# Supporting information

for

# Switch-on luminescent sensing of unlabelled bacterial lectin by terbium(III) glycoconjugate systems

Karolina Wojtczak,[a] Eva Zahorska,[b,c,d] Ian J. Murphy, [a] Finnja Koppel,[e] Gordon Cooke,[e] Alexander Titz[b,c,d] and Joseph P. Byrne*[a,f]

[a.] *School of Biological and Chemical Sciences, University of Galway, University Road, Galway, Ireland.*
[b.] *Chemical Biology of Carbohydrates, Helmholtz Institute for Pharmaceutical Research Saarland, Helmholtz Centre for Infection Research, D-66123 Saarbrücken, Germany.*
[c.] *Department of Chemistry, Saarland University, D-66123 Saarbrücken, Germany.*
[d.] *Deutsches Zentrum für Infektionsforschung (DZIF), Standort Hannover-Braunschweig.*
[e.] *School of Chemical & BioPharmaceutical Sciences, Technological University Dublin, Ireland*
[f.] *School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland.*
**Joseph.byrne@ucd.ie*

## General experimental details:

NMR spectra were obtained on a Varian VNMRS 500 MHz 54mm AR Spectrometer operating under VnmrJ software. Chemical shifts reported relative to an internal TMS standard ($\delta$ 0.00 ppm). Deuterated solvents $CDCl_3$, $D_2O$, $CD_3OD$ were obtained commercially and used without further purification. Spectra were visualised and analysed using MestReNova v14.2.1-27684 software. Infrared spectra were obtained on a Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer equipped with a universal ATR accessory. High resolution mass spectroscopy was performed on an Agilent 6530 Accurate Mass Q-TOF LC/MS coupled to an Agilent 1290 Infinity UPLC system and on a Bruker 'autoflex maX' MALDI TOF/TOF system equipped with a HTX Technologies TM-Sprayer. TLC experiments were performed using aluminium sheets pre-coated with silica gel 60 (HF254, E. Merck, Darmstadt, Germany). Chromatography was performed with silica gel 60 mesh (Sigma Aldrich, Wicklow, Ireland). Reactions performed under microwave irradiation were carried out in a CEM Discover-SP Microwave Reactor, 2012 model, in the appropriate bespoke vessels. All starting materials were obtained from commercial sources and used without further purification. Recombinant LecA, PNA, ConA and WGA for sensing experiments were purchased as lyophilised powders from Sigma Aldrich and used without further purification to make stock solutions. Where denaturation of proteins was required, this was performed by heating a stock solution at 85°C for 30 min.

## Synthetic procedures:

Dialkyne **1**, and glycoconjugate azides, 1-Azido-3,6-dioxaoct-8-yl-2,3,4,6-tetra-o-acetyl-β-D-galactopyranoside and 1-Azido-3,6-dioxaoct-8-yl-2,3,4,6-tetra-o-acetyl-α-D-mannopyranoside, were prepared as previously reported.[1,2]

**2Gal$^{OAc}$**. Dialkyne **1** (0.042 g, 0.15 mmol), $CuSO_4{\cdot}5H_2O$ (0.015 g, 0.06 mmol) and sodium ascorbate (0.023 g, 0.12 mmol) were added to a round-bottomed flask. 1-Azido-3,6-dioxaoct-8-yl-2,3,4,6-tetra-o-acetyl-β-D-galactopyranoside (0.15 g, 0.31 mmol) was dissolved in DMF-$H_2O$ (20 mL. 4:1 mixture) and added to the reaction vessel, and the mixture stirred at room temperature for 20 h. The reaction mixture was extracted three times with DCM, and the combined organic layers washed with $H_2O$ (×2), brine (×2) and dried over $Na_2SO_4$. The reaction mixture was concentrated under reduced pressure and co-evaporation with toluene. Flash chromatography ($SiO_2$, EtOAc → $CH_3OH$/EtOAc) yielded **3Gal$^{OAc}$** as a very hygroscopic white solid (0.162 g, 0.129 mmol, 86%). **M.p.** 77-81°C; **HRMS** (ESI+) (Q-TOF) (*m/z*): Calculated for $C_{53}H_{74}N_9O_{26}^+$ $[M+H]^+$ *m/z* = 1252.4745. Found *m/z* = 1252.4765; **$^1$H NMR** (500 MHz, $CDCl_3$): δ = 9.01 (br s, 2H, triazolyl CH), 8.30 (d, 2H, *J* = 7.7 Hz, 3-,5-pyr CH), 8.00 (t, 1H, *J* = 7.7 Hz, 4-pyr CH), 7.80 (br s, 2H, NH), 5.39 (dd, 2H, *J* = 3.4, 0.8 Hz, Gal C$^4$H), 5.19 (dd, 2H, *J* = 10.5, 8.0 Hz, Gal C$^2$H), 5.04 (dd, 2H, *J* = 10.5, 3.4 Hz, Gal C$^3$H), 4.74 (br s, 4H, $CH_2$), 4.57 (d, 2H, *J* = 8.0 Hz, Gal C$^1$H), 4.47–4.55 (m, 4H, $CH_2$), 4.25 – 4.07 (m, 4H, $CH_2$), 4.02–3.92 (m, 4H, Gal C$^5$H and Gal C$^6$*H*H), 3.86 (t, 4H, *J* = 4.8 Hz, C*H*$_2$–NH), 3.75–3.68 (m, 2H, Gal C$^6$H*H*), 3.65 – 3.54 (m, 6H, $CH_2$), 2.14, 2.03, 2.02, 1.97 (4 × s, 12H, C(O)$CH_3$).; **$^{13}$C NMR** (126 MHz, $CDCl_3$): δ = 170.4, 170.2, 170.2, 169.5 (4 × C=O), 163.5 (qt), 148.7 (qt), 138.8 (pyr CH), 124.8 (pyr CH), 101.3 (Gal C$^1$H), 70.9 (Gal C$^3$H), 70.7 (Gal C$^5$H), 70.6 ($CH_2$), 70.5 ($CH_2$), 70.4 ($CH_2$), 70.2 ($CH_2$), 69.4 ($CH_2$), 69.1 ($CH_2$), 68.8 (Gal C$^2$H), 67.1 (Gal C$^4$H), 63.5, 61.3 ($CH_2$), 50.3 ($CH_2$), 34.4 ($CH_2$), 20.8, 20.7, 20.7, 20.6 (4 × C(O)$CH_3$); **FT-IR (ATR, cm$^{-1}$):** 3333 (br), 2929, 1742 (s), 1669 (s), 1530 (s), 1429, 1370, 1219 (s), 1173, 1044 (s), 957, 920, 904, 801, 771.

**2Man$^{OAc}$**. Dialkyne **1** (0.053 g, 0.22 mmol), $CuSO_4{\cdot}5H_2O$ (0.023 g, 0.09 mmol), sodium ascorbate (0.058 g, 0.29 mmol) and $NaN_3$ (0.026 g, 0.4 mmol) were added to a round-bottomed flask. A solution of 1-azido-3,6-dioxaoct-8-yl-2,3,4,6-tetra-o-acetyl-α-D-mannopyranoside (0.225 g, 0.45 mmol) in DMF-$H_2O$ (10 mL, 4:1 mixture) was added to the reaction vessel and the reaction stirred at room temperature under inert atmosphere for 18 h. The reaction mixture was extracted into DCM, washed with $H_2O$ (×4) and brine. The organic layers were dried over

$Na_2SO_4$, filtered and concentrated under reduced pressure. The product was purified by flash chromatography ($SiO_2$, EtOAc → $CH_3OH$/EtOAc). Eluent was concentrated under reduced pressure, dissolved in DCM and filtered before concentrated under reduced pressure again to yield **2Man$^{OAc}$** as a hygroscopic white solid (0.160 g, 0.136 mmol, 62%). **M.p.** 61-67 °C. **HRMS** (ESI+) (Q-TOF) (*m/z*): Calculated for $C_{53}H_{74}N_9O_{26}^+$ $[M+H]^+$ *m/z* = 1252.4745. Found *m/z* = 1252.4735; **$^1$H NMR** (500 MHz, $CDCl_3$): δ = 8.90 (br s, 2H, NH), 8.31 (d, 2H, *J* = 7.7 Hz, pyr CH), 8.00 (t, 1H, *J* = 7.7 Hz, pyr CH ), 7.78 (s, 2H, triazolyl CH), 5.37-5.22 (m, 6H, Man C$^2$H, C$^3$H and C$^4$H), 4.87 (d, 2H, *J* = 1.8 Hz, Man C$^1$H), 4.75 (d, 4H, *J* = 6.2 Hz, NHC*H*$_2$), 4.56-4.51 (m, 4H, $CH_2$), 4.27 (dd, 2H, *J* = 12.2, 5.0 Hz, Man C$^6$H*H*), 4.11 (dd, 2H, *J* = 12.2, 2.6 Hz, Man C$^6$*H*H), 4.05 (ddd, 2H, *J* = 9.7, 5.0, 2.6 Hz, Man C$^5$H), 3.91-3.85 (m, 4H, $CH_2$), 3.83-3.75 (m, 2H, CH*H*), 3.70-3.55 (m, 14H, 3 × $CH_2$, C*H*H), 2.15, 2.09, 2.03, 1.98 (4 × s, 6H, C(O)$CH_3$); **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ = 170.7, 170.1, 170.0, 169.7 (4 × C=O), 163.5 (triazolyl qt), 148.7 (pyr qt), 138.8 (pyr CH), 124.9 (pyr CH), 124.4 (triazolyl CH), 97.7 (Man C$^1$H), 70.6 ($CH_2$), 70.5 ($CH_2$), 70.0 ($CH_2$), 69.5 (Man CH), 69.4 ($CH_2$), 69.1 (Man CH), 68.4 (Man C$^5$H), 67.3 ($CH_2$), 66.1 (Man CH), 62.4 (Man C$^6$H$_2$), 50.3 ($CH_2$), 34.6 ($CH_2$), 20.9, 20.8, 20.71, 20.69 (4 × C(O)*C*H$_3$); **FT-IR (ATR, cm$^{-1}$):** 3337 (br), 2926, 2865, 1741 (s), 1671 (s), 1528, 1443, 1367, 1217, 1134, 1080, 1043, 977, 935, 846, 792.

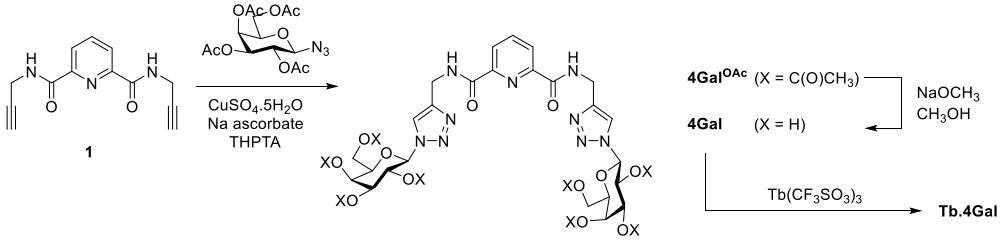
**General procedure for deprotection of ligands 2:**

To a solution of the relevant ligand (0.1 mmol) was added 0.5 M $NaOCH_3$ in $CH_3OH$ (3 mL) and stirred at room temperature. Reaction mixture was neutralised with DOWEX® 50X8 $H^+$ resin, filtered, and filtrate concentrated under reduced pressure to yield **3** as amber oils.

**3Gal.** Yield: 0.090 g, 0.098 mmol, 76%. **HRMS** (ESI+) (Q-TOF) (*m/z*): Calculated for $C_{37}H_{57}N_9O_{18}^+$ $[M+H]^+$ *m/z* = 916.3894. Found *m/z* = 916.3925; Calculated for $C_{37}H_{56}N_9O_{18}Na^+$ $[M+Na]^+$ *m/z* = 938.3714. Found *m/z* = 938.3744; **$^1$H NMR** (500 MHz, $CD_3OD$): δ = 8.31 (d, 2H, pyr CH), 8.22–8.12 (m, 1H, 4-pyr CH), 8.02 (br s, 2H, trizolyl CH), 4.76–4.68 (m, 4H, $CH_2$), 4.59–4.49 (m, 4H, $CH_2$), 4.20 (d, 2H, *J* = 7.5 Hz, Gal C$^1$H), 3.93-3.85 (m, 6H, C*H*H, $CH_2$), 3.81 (d, 2H, Gal CH), 3.77-3.67 (m, 4H, Gal C$^6$H$_2$), 3.64-3.52 (m, 14H, 3 × $CH_2$, CH*H*), 3.52-3.43 (m, 6H, 3 × Gal CH); **$^{13}$C NMR** (126 MHz, $CD_3OD$): δ = 164.4 (qt), 164.3 (qt), 148.6 (triazolyl CH), 139.2 (pyr CH), 124.6 (pyr CH), 103.6 (Gal C$^1$H), 75.3 (CH), 73.5 (Gal CH), 71.1 (Gal CH), 70.0 ($CH_2$), 69.95 ($CH_2$), 69.85 ($CH_2$), 68.9 (Gal CH), 68.1 (Gal $CH_2$), 61.2 (Gal $CH_2$), 60.8 ($CH_2$), 50.0 ($CH_2$), 34.4 ($CH_2$); **FT-IR (ATR, cm$^{-1}$)**: 3316 (s, br), 2876, 1662 (s), 1534 (s), 1446, 1351, 1304, 1239, 1059 (s), 922, 892, 847, 751.

