# Supplementary Information

# Chemistry-driven translocation of glycosylated proteins in mice

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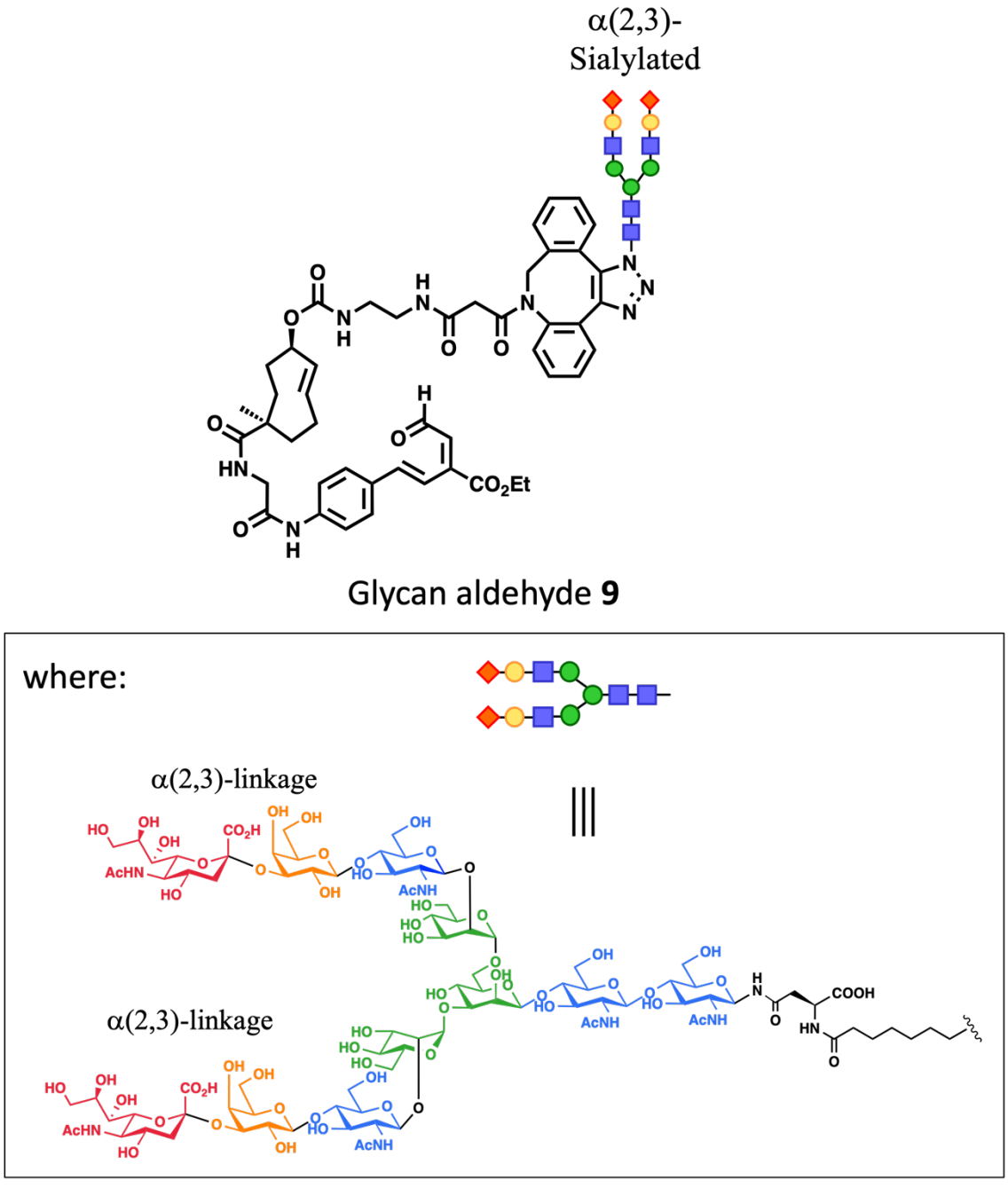
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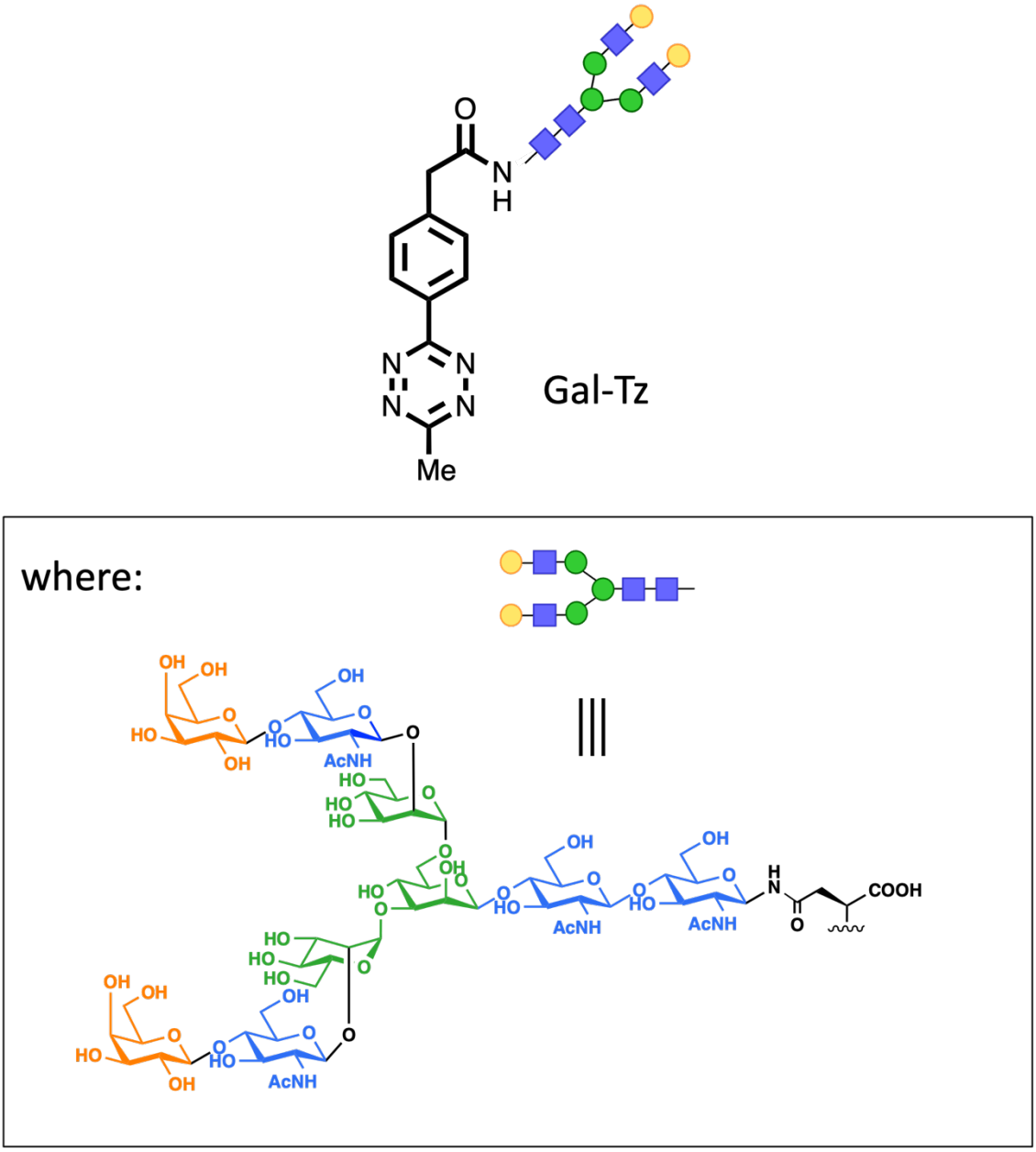
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# 1. Supplementary Methods

## 1.1 Structure of glycan aldehyde **9** and Gal-Tz



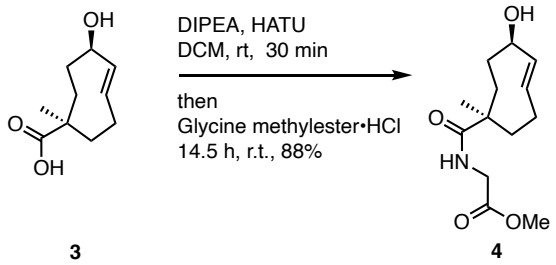

**Supplementary Figure 1.** The structure of glycan aldehyde **9**.




**Supplementary Figure 2.** The structure of Gal-Tz.

## 1.2 Synthetic protocols and characterizations

Synthesis of **4**




**3** **4**

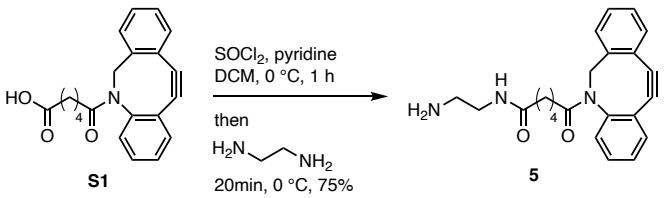
To a stirred solution of the reported carboxylic acid **3**[1] (51.7 mg, 283.5 μmol, 1.0 eq.) and DIPEA (60.0 μL, 343.5 μmol, 1.2 eq.) in anhydrous DCM (10.0 ml) was added by HATU (129.3 mg, 340.0 μmol, 1.2 eq.) at room temperature. After stirring the mixture for 30 minutes, the mixture solution of glycine methyl ester hydrochloride (70.2 mg, 850.5 μmol, 3.0 eq.) and DIPEA (198.0 μl, 1133.6 μmol, 4.0 eq.) in anhydrous DCM was added dropwise into the above solution. The resulted mixture was stirred for 14.5 hours at room temperature, followed by removing solvent under vacuum, and purified by silica gel column chromatography (eluent Hexane/AcOEt gradient from 50% to 100%) to give methyl ester **4** as a colorless viscous oil at a 88% yield.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.08 (s, 1H), 6.05 (ddd, $J$ = 16.2, 11.1, 3.7 Hz, 1H), 5.63 (dd, $J$ = 16.6, 2.6 Hz, 1H), 3.96 (d, $J$ = 5.2 Hz, 2H), 3.73 (s, 3H), 2.34–2.28 (m, 1H), 2.24 (qd, $J$ = 11.8, 3.9 Hz, 1H), 2.14–1.74 (m, 7H), 1.58 (dd, $J$ = 15.4, 6.2 Hz, 1H), 1.11 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 180.9, 170.9, 135.2, 130.3, 69.7, 52.4, 45.9, 44.5, 41.5, 38.5, 31.1, 30.2, 17.8.

HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{13}H_{21}NNaO_4$: 278.1363; found 278.1361.

Synthesis of **5**




**S1** **5**

To a stirred solution of the reported carboxylic acid **S1**[2] (200.0 mg, 600.6 μmol, 1.0 eq.) and pyridine (52.7 μl, 660.7 μmol, 1.1 eq.) in anhydrous DCM (10.0 ml) was added by $SOCl_2$ (47.9 μl, 660.7 μmol, 1.1 eq.) in 0 °C. After stirring the mixture at 0 °C for 1 hour,

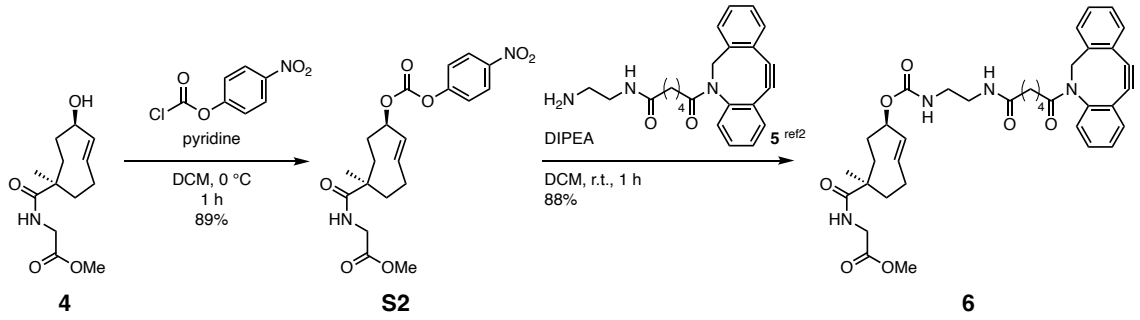
the solution was added by ethylene diamine dropwise (80.0 µl, 1.2 µmol, 20 eq.). The obtained solution was stirred for 20 minutes at 0 °C, followed by removing solvent under vacuum and purified by silica gel column chromatography (eluent $CHCl_3$/MeOH gradient from 5% to 30%) to give amine **5**, as a dark brown amorphous solid at a 75% yield.

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.69 (d, $J$ = 7.4 Hz, 1H), 7.43–7.24 (m, 7H), 5.16 (d, $J$ = 14.0 Hz, 1H), 3.66 (d, $J$ = 14.0 Hz, 1H), 2.27–2.19 (m, 2H), 2.12 (td, $J$ = 7.4, 1.4 Hz, 2H), 1.96–1.86 (m, 2H), 1.54–1.28 (m, 6H).

$^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 173.5, 173.3, 151.8, 148.2, 132.4, 129.1, 128.6, 128.3, 128.2, 127.9, 127.3, 125.6, 123.2, 122.6, 115.1, 108.0, 55.5, 41.6, 41.3, 35.9, 34.6, 25.1, 24.5.

HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{23}H_{26}N_3O_2$ 376.2020; found 376.2019

Synthesis of **6**




To a stirred solution of alcohol **4** (47.7 mg, 186.8 µmol, 1.0 eq.) and anhydrous pyridine (150.0 µl, 1865.0 µmol, 10.0 eq.) in anhydrous DCM (5.0 ml) was added by 4-nitrophenyl chloroformate (180.0 mg, 932.5 µmol, 5.0 eq.) at 0 °C. The obtained solution was stirred for 1 hours, followed by removing solvent under vacuum, and purified by silica gel column chromatography (eluent Hexane/AcOEt gradient from 10% to 100%) to give carbonate intermediate **S2** as an amorphas solid at a 89% yield.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.28 (d, $J$ = 9.3 Hz, 2H), 7.40 (d, $J$ = 9.3 Hz, 2H), 6.08 (ddd, $J$ = 16.8, 11.3, 3.6 Hz, 1H), 6.01 (s, 1H), 5.65 (dd, $J$ = 16.8, 2.6 Hz, 1H), 5.29 (s, 1H), 4.00 (dd, $J$ = 5.1, 1.9 Hz, 2H), 3.76 (s, 3H), 2.44–2.37 (m, 1H), 2.30 (qd, $J$ = 11.8, 3.9 Hz, 1H), 2.24–2.13 (m, 2H), 2.02–1.86 (m, 3H), 1.75 (dd, $J$ = 15.1, 6.3 Hz, 1H), 1.18 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 180.2, 170.9, 155.7, 151.7, 145.5, 133.0, 129.5, 125.5, 121.9, 77.0, 52.5, 45.7, 44.5, 41.5, 35.9, 31.3, 31.2, 18.0.

HRMS (ESI): m/z $[M+Na]^+$ calculated for $C_{20}H_{24}N_2NaO_8$ 443.1425; found 443.1427.

To a mixture solution of carbonate intermediate **S2** (11.1 mg, 26.4 µmol, 1.0 eq.) and

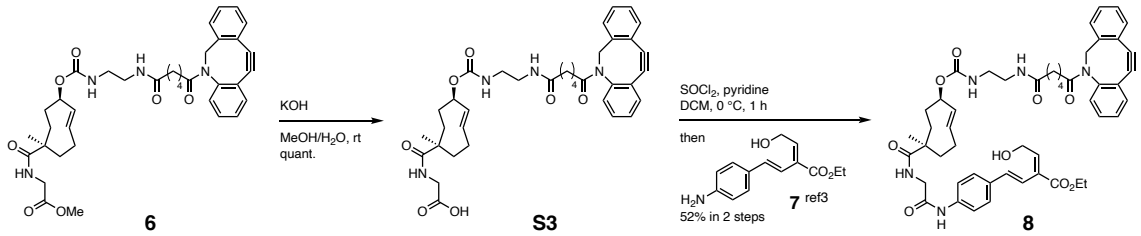
amine **5** (15.7 mg, 41.8 μmol, 1.6 eq.) in anhydrous DCM (1.0 ml) was added by DIPEA (9.3 μl, 52.8 μmol, 2.0 eq.) at 35 °C. After stirring the mixture for 1 hours, solvent was removed under vacuum and purified by silica gel column chromatography (eluent $CHCl_3$/MeOH gradient from 1% to 7%) to give methyl ester **6** as a yellowish amorphous solid at a 88% yield.

$^1$H NMR (400 MHz, $CDCl_3$) : $\delta$ 7.72 (d, $J$ = 7.5 Hz, 1H), 7.43–7.24 (m, 7H), 6.09 (s, 1H), 5.91 (m, 2H), 5.73–5.67 (m, 1H), 5.61 (dd, $J$ = 16.7, 2.6 Hz, 1H), 5.17 (s, 1H), 5.16 (d, $J$ = 13.9 Hz, 1H), 3.96 (d, $J$ = 5.1 Hz, 2H), 3.43–3.05 (m, 4H), 3.25 (q, $J$ = 5.6 Hz, 2H), 3.20–3.05 (m, 1H), 2.35–2.19 (m, 3H), 2.08 (m, 2H), 1.99–1.75 (m, 7H), 1.64 (dd, $J$ = 15.5, 6.1 Hz, 1H), 1.52–1.37 (m, 3H), 1.14 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) : $\delta$ 180.6, 173.7, 173.6, 170.9, 156.3, 151.7, 148.2, 132.5, 131.7, 131.6, 131.5, 129.2, 128.6, 128.4, 128.3, 128.0, 127.3, 125.7, 123.3, 122.6, 115.0, 108.1, 72.3, 55.6, 52.4, 45.7, 44.5, 41.5, 41.2, 40.0, 36.1, 35.7, 34.7, 31.2, 25.1, 24.1, 17.9.

HRMS (ESI) : m/z $[M+Na]^+$ calculated for $C_{37}H_{44}N_4NaO_7$ 679.3102; found 679.3120.

Synthesis of **8**




To a stirred solution of **6** (7.6 mg, 11.6 μmol, 1.0 eq.) in mixture of MeOH (1.0 ml) and $H_2O$ (1.0 ml) was added by 30% KOH aqueous solution at room temperature. After stirring the mixture for 1 hours, the mixture was cooled to 0 °C, followed by acidifying to a pH of 1.0 using a 2 M HCl aqueous solution. The mixture solution was diluted with $H_2O$ (10 ml) and then extracted with AcOEt (20 ml × 3). The combined organic layers were washed with sat. NaCl aqueous solution (10 ml × 2), dried over by $Na_2SO_4$, filtered, and concentrated under vacuum. The obtained crude carboxylic acid **S3** was an amorphous solid and used in the next step without further purification.

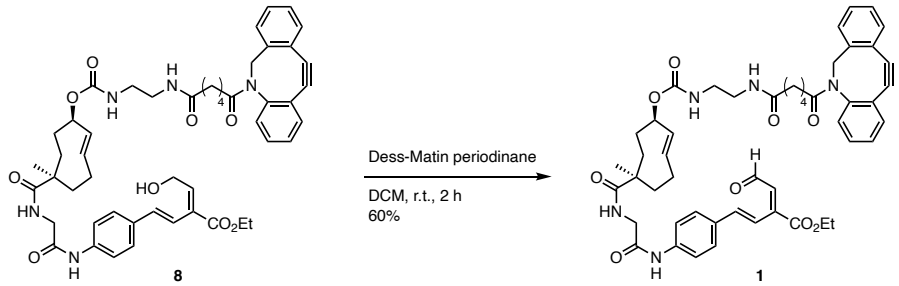
$^1$H NMR (400 MHz, $CDCl_3$) : $\delta$ 7.67 (d, $J$ = 7.1 Hz, 1H), 7.48–7.21 (m, 7H), 6.55 (s, 1H), 6.51–6.44 (m, 1H), 6.08–5.99 (m, 1H), 5.86–5.79 (m, 1H), 5.57 (d, $J$ = 16.4 Hz, 1H), 5.14 (d, $J$ = 13.8 Hz, 1H), 5.12 (s, 1H), 3.94 (dd, $J$ = 18.3, 5.3 Hz, 2H), 3.68 (d, J = 13.8 Hz, 1H), 3.43–3.07 (m, 4H), 2.26–1.76 (m, 12H), 1.59 (dd, J = 14.8, 5.9 Hz, 1H), 1.45–1.35 (m, 3H), 1.11 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) : $\delta$ 181.4, 174.5, 174.0, 172.1, 156.6, 151.6, 148.0, 132.5,

131.7, 131.5, 129.1, 128.7, 128.5, 128.3, 128.0, 127.3, 125.7, 123.2, 122.6, 115.1, 107.9, 72.3, 55.7, 45.6, 44.5, 41.9, 40.9, 40.1, 36.1, 35.8, 34.5, 31.2, 29.8, 24.9, 24.4, 17.9.
HRMS (ESI) : m/z $[M+Na]^+$ calculated for $C_{36}H_{42}N_4NaO_7$ 665.2946; found 665.2958

To a stirred solution of carboxylic acid **S3** (7.4 mg, 11.6 μmol, 1.0 eq.) and pyridine (2.3 μl, 29.0 μmol, 2.5 eq.) in anhydrous DCM (5.0 ml) was added by $SOCl_2$ (1.0 μl, 13.9 μmol, 1.2 eq.) in 0 °C. After stirring the mixture at 0 °C for 10 minutes, aniline **7**[3] (8.6 mg, 34.8 μmol, 3.0 eq.) in anhydrous DCM (1.0 ml) was added into the above solution. The obtained mixture solution was stirred for 1 hour at 0 °C, followed by removing solvent under vacuum, and purified by silica gel column chromatography (eluent $CHCl_3$/MeOH gradient from 1% to 10%) to give the allylalcohol **8** at a 52% yield in 2 steps.
$^1$H NMR (400 MHz, $CDCl_3$) : $\delta$ 7.68 (d, $J$ = 7.1 Hz, 1H), 7.49 (d, $J$ = 8.3 Hz, 2H), 7.44–7.19 (m, 9H), 6.86–6.75 (m, 3H), 6.28–6.11 (m, 1H), 6.06–5.92 (s, 1H), 5.92–5.80 (m, 1H), 5.58 (d, $J$ = 16.5 Hz, 1H), 5.13 (d, $J$ = 13.5 Hz, 1H), 5.12 (s, 1H), 4.51 (d, $J$ = 6.1 Hz, 2H), 4.26 (q, $J$ = 7.1 Hz, 2H), 4.11–3.86 (m, 2H), 3.67 (d, $J$ = 13.5. Hz, 1H), 3.38–3.05 (m, 4H), 2.33–2.10 (m, 6H), 1.98–1.90 (m, 2H), 1.87–1.74 (m, 4H), 1.58 (dd, J = 15.5, 6.3 Hz, 1H), 1.45–1.36 (m, 3H), 1.32 (t, $J$ = 7.1 Hz, 3H), 1.15 (s, 3H).
$^{13}$C NMR (101 MHz, $CDCl_3$) : $\delta$ 181.6, 173.8, 173.7, 167.7, 167.1, 156.5, 151.7, 148.2, 140.9, 138.0, 134.3, 133.1, 132.5, 131.8, 130.9, 129.1, 128.7, 128.4, 128.3, 128.3, 128.0, 127.4, 127.3, 125.7, 123.2, 122.6, 120.2, 120.0, 115.0, 108.1, 72.3, 70.7, 61.2, 59.8, 55.6, 44.6, 40.9, 40.1, 36.0, 35.9, 34.6, 31.5. 31.2, 31.1, 25.1, 24.3, 18.0, 14.4.
HRMS (ESI) : m/z $[M+H]^+$ calculated for $C_{50}H_{58}N_5O_9$ 872.4229; found 872.4226.

Synthesis of **1**




To a stirred solution of allyalcohol **8** (0.87 mg, 1.0 μmol, 1.0 eq.) in DCM (5.0 ml) was added by Dess-Matin periodinane (1.6 mg, 4.0 μmol, 4.0 eq.) at room temperature. After stirring the mixture for 3 hours, the reaction was quenched by adding saturated $NaHCO_3$ aqueous solution (1.0 ml) and 10% $Na_2SO_3$ aqueous solution (1.0 ml). The obtained solution was stirred for 30 min and then added by brine (10ml). The mixture solution was

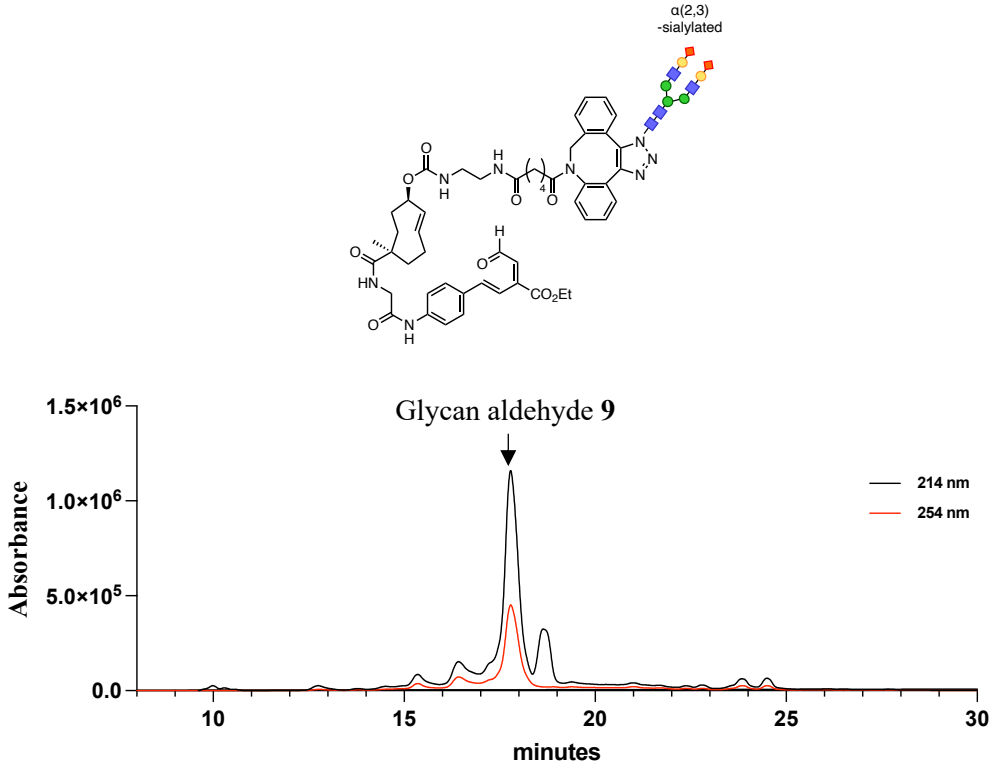
extracted with DCM (10 ml × 2), and the combined organic layer was dried over by $Na_2SO_4$ and filtered. The obtained aldehyde product was unstable at room temperature after removal of solvent. Therefore, the solvent evaporation was performed at 0 °C. Crude aldehyde **1** was obtained as a yellowish oil and the reaction yield was determined by NMR (60%-calculated; DMF was used as reference), which was used in the next step without further purification.

$^1$H NMR (400 MHz, $CD_2Cl_2$) : $\delta$ 10.08 (d, $J$ = 7.1 Hz, 1H), 7.60 (d, $J$ = 7.4 Hz, 1H), 7.16–7.47 (m, 12H), 6.99 (d, $J$ = 15.9 Hz, 1H), 6.49 (d, J = 7.1 Hz, 1H), 5.72–5.91 (m, 2H), 5.53 (d, $J$ = 16.4 Hz, 1H), 5.06 (d, $J$ = 14.0 Hz, 2H), 4.25 (q, $J$ = 7.2 Hz, 2H), 3.60 (d, $J$ = 14.0 Hz, 1H), 3.04–3.25 (m, 4H), 2.09–2.23 (m, 4H), 1.71–1.88 (m, 6H), 1.47–1.60 (m, 6H), 1.27 (t, $J$ = 7.2 Hz, 3H), 1.07 (s, 3H).

