Supplementary Information

Development of Site- and Stereoselective Continuous Flow Deuterium Labelling Method for Carbohydrates Utilising High Dispersion Effect towards Ru/C of Hydrogen Flow

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1. General considerations:

Reagents: D_2O (>99.9% D atom) was purchased from Silantes Inc., Germany. 10% Ru/C were obtained from N.E. CHEMCAT CORPORATION. Acetic anhydride (Guaranteed Reagent) and anhydrous pyridine (Guaranteed Reagent) were purchased from FUJIFILM Wako Pure Chemical Corporation. Unless otherwise stated, the substrates and solvents were purchased from commercial suppliers and used without further purification. Flash column chromatography was carried out on Silica Gel 60 N (Kanto Chemical Corporation., Inc., 63–210 μm spherical, neutral). All reactions were monitored by thin-layer chromatography (TLC) on glass-backed silica gel 60 F^{254}, 0.2 mm plates (Merck), and compounds were visualisesd using *p*-anisaldehyde solution as a TLC stain.

Analytical methods: Optical rotations were measured by ATAGO AP300 automatic polarimeter. ^1H and ^2H NMR spectra were recorded on a JEOL ECZ 400 (^1H: 400 MHz, ^2H: 61 MHz) or ECA 500 spectrometer (^1H: 500 MHz, ^2H: 77 MHz) at room temperature in $CDCl_3$ or D_2O as a solvent and an internal standard (^1H NMR: δ = 7.26 ppm for $CHCl_3$; ^2H NMR: δ = 7.26 ppm for $CDCl_3$, 4.79 for D_2O). The ^1H NMR spectra of known products are identical to those reported in the literature.[1,2)] Inductivily coupled plasma atomic emission spectrometry (ICP-AES) analysis was performed on HORIBA Jobin Yvon ULTIMA2. The deuterium contents were determined by the ^1H NMR on the basis of the integration of the protons at C1 (anomeric carbon), since protons on anomeric carbons in *O*-methyl carbohydrates have never been deuterated. The deuterium incorporation was also confirmed by the ^2H NMR spectra.

Flow reactor: The intelligent pump (UI-22 110P, GL Sciences Inc.), mass flow controller (MUC-11GU, TOKYO RIKAKIKAI CO. LTD.), hydrogen storage canister (KHCS-500LF, KOFLOC), gas-liquid column end (CLM-U2G, TOKYO RIKAKIKAI CO. LTD.), catalyst flow cartridge (φ 3.0 × 200 mm or φ 5.0 × 200 mm, special order form TOKYO RIKAKIKAI CO. LTD.), heating block (LCR-1300, TOKYO RIKAKIKAI CO. LTD.), and fraction collector (DC-1000, TOKYO RIKAKIKAI CO. LTD.) were assembled and operated as an integrated flow reactor (see Figure S1).

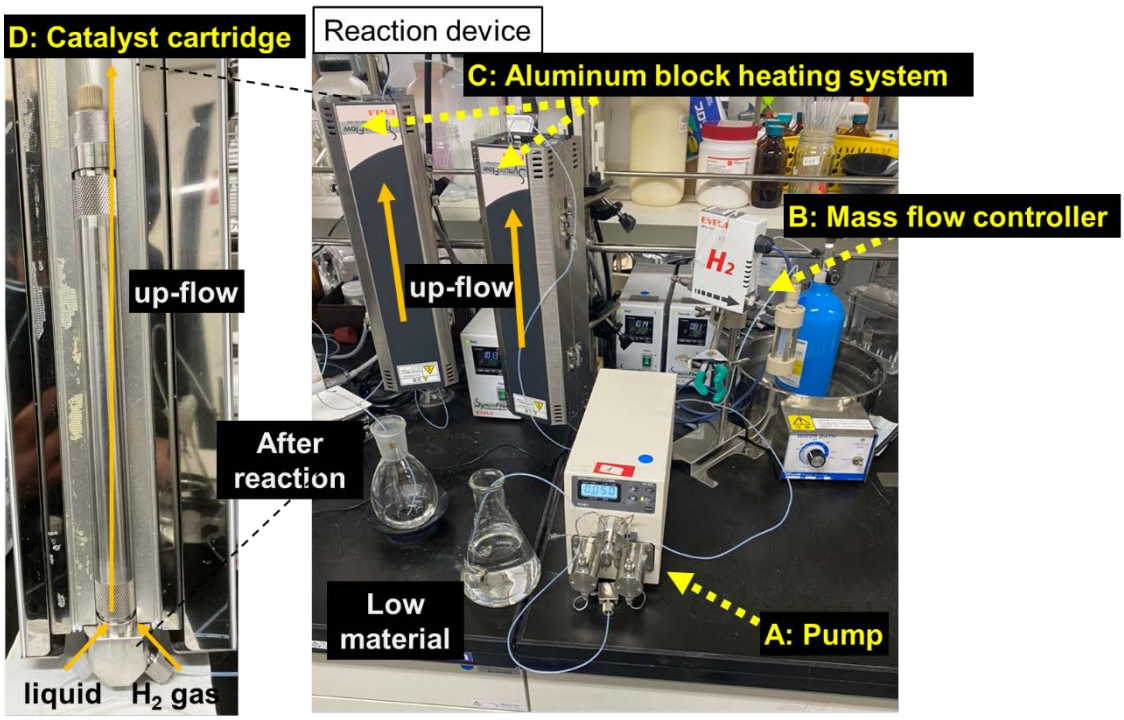


Figure S1. Appearance of flow reactor: A) Pump (UI-22); B) Mass flow controller (MUC-11GU); C) Aluminum block heating system (LCR-1300); D) Catalyst cartridge and gas-liquid column end (Special order from TOKYO RIKAKIKAI CO. LTD.).

Table S1. Residence time of catalyst cartridge (φ 3.0 × 200 mm).

