Carbocations in Action. Design, Synthesis and Evaluation of a Highly Acid Sensitive Naphthalene Based Backbone Amide Linker for Solid-Phase Synthesis

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SUPPORTING INFORMATION

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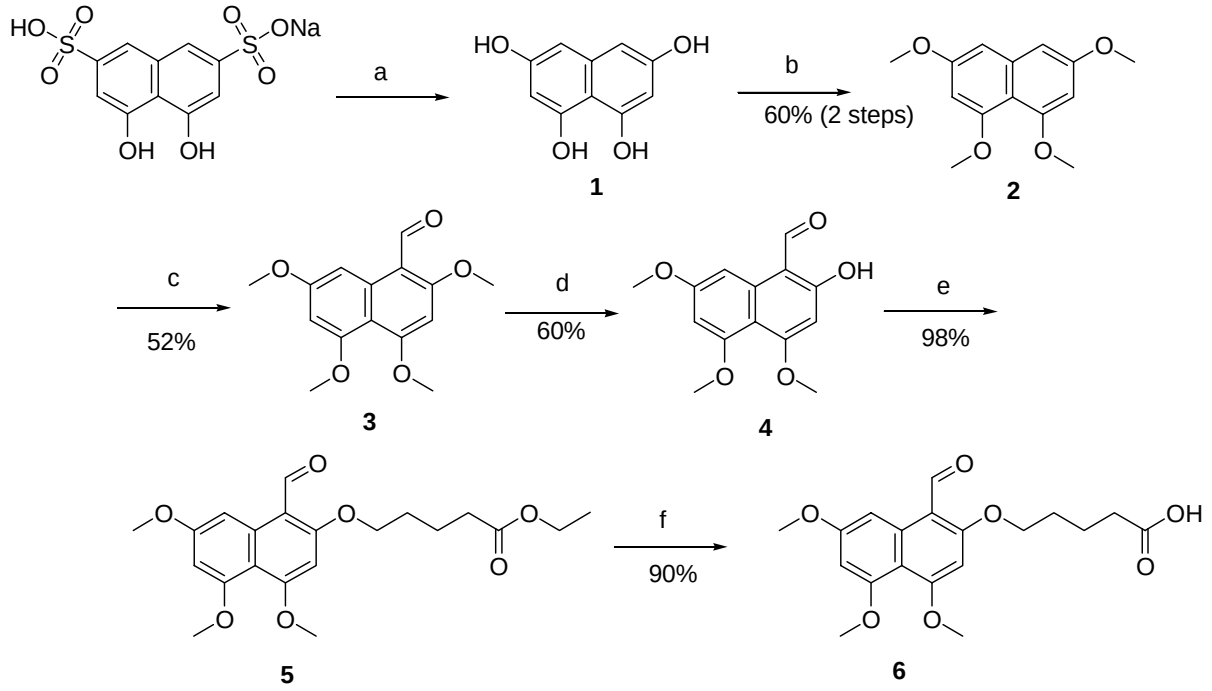
General experimental Section:

General comments. High-loading aminomethylated PS resin, all amino acids, and HBTU were obtained from NovaBioChem, HOBt from Quantum Richelieu. Solid phase reactions were performed in polypropylene syringes equipped with a polyethylene filter, placed on a shaker. HPLC-MS analysis was performed on a Shimadzu 2010, using a Phenomenex Jupiter C5 column (5μ, 300Å). Gradient: Linear 1 mL/min from 3 % to 95 % buffer B over 18 min. (buffer A: 0.025 % TFA in H_2O; buffer B: 0.025 % TFA in 90 % aq. CH_3CN). UV analysis was performed at a Perkin Elmer Lambda 2 UV/VIS spectrometer. Solvents were HPLC grade and were used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 MHz NMR (Varian or Bruker Avance) instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) or on a 400 MHz NMR (Bruker) instrument (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Proton chemical shifts are reported in ppm downfield from tetramethylsilane (TMS) and carbon chemical shifts in ppm downfield of TMS using the resonance of the deuterated solvent as internal standard. Assignments of ^{1}H NMR signals were based on NOESY and COSY spectra. Melting points were measured on a Büchi B-140 apparatus and are uncorrected. Elemental analysis was performed by Mrs Birgitta Kegel. Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol JMS-HX 110A Tandem Mass Spectrometer in the positive ion mode using m-NBA as the matrix. HRMS were recorded on a Micromass Q-TOF apparatus using electrospray ionisation (ESI) technique. All column chromatography was performed on Merck Kiselgel 60 (0.015 – 0.040 mm) using the DCVC technique.[1]

Synthesis:



Scheme 1. Reagents and conditions: a) $Ba(OH)_2 \times 8H_2O$, KOH, NaOH, 260°C, 12 hours then b) Dimethylsulphate, acetone, K_2CO_3, 48 hours, 60 % (two steps) c) $POCl_3$, DMF, -5 °C, 52 % d) BBr_3, CH_2Cl_2, 60 % e) 5-bromovaleric acid ethyl ester, DMF, K_2CO_3, 98 % f) NaOH, THF, 90 %.

1,3,6,8-Tetrahydroxynaphthalene (1).[2] Chromotropic acid sodium salt (tech., 60%, 10.0 g, 17.5 mmol), KOH (36 g), NaOH (24 g) and $Ba(OH)_2 \times 8H_2O$ (36 g) was placed in a Ag container (200 mL) fitted with a magnetic stir bar. The reaction vessel was covered with aluminum foil and a continuous stream of N_2 was flushed over the mixture during the entire course of the reaction. The oil bath was heated to 250°C over night, and then allowed to cool to room temperature under N_2. After cooling to room temperature the solid was crushed with a mortar and added carefully to ice (1000 mL) containing concentrated H_2SO_4 (200 mL) and stirred vigorously for 1 hour. The aqueous slurry was extracted with EtOAc (4×400 mL). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness yielding a dark solid material, which was purified by dry column vacuum chromatography (heptane to EtOAc with 10% increments, and then additional EtOAc until

all the compound was off the column). The resulting pale yellow solid was soluble in EtOAc but not in CH_2Cl_2 or $CHCl_3$. Yield: 1.89 g, 56 %. ^{1}H NMR (DMSO) δ 10.57 (br s, 2H), 9.31 (s, 2H), 6.28 (d, 2H), 6.07 (d, 2H); ^{13}C NMR (DMSO) δ 156.4, 155.4, 138.8, 104.2, 99.6, 97.8; Anal. Calcd. for $C_{10}H_8O_4$: C, 62.49; H, 4.20. Found: C, 62.35; H, 4.32.

1,3,6,8-Tetramethoxynaphthalene (2) (from 1).[3] Tetrahydroxy naphthalene (**1**) (1.51 g, 7.81 mmol) was dissolved in anhydrous acetone (100 mL). K_2CO_3 (10.78 g, 78.1 mmol, 10 eqv) and dimethylsulfat (9.85 g, 7.4 mL, 78.1 mol, 10 eqv) was added. The reaction mixture was stirred at reflux temperature for 48 hours. Water (150 mL) was added and the mixture was extracted with CH_2Cl_2 (3×100 mL), the combined organic extracts were dried (Na_2SO_4), filtered through paper and evaporated to dryness *in vacuo*. The crude material was purified by dry column vacuum chromatography (heptane to CH_2Cl_2 with 10 % increments). Yield: 1.66 g, 85 %. See below for analytical data.

1,3,6,8-Tetramethoxynaphthalene (2) (from chromotropic acid sodium salt).[3] Chromotropic acid sodium salt (tech., 60%, 8.33 g, 14.6 mmol), KOH (18 g), NaOH (12 g) and $Ba(OH)_2×8H_2O$ (18 g) was placed in a Ag container (200 mL) fitted with a magnetic stir bar. The reaction vessel was covered with aluminum foil and a continuous stream of N_2 was flushed over the mixture during the entire course of the reaction. The oil bath was heated to 250°C over night, and then allowed to cool to room temperature under N_2. After cooling to room temperature the solid was crushed with a mortar and added carefully to ice (1000 mL) containing concentrated H_2SO_4 (200 mL) and stirred vigorously for 1 hours. The aqueous slurry was extracted with EtOAc (4×400 mL). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness yielding a dark solid material. This was dissolved in anhydrous acetone and K_2CO_3 (20.2 g, 146 mmol, 10 eqv.) and

DMS (13.9 mL, 146 mmol, 10 eqv.) was added. The reaction mixture was heated to reflux for 48 hours, cooled to room temperature and hydrolyzed with water (100 mL). The crude material was extracted with CH_2Cl_2 (3×150 mL), the combined organic extracts were dried (Na_2SO_4), filtered through paper and evaporated to dryness *in vacuo*. The crude was purified by dry column vacuum chromatography (heptane to CH_2Cl_2 with 5% increments) to yield a white solid; Yield 2.18 g, 60 %. Mp. 101-102 °C; ^{1}H NMR ($CDCl_3$) δ 6.62 (d, 2H), 6.36 (d, 2H), 3.92 (s, 6H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.6, 158.4, 139.0, 98.4, 96.5, 56.0, 55.1; MS (FAB^+) *m/z* 248.06 (M^+); HRMS (FAB^+): Anal. Calcd. for $C_{14}H_{16}O_4$: *m/z* 248.1049. Found: *m/z* 248.1056; Anal. Calcd. for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.67; H, 6.56.

