Supplementary Material (ESI) for Chemical Communications

Supplemental Information

Identification of Dihydropyridines That Reduce Cellular Tau Levels

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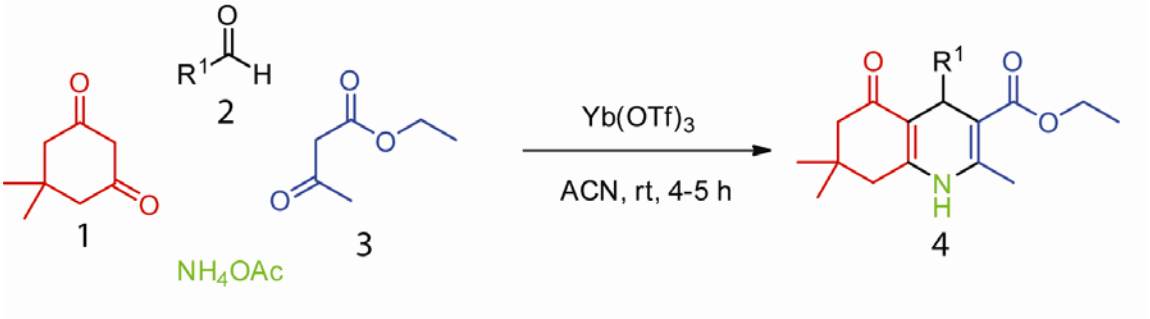
Synthesis of Compounds 4a-4r.



Component 1 dimedone (1.5eq, 0.6mmol) and component 3 ethylacetoacetate (1eq, 0.4mmol) were suspended along with 10mol% VII in 1mL of acetonitrile. After stirring under inert gas for 10min, component 2 (1eq, 0.4mmol) and ammonium acetate (1eq, 0.4mmol) were added. The reaction was allowed to stir for 3-5h. All reactions except for 4d, 4f, 4k, 4l, and 4o were precipitated by the addition of ice/water. Following precipitation the reactions were recrystallized with an ethanol/water system. For the other reactions (4d, 4f, 4k, 4l, and 4o), the reactions were added to brine and extracted with ethylacetate. Following concentration, these products were also recrystallized with an ethanol/water system.

Characterization of compounds 4a-4r.

All NMR collected and analyzed on Varian 400MHz system using VnmrJ™ version 2.2 revision C. Samples diluted in $CDCl_3$ immediately prior to data collection. Mass spectrometry data collected on and Micromass LCT time-of-flight mass spectrometer in the ES+ mode.

ethyl 4-(2,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4a): ^{1}H δ ($CDCl_3$) 7.33-7.31 (1H, d), 7.24 (1H, s), 7.11-7.08 (1H, d), 6.53 (1H, s), 5.33 (1H,s), 4.07-3.99 (2H, m), 2.30 (3H, s), 2.29-2.21 (2H, dd), 2.18-2.09 (2H, dd), 1.19-1.16 (3H, t), 1.06 (3H, s), 0.94 (3H, s). [M+H] e: 408.11, o: 408.1. Yield 89-94%.

ethyl 2,7,7-trimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4b): ^{1}H δ ($CDCl_3$) 7.3-7.29 (2H, d), 7.21-7.17 (2H, t), 7.11-7.07 (1H, t),5.93 (1H, s), 5.05 (1H, s), 4.08-4.03 (2H, m), 2.37 (3H, s),2.34-2.2.22 (2H, dd), 2.21-2.13 (2H, dd), 1.21-1.17 (3H, t), 1.07 (3H, s), 0.93 (3H, s). [M+H] e: 340.18, o: 340.2. Yield 82-85%.

ethyl 4-(4-bromophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4c): ^{1}H δ ($CDCl_3$) 7.32-7.30 (2H, d), 7.18-7.16 (2H, d), 6.29 (1H, s), 5.00 (1H, s), 4.08-4.03 (2H, m), 2.37 (3H, s), 2.35-2.23 (2H, dd), 2.21-2.12 (2H, dd), 1.20-1.17 (3H, t), 1.07 (3H, s), 0.92 (3H, s). [M+H] e: 418.09, o: 418.1. Yield 80-84%.

ethyl-(3,5-dimethoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4d): ^{1}H δ ($CDCl_3$) 6.55 (1H, s), 6.47 (2H, s), 6.23-6.22 (1H, t), 5.01 (1H, s), 4.10-4.05 (2H, m),

3.72 (6H, s), 2.34 (3H, s), 2.31-2.25 (2H, dd), 2.22-2.15 (2H, dd), 1.24-1.20 (3H, t), 1.05 (3H, s), 0.96 (3H, s). [M+H] e: 400.20, o: 400.2. Yield 72-74%.

ethyl 4-(3-fluorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4e): ^{1}H δ ($CDCl_3$) 7.17-7.09 (2H, m), 6.99-6.95 (1H, d), 6.80-6.76 (2H, t), 6.28 (1H, s), 5.06 (1H, s), 4.09-4.04 (2H, m), 2.38 (3H, s), 2.33-2.26 (2H, d), 2.22-2.14 (2H, dd), 1.21-1.17 (3H, t), 1.07 (3H, s), 0.93 (3H, s). [M+H] e: 358.17, o: 358.2. Yield 89-92%.

ethyl-(4-(dimethylamino)phenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4f): ^{1}H δ ($CDCl_3$)7.18-7.16 (2H, d), 6.64-6.62 (2H, d), 6.44 (1H, s), 4.95 (1H, s), 4.09-4.04 (2H, m), 2.86 (6H, s), 2.34 (3H, s), 2.28-2.23 (2H, dd), 2.19-2.11 (2H, dd), 1.24-1.20 (3H, t), 1.05 (3H, s), 0.94 (3H, s). [M+H] e: 383.23, o: 383.2. Yield 75-78%.

ethyl-2,7,7-trimethyl-5-oxo-4-(2-(trifluoromethyl)phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4g): ^{1}H δ ($CDCl_3$) 7.50-7.48 (1H, d), 7.45-7.43 (1H, d), 7.39-7.35 (1H, t), 7.22-7.18 (1H, t), 6.48 (1H, s), 5.60 (1H, s), 4.15-3.93 (2H, m), 2.36-2.22 (2H, d), 2.28 (3H, t), 2.21-2.07 (2H, dd), 1.14-1.11 (3H, t), 1.05 (3H, s), 0.90 (3H, s). [M+H] e: 408.17, o: 408.2. Yield 79-85%.

ethyl -4-(2,5-difluorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4h): ^{1}H δ ($CDCl_3$) 7.02 (1H, m), 6.84-6.74 (2H, m), 6.33 (1H, s), 5.19 (1H, s), 4.07-4.02 (2H, m), 2.38-2.26 (2H, dd), 2.34 (3H, s), 2.21-2.13 (2H, dd), 1.21-1.17 (3H, t), 1.08 (3H, s), 0.95 (3H, s). [M+H] e: 376.16, o: 376.2.. Yield 90-94%.

ethyl -4-(4-tert-butylphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4i): ^{1}H δ ($CDCl_3$) 7.18 (4H, s), 6.22, (1H, s), 5.02 (1H, s), 4.11-4.04 (2H, m), 2.37-2.26 (2H, dd), 2.34 (3H, s), 2.24-2.16 (2H, dd), 1.26 (9H, s), 1.21-1.19 (3H, t), 1.07 (3H, s), 0.97 (3H, s). [M+H] e: 396.25, o: 396.3. Yield 84-88%.

ethyl 2,7,7-trimethyl-4-(naphthalen-2-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4j): ^{1}H δ ($CDCl_3$) 7.74-7.67 (4H, m), 7.50 (1H, d), 7.38-7.35 (2H, t), 6.52 (1H, s), 5.22 (1H, s), 4.07-4.02 (2H, m), 2.41 (3H, s), 2.21-2.19 (2H, dd), 2.14-2.06 (2H, dd), 1.21-1.17 (3H, t), 1.10 (3H, s), 0.87 (3H, s). [M+H] e: 390.20, o: 390.2. Yield 81-83%.

ethyl 4-(4-cyanophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4k): ^{1}H δ ($CDCl_3$) 7.51-7.49 (2H, d), 7.43-7.41 (2H, d), 6.29 (1H, s), 5.09 (1H, s), 4.07-4.01 (2H, m), 2.40 (3H, s), 2.34-2.25 (2H, d), 2.21-2.12 (2H, dd), 1.18-1.14 (3H, t), 1.07 (3H, s), 0.90 (3H, s). [M+H] e: 365.18, o:365.2. Yield 72%.

ethyl -4-(3,4-dihydroxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4l): ^{1}H δ (CD_3OD) 6.68 (1H, s), 6.54 (2H, s), 4.84 (2H, s), 4.81 (1H, s), 4.06-4.00 (2H, m), 3.28 (1H, s), 2.42-2.24 (2H, dd), 2.31 (3H, s), 2.19-2.06 (2H, dd), 1.20-1.17 (3H, t), 1.04 (3H, s), 0.91 (3H, s). [M+H] e: 371.17, o: 372.2. Yield 69%.

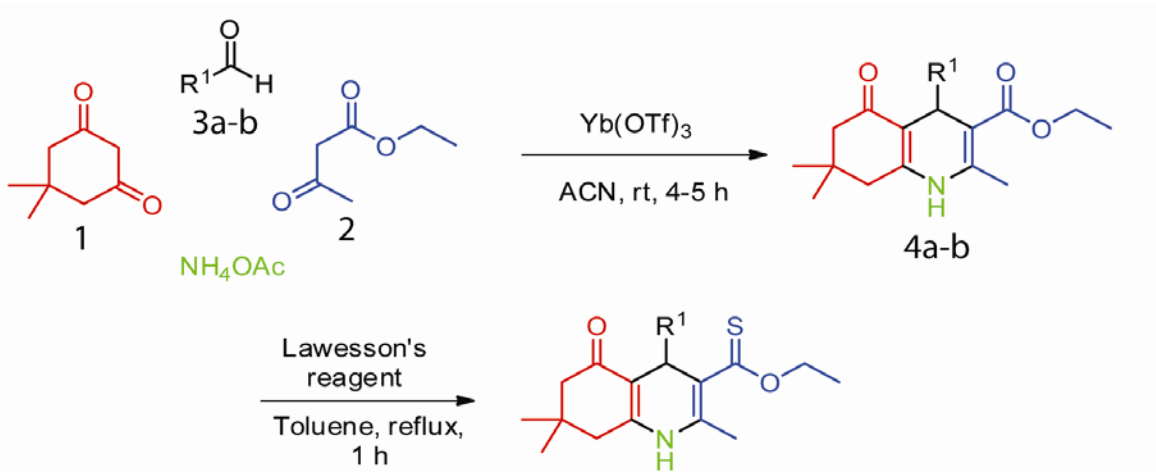
ethyl 2,7,7-trimethyl-5-oxo-4-phenethyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4m): ^{1}H δ ($CDCl_3$) 7.25-7.08 (5H, m), 6.33 (1H, s), 4.21-4.00 (3H, m), 2.57-2.48 (2H, m), 2.23 (3H, s), 2.30-2.25 (2H, d), 2.20-2.16 (2H, d), 1.59-1.82 (2H, m), 1.29-1.25 (3H, t), 1.10 (3H, s), 1.08 (3H, s). [M+H] e: 368.21, o: 368.2. Yield 92-94%.

ethyl 4-(2-chlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4n): ^{1}H δ ($CDCl_3$) 7.39-7.37 (1H, d), 7.24-7.22 (1H, d), 7.13-7.09 (1H, t), 7.03-7.00 (1H, t), 6.76 (1H, s), 5.38 (1H, s), 4.07-4.00 (2H, m), 2.33-2.23 (2H, dd), 2.27 (3H, s), 2.18-2.08 (2H, dd), 1.19-1.15 (3H, t), 1.05 (3H, s), 0.93 (3H, s). [M+H] e: 374.14, o: 374.1. Yield 89-94%.

ethyl 4-(biphenyl-4-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4o): ^{1}H δ ($CDCl_3$) 7.53-7.51 (2H, d), 7.44-7.36 (6H, m), 7.30-7.28 (1H, d), 6.50 (1H, s), 5.10 (1H, s), 4.10-4.05 (2H, m), 2.38 (3H, s), 2.36-2.26 (2H, dd), 2.22-2.14 (2H, dd), 1.23-1.20 (3H, t), 1.07 (3H, s), 0.95 (3H, s). [M+H] e: 416.21, o: 416.2. Yield 72-77%.

ethyl 2,7,7-trimethyl-5-oxo-4-(1,4-dioxaspiro[4.5]decan-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (4r): ^{1}H δ ($CDCl_3$) 5.83-5.66 (1H, d), 4.32-4.30 (1H, m), 4.26-4.11 (2H, m), 3.99-3.92 (1H, m), 3.84-3.79 (1H, m), 3.73-3.66 (1H, m), 2.44-2.16 (4H, t), 1.55-1.43 (6H, dd), 1.38-1.20 (4H, m), 1.15-1.05 (3H, t). [M+H] e: 404.24, o: 404.2. Yield 75%.

Synthesis of Compounds 5a-b.



Component 1 dimedone (1.5eq, 0.6mmol) and component 3 ethylacetoacetate (1eq, 0.4mmol) were suspended along with 10mol% $Yb(OTf)_3$ in 1mL of acetonitrile. After stirring under inert gas for 10min, component 2 (1eq, 0.4mmol) and ammonium acetate (1eq, 0.4mmol) were added. The reaction was allowed to stir for 3-5h. All reactions were precipitated by the addition of ice/water. Following precipitation the reactions were recrystallized with an ethanol/water system. The product was then added to a round bottom flask diluted with toluene and Lawesson's reagent (2.2 equiv) was added and the reaction was refluxed for 1-2 hours. The resulting product was purified by column chromatography (eluting with 25/75

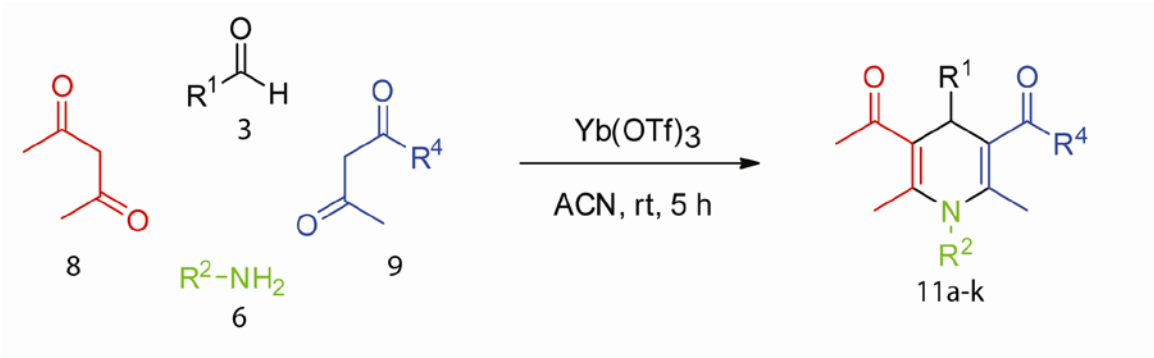
ethylactetate/hexanes).

Characterization of Compounds 5a-b.

O-ethyl 4-(2,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbothioate (5a): ^{1}H □ (CDCl3) 7.33-7.31 (1H, d), 7.24 (1H, s), 7.11-7.08 (1H, d), 6.53 (1H, s), 5.33 (1H,s), 4.07-3.99 (2H, m), 2.30 (3H, s), 2.29-2.21 (2H, dd), 2.18-2.09 (2H, dd), 1.19-1.16 (3H, t), 1.06 (3H, s), 0.94 (3H, s). [M+H] e: 424.08, o: 424.1. Yield 89-94%.

O-ethyl 2,7,7-trimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbothioate (5b): ^{1}H □ (CDCl3) 7.3-7.29 (2H, d), 7.21-7.17 (2H, t), 7.11-7.07 (1H, t),5.93 (1H, s), 5.05 (1H, s), 4.08-4.03 (2H, m), 2.37 (3H, s),2.34-2.2.22 (2H, dd), 2.21-2.13 (2H, dd), 1.21-1.17 (3H, t), 1.07 (3H, s), 0.93 (3H, s). [M+H] e: 356.16, o: 356.2. Yield 82-85%.

Synthesis of Compounds 7a-d, 10a-d, and 11a-k.



The synthesis of compounds 7, 10, and 11 necessitated adding the amine (1 eq, 0.4 mmol) component to the beta-keto ester (1 eq, 0.4 mmol) in acetonitrile. The two components were then stirred for 30 minutes. The catalyst $Yb(OTf)_3$ (10 mol %), the aldehyde (1 eq, 0.4 mmol), and 1,3-diketone (1.5 eq, 0.6 mmol) were then added to the reaction vessel and allowed to stir for the required amount of time. The reactions were added to brine and extracted with ethylacetate. Following concentration, these products were also recrystallized from ethanol.

Characterization of Compounds 7a-d, 10a-d, and 11a-k.

4-(4-(2,4-dichlorophenyl)-3-(ethoxycarbonyl)-2,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinolin-1(4H)-yl)butanoic acid (7a): ^{1}H δ ($CDCl_3$) 11.87, 7.39-7.09 (2H, dd), 7.06-6.95 (1H, d), 5.61-5.51 (1H, t), 4.84- 4.74 (1H, t), 3.21-3.11 (2H, t), 2.52-2.38 (6H, m), 2.35-2.29 (3H, t), 2.27-2.17 (2H, dd), 2.12-1.94 (2H, dd), 1.25-1.20 (4H, t), 1.15-0.99 (12H, d), 0.99-0.88 (3H), t). [M+H] e: 494.14, o: 491.1. Yield 71%.

ethyl 4-(2,4-dichlorophenyl)-1-(4-hydroxybutyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (7b): ^{1}H δ ($CDCl_3$) 7.32-7.25 (2H, m), 7.24-6.98 (1H, m), 4.91 (1H, bs), 4.15-3.97 (2H, m), 3.09-2.82 (2H, bd), 2.47 (3H, m), 2.42-2.15 (2H,m), 2.07-1.97 (1H, t), 1.58-1.46 (3H, t), 1.30-1.25 (2H, d), 1.24-0.83 (10H, m). [M+H] e: 480.16, o: 480.2. Yield 74%.

ethyl 4-(2,4-dichlorophenyl)-1-(3-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (7c): ^{1}H δ ($CDCl_3$) 7.60-7.58 (1H, d), 7.38-7.29 (1H, m), 7.25-7.04 (2H, m), 7.04-6.93 (2H, m), 6.78-6.53 (1H, dd), 6.44-6.34 (1H,dd), 5.63 (1H, s), 5.46 (1H, d), 4.15-4.05 (2H, m), 2.39-2.19 (2H, dd), 2.15-2.08 (2H, d), 2.08-2.05 (3H, s), 1.97-1.89 (1H, t), 1.28-1.24 (2H, t), 1.19-1.02 (3H, t), 0.98-0.84 (6H, d). [M+H] e: 500.13, o: 500.1. Yield 82%.

ethyl 1-benzyl-4-(2,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (7d): ^{1}H δ ($CDCl_3$) 7.31-6.72 (9H), 5.31 (1H, s), 5.06-5.00 (2H, q), 2.32-2.25 (3H, s), 2.24-2.12 (3H, dd), 2.10-2.04 (2H, dd), 1.08 (3H, s), 0.96(3H, s). [M+H] e: 498.15, o: 498.2. Yield 77%.

ethyl 4-(2,4-dichlorophenyl)-2-methyl-5-oxo-4,5-dihydro-1H-indeno[1,2-b]pyridine-3-carboxylate (10a): ^{1}H δ ($CDCl_3$) 7.93-7.10 (7H), 5.36 (1H, t), 4.09-3.98 (2H, m), 2.78-2.68 (3H, t), 2.39-2.24 (2H, dt), 1.34-1.28 (3H, m), 1.10-0.86 (3H, m). [M+H] e: 414.06, o: 414.1. Yield 71%.

ethyl 5-acetyl-4-(2,4-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (10b): ^{1}H δ ($CDCl_3$) δ 7.31-7.09 (3H), 6.15 (1H, s), 5.43-5.33 (1H, t), 4.13-4.07 (2H, m), 2.32-2.28 (3H, t), 2.22-2.12 (6H, t),1.32-1.22 (3H, t). [M+H] e: 368.07, o: 368.1. Yield 75%.

methyl 4-(2,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (10c): ^{1}H δ ($CDCl_3$) 7.35-7.16 (2H, m), 7.11-7.04 (1H, m), 6.44 (1H, bs), 5.38-5.28 (1H, t), 3.83-3.54 (3H, t), 2.37-2.27 (3H, t), 2.24-2.05 (4H, d), 1.24-1.02 (3H, t), 1.00-0.80 (3H, t). [M+H] e: 394.09, o: 394.1. Yield 82%.

benzyl 4-(2,4-dichlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (10d): ^{1}H δ ($CDCl_3$) 7.31-6.99 (7H, m), 6.98 (1H, d), 6.72 (1H, s), 5.31 (1H, s), 5.03-5.00 (2H, q), 2.27 (3H, t), 2.25-2.16 (4H, d), 2.12-2.04 (2H,dd), 1.08-1.03 (3H, t), 0.98-0.91 (3H, t). [M+H] e: 470.12, o: 470.1. Yield 85%.

benzyl 5-acetyl-4-(2,4-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (11a): ^{1}H δ ($CDCl_3$) 7.38-7.10 (8H), 6.28 (1H, t), 5.43 (1H, t), 5.21-5.11 (2H, t), 2.39-2.34 (3H, t), 2.33-2.21 (6H, t). [M+H] e: 430.09, o: 430.1. Yield 81%.

5-acetyl-N-(cyclohexylmethyl)-4-(2,4-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxamide (11b): ^{1}H δ ($CDCl_3$) 8.20 (1H, s), 7.31-7.21 (3H), 6.90 (1H, m), 5.39 (1H, s), 3.77-3.60 (2H, dq), 3.18-2.96 (6H, m), 2.81 (3H, t), 2.37-2.30 (3H, dd), 1.99-1.58 (10H, m), 1.30-1.17 (3H, m). [M+H] e: 435.15, o: 435.2. Yield 82%.

5-acetyl-N-(4-(tert-butyl)benzyl)-4-(2,4-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxamide (11c): ^{1}H δ ($CDCl_3$) 7.34-7.09 (7H, m), 5.37 (1H, t), 4.12-4.09 (2H, m), 3.08-2.66 (2H, dd), 2.54-2.28 (3H, m), 2.27-2.13 (6H, m), 1.41-1.15 (9H, m). [M+H] e: 485.17, o: 485.2. Yield 76%.

ethyl 5-acetyl-4-(2,4-dichlorophenyl)-1-(4-hydroxybutyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (11d): ^{1}H δ ($CDCl_3$) 7.41-7.09 (3H, m), 4.63- 4.55 (1H, m), 4.09 (2H, m), 2.56 (2H, m), 2.26-1.80 (4H, m), 1.76-1.59 (6H, t), 1.30-1.22 (6H, t), 1.18-1.03 (3H, m). [M+H] e: 440.13, o: 440.1. Yield 69%.

ethyl 5-acetyl-1-benzyl-4-(2,4-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (11e) ^{1}H δ ($CDCl_3$) 7.36-7.19 (8H), 4.49 (1H, d), 4.44-4.40 (2H, m), 4.38 (2H, m), 3.47-3.37 (3H, t), 2.29 (6H, t), 1.99 (3H, t). [M+H] e: 458.12, o: 458.1. Yield 82%.

ethyl 5-acetyl-2,6-dimethyl-4-(naphthalen-2-yl)-1,4-dihydropyridine-3-carboxylate (11f): ^{1}H δ ($CDCl_3$) 7.76-7.39 (7H), 6.23 (1H, t), 5.20 (1H, t), 4.19-4.14 (3H, m), 2.39-2.33 (3H, t), 2.28-2.19 (6H, t), 1.34-1.24 (3H, m). [M+H] e: 350.17, o: 350.2. Yield 71%.