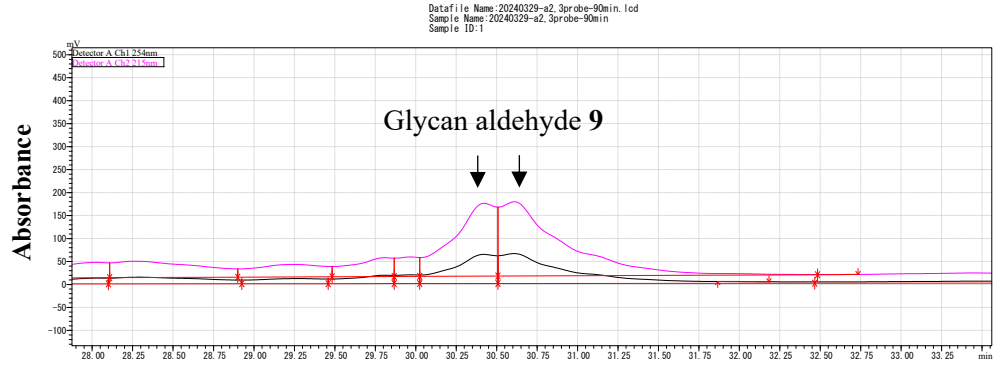
$^{13}$C NMR (101 MHz, $CD_2Cl_2$) : $\delta$ 191.6, 173.7, 173.7, 168.2, 166.9, 162.7, 156.5, 148.8, 146.5, 141.0, 132.7, 131.7, 131.7, 131.6, 131.0, 130.9, 129.6, 128.9, 128.8, 128.5, 128.5, 128.4, 128.2, 128.1, 127.5, 125.9, 123.6, 122.8, 120.1, 115.0, 108.5, 72.5, 62.4, 55.8, 44.8, 41.4, 36.6, 36.2, 36.1, 34.9, 32.3, 31.4, 30.1, 29.8, 25.4, 23.1, 18.1, 14.3.

HRMS (ESI) : m/z $[M+H]^+$ calculated for $C_{50}H_{56}N_5O_9$ 870.4073; found 870.4091.

Characterization of glycan aldehyde **9**



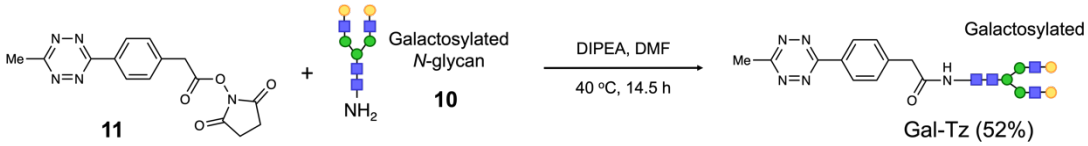

**Supplementary Figure 3.** HPLC analysis (column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 100%B over 30 min; flow rate: 1 ml/min; ) of the reaction mixture of SPAAC reaction between α(2,3)-sialylated glycan azide **2** and aldehyde **1** after 3 hours. The glycan aldehyde **9** was observed at 18 min and confirmed by ESI-MS (HRMS (ESI) : m/z $[M-2H]^{-2}$ calculated for $C_{145}H_{210}N_{16}O_{74}$ 1679.6586; found 1679.6545).




**Supplementary Figure 4.** HPLC analysis (column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 100%B over 90 min; flow rate: 1

ml/min) of the purified glycan aldehyde **9** showed two peaks at 30.3 and 30.7 min to indicate two regioisomers of **9** as a result of the SPAAC reaction.

Synthesis of Gal-Tz

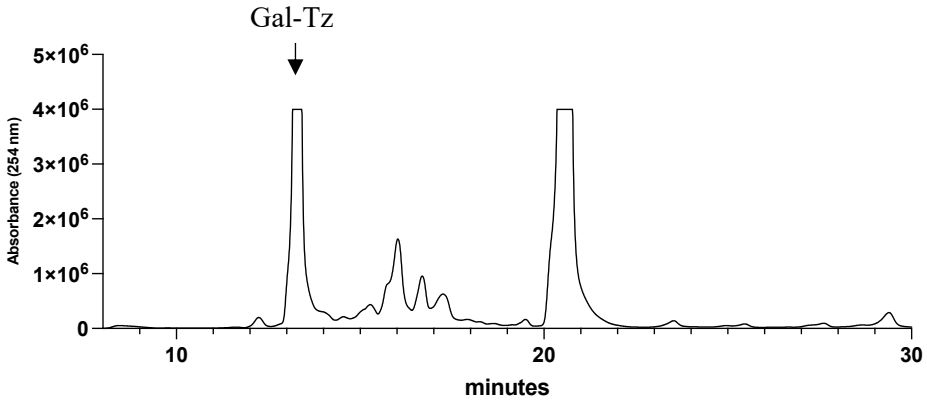



To a mixture solution of ester **11** (10.2 mg, 25.8 μmol, 5.0 eq.) and Gal-amine **10** (8.9 mg, 5.1 μmol, 1.0 eq.) in DMF (3.0 ml) was added by DIPEA (9.0 μl, 51.0 μmol, 10.0 eq.). After stirring the mixture for 5 hours at 35 °C, the solvent was removed under vacuum and purified by HPLC (column was an preparative 20.0 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 100%B over 30 min; flow rate: 8 ml/min; the product was observed at 13.5 min as shown in Supplementary Figure 5) to give the Gal-Tz at a 53% yield.

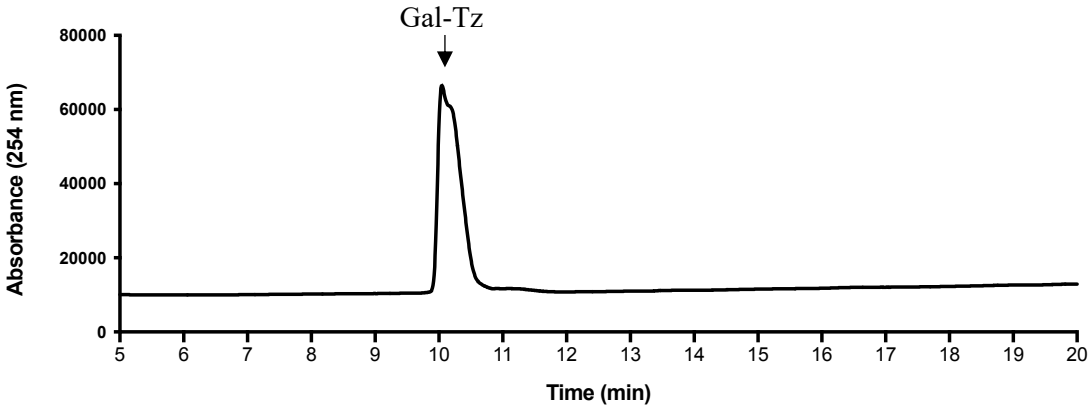
$^1$H NMR (400 MHz, $D_2O$) : $\delta$ 8.39 (d, $J$ = 8.4 Hz, 2H), 7.58 (d, $J$ = 8.4 Hz, 2H), 5.12 (s, 1H), 5.04 (d, $J$ = 9.5 Hz, 1H), 4.93 (s, 1H), 4.76 (s, 1H), 4.61 (d, $J$ = 7.9 Hz, 1H), 4.58 (d, $J$ = 8.1 Hz, 1H), 4.47 (dd, $J$ = 7.8, 2.5 Hz, 2H), 4.25 (d, $J$ = 2.0 Hz, 1H), 4.19 (dd, $J$ = 3.6, 1.5 Hz, 1H), 4.11 (dd, $J$ = 3.7, 1.7 Hz, 1H), 4.02–3.44 (m, 56H), 3.07 (s, 3H), 2.88 (dd, $J$ = 5.8, 2.1 Hz, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 1.97 (s, 3H).

$^{13}$C NMR (101 MHz, $D_2O$) : $\delta$ 174.7, 174.6, 173.8, 173.6, 172.6, 167.2, 164.9, 164.0,163.1, 162.8, 139.7, 130.2, 128.3, 117.8, 102.9, 101.3, 100.4, 99.4, 97.0, 80.4, 78.5, 78.3, 76.2, 75.3, 74.7, 74.4, 73.5, 72.8, 72.7, 72.5, 71.9, 70.9, 70.2, 69.4, 68.5, 67.3, 61.7, 60.9, 59.9, 54.9, 53.7, 49.2, 41.9, 36.9, 31.4, 22.3, 22.2, 21.9, 20.1, 10.3.

HRMS (ESI) : m/z $[M+Na]^+$ calculated for 1989.6941 $C_{77}H_{118}N_{10}NaO_{49}$; found 1989.6959.



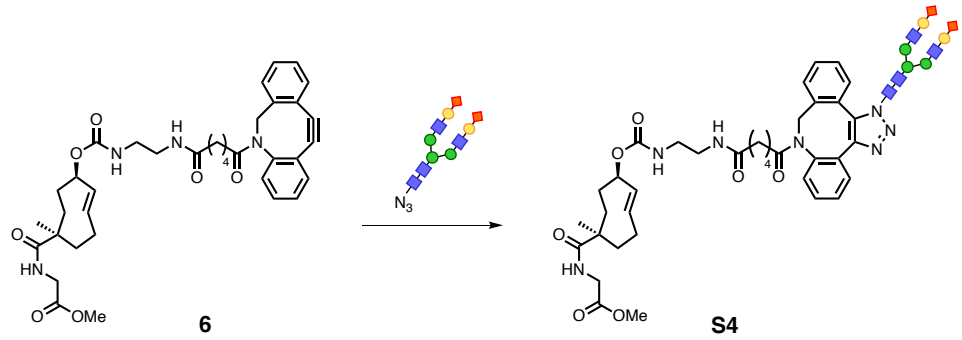

**Supplementary Figure 5.** HPLC analysis of the crude Gal-Tz. The Gal-Tz was observed at 13.5 min.




**Supplementary Figure 6.** HPLC analysis (column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 70%B/30%A over 20 min; flow rate: 1 ml/min) of the purified Gal-Tz. The Gal-Tz was observed at 10.5 min.

Preparation compounds **S4-5** for evaluation of the click-to-release reaction

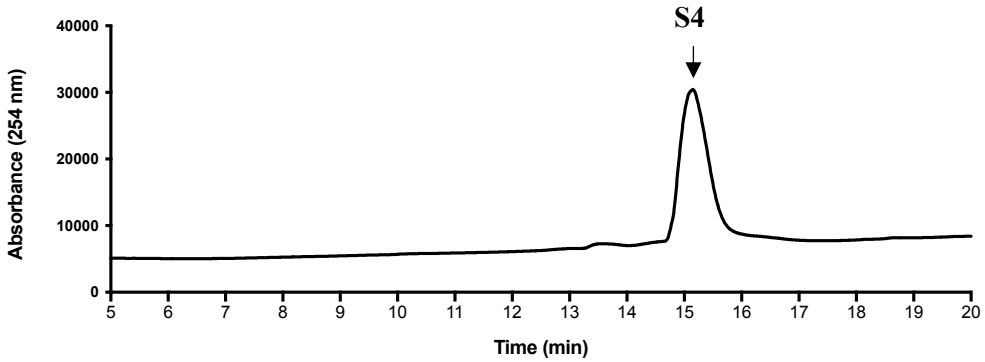
Synthesis of **S4**



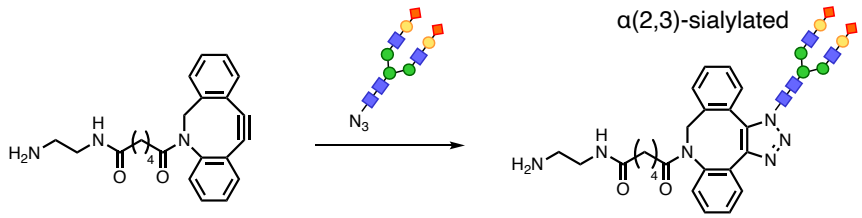

To a stirred solution of glycan-azide **2** (10.0 mg, 4.0 µmol, 1.0 eq.) in DMSO (803 µl) was added by the TCO-ester **6** (2.9 mg, 4.4 µmol, 1.1 eq.). After stirring the mixture for 3 hours at 70 °C, the reaction mixture was purified by HPLC (column was an preparative 20.0 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; B: MeCN + 0.1% TFA; gradient: from 10% B/100% A to 100% B over 30 min; flow rate: 8 ml/min; the product **S4** was observed at 17.0 min (mixture of regioisomers)). The **S4** was collected and followed by lyophilization to give a white solid at a 67% yield.

$^1$H-NMR (400 MHz, $D_2O$) : δ 7.62–7.51 (m, 4H), 7.39–7.34 (m, 3H), 7.18 (d, $J$ =7.5 Hz, 1H), 5.92 (d, $J$ = 16.4 Hz, 1H), 5.78–5.55 (m, 6H), 5.27 (d, $J$ = 3.6 Hz, 1H), 5.06 (s, 2H), 4.99 (dd, $J$ = 17.3, 7.9 Hz, 2H), 4.92 (s, 1H), 4.87 (s, 2H), 4.53–4.51 (m, 7H), 4.42 (d, $J$ = 7.7 Hz, 2H), 4.19 (s, 2H), 4.14 (s, 2H), 4.06–3.27 (m, 72H), 3.26–3.14 (m, 5H), 2.73–2.67 (m, 4H), 2.23–1.64 (m, 36H), 1.56–0.97 (m, 23H).

LRMS (ESI) : m/z $[M-2H+2Na]^{2+}$ calculated for 1574.5782 $C_{129}H_{191}N_{15}Na_2O_{72}$; found 1574.6251.




**Supplementary Figure 7.** HPLC analysis (column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 70%B/30%A over 20 min; flow rate: 1 ml/min) of the purified **S4**. The **S4** was observed at 15.2 min.

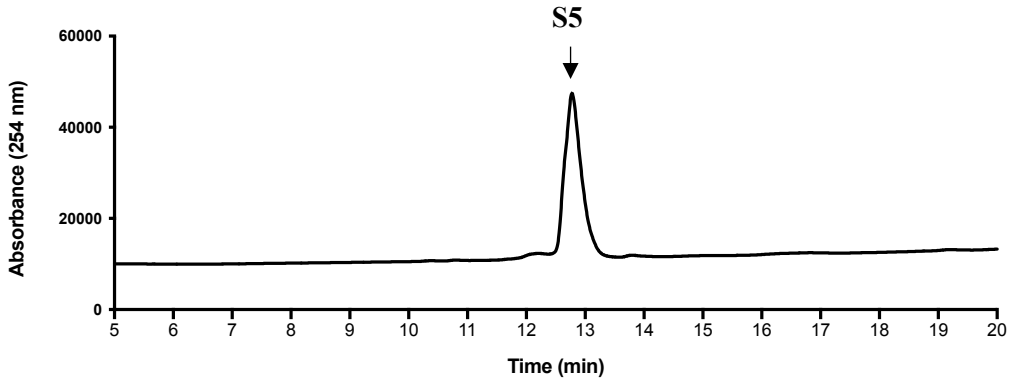



**5** **S5**

To a stirred solution of glycan-azide **2** (1.8 mg, 723 nmol, 1.0 eq.) in DMSO (140 µl) was added by amine **5** (1.0 mg, 2.7 µmol, 3.7 eq.) at room temperature. After stirring the mixture for 3 hours at 70 °C, the reaction mixture was purified by HPLC (column was an preparative 20.0 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; B: MeCN + 0.1% TFA; gradient: from 10% B/100% A to 100% B over 30 min; flow rate: 8 ml/min; the product **S5** was observed at 16.0 min (mixture of regioisomers)). The **S5** was collected and followed by lyophilization to give a white solid at a 53% yield.

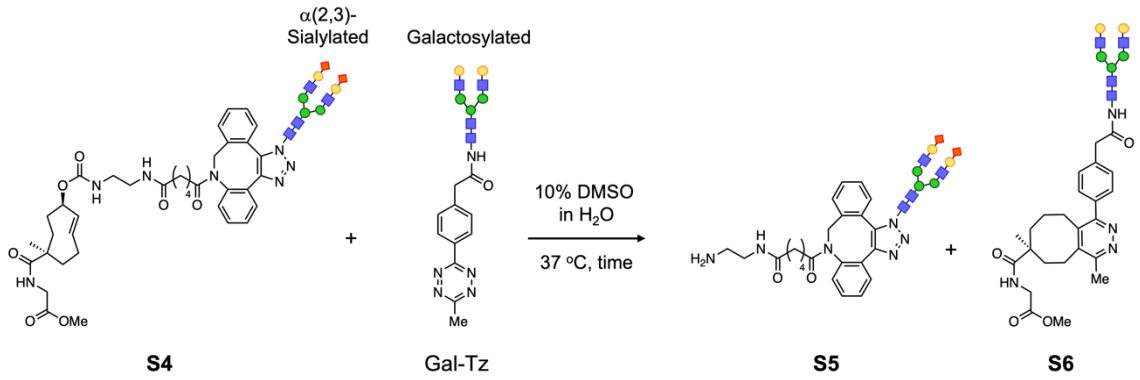
$^1$H-NMR (400 MHz, $D_2O$) : $\delta$ 7.68–7.53 (m, 4H), 7.47–7.34 (m, 4H), 7.29 (d, $J$ = 8.3 Hz, 1H), 7.22 (d, $J$ = 8.3 Hz, 1H), 5.94 (d, $J$ = 17.1 Hz, 1H), 5.64 (d, $J$ = 17.1 Hz, 1H), 5.10 (s, 1H), 5.05–4.97 (m, 1H), 4.95–4.84 (m, 4H), 4.64–4.49 (m, 8H), 4.45 (d, $J$ = 7.6 Hz, 1H), 4.40–4.26 (m, 3H), 4.23 (s, 1H), 4.17 (s, 1H), 4.12 (s, 1H), 4.09 (s, 2H), 4.03–3.31 (m, 60H), 3.12–3.08 (m, 3H), 2.80 (d, $J$ = 4.7 Hz, 2H), 2.74 (dd, $J$ = 12.6, 4.4 Hz, 2H), 2.31–2.24 (m, 2H), 2.18–1.93 (m, 20H), 1.90–1.72 (m, 4H), 1.61–1.52 (m, 2H), 1.48–1.09 (m, 11H).

LRMS (ESI) : m/z $[M-2H]^{2-}$ calculated for 1431.5481 $C_{118}H_{178}N_{14}O_{67}$; found 1431.9180.



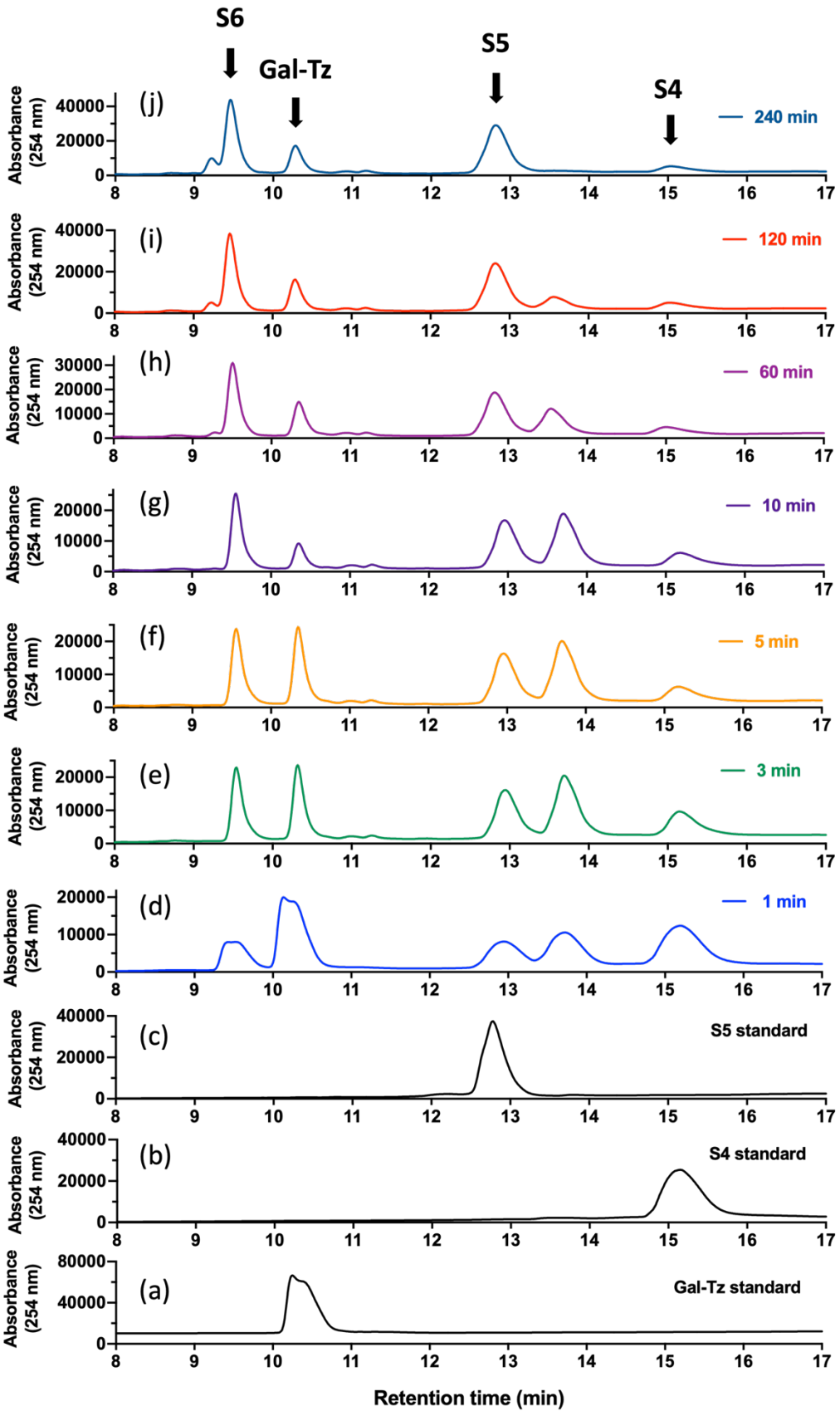

**Supplementary Figure 8.** HPLC analysis (column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 70%B/30%A over 20 min; flow rate: 1 ml/min) of the purified **S5**. The **S5** was observed at 12.8 min.

Kinetics analysis

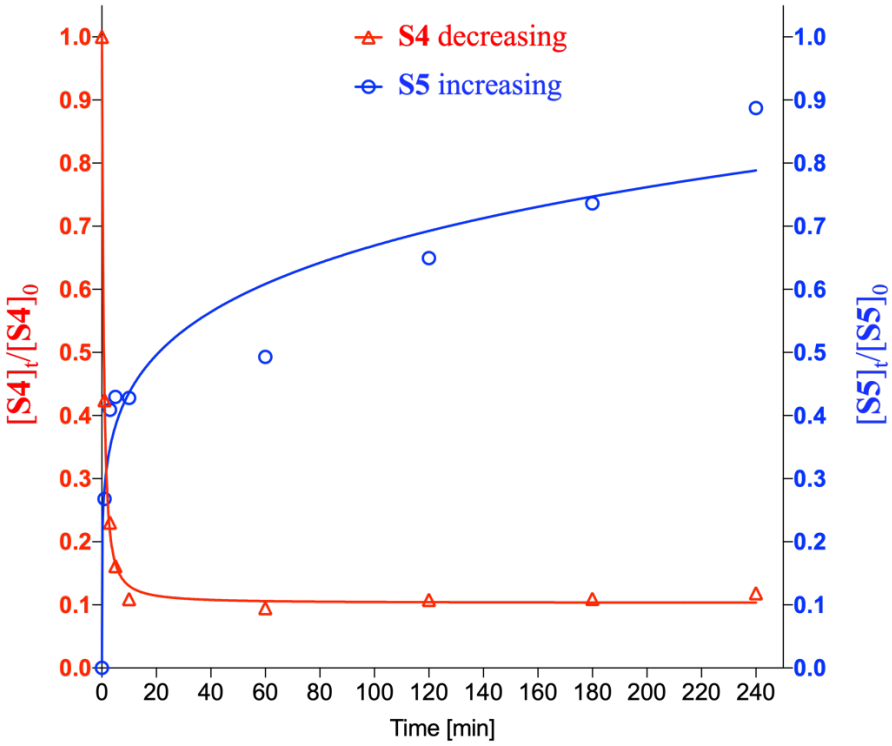



A reaction consisted of the **S4** (100 μM) and 1 equivalent of Gal-Tz (100 μM) in the 10% DMSO/$H_2O$ solution. Following initiation by Gal-Tz addition, the reactions mixture was mixed and incubated at 37 °C. As shown in Supplementary Figure 9, the result was monitored at the indicated times (1, 3, 5, 10, 60, 120, and 240 min) by HPLC analysis and UV-visible detection. HPLC conditions: column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 70%B/30%A over 20 min; flow rate: 1 ml/min. The Gal-Tz conjugation progress was observed by monitoring the peak area of **S4** decrease at 15.2 minutes of retention times, whereas the α(2,3)-sialylated glycan release progress was observed by monitoring the peak area of **S5** increase at 12.8 minutes of retention times. As depicted in Supplementary Figure 10, the peak area ratios ($[\mathbf{S4}]_t/[\mathbf{S4}]_0$ and $[\mathbf{S5}]_t/[\mathbf{S5}]_0$) were plotted versus reaction time using GraphPad Prism (version 8.0.2). $[\mathbf{S4}]_t$ = HPLC chromatogram peak area of **S4** at *t* minutes. $[\mathbf{S4}]_0$ = HPLC chromatogram peak area of **S4** at 0 minutes. $[\mathbf{S5}]_t$ = HPLC chromatogram peak area of **S5** at *t* minutes. $[\mathbf{S5}]_0$ = HPLC chromatogram peak area of **S5** at 0 minutes.

The results of Supplementary Figures 9 and 10 indicated that the click conjugation progress finished within 10 min and the glycan release progress takes over 4 h in line with the click-to-release progress on albumin, as shown in Figure 4.


S6
Gal-Tz
S5
S4
(j)
240 min
(i)
120 min
(h)
60 min
(g)
10 min
(f)
5 min
(e)
3 min
(d)
1 min
(c)
S5 standard
(b)
S4 standard
(a)
Gal-Tz standard
Absorbance (254 nm)
Retention time (min)

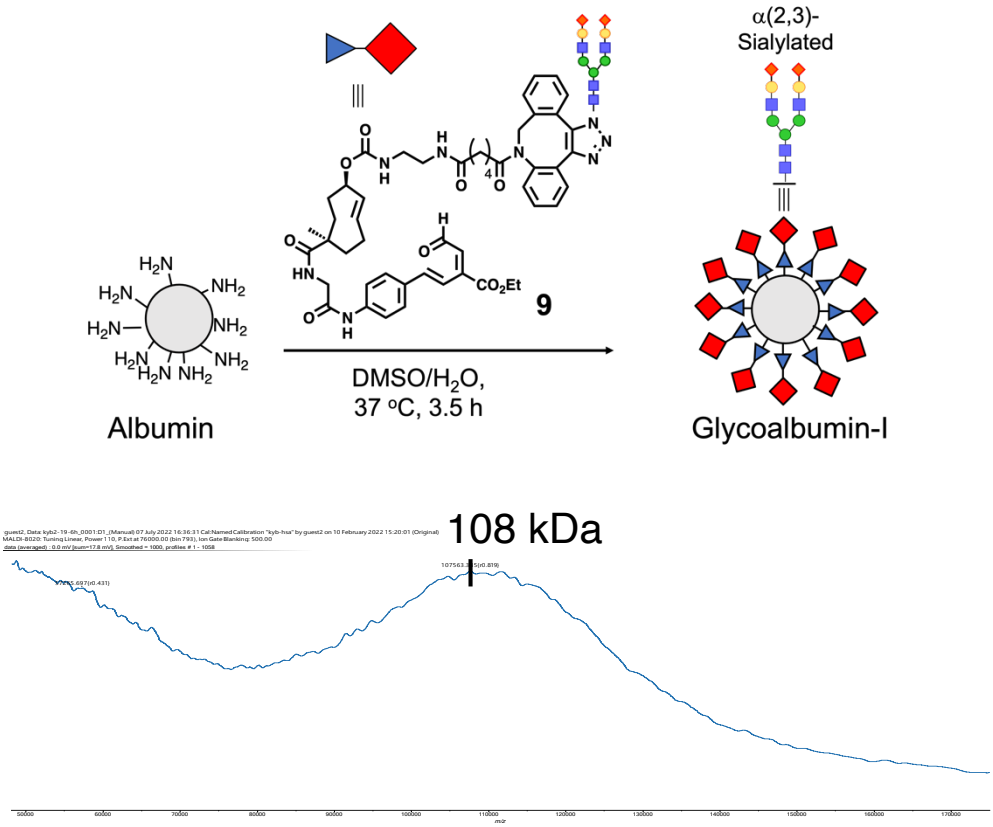
**Supplementary Figure 9.** HPLC time trace of the click-to-release reaction between **S4** (100 μM) and Gal-Tz (100 μM) in the 10% DMSO/$H_2O$ solution at 37 °C. Standard runs of known compounds include (a) Gal-Tz standard, (b) **S4** standard, and (c) **S5** standard. Example HPLC traces of the reactions at (d) 1 min, (e) 3 min, (f) 5 min, (g) 10 min, (h) 60 min (i) 120 min, and (j) 240 min. HPLC conditions: column was an analytical 4.6 × 250 mm Cosmosil 5C18-AR-300 from Nacalai Tesque; solvent A: $H_2O$ + 0.1% TFA; solvent B: MeCN + 0.1% TFA; gradient: from 10%B/90%A to 70%B/30%A over 20 min; flow rate: 1 ml/min. The release **S6** was observed at 9.5 min and confirmed by ESI-MS (LRMS (ESI) : m/z $[M-H]^{-1}$ calculated for $C_{90}H_{136}N_9O_{52}$ 2175.8313; found 2175.7168.




