

Supporting Information

Synthesis and Biological Evaluation of D-gluconhydroximo-1, 5-lactam and Its Oxime-substituted Derivatives as Pharmacological Chaperons for the Treatment of Gaucher Disease

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Experimental section

Cytotoxicity assay in wild-type human fibroblasts.

CCC-ESF-1 cells were seeded at a density of 4000 cells per well in 96-well plates and incubated overnight. The cells were treated with a range of concentrations from 100 to 3.125 μM at 37°C in 5% CO_2 for 24h. Then 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT, 20 μL , 5 mg/mL in PBS; Sigma–Aldrich) was added to each well, and the mixtures were incubated for additional 4 h at 37°C. After removal of the medium, formazan was dissolved in DMSO (150 μL) and quantified by using a microplate reader (570 nm). Experiments were performed in triplicate. Herein, only show the results at 100 μM , the highest concentration in activation assays, and no cytotoxicity was observed (Table S1)

Compound	% Cell inhibition
27	1.67
28	4.62
29	1.52
30	4.52
31	1.96
32	2.40
33	2.56
34	3.29
35	2.85
36	1.82
37	0.53
38	2.31
NN-DNJ	1.19
IFG	0.45

Table S1. Cytotoxicity of compounds **27–38**, IFG and NN-DNJ at 100 μM in wild-type human fibroblasts.

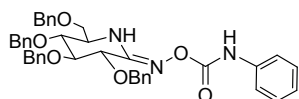
General procedures. All reagents for synthesis were obtained from commercial sources and were of reagent grade. All solvents were available commercially, dried or freshly dried and distilled prior to use. Thin-layer chromatography (TLC) was performed on silica gel GF254 plates with detection using shortwave UV light ($\lambda=254$ nm) and staining with 10% phosphomolybdic acid in EtOH or a p-anisaldehyde solution (EtOH/p-anisaldehyde/AcOH/H₂SO₄, 135:5:4:1.5), followed by heating on a hot plate. Flash chromatography was performed with silica gel (100–200 mesh) with EtOAc/petroleum ether or EtOAc (or CH₂Cl₂)/MeOH or EtOAc/acetone/NH₄OH/H₂O as eluent. ¹H and ¹³C NMR spectra were recorded on a Bruker AV 400 spectrometer at 400 MHz (¹H NMR) and 100 MHz (¹³C NMR), using CDCl₃ or CD₃OD as solvents. Coupling constants are reported in Hertz. High-resolution mass spectra (HRMS) were obtained on a Varian QFT-ESI mass spectrometer.

2,3,4,6-tetra-O-benzyl-D-glucothionolactam (14). 2,3,4,6-Tetra-O-benzyl-D-gluconolactam **13** (2.56 g, 5.7 mmol) (obtained in eight steps from Methyl α -D-glucopyranose **12** using established protocols) and Lawesson's reagent (3.47 g, 8.6 mmol) in toluene (100 mL) was stirred at room temperature for 36 h. The mixture was concentrated in vacuo and extracted with CH₂Cl₂ (150 mL) and washed with water (2 \times 50 mL), the organic phase was collected and dried with anhydrous Na₂SO₄, the solvent was removed and the crude product subjected to flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give **14** a white solid (2.91 g, 92%). ¹H NMR (400 MHz, CDCl₃): δ 7.39–7.41 (m, 2H), 7.24–7.35 (m, 16H), 7.13–7.14 (m, 2H), 5.02 (dd, J = 11.6, 2.0 Hz, 1H), 4.74 (d, J = 12.0, 2.0 Hz, 1H), 4.67 (d, J = 11.6 Hz, 1H), 4.58 (d, J = 11.6, 1.6 Hz, 1H), 4.41–4.48 (m, 4H), 4.35 (d, J = 11.6, 2.0 Hz, 1H), 3.85–3.90 (m, 2H), 3.61–3.64 (m, 1H), 3.55–3.59 (m, 1H), 3.35–3.40 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 200.43, 137.45, 128.63, 128.51, 128.46, 128.44, 128.36, 128.24, 128.14, 128.05, 127.93, 82.54, 81.37, 78.43, 73.44, 72.82, 72.65, 72.57, 68.37, 56.01. HRMS (ESI): m/z [M+ H]⁺ calcd for C₃₄H₃₅NO₅: 538.2594, found 538.2593

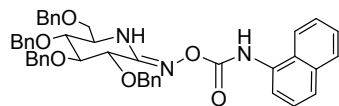
for **13**; HRMS (ESI): m/z $[M+Na]^+$ calcd for $C_{34}H_{35}NO_4S$: 576.2179, found 576.2183 for **14**.

2,3,4,6-tetra-O-benzyl-D-gluconhydroximo lactam (15). A mixture of **14** (2.91 g, 5.25 mmol), hydroxylamine hydrochloride (365 mg, 5.25 mmol) and sodium bicarbonate (365 mg, 5.25 mmol) in 50 mL methanol was refluxed at 75°C for 6 h. TLC analysis indicated the starting material was consumed completely, the mixture was concentrated in vacuo and extracted with 150 mL CH_2Cl_2 , washed with 50 mL H_2O and 50 mL saturated brines respectively, dried over Na_2SO_4 and concentrated in vacuo using rotary evaporator to get the crude product. The crude mixture was purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to afford the product as a colorless oil (2.41 g, 83%). 1H NMR (400 MHz, $CDCl_3$) δ 7.38-7.28 (m, 18H), 7.16-7.14 (m, 2H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.64-4.44 (m, 6H), 4.41 (d, $J = 11.6$ Hz, 1H), 4.33 (d, $J = 11.6$ Hz, 1H), 4.23 (s, 1H), 3.92 (m, 1H), 3.78-3.75 (m, 1H), 3.70 (dd, $J = 9.2, 2.4$ Hz, 1H), 3.53 (dd, $J = 9.6, 3.7$ Hz, 1H), 3.48 (dd, $J = 9.2, 7.2$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.76, 137.79, 137.68, 137.55, 137.44, 128.62, 128.58, 128.50, 128.46, 128.22, 128.11, 128.00, 127.96, 127.92, 81.55, 80.26, 73.47, 73.28, 72.47, 72.01, 71.26, 69.09, 51.75. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{34}H_{36}N_2O_5$: 553.2702, found 553.2697.

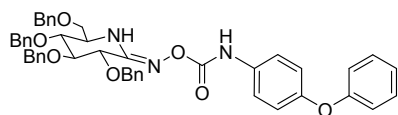
General procedure for the preparation of 16-26. To a stirred solution of **15** (100 mg, 0.18 mmol) and trimethylamine (47 μ L, 0.36 mmol) in 5 mL of dry THF at 0°C was added a solution of various of substituted isocyanate (0.22 mmol) in 1 mL of dry THF. The reaction was allowed to warm to room temperature and was stirred for 8 h. The mixture was concentrated in vacuo and extracted with 50 mL, subsequently washed with 20 mL 1M HCl and 20 mL saturated sodium bicarbonate, dried over anhydrous Na_2SO_4 . The solution was concentrated under vacuum and purified by flash column chromatography (petroleum ether/ethyl acetate = 10:1 or 15:1) to afford the desired oxime substituted of 2,3,4,6-tetra-O-benzyl-D-gluconhydroximo lactam.



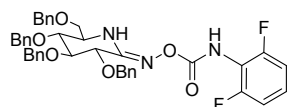
O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-phenylcarbamate (**16**) A white solid (91.2 mg, 75%). 1H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, $J = 8.4$ Hz, 1H), 7.37-7.27 (m, 20H), 7.11-7.14 (m, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.61-4.45 (m, 6H), 4.34 (d, $J = 11.6$ Hz, 1H), 4.08 (s, 1H), 3.96 (s, 1H), 3.77 (m, 1H), 3.66 (dd, $J = 9.6, 2.4$ Hz, 1H), 3.58 (dd, $J = 9.6, 3.6$ Hz, 1H), 3.49 (dd, $J = 9.6, 6.4$ Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.55, 151.43, 137.57, 137.44, 137.40, 137.31, 137.16, 129.11, 128.63, 128.60, 128.45, 128.15, 128.13, 128.08, 127.95, 124.02, 119.60, 81.56, 79.94, 73.50, 73.31, 72.56, 72.03, 71.07, 68.41, 51.93. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{32}N_2O_5$: 672.3074, found: 672.3075.



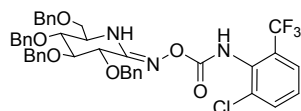
O-(2,3,4,6-tetra-O-benzyl-D-glucopyranosylidene) amino-Z-N-Naphthylcarbamate (**17**) A white solid (97.4 mg, 75%). 1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, $J = 7.6$ Hz, 1H), 7.93-7.83 (m, 2H), 7.68 (d, $J = 8.4$ Hz, 1H), 7.55-7.43 (m, 3H), 7.43-7.21 (m, 18H), 7.18-7.06 (m, 2H), 4.82 (d, $J = 12.0$ Hz, 1H), 4.67-4.43 (m, 6H), 4.38-4.28 (m, 1H), 4.13 (s, 1H), 4.03-3.91 (m, 1H), 3.86-3.73 (m, 1H), 3.69 (dd, $J = 9.8, 2.8$ Hz, 1H), 3.61 (dd, $J = 9.8, 3.6$ Hz, 1H), 3.51 (dd, $J = 9.8, 6.4$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.31, 151.62, 137.59, 137.45, 137.30, 137.10, 134.16, 132.27, 130.96, 128.88, 128.82, 128.62, 128.57, 128.44, 128.26, 128.14, 128.08, 127.94, 126.91, 126.28, 126.02, 125.16, 120.56, 119.23, 81.67, 79.99, 73.50, 73.33, 72.58, 72.13, 71.10, 68.43, 65.61, 51.99. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{45}H_{43}N_3O_6$: 722.3230, found: 722.3229.



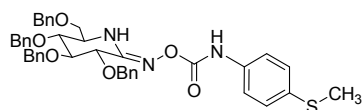
O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-4-phenoxyphenylcarbamate (**18**) A white solid (115.5 mg, 84%). ^1H NMR (400 MHz, CDCl_3) δ 8.43 (s, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.40-7.24 (m, 20H), 7.13 (d, J = 4.4 Hz, 2H), 7.08 (d, J = 7.6 Hz, 1H), 7.00 (t, J = 8.1 Hz, 4H), 5.88 (s, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.64-4.43 (m, 6H), 4.34 (d, J = 11.8 Hz, 1H), 4.08 (s, 1H), 3.96 (s, 1H), 3.77 (s, 1H), 3.67 (d, J = 9.6 Hz, 1H), 3.62-3.55 (m, 1H), 3.49 (dd, J = 9.2, 6.8 Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 157.73, 153.30, 152.83, 151.48, 137.56, 137.43, 137.31, 137.16, 132.97, 129.78, 128.60, 128.45, 128.12, 128.05, 127.96, 123.04, 121.45, 119.89, 118.37, 81.55, 79.93, 73.55, 73.31, 72.56, 72.03, 71.08, 68.39, 51.93. HRMS (ESI): m/z [M+H]⁺ calcd for $\text{C}_{47}\text{H}_{45}\text{N}_3\text{O}_7$: 764.3336, found: 764.3315.



O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-2,4-difluorophenylcarbamate (**19**) A white solid (87.8 mg, 69%). ^1H NMR (400 MHz, CDCl_3) δ 7.96 (s, 1H), 7.42-7.06 (m, 21H), 6.96 (t, J = 7.6 Hz, 2H), 5.85 (s, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.50 (m, 6H), 4.33 (d, J = 11.2 Hz, 1H), 4.06 (s, 1H), 3.94 (s, 1H), 3.77 (s, 1H), 3.66 (d, J = 8.8 Hz, 1H), 3.58 (d, J = 7.2 Hz, 1H), 3.53-3.42 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 159.33, 159.29, 156.83, 156.79, 153.00, 151.66, 137.60, 137.45, 137.30, 137.12, 128.65, 128.58, 128.45, 128.21, 128.09, 127.95, 127.65, 114.07, 111.99, 111.77, 81.53, 80.01, 73.30, 72.54, 72.00, 71.09, 68.40, 51.88. HRMS (ESI): m/z [M+H]⁺ calcd for $\text{C}_{41}\text{H}_{39}\text{F}_2\text{N}_3\text{O}_6$: 708.2885, found: 708.2862.

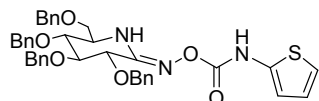


O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-2-chloro-6-(trifluoromethyl)phenylcarbamate (**20**) A white solid (108.7 mg, 78%). ^1H NMR (400 MHz, CDCl_3) δ 9.40 (s, 1H), 8.66 (d, J = 1.2 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.40-7.20 (m, 20H), 7.14 (dd, J = 6.4, 2.8 Hz, 2H), 4.78 (d, J = 12.0 Hz, 1H), 4.58-4.47 (m, 5H), 4.41 (d, J = 11.6 Hz, 1H), 4.34 (d, J = 11.6 Hz, 1H), 4.07 (s, 1H), 3.95-3.89 (m, 1H), 3.82-3.72 (m, 1H), 3.68 (dd, J = 9.8, 2.8 Hz, 1H), 3.56 (dd, J = 9.6, 3.6 Hz, 1H), 3.48 (dd, J = 9.8, 6.4 Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 151.94, 151.92, 137.51, 137.36, 137.12, 136.92, 135.20, 129.49, 128.62, 128.53, 128.40, 128.25, 128.18, 128.13, 128.03, 127.93, 126.14, 120.59, 117.25, 81.41, 79.84, 73.29, 73.27, 72.51, 72.05, 71.14, 68.38, 51.88. HRMS (ESI): m/z [M+H]⁺ calcd for $\text{C}_{42}\text{H}_{39}\text{ClF}_3\text{N}_3\text{O}_6$: 774.2598, found: 774.2564.

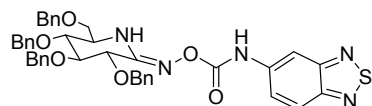


O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-4-(methylthio)phenylcarbamate (**21**) A white solid (82.7 mg, 64%). ^1H NMR (400 MHz, CDCl_3) δ 7.42 (d, J = 8.8 Hz, 2H), 7.39-7.23 (m, 20H), 7.13 (dd, J = 6.4, 2.8 Hz, 2H), 4.75 (d, J = 12.0 Hz, 1H), 4.75 (d, J = 12.0 Hz, 4H), 4.62-4.49 (m, 2H), 4.47 (dd, J = 11.8, 2.0 Hz, 1H), 4.34 (d, J = 11.2 Hz, 1H), 4.08 (s, 1H), 4.00-3.91 (m, 1H), 3.82-3.72 (m, 1H), 3.66 (dd, J = 9.6, 2.8 Hz, 1H), 3.58 (dd, J = 9.6, 3.6 Hz, 1H), 3.49 (dd, J = 9.8, 6.4 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 152.52, 151.52, 137.57, 137.43, 137.31, 137.16, 135.14, 133.18, 130.95, 128.88, 128.57, 128.42, 128.29, 128.08, 128.02, 127.92, 120.25, 81.56,

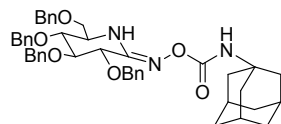
79.94, 73.59, 73.30, 72.56, 72.04, 71.09, 68.41, 65.60, 51.93. HRMS (ESI): m/z [M+H]⁺ + calcd for C₄₂H₄₃N₃O₆S: 718.2951, found: 718.2951.



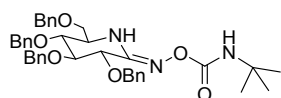
O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-1-Thienylcarbamate (**22**) A white solid (75.6 mg, 62%). ¹H NMR (400 MHz, CDCl₃) δ 7.45-7.21 (m, 9H), 7.20-7.05 (m, 2H), 6.89 (dd, *J* = 14.8, 4.4 Hz, 2H), 6.71 (d, *J* = 2.8 Hz, 1H), 5.84 (s, 1H), 4.74 (d, *J* = 12.0 Hz, 1H), 4.65-4.38 (m, 6H), 4.33 (d, *J* = 11.4 Hz, 1H), 4.06 (s, 1H), 4.00-3.86 (m, 1H), 3.84-3.71 (m, 1H), 3.66 (dd, *J* = 9.8, 2.8 Hz, 1H), 3.57 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.48 (dd, *J* = 9.6, 6.4 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 152.53, 151.68, 139.40, 137.51, 137.37, 137.25, 137.06, 128.63, 128.58, 128.42, 128.09, 128.02, 127.93, 124.72, 118.13, 113.40, 81.47, 79.93, 73.39, 73.28, 72.50, 71.99, 71.05, 68.28, 51.83, 29.73. HRMS (ESI): m/z [M+H]⁺ + calcd for C₃₉H₃₉N₃O₆S: 678.2638, found: 678.2629.



O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene)amino-*Z*-*N*-2,1,3-Benzothiadiazol-4-ylcarbamate (**23**) A white solid (95.8 mg, 73%). ¹H NMR (400 MHz, CDCl₃) δ 9.89 (s, 1H), 7.44-7.25 (m, 15H), 7.14 (dd, *J* = 6.4, 2.4 Hz, 2H), 5.91 (s, 1H), 4.87 (d, *J* = 12.0 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.62-4.28 (m, 7H), 4.22 (s, 1H), 3.98-3.92 (m, 1H), 3.83-3.75 (m, 1H), 3.69 (dd, *J* = 9.6, 2.8 Hz, 1H), 3.58 (dd, *J* = 9.8, 4.0 Hz, 1H), 3.49 (dd, *J* = 9.8, 6.4 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 154.99, 152.17, 152.13, 148.15, 137.58, 137.45, 137.24, 137.14, 131.15, 129.80, 128.89, 128.64, 128.55, 128.43, 128.25, 128.16, 128.07, 127.94, 115.37, 113.88, 81.49, 79.76, 73.31, 72.60, 72.12, 71.37, 68.57, 52.06. HRMS (ESI): m/z [M+H]⁺ + calcd for C₄₁H₃₁N₅O₆S: 730.2699, found: 730.2693.

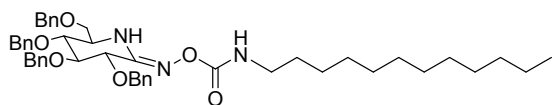


O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-adamantylcarbamate (**24**) A white solid (106.4 mg, 81%). ¹H NMR (400 MHz, CDCl₃) δ 7.39-7.23 (m, 18H), 7.14-7.09 (m, 2H), 6.34 (s, 1H), 5.76 (s, 1H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.50 (m, 6H), 4.33 (d, *J* = 11.6 Hz, 1H), 4.01 (s, 1H), 3.91 (s, 1H), 3.70 (d, *J* = 7.2 Hz, 1H), 3.64 (d, *J* = 9.8 Hz, 1H), 3.54 (dd, *J* = 9.8, 4.0 Hz, 1H), 3.46 (dd, *J* = 9.6, 6.4 Hz, 1H), 2.11 (s, 3H), 2.02 (s, 6H), 1.70 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 153.35, 150.64, 137.65, 137.49, 137.40, 137.28, 128.58, 128.52, 128.39, 128.20, 128.09, 128.01, 127.95, 127.87, 81.81, 79.92, 73.58, 73.24, 72.62, 71.96, 70.93, 68.51, 51.89, 50.94, 41.65, 36.37, 29.51. HRMS (ESI): m/z [M+H]⁺ + calcd for C₄₅H₅₁N₃O₆: 730.3856, found: 730.3860.



O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-tert-Butylcarbamate (**25**) A white solid (84.5 mg, 72%). ¹H NMR (400 MHz, CDCl₃) δ 7.56-7.22 (m, 20H), 7.18-7.02 (m, 2H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.51 (m, 6H), 4.34 (d, *J* = 11.6 Hz, 1H), 4.02 (d, *J* = 2.4 Hz, 1H), 3.91 (dd, *J* = 4.0, 3.2 Hz, 1H), 3.77-3.68 (m, 1H), 3.65 (dd, *J* = 9.6, 2.4 Hz, 1H), 3.55 (dd, *J* = 9.6, 4.2 Hz, 1H), 3.47 (dd, *J* = 9.6, 6.4 Hz, 1H), 1.39 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ

153.71, 150.85, 137.63, 137.47, 137.39, 137.23, 128.56, 128.49, 128.38, 128.13, 128.02, 127.85, 81.84, 79.89, 73.63, 73.27, 72.63, 72.03, 71.02, 68.57, 51.99, 50.58, 28.83. HRMS (ESI): m/z [M+H]⁺ calcd for C₃₉H₄₅N₃O₆: 652.3387, found: 652.3395.

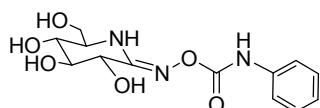


O-(2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosylidene) amino-*Z*-*N*-1-Dodecylcarbamate (**26**) A white solid (105.9 mg, 77%). ¹H NMR (400 MHz, CDCl₃) δ 7.41-7.24 (m, 18H), 7.15-7.09 (m, 2H), 6.44 (t, J = 5.6 Hz, 1H), 5.78 (s, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.61-4.41 (m, 6H), 4.33 (d, J = 11.6 Hz, 1H), 4.01 (s, 1H), 3.92 (d, J = 2.8 Hz, 1H), 3.72 (dd, J = 9.2, 5.6 Hz, 1H), 3.66-3.60 (m, 1H), 3.55 (dd, J = 9.8, 3.6 Hz, 1H), 3.47 (dd, J = 9.8, 6.4 Hz, 1H), 3.32-3.23 (m, 2H), 1.62-1.51 (m, 2H), 1.29 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 155.86, 151.03, 137.62, 137.46, 137.36, 137.26, 128.53, 128.40, 128.07, 128.01, 127.89, 81.72, 79.95, 73.64, 73.28, 72.56, 71.92, 70.91, 68.43, 51.86, 41.16, 31.97, 29.88, 29.69, 29.41, 26.93, 22.75, 14.21. HRMS (ESI): m/z [M+H]⁺ calcd for C₄₇H₆₁N₃O₆: 764.4639, found: 764.4622.

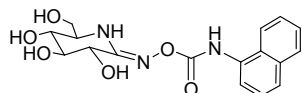
General procedure for removal Benzyl protective groups

Each compound of **15-26** (50 mg) was dissolved in 5 mL dry CH₂Cl₂ at -78°C for 20 min, then 2.5 mL BCl₃ (1M in hexane) was added and continually stirred at -78°C for 3 h and then 0°C for 12 h. The mixture was moved to warm and concentrated in vacuo and the crude product purified by flashing column chromatography (EtOAc/acetone/NH₄OH/H₂O = 8:2:0.5:0.5) to get the target product.

Data for D-glucohydroximo-lactam (27). A colorless oil (14.8 mg, 85%). ¹H NMR (400 MHz, CDCl₃) δ 4.04 (d, J = 8.4 Hz, 1H), 3.98-3.91 (dd, J = 2.0 Hz, J = 10.8 Hz, 1H), 3.60-3.48 (m, 2H), 3.45-3.35 (m, 1H), 3.16 (m, 1H), 2.56 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 153.07, 76.06, 70.09, 69.43, 62.67, 57.37. HRMS (ESI): m/z [M+H]⁺ calcd for C₆H₁₂N₂O₅: 193.0824, found: 193.0821.

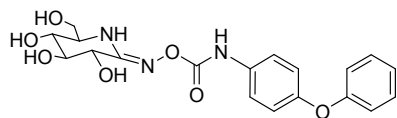


Data for O-(*D*-glucopyranosylidene) amino-*Z*-*N*-phenylcarbamate (**28**). A colorless oil (19.0 mg, 82%). ¹H NMR (400 MHz, CD₃OD) δ 3.30-3.25 (m, 1H), 3.51 (t, J = 8.8 Hz, J = 18 Hz, 1H), 3.62 (dd, J = 6.8 Hz, J = 11.2 Hz, 1H), 3.68 (t, J = 8.8 Hz, 1H), 3.94 (dd, J = 2.8 Hz, J = 11.2 Hz, 1H), 4.21 (d, J = 8.8 Hz, 1H), 7.07 (t, J = 7.2 Hz, J = 14.4 Hz, 1H), 7.33 (t, J = 7.6 Hz, J = 15.6 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H); ¹³C NMR (100 MHz, CD₃OD) δ: 155.52, 153.95, 137.89, 128.52, 123.48, 119.32, 75.12, 69.39, 61.96, 57.92; HRMS (ESI): m/z [M+H]⁺ calcd for C₁₀H₂₁NO₄: 312.1190, found: 312.1192.

