Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Selective fluorogenic imaging of hepatocellular H_2S by a galactosyl azidonaphthalimide probe

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S1. Additional figures

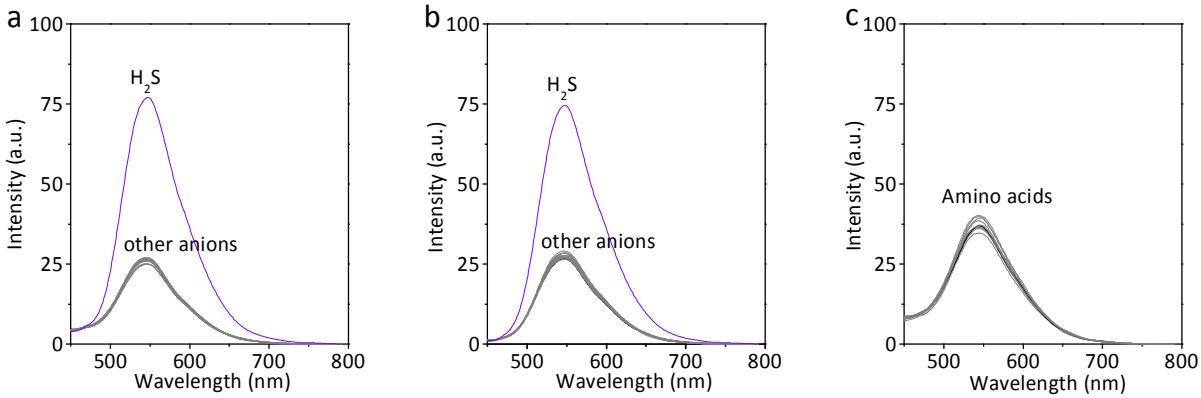


Figure S1. Fluorescence intensity of (a) **DT-OH** (10 μM) and (b) **DT-Gal** (10 μM) in the absence and presence of a variety of anions including F^-, Cl^-, Br^-, I^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, HSO_4^-, $S_2O_3^-$, PO_4^{3-}, HPO_4^{2-} and $H_2PO_4^-$ (100 μM). (c) Fluorescence intensity of **DT-Gal** (10 μM) in the absence and presence of a variety of amino acids including Phe, Ser, Thr, Pro, Val, His, Gly, Cys and GSH (100 μM). All fluorescence spectra were recorded in PBS (0.05 M, pH 7.4 or varied pH as indicated) with an excitation at 426 nm.

