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

Supporting Information

Two short approaches to the COVID-19 drug β-D-N^4-hydroxycytidine and its prodrug molnupiravir

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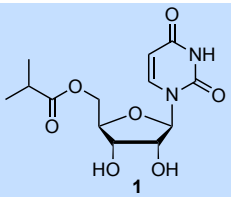
Table of Contents

General experimental considerations

CH_2Cl_2 and MeCN were distilled over CaH_2 before use. THF and 1,4-dioxane were distilled from $LiAlH_4$ and then from Na prior to use. CCl_4 was distilled from P_2O_5 and stored over 5 Å molecular sieves. Commercially obtained petroleum ether was distilled, EtOAc and hexanes were distilled over $CaSO_4$. All other reagents were obtained from commercial sources and no additional purification was performed. The reaction temperatures recorded were of a pre-equilibrated sand, or oil, or ice bath prior to their use. Thin-layer chromatography was performed on 200 μm aluminium-foil-backed silica plates. Column chromatographic purifications were conducted with either 60–120 or 200–300 mesh silica gel, and the specific mesh-size used is indicated in each procedure. ^{1}H NMR spectra were obtained either at 400 or 500 MHz and are referenced to the residual protiated solvent resonance. ^{13}C NMR spectra were obtained at 100 or 125 MHz and are referenced to the solvent resonance. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are in hertz (Hz).

Uridine 5'-isobutyrate ester (1)



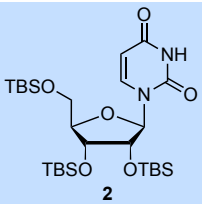
1

On a 0.20 mmol scale. Uridine (49.8 mg, 0.20 mmol, 1.0 eq.) was placed in an oven-dried 25 mL three-neck flask, equipped with a stir bar. One arm was connected to a gas inlet, the centre neck was stoppered, and the remaining arm was connected to a vacuum outlet. All the joints were sealed with Parafilm, and the flask was placed vacuum for 6.5 h at room temperature. A solution of $(iBuCO)_2O$ (0.10 mL, 0.61 mmol, 3.0 eq.) in 1,4-dioxane (3.0 mL, 0.20 M) was prepared under a nitrogen atmosphere (in a glove bag). The three-neck flask was removed from under vacuum and filled with nitrogen gas from a balloon. Under a nitrogen atmosphere, 1,4-dioxane (1.7 mL) was added followed by Novozyme 435 (74.7 mg, 1.50 eq.). The centre neck was stoppered with a septum and sealed with Parafilm. The flask was evacuated under vacuum and refilled with nitrogen gas. To this stirred reaction mixture, the previously prepared solution of $(iBuCO)_2O$ was added dropwise. The mixture was allowed to stir for 15 h at room temperature, filtered, and washed with 1,4-dioxane (15 mL). The filtrate was evaporated under reduced pressure and then applied to a silica gel (200–300 mesh) column packed in 1% MeOH in EtOAc. Elution with 1% MeOH in EtOAc gave 48.5 mg (77% yield) of product **1** as a white solid. R_f (SiO_2/3% MeOH in EtOAc) = 0.24.

^{1}H NMR (500 MHz, CD_3OD): δ 7.69 (d, *J* = 8.1 Hz, 1H), 5.83 (d, *J* = 3.9 Hz, 1H), 5.71 (d, *J* = 8.1 Hz, 1H), 4.37–4.30 (m, 2H), 4.19 (app t, J_{app} ≈ 4.6 Hz, 1H), 4.15 (app q, J_{app} ≈ 4.5 Hz, 1H), 4.10 (app t, J_{app} ≈ 5.6 Hz, 1H), 2.62 (sept, *J* = 7.0 Hz, 1H), 1.18 (d, *J* = 7.0 Hz, 6H). $^{13}C\{^1H\}$ NMR (125 MHz, CD_3OD): δ 178.4, 166.2, 152.3, 142.4, 103.0, 91.9, 83.1, 75.3, 71.3, 64.8, 35.3, 19.5, 19.4. HRMS (ESI/TOF) *m/z* calculated for $C_{13}H_{18}N_2O_7$ $[M]^+$: 314.1109, found 314.1093.

On a 2.06 mmol scale. Uridine (503.6 mg, 2.06 mmol, 1.0 eq.) was placed an oven-dried 100 mL three-neck flask, equipped with a stir bar. One arm was connected to a nitrogen gas inlet, the centre neck was stoppered, and the other arm was connected to a vacuum outlet. All the joints were sealed with Parafilm, and the three-neck flask was maintained under vacuum for 6.5 h at room temperature. A solution of $(iBuCO)_2O$ (1.1 mL, 6.2 mmol, 3.0 eq.) in 1,4-dioxane (30.0 mL, 0.21 M) was prepared under nitrogen atmosphere (in a glove bag). Next, the three-neck flask was removed from under vacuum and refilled with nitrogen gas. Under a nitrogen atmosphere, 1,4-dioxane (18.0 mL) and Novozyme 435 (750.0 mg, 1.5 eq.) were introduced in that sequential order. The centre neck was stoppered with a septum and sealed with Parafilm. The flask was evacuated under vacuum and recharged with nitrogen gas. To this stirred reaction mixture, the previously prepared solution of $(iBuCO)_2O$ was added dropwise. The reaction mixture was stirred for 15 h at room temperature, filtered, and washed with 1,4-dioxane (30 mL). The filtrate was evaporated under reduced pressure and then applied to a silica gel (200–300 mesh) column packed in 1% MeOH in EtOAc. Gradient elution with 1–8% MeOH in EtOAc gave 479.4 mg (74% yield) of product **1** as a white solid.

2',3',5'-Tri-*O*-(*t*-butyldimethylsilyl)uridine (2)[1–3]

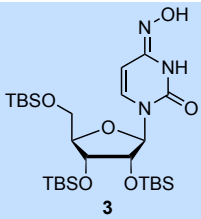


2

In a clean, dry 250 mL round-bottom flask equipped with a stir bar was placed *t*-$BuMe_2SiCl$ (7.46 g, 49.5 mmol, 5.50 eq.) and DMF (30.0 mL). The flask was stoppered, and mixture was allowed to stir at room temperature for 5 min. Imidazole (6.74 g, 99.0 mmol, 11.0 eq.) was added to the mixture, once again the flask was stoppered, and the mixture was allowed to stir at room temperature for 10 min. Uridine (2.2 g, 9.0 mmol, 1.0 eq.) was added followed by DMF (30.0 mL). The reaction flask was stoppered, sealed with Parafilm, and the mixture was allowed to stir at 28 °C for 16 h. The reaction was quenched by addition of deionized H_2O (100 mL), the mixture was transferred to a

separatory funnel, and extracted with EtOAc (150 mL). The aqueous layer was back extract with EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield a thick, colourless liquid. This solidified to 3.84 g (73% yield) of a white solid when left at –22 °C for 7 days. R_f (SiO_2/30% EtOAc in petroleum ether) = 0.70. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.05 (br s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 5.85 (d, *J* = 3.5 Hz, 1H), 5.68 (d, *J* = 8.1 Hz, 1H), 4.09–4.05 (m, 3H), 3.99 (dd, *J* = 11.7, 1.4 Hz, 1H), 3.75 (d, *J* = 11.3 Hz, 1H), 0.94 (s, 9H), 0.90 and 0.88 (2s, 18H), 0.12 and 0.11 (2s, 6H), 0.084, 0.080, 0.07, and 0.06 (4s, 12H). ^{13}C{^{1}H} NMR (125MHz, $CDCl_3$): δ 163.7, 150.5, 140.6, 102.1, 89.2, 84.7, 76.4, 70.9, 62.0, 26.2, 26.04, 25.97, 18.7, 18.3, 18.2, –4.0, –4.3, –4.6, –4.7, –5.2, –5.4.

2',3',5'-Tri-*O*-(*t*-butyldimethylsilyl)-β-D-N^4-hydroxycytidine (3)



3

Using BOP on a 0.09 mmol scale. In an oven-dried 4-dram, screw-cap glass vial equipped with a stir bar, a mixture of tri-*O*-TBS-protected uridine **2** (50.3 mg, 0.09 mmol, 1.0 eq.), BOP (79.4 mg, 0.18 mmol, 2.0 eq.), and Cs_2CO_3 (58.3 mg, 0.18 mmol, 2.0 eq.) was prepared in MeCN (0.68 mL, 0.132 M solution based on the nucleoside). The vial was purged with nitrogen gas, capped, and sealed with Parafilm. The mixture was allowed to stir at room temperature for 30 min, at which time complete formation of the less polar O^4-benzotriazolyl intermediate was observed by TLC. Then, 50% NH_2OH in H_2O (20 μL, 0.36 mmol, 4.0 eq.) was added to the mixture. The vial was purged with nitrogen gas, capped, and sealed with Parafilm. The reaction mixture was allowed to stir at room temperature for 3.5 h, at which time consumption of the intermediate was observed by TLC. The mixture was diluted with EtOAc (10 mL), transferred to a separatory funnel, and extracted. The organic phase was washed with deionized water (3 × 15 mL) followed by brine (15 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was applied to a silica gel (200–300 mesh) column packed in 25% EtOAc in hexanes. Elution with 25% EtOAc in hexanes gave 45.0 mg (83% yield) of compound **3** as a pale yellow, glass-like solid material. R_f (SiO_2/20% EtOAc in hexanes) = 0.22. ^{1}H NMR (500 MHz, CD_2Cl_2): δ 8.28 (br s, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 7.12 (br s, 1H), 5.88 (d, *J* = 5.0, 1H), 5.56 (d, *J* = 8.2 Hz, 1H), 4.10–4.07 (m, 2H), 4.01–4.00 (m, 1H), 3.88 (dd, *J* = 11.5, 2.3 Hz, 1H), 3.73 (dd, *J* = 11.5, 1.7 Hz, 1H), 0.95, 0.92, and 0.88 (3s, 27H), 0.12 (s, 6H), 0.11, 0.10, 0.05, and 0.04 (4s, 12H). ^{13}C{^{1}H} NMR (125 MHz,

CD_2Cl_2): δ 150.1, 145.9, 131.5, 98.5, 87.9, 85.9, 75.8, 72.9, 63.5, 26.4, 26.2, 26.1, 18.9, 18.5, 18.4, –4.1, –4.2, –4.4, –5.2. HRMS (ESI/TOF) *m/z* calculated for $C_{27}H_{55}N_3O_6Si_3K$ $[M + K]^+$: 640.3030, found 640.3033.

Using BOP on a 0.50 mmol scale. In an oven-dried 8-dram screw-cap glass vial equipped with a stir bar, a mixture of tri-*O*-TBS-protected uridine **2** (293.4 mg, 0.50 mmol, 1.0 eq.), BOP (442.2 mg, 1.00 mmol, 2.0 eq.), and Cs_2CO_3 (325.8 mg, 1.00 mmol, 2.0 eq) was prepared in MeCN (3.8 mL, 0.132 M solution based on the nucleoside). The vial was purged with nitrogen gas, capped, and sealed with Parafilm. The mixture was allowed to stir at room temperature for 30 min, at which time complete formation of the less polar O^4-benzotriazolyl intermediate was observed by TLC. Then, 50% NH_2OH in H_2O (120 µL, 2.00 mmol, 4.0 eq.) was added to the mixture. The vial was purged with nitrogen gas, capped, and sealed with Parafilm. The reaction mixture was allowed to stir for 2.5 h at room temperature, at which time consumption of the intermediate was observed by TLC. The mixture was diluted with EtOAc (15 mL), transferred to a separatory funnel, and extracted. The organic phase was washed with deionized water (3 × 20 mL) followed by brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was applied to a silica gel (200–300 mesh) column packed in 15% EtOAc in hexanes. Gradient elution with 15–25% EtOAc in hexanes gave 258.1 mg of a mixture composed of product **3** and a trace of by-product **4**. [On the basis of ^{1}H NMR analysis, the by-product was estimated to be 4.4%. At 500 MHz, in CD_2Cl_2, the ^{1}H chemical shifts of the benzotriazolyl protons in by-product **4** were δ 8.91 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.58 (t, *J* = 7.9 Hz, 1H). These shifts are comparable to those of an analogous product obtained from 3',5'-di-*O*-TBS thymidine.[4]] This mixture was then subjected to the desilylation and purification (*vide infra*) resulting in 99.0 mg (95% yield) of only product NHC as a white solid.