Entry	Catalyst (mg)	Celite (mg)	Void volume (mL)	Void fraction (%)	Residence time (min)[a)]
1	130	300	0.69	48.5	13.7
2	130	-	1.32	93.7	26.4
3	250	-	1.24	87.8	24.8
4	400	-	1.13	80.5	22.7
5[b)]	125	-	1.33	93.9	26.5

a) Residence time (min) = void volume (mL) / 0.05 mL/min. b) Using two connected cartridges.

2. General procedures for Figures 2–5:

General Procedure A

Optimisation of conditions for continuous flow deuteration (Figures 2 and 3)

A Ru/C catalyst (125–400 mg) was packed into a column cartridge (φ 3.0 × 200 mm). The catalyst column cartridge was installed onto the reactor, and D_2O was used as a blank solvent at a flow rate of 0.05 mL/min for 30 minutes to flush air out. The catalyst column cartridge was then heated to 80 or 100 °C whilst maintaining a D_2O flow rate of 0.05 mL/min along with hydrogen gas at a flow rate of 0.8 mL/min. After stabilisation, the reaction was initiated by switching the feed solution to a 0.125 M solution of carbohydrates (**1a** or **1b**) in D_2O. The resulting solution was fractionated every 20 min (1 mL per fraction) using a fraction collector. Selected fractions were analysed by TLC using phosphomolybdic acid reagent as the visualisation reagent. After collecting the fractions over the designated period, D_2O was evaporated from the combined fractions. To the resulting residue, 5 equiv of acetic anhydride and 10 equiv of pyridine were added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure, and purification was performed using silica gel column chromatography (eluent: chloroform) to obtain spectrally pure deuterated *O*-acetylated carbohydrate **2a** or **2b**, and the deuterium content was determined by ^{1}H NMR analysis.

General Procedure B

Ru/C-catalyzed site- and stereoselective H–D exchange reaction of 1a and 1b (Figure 4)

A Ru/C catalyst (125 mg) was packed into two column cartridges (φ 3.0 × 200 mm). The catalyst column cartridges were installed onto the reactor, and D_2O was used as a blank solvent, which was flowed at a rate of 0.05 mL/min for 30 minutes to flush air out. The catalyst column was then heated to 100 °C whilst maintaining a D_2O flow rate of 0.05 mL/min along with hydrogen gas at a flow rate of 0.8 mL/min. After stabilisation, the reaction was initiated by switching the feed solution to a 0.125 M solution of carbohydrates (**1a** or **1b**) in D_2O. The resulting solution was fractionated every 20 min (1 mL per fraction) using a fraction collector. Selected fractions were analysed by TLC using phosphomolybdic acid reagent as the visualisation reagent. After the collection of the fractions over the designated period, D_2O was evaporated from the combined fractions. To the resulting residue, 5 equiv of acetic anhydride and 10 equiv of pyridine were added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure, and purification was performed using silica gel column chromatography (eluent: chloroform) to obtain spectrally pure deuterated *O*-acetylated carbohydrate **2a** or **2b**, and the deuterium content was determined by ^{1}H NMR analysis. Fractions corresponding to a three-hour period (from the second to

the fourth hour after the start of the reaction, excluding the first hour) were combined, concentrated, and the isolated yield and average deuterium content were calculated.

General Procedure C

Ru/C-catalyzed site- and stereoselective H–D exchange reaction of 1c and 1d (Figure 4)

A Ru/C catalyst (125 mg) was packed into two column cartridges (φ 3.0 × 200 mm for **1c**, φ 5.0 × 200 mm for **1d**). The catalyst column cartridges were installed onto the reactor, and D_2O was used as a blank solvent, which was flowed at a rate of 0.05 mL/min for 30 minutes to flush air out. The catalyst column was then heated to 100 °C whilst maintaining a D_2O flow rate of 0.05 mL/min along with hydrogen gas at a flow rate of 0.8 mL/min. After stabilisation, the reaction was initiated by switching the feed solution to a solution of carbohydrates (**1c** or **1d**, 0.125 M) and LiOH (0.25 M) in D_2O. The resulting solution was fractionated every 20 min (1 mL per fraction) using a fraction collector. Selected fractions were analysed by TLC using phosphomolybdic acid reagent as the visualisation reagent. After the collection of the fractions over the designated period, D_2O was evaporated from the combined fractions. To the resulting residue, 10 equiv of acetic anhydride and 20 equiv of pyridine were added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure, and purification was performed using silica gel column chromatography (eluent: chloroform) to obtain spectrally pure deuterated *O*-acetylated carbohydrate **2c** or **2d**, and the deuterium content was determined by ^{1}H NMR analysis. Fractions corresponding to a three-hour period (from the second to the fourth hour after the start of the reaction, excluding the first hour) were combined, concentrated, and the isolated yield and average deuterium content were calculated.

General Procedure D

Ru/C-catalyzed long-term continuous flow deuterium labeling of 1a (Figure 5)

A Ru/C catalyst (125 mg) was packed into two column cartridges (φ 3.0 × 200 mm). The catalyst column cartridges were installed onto the reactor, and D_2O was used as a blank solvent, which was flowed at a rate of 0.05 mL/min for 30 minutes to flush air out. The catalyst column was then heated to 100 °C whilst maintaining a D_2O flow rate of 0.05 mL/min along with hydrogen gas at a flow rate of 0.8 mL/min. After stabilisation, the reaction was initiated by switching the feed solution to a 0.125 M solution of carbohydrates **1a** in D_2O. The resulting solution was fractionated every 1 day (72 mL per fraction) using a round-bottom flask. Selected fractions were analysed by TLC using phosphomolybdic acid reagent as the visualisation reagent. Deuterium content in the fractions was determined by ^{1}H NMR analysis. After the collection of the fractions over the designated period, D_2O was evaporated from the combined fractions. To the resulting residue, 5 equiv of acetic anhydride and 10 equiv of pyridine were added, and the mixture was stirred at room temperature for 24 h. The

reaction mixture was then concentrated under reduced pressure, and purification was performed using silica gel column chromatography (eluent: chloroform) to obtain spectrally pure deuterated *O*-acetylated carbohydrate **2a**, and the deuterium content was determined by ^{1}H NMR analysis. Fractions corresponding to the entire 150-hour period were combined, concentrated, and the isolated yield and average deuterium content were calculated.

