Rapid and Modular Assembly of Click Substrates to Assay Enzyme Activity in the Newborn Screening of Lysosomal Storage Disorders

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1) General methods

All reagents, unless otherwise noted, were purchased from commercial sources without further purification. Dichloromethane, methanol, THF and diethyl ether were dried using PURESORT columns (Inert Corporation, USA). Solvents used for flash column chromatography were purchased from Donau Chemie AG (Austria). Dry acetonitrile and dry DMF were obtained from Sigma-Aldrich (Germany) and ACROS Organics (Belgium), respectively, and stored under argon. All reactions were carried out under argon in air-dried glassware. Thin layer chromatography was performed using TLC plates on aluminum support (Merck, silica gel 60, fluorescent indicator 254). Column chromatography was performed using a BUCHI Sepacore Flash System (2 x BUCHI Pump Module C-605, BUCHI Pump Manager C-615, BUCHI UV Photometer C-635, and BUCHI Fraction Collector C-660) and a Reveleris® X2 Flash Chromatography/Prep Purification Systems (BUCHI). Silica gel 60 (40-63 µm) was obtained from Merck. A Kinetex® 5 µm C18 100 Å, AXIA LC column (100 x 30.0 mm, Phenomenex) was used for preparative HPLC. HPLC grade solvents were purchased from VWR (USA). 1H and 13C NMR spectra were recorded on a Bruker AC 200 MHz, Bruker Avance UltraShield 400 MHz or Bruker Ascend 600 MHz spectrometer at 20 °C. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using solvent residual peaks. Data are shown as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, b = broad signal), coupling constants (J, Hz) and integration. HPLC analysis for reaction monitoring was performed on a 1200 series system (Agilent Technologies, USA) using a Kinetex® (5µm C18 100 Å, 50 x 4.6 mm, Phenomenex, USA) column and water/acetonitrile gradient elution. Peak detection was enabled via DAD (Agilent Technologies) and a Bruker HCT Esquire Ion Trap MS.

In general, no unexpected or unusually high safety hazards were encountered.
2) Synthesis

Clickable linker and click markers (CM)

Compounds 2a, 3a, 4a and 5a have been prepared using previously published methods. Analytical data matched that reported.

4-(Hydroxy-\(d_2\)-methyl) benzoic acid methyl ester (2b)

\[ \text{To a solution of triphenylphosphine (3.90 g, 14.85 mmol) in dichloromethane (40 mL) cooled to 0°C was added bromine (0.76 mL, 15.85 mmol) and trimethylamine (2.37 mL, 17.14 mmol). After 15 min a solution of 2b (1.91 g, 11.43 mmol) in dichloromethane (50 mL) was added and the mixture was stirred for 10 min. The reaction was quenched by addition of aqueous HCl (100 mL, 0.1N) and phases were separated. The organic layer was washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated. The residue was purified by column chromatography (90 g SiO\(_2\), 0-20% EtOAc in hexanes) to obtain 3b (2.46 g, 93%) as a pale yellow solid; } \]

\[ \text{\textsuperscript{1}H NMR (400 MHz, CDCl\(_3\)) } \delta \text{ 8.01 (d, } J = 8.4 \text{ Hz, 2H), 7.45 (d, } J = 8.3 \text{ Hz, 2H), 4.48 (s, 1H), 3.91 (s, 3H); } \]

\[ \text{\textsuperscript{13}C NMR (100 MHz, CDCl\(_3\)) } \delta \text{ 166.64 (s, 1C), 142.68 (s, 2C), 130.20 (d, 2C), 129.14 (d, 2C), 52.35 (q, 1C), 32.14 (t, } J_{CD} = 23.43 \text{ Hz, 1C); } \]

\[ \text{HR-ESI-ORBITRAP [M+H]\(^+\) m/z calcd. 229.9922 for C\(_9\)H\(_{13}\)DBrO\(_2\), found 229.9924. } \]

4-(Bromo-\(d_2\)-methyl) benzoic acid methyl ester (3b)

\[ \text{To a solution of triphenylphosphine (3.90 g, 14.85 mmol) in dichloromethane (40 mL) cooled to 0°C was added bromine (0.76 mL, 15.85 mmol) and trimethylamine (2.37 mL, 17.14 mmol). After 15 min a solution of 2b (1.91 g, 11.43 mmol) in dichloromethane (50 mL) was added and the mixture was stirred for 10 min. The reaction was quenched by addition of aqueous HCl (100 mL, 0.1N) and phases were separated. The organic layer was washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated. The residue was purified by column chromatography (90 g SiO\(_2\), 0-20% EtOAc in hexanes) to obtain 3b (2.46 g, 93%) as a pale yellow solid; } \]

\[ \text{\textsuperscript{1}H NMR (400 MHz, CDCl\(_3\)) } \delta \text{ 8.01 (d, } J = 8.4 \text{ Hz, 2H), 7.45 (d, } J = 8.3 \text{ Hz, 2H), 4.48 (s, 1H), 3.91 (s, 3H); } \]

\[ \text{\textsuperscript{13}C NMR (100 MHz, CDCl\(_3\)) } \delta \text{ 166.64 (s, 1C), 142.68 (s, 2C), 130.20 (d, 2C), 129.14 (d, 2C), 52.35 (q, 1C), 32.14 (t, } J_{CD} = 23.43 \text{ Hz, 1C); } \]

\[ \text{HR-ESI-ORBITRAP [M+H]\(^+\) m/z calcd. 229.9922 for C\(_9\)H\(_{13}\)DBrO\(_2\), found 229.9924. } \]
4-[[Azido-d₅-methyl]phenyl]-d₅-methanol (5b)

4b (1.78 g, 8.72 mmol), sodium azide (0.74 g, 11.3 mmol) and potassium iodide (0.29 g, 1.79 mmol) were dissolved in DMF (50 mL) and stirred overnight at room temperature. The mixture was diluted with water (100 mL) and extracted with diethyl ether. The combined organic layer was washed with saturated aqueous ammonium chloride solution and dried over sodium sulfate. The solvent was evaporated and the residue was purified by column chromatography (90 g SiO₂, 0-20% EtOAc in hexanes) to obtain 5b (1.05 g, 84%) as a colorless oil; ¹H NMR (400 MHz, CDCl₃) δ 7.39 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 4.32 (bs, 1H), 1.72 (bs, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 153.53 (s, 1C), 135.98 (s, 1C), 135.62 (s, 1C), 128.82 (d, 2C), 127.54 (q, 3C) 54.58 (t, JCD = 21.87 Hz, 1C), 54.20 (t, JCD = 21.87 Hz, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 167.1007 for C₆H₇D₃N₃O⁺, found 167.0928.

(1,1-Dimethyl)acetic acid, [4-[[acidomethyl]phenyl]methyl]ester (CM1a)

To a solution of 5a (2 g, 12.26 mmol) and di-tert-butyl dicarbonate (2.94 g, 13.48 mmol) in dry dichloromethane (20 mL) was added zinc acetate (0.27 g, 1.23 mmol). The mixture was heated to reflux for 11 h, cooled to room temperature and washed with water. The organic layer was dried over sodium sulfate and concentrated. The residue was purified by column chromatography (12 g SiO₂, 0-10% EtOAc in hexanes) to afford CM1a (2.76 g, 86%) as a colorless liquid; ¹H NMR (400 MHz, CDCl₃) δ 7.40 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 5.09 (s, 2H), 4.33 (s, 2H), 1.49 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 153.53 (s, 1C), 135.98 (s, 1C), 135.62 (s, 1C), 128.82 (d, 2C), 128.51 (d, 2C), 68.32 (t, 1C), 54.58 (t, 1C), 27.54 (q, 3C); HR-ESI-ORBITRAP [M+Na]+ m/z calcd. 286.1162 for C₁₃H₁₄D₃N₃O₃Na⁺, found 286.1166.

(1,1-Dimethyl)acetic acid, [4-[[acidomethyl-d₅]phenyl]methyl-d₅]ester (CM1b)

To a solution of 5b (1 g, 6.02 mmol) and di-tert-butyl dicarbonate (1.44 g, 6.62 mmol) in dry dichloromethane (10 mL) was added zinc acetate (0.13 g, 0.6 mmol) and washed with water. The mixture was heated to reflux for 11 h, cooled to room temperature and washed with water. The organic layer was dried over sodium sulfate and concentrated. Column chromatography (12 g SiO₂, 0-10% EtOAc in hexanes) gave CM1b (1.29 g, 81%) as a colorless liquid; ¹H NMR (400 MHz, CDCl₃) δ 7.40 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 7.7 Hz, 2H), 4.31 (s, 1H), 1.49 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 153.48 (s, 1C), 135.79 (s, 1C), 135.53 (s, 1C), 128.84 (d, 2C), 128.49 (d, 2C), 82.49 (s, 1C), 67.68 (quint, JCD = 22.55 Hz, 1C), 54.20 (t, JCD = 21.85 Hz, 1C), 27.84 (q, 3C); HR-ESI-ORBITRAP [M+Na]+ m/z calcd. 289.135 for C₁₃H₁₄D₃N₃O₃Na⁺, found 289.1352.
General procedure A (Carbamate formation)

To a solution of alcohol (1 eq) in THF (1.67 mL/mmol) cooled to 0°C was added sodium carbonate (1 eq) and triphosgene (0.5 eq). The reaction mixture was stirred at room temperature overnight, filtered and concentrated under reduced pressure. The residue was dissolved in THF (0.56 mL/mmol) and added to a solution of amine (1.1 eq) in THF (1.11 mL/mmol) and aqueous NaOH (1N, 1.67 mL/mmol). The reaction was stirred overnight and then extracted with dichloromethane. The combined organic layer was concentrated and the residue was purified by column chromatography (90 g SiO₂, 10-30 % EtOAc in hexanes).