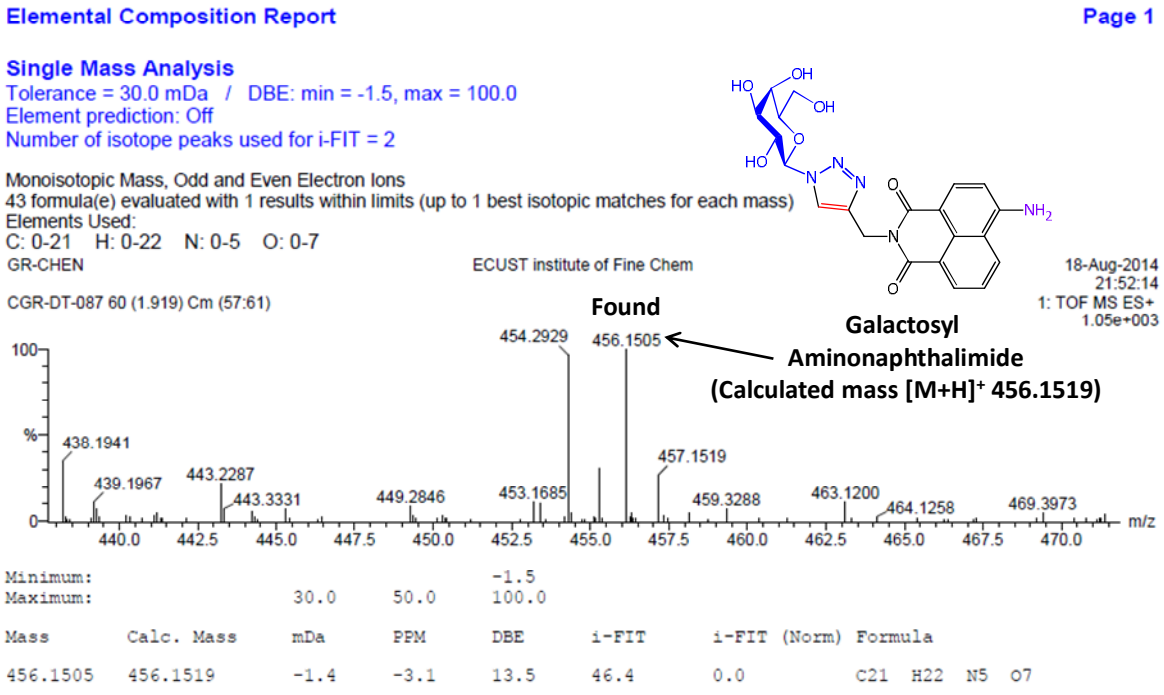


Figure S2. Mass spectrum of **DT-Gal** after reduction by H_2S to produce the fluorescent galactosyl aminonaphthalimide derivative (structure as shown in the figure) recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV).