3. Calculation of TON, TOF, and STY (Figure 5)

TON (turn over number) = 53.288 (product **2a**, mmol) × (0.95 × 4 + 0.94 × 1) / 0.2474 (Ru, mmol)
= ca. 1021

TOF (turn over frequency) = 1021 / 150 h = 6.8 h^{-1}

STY (space time yield) = 53.288 (product **2a**, mmol) × (0.95 × 4 + 0.94 × 1) / (1.33 × 2 mL × 150 h)
= 4.5 mol $Lcat^{-1}$ h^{-1}

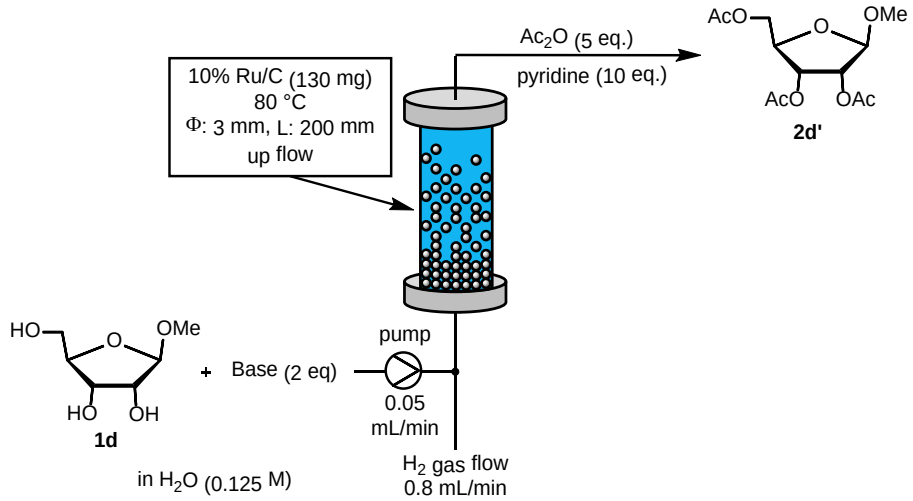
4. Optimisation of basic additives for deuteration of furanoside 2d:

General Procedure E

A Ru/C catalyst (130 mg) was packed into a column cartridge (φ 3.0 × 200 mm). The catalyst column cartridge was installed onto the reactor, and H_2O^* was used as a blank solvent, which was flowed at a rate of 0.05 mL/min for 30 minutes to flush air out. The catalyst column was then heated to 80 °C whilst maintaining a H_2O flow rate of 0.05 mL/min along with hydrogen gas at a flow rate of 0.8 mL/min. After stabilisation, the reaction was initiated by switching the feed solution to a solution of methyl-*β*-D-ribofuranoside (**1d**, 0.25 mmol, 0.125 M) and base (none or NaOH or LiOH, 0.5 or 2.5 mmol, 0.25 or 1.25 M) in H_2O^*. After the entire solution in the flask had passed through the catalyst cartridge, the flask was rinsed with 1.0 mL of H_2O three times to clean the inner walls of the flask, followed by 50 mL of H_2O to flush the flow path. After collecting the entire solution into a single round-bottom flask, the reaction mixture was concentrated under reduced pressure. To the resulting residue, 10 equiv of acetic anhydride and 20 equiv of pyridine were added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure, and purification was performed using silica gel column chromatography (eluent: chloroform), and the degree of isomerisation and yield were determined by ^{1}H NMR analysis.

$^*H_2O$ was used instead of D_2O for this optimisation of basic additive to evaluate the stability of **1d** under the flow reaction conditions.

Table S2. Optimisation of basic additives for the flow deuteration of 1d.



Entry	Basic additive	Equiv	Yield (%)	Epimerisation
1	-	-	-	occurred
2	NaOH	2	82	controlled
3	LiOH	2	88	controlled
4[a)]	LiOH	10	13	controlled

a) Flow velocity drops and pump stops.

In the absence of a base, the peak associated with the methoxy group at the C1 position of **2d'** diminished in the ^{1}H NMR spectra [Table S2; entry 1, Figure S2(b); red box], and the peaks within the range of 4.28–4.38 ppm, corresponding to the target compound, were completely replaced by distinct peaks [Figure S2(b), blue box]. These findings suggest that partial epimerisation of methyl-2,3,5-triacetyl-*β*-D-ribofuranoside (**2d'**) occurred at the C1 position. Upon the addition of NaOH (2 equiv), the singlet peak attributed to the methoxy group and the peaks within the range of 4.28–4.38 ppm were preserved, and no epimerisation was observed. However, a minor peak unrelated to the target compound emerged at 3.48 ppm [Table S2; entry 2, Figure S2(c), red box]. When a drop of D_2O was introduced into this NMR tube, this peak at 3.48 ppm disappeared [Figure S2(d), red box], indicating that it originated from hydroxyl groups present in a by-product. Subsequently, the addition of LiOH (2 equiv) completely retained the methoxy group peak, and no new peaks were observed [Table S2; entry 3, Figure S2(e)]. Furthermore, both isomerisation and epimerisation were entirely suppressed, demonstrating that the inclusion of 2 equiv of LiOH was highly effective in mitigating side reactions. However, when the amount of LiOH was incrased to 10 equiv, the flow rate decreased substantially, resulting in the pump failure during the process (Table S2; entry 4).

