Electronic Supplementary Material (ESI) for RSC Advances.


# New total synthesis and structure confirmation of putative (+)-hyacinthacine $C_3$ and (+)-5-*epi*-hyacinthacine $C_3$

Lívia Dikošová,[a] Barbora Otočková,[a] Tomáš Malatinský,[a] Jana Doháňošová,[b] Mária Kopáčová,[c] Anna Ďurinová,[d] Lucie Smutná,[d] František Trejtnar,*[d] and Róbert Fischer*[a]

[a] *Institute of Organic Chemistry, Catalysis and Petrochemistry, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic*

[b] *Central Laboratories, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic*

[c] *Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovak Republic*

[d] *Charles University, Faculty of Pharmacy in Hradec Kralove, Heyrovskeho 1203, 50005 Hradec Kralove, Czech Republic*

E-mail: robert.fischer@stuba.sk

E-mail: trejtnarf@faf.cuni.cz

# Supporting Information

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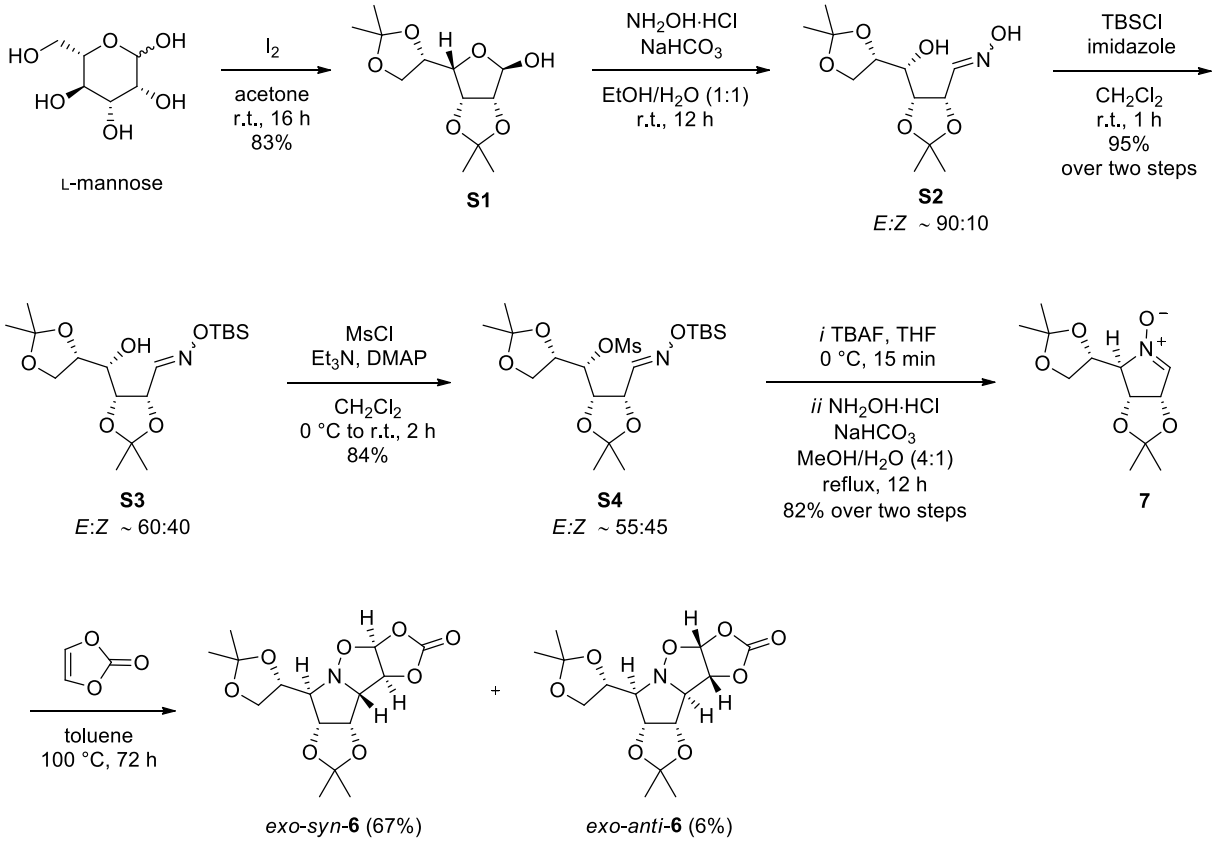
# Experimental section

## General methods

Melting points were measured with a Melting Point B-540 apparatus (Büchi). Flash column chromatography (FCC) was carried out with a Büchi system (Pump Manager C-615 and Fraction Collector C-660) using Normasil 60 silica gel (0.040–0.063 mm; VWR). Thin Layer Chromatography (TLC) analysis was carried out using TLC silica gel 60 $F_{254}$ (aluminium sheets, Merck), and plates were visualized with UV light or by treatment with permanganate solution followed by heating. Optical rotations were measured with a JASCO P-2000 digital polarimeter with a Na-D lamp (10 cm cell length). Concentrations (*c*) are given in gram per 100 mL. Infrared (IR) spectra were recorded as neat samples with a Nicolet 5700 FTIR spectrometer with an ATR Smart Orbit Diamond adapter (Thermo Electron Corporation). NMR spectra were recorded with a Varian VNMRS-600 instrument ($^1$H, 599.75 MHz, and $^{13}$C, 150.81 MHz) in $CDCl_3$ using tetramethylsilane as the internal standard. Data are presented as follows: chemical shift, multiplicity, coupling constants and integration. MS analysis was carried out with an Agilent 1260B LCMS system with a multimode ion source (ESI + APCI) in positive mode, 50% scan and 50% SIM (selected-ion monitoring). HRMS analysis was carried out with an Orbitrap Velos Pro spectrometer (Thermo Fisher Scientific). Acetone was distilled in the presence of anhydrous calcium chloride and stored over 4A° molecular sieves. Dichloromethane was distilled in the presence of calcium hydride. Tetrahydrofuran and toluene were distilled in the presence of sodium. Simple distillation was used to purify other solvents prior to use if not stated otherwise.

## Synthesis of isoxazolidine intermediate *exo-syn*-6 from L-mannose



## 2,3:5,6-Di-*O*-isopropylidene-α-L-mannofuranose (S1)

L-Mannose (10.0 g, 55.5 mmol, 1 equiv.) was dissolved in acetone (500 mL). Iodine (2.8 g, 11 mmol, 0.2 equiv.) was added, and the mixture was stirred at room temperature for 16 h. After this time, saturated aqueous solution of $Na_2S_2O_3$ (150 mL) was added until complete decolouration of the solution occurred, followed by addition of saturated aqueous solution of $NaHCO_3$ (150 mL) to reach pH ~ 8. The mixture was vigorously stirred for 5 min, and acetone was evaporated under reduced pressure. The residue was extracted with $CH_2Cl_2$ (4 × 150 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by crystallisation (acetone/hexanes) to give pure α-anomer of protected mannofuranose **S1** (12 g, 46.1 mmol, 83%) as a white crystalline solid with analytical data[1a] and $^1$H and $^{13}$C NMR spectral data for D-enantiomer[1b] in good agreement with those published in the literature.

**R*f*** = 0.19 (*n*-hexane/EtOAc, 7:3), m.p. = 122–124 °C, $[\alpha]_D^{25}$ = –8.78 (*c* = 1.01, $CHCl_3$). **IR** (ATR) $\nu_{max}$: 3427, 2984, 2949, 2899, 1372, 1202, 1059, 975, 953, 890, 837, 755, 683, 515 cm$^{-1}$. **$^1$H NMR** (300 MHz, $CDCl_3$) δ (ppm) 1.33 (s, 3 H, $CH_3$), 1.38 (s, 3 H, $CH_3$), 1.46 (s, 3 H, $CH_3$), 1.47 (s, 3 H, $CH_3$), 3.25 (d, *J* = 2.6 Hz, 1 H, OH), 4.05 (dd, *J* = 4.8, 8.7 Hz, 1 H, $CH_2O$), 4.08 (dd, *J* = 6.2, 8.7 Hz, 1 H, $CH_2O$), 4.19 (dd, *J* = 3.6, 7.1 Hz, 1 H, H-4′), 4.41 (ddd, *J* = 5.0, 5.9, 7.1 Hz, 1 H, CHO), 4.61 (d, *J* = 5.9 Hz, 1 H, H-2′), 4.81 (dd, *J* = 3.6, 5.9 Hz, 1 H, H-3′), 5.38 (d, *J* = 2.6 Hz, 1 H, H-1′). **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ (ppm) 24.6, 25.3, 26.0, 26.9, 66.6, 73.4, 79.8, 80.3, 85.6, 101.4, 109.2, 112.8. **MS** (ESI+APCI) *m/z* 261 ([M+H]$^+$, 100%), 235 (37), 221 (28), 203 (29), 185 (31), 163 (63).

## (*E*, *Z*)-2,3:5,6-Di-*O*-isopropylidene-L-mannose Oxime (S2)

Hydroxylamine hydrochloride (14.7 g, 212 mmol, 4.6 equiv.) was dissolved in EtOH/$H_2O$ (1:1, v/v, 230 mL). Afterwards, solid $NaHCO_3$ (15.4 g, 184 mmol, 4 equiv.) was added in portions, followed by addition of protected α-L-mannofuranose **S1** (12 g, 46.1 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 12 h and the progress of the reaction was monitored by TLC ($CH_2Cl_2$/MeOH, 95:5). After the starting material was no longer present in the reaction mixture, the solvent was partially removed under reduced pressure, and the residue was diluted with brine (100 mL). The mixture was extracted with EtOAc (5 × 100 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The obtained oximes **S2** (15 g, *E*/*Z* ~ 90:10 ratio) were used in the next reaction without further purification.

**R*f*** (*E*, *Z*) = 0.30 (*n*-hexane/EtOAc, 1:1). **IR** (ATR) $\nu_{max}$ (*E*, *Z*): 3372, 3276, 2997, 2942, 2895, 1375, 1257, 1209, 1143, 1052, 894, 865, 571, 513 cm$^{-1}$. $^1$H NMR spectra of the major *E*-isomer extracted from the corresponding mixture: **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 1.34 (s, 3 H, $CH_3$), 1.41 (s, 3 H, $CH_3$), 1.43 (s, 3 H, $CH_3$), 1.52 (s, 3 H, $CH_3$), 3.72 (d, *J* = 6.3 Hz, 1 H, H-4), 4.04–4.08 (m, 1 H, H-6$_a$), 4.16–4.20 (m, 2 H, H-6$_b$, H-5), 4.57 (d, *J* = 7.6 Hz, 1 H, H-3), 4.66 (d, *J* = 6.4 Hz, 1 H, OH), 5.25 (dd, *J* = 3.5, 7.6 Hz, 1 H, H-2), 7.12 (d, *J* = 3.5 Hz, 1 H, H-1), 9.82 (s, 1 H, N-OH). $^{13}$C NMR spectra of the major *E*-isomer extracted from

the corresponding mixture: **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ (ppm) 24.9, 26.0, 26.1, 26.2, 65.1, 67.6, 73.1, 78.1, 78.7, 108.6, 109.8, 152.3. The NMR spectra of the minor *Z*-isomer [7.61 ppm (d, *J* = 7.7 Hz, 1 H, H-1)] are not reported due to the low clarity of the signals in the spectra of the corresponding mixture. **MS** (ESI+APCI) *m/z* 276 ($[M+H]^+$, 100%), 258 (10), 236 (5), 218 (6).

## (*E, Z*)-2,3:5,6-Di-*O*-isopropylidene-L-mannose-*O*-(*tert*-butyldimethylsilyl) Oxime (S3)

Oximes **S2** (15 g) from the previous reaction were dissolved in $CH_2Cl_2$ (230 mL) and the solution was cooled down to 0 °C in an ice bath. Imidazole (7.5 g, 110 mmol, 2.4 equiv.) and a solution of *tert*-butyldimethylsilyl chloride (50 wt% in toluene, 19.2 mL, 55.3 mmol, 1.2 equiv.) were added. The ice bath was removed, and the reaction mixture was stirred at room temperature for 1 h. The reaction progress was monitored by TLC (hexanes/EtOAc, 2:3). After this time, water (100 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous layer was extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by FCC (hexanes/EtOAc, 85:15) to give an inseparable *E*/*Z* mixture of silylated oximes **S3** (17 g, 43.6 mmol, 95% over two steps, *E*/*Z* ~ 60:40 ratio) as a colourless oil.

**R*f*** (*E*, *Z*) = 0.65 (*n*-hexane/EtOAc, 2:3). **IR** (ATR) $\nu_{max}$ (*E*, *Z*): 3508, 2931, 1471, 1371, 1251, 1211, 1159, 1063, 914, 838, 784, 688, 515 $cm^{-1}$. $^1$H NMR spectra of the major *E*-isomer extracted from the corresponding mixture: **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 0.17 (s, 3 H, $SiCH_3$), 0.18 (s, 3 H, $SiCH_3$), 0.94 (s, 9 H, Si*t*-Bu), 1.35 (s, 3 H, $CH_3$), 1.38 (s, 3 H, $CH_3$), 1.40 (s, 3 H, $CH_3$), 1.54 (s, 3 H, $CH_3$), 1.95 (d, *J* = 10.0 Hz, 1 H, OH), 3.34 (ddd, *J* = 0.9, 8.2, 9.9 Hz, 1 H, H-4), 3.91 (dd, *J* = 5.8, 8.5 Hz, 1 H, H-$6_a$), 3.97–4.11 (m, 2 H, H-5, H-$6_b$), 4.67 (dd, *J* = 1.0, 7.8 Hz, 1 H, H-3), 5.34 (dd, *J* = 4.1, 7.8 Hz, 1 H, H-2), 7.20 (d, *J* = 4.1 Hz, 1 H, H-1). $^1$H NMR spectra of the minor *Z*-isomer extracted from the corresponding mixture: **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 0.16 (2 × s, 2 × 3 H, $SiCH_3$), 0.93 (s, 9 H, Si*t*-Bu), 1.35 (s, 3 H, $CH_3$), 1.39 (s, 3 H, $CH_3$), 1.41 (s, 3 H, $CH_3$), 1.53 (s, 3 H, $CH_3$), 2.25 (d, *J* = 9.3 Hz, 1 H, OH), 3.46 (ddd, *J* = 1.4, 7.8, 9.2 Hz, 1 H, H-4), 3.97–4.11 (m, 3 H, H-5, H-$6_{a,b}$), 4.50 (dd, *J* = 1.4, 7.7 Hz, 1 H, H-3), 4.82 (pseudo t, *J* = 7.8 Hz, 1 H, H-2), 7.67 (d, *J* = 7.9 Hz, 1 H, H-1). **$^{13}$C NMR** (150 MHz, $CDCl_3$) carbon signals were observed at δ (ppm) –5.2 (3 × C), –5.0, 18.1, 18.3, 24.1, 24.6, 25.4 (2 × C), 26.2 (2 × C), 26.5, 26.8, 26.9 (2 × C), 67.1, 67.5, 70.1, 71.2, 72. 6, 75.2, 76.0, 76.2, 76.7, 77.0, 109.2, 109.5, 109.6, 109.8, 153.1, 155.2. **MS** (ESI+APCI) *m/z* 390 ($[M+H]^+$, 100%), 350 (6), 258 (11).

## (*E, Z*)-2,3:5,6-Di-*O*-isopropylidene-4-*O*-methanesulfonyl-L-mannose-*O*-(*tert*-butyldimethylsilyl) Oxime (S4)

Oximes **S3** (16.9 g, 43.4 mmol, 1 equiv.) were dissolved in $CH_2Cl_2$ (220 mL) and the stirring solution was cooled down in an ice bath to 0 °C. Afterwards, DMAP (1.59 g, 13.0 mmol, 0.3 equiv.) and $Et_3N$ (18.1 mL, 130 mmol, 3 equiv.) were added. Subsequently, MsCl (5.1 mL, 65.9 mmol, 1.5 equiv.) was added dropwise.

The ice bath was removed, and the reaction was stirred at room temperature for 2 h. The reaction progress was monitored by TLC ($CH_2Cl_2$/MeOH, 98:2). After the starting material was no longer present in the reaction mixture, saturated aqueous solution of $NH_4Cl$ (150 mL) was added, and the organic layer was separated. The aqueous layer was washed $CH_2Cl_2$ (150 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by FCC (hexanes/EtOAc, 9:1) to give an inseparable *E*/*Z* mixture of mesylated oximes **S4** (17 g, 36.4 mmol, 84%, *E*/*Z* ~ 55:45 ratio) as a colourless oil.

**R*f*** (*E*, *Z*) = 0.30 ($CH_2Cl_2$). **IR** (ATR) $\nu_{max}$ (*E*, *Z*): 2933, 2858, 1356, 1252, 1217, 1174, 1061, 945, 832, 777, 669, 514 $cm^{-1}$. $^1H$ NMR spectra of the major *E*-isomer extracted from the corresponding mixture: **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 0.20 (s, 6 H, 2 × $SiCH_3$), 0.95 (s, 9 H, Si*t*-Bu), 1.35 (s, 3 H, $CH_3$), 1.39 (s, 3 H, $CH_3$), 1.42 (s, 3 H, $CH_3$), 1.54 (s, 3 H, $CH_3$), 3.10 (s, 3 H, OMs), 3.96 (dd, *J* = 7.6, 8.6 Hz, 1 H, H-$6_a$), 4.11 (dd, *J* = 6.3, 8.6 Hz, 1 H, H-$6_b$), 4.17–4.22 (m, 1 H, H-5), 4.63 (dd, *J* = 2.5, 6.9 Hz, 1 H, H-3), 4.68 (dd, *J* = 2.6, 5.1 Hz, 1 H, H-4), 5.25 (dd, *J* = 3.8, 6.9 Hz, 1 H, H-2), 7.24 (d, *J* = 3.9 Hz, 1 H, H-1). $^1H$ NMR spectra of the minor *Z*-isomer extracted from the corresponding mixture: **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 0.17 (2 × s, 2 × 3 H, $SiCH_3$), 0.93 (s, 9 H, Si*t*-Bu), 1.34 (s, 3 H, $CH_3$), 1.40 (s, 3 H, $CH_3$), 1.43 (s, 3 H, $CH_3$), 1.53 (s, 3 H, $CH_3$), 3.12 (s, 3 H, OMs), 4.01 (dd, *J* = 7.3, 8.4 Hz, 1 H, H-$6_a$), 4.08 (dd, *J* = 6.4, 8.4 Hz, 1 H, H-$6_b$), 4.17–4.22 (m, 1 H, H-5), 4.27 (dd, *J* = 5.9, 8.2 Hz, 1 H, H-3), 4.72 (dd, *J* = 5.9, 7.6 Hz, 1 H, H-2), 4.92 (dd, *J* = 4.7, 8.2 Hz, 1 H, H-4), 7.54 (d, *J* = 7.6 Hz, 1 H, H-1). **$^{13}$C NMR** (150 MHz, $CDCl_3$) carbon signals were observed at δ (ppm) –5.2, –5.1 (2 × C), –5.0, 18.1, 18.2, 25.3 (2 × C), 25.4, 25.9, 26.1, 26.2 (2 × C), 26.4, 26.5, 27.7, 39.5, 40.0, 65.4, 66.2, 72.6, 73.9, 74.9, 75.0, 76.7, 77.2, 78.7, 79.0, 109.4, 109.8, 110.2 (2 × C), 150.8, 152.3. **MS** (ESI+APCI) *m/z* 468 ($[M+H]^+$, 100%), 428 (6), 258 (10).

## (3a*R*,4*R*,6a*S*)-4-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-2,2-dimethyl-4,6a-dihydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyrrole *N*-oxide (7)

A mixture of oximes **S4** (16.8 g, 35.9 mmol, 1 equiv.) was placed into a reaction flask which was sealed with a rubber septum, evacuated, and filled with argon. The oximes were dissolved in anhydrous THF (240 mL), and the solution was cooled down to 0 °C in an ice bath. Subsequently, TBAF (1 M solution in THF, 39.5 mL, 39.5 mmol, 1.1 equiv.) was added, and the mixture was stirred at 0 °C for 15 minutes. The progress of the reaction was monitored by TLC (hexanes/EtOAc, 1:4). After this time, THF was evaporated under reduced pressure. The residue was washed with brine (120 mL), and the mixture was extracted with EtOAc (6 × 120 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure to give the desilylated oxime together with a small amount of nitrone **7**. The residue was dissolved in MeOH/$H_2O$ (4:1, v/v, 360 mL), and solid $NaHCO_3$ (22.9 g, 273 mmol, 7.6 equiv.) was added, together with hydroxylamine hydrochloride (19.9 g, 287 mmol, 8 equiv.). The reaction mixture was heated under reflux for 12 h. After the TLC (EtOAc) showed complete consumption of the starting material, the solvent was partially

evaporated under reduced pressure. Brine (100 mL) was added, and the product was extracted with EtOAc (4 × 150 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated to dryness. The residue was purified by FCC (hexanes/EtOAc, 1:4) to give nitrone **7** (7.57 g, 29.4 mmol, 82% over two steps) as a colourless white solid with spectroscopic properties for enantiomer.[2]

**R*f*** = 0.15 (*n*-hexane/EtOAc, 1:4), **m.p.** = 86–88 °C, $[\alpha]_D^{25}$ = –1.91 (*c* = 1.02, $CHCl_3$). **IR** (ATR) $\nu_{max}$: 3080, 2986, 2943, 1570, 1371, 1261, 1202, 1156, 1052, 892, 860, 832, 789, 665, 506, 434 $cm^{-1}$. **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 1.37 (s, 6 H, 2 × $CH_3$), 1.38 (s, 3 H, $CH_3$), 1.45 (s, 3 H, $CH_3$), 4.09 (dd, *J* = 6.6, 8.8 Hz, 1 H, H-$7_a$), 4.19–4.21 (m, 1 H, H-5), 4.46 (dd, *J* = 6.6, 8.8 Hz, 1 H, H-$7_b$), 4.53 (td, *J* = 2.5, 6.6 Hz, 1 H, H-6), 4.80 (d, *J* = 5.9 Hz, 1 H, H-4), 5.25 (dt, *J* = 1.3, 5.9 Hz, 1 H, H-3), 6.91 (s, 1 H, H-2). **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ (ppm) 25.4 ($CH_3$), 26.1 (2 × $CH_3$), 27.5 ($CH_3$), 65.0 (C-7), 74.3 (C-6), 78.1 (C-4), 78.7 (C-5), 79.3 (C-3), 109.9 [$\underline{C}(CH_3)_2$], 111.9 [$\underline{C}(CH_3)_2$], 134.0 (C-2). **HRMS** (ESI) *m/z*: for $C_{12}H_{20}NO_5$ $[M+H]^+$, calcd: 258.1341; found: 258.1336.

## (4*S*,5*R*,6*R*)-6-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-4,5-isopropylidenedioxyhexahydropyrrolo[1,2-*b*]isoxazol-2,3-diyl carbonate (6)

Nitrone **7** (2.92 g, 11.35 mmol, 1 equiv.) was dissolved in toluene (80 mL) and vinylene carbonate (2.93 g, 34.05 mmol, 3 equiv.) was added. The reaction flask was sealed with a rubber septum and the mixture was stirred at 100 °C for 72 h. After TLC (EtOAc) showed complete disappearance of the starting material, the solvent was removed under reduced pressure. The residue was purified by FCC (hexanes/EtOAc 3:2) to give two pure isoxazolidines (2*R*,3*S*,3a*S*)-**6** (2.60 g, 7.57 mmol, 67%) and (2*S*,3*R*,3a*R*)-**6** (235 mg, 0.68 mmol, 6%) as white solids.

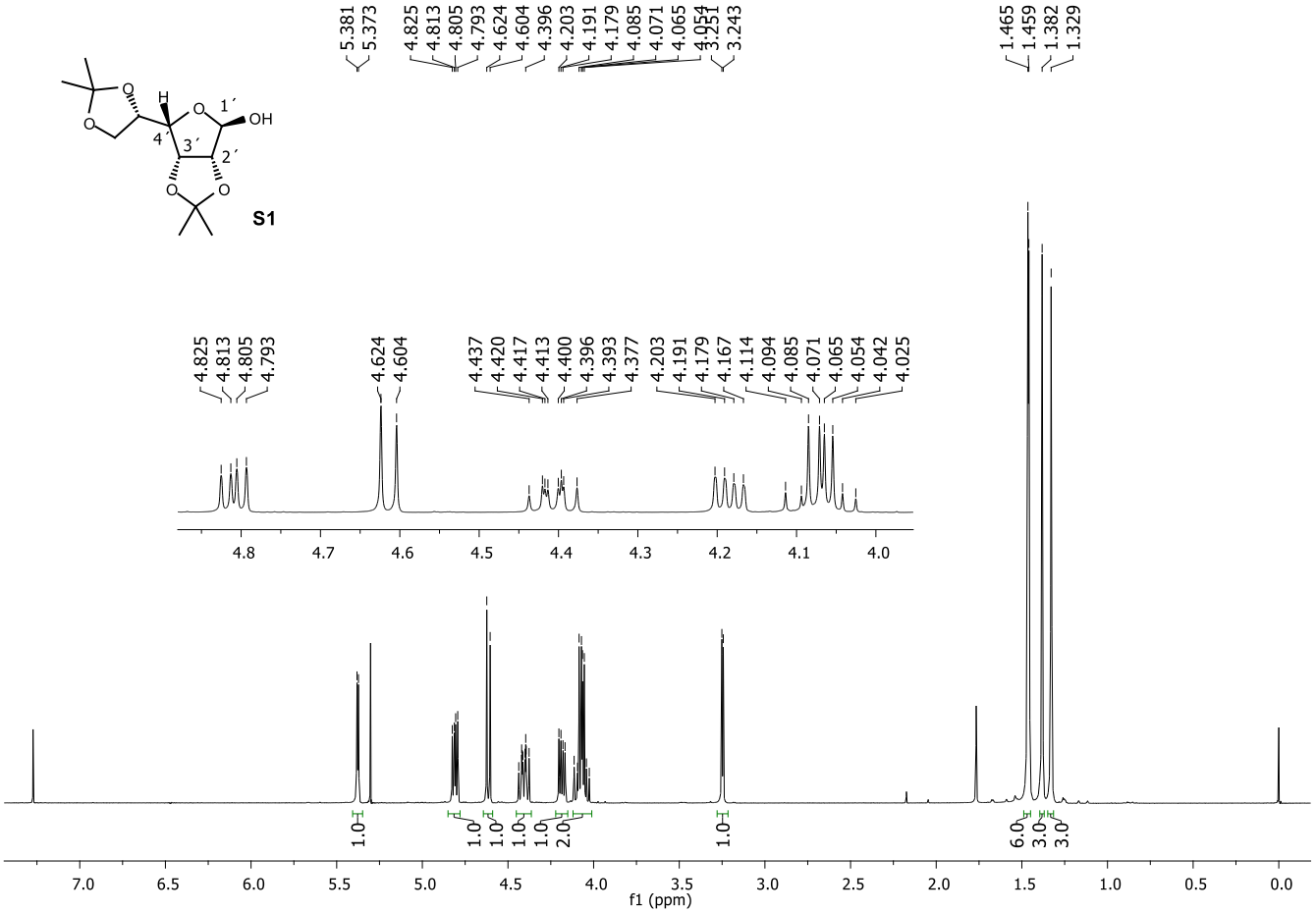
Data for (2*R*,3*S*,3a*S*)-**6** (*exo-syn*)

**$R_f$** = 0.29 (*n*-hexane/EtOAc 3:2), **m.p.** = 190–192 °C, $[\alpha]_D^{25}$ = –104.9 (*c* 0.99, $CHCl_3$). **IR** (ATR) $\nu_{max}$: 2990; 2940; 1794; 1381; 1220; 1082; 1055; 987; 863; 771; 724; 505 $cm^{-1}$. **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 1.29 (s, 3H, $CH_3$), 1.32 (s, 3H, $CH_3$), 1.33 (s, 3H, $CH_3$), 1.42 (s, 3H, $CH_3$), 3.91 (d, *J* = 2.0 Hz, 1H, H-6), 4.03 (d, *J* = 5.7 Hz, 1H, H-3a), 4.02–4.06 ( m, 2H, H-5′$_a$, H-5′$_b$), 4.23 (ddd, *J* = 2.3, 7.0, 7.9 Hz, 1H, H-4′), 4.88 (pseudo t, *J* = 5.7, 5.9 Hz, 1H, H-4), 4.97 (dd, *J* = 0.9, 6.2 Hz, 1H, H-5), 5.58 (d, *J* = 5.2 Hz, 1H, H-3), 6.01 (d, *J* = 5.2 Hz, 1H, H-2). **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ (ppm) 23.7 ($CH_3$), 25.7 ($CH_3$), 25.8 ($CH_3$), 26.1 ($CH_3$), 66.2 (C-5′), 71.0 (C-6), 74.5 (C-3a), 76.1 (C-4′), 81.6 (C-4), 84.9 (C-3), 87.1 (C-5), 100.9 (C-2), 109.7 [$\underline{C}(CH_3)_2$], 113.4 [$\underline{C}(CH_3)_2$], 153.1 (C=O). **HRMS** (ESI) *m/z*: for $C_{15}H_{21}NNaO_8$ $[M+Na]^+$, calcd: 366.1160; found: 366.1158.