Using the 4-chloropyrimidinone nucleoside on a 0.09 mmol scale. In a clean, dry 10 mL round bottom flask equipped with a stir bar was placed tri-*O*-TBS-protected uridine **2** (50.4 mg, 0.09 mmol, 1.0 eq.). CH_2Cl_2 (0.55 mL, 6.0 mL/mmol), CCl_4 (0.55 mL, 6.0 mL/mmol), and PPh_3 (60.5 mg, 0.23 mmol, 2.5 eq.) were added to the flask. The flask was capped and sealed with Teflon tape. The reaction mixture was stirred and heated in a sand bath that was pre-equilibrated at 67 °C for

3 h, at which time the mixture had turned orange. TLC indicated the formation of a less-polar compound, presumably the C4 chloropyrimidinone nucleoside. The volatiles were evaporated under reduced pressure and MeCN (0.68 mL, 0.132 M solution based on the nucleoside) was added. To the resulting solution 50% NH_2OH in H_2O (20 µL, 0.36 mmol, 4.0 eq.) was added and the flask was purged with nitrogen gas. The flask was capped, sealed with Parafilm, and the mixture was allowed to stir at room temperature for 2.5 h, at which time complete consumption of the intermediate was observed by TLC. The reaction mixture was diluted with EtOAc (10 mL), transferred to a separatory funnel, and extracted. The organic layer was washed with deionized H_2O (3 × 10 mL) and then with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was applied to a silica gel (200–300 mesh) column packed in 10% EtOAc in hexanes. Gradient elution with 10–22% EtOAc in hexanes gave 49.0 mg (90% yield) of **3** as a pale yellow, glass-like solid material. R_f (SiO_2/20% EtOAc in hexanes) = 0.15.

Using the 4-chloropyrimidinone nucleoside on a 0.50 mmol scale. In a clean, dry 25 mL round bottom flask equipped with a stir bar was placed tri-*O*-TBS-protected uridine **2** (293.1 mg, 0.50 mmol, 1.0 eq.). CH_2Cl_2 (3.0 mL, 6.0 mL/mmol), CCl_4 (3.0 mL, 6.0 mL/mmol), and PPh_3 (328.0 mg, 1.25 mmol, 2.5 eq.) were added to the flask. A reflux condenser was attached to the flask, and the reaction mixture was stirred and heated in a sand bath that was pre-equilibrated at 65 °C for 2 h, at which time the mixture had turned orange. TLC indicated the formation of a less-polar compound, presumably the C4 chloropyrimidinone nucleoside. The volatiles were evaporated under reduced pressure and MeCN (3.80 mL, 0.132 M solution based on the nucleoside) was added. To the resulting solution 50% NH_2OH in H_2O (120 µL, 2.00 mmol, 4.0 eq.) was added and the flask was purged with nitrogen gas. The flask was capped, sealed with Parafilm, and the mixture was allowed to stir at room temperature for 1 h, at which time complete consumption of the intermediate was observed by TLC. The reaction mixture was diluted with EtOAc (15 mL), transferred to a separatory funnel, and extracted. The organic layer was washed with deionized H_2O (3 × 25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was applied to a silica gel (200–300 mesh) column packed

in 10% EtOAc in hexanes. Gradient elution with 10–35% EtOAc in hexanes gave 198.0 mg (66% yield) of compound **3** as a pale yellow, glass-like solid.

β-D-*N*^{4}-Hydroxycytidine (NHC)

NHC

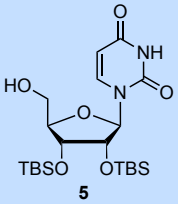
In a clean 6 mL HDPE screwcap vial equipped with a stir bar 2',3',5'-tri-*O*-TBS-protected *N*-hydroxycytidine **3** (87.4 mg, 0.15 mmol, 1.0 eq.) was dissolved in THF (1.5 mL, 0.10 M solution based on the nucleoside). $Et_3N{\bullet}3HF$ (0.18 mL, 1.13 mmol, 7.5 eq.) was added and the mixture was stirred at room temperature for 26 h. The volatiles were evaporated under a gentle stream of nitrogen gas. CH_2Cl_2 was added to the vial and a white precipitate formed. The mixture was sonicated, centrifuged, and the supernatant was removed (after allowing the mixture to rest for a few minutes). The procedure of adding CH_2Cl_2, sonication, and centrifugation was performed six times using 4 mL of CH_2Cl_2 each time. The residual CH_2Cl_2 was evaporated under a gentle stream of nitrogen gas. Final drying of the product under vacuum gave 34.9 mg (90% yield) of NHC as a white solid. R_f (SiO_2/100% EtOAc) = 0.05 (material tails on TLC). ^{1}H NMR (500 MHz, CD_3OD): δ 7.16 (d, J = 8.3 Hz, 1H), 5.86 (d, J = 5.9 Hz, 1H), 5.59 (d, J = 8.2 Hz, 1H), 4.15 (app t, $J_{app} \approx$ 5.5 Hz, 1H), 4.12–4.10 (m, 1H), 3.94 (app q, $J_{app} \approx$ 3.3 Hz, 1H), 3.78 (dd, J = 12.1, 2.8 Hz, 1H), 3.69 (dd, J = 12.1, 3.4 Hz, 1H). ^{13}C{^{1}H} NMR (125 MHz, CD_3OD): δ 151.9, 146.5, 132.4, 99.4, 89.9, 86.2, 74.8, 71.9, 63.0. HRMS (ESI/TOF) *m/z* calculated for $C_9H_{12}N_3O_6$ $[M - H]^-$: 258.0732, found 258.0696.

Recrystallization of β-D-*N*^{4}-hydroxycytidine

MeCN (3 mL) was added β-D-*N*^{4}-Hydroxycytidine (18 mg) in an 8-dram clear glass vial**.** The mixture was heated with swirling to 75 °C. MeOH was added dropwise (1 mL was required) at which point the white solid had dissolved. The solution was left at room temperature and after 2.5 days, tiny particulates were observed. The crystallizing solution was then left at 3 °C for 1 day and further cooled at –13 °C for 2 days, which resulted in a single large crystal (14.6 mg). The remaining mother liquor was removed and evaporated under a gentle stream of nitrogen gas and further evaporated under vacuum.

2',3'-Di-*O*-(*t*-butyldimethylsilyl)uridine (5)

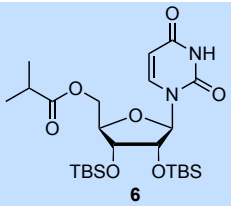
In a 250 mL dry round bottom flask equipped with a magnetic stir bar was placed 2',3',5'-tri-*O*-TBS-protected uridine **2** (5.047 g, 8.59 mmol, 1.0 eq.), and THF (102.0 mL) was added. The stirred



5

solution was cooled to 0 °C in an ice bath and 50% aqueous TFA (51.0 mL) was added dropwise to the reaction mixture. The flask was then capped, sealed with Parafilm, and the mixture was allowed to stir at 0 °C for 6 h. The reaction was neutralized with saturated aqueous $NaHCO_3$ (100 mL), transferred to a separatory funnel, and extracted, with EtOAc (100 mL). The aqueous layer was back extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude material was applied to a silica gel (60–120 mesh) column packed in petroleum ether. Gradient elution with 0–40% EtOAc in petroleum ether gave 2.7 g (66% yield) of compound **5** as a white solid. R_f (SiO_2/50% EtOAc in petroleum ether) = 0.50. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.02 (br s, 1H), 7.63 (d, J = 8.1 Hz, 1H), 5.73 (d, J = 8.1 Hz, 1H), 5.47 (d, J = 5.3 Hz, 1H), 4.54 (app t, $J_{app} \approx$ 4.9 Hz, 1H), 4.16 (app t, $J_{app} \approx$ 4.0 Hz, 1H), 4.09–4.08 (m, 1H), 3.94 (app dt, $J_{app} \approx$ 12.2, 2.2 Hz, 1H), 3.72 (ddd, J = 12.2, 7.4, 1.8 Hz, 1H), 3.11 (dd, J = 7.3, 2.8 Hz, 1H), 0.90 and 0.87 (2s, 18H), 0.09, 0.08, 0.06, and 0.03 (4s, 12H). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ 163.3, 150.5, 143.2, 102.4, 94.1, 86.3, 73.9, 71.9, 61.9, 26.0, 18.3, 18.2, –4.2, –4.4, –4.56, –4.63. HRMS (ESI/TOF) *m/z* calculated for $C_{21}H_{41}N_2O_6Si_2$ $[M + H]^+$: 473.2498, found 473.2477.

2',3'-Di-*O*-(*t*-butyldimethylsilyl)-5'-*O*-isobutyryluridine (6)

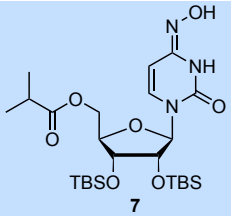


6

In a 150 mL oven-dried, round bottom flask equipped with a magnetic stir bar was placed 2',3'-di-*O*-TBS-protected uridine **5** (2.36 g, 5.00 mmol, 1.0 eq.) and DMAP (152.7 mg, 1.25 mmol, 0.25 eq.). MeCN (30.0 mL), $(iBuCO)_2O$ (915.9 μL, 5.50 mmol, 1.1 eq.), and Et_3N (766.6 μL, 5.50 mmol, 1.1 eq.) were added in that sequence. The flask was capped, sealed with Parafilm, and the mixture was allowed to stir at 28 °C for 1 h. The reaction was neutralized with saturated aqueous $NaHCO_3$ (50 mL), transferred to a separatory funnel, and extracted with EtOAc (75 mL). The aqueous layer was back extracted with EtOAc (3 × 75 mL). The combined organic layer was washed with brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to obtain 2.25 g (83% yield) of compound **6** as a white solid. R_f (SiO_2/40% EtOAc in petroleum ether) = 0.34. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.19 (br s, 1H), 7.75 (d, J = 8.2 Hz, 1H), 5.71 (d, J = 8.1 Hz, 1H), 5.67 (d, J = 1.9 Hz, 1H), 4.36 (dd, J = 12.7, 2.8 Hz, 1H), 4.31–4.27 (m, 2H), 4.17

(dd, *J* = 4.0, 2.4 Hz, 1H), 3.97 (dd, *J* = 7.1, 4.1 Hz, 1H), 2.57 (septet, *J* = 7.0 Hz, 1H), 1.22 (d, *J* = 6.1 Hz, 3H), 1.20 (d, *J* = 6.7 Hz, 3H), 0.91 and 0.88 (2s, 18H), 0.16, 0.10, 0.06, and 0.03 (4s, 12H). $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$): δ 176.6, 163.6, 150.3, 139.9, 102.0, 90.9, 81.1, 75.8, 70.6, 62.5, 34.3, 26.01, 26.00, 19.22, 19.19, 18.25, 18.24, –4.0, –4.2, –4.7, –4.9. HRMS (ESI/TOF) *m/z* calculated for $C_{25}H_{47}N_2O_7Si_2$ $[M + H]^+$: 543.2916, found 543.2918.

2',3'-Di-*O*-(*t*-butyldimethylsilyl) molnupiravir (7)



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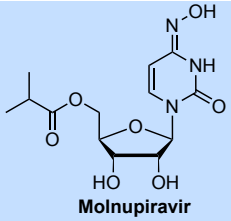
Using BOP. In a clean, dry 100 mL round bottom flask equipped with a magnetic stir bar was placed the 2',3'-di-*O*-silyl-5'-isobutyryl ester of uridine **6** (1.09 g, 2.00 mmol, 1.0 eq.) in MeCN (15.0 mL). Cs_2CO_3 (608.9 mg, 4.00 mmol, 2.0 eq.) and BOP (1.769 g, 4.00 mmol, 2.0 eq.) were added. The reaction flask was stoppered, sealed with Parafilm, and the mixture was allowed to stir at 28 °C for 30 min, at which time complete formation of the less polar O^4-benzotriazolyl intermediate was observed by TLC. Then, 50% NH_2OH in H_2O (332.8 μL, 8.00 mmol, 4.0 eq.) was added to the reaction mixture, the flask was stoppered, and sealed with Parafilm. The mixture was allowed to stir for an additional 1 h at 28 °C, at which time consumption of the intermediate was observed by TLC. Deionized H_2O (50 mL) was added to the mixture, the mixture was transferred to a separatory, and extracted with EtOAc (50 mL). The aqueous layer was back extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was applied to a silica gel (60–120 mesh) column packed in petroleum ether. Gradient elution with 0–35% EtOAc in petroleum ether gave 747.5 mg (67% yield) of product **7** as a white solid. R_f (SiO_2/40% EtOAc in petroleum ether) = 0.30. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.88–8.16 (2 br s, 2H, *note:* 1s is observable at 8.66 ppm, whereas the other s is broadened into the baseline), 6.97 (d, *J* = 8.3 Hz, 1H), 5.72 (d, *J* = 3.7 Hz, 1H), 5.62 (d, *J* = 8.3 Hz, 1H), 4.30 (dd, *J* = 12.5, 3.2 Hz, 1H), 4.25 (dd, *J* = 12.5, 3.5 Hz, 1H), 4.21–4.19 (m, 1H), 4.11 (app t, $J_{app} \approx 4.0$ Hz, 1H), 3.99 (app t, $J_{app} \approx 4.9$ Hz, 1H), 2.58 (septet, *J* = 7.0 Hz, 1H), 1.21 (d, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 7.0 Hz, 3H), 0.90 (s, 18H), 0.11 (s, 3H), 0.07 (s, 6H), 0.05 (s, 3H). $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$): δ 176.7, 149.5, 145.5, 130.6, 98.2, 89.8, 81.3, 75.3, 71.4, 63.1, 34.4, 26.00, 25.97, 19.21, 19.18, 18.25, 18.21, –4.1, –4.3, –4.6, –4.8. HRMS (ESI/TOF) *m/z* calculated for $C_{25}H_{48}N_3O_7Si_2$ $[M + H]^+$: 558.3025, found 558.3020.

