Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

# Supporting Information

# Hydrosoluble and Solvatochromic Naphthalene Diimides with NIR Absorption.

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**Supplementary Figures, Table and Data:**

## Experimental Section

**General Methods.** All commercials were used as received without further purification. $^{1}H$ and $^{13}C$ NMR spectra were collected on a 200 or 300 MHz spectrometer. UV−vis and fluorescence spectra were registered on a UV or fluorescence spectrophotometer, respectively. Mass spectra were performed on ESI-MS. Elemental analyses were performed by investigation of C, H, N. HPLC analysis and purifications were performed using two different HPLCs for preparative and analytical purposes. The analytical columns were: HSS C18 (40 x 4.6 mm) for method A; CSH Phenyl-Hexyl (150 x 4.6 mm) for method B. The preparative column was CSH Prep Phenyl-Hexyl 5µm (150 x 30 mm). Flows were 1.4 ml/min for analytical method A, 1.0 ml/min for analytical method B and 27 ml/min for preparative. The analytical methods used were: method A (Aqueous solvent: 0.1% trifluoroacetic acid in water; organic solvent: ACN; gradient: 95% aqueous, gradually to 40% aqueous over 8 minutes and at the end an isocratic flow over 4 minutes); method B (Aqueous solvent: 0.1% trifluoroacetic acid in water; organic solvent: ACN; gradient: 95% aqueous, gradually to 20% aqueous over 14 minutes and at the end an isocratic flow over 2 minutes). Preparative HPLC was performed using method C (Aqueous solvent: 0.1% trifluoroacetic acid in water; organic solvent: ACN; gradient: 95% aqueous, gradually to 20% aqueous over 28 minutes and at the end an isocratic flow over 4 minutes).

### Synthesis of the ethynyl-aryl building block 11-15.

The intermediates **9**, **12** and **13** were synthesized according to the literature procedures starting respectively from the commercially available 4-iodophenol and 5-bromo2-hydroxybenzaldehyde.[S1,S2]

**4-((trimethylsilyl)ethynyl)phenyl acetate (10).** Acetyliodophenol **9** (300 mg, 1.14 mmol) was dissolved in 10 mL of dry Toluene. To the resulting solution (24 mg, 0.034 mmol) of $PdCl_2(PPh_3)_2$, (22 mg, 0.114 mmol) of Copper Iodide, (0.4 mL, 1.14 mmol) of tert-butylamine and (161µL, 1.14 mmol) of trimethylsilylacetylene were subsequently added. The reaction was stirred at 40°C for 24h under argon atmosphere. Evaporation of the solvent yielded a crude product, which was purified by column chromatography (AcOEt: Hexane = 1:9) affording the product **10** as yellow oil (238 mg, 90% yield). Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 2.31 (s, 3H), 0.27 (s, 9H). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 168.6, 150.2, 132.7, 121.1, 120.4, 103.8, 89.4, 20.6, -0.47. Anal Calcd for $C_{13}H_{16}O_2Si$: C, 67.20; H, 6.94. Found: C, 67.03; H, 7.01.

**2,2-dimethyl-6-((trimethylsilyl)ethynyl)benzo[1,3]dioxin (14). 13** (500 mg, 2.07 mmol) was dissolved in 20 mL of triethylamine. To the resulting solution (54 mg, 0.21 mmol) of $PPh_3$, (58 mg, 0.09 mmol) of $PdCl_2(PPh_3)_2$, (39 mg, 0.21 mmol) of Copper Iodide, and (437 μL, 3.10 mmol) of trimethylsilylacetylene were subsequently added. The reaction was heated to reflux under argon atmosphere for 10 hours. Evaporation of the solvent yielded a crude product, which was purified by column chromatography (AcOEt: Hexane = 1:9) affording the product **14** as yellow oil (441 mg, 82% yield). Yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 4.80 (s, 2H), 1.54 (s, 6H), 0.26 (s, 9H). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 151.2, 131.5, 128.1, 118.9, 116.7, 114.5, 104.6, 99.5, 92.0, 60.1, 26.5, 24.3, -0.38. Anal Calcd for $C_{15}H_{20}O_2Si$: C, 69.19; H, 7.74. Found: C, 69.31; H, 7.69.

**General Synthetic Procedure for the deprotection of the trimethylsilyl group. Synthesis of 11 and 15.**

TBAF sol. 1 M in THF (1.2 mmol,) was added slowly to a solution containing the trimethylsilyl(ethynyl)phenol derivative (1 mmol) dissolved in dry THF (15 mL) cooled at 0°C. The resulting solution was stirred at this temperature for 30 minutes. The reaction was quenched by addition of sat. aq. NH4Cl and the mixture extracted with $CHCl_3$. The organic layer was washed with water (x1) and brine (x1), dried over sodium sulphate and evaporated under vacuum, to give the corresponding ethynylphenol as brown oil (99% yield).

**4-ethynylphenyl acetate (11).** Brown oil (158 mg, 99%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 3.08 (s, 1H), 2.31 (s, 3H). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 168.8, 150.4, 132.9, 121.3, 119.4, 115.1, 82.4, 20.7. Anal Calcd for $C_{10}H_8O_2$: C, 74.99; H, 5.03. Found: C, 75.01; H, 5.11.

**6-ethynyl-2,2-dimethylbenzo[1,3]dioxin (15).** Brown oil (186 mg, 99%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.8 Hz, 1H), 7.15 (s, 1H); 6.78 (d, *J* = 8.8 Hz, 1H), 4.83 (s, 2H), 3.00 (s, 1H), 1.56 (s, 6H). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 151.4, 131.7, 128.3, 119.1, 116.8, 113.4, 99.6, 83.1, 60.1, 24.3. Anal Calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.67; H, 6.41.

The compounds **16**, **17** and the 2,6-dibromo-naphthalenetetracarboxylic dianhydride were synthesized according to the literature procedures.[13,20]

### General Synthetic Procedure for the NDIs-Sonogashira Coupling Reaction. Synthesis of 18-21.

Under an argon atmosphere the NDIs mixture **16**, **17** (0.530 mmol), is suspended in 30 ml of dry THF. In this sequential order: $Pd(PPh_3)_4$ (30 mg, 0.026 mmol), CuI (4.9 mg, 0.026 mmol) and triethylamine (30 ml) were added. 1.06 mmol fo the 4-ethynyl-aryl derivative (**11** or **15**) were added and the solution was stirred at 40°C for 1.5h. After this period the resulting dark-brown solution was dried under vacuum and the crude residue was purified by chromatographic separation (Chloroform: methanol = 95:5) afferding **18**, **19** (108 mg, 36%; 188 mg, 49% yields) and **20**, **21** (88.5 mg, 28%; 252 mg, 61% yields), starting from the ethynyl-aryl **11** and **15** respectively.

**N,N'-Bis-[(dimethylamino)ethylamino]-2-[2-(4-phenyl-acetate)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide (18):** Green solid (108 mg, 36%); m.p. dec > 350°C. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.83-8.71 (m, 3H) 7.29 (d, *J* = 8.6 Hz, 2H), 7.21 (d, *J* = 8.6 Hz, 2H), 4.43-4.36 (m, 4H), 2.78-2.72 (m, 4H), 2.42 (s, 6H), 2.38 (s, 6H), 2.36 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 168.8, 162.7, 162.4, 161.5, 151.7, 136.6, 134.3, 133.8, 131.3, 130.5, 127.5, 127.3, 126.3, 126.1, 125.5, 125.4, 121.9, 119.9, 115.2, 101.9, 89.4, 56.7, 45.6, 38.4, 21.1. Anal Calcd for $C_{32}H_{30}N_4O_6$: C, 67.83; H, 5.34; N, 9.89. Found: C, 67.88; H, 5.22; N, 9.91.

**N,N'-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(4-phenyl-acetate)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide (19):** Orange solid (188 mg, 49%); m.p. dec > 350°C. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.87 (s, 2H) 7.72 (d, *J* = 8.7 Hz, 4H), 7.21 (d, *J* = 8.7 Hz, 4H), 4.41 (t, *J* = 6.8 Hz, 4H), 2.75 (t, *J* = 6.8 Hz, 4H), 2.42 (s, 12H), 2.36 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 168.8, 161.9, 161.4, 137.0, 133.8, 126.9, 126.4, 125.3, 125.0, 121.9, 120.0, 102.0, 89.5, 56.7, 45.6, 38.5, 21.1. Anal Calcd for $C_{42}H_{36}N_4O_8$: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.49; H, 5.12; N, 7.68.

**(20):** Orange solid (88.5 mg, 28%); m.p. dec > 350°C. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.73-8.63 (m, 3H) 7.54-7.51 (m, 1H), 7.38 (s, 1H), 6.88-6.84 (m, 1H), 4.90 (s, 2H), 4.31-4.30 (m, 4H), 2.76-2.68 (m, 4H), 2.41 (s, 6H), 2.37 (s, 6H), 1.58 (s, 6H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 162.7, 162.4, 161.5, 160.6, 153.0, 138.8, 136.5, 132.8, 131.1, 130.1, 129.5, 128.1, 127.7, 127.4, 126.2, 126.0, 125.2, 124.7, 119.8, 117.6, 113.9, 103.7, 100.2, 89.0, 60.4, 56.7, 47.9, 45.6, 38.4, 24.7, 19.1. Anal Calcd for $C_{34}H_{34}N_4O_6$: C, 68.67; H, 5.76; N, 9.42. Found: C, 68.61; H, 5.80; N, 9.38.

**(21):** Violet solid (252 mg, 49%); m.p. dec > 350°C. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 2H) 7.56 (d, *J* = 8.4 Hz, 2H), 7.41 (s, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 4.94 (s, 4H), 4.39 (t, *J* = 6.7 Hz, 4H), 2.74 (t, *J* = 6.7 Hz, 4H), 2.41 (s, 12H), 1.59 (s, 12H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 162.0, 161.5, 153.0, 136.9, 132.8, 129.4, 127.0, 126.2, 124.8, 124.5, 119.8, 117.6, 114.0, 103.6, 100.2, 89.0, 60.5, 56.5, 45.4, 38.3, 29.6, 24.7. Anal Calcd for $C_{46}H_{44}N_4O_8$: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.81; H, 5.64; N, 7.22.

**General Synthetic Procedure for the deprotection of the acetyl group. Synthesis of 1 and 5.**

The acetyl-phenol NDI (**18** or **19**, 1 mmol) was dissolved in a mixture of MeOH/$H_2O$ 4:1 (80:20 ml). $K_2CO_3$ (1.68 g, 12 mmol) was added at the solution and the suspension was stirred at room temperature for 1h. The solution was poured in 50 ml of water and the methanol was eliminated under vacuum. The aqueous solution was acidified with HCl 10% and extracted with $CHCl_3$ (3x250 ml). The organic phases were collected and dried over $Na_2SO_4$. The solvent was removed under reduced pressure to yield the crude product, subsequently purified by preparative HPLC chromatography, using a C-18 reverse phase column, ($CH_3CN$:$H_2O$ 0.1%TFA) according to method C. HCl 1 M solution was added to each chromatographic portion. Solvent evaporation under vacuum afforded the adducts **1** (387.5 mg, 65% yield), and **5** (427.3 mg, 60%) as hydrochlorides.

**N,N'-Bis-[(dimethylamino)ethylamino]-2-[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl (1):** Red solid (387.5 mg, 65%); m.p. dec > 350°C. $^1H$ NMR (300 MHz, DMSO-d$_6$) δ 10.46 (s, 1H), 8.72-8.57 (m, 3H) 7.62 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 4.43 (bs, 4H), 3.49 (bs, 4H), 2.93 (s, 12H). $^{13}C$ NMR (75 MHz, DMSO) δ 163.2, 163.1, 162.8, 161.9, 159.8, 134.8, 134.3, 130.8, 130.3, 129.8, 127.3, 126.6, 126.5, 126.3, 126.2, 126.1, 126.0, 125.1, 124.8, 116.2, 111.8, 103.2, 88.9, 57.5, 45.2, 35.5. Anal Calcd for $C_{30}H_{30}Cl_2N_4O_5$: C, 60.31; H, 5.06; N, 9.38. Found: C, 60.49; H, 5.12; N, 9.41.

**N,N'-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl (5):** Violet solid (427.3 mg, 60%); m.p. dec > 350°C. $^1H$ NMR (300 MHz, $CD_3OD$) δ 8.80 (s, 2H) 7.62 (d, *J* = 8.7 Hz, 4H), 6.89 (d, *J* = 8.7 Hz, 4H), 4.64-4.62 (m, 4H), 3.66-3.60 (m, 4H), 3.10 (s, 12H). Anal Calcd for $C_{38}H_{34}Cl_2N_4O_6$: C, 63.96; H, 4.80; N, 7.85. Found: C, 64.01; H, 4.82; N, 7.81.

**General Synthetic Procedure for the deprotection of the isopropilidene acetals. Synthesis of 2 and 6.**

The NDI (**20**, **21**, 1.7 mmol) was dissolved in 20 ml of THF. HCl (1.5 ml, 10%) was added at the solution and the suspension was stirred at room temperature for 30 min. The reaction was quenched with 20 ml of a saturated solution of $NaHCO_3$, then the THF was eliminated under vacuum and the aqueous solution was neutralized and extracted with $CHCl_3$ (3 x 250 ml). The organic phases were collected and dried over $Na_2SO_4$. The solvent was removed under pressure to yield the crude product, subsequently purified by preparative HPLC chromatography, using a C-18 reverse phase column, ($CH_3CN$:$H_2O$ 0.1%TFA) according to method C. HCl 1 M solution was added to each chromatographic portion. Solvent evaporation under vacuum afforded the adducts **2** (926 mg, 87% yield), and **6** (945 mg, 72%) as hydrochlorides.

**N,N'-Bis-[(dimethylamino)ethylamino]-2-[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl (2):** Red solid (926 mg, 87%); m.p. dec > 350°C. $^1$H NMR (300 MHz, DMSO) δ 10.68 (bs, 1H), 10.49 (bs, 2H), 8.72-8.63 (m, 2H) 8.58 (s, 1H), 7.72 (s, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 4.52 (s, 2H), 4.47-4.43 (m, 4H), 3.48 (bs, 4H), 2.90 (s, 6H), 2.86 (s, 6H). $^{13}$C NMR (DMSO) δ 163.1, 162.8, 161.9, 158.2, 156.5, 134.9, 132.3, 131.5, 130.8, 129.8, 129.7, 127.3, 126.5, 126.4, 126.1, 126.0, 125.1, 124.6, 115.2, 111.6, 103.9, 88.8, 57.6, 54.3, 54.2, 42.25, 35.5. Anal Calcd for $C_{31}H_{32}Cl_2N_4O_6$: C, 59.33; H, 5.14; N, 8.93. Found: C, 59.49; H, 5.12; N, 8.84.

**N,N'-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl (6):** Violet solid (945 mg, 72%); m.p. dec > 350°C. $^1$H NMR (300 MHz, DMSO $d_6$) δ 10.20 (s, 2H), 8.67 (s, 2H), 7.74 (s, 2H), 7.52 (d, *J* = 8.3 Hz, 2H), 6.93 (d, *J* = 8.3 Hz, 2H), 5.20 (bs, 2H), 4.53 (s, 4H), 4.45 (bs, 4H), 2.90 (bs, 4H), 2.50 (s, 12H). $^{13}$C NMR (75 MHz, DMSO) δ 162.2, 161.7, 156.5, 135.6, 132.4, 131.5, 129.8, 126.1, 125.8, 124.4, 120.7, 115.2, 111.7, 104.1, 88.7, 57.6, 54.9, 43.1, 30.7. Anal Calcd for $C_{40}H_{38}Cl_2N_4O_8$: C, 62.10; H, 4.95; N, 7.24. Found: C, 62.19; H, 4.88; N, 7.21.

**General protocol for exhaustive methylation of NDIs 1,2-5,6:**

The NDI purified as hydrochloride was dissolved in a $Na_2CO_3$ solution and extracted 3 times with $CH_2Cl_2$. The recovered organic layers were dried on $Na_2SO_4$ and the solvent evaporated under reduced pressure. The collected amine (1 mmol) was dissolved in 50 ml of $CH_3CN$ and 0.85 g (6 mmol) of $CH_3I$ were added. This suspension was stirred under nitrogen atmosphere for 12 h. After this period the solvent was removed under vacuum to give the quaternary ammonium salts **3**, **4**, **7** and **8** (99%, yield).

**N,N'-Bis-[(trimethylamino)ethylamino]-2-[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (3):** Orange solid (618 mg, 99%); m.p. dec > 350°C. $^1$H NMR (300 MHz, $CD_3CN$) δ 8.80-8.73 (m, 3H) 7.66 (d, *J* = 8.5 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 2H), 4.68-4.55 (m, 4H), 3.67-3.64 (m, 4H), 3.25-3.23 (s, 18H). $^{13}$C NMR (75 MHz, DMSO) δ 162.2, 161.5, 159.7, 134.9, 130.5, 126.5, 126.0, 125.9, 120.1, 117.8, 116.2, 111.8, 109.7, 103.4, 86.9, 52.5, 45.7, 42.6. Anal Calcd for $C_{32}H_{34}I_2N_4O_5$: C, 47.54; H, 4.24; N, 6.93. Found: C, 47.49; H, 4.20; N, 7.01.

**N,N'-Bis-[(trimethylamino)ethylamino]-2-[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (4):** Dark red solid (647.6 mg, 99%); m.p. dec > 350°C. $^1$H NMR (300 MHz, $CD_3OD$) δ 8.86-8.76 (m, 3H), 7.78 (s, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.75 (s, 2H), 4.71-4.60 (m, 4H), 3.58-3.37 (m, 4H), 3.09 (s, 9H), 3.06 (s, 9H). Anal Calcd for $C_{33}H_{36}I_2N_4O_6$: C, 47.27; H, 4.33; N, 6.68. Found: C, 47.32; H, 4.25; N, 6.71.

**N,N'-Bis-[(trimethylamino)ethylamino]-2,6-bis[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (7):** Violet solid (733 mg, 99%); m.p. dec > 350°C. $^1$H NMR (300 MHz, $CD_3OD$) δ 8.70 (s, 2H), 7.62 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 4.61 (bs, 4H), 3.62 (bs, 4H), 3.08 (s, 18H). $^{13}$C NMR (75 MHz, $CD_3OD$) δ 164.4, 163.8, 163.2, 161.5, 154.0, 137.9, 136.5, 136.0, 128.8, 126.7, 125.0, 123.8, 117.3, 114.8, 114.5, 96.4, 90.1, 57.5, 44.5, 31.0. Anal Calcd for $C_{40}H_{38}I_2N_4O_6$: C, 51.96; H, 4.14; N, 6.06. Found: C, 52.03; H, 4.12; N, 6.18.

**N,N'-Bis-[(trimethylamino)ethylamino]-2,6-bis[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (8):** Violet-blue solid (792 mg, 99%); m.p. dec > 350°C. $^1$H NMR (300 MHz, $CD_3OD$) δ 8.77 (s, 2H), 7.78 (s, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 6.88 (d, *J* = 8.3 Hz, 2H), 4.70-4.63 (m, 8H), 3.75-3.72 (m, 4H), 3.36 (s, 18H). $^{13}$C NMR (75 MHz, $CD_3OD$) δ 164.4, 163.8, 163.4, 162.5, 154.8, 138.2, 137.9, 134.4, 133.9, 130.4, 129.9, 129.2, 128.9, 128.2, 126.8, 126.2, 125.6, 118.5, 116.5, 114.4, 101.0, 96.3, 60.5, 54.3, 44.6 32.7. Anal Calcd for $C_{42}H_{42}I_2N_4O_8$: C, 51.23; H, 4.30; N, 5.69. Found: C, 51.29; H, 4.22; N, 5.71.

**Spectrophotometric and Potentiometric titrations.** Spectrophotometric titrations were performed with a commercial spectrophotometer. The pH-metric titrations were carried out with a commercial titration system. All titrations were performed at 25.0 ± 0.1 °C. Protonation constants of ligand L were determined in a water mixture, 0.1 M in $NaNO_3$. In a typical experiment, 15 mL of a $5\times10^{-4}$ M ligand solution were treated with an excess of a 1.0 M $HNO_3$ standard solution. Titrations were run by addition of 10 μL aliquots of carbonate-free standard 0.1 M NaOH, recording 80-100 points for each titration. Prior to each potentiometric titration, the standard electrochemical potential (E°) of the glass electrode was determined in water, by a titration experiment according to the Gran method.[S3] Protonation  titration data (emf *vs.* mL of NaOH) were processed with the Hyperquad package,[S4] to determine the equilibrium constants.