N,N-(Diethyl)carbamic acid, [4-[acidomethyl]phenyl]methyl]ester (CM2a)

Following general procedure A, 5a (2 g, 12.26 mmol) was reacted with diethylamine (0.99 g, 13.49 mmol) to obtain CM2a (1.62 g, 50%) as a colorless liquid; ¹H NMR (400 MHz, CDCl₃) δ 7.38 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 5.13 (s, 2H), 4.34 (s, 2H), 3.30 (s, 4H), 1.13 (t, J = 7.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 155.84 (s, 1C), 137.49 (s, 1C), 135.09 (s, 1C), 128.43 (d, 2C), 128.30 (d, 2C), 66.41 (t, 1C), 54.66 (t, 1C), 42.05 (t, 1C), 41.39 (t, 1C), 14.25 (q, 1C), 13.60 (q, 1C); HR-ESI-ORBITRAP [M+H]⁺ m/z calcd. 263.1503 for C₁₃H₁₉N₄O₂⁺, found 263.1502.

N,N-(Diethyl)carbamic acid, [4-[acidomethyl-d₂]phenyl]methyl-d₂]ester (CM2b)

Following general procedure A, 5b (0.875 g, 5.26 mmol) was reacted with diethylamine (0.42 g, 5.79 mmol) to obtain CM2b (0.99 g, 71%) as a colorless liquid; ¹H NMR (400 MHz, CDCl₃) δ 7.38 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 4.32 (s, 1H), 3.30 (s, 4H), 1.13 (t, J = 7.02 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 155.84 (s, 1C), 137.38 (s, 1C), 135.05 (s, 1C), 128.44 (d, 2C), 128.35 (d, 2C), 65.80 (quin, J = 23.14 Hz, 1C), 54.34 (t, J = 21.87 Hz, 1C), 42.02 (t, 1C), 41.39 (t, 1C), 14.25 (q, 1C), 13.61 (q, 1C); HR-ESI-ORBITRAP [M+H]⁺ m/z calcd. 266.1691 for C₁₃H₁₆D₃N₄O₂⁺, found 266.1694.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]carbamic acid, [4-[acidomethyl]phenyl]methyl]ester (CM3a)

Following general procedure A, 5a (3.2 g, 19.18 mmol) was reacted with N-boc-ethylenediamine (3.38 g, 21.1 mmol) to obtain CM3a (4.58 g, 67%) as a white solid; ¹H NMR (400 MHz, CDCl₃) δ 7.37 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 5.21 (bs, 1H), 5.10 (s, 2H), 4.84 (bs, 1H), 4.33 (s, 2H), 3.22-3.23 (m, 4H), 1.43 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 156.78 (s, 1C), 156.53 (s, 1C), 136.82 (s, 1C), 135.42 (s, 1C), 128.64 (d, 2C), 128.50 (d, 2C), 79.81 (s, 1C), 66.40 (t, 1C), 54.61(t, 1C), 41.72 (t, 1C), 40.72 (t, 1C), 28.49 (q, 3C), HR-ESI-ORBITRAP [M+H]⁺ m/z calcd. 350.1823 for C₁₃H₂₈N₅O₄⁺, found 350.1823.
N-[[[1,1-Dimethylethoxy]carbonyl][amino][ethyl]carbamic acid, [4-[[acidomethyl-\text{\textit{d}}_3]phenyl]methyl-\text{\textit{d}}_2]ester (CM3b)

Following general procedure A, 5b (1.3g, 7.79 mmol) was reacted with N-boc-ethylenediamine (1.37 g, 8.57 mmol) to obtain CM3b (1.88 g, 68%) as a white solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.37 (d, \(J = 8.1\) Hz, 2H), 7.30 (d, \(J = 8.1\) Hz, 2H), 5.21 (bs, 1H), 4.84 (bs, 1H), 4.32 (s, 1H), 3.32-3.23 (m, 4H), 1.43 (s, 9H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 156.78 (s, 1C), 156.55 (s, 1C), 136.71 (s, 1C), 135.38 (s, 1C), 128.69 (d, 2C), 128.50 (d, 2C), 79.81 (s, 1C), 54.30 (t, \(\text{j}_{\text{CD}} = 21.85\) Hz, 1C), 41.71 (t, 1C), 40.72 (t, 1C), 28.48 (q, 3C); HR-ESI-ORBITRAP [M+H]\(^+\) m/z calcd. 353.2111 for C\(_{18}\)H\(_{23}\)N\(_{3}\)O\(_{4}\)^+, found 353.2105.

N-[[[1,1-Dimethylethoxy]carbonyl][amino][propyl]carbamic acid, [4-[[acidomethyl]phenyl]methyl] ester (CM4a)

Following general procedure A, 5a (2.4 g, 14.71 mmol) was reacted with N-boc-propanediamine (2.82 g, 16.18 mmol) to obtain CM4a (3.96 g, 74%) as a white solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.38 (d, \(J = 8.1\) Hz, 2H), 7.31 (d, \(J = 8.1\) Hz, 3H), 5.28 (bs, 1H), 5.10 (s, 2H), 4.80 (bs, 1H), 4.33 (s, 2H), 3.24 (q, \(J = 6.15\) Hz, 2H), 3.14 (q, \(J = 5.59\) Hz, 2H), 1.63 (quint, \(J = 6.24\) Hz, 2H), 1.43 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.76 (s, 1C), 156.56 (s, 1C), 156.07 (s, 1C), 137.01 (s, 1C), 139.35 (s, 1C), 128.62 (d, 2C), 128.50 (d, 2C), 79.55 (s, 1C), 66.28 (t, 1C), 54.64 (t, 1C), 37.88 (t, 1C), 37.34 (t, 1C), 30.75 (t, 1C), 28.53 (s, 3C); HR-ESI-ORBITRAP [M+H]\(^+\) m/z calcd. 364.1979 for C\(_{17}\)H\(_{28}\)N\(_{3}\)O\(_{4}\)^+, found 364.1978.

N-[[[1,1-Dimethylethoxy]carbonyl][amino][propyl]carbamic acid, [4-[[acidomethyl-\text{\textit{d}}_3]phenyl]-methyl-\text{\textit{d}}_2]ester (CM4b)

Following general procedure A, 5b (1.05 g, 6.32 mmol) was reacted with N-boc-propanediamine (1.21 g, 6.95 mmol) to obtain CM4b (1.35 g, 58%) as a white solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.38 (d, \(J = 8.0\) Hz, 2H), 7.30 (d, \(J = 8.2\) Hz, 2H), 5.30 (bs, 1H), 4.81 (bs, 1H), 4.32 (s, 1H), 3.23 (q, \(J = 6.1\) Hz, 2H), 3.17 (q, \(J = 5.5\) Hz, 2H), 1.62 (quint, \(J = 6.3\) Hz, 2H), 1.43 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.76 (s, 1C), 156.56 (s, 1C), 156.06 (s, 1C), 138.9 (s, 1C), 135.3 (s, 1C), 128.59 (d, 2C), 128.49 (d, 2C), 79.51 (s, 1C), 54.31 (t, \(\text{j}_{\text{CD}} = 21.89\) Hz, 1C), 37.86 (t, 1C), 37.29 (t, 1C), 30.72 (t, 1C), 28.51 (s, 3C); HR-ESI-ORBITRAP [M+H]\(^+\) m/z calcd. 367.2168 for C\(_{17}\)H\(_{23}\)D\(_{2}\)N\(_{3}\)O\(_{4}\)+, found 367.2167.

N-[[[1,1-Dimethylethoxy]carbonyl][amino][butyl]carbamic acid, [4-[[acidomethyl]phenyl]methyl] ester (CM5a)

Following general procedure A, 5a (0.4 g, 2.45 mmol) was reacted with N-boc-butane diamine (0.51 g, 2.70 mmol) to obtain CM5a (0.67 g, 72%) as a white solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.37 (d, \(J = 8.1\) Hz, 2H), 7.30 (d, \(J = 8.1\) Hz, 2H), 5.09 (s, 2H), 4.85 (bs, 1H), 4.56 (bs, 1H), 4.33 (s, 2H), 3.24-3.16 (m, 2H), 3.11 (s, 2H), 1.55-1.47 (m, 4H), 1.43 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.45 (s, 1C), 156.13 (s, 1C), 136.96 (s, 1C), 135.37 (s, 1C), 128.65 (d, 2C), 128.49 (d, 2C), 79.38 (s, 1C), 66.27 (t, 1C), 54.61 (t, 1C), 40.86 (t, 1C), 40.32 (t, 1C), 28.54 (q, 3C), 27.52 (t, 1C), 27.38 (t, 1C); HR-ESI-ORBITRAP [M+H]\(^+\) m/z calcd. 378.2136 for C\(_{18}\)H\(_{28}\)N\(_{3}\)O\(_{4}\)^+, found 378.2138.
N-[[[1,1-Dimethylethoxy]carbonyl]amino]butyl]carbamic acid, [4-[[acidomethyl-$d_3$]phenyl]methyl-$d_3$]ester (CM5b)

Following general procedure A, 5b (0.28 g, 1.68 mmol) was reacted with N-boc-butanediamine (0.35 g, 1.85 mmol) to obtain CM5b (0.53 g, 83%) as a white solid; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.37 (d, $J$ = 8.12 Hz, 2H), 7.30 (d, $J$ = 8.04 Hz, 2H), 4.84 (bs, 1H), 4.55 (bs, 1H), 4.32 (s, 1H), 3.25-3.16 (m, 2H), 3.11 (bs, 2H), 1.54-1.46 (m, 4H), 1.43 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.45 (s, 1C), 156.12 (s, 1C), 136.84 (s, 1C), 135.32 (s, 1C), 128.69 (d, 2C), 128.49 (d, 2C), 79.36 (s, 1C), 54.29 (t, $J_{CD} = 21.86$ Hz, 1C), 40.85 (t, 1C), 40.28 (t, 1C), 28.53 (q, 3C), 27.51 (t, 1C), 27.37 (t, 1C), HR-ESI-ORBITRAP [M+H]+$^-$ m/z calcd. 381.2324 for C$_{18}$H$_{23}$D$_3$N$_5$O$_7^-$, found 381.2321.