**3Man.** Yield: 0.051 g, 0.055 mmol, 98%. **HRMS** (ESI+) (Q-TOF) (*m/z*): Calculated for $C_{37}H_{57}N_9O_{18}^+$ $[M+H]^+$ *m/z* = 916.3894. Found *m/z* = 916.3959; Calculated for $C_{37}H_{56}N_9O_{18}Na^+$ $[M+Na]^+$ *m/z* = 938.3714. Found *m/z* = 938.3807; **$^1$H NMR** (500 MHz, $CD_3OD$): δ = 8.31 (d, 2H, *J* = 7.6 Hz, pyr CH), 8.20-8.15 (llm, 1H, 4-pyr CH), 8.00 (s, 2H, triazolyl CH), 4.75 (d, 2H, *J* = 1.8 Hz, Man C$^1$H), 4.73 (br s, 4H, $CH_2$), 4.57 (app t, 4H, $CH_2$), 3.88 (app t, 4H, $CH_2$), 3.82 (dd, 2H, *J* = 11.7, 2.3 Hz, Man C$^6$*H*H), 3.78 (dd, 2H, *J* = 3.4, 1.8 Hz, Man C$^2$H), 3.76-3.72 (m, 2H, C*H*H), 3.72-3.65 (m, 4H, Man C$^6$H*H* and C$^3$H), 3.63-3.45 (m, 18H, Man C$^4$H, C$^5$H, CH*H*, 3 × $CH_2$); **$^{13}$C NMR** (126 MHz, $CD_3OD$): δ = 166.6 (qt), 165.9 (qt), 150.1 (qt), 140.7 (pyr CH), 126.1 (pyr CH), 125.1 (triazolyl CH), 101.7 (Man C$^1$H), 74.7 (Man CH), 72.6 (Man C$^3$H), 72.1 (Man C$^2$H), 71.6 ($CH_2$), 71.5 ($CH_2$), 71.4 ($CH_2$), 70.4 ($CH_2$), 68.7 (Man CH), 67.7 ($CH_2$), 6ii3.0 (Man C$^6$H$_2$), 51.5 ($CH_2$), 35.8 ($CH_2$); **FT-IR (ATR, cm$^{-1}$):** 3313, 2923, 1942, 1663, 1591, 1536, 1446, 1350, 1233, 1131, 1056, 1028, 974, 916, 882, 845, 808, 748, 674.

**4Gal$^{OAc}$**. Dialkyne **1** (0.200 g, 0.83 mmol) and 2,3,4,6-*O*-acetyl-β-D-galactopyranose azide (0.620 g, 1.6 mmol) were dissolved in a small amount of THF. Separately, $CuSO_4{\cdot}5H_2O$ (0.042 g, 0.16 mmol), tris-hydroxypropyltriazolylmethylamine (THPTA) (0.040 g, 0.09 mmol) and sodium ascorbate (0.068 g, 0.32 mmol) were dissolved in an equivalent amount of water. The solutions were combined in a microwave reactor tube and heated at 80 ˚C for 45 min under microwave irradiation. The reaction mixture was left to cool to room temperature and subsequently ~5 mL of ethylyenediaminetetraacetic acid (EDTA) solution were added. The mixture was then extracted with EtOAc (×2), the organic layers combined and dried over $MgSO_4$. The solvent was removed *in vacuo* to yield a crude pale yellow solid. This was purified by column chromatography as follows: unreacted dialkyne starting material was eluted with 100% EtOAc, the solvent system was then changed to 2:1 EtOAc:acetone until the product was fully eluted. Fractions were combined and solvent removed under reduced pressure to yield a colourless residue which upon the addition of >5 mL of ACN and its subsequent removal yielded a white solid, **4Gal$^{OAc}$** (0.563 g, 0.57 mmol, 68.6%). **$^1$H NMR** (500 MHz, DMSO-$d_6$) δ 9.92 (t, 2H, *J* = 6.3 Hz, NH), 8.26 – 8.14 (m, 5H, Pyr and Tz H), 6.22 (d, 2H, *J* = 9.2 Hz, Gal C$^1$H), 5.61 (t, 2H, *J* = 9.6 Hz, Gal C$^2$H), 5.46 – 5.37 (m, 4H, $CH_2$), 4.67 – 4.60 (m, 4H, $CH_2$), 4.55 (dd, 2H, *J* = 7.2, 5.3 Hz, Gal CH), 4.11 (dd, 2H, *J* = 11.6, 5.0 Hz, Gal CH), 3.99 (dd, 2H, *J* = 11.6, 7.3 Hz, Gal CH), 2.17 (s, 6H, C(O)$CH_3$), 1.97 (s, 6H, C(O)$CH_3$), 1.93 (s, 6H, C(O)$CH_3$), 1.79 (s, 6H, C(O)$CH_3$). **$^{13}$C NMR** (126 MHz, dmso) δ 170.44 (OAc C=O), 170.36 (OAc C=O), 169.89 (OAc C=O), 168.94 (OAc C=O), 163.75 (Amide C=O), 148.93 (Tz C-1), 145.86 (Pyr C-3,5), 140.02 (Pyr C-1), 124.96 (Pyr C-2,6), 122.94 (Tz C-2), 84.65 (Gal C-1), 73.42 (Gal C-3), 70.92 (Gal C-5), 68.07 (Gal C-2), 67.76 (Gal C-4), 62.01 (Gal C-6), 40.48-39.48 (DMSO), 34.82 ($CH_2$), 20.92 (OAc $CH_3$), 20.86 (OAc $CH_3$), 20.74 (OAc $CH_3$), 20.43 (OAc $CH_3$). **FT-IR (ATR, cm$^{-1}$):** 3330, 2988, 1743, 1660, 1532, 1446, 1368, 1211, 1096, 1045, 984, 958, 945, 919, 844, 818, 751, 676.




Scheme S1. Synthesis of spacerless analogue of **3**, ligand **4Gal** and its Tb(III) complex.

**4Gal.** Protected galactoside **4Gal$^{OAc}$** (0.080 g, 0.081 mmol) was deacetylated following the standard procedure described above. Crude **4Gal** was purified by precipitation from EtOH as a white solid (0.053g, 0.081 mmol, quantitative yield). **HRMS** (ESI+) (Q-TOF) (m/z): Calculated for $C_{25}H_{33}N_9O_{12}^+$ 652.2326, found 652.2320. **$^1$H NMR** (500MHz, $D_2O$): δ 8.23 (s, 2H, Triazole), 8.16 – 8.11 (d, 2H, Pyr H-2,6), 8.10 – 8.03 (m, 1H, Pyr H-1), 5.69 (d, 2H, *J* = 9.2 Hz, Gal C$^1$H), 4.75–4.65 (m, 4H, $CH_2$), 4.22 (t, 2H, *J* = 9.5 Hz, Gal C$^2$H), 4.10 (d, 2H, *J* = 3.1 Hz, Gal C$^4$H), 4.02 (t, 2H, *J* = 6.0 Hz, Gal C$^5$H), 3.89 (dd, 2H, *J* = 9.8, 3.3 Hz, Gal C$^3$H), 3.80 (app d, 4H, *J* = 6.1 Hz, Gal C$^6H_2$) ppm. **$^{13}$C NMR** (126 MHz, $D_2O$): δ 165.59 (Amide C), 147.60 (Triazole C-1), 144.83 (Pyr C-5,3), 139.81 (Pyr C-1), 125.02 (Pyr C-2,6), 122.90 (Triazole C-2), 87.97 (Gal C-1), 78.20 (Gal C-5), 72.85 (Gal C-3), 69.67 (Gal C-2), 68.50 (Gal C-4), 60.79 (Gal C-6), 34.36 ($CH_2$) ppm. **FT-IR (ATR, cm$^{-1}$)** : 3301, 2926, 1658, 1536, 1447, 1237, 1091, 1049, 884, 819, 747, 700, 643, 606.

**1:1 Tb(III) self-assembly complexes of 3 in solution:**

**Tb.3Gal.** $^1$H NMR (400 MHz, $D_2O$): δ = 3.15-3.66 (br m), 3.67-3.96 (br m), 4.11 (br app s), 4.46 (br app s, [overlaps with $H_2O$ peak]), 7.70-7.96 (br m), 7.98-8.25 (br m).

**Tb.3Man.** $^1$H NMR (400 MHz, $D_2O$): δ =3.09-3.88 (br m), 4.17 (br app s), 4.45 (br app s [overlaps with $H_2O$ peak]), 4.82 (br app s [overlaps with $H_2O$ peak]), 7.76-7.94 (br m), 7.94-8.22 (br m).

**Tb.4Gal.** $^1$H NMR (400 MHz, $D_2O$): δ = 3.58-4.33 (br m), 4.64 (br app s), 7.70-7.96 (br m), 7.86-8.45 (br m).

## Photophysical experiments

All photophysical experiments were performed on an Agilent Cary Eclipse Fluorescence Spectrophotometer equipped with a single cell holder, using quartz cuvettes of 3500 and 700 μL capacity. UV/Vis spectra were obtained on Agilent Cary 5000 UV-Vis-NIR and Varian Cary 100 Scan UV-Vis spectrophotometers, quantum yield determinations were performed using a Varian Cary 50 Scan UV-Vis instrument.

**Table S1.** Photophysical properties of Tb(iii) complexes of ligands **2–4**: luminescence lifetimes, hydration states and total quantum yields in aqueous solution.

| | $\tau_{H2O}$ (ms) | $\tau_{D2O}$ (ms) | *q* (±0.5) [a] | Φ (%) [b] |
|---|---|---|---|---|
| **Tb.3Gal** | 0.37 ±0.01 | 1.65 ±0.03 | 10.1 | 7.5 |
| **Tb.3Man** | 0.44±0.01 | 2.54±0.04 | 9.2 | 13.0 |
| **Tb.2Gal$^{OAc}$** | 0.38±0.01 | 0.73±0.01 | 5.9 | 10.5 |
| **Tb.2Man$^{OAc}$** | 0.43±0.01 | 0.89±0.01 | 5.8 | 8.9 |
| **Tb.4Gal** | 0.48±0.01 | 1.62±0.02 | 6.9 | 2.5 |

[a] Apparent hydration states of complexes were determined by measuring lifetimes of complexes assembled in both $H_2O$ and $D_2O$ solution, using the modified Horrocks equation: $q = A\{(\tau_{H2O}^{-1} - \tau_{D2O}^{-1}) - B - Cx\}$. For Tb(III) complexes, *A* = 5.0, *B* = 0.06 and *C* = 0.[3]

[b] Luminescent quantum yields were determined by a relative method, in buffer solution (10 mM Tris, pH 7.2, 150 mM NaCl, 1 mM $CaCl_2$), using $Cs_3$[Tb·(**dpa**)$_3$] as a standard. (**dpa** = 2,6-dipicolinate). The following equation was used to compare absorption and emission spectra of the standard and the new complexes, where refractive index, $n = n_{ref} = 1.35$, $A_{ref} = 0.645$ and $\Phi_{ref} = 22\pm2.5$ % for the reference complex.[4]

$$\Phi = \frac{n^2}{n_{ref}^2} \times \frac{A_{ref}}{A} \times \frac{I}{I_{ref}} \times \Phi_{ref}$$

## Lifetimes of Complex Tb.3Gal in presence of LecA lectin

**Table S2.**Phosphorescence lifetimes, τ, for **Tb.3Gal** in buffer solution (10 mM Tris, pH 7.2, 150 mM NaCl, 1 mM $CaCl_2$), in the absence and in the presence of 1 equivalent of LecA, and in the presence of 1 equivalent of denatured LecA (dLecA).

| *Name* | *τ (ms)* |
|---|---|
| **Tb.3Gal** | 0.24 ± 0.02 |
| **Tb.3Gal** + 1 equiv. of LecA | 0.28 ± 0.01 |
| **Tb.3Gal** + 1 equiv. of dLecA | 0.27 ± 0.03 |

## Self-Assembly Titration of 3Gal

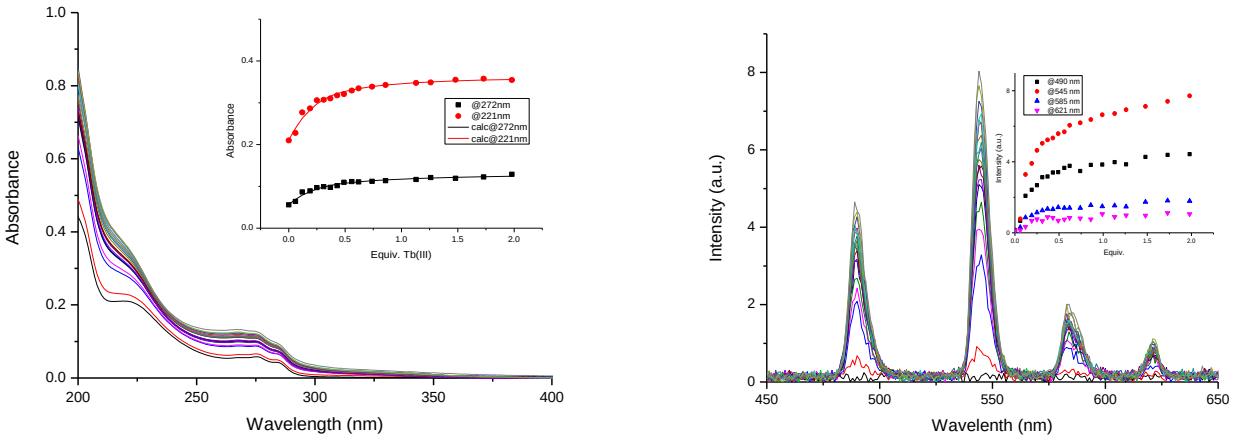



**Figure S1.** Self-assembly titration plots of spectral changes to **3Gal** (concentration 1 × $10^{-5}$ M) upon addition of aliquots of $Tb(CF_3SO_3)_3$ in distilled water. (*left*) UV-Vis spectral changes; inset: experimental binding isotherms (dots) and calculated isotherms (solid lines) at two wavelengths; (*right*) Changes in Tb(III) luminescence; inset: experimental binding isotherms at various wavelengths.

