Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information for

N-Methoxy-1,3-Oxazinane Nucleic Acids (MOANAs) – a Configurationally Flexible Backbone Modification Allows Post-Synthetic Incorporation of Base Moieties

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General experimental methods

All chemicals used were purchased from commercial suppliers and used as received. Pyridine, toluene, and CH_2Cl_2 were dried over activated 4 Å molecular sieves while Et_3N was dried over CaH_2. Reactions that were sensitive to moisture were performed under N_2 atmosphere using anhydrous solvents. Thin layer chromatography was performed on silica aluminum sheets (Merck, Silica gel 60 F_{254}) and visualized with UV light. Preparative column chromatography was performed using Sigma-Aldrich silica gel (60 Å pore size, 40—63 μm particle size). NMR spectra (^1H, ^{13}C and ^{31}P NMR) were recorded on Bruker Biospin 500 and 600 MHz NMR spectrometers. Chemical shifts (δ) are reported as parts per million (ppm) referenced to the residual proton signal of the deuterated solvent as an internal standard. High-resolution mass spectra were recorded on a Bruker Daltonics micrOTOF-Q mass spectrometer using electrospray ionization.

(*E*)-2-Hydroxy-2-((4-((*E*)-(methoxyimino)methyl)-2-phenyl-1,3-dioxan-5-yl)oxy) acetaldehyde *O*-methyl oxime (4)

(4*R*,5*R*)-5-((*S*)-1-Methoxy-2-oxoethoxy)-2-phenyl-1,3-dioxane-4-carbaldehyde (3, 3.70 g, 13.2 mmol) was dissolved in dry pyridine (60 mL). *O*-Methylhydroxylammonium chloride (7.38 g, 88.4 mmol) was added and the resulting mixture stirred at 80 °C for 16 h, after which TLC (Et_3N:CH_2Cl_2 = 1:99, *v*/*v*) showed complete conversion of the starting material. The product mixture was allowed to cool to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL). The aqueous phase was extracted with dichloromethane (2 × 100 mL), the combined organic phases were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was used in the following step without further purification. Yield: 4.01 g (11.9 mmol, 89%). ^1H NMR (500 MHz, $CDCl_3$) δ_H: 3.44 (s, 3H, CHOC<u>H</u>$_3$), 3.73 (dd, *J* = 9.8 Hz, 9.4 Hz, 1H, OCH_2), 3.915 (s, 3H, $NOCH_3$), 3.917 (s, 3H, $NOCH_3$), 3.94 (m, 1H, OCH_2C<u>H</u>), 4.37 (dd, *J* = 9.4 Hz, 7.2 Hz, 1H, OCH_2CHC<u>H</u>), 4.41 (dd, *J* = 11.0 Hz, 5.2 Hz, 1H, OCH_2), 4.96 (d, *J* = 6.0 Hz, 1H, C<u>H</u>OCH_3), 5.56 (s, 1H, PhCH), 7.26 (d, *J* = 6.0 Hz, 1H, C<u>H</u>CHOCH_3), 7.35—7.41 (m, 3H, Ph-H3, H4 and H5), 7.44 (d, *J* = 7.1 Hz, NC<u>H</u>CHCH), 7.47—7.52 (m, 2H, Ph-H2 and H6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 54.9 (CHO<u>C</u>H_3), 62.0 ($NOCH_3$), 62.1 ($NOCH_3$), 66.9 (CH_2), 69.5 (CH_2<u>C</u>H), 77.8 (CH_2CH<u>C</u>H), 100.2 (<u>C</u>HOCH_3), 101.0 (PhCH), 126.1 (Ph-C2 and C6), 128.3 (Ph-C3 and C5), 129.2 (Ph-C4), 137.0 (Ph-C1), 146.2 (CHN), 146.4 (CHN). HRMS (ESI) *m*/z [M + Na]$^+$ calcd for $C_{16}H_{22}N_2NaO_6^+$: 361.1370; found: 361.1358.

N-((2*S*)-2-methoxy-2-(((4*S*,5*R*)-4-((methoxyamino)methyl)-2-phenyl-1,3-dioxan-5-yl)oxy)ethyl)-*O*-methylhydroxylamine (5)

(*E*)-2-Hydroxy-2-((4-((*E*)-(methoxyimino)methyl)-2-phenyl-1,3-dioxan-5-yl) oxy) acetaldehyde *O*-methyl oxime (4, 4.01 g, 11.9 mmol) was dissolved in a mixture of CH_2Cl_2 (30 mL) and MeOH (30 mL). $NaCNBH_3$ (4.50 g, 71.6 mmol) was added and the reaction mixture stirred at 25 °C. AcOH (5.0 mL, 87 mmol) was added dropwise over a period of 16 h, after which TLC (Et_3N:MeOH:CH_2Cl_2 = 1:3:96, *v*/*v*) showed complete conversion of the starting material. The mixture was neutralized with saturated aqueous $NaHCO_3$ (100 mL) and extracted with dichloromethane (3 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography (Et_3N:MeOH:CH_2Cl_2 = 1:3:96, *v*/*v*). Yield: 2.50 g (7.30 mmol, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 2.99 (dd, *J* = 13.7 Hz, 5.5 Hz, 1H, NHC$\underline{H}_2$CHOCH$_3$), 3.05 (dd, *J* = 13.7 Hz, 5.4 Hz, 1H, NHC$\underline{H}_2$CHOCH$_3$), 3.11 (dd, *J* = 13.3 Hz, 7.7 Hz, 1H, NHC$\underline{H}_2$CHCH), 3.47 (s, 3H, CHOC$\underline{H}_3$), 3.53 (m, 1H, NHC$\underline{H}_2$CHCH), 3.53 (s, 3H, NOC$\underline{H}_3$), 3.55 (s, 3H, NOC$\underline{H}_3$), 3.63 (dd, *J* = 10.6 Hz, 10.3 Hz, 1H, OC$\underline{H}_2$), 3.76 (m, 1H, OCH$_2$C$\underline{H}$), 3.97 (m, 1H, OCH$_2$CHC$\underline{H}$), 4.44 (dd, *J* = 10.7 Hz, 4.9 Hz, 1H, OC$\underline{H}_2$), 4.79 (t, *J* = 5.4 Hz, 1H, NHCH$_2$C$\underline{H}$OCH$_3$), 5.51 (s, 1H, PhCH), 7.34—7.40 (m, 3H, Ph-H3, H4 and H5), 7.46—7.51 (m, 2H, Ph-H2 and H6). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 52.5 (NH$\underline{C}$H$_2$CHCH), 53.8 (NH$\underline{C}$H$_2$CHOCH$_3$), 55.1 (CHO$\underline{C}$H$_3$), 61.2 (NHO$\underline{C}$H$_3$), 61.5 (NHO$\underline{C}$H$_3$), 68.4 (OCH$_2$$\underline{C}$H), 69.6 (O$\underline{C}H_2$), 76.5 (OCH$_2CH\underline{C}$H), 100.1 ($\underline{C}$HOCH$_3$), 101.0 (PhCH), 126.1 (Ph-C2 and C6), 128.2 (Ph-C3 and C5), 129.0 (Ph-C4), 137.6 (Ph-C1). HRMS (ESI) *m*/z [M + H]$^+$ calcd for $C_{16}H_{27}N_2O_6^+$: 343.1864; found: 343.1868.

(2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol (6)

N-((2*S*)-2-Methoxy-2-(((4*S*,5*R*)-4-((methoxyamino)methyl)-2-phenyl-1,3-dioxan-5-yl)oxy)ethyl)-*O*-methylhydroxylamine (5, 2.50 g, 7.30 mmol) was dissolved in 6 M hydrochloric acid (10 mL). 1,3-Propanedithiol (2 mL) was added and the reaction mixture stirred at 40 °C for 16 h, after which it was diluted with H_2O (30 mL) and washed with CH_2Cl_2 (3 × 30 mL). The pH of the aqueous phase was adjusted to approximately 9 with OH-form anion exchange resin. The aqueous phase was washed again with CH_2Cl_2 (3 × 30 mL), the resin was removed by filtration and the filtrate was evaporated to dryness. Yield: 1.00 g, (6.62 mmol, 90%). ^{1}H NMR (600 MHz, D_2O) δ_H: 2.77 (dd, *J* = 13.7 Hz, 9.2 Hz, 1H, C$\underline{H}_2$NH), 3.14 (dd, *J* = 13.7 Hz, 2.8 Hz, 1H, C$\underline{H}_2$NH), 3.47 (s, 3H, OCH$_3$), 3.53 (m, 1H, C$\underline{H}_2$OH), 3.56 (m, 1H, C$\underline{H}$CH$_2$OH), 3.67 (dd, *J* = 11.1 Hz, 2.6 Hz, 1H, C$\underline{H}_2$OH), 3.73 (ddd, *J* = 9.1 Hz, 6.2 Hz, 2.9 Hz, 1H, C$\underline{H}$CH$_2$NH). ^{13}C NMR (150 MHz, D_2O), δ_C: 52.4 (CH_2NH), 60.5 (OCH_3), 62.4 (CH_2OH), 68.2 ($\underline{C}$HCH$_2$NH), 73.5 ($\underline{C}$HCH$_2$OH). HRMS (ESI) *m*/z [M + Na]$^+$ calcd for $C_5H_{13}NNaO_4^+$: 174.0737; found: 174.0744.

(2*R*,3*S*)-*N*-(9-Fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol (7)

(2*R*,3*S*)-4-(Methoxyamino)butane-1,2,3-triol (6, 1.00 g, 6.62 mmol) was dissolved in a mixture of H_2O (0.80 mL) and 1,4-dioxane (19.2 mL). K_2CO_3 (3.81 g, 27.6 mmol) was added and stirred for 1 min. 9-Fluorenylmethyl chloroformate (3.57 g, 13.8 mmol) was added and the reaction mixture stirred at 25 °C for 16 h, after which TLC (MeOH:CH_2Cl_2 = 1:9, *v*/*v*) showed complete conversion of the starting material. The reaction mixture was concentrated to approximately 10% volume under reduced pressure, the residue was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (40 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography (MeOH: CH_2Cl_2, 1:9, *v*/*v*). Yield: 1.91 g, (5.12 mmol, 77%). ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 3.34 (m, 1H, C<u>H</u>CH_2OH), 3.37 (m, 1H, CH_2N), 3.51 (s, 3H, OCH_3), 3.52 (m, 1H, CH_2N), 3.53 (m, 1H, C<u>H</u>$_2$OH), 3.60 (m, 1H, C<u>H</u>$_2$OH), 3.64 (m, 1H, C<u>H</u>CH_2N), 4.30 (t, *J* = 6.7 Hz, 1H, fluorenyl-H9), 4.40 (d, *J* = 6.9 Hz, 2H, fluorenyl-C<u>H</u>$_2$), 4.42 (t, 1H, *J* = 5.7 Hz, CH_2O<u>H</u>), 4.64 (d, *J* = 5.0 Hz, 1H, <u>H</u>OCHCH_2OH), 4.73 (d, *J* = 6.3 Hz, <u>H</u>OCHCH_2N), 7.34 (t, *J* = 7.5 Hz, 2H, fluorenyl-H2 and H7), 7.42 (t, *J* = 7.6 Hz, 2H, fluorenyl-H3 and H6), 7.71 (d, *J* = 7.2 Hz, 2H, fluorenyl-H1 and H8), 7.90 (d, *J* = 7.5 Hz, 2H, fluorenyl-H4 and H5). ^{13}C NMR (150 MHz, DMSO-d_6) δ_C: 47.1 (fluorenyl-C9), 52.3 (CH_2OH), 61.8 (Ar-OCH_3), 63.5 (CH_2N), 67.3 (fluorenyl-<u>C</u>H_2), 68.5 (<u>C</u>HCH_2N), 74.2 (<u>C</u>HCH_2OH), 120.6 (fluorenyl-C4 and C5), 125.6 (fluorenyl-C1 and C8), 127.6 (fluorenyl-C2 and C7), 128.2 (fluorenyl-C3 and C6), 141.3 (fluorenyl bridgehead), 144.2 (fluorenyl bridgehead), 156.9 (carbonyl). HRMS (ESI) *m*/z [M + Na]$^+$ calcd for $C_{20}H_{23}NNaO_6^+$: 396.1418; found: 396.1393.

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-*N*-(9-fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol (8)

(2*R*,3*S*)-*N*-(9-Fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol (7, 1.91 g, 5.12 mmol) was coevaporated from dry pyridine (3 × 20 mL) and the residue was dissolved in dry pyridine (10 mL) under N_2 atmosphere. A solution of 4,4´-dimethoxytrityl chloride (1.91 g, 5.65 mmol) in dry CH_2Cl_2 (2 mL) was added and the reaction mixture stirred at 25 °C under N_2 atmosphere for 16 h. The reaction mixture was concentrated to approximately 30% volume under reduced pressure, the residue was diluted with CH_2Cl_2 (50 mL) and washed with evaporated to dryness and extracted with saturated aqueous $NaHCO_3$ (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography (Et_3N:MeOH:CH_2Cl_2 =

1:3:96, *v*/*v*). Yield 2.34 g (3.46 mmol, 67%). ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 2.99 (dd, *J* = 9.1 Hz, 6.9 Hz, 1H, CH_2N), 3.06 (dd, *J* = 9.3 Hz, 4.2 Hz, 1H, CH_2N), 3.49 (s, 3H, $NOCH_3$), 3.53 (m, 2H, C<u>H</u>$_2$O), 3.63 (m, 1H, C<u>H</u>CH_2N), 3.72 (m, 7H, Ar-OCH_3 and C<u>H</u>CH_2O), 4.26 (t, *J* = 6.7 Hz, 1H, fluorenyl-H9), 4.36 (d, *J* = 6.7 Hz, 2H, fluorenyl-CH_2), 4.72 (d, *J* = 6.4 Hz, 1H, <u>H</u>$OCHCH_2O$), 4.98 (d, *J* = 5.4 Hz, 1H, <u>H</u>$OCHCH_2N$), 6.86 (d, *J* = 7.8 Hz, 4H, MeOPh-H3 and H5), 7.21 (t, *J* = 7.2 Hz, 1H, Ph-H4), 7.25—7.35 (m, 8H, Ph-H3 and H5, MeOPh-H2 and H6 and fluorenyl-H2 and H7), 7.35—7.45 (m, 4H, Ph-H2 and H6 and fluorenyl-H3 and H6), 7.69 (m, 2H, fluorenyl-H1 and H8), 7.90 (d, *J* = 7.5 Hz, 2H, fluorenyl-H4 and H5). ^{13}C NMR 150 MHz, CD_3OD) δ_C: 46.9 (fluorenyl-C9), 51.2 (CH_2O), 54.3 (Ar-OCH_3), 61.0 ($NOCH_3$), 64.8 (CH_2N), 67.6 (fluorenyl-CH_2), 69.0 (<u>C</u>HCH_2O), 72.5 (<u>C</u>HCH_2N), 86.1 (Ar_3C), 112.67 (MeOPh-C3 and C5), 119.6 (fluorenyl-C4 and C5), 124.9 (fluorenyl-C1 and C8), 126.3 (fluorenyl-C2 and C7), 126.8 (Ph-C4), 127.3 (Ph-C2 and C6), 127.5 (Ph-C3 and C5), 128.0 (fluorenyl-C3 and C6), 129.95 (MeOPh-C2 and C6), 129.98 (MeOPh-C2 and C6), 136.0 (MeOPh-C1), 141.2 (fluorenyl bridgehead), 143.7 (fluorenyl bridgehead), 145.2 (Ph-C1), 157.6 (carbonyl), 158.7 (MeOPh-C4). HRMS (ESI) *m*/z [M + Na]$^+$ calcd for $C_{41}H_{41}NNaO_8^+$: 698.2724; found: 698.2720.

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-4-(methoxyamino)butane-1,2,3-triol (9)

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-*N*-(9-fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol (8, 2.34 g, 3.46 mmol) was dissolved in a mixture of Et_2NH (30 mL) and CH_2Cl_2 (30 mL) and the resulting mixture stirred at 25 °C for 45 min. The reaction mixture was evaporated to dryness and the residue was purified by silica gel column chromatography (Et_3N:MeOH:CH_2Cl_2 = 1:6:93, *v*/*v*). Yield: 1.57 g (3.46 mmol, 99%). ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 2.61 (m, 1H, CH_2N), 2.97 (dd, *J* = 9.2 Hz, 6.4 Hz, 1H, CH_2O), 3.01(m, 1H, CH_2N), 3.04 (dd, *J* = 9.2 Hz, 3.6 Hz, 1H, CH_2O), 3.36 (s, 3H, $NOCH_3$), 3.57 (m, 1H, C<u>H</u>CH_2N), 3.58 (m, 1H, C<u>H</u>CH_2O), 3.74 (s, 6H, Ar-OCH_3), 4.50 (d, *J* = 5.3 Hz, 1H, <u>H</u>$OCHCH_2N$), 4.87 (d, *J* = 5.0 Hz, 1H, <u>H</u>$OCHCH_2O$), 6.34 (m, 1H, NH), 6.88 (d, *J* = 8.8 Hz, MeOPh-H3 and H5), 7.21 (t, *J* = 7.3 Hz, 1H, Ph-H4), 7.25—7.33 (m, 6H, Ph-H3 and H5, MeOPh-H2 and H6), 7.43 (d, *J* = 7.7 Hz, 2H, Ph-H2 and H6). ^{13}C NMR (150 MHz, DMSO-d_6) δ_C: 54.4 (CH_2N), 55.4 (Ar-OCH_3), 61.0 ($NOCH_3$), 65.9 (CH_2O), 68.6 (<u>C</u>HCH_2N), 73.0 (<u>C</u>HCH_2O), 85.6 (Ar_3C), 113.5 (MeOPh-C3 and C5), 126.9 (Ph-C4), 128.2 (Ph-C2 and C6), 128.4 (Ph-C3 and C5), 130.3 (MeOPh-C2 and C6), 136.45 (MeOPh-C1), 136.54 (MeOPh-C1), 145.7 (Ph-C1), 158.4 (MeOPh-C4). HRMS (ESI) *m*/z [M + Na]$^+$ calcd for $C_{26}H_{31}NNaO_6^+$: 476.2044; found: 476.2036.

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-2-*O*-(*tert*-butyldimethylsilyl)-4-(methoxyamino)butane-1,2,3-triol (10)

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-4-(methoxyamino)butane-1,2,3-triol (9, 1.57 g, 3.46 mmol) was coevaporated from dry pyridine (3 × 20 mL) and the residue was dissolved in dry DMF (15 mL). Imidazole (0.473 g, 6.94 mmol) was added and the resulting solution stirred at 25 °C for 1 min. *Tert*-butyldimethylsilyl chloride (0.529 g, 3.51 mmol) was added and the resulting mixture stirred at 25 °C for 16 h. The reaction mixture was concentrated to approximately 10% volume under reduced pressure, diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The aqueous phase was extracted three times with CH_2Cl_2, the combined organic extracts dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography (Et_3N:EtOAc:hexane = 1:40:59, *v*/*v*). Yield: 0.560 g (0.986 mmol, 28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: -0.04 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$), 0.83 (s, 9H, $SiC(CH_3)_3$), 2.58 (ddd, *J* = 12.8 Hz, 8.6 Hz, 4.0 Hz, 1H, CH_2N), 2.93 (ddd, *J* = 12.8 Hz, 9.5 Hz, 3.3 Hz, 1H, CH_2N), 3.00 (dd, *J* = 9.6 Hz, 5.5 Hz, 1H, CH_2O), 3.05 (dd, *J* = 9.6 Hz, 4.6 Hz, 1H, CH_2O), 3.37 (s, 3H, $NOCH_3$), 3.74 (s, 6H, $ArOCH_3$), 3.75 (m, 1H, C<u>H</u>CH_2N), 3.79 (m, 1H, C<u>H</u>CH_2O), 4.64 (d, *J* = 5.3 Hz, OH), 6.35 (dd, 1H, *J* = 9.4 Hz, 3.9 Hz, NH), 6.88 (m, 4H, MeOPh-H3 and H5), 7.22 (m, 1H, Ph-H4), 7.24—7.34 (m, 6H, Ph-H3 and H5, MeOPh-H2 and H6), 7.40 (m, 2H, Ph-H2 and H6). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: -4.2 ($SiCH_3$), -4.0 ($SiCH_3$), 18.3 (Si<u>C</u>$(CH_3)_3$), 26.3 ($SiC(\underline{C}H_3)_3$), 53.4 (CH_2N), 55.5 ($Ar\text{-}OCH_3$), 61.0 ($NOCH_3$), 65.4 (CH_2O), 68.8 (<u>C</u>HCH_2N), 75.1 (<u>C</u>HCH_2O), 86.0 (Ar_3C), 113.5 (MeOPh-C3 and C5), 127.1 (Ph-C4), 128.2 (Ph-C2 and C6), 128.3 (Ph-C3 and C5), 130.2 (MeOPh-C2 and C6), 136.25 (MeOPh-C1), 136.29 (MeOPh-C1), 145.4 (Ph-C1), 158.5 (MeOPh-C4). HRMS (ESI) *m*/z $[M + Na]^+$ calcd for $C_{32}H_{45}NNaO_6Si^+$: 590.2908; found: 590.2875.

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-2-*O*-(*tert*-butyldimethylsilyl)-*N*-(9-fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol (11)

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-2-*O*-(*tert*-butyldimethylsilyl)-4-(methoxyamino)butane-1,2,3-triol (10, 0.560 g, 0.986 mmol) was dissolved in a mixture of H_2O (0.80 mL) and 1,4-dioxane (19.2 mL). K_2CO_3 (0.294 g, 2.13 mmol) was added and the resulting mixture stirred for 1 min. 9-Fluorenylmethyl chloroformate (0.275 g, 1.06 mmol) was added and the reaction mixture stirred at 25 °C for 16 h, after which it was concentrated to approximately 10% volume. The residue was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (40 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography (Et_3N:EtOAc:hexane = 1:40:59, *v*/*v*). Yield:

0.604 g, (0.765 mmol, 77%). ^{1}H NMR (600 MHz, CD_3OD) δ_H: 0.00 (s, 3H, $SiCH_3$), 0.09 (s, 3H, $SiCH_3$), 0.88 (s, 9H, $SiC(CH_3)_3$), 3.20 (d, J = 5.5 Hz, 2H, CH_2N), 3.60 (dd, J = 14.9 Hz, 3.3 Hz, 1H, CH_2O), 3.62 (s, $NOCH_3$), 3.67 (m, 1H, CH_2O), 3.728 (s, 3H, $ArOCH_3$), 3.731 (s, 3H, $ArOCH_3$), 3.89 (m, 1H, $CHCH_2N$), 4.13 (dt, J = 9.0 Hz, 3.4 Hz, 1H, $CHCH_2O$), 4.20 (t, J = 6.7 Hz, 1H, fluorenyl-H9), 4.41 (dd, J = 6.8 Hz, 2.6 Hz, 2H, fluorenyl-CH_2), 6.81 (m, 4H, MeOPh-H3 and H5), 7.19 (t, J = 7.3 Hz, 1H, Ph-H4), 7.23—7.35 (m, 8H, Ph-H2, H3, H5 and H6 and MeOPh-H2 and H6), 7.38 (t, J = 7.3 Hz, 2H, fluorenyl-H2 and H7), 7.45 (d, J = 7.5 Hz, 2H, fluorenyl-H3 and H6), 7.64 (t, J = 7.8 Hz, 2H, fluorenyl-H1 and H8), 7.80 (d, J = 7.5 Hz, 2H, fluorenyl-H4 and H5). ^{13}C NMR (150 MHz, CD_3OD) δ_C: -5.8 ($SiCH_3$), -5.6 ($SiCH_3$), 17.6 ($SiC(CH_3)_3$), 25.1 ($SiC(CH_3)_3$), 46.9 (fluorenyl-C9), 50.8 (CH_2O), 54.3 ($ArOCH_3$), 61.0 ($NOCH_3$), 64.9 (CH_2N), 67.7 (fluorenyl-CH_2), 69.4 ($CHCH_2O$), 74.5 ($CHCH_2N$), 86.3 (Ar_3C), 112.7 (MeOPh-C3 and C5), 119.6 (fluorenyl-C4 and C5), 124.8 (fluorenyl-C1 and C8), 124.9 (fluorenyl-C1 and C8), 126.9 (Ph-C4), 127.3 (Ph-C2 and C6), 127.5 (Ph-C3 and C5), 128.0 (fluorenyl-C3 and C6), 129.93 (MeOPh-C2 and C6), 129.97 (MeOPh-C2 and C6), 135.9 (MeOPh-C1), 141.2 (fluorenyl bridgehead), 143.6 (fluorenyl bridgehead), 143.7 (fluorenyl bridgehead), 145.0 (Ph-C1), 157.5 (carbonyl), 158.68 (MeOPh-C4), 158.70 (MeOPh-C4). HRMS (ESI) *m*/z $[M + Na]^+$ calcd for $C_{47}H_{55}NNaO_8Si^+$: 812.3589; found: 812.3564.

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-2-*O*-(*tert*-butyldimethylsilyl)-*N*-(9-fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol-3-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (1)

(2*R*,3*S*)-*1*-*O*-(4,4´-Dimethoxytrityl)-2-*O*-(*tert*-butyldimethylsilyl)-*N*-(9-fluorenylmethoxycarbonyl)-4-(methoxyamino)butane-1,2,3-triol (11, 0.604 g, 0.765 mmol) was coevaporated from dry toluene (3 × 20 mL). The residue was dissolved in dry CH_2Cl_2 (10 mL) and dry Et_3N (320 µL, 2.30 mmol) was added under N_2 atmosphere. 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (205 µL, 0.918 mmol) was added and the resulting mixture stirred at 25 °C under N_2 atmosphere for 45 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), the combined organic extracts dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography (Et_3N:EtOAc:hexane = 1:30:69, *v*/*v*). Yield: 0.387 g (0.391 mmol, 51%). ^{1}H NMR (500 MHz, CD_3CN, major diastereomer) δ_H: 0.00 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.87 (s, 9H, $SiC(CH_3)_3$), 1.14 (d, J = 6.8 Hz, 6H, $NCH(CH_3)_2$), 1.17 (d, J = 6.8 Hz, 6H, $NCH(CH_3)_2$), 2.52 (m, 2H, CH_2CN), 3.10 (m, 2H, CH_2ODMTr), 3.49 (s, 3H, $NOCH_3$), 3.54 (m, 1H, CH_2N), 3.62 (m, 2H, $NCH(CH_3)_2$), 3.71 (m, 2H, $POCH_2$), 3.763 (s, 3H, $ArOCH_3$), 3.765 (s, 3H, $ArOCH_3$), 3.81 (m, 1H, CH_2N), 4.24 (m, 2H, fluorenyl-H9 and $CHCH_2ODMTr$), 4.43 (m, 2H, fluorenyl-CH_2), 4.45 (m, 1H, $CHCH_2N$), 6.86 (m, 4H, MeOPh-H3 and H5), 7.24 (m, 1H, Ph-H4), 7.27—7.39 (m, 8H, Ph-H2, H3, H5 and H6 and MeOPh-H2 and H6), 7.40—7.50 (m,

4H, fluorenyl-H2, H3, H6 and H7), 7.66 (m, 2H, fluorenyl-H1 and H8), 7.85 (m, 2H, fluorenyl-H4 and H5). ^{13}C NMR (125 MHz, CD_3CN, major diastereomer) δ_C: -5.3 ($SiCH_3$), -5.2 ($SiCH_3$), 17.8 ($SiC(CH_3)_3$), 20.1 (d, J = 7.4 Hz, CH_2CN), 23.96 (d, J = 6.6 Hz, $NCH(CH_3)_2$), 24.05 (d, J = 7.9 Hz, $NCH(CH_3)_2$), 25.3 ($SiC(CH_3)_3$), 43.0 (d, J = 12.5 Hz, $NCH(CH_3)_2$), 47.04 (fluorenyl-C9), 54.9 ($ArOCH_3$), 58.2 (d, J = 19.6 Hz, $POCH_2$), 61.1 ($NOCH_3$), 64.8 (CH_2N), 67.2 (fluorenyl-CH_2), 73.5 (d, J = 16.4 Hz, CH_2ODMTr), 73.8 (d, J = 1.5 Hz, $CHCH_2N$), 86.2 (Ar_3C), 113.0 (MeOPh-C3 and C5), 118.4 (CN), 120.0 (fluorenyl-C4 and C5), 125.09 (fluorenyl-C1 and C8), 125.13 (fluorenyl-C1 and C8), 126.8 (Ph-C4), 127.2 (Ph-C2 and C6), 127.3 (Ph-C2 and C6), 127.78 (Ph-C3 and C5), 127.84 (Ph-C3 and C5), 128.0 (fluorenyl-C3 and C6), 129.99 (MeOPh-C2 and C6), 130.02 (MeOPh-C2 and C6), 136.05 (MeOPh-C1), 136.06 (MeOPh-C1), 141.2 (fluorenyl bridgehead), 144.0 (fluorenyl bridgehead), 144.1 (fluorenyl bridgehead), 145.1 (Ph-C1), 156.1 (carbonyl), 158.66 (MeOPh-C4), 158.67 (MeOPh-C4). ^{31}C NMR (202 MHz, CD_3CN, major diastereomer) δ_P: 150.8. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{56}H_{73}N_3O_9PSi^+$: 989.4775; found 990.4821.

