Electronic Supplementary Information

Mechanism of the alkali degradation of (6–4) photoproduct-containing DNA

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The method for the preparation of dinucleoside monophosphate (from **13** to **18**) was basically the same as that of thymidylyl(3'–5')thymidine, which was used in our previous study.[29]

1-[5-*O*-(4,4'-dimethoxytrityl)-2-deoxy-β-D-ribofuranosyl]-5-methyl-2-pyrimidinone (14)

The starting material, 1-(2-deoxy-β-D-ribofuranosyl)-5-methyl-2-pyrimidinone (**13**), prepared as described previously[30] (810 mg, 3.58 mmol), was coevaporated with pyridine, dissolved in anhydrous pyridine (20 ml), and mixed with 4,4'-dimethoxytrityl chloride (2.02 g, 5.97 mmol). After stirring at room temperature for 1 h, methanol (1.0 ml) was added, and the mixture was concentrated. The residue was dissolved in chloroform (30 ml), and was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4, followed by filtration and concentration. After coevaporation with toluene, the crude product was chromatographed on silica gel with a stepwise gradient of 0–3% methanol in chloroform to give **14**. Yield: 852 mg (1.61 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ (ppm) 8.41 (d, J = 3.2 Hz, 1H, H4), 8.18 (d, J = 3.2 Hz, 1H, H6), 7.50−6.72 (m, 13H, DMT), 6.31 (t, J = 6.2 Hz, 1H, H1'), 4.56 (dd, J = 3.9, 2.1 Hz, 1H, H3'), 4.19 (d, J = 3.5 Hz, 1H, H4'), 3.79 (s, 6H, DMT-OMe), 3.50 (dd, J = 10.7, 3.1 Hz, 1H, H5'), 3.39 (dd, J = 10.7, 3.4 Hz, 1H, H5'), 2.80 (ddd, J = 13.9, 6.1, 4.3 Hz, 1H, H2'), 2.30 (dt, J = 13.8, 6.3 Hz, 1H, H2'), 1.72 (s, 3H, Me). FAB-HRMS m/z: 529.2339 ($[M+H]^+$ calcd. for $C_{31}H_{33}N_2O_6$: 529.2338).

1-[5-*O*-(4,4'-dimethoxytrityl)-3-*O*-levulinyl-2-deoxy-β-D-ribofuranosyl]-5-methyl-2-pyrimidinone (15)

Compound **14** (798 mg, 1.51 mmol) was coevaporated with pyridine and dissolved in anhydrous 1,4-dioxane (10 ml). To this solution, 4-(*N*,*N*-dimethylamino)pyridine (16.0 mg, 131 μmol), *N*,*N*'-dicyclohexylcarbodiimide (779 mg, 3.77 mmol), and levulinic acid (308 μl, 3.00 mmol) were added. The mixture was stirred at room temperature for 2 h, and then saturated aqueous $NaHCO_3$ (1.0 ml) was added. The precipitates were removed by filtration. After evaporation, the residue was dissolved in chloroform (10 ml) and washed with saturated aqueous $NaHCO_3$ and brine. The

organic layer was dried with Na_2SO_4. After the solvent was evaporated, the residue was coevaporated with toluene. To this residue, acetonitrile (5.0 ml) was added, and the insoluble materials were removed by filtration. After concentration, the product was purified by column chromatography with a stepwise gradient of 0–2% methanol in chloroform to give **15**, as a yellow foamy solid. Yield: 698 mg (1.11 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ (ppm) 8.41 (d, *J* = 3.2 Hz, 1H, H4), 8.10 (m, 1H, H6), 7.37−6.79 (m, 13H, DMT), 6.34 (dd, *J* = 7.9, 5.7 Hz, 1H, H1'), 5.42 (dt, *J* = 4.1, 2.0 Hz, 1H, H3'), 4.25 (q, *J* = 2.5 Hz, 1H, H4'), 3.77 (d, *J* = 2.5 Hz, 6H, DMT-OMe), 3.46 (dd, *J* = 10.2, 2.9 Hz, 2H, H5'), 2.84 (ddd, *J* = 14.3, 5.7, 2.0 Hz, 1H, H2'), 2.75 (dt, *J* = 10.2, 6.4 Hz, 2H, -OCOCH$_2$C*H*$_2$CO-), 2.58 (m, 2H, -OCOC*H*$_2$CH$_2$CO-), 2.34 (ddd, *J* = 14.3, 7.9, 6.3 Hz, 1H, H2'), 2.19 (s, 3H, Lev-Me), 1.69 (s, 3H, Me). FAB-HRMS *m/z*: 649.2510 ([M+Na]$^+$ calcd. for $C_{36}H_{38}N_2O_8Na$: 649.2525).

1-(3-*O*-levulinyl-2-deoxy-β-D-ribofuranosyl)-5-methyl-2-pyrimidinone (16)

Compound **15** (698 mg, 1.11 mmol) was dissolved in 80% aqueous acetic acid (10 ml). After 2 h, the mixture was concentrated and coevaporated with toluene. The product was purified by column chromatography on silica gel with a stepwise gradient of 0–4% methanol in chloroform to give **16**. Yield: 331 mg (1.02 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ (ppm) 8.46 (d, *J* = 3.2 Hz, 1H, H4), 8.10 (d, *J* = 3.1 Hz, 1H, H6), 6.21 (t, *J* = 6.5 Hz, 1H, H1'), 5.35 (dt, *J* = 6.5, 3.2 Hz, 1H, H3'), 4.19 (q, *J* = 2.8 Hz, 1H, H4'), 4.00−3.88 (m, 2H, H5'), 2,80-2.33 (m, 6H, -OCOCH$_2$C*H*$_2$CO-, -OCOC*H*$_2$CH$_2$CO- and H2'), 2.20 (s, 3H, Lev-Me), 2.12 (s, 3H, Me). FAB-HRMS *m/z*: 325.1399 ([M+H]$^+$ calcd. for $C_{15}H_{21}N_2O_6$: 325.1339).

Dinucleoside monophosphate containing 5-methyl-2-pyrimidinone (4)

Compound **16** (267 mg, 823 μmol) and 5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (657 mg, 884 μmol) were dissolved in anhydrous acetonitrile (4.2 ml). To this solution, 1*H*-tetrazole (217 mg, 3.10 mmol) dissolved in anhydrous acetonitrile (7.5 ml) was added, and the mixture was stirred for 10 min. Chloroform (30 ml) was then added, and the

solution was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4 and concentrated. To this residue, 0.15 M iodine in THF-pyridine-water (80:18:2, v/v/v) (17.6 ml) was added, and the mixture was stirred for 1 h. A 1 M aqueous Na_2SO_3 solution (30 ml) was then added, and the mixture was extracted with ethyl acetate (10 ml) three times. The organic layer was dried with Na_2SO_4 and concentrated. The residue was coevaporated with toluene, and the product was purified by column chromatography on silica gel with a stepwise gradient of 0–5% methanol in chloroform, to give the fully protected dimer (**17**). Yield: 462 mg (470 μmol, 57%).

The fully protected dimer **17** (462 mg, 470 μmol) was dissolved in 80% aqueous acetic acid (10 ml). After 1 h, the mixture was concentrated and coevaporated with toluene. The product was purified by column chromatography on silica gel with a stepwise gradient of 0–7% methanol in chloroform. Compound **18** was obtained as a white foam by evaporation. Yield: 222 mg (326 μmol, 69%). FAB-HRMS *m/z*: 682.2133 ($[M+H]^+$ calcd. for $C_{28}H_{37}N_5O_{13}P$: 682.2125).

Compound **18** (126 mg, 185 μmol) was dissolved in 28% aqueous ammonia (2.0 ml). After 2 h, the solution was concentrated, and the product was purified by reversed-phase HPLC. The HPLC purification was performed with a linear gradient of 7.5–12.5% acetonitrile during 30 min. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 8.49 (d, *J* = 3.0 Hz, 1H, m5K-H4), 8.31 (d, *J* = 3.0 Hz, 1H, m5K-H6), 7.64 (d, *J* = 1.0 Hz, 1H, Tp-H6), 6.24 (t, *J* = 6.2 Hz, 1H, m5K-H1'), 6.20 (t, *J* = 6.9 Hz, 1H, Tp-H1'), 4.76 (td, *J* = 6.7, 3.4 Hz, 1H, Tp-H3'), 4.53 (q, *J* = 5.5 Hz, 1H, m5K-H3'), 4.26−4.19 (m, 2H, m5K-H5'), 4.17 (q, *J* = 3.9 Hz, 1H, m5K-H4'), 4.09 (dt, *J* = 11.7, 4.1 Hz, 1H, Tp-H4'), 3.79 (qd, *J* = 12.2, 4.1 Hz, 2H, Tp-H5'), 2.61 (ddd, *J* = 14.1, 6.5, 5.0 Hz, 1H, m5K-H2'), 2.49 (ddd, *J* = 14.2, 6.2, 3.6 Hz, 1H, m5K-H2'), 2.39−2.30 (m, 2H, Tp-H2'), 2.17 (s, 3H, m5K-Me), 1.87 (s, 3H, Tp-Me). FAB-HRMS *m/z*: 531.1487 ($[M+H]^+$ calcd. for $C_{20}H_{28}N_4O_{11}P$: 531.1512).

