**Supporting Information**

for

# Sequential one-pot multienzyme (OPME) synthesis of lacto-*N*-neotetraose and its sialyl and fucosyl derivatives

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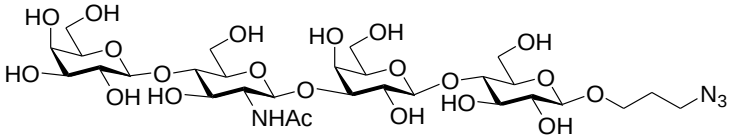
## General methods

All chemicals were obtained from commercial suppliers and used without further purification unless noted. Thin layer chromatography (TLC) was performed on silica gel plates 60 $F_{254}$ (Merck, Billerica MA). Plates were visualized under UV light and/or by treatment with 5% sulfuric acid in ethanol or *p*-anisaldehyde sugar stain followed by heating. Silica gel 60 (300-400 mesh, Haiyang, Qingdao, China) was used for flash column chromatography. Gel filtration chromatography was performed using a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA). $^{1}$H NMR (600 MHz) and $^{13}$C NMR (151 MHz) spectra were recorded on Bruker AVANCE-600 spectrometer, or Agilent VNMRS-600 spectrometer at 25 ℃. NMR spectra were calibrated using solvent signals ($^{1}$H: δ 7.26 for $CDCl_3$ or δ 3.34 for $CD_3OD$, $^{13}$C: δ 77.0 for $CDCl_3$). High resolution electrospray ionization (ESI) mass spectra were obtained at the National Glycoengineering Research Center and Drug Testing and Analysis Center in Shandong University.

NahK/GlmU[1], a fusion enzyme from *Bifidobacterium longum N*-acetylhexosamine-1-kinase (NahK) and *Escheerichia coli N*-acetylglucosamine uridylyltransferase (GlmU), *Helicobacter pylori* β1–3-*N*-acetylglucosaminyltransferase (HpLgtA)[2], *Escherichia coli* UDP-galactose-4-epimerase (EcGalE)[3], *Neisseria meningitides* β1–4-galactosyltransferase (NmLgtB)[1], *Neisseria meningitides* CMP-sialic acid synthetase (NmCSS)[4], *Photobacterium damselae* α2–6-sialyltransferase (Pd2,6ST)[5], *Pasteurella multocida* multifunctional α2–3-sialyltransferase 1 M144D mutant (PmST1 M144D)[6], *Bacteroides fragilis* bifunctional L-fucokinase/GDP-L-fucose pyrophosphorylase (FKP)[7], *Helicobacter pylori* α1–3-fucosyltransferase (Hpα1–3FTΔ66)[8] were expressed and purified as described previously.

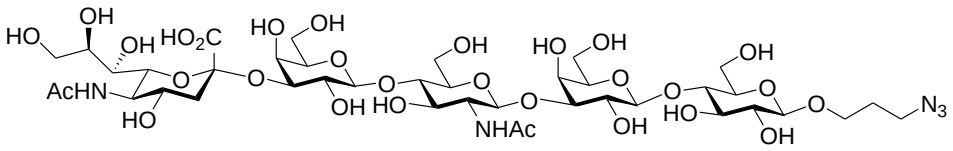
## Experimental Procedures




**Galβ1–4GlcNAcβ1–3Galβ1–4GlcβProN$_3$ (1)**

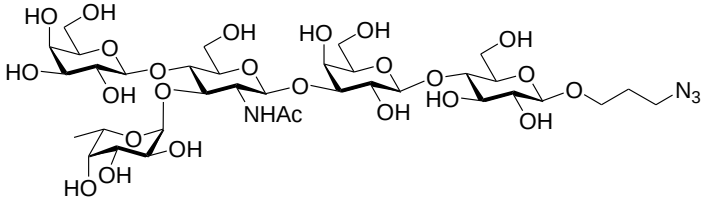
Trisaccharide **9** (126 mg, 0.20 mmol) and UDP-glucose (148 mg, 0.26 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mmol, pH 7.5) and $MgCl_2$ (20 mmol). After the addition of appropriate amount of EcGalE (3.5 mg) and NmLgtB (2.0 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction mixture was incubated in a shaking incubator at 37 ℃ for 12 hrs with agitation at 140 rpm. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). To stop the reaction, the reaction mixture was added with same volume of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 6:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide tetrasaccharide **1** (148 mg, 93%) as a

white solid. $^{1}$H NMR (600 MHz, $D_2O$) δ 4.69 (d, *J* = 8.4 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.46 (d, *J* = 7.7 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.14 (d, *J* = 3.0 Hz, 1H), 4.03–3.93 (m, 3H), 3.91 (d, *J* = 3.0 Hz, 1H), 3.83 (dd, *J* = 4.5, 12.4 Hz, 1H), 3.80–3.51 (m, 19H), 3.45 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 8.4 Hz, 1H), 2.02 (s, 3H), 1.90 (p, *J* = 6.6 Hz, 2H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.78, 102.83, 102.76, 102.64, 102.01, 81.94, 78.25, 78.06, 75.24, 74.77, 74.67, 74.44, 74.26, 72.69, 72.41, 72.07, 70.87, 69.86, 68.45, 68.23, 67.26, 60.94, 60.86, 59.96, 59.77, 55.09, 47.78, 28.15, 22.07; HRMS (ESI) *m/z* calcd for $C_{29}H_{50}N_4O_{21}Na$ $[M+Na]^+$ 813.2865, found 813.2831.



**Neu5Acα2–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcβProN$_3$ (2)**

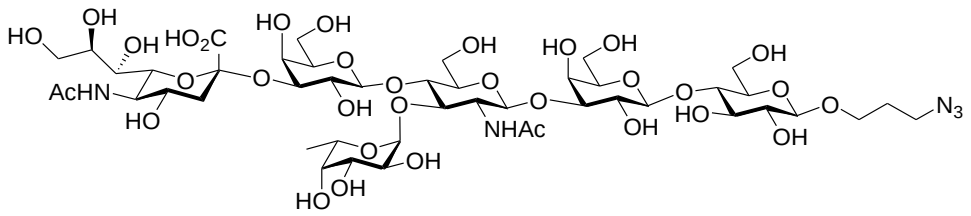
Tetrasaccharide **1** (100 mg, 0.13 mmol), Neu5Ac (53 mg, 0.17 mmol), and cytidine 5'-triphosphate (CTP) (89 mg, 0.17 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mmol, pH 8.5) and $MgCl_2$ (20 mmol). After the addition of appropriate amount of NmCSS (2.0 mg) and PmST1 M144D (0.5 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction mixture was incubated in a shaking incubator at 37 ℃ for 1 h with agitation at 140 rpm. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). The reaction was stopped by adding 10 mL of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 5:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide pentasaccharide **2** (130 mg, 95%) as a white solid. $^{1}$H NMR (600 MHz, $D_2O$) δ 4.68 (d, *J* = 8.4 Hz, 1H), 4.54 (d, *J* = 7.8 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.14 (d, *J* = 3.6 Hz, 1H), 4.10 (dd, *J* = 3.1, 9.9 Hz, 1H), 4.03 – 3.50 (m, 31H), 3.45 (t, *J* = 6.9 Hz, 2H), 3.30 (m, 1H), 2.74 (dd, *J* = 4.8, 12.6 Hz, 1H), 2.02 (s, 6H), 1.90 (p, *J* = 6.6 Hz, 2H), 1.80 (t, *J* = 12.0 Hz, 1H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.90, 174.76, 173.41, 102.84, 102.69, 102.46, 102.01, 99.53, 81.96, 78.27, 77.92, 75.38, 75.04, 74.79, 74.67, 74.44, 74.26, 72.84, 72.70, 72.03, 71.55, 69.87, 69.27, 68.23, 68.10, 68.01, 67.41, 67.26, 62.56, 60.93, 60.89, 59.98, 59.75, 55.09, 51.60, 47.79, 39.44, 28.16, 22.14, 22.02; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}$ $[M-H]^-$ 1080.3849, found 1080.3800.



**Gal β1–4(Fucα1-3)GlcNAcβ1–3Galβ1–4GlcβProN$_3$ (3)**

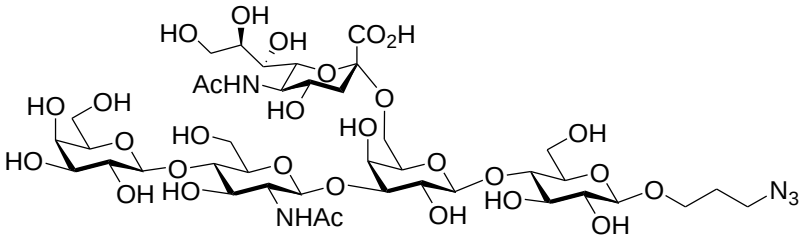
Tetrasaccharide **1** (160 mg, 0.20 mmol), L-fucose (49 mg, 0.30 mmol), ATP (152 mg, 0.30 mmol) and guanosine 5'-triphosphate (GTP) (180 mg, 0.30 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mmol, pH 7.5) and

$MnCl_2$ (20 mmol). After the addition of appropriate amount of recombinant FKP (2 mg) and Hpα1–3FTΔ66 (1.5 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction mixture was incubated in a shaking incubator at 37 ℃ for 12 hrs with agitation at 140 rpm. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). The reaction was stopped by adding 10 mL of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 5:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide Lewis x-containing petasaccharide **3** (109 mg, 92 %) as a white solid. $^1$H NMR (600 MHz, $D_2O$) δ 5.12 (d, *J* = 4.0 Hz, 1H), 4.84 (m, 1H)，4.70 (d, *J* = 8.4 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 8.4 Hz, 1H), 4.15 (d, *J* = 3.0 Hz, 1H), 4.05–3.56 (m, 26H), 3.48 (dd, *J* = 7.9, 9.6 Hz, 1H), 3.46 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 8.5 Hz, 1H), 2.02 (s, 3H), 1.91 (p, *J* = 6.5 Hz, 2H), 1.17 (d, *J* = 6.6 Hz, 3H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.58, 102.84, 102.43, 102.01, 101.67, 98.51, 81.97, 78.24, 75.01, 74.80, 74.76, 74.66, 74.26, 72.96, 72.69, 72.38, 71.81, 70.95, 69.85, 69.10, 68.25, 68.20, 67.61, 67.26, 66.59, 61.40, 60.87, 59.97, 59.54, 55.86, 47.78, 28.15, 22.18, 15.25; HRMS (ESI) *m/z* calcd for $C_{35}H_{60}N_4O_{25}Na$ $[M+Na]^+$ 959.3444, found 959.3418.