Oligonucleotide synthesis

The oligonucleotide scaffold ON1 was assembled by an ÄKTA oligopilot plus 10 DNA/RNA synthesizer in 2 µmol scale on CPG support following conventional phosphoramidite strategy. A prolonged coupling time of 15 min was used for the sterically crowded (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol building block 1. Despite this precaution, the coupling yield was only approximately 64%. The other couplings, including the one immediately after the coupling of 1, proceeded with typical (>99%) efficiency. After chain assembly, the solid support was incubated in 25% aqueous NH_3 at 25 °C for 16 h to cleave the succinyl linker and to remove the base and phosphate protections, as well as the Fmoc protection of the methoxyamino group of 1. The crude product was purified by RP-HPLC on a Hypersil ODS C18 column (250 × 10 mm, 5 µm) eluting with a linear gradient (5—20% over 25 min, flow rate = 3.0 mL min^{-1}) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0), the detection wavelength being 260 nm (Figure S20). The TBDMS protection was removed by treatment with a mixture of $Et_3N \bullet 3HF$ (125 µL) and DMSO (100 µL) at 65 °C for 2.5 h, after which a second HPLC purification under the aforementioned conditions afforded pure ON1, as verified by ESI-TOF-MS. Concentration of ON1 was determined UV spectrophotometrically using a molar absorptivity calculated by an implementation of the nearest-neighbors method. Contribution of the (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol residue to molar absorptivity of ON1 was assumed to be negligible.

Stability of ON1 under conditions used for introduction of aldehydes and isolation of the products was tested by incubating ON1 (4.0 µM) at either 25 or 55 °C and pH of either 5.0 (30 mM

acetate buffer) or 7.4 (30 mM cacodylate buffer). Samples were withdrawn from the solutions at 0, 4, 15 and 49 h and analyzed by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 µm) eluting with a linear gradient (8—23% over 25 min, flow rate = 1.0 mL min^{-1}) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0), the detection wavelength being 260 nm (Figures S23—S26). The observed peaks were identified by spiking with authentic samples. In each case, the only reaction detected was the interconversion of naked ON1 and its formaldehyde and acetaldehyde adducts.

Derivatization of the oligonucleotide scaffold with aldehydes

Method A: The aldehyde (2.0 mg) was suspended in a mixture of MeCN (10 µL) and acetic acid / sodium acetate buffer (10 µL, 30 mM, pH = 5.0). Aqueous solution of the oligonucleotide scaffold ON1 (4 µL, 250 µM) was added and the resulting mixture incubated at 55 °C for 16 h. Aqueous triethylammonium acetate (200 µL, 50 mM) was added, the mixture washed with CH_2Cl_2 (1000 µL) and fractioned by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 µm) eluting with a linear gradient (8—23% over 25 min, flow rate = 1.0 mL min^{-1}) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0), the detection wavelength being 260 nm (Figures S27, S29, S31, S33, S35, S37, S39, S41, S43 and S45). The major peaks were recovered and analyzed by ESI-TOF-MS.

Method B: A solution of the aldehyde (50 µL, 10 mM) was mixed with acetic acid / sodium acetate buffer (50 µL, 30 mM, pH = 5.0). Aqueous solution of the oligonucleotide scaffold ON1 (20 µL, 250 µM) was added and the resulting mixture incubated at 55 °C for 16 h. The solution was split into two equal parts, aqueous triethylammonium acetate (500 µL, 50 mM) was added, and the mixture was analyzed by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 µm) eluting with a linear gradient (8—23% over 25 min, flow rate = 1.0 mL min^{-1}) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0), the detection wavelength being 260 nm (Figures S47—S49).

Method C: Oligonucleotide scaffold ON1 (5 nmol) was dissolved in a solution of the aldehyde (1.67 mM, 6.0 µL). AcOH (2.0 µL) was added and the resulting mixture incubated at 55 °C for 16 h. The solution was split into two equal parts and Et_3N (2.5 µL) was added, followed by H_2O (500 µL). The mixture thus obtained was analyzed by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 µm) eluting with a linear gradient (8—23% over 25 min, flow rate = 1.0 mL min^{-1}) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0), the detection wavelength being 260 nm. Only traces of the expected product could be detected (data not shown).

NMR spectrometric assessment of the pseudoanomer ratio of an *N*-methoxy-1,3-oxazinane

(2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol (6, 24.8 mg, 0.164 mmol) and 3-hydroxybenzaldehyde (40.1 mg, 0.328 mmol) were dissolved in DMSO-d_6 (375 µL). AcOH-d_4 (125 µL) was added and the resulting mixture incubated at 55 °C for 16 h. NMR analysis of the product mixture (Figure S50) revealed the formation of four products, in all likelihood two pseudoanomers of a six-membered *N*-methoxy-1,3-oxazinane and two pseudoanomers of a five-membered *N*-methoxy-1,3-oxazolidine. Signals originating from the 4-(methoxyamino)butane-1,2,3-triol part overlapped too badly to allow reliable characterization of the products. Signals of the pseudoanomeric protons were broad, suggesting a relatively rapid equilibrium. Attempts to separate the products chromatographically were not successful.

Hydrolysis of the oligonucleotide products

An HPLC-purified sample of a product of functionalization of oligonucleotide scaffold ON1 with an aldehyde (approximately 20 µL) was diluted with acetic acid / sodium acetate buffer (200 µL, 30 mM, pH = 5.0) and incubated at 55 °C for 16 h. The product mixture was analyzed by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 µm) eluting with a linear gradient (8—23% over 25 min, flow rate = 1.0 mL min^{-1}) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0), the detection wavelength being 260 nm (Figures S51 and S52). The observed peaks were identified by spiking with authentic samples.

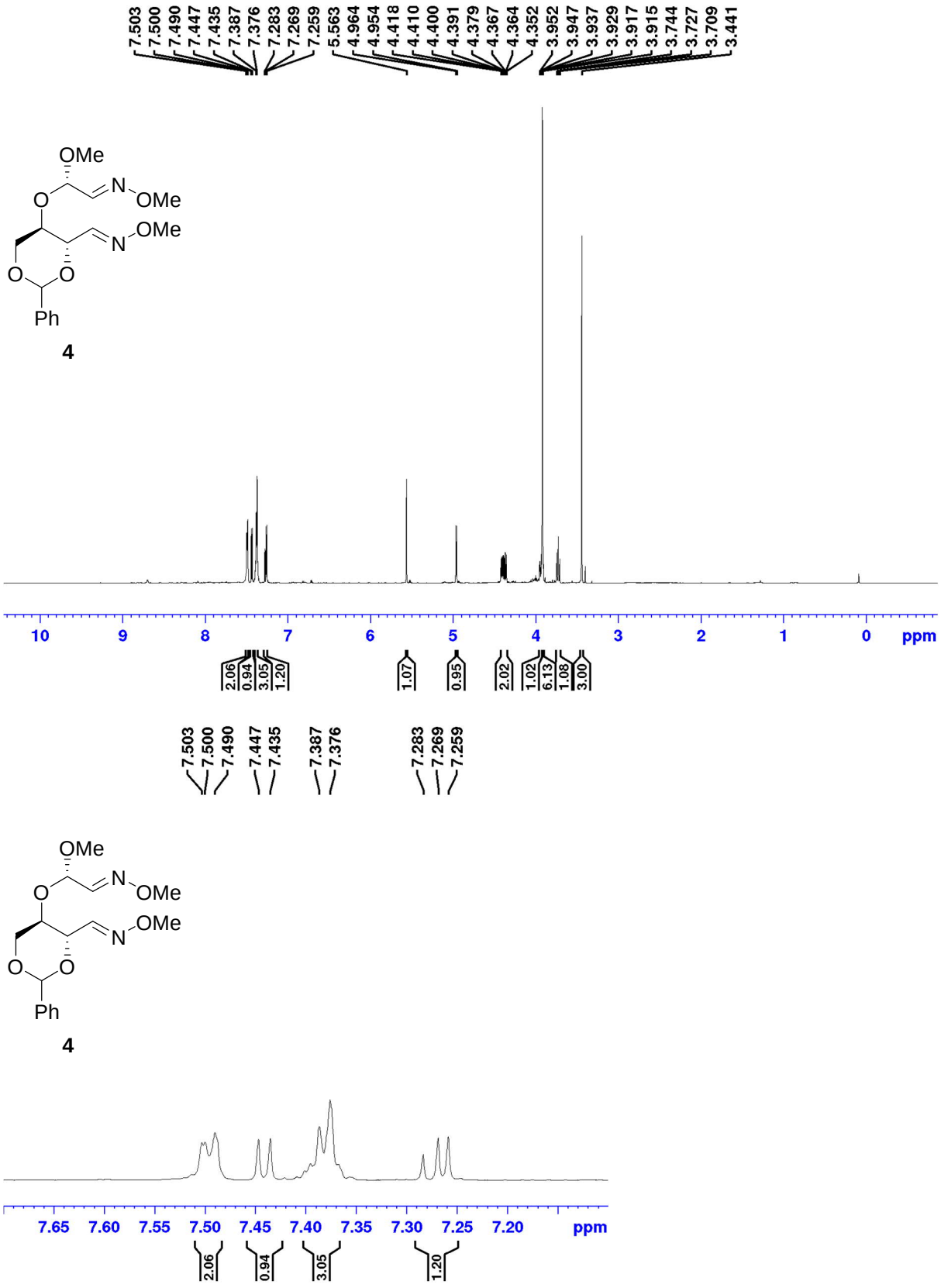


Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound 4.

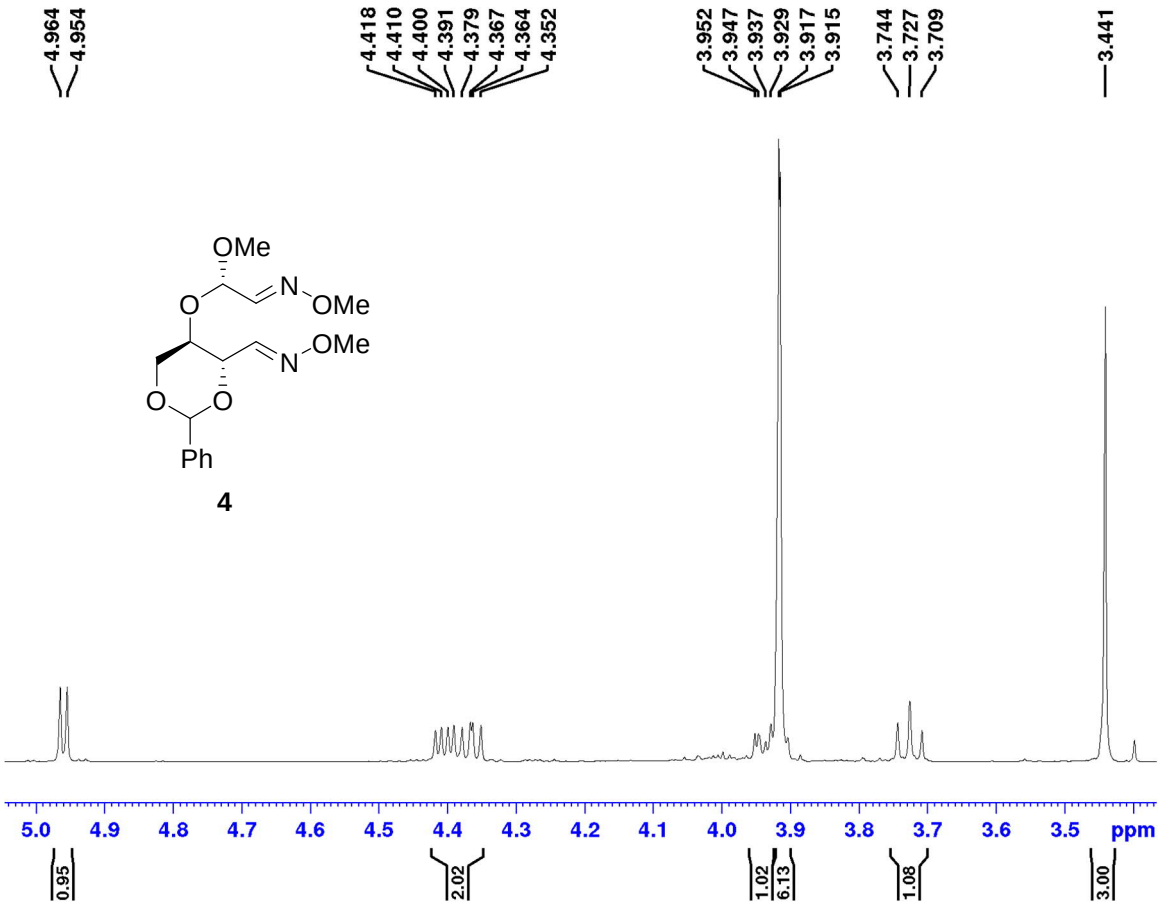


Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound 4 (continued).

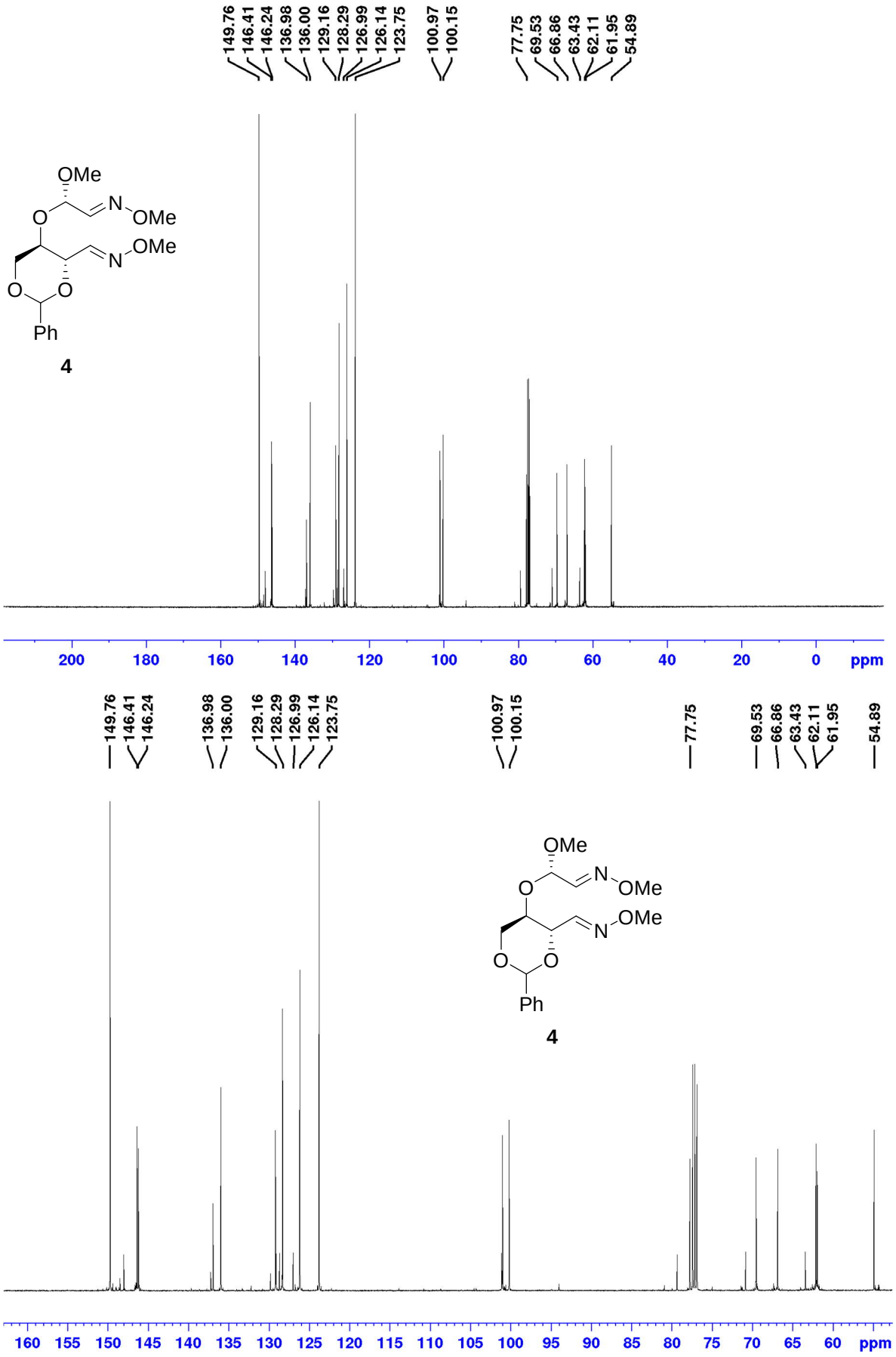


Figure S2. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound 4.

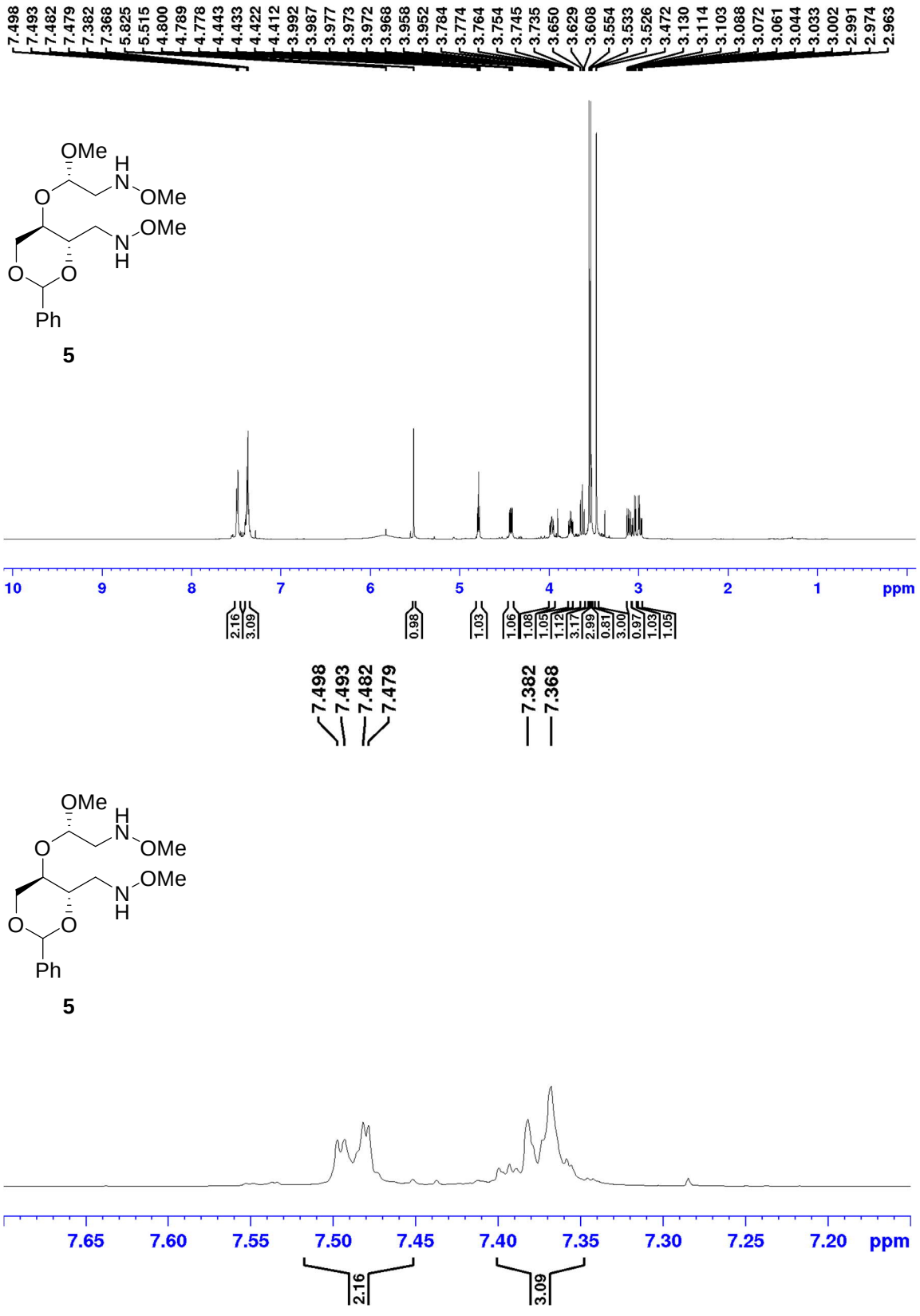


Figure S3. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound 5.

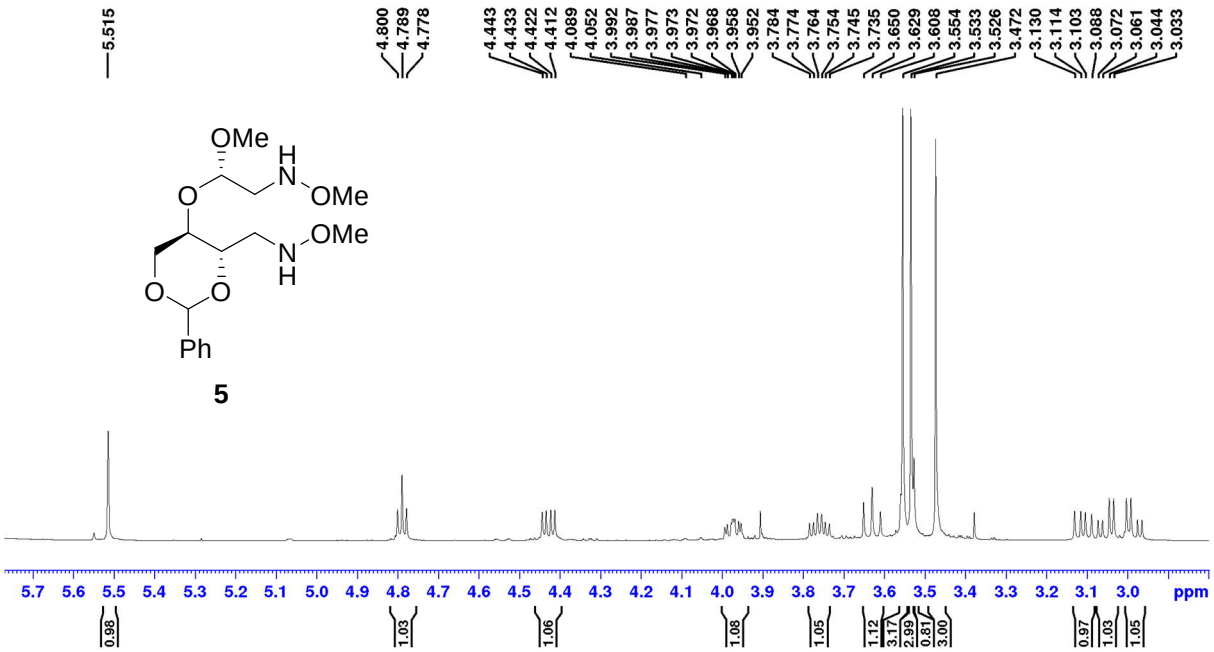


Figure S3. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound 5 (continued).

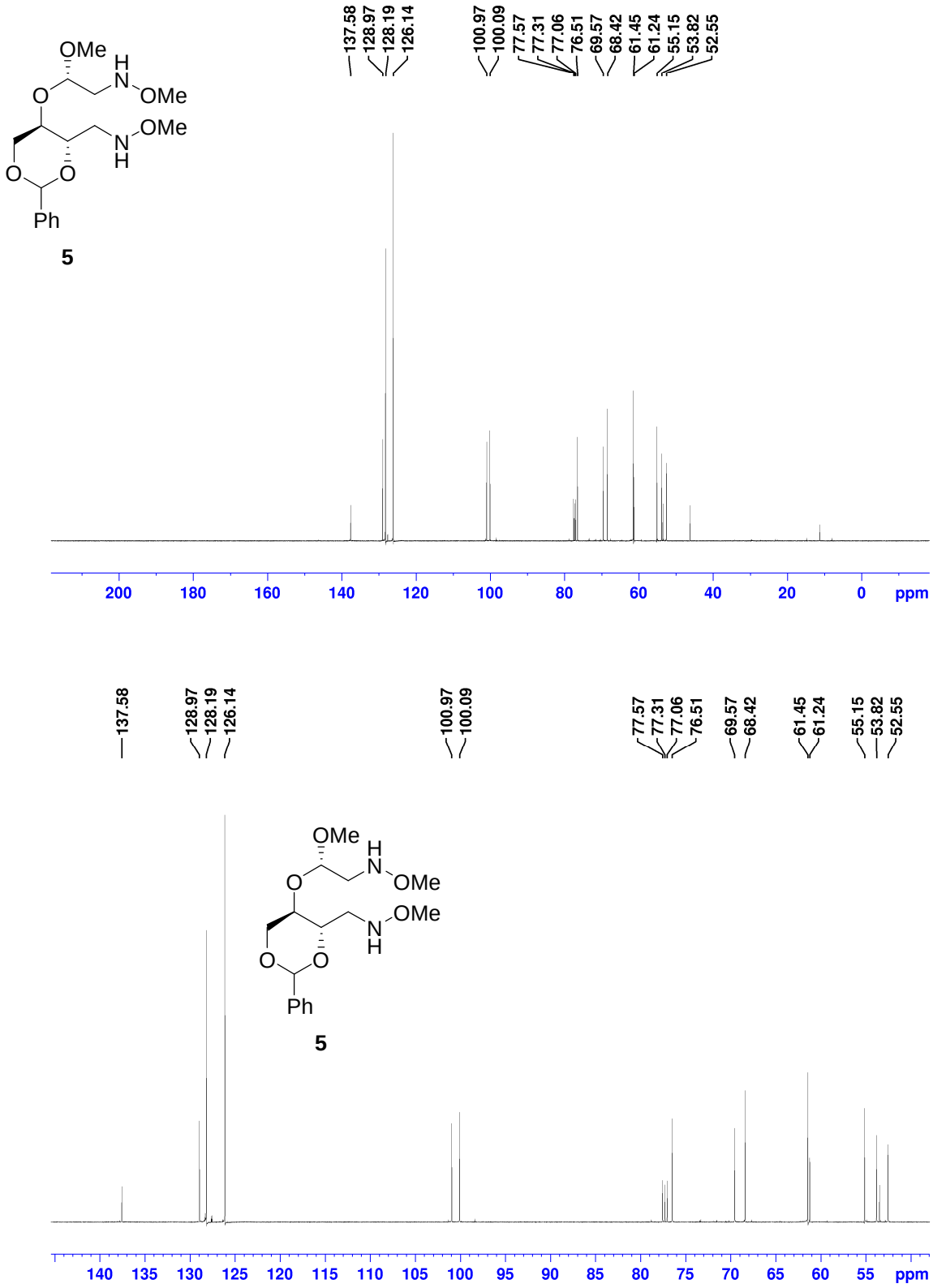


Figure S4. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of compound 5.

