Supplementary Information

Gaining insight into the catalysis by GH20 lacto-*N*-biosidase using small molecule inhibitors and structural analysis

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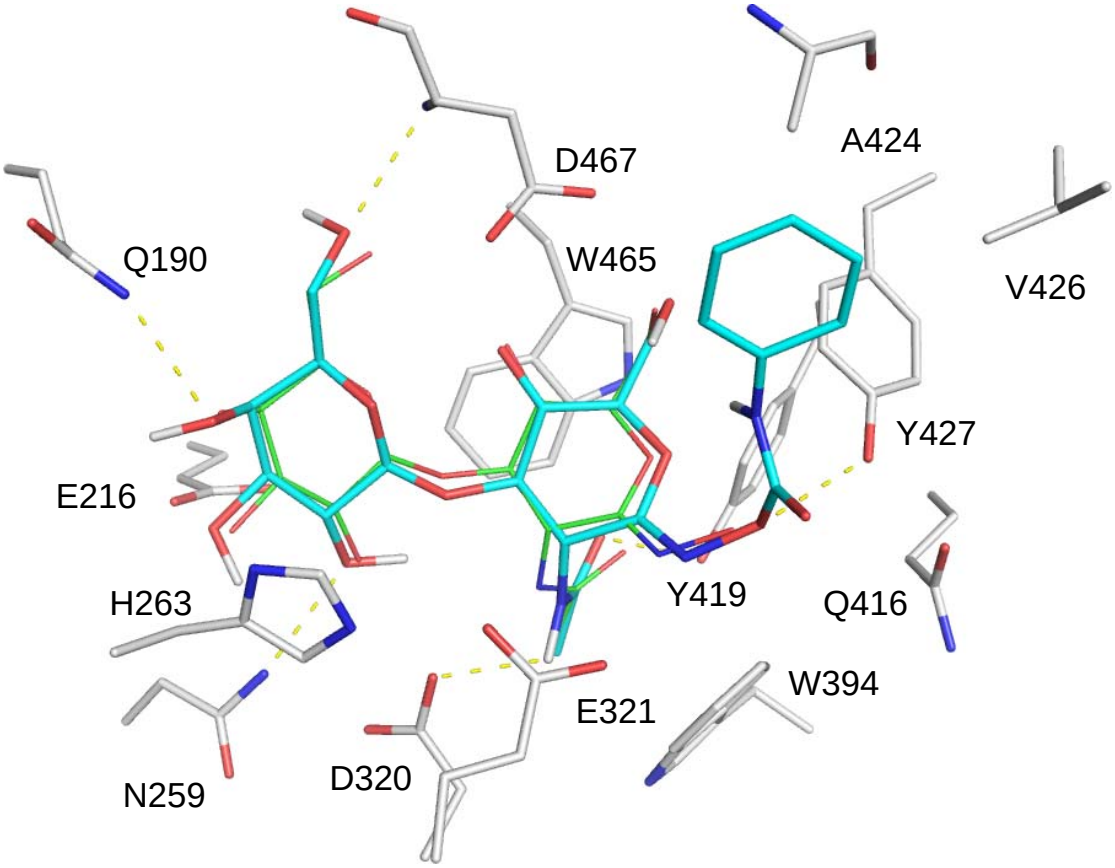

Supporting Figure. Results of docking study of compound **3** (shown in cyan) with *Bb*LNBase using Autodock Vina. The inhibitor **2** is shown in green as a comparison.

Supporting Table. Data collection and refinement statistics

Dataset	Compound **2**	Compound **4**	Compound **5**	Compound **6**
PDB entry	5BXP	5BXR	5BXS	5BXT
Data collection				
Wavelength (Å)	1.00000	1.00000	1.00000	1.00000
Space group	$P2_12_12$	$P2_12_12$	$P2_12_12$	$P2_12_12$
Unit cell (Å)	a = 116.8	a = 116.9	a = 116.7	a = 117.0
	b = 131.6	b = 131.3	b = 131.7	b = 131.5
	c = 104.6	c = 104.3	c = 104.5	c = 104.6
Resolution (Å)[a]	50.0–1.70 (1.73–1.70)	50.0–1.60 (1.63–1.60)	50.0–2.20 (2.24–2.20)	50.0–1.80 (1.83–1.80)
Total reflections	1,294,889	467,217	609,300	1,106,617
Unique reflections	175,974 (8,719)	189,858 (9,912)	82,468 (4,077)	148,686 (7,337)
Completeness (%)[a]	99.9 (100)	90.0 (95.2)	100 (100)	100 (100)
Redundancy[a]	7.4 (7.3)	2.5 (2.3)	7.4 (7.5)	7.4 (7.4)
Mean $I/\sigma(I)$[a]	29.4 (3.48)	18.0 (2.92)	12.4 (3.11)	25.9 (4.26)
R_{merge} (%)[a]	8.3 (51.3)	5.5 (27.1)	16.9 (62.5)	8.6 (45.7)
Refinement				
Resolution range (Å)	29.94-1.70	28.19-1.60	44.85-2.20	30.81-1.80
No. of reflections	167,079	180,311	78,289	141,167
R-factor/R_{free} (%)	17.5/20.6	18.1/21.2	19.1/23.8	17.7/20.9
RMSD from ideal values				
Bond lengths (Å)	0.0252	0.0247	0.0171	0.0214
Bond angles (°)	2.20	2.26	1.79	2.00
Average B-factor (Å^2)				
Protein	20.0/19.9	17.1/17.6	21.6/21.4	17.4/18.1
Ligand	22.0/19.8	16.0/14.8	27.1/25.3	18.3/16.9
Water	27.2	25.4	23.9	27.4
SO_4^{2-}	27.6	24.0	24.5	32.6
Ramachandran plot (%)				
Favored	96.8	96.9	95.5	97.3
Allowed	2.6	2.3	3.5	2.0
Outlier (chain A/B)	0.63	0.79	1.0	0.71
Cremer-Pople parameters of sugar ring at subsite –1				
φ (°)/θ (°)/Q (chain A)	244/64.7/0.596	246/83.7/0.735	234/80.8/0.771	
φ (°)/θ (°)/Q (chain B)	247/59.0/0.611	243/85.1/0.743	238/75.0/0.692	

[a] Values in parentheses are for the highest resolution shell.

Experimental

General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV600 (600 MHz for ^{1}H and 151 MHz for ^{13}C) spectrometer. Solvents used for NMR were: deuteriochloroform ($CDCl_3$) with $CHCl_3$ (^{1}H, δ 7.26 $CDCl_3$ (^{13}C, δ 77.16) used as an internal standard, tetradeuteriomethanol (CD_3OD) with CD_2HOD (^{1}H, δ 3.31) or CD_3OD (^{13}C, δ 49.00) used as an internal standard, deuterium oxide (D_2O) with HOD (^{1}H, δ 4.79) or CH_3OH (^{13}C, δ 49.50) used as an internal standard. High resolution mass spectra (HR-MS) were recorded with a Waters LCT Premier XE spectrometer, run in W-mode, using ESI ionisation method, with MeCN:water (9:1) as a matrix. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One ATR-FT-IR spectrometer. Flash chromatography was performed on BDH silica gel with the specified solvents. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} aluminium-backed plates that were stained by heating (>200 °C) with 5% solution of sulfuric acid in EtOH. All solvents except DMF and DMSO were distilled prior to use. Dimethylformamide was dried over 4Å sieves before use. Dichloromethane and THF were dried over, and distilled from, CaH_2 and Na, respectively, before use.

Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside **9**

Dried powdered 4Å molecular sieves (2.5 g) were added to a stirred solution of **8**[1]

(1.82 g, 3.32 mmol) and **7** (680 mg, 2.14 mmol) in CH_2Cl_2 (18 mL). After 15 min., TMSOTf (100 μL, 0.553 mmol) was added dropwise to the resulting suspension at -30°C. After 5 min. at -30°C, the suspension was stirred at room temperature for 1.5 h., then made neutral with Et_3N at 0°C, filtered through Celite, and concentrated. Flash chromatography (EtOAc/Hex, 3:7) of the resulting oil gave **9** as a white foam (1.29 g, 91%). R_f 0.30 (EtOAc/Hex, 2:3). ATR-IR (neat) ν_{max} 2114 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.50 – 7.43 (m, 2H), 7.41 – 7.32 (m, 3H), 5.55 (s, 1H), 5.32 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.25 (dd, *J* = 8.1, 10.5 Hz, 1H), 5.04 – 4.92 (m, 1H), 4.73 (d, *J* = 8.1 Hz, 1H), 4.33 (dd, *J* = 4.9, 10.5 Hz, 1H), 4.29 (dd, *J* = 0.9, 8.1 Hz, 1H), 4.07 (dd, *J* = 7.7, 11.2 Hz, 1H), 3.88 (dd, *J* = 5.9, 11.0 Hz, 1H), 3.79 (dd, *J* = 10.3, 10.3 Hz, 1H), 3.74 – 3.60 (m, 3H), 3.58 (s, 3H), 3.42 – 3.29 (m, 2H), 2.11 (s, 3H), 2.07 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.36, 170.30, 170.25, 169.57, 137.06, 129.32, 128.39, 126.05, 103.82, 101.73, 101.38, 79.98, 79.48, 71.10, 70.84, 69.40, 68.57, 66.95, 66.41, 66.15, 61.04, 57.64, 20.82, 20.76, 20.70, 20.67. HR-MS (ESI) *m/z* 660.2021; $[M+Na]^+$ requires 660.2017.

Methyl

2-azido-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside

10

The disaccharide **9** (2.64 g, 4.14 mmol) was added to a stirred 80% AcOH solution (80 mL). After 1 h. at 80°C, the solution was concentrated and coevaporated with PhMe (3 X 40 mL). Flash chromatography (EtOAc/Hex, 7:3) of the resulting residue gave **10** as a white foam (1.70 g, 75%). R_f 0.16 (EtOAc/Hex, 7:3). ATR-IR (neat) ν_{max}

2111 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.38 (dd, *J* = 1.1, 3.4 Hz, 1H), 5.24 (dd, *J* = 8.0, 10.5 Hz, 1H), 5.03 (dd, *J* = 3.4, 10.5 Hz, 1H), 4.56 (d, *J* = 8.1 Hz, 1H), 4.24 (d, *J* = 8.0 Hz, 1H), 4.14 (dd, *J* = 4.8, 11.5 Hz, 1H), 4.11 – 4.06 (m, 1H), 4.03 – 3.95 (m, 2H), 3.95 – 3.84 (m, 1H), 3.80 – 3.71 (m, 1H), 3.57 (s, 3H), 3.53 (ddd, *J* = 1.4, 8.3, 9.7 Hz, 1H), 3.34 – 3.26 (m, 2H), 3.21 (dd, *J* = 8.4, 10.0 Hz, 1H), 2.22 – 2.17 (m, 1H), 2.15 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.50, 170.19, 170.13, 169.62, 103.33, 102.56, 86.60, 75.32, 71.36, 70.68, 69.57, 68.58, 66.93, 64.82, 62.67, 61.83, 57.42, 20.70, 20.64, 20.62, 20.59. HR-MS (ESI) *m/z* 572.1700; $[M+Na]^+$ requires 572.1704.

Methyl 4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside **11**

The disaccharide **10** (1.11 g, 2.02 mmol) was in dissolved in C_5H_5N (20 mL, 240 mmol) and treated with Ac_2O (2.50 mL, 26.5 mmol) and DMAP (100 mg, 0.820 mmol) 0°C. After 2 h. at room temperature, the mixture was diluted with MeOH (30 mL), concentrated, dissolved in EtOAc (40 mL) and washed with H_2O, 1M HCl solution, H_2O, saturated $NaHCO_3$ solution, brine (30 mL each), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc/Hex, 1:1) of the resulting residue gave **11** as a colourless oil (1.17 g, 91%). R_f 0.15 (EtOAc/Hex, 2:3). ATR-IR (neat) ν_{max} 2114 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.11 (dd, *J* = 7.9, 10.5 Hz, 1H), 5.01 (dd, *J* = 3.5, 10.4 Hz, 1H), 4.92 (dd, *J* = 9.7, 9.7 Hz, 1H), 4.74 (d, *J* = 7.9 Hz, 1H), 4.24 – 4.18 (m, 2H), 4.17 – 4.09 (m, 2H), 4.04 (dd,

J = 7.4, 11.1 Hz, 1H), 3.87 (ddd, *J* = 1.2, 6.2, 7.4 Hz, 1H), 3.61 – 3.51 (m, 5H), 3.34 (dd, *J* = 8.1, 10.0 Hz, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.85, 170.48, 170.29, 170.27, 169.43, 169.33, 103.20, 101.36, 78.96, 71.91, 71.14, 70.64, 69.13, 66.97, 66.38, 62.27, 61.08, 57.50, 20.90, 20.87, 20.85, 20.79, 20.77, 20.68. HR-MS (ESI) *m/z* 656.1917; $[M+Na]^+$ requires 656.1915.

4,6-Di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-D-α/β-glucopyranose **12**

A 5% solution of H_2SO_4 in Ac_2O (20 mL) was added to a solution of **11** (1.30 g, 2.05 mmol) in Ac_2O (20 mL) at 0°C. After 2 h. at 0°C, the reaction mixture was carefully quenched with ice and the aqueous layer thoroughly extracted with EtOAc (5 X 20 mL). The organic layer was washed thoroughly with saturated $NaHCO_3$ solution until washings remained basic, brine (30 mL), dried over $MgSO_4$, filtered, and concentrated to give presumed acetate as a white foam (990 mg, 73%) which was used without further purification. 40% Methylamine solution (0.15 mL, 1.8 mmol) was added to a solution of presumed acetate (890 mg, 1.35 mmol) in THF (20 mL). After 1 h. at room temperature, the solution was concentrated. Flash chromatography (EtOAc/Hex, 11:9) of the resulting residue gave **12** as a white foam (652 mg, 78%). R_f 0.32 (EtOAc/Hex, 3:2). ATR-IR (neat) ν_{max} 2112 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.38 – 5.37 (m, 1H), 5.36 – 5.35 (m, 2H), 5.12 – 5.09 (m, 2H), 5.02 – 4.97 (m, 3H), 4.93 (dd, *J* = 9.7, 9.7 Hz, 1H), 4.74 (d, *J* = 7.9 Hz, 1H), 4.70 (d, *J* = 7.9 Hz, 1H), 4.65 (d, *J* = 8.0 Hz, 1H), 4.30 (br. s, 1H), 4.18 – 4.02 (m, 9H), 3.95 – 3.83 (m, 3H), 3.63

(ddd, *J* = 2.8, 4.5, 10.1 Hz, 1H), 3.57 – 3.54 (m, 2H), 3.39 (dd, *J* = 3.4, 10.3 Hz, 1H), 3.35 (dd, *J* = 8.0, 10.0 Hz, 1H), 2.14 (s, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.88, 170.79, 170.39, 170.38, 170.19, 170.16, 169.50, 169.40, 169.38, 169.35, 101.14, 101.11, 96.37, 91.92, 78.69, 76.33, 71.89, 71.00, 70.95, 70.51, 70.46, 69.09, 68.99, 68.38, 68.23, 67.81, 67.36, 66.82, 66.80, 63.93, 62.22, 61.16, 60.90, 20.73, 20.72, 20.69, 20.59, 20.49. HR-MS (ESI) *m/z* 641.1757; $[M+Na]^+$ requires 642.1759.

4,6-Di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-D-gluco nohydroxyimino-1,5-lactone **13**

Pyridine (36 μL, 0.45 mmol) was added to a stirred solution of **12** (110 mg, 0.178 mmol) and $NH_2OH.HCl$ (19 mg, 0.27 mmol) in MeOH (5 mL). After 2 h. at relux, the mixture was concentrated, the resulting residue dissolved in EtOAc (15 mL) and washed with H_2O, 1M HCl solution, H_2O, saturated $NaHCO_3$ solution, brine (10 mL each), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc/Hex, 11:9) of the resulting residue gave a white foam (103 mg, 92%), which was quickly used for the next reaction. *N*-Chlorosuccinimide (25 mg, 0.19 mmol) was added to a stirred solution the white foam (103 mg, 0.162 mmol) in CH_2Cl_2 (5 mL) at -20°C. The resulting solution quickly cooled to -40°C and DBU (28 μL, 0.19 mmol) was added in such a way that the temperature was kept between -45°C and -40°C. After 1 h. at -40°C, the reaction mixture was slowly allowed to warm to room temperature where it was then diluted with H_2O (5 mL) and CH_2Cl_2 (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. Flash

chromatography (EtOAc/Hex, 11:9) of the resulting residue gave **13** as a white foam (50 mg, 49%). R_f 0.31 (EtOAc/Hex, 3:2). ATR-IR (neat) ν_{max} 2113 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 6.83 (s, 1H), 5.38 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.21 (dd, *J* = 5.7, 8.5 Hz, 1H), 5.15 (dd, *J* = 8.0, 10.4 Hz, 1H), 5.02 (dd, *J* = 3.5, 10.4 Hz, 1H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.38 (ddd, *J* = 4.1, 4.1, 8.0 Hz, 1H), 4.34 – 4.28 (m, 2H), 4.24 (d, *J* = 5.7 Hz, 1H), 4.21 – 4.04 (m, 2H), 3.91 (m, 2H), 2.17 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.73, 170.59, 170.32, 170.28, 169.51, 169.24, 148.71, 100.83, 77.67, 75.82, 71.01, 70.96, 68.75, 67.85, 66.93, 61.97, 61.12, 59.37, 20.85, 20.79, 20.78, 20.76, 20.66. HR-MS (ESI) *m/z* 633.1891; $[M+H]^+$ requires 633.1892.

4,6-Di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-D-gluco noacetoxyimino-1,5-lactone **14**

Acetic anhydride (0.23 mL, 2.4 mmol) was added to a stirred solution of **13** (275 mg, 0.434 mmol) and C_5H_5N (0.46 mL, 5.7 mmol) in CH_2Cl_2 (5 mL) at 0°C. After 2 h. at room temperature, the mixture was diluted with MeOH (3 mL), concentrated, dissolved in EtOAc (15 mL) and washed with H_2O, 1M HCl solution, H_2O, saturated $NaHCO_3$ solution, brine (7.5 mL each), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc/Hex, 1:1) of the resulting residue gave **14** as a colourless oil (250 mg, 85%). R_f 0.37 (EtOAc/Hex, 3:2). ATR-IR (neat) ν_{max} 2114 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 5.38 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.23 (dd, *J* = 4.2, 8.7 Hz, 1H), 5.15 (dd, *J* = 8.0, 10.4 Hz, 1H), 4.99 (dd, *J* = 3.4, 10.4 Hz, 1H), 4.65 (d, *J* = 8.0 Hz, 1H), 4.53 (ddd, *J* = 4.0, 4.0, 8.4 Hz, 1H), 4.44 (d, *J* = 4.2 Hz, 1H), 4.32 –

4.25 (m, 2H), 4.19 – 4.11 (m, 2H), 4.01 (dd, *J* = 4.2, 4.2 Hz, 1H), 3.93 (ddd, *J* = 1.2, 6.6, 6.6 Hz, 1H), 2.18 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.55, 170.48, 170.28, 170.15, 169.54, 169.15, 167.60, 153.99, 100.61, 77.37, 76.02, 71.33, 70.90, 68.42, 68.22, 66.93, 61.91, 61.14, 58.28, 20.84, 20.80, 20.78, 20.66, 19.43. HR-MS (ESI) *m/z* 697.1818; $[M+Na]^+$ requires 697.1817.