Enzyme responsive units

1-O-Propargyl-2,3,4-tri-O-acetyl-$\alpha$-L-idopyranuronic acid, methyl ester (7)

To a solution of 6 (2 g, 5.95 mmol) in degassed dry acetonitrile (140 mL) was added molecular sieve (100 mg). After cooling to -20°C propargyl alcohol (3.44 mL, 59.5 mmol) was added followed by dropwise addition of TMSOTf (1.08 mL, 5.95 mmol). The mixture was allowed to reach room temperature and stirred for 24h. The mixture was diluted with CH$_2$Cl$_2$ (200 mL) and washed with saturated aqueous sodium bicarbonate solution (2 x 200 mL). The organic layer was dried over sodium sulfate and concentrated. The residue was purified by column chromatography (120 g SiO$_2$, 10-50% EtOAc in hexanes) to obtain 7 (0.491 g, 22%) as a colorless oil; $^1$H NMR (200 MHz, CDCl$_3$) $\delta$ 5.19 (bs, 1H), 5.16–5.09 (m, 1H), 5.09-5.01 (m, 1H), 4.85 (d, $J$ = 2.4 Hz, 1H), 4.83–4.78 (m, 1H), 4.20 (d, $J$ = 2.3 Hz, 2H), 3.78 (s, 3H), 2.44 (t, $J$ = 2.4 Hz, 1H), 2.19-1.92 (m, 9H); $^{13}$C NMR (50 MHz, CDCl$_3$) $\delta$ 169.59 (s, 1C), 169.34 (s, 1C), 169.10 (s, 1C), 168.30 (s, 1C), 96.79 (d, 1C), 75.38 (s, 1C), 67.27 (d, 1C), 66.89 (d, 1C), 66.74 (d, 1C), 66.65 (d, 1C), 55.76 (t, 1C), 52.72 (q, 1C), 20.89 (q, 2C), 20.71 (q, 1C); HR-ESI-ORBITRAP [M+Na]+$^-$ m/z calcd. 395.0949 for C$_{16}$H$_{20}$O$_{10}$Na$,^+$, found 395.0948.

2-O-Propynyl-2,3,4-tri-O-acetyl-6-O-(triisopropylsilyl)-$\beta$-$d$-galactopyranoside

1-O-Propargyl-$\beta$-$d$-galactoside (13)$^5$ (1.1 g, 5 mmol, 1 eq.) was dissolved dry DMF (21 mL) and imidazole (1.0 g, 15 mmol, 3 eq) was added. Trisopropylsilyl chloride was added dropwise at 0°C and the reaction was allowed to warm to room temperature and stirred overnight. The solvent was removed under high vacuum. The crude product (Rf: 0.83, CH$_2$Cl$_2$/MeOH = 5:1), which was used without further purification, was dissolved in pyridine (50 mL) and DMAP (18 mg, 150 µmol, 0.05 eq.) was added. Acetic anhydride (1.5 g, 15 mmol, 5 eq.) was added to the cooled, homogeneous solution. After stirring overnight, the solvent was removed under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$ and washed with saturated CuSO$_4$ solution (to remove remaining pyridine), brine and dried over Na$_2$SO$_4$. Column chromatography (SiO$_2$, hexanes/EtOAc = 5:1) afforded the title compound as a white solid (1.2 g, 80% over 2 steps); Rf: 0.83, hexanes/EtOAc = 2:1; $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 5.50 (d, $J$ = 3.3 Hz, 1H), 5.20 (dd, $J_1 = 10.4$ Hz, $J_2 = 7.8$ Hz, 1H), 5.06 (dd, $J_1 = 10.4$ Hz, $J_2 = 3.3$ Hz, 1H), 4.72 (d, $J$ = 8.0 Hz, 1H), 4.36 (d, $J$ = 2.3 Hz, 2H), 3.87-3.68 (m, 3H), 2.44 (t, $J$ = 2.3 Hz, 1H), 2.11 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.07-0.97 (m, 21H); $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta$ 170.14 (s, 1C), 170.00 (s, 1C), 169.65 (s, 1C), 98.62 (d, 1C), 78.33 (s, 1C), 75.19 (d, 1C), 73.71 (d, 1C), 71.18 (d, 1C), 68.83 (d, 1C), 67.00 (d,
1C), 60.91 (t, 1C), 55.76 (t, 1C), 20.81 (q, 1C), 20.71 (q, 1C), 20.63 (q, 1C) 17.84 (q, 6C), 11.78 (d, 3C); HR-ESI-ORBITRAP, m/z [M+Na]+ calcd. 523.2334 for C_{25}H_{40}O_{9}NaS, found 523.2334.

2-O-Propynyl-2,3,4-tri-O-acetyl-β,D-galactopyranoside

2-Propynyl-2,3,4-tri-O-acetyl-6-O-(trisopropylsilyl)-β,D-galactopyranoside (5 g, 10 mmol, 1 eq) was dissolved in dry THF (150 mL). The reaction mixture was cooled to -14 °C using a cryostat and glacial acetic acid (2.3 mL, 40 mmol, 4 eq.) was added in one portion. Subsequently, tetrabutylammonium fluoride (1 M in THF, 50 mL, 50 mmol, 5 eq) was added dropwise and the reaction was kept at -10 °C overnight until complete conversion was verified by TLC. Saturated ammonium chloride solution (250 mL) was added under vigorous stirring and the layers were separated. The aqueous layer was extracted with ethyl acetate (3 x 200 mL). The combined organic layer was re-extracted with saturated sodium bicarbonate solution (2 x 100 mL), dried over Na_{2}SO_{4} and concentrated. The residue was purified by column chromatography (SiO₂, hexanes/EtOAc = 1:1) to afford the title compound as a white solid (2.53 g, 73%); Rf: 0.4, hexanes/EtOAc = 1:1; ¹³C NMR (200 MHz, CDCl₃): δ 5.36 (d, J = 3.3 Hz), 5.22 (dd, J₁ = 10.4 Hz, J₂ = 7.8 Hz, 1H), 5.06 (dd, J₁ = 10.4 Hz, J₂ = 3.3 Hz, 1H), 4.73 (d, J = 7.8 Hz, 1H), 4.37 (dd, J₁ = 2.3 Hz, J₂ = 0.5 Hz, 2H), 3.81-3.47 (m, 3H), 2.46 (t, J = 2.3 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H); ¹³C NMR (50 MHz, CDCl₃): δ 171.12 (s, 1C), 170.05 (s, 1C), 169.61 (s, 1C), 98.98 (d, 1C), 78.35 (s, 1C), 75.35 (d, 1C), 73.64 (d, 1C), 70.88 (d, 1C), 68.81 (d, 1C), 67.81 (d, 1C), 60.53 (t, 1C), 56.12 (t, 1C), 20.75 (q, 1C), 20.66 (q, 1C), 20.58 (q, 1C); HR-ESI-ORBITRAP, m/z [M+Na]+ calcd. 367.1000 for C_{15}H_{20}O_{5}Na, found 367.1005.

2-O-Propynyl-2,3,4-tri-O-acetyl-β,D-galactopyranose-6-O-sulfate, triethylammonium salt (14)

2-O-Propynyl-2,3,4-tri-O-acetyl-β,D-galactopyranoside (200 mg, 581 µmol, 1 eq) and sulfur trioxide trimethylamine complex (809 mg, 5.81 mmol, 10 eq) were dissolved in dry DMF (6 mL) and the mixture was stirred at 55 °C overnight. After completion as verified by LCMS the solvent was evaporated under high vacuum at 40 °C and the residue was dissolved/suspended in MeOH. The suspension was filtered to remove excess SO₃-NMe₃-complex. Et₃N (1 mL) was added and the solution was concentrated. The residue was purified by column chromatography (SiO₂, MeOH in CH₂Cl₂, 0-20% gradient elution) to obtain 14 (271 mg, 90%); RF: 0.52, CHCl₃/MeOH 4:1; ¹'H NMR (200 MHz, CDCl₃): δ 5.47 (d, J = 3.14 Hz, 1H), 5.18 (dd, J₁ = 10.4 Hz, J₂ = 7.8 Hz, 1H), 4.99 (dd, J₁ = 10.4 Hz, J₂ = 3.3 Hz, 1H), 4.67 (dd, J₁ = 16.0 Hz, J₂ = 7.9 Hz, 1H), 4.35 (d, J = 2.3 Hz, 2H), 4.27-4.16 (m, 1H), 4.15-3.95 (m, 2H) 3.14 (q, J = 7.37 Hz, 6H), 2.45 (t, J = 2.24 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 3H), 1.94 (s, 3H), 1.34 (t, J = 7.34 Hz, 9H); ¹³C NMR (50 MHz, CDCl₃): δ 170.43 (s, 1C), 170.15 (s, 1C), 169.59 (s, 1C), 98.70 (d, 1C), 78.32 (s, 1C), 75.31 (d, 1C), 71.25 (d, 1C), 71.03 (d, 1C), 68.55 (d, 1C), 66.97 (d, 1C), 64.13 (t, 1C), 55.93 (t, 1C), 46.56 (t, 3C), 20.78 (q, 1C), 20.73 (q, 1C), 20.62 (q, 1C), 8.66 (q, 3C); HR-ESI-ORBITRAP [M-H]- m/z calcd. 423.0591731 for C_{15}H_{19}O_{12}S, found 423.06049.
**IDUA Sets (Substrates, internal standards, products)**