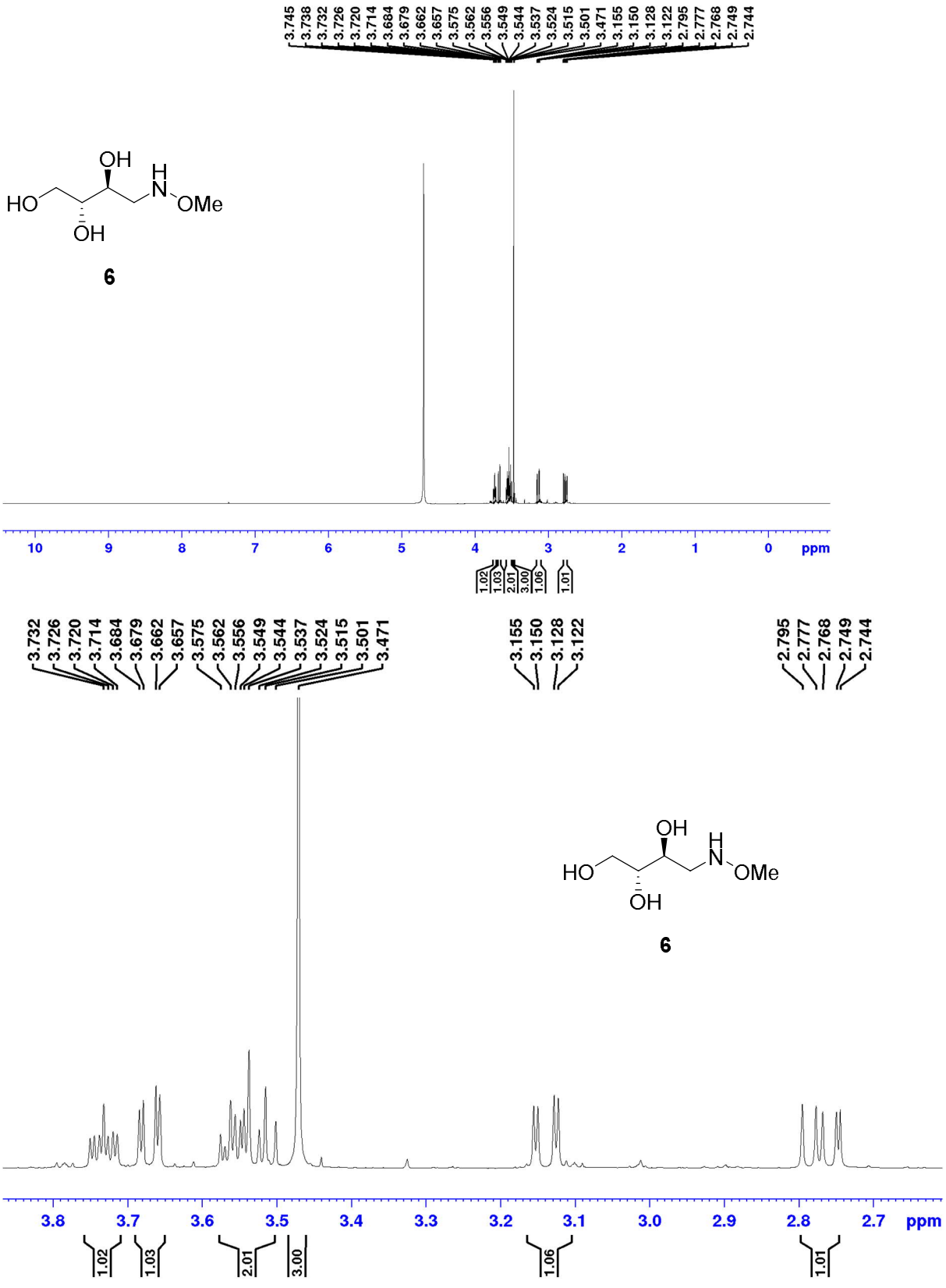


Figure S5. ^{1}H NMR (600 MHz, D_2O) spectrum of compound 6.

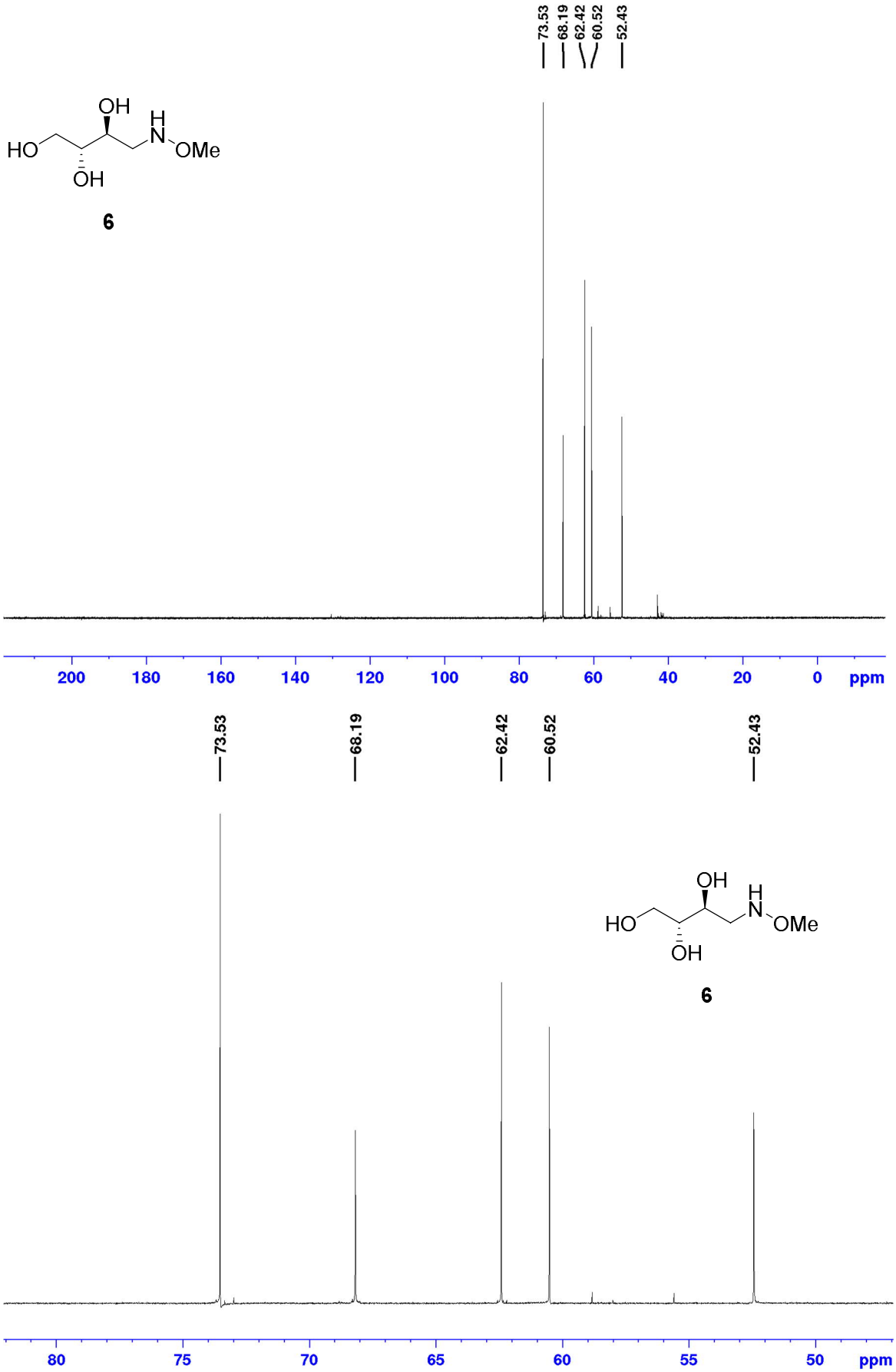
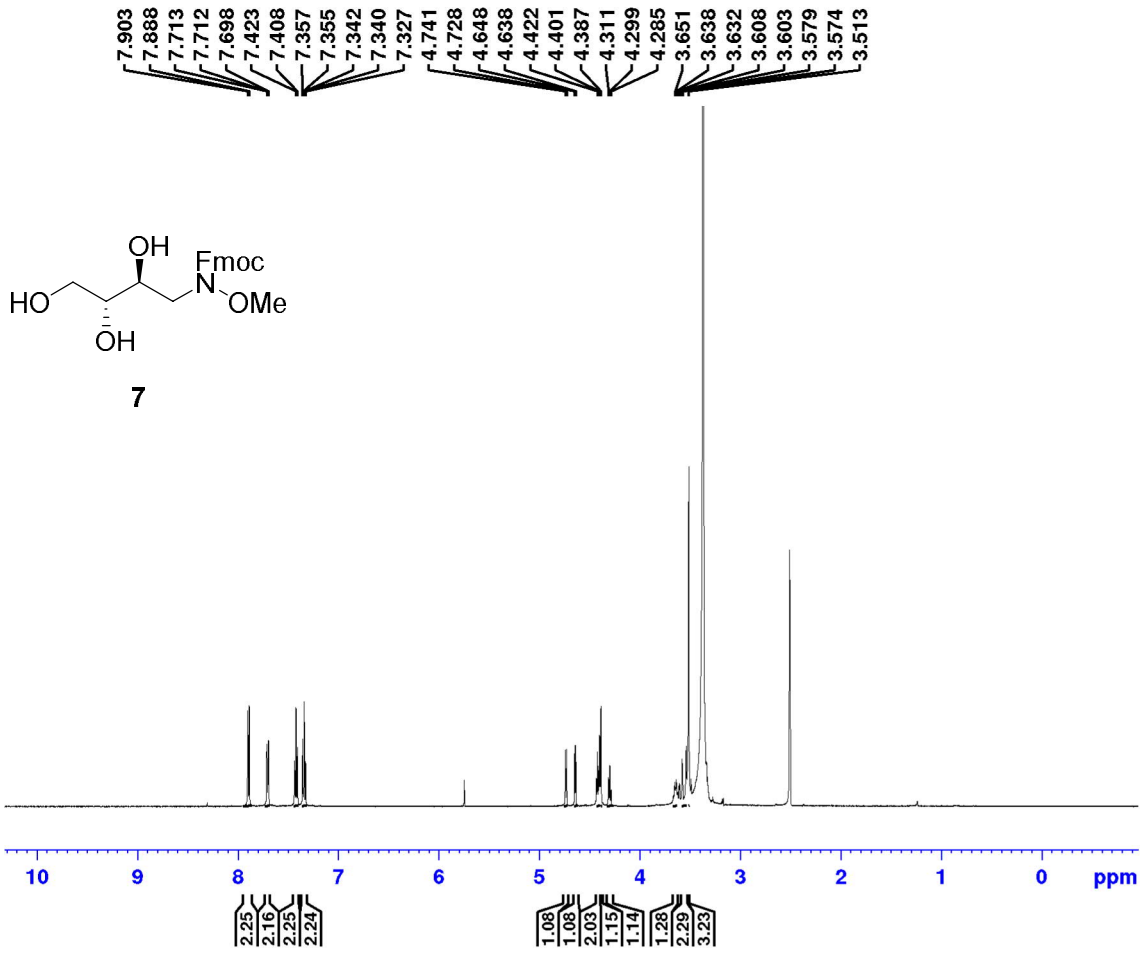


Figure S6. ^{13}C NMR (150 MHz, D_2O) spectrum of compound 6.



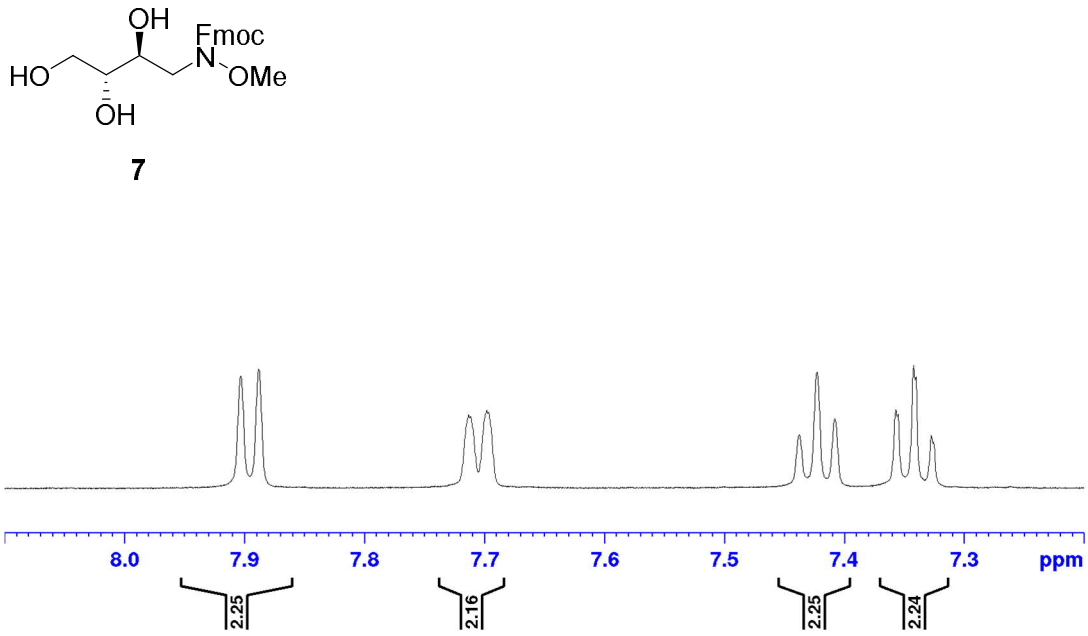


Figure S7. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 7.

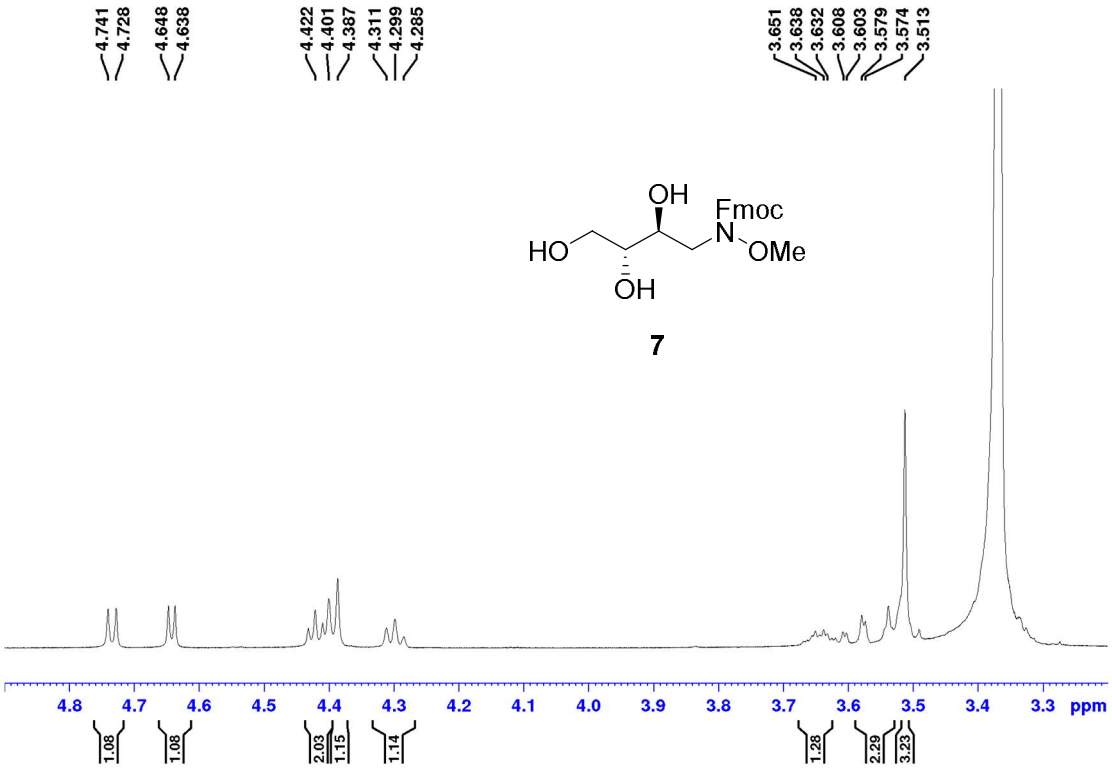


Figure S7. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 7 (continued).

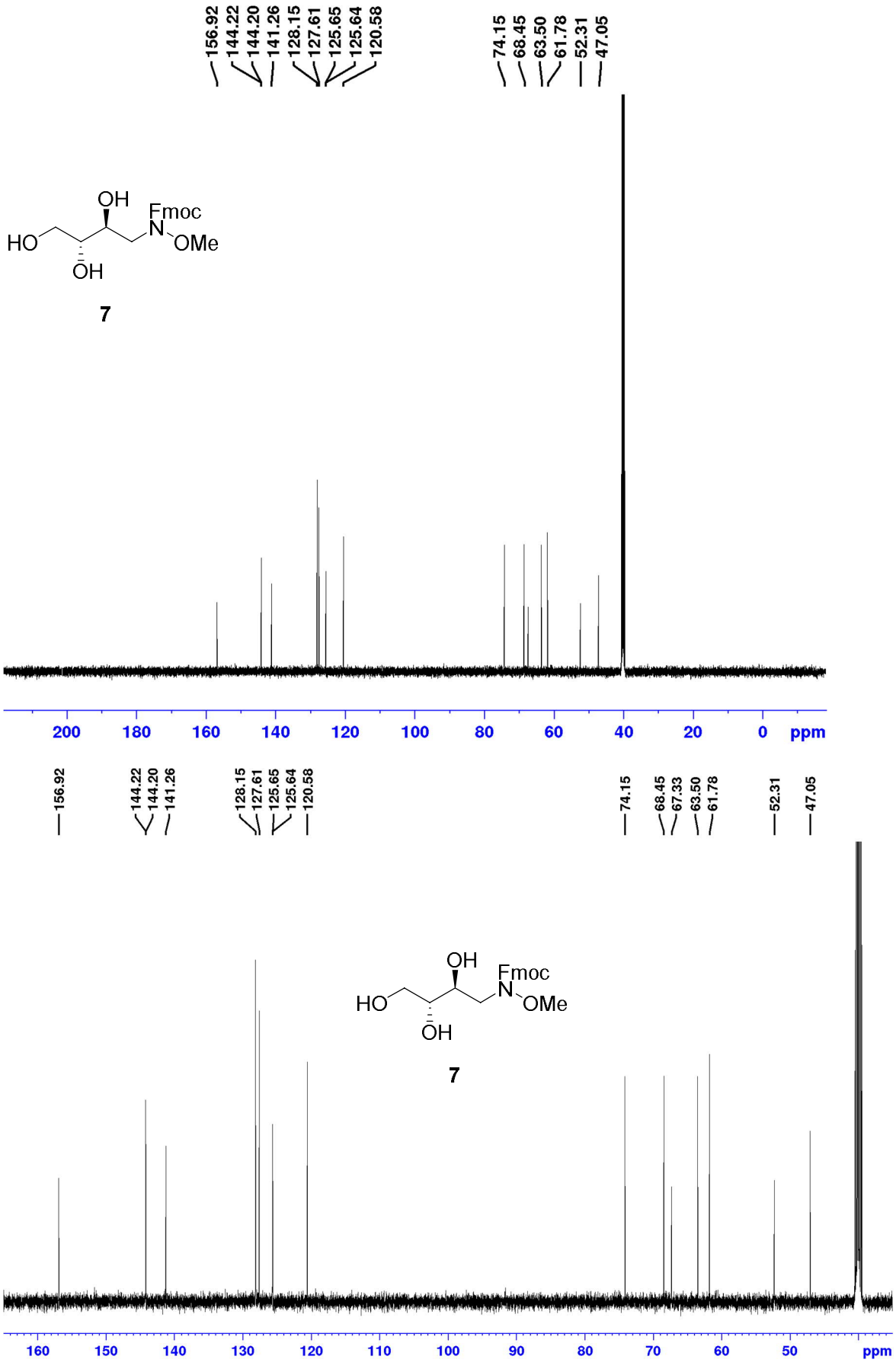


Figure S8. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound 7.

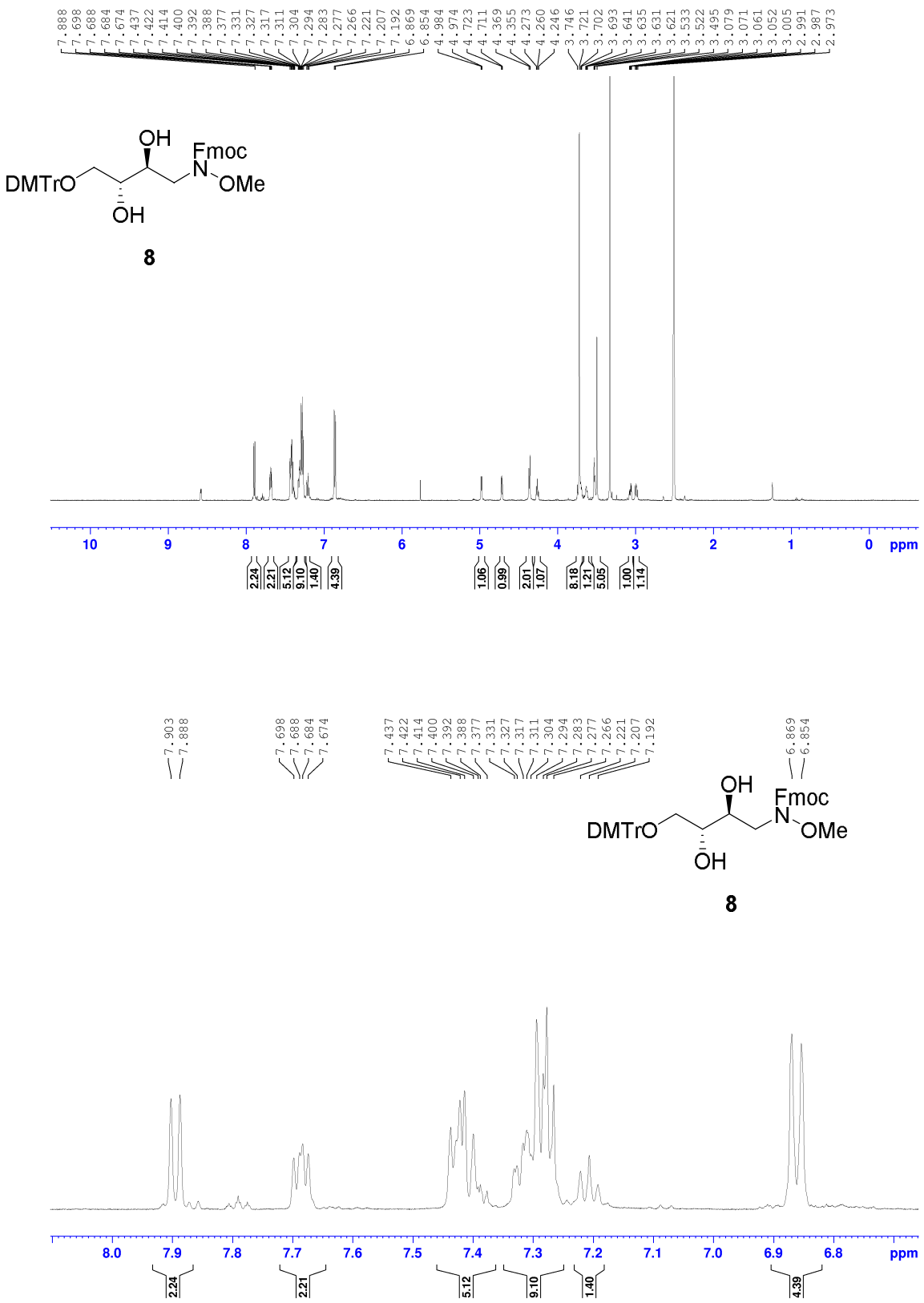


Figure S9. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 8.

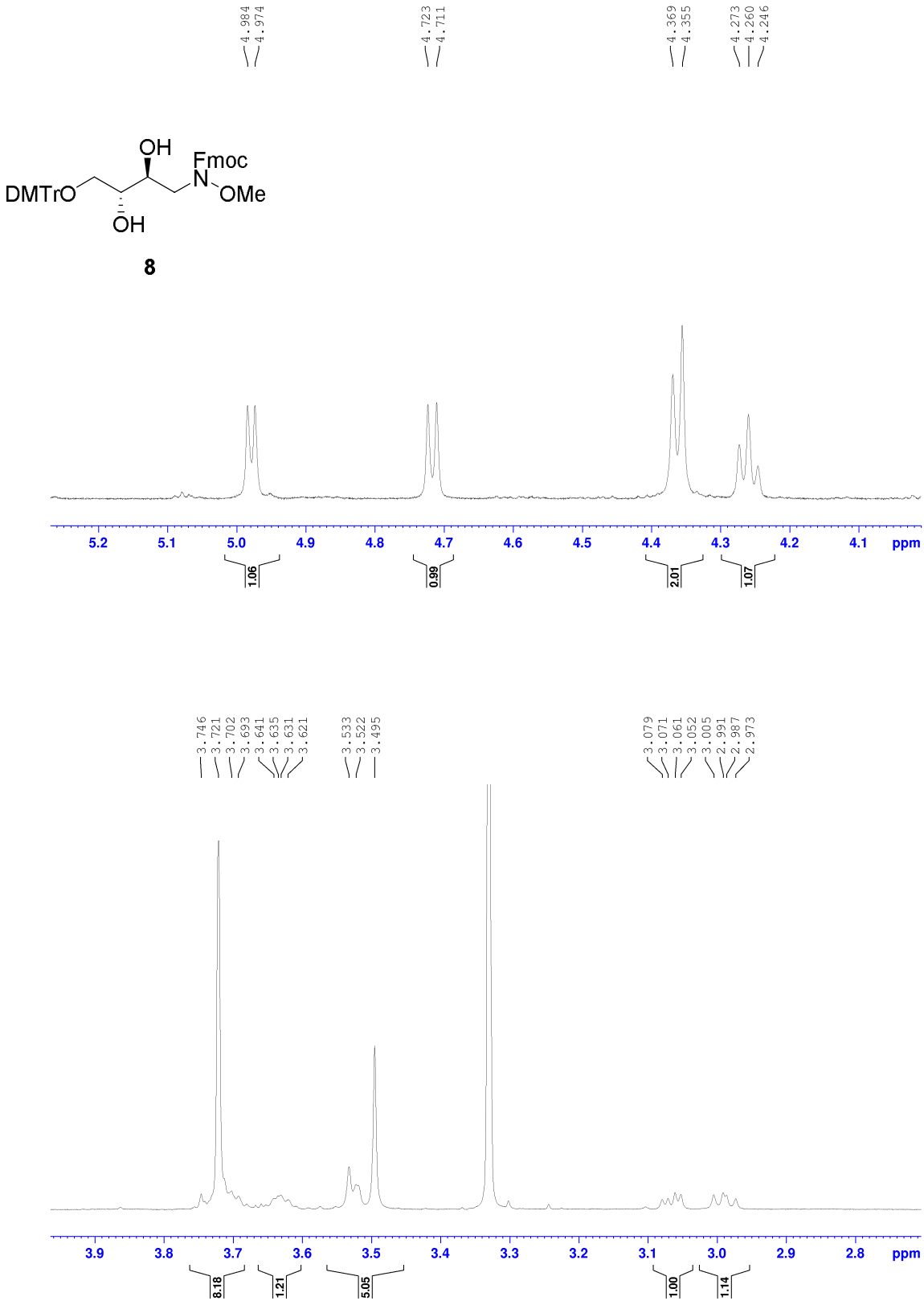


Figure S9. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 8 (continued).