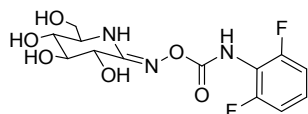


Data for O-(*D*-glucopyranosylidene) amino-*Z*-*N*-Naphthylcarbamate (**29**). A brown oil (19.1 mg, 76%).. ¹H NMR (400 MHz, CDOD₃) δ 8.06 (d, J = 7.6 Hz, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.74 (dd, J = 21.0, 7.4 Hz, 2H), 7.52 (dt, J = 15.6, 10.0 Hz, 3H), 4.25 (dd, J = 2.8, 2.4 Hz, 1H), 3.95 (d, J = 10.0 Hz, 1H), 3.76-3.51 (m, 3H), 3.35 (d, J = 15.6 Hz, 1H); ¹³C NMR (100 MHz, CDOD₃) δ 155.74, 155.44, 134.30, 132.50, 128.23, 128.04, 127.93, 125.96, 125.83, 125.77, 125.68, 125.18, 121.74, 121.54, 121.19, 121.12, 75.13, 69.48, 61.93, 57.97. HRMS (ESI): m/z [M+H]⁺ calcd

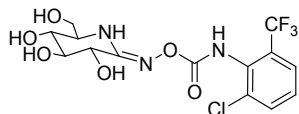
for C₁₇H₁₉N₃O₆: 362.1352, found: 362.1350.



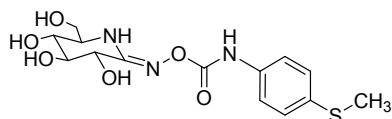
Data for *O*-(*D*-glucopyranosylidene) amino-*Z*-*N*-4-Phenoxyphenylcarbamate (**30**). A colorless oil (20.6 mg, 78%). ¹H NMR (400 MHz, CDOD₃) δ 7.52 (d, *J* = 8.8 Hz, 2H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.18 (dd, *J* = 12.8, 7.6 Hz, 1H), 7.09 (t, *J* = 7.2 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 3H), 4.21 (d, *J* = 8.8 Hz, 1H), 3.94 (dd, *J* = 11.2, 2.8 Hz, 1H), 3.72-3.59 (m, 2H), 3.52 (t, *J* = 8.8 Hz, 1H), 3.27 (m, 1H). ¹³C NMR (100 MHz, CDOD₃) δ 157.73, 155.46, 154.20, 153.28, 133.48, 129.45, 122.75, 121.12, 119.11, 118.13, 117.97, 75.12, 69.41, 61.98, 57.95. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₉H₂₁N₃O₇: 404.1458, found: 404.1455.



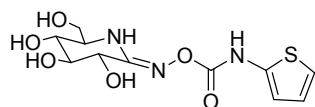
Data for *O*-(*D*-glucopyranosylidene) amino-*Z*-*N*-2,4-Difluorophenylcarbamate (**31**). A colorless oil (19.8 mg, 81%). ¹H NMR (400 MHz, CDOD₃) δ 7.35 (m, 1H), 7.07 (t, *J* = 8.0 Hz, 2H), 4.20 (d, *J* = 8.4 Hz, 1H), 3.93 (dd, *J* = 11.2, 2.8 Hz, 1H), 3.68 (t, *J* = 8.4 Hz, 1H), 3.62 (dd, *J* = 11.2, 6.8 Hz, 1H), 3.52 (t, *J* = 8.8 Hz, 1H), 3.29 (m, 1H); ¹³C NMR (100 MHz, CDOD₃) δ 159.86, 159.82, 157.38, 157.33, 155.63, 154.88, 128.21, 128.12, 128.02, 111.48, 111.43, 111.30, 111.25, 75.11, 69.49, 69.39, 61.92, 57.88. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₃H₁₅F₂N₃O₆: 348.1007, found: 348.1009.



Data for *O*-(*D*-glucopyranosylidene) amino-*Z*-*N*-2-Chloro-6-(trifluoromethyl) phenylcarbamate (**32**). A brown oil (22.4 mg, 84%). ¹H NMR (400 MHz, CDOD₃) δ 8.45 (s, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 4.20 (d, *J* = 7.6 Hz, 1H), 3.94 (d, *J* = 10.4 Hz, 1H), 3.76-3.62 (m, 2H), 3.56 (t, *J* = 8.4 Hz, 1H), 3.35 (d, *J* = 15.2 Hz, 1H). ¹³C NMR (100 MHz, CDOD₃) δ 156.20, 153.24, 135.35, 130.01, 129.76, 129.44, 127.65, 125.05, 122.35, 120.95, 117.90, 75.09, 69.70, 69.44, 61.75, 57.77. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₄₂H₄₀ClF₃N₃O₆: 414.0680, found: 414.0673.

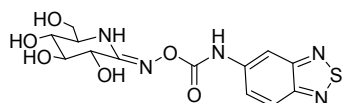


Data for *O*-(*D*-glucopyranosylidene) amino-*Z*-*N*-4-(Methylthio)phenylcarbamate (**33**). A colorless oil (21.1 mg, 85%). ¹H NMR (400 MHz, CDOD₃) δ 7.49 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 4.19 (d, *J* = 8.4 Hz, 1H), 3.93 (dd, *J* = 11.2, 2.8 Hz, 1H), 3.71-3.55 (m, 1H), 3.51 (t, *J* = 8.9 Hz, 1H), 3.29-3.22 (m, 1H), 2.47 (s, 1H); ¹³C NMR (100 MHz, CDOD₃) δ 155.48, 153.91, 135.57, 133.22, 127.57, 119.94, 75.12, 69.39, 61.95, 57.91, 15.20. HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₄H₁₉N₃O₆S: 358.1073, found: 358.1073.

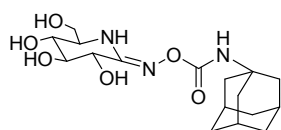


Data for *O*-(*D*-glucopyranosylidene) amino-*Z*-*N*-1-Thienylcarbamate (**34**). A red oil (17.3 mg, 74%). ¹H NMR (400 MHz, CDOD₃) δ 6.94 (d, *J* = 5.3 Hz, 1H), 6.86 (t, *J* = 4.4 Hz, 1H), 6.78 (s, 1H), 4.20 (d, *J* = 8.8 Hz, 1H), 3.93 (d, *J* = 11.2

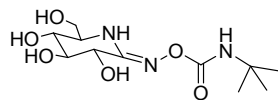
Hz, 1H), 3.70-3.58 (m, 2H), 3.51 (t, $J = 8.8$ Hz, 1H), 3.30-3.23 (m, 1H). ^{13}C NMR (100 MHz, CDOD_3) δ 155.62, 153.81, 139.81, 124.22, 117.02, 112.46, 75.07, 69.38, 69.33, 61.94, 58.02. HRMS (ESI): m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{39}\text{H}_{39}\text{N}_3\text{O}_6\text{S}$: 340.0579, found: 340.0569.



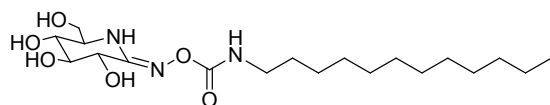
Data for O-(D-glucopyranosylidene) amino-Z-N-2,1,3-Benzothiadiazol-4-ylcarbamate (35). A yellow oil (20.5 mg, 81%). ^1H NMR (400 MHz, CDOD_3) δ 8.04 (dd, $J = 6.8, 1.2$ Hz, 1H), 7.59-7.48 (m, 2H), 4.13 (d, $J = 7.6$ Hz, 1H), 3.85 (dd, $J = 11.2, 2.8$ Hz, 1H), 3.64 (t, $J = 8.0$ Hz, 1H), 3.57 (dd, $J = 11.2, 6.4$ Hz, 1H), 3.46 (t, $J = 8.4$ Hz, 1H), 3.29-3.23 (m, 1H). ^{13}C NMR (100 MHz, CDOD_3) δ 156.33, 154.91, 153.04, 147.83, 130.47, 129.73, 115.08, 114.04, 75.18, 69.84, 69.50, 61.79, 57.67. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{15}\text{N}_5\text{O}_6\text{S}$: 370.0821, found: 370.0824.



Data for O-(D-glucopyranosylidene) amino-Z-N-adamantylcarbamate (36). A colorless oil (18.5 mg, 73%). ^1H NMR (400 MHz, CDOD_3) δ 4.01 (d, $J = 8.8$ Hz, 1H), 3.79 (dd, $J = 11.2, 2.8$ Hz, 1H), 3.56-3.41 (m, 2H), 3.35 (t, $J = 8.8$ Hz, 1H), 3.15-3.01 (m, 1H), 1.98 (s, 3H), 1.92 (s, 6H), 1.63 (s, 6H); ^{13}C NMR (100 MHz, CDOD_3) δ 154.89, 154.77, 75.16, 69.44, 69.37, 62.01, 57.86, 50.71, 41.10, 36.02, 29.55. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{27}\text{N}_3\text{O}_6$: 370.1978, found: 370.1974.



Data for O-(D-glucopyranosylidene) amino-Z-N-tert-Butylcarbamate (37). An orange oil (18.6 mg, 83%). ^1H NMR (400 MHz, CDOD_3) δ 4.01 (d, $J = 8.4$ Hz, 1H), 3.80 (dd, $J = 11.2, 2.8$ Hz, 1H), 3.49 (dt, $J = 11.2, 7.6$ Hz, 2H), 3.36 (t, $J = 8.8$ Hz, 1H), 3.12 (m, 1H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, CDOD_3) δ 155.23, 154.87, 75.20, 69.50, 69.36, 62.01, 57.78, 50.24, 27.60. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_6$: 292.1509, found: 292.1508.

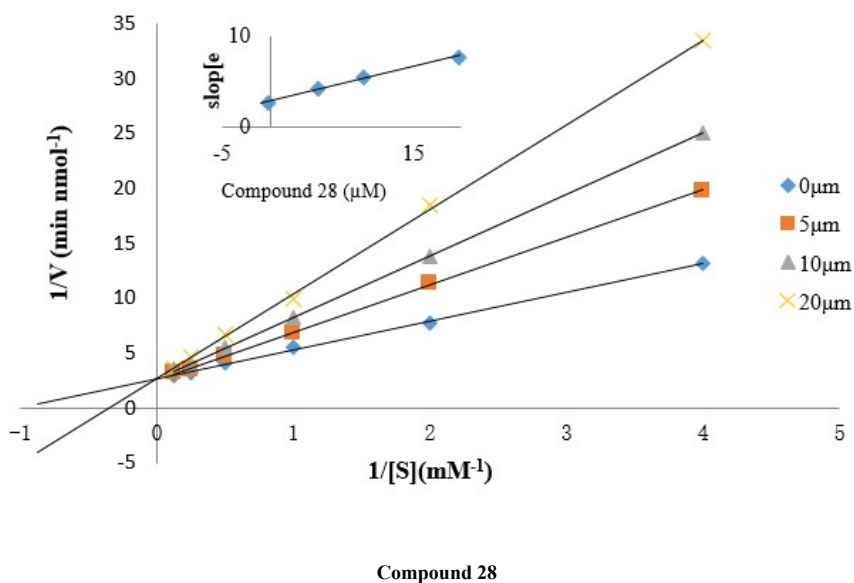
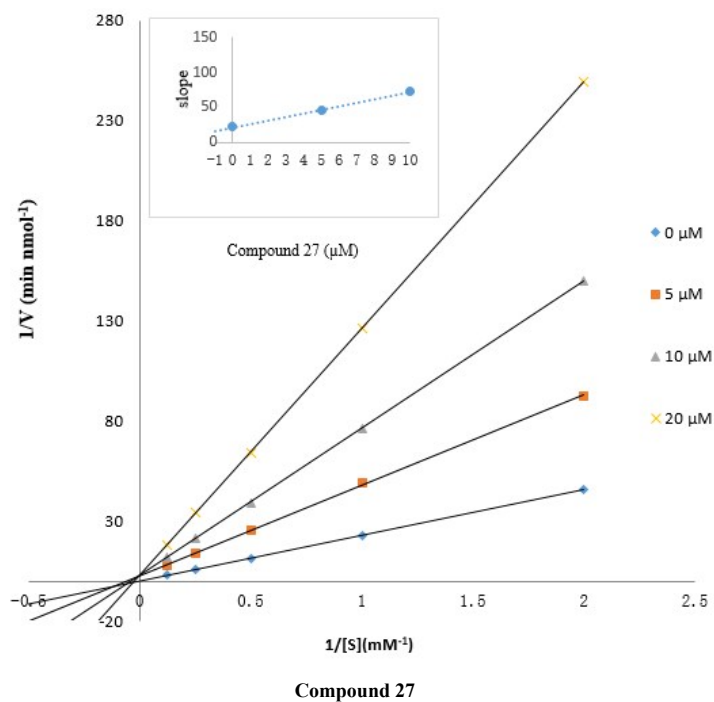


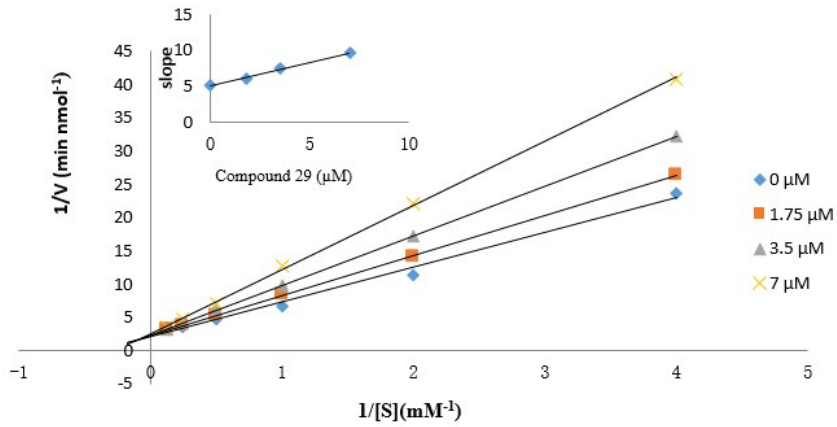
Data for O-(D-glucopyranosylidene) amino-Z-N-1-Dodecylcarbamate (38). A white solid (22.4 mg, 85%). ^1H NMR (400 MHz, CDOD_3) δ 4.13 (d, $J = 8.8$ Hz, 1H), 3.91 (dd, $J = 11.2, 2.8$ Hz, 1H), 3.60 (dt, $J = 11.2, 7.6$ Hz, 2H), 3.48 (t, $J = 8.8$ Hz, 1H), 3.22 (dt, $J = 11.6, 5.2$ Hz, 3H), 1.60-1.52 (m, 2H), 1.33 (d, $J = 13.2$ Hz, 18H), 0.92 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CDOD_3) δ 157.33, 154.91, 75.18, 69.37, 61.94, 57.87, 40.58, 31.68, 29.36, 29.08, 26.47, 22.34, 13.05. HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{37}\text{N}_3\text{O}_6$: 404.2761, found: 404.2763.

Recombinant GCCase (imiglucerase, Cerezyme) inhibition assay

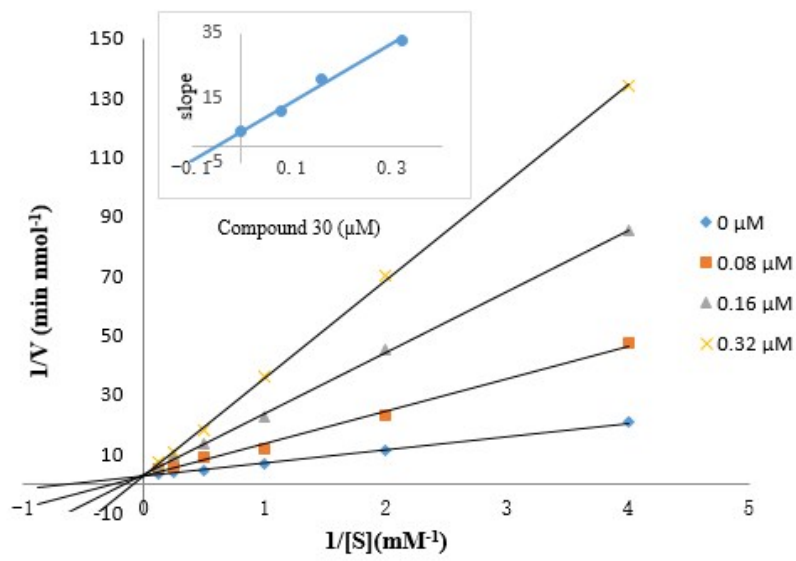
Recombinant GCCase was obtained from Genzyme (Cambridge, MA). Imiglucerase in vitro activity was determined with 4-MU- β -D-Glu as the substrate in McIlvaine buffer (100 mM sodium citrate, pH 5.2 or 200 mM sodium phosphate buffer, pH 7.0) containing sodium taurocholate (0.25% w/v) and Triton X-100 (0.1% v/v). To assay IC_{50} ,

enzyme solution (12.5 μL , 0.1 mg/mL) without (control) or with inhibitor (7.5 μL , various concentrations) were incubated at 37°C for 30 min. Then, substrate (30 μL , 4.0 mM, McIlvaine buffer, pH 5.2 or 7.0) was added, and the samples were incubated for another 10 min at 37°C. Enzymatic reactions were stopped by adding of aliquots 150 μL of 100 mM glycine/NaOH buffer (pH 10.6). The amount of 4-methylumbelliferon formed was determined with a fluorometer at 355 nm (excitation) and 460 nm (emission). IC_{50} values were determined by plotting percent activity versus $\log [I]$ with eight different inhibitors concentrations. The type of inhibition and constant K_i values were determined by Lineweaver-Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate. Lineweaver-Burk double reciprocal plots of compounds **27-38** were shown below:

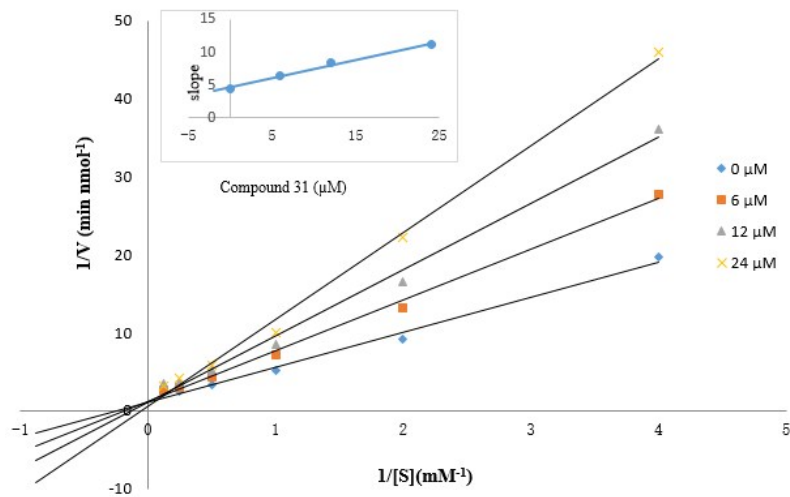




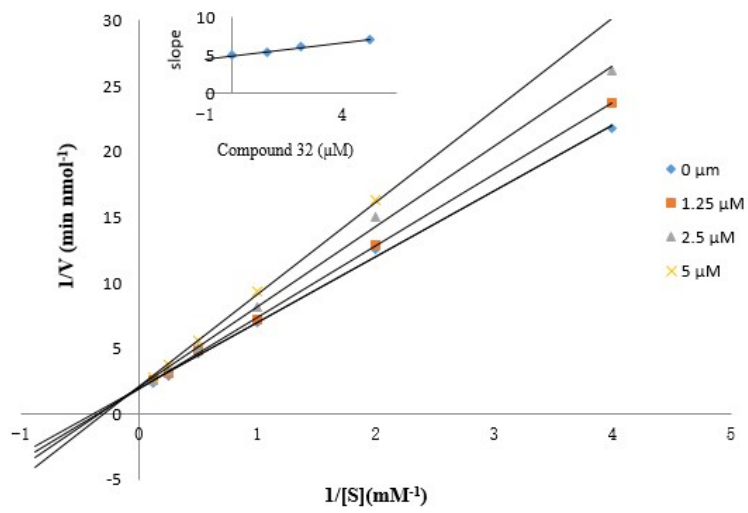
Compound 29



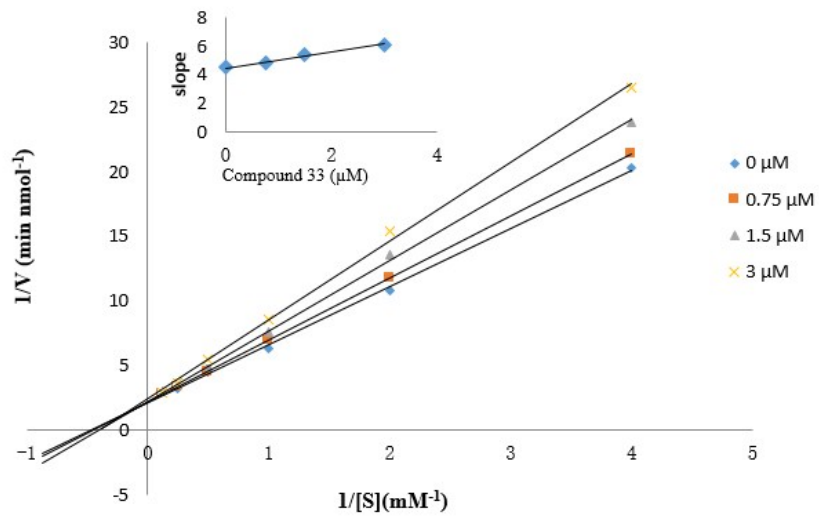
Compound 30



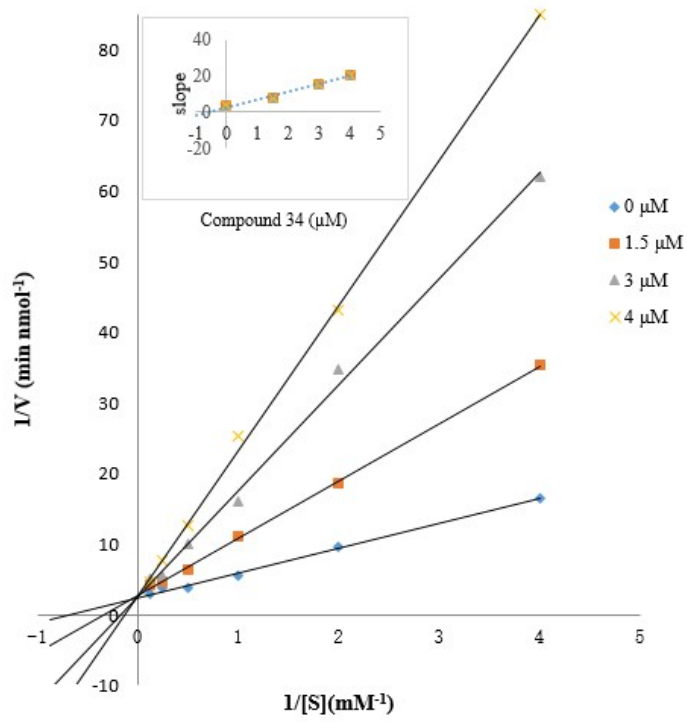
Compound 31



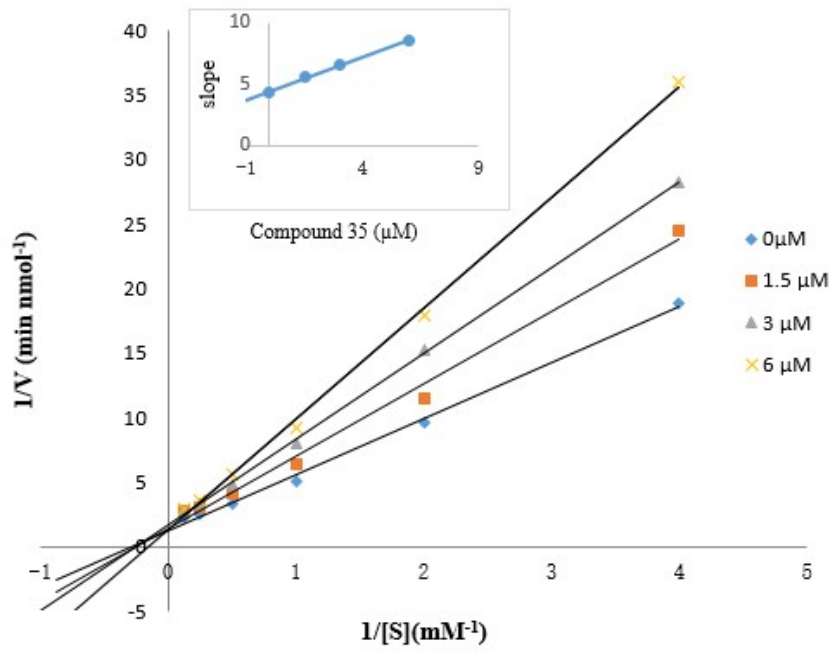
Compound 32



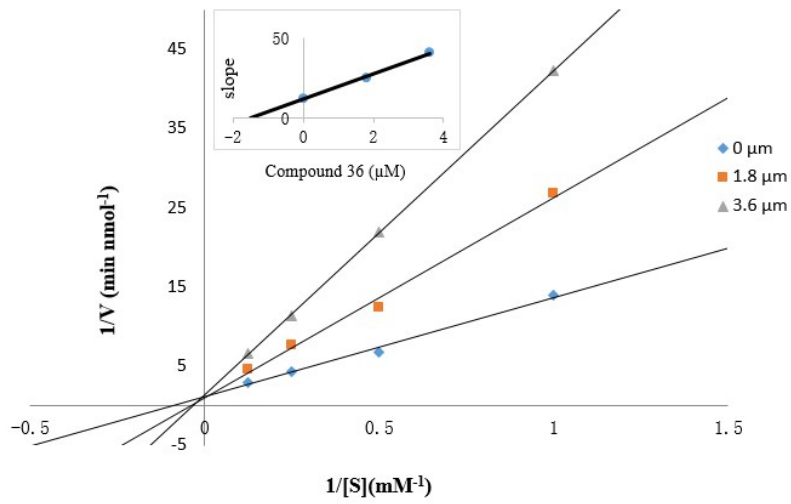
Compound 33



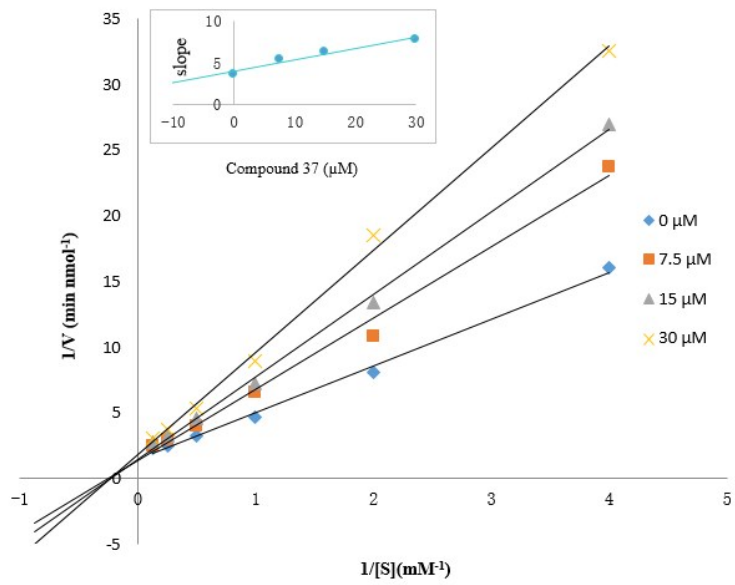
Compound 34



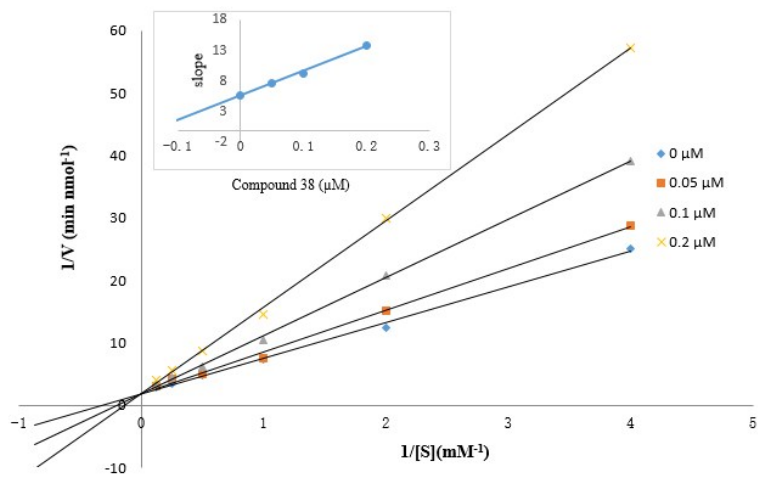
Compound 35



Compound 36



Compound 37



Compound 38

Cell lines and culture

Gaucher lymphoblasts cell lines homozygous for the N370S GCase (GM10873) and wild-type CCC-ESF-1 fibroblasts were obtained from the National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China). The lymphoblasts were cultured in RPMI-1640 medium (Invitrogen) contained with 15% FBS and 1% penicillin/streptomycin. The wild-type CCC-ESF-1 fibroblasts were cultured in DMEM (Invitrogen) contained with 10% FBS and 1% penicillin-streptomycin. All cell lines were maintained in a humidified atmosphere containing 5% CO₂ at 37°C.

Measurement of N370S GCase activity in lymphoblasts derived from patients with GD

Patient fibroblasts homozygous for the N370S mutation were seeded at a density of 5×10⁵ cells per well in 12-well plates for 24h. Then cells were incubated with or without various concentrations of compounds from 100 to 3.25 μM for 3 days. The cells were washed with PBS twice and lysed in 0.1 M citrate phosphate buffer (0.4% v/v sodium taurocholate and 0.4% Triton X-100) for 30 min at 4°C. Total protein was determined with a BCA protein assay kit (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) referred to the manufacturer's instructions. Sample (30 mg) and 4-MU-β-D-Glu (5.0 mM) were incubated in 0.1M pH 5.2 citrate phosphate buffer (0.25% sodium taurocholate and 0.1 %Triton X-100) at 37°C for 2 h. The reactions were stopped by adding 150 μL glycine/NaOH buffer (200 mM, pH 10.6) and fluorescence was measured (excitation wavelength 355 nm, emission wavelength 460 nm) with a fluorimeter. Nonspecific GCase activity was evaluated by addition of 2.5 mM Conduritol B Epoxide (Enzo Life Sciences, Ann Arbor, MI) to control wells and identified not more than 2% (in control cells). All experiments were performed in triplicate.

Cytotoxicity assay in wild-type human fibroblasts

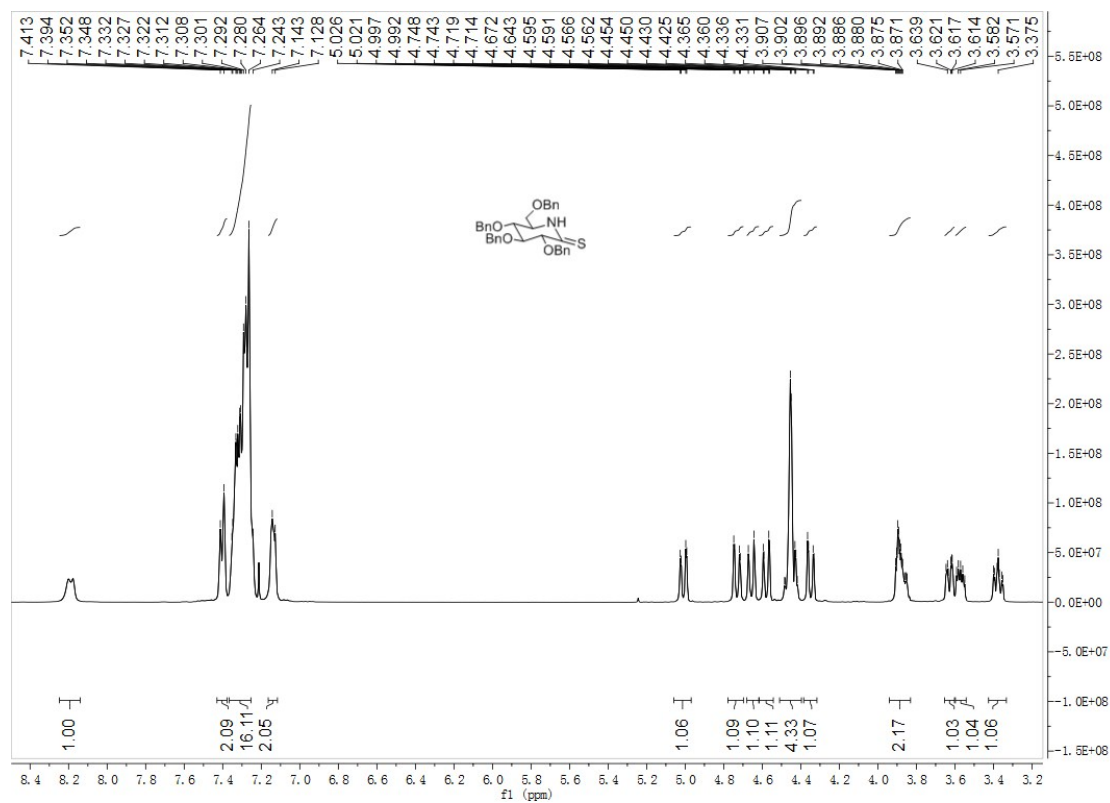
CCC-ESF-1 cells were seeded at a density of 4000 cells per well in 96-well plates and incubated overnight. The cells were treated with a range of concentrations from 100 to 3.125 μM at 37°C in 5% CO₂ for 24h. Then each well was added 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT, 20 μL, 5 mg/mL in PBS; Solarbio) and the mixtures were incubated for additional at 37°C for 4 h. After removal of the medium, DMSO (150 μL) was added and quantified by microplate reader at 570 nm.

Molecular docking study

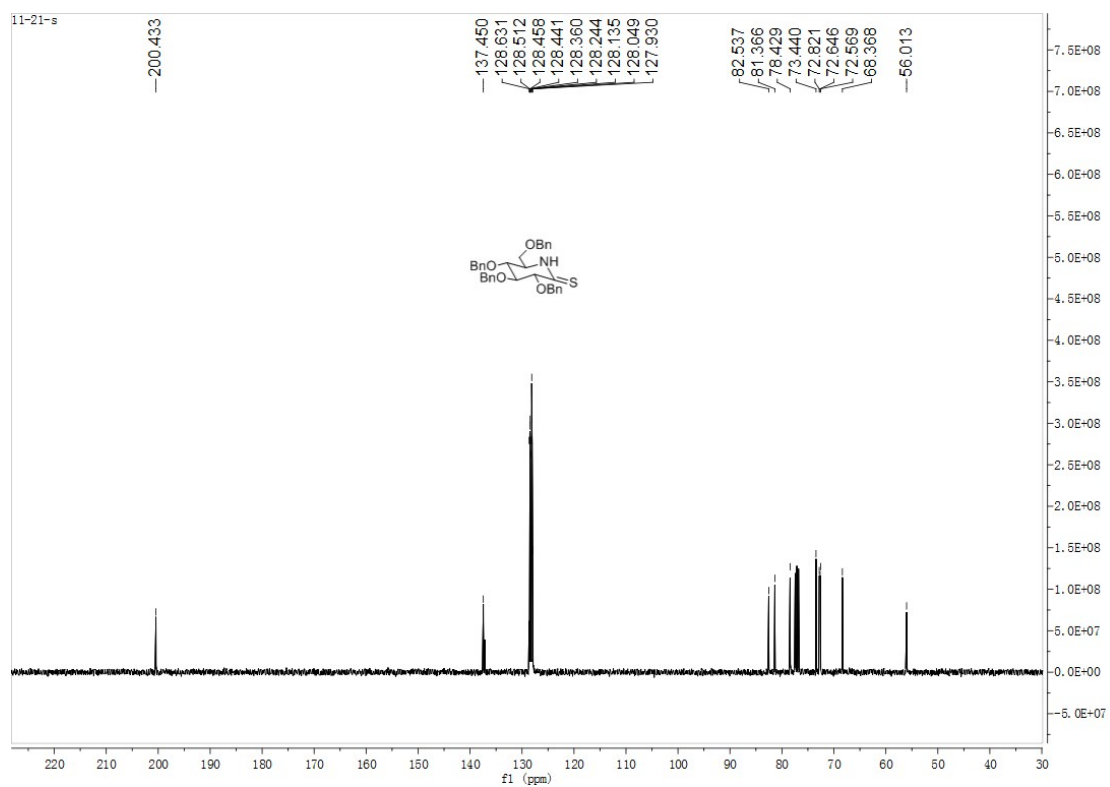
The AutoDock 4.0 package was used to further evaluate the binding mode between **38** and GCase. The crystal structures of GCase was obtained from the Protein Data Bank (ID: 2V3E). At this stage, the structure of human GCase was preprocessed and prepared by deleting all of the water molecules, and hydrogen atoms were then added. Then a grid box was generated before docking. PDBQT files of targets and ligands were prepared using AutoDockTools. The center of the grid box was placed in the active site of GCase. A genetic algorithm (GA) was used to simulate ligand-receptor binding. The number of GA runs was 200. The step size parameters of quaternion and torsion were set to 200. For compound **38**, 200 independent runs were performed. Default values were used for all other parameters.

[1] L. DÍaz, J. Casas, J. Bujons, A. Llebaria, A. Delgado, *J. Med. Chem.* 2011, **54**, 2069–2079.