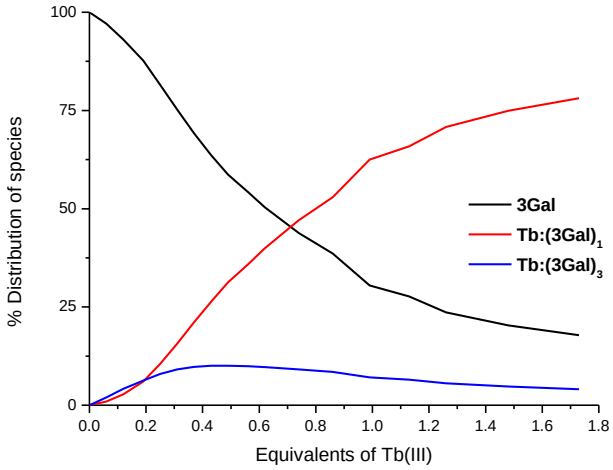



**Figure S2.** Calculated speciation distribution diagram for the UV-Vis absorption titration data in Figure S1, as a function of [Tb(III)]; the various traces show the percentage formation of the ligand (black), 1:3 stoichiometry (blue), and 1:1 stoichiometry (red), which are calculated at various concentrations, when these data were fit using ReactLab Equilibria (Jplus Consulting Pty Ltd).

## Method for the determination of sensing behaviour

Quartz cuvettes (3500 µL capacity) were thoroughly cleaned by immersion in 10% aqueous Nitric Acid solution, followed by rinsing ×6 with running water and ×6 with distilled water; then left to dry completely and the outer surface wiped with Kimtech precision wipes. Buffer solution (10 mM Tris pH 7.2, 150 mM NaCl, 1 mM $CaCl_2$) was added to two cuvettes per replicate, where cuvette A (*CuvA*) served as the control and cuvette B (*CuvB*) as the experiment. Spectra of blank cuvettes were taken to ascertain the absence of Tb(III) or other phosphorescent entities using the standardised method (excitation at 236 nm, emission range 450-750 nm, high voltage, wide slits). To *CuvA* was added the appropriate amount of stock solution of Tb complex (in distilled water) to achieve a working concentration *ca.* 1.3 × $10^{-6}$ M, and the spectrum of this was measured as the reference for the emission in the absence of any protein. To *CuvB* was added the appropriate amount of protein stock solution corresponding to 1 molar equivalent of each protein monomer with respect to the complex. Emission spectra of the lectin solution confirmed no intrinsic phosphorescence from the lectin, after this the same amount of Tb(III) complex solution was added as had been added to the control and the spectrum taken again. All experiments were carried out in triplicate and the raw spectra are reported below. After use the solutions were disposed of as appropriate and the cuvettes cleaned as described above, before the next use.

## Luminescence sensing experiments raw data

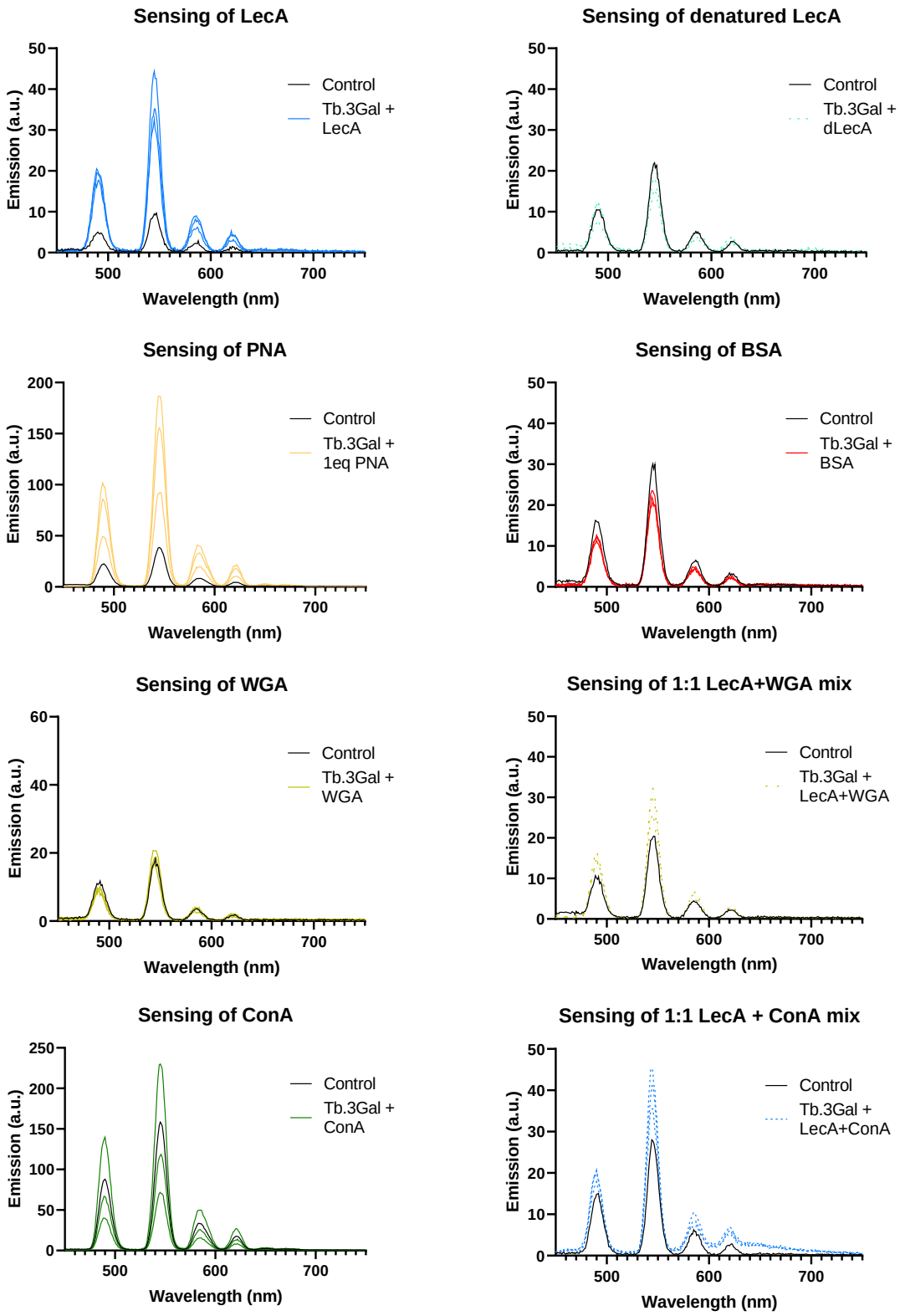



**Figure S3.** Spectra of changes in Tb(III)-centred emission of sensors **Tb.3Gal** in the presence of 1 equivalent of various proteins (1 protein monomer/carbohydrate binding domain per metal ion). All measurements were carried out in triplicate and emission changes are compared to a control. Complexes, at a concentration of *ca.* $1.3 \times 10^{-6}$ M, were excited at 236 nm.

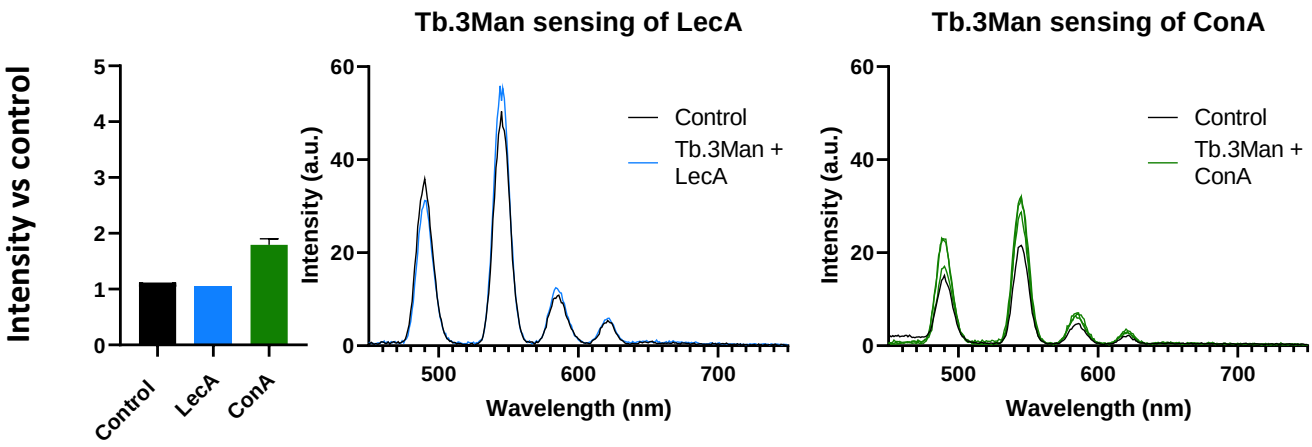



**Figure S4.** Spectra of changes in Tb(III)-centred emission of complex **Tb.3Man** in the presence of 1 equivalent of LecA and ConA. Measurements were carried out in triplicate for the ConA titration, and once for LecA. Emission changes are compared to a control. Complexes, at a concentration of *ca.* $1.3 \times 10^{-6}$ M, were excited at 236 nm.

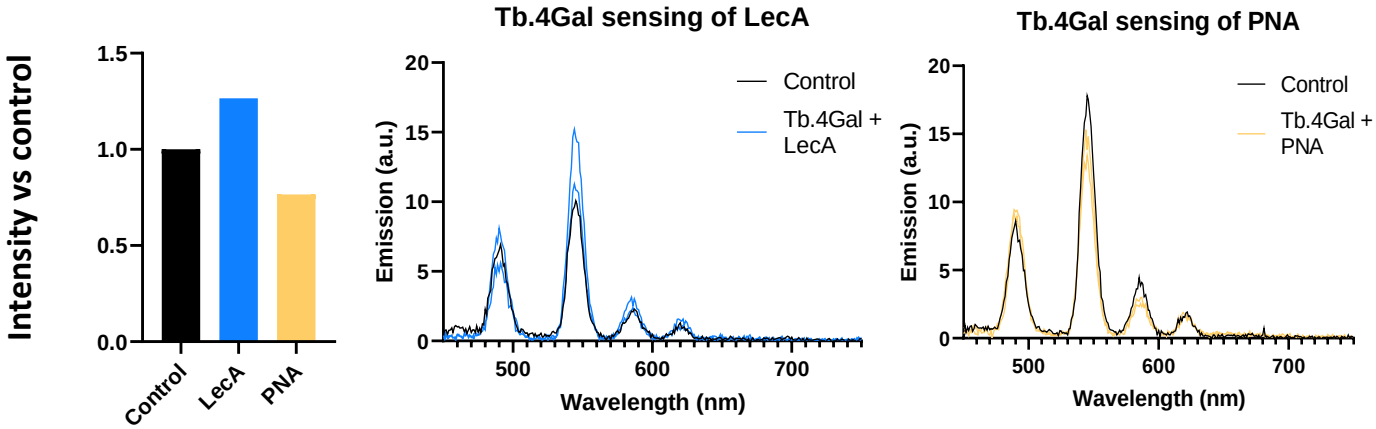



**Figure S5.** Spectra of changes in Tb(III)-centred emission of linkerless **Tb.4Gal** in the presence of 1 equivalent of LecA and PNA. All measurements were carried out in duplicate and emission changes are compared to a control. Complexes, at a concentration of *ca.* $1.3 \times 10^{-6}$ M, were excited at 236 nm.

## UV-Vis absorbance spectra

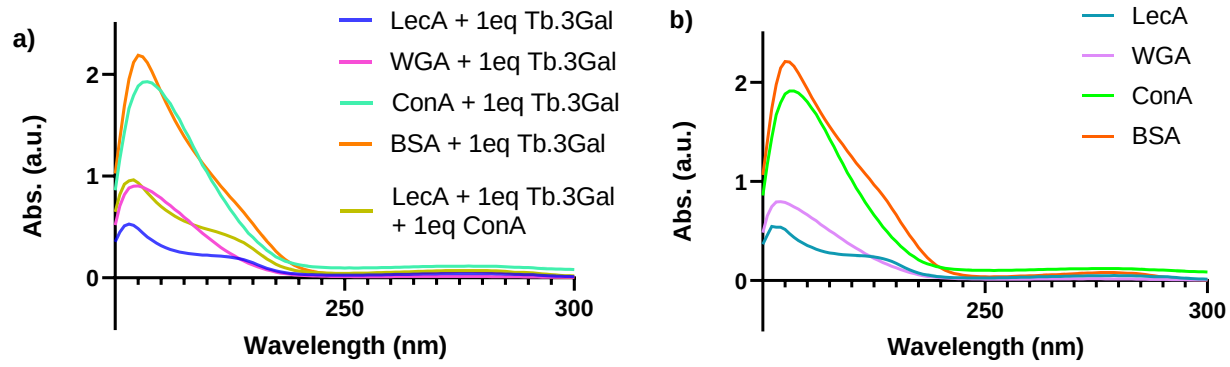



**Figure S6. (a)** UV-Vis spectra of **Tb.3Gal** in the presence of various proteins and **(b)** the corresponding protein absorption spectrum at the same concentration.