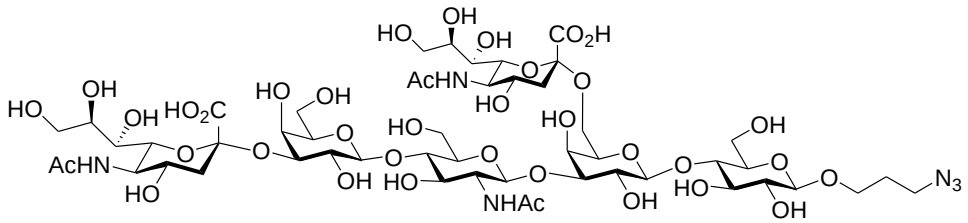
**Neu5Acα2–3Gal β1–4(Fucα1-3)GlcNAcβ1–3Galβ1–4GlcβPro$N_3$ (4)**

The enzymatic fucosylation of sialyl petasaccharide **2** (80 mg, 0.074mmol) was performed following the same procedure as described above for **3**. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 3:2:1:0.2, v/v). The reaction was stopped by adding 10 mL of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 4:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to give sialyl Lewis x-containing hexasaccharide **4** (55 mg, 61%) as a white solid. $^1$H NMR (600 MHz, $D_2O$) δ 5.08 (d, *J* = 4.2 Hz, 1H), 4.67 (d, *J* = 7.8 Hz, 1H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.45 (d, *J* = 8.4 Hz, 1H), 4.40 (d, *J* = 7.8 Hz, 1H), 4.13 (d, *J* = 3.0 Hz, 1H), 4.05 (dd, *J* = 2.6, 9.8 Hz, 1H), 4.04 (d, *J* = 2.4 Hz, 1H), 4.00–3.52 (m, 33H), 3.49 (t, *J* = 8.8 Hz, 1H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 8.4 Hz, 1H), 2.73 (dd, *J* = 4.2, 12.6 Hz, 1H), 2.00 (s, 3H), 1.99 (s, 3H), 1.88 (p, *J* = 6.6 Hz, 2H), 1.76 (t, *J* = 12.0 Hz, 1H), 1.13 (d, *J* = 6.6 Hz, 3H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.88, 174.55, 173.74, 102.81, 102.47, 101.97, 101.42, 99.51, 98.46, 81.97, 78.19, 75.52, 74.89, 74.79, 74.76, 74.64, 74.51, 74.23, 72.88, 72.78, 72.65, 71.77, 71.73, 69.82, 69.13, 69.04, 68.17, 67.96, 67.57, 67.23, 67.18, 66.53, 62.46, 61.37, 60.85, 59.92, 59.37, 59.18, 55.83, 51.56, 47.73, 39.65, 28.11, 22.12, 21.90, 15.16; HRMS (ESI) *m/z* calcd for $C_{46}H_{76}N_5O_{33}$ $[M-H]^-$ 1226.4428, found 1226.4580.



**Galβ1–4 GlcNAcβ1–3(Neu5Acα2–6)Galβ1–4GlcβPro$N_3$(5)**

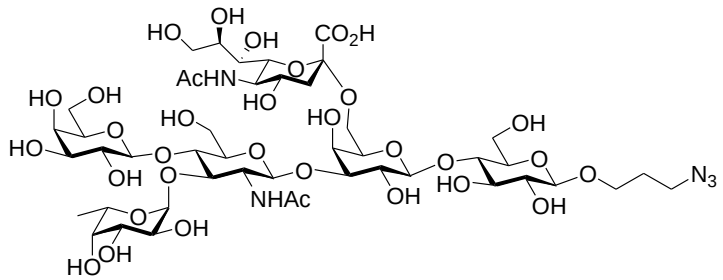
The enzymatic galactosylation of sialyl tetrasaccharide **10** (92 mg, 0.10 mmol) was performed following the same procedure as described above for **1**. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). The reaction was stopped by adding 10 mL of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 5:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide sialyl pentasaccharide **5** (97 mg, 90%) as a white solid.$^1$H NMR (600 MHz, $D_2O$) δ 4.66 (d, *J* = 8.4 Hz, 1H), 4.46 (d, *J* = 8.4 Hz, 1H), 4.44 (d, *J* = 8.2 Hz, 1H), 4.39 (d, *J* = 8.4 Hz, 1H), 4.15 (d, *J* = 3.0 Hz, 1H), 4.02–3.47 (m, 32H), 3.44 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 8.4 Hz, 1H), 2.67 (dd, *J* = 4.2, 12.6 Hz, 1H), 2.00 (s, 6H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.79 (t, *J* = 12.3Hz, 1H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.78, 174.75, 171.70, 103.13, 102.75, 102.67, 101.88, 99.18, 81.92, 79.53, 78.06, 75.23, 74.49, 74.47, 74.44, 72.96, 72.66, 72.61, 72.40, 72.10, 71.00, 70.86, 69.57, 68.45, 68.29, 68.06, 67.64, 67.23, 63.22, 62.81, 60.93, 60.10, 59.78, 55.08, 51.61, 47.78, 39.24, 28.11, 22.09, 22.00; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}$ [M-H]$^-$ 1080.3849, found 1080.3824.



**Neu5Acα2–3Gal β1–4 GlcNAcβ1–3(Neu5Acα2–6)Galβ1–4GlcβPro$N_3$ (6)**

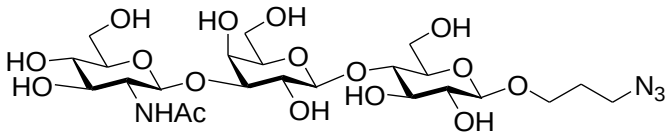
The α2–3-sialylation of pentasaccharide **5** (100 mg, 0.093 mmol) was performed following the same procedure as described above for **2**. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). The reaction was stopped by adding 10 mL of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc:MeOH:$H_2O$ =3:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide disialyl hexasaccharide **6** (121 mg, 95 %) as a white solid. $^1$H NMR (600 MHz, $D_2O$) δ 4.66 (d, *J* = 8.4 Hz, 1H), 4.52 (d, *J* = 7.8 Hz, 1H), 4.46 (d, *J* = 8.4 Hz, 1H), 4.39 (d, *J* = 7.8 Hz, 1H), 4.15 (d, *J* = 3.0 Hz, 1H), 4.11 (dd, *J* = 3.0, 9.6 Hz, 1H), 4.02–3.48 (m, 39H), 3.44 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 8.4 Hz, 1H), 2.73 (dd, *J* = 4.8, 12.6 Hz, 1H), 2.67 (dd, *J* = 4.2, 12.6 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.89 (p, *J* = 6.8 Hz, 2H), 1.82 (t, *J* = 12.0 Hz, 1H), 1.76 (t, *J* = 12.0 Hz, 1H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.88, 174.79, 174.75, 172.84, 172.27,

103.12, 102.72, 102.43, 101.88, 99.52, 99.20, 81.97, 79.53, 77.90, 75.36, 74.98, 74.51, 74.48, 74.43, 73.04, 72.91, 72.61, 72.58, 72.07, 71.33, 71.22, 69.58, 69.27, 68.29, 68.07, 68.02, 67.89, 67.85, 67.44, 67.23, 63.29, 62.73, 62.64, 60.88, 60.12, 59.76, 55.09, 51.64, 51.57, 47.79, 39.51, 39.24, 28.15, 22.11, 22.01, 22.00; HRMS (ESI) *m/z* calcd for $C_{51}H_{83}N_6O_{37}$ $[M-H]^-$ 1371.4803, found 1371.4627.



**Gal β1–4(Fucα1-3)GlcNAcβ1–3(Neu5Acα2–6)Galβ1–4GlcβProN$_3$ (7)**

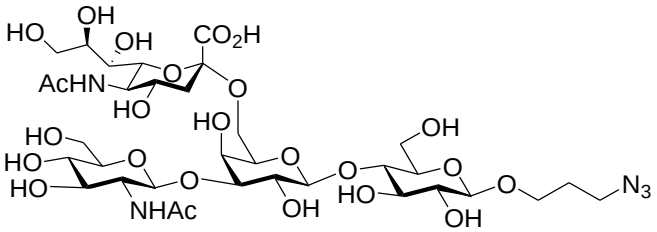
The enzymatic fucosylation of sialyl petasaccharide **5** (100 mg, 0.093 mmol) was performed following the same procedure as described above for **3**. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 3:2:1:0.2, v/v). The reaction was stopped by adding 10 mL of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 4:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to give Lewis x-containing hexasaccharide **7** (101 mg, 89%) as a white solid. $^1$H NMR (600 MHz, $D_2O$) δ 5.08 (d, *J* = 4.2 Hz, 1H), 4.65 (d, *J* = 8.4 Hz, 1H), 4.45 (d, *J* = 8.4 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.37 (d, *J* = 7.8 Hz, 1H), 4.14 (d, *J* = 3.6 Hz, 1H), 3.99–3.51 (m, 35H), 3.45 (dd, *J* = 7.8, 9.8 Hz, 1H), 3.43 (t, *J* = 6.8 Hz, 2H), 3.29 (dd, *J* = 8.4, 9.0 Hz, 1H), 2.67 (dd, *J* = 4.8, 12.6 Hz, 1H), 1.99 (s, 3H), 1.98 (s, 3H), 1.88 (p, *J* = 6.6 Hz, 2H), 1.69 (t, *J* = 12.0 Hz, 1H), 1.14 (d, *J* = 6.6 Hz, 3H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.77, 174.53, 173.33, 103.09, 102.48, 101.85, 101.66, 100.14, 98.48, 82.09, 79.46, 74.98, 74.76, 74.64, 74.51, 74.47, 73.09, 72.96, 72.58, 72.40, 72.32, 71.79, 71.63, 70.92, 69.52, 69.06, 68.21, 68.01, 67.58, 67.19, 66.55, 63.31, 62.50, 61.38, 60.10, 59.49, 59.19, 55.82, 51.66, 47.74, 39.97, 28.11, 22.14, 21.95, 15.20; HRMS (ESI) *m/z* calcd for $C_{46}H_{76}N_5O_{33}$ $[M-H]^-$ 1226.4428, found 1226.4569.



**GlcNAcβ1–3Galβ1–4GlcβProN$_3$ (9)**

LacβProN$_3$ (**8**)[9] (100 mg, 0.24 mmol), *N*-acetylglucosamine (GlcNAc, 69 mg, 0.31 mmol), adenosine 5'-triphosphate (ATP, 171 mg, 0.31 mmol) and uridine 5'-triphosphate (UTP, 157 mg, 0.31 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mmol , pH 8.0) and $MgCl_2$ (20 mmol).After the addition of appropriate amount of NahK/GlmU (1.5 mg) and Hp LgtA (0.7 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction mixture

was incubated in a shaking incubator at 37 ℃ for 18 hrs with agitation at 140 rpm. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). To stop the reaction, the reaction mixture was added with same volume of ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 7:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide trisaccharide **9** (130 mg, 88%) as a white solid. $^1$H NMR (600 MHz, $D_2O$) δ 4.65 (d, *J* = 8.4 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.41 (d, *J* = 8.4 Hz, 1H), 4.12 (d, *J* = 2.6 Hz, 1H), 3.98–3.94 (m, 2H), 3.86 (d, *J* = 12.1 Hz, 1H), 3.78–3.40 (m, 17H), 3.28 (t, *J* = 8.2 Hz, 1H), 2.01 (s, 3H), 1.88 (p, *J* = 6.4 Hz, 2H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.82, 102.82, 102.74, 102.00, 81.85, 78.24, 75.54, 74.78, 74.66, 74.25, 73.45, 72.68, 69.89, 69.58, 68.23, 67.25, 60.86, 60.38, 59.96, 55.55, 47.77, 28.14, 22.09; HRMS (ESI) *m/z* calcd for $C_{23}H_{40}N_4O_{16}Na$ [M+Na]1$^+$ 651.2337, found 651.2336.