# Absorption spectra

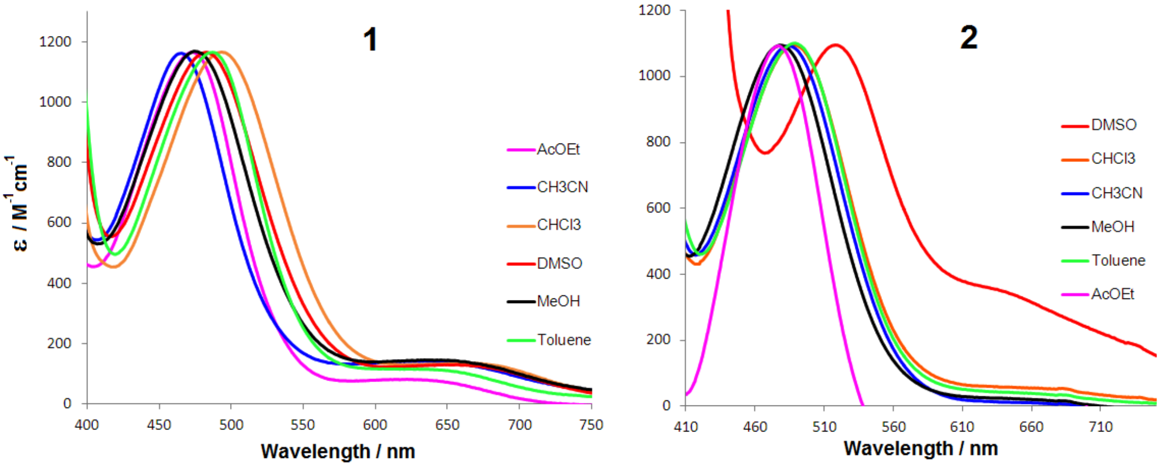


**Figure 1S:** Absorption spectra of the NDI **1** and **2**, measured in different organic solvents.

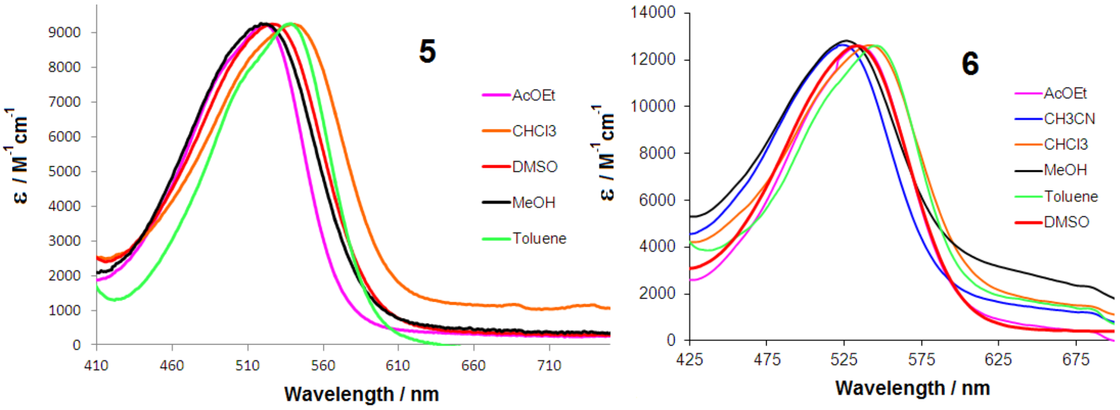


**Figure 2S:** Absorption spectra of the NDI **5** and **6**, measured in different organic solvents.

# Titration

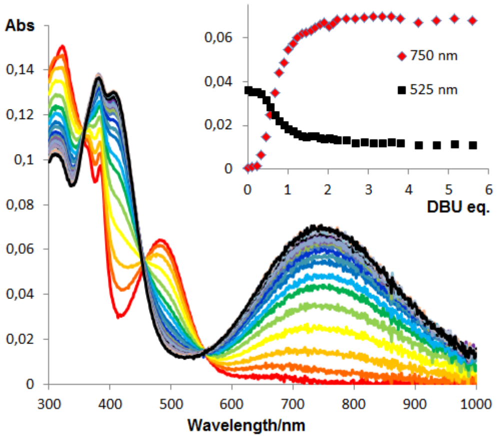


**Figure 3S.** Titration of **2** by DBU in DMSO. Inset: absorbance change monitored at 525, and 750 nm as a function of the BDU equivalents.

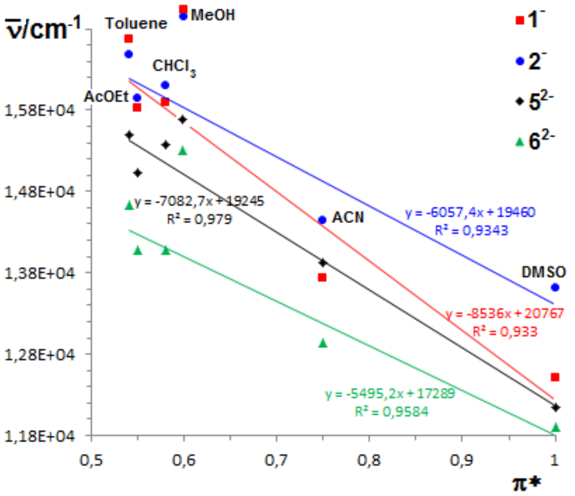


**Figure 4S.** LFER between the absorption maximum wavenumbers (ῡ/cm$^{-1}$) and the π* (Taft) polarity scale, for the NDIs **1**$^{-}$, **2**$^{-}$, **5**$^{2-}$ and **6**$^{2-}$.

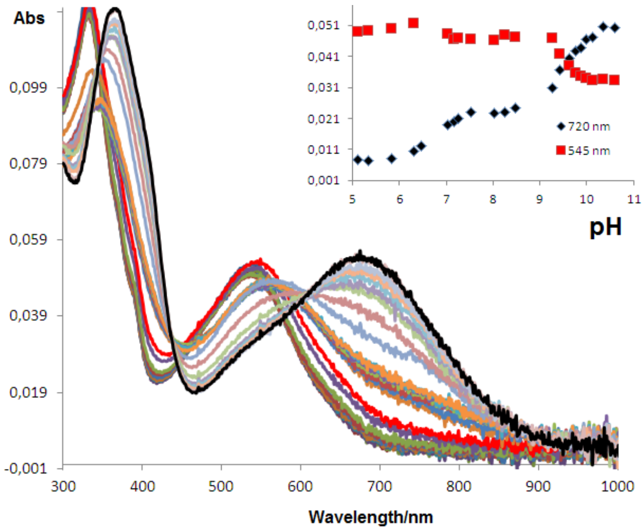


**Figure 5S.** Spectrophotometric titration of the mono-functional NDIs **2** in water.

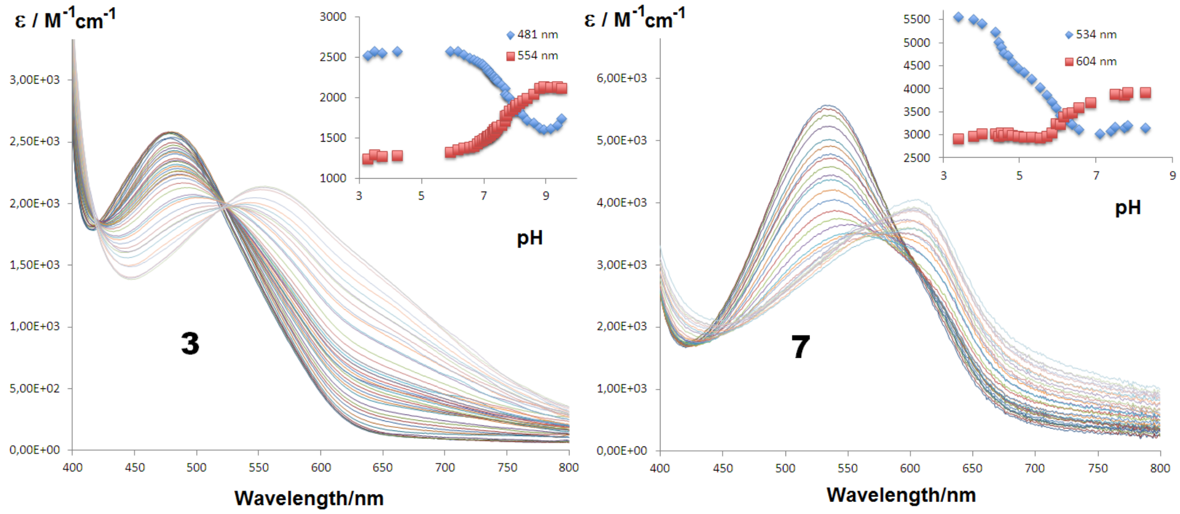


**Figure 6S:** Spectrophotometric titration of **3** and **7** in water.

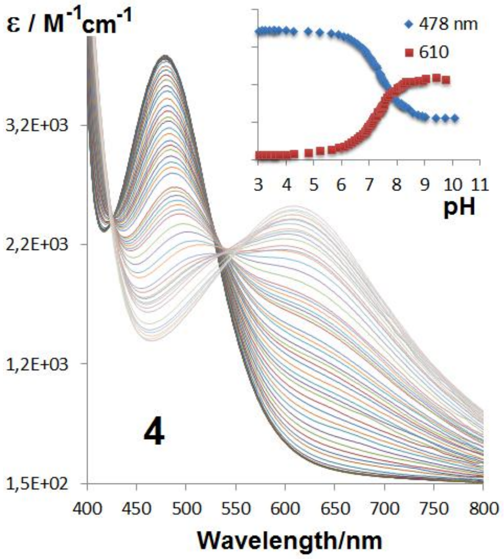


**Figure 7S:** Spectrophotometric titration of **4,** in water.

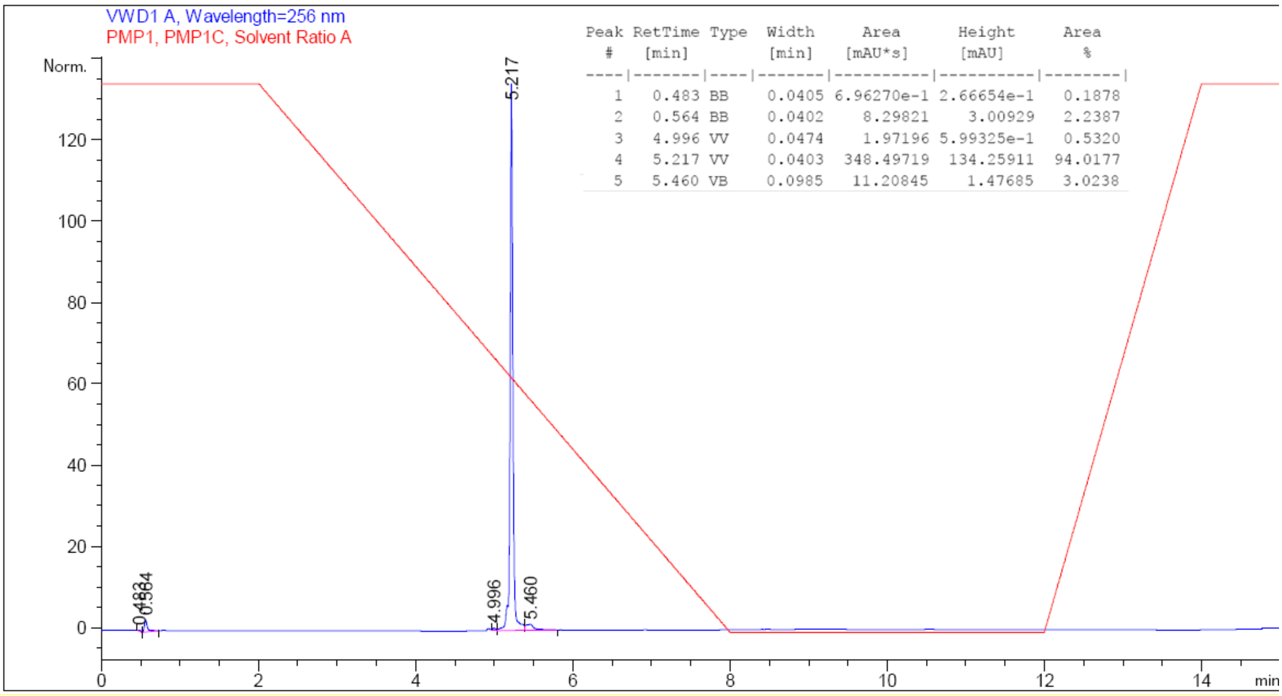
**Table 1S.** Wavelength of maximum absorbance ($\lambda_{max}$/nm) for the NDIs **4**, **8** and their conjugate bases **4**$^{-}$, **8**$^{-}$ and **8**$^{2-}$, in water.

| **$\lambda_{max}$/nm** | | | | |
|---|---|---|---|---|
| **4** | **4**$^{-}$ | **8** | **8**$^{-}$ | **8**$^{2-}$ |
| 478 | 610 | 538 | 629 | 704 |

# HPLC PURITY DATA:

## N,N’-Bis-[(dimethylamino)ethylamino]-2-[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl (2).

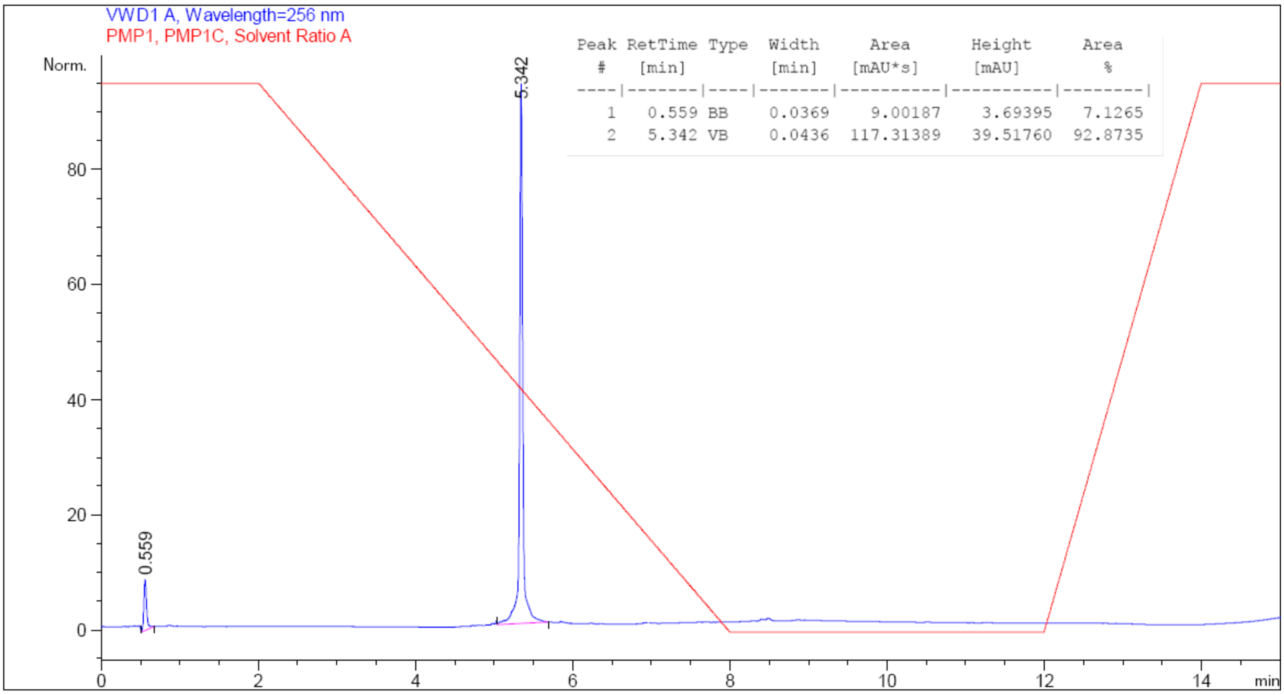
Method A



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   0.483 BB    0.0405 6.96270e-1 2.66654e-1   0.1878
   2   0.564 BB    0.0402    8.29821    3.00929   2.2387
   3   4.996 VV    0.0474    1.97196 5.99325e-1   0.5320
   4   5.217 VV    0.0403  348.49719  134.25911  94.0177
   5   5.460 VB    0.0985   11.20845    1.47685   3.0238
```

## N,N’-Bis-[(trimethylamino)ethylamino]-2-[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (3).

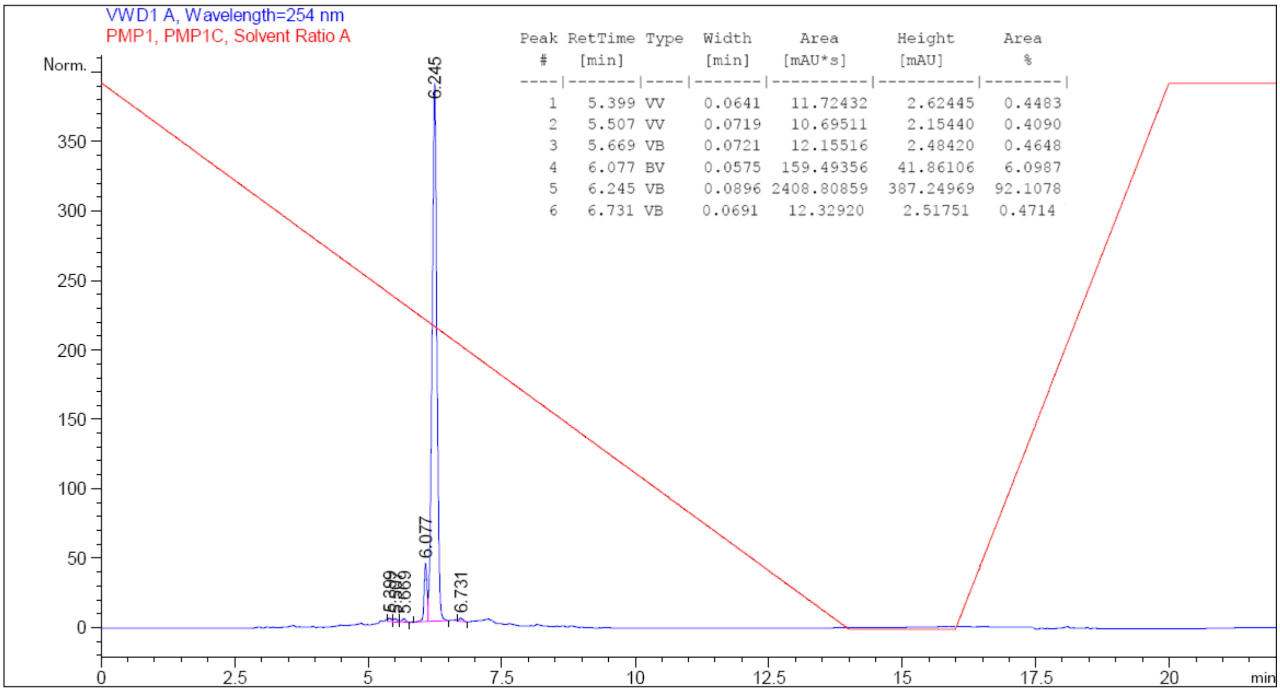
Method A



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   0.559 BB    0.0369    9.00187    3.69395   7.1265
   2   5.342 VB    0.0436  117.31389   39.51760  92.8735
```