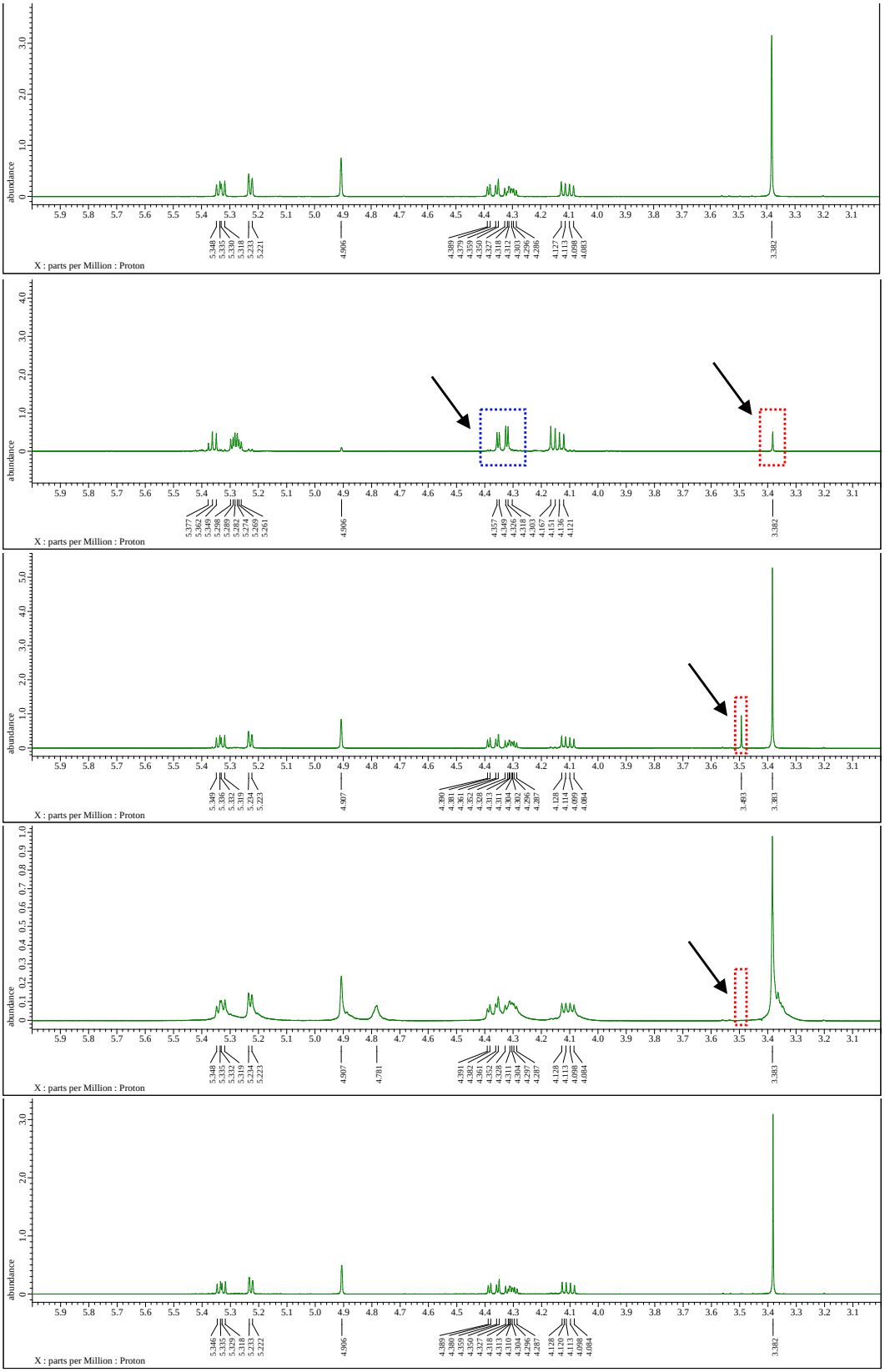
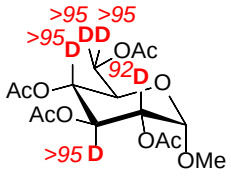


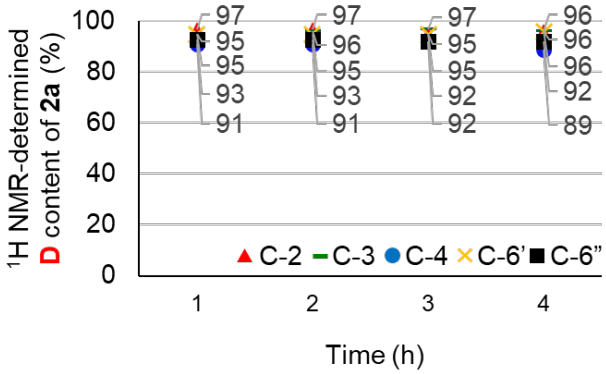
Figure S2. Effect of inorganic base under deuterium labeling reaction conditions.

5. Spectroscopic data of deuterated products:

Methyl 2,3,4,6-tetraacetyl-α-D-glucopyranoside-d_5 (2a):[1,2]

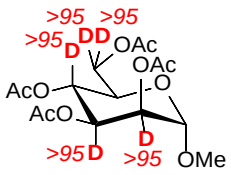


According to General Procedure B, a solution of methyl-α-D-glucopyranoside (1a, 0.125 M) in D_2O (20 mL) was prepared. The D content of 2a was analysed as described below. Fractions were collected from 2 to 4 hours. After evaporation of the solvent and purification using silica gel column chromatography (eluent: chloroform), the isolated product was weighed. The yield was determined by comparing the observed mass with the theoretical mass corresponding to complete conversion of **1a** to **2a**, giving the titled compound **2a** (379.6 mg, 1.125 mmol, 92%) as a colorless oil.

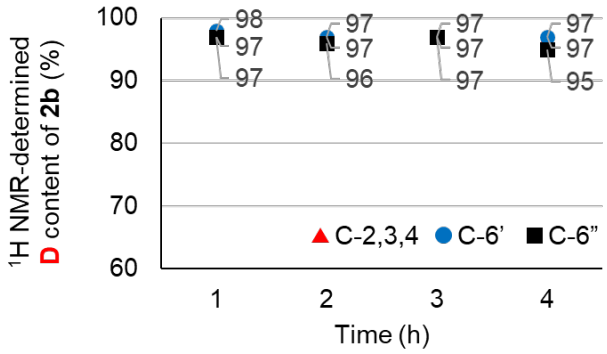


$[\alpha]_D^{20}$ = +126.6 (c 1.0, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.46 (m, 0.03H), 5.04–5.09 (m, 0.02H), 4.94 (s, 1H), 4.89–4.86 (m, 0.08H), 4.23–4.28 (m, 0.04H), 4.08–4.16 (m, 0.05H), 3.96 (s, 1H), 3.40 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H); ^{2}H NMR (61 MHz, $CHCl_3$): δ 5.43 (brs), 4.99 (brs), 4.84 (brs), 4.18 (brs), 4.07 (brs).