**GlcNAcβ1–3(Neu5Acα2–6)Galβ1–4GlcβProN$_3$ (10)**

Trisaccharide **9** (100 mg, 0.16 mmol), Neu5Ac (98 mg, 0.32 mmol), and CTP (168 mg, 0.32 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mmol , pH 8.5) and $MgCl_2$ (20 mmol). After the addition of appropriate amount of NmCSS (2.0 mg) and Pdα2,6ST (1.5 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction mixture was incubated in a shaking incubator at 37 ℃ for 5 hrs with agitation at 140 rpm. The product formation was monitored by TLC (EtOAc/MeOH/$H_2O$/HOAc, 4:2:1:0.2, v/v). The reaction was stopped by adding 10 mL ice-cold ethanol and incubated at 4 ℃ for 30 min. After centrifugation, the supernatant containing the product was concentrated, purified by silica gel flash column chromatography (EtOAc/MeOH/$H_2O$, 6:2:1, v/v) and Bio-Gel P-2 column (eluted with $H_2O$) to provide sialyl tetrasaccharide **10** (137 mg, 94%) as a white solid. $^1$H NMR (600 MHz, $D_2O$) δ 4.64 (d, *J* = 8.4 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.39 (d, *J* = 7.8 Hz, 1H), 4.15 (d, *J* = 3.6 Hz, 1H), 4.02–3.37 (m, 28H), 3.30 (t, *J* = 8.2 Hz, 1H), 2.67 (dd, *J* = 4.8, 12.6 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.72 (t, *J* = 12.0 Hz, 1H); $^{13}$C NMR (151 MHz, $D_2O$) δ 174.79, 172.99, 103.10, 102.79, 101.89, 99.96, 81.98, 79.48, 75.54, 74.53, 74.49, 73.50, 73.10, 72.62, 72.47, 71.52, 69.62, 69.61, 68.26, 68.10, 68.07, 67.22, 63.29, 62.60, 60.41, 60.13, 55.55, 51.68, 47.78, 39.86, 28.15, 22.11,22.01; HRMS (ESI) *m/z* calcd for $C_{34}H_{56}N_5O_{24}$ [M-H]918.3321, found 918.3440.

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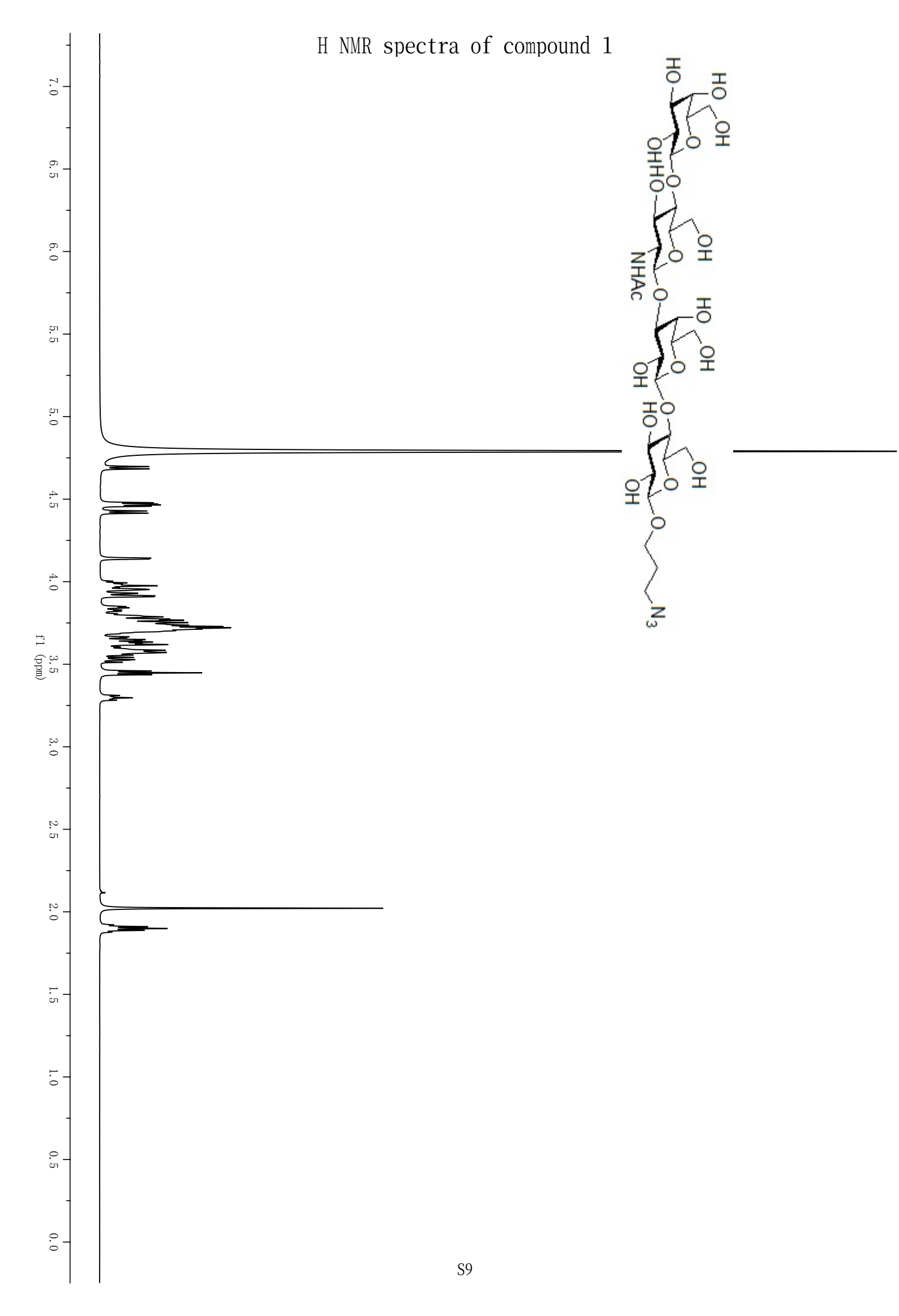
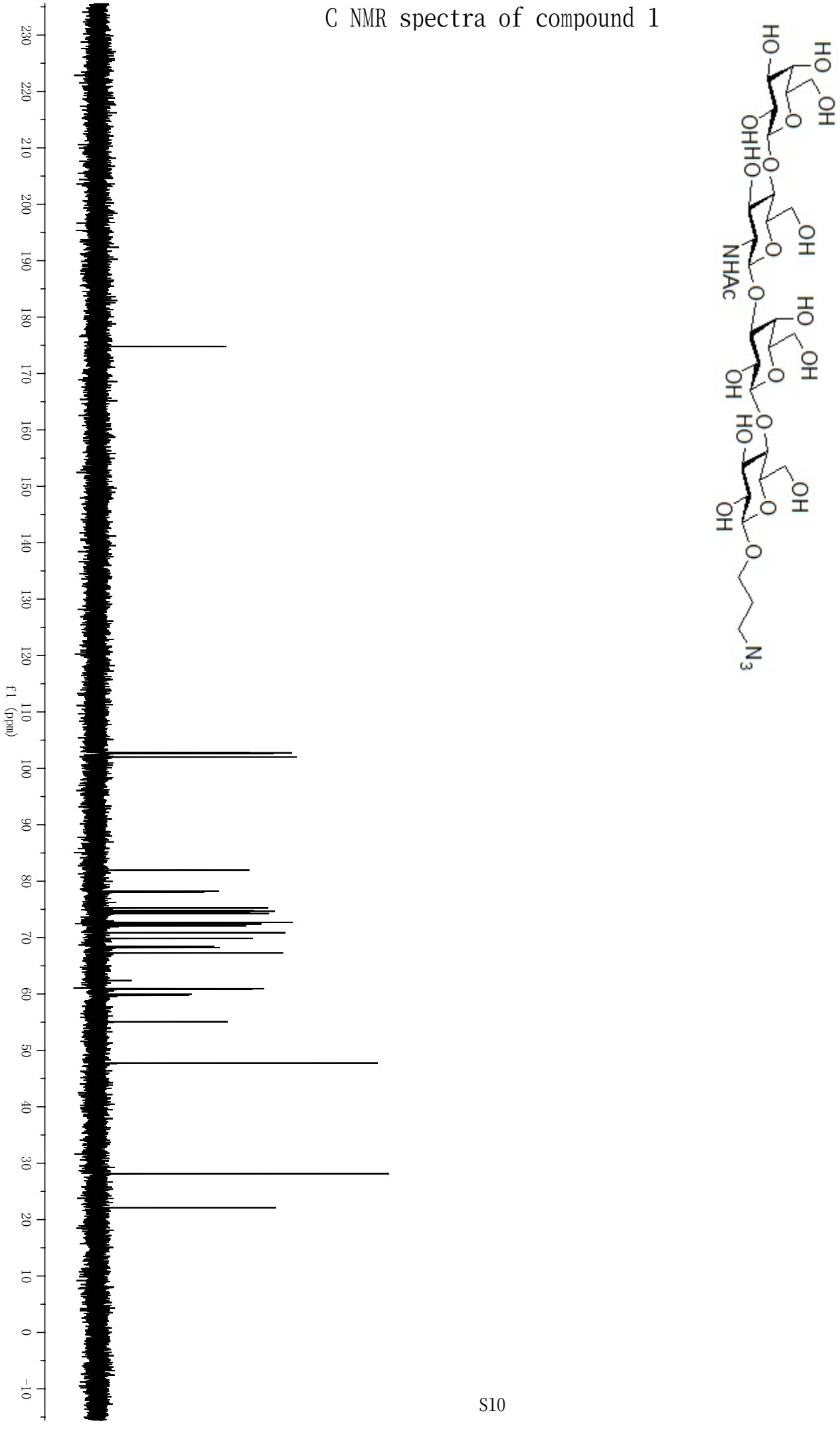
H NMR spectra of compound 1



