Supplementary Data for:

Synthesis and DNA incorporation of an ethynyl-bridged cytosine *C*-nucleoside as guanosine surrogate

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Table of contents

Section A Experimental Procedures

General Methods and Materials S4

1'-Chloro-3',5'-di-*O*-(*p*-chlorbenzoyl)-2'-deoxy-*α*/*β*-ribose (**3**) S4

1'-Ethynyl-2'-deoxy-*α*/*β*-ribose (**4**) S5

3',5'-Di(*tert*.-butyldimethylsiloxy)-1'-ethynyl-2'-deoxy-*α*/*β*-ribose (**5**) S5

2-(Cytosine-5-yl)-1-(3',5'-di(*tert.*-butyldimethylsiloxy)-2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (**6**) S5

2-(Cytosine-5-yl)-1-(3',5'-dihydroxy)- 2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (**7**) S6

2-(Cytosine-5-yl)-1-(5'-dimethoxytrityl-3'-hydroxy)- 2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (**8**) S6

2-(*N,N*-Dimethyl amino methylene cytosine-5-yl)-1-(5'-dimethoxytrityl-3'-hydroxy)-2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (**9**) S6

2-(*N,N*-Dimethyl amino methylene cytosine-5-yl)-1-(5'-dimethoxytrityl-3'-*O*-yl-cyanoethyl-*N,N*-diisopropylphosphoramidite) (**2**) S7

Oligonucleotide synthesis, purification, and characterization S7

Section B NMR Spectra

Compound **3** ^{1}H-NMR S8

Compound **3** ^{13}C-NMR S9

Compound **4** ^{1}H-NMR S10

Compound **4** ^{13}C-NMR S11

Compound **5** ^{1}H-NMR S12

Compound **5** ^{13}C-NMR S13

Compound **6** ^{1}H-NMR S14

Compound **6** ^{13}C-NMR S15

Compound **7** ^{1}H-NMR S16

Compound **7** ^{13}C-NMR S17

Compound **8** ^{1}H-NMR S18

Compound **8** ^{13}C-NMR S19

Compound **9** ^{1}H-NMR S20

Compound **9** ^{13}C-NMR S21

Compound **2** ^{1}H-NMR S22

Compound **2** ^{13}C-NMR S23

Compound **2** ^{31}P-NMR S24

Section C UV-melting spectra

Modified oligomer **11** 0M NaCl S25

Modified oligomer **11** 150 mM NaCl S25

Modified oligomer **11** 4 M NaCl S25

Reference oligomer **13** 0 M NaCl S26

Reference oligomer **13** 150 mM NaCl S26

Reference oligomer **13** 4 M NaCl S26

Modified oligomer **12** 0 M NaCl S27

Modified oligomer **12** 150 mM NaCl S27
Modified oligomer **12** 4 M NaCl S27
Reference oligomer **14** 0 M NaCl S28
Reference oligomer **14** 150 mM NaCl S28
Reference oligomer **14** 4 M NaCl S28
Melting curves of oligomers **11**, **12** and references **13** and **14** at 0 M NaCl S29
Melting curves of oligomers **11**, **12** and references **13** and **14** at 150 mM NaCl S30
Melting curves of oligomers **11**, **12** and references **13** and **14** at 4 M NaCl S31

Section D CD spectra

Modified oligomer **11** 0 M NaCl S32
Modified oligomer **11** 1 M NaCl S32
Modified oligomer **11** 4 M NaCl S32
Reference oligomer **13** 0 M NaCl S33
Reference oligomer **13** 1 M NaCl S33
Reference oligomer **13** 4 M NaCl S33
Modified oligomer **12** 0 M NaCl S34
Modified oligomer **12** 1 M NaCl S34
Modified oligomer **12** 4 M NaCl S34
Reference oligomer **14** 0 M NaCl S35
Reference oligomer **14** 1 M NaCl S35
Reference oligomer **14** 4 M NaCl S35
CD spectra of oligomer **11** and reference DNA **13** at 150 mM NaCl S36
CD spectra of oligomer **12** and reference DNA **14** at 150 mM NaCl S37

General Methods and Materials:

Electrospray ionization mass spectra (ESI-MS) were obtained using a FINNIGAN LCQ instrument. High-resolution mass spectra (HRMS-ESI) were obtained on a BRUKER Apex-Q IV FT-ICR-MS instrument. Infrared spectra were obtained from a PERKIN ELMER type 1600 Series FT-IR. ^{1}H-NMR spectra were recorded at 300 MHz on a BRUKER Unity 300. ^{13}C-NMR spectra were recorded at 75 MHz. Chemical shifts are quoted in parts per million (ppm) downfield of tetramethylsilane (TMS). Abbreviations for multiplicities are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants (*J*) are given in Hz. Flash-column chromatography was carried out using MERCK silica gel 60 (40-60 mesh). Thin layer chromatography (TLC) was carried out using MERCK aluminium sheets of silica gel 60 F_{254}. Non-fluorescent compounds were detected using acidic solution of vanillin in ethanol. All reagents were obtained commercially. Organic solvents were distilled and/or dried prior to use. Dry tetrahydrofurane, dichloromethane, dimethylformamide, methanol were obtained commercially from FLUKA. All reactions requiring anhydrous conditions were carried out in heating-fan dried flasks under argon. Lyophylization was carried out on a CHRIST Alpha-2-4-lyophilizer or on a CHRIST RVC 2-18 vacuum centrifuge. HPLC purification of oligomers was carried out on a DuPont Series 8800 chromatograph, using a Gemini 5μ C18 (150 x 4.6mm) column with a gradient 0-60/15' (A = 50 mM TEAA, B = 50 mM TEAA/70%MeCN). UV-melting spectra of oligomers were obtained from a JASCO V-550 UV/VIS spectrometer connected to a JASCO ETC-505S/ETC-505T peltier-temperature controller using a silica macro cuvette (length = 1 cm); 3.3 µM solution of oligomer in sodium phosphate buffer. The spectrometer was permanently purged with a constant stream of nitrogen. The following temperature program was used: 20°C→85°C (6 degrees/min), 85°C→0°C (3 degrees/min), 0°C→85°C (0.4 degrees/min). CD spectra were carried out on a JASCO J-800A spectrometer, connected to a JASCO ETC-505S/ETC-505T peltier temperature controller. Parameters used for measurement: Data mode: CD, band width: 1.0 nm, response: 2 s, sensitivity: 50-100 mdeg, data pitch: 0.2 nm, scanning speed: 100 nm/min, length of silica cuvette: 1 cm, 3 accumulations; 20 µM solution of oligomer in sodium phosphate buffer. The spectrometer was permanently purged with a constant stream of nitrogen. Concentrations were calculated from UV-absorption spectra. Extinction coefficients: oligomer **11** (260 nm, 20°C): ε = 0.77576 m^2/mol; oligomer **12** (260 nm, 20°C): ε = 14.752 m^2/mol.

1'-Chloro-3',5'-di-*O*-(*p*-chlorbenzoyl)-2'-deoxy-*α*/*β*-ribose (3):

(Synthesis in analogy to Wang, Z.-X.; Duan, W.; Wiebe, L. I.; Balzarini, J.; de Clercq, E.; Knaus, E. E. *Nucleosides Nucleotides* **2001**, *20*, 11-40.)

Under an argon atmosphere 2'-deoxy-D-ribose (6.00 g) was dissolved in dry methanol (100 mL), HCl (1.50 mL of 1.25 M HCl in MeOH) was added and the resulting solution was stirred for 1 h at r.t. After neutralization with pyridine (40 mL) the solution was evaporated close to dryness and further co-evaporated with pyridine (15 mL). The residue was dissolved in pyridine (100 mL), cooled to 0°C, treated with *p*-chlorbenzoyl chloride (13.6 mL 107 mmol, 2.4 eq.) and stirred for 18 h at r.t. The slurry solution was poured into water/dichloromethane (2:3, 250 mL), the organic phase washed with sat. $NaHCO_3$ solution (2 x 20 mL), 3N H_2SO_4 (2 x 20 mL), water (2 x 20 mL), dried with $MgSO_4$ and the solvents evaporated *in vacuo*. The resulting slurry was dissolved in ether (45 mL) and AcOH (60 mL) saturated with $HCl_{(g)}$ at 0°C. The solution was treated with gaseous HCl for approximately 1 h at 0°C. A white, foamy solid precipitated which was washed with cold ether and dried *in vacuo* for 18 h, yielding **3** (13. 6 g, 32 mmol, 71 %). **^{1}H-NMR** (300 MHz, $CDCl_3$, r.t.): δ = 8.03-7.90 (m, 4 H, Ph), 7.44-7.37 (m, 4 H, Ph), 6.45 (d, $^{3}J_{H,H}$ = 5 Hz, 1 H, H1'), 5.53 (ddd, $^{3}J_{H,H}$ = 7 Hz, $^{3}J_{H,H}$ = 3 Hz, $^{3}J_{H,H}$ = 2 Hz, 1 H, H3'), 4.84-4.80 (m, 1 H, H4'), 4.66 (dd, $^{2}J_{H,H}$ = 12 Hz, $^{3}J_{H,H}$ = 4 Hz, 1 H, H5'), 4.57 (dd, $^{2}J_{H,H}$ = 12 Hz, $^{3}J_{H,H}$ = 4 Hz, 1 H, H5'), 2.83 (ddd, $^{2}J_{H,H}$ = 15 Hz, $^{3}J_{H,H}$ = 7 Hz, $^{3}J_{H,H}$ = 5 Hz, 1 H, H2'), 2.77-2.68 (m, 1 H, H2') ppm; **^{13}C-NMR** (75 MHz, $CDCl_3$, r.t.): δ = 165.4, 165.1 (**C**OO), 140.1, 139.8 (**C**$_{Ph}$-Cl), 131.4, 131.2, 131.2, 131.1, 131.0, 128.9, 128.8, 128.7, 127.8, 127.7 (Ph), 95.2, 95.1 (C1'), 84.5, 84.3 (C4'), 73.8, 73.7 (C3'), 64.4, 63.7 (C5'), 44.5, 44.3 (C2') ppm.

1'-Ethynyl-2'-deoxy-*α*/*β*-ribose (4):

Under an argon atmosphere **3** (2.00 g, 4.65 mmol) was dissolved in ethynyl magnesium bromide solution in THF (0.5 M, 112 mL), heated to reflux and stirred for 18 h at r.t. The resulting brown solution was poured into dichloromethane/sat. NH_4Cl-solution (340 mL, 1:1). The phases were separated and the water phase extracted with dichloromethane (5 x 50 mL), the combined organic phases dried over $MgSO_4$ and purified *via* flash-column chromatography, using ethyl acetate as eluent, yielding **4** as a slurry brown solid (212 mg, 1.49 mmol, 32 %). **IR** (KBr): 3398, 3290, 2931, 1684, 1592, 1424, 1323, 1176, 1092, 1044, 897, 853, 762, 669, 549, 474 cm^{-1}; **DCI-MS** *m/z*: 160.0 $[M+Na]^+$, 177.0 $[M+NH_3+NH_4]^+$, 302.1 $[2M+Na]^+$; **UV/VIS** (MeOH): λ_{max} (ε) = 236 nm; **TLC** (ethyl acetate): R_f = 0.49; **^{1}H-NMR** (300 MHz, [D_6]DMSO, 35°C): δ = 4.99 (s br, 1 H, 5'-OH), 4.62-4.53 (m, 2 H, 3'-OH, H1'), 4.12-4.07 (m, 0.5 H, H3'), 4.04-3.98 (m, 0.5 H, H3'), 3.70-3.65 (m, 1 H, H4'), 3.63-3.58 (m, 0.5 H, H4'), 3.48-3.19 (m, 3 H, H2, H5'), 2.44-2.34 (m, 0.5 H, H2'), 2.03-1.87 (m, 1 H, H2'), 1.83-1.74 (m, 0.5 H, H2') ppm; **^{13}C-NMR** (75 MHz, [D_6]DMSO, 35°C): δ = 87.6, 85.8 (C4'), 84.7, 83.8 (C1), 75.8, 75.4 (C2), 71.7, 70.9 (C3'), 66.3, 66.0 (C1'), 62.1, 61.3 (C5'), 41.8, 41.7 (C2') ppm.

3',5'-Di(*tert.*-butyldimethylsiloxy)-1'-ethynyl-2'-deoxy-*α*/*β*-ribose (5):

Under an argon atmosphere **4** (151 mg, 1.06 mmol) was dissolved in dry DMF (2.6 mL), treated with imidazolde (362 mg, 5.31 mmol, 5 eq.), cooled to 0°C, treated with TBDMS-Cl (480 mg, 3.19 mmol, 3 eq.) and strirred for 12 h at r.t. The reaction was quenched with water (30 mL), extracted with ether (5 x 30 ml), the combined organic phases dried over $MgSO_4$ and the solvent evaporated *in vacuo*, yielding **5** as a brown oil (429 mg, 1.06 mmol, 100 %). **IR** (KBr): 3425, 2939, 2858, 2372, 2086, 1722, 1684, 1590, 1470, 1401, 1256, 1093, 934, 838, 779, 710, 672, 549 cm^{-1}; **ESI-MS** *m/z*: 393.3 $[M+Na]^+$, 763.0 $[2M+Na]^+$; **^{1}H-NMR** (300 MHz, $CDCl_3$, r.t.): δ = 4.74-4.66 (m, 1 H, H1'), 4.38-4.32 (m, 1 H, H3'), 4.91 (dd, $^3J_{H,H}$ = 8 Hz, $^3J_{H,H}$ = 4 Hz, 0.5 H, H4'), 3.80 (ddd, $^3J_{H,H}$ = 7 Hz, $^3J_{H,H}$ = 4 Hz, $^3J_{H,H}$ = 2 Hz, 0.5 H, H4'), 3.69-3.63 (m, 1.5 H, H5'), 3.45 (dd, $^2J_{H,H}$ = 11 Hz, $^3J_{H,H}$ = 7 Hz, 0.5 H, H5'), 2.44 (d, $^3J_{H,H}$ = 2 Hz, 0.5 H, H2'), 2.43 (d, $^4J_{H,H}$ = 2 Hz, 0.5 H, H2'), 2.38 (ddd, $^2J_{H,H}$ = 14 Hz, $^3J_{H,H}$ = 7 Hz, $^3J_{H,H}$ = 7 Hz, 0.5 H, H2'), 2.06-1.98 (m, 1.5 H, H2'), 0.88-0.86 (m, 18 H, *t*-Bu), 0.05-0.03 (m, 12 H, CH_3Si) ppm; **^{13}C-NMR** (75 MHz, $CDCl_3$, r.t.): δ = 86.7, 86.2 (C4'), 84.1, 82.8 (C1), 74.4, 74.3 (C2), 73.8, 73.4 (C3'), 67.4, 67.2 (C1'), 64.0, 63.8 (C5'), 41.8, 41.6 (C2'), 25.9, 25.6 (C**C**H_3), 18.3, 18.2 (**C**CH_3), -3.6, -5.5 (CH_3Si) ppm.

2-(Cytosine-5-yl)-1-(3',5'-di(*tert.*-butyldimethylsiloxy)-2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (6):

Under an argon atmosphere 5-iodocytidine (756 mg, 3.19 mmol) was suspended in HMDS (32 mL), treated with $(NH_4)_2SO_4$ (100 mg) and refluxed until it got a clear colorless solution. The solution was cooled to -20°C, the precipitated residue filtered off and dried *in vacuo* for 1 h. Under argon atmosphere **5** (1.42 g, 3.83 mmol, 1.2 eq.) was dissolved in dry DMF (6.38 mL), treated with DIPEA (536 μl 4.15 mmol, 1.3 eq.), CuI (121.6 mg, 638 μmol, 0.2 eq.), Pd[0]$(Ph_3)_4$ (369 mg, 319 μmol, 0.1 eq) and the silylated cytidine was added to the solution. The solution was briefly heated with a hot fan and then stirred for 12 h at r.t. The solvent was evaporated *in vacuo* and the residue purified *via* flash-column chromatography, using a gradient of MeOH:DCM 0:100, 2:98, 5:95, 10:90, yielding **6** as a bright yellow solid (847 mg, 1.76 mmol, 55 %). **IR** (KBr): 3439, 3264, 3058, 3010, 2912, 2874, 1655, 1550, 1508, 1450, 1400, 1295, 1225, 1144, 1093, 1030, 987, 954, 922, 799, 719, 654, 532 cm^{-1}; **ESI-MS** *m/z*: 480.5 $[M+H]^+$, 502.5 $[M+Na]^+$, 959.3 $[2M+H]^+$, 981.3 $[2M+Na]^+$, 1438.2 $[3M+Na]^+$; **HR-ESI-MS**: *m/z* $[M+H]^+$ = calc.: 480.27084, found: 480.27072; **UV/VIS** (MeOH): λ_{max} (ε) = 239, 295 nm; **TLC** (ethyl acetate : methanol 8:1): R_f = 0.44; **^{1}H-NMR** (300 MHz, [D_6]DMSO, 35°C): δ = 7.71-7.60 (m, 2 H, NH, H6), 7.43 (s br, 2 H, NH_2), 4.91-4.84 (m, 1 H, H1'), 4.42-4.27 (m, 1 H, H3'), 3.79 (ddd, $^3J_{H,H}$ = 4 Hz, $^3J_{H,H}$ = 4 Hz, $^3J_{H,H}$ = 4 Hz, 0.5 H, H4'), 3.71-3.65 (m, 0.5 H, H4'), 3.63-3.45 (m, 2 H, H5'), 2.45 (ddd, $^2J_{H,H}$ = 14 Hz, $^3J_{H,H}$ = 7 Hz, $^3J_{H,H}$ = 7 Hz, 0.5 H, H2'), 2.21-1.96 (m, 1.5 H, H2'), 0.87 (s, 18 H, *t*-Bu), 0.07-0.04 (m, 12 H, CH_3Si) ppm; **^{13}C-NMR** (75 MHz, [D_6]DMSO, 35°C): δ = 162.2 (C4), 154.9 (C2), 146.6

(C6), 102.5 (C5), 94.9, 93.6 (C1''), 87.1, 85.6 (C4'), 76.9, 76.3 (C2''), 73.5, 72.3 (C3'), 67.5, 67.2 (C1'), 63.2, 62.5 (C5'), 41.6, 41.4 (C2'), 25.7, 25.6 (C**C**H_3), 17.9, 17.6 (**C**CH_3), -4.8, -5.0, -5.0, -5.5, -5.5, -5.6 (CH_3Si) ppm.