Methyl 2,3,4,6-tetraacetyl-α-D-mannopyranoside-d_5 (2b):[1,2]

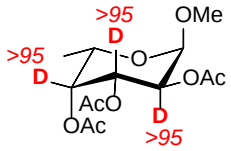


According to General Procedure B, a solution of methyl-α-D-mannopyranoside (**1b**, 0.125 M) in D_2O (20 mL) was prepared. The D content of **2b** was analysed as described below. Fractions were collected from 2 to 4 hours. After evaporation of the solvent and purification using silica gel column chromatography (eluent: chloroform), the isolated product was weighed. The yield was determined by comparing the observed mass with the theoretical mass corresponding to complete conversion of **1b** to **2b**, giving the titled compound **2b** (387.5 mg, 1.125 mmol) as a colorless oil.

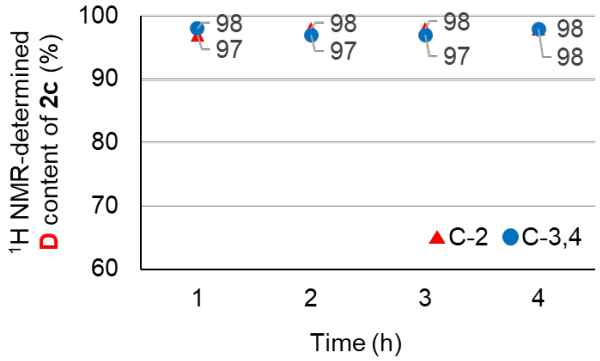


[α]D^{20} = +57.5 (c 1.0, MeOH).^{1}H NMR (500 MHz, $CDCl_3$): δ 5.31–5.21 (m, 0.07H), 4.70 (s, 1H), 4.26–4.25 (m, 0.07H), 4.18–4.09 (m, 0.04H), 3.95 (s, 1H), 3.40 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H); ^{2}H NMR (77 MHz, $CHCl_3$): δ 5.25 (brs), 4.24 (brs), 4.08 (brs).

Methyl 2,3,4-triacetyl-α-L-fucopyranoside-*d*$_3$ (2c):[1,2]

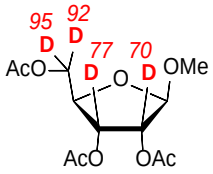


According to General Procedure C using catalyst cartridges (φ 3.0 × 200 mm), a solution of methyl-α-L-fucopyranoside (**1c**, 0.125 M) and LiOH (0.25 M) in D_2O (20 mL) was prepared. The D content of **2c** was analysed as described below. Fractions were collected from 2 to 4 hours. After evaporation of the solvent and purification using silica gel column chromatography (eluent: chloroform), the isolated product was weighed. The yield was determined by comparing the observed mass with the theoretical mass corresponding to complete conversion of **1c** to **2c**, giving the titled compound **2c** (319.4 mg, 1.125 mmol) as a colorless oil.

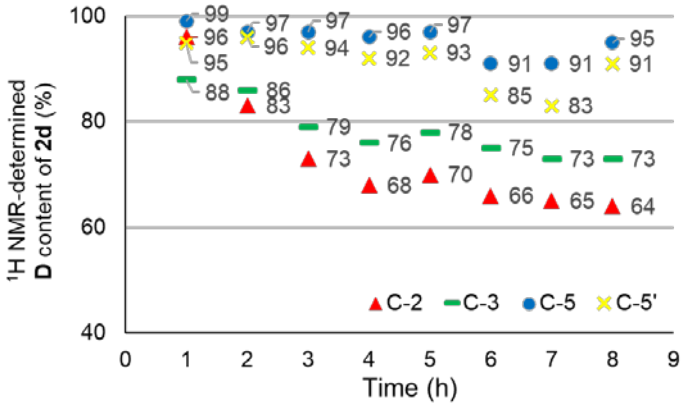


[α]D^{20} = −112.6 (c 1.0, MeOH).^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34–5.29 (m, 0.06H), 5.15–5.14 (m, 0.02H), 4.93 (s, 1H), 4.14–4.09 (m, 1H), 3.39 (s, 3H), 2.17 (s, 3H), 2.09 (s, 3H), 1.98 (s, 3H), 1.16–1.14 (m, 3H); ^{2}H NMR (61 MHZ, $CHCl_3$): δ 5.32 (brs), 5.14 (brs).

Methyl-2,3,5-triacetyl-β-D-ribofuranoside-d_4 (2d):[1,2]



According to General Procedure C using catalyst cartridges (φ 5.0 × 200 mm), a solution of methyl-*β*-D-ribofuranoside (**1d**, 0.125 M) and LiOH (0.25 M) in D_2O (20 mL) was prepared. The D content of **2d** was analysed as described below. Fractions were collected from 4 to 8 hours. After evaporation of the solvent and purification using silica gel column chromatography (eluent: chloroform), the isolated product was weighed. The yield was determined by comparing the observed mass with the theoretical mass corresponding to complete conversion of **1d** to **2d**, giving the titled compound **2d** (459.6 mg, 1.57 mmol) was obtained in 84% yield as a colorless oil. as a colorless oil.



$[\alpha]_D^{20}$ = −19.2 (c 1.0, MeOH).^{1}H NMR (400 MHz, $CDCl_3$): *δ* 5.32–5.30 (m, 0.34H), 5.20–5.19 (m, 0.46H), 4.88 (s, 1H), 4.32 (s, 0.09H), 4.28 (s, 1H), 4.07–4.05 (m, 0.13H), 3.36 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); ^{2}H NMR (61 MHz, $CHCl_3$): *δ* 5.28 (brs), 4.29 (brs), 4.04 (brs).