**Supplementary Figure 10.** The click-to-release reaction between **S4** (100 μM) and Gal-Tz (100 μM) in the 10% DMSO/$H_2O$ solution at 37 °C was monitored at the indicated times (1, 3, 5, 10, 60, 120, and 240 min) by HPLC and UV-visible detection. The HPLC chromatogram peak area-changing ratios of **S4** (red line) and **S5** (blue line) were plotted against reaction time. $[\mathbf{S4}]_t$ = HPLC chromatogram peak area of **S4** at *t* minutes. $[\mathbf{S4}]_0$ = HPLC chromatogram peak area of **S4** at 0 minutes. $[\mathbf{S5}]_t$ = HPLC chromatogram peak area of **S5** at *t* minutes. $[\mathbf{S5}]_0$ = HPLC chromatogram peak area of **S5** at 0 minutes.

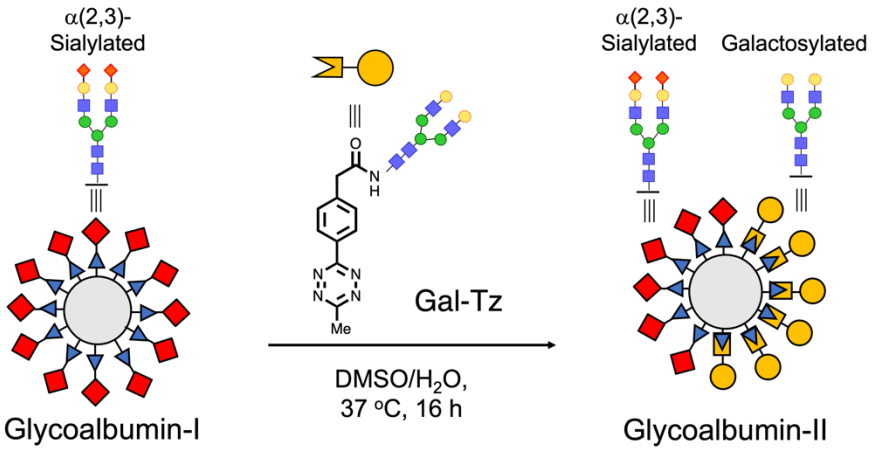
## 1.3 Preparation of glycoalbumins

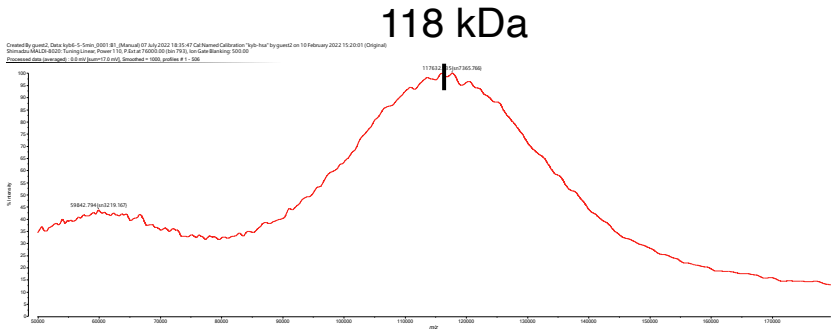
Preparation of glycoalbumin-I



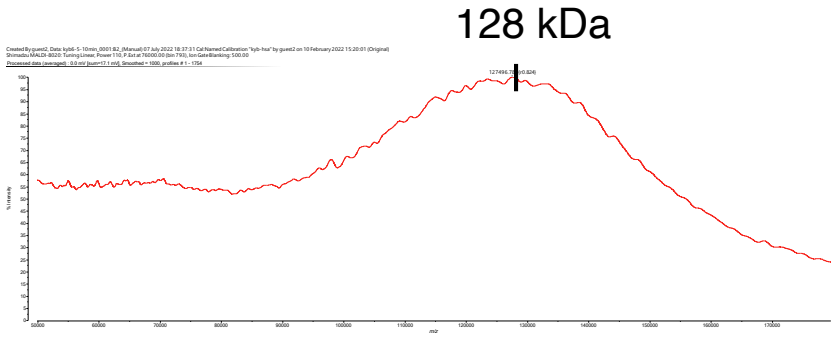

**Supplementary Figure 11.** MALDI-TOF-MS of glycoalbumin-I

Glycan remodeling reaction of glycoalbumin-I to give glycoalbumin-II

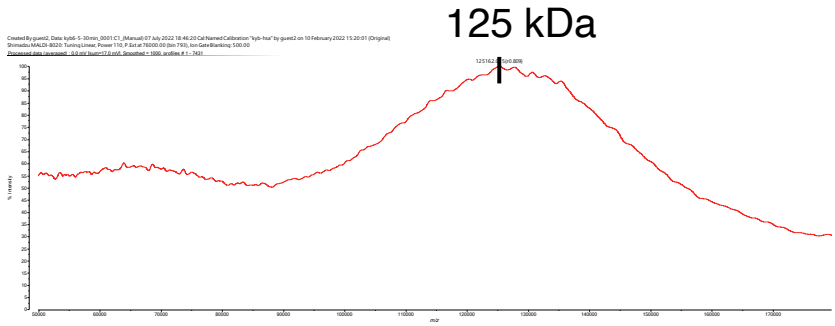




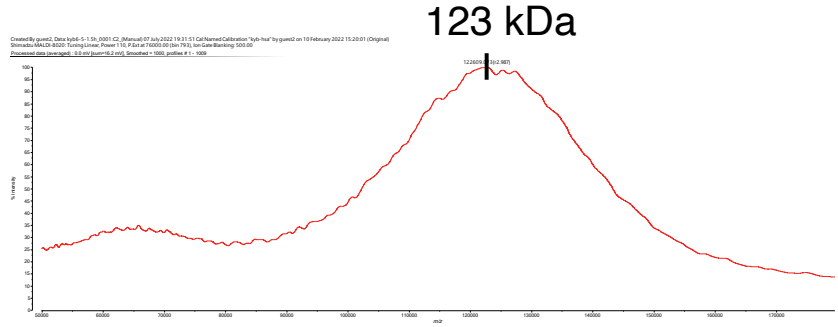

**Supplementary Figure 12.** MALDI-TOF-MS data of glycoalbumin-II for 5 min after starting the reaction.



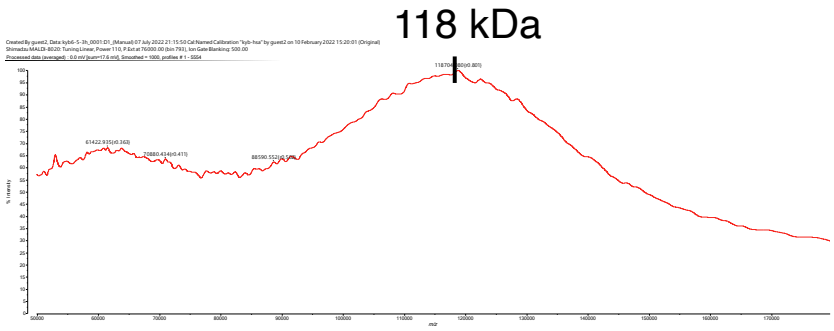

**Supplementary Figure 13.** MALDI-TOF-MS data of glycoalbumin-II for 10 min after starting the reaction.



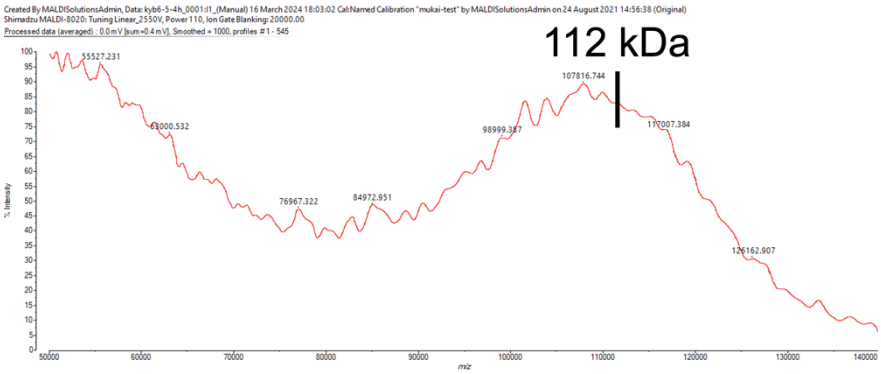

**Supplementary Figure 14.** MALDI-TOF-MS data of glycoalbumin-II for 30 min after starting the reaction.



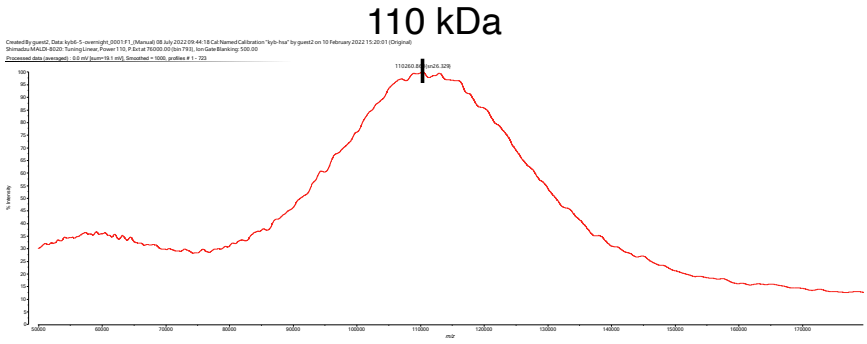
**Supplementary Figure 15.** MALDI-TOF-MS data of glycoalbumin-II for 1.5 h after starting the reaction.




**Supplementary Figure 16.** MALDI-TOF-MS data of glycoalbumin-II for 3 h after starting the reaction.

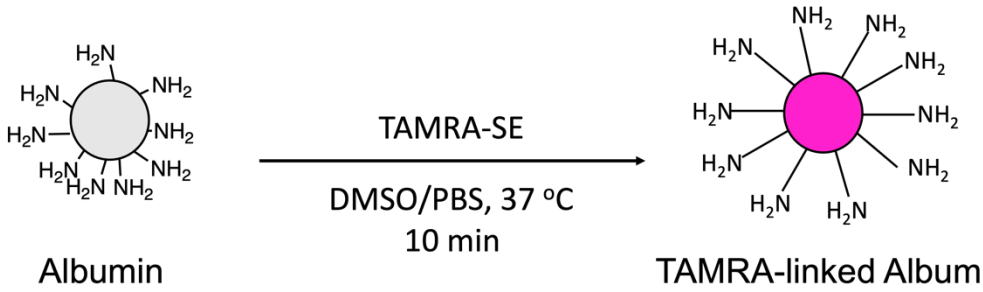



**Supplementary Figure 17.** MALDI-TOF-MS data of glycoalbumin-II for 4 h after starting the reaction.

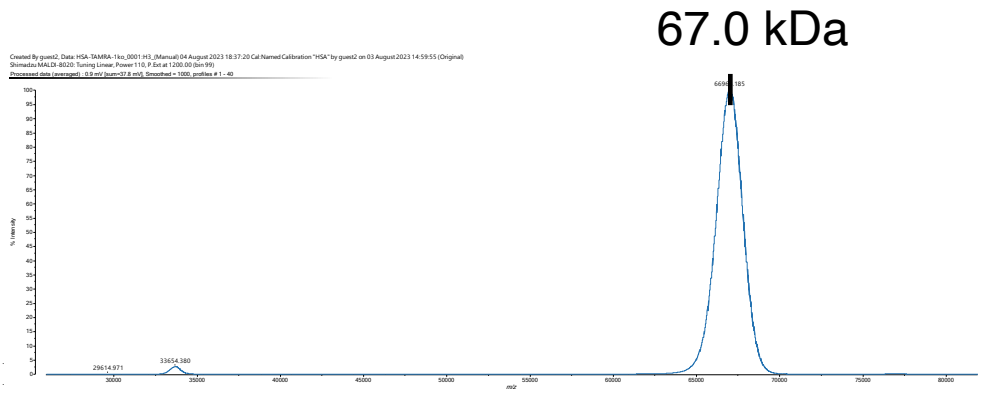



**Supplementary Figure 18.** MALDI-TOF-MS data of glycoalbumin-II for 16 h after starting the reaction.

Preparation of TAMRA-linked albumin

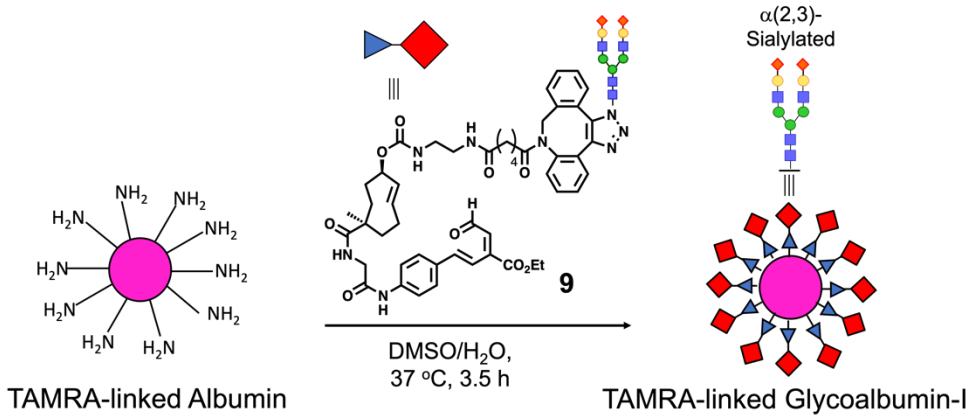


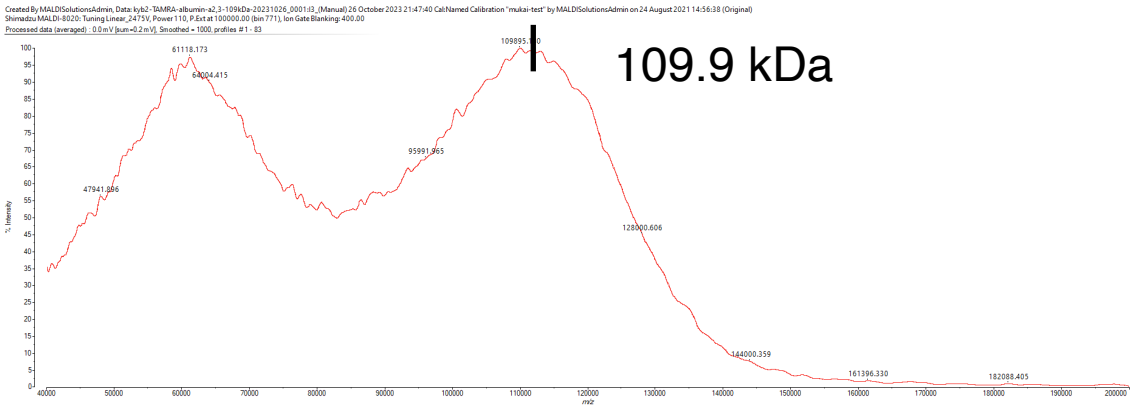

To a solution of human serum albumin (6.7 mg, 100 nmol) in PBS buffer (pH 7.4, 1.0 ml) was added 5(6)-carboxytetramethylrhodamine succinimidyl ester (0.13 mg, 250 nmol) in DMSO (13 µl). The resulted mixture solution was reacted at 37 °C. After 10 min, the solution was concentrated and washed with milli-Q water using Amicon 10K® ultra centrifugal filters (10 kDa cut-off) to remove the unreacted TAMRA. To confirm formation of TAMRA-linked albumin, MALDI-TOF-MS was done. Analysis under positive mode, an average molecular weight of 67.0 kDa was detected, which indicated 1.3 molecules of TAMRA fluorophore were conjugated per albumin.




**Supplementary Figure 19.** MALDI-TOF-MS data of TAMRA-linked albumin.

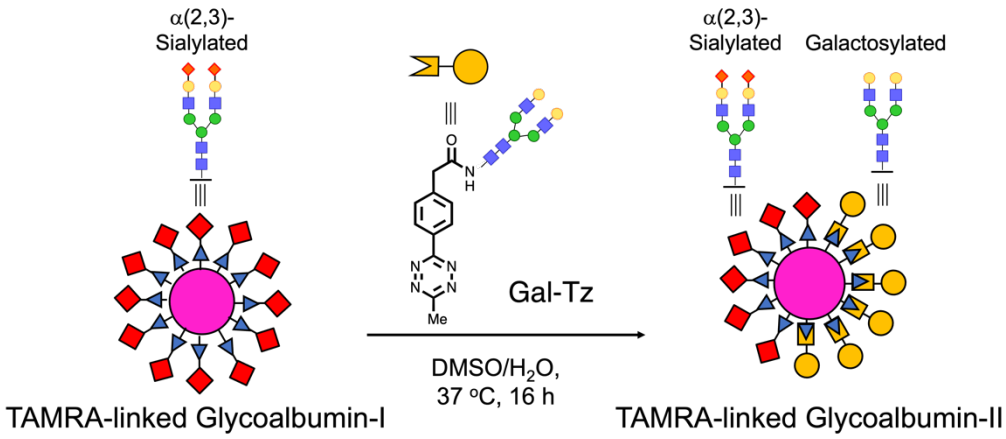
Preparation of TAMRA-linked glycoalbumin-I



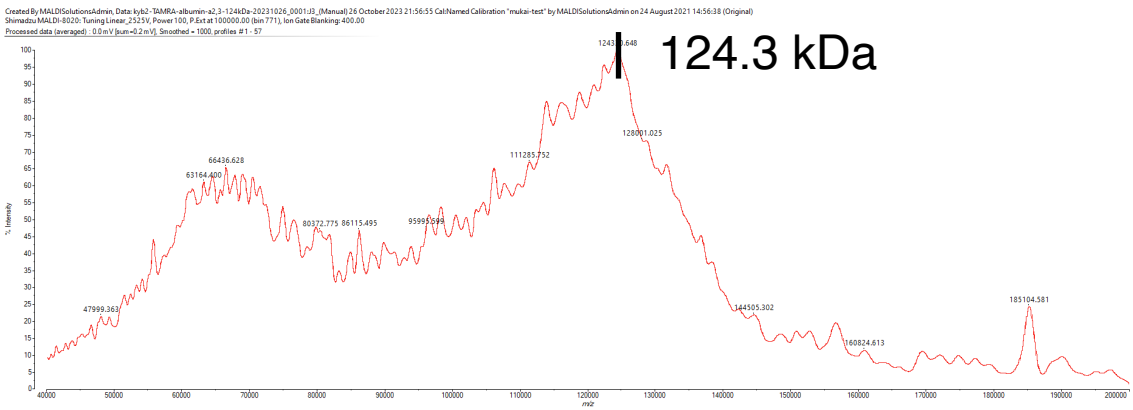




**Supplementary Figure 20.** MALDI-TOF-MS of TAMRA-linked glycoalbumin-I

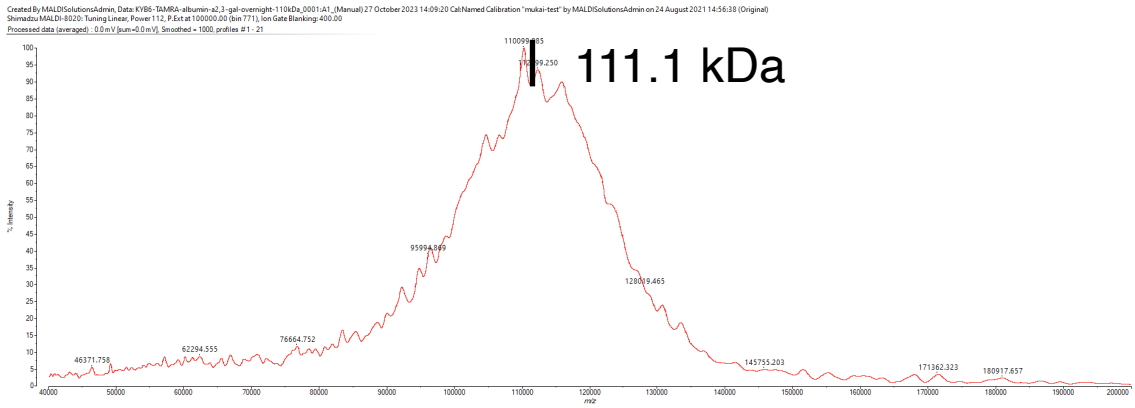
Glycan remodeling reaction of TAMRA-linked glycoalbumin-I to give TAMRA-linked glycoalbumin-II




The protocol for the glycan remodeling reaction of TAMRA-linked glycoalbumin-I to give TAMRA-linked glycoalbumin-II was described in the main text. After starting the reaction at 30 min, the average molecular weight of TAMRA-linked glycoalbumin-I was measured by MALDI-TOF-MS (positive mode) to reveal an increase to 124.3 kDa, indicating that around 7.6 galactosylated glycans were conjugated. After 16 h, the average molecular weight of TAMRA-linked glycoalbumin-I was decreasing to 111.1 kDa, indicating that around 4.6 α(2,3)-sialylated glycans were released. As a result, TAMRA-linked glycoalbumin-II consisted of 8.1 α(2,3)-sialylated glycans and 7.6 galactosylated glycans.

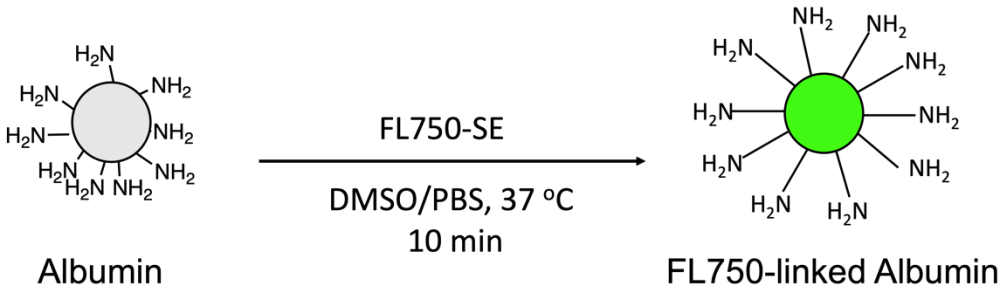



**Supplementary Figure 21.** MALDI-TOF-MS of TAMRA-linked glycoalbumin-I for 30 min after starting the reaction.

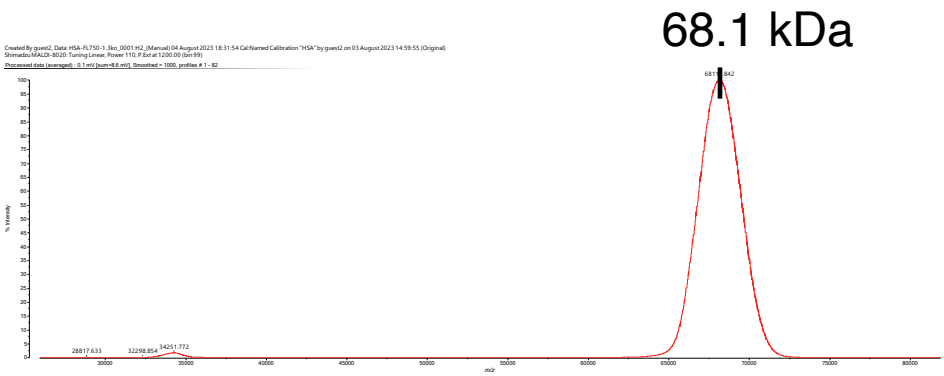



**Supplementary Figure 22.** MALDI-TOF-MS of TAMRA-linked glycoalbumin-I for 16 h after starting the reaction.

Preparation of FL750-linked albumin.

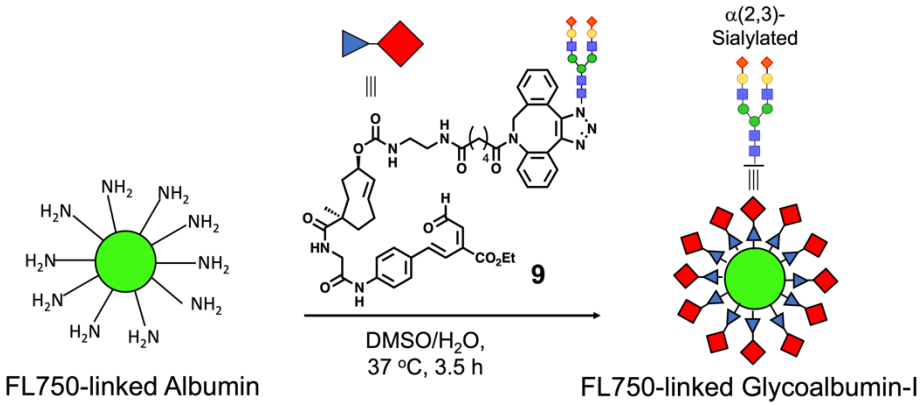


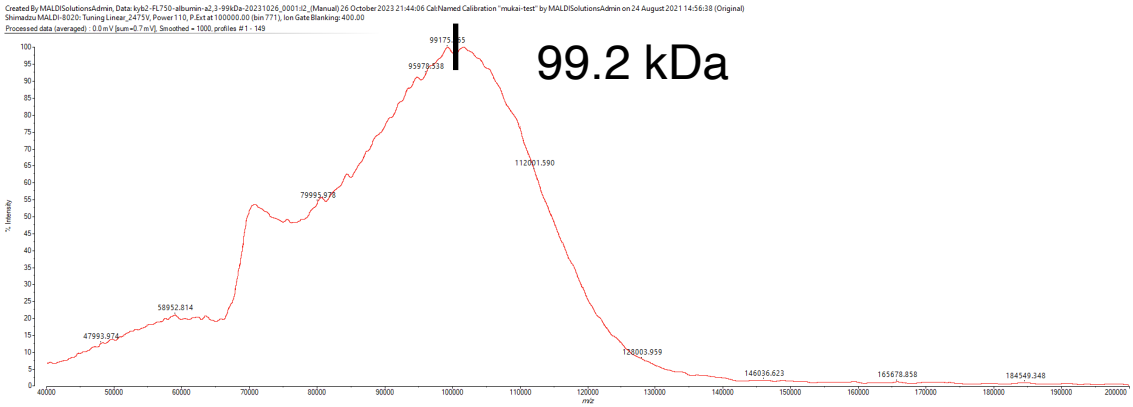

To a solution of human serum albumin in phosphate saline buffer (pH 7.4, 300 µl) was added by HiLyte Fluor 750 acid SE® (0.25 mg, 0.19 µmol) in DMSO (10 µl). The resulted mixture solution was reacted at 37 °C. After 10 min, the solution was concentrated and washed with milli-Q water using amicon 10K® ultra centrifugal filters (10 kDa cut-off) to remove the unreacted Fluor 750 dye. To confirm formation of FL750-linked albumin, MALDI-TOF-MS was done. Analysis under positive mode, an average molecular weight of 68.1 kDa was detected , which indicated 1.3 molecules of FL750 fluorophore were conjugated per albumin.




