Supplementary Material (ESI) for Chemical Science

Supporting Information

^{19}F MRI Detection of β-Galactosidase Activity for Imaging of Gene Expression

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CONTENTS:

1. Materials and general methods

General chemicals were of the best grade available, supplied by Tokyo Chemical Industries, Wako Pure Chemical, Aldrich Chemical Co., and Novabiochem. They were used without further purification. β-galactosidase (G6008), β-glucuronidase (G7396), and α-galactosidase (G8507) were purchased from Sigma Aldrich Life Science. The pcDNA^{TM}4/TO/*myc*-His/*lac*Z plasmid was purchased from Invitrogen (35-1614). Silica gel column chromatography was performed using BW-300 (Fuji Silysia Chemical Ltd.).

NMR spectra were recorded using a JEOL JNM-AL400 instrument at 400 MHz for ^{1}H, at 100.4 MHz for ^{13}C NMR using tetramethylsilane as an internal standard, and for ^{19}F NMR at 376 Hz using sodium trifluoroacetate as an internal standard. ^{19}F NMR spectra were recorded using a JEOL JNM-AL400 instrument at 376 MHz using sodium trifluoroacetate as an internal standard. The longitudinal relaxation time T_1 and the transverse relaxation time T_2 was measured by inversion recovery method and spin-echo method, respectively. ESI-MS were measured using a Waters LCT-Premier XE.

MRI images were recorded using a Bruker Avance DRX-500 spectrometer equipped with a standard bore (54 mm), an 11.7 T magnet, and a Micro-5-imaging probe head with an insert coil 8 mm in diameter. The rapid acquisition with refocused echoes (RARE) method was used for ^{1}H and ^{19}F MRI. For ^{19}F MRI, the matrix size was 32 × 32 (zero filled to 64 × 64) with a field of view of 16 × 16 mm, a slice depth of 15 mm, and a RARE factor of 32. The sweep width was 59523.8 Hz and the repetition time, and effective echo time were 250 and 28.4 ms, respectively. Gaussian-shaped pulses were used for excitation and refocusing. The number of accumulations was 3600. MRI image acquisition and processing were carried out using Para Vision software (Bruker BioSpin).

2. Synthesis of compounds

Ethyl 4-hydroxy-2-(trifluoromethyl)benzoate (1). To the acetonitrile solution of 4-carbethoxy-3-trifluoromethylcyclohex-2-enone[S1] (200 mg, 0.85 mmol, 1.0 eq.) were added $CuBr_2$ (380 mg, 1.7 mmol, 2.0 eq.) and NaBr (86.7 mg, 0.85 mmol, 1.0 eq.). The mixture was stirred at 60 °C for 1 h. The solvent was removed under vacuum, and the crude product was diluted with ethyl acetate, and washed with 2 M hydrochloric acid and brine. The organic layer was dried over $MgSO_4$ and removed under vacuum to give the crude product. The product was dissolved in CH_2Cl_2, then purified with silica gel chromatography, eluted with CH_2Cl_2 to afford **1** (167 mg, 0.71 mmol, y. 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (t, J = 7.2 Hz, 3H), 4.36 (q, 2H, J = 7.2 Hz), 5.47(s, 1H), 7.01 (dd, J = 2.4, 8.6 Hz, 1H), 7.20 (d, J = 2.4 Hz, 1H), 7.80 (d, J = 8.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 62.0, 115.4, 119.0, 123.3, 124.0, 132.1, 137.2, 159.0, 168.1; MS (ESI^{+}) *m*/*z*: 235 ([M+H]$^{+}$).

4-Hydroxymethyl-3-trifluoromethyl-phenol (2). A solution of **1** (124 mg, 0.53 mmol, 1.0 eq.) in dry THF was cooled to 0 °C. DIBALH (3.25 mL, 3.25 mmol, 6.0 eq. in toluene) was added and the solution was stirred at 0 °C for 30 min. Then, the solution was allowed to warm RT and stirred for 5 h. The mixture was added to 10% NH_4Cl aq. (1.0 mL) and extracted with diethyl ether. The organic layer was dried over $MgSO_4$ and removed under vacuum to give the pure product **2** (98 mg, 0.51 mmol, y. 97%). ^{1}H NMR (400 MHz, CD_3OD): δ 4.66 (s, 2H), 6.99 (dd, J = 2.8, 8.4 Hz, 1H), 7.04 (d, J = 2.8 Hz), 7.50 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz,

CD_3OD) δ 61.0, 113.5, 119.7, 127.1, 129.4, 131.3, 132.1, 158.0; HRMS (EI^+) *m/z*: 192.0395 (Calcd for $[M]^+$ 192.0398).

