H, t, J 7.3, CH_2CH_3); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 25.6 (app qn, J 10.6, NHPS); ^{13}C NMR (101 MHz; D_2O) δ 140.7 (d, $^{3}J_{C\text{-}P}$ 7.1, i-C_6H_5), 129 (m-C_6H_5), 128.1 (o-C_6H_5), 127.5 (p-C_6H_5), 45.4 (CH$_2$NH), 32.2 (SCH$_2$), 23.7 (d, $^{3}J_{C\text{-}P}$ 5.8, $CH_2CH_2CH_3$), 12.8 (CH_2CH_3); HRMS (ES$^-$, *m/z*): calcd for $C_{10}H_{15}NO_2PS^-$: 244.0566, found 244.0565.

Table 3, entry 4 (two sets of ^{1}H and ^{31}P spectra available):



^{1}H NMR (400 MHz; D_2O) δ 7.28-7.20 (5 H, m, C_6H_5), 3.98 (2 H, d, J 10.5, CH_2NH), 3.14-3.01 (1 H, m, CH(CH_3)$_2$), 1.42 (6 H, d, J 6.8, CH(CH_3)$_2$); δ_P[^{1}H](162 MHz; D_2O) 24.8 (app q, J 10.3, NHPS); ^{13}C NMR (101 MHz; D_2O) δ 140.8 (d, $^{3}J_{C\text{-}P}$ 8.1, i-C_6H_5), 128.7 (m-C_6H_5), 128.1 (o-C_6H_5), 127.5 (p-C_6H_5), 45.6 (CH$_2$NH), 36.2 (CH(CH_3)$_2$), 25.2 (d, $^{3}J_{C\text{-}P}$ 5.1, CH(CH$_3$)$_2$); HRMS (ES$^-$, *m/z*): calcd for $C_{10}H_{15}NO_2PS^-$: 244.0566, found 244.0567.

Table 3, entry 5:



^{1}H NMR (400 MHz; D_2O) δ 7.23-7.01 (5 H, m, C_6H_5), 3.87 (2 H, d, J 11.0, CH_2NH), 3.48-3.40 (1 H, m, CH_2CHOH CH_2), 2.53-2.40 (2 H, m, SCH_2CH), 1.27-0.90 (6 H, m, OHCHCH_2 and OHCH(CH_2)$_2$), 0.70 (3 H, t, J 7.0, CH_2CH_3); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 24.8 (app qn, J 10.6, NHPS); ^{13}C NMR (101 MHz; D_2O) δ 140.7 (d, $^{3}J_{C\text{-}P}$ 7.3, i-C_6H_5), 128.5 (m-C_6H_5), 127.8 (o-C_6H_5), 126.9 (p-C_6H_5), 71.3 (d, $^{3}J_{C\text{-}P}$ 5.8, CH_2CHOH), 45.6 (CH$_2$NH), 36.8 (SCH_2-

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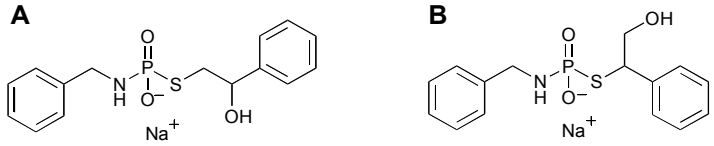
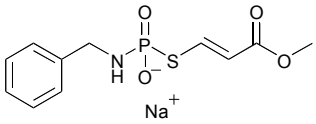
CH), 35.3 (OHCH*C*H$_2$), 27.4 (CHCH$_2$*C*H$_2$), 22.3 (CH$_2$*C*H$_2$CH$_2$), 13.8 (CH$_2$*C*H$_3$); HRMS (ES$^-$, *m/z*): calcd for $C_{13}H_{21}NO_3PS^-$: 302.0984, found 302.0984.

Table 3, entry 6:



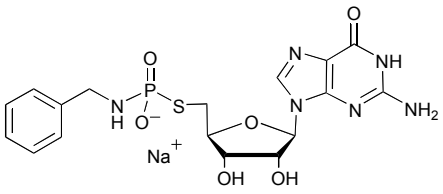
^{1}H NMR (400 MHz; D$_2$O) δ 7.33-6.67 (10 H$_A$+10 H$_B$, m, 2 × C$_6$*H*$_5$), 4.56-4.51 (1 H$_A$, m, OHCH$_2$C*H*), 3.68 (2 H$_A$+2 H$_B$ overlapping, both d, *J* 10.8, C*H*$_2$NH), 2.88-2.69 (2 H$_A$, m, OHC*H*$_2$CH*)*, the other signals were not resolved; ^{31}P NMR [^{1}H] (162 MHz; D$_2$O) δ 25.6 (A: app qn, *J* 10.8, NH*P*S), 23.8 (B: app q, *J* 9.2, NH*P*S; ^{13}C NMR (101 MHz; D$_2$O) δ 142.5 (*i*$_A$-C$_6$H$_5$), 141.1 (*i*$_B$-C$_6$H$_5$), 140.6 (d, $^3J_{C\text{-}P}$ 7.2, *i*$_A$-C$_6$H$_5$CH$_2$NH), 140.1 (d, $^3J_{C\text{-}P}$ 9.6, *i*$_B$-C$_6$H$_5$CH$_2$NH), 128.7-126.3 (4 × *C*$_6$H$_5$), 73.4 (d, $^3J_{C\text{-}P}$ 5.8, Ph*C*$_A$HOHCH$_2$), 66.2 (d, $^3J_{C\text{-}P}$ 6.7, HO*C*$_B$H$_2$CH), 50.8 (*C*$_B$H$_2$NH), 45.6 (*C*$_A$H$_2$NH), 45.4 (S*C*$_A$HPh), 38.0 (S*C*$_B$HCH$_2$); HRMS (ES$^-$, *m/z*): calcd for $C_{15}H_{17}NO_3PS^-$: 322.0672, found 322.0674.

Table 3, entry 7:



^{1}H NMR (400 MHz; D$_2$O) δ 7.28-7.05 (6 H, m, C$_6$*H*$_5$ and SC*H*=CH), 5.82 (1 H, d, *J* 10.3, SCH=C*H*CO$_2$Me), 3.90 (2 H, d, *J* 12.0, C*H*$_2$NH), 3.55 (3 H, s, OC*H*$_3$); ^{31}P NMR [^{1}H] (162 MHz; D$_2$O) δ 21.5 (dt, *J* 14.9 and 12.0, NH*P*S); ^{13}C NMR (101 MHz; D$_2$O) δ 168,7 (C=O), 145.2 (S*C*H=CH), 140.2 (d, $^3J_{C\text{-}P}$ 6.3, *i*-C$_6$H$_5$CH$_2$NH), 128.7 (*m*-C$_6$H$_5$), 127.8 (*o*-C$_6$H$_5$), 127.2 (*p*-C$_6$H$_5$), 115.7 (d, $^3J_{C\text{-}P}$ 7.7, SCH=*C*H), 45.7 (*C*H$_2$NH), 51.8 (O*C*H$_3$); HRMS (ES$^-$, *m/z*): calcd for $C_{11}H_{13}NO_4PS^-$: 286.0308, found 286.0308.

Table 3, entry 8:



The purity of the material after ion exchange chromatography was estimated using ^{31}P NMR (95% + 5% inorganic phosphate) and ^{1}H NMR (100%) spectroscopies. ^{1}H NMR (700 MHz; D$_2$O) δ 7.78 (1 H, s, 8-*H*), 7.14-6.99 (5 H, m, C$_6$*H*$_5$), 5.67 (1 H, d, *J* 5.6, 1′-*H*, 2′-*H*), 4.64 (1 H, 2′-*H*, covered with the HOD signal), 4.17 (1 H, d, *J* 4.2, 3′-*H*), 4.13-4.09 (1 H, m, 4′-*H*),

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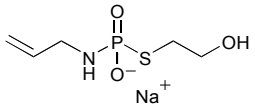
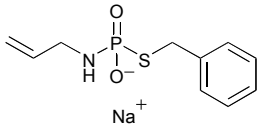
3.75-3.69 (2 H, m, CH_2S), 2.95-2.83 (2 H, m, 5′-*H*); ^{31}P NMR [^{1}H] (283 MHz; D_2O) δ 24.5 (m, NH*P*S); ^{13}C NMR (176 MHz; D_2O) δ 161.0 (6-*C*), 154.8 (br s, 2-*C*), 151.7 (4-*C*), 140.3 (8-*C*), 137.8 (*C*H_2S), 128.6 (*o*-$C_6H_5CH_2S$), 127.4 (*m*-$C_6H_5CH_2S$), 127.2 (*p*-$C_6H_5CH_2S$), 117.0 (5-*C*), 87.5 (1′-*C*), 84.1 (d, $^3J_{C\text{-}P}$ 4.9, 4′-*C*), 73.1 (2′-*C*), 72.6 (3′-*C*), 45.6 (NH*C*H_2Ph), 32.7 (*C*H_2S); HRMS (ES$^-$, *m/z*): calcd for $C_{17}H_{20}N_6O_6PS^-$: 467.0908, found 467.0916.

Table 3, entry 9:



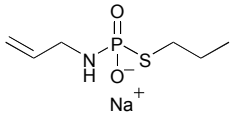
^{1}H NMR (400 MHz; D_2O) δ 5.92-5.81 (1 H, m, CH_2=C*H*), 5.20-5.12 (1 H, m, C*H*H=CH), 5.05-4.99 (1 H, m, CH*H*=CH), 3.67 (2 H, t, *J* 6.2, CH_2OH), 3.40-3.34 (2 H, m, CH_2NH), 2.74 (2 H, dt, *J* 12.3 and 6.2, SCH_2); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 27.6 (app qn, *J* 11.5, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 137.2 (d, $^3J_{C\text{-}P}$ 8.1, CH_2=*C*H), 115.3 (*C*H_2=CH), 61.9 (d, $^3J_{C\text{-}P}$ 7.3, *C*H_2OH), 44.3 (*C*H_2NH), 32.2 (S*C*H_2); HRMS (ES$^-$, *m/z*): calcd for $C_5H_{11}NO_3PS^-$: 196.0203, found 196.0201.

Table 3, entry 10:



^{1}H NMR (400 MHz; D_2O) δ 7.29-7.13 (5 H, m, C_6H_5), 5.74-5.63 (1 H, m, CH_2=C*H*), 5.01 (1 H, dq, *J* 7.2 and 1.7, CH*H*=CH), 4.94-4.89 (1 H, m, C*H*H=CH), 3.75 (2 H, d, *J* 10.7, SCH_2), 3.13 (2 H, ddt, *J* 10.0, 5.7 and 1.5, CH_2NH); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 25.0 (app qn, *J* 10.3, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 139.2 (d, $^3J_{C\text{-}P}$ 6.1, *i*-$C_6H_5CH_2S$), 136.8 (d, $^3J_{C\text{-}P}$ 8.1, CH_2=*C*H), 128.9 (*m*-C_6H_5), 128.8 (*o*-C_6H_5), 127.4 (*p*-C_6H_5), 115.3 (*C*H_2=CH), 44.2 (*C*H_2NH), 34.2 (d, $^3J_{C\text{-}P}$ 2.9, S*C*H_2); HRMS (ES$^-$, *m/z*): calcd for $C_{10}H_{13}NO_2PS^-$: 242.0410, found 242.0411.

Table 3, entry 11:



^{1}H NMR (400 MHz; D_2O) δ 6.03-5.92 (1 H, m, CH_2=C*H*), 5.31-5.24 (1 H, m, CH*H*=CH), 5.16-5.11 (1 H, m, C*H*H=CH), 3.50-3.44 (2 H, m, CH_2NH), 2.67 (2 H, dt, *J* 10.9 and 7.4, SCH_2), 1.64 (2 H, sx, *J* 7.4, CH_2CH$_3$), 0.97 (3 H, t, *J* 7.4 CH$_2$CH_3); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 26.9 (app qn, *J* 10.7, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 137.4 (d, $^3J_{C\text{-}P}$ 8.1,

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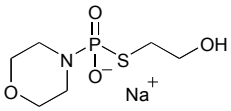
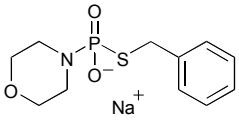
CH_2=*C*H), 115.3 (*C*H_2=CH), 44.5 (*C*H_2NH), 32.5 (S*C*H_2), 24.1 (d, $^3J_{C\text{-}P}$ 5.8, *C*H_2CH_3), 13.1 (CH_2*C*H_3); HRMS (ES$^-$, *m/z*): calcd for $C_6H_{13}NO_2PS^-$: 194.0410, found 194.0410.

Table 3, entry 12:



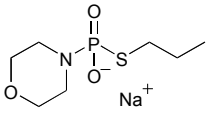
^{1}H NMR (400 MHz; D_2O) δ 3.72 (2 H, t, *J* 6.3, C*H*$_2$OH), 3.65-3.63 (4 H, m, O(C*H*$_2$)$_2$), 3.06-3.02 (4 H, m, (CH_2)$_2$N), 2.83 (2 H, dt, *J* 11.8 and 6.2, SC*H*$_2$).; ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 24.3-24.0 (m, N*P*S); ^{13}C NMR (101 MHz; D_2O) δ 67.3 (d, $^3J_{C\text{-}P}$ 11.3, O(*C*H$_2$)$_2$), 62.1 (d, $^3J_{C\text{-}P}$ 11.3, *C*H$_2$OH), 45.1 ((*C*H$_2$)$_2$N), 31.8 (d, $^3J_{C\text{-}P}$ 2.9, S*C*H$_2$); HRMS (ES$^-$, *m/z*): calcd for $C_6H_{13}NO_4PS^-$: 226.0308, found 226.0308.

Table 3, entry 13:



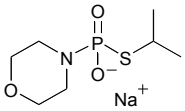
^{1}H NMR (400 MHz; D_2O) δ 7.20-7.15 (2 H, m, *o*-$C_6H_5CH_2S$), 7.07 (2 H, t, *J* 7.4, *m*-$C_6H_5CH_2S$), 7.01-6.95 (1 H, m, *p*-$C_6H_5CH_2S$), 3.75 (2 H, d, *J* 9.9, SC*H*$_2$), 3.52-3.40 (4 H, m, O(C*H*$_2$)$_2$), 2.92-2.79 (4 H, m, (CH_2)$_2$N); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 23.9-23.6 (m, N*P*S); ^{13}C NMR (101 MHz; D_2O) δ 139.2 (d, $^3J_{C\text{-}P}$ 6.1, *i*-*C*$_6$H$_5$CH$_2$S), 128.9 (*m*-*C*$_6$H$_5$), 128.8 (*o*-*C*$_6$H$_5$), 127.2 (*p*-*C*$_6$H$_5$), 67.2 (d, $^3J_{C\text{-}P}$ 7.7, O(*C*H$_2$)$_2$), 45.4 ((*C*H$_2$)$_2$N) , 34.4 (S*C*H$_2$); HRMS (ES$^-$, *m/z*): calcd for $C_{11}H_{15}NO_3PS^-$: 272.0516, found 272.0514.

Table 3, entry 14:



^{1}H NMR (400 MHz; D_2O) δ 3.75-3.72 (4 H, m, O(C*H*$_2$)$_2$), 3.13-3.09 (4 H, m, (CH_2)$_2$N), 2.74 (2 H, dt, *J* 11.6 and 7.4, SC*H*$_2$), 1.66 (2 H, sx, *J* 7.4, CH$_2$C*H*$_2$CH$_3$), 0.99 (3 H, t, *J* 7.4, CH$_2$C*H*$_3$); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 24.8-24.4 (m, N*P*S); ^{13}C NMR (101 MHz; D_2O) δ 67.3 (d, $^3J_{C\text{-}P}$ 7.3, O(*C*H$_2$)$_2$), 45.2 ((*C*H$_2$)$_2$N) , 32.0 (d, $^3J_{C\text{-}P}$ 2.9, S*C*H$_2$), 24.4 (d, $^3J_{C\text{-}P}$ 5.8, *C*H$_2$CH$_2$), 13.1 (CH$_2$*C*H$_3$); HRMS (ES$^-$, *m/z*): calcd for $C_7H_{15}NO_3PS^-$: 224.0515, found 224.0516.

Table 3, entry 15:



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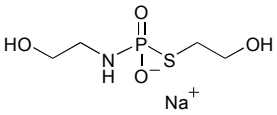
^{1}H NMR (400 MHz; D_2O) δ 3.77-3.75 (4 H, m, O(CH_2)$_2$), 3.36-3.26 (1 H, m, SC*H*), 3.15-3.11 (4 H, m, (CH_2)$_2$N), 1.40 (6 H, d, *J* 6.8, CH(CH_3)$_2$); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 23.8-23.5 (m, N*P*S); ^{13}C NMR (101 MHz; D_2O) δ 67.3 (d, $^{3}J_{C\text{-}P}$ 7.3, O(CH_2)$_2$), 45.2 ((CH_2)$_2$N), 36.1 (*CH*CH_3), 25.9 (d, $^{3}J_{C\text{-}P}$ 5.1, CH*C*H_3), HRMS (ES^-, *m/z*): calcd for $C_7H_{15}NO_3PS^-$: 224.0515, found 224.0516.

Table 3, entry 16:



^{1}H NMR (400 MHz; D_2O) δ 3.67 (2 H, t, *J* 6.4, HOCH_2CH_2NH), 3.54 (2 H, t, *J* 5.6, HOCH_2CH_2S), 2.88 (2 H, app dt, *J* 10.7 and 5.6, CH_2NH), 2.74 (2 H, app dt, *J* 12.2 and 6.4, SCH_2); ^{31}P NMR [^{1}H] (162 MHz; D_2O) δ 26.6 (app qn, *J* 11.5, NH*P*S); ^{13}C NMR (101 MHz; D_2O) δ 62.3 (d, $^{3}J_{C\text{-}P}$ 8.1, HO*C*H_2CH_2NH), 61.8 (d, $^{3}J_{C\text{-}P}$ 5.0, HO*C*H_2CH_2S), 43.5 (NH*C*H_2CH_2OH), 32.2 (S*C*H_2CH_2OH); HRMS (ES^-, *m/z*): calcd for $C_4H_{11}NO_4PS^-$: 200.0151, found 200.0151.

NMR Spectra

^{1}H, ^{13}C, ^{31}P NMR spectra were recorded on the following spectrometers, operating at the quoted frequencies: Varian Mercury 200 MHz (^{1}H 199.991 MHz; ^{31}P 80.957 MHz), Varian Mercury 400 MHz (^{1}H 399.958 MHz; ^{31}P 161.906 MHz), Varian Mercury 500 MHz (^{1}H 499.768 MHz; ^{13}C 125.666 MHz), Varian Mercury 700 MHz (^{1}H 699.737 MHz; ^{31}P 283.257 MHz; ^{13}C 175.948 MHz), Bruker 400 MHz (^{1}H 400.130 MHz; ^{31}P 161.943 MHz; ^{13}C 100.612 MHz). ^{1}H chemical shifts (δ) are reported as parts per million (ppm) upfield relative to tetramethylsilane (δ = 0.00 ppm) and are referenced to the residual protic solvent (HOD, δ = 4.79 ppm, CHD_2Cl_3, δ = 7.26 ppm and (CHD_2)$SOCD_3$, δ = 2.50 ppm). ^{31}P NMR spectra were recorded both in proton decoupled and coupled modes where the multiplicities of the signals are presented along with the coupling constant *J* (Hz). ^{13}C NMR spectra were proton decoupled and unless stated otherwise, the multiplicity was singlet. Sample purity was assessed using ^{1}H NMR spectroscopy and ^{31}P NMR spectroscopy.

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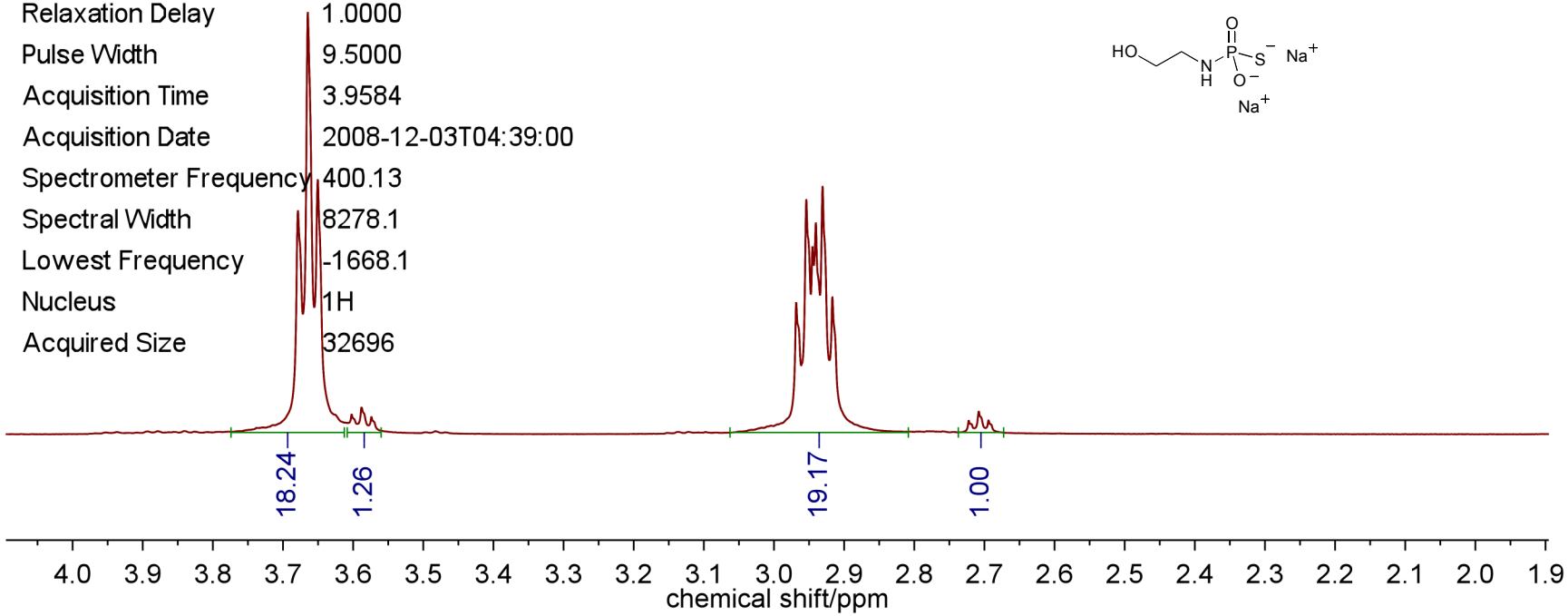
Table 1, entry 1, ^{1}H NMR spectrum

Parameter	Value
Data File Name	/Users/davidhodgson/Desktop/ChemComm/Spectra/Table 1/Table 1 entry 1/02174501/10/fid
Title	2174501
Comment	RGL drwh Owner milena trmcic MT367 thiophosph check
Origin	Bruker BioSpin GmbH
Solvent	D2O
Pulse Sequence	zg30
Number of Scans	16
Relaxation Delay	1.0000
Pulse Width	9.5000
Acquisition Time	3.9584
Acquisition Date	2008-12-03T04:39:00
Spectrometer Frequency	400.13
Spectral Width	8278.1
Lowest Frequency	-1668.1
Nucleus	1H
Acquired Size	32696



100*18.24/(18.24+1.26)=94%

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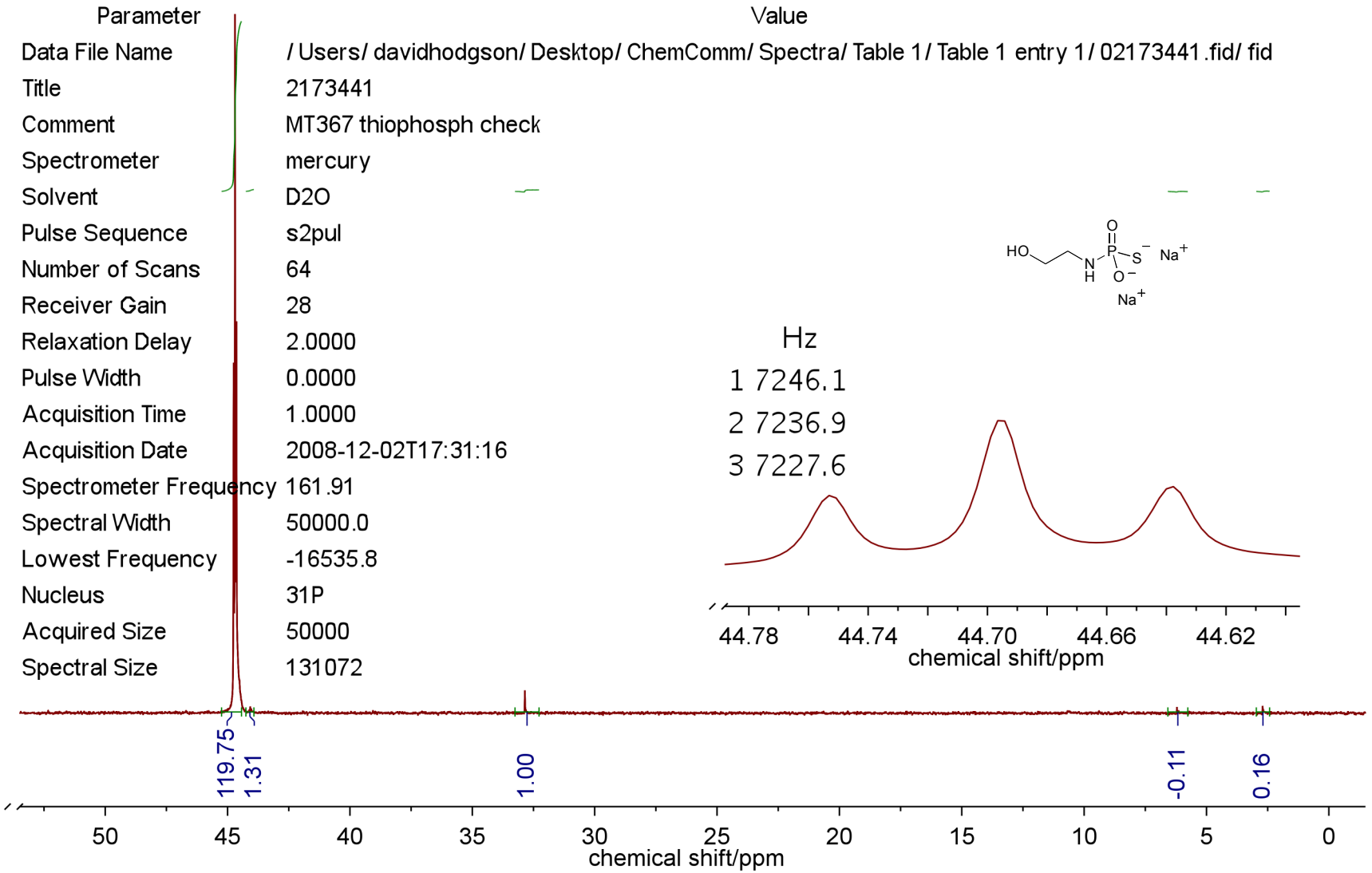
Table 1, entry 1 ^{31}P NMR spectrum