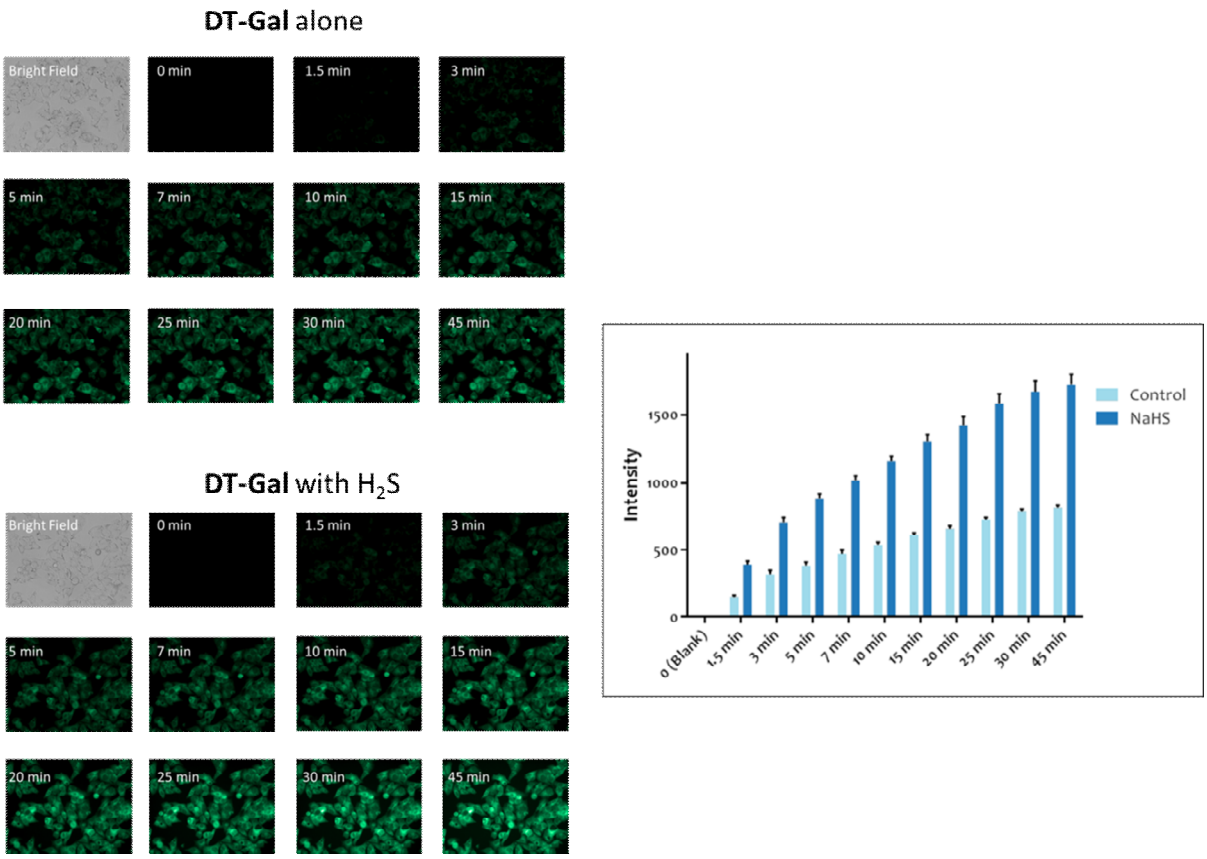


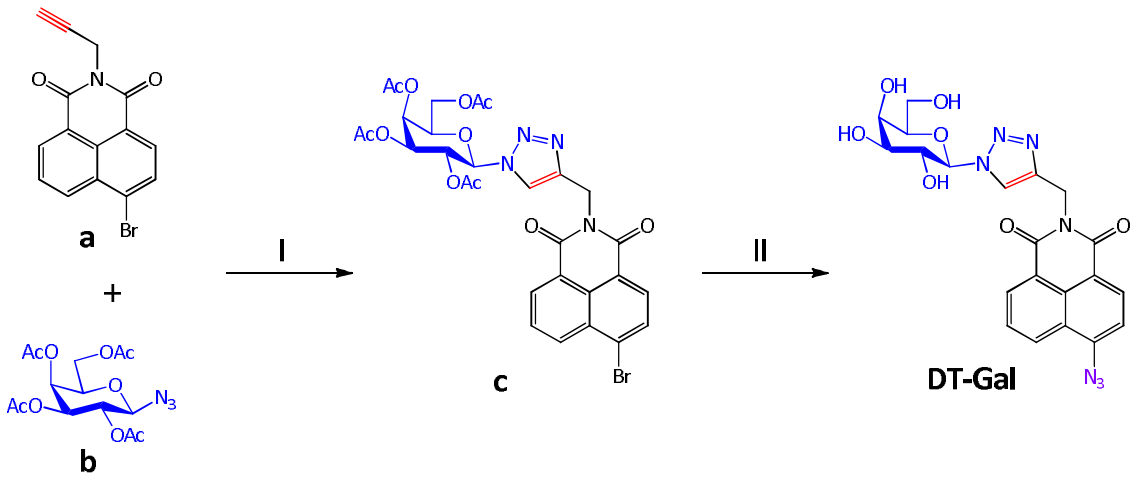
Figure S3. Fluorescence imaging (left) and quantification (right) of Hep-G2 cells with **DT-Gal** in the absence and presence of H_2S.

S2. Experimental section

General

All purchased chemicals and reagents are of analytical grade. Solvents were purified by standard procedures. Reactions were monitored by TLC (thin-layer chromatography) using E-Merck aluminum precoated plates of Silica Gel. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using tetramethylsilane (TMS) as the internal standard (chemical shifts in parts per million). High resolution mass spectra were recorded on a Waters LCT Premier XE spectrometer using standard conditions (ESI, 70 eV).

S2.1. Synthesis



Scheme S1. Reagents and conditions: (I) $CuSO_4 \cdot H_2O$ and Na ascorbate in H_2O/CH_2Cl_2. (II) NaN_3 in DMF, and then Et_3N.

Intermediate **c**: To a suspension of **a** (500 mg, 1.59 mmol)[1] and **b** (593.5 mg, 1.59 mmol)[2] in deionized water/CH_2Cl_2 (10 mL, v/v = 1:1) were added sodium ascorbate (126.8 mg, 0.64 mmol) and $CuSO_4 \cdot 5H_2O$ (79.5 mg, 0.32 mmol). The mixture was stirred at room temperature overnight. Then the mixture was diluted with CH_2Cl_2 and washed with water. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated and then purified by column chromatography (petroleum ether/EtOAc = 1:1, v/v) to give **c** (710.5 mg) in 65% yield. ^{1}H NMR (400 Hz, $CDCl_3$): δ 8.65 (d, J = 7.0 Hz, 1H), 8.52 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 7.7 Hz,

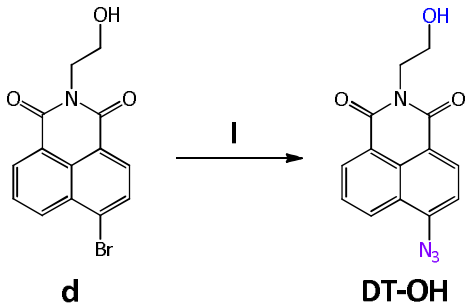
1H), 8.02–7.97 (m, 2H), 7.82 (t, *J* = 7.8 Hz, 1H), 5.84 (d, *J* = 8.6 Hz, 1H), 5.60-5.53 (m, 3H), 5.43 (d, *J* = 14.3 Hz, 1H), 5.23 (dd, *J* = 2.8, 10.4 Hz, 1H), 4.22–4.01 (m, 3H), 2.23 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): *δ* 170.3, 170.0, 169.8, 169.0, 163.2(2), 144.0, 133.5, 132.3, 131.5, 131.1, 130.6, 130.5, 129.0, 128.1, 122.8, 122.1, 122.0, 86.3, 74.0, 70.8, 67.8, 66.9, 61.2, 35.2, 20.7, 20.6, 20.4, 20.2.