Using the 4-chloropyrimidinone nucleoside. In a clean, dry 50 mL round bottom flask equipped with a magnetic stir bar was placed the 2',3'-di-*O*-TBS-5'-isobutyryl ester of uridine **6** (271.4 mg, 0.50 mmol, 1.0 eq.). CH_2Cl_2 (3.0 mL, 6.0 mL/mmol), CCl_4 (3.0 mL, 6.0 mL/mmol), and PPh_3 (328.0 mg, 1.25 mmol, 2.5 eq.), were added to the flask. A reflux condenser was attached to the flask, and the reaction mixture was stirred and heated in an oil bath that was pre-equilibrated at 65 °C for 2 h, at which time the mixture had turned orange. TLC indicated the formation of a less-polar compound, presumably the C4 chloropyrimidinone nucleoside. The resulting orange reaction mixture was evaporated under reduced pressure and MeCN (3.80 mL) was added. To the resulting solution was added 50% NH_2OH in H_2O (120 µL, 2.00 mmol, 4.0 eq.). The reaction flask was capped, sealed with Parafilm, and allowed to stir at room temperature for 1 h. Deionized H_2O (20 mL) was added to the mixture, the mixture was transferred to a separatory funnel, and extracted with EtOAc (50 mL). The aqueous layer was back extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was applied to a silica gel (60–120 mesh) column packed in petroleum ether. Gradient elution with 0–35% EtOAc in petroleum ether afforded 178.5 mg (64% yield) of compound **7** as a white solid.

Molnupiravir



Molnupiravir

Using TBAF. In an oven-dried Schleck tube equipped with a stir bar the 2',3'-di-*O*-TBS-5'-isobutyryl ester of molnupiravir **7** (111.5 mg, 0.20 mmol, 1.0 eq.) was dissolved in THF (2.0 mL). *n*-Bu_4NF in THF (0.51 mL of a 1 M solution, 0.50 mmol, 2.5 eq.) was added. The tube was stoppered, sealed with Parafilm, and the mixture was allowed to stir at room temperature for 40 h. The volatiles were removed under reduced pressure. The crude material was applied to a silica gel (60–120 mesh) column packed in CH_2Cl_2. Gradient elution with 0–6% MeOH in CH_2Cl_2 gave 27.6 mg (42% yield) of molnupiravir as a white solid material. R_f (SiO_2/10% MeOH in DCM) = 0.29. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.01 (br s, 1H), 9.53 (br s, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 5.71 (d, *J* = 5.5 Hz, 1H), 5.58 (d, *J* = 8.2 Hz, 1H), 5.37 (br s, 1H), 5.23 (br s, 1H), 4.21 (dd, *J* = 11.9, 2.9 Hz, 1H), 4.13 (dd, *J* = 12.0, 5.0 Hz, 1H), 4.01–3.99 (m, 1H), 3.94–3.89 (m, 2H), 2.57 (septet, *J* = 7.0 Hz, 1H), 1.10 (d, *J* = 6.9 Hz, 6H). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): δ 175.9, 149.3, 143.2, 129.8, 98.7, 87.8, 80.7, 71.9, 69.9, 63.8, 33.1,

18.74, 18.71. HRMS (ESI/TOF) *m/z* calculated for $C_{13}H_{19}N_3O_7Na$ $[M + Na]^+$: 352.1115, found 352.1084.

Using $Et_3N•3HF$. In a clean 10 mL HDPE screwcap vial equipped with a stir bar the 2',3'-di-*O*-TBS-5'-isobutyryl ester of molnupiravir **7** (278.9 mg, 0.50 mmol, 1.0 eq.) was dissolved in THF (5.0 mL, 0.10 M solution based on the nucleoside). $Et_3N•3HF$ (0.56 mL, 3.75 mmol, 7.5 eq.) was added dropwise. The vial was capped, sealed with Parafilm, and the reaction mixture was allowed to stir at room temperature for 30 h. The volatiles were evaporated under a gentle stream of nitrogen gas and further evaporated under vacuum. The resulting crude material was applied to a silica gel (60–120 mesh) column packed in CH_2Cl_2. Sequential elution with 0 to 6% MeOH in CH_2Cl_2 gave 102.0 mg (62% yield) of molnupiravir as a white solid.

X-ray structure determination

X-ray diffraction data were collected on a Bruker D8 VENTURE diffractometer using Cu Kα radiation. Crystal data, data collection and refinement parameters are summarized in Table S1.

The structure was solved using a dual-space method and standard difference map techniques, and was refined by full-matrix least-squares procedures on F^2 with SHELXTL (Version 2018/3).[5,6] All hydrogen atoms bound to carbon were placed in calculated positions and refined with a riding model, while hydrogen atoms bound to nitrogen and oxygen were located on the difference map and freely refined [U_{iso}(H) = 1.2U_{eq}(C, N, or O)]. CCDC reference number 2307658 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

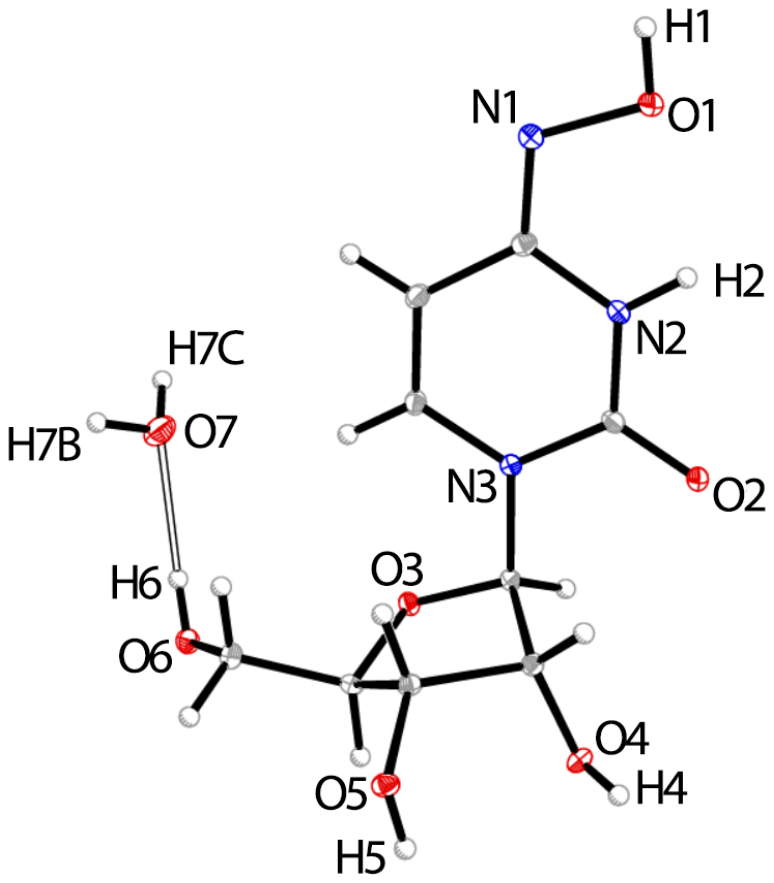


Fig. S1 Molecular structure of NHC•OH_2 displaying the hydrogen bond from NHC to water (atomic displacement parameters are displayed at the 30% probability level).

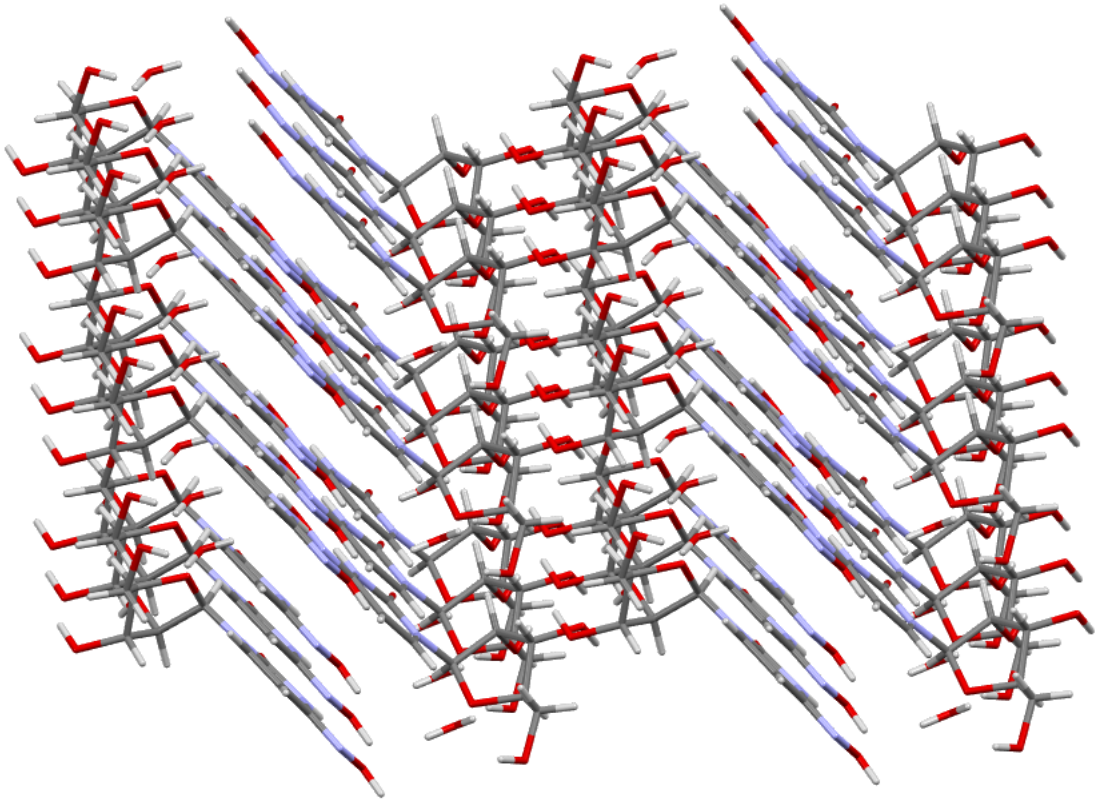


Fig. S2 Packing diagram of NHC•OH_2.