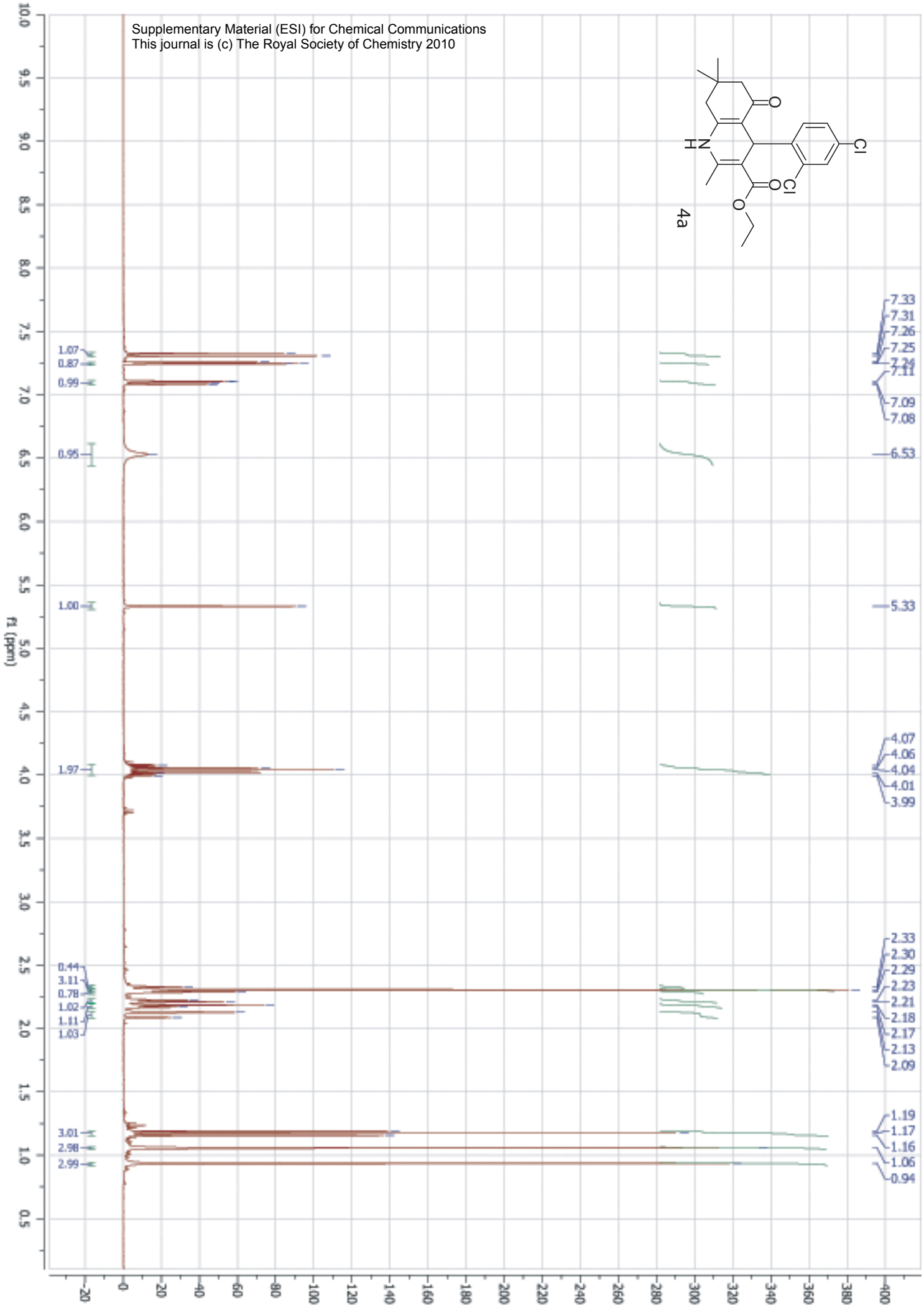
ethyl 5-acetyl-4-(4-bromophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (11g): ^{1}H δ ($CDCl_3$) 7.35-7.24 (2H, m), 7.18-7.08 (2H, m), 6.19 (1H, t), 5.01-4.96 (1H, m), 4.17-4.08 (2H, m), 2.36-2.25 (6H, t), 2.23-2.18 (3H, t), 1.30-1.18 (3H, m). [M+H] e: 378.06, o: 378.1. Yield 84%.

ethyl 5-acetyl-4-(3-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (11h): ^{1}H δ ($CDCl_3$) 7.31-7.13 (, 7.01, 7.00, 6.99, 6.98, 6.72, 5.31, 5.06, 5.03, 5.03, 5.00, 4.12, 4.11, 2.32, 2.27, 2.25, 2.24, 2.22, 2.20, 2.17, 2.16, 2.12, 2.10, 2.08, 2.07, 2.07, 2.06, 2.04, 2.04, 1.27, 1.27, 1.26, 1.25, 1.24, 1.24, 1.08, 1.07, 1.03, 0.98, 0.96, 0.94, 0.93, 0.91. [M+H] e: 318.14, o: 318.1. Yield 82%.

ethyl 5-acetyl-2,6-dimethyl-4-(4-(pyridin-2-yl)phenyl)-1,4-dihydropyridine-3-carboxylate (11i): ^{1}H δ ($CDCl_3$) 7.76-7.39 (7H), 6.23 (1H, t), 5.20 (1H, t), 4.19-4.14 (3H, m), 2.39-2.33 (3H, t), 2.28-2.19 (6H, t), 1.34-1.24 (3H, m). [M+H] e: 377.18, o: 377.2. Yield 79%.

ethyl 5-acetyl-2,4,6-trimethyl-1,4-dihydropyridine-3-carboxylate (11j): ^{1}H δ ($CDCl_3$) 5.86 (1H, s), 4.24-4.15 (2H, m), 3.81 (1H, m), 2.32-2.14 (9H, t), 1.36-1.23 (3H, m), 1.01-0.90 (3H, t). [M+H] e: 238.14, o: 238.1. Yield 71%.

ethyl 5-acetyl-4-(sec-butyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (11k): ^{1}H NMR δ ($CDCl_3$) 5.72 (1H, t), 4.25-4.12 (2H, m), 3.93-3.89 (1H, m), 2.63-2.51 (1H, m), 2.37-2.18 (9H, t), 1.44-1.17 (6H, m), 1.01-0.77 (3H, m), 0.77-0.65 (2H, m). [M+H] e: 280.18, o: 280.2. Yield 74%.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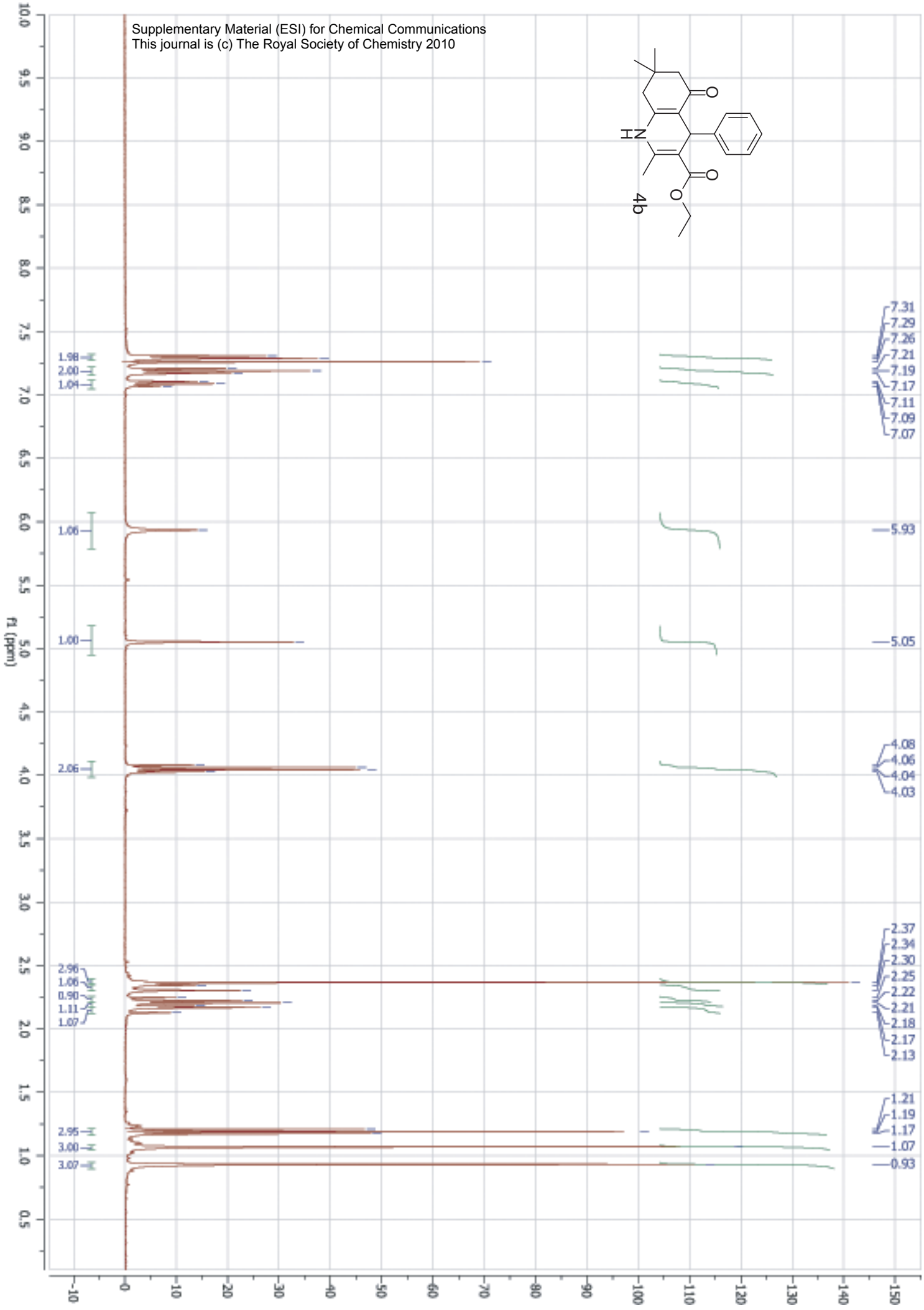
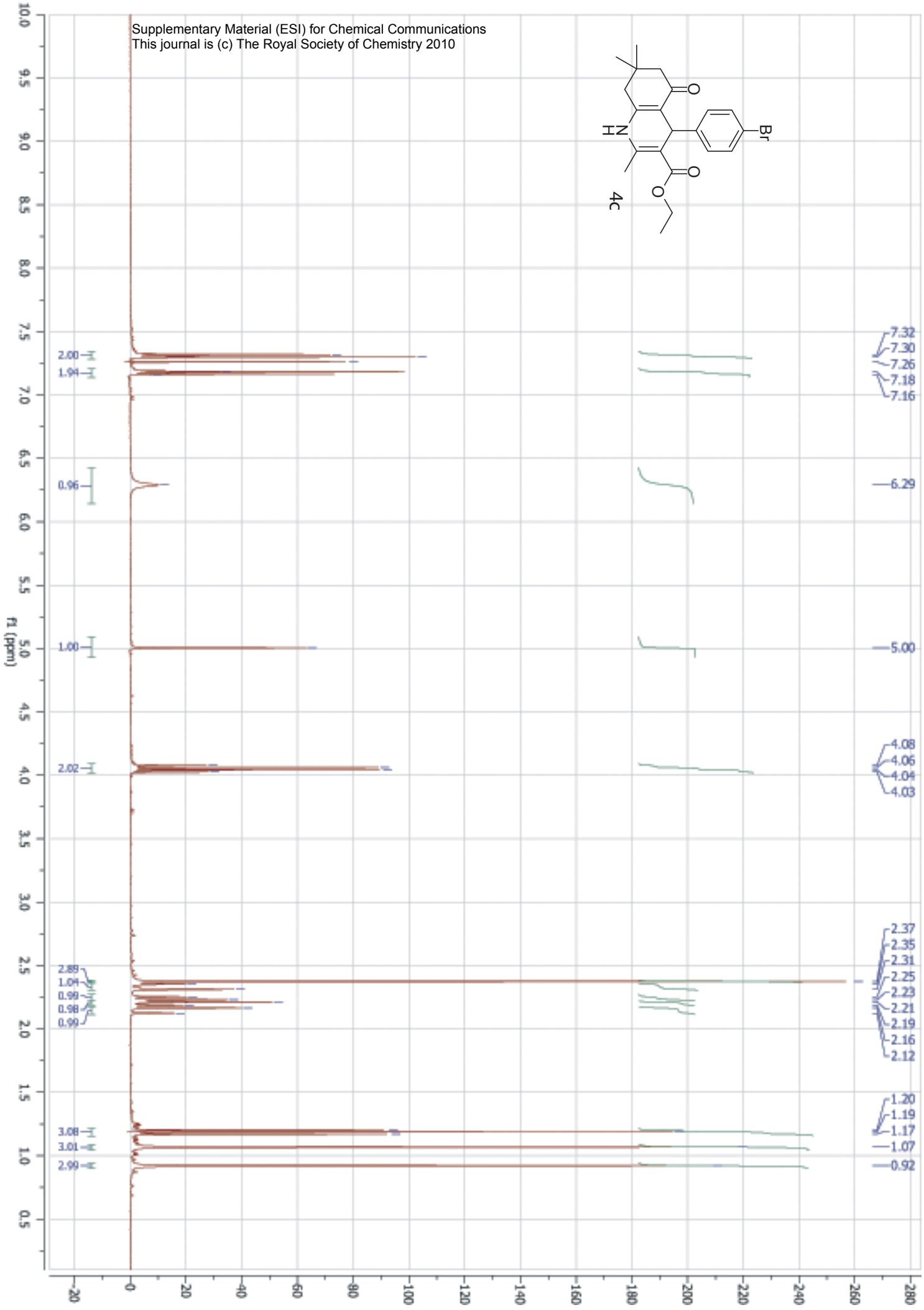
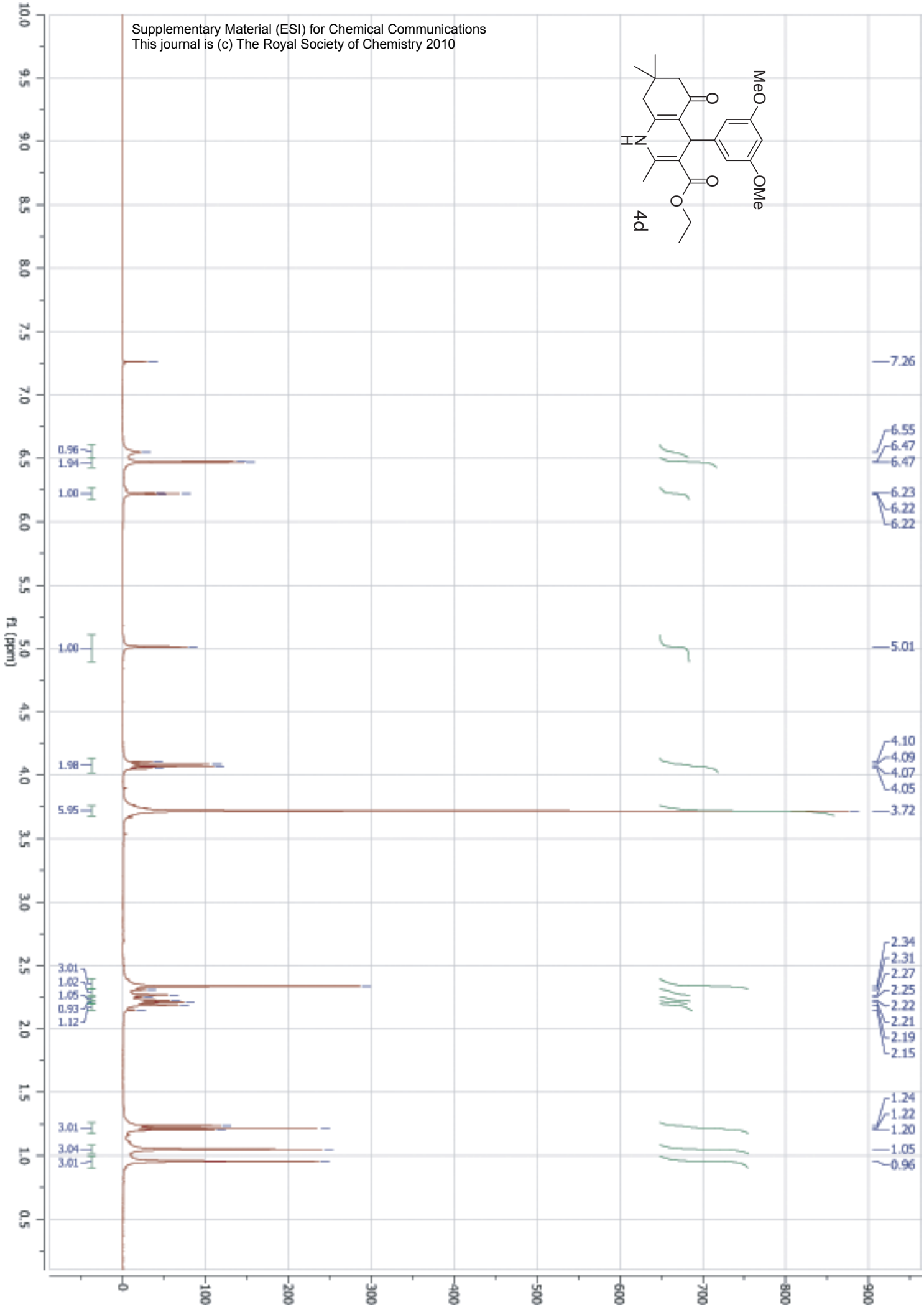
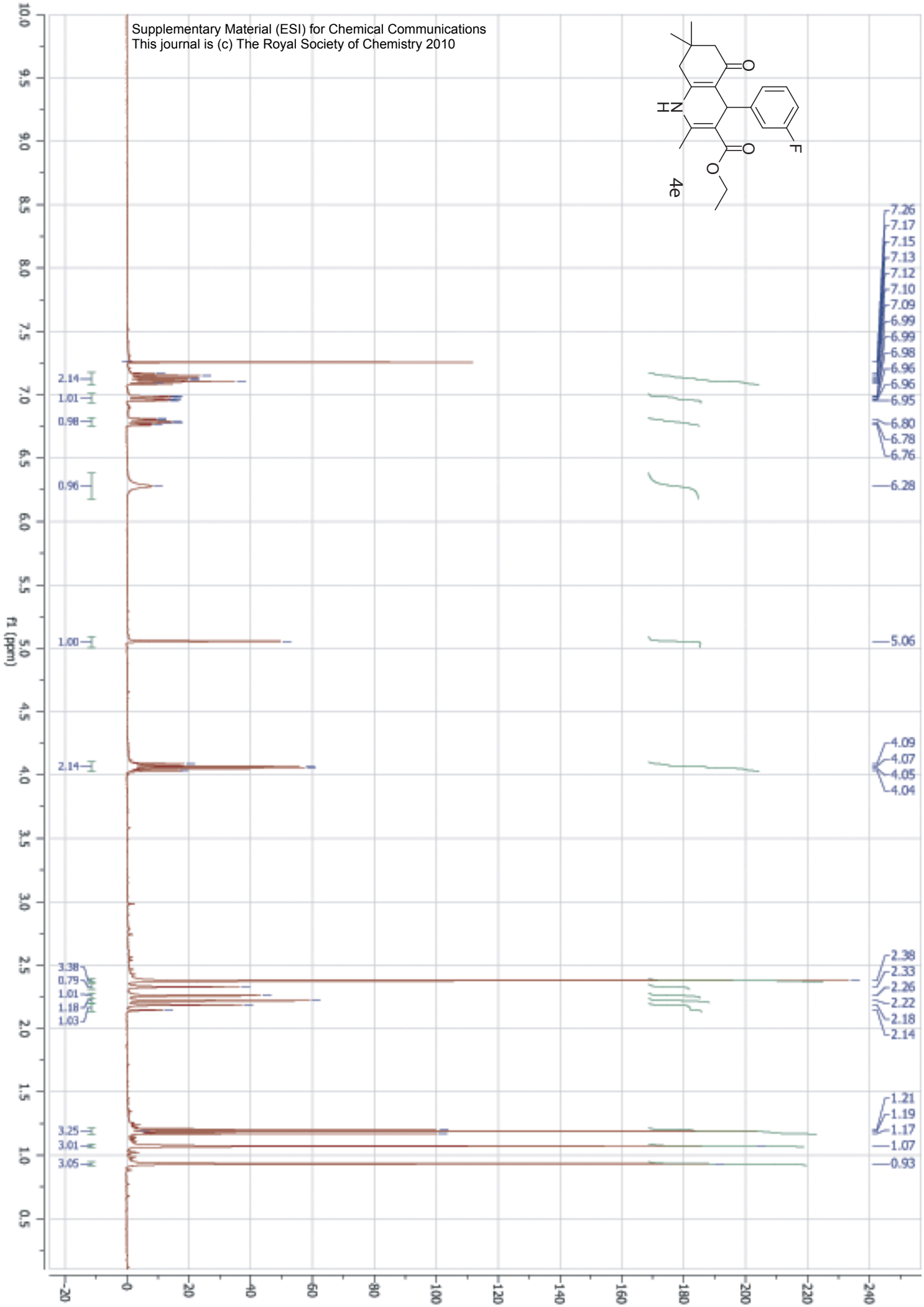
1.07
0.87
0.99
0.95
1.00
1.97
0.44
3.11
0.78
1.02
1.11
1.03
3.01
2.98
2.99
7.33
7.31
7.26
7.25
7.24
7.11
7.09
7.08
6.53
5.33
4.07
4.06
4.04
4.01
3.99
2.33
2.30
2.29
2.23
2.21
2.18
2.17
2.13
2.09
1.19
1.17
1.16
1.06
0.94




4c
Br
O
N
H
O
O
f1 (ppm)
2.00
1.94
0.96
1.00
2.02
2.89
1.04
0.99
0.98
0.99
3.08
3.01
2.99
7.32
7.30
7.26
7.18
7.16
6.29
5.00
4.08
4.06
4.04
4.03
2.37
2.35
2.31
2.25
2.23
2.21
2.19
2.16
2.12
1.20
1.19
1.17
1.07
0.92