**General procedure B** (Click reaction, IDUA substrates)

Cul (0.1 eq) was added to a solution of 7 (1 eq), azide (1.1 eq) and Et₃N (1.2 eq) in THF (55 mL/mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was filtrated and concentrated. The residue was purified by column chromatography (40 g SiO₂, 20-100% EtOAc in hexanes)

[1-[[1,1-Dimethylethoxy]carbonyl[methyl]phenyl]methyl]-1H-1,2,3-triazol-4-yl]methyl] 3,4,6-tri-O-acetyl α-L-idopyranuric acid, methyl ester (8)

Following general procedure B, 7 (0.1 g, 0.27 mmol) was reacted with CM1a (0.078 g, 0.295 mmol) to afford 8 (157 mg, 92%) as a white solid; ¹H NMR (600 MHz, CDCl₃) δ 7.45 (s, 1H), 7.38 (d, J = 8.1 Hz, 2H), 7.28-7.25 (m, 2H), 5.51 (q, J = 14.5 Hz, 2H), 5.13-5.09 (m, 4H), 5.02 (t, J = 4.0 Hz, 1H), 4.89-4.83 (m, 3H), 4.80-4.75 (m, 1H), 4.70 (d, J = 12.2 Hz, 1H), 3.77 (s, 3H), 3.29 (bs, 4H), 2.06 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H), 1.12 (bs, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 169.53 (s, 1C), 169.33 (s, 1C), 169.01 (s, 1C), 168.32 (s, 1C), 155.68 (s, 1C), 144.36 (s, 1C), 138.12 (s, 1C), 134.10 (s, 1C), 128.42 (d, 2C), 128.31 (d, 2C), 122.07 (d, 1C), 97.49 (d, 1C), 67.25 (d, 1C), 66.74 (d, 1C), 66.72 (d, 1C), 66.69 (d, 1C), 66.13 (t, 1C), 62.03 (t, 1C), 53.96 (t, 1C), 52.64 (q, 1C), 42.01 (t, 1C), 41.34 (t, 1C), 20.81 (q, 1C), 20.72 (q, 1C), 20.65 (q, 1C), 14.26 (q, 1C), 13.53 (q, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 635.2559 for C₂₉H₃₉N₄O₁₂⁺, found 635.2558.
[1-[[4-[[[[[1,1-Dimethylethoxy]carbonyl]amino]ethyl]amino]carbonyl]oxy]methyl]phenylmethyl]-1H-1,2,3-triazol-4-yl]methyl] 3,4,6-tri-O-acetyl α-L-idopyranuric acid, methyl ester (10)

Following general procedure B, 7 (0.1 g, 0.27 mmol) was reacted with CM3a (0.103 g, 0.295 mmol) to obtain 10 (166 mg, 86%) as a white solid; 1H NMR (600 MHz, CDCl3) δ 7.45 (s, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.34 (d, J = 15.0 Hz, 2H), 5.33 (bs, 1H), 5.03-5.00 (m, 4H), 5.01 (t, J = 5.9 Hz, 2H), 4.88-4.83 (m, 2H), 4.81-4.74 (m, 2H), 4.71-4.67 (m, 1H), 3.78 (s, 3H), 3.23 (s, J = 6.2 Hz, 2H), 1.43 (s, 9H). 13C NMR (100 MHz, CDCl3) δ 169.52 (s, 1C), 169.47 (s, 1C), 156.59 (s, 2C), 156.51 (s, 1C), 144.36 (s, 1C), 134.39 (s, 1C), 66.70 (d, 1C), 66.65 (d, 1C), 66.62 (d, 1C), 53.88 (t, 1C), 52.61 (d, 1C), 40.58 (t, 1C), 28.40 (q, 1C), 20.78 (q, 1C), 20.70 (q, 1C), 20.62 (q, 1C), 14.23 (q, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 722.2880 for C32H46N7O14+, found 722.2883.

[1-[[4-[[[[[1,1-Dimethylethoxy]carbonyl]amino]propyl]amino]carbonyl]oxy]methyl]phenylmethyl]-1H-1,2,3-triazol-4-yl]methyl] 3,4,6-tri-O-acetyl α-L-idopyranuric acid, methylester (11)

Following general procedure B, 7 (0.1 g, 0.27 mmol) was reacted with CM4a (0.107 g, 0.295 mmol) to give 11 (147 mg, 74%) as a white solid; 1H NMR (600 MHz, CDCl3) δ 7.45 (s, 1H), 7.36 (d, J = 8.04 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 5.37 (q, J = 15.0 Hz, 2H), 5.36 (bs, 1H), 5.11-5.07 (m, 4H), 5.02 (t, J = 3.66 Hz, 1H), 4.88-4.83 (m, 2H), 4.81-4.75 (m, 2H), 4.71-4.67 (m, 1H), 3.78 (s, 3H), 3.23 (q, J = 6.2 Hz, 2H), 1.43 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 169.52 (s, 1C), 169.47 (s, 1C), 156.59 (s, 2C), 156.51 (s, 1C), 144.36 (s, 1C), 134.39 (s, 1C), 66.70 (d, 1C), 66.65 (d, 1C), 53.88 (t, 1C), 52.61 (d, 1C), 40.58 (t, 1C), 28.40 (q, 1C), 20.78 (q, 1C), 20.70 (q, 1C), 20.62 (q, 1C), 14.23 (q, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 736.3036 for C33H48N7O14+, found 736.3033.

[1-[[4-[[[[[1,1-Dimethylethoxy]carbonyl]amino]butyl]amino]carbonyl]oxy]methyl]phenylmethyl]-1H-1,2,3-triazol-4-yl]methyl] 3,4,6-tri-O-acetyl α-L-idopyranuric acid, methylester (12)

Following general procedure B, 7 (0.1 g, 0.27 mmol) was reacted with CM5a (0.111 g, 0.295 mmol) to obtain 12 (169 mg, 84%) as a white solid; 1H NMR (600 MHz, CDCl3) δ 7.45 (s, 1H), 7.36 (d, J = 15.4 Hz, 2H), 7.26 (d, J = 3.7 Hz, 1H), 4.88-4.83 (m, 3H), 4.79-4.74 (m, 1H), 4.69 (d, J = 12.5 Hz, 1H), 4.57 (bs, 1H), 3.77 (s, 3H), 3.20 (q, J = 6.3 Hz, 2H), 3.15-3.08 (m, 2H), 2.06 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H), 1.51 (s, 4H), 1.43 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 169.46 (s, 1C), 169.26 (s, 1C), 168.98 (s, 1C), 168.23 (s, 1C), 156.29 (s, 1C), 156.05 (s, 1C), 144.30 (s, 1C), 137.58 (s, 1C), 134.32 (s, 1C), 128.70 (d, 2C), 128.28 (d, 2C), 122.69 (d, 1C), 79.39 (d, 1C), 79.10 (s, 1C), 67.16 (d, 1C), 66.64 (d, 2C), 66.59 (d, 1C), 65.83 (t, 1C), 61.91 (t, 1C), 53.83 (t, 1C), 52.56 (q, 1C), 40.73 (t, 1C), 40.13 (t, 1C), 28.41 (q, 3C), 27.35 (t, 1C), 27.17 (t, 1C), 20.73 (q, 1C), 20.64 (q, 1C), 20.57 (q, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 750.3192 for C34H48N7O14+, found 750.3186.
General procedure C (Deprotection)
To a solution of the protected glycoside (1 eq) in THF (216 mL/mmol) and water (22 mL/mmol) cooled to 0°C was added aqueous NaOH (5 eq, 0.1M). The reaction mixture was stirred for 4h and then quenched by addition of aqueous HCl (0.1 M) to adjust the pH to 7. The reaction was concentrated and the residue was purified by preparative HPLC (C18-reversed phase, 100 x 30, 5 μm, 50 mL/min, H2O/McCN 5-30%).

[1-[4-[[[1,1-Dimethylethoxy]carbonyl]methyl]phenyl[methyl]-1H-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS1)

Following general procedure C, starting from 8 (70 mg, 0.11 mmol) CS1 was obtained as a white solid (54 mg, 98%); 1H NMR (600 MHz, CD3OD) δ 8.04 (s, 1H), 7.36 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 5.58 (s, 2H), 5.05 (s, 2H), 4.88 (d, J = 4.7 Hz, 1H), 4.76 (d, J = 12.7 Hz, 1H), 4.35 (d, J = 4.1 Hz, 1H), 3.79 (dd, J1 = 6.2, J2 = 4.0 Hz, 1H), 3.64 (t, J = 6.2 Hz, 1H), 3.37 (dd, J1 = 6.3, J2 = 4.5 Hz, 1H), 1.45 (s, 9H); 13C NMR (150 MHz, CD3OD) δ 176.98 (s, 1C), 154.91 (s, 1C), 145.88 (s, 1C), 123.92 (s, 1C), 136.7 (s, 1C), 129.3 (d, 2C), 129.3 (d, 2C), 129.3 (d, 2C), 125.60 (d, 1C), 101.4 (d, 1C), 83.11 (s, 1C), 73.57 (d, 1C), 72.28 (d, 2C), 72.23 (d, 1C), 71.65 (d, 1C), 68.97 (t, 1C), 62.60 (t, 1C), 54.53 (t, 1C), 27.97 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 496.1926 for C20H30N3O16+, found 496.1921.