A trimer containing 5-methyl-2-pyrimidinone (8)

The 5'-protected nucleoside (**14**) (256 mg, 485 μmol) was dissolved in anhydrous THF (4.8 ml) and mixed with *N*,*N*-diisopropylethylamine (330 μl, 1.90 mmol). To this solution, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (210 μl, 948 μmol) was added. The mixture was stirred at

room temperature for 40 min, diluted with ethyl acetate (90 ml), and washed with 2% $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4 and concentrated. The product was purified by column chromatography on silica gel and eluted with hexane/ethyl acetate (1:4, v/v) containing 0.1% pyridine. After coevaporation with acetonitrile, the phosphoramidite building block was obtained as a yellow solid. Yield: 124 mg (170 μmol, 35%).

This building block (81.5 mg, 110 μmol) was dissolved in anhydrous acetonitrile (1.1 ml), and the vial containing the solution was attached to the DNA synthesizer (Applied Biosystems 3400). The trimer **8** was synthesized using five 1.0 μmol columns. After the chain assembly, the CPG support was treated with 28% aqueous ammonia at room temperature for 2 h. The ammonia and water were then evaporated, and the product was purified by reversed-phase HPLC. The HPLC purification was performed with a linear gradient of 5–15% acetonitrile during 30 min. The eluate was concentrated and coevaporated with water several times.

A tetramer containing the Dewar valence isomer of the (6–4) photoproduct (12)

A 20 μM solution (12 ml) of a tetramer, d(ATTG), was placed in an ice-cooled petri dish with an internal diameter of 9 cm, and was irradiated at a total UV dose of 8 J/cm^2 on a SpectroLinker XL-1500 UV crosslinker (Spectronics Corporation). After repeating this irradiation 9 times, the solutions were combined and concentrated *in vacuo*. The product with an absorption maximum at 325 nm (**11**) was purified by HPLC using an Inertsil ODS-3 column (7.6 × 250 mm) with a linear gradient of 7–13% acetonitrile for 20 min. The eluate was evaporated and coevaporated with water several times. A 1.7 μM solution of **11** (100 ml) was irradiated in an ice-cooled aluminum tray (28 × 20 cm) for 1.5 h with a 450 W high-pressure mercury lamp (Ushio UM-452) covered with a Pyrex jacket. The product without absorption at wavelengths longer than 300 nm (**12**) was purified by HPLC using a Waters XBridge C18 5 μm column (4.6 × 250 mm) with a linear gradient of 0–25 % acetonitrile for 20 min, followed by concentration and coevaporation with water.

Table 1. ^{13}C chemical shifts of the compounds shown in Fig. 1

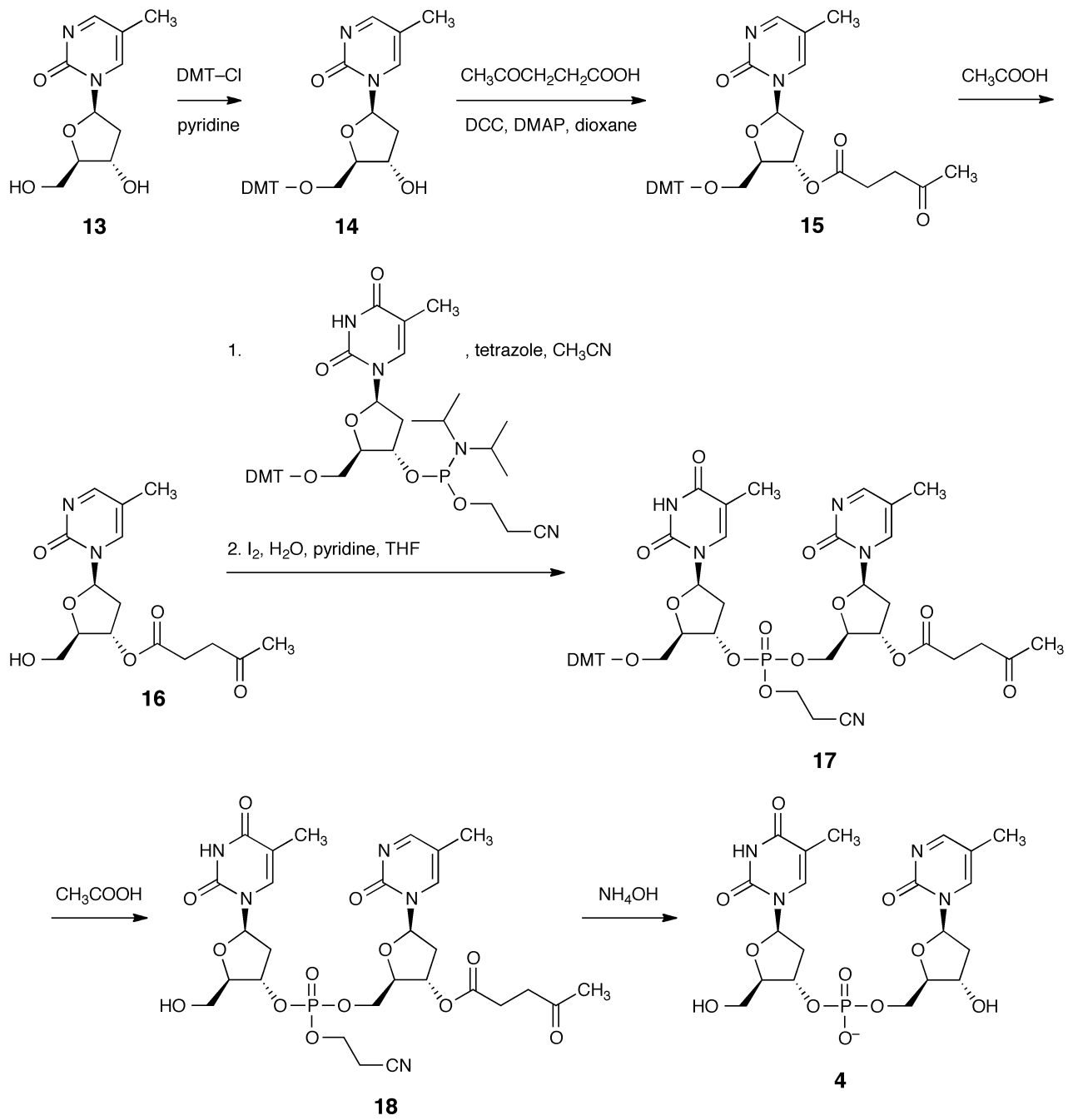
Compounds	5' Base					3' Base					5' Sugar				
	C2	C4	C5	C6	Me	C2	C4	C5	C6	Me	C1'	C2'	C3'	C4'	C5'
1 [a]	156.4	176.7	74.9	60.4	27.7	159.9	176.9	119.0	146.8	16.2	84.9	37.5	72.3	84.8	61.4
2 [b]	160.7	179.3	86.3	61.7	21.4	159.7	175.2	117.5	148.1	15.7	84.4	36.9	72.7	84.8	62.3
3 [c]	161.4	179.8	87.1	61.8	21.0	161.6	174.8	117.3	152.5	15.9	86.8	39.4	77.0	87.9	64.8

[a] J.-S. Taylor, D. S. Garrett and M. P. Cohrs, *Biochemistry*, 1988, **27**, 7206–7215.

[b] M. Higurashi, T. Ohtsuki, A. Inase, R. Kusumoto, C. Masutani, F. Hanaoka and S. Iwai, *J. Biol. Chem.*, 2003, **278**, 51968–51973.

[c] This study.