## **N,N'-Bis-[(trimethylamino)ethylamino]-2-[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide** (4).

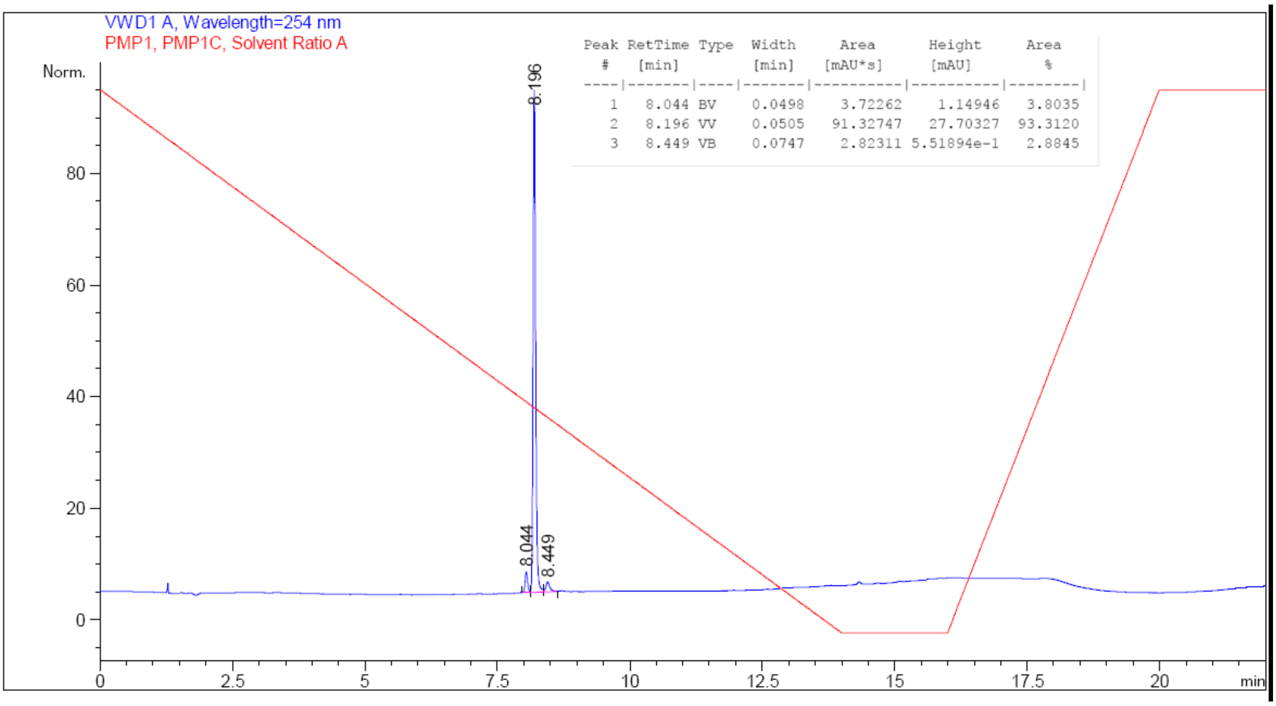
Method B



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   5.399 VV    0.0641   11.72432    2.62445   0.4483
   2   5.507 VV    0.0719   10.69511    2.15440   0.4090
   3   5.669 VB    0.0721   12.15516    2.48420   0.4648
   4   6.077 BV    0.0575  159.49356   41.86106   6.0987
   5   6.245 VB    0.0896 2408.80859  387.24969  92.1078
   6   6.731 VB    0.0691   12.32920    2.51751   0.4714
```

## **N,N'-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl** (5).

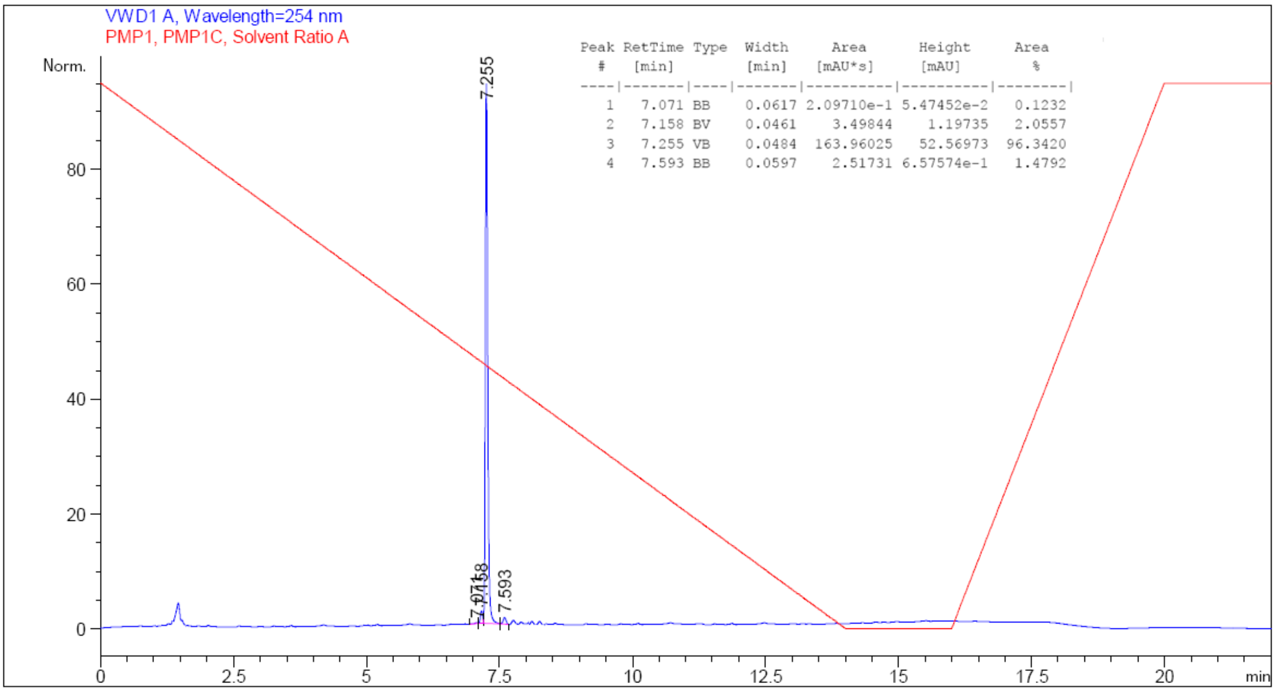
Method B



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   8.044 BV    0.0498    3.72262    1.14946   3.8035
   2   8.196 VV    0.0505   91.32747   27.70327  93.3120
   3   8.449 VB    0.0747    2.82311 5.51894e-1   2.8845
```

### **N,N’-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl** (**6**).

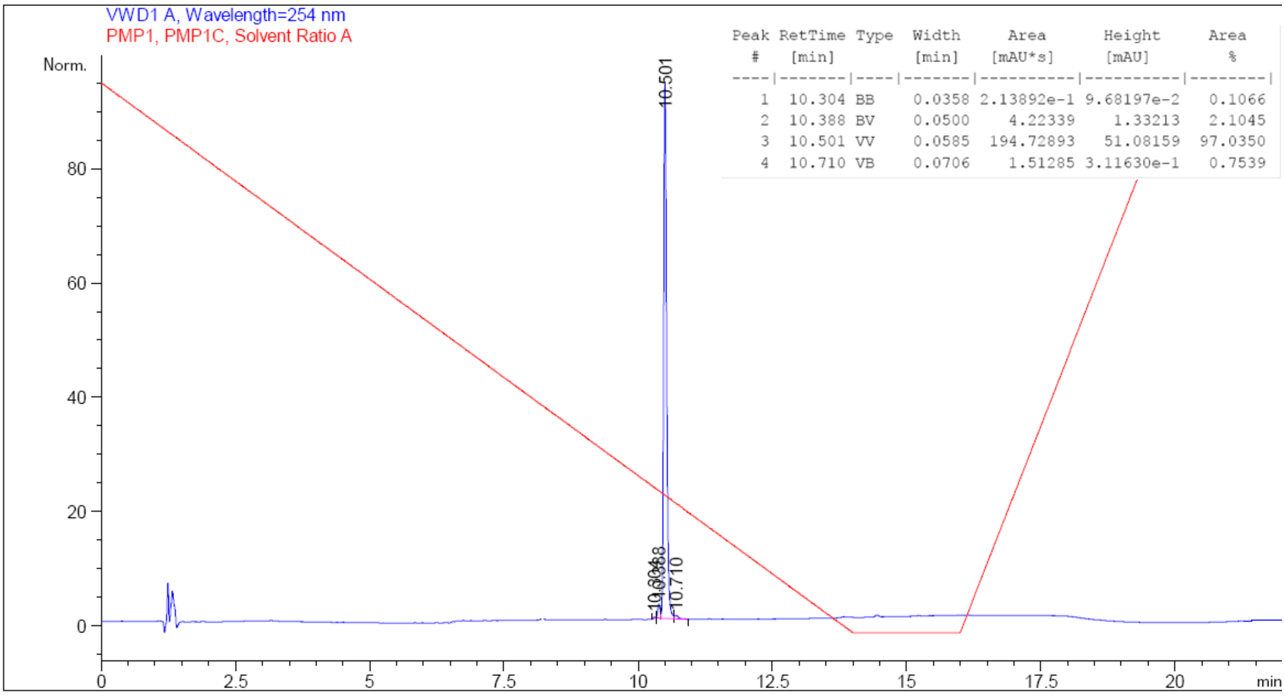
Method B



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   7.071 BB    0.0617 2.09710e-1 5.47452e-2   0.1232
   2   7.158 BV    0.0461    3.49844    1.19735   2.0557
   3   7.255 VB    0.0484  163.96025   52.56973  96.3420
   4   7.593 BB    0.0597    2.51731 6.57574e-1   1.4792
```

### **N,N’-Bis-[(trimethylamino)ethylamino]-2,6-bis[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide** (**7**).

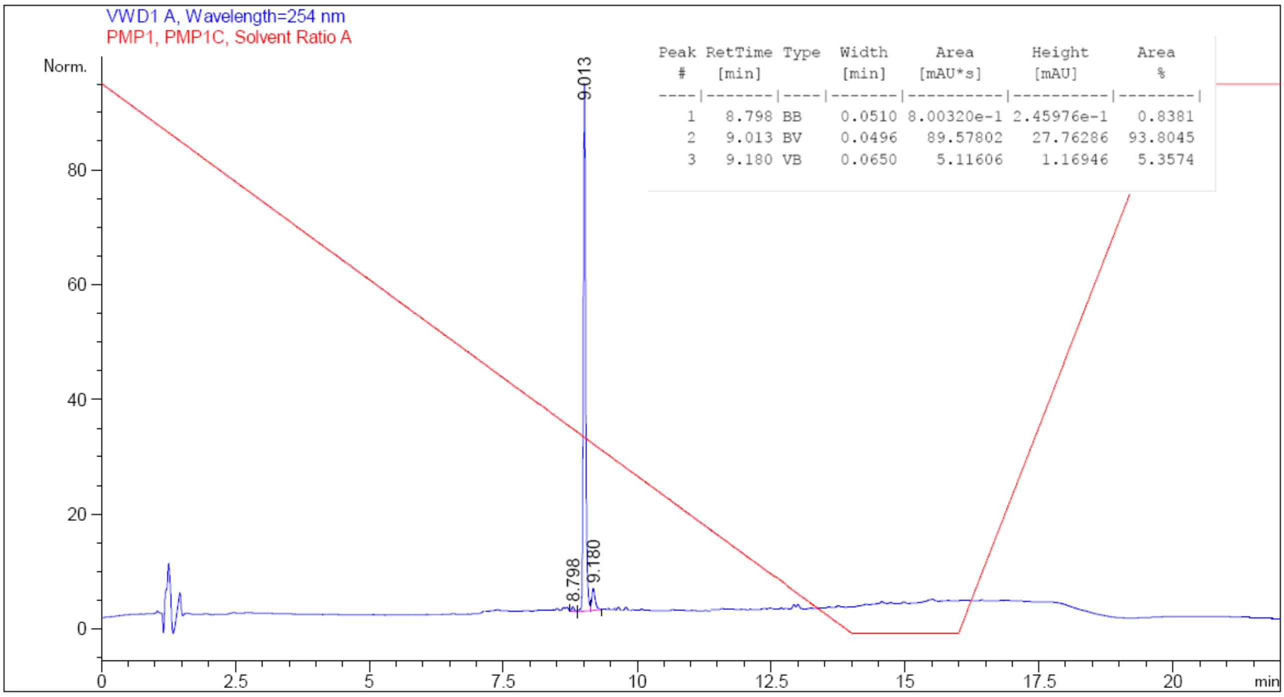
Method B



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1  10.304 BB    0.0358 2.13892e-1 9.68197e-2   0.1066
   2  10.388 BV    0.0500    4.22339    1.33213   2.1045
   3  10.501 VV    0.0585  194.72893   51.08159  97.0350
   4  10.710 VB    0.0706    1.51285 3.11630e-1   0.7539
```

## **N,N'-Bis-[(trimethylamino)ethylamino]-2,6-bis[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (8).**

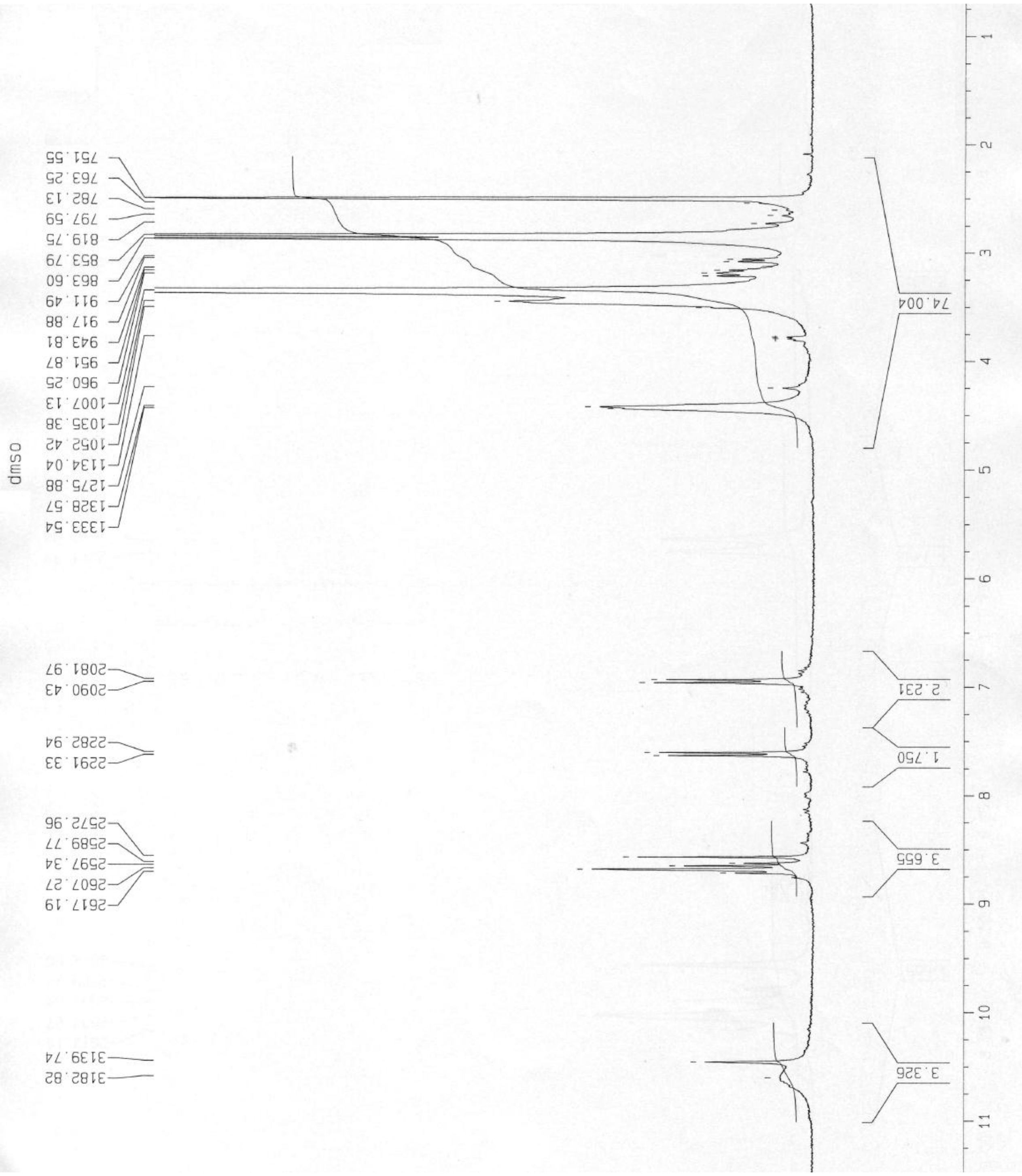
Method B



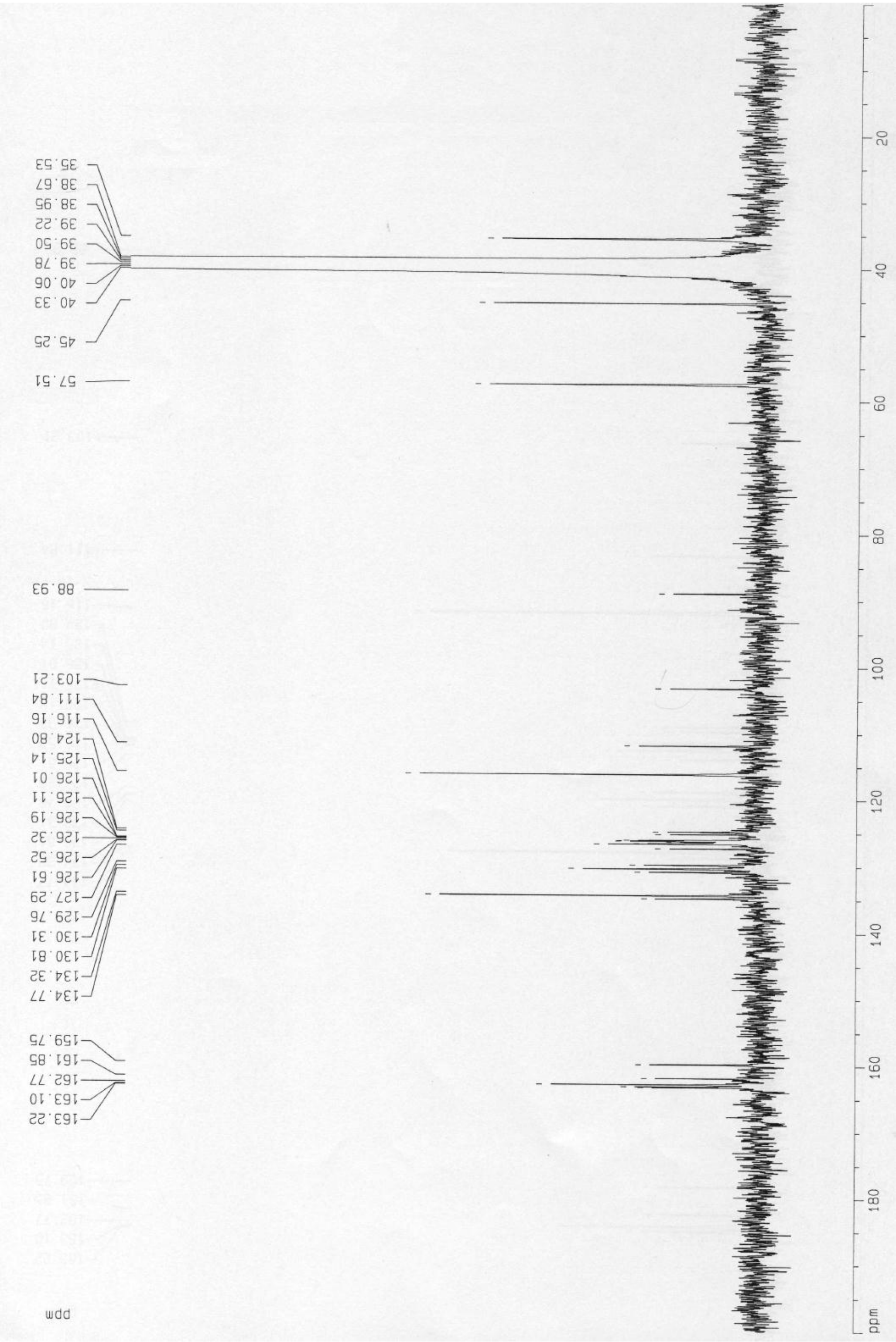
```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.798 BB    0.0510 8.00320e-1 2.45976e-1   0.8381
   2   9.013 BV    0.0496   89.57802   27.76286  93.8045
   3   9.180 VB    0.0650    5.11606    1.16946   5.3574
```

## NMR spectra :

**N,N’-Bis-[(dimethylamino)ethylamino]-2-[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl** (**1**).
(DMSO) $^1$H NMR 300MHz