Data for (2*S*,3*R*,3a*R*)-**6** (*exo-anti*)

**$R_f$** = 0.13 (*n*-hexane/EtOAc 3:2), **m.p.** = 167–169 °C, $[\alpha]_D^{25}$ = +132.0 (*c* 1.02, $CHCl_3$). **IR** (ATR) $\nu_{max}$: 2981; 1794; 1369; 1184; 1063; 1022; 984; 841; 767; 714; 506 $cm^{-1}$. **$^1$H NMR** (600 MHz, $CDCl_3$) δ (ppm) 1.32 (s, 3H, $CH_3$), 1.40 (s, 3H, $CH_3$), 1.48 (s, 3H, $CH_3$), 1.54 (s, 3H, $CH_3$), 3.47 (ddd, *J* = 1.7, 7.0, 7.9 Hz, 1H, H-6),

3.90 (dd, *J* = 6.7, 8.7 Hz, 1H, H-5′$_a$), 3.98 ( d, *J* = 4.5 Hz, 1H, H-3a), 4.11 (dd, *J* = 6.4, 8.7 Hz, 1H, H-5′$_b$), 4.50 (dt, *J* = 6.5, 7.9 Hz, 1H, H-4′), 4.57 (dd, *J* = 4.5, 6.9 Hz, 1H, H-4), 4.68 (t, *J* = 6.9 Hz, 1H, H-5), 5.50 (d, *J* = 5.2 Hz, 1H, H-3), 6.13 (ddd, *J* = 0.5, 1.7, 5.2 Hz, 1H, H-2). **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ (ppm) 23.7 ($CH_3$), 25.7 ($CH_3$), 25.8 ($CH_3$), 26.1 ($CH_3$), 66.2 (C-5′), 71.0 (C-6), 74.5 (C-3a), 76.1 (C-4′), 81.6 (C-4), 84.9 (C-3), 87.1 (C-5), 100.9 (C-2), 109.7 [C($CH_3$)$_2$], 113.4 [C($CH_3$)$_2$], 153.1 (C=O). **HRMS** (ESI) *m/z*: for $C_{15}H_{21}NNaO_8$ $[M+Na]^+$, calcd: 366.1160; found: 366.1160.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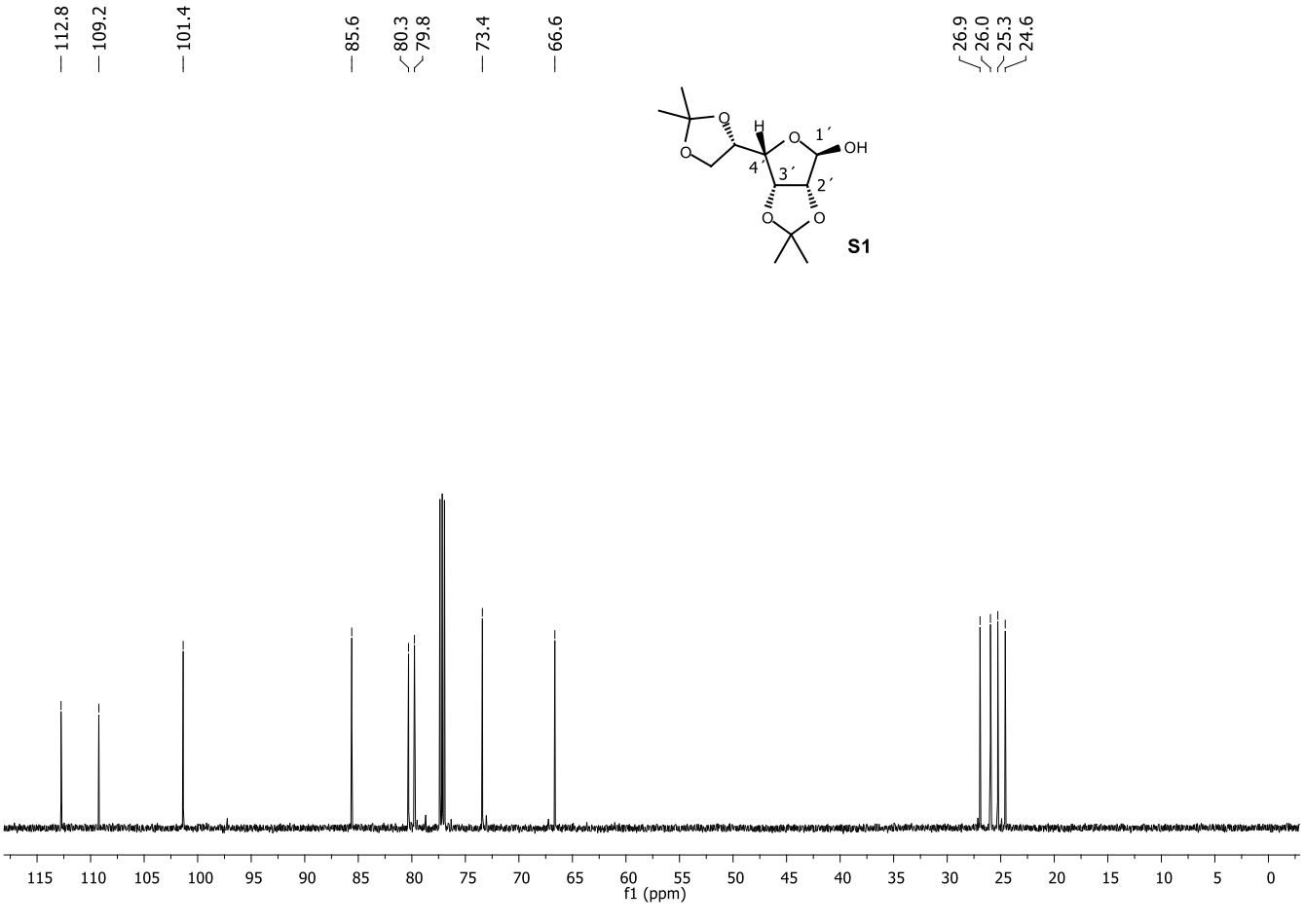
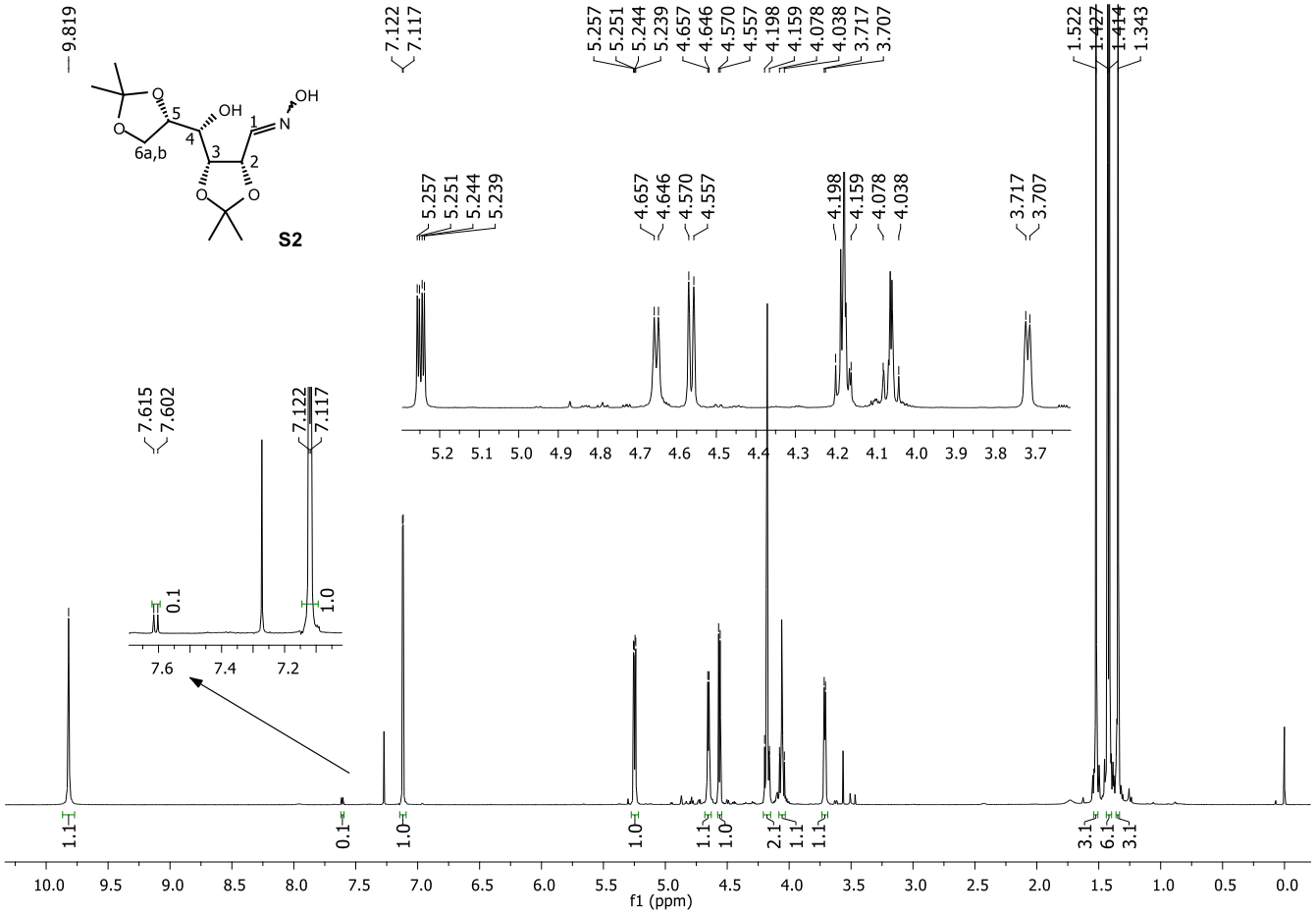
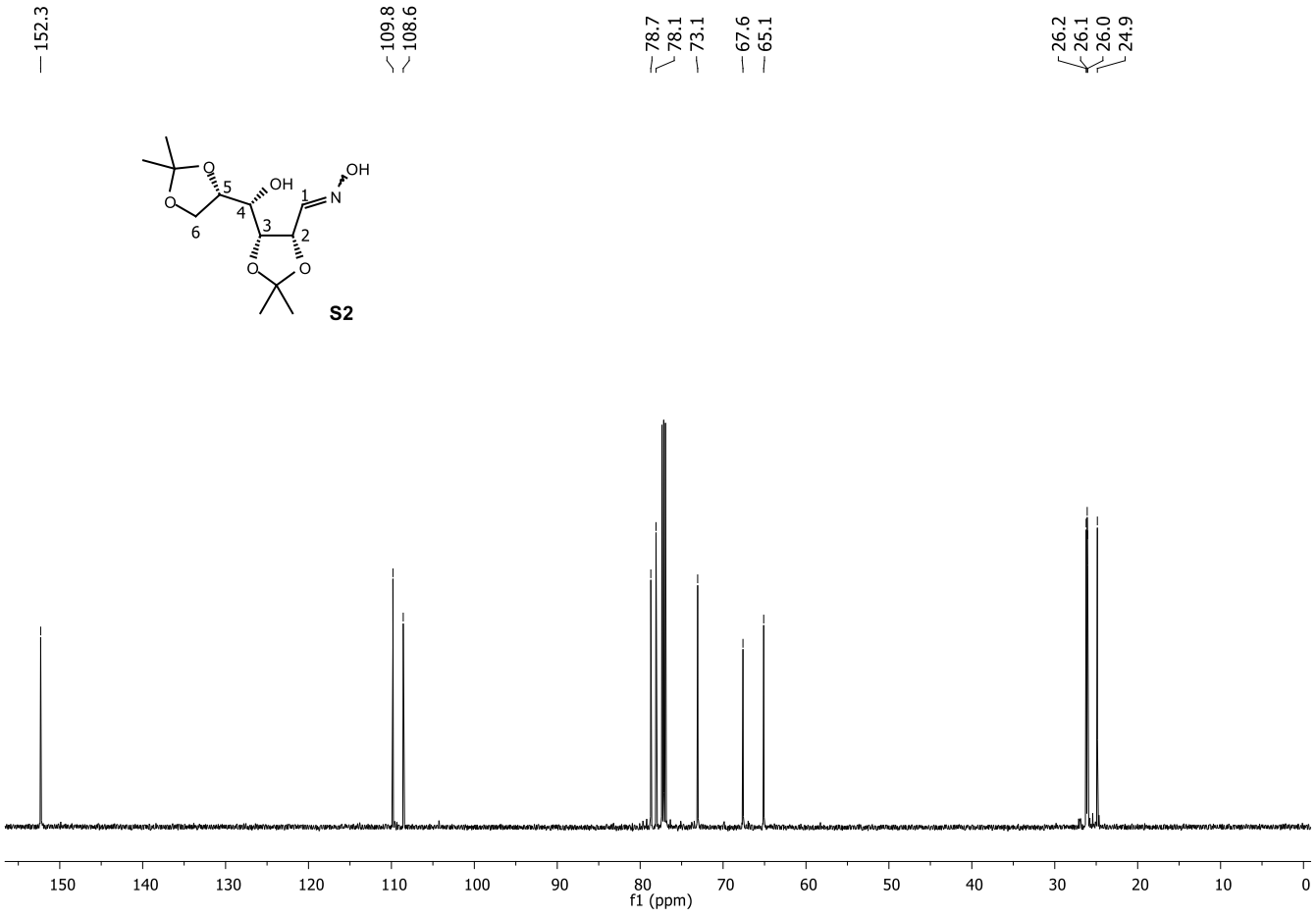
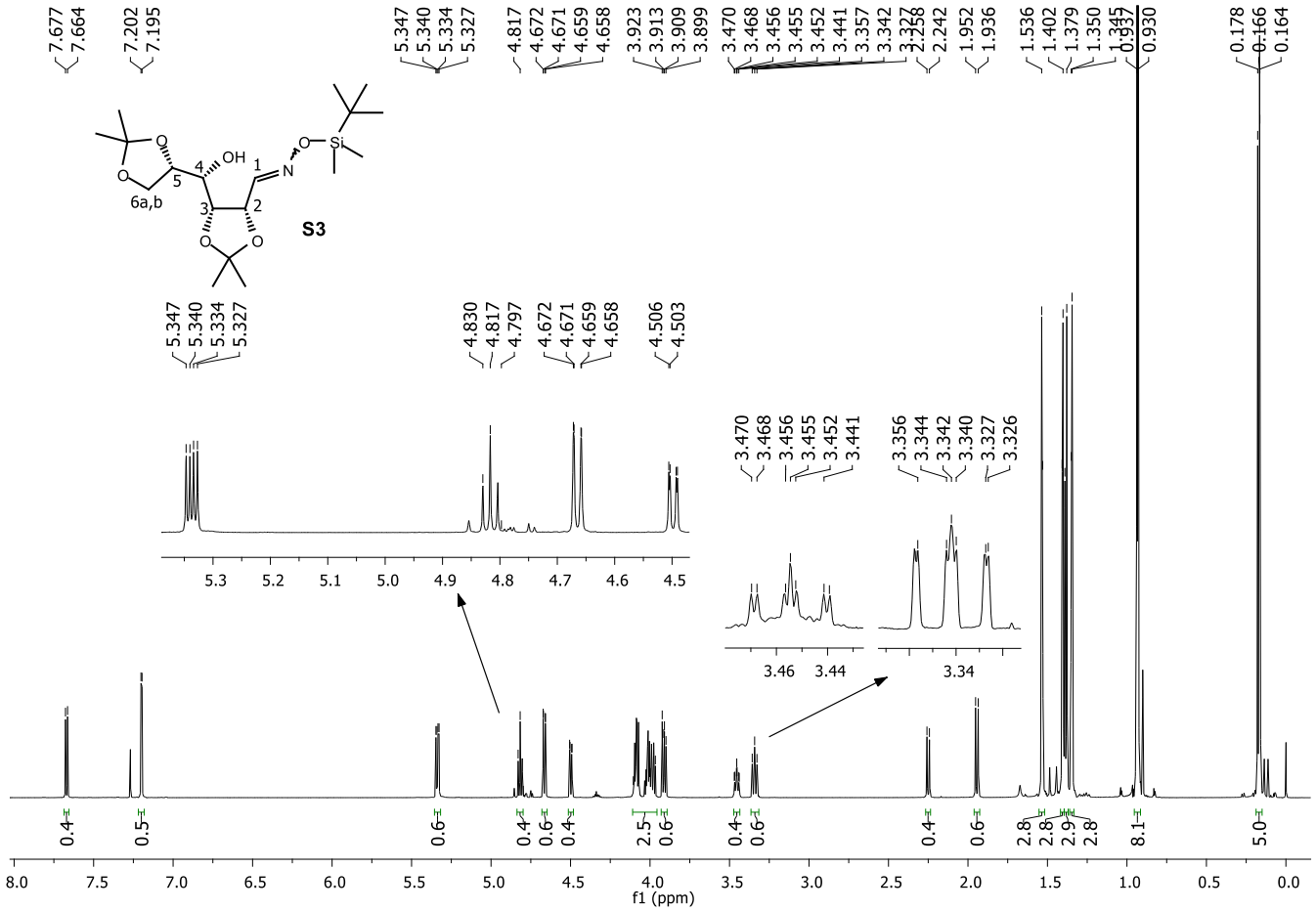
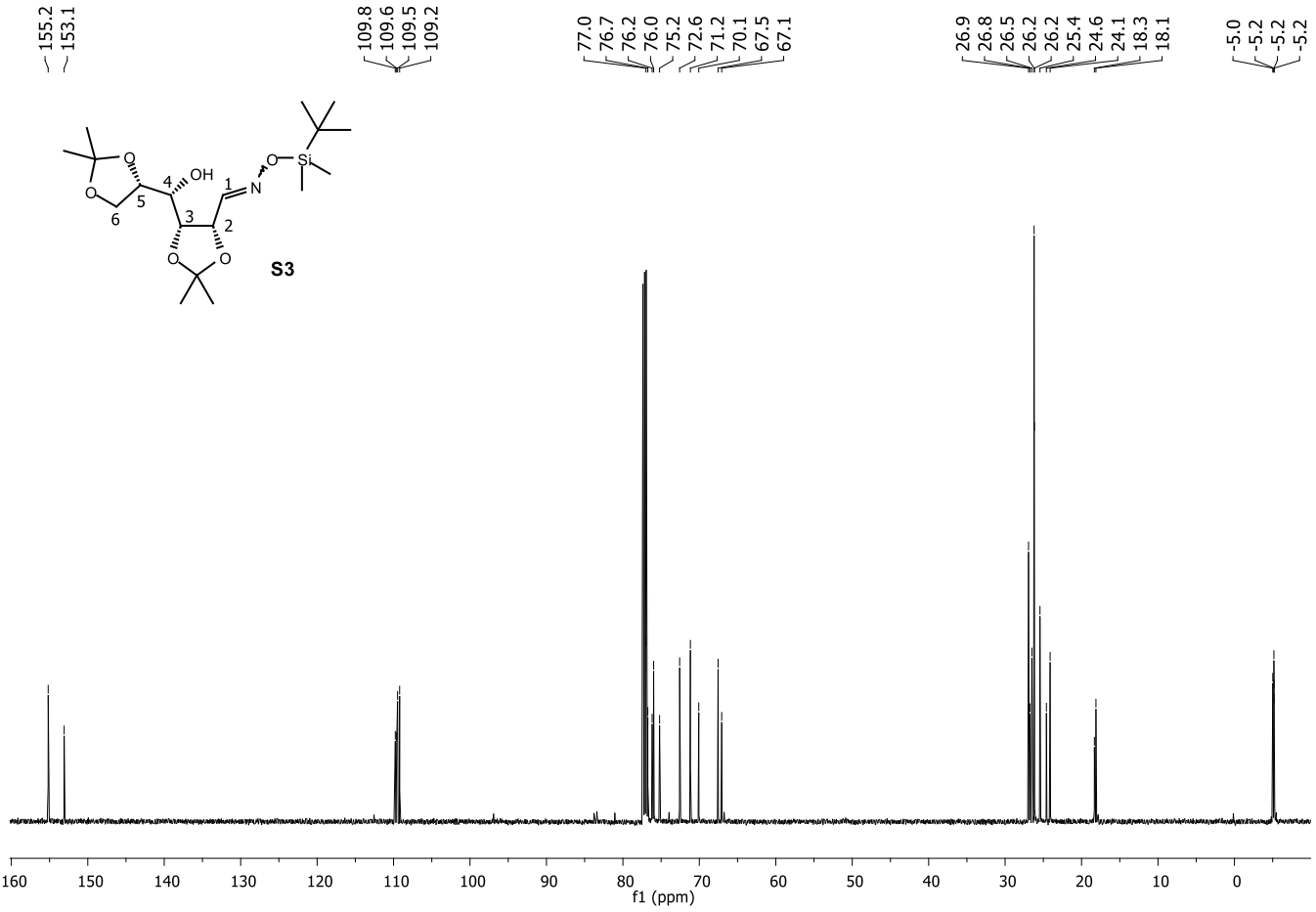
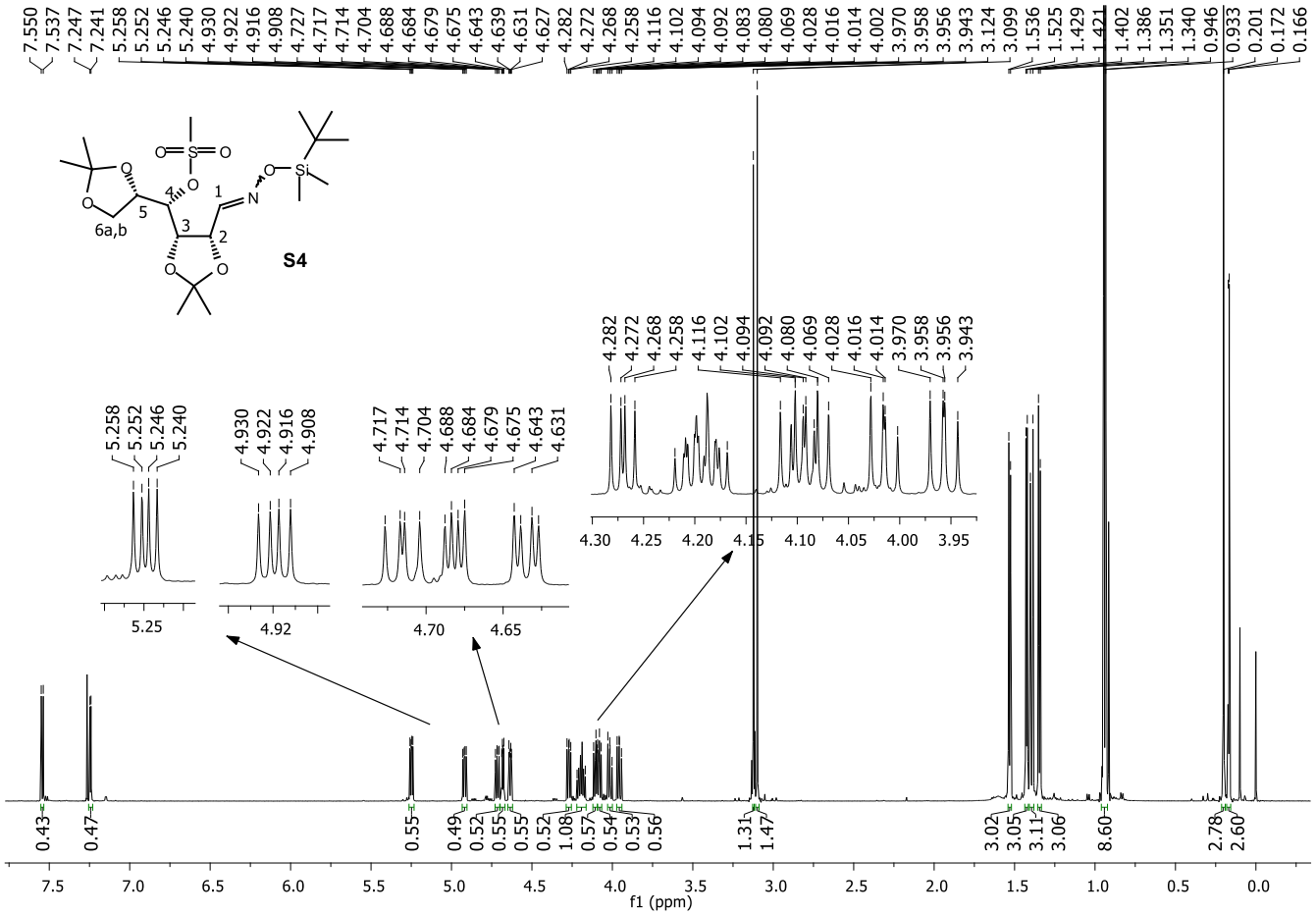

112.8
109.2
101.4
85.6
80.3
79.8
73.4
66.6
26.9
26.0
25.3
24.6
S1
f1 (ppm)

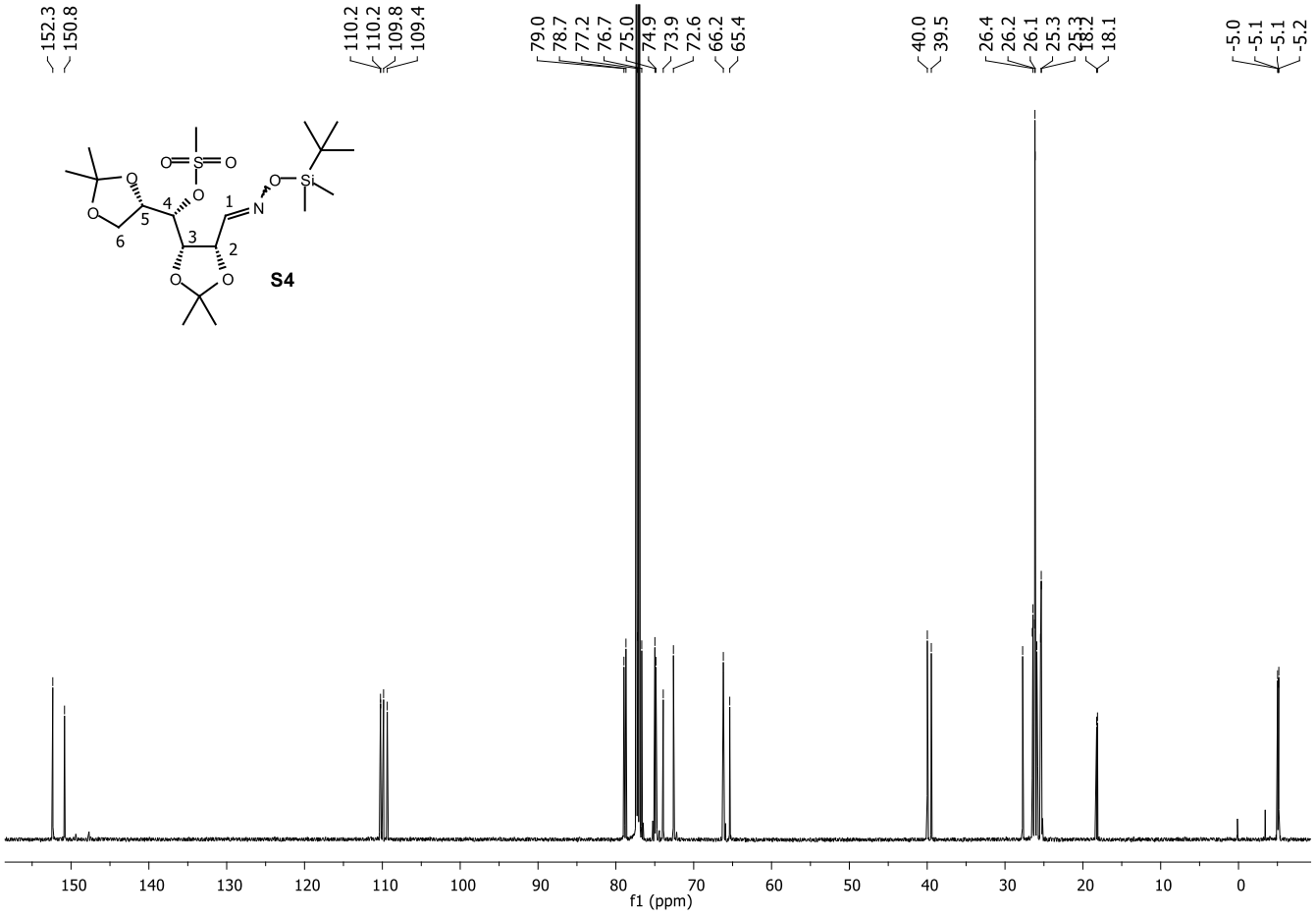

9.819
7.122
7.117
5.257
5.251
5.244
5.239
4.657
4.646
4.570
4.557
4.198
4.159
4.078
4.038
3.717
3.707
1.522
1.427
1.414
1.343
7.615
7.602
0.1
1.0
1.1
0.1
1.0
1.0
1.1
1.0
2.1
1.1
1.1
3.1
6.1
3.1
f1 (ppm)
S2