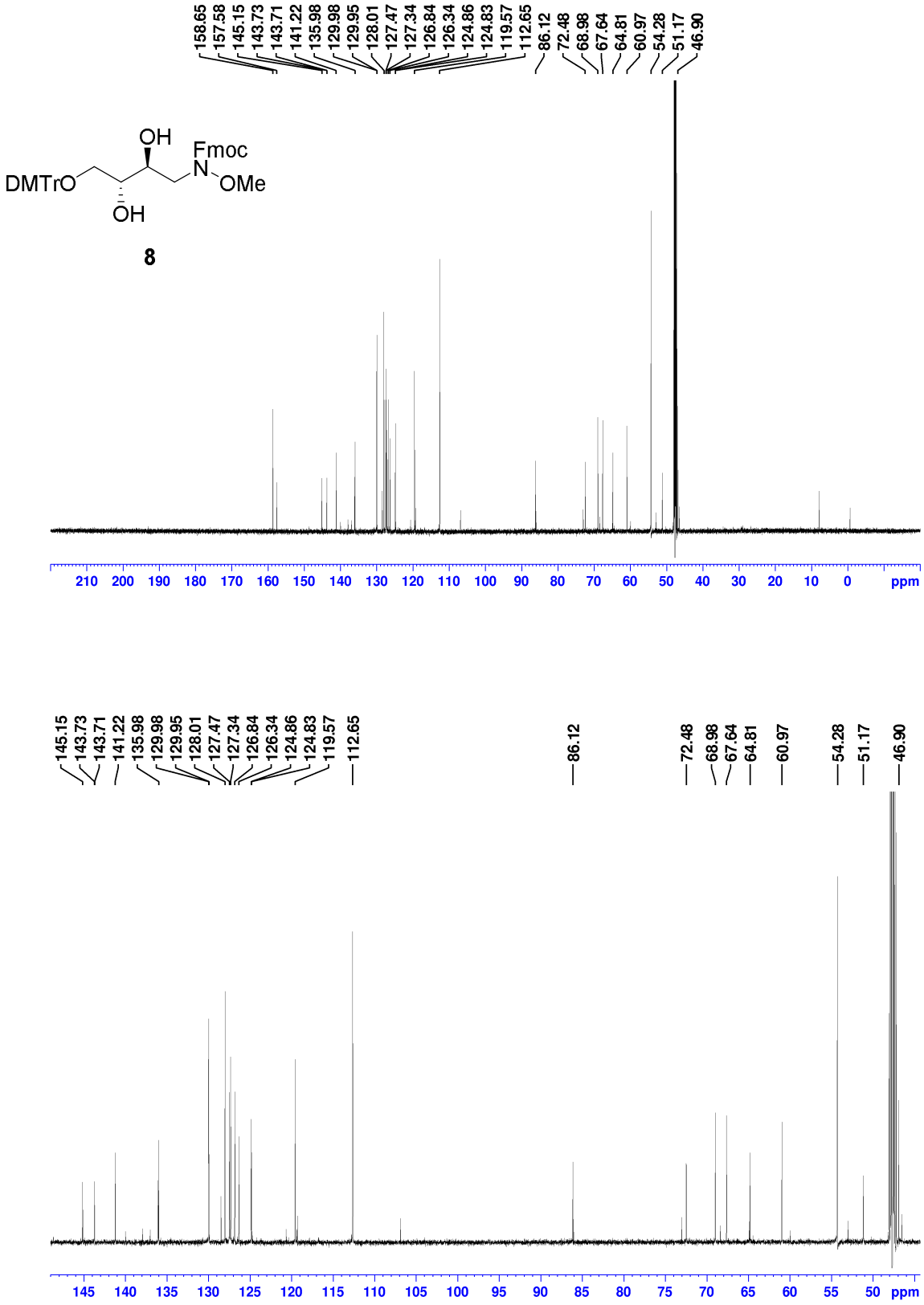


Figure S10. ^{13}C NMR (150 MHz, CD_3OD) spectrum of compound 8.

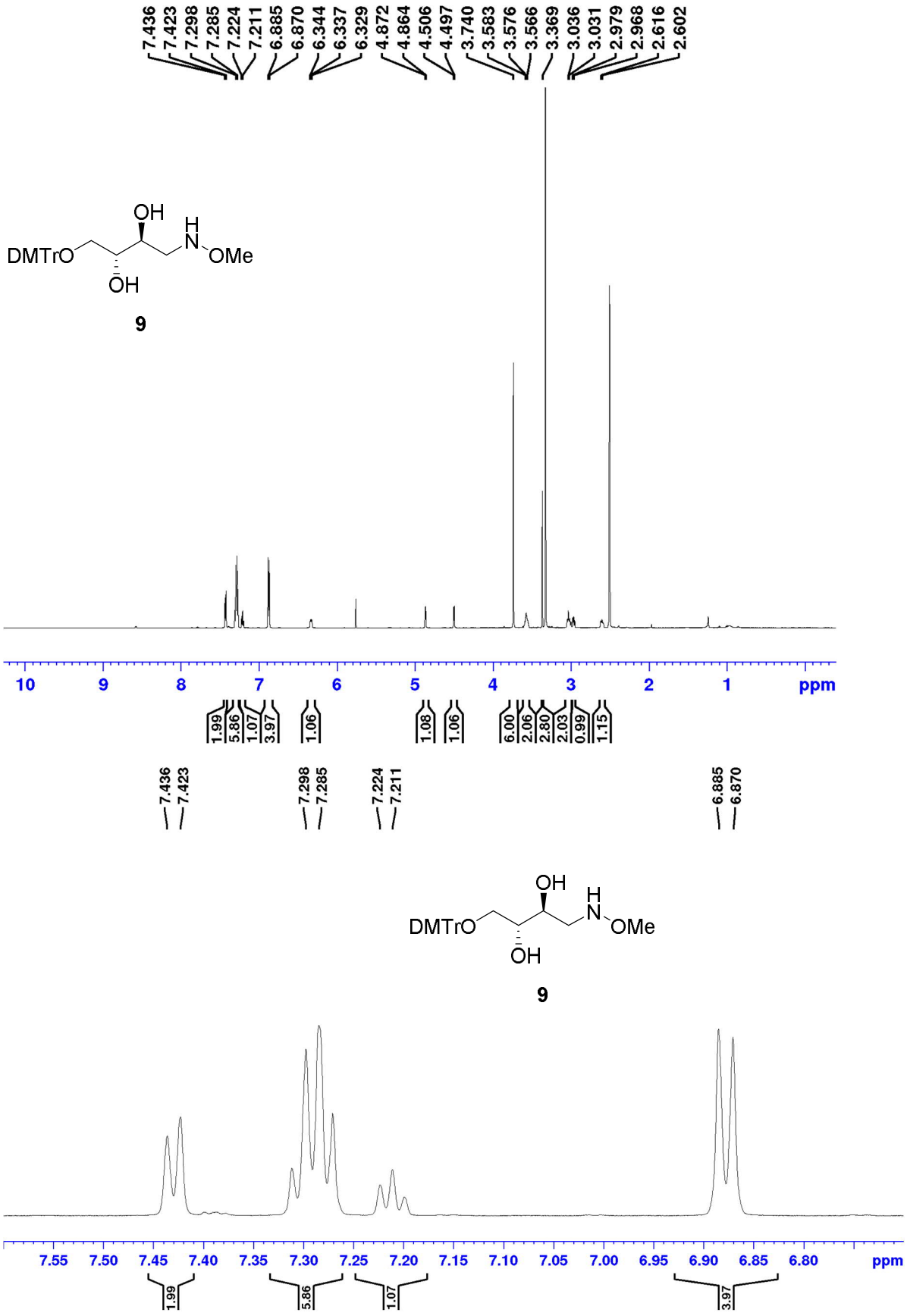


Figure S11. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 9.

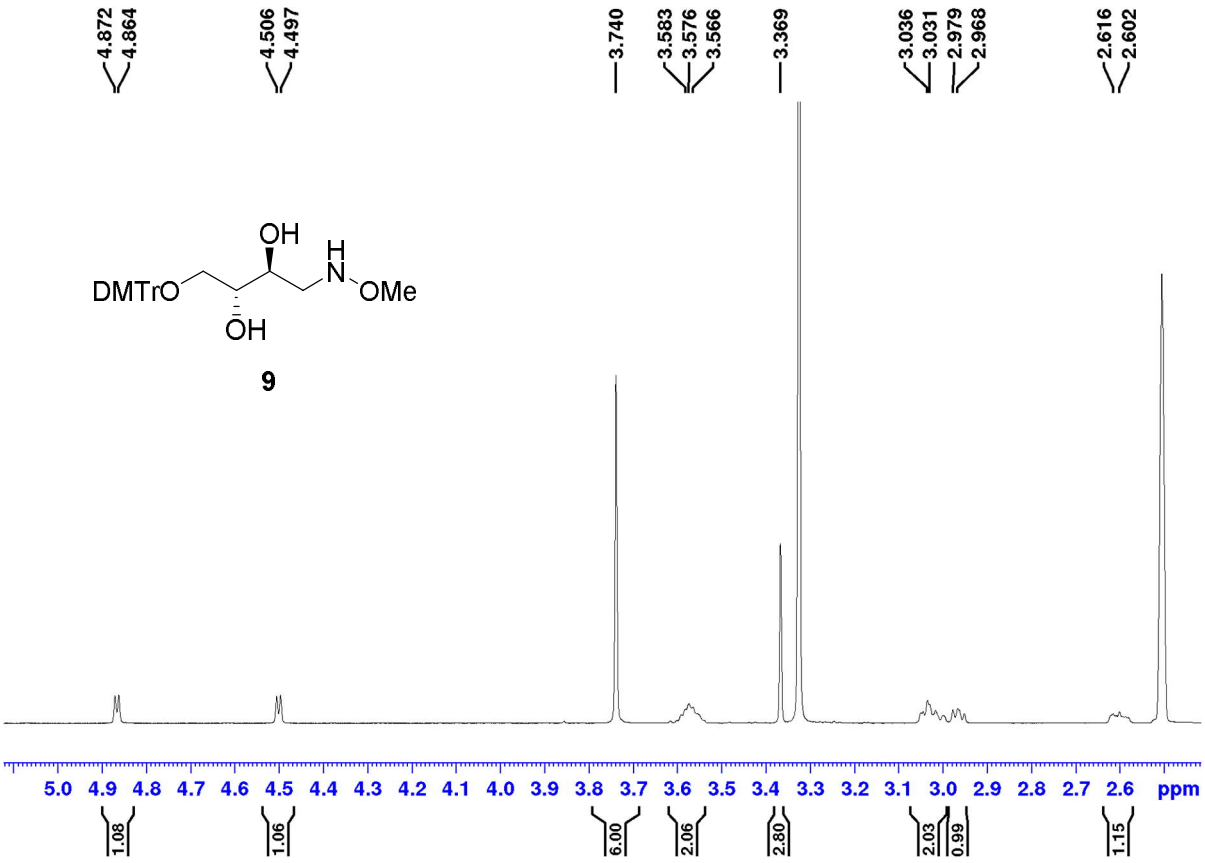


Figure S11. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 9 (continued).

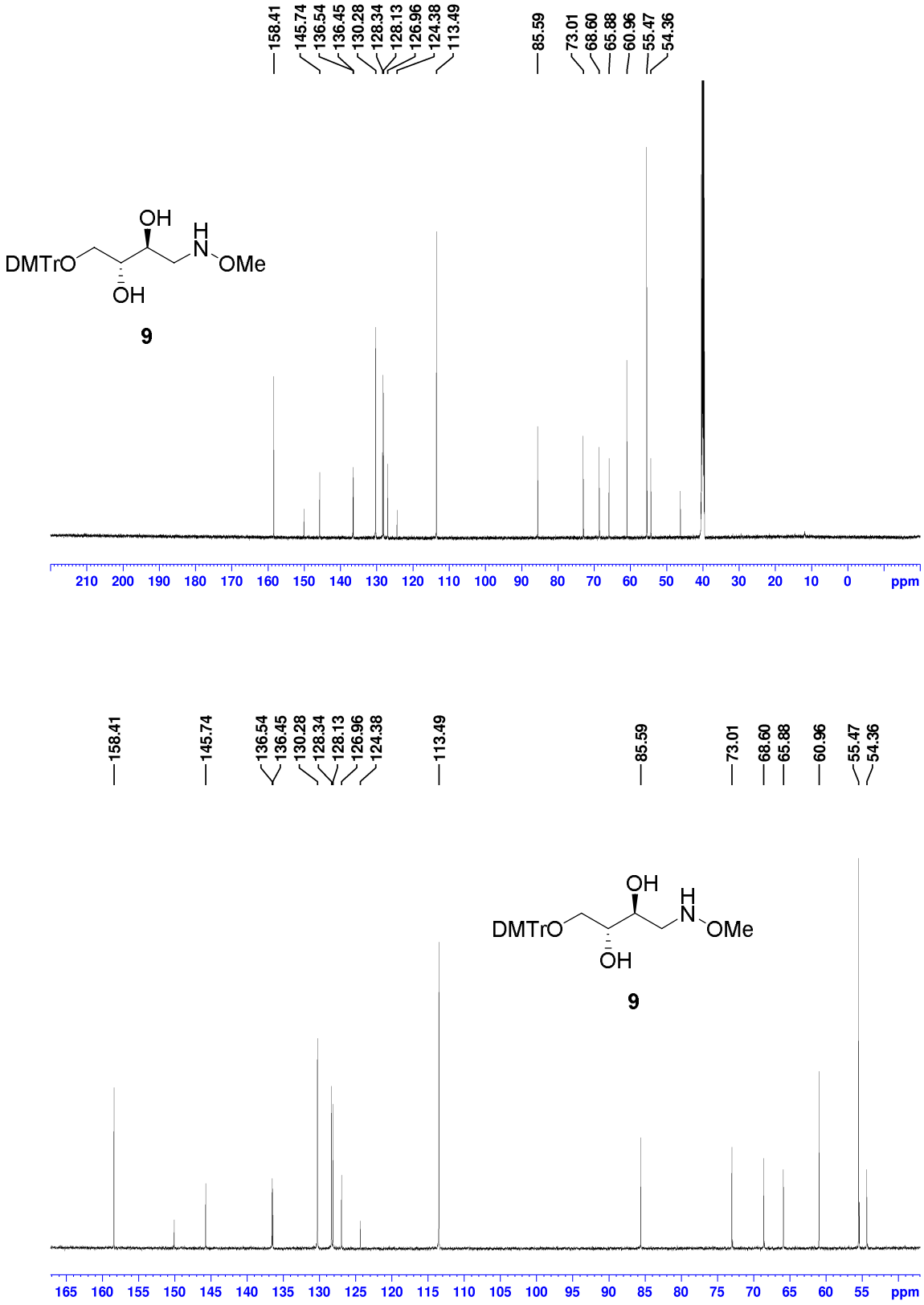


Figure S12. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound 9.

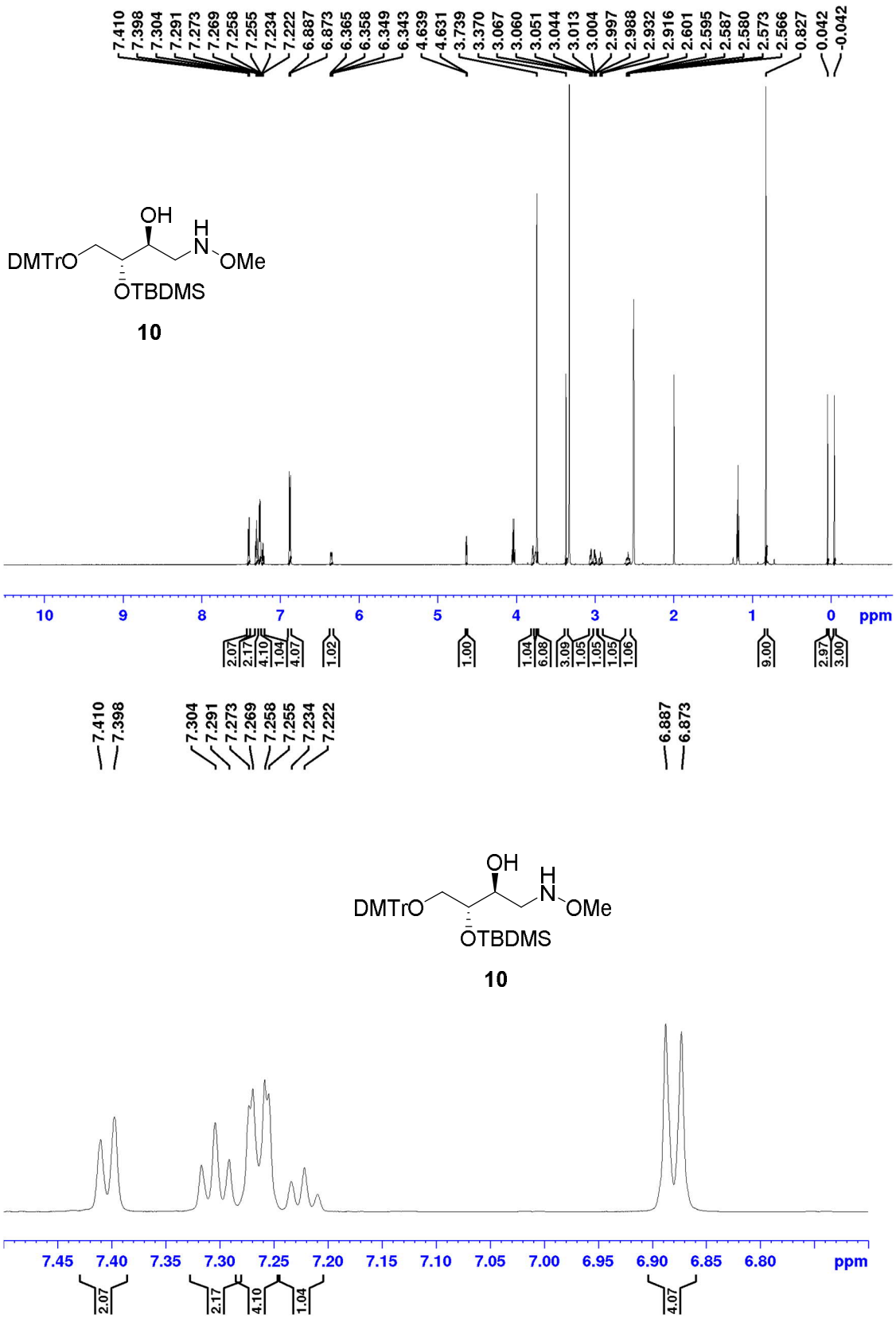


Figure S13. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 10.

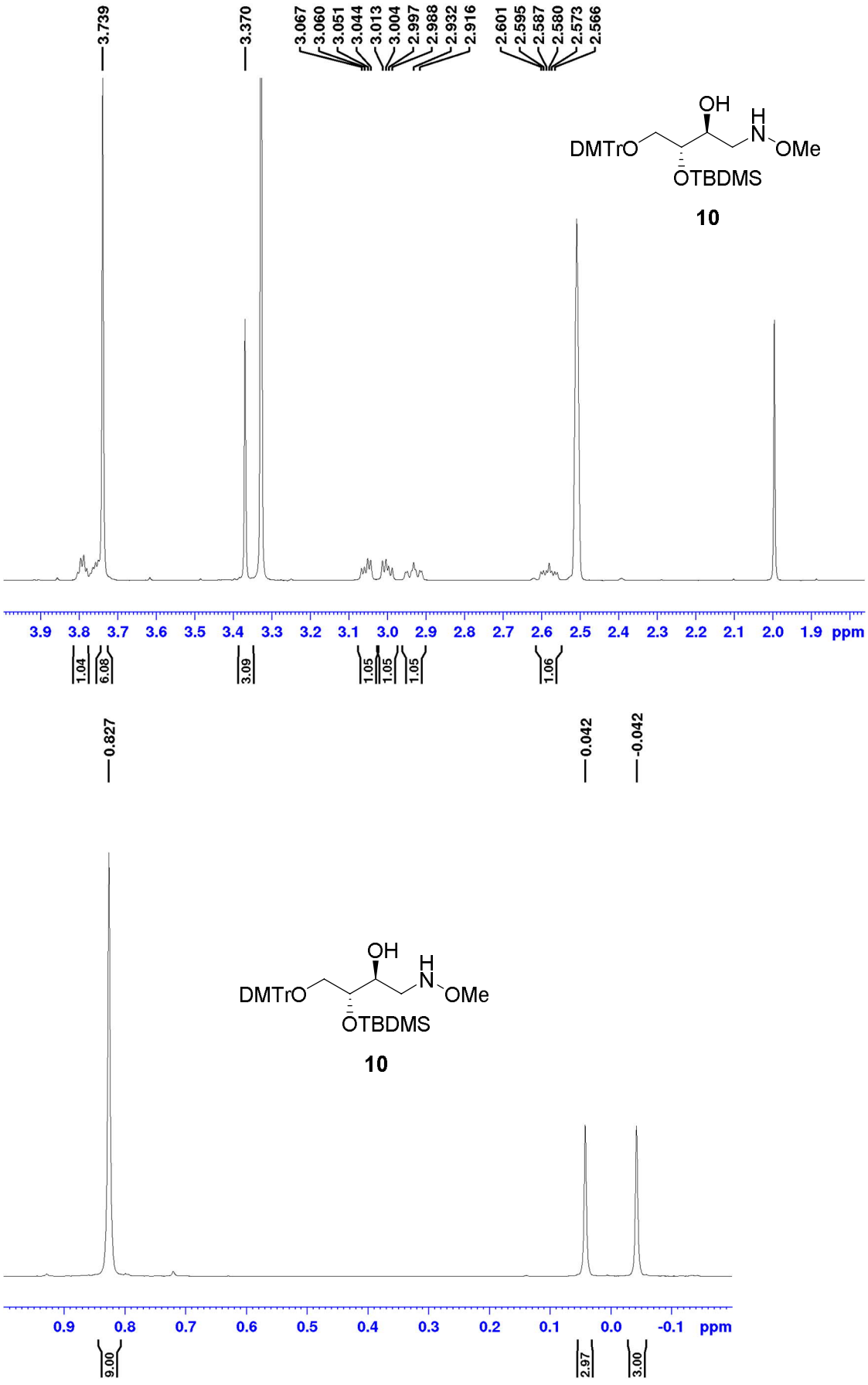


Figure S13. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 10 (continued).

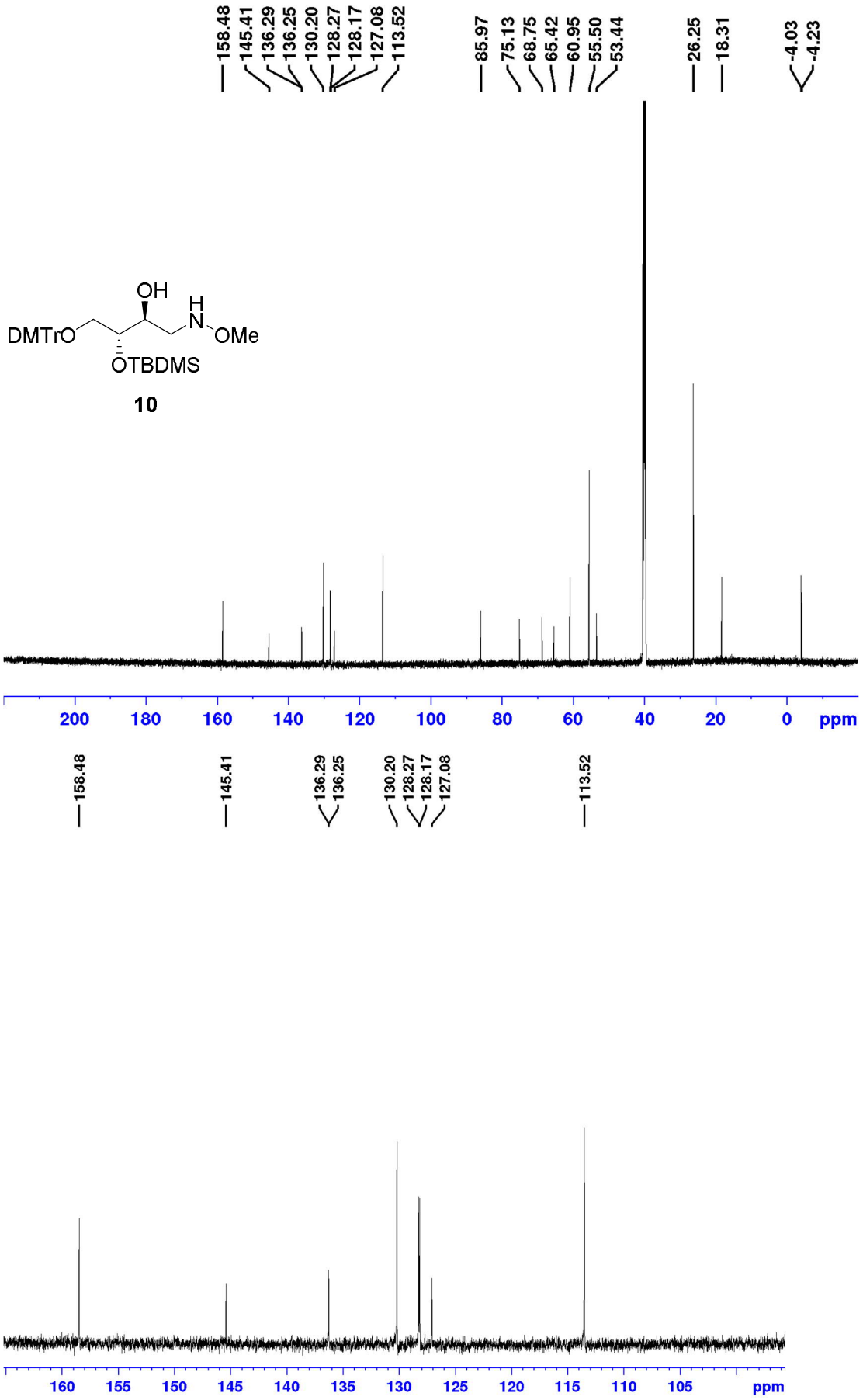


Figure S14. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound 10.

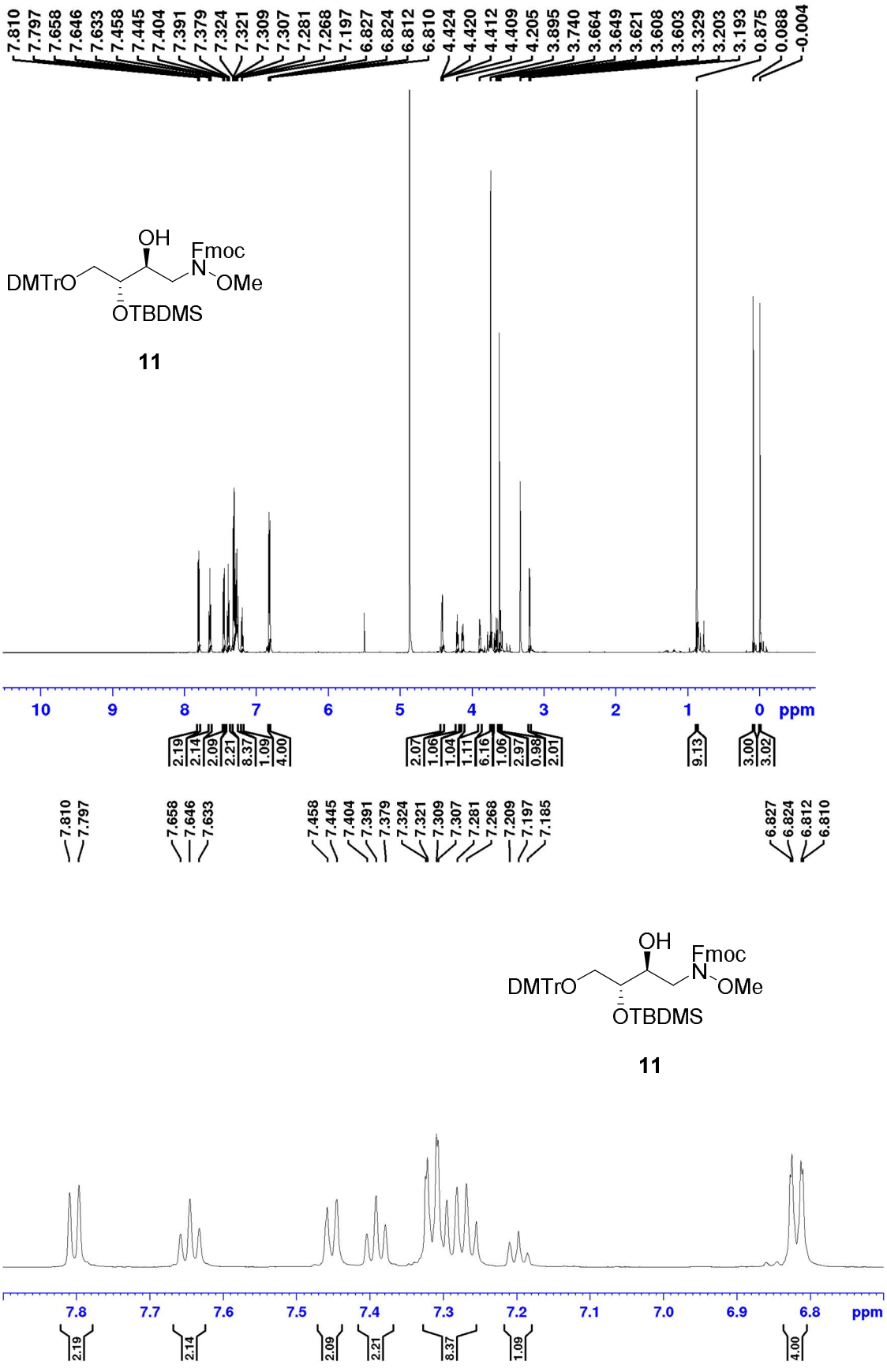


Figure S15. ^{1}H NMR (600 MHz, MeOD) spectrum of compound 11.

