

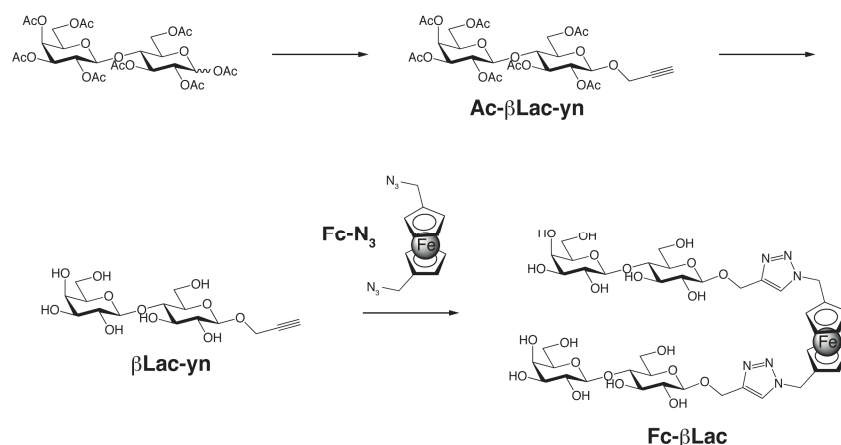
Ferrocenes having two carbohydrate appendages at upper and lower rings are useful for investigating carbohydrate-carbohydrate interactions

Atsushi Otsuka, Kunihiro Sakurai and Teruaki Hasegawa

Synthetic procedures

General

^1H and ^{13}C NMR spectra were acquired on a JEOL AL300 (JEOL DATUM, Ltd) in CDCl_3 or D_2O at 300 MHz. ^1H - ^1H COSY and ^{13}C - ^1H COSY (HMQC) were also recorded to support 1D (^1H and ^{13}C) NMR assignments. IR spectra were recorded on a JASCO FT/IR-4100 fourier transform infrared spectrometer (JASCO Co., Ltd) or on a FT/IR-230 fourier transform infrared spectrometer (JASCO Co., Ltd). Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on a SHIMAZU AXIMA-CFR+ (SHIMAZU, Ltd). HPLC analyses were carried out on HEWLETT PACKARD 1100 SERIES equipped with SHISEIDO CAPCELL PAK C18 (Conditions: 40 °C, Flow rate = 2 ml/min, $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ = 50/50 isocratic, λ = 254 nm). Circular Dichroism (CD) spectra were acquired on a JASCO J-820 spectropolarimeter (JASCO Co.). Silica gel 60 N (particle size 63-210 mm) for column chromatography was purchased from KANTO CHEMICAL Co. INC. Thin layer chromatography (TLC) was carried out with Whatman TLC glass plates pre-coated with silica gel 60 F_{254} . All other chemicals were purchased from Wako Pure Chemical Industries Ltd., Kishida Chemicals Co. Ltd., or Funakoshi Co. Ltd..



Scheme S1. Synthesis of Fc- β Lac

Synthesis of 1,1'-diazidomethylferrocene (Fc- N_3)

Sodium azide (120 mg) was added to 1,1'-ferrocenedimethanol (15 mg) in acetic acid (2.0 ml) and stirred at ambient temperature for 3 h. The resultant mixture was diluted with ethyl acetate and washed with NaHCO_3 saturated aqueous solution. The organic layer was evaporated and the residue was subjected to the purification by silica-gel column chromatography (toluene/ethyl acetate (15/1)) to give 1,1'-diazidomethylferrocene as pale yellow syrup: ^1H NMR (CDCl_3 , TMS): 4.23 (m, 4H, Cp), 4.21 (m, 4H, Cp), 4.12 (brs, 4H, $-\text{CH}_2\text{N}_3$); ^{13}C NMR (CDCl_3 , TMS): 82.878 (C_{ipso}), 69.477 (Cp), 69.213 (Cp), 50.587 ($-\text{CH}_2\text{N}_3$); $[\text{M}+\text{H}]^+ = 296.78$ (calc. 297.05); IR (KBr, cm^{-1}) 2097.21 (azide).