Table S1 Crystal, intensity collection, and refinement data

	Compound 5 (NHC)•OH_2
lattice	Monoclinic
formula	$C_9H_{15}N_3O_7$
formula weight	277.24
space group	$P2_1$
a/Å	5.13410(10)
b/Å	8.4181(2)
c/Å	13.3376(3)
α/°	90
β/°	93.2350(10)
γ/°	90
V/Å^3	575.52(2)
Z	2
temperature (K)	130(2)
radiation (λ, Å)	1.54178
ρ (calcd.) g cm^{-3}	1.600
μ (Cu Kα), mm^{-1}	1.204
θ max, deg.	74.378
no. of data collected	10465
no. of data	2288
no. of parameters	194
R_1 [$I > 2\sigma(I)$]	0.0281
wR_2 [$I > 2\sigma(I)$]	0.0745
R_1 [all data]	0.0288
wR_2 [all data]	0.0747
GOF	1.051
R_{int}	0.0278

Table S2 Bond lengths [Å] and angles [º] for compound **5** (NHC)•OH_2

O(1)-N(1)	1.427(2)
O(1)-H(1)	0.90(3)
O(2)-C(4)	1.234(3)
O(3)-C(5)	1.413(2)
O(3)-C(8)	1.448(2)
O(4)-C(6)	1.423(2)
O(4)-H(4)	0.79(3)
O(5)-C(7)	1.412(2)
O(5)-H(5)	0.89(3)
O(6)-C(9)	1.430(2)
O(6)-H(6)	0.81(3)
N(1)-C(1)	1.295(3)
N(2)-C(4)	1.365(3)
N(2)-C(1)	1.389(3)
N(2)-H(2)	0.90(3)
N(3)-C(4)	1.366(3)
N(3)-C(3)	1.392(2)
N(3)-C(5)	1.486(2)
C(1)-C(2)	1.439(3)
C(2)-C(3)	1.338(3)
C(2)-H(2A)	0.9500
C(3)-H(3A)	0.9500
C(5)-C(6)	1.538(2)
C(5)-H(5A)	1.0000
C(6)-C(7)	1.532(2)
C(6)-H(6A)	1.0000
C(7)-C(8)	1.527(2)
C(7)-H(7A)	1.0000
C(8)-C(9)	1.511(3)
C(8)-H(8A)	1.0000
C(9)-H(9A)	0.9900
C(9)-H(9B)	0.9900
O(7)-H(7B)	0.88(4)
O(7)-H(7C)	0.85(4)

N(1)-O(1)-H(1)	101(2)
C(5)-O(3)-C(8)	110.03(14)
C(6)-O(4)-H(4)	109(2)
C(7)-O(5)-H(5)	109.7(18)
C(9)-O(6)-H(6)	107(2)
C(1)-N(1)-O(1)	109.74(16)
C(4)-N(2)-C(1)	124.37(17)
C(4)-N(2)-H(2)	120.6(19)
C(1)-N(2)-H(2)	114.9(19)
C(4)-N(3)-C(3)	121.16(17)
C(4)-N(3)-C(5)	115.51(16)
C(3)-N(3)-C(5)	123.32(16)
N(1)-C(1)-N(2)	122.50(18)
N(1)-C(1)-C(2)	121.38(18)
N(2)-C(1)-C(2)	116.12(18)
C(3)-C(2)-C(1)	119.50(19)
C(3)-C(2)-H(2A)	120.3
C(1)-C(2)-H(2A)	120.3
C(2)-C(3)-N(3)	121.51(19)
C(2)-C(3)-H(3A)	119.2
N(3)-C(3)-H(3A)	119.2
O(2)-C(4)-N(2)	122.33(19)
O(2)-C(4)-N(3)	120.46(18)
N(2)-C(4)-N(3)	117.22(18)
O(3)-C(5)-N(3)	109.38(15)
O(3)-C(5)-C(6)	107.21(15)
N(3)-C(5)-C(6)	111.38(14)
O(3)-C(5)-H(5A)	109.6
N(3)-C(5)-H(5A)	109.6
C(6)-C(5)-H(5A)	109.6
O(4)-C(6)-C(7)	112.20(15)
O(4)-C(6)-C(5)	107.66(14)
C(7)-C(6)-C(5)	99.52(14)
O(4)-C(6)-H(6A)	112.2
C(7)-C(6)-H(6A)	112.2

C(5)-C(6)-H(6A)	112.2
O(5)-C(7)-C(8)	114.80(15)
O(5)-C(7)-C(6)	117.21(15)
C(8)-C(7)-C(6)	101.93(14)
O(5)-C(7)-H(7A)	107.4
C(8)-C(7)-H(7A)	107.4
C(6)-C(7)-H(7A)	107.4
O(3)-C(8)-C(9)	111.13(15)
O(3)-C(8)-C(7)	103.86(14)
C(9)-C(8)-C(7)	111.41(15)
O(3)-C(8)-H(8A)	110.1
C(9)-C(8)-H(8A)	110.1
C(7)-C(8)-H(8A)	110.1
O(6)-C(9)-C(8)	112.81(15)
O(6)-C(9)-H(9A)	109.0
C(8)-C(9)-H(9A)	109.0
O(6)-C(9)-H(9B)	109.0
C(8)-C(9)-H(9B)	109.0
H(9A)-C(9)-H(9B)	107.8
H(7B)-O(7)-H(7C)	105(3)

Symmetry transformations used to generate equivalent atoms:

Table S3 Hydrogen bonds for compound **5** (NHC)•OH_2 [Å and º]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(2)#1	0.90(3)	1.72(3)	2.620(2)	171(3)
N(2)-H(2)...N(1)#2	0.90(3)	2.07(3)	2.900(2)	152(3)
O(4)-H(4)...O(5)#3	0.79(3)	2.01(3)	2.787(2)	165(3)
O(5)-H(5)...O(6)#4	0.89(3)	1.82(3)	2.684(2)	165(3)
O(6)-H(6)...O(7)	0.81(3)	1.98(3)	2.731(2)	153(3)
O(7)-H(7B)...O(4)#5	0.88(4)	2.10(4)	2.958(2)	164(3)
O(7)-H(7C)...O(1)#6	0.85(4)	2.00(4)	2.852(2)	175(3)

Symmetry transformations used to generate equivalent atoms:

#1 -x+2,y+1/2,-z+1 #2 -x+2,y-1/2,-z+1 #3 -x+1,y-1/2,-z+2

#4 -x,y-1/2,-z+2 #5 x,y+1,z #6 -x+1,y+1/2,-z+1

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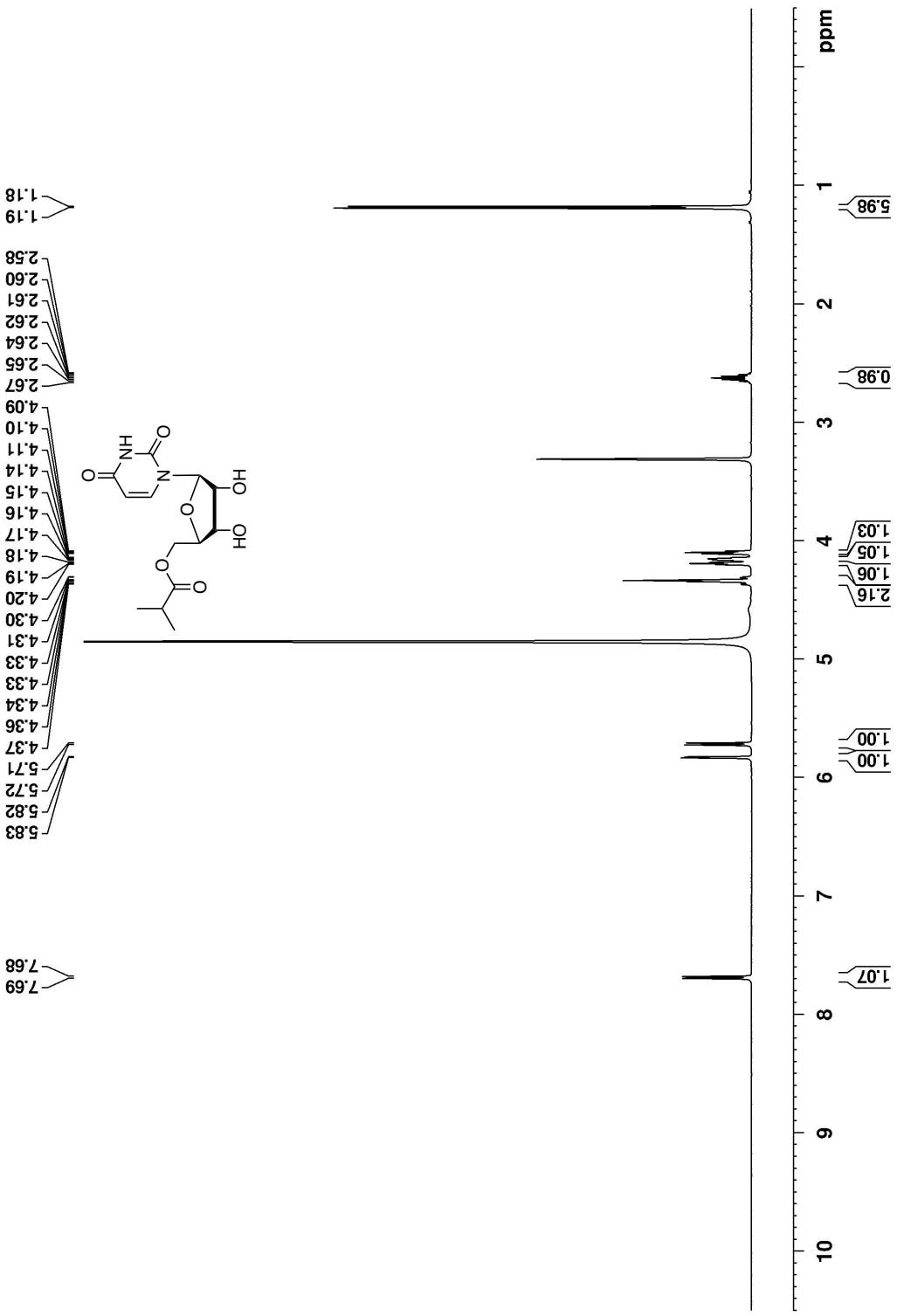
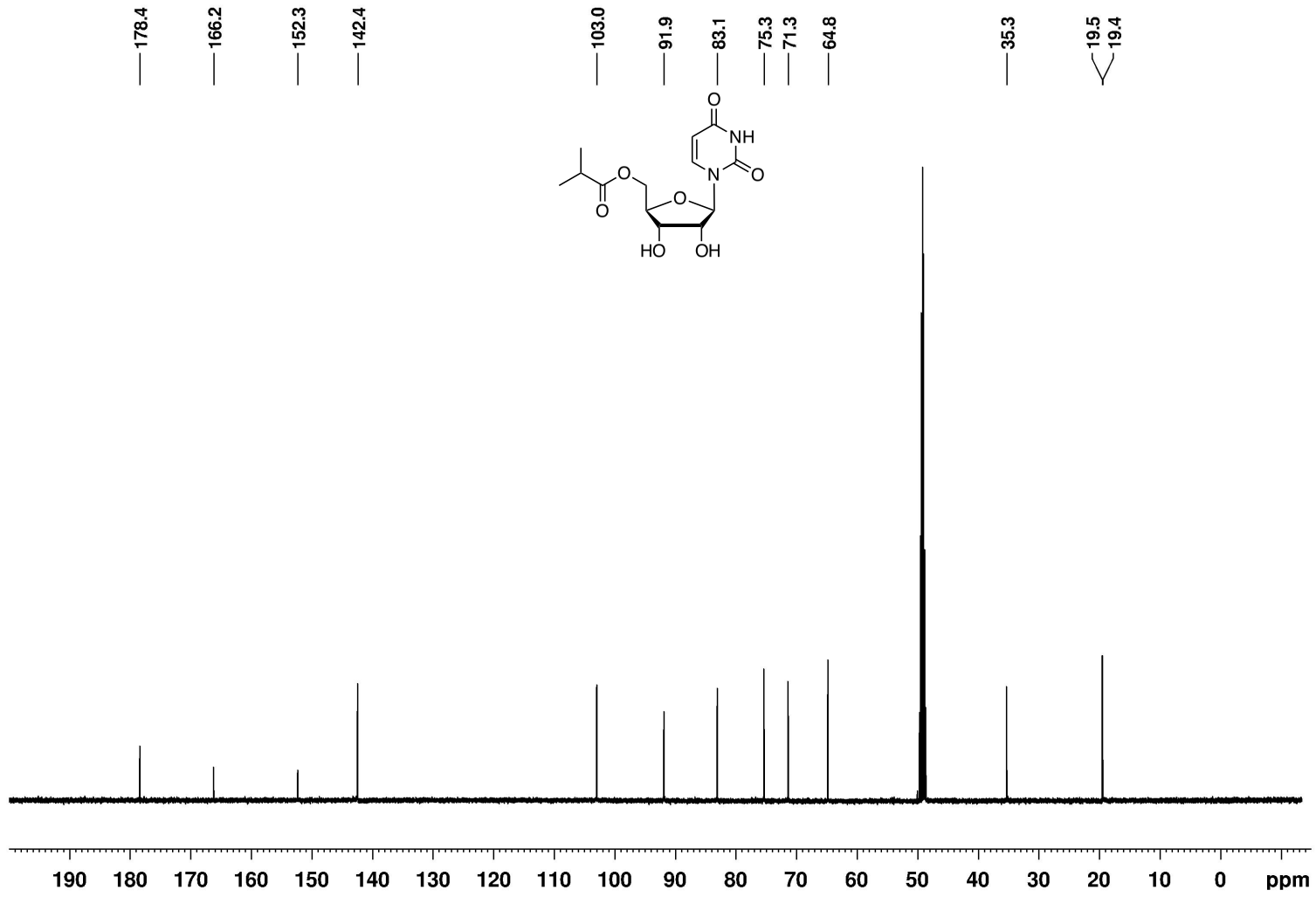
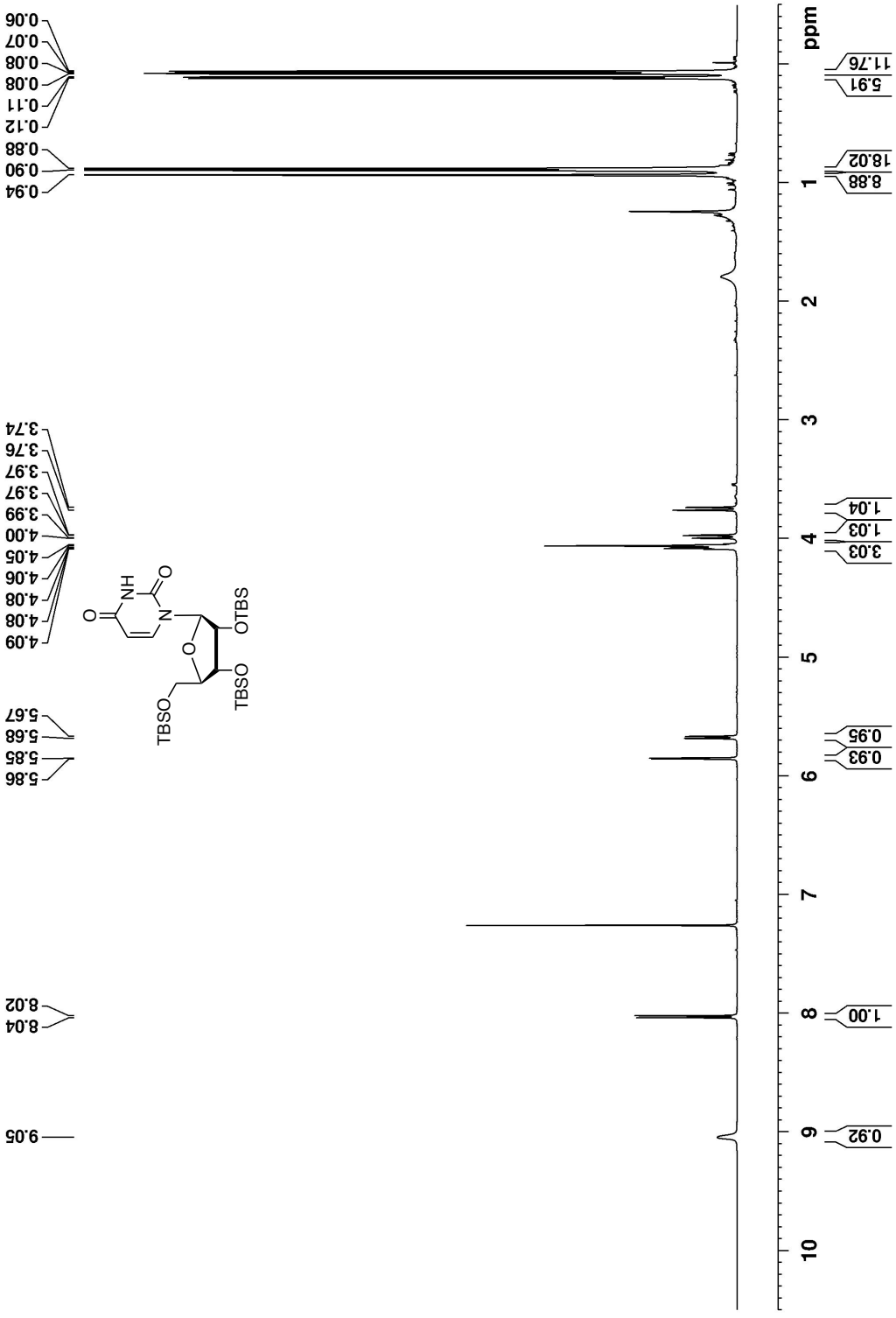
500 MHz ^{1}H NMR spectrum of compound **1** in CD_3OD