Scheme 1



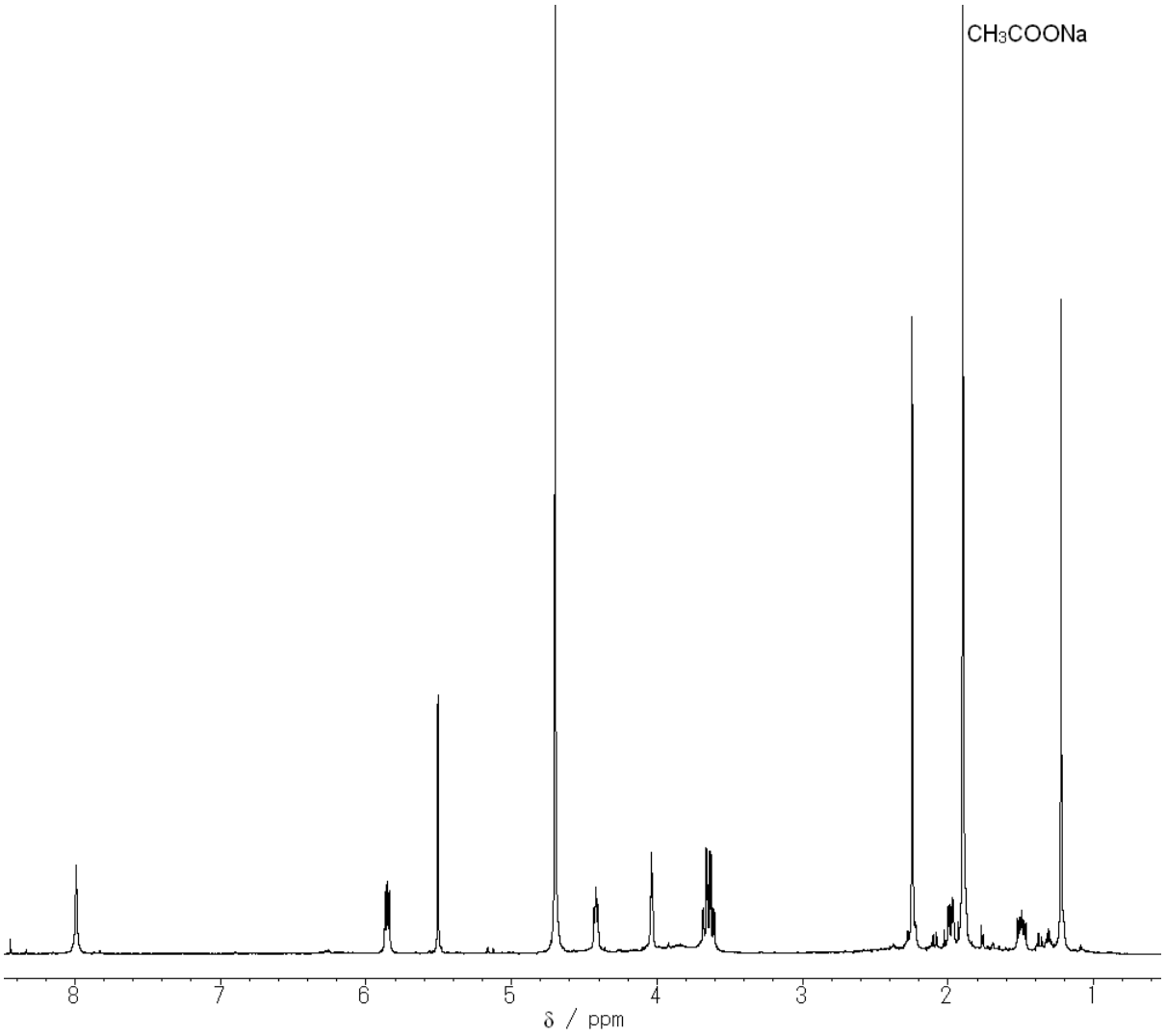


Fig. S1 A ^{1}H NMR spectrum (500 MHz, D_2O) of the sodium salt of **3**. Since this compound was isolated by HPLC using triethylammonium acetate buffer, followed by cation exchange to sodium, the sample was contaminated with sodium acetate.

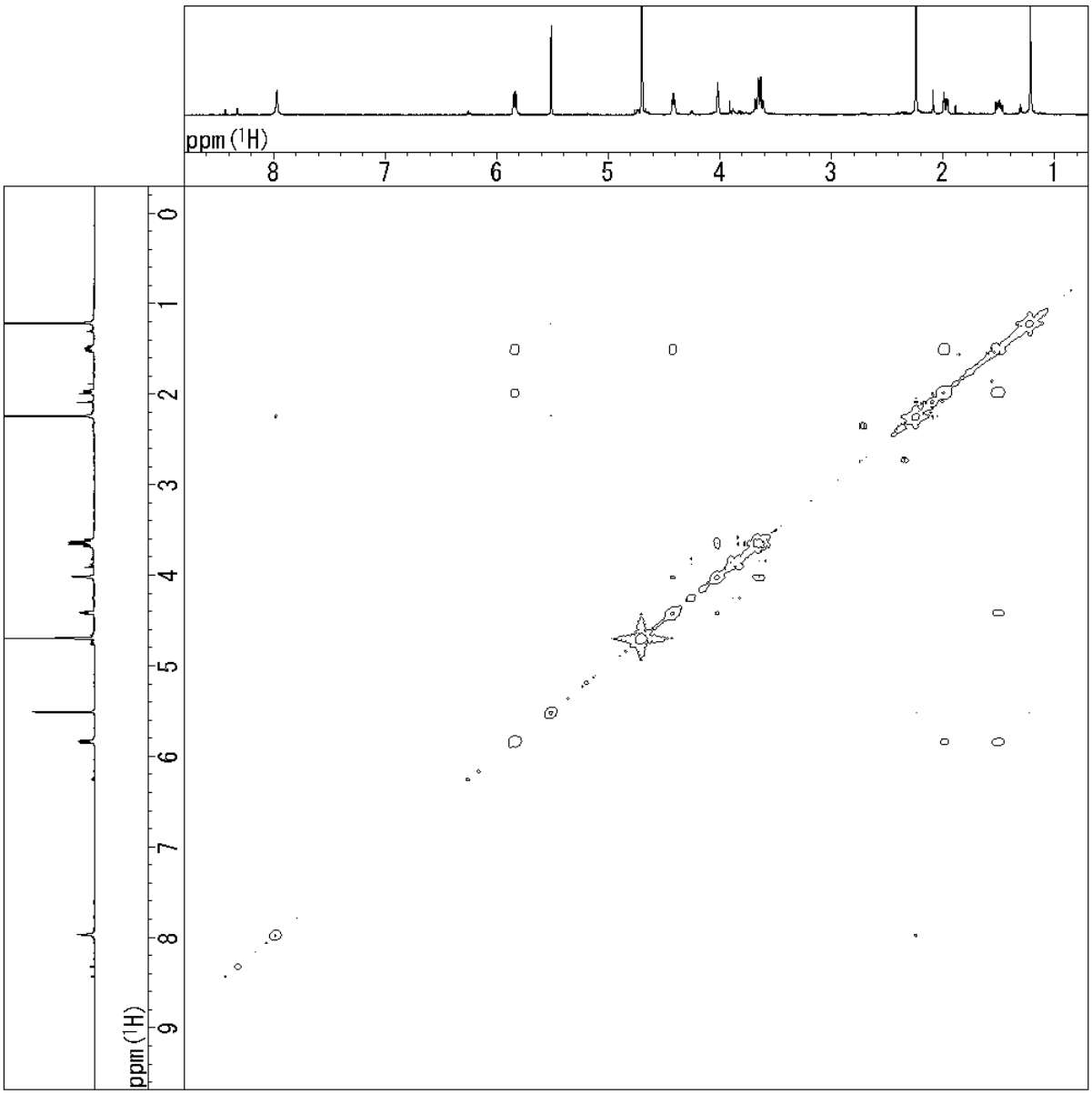


Fig. S2 A COSY spectrum of the sodium salt of **3**.

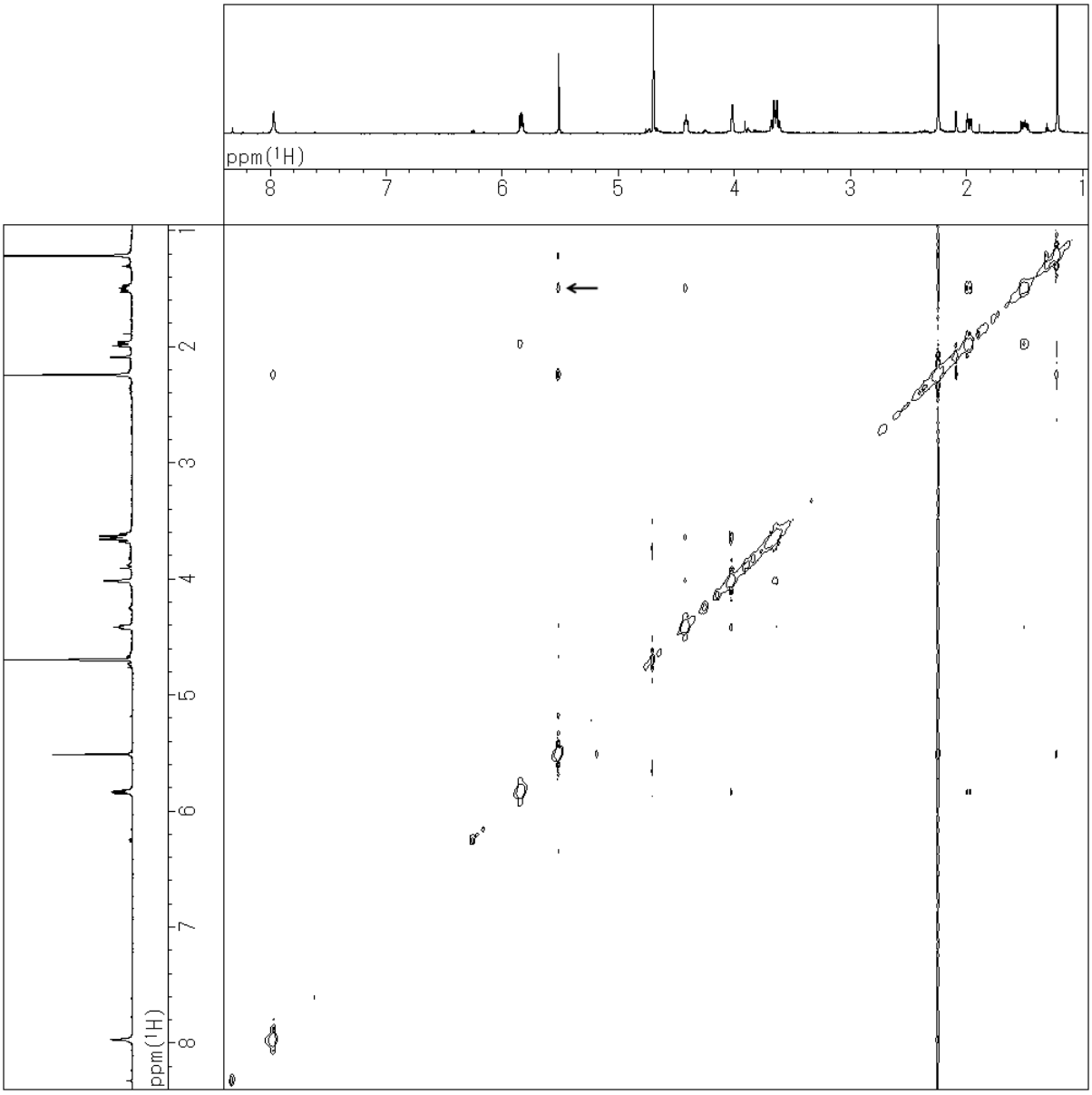


Fig. S3 A NOESY spectrum of the sodium salt of **3**. The cross-peak between the H6 of the 5' component and the H2' of the sugar moiety is indicated by an arrow.