2,4,5,7-Tetramethoxy-1-naphthaldehyde (3). 1,3,6,8-Tetramethoxynaphthalene (**2**) (2.39 g, 9.63 mmol) was dissolved in anhydrous DMF (350 mL) and the mixture was cooled to -5 °C. $POCl_3$ (1.5 mL, 2.47 g, 16.1 mmol) was added drop wise during 5 minutes. The reaction mixture was stirred for 30 minutes at -5 °C and then water (300 mL) was added. The yellow mixture was extracted with CH_2Cl_2 (4×500 mL), dried ($MgSO_4$), filtered through paper and evaporated to dryness *in vacuo*. Dry column vacuum chromatography (heptane to EtOAc with 10 % increments) yields the target aldehyde as a white solid. Yield: 1.38 g, 52 %. Mp. 163-164 °C; ^{1}H NMR ($CDCl_3$) δ 10.67 (s, 1H), 8.72 (d, 1H), 6.44 (d, 1H), 6.37 (s, 1H), 4.05 (s, 3H), 4.03 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 189.8, 167.0, 165.0, 162.1, 158.7, 137.4, 109.6, 107.7, 97.6, 96.6, 89.8, 56.1, 56.0, 55.9, 55.3; MS (GC-MS) 276 *m/z*; Anal. Calcd. for $C_{15}H_{16}O_5$: C, 65.20; H, 5.85. Found: C, 65.18; H, 5.91.

2-Hydroxy-4,5,7-trimethoxy-1-naphthaldehyde (4). Aldehyde (**3**) (1.07 g, 3.87 mmol) was dissolved in anhydrous CH_2Cl_2 (200 mL) and BBr_3 (7.75 mL, 1M, 7.75 mmol) was added during 5

minutes at room temperature under an N_2 atmosphere. The reaction mixture was heated to reflux for 4 hours and the cooled to room temperature. Water was added (200 mL) and the phases were separated. The aqueous phase was extracted with additional CH_2Cl_2 (2×100 ml), the combined organic extracts were dried (Na_2SO_4), filtered through paper and evaporated to dryness *in vacuo*. The crude material was purified by dry column vacuum chromatography (heptane to EtOAc with 5 % increments) to give the target compound as a white solid material. Yield: 610 mg, 60 %. Mp. 170-171 °C; ^{1}H NMR ($CDCl_3$) δ 13.74 (O*H*, br s, 1H), 10.49 (C*H*O, s, 1H), 7.19 (*H*8, d, 1H), 6.44 (*H*6, d, 1H), 6.27 (*H*3, s, 1H), 3.99 (OC*H*$_3$4, s, 3H), 3.92 (OC*H*$_3$5, s, 3H), 3.91 (OC*H*$_3$*7*, s, 3H); ^{13}C NMR ($CDCl_3$) δ 190.6, 168.3, 166.3, 160.9, 159.9, 138.6, 107.4, 106.3, 96.7, 95.2, 91.9, 56.2, 56.0, 55.2; MS (GC-MS) 262 *m/z*; Anal. Calcd. for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38. Found: C, 63.96; H, 5.54.

Ethyl 5-(1-formyl-4,5,7-trimethoxynaphthalen-2-yloxy)pentanoate (5). Naphthol (**4**) (0.42 g, 1.6 mmol) was dissolved in anhydrous DMF (100 mL) and K_2CO_3 (0.44 g, 3.2 mmol) was added. Ethyl 5-bromovalerate (0.67 g, 3.2 mmol, 0.51 mL) was added and the reaction was stirred at 60 °C for 3 hours. Water (200 mL) was added and the mixture was extracted with CH_2Cl_2 (3×150 mL), the combined organic extracts were dried ($MgSO_4$), filtered through paper and evaporated to dryness *in vacuo* (1 mmHg). The resulting material was purified by dry column vacuum chromatography (heptane to EtOAc with 5 % increments) to give a white solid material. Yield: 0.62 g, 98 %. Mp. 103-105 °C; ^{1}H NMR ($CDCl_3$) δ 10.72 (s, 1H), 8.72 (d, 1H), 6.44 (d, 1H), 6.34 (s, 1H), 4.19 (t, 2H), 4.13 (q, 2H), 4.02 (s, 3H), 3.95 (s, 3H), 3.90 (s, 3H), 2.41 (t, 2H), 1.82-1.94 (m, 4H), 1.26 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 189.7, 173.1, 166.5, 165.0, 162.1, 158.7, 137.6, 109.7, 107.8, 97.6, 96.6, 90.6, 68.5, 60.3, 56.1, 55.9, 55.3, 33.7, 28.6, 21.5, 14.1; MS (FAB$^+$) *m/z* 391.13 (M+H$^+$); HRMS (FAB$^+$):

Anal. Calcd. for $C_{21}H_{27}O_7$: *m/z* 391.1757. Found: *m/z* 391.7651; Anal. Calcd. for $C_{21}H_{26}O_7$: C, 64.60; H, 6.71. Found: C, 64.34; H, 7.09.

5-(1-Formyl-4,5,7-trimethoxynaphthalen-2-yloxy)pentanoic acid (6), NALdehyde-4. Ester (530 mg, 1.35 mmol) was dissolved in THF (50 mL) and 2M NaOH (30 mL) was added. The mixture was heated to reflux for 5 hours. The reaction was cooled to room temperature and 2M HCl was added (until acidic, pH ~ 1-2). The reaction mixture was extracted with CH_2Cl_2 (3×200 mL), dried ($MgSO_4$), filtered through paper and concentrated *in vacuo* to yield the crude carboxylic acid as an off-white solid. Trituation with cold ether (50 mL, 0 °C) produced the pure NALdehyde-4. Yield: 0.44 g, 90 %. Mp. 198-200 °C; ^{1}H NMR ($CDCl_3$) δ 10.72 (s, 1H), 8.72 (d, 1H), 6.43 (d, 1H), 6.34 (s, 1H), 4.21 (t, 2H), 4.02 (s, 3H), 3.95 (s, 3.95), 3.90 (s, 3H), 2.48 (t, 2H), 1.82-1.99 (m, 4H); ^{1}H NMR (DMSO) δ 12.04 (br s, 1H), 10.60 (s, 1H), 8.59 (d, 1H), 6.62 (s, 1H), 6.50 (d, 1H), 4.32 (t, 2H), 4.00 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 2.33 (t, 2H), 1.82-1.86 (m, 2H), 1.68-1.75 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 189.7, 177.3, 166.5, 165.1, 162.1, 158.7, 137.4, 109.7, 107.8, 97.7, 96.7, 90.6, 68.5, 56.1, 55.9, 55.3, 33.1, 28.5, 21.3; MS (FAB^+) *m/z* 363.07 ($M+H^+$); HRMS (FAB^+): Anal. Calcd. for $C_{19}H_{23}O_7$: *m/z* 363.1444. Found: *m/z* 363.1451; Anal. Calcd. for $C_{19}H_{22}O_7$: C, 62.97; H, 6.12. Found: C, 62.89; H, 5.86.

Solid-phase anchoring of NALdehyde-4:

NALdehyde-4 (**6**) (0.117 g, 0.324 mmol) and PyBOP (0.169 g, 0.324 mmol) were dissolved in DMF (4 mL) and DIEA (0.112 mL, 0.648 mmol) was added and the mixture was gently shaken for 5 minutes at room tempreature. The clear solution was added to aminomethyl-polystyrene resin (0.300 g, 0.108 mmol, loading 0.36 mmol/g) and the suspension was shaken for 16 hours at room temperature. The resin was washed with DMF (10 times) and DCM (10 times). Residual free amino groups on the resin were capped using 20 % acetic anhydride in DMF (5 mL) together with a catalytic amount of DIEA followed by shaking for 2 hours at room temperature. Finally, the resin was washed with DMF (10 times) and DCM (10 times), shrunk with methanol and air dried.

Solid-phase synthesis of Fmoc-Phe-Ala-OH:

NALdehyde-4 derivatised resin (0.150 g, 0.054 mmol, theoretical load 0.36 mmol/g) was swelled in DMF (2 mL), followed by addition of H-Ala-O*t*Bu×HCl (98 mg, 0.54 mmol) and $NaBH_3CN$ (34 mg, 0.54 mmol). The mixture was shaken for 16 hours at room temperature. Hereafter, the resin was washed with DMF (10 times) and DCM (10 times). The resin was swelled in a DCM-DMF 9:1 mixture (2 mL) and Fmoc-Phe-OH (0.209 g, 0.54 mmol) and DIPCDI (42 μL, 0.27 mmol) were added and the mixture was shaken 16 hours at room temperature creating a viscous suspension. The peptidyl-resin was washed with DMF (10 times) and CH_2Cl_2 (10 times), shrunk with methanol and air dried.