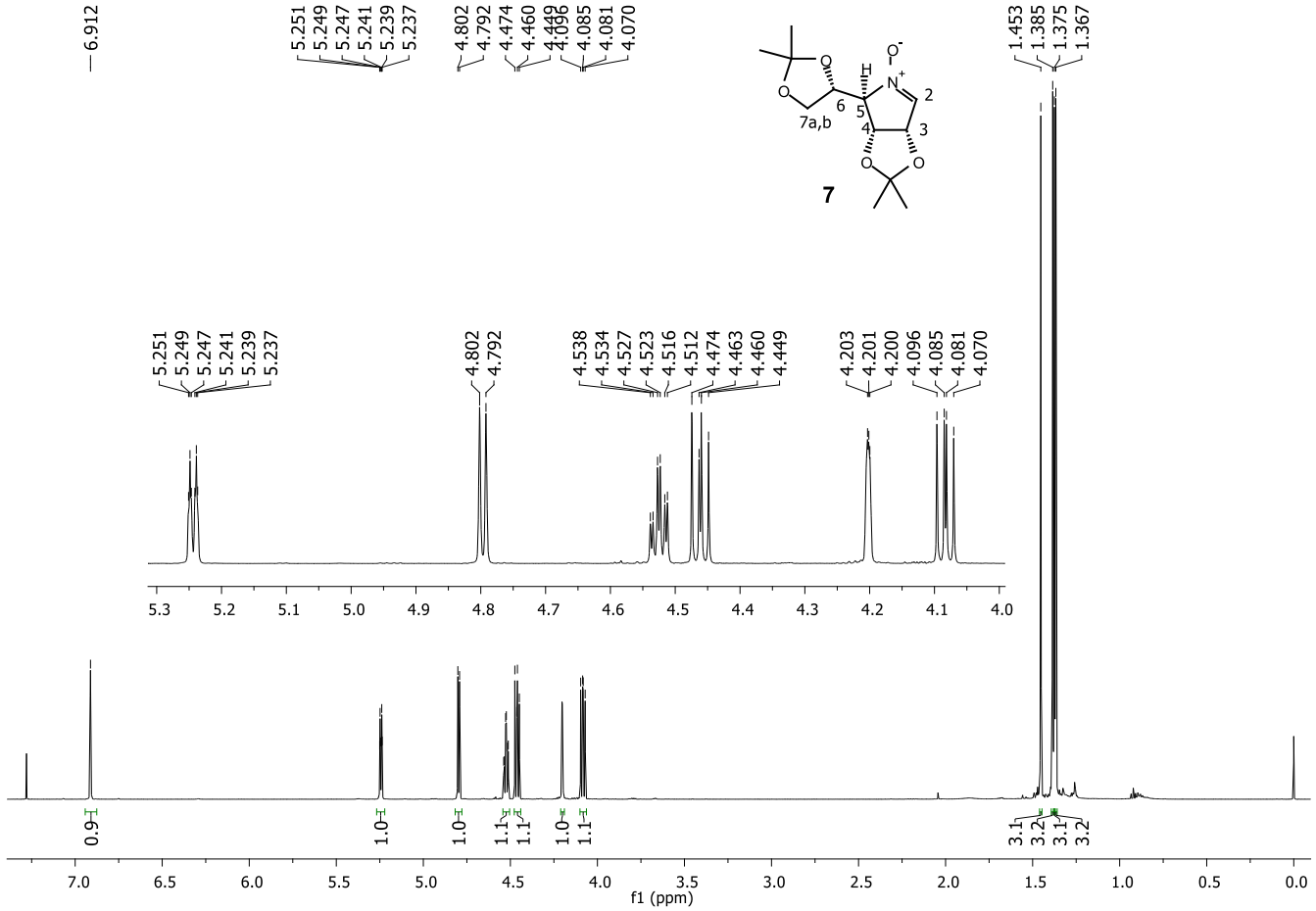

152.3
109.8
108.6
78.7
78.1
73.1
67.6
65.1
26.2
26.1
26.0
24.9
f1 (ppm)
S2

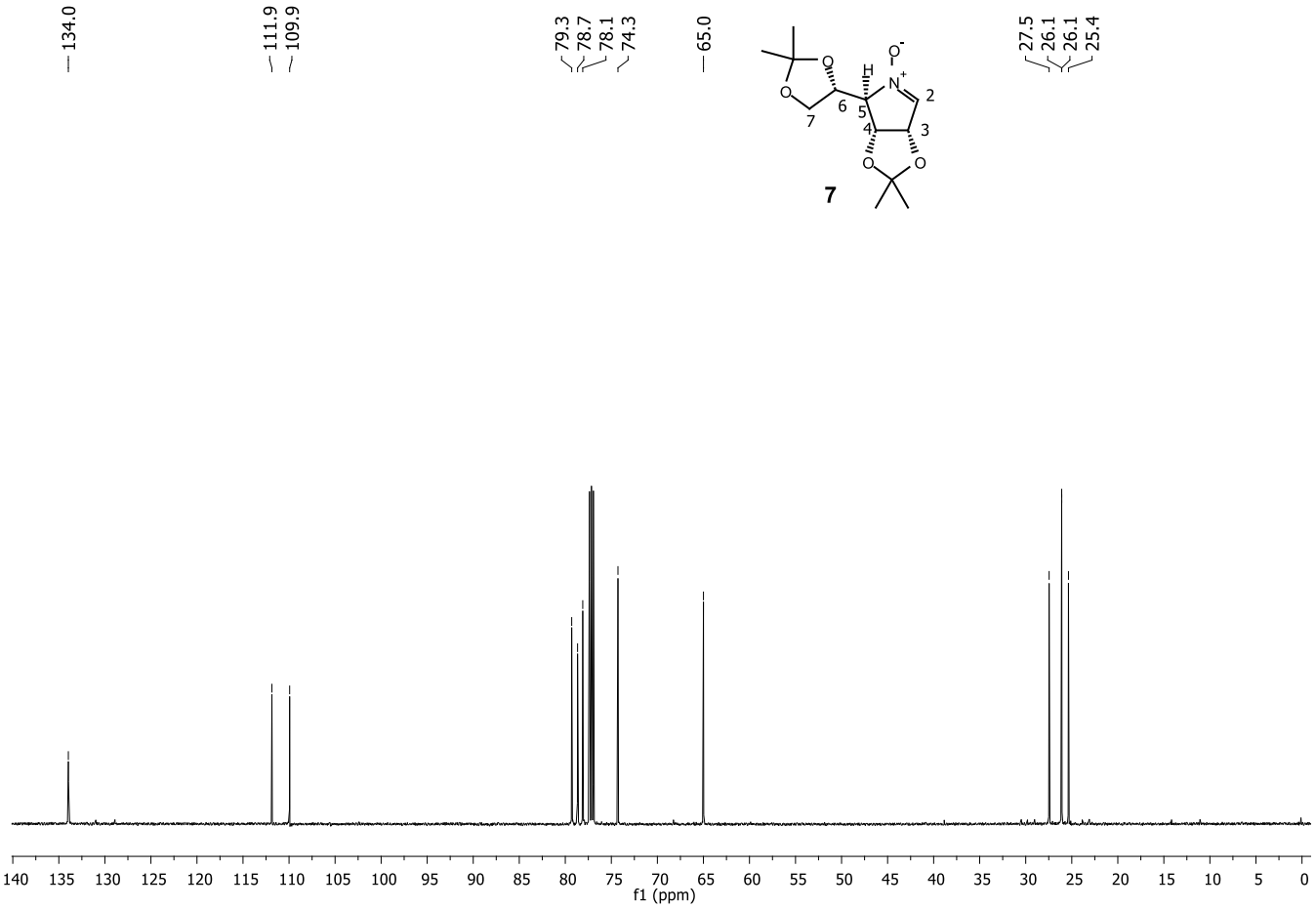

S3
f1 (ppm)

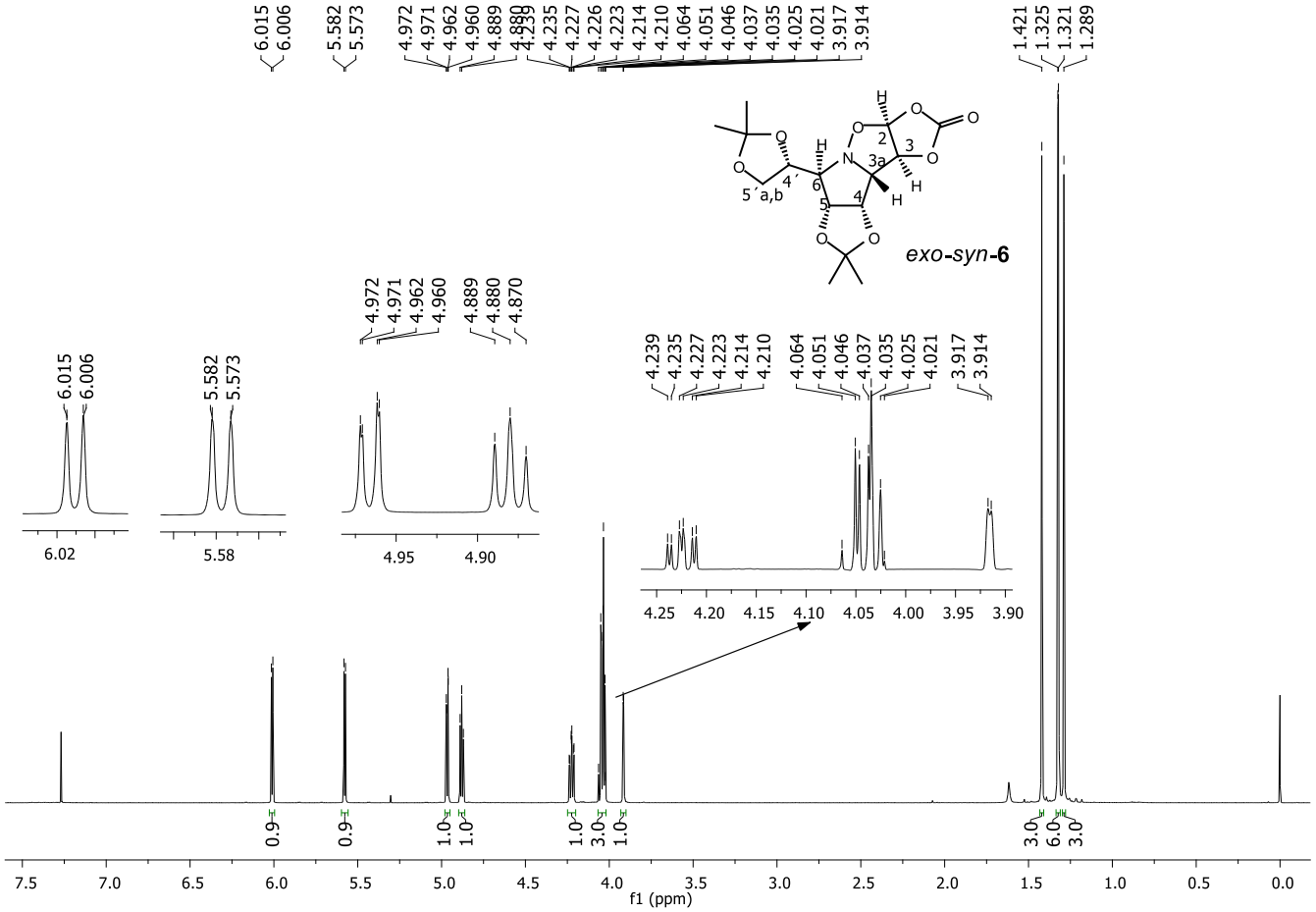

S3
f1 (ppm)

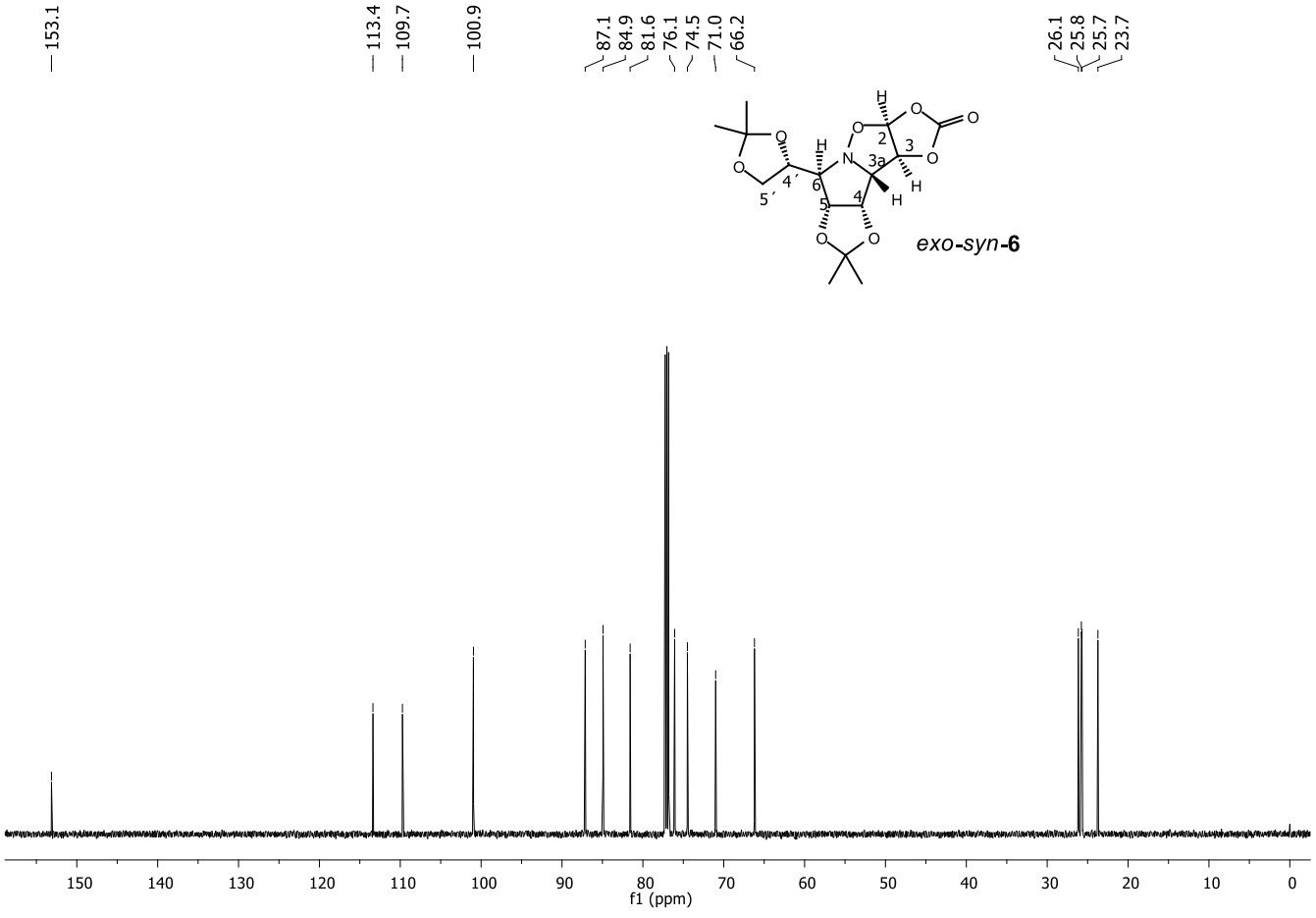

S4
f1 (ppm)

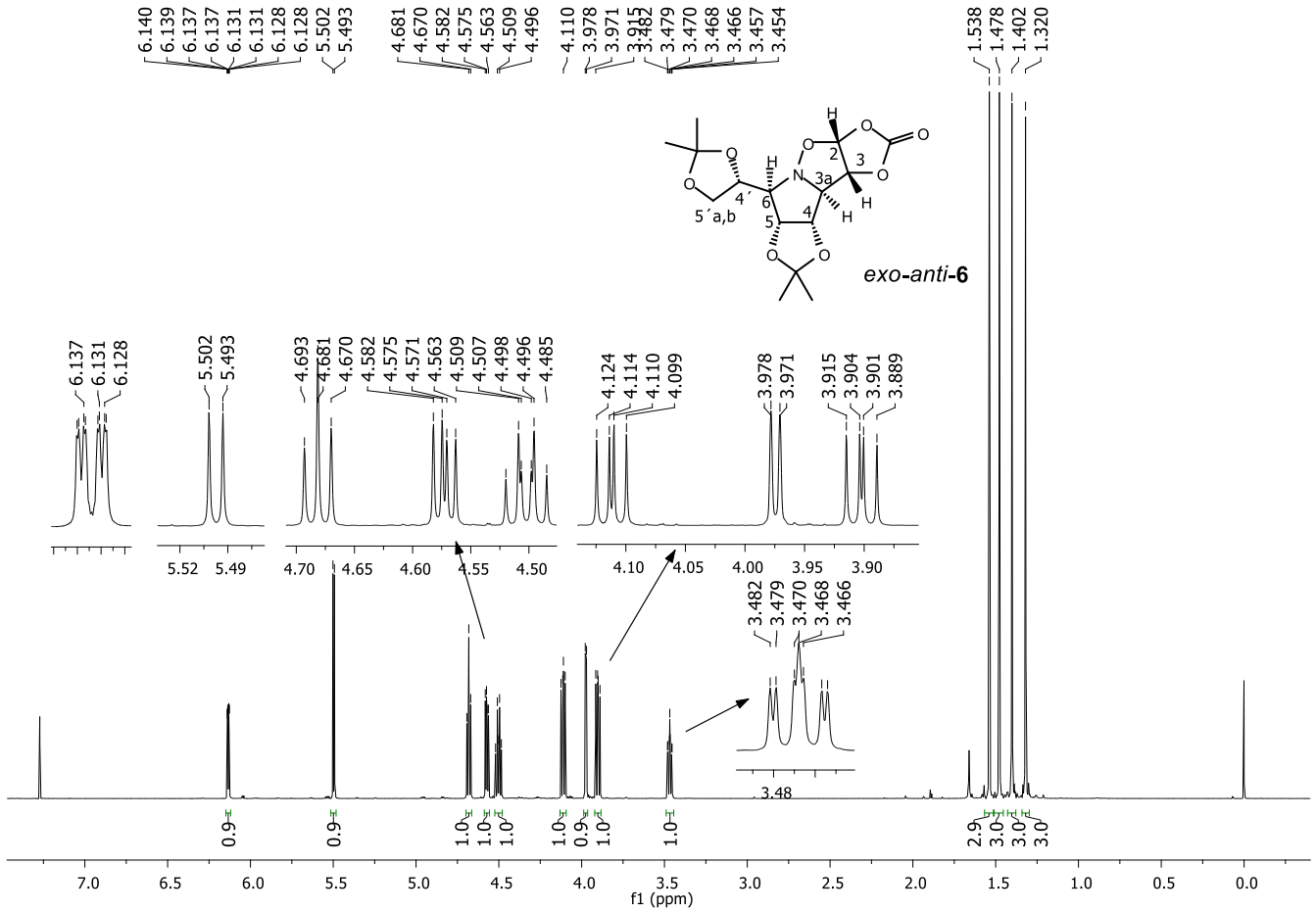

S4
f1 (ppm)

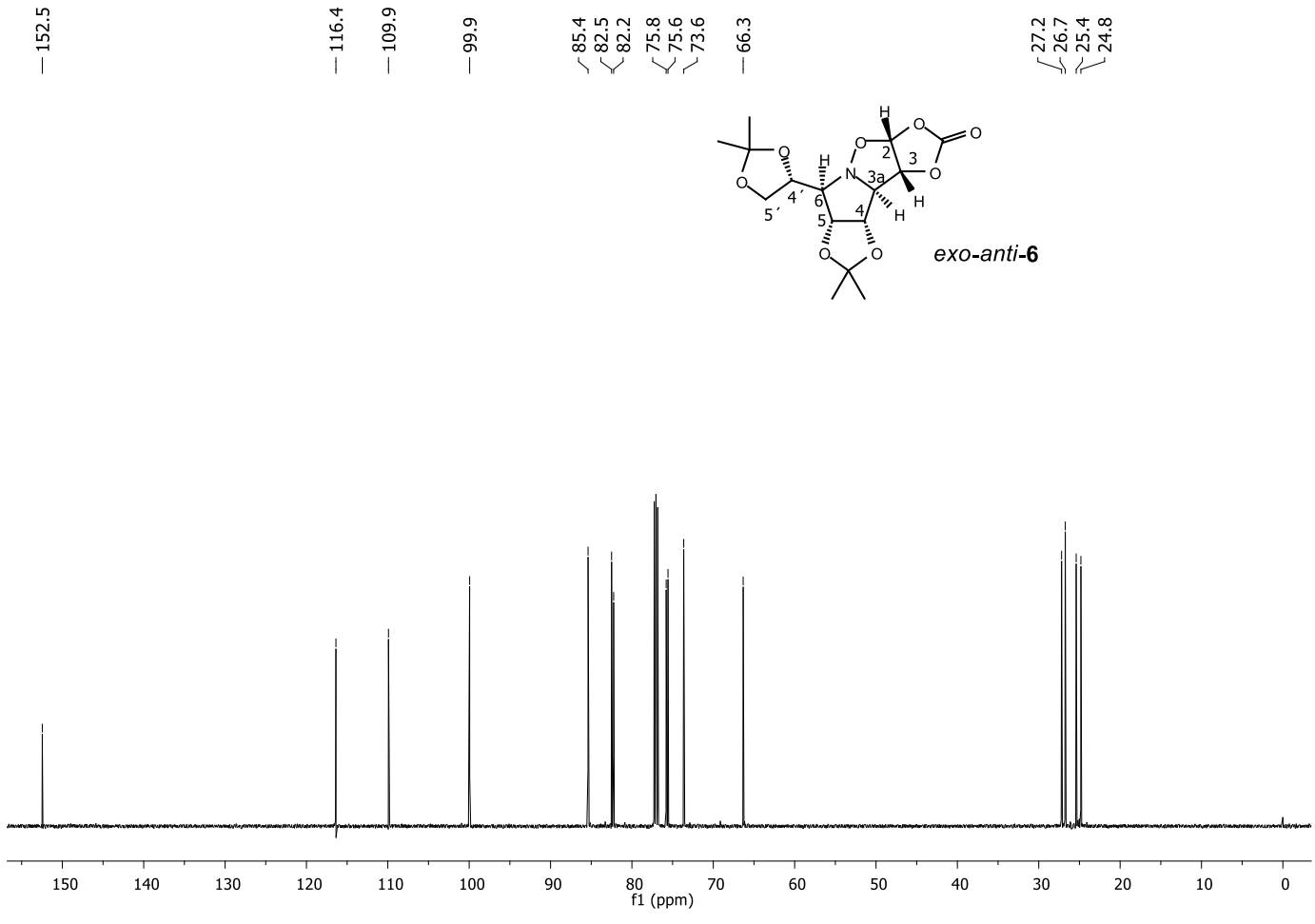

6.912
5.251
5.249
5.247
5.241
5.239
5.237
4.802
4.792
4.474
4.460
4.449
4.096
4.085
4.081
4.070
1.453
1.385
1.375
1.367
7a,b
7
0.9
1.0
1.0
1.1
1.1
1.0
1.1
3.1
3.2
3.1
3.2
f1 (ppm)

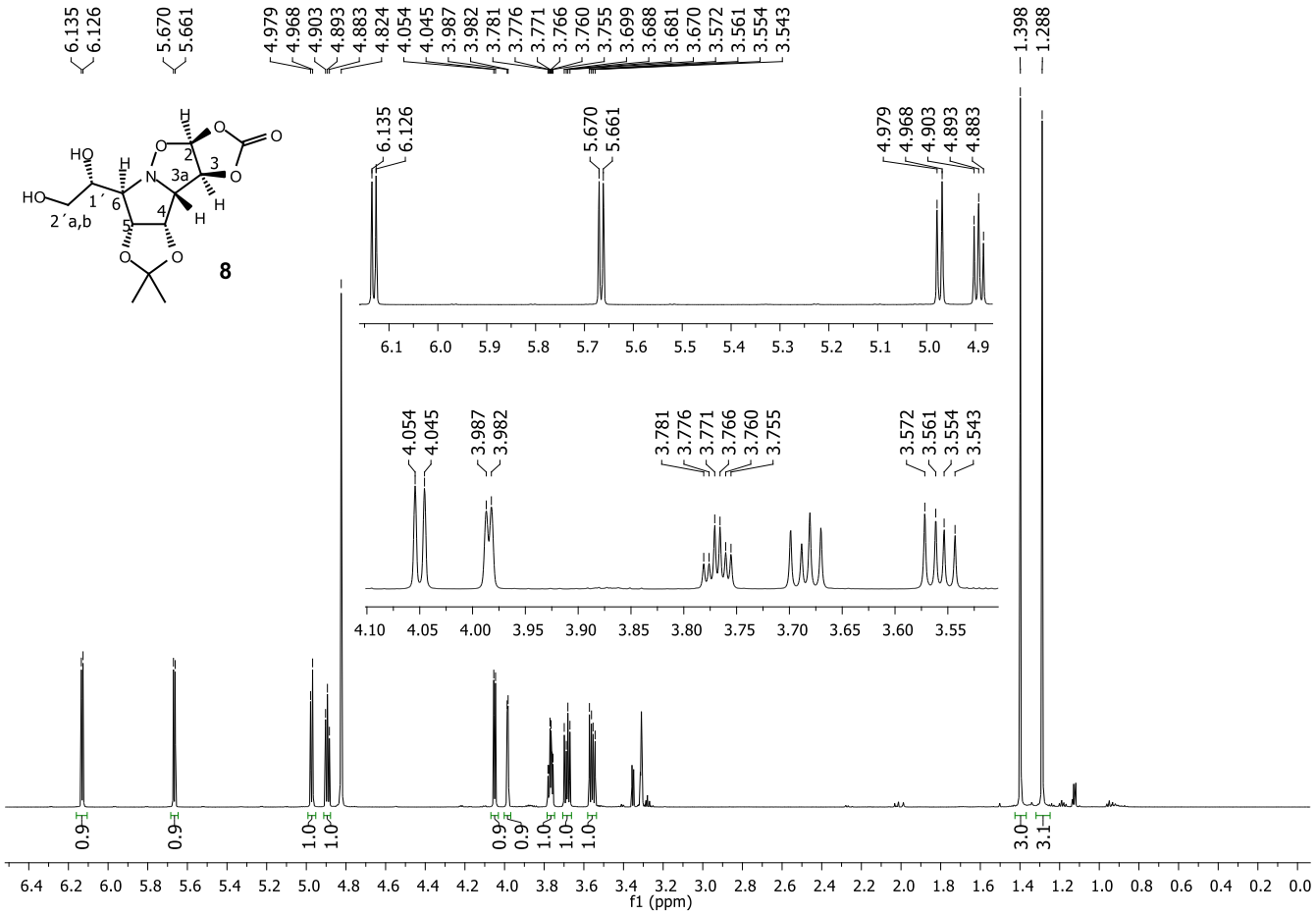

134.0
111.9
109.9
79.3
78.7
78.1
74.3
65.0
27.5
26.1
26.1
25.4
7
f1 (ppm)