**Supplementary Figure 23.** MALDI-TOF-MS of FL750-linked albumin.

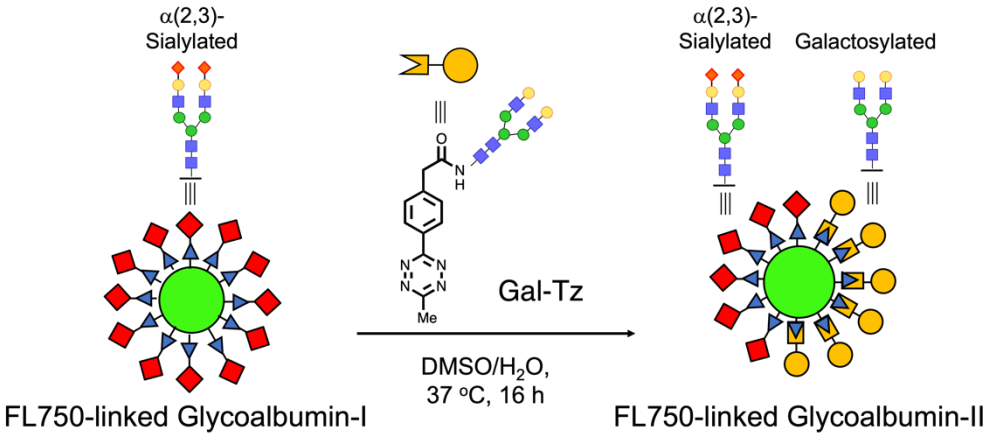
Preparation of FL750-linked glycoalbumin-I



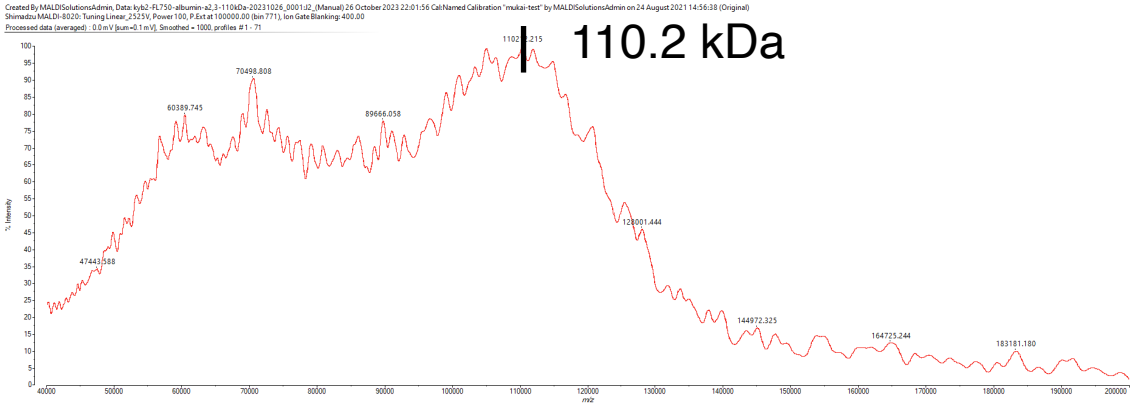




**Supplementary Figure 24.** MALDI-TOF-MS of FL750-linked glycoalbumin-I

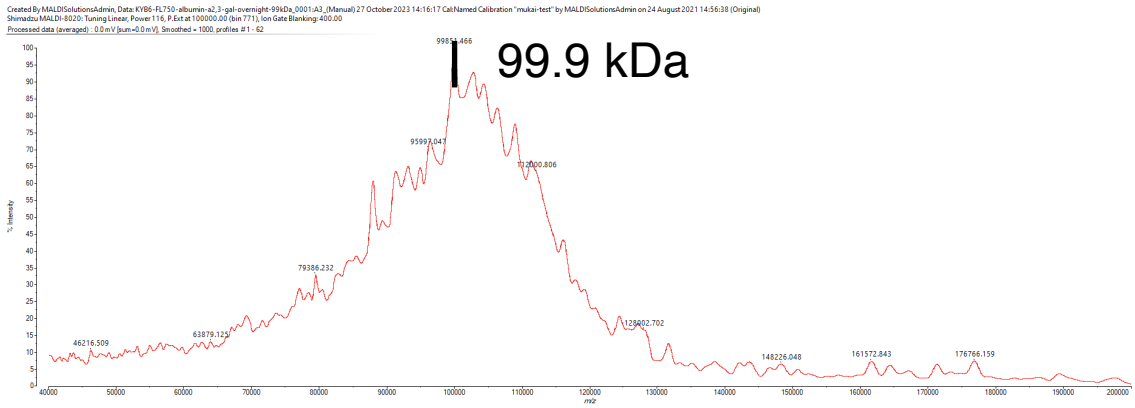
Glycan remodeling reaction of FL750-linked glycoalbumin-I to give FL750-linked glycoalbumin-II




The protocol for the glycan remodeling reaction of FL750-linked glycoalbumin-I to give FL750-linked glycoalbumin-II was described in the main text. After starting the reaction at 30 min, the average molecular weight of FL750-linked glycoalbumin-I was measured by MALDI-TOF-MS (positive mode) to reveal an increase to 110.2 kDa, indicating that around 5.8 galactosylated glycans were conjugated. After 16 h, the average molecular weight of FL750-linked glycoalbumin-I was decreasing to 99.9 kDa, indicating that around 3.6 α(2,3)-sialylated glycans were released. As a result, FL750-linked glycoalbumin-II consisted of 5.7 α(2,3)-sialylated glycans and 5.8 galactosylated glycans.

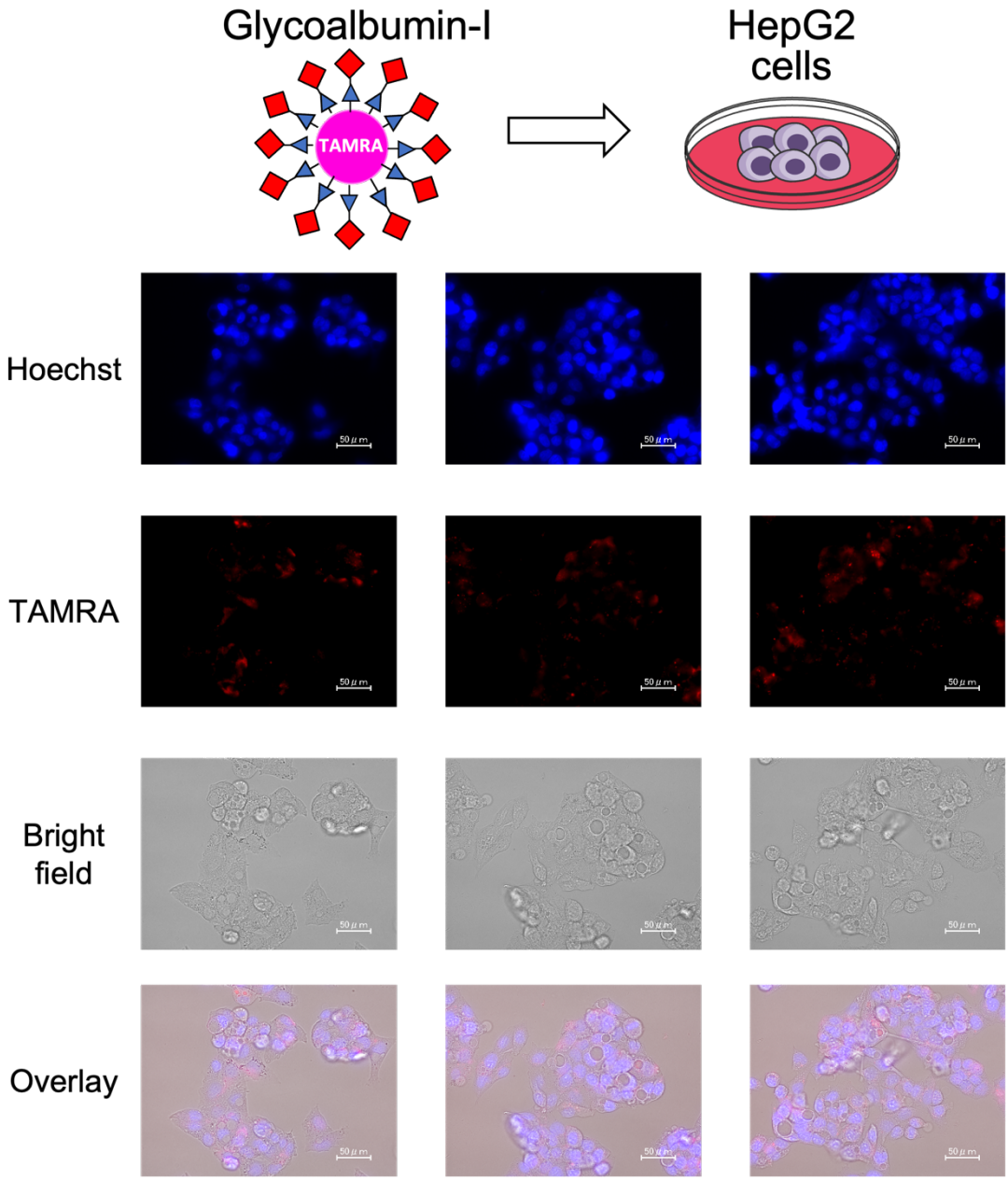



**Supplementary Figure 25.** MALDI-TOF-MS of FL750-linked glycoalbumin-I for 30 min after starting the reaction.

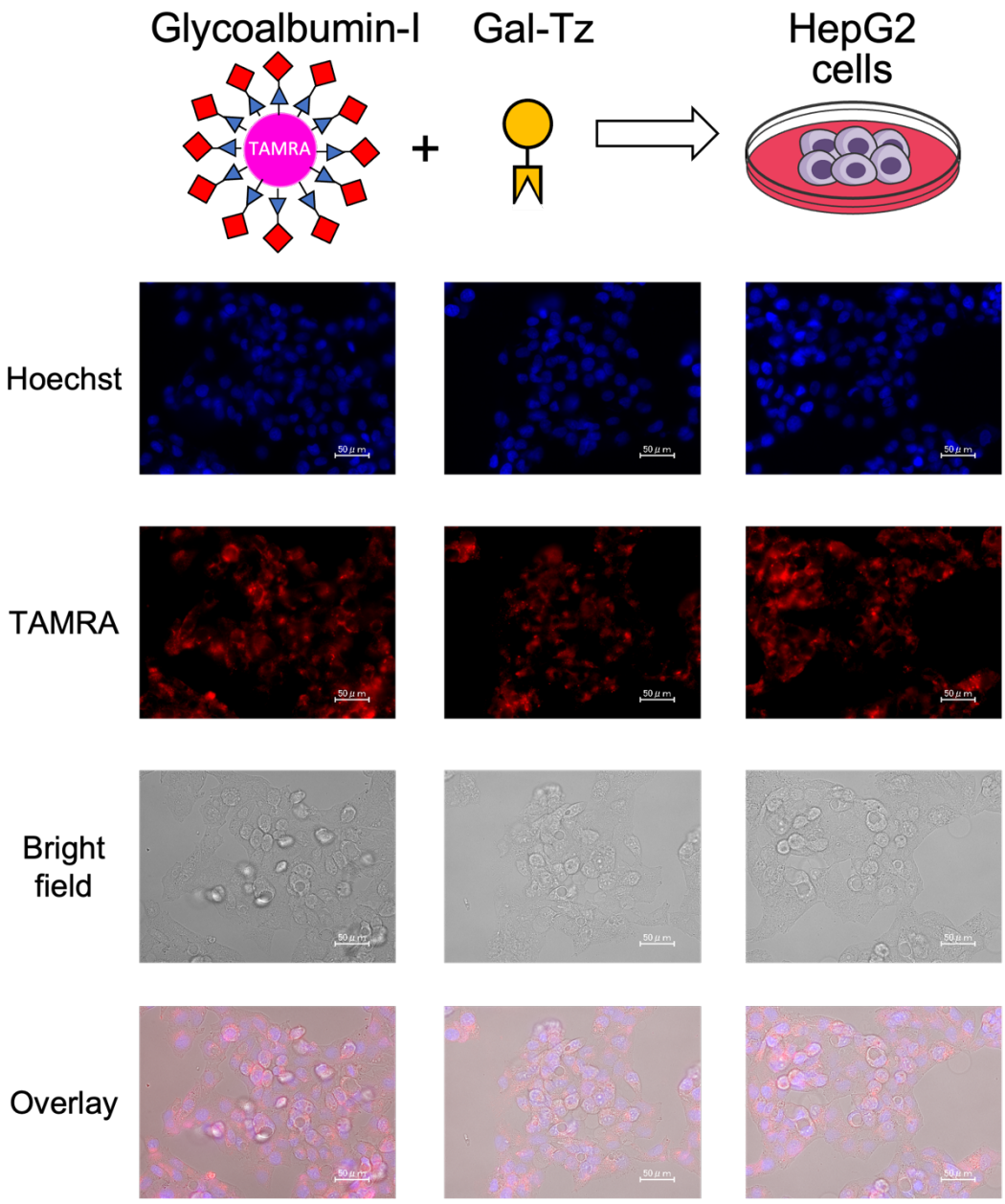



**Supplementary Figure 26.** MALDI-TOF-MS of FL750-linked glycoalbumin-I for 16 h after starting the reaction.

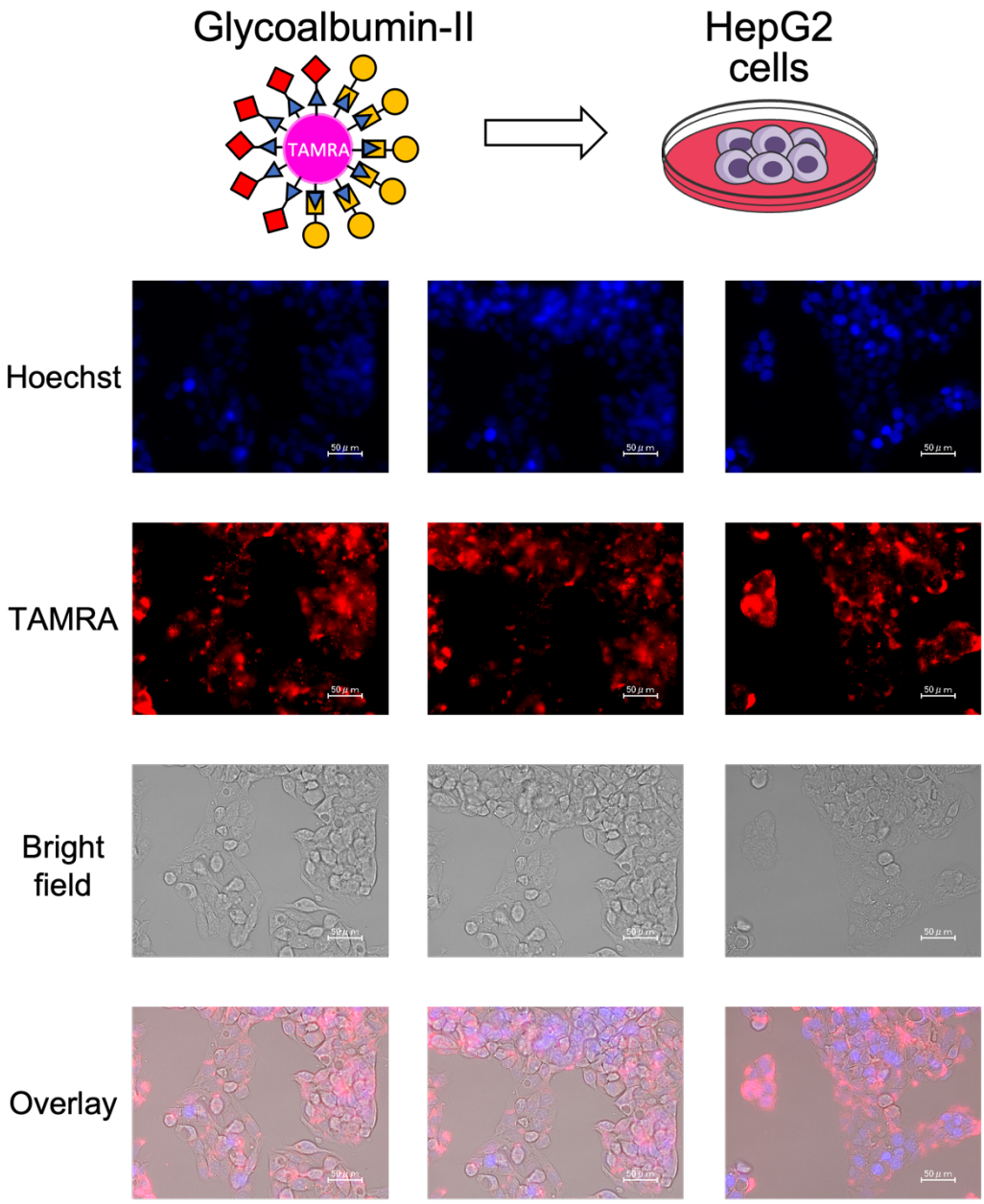
## 1.4 Cell imaging studies



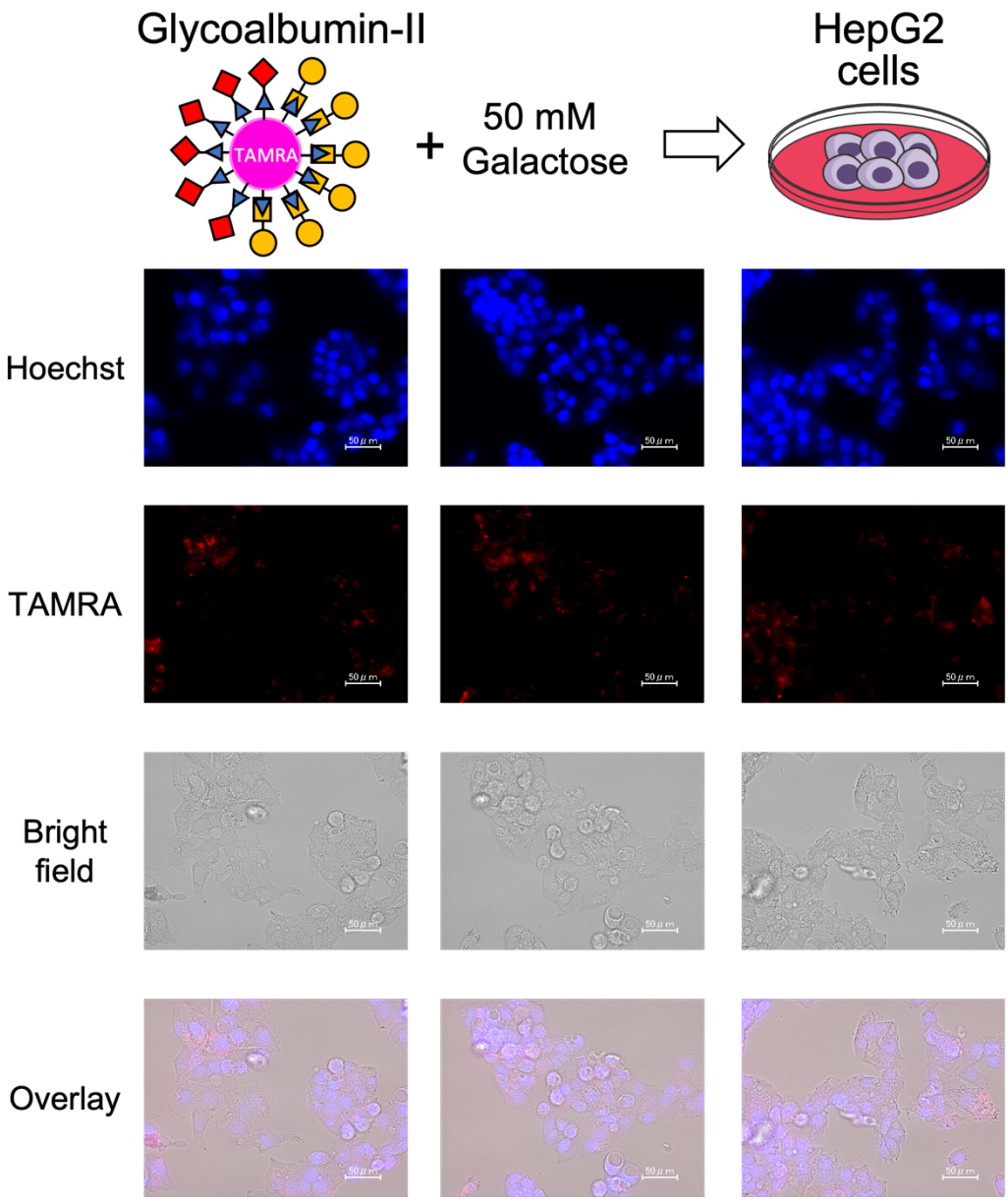

**Supplementary Figure 27**. HepG2 cell imaging results following incubation (3 hours, 37 °C) with TAMRA-labeled glycoalbumin-I (1 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (3s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.



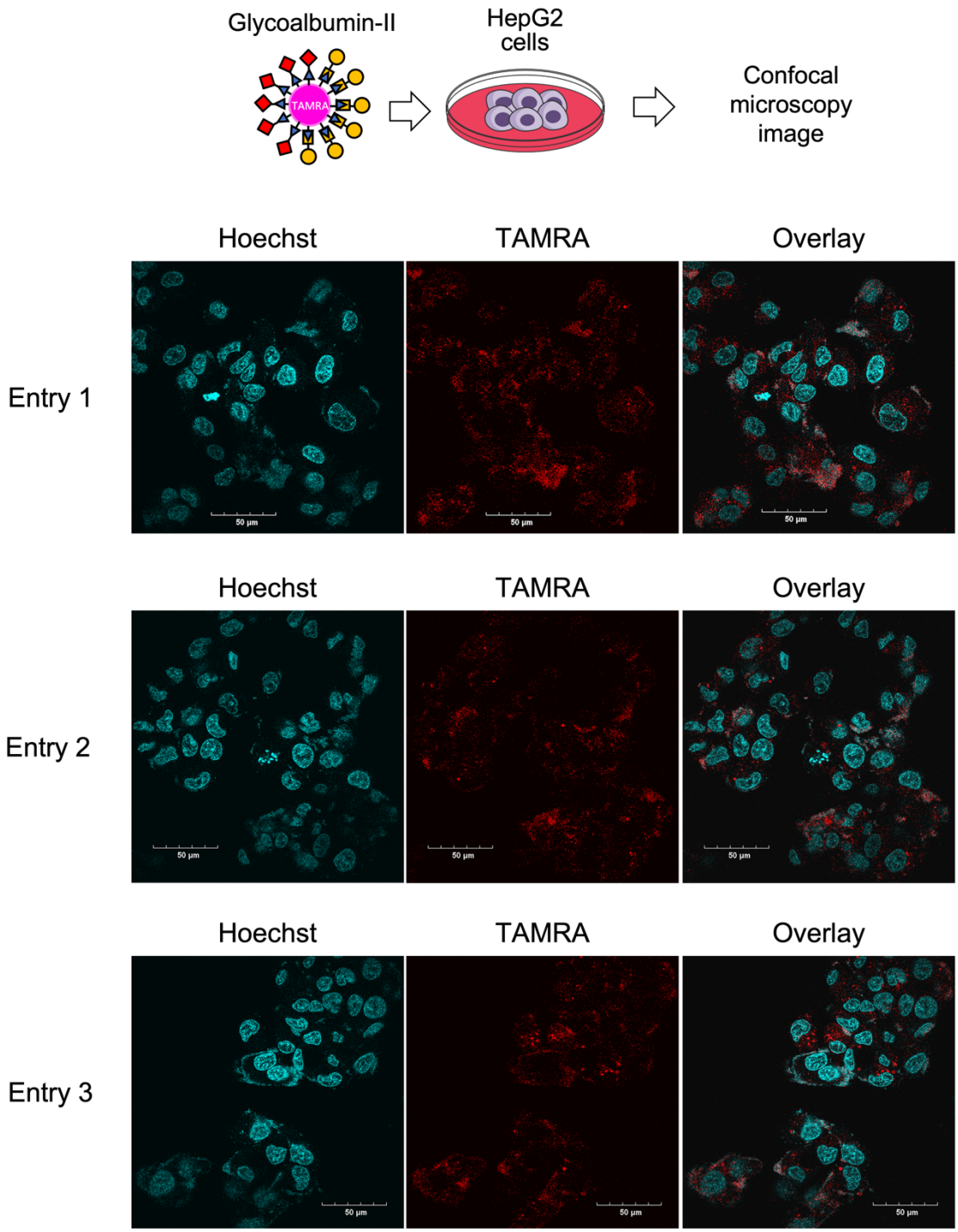

**Supplementary Figure 28**. HepG2 cell imaging results following incubation (3 hours, 37 °C) with TAMRA-labeled glycoalbumin-I (1 µM) and Gal-Tz (100 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (3s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.



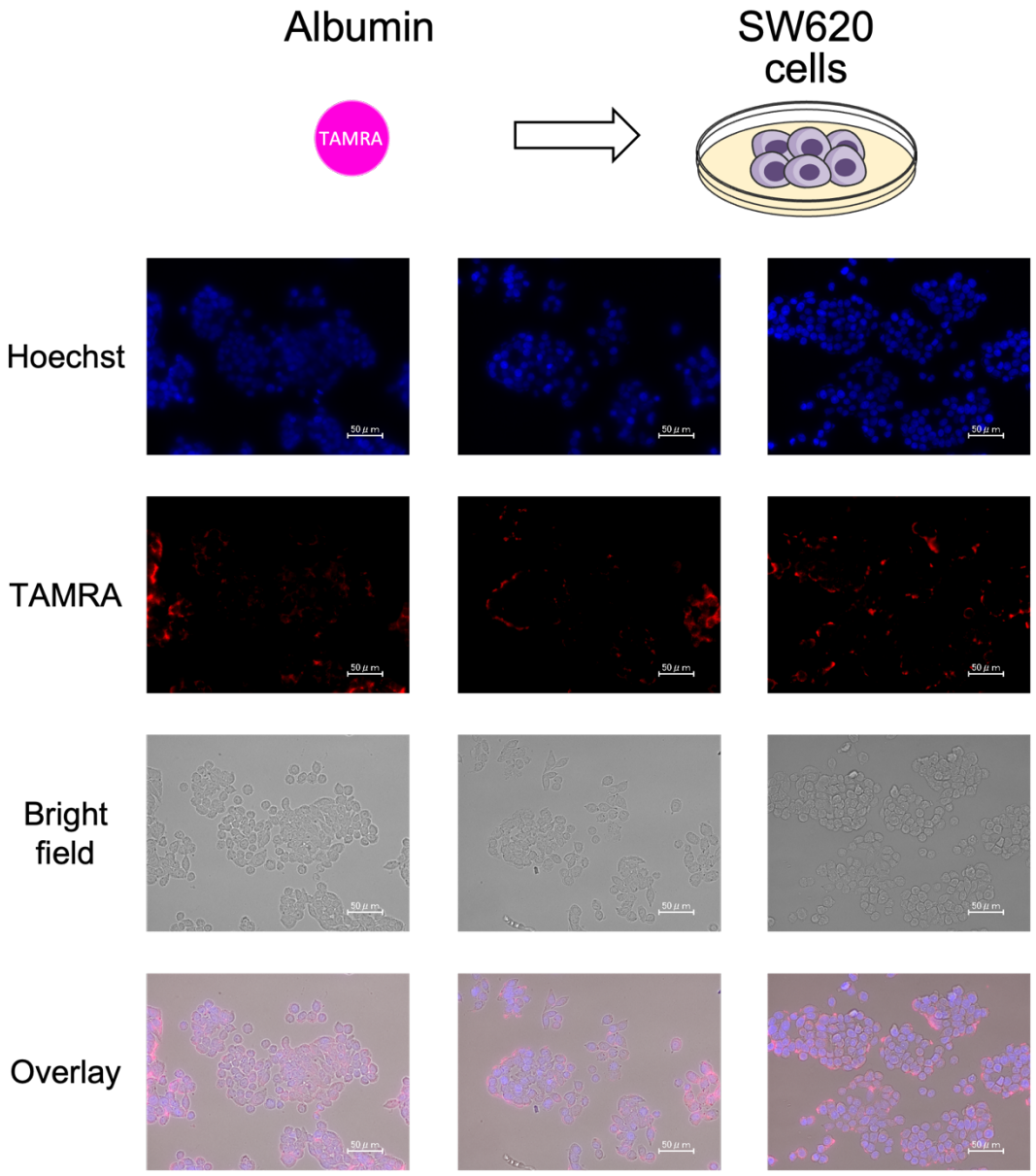

**Supplementary Figure 29**. HepG2 cell imaging results following incubation (3 hours, 37 °C) with TAMRA-labeled glycoalbumin-II (1 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (3s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.



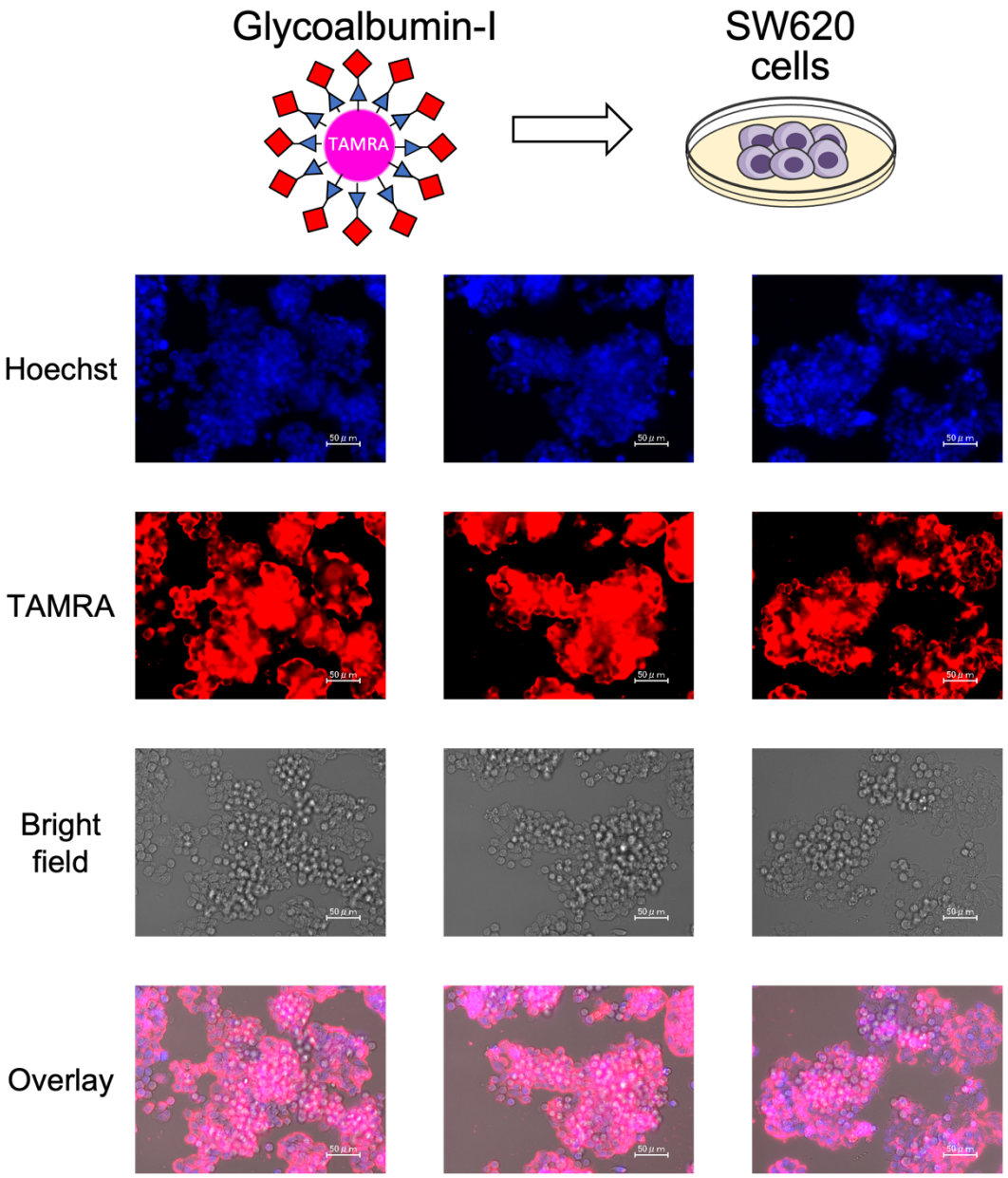

**Supplementary Figure 30**. HepG2 cell imaging results following incubation (3 hours, 37 ºC) with TAMRA-labeled glycoalbumin-II (1 µM) and 50 mM of galactose in cell medium (n = 3). Shown are images obtained via TAMRA (3s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.