2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-D-gluconoacetoxyimino-1,5-lactone **15**

20% Palladium hydroxide on carbon (30 mg) was added to a solution of **14** (230 mg, 0.341 mmol), and Ac_2O (53 μL, 0.57 mmol) in EtOH (4 mL) which was then stirred under an atmosphere of H_2. After 1.5 h. at room temperature, the reaction mixture was filtered through Celite and concentrated. Flash chromatography (EtOAc) of the resulting residue gave **15** as a white solid (106 mg, 45%). R_f 0.33 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (br. s, 1H), 5.34 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.17 (dd, *J* = 7.5, 7.5 Hz, 1H), 5.06 (dd, *J* = 8.0, 10.4 Hz, 1H), 4.96 (dd, *J* = 3.5, 10.5 Hz, 1H), 4.77 (d, *J* = 8.0 Hz, 1H), 4.63 – 4.51 (m, 2H), 4.39 (dd, *J* = 4.7, 12.8 Hz, 1H), 4.32 – 4.21 (m, 2H), 4.15 (dd, *J* = 6.3, 11.2 Hz, 1H), 4.06 (dd, *J* = 7.2, 11.1 Hz, 1H), 3.91 (ddd, *J* = 1.1, 7.3, 7.3 Hz, 1H), 2.15 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 170.76, 170.48, 170.44, 170.24, 170.18, 169.57, 169.12, 168.61, 158.28, 100.47, 77.25, 76.65, 70.90, 70.81, 68.83, 67.74, 67.05, 61.86, 60.97, 50.79, 22.92, 20.80, 20.73, 20.63, 19.58. HR-MS (ESI) *m/z* 713.2015; $[M+Na]^+$ requires 713.2017.

2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-gluconohydroxyimino-1,5-lacto ne **2**

A saturated solution of NH_3 in MeOH (2 mL) was prepared at 0°C, cooled to -30°C, then added to a solution of **15** (104 mg, 0.151 mmol) in MeOH (2 mL) at -30°C. After 8 h. between -25°C and -15°C, the reaction mixture was concentrated at room temperature. Flash chromatography (EtOAc/MeOH/H_2O, 7:2:1) of the resulting residue gave **2** as a white solid (38 mg, 64%). R_f 0.14 (EtOAc/MeOH/H_2O, 7:2:1). ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, *J* = 9.6 Hz, 1H), 4.49 (d, *J* = 7.7 Hz, 1H), 4.09 – 3.97 (m, 3H), 3.95 – 3.86 (m, 3H), 3.80 – 3.68 (m, 3H), 3.64 (dd, *J* = 3.4, 10.0 Hz, 1H), 3.53 (dd, *J* = 7.7, 9.9 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (D_2O, 151 MHz) δ 175.06, 153.72, 103.87, 81.23, 80.98, 75.94, 73.12, 71.27, 69.19, 67.69, 61.69, 60.68, 50.71, 22.77. HR-MS (ESI) *m/z* 419.1278; $[M+Na]^+$ requires 419.1278.

O-(4,6-Di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-D-gl ucopyranosylidene)amino *N*-phenyl carbamate **16**

Phenyl isocyanate (69 μL, 0.63 mmol) was added dropwise to a stirred solution of **13** (375 mg, 0.593 mmol) in THF (8 mL) at 0°C, followed by Et_3N (103 μL, 0.74 mmol) dropwise at 0°C. After 1 h. at 0°C the reaction mixture was diluted with PhMe (1.5 mL) and concentrated. Flash chromatography (EtOAc/Hex, 1:1) of the resulting residue gave **16** as a white foam (260 mg, 58%). R_f 0.28 (EtOAc/Hex, 3:2). ATR-IR (neat) ν_{max} 2114 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (br. s, 1H), 7.52 – 7.44 (m, 2H), 7.37 – 7.31 (m, 2H), 7.16 – 7.07 (m, 1H), 5.38 (dd, *J* = 1.2, 3.4 Hz, 1H), 5.26 (dd, *J* = 4.6, 8.5 Hz, 1H), 5.15 (dd, *J* = 8.0, 10.4 Hz, 1H), 5.02 (dd, *J* = 3.4, 10.4 Hz,

1H), 4.70 (d, *J* = 8.0 Hz, 1H), 4.53 (ddd, *J* = 2.8, 5.1, 8.3 Hz, 1H), 4.45 (d, *J* = 5.2 Hz, 1H), 4.35 (dd, *J* = 5.1, 12.8 Hz, 1H), 4.29 (dd, *J* = 2.8, 12.9 Hz, 1H), 4.16 (dd, *J* = 6.5, 11.2 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.00 (dd, *J* = 4.9, 4.9 Hz, 1H), 3.94 (ddd, *J* = 1.3, 6.5, 6.6 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.61, 170.46, 170.24, 170.13, 169.46, 169.17, 152.36, 151.16, 137.05, 129.25, 124.37, 119.45, 100.97, 77.28, 76.57, 71.31, 70.81, 68.60, 67.89, 66.91, 61.76, 61.14, 58.65, 20.82, 20.80, 20.77, 20.75, 20.64. HR-MS (ESI) *m/z* 774.2081; $[M+Na]^+$ requires 774.2082.

O-(2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-D-glucopyranosylidene)amino *N*-phenyl carbamate **17**

20% Palladium hydroxide on carbon (15 mg) was added to a solution of **16** (160 mg, 0.213 mmol), and Ac_2O (30 μL, 0.32 mmol) in EtOH (2 mL) which was then stirred under an atmosphere of H_2. After 2 h. at room temperature, the reaction mixture was filtered through Celite and concentrated. Flash chromatography (EtOAc/Hex, 17:3) of the resulting residue gave **17** as a white foam (83 mg, 51%). R_f 0.17 (EtOAc/Hex, 7:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (br. s, 1H), 7.46 – 7.38 (m, 2H), 7.36 – 7.29 (m, 2H), 7.16 – 7.02 (m, 2H), 5.37 (dd, *J* = 0.9, 3.5 Hz, 1H), 5.27 (dd, *J* = 5.2, 7.1 Hz, 1H), 5.11 (dd, *J* = 7.9, 10.4 Hz, 1H), 5.02 (dd, *J* = 3.5, 10.4 Hz, 1H), 4.77 (d, *J* = 8.0 Hz, 1H), 4.75 (dd, *J* = 6.4, 6.4 Hz, 1H), 4.62 (ddd, *J* = 3.4, 5.8, 7.4 Hz, 1H), 4.41 (dd, *J* = 4.8, 12.7 Hz, 1H), 4.31 (dd, *J* = 3.2, 12.7 Hz, 1H), 4.25 (dd, *J* = 5.5, 5.5 Hz, 1H), 4.17 – 4.06 (m, 2H), 3.93 (dd, *J* = 6.8, 6.8 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (151 MHz,

$CDCl_3$) δ 170.68, 170.56, 170.54, 170.30, 170.22, 169.69, 169.18, 155.22, 152.59, 136.83, 129.33, 124.73, 119.72, 101.19, 77.47, 76.93, 76.77, 71.05, 70.87, 68.88, 68.37, 67.01, 62.03, 61.11, 49.71, 23.08, 20.87, 20.84, 20.83, 20.78, 20.69. HR-MS (ESI) *m/z* 790.2283; $[M+Na]^+$ requires 790.2283.

O-(2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-glucopyranosylidene)amino *N*-phenyl carbamate **3**

A saturated solution of NH_3 in MeOH (2 mL) was prepared at 0°C, cooled to -30°C, then added to a solution of **17** (80 mg, 0.10 mmol) in MeOH (2 mL) at -30°C. After 6 h. between -25°C and -15°C, the reaction mixture was concentrated at room temperature. Flash chromatography (MeOH/$CHCl_3$, 13:37) of the resulting residue gave **3** as a white solid (18 mg, 34%). R_f 0.27 (EtOAc/MeOH/H_2O, 78:15:7). ^1H NMR (600 MHz, D_2O) δ 7.52 – 7.28 (m, 4H), 7.24 – 7.11 (m, 1H), 4.83 – 4.77 (m, 1H), 4.47 (d, *J* = 7.7 Hz, 1H), 4.19 – 4.14 (m, 1H), 4.12 (dd, *J* = 8.9, 8.9 Hz, 1H), 3.99 (dd, *J* = 2.3, 13.0 Hz, 1H), 3.94 – 3.85 (m, 3H), 3.72 (m, 2H), 3.65 (dd, *J* = 3.9, 8.1 Hz, 1H), 3.61 (dd, *J* = 3.4, 10.0 Hz, 1H), 3.50 (dd, *J* = 7.7, 9.9 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (151 MHz, D_2O) δ 175.14, 159.15, 155.07, 137.15, 129.94, 125.66, 121.54, 103.84, 82.13, 80.62, 75.98, 73.12, 71.29, 69.19, 67.38, 61.70, 60.56, 50.86, 22.75. HR-MS (ESI) *m/z* 538.1653; $[M+Na]^+$ requires 538.1649.

Methyl

4-*O*-acetyl-2-azido-2,6-dideoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-6-iodo-β-D-glucopyranoside **18**

4-Toluene-sulfonyl chloride (370 mg, 1.94 mmol) was added to stirred solution of **10** (870 mg, 1.58 mmol) and C_5H_5N (2.6 mL, 32 mmol) in CH_2Cl_2 (9 mL) at 0°C. After 6 h. at room temperature, the reaction mixture was diluted with saturated $NaHCO_3$ solution (15 mL) and CH_2Cl_2 (20 mL). The organic layer was washed with 1M HCl solution, H_2O, saturated $NaHCO_3$ solution, brine (10 mL each), dried over $MgSO_4$, filtered, and concentrated. The resulting residue was treated with NaI (1.36 g, 9.07 mmol) in DMF (9 mL). After 1.5 h. at 100°C, the reaction mixture was allowed to cool and diluted with H_2O (20 mL) and extracted thoroughly with EtOAc (3 X 15 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. The resulting residue treated with C_5H_5N (3.0 mL, 37 mmol) and Ac_2O (1.0 mL, 11 mmol) in CH_2Cl_2 (5 mL). After 16 h. at room temperature, the mixture was diluted with MeOH (3 mL), concentrated, dissolved in EtOAc (25 mL) and washed with H_2O, 1M HCl solution, H_2O, saturated $NaHCO_3$ solution, brine (15 mL each), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc/Hex, 3:7) of the resulting residue gave **18** as a white solid (577 mg, 52% over three steps). R_f 0.37 (EtOAc/Hex, 1:1). ATR-IR (neat) ν_{max} 2113 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 1.1, 3.5 Hz, 1H), 5.10 (dd, *J* = 7.9, 10.4 Hz, 1H), 5.01 (dd, *J* = 3.4, 10.4 Hz, 1H), 4.73 (d, *J* = 8.0 Hz, 1H), 4.70 (dd, *J* = 9.4, 9.4 Hz, 1H), 4.23 (d, *J* = 8.1 Hz, 1H), 4.13 (dd, *J* = 6.3, 11.2 Hz, 1H), 4.04 (dd, *J* = 7.2, 11.2 Hz, 1H), 3.87 (dd, 1H), 3.62 (s, 3H), 3.53 (dd, *J* = 9.5, 9.5 Hz, 1H), 3.41

(ddd, J = 2.6, 9.3, 9.4 Hz, 1H), 3.34 (dd, J = 8.0, 9.9 Hz, 1H), 3.27 (dd, J = 2.6, 10.9 Hz, 1H), 3.11 (dd, J = 9.0, 11.0 Hz, 1H), 2.14 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.96 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 170.47, 170.26, 170.24, 169.56, 169.39, 102.83, 101.30, 78.53, 73.79, 72.25, 71.10, 70.67, 69.08, 66.96, 66.52, 61.10, 57.54, 20.95, 20.83, 20.79, 20.76, 20.66, 3.23. HR-MS (ESI) *m/z* 724.0871; $[M+Na]^+$ requires 724.0827.

Methyl 4-*O*-acetyl-2-azido-2,6-dideoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-*xylo*-hex-5-enoside **19**

1,8-Diazabicyclo[5.4.0]undec-7-ene (0.88 mL, 3.1 mmol) was added to a stirred solution of **18** (1.10 g, 1.59 mmol). After 10 h. at reflux, the reaction mixture was concentrated, dissolved in EtOAc (40 mL) and washed with H_2O, ice cold 1M HCl solution, H_2O, saturated $NaHCO_3$ solution, brine (15 mL each), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc/Hex, 3:7) of the resulting residue gave **19** as a white solid (739 mg, 81%). R_f 0.38 (EtOAc/Hex, 1:1). ATR-IR (neat) ν_{max} 2114 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.41 (ddd, J = 1.5, 1.6, 7.8 Hz, 1H), 5.37 (dd, J = 1.0, 3.4 Hz, 1H), 5.15 (dd, J = 7.9, 10.4 Hz, 1H), 5.02 (dd, J = 3.5, 10.4 Hz, 1H), 4.79 (dd, J = 1.6, 1.6 Hz, 1H), 4.71 (d, J = 8.0 Hz, 1H), 4.57 (dd, J = 1.6, 1.6 Hz, 1H), 4.36 (d, J = 7.2 Hz, 1H), 4.18 (dd, J = 6.4, 11.2 Hz, 1H), 4.06 (dd, J = 7.3, 11.2 Hz, 1H), 3.90 (ddd, J = 0.9, 6.8, 6.8 Hz, 1H), 3.61 (s, 3H), 3.56 – 3.44 (m, 2H), 2.16 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.52, 170.34, 170.28, 169.44, 169.23, 150.96, 103.28, 101.50,

97.69, 78.85, 71.10, 70.79, 69.99, 69.08, 66.98, 65.72, 61.14, 57.44, 20.95, 20.84, 20.80, 20.79, 20.70. HR-MS (ESI) *m/z* 596.1708; $[M+Na]^+$ requires 596.1704.

Methyl

2-acetamido-4-*O*-acetyl-2,6-dideoxy-3-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-*xylo*-hex-5-enoside **20**

Tributyl phosphine (0.17 mL, 0.69 mmol) and H_2O (22 μL) were added to a stirred solution of **19** (350 mg, 0.610 mmol) in THF (5 mL) at 0°C. After 1 h. at 0°C, Ac_2O (100 μL, 1.06 mmol) and C_5H_5N (1.0 mL, 12 mmol) were added to the resulting solution. After 2 h. at room temperature, the reaction mixture was and concentrated. Flash chromatography (EtOAc/Hex, 7:3) of the resulting residue gave **20** as a white solid (187 mg, 52%). R_f 0.28 (EtOAc/Hex, 7:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.89 (d, *J* = 8.8 Hz, 1H), 5.57 (d, *J* = 5.1 Hz, 1H), 5.38 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.16 (dd, *J* = 7.9, 10.4 Hz, 1H), 5.03 (dd, *J* = 3.5, 10.4 Hz, 1H), 4.82 – 4.77 (m, 2H), 4.76 – 4.72 (m, 1H), 4.60 – 4.51 (m, 1H), 4.16 (dd, *J* = 6.2, 11.2 Hz, 1H), 4.10 (dd, *J* = 7.3, 11.2 Hz, 1H), 4.05 (ddd, *J* = 2.7, 2.7, 8.9 Hz, 1H), 3.92 (ddd, *J* = 0.9, 1.2, 6.9 Hz, 1H), 3.87 (ddd, *J* = 0.8, 2.7, 4.9 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 170.54, 170.44, 170.24, 169.53, 169.39, 168.80, 150.25, 101.37, 99.99, 98.25, 77.18, 71.10, 70.89, 69.89, 68.72, 67.12, 61.14, 55.94, 50.81, 23.51, 21.20, 20.85, 20.80, 20.74. HR-MS (ESI) *m/z* 612.1909; $[M+Na]^+$ requires 612.1904.

2-Acetamido-3-*O*-(β-D-galactopyranosyl)-1,5-imino-1,2,5-trideoxy-D-glucitol **4**

3-Chloroperoxybenzoic acid (90 mg, 0.52 mmol) was added to a stirred solution of **20** (190 mg, 0.322 mmol) and BnOH (4.0 mL, 38 mmol) in CH_2Cl_2 (4 mL). After 2 h. at room temperature, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated $NaHCO_3$ solution (15 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc) gave a colourless oil which was treated with NaOMe (10 mg, 0.19 mmol) in MeOH (5 mL). After 30 min. at room temperature, the solution was made neutral with Amberlite IR-120 resin (H^+), filtered, and concentrated. Flash chromatography (MeOH/EtOAc, 1:3) of the resulting residue gave a colourless oil (65 mg 0.13 mmol) which was treated with 20% $Pd(OH)_2/C$ (8.0 mg) and NH_4HCOO (8.0 mg, 0.13 mmol) in 15:1 MeOH/H_2O (4.3 mL). After stirring for 2 days at room temperature under an atmosphere of H_2, further 20% $Pd(OH)_2/C$ (6.0 mg) and NH_4HCOO (6.0 mg, 95 μmol) were added. After a further 2 days at room temperature under an atmosphere of H_2, the reaction mixture was filtered through Celite and concentrated. Flash chromatography (MeOH/$CHCl_3$/NH_3, 5:5:1) of the resulting residue gave **4** as a white solid (39 mg, 33% over three steps). The ^1H NMR spectrum was consistent with that found in the literature.[2]

6-*O*-Carbobenzyloxy-7-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-1,8-isopropylidenec astanospermine **22**

Dried powdered 4Å molecular sieves (2.3 g) were added to a stirred solution of **8**[1] (1.70 g, 3.10 mmol) and **21**[3] (570 mg, 1.57 mmol) in CH_2Cl_2 (15 mL). After 15 min., TMSOTf (0.71 mL, 3.9 mmol) was added dropwise to the resulting suspension at

-30°C. After 5 min. at -30°C, the suspension was stirred at room temperature for 1.5 h., then made basic with Et_3N at 0°C, filtered through Celite, and concentrated. Flash chromatography (EtOAc/Hex, 13:7) of the resulting residue gave **22** as a white foam (550 mg, 50%). R_f 0.40 (EtOAc/Hex, 7:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 – 7.28 (m, 5H), 5.35 (dd, *J* = 1.2, 3.4 Hz, 1H), 5.19, 5.10 (ABq, J_{AB} = 12.0 Hz, 2H), 5.18 (dd, *J* = 8.1, 10.6 Hz, 1H), 4.98 (dd, *J* = 3.4, 10.5 Hz, 1H), 4.89 (ddd, *J* = 5.1, 7.2, 9.1 Hz, 1H), 4.79 (d, *J* = 8.1 Hz, 1H), 4.47 (ddd, *J* = 1.8, 6.9, 8.3 Hz, 1H), 4.21 – 4.06 (m, 2H), 3.89 (ddd, *J* = 1.3, 5.8, 7.4 Hz, 1H), 3.84 (dd, *J* = 9.6 Hz, 1H), 3.75 (dd, *J* = 7.2, 9.5 Hz, 1H), 3.22 (dd, *J* = 5.1, 13.4 Hz, 1H), 2.99 – 2.89 (m, 2H), 2.85 (dd, *J* = 9.1, 13.4 Hz, 1H), 2.80 (ddd, *J* = 2.3, 7.6, 8.8 Hz, 1H), 2.24 – 2.16 (m, 1H), 2.13 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.93 – 1.88 (m, 1H), 1.38 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.42, 170.40, 170.33, 169.62, 154.37, 135.10, 128.65, 128.61, 101.23, 100.88, 81.85, 74.01, 71.19, 71.07, 70.85, 70.08, 69.63, 67.08, 66.49, 62.41, 61.05, 49.61, 49.57, 33.12, 27.86, 24.99, 20.79, 20.75, 20.72. HR-MS (ESI) *m/z* 716.2529; $[M+Na]^+$ requires 716.2530.