[1-[4-[[[N,N-Diethylamino]carbonyl]oxy]methyl]phenyl[methyl]-1H-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS2)

Following general procedure C, starting from 9 (70 mg, 0.11 mmol) CS2 was obtained as a white solid (52 mg, 95%); 1H NMR (600 MHz, CD3OD) δ 8.07 (s, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 5.60 (s, 2H), 5.11 (s, 2H), 4.87 (d, J = 4.4 Hz, 1H), 4.78 (d, J = 12.6 Hz, 1H), 4.36 (d, J = 4.0 Hz, 1H), 3.81 (dd, J1 = 6.2, J2 = 4.0 Hz, 1H), 3.66 (t, J = 6.2 Hz, 1H), 3.39 (dd, J1 = 6.5, J2 = 4.4 Hz, 1H), 3.35-3.28 (m, 4H); 13C NMR (150 MHz, CD3OD) δ 176.97 (s, 1C), 157.40 (s, 1C), 145.86 (s, 1C), 138.72 (s, 1C), 136.52 (s, 1C), 129.34 (d, 4C), 125.60 (d, 1C), 101.39 (d, 1C), 73.58 (d, 1C), 72.29 (d, 1C), 72.22 (d, 1C), 71.65 (d, 1C), 67.50 (t, 1C), 62.05 (t, 1C), 54.54 (t, 1C), 43.08 (t, 1C), 42.56 (t, 1C), 14.37 (q, 1C), 13.71 (q, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 495.2086 for C22H31N4O7+, found 495.2092.

[1-[4-[[[1,1-Dimethylethoxy]carbonyl]amino]ethyl]amino]carbonyl]oxy]methyl]phenyl[methyl]-1H-1,2,3-triazol-4yl]methyl] α-L-idopyranuric acid (CS3)

Following general procedure C, starting from 10 (100 mg, 0.139 mmol) CS3 was obtained as a white solid (45 mg, 56%); 1H NMR (600 MHz, CD3OD) δ 8.04 (s, 1H), 7.36 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 5.58 (s, 2H), 5.06 (s, 2H), 4.82 (d, J = 4.7 Hz, 1H), 4.76 (d, J = 12.6 Hz, 1H), 4.32 (d, J = 4.40Hz, 1H), 3.76 (dd, J1 = 6.8, J2 = 4.4 Hz, 1H), 3.61 (t, J = 6.6 Hz, 1H), 3.34 (dd, J1 = 6.5, J2 = 5.3 Hz, 1H), 3.20-3.10 (m, 4H), 1.42 (s, 9H); 13C NMR (150 MHz, CD3OD) δ 176.96 (s, 1C), 158.84 (s, 1C), 158.56 (s, 1C), 145.91 (s, 1C), 138.84 (s, 1C), 136.42 (s, 1C), 129.31 (d, 2C), 129.28 (d, 2C), 125.59 (d, 1C), 101.36 (d, 1C), 80.10 (s, 1C), 73.83 (d, 1C), 72.63 (d, 1C), 72.38 (d, 1C), 71.83 (d, 1C), 66.86 (t, 1C), 62.13 (t, 1C), 54.56 (t, 1C), 41.79 (t, 1C), 41.18 (t, 1C), 28.74 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 582.2406 for C32H36N3O11+, found 582.2378.
Following general procedure C, starting from 11 (70 mg, 0.095 mmol) CS4 was obtained as white solid (33 mg, 58%); \( ^1H \) NMR (600 MHz, CD\(_3\)OD) \( \delta \) 8.04 (s, 1H), 7.35 (d, \( J = 8.1 \) Hz, 2H), 7.32 (d, \( J = 8.1 \) Hz, 4H), 5.58 (s, 2H), 5.05 (s, 2H), 4.82 (d, \( J = 5.0 \) Hz, 1H), 4.76 (d, \( J = 12.6 \) Hz, 1H), 4.32 (d, \( J = 4.1 \) Hz, 1H), 3.76 (dd, \( J_1 = 6.6, J_2 = 4.2 \) Hz, 1H), 3.61 (t, \( J = 6.6 \) Hz, 1H), 3.34 (dd, \( J_1 = 6.3, J_2 = 4.8 \) Hz, 1H), 3.12 (t, \( J = 7.1 \) Hz, 2H), 3.05 (t, \( J = 6.8 \) Hz, 2H), 1.62 (quint, \( J = 6.7 \) Hz, 2H), 1.42 (s, 9H); \(^13C\) NMR (150 MHz, CD\(_3\)OD) \( \delta \) 176.96 (s, 1C), 158.78 (s, 1C), 158.54 (s, 1C), 145.91 (s, 1C), 138.90 (s, 1C), 136.42 (s, 1C), 129.29 (d, 4C), 125.58 (d, 1C), 101.36 (d, 1C), 79.96 (s, 1C), 73.80 (d, 1C), 72.59 (d, 1C), 72.36 (d, 1C), 71.81 (d, 1C), 66.82 (t, 1C), 62.12 (t, 1C), 54.56 (t, 1C), 39.15 (t, 1C), 38.60 (t, 1C), 31.13 (t, 1C), 28.76 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 596.2562 for C\(_{26}\)H\(_{38}\)N\(_5\)O\(_{11}\)\(^+\), found 596.2544.

Following general procedure C, starting from 12 (70 mg, 0.093 mmol) CS5 was obtained as white solid (37 mg, 64%); \(^1H\) NMR (600 MHz, CD\(_3\)OD) \( \delta \) 8.04 (s, 1H), 7.36 (d, \( J = 8.1 \) Hz, 2H), 7.31 (d, \( J = 8.1 \) Hz, 2H), 5.58 (s, 2H), 5.05 (s, 2H), 4.83 (d, \( J = 4.7 \) Hz, 1H), 4.76 (d, \( J = 12.6 \) Hz, 1H), 4.33 (d, \( J = 4.7 \) Hz, 1H), 3.77 (dd, \( J_1 = 6.6, J_2 = 4.0 \) Hz, 1H), 3.62 (t, \( J = 6.5 \) Hz, 1H), 3.35 (dd, \( J_1 = 6.3, J_2 = 4.8 \) Hz, 1H), 3.10 (t, \( J = 6.3 \) Hz, 2H), 3.03 (t, \( J = 6.4 \) Hz, 2H), 1.50-1.45 (m, 4H), 1.42 (s, 9H); \(^13C\) NMR (150 MHz, CD\(_3\)OD) \( \delta \) 176.96 (s, 1C), 158.75 (s, 1C), 158.53 (s, 1C), 145.90 (s, 1C), 138.95 (s, 1C), 136.39 (s, 1C), 129.27 (d, 4C), 125.58 (d, 1C), 101.37 (d, 1C), 79.83 (s, 1C), 73.76 (d, 1C), 72.55 (d, 1C), 72.34 (d, 1C), 71.78 (d, 1C), 66.75 (t, 1C), 62.11 (t, 1C), 54.56 (t, 1C), 41.45 (t, 1C), 40.94 (t, 1C), 28.78 (q, 3C), 28.17 (t, 1C), 28.13 (t, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 610.2719 for C\(_{27}\)H\(_{40}\)N\(_5\)O\(_{11}\)\(^+\), found 610.2696.

[1-[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]propyl]amino]carbonyl]oxy]methyl]phenyl]-1H-1,2,3-triazol-4-yl)methyl α-L-idopyranuric acid (CS4)

[1-[4-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]amino]carbonyl]oxy]methyl]phenyl]-1H-1,2,3-triazol-4-yl)methyl α-L-idopyranuric acid (CS5)
General procedure D (Click chemistry, IDUA products and IS)

Cul (0.1 eq) was added to a solution of azide (1 eq), propargyl alcohol (1.5 eq) and Et₃N (1.2 eq) in THF (55 mL/mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was filtered and concentrated. The residue was purified by column chromatography (8 g SiO₂, 0-10% MeOH in CH₂Cl₂).

**[(1,1-Dimethyl)acetic acid, [4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl] ester (P1)**

Following general procedure D, starting from CM1a (25 mg, 0.095 mmol) P1 was obtained as a white solid (13 mg, 45%); ¹H NMR (400 MHz, CDCl₃) δ 7.61 (s, 1H), 7.36 (d, J = 7.2 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 5.49 (s, 2H), 5.1 (s, 2H), 4.79 (bs, 2H), 1.47 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 135.45 (s, 1C), 136.62 (s, 1C), 134.61 (s, 1C), 128.98 (d, 2C), 128.46 (d, 2C), 82.45 (s, 2C), 82.07 (t, 1C), 78.65 (t, 1C), 54.24 (t, 1C), 27.87 (q, 3C); HR-ESI-ORBITRAP [M+H]^+ m/z calcd. 320.1605 for C₁₆H₂₂N₃O₄, found 320.1609.

**(1,1-Dimethyl)acetic acid, [4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl-2,3-dij phenyl]methyl] ester (IS1)**

Following general procedure D, starting from CM1b (50 mg, 0.188 mmol) IS1 was obtained as a white solid (47 mg, 81%); ¹H NMR (400 MHz, CDCl₃) δ 7.57 (s, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.2 Hz, 2H), 5.47 (s, 1H), 4.77 (bs, 2H), 1.46 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 135.45 (s, 1C), 136.46 (s, 1C), 134.61 (s, 1C), 128.98 (d, 2C), 128.45 (d, 2C), 82.62 (s, 1C), 67.48 (t, 1C), 53.87 (t, 1C), 20.97 Hz, 1C), 27.86 (q, 3C); HR-ESI-ORBITRAP [M+H]^+ m/z calcd. 323.1793 for C₁₆H₂₃D₂N₃O₄, found 323.1798.

**N,N-(Diethyl)carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl ester (P2)**

Following general procedure D, starting from CM2a (25 mg, 0.095 mmol, 1 eq) P2 was obtained as a white solid (27 mg, 93%); ¹H NMR (400 MHz, CDCl₃) δ: 7.72 (bs, 1H), 7.34 (d, J = 7.6 Hz, 2H), 7.23 (d, J = 7.8 Hz, 1H), 5.49 (s, 1H), 5.1 (s, 1H), 4.82 (bs, 1H), 3.28 (s, 4H), 1.11 (t, J = 7.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 155.75 (s, 1C), 138.10 (s, 1C), 134.09 (s, 1C), 128.42 (d, 2C), 128.40 (d, 2C), 66.19 (t, 1C), 54.40 (t, 1C), 42.03 (t, 1C), 41.42 (t, 1C), 14.22 (q, 1C), 13.56 (q, 1C); HR-ESI-ORBITRAP [M+H]^+ m/z calcd. 319.1765 for C₁₆H₂₃N₂O₄, found 319.1763.