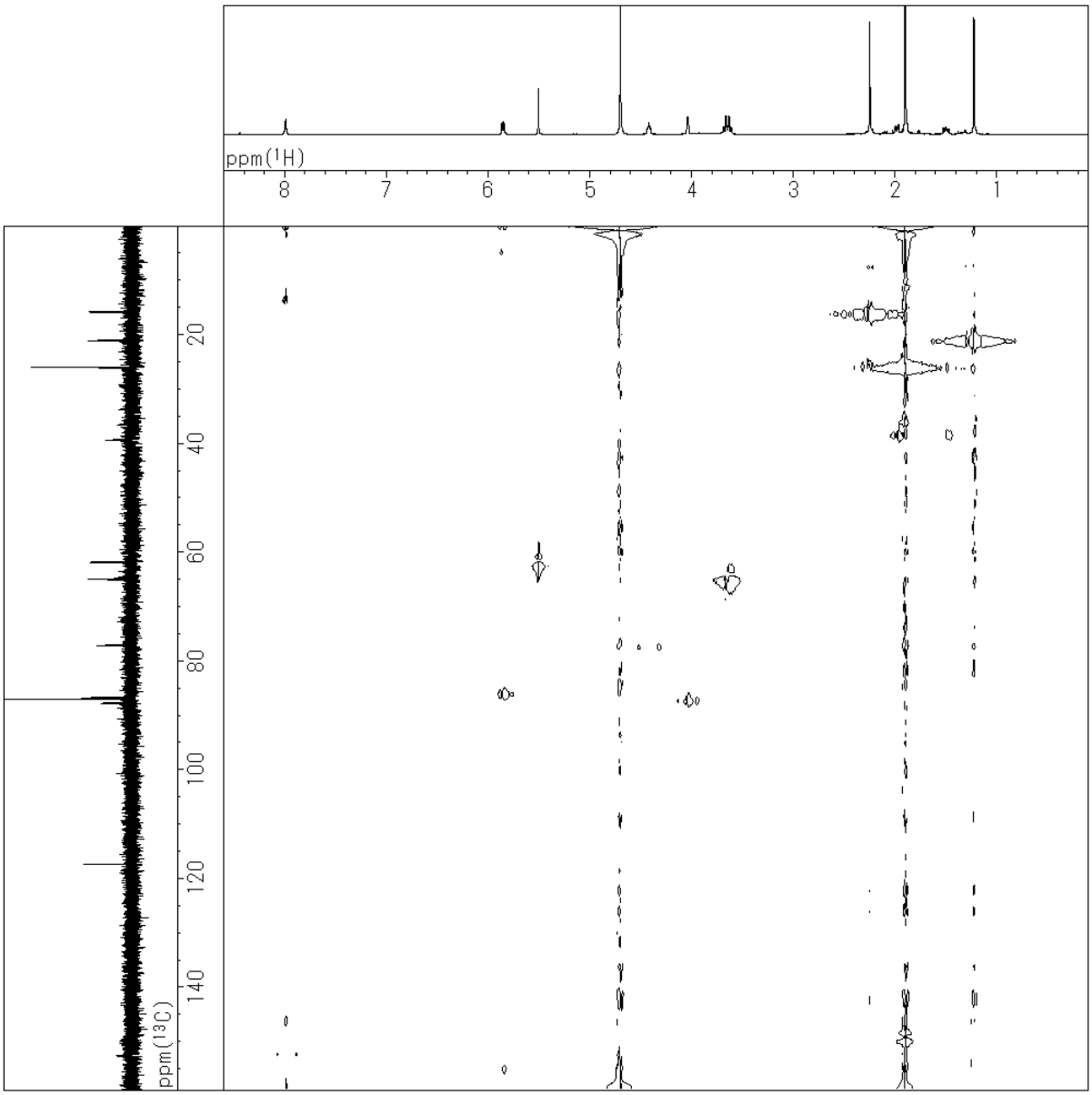


Fig. S4 An HMQC spectrum of the sodium salt of **3**.

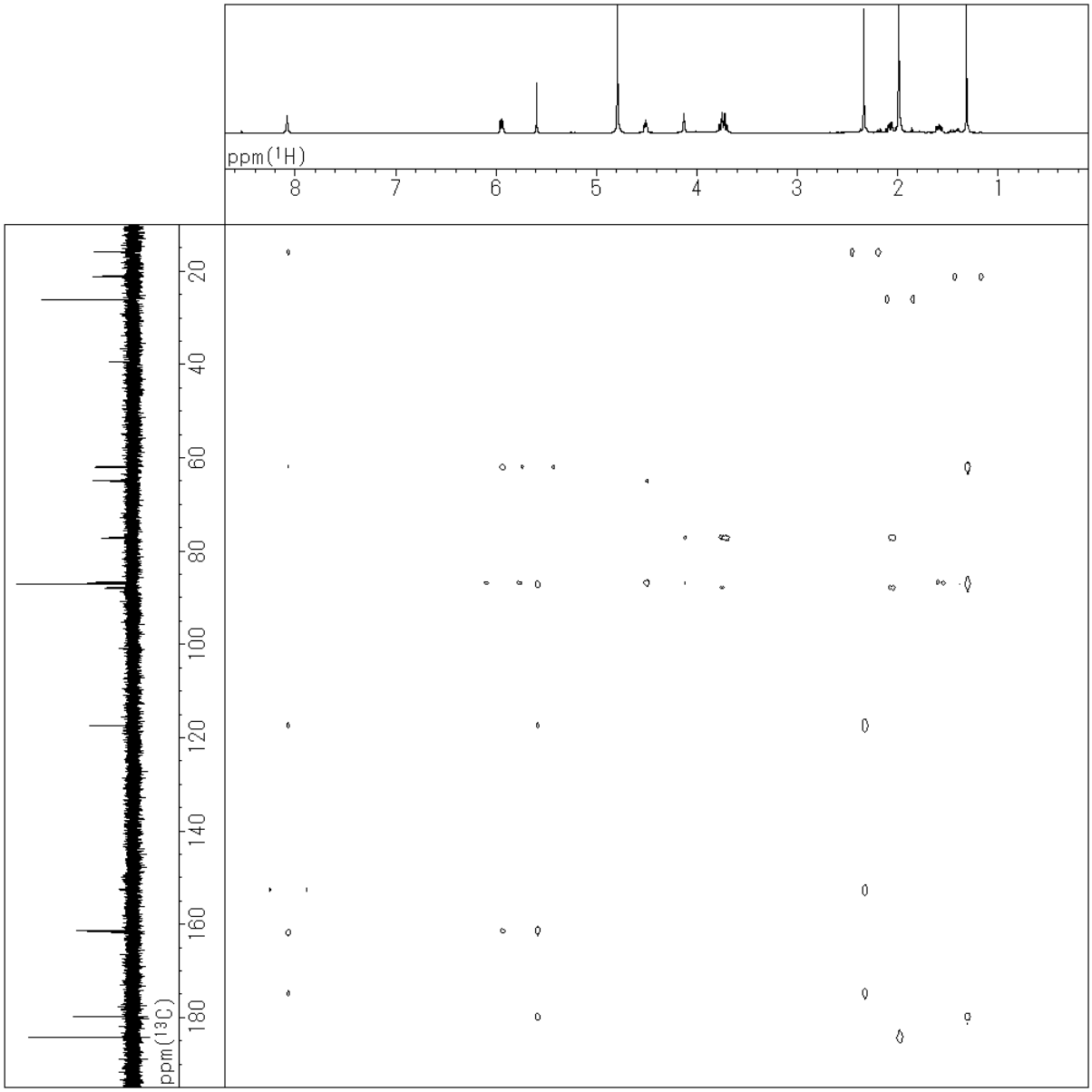


Fig. S5 An HMBC spectrum of the sodium salt of **3**.