Synthesis of 2,3,6,2',3',4',6'-hepta-*O*-acetyl-1-(2"-propargyl)- β -lactoside (Ac- β Lac-yn)

BF_3OEt_2 (4.0 ml) was added to 1,2,3,6,2',3',4',6'-octa-*O*-acetyl-lactose (3.0 g) and propargyl alcohol (4.0 ml) in anhydrous CH_2Cl_2 (10 ml) at room temperature and the stirring was continued for 44 h under nitrogen atmosphere. The resultant mixture was diluted with ethyl acetate and washed with NaHCO_3 saturated aqueous solution. The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to dryness. Although the residue was subjected to the purification by silica-gel column chromatography (hexane only ~ hexane/ethyl acetate (1/1)), the R_f values of the starting material and the product were so close that the pure product could not be obtained. Thus, a deacetylation (in a mixture of aqueous ammonia, MeOH and THF) of this product was performed for purification purpose (silica-gel, $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (4/5/1)). Re-acetylation of the compound was carried out by treating with a pyridine (200 ml) /acetic acid (150 ml) mixture. An excess amount of EtOH was added to the

resultant mixture to quench the reaction and the solution was concentrated in vacuo. The mixture was diluted with ethyl acetate and the organic layer was washed with 0.5 N HCl aqueous solution and NaHCO₃ saturated aqueous solution several times. The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness to give 2,3,6,2',3',4',6'-hepta-*O*-acetyl-1-(2''-propargyl)- β -lactoside (Ac- β Lac-yn) as white powder in 20 % yield. ¹H NMR (CDCl₃, TMS): 5.35 (d, *J* = 2.7 Hz, 1H), 5.23 (t, *J* = 9.3 Hz, 1H), 5.11 (dd, *J* = 8.1 and 10.2 Hz, 1H), 4.96 (dd, *J* = 3.3 and 7.8 Hz, 1H), 4.75 (d, *J* = 7.8 Hz, 1H), 4.34 (d, *J* = 2.4 Hz, 2H), 4.14-4.05 (m, 2H), 3.90-3.79 (m, 2H), 3.66-3.62 (m, 1H), 2.47 (t, *J* = 2.1 Hz, 1H), 2.18 (s, 3H), 2.13 (s, 3H), 2.07-2.05 (m, 12H), 1.97 (s, 3H); [M+Na]⁺ = 697.26 (calc. 697.21); IR (KBr, cm⁻¹) 1753 (acetyl).

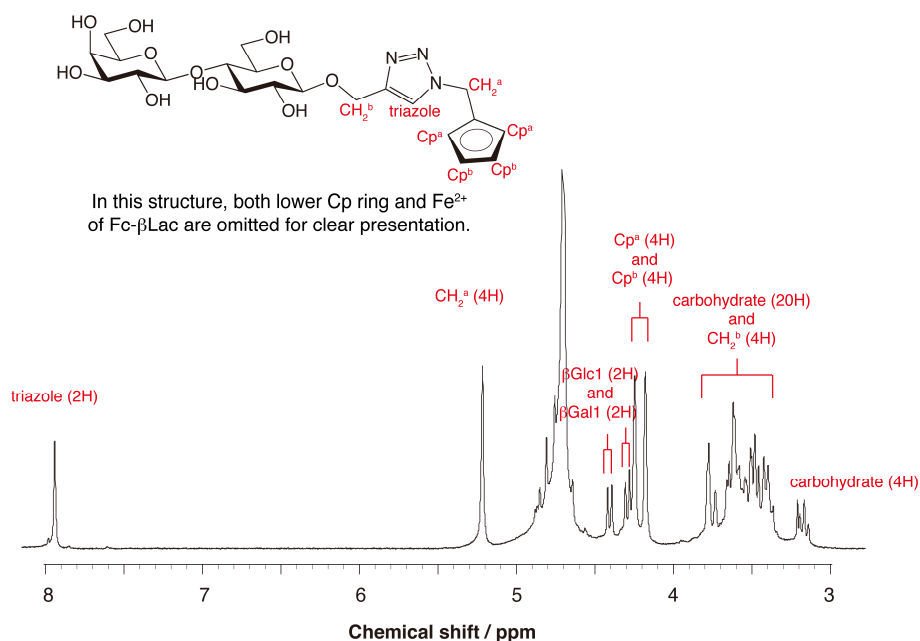
Synthesis of 1-*O*-(2''-propargyl)- β -lactoside (β Lac-yn)

