REVISED

9/12/0

Total Synthesis and Structural Confirmation of Malayamycin A – A Novel Bicyclic *C*-Nucleoside from *Streptomyces malaysiensis*

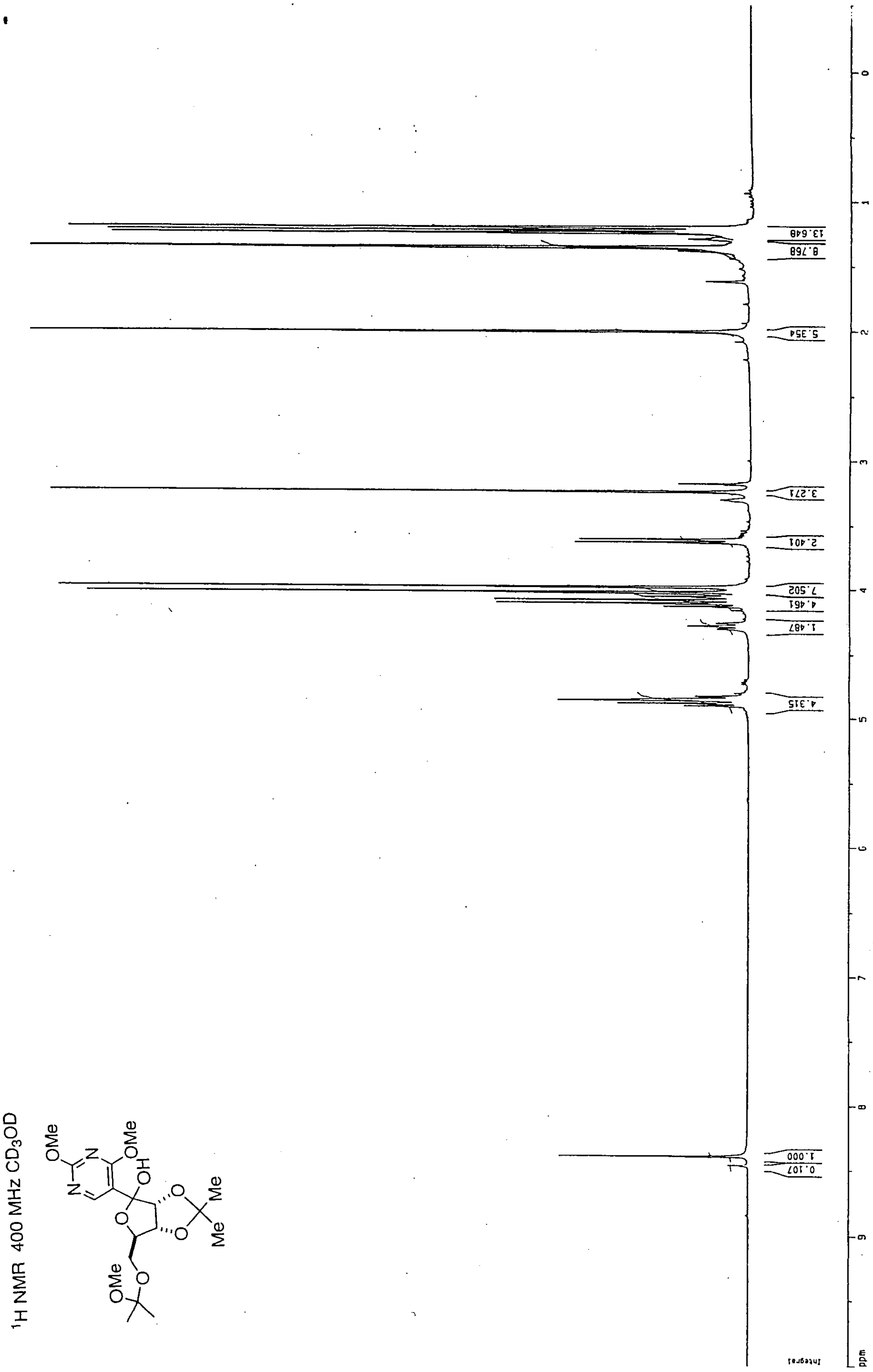
Stephen Hanessian*, Stéphane Marcotte, Roger Machaalani, Guobin Huang

Department of Chemistry, Université de Montréal, P.O. Box 6128, Station Centre-ville, Montréal, QC, H3C 3J7, stephen.hanessian@umontreal.ca

Supporting Information

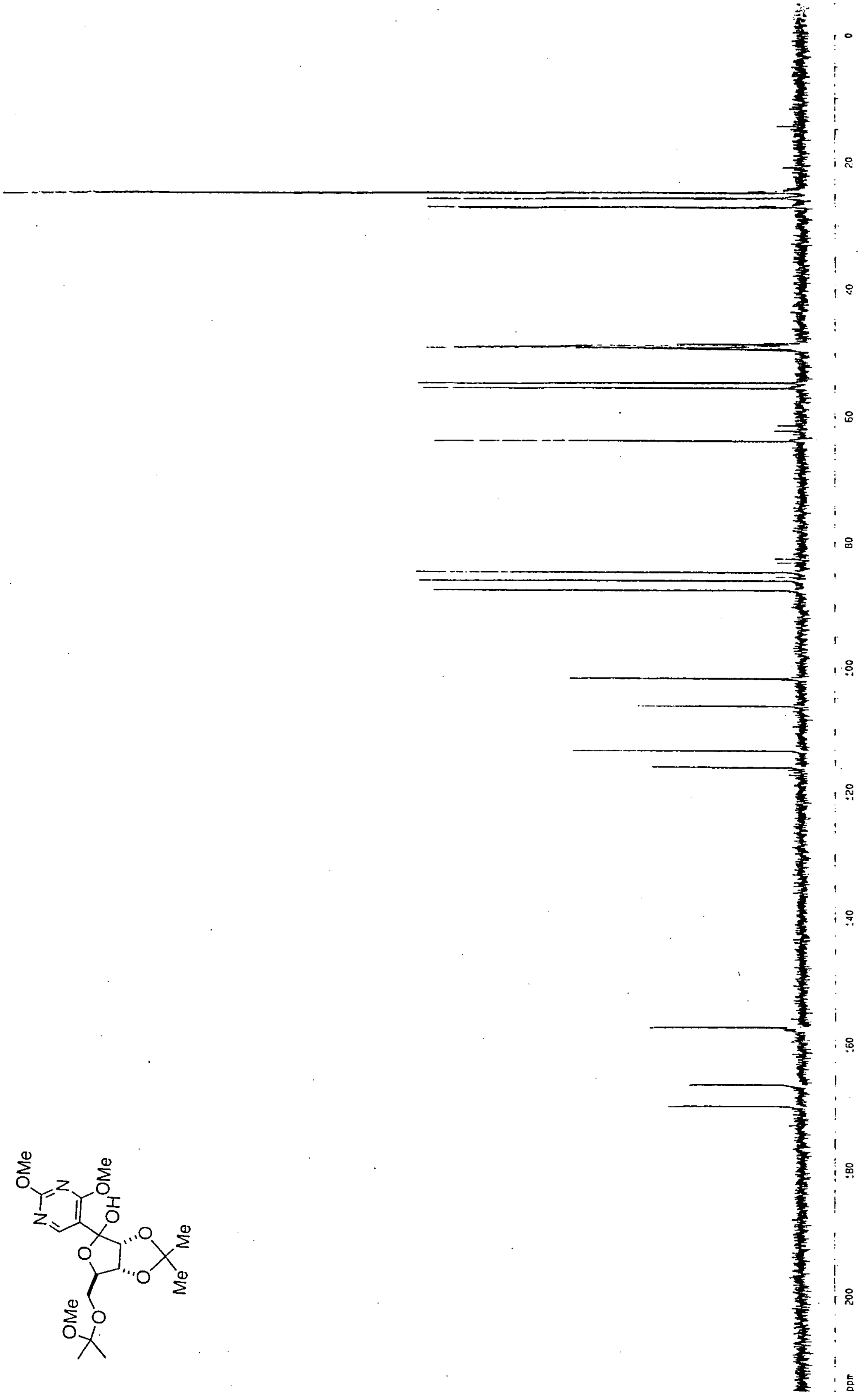
Compound number	**Proton NMR**	**IR**	**Carbon NMR**	**HRMS**	**[α]D**	**Melting Point**
4	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✓	
7	✓	✓	✓	✓	✓	
8	✓	✓	✓	✓	✓	
9	✓	✓	✓	✓	✓	✓
10	✓	✓	✓	✓	✓	
11	✓	✓	✓	✓	✓	✓
14	✓	✓	✓	✓	✓	✓
16	✓	✓	✓	✓	✓	✓
18	✓	✓	✓	✓	✓	
1	✓	✓	✓	✓	✓	✓