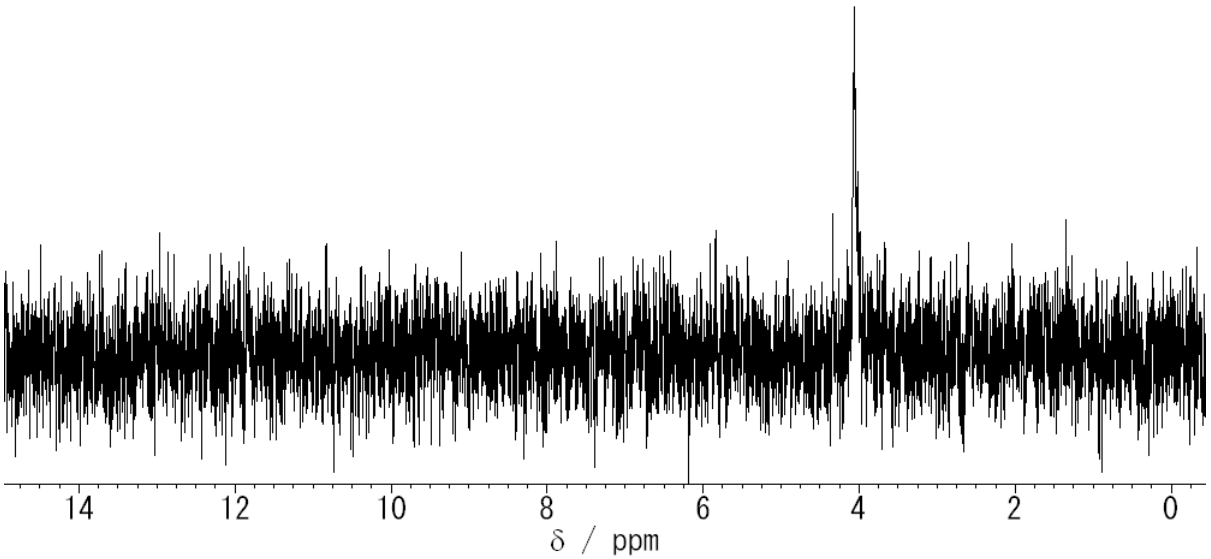


Fig. S6 A ^{31}P NMR spectrum (203 MHz, D_2O) of the sodium salt of **3**.

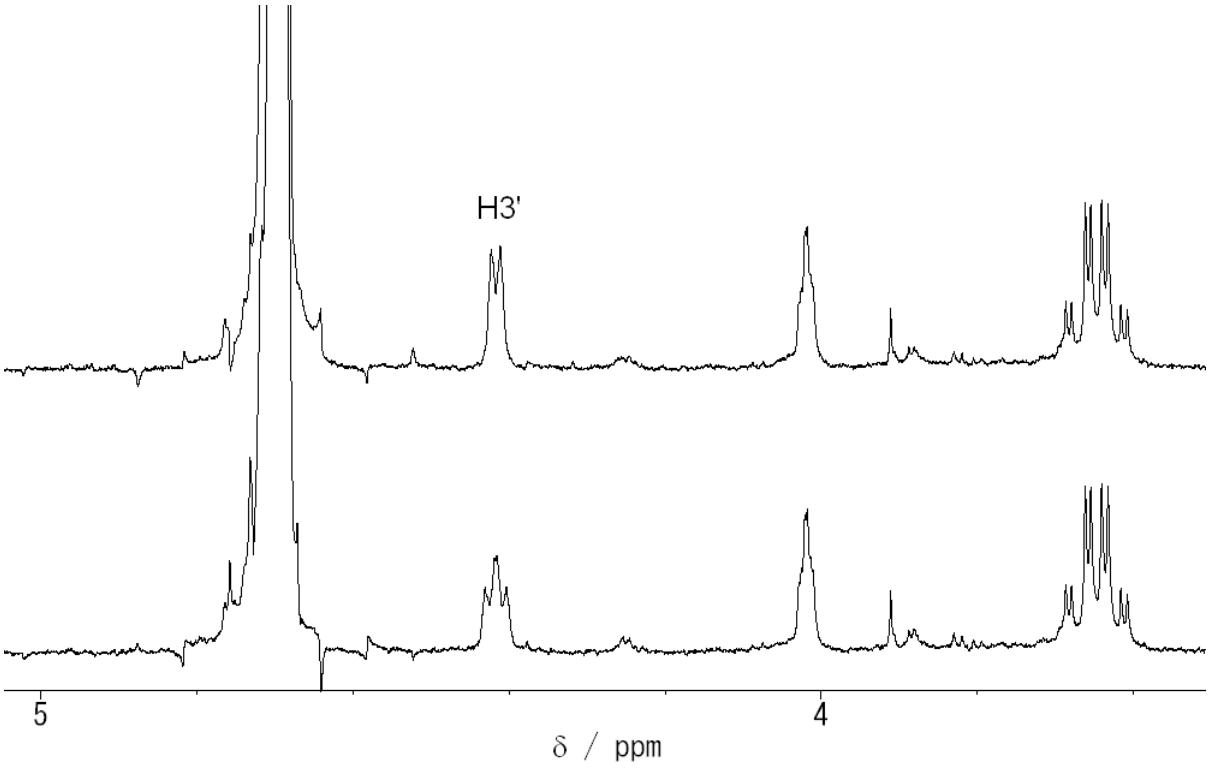


Fig. S7 ^{1}H NMR spectra of the sodium salt of **3** with (upper) and without (lower) the ^{31}P decoupling.

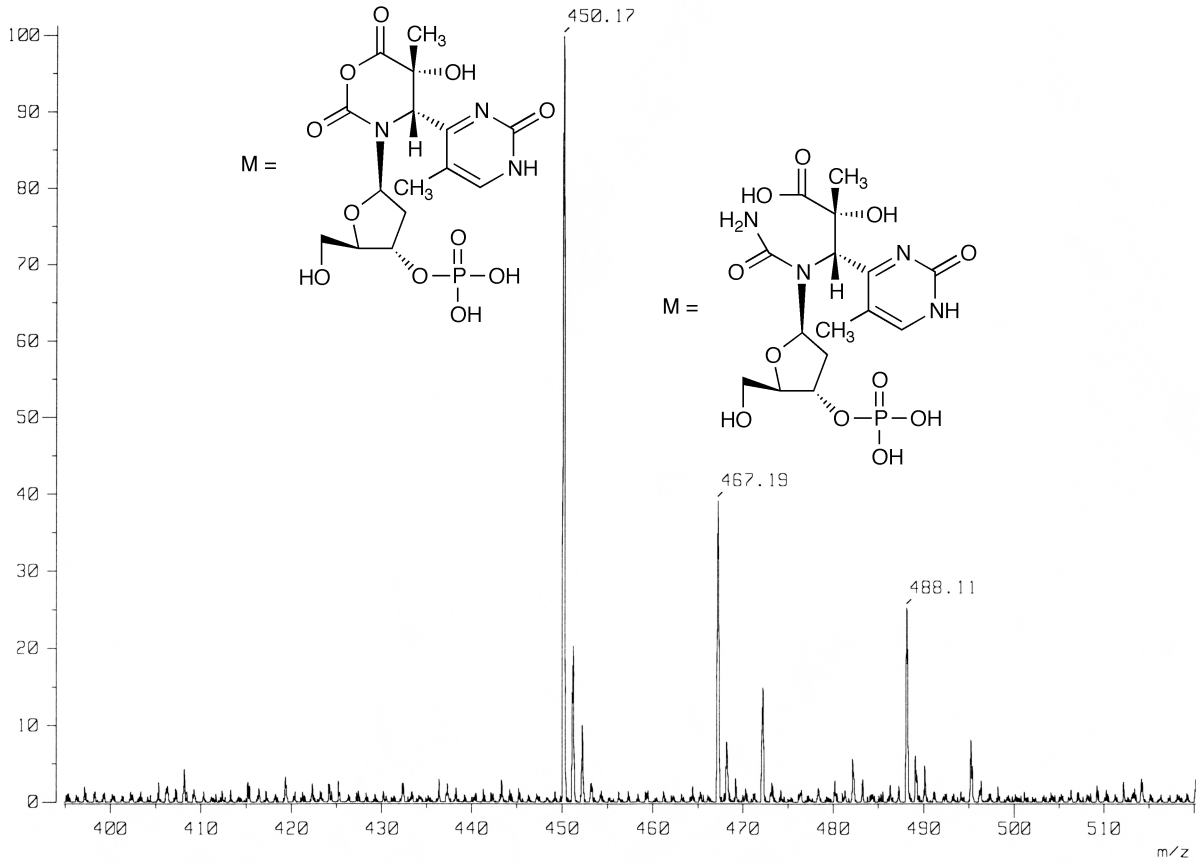


Fig. S8 An ESI-MS spectrum (positive ion mode) of **3**.