4d
MeO
OMe
O
O
O
H
N
f1 (ppm)
7.26
6.55
6.47
6.47
6.23
6.22
6.22
5.01
4.10
4.09
4.07
4.05
3.72
2.34
2.31
2.27
2.25
2.22
2.21
2.19
2.15
1.24
1.22
1.20
1.05
0.96
0.96
1.94
1.00
1.00
1.98
5.95
3.01
1.02
1.05
0.93
1.12
3.01
3.04
3.01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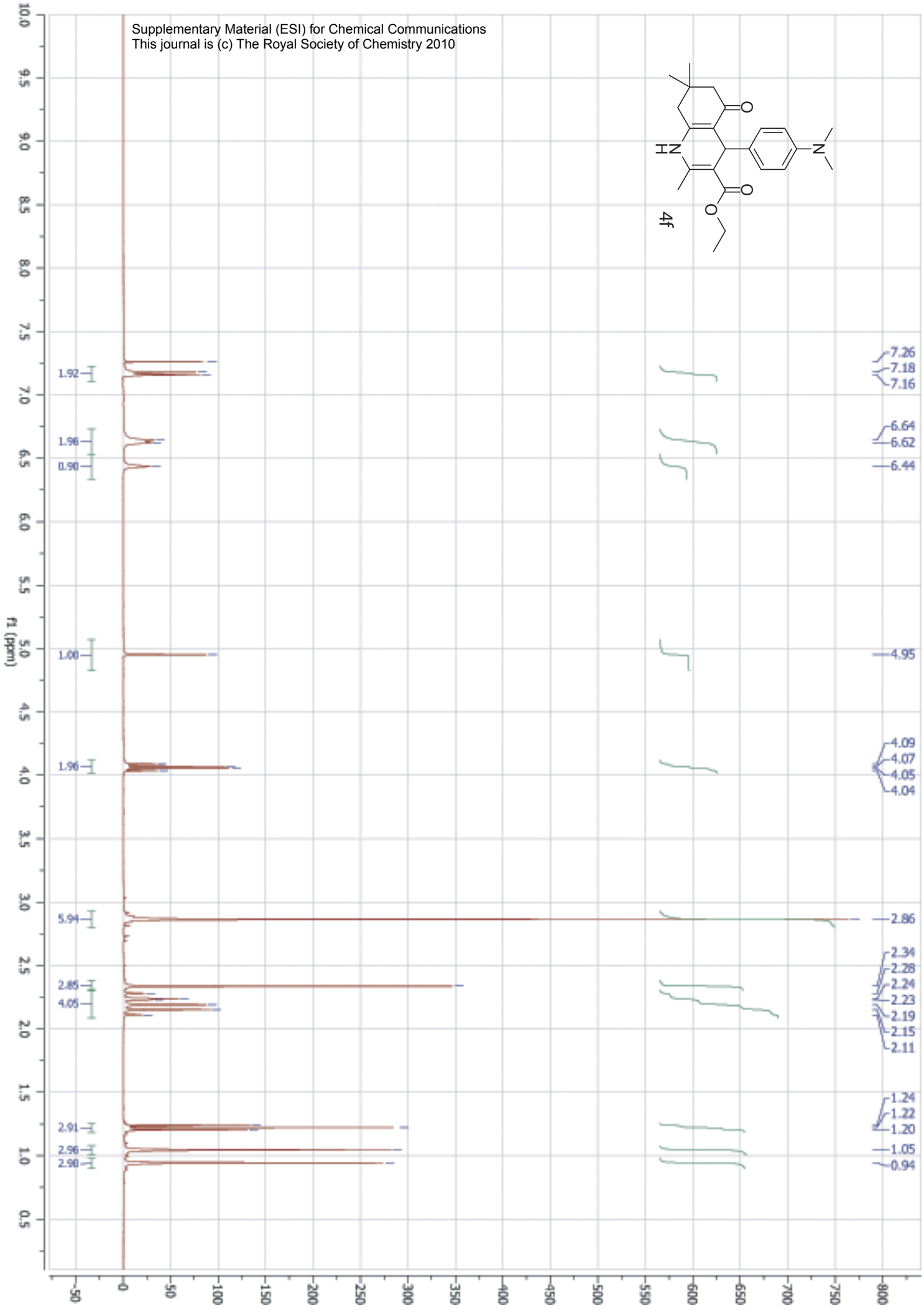
f1 (ppm)
7.26
7.17
7.15
7.13
7.12
7.10
7.09
6.99
6.99
6.98
6.96
6.96
6.95
6.80
6.78
6.76
6.28
5.06
4.09
4.07
4.05
4.04
2.38
2.33
2.26
2.22
2.18
2.14
1.21
1.19
1.17
1.07
0.93
2.14
1.01
0.98
0.96
1.00
2.14
3.38
0.79
1.01
1.18
1.03
3.25
3.01
3.05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f1 (ppm)
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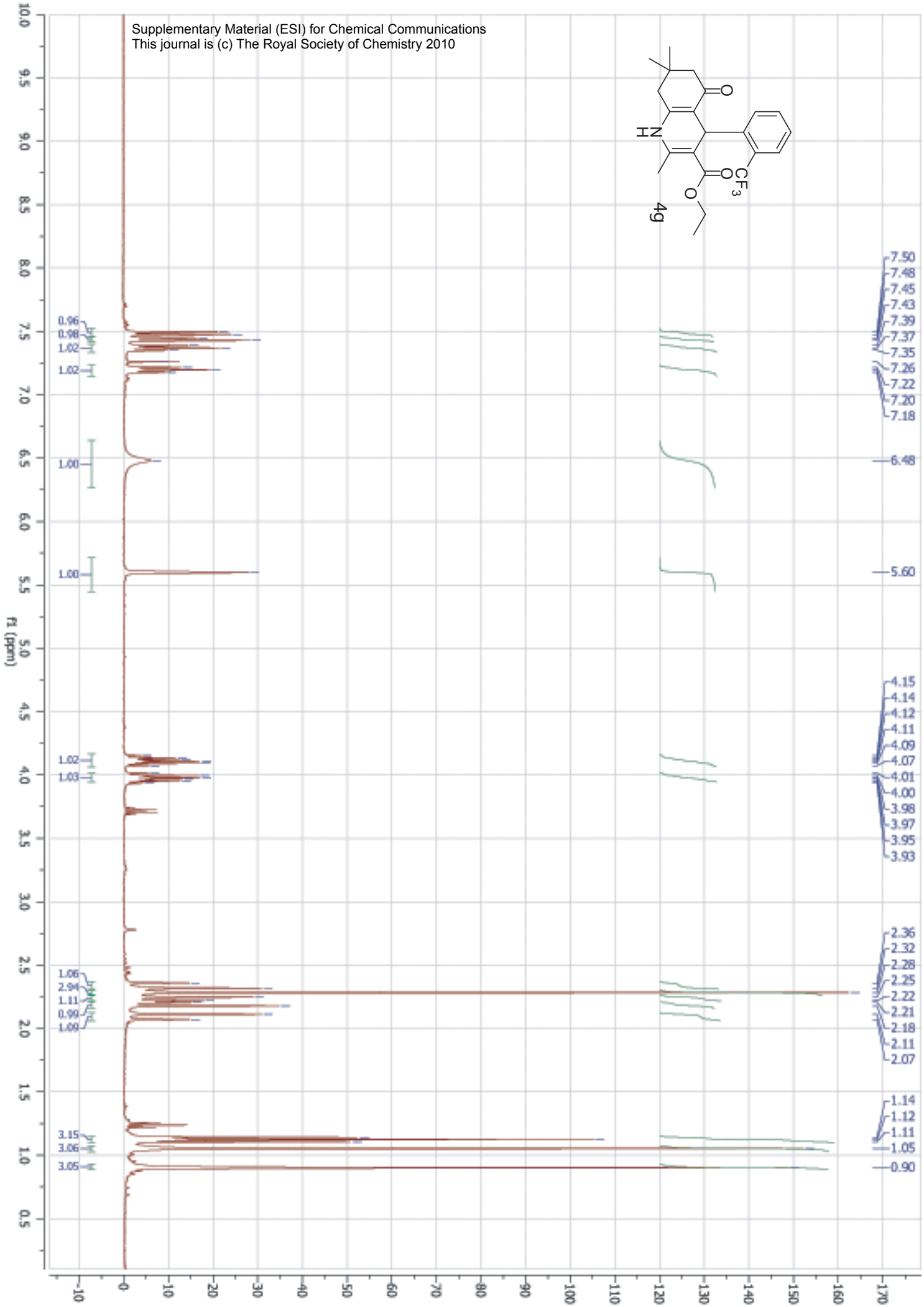
1.96
0.90
1.00
1.96
5.94
2.85
4.05
2.91
2.96
2.90
7.26
7.18
7.16
6.64
6.62
6.44
4.95
4.09
4.07
4.05
4.04
2.86
2.34
2.28
2.24
2.23
2.19
2.15
2.11
1.24
1.22
1.20
1.05
0.94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f1 (ppm)
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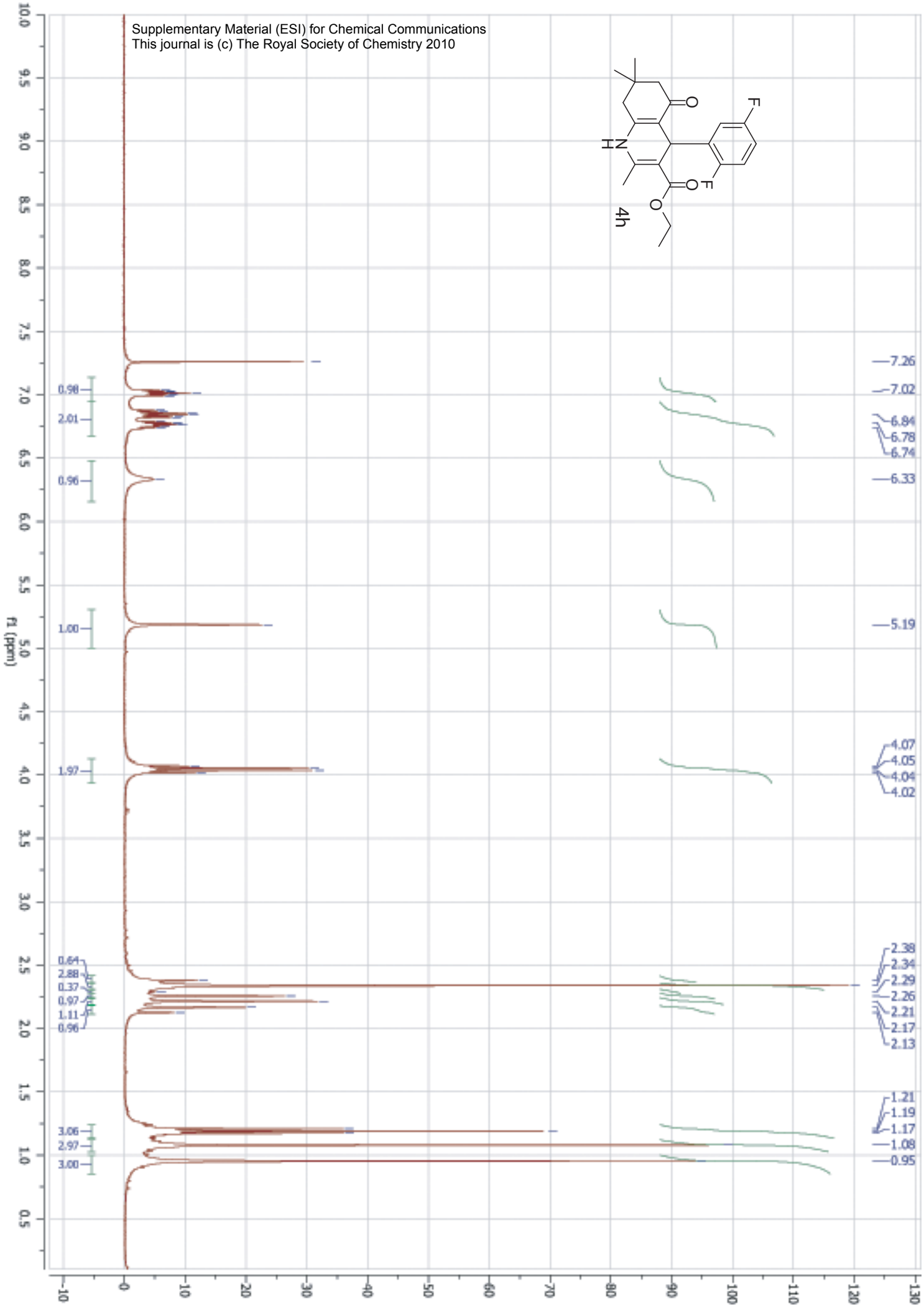
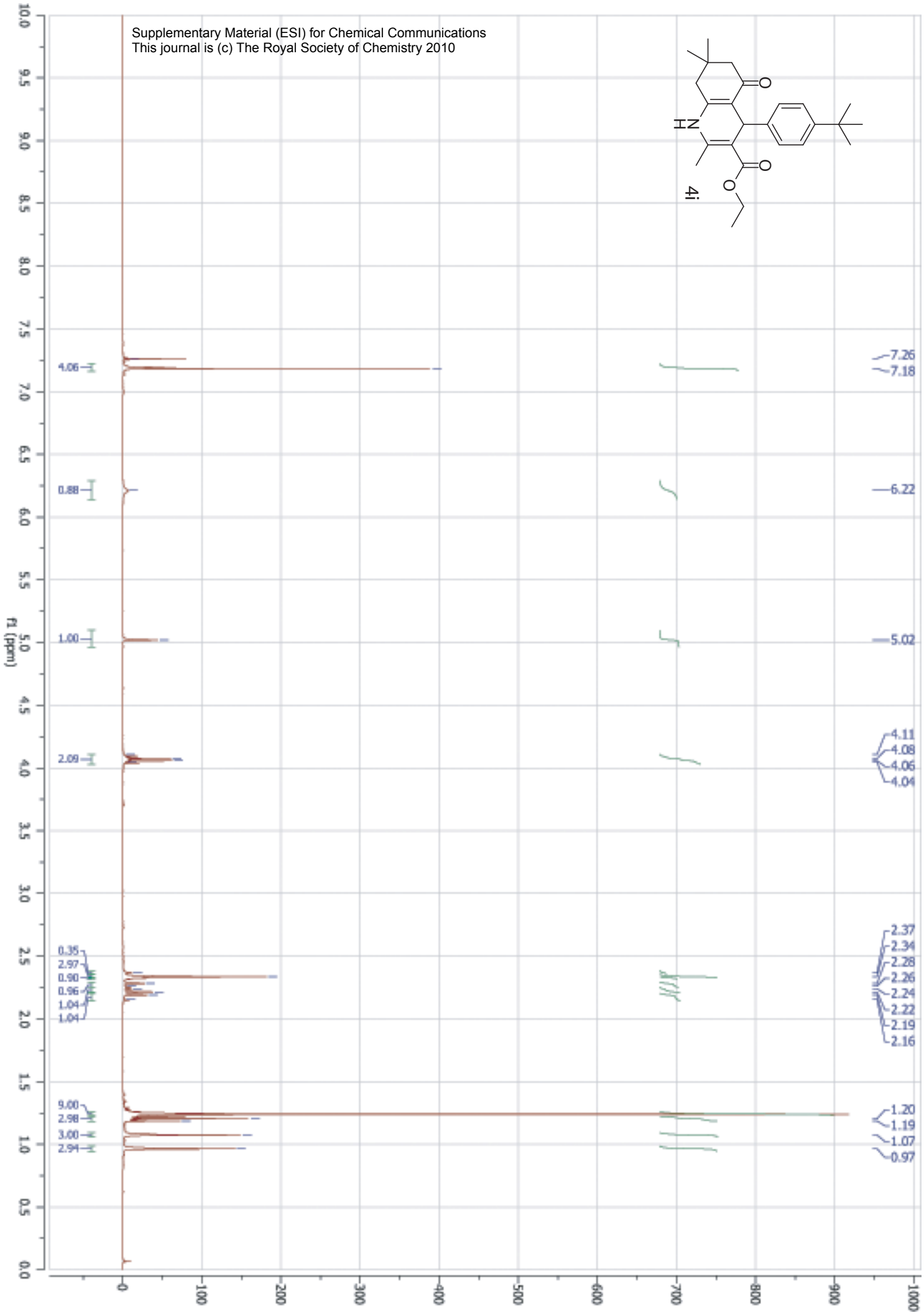
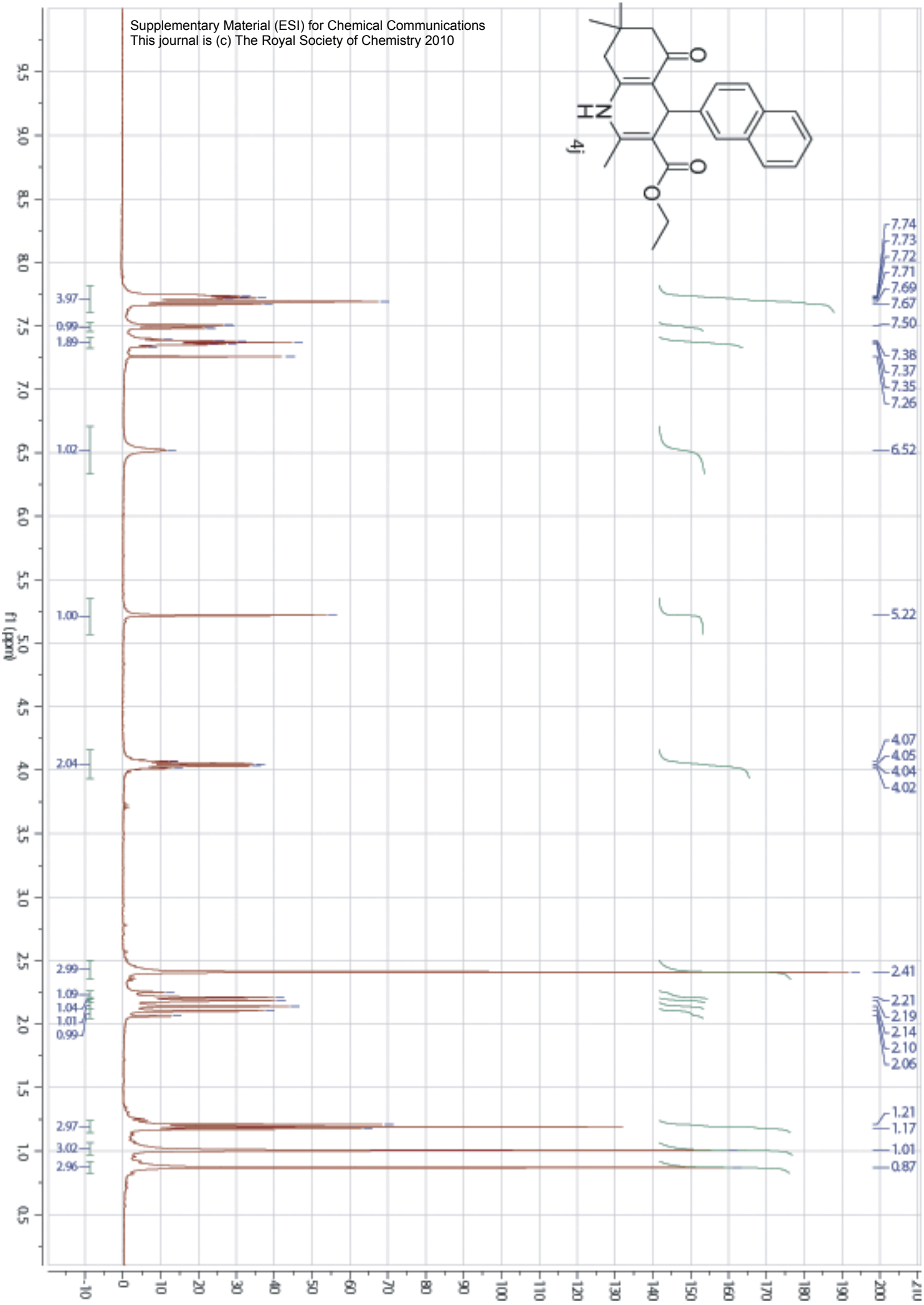
7.48
7.45
7.43
7.39
7.37
7.35
7.26
7.22
7.20
7.18
6.48
5.60
4.15
4.14
4.12
4.11
4.09
4.07
4.01
4.00
3.98
3.97
3.95
3.93
2.36
2.32
2.28
2.25
2.22
2.21
2.18
2.11
2.07
1.14
1.12
1.11
1.05
0.90


4h
f1 (ppm)
7.26
7.02
6.84
6.78
6.74
6.33
5.19
4.07
4.05
4.04
4.02
2.38
2.34
2.29
2.26
2.21
2.17
2.13
1.21
1.19
1.17
1.08
0.95
0.98
2.01
0.96
1.00
1.97
0.64
2.88
0.37
0.97
1.11
0.96
3.06
2.97
3.00


4i
f1 (ppm)
4.06
0.88
1.00
2.09
0.35
2.97
0.90
0.96
1.04
1.04
9.00
2.98
3.00
2.94
7.26
7.18
6.22
5.02
4.11
4.08
4.06
4.04
2.37
2.34
2.28
2.26
2.24
2.22
2.19
2.16
1.20
1.19
1.07
0.97


4j
f1 (ppm)
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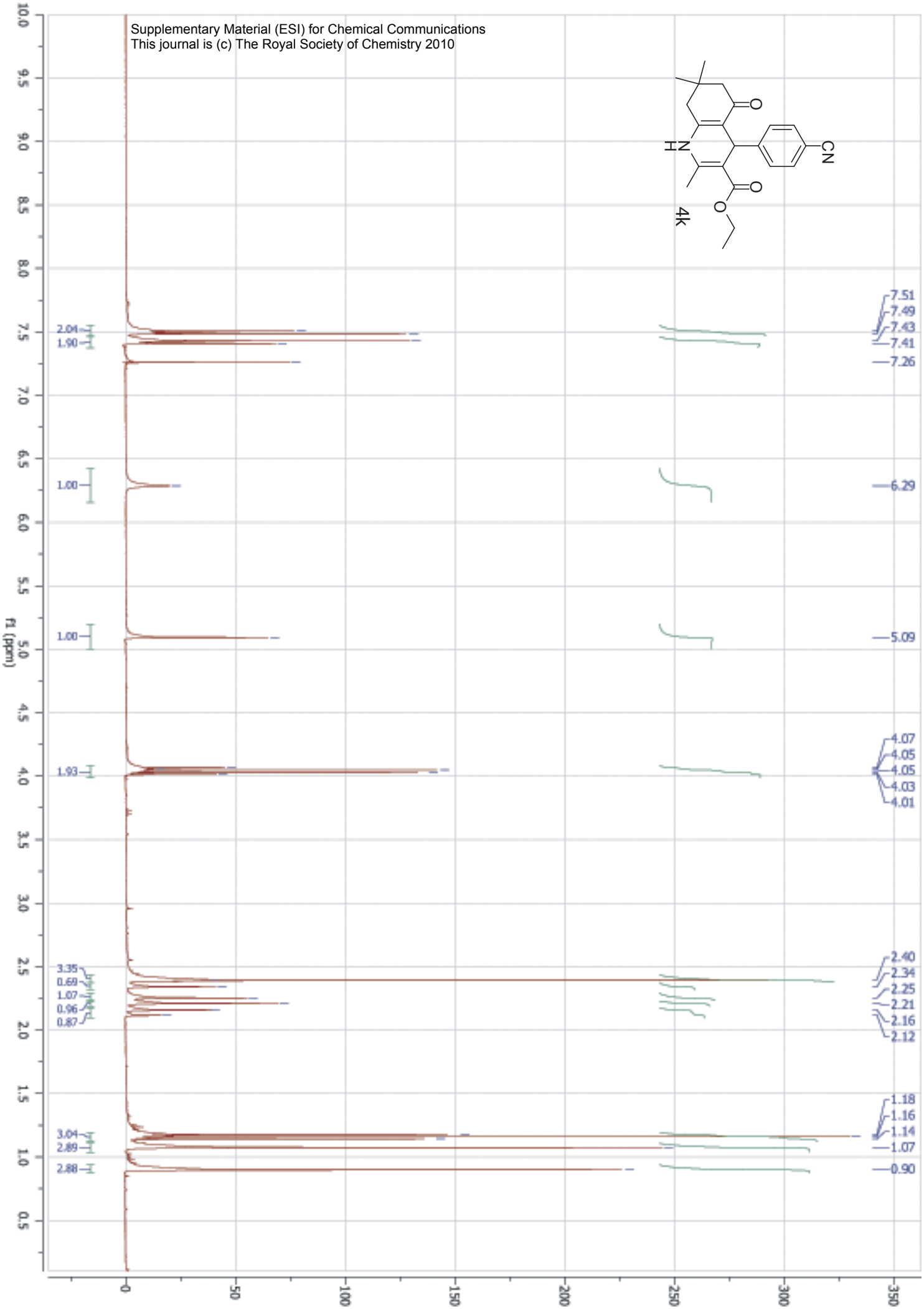
7.73
7.72
7.71
7.69
7.67
7.50
7.38
7.37
7.35
7.26
6.52
5.22
4.07
4.05
4.04
4.02
2.41
2.21
2.19
2.14
2.10
2.06
1.21
1.17
1.01
0.87
3.97
0.99
1.89
1.02
1.00
2.04
2.99
1.09
1.04
1.01
0.99
2.97
3.02
2.96