## NMR Spectra

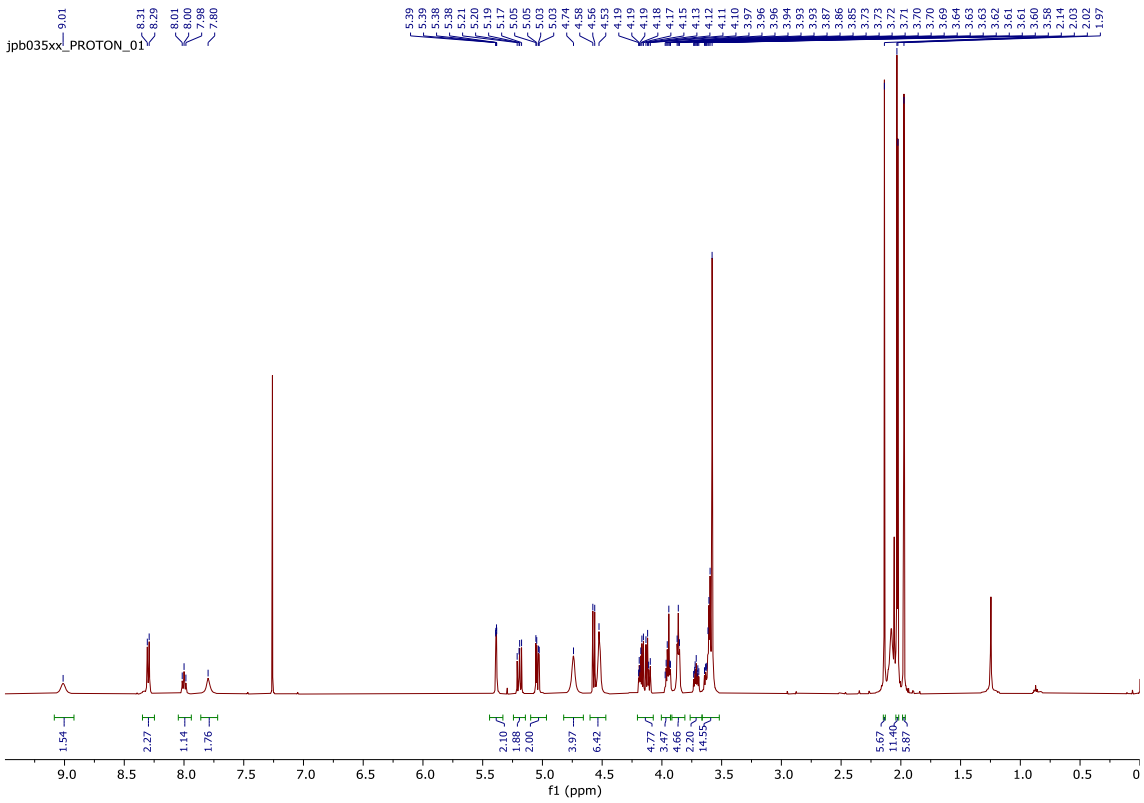



**Figure S7.** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$) of **2Gal$^{OAc}$**.

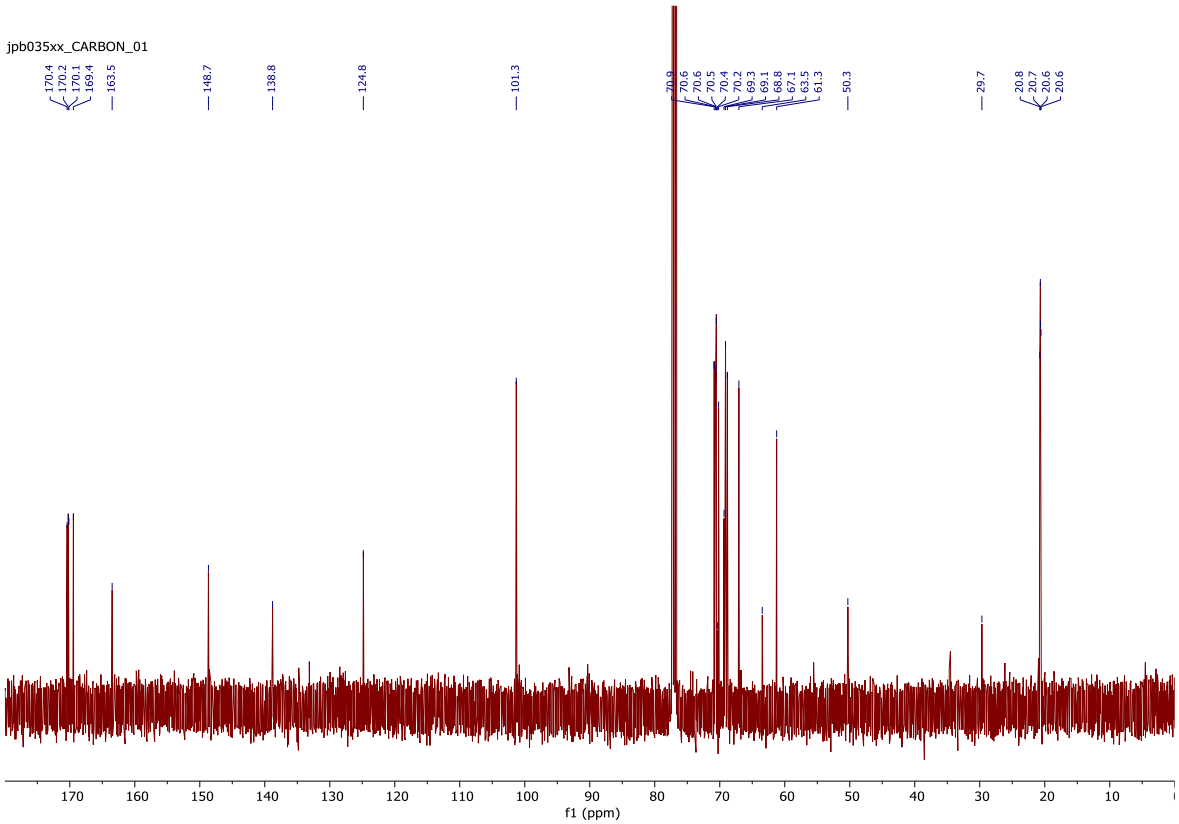



**Figure S7.** $^{13}$C NMR spectrum (126 MHz, $CDCl_3$) of **2Gal$^{OAc}$**.

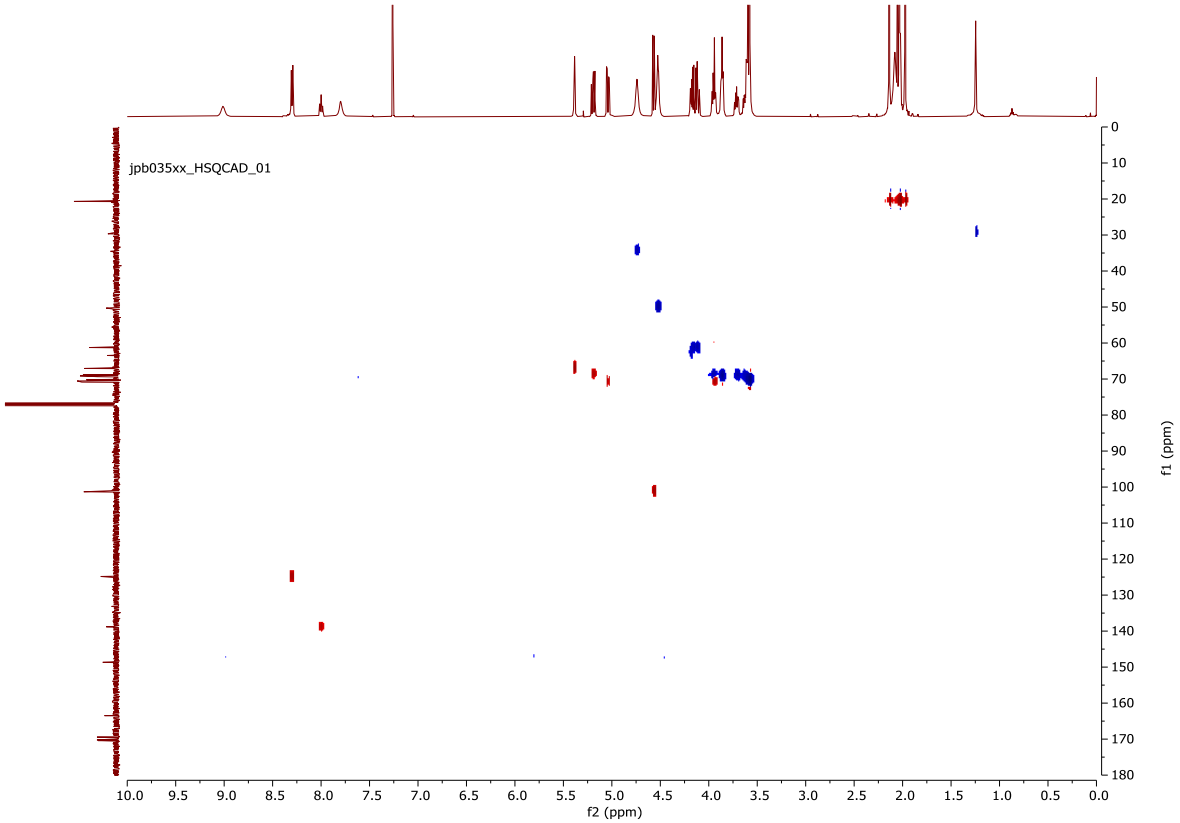



**Figure S8.** HSQC of **2Gal$^{OAc}$**.

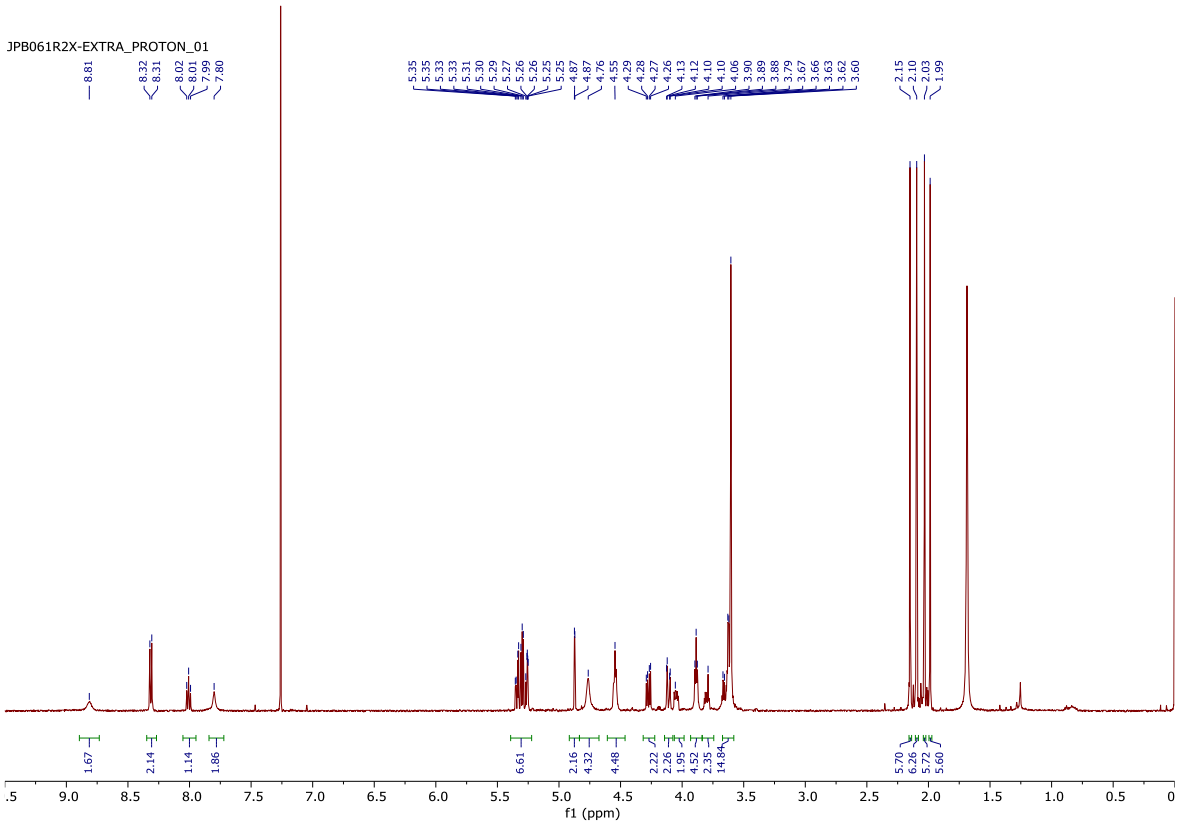



**Figure S9.** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$) of **2Man$^{OAc}$**.

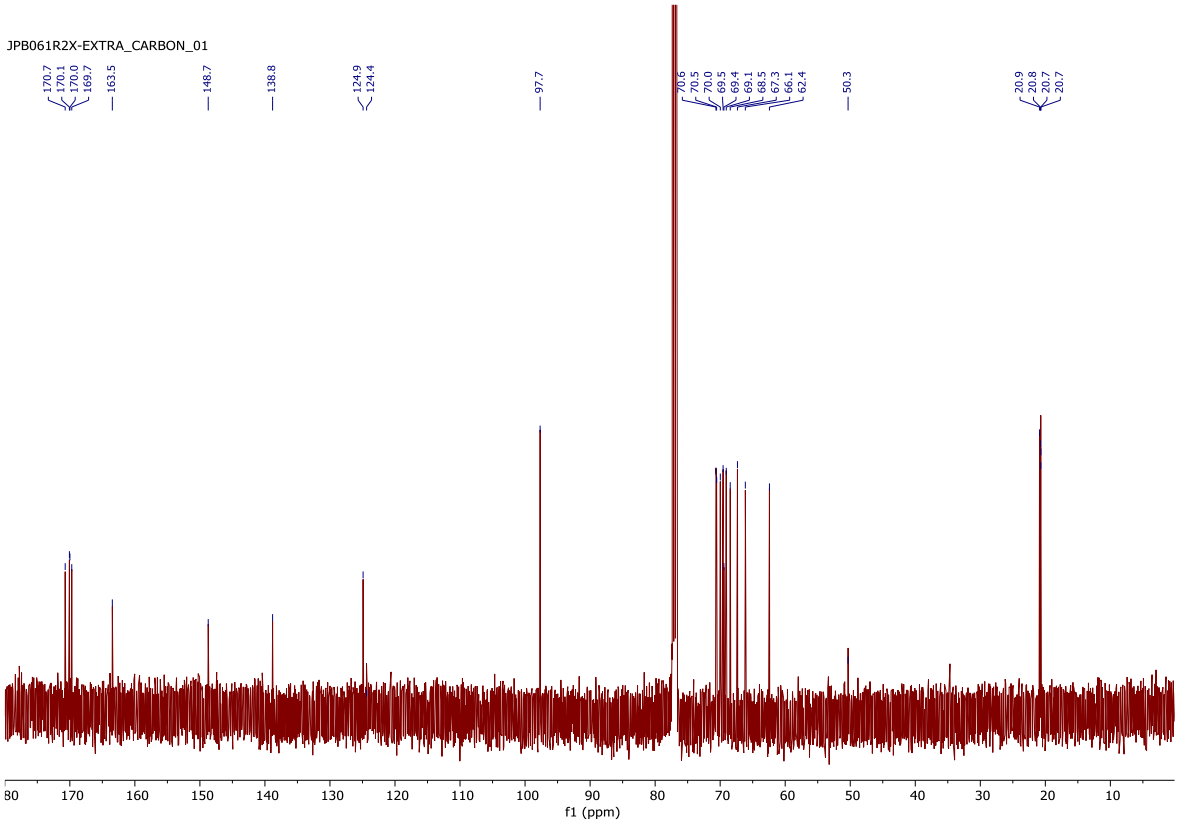



**Figure S10.** $^{13}C$ NMR spectrum (126 MHz, $CDCl_3$) of **2Man$^{OAc}$**.

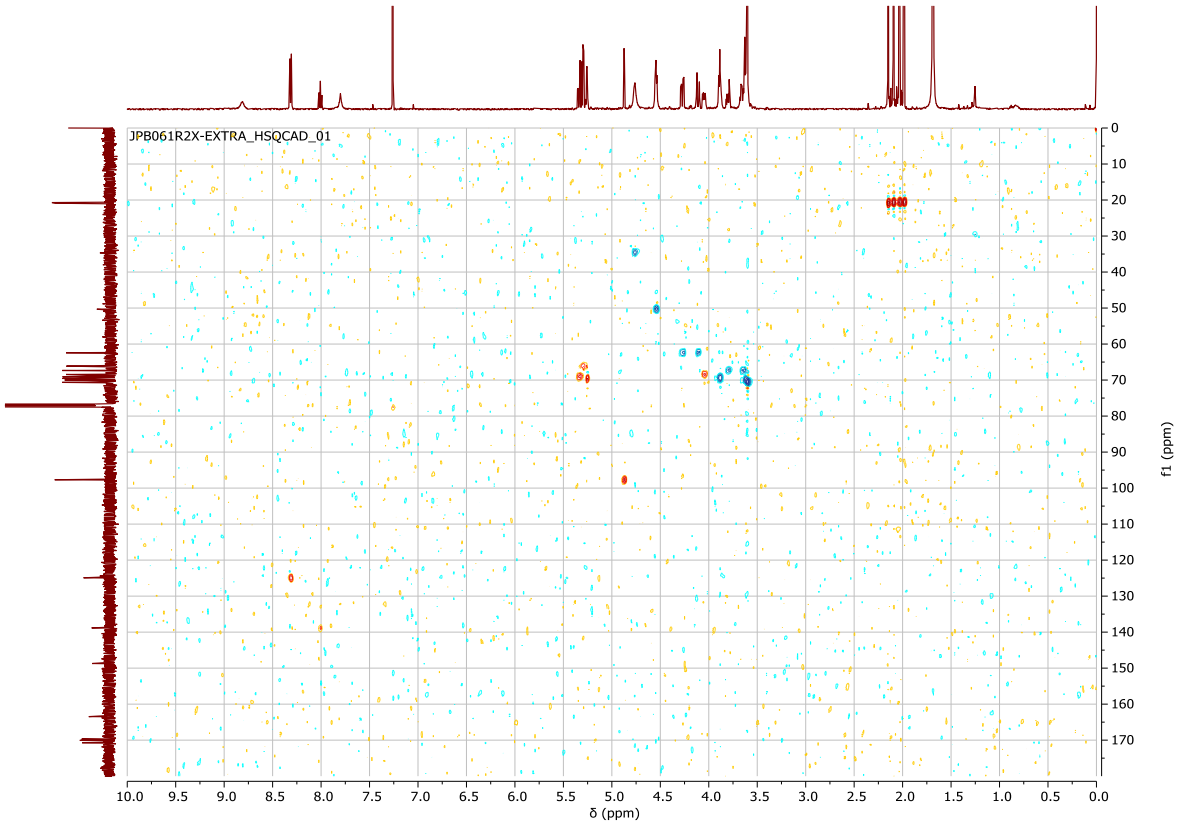



**Figure S11.** HSQC of **3Gal**.

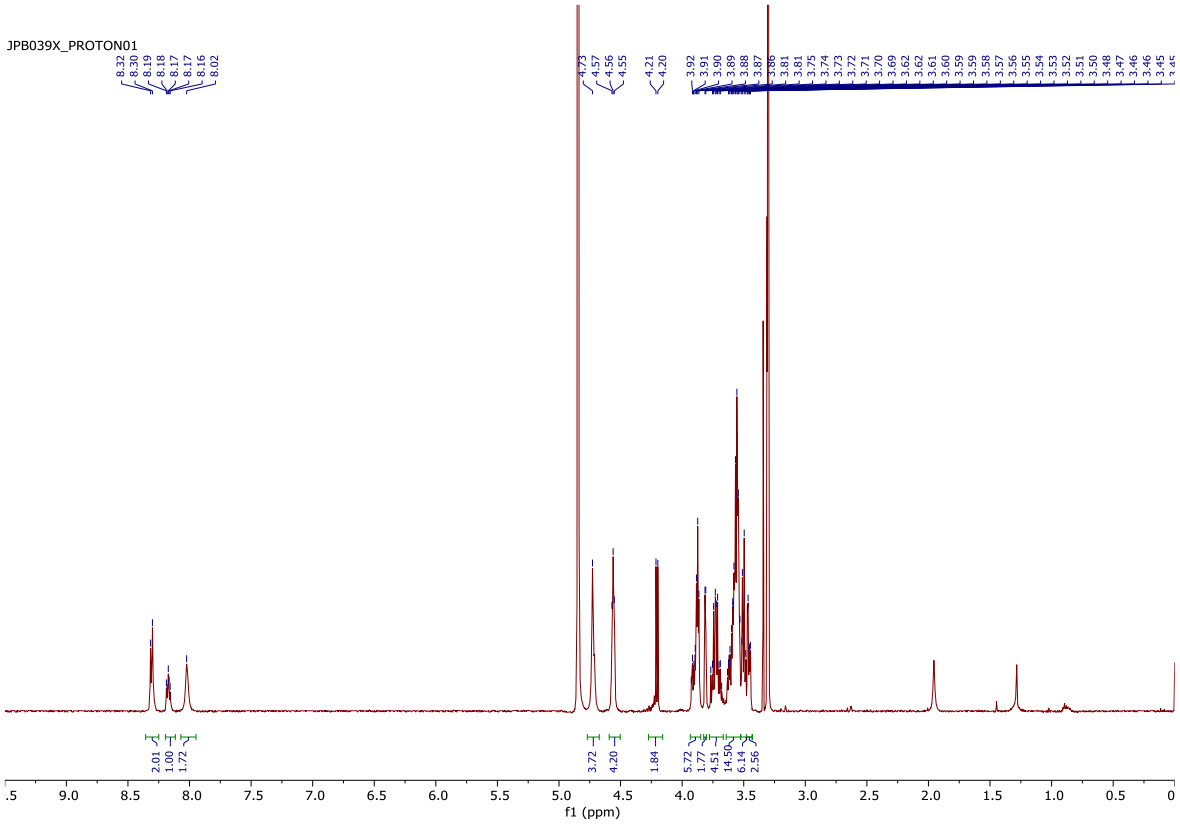



**Figure S12.** $^{1}$H NMR spectrum (500 MHz, $CD_3OD$) of **3Gal**.

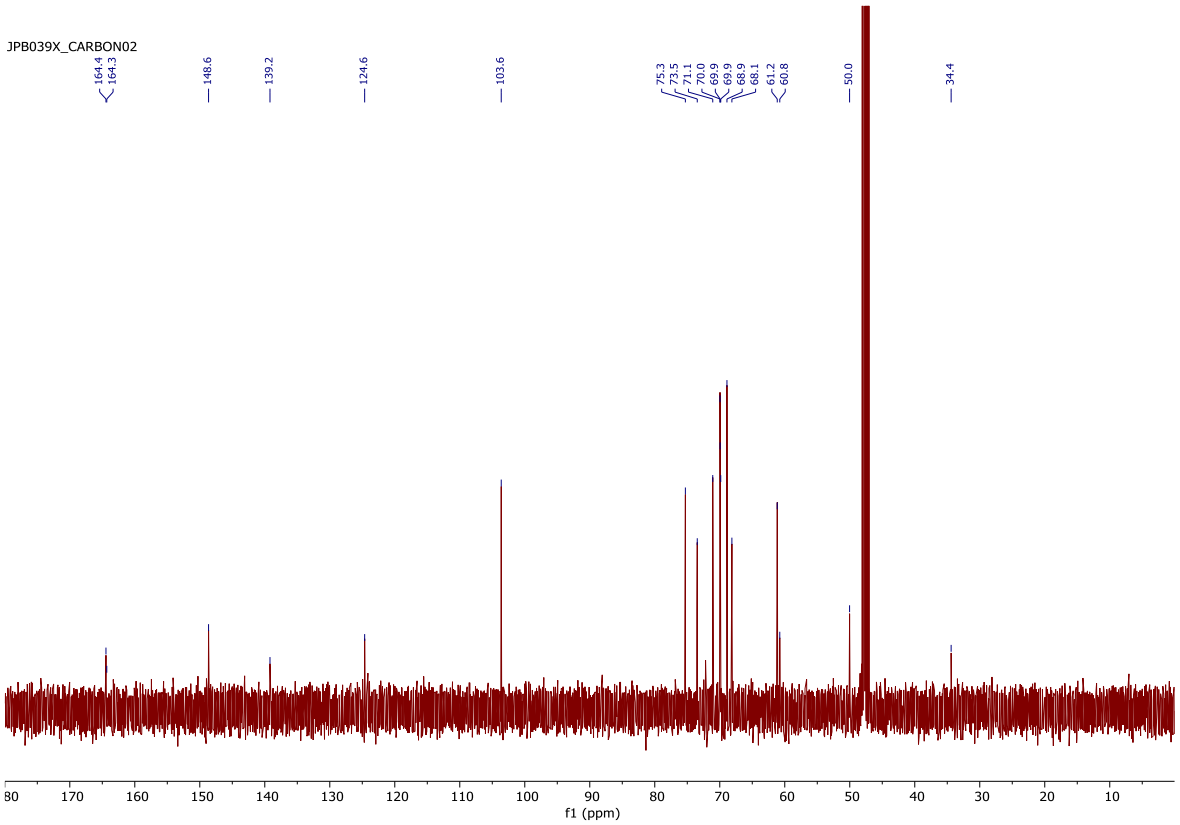



**Figure S13.** $^{13}$C NMR spectrum (126 MHz, $CD_3OD$) of **3Gal**.

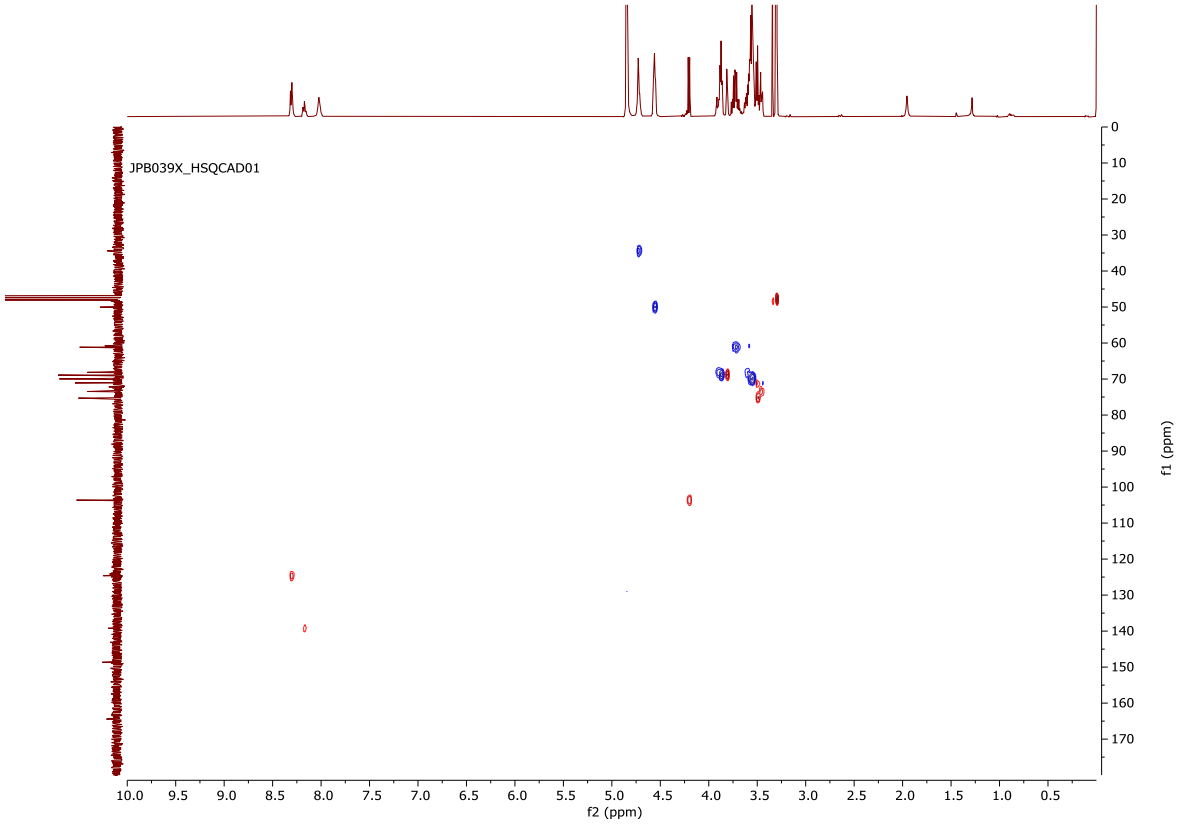



**Figure S14.** HSQC of **3Gal**.

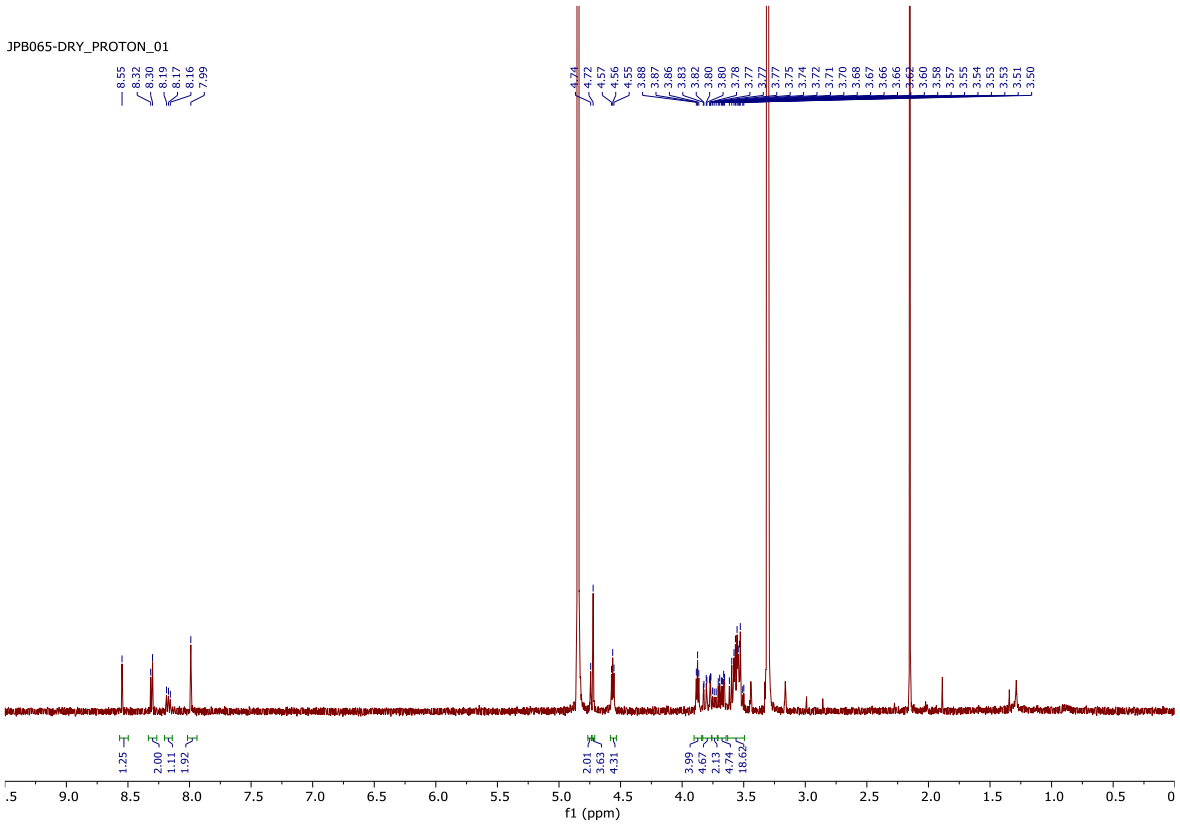



**Figure S15.** $^{1}$H NMR spectrum (500 MHz, $CD_3OD$) of **3Man**.

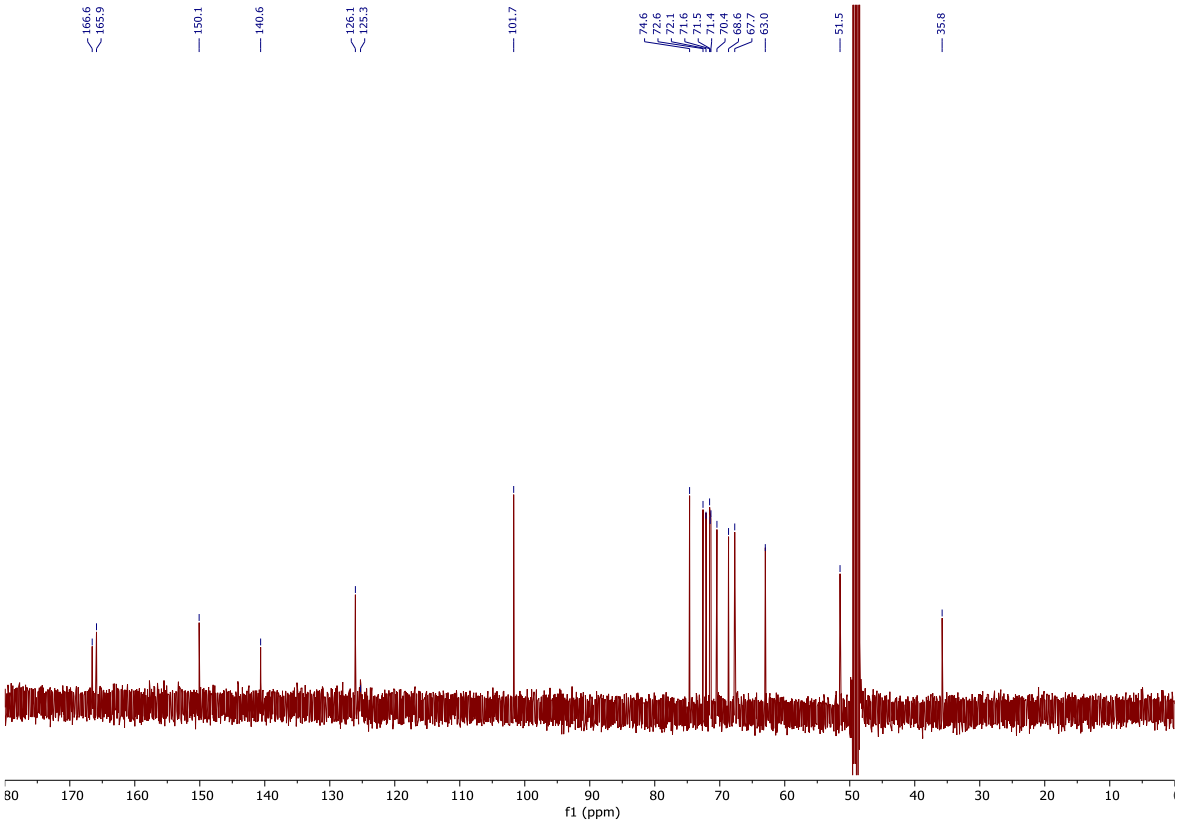



**Figure S15.** $^{13}C$ NMR spectrum (126 MHz, $CD_3OD$) of **3Man**.

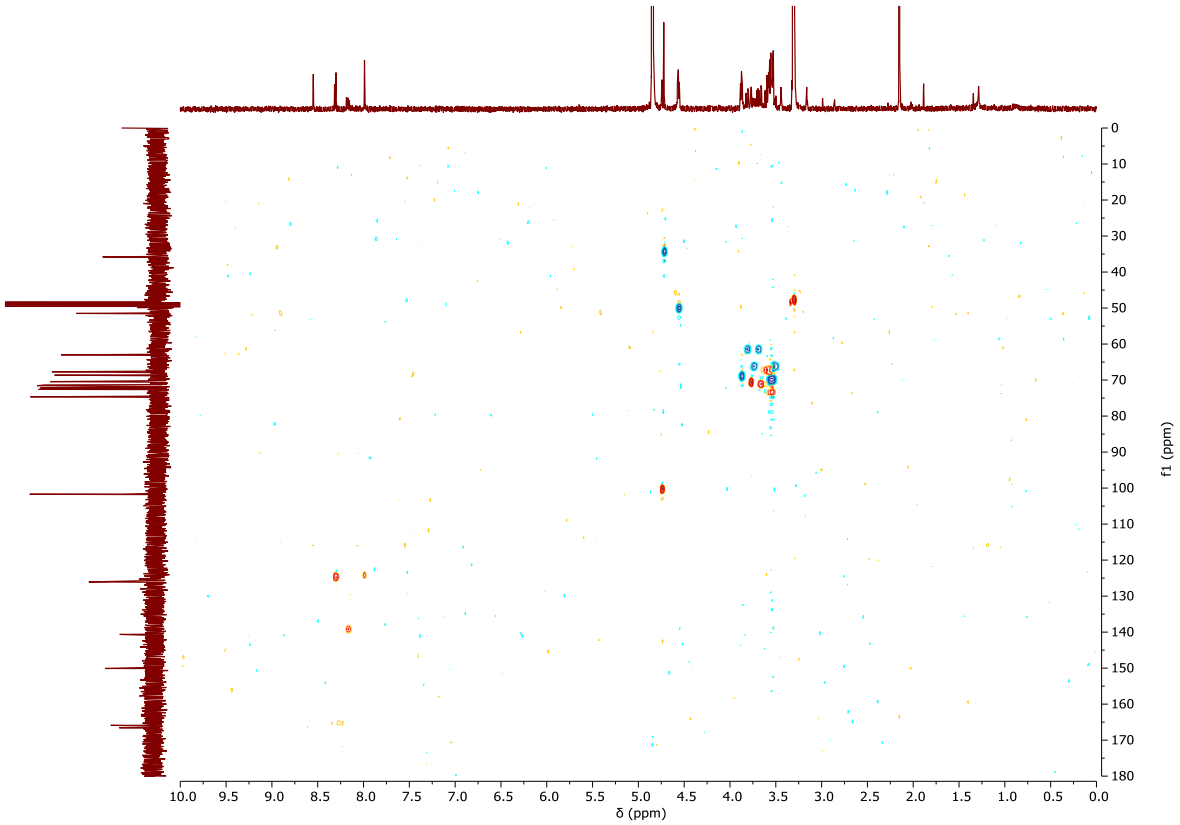



**Figure S16.** HSQC of **3Man**.

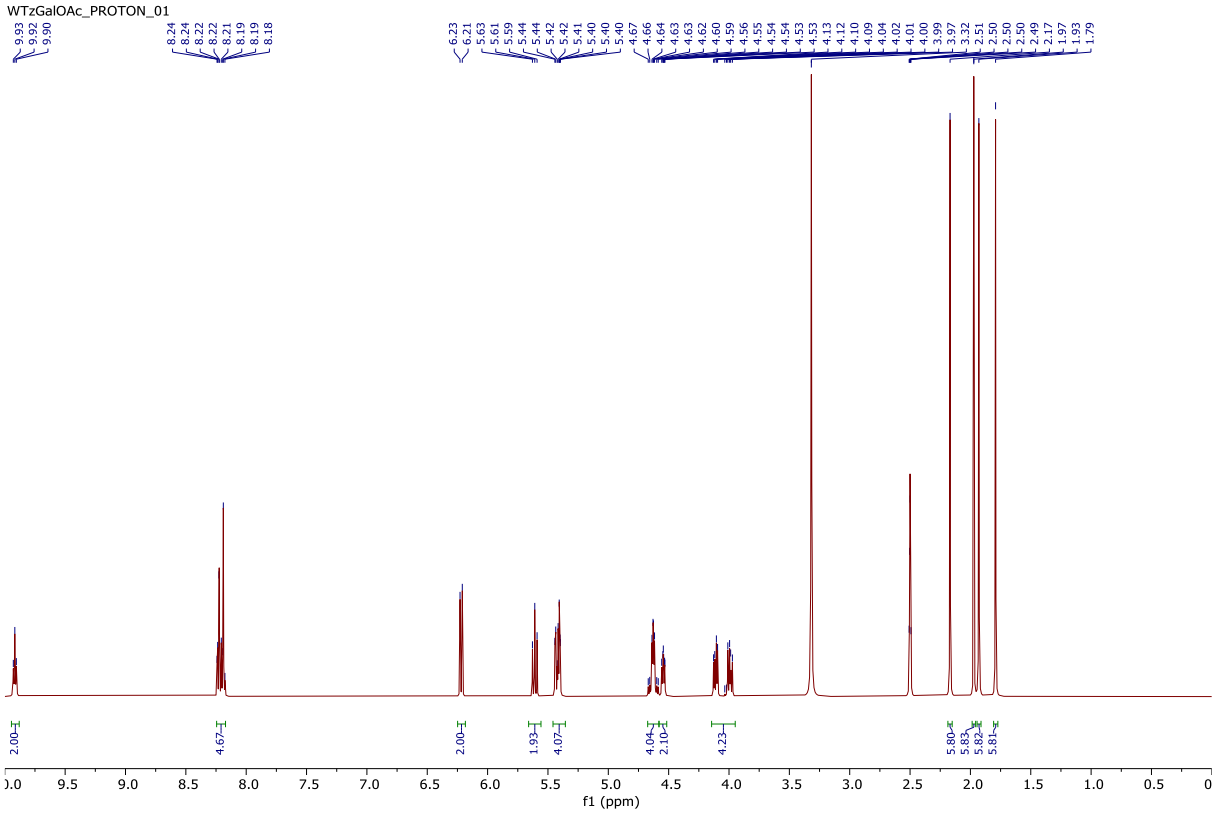