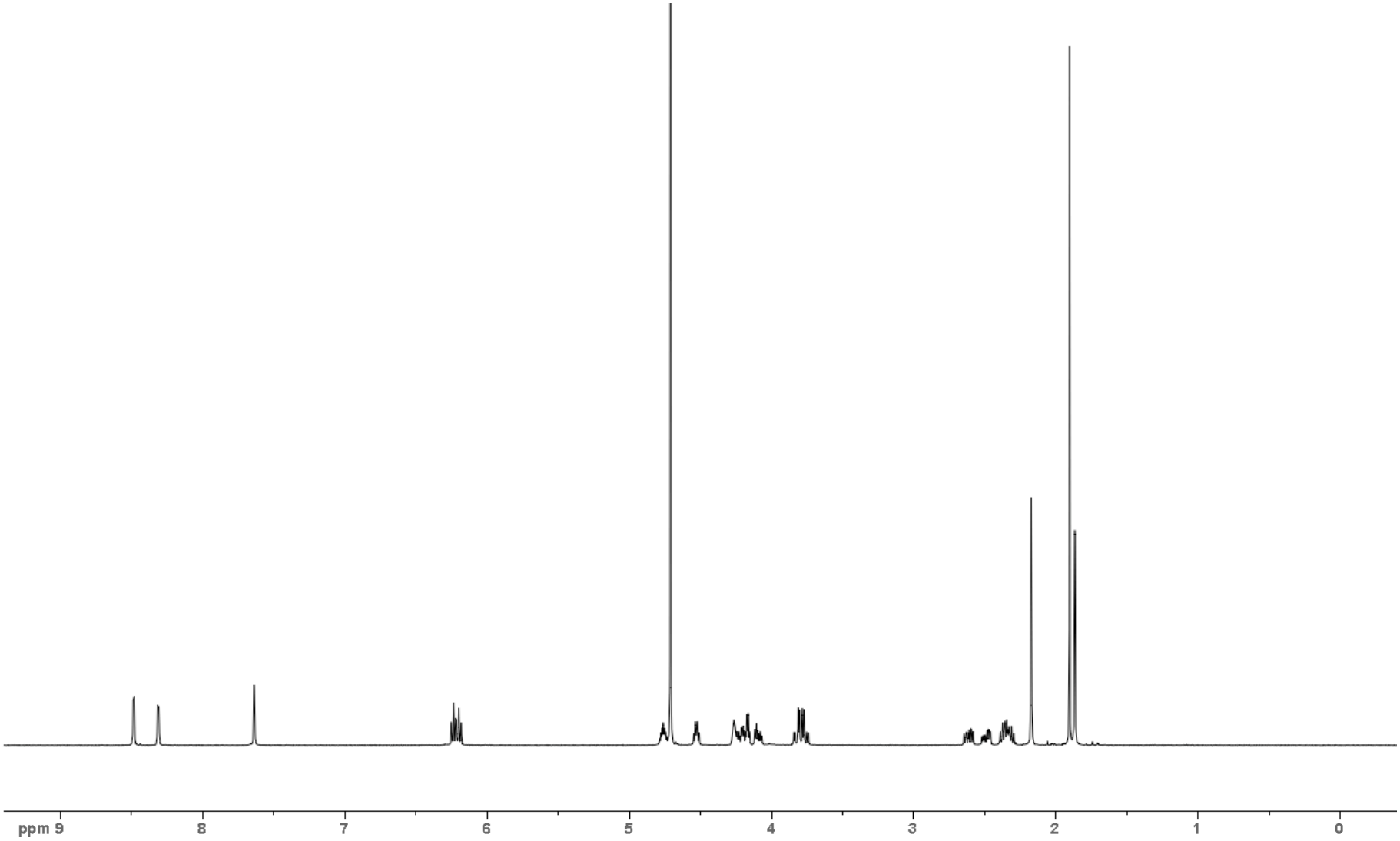


Fig. S9 A ^{1}H NMR spectrum (400 MHz, D_2O) of **4**.

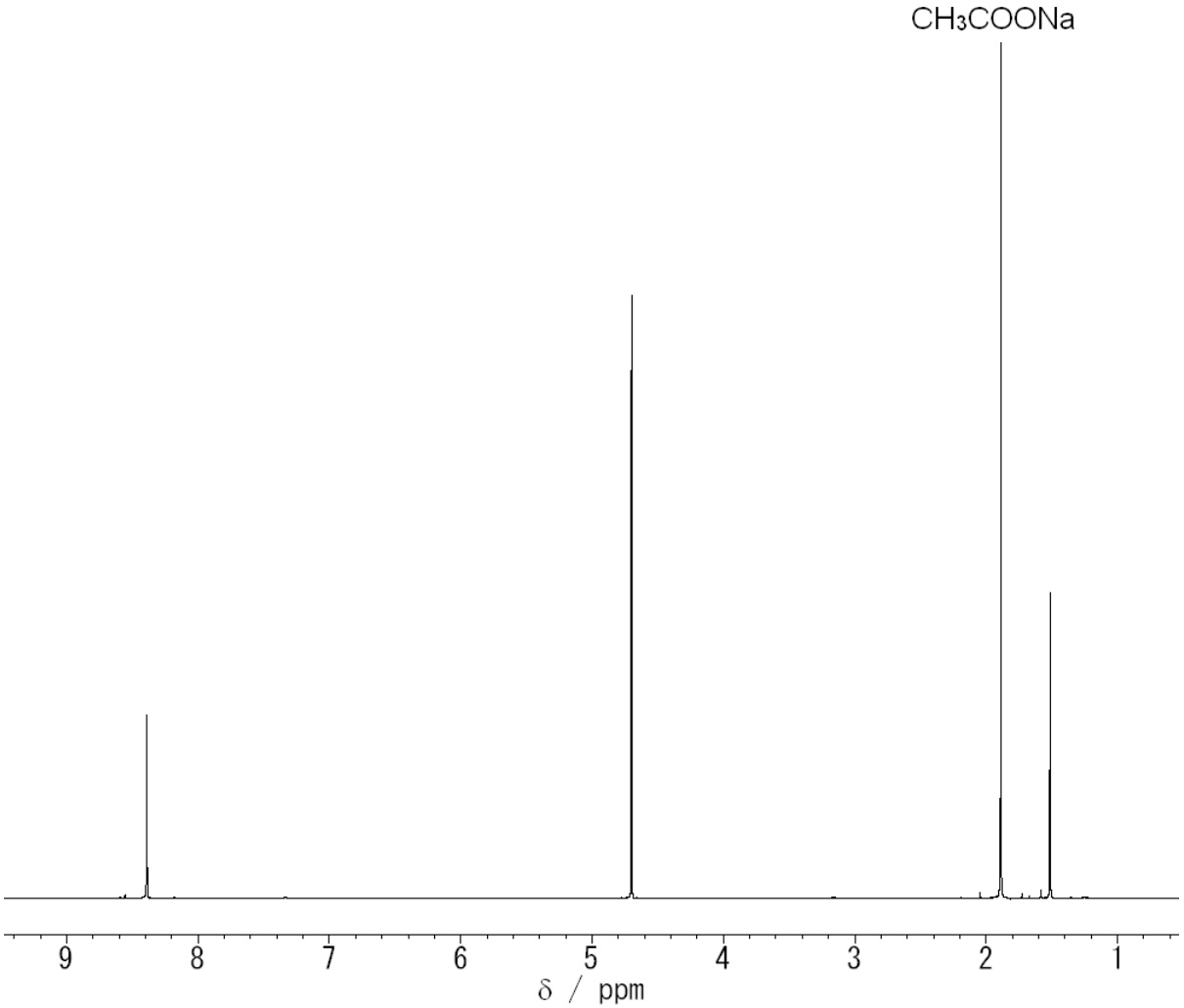


Fig. S10 A ^{1}H NMR spectrum (400 MHz, D_2O) of **7**. Since this compound was isolated by HPLC using triethylammonium acetate buffer, followed by cation exchange to sodium, the sample was contaminated with sodium acetate.

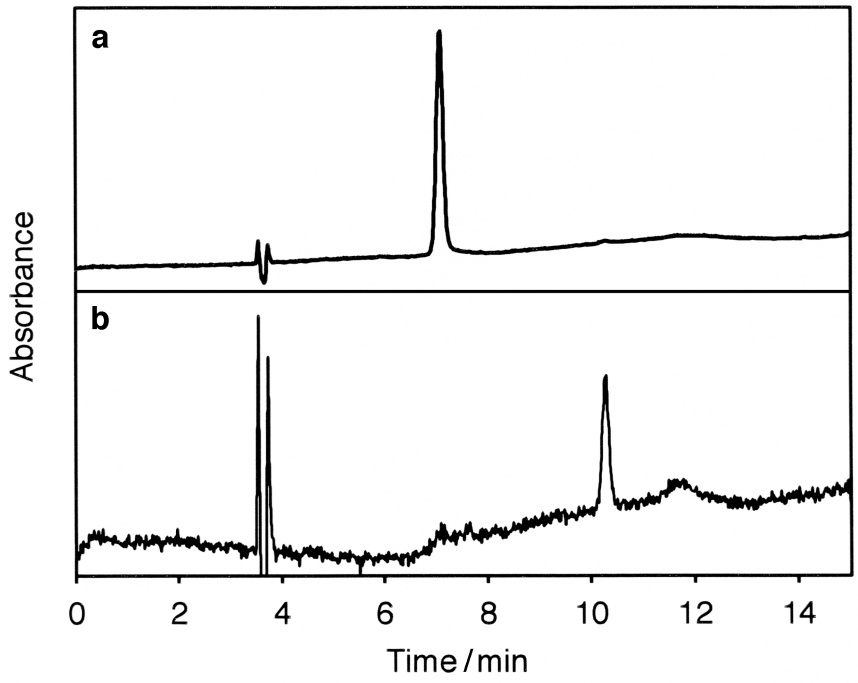


Fig. S11 Conversion of **7** to **6** by treatment with 1 M HCl at room temperature for 2 h. Elution was monitored at 275 (a) and 310 (b) nm. The peaks at 7.1 and 10.3 min are the remaining **7** and the produced **6**, respectively, as determined by their UV absorption spectra.