2-(Cytosine-5-yl)-1-(3',5'-dihydroxy)- 2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (7):

C-Nucleoside **6** (200 mg, 418 μmol) was dissolved in AcOH (80 % in water, 5 mL) and stirred for 3 d at 40°C. The solvent was evaporated *in vacuo* and the residue purified *via* flash-column chromatography, using MeOH : DCM 1:4 as eluent, yielding **7** as a yellow solid (76.0 mg, 304 μmol, 73 %), **7** (24.5 mg, 51 μmol, 12 %), and 5' deprotected nucleoside (39.0 mg, 10 μmol, 2 %). **IR** (KBr): 3385, 3070, 2975, 2938, 2879, 2803, 2757, 2739, 2677, 2602, 2529, 2491, 2355, 2238, 2136, 1938, 1649, 1566, 1509, 1476, 1435, 1398, 1384, 1365, 1331, 1290, 1249, 1216, 1172, 1072, 1036, 942, 904, 850, 806, 760, 745, 704, 587, 532, 509, 470, 461 cm^{-1}; **ESI-MS**: *m/z* = 252.10 $[M+H]^+$, 503.19 $[2M+H]^+$; **HR-ESI-MS**: *m/z* $[M+H]^+$ = calc.: 252.09788, found: 252.09784; **UV/VIS** (MeOH): λ_{max} (ε) = 256, 280 nm; **TLC** (MeOH : DCM 1:9): R_f = 0.19; **^{1}H-NMR** (300 MHz, [D_6]DMSO, 35°C): δ = 7.86 (d, $^3J_{H,H}$ = 6.0 Hz, 1 H, H6), 6.78 (s, 1 H, NH), 4.87 (m, 1 H, H1'), 4.15-4.11 (m, 1 H, H3'), 3.85-3.80 (m, 0.66 H, H4'), 3.66-3.62 (m, 0.33 H, H4'), 3.39-3.33 (m, 2 H, H5'), 2.30-2.26 (m, 0.61 H, H2'), 2.23 (m, 1.39 H, H2') ppm; **^{13}C-NMR** (75 MHz, [D_6]DMSO, 35°C): δ = 162.5 (C4), 155.7 (C2), 147.4, 146.6 (C6), 104.4, 102.0 (C5) 94.6 (C1''), 88.1, 87.5, 86.7 (C4'), 76.6, 76.5 (C2''), 71.8 (C3'), 67.3, 67.2 (C1'), 63.2, 63.0, 62.9 (C5'), 41.8 (C2') ppm.

2-(Cytosine-5-yl)-1-(5'-dimethoxytrityl-3'-hydroxy)-2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (8):

Under an argon atmosphere **7** (468 mg, 1.87 mmol) was co-evaporated with dry pyridine (20 mL), dissolved in dry pyridine (11 mL), treated with DMAP (46 mg, 373 μmol, 0.2 eq.), DMT-Cl (814 mg, 2.43 mmol, 1.3 eq.), and stirred for 6 h at r.t. and the solvent evaporated *in vacuo*. The residue was purified *via* flash-column chromatography, using MeOH : DCM : TEA 1:9:0.2 as eluent, yielding **8** as a bright yellow solid (905 mg, 1.64 mmol, 88 %). **IR** (KBr): 3418, 3192, 2959, 2941, 2878, 2740, 2677, 2624, 2603, 2531, 2493, 2358, 2250, 2139, 1941, 1915, 1645, 1609, 1561, 1508, 1476, 1445, 1398, 1384, 1365, 1331, 1290, 1250, 1214, 1173, 1074, 1036, 943, 882, 851, 828, 807, 762, 740, 701, 685, 673, 660, 647, 634, 621, 595, 583, 570, 557, 544, 532, 517, 507, 494, 462, 443, 430, 418 cm^{-1}; **ESI-MS**: *m/z* = 552.3 $[M-H]^-$, 588.1 $[M+Cl]^-$, 1105.2 $[2M-H]^-$, 1140.9 $[2M+Cl]^-$, 576.2 $[M+Na]^+$, 1129.1 $[2M+Na]^+$; **HR-ESI-MS**: *m/z* $[M+H]^+$ = calc. 554.22856, found 554.22855; $[M+Na]^+$ = calc. 576.21051, found: 576.21043; $[M+K]^+$ = calc.: 592.18444, found: 592.18439; **UV/VIS** (MeOH): λ_{max} (ε) = 255, 280 nm; **TLC** (MeOH : DCM : TEA 1:9:0.2): R_f = 0.42; **^{1}H-NMR** (300 MHz, [D_6]DMSO, 35°C): δ = 10.80 (s br, 1 H, NH), 7.52 (s, 1 H, H6), 7.48-7.18 (m, 9 H, Ph), 6.88 (d, $^3J_{H,H}$ = 3 Hz, 4 H, Ph), 5.31 (s br, 1 H, 3'OH), 4.96-4.91 (m, 1 H, H1'), 4.14 (m, 1 H, H3'), 3.84-3.80 (m, 1 H, H4'), 3.72 (d, $^3J_{H,H}$ = 1.2 Hz, 6 H, PhO**C**H_3), 3.07-2.95 (m, 2 H, H5'), 2.20-2.45 (m, 2 H, H2') ppm; **^{13}C-NMR** (75 MHz, [D_6]DMSO, 35°C): δ = 165.0, 162.2 (C4), 158.0, 154.9 (C2), 146.7, 144.8 (C6), 135.7, 135.6, 129.6, 129.6, 127.7, 127.7, 126.5, 113.0 (Ph), 94.2 (C1''), 87.7 (C5), 85.5 (C4'), 85.3 (**C**$(Ph)_3$), 76.7 (C2''), 72.1 (C3'), 67.5 (C1'), 64.4 (C5'), 54.9 (O**C**H_3), 41.7 (C2') ppm.

2-(*N,N*-Dimethyl amino methylene cytosine-5-yl)-1-(5'-dimethoxytrityl-3'-hydroxy)-2'-deoxy-*α*/*β*-pentofuranosyl)ethyne (9):

Under an argon atmosphere **8** (104 mg, 200 μmol) was dissolved in dry DMF (1.06 mL), treated with *N,N*-dimethylformamide dimethylacetal (250 μL, 2.00 mmol, 10 eq.), stirred for 4 h at r.t., cooled to 0°C, quenched with ice water and the solvents were evaporated *in vacuo*. The residue was purified *via* flash-column chromatography, using MeOH : DCM : TEA 5:95:0.2 as eluent, yielding **9** as a bright yellow solid (93 mg, 153 μmol, 76 %). **IR** (KBr): 3385, 3061, 2931, 2836, 2229, 2044, 1908, 1732, 1651, 1603, 1509, 1488, 1449, 1425, 1411, 1378, 1331, 1303, 1249, 1176, 1114, 1081, 1033, 904, 880, 830, 791, 756, 727, 703, 665, 635, 584, 475 cm^{-1}; **ESI-MS**: *m/z* = 609.27 $[M+H]^+$; **HR-ESI-MS**: *m/z* $[M+H]^+$ = calc.: 609.27076, found: 609.27081;

UV/VIS (MeOH): λ_{max} (ε) = 238, 311 nm; **TLC** (MeOH : DCM : TEA 5:95:0.2): R_f = 0.10; **^{1}H-NMR** (300 MHz, [D_6]DMSO, 35°C): δ = 11.40 (s br, 1 H, NH), 8.54 (s, 1 H, Me_2N**CH**=NR, 7.69 (s, 1 H, H6), 7.42-7.20 (m, 9 H, Ph), 6.87 (d, $^3J_{H,H}$ = 1.8 Hz, 4 H, Ph), 5.07 (d, $^3J_{H,H}$ = 3.9 Hz, 1 H, 3'OH), 4.90 (t, $^3J_{H,H}$ = 7.5 Hz, 1 H, H1'), 4.17-4.15 (m, 1 H, H3'), 3.84-3.80 (m, 1 H, H4'), 3.71 (d, $^3J_{H,H}$ = 2.1 Hz, 6 H, PhO**CH$_3$**), 3.13 (t, $^2J_{H,H}$ = 12.6 Hz, 3 H, **CH$_3$**NMeR), 3.02-2.98 (m, 2 H, H5'), 2.93 (s, 3 H, **CH$_3$**NMeR), 2.09 (dd, $^3J_{H,H}$ = 7.8 Hz, 4.5 Hz, 2 H, H2') ppm; **^{13}C-NMR** (75 MHz, [D_6]DMSO, 35°C): δ = 158.0 (C4), 157.5 (C=**C**HNMe$_2$), 157.4 (C2), 144.9 (C6), 135.7, 135.6, 129.7, 129.6, 127.7, 126.5, 113.1 (Ph), 113.0 (C5) 91.5 (C1''), 85.4 (C4'), 85.3 (**C**(Ph)$_3$), 79.4 (C2''), 72.1 (C3'), 67.4 (C1'), 64.5 (C5'), 54.9 (O**C**H$_3$), 42.1 (C2'), 40.6 (**C**H$_3$NMeR), 34.4 (**C**H$_3$NMeR) ppm.

2-(*N,N*-Dimethyl amino methylene cytosine-5-yl)-1-(5'-dimethoxytrityl-3'-*O*-yl-cyanoethyl-*N,N*-diisopropylphosphoramidite) (2):

Under an argon atmosphere **9** (65 mg, 107 μmol) was dissolved in dry DCM (1.00 mL), treated with DIPEA (26 μl, 150 μmol, 1.3 eq.), *N,N*-diisopropylidenephosphoramidite (26 μl, 117 μmol, 1.1 eq.), stirred for 3 h at r.t. and the solvent evaporated *in vacuo*. The residue was purified *via* flash-column chromatography, using MeOH : DCM : TEA 5:95:0.2 as eluent, yielding **2** as a bright yellow solid (38.1 mg, 47 μmol, 44 %, 30 % of **9** could be recovered). **IR** (KBr): 3393, 3049, 2987, 2942, 2880, 2778, 2741, 2584, 2505, 2409, 2347, 2250, 2126, 1871, 1794, 1677, 1609, 1509, 1492, 1468, 1447, 1427, 1399, 1317, 1296, 1248, 1207, 1180, 1154, 1126, 1102, 1074, 1063, 998, 935, 830, 800, 768, 721, 703 cm^{-1}; **ESI-MS**: *m/z* = 809.38 [M+H]$^+$; **HR-ESI-MS**: *m/z* [M+H]$^+$ = calc.: 809.37861, found: 809.37825; **UV/VIS** (MeOH): λ_{max} (ε) = 229, 276, 283 nm; **$[\alpha]_D^{20}$** = +12.4°; **TLC** (MeOH : DCM : TEA 1:9:0.2): R_f = 0.69; 11.40 (s br, 1 H, NH), 8.58 (s, 0.5 H, Me_2N**CH**=NR), 8.54 (s, 0.5 H, Me_2N**CH**=NR), 7.78 (s, 0.5 H, H6), 7.70 (s, 0.5 H, H6), 7.44-7.14 (m, 9 H, Ph), 6.93-6.80 (m, 4 H, Ph), 5.00 (t, $^3J_{H,H}$ = 7.5 Hz, 0.5 H, H1'), 4.90 (t, $^3J_{H,H}$ = 7.5 Hz, 0.5 H, H1'), 4.13-4.00 (m, 1 H, H3'), 4.00-3.80 (m, 1 H, H4'), 3.72 (d, $^3J_{H,H}$ = 2.1 Hz, 6 H, PhO**CH$_3$**), 3.60-3.44 (m, 2 H, O**CH$_2$**CH$_2$CN), 3.13 (t, $^2J_{H,H}$ = 12.6 Hz, 3 H, **CH$_3$**NMeR), 3.05-2.97 (m, 2 H, H5'), 2.92 (s, 3 H, **CH$_3$**NMeR), 2.77-2.42 (m, 2 H, H2'), 1.26-0.90 (m, 14 H, *i*-Pr) ppm; **^{13}C-NMR** (75 MHz, [D_6]DMSO, 35°C): δ = 158.0, 158.0 (C4), 157.5 (C=**C**HNMe$_2$), 157.4 (C=**C**HNMe$_2$) 155.1 (C2), 144.7, 144.6 (C6), 135.6, 135.5, 135.5, 135.4, 129.6, 129.6, 127.7, 127.7, 126.6, 118.8, 118.8 (**C**N), 113.1 (Ph), 112.9 (C5) 92.0, 91.6 (C1''), 85.5, 85.4 (**C**(Ph)$_3$), 83.1 (C4'), 79.8, 79.5 (C2''), 74.6, 74.5 (C3'), 67.7, 67.5 (C1'), 63.8, 63.4 (C5'), 58.3, 58.2 (O**C**H$_2$CH$_2$CN), 55.0, 54.9 (O**C**H$_3$), 42.6, 42.6, 42.5, 42.5 (N**C**(CH$_3$)$_2$), 41.2, 41.1 (C2'), 40.6, 40.6 (**C**H$_3$NMeR), 34.6, 34.5 (**C**H$_3$NMeR), 24.2, 24.1 (OCH$_2$**C**H$_2$CN), 19.8, 19.7, 19.7, 19.6 (C(**C**H$_3$)$_2$) ppm; **^{31}P-NMR** (122 MHz, [D_6]DMSO, 35°C): δ = 148.7, 149.6 ppm.

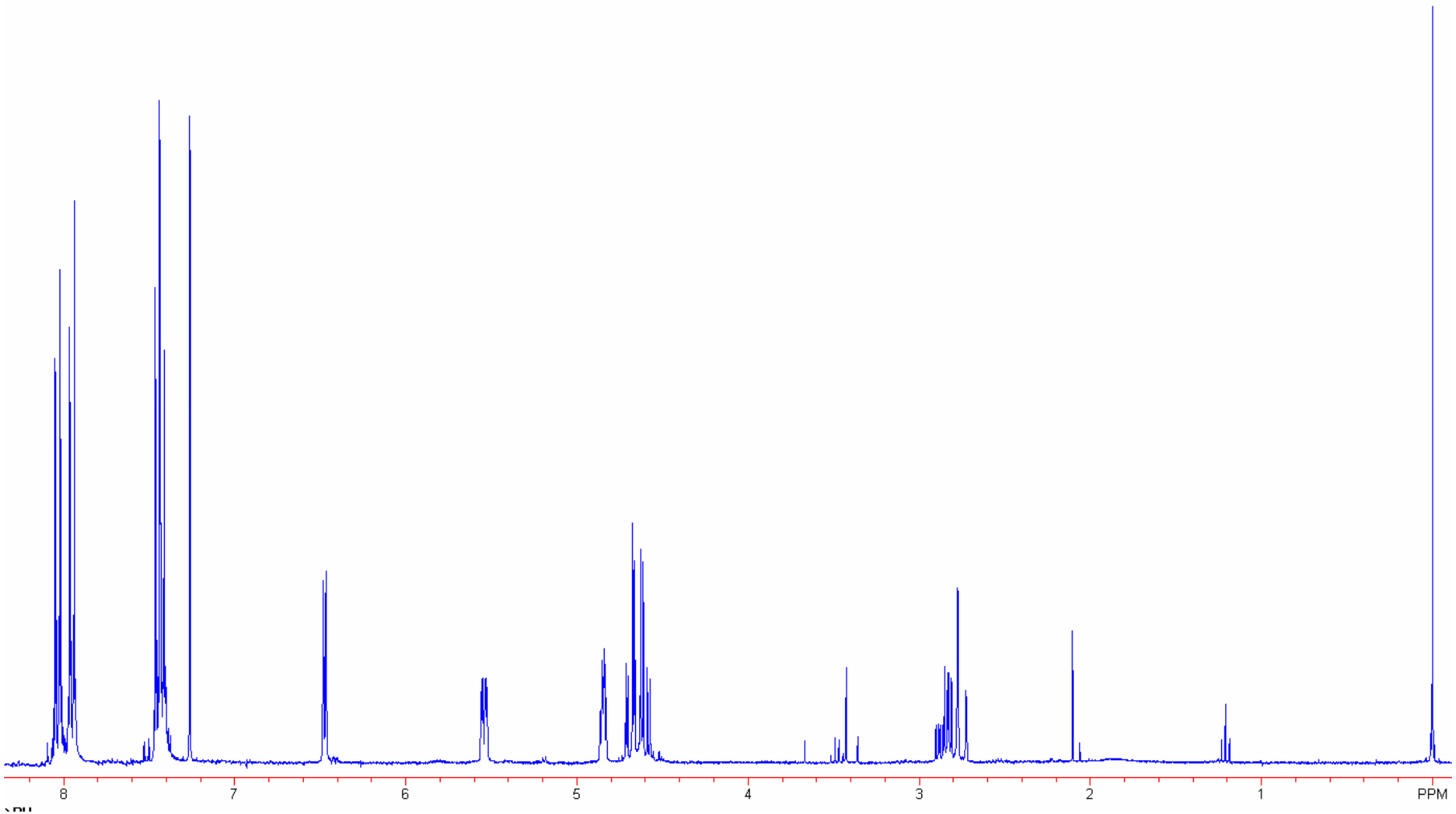
Oligomer Synthesis, purification and characterization

Oligomer synthesis was done by IBA BioTAGnology, Göttingen *via* solid-phase DNA synthesis. Purification was done *via* HPLC (see above) by IBA.

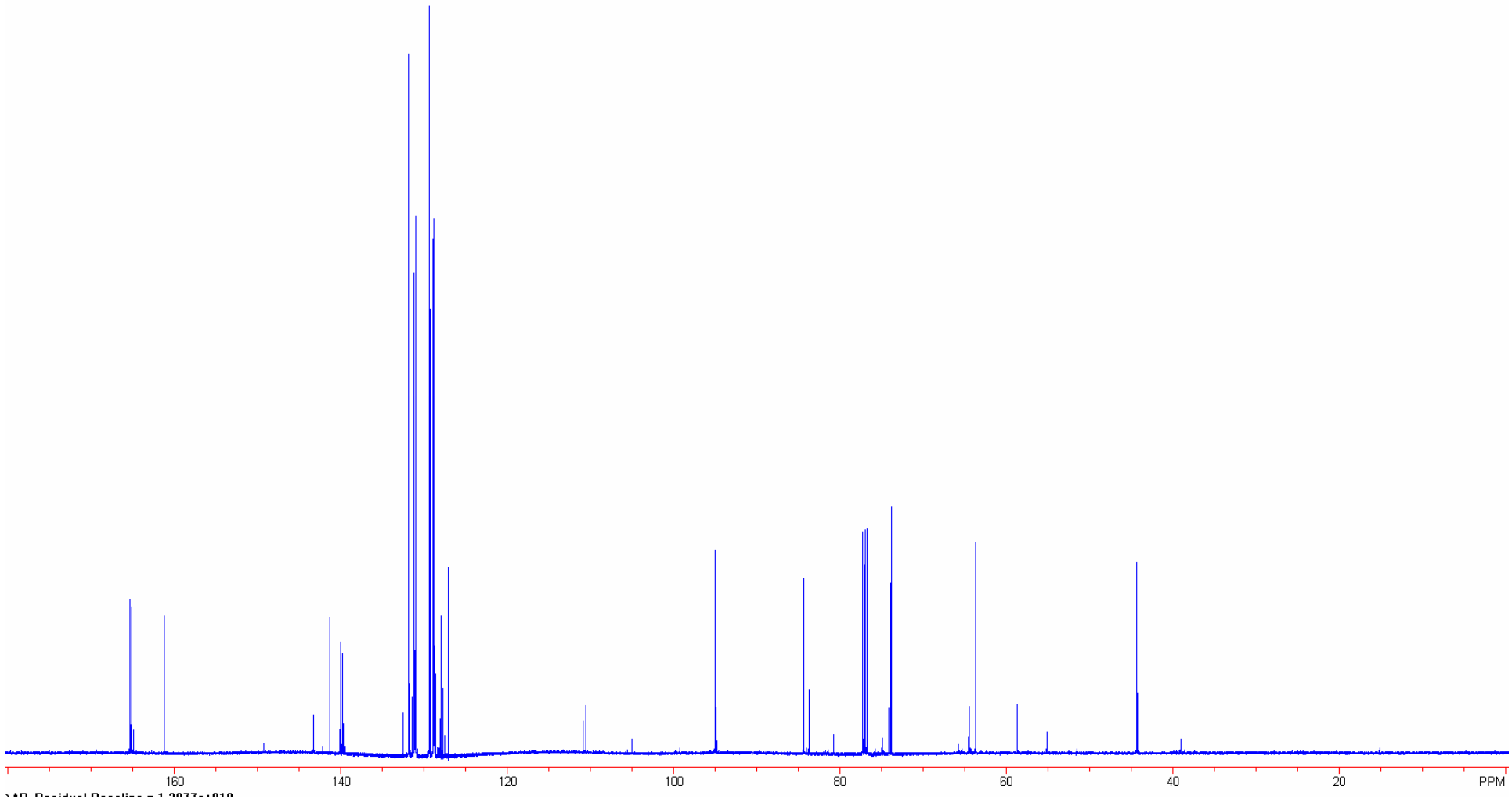
Oligomer **11**: **ESI-MS**: *m/z* = 920.14 [M+Na]$^{2-}$; **HR-ESI-MS**: *m/z* [M]$^{2-}$ = calc.: 920.13302, found: 920.13434; R_t = 11.90 min.