100*119.75/(119.75+1.31+1.00+0.16)=98%

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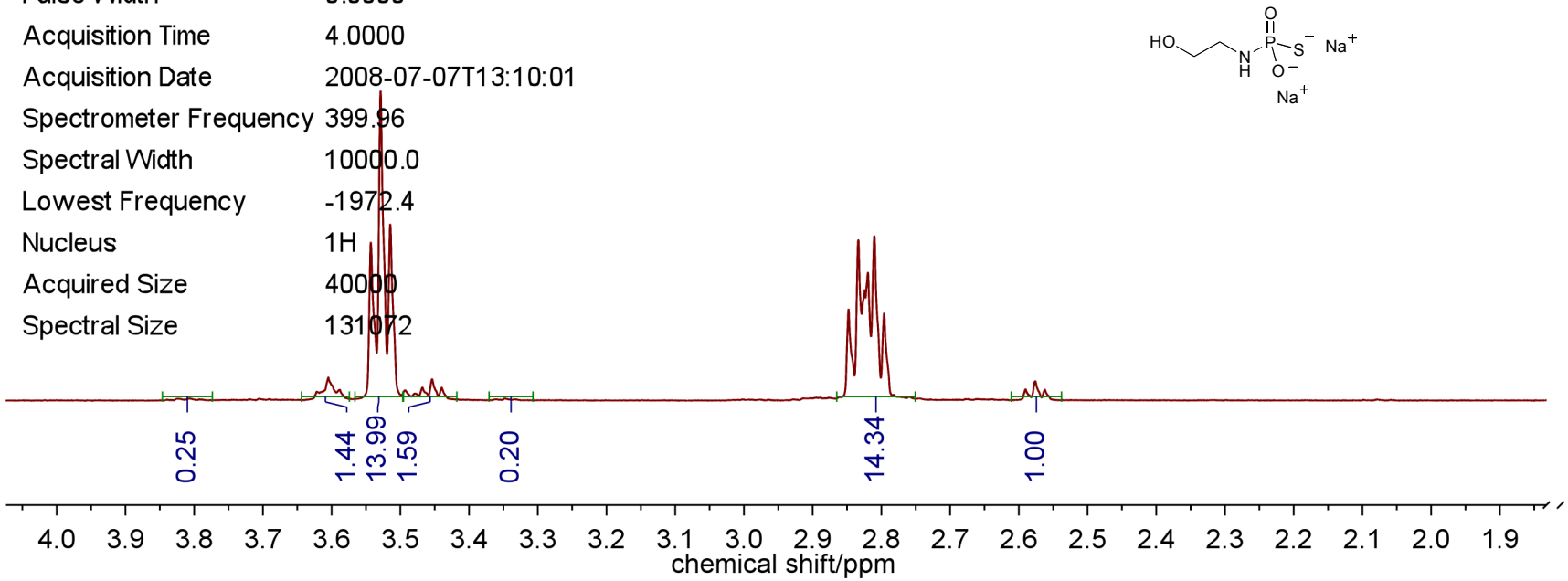
Table 1, entry 2 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 1/ Table 1 entry 2/ 07131111.fid/ fid
Title	7131111
Comment	STANDARD 1H OBSERVE
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	26
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-07-07T13:10:01
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



100*14.34/(14.34+1.00)=93%

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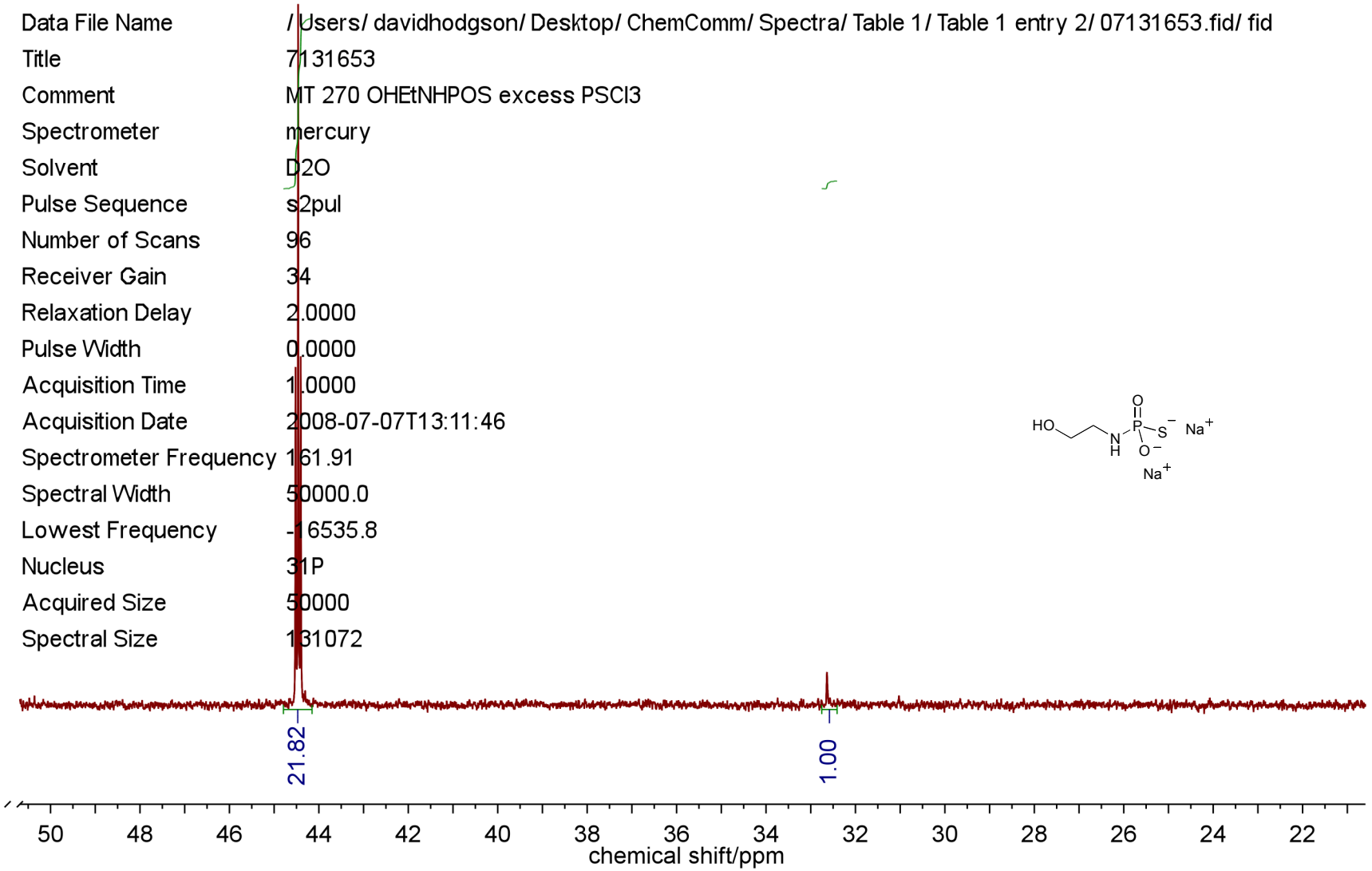
Table 1, entry 2 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/Users/davidhodgson/Desktop/ChemComm/Spectra/Table 1/Table 1 entry 2/07131653.fid/fid
Title	7131653
Comment	MT 270 OHEtNHPOS excess PSCl3
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	96
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-07-07T13:11:46
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*21.82/(21.82+1.00)=96%

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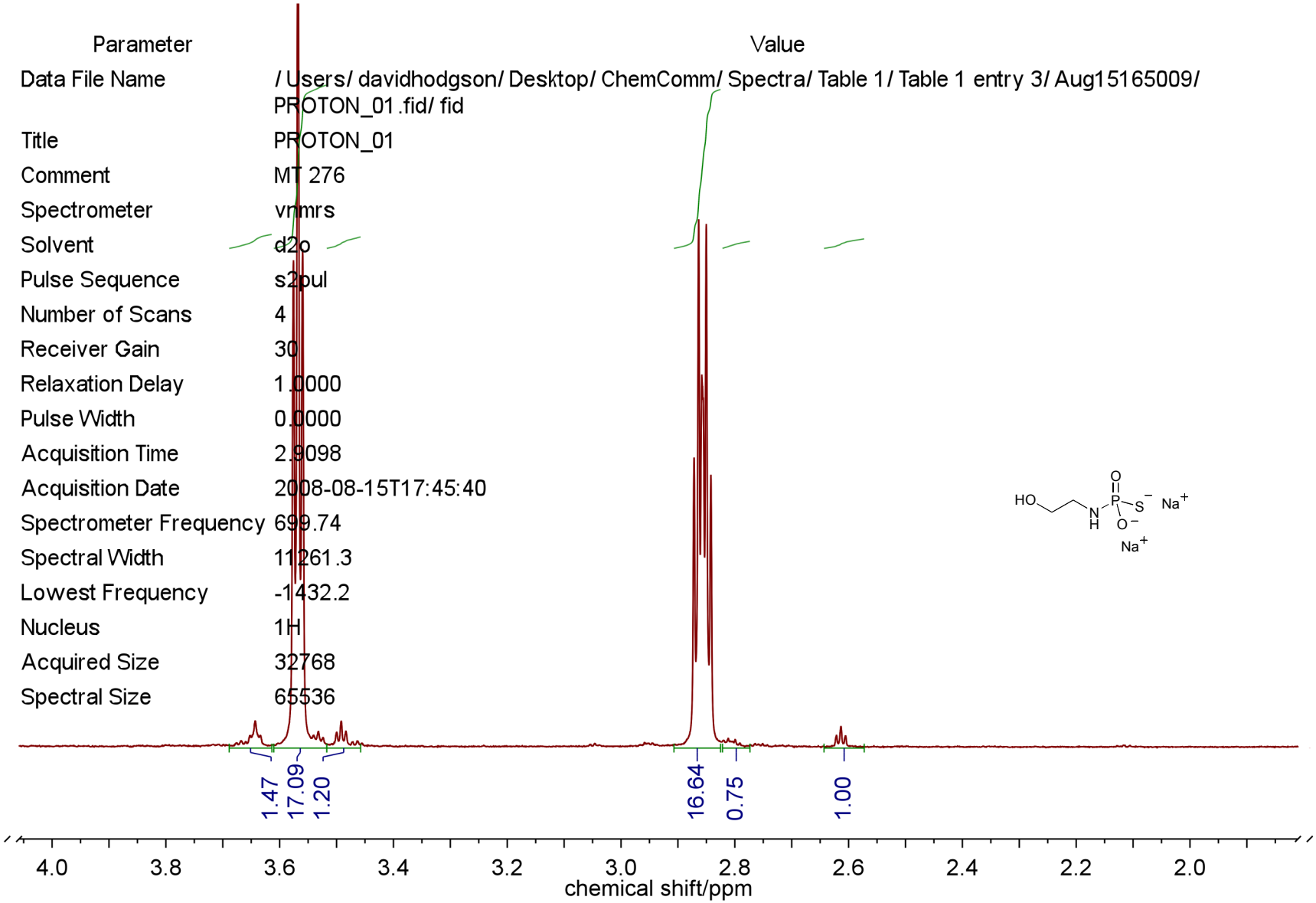
Table 1, entry 3 ^{1}H NMR spectrum



100*17.09/(17.09+1.47+1.20)=86%

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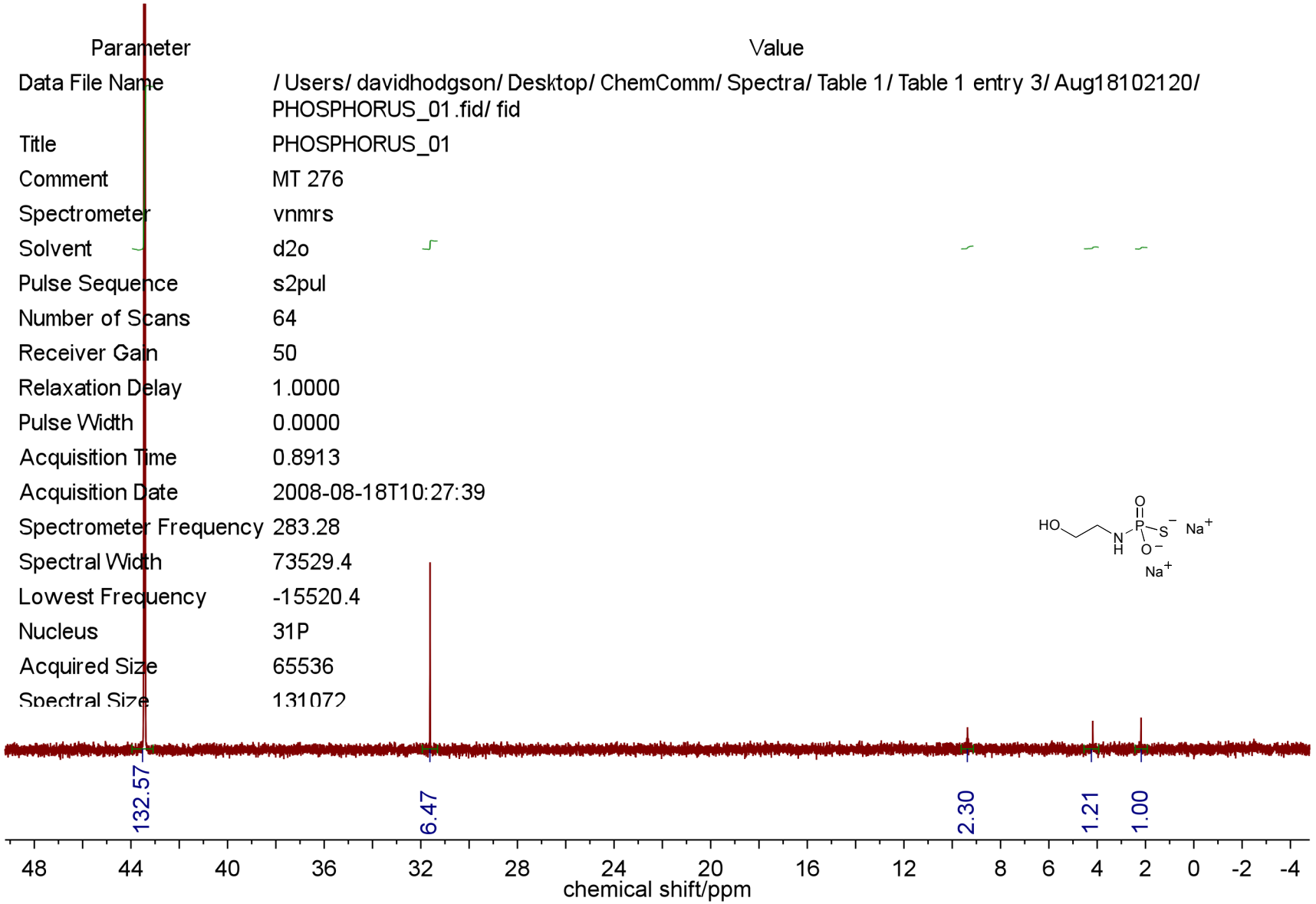
Table 1, entry 3 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 1/ Table 1 entry 3/ Aug18102120/ PHOSPHORUS_01.fid/ fid
Title	PHOSPHORUS_01
Comment	MT 276
Spectrometer	vnmrs
Solvent	d2o
Pulse Sequence	s2pul
Number of Scans	64
Receiver Gain	50
Relaxation Delay	1.0000
Pulse Width	0.0000
Acquisition Time	0.8913
Acquisition Date	2008-08-18T10:27:39
Spectrometer Frequency	283.28
Spectral Width	73529.4
Lowest Frequency	-15520.4
Nucleus	31P
Acquired Size	65536
Spectral Size	131072



100*132.57/(132.57+6.47+2.30+1.21+1.00)=92%

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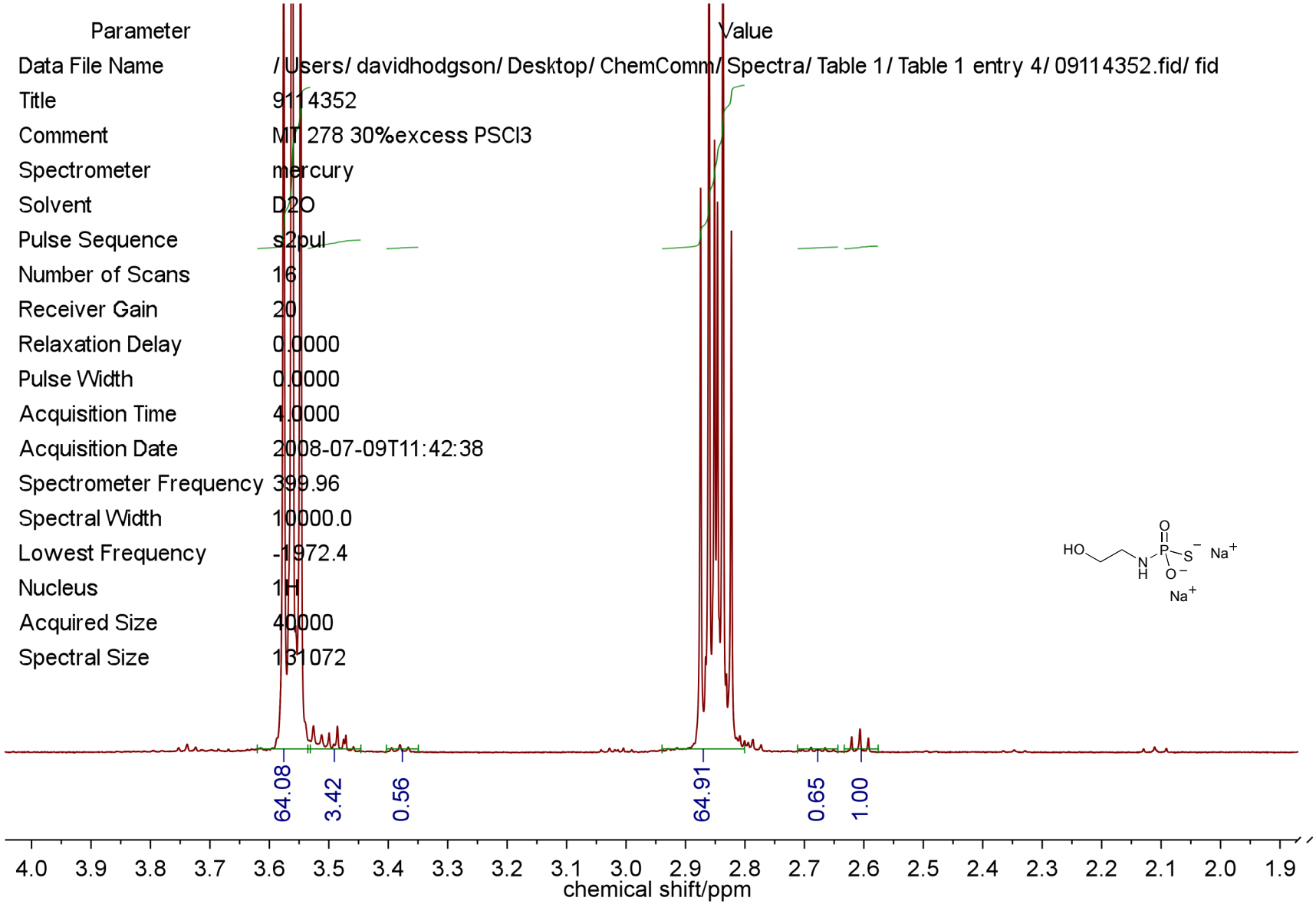
Table 1, entry 4 ^{1}H NMR spectrum



100*64.08/(64.08+3.42+0.56)=94%

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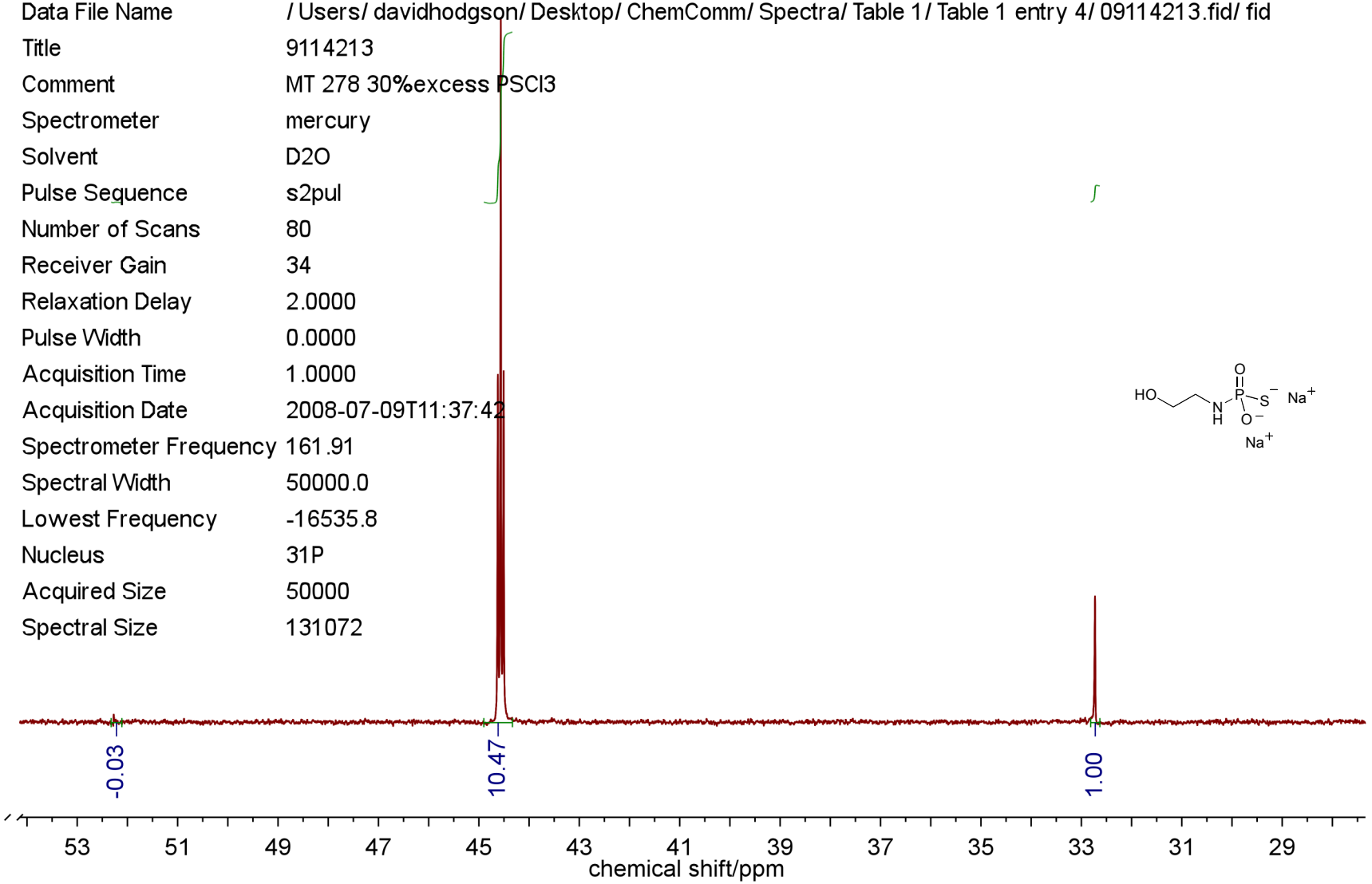
Table 1, entry 4 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 1/ Table 1 entry 4/ 09114213.fid/ fid
Title	9114213
Comment	MT 278 30%excess PSCl3
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	80
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-07-09T11:37:42
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*10.47/(10.47+1.00)=91%

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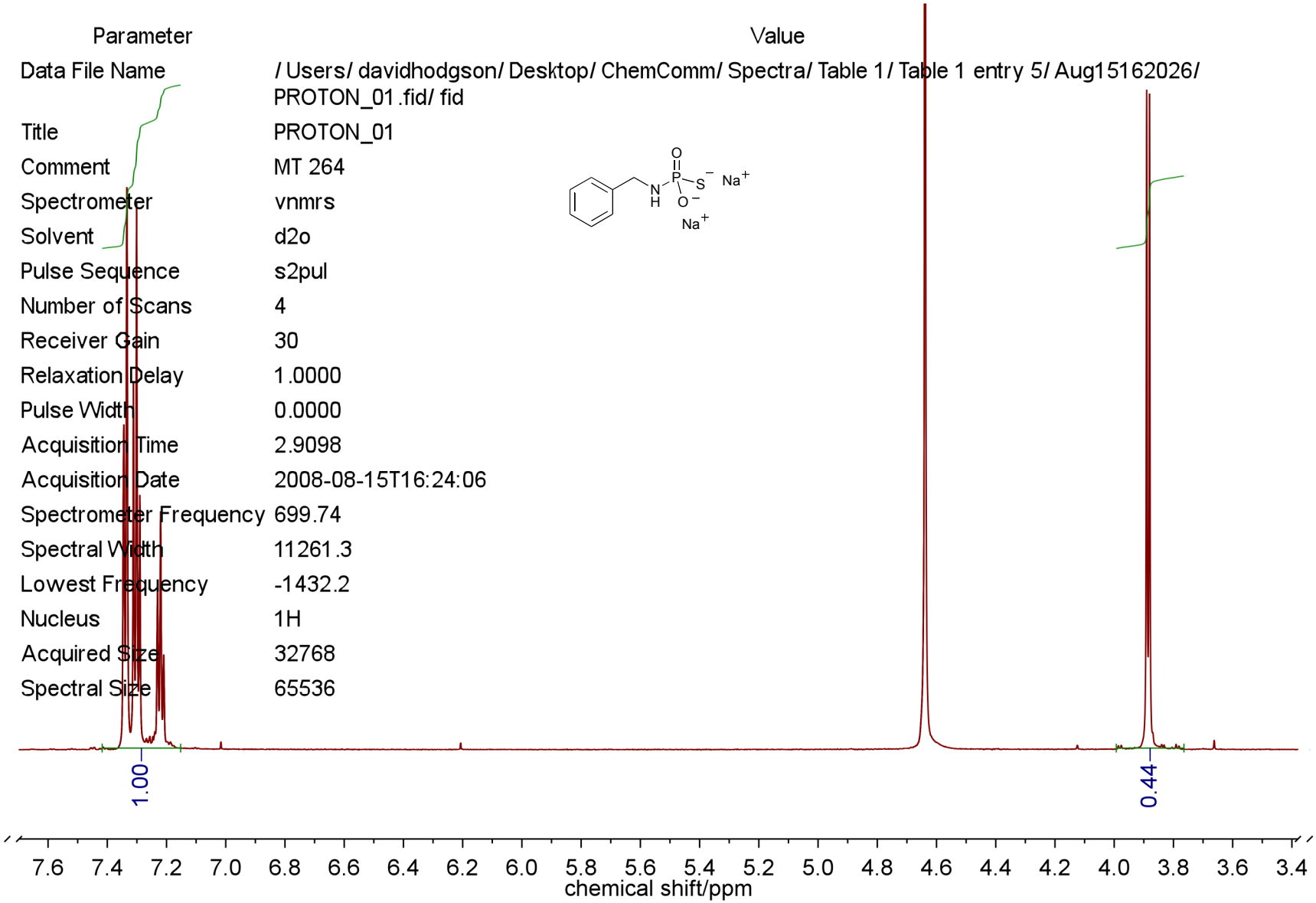
Table 1, entry 5 ^{1}H NMR spectrum