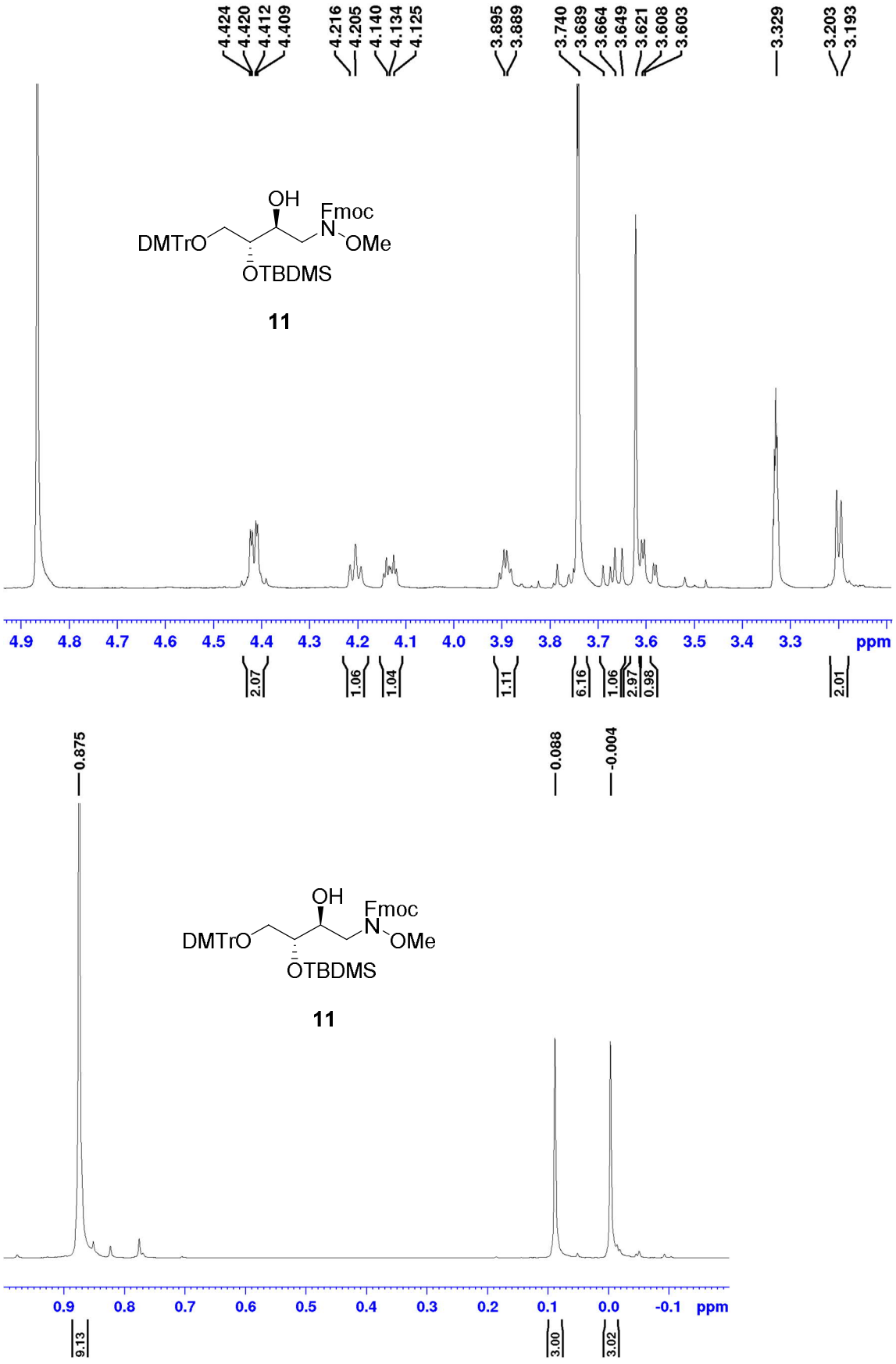


Figure S15. ^{1}H NMR (600 MHz, MeOD) spectrum of compound 11 (continued).

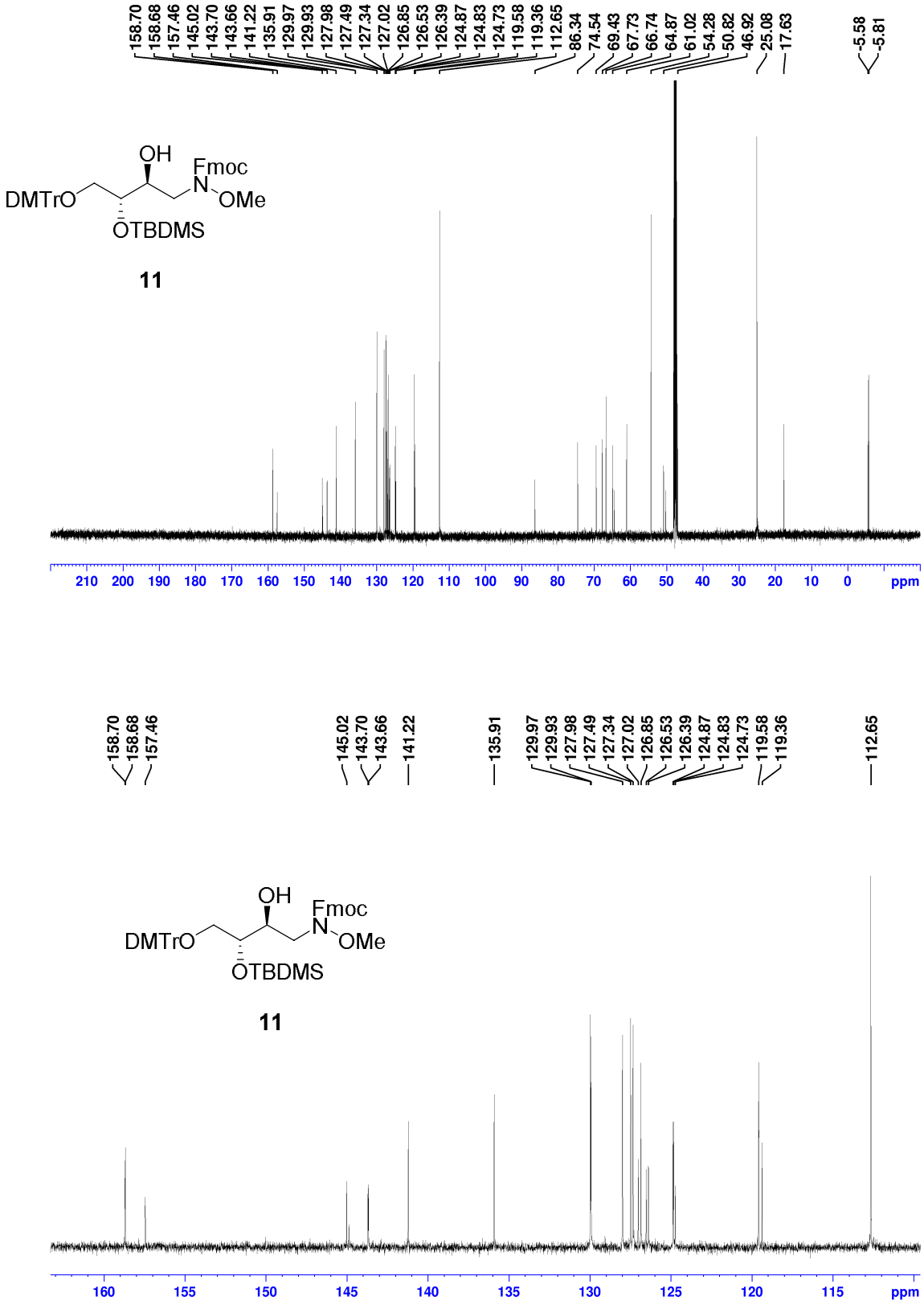


Figure S16. ^{13}C NMR (150 MHz, MeOD) spectrum of compound 11.

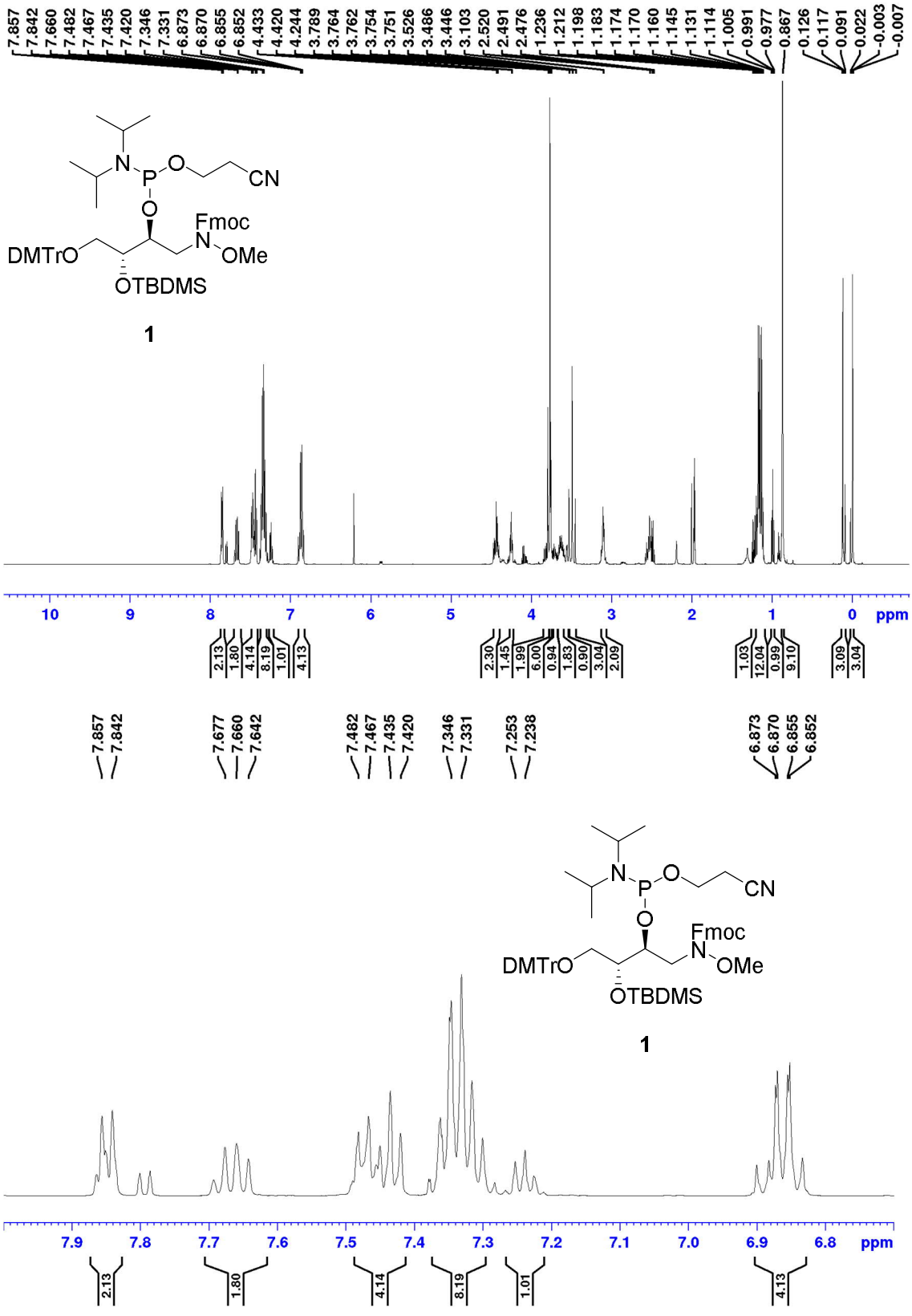


Figure S17. ^{1}H NMR (500 MHz, CD_3CN) spectrum of compound 1.

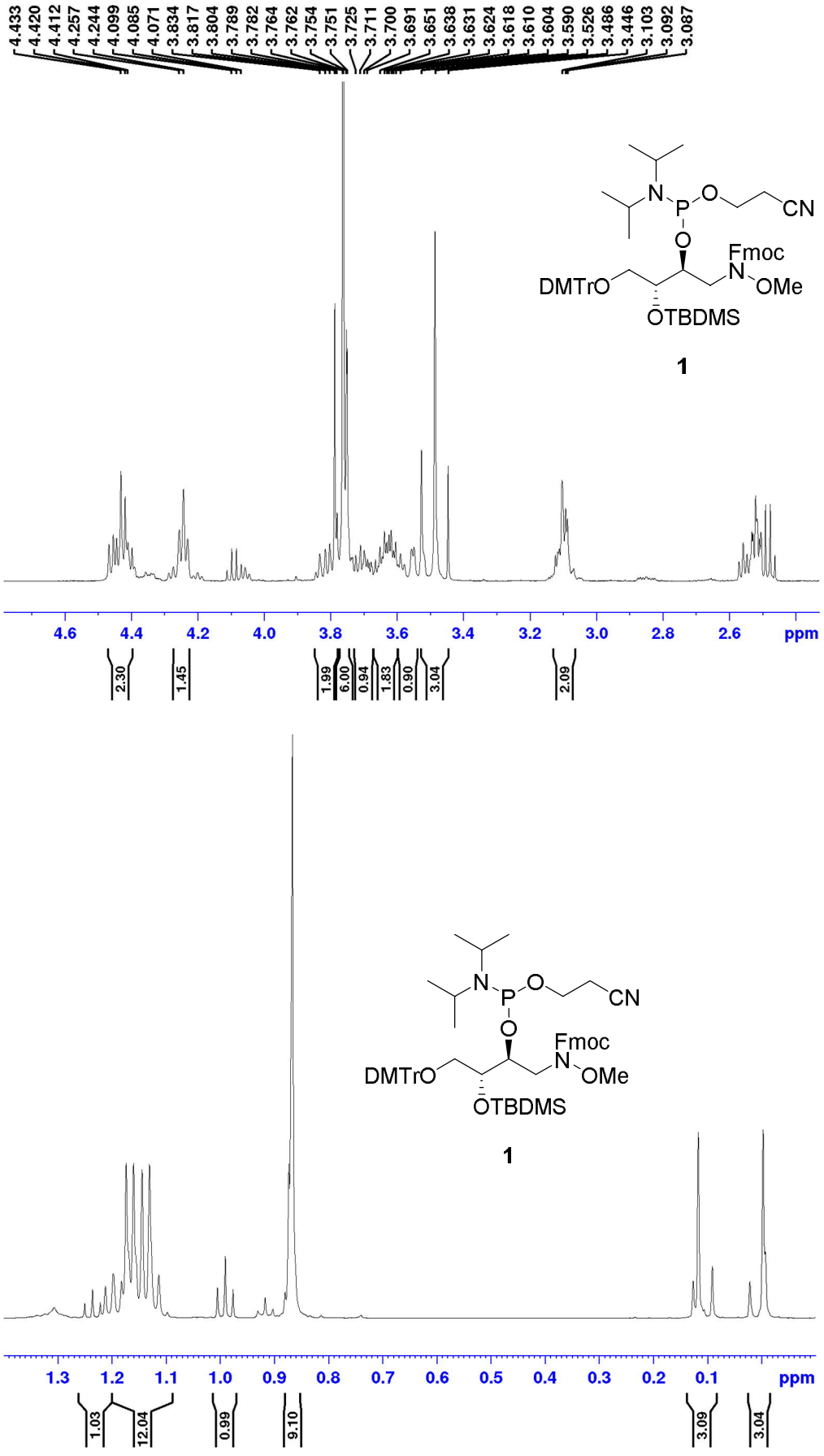


Figure S17. ^{1}H NMR (500 MHz, CD_3CN) spectrum of compound 1 (continued).

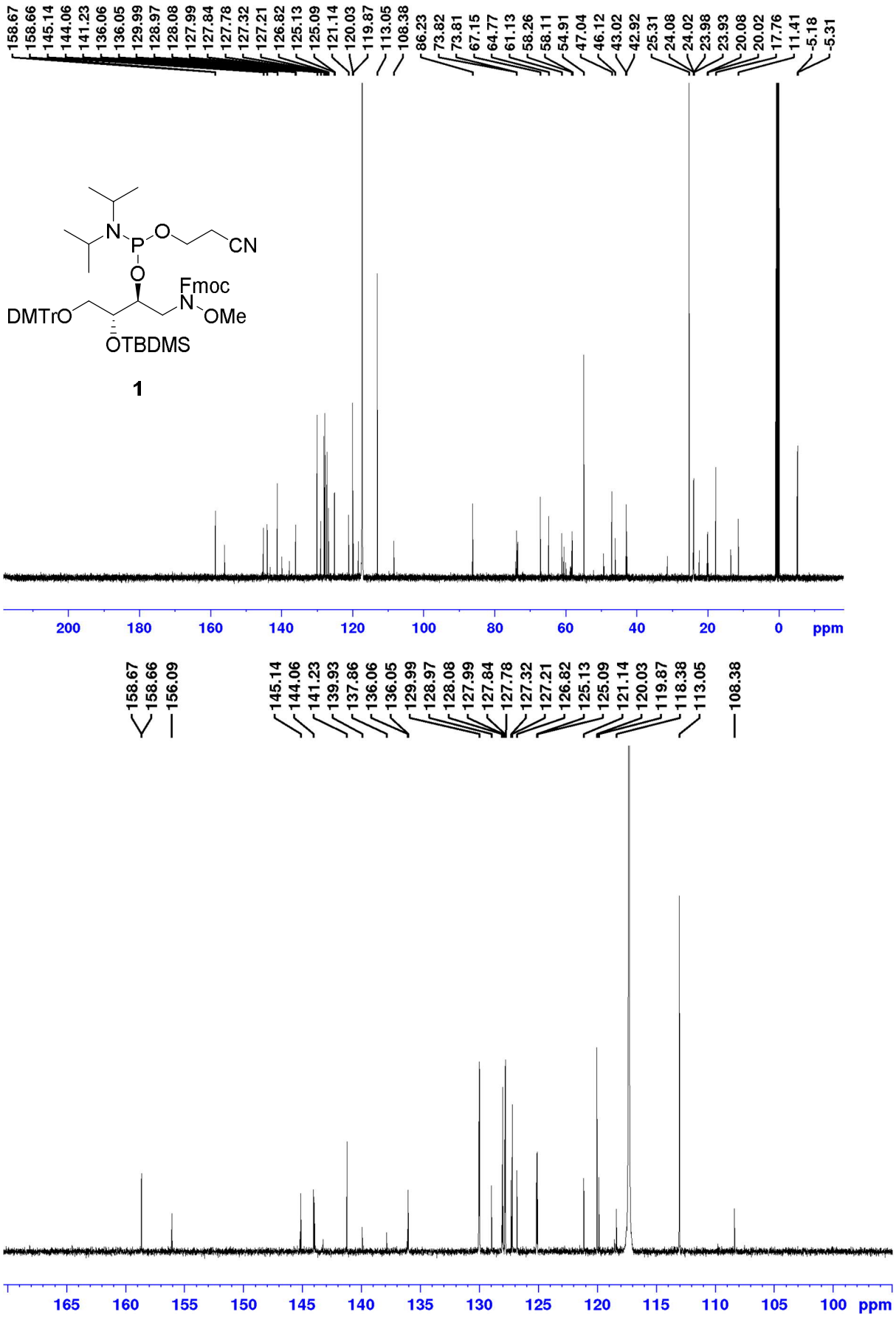


Figure S18. ^{13}C NMR (125 MHz, CD_3CN) spectrum of compound 1.

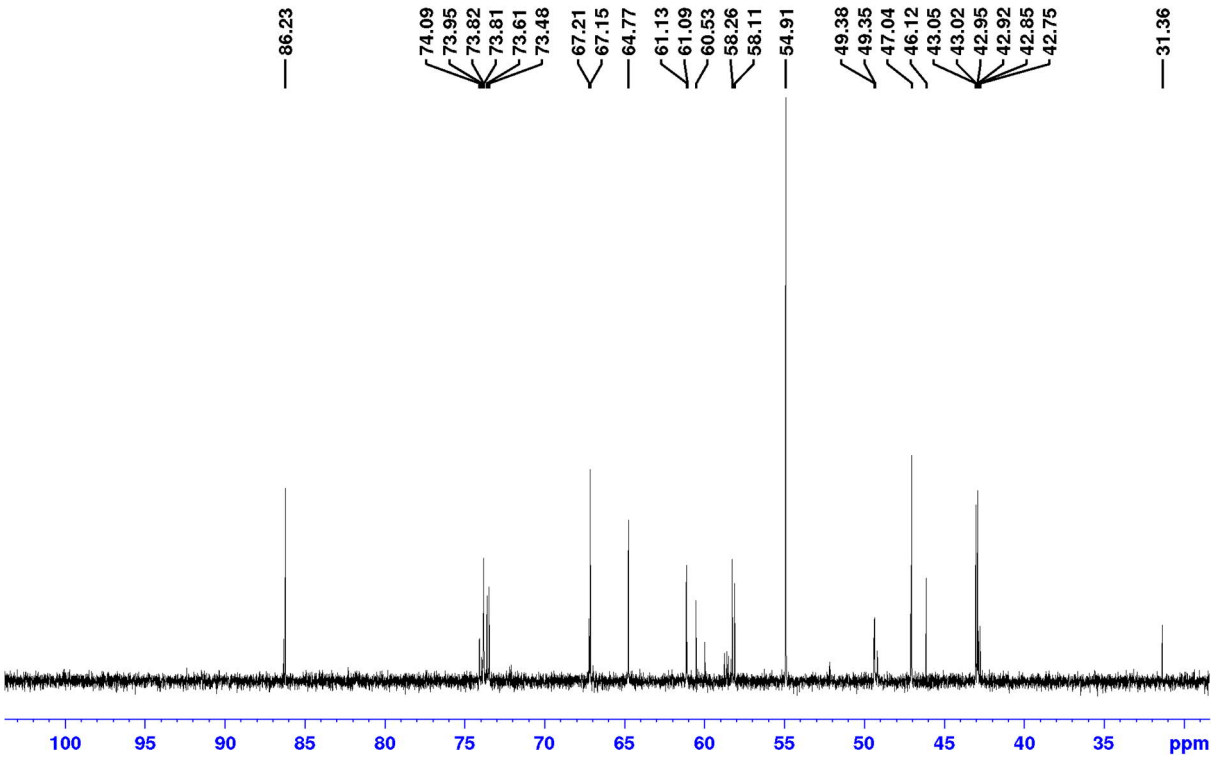


Figure S18. ^{13}C NMR (125 MHz, CD_3CN) spectrum of compound 1 (continued).

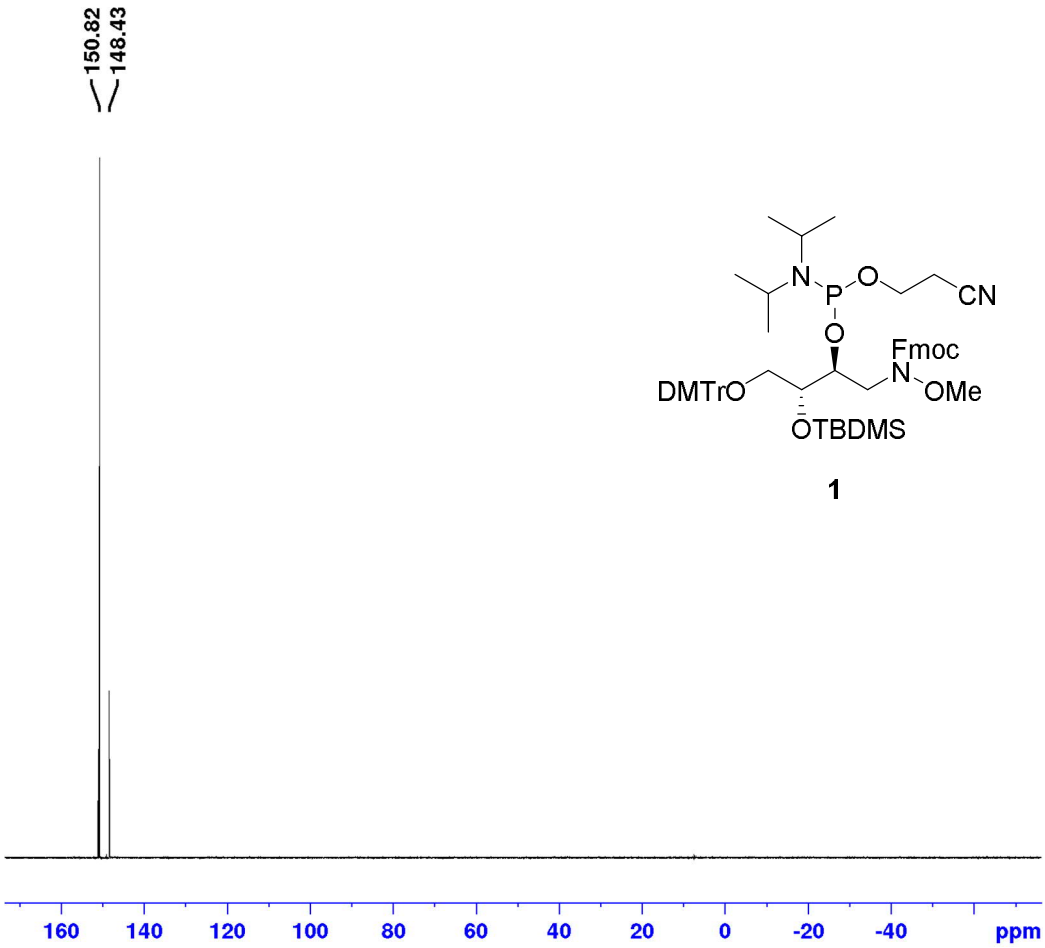


Figure S19. ^{31}P NMR (202 MHz, CD_3CN) spectrum of compound 1.

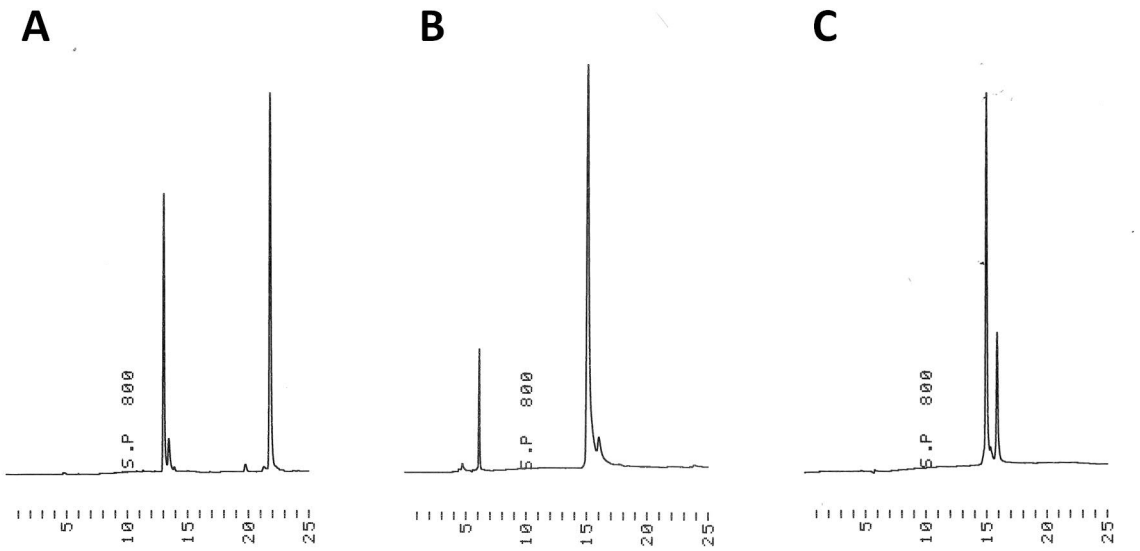


Figure S20. HPLC traces of A) the crude product mixture of the synthesis of ON1, B) crude ON1 after removal of the TBDMS protection and C) purified ON1; Hypersil ODS C18 column (250 × 10 mm, 5 µm); detection wavelength = 260 nm; flow rate = 3.0 mL min^{-1}; linear gradient (5—20% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). The smaller peak eluting immediately after the desired product ON1 corresponds to an acetaldehyde adduct, presumably resulting from impurities in the solvents used.