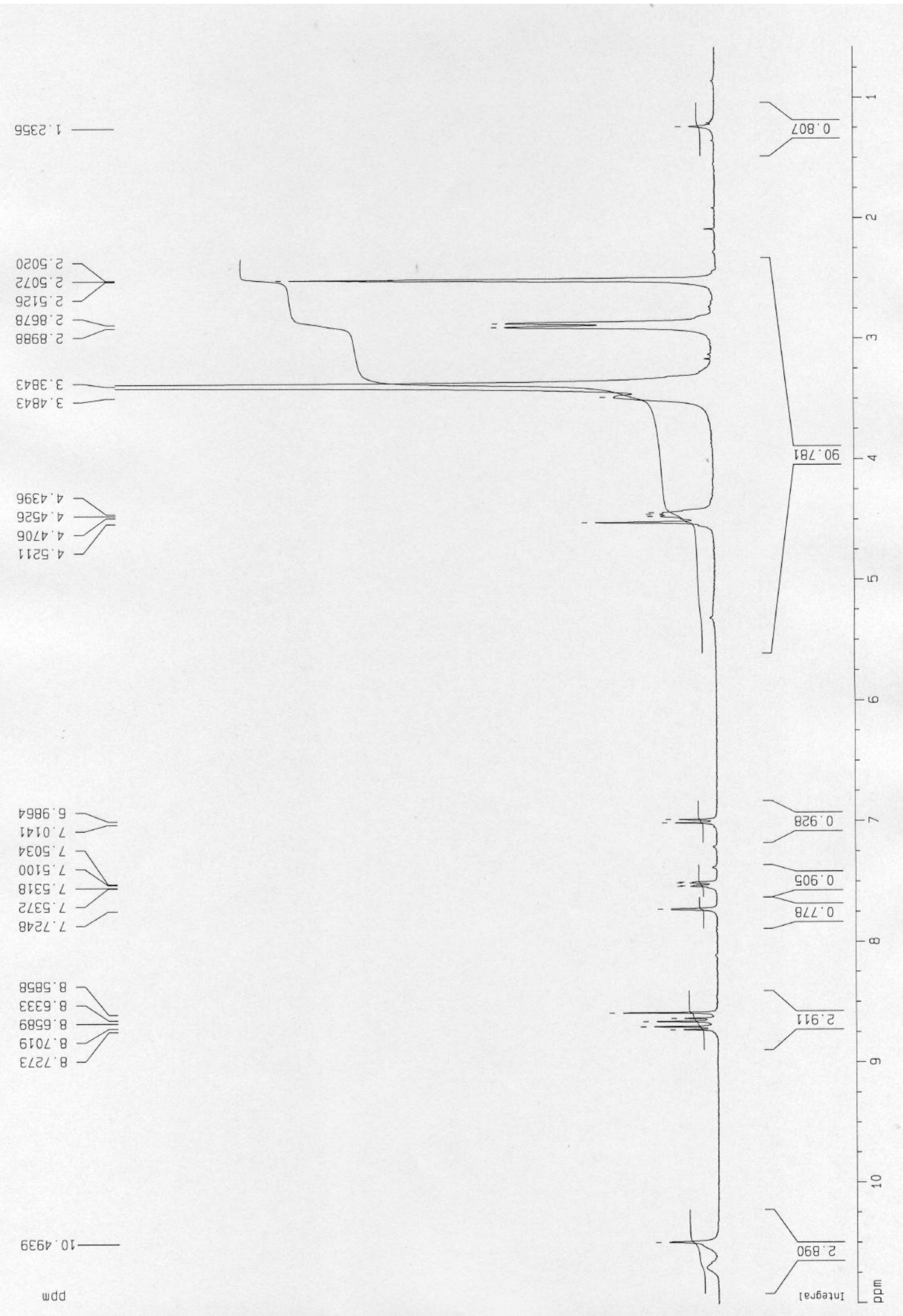


(DMSO) $^{13}$C NMR 75MHz

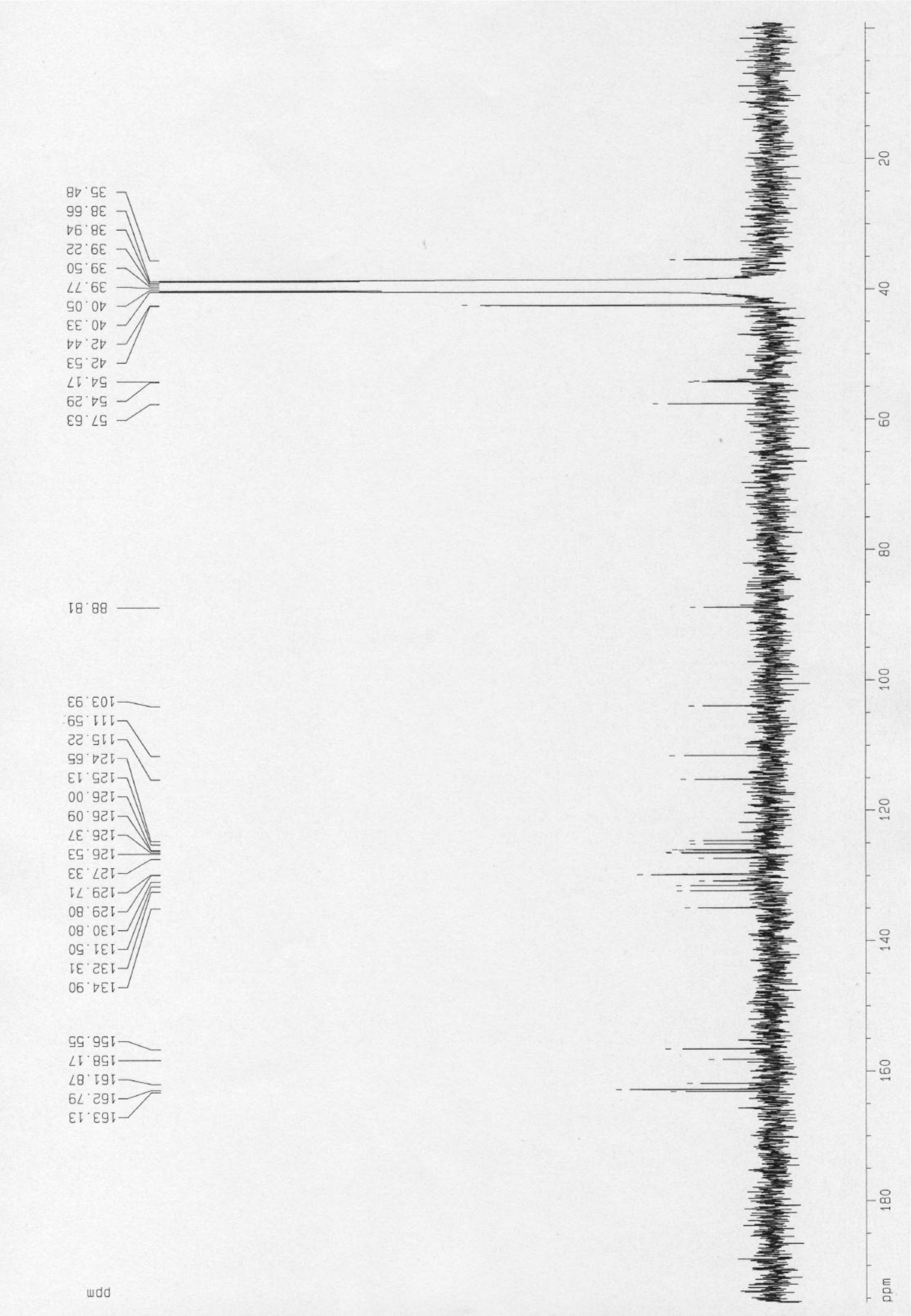


**N,N’-Bis-[(dimethylamino)ethylamino]-2-[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl** (**2**).

(DMSO) $^1$H NMR 300MHz

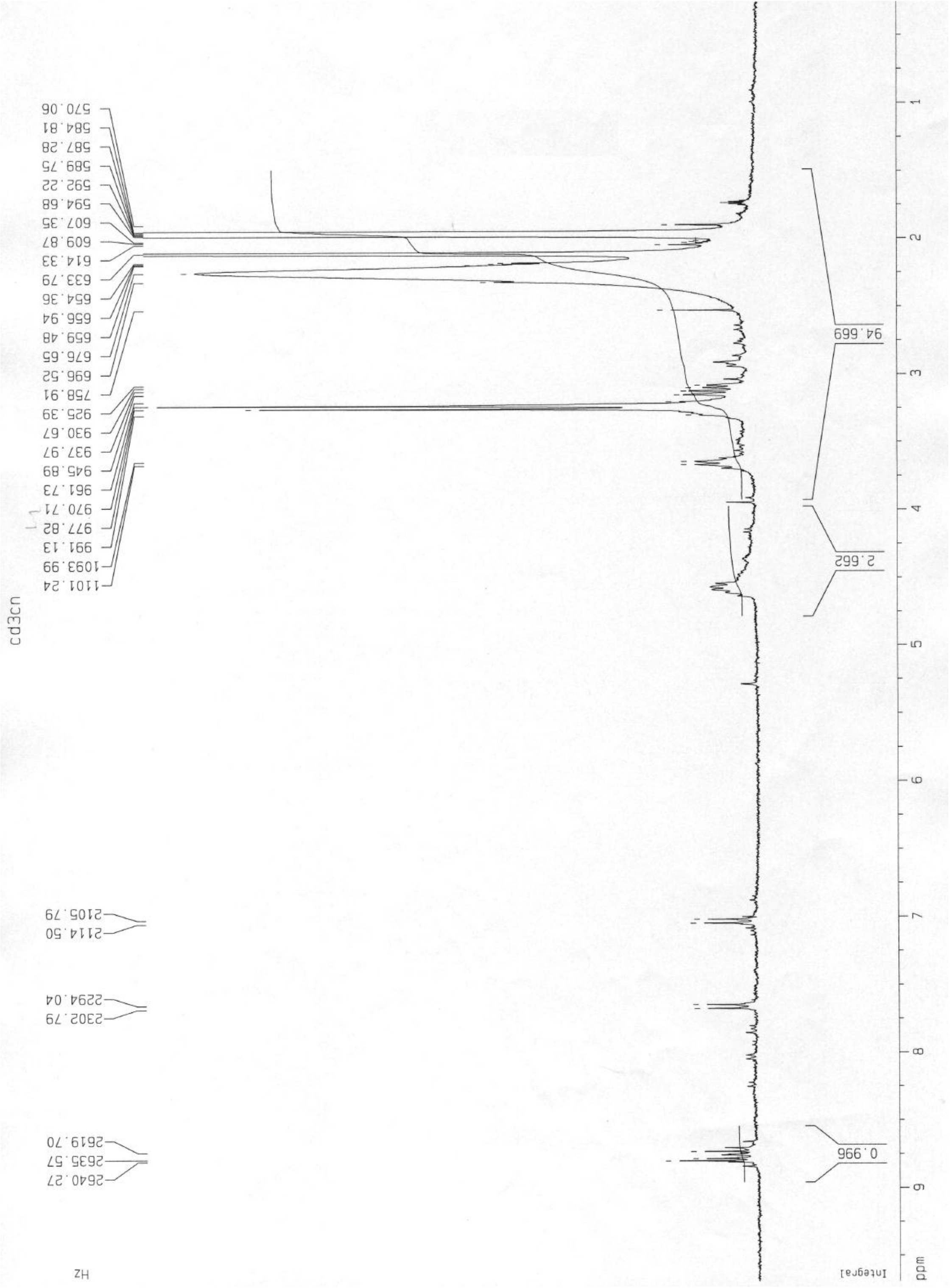


(DMSO) $^{13}$C NMR 75MHz



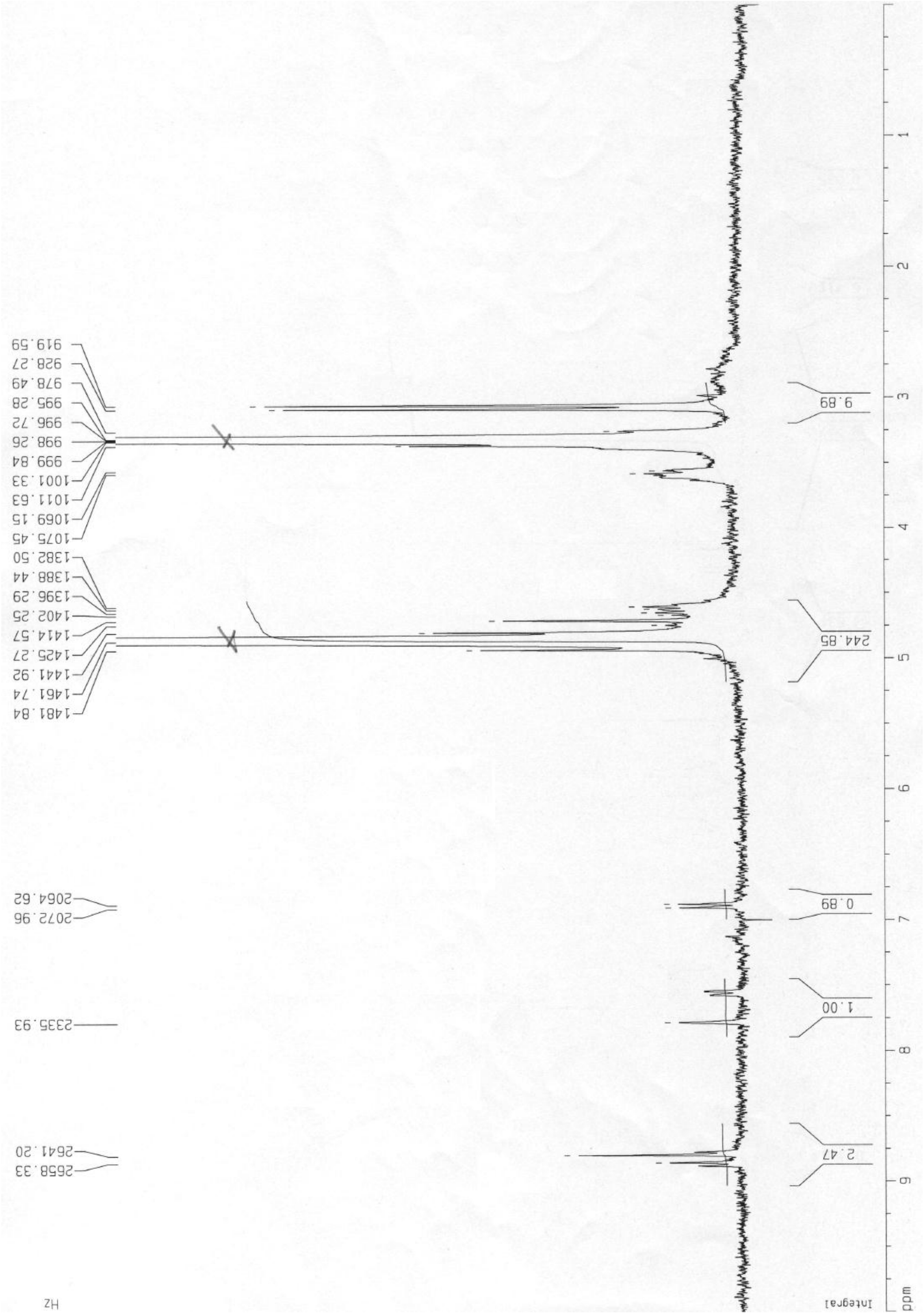
**N,N'-Bis-[(trimethylamino)ethylamino]-2-[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (3).**

($CD_3CN$) $^1$H NMR 300MHz



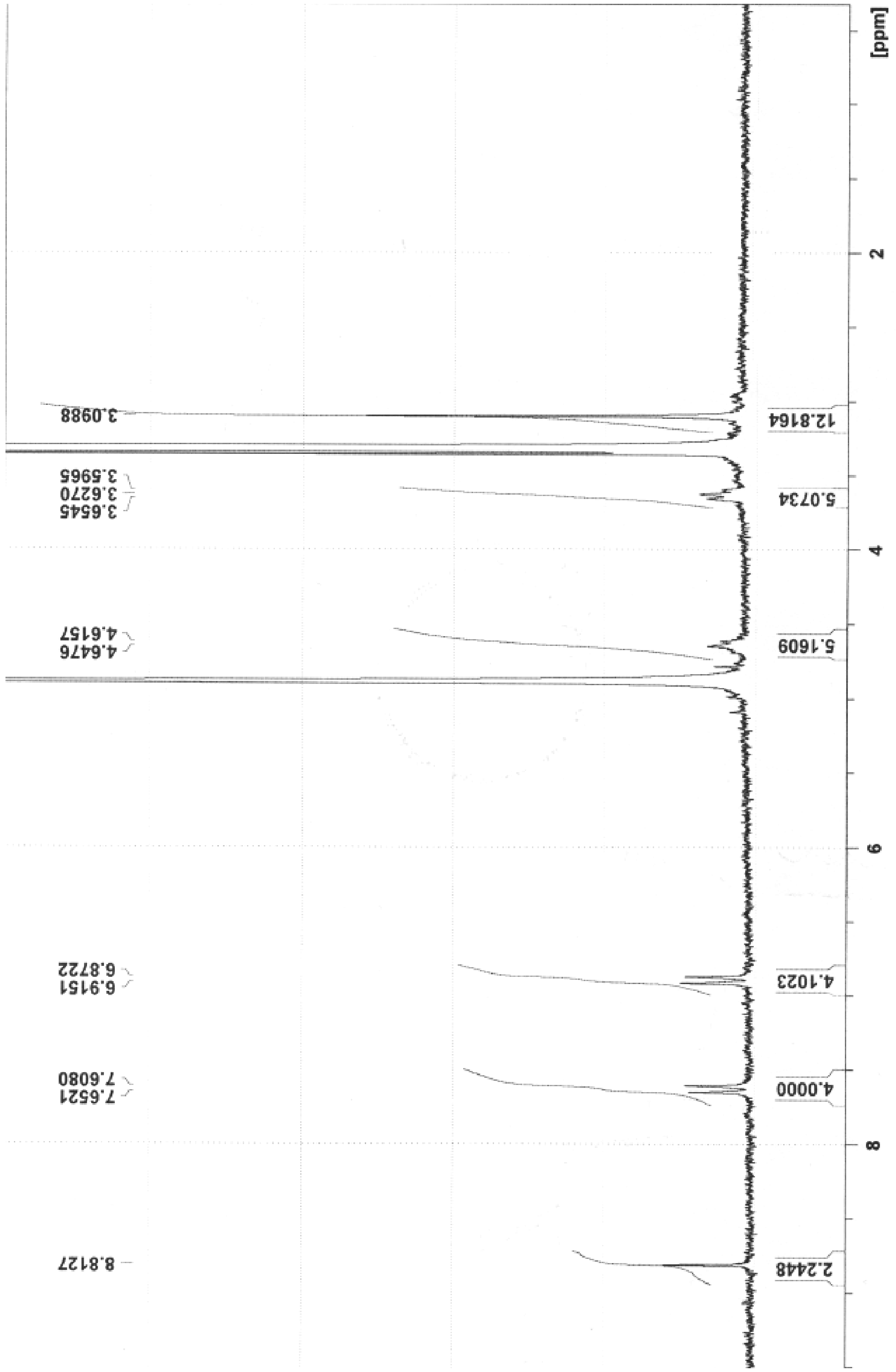
## N,N'-Bis-[(trimethylamino)ethylamino]-2-[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (4).