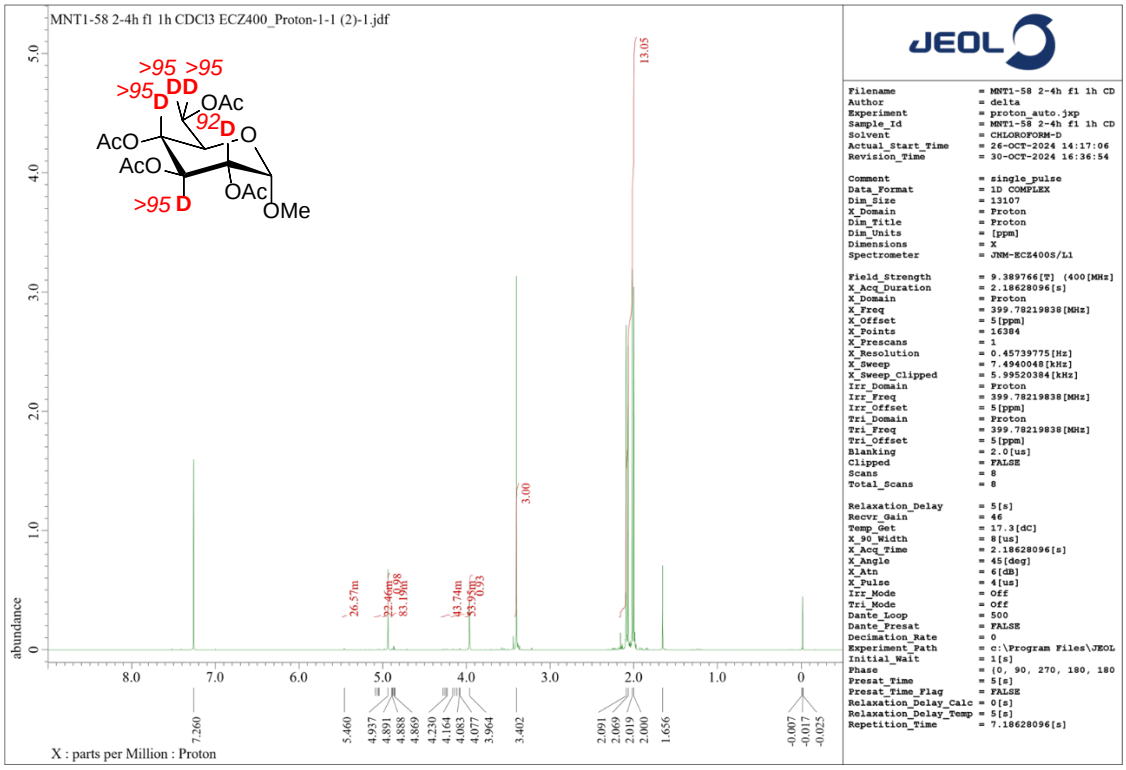
6. References

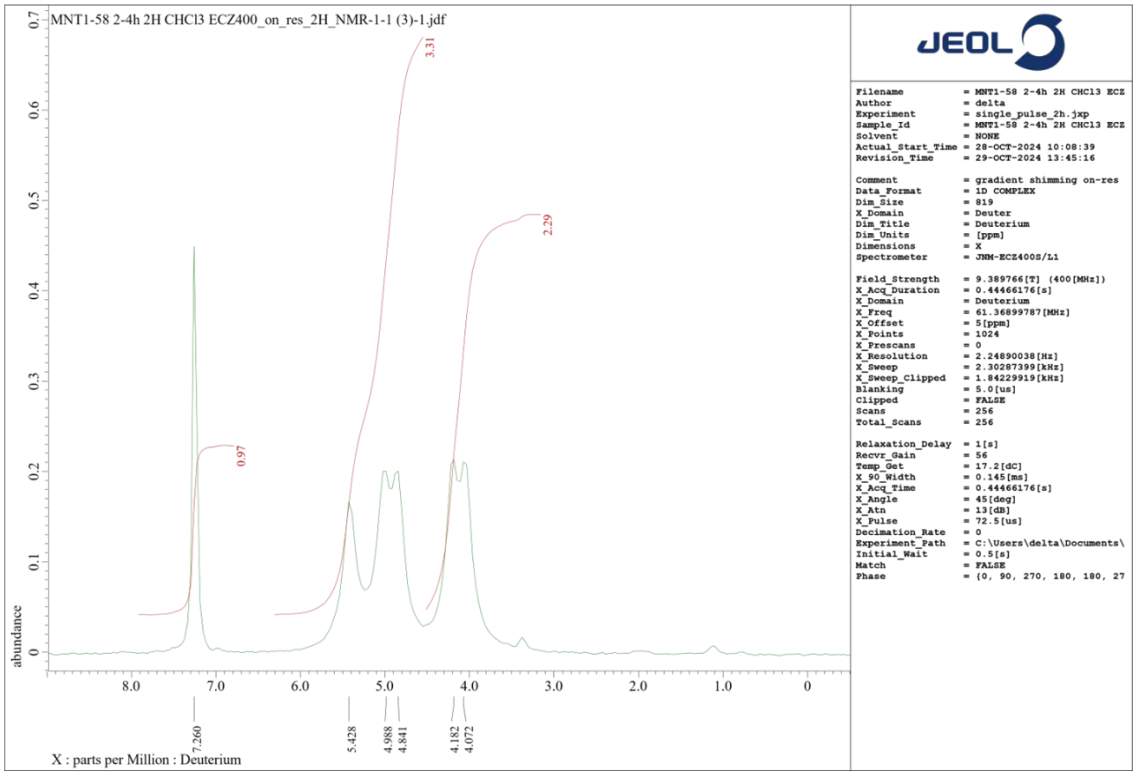
1) Y. Fujiwara, H. Iwata, Y. Sawama, Y. Monguchi, H. Sajiki, *Chem. Commun.*, 2010, **46**, 4977–4979.