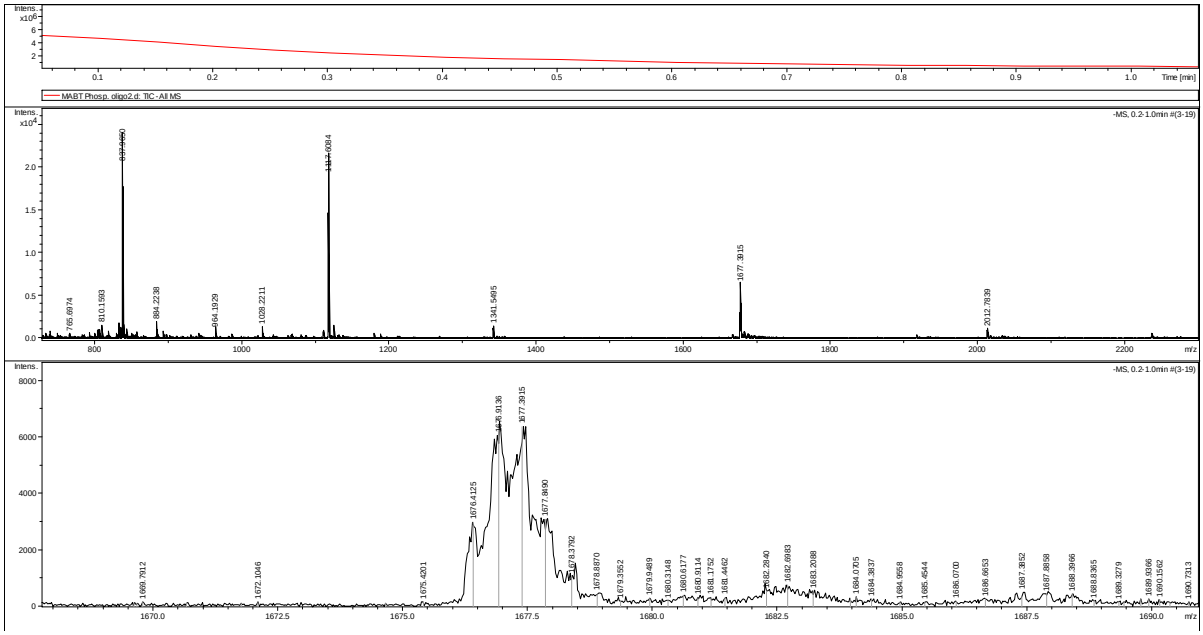


Figure S21. Mass spectrum of the purified TBDMS-protected oligonucleotide intermediate.

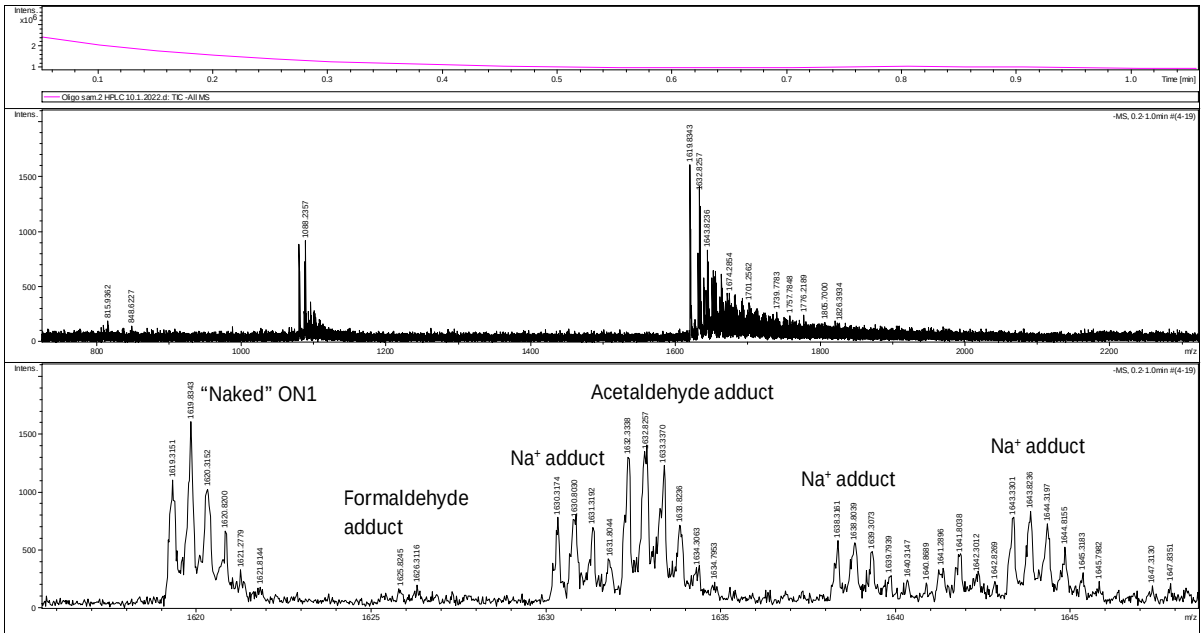


Figure S22. Mass spectrum of the purified oligonucleotide scaffold ON1 (*m/z* [M-2H]$^{2-}$ calcd for $C_{101}H_{132}N_{40}O_{64}P_{10}$: 1619.8; found: 1619.8). The 12 and 26 Da higher masses correspond to formaldehyde (*m/z* [M-2H]$^{2-}$ calcd for $C_{102}H_{132}N_{40}O_{64}P_{10}$: 1625.8; found: 1625.8) and acetaldehyde (*m/z* [M-2H]$^{2-}$ calcd for $C_{103}H_{134}N_{40}O_{64}P_{10}$: 1632.8; found: 1632.8) adducts, presumably resulting from impurities in the solvents used.

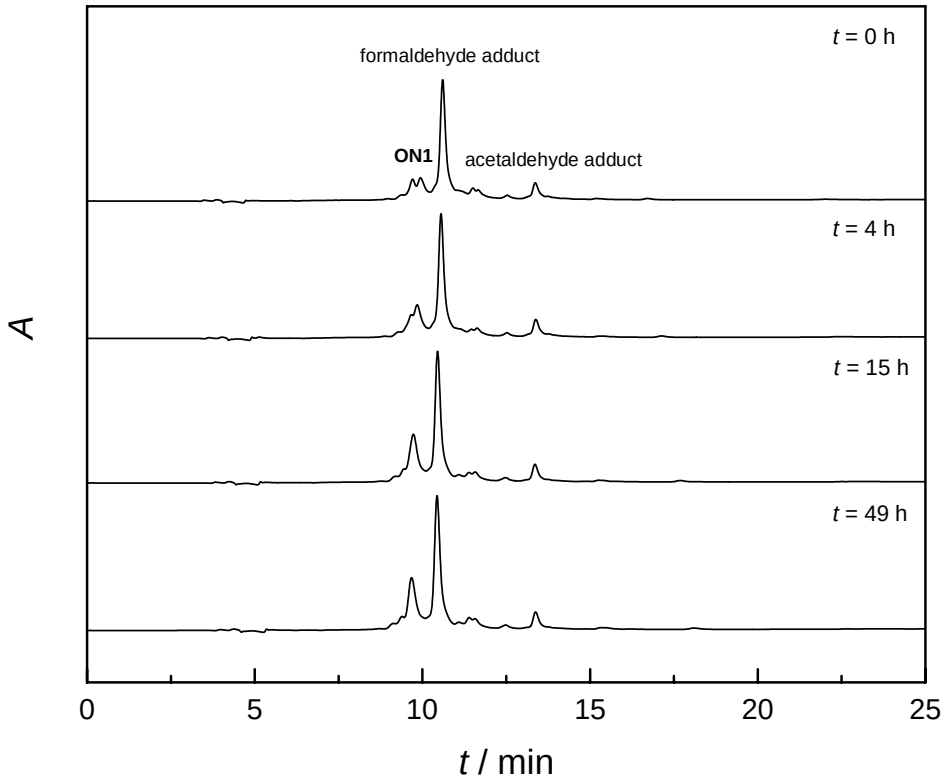


Figure S23. RP-HPLC traces of an aqueous solution of the oligonucleotide scaffold ON1 and its formaldehyde and acetaldehyde adducts at 0, 4, 15 and 49 h; T = 25 °C; pH = 5.0 (30 mM acetate buffer); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0).

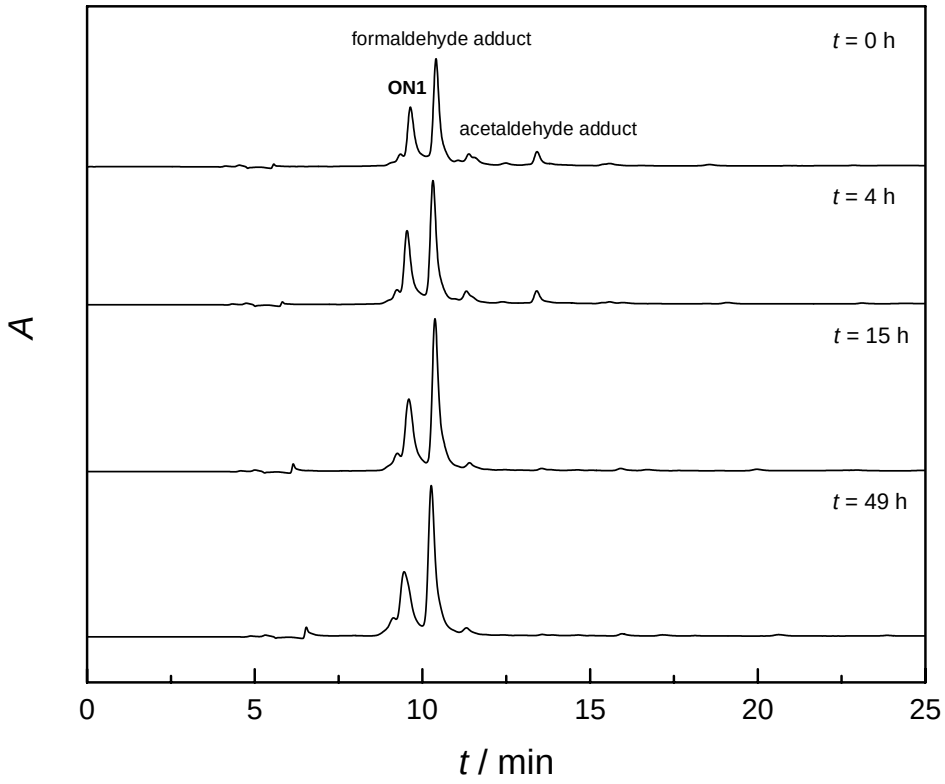


Figure S24. RP-HPLC traces of an aqueous solution of the oligonucleotide scaffold ON1 and its formaldehyde and acetaldehyde adducts at 0, 4, 15 and 49 h; T = 55 °C; pH = 5.0 (30 mM acetate buffer); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0).

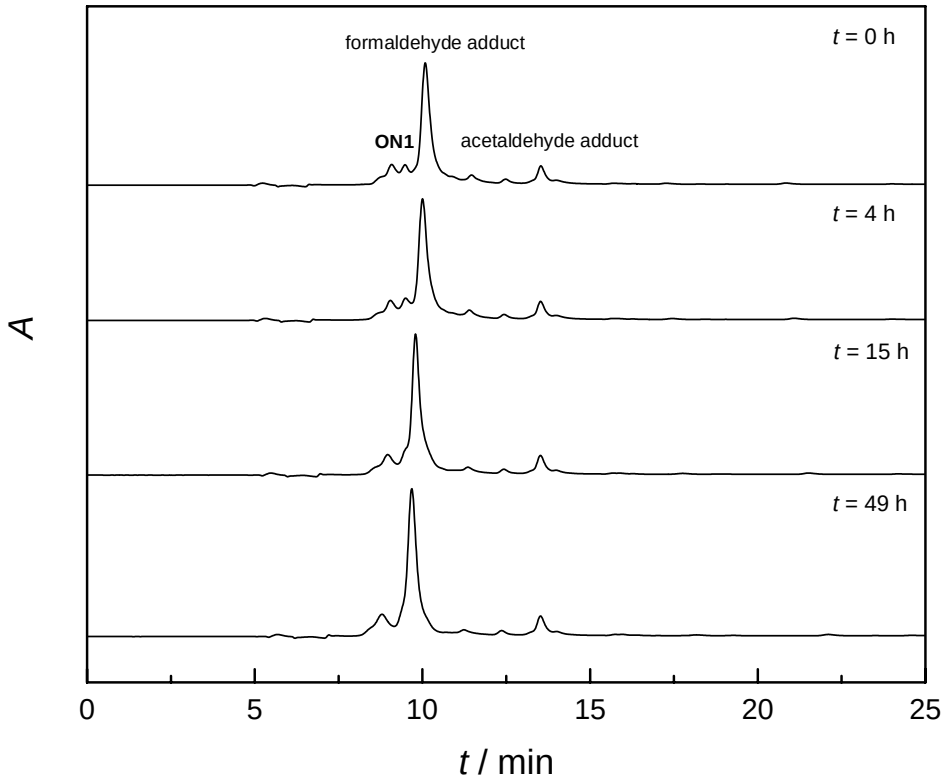


Figure S25. RP-HPLC traces of an aqueous solution of the oligonucleotide scaffold ON1 and its formaldehyde and acetaldehyde adducts at 0, 4, 15 and 49 h; T = 25 °C; pH = 7.4 (30 mM cacodylate buffer); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0).

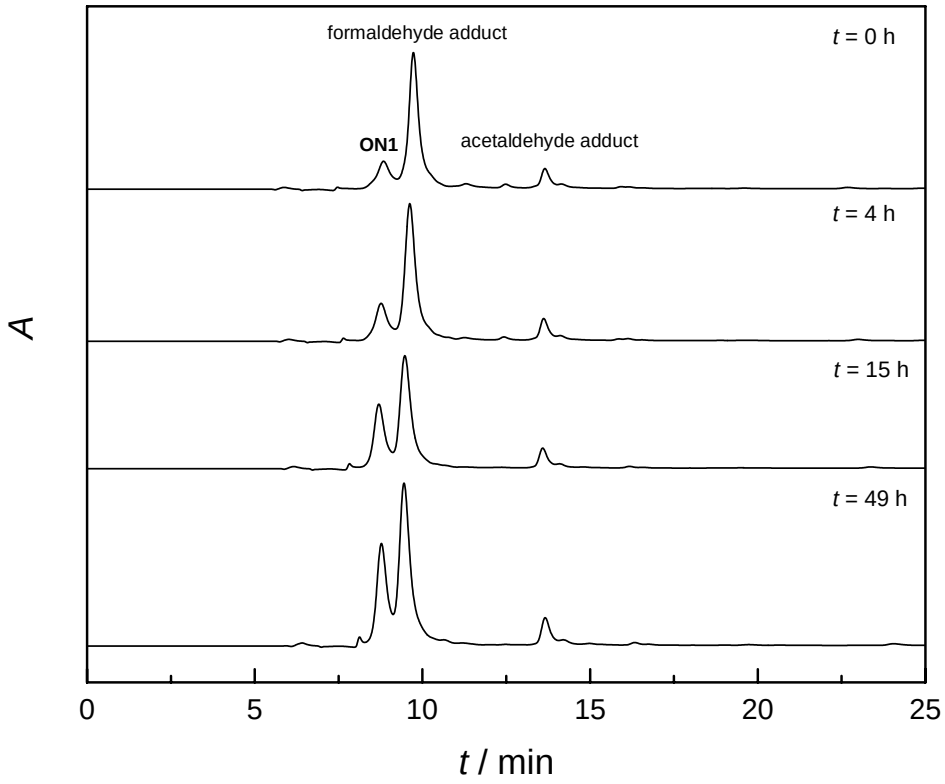


Figure S26. RP-HPLC traces of an aqueous solution of the oligonucleotide scaffold ON1 and its formaldehyde and acetaldehyde adducts at 0, 4, 15 and 49 h; T = 55 °C; pH = 7.4 (30 mM cacodylate buffer); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0).

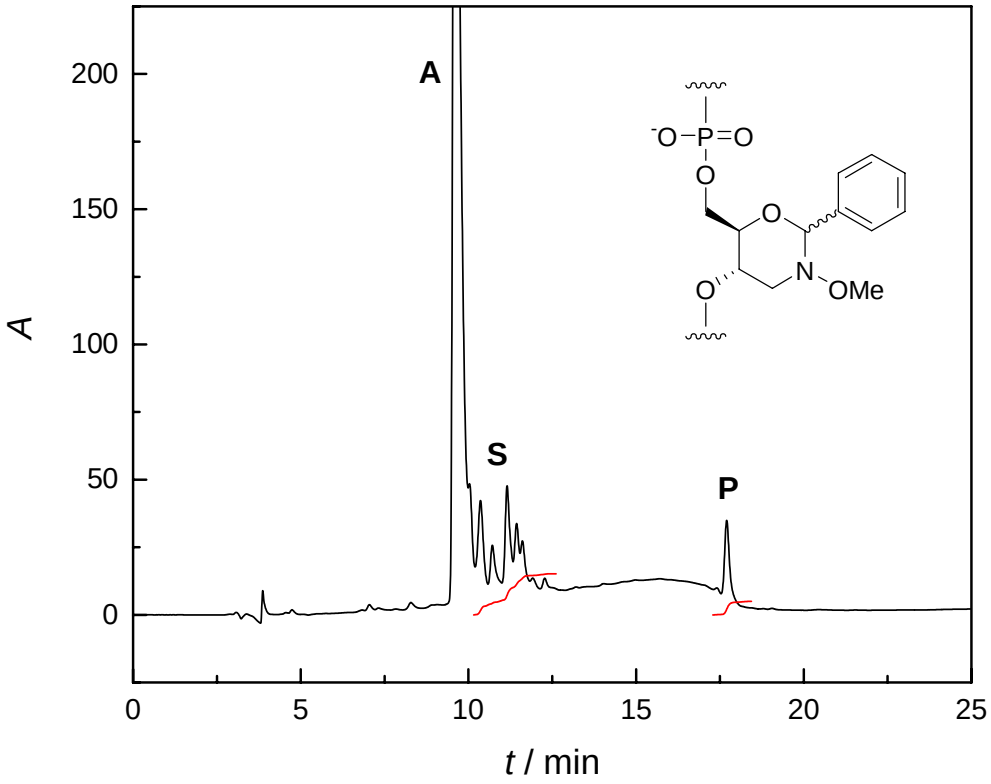


Figure S27. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with benzaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). "S" refers to ON1 (and its formaldehyde and acetaldehyde adducts), "P" to the product and "A" to residual aldehyde.

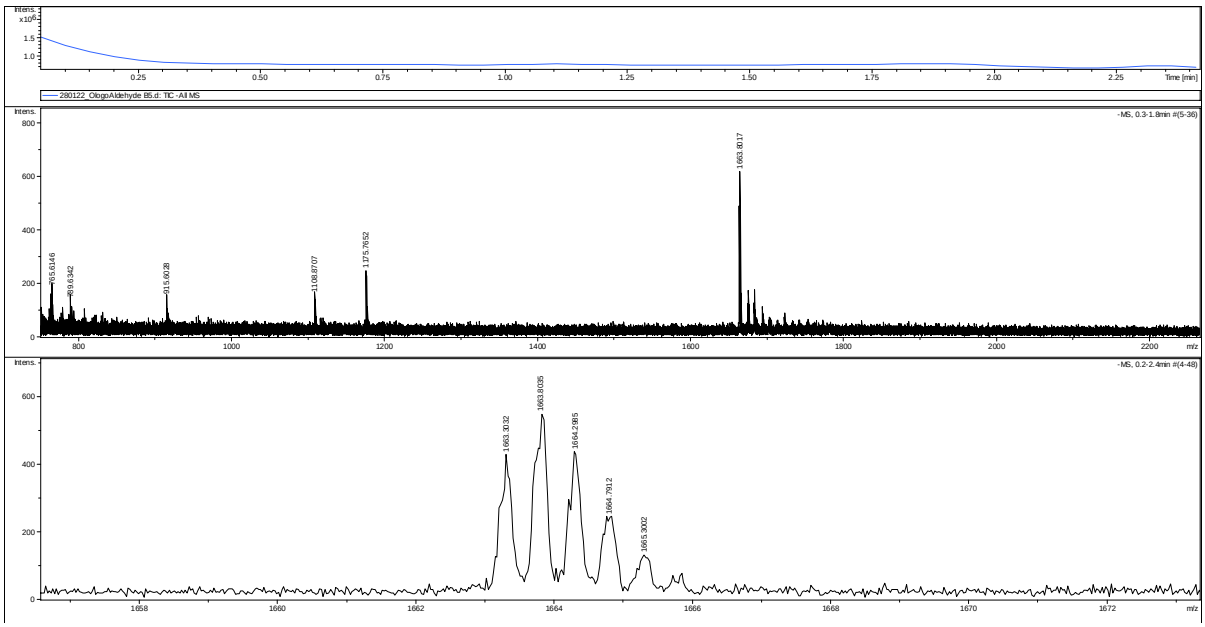


Figure S28. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with benzaldehyde (method A).

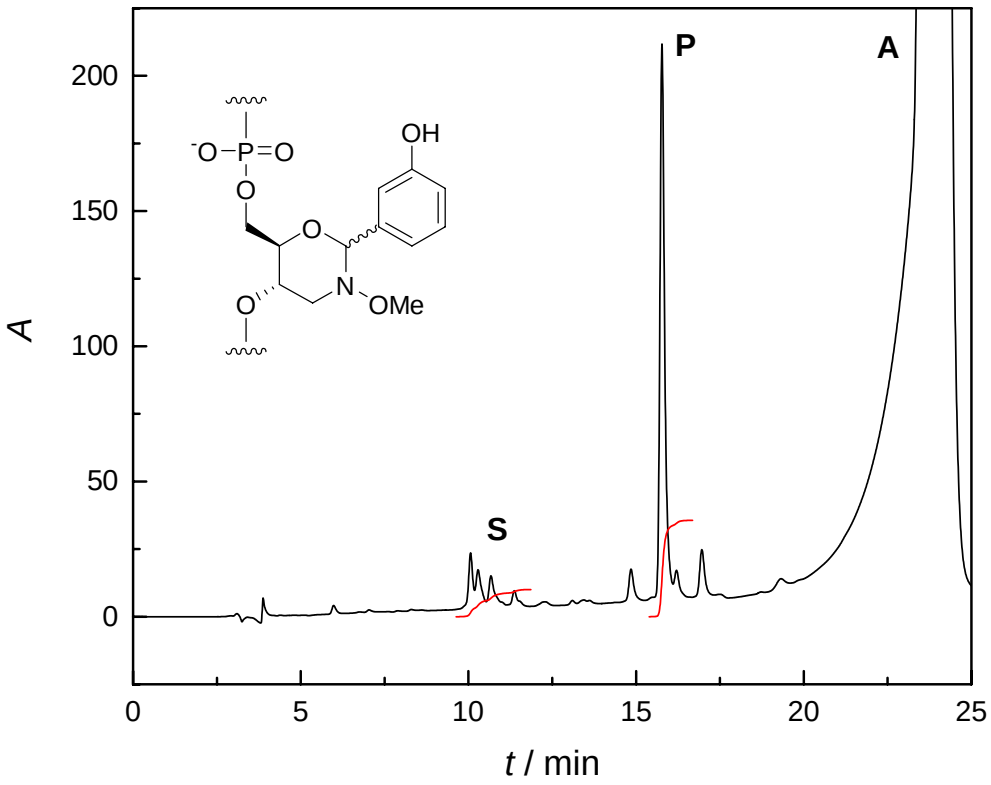


Figure S29. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with 3-hydroxybenzaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts), “P” to the product and “A” to residual aldehyde.

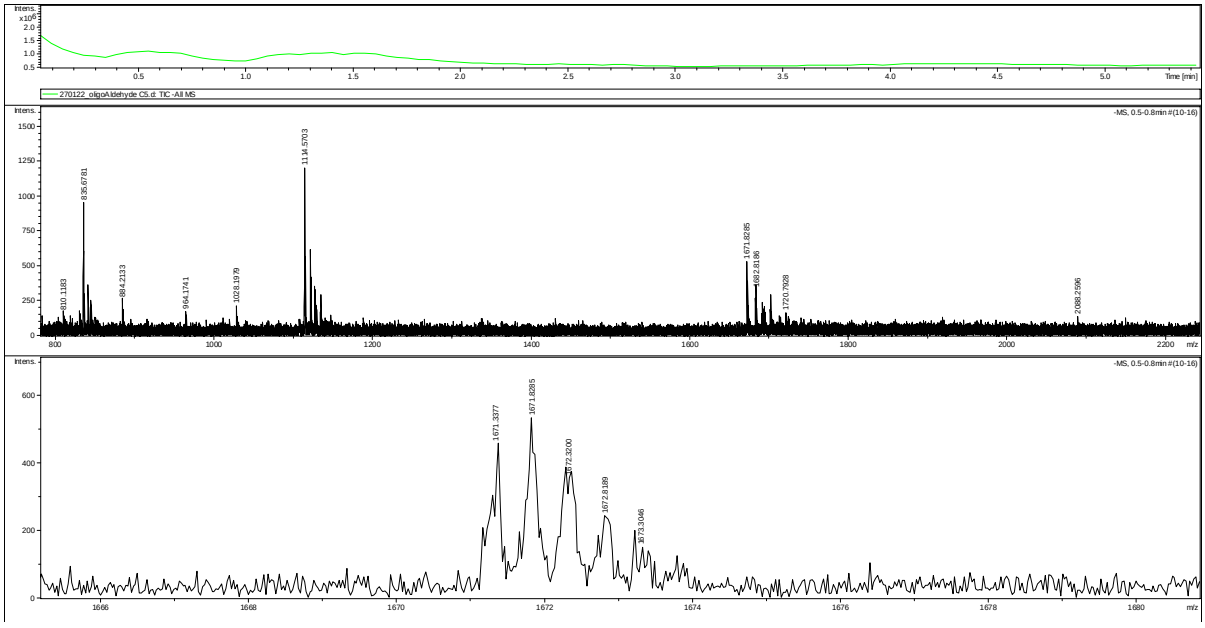


Figure S30. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with 3-hydroxybenzaldehyde (method A).

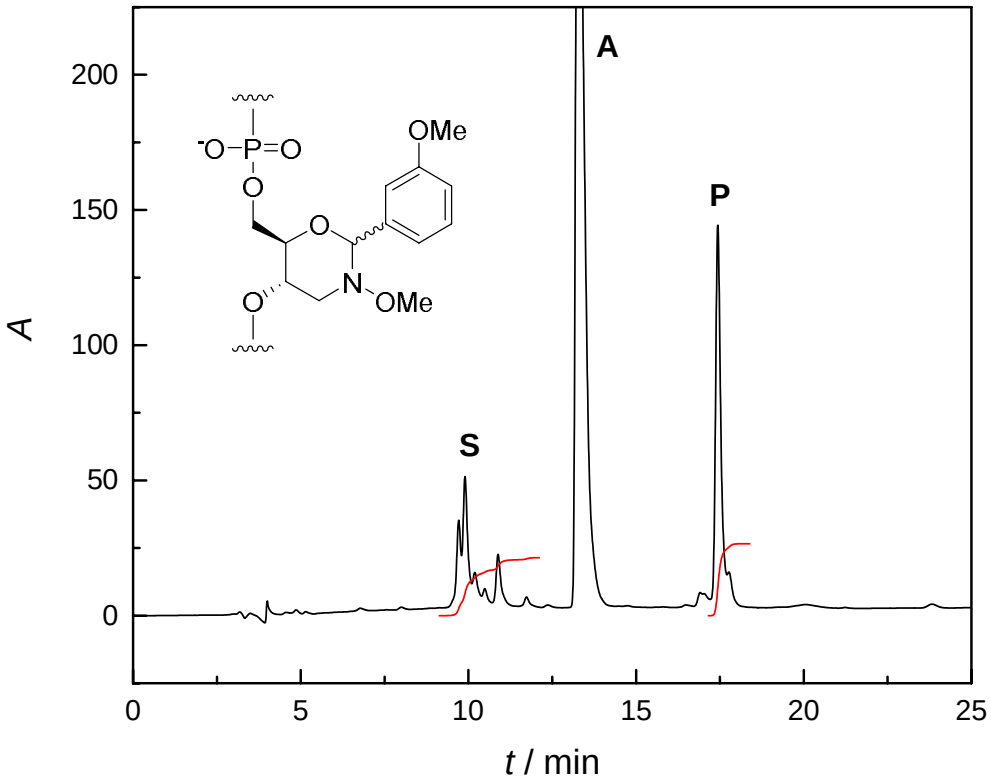


Figure S31. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with 3-methoxybenzaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts), “P” to the product and “A” to residual aldehyde.