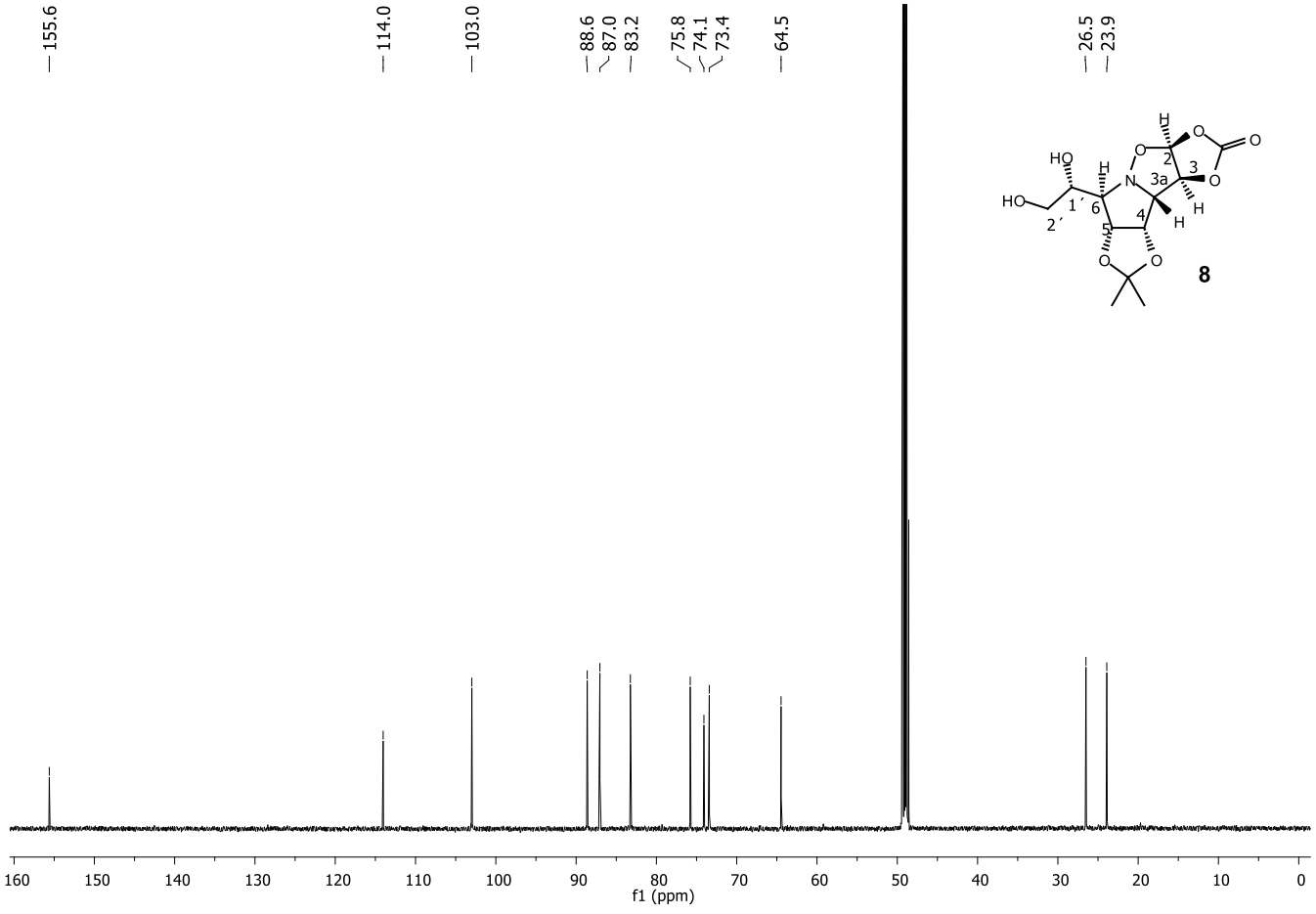

6.015
6.006
5.582
5.573
4.972
4.971
4.962
4.960
4.889
4.880
4.870
4.239
4.235
4.227
4.226
4.223
4.214
4.210
4.064
4.051
4.046
4.037
4.035
4.025
4.021
3.917
3.914
1.421
1.325
1.321
1.289
exo-syn-6
0.9
0.9
1.0
1.0
1.0
3.0
1.0
3.0
6.0
3.0
f1 (ppm)

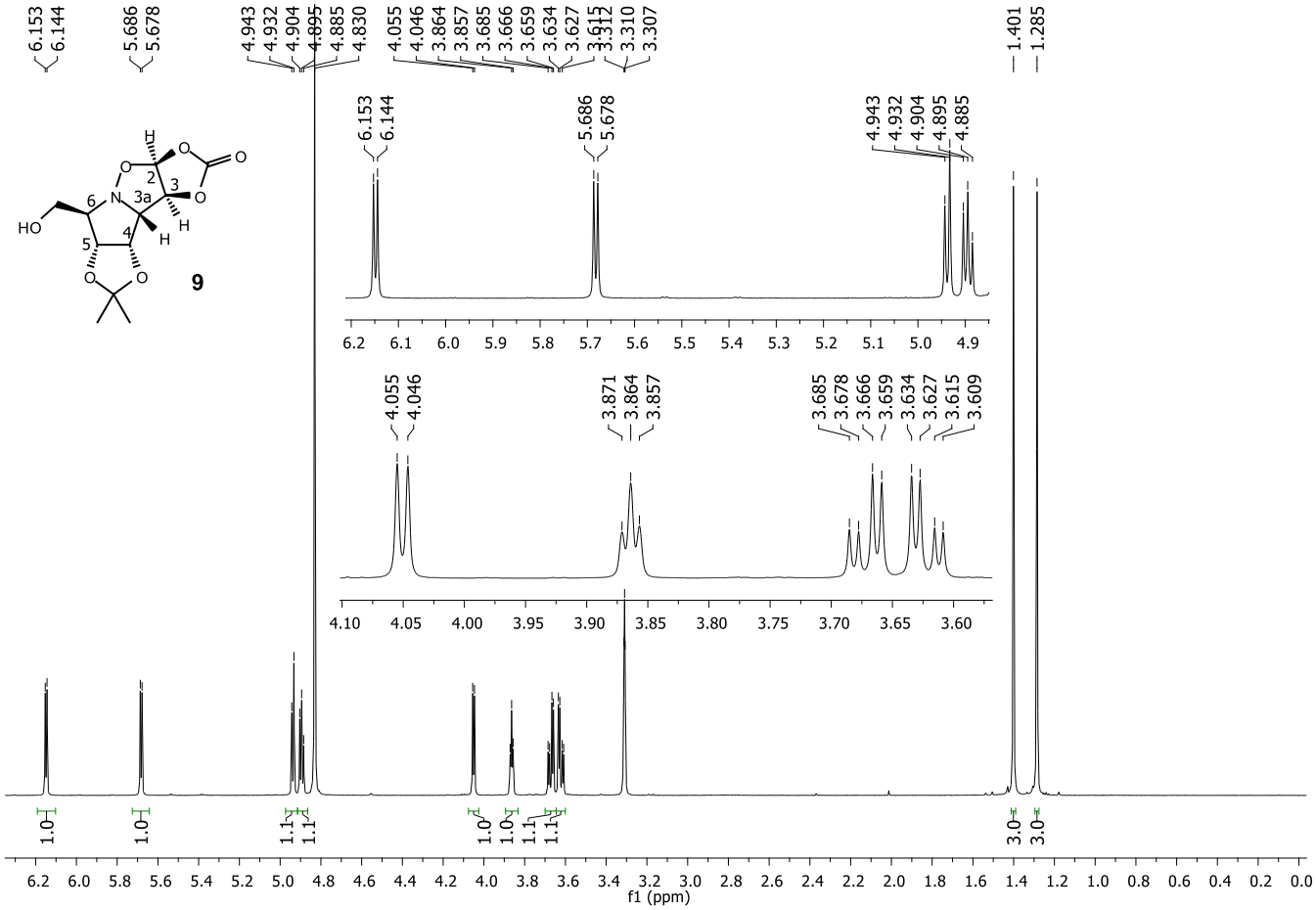

153.1
113.4
109.7
100.9
87.1
84.9
81.6
76.1
74.5
71.0
66.2
26.1
25.8
25.7
23.7
exo-syn-6
f1 (ppm)

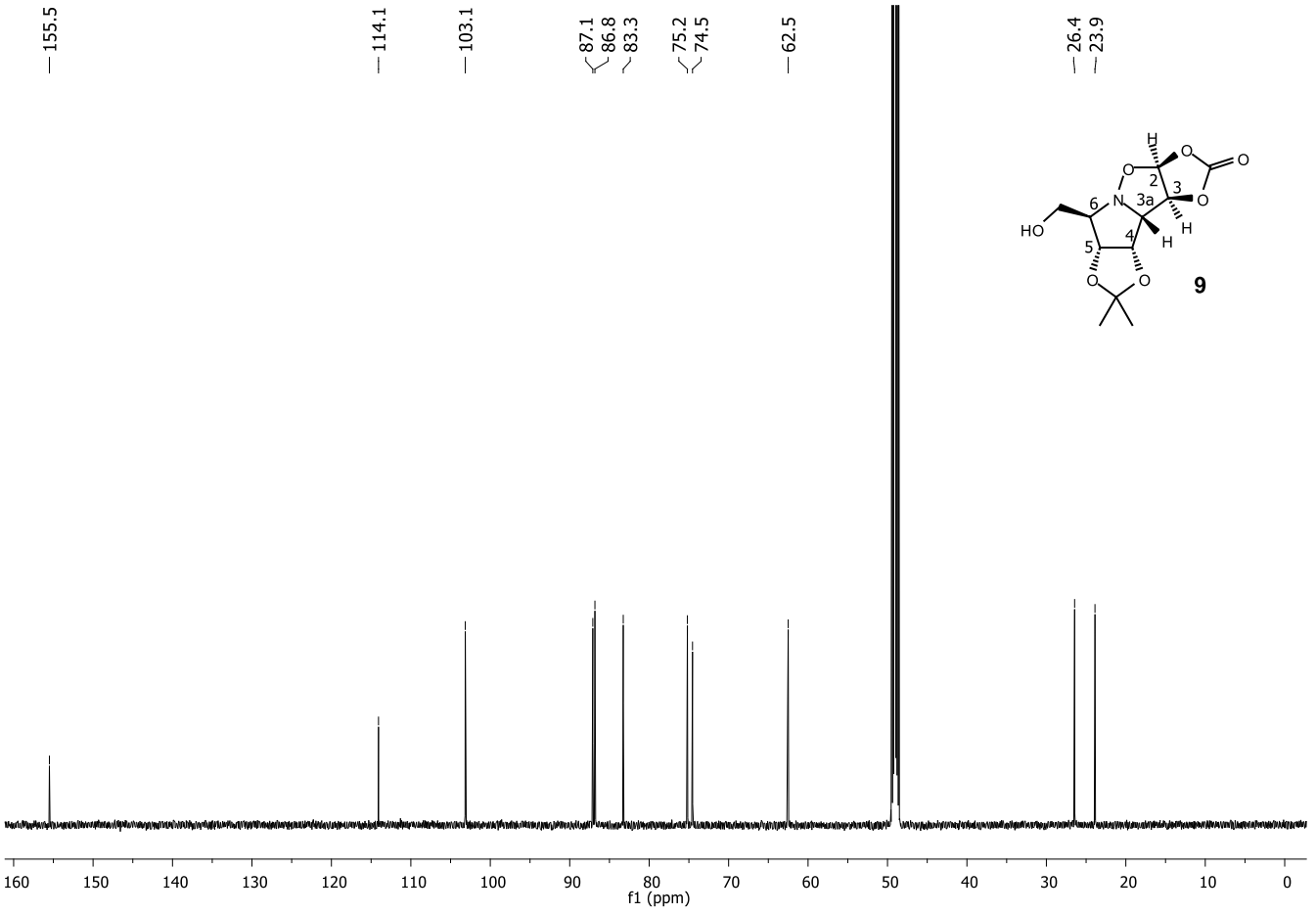

6.140
6.139
6.137
6.137
6.131
6.131
6.128
6.128
5.502
5.493
4.681
4.670
4.582
4.575
4.563
4.509
4.496
4.110
3.978
3.971
3.915
3.482
3.479
3.470
3.468
3.466
3.457
3.454
1.538
1.478
1.402
1.320
5´a,b
exo-anti-6
f1 (ppm)

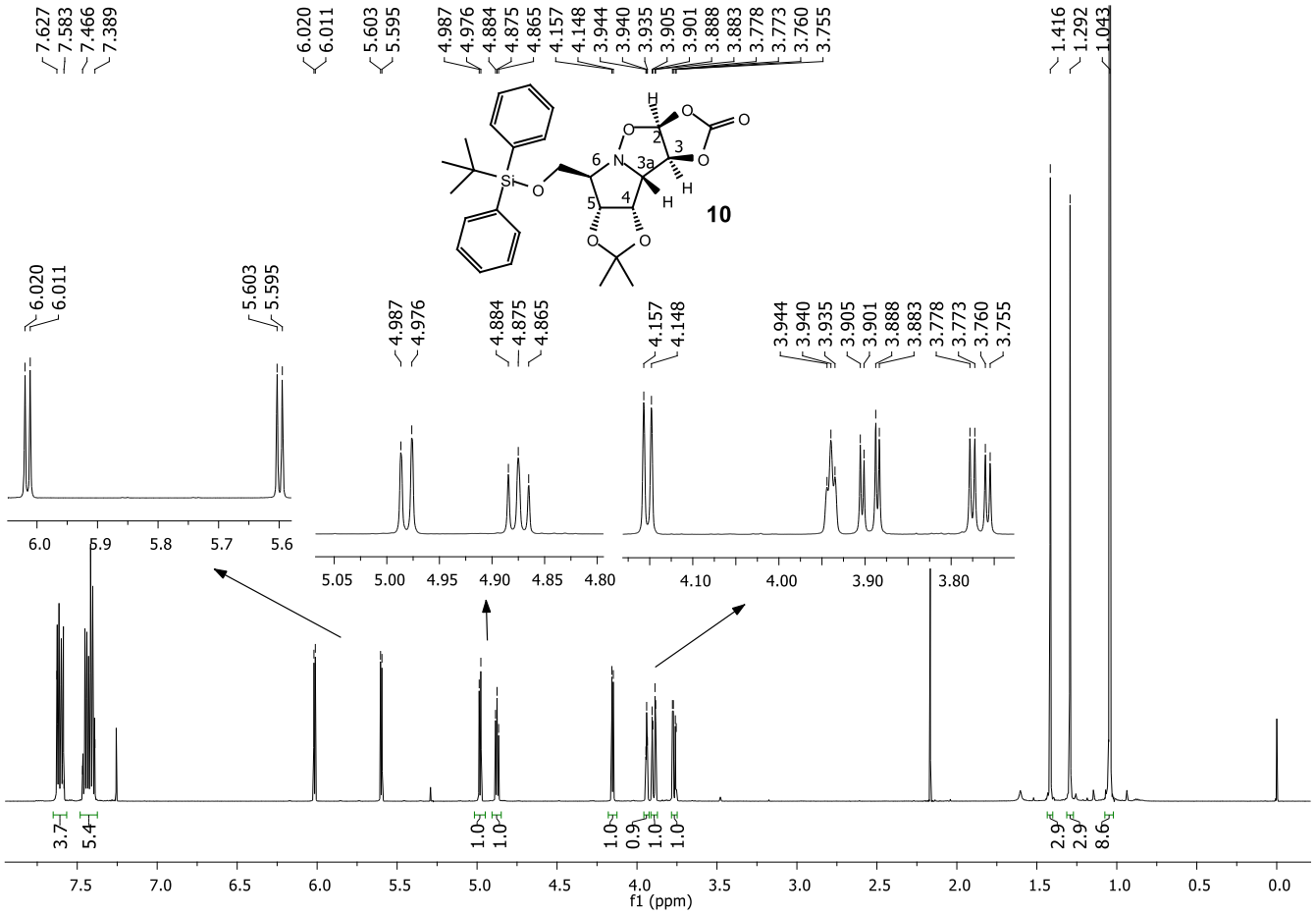

152.5
116.4
109.9
99.9
85.4
82.5
82.2
75.8
75.6
73.6
66.3
27.2
26.7
25.4
24.8
5´a,b
exo-anti-6
f1 (ppm)

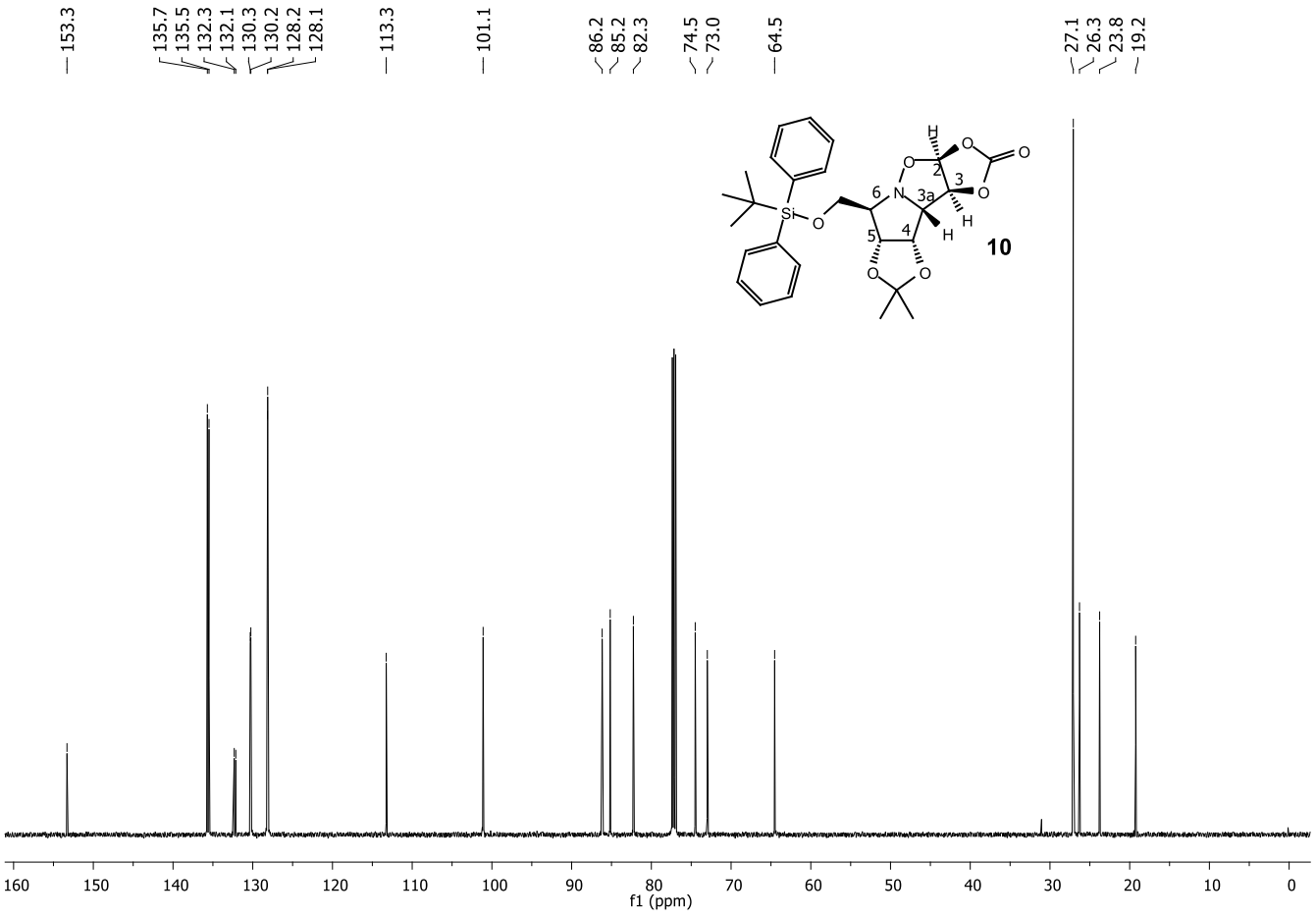

6.135
6.126
5.670
5.661
4.979
4.968
4.903
4.893
4.883
4.824
4.054
4.045
3.987
3.982
3.781
3.776
3.771
3.766
3.760
3.755
3.699
3.688
3.681
3.670
3.572
3.561
3.554
3.543
1.398
1.288
8
f1 (ppm)

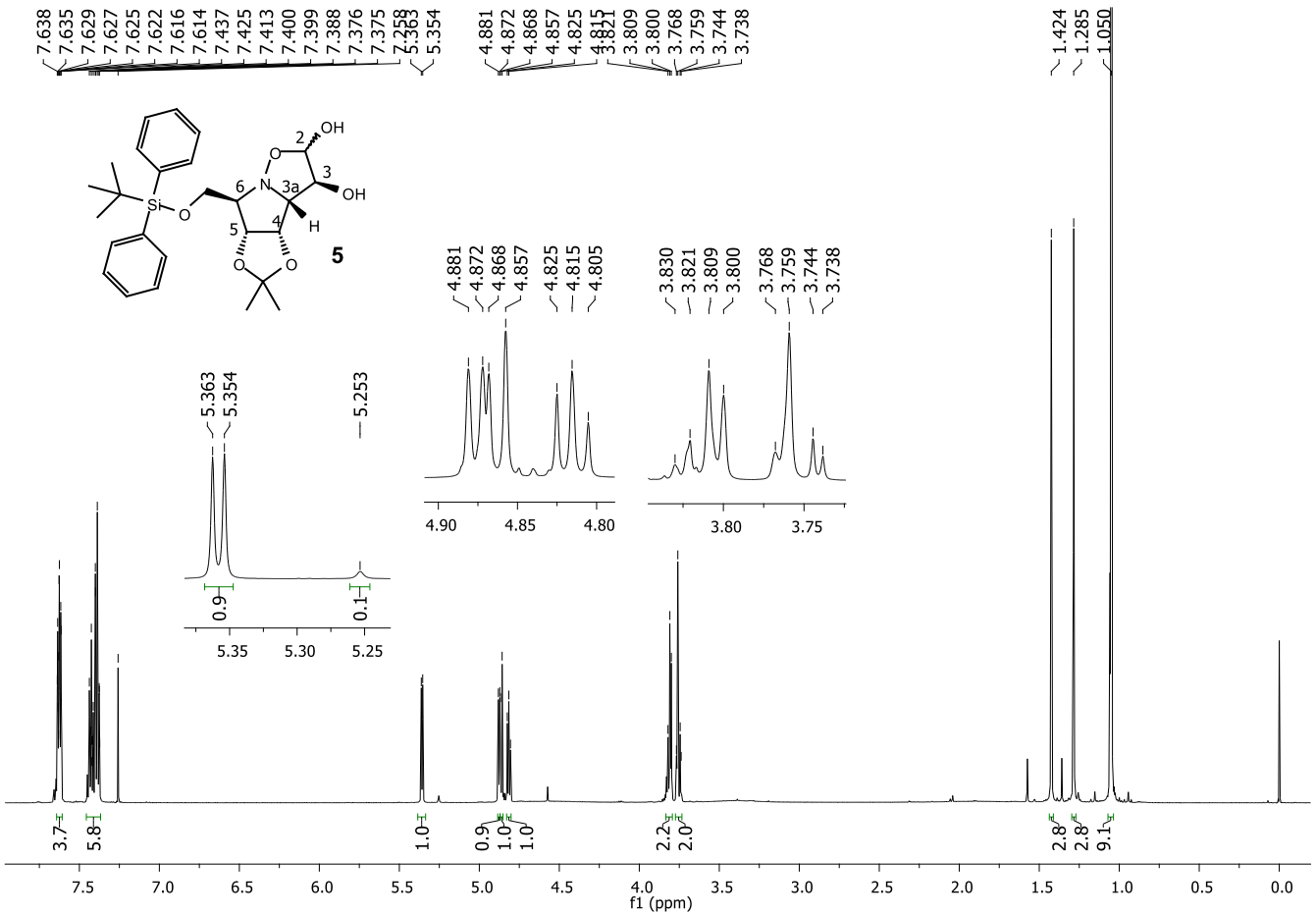

155.6
114.0
103.0
88.6
87.0
83.2
75.8
74.1
73.4
64.5
26.5
23.9
8
f1 (ppm)

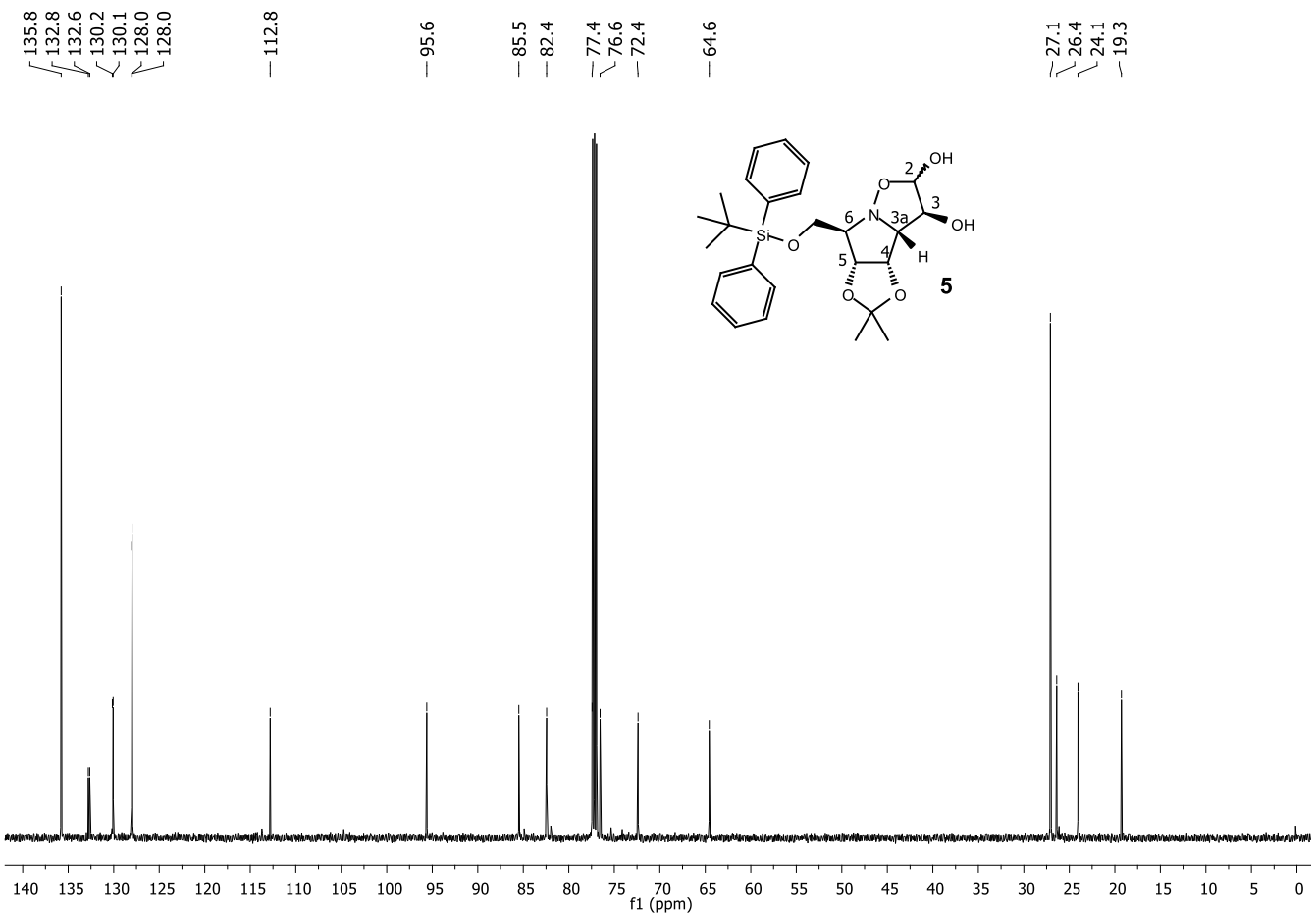

6.153
6.144
5.686
5.678
4.943
4.932
4.904
4.895
4.885
4.830
4.055
4.046
3.864
3.857
3.685
3.666
3.659
3.634
3.627
3.615
3.312
3.310
3.307
1.401
1.285
HO
N
3a
H
9
f1 (ppm)