2) Y. Sawama, Y. Yabe, H. Iwata, Y. Fujiwara, Y. Monguchi, H. Sajiki, *Chem. Eur. J.*, 2012, **18**, 16436–16442.

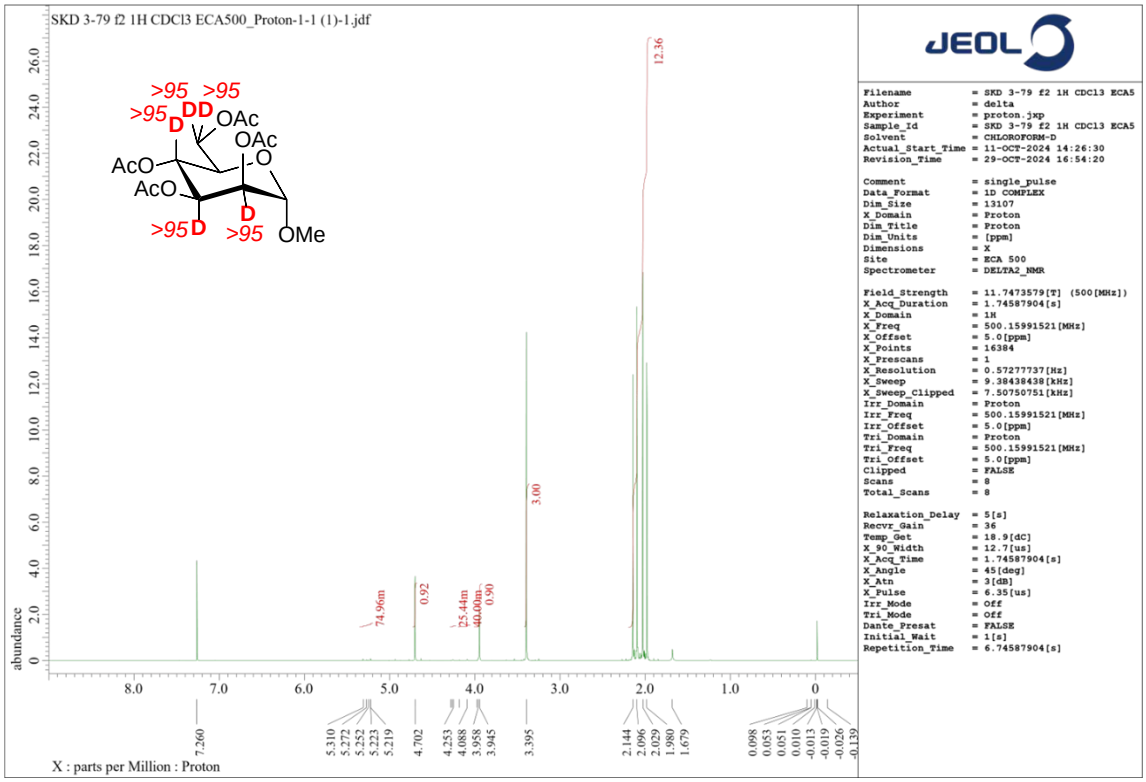
7. ^{1}H and ^{2}H NMR spectra of deuterated products

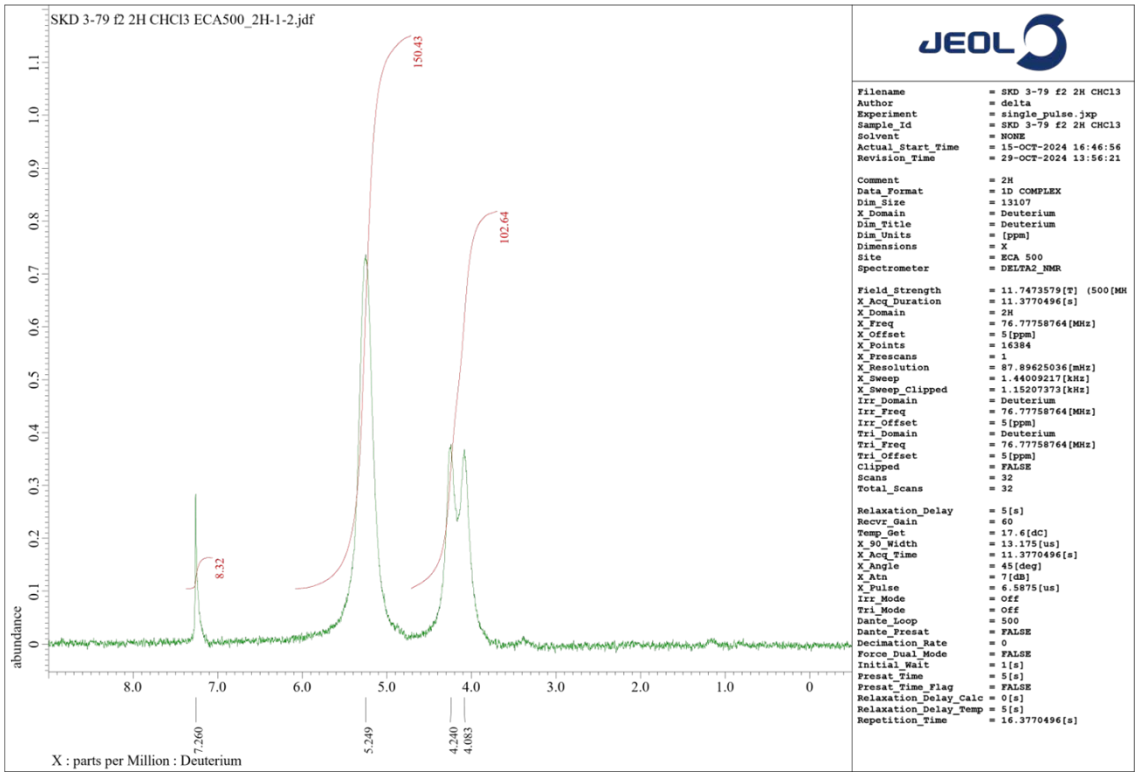
Methyl 2,3,4,6-tetraacetyl-α-D-glucopyranoside-d_5 (**2a**)