>99%

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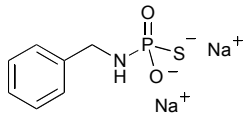
Table 1, entry 5 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 1/ Table 1 entry 5/ Aug18101914/ PHOSPHORUS_01.fid/ fid
Title	PHOSPHORUS_01
Comment	MT 264
Spectrometer	vnmrs
Solvent	d2o
Pulse Sequence	s2pul
Number of Scans	64
Receiver Gain	50
Relaxation Delay	1.0000
Pulse Width	0.0000
Acquisition Time	0.8913
Acquisition Date	2008-08-18T10:22:22
Spectrometer Frequency	283.28
Spectral Width	73529.4
Lowest Frequency	-15520.4
Nucleus	31P
Acquired Size	65536
Spectral Size	131072



100*35.10/(35.10+1.00+0.14+0.06)=97%

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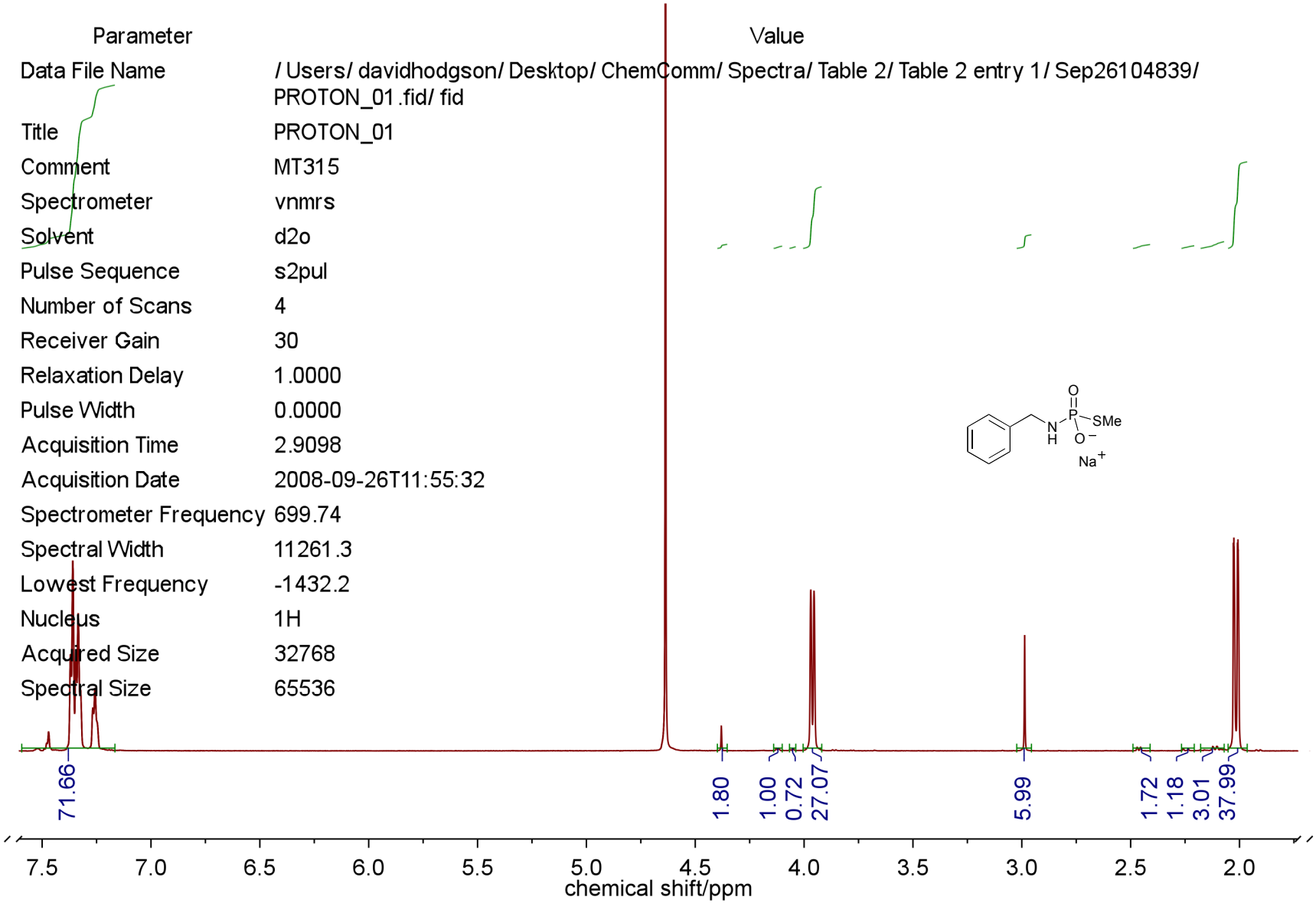
Table 2, entry 1 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 2/ Table 2 entry 1/ Sep26104839/ PROTON_01.fid/ fid
Title	PROTON_01
Comment	MT315
Spectrometer	vnmrs
Solvent	d2o
Pulse Sequence	s2pul
Number of Scans	4
Receiver Gain	30
Relaxation Delay	1.0000
Pulse Width	0.0000
Acquisition Time	2.9098
Acquisition Date	2008-09-26T11:55:32
Spectrometer Frequency	699.74
Spectral Width	11261.3
Lowest Frequency	-1432.2
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



100*27.07/(27.07+1.00+0.72)=94%

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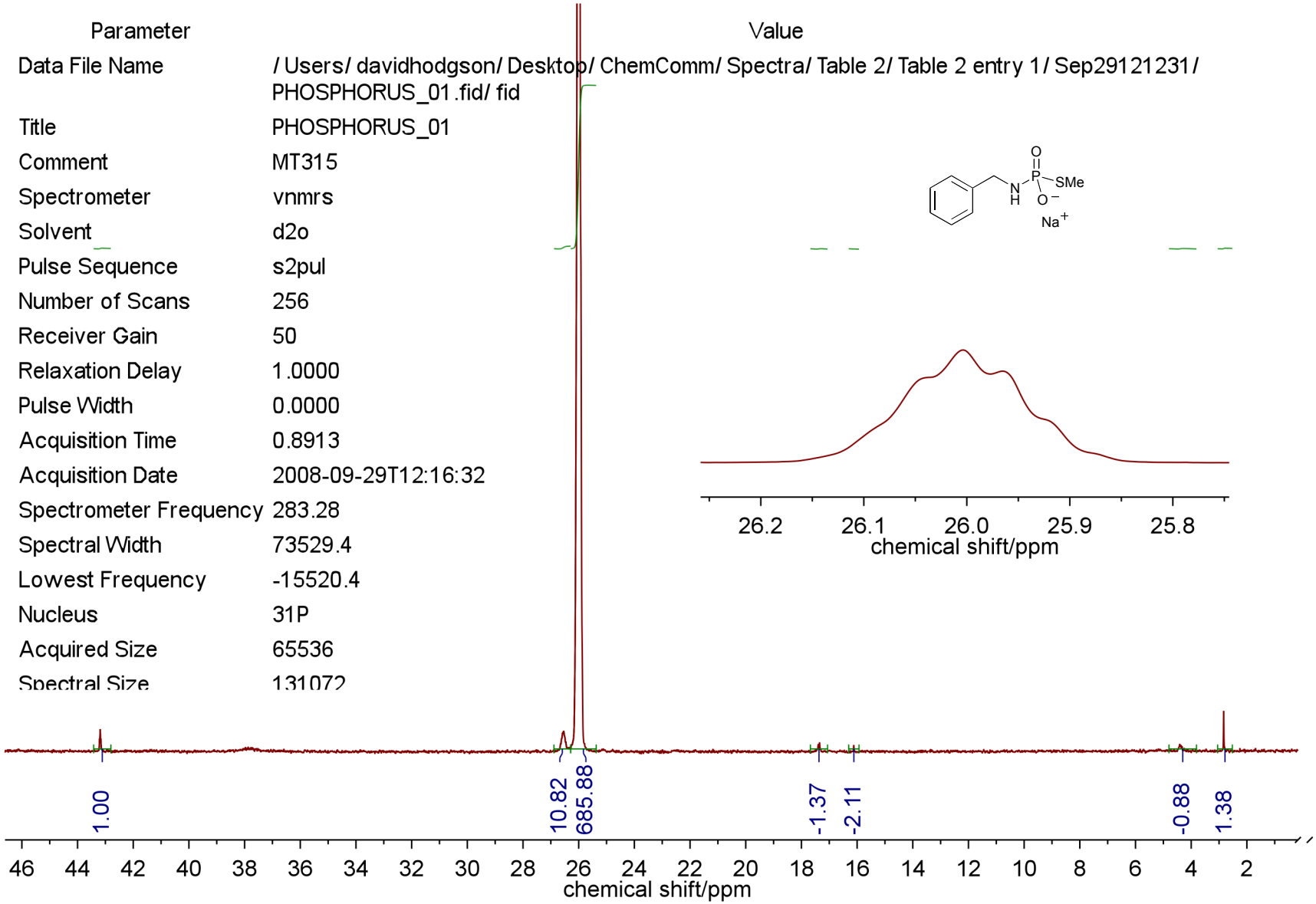
Table 2, entry 1 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/Users/davidhodgson/Desktop/ChemComm/Spectra/Table 2/Table 2 entry 1/Sep29121231/PHOSPHORUS_01.fid/fid
Title	PHOSPHORUS_01
Comment	MT315
Spectrometer	vnmrs
Solvent	d2o
Pulse Sequence	s2pul
Number of Scans	256
Receiver Gain	50
Relaxation Delay	1.0000
Pulse Width	0.0000
Acquisition Time	0.8913
Acquisition Date	2008-09-29T12:16:32
Spectrometer Frequency	283.28
Spectral Width	73529.4
Lowest Frequency	-15520.4
Nucleus	31P
Acquired Size	65536
Spectral Size	131072



100*685.88/(1.00+10.82+685.88+1.38)=98%

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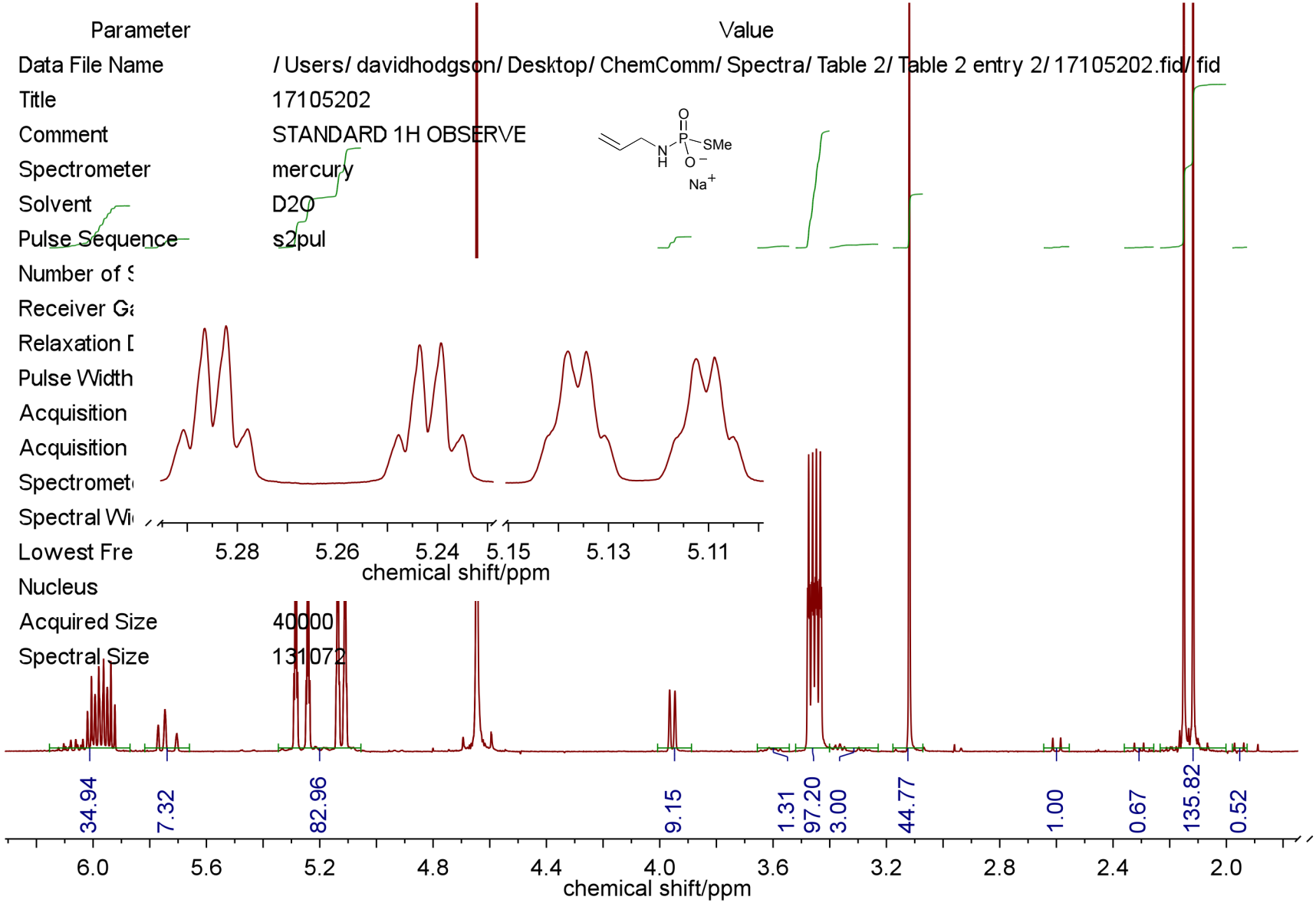
Table 2, entry 2 ^{1}H NMR spectrum



100*97.20/(97.20+1.31+3.00)=96%

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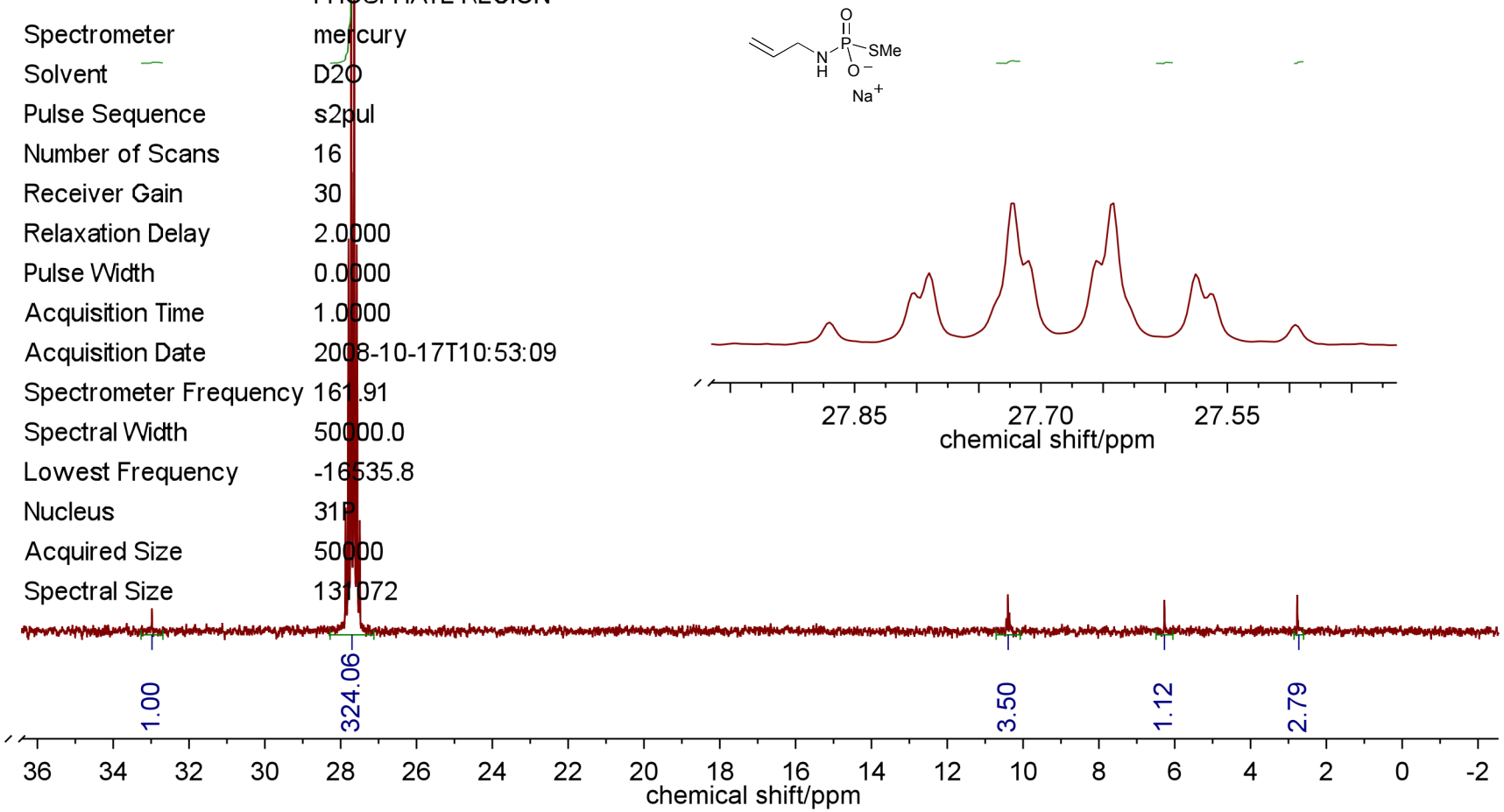
Table 2, entry 2 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 2/ Table 2 entry 2/ 17105420.fid/ fid
Title	17105420
Comment	PHOSPHORUS OBSERVE STANDARD PARAMETERS PHOSPHATE REGION
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	30
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-10-17T10:53:09
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*324.06/(324.06+1.00+3.50+1.12+2.79)=97%

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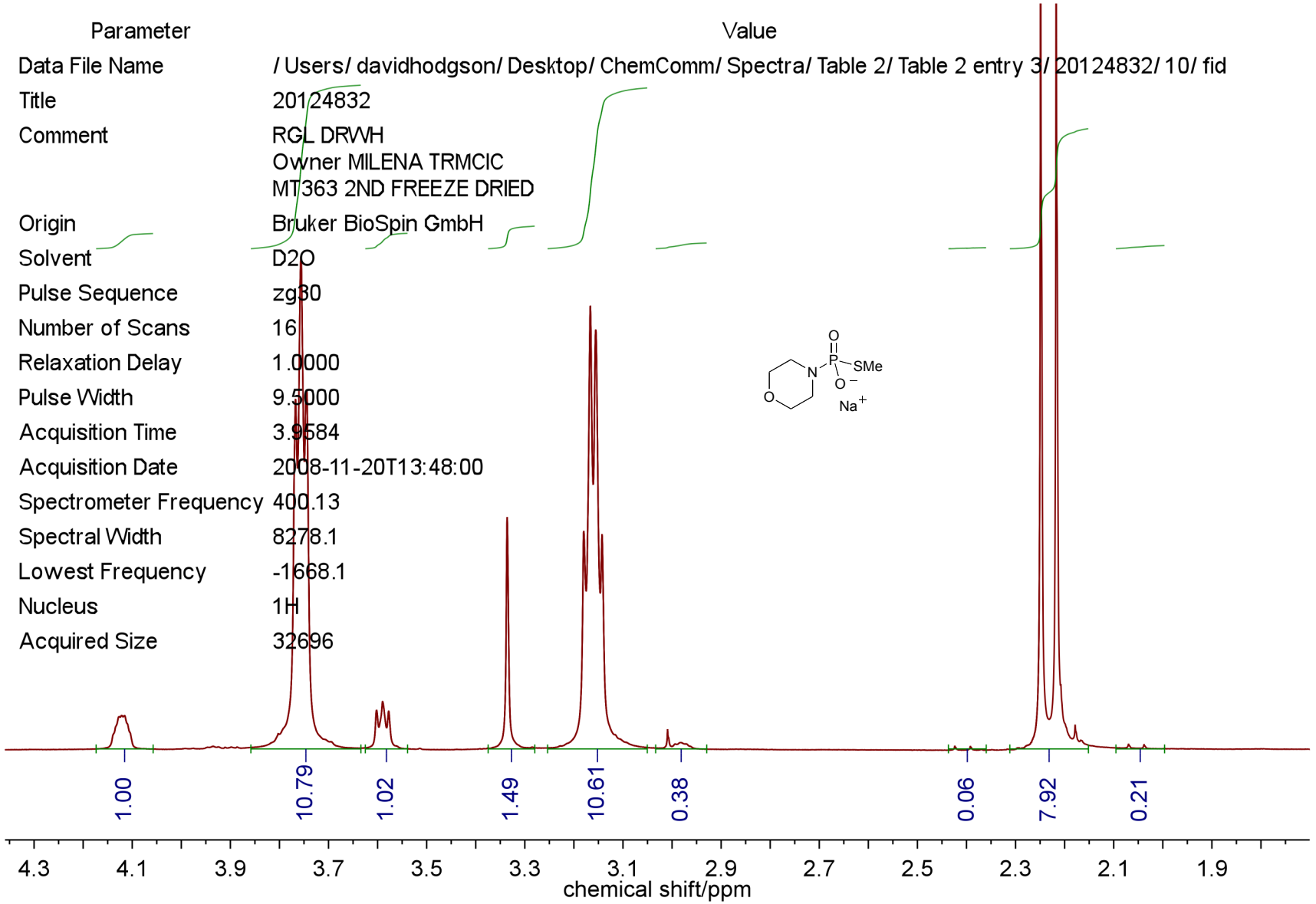
Table 2, entry 3 ^{1}H NMR spectrum



100*10.79/(10.79+1.02)=91%

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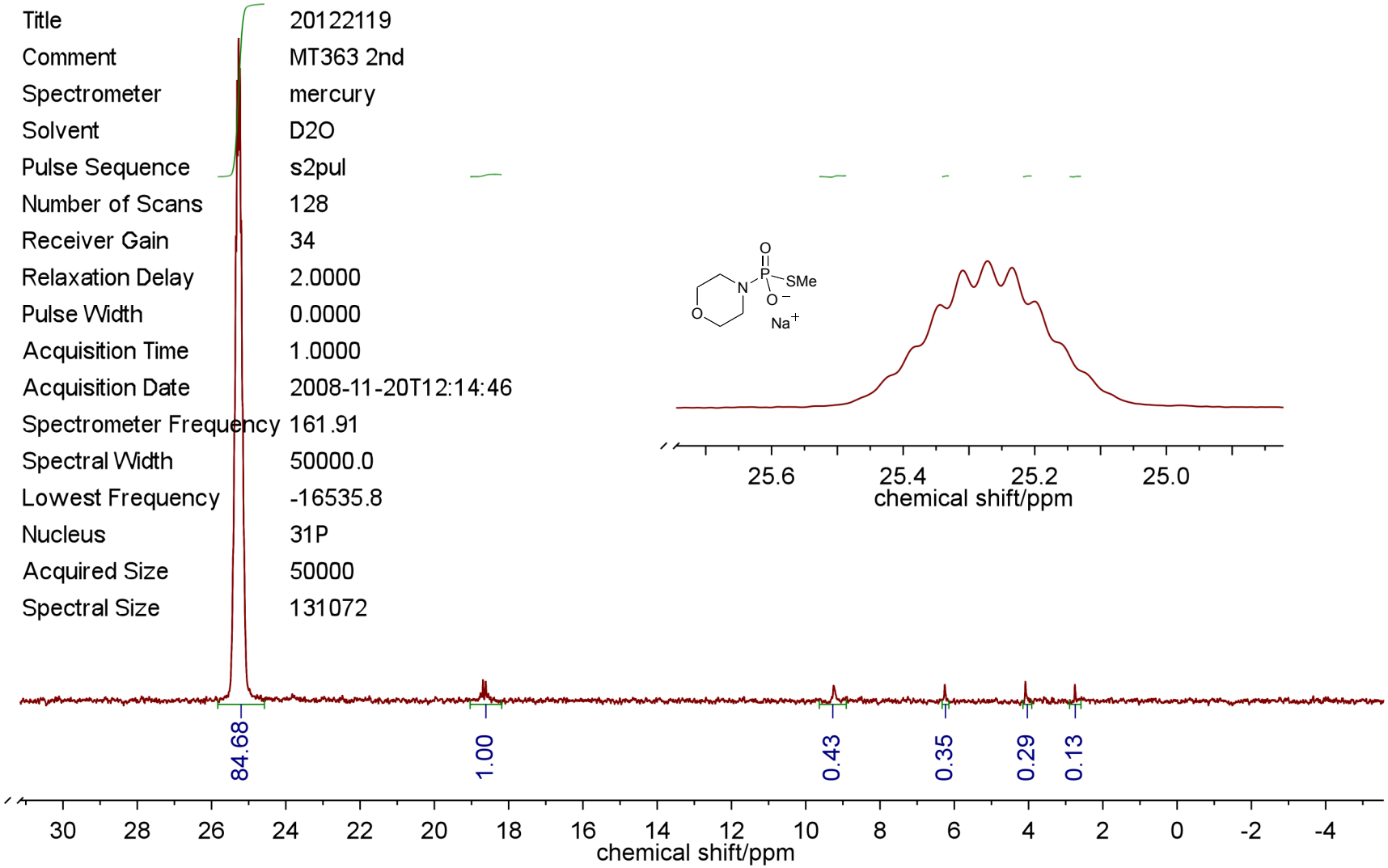
Table 2, entry 3 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 2/ Table 2 entry 3/ 20122119.fid/ fid
Title	20122119
Comment	MT363 2nd
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	128
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-11-20T12:14:46
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*84.68/(84.68+1.00+0.43+0.35+0.29+0.13)=97%