HRMS (ESI) *m/z*: calcd. for $[M + H]^+$ $C_{29}H_{28}BrN_4O_{11}$ 687.0938, found 687.0934.

DT-Gal: An aqueous solution of NaN_3 (175 mg, 2.69 mmol) was added dropwise to a DMF solution of **c** (500 mg, 0.72 mmol). The reaction mixture was stirred at 100 °C for 8 h. Then the mixture was poured into iced water to generate a yellow solid which was filtered and dried in a vacuum dried oven. The dried precipitant was dissolved in MeOH/H_2O (8:1, v/v), followed by addition of excessive Et_3N. The mixture was stirred at room temperature for 1 h. Then solvent was removed and the resulting crude product was purified by column chromatography (CH_2Cl_2/MeOH = 4:1, v/v) to give **DT-Gal** (135 mg) in 38% yield.

^{1}H NMR (400 Hz, DMSO-d_6): *δ* 8.58 (dd, *J* = 1.2, 7.2 Hz, 1H), 8.53 (d, *J* = 8.0 Hz, 1H), 8.47 (dd, *J* = 1.2, 7.2 Hz, 1H), 8.18 (s, 1H), 7.90 (dd, *J* = 7.2, 8.4 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 5.42 (d, *J* = 9.2 Hz, 1H), 5.33–5.28 (m, 2H), 5.24 (d, *J* = 6.0 Hz, 1H), 5.09 (d, *J* = 5.2 Hz, 1H), 4.79 (t, *J* = 6.0 Hz, 1H), 4.71 (d, *J* = 5.2 Hz, 1H), 3.74 (t, *J* = 4.0 Hz, 1H), 3.65 (t, *J* = 6.0 Hz, 1H), 3.53-3.41 (m, 4H, OH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 163.0, 162.5, 143.1, 143.0, 131.8, 131.7, 128.5, 128.4, 127.3, 123.5, 122.0, 121.9, 118.0, 116.0, 88.0, 78.3, 73.7, 69.3, 68.1, 60.1, 45.1.

HRMS (ESI) *m/z*: calcd. for $[M + Na]^+$ $C_{21}H_{19}N_7O_7Na$ 504.1244, found 504.1238.



d **DT-OH**

Scheme S2. Reagents and conditions: (I) NaN_3 in DMF.

To a DMF solution of **d** (500 mg, 1.56 mmol)[3] was added NaN_3 (175mg, 2.69 mmol). The reaction mixture was stirred at 100 °C for 8 h. Then the mixture was poured into iced water to precipitate a yellow solid which was filtered and dried in a vacuum dried oven. The dried precipitant was purified by column chromatography (petroleum ether/EtOAc = 1:1, v/v) to give **DT-OH** (375 mg) in 85% yield.

HRMS (ESI) *m/z*: calcd. for $[M + H]^+$ $C_{14}H_{11}N_4O_3$: 283.0831, found: 283.0833.[4]

S2.2. Fluorescence spectroscopy

All fluorescence spectra were measured on a Varian Cary Eclipse Fluorescence spectrometer. Stock solutions of **DT-Gal** and **DT-OH** were prepared in DMSO (1 mM), which were then transferred to 10 mL volumetric flasks. Then, phosphate buffered saline (PBS, 0.05 M, pH 7.4) was added dropwise under vigorous stirring to furnish 1.0×10^{-5} M solutions (DMSO/PBS = 1:99, v/v). For titration experiments, increasing NaHS (0-200 μM) was added to the solution of **DT-Gal** and **DT-OH** (10 μM), incubated for 5 min, and then were the fluorescence spectra recorded. For selectivity measurements, various selected anions and amino acids (100 μM) were added into the solution of **DT-Gal** and **DT-OH** (10 μM), incubated for 5 min, and then were the fluorescence spectra recorded.