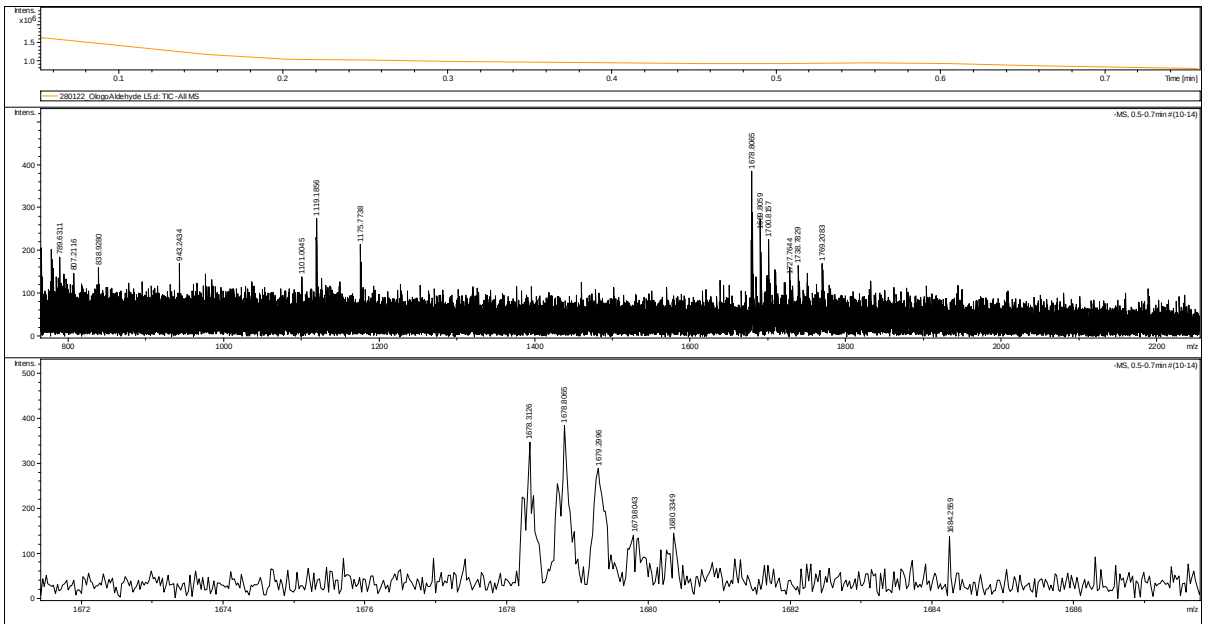


Figure S32. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with 3-methoxybenzaldehyde (method A).

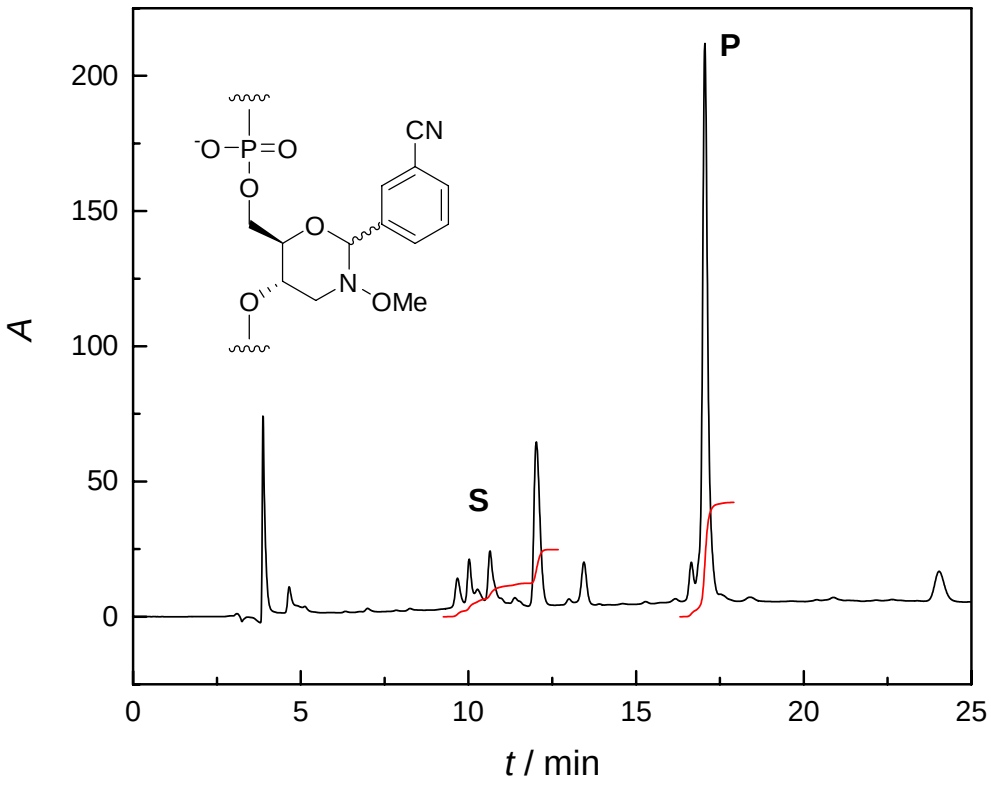


Figure S33. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with 3-cyanobenzaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts) and “P” to the product.

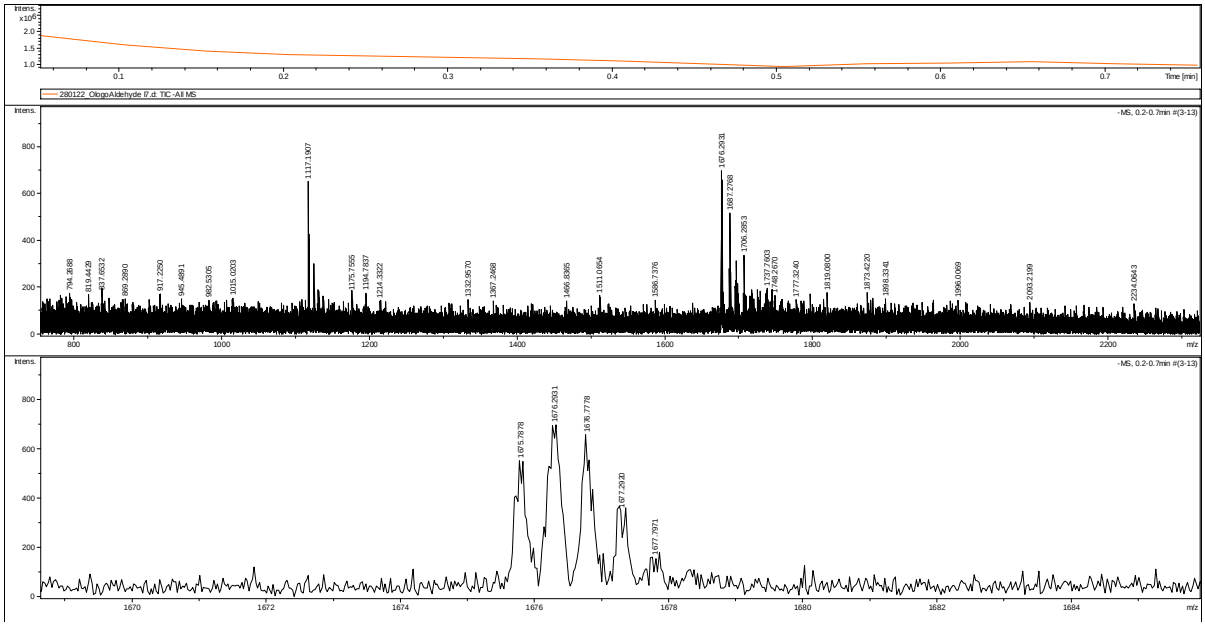


Figure S34. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with 3-cyanobenzaldehyde (method A).

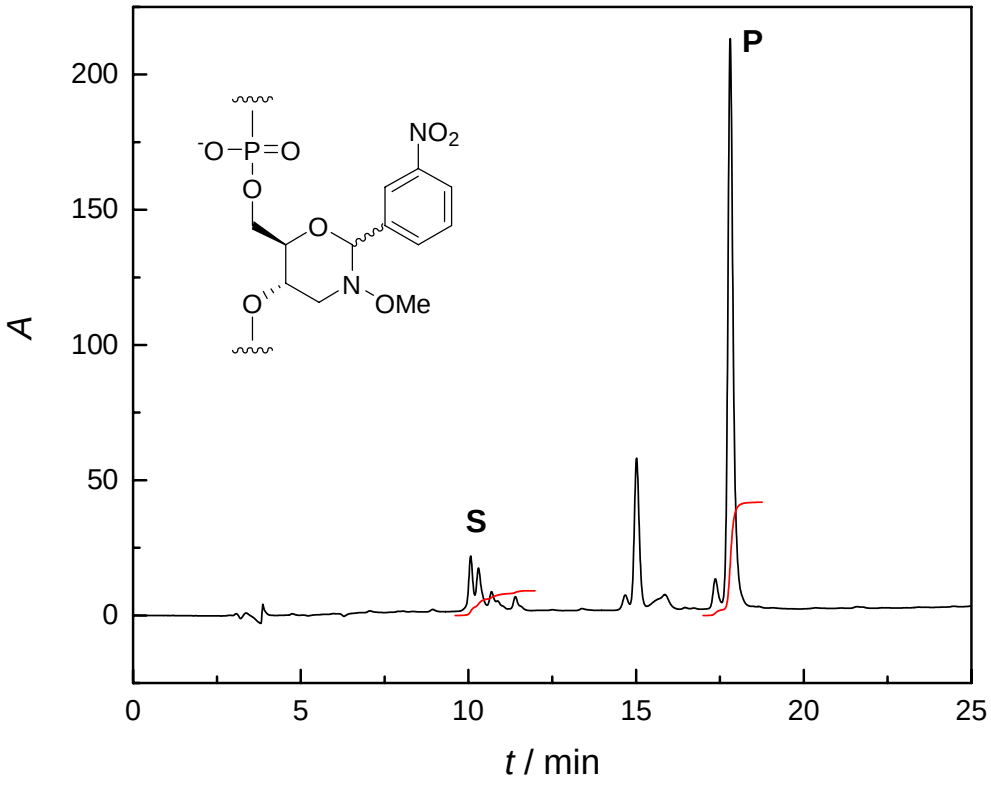


Figure S35. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with 3-nitrobenzaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts) and “P” to the product.

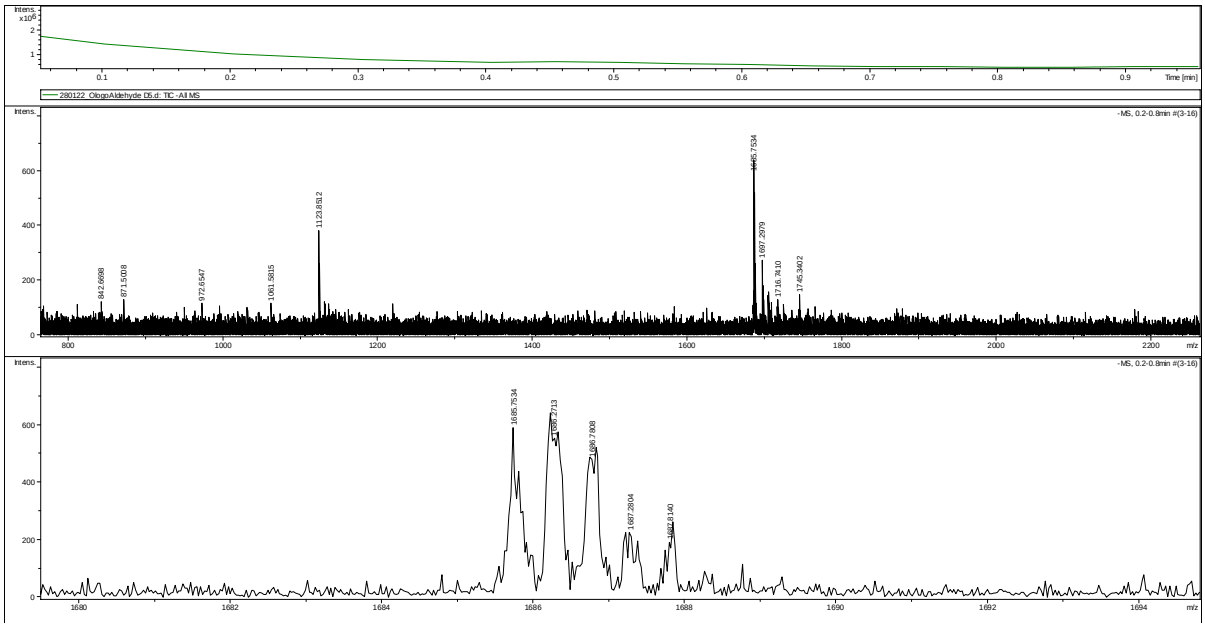


Figure S36. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with 3-nitrobenzaldehyde (method A).

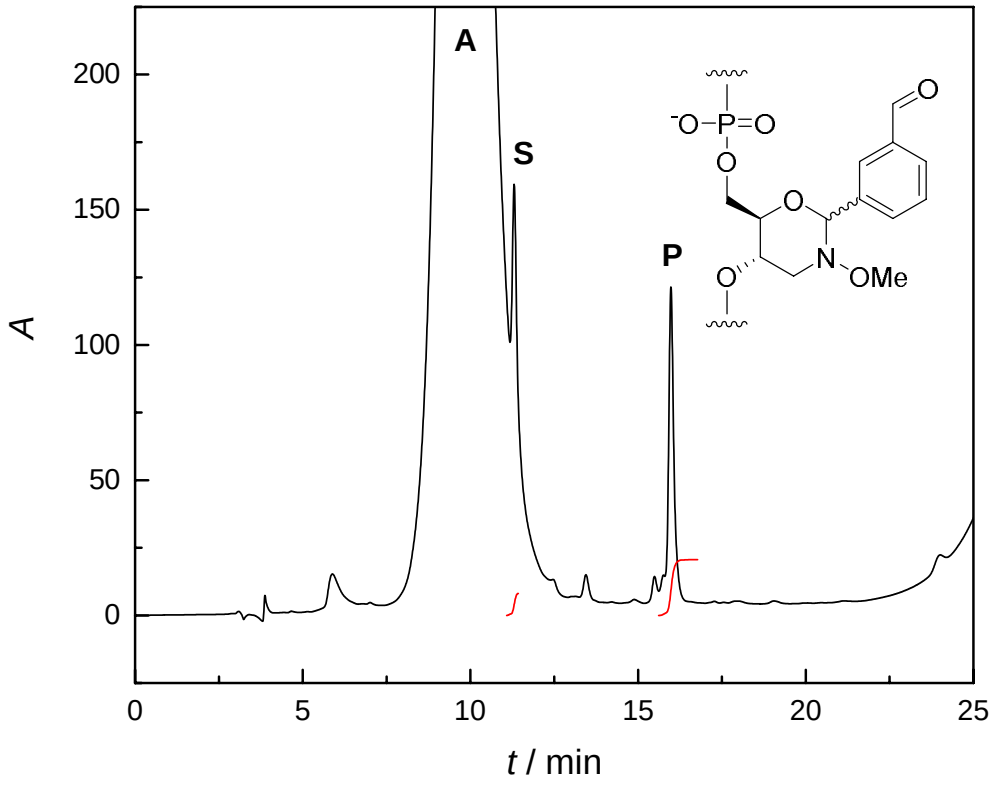


Figure S37. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with isophthaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts), “P” to the product and “A” to residual aldehyde.

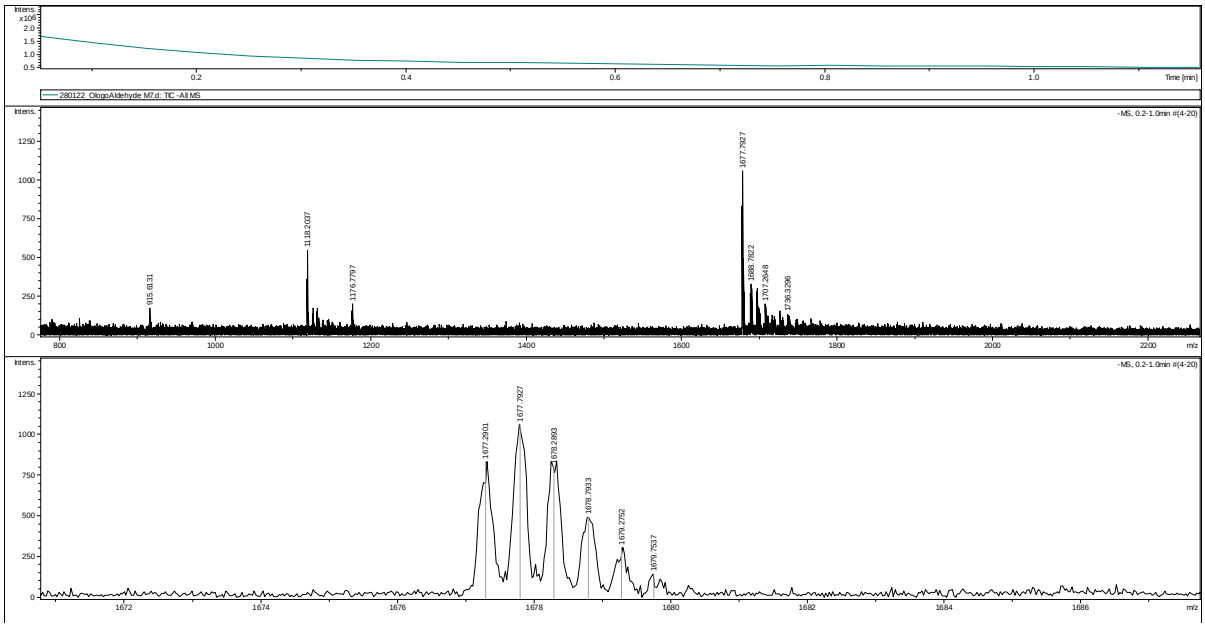


Figure S38. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with isophthaldehyde (method A).

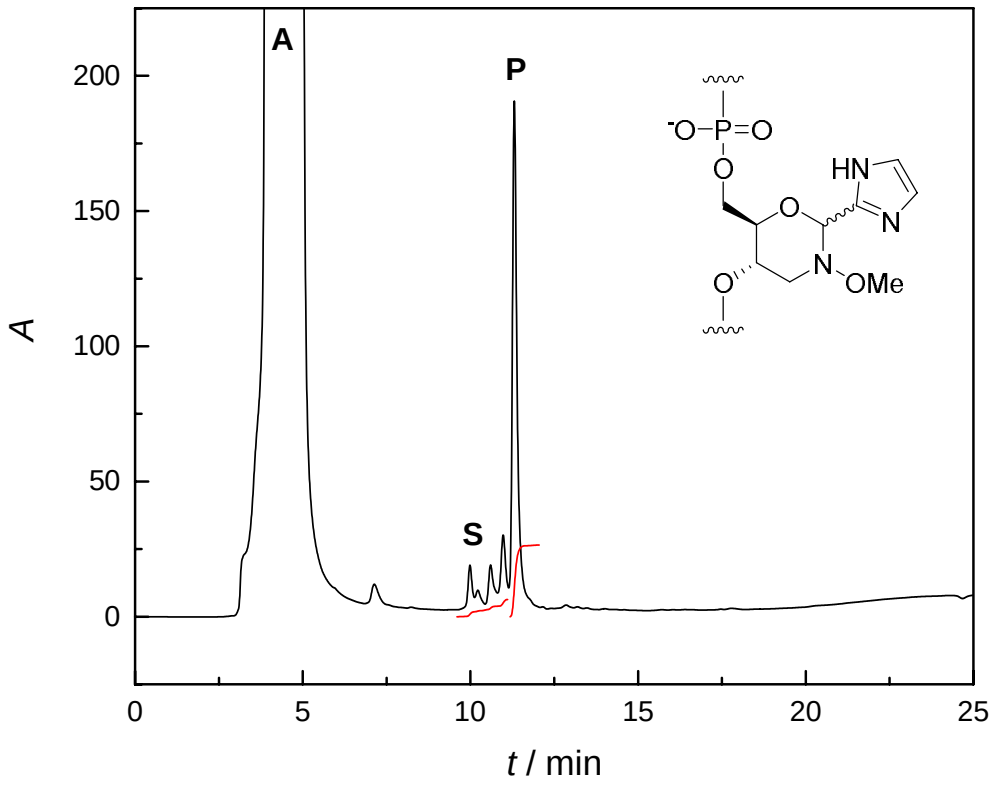


Figure S39. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with imidazole-2-carbaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). "S" refers to ON1 (and its formaldehyde and acetaldehyde adducts), "P" to the product and "A" to residual aldehyde.

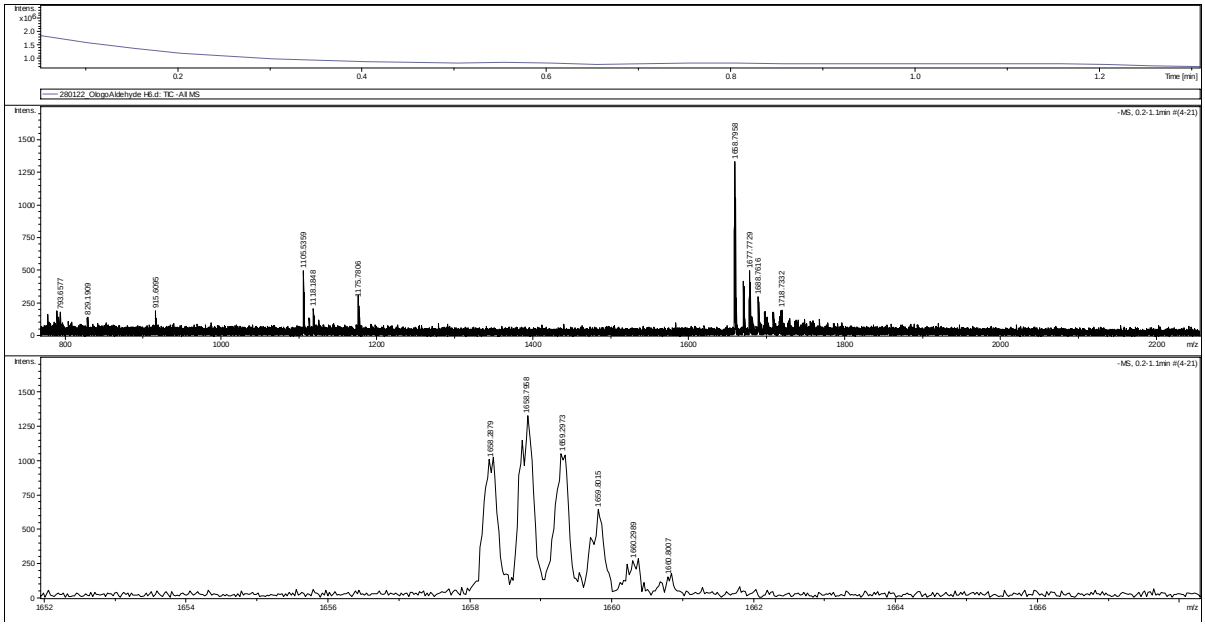


Figure S40. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with imidazole-2-carbaldehyde (method A).

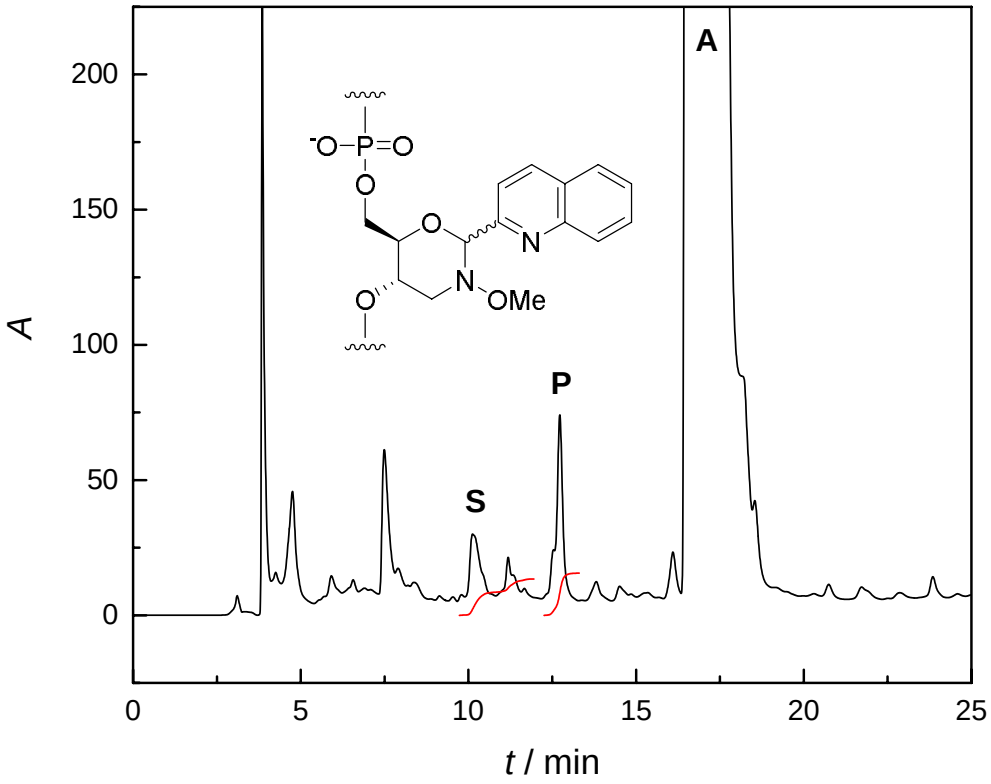


Figure S41. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with quinoline-2-carbaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts), “P” to the product and “A” to residual aldehyde.

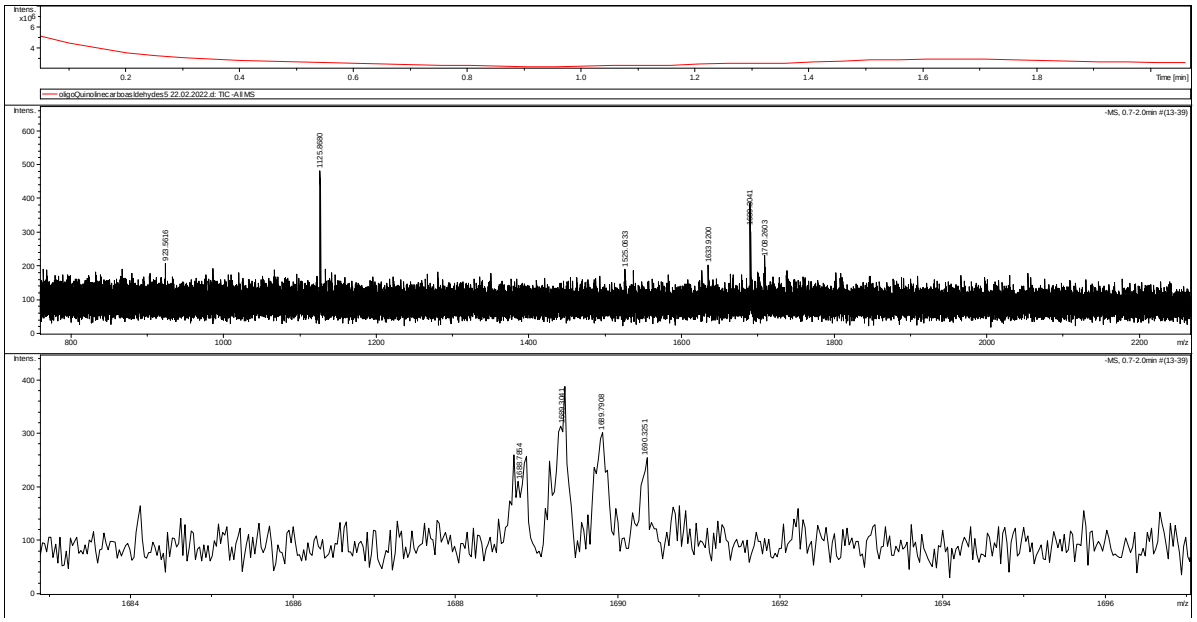


Figure S42. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with quinoline-2-carbaldehyde (method A).