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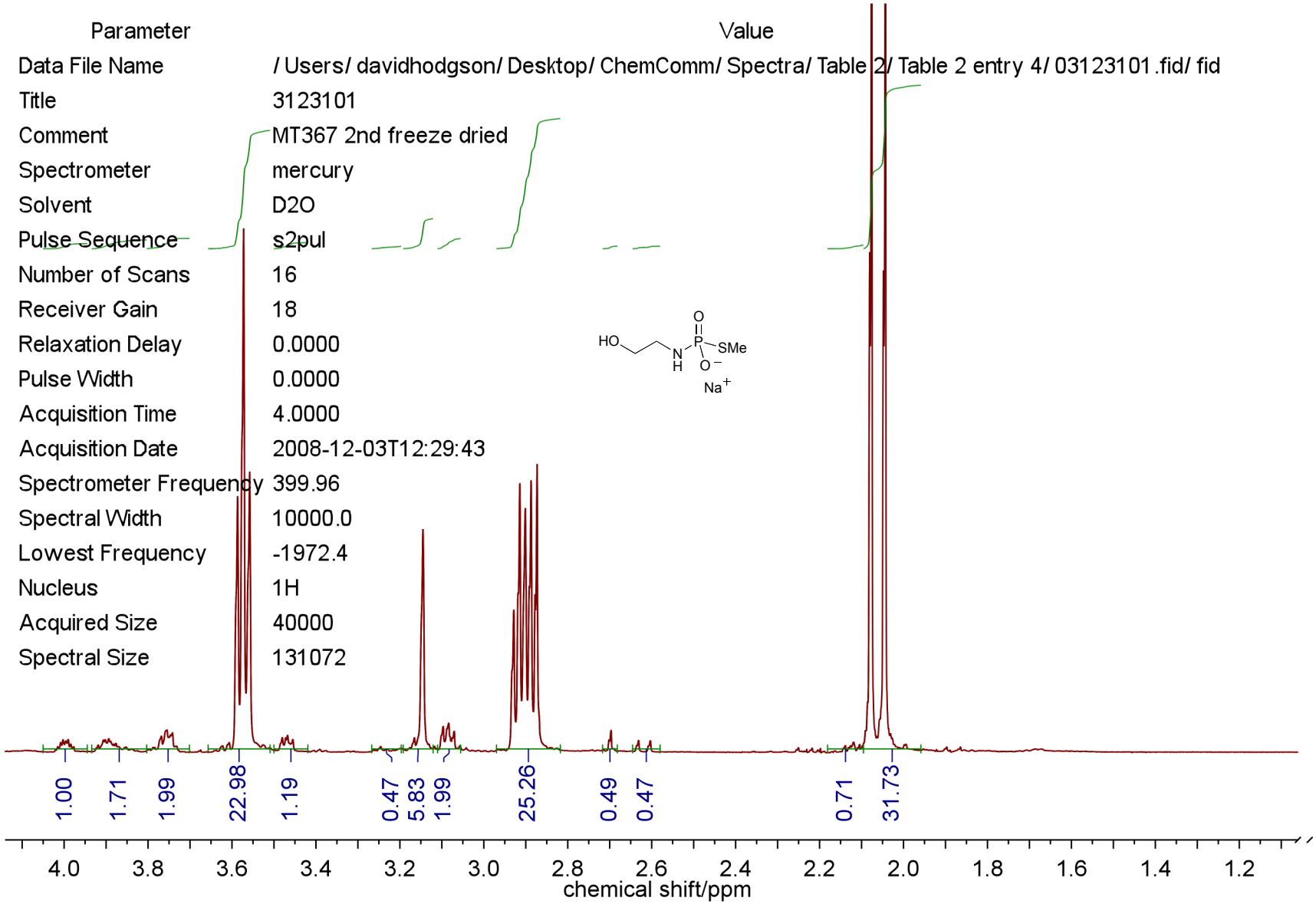
Table 2, entry 4 ^{1}H NMR spectrum



100*22.98/(1.00+1.71+1.99+22.98+1.19)=80%

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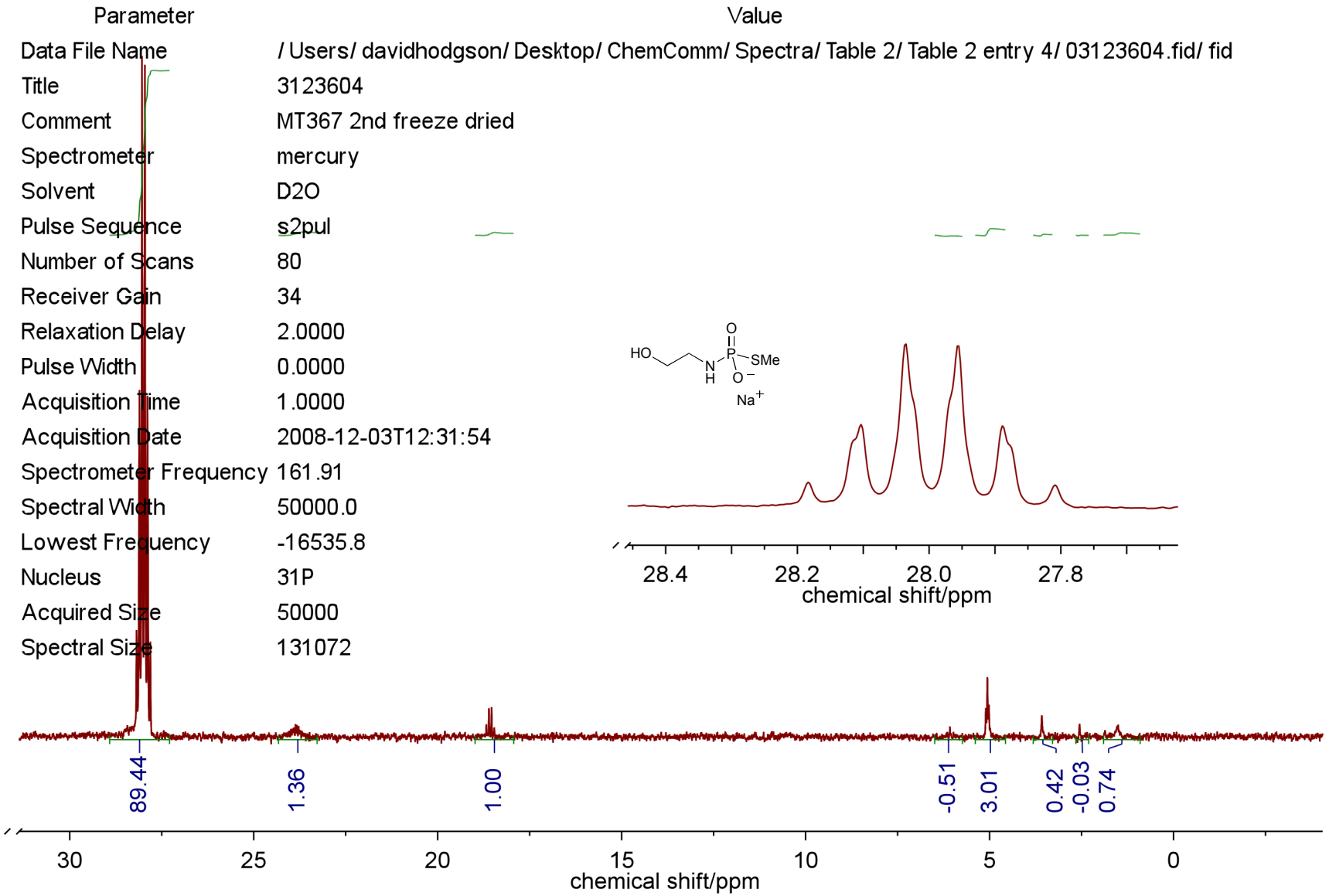
Table 2, entry 4 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 2/ Table 2 entry 4/ 03123604.fid/ fid
Title	3123604
Comment	MT367 2nd freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	80
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-12-03T12:31:54
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*89.44/(89.44+1.36+1.00+3.01+0.74)=93%

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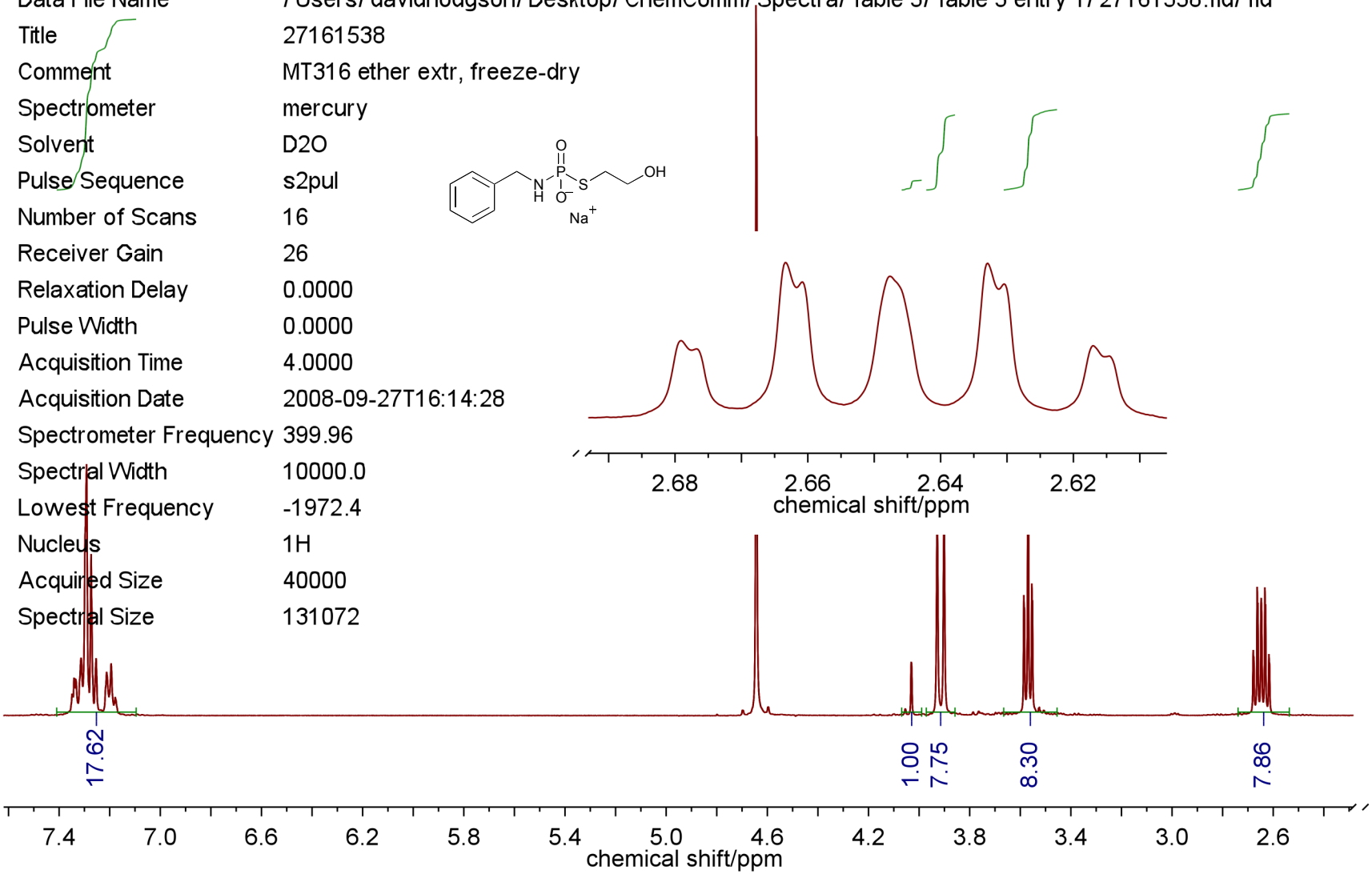
Table 3, entry 1 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 1/ 27161538.fid/ fid
Title	27161538
Comment	MT316 ether extr, freeze-dry
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	26
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-09-27T16:14:28
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



estimated>99%

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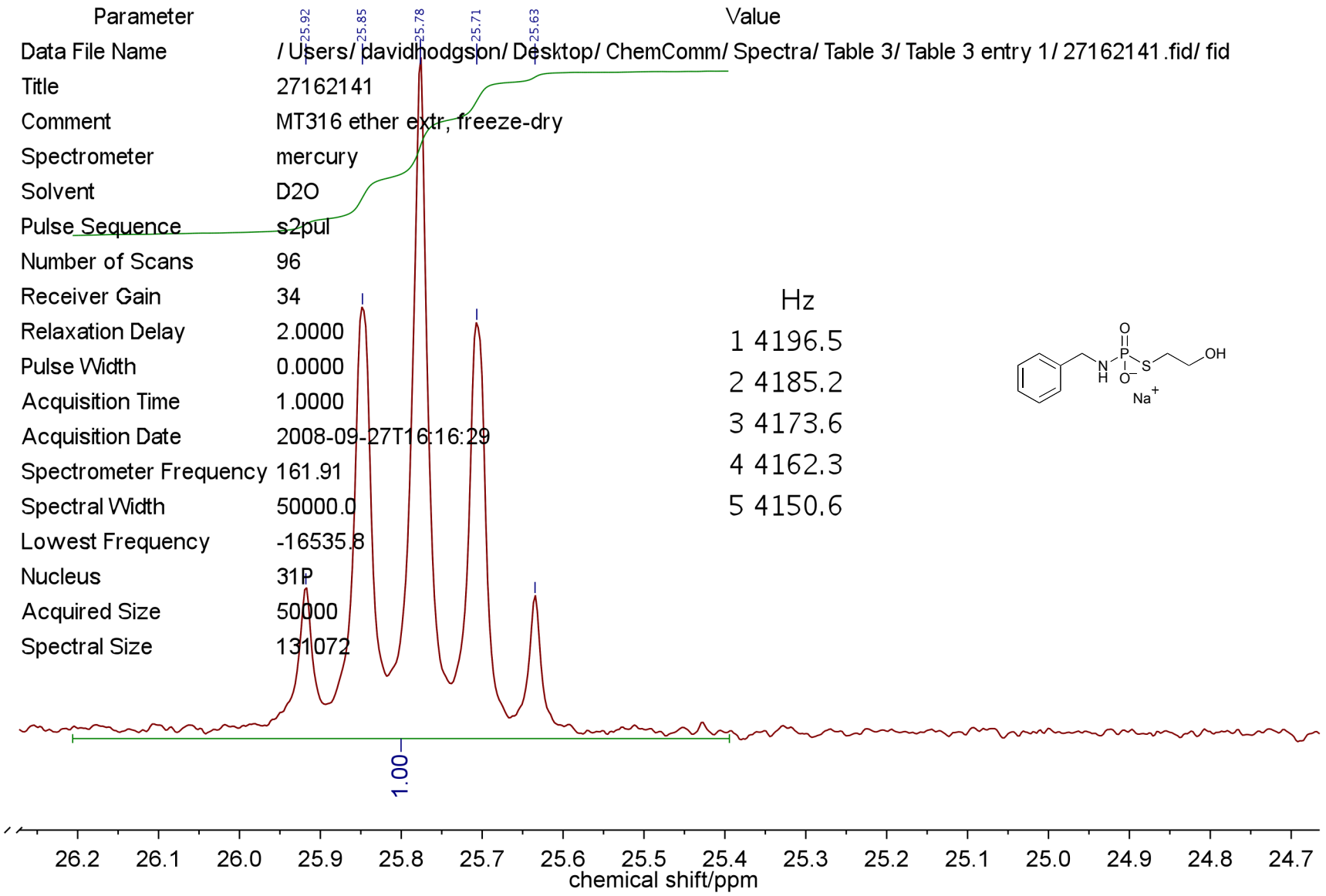
Table 3, entry 1 ^{31}P NMR spectrum



estimated >99%

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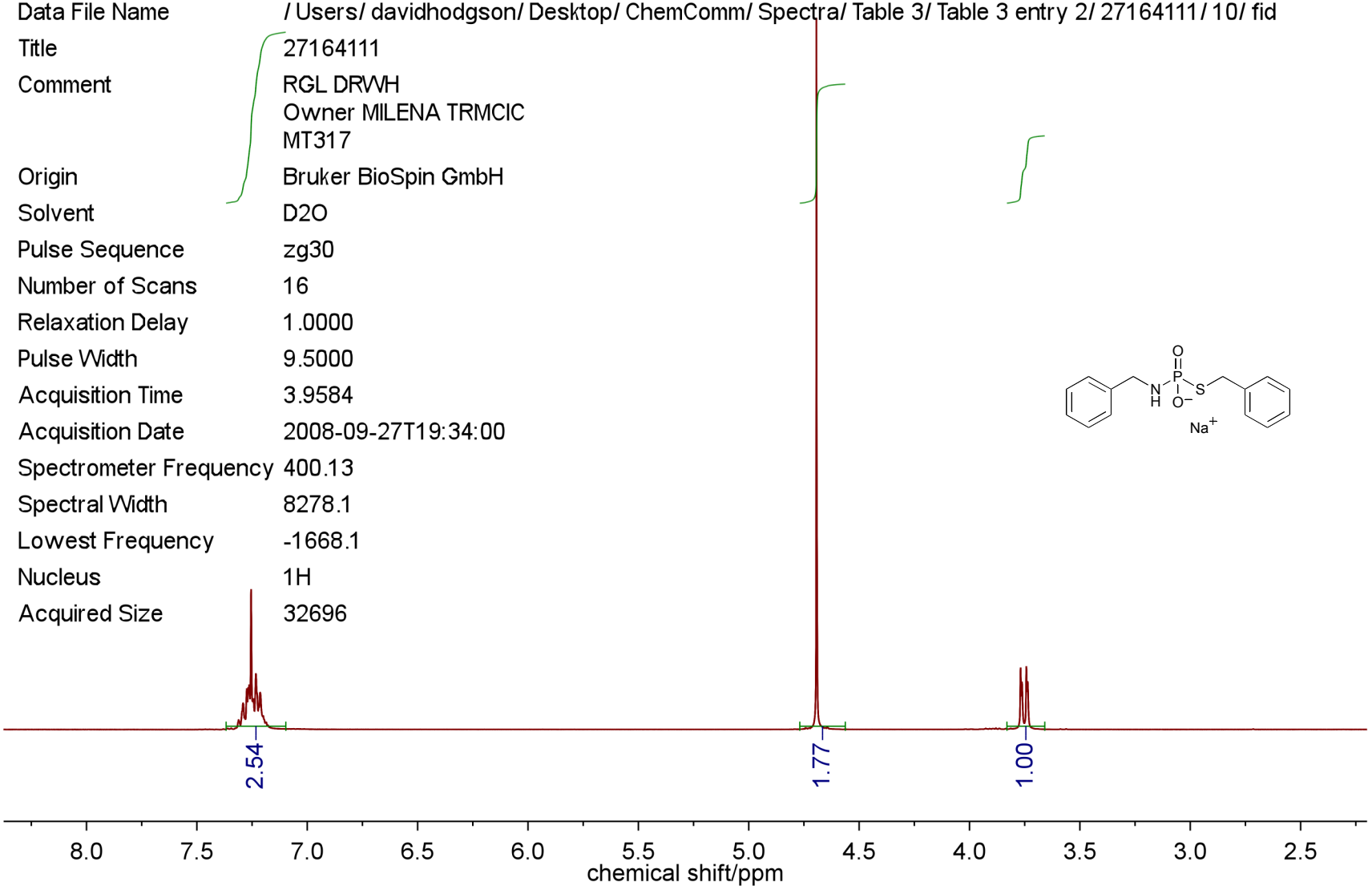
Table 3, entry 2 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 2/ 27164111/ 10/ fid
Title	27164111
Comment	RGL DRWH Owner MILENA TRMCIC MT317
Origin	Bruker BioSpin GmbH
Solvent	D2O
Pulse Sequence	zg30
Number of Scans	16
Relaxation Delay	1.0000
Pulse Width	9.5000
Acquisition Time	3.9584
Acquisition Date	2008-09-27T19:34:00
Spectrometer Frequency	400.13
Spectral Width	8278.1
Lowest Frequency	-1668.1
Nucleus	1H
Acquired Size	32696



estimated>99%

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Table 3, entry 2 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 2/ 27162946.fid/ fid
Title	27162946
Comment	MT317 ether extr, freeze-dry
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	96
Receiver Gain	36
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-09-27T16:24:35
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072

	Hz
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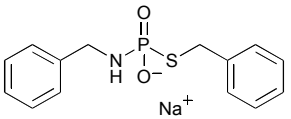
2	3986.6
3	3976.2
4	3965.8
5	3955.5



estimated>99%

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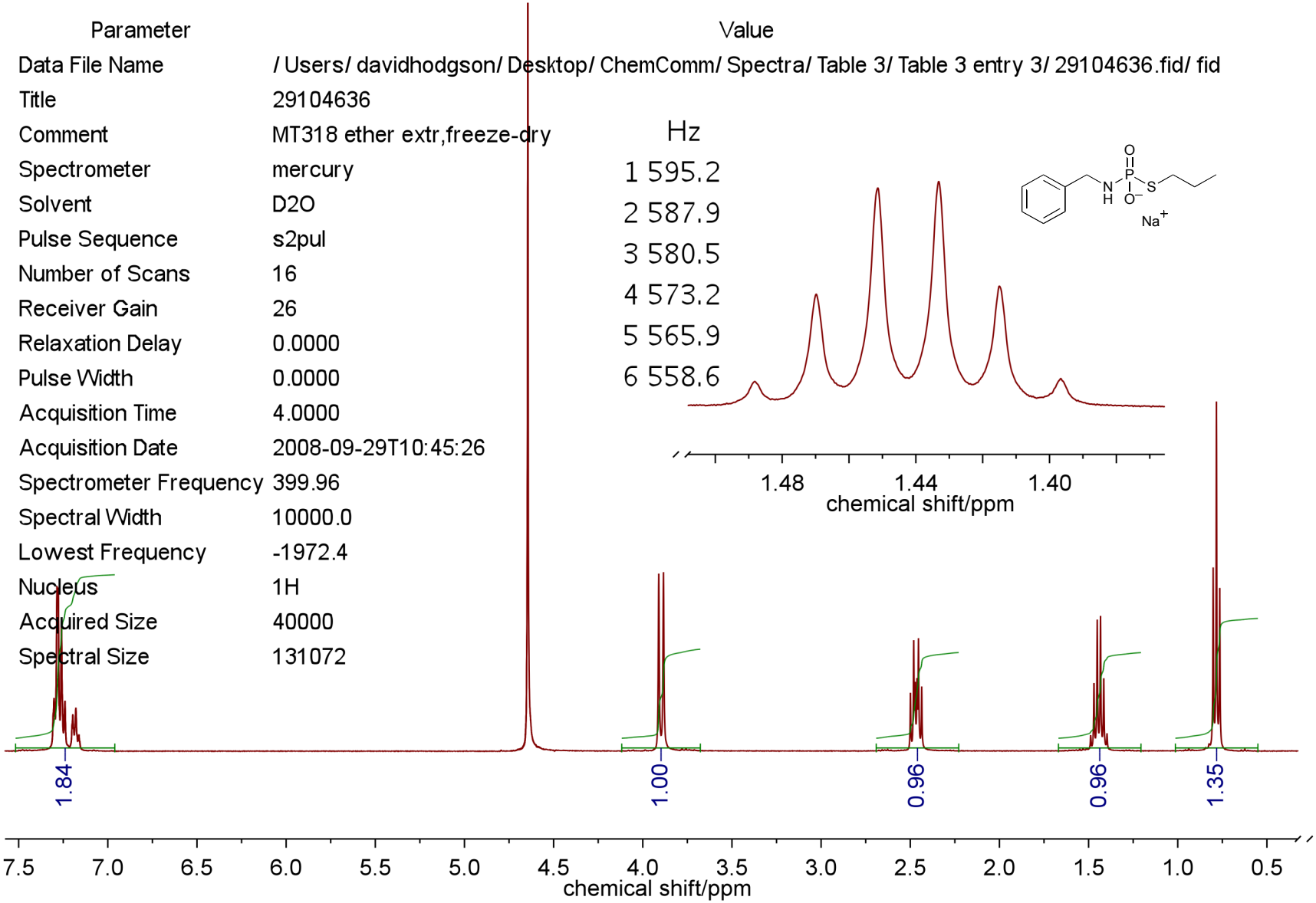
Table 3, entry 3 ^{1}H NMR spectrum



estimated>99%

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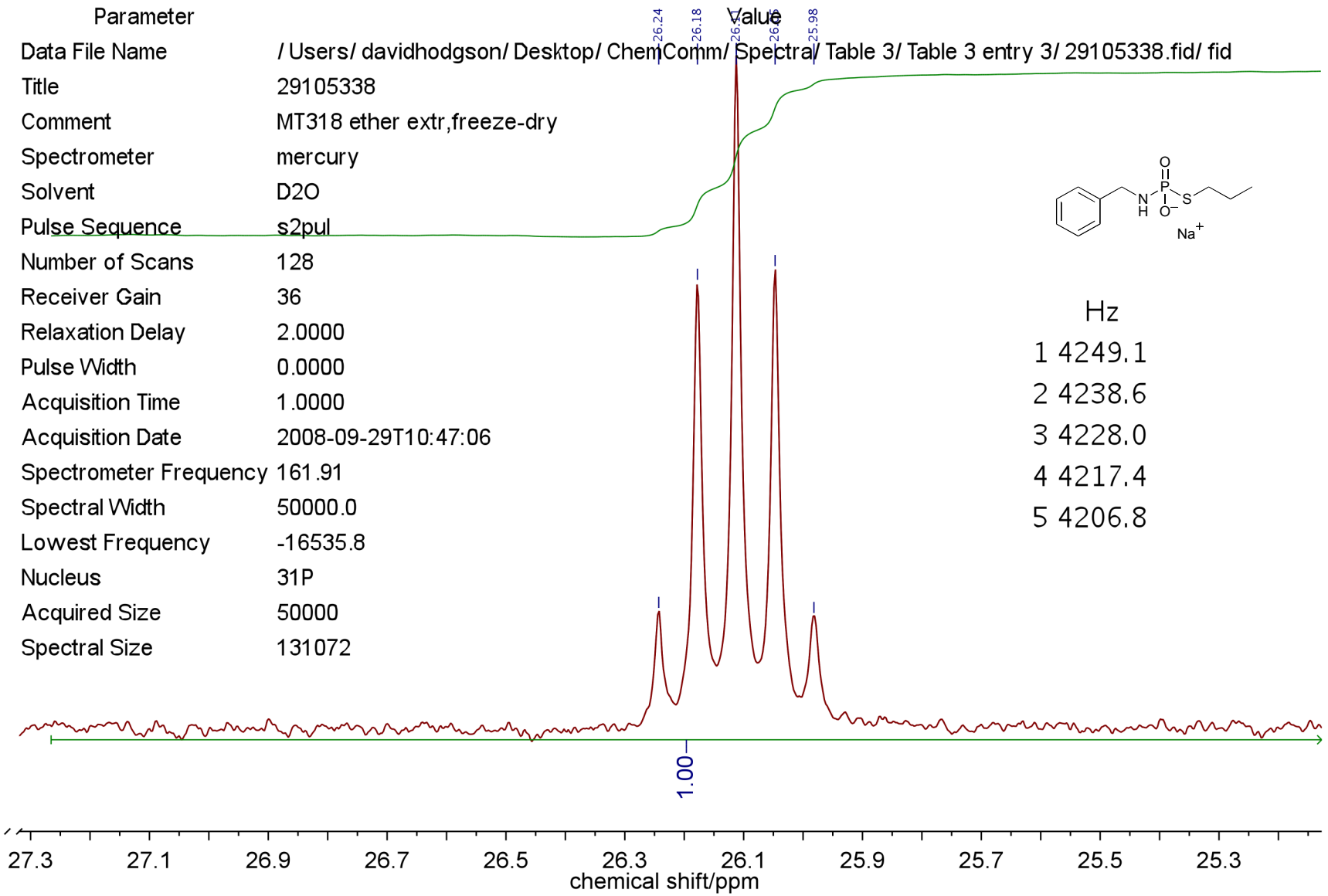
Table 3, entry 3 ^{31}P NMR spectrum