S2



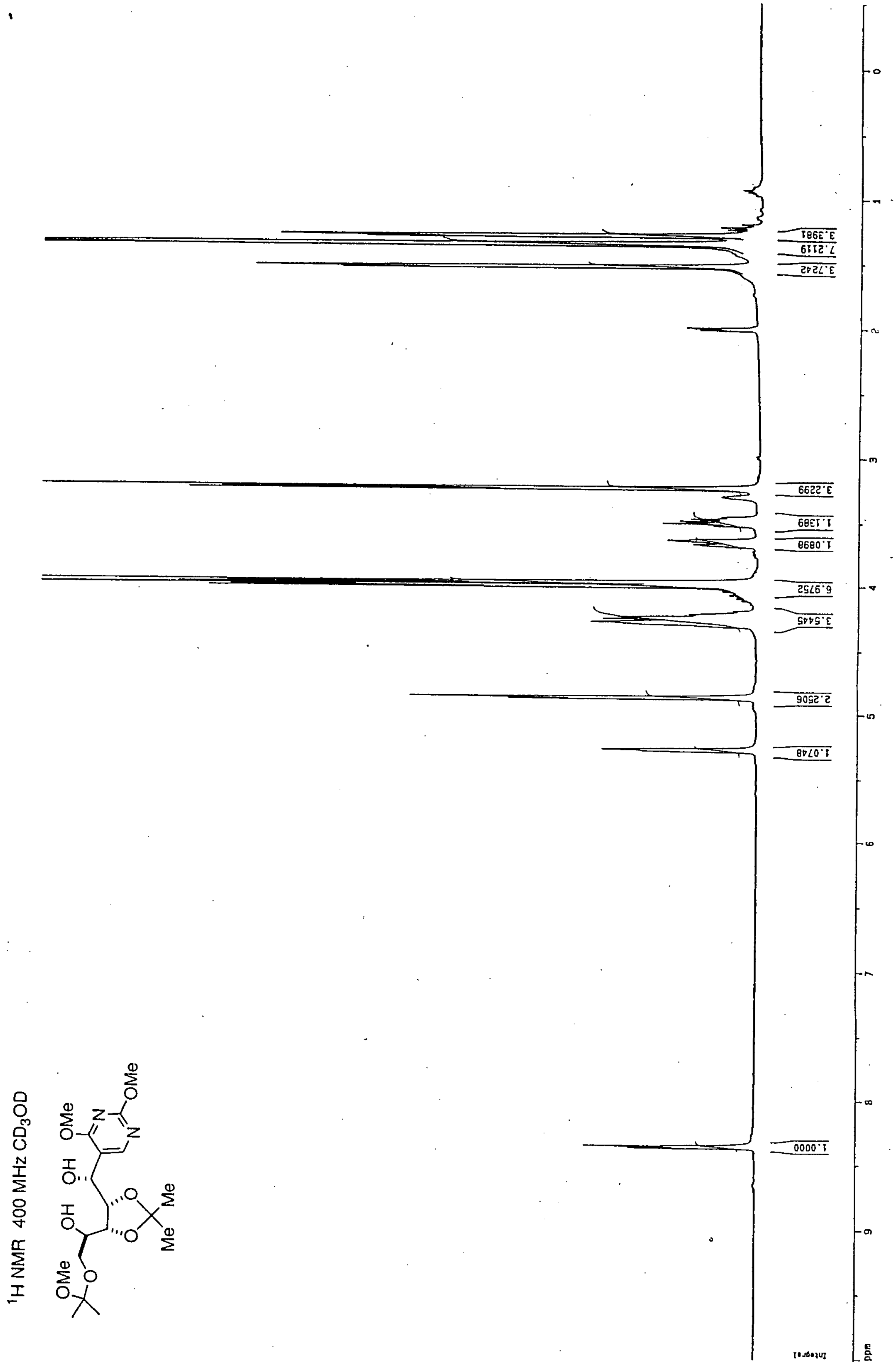
^{1}H NMR 400 MHz CD_3OD

S3



^{13}C NMR 400 MHz CD$_3$OD

S4



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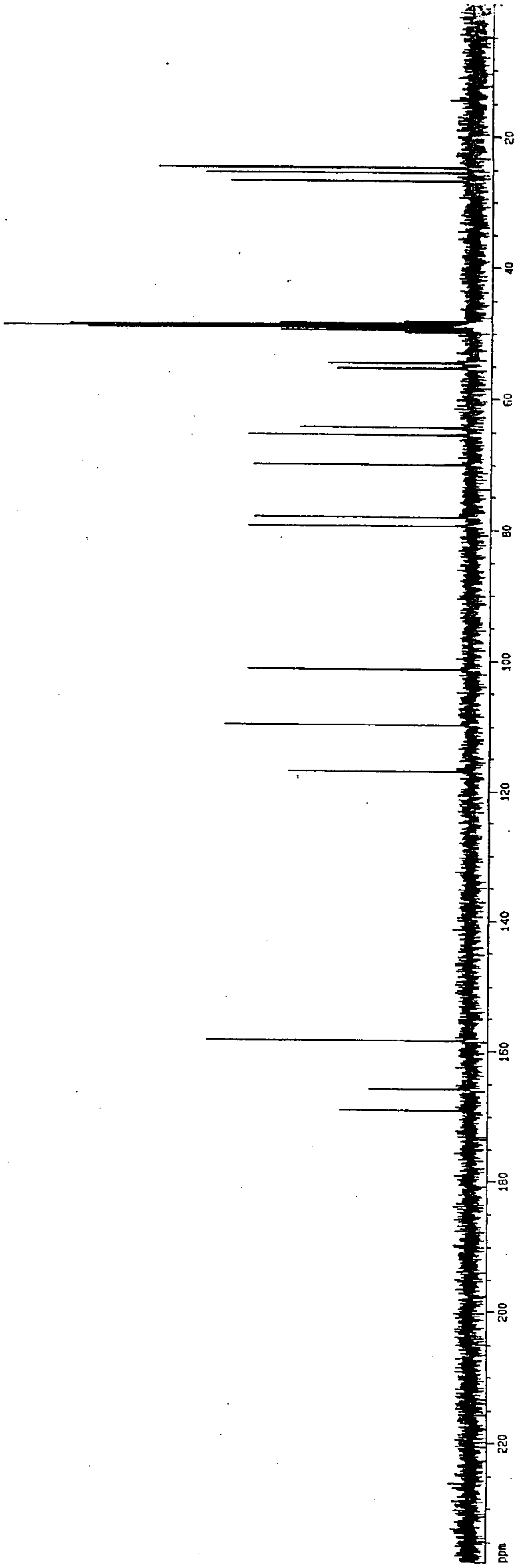
S5



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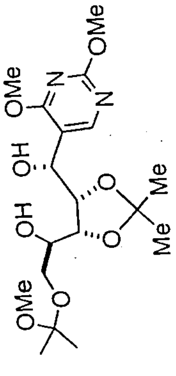

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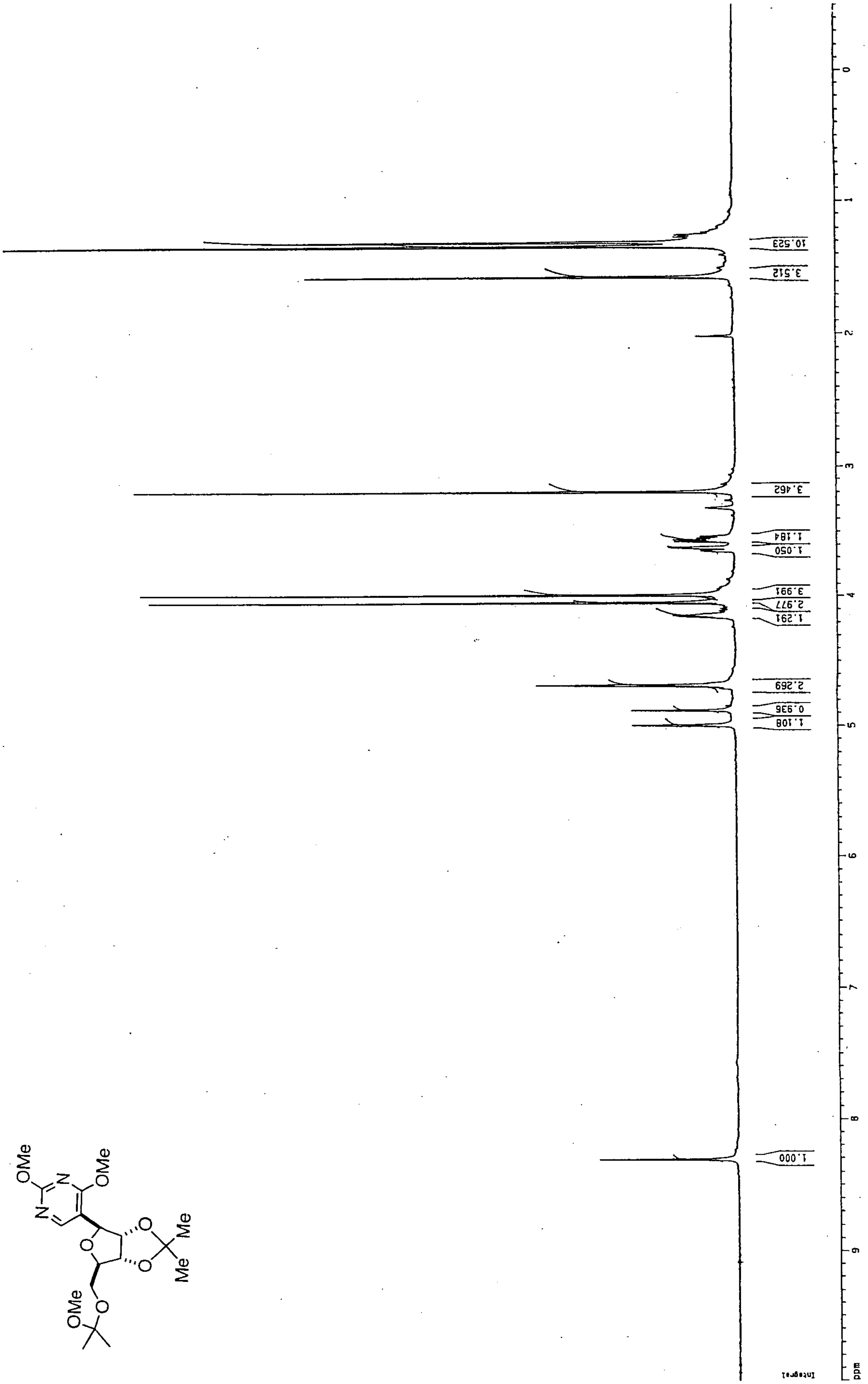

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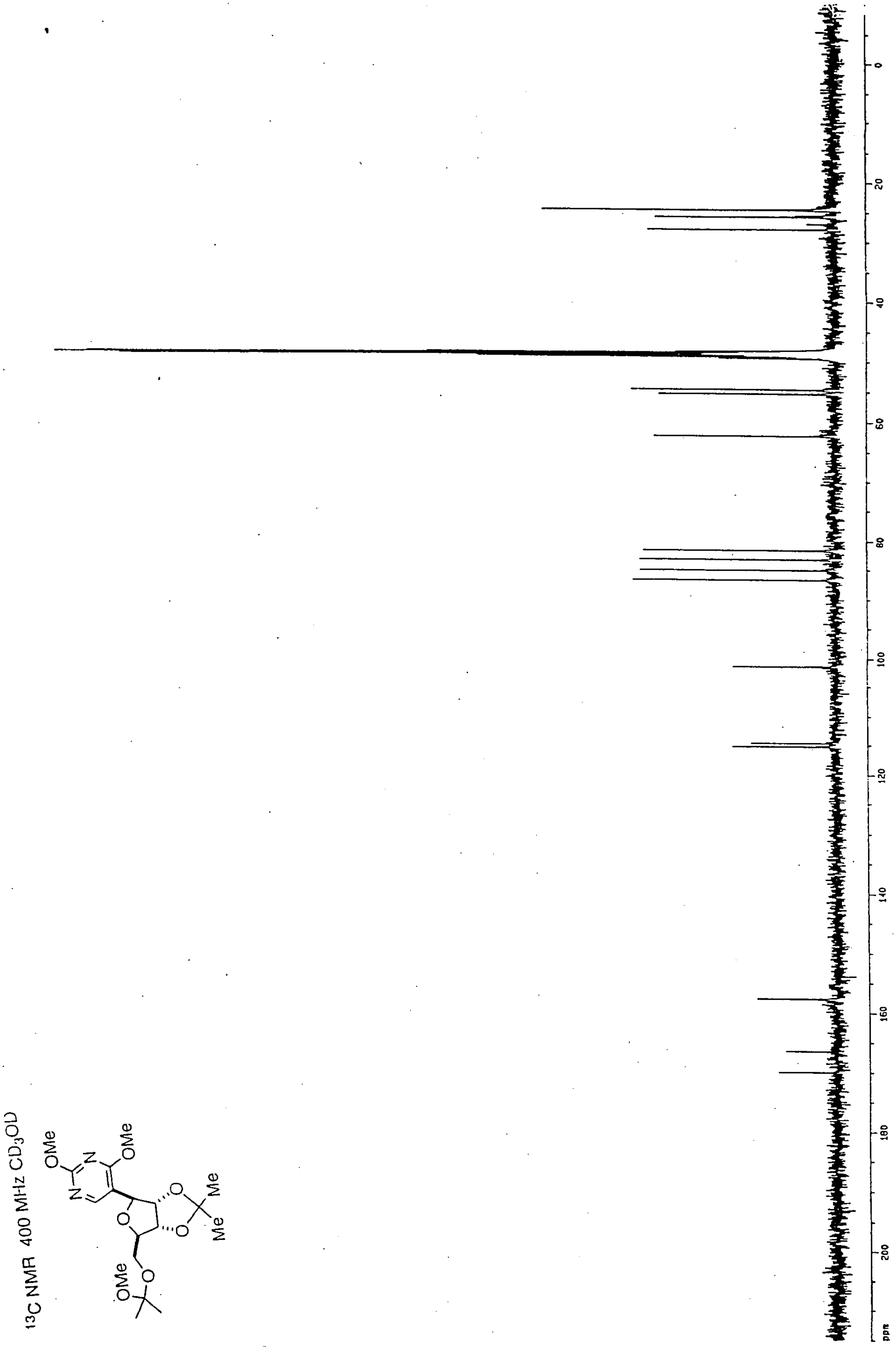
S7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OMe
N
N
OMe
O
O
O
Me
Me
OMe
O
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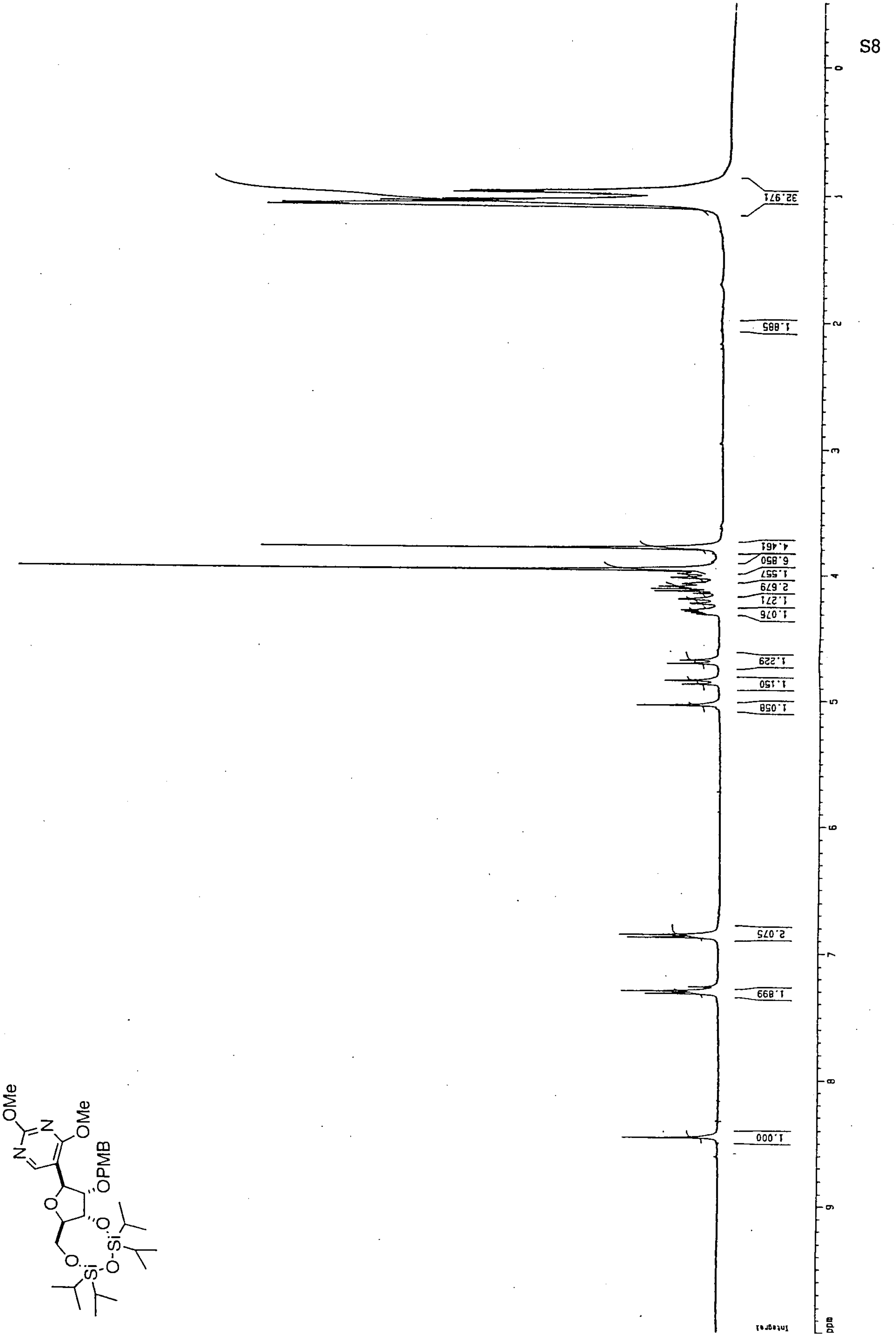
60
80
100
120
140
160
180
200
ppm