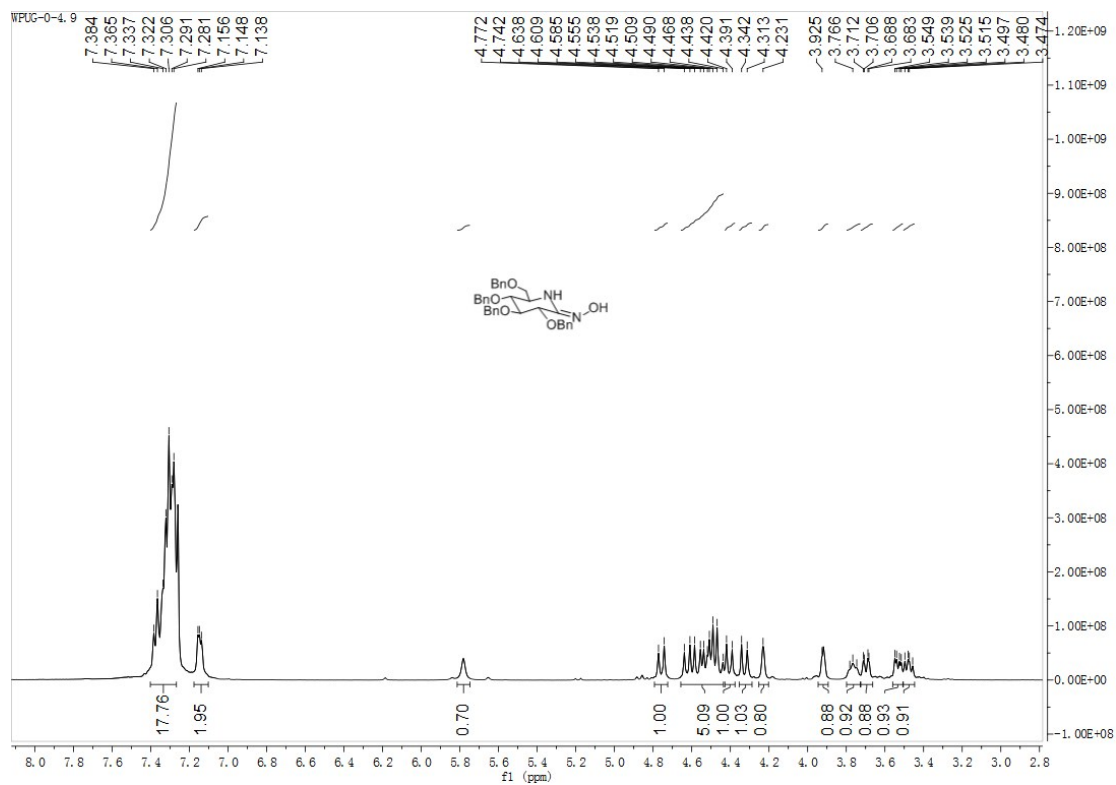
¹H NMR spectrum of 14



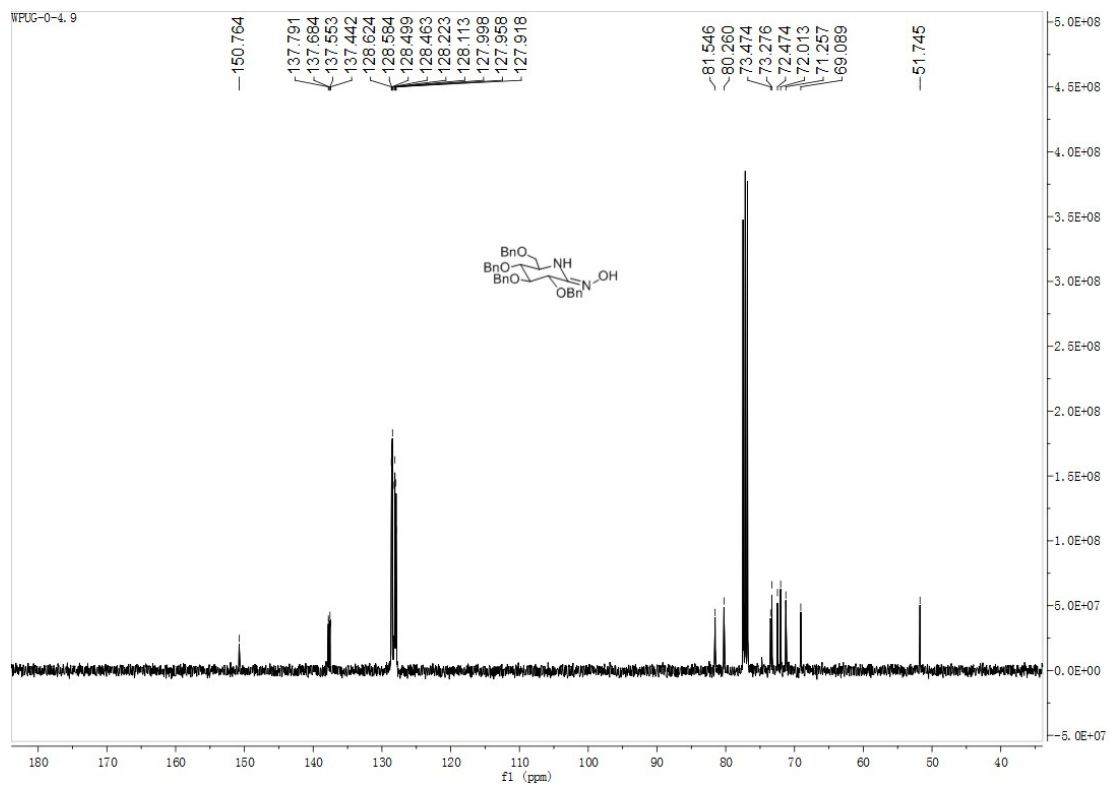
¹³C NMR spectrum of 14



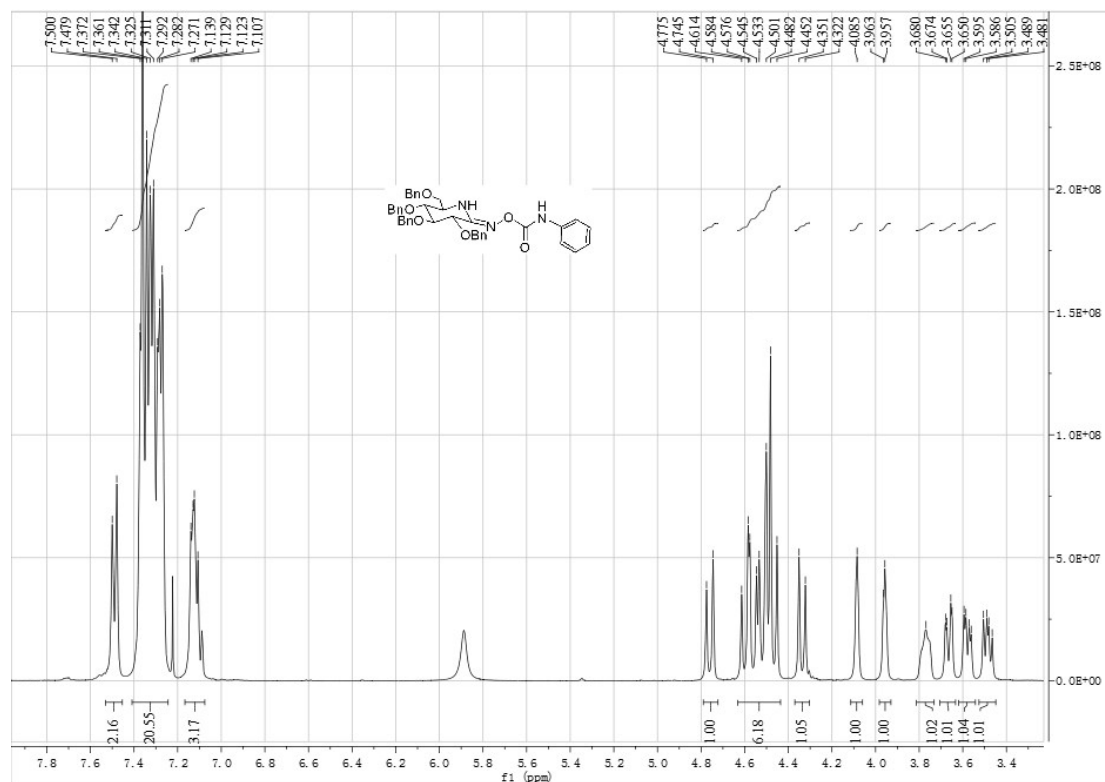
¹H NMR spectrum of 15



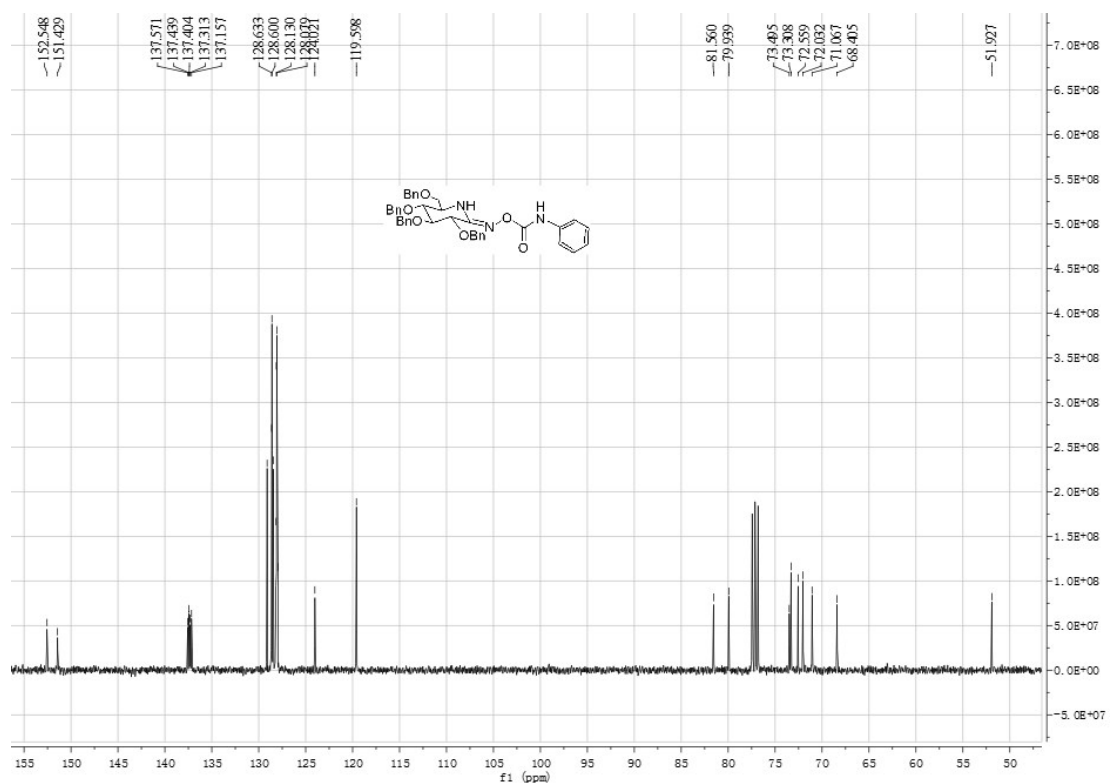
¹³C NMR spectrum of 15



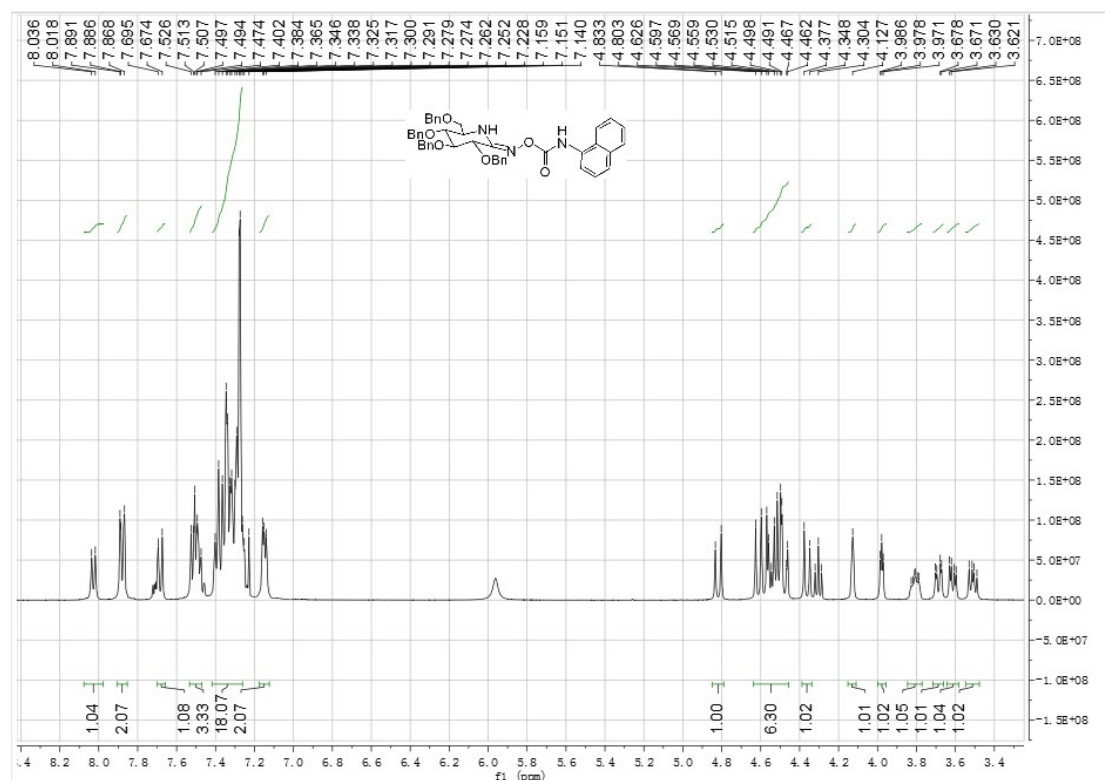
¹H NMR spectrum of 16



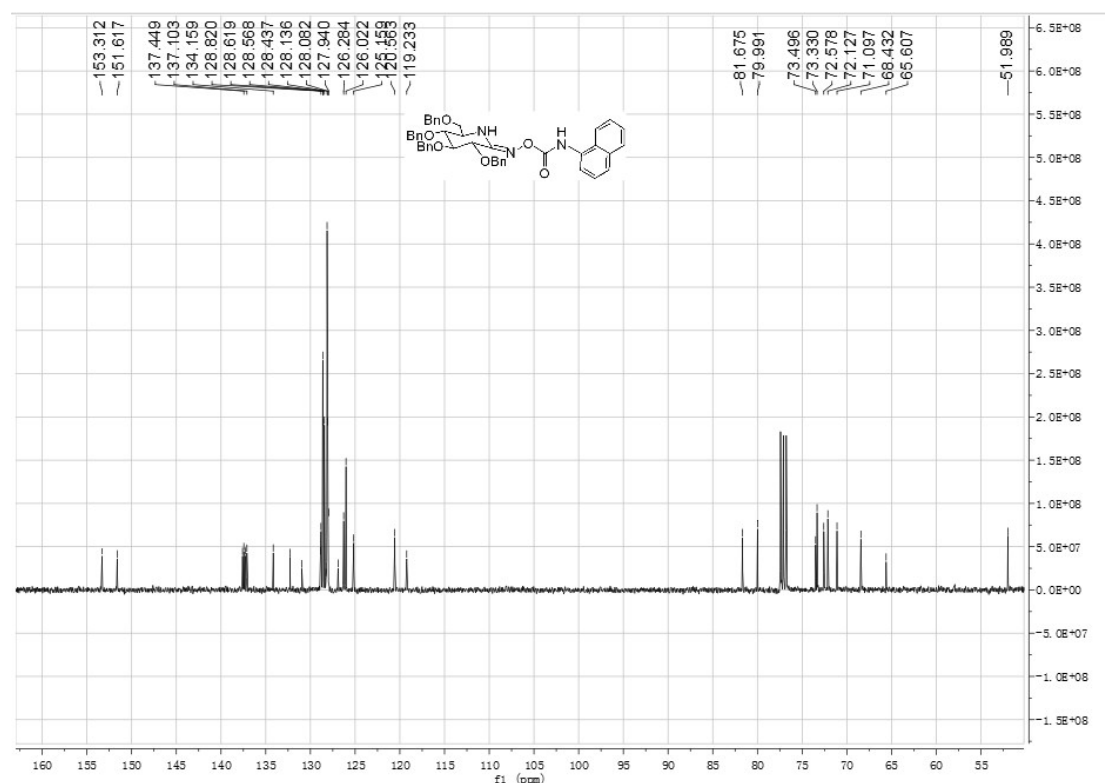
¹³C NMR spectrum of 16



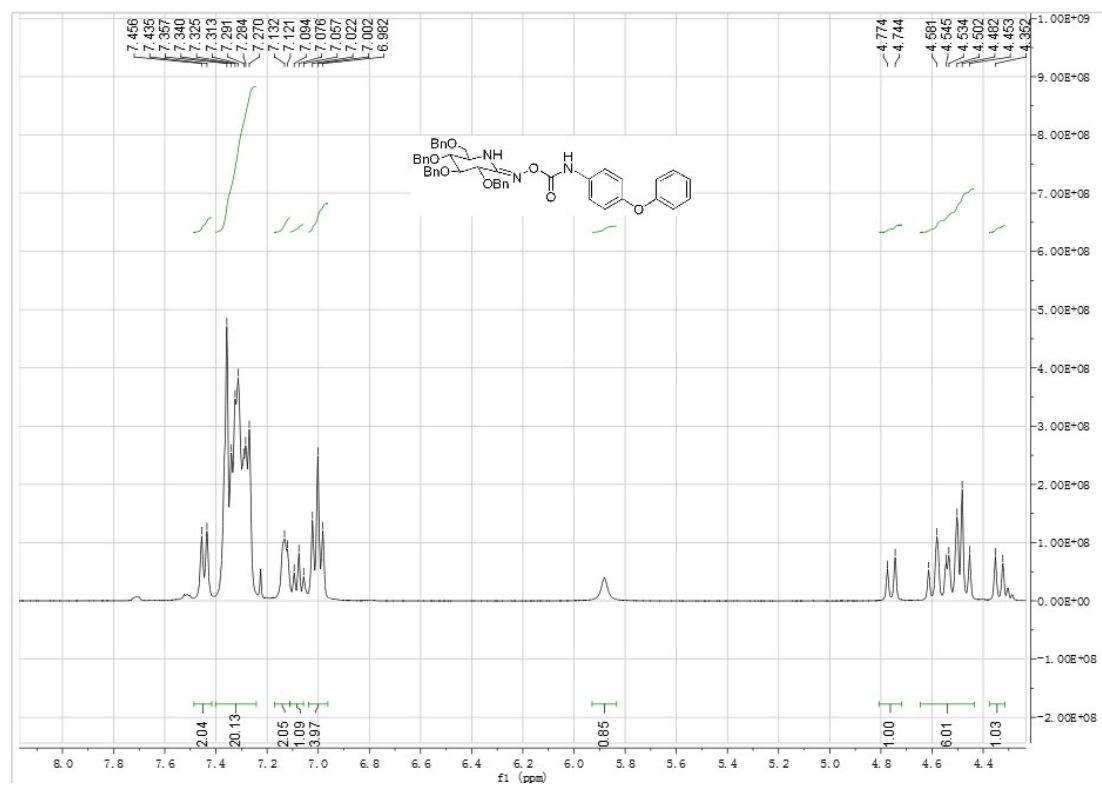
¹H NMR spectrum of 17



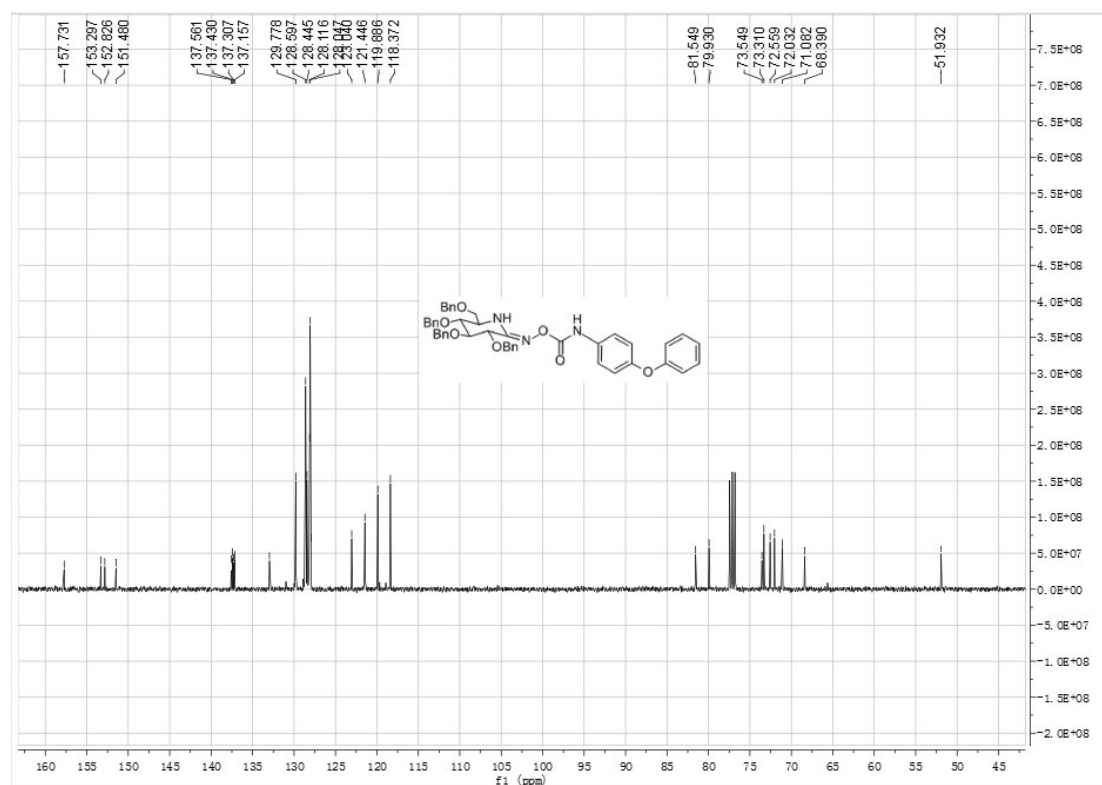
¹³C NMR spectrum of 17



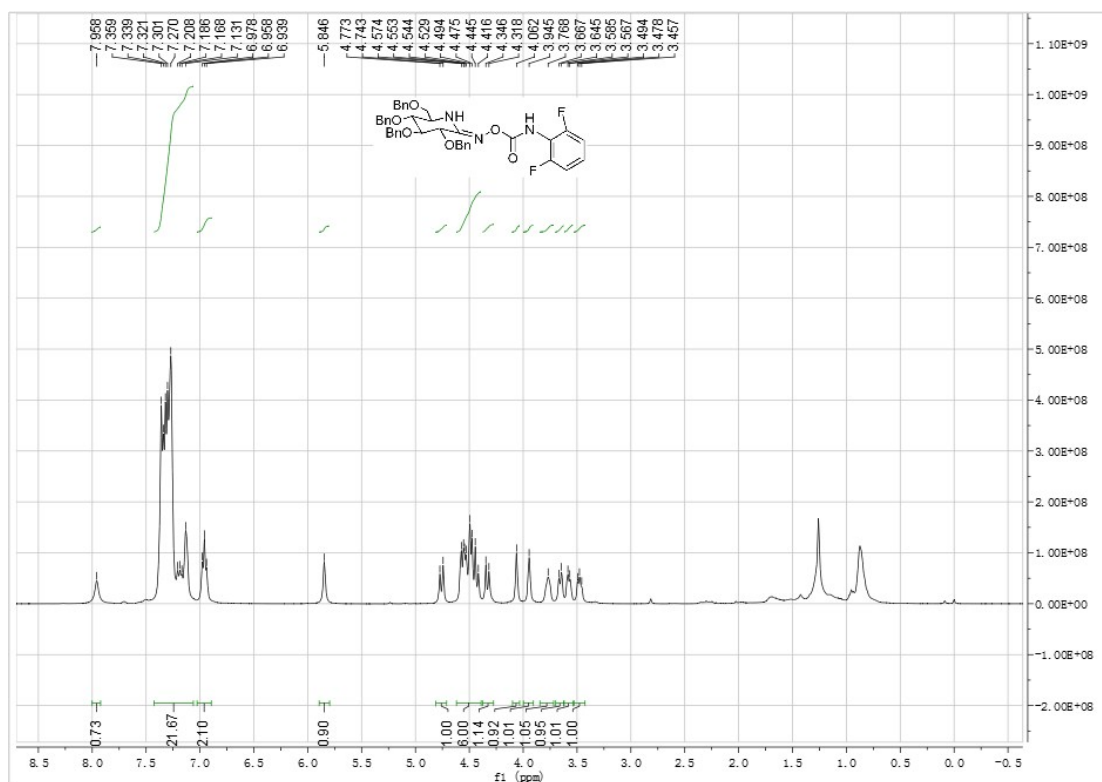
¹H NMR spectrum of 18



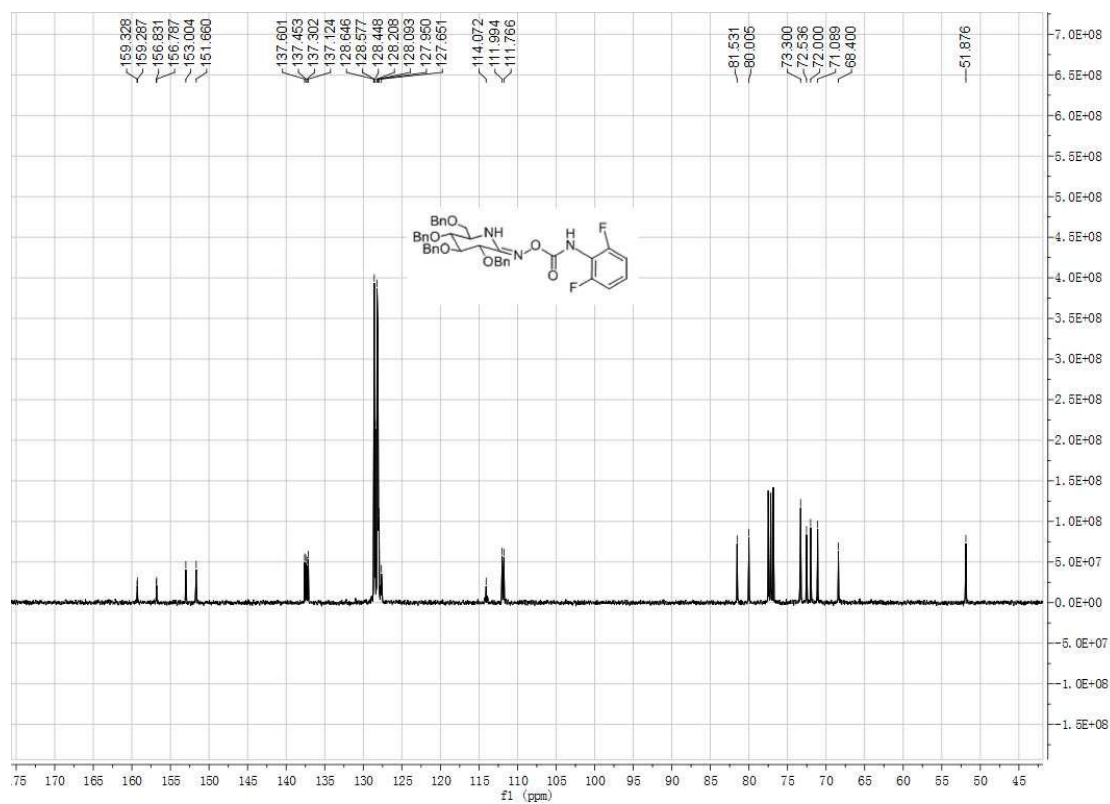
¹³C NMR spectrum of 18