Oligomer **12**: **ESI-MS**: *m/z* = 3614.67 [M]$^-$, 3636.65 [M-H+Na]$^-$, 3658.63 [M-2H+2Na]$^-$, 3680.62 [M-3H+3Na]$^-$, 3702.60 [M-4H+4Na]$^-$, 3724.59 [M-5H+5Na]$^-$, 3747.58 [M-6H+6Na]$^-$; R_t = 11.77 min.

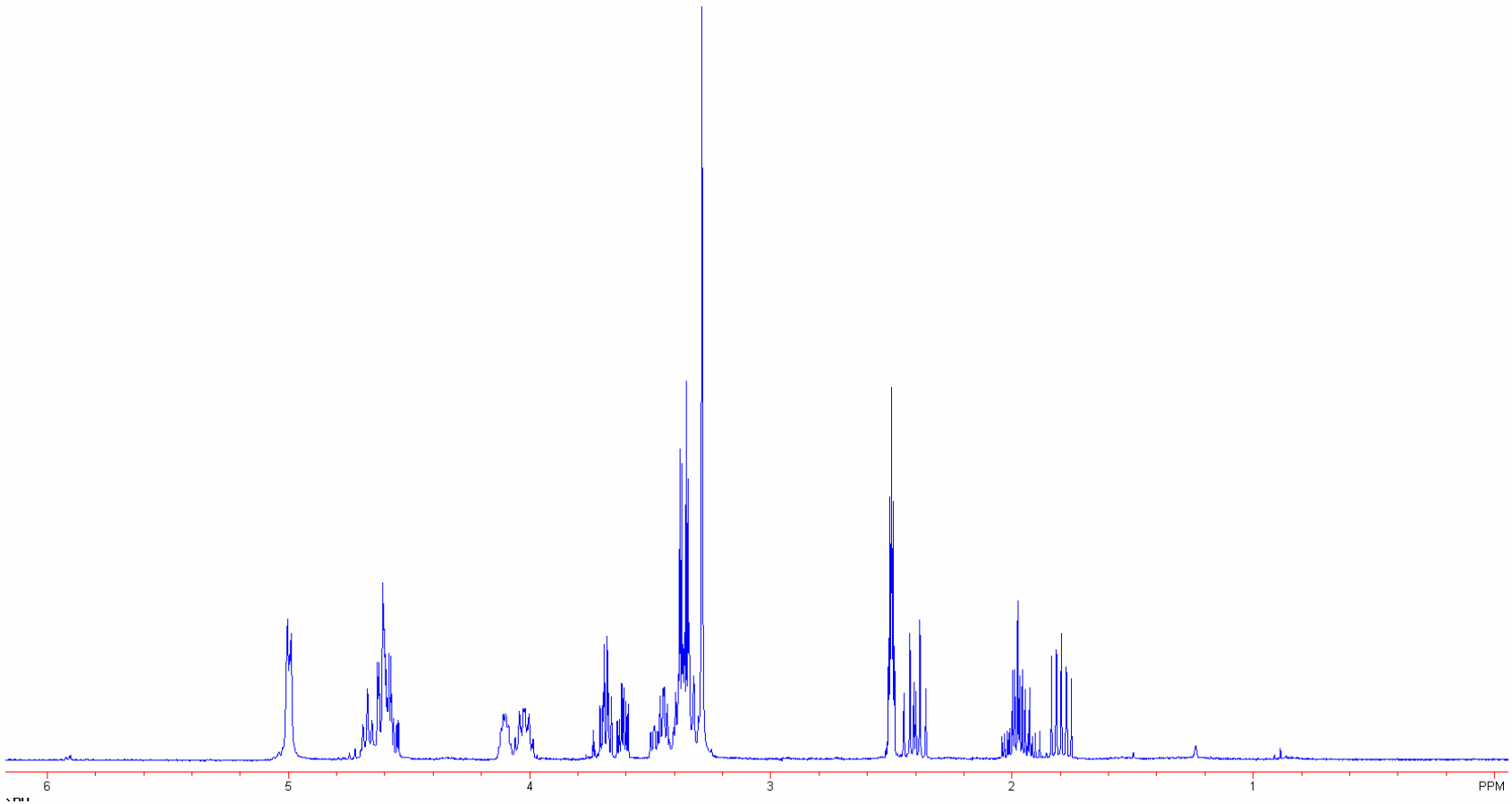
^{1}H-NMR spectrum of **3**; Solvent: $CDCl_3$; 300.54 MHz Instrument: Bruker Unity 300



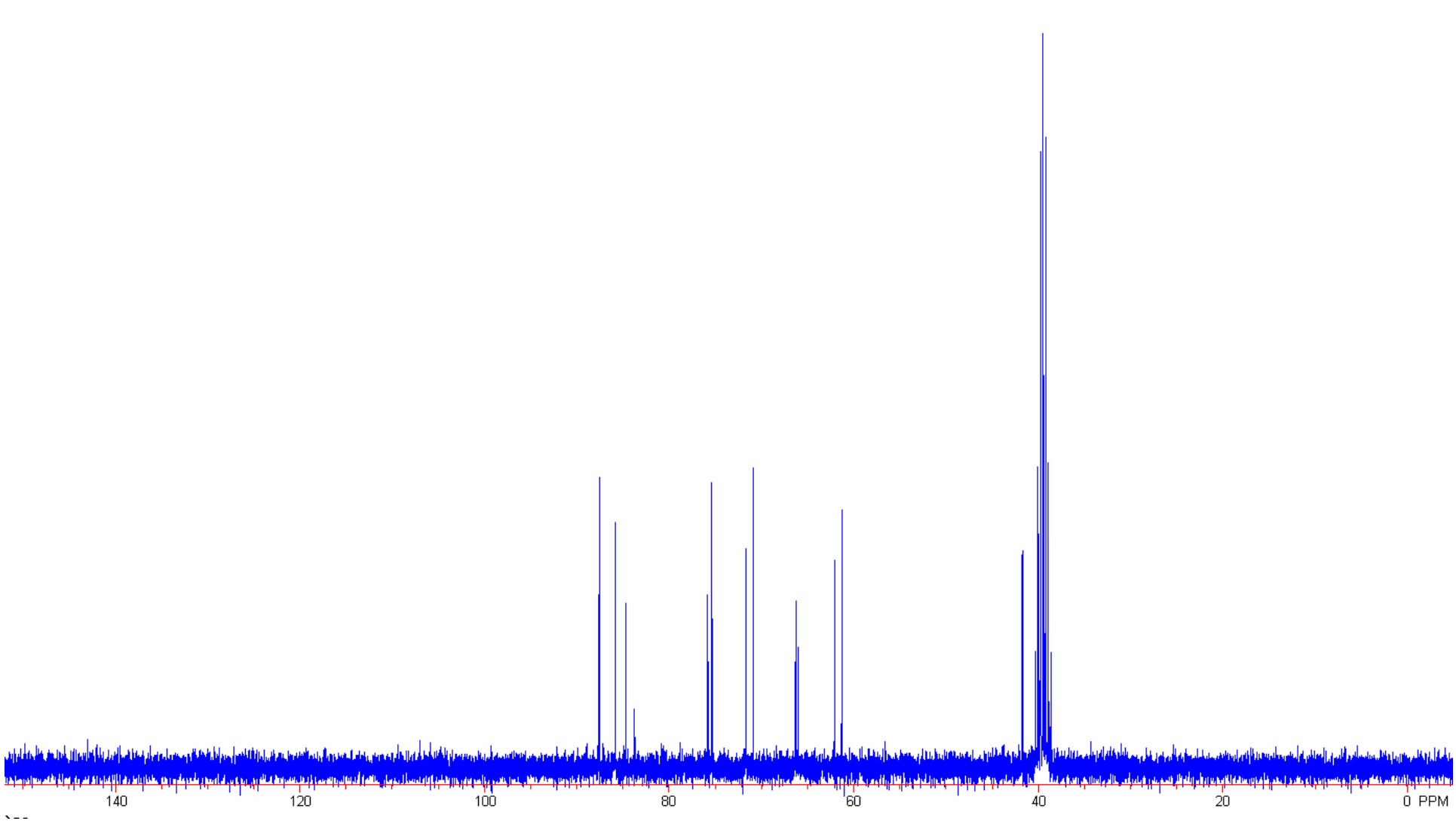
^{13}C-NMR spectrum of **3**; Solvent: $CDCl_3$: 75.58 MHz; Instrument: Bruker Unity 300



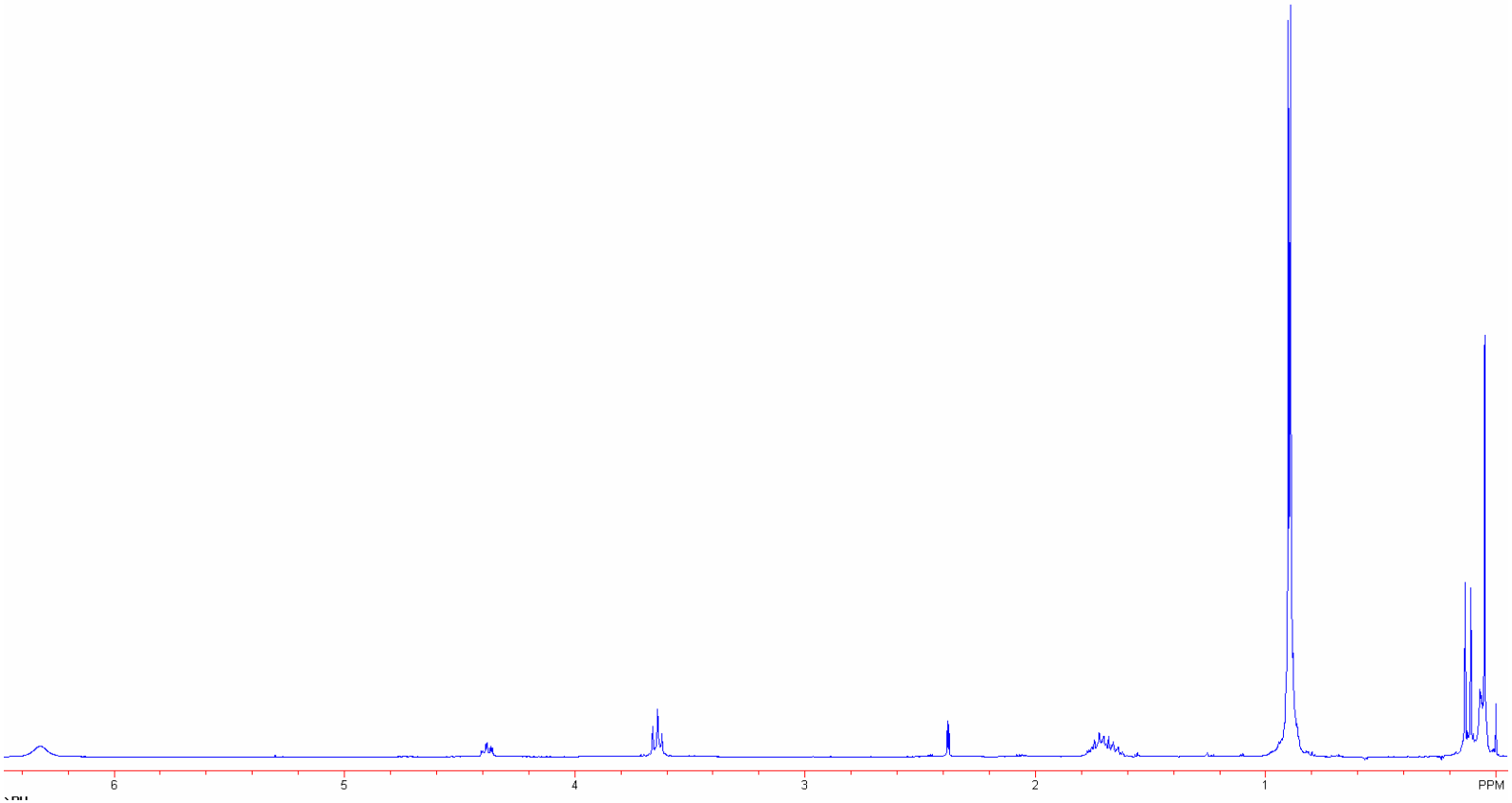
^{1}H-NMR spectrum of **4**; Solvent: DMSO; 300.52 MHz Instrument: Bruker Unity 300



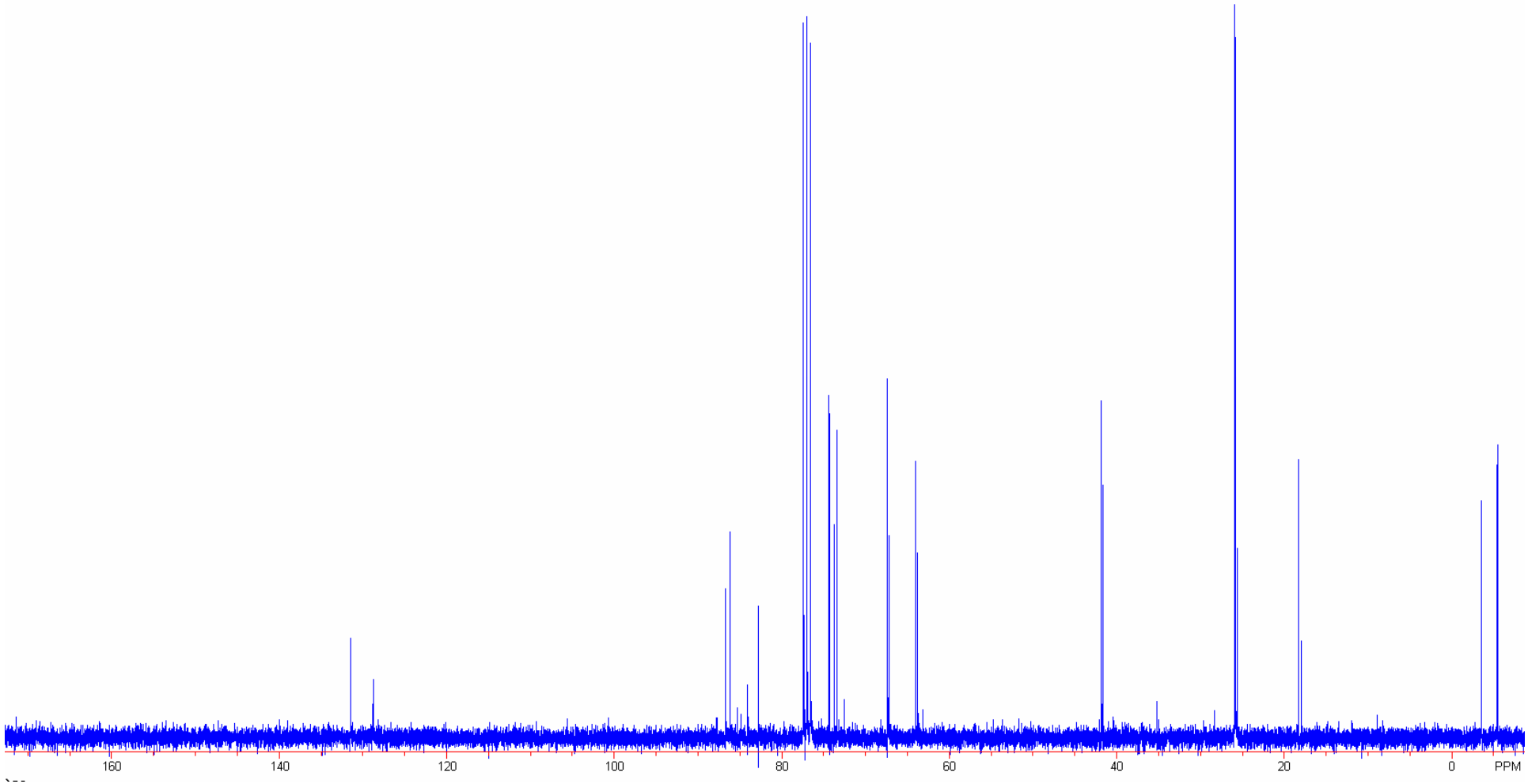
^{13}C-NMR spectrum of **4**; Solvent: DMSO: 75.54 MHz; Instrument: Bruker Unity 300