^{1}H NMR 400MHz CDCl$_3$



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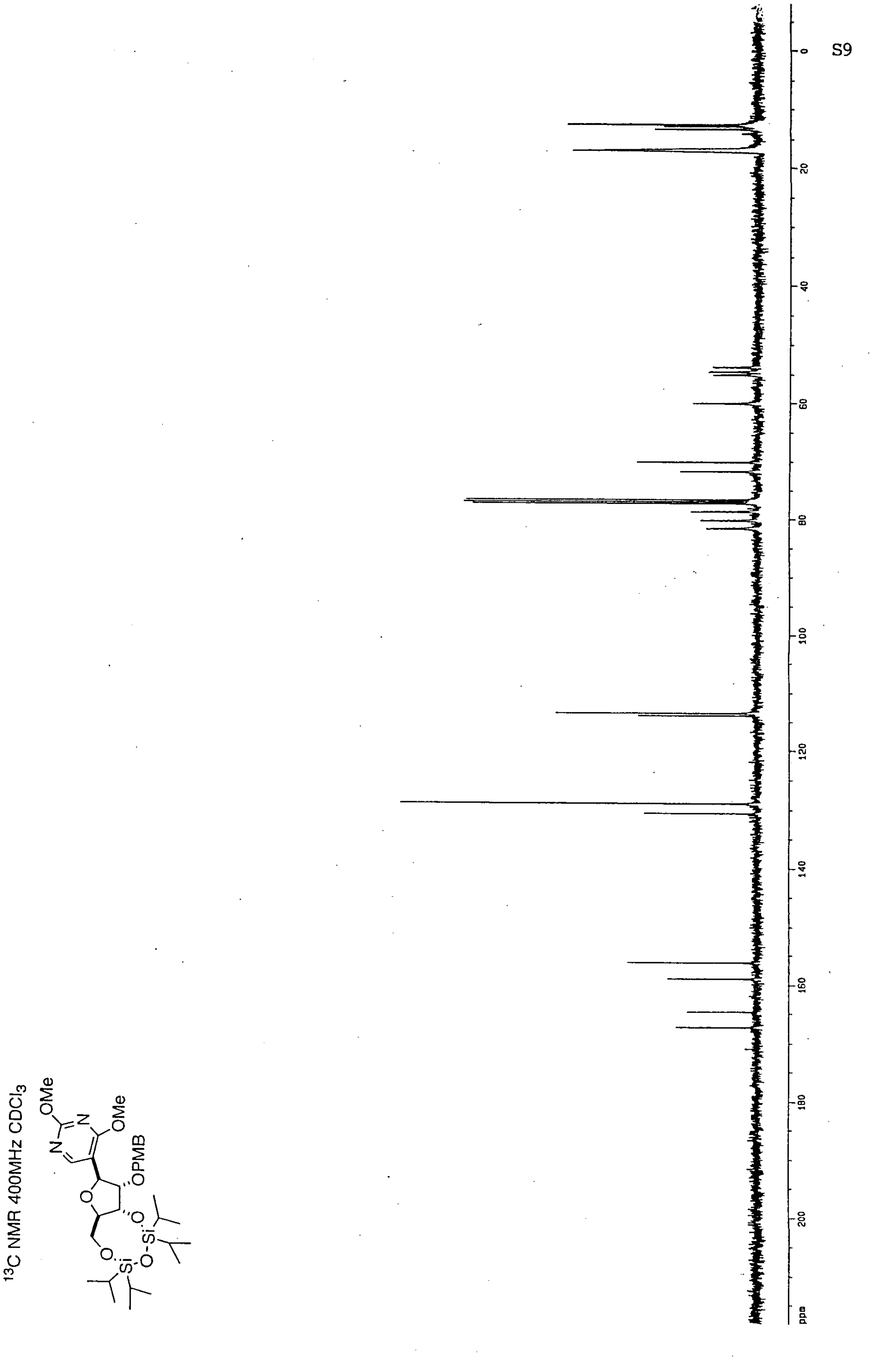
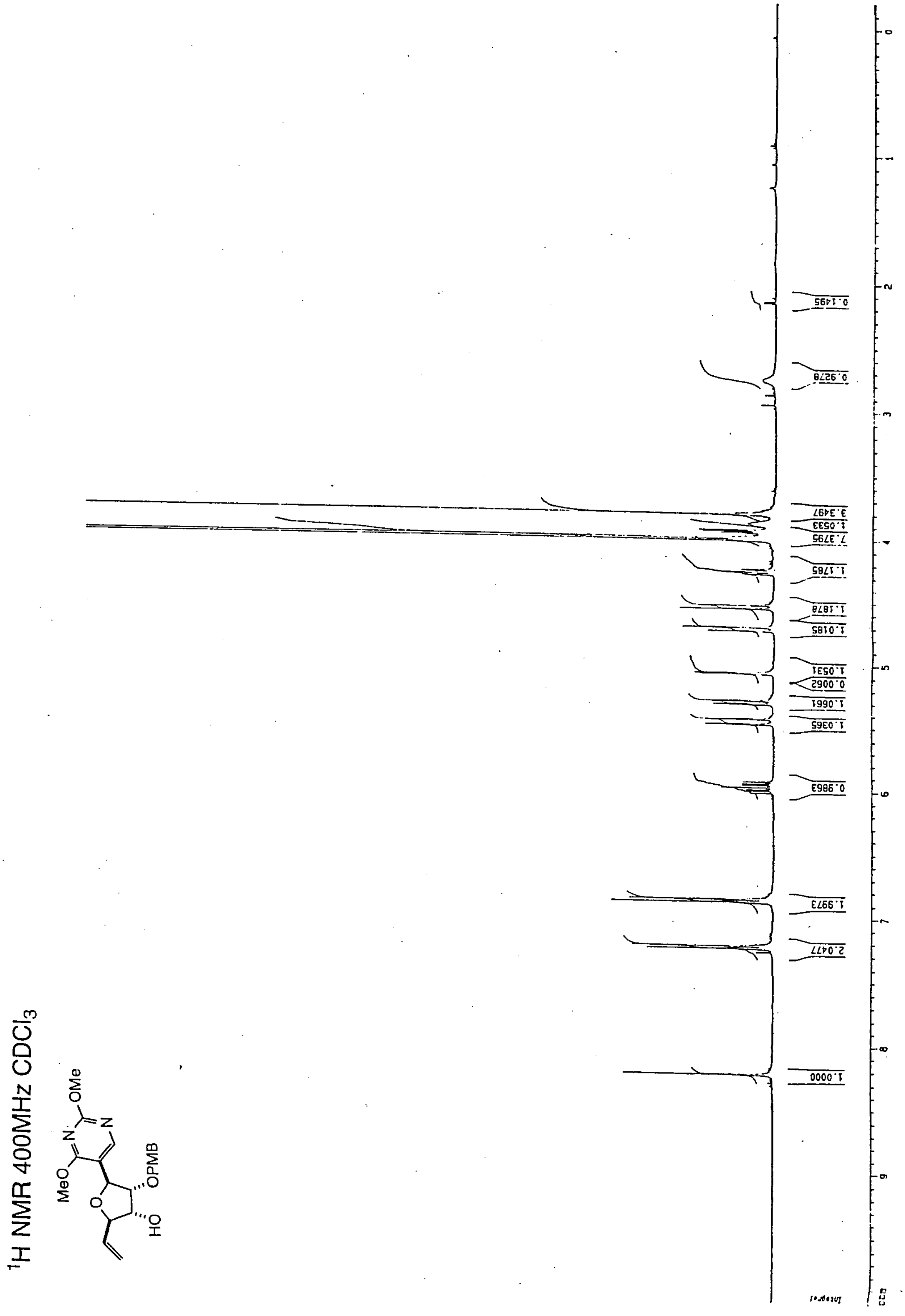

S9
13C NMR 400MHz CDCl3
OMe
N
N
OMe
OPMB
O
O
Si
O
Si
O
O
0
20
40
60
80
100
120
140
160
180
200
ppm