Determination of resin substitution by Fmoc-quantisation:[4]

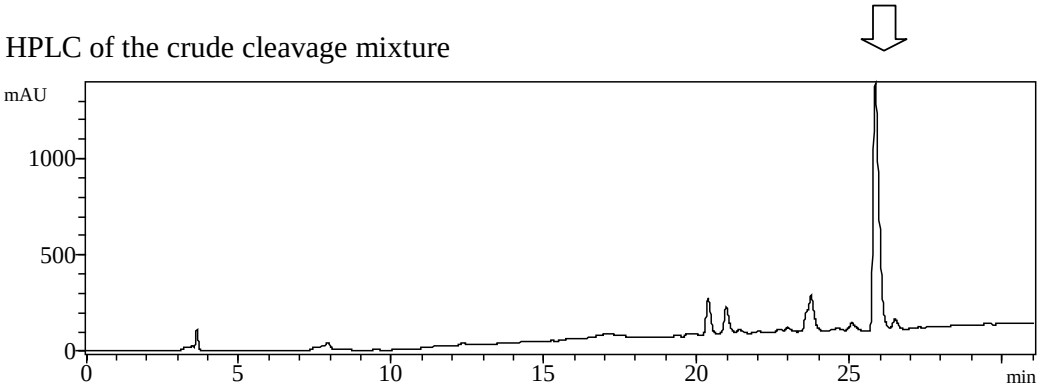
Three portions of resin (approximately 5 mg, 2 μmol theoretical amount of peptide) were suspended in a 20% piperidine-DMF solution (25 mL). The mixtures were shaken for 30 minutes, and the absorbance (290 nm) was measured using piperidine-DMF (1:4) as blank scan reference. The loading of the peptidyl-resin was measured to be 0.173 mmol/g.

Fmoc-Tyr(O*t*Bu)Gly-Gly-Phe-Leu-O*t*Bu:

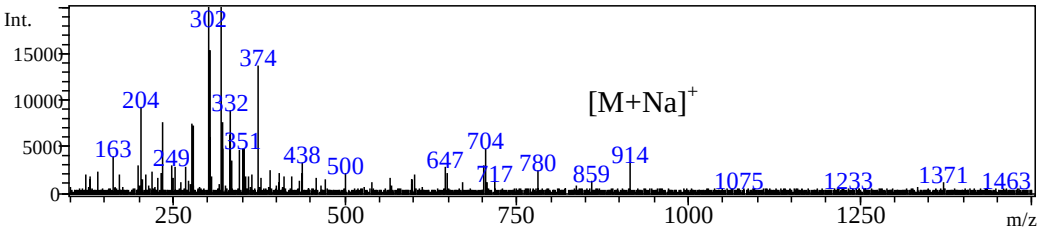
The NAL-4 derivatised resin (0.160g, 0.058 mmol, theoretical loading) were swelled in dry DMF (2 mL) and H-Leu-O*t*Bu×HCl (0.130 g, 0.580 mmol) and $NaBH_3CN$ (0.036 g, 0.58 mmol) were added. The mixture was shaken for 16 hours, washed with DMF (8×10 ml), CH_2Cl_2 (4×10 mL), this reaction was repeated twice. Fmoc-Phe-OH (0.160 g, 0.58 mmol) was dissolved in DMF-CH_2Cl_2 (1:9) (8 mL) and DIPCDI (45 μL, 0.29 mmol) was slowly added under continuous shaking. The slurry was then added to the derivatised resin (0.058 mmol) and shaken for 16 hours (this reaction was repeated twice). The resin was washed with DMF (8×10 ml), CH_2Cl_2 (4×10 mL). Performing Fmoc quantisation test on the derivatised resin gave a loading of 0.35 mmol/g.[4] The Fmoc protected resin was treated with piperidine-DMF (1:4) (5 mL) for 5+30 minutes and then washed with DMF (5×10 mL), CH_2Cl_2 (2×10 ml). Subsequent derivatisation of the peptidyl resin was performed using standard SPPS protocol, coupling reactions were carried out as follows: Fmoc-AA-OH (4 equiv.) was dissolved in DMF and pre-activated for 5 minutes with HBTU (3.8 equiv.), HOBt (4 equiv.) and DIPEA (7.8 equiv.) and transferred to a filter syringe with the derivatised resin (1 equiv. based on loading measurement).

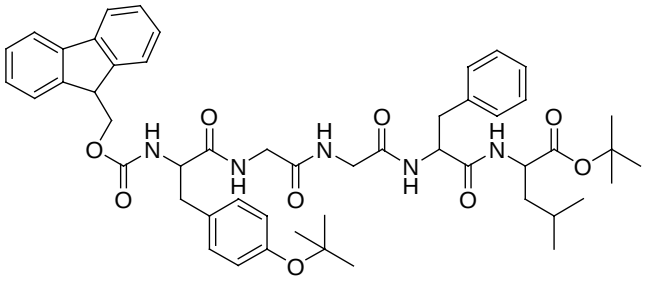
A part of the derivatised resin (0.070 g, 0.025 mmol) was subjected to very mild acidolytic treatment (0.5% TFA/1%TIS/DCM for 2 hours) which released 4 mg (20%) of the pure protected Leu-Enkephalin. HPLC (t_R: 25.9 min (one major peak); MS (ESI^+): 914 (MNa^+).

HPLC of the crude cleavage mixture



MS (peak at 25.9 minutes)





$C_{51}H_{63}N_5O_9$
Exact Mass: 889,46
Mol. Wt.: 890,07
C. 68,82; H. 7,13; N. 7,87; O. 16,18

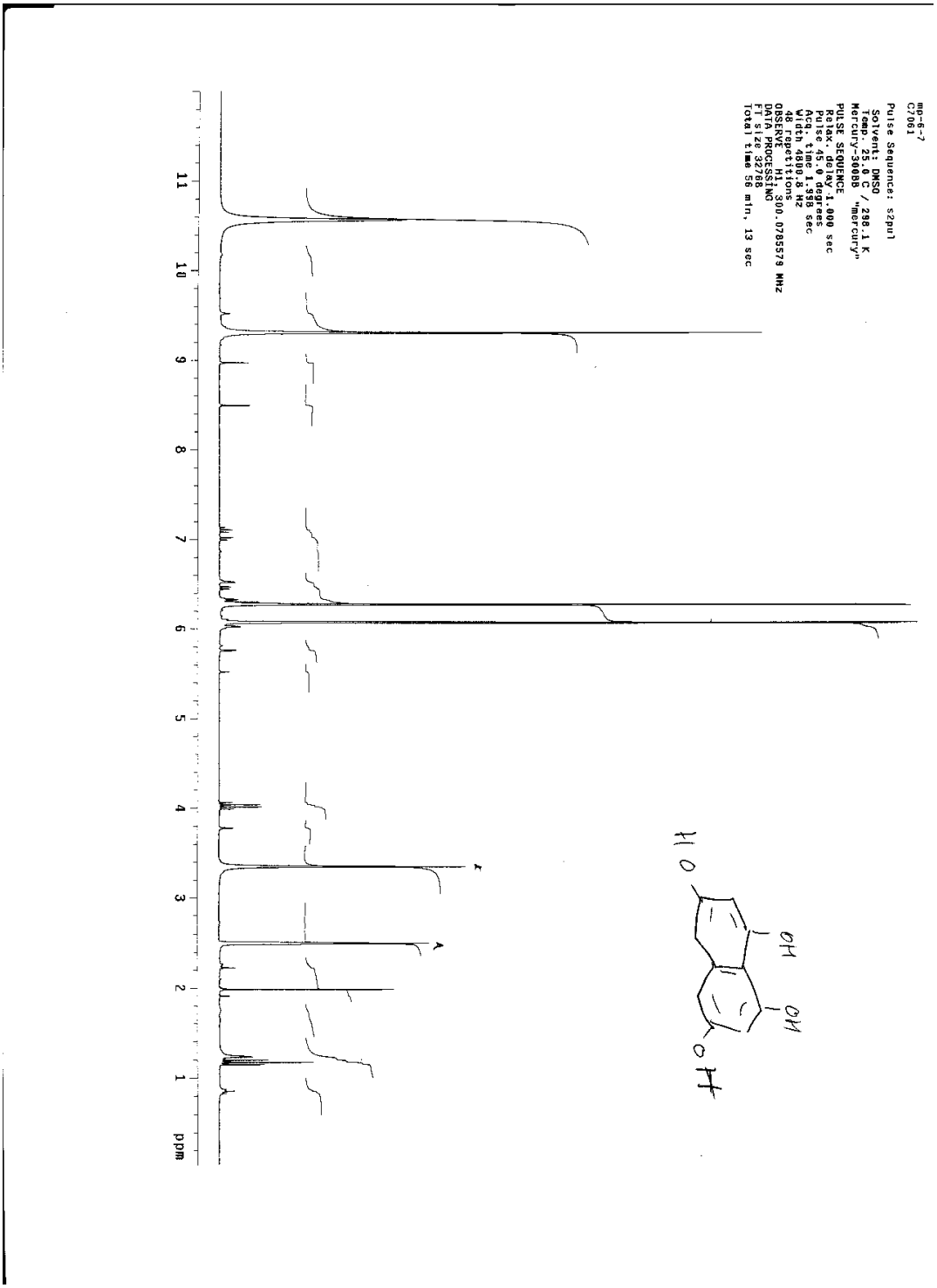
Fmoc-Tyr(OtBu)Gly-Gly-Phe-Leu-OtBu

Cleavage studies of Fmoc-Phe-Ala-OH:

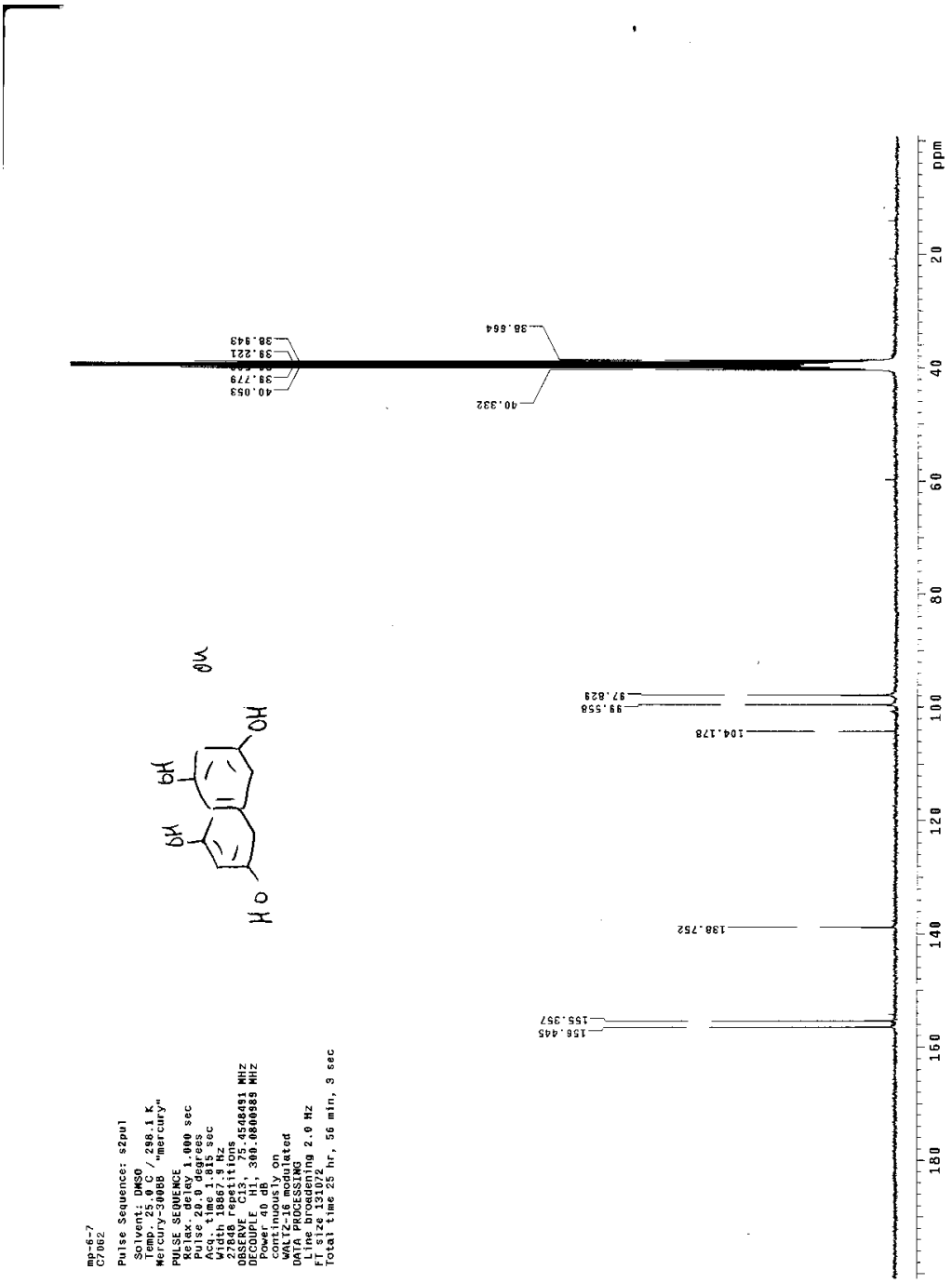
Portions of peptidyl-resin (approximately 5 mg) were placed in filter syringes and subjected to acidolytic cleavage for the time stated in Table 1 by 0.5 mL of a premixed cleavage mixture as stated in Table 1. After collection of the supernatant from the resin and removal of the solvents *in vacuo* the residual material were dissolved in a 1:1 acetonitrile-H_2O mixture (0.5 mL) and analysed by HPLC-MS at 220 nm. The cleavage yields were determined from the HPLC areas of released Fmoc-Phe-Leu-OH in comparison with a Fmoc-Phe-OH standard curve having an initial concentration of 0.25 mg/mL Fmoc-Phe-OH in acetonitrile-H_2O (1:1). The results are shown in Table 1 in the main manuscript.

NMR Spectra.