C NMR spectra of compound 1



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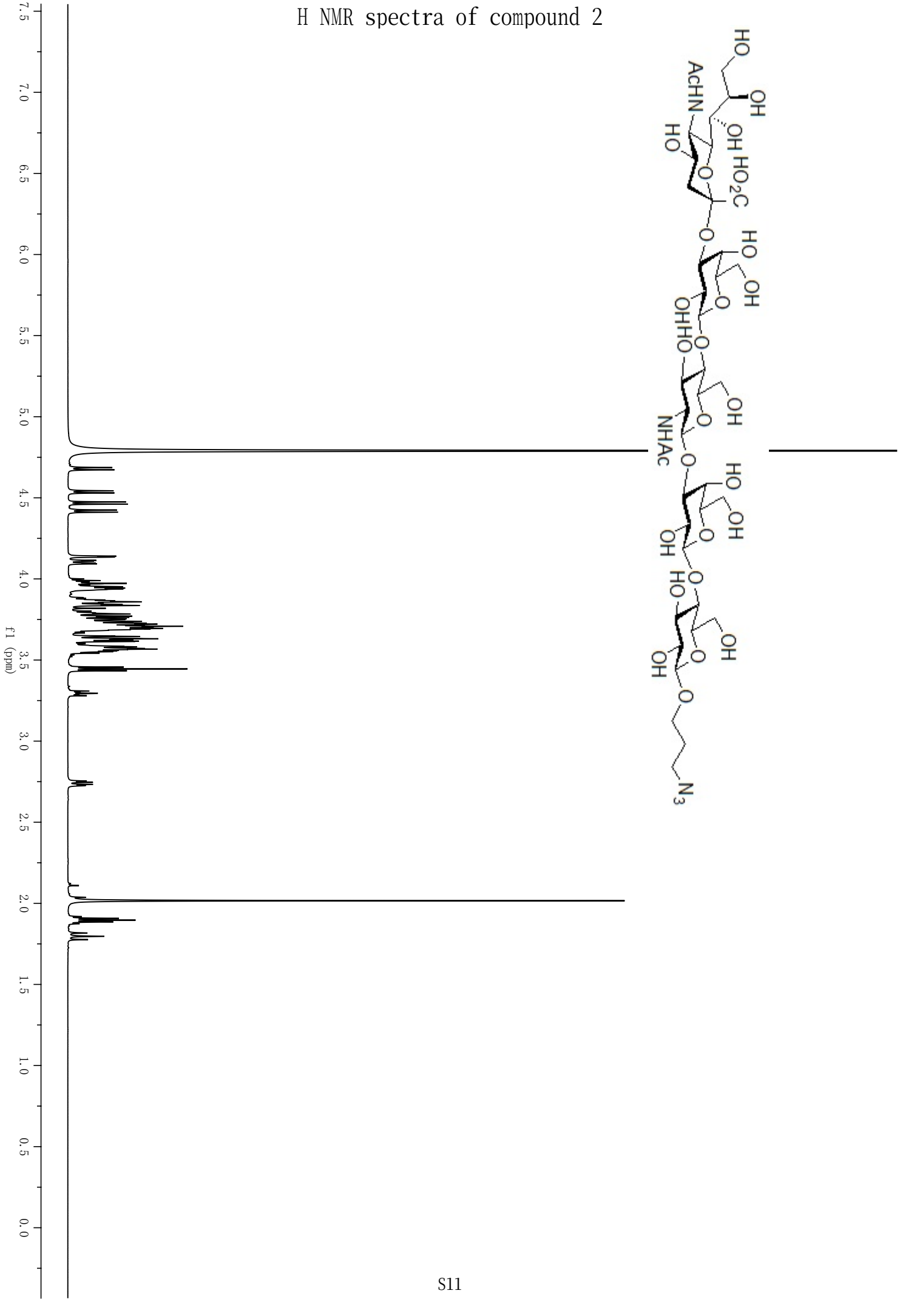

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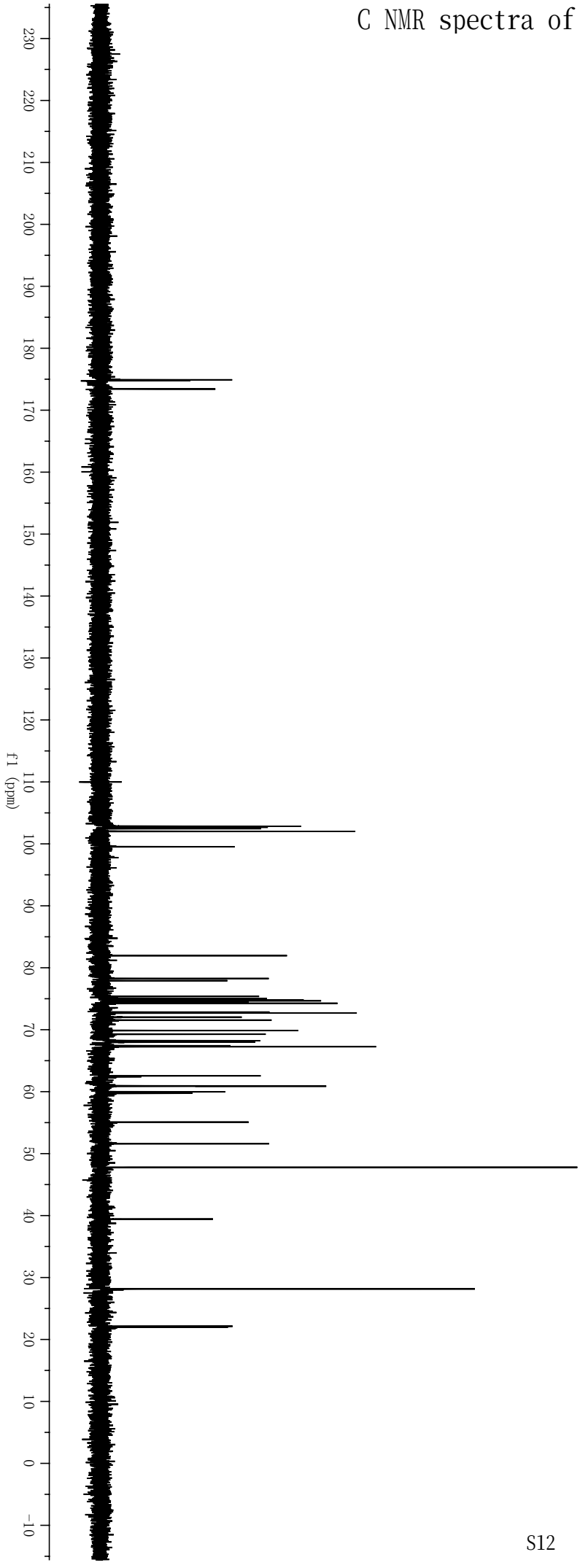
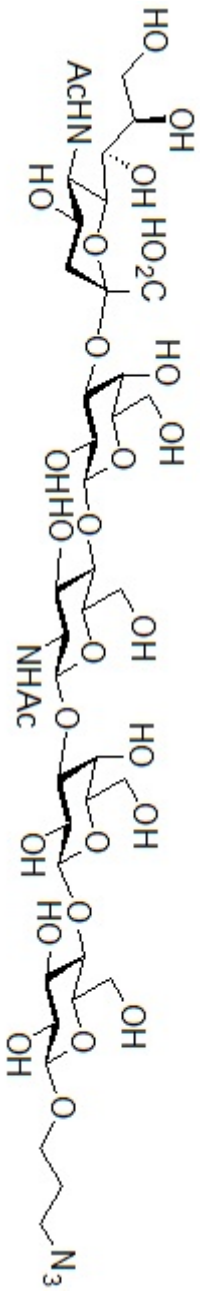
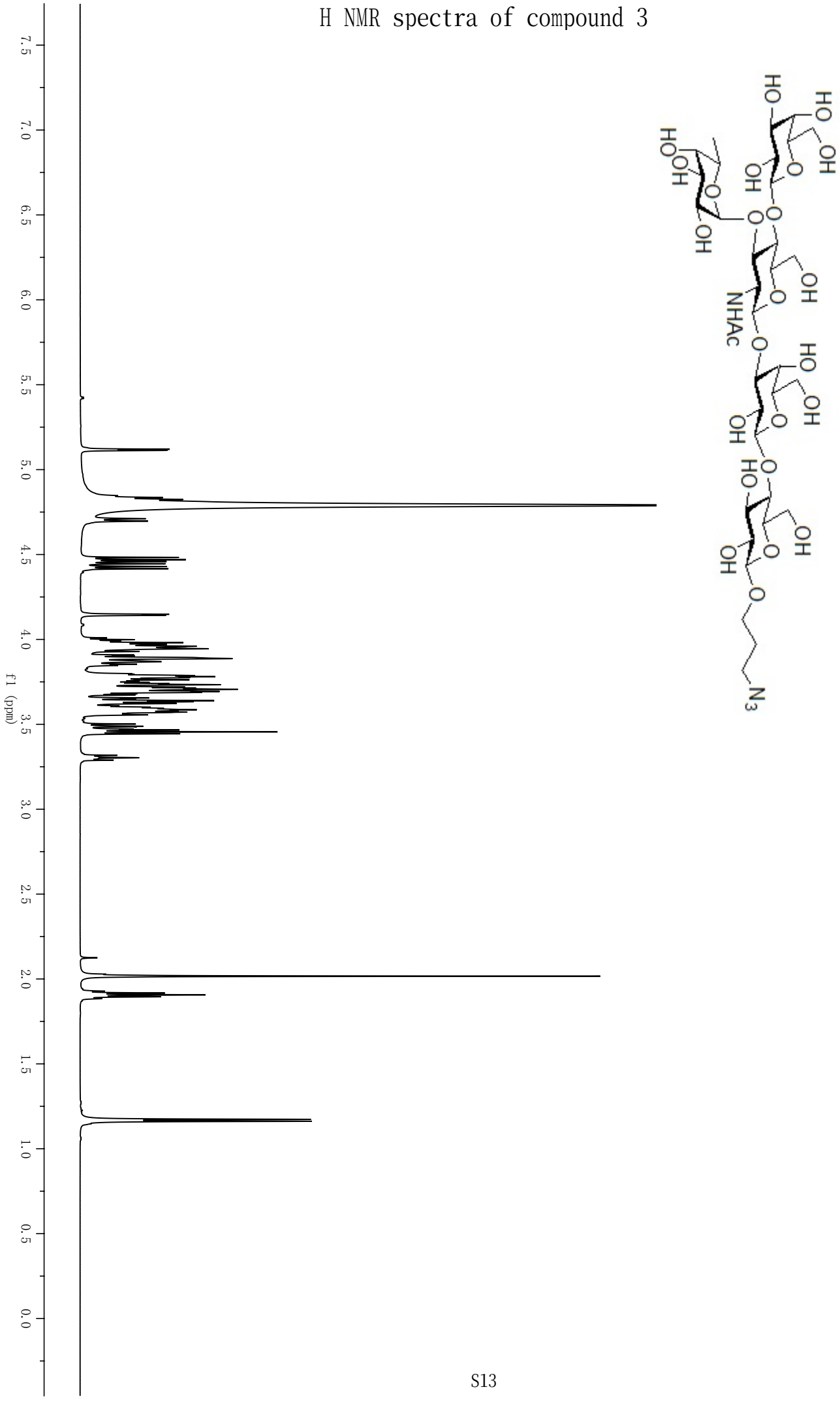