^{1}H NMR 400MHz CDCl$_3$
MeO
N
OMe
N
O
HO
OPMB
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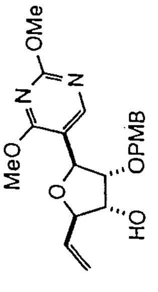
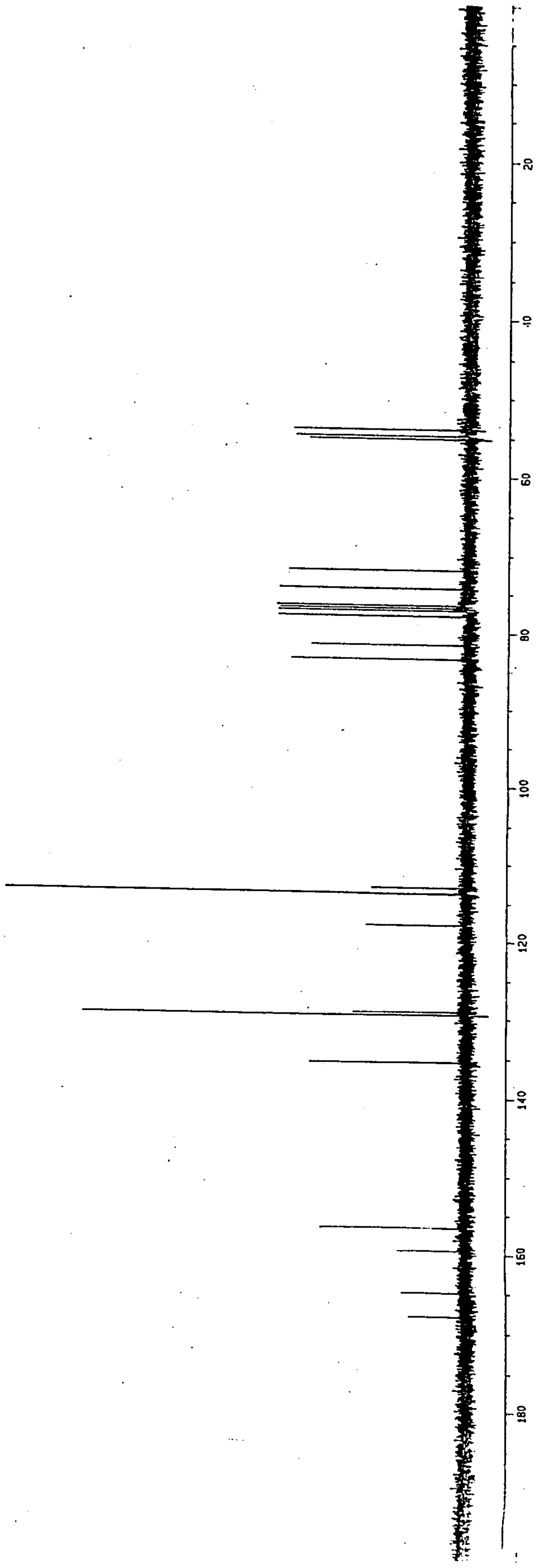
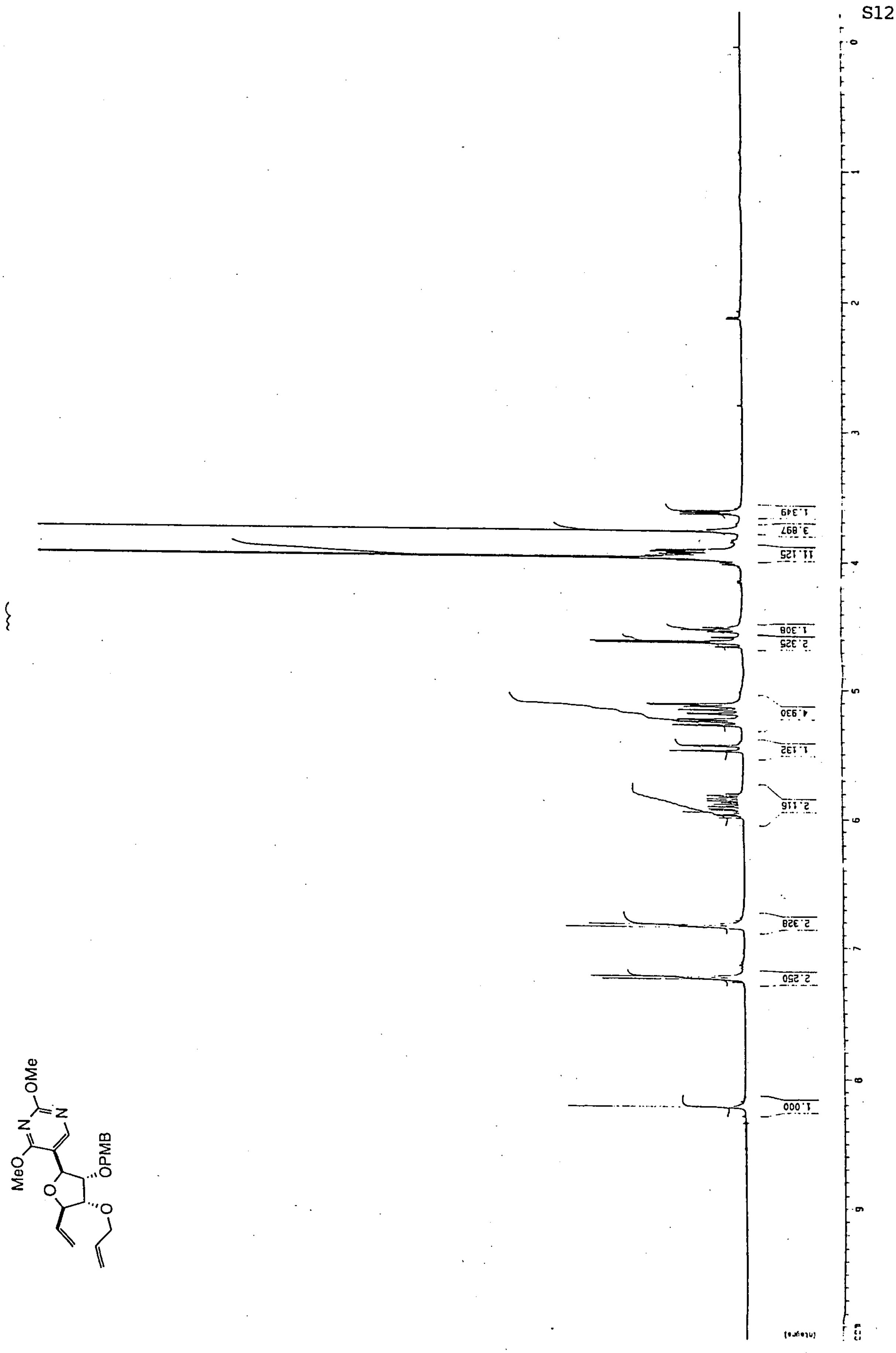
7.3795
1.1785
1.1878
1.0185
1.0531
0.0062
1.0661
1.0365
0.9863
1.9973
2.0477
1.0000
Integral
ppm
0
1
2
3
4
5
6
7
8
9