**Figure S17.** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$) of **4Gal$^{OAc}$**.

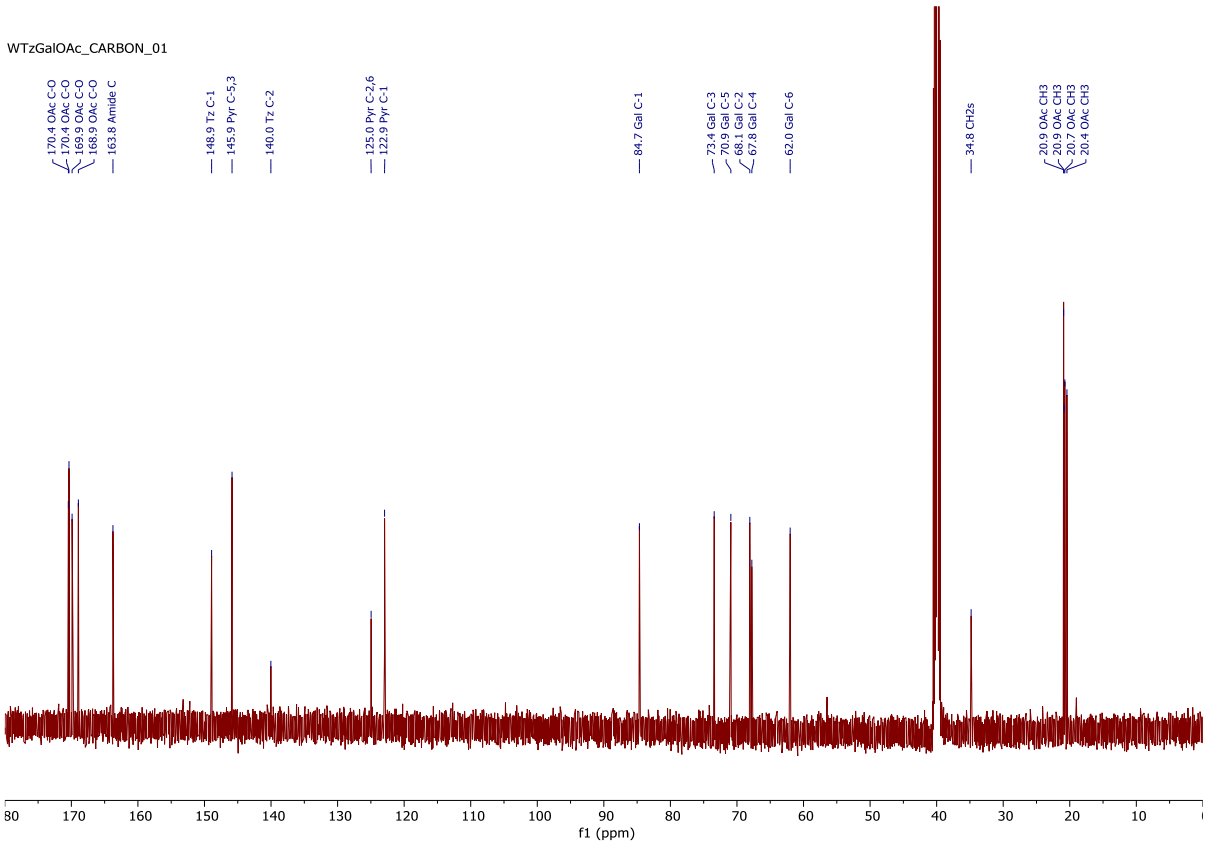



**Figure S18.** $^{13}$C NMR spectrum (126 MHz, $CDCl_3$) of **4Gal$^{OAc}$**.

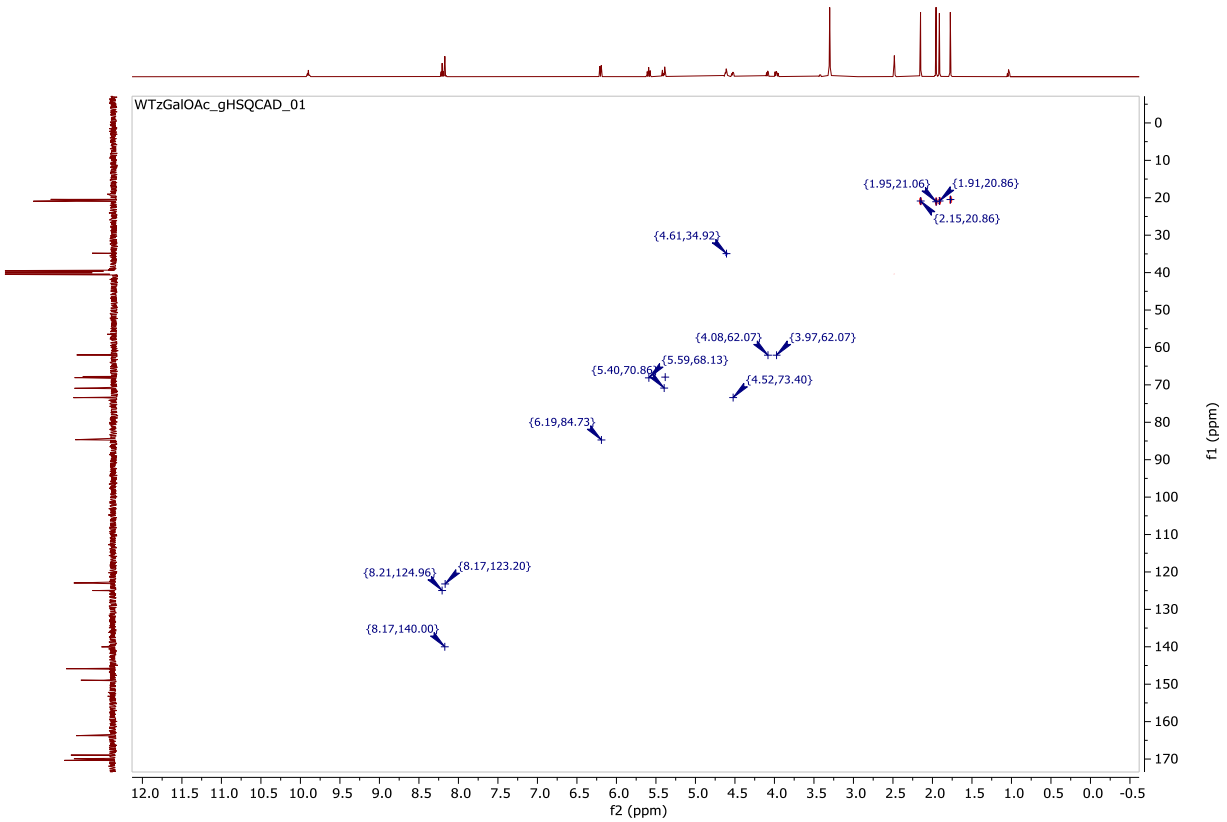



**Figure S19.** HSQC of **4Gal$^{OAc}$**.

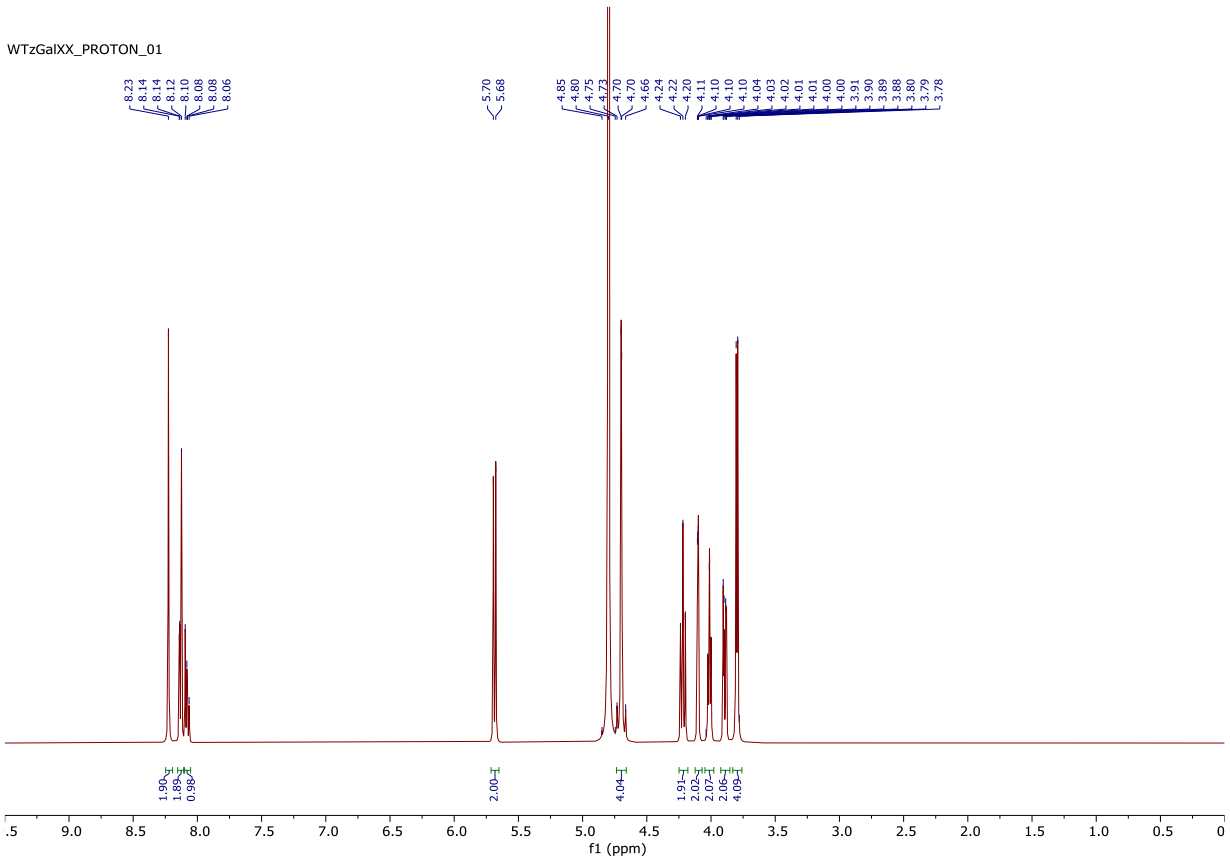



**Figure S20.** $^{1}$H NMR spectrum (500 MHz, $D_2O$) of deprotected **4Gal**.

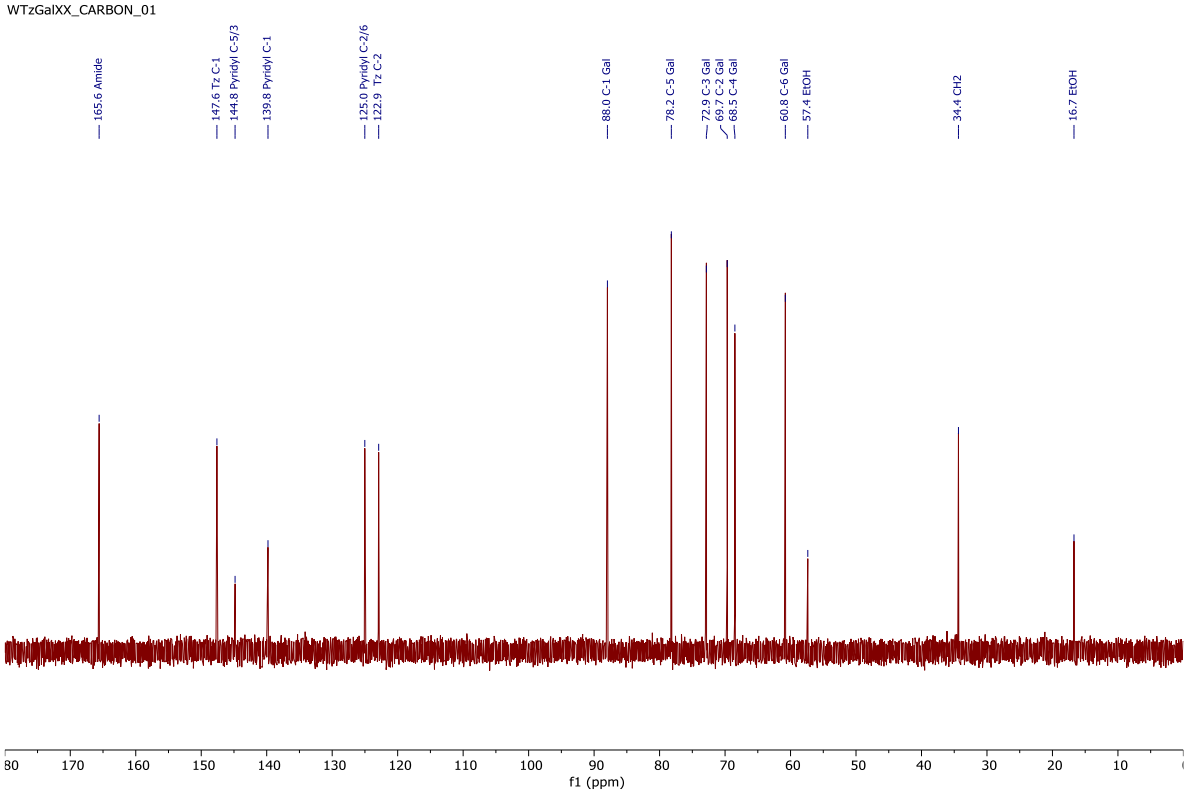



**Figure S21.** $^{13}C$ NMR spectrum (126 MHz, $D_2O$) of deprotected **4Gal**.

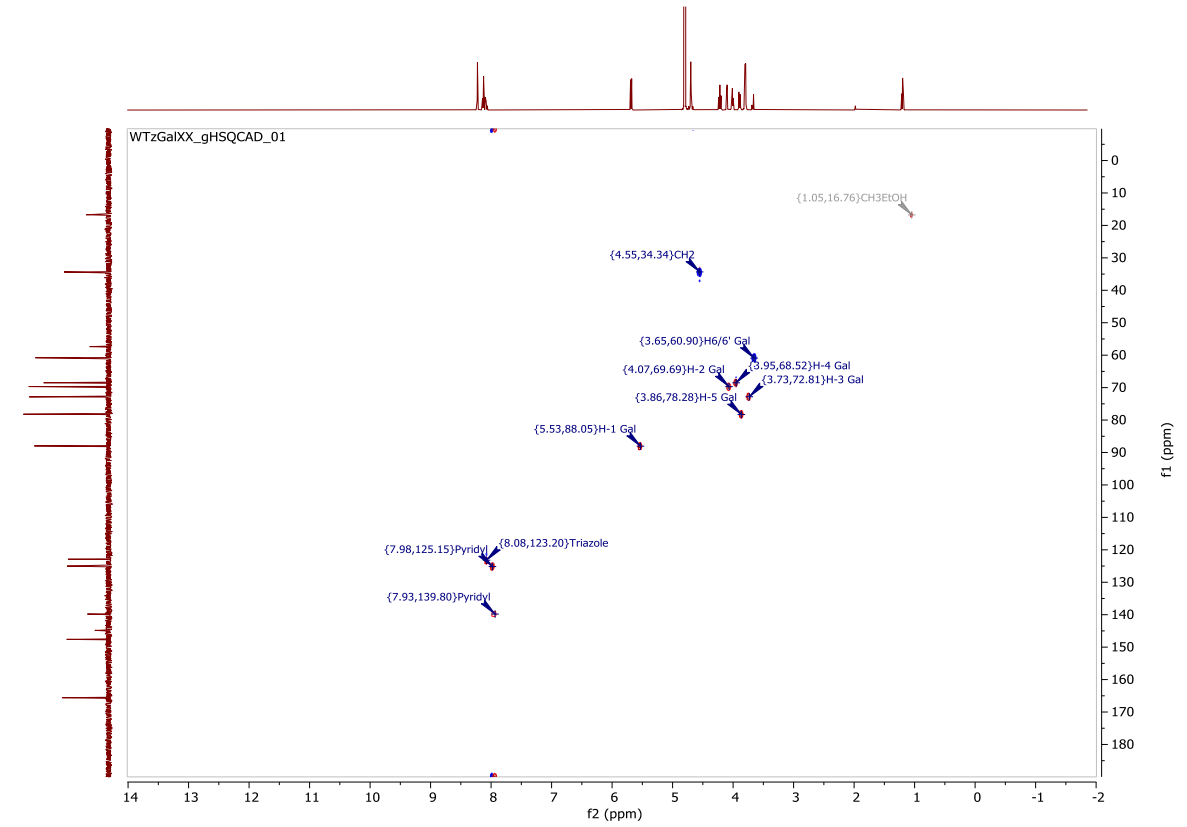



**Figure S22.** HSQC of **4Gal**.

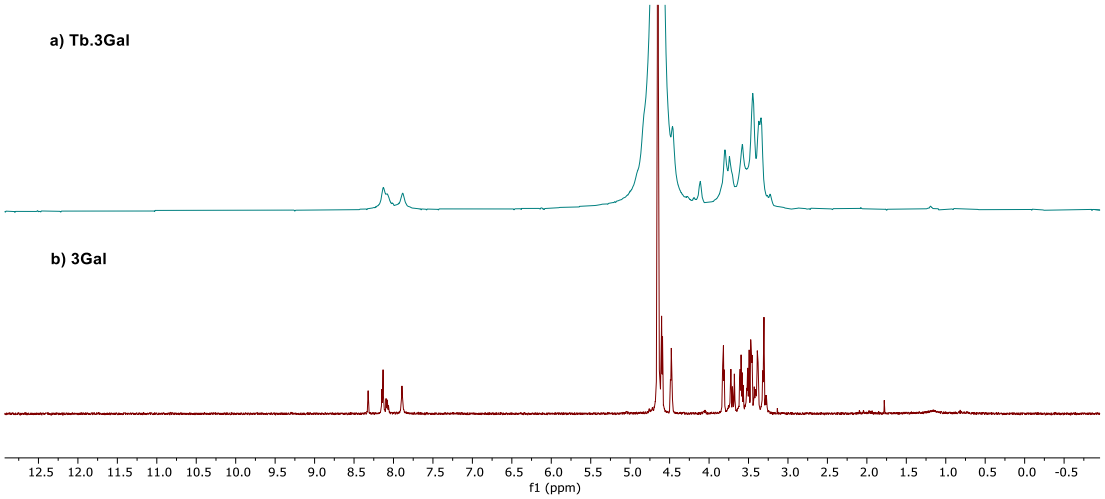



**Figure S23.** Comparison of $^{1}H$ NMR of Tb(III) complex **Tb.3Gal** and the ligand **3Gal** ($D_2O$).

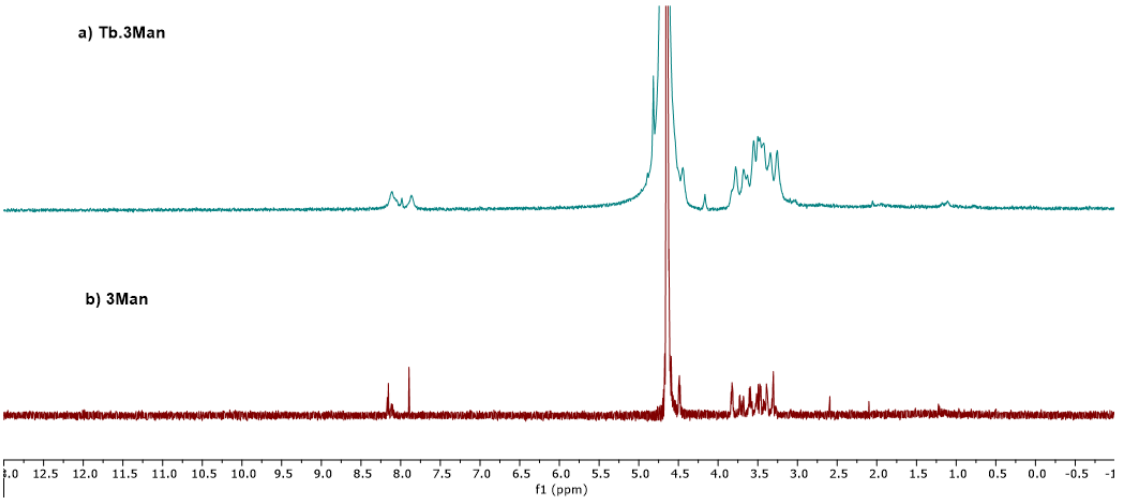



**Figure S24.** Comparison of $^{1}H$ NMR spectrum of Tb(III) complex **Tb.3Man** and ligand **3Man** ($D_2O$).

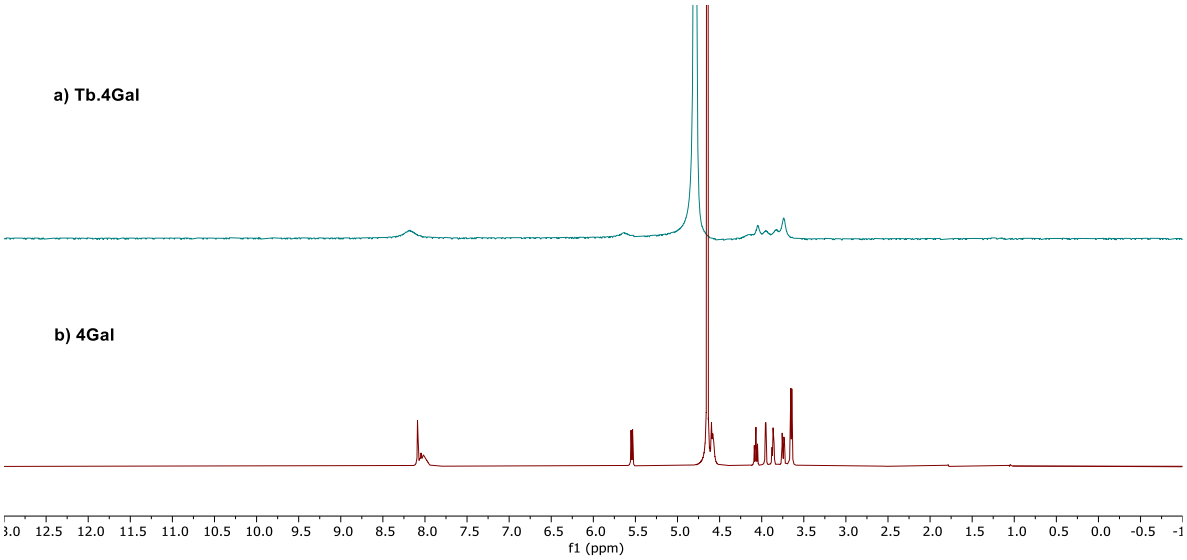



**Figure S25.** Comparison of $^{1}H$ NMR spectrum of Tb(III) complex **Tb.4Gal** and ligand **4Gal** ($D_2O$).

## *P. aeruginosa* MIC assay and biofilm assay data

The Gram-negative bacterium *Pseudomonas aeruginosa* has been highlighted by the World Health Organization as a critical priority pathogen for which new antimicrobials and diagnostics are urgently needed.[5] As a leading cause of nosocomial infections, its societal and economic burden is increasing in line with rising antimicrobial resistance around the world.[6]