H NMR spectra of compound 3



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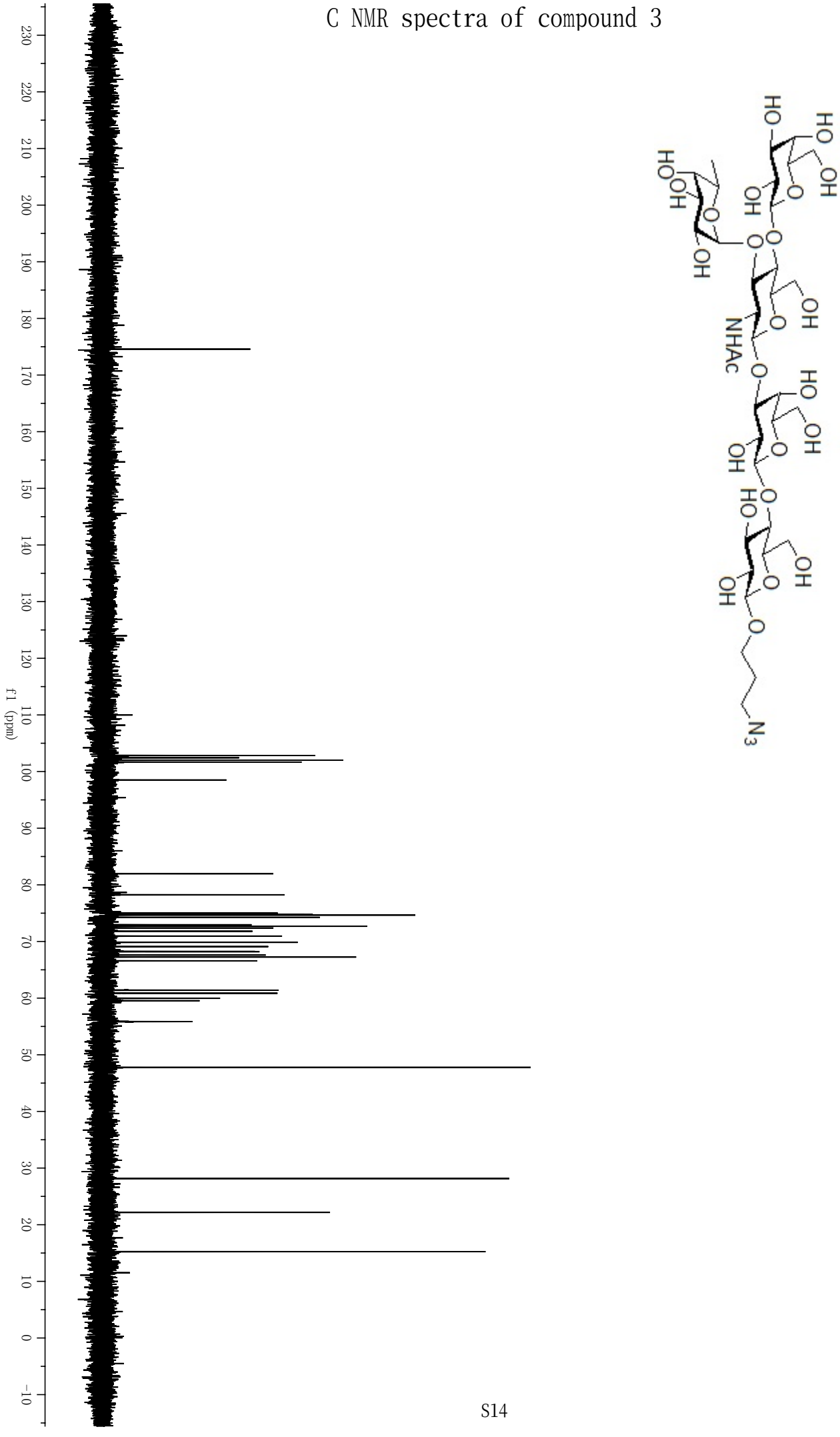
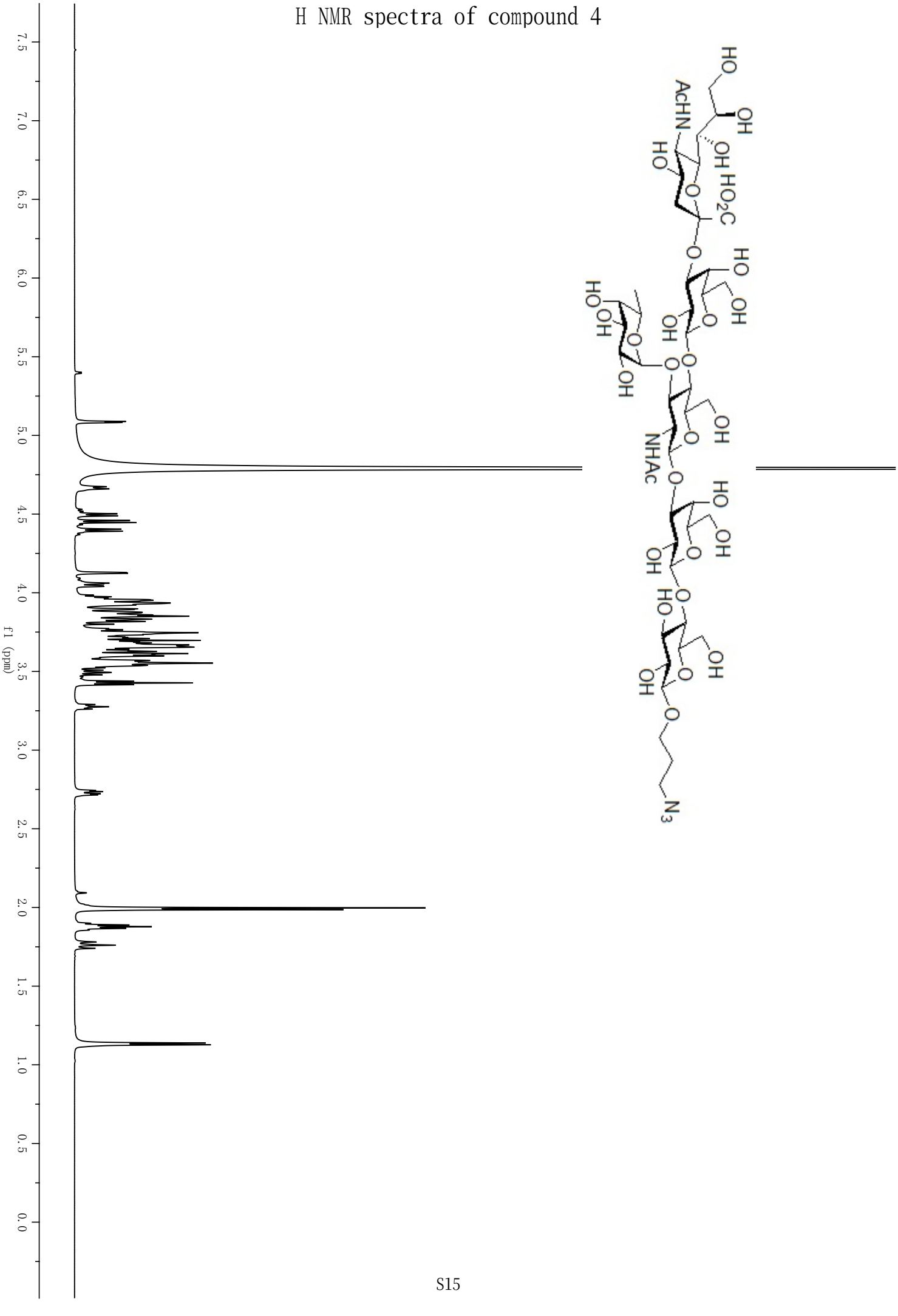
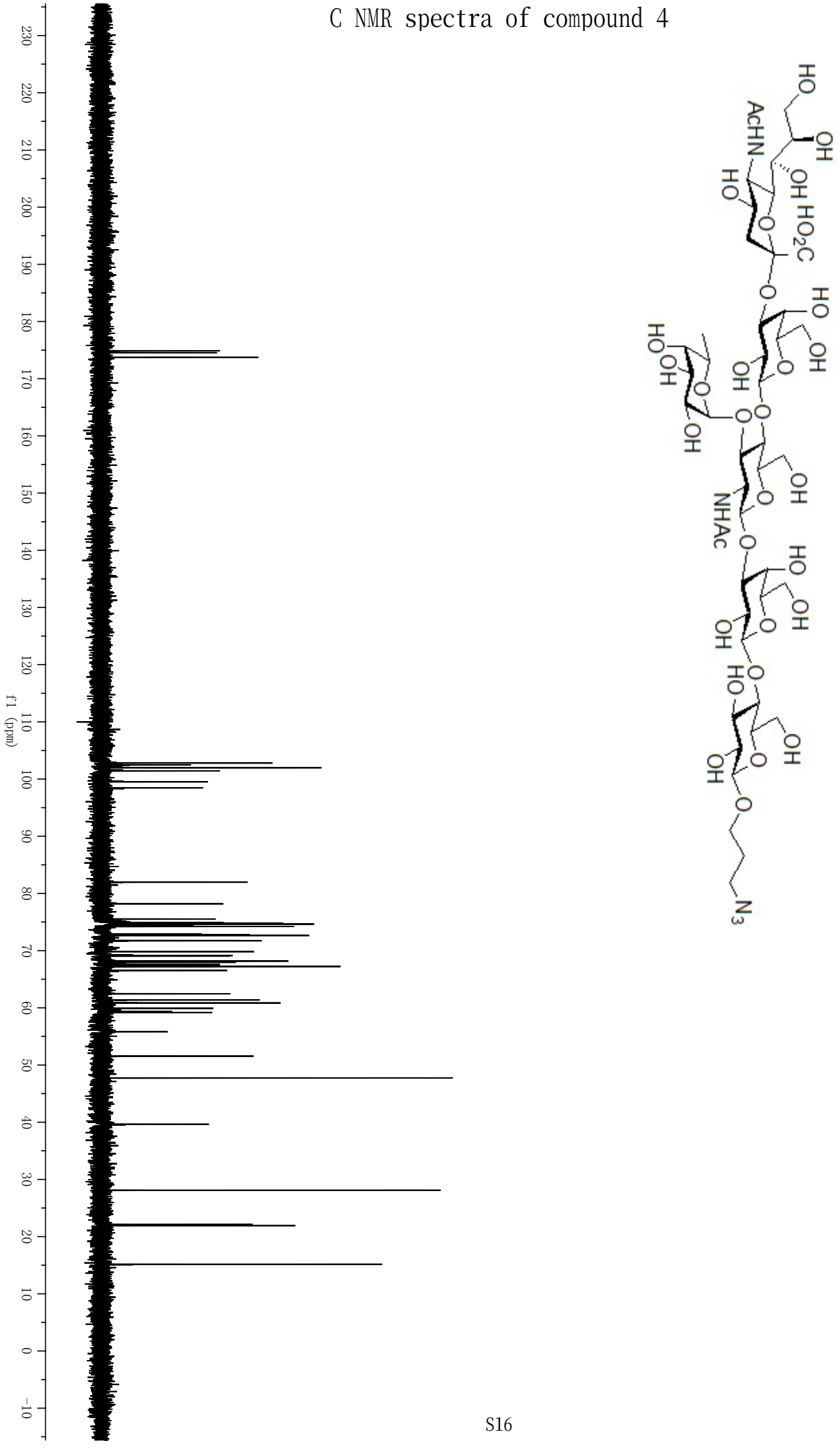