S11

^{13}C NMR 400MHz CDCl$_3$

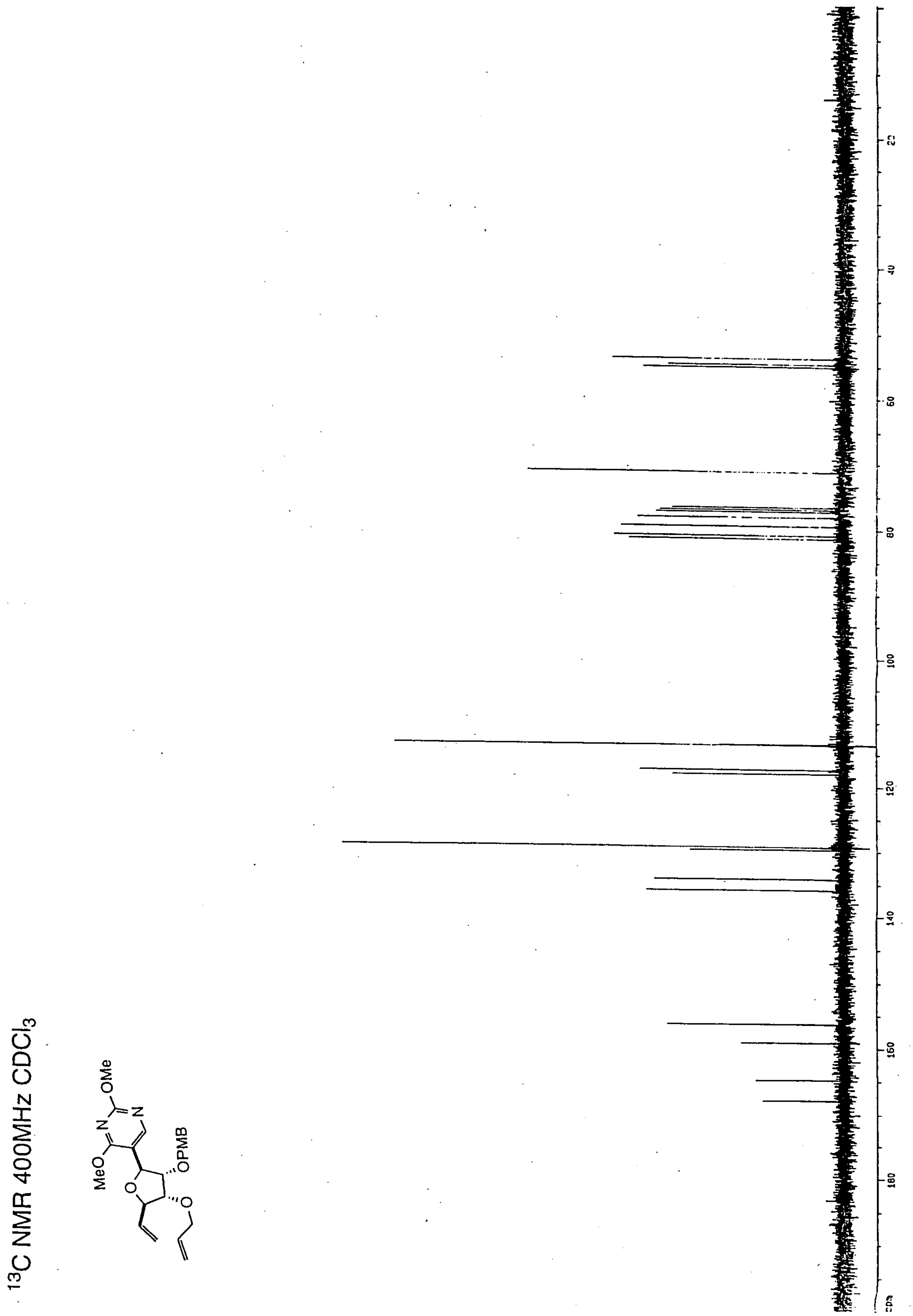






^{1}H NMR 400MHz $CDCl_3$

S13



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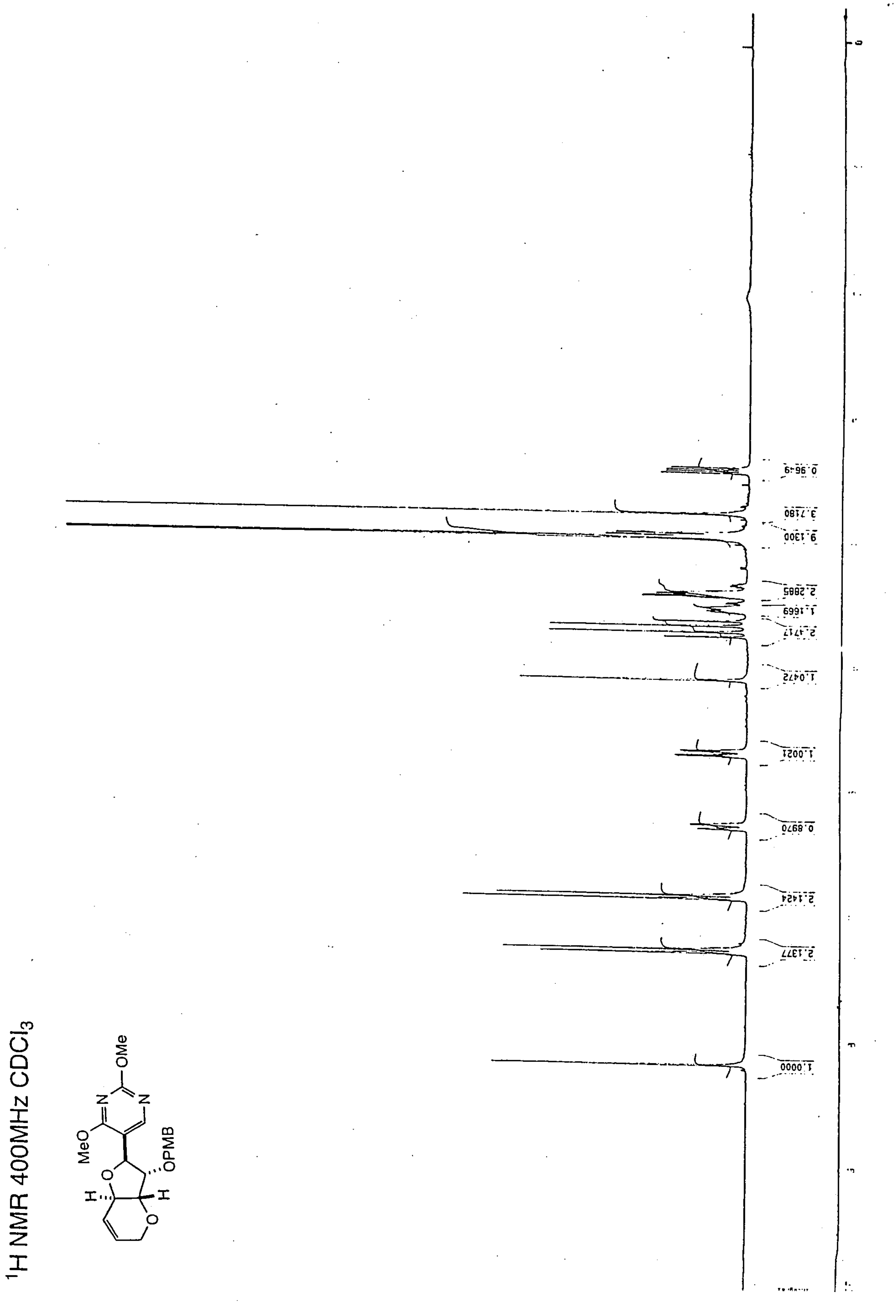
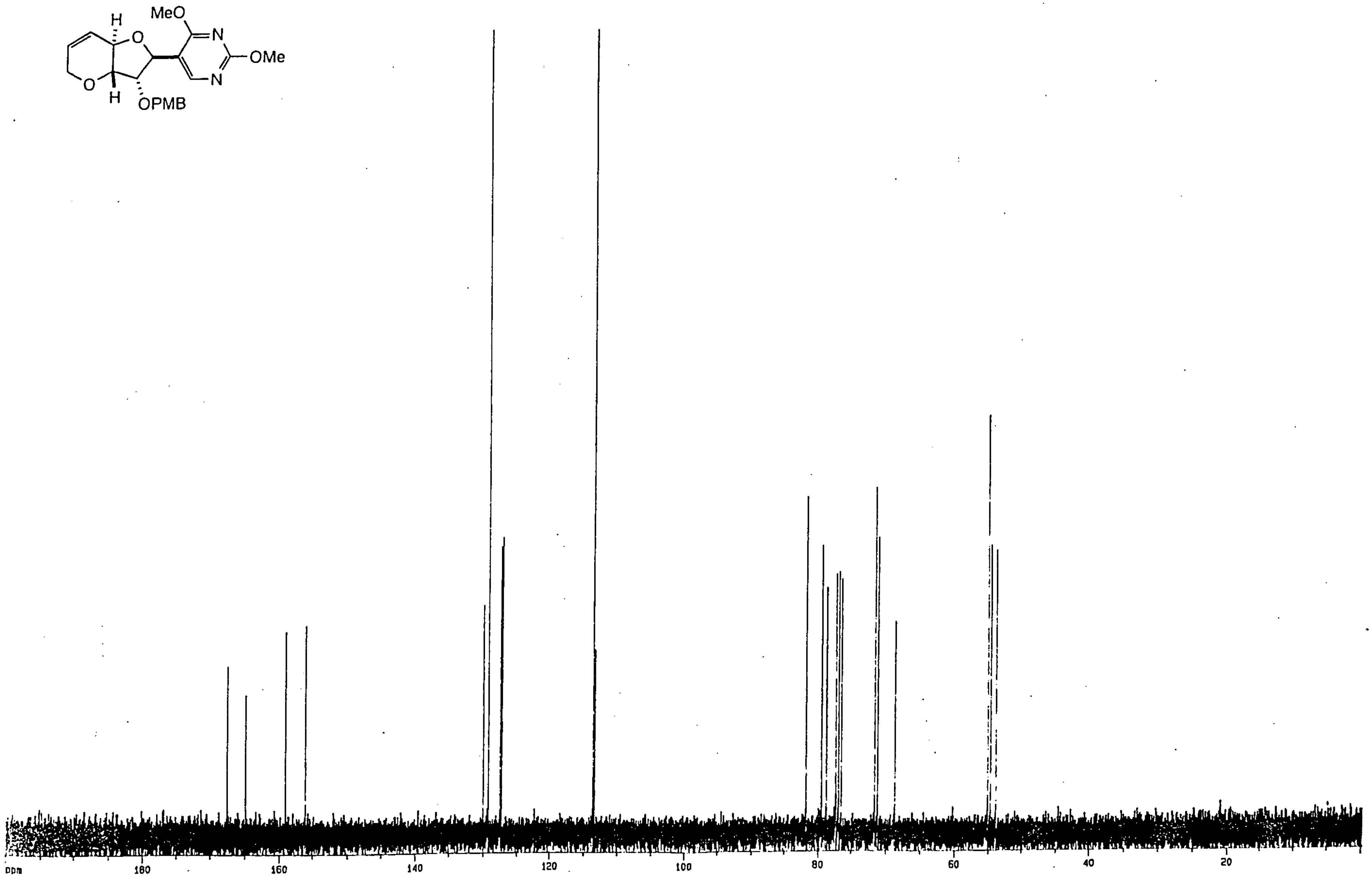