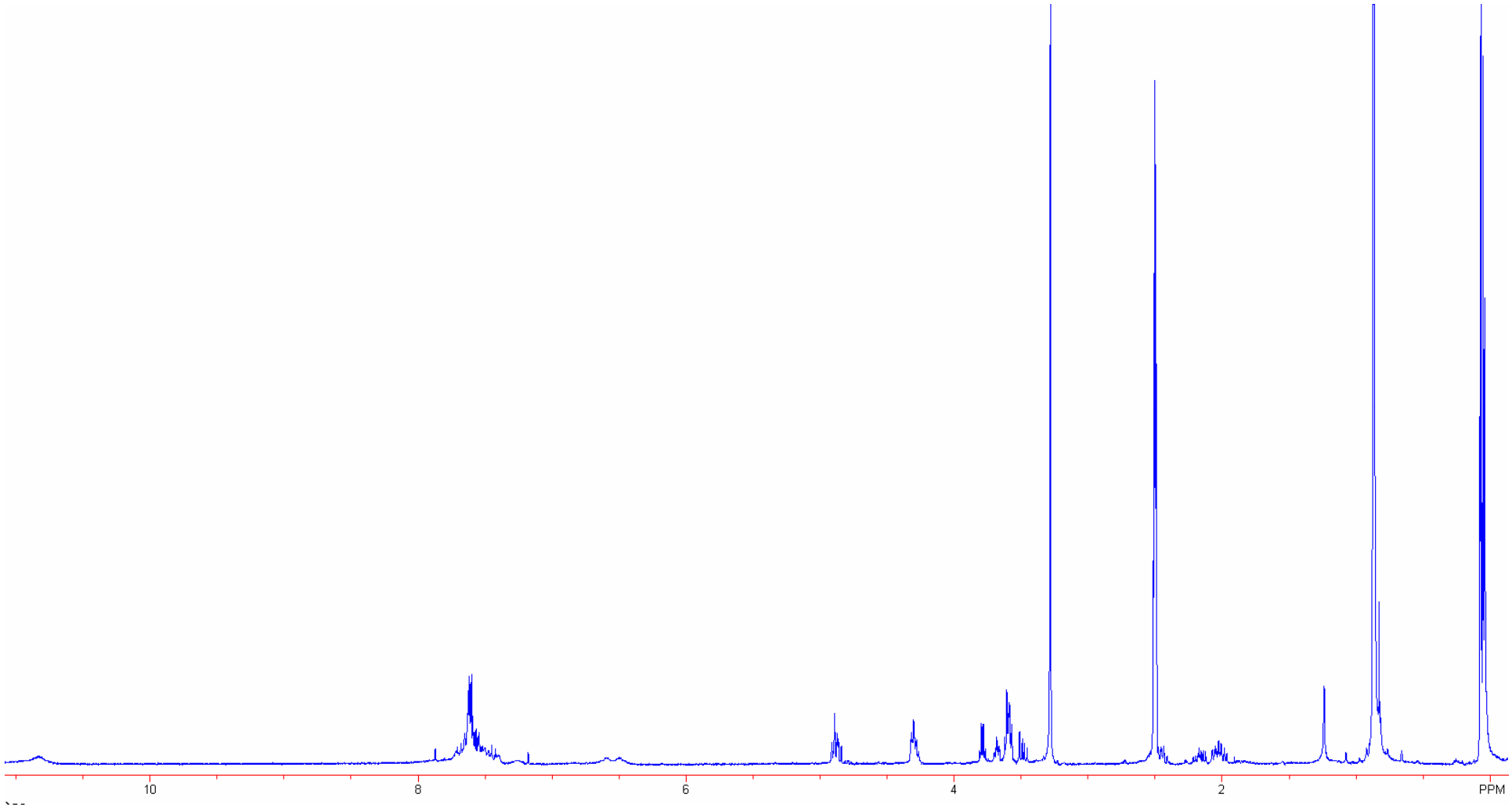
^{1}H-NMR spectrum of **5**; Solvent: $CDCl_3$; 300.48 MHz Instrument: Bruker Unity 300



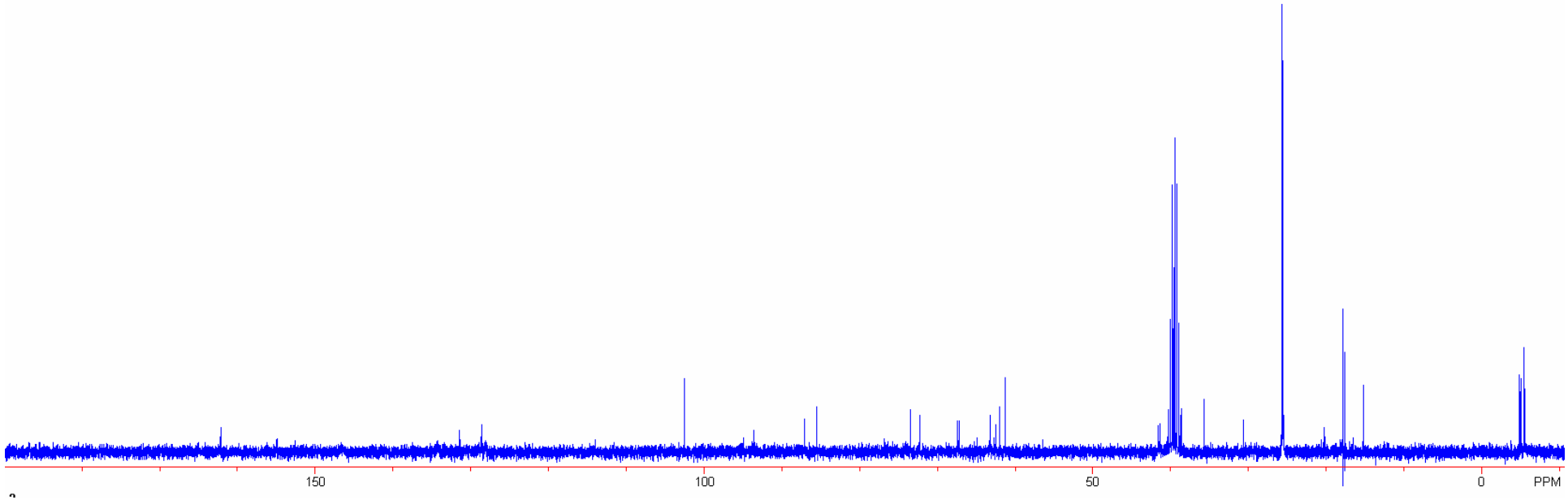
^{13}C-NMR spectrum of **5**; Solvent: $CDCl_3$: 75.48 MHz; Instrument: Bruker Unity 300



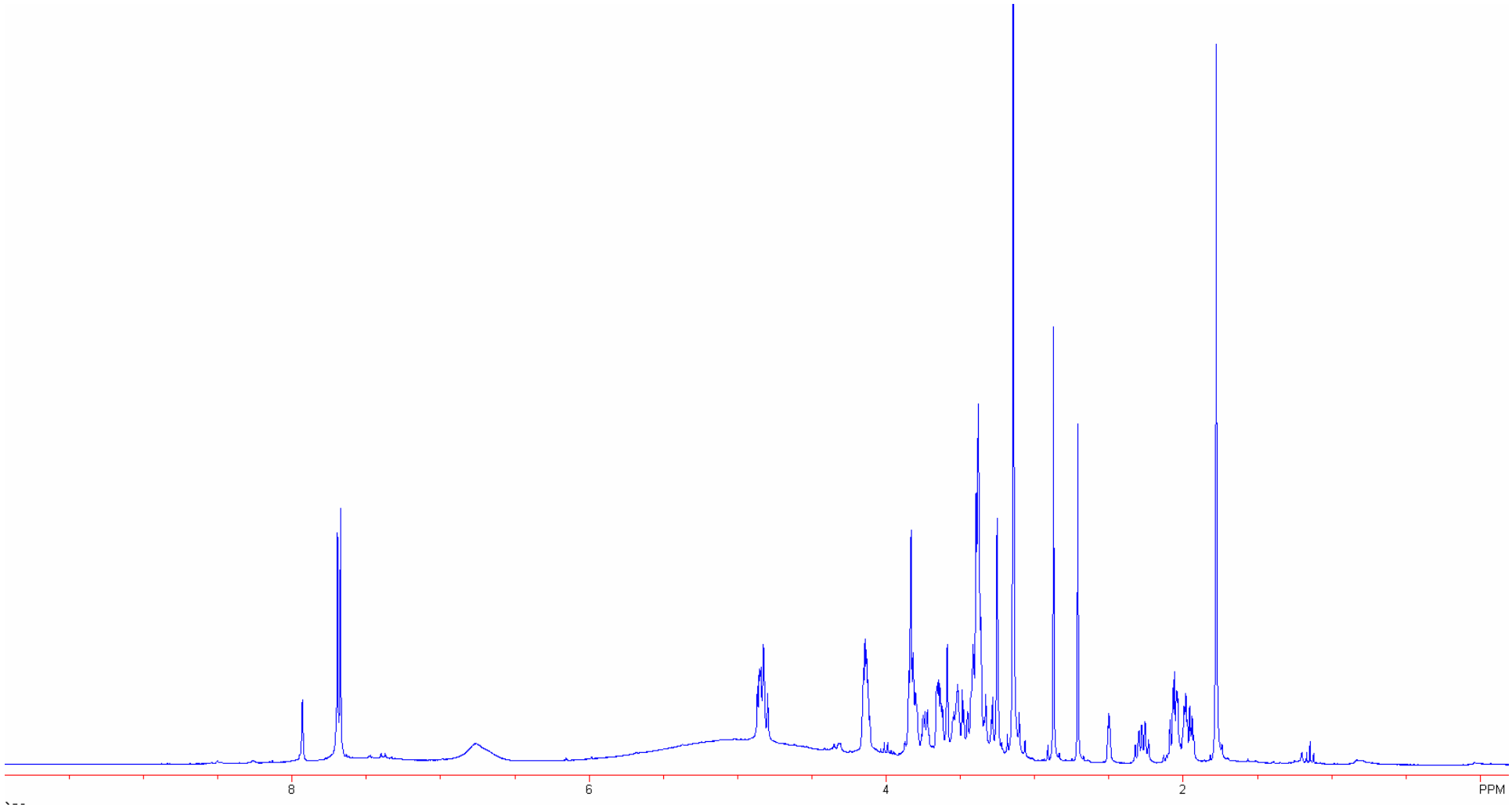
^{1}H-NMR spectrum of **6**; Solvent: DMSO; 300.58 MHz Instrument: Bruker Unity 300



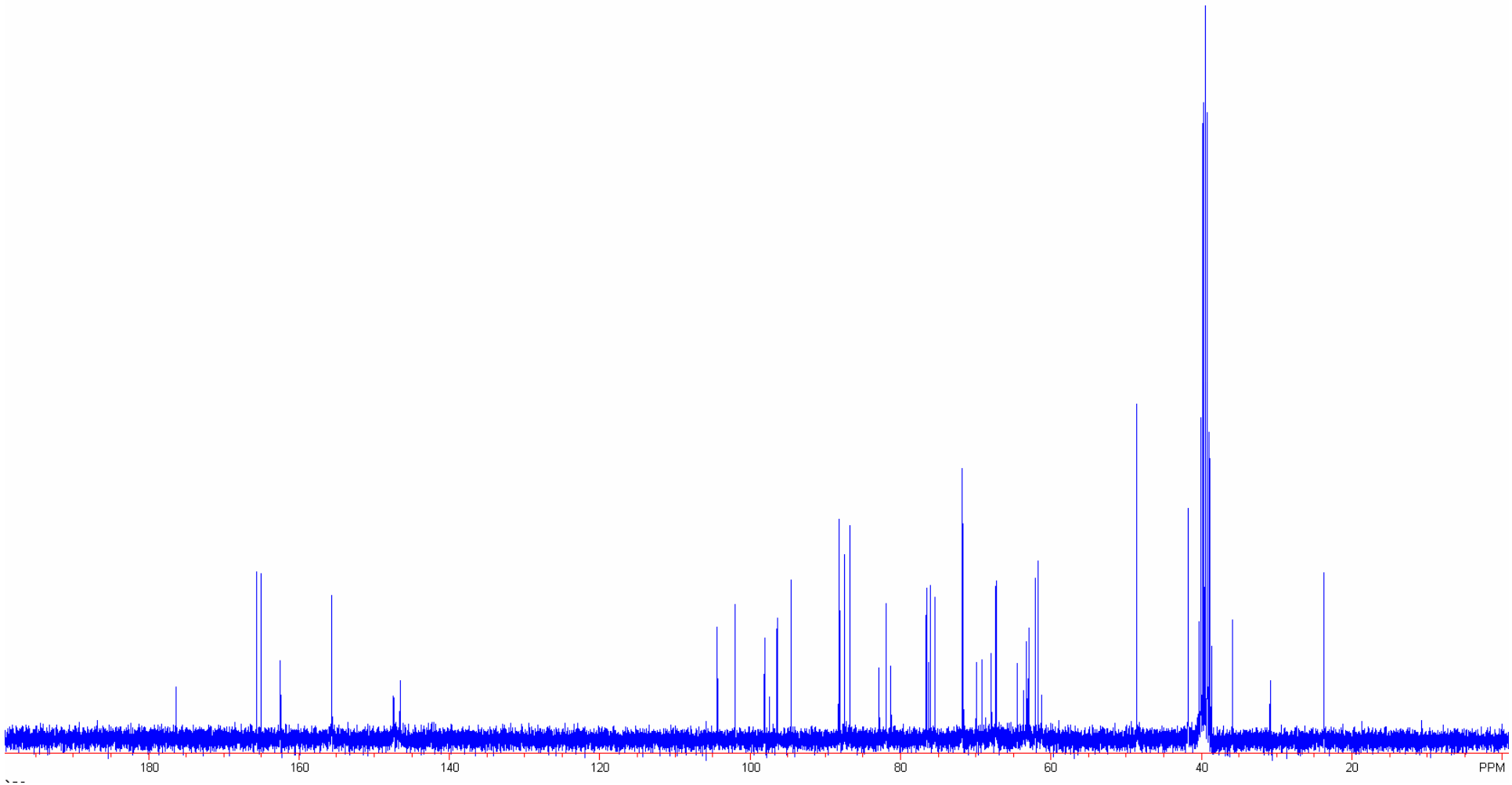
^{13}C-NMR spectrum of **6**; Solvent: DMSO: 75.50 MHz; Instrument: Bruker Unity 300



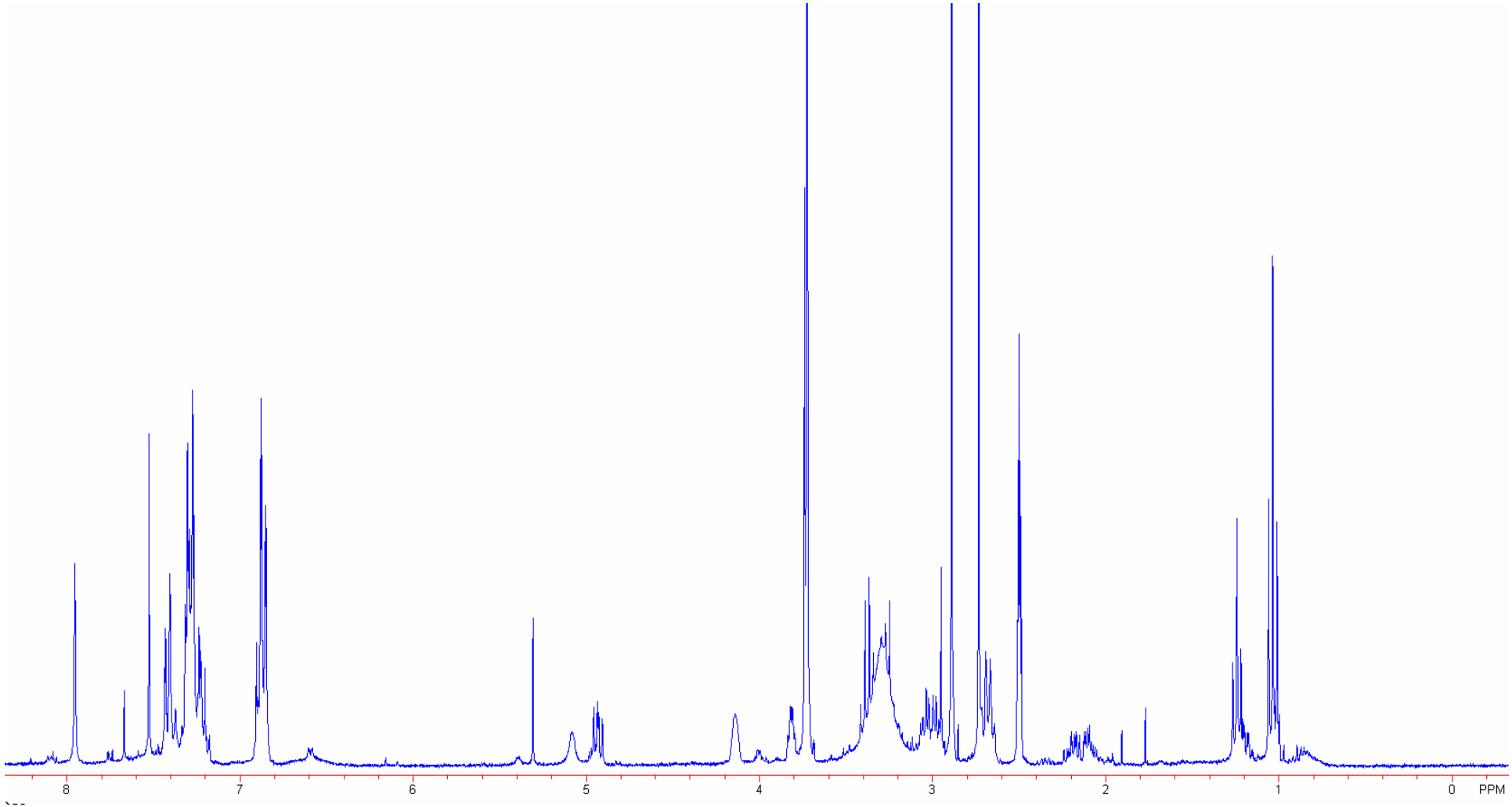
^{1}H-NMR spectrum of **7**; Solvent: DMSO; 300.54 MHz Instrument: Bruker Unity 300



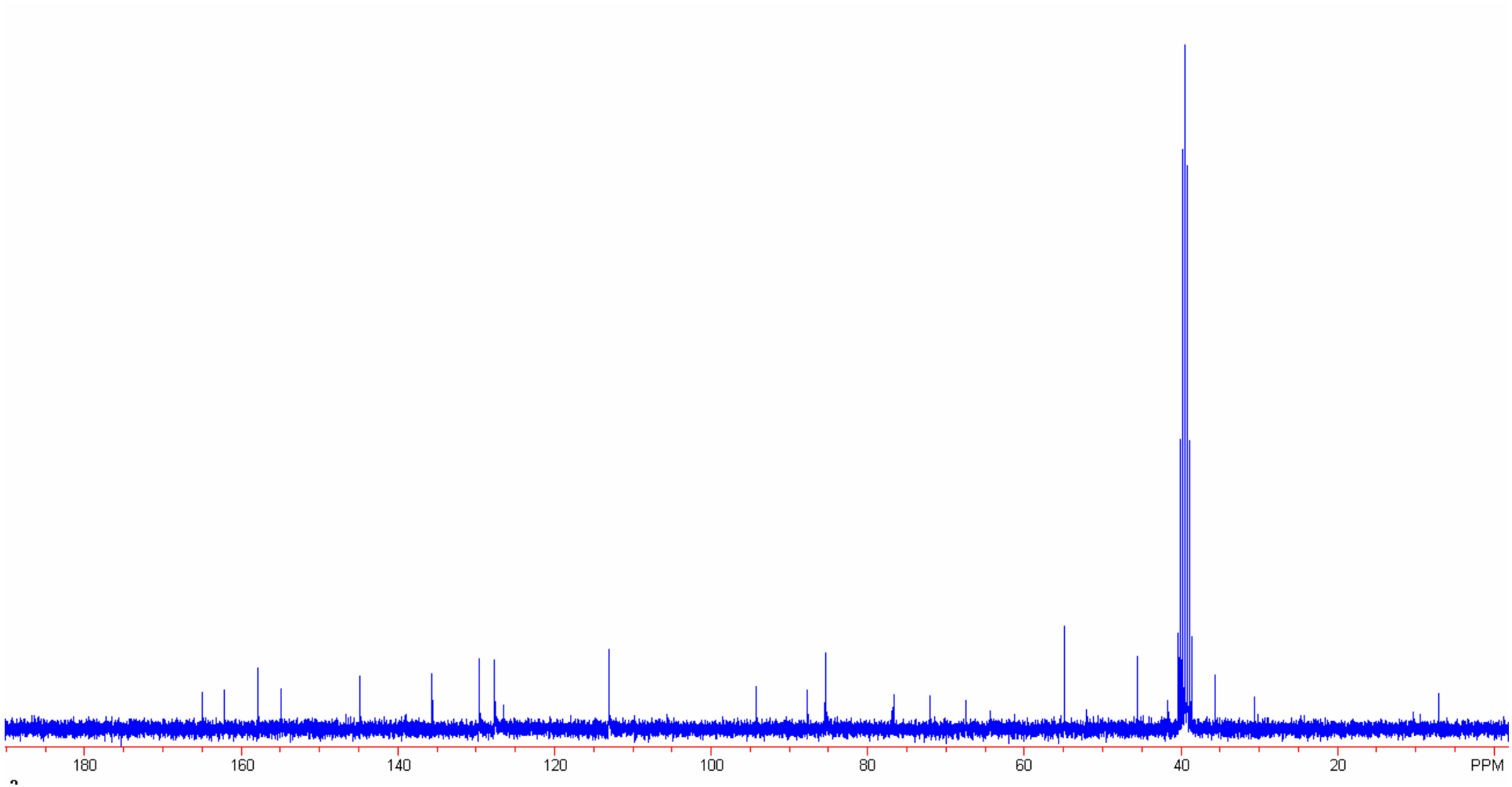
^{13}C-NMR spectrum of **7**; Solvent: DMSO: 75.00 MHz; Instrument: Bruker Unity 300