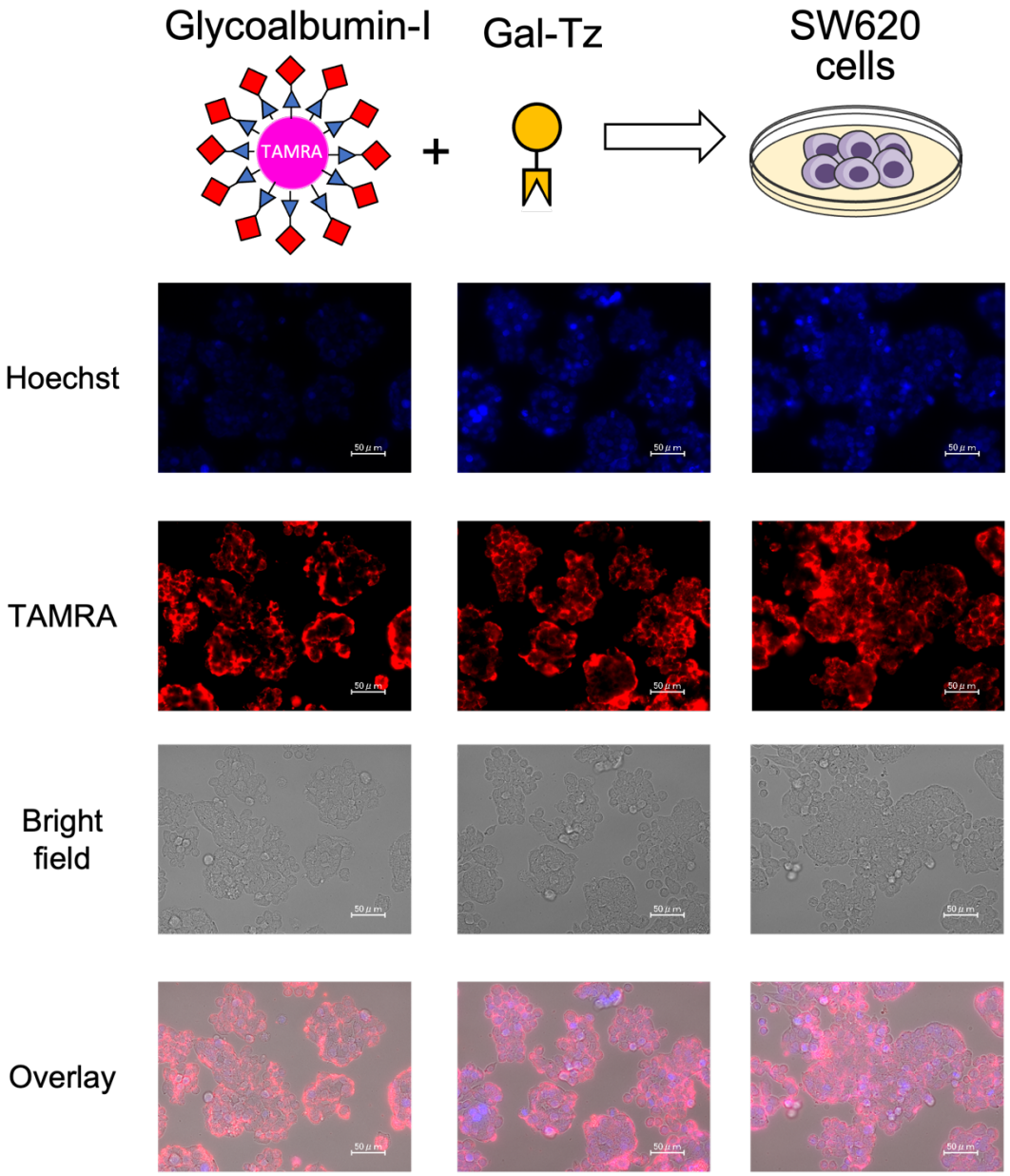

**Supplementary Figure 31**. Confocal microscopy fluorescence images of HepG2 cells incubated with galactosylated glycoalbumin-II (1 µM) at 37°C for 3 h. The shown images obtained at 60x magnification via Hoechst fluorescence (cell nuclei staining), TAMRA fluorescence (glycoalbumins), and the combined overlay. Three independent replicates were presented. The scale bar corresponds to 50 µm.



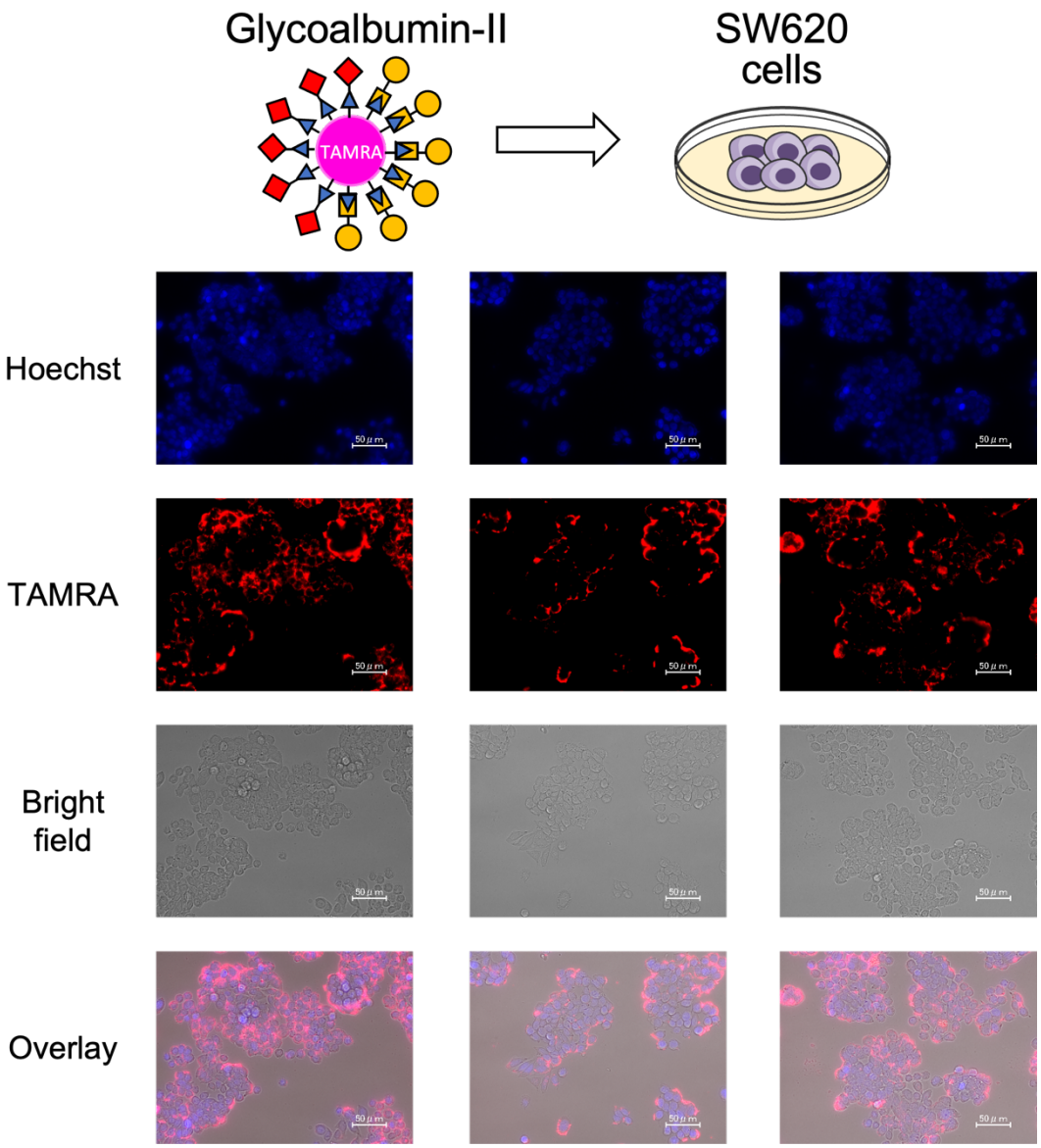

**Supplementary Figure 32**. SW620 cell imaging results following incubation (30 min, 37 ºC) with TAMRA-labeled albumin (5 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (2s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.



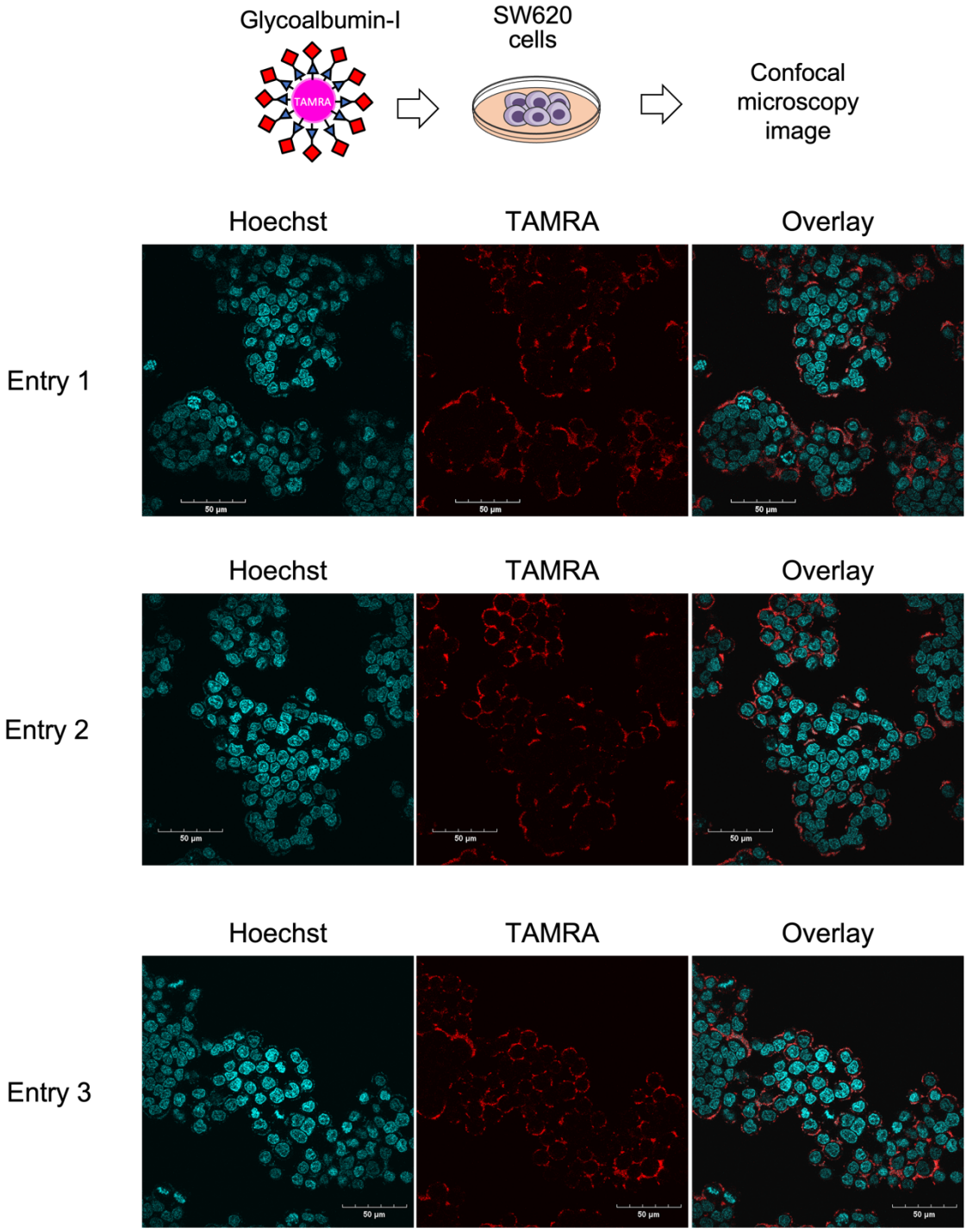

**Supplementary Figure 33**. SW620 cell imaging results following incubation (30 min, 37 ºC) with TAMRA-labeled glycoalbumin-I (5 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (2s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.



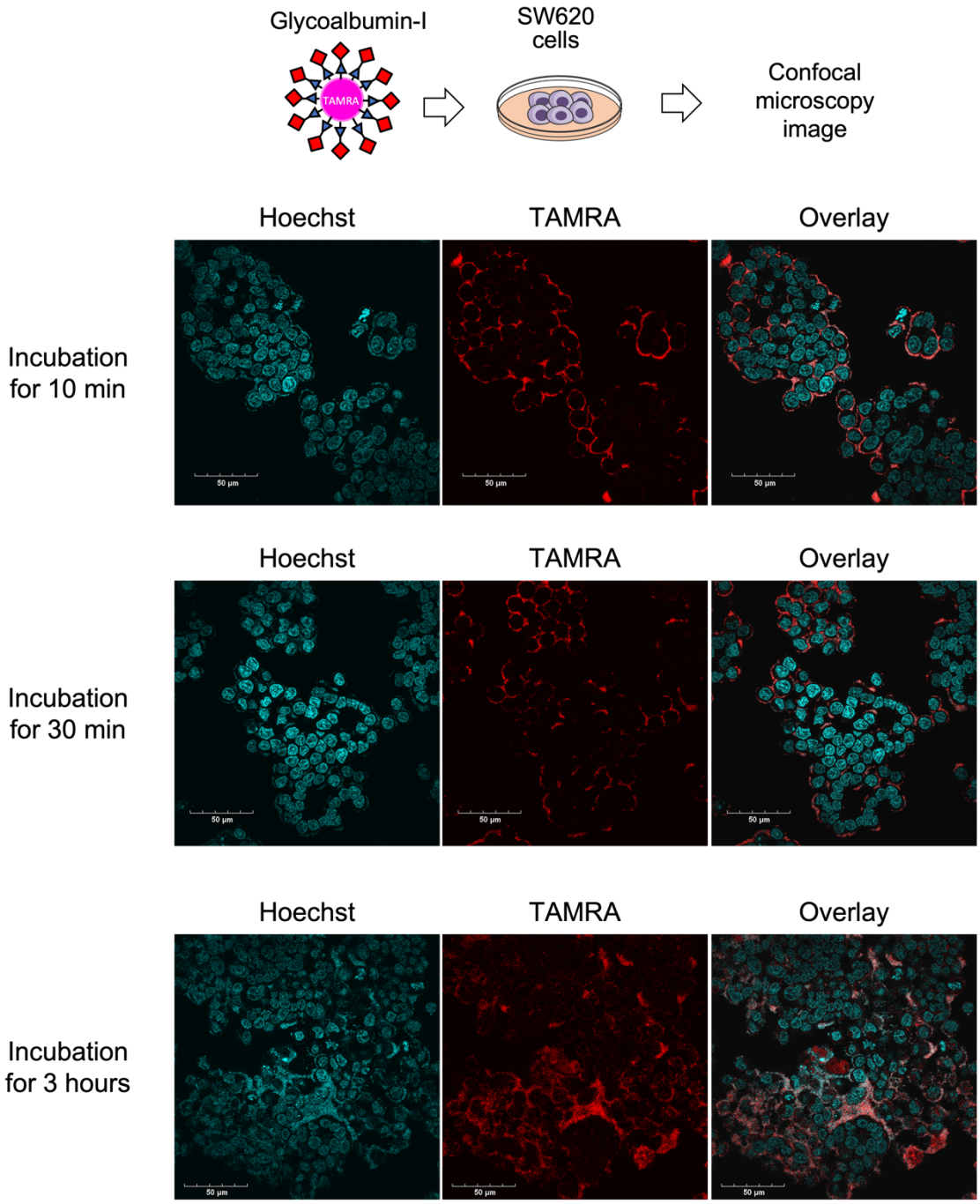

**Supplementary Figure 34**. SW620 cell imaging results following incubation (30 min, 37 °C) with TAMRA-labeled glycoalbumin-I (5 µM) and Gal-Tz (300 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (2s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.




**Supplementary Figure 35**. SW620 cell imaging results following incubation (30 min, 37 °C) with TAMRA-labeled glycoalbumin-II (5 µM) in cell medium (n = 3). Shown are images obtained via TAMRA (2s exposure time), Hoechst (1/10s exposure time), Brightfield (1/500s exposure time), and the combined Overlay. The scale bar corresponds to 50 µm.

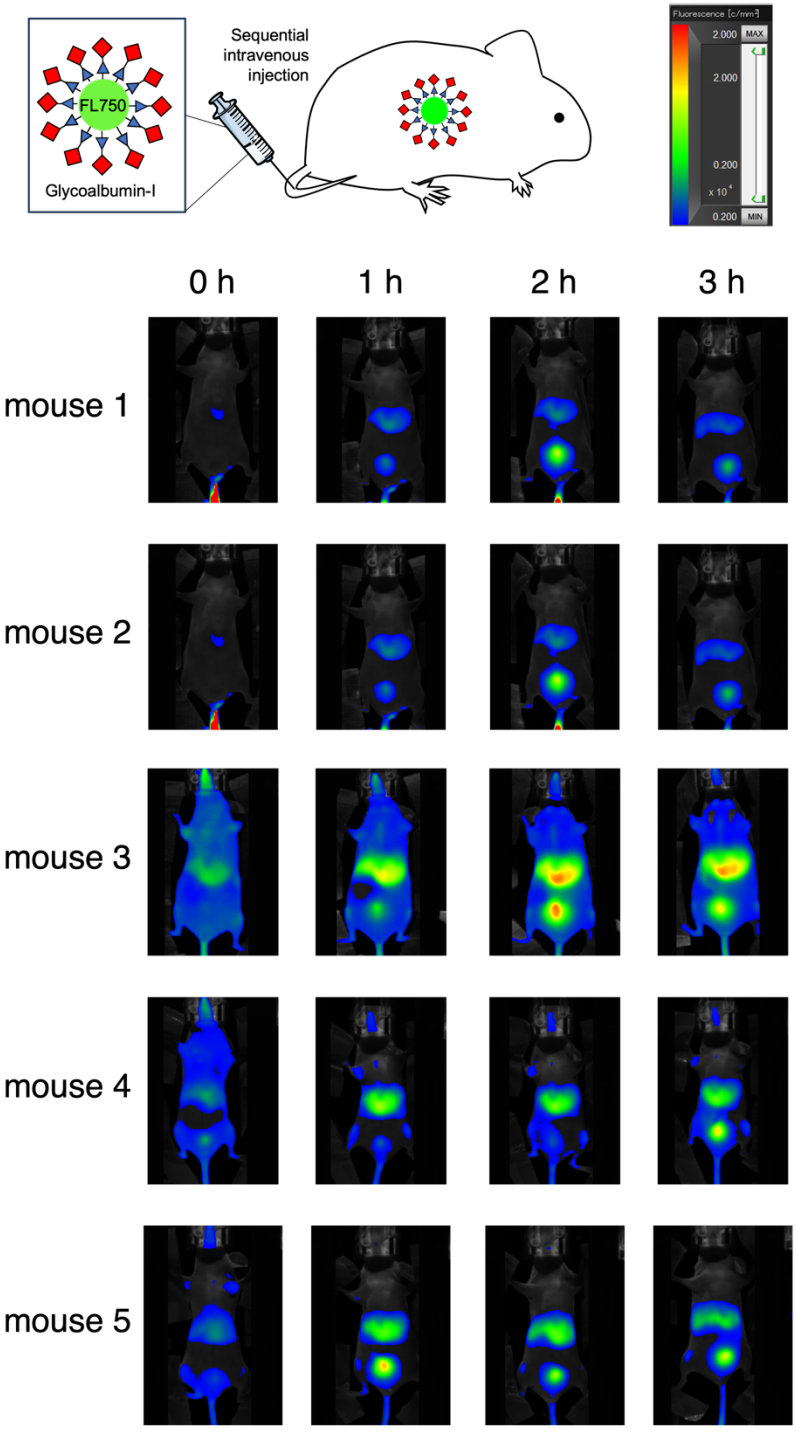



**Supplementary Figure 36**. Confocal microscopy images of SW620 cells incubated with α(2,3)-sialylated glycoalbumin-I (5 µM) at 37°C for 30 min. The shown images obtained at 60x magnification via Hoechst fluorescence (cell nuclei staining), TAMRA fluorescence (glycoalbumins), and the combined Overlay. Three independent replicates were presented. The scale bar corresponds to 50 µm.

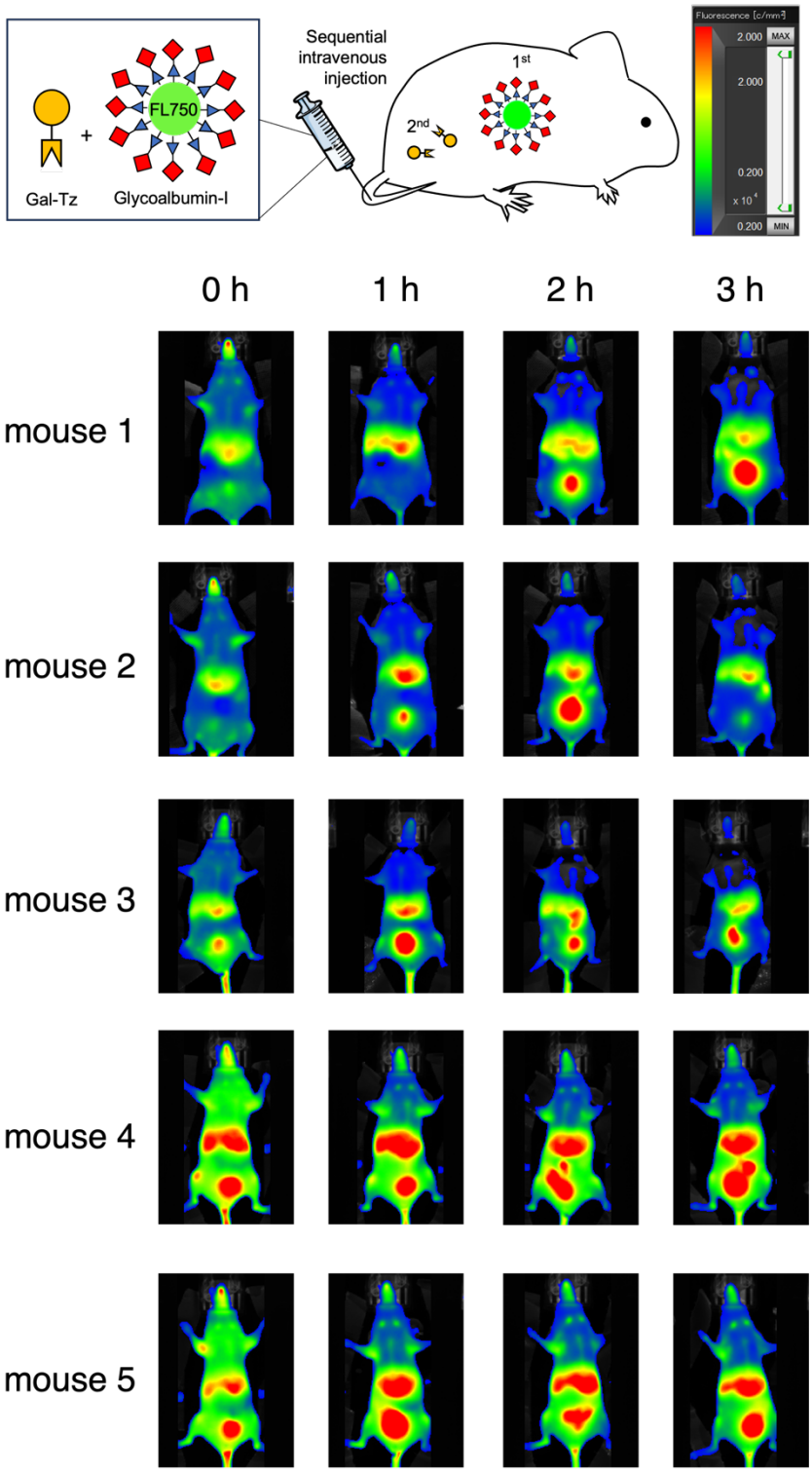



**Supplementary Figure 37**. Confocal microscopy images of SW620 cells incubated with α(2,3)-sialylated glycoalbumin-I (5 μM) at 37°C for 10, 30 min and 3 hours (n = 1). The shown images obtained at 60x magnification *via* Hoechst fluorescence (cell nuclei staining), TAMRA fluorescence (glycoalbumins), and the combined Overlay. The scale bar corresponds to 50 μm.