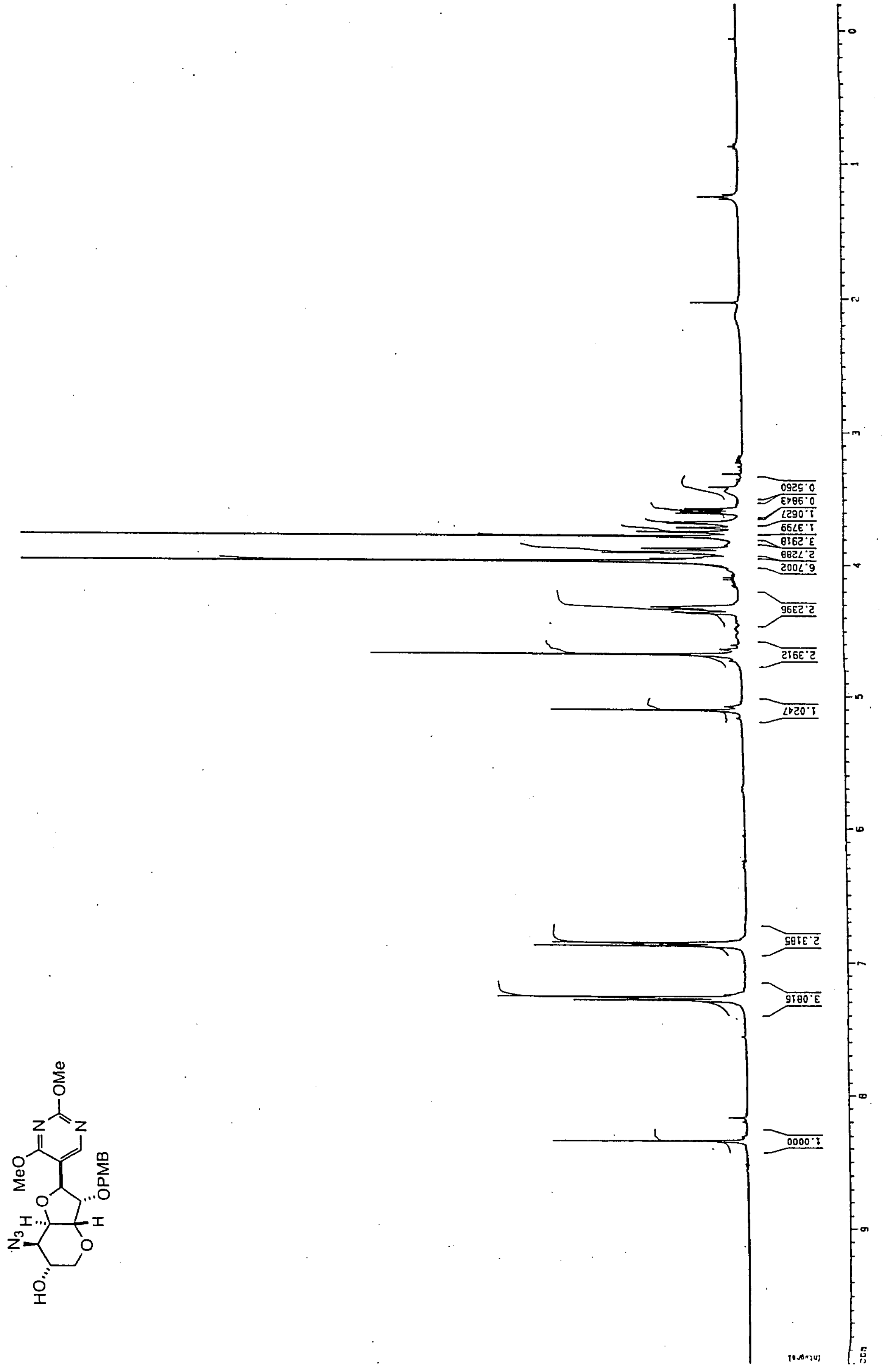
^{1}H NMR 400MHz CDCl$_3$

^{13}C NMR 400MHz CDCl$_3$

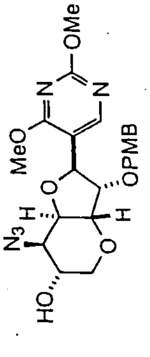


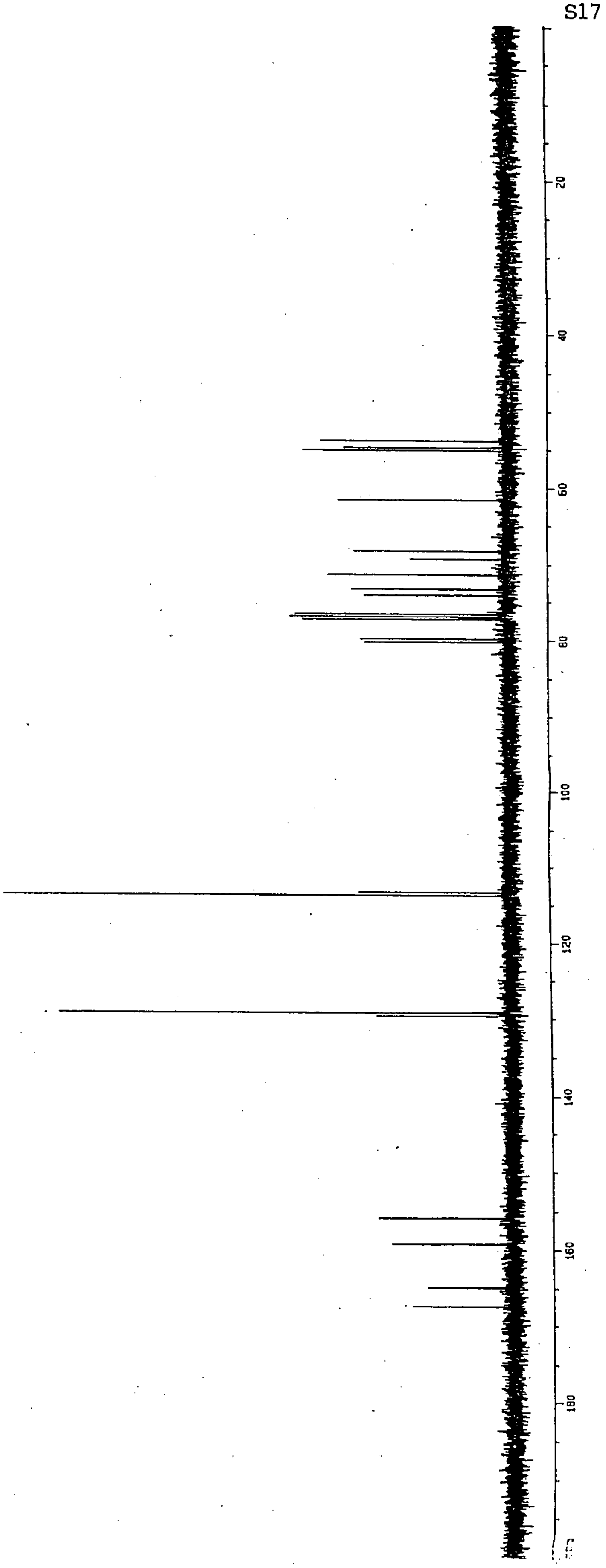
S16

^1H NMR 400MHz $CDCl_3$



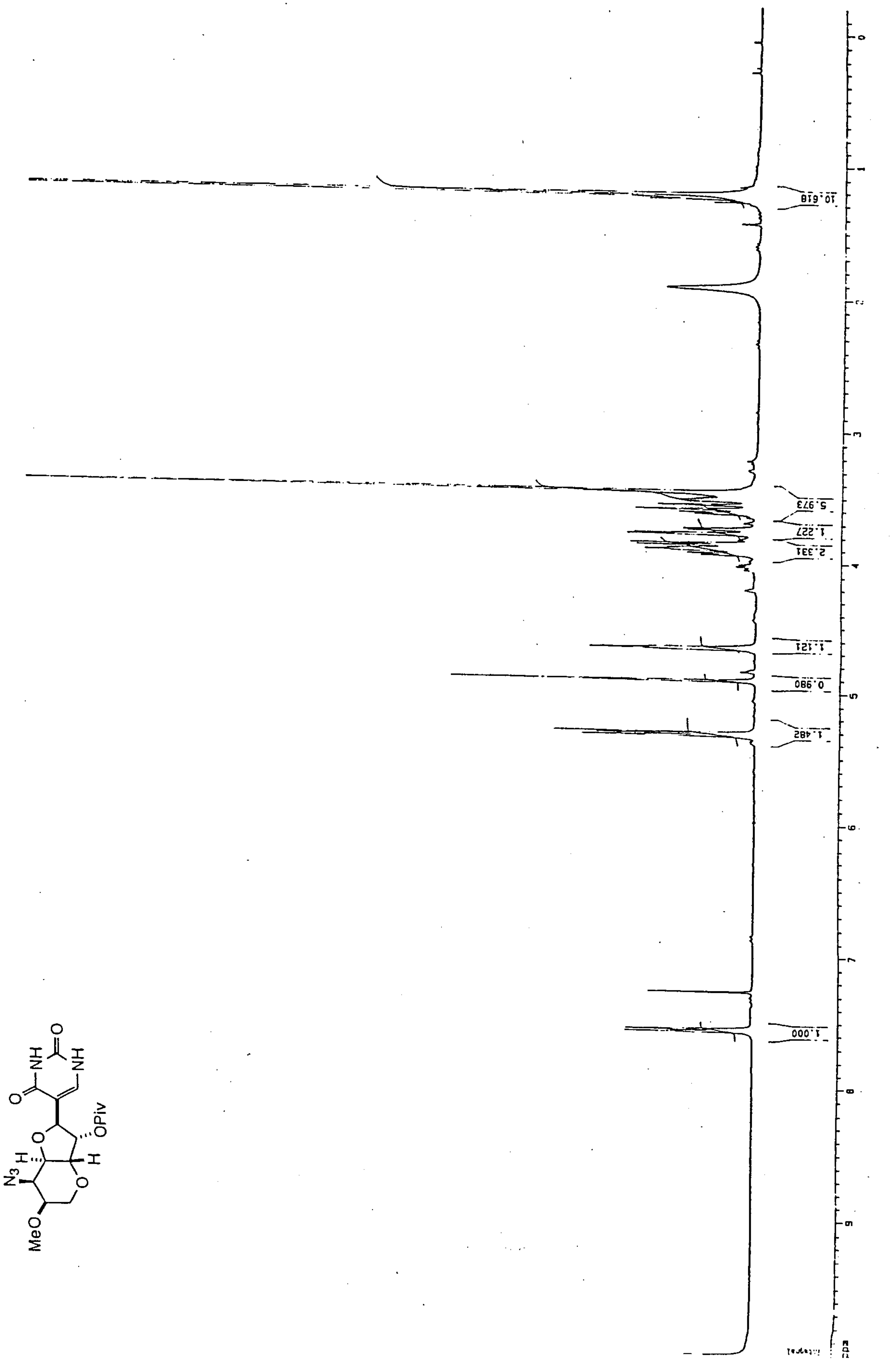
^{13}C NMR 400MHz $CDCl_3$



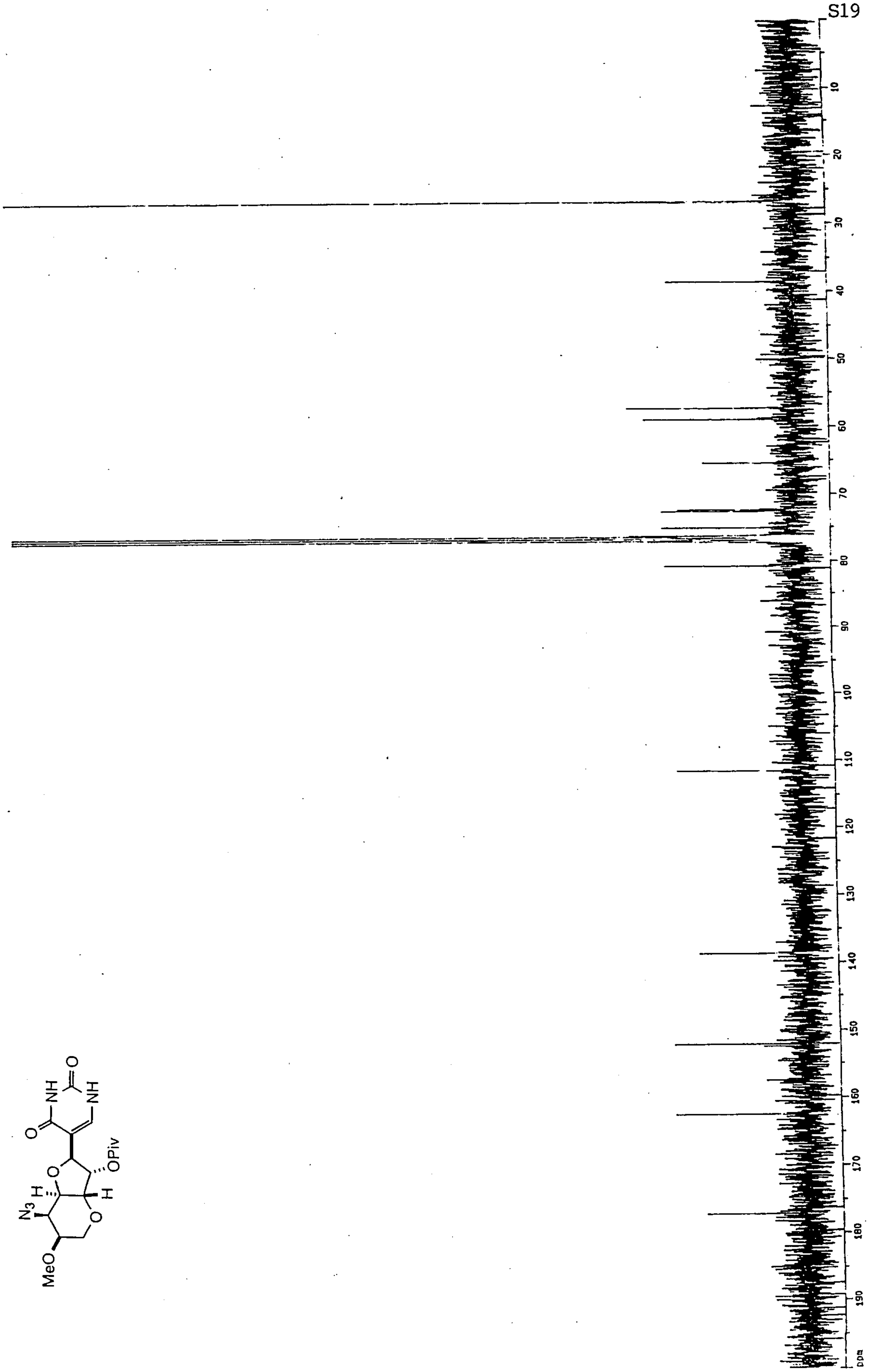


S18

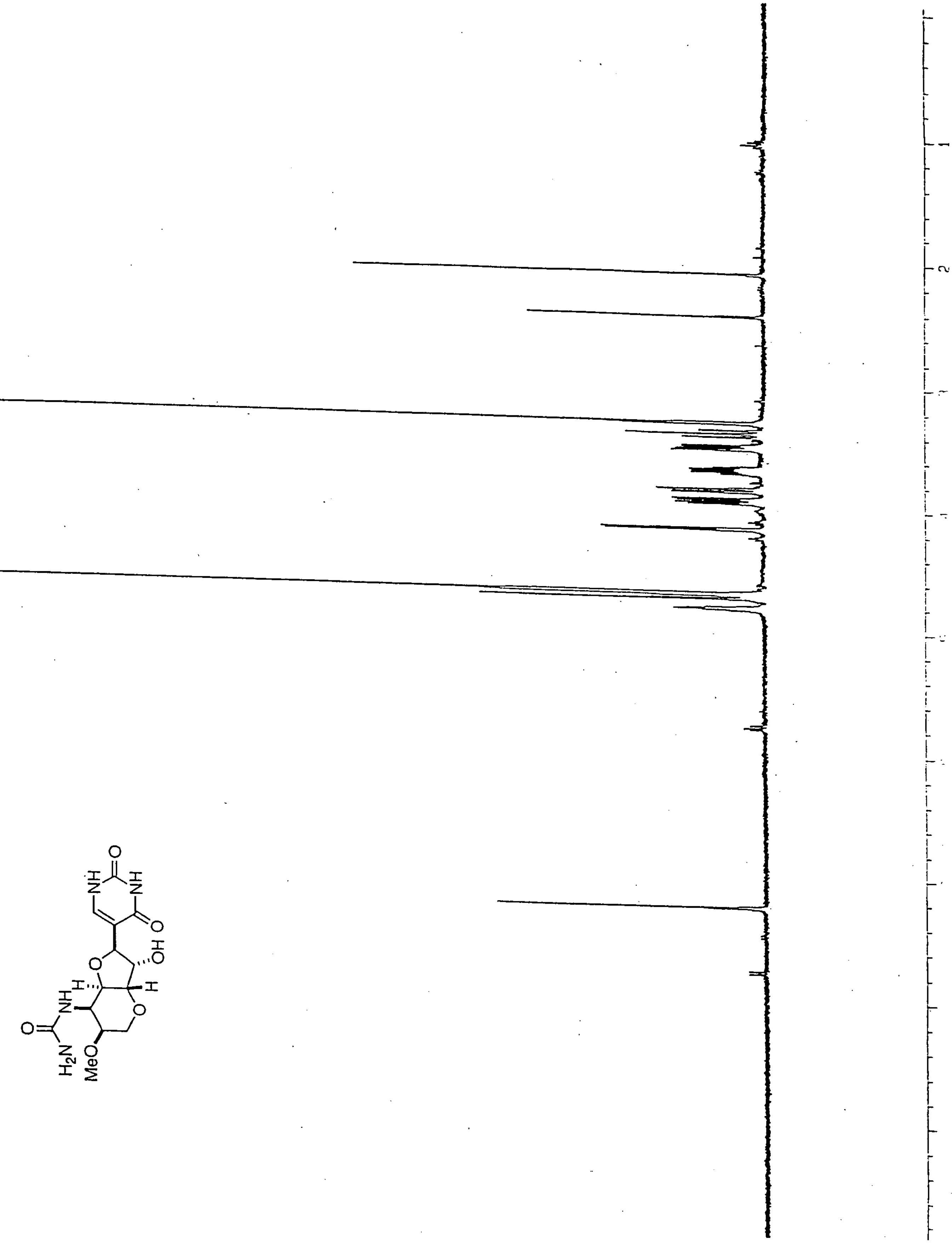
^{1}H NMR 400MHz CDCl$_3$



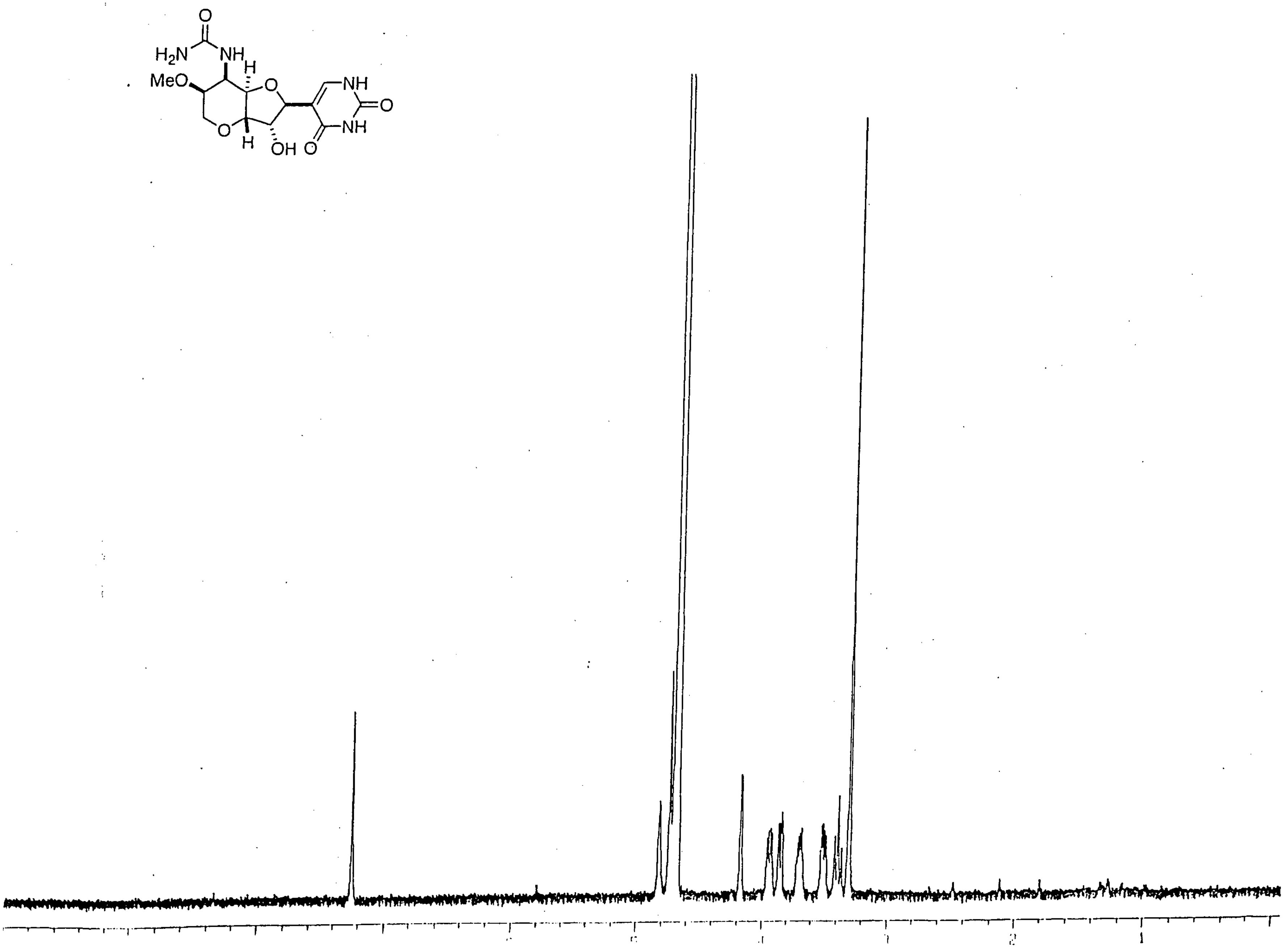
^{13}C NMR 400MHz $CDCl_3$



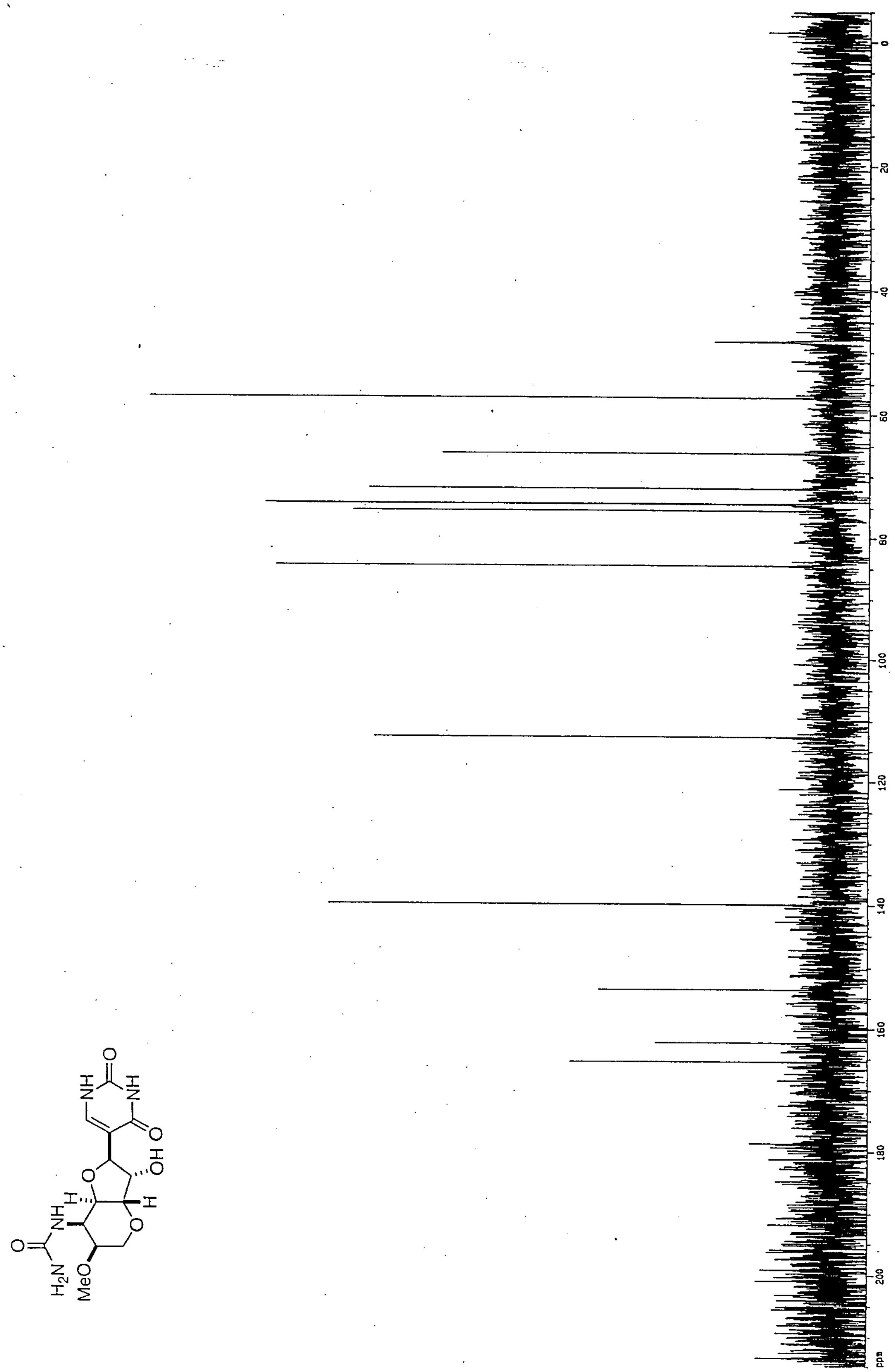
Natural Malayamycin A, 400MHz ^1H NMR in D_2O



Synthetic Malayamycin A, 400MHz ^{1}H NMR in D_2O



S22



Malayamycin A, 400 MHz ^{13}C NMR in D_2O

Supplementary Material

Solvents were distilled under positive pressure of dry argon before use and dried by standard methods: toluene from sodium; THF and ether from sodium/benzophenone ketyl; CH_2Cl_2 from calcium hydride. All commercially available reagents were used without further purification. All non aqueous reactions were performed under argon atmosphere with oven-dried glassware. NMR (^1H and ^{13}C) spectra were recorded on AV-400 and ARX-400 spectrometers. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. High-resolution mass spectra were recorded on VG Micromass, Ael-MS902 or Kratos MS-50 spectrometers using fast atom bombardment (FAB), (TOF CI +). Melting points were determined on a Buchi Type S melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in a 1 dm cell at ambient temperature with a sodium lamp (wavelength of 589 nm). Analytical thin-layer chromatography was performed on Merck 60F_{254} pre-coated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate or potassium permanganate. Chromatographic purifications were performed on a column with 230-400 mesh silica gel (Merck 9385) with the indicated solvent system.

2,4-Dimethoxy-5-[3-*O*-Allyl-5,6-dideoxy-2-*O*-paramethoxybenzyl -D-ribo-hex-5-enofuranosyl]-pyrimidine (10)