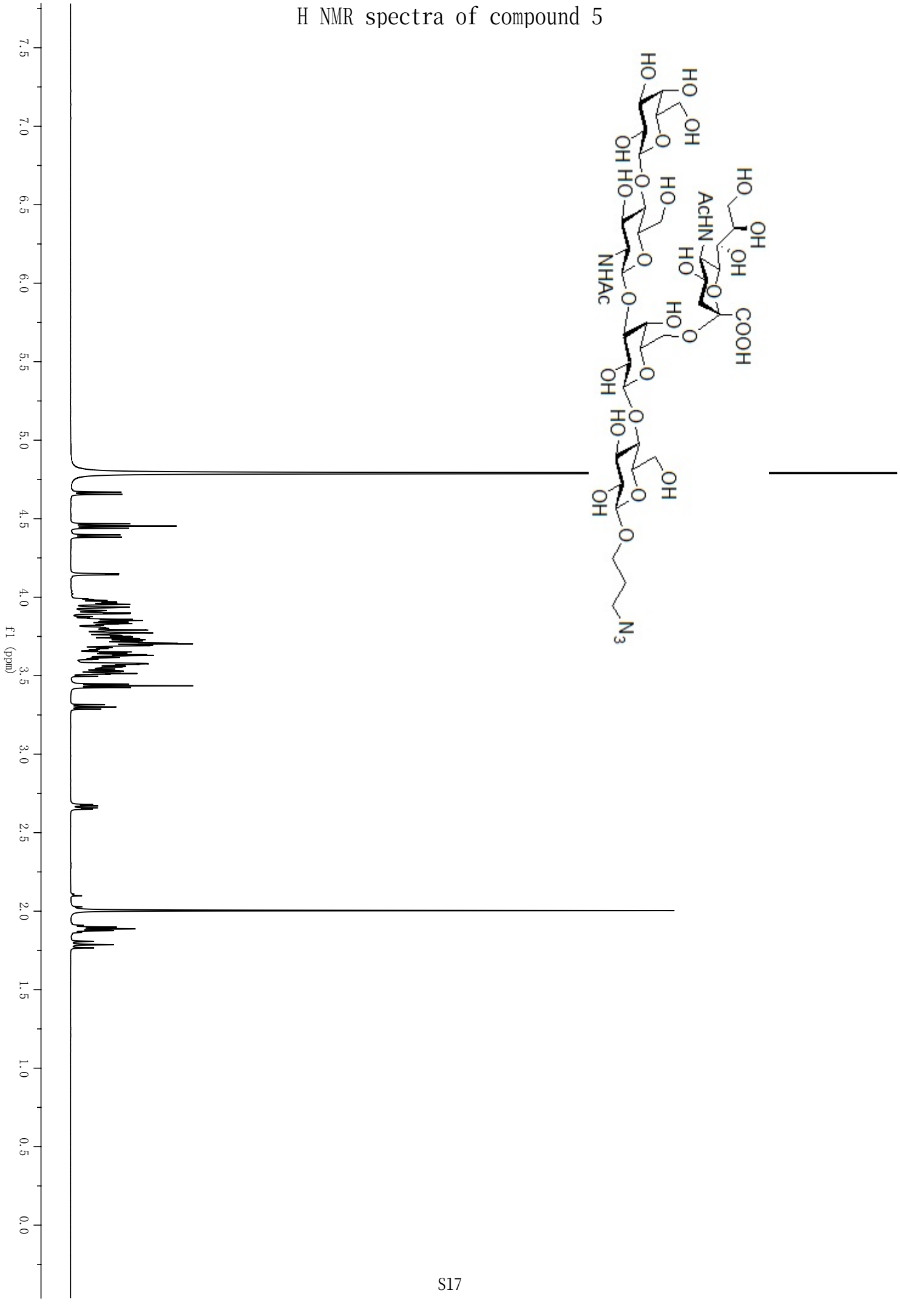
H NMR spectra of compound 4



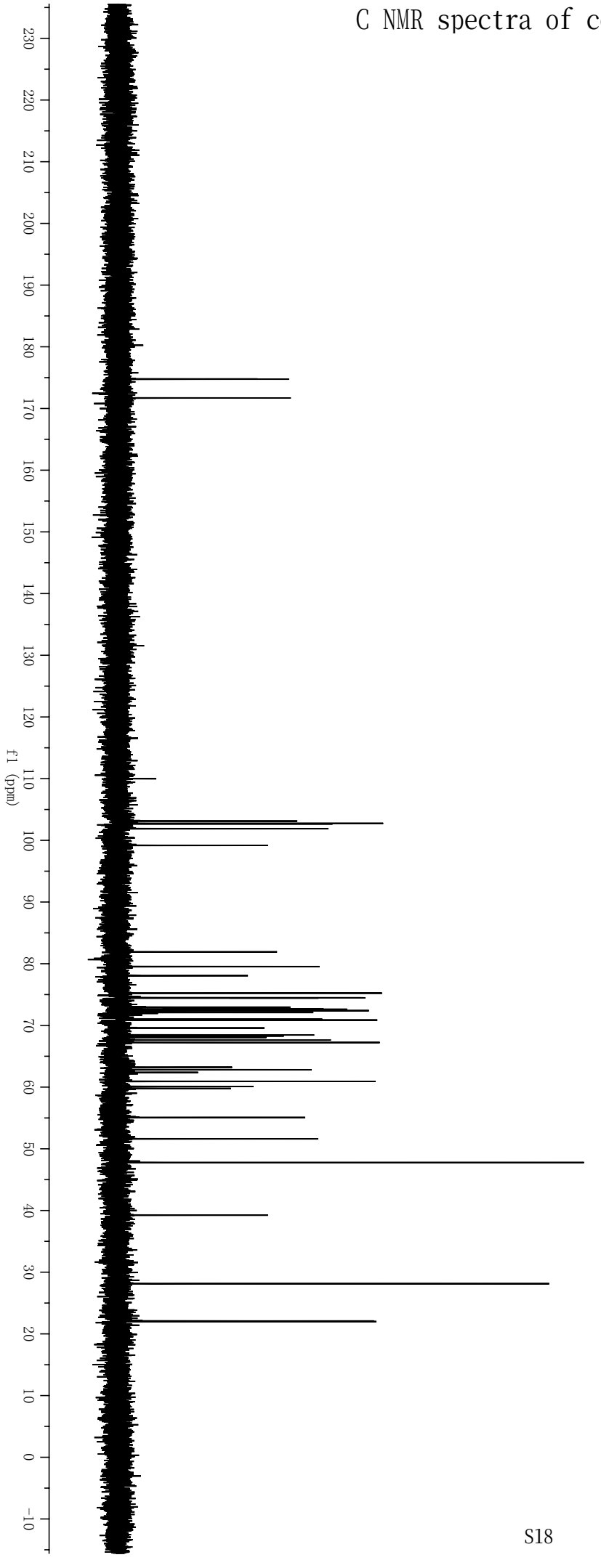
C NMR spectra of compound 4

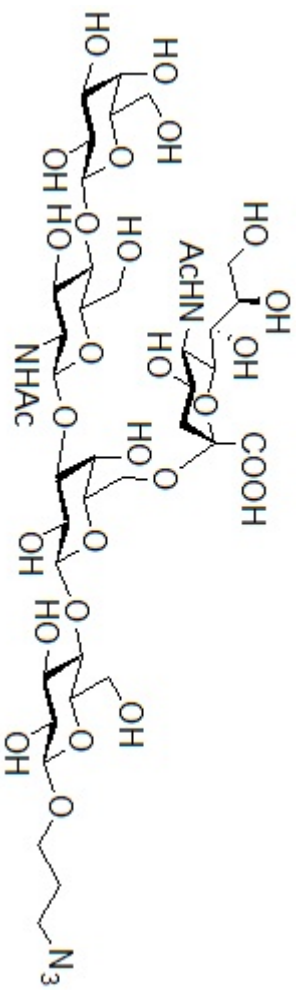


H NMR spectra of compound 5



C NMR spectra of compound 5



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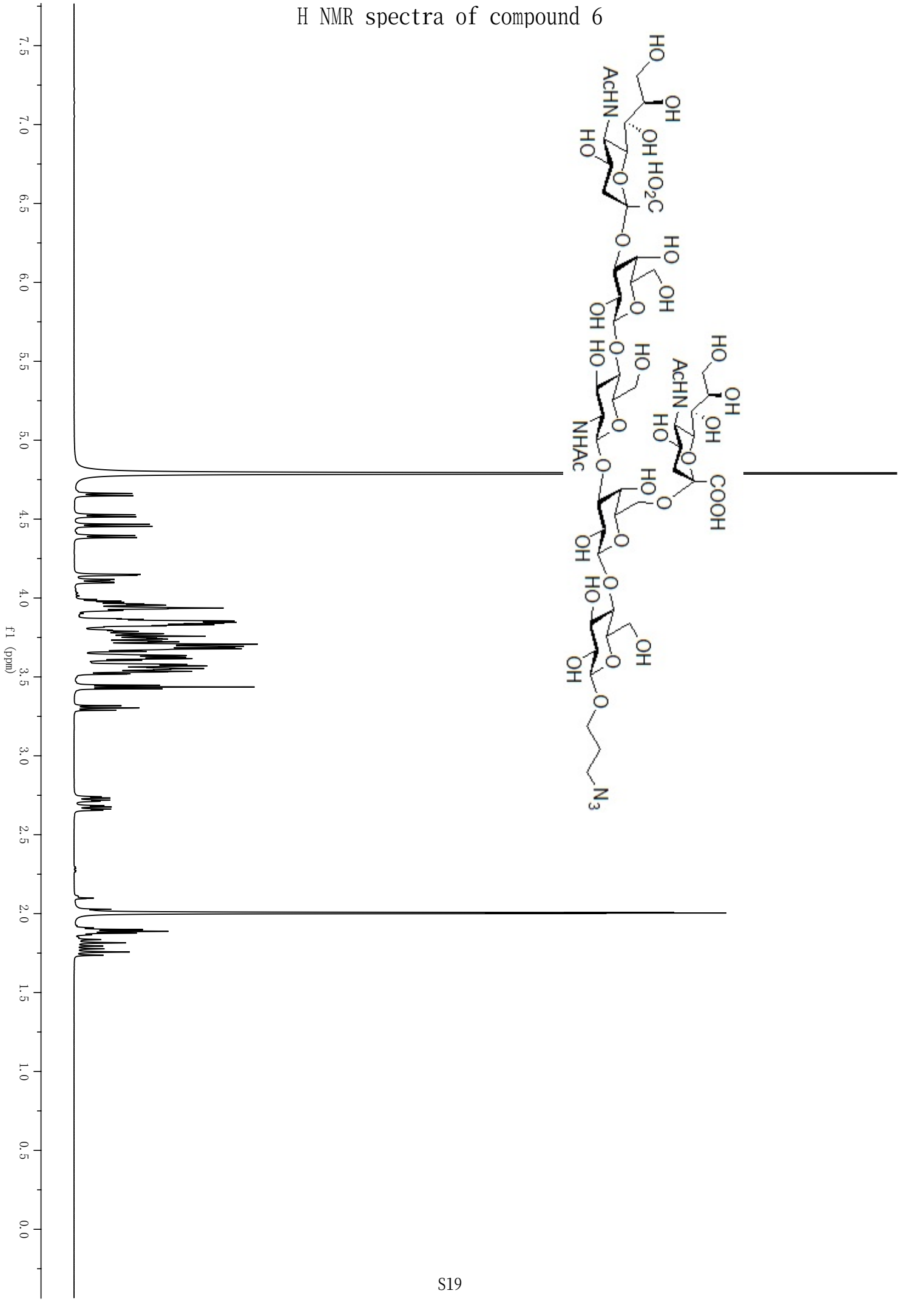