estimated>99%

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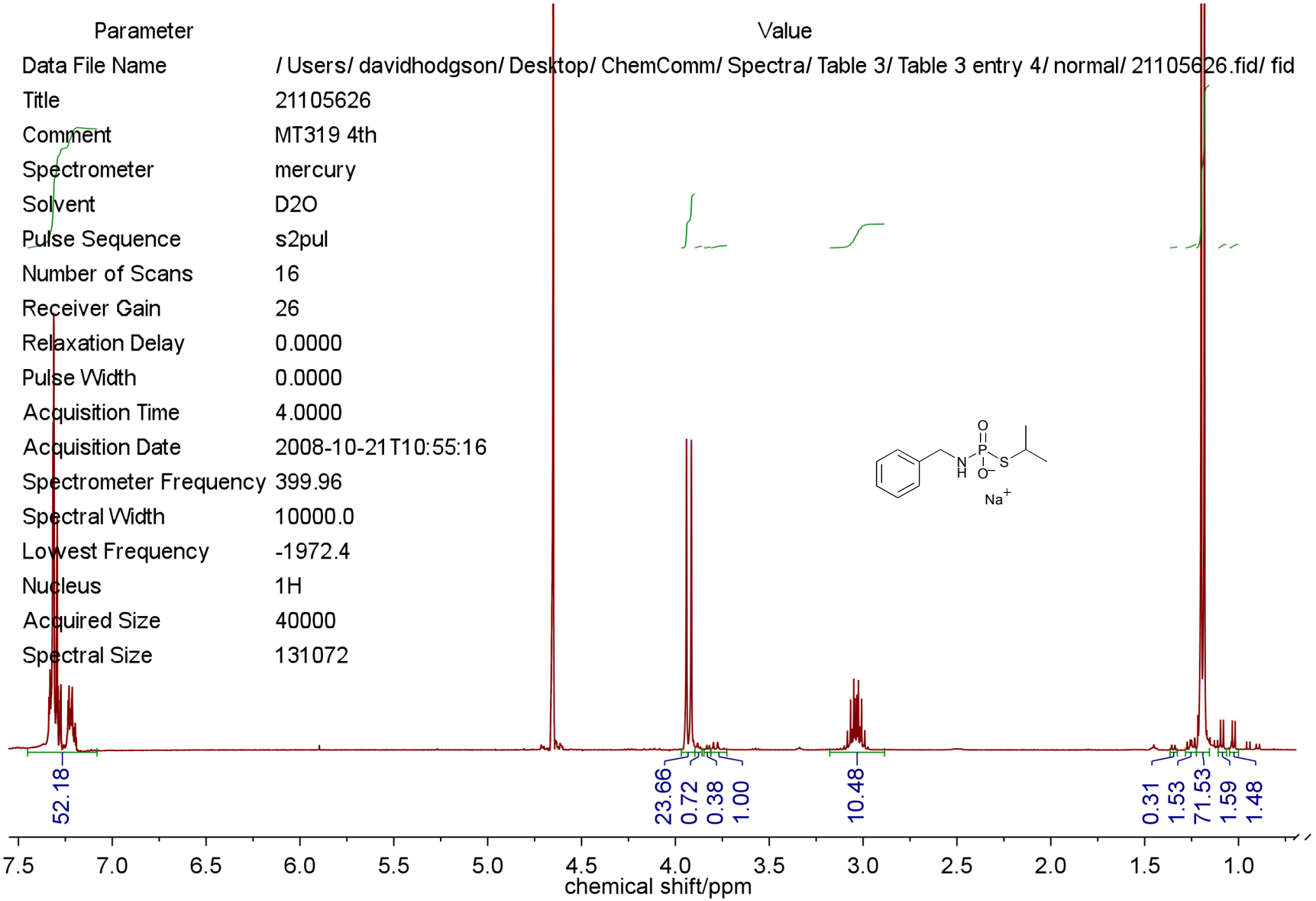
Table 3, entry 4 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 4/ normal/ 21105626.fid/ fid
Title	21105626
Comment	MT319 4th
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	26
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-10-21T10:55:16
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



100*23.66/(23.66+0.72+0.38+1.00)=92%

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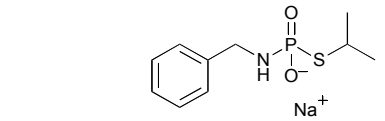
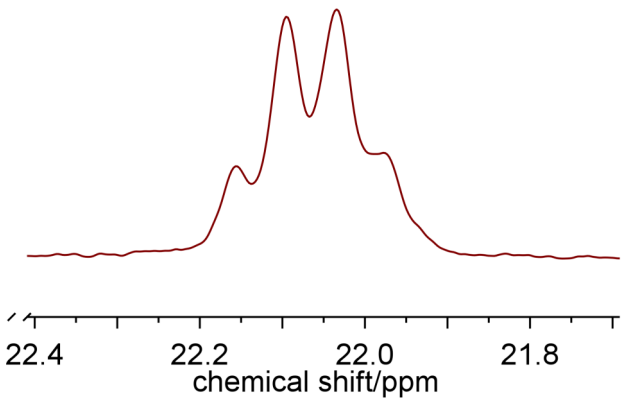
Table 3, entry 4 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 4/ normal/ 17182422.fid/ fid
Title	17182422
Comment	MT319 4th
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	96
Receiver Gain	36
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-10-17T18:19:24
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072





100*59.73/(1.31+2.21+59.73+1.00+0.65)=92%

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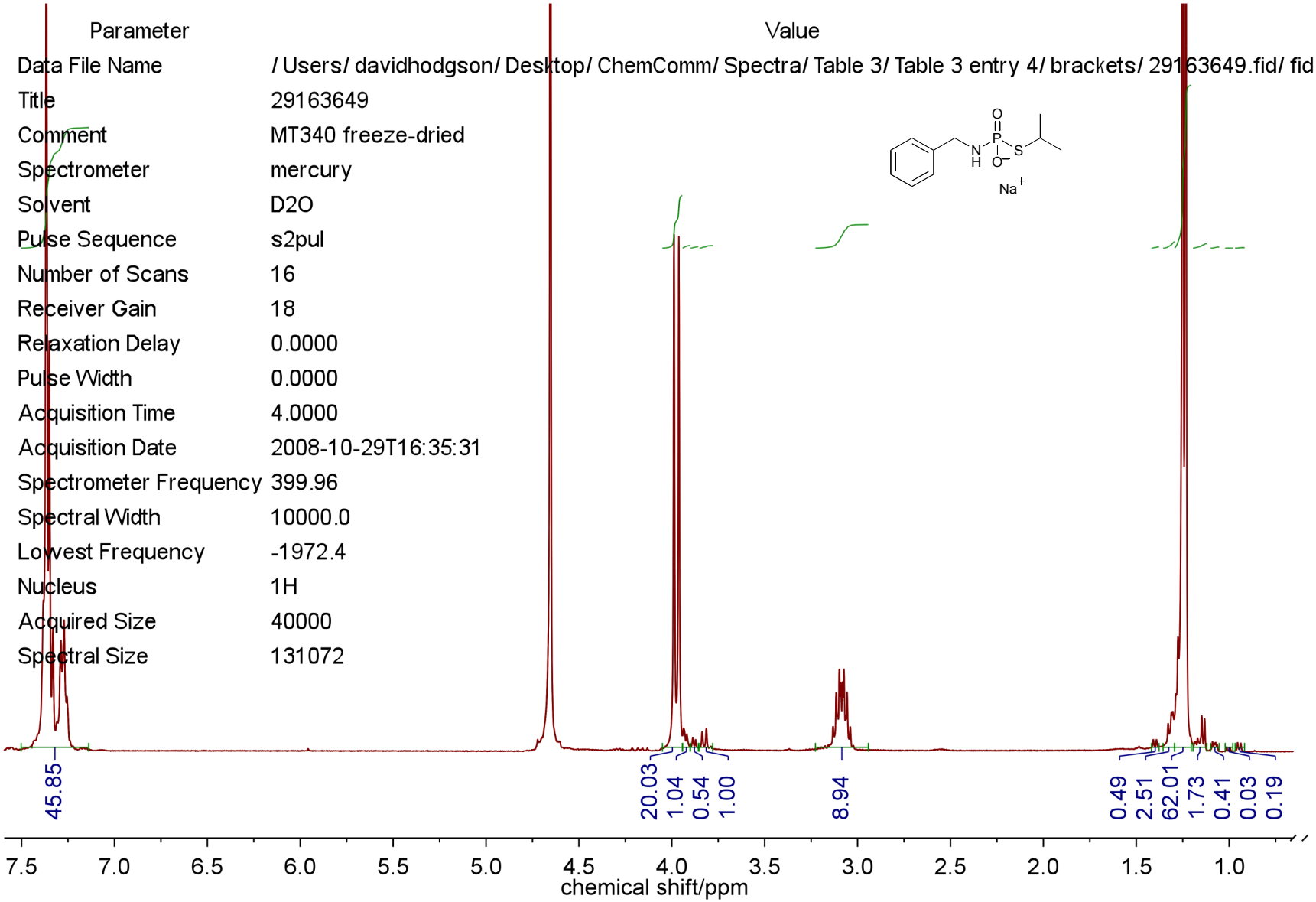
Table 3, entry 4 brackets ^{1}H NMR spectrum



100*20.03/(20.03+1.04+0.54+1.00)=89%

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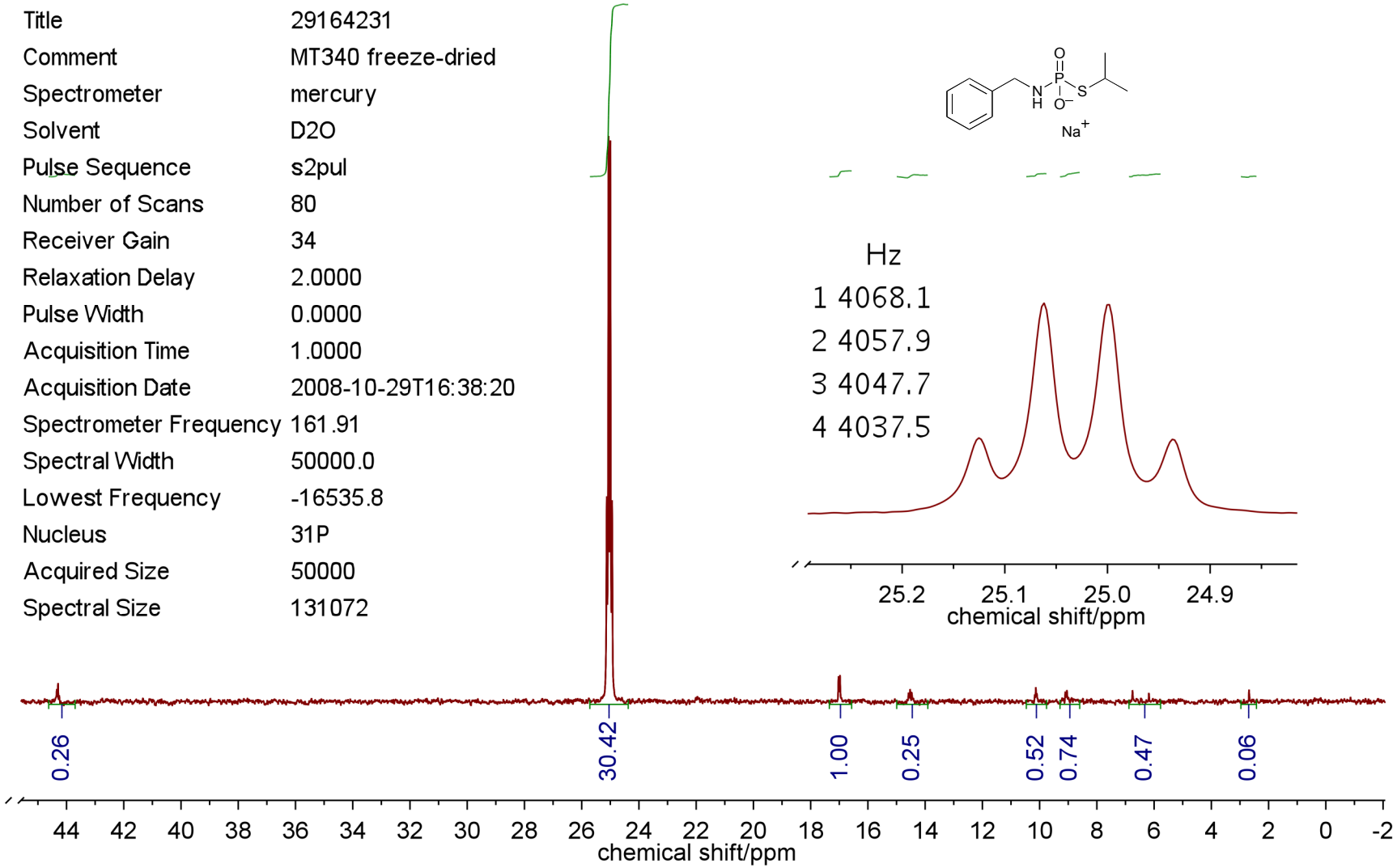
Table 3, entry 4 brackets ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 4/ brackets/ 29164231.fid/ fid
Title	29164231
Comment	MT340 freeze-dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	80
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-10-29T16:38:20
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*30.42/(0.26+30.42+1.00+0.25+0.52+0.74+0.47+0.06)=90%

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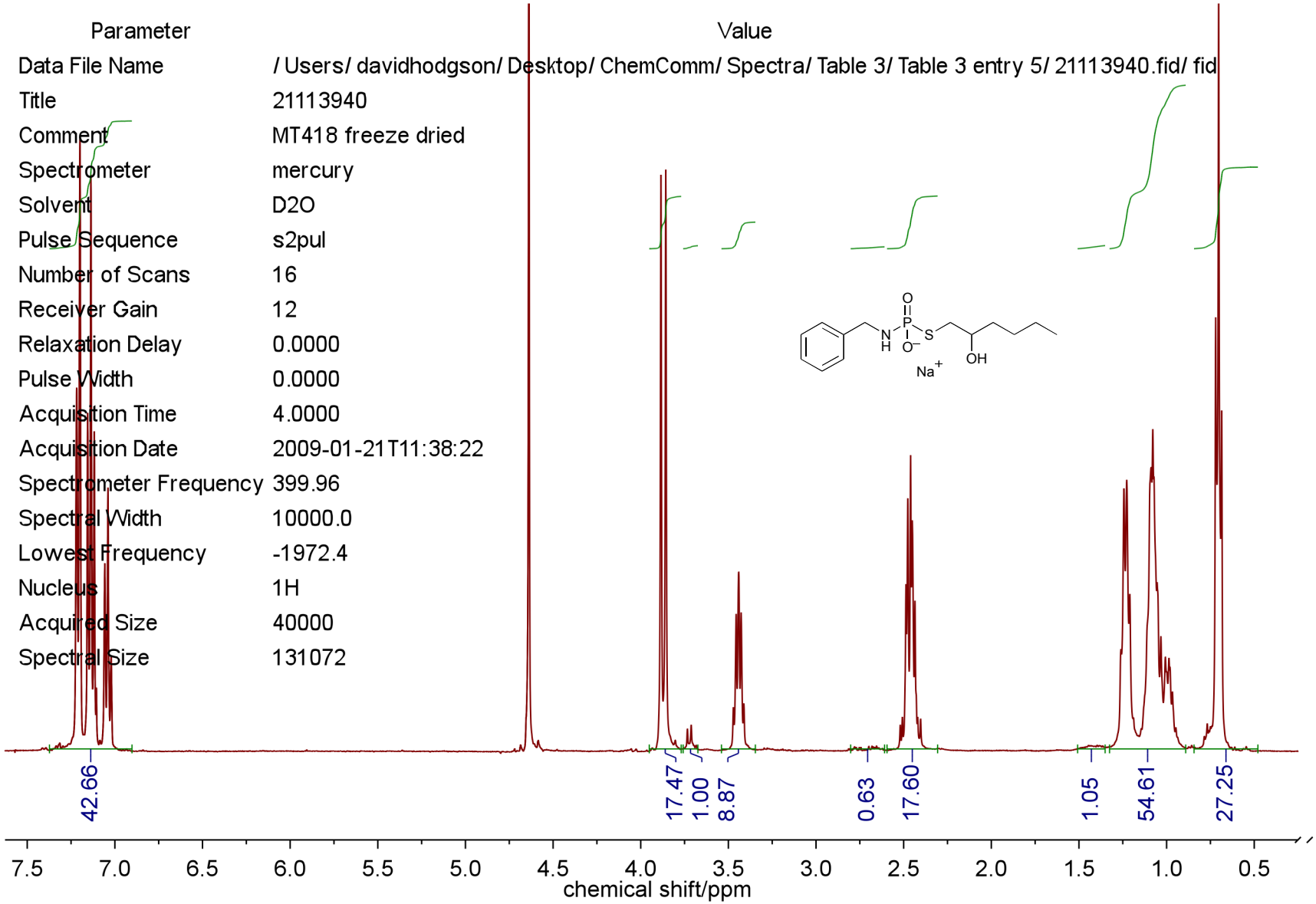
Table 3, entry 5 ^{1}H NMR spectrum



100*17.47/(17.47+1.00)=95%

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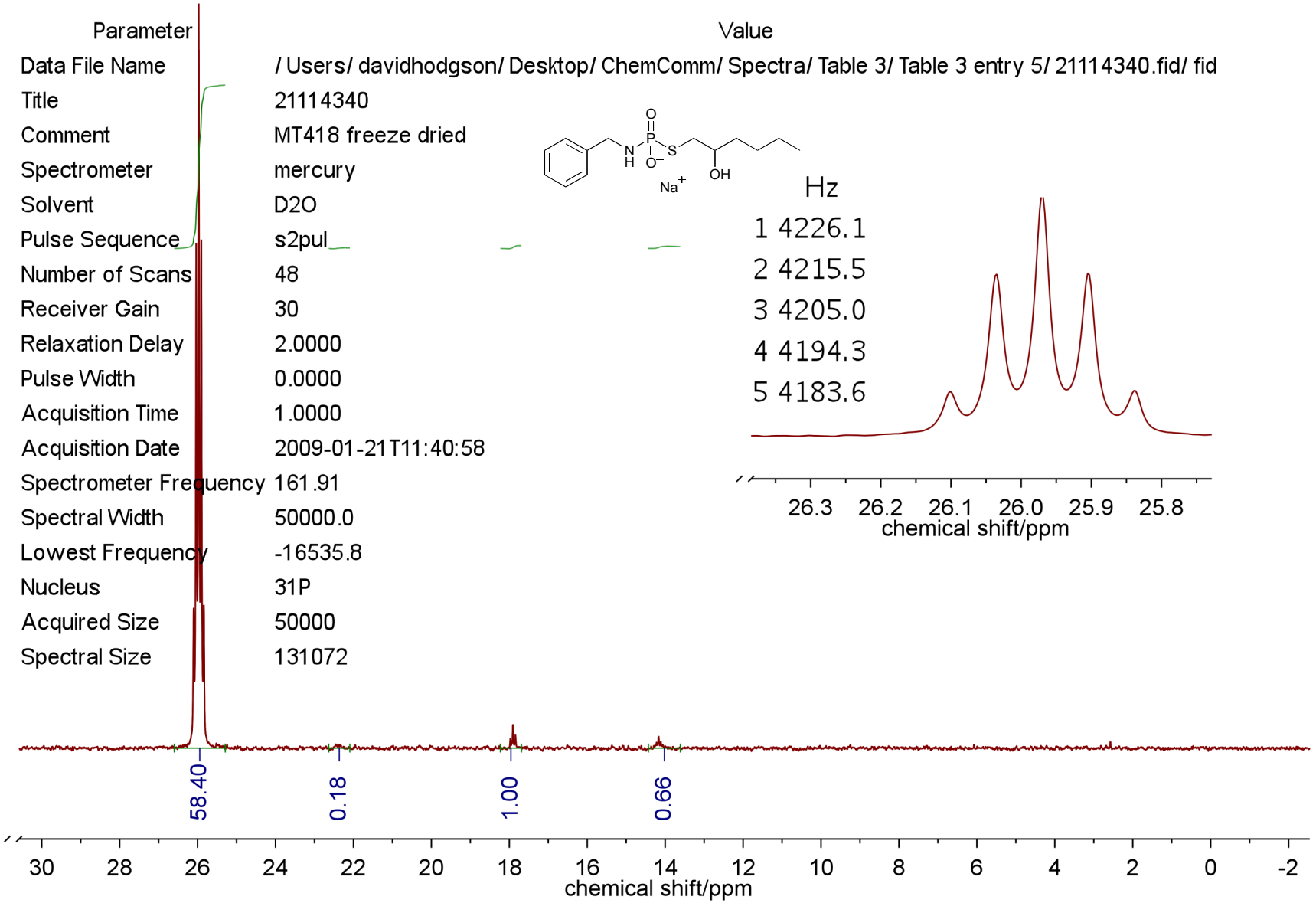
Table 3, entry 5 ^{31}P NMR spectrum



100*58.40/(58.40+0.18+1.00+0.66)=97%

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Table 3, entry 6 ^{1}H NMR spectrum

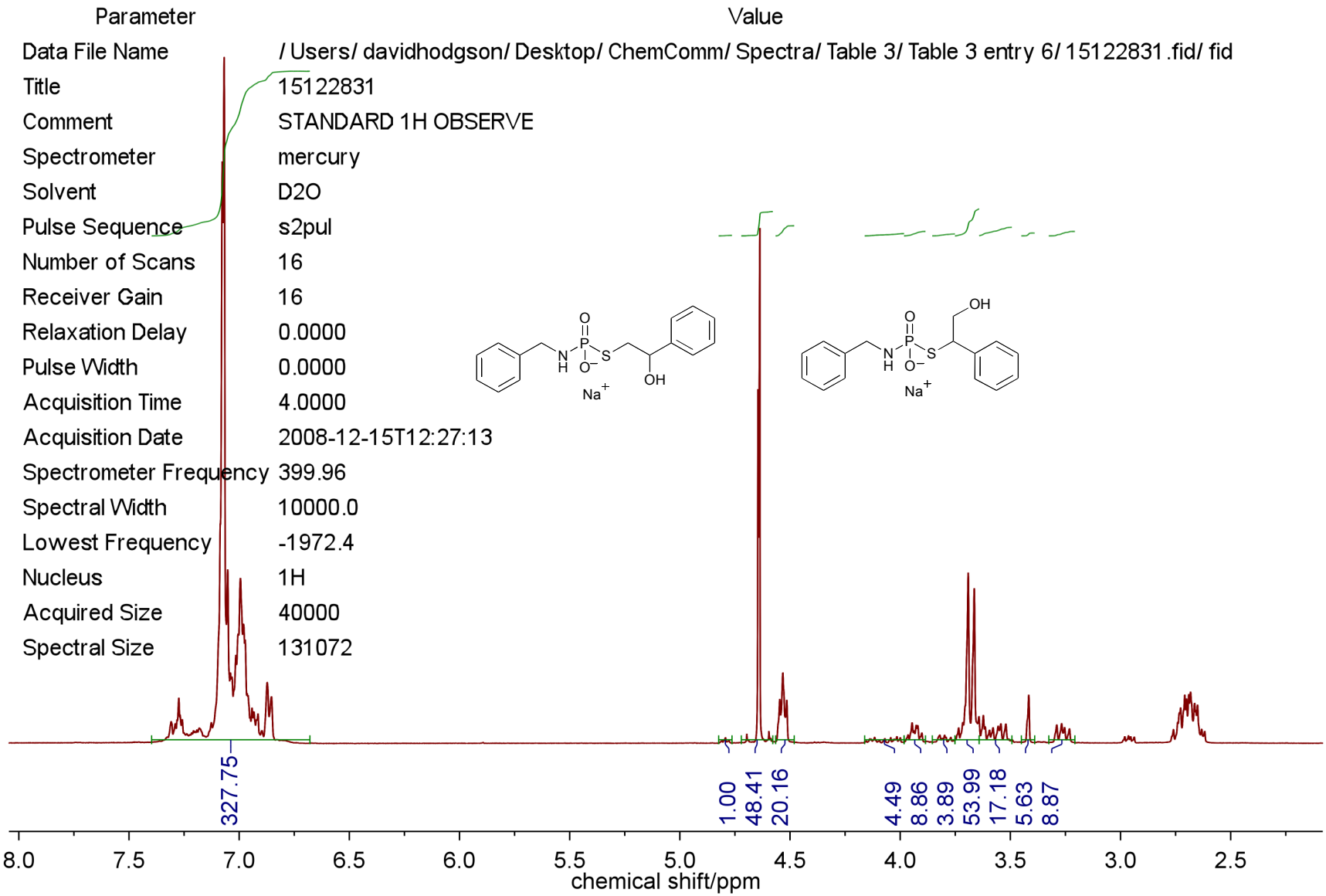

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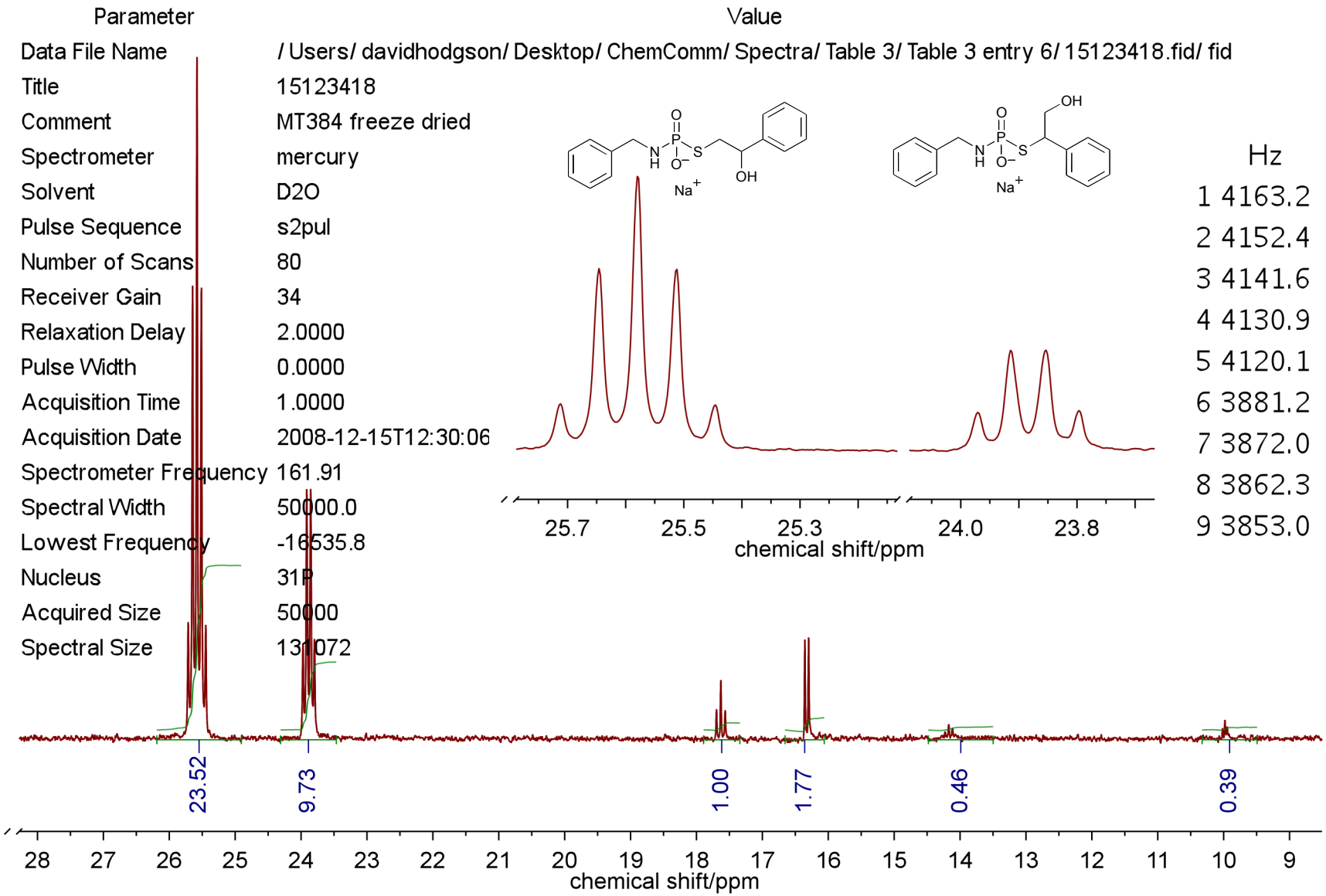
Table 3, entry 6 ^{31}P NMR spectrum



total for mixture of isomers: 100*(23.52+9.73)/(23.52+9.73+1.00+1.77+0.46+0.39)=90%