4k
f1 (ppm)
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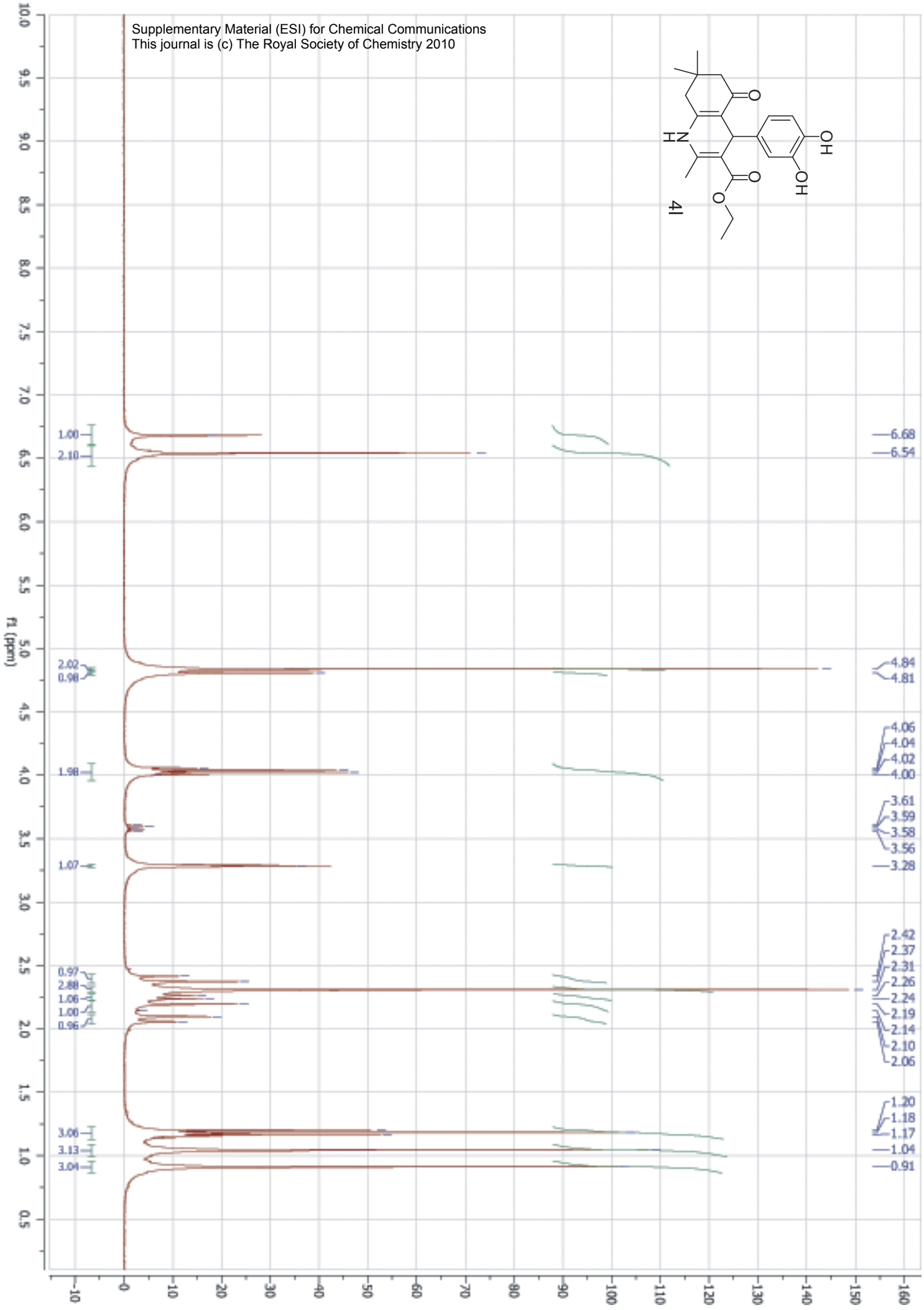
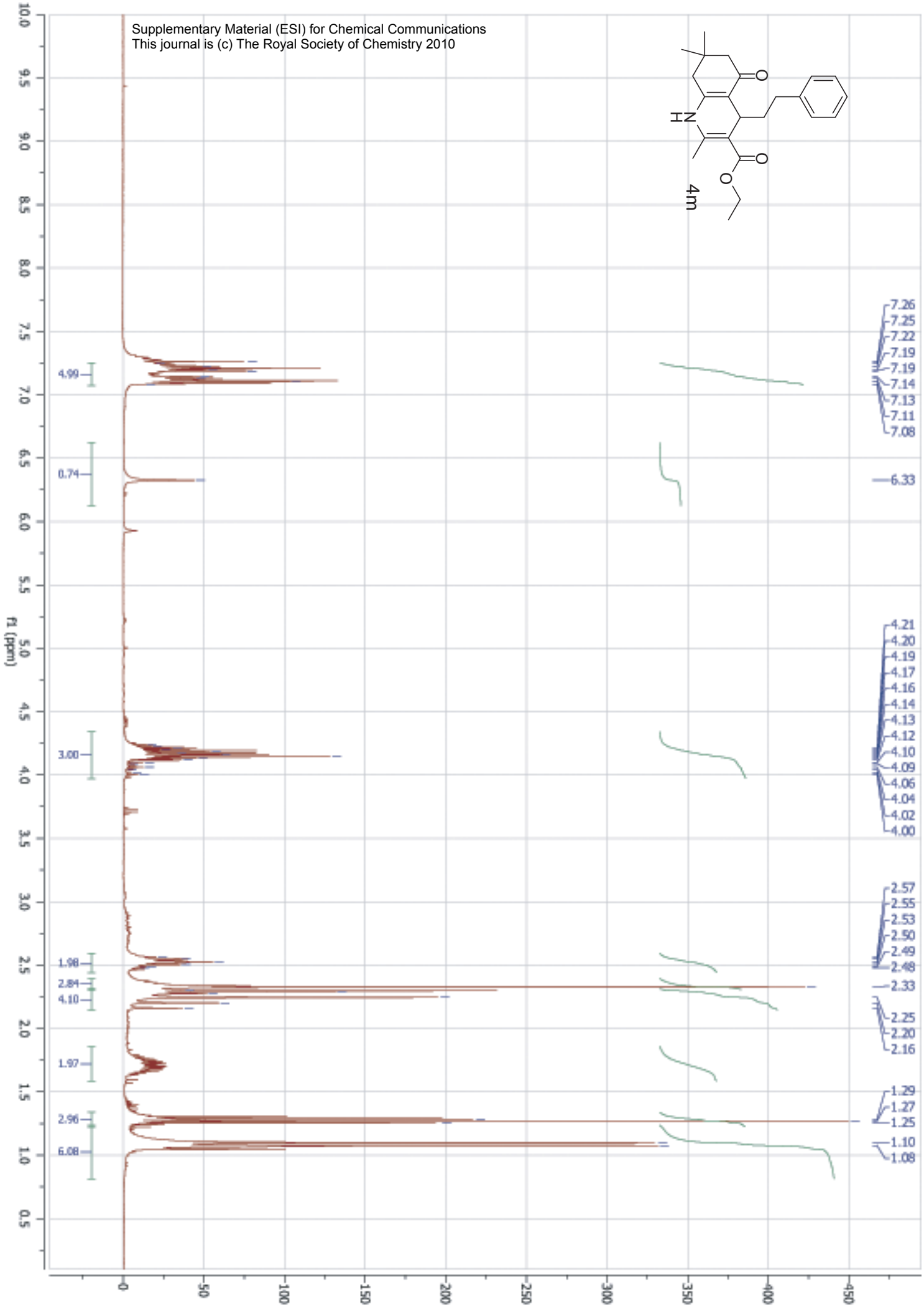
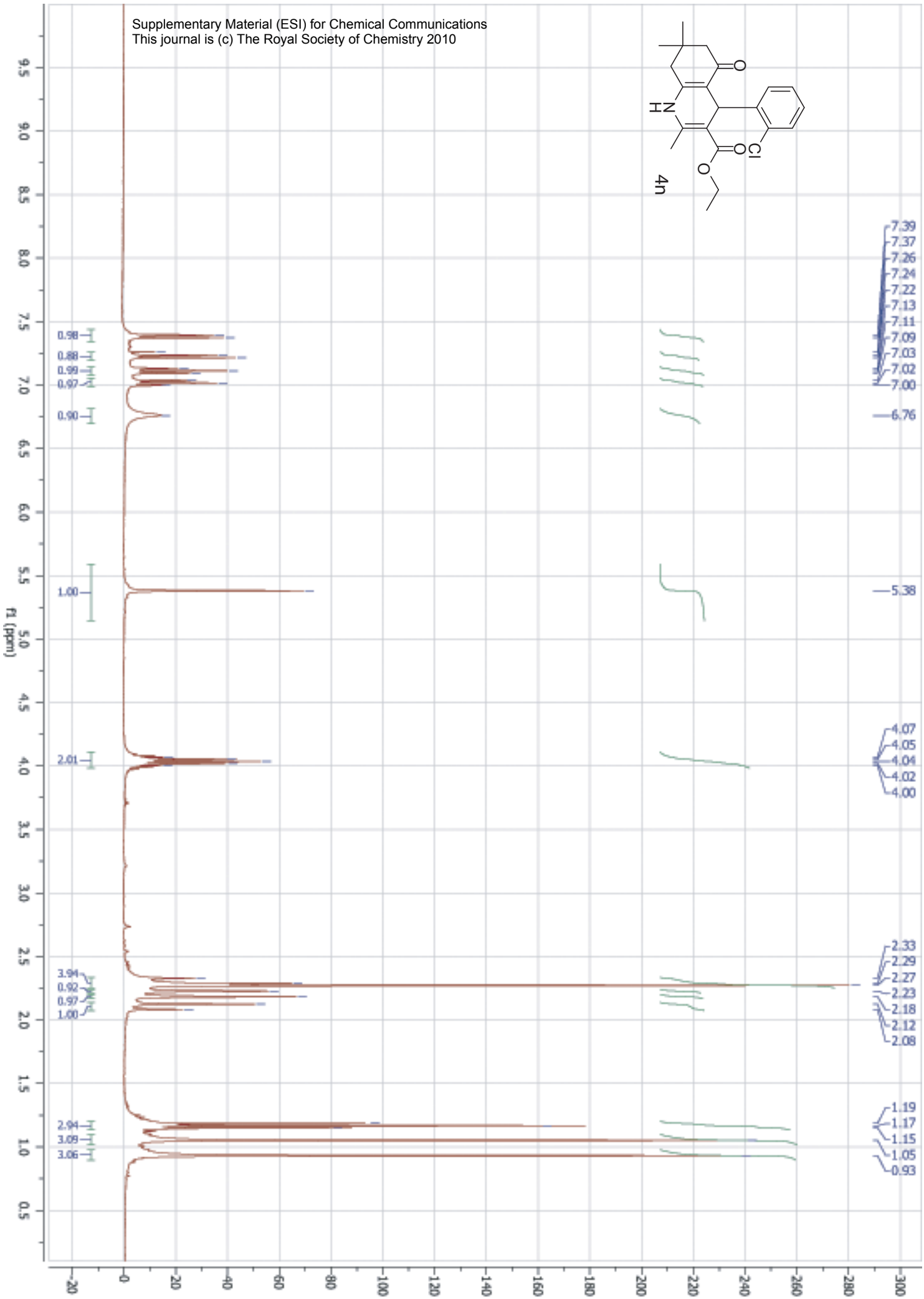
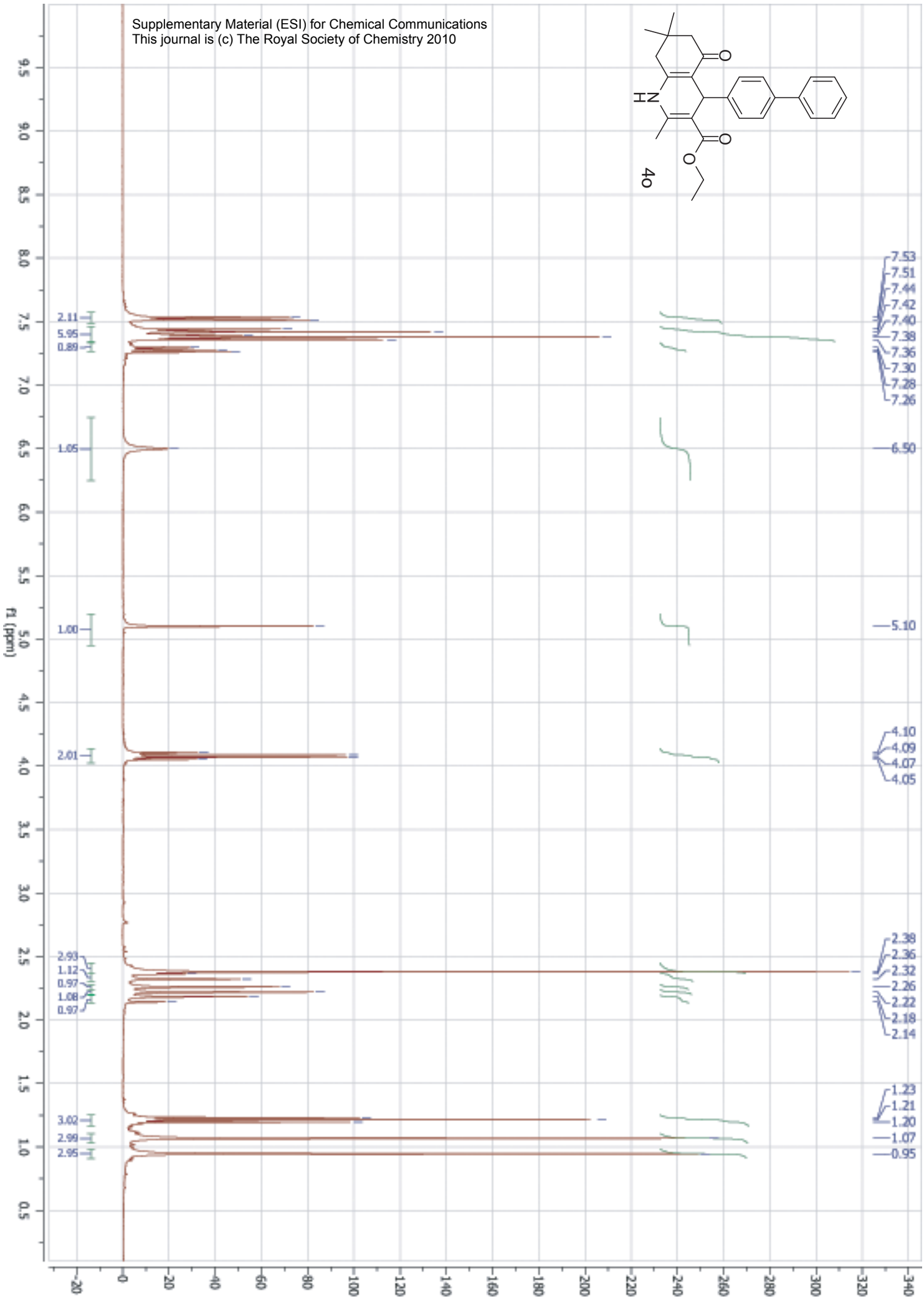
7.49
7.43
7.41
7.26
6.29
5.09
4.07
4.05
4.05
4.03
4.01
2.40
2.34
2.25
2.21
2.16
2.12
1.18
1.16
1.14
1.07
0.90
2.04
1.90
1.00
1.00
1.93
3.35
0.68
1.07
0.96
0.87
3.04
2.89
2.88


4l
f1 (ppm)
1.00
2.10
2.02
0.98
1.98
1.07
0.97
2.88
1.06
1.00
0.96
3.06
3.13
3.04
6.68
6.54
4.84
4.81
4.06
4.04
4.02
4.00
3.61
3.59
3.58
3.56
3.28
2.42
2.37
2.31
2.26
2.24
2.19
2.14
2.10
2.06
1.20
1.18
1.17
1.04
0.91


4m
f1 (ppm)
4.99
0.74
3.00
1.98
2.84
4.10
1.97
2.96
6.08
7.26
7.25
7.22
7.19
7.19
7.14
7.13
7.11
7.08
6.33
4.21
4.20
4.19
4.17
4.16
4.14
4.13
4.12
4.10
4.09
4.06
4.04
4.02
4.00
2.57
2.55
2.53
2.50
2.49
2.48
2.33
2.25
2.20
2.16
1.29
1.27
1.25
1.10
1.08




4o
f1 (ppm)
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7.51
7.44
7.42
7.40
7.38
7.36
7.30
7.28
7.26
6.50
5.10
4.10
4.09
4.07
4.05
2.38
2.36
2.32
2.26
2.22
2.18
2.14
1.23
1.21
1.20
1.07
0.95
2.11
5.95
0.89
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2.01
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1.12
0.97
1.08
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3.02
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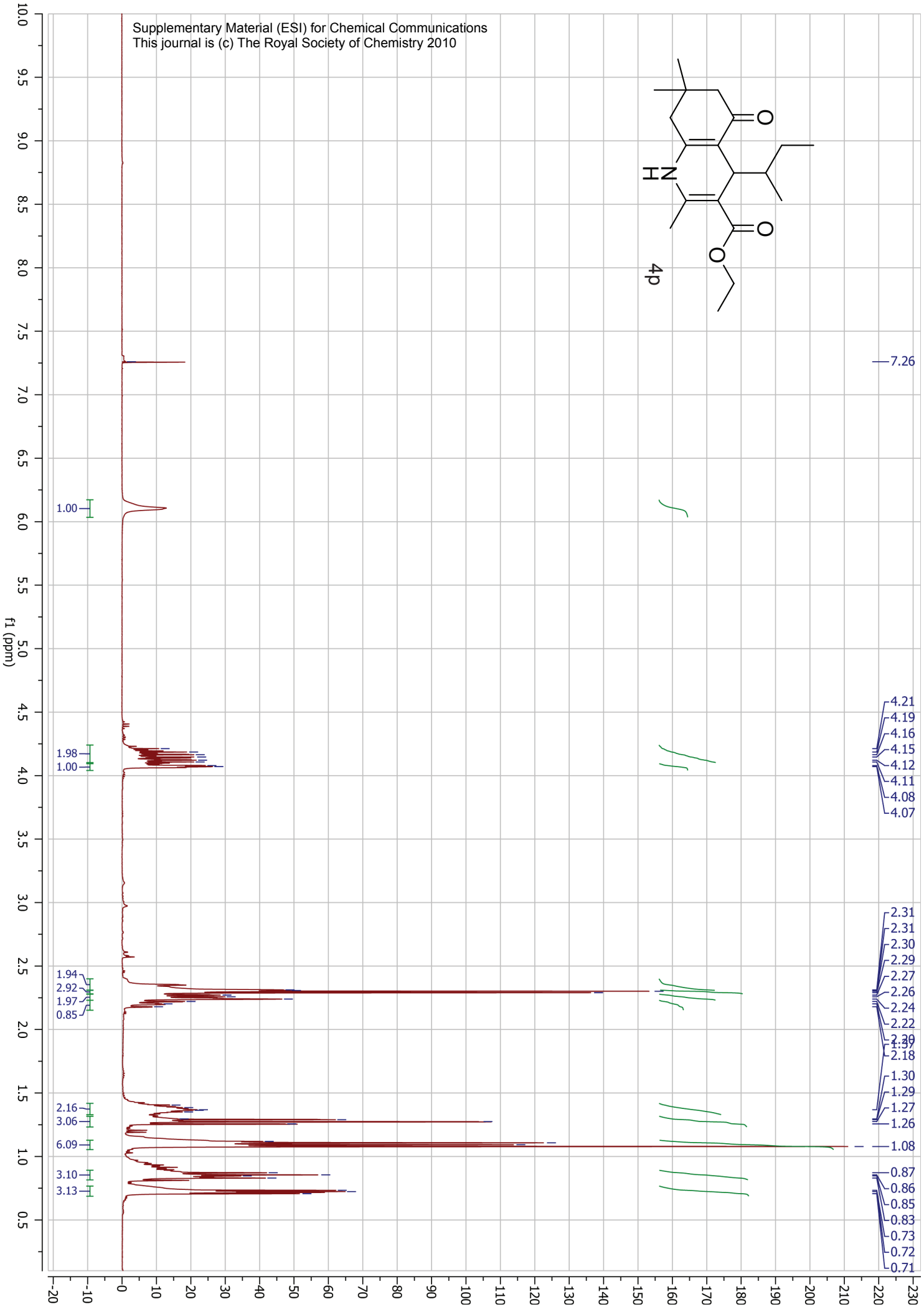
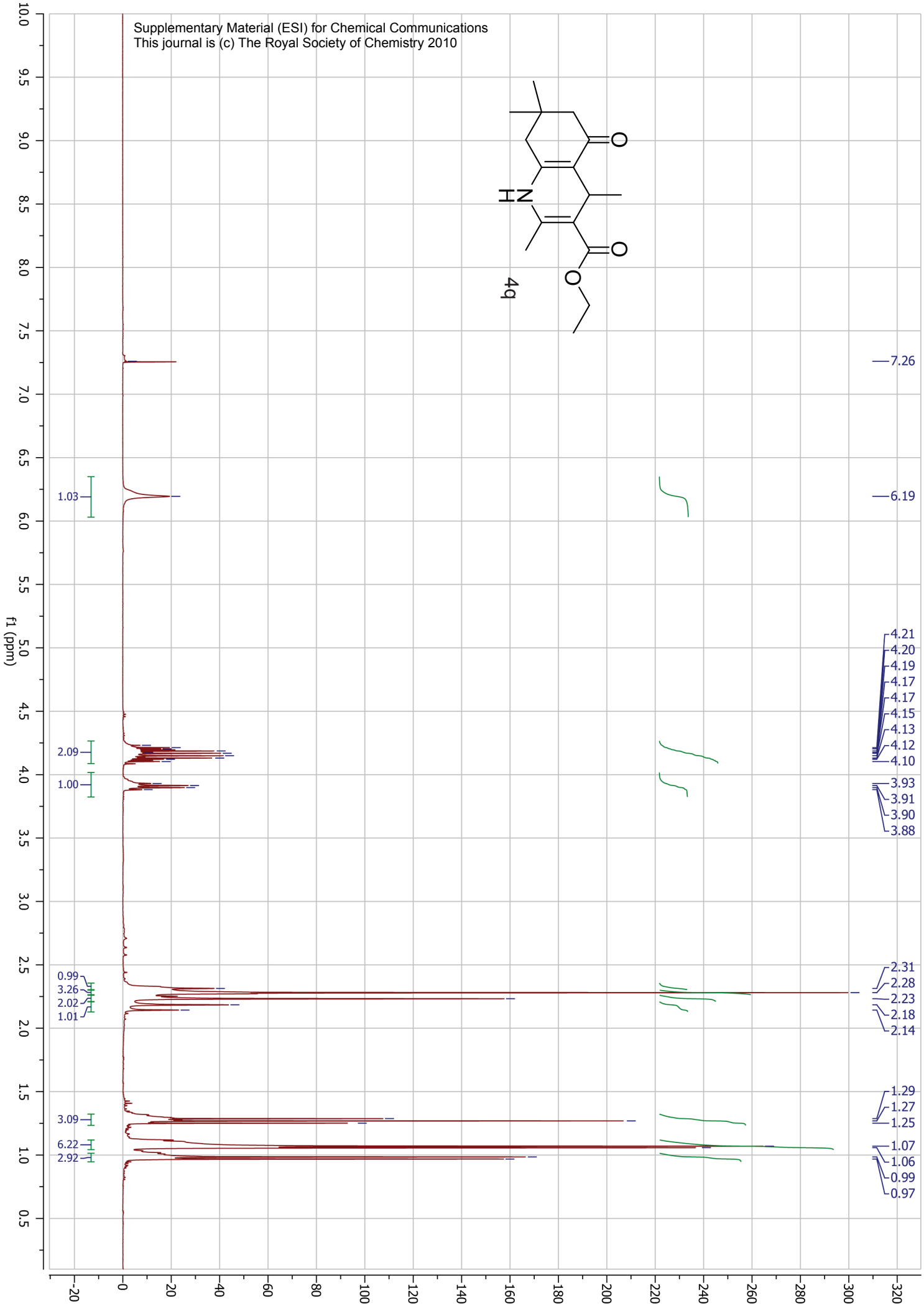
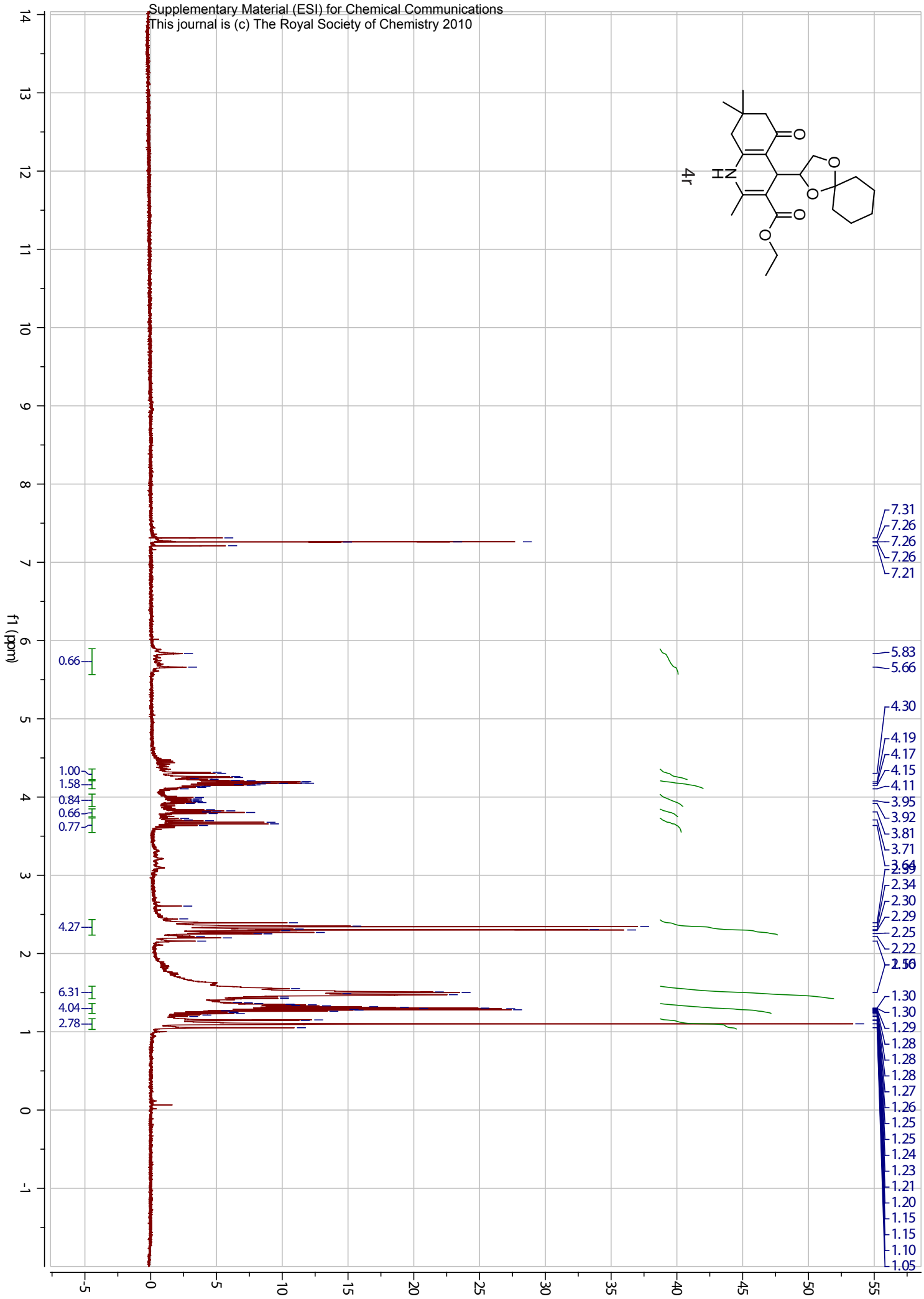
2.95