Ligand **3Gal** and complex **Tb.3Gal** were tested for their ability to inhibit growth of *P. aeruginosa* (PAO1) at various concentrations (Figure S26). In brief: PAO1 was seeded into wells at $10^6$ CFU/mL. Serial dilutions of each of the compounds was then added to the wells and a set of control wells with no compound was also set up. Plates were incubated for 24 h at 37°C before absorption readings at 590 nm were taken. The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that inhibits visible *in-vitro* growth of microorganisms. Each experiment was performed in four replicates. Compounds at high concentration were very coloured but turbidity could be observed in all wells.

Biofilm formation assays were based on methodology from previously published work.[7] In brief, starting from an overnight liquid culture, a dilution containing approximately $10^8$ CFU/mL was made of PAO1 strain (kind donation from Prof. Seamas Donnelly, School of Medicine, Trinity College Dublin). For each biofilm experiment, 8 wells of a round-bottomed polypropylene 96-well micro plate (Corning Costar, Sigma) were inoculated with 100 µl of this dilution, 8 wells were inoculated with the dilution and treated with the test compounds (in concentrations from 0 to 10 mM) and 8 control wells were filled with sterile medium (bacteria alone). Following 4 hours of adhesion, the supernatant, containing non-adhered cells, was removed from each well and plates rinsed using PBS solution. Following this 100 µl of fresh media was added to the control wells and fresh media with each concentration of **3Gal** or **Tb.3Gal** was added to the appropriate wells, the plate was then incubated for a further 24 h. After 24 h biofilm formation, the supernatants were again removed, and the wells rinsed with PBS again. Once the wells were washed, 100 µl of a 0.5% crystal violet (CV) solution was added to all wells. After 20 min, the excess CV was removed by washing the plates under running tap water. Finally, bound CV was released by adding 150 µl of 33% acetic acid (Sigma). The absorbance was measured at 600 nm (Figure S27).

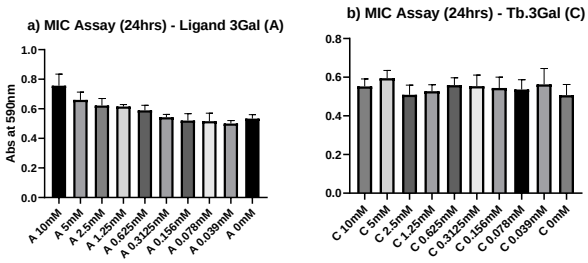



**Figure S26.** Minimum Inhibitory Concentration assay for (a) digalactoside **3Gal**; and (b) its Tb(III) complex. Neither compound showed significant inhibition of *P. aeruginosa* up to 10 mM

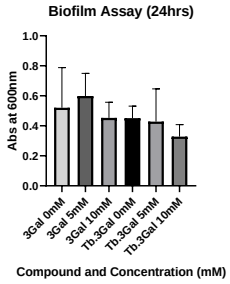
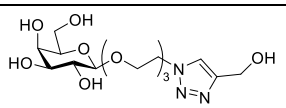


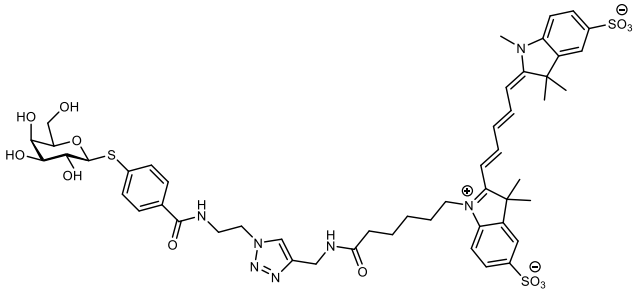