($CD_3OD$) $^1H$ NMR 300MHz

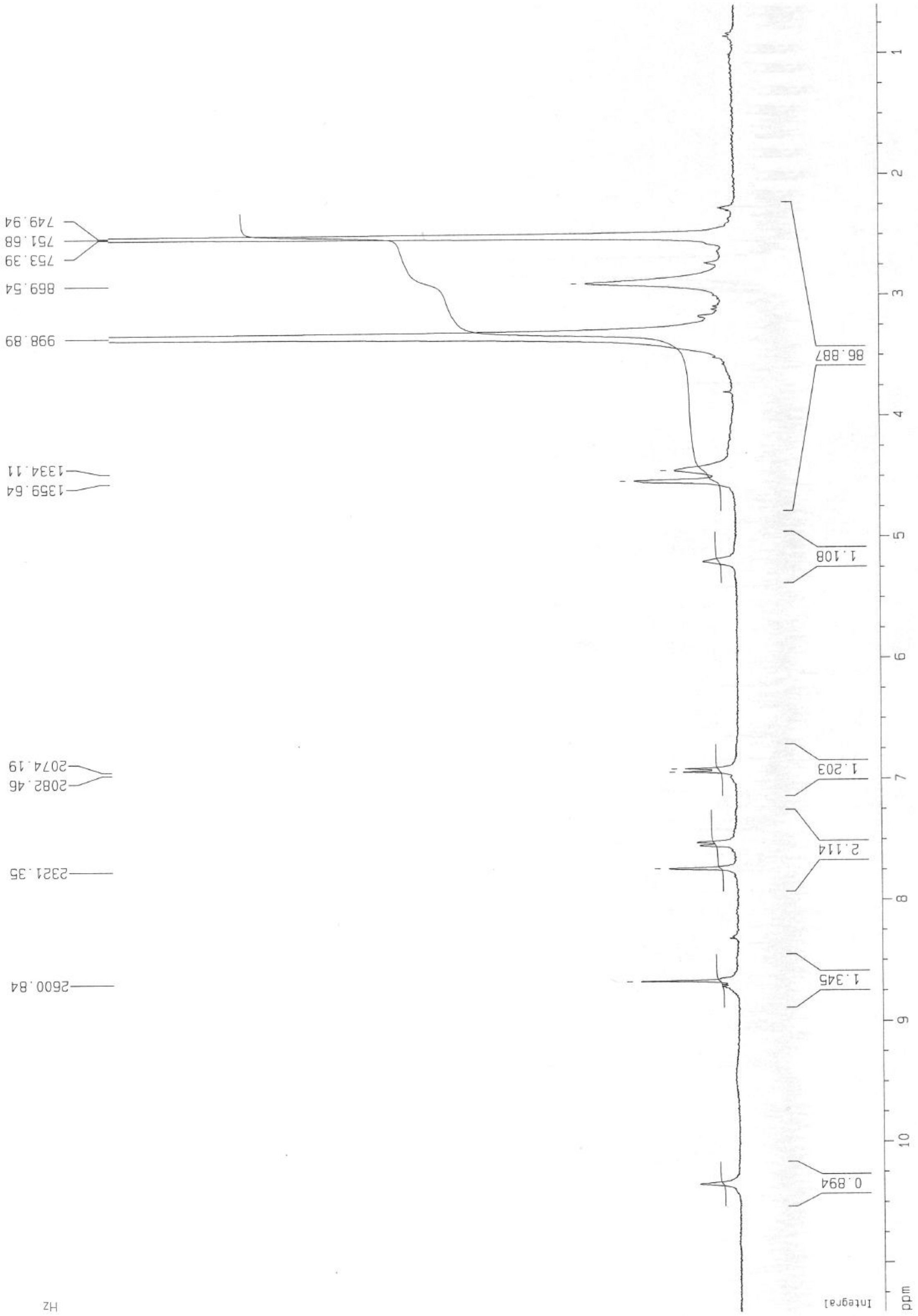


**N,N'-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl** (5).

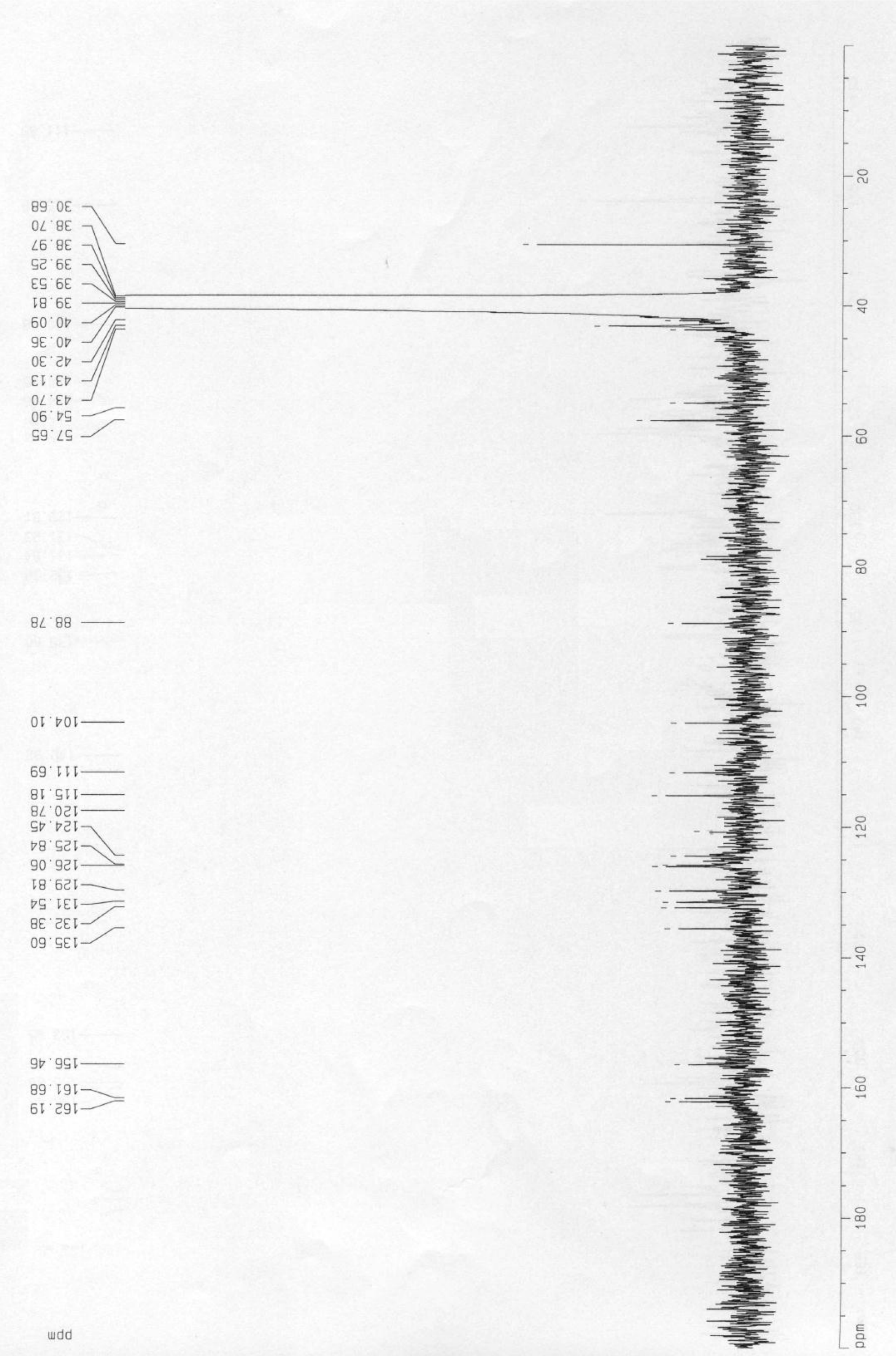
($CD_3OD$) $^1H$ NMR 300MHz



**N,N’-Bis-[(dimethylamino)ethylamino]-2,6-bis[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide 2 HCl (6).**
(DMSO) $^{1}$H NMR 300MHz

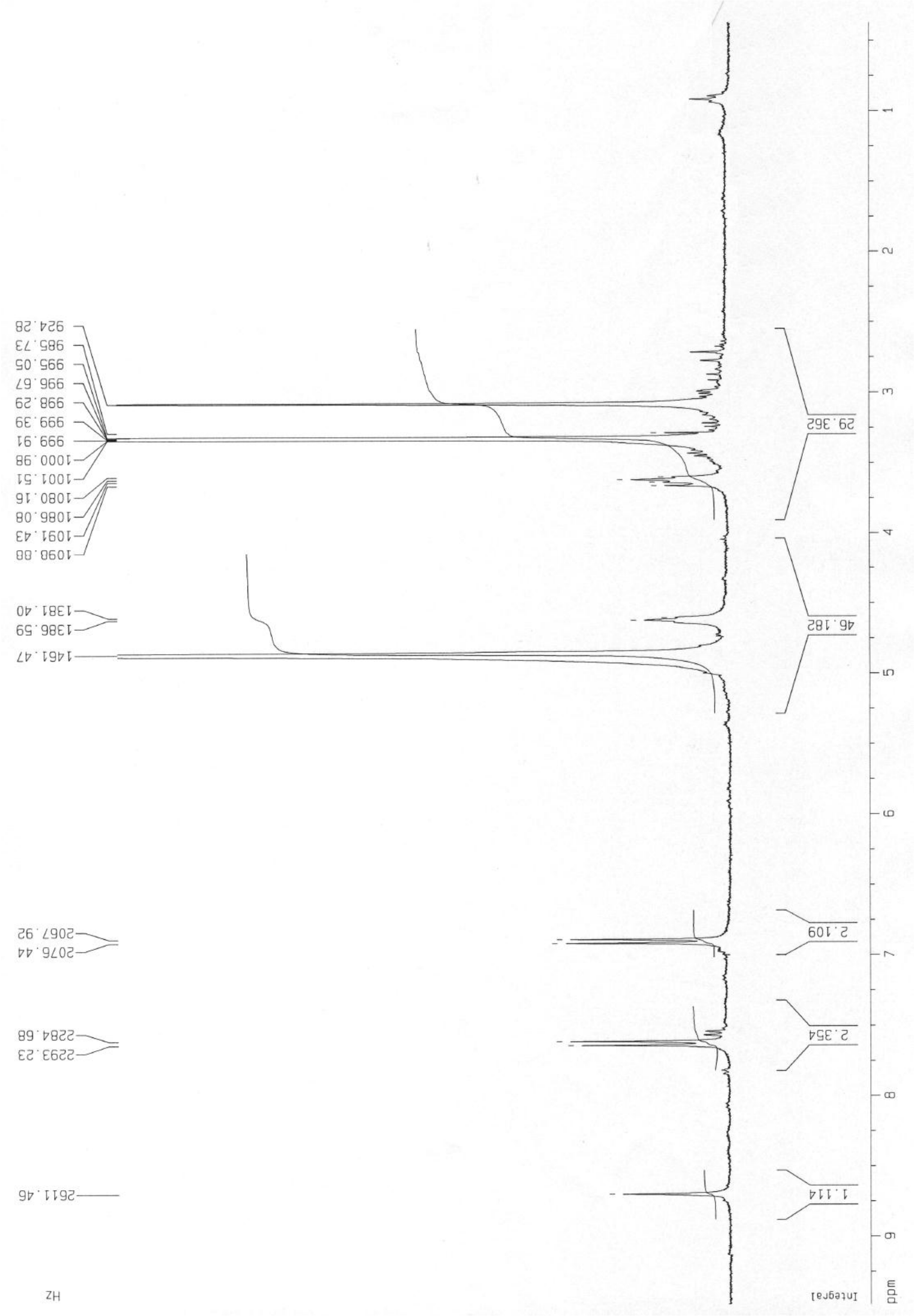


(DMSO) $^{13}$C NMR 75MHz

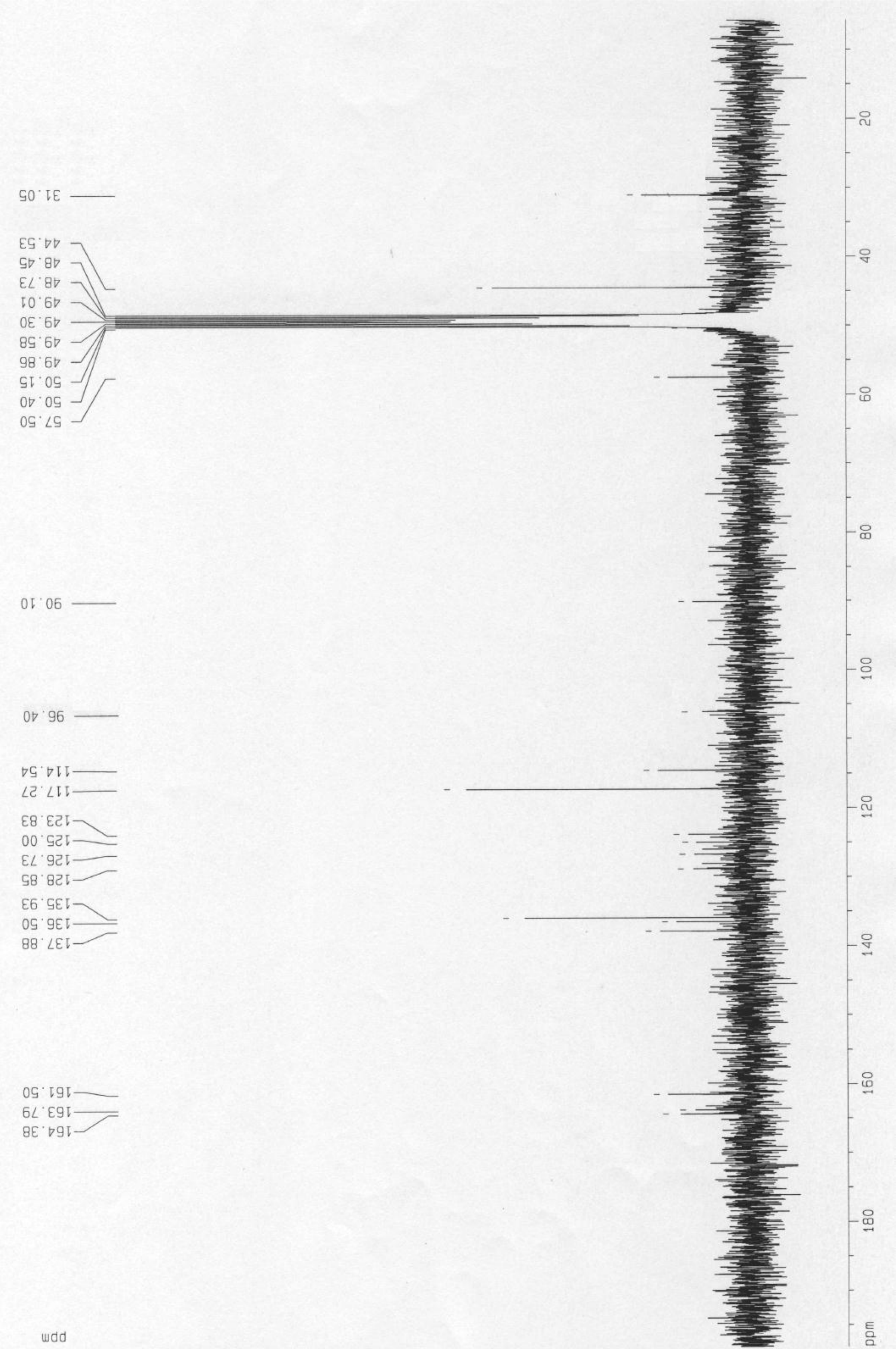


## N,N’-Bis-[(trimethylamino)ethylamino]-2,6-bis[2-(4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (7).

(CD$_3$OD) $^1$H NMR 300MHz

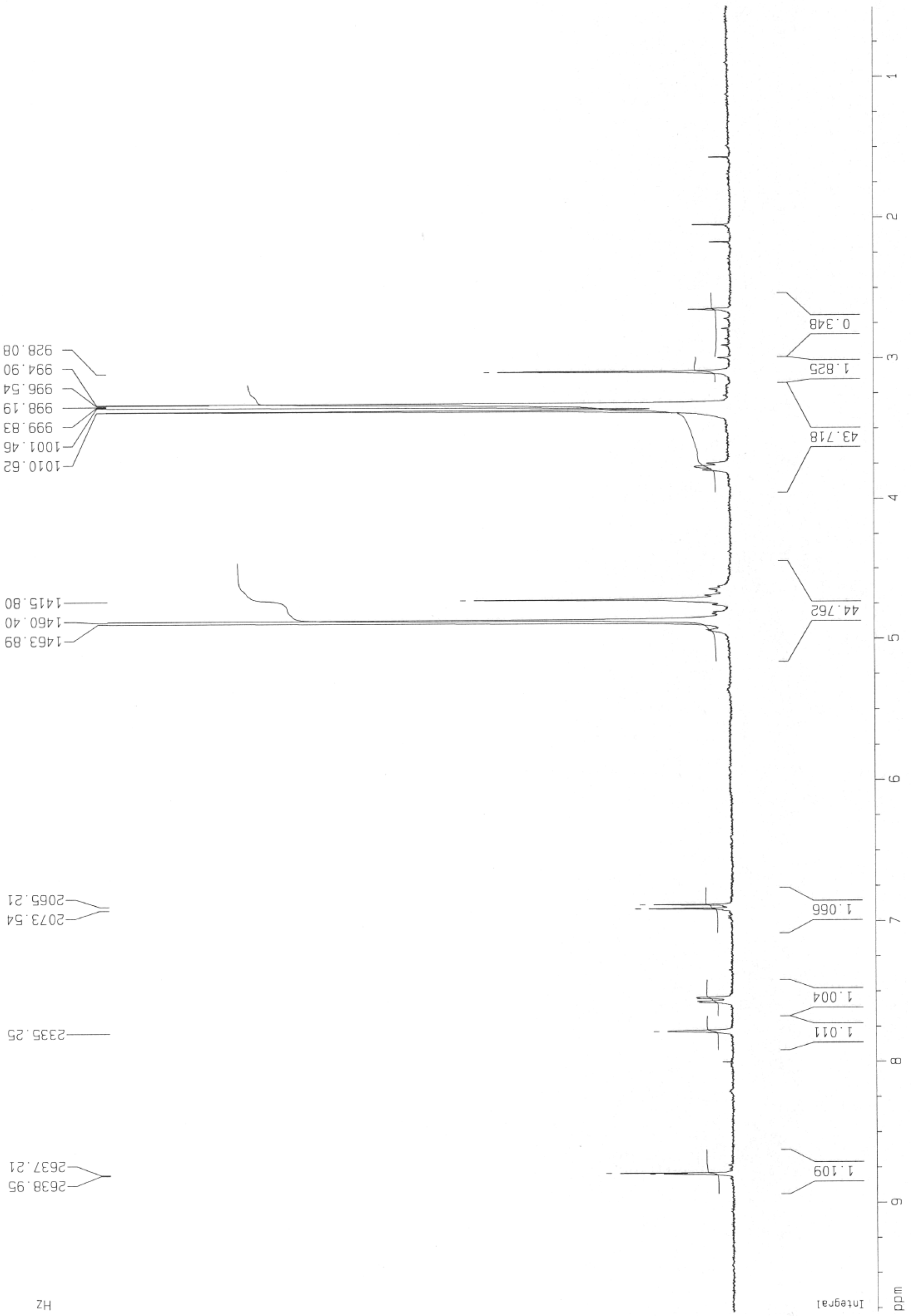


(CD$_3$OD) $^{13}$C NMR 75MHz

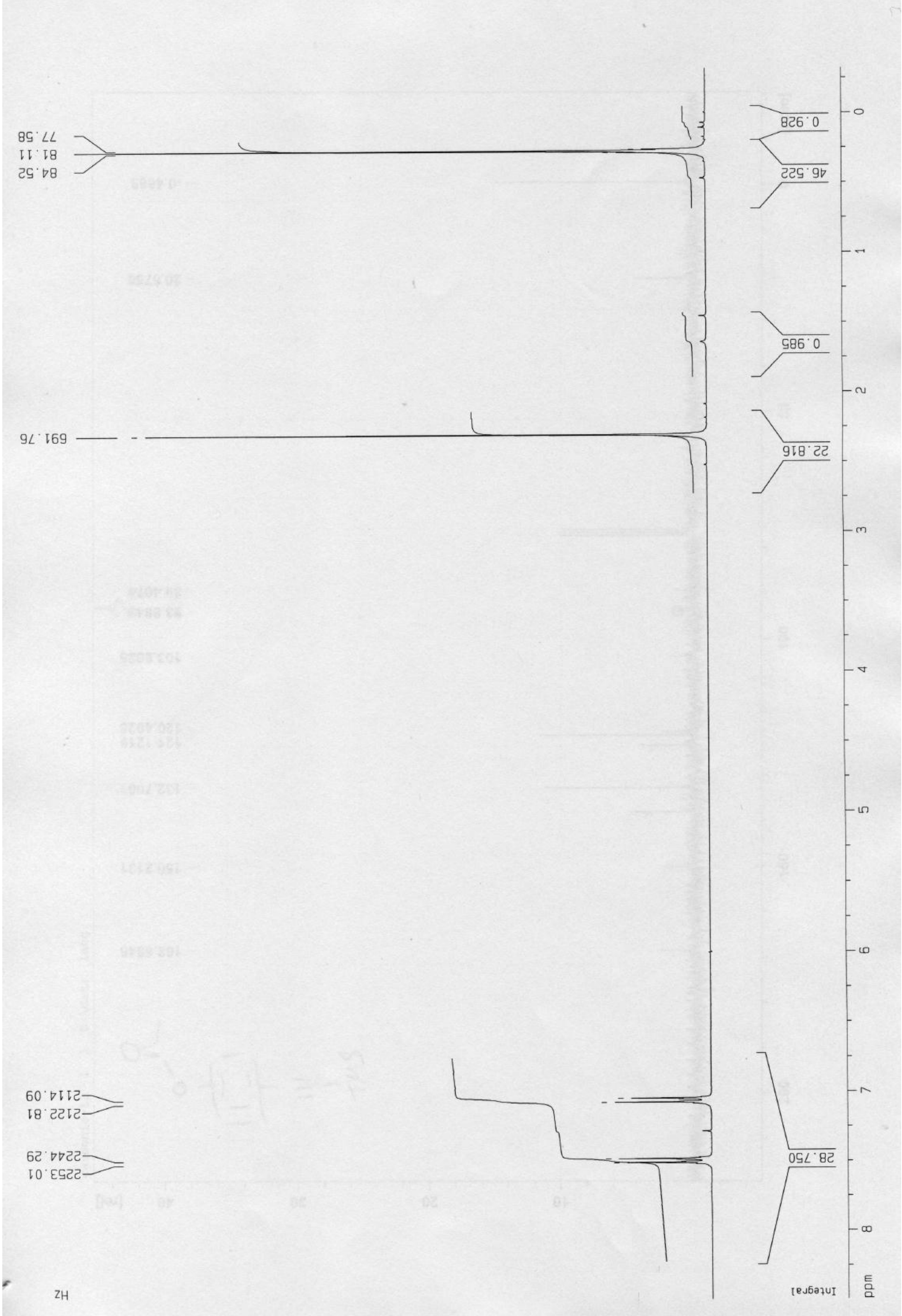


## **N,N’-Bis-[(trimethylamino)ethylamino]-2,6-bis[2-(3-hydroxymethyl-4-hydroxyphenyl)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide diiodide (8)**.