Aqueous ammonia (50 ml) was added to 2,3,6,2',3',4',6'-hepta-*O*-acetyl-1-(2''-propargyl)- β -lactoside (1.1 g) in a THF/MeOH mixture (200 ml, 1/1 v/v) and stirring was continued for 3 h at room temperature. The solvent was evaporated and the resultant residue was subjected to the purification by silica-gel column chromatography (CHCl₃/MeOH (4/1) ~ CHCl₃/MeOH/H₂O (4/5/1)) to obtain 1-(2''-propargyl)- β -lactoside (β Lac-yn) in 93 % yield as white powder after lyophilization. ¹H NMR (D₂O, HOD): 4.53 (d, *J* = 7.93 Hz 1H), 4.35 (dd, *J* = 1.90 and 8.66 Hz, 1H), 4.32 (d, *J* = 7.72 Hz, 1H), 3.85 (d, *J* = 11.5 Hz, 1H), 3.80 (d, *J* = 3.03 Hz, 1H), 3.70-3.58 (m, 5H), 3.55-3.52 (m, 3H), 3.49-3.48 (m, 1H), 3.42 (t, *J* = 8.11 Hz, 1H), 3.32 (t, *J* = 8.28 Hz, 2H), 2.82 (s, 1H); ¹³C NMR (D₂O): 103.282, 100.739, 79.136, 78.602, 76.769, 75.707, 75.206, 74.711, 72.959, 72.880, 71.311, 68.911, 61.403, 60.359, 56.958; [M+Na]⁺ = 403.09 (calc. 403.12); IR (KBr, cm⁻¹) 3397, 2919.

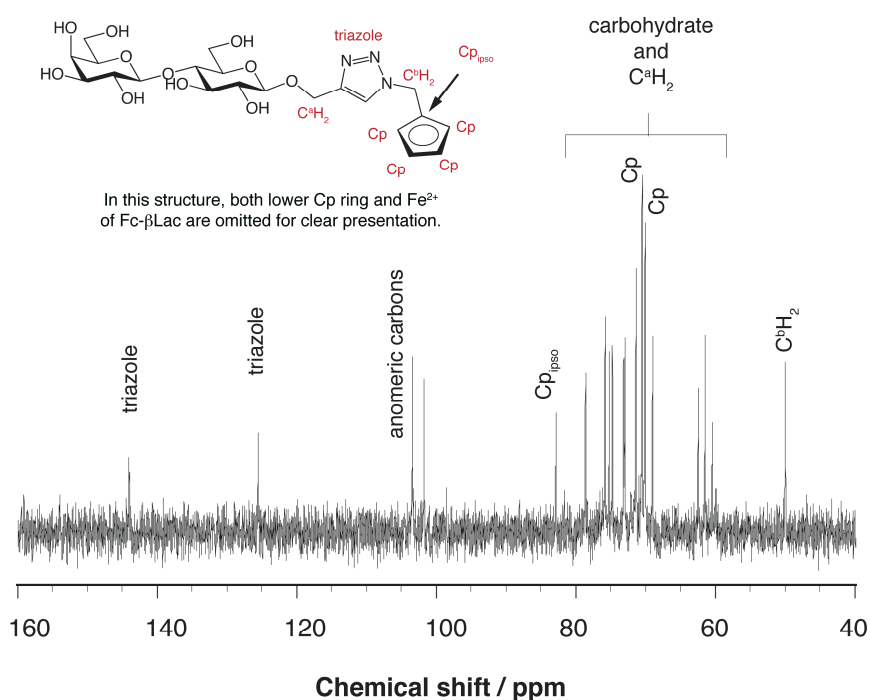
Synthesis of Fc- β Lac

1-*O*-Propargyl- β -lactoside (β Lac-yn, 75 mg), CuBr₂ (9 mg), ascorbic acid (12 mg), and propylamine (15 ml) were added to 1,1'-azidomethylferrocene (10 mg) in DMSO (0.50 ml), and the mixture was incubated at room temperature for 3 h. The resultant mixture was dialyzed (water, MWCO500) and concentrated by evaporation. The resultant aqueous solution was subjected to purification on Diaion HP-20 column chromatography (eluent: water only and then 20 % aqueous methanol) followed by lyophilization to give Fc- β Lac in 93 % yield: ¹H NMR (D₂O, HOD): 7.90 (s, 2H, triazole), 5.19 (s, 4H, CH^a₂), 4.38 (d, *J* = 7.8 Hz, 2H), 4.27 (d, *J* = 7.8 Hz, 2H), 4.22 (s, 4H, Cp), 4.15 (s, 4H, Cp), 3.77-3.37 (m, 24H), 3.17-3.11 (m, 4H); ¹³C NMR (D₂O): 144.054, 125.584, 103.439, 101.824, 82.917 (Cp_{ipso}), 78.690, 75.879, 75.294, 74.841, 73.225, 73.036, 71.470, 71.033, 70.555 (Cp), 70.151 (Cp), 69.072, 62.536, 61.563, 60.525, 50.083 (Fc-CH₂-); [M + Na]⁺ = 1079.07 (calc. 1079.31); IR (KBr, cm⁻¹): 3389 (OH).