125 MHz $^{13}C\{^{1}H\}$ NMR spectrum of compound **1** in CD_3OD



500 MHz ^{1}H NMR spectrum of compound **2** in $CDCl_3$



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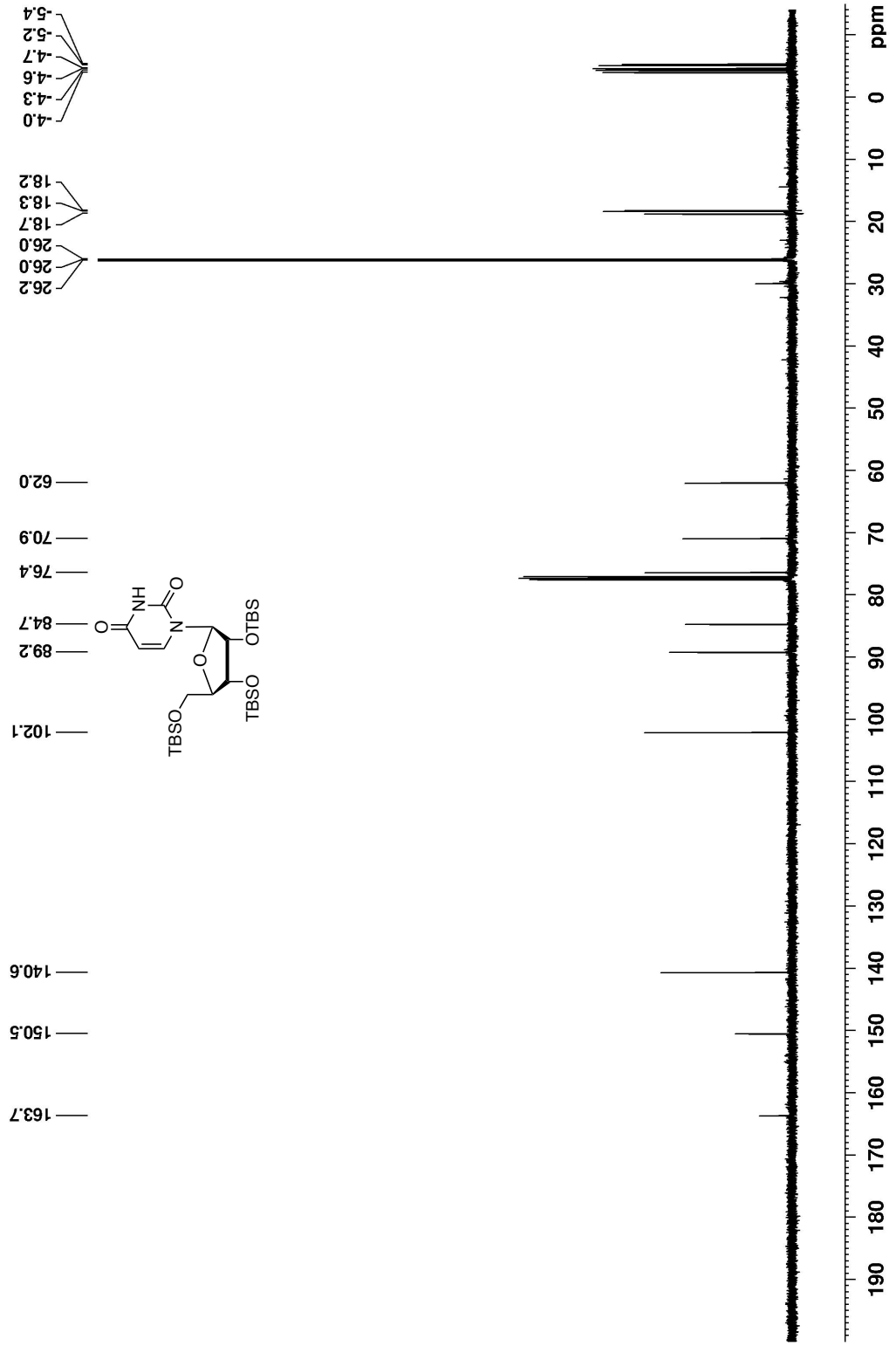
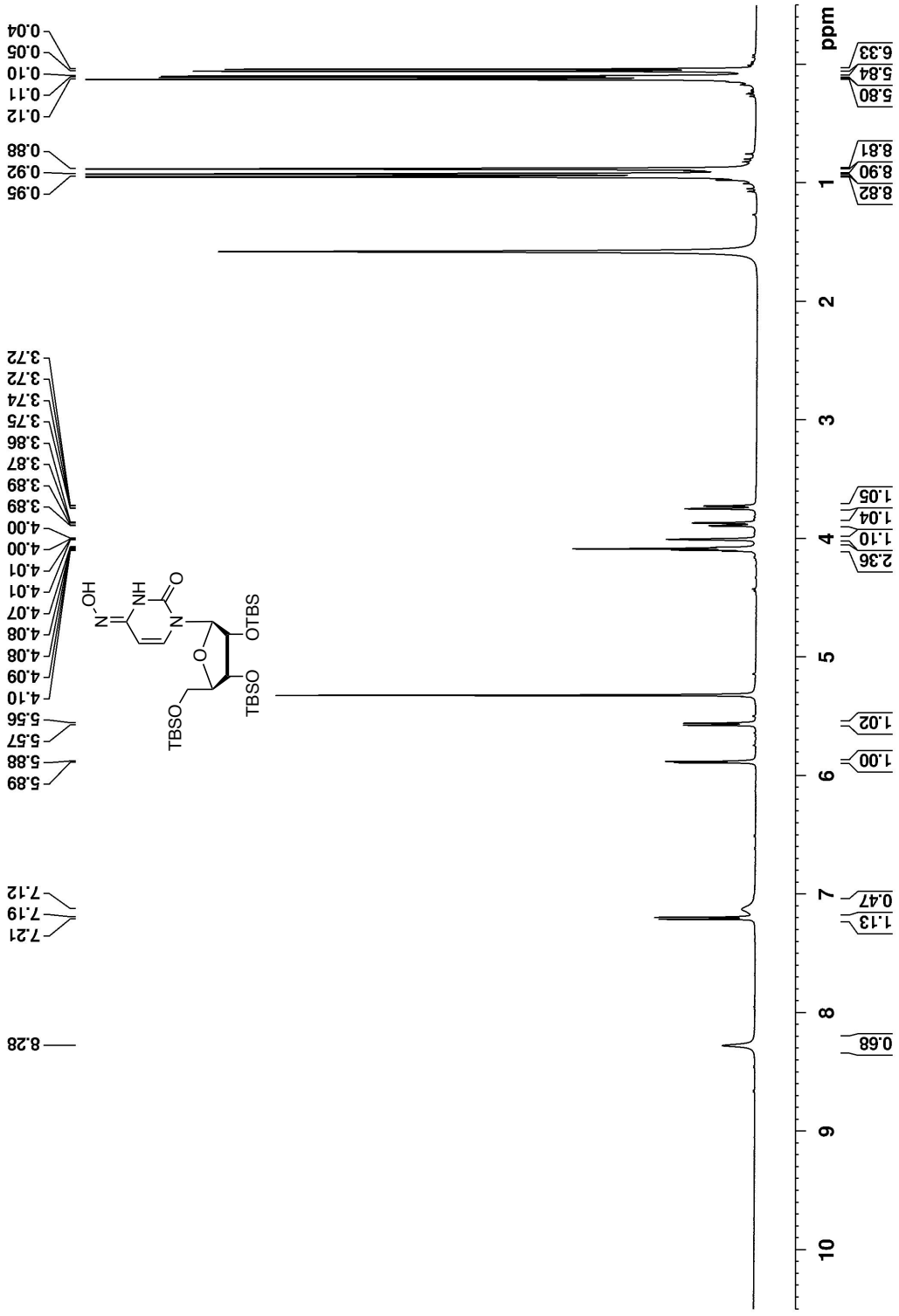