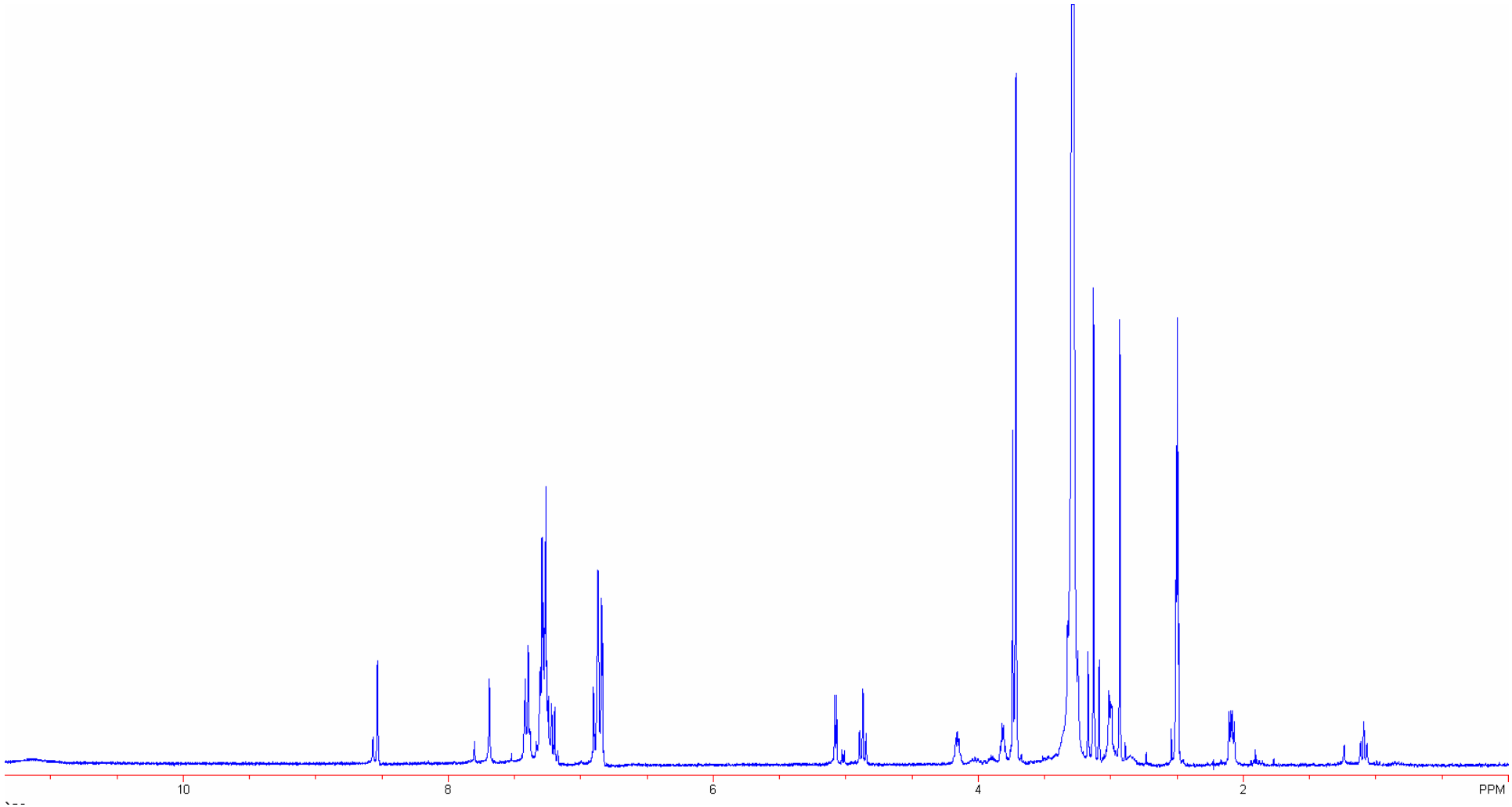
^{1}H-NMR spectrum of **8**; Solvent: DMSO; 300.03 MHz Instrument: Bruker Unity 300



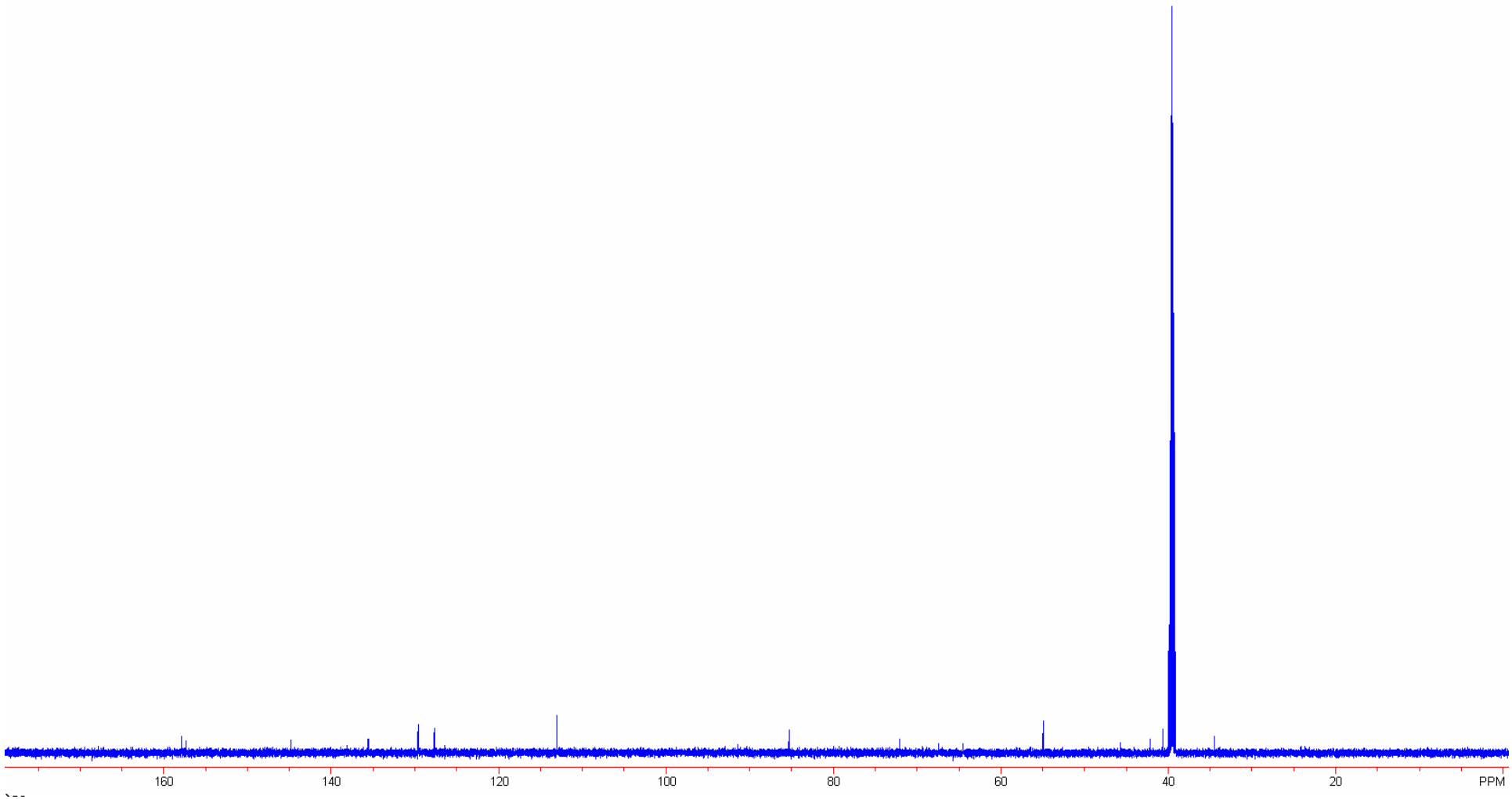
^{13}C-NMR spectrum of **8**; Solvent: DMSO: 75.60 MHz; Instrument: Bruker Unity 300



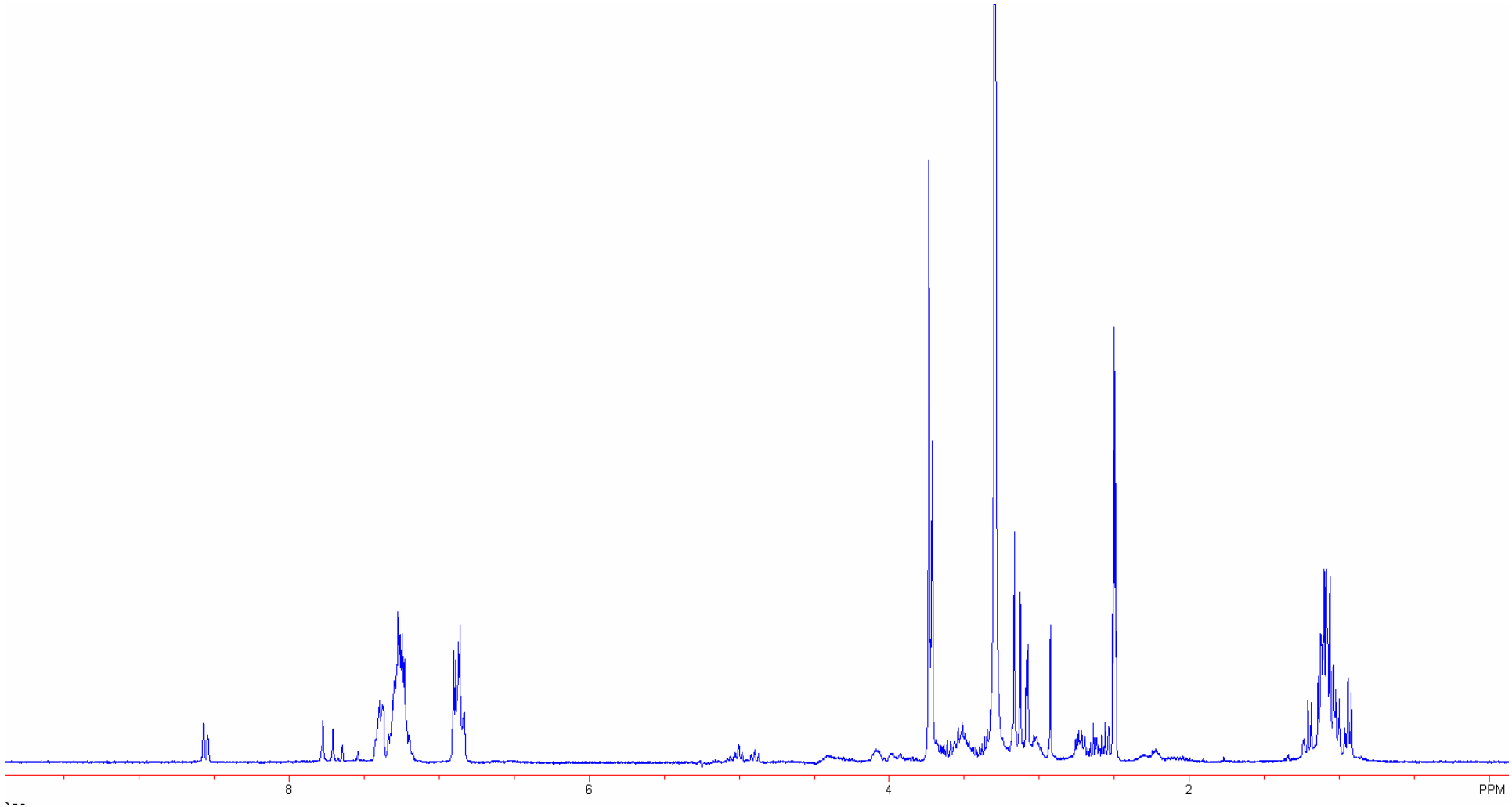
^{1}H-NMR spectrum of **9**; Solvent: DMSO; 300.17 MHz Instrument: Bruker Unity 300



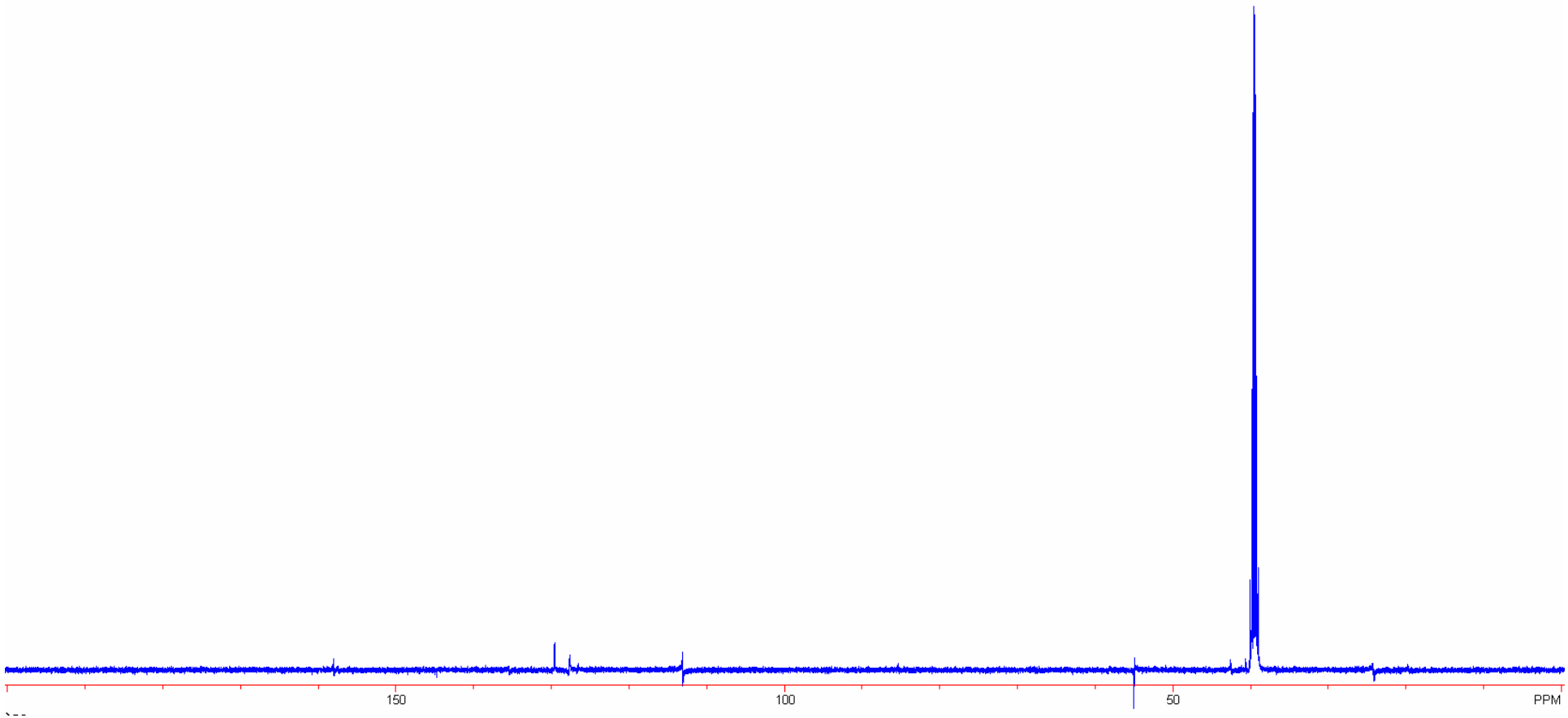
^{13}C-NMR spectrum of **9**; Solvent: DMSO: 75.49 MHz; Instrument: Bruker Unity 300



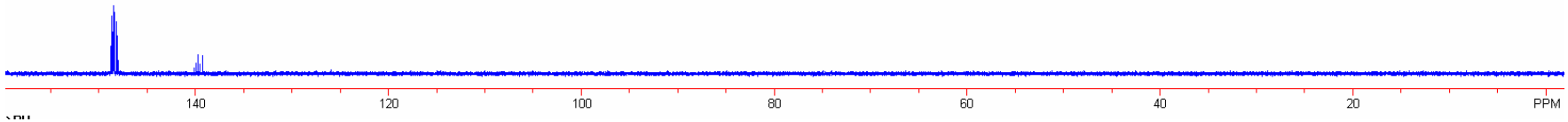
^{1}H-NMR spectrum of **2**; Solvent: DMSO; 300.20 MHz Instrument: Bruker Unity 300