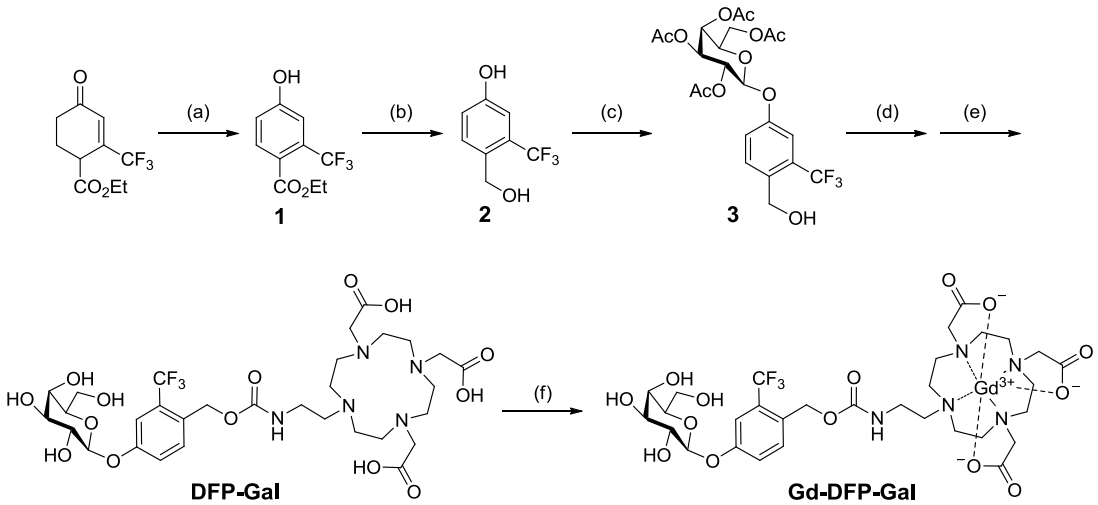
Tetraacetyl-β-galactopylanocyl-2-trifluoromethyl-benzylarcohol (3). 1-Bromo-β-galactose tetraacetate was prepared according to the literature.[S2] Compound **2** (1.1 g, 5.7 mmol, 1.0 eq.) was dissolved in DMF, and cesium carbonate (7.41 g, 22.8 mmol, 4.0 eq.) was added to the solution at 0 °C. 1-Bromo-β-galactose tetraacetate (10.6 g, 25.9 mmol, 4.5 eq.) in DMF was added dropwisely under Ar atmosphere, and the solution was stirred at RT for 3 h. Cooled 2 M hydrochloric acid was poured into the reaction mixture on ice bath. The product was extracted with AcOEt, and dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by silica gel chromatography, eluted with AcOEt / hexane to afford **3** (1.7 g, 3.2 mmol, y. 57%). ^1H NMR (400 MHz, $CDCl_3$): δ 2.02 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 2.19 (s, 3H), 4.08–4.11 (m, 1H), 4.18–4.20 (m, 2H), 4.82–4.83 (m, 2H), 5.06-5.14 (m, 2H), 5.46–5.52 (m, 2H), 7.19 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.30 (d, *J* = 2.4 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H); MS (ESI^+) *m/z*: 545 ($[M+Na]^+$).

DFP-Gal. Compound **3** (513 mg, 0.98 mmol, 1.0 eq.) with one drop of anhydrous pyridine was dissolved in dry CH_2Cl_2 and placed under argon. Then, 4-nitrophenyl chloroformate (590 mg, 2.94 mmol, 3.0 eq.) were poured onto the mixture. After leaving for 3 h at RT, water was added and organic layer was carefully washed with 100 mM Na_2CO_3 solution, and with H_2O, then dried Na_2SO_4, filtered, and evaporated. The crude product was partly purified by silica gel chromatography, eluted with 2% MeOH/CH_2Cl_2 to afford activated carbonate. The activated carbonate (762 mg, 1.09 mmol, 1.0 eq.) and 1-(2-aminoethyl)-4,7,10-(triscarboxymethyl)-(1,4,7,10-tetraazacyclododecane) TFA Salt[S3] (850 mg, 2.18 mmol, 2.0 eq.) with anhydrous triethylamine (3.0 mL, 21.8 mmol, 20 eq.) were dissolved in dry DMF and placed under argon. The mixture was stirred at 50 °C for overnight. Then, the solvent was removed under vacuum to give the crude product. It was dissolved in MeOH, and sodium methoxide (2.9 g, 54.5 mmol, 50 eq.) in MeOH was slowly added on ice. Then, the solution was stirred on ice for 2 h. After neutralization by formic acid, the solvent was evaporated. The residue was dissolved in water containing 0.1% formic acid. Reversed-phase HPLC, eluted with H_2O/acetonitrile containing 100 mM ammonium formate to afford pure DFP-Gal (Figure S1a, y. 16%). ^1H NMR (400 MHz, CD_3OD): δ 2.87–3.90 (m, 36H), 5.19 (s, 2H), 7.36 (dd, *J* = 2.6, 8.0 Hz, 1H), 7.41 (d, *J* = 2.6 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 8.27 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 9.58, 28.5, 60.8, 62.3, 70.1, 72.1, 74.7, 77.1, 103.0, 115.4, 115.5, 121.0, 131.4, 134.6, 158.0; ^{19}F NMR (376 MHz, CD_3OD with TFANa): δ 14.81 (s, 3F); HRMS (ESI^+) *m/z*: 770.2769 (Calcd for $[M+H]^+$ 770.3072).