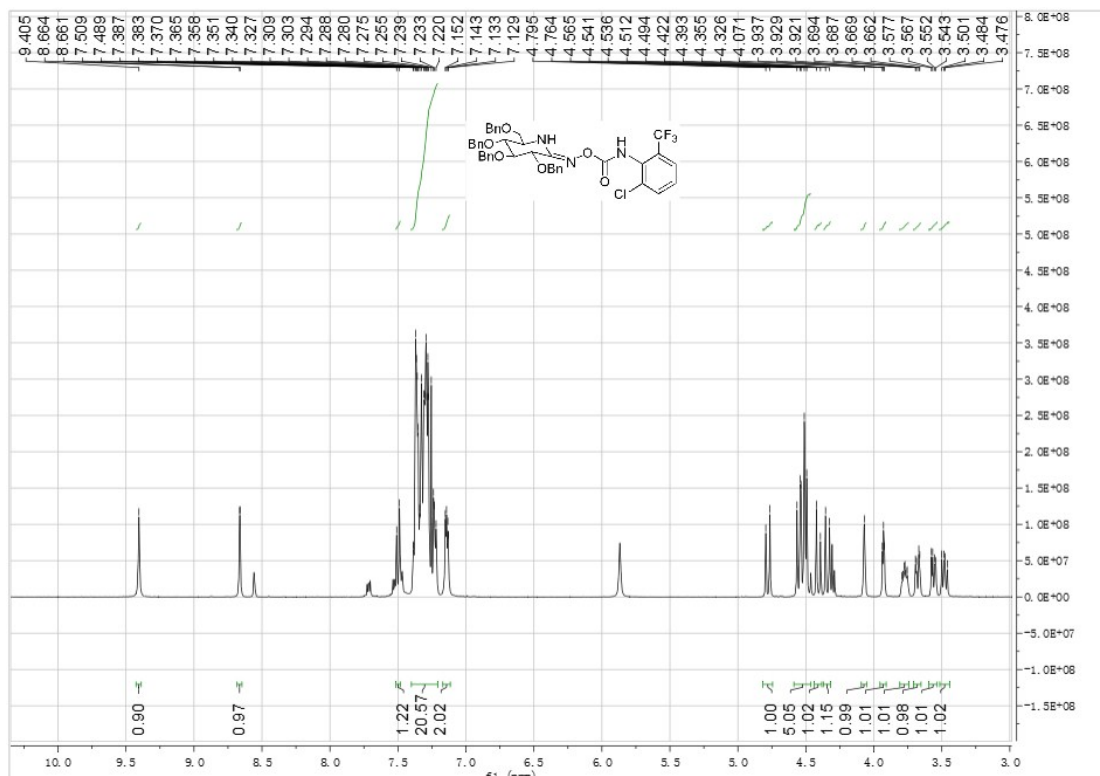
¹H NMR spectrum of **19**



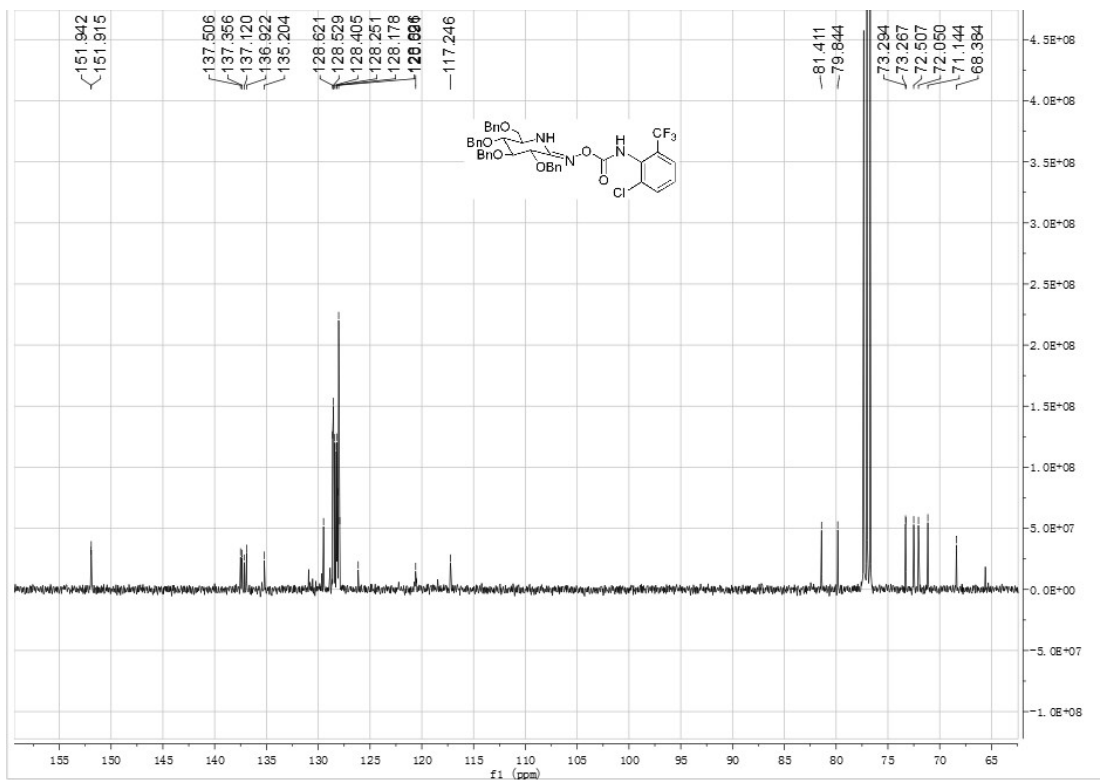
¹³C NMR spectrum of **19**



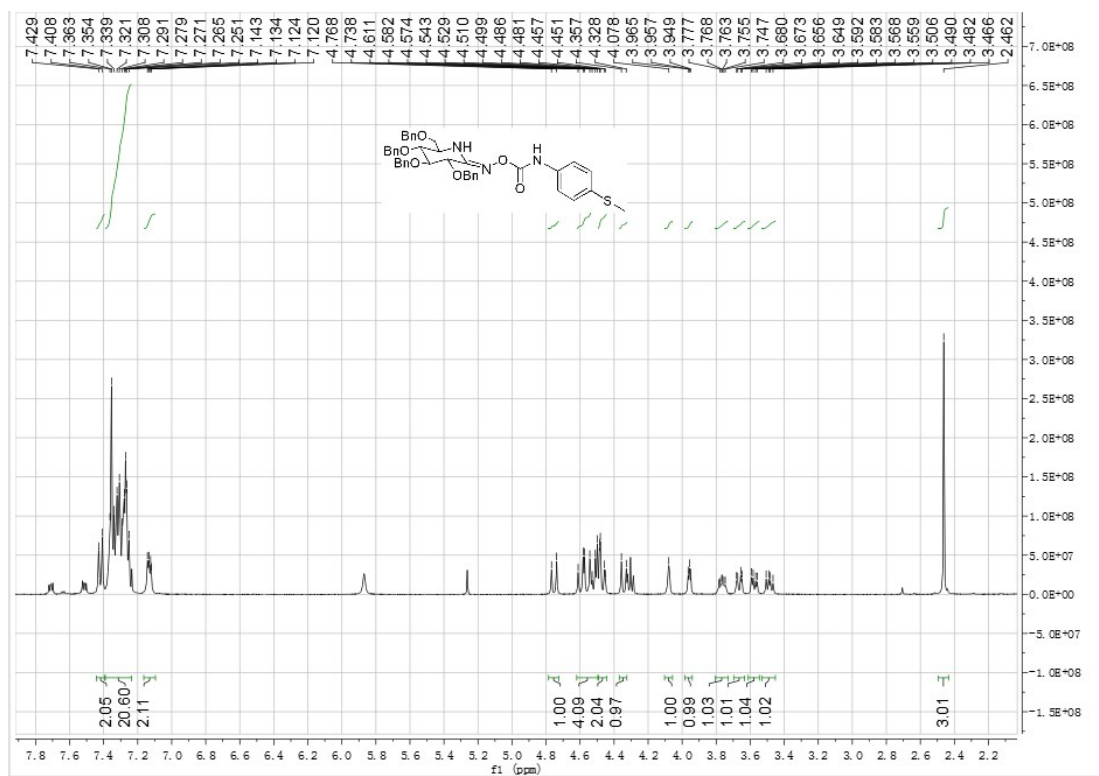
¹H NMR spectrum of **20**



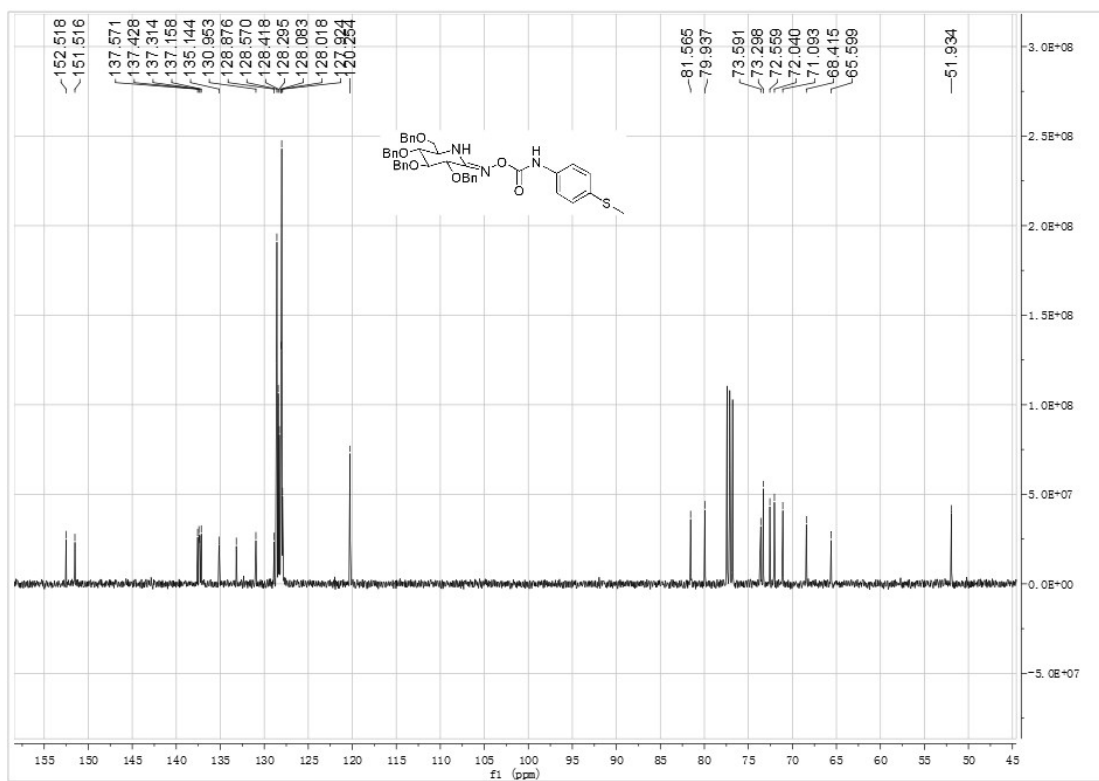
¹³C NMR spectrum of 20



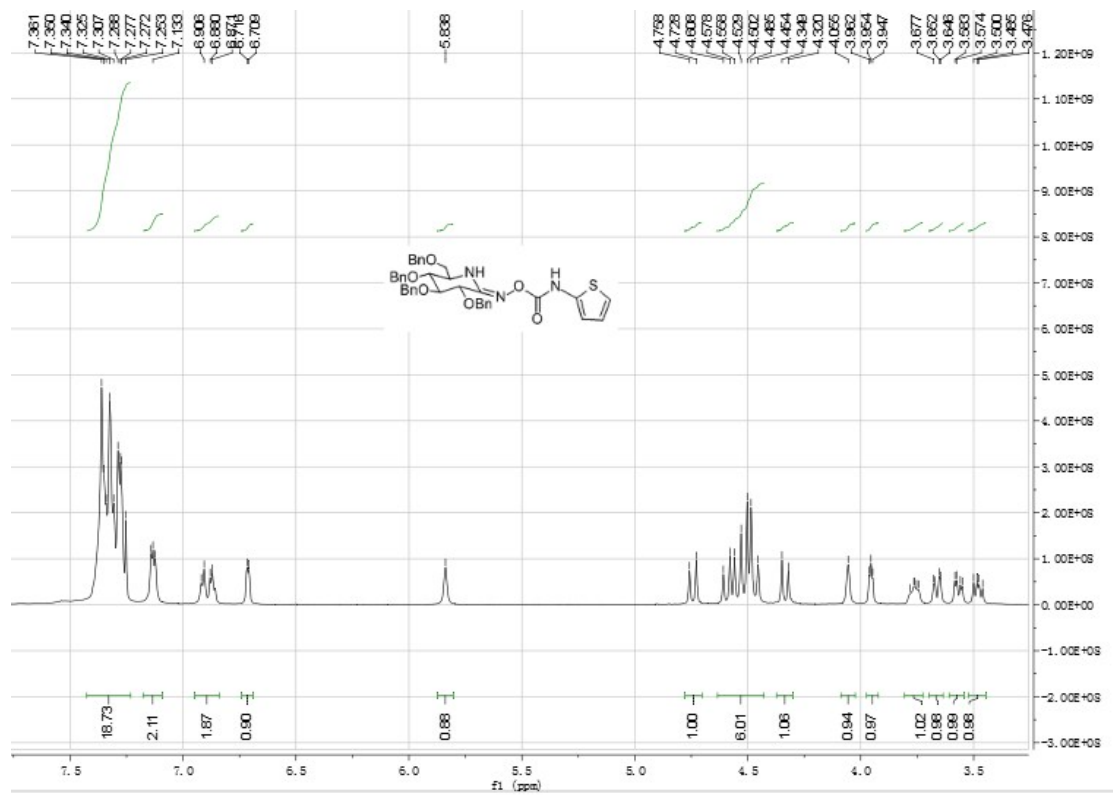
¹H NMR spectrum of 21



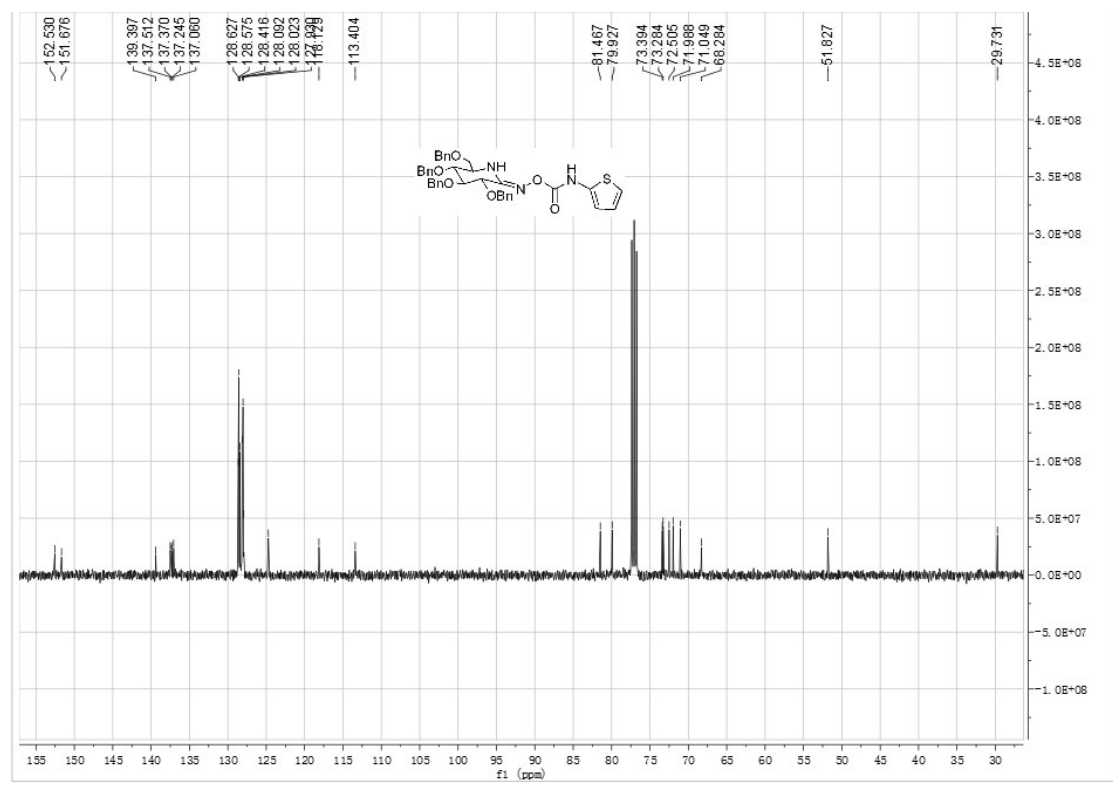
¹³C NMR spectrum of **21**



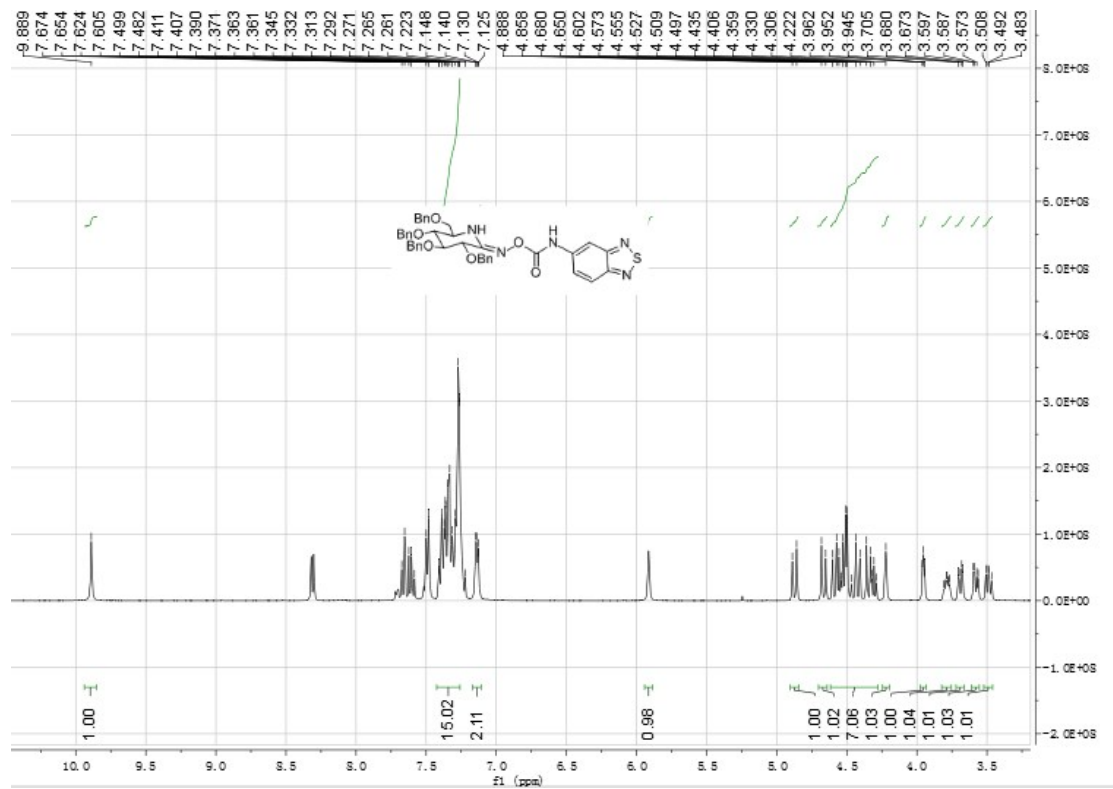
¹H NMR spectrum of **22**



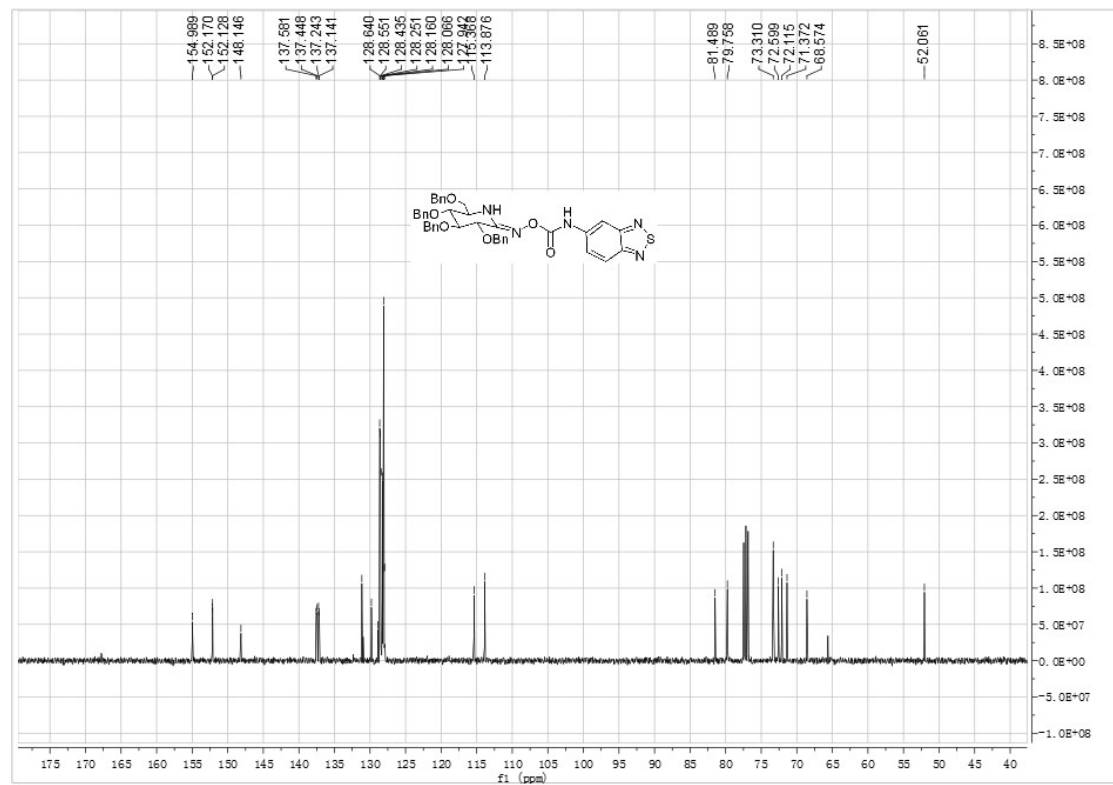
¹³C NMR spectrum of 22



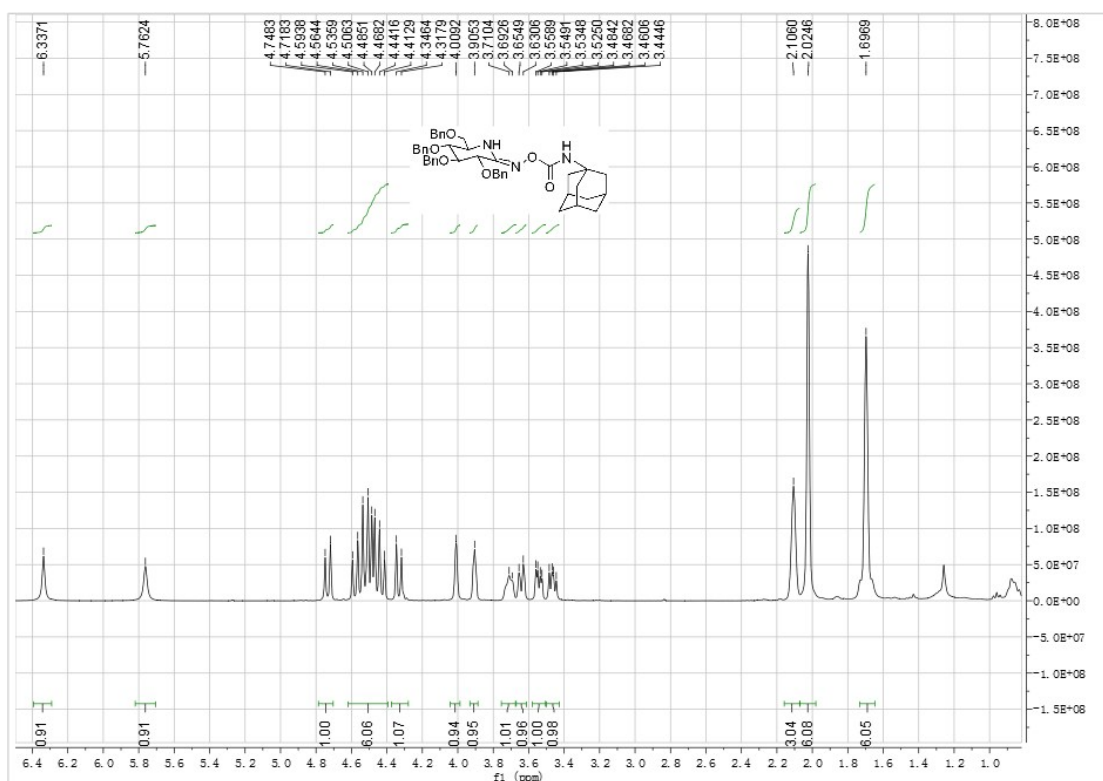
¹H NMR spectrum of 23



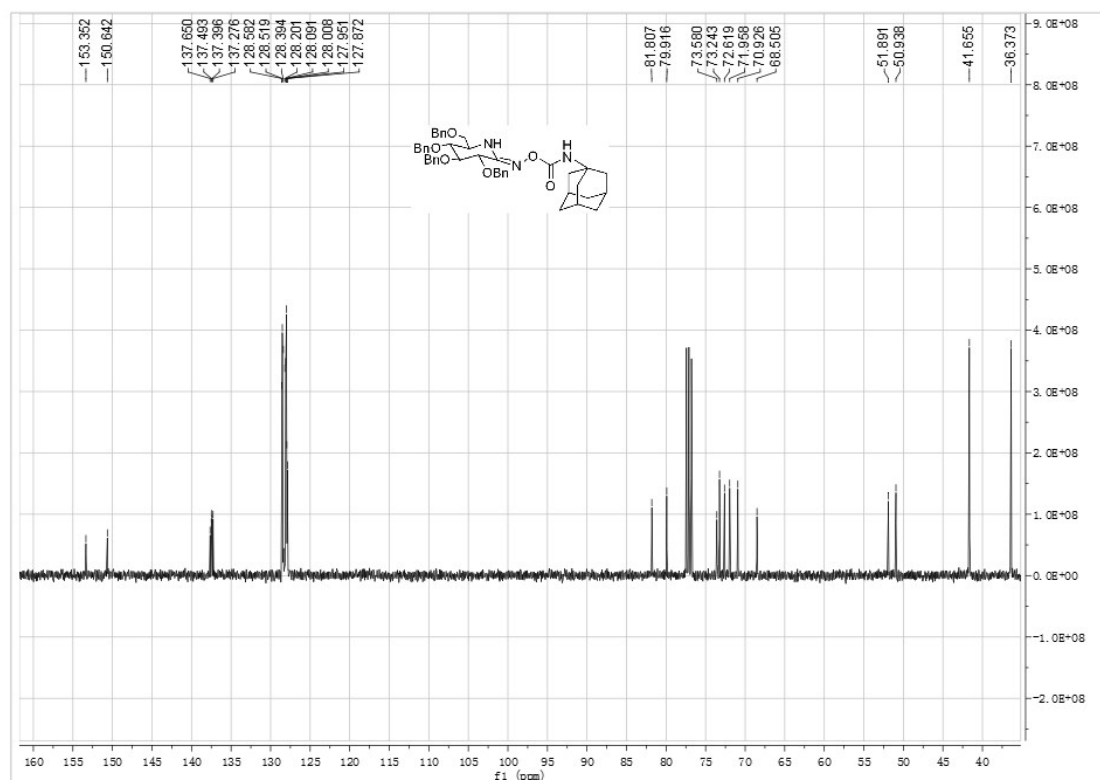