4q




4r
f1 (ppm)
7.31
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7.26
7.26
7.21
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5.66
4.30
4.19
4.17
4.15
4.11
3.95
3.92
3.81
3.71
3.64
2.99
2.34
2.30
2.29
2.25
2.22
2.16
1.30
1.30
1.29
1.28
1.28
1.28
1.27
1.26
1.25
1.25
1.24
1.23
1.21
1.20
1.15
1.15
1.10
1.05
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1.00
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0.66
0.77
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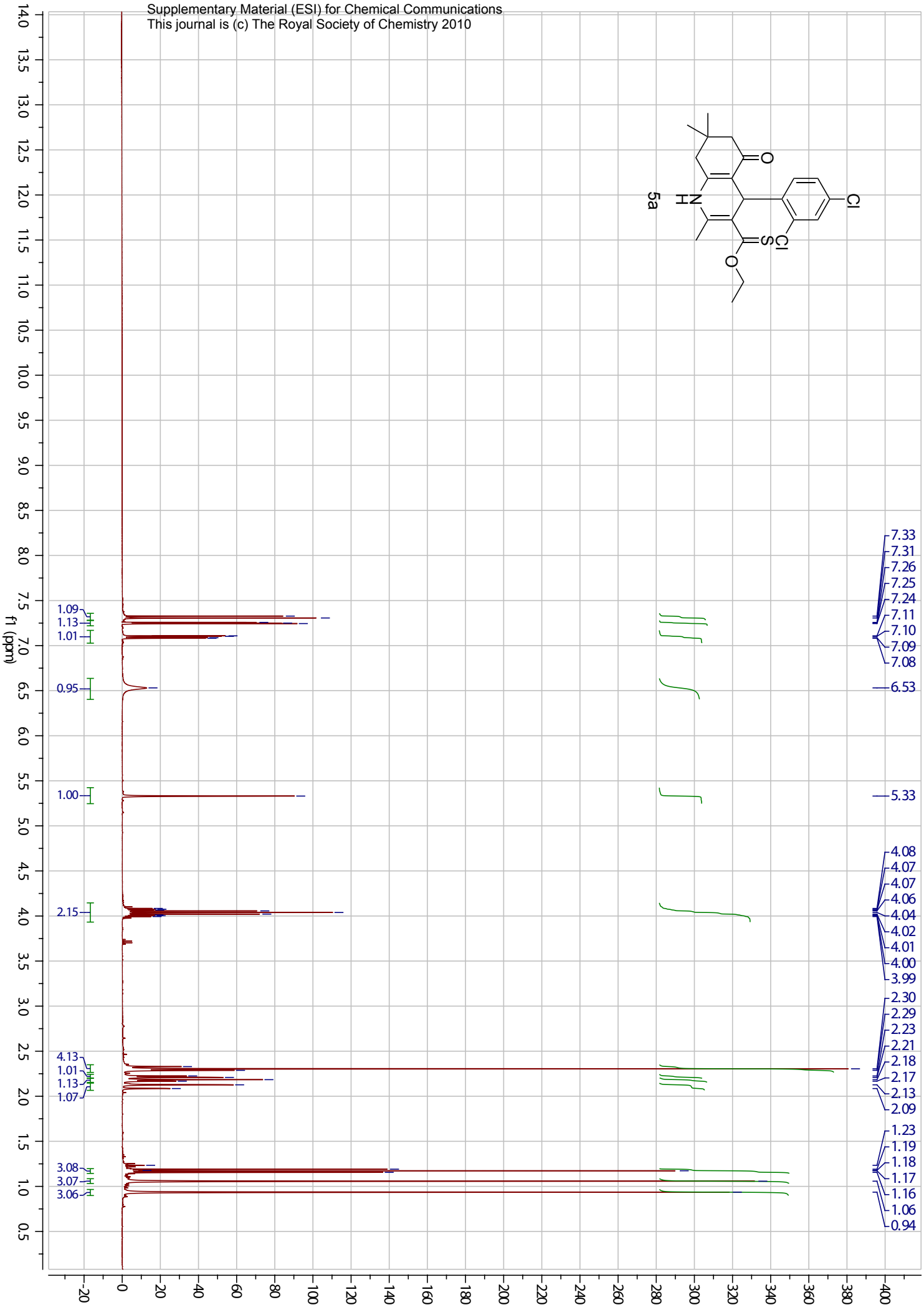
6.31
4.04
2.78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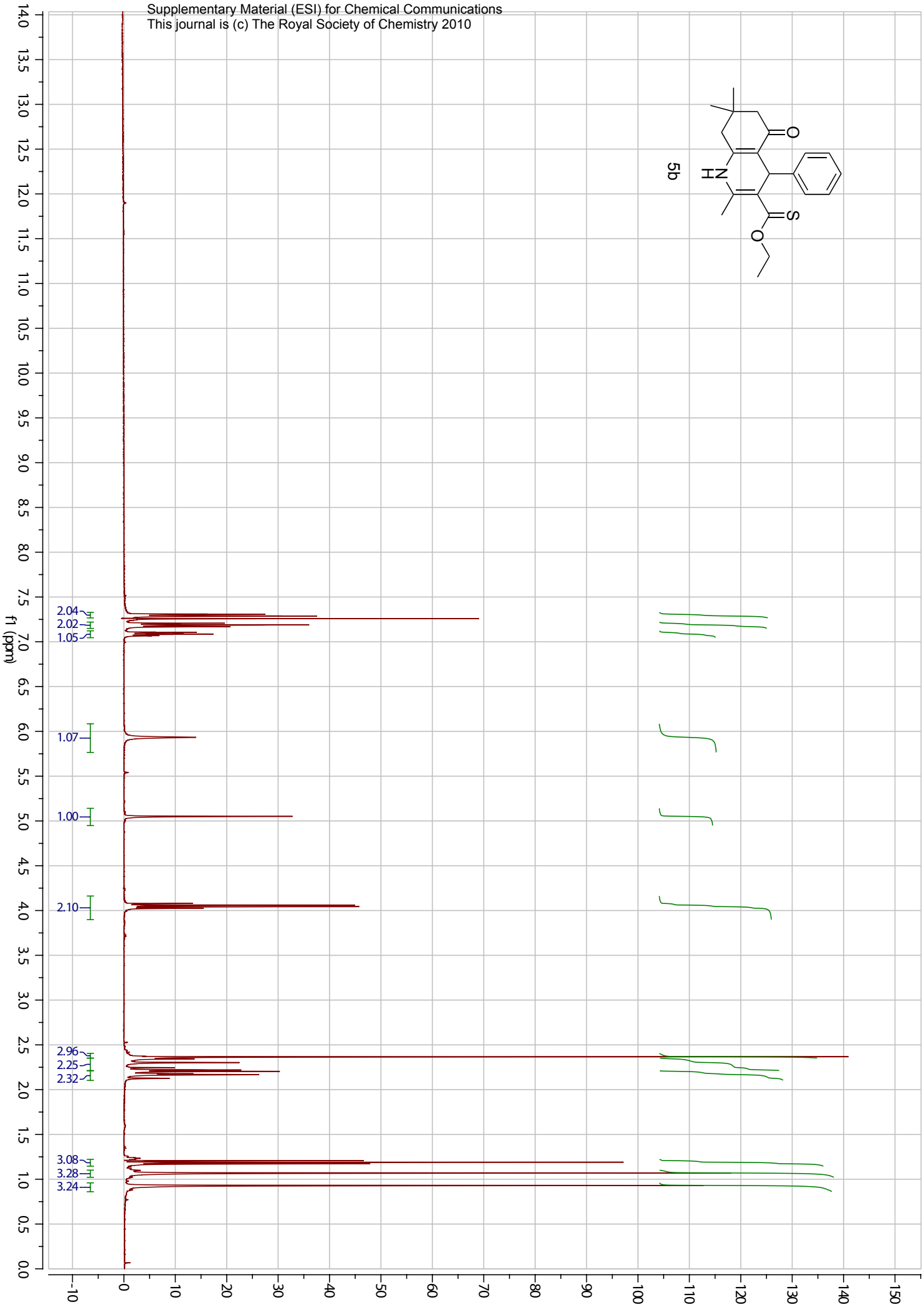
1.01
1.13
1.07
3.08
3.07
3.06
7.33
7.31
7.26
7.25
7.24
7.11
7.10
7.09
7.08
6.53
5.33
4.08
4.07
4.07
4.06
4.04
4.02
4.01
4.00
3.99
2.30
2.29
2.23
2.21
2.18
2.17
2.13
2.09
1.23
1.19
1.18
1.17
1.16
1.06
0.94


5b
f1 (ppm)
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2.02
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1.07
1.00
2.10
2.96
2.25
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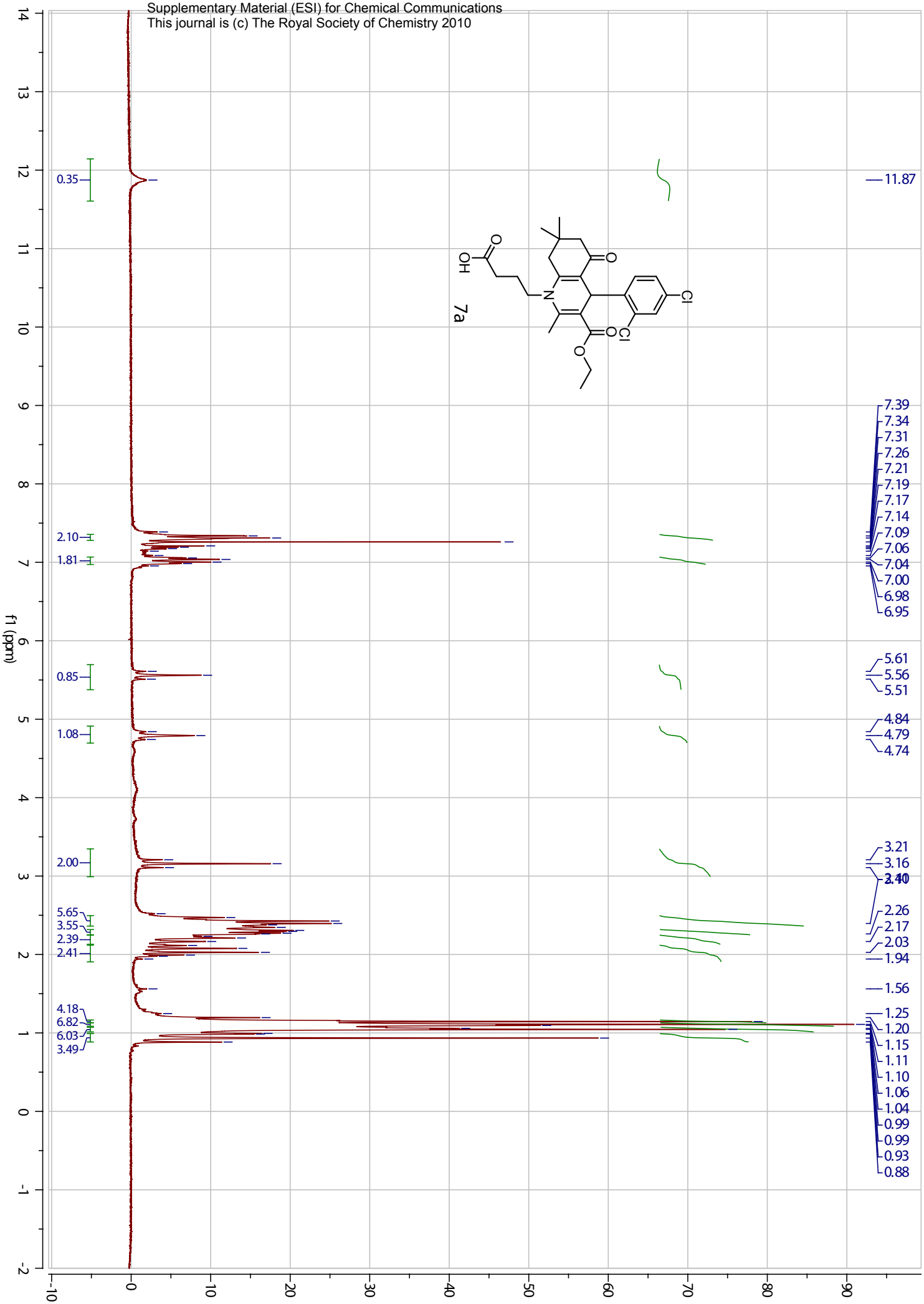
3.08
3.28
3.24


7a
f1 (ppm)
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2.10
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0.85
1.08
2.00
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3.55
2.39
2.41
4.18
6.82
6.03
3.49
11.87
7.39
7.34
7.31
7.26
7.21
7.19
7.17
7.14
7.09
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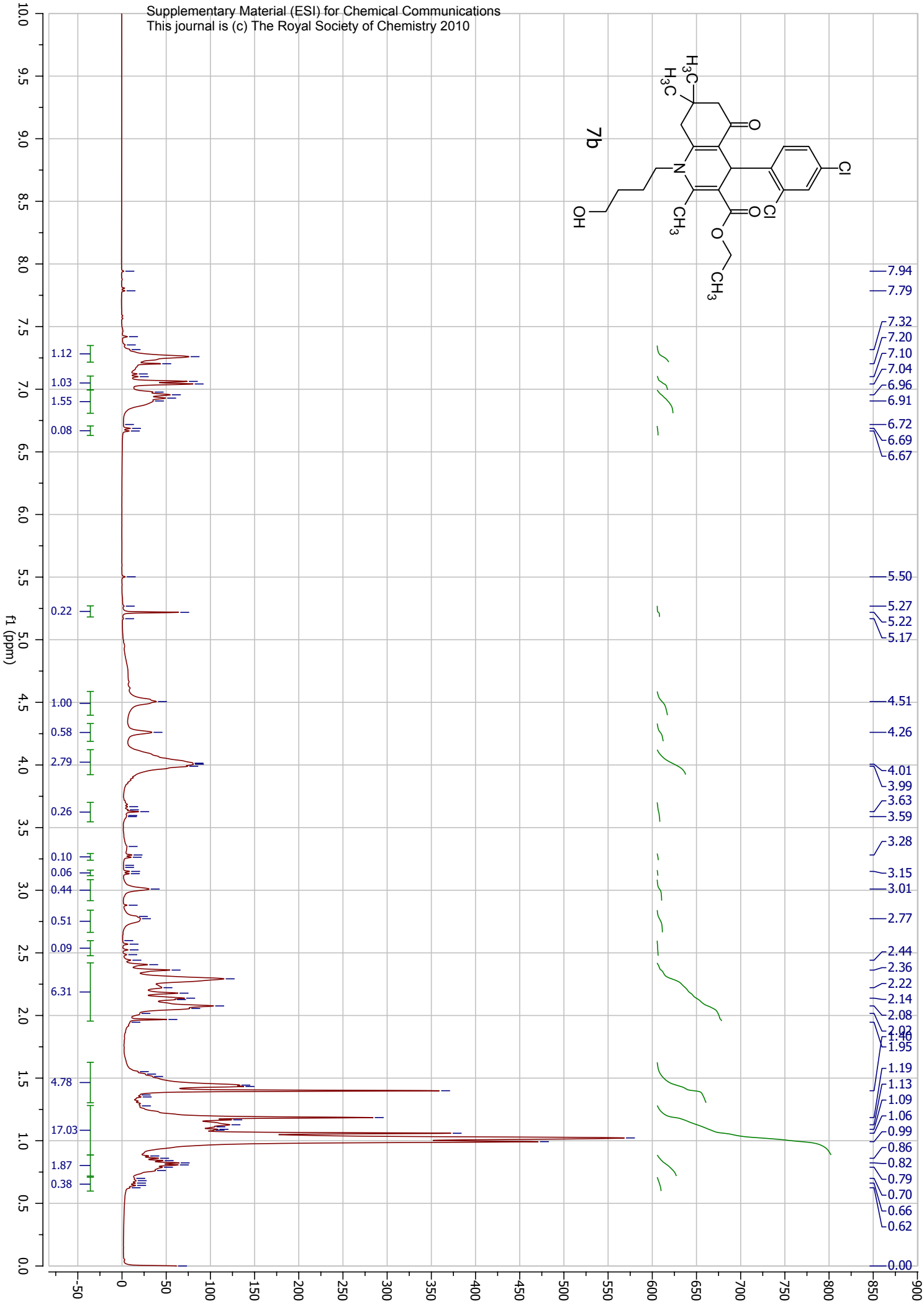
3.10
2.26
2.17
2.03
1.94
1.56
1.25
1.20
1.15
1.11
1.10
1.06
1.04
0.99
0.99
0.93
0.88


7b
f1 (ppm)
1.12
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0.08
0.22
1.00
0.58
2.79
0.26
0.10
0.06
0.44
0.51
0.09
6.31
4.78
17.03
1.87
0.38
7.94
7.79
7.32
7.20
7.10
7.04
6.96
6.91
6.72
6.69
6.67
5.50
5.27
5.22
5.17
4.51
4.26
4.01
3.99
3.63
3.59
3.28
3.15
3.01
2.77
2.44
2.36
2.22
2.14
2.08
2.02
1.95
1.19
1.13
1.09
1.06
0.99
0.86
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0.79
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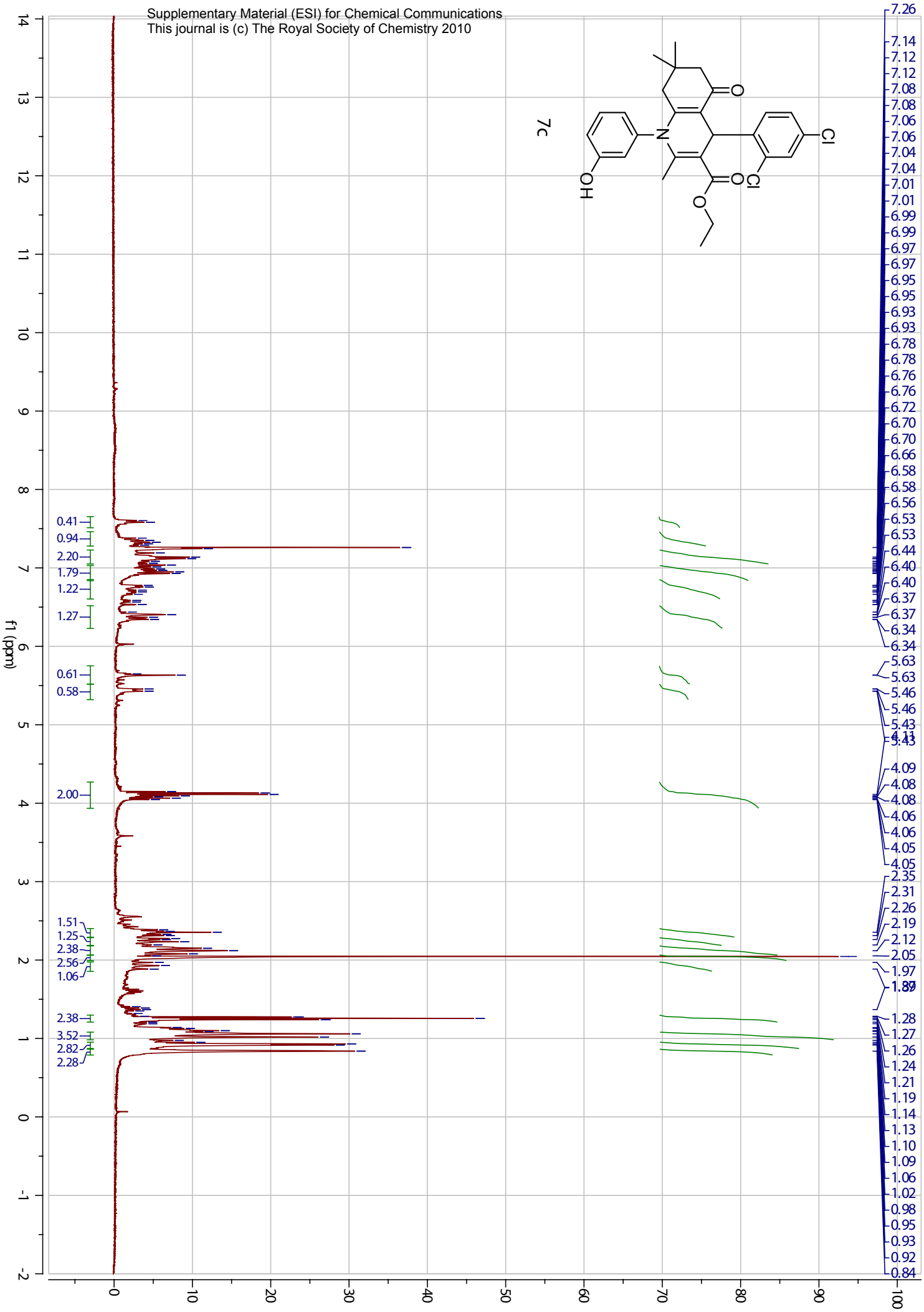
0.62
0.00