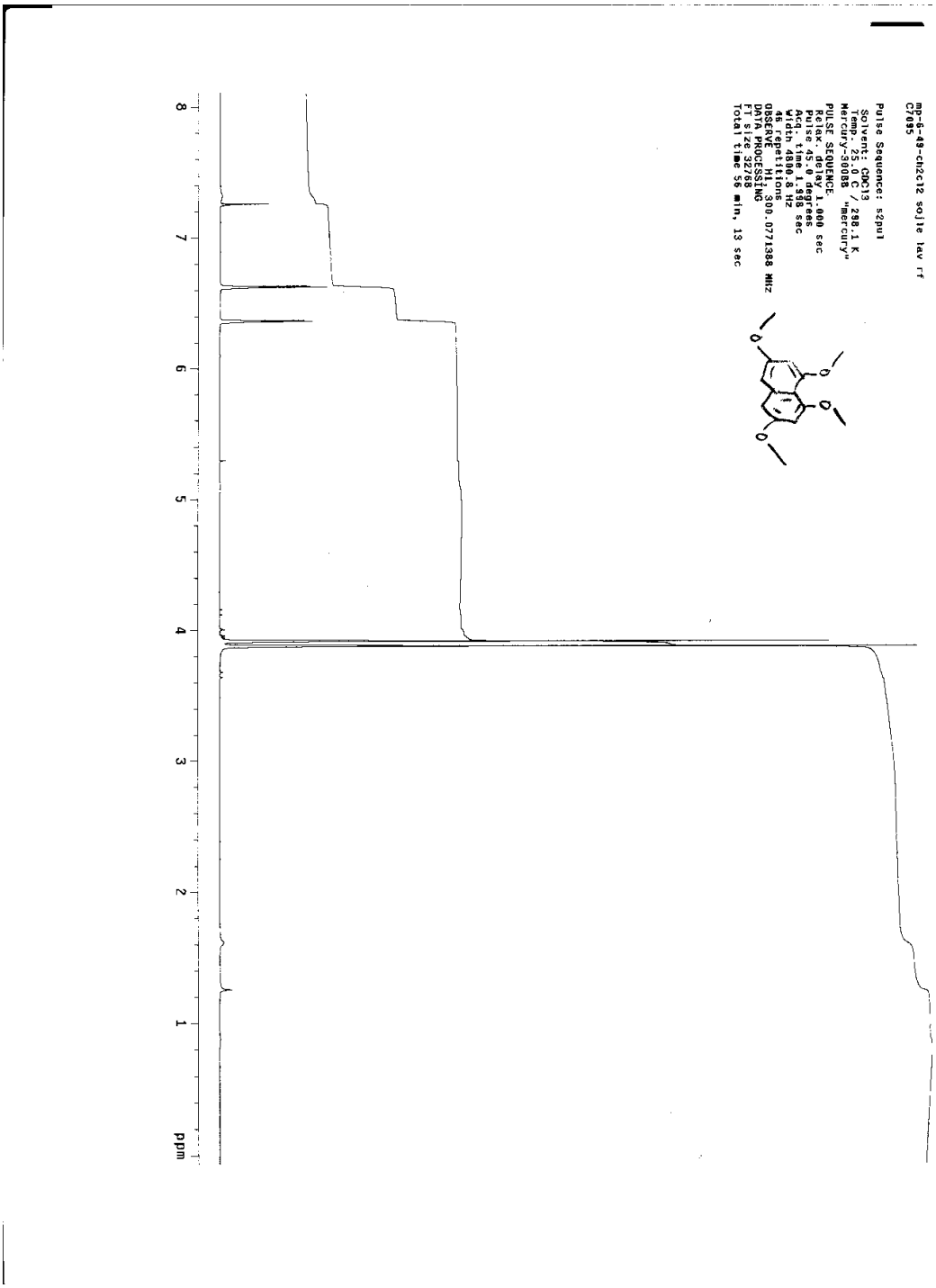
Compound **1**: 1H NMR



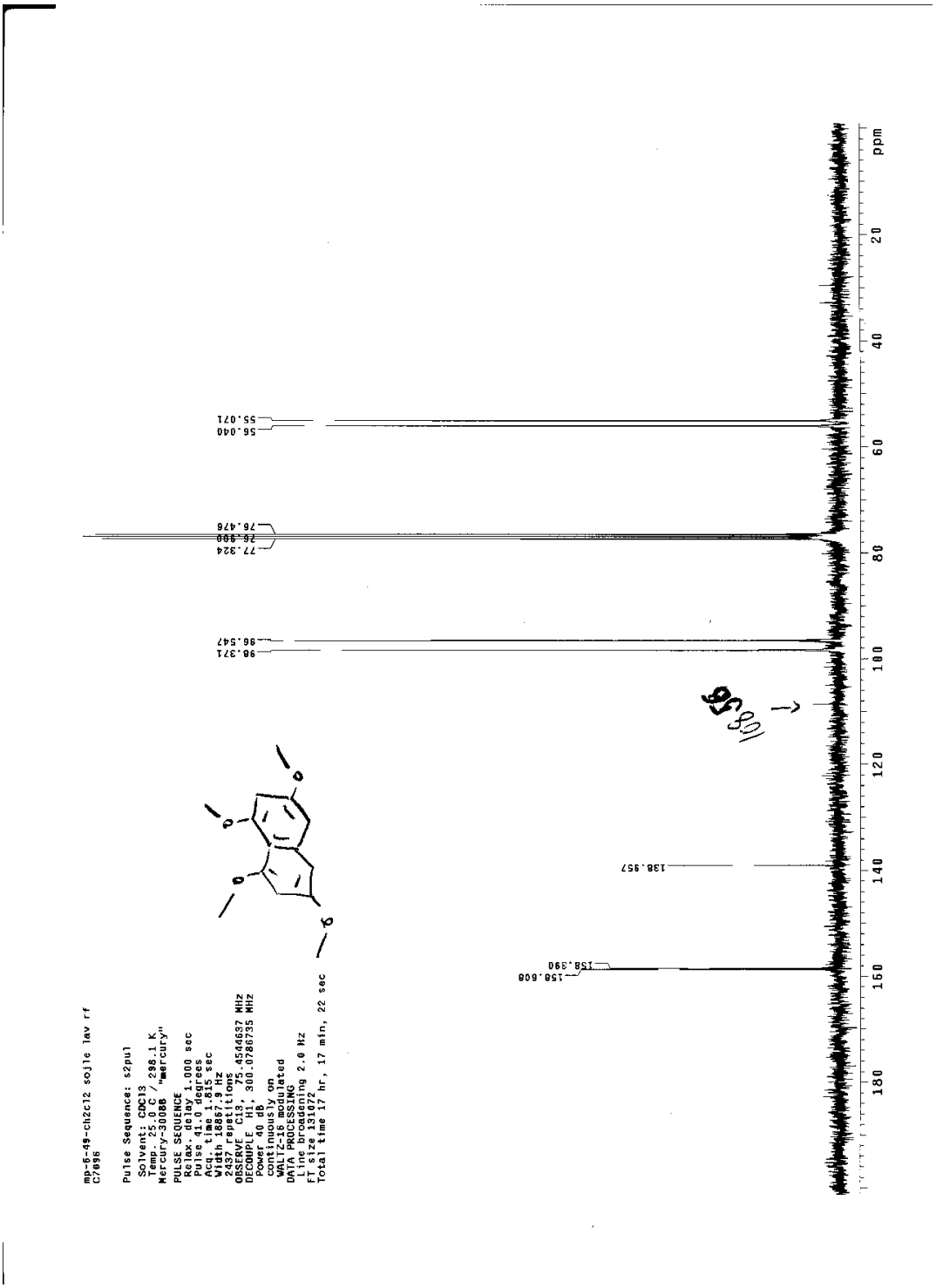
Compound **1**: 13C NMR



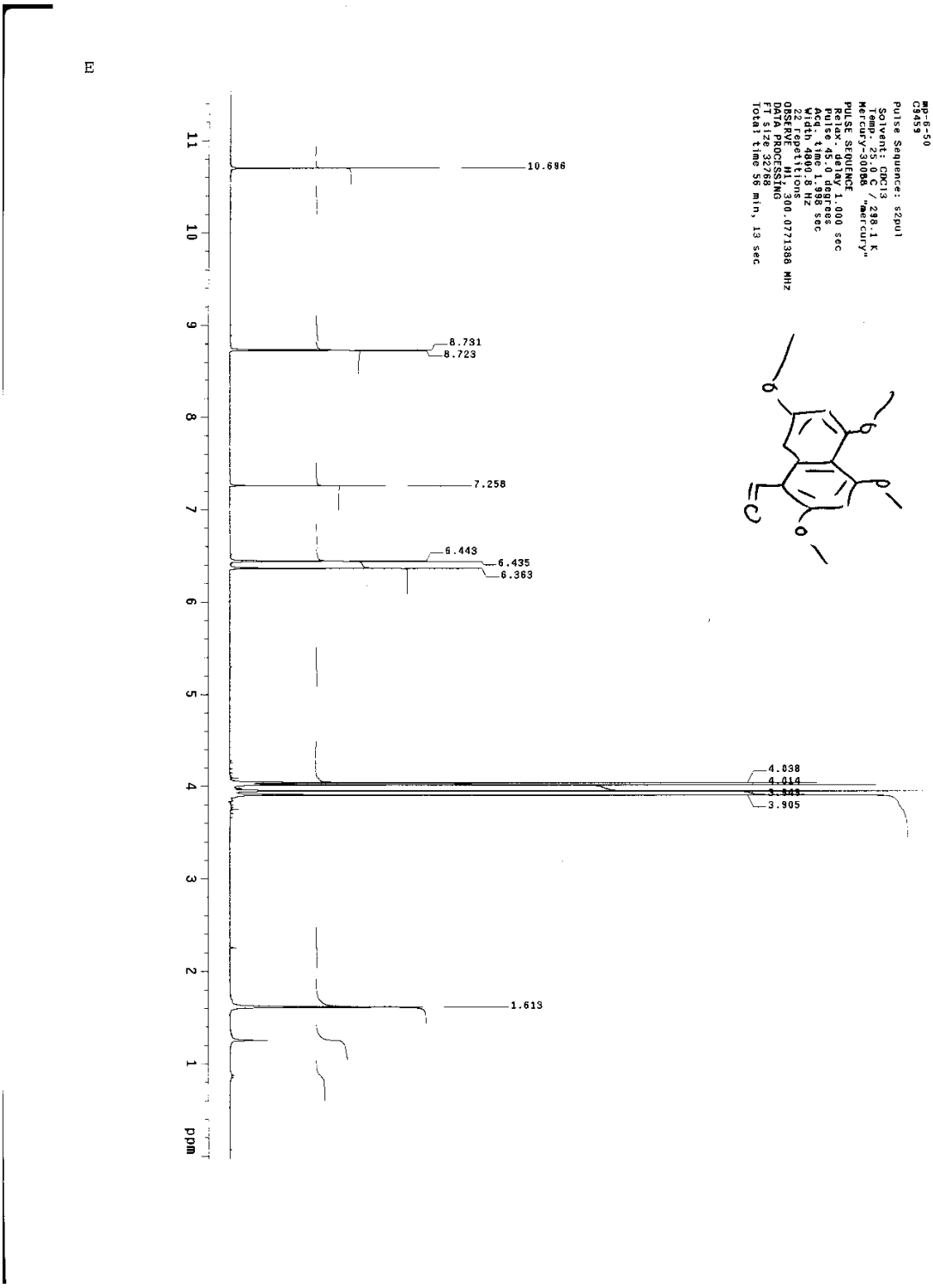
Compound **2**: 1H NMR



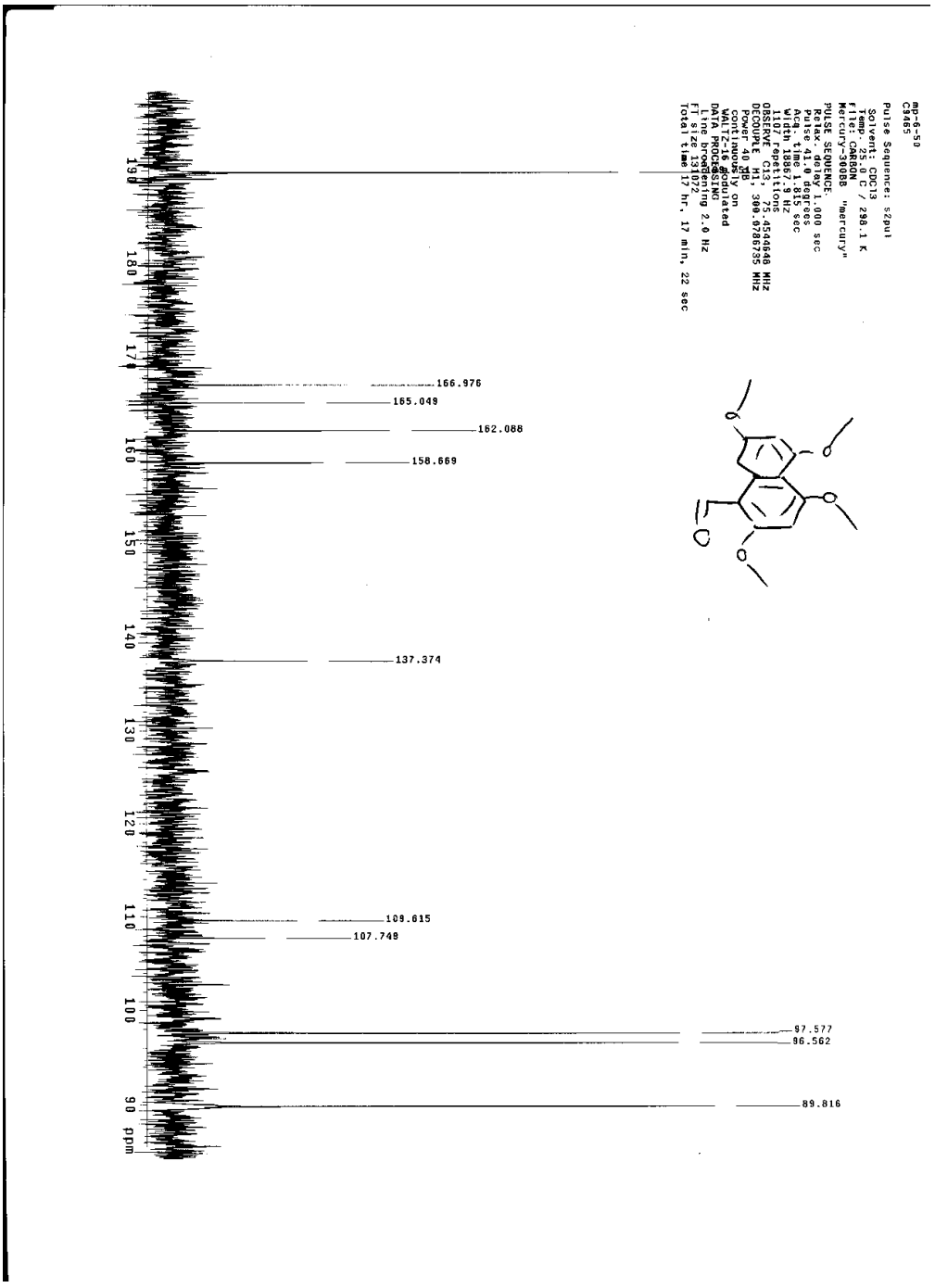
Compound **2**: 13C NMR



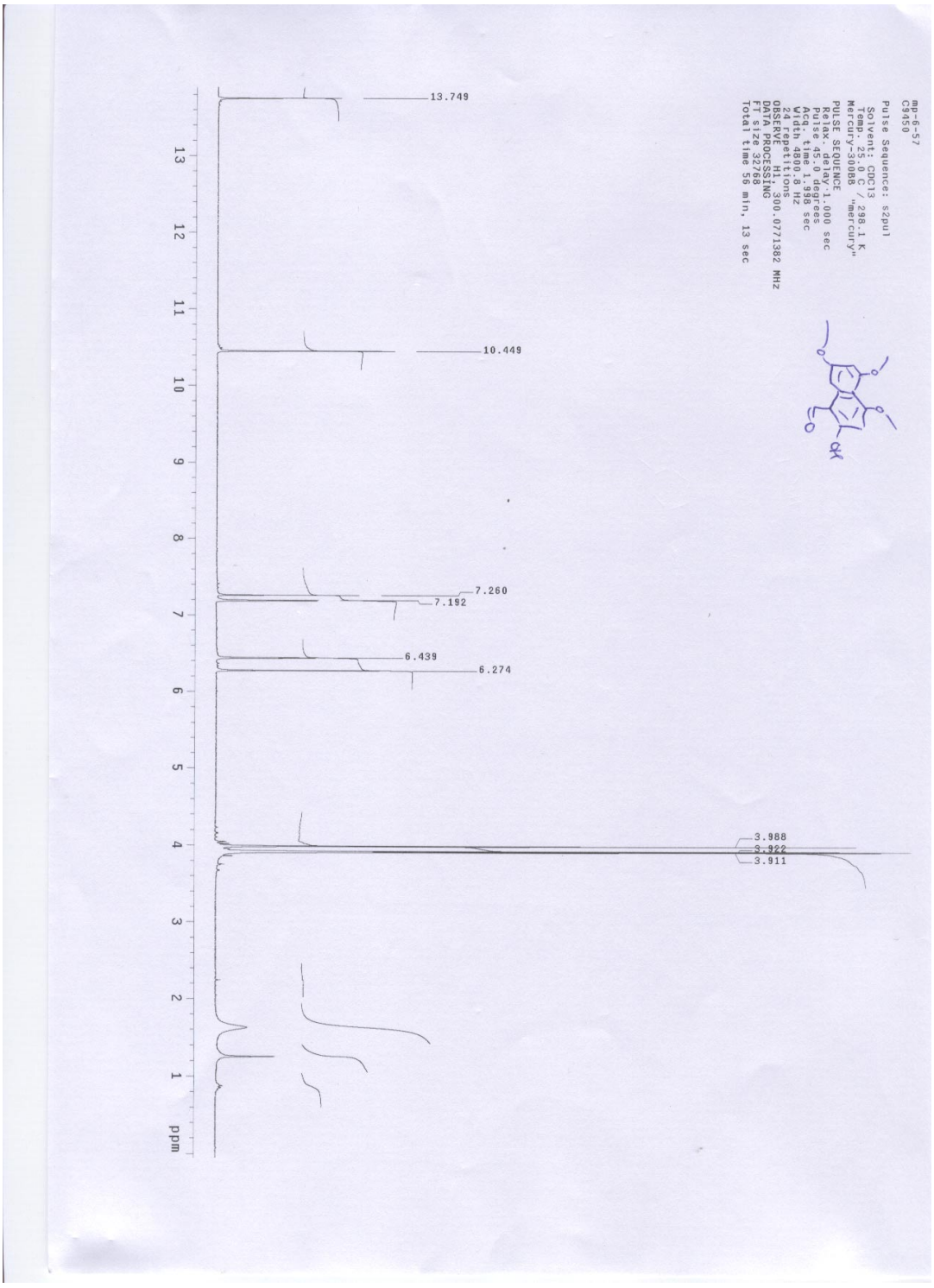