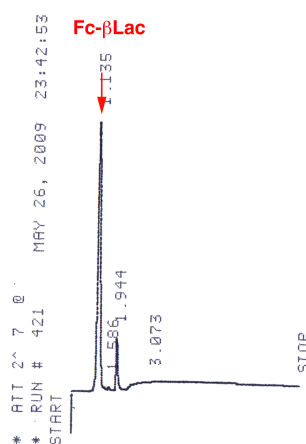
¹H NMR spectrum of Fc- β Lac



^{13}C NMR spectrum of Fc- β Lac



RP-HPLC analysis of Fc- β Lac



Synthesis of 2,3,6,2',3',4',6'-hepta-*O*-acetyl-1-(2''-propargyl)- β -maltoside (Ac- β Mal-yn)

BF $_3$ OEt $_2$ (5.0 ml) was added to 1,2,3,6,2'3'4'6'-octa-*O*-acetyl-maltose (3.2 g) and propargyl alcohol (5.0 ml) in anhydrous CH $_2$ Cl $_2$ (10 ml) at room temperature and the stirring was continued for 45 h under nitrogen atmosphere. The resultant mixture was diluted with ethyl acetate and washed with NaHCO $_3$ saturated aqueous solution. The organic layer was dried over anhydrous MgSO $_4$, filtered and concentrated to dryness. Since MALDI-TOF-MS and TLC analysis of the residue showed partially deacetylated product, the residue was subjected to full deacetylation (next step) without purification and characterization at this step: [M+Na] $^+$ = 696.85 (calc. 697.21).

Synthesis of 1-*O*-(2''-propargyl)- β -maltoside (β Mal-yn)

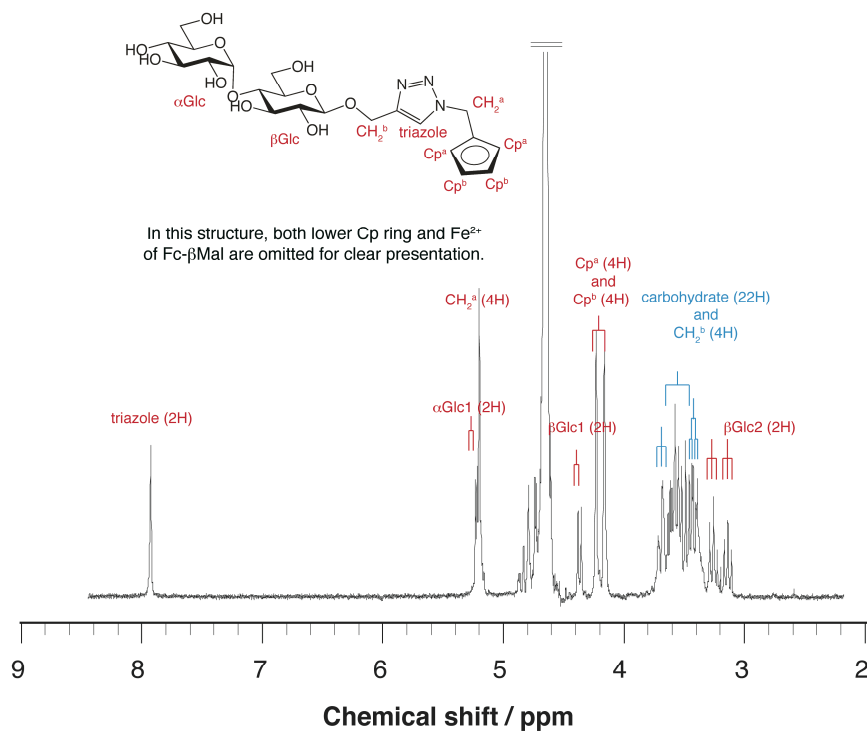
A catalytic amount of sodium methoxide was added to crude Ac-Mal-yn in a MeOH (50 ml)/ THF (50 ml) mixed solvent system and the resultant mixture was stirred for 6 h. The resultant mixture was neutralized on DOWEX and evaporated to dryness. The resultant residue was subjected to the purification by silica-gel column chromatography (CHCl $_3$ /MeOH (2/1)) to obtain 1-(2''-propargyl)- β -maltoside (β Mal-yn) in 38 % yield (2 steps) as white powder after lyophilization: ^1H NMR (D $_2$ O,

HOD): 5.26 (d, $J = 3.6$ Hz, 1H), 4.52 (d, $J = 8.1$ Hz, 1H), 4.33 (s, 2H), 3.78 (t, $J = 12.9$ Hz, 1H), 3.70-3.48 (m, 8H), 3.43 (dd, $J = 3.6$ and 9.9 Hz, 1H), 3.27 (t, $J = 9.3$ Hz, 1H), 3.19 (t, $J = 8.7$ Hz, 1H), 2.78-2.75 (m, 1H); ^{13}C NMR (D_2O): 101.016 (αGlc1), 100.200 (βGlc1), 79.390 ($-\text{CH}_2-\text{CCH}$), 77.231, 77.000, 76.843 ($-\text{CH}_2-\text{CCH}$), 76.643, 75.294, 73.448, 73.357, 72.294, 69.978, 61.308 (Glc6), 61.127 (Glc6), 57.204 ($-\text{CH}_2-\text{CCH}$); $[\text{M}+\text{Na}]^+ = 402.66$ (calc. 403.12); IR (KBr, cm^{-1}) 3381 (OH).