**N,N-(Diethyl)carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl-2,3-dij phenyl]methyl-2,3-dij ester (IS2)**

Following general procedure D, starting from CM2b (50 mg, 0.188 mmol) IS2 was obtained as a white solid (50 mg, 86%); ¹H NMR (400 MHz, CDCl₃) δ 7.56 (bs, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.1 Hz, 2H), 5.45 (s, 1H), 4.76 (bs, 2H), 3.61 (s, 1H), 3.26 (s, 4H), 1.09 (t, J = 7.2 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 155.75 (s, 1C), 137.90 (s, 1C), 134.07 (s, 1C), 128.44 (d, 2C), 128.34 (d, 2C), 65.58 (quint, J = 22.64 Hz, 1C), 53.86 (t, J = 21.32 Hz, 1C), 41.99 (t, 1C), 41.37 (t, 1C), 14.18 (q, 1C), 13.54 (q, 1C); HR-ESI-ORBITRAP [M+H]^+ m/z calcd. 322.1953 for C₁₆H₂₃D₃N₂O₄, found 322.1953.
N-[[1,1-Dimethylethoxy]carbonyl]amino[ethyl]carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl]phenyl)methyl ester (P3)

Following general procedure D, starting from CM3a (25 mg, 0.072 mmol) P3 was obtained as a white solid (24 mg, 83%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.44 (s, 1H), 7.32 (d, \(J = 7.9\) Hz, 2H), 7.23 (d, \(J = 7.9\) Hz, 2H), 5.48 (s, 2H), 5.38 (bs, 1H), 5.06 (s, 2H), 4.95 (bs, 1H), 4.73 (s, 2H), 3.30-3.20 (m, 4H), 1.41 (s, 9H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.74 (s, 1C), 156.63 (s, 1C), 148.36 (s, 1C), 137.52 (s, 1C), 134.45 (s, 1C), 128.81 (d, 2C), 128.42 (d, 2C), 121.79 (d, 1C), 77.48 (s, 1C), 66.15 (t, 1C), 56.59 (t, 1C), 53.99 (t, 1C), 41.71 (t, 1C), 40.65 (t, 1C), 28.49 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 406.2085 for C\(_{19}\)H\(_{28}\)N\(_2\)O\(_5\), found 406.2084.

N-[[1,1-Dimethylethoxy]carbonyl]amino[ethyl]carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl-d\(_2\)]phenyl)methyl-d\(_2\) ester (IS3)

Following general procedure D, starting from CM3b (50 mg, 0.142 mmol) IS3 was obtained as a white solid (40 mg, 69%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.43 (s, 1H), 7.33 (d, \(J = 8.0\) Hz, 2H), 7.23 (d, \(J = 8.0\) Hz, 2H), 5.47 (s, 1H), 5.36 (bs, 1H), 4.93 (bs, 1H), 4.73 (s, 2H), 3.33-3.18 (m, 4H), 1.41 (s, 9H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.76 (s, 1C), 156.61 (s, 1C) 148.33 (s, 1C), 137.38 (s, 1C), 134.42 (s, 1C), 128.86 (d, 2C), 128.42 (d, 2C), 121.73 (d, 1C), 79.79 (s, 1C), 56.59 (t, 1C), 53.71 (t, \(J_{CO} = 21.11\) Hz, 1C), 41.71 (t, 1C), 40.63 (t, 1C), 28.48 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 409.2273 for C\(_{19}\)H\(_{25}\)D\(_3\)N\(_2\)O\(_5\), found 409.2251.

N-[[1,1-Dimethylethoxy]carbonyl]amino[propyl]carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl]phenyl)methyl ester (P4)

Following general procedure D, starting from CM4a (25 mg, 0.069 mmol) P4 was obtained as a white solid (17 mg, 59%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.44 (s, 1H), 7.35 (d, \(J = 8.1\) Hz, 2H), 7.24 (d, \(J = 8.1\) Hz, 2H), 5.49 (s, 2H), 5.41 (bs, 1H), 5.07 (s, 2H), 4.85 (bs, 1H), 4.75 (s, 2H), 3.21 (q, \(J = 6.2\) Hz, 2H), 3.14 (q, \(J = 5.9\) Hz, 2H), 1.60 (quint, \(J = 6.4\) Hz, 2H), 1.42 (s, 9H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.69 (s, 2C), 148.31 (s, 1C), 137.72 (s, 1C), 134.35 (s, 1C), 128.82 (d, 2C), 128.44 (d, 2C), 121.73 (d, 1C), 79.54 (s, 1C), 66.04 (t, 1C), 56.69 (t, 1C), 54.05 (t, 1C), 37.80 (t, 1C), 37.17 (t, 1C), 30.64 (t, 1C), 28.52 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 420.2242 for C\(_{20}\)H\(_{38}\)N\(_2\)O\(_5\), found 420.2237.

N-[[1,1-Dimethylethoxy]carbonyl]amino[propyl]carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl-d\(_2\)]phenyl)methyl-d\(_2\) ester (IS4)

Following general procedure D, starting from CM4b (50 mg, 0.136 mmol) IS4 was obtained as a white solid (32 mg, 55%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.43 (s, 1H), 7.33 (d, \(J = 8.0\) Hz, 2H), 7.23 (d, \(J = 8.0\) Hz, 2H), 5.52-5.42 (m, 2H), 4.90 (s, 1H), 4.72 (s, 2H), 3.19 (q, \(J = 6.1\) Hz, 2H), 3.12 (q, \(J = 5.8\) Hz, 2H), 2.70 (s, 6H), 1.59 (quint, \(J = 6.1\) Hz, 2H), 1.41 (s, 9H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.72 (s, 1C), 156.62 (s, 1C), 148.40 (s, 1C), 137.56 (s, 1C), 134.34 (s, 1C), 128.82 (d, 2C), 128.41 (d, 2C), 121.76 (d, 1C), 79.49 (s, 1C), 56.56 (t, 1C), 53.73 (t, \(J_{CO} = 21.71\) Hz, 1C), 37.78 (s, 1C), 37.18 (t, 1C), 30.57 (t, 1C), 28.50 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 423.2430 for C\(_{20}\)H\(_{37}\)D\(_3\)N\(_2\)O\(_5\), found 423.2430.
N-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]carbamic acid, 4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl[phenyl]methyl ester (P5)

Following general procedure D, starting from CM5a (25 mg, 0.066 mmol) P5 was obtained as a white solid (16 mg, 55%). 1H NMR (400 MHz, CDCl3) δ 7.43 (s, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 7.9 Hz, 2H), 5.48 (s, 2H), 5.06 (s, 2H), 4.99 (bs, 1H), 4.74 (s, 2H), 4.63 (bs, 1H), 4.63 (bs, 1H), 4.30 (s, 1H), 4.19 (s, 1H), 3.21-3.13 (m, 2H), 3.13-3.06 (m, 2H), 1.55-1.45 (m, 4H), 1.42 (s, 9H); 13C NMR (101 MHz, CDCl3) δ 156.40 (s, 1C), 156.17 (s, 1C), 148.35 (s, 1C), 137.60 (s, 1C), 134.41 (s, 1C), 128.81 (d, 2C), 128.41 (d, 2C), 121.73 (d, 1C), 79.37 (s, 1C), 66.01 (t, 1C), 56.61 (t, 1C), 53.97 (t, 1C), 40.22 (t, 1C), 28.52 (q, 3C), 27.43 (t, 1C), 27.26 (t, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 434.2398 for C21H32N5O5+, found 434.2422.

N-[[[(1,1-Dimethylethoxy)carbonyl]amino]butyl]carbamic acid, [4-[[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]methyl-d1]phenyl]methyl-d2] ester (IS5)

Following general procedure D, starting from CM5b (50 mg, 0.131 mmol) IS5 was obtained as a white solid (42 mg, 72%); 1H NMR (400 MHz, CDCl3) δ 7.43 (s, 1H), 7.34 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 5.47 (s, 1H), 4.97 (bs, 1H), 4.74 (s, 2H), 4.62 (bs, 1H), 4.55-1.45 (m, 4H), 1.42 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 156.43 (s, 1C), 156.19 (s, 1C), 148.38 (s, 1C), 137.54 (s, 1C), 134.39 (s, 1C), 128.86 (d, 12C), 128.43 (d, 2C), 121.72 (d, 1C), 79.38 (s, 1C), 56.64 (t, 1C), 53.72 (t, JCD = 21.5 Hz, 1C), 40.83 (t, 1C), 40.25 (t, 1C), 28.54 (q, 3C), 27.45 (t, 1C), 27.29 (t, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 437.2586 for C21H29D3N5O5+, found 437.2570.