1,8-Di-*O*-acetyl-6-*O*-carbobenzyloxy-7-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)castanospermine **23**

The disaccharide **22** (550 mg, 0.793 mmol) was added to a stirred AcOH/H_2O solution (4:1, 15 mL). After 1 h. at 70°C, the solution was concentrated and coevaporated with PhMe (2 X 25 mL) and C_5H_5N (25 mL). The resulting residue was cooled to 0°C, dissolved in C_5H_5N (10 mL, 120 mmol) and treated with Ac_2O (0.50 mL, 5.3 mmol). After 16 h. at room temperature, the mixture was diluted with MeOH

(10 mL), concentrated and coevaporated with PhMe (3 X 25 mL). Flash chromatography (EtOAc/Hex, 11:9) of the resulting residue gave **23** as a white foam (520 mg, 89%). R_f 0.92 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.31 (m, 5H), 5.35 – 5.28 (m, 2H), 5.20 – 5.14 (m, 2H), 5.11 (dd, *J* = 10.1, 10.1 Hz, 1H), 5.05 (dd, *J* = 8.0, 10.5 Hz, 1H), 4.94 (dd, *J* = 3.4, 10.5 Hz, 1H), 4.87 (ddd, *J* = 5.4, 9.9, 9.9 Hz, 1H), 4.66 (d, *J* = 8.0 Hz, 1H), 4.15 – 4.08 (m, 1H), 4.02 (dd, *J* = 7.3, 11.1 Hz, 1H), 3.85 (dd, *J* = 1.2, 6.4, 6.8 Hz, 1H), 3.73 (dd, *J* = 9.2, 9.2 Hz, 1H), 3.37 (dd, *J* = 5.4, 10.4 Hz, 1H), 3.17 (ddd, *J* = 2.3, 9.0, 9.0 Hz, 1H), 2.30 (dddd, *J* = 2.2, 7.3, 9.4, 14.3 Hz, 1H), 2.24 – 2.15 (m, 2H), 2.11 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.84 (s, 3H), 1.83 – 1.77 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.77, 170.40, 170.30, 170.23, 169.27, 169.21, 154.14, 134.86, 128.86, 128.83, 128.40, 101.02, 80.91, 76.47, 71.10, 70.48, 70.04, 68.98, 68.66, 67.90, 67.06, 61.08, 53.06, 52.06, 31.87, 21.21, 20.88, 20.72, 20.70, 20.66, 20.49. HR-MS (ESI) *m/z* 760.2422; $[M+Na]^+$ requires 760.2429.

1,8-Di-*O*-acetyl-7-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)castanospermine **24**

10% Palladium on carbon (80 mg) was added to a solution of **23** (520 mg, 0.705 mmol) in MeOH (5 mL) which was then stirred under an atmosphere of H_2. After 8 h., the suspension was filtered through Celite and concentrated. Flash chromatography (EtOAc) of the resulting oil gave **24** as a white solid (290 mg, 68%). R_f 0.34 (EtOAc). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 1.2, 3.4 Hz, 1H), 5.31 (ddd, *J* = 1.7, 4.4, 7.4 Hz, 1H), 5.22 – 5.11 (m, 2H), 5.00 (dd, *J* = 3.4, 10.5 Hz, 1H), 4.77 (d, *J* = 8.0 Hz, 1H), 4.18 – 4.02 (m, 2H), 3.93 – 3.79 (m, 2H), 3.51 (dd, *J* = 9.2, 9.2 Hz, 1H), 3.27

(dd, *J* = 5.1, 10.7 Hz, 1H), 3.20 (ddd, *J* = 2.2, 9.0, 9.1 Hz, 1H), 2.73 (br. s, 1H), 2.34 – 2.26 (m, 1H), 2.23 – 2.17 (m, 2H), 2.14 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.82 (dddd, *J* = 1.7, 8.8, 8.9, 14.6 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.84, 170.45, 170.29, 170.19, 169.43, 169.27, 101.54, 87.28, 71.15, 70.86, 70.41, 69.91, 69.43, 67.80, 66.96, 61.15, 55.48, 52.16, 31.79, 21.26, 21.02, 20.87, 20.74, 20.65. HR-MS (ESI) *m/z* 626.2058; $[M+Na]^+$ requires 626.2061.

1,8-Di-*O*-acetyl-6-azido-6-deoxy-7-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)castanospermine **25** and

1,7-Di-*O*-acetyl-8-azido-8-deoxy-2-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)australine **26**

Methanesulfonyl chloride (55 μL, 0.71 mmol) was added dropwise to a stirred solution of **24** (355 mg, 0.588 mmol) in C_5H_5N (5 mL) at 0°C. After 16 h. at room temperature, further MsCl (55 μL, 0.71 mmol) was added to the stirred solution at 0°C. After a further 24 h. at room temperature, the solution was diluted with MeOH (5 mL), concentrated, and coevaporated with PhMe (2 X 10 mL). The resulting residue was dissolved in CH_2Cl_2 (15 mL) and washed with saturated $NaHCO_3$ solution (10 mL) brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. The resulting residue was dissolved in DMSO (3 mL) and treated with NaN_3 (181 mg, 2.78 mmol). After 1 h. at 80°C and a further 5 h. at 60°C, the reaction mixture was diluted with H_2O (15 mL) and thoroughly extracted with CH_2Cl_2 (4 X 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (EtOAc/Hex, 3:2) of the resulting residue gave two compounds. First

to elute was **25** to give a colourless glass (113 mg, 27%). R_f 0.62 (EtOAc/Hex, 4:1). ATR-IR (neat) ν_{max} 2107 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.34 – 5.26 (m, 1H), 5.15 – 5.07 (m, 2H), 5.01 (dd, *J* = 3.5, 10.4 Hz, 1H), 4.76 (d, *J* = 7.8 Hz, 1H), 4.16 (dd, *J* = 6.2, 11.1 Hz, 1H), 4.05 (dd, *J* = 7.5, 11.1 Hz, 1H), 3.88 (ddd, *J* = 1.2, 6.2, 7.4 Hz, 1H), 3.65 (ddd, *J* = 5.2, 9.7, 10.8 Hz, 1H), 3.45 (dd, *J* = 9.5, 9.5 Hz, 1H), 3.30 (dd, *J* = 5.2, 11.0 Hz, 1H), 3.20 (ddd, *J* = 2.3, 9.0, 9.1 Hz, 1H), 2.31 (dddd, *J* = 2.2, 7.3, 9.4, 14.5 Hz, 1H), 2.23 – 2.15 (m, 2H), 2.13 (s, 3H), 2.06 – 2.02 (m, 10H), 1.98 (s, 3H), 1.96 (s, 3H), 1.82 (dddd, *J* = 1.9, 8.8, 8.8, 16.9 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.73, 170.43, 170.27, 169.42, 169.25, 101.07, 82.12, 71.25, 71.17, 70.56, 69.31, 68.88, 68.07, 66.99, 62.79, 61.00, 54.24, 52.12, 31.80, 21.20, 20.94, 20.88, 20.75, 20.71, 20.65. HR-MS (ESI) *m/z* 651.2139; $[M+Na]^+$ requires 651.2126.

Next to elute was **26** as a colourless glass (62 mg) which was used without any further purification.

6-Acetamido-1,8-di-*O*-acetyl-6-deoxy-7-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)castanospermine **27**

10% Palladium on carbon (50 mg) was added to a solution of **26** (110 mg, 0.174 mmol) in MeOH (3 mL) which was then stirred under an atmosphere of H_2. After 2 h., the suspension was filtered through Celite and concentrated. The resulting residue was cooled to 0°C, dissolved in C_5H_5N (3.0 mL, 37 mmol) and treated with Ac_2O (100 μL, 1.04 mmol). After 2 h. at room temperature, the mixture was diluted with MeOH (1 mL), concentrated and coevaporated with PhMe (5 X 5 mL). Flash chromatography

(MeOH/EtOAc, 1:9) of the resulting residue gave **27** as a white solid (35 mg, 31%). R_f 0.55 (MeOH/EtOAc, 1:4). ^{1}H NMR (600 MHz, CD_3OD) δ 5.43 – 5.28 (m, 2H), 5.07 (dd, *J* = 3.6, 10.4 Hz, 1H), 5.02 (dd, *J* = 9.0, 9.9 Hz, 1H), 4.96 (dd, *J* = 7.9, 10.4 Hz, 1H), 4.22 – 4.13 (m, 3H), 4.12 – 4.03 (m, 1H), 3.75 – 3.63 (m, 1H), 3.15 (ddd, *J* = 2.1, 8.9, 9.1 Hz, 1H), 3.07 (dd, *J* = 5.0, 10.9 Hz, 1H), 2.38 – 2.26 (m, 2H), 2.22 (ap. q, *J* = 9.2 Hz, 1H), 2.13 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 – 1.98 (m, 10H), 1.92 (s, 3H), 1.78 (dddd, *J* = 2.0, 8.8, 8.8, 14.2 Hz, 1H). ^{13}C NMR (151 MHz, CD_3OD) δ 172.88, 172.11, 172.00, 171.91, 171.74, 171.58, 171.46, 101.99, 81.92, 72.85, 72.60, 71.59, 70.61, 70.15, 69.91, 68.76, 62.40, 55.50, 53.00, 51.77, 32.50, 23.09, 21.32, 21.11, 20.80, 20.60, 20.47. HR-MS (ESI) *m/z* 645.2507; $[M+H]^+$ requires 645.2507.

6-Acetamido-6-deoxy-7-*O*-(β-D-galactopyranosyl)castanospermine **5**

Freshly prepared 0.83 M NaOMe solution in MeOH (0.1 mL) was added to a solution of **27** (30 mg, 0.047 mmol) in MeOH (3mL). After 2 h. at room temperature, the solution was made neutral with 1 M HCl solution and concentrated. The resulting solid was dissolved in H_2O (0.5 mL) and purified on AG50W-X4 resin (H^+), eluted first with H_2O, then 1 M NH_3 solution. Lyophilisation gave **5** as a white solid (16 mg, 88%). R_f 0.07 (MeOH/CH_2Cl_2, 3:7). ^{1}H NMR (600 MHz, D_2O) δ 4.54 – 4.40 (m, 2H), 4.07 (ddd, *J* = 5.0, 10.1, 11.3 Hz, 1H), 3.94 (dd, *J* = 1.0, 3.4 Hz, 1H), 3.84 – 3.73 (m, 4H), 3.67 (dd, *J* = 3.4, 9.9 Hz, 1H), 3.64 (dd, *J* = 8.6, 10.1 Hz, 1H), 3.56 (dd, *J* = 7.7, 10.0 Hz, 1H), 3.13 (ddd, *J* = 3.7, 8.1, 13.7 Hz, 2H), 2.36 (dddd, *J* = 2.4, 7.3, 9.6, 14.2 Hz, 1H), 2.23 (ap. q, *J* = 9.2 Hz, 1H), 2.13 – 2.05 (m, 2H), 2.02 (s, 3H), 1.75 (dddd, *J* = 1.9, 8.8, 8.8, 14.3 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.93, 104.20, 86.19,

75.91, 73.16, 71.47, 71.37, 70.30, 69.15, 61.61, 54.05, 52.06, 50.53, 33.20, 22.78. HR-MS (ESI) *m/z* 415.1692; $[M+Na]^+$ requires 415.1693.

8-Acetamido-1,7-di-*O*-acetyl-8-deoxy-2-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)australine **28**

10% Palladium on carbon (30 mg) was added to a solution of crude **26** (60 mg) in MeOH (2 mL) which was then stirred under an atmosphere of H_2. After 2 h., the suspension was filtered through Celite and concentrated. The resulting residue was cooled to 0°C, dissolved in C_5H_5N (3.0 mL, 37 mmol) and treated with Ac_2O (100 μL, 1.04 mmol). After 2 h. at room temperature, the mixture was diluted with MeOH (1 mL), concentrated and coevaporated with PhMe (5 X 5 mL). Flash chromatography (MeOH/EtOAc, 1:9) of the resulting residue gave **28** as a white solid (21 mg, 6% over two steps). R_f 0.11 (MeOH/EtOAc, 1:9). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.07 (d, *J* = 8.7 Hz, 1H), 5.37 (dd, *J* = 1.2, 3.5 Hz, 1H), 5.31 (ddd, *J* = 1.4, 4.1, 4.1 Hz, 1H), 5.23 – 5.11 (m, 2H), 5.03 (dd, *J* = 3.5, 10.5 Hz, 1H), 4.63 (d, *J* = 8.0 Hz, 1H), 4.12 (dd, *J* = 6.2, 11.3 Hz, 1H), 4.09 – 3.99 (m, 2H), 3.88 (ddd, *J* = 1.3, 6.2, 7.5 Hz, 1H), 3.82 – 3.73 (m, 1H), 3.29 (dd, *J* = 4.1, 5.4 Hz, 1H), 3.08 (ddd, *J* = 4.1, 7.2, 7.7 Hz, 1H), 2.94 – 2.84 (m, 2H), 2.62 (ddd, *J* = 6.5, 10.2, 10.2 Hz, 1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 – 2.05 (m, 4H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 – 1.95 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.56, 170.53, 170.28, 170.15, 170.10, 101.53, 86.59, 75.52, 73.92, 71.24, 70.86, 70.62, 68.98, 68.15, 66.94, 61.08, 50.98, 38.92, 33.94, 23.40, 21.34, 20.89, 20.83, 20.77, 20.70, 20.64. HR-MS (ESI) *m/z* 645.2509; $[M+H]^+$ requires 645.2507.

8-Acetamido-8-deoxy-2-*O*-(β-D-galactopyranosyl)australine **6**

Freshly prepared 0.83 M NaOMe solution in MeOH (0.1 mL) was added to a solution of **28** (21 mg, 0.033 mmol) in MeOH (2 mL). After 2 h. at room temperature, the solution was made neutral with HCl solution (1 M) and concentrated. The resulting solid was dissolved in H_2O (0.5 mL) and purified on AG50W-X4 resin (H^+), eluted first with H_2O, then NH_3 solution (1 M). Lyophilisation gave **6** as a white solid (11 mg, 85%). R_f 0.05 (MeOH/CH_2Cl_2, 3:7). ^{1}H NMR (600 MHz, D_2O) δ 4.50 (d, *J* = 7.8 Hz, 1H), 4.42 – 4.32 (m, 2H), 3.98 (dd, *J* = 7.2, 9.5 Hz, 1H), 3.94 (dd, *J* = 0.5, 3.4 Hz, 1H), 3.87 – 3.74 (m, 3H), 3.70 (dd, *J* = 3.5, 10.0 Hz, 1H), 3.61 (dd, *J* = 7.9, 9.9 Hz, 1H), 3.48 (dd, *J* = 4.8, 14.1 Hz, 1H), 3.39 (dd, *J* = 6.4, 14.1 Hz, 1H), 3.25 (dd, *J* = 4.1, 7.1 Hz, 1H), 3.18 – 3.12 (m, 1H), 3.02 – 2.90 (m, 1H), 2.73 (ddd, *J* = 6.0, 10.7, 11.0 Hz, 1H), 2.10 – 2.04 (m, 1H), 2.03 (s, 3H), 2.01 – 1.93 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 175.12, 104.23, 91.43, 76.33, 73.58, 73.20, 72.38, 71.73, 70.57, 69.43, 68.29, 61.97, 52.59, 42.52, 36.23, 22.87. HR-MS (ESI) *m/z* 415.1692; $[M+Na]^+$ requires 415.1693.

Enzyme kinetics

All assays were carried out in triplicate at 37 °C for 60 minutes by using a stopped assay procedure in which the enzymatic reactions (50 μl) were quenched by the addition of a 4-fold excess (200 μl) of quenching buffer (200 mM glycine, pH 10.75). Assays were initiated by the careful addition, *via* pipette, of enzyme (5 μl), and in all cases the final pH of the resulting quenched solution was greater than 10. Time-dependent assay of the enzyme revealed that the enzyme was stable in the

buffer over the period of the assay (50 mM citrate-phosphate buffer, pH 4.5). The progress of the reaction at the end of 60 minutes was determined by measuring the extent of 4-methylumbelliferone liberated as determined by fluorescence measurements using a Varian CARY Eclipse Fluorescence Spectrophotometer 96-well plate system and comparison to a standard curve of 4-methylumbelliferone under identical buffer conditions. Excitation and emission wavelengths of 368 and 450 nM were used, respectively, with 5 mm slit openings. The enzyme was expressed fresh before use as by literature procedures[4] and the concentration used in assays was 0.0011 μg/μl, with the substrate 4-methylumbelliferyl 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)glucopyranoside. The inhibitors were tested at six concentrations with K_i values determined by linear regression of data from Dixon plots.

Crystallography methods

Expression and purification of *Bb*LNBase were carried out as described previously.[5] Co-crystals of *Bb*LNBase complexed with the inhibitors (**2**, **4**, **5**, and **6**) were grown at 293 K using the sitting drop vapor diffusion method, by mixing 1.0 μl of 10 mg/ml protein solution containing 0.1 mM the inhibitors, with an equal volume of a reservoir solution which contained 0.2 M potassium sodium tartrate tetrahydrate, 0.1 M sodium citrate (pH 5.6) and 2.0 M ammonium sulfate. For cryoprotectant, 20% glycerol was used. The crystals were flash-cooled in a nitrogen stream at 100 K. Diffraction images were collected at the beamline AR-NE3A of the Photon Factory in the High Energy Accelerator Research Organization (KEK, Tsukuba, Japan), and

processed using HKL2000.[6] Phasing and initial model building were performed using rigid body refinement in Refmac5,[7] for which the coordinates of the LNB complex (PDB ID: 4H04) were used as a structural template. Library files for Refmac5 of the inhibitors were generated using Sketcher in the CCP4 suite.[8] The models were further built manually using COOT,[9] and refined using Refmac5. Molecular graphic figures were prepared using PyMol (Delano Scientific, Palo Alto, CA).

Automated docking studies

The inhibitor **3** was generated using GaussView (Gaussian, Inc., Wallingford, CT). The crystal structure of *Bb*LNBase in complex with **2** was used for the analysis by removing all water and ligand molecules. The docking studies were carried out using the AutoDock Vina program.[10] Using AutoDockTools, polar hydrogen atoms were added to amino acid residues, and Gasteiger charges were assigned to all atoms of the enzyme. The grid map was prepared with 18 × 18 × 18 $Å^3$. The grid box was centered on the C2 atom of the GlcNAc bound in the -1 subsite. The ligand structure was docked with flexible torsion angles, whereas the protein structure was fixed. Fourteen torsion angles of **3** were rotatable but the sugar ring was fixed in the ^4E conformation.