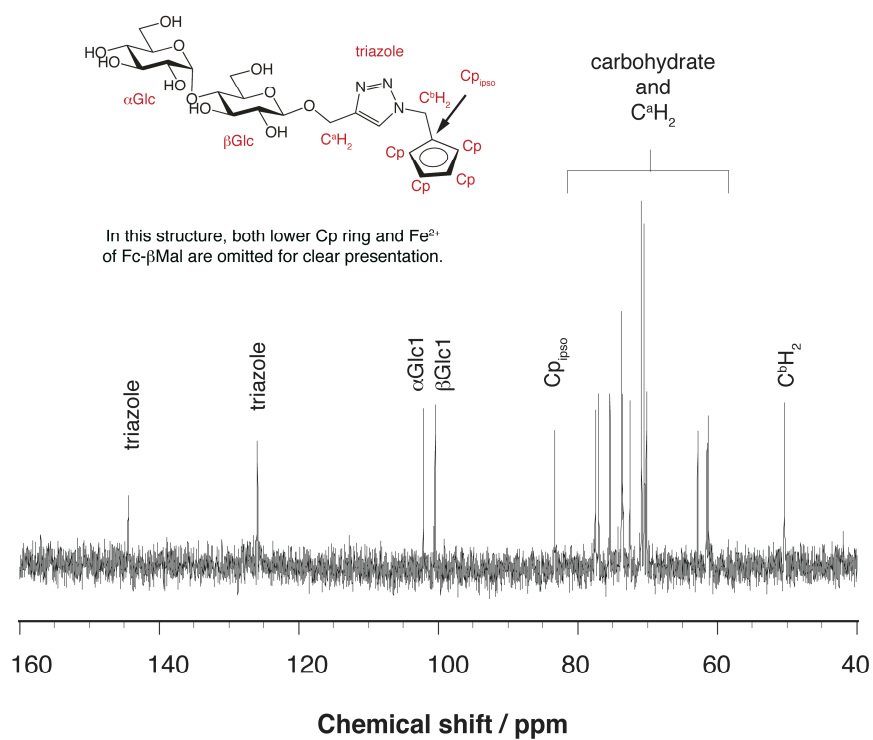
Synthesis of Fc- β Mal

1-*O*-Propargyl- β -maltoside ($\beta\text{Mal-yn}$, 75 mg), CuBr_2 (9.0 mg), ascorbic acid (12 mg), and propylamine (15 μl) were added to 1,1'-azidomethylferrocene (10 mg) in DMSO (0.36 ml), and the mixture was incubated at room temperature for 14 h. The resultant mixture was dialyzed (water, MWCO500) followed by lyophilization to give Fc- β Mal in 51 % yield: ^1H NMR (D_2O , HOD): 7.91 (s, 2H, triazole), 5.21 (d, $J = 4.2$ Hz, 2H, αGlc1), 5.18 (s, 4H, CH^{a_2}), 4.35 (d, $J = 7.8$ Hz, 2H, βGlc1), 4.21 (s, 4H, Cp), 4.14 (s, 4H, Cp), 3.72-3.39 (m, 24H), 3.25 (t, $J = 9.3$ Hz, 2H), 3.12 (t, $J = 8.4$ Hz, 2H, βGlc2); ^{13}C NMR (D_2O): 144.31 (triazole), 125.906 (triazole), 102.129 (αGlc1), 100.415 (βGlc1), 83.255 (Cp_{ipso}), 77.462, 77.000, 76.588, 75.368, 73.708, 73.698, 73.537, 72.492, 77.885 (Cp), 70.481 (Cp), 70.143, 62.816, 61.497, 61.300, 50.396 (Fc- CH_2-); $[\text{M}+\text{Na}]^+ = 1079.22$ (calc. 1079.31); IR (KBr, cm^{-1}): 3376 (OH).

^1H NMR spectrum of Fc- β Mal



^{13}C NMR spectrum of Fc- β Mal



RP-HPLC analysis of Fc- β Mal