¹³C NMR spectrum of 23



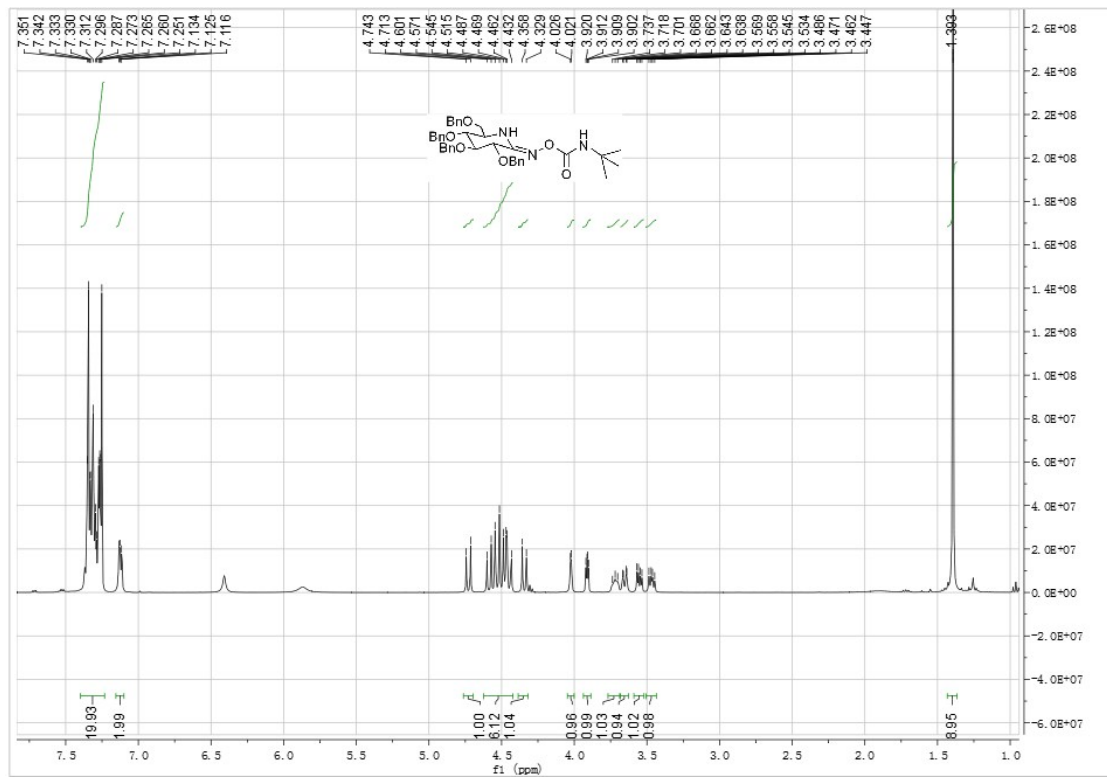
¹H NMR spectrum of 24



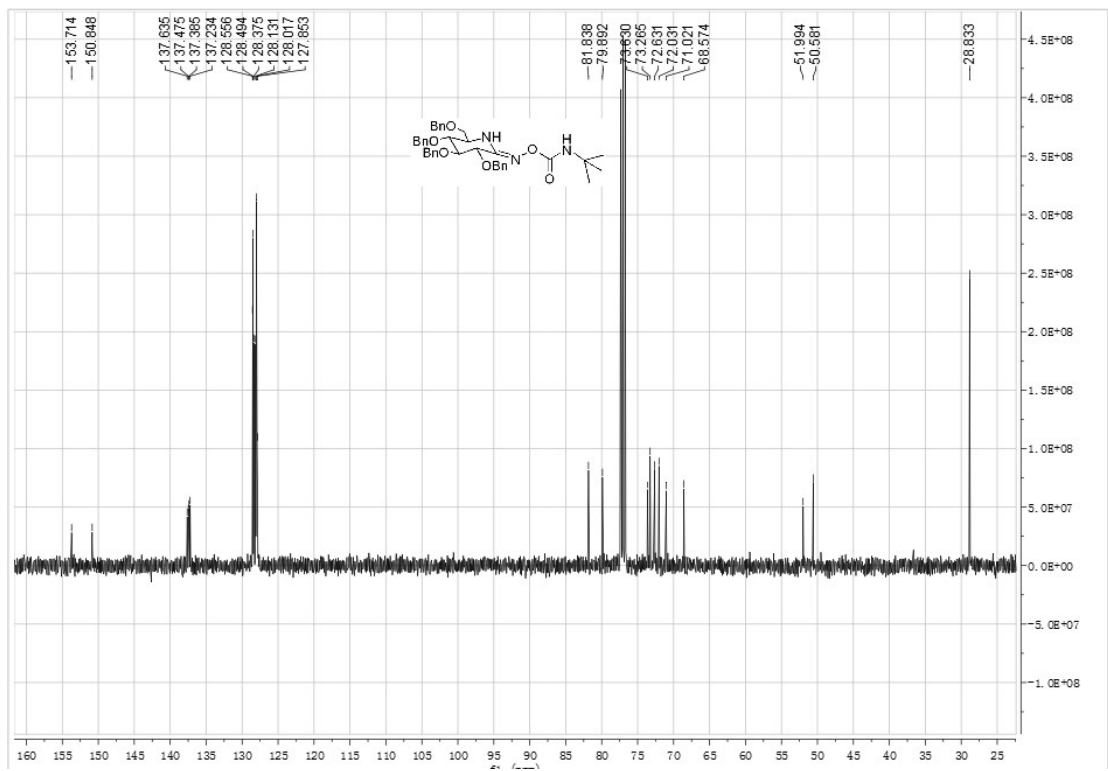
¹³C NMR spectrum of 24



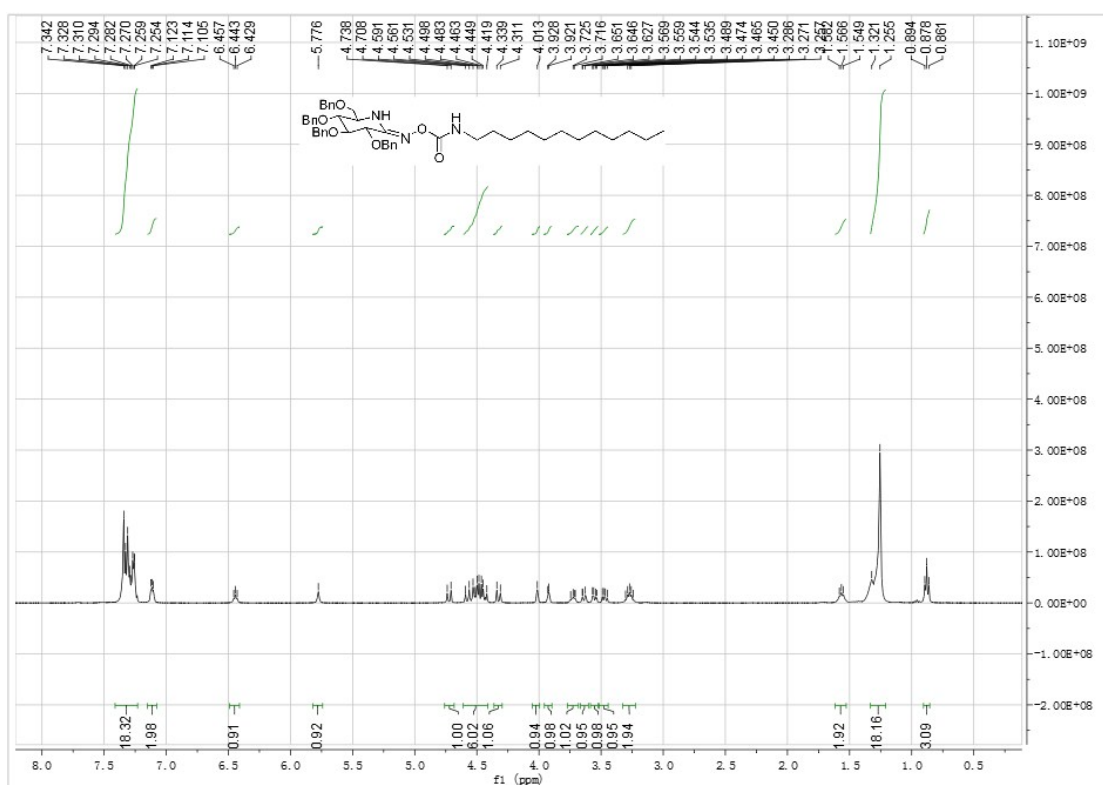
¹H NMR spectrum of 25



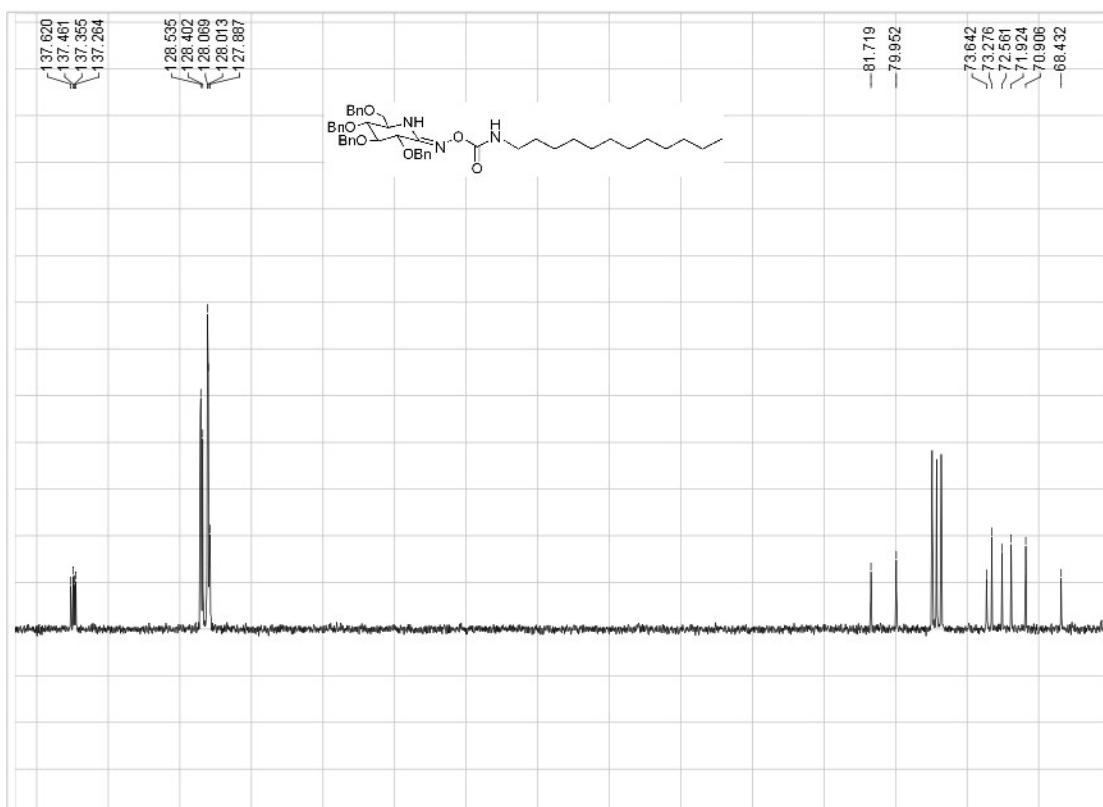
^{13}C NMR spectrum of **25**



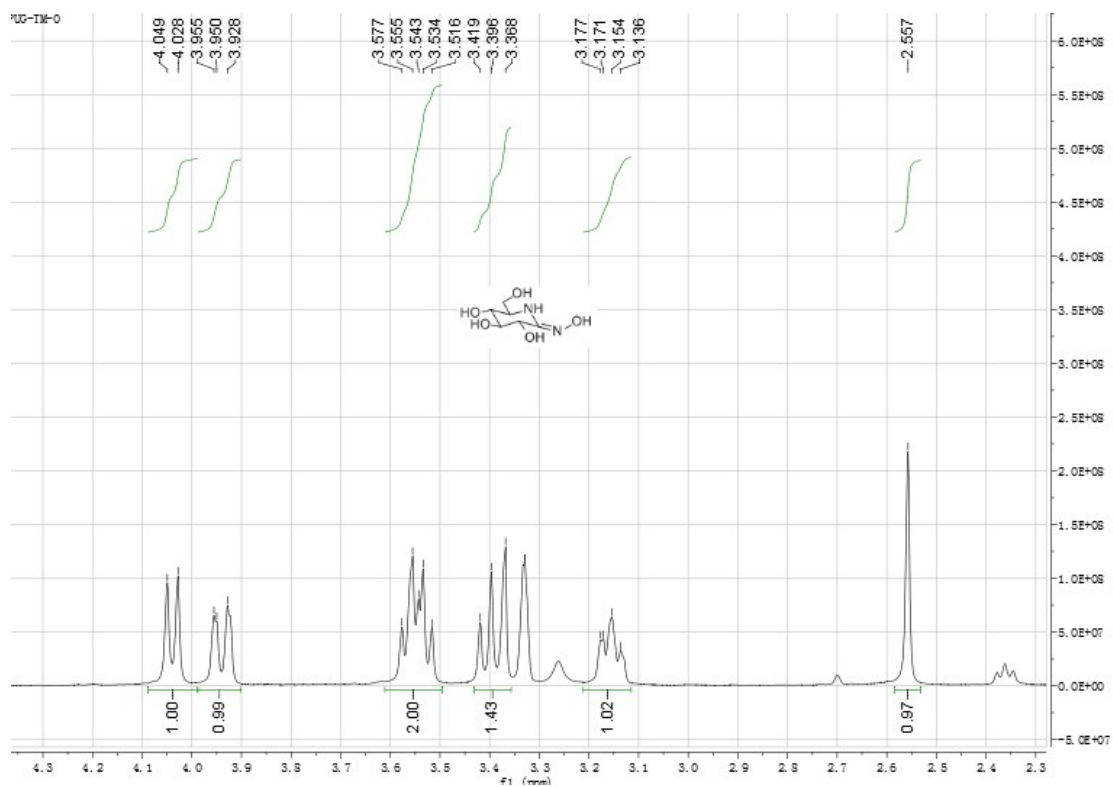
^1H NMR spectrum of **26**



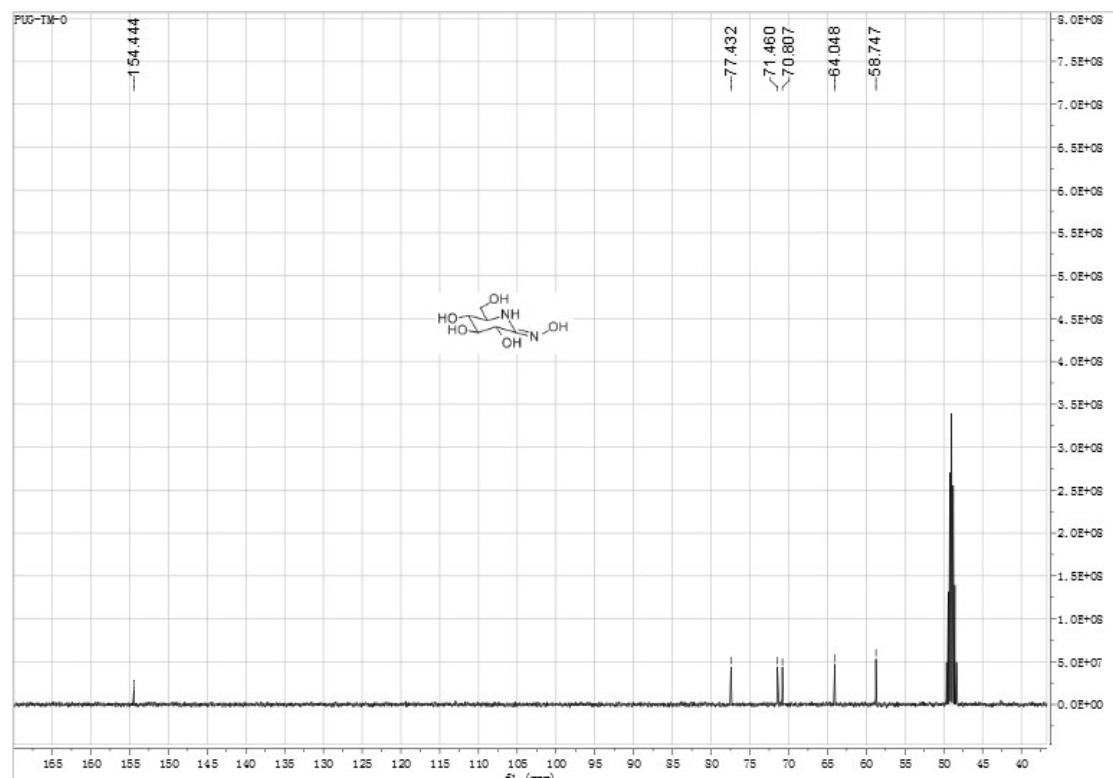
¹³C NMR spectrum of 26



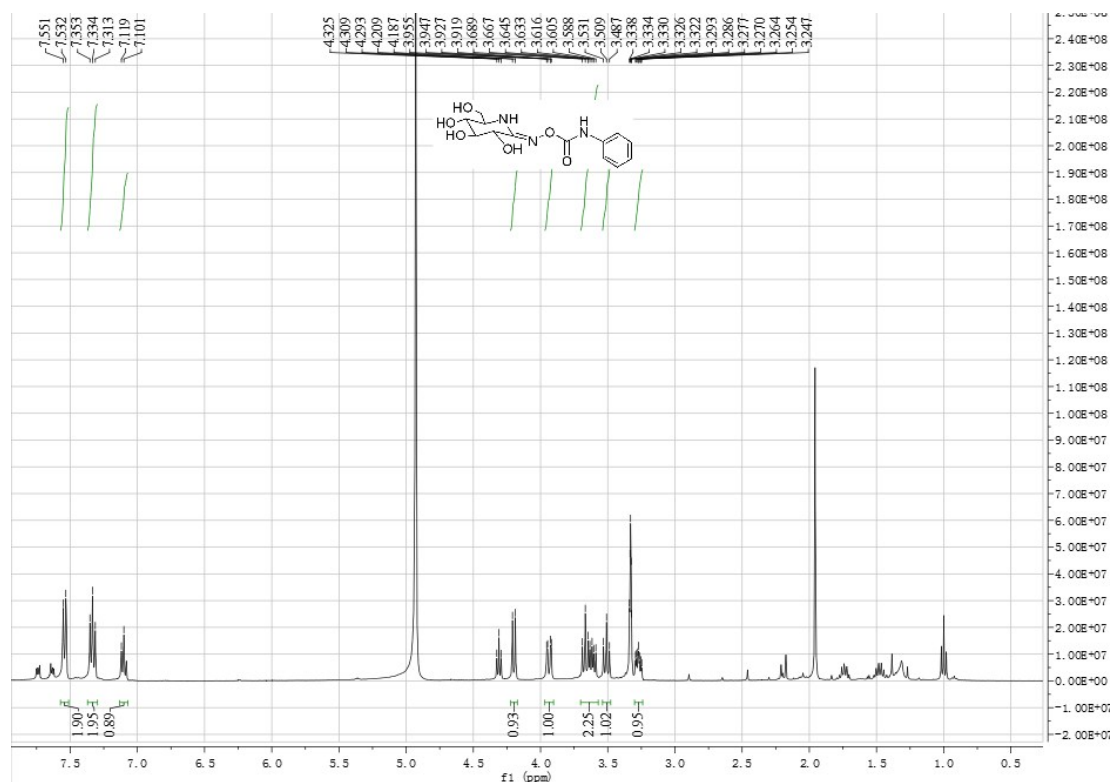
¹H NMR spectrum of 27



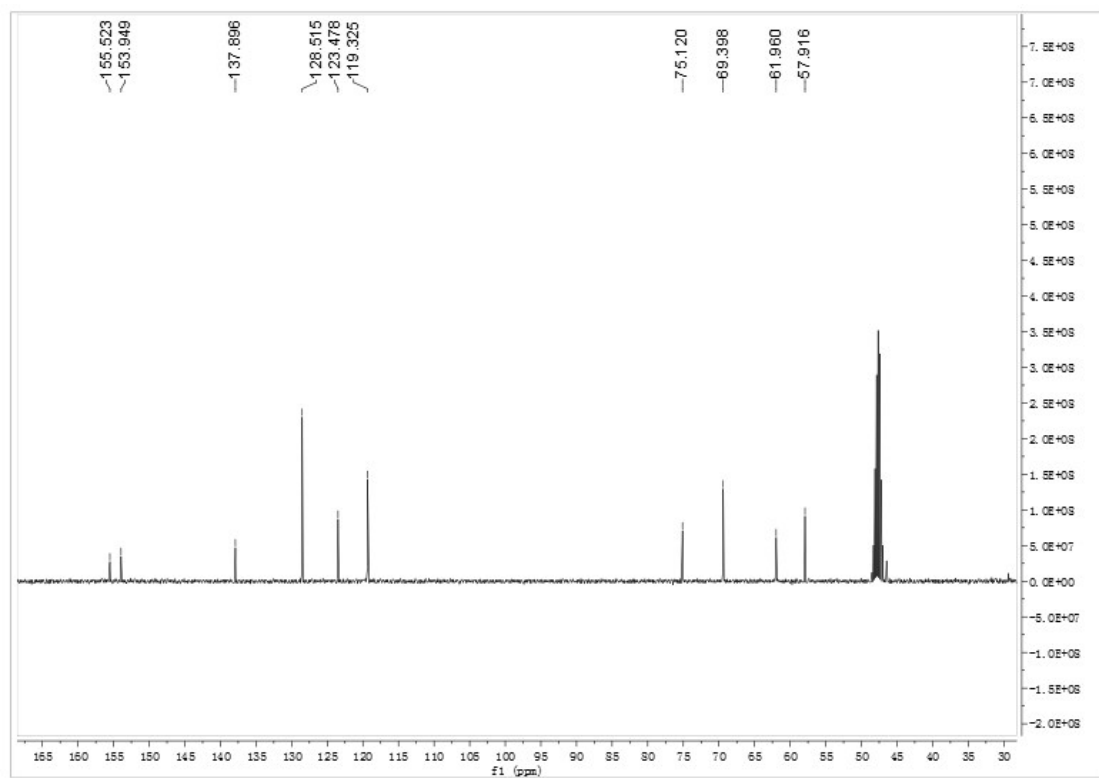
¹³C NMR spectrum of 27



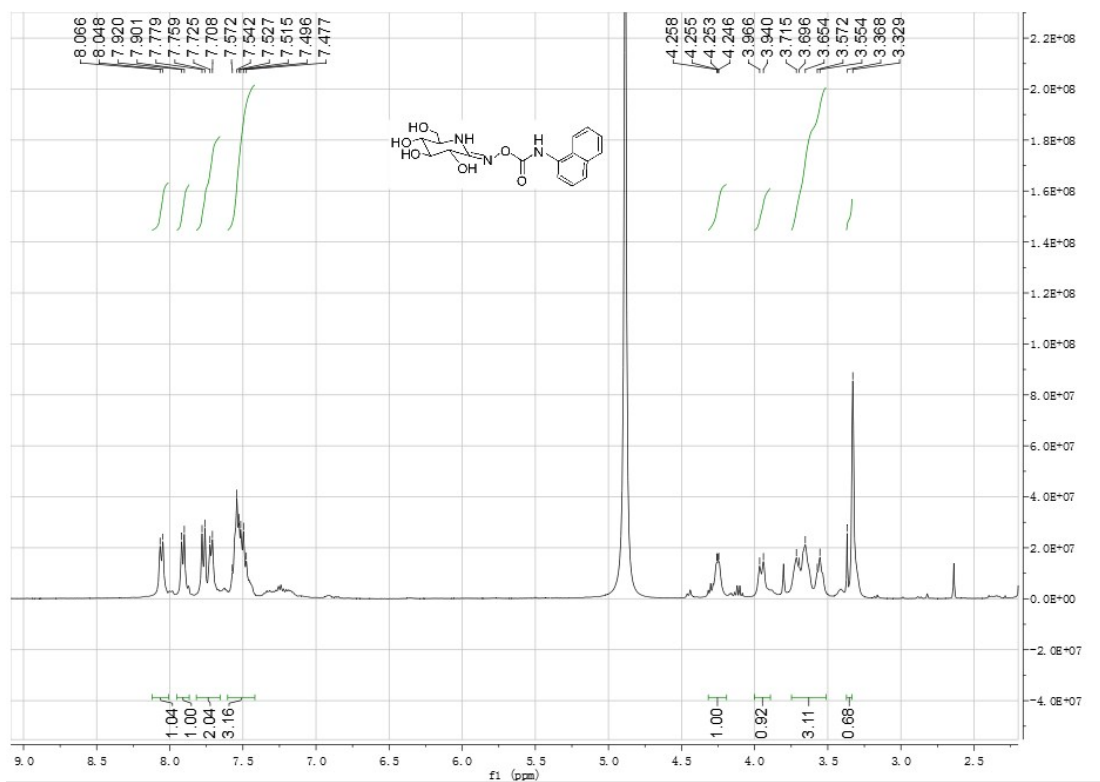
¹H NMR spectrum of **28**