Compound **3**: 1H NMR



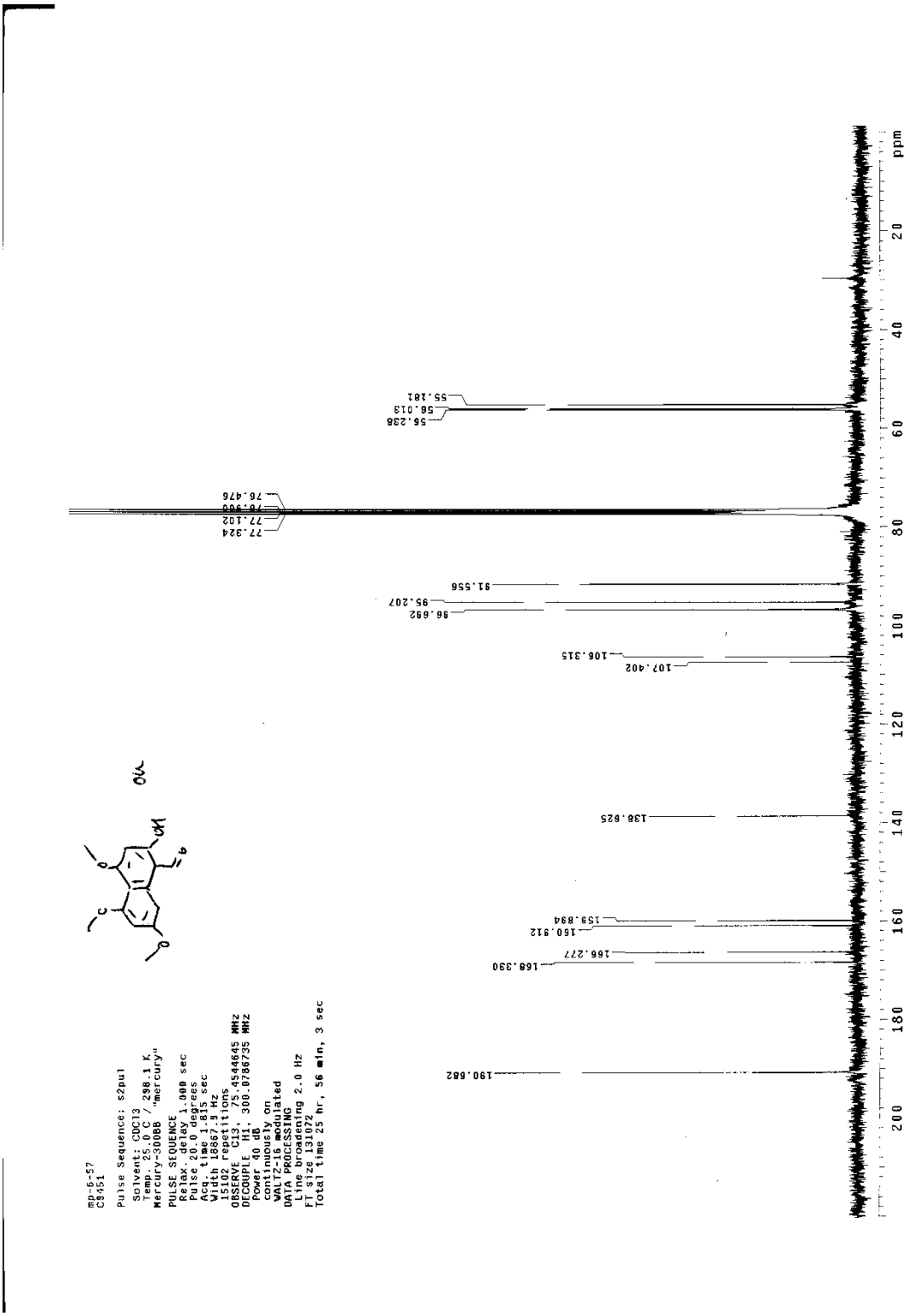
Compound **3**: 13C NMR



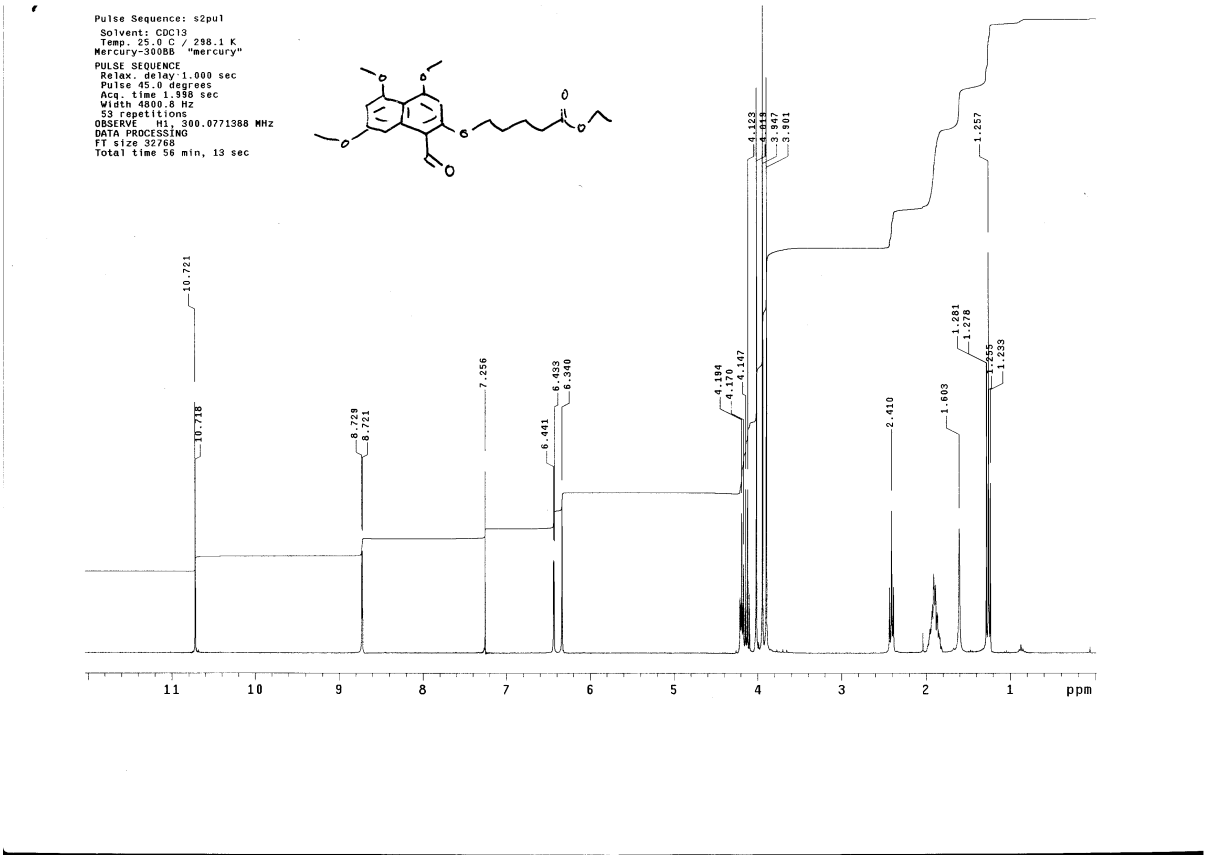
Compound **4**: 1H NMR



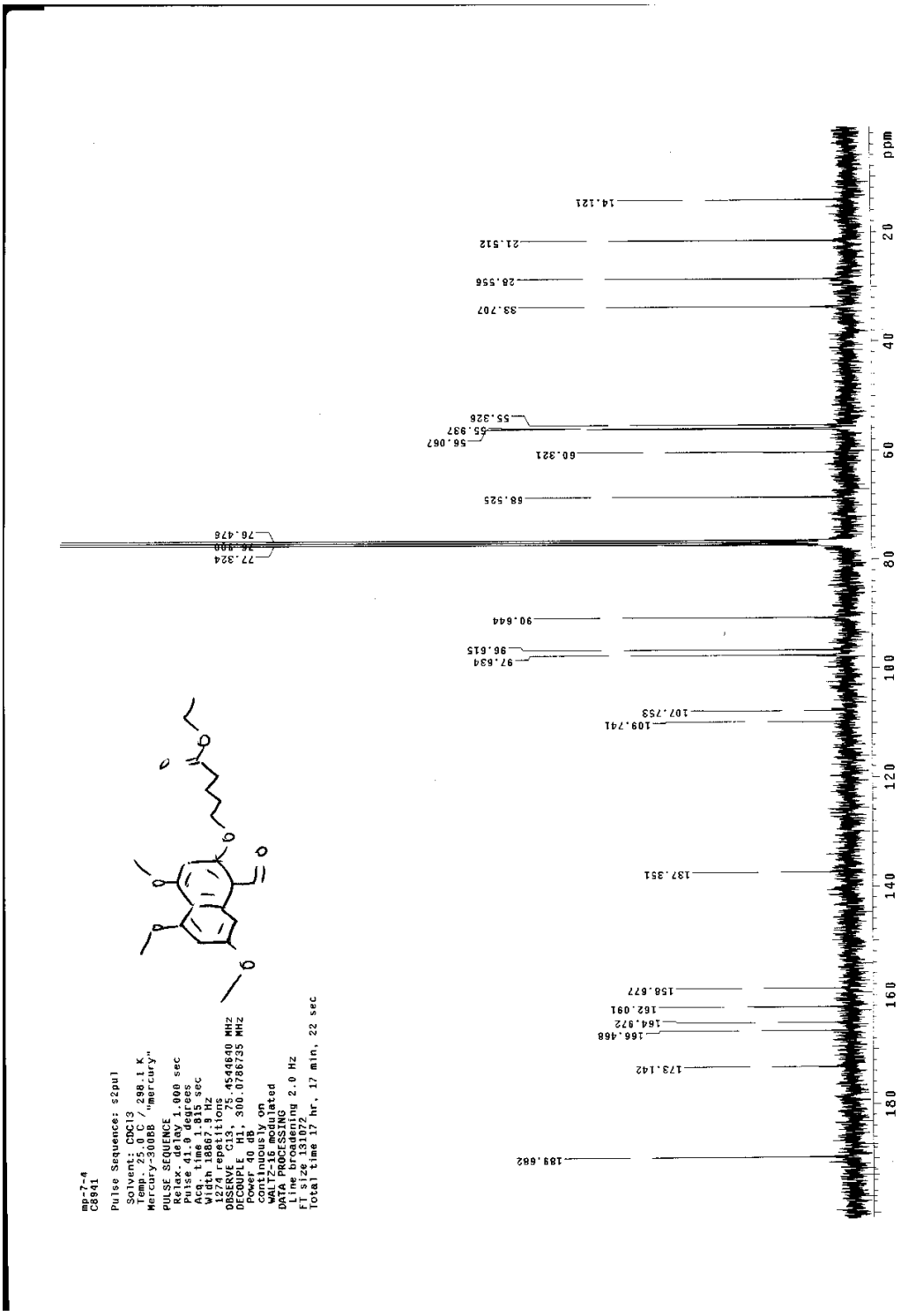
Compound **4**: 13C NMR



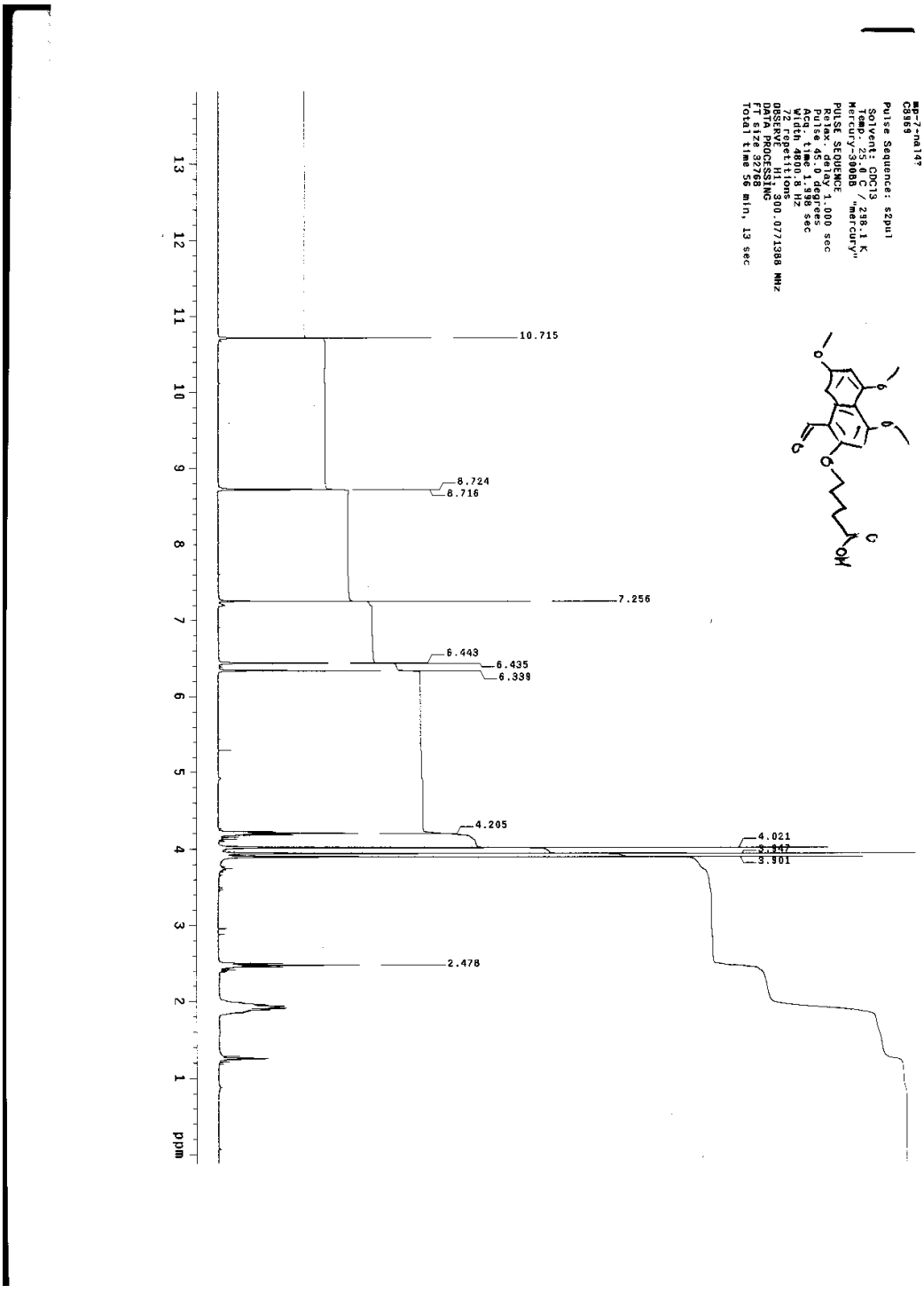