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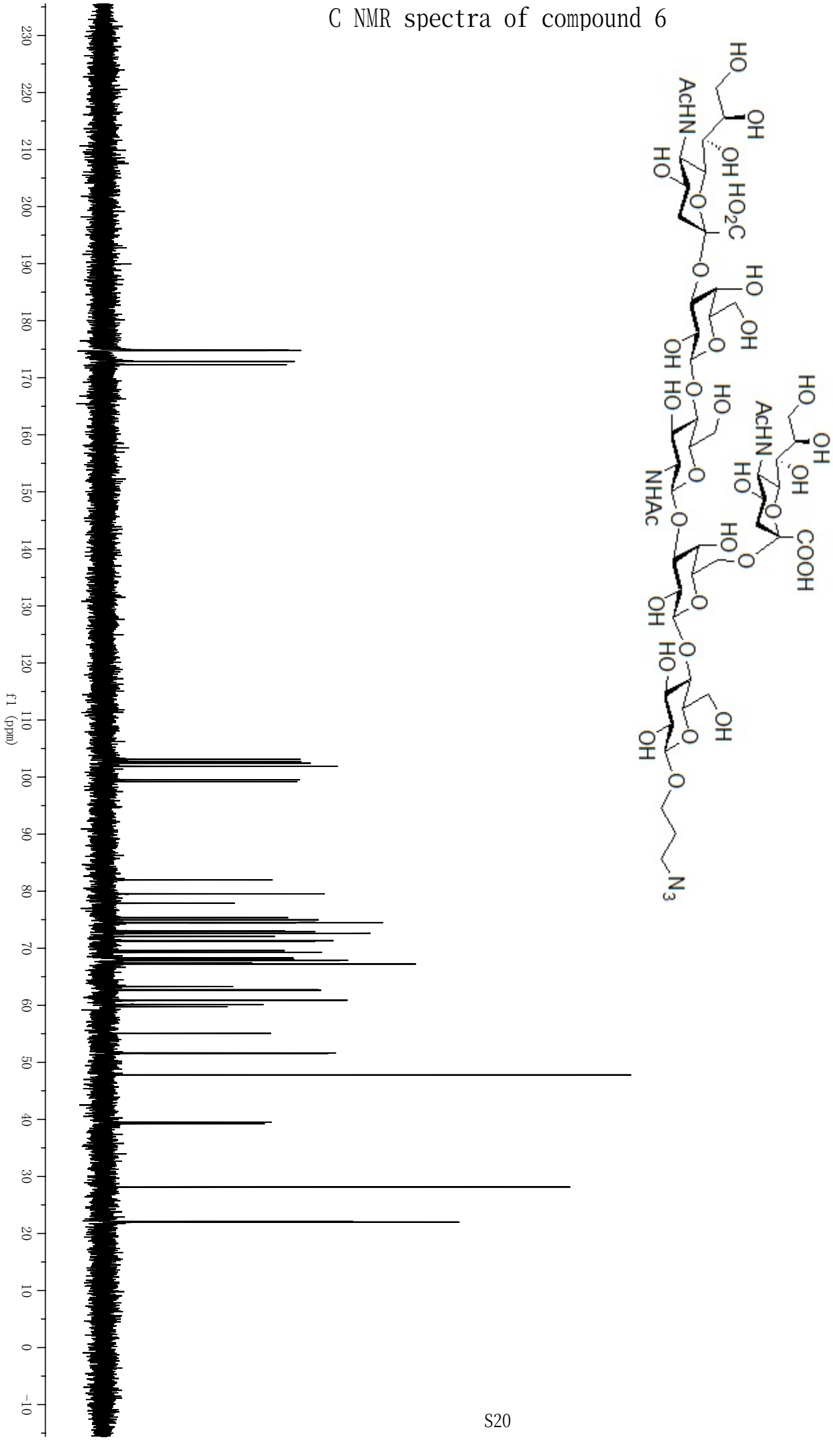

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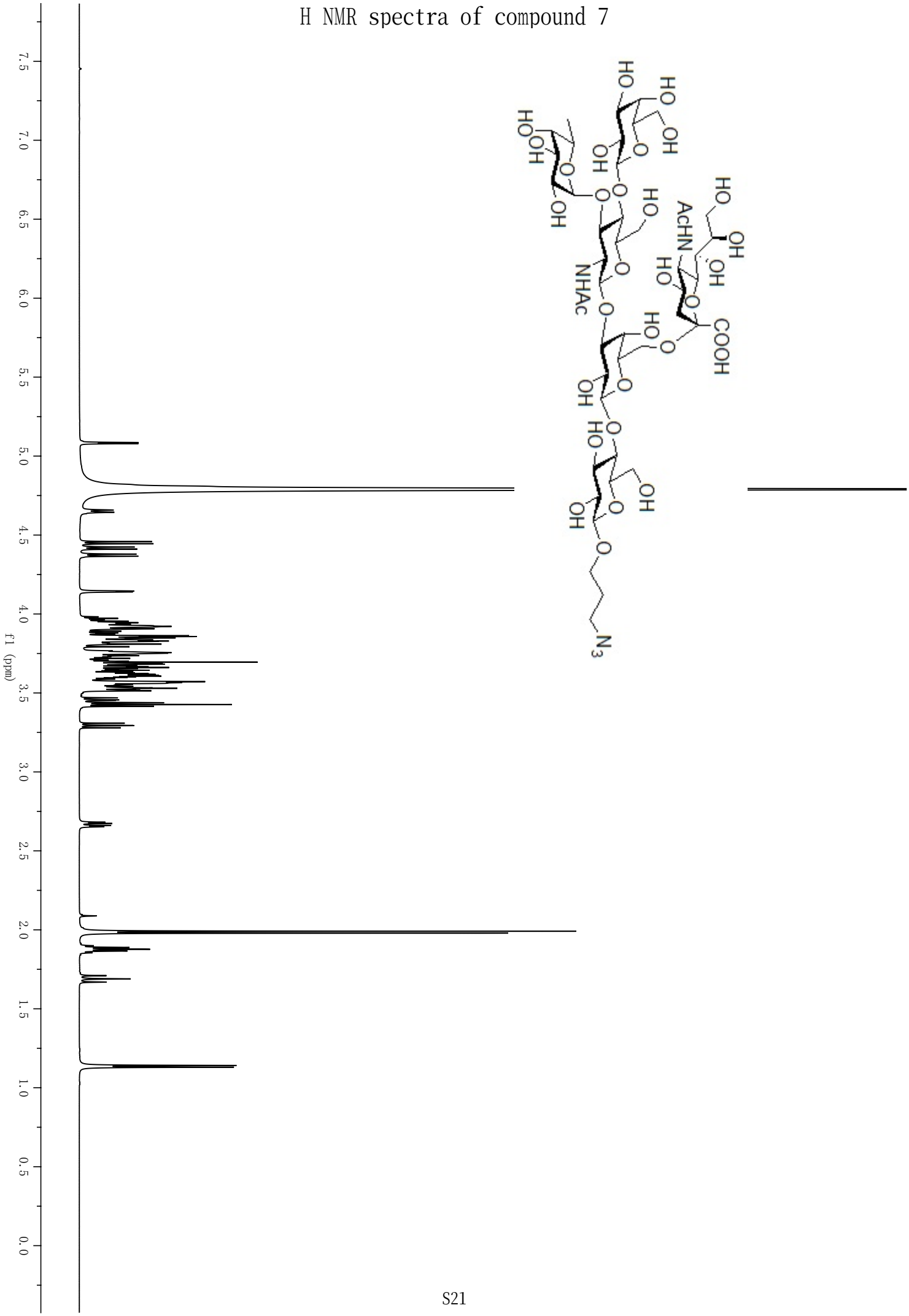
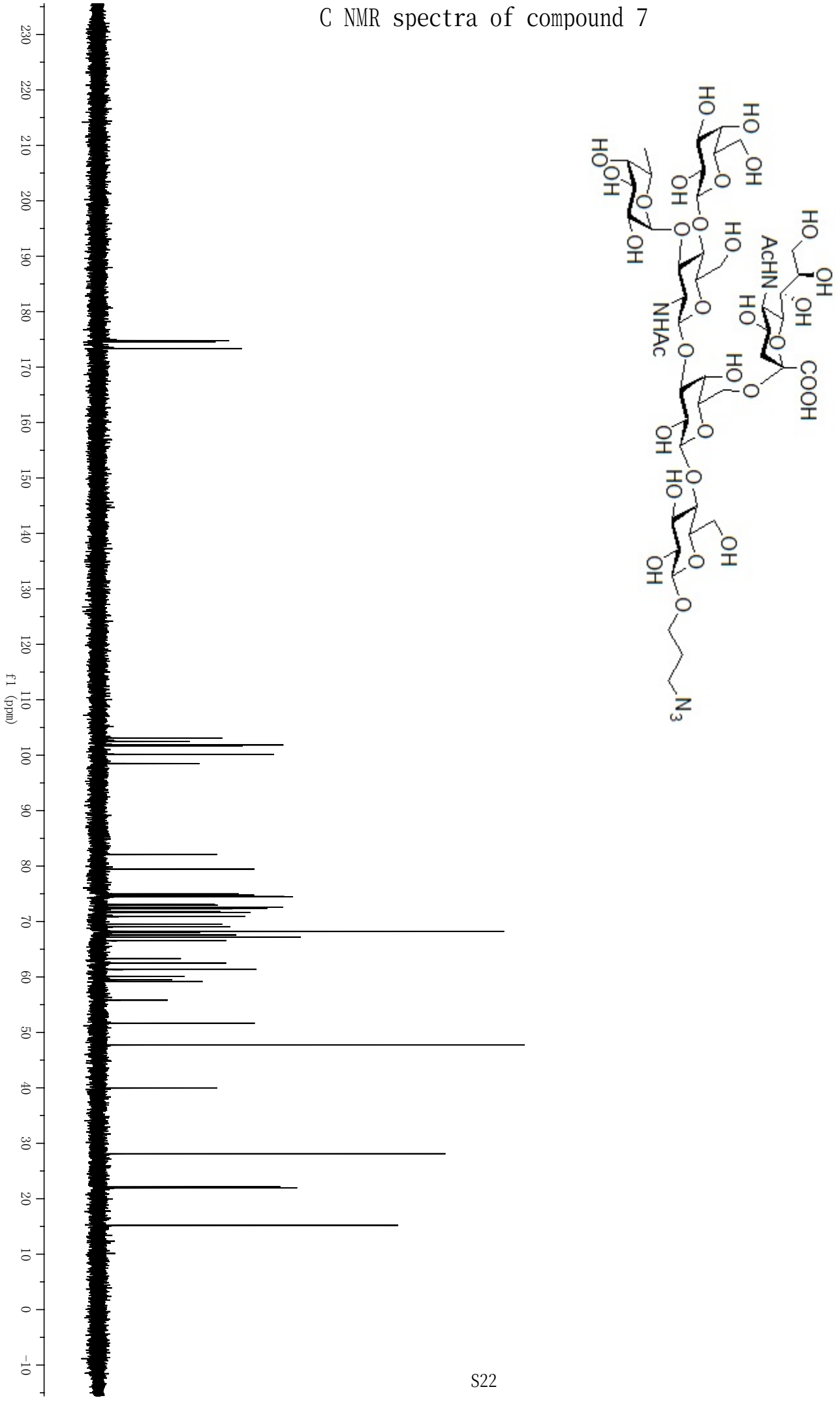
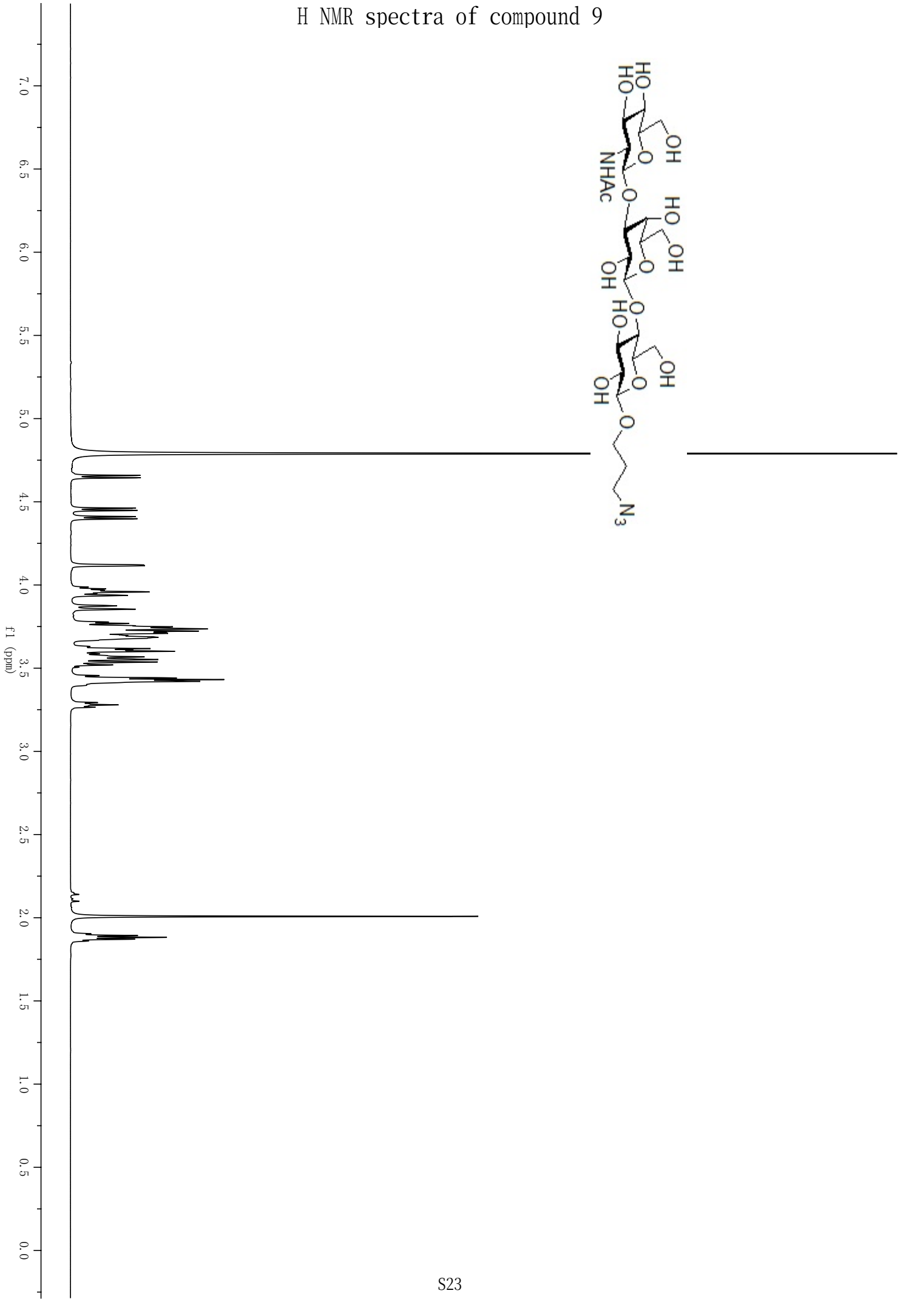