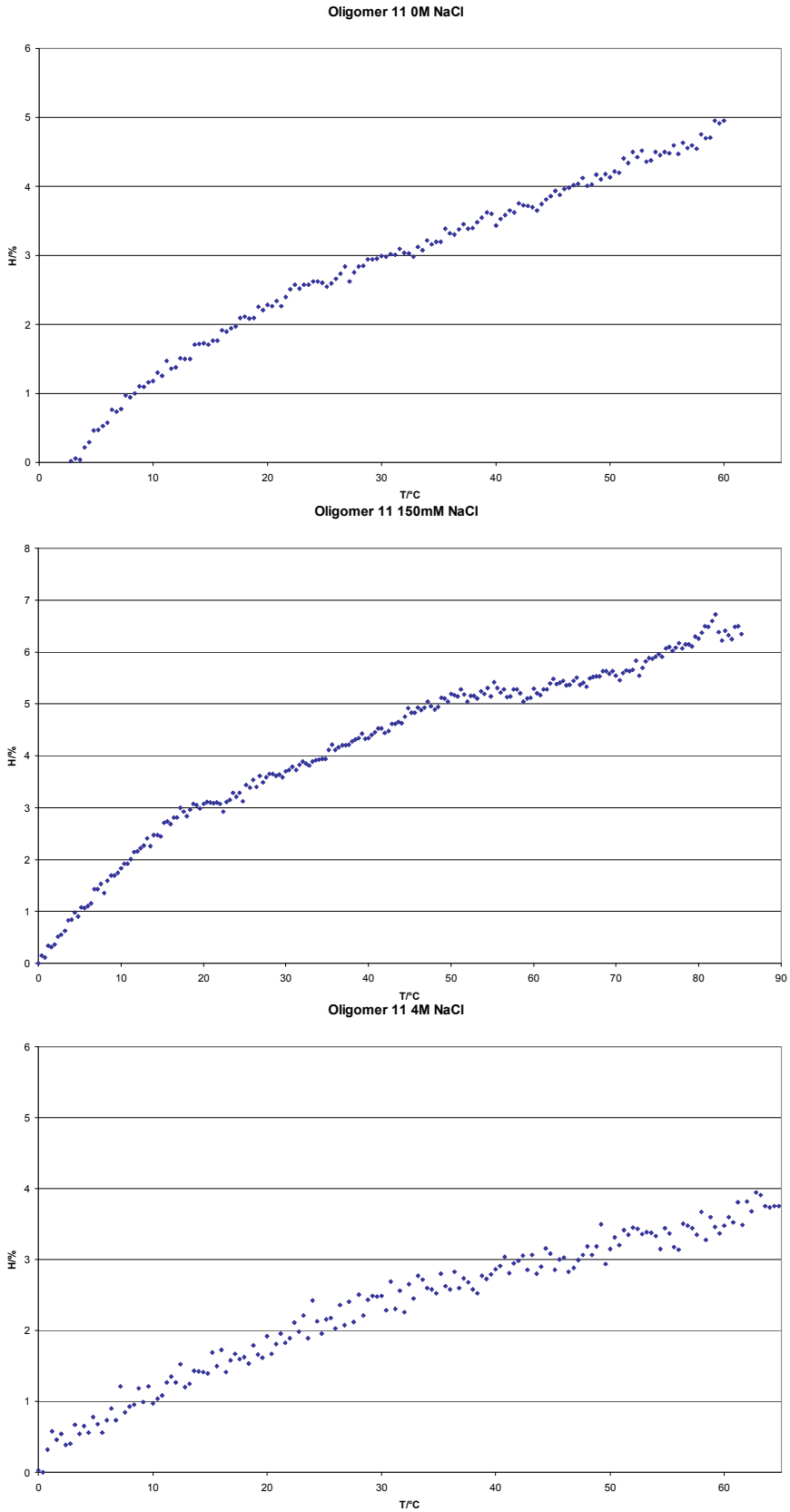


^{13}C-NMR spectrum of **2**; Solvent: DMSO: 75.38 MHz; Instrument: Bruker Unity 300

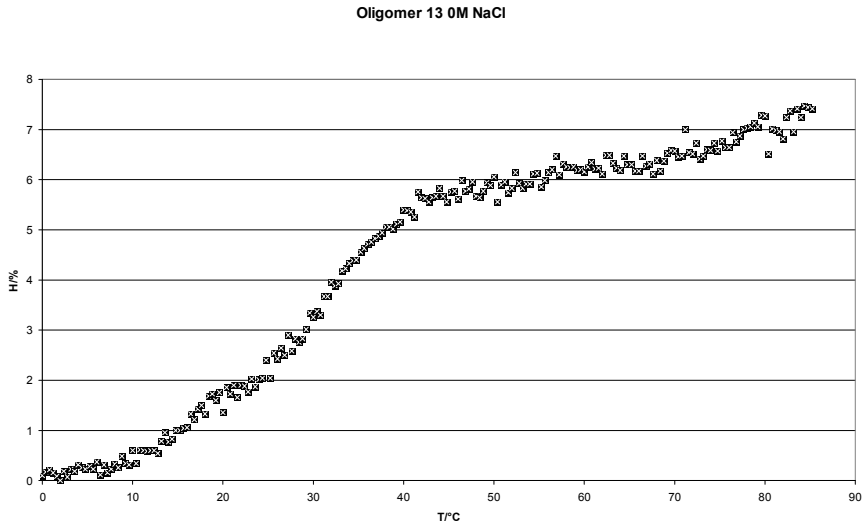


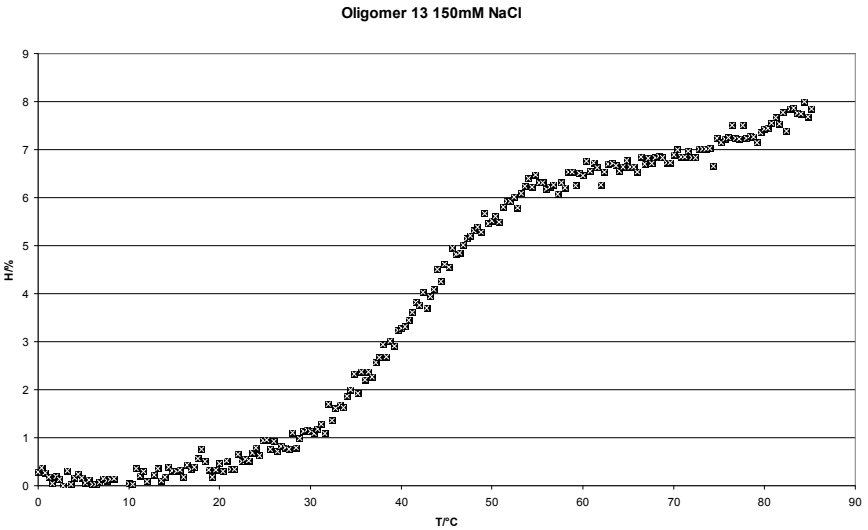
^{31}P-NMR of **2**; Solvent: DMSO; 121.67 MHz; Instrument: Bruker Unity 300

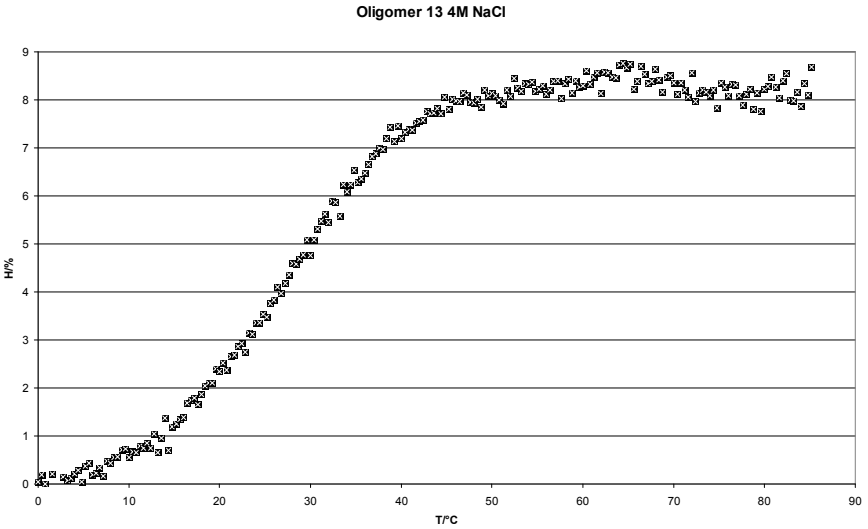




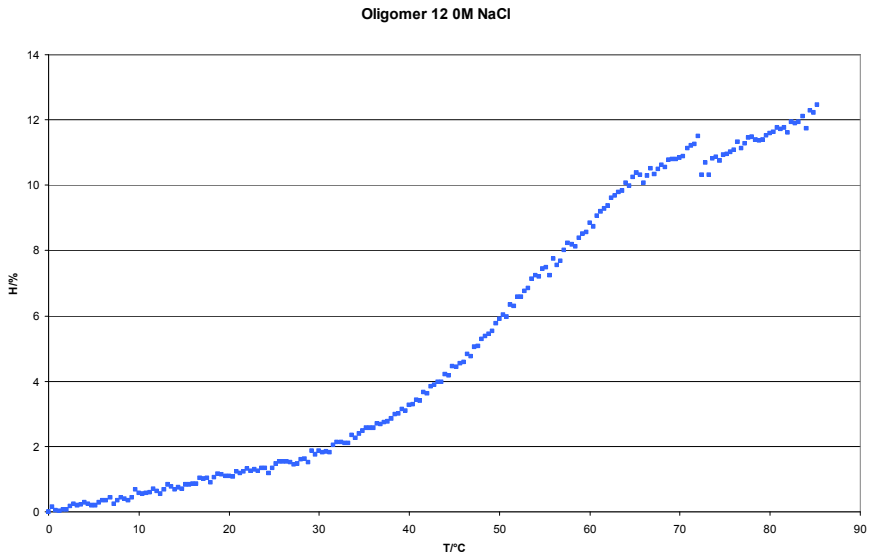
Salt-concentration dependency of the melting temperature of oligomer **11**. A change of the melting temperature of oligomer **11** due to increased salt concentration could not be measured. Concentration of oligomer **11** in NaH_2PO_4/Na_2HPO_4 (150 mM NaCl/4 M NaCl) buffer: 3.3 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

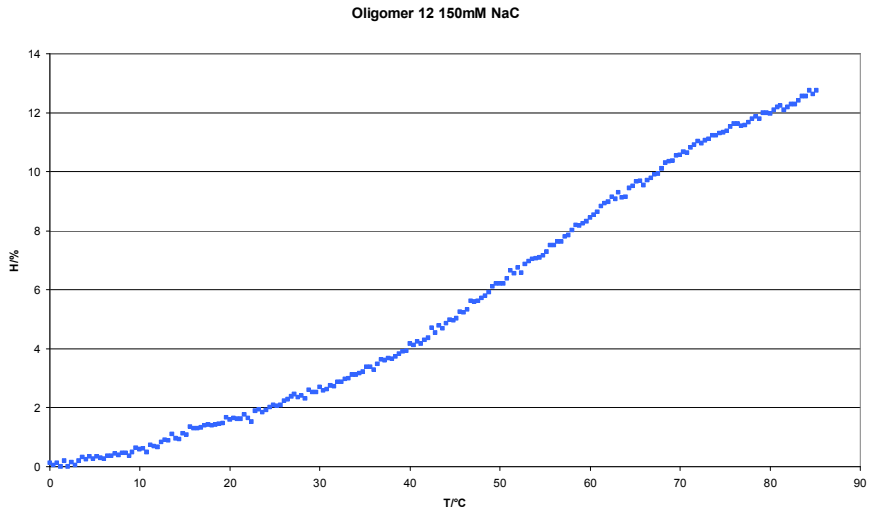


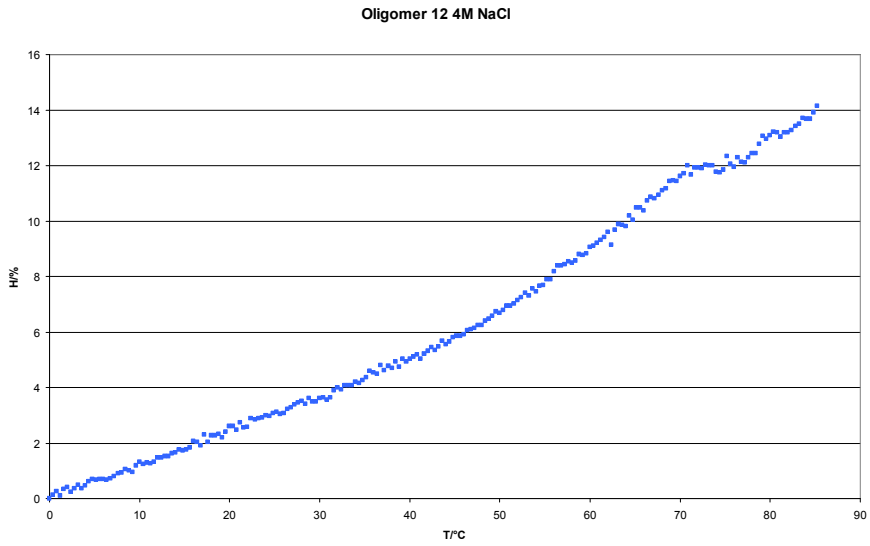




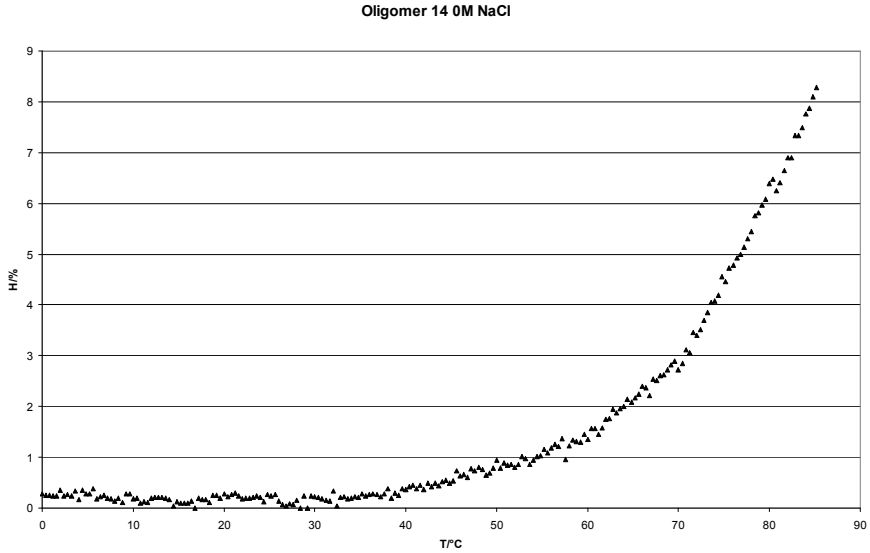
Salt-concentration dependency of the melting temperature of oligomer **13**. With increasing NaCl-concentration (0 M - 150 mM) the melting temperature rises, but further increase of the salt concentration to 4 M NaCl lets the melting temperature drop to 27 °C below the first measured value. Concentration of oligomer **13** in NaH_2PO_4/Na_2HPO_4 (150 mM NaCl/4 M NaCl) buffer: 3.3 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

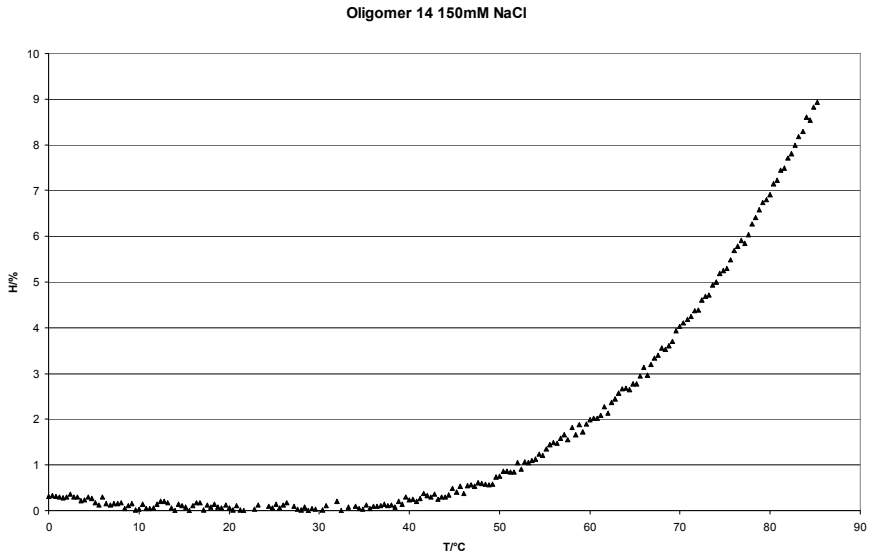


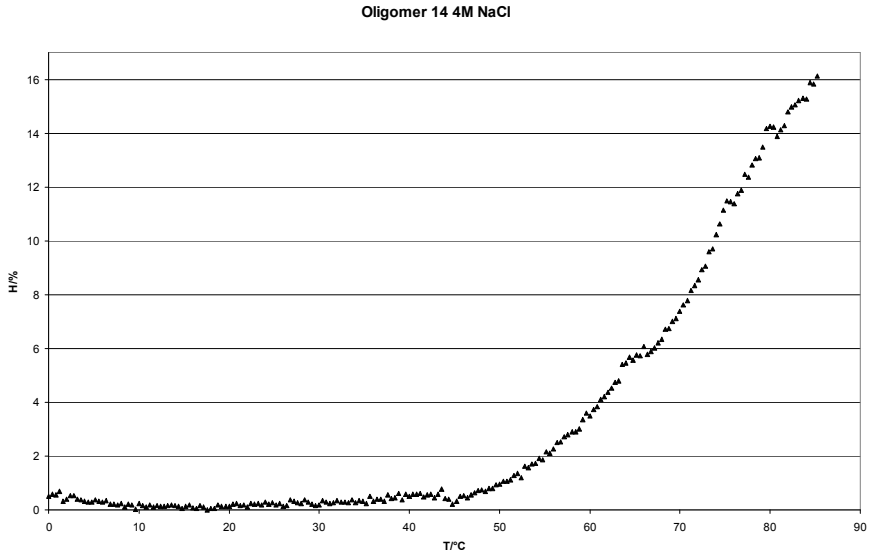




Salt-concentration dependency of the melting temperature of oligomer **12**. With increasing NaCl-concentration (0 M - 4 M) the melting temperature rises to 56 °C. Concentration of oligomer **12** in NaH_2PO_4/Na_2HPO_4 (150 mM NaCl/4 M NaCl) buffer: 3.3 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