S2.3. Cellular assays

Cell culture. Hep-G2 and Hela cells were maintained in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% Fetal bovine serum (Gibco, Gland Island, NY, USA), HCT-116 were maintained in McCoy's 5A Medium (sigma-aldrich (Shanghai), Shanghai, China) supplemented with 10% FBS, and A549 were maintained in Ham's F-12 Nutrient Mixture (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS. All the cell lines were passaged every 3-4 days and cultured in a 37 ^oC humidified incubator under an atmosphere of 5% CO_2 in air.

Cellular imaging assay. For the experiments, cells (2.0×10^5) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) and were allowed to

adhere overnight. Cells were incubated with **DT-Gal** (30 μM, in PBS) or **DT-OH** (30 μM, in PBS) for 30 min with or without pretreatment of NaHS (500 μM, 15 min, in PBS) at 37 ^{o}C, and then washed with PBS three times. The fluorescent images were recorded using Operetta high content imaging system (Perkinelmer, US). The excitation channel of 410 to 430 nm and the emission channel of 460 to 540 nm were used. Data were mean of three independent experiments and at least 500 cells for each condition were analyzed and plotted by columbus analysis system (Perkinelmer, US).

Cell viability assay. Cell viability was measured with CellTiter 96®AQueous Non-Radioactive Cell Proliferation Assay (Promega, USA). After plated overnight on 96-well plates at 1×10^4 cells per well in growth medium, cells were treated with increasing concentrations (12.5 μM, 25 μM, 50 μM, 100 μM, 200 μM) of **DT-Gal** or **DT-OH** (dissolved in DMSO, diluted with PBS to final concentration) for 24 h. Then 20 μL MTS/PMS solution was added to each well. Absorbances were measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 490 nm after 2 h of incubation at 37 ^{o}C, and readings were expressed as viability percentages using the medium as negative controls (100% of viability). Each experiment was done in triplicate.

Real-time quantitative PCR. Total RNA was isolated from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR® Premix Ex TaqTM. Real-time PCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, CA, USA). GAPDH was detected as the housekeeping gene. Primers used for qPCR analysis of human cell lines were as follows:

Human GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'

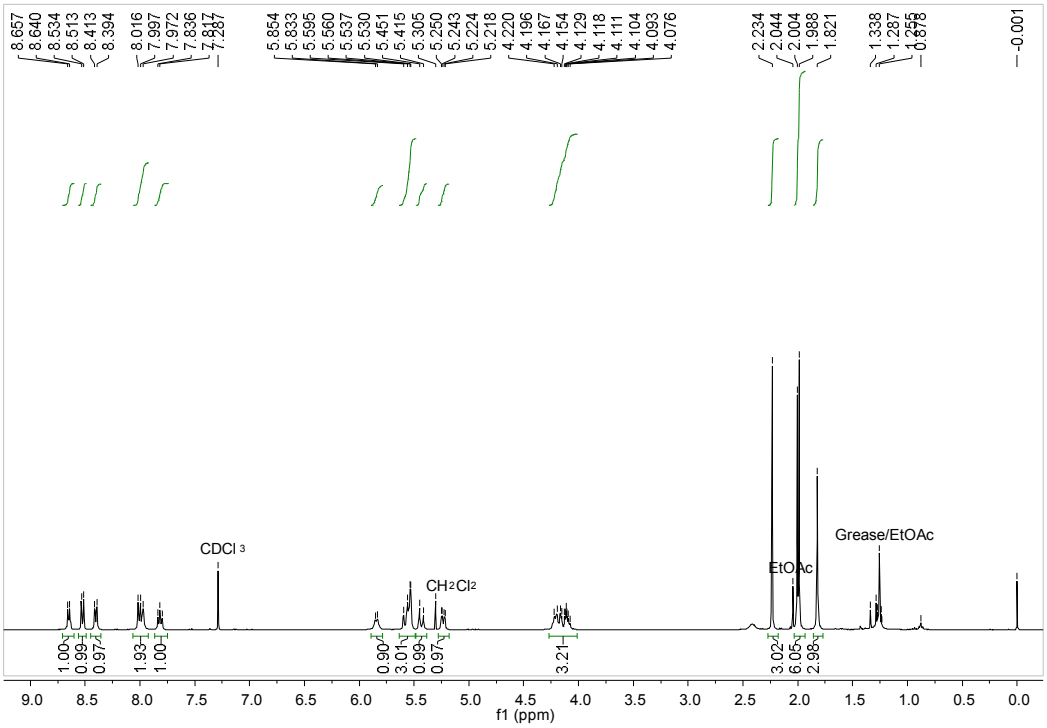
and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'