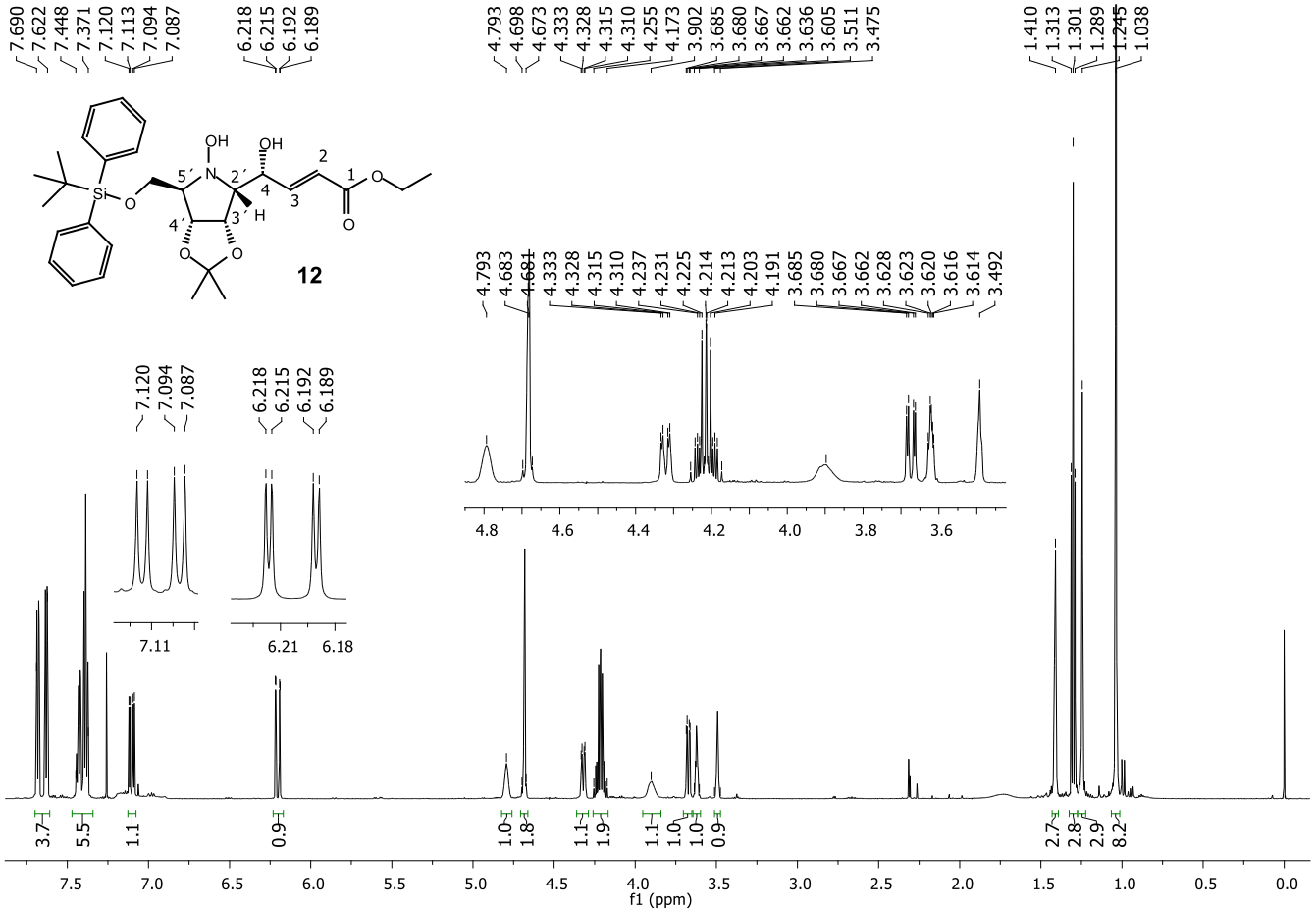

155.5
114.1
103.1
87.1
86.8
83.3
75.2
74.5
62.5
26.4
23.9
HO
N
3a
H
9
f1 (ppm)

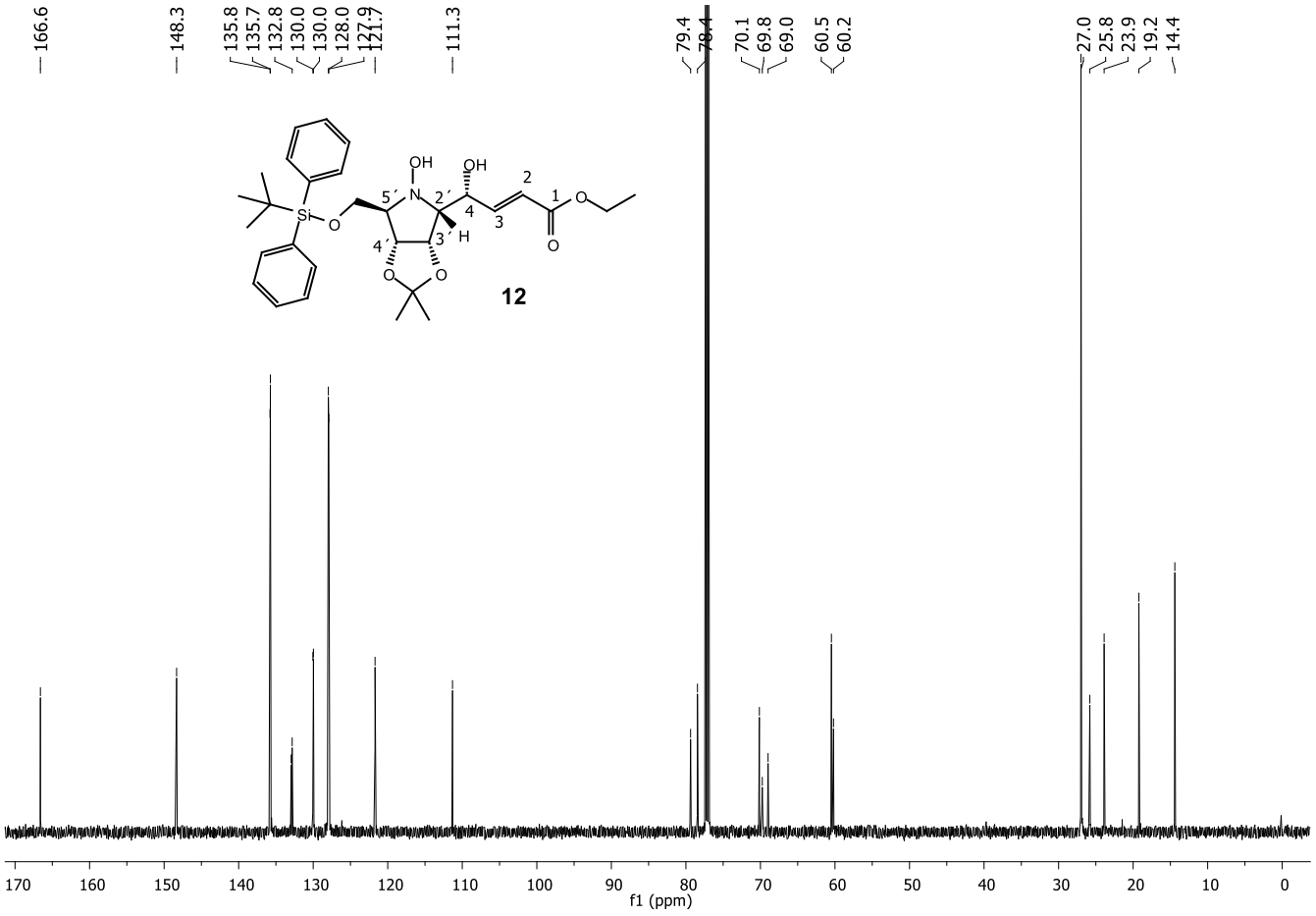

7.627
7.583
7.466
7.389
6.020
6.011
5.603
5.595
4.987
4.976
4.884
4.875
4.865
4.157
4.148
3.944
3.940
3.935
3.905
3.901
3.888
3.883
3.778
3.773
3.760
3.755
1.416
1.292
1.043
10
f1 (ppm)

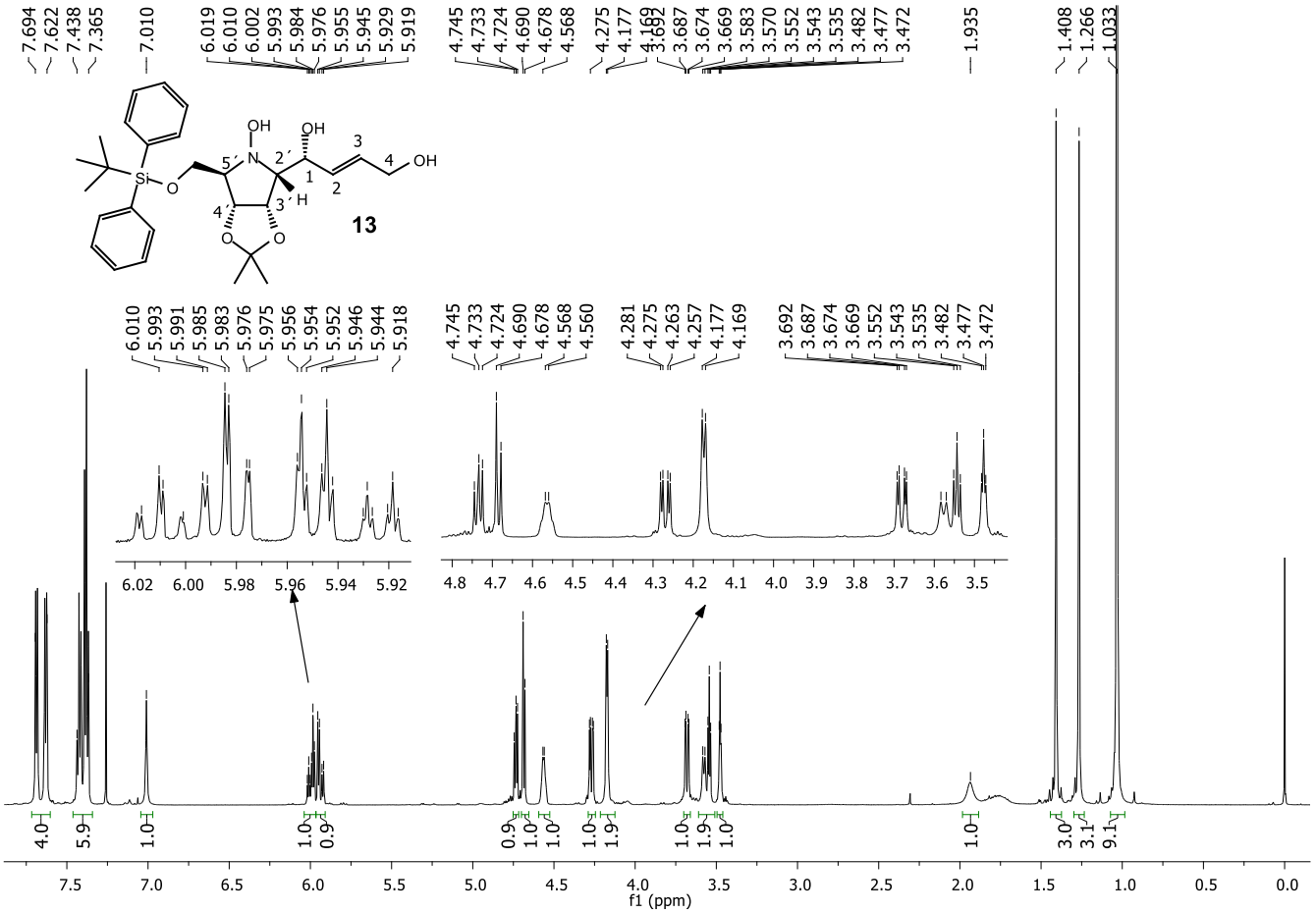

153.3
135.7
135.5
132.3
132.1
130.3
130.2
128.2
128.1
113.3
101.1
86.2
85.2
82.3
74.5
73.0
64.5
27.1
26.3
23.8
19.2
10
f1 (ppm)


7.638
7.635
7.629
7.627
7.625
7.622
7.616
7.614
7.437
7.425
7.413
7.400
7.399
7.388
7.376
7.375
5.363
5.354
4.881
4.872
4.868
4.857
4.825
4.815
4.805
3.830
3.821
3.809
3.800
3.768
3.759
3.744
3.738
1.424
1.285
1.050
5.253
OH
OH
H
5
0.9
0.1
3.7
5.8
1.0
0.9
1.0
1.0
2.2
2.0
2.8
2.8
9.1
f1 (ppm)


135.8
132.8
132.6
130.2
130.1
128.0
128.0
112.8
95.6
85.5
82.4
77.4
76.6
72.4
64.6
27.1
26.4
24.1
19.3
OH
OH
H
5
f1 (ppm)


12
f1 (ppm)


12
f1 (ppm)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7.365
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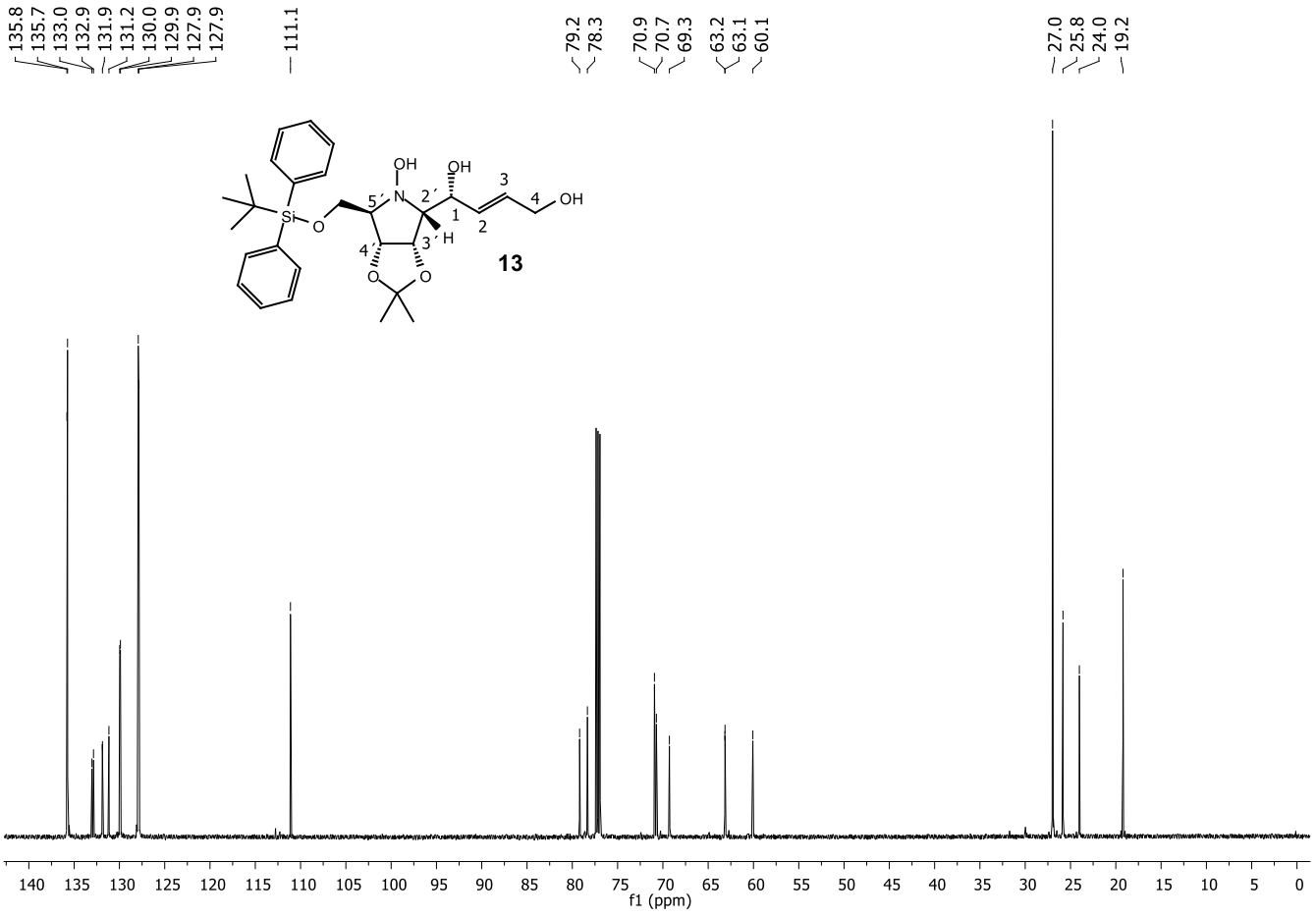
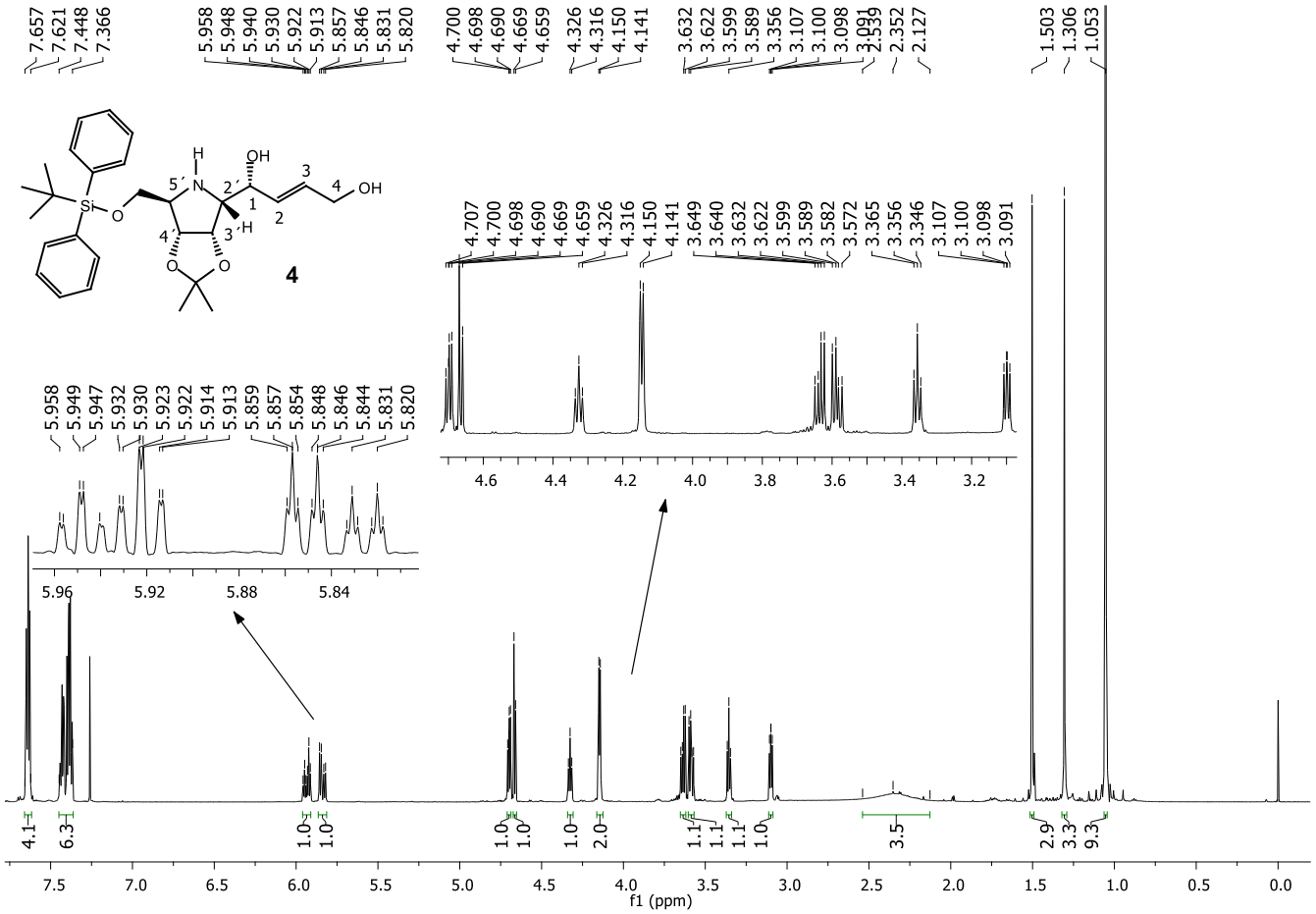
6.019
6.010
6.002
5.993
5.984
5.976
5.955
5.945
5.929
5.919
4.745
4.733
4.724
4.690
4.678
4.568
4.275
4.177
4.169
3.692
3.687
3.674
3.669
3.583
3.570
3.552
3.543
3.535
3.482
3.477
3.472
1.935
1.408
1.266
1.033
13
f1 (ppm)


135.8
135.7
133.0
132.9
131.9
131.2
130.0
129.9
127.9
127.9
111.1
79.2
78.3
70.9
70.7
69.3
63.2
63.1
60.1
27.0
25.8
24.0
19.2
13
f1 (ppm)


7.657
7.621
7.448
7.366
5.958
5.948
5.940
5.930
5.922
5.913
5.857
5.846
5.831
5.820
4.700
4.698
4.690
4.669
4.659
4.326
4.316
4.150
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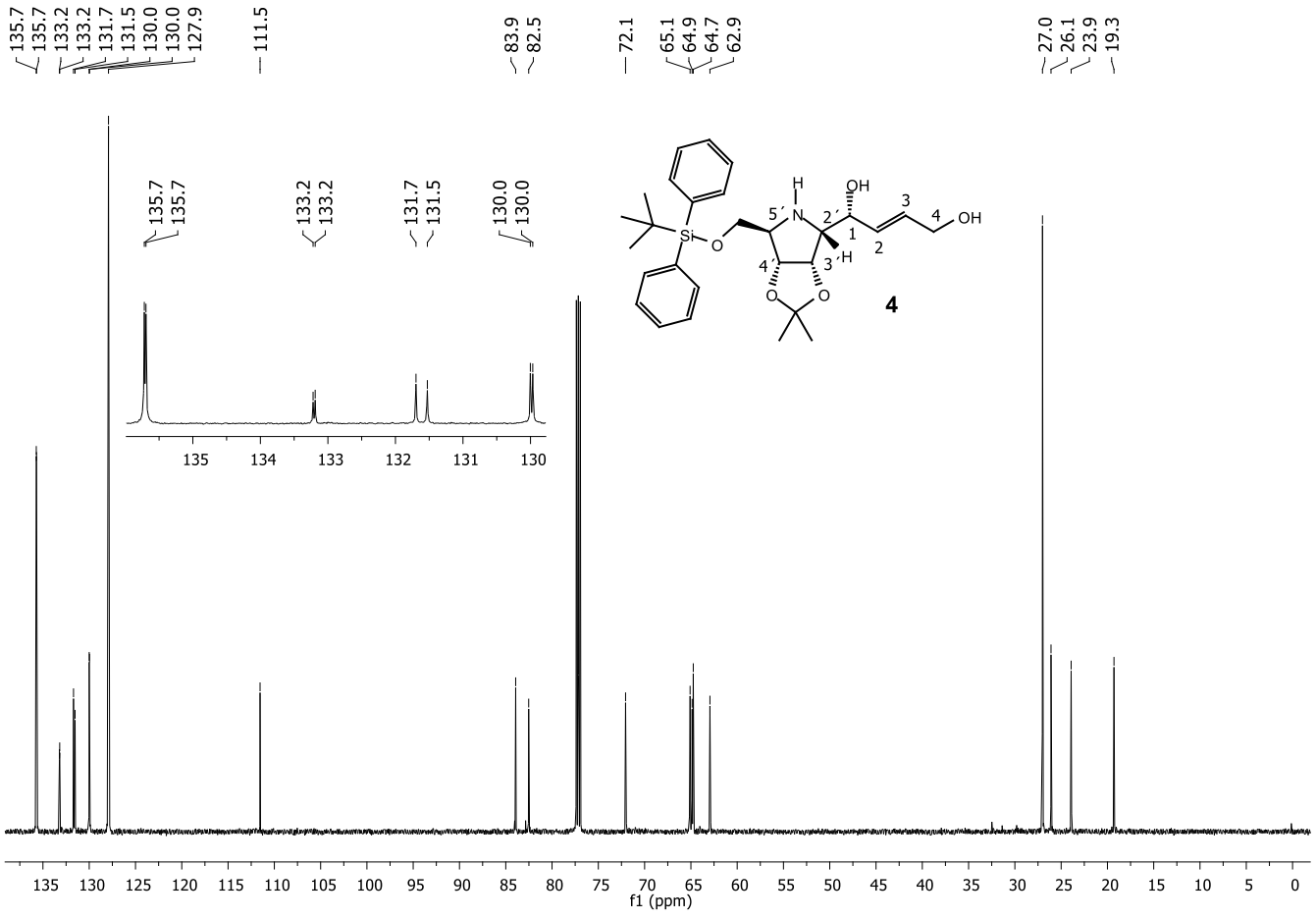
3.632
3.622
3.599
3.589
3.356
3.107
3.100
3.098
3.091
2.539
2.352
2.127
1.503
1.306
1.053
4
4.1
6.3
1.0
1.0
1.0
1.0
1.0
2.0
1.1
1.1
1.1
1.0
3.5
2.9
3.3
9.3
f1 (ppm)