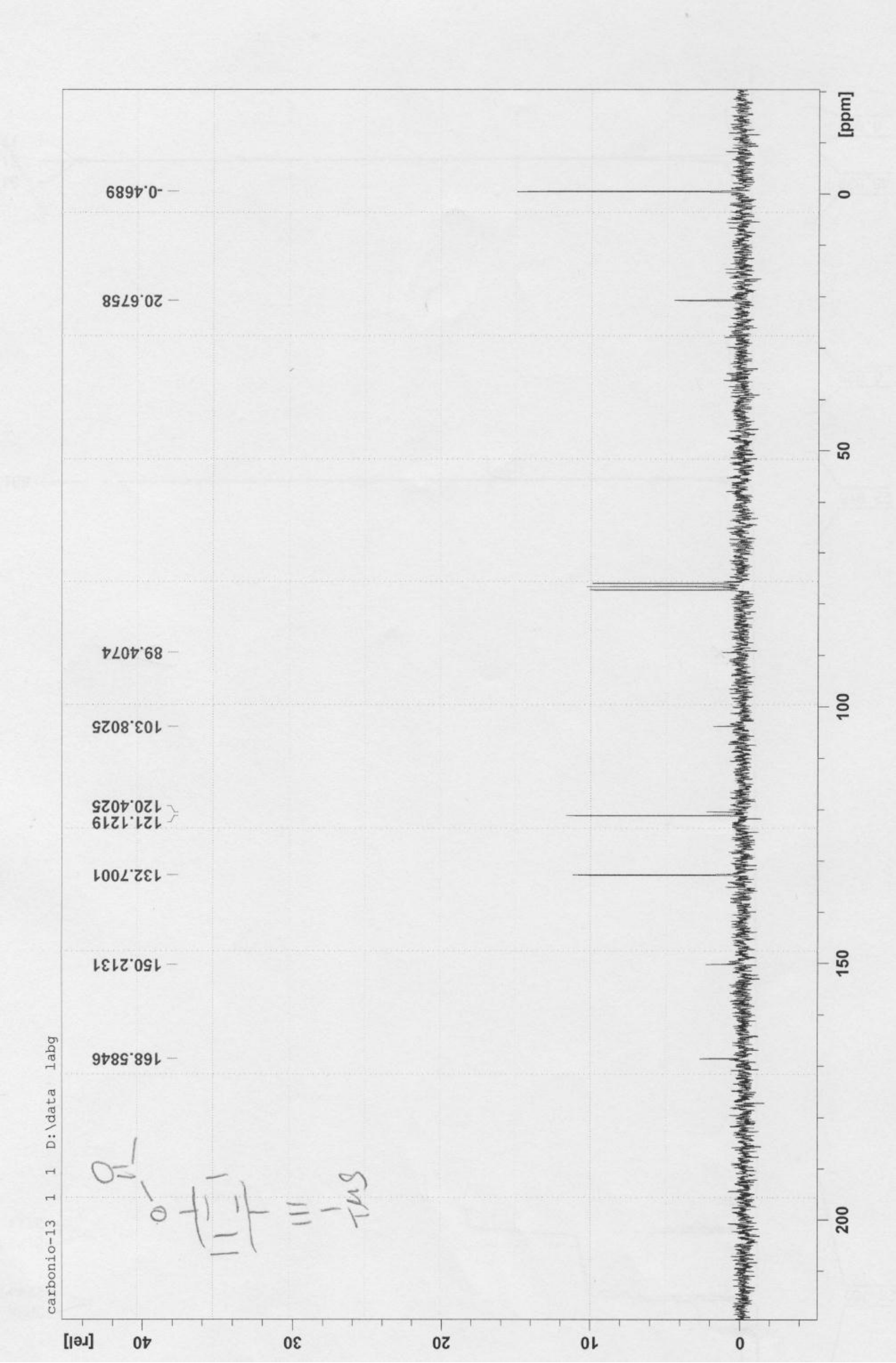
($CD_3OD$) $^1H$ NMR 300MHz



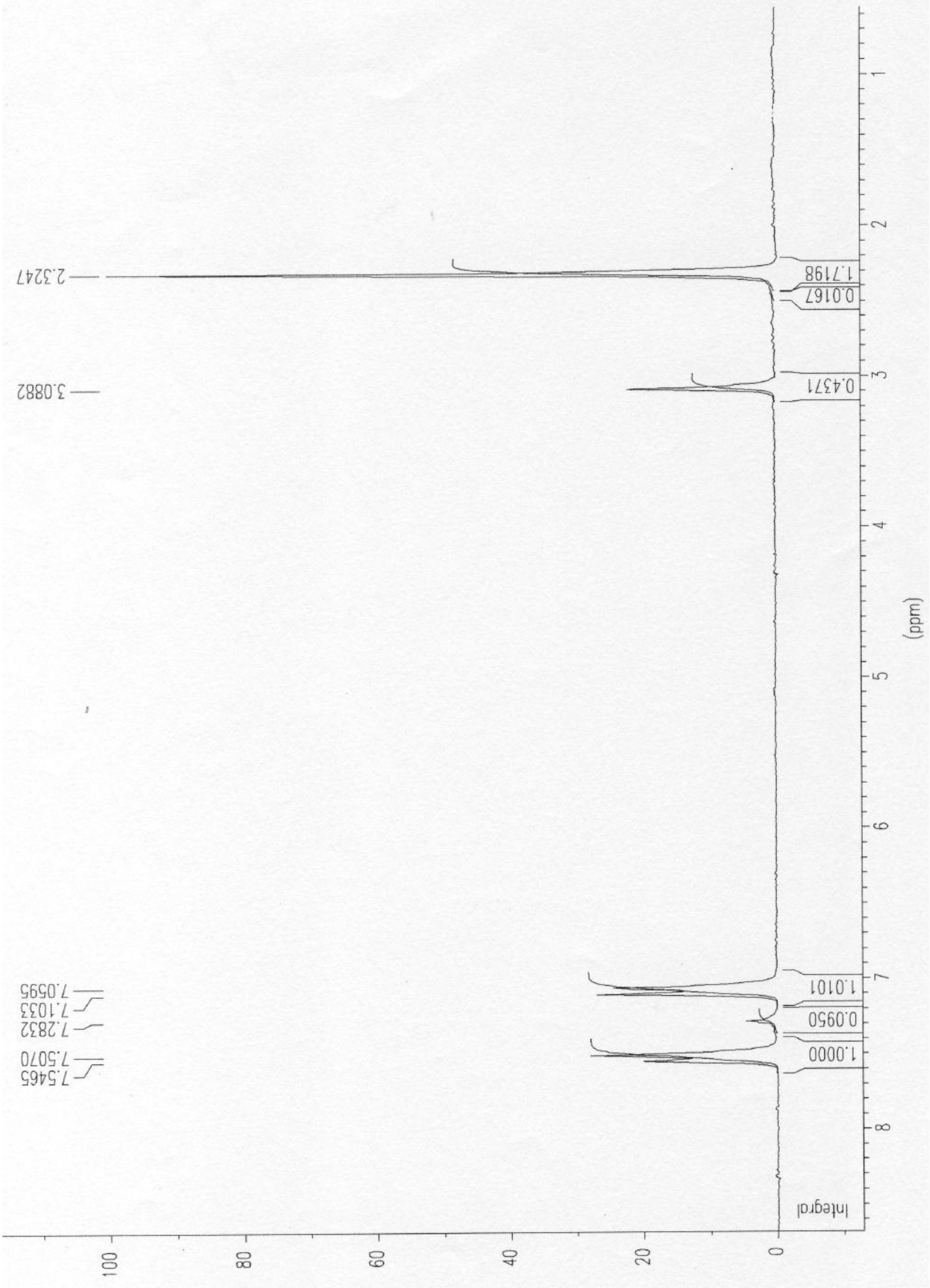
**4-((trimethylsilyl)ethynyl)phenyl acetate (10).**
($CDCl_3$) $^1H$ NMR 300MHz