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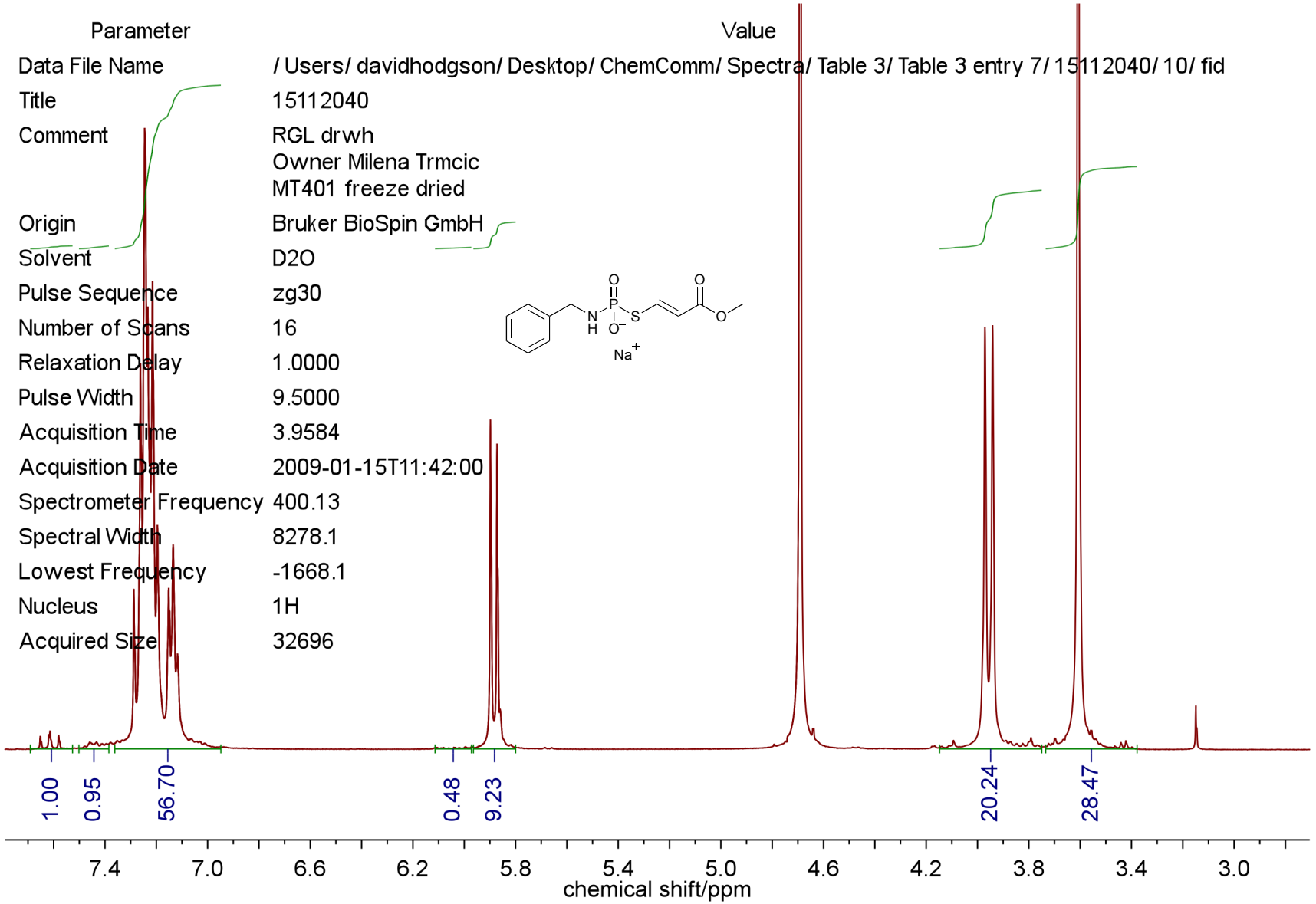
Table 3, entry 7 ^{1}H NMR spectrum



100*9.28/(9.23+0.48)=96%

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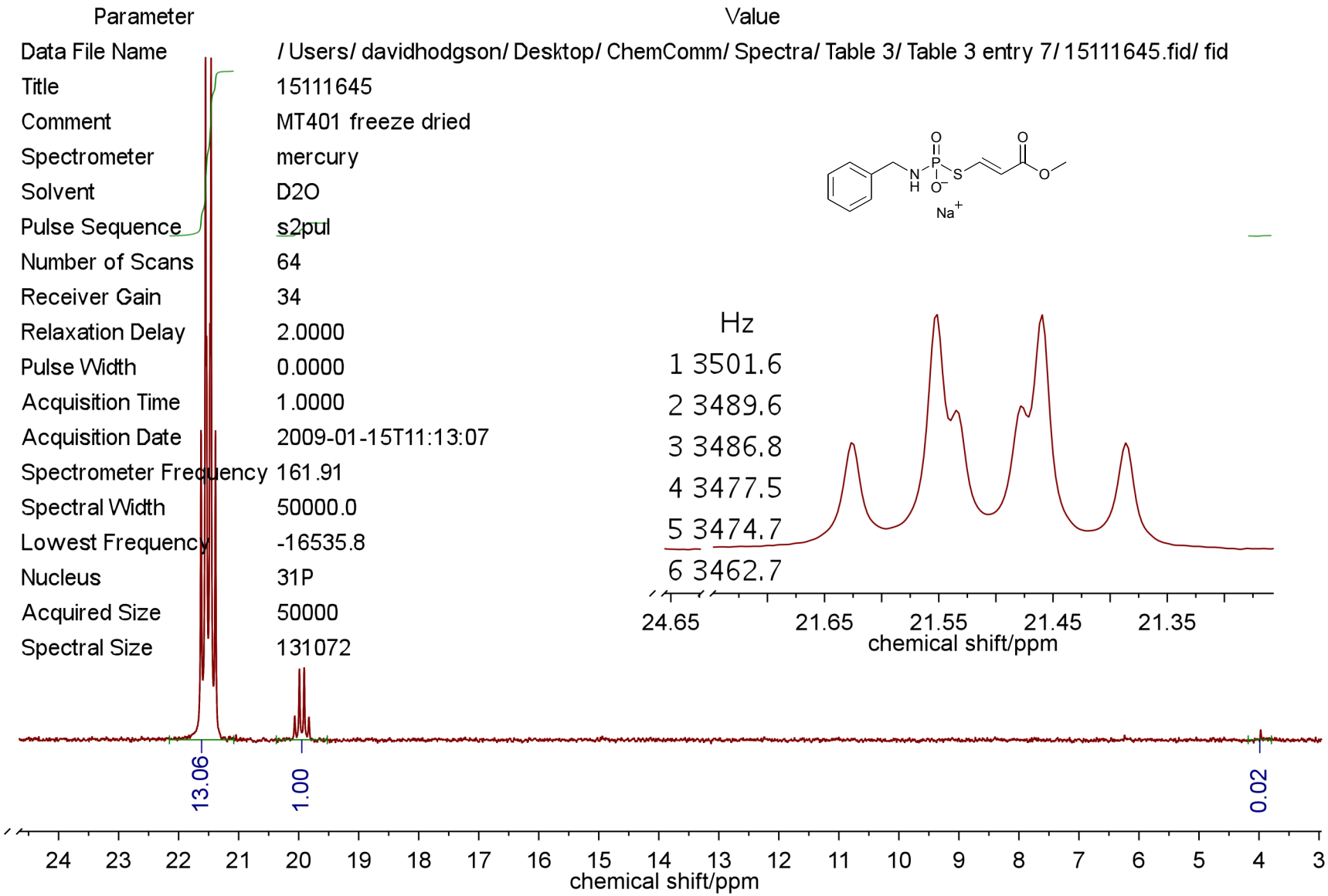
Table 3, entry 7 ^{31}P NMR spectrum



100*13.06/(13.06+1.00+0.02)=93%

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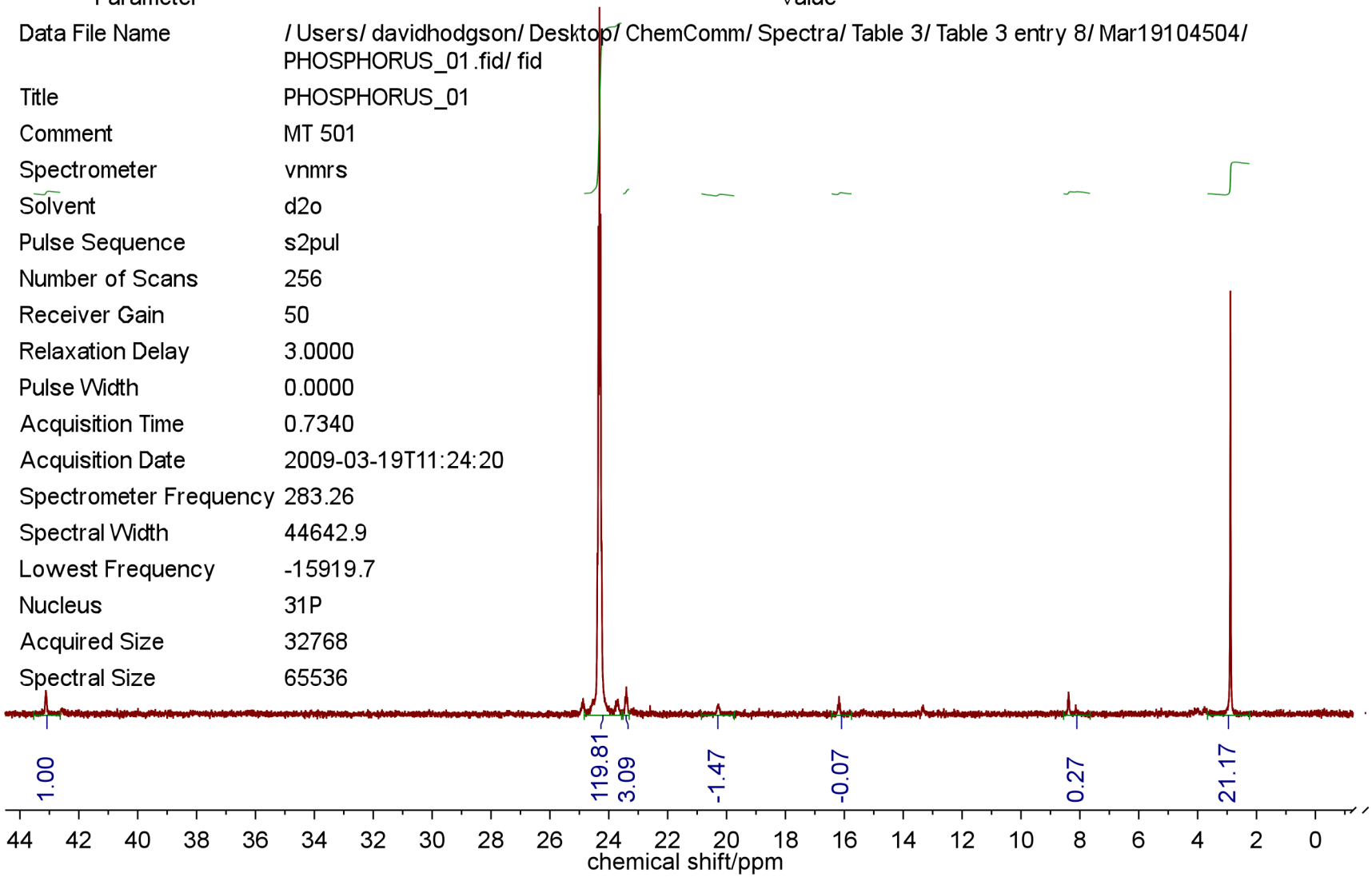
Table 3, entry 8 crude ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 8/ Mar19104504/ PHOSPHORUS_01.fid/ fid
Title	PHOSPHORUS_01
Comment	MT 501
Spectrometer	vnmrs
Solvent	d2o
Pulse Sequence	s2pul
Number of Scans	256
Receiver Gain	50
Relaxation Delay	3.0000
Pulse Width	0.0000
Acquisition Time	0.7340
Acquisition Date	2009-03-19T11:24:20
Spectrometer Frequency	283.26
Spectral Width	44642.9
Lowest Frequency	-15919.7
Nucleus	31P
Acquired Size	32768
Spectral Size	65536



100*119.81/(1.00+119.81+3.09+0.27+21.17)=82%

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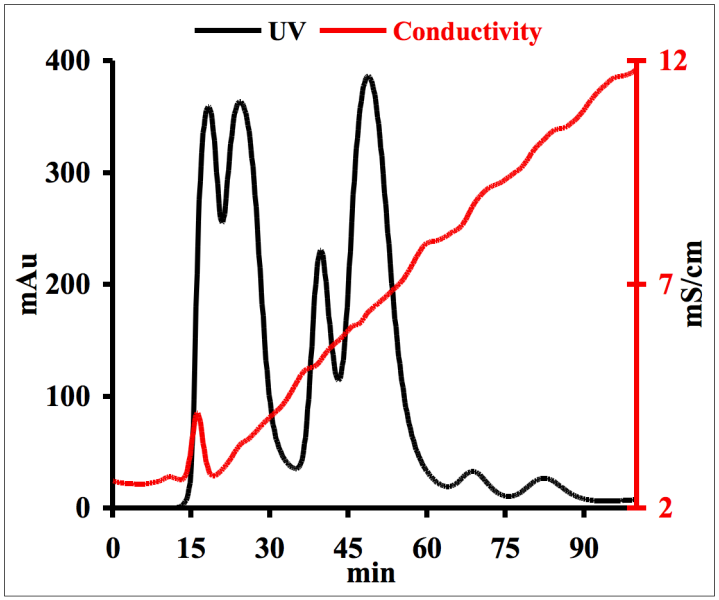
Anion exchange chromatogram of Table 3, entry 8.



fractions collected between retention times of 45 and 60 minutes were combined and lyophilised. Thereafter, triethyalammonium ions were exchanged for sodium ions using a strong cation exchange resin. The lyophilised solid was dissolved in D_2O and subjected to NMR analyses.

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Table 3, entry 8 ^{1}H NMR spectrum after weak anion exchange chromatography and cation exchange

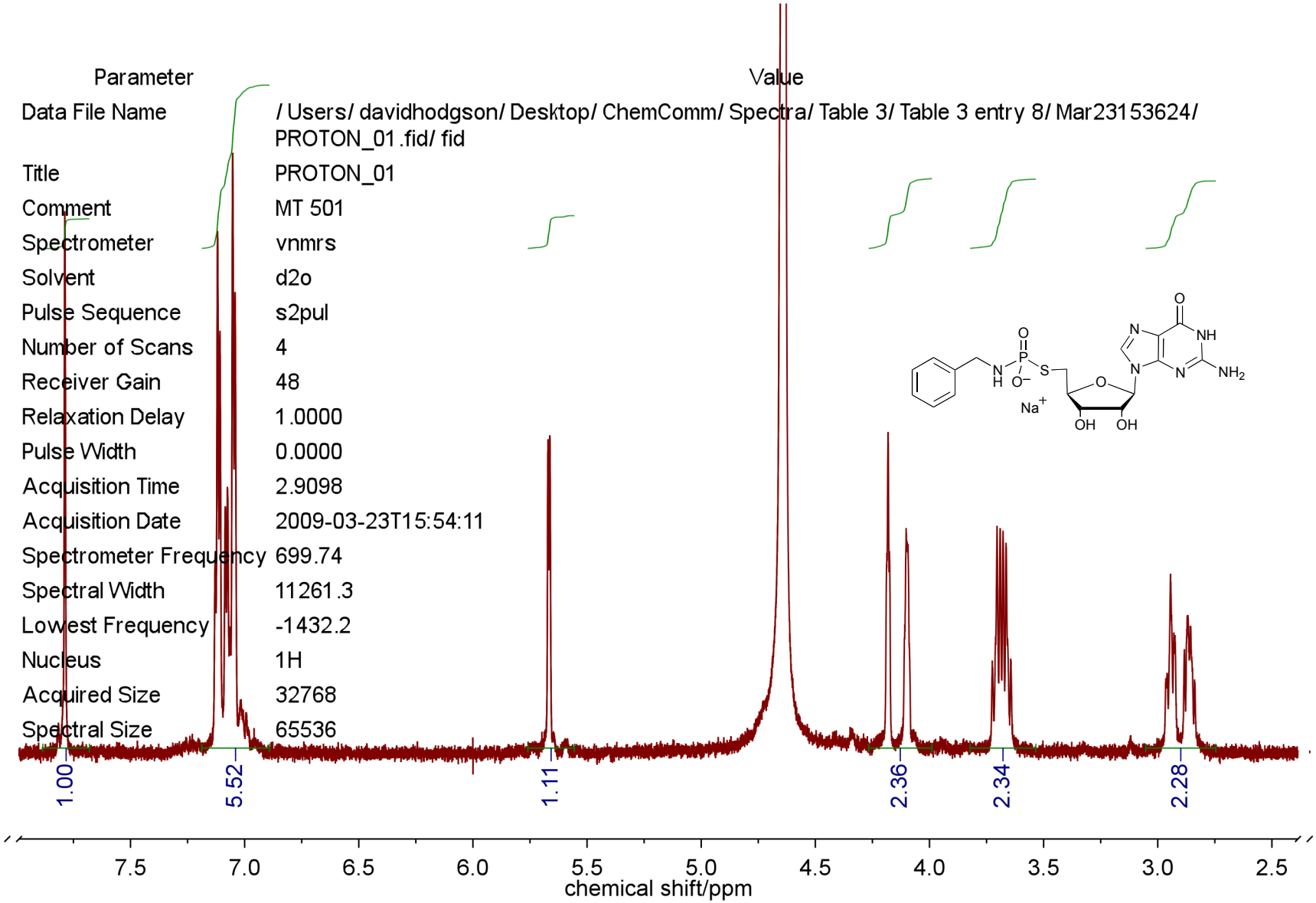

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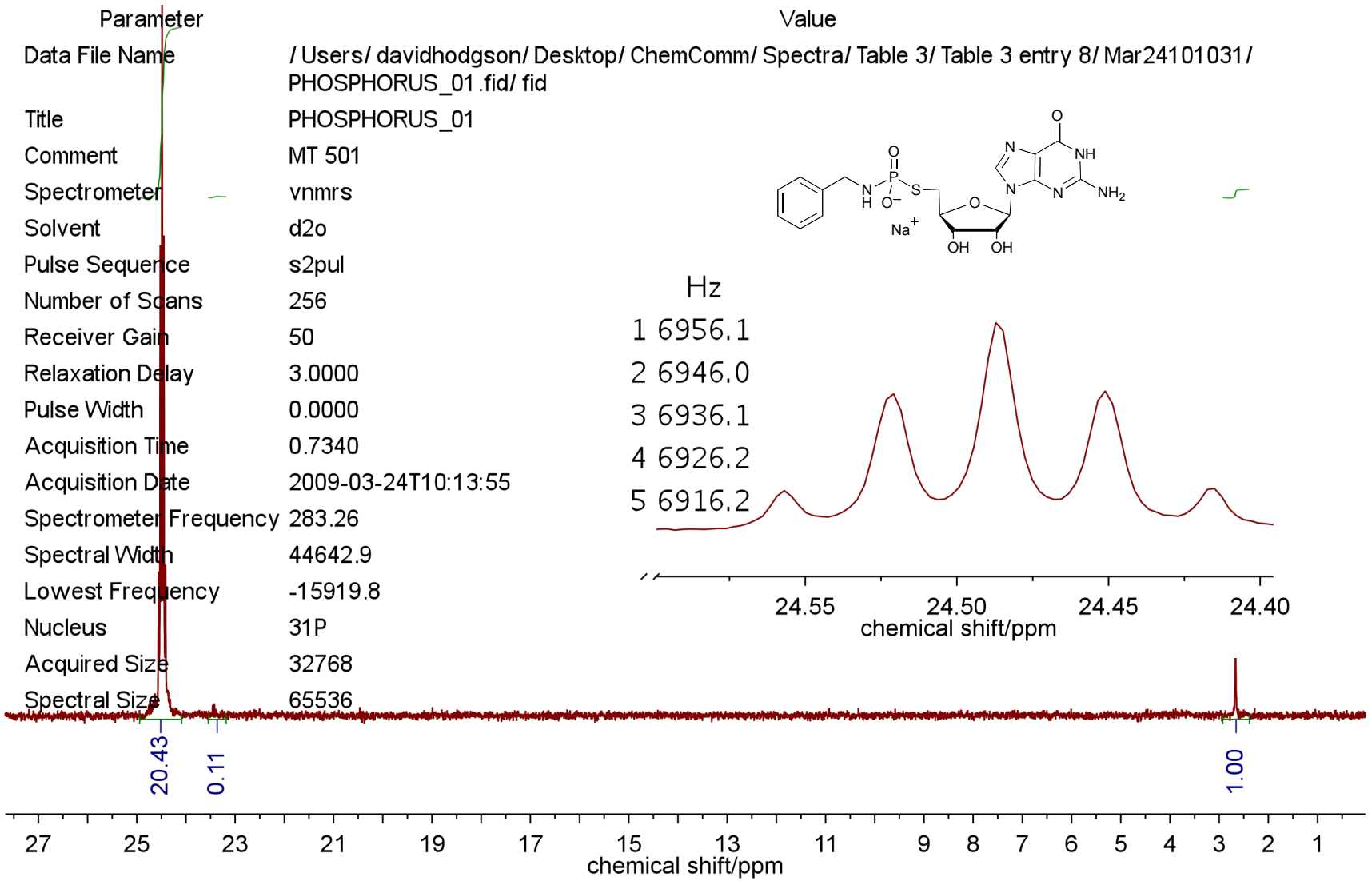
Table 3, entry 8 ^{31}P NMR spectrum after weak anion exchange chromatography and cation exchange