7c
f1 (ppm)
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1.79
1.22
1.27
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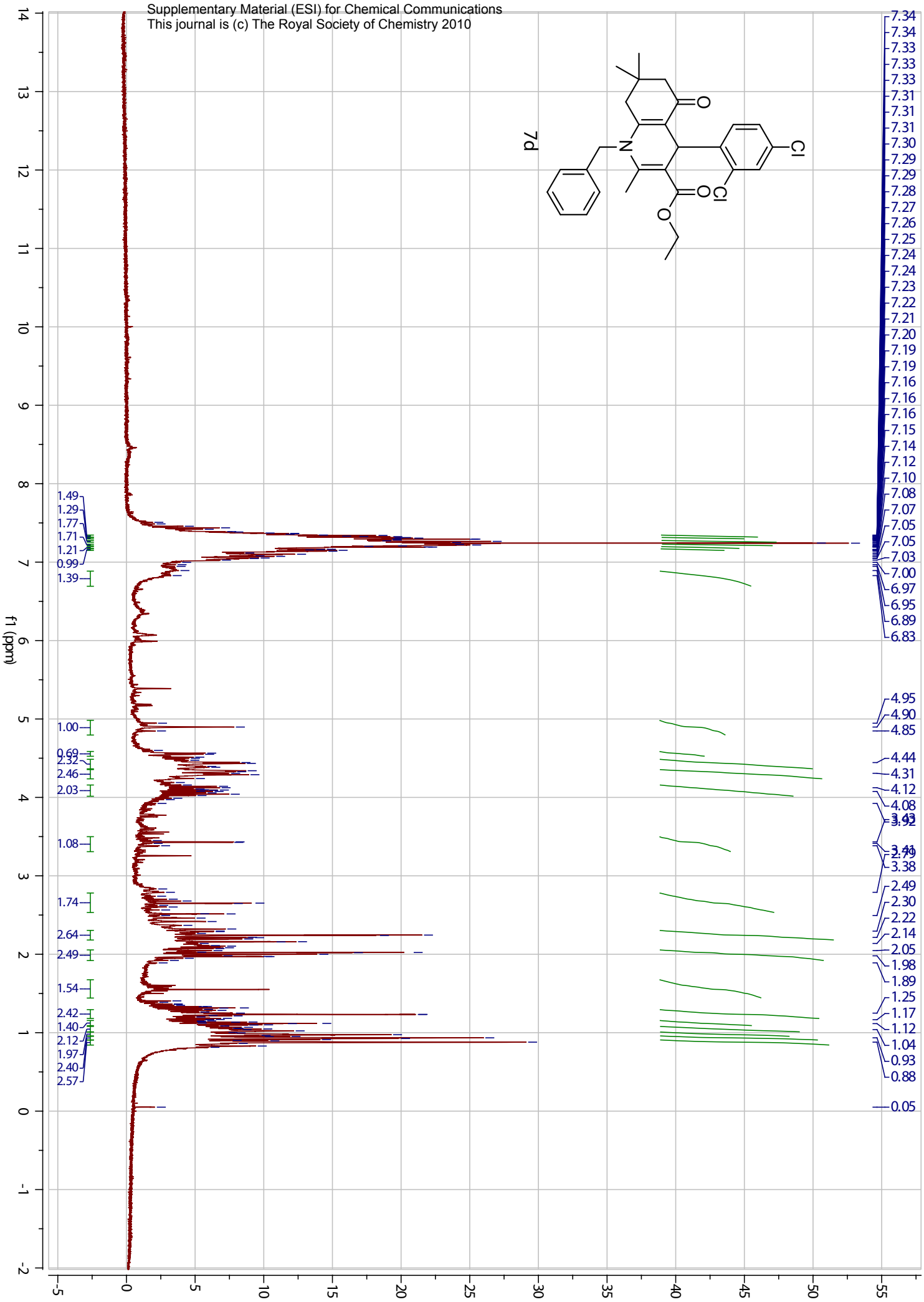
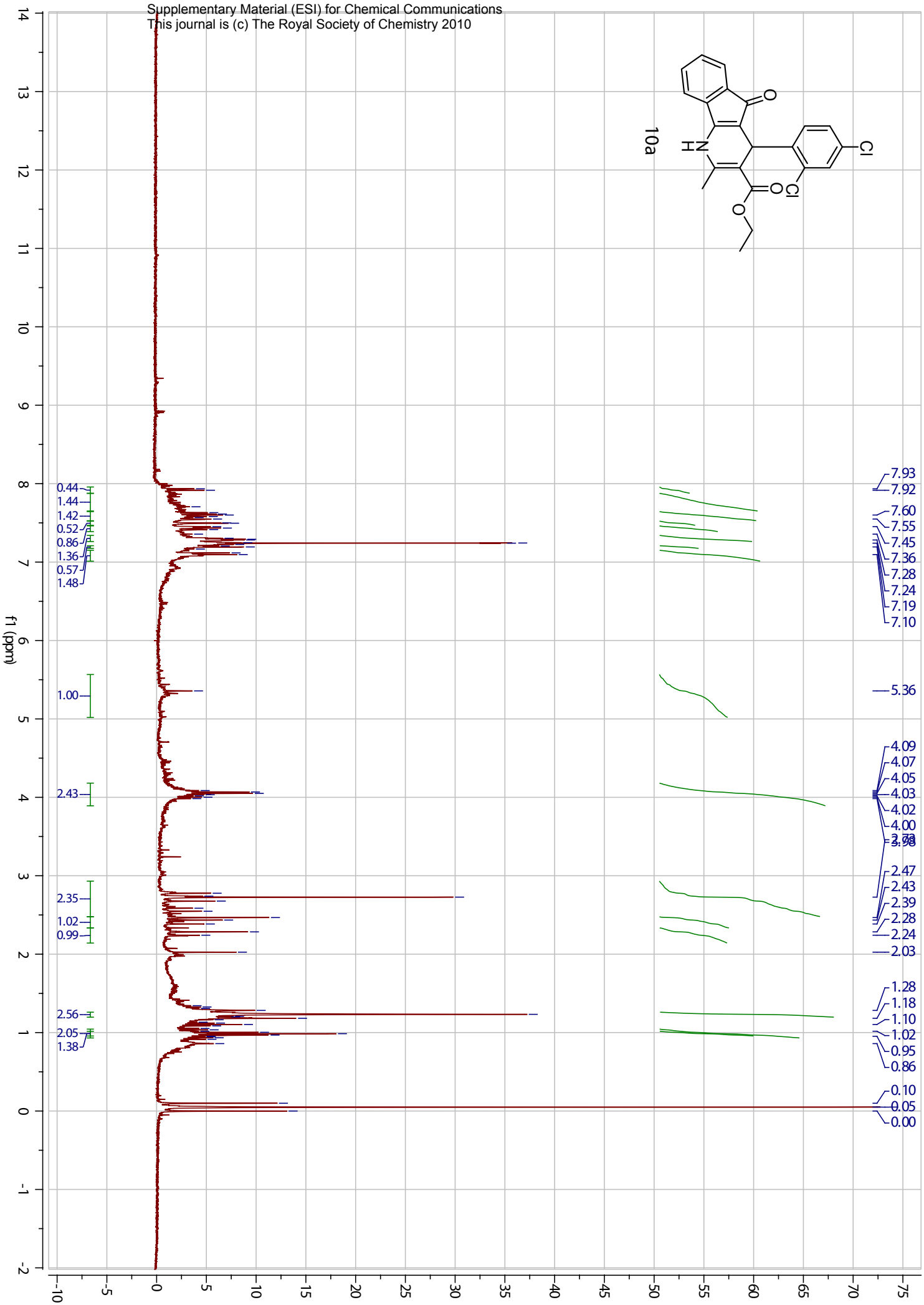
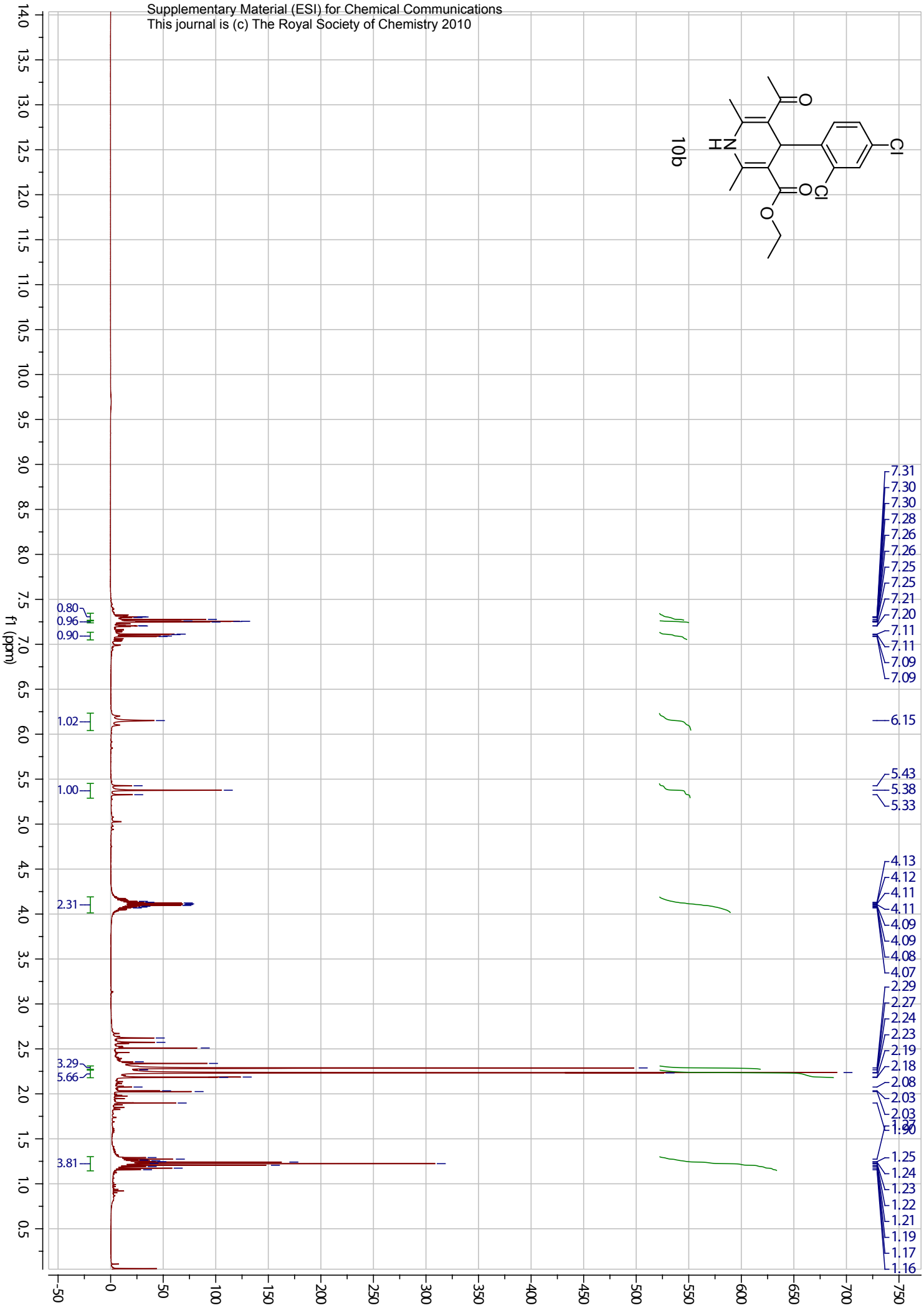
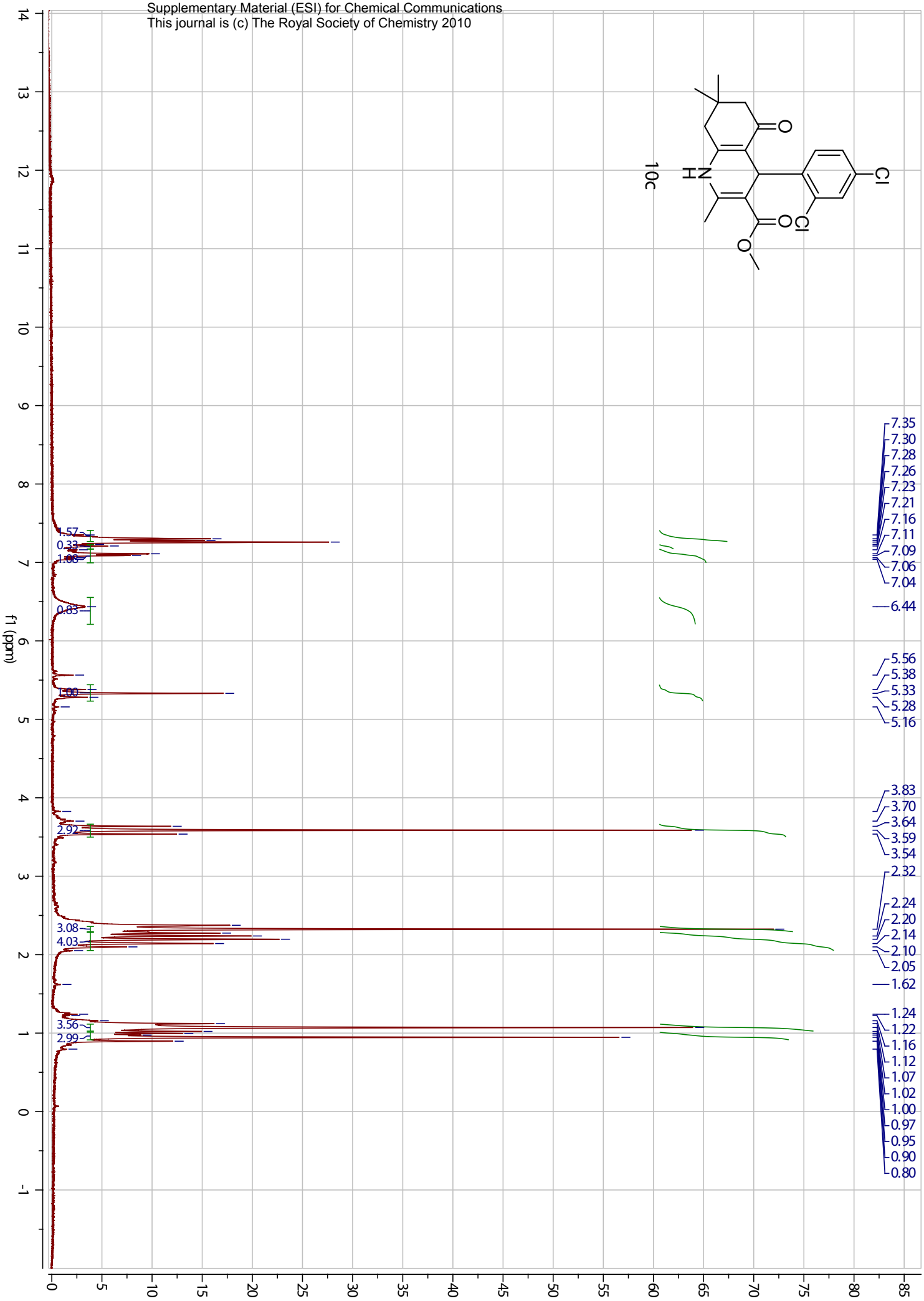
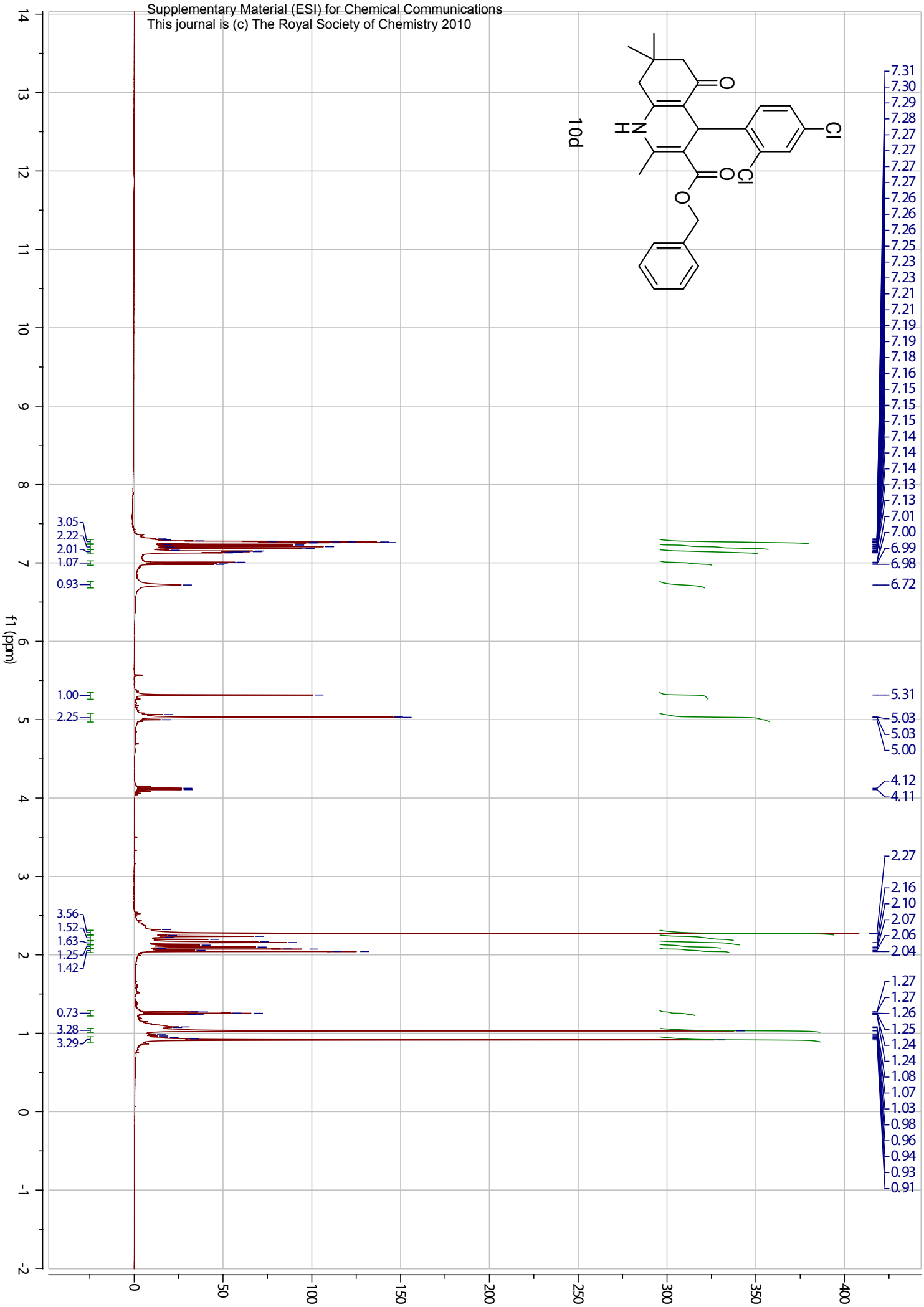
0.58
2.00
1.51
1.25
2.38
2.56
1.06
2.38
3.52
2.82
2.28

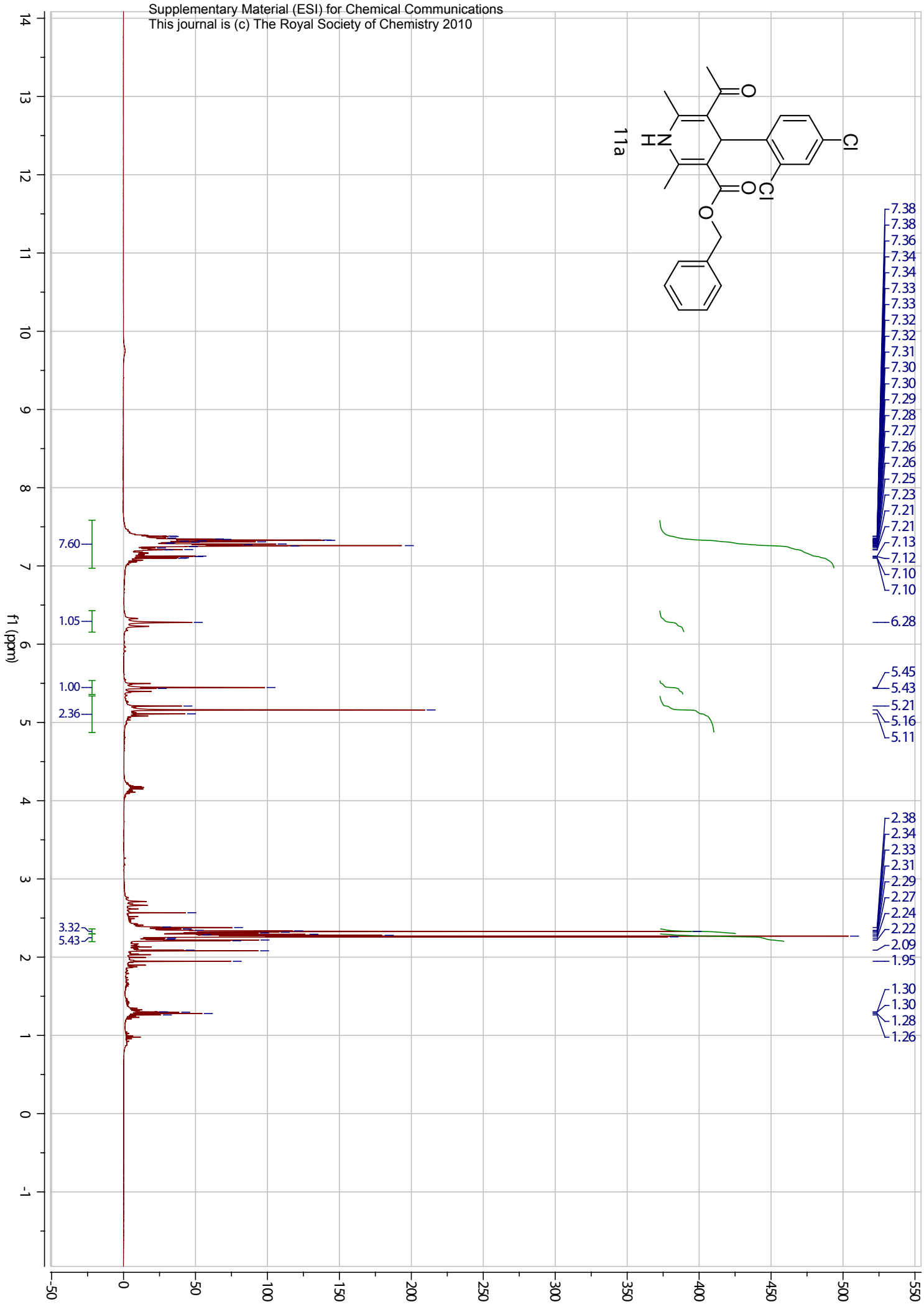

7d
f1 (ppm)