Gd-DFP-Gal. Crude DFP-Gal was dissolved in 100 mM HEPES buffer (pH 7.4), and $GdCl_3 \cdot 6H_2O$ (1.2 eq.) was added. Then, the mixture was stirred at RT for 12 h. The product was purified with reversed-phase HPLC, eluted with H_2O/acetonitrile containing 100 mM ammonium formate to yield Gd-DFP-Gal （Figure S1b, y. 23%). HRMS (ESI^+) m/z: 925.1270 (Calcd for $[M+H]^+$ 925.2078).

3. Supplementary scheme, figures and tables

Scheme S1. Synthetic route to Gd-DFP-Gal.



(a) $CuBr_2$, NaBr, CH_3CN, (b) DIBAL-H, THF, (c) 1-bromo-β-galactose tetraacetate, Cs_2CO_3, DMF, (d) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2, (e) 1) 1-(2-aminoethyl)-4,7,10-(triscarboxymethyl)-(1,4,7,10-tetraazacyclododecane), triethylamine, DMF, 2) NaOMe, MeOH, (f) $GdCl_3 \cdot 6H_2O$, 100 mM HEPES buffer (pH 7.4).

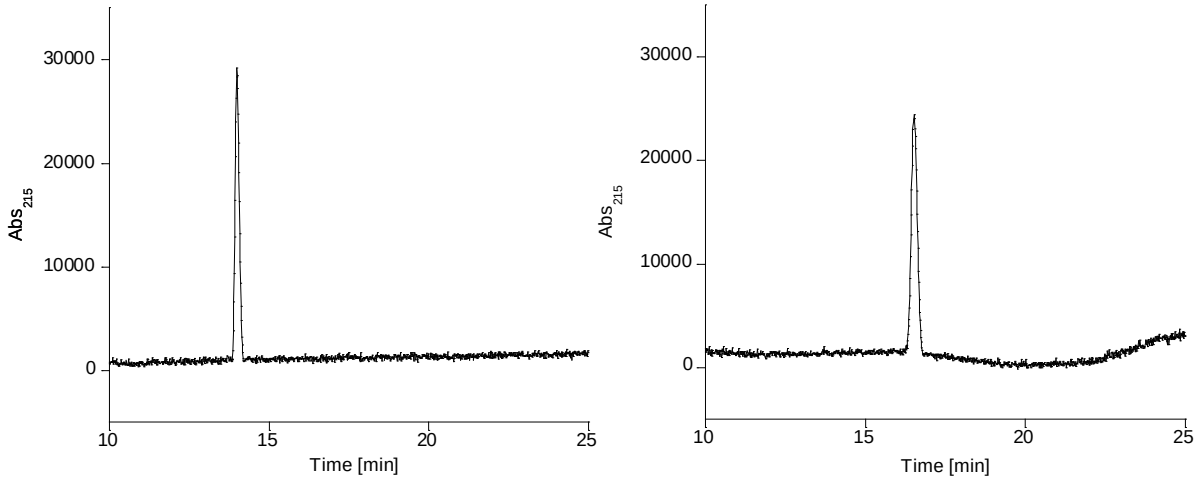


Figure S1. Reversed-phase HPLC diagrams of (a) DFP-Gal and (b) Gd-DFP-Gal. Eluent: H_2O/acetonitrile containing 100 mM ammonium formate.

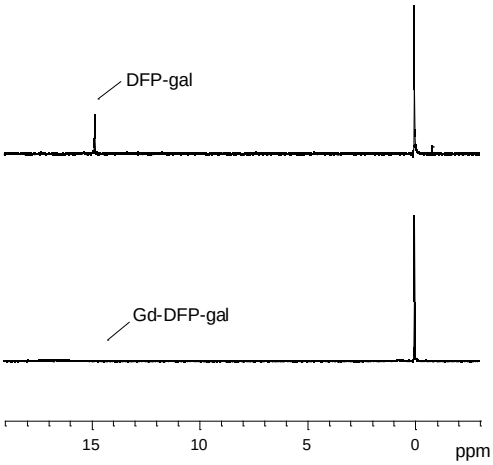


Figure S2. ^{19}F NMR spectra of DFP-Gal (1 mM) and Gd-DFP-Gal (1 mM). Sodium trifluoroacetate was added as an internal standard (0 ppm).