100*20.43/(20.43+0.11+1.00)=95% after chromatography and cation exchange

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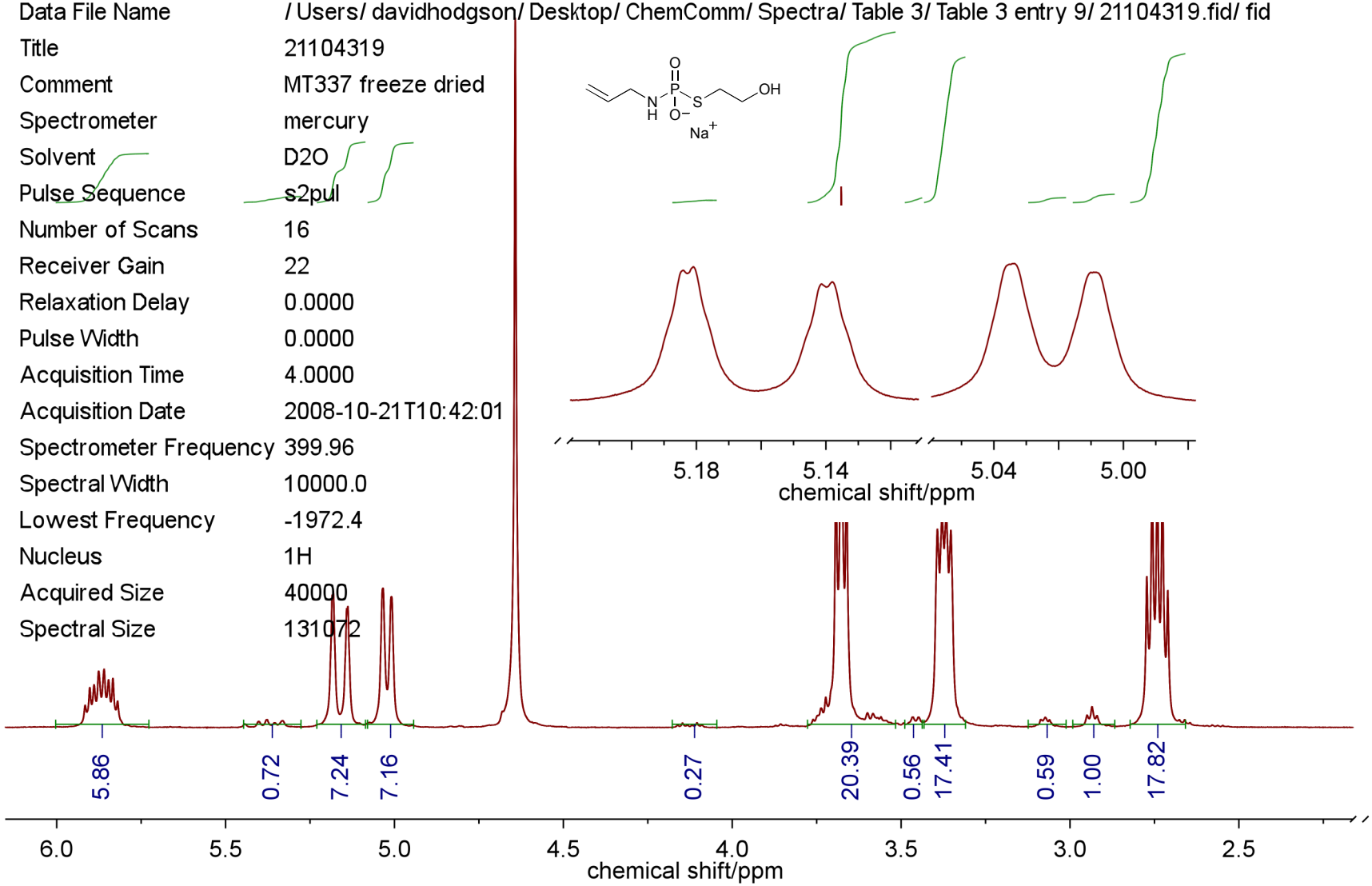
Table 3, entry 9 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 9/ 21104319.fid/ fid
Title	21104319
Comment	MT337 freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	22
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-10-21T10:42:01
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



100*17.82/(17.82+1.00)=95%

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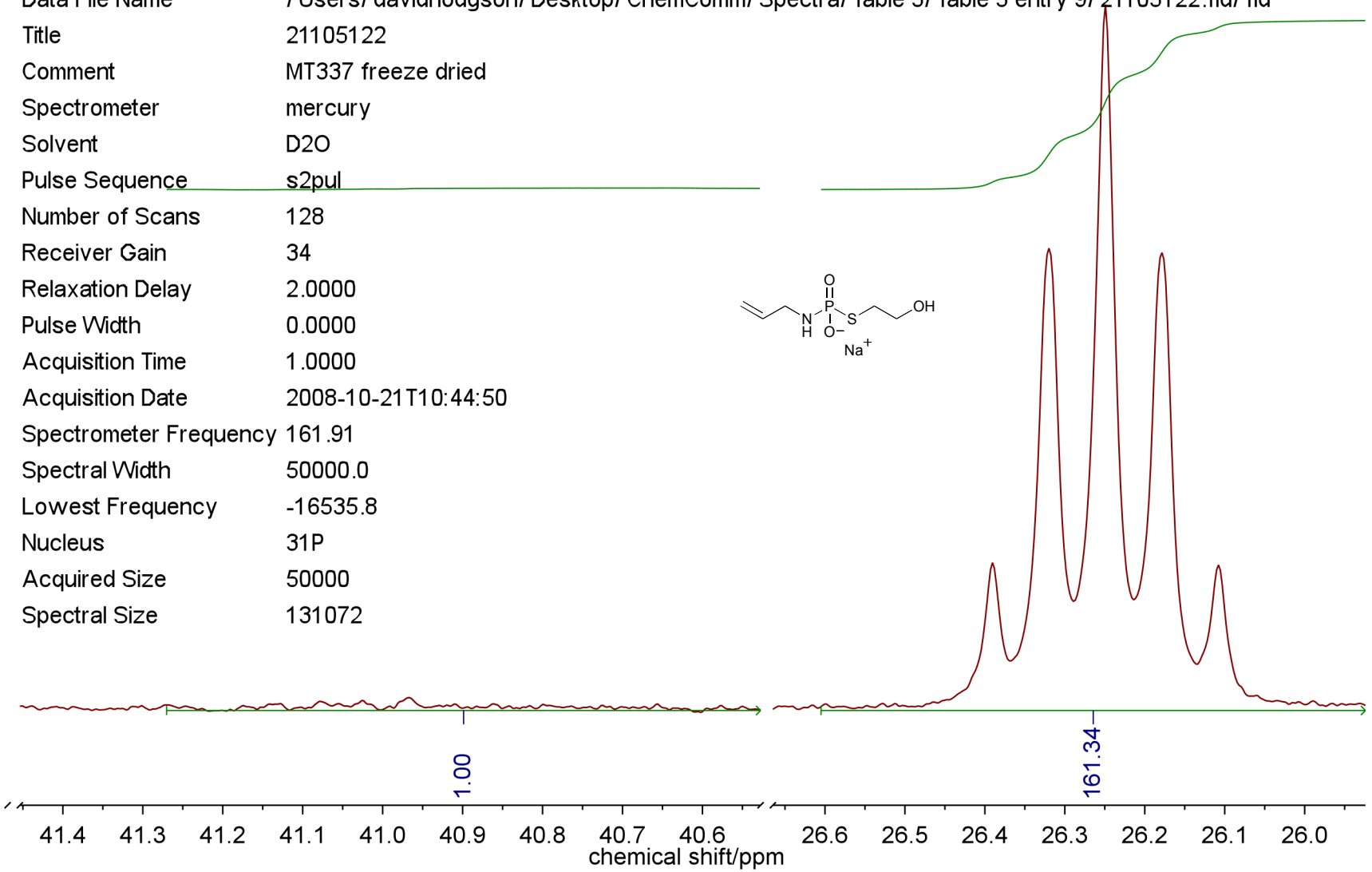
Table 3, entry 9 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 9/ 21105122.fid/ fid
Title	21105122
Comment	MT337 freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	128
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-10-21T10:44:50
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*161.34/(1.0+161.34)=99.4%

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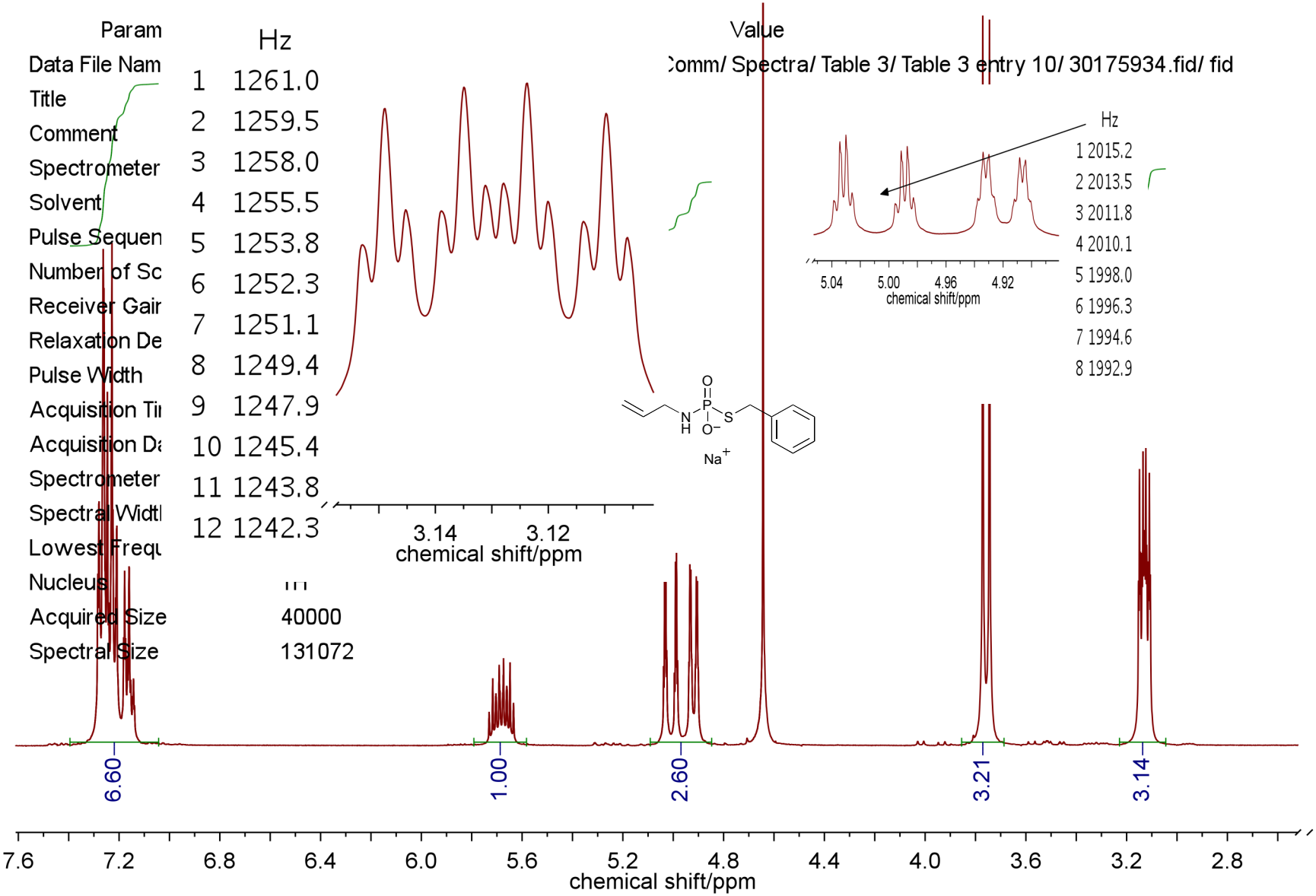
Table 3, entry 10 ^{1}H NMR spectrum



estimated>99%

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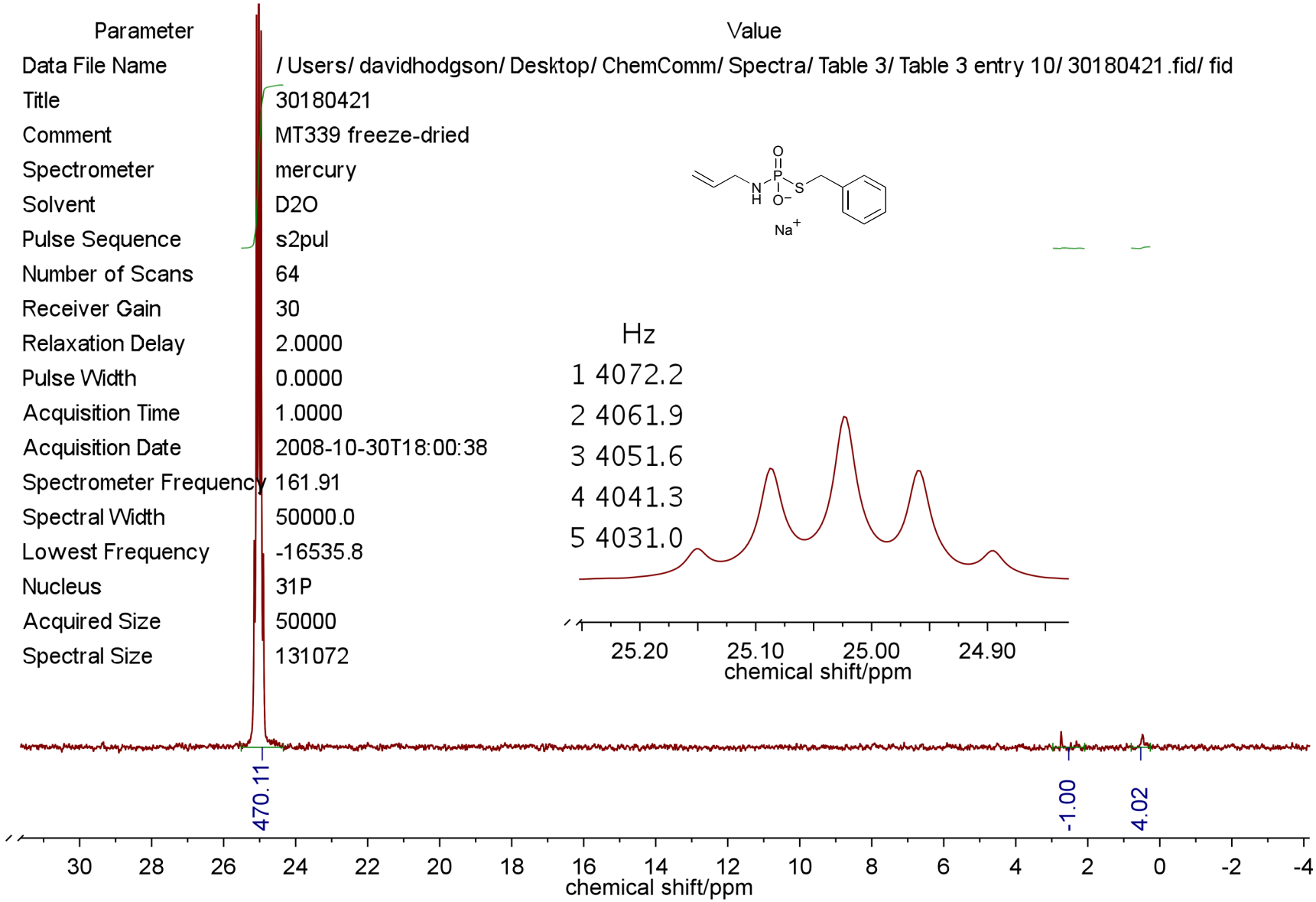
Table 3, entry 10 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 10/ 30180421.fid/ fid
Title	30180421
Comment	MT339 freeze-dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	64
Receiver Gain	30
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-10-30T18:00:38
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



estimated>99%

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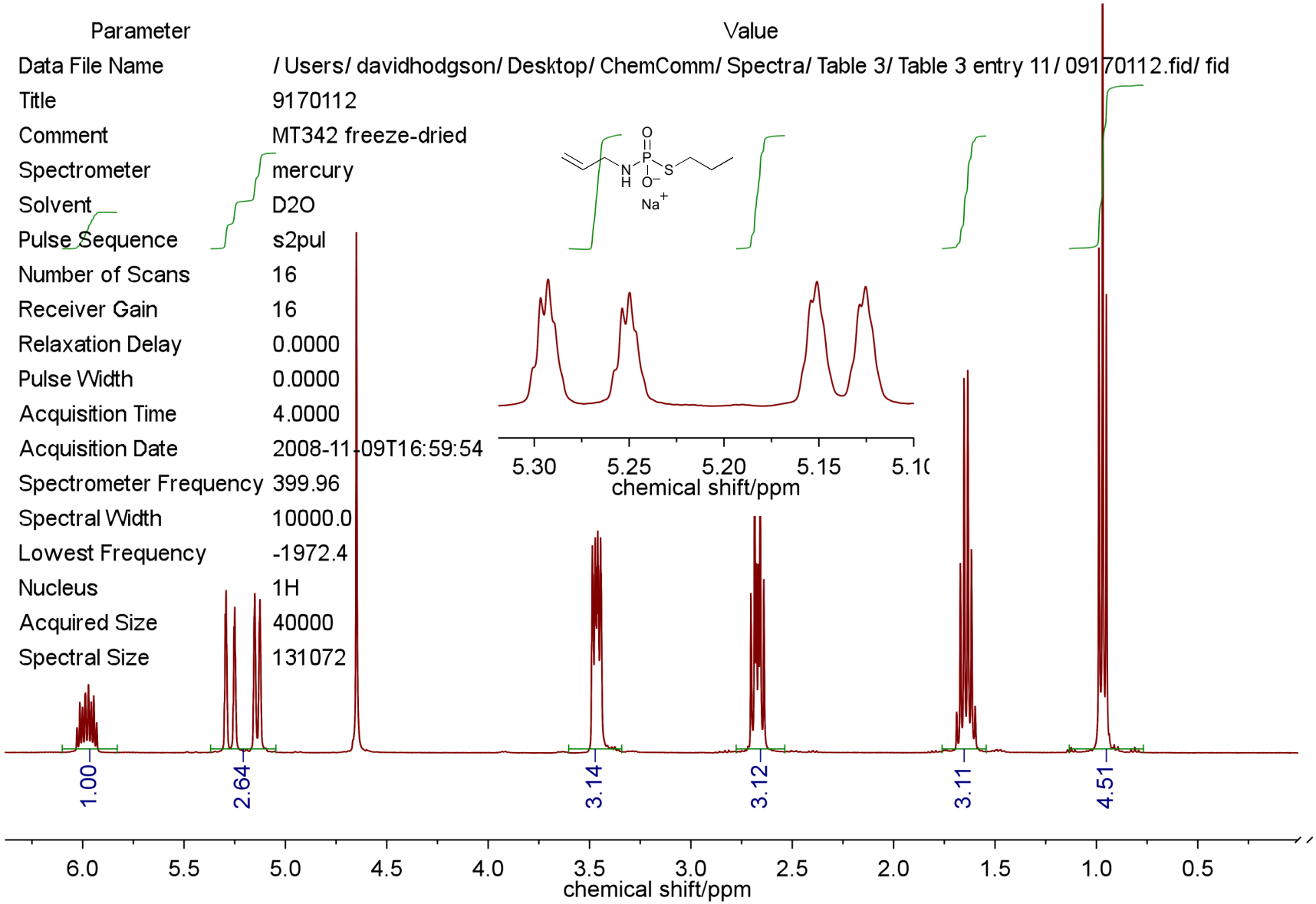
Table 3, entry 11 ^{1}H NMR spectrum



estimated>99%

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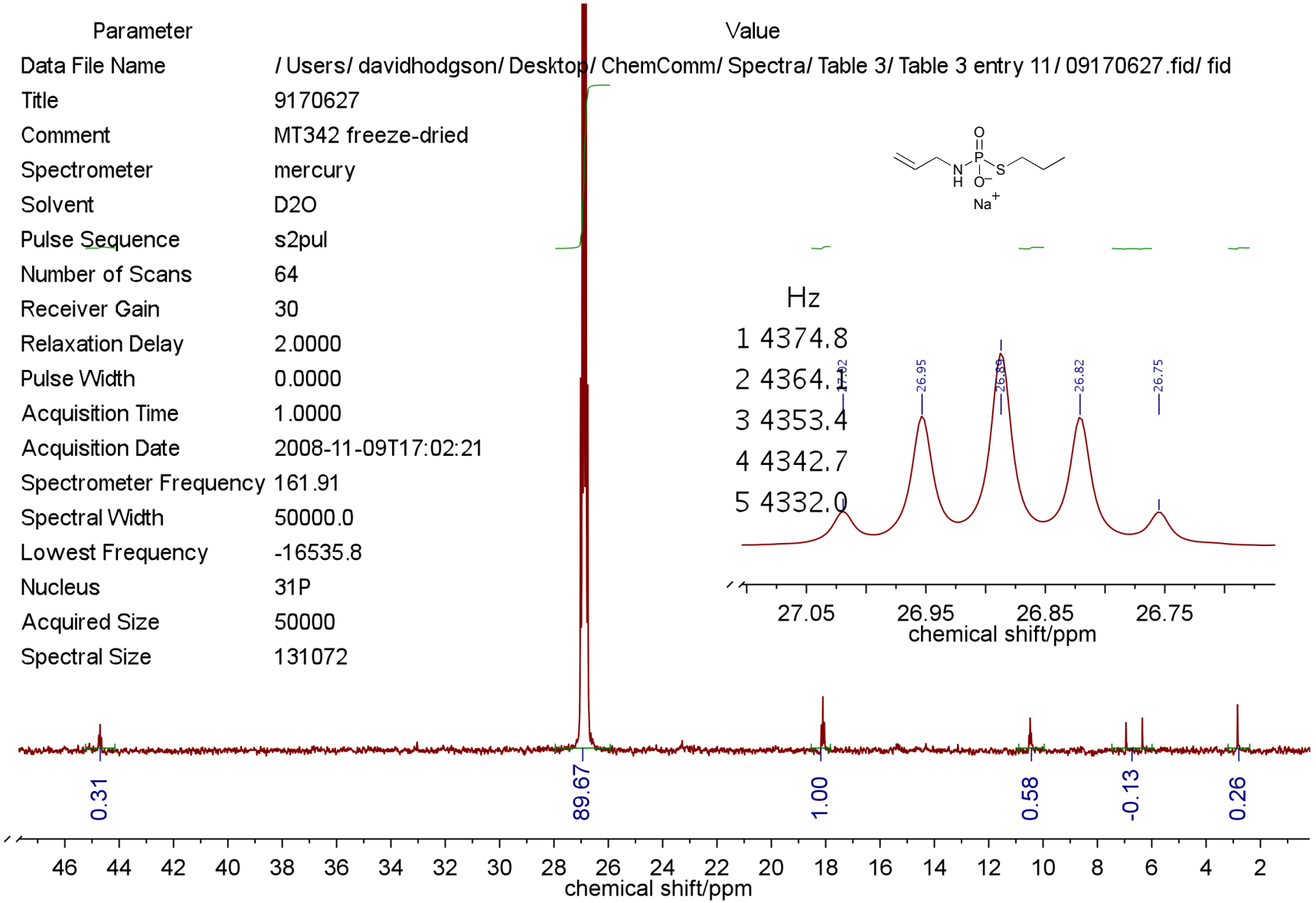
Table 3, entry 11 ^{31}P NMR spectrum



100*89.67/(0.31+89.67+1.00+0.58+0.26)=98%

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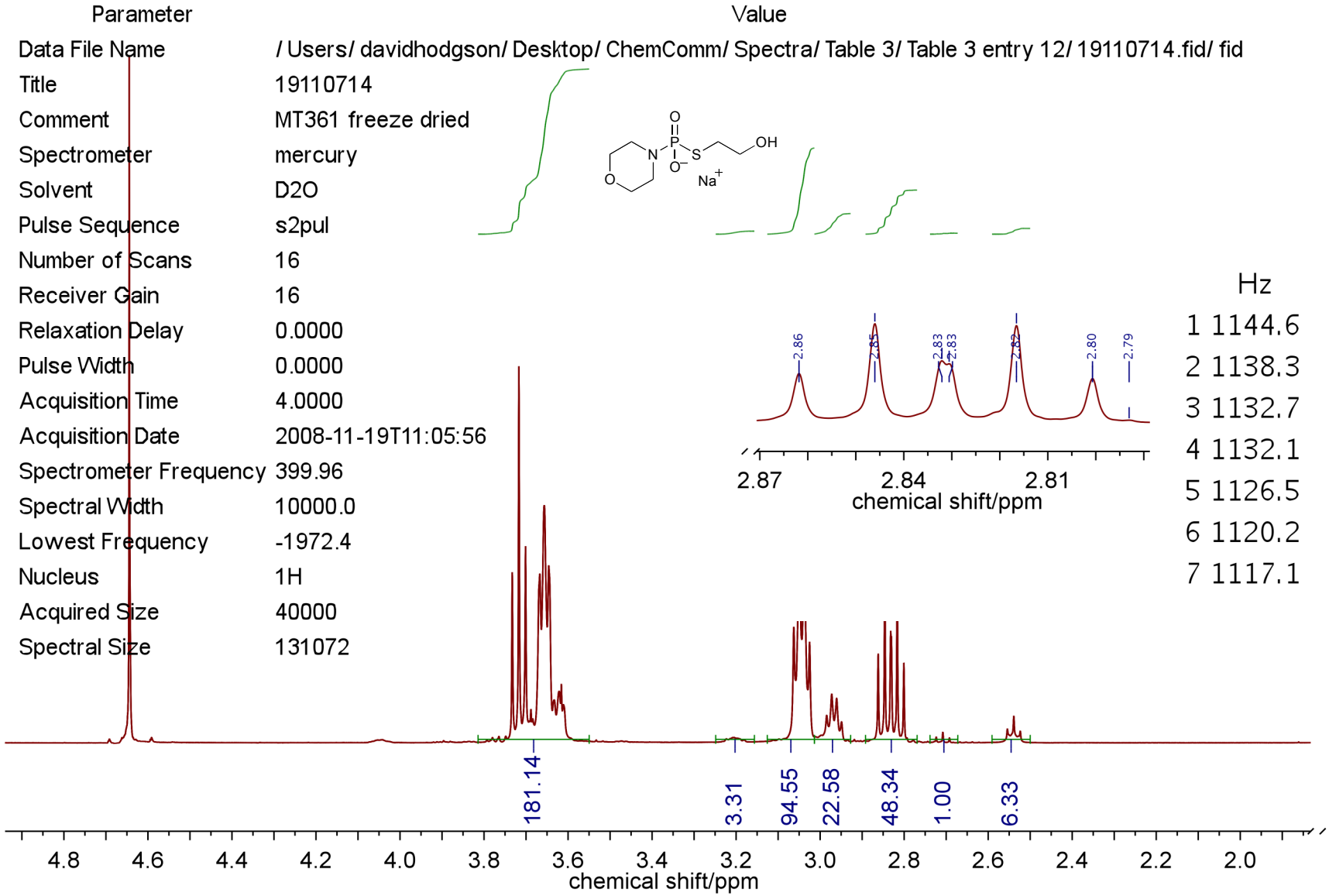
Table 3, entry 12 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 12/ 19110714.fid/ fid
Title	19110714
Comment	MT361 freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	16
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-11-19T11:05:56
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