500 MHz ^{1}H NMR spectrum of compound **3** in CD_2Cl_2



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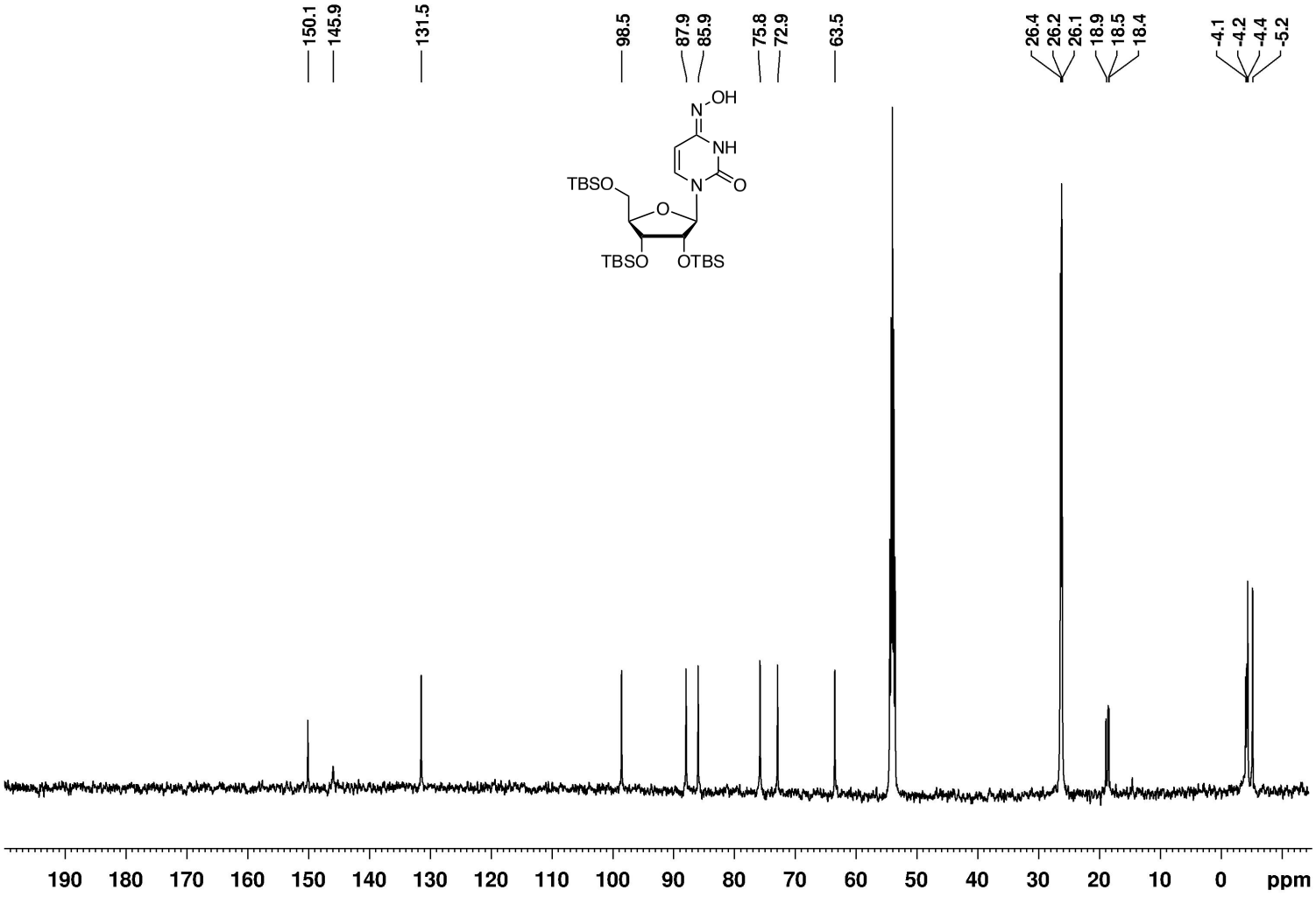
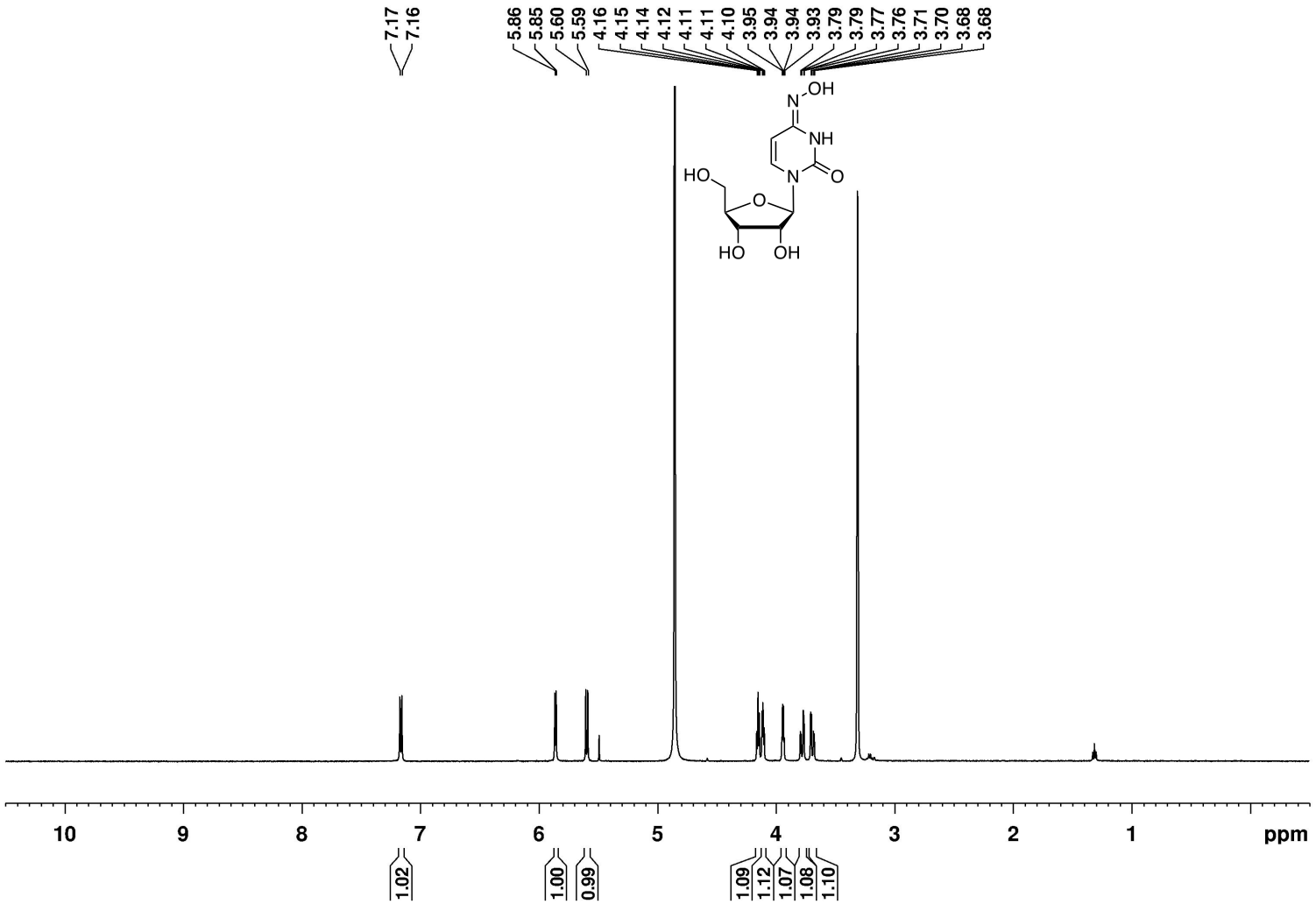
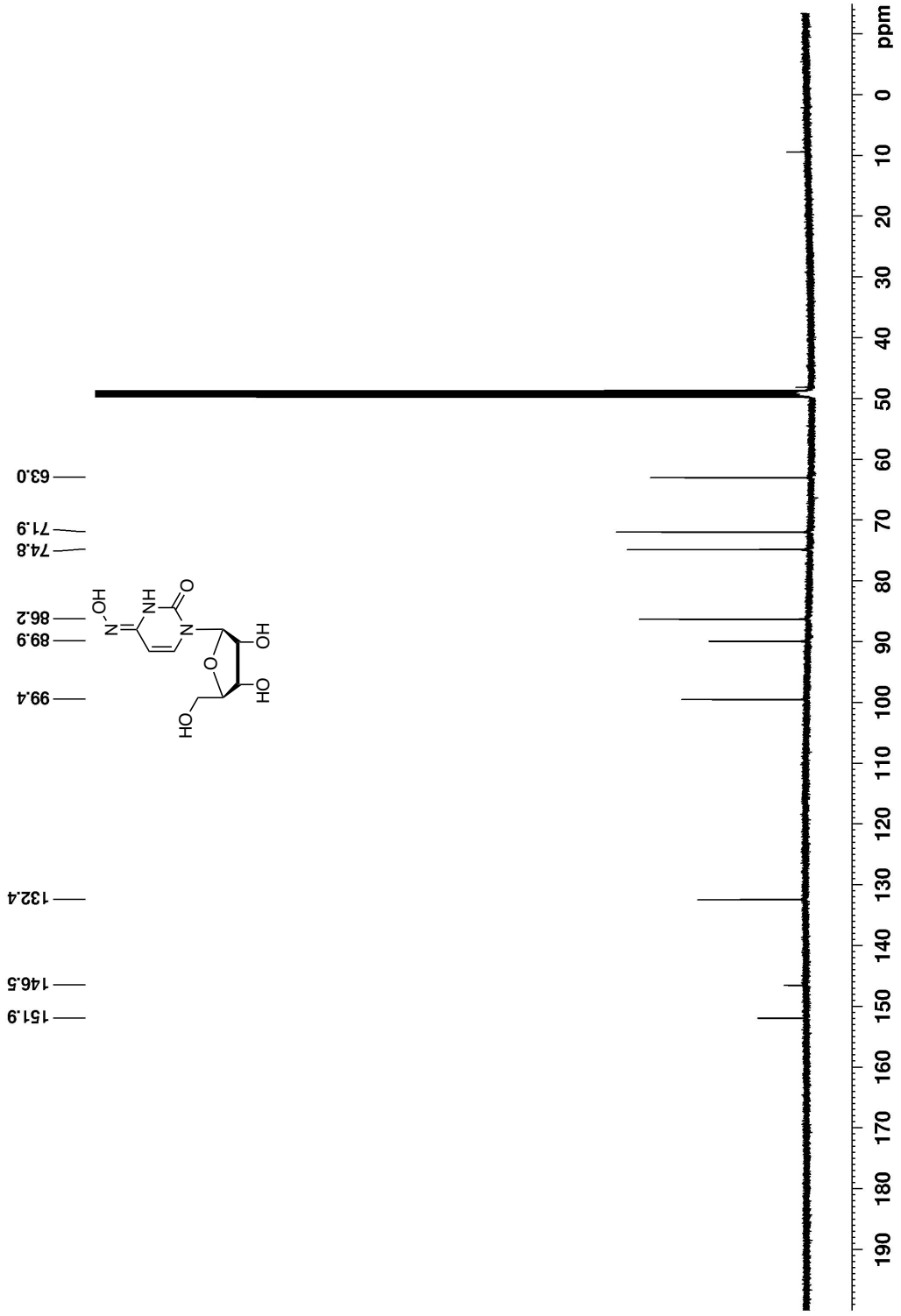


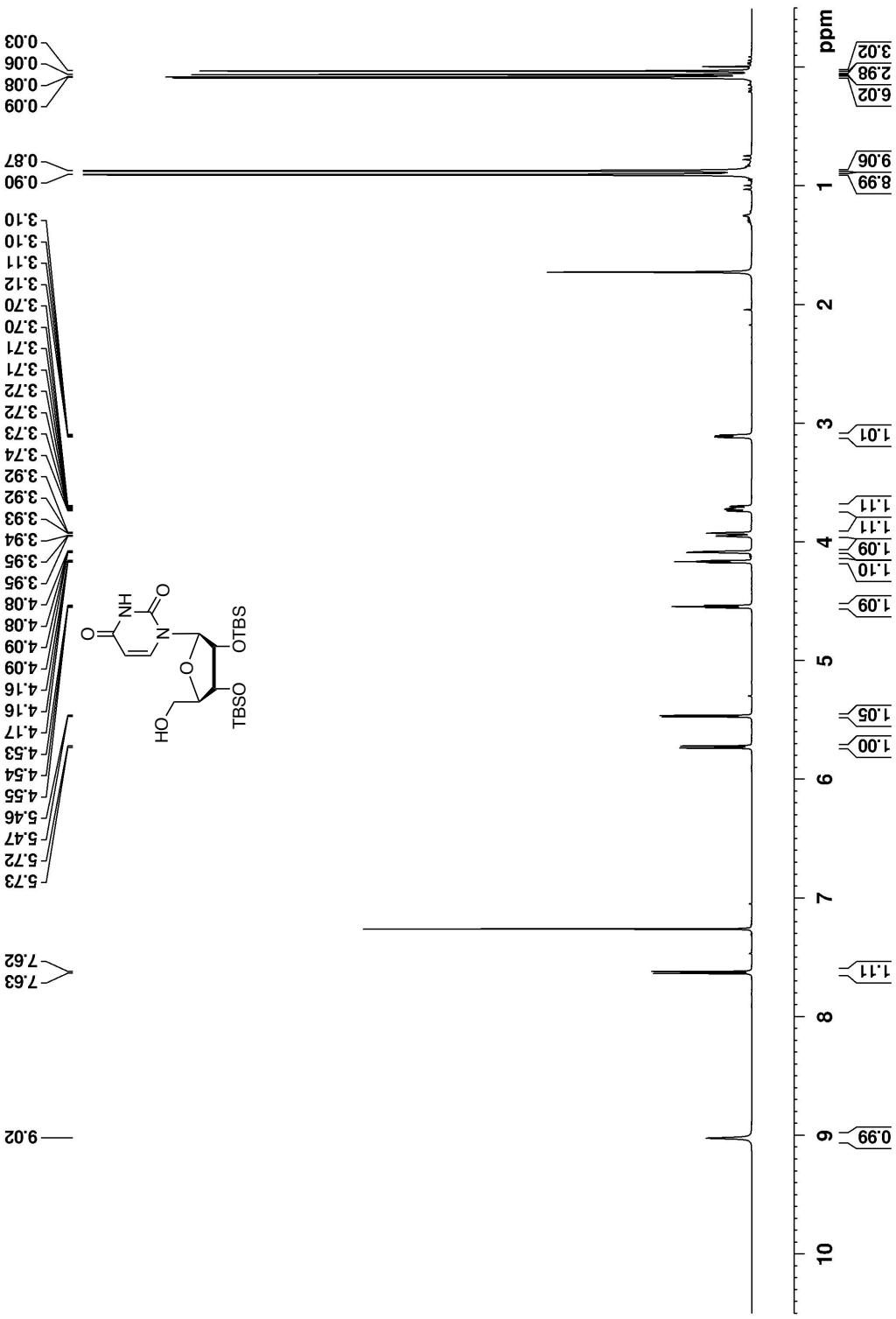
500 MHz ^{1}H NMR spectrum of β-D-*N*^{4}-hydroxycytidine in CD_3OD