Compound **5**: 1H NMR



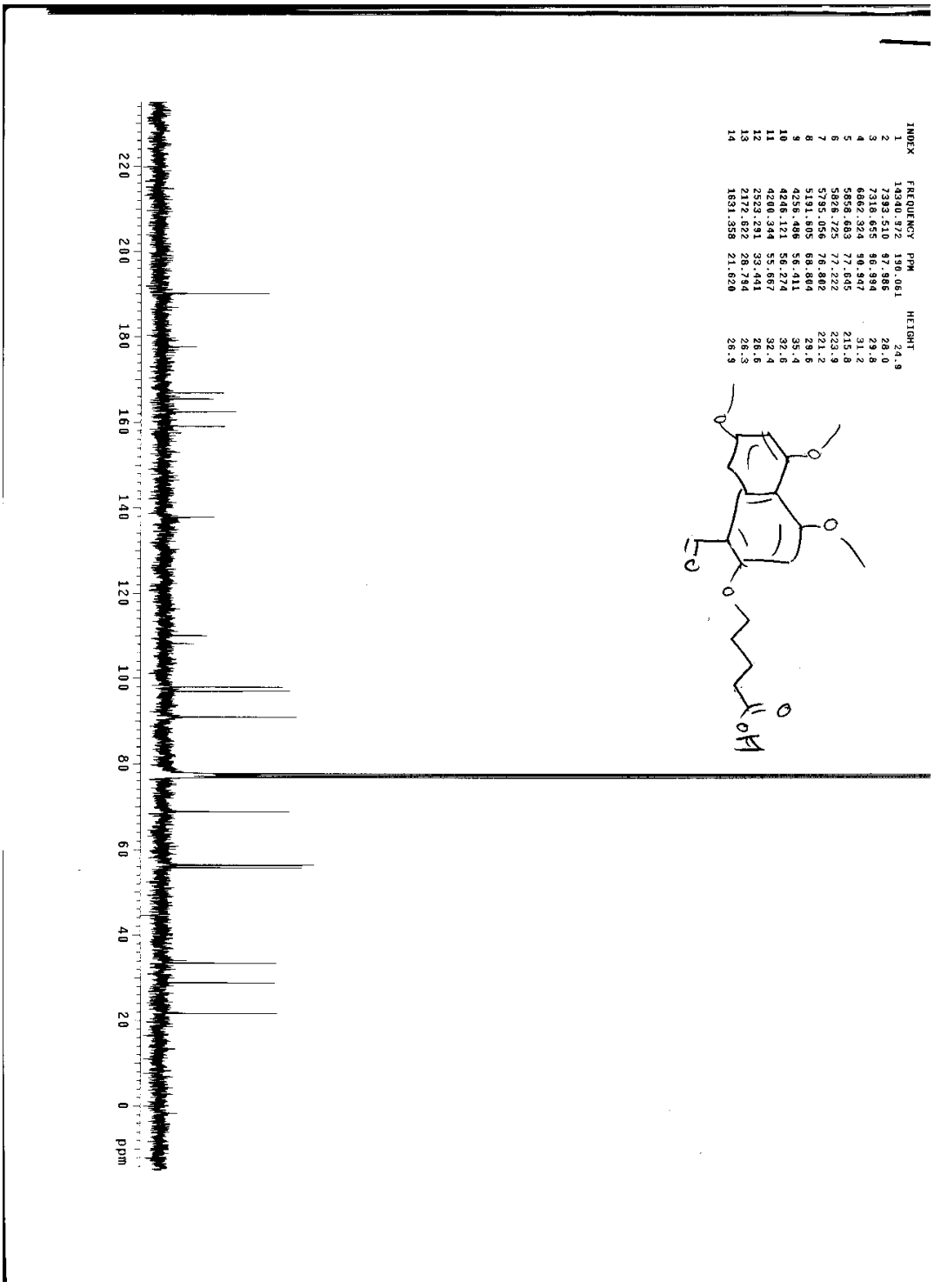
Compound **5**: 13C NMR



Compound **6**: 1H NMR



Compound **6**: 13C NMR



References:

(1) Pedersen, D. S.; Rosenbohn, C.; *Synthesis* 2001, *16*, 2431.

(2) Our procedure for the synthesis of **1** is similar to: Tanaka, H.; Ohne, Y.;. Ogawa, N; Tamura, T. *Agr. Biol. Chem.* 1963, *27*, 48.

(3) For a recent and different strategy to **2**: McCulloch, M. W. B.; Barrow, R. A. *Tetrahedron Lett.* 2005, *46*, 7619.

(4) Boas, U.; Gertz, H., Christensen, J. B.; Heegaard, P. M. H. *Tetrahedron Lett.* 2004, *45*, 269.