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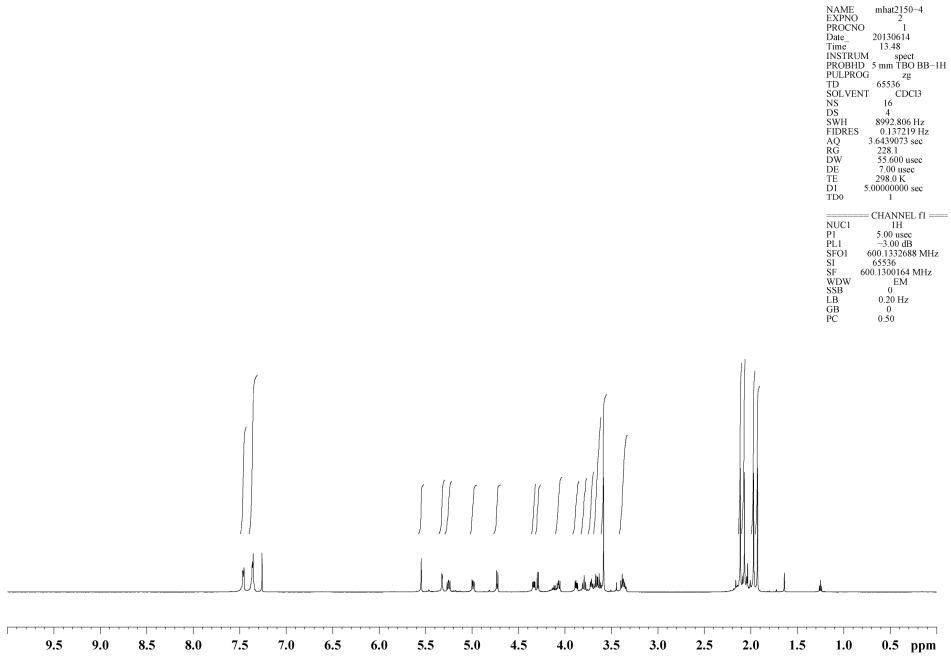
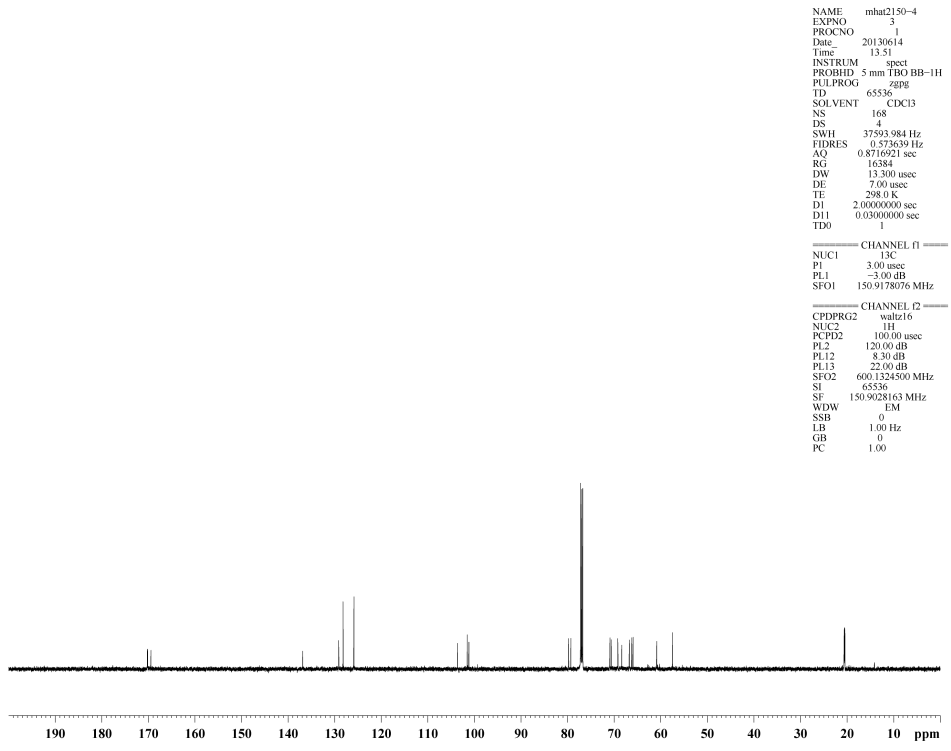
^{1}H NMR spectrum of **9**



^{13}C NMR spectrum of **9**



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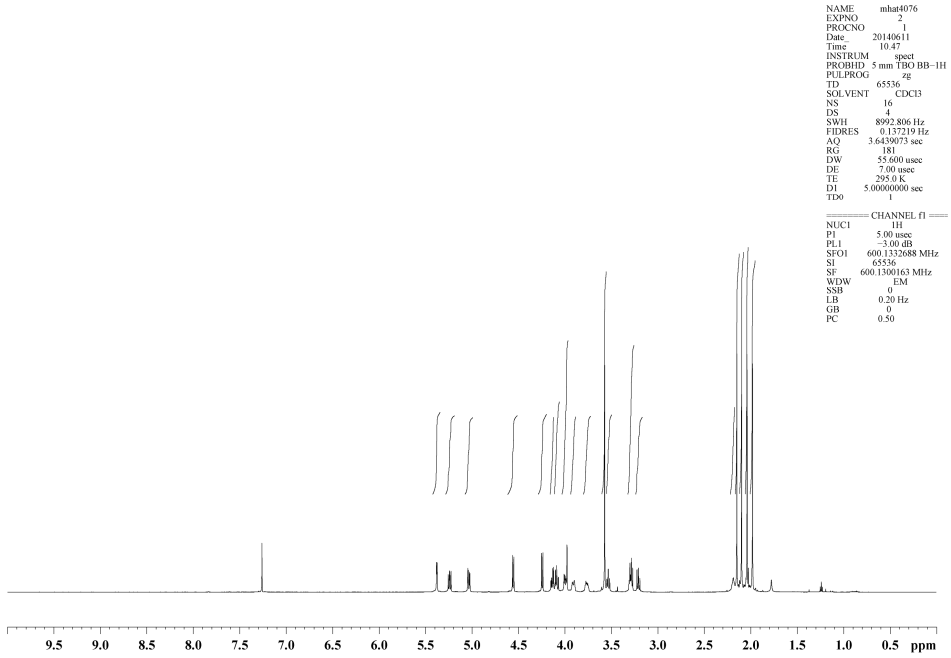
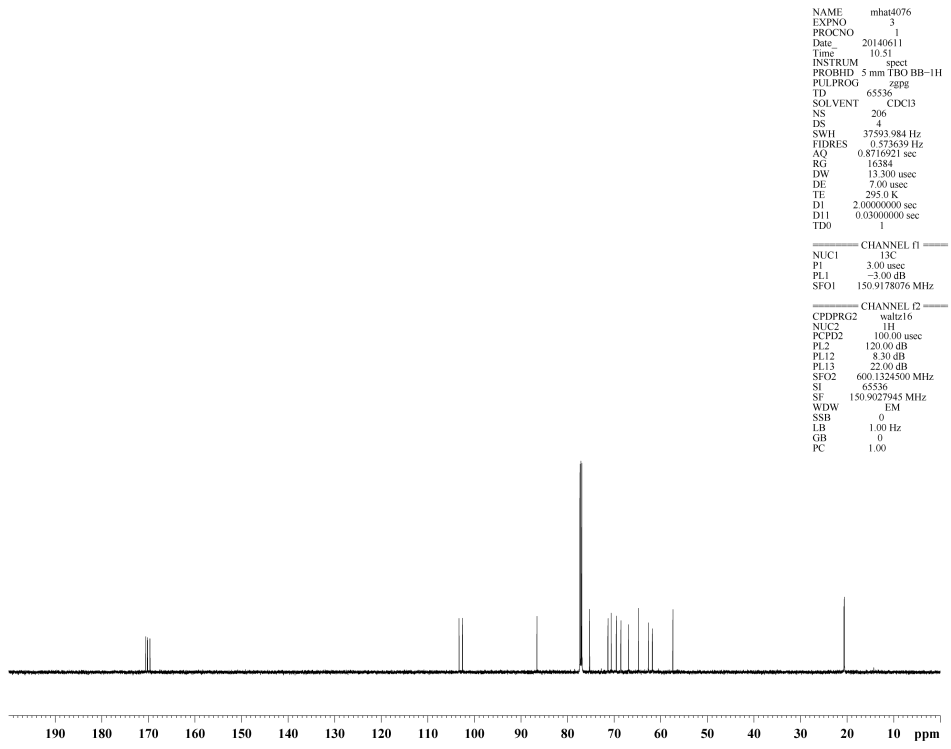
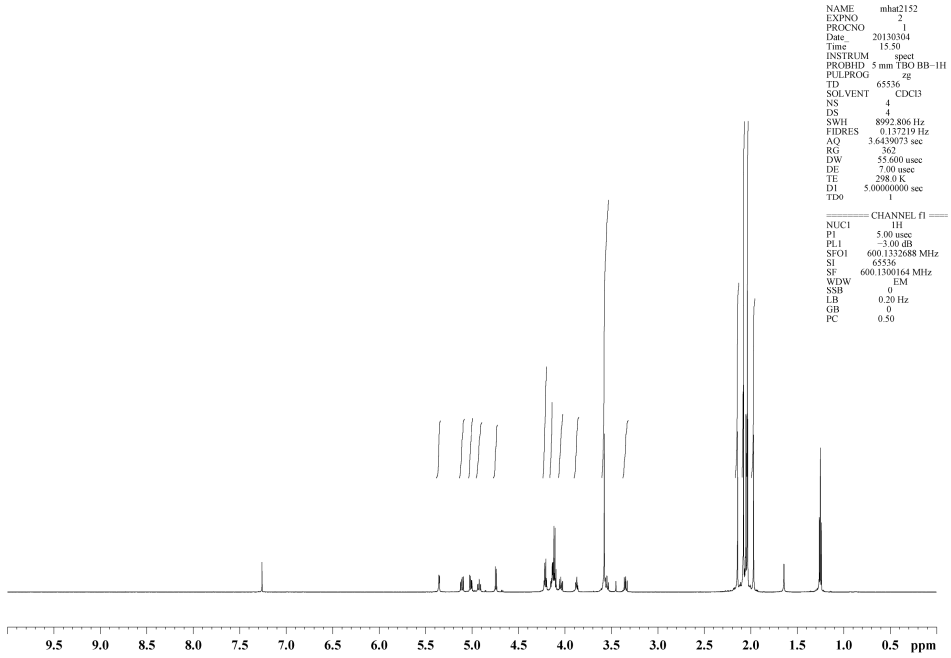


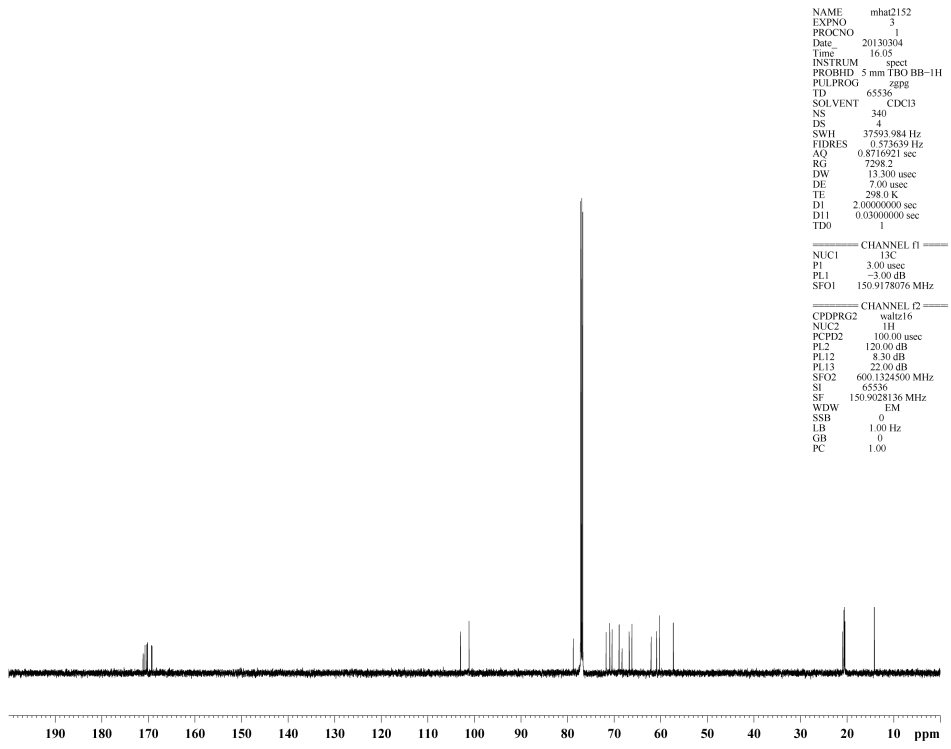
^{13}C NMR spectrum of **10**



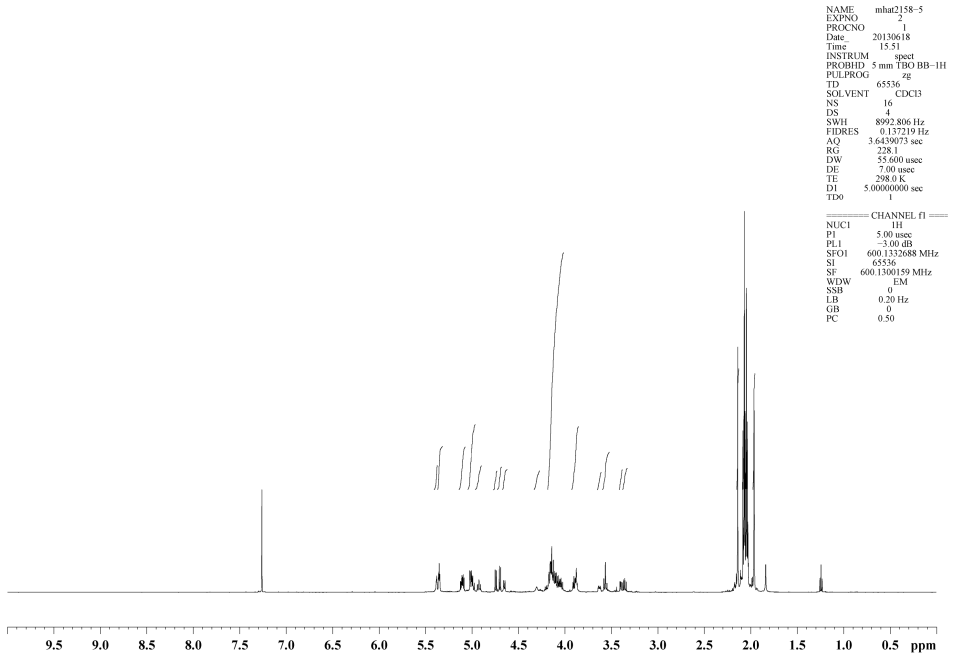
^{1}H NMR spectrum of **11**



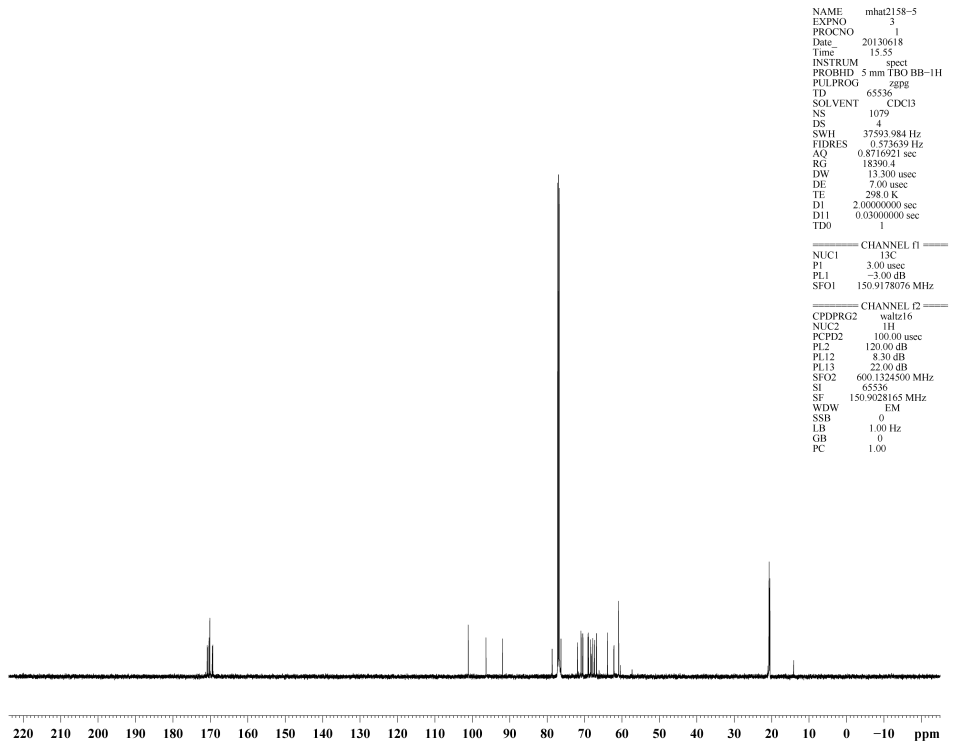
^{13}C NMR spectrum of **11**



^{1}H NMR spectrum of **12**



^{13}C NMR spectrum of **12**



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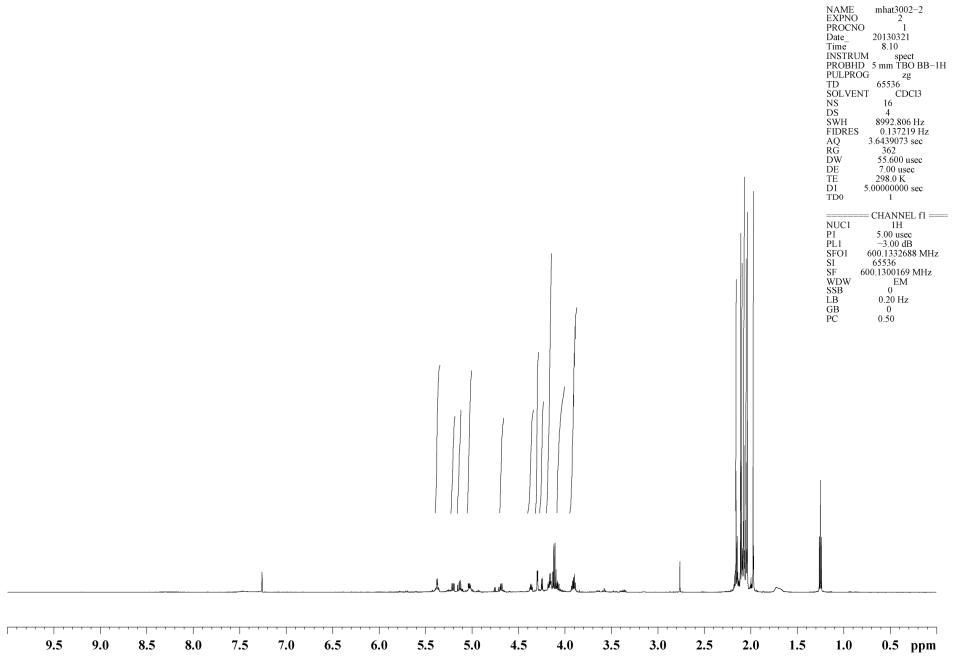