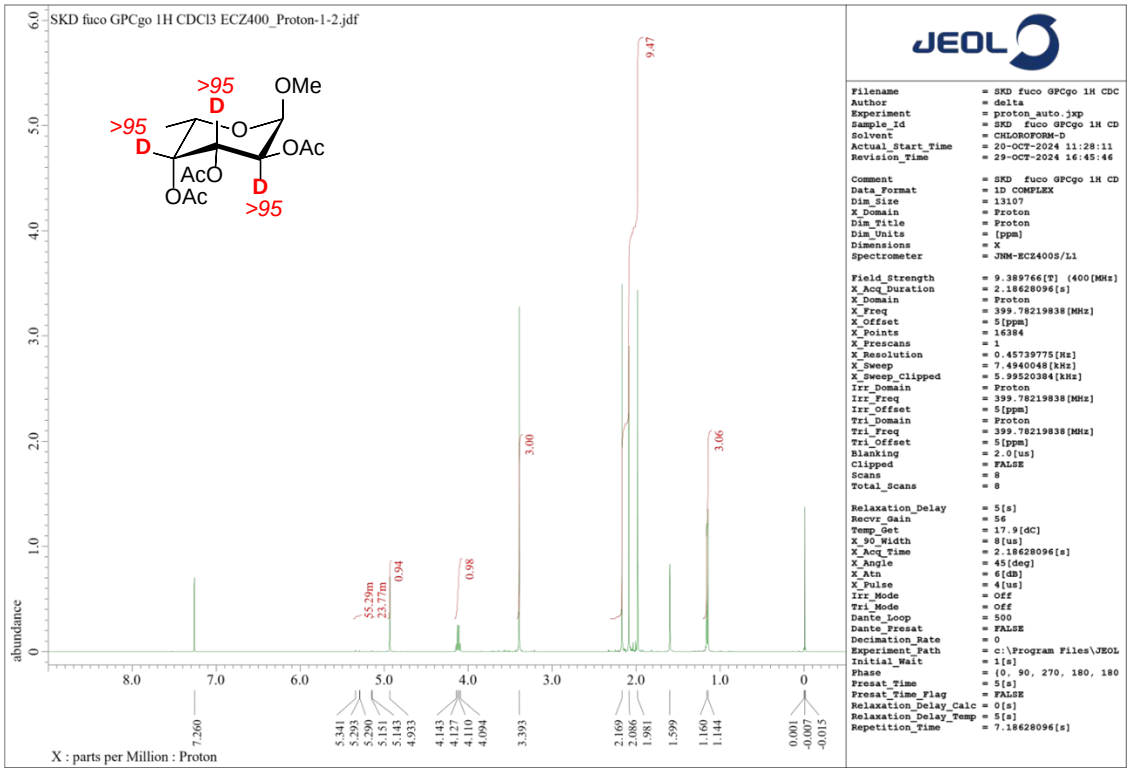


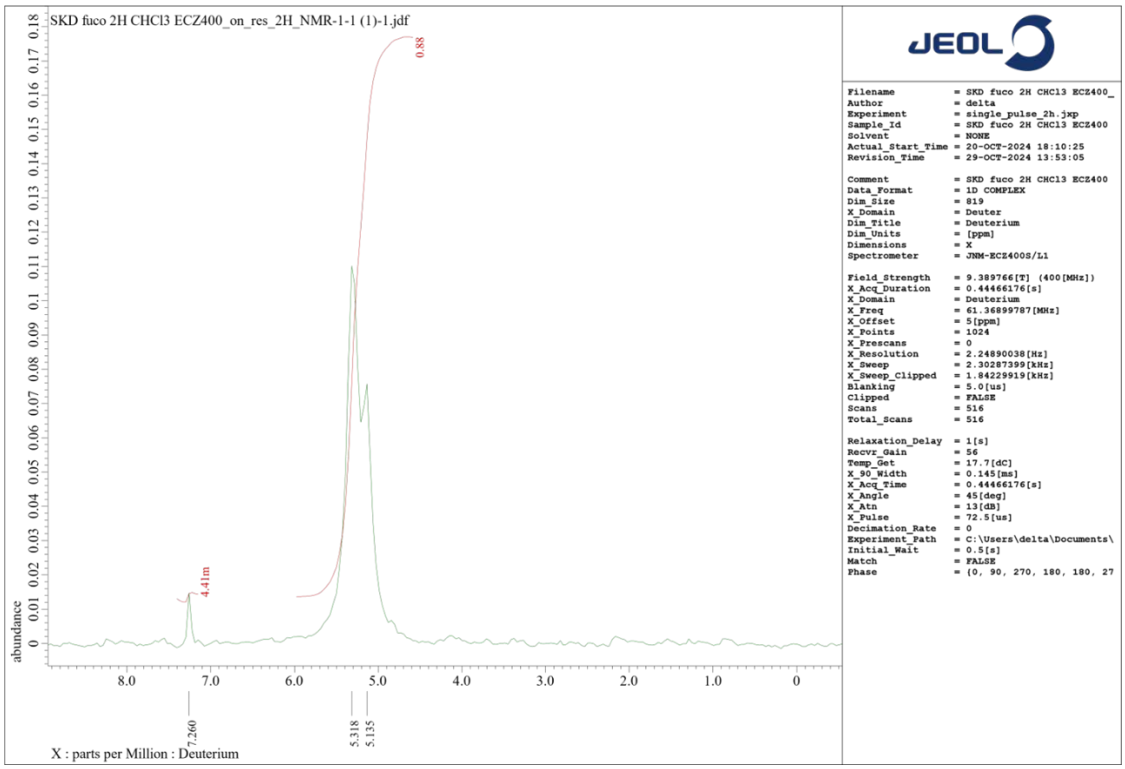
Methyl 2,3,4,6-tetraacetyl-α-D-mannopyranoside-d_5 (**2b**)





Methyl 2,3,4-triacetyl-α-L-fucopyranoside-d_3 (**2c**)





Methyl-2,3,5-triacetyl-β-D-ribofuranoside-d_4 (**2d**)