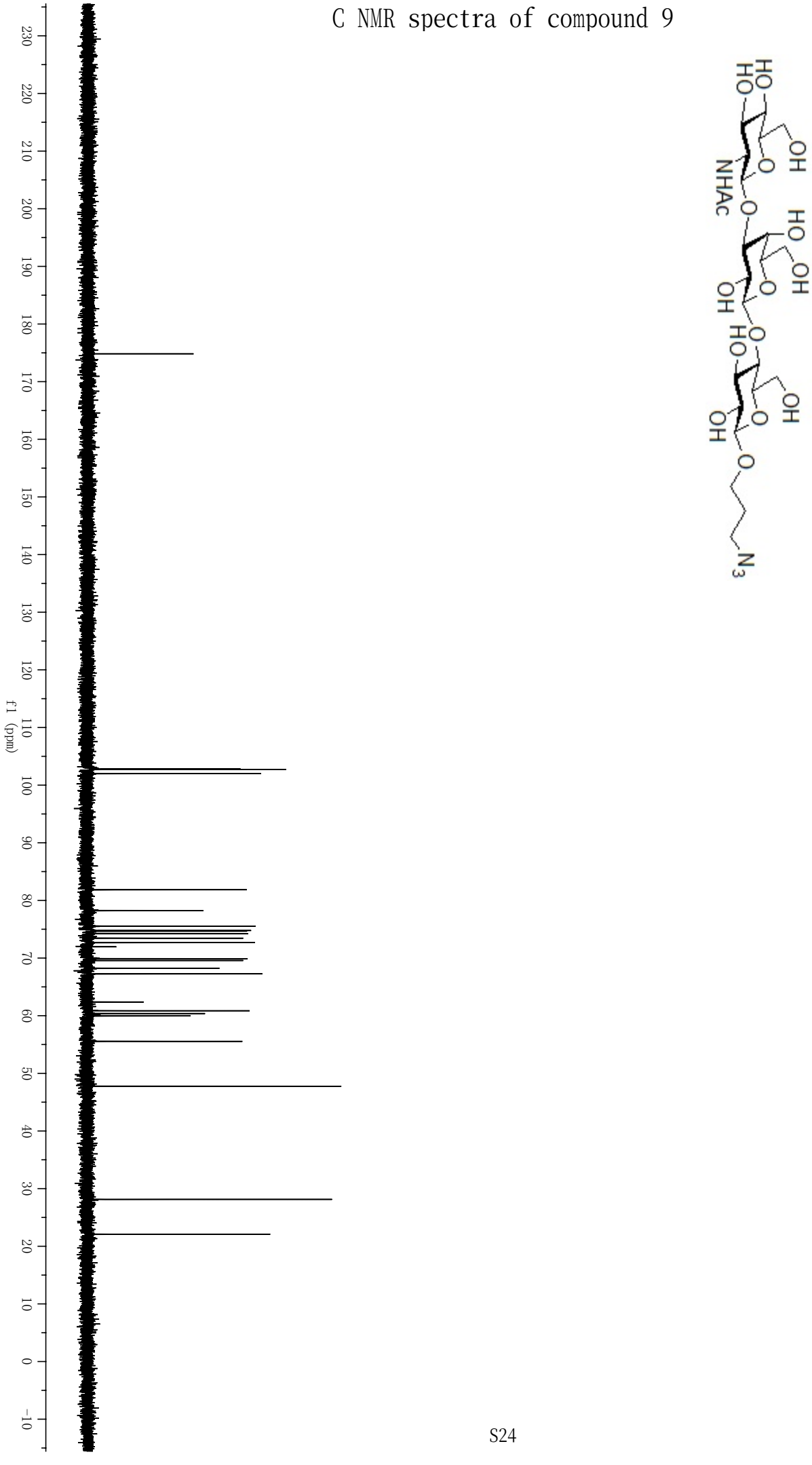
C NMR spectra of compound 7



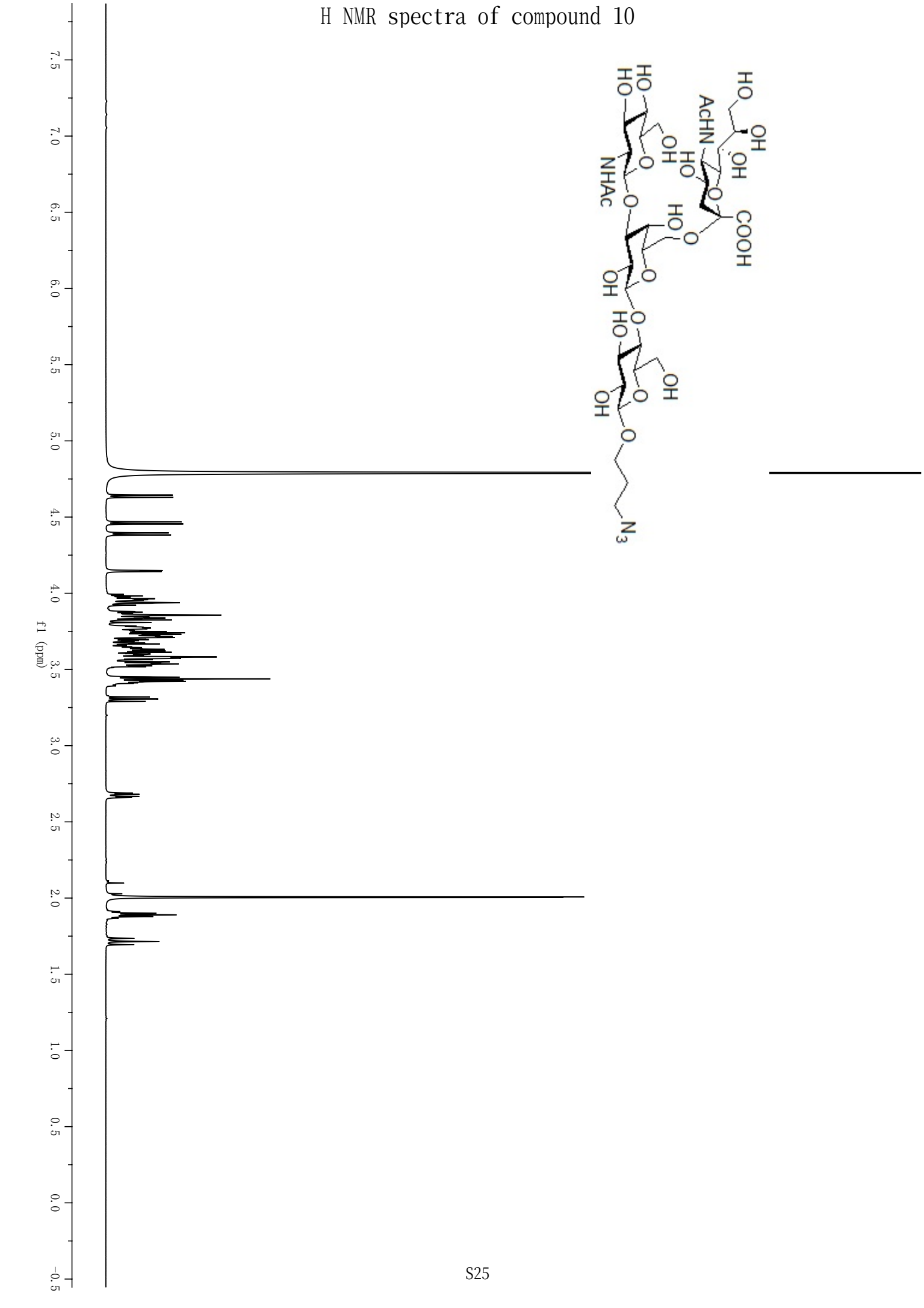
H NMR spectra of compound 9



C NMR spectra of compound 9



H NMR spectra of compound 10



C NMR spectra of compound 10