To a stirred mixture of dry DMF (1.6 mL) and NaH (67 mg, 1.67 mmol, 60% in a mineral oil) was added dropwise a solution of the alcohol **9** (334 mg, 0.861 mmol) in dry DMF (4.6 mL) and allyl bromide (161 µL, 1.86 mmol)at 0 °C. When the addition was completed, the solution was stirred at room temperature under argon for 2h. A few drops of MeOH were added at 0 °C and the solution was poured into water (50 mL). The aqueous phase was extracted with ether (4x50 mL), the combined organic phase were dried over Na_2SO_4, filtered and concentrated. The oil was purified by flash column chromatography to give a colorless oil (344 mg, 0.80 mmol, 93%).

$[\alpha]_D^{20}$+37.7° (c 0.97, $CHCl_3$), I.R. 2956, 1603, 1571, 1514. ^1H NMR ($CDCl_3$, 400MHz) δ 3.62 (dd, 1H, J=4.8, J=7.5, H-3'), 3.77 (s, 3H, OMe), 3.92 (m, 3H, H-2, CH_2allyl), 3.97 (s, 6H, 2xOMe), 4.52 (t, 1H, J=7.5, H-4), 4.59 (d, 1H, J=11.9, CH_2Ar), 4.65 (d, 1H, J=11.9, CH_2Ar), 5.15-5.3 (m, 5H, H-1, 3xCH vinyl), 5.45 (d, 1H, J=17.1, CH ethylenic), 5.75-6.0 (m, 2H, vinyl), 6.82 (d, 2H, J=8.6, Ar), 7.23 (d, 2H, J=8.6, Ar), 8.21 (s, 1H, H-6). ^{13}C NMR ($CDCl_3$, 400MHz) δ 53.81, 54.67, 55.10, 71.20 (2C), 78.09, ,79.43, 80.81, 81.28, 113.48, 113.54, 117.28, 117.94, 129.31, 129.74, 134.14, 135.87, 156.34, 159.14, 164.81, 167.85. HRMS (FAB) Calcd 429.2025; Found 429.2010

2,4-Dimethoxy-5-[3,7-anhydro-5,6-dideoxy-2-*O*-paramethoxybenzyl -D-*glycero*-β-D-*allo*-hepto-5-enofuranosyl]-pyrimidine (11)

In a 250 mL round-bottomed flask was added dry dichloromethane (160 mL) and the olefin **10** (344 mg, 0.80 mmol). Argon was bubbled for 20 minutes in the solution, Grubb's catalyst (39 mg, 0.047 mmol) was added, a slight flow of argon was applied. The solution was refluxed for 5h30. When the reaction was complete, the mixture was concentrated to give a brown oil. Flash chromatography (8/2 hexanes/ ethyl acetate) gave a white crystalline solid (286 mg, 0.71 mmol, 89%).