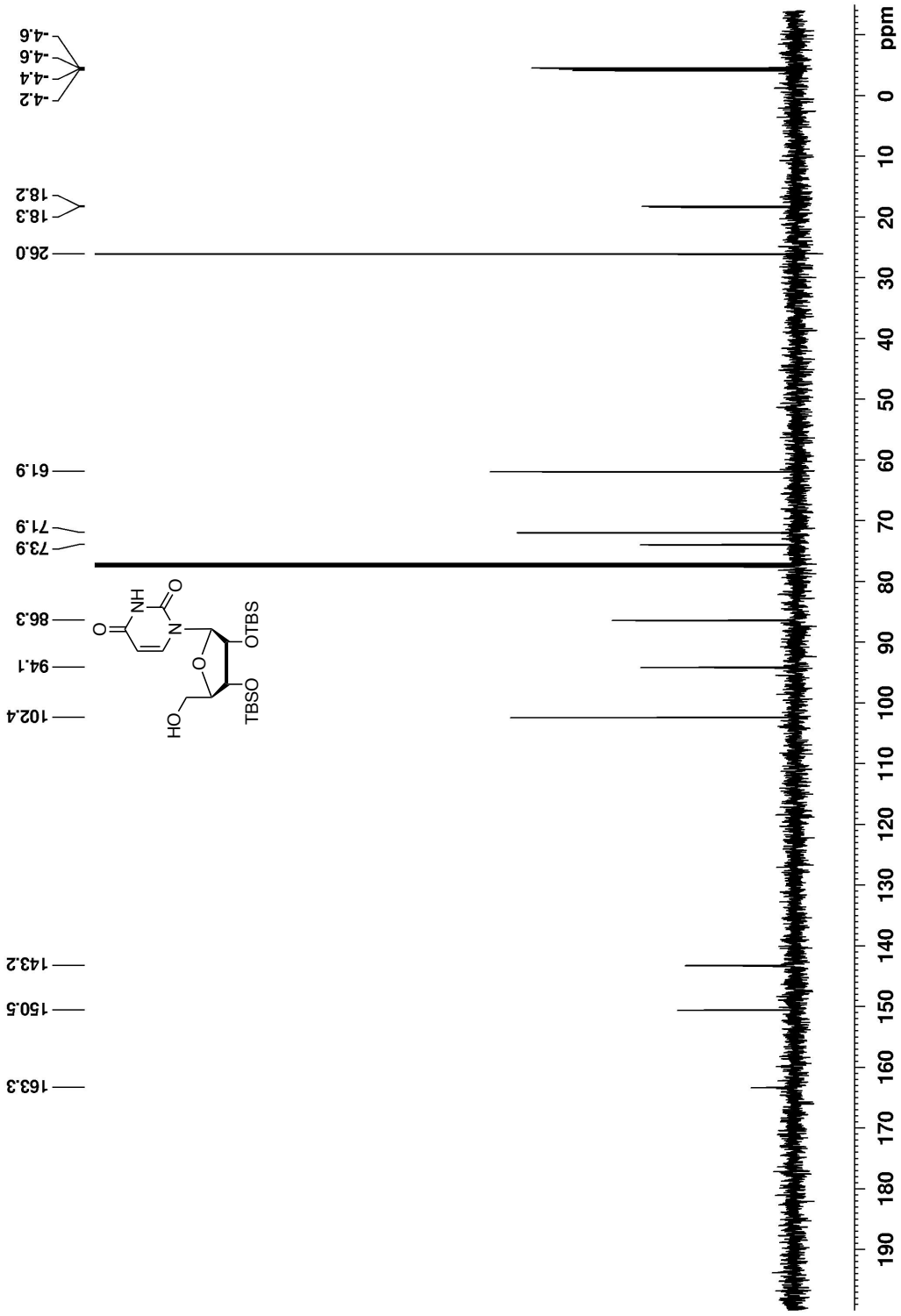
125 MHz $^{13}C\{^{1}H\}$ NMR spectrum of β-D-N^4-hydroxycytidine in CD_3OD



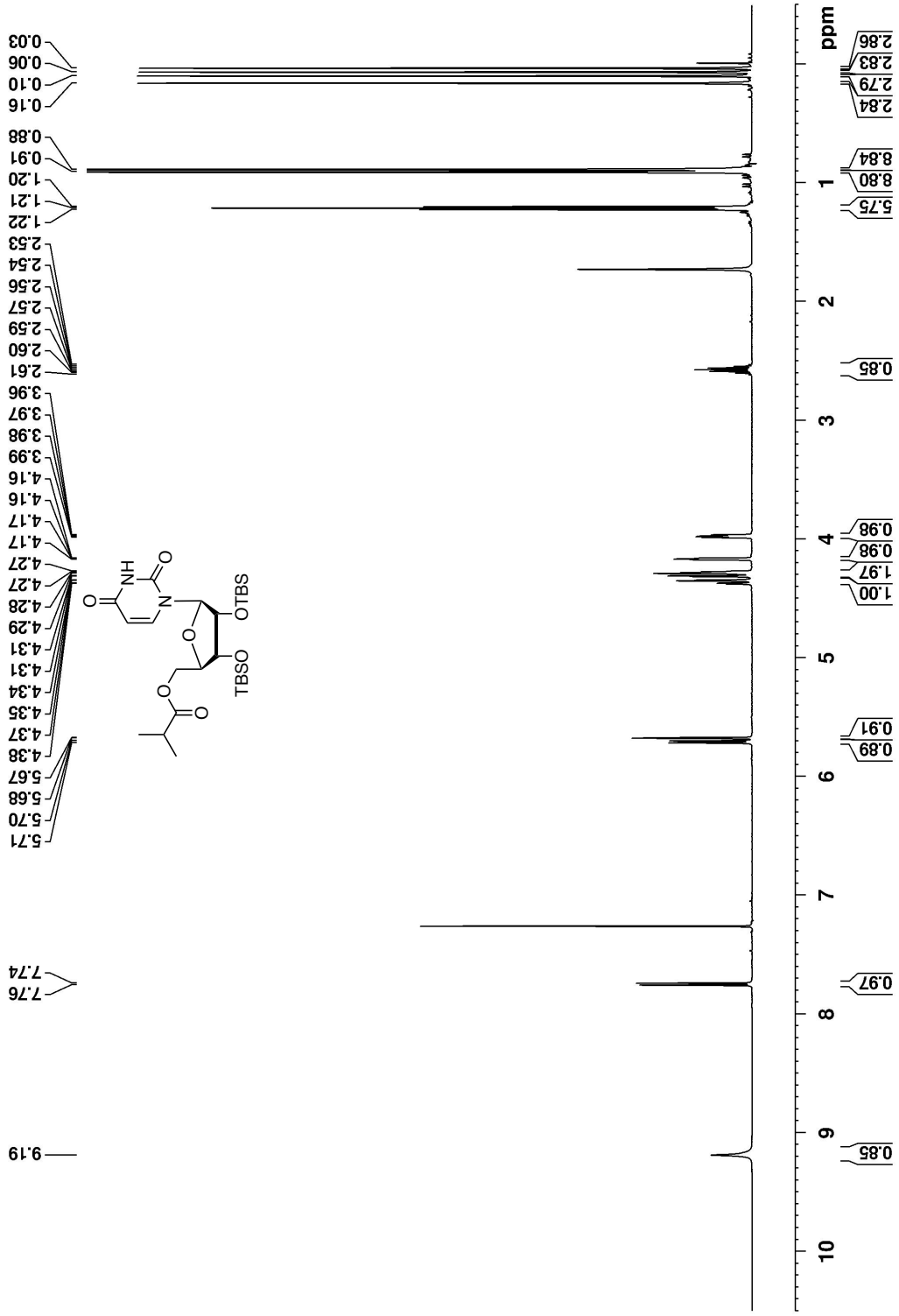
500 MHz ^{1}H NMR spectrum of compound **5** in $CDCl_3$



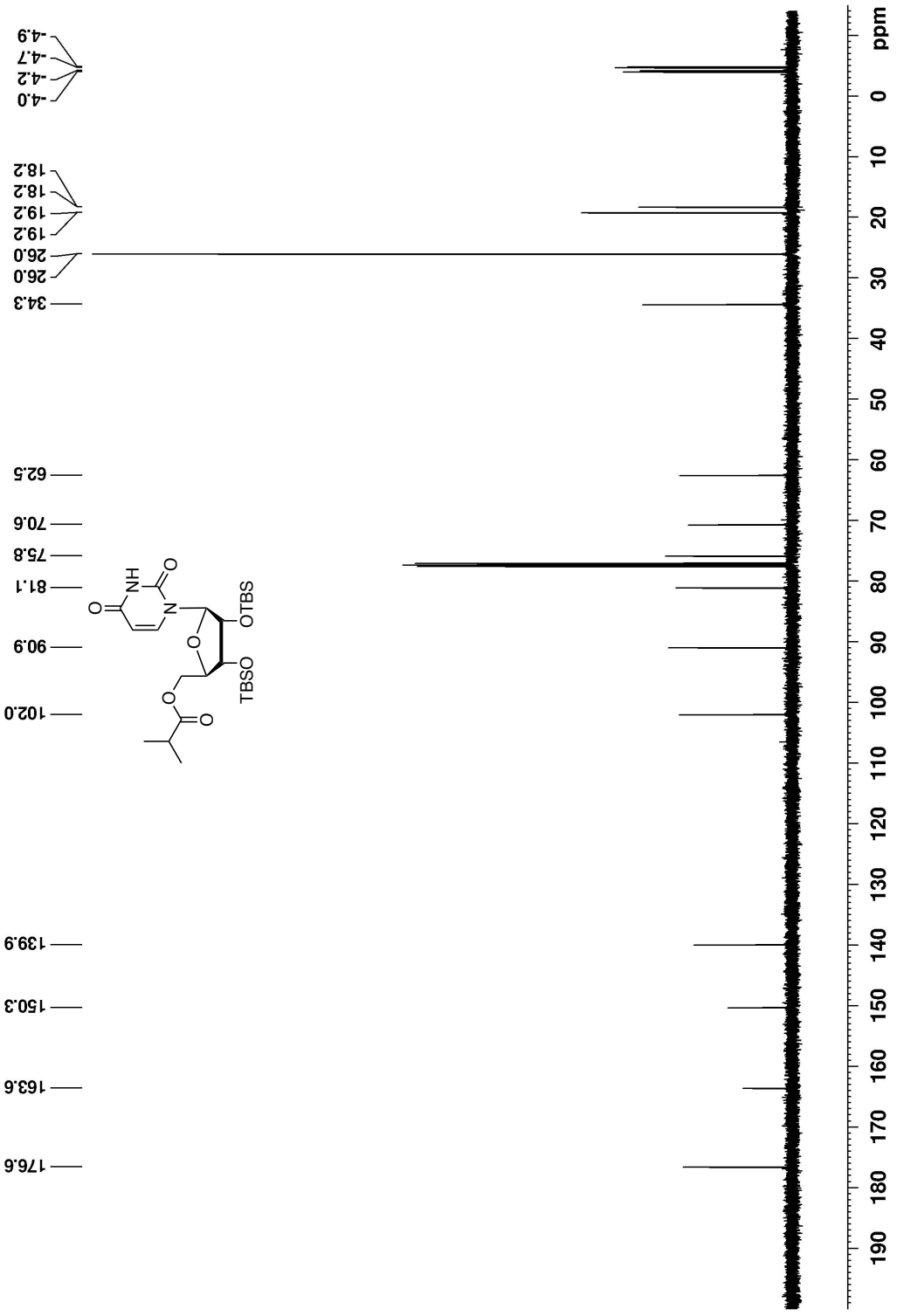
125 MHz $^{13}C\{^{1}H\}$ NMR spectrum of compound **5** in $CDCl_3$