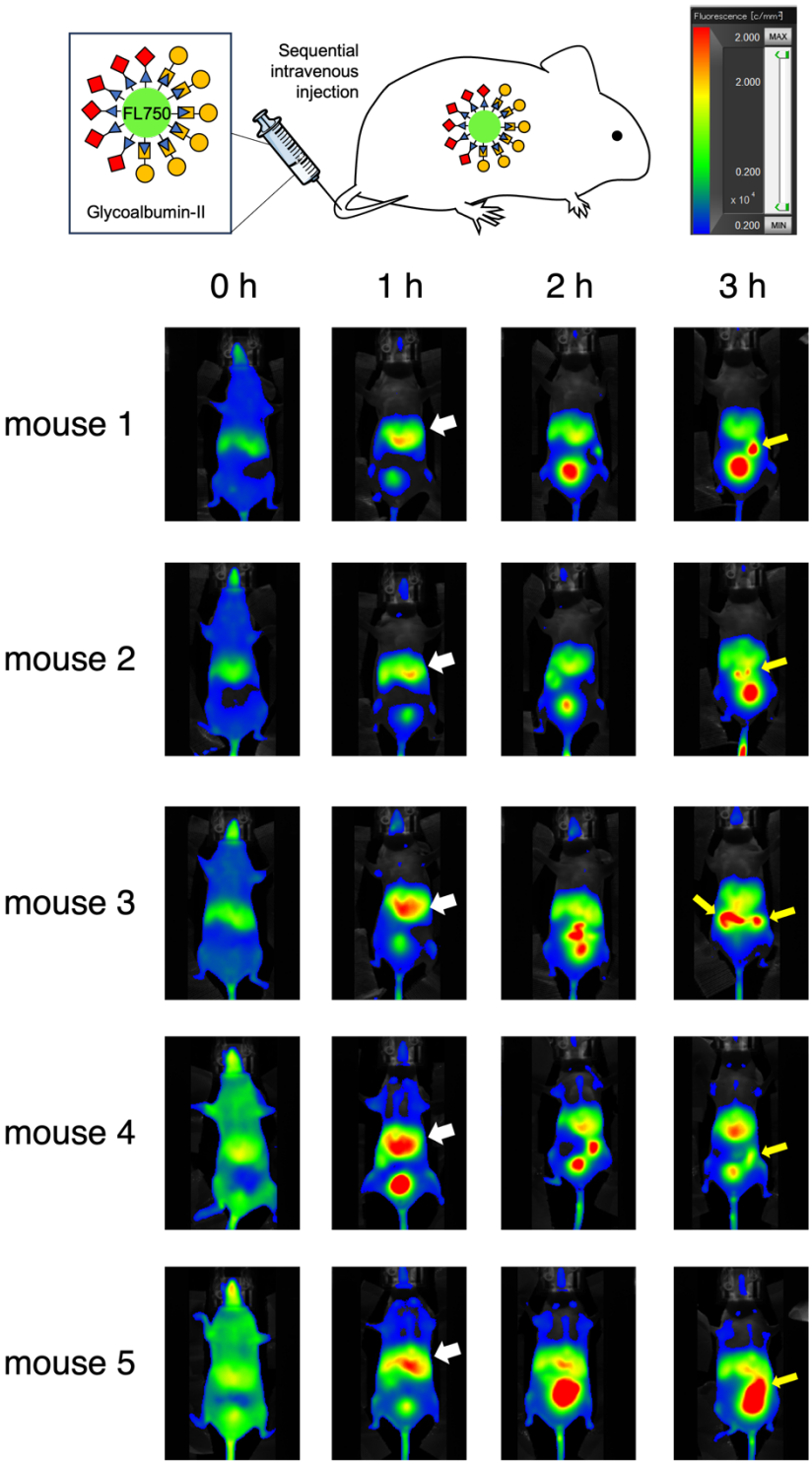
## 1.5 Animal studies



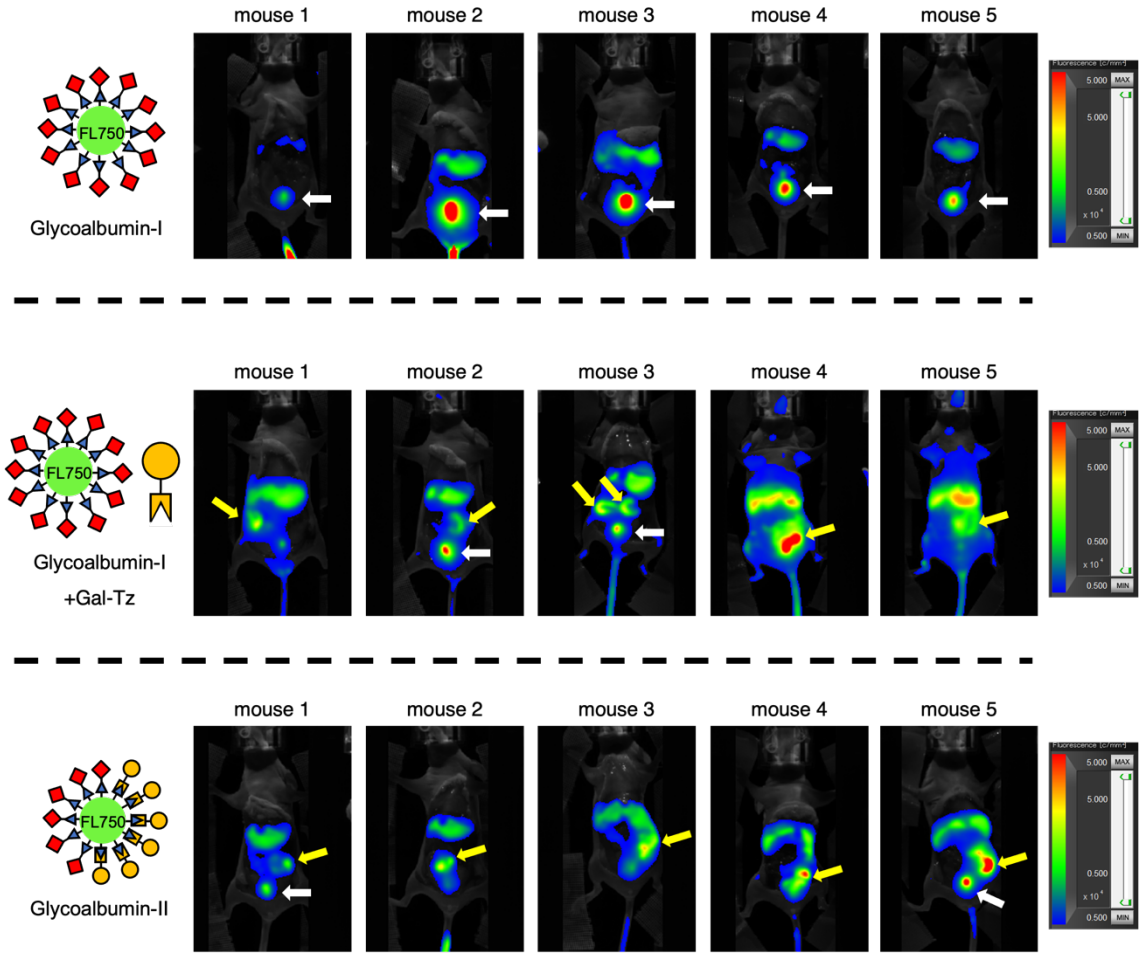

**Supplementary Figure 38.** Replicate data (n = 5) for whole-body fluorescence imaging of mice (abdominal side) at 0 to 3 hours following intravenous injection of glycoalbumin-I. The fluorescence intensity ranged from 0.2 to 2.0 × $10^4$.



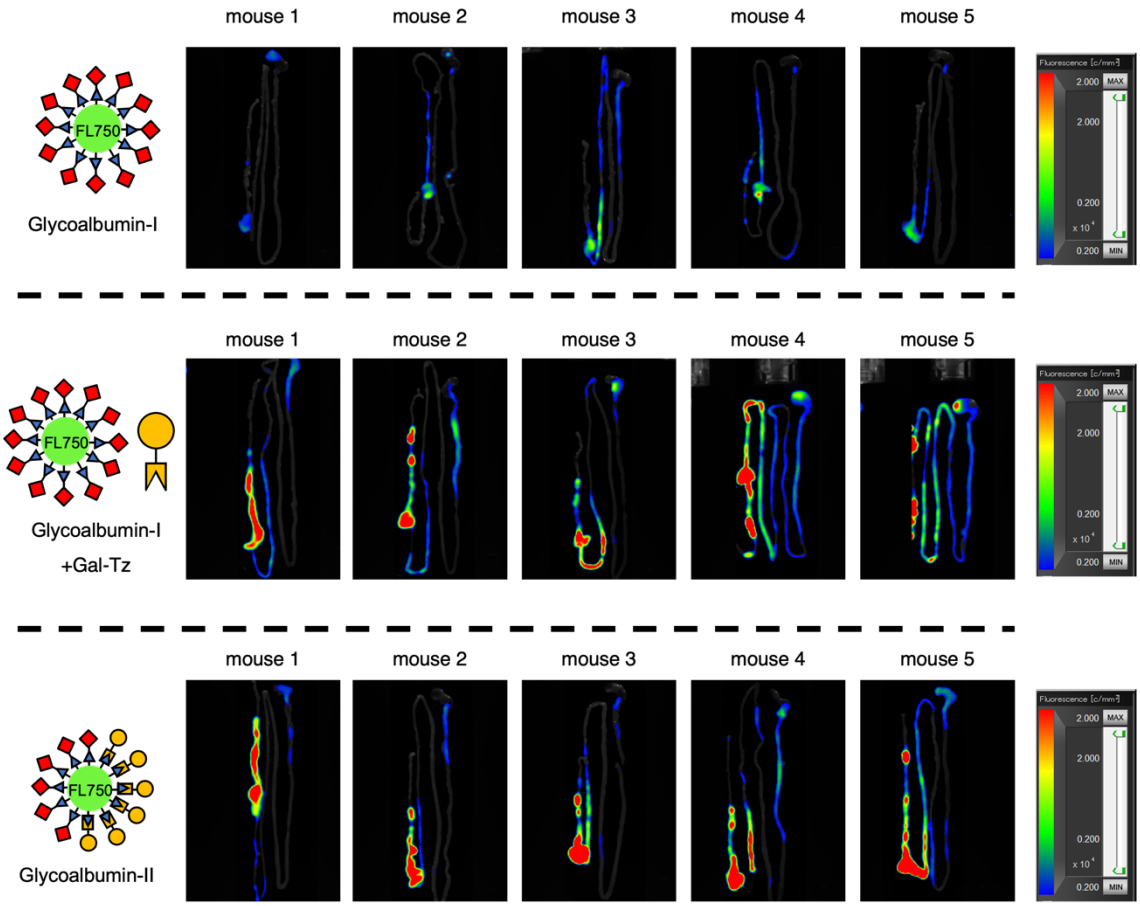

**Supplementary Figure 39.** Replicate data (n = 5) for whole-body fluorescence imaging of mice (abdominal side) at 0 to 3 hours following intravenous co-injection of glycoalbumin-I and Gal-Tz. The fluorescence intensity ranged from 0.2 to 2.0 × $10^4$.



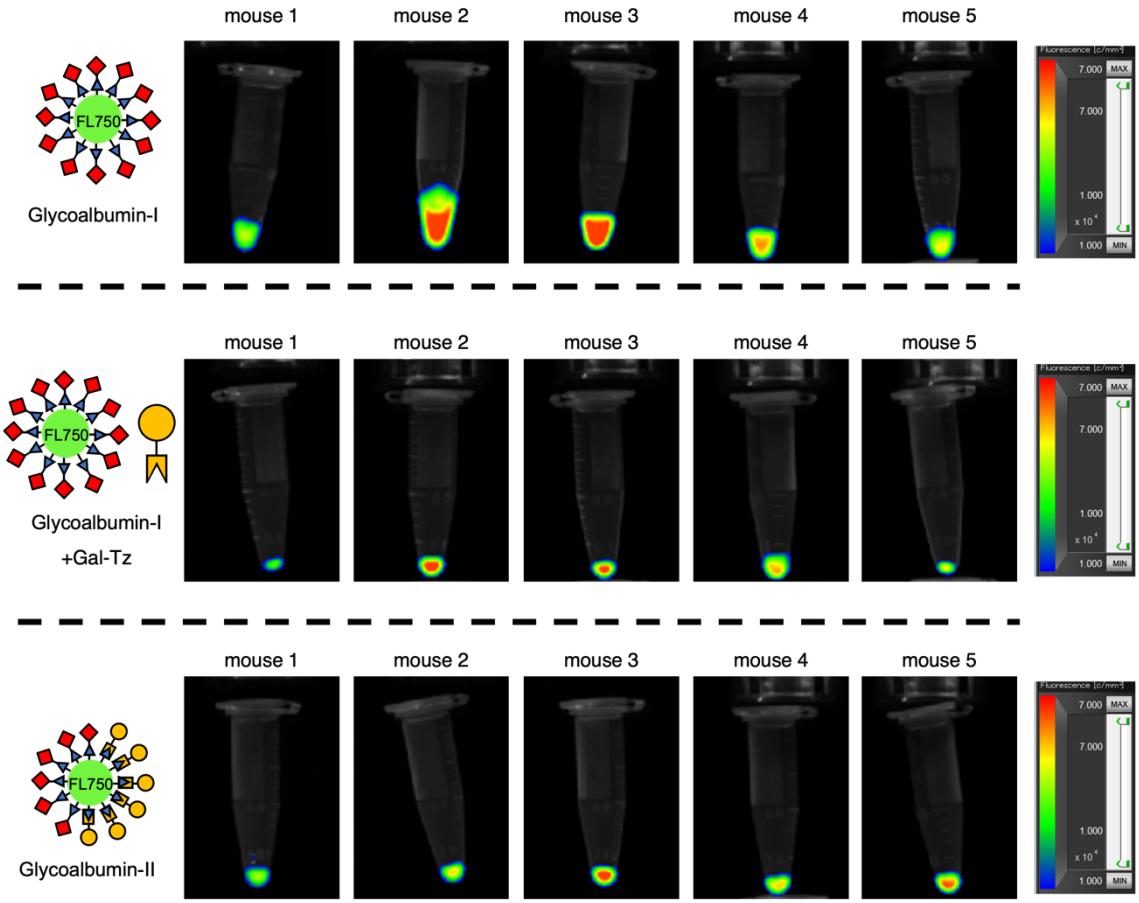

**Supplementary Figure 40.** Replicate data (n = 5) for whole-body fluorescence imaging of mice (abdominal side) at 0 to 3 hours following intravenous co-injection of glycoalbumin-II. The fluorescence intensity ranged from 0.2 to 2.0 × $10^4$. White arrow: liver; yellow arrow: intestine.



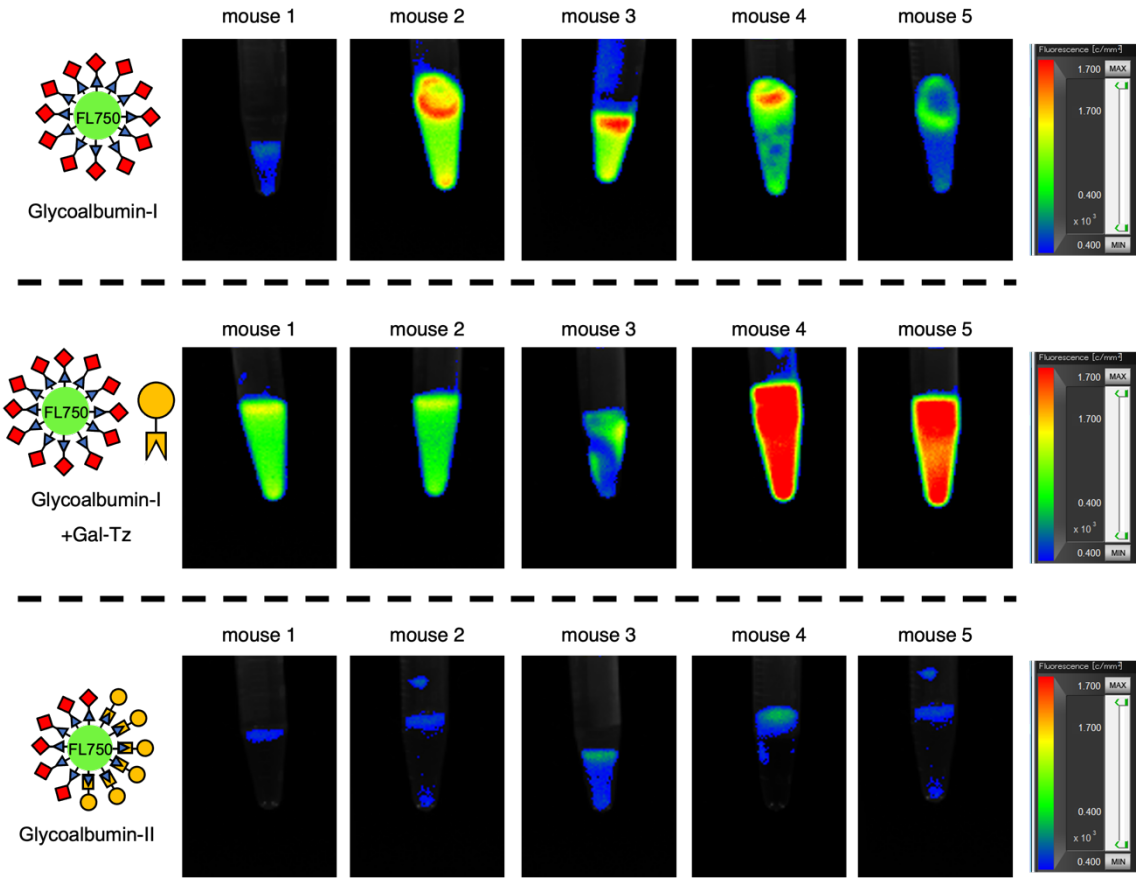

**Supplementary Figure 41.** Replicate data (n = 5) for whole-body fluorescence imaging of mice (abdominal side, open) at 3 hours following intravenous co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. White arrow: excretion to urinary bladder; yellow arrow: excretion to intestine. The fluorescence intensity ranged from 0.5 to 5.0 × $10^4$.



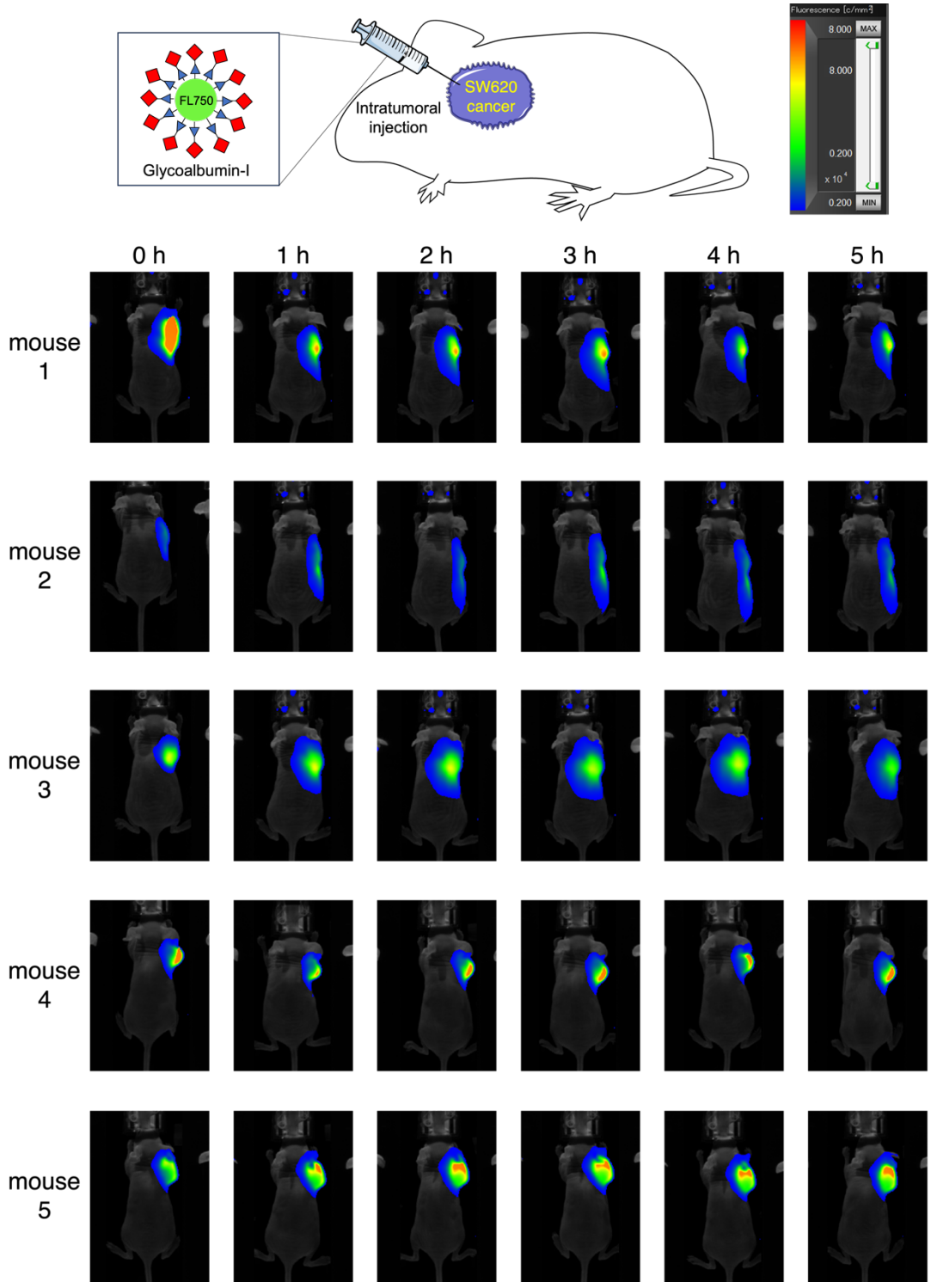

**Supplementary Figure 42.** Replicate data (n = 5) for fluorescence imaging of the collected intestines in mice at 3 hours following intravenous co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 0.2 to 2.0 × $10^4$.



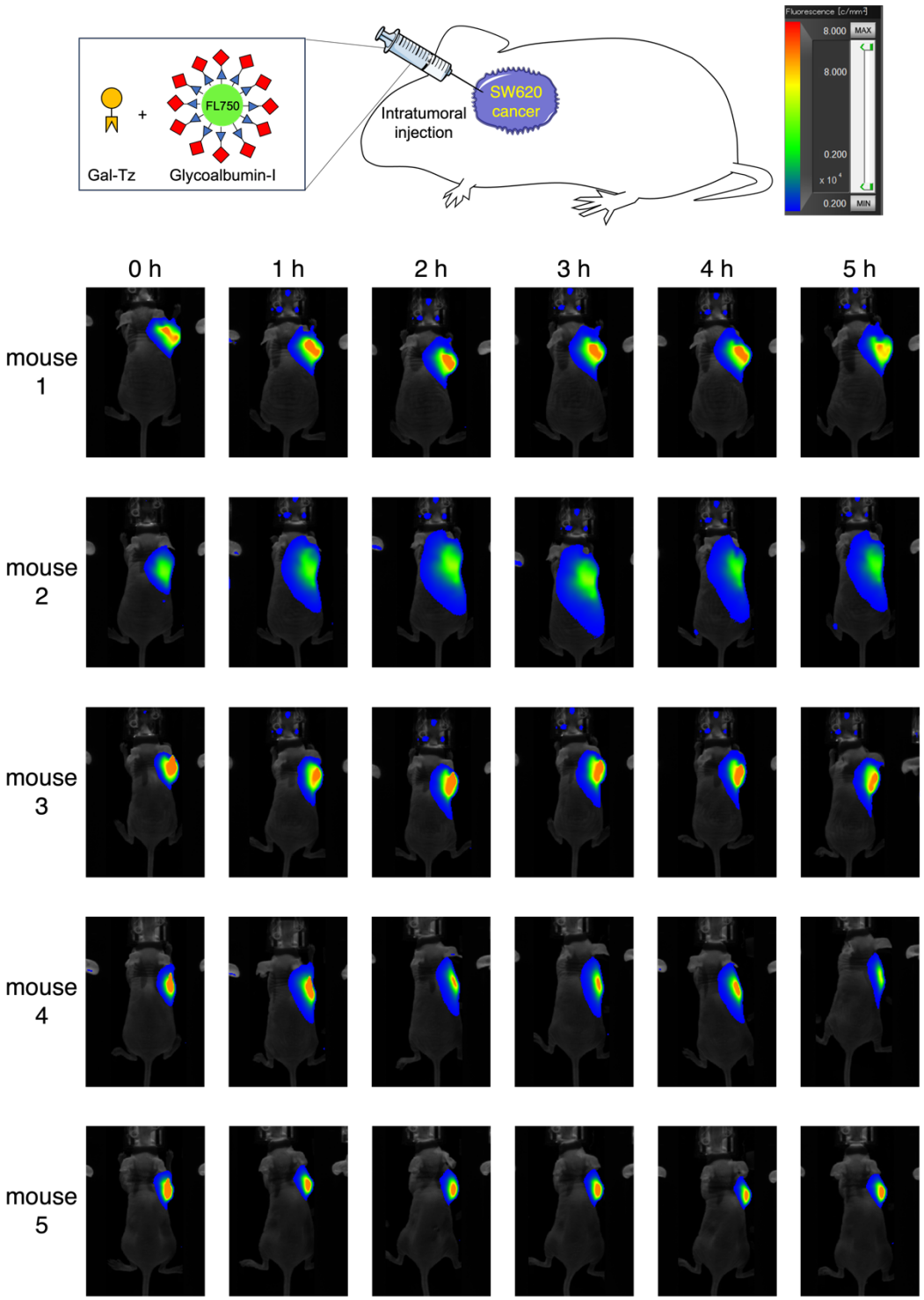

**Supplementary Figure 43.** Replicate data (n = 5) for fluorescence images of the collected urine samples from the mice at 3 hours following intravenous co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 1.0 to 7.0 × $10^4$.



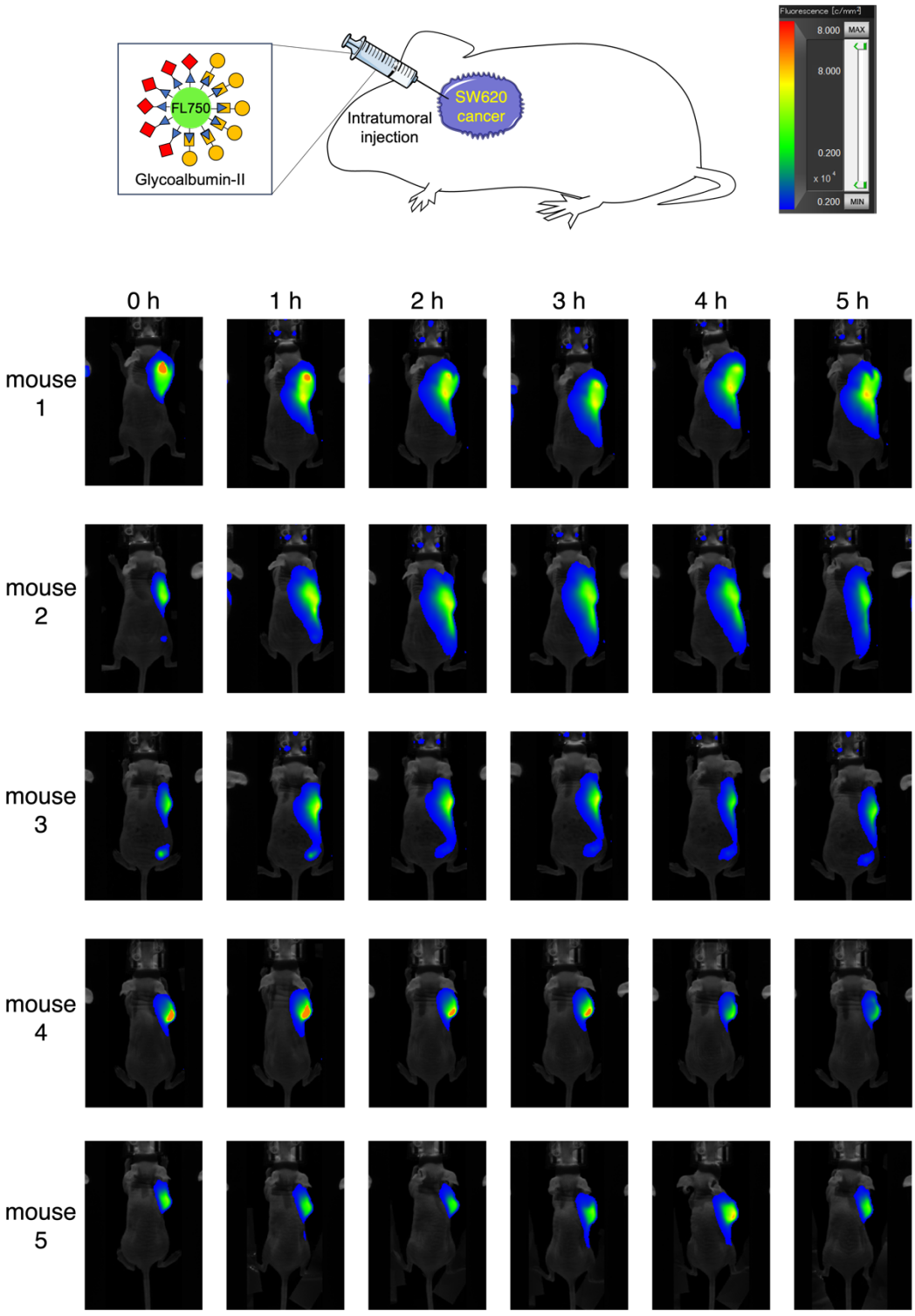

**Supplementary Figure 44.** Replicate data (n = 5) for fluorescence images of the collected blood samples from the mice at 3 hours following intravenous co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 0.4 to 1.7 × $10^3$.



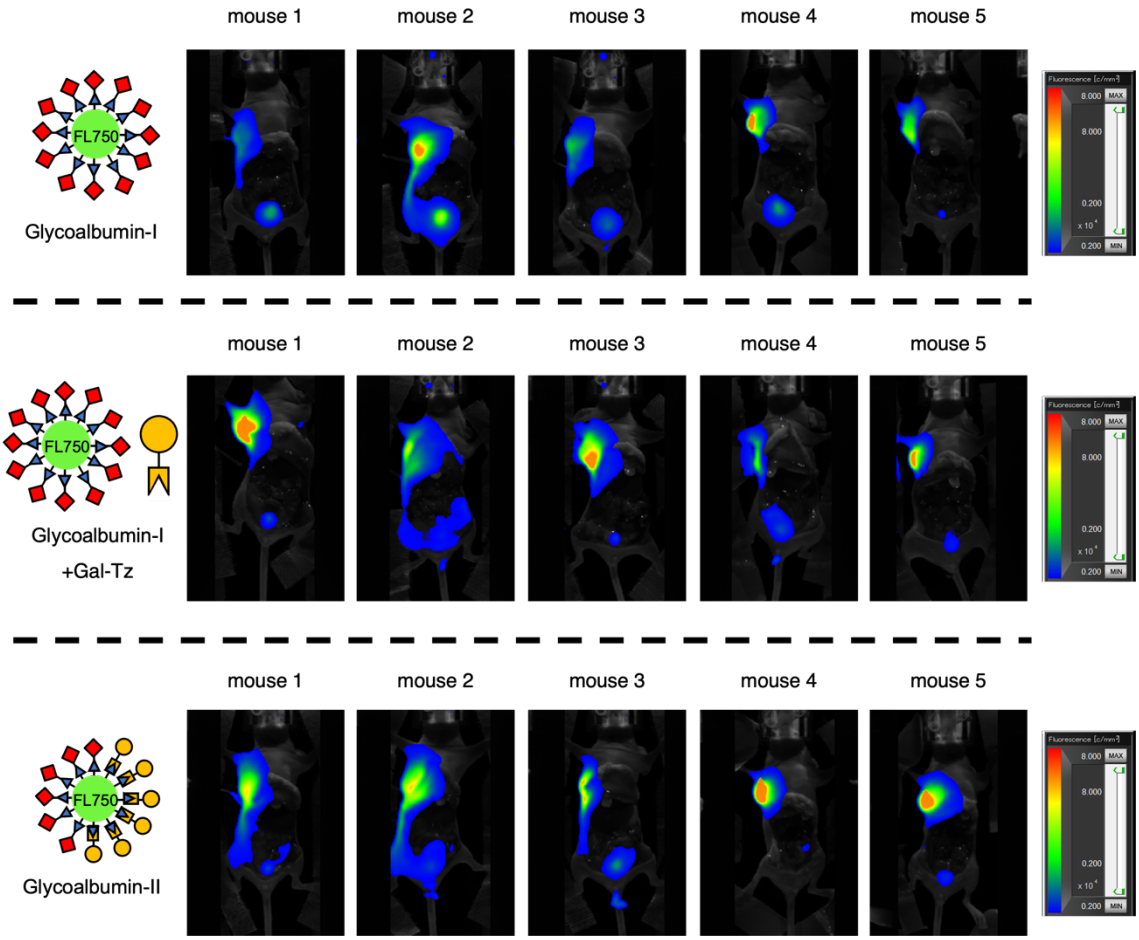

**Supplementary Figure 45.** Replicate data (n = 5) for whole-body fluorescence imaging of the SW620 bearing tumor mice (dorsal side) at 0 to 5 hours following intratumoral injection of glycoalbumin-I. The fluorescence intensity ranged from 0.2 to 8.0 × $10^4$.