M.p. 80°C $[\alpha]_D^{20}$-33.18° (c 1.1, $CHCl_3$), I.R. 2956, 1603, 1602, 1575. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.42 (dd, 1H, J=4.5, J=8.9, H-3'), 3.77 (s, 3H, OMe), 3.93 (m, 1H, H-2'), 3.95 (s, 3H, OMe), 3.96 (s, 3H, OMe), 4.40 (m, 2H, H-7', H-7''), 4.54 (m, 1H, H-4'), 4.65 (d, 1H, J=11.9, CH_2Ar), 4.74 (d, 1H, J=11.9, CH_2Ar), 5.11 (m, 1H, H-1'), 5.69 (dm, 1H, J=10.3, H-5'), 6.28 (d, 1H, d=9.7, H-6'), 6.85 (d, 2H, J=8.7, Ar), 7.28 (d, 2H, J=8.7, Ar), 8.18 (s, 1H, H-6) ^{13}C NMR ($CDCl_3$, 400MHz) δ 53.87, 54.69, 55.11, 68.61, 71.28, 71.63, 78.78, 79.49, 81.72, 113.42, 113.62, 127.14, 127.40, 129.14, 130.01, 156.11, 159.06, 164.84, 167.55. HRMS (MAB) Calcd. 401.1710; Found 401.1699

2,4-Dimethoxy-5-[3,7-anhydro-5-azido -2-*O*-paramethoxybenzyl -L-*glycero*-β-D-*allo*-heptofuranosyl]-pyrimidine (14)

The olefin **11** (133 mg, 0.33 mmol) was dissolved in THF (7.4 mL) and water (7.4 mL). NBS (70 mg, 0.39 mmol) was added to the reaction mixture which was stirred vigorously for 2h in the dark. Then the mixture was poured into water (50 mL) (with one crystal of sodium thiosulfate) and extracted with diethyl ether (5x 25 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. The white solid **12** was dissolved THF (14 mL) and NaOH (3.55 mL, 1M in water) was added. The solution was refluxed for 45 minutes, then poured into water (50 mL). The solution was extracted with diethyl ether (4x 40 mL), the combined organic phase were dried over Na_2SO_4, filtered and concentrated. The oil **13** was then dissolved in methoxyethanol (30 mL) and sodium azide (327 mg, 5.02 mmol) was added. The solution was heated at 126 °C for 1h15. After cooling, the solution was poured into brine (50 mL) and extracted with diethyl ether (4x20 mL). The combined organic phase were dried over Na_2SO_4, filtered and concentrated. The oil was purified by chromatography on silica (7/3 hexanes/ ethyl acetate) to give a white amorphous solid **14** (63 mg, 0.132 mmol, 41%).

M.p. 119°C; $[\alpha]_D^{20}$+93.1° (c 0.98, $CHCl_3$), I.R. 3400, 2915, 2105 (N_3), 1604, 1572. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.40 (b, 1H, OH), 3.58(dd, 1H, J=4.7, J=10.0, H-3'), 3.68 (m, 1H, H-6'), 3.73 (d, J=12.7, 1H, H-7), 3.78 (s, 3H, OMe), 3.89 (m, 2H, H-2', H-7'), 3.97 (s, 6H, 2xOMe), 4.33 (m, 2H, H-5', H-4'), 4.67 (s, 2H, CH_2Ar), 5.10 (m, 1H, H-1'), 6.86 (d, 2H, J=8.7, Ar), 7.27 (d, 2H, J=8.7, Ar), 8.33 (s, 1H, H-6) ^{13}C NMR ($CDCl_3$, 400MHz) δ 53.87, 54.77, 55.12, 61.62, 68.26, 69.25, 71.41, 73.32, 74.16, 79.80, 80.25, 113.26, 113.66, 129.24, 129.65, 155.76, 159.18, 164.83, 167.35. HRMS (FAB) MH^+ Calcd 460.1832; Found 460.1842

2,4-Dimethoxy-5-[3,7-anhydro-5-azido –6-*O*-methyl-2-*O*-paramethoxybenzyl -D-*glycero*-β-D-*allo*-heptofuranosyl]-pyrimidine (16)

The azide **14** (60 mg, 0.129 mmoL) was dissolved in dry DCM (3 mL) under an argon atmosphere, and the Dess-Martin reagent (133 mg, 0.42 mmol) was added. After 3h30, a saturated solution of $NaHCO_3$ (2.5 mL) and $Na_2S_2O_3$ (2.5 mL) were added and the mixture was stirred for 10 minutes. DCM (25 mL) was added, the organic phase was separated and washed with $NaHCO_3$ sat (2x10 mL). After drying over Na_2SO_4, the solution was filtered and concentrated. The oily residue **15** was dissolved in dry MeOH

(6 mL), treated with $NaBH_4$ (50 mg, 1.32 mmol) at 0 °C and the solution was stirred at room temperature for 10 minutes. The solution was concentrated under vacuum without heating. It was then dissolved in ethyl acetate (20 mL) and washed with water (20 mL). The organic phase was separated and the aqueous layer was extracted with ethyl acetate (2x20 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give an oil. To a stirred mixture of DMF (0.5 mL) and NaH (19 mg, 60%, 0.49 mmol) at 0 °C, was added a solution of the alcohol, MeI (38 μL, 5.9 mmol) in DMF 2 mL dropwise. The mixture was left at room temperature for 2h, a few drops of methanol were added at 0 °C and the solution was poured into cold water (20 mL). It was then extracted with ether (5x10 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated. The oil was purified by flash column chromatograpy under silica (8/2 hexanes / ethyl acetate) to give a white powder **16** (56 mg, 0.121 mmol, 93%, 3 steps).

M.p. 89°C; $[\alpha]_D^{20}$+60.56° (c 0.88, $CHCl_3$), I.R. 2913, 2105 (N_3), 1602, 1572. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.47 (s, 3H, OMe), 3.61 (m, 2H, H-7', H-6'.), 3.76 (m, 4H, OMe, H-3'), 3.85 (d, J=4.63, 1H, H-2'), 3.96 (s, 3H, OMe), 3.97 (s, 3H, OMe), 4.01 (dd, J=10.04, J=2.91, 1H, H-4'), 4.67 (m, 3H, CH_2Ar, H-5'), 5.16 (s, 1H, H-1'), 6.86 (d, 2H, J=8.6, Ar), 7.26 (d, 2H, J=8.6, Ar), 8.44 (s, 1H, H-6) ^{13}C NMR ($CDCl_3$, 400MHz) δ 53.86, 54.76, 55.13, 57.13, 57.44, 59.72, 65.69, 71.17, 74.02, 75.01, 76.41, 78.89, 81.54, 113.31, 113.66, 129.76, 156.02, 159.10, 164.88, 167.23. HRMS (FAB) MH^+ Calcd. 474.1988; Found 474.2002

2,4-Dimethoxy-5-[3,7-anhydro-5-azido –6-*O*-methyl -D-*glycero*-β-D-*allo*-heptofuranosyl]-pyrimidine. (16 a)

Compound **16** (43 mg, 0.09 mmol) was dissolved in DCM (8 mL) and water (0.8 mL) and DDQ (80 mg, 0.35 mmol) was added. After 1h, saturated $NaHCO_3$ (30 mL) was added, the organic layer was separated and the aqueous phase was extracted with EtOAc (3x10 mL). The combined organic phases were washed with water (10 mL) and brine (10 mL). After drying over Na_2SO_4 and concentrating, the oil was purified by flash chromatography (DCM then DCM/MeOH, 95/5) to give a colorless oil **16 a** (27 mg, 0.076 mmol, 84%).

$[\alpha]_D^{20}$+93° (c 0.3, $CHCl_3$). IR (Neat) 3540, 2920, 2105, 1603, 1574. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.49 (s, 3H, OMe), 3.57 (ddd, 1H, J=2.91, J=4.85, J=10.4, H-6'.), 3.65 (t, J=10.4, 1H, H-7'ax), 3.76 (dd, J=4.64, J=9.8, 1H, H-3'), 3.94 m(m, 2H, H-4', H-7'eq), 3.98 (m, 4H, OMe), 4.02 (s, 3H, OMe), 4.10 (d, , J=4.6, 1H, H-2'), 4.67 (m, 2H, CH_2Ar), 5.06 (s, 1H, H-1'), 8.41 (s, 1H, H-6) ^{13}C NMR ($CDCl_3$, 400MHz) δ 54.06, 54.79, 57.43, 59.40, 65.67, 73.05, 73.65, 74,67, 76.28, 83.53, 112.91, 155.77, 164.41, 167.61. HRMS (FAB) MH^+ Calcd. 353.1335; Found 353.1341

5-[3,7-anhydro-5-azido –2-*O*-(2,2-dimethylpropanoyl)-6-*O*-methyl -D-*glycero*-β-D-*allo*-heptofuranosyl]-pyrimidin-3,4-dione (18).

The above compound (33 mg, 0.0926 mmol) was dissolved in dry pyridine (1.1 mL). To this solution was added DMAP (16 mg, 0.13 mmol), NEt_3 (31 μL, 0.265 mmol) and pivaloyl chloride (80 μL, 0.649 mmol) and the solution was stirred for 36 h at room temperature. It was then concentrated, the residue was dissolved EtOAc (20 mL), the organic layer was washed with 1% HCl solution (5 mL), then a saturated solution of $NaHCO_3$ (5 mL) and brine (5 mL). After drying over Na_2SO_4, the solution filtered, concentrated, and the oil was dissolved in dry acetonitrile (0.5 mL). Dry NaI (32 mg, 0.21 mmol), and TMSCl (29 µL, 0.21 mmol) were added and the solution was stirred at room temperature for 16 h. Several drops of a 10% sodium metabisulfite solution were added until a colorless solution was obtained. Then a saturated solution of $NaHCO_3$ (10 mL) was added, the aqueous layer was extracted with EtOAc (5x10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated. The oily residue was purified by flash chromatography to give a colorless oil **18** (0.0388 mmol, 2 steps).

$[\alpha]_D^{20}$+100.75° (c 0.4, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.22, (s, 9H, tBu), 3.46 (s, 3H, OMe), 3.47 (ddd, 1H, J=2.9, J=4.0, J=10.7, H-6'.), 3.59 (t, J=10.7, 1H, H-7'ax), 3.75 (dd, J=2.7, J=10.0, 1H, H-4'), 3.86 (m, 2H, H-3', H-7'eq), 4.65 (s, 1H, H-5'), 4.91 (s, 1H, H-1'), 5.31 (d, 1H, J=4.7, H-2'), 7.55 (s, 1H, H-6), 10.00 (br, 2H, 2xNH). ^{13}C NMR ($CDCl_3$, 400MHz) δ 26.97, 38.70, 57.38, 59.07, 65.55, 72.56, 72.81, 75.19, 76.35, 80.87, 111.63, 138.82, 152.17, 162.47, 171.14. HRMS (FAB) MH^+ Calcd. 410.1675; Found 410.1693

Malayamycin A (1).

Compound **18** (16 mg, 0.0388 mmol) was dissolved in dry THF (3 mL) and argon was bubbled in the solution for 10 minutes. Water (3 µL) and PMe_3 (44 µL, 1M solution in toluene, 0.044 mmol) were added. After 5 minutes at room temperature, the solution was refluxed for 30 minutes, then concentrated and held under vacuum for 1h. It was then dissolved in dry DCM (4 mL) and trichloroacetylisocyanate (5mL, 0.041 mmol) was added. After 30 minutes, the solution was concentrated, the oily residue was dissolved in MeOH (1 mL), 40% $MeNH_2$ in water (2 mL) was added, and the solution was stirred for 52 h. Concentration gave a solid that was purified by flash chromatography (9/1 DCM/MeOH) to give pure Malayamycin A as a white solid (8 mg, 0.0233 mmol, 60%).

M.p. 158°C (dec) $[\alpha]_D^{20}$+120° (c 0.19, MeOH); (authentic sample $[\alpha]_D^{20}$+126° (c 0.36, MeOH)) ^{1}H NMR (D_2O, 400MHz) identical to the authentic sample δ 3.30 (s, 3H, OMe), 3.38 (t, 1H, J=10.7, H-7ax'.), 3.51 (dd, J=10.7, J=5.1, 1H, H-3'), 3.69 (ddd, J=5.2, J=3.5, J=10.7, 1H, H-6'), 3.85 (dd, J=3.5, J=11.8, 1H, H-7eq'), 3.93 (dd, J=10.7, J=5.4, 1H, H-6'), 4.16 (d, 1H, J=2.1, H-2'), 4.74 (s, 1H, H-1'), 4.82 (s, 1H, H-5'), 7.24 (s, 1H, H-6). HRMS (FAB) MH^+ Calcd. 342.1176; Found 342.1174.