**Figure S27.** Crystal violet biofilm assay of PA in presence of **3Gal** and **Tb.3Gal**

## Biophysical evaluation of LecA binding

In order to investigate the binding affinity of **3Gal** and **Tb.3Gal** for protein LecA, competitive binding by fluorescence polarisation was performed as previously described by Titz and co-workers.[8a] LecA used in the binding assay was expressed and purified in analogy to previously published protocols.[8a-c] In brief, the assay was performed with 2.5% DMSO in TBS/$Ca^{2+}$ buffer (20 mM Tris, 137 mM NaCl, 2.6 mM KCl, pH 7.4, 1 mM $CaCl_2$), with a concentration of 20 µM LecA and 10 nM **Gal-Cy5** (as fluorescent reporter ligand[9]). Averages and standard deviations were calculated from at least three experiments.

**Table S3.** Results from evaluation of galactosides as LecA binders in competitive fluorescent polarisation assay: $IC_{50}$ values; relative potency per galactoside (vs monovalent control). Structure of fluorescent reporter ligand **Gal-Cy5** is also shown.

| ***Compound*** |  | **3Gal** | **Tb.3Gal** |
|---|---|---|---|
| ***$IC_{50}$ (µM)*** | 1068±192 | 163±41 | 131±26 |
| **r.p./Gal** | 1 | 3.3 | 4.1 |



**Gal-Cy5**

## Author Contributions

Project was conceptualised and funding acquired by JPB. Manuscript written and reviewed by KW and JPB. Investigation and analysis by KW, EZ, JPB, IJM and FK. GC and AT provided resources, supervision, analysis, and reviewed the manuscript.

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