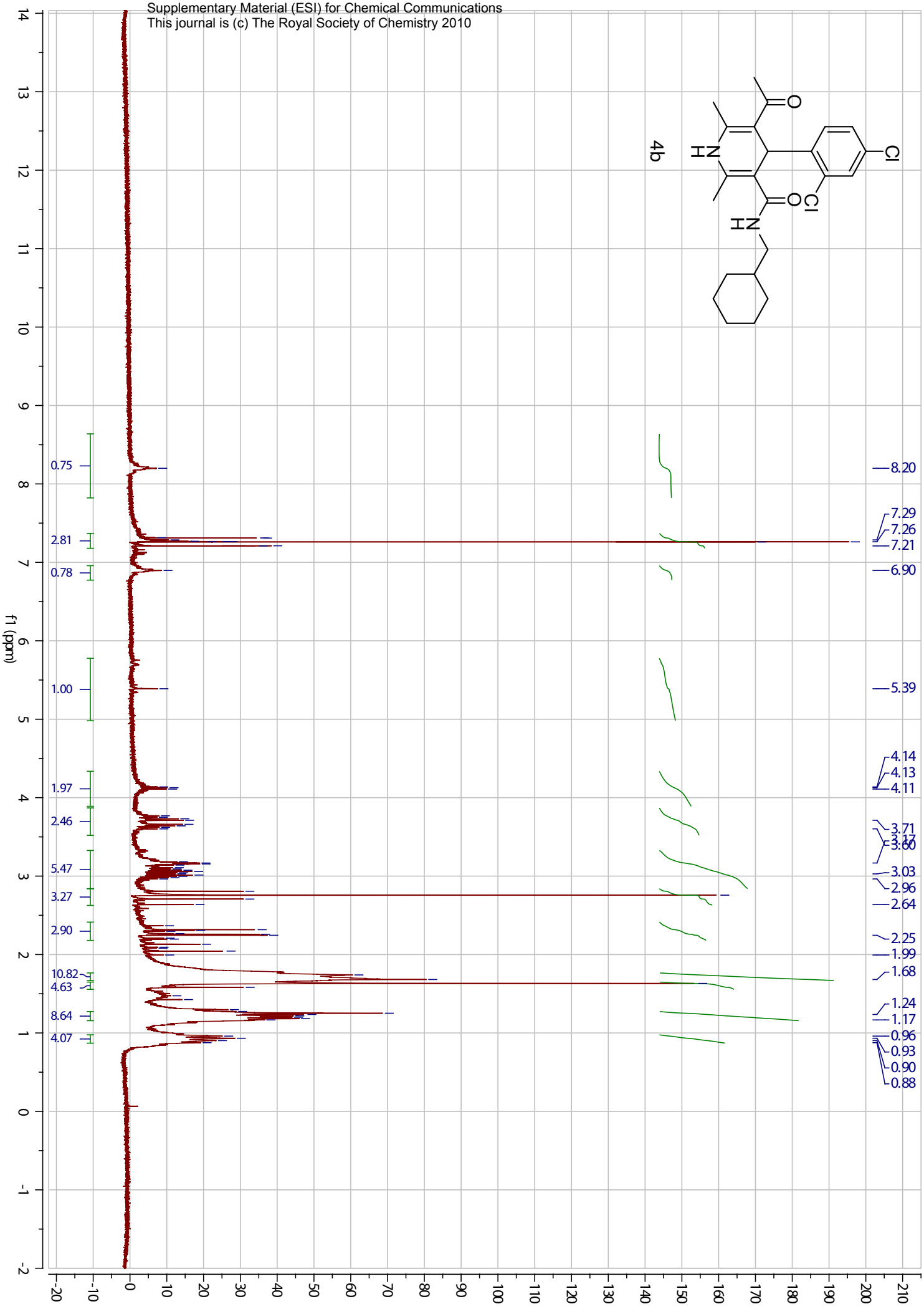

10a
f1 (ppm)
0.44
1.44
1.42
0.52
0.86
1.36
0.57
1.48
1.00
2.43
2.35
1.02
0.99
2.56
2.05
1.38
7.93
7.92
7.60
7.55
7.45
7.36
7.28
7.24
7.19
7.10
5.36
4.09
4.07
4.05
4.03
4.02
4.00
2.47
2.43
2.39
2.28
2.24
2.03
1.28
1.18
1.10
1.02
0.95
0.86
0.10
0.05
0.00

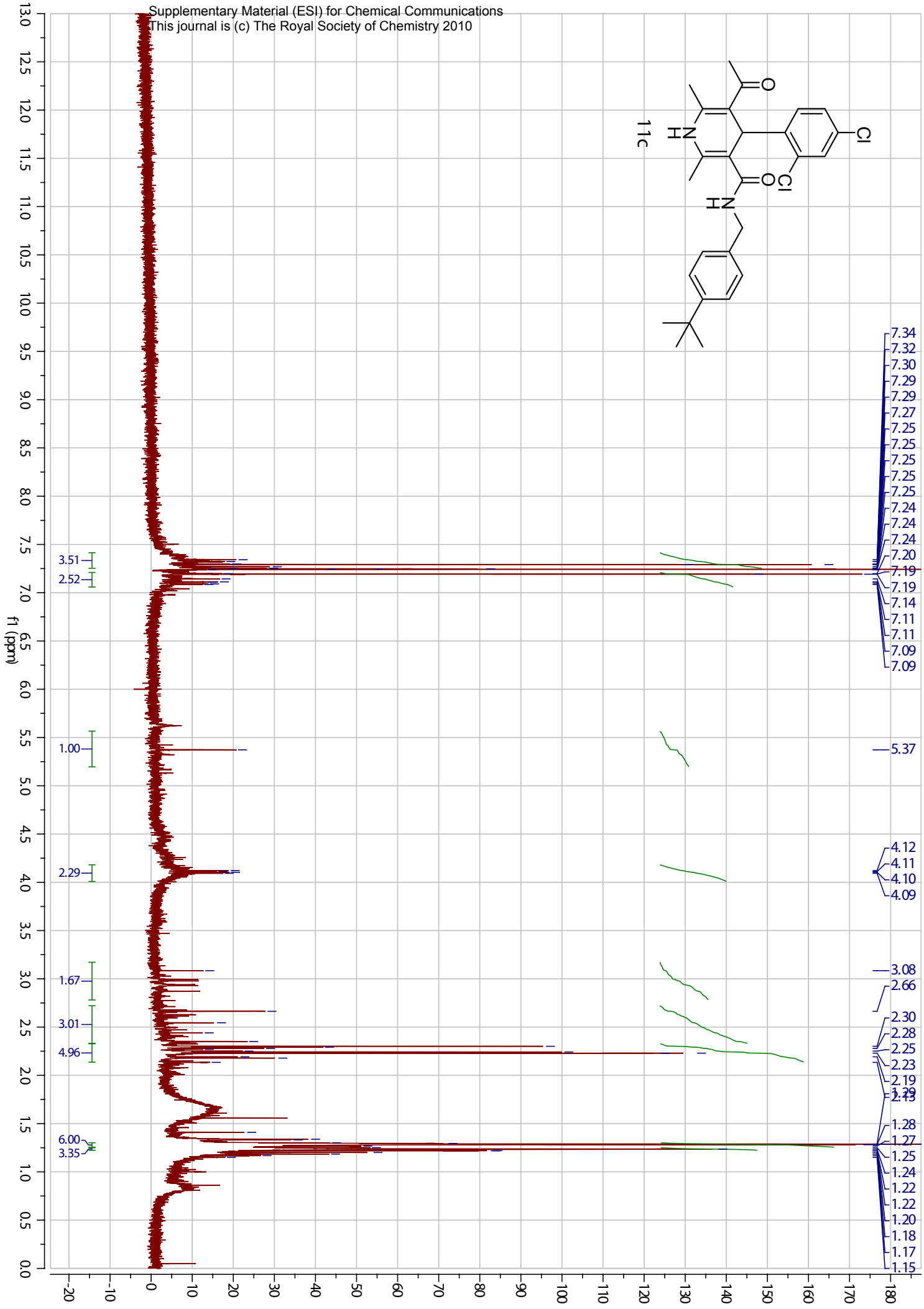

10b
f1 (ppm)
0.80
0.96
0.90
1.02
1.00
2.31
3.29
5.66
3.81
7.31
7.30
7.30
7.28
7.26
7.26
7.25
7.25
7.21
7.20
7.11
7.11
7.09
7.09
6.15
5.43
5.38
5.33
4.13
4.12
4.11
4.11
4.09
4.09
4.08
4.07
2.29
2.27
2.24
2.23
2.19
2.18
2.08
2.03
2.03
1.27
1.90
1.25
1.24
1.23
1.22
1.21
1.19
1.17
1.16

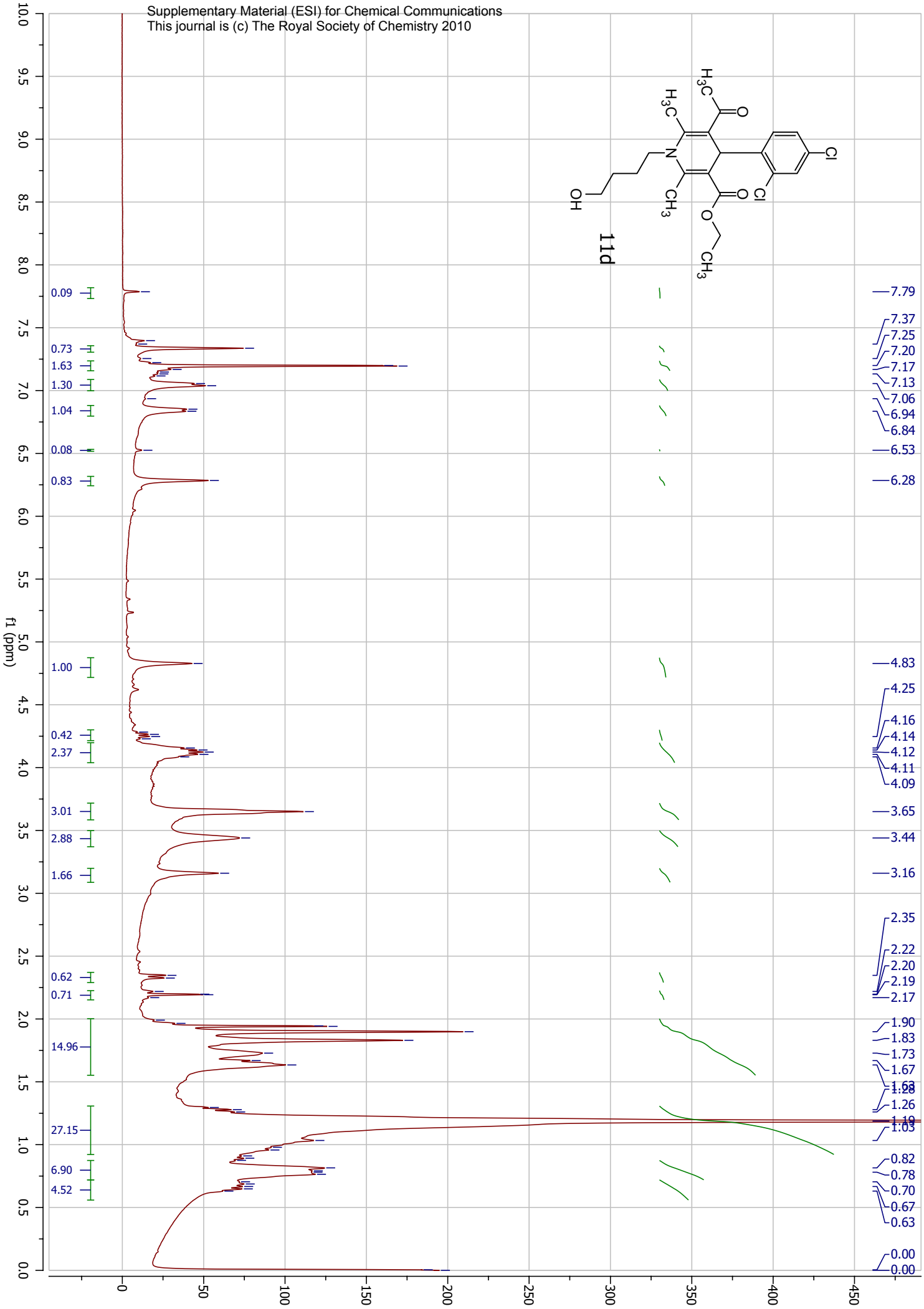

10c
f1 (ppm)
7.35
7.30
7.28
7.26
7.23
7.21
7.16
7.11
7.09
7.06
7.04
6.44
5.56
5.38
5.33
5.28
5.16
3.83
3.70
3.64
3.59
3.54
2.32
2.24
2.20
2.14
2.10
2.05
1.62
1.24
1.22
1.16
1.12
1.07
1.02
1.00
0.97
0.95
0.90
0.80
1.57
0.33
1.08
0.83
1.00
2.92
3.08
4.03
3.56
2.99

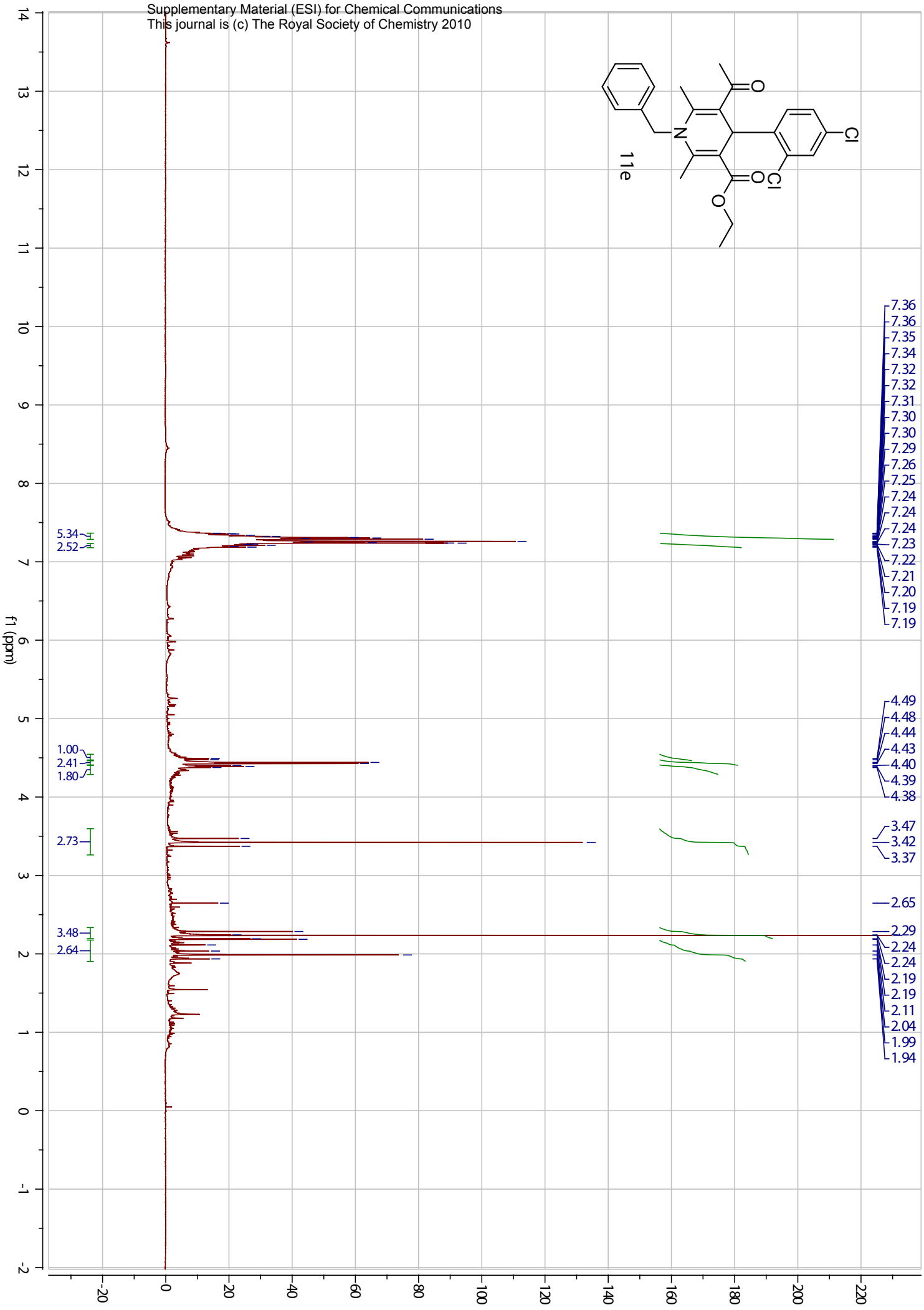

10d
f1 (ppm)
3.05
2.22
2.01
1.07
0.93
1.00
2.25
3.56
1.52
1.63
1.25
1.42
0.73
3.28
3.29
7.31
7.30
7.29
7.28
7.27
7.26
7.25
7.23
7.21
7.19
7.18
7.16
7.15
7.14
7.13
7.01
7.00
6.99
6.98
6.72
5.31
5.03
5.00
4.12
4.11
2.27
2.16
2.10
2.07
2.06
2.04
1.27
1.26
1.25
1.24
1.08
1.07
1.03
0.98
0.96
0.94
0.93
0.91

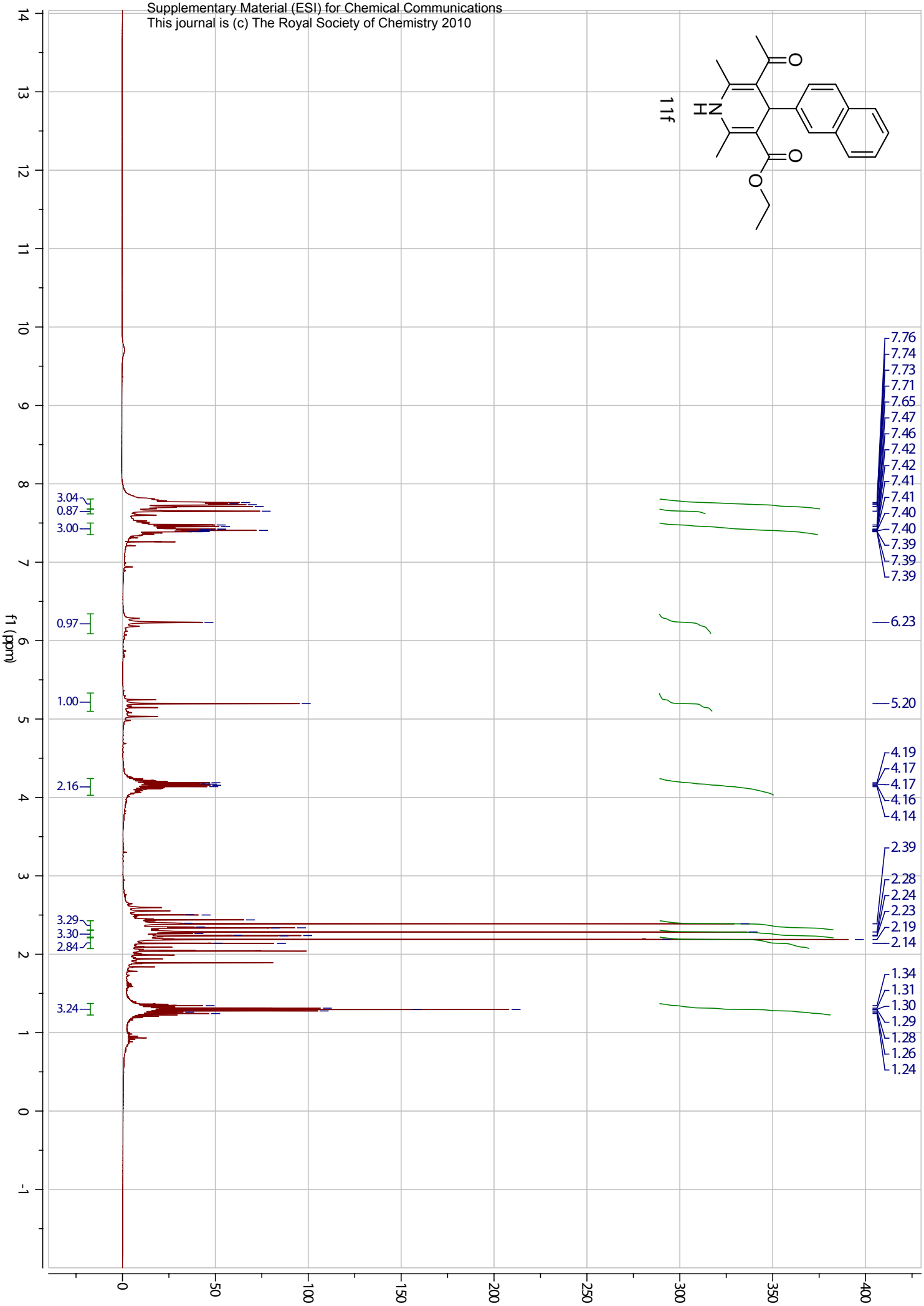

11a
f1 (ppm)
7.60
1.05
1.00
2.36
3.32
5.43
7.38
7.38
7.36
7.34
7.34
7.33
7.33
7.32
7.32
7.31
7.30
7.30
7.29
7.28
7.27
7.26
7.26
7.25
7.23
7.21
7.21
7.13
7.12
7.10
7.10
6.28
5.45
5.43
5.21
5.16
5.11
2.38
2.34
2.33
2.31
2.29
2.27
2.24
2.22
2.09
1.95
1.30
1.30
1.28
1.26