135.7
135.7
133.2
133.2
131.7
131.5
130.0
130.0
127.9
111.5
83.9
82.5
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64.9
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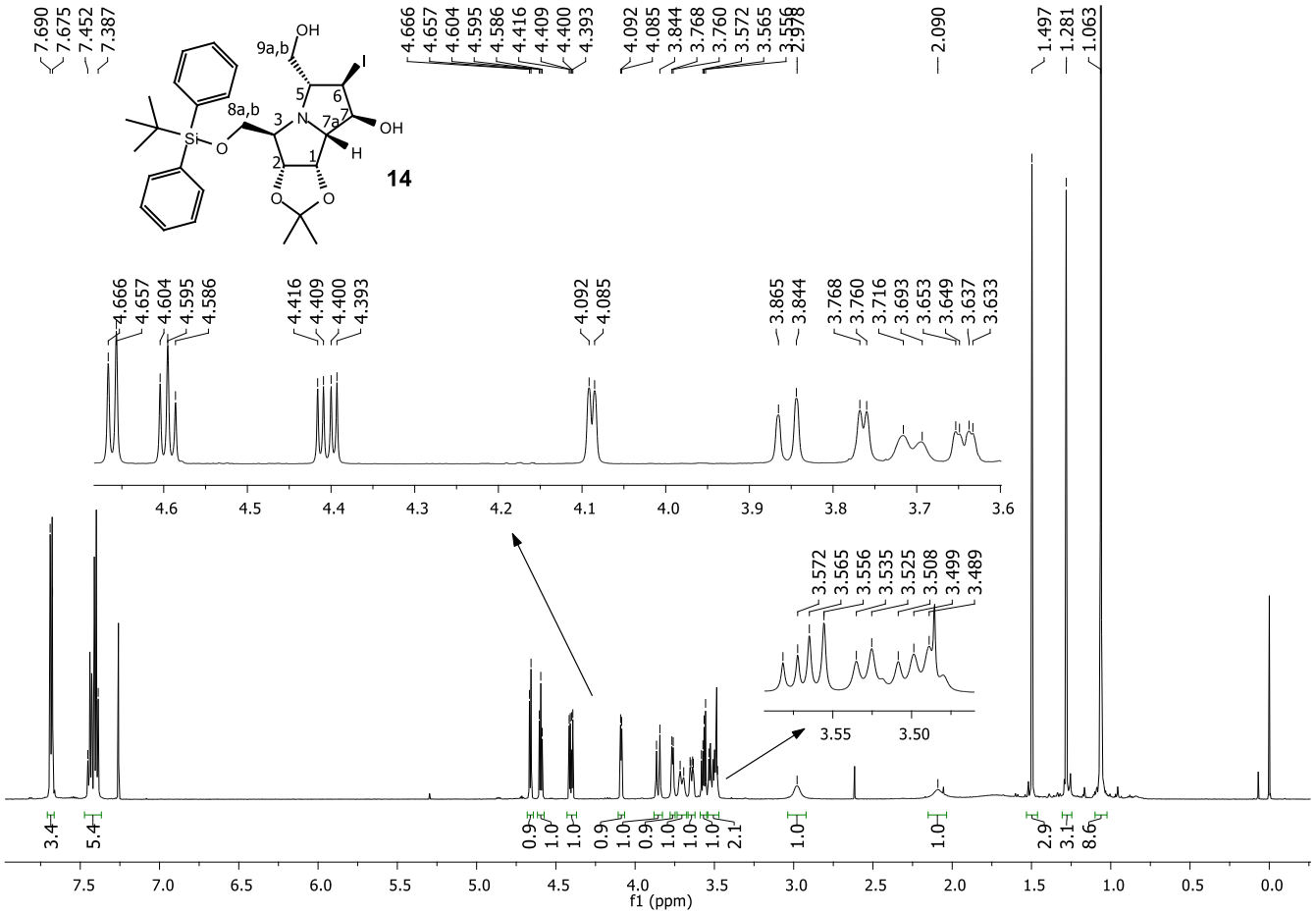
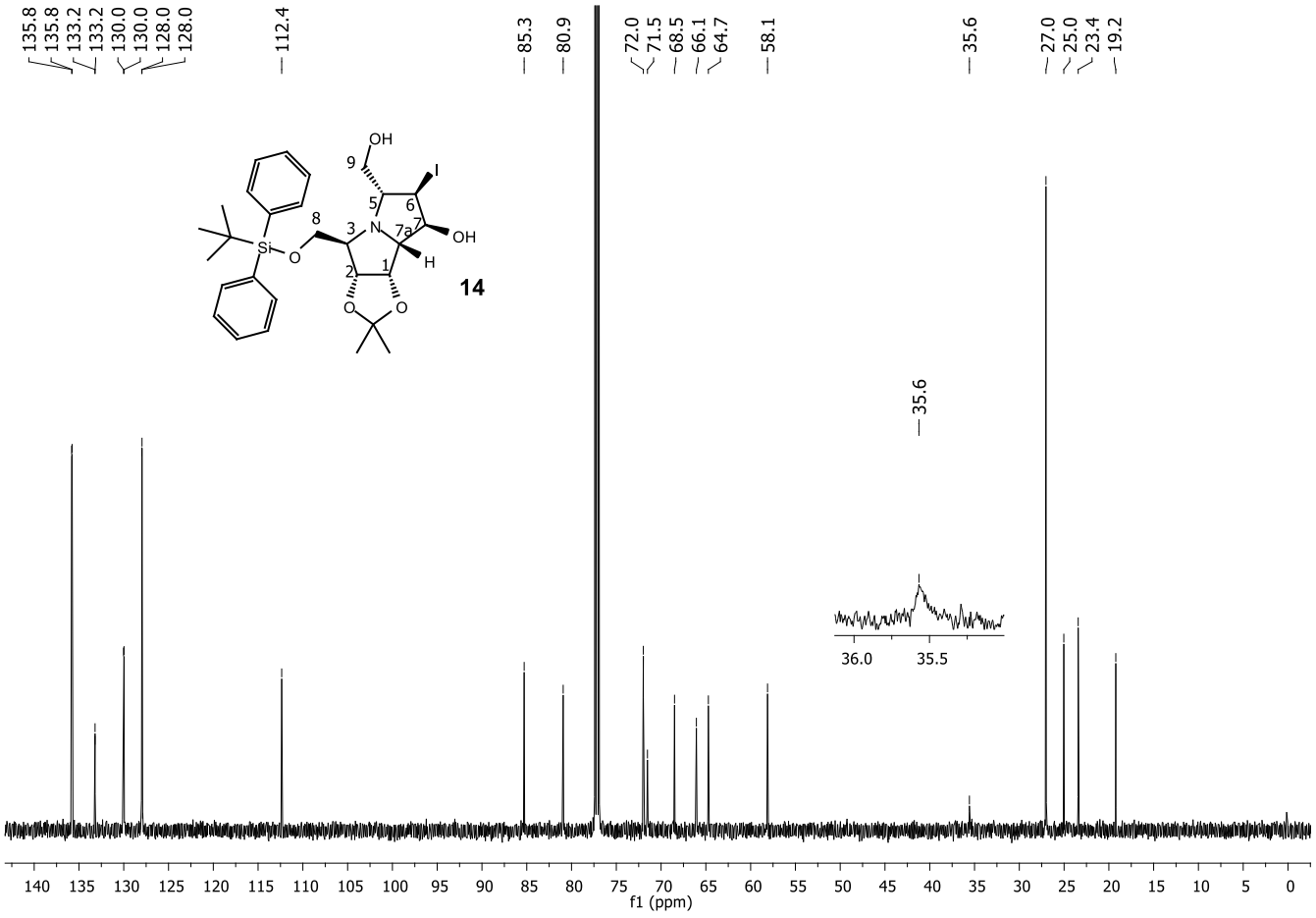
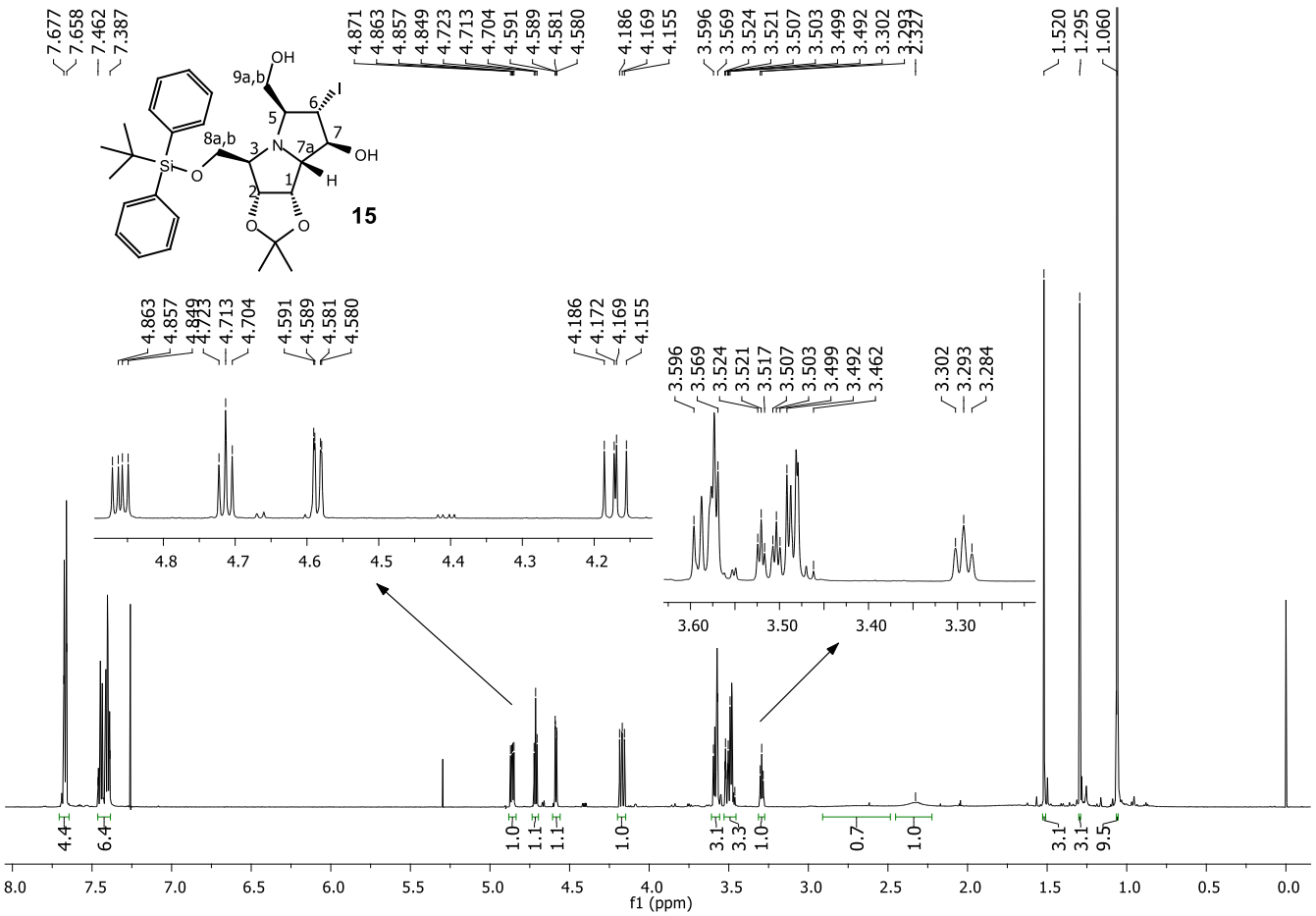
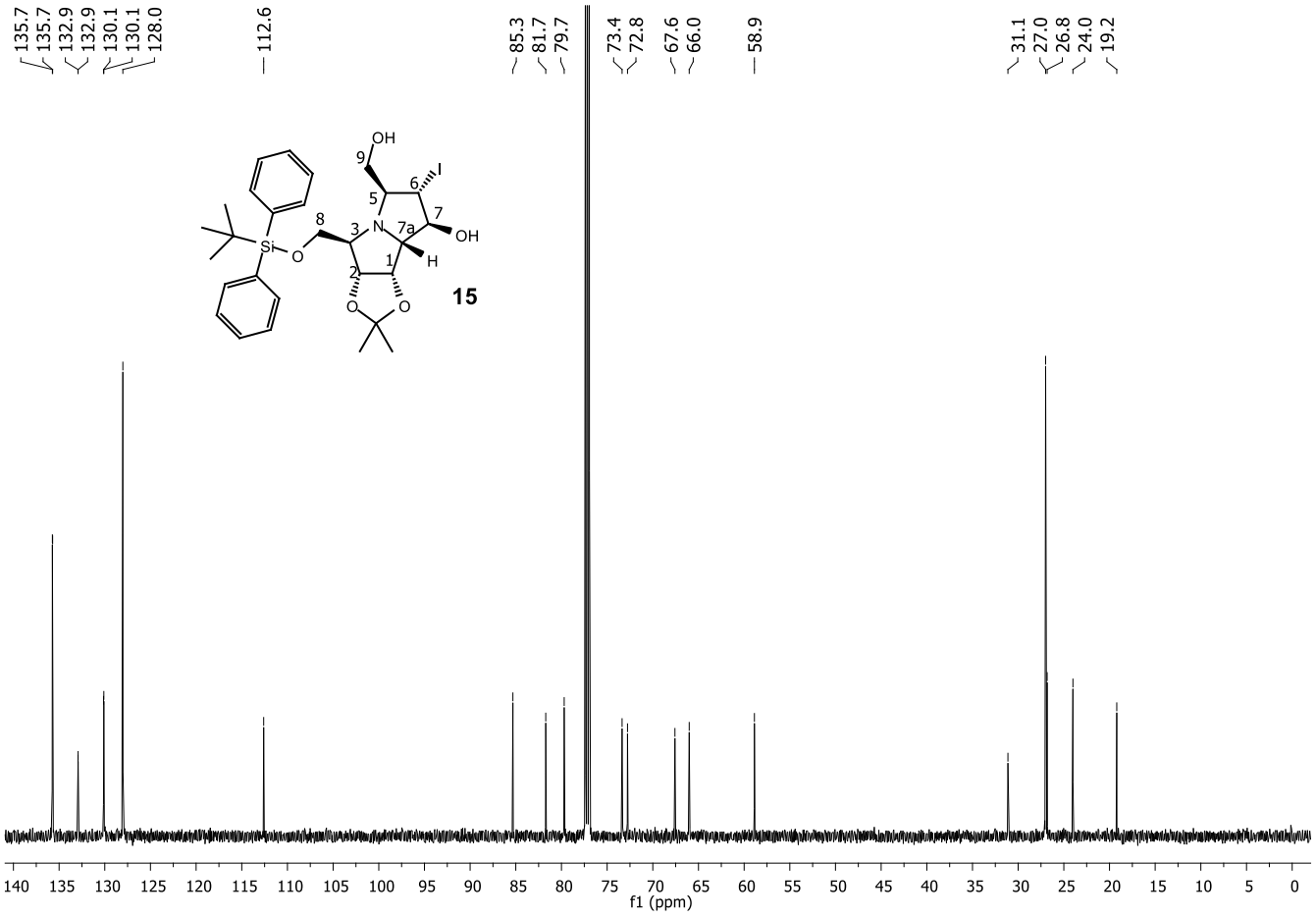
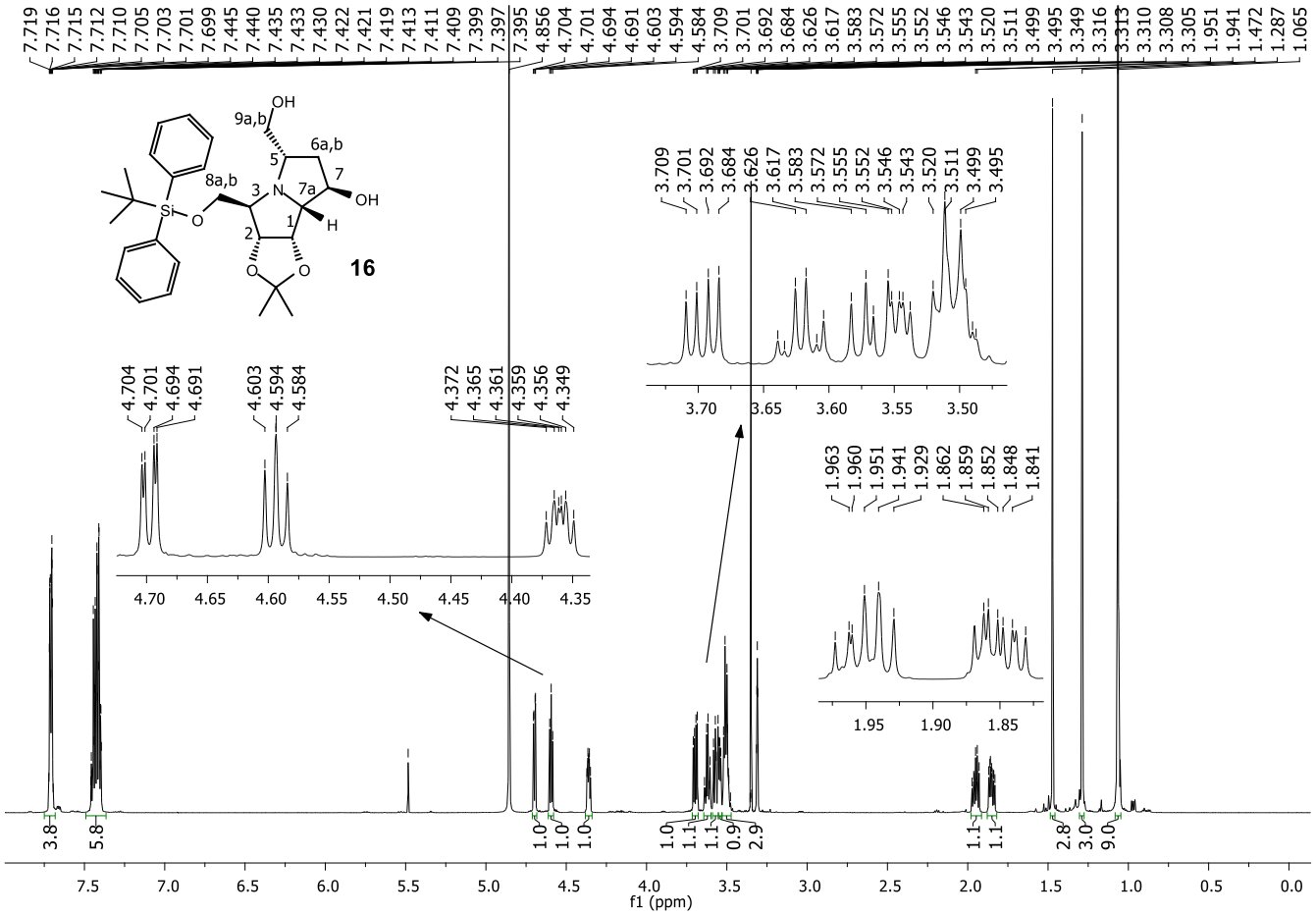
62.9
27.0
26.1
23.9
19.3
4
f1 (ppm)

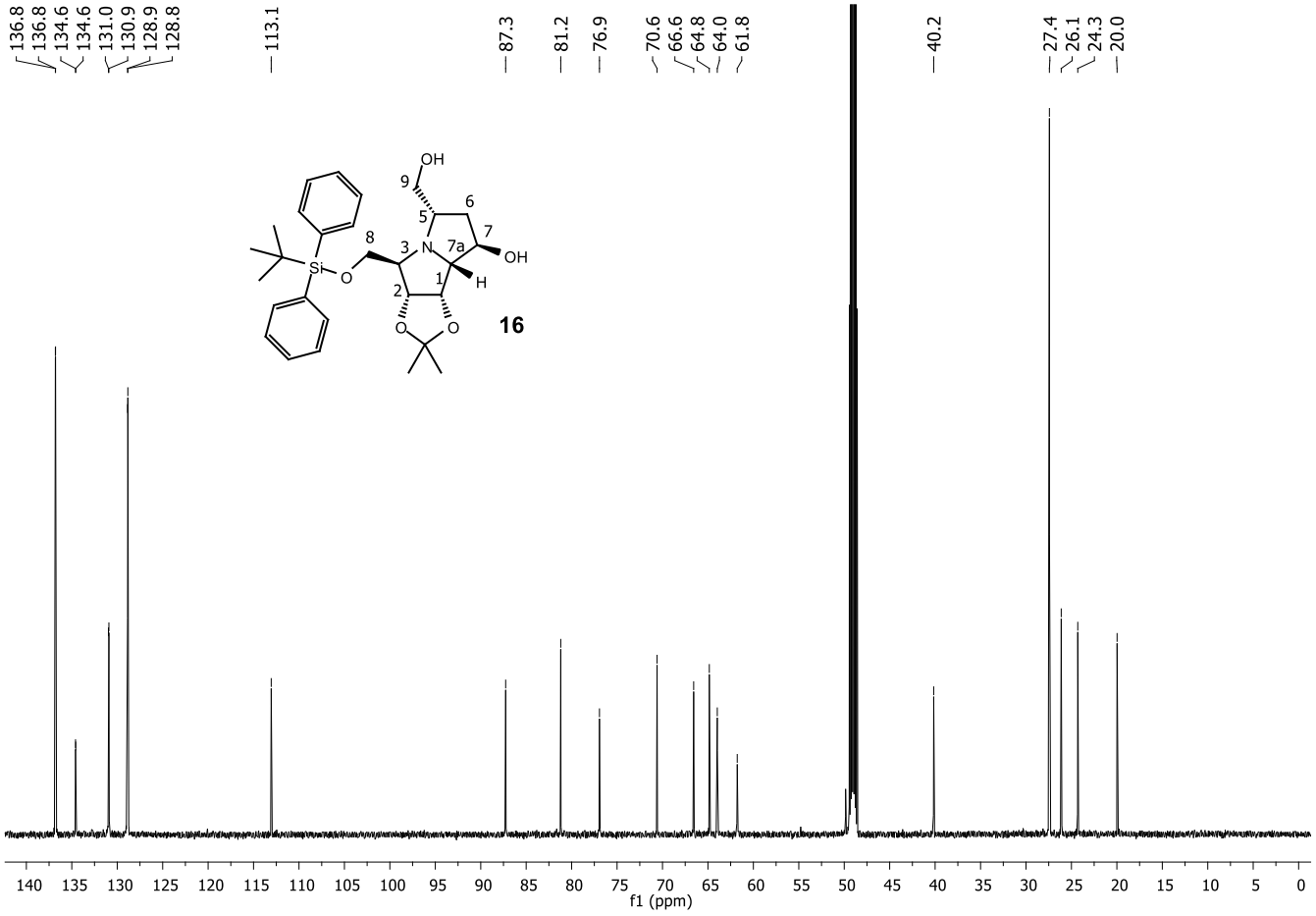

14
f1 (ppm)

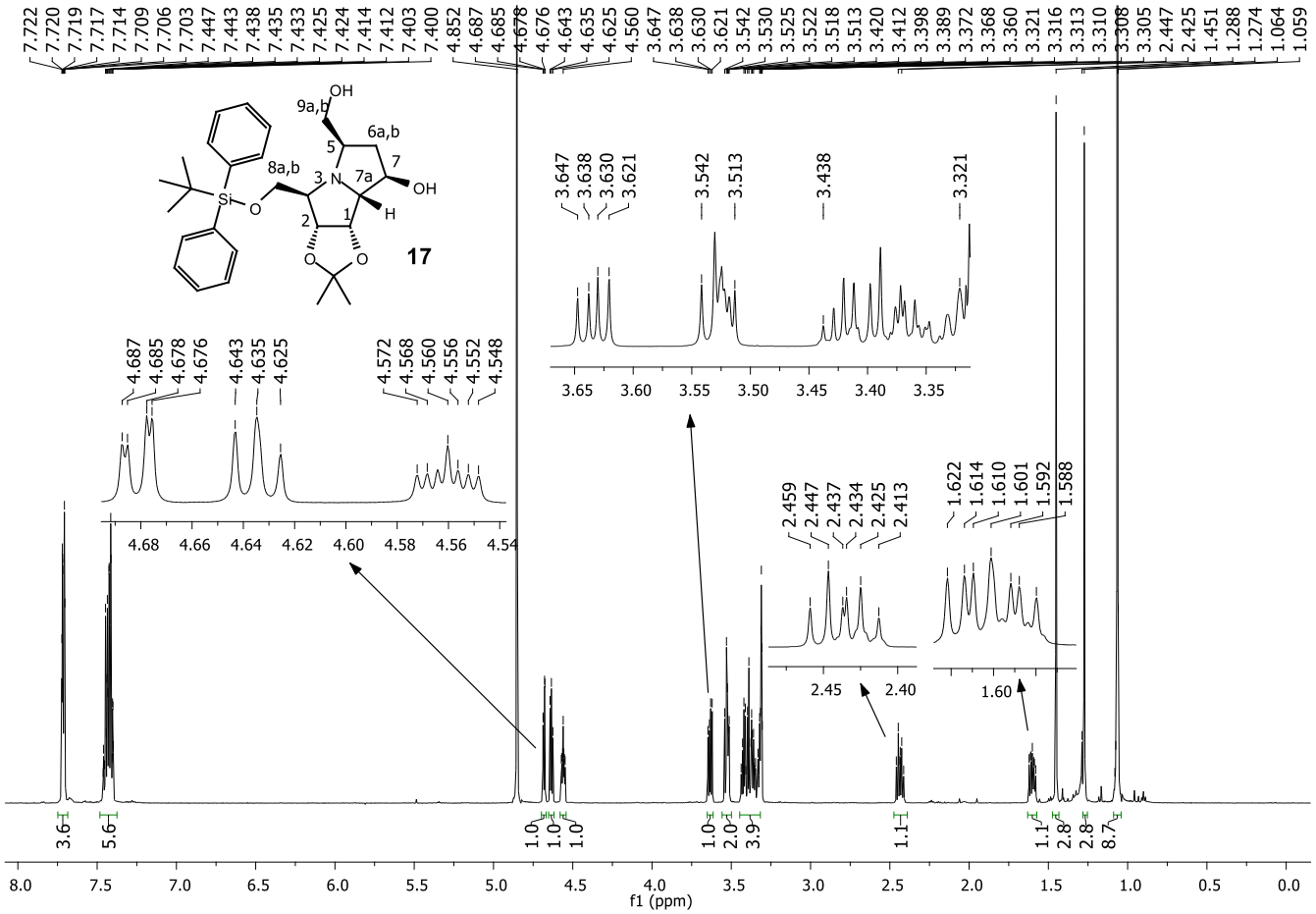

14
f1 (ppm)

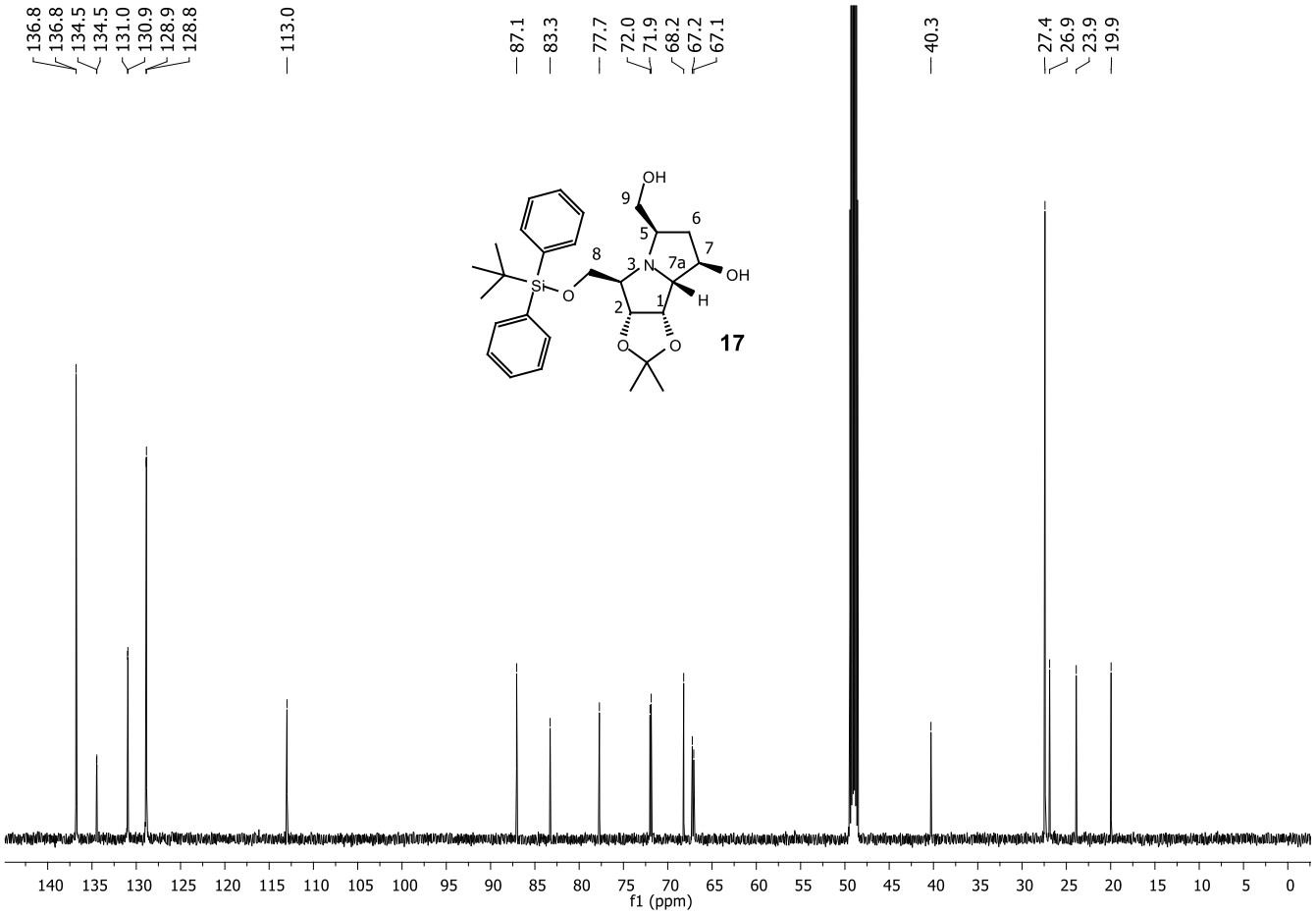

15
f1 (ppm)

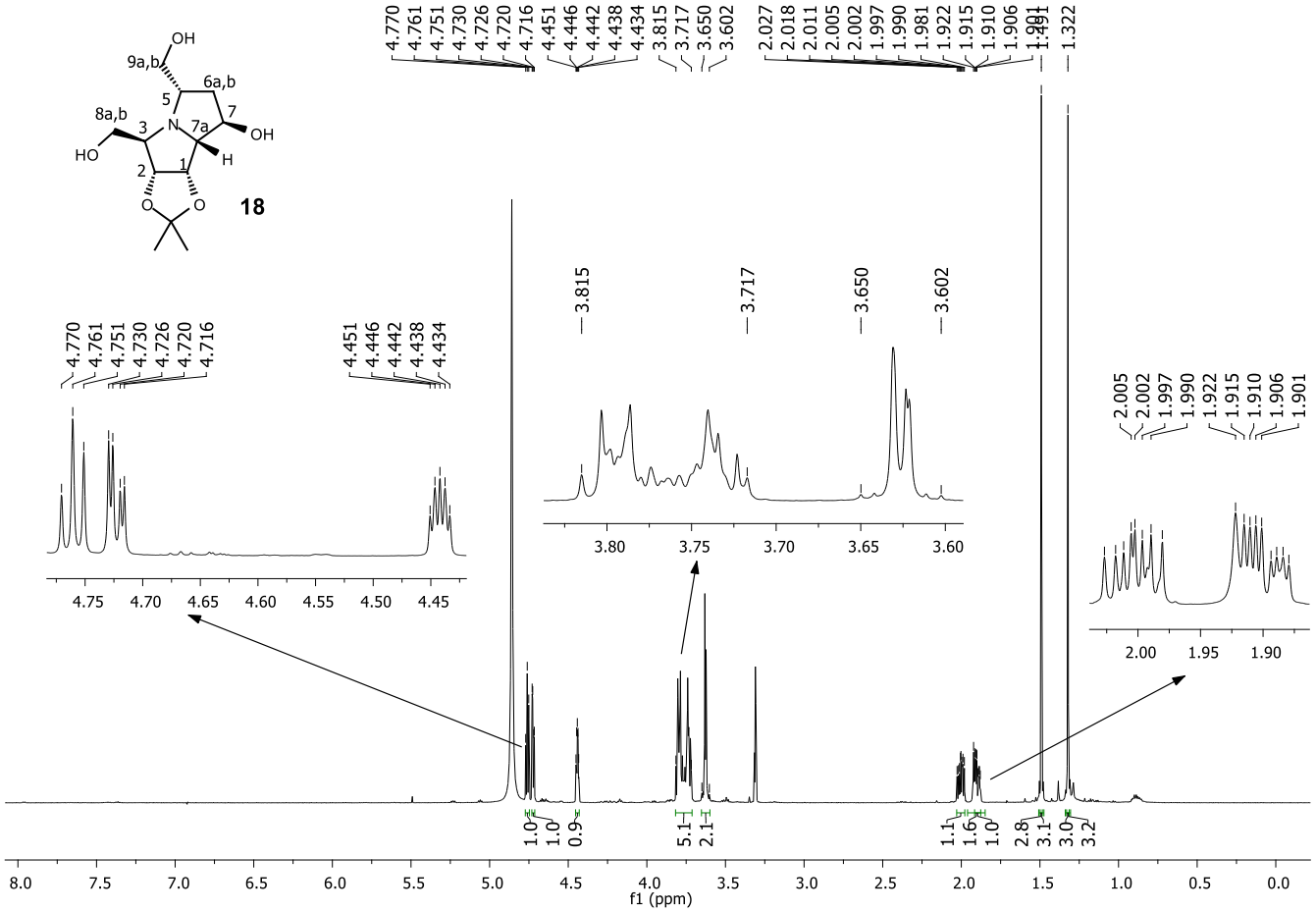

15
f1 (ppm)