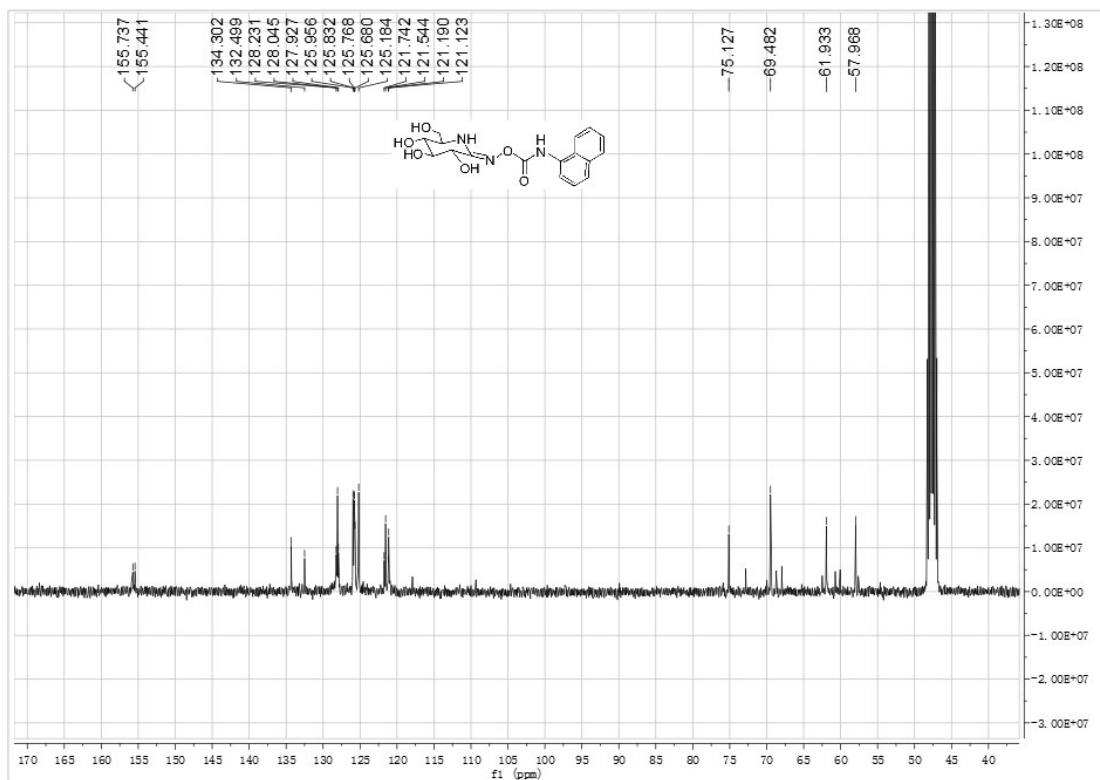
¹³C NMR spectrum of **28**



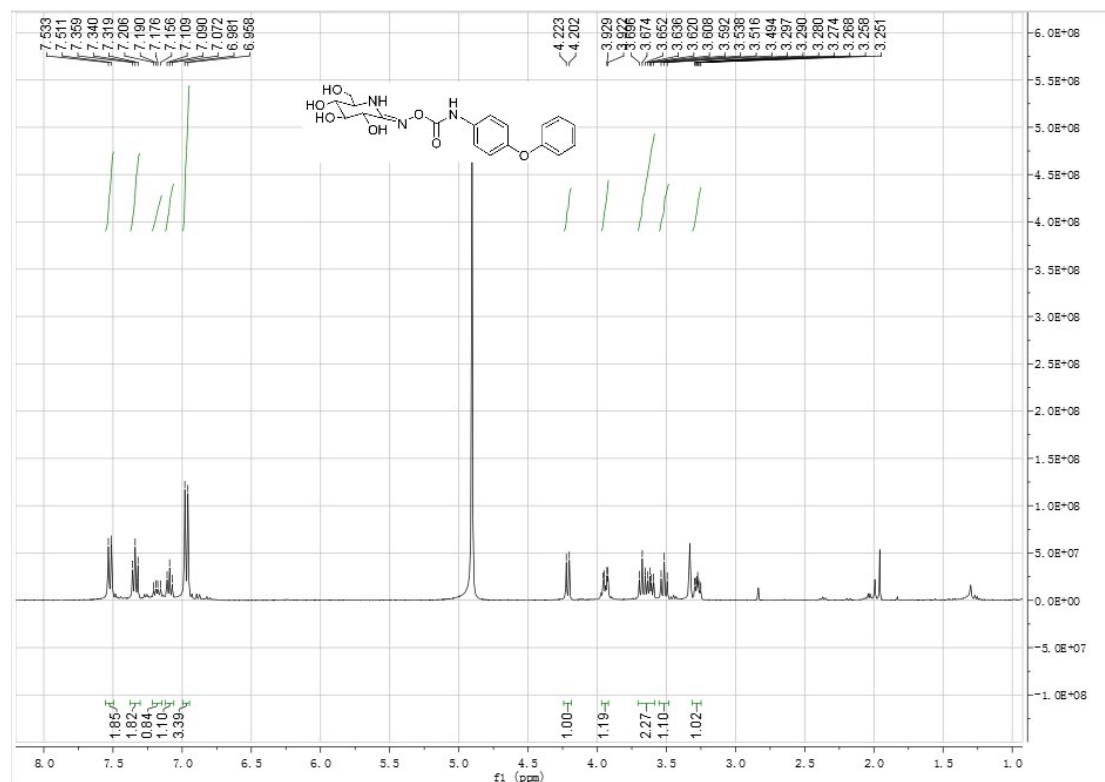
¹H NMR spectrum of 29



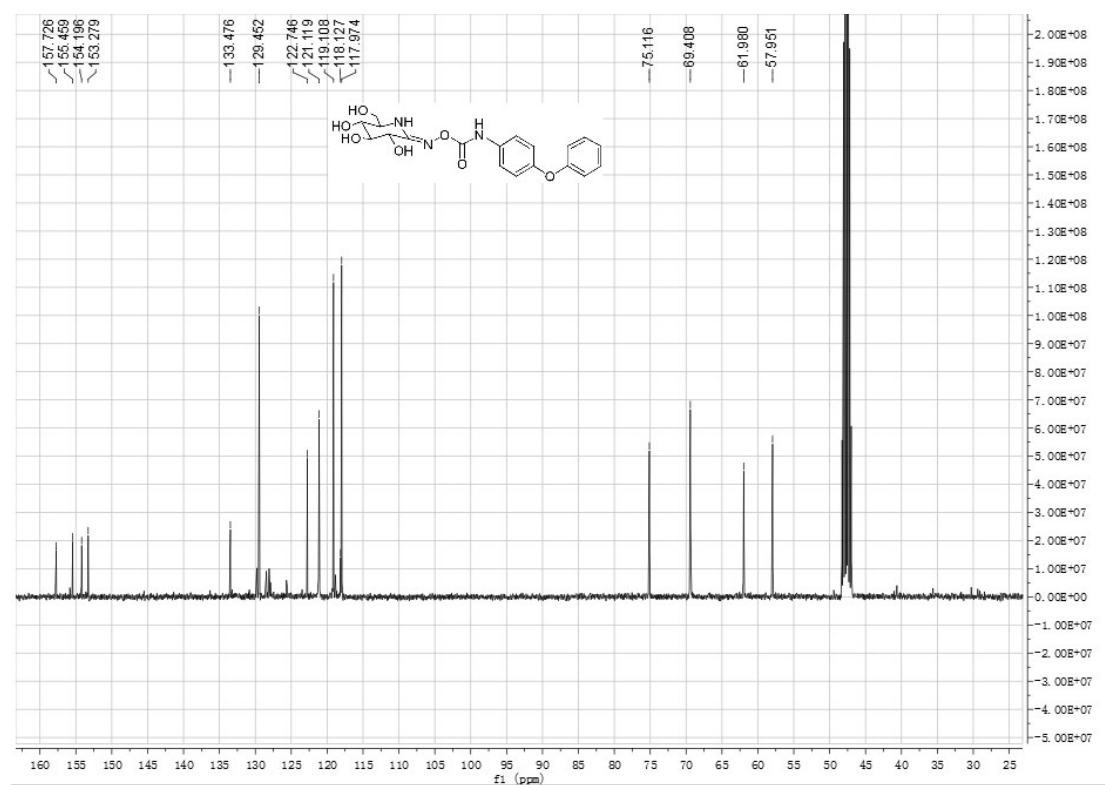
¹³C NMR spectrum of 29



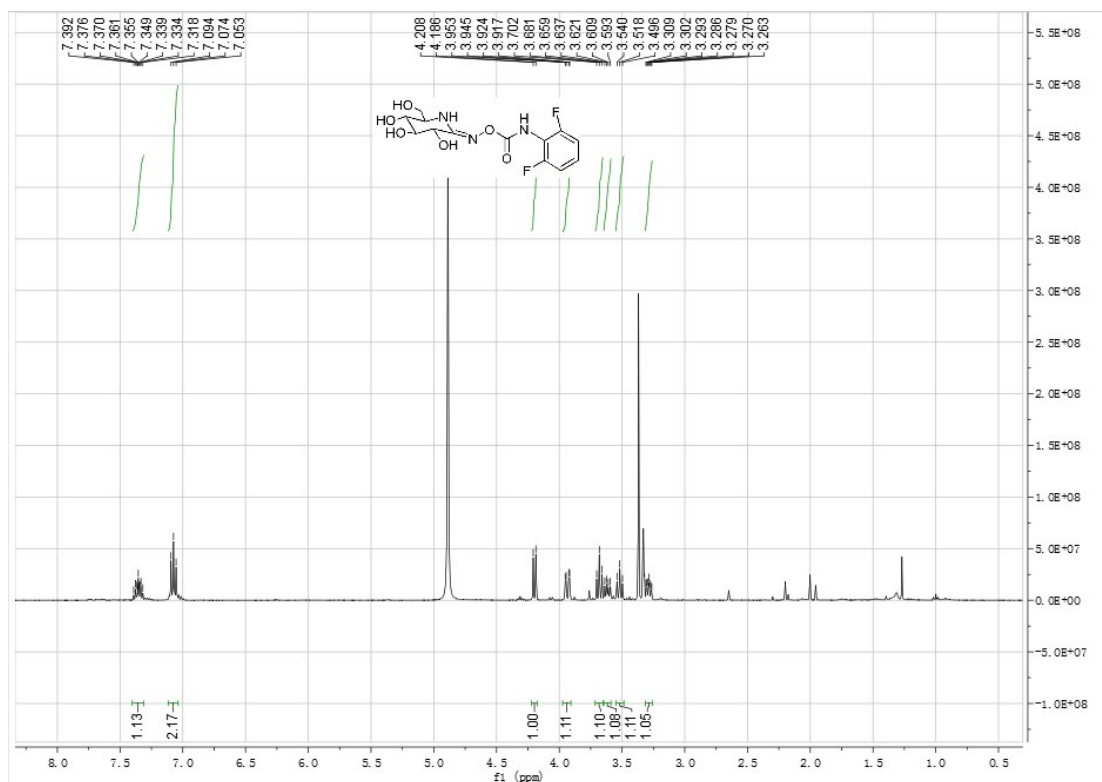
¹H NMR spectrum of **30**



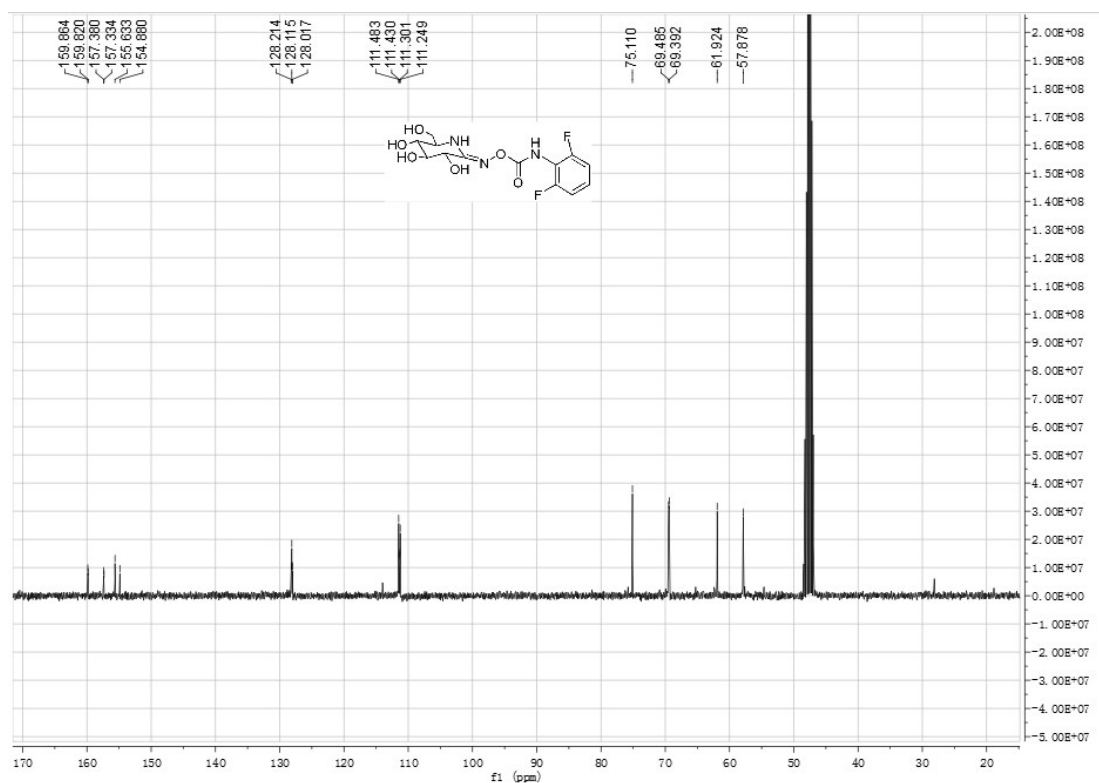
¹³C NMR spectrum of **30**



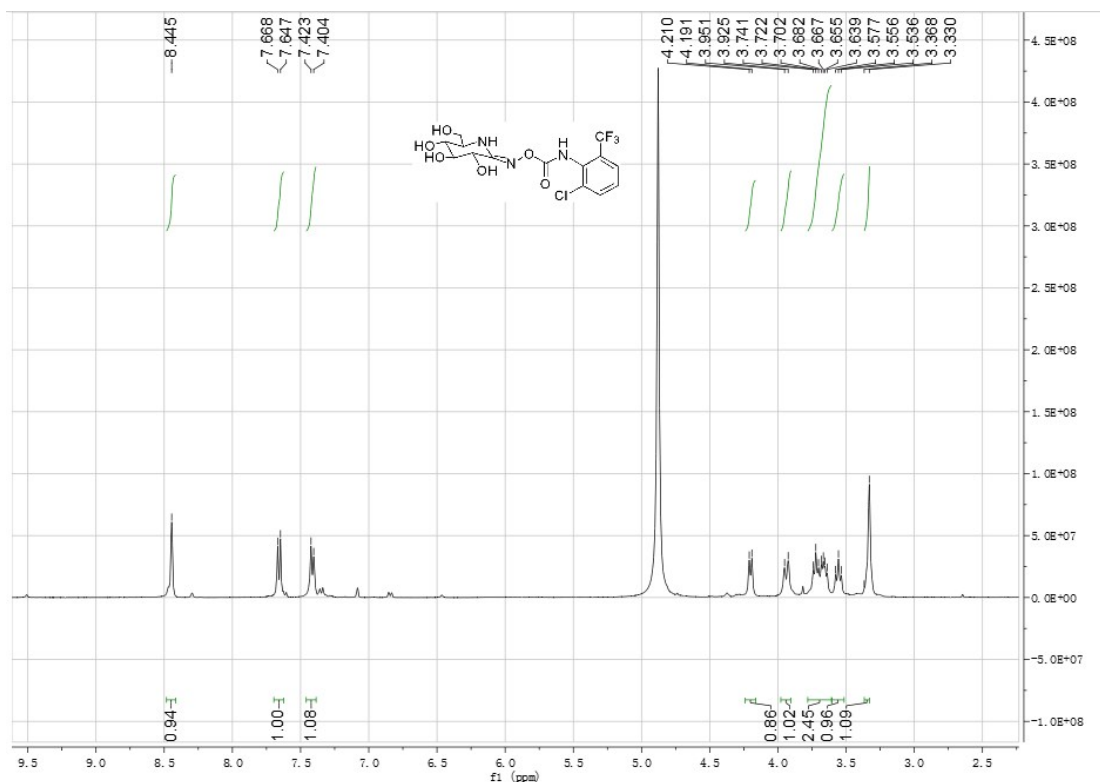
¹H NMR spectrum of **31**



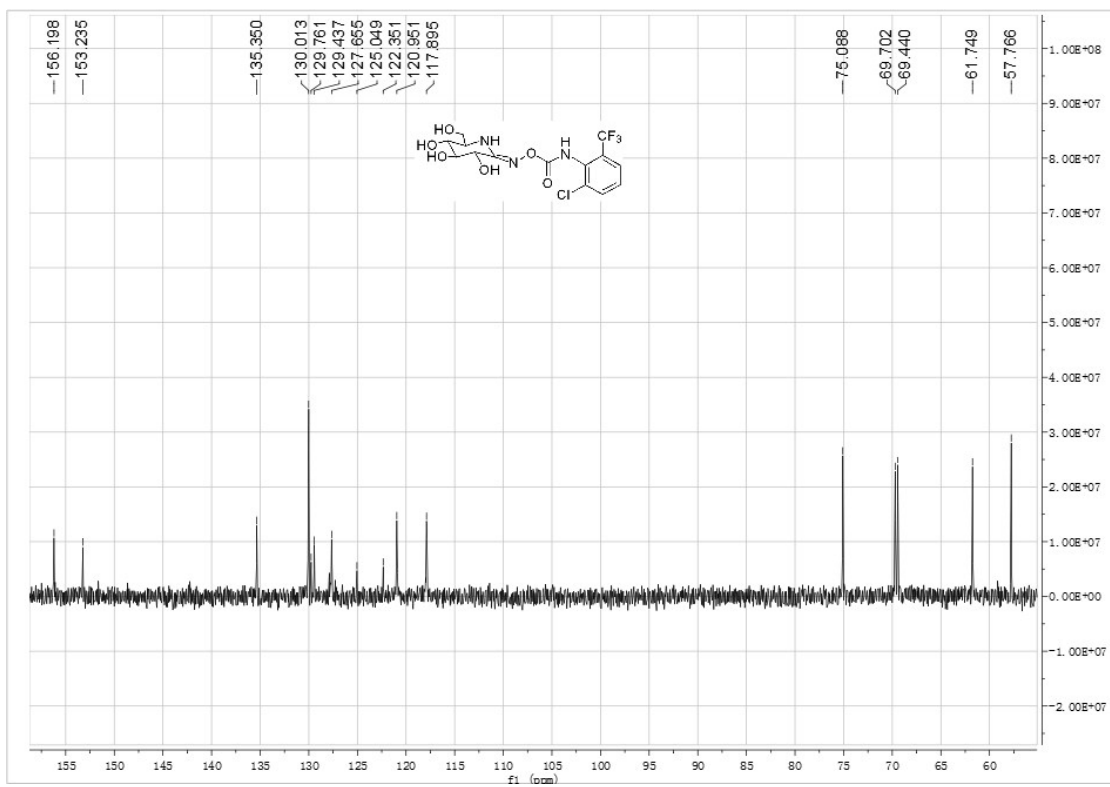
¹³C NMR spectrum of **31**



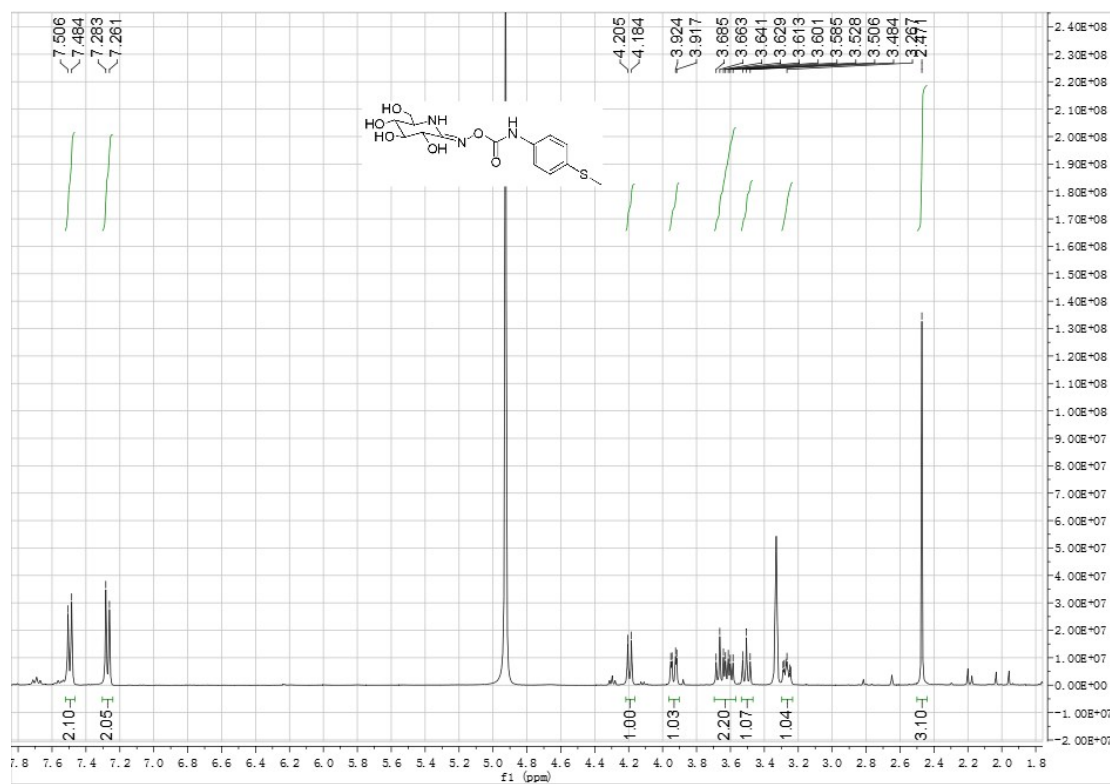
¹H NMR spectrum of **32**



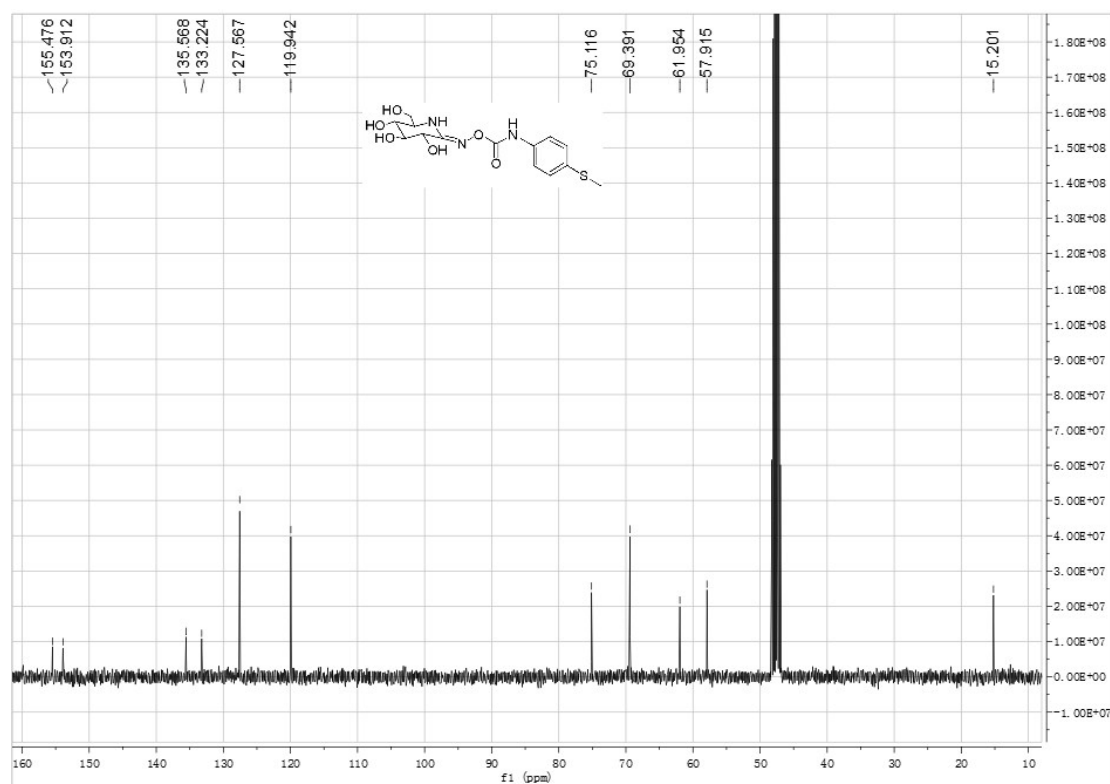
¹³C NMR spectrum of **32**



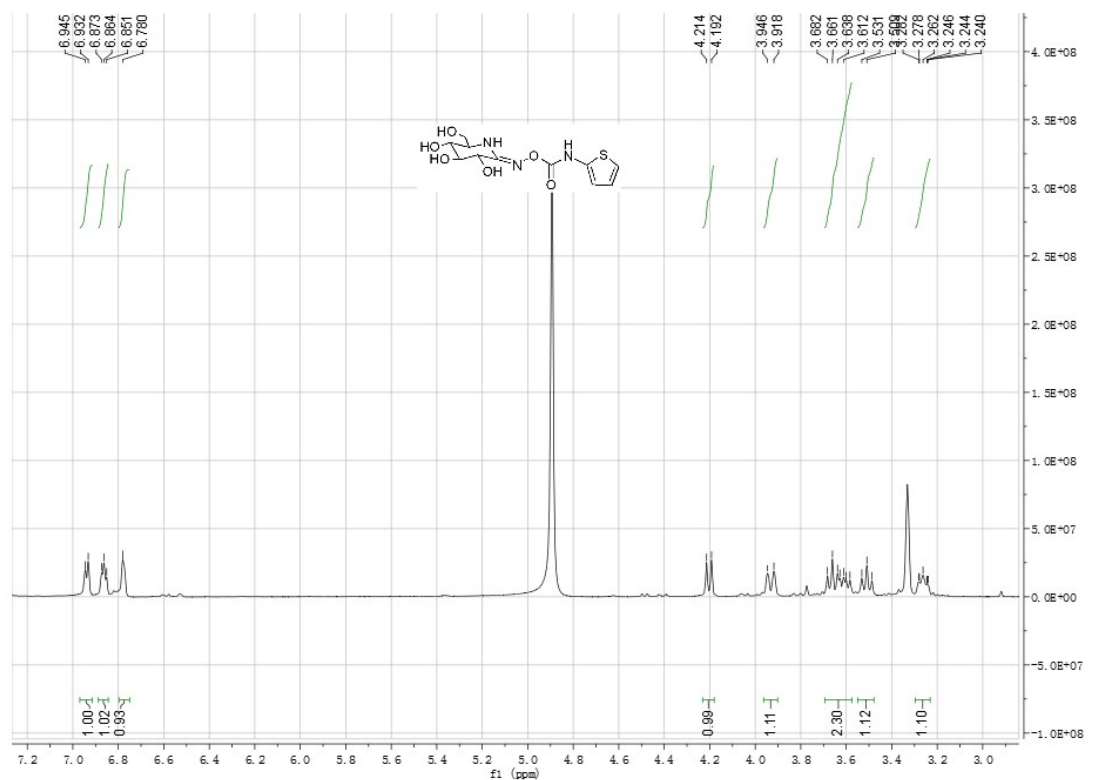