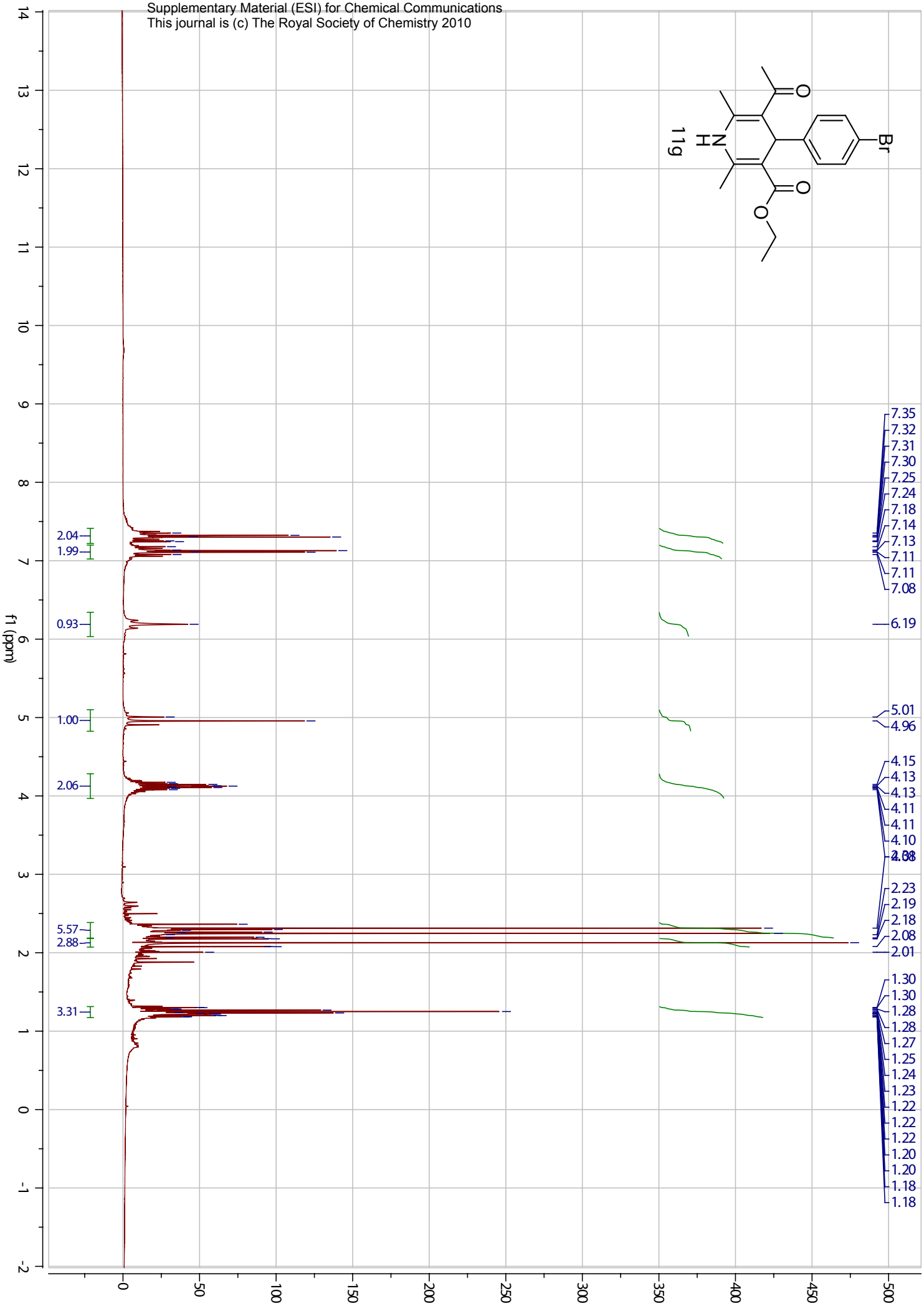

4b
f1 (ppm)
0.75
2.81
0.78
1.00
1.97
2.46
5.47
3.27
2.90
10.82
4.63
8.64
4.07
8.20
7.29
7.26
7.21
6.90
5.39
4.14
4.13
4.11
3.71
3.17
3.60
3.03
2.96
2.64
2.25
1.99
1.68
1.24
1.17
0.96
0.93
0.90
0.88

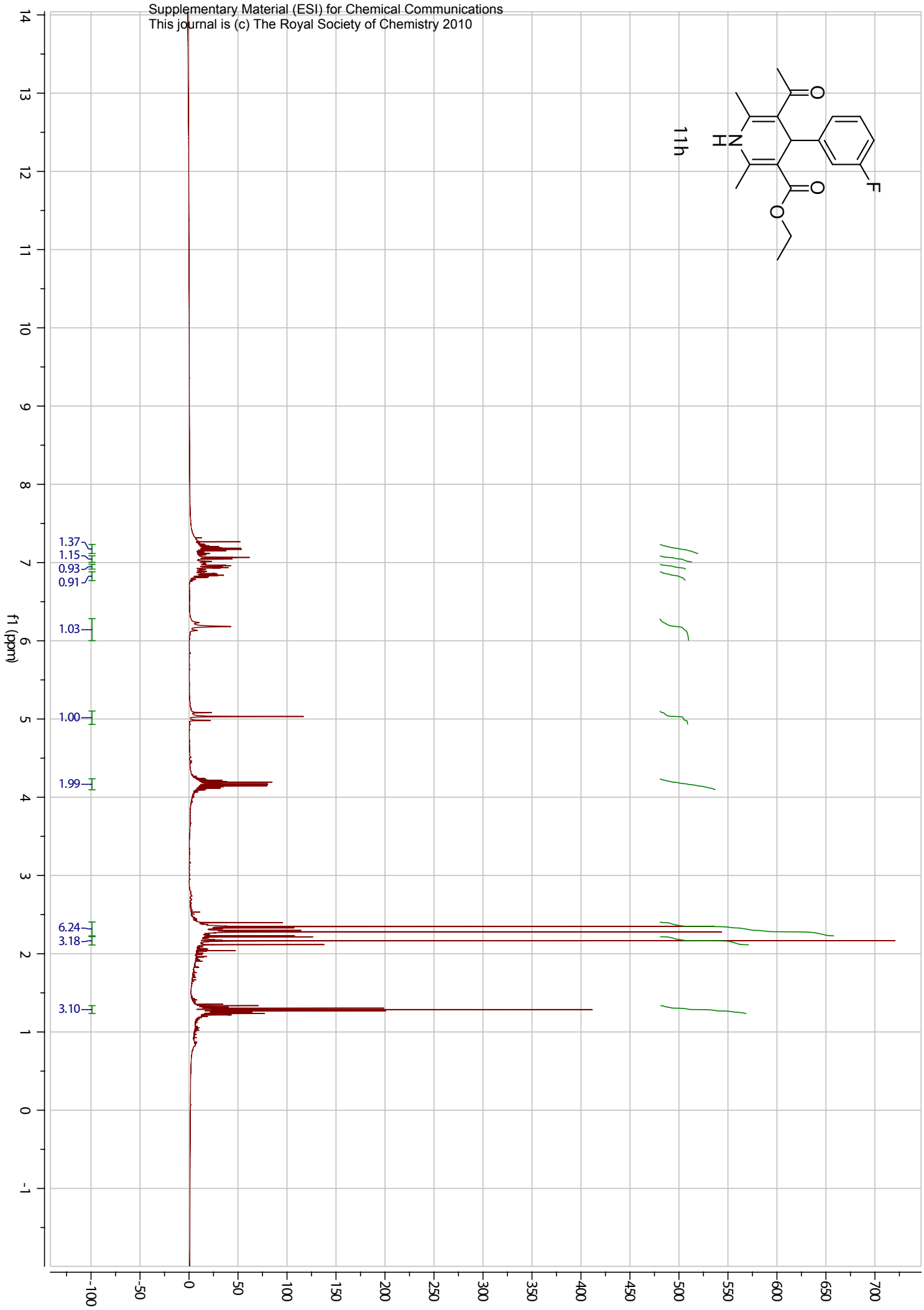

11c
f1 (ppm)
3.51
2.52
1.00
2.29
1.67
3.01
4.96
6.00
3.35
7.34
7.32
7.30
7.29
7.29
7.27
7.25
7.25
7.25
7.25
7.25
7.24
7.24
7.24
7.20
7.19
7.19
7.14
7.11
7.11
7.09
7.09
5.37
4.12
4.11
4.10
4.09
3.08
2.66
2.30
2.28
2.25
2.23
2.19
1.28
1.27
1.25
1.24
1.22
1.22
1.20
1.18
1.17
1.15

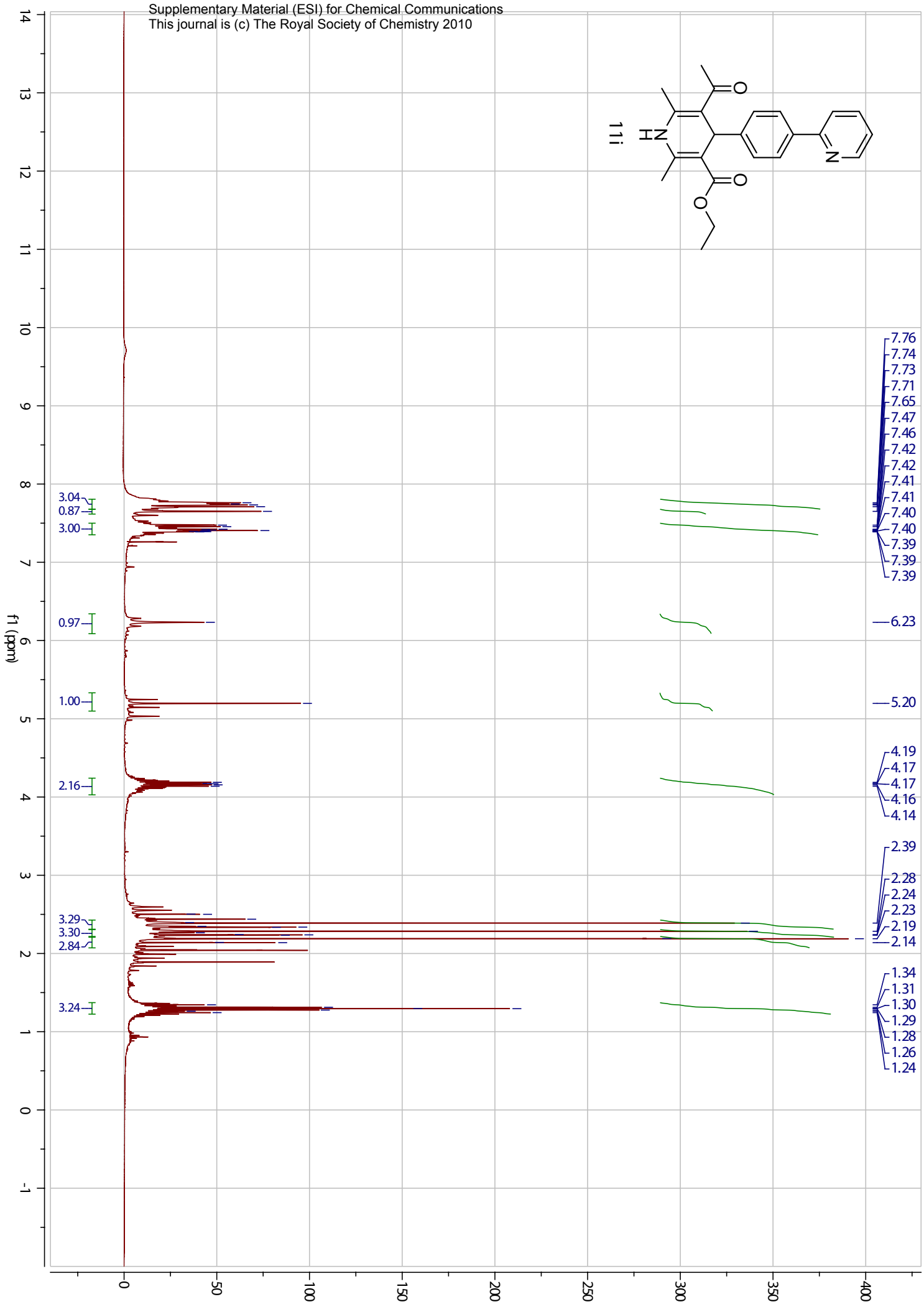

11d
f1 (ppm)
0.09
0.73
1.63
1.30
1.04
0.08
0.83
1.00
0.42
2.37
3.01
2.88
1.66
0.62
0.71
14.96
27.15
6.90
4.52
7.79
7.37
7.25
7.20
7.17
7.13
7.06
6.94
6.84
6.53
6.28
4.83
4.25
4.16
4.14
4.12
4.11
4.09
3.65
3.44
3.16
2.35
2.22
2.20
2.19
2.17
1.90
1.83
1.73
1.67
1.63
1.26
1.19
1.03
0.82
0.78
0.70
0.67
0.63
0.00
0.00

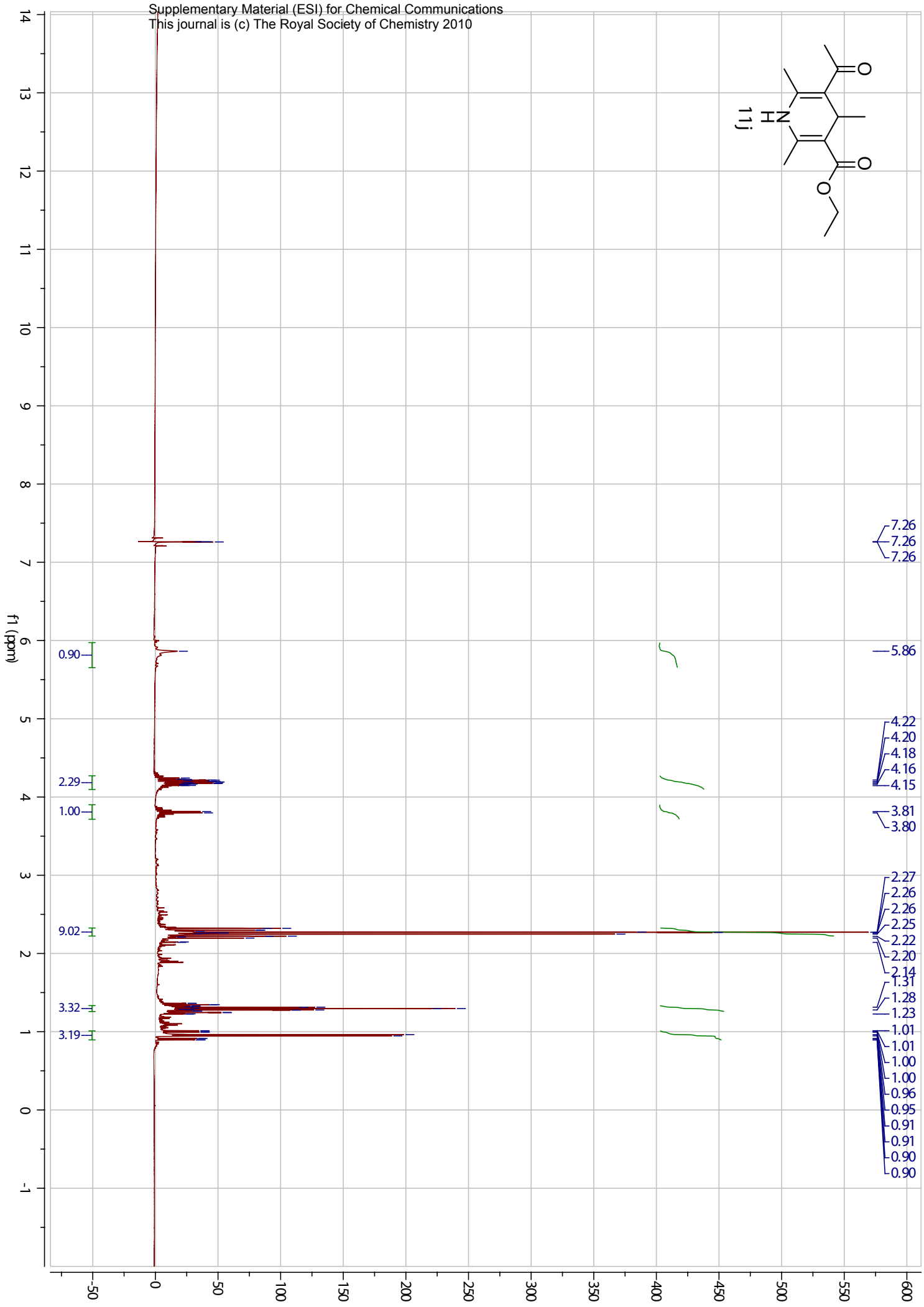

11e
f1 (ppm)
5.34
2.52
1.00
2.41
1.80
2.73
3.48
2.64
7.36
7.36
7.35
7.34
7.32
7.32
7.31
7.30
7.30
7.29
7.26
7.25
7.24
7.24
7.24
7.23
7.22
7.21
7.20
7.19
7.19
4.49
4.48
4.44
4.43
4.40
4.39
4.38
3.47
3.42
3.37
2.65
2.29
2.24
2.24
2.19
2.19
2.11
2.04
1.99
1.94

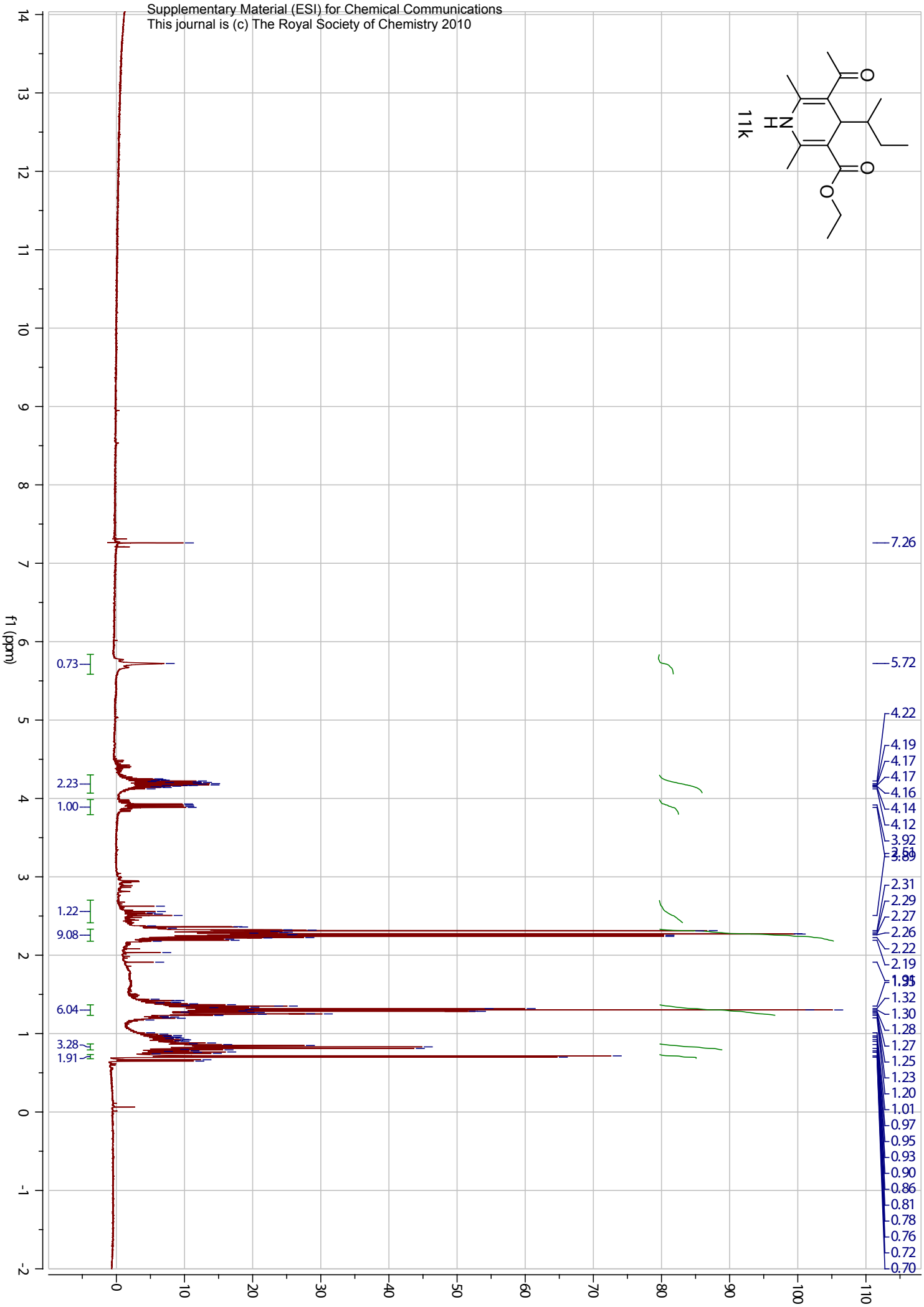

11f
f1 (ppm)
3.04
0.87
3.00
0.97
1.00
2.16
3.29
3.30
2.84
3.24
7.76
7.74
7.73
7.71
7.65
7.47
7.46
7.42
7.42
7.41
7.41
7.40
7.40
7.39
7.39
7.39
6.23
5.20
4.19
4.17
4.17
4.16
4.14
2.39
2.28
2.24
2.23
2.19
2.14
1.34
1.31
1.30
1.29
1.28
1.26
1.24


11g
Br
O
O
O
N
H
f1 (ppm)
2.04
1.99
0.93
1.00
2.06
5.57
2.88
3.31
7.35
7.32
7.31
7.30
7.25
7.24
7.18
7.14
7.13
7.11
7.11
7.08
6.19
5.01
4.96
4.15
4.13
4.13
4.11
4.11
4.10
2.23
2.19
2.18
2.08
2.01
1.30
1.30
1.28
1.28
1.27
1.25
1.24
1.23
1.22
1.22
1.22
1.20
1.20
1.18
1.18


11h
1.37
1.15
0.93
0.91
1.03
1.00
1.99
6.24
3.18
3.10
f1 (ppm)


11i
f1 (ppm)
3.04
0.87
3.00
0.97
1.00
2.16
3.29
3.30
2.84
3.24
7.76
7.74
7.73
7.71
7.65
7.47
7.46
7.42
7.42
7.41
7.41
7.40
7.40
7.39
7.39
7.39
6.23
5.20
4.19
4.17
4.17
4.16
4.14
2.39
2.28
2.24
2.23
2.19
2.14
1.34
1.31
1.30
1.29
1.28
1.26
1.24


11j
f1 (ppm)
0.90
2.29
1.00
9.02
3.32
3.19
7.26
7.26
7.26
5.86
4.22
4.20
4.18
4.16
4.15
3.81
3.80
2.27
2.26
2.26
2.25
2.22
2.20
2.14
1.31
1.28
1.23
1.01
1.01
1.00
1.00
0.96
0.95
0.91
0.91
0.90
0.90


11k
f1 (ppm)
0.73
2.23
1.00
1.22
9.08
6.04
3.28
1.91
7.26
5.72
4.22
4.19
4.17
4.17
4.16
4.14
4.12
3.92
2.31
2.29
2.27
2.26
2.22
2.19
1.32
1.30
1.28
1.27
1.25
1.23
1.20
1.01
0.97
0.95
0.93
0.90
0.86
0.81
0.78
0.76
0.72
0.70

Cells and Antibodies. IMR32 cells were maintained in Opti-MEM plus 10% bovine serum albumin and 2% 200 mM L-glutamine (Cellgro, Mediatech). Anti-tau antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and used at 1:1000. Anti-Hsp70 antibody was from Stressgen (Ann Arbor, MI) and used at 1:1000. Secondary antibody was from Molecular Probes (Eugene, OR).

Estimation of Cellular Toxicity. Immediately prior to preparation of cell lysates (below), the viability of the IMR32 cells was visually estimated by light microscopy. Briefly, at least five random fields were inspected for each treatment. Cells that were visually undergoing apoptosis or necrosis (e.g. rounded cells, membrane blebs, failure to adhere, etc.) were counted. After counting ~100 cells, compounds were considered as toxic if more than 10% of the cells displayed abnormal morphology. Under these conditions, MB and 17-AAG were non-toxic. Toxic compounds are signified by an asterisk in Figure 1A.

Western Blots. IMR32 cells were grown to near confluence and treated with either compound or 1% DMSO in Opti-MEM (as above). After 24 hrs at 37 °C, the cells were harvested with cold M-PER (Pierce, Rockford, IL) and Western blots prepared as described (1, 2). The blots were then quantified by densitometry using NIH Image J. All the results were normalized to the actin-loading controls. In Figure 1A, the screening experiments were performed in either singlet or duplicates. When appropriate, the error in these studies was approximately 9 to 14%. For Figure 1B and 1C, the experiments were performed in triplicate. The error bars are the standard errors of the mean (SEM). The chosen images are representative. From the quantifications, the dose dependence curves were fit with variable slope in GraphPad PRISM.

References

(1) Jinwal *et al.* (2010) *J. Biol. Chem.* 285:16798.
(2) Jinwal *et al.* (2009) *J. Neurosci.* 29:12079.