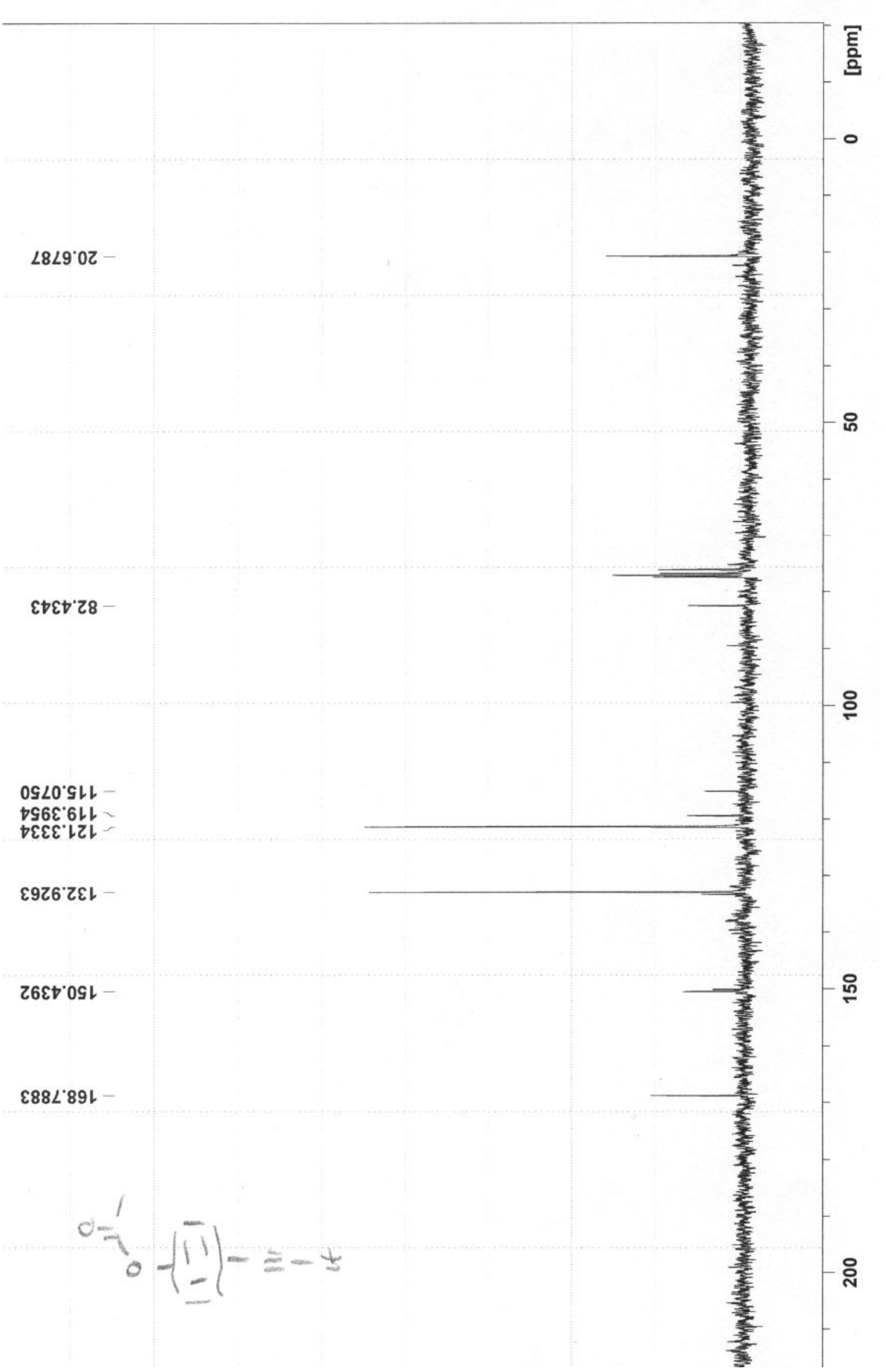
(CDCl$_3$) $^{13}$C NMR 50MHz



**4-ethynylphenyl acetate** (**11**).
($CDCl_3$) $^1$H NMR 300MHz

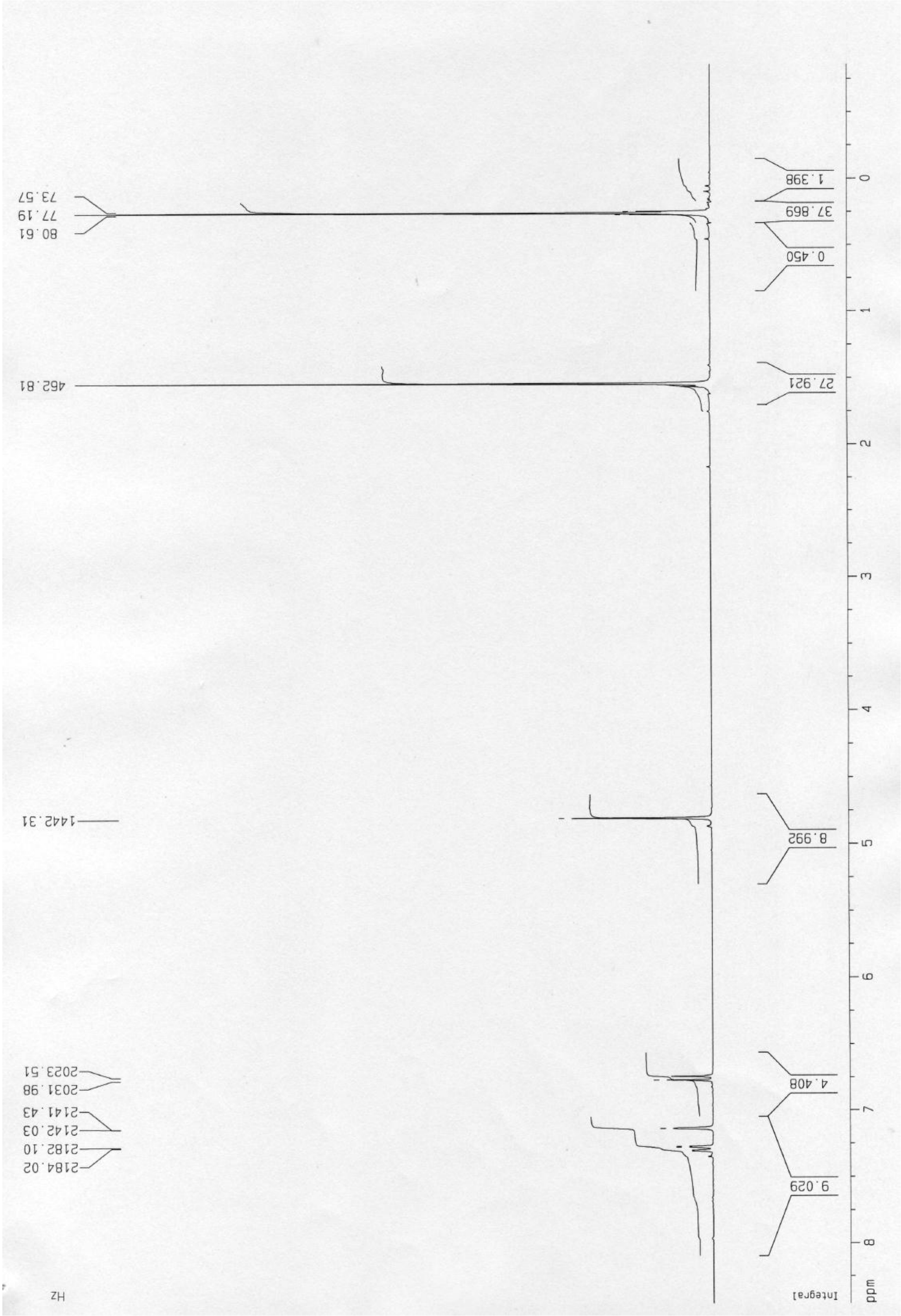


(CDCl$_3$) $^{13}$C NMR 50MHz

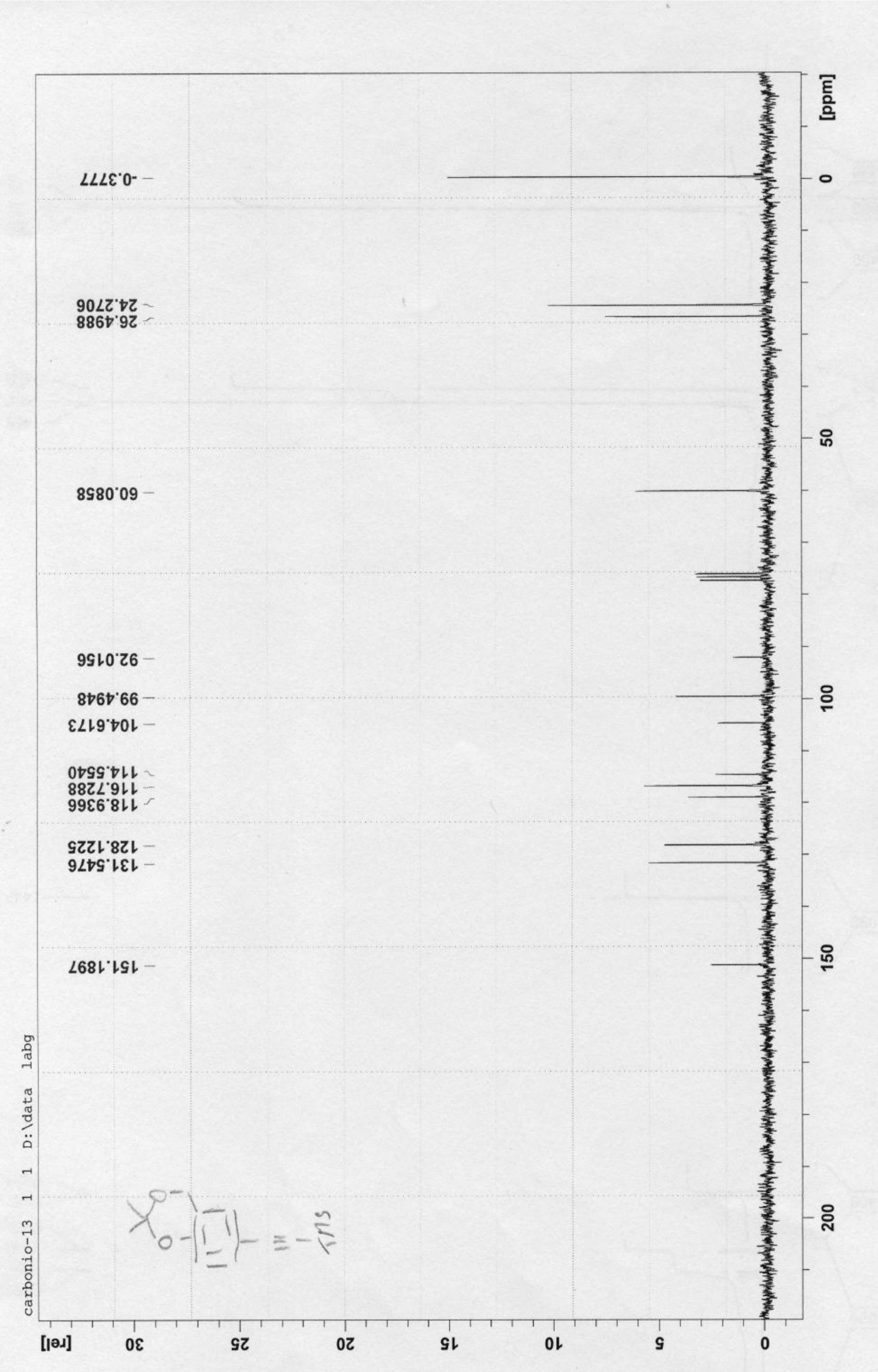


## **2,2-dimethyl-6-((trimethylsilyl)ethynyl)benzo[1,3]dioxin** (**14**).

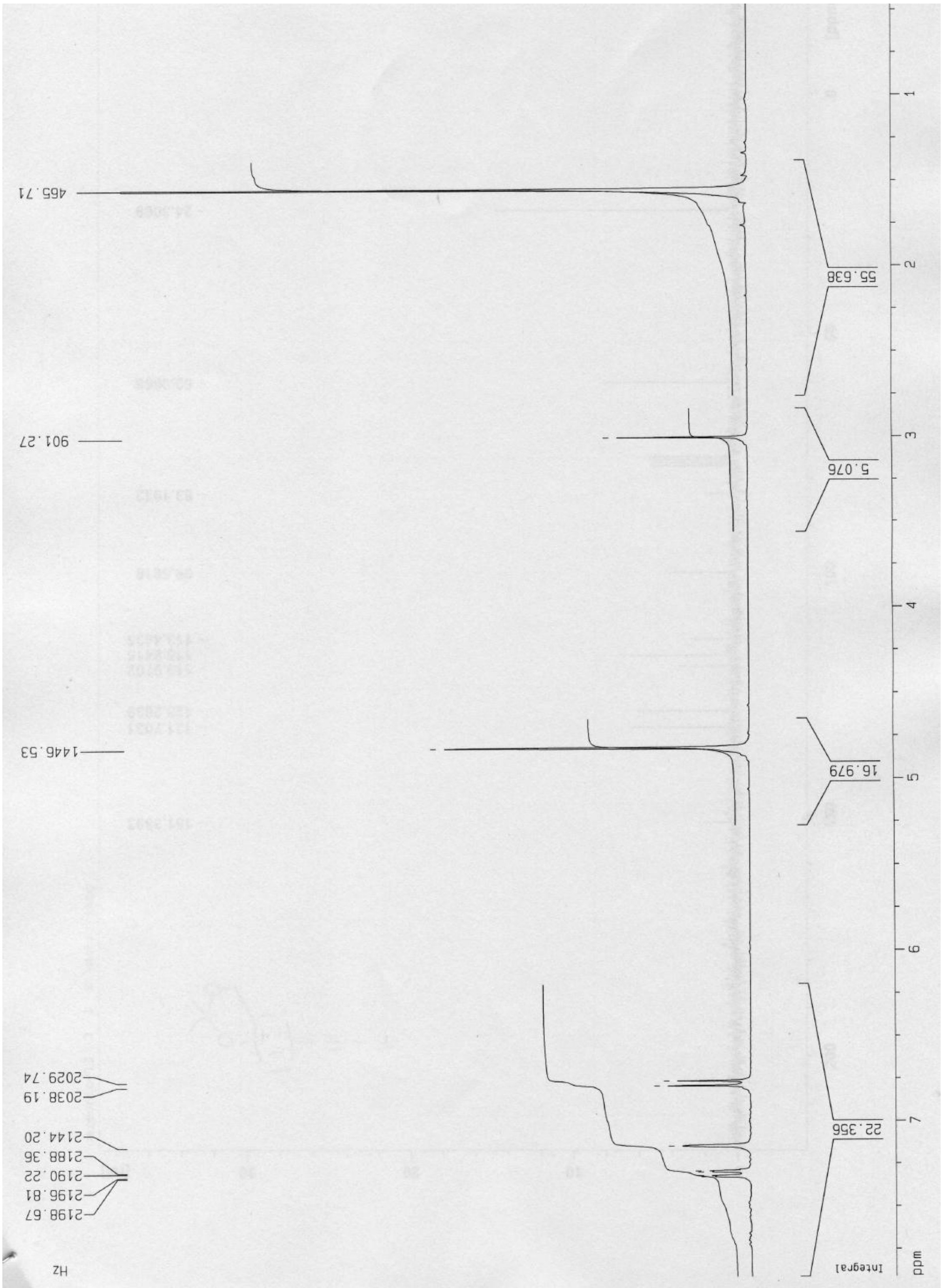
(CDCl$_3$) $^1$H NMR 300MHz



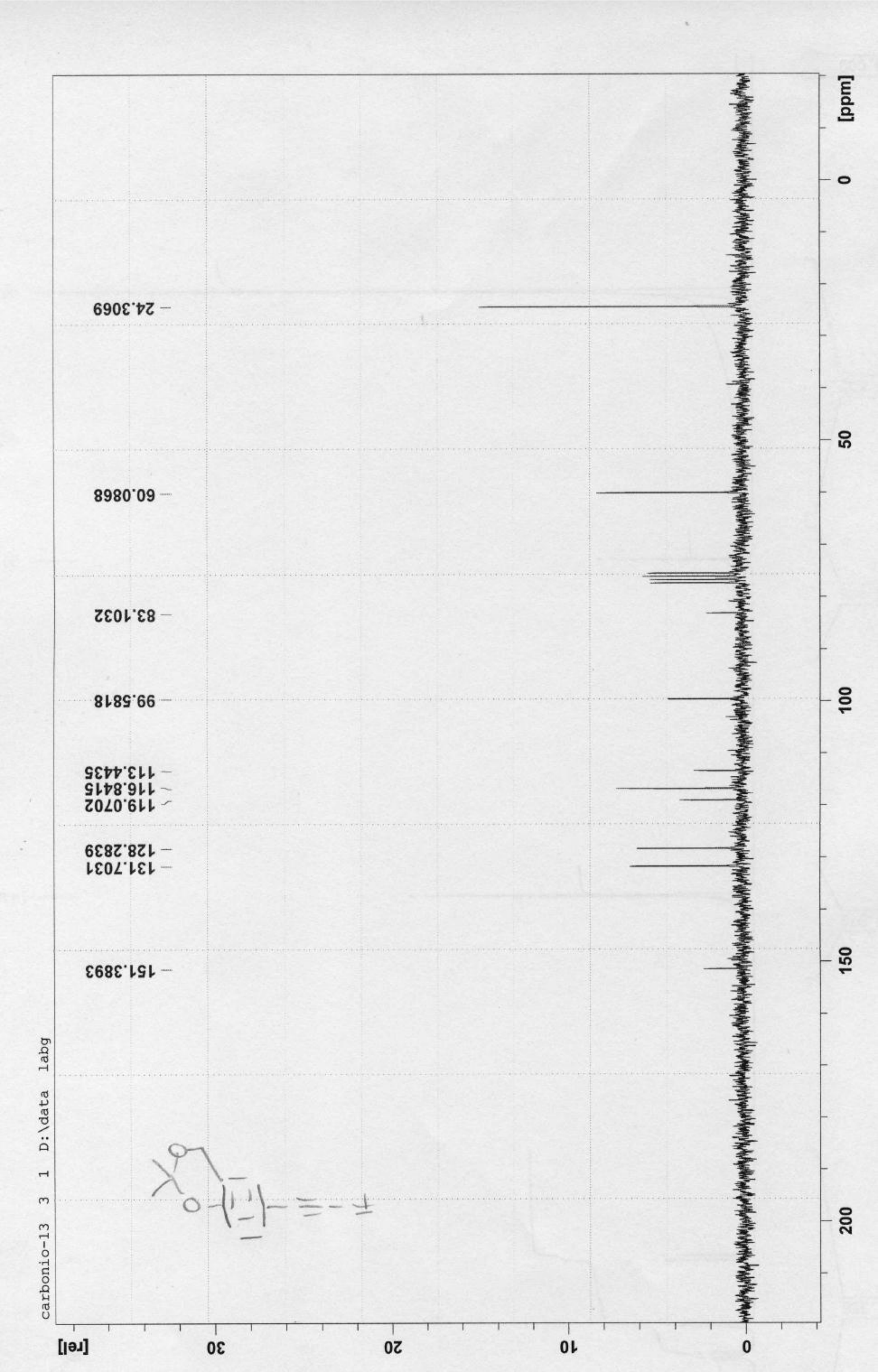
($CDCl_3$) $^{13}C$ NMR 50MHz



**6-ethynyl-2,2-dimethylbenzo[1,3]dioxin** (**15**).
($CDCl_3$) $^1$H NMR 300MHz

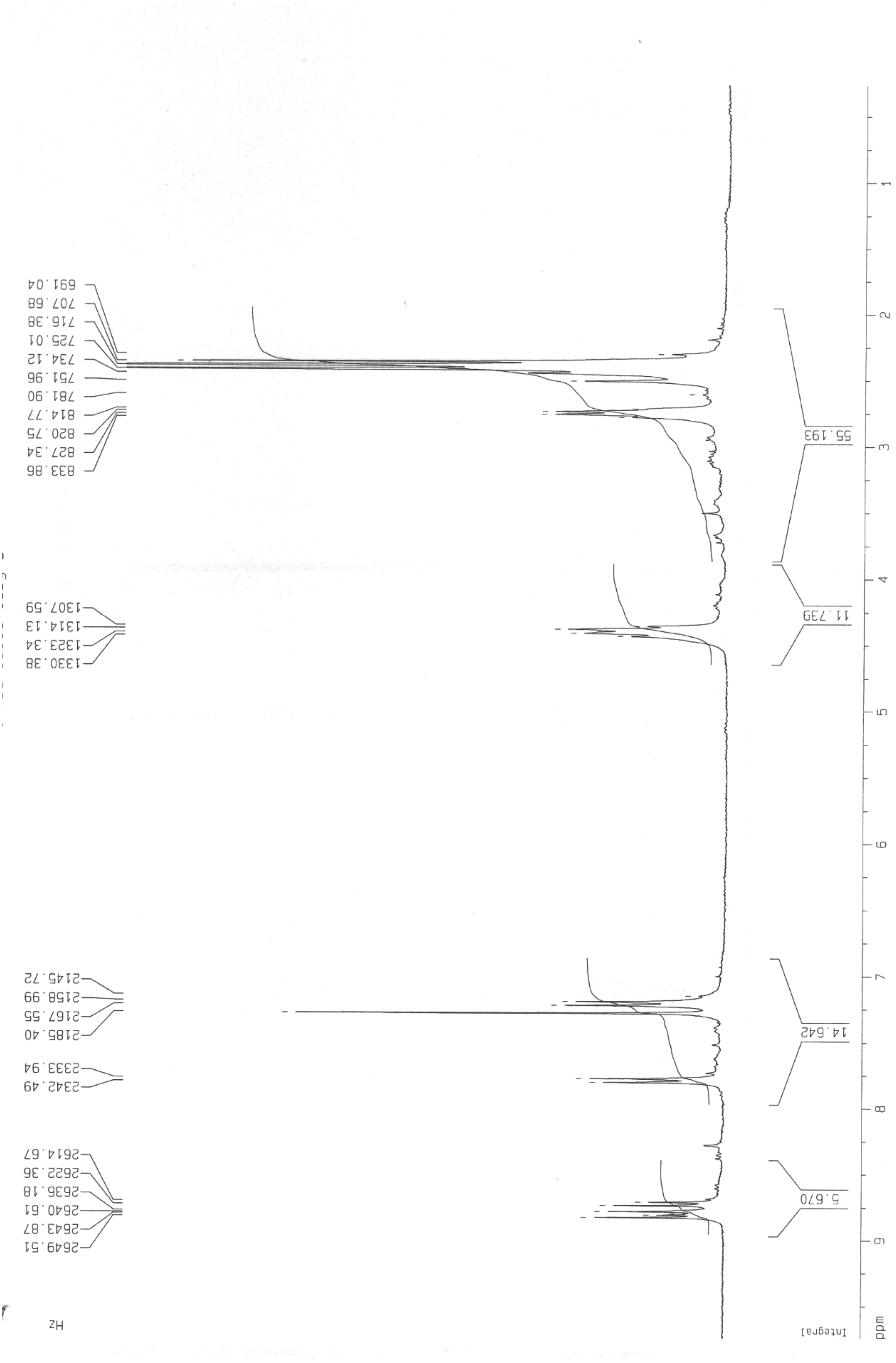


(CDCl$_3$) $^{13}$C NMR 50MHz

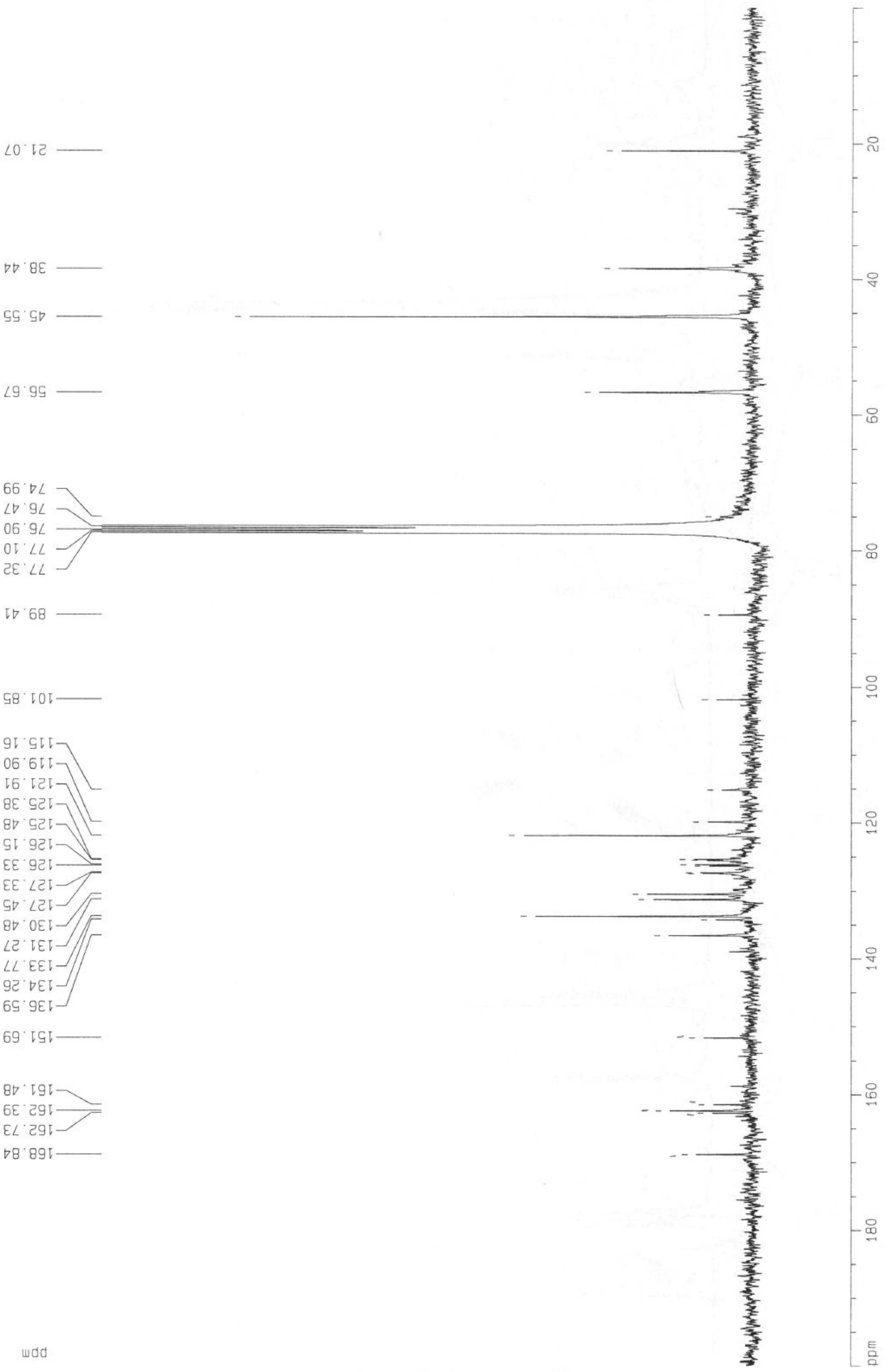


**N,N’-Bis-((dimethylamino)ethylamino)-2-[2-(4-phenyl-acetate)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide (18).**