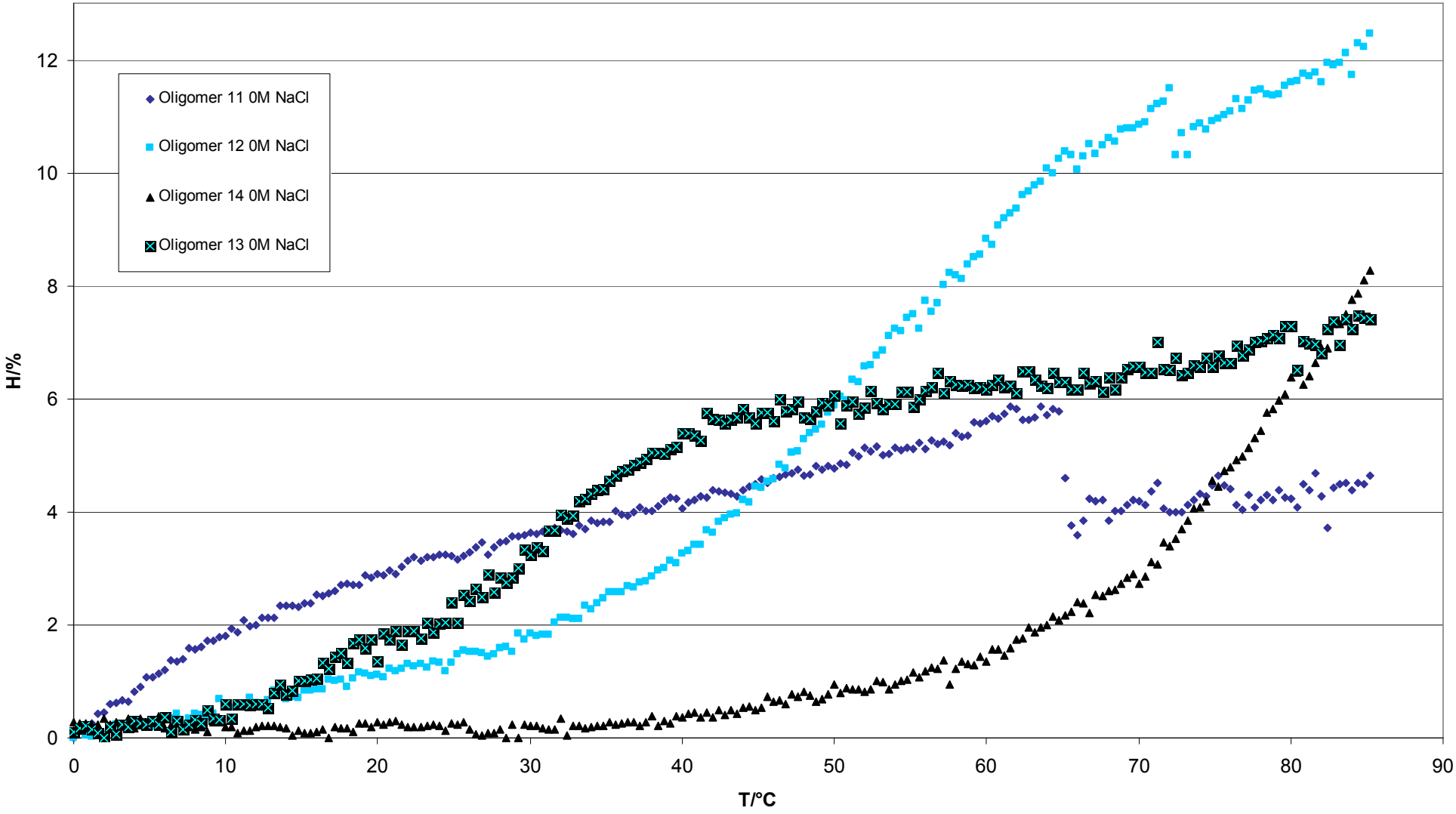




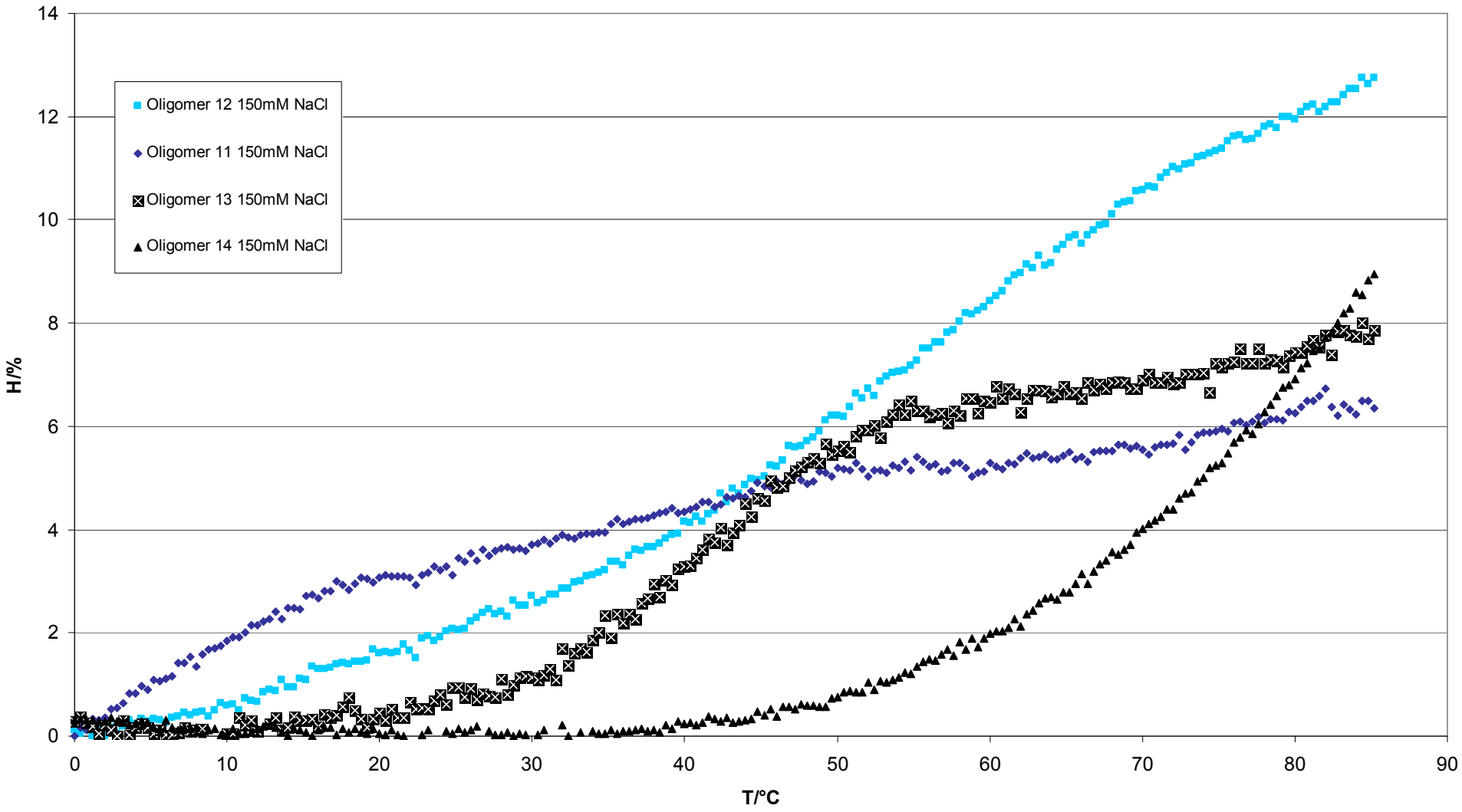


Salt-concentration dependency of the melting temperature of oligomer **14**. With increasing NaCl-concentration (0M-4M) the melting temperature drops to 71°C. Concentration of oligomer **12** in NaH_2PO_4/Na_2HPO_4 (150mM NaCl/4M NaCl) buffer: 6 μM; pH: 7. Details for accumulation of data see above at "General Methods and Materials".

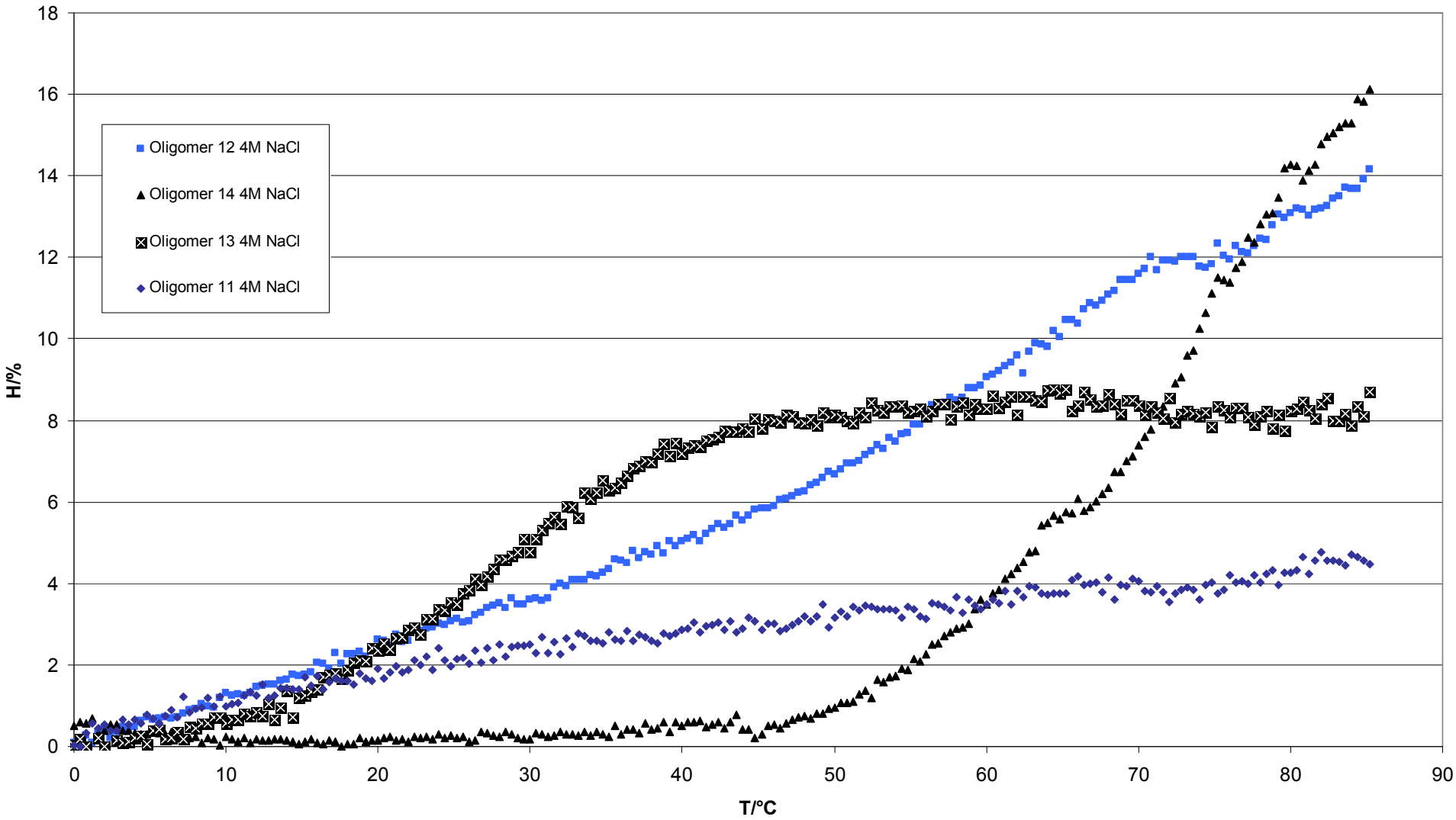
Comparison of melting temperatures of oligomers 11-14 at 0M NaCl

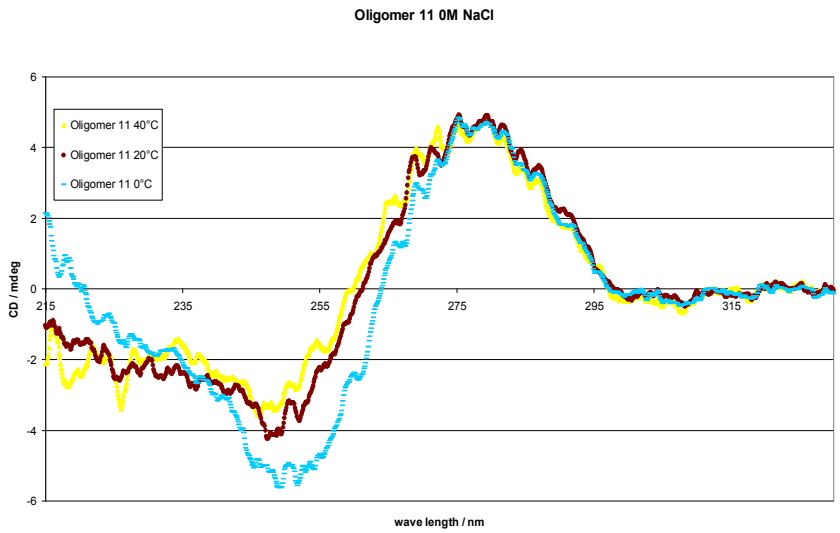


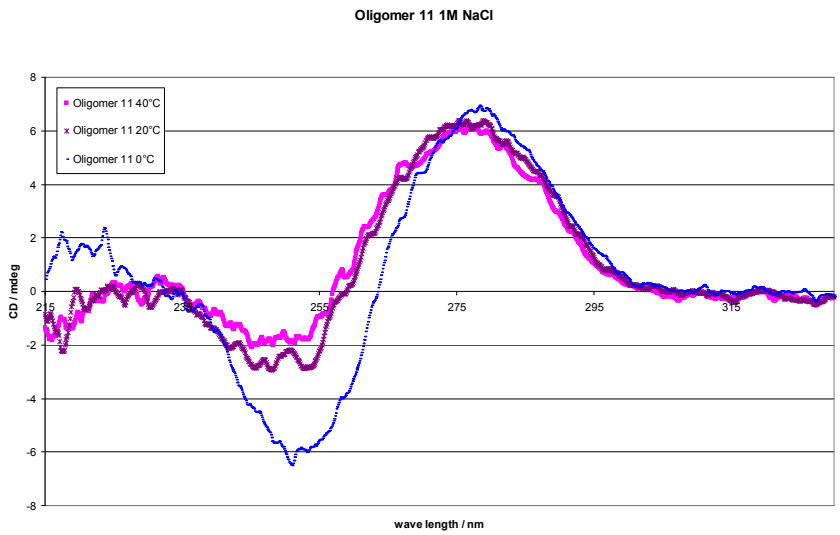
Comparison of melting temperatures of oligomers 11-14 at 150mM NaCl

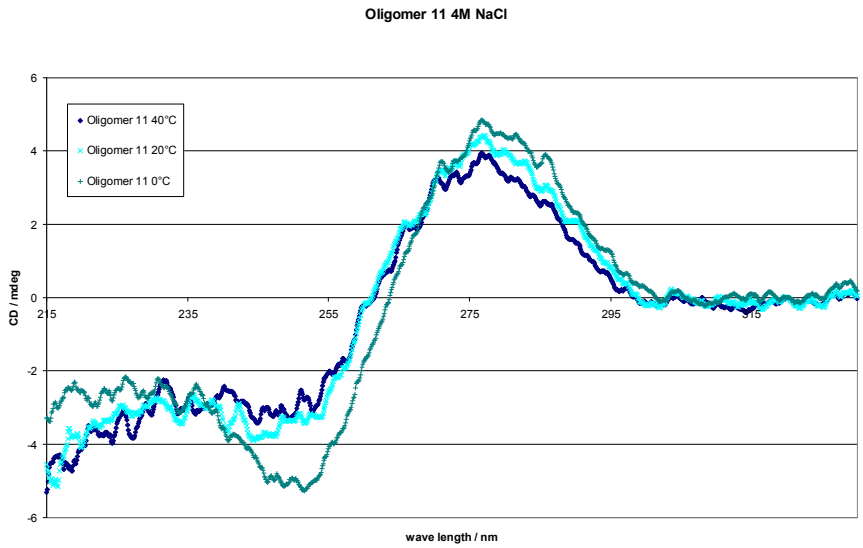


Comparison of melting temperatures of oligomers 11-14 at 4M NaCl

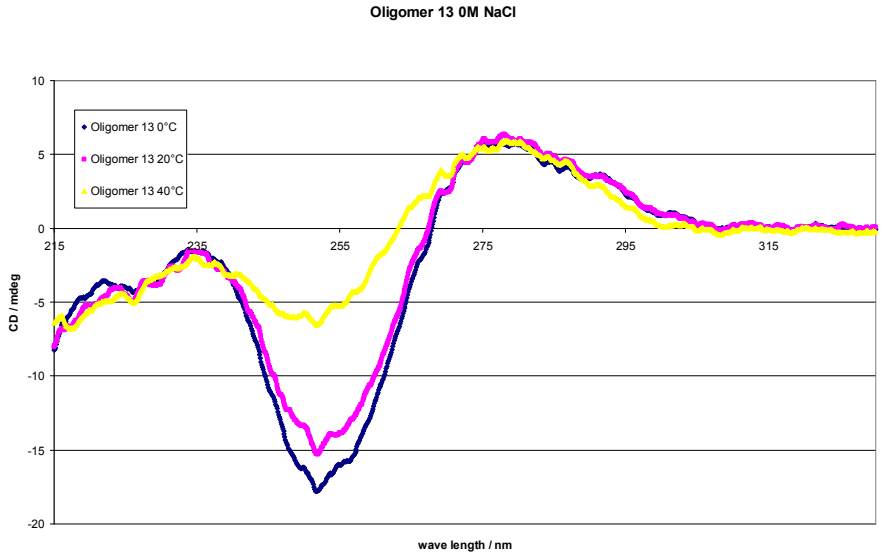


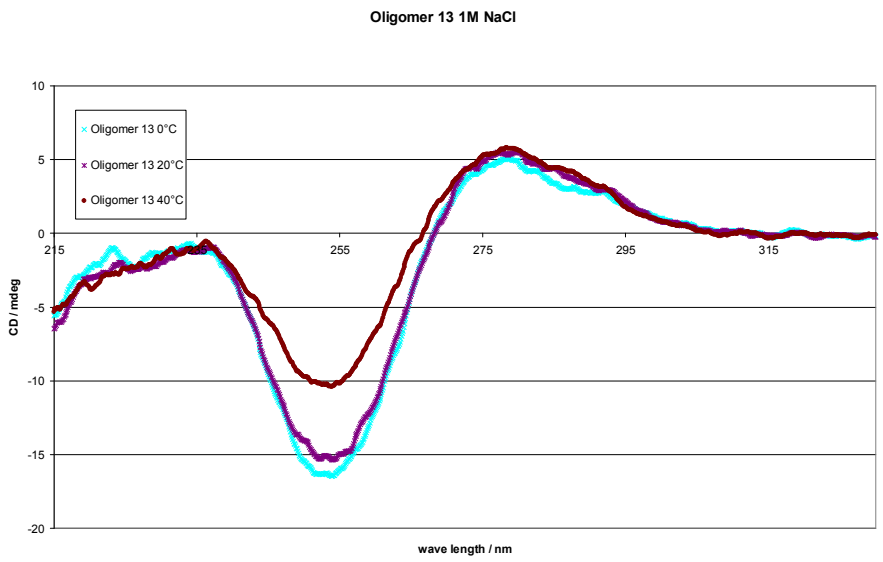


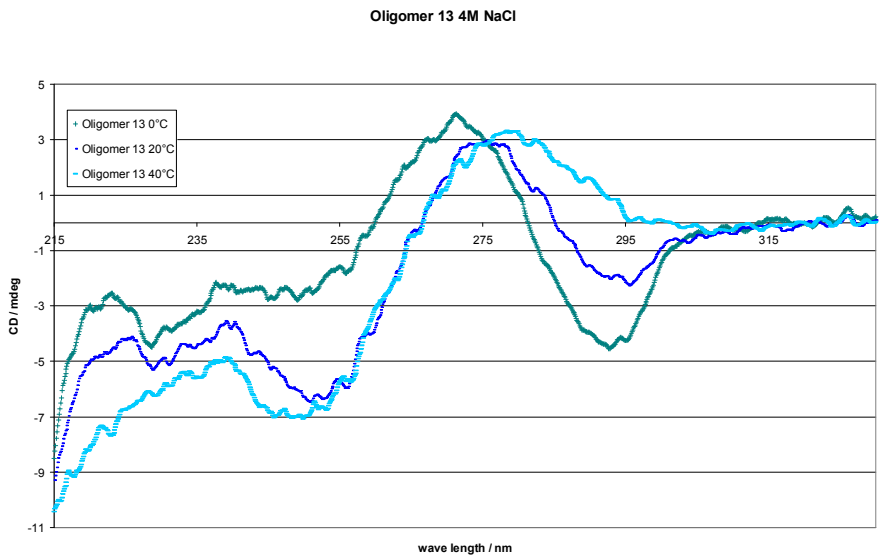




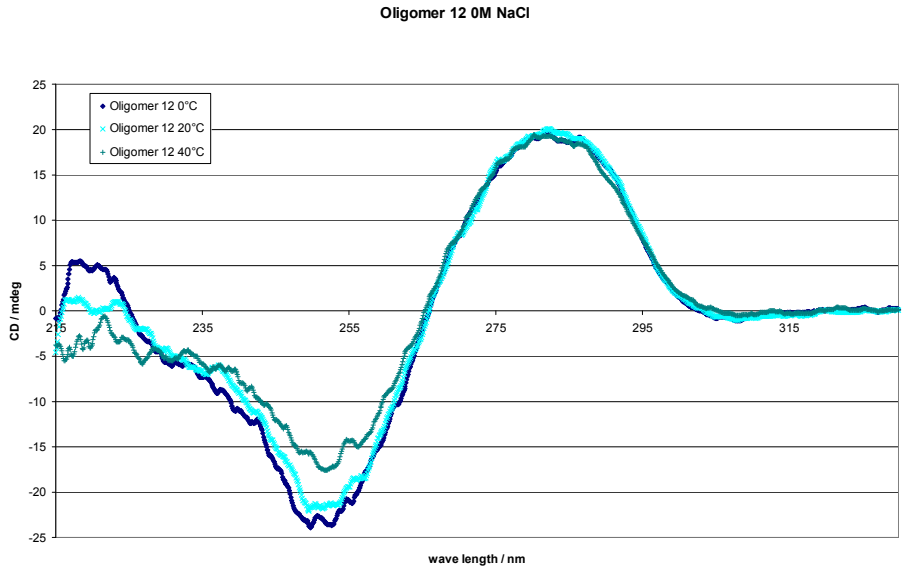
Salt-concentration and temperature dependency of modified oligomer **11**. Double strand formation is visible at low temperatures independent of salt concentration. No B to Z-form transition occurs at high salt-concentrations. Concentration of oligomer **11** in NaH_2PO_4/Na_2HPO_4 (150mM NaCl/4M NaCl) buffer: 20 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