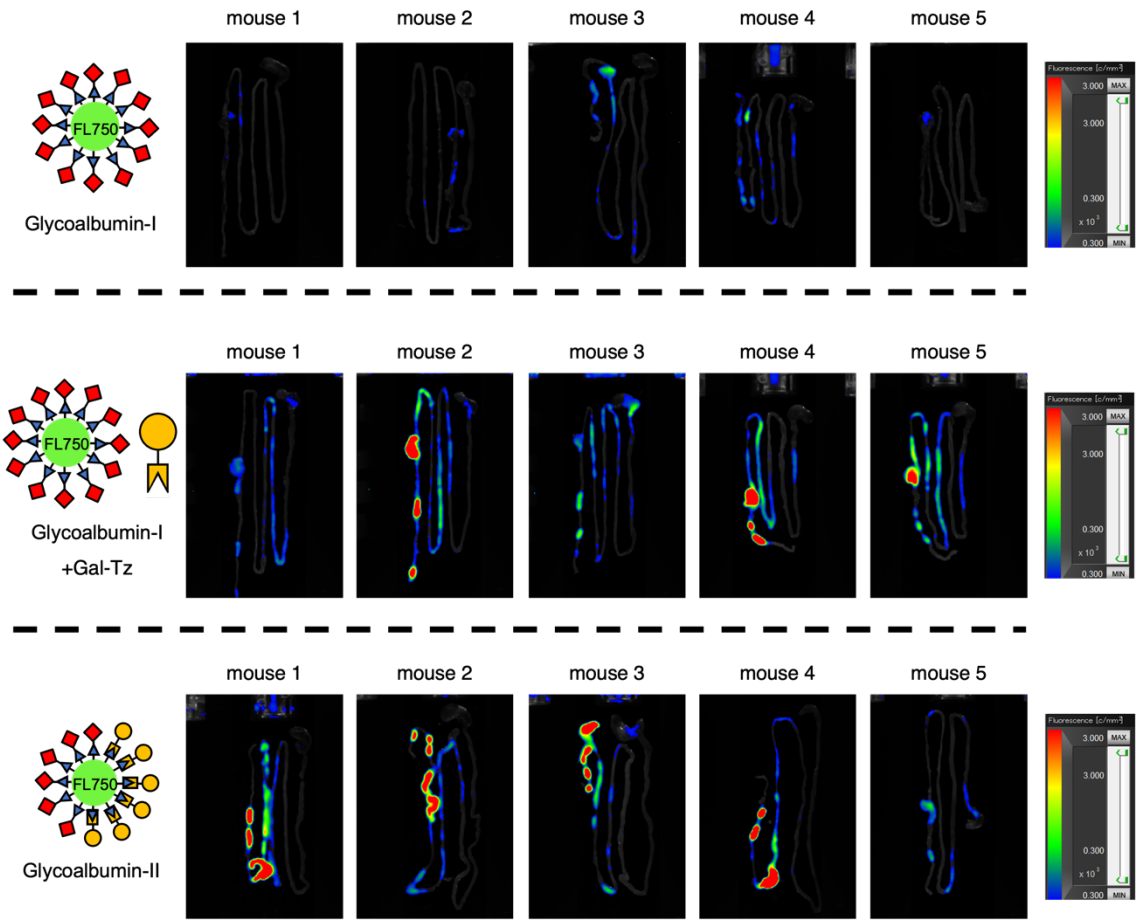

**Supplementary Figure 46.** Replicate data (n = 5) for whole-body fluorescence imaging of the SW620 bearing tumor mice (dorsal side) at 0 to 5 hours following intratumoral injection of glycoalbumin-I and Gal-Tz. The fluorescence intensity ranged from 0.2 to $8.0 \times 10^4$.



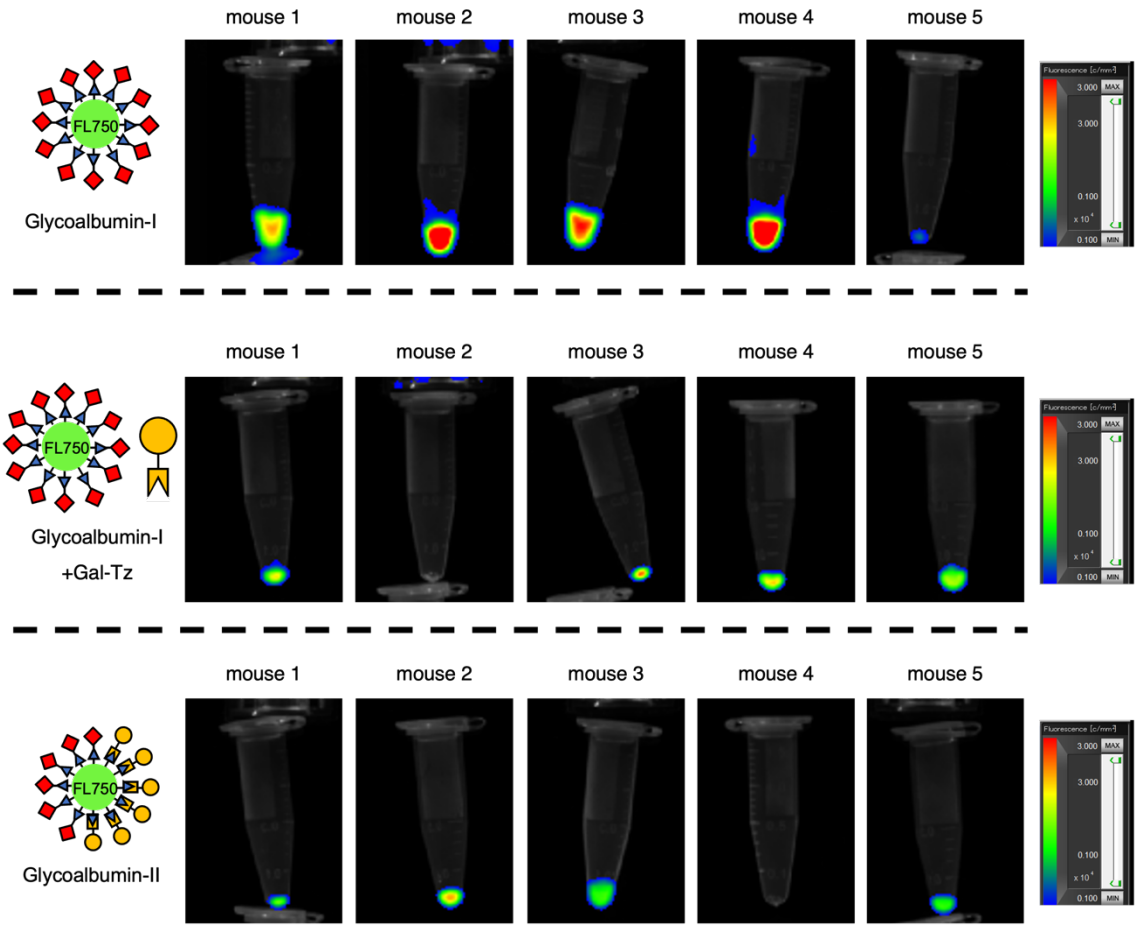

**Supplementary Figure 47.** Replicate data (n = 5) for whole-body fluorescence imaging of the SW620 bearing tumor mice (dorsal side) at 0 to 5 hours following intratumoral injection of glycoalbumin-II. The fluorescence intensity ranged from 0.2 to 8.0 × $10^4$.



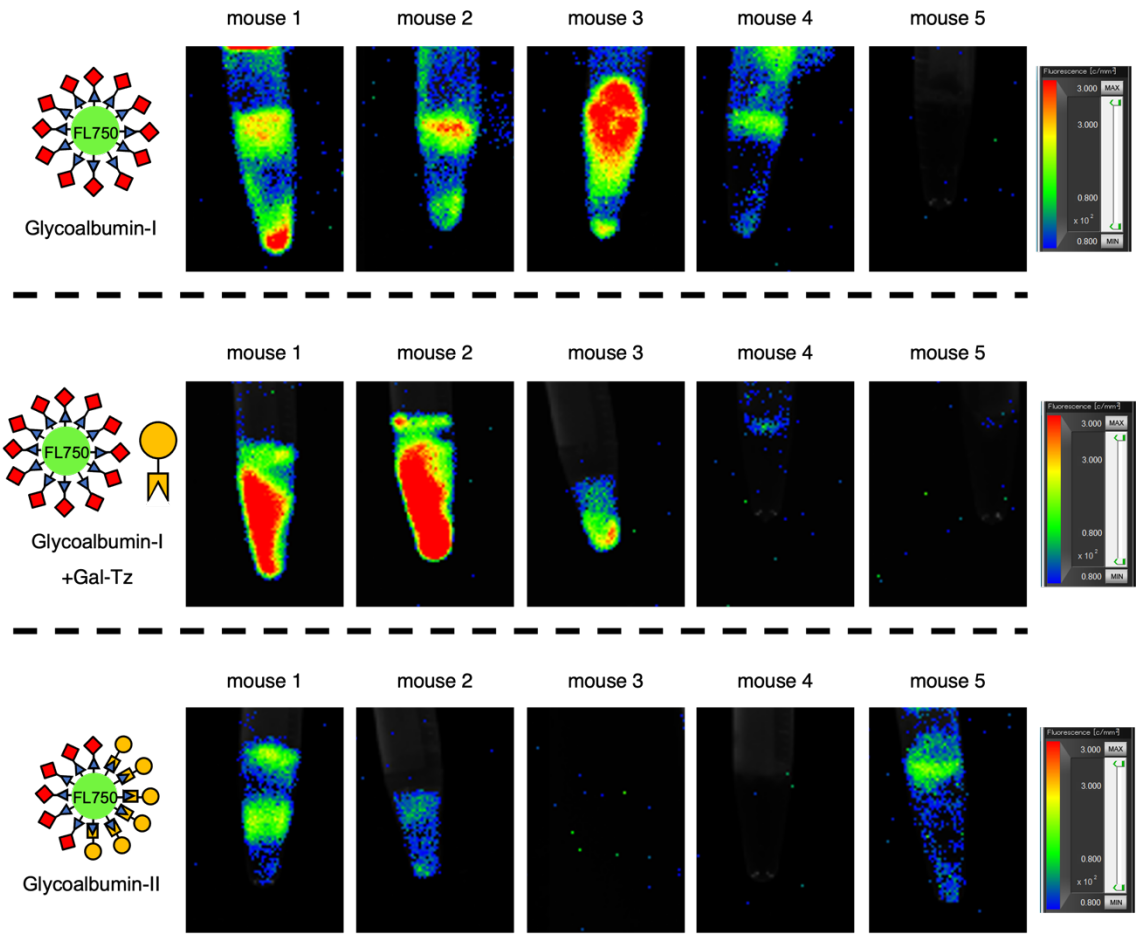

**Supplementary Figure 48.** Replicate data (n = 5) for whole-body fluorescence imaging of the SW620 bearing mice (abdominal side, open) at 5 hours following intratumoral injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 0.2 to 8.0 × $10^4$.



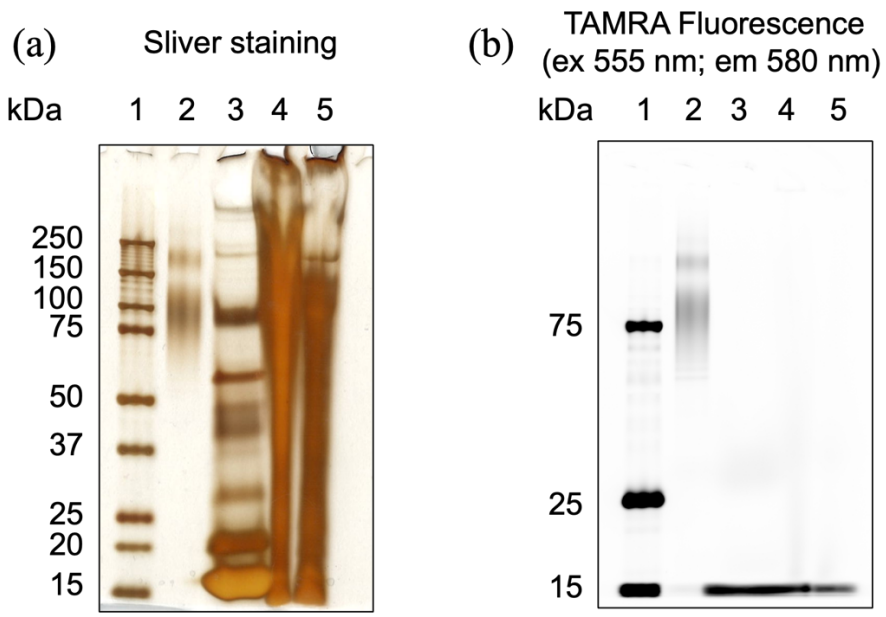

**Supplementary Figure 49.** Replicate data (n = 5) for fluorescence images of the collected intestines from the mice at 5 hours following intratumoral co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 0.3 to 3.0 × $10^3$.



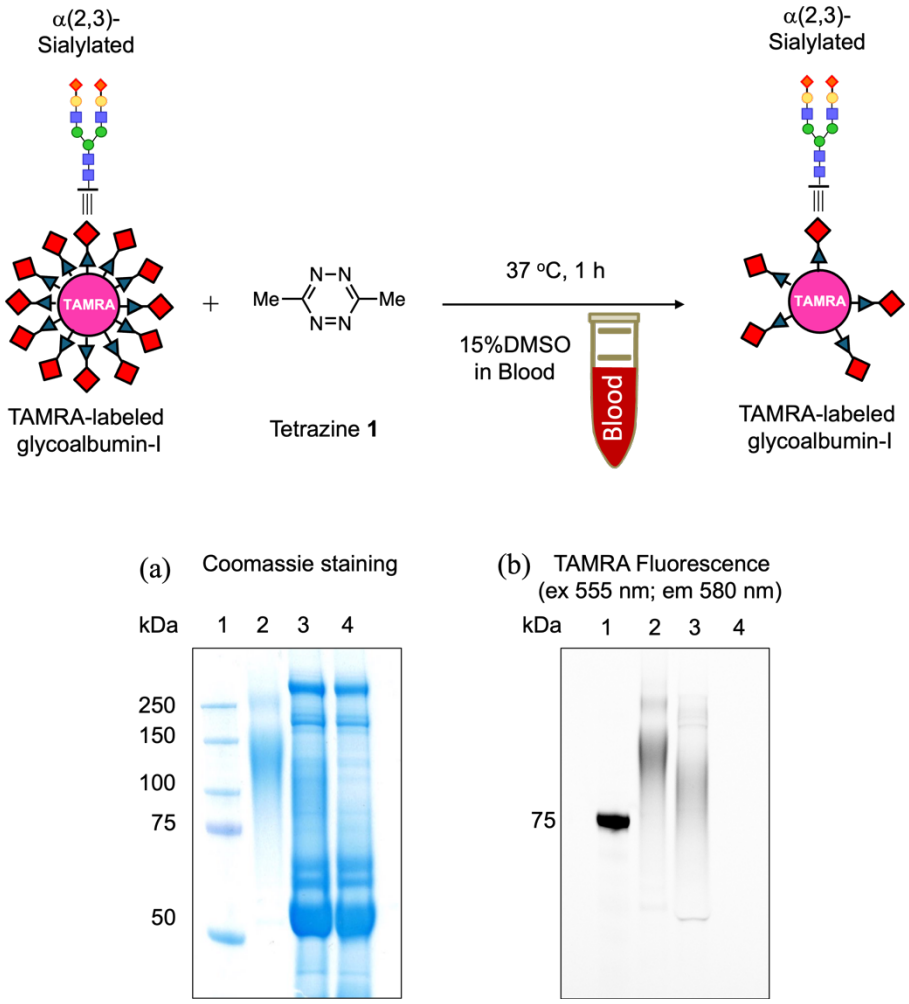

**Supplementary Figure 50.** Replicate data (n = 5) for fluorescence images of the collected urine samples from the mice at 5 hours following intratumoral co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 0.1 to 3.0 × $10^4$.




**Supplementary Figure 51.** Replicate data (n = 5) for fluorescence images of the collected blood samples from the mice at 5 hours following intratumoral co-injection of glycoalbumin-I and Gal-Tz. As control groups, glycoalbumin-I or -II only was used. The fluorescence intensity ranged from 0.8 to 3.0 × $10^2$.

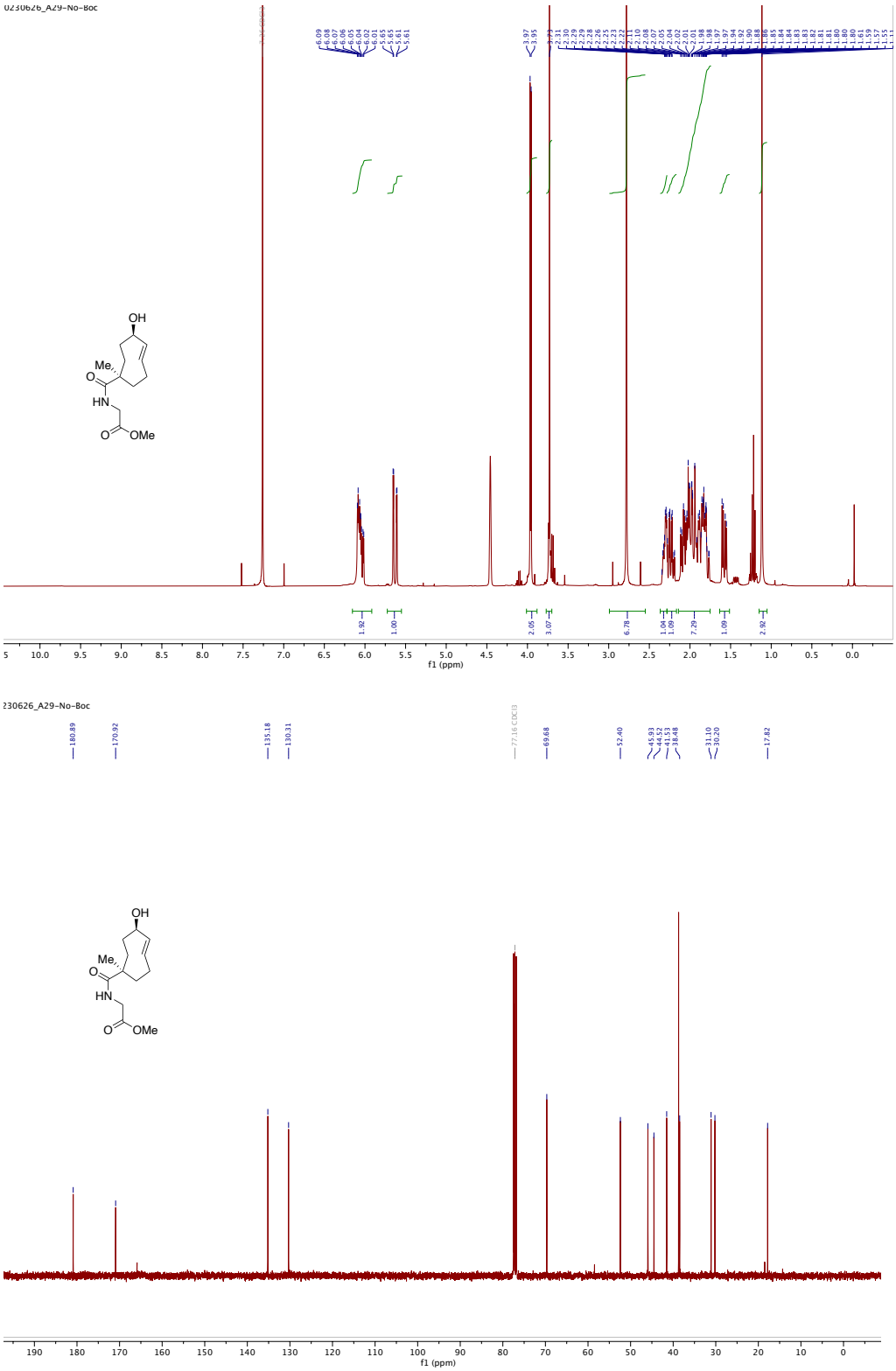



**Supplementary Figure 52.** Mice received the TAMRA-labeled glycoalbumin-II (1.5 nmol in 200 μL saline). The indicated compound was injected intravenously into 8–10-week-old BALB/cAJcl-nu/nu mice (n = 1). After 3 hours, the mice were dissected, and we analyzed the urine and the PBS buffer extractions from the cecum and inner feces of the colon by SDA-PAGE. The SDA-PAGE gel images showed silver staining (panel a) and TAMRA fluorescence (panel b) for the same gel. Lane 1 is protein MW standards. Lane 2 is the TAMRA-labeled glycoalbumin-II. Lane 3 is the urine. Lane 4 is the extraction from the cecum. Lane 5 is the extraction from inner feces of the colon. The ChemiDoc™ Touch (Bio-Rad) was used to record the TAMRA fluorescent image using a Blot/UV/Stain-free tray with Pro-Q Diamond setting. The TAMRA fluorescent image was obtained at a 6.0 s exposure setting. Based on the result (Supplementary Figure 52), This indicated that glycoalbumin-II was degraded into TAMRA-labeled peptides in the liver. Thus, the glycoalbumin-II was initially taken up by the liver, digested into small peptides, and eventually excreted through intestine.

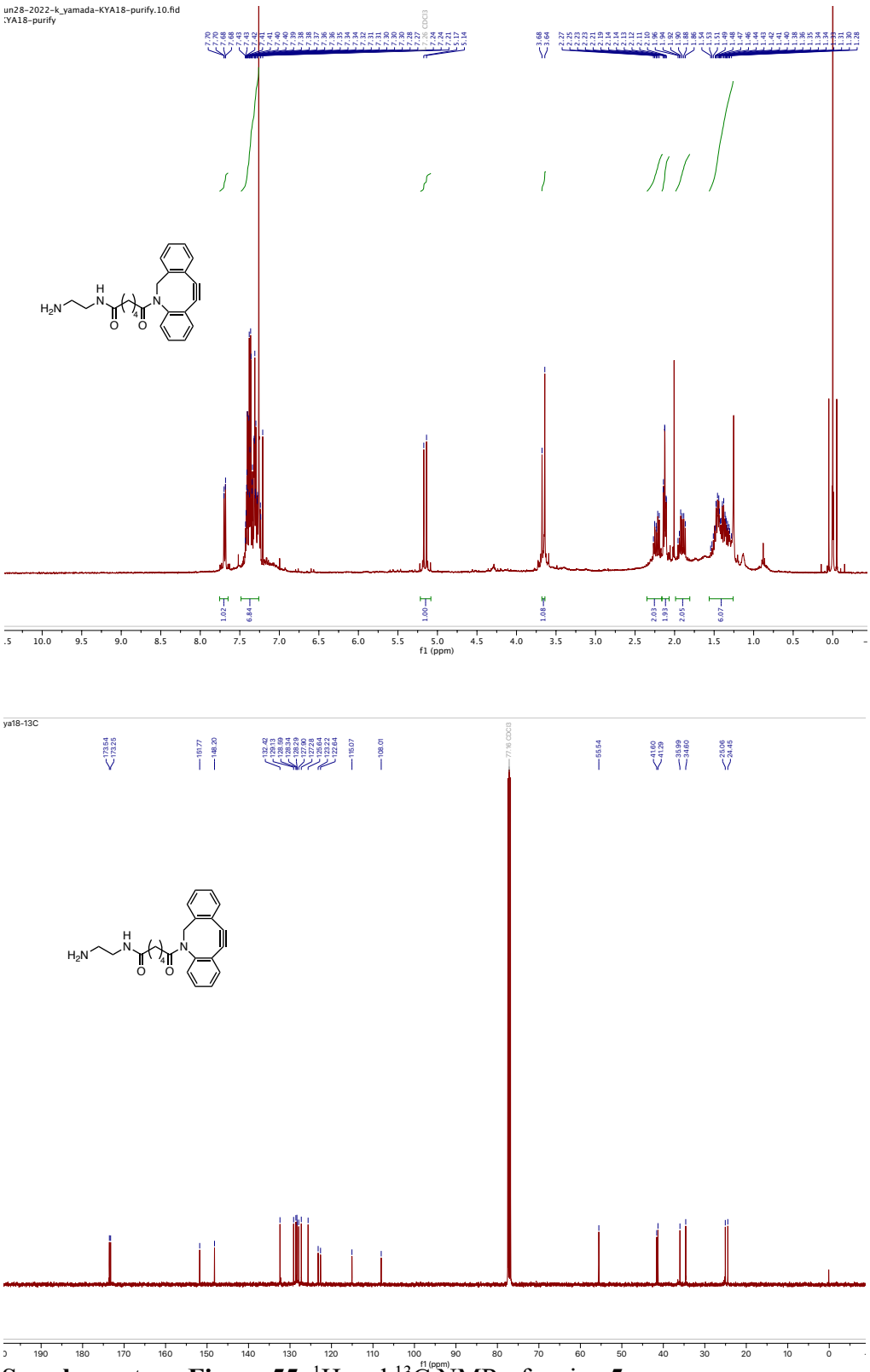



**Supplementary Figure 53.** TAMRA-labeled glycoalbumin-I (20 μM) and tetrazine **1** (1 mM) were reacted in a mixture of blood solution (15% DMSO in mouse blood) at 37 °C for 1 h. After reaction, the solution was centrifuged for 10 min at 16900 x *g* and the generated supernatant was analyzed by SDS-PAGE. SDS-PAGE gel images showing Coomassie staining (panel a) and TAMRA fluorescence (panel b) for the same gel. Lane 1 is protein MW standards. Lane 2 is the TAMRA-linked glycoalbumin-I. Lane 3 is the mixture of TAMRA-labeled glycoalbumin-I and tetrazine **1** in a mixture blood solution. Lane 4 is mouse blood. The ChemiDoc™ Touch (Bio-Rad) was used to record the TAMRA fluorescent images using a Blot/UV/Stain-free tray with Pro-Q Diamond setting. The TAMRA fluorescent images were obtained at a 0.1s exposure setting.