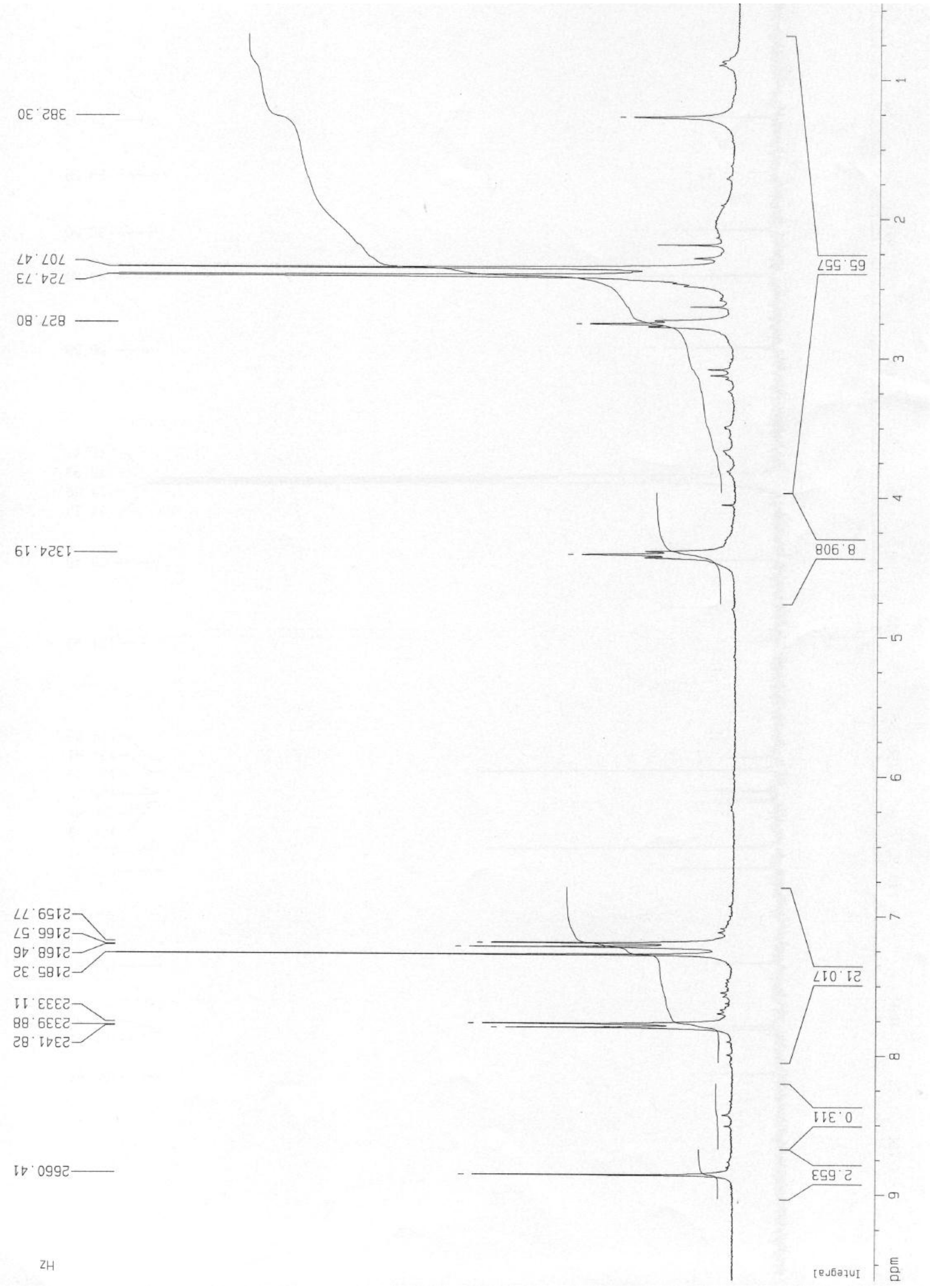
($CDCl_3$) $^1$H NMR 300MHz



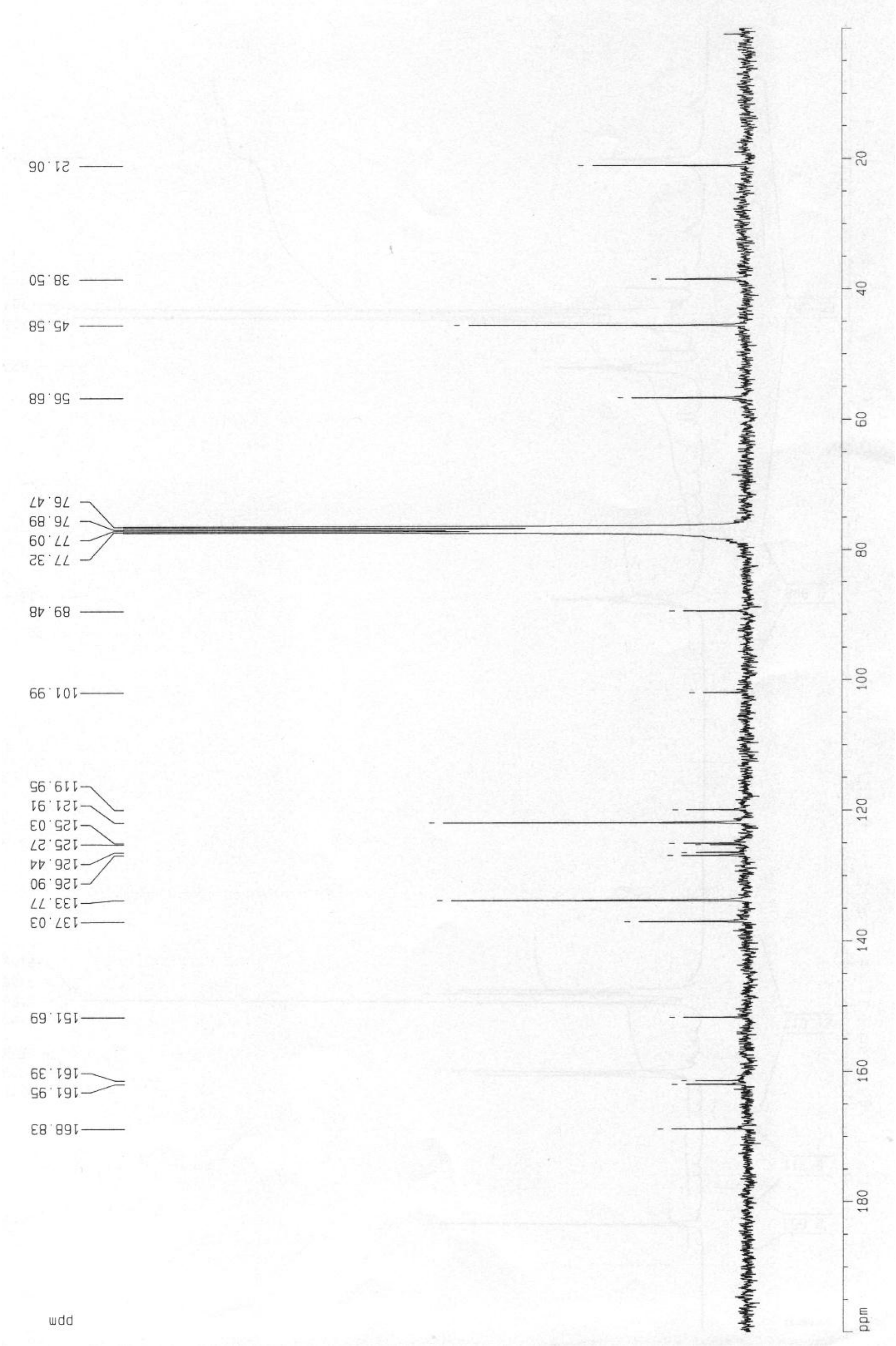
(CDCl$_3$)  $^{13}$C NMR 75MHz



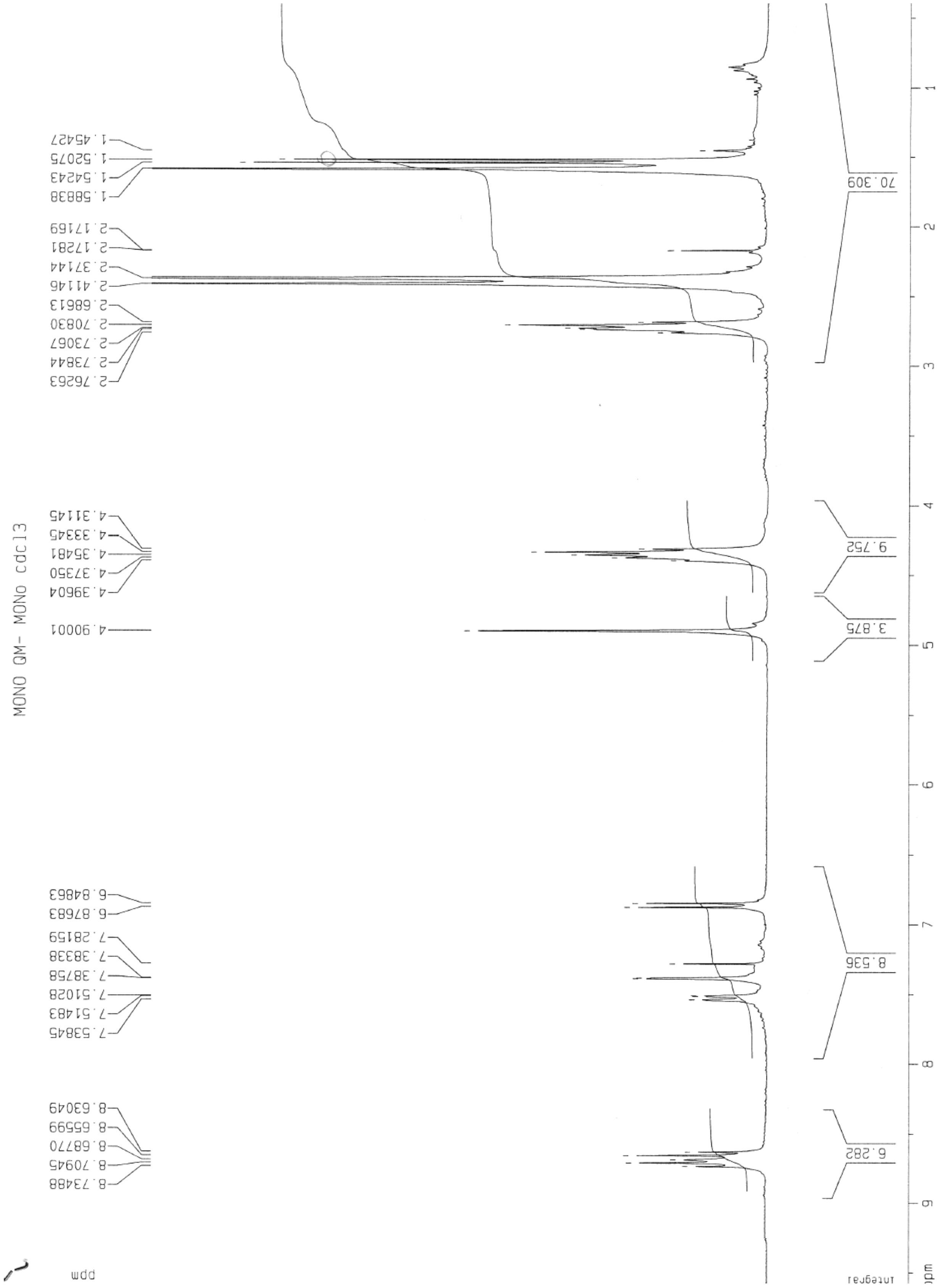
**N,N'-Bis-((dimethylamino)ethylamino)-2,6-bis[2-(4-phenyl-acetate)ethynyl]-1,4-5,8-naphtalenetetracarboxylic bisimide** (**19**).
($CDCl_3$) $^1H$ NMR 300MHz



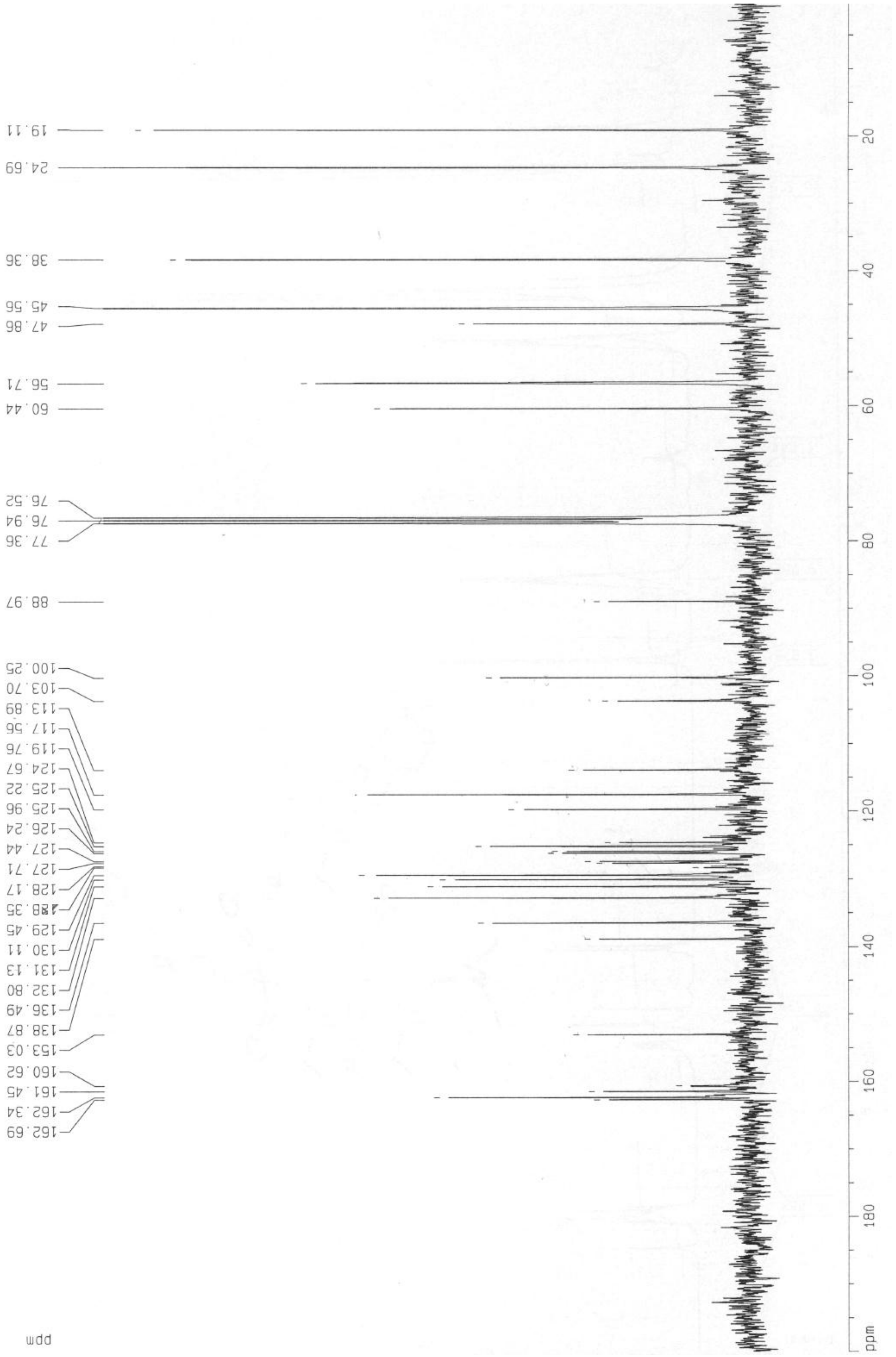
(CDCl$_3$) $^{13}$C NMR 75MHz



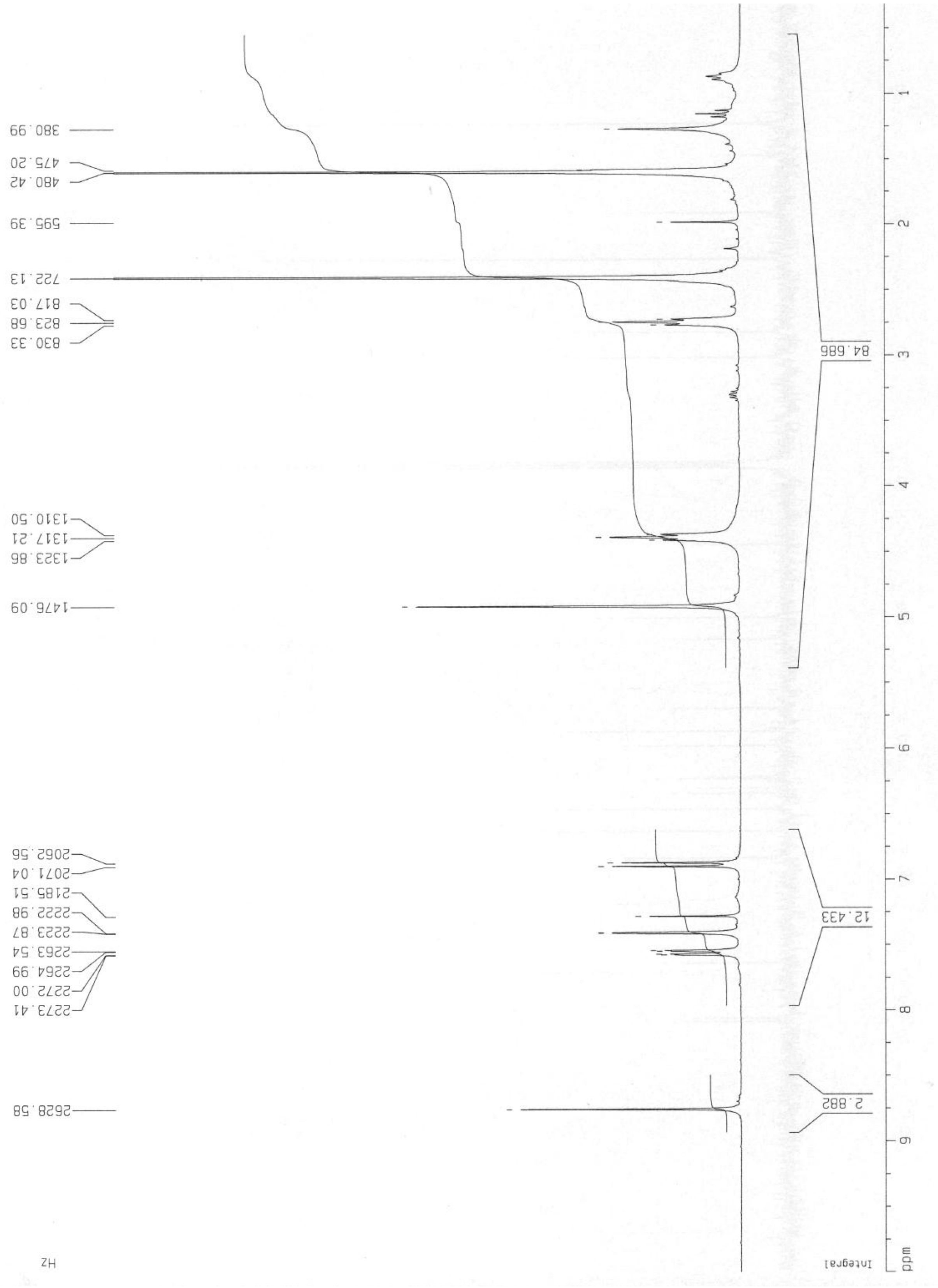
(**20**).
($CDCl_3$) $^1H$ NMR 300MHz



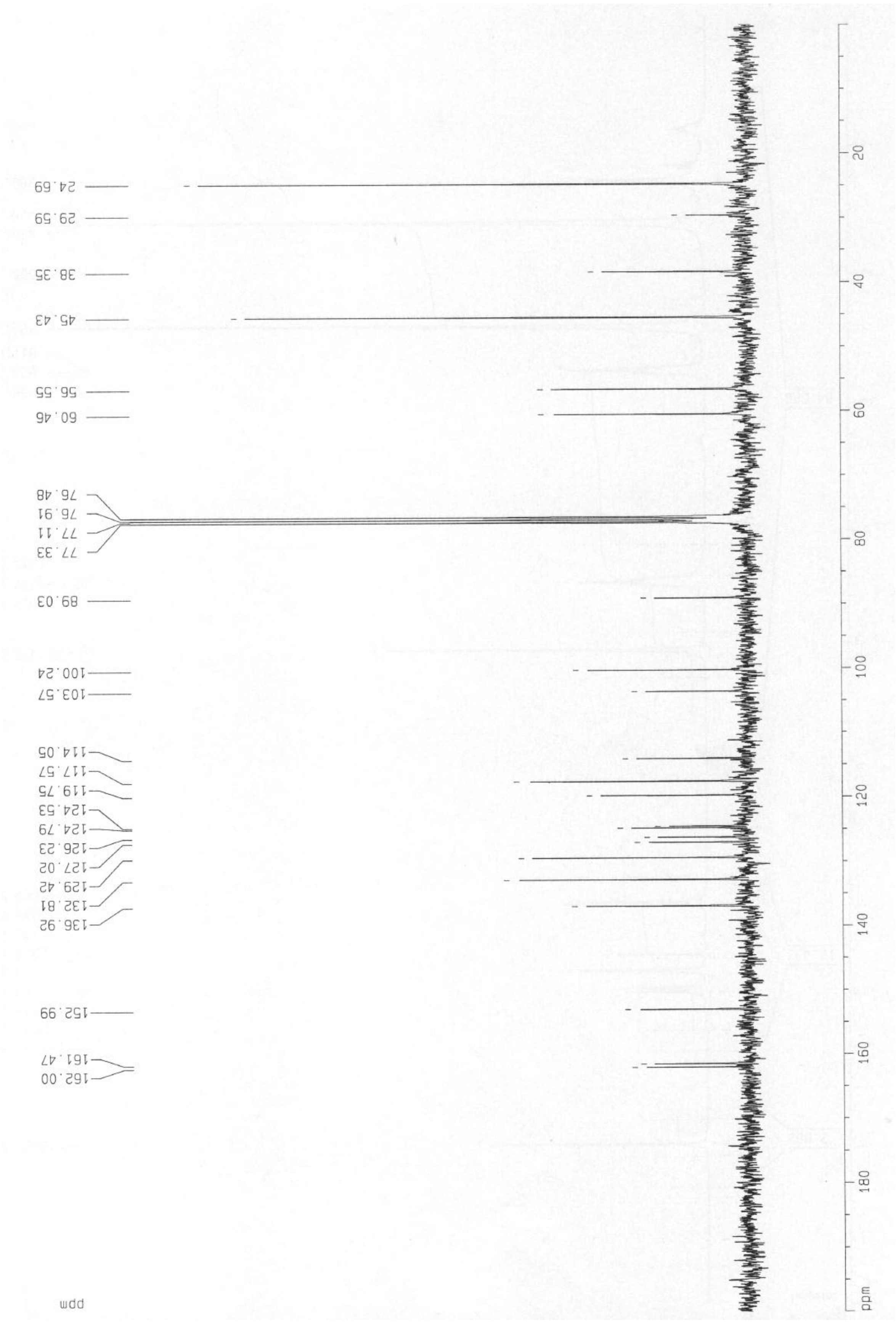
(CDCl$_3$) $^{13}$C NMR 75MHz



(**21**).
(CDCl$_3$) $^1$H NMR 300MHz



($CDCl_3$) $^{13}C$ NMR 75MHz



## Supplementary References

S1 N. Gisch, J. Balzarini and C. Meier, *J. Med. Chem.* 2007, **50**, 1658.
S2 N. Deka, A.-M. Mariotte and A. Boumendjel, *Green Chem.*, 2001, **3**, 263.
S3 L. Pehrsson, F. Ingman and A. Johansson, *Talanta* 1976, **23**, 769.
S4 P. Gans, A. Sabatini and A. Vacca, *Talanta* 1996, **43**, 1739.