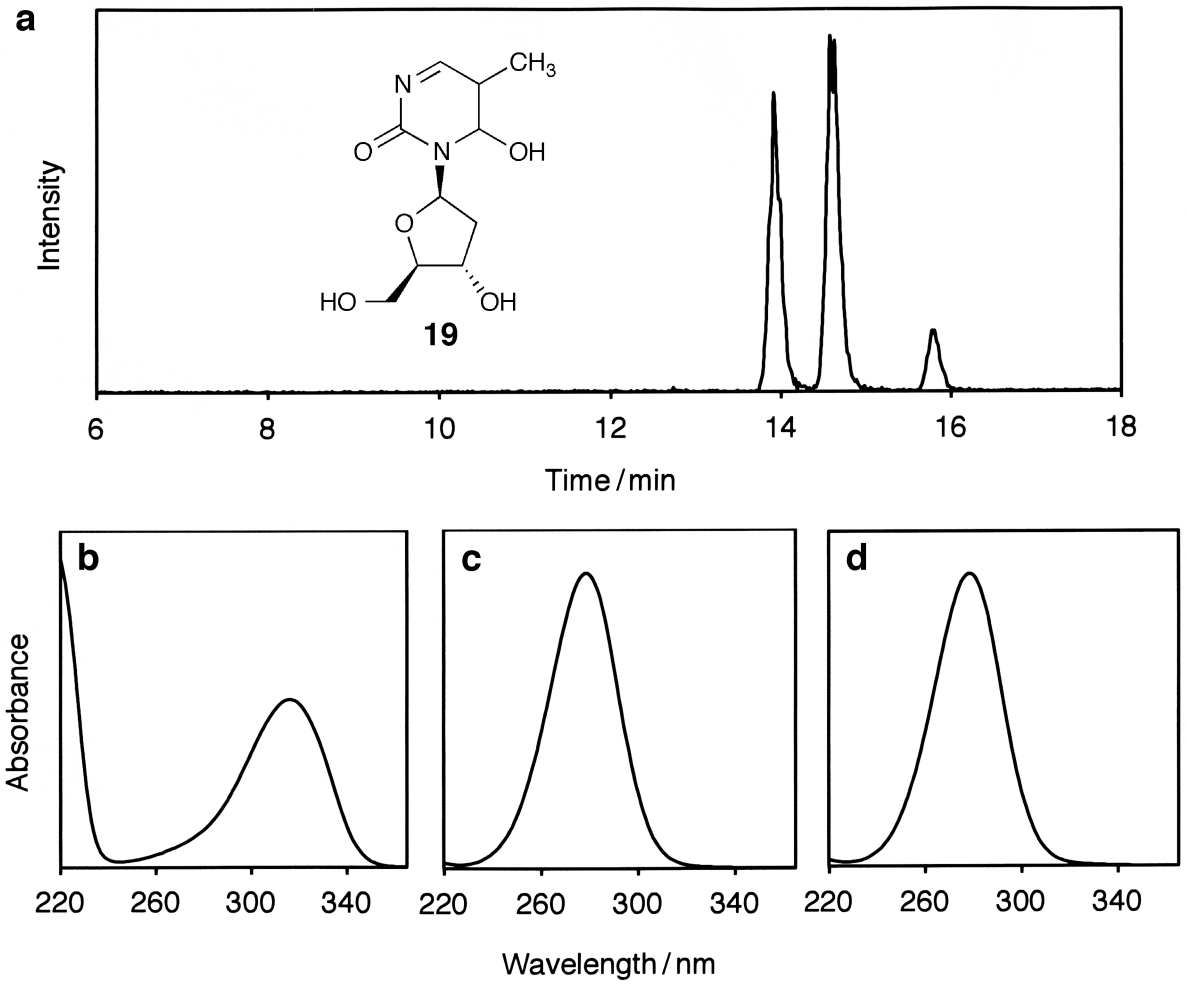


Fig. S12 Mass chromatogram ($[M–H]^-$ = 243) of the alkali-treated sample of **13** (a) and absorption spectra of **13** (b), the 13.9 min peak (c), and the 14.6 min peak (d). Alkali treatment was performed with 1 M NaOH at 60°C for 1 h, and the mass of **19** is 244.11.

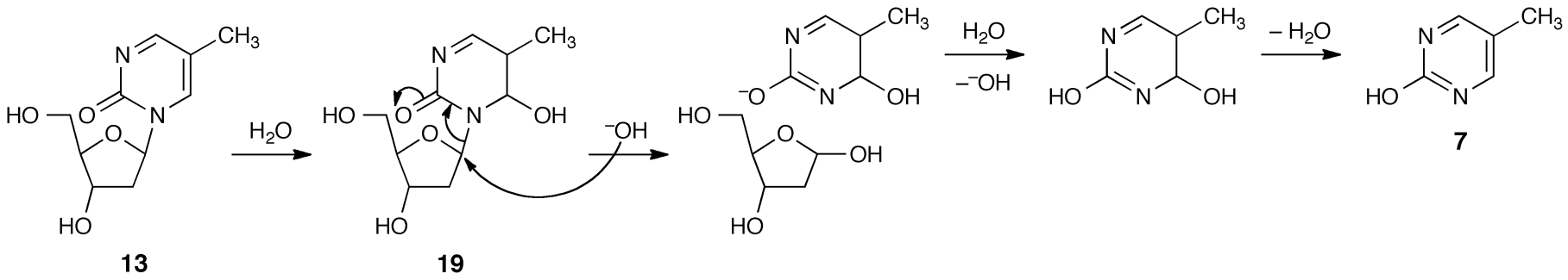


Fig. S13 Proposed mechanism of the alkali hydrolysis of **13** to form **7**.

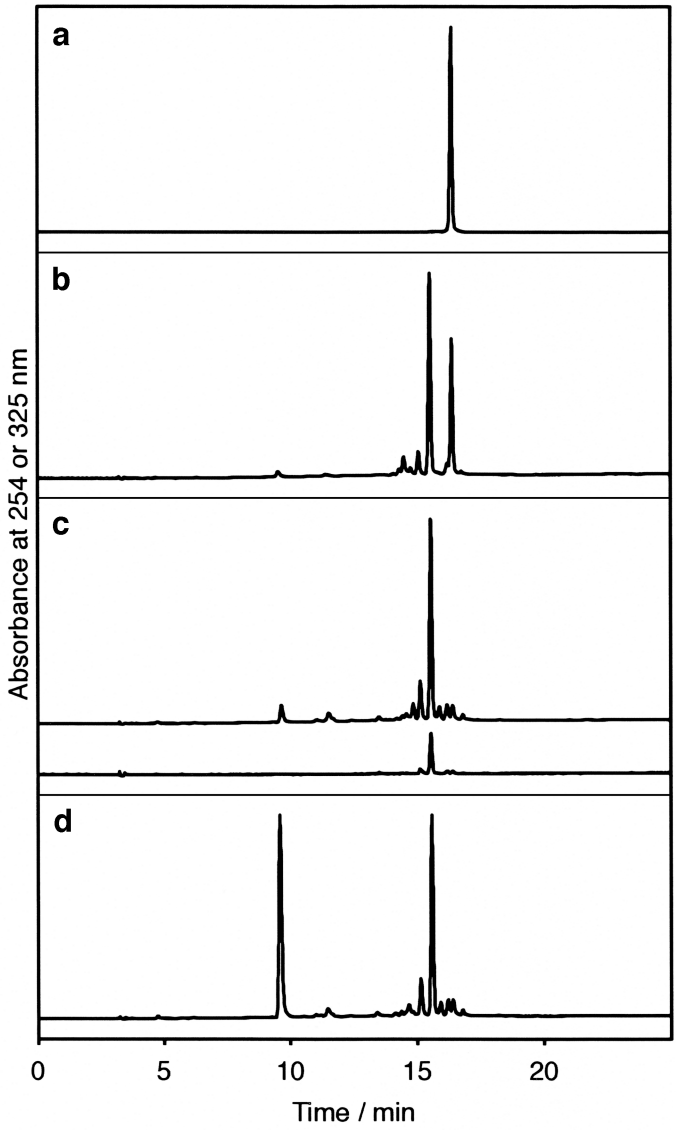


Fig. S14 HPLC analysis of the treatment of a tetramer containing the (6–4) photoproduct (**11** in Fig. 7) with 0.1 M KOH at 60°C for 2 h (b) and at 90°C for 30 min (c: upper, 254 nm; lower, 325 nm). Compound **11** without the alkali treatment (a) and coinjection of sample c with 2'-deoxyguanosine 5'-phosphate (d) are also shown.