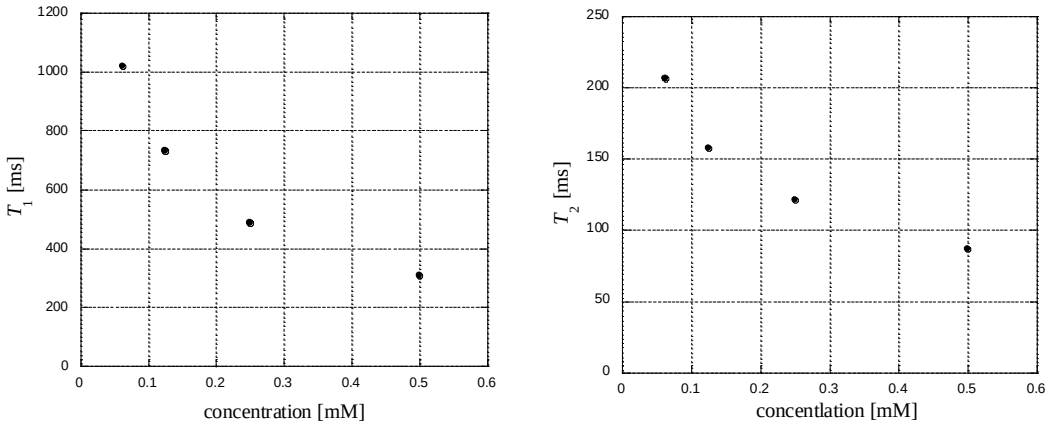


Figure S3. Plots of longitudinal and transverse relaxation times *vs.* concentration of Gd-DFP-Gal after the complete cleavage by β-galactosidase.

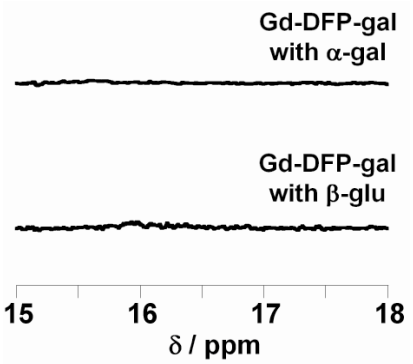


Figure S4. ^{19}F NMR spectra of Gd-DFP-Gal (1 mM) under incubation with α-galactosidase (top) or β-glucuronidase (bottom). Sodium trifluoroacetate was added as an internal standard (0 ppm). After Gd-DFP-Gal (1 mM) was incubated with α-galactosidase or β-glucuronidase at 37 °C in the 100 mM phosphate buffer (pH 6.4) containing 5% D_2O for 2 h, the ^{19}F NMR spectra were obtained

Table S1. Longitudinal and transverse relaxation times of synthesized compounds (500 μM) in PBS (pH 7.3) containing 10 mM magnesium chloride and 5% D_2O.

	T_1 (s)[a]	T_2 (s)[a]
DFP-Gal	1.29(1)	0.271(2)
Gd-DFP-Gal	N.D.[b]	N.D.[b]
4-hydroxymethyl-3-trifluoromethylphenol	1.79(1)	0.401(3)

[a] Parenthesis denotes the standard deviation (n = 3).
[b] The relaxation time was too short to be estimated.

Table S2. T_1 and T_2 of Gd-DFP-Gal at the various concentrations after the complete cleavage by β-galactosidase.

[Gd-DFP-Gal] /mM	T_1 /s [a]	T_2 /s [a]
0.50	0.31(1)	0.086(1)
0.25	0.49(3)	0.12(1)
0.13	0.73(3)	0.16(1)
0.063	1.0(1)	0.21(2)

[a] Parenthesis denotes the standard deviation (n = 3).

4. References

(S1) Bégué, J. -P.; Bonnet-Delpon, D.; Dogbeavou A. *Synth. Commun.* **1992,** *22*, 573–579.

(S2) Jean-Lowis, M.; Jean-Yves, W.; Alan, L., Mehrnaz, K., Andre, A. P., Jean-Pierre, R. *Carbohydrate Res.* **1997**, *297*, 175-180.

(S3) Duimstra, J. A.; Femia, F. J.; Meade, T. J. *J. Am. Chem. Soc.,* **2005**, *127*, 12847–12855.