GALNS Sets (Substrates, internal standards, products)

General procedure E (click chemistry & deprotection, GALNS substrates)

Azide (1 eq) and alkyne 14 (1 eq) were dissolved in dry THF (19 mL/mmol alkyne) under argon and Et₃N (1.2 eq) was added in one portion at room temperature. The reaction was initiated by the addition of Cul (0.1 eq). After 10 minutes the formation of a bright, yellow complex was observed. The reaction was stirred at room temperature for 4 hours. After completion, the mixture was concentrated and directly subjected to Zemplén conditions by re-suspending the crude mixture in dry methanol (30 mmol/mL) and addition of sodium methoxide in methanol (4.88 M, 2 eq). The reaction was stirred overnight and completion was verified by LCMS. Sodium bicarbonate (20 eq) was added in one portion and the mixture was stirred for 30 minutes. The suspension was filtered and the residue was washed three times with methanol. The combined filtrate was concentrated, re-dissolved in water containing 3% MeCN and subjected to prep-HPLC (AXIA 30x100 mm, 5µm coreshell C18XB on a Reveleris X2, 50 mL/min, 5-70% MeCN in H2O).
N-[[4-[[6-O-Sulfato-β-D-galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl][methyl]phenyl][methyl]oxy][carbonyl][amino][ethyl]carbamic acid, 1,1-dimethylethyl ester (CS6)

Following general procedure E, 14 (40 mg, 83 µmol) was reacted with CM3a (29 mg, 83 µmol) to obtain CS6 (40 mg, 75%) as a white solid; 1H NMR (400 MHz, CD3OD): δ 8.14 (s, 1H), 7.43-7.35 (m, 4H), 5.62 (s, 2H), 5.08 (s, 2H), 4.31 (d, J = 7.4, 1H), 4.19 (d, J = 6.1, 2H), 3.86 (d, J = 3.1, 1H), 3.78 (t, J = 6.1, 1H), 3.57-3.50 (m, 1H), 3.47 (dd, J1 = 9.7, J2 = 3.3, 1H), 3.18 (d, J = 5.0, 2H), 3.14 (d, J = 4.9, 2H), 1.44 (s, 9H); 13C NMR (100 MHz, CD3OD): δ 157.49 (s, 1C), 157.20 (s, 1C), 144.68 (s, 1C), 137.35 (s, 1C), 135.23 (s, 1C), 128.09 (d, 2C), 127.88 (d, 2C), 124.86 (d, 1C), 103.30 (d, 1C), 78.72 (s, 1C), 73.24 (d, 1C), 73.16 (d, 1C), 70.94 (d, 1C), 68.78 (d, 1C), 66.73 (t, 1C), 65.54 (t, 1C), 62.16 (t, 1C), 53.16 (t, 1C), 40.41 (t, 1C), 39.83 (t, 1C), 27.34 (q, 3C); HR-ESI-ORBITRAP [M-H]⁻ m/z calcd. 646.20248 for C26H38N5O13S, found 646.20271.

N-[[4-[[6-O-Sulfato-β-D-galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl][methyl]phenyl][methyl]oxy][carbonyl][amino][propyl]carbamic acid, 1,1-dimethylethyl ester (CS7)

Following general procedure E, 14 (40 mg, 83 µmol) was reacted with CM4a (30 mg, 83 µmol) to obtain CS7 (44 mg, 80%) as a white solid; 1H NMR (600 MHz, CD3OD): δ 8.14 (s, 1H), 7.39-7.36 (m, 4H), 5.62 (s, 2H), 5.07 (s, 2H), 4.90 (d, J = 12.7, 1H), 4.85 (d, J = 12.8, 1H), 4.32 (d, J = 7.9, 1H), 4.19 (d, J = 6.2, 2H), 3.87 (dd, J1 = 3.4, J2 = 1.1, 1H), 3.79 (t, J = 6.1, 1H), 3.54 (dd, J1 = 9.7, J2 = 7.7, 1H), 3.49 (dd, J1 = 9.72, J2 = 3.3, 1H), 3.14 (t, J = 6.8, 2H), 3.07 (t, J = 6.7, 2H), 1.64 (quint, J = 6.8, 2H), 1.45 (s, 9H); 13C NMR (150 MHz, CD3OD): δ 157.43 (s, 1C), 157.17 (s, 1C), 144.66 (s, 1C), 137.42 (s, 1C), 135.20 (s, 1C), 128.09 (d, 2C), 127.86 (d, 2C), 124.78 (d, 1C), 103.23 (d, 1C), 78.59 (s, 1C), 73.20 (d, 1C), 73.13 (d, 1C), 70.93 (d, 1C), 68.76 (d, 1C), 66.67 (t, 1C), 65.50 (t, 1C), 62.14 (t, 1C), 53.18 (t, 1C), 37.78 (t, 1C), 37.25 (t, 1C), 29.75 (t, 1C), 27.39 (q, 3C); HR-ESI-ORBITRAP [M-H]⁻ m/z calcd. 660.21813 for C26H38N5O13S, found 660.21842.

N-[[4-[[6-O-Sulfato-β-D-galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl][methyl]phenyl][methyl]oxy][carbonyl][amino][butyl]carbamic acid, 1,1-dimethylethyl ester (CS8)

Following general procedure E, 14 (40 mg, 83 µmol) was reacted with CM5a (31 mg, 83 µmol) to obtain CS8 (44 mg, 78%) as a white solid; 1H NMR (600 MHz, CD3OD): δ 8.15 (s, 1H), 7.41-7.36 (d, 4H), 5.62 (s, 2H), 5.07 (s, 2H), 4.90-4.84 (m, 2H), 4.32 (d, J = 7.6, 1H), 4.19 (d, J = 6.2, 2H), 3.87-3.85 (m, 1H), 3.79 (t, J = 6.1, 1H), 3.53 (dd, J1 = 9.8, J2 = 7.5, 1H), 3.48 (dd, J1 = 9.7, J2 = 3.6, 1H), 3.12 (t, J = 6.4, 2H), 3.05 (t, J = 6.4, 2H), 1.53-1.47 (m, 4H), 1.44 (s, 9H); 13C NMR (150 MHz, CD3OD): δ 157.41 (s, 1C), 157.16 (s, 1C), 144.67 (s, 1C), 137.47 (s, 1C), 135.20 (s, 1C), 128.09 (d, 2C), 127.83 (d, 2C), 124.85 (d, 1C), 103.30 (d, 1C), 78.45 (s, 1C), 73.22 (d, 1C), 73.14 (d, 1C), 70.92 (d, 1C), 68.78 (d, 1C), 66.73 (t, 1C), 65.42 (t, 1C), 62.16 (t, 1C), 53.16 (t, 1C), 40.07 (t, 1C), 39.57 (t, 1C), 27.39 (q, 3C), 26.79 (t, 1C), 26.75 (t, 1C); HR-ESI-ORBITRAP [M-H]⁻ m/z calcd. 674.23378 for C32H40N6O13S, found 674.2338.
General procedure F (Click chemistry, GALNS products and IS)

Azide (1 eq) and alkyne 13 (1 eq) were dissolved in dry THF (19 mL/mmol alkyne) under argon and Et₃N (1.2 eq) was added in one portion at room temperature. The reaction was initiated by the addition of Cul (0.1 eq). After 10 minutes the formation of a bright, yellow complex was observed. The reaction was stirred at room temperature for 4 hours. After completion, the mixture was concentrated and directly subjected to column chromatography (SiO₂, MeOH in CH₂Cl₂, 0-20% gradient elution).

N-[[[[4-[[β-D-Galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl-dJphenyl]methyl-dJoxy]carbonyl] aminoethyl]carboxylic acid, 1,1-dimethylethyl ester (IS6)

Following general procedure F, 13 (10 mg, 46 µmol) was reacted with CM3b (16 mg, 46 µmol) to obtain IS6 (20 mg, 76%) as a white solid; ¹H NMR (200 MHz, CD₂OD): ½ 8.00 (s, 1H), 7.38 (d, J = 8.1, 2H), 7.31 (d, J = 8.4, 2H), 5.57 (s, 1H), 4.96 (d, J = 12.5, 1H), 4.76 (d, J = 12.5, 1H), 4.32 (d, J = 7.0, 1H), 3.85-3.80 (m, 1H), 3.77-3.70 (m, 2H), 3.56-3.45 (m, 3H), 3.19-3.10 (m, 4H), 1.42 (s, 9H); ¹³C NMR (50 MHz, CD₂OD): ½ 158.85 (s, 1C), 158.57 (s, 1C), 146.27 (s, 1C), 138.89 (s, 1C), 136.39 (s, 1C), 129.38 (d, 2C), 129.31 (d, 2C), 125.34 (d, 1C), 104.30 (d, 1C), 80.15 (s, 1C), 76.80 (d, 1C), 74.87 (d, 1C), 72.42 (d, 1C), 70.29 (d, 1C), 63.06 (t, 1C), 62.55 (t, 1C), 41.84 (t, 1C), 41.22 (t, 1C), 28.75 (q, 3C); HR-ESI-ORBITRAP [M+H]⁺ m/z calcd. 571.28015 for C₂₅H₃₈D₇NO₁₀⁺, found 571.27863.

N-[[[[4-[[β-D-Galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl]oxy]carbonyl] aminoethyl]carboxylic acid, 1,1-dimethylethyl ester (P6)

Following general procedure F, 13 (30 mg, 138 µmol) was reacted with CM3a (48 mg, 138 µmol) to obtain P6 (62 mg, 79%) as a white solid; ¹H NMR (200 MHz, CD₂OD): ½ 7.99 (s, 1H), 7.37 (d, J = 8.3, 2H), 7.30 (d, J = 8.3, 2H), 5.58 (s, 2H), 5.06 (s, 2H), 4.95 (d, J = 12.5, 1H), 4.76 (d, J = 12.5, 1H), 4.33 (d, J = 7.3, 1H), 3.85-3.80 (m, 1H), 3.78-3.69 (m, 2H), 3.57-3.45 (m, 3H), 3.22-3.08 (m, 4H), 1.42 (s, 9H); ¹³C NMR (50 MHz, CD₂OD): ½ 158.84 (s, 1C), 158.56 (s, 1C), 146.26 (s, 1C), 138.92 (s, 1C), 136.42 (s, 1C), 129.33 (m, 4C), 125.40 (d, 1C), 104.31 (d, 1C), 80.14 (s, 1C), 76.80 (d, 1C), 74.87 (d, 1C), 72.43 (d, 1C), 70.30 (d, 1C), 66.88 (t, 1C), 63.07 (t, 1C), 62.57 (t, 1C), 54.63 (t, 1C), 41.86 (t, 1C), 41.24 (t, 1C), 28.77 (q, 3C); HR-ESI-ORBITRAP [M+H]⁺ m/z calcd. 568.26132 for C₂₅H₃₈D₇NO₁₀⁺, found 568.2601.