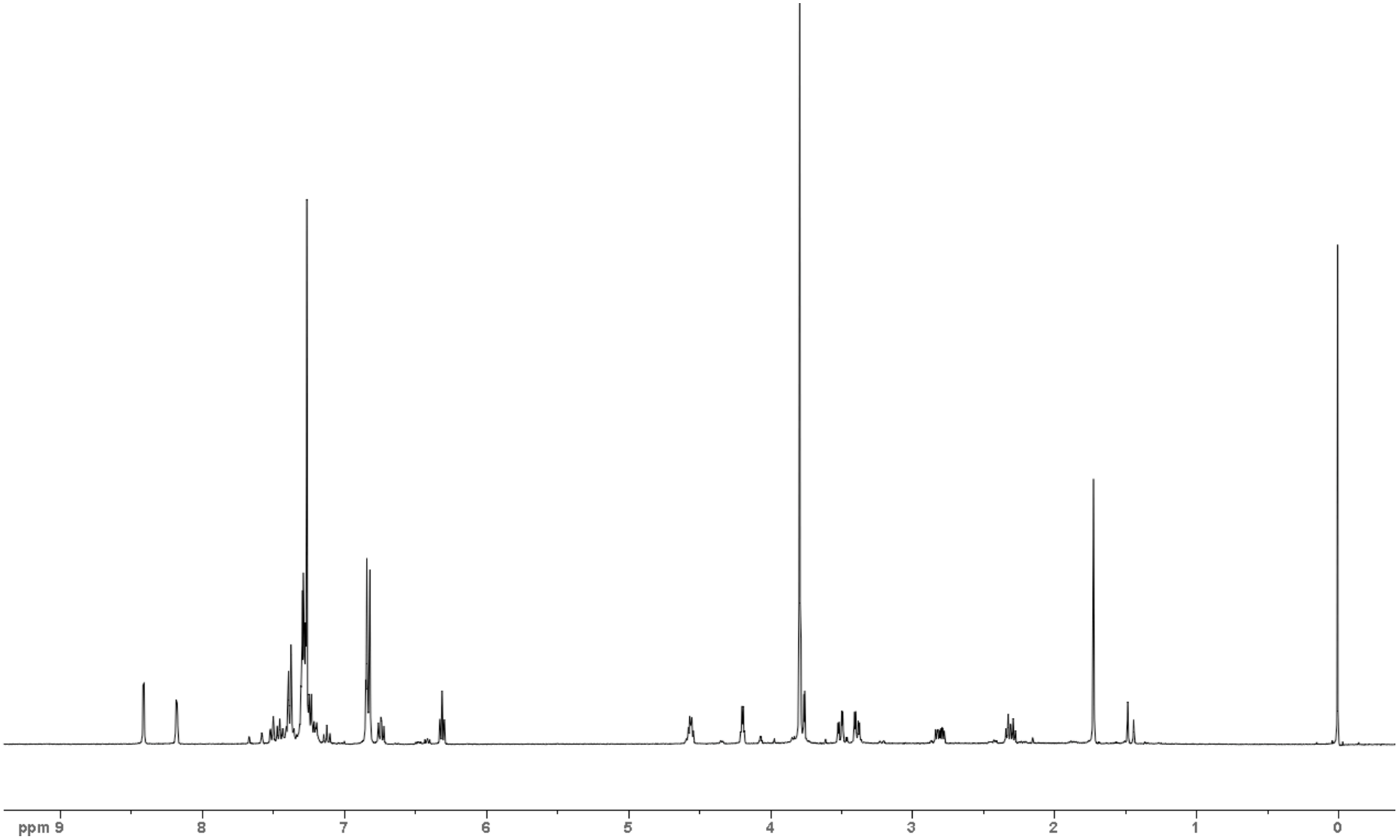


Fig. S15 A ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **14**.

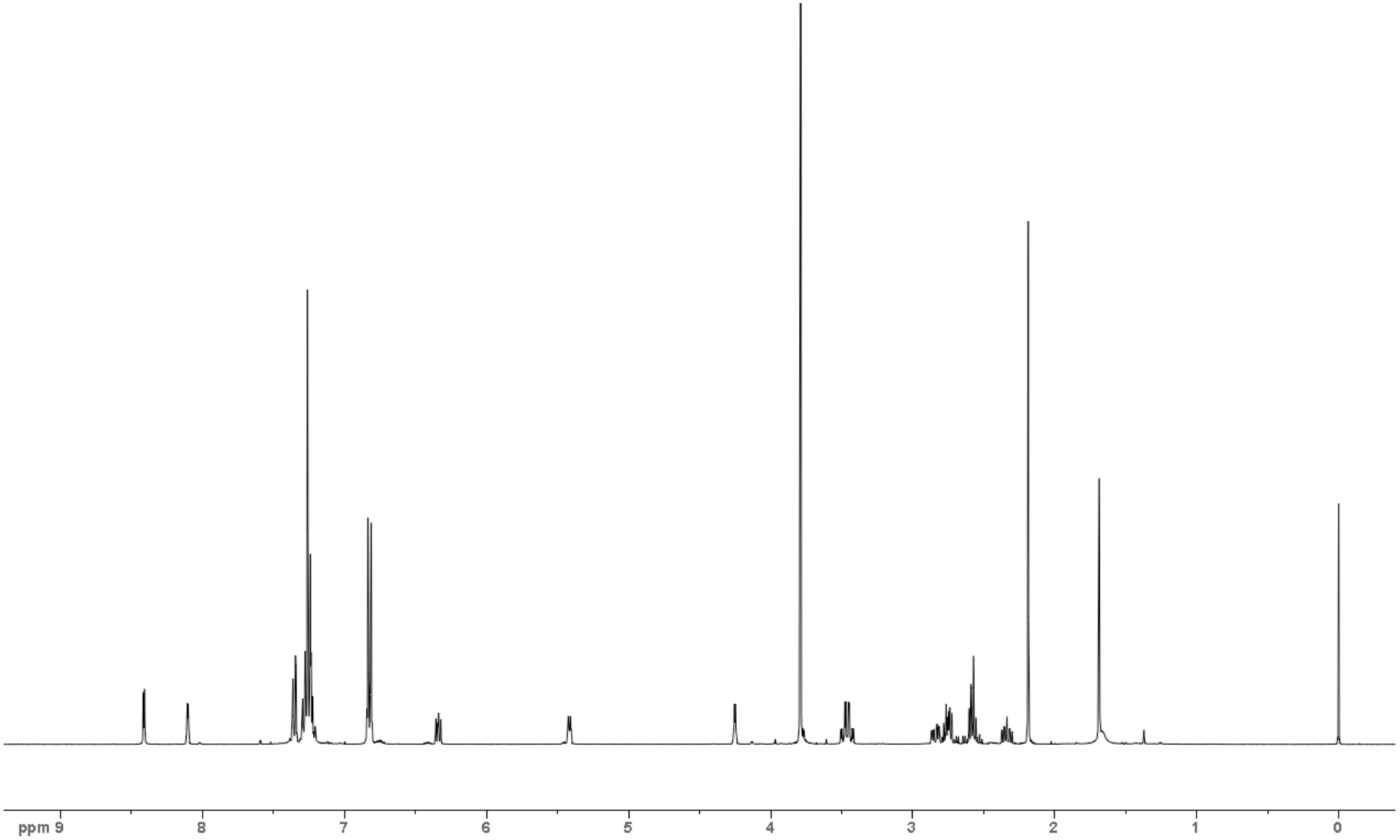


Fig. S16 A ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **15**.

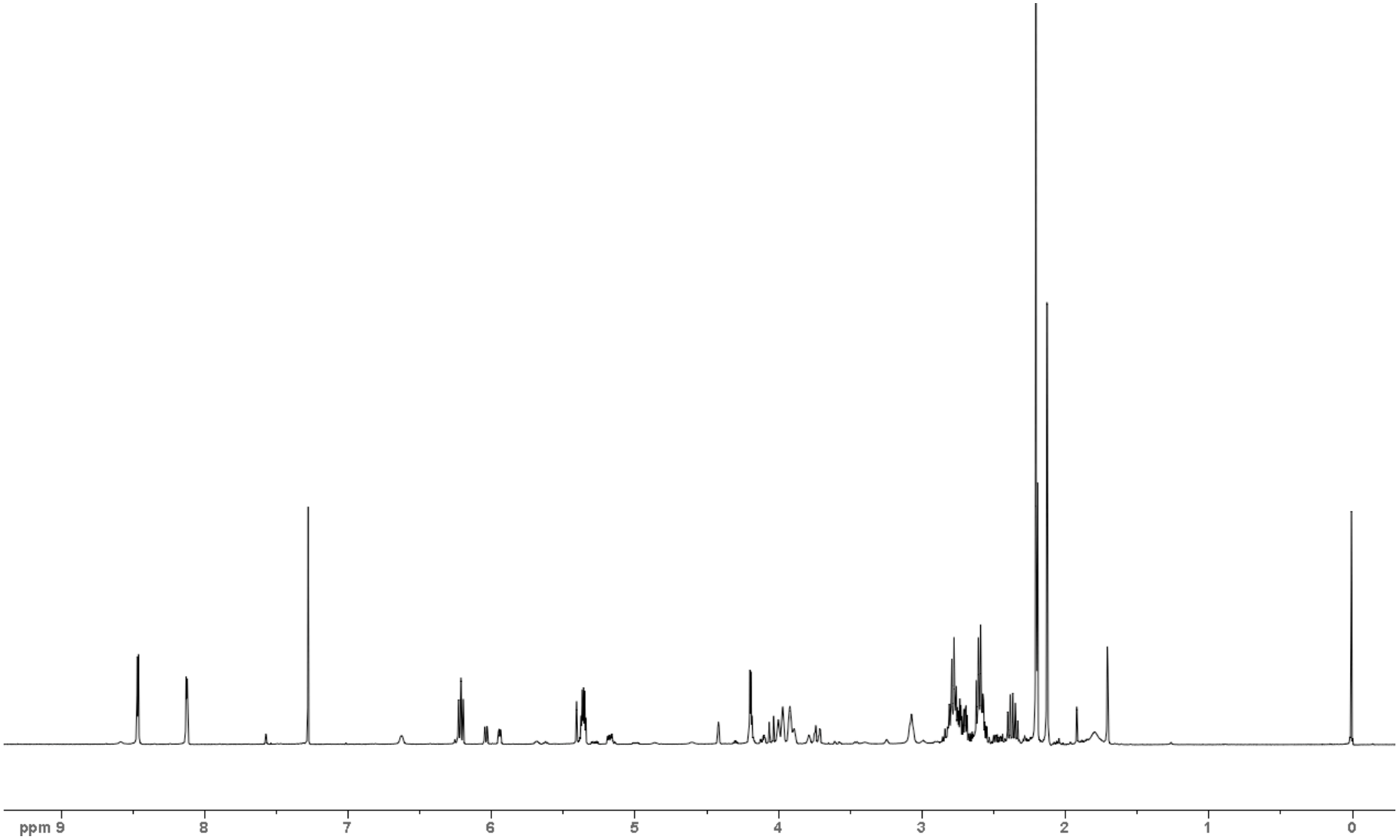


Fig. S17 A ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **16**.