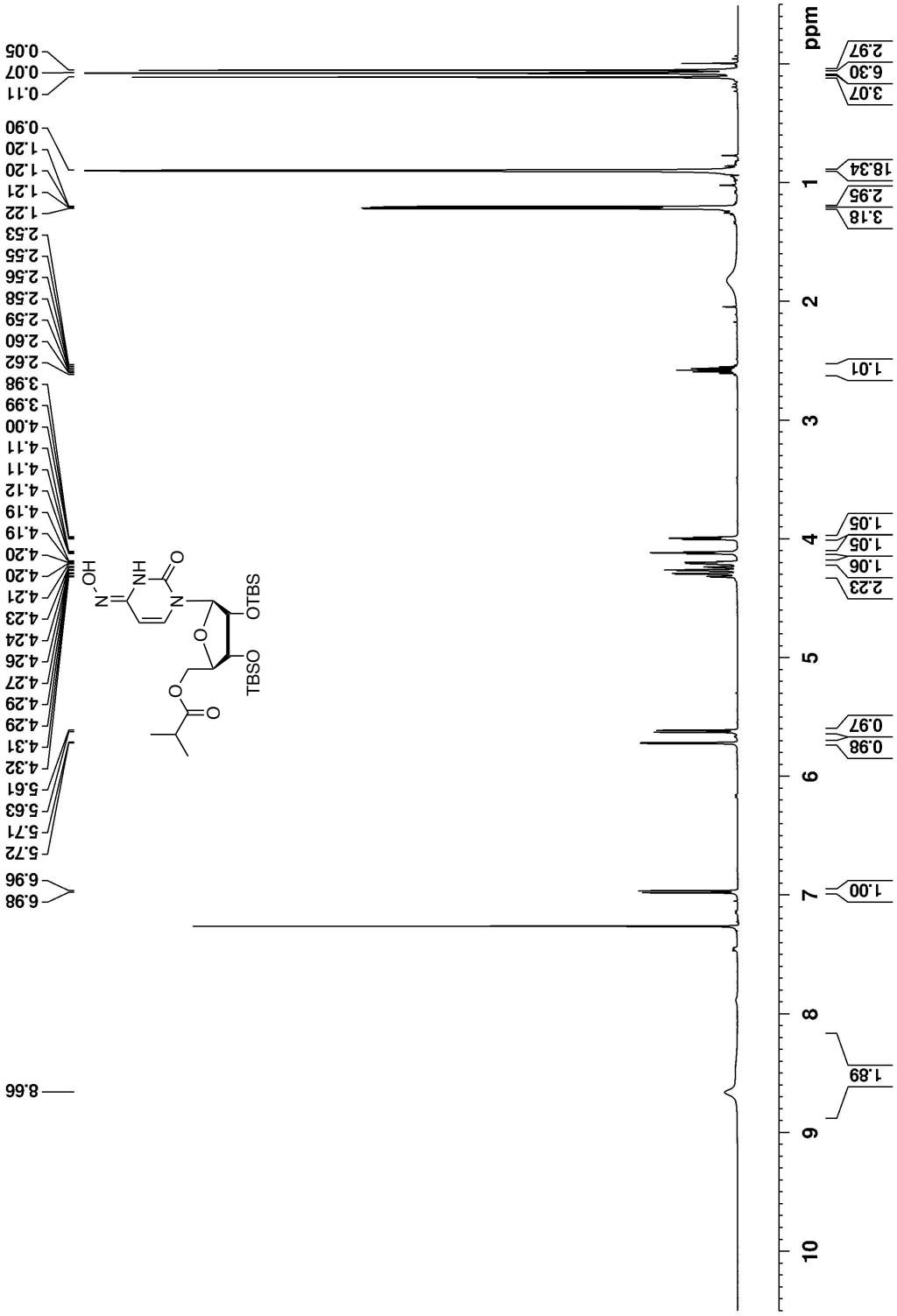
500 MHz ^{1}H NMR spectrum of compound **6** in $CDCl_3$



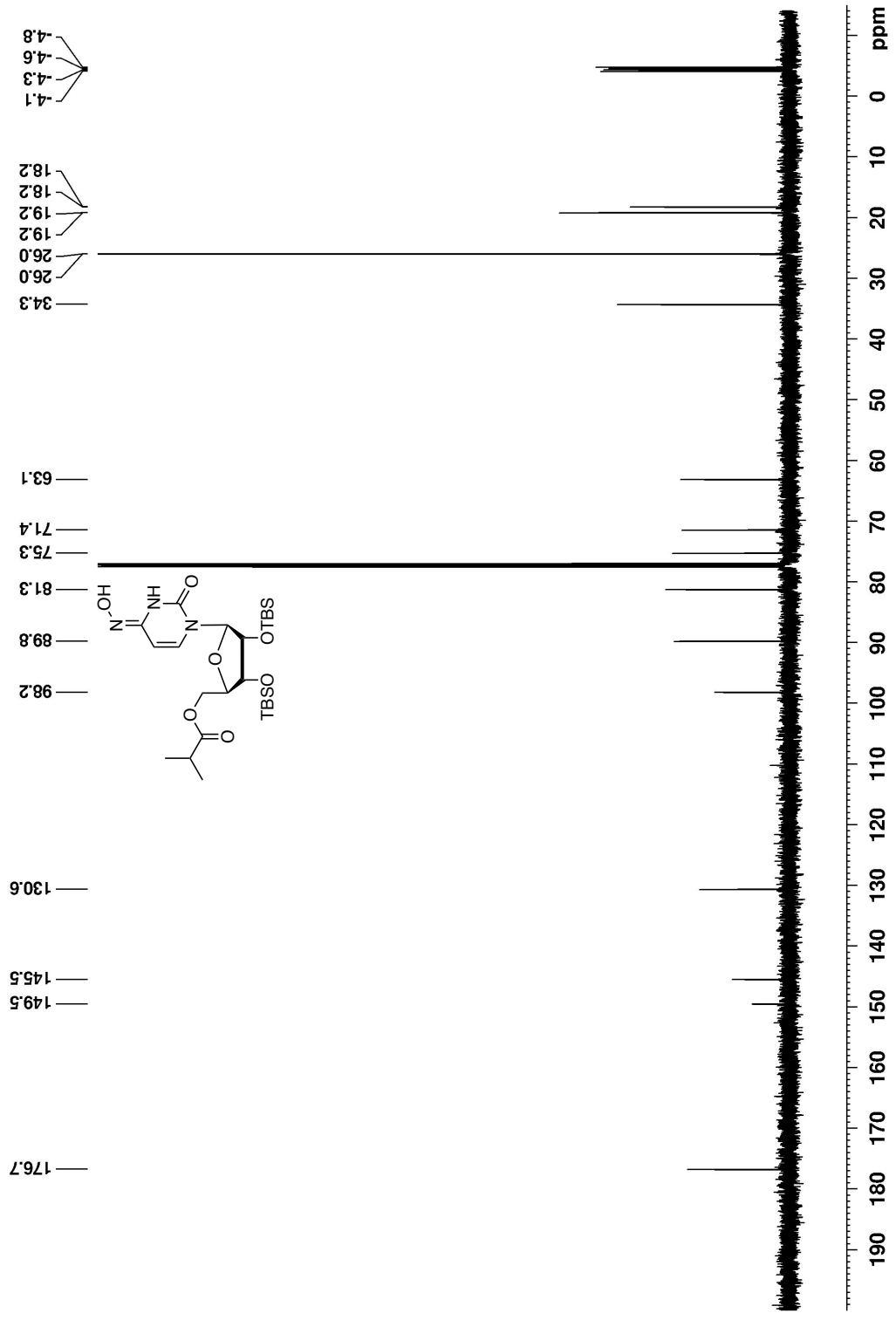
125 MHz $^{13}C\{^1H\}$ NMR spectrum of compound **6** in $CDCl_3$



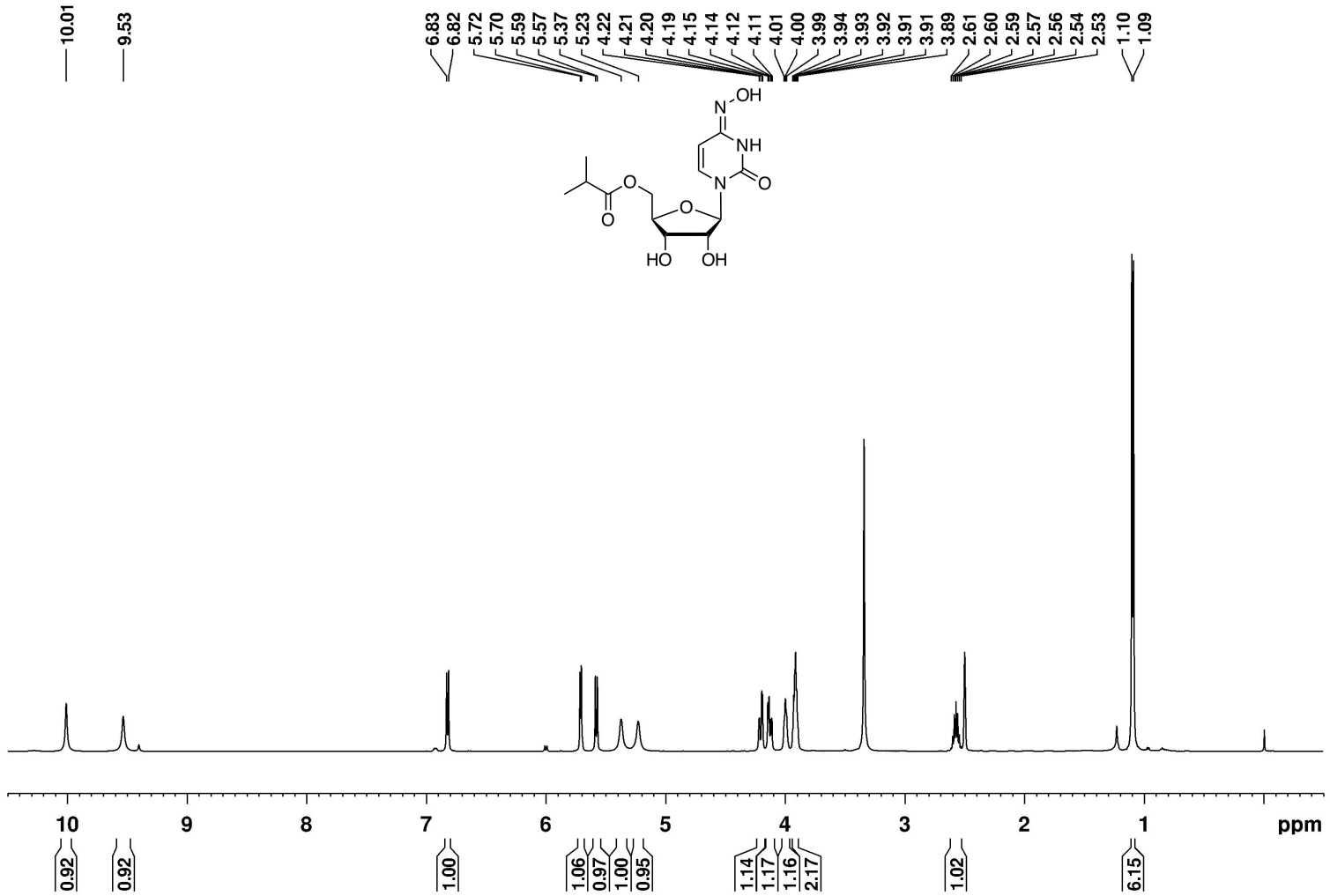
500 MHz ^{1}H NMR spectrum of compound **7** in $CDCl_3$



125 MHz $^{13}C\{^{1}H\}$ NMR spectrum of compound **7** in $CDCl_3$



400 MHz ^{1}H NMR spectrum of molnupiravir in DMSO-d_6



100 MHz $^{13}C\{^{1}H\}$ NMR spectrum of molnupiravir in DMSO-d_6