Human ASGPR1 forward, 5'-CTGGACAATGAGGAGAGTGAC-3'

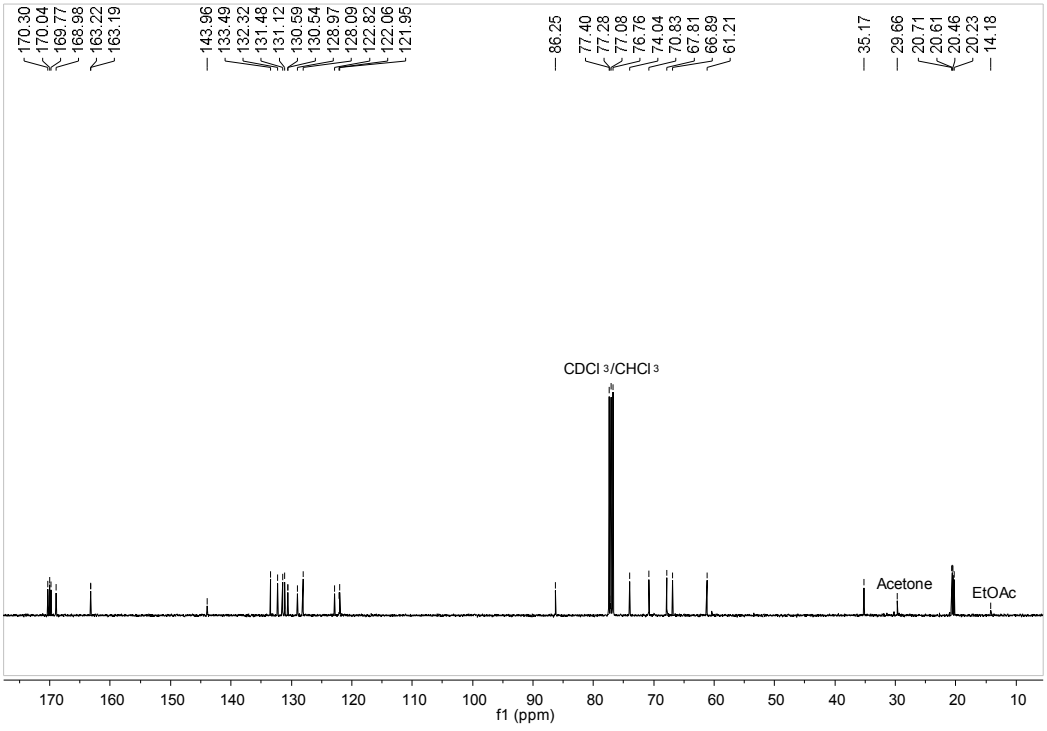
and reverse, 5'-TTGAAGCCCGTCTCGTAGTC-3'