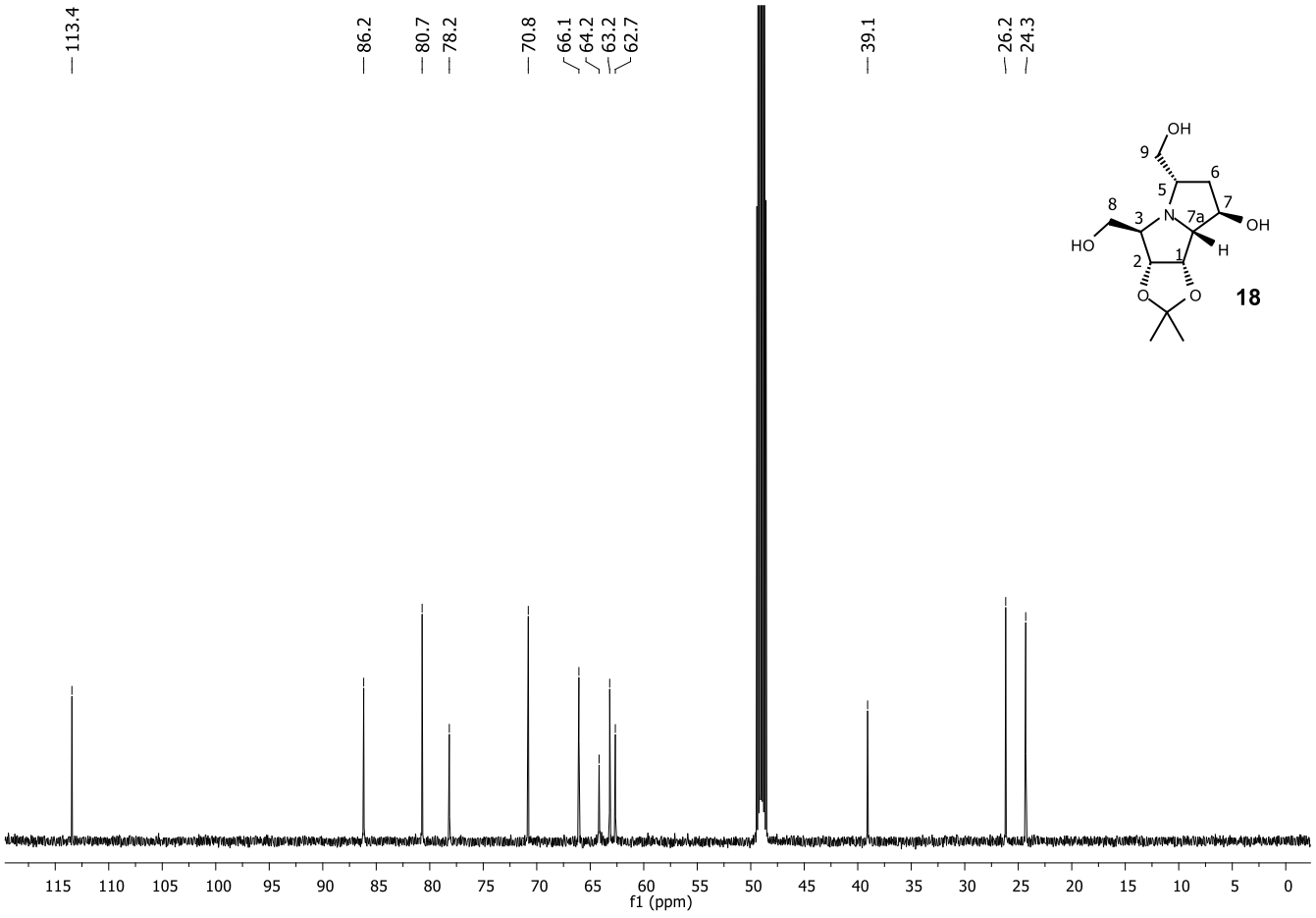

16
f1 (ppm)

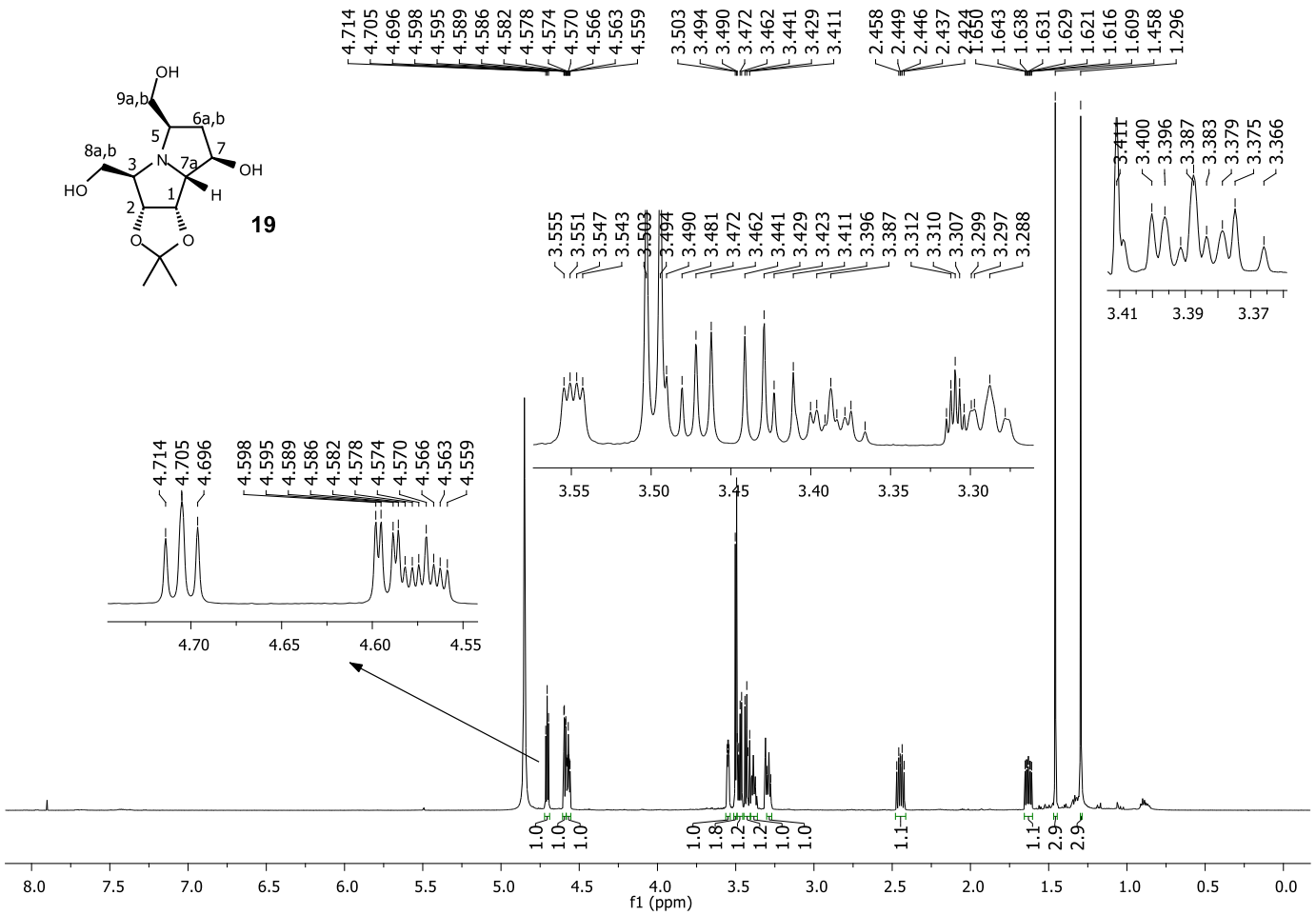

16
f1 (ppm)

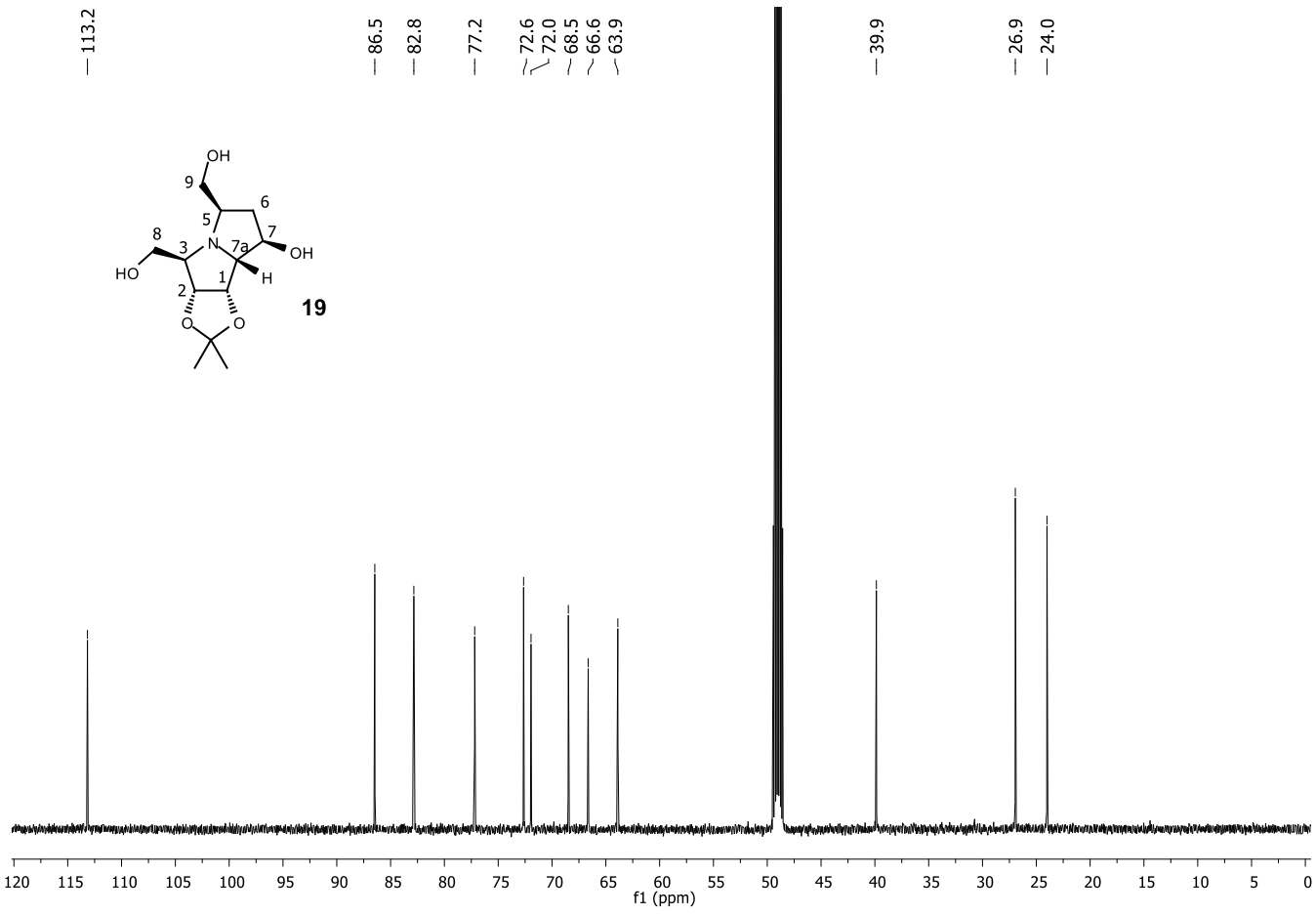

17
f1 (ppm)

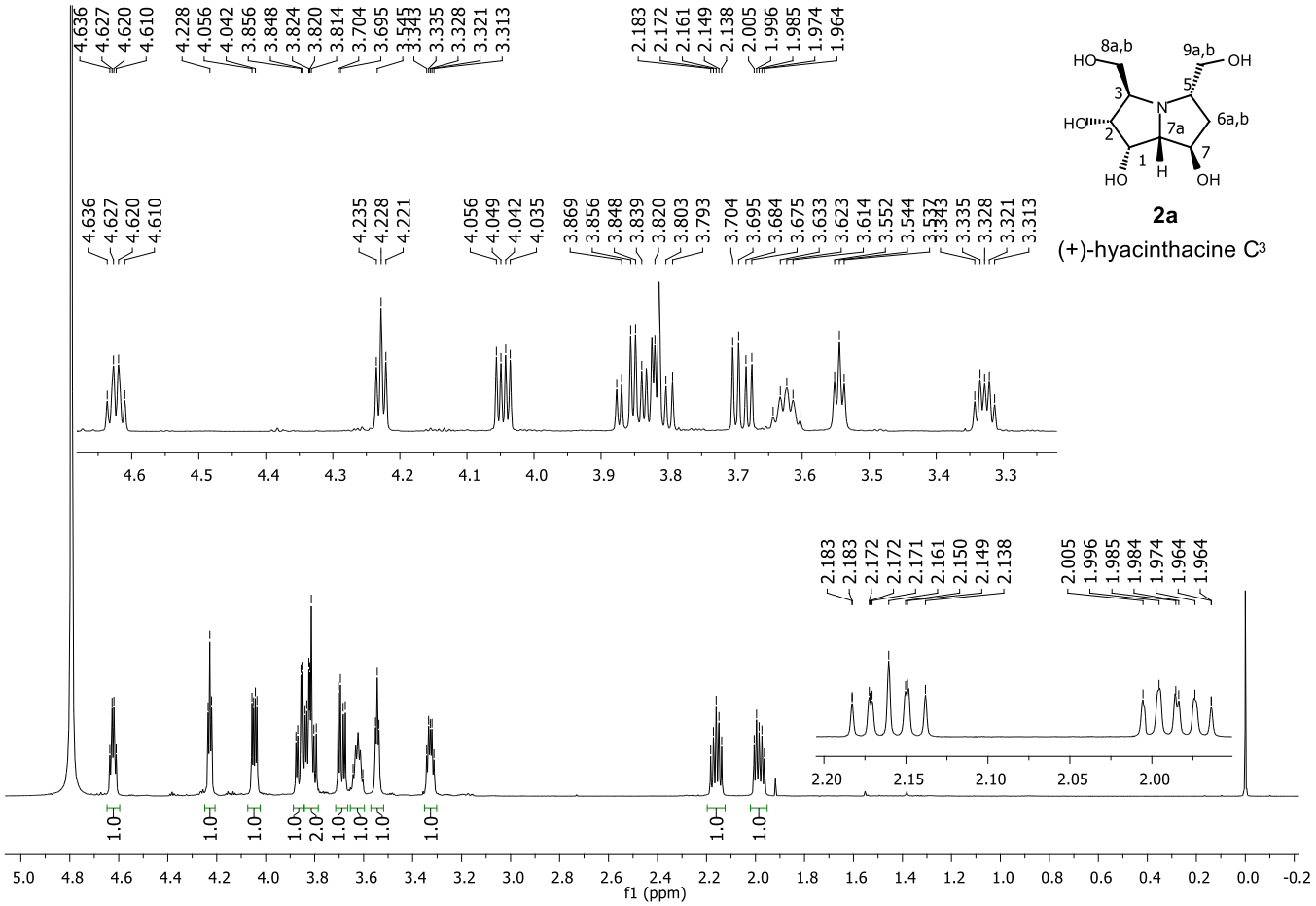

17
f1 (ppm)


18
f1 (ppm)


18
f1 (ppm)


19
f1 (ppm)


19
f1 (ppm)

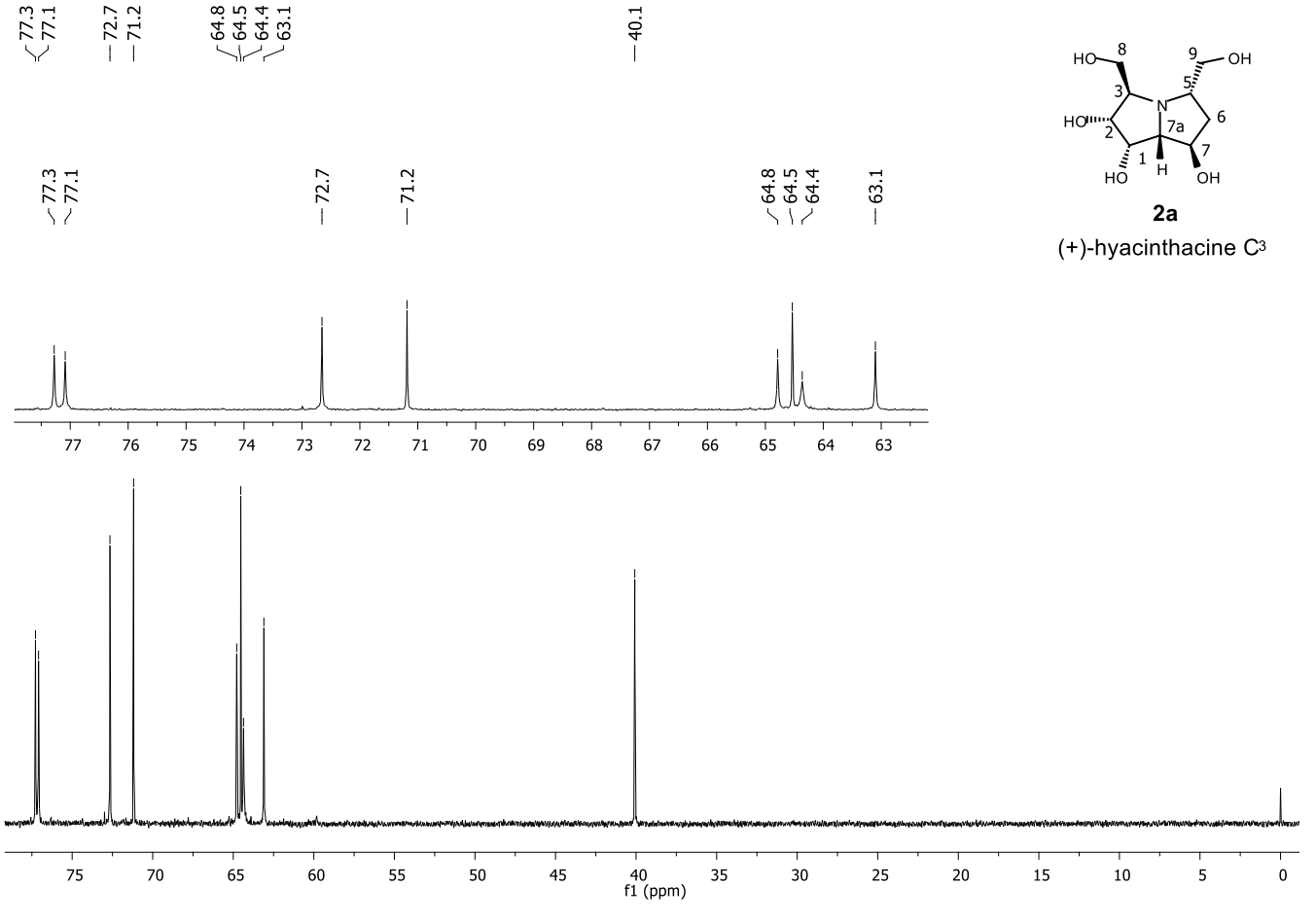






**2a**

(+)-hyacinthacine C3

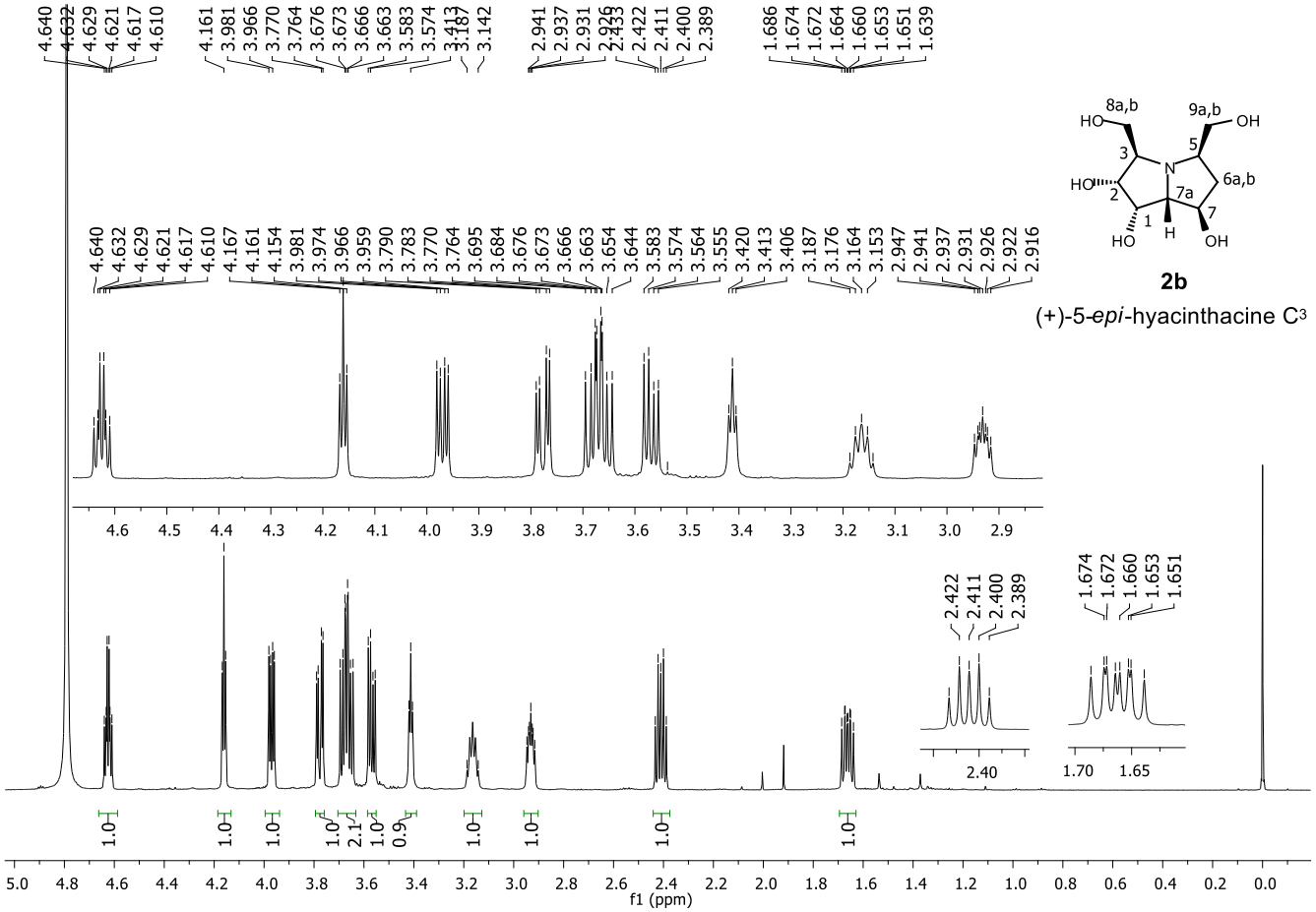






**2a**

(+)-hyacinthacine C3


4.640 4.632 4.629 4.621 4.617 4.610 4.161 3.981 3.966 3.770 3.764 3.676 3.673 3.666 3.663 3.583 3.574 3.413 3.187 3.142 2.941 2.937 2.931 2.926 2.433 2.422 2.411 2.400 2.389 1.686 1.674 1.672 1.664 1.660 1.653 1.651 1.639
8a,b HO 9a,b OH 3 5 N 6a,b HO 2 7a 1 7 H HO OH
2b
(+)-5-epi-hyacinthacine C3
4.640 4.632 4.629 4.621 4.617 4.610 4.167 4.161 4.154 3.981 3.974 3.966 3.959 3.790 3.783 3.770 3.764 3.695 3.684 3.676 3.673 3.666 3.663 3.654 3.644 3.583 3.574 3.564 3.555 3.420 3.413 3.406 3.187 3.176 3.164 3.153 2.947 2.941 2.937 2.931 2.926 2.922 2.916
4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9
2.422 2.411 2.400 2.389
1.674 1.672 1.660 1.653 1.651
2.40
1.70 1.65
1.0 1.0 1.0 1.0 2.1 1.0 0.9 1.0 1.0 1.0 1.0
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0
f1 (ppm)

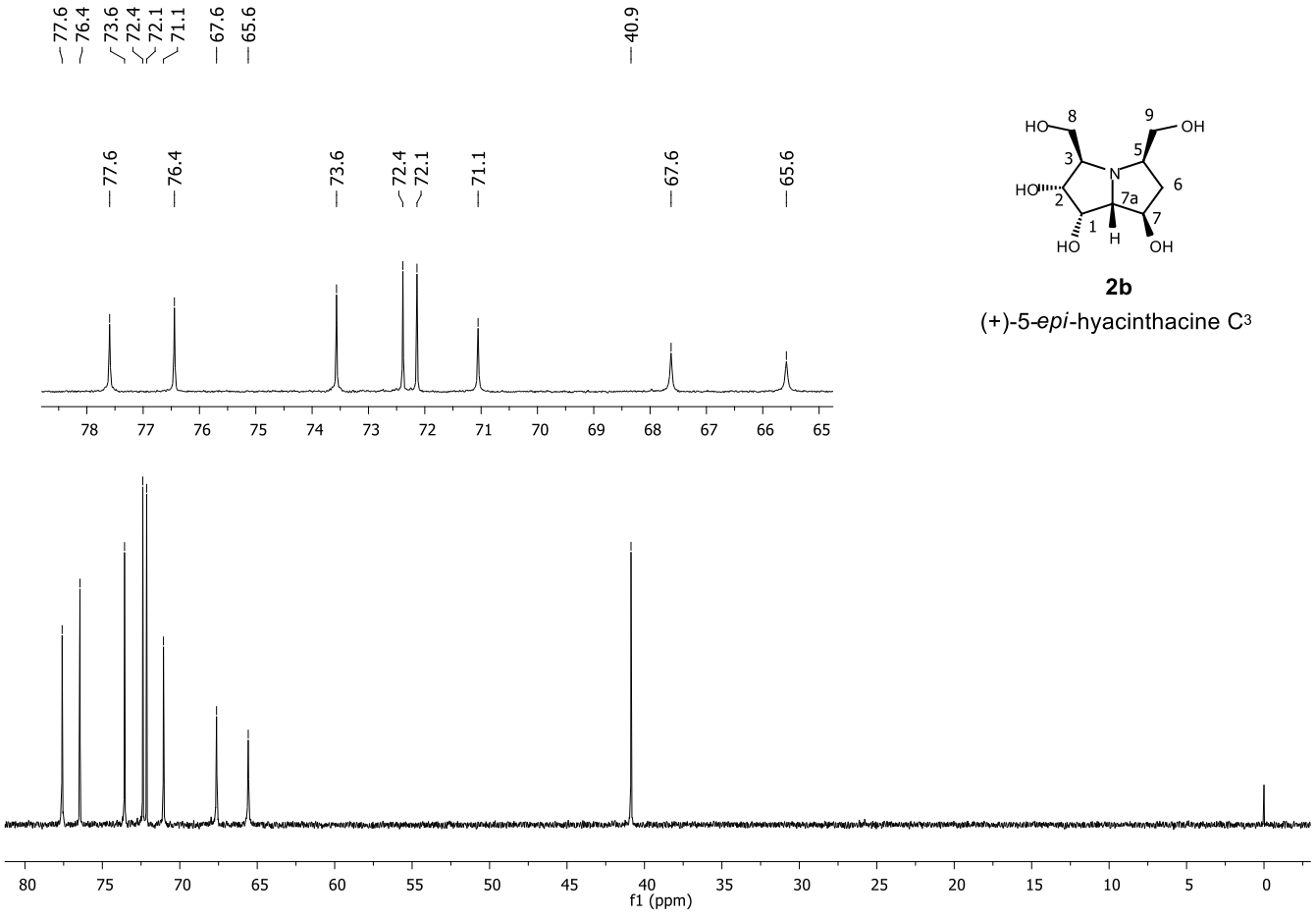

77.6 76.4 73.6 72.4 72.1 71.1 67.6 65.6 40.9
77.6 76.4 73.6 72.4 72.1 71.1 67.6 65.6
78 77 76 75 74 73 72 71 70 69 68 67 66 65
8 HO 9 OH 3 5 N 6 HO 2 7a 1 7 H HO OH
2b
(+)-5-epi-hyacinthacine C3
80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
f1 (ppm)

**Table S1.** $^{1}$H NMR spectroscopic data (in $D_2O$) of the synthetic (+)-**2a** (**x**) prepared by Fischer et al., the synthetic (+)-**2a** (**y**) prepared by Yoda et al.,[3] and the natural sample (**z**) isolated by Kato et al.[4]

| Entry | Proton | Synthetic (+)-**2a**[a,b] (**x**) | Synthetic (+)-**2a**[c,d,e] (**y**) | (**x**-**y**) | Natural Sample[a,f] (**z**) | (**x**-**z**) |
|---|---|---|---|---|---|---|
| 1 | H-7 | 4.63 (td, *J* = 5.8, 4.2 Hz) | 4.58 (m) | +0.05 | 4.56 (ddd, *J* = 2.5, 4.4, 2.5 Hz) | +0.07 |
| 2 | H-1 | 4.23 (pseudo t, *J* = 4.3 Hz) | 4.16 (t, *J* = 4.2 Hz) | +0.07 | 4.32 (t, *J* = 4.4 Hz) | –0.09 |
| 3 | H-2 | 4.05 (dd, *J* = 8.3, 4.0 Hz) | 3.98 (dd, *J* = 7.8, 4.2 Hz) | +0.07 | 4.04 (dd, *J* = 9.5, 4.4 Hz) | +0.01 |
| 4 | H-$9_b$ | 3.86 (dd, *J* = 12.5, 4.3 Hz) | 3.71–3.83 (m) | --- | 3.84 (ol)[g] | +0.02 |
| 5 | H-$8_b$ | 3.83 (dd, *J* = 11.8, 4.2 Hz) | | --- | 3.85 (dd, *J* = 12.6, 3.2 Hz) | –0.02 |
| 6 | H-$9_a$ | 3.81 (dd, *J* = 12.2, 6.1 Hz) | | --- | 3.79 (dd, *J* = 12.0, 6.2 Hz) | +0.02 |
| 7 | H-$8_a$ | 3.69 (dd, *J* = 11.8, 5.3 Hz) | 3.62 (dd, *J* = 11.2, 5.8 Hz) | +0.07 | 3.69 (dd, *J* = 12.6, 3.2 Hz) | 0.00 |
| 8 | H-5 | 3.60–3.64 (m) | 3.49 (m) | +0.13 | 3.84 (ol)[g] | –0.22 |
| 9 | H-7a | 3.54 (pseudo t, *J* = 4.3 Hz) | 3.38 (t, *J* = 4.2 Hz) | +0.16 | 3.85 (ol)[g] | –0.31 |
| 10 | H-3 | 3.33 (dt, *J* = 8.3, 4.7 Hz) | 3.20 (dt, *J* = 7.8, 5.1 Hz) | +0.13 | 3.50 (m) | –0.17 |
| 11 | H-$6_b$ | 2.16 (dt, *J* = 13.2, 6.6 Hz) | 2.13 (m) | +0.03 | 2.07 (m) | +0.09 |
| 12 | H-$6_a$ | 1.99 (dt, *J* = 13.1, 6.0 Hz) | 1.92 (m) | +0.07 | 1.93 (m) | +0.06 |

[a] Chemical shifts are expressed in ppm and referenced to sodium 3-(trimethylsilyl)propionate (TSP). [b] NMR spectra have been recorded on 600 MHz instrument. [c] Spectrometer frequency was not specified. [d] $^{1}$H NMR spectrum has been recorded without the use of TSP as an internal standard. [e] The corresponding protons were not assigned. [f] NMR spectra have been recorded on 500 MHz instrument. [g] Overlapped (ol).