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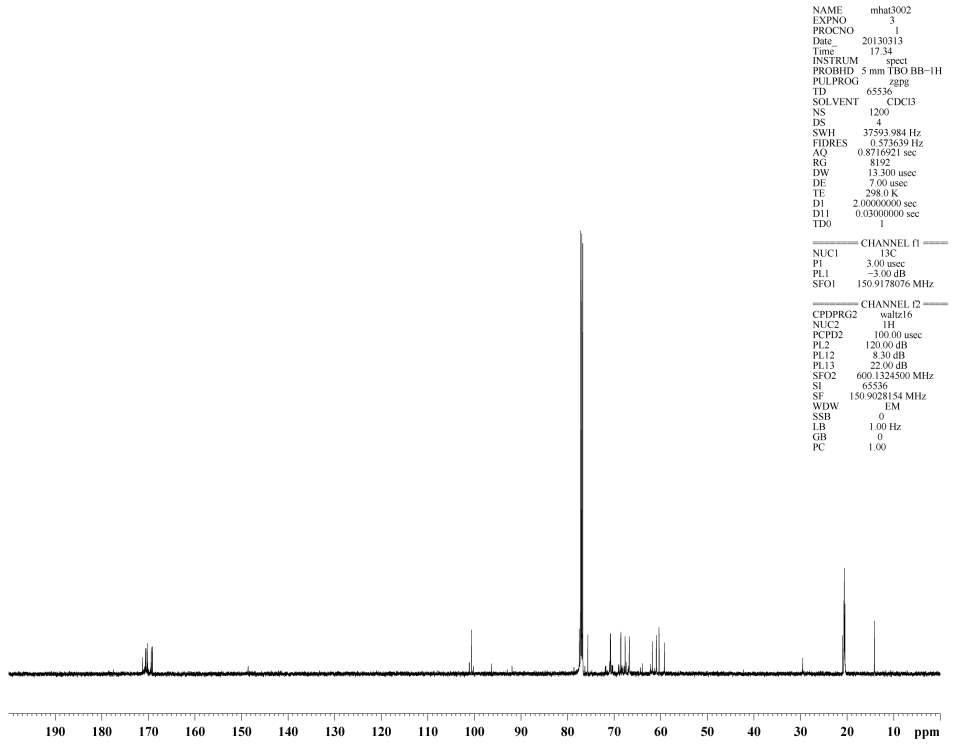

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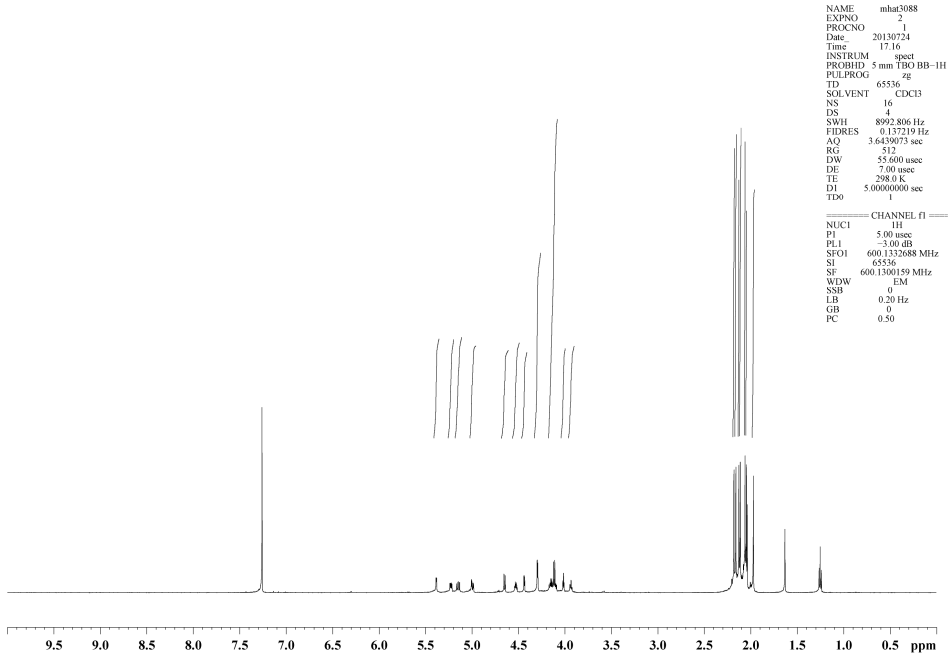
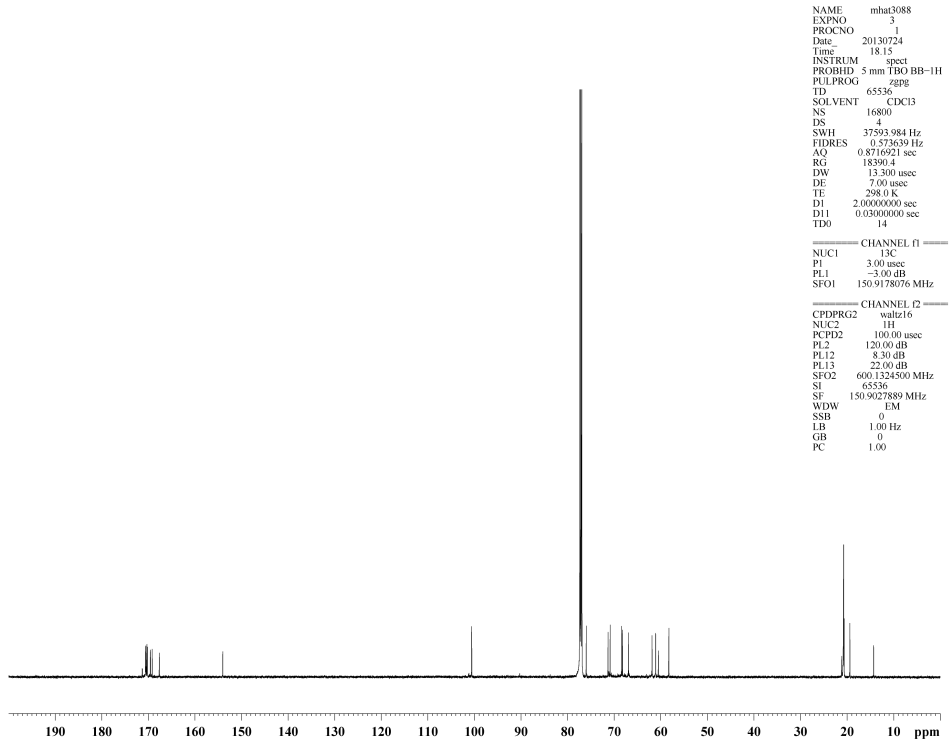


^{13}C NMR spectrum of **14**



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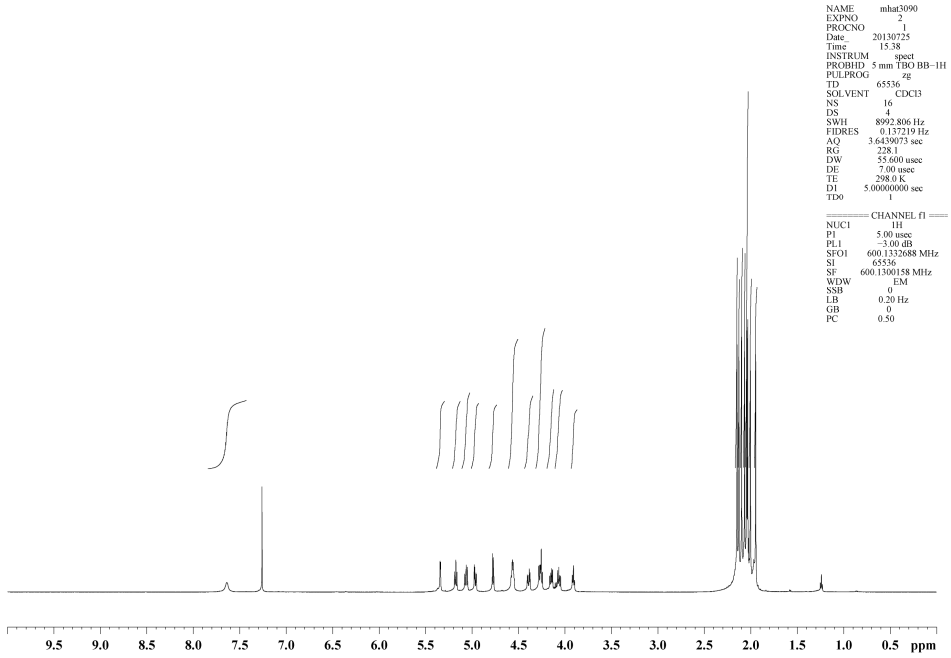

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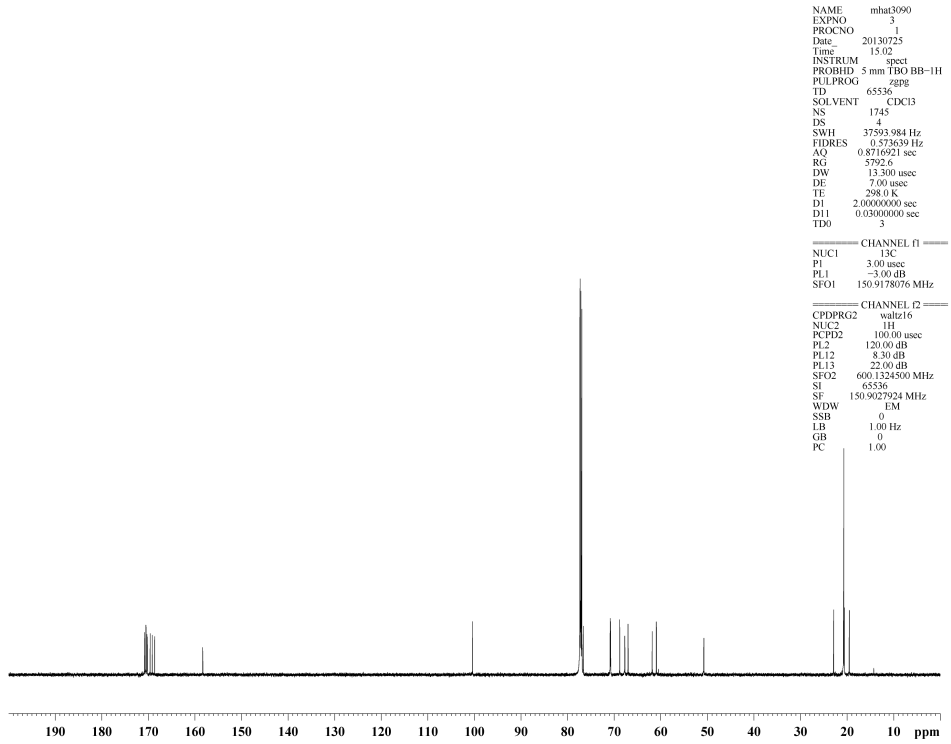
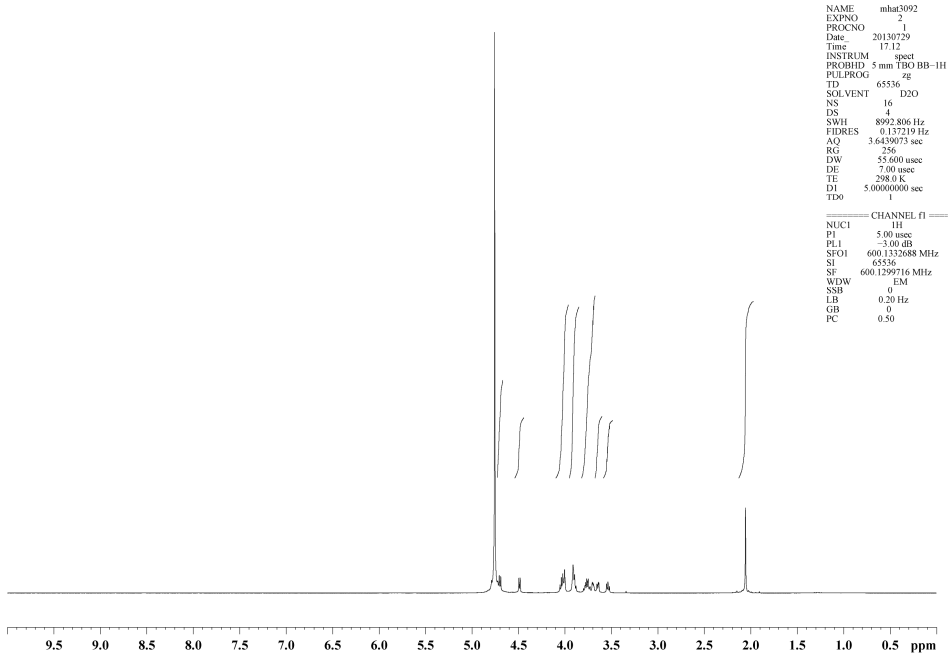
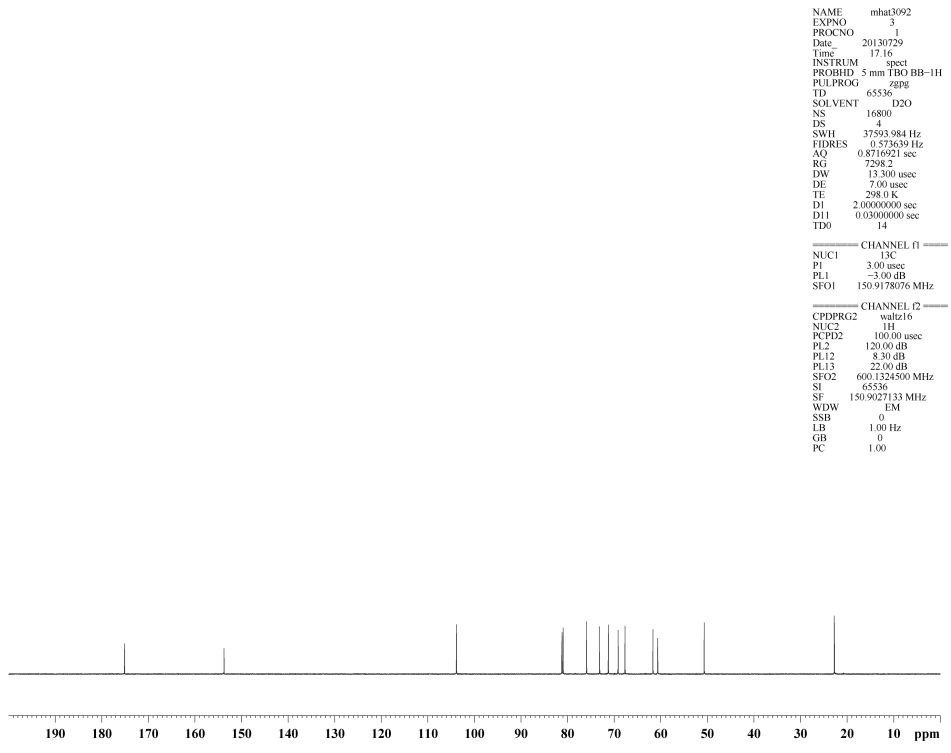


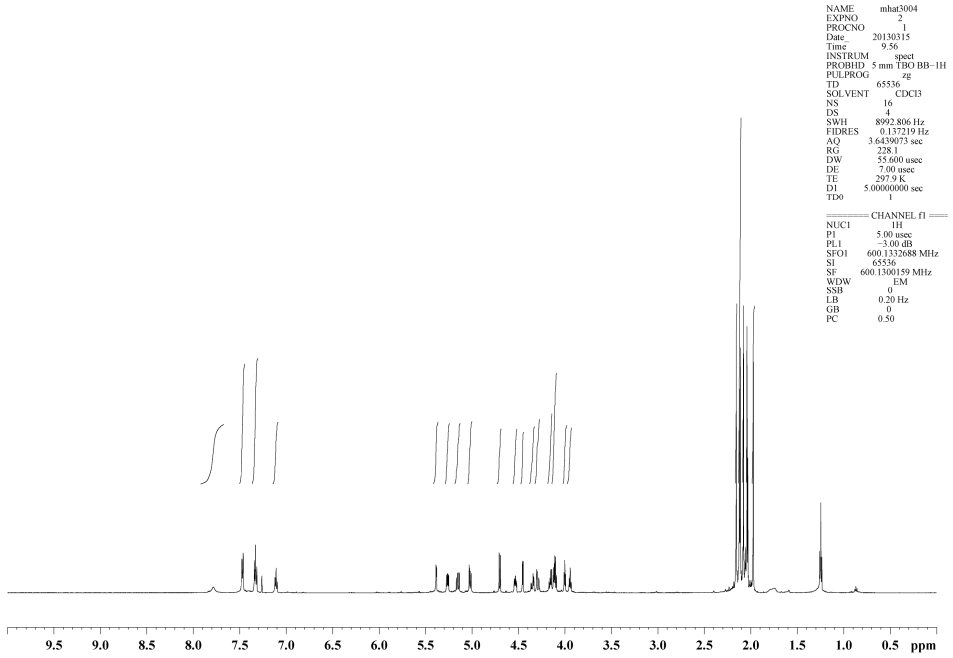
^{1}H NMR spectrum of **2**



^{13}C NMR spectrum of **2**



^{1}H NMR spectrum of **16**



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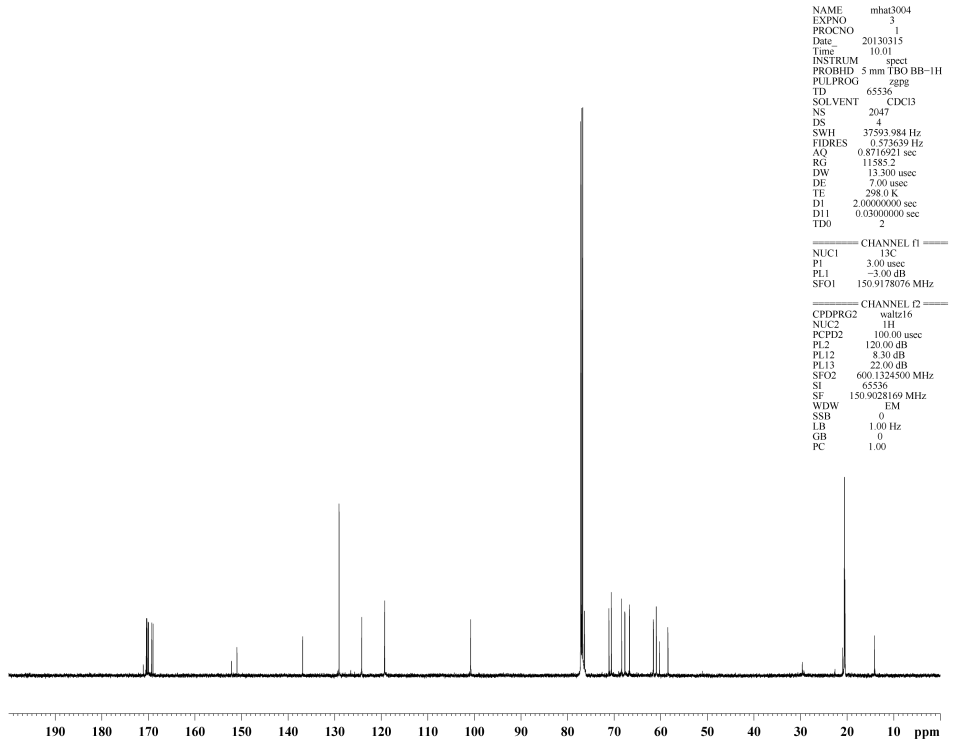
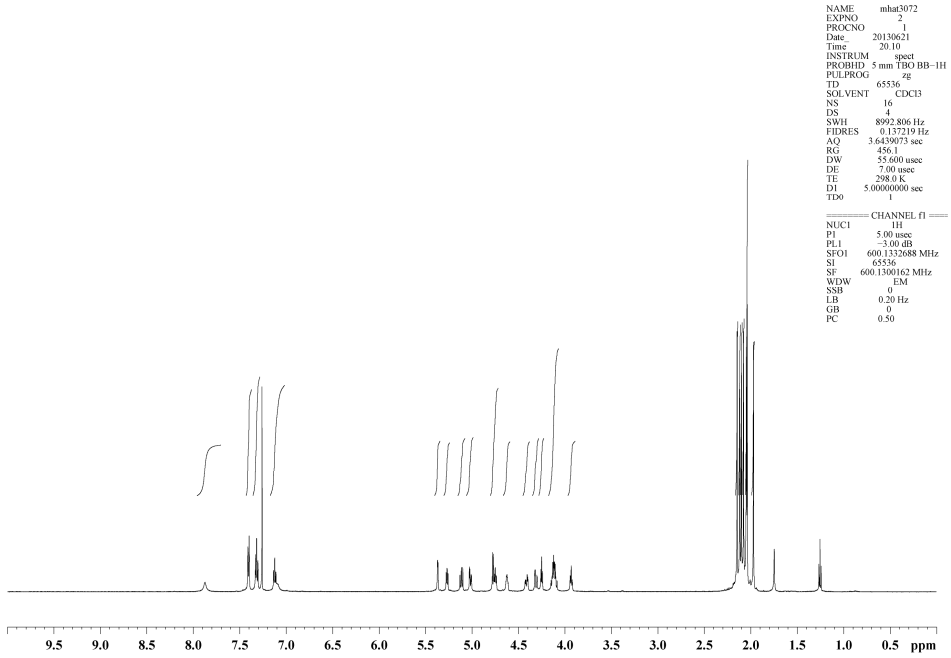