S3. Original spectra of new compounds

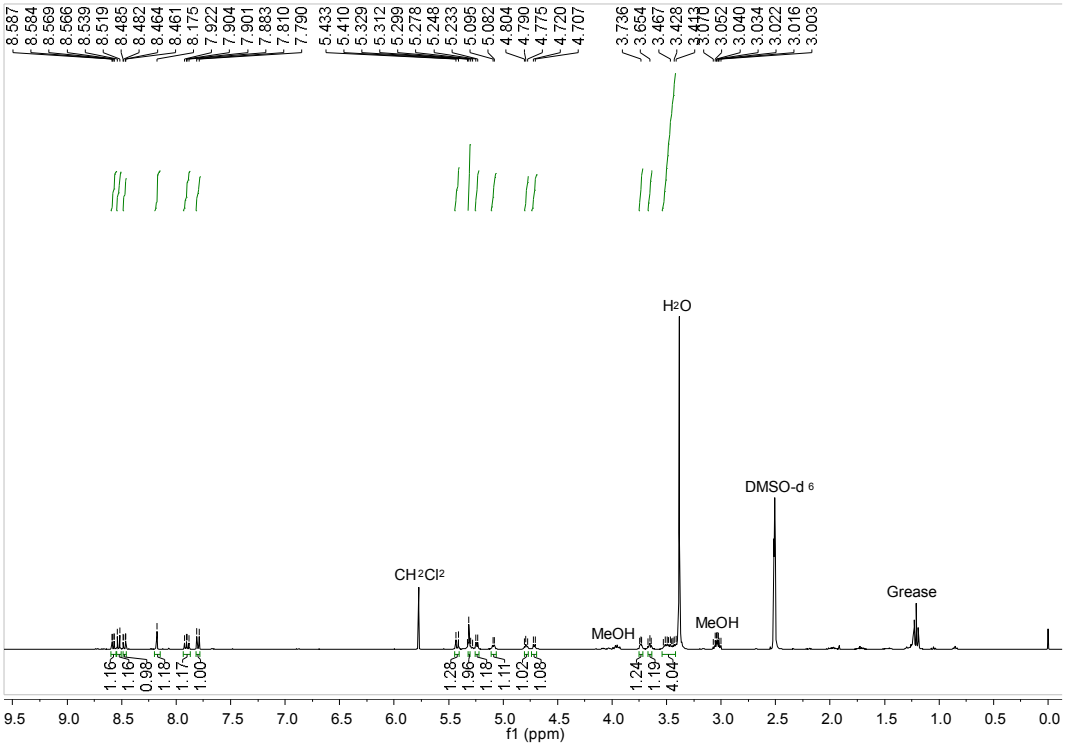
^{1}H NMR of **c**



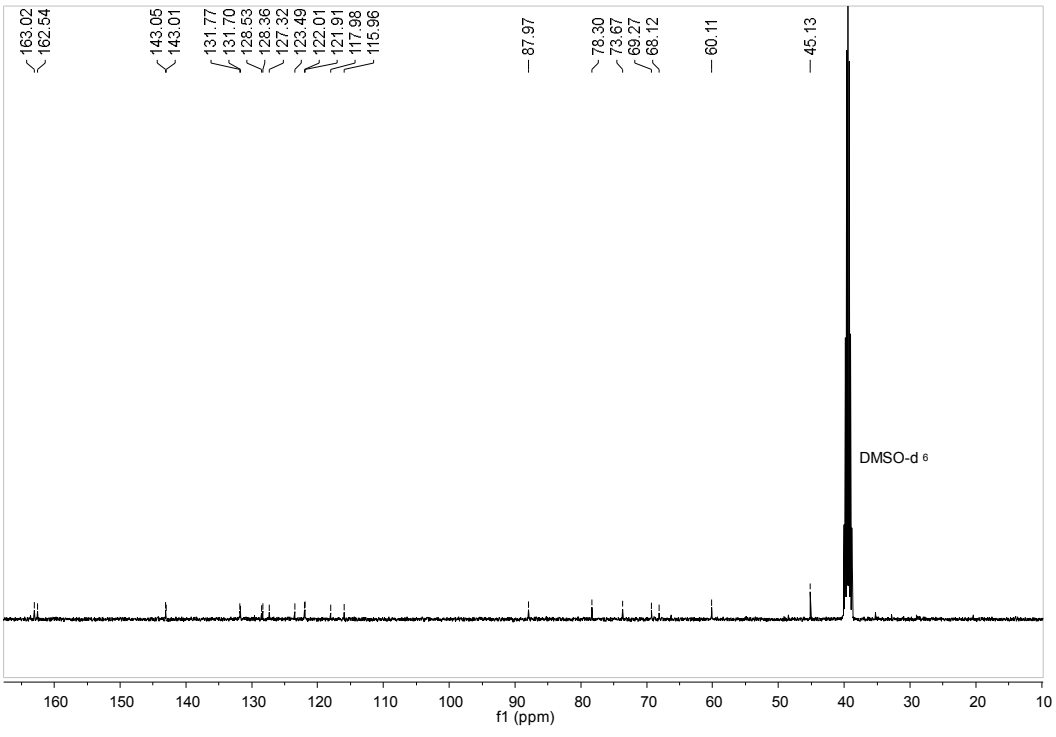
^{13}C NMR of **c**



^{1}H NMR of **DT-Gal**



^{13}C NMR of **DT-Gal**



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