100*48.34/(48.34+1.00+6.33)=87%

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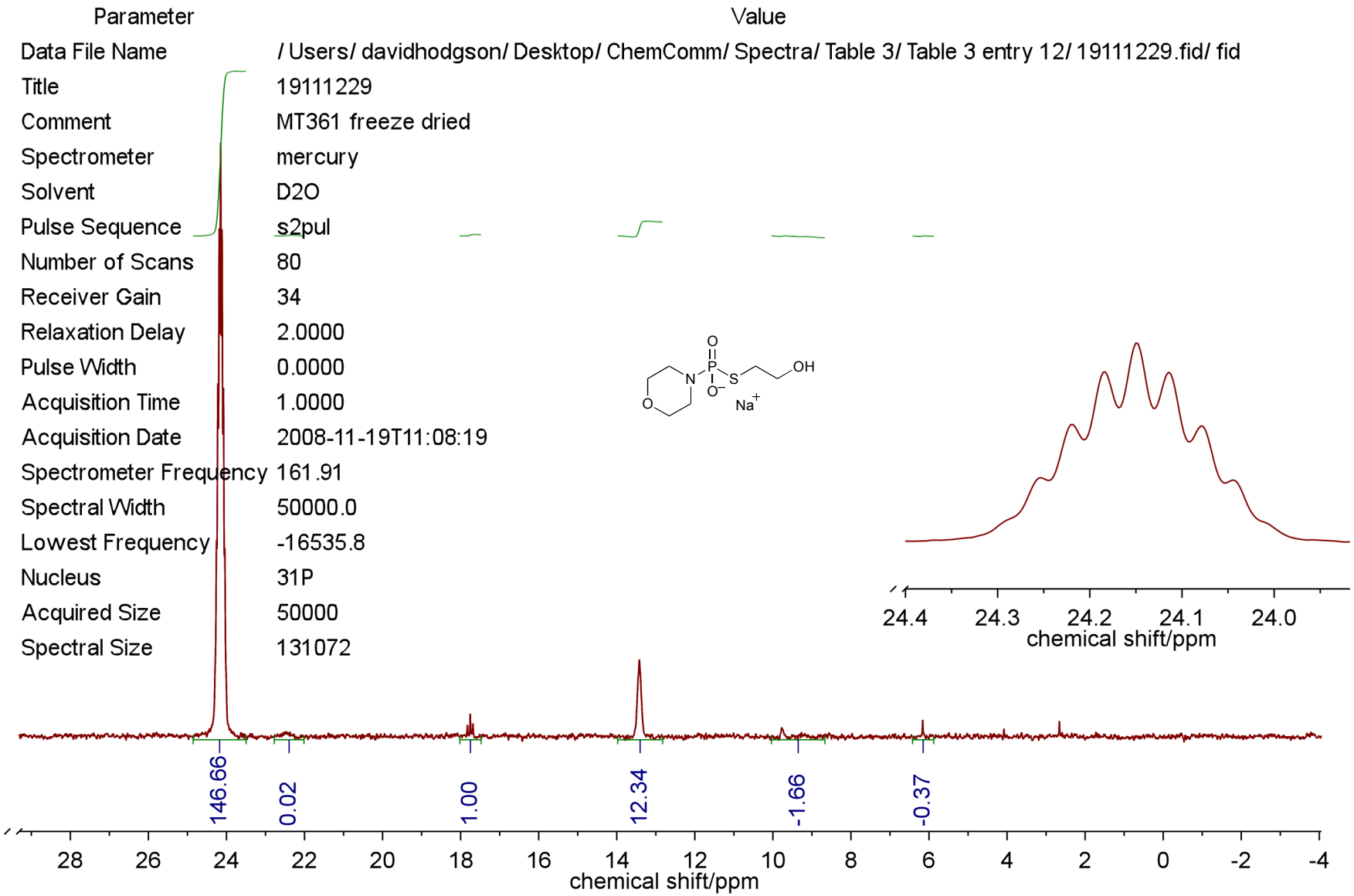
Table 3, entry 12 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 12/ 19111229.fid/ fid
Title	19111229
Comment	MT361 freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	80
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-11-19T11:08:19
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*146.66/(146.66+0.02+1.00+12.34)=92%

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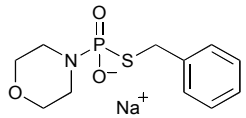
Table 3, entry 13 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 13/ 22133709.fid/ fid
Title	22133709
Comment	MT359 3rd freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	12
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-11-22T13:35:51
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



100*5.77/(5.77+1.00)=85%

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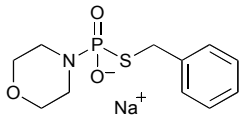
Table 3, entry 13 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 13/ 22134446.fid/ fid
Title	22134446
Comment	MT359 3rd freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	128
Receiver Gain	28
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-11-22T13:38:14
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*243.63/(243.63+0.92+1.00+14.49+1.10+0.88+0.90)=93%

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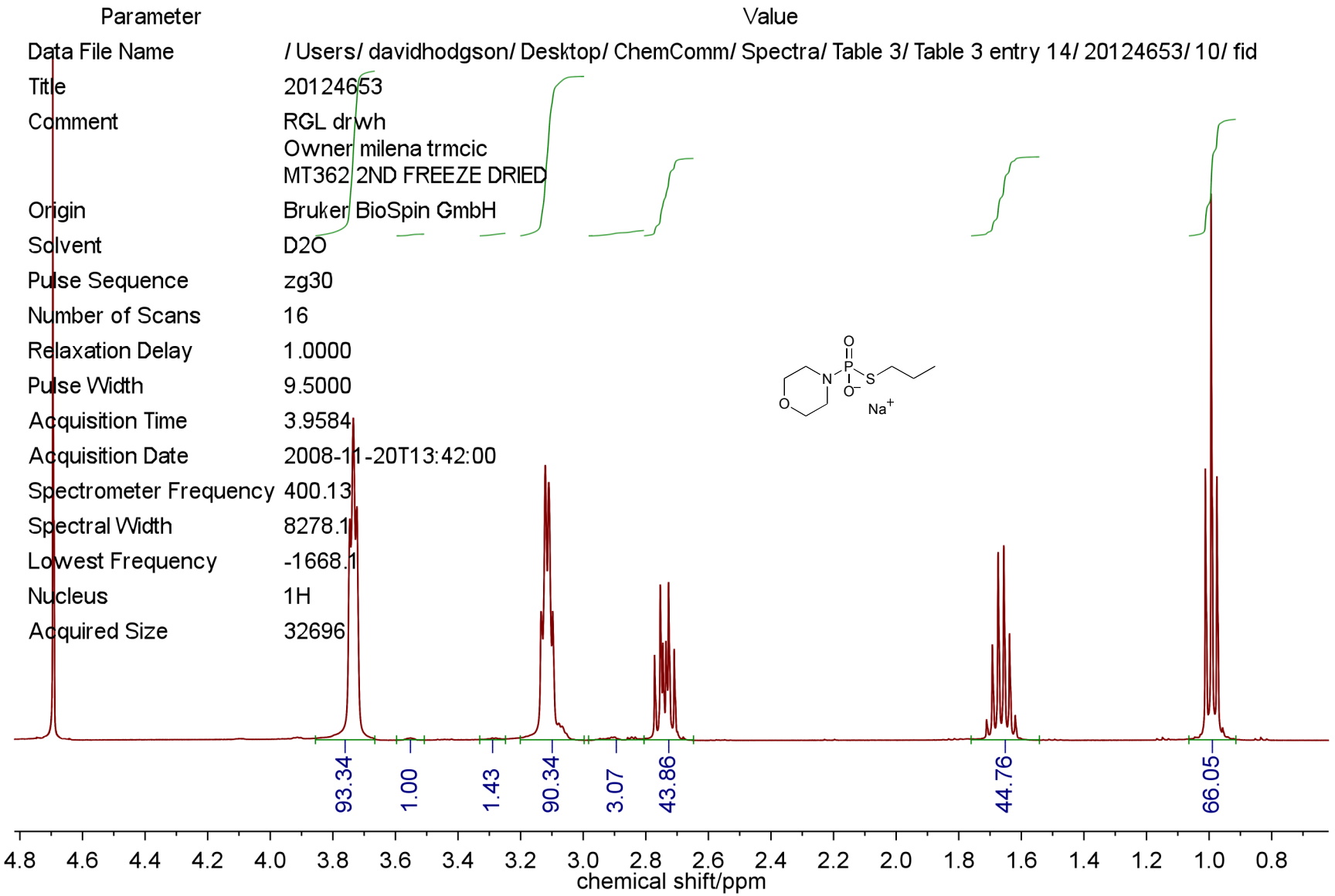
Table 3, entry 14 ^{1}H NMR spectrum



100*93.34/(93.34+1.00)=99%

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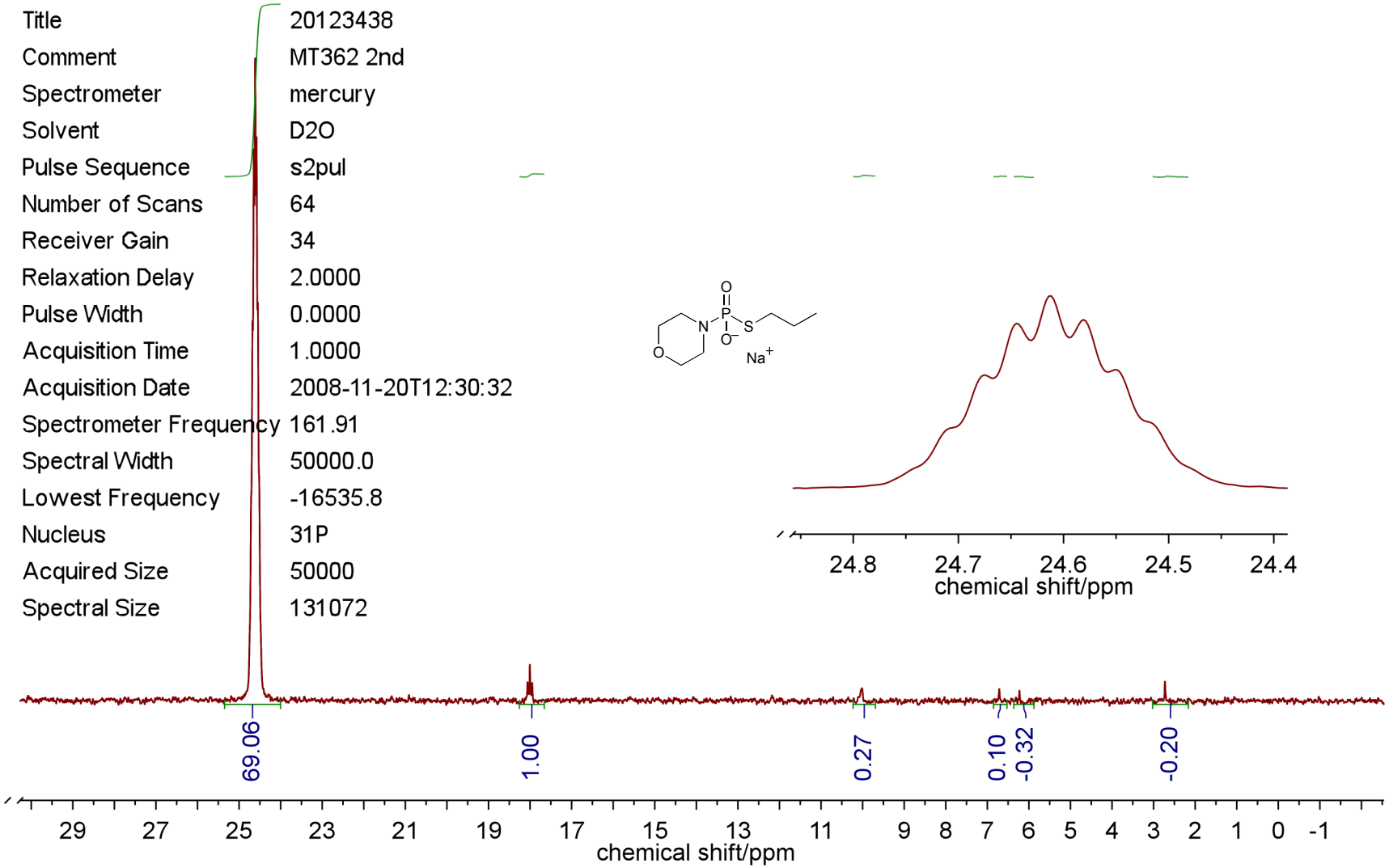
Table 3, entry 14 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 14/ 20123438.fid/ fid
Title	20123438
Comment	MT362 2nd
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	64
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-11-20T12:30:32
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*69.06/(69.06+1.00+0.27+0.10)=98%

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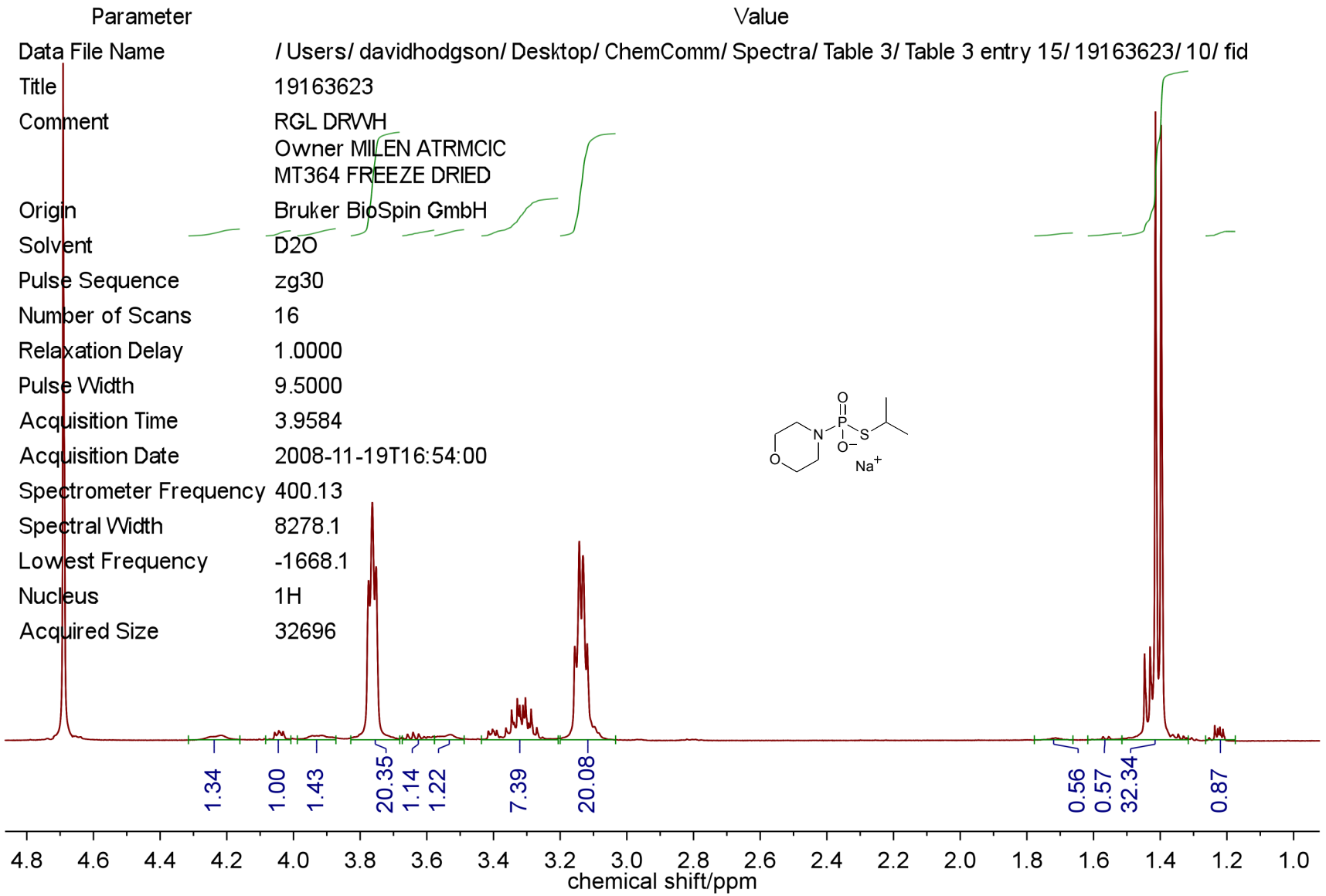
Table 3, entry 15 ^{1}H NMR spectrum



100*20.35/(1.00+1.43+20.35)=89%

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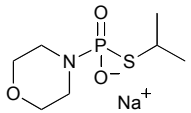
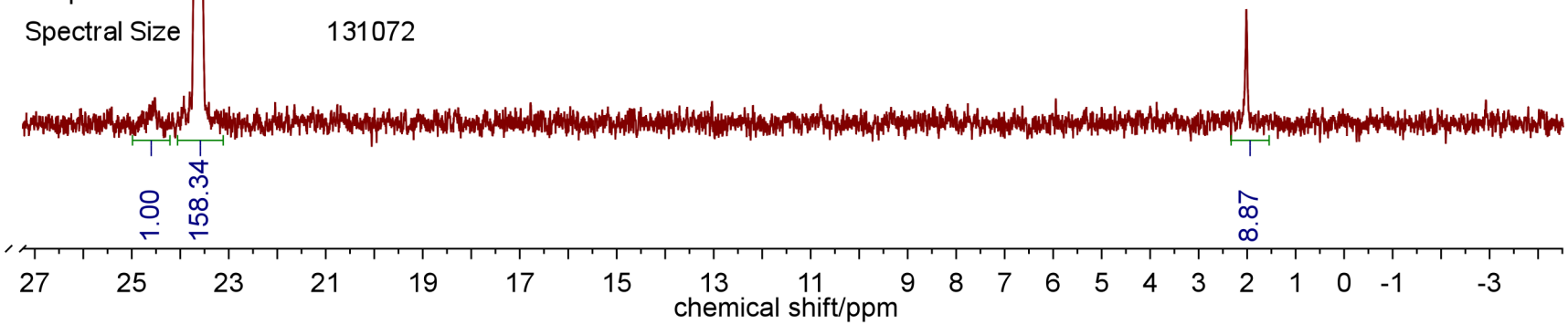
Table 3, entry 15 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 15/ 19114150.fid/ fid
Title	19114150
Comment	MT364 freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	64
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-11-19T11:38:26
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072





100*158.34/(1.00+158.34+8.87)=94%

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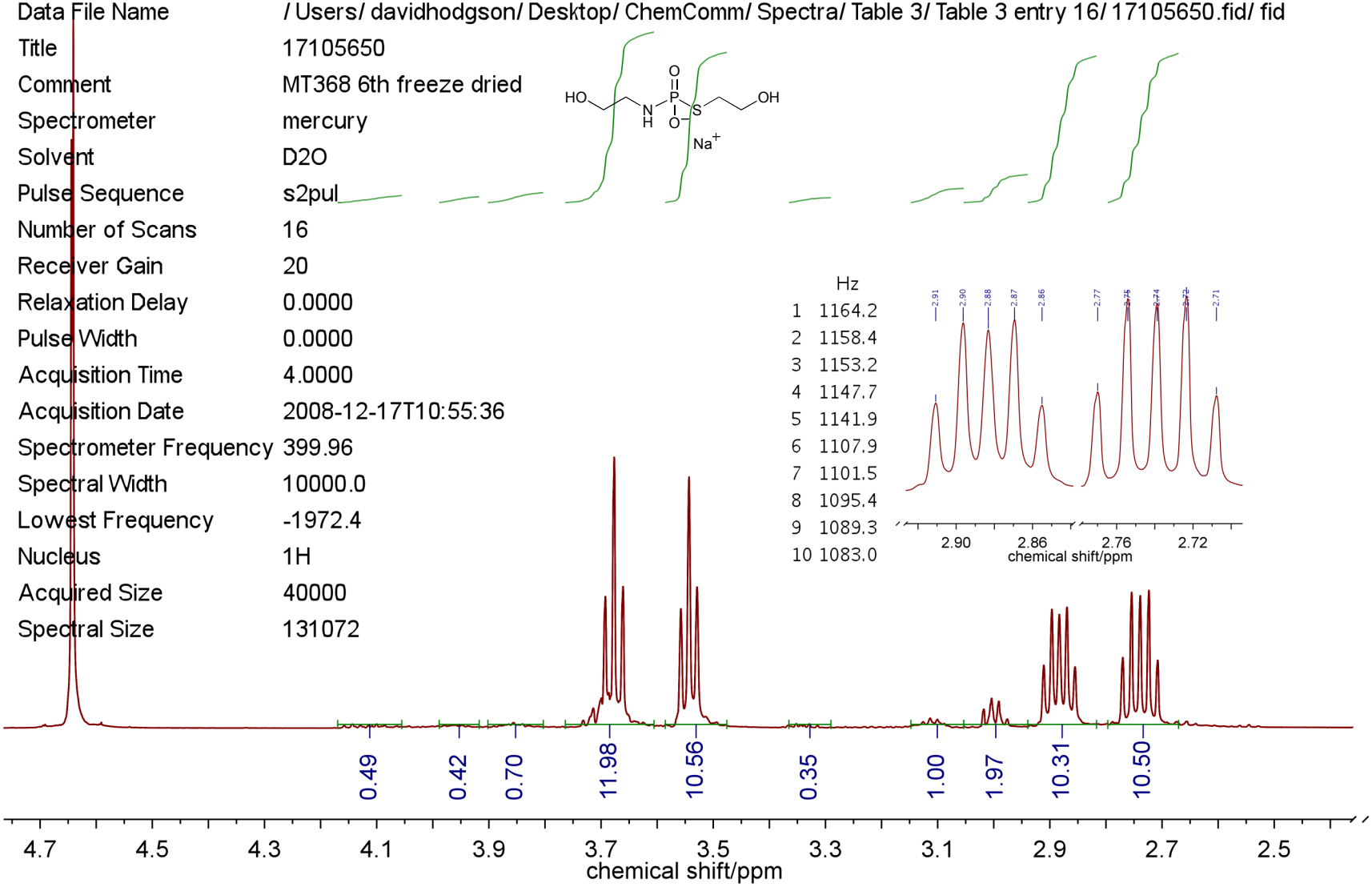
Table 3, entry 16 ^{1}H NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 16/ 17105650.fid/ fid
Title	17105650
Comment	MT368 6th freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	16
Receiver Gain	20
Relaxation Delay	0.0000
Pulse Width	0.0000
Acquisition Time	4.0000
Acquisition Date	2008-12-17T10:55:36
Spectrometer Frequency	399.96
Spectral Width	10000.0
Lowest Frequency	-1972.4
Nucleus	1H
Acquired Size	40000
Spectral Size	131072



100*10.31/(10.31+1.00+1.97)=78%

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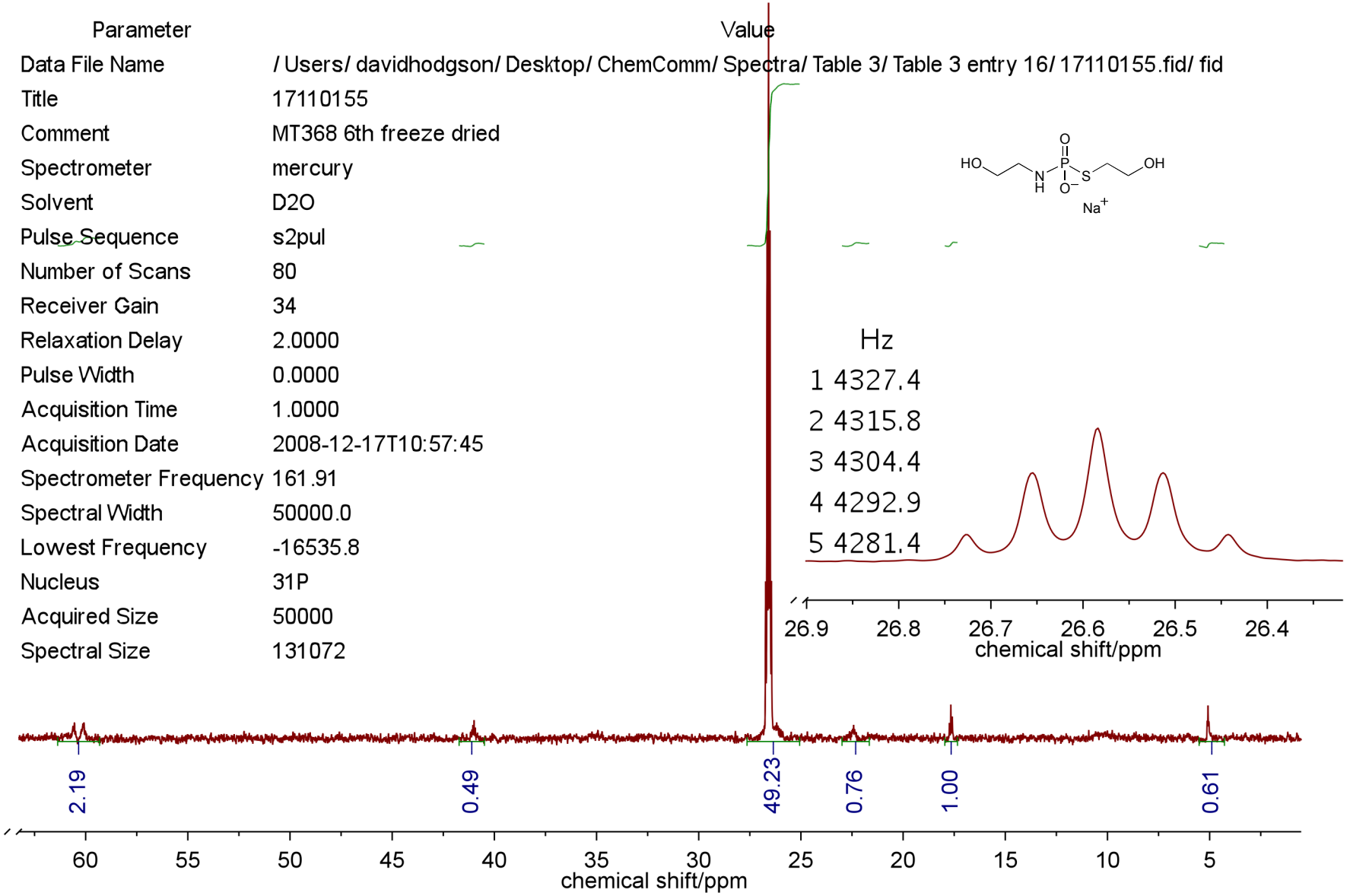
Table 3, entry 16 ^{31}P NMR spectrum

Parameter	Value
Data File Name	/ Users/ davidhodgson/ Desktop/ ChemComm/ Spectra/ Table 3/ Table 3 entry 16/ 17110155.fid/ fid
Title	17110155
Comment	MT368 6th freeze dried
Spectrometer	mercury
Solvent	D2O
Pulse Sequence	s2pul
Number of Scans	80
Receiver Gain	34
Relaxation Delay	2.0000
Pulse Width	0.0000
Acquisition Time	1.0000
Acquisition Date	2008-12-17T10:57:45
Spectrometer Frequency	161.91
Spectral Width	50000.0
Lowest Frequency	-16535.8
Nucleus	31P
Acquired Size	50000
Spectral Size	131072



100*49.23/(2.19+0.49+49.23+0.76+1.00+0.61)=91%

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