^{1}H NMR spectrum of **17**



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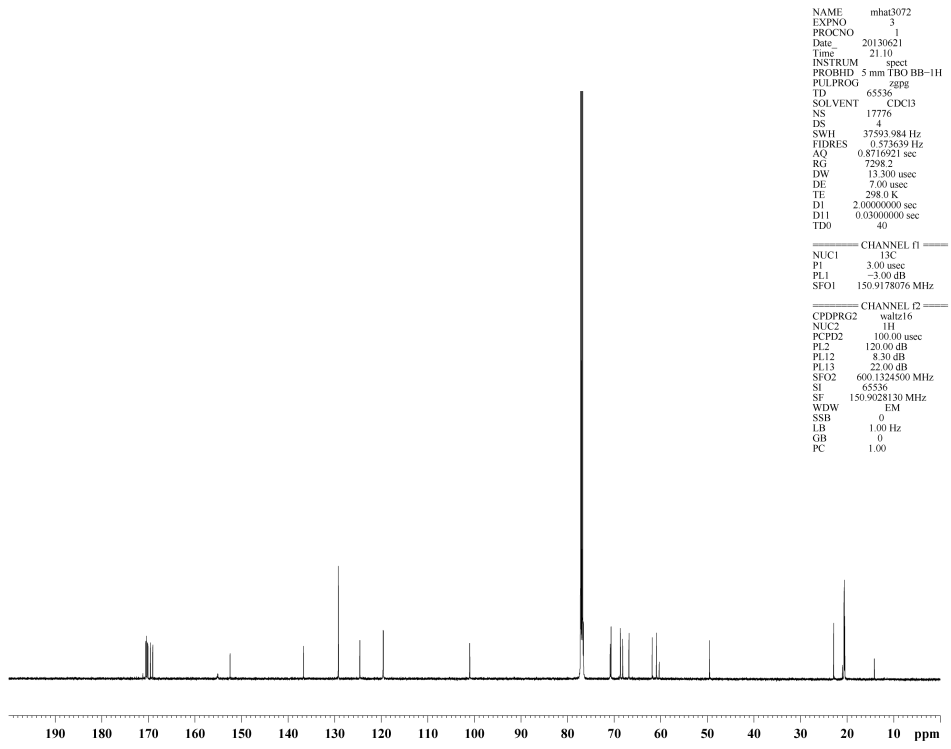
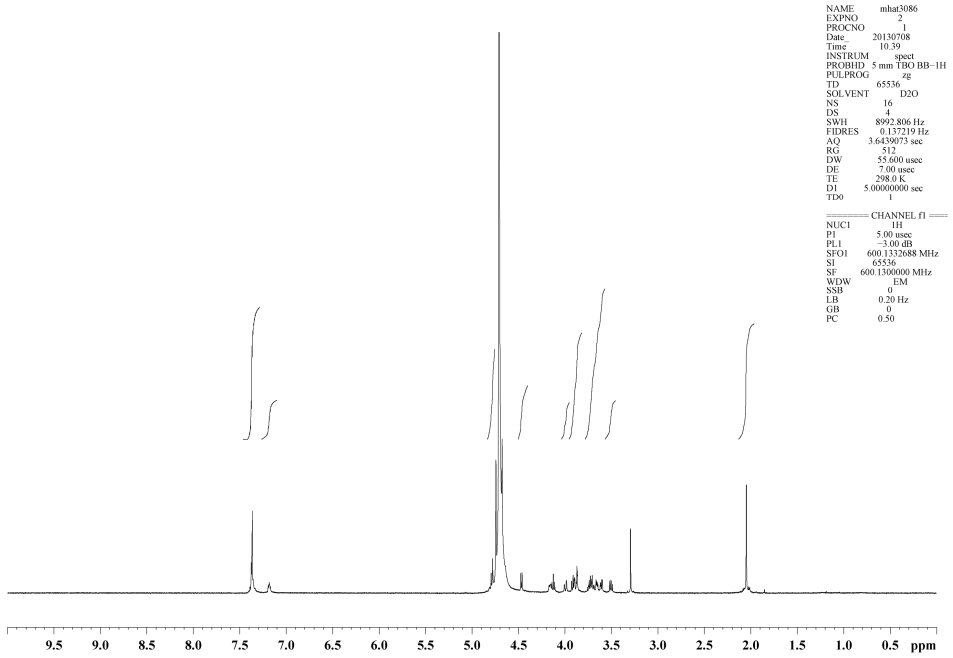


^{1}H NMR spectrum of **3**



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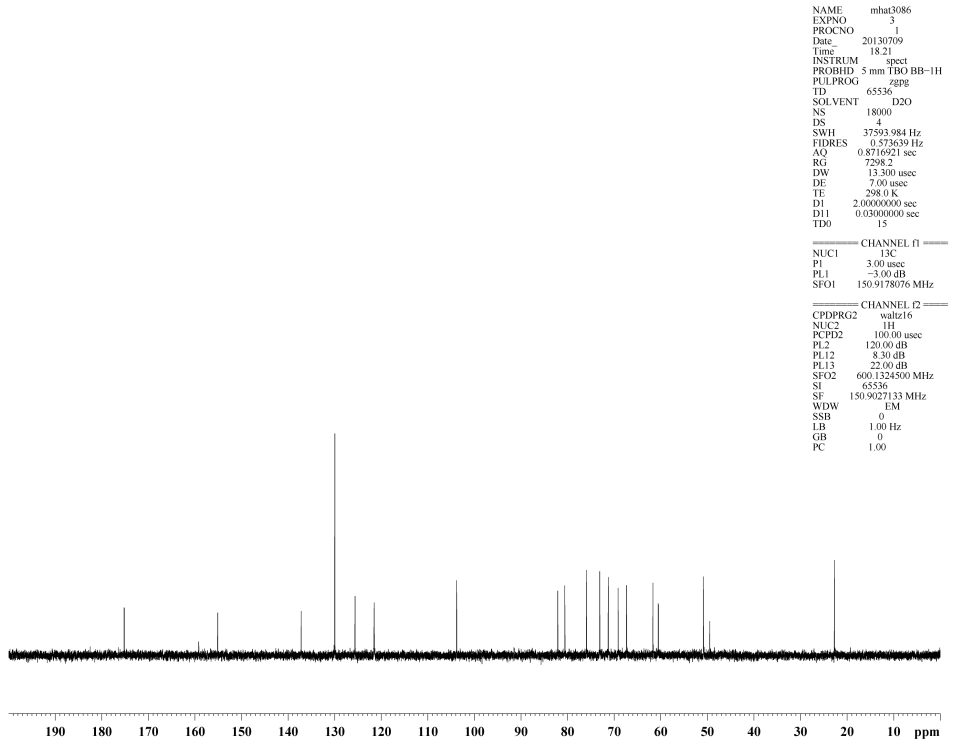
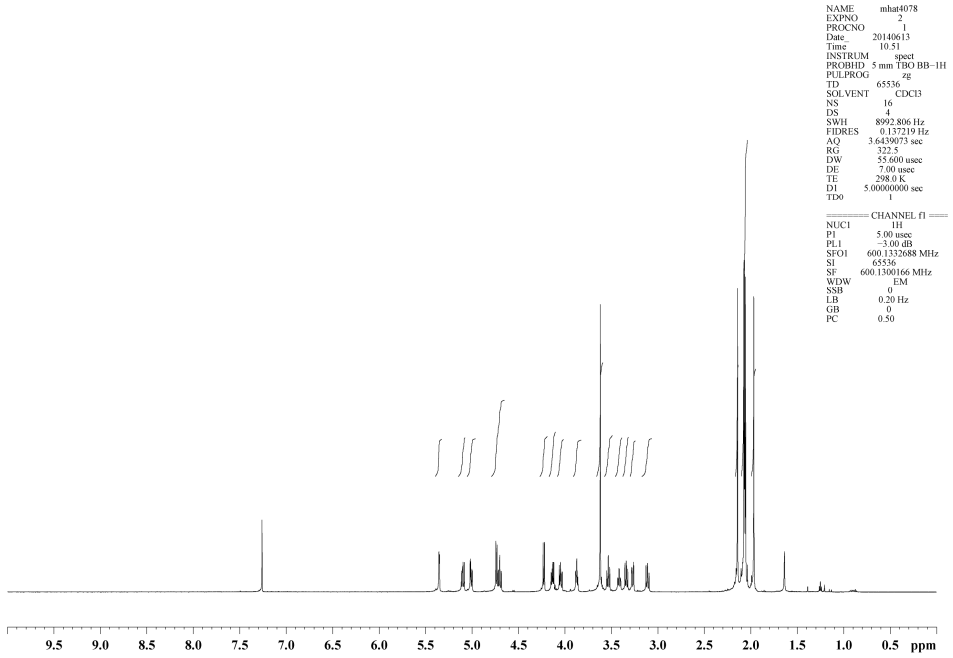
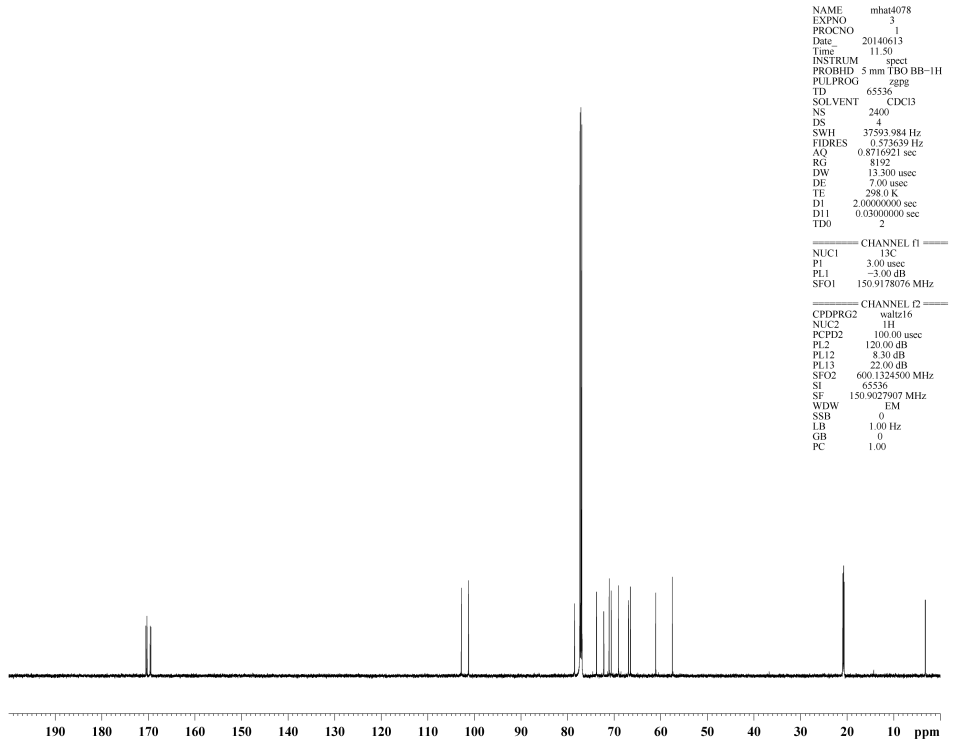