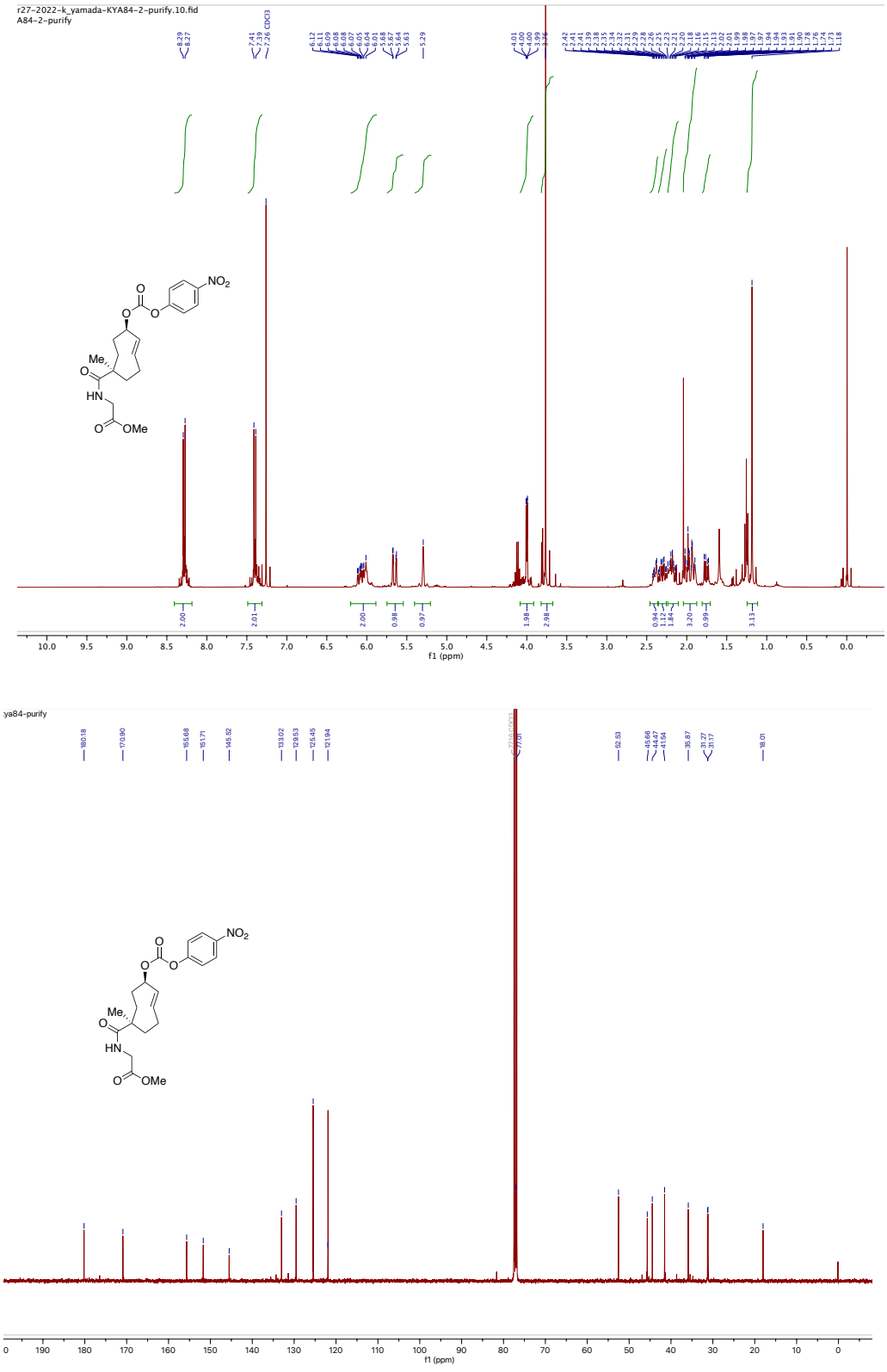
# 2. Supplementary Figures ($^{1}$H and $^{13}$C NMR spectra)



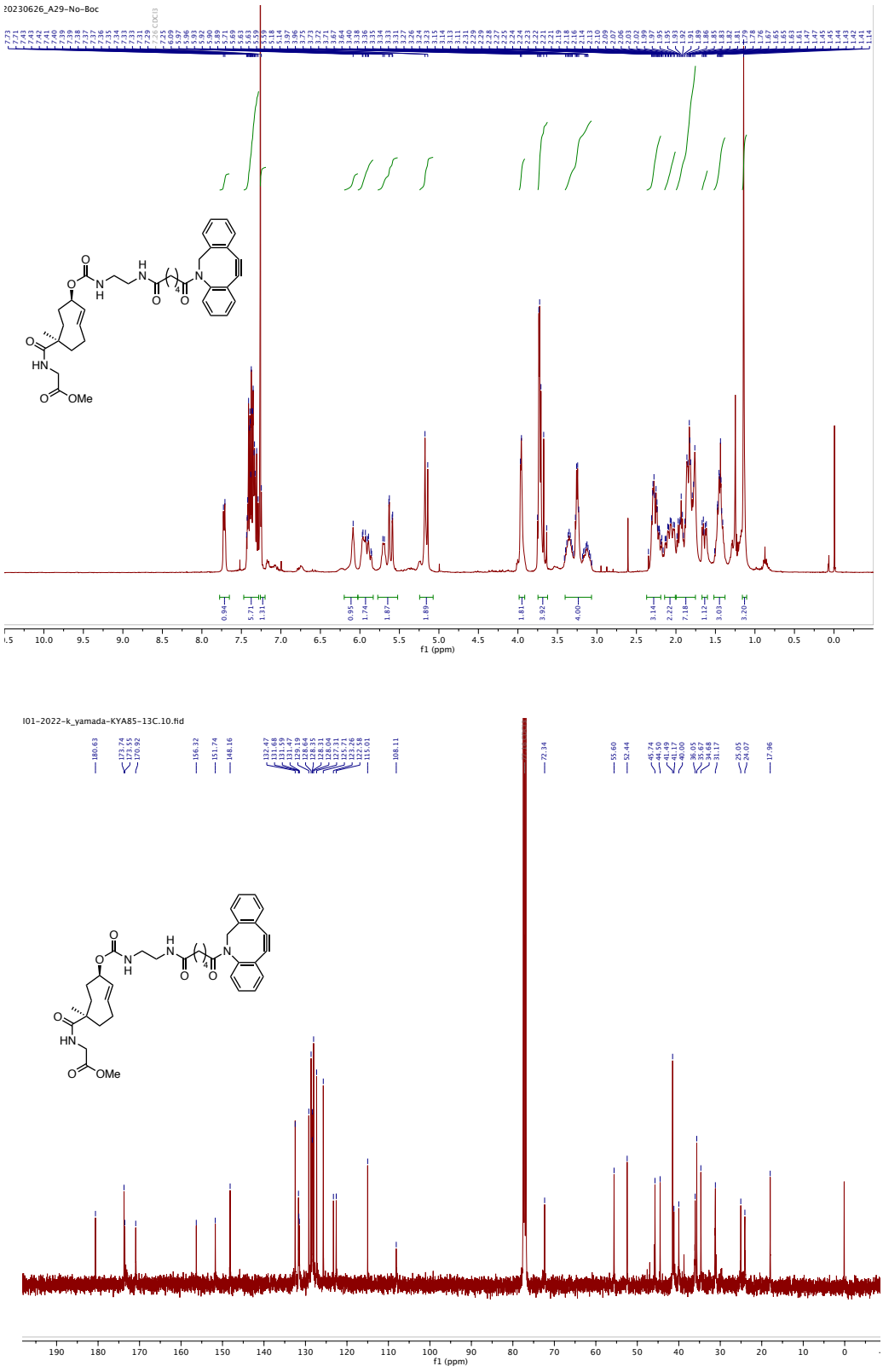

**Supplementary Figure 54.** $^{1}$H and $^{13}$C NMR spectra of carbonate **4**.



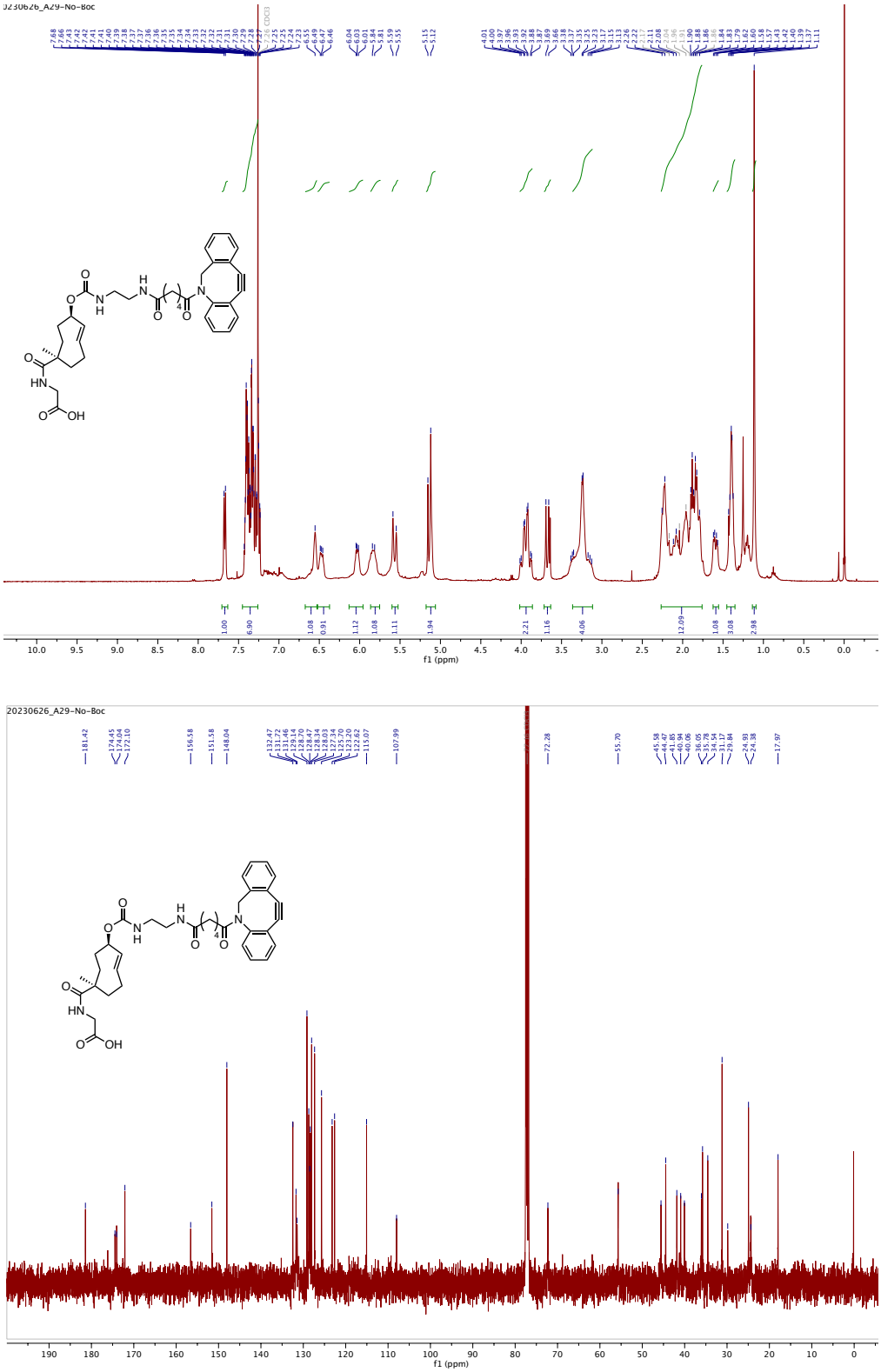

**Supplementary Figure 55.** $^{1}H$ and $^{13}C$ NMR of amine **5**.



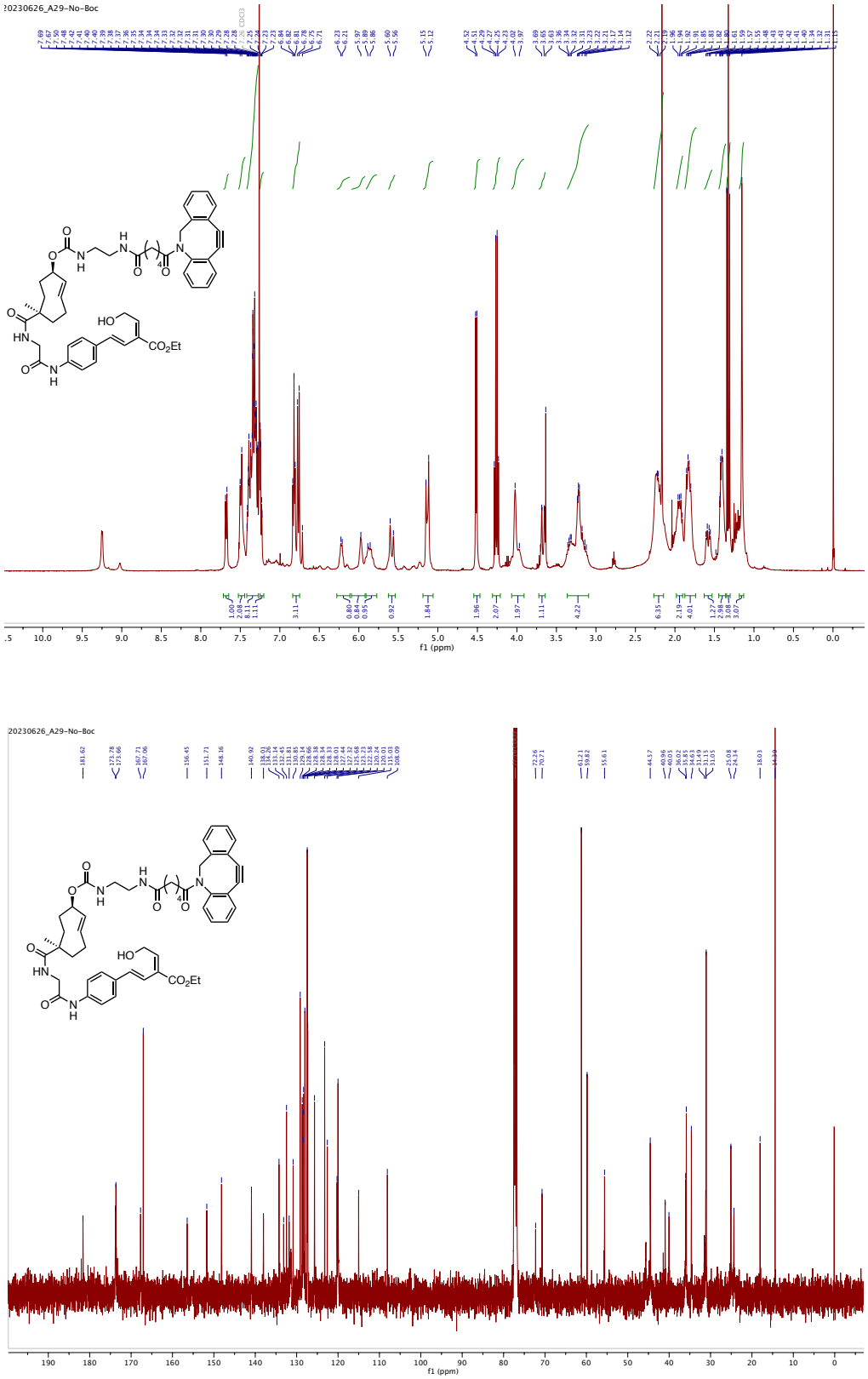

**Supplementary Figure 56.** $^{1}H$ and $^{13}C$ NMR spectra of carbonate intermediate **S1**.



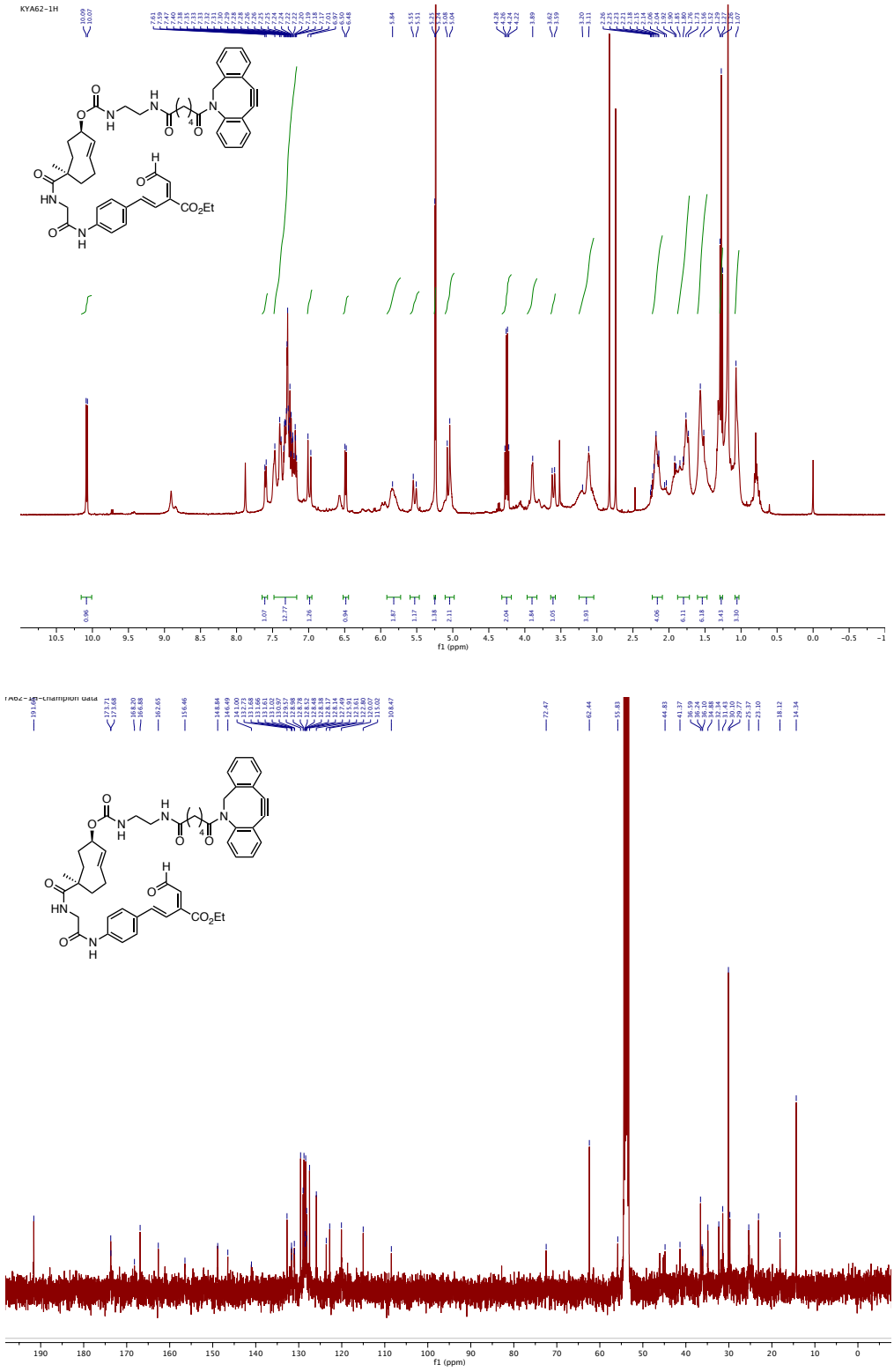

**Supplementary Figure 57.** $^{1}$H and $^{13}$C NMR spectra of methyl ester **6**.



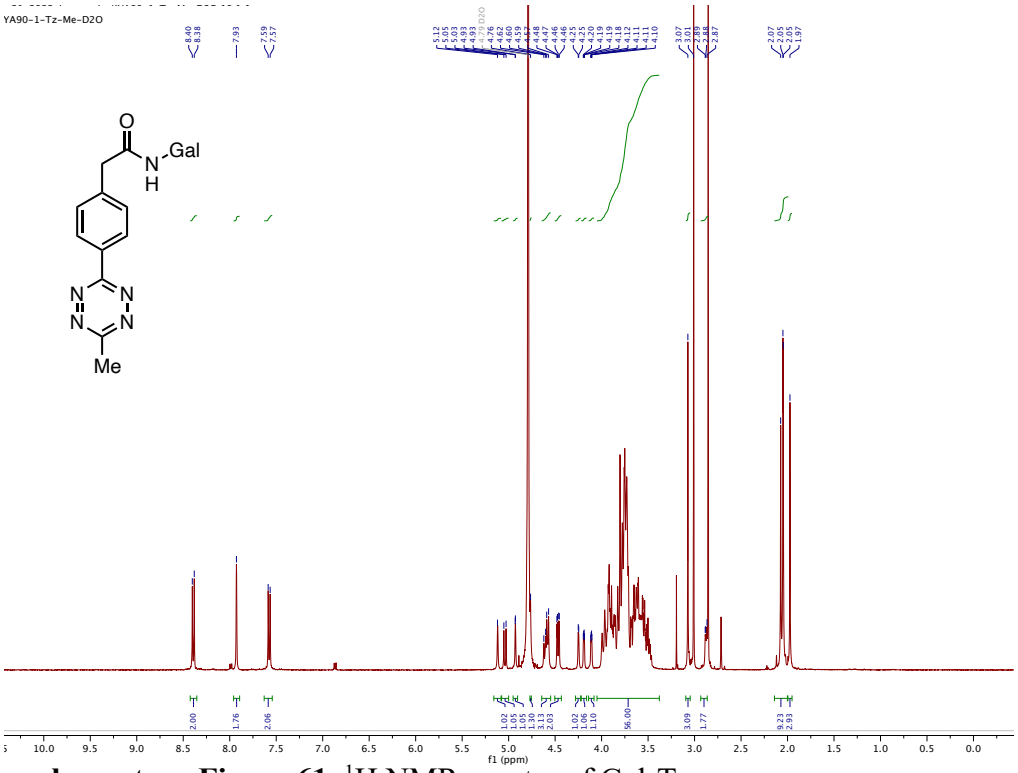
**Supplementary Figure 58.** $^{1}H$ and $^{13}C$ NMR spectra of carboxylic acid **S2**.



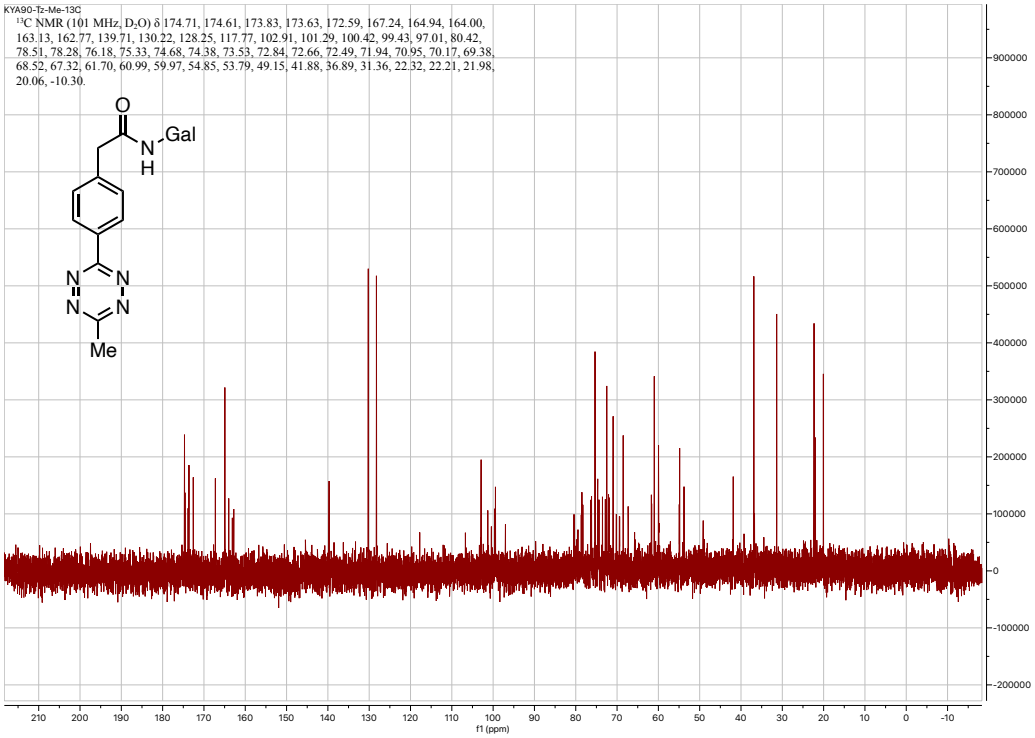
**Supplementary Figure 59.** $^{1}H$ and $^{13}C$ NMR spectra of allyalcohol **8**.



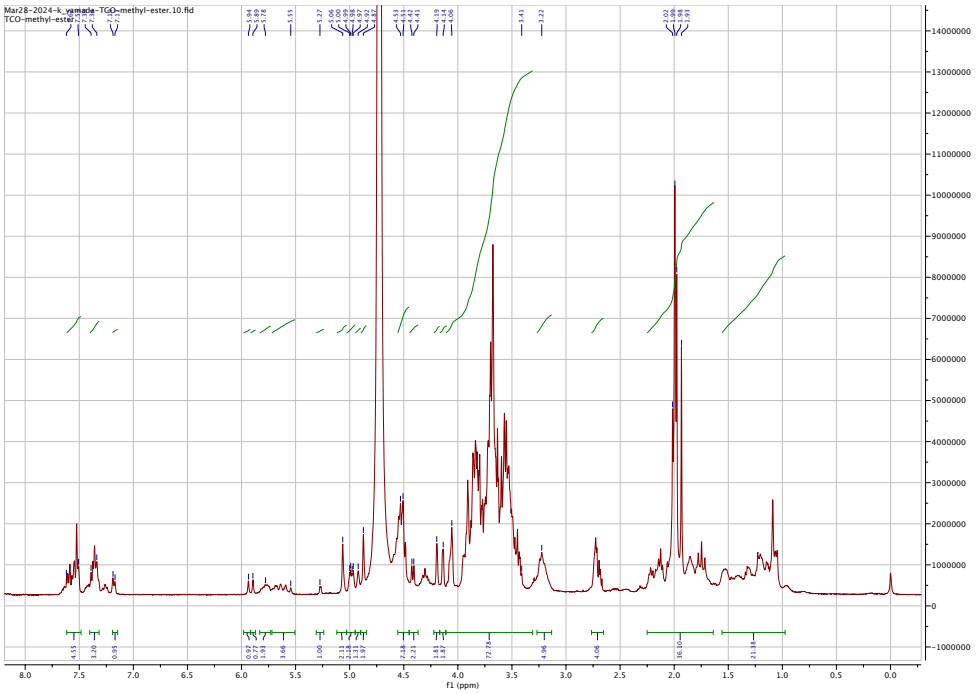
**Supplementary Figure 60.** $^{1}H$ and $^{13}C$ NMR spectra of aldehyde **1**.



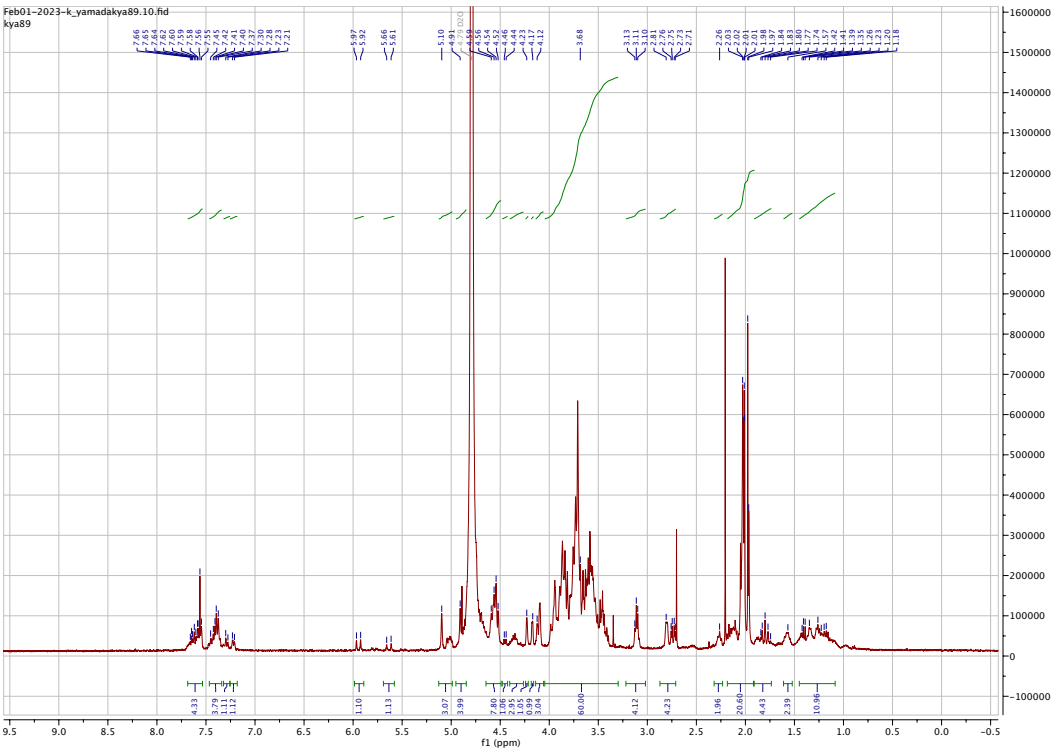

**Supplementary Figure 61.** $^{1}$H NMR spectra of Gal-Tz.




**Supplementary Figure 62.** $^{13}$C NMR spectra of Gal-Tz.



**Supplementary Figure 63.** $^{1}$H NMR spectra of **S4**.



**Supplementary Figure 64.** $^{1}$H NMR spectra of **S5**.

# 3. Supplementary References


1. Geus, M. A. R. D., Maurits, E., Sarris, A. J. C., Hansen, T., Kloet, M. S., Kamphorst, K., Hoeve, W. T., Robillard, M. S., Pannwitz, A., Bonnet, S. A., Codée, J. D. C., Filippov, D. V. Overkleeft, H. S., Kasteren, S. I. V., Fluorogenic bifunctional *trans*-cyclooctenes as efficient tools for investigating click-to-release kinetics. *Chemistry* **26,** 9900-9904 (2020).
2. Debets, M. F., Berkel, S. S. V., Schoffelen, S., Rutjes, F. P. J. T., Hest, J. C. M. V., Delft, F. L. V. Aza-dibenzocyclooctynes for fast and efficient enzyme PEGylation via copper-free (3+2) cycloaddition. *Chem. Commun.* **46**, 97-99 (2010).
3. Nakamura, K., Tsubokura, K., Kurbangalieva, A., Nakao, Y., Murase, T., Shimoda, T., Tanaka, K. Efficient route to RIKEN click probes for glycoconjugation. *J. Carbohydr. Chem.* ***38***, 127-138 (2019).