¹H NMR spectrum of **33**



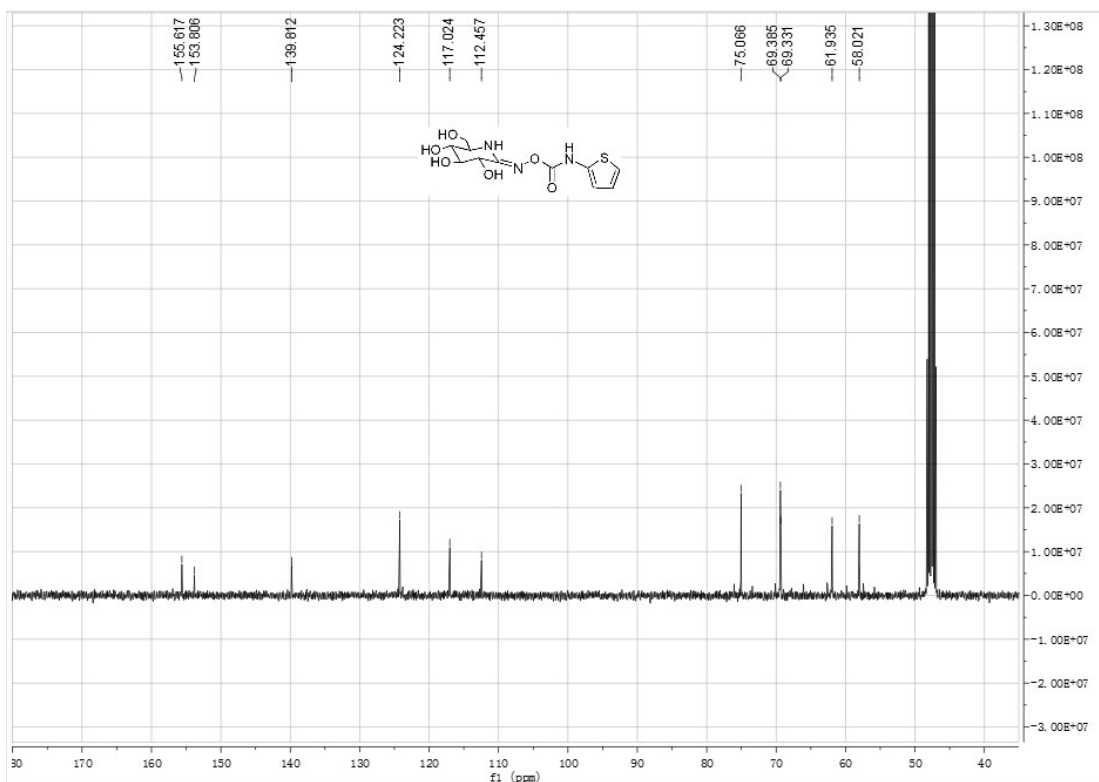
¹³C NMR spectrum of **33**



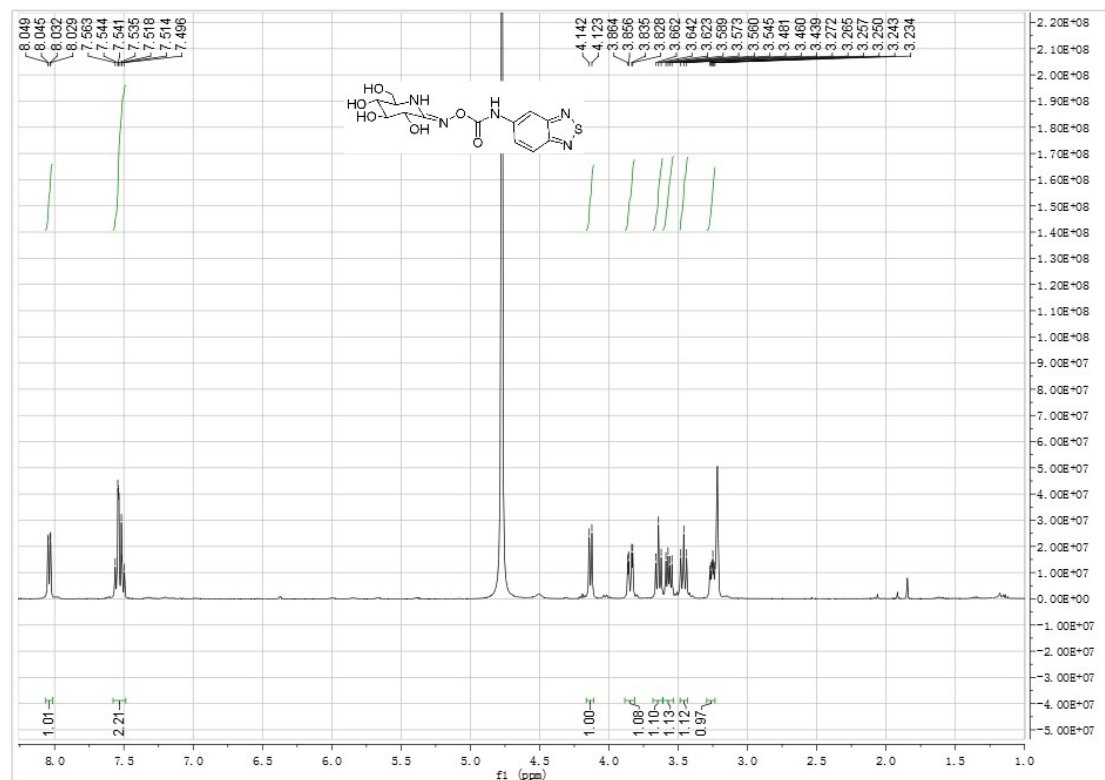
¹H NMR spectrum of **34**



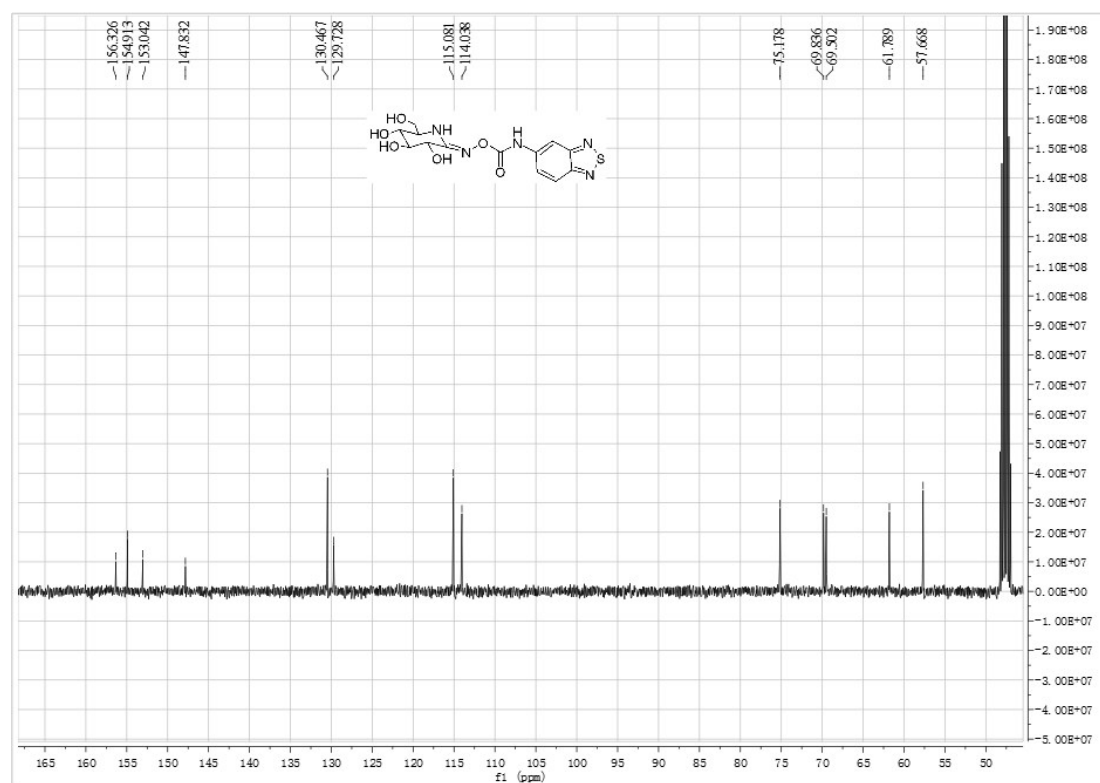
¹³C NMR spectrum of **34**



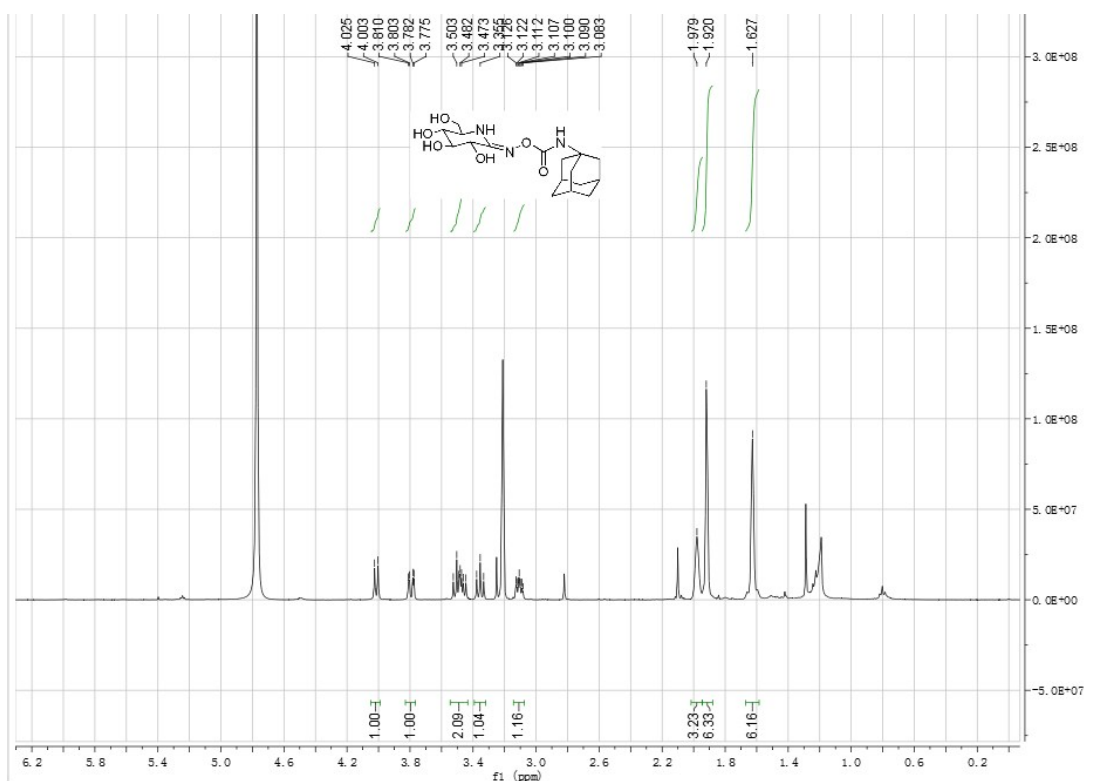
¹H NMR spectrum of **35**



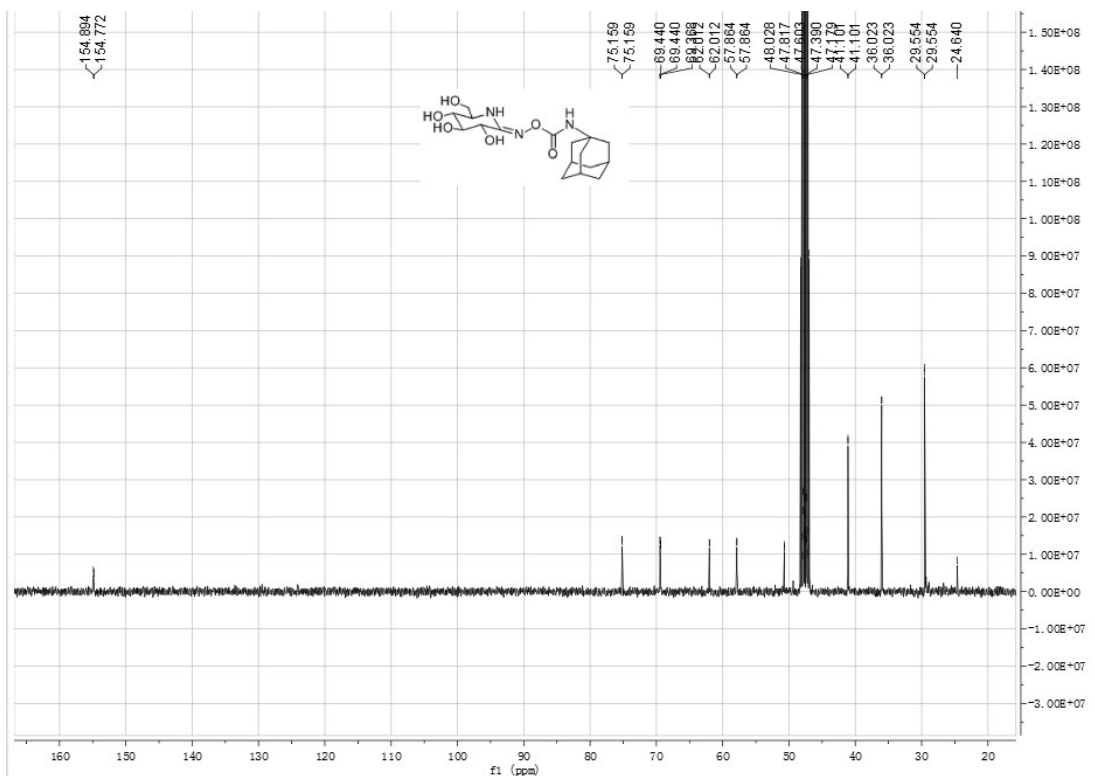
¹³C NMR spectrum of **35**



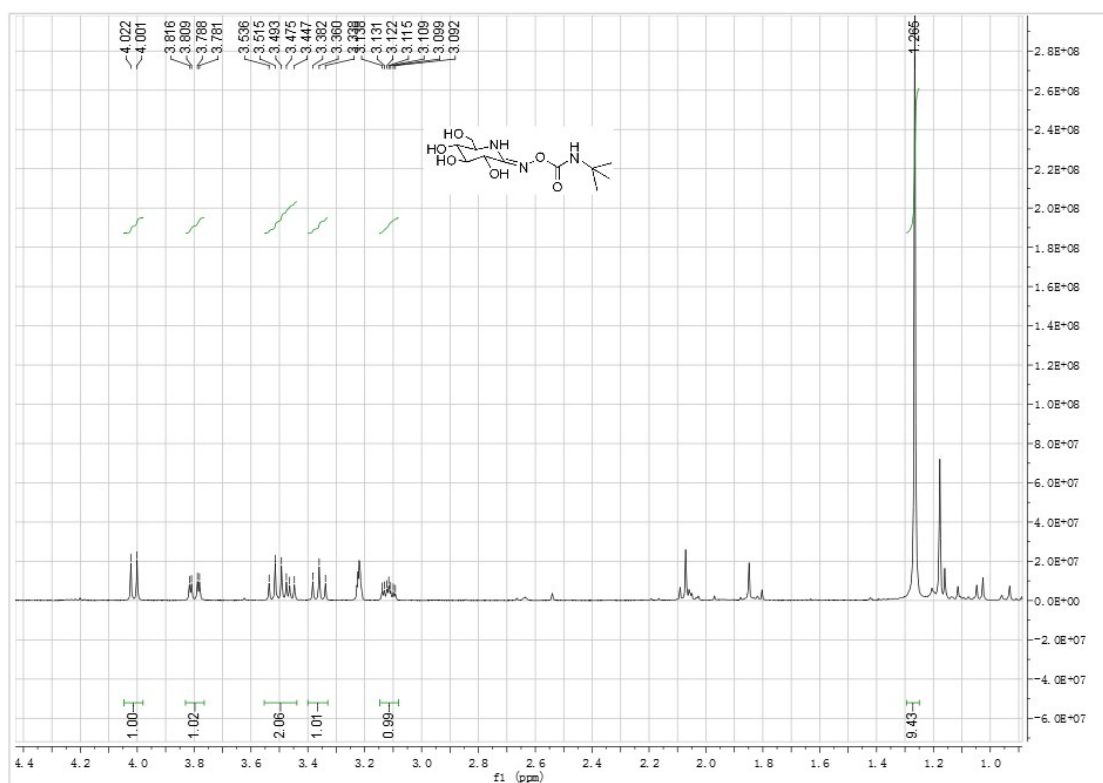
¹H NMR spectrum of **36**



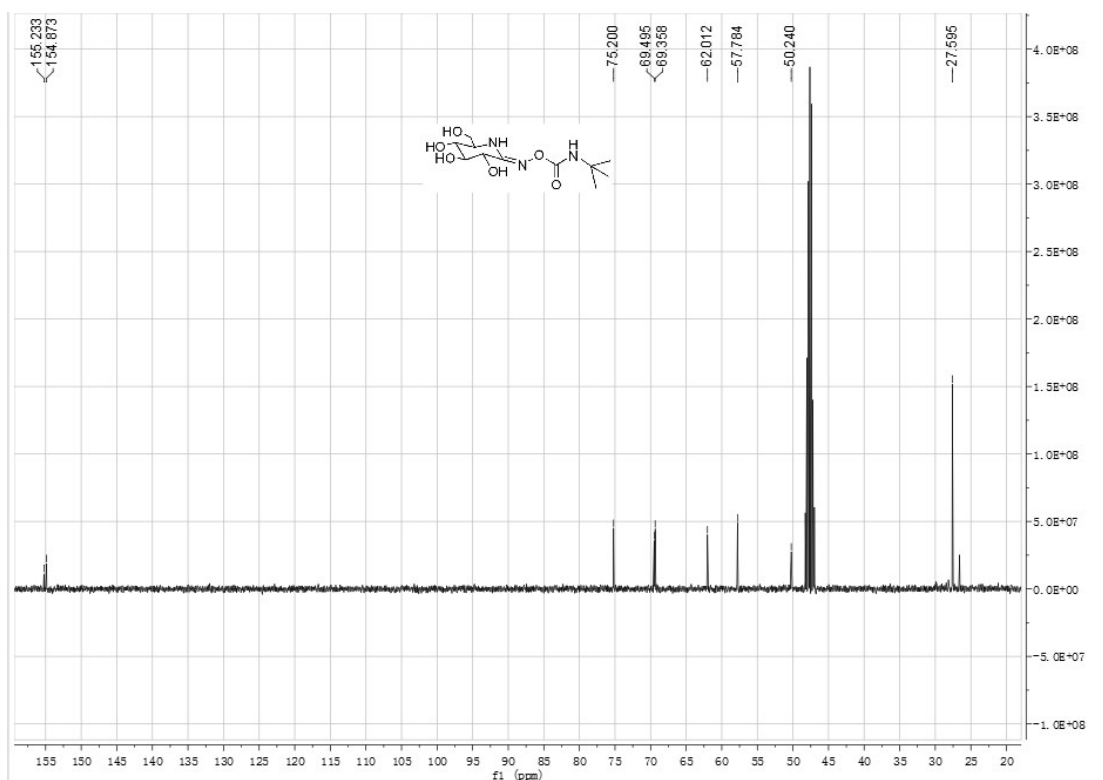
¹³C NMR spectrum of 36



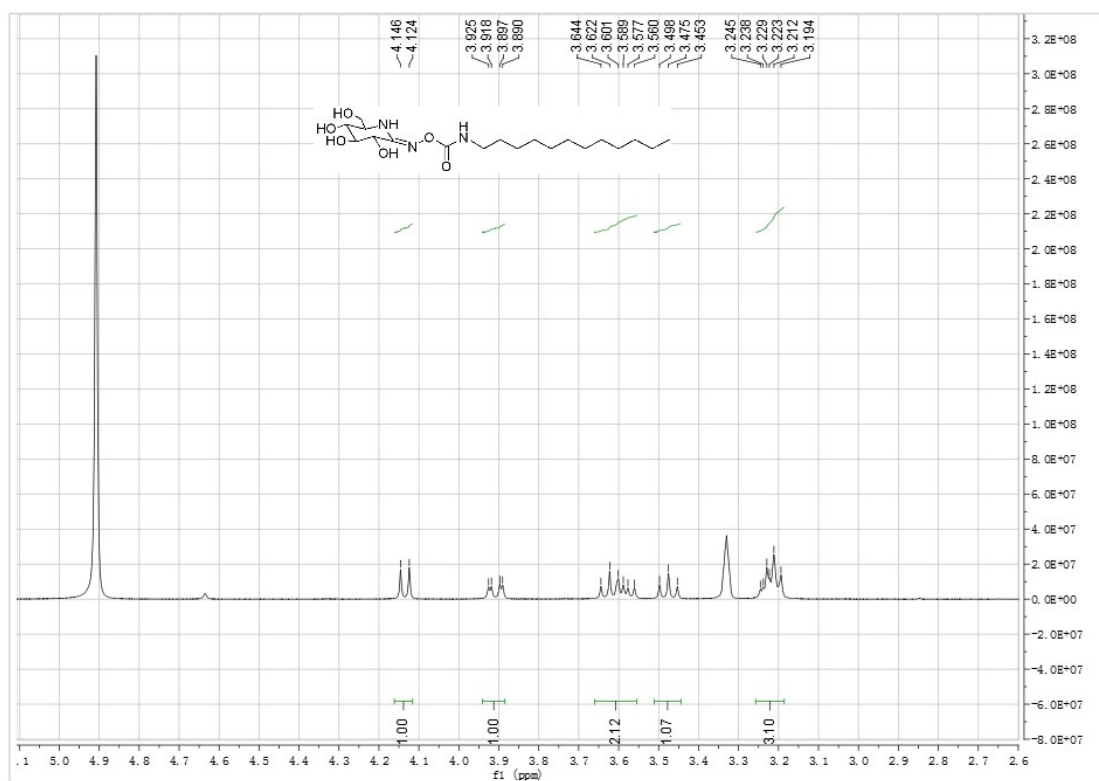
¹H NMR spectrum of 37



¹³C NMR spectrum of **37**



¹H NMR spectrum of **38**



^{13}C NMR spectrum of **38**