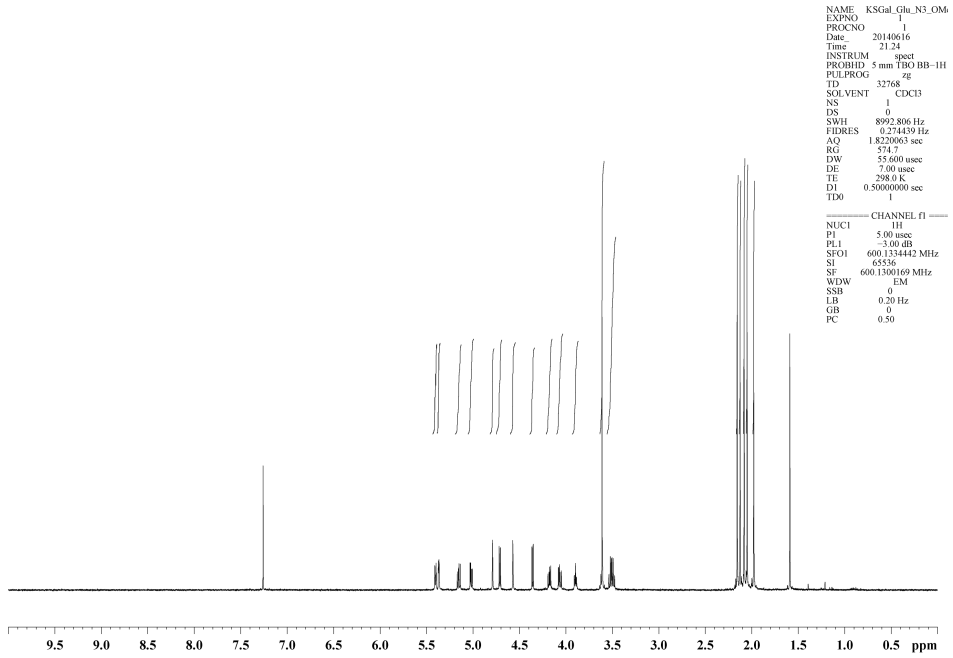
^{1}H NMR spectrum of **18**



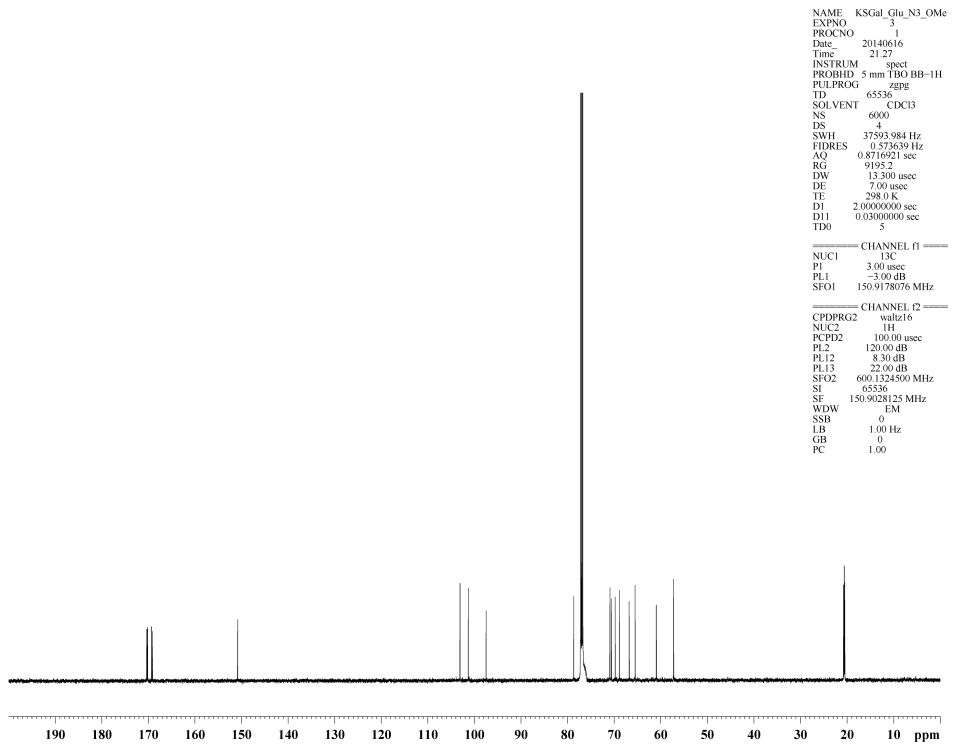
^{13}C NMR spectrum of **18**



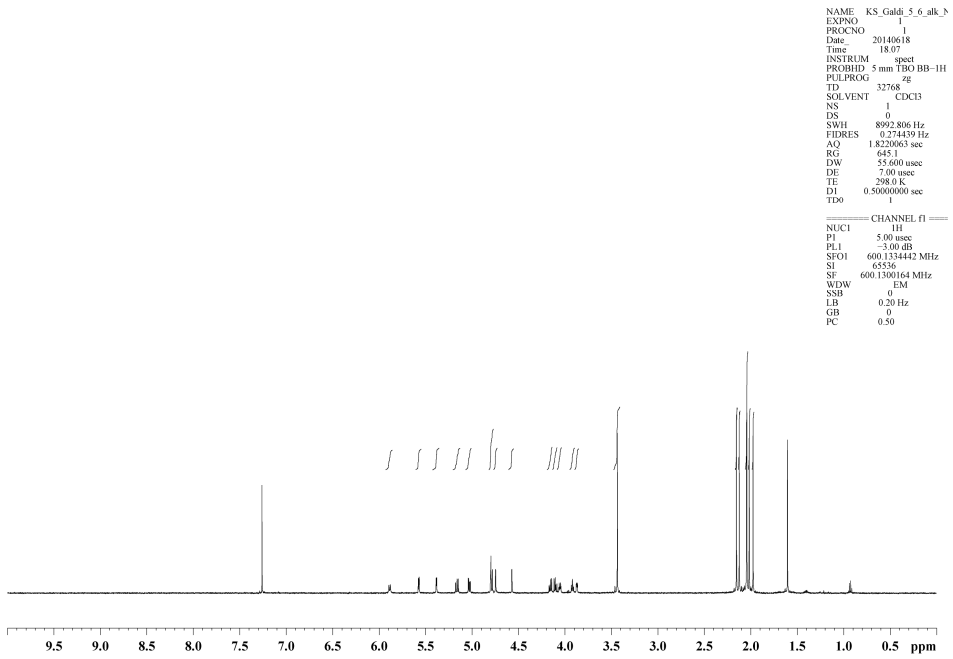
^{1}H NMR spectrum of **19**



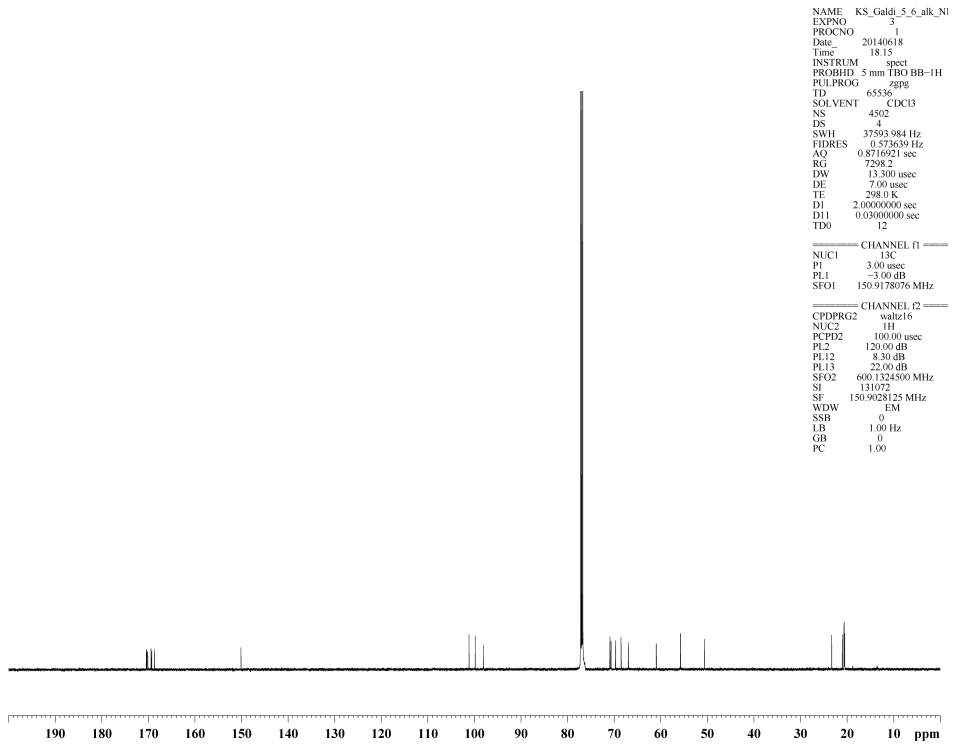
^{13}C NMR spectrum of **19**



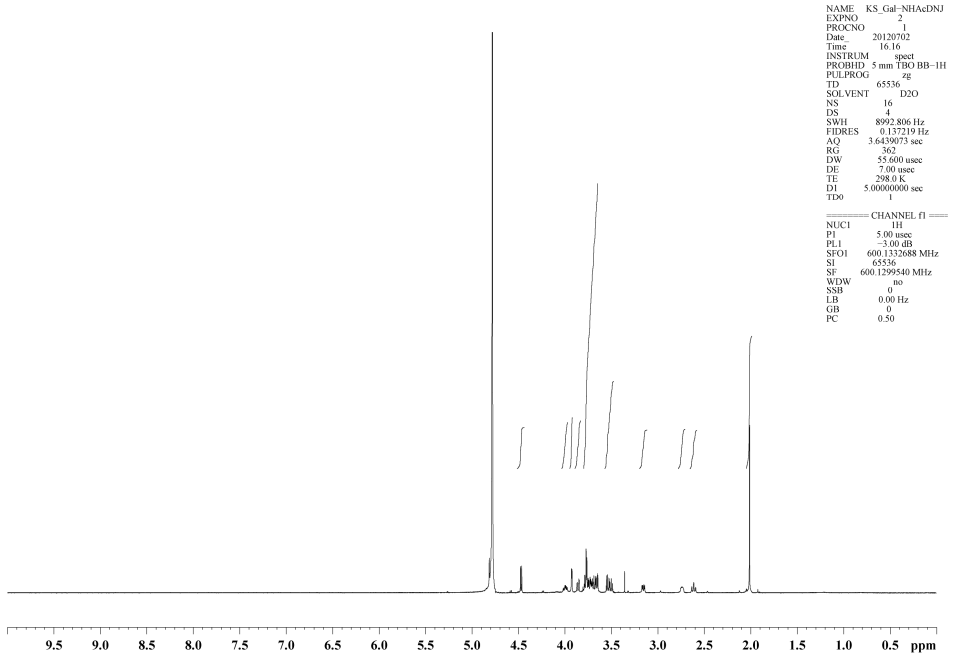
^{1}H NMR spectrum of **20**



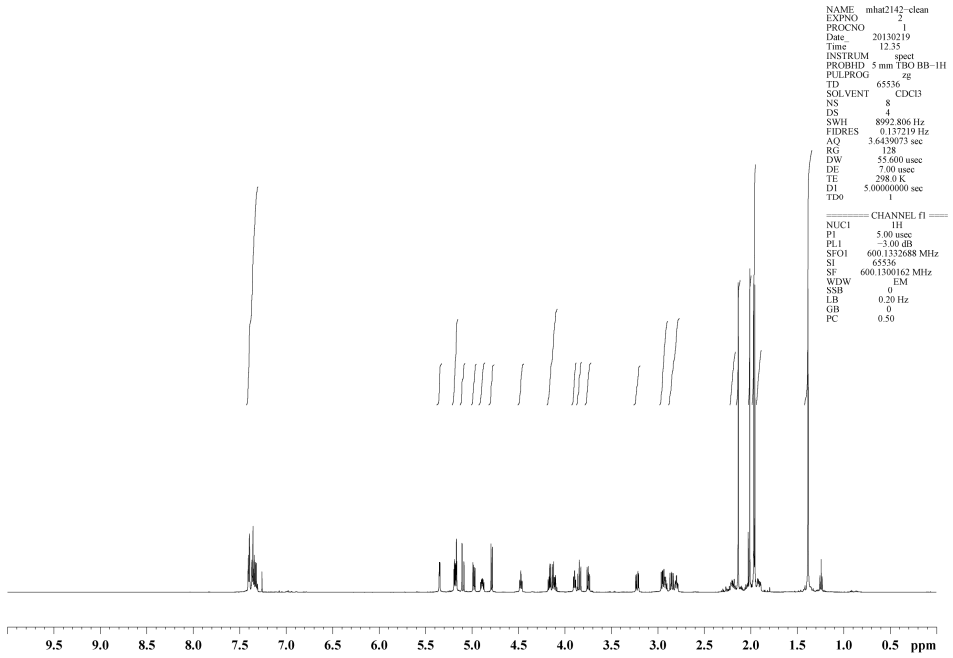
^{13}C NMR spectrum of **20**



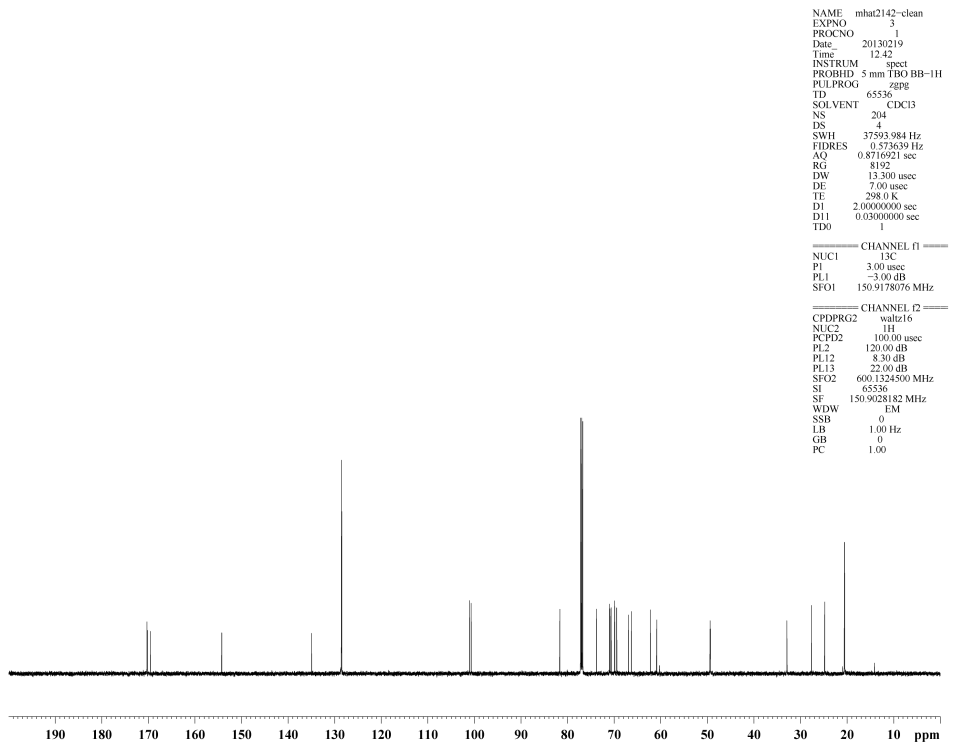
^{1}H NMR spectrum of **4**



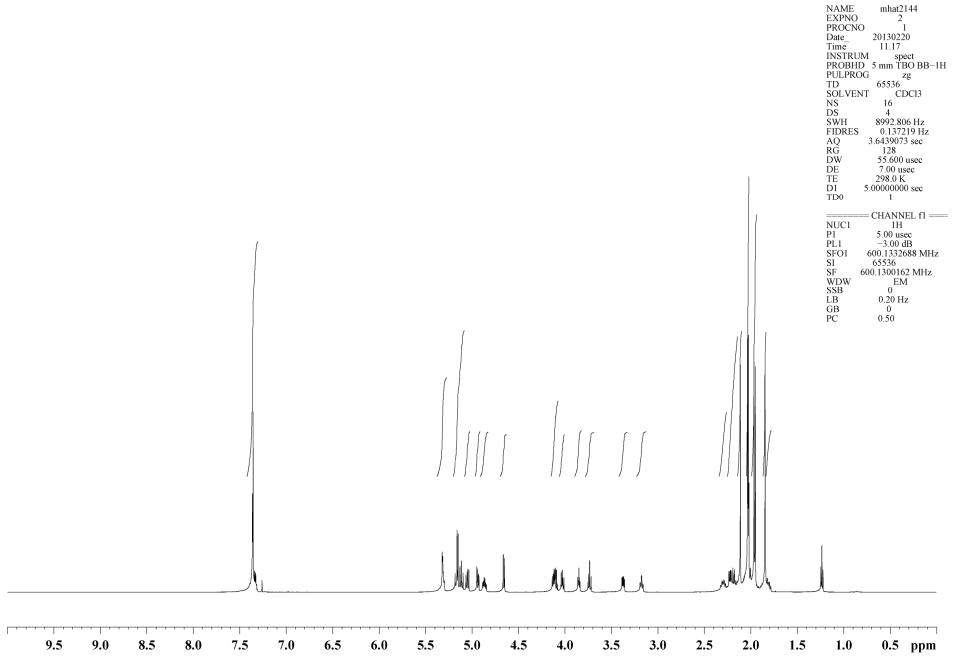
^{1}H NMR spectrum of **22**



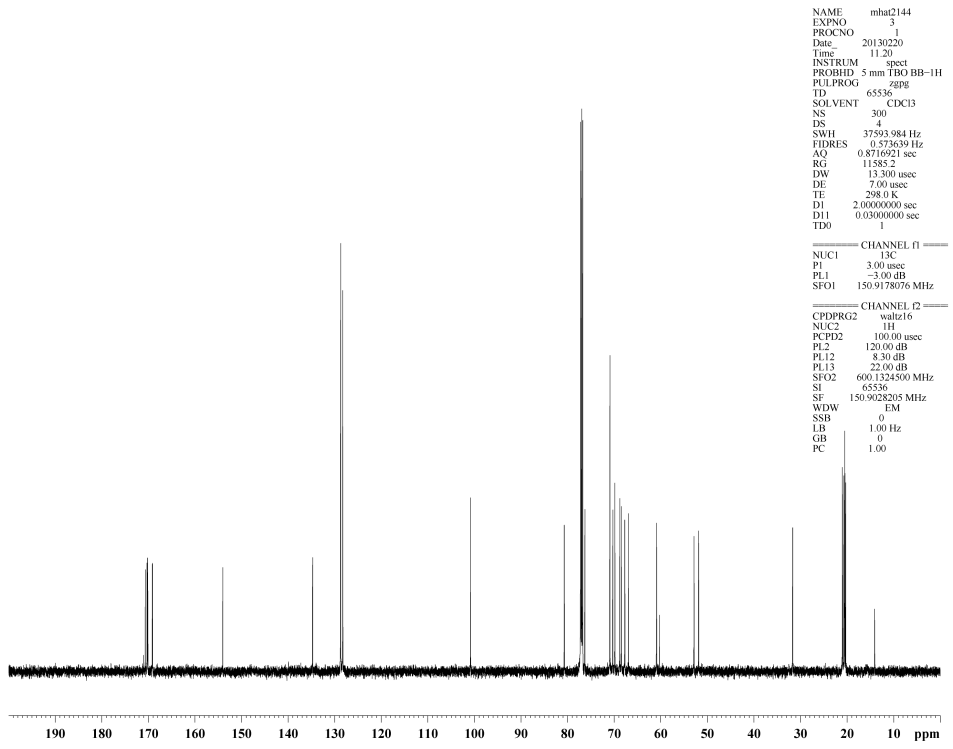
^{13}C NMR spectrum of **22**



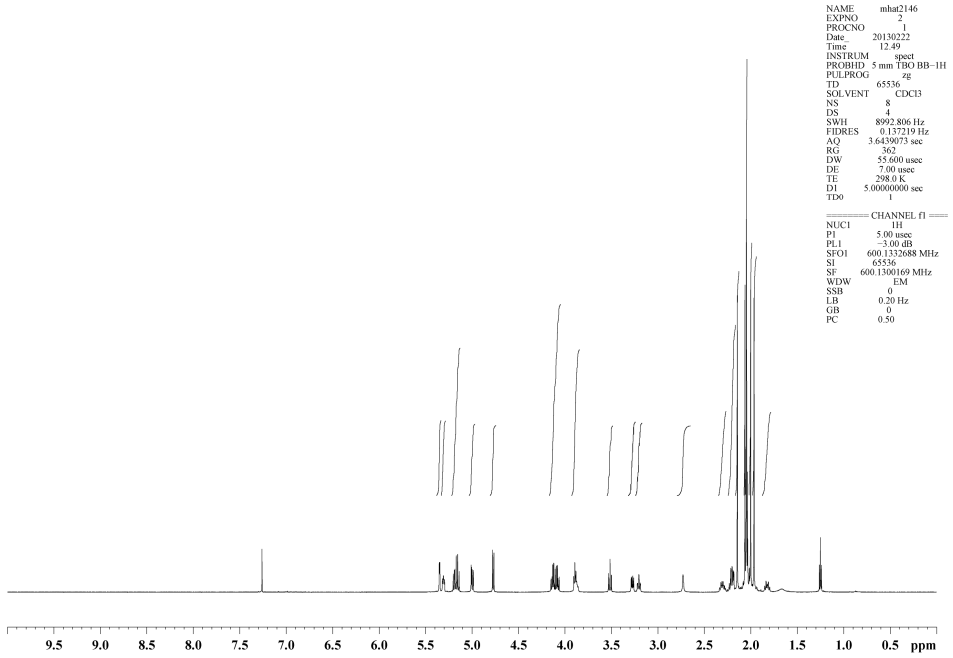
^{1}H NMR spectrum of **23**



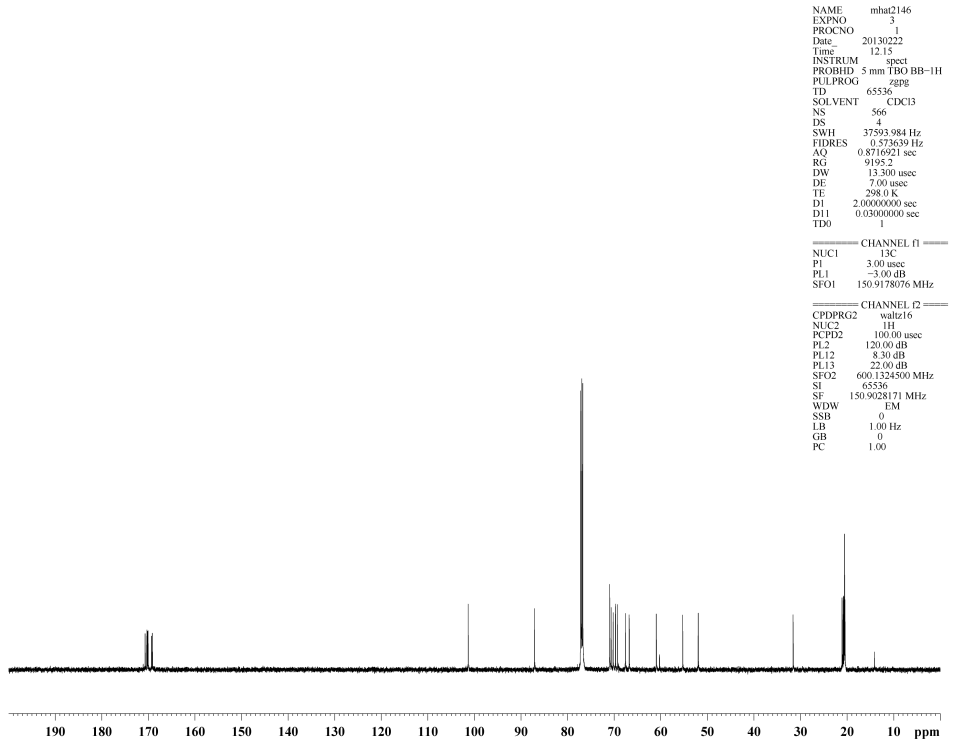