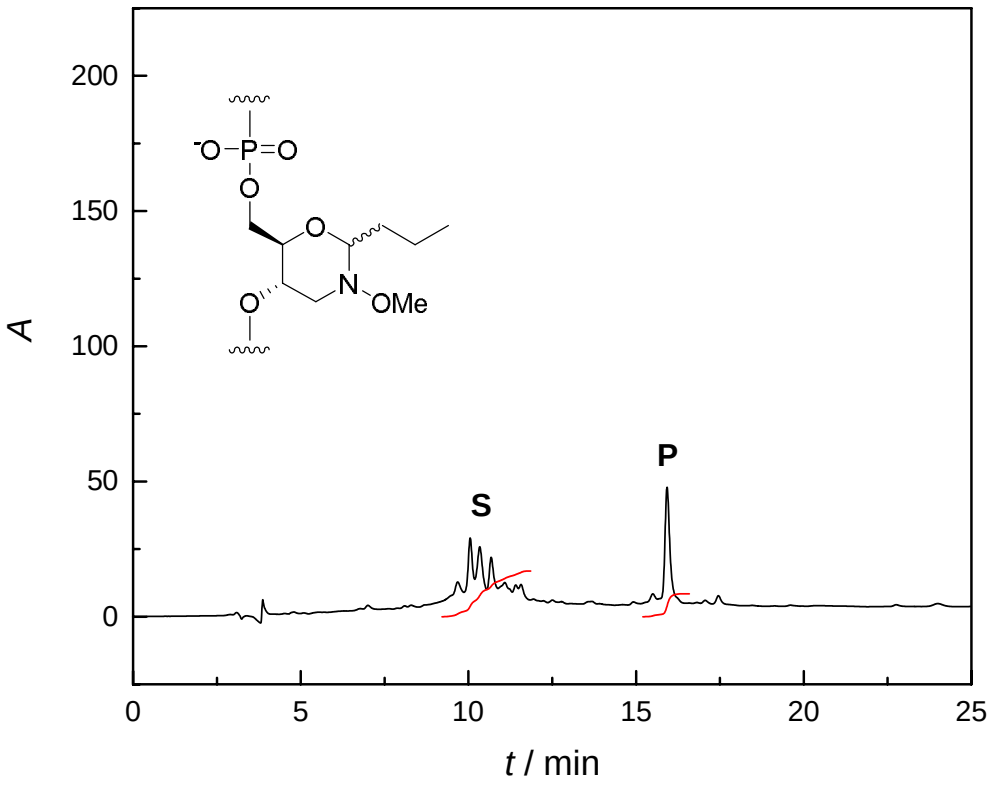


Figure S43. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with butyraldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts) and “P” to the product.

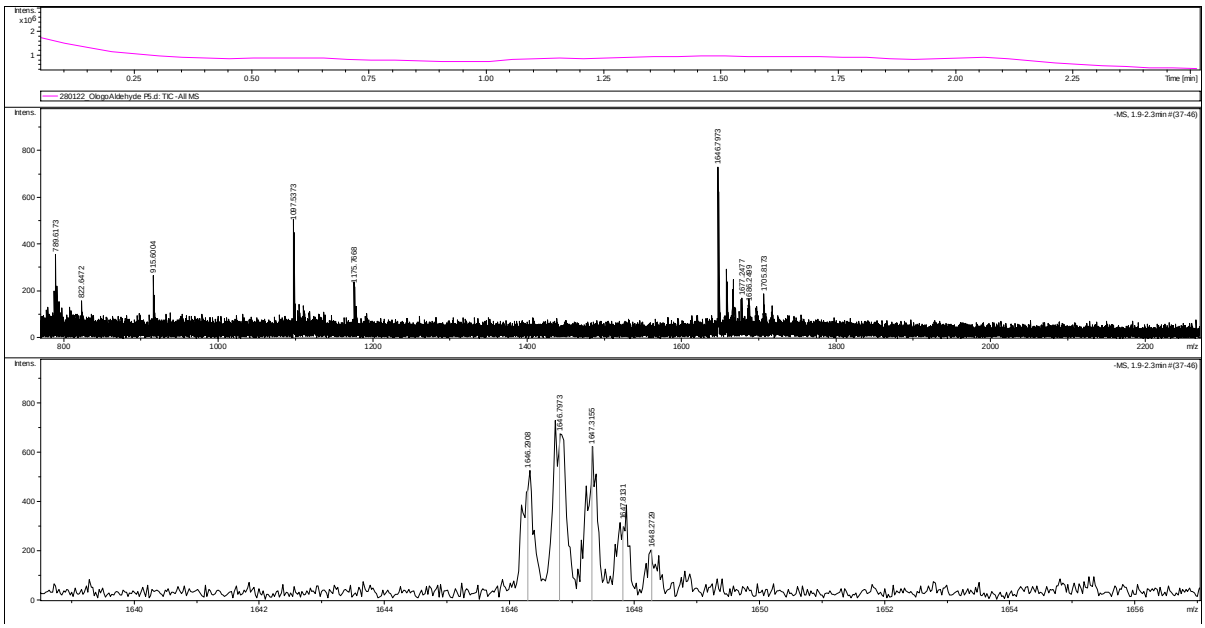


Figure S44. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with butyraldehyde (method A).

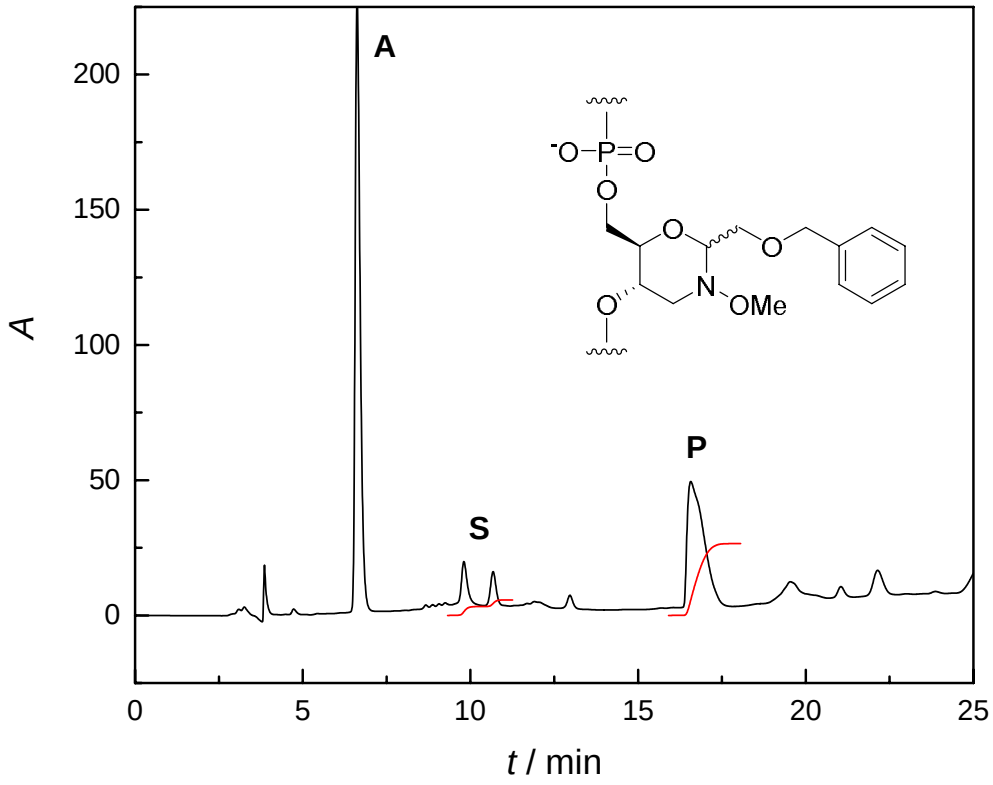


Figure S45. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with benzyloxyacetaldehyde (method A); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). “S” refers to ON1 (and its formaldehyde and acetaldehyde adducts), “P” to the product and “A” to residual aldehyde.

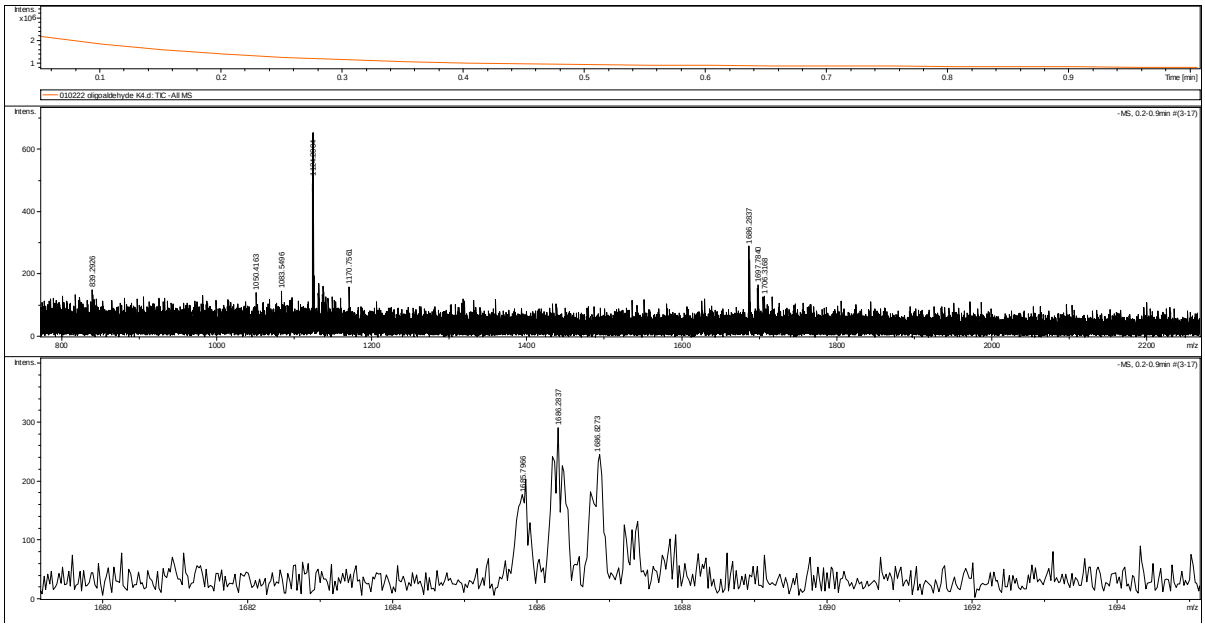


Figure S46. Mass spectrum of the purified product of the reaction of the oligonucleotide scaffold ON1 with benzyloxyacetaldehyde (method A).

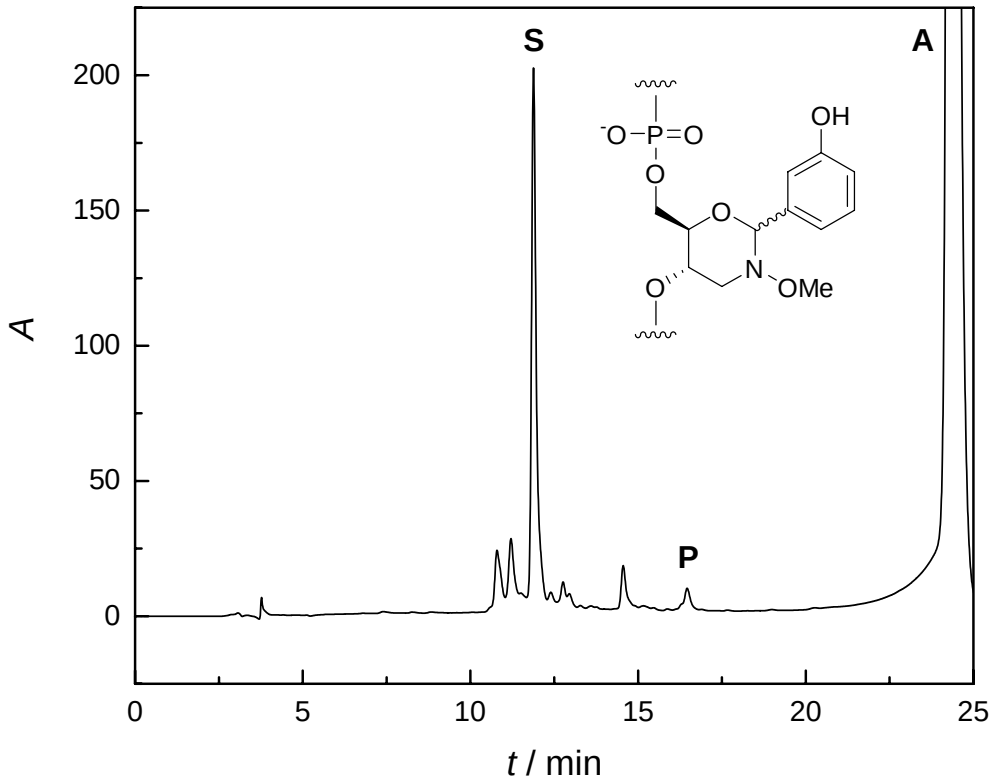


Figure S47. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with 3-hydroxybenzaldehyde (method B); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). "S" refers to ON1 (and its formaldehyde and acetaldehyde adducts), "P" to the product and "A" to residual aldehyde.

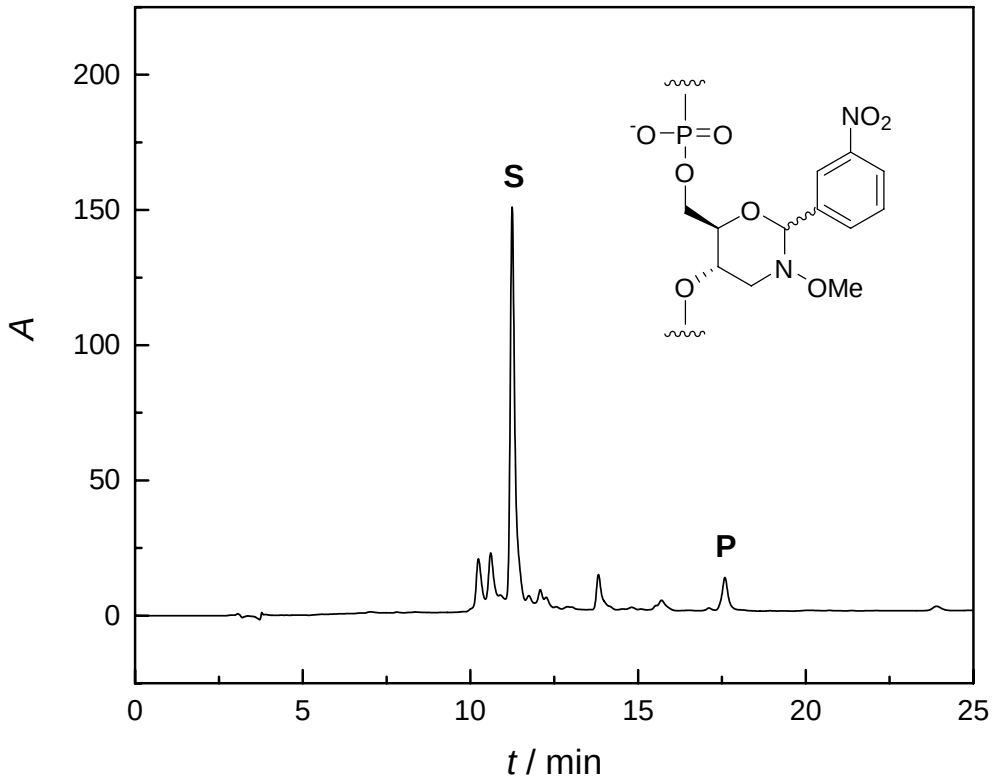


Figure S48. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with 3-nitrobenzaldehyde (method B); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). "S" refers to ON1 (and its formaldehyde and acetaldehyde adducts) and "P" to the product.

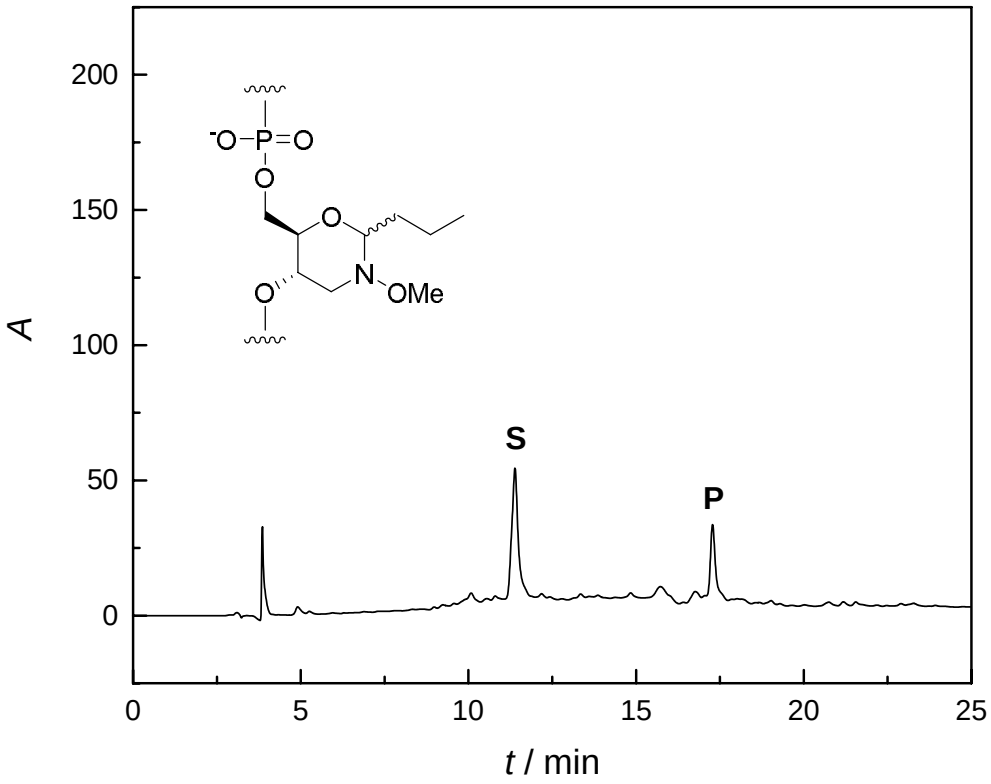


Figure S49. RP-HPLC trace of the product mixture of the reaction of the oligonucleotide scaffold ON1 with butyraldehyde (method B); Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0). "S" refers to ON1 (and its formaldehyde and acetaldehyde adducts) and "P" to the product.

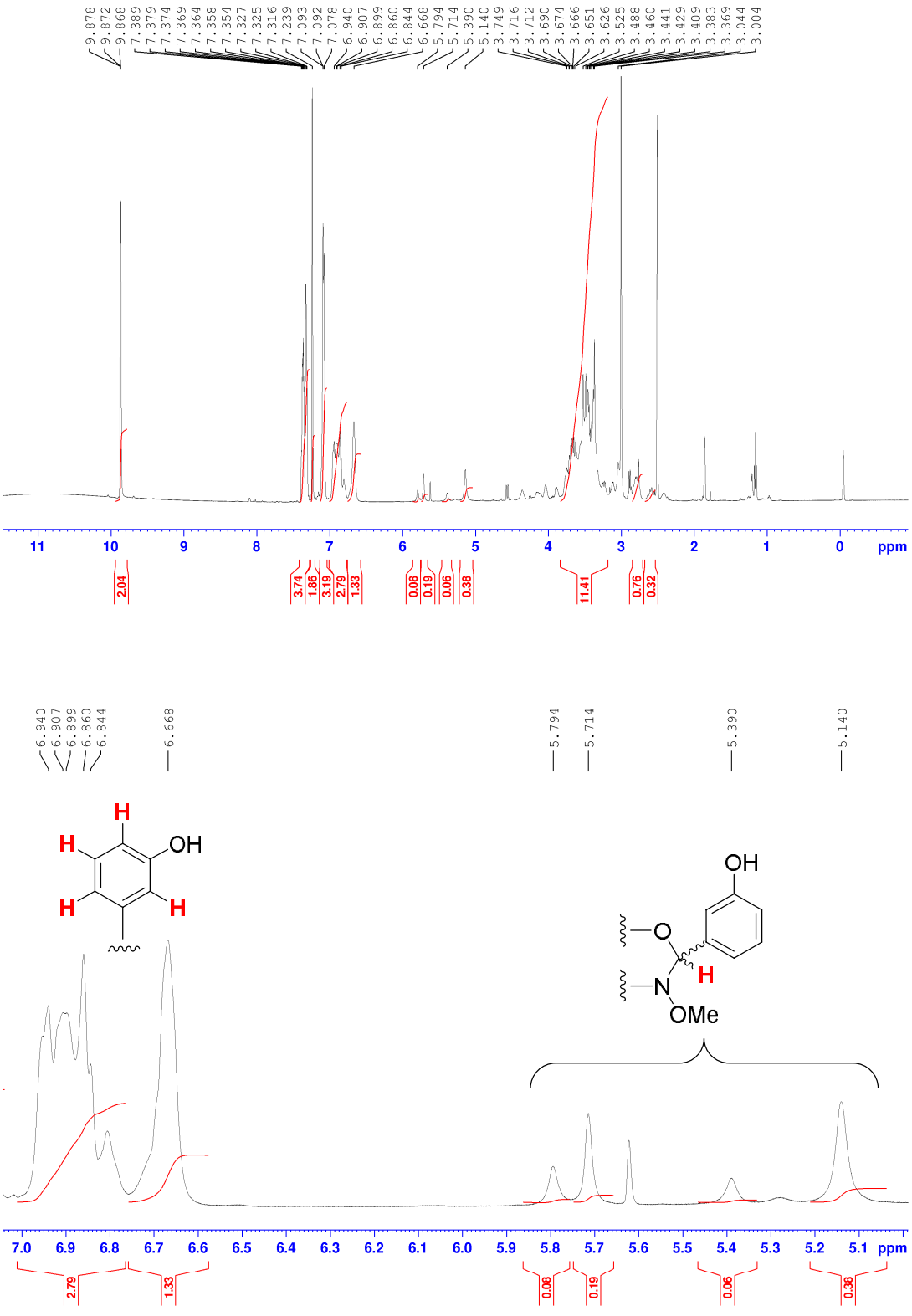


Figure S50. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of the reaction mixture of (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol (6) and 3-hydroxybenzaldehyde under acidic conditions.

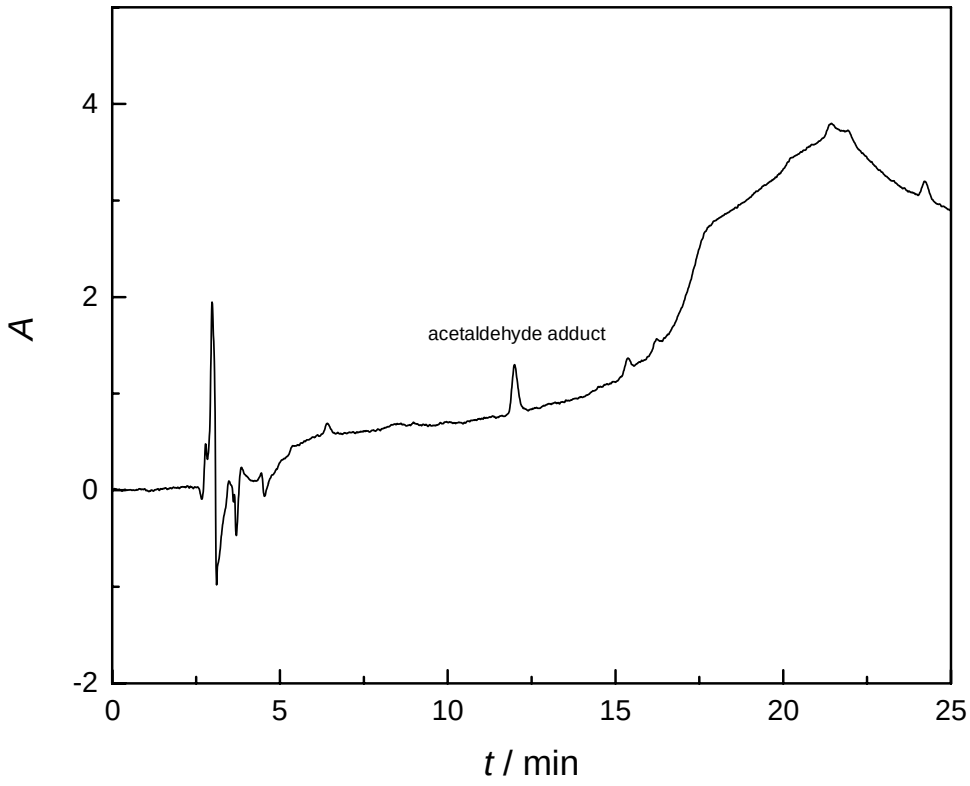


Figure S51. RP-HPLC trace of the product mixture after incubation of the 3-hydroxybenzaldehyde derivative of ON1 at pH 5.0 (30 mM acetate buffer) for 16 h at 55 °C; Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0).

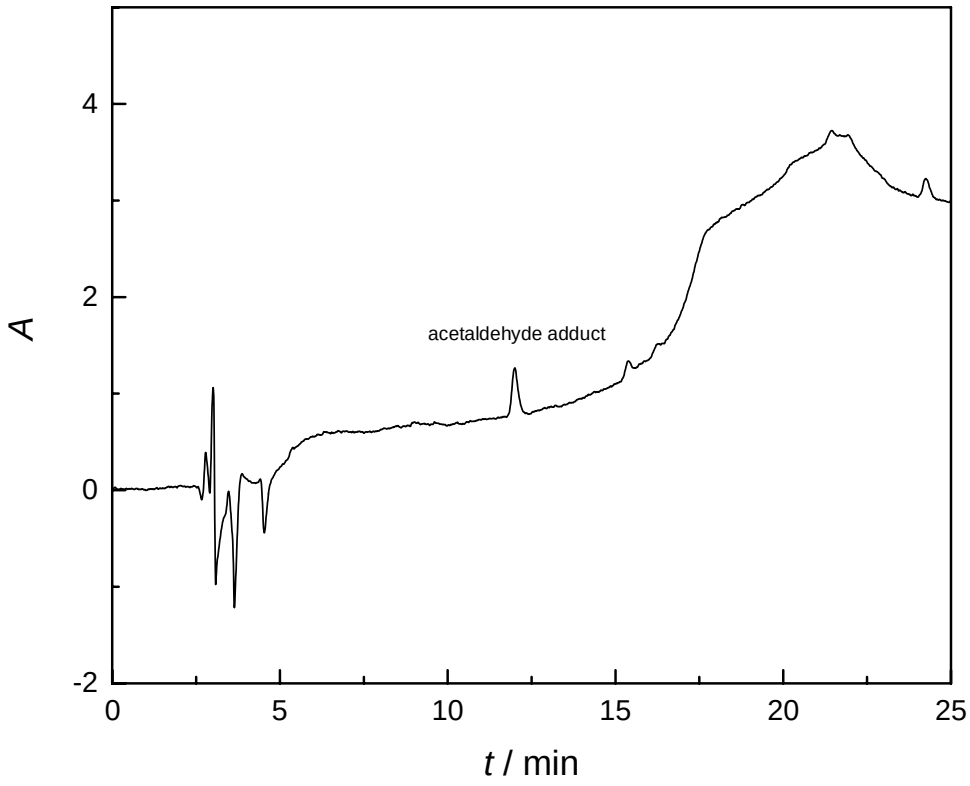


Figure S52. RP-HPLC trace of the product mixture after incubation of the 3-cyanobenzaldehyde derivative of ON1 at pH 5.0 (30 mM acetate buffer) for 16 h at 55 °C; Hypersil ODS C18 column (250 × 4.6 mm, 5 µm); detection wavelength = 260 nm; flow rate = 1.0 mL min^{-1}; linear gradient (8—23% over 25 min) of acetonitrile in 50 mM aqueous triethylammonium acetate (pH = 7.0).