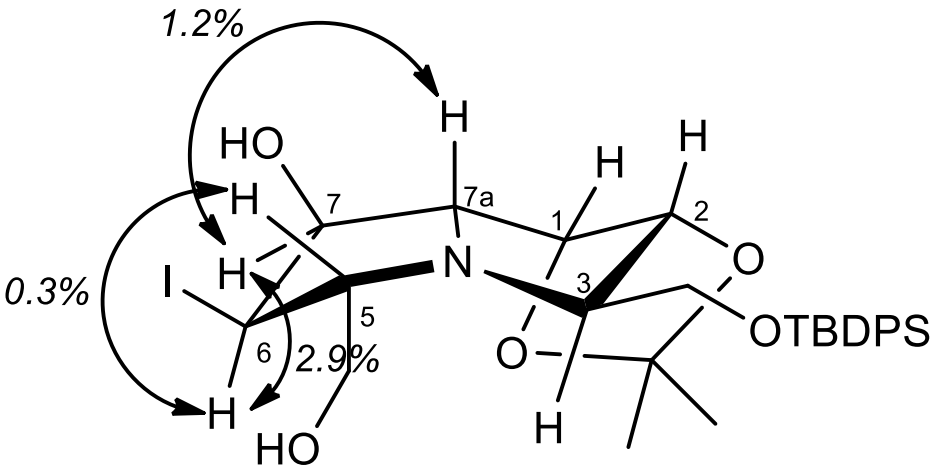
**Table S2.** $^{13}C$ NMR spectroscopic data (in $D_2O$) of the synthetic (+)-**2a** (**x**) prepared by Fischer et al., the synthetic (+)-**2a** (**y**) prepared by Yoda et al.,[3] and the natural sample (**z**) isolated by Kato et al.[4]

| Entry | Carbon | Synthetic (+)-**2a**[a,b] (**x**) | Synthetic (+)-**2a**[c,d,e] (**y**) | (**x**-**y**) | Natural Sample[a,f] (**z**) | (**x**-**z**) |
|---|---|---|---|---|---|---|
| 1 | C-7a | 77.3 | 75.5 (CH) | +1.8 | 79.9 | –2.6 |
| 2 | C-2 | 77.1 | 75.0 (CH) | +2.1 | 75.4 | +1.7 |
| 3 | C-1 | 72.7 | 70.9 (CH) | +1.8 | 72.2 | +0.5 |
| 4 | C-7 | 71.2 | 69.2 (CH) | +2.0 | 71.7 | –0.5 |
| 5 | C-5 | 64.8 | 62.5 (CH) | +2.3 | 67.5 | –2.7 |
| 6 | C-3 | 64.5 | 62.5 (CH) | +2.0 | 65.5 | –1.0 |
| 7 | C-8 | 64.4 | 63.0 ($CH_2$) | +1.4 | 61.7 | +2.7 |
| 8 | C-9 | 63.1 | 61.4 ($CH_2$) | +1.7 | 61.8 | +1.3 |
| 9 | C-6 | 40.1 | 38.3 ($CH_2$) | +1.8 | 39.4 | +0.7 |

[a] Chemical shifts are expressed in ppm and referenced to sodium 3-(trimethylsilyl)propionate (TSP). [b] NMR spectra have been recorded on 600 MHz instrument ($^{13}C$ NMR, 150 MHz). [c] Spectrometer frequency was not specified. [d] $^{1}H$ NMR spectrum has been recorded without the use of TSP as an internal standard. [e] The corresponding carbon atoms were not assigned. [f] NMR spectra have been recorded on 500 MHz instrument ($^{13}C$ NMR, 125 MHz).

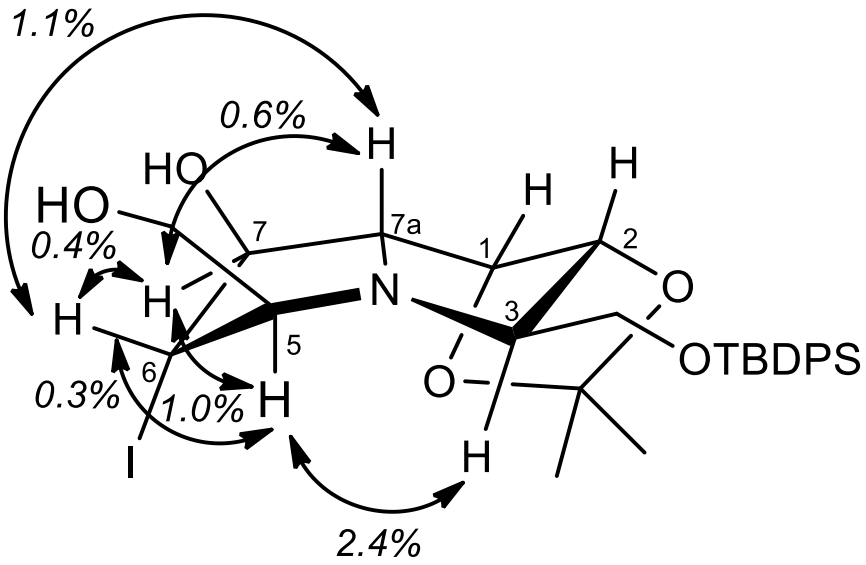
**Important nOe enhancements observed in iodo-pyrrolizidines. Arrows show the NOESY correlations.**

The structure of **14**:




**14**

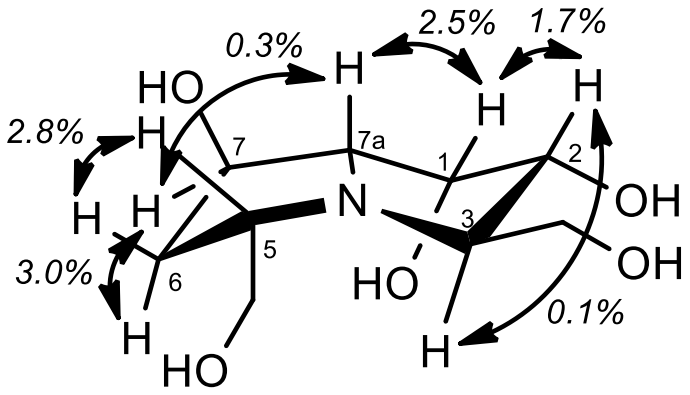
The structure of **15**:




**15**

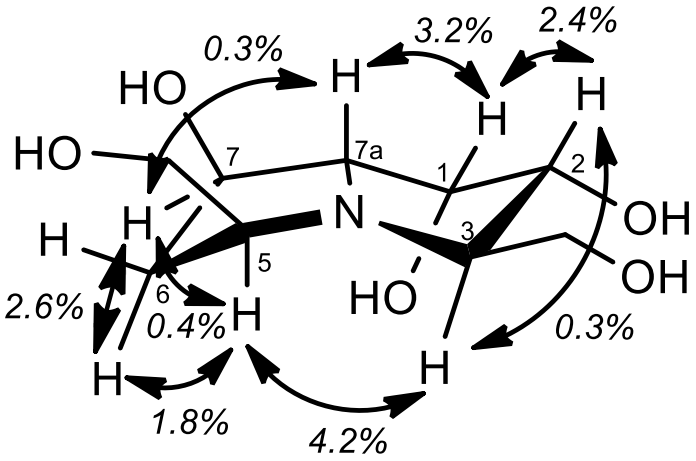
**Important nOe enhancements observed in the (+)-hyacinthacine $C_3$ and the (+)-5-*epi*-hyacinthacine $C_3$. Arrows show the NOESY correlations.**

The structure of **2a**:




**2a**

The structure of **2b**:




**2b**

**Table S3.** Summarization of $IC_{50}$ values for compounds **2a** and **2b**.

| **Tested compound** | **Cell line** | **The concentration range (µM)** | **$IC_{50}$ (µM)** treatment 24 h | **$IC_{50}$ (µM)** treatment 48 h | **$IC_{50}$ (µM)** treatment 72 h |
|---|---|---|---|---|---|
| **2a** | U87-MG | 1 – 1000 | > 1000 | > 1000 | > 1000 |
| **2b** | U87-MG | 1 – 1000 | > 1000 | > 1000 | > 1000 |
| **2a** | HK-2 | 1 – 1000 | > 1000 | > 1000 | > 1000 |
| **2b** | HK-2 | 1 – 1000 | > 1000 | > 1000 | > 1000 |
| **2a** | HepG2 | 1 – 1000 | > 1000 | > 1000 | > 1000 |
| **2b** | HepG2 | 1 – 1000 | > 1000 | > 1000 | > 1000 |
| **2a** | JEG-3 | 1 – 1000 | > 1000 | > 1000 | -* |
| **2b** | JEG-3 | 1 – 1000 | > 1000 | > 1000 | -* |

*Note:* The viability of JEG-3 cell line was not tested after 72 hours treatment of compounds.*

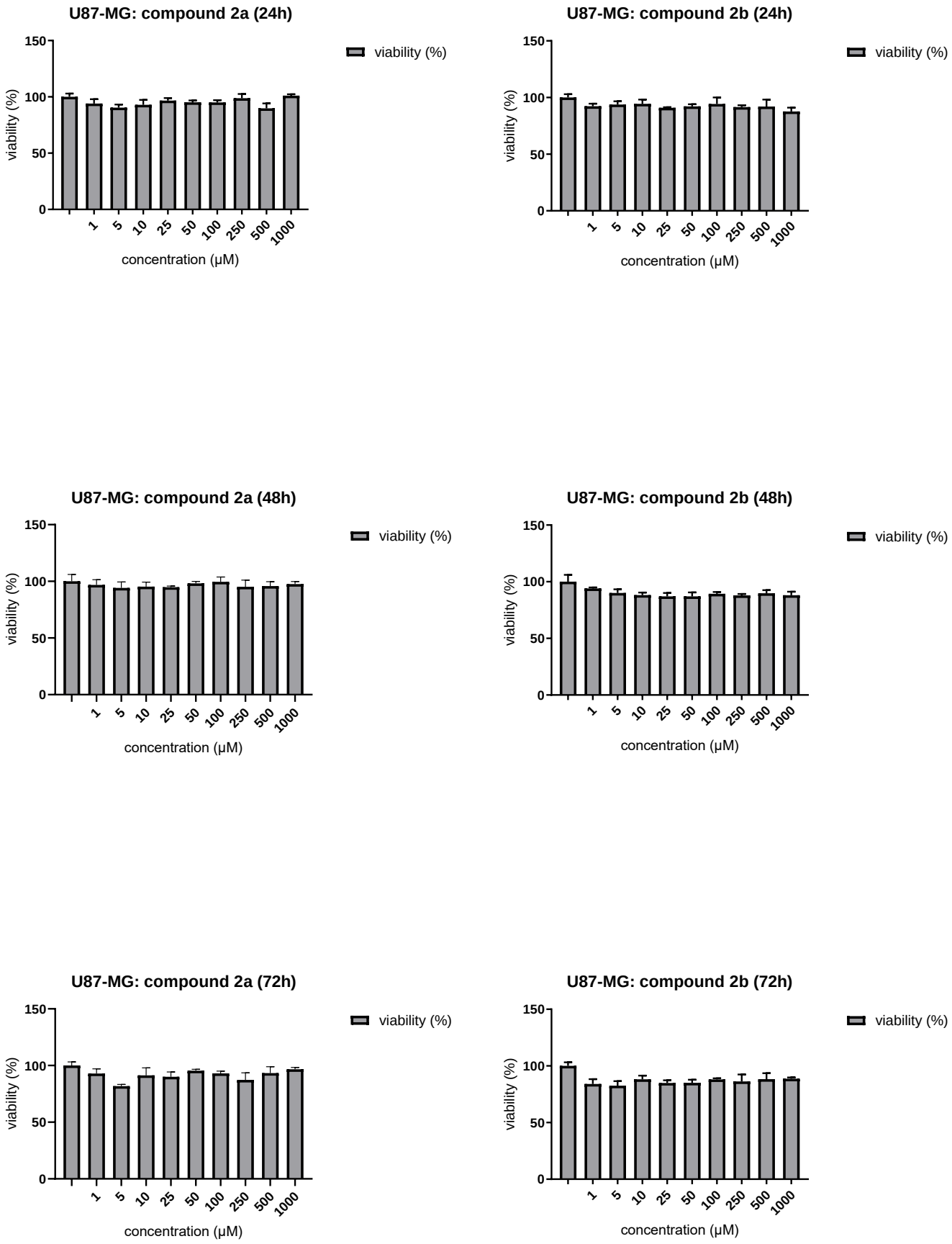



**Figure S1.** Antiproliferative effect of the tested compounds **2a** and **2b** on U87-MG cells after 24, 48, and 72 h treatment.

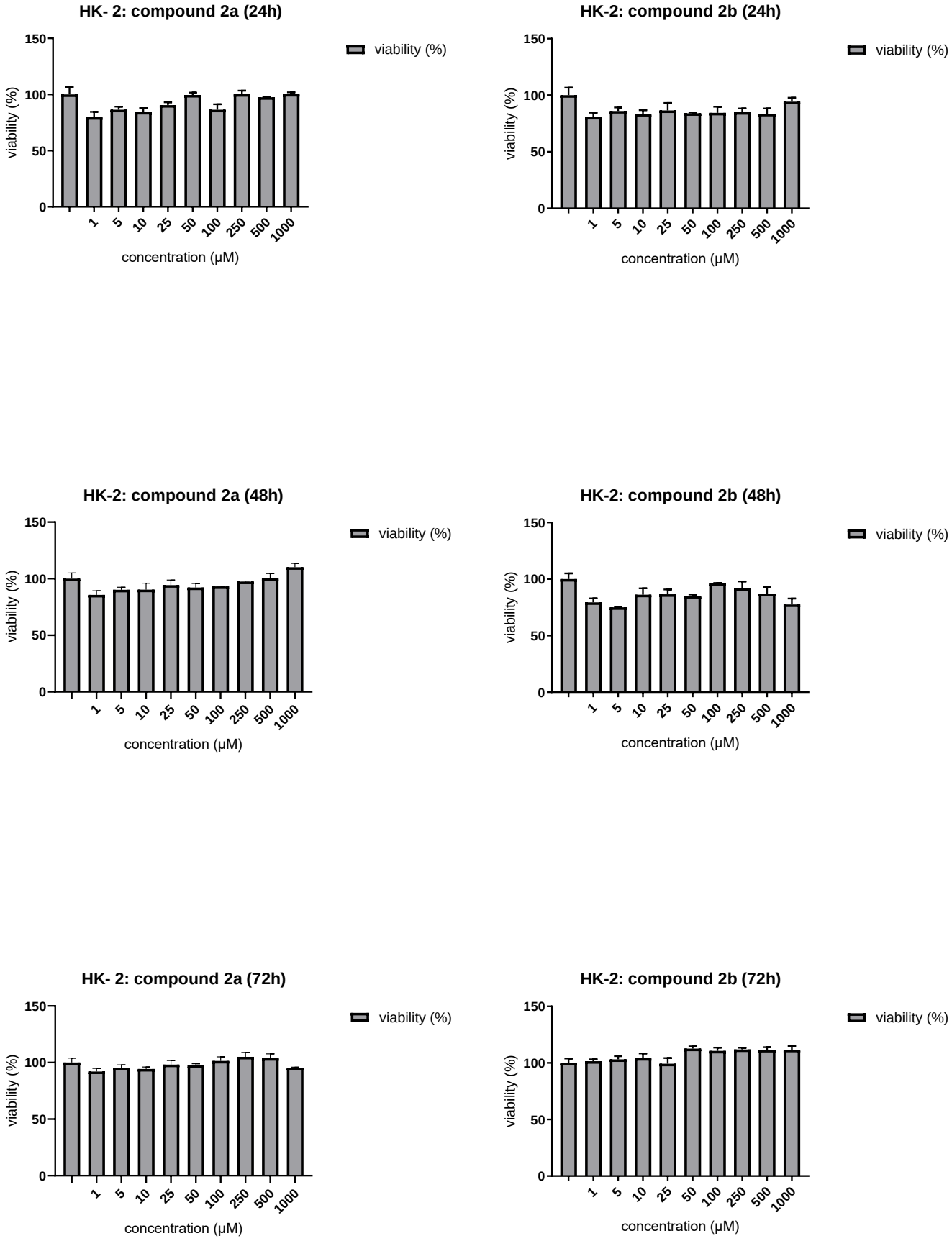



**Figure S2.** Antiproliferative effect of the tested **2a** and **2b** compounds on HK-2 cells after 24, 48, and 72 h treatment.

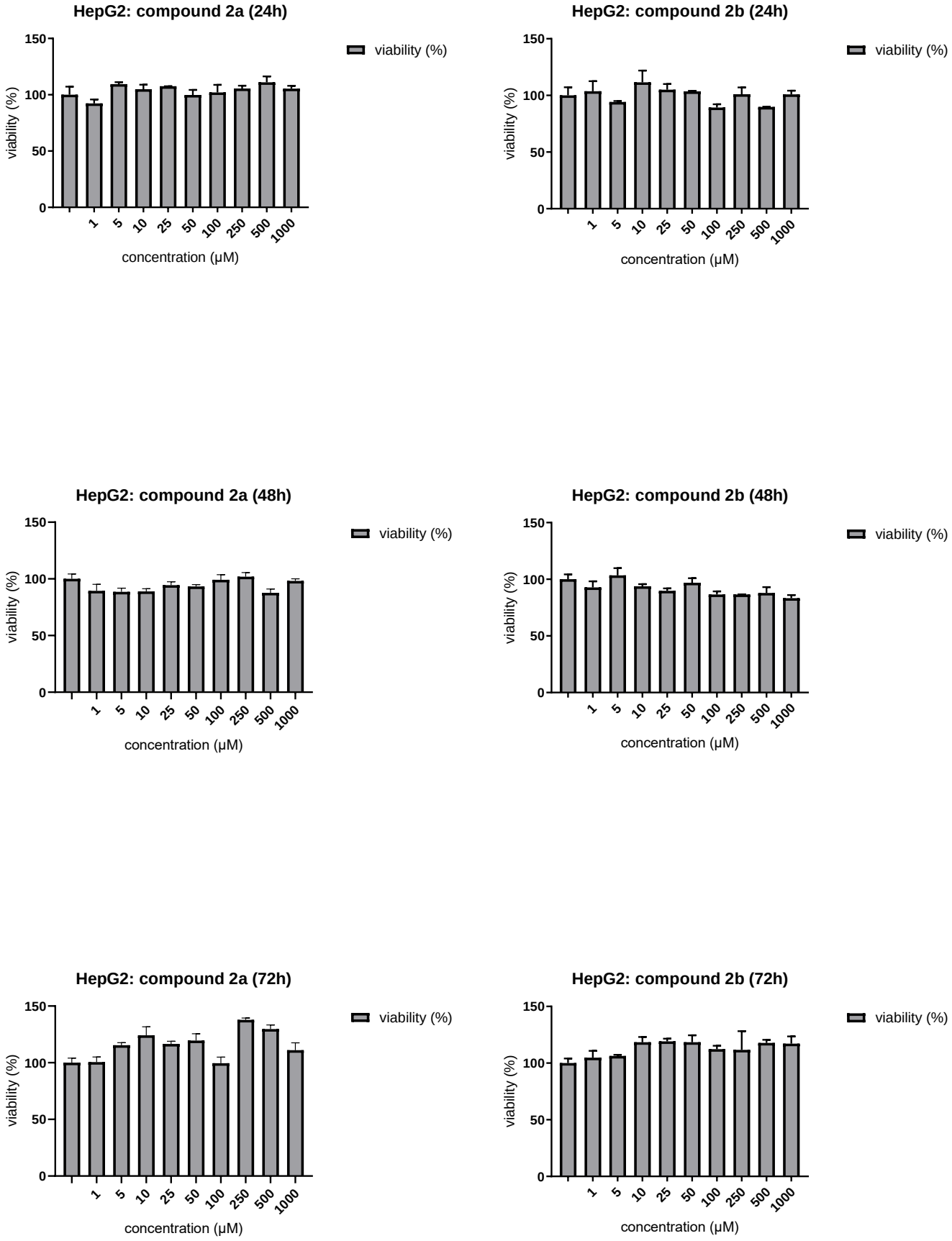



**Figure S3.** Antiproliferative effect of the tested compounds **2a** and **2b** on HepG2 cells after 24, 48, and 72 h treatment.

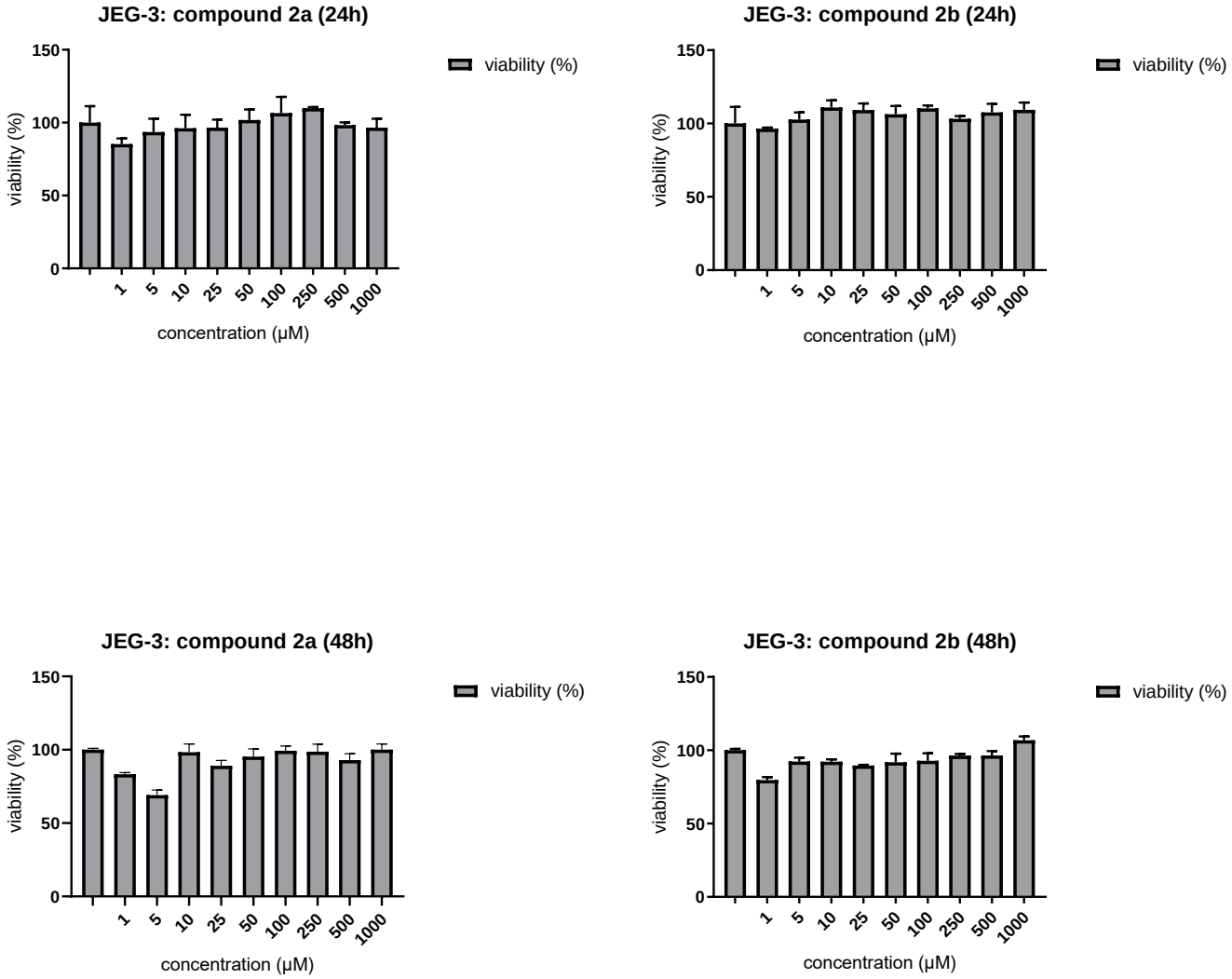



**Figure 4.** Antiproliferative effect of the tested compounds **2a** and **2b** on JEG-3 cells after 24 or 48 h treatment.

## References


1. (*a*) J. G. Buchanan, K. W. Lumbard, R. J. Sturgeon, D. K. Thompson and R. H. Wightman, *J. Chem. Soc., Perkin Trans. 1*, 1990, 699; (*b*) K. K. Somarathne, J. A. J. McCone, A. Brackovic, J. Luis Pinedo Rivera, J. Robin Fulton, E. Russell, J. J. Field, C. L. Orme, H. L. Stirrat, J. Riesterer, P. H. Teesdale-Spittle, J. H. Miller and J. E. Harvey, *Chem. Asian J.*, 2019, **14**, 1230.
2. X. Li, Z. Qin, R. Wang, H. Chen and P. Zhang, *Tetrahedron*, 2011, **67**, 1792.
3. T. Sengoku, Y. Satoh, M. Takahashi and H. Yoda, *Tetrahedron Lett.*, 2009, **50**, 4937.
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