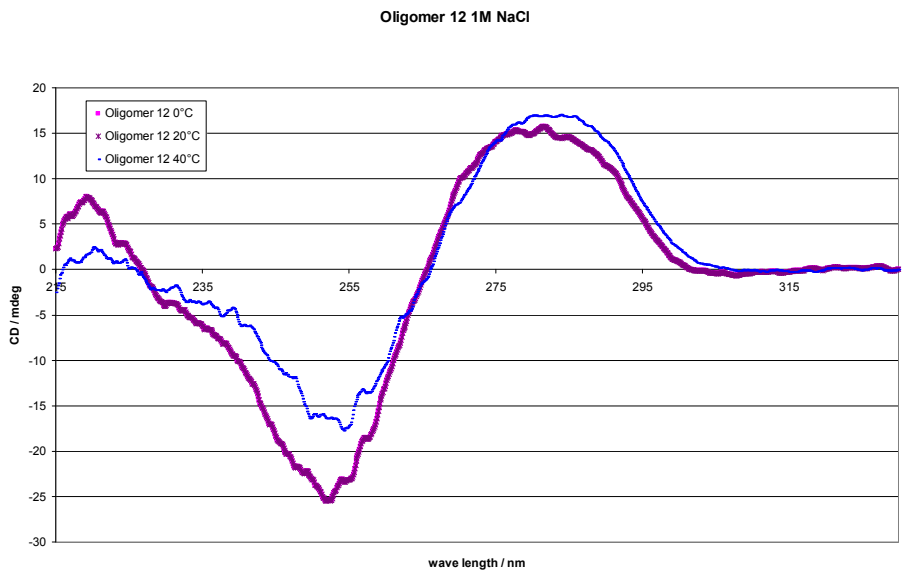


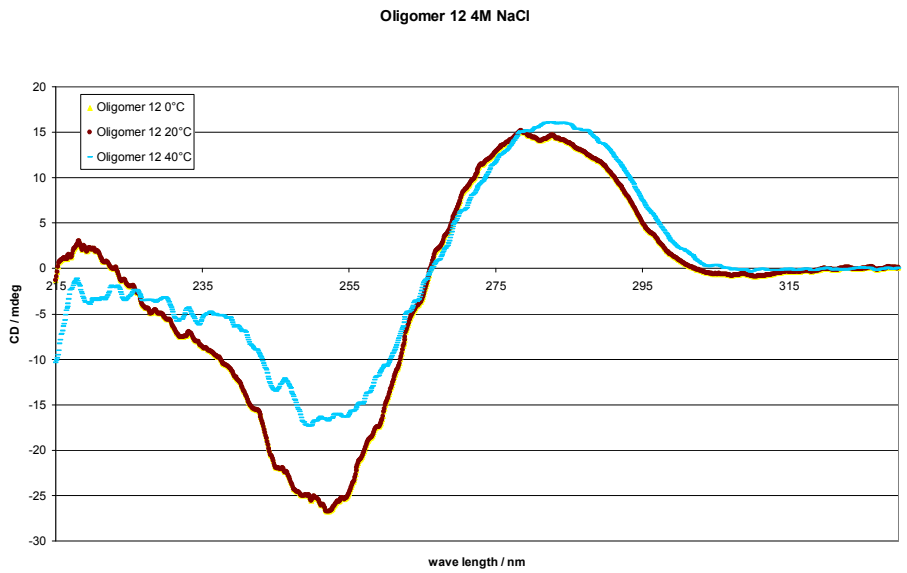




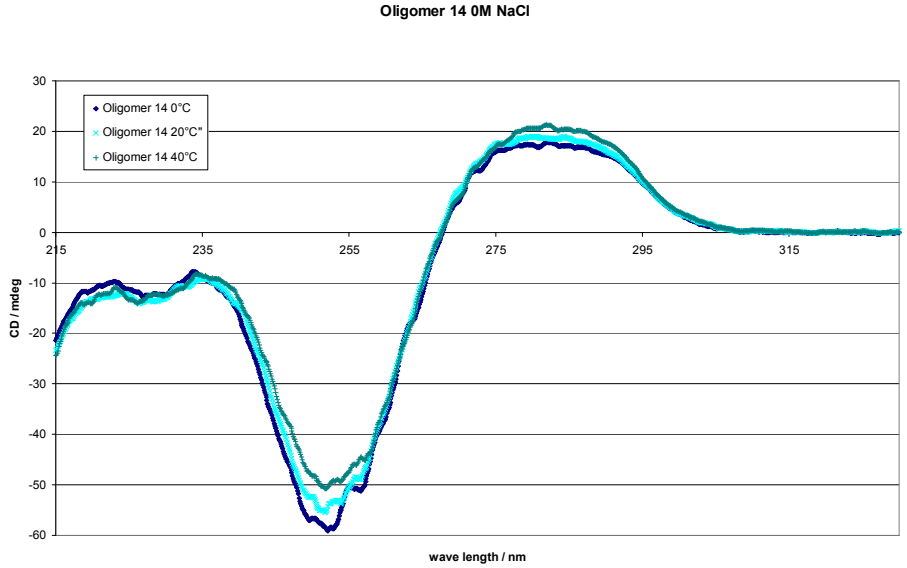
Salt-concentration and temperature dependency of reference oligomer **13**. A clear B to Z-form transition is visible at high salt-concentrations. Concentration of oligomer **13** in NaH_2PO_4/Na_2HPO_4 (150 mM NaCl/4 M NaCl) buffer: 20 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

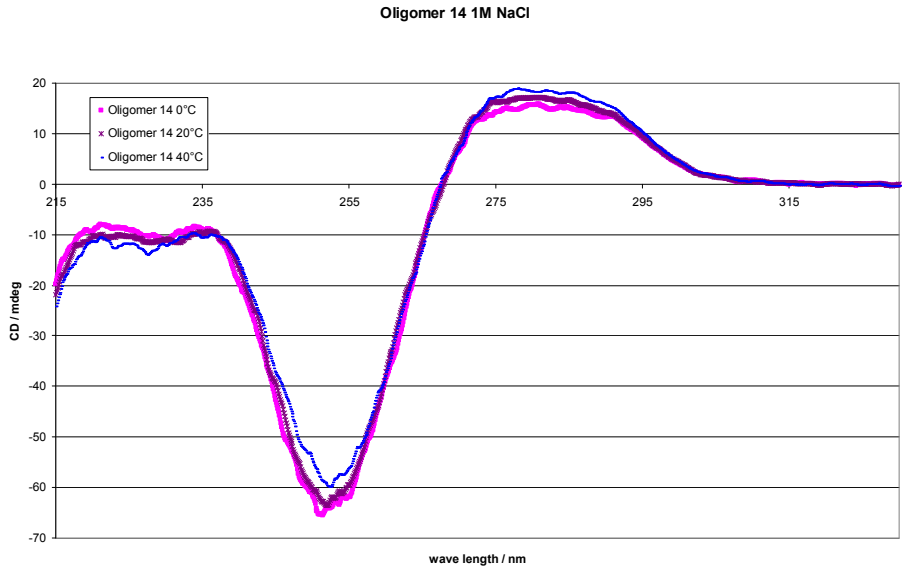


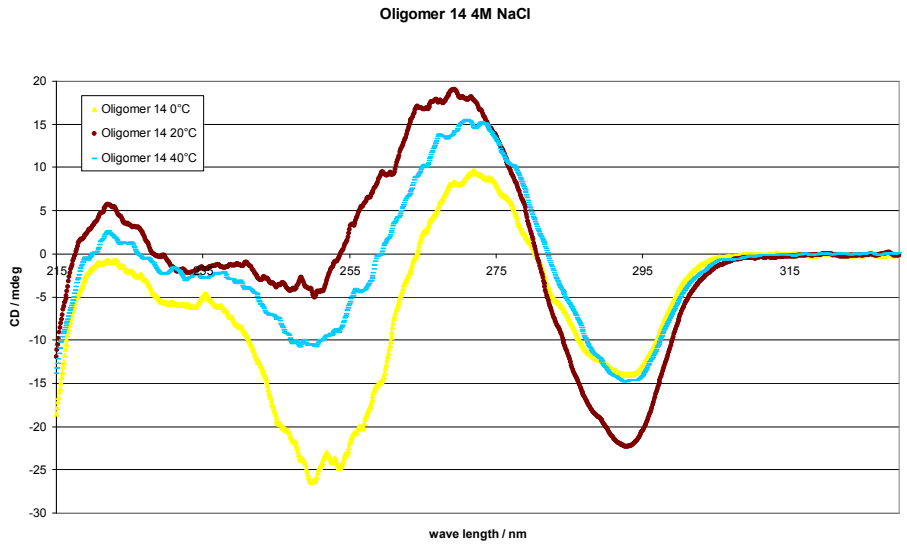




Salt-concentration and temperature dependency of modified oligomer **12**. No B to Z-form transition occurs at high salt-concentrations. Concentration of oligomer **12** in NaH_2PO_4/Na_2HPO_4 (150 mM NaCl/4 M NaCl) buffer: 20 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

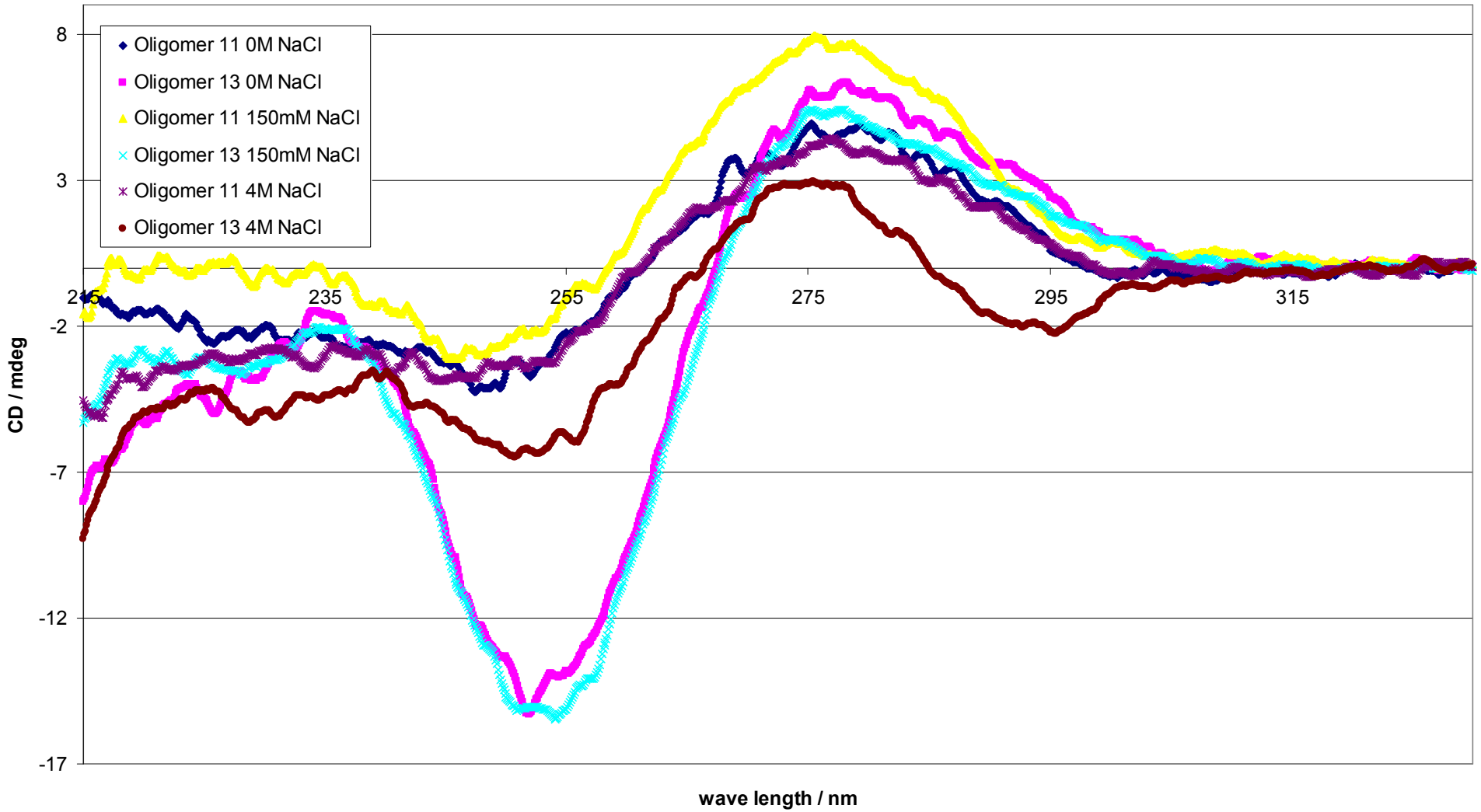


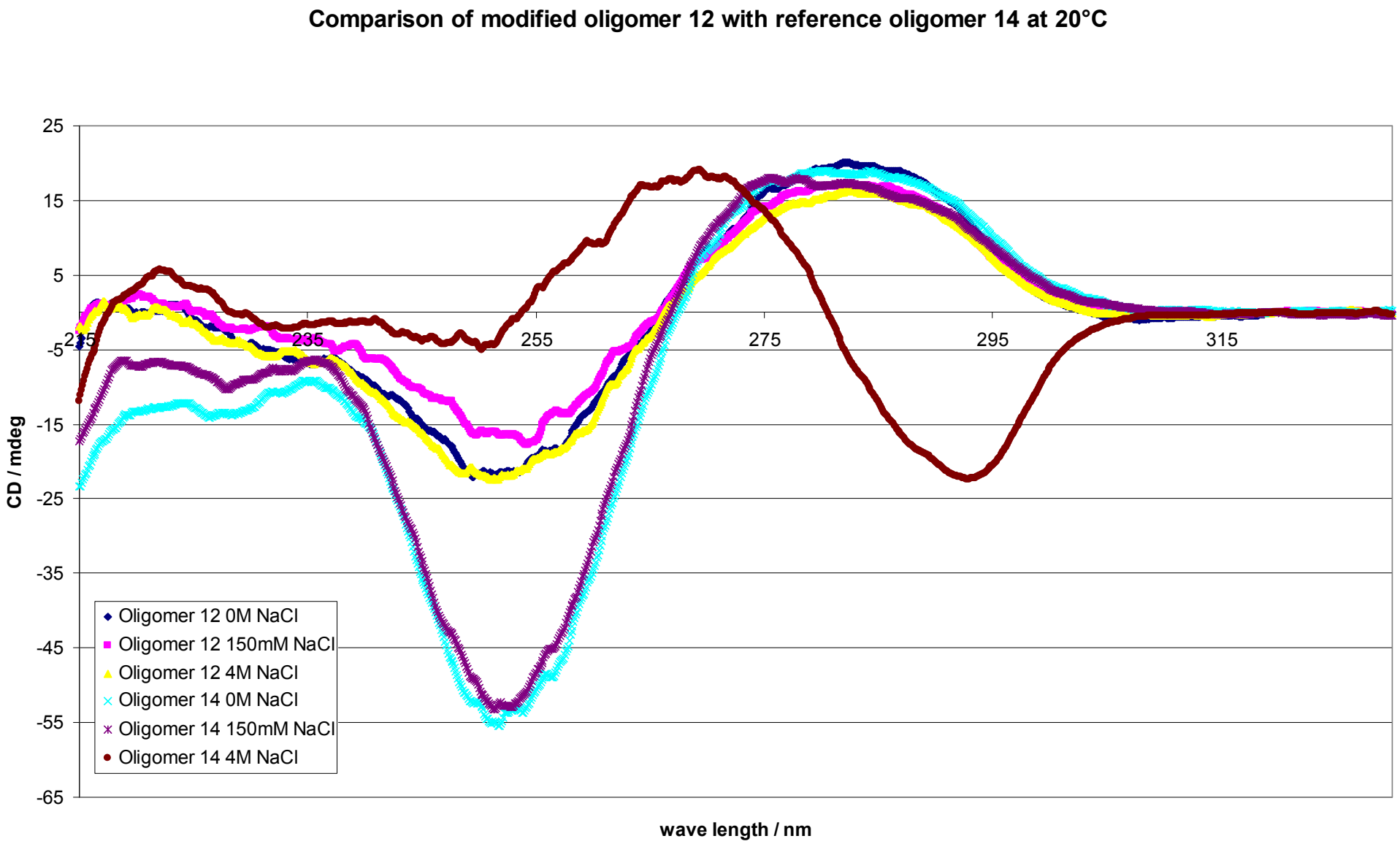




Salt-concentration and temperature dependency of reference oligomer **14**. A clear B to Z-form transition is visible at high salt-concentrations. Concentration of oligomer **14** in NaH_2PO_4/Na_2HPO_4 (150 mM NaCl/4 M NaCl) buffer: 20 μM; pH 7. Details for accumulation of data see above at 'General Methods and Materials'.

Comparison of modified oligomer 11 with reference oligomer 13 at 20°C




Comparison of modified oligomer 12 with reference oligomer 14 at 20°C
25
15
5
-5
-15
-25
-35
-45
-55
-65
CD / mdeg
215
235
255
275
295
315
wave length / nm
Oligomer 12 0M NaCl
Oligomer 12 150mM NaCl
Oligomer 12 4M NaCl
Oligomer 14 0M NaCl
Oligomer 14 150mM NaCl
Oligomer 14 4M NaCl