^{13}C NMR spectrum of **23**



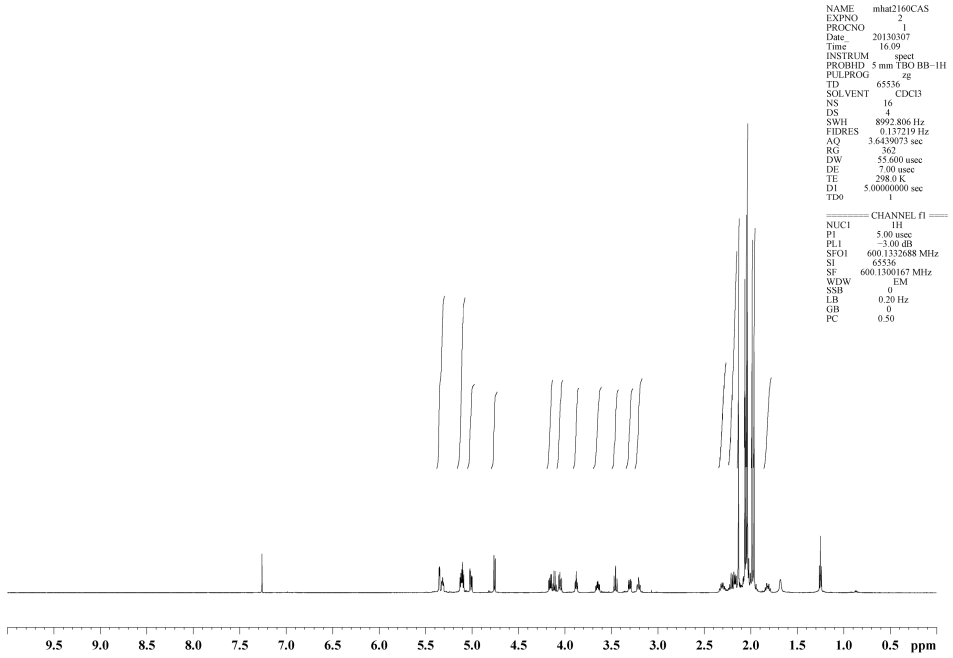
^{1}H NMR spectrum of **24**



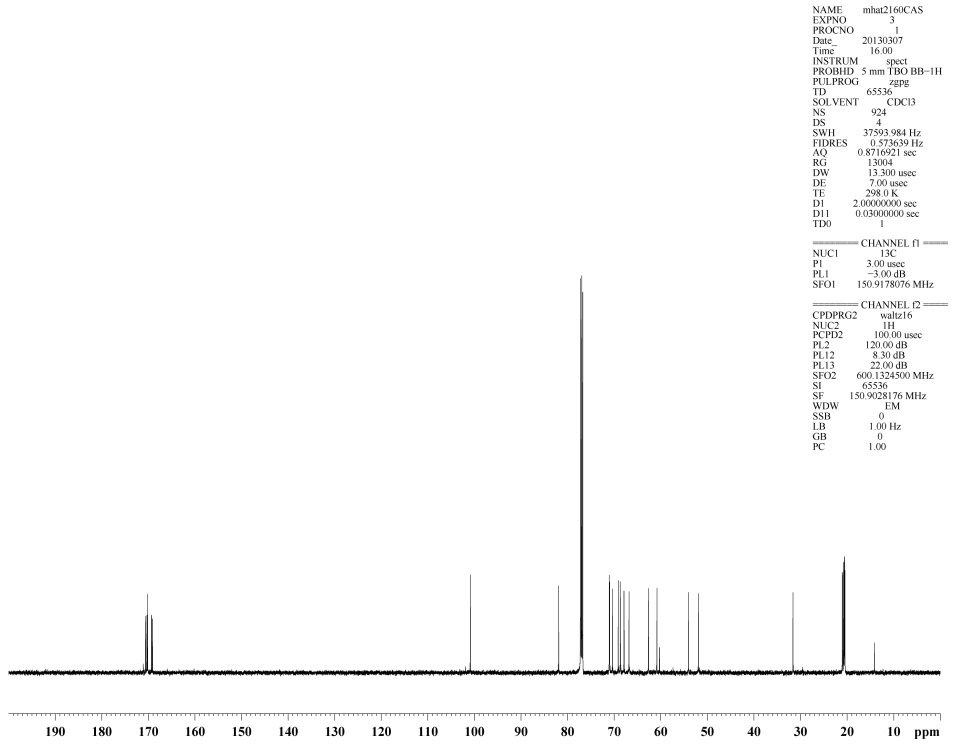
^{13}C NMR spectrum of **24**



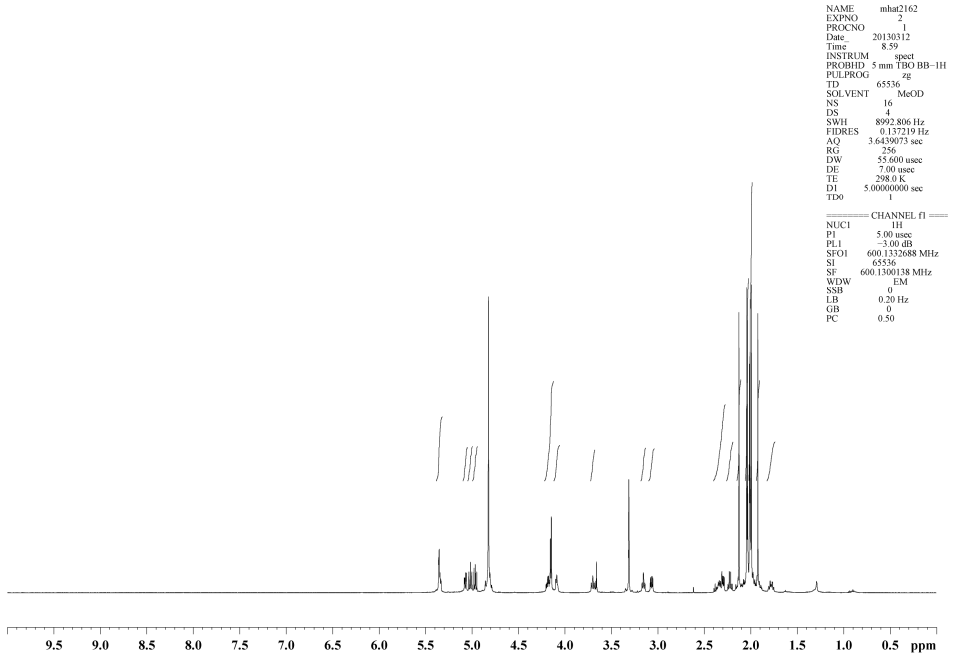
^{1}H NMR spectrum of **25**



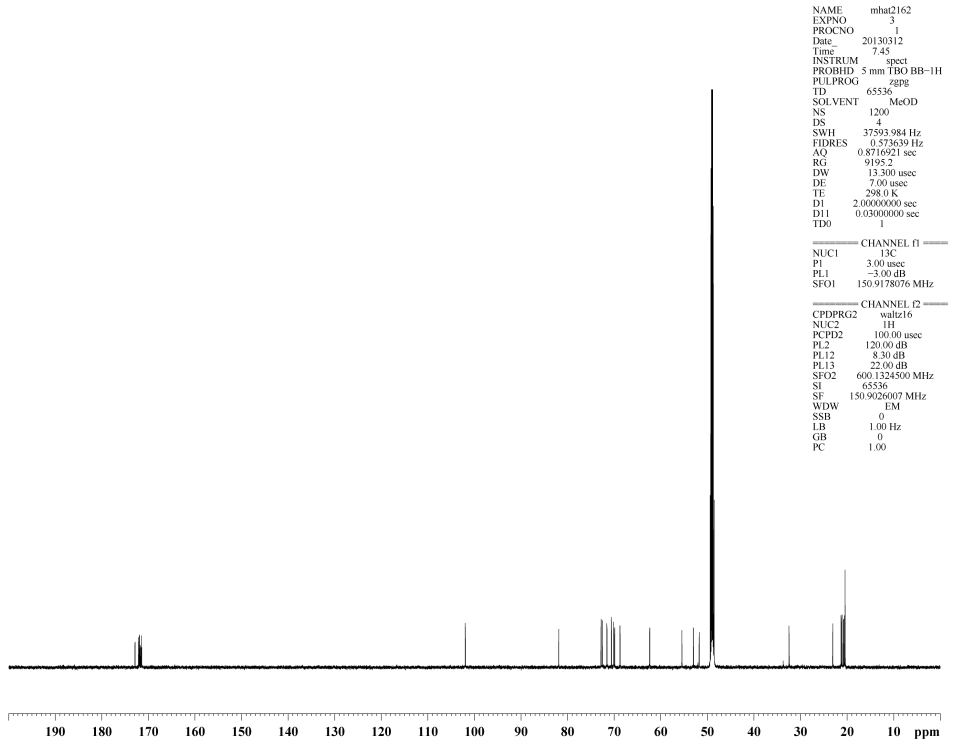
^{13}C NMR spectrum of **25**



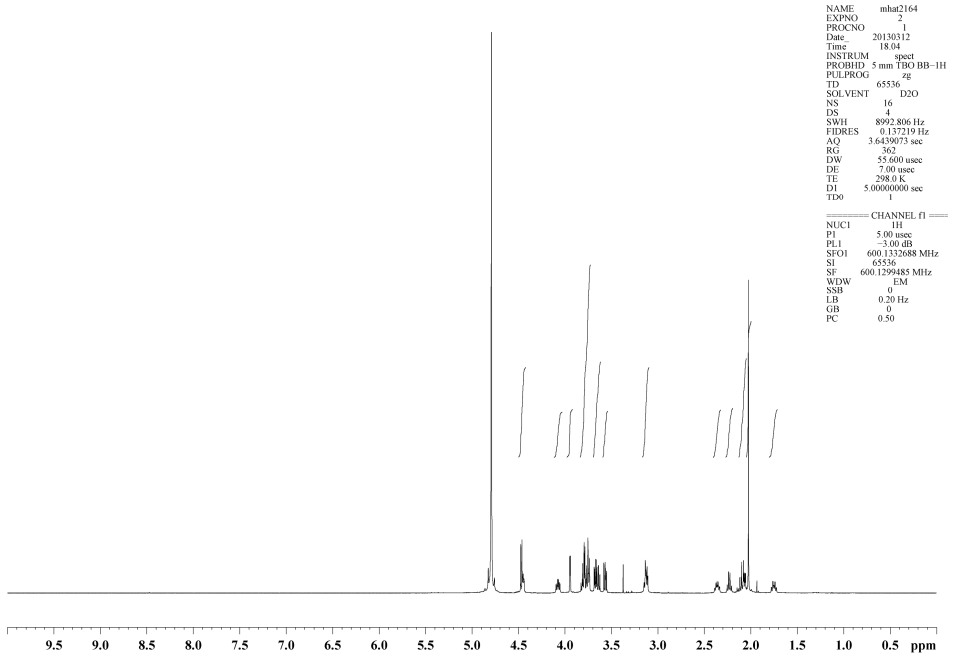
^{1}H NMR spectrum of **27**



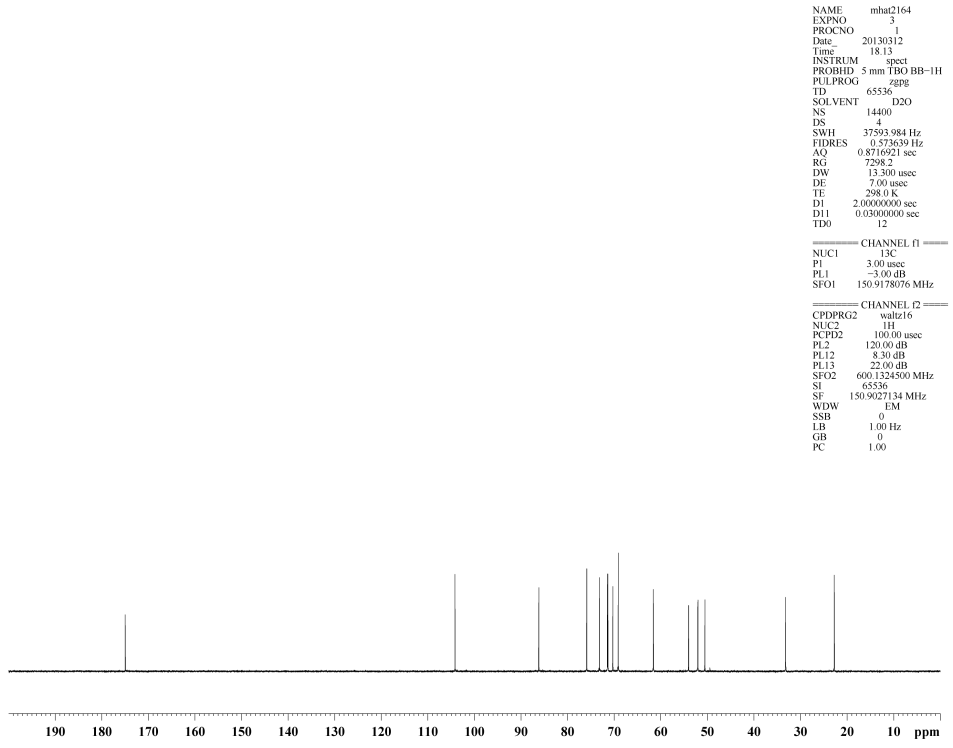
^{13}C NMR spectrum of **27**



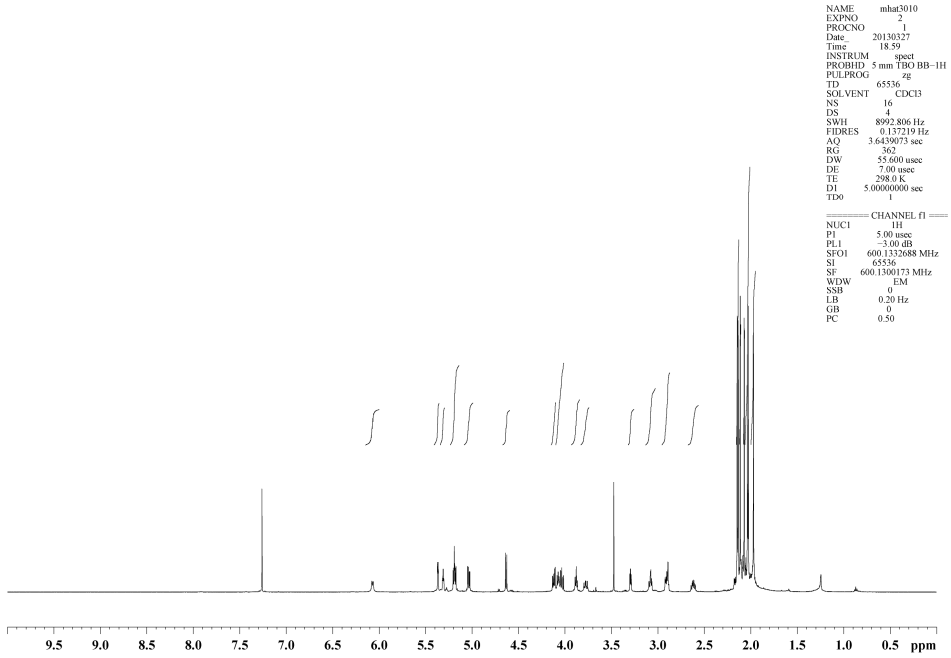
^{1}H NMR spectrum of **5**



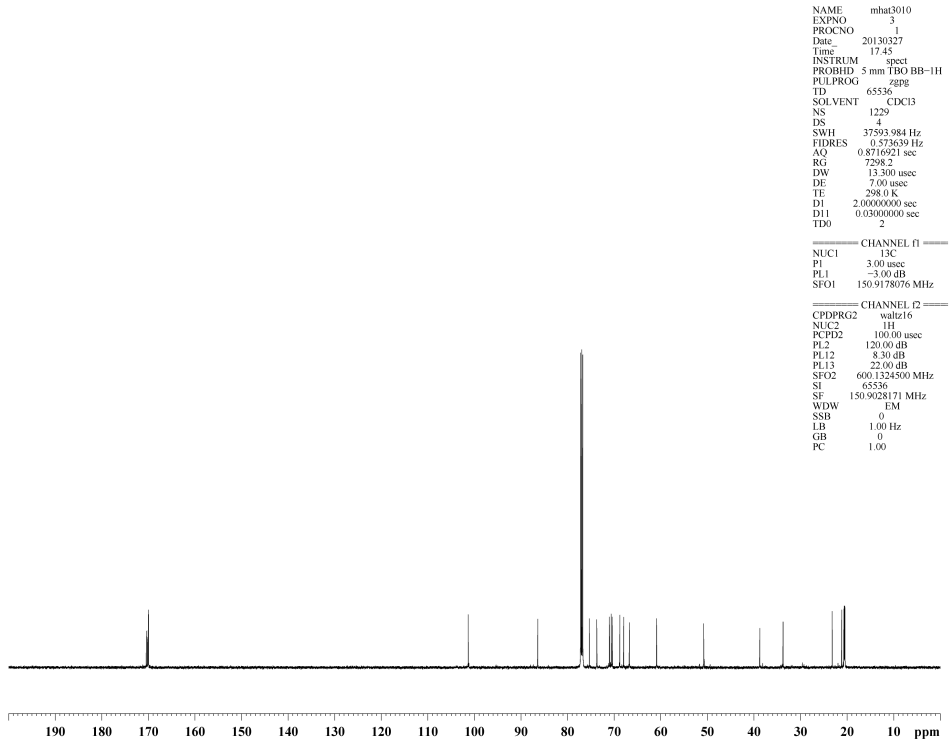
^{13}C NMR spectrum of **5**



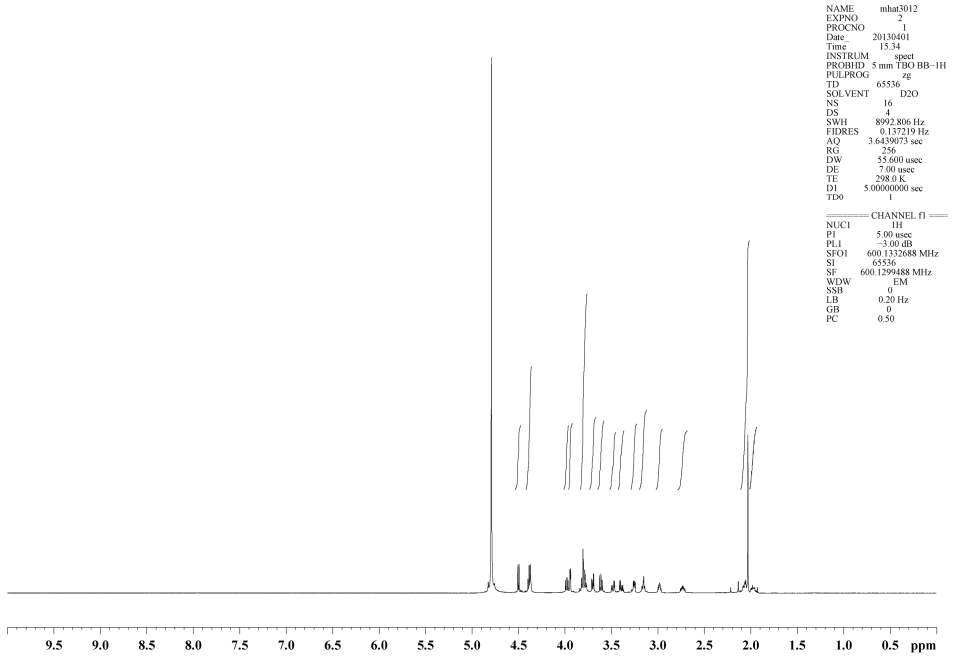
^{1}H NMR spectrum of **28**



^{13}C NMR spectrum of **28**



^{1}H NMR spectrum of **6**



^{13}C NMR spectrum of **6**