N-[[[[4-[[β-D-Galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl-dJphenyl]methyl-dJoxy]carbonyl] amino]propyl]carboxylic acid, 1,1-dimethylethyl ester (IS7)

Following general procedure F, 13 (10 mg, 46 µmol) was reacted with CM4b (17 mg, 46 µmol) to give IS7 (19 mg, 70%) as a white solid; ¹H NMR (600 MHz, CD₂OD): ½ 8.00 (s, 1H), 7.38 (d, J = 8.1, 2H), 7.33 (d, J = 8.1, 2H), 5.58 (d, J = 2.9, 1H), 4.97 (d, J = 12.1, 2H), 4.78 (d, J = 12.5, 1H), 4.34 (d, J = 7.6, 1H), 3.85-3.83 (m, 1H), 3.77 (dd, J₁ = 11.4, J₂ = 7.1, 1H), 3.72 (dd, J₁ = 11.3, J₂ = 5.2, 1H), 3.57-3.51 (m, 2H), 3.47 (dd, J₁ = 9.7, J₂ = 3.4, 1H), 3.14 (t, J = 6.8, 2H), 3.07 (t, J = 6.6, 2H), 1.63 (quint, J = 6.8, 2H), 1.44 (s, 9H); ¹³C NMR (150 MHz, CD₂OD): ½ 157.41 (s, 1C), 157.17 (s, 1C), 144.88 (s, 1C), 137.49 (s, 1C), 137.45 (s, 1C), 134.98 (s, 1C), 127.98 (d, 2C), 127.93 (d, 2C), 123.96 (d, 1C), 102.91 (d, 1C), 78.60 (s, 1C), 75.41 (d, 1C), 73.48 (d, 1C), 71.03 (d, 1C), 68.91 (d, 1C), 64.86 (quint, 1CD₂), 61.67 (t, 1C), 61.17 (t, 1C), 52.98 (t,
N-[[[4-[[6-D-Galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl]oxy]carbonyl]amino]propyl]carbamic acid, 1,1-dimethylethyl ester (P7)

Following general procedure F, 13 (30 mg, 138 µmol) was reacted with CM4a (50 mg, 138 µmol) to give P7 (66 mg, 82%) as a white solid; 1H NMR (600 MHz, CD3OD): δ 8.01 (s, 1H), 7.39 (d, J = 8.0, 2H), 7.34 (d, J = 8.1, 2H), 5.61 (s, 2H), 5.08 (s, 2H), 4.97 (d, J = 12.5, 1H), 4.79 (d, J = 12.5, 1H), 4.33 (d, J = 7.7, 1H), 3.84 (dd, J1 = 3.4, J2 = 0.8, 1H), 3.77 (dd, J1 = 11.3, J2 = 7.0, 1H), 3.72 (dd, J1 = 11.3, J2 = 5.1, 1H), 3.56-3.51 (m, 2H), 3.47 (dd, J1 = 9.7, J2 = 3.4, 1H), 3.14 (t, J = 6.8, 2H), 3.07 (t, J = 6.7, 2H), 1.64 (quint, J = 6.8, 2H), 1.45 (s, 9H); 13C NMR (150 MHz, CD3OD): δ 157.40 (s, 1C), 157.17 (s, 1C), 144.95 (s, 1C), 137.62 (s, 1C), 135.01 (s, 1C), 127.90 (d, 4C), 123.98 (d, 1C), 102.91 (d, 1C), 78.58 (s, 1C), 75.41 (d, 1C), 73.48 (d, 1C), 71.01 (d, 1C), 68.90 (d, 1C), 65.43 (t, 1C), 61.66 (t, 1C), 61.16 (t, 1C), 53.23 (t, 1C), 37.76 (t, 1C), 37.21 (t, 1C), 29.75 (t, 1C), 27.36 (q, 3C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 582.2770 for C26H30NO15, found 582.2760.

N-[[[4-[[6-D-Galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl]-d2]phenyl]methyl]d2]oxy]carbonyl] amino]butyl]carbamic acid, 1,1-dimethylethyl ester (IS8)

Following general procedure A, 13 (10 mg, 46 µmol) was reacted with CM5b (18 mg, 46 µmol) to give IS8 (20 mg, 72%) as a white solid; 1H NMR (400 MHz, CD3OD): δ 8.03 (s, 1H), 7.38 (d, J = 8.8, 2H), 7.33 (d, J = 9.0, 2H), 5.59 (s, 1H), 4.97 (d, J = 12.5, 1H), 4.79 (d, J = 12.4, 1H), 4.46-4.44 (m, 1H), 4.35 (d, J = 7.5, 1H), 3.88-3.84 (m, 1H), 3.79-3.72 (m, 1H), 3.56-3.52 (m, 1H), 3.49 (dd, J1 = 9.8, J2 = 3.3, 1H), 3.12 (t, J = 6.4, 2H), 3.04 (t, J = 6.1, 2H), 1.52-1.47 (m, 4H), 1.44 (s, 9H); 13C NMR (100 MHz, CD3OD): δ 157.40 (s, 1C), 157.17 (s, 1C), 144.82 (s, 1C), 137.54 (s, 1C), 134.96 (s, 1C), 127.94 (d, 4C), 124.02 (d, 1C), 102.87 (d, 1C), 78.79 (s, 1C), 75.35 (d, 1C), 73.45 (d, 1C), 71.04 (d, 1C), 70.89 (d, 1C), 68.92 (d, 1C), 61.13 (t, 1C), 55.16 (t, 1C), 40.11 (t, 1C), 39.60 (t, 1C), 27.44 (q, 3C), 26.82 (t, 1C), 26.76 (t, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 599.311449 for C27H31D6N2O15, found 599.31016.

N-[[[4-[[6-D-Galactopyranosyl]methyl]-1H-1,2,3-triazol-1-yl]methyl]phenyl]methyl]oxy]carbonyl]amino]butyl]carbamic acid, 1,1-dimethylethyl ester (P8)

Following general procedure E, 13 (30 mg, 138 µmol) was reacted with CM4a (52 mg, 138 µmol) to give P8 (72 mg, 87%) as a white solid; 1H NMR (400 MHz, CD3OD): δ 8.02 (s, 1H), 7.39 (d, J = 8.0, 2H), 7.34 (d, J = 8.0, 2H), 5.61 (s, 2H), 5.08 (s, 2H), 4.97 (d, J = 12.5, 1H), 4.79 (d, J = 12.4, 1H), 4.44 (dd, J1 = 5.0, J2 = 2.3, 1H), 4.34 (d, J = 7.6, 1H), 3.87-3.83 (m, 1H), 3.79-3.70 (m, 1H), 3.56-3.50 (m, 1H), 3.47 (dd, J1 = 9.8, J2 = 3.1, 1H), 3.12 (t, J = 5.9, 2H), 3.05 (t, J = 5.7, 2H), 1.52-1.47 (m, 4H), 1.44 (s, 9H); 13C NMR (100 MHz, CD3OD): δ 157.38 (s, 1C), 157.16 (s, 1C), 144.86 (s, 1C), 137.67 (s, 1C), 134.99 (s, 1C), 127.90 (d, 2C), 127.87 (d, 2C), 123.97 (d, 1C), 102.89 (d, 1C), 78.46 (s, 1C), 75.38 (d, 1C), 71.01 (d, 1C), 70.86 (d, 1C), 68.92 (d, 1C), 61.67 (t, 1C), 61.11 (t, 1C), 55.09 (t, 1C), 53.23 (t, 1C), 40.08 (t, 1C), 39.58 (t, 1C), 27.39 (q, 3C), 26.80 (t, 1C), 26.75 (t, 1C); HR-ESI-ORBITRAP [M+H]+ m/z calcd. 596.2926 for C27H42N5O16, found 596.2918.
3) Analytical methods and materials used for enzyme assays and DBS analysis

Materials and reagents

Acetonitrile (LC-MS Grade), methanol (Ultra LC-MS Grade) and water (Ultra LC-MS Grade) were obtained from Carl Roth GmbH (Austria). α-L-iduronidase (IDUA) and all other chemicals were obtained from Sigma-Aldrich (Germany). Equipment used to perform the enzyme assays: MT-plate shaker TiMix 5 control equipped with incubation hood TH15 (Edmund Bühler GmbH, Germany), Centrifuge 5810 from Eppendorf (Germany), DBS Puncher® Instrument from Perkin Elmer (USA), balance (PCB 200-2) from Kern&Sohn GmbH (Germany), pH-meter (seven compact) from Mettler Toledo (USA). DBS quality control cards were kindly provided by the CDC (Center for Disease Control and Prevention, USA), including permission for their use in this and related studies. DBS samples of newborns were provided by the Medical University of Vienna and the Vienna General Hospital (Newborn Screening Program) and anonymized DBS samples of affected patients (non-newborns) were kindly provided by the University Medical Center of the Johannes Gutenberg-University Mainz (Germany). The institutional ethics committee approved the study (EK 478/2009 and EK 1687/2014).

Assaying IDUA

IDUA assay buffer

Sodium formate (1.9 g) was dissolved in water (250 mL). The pH was adjusted to 4.04 by addition of formic acid. The buffer solution was partitioned to give 14 vials, each containing 17.5 mL. The buffer was stored at -20 °C. A D-saccharolactone inhibitor solution (3 mmol/L) was prepared by dissolving D-saccharolactone (CAS 61278-30-6, 31.5 mg) in water (40 mL) followed by vortexing for 2 min. Water was added to reach an end volume of 50 mL. 500 µL of the inhibitor solution (3 mmol/L) were added to 17.5 mL assay buffer and the final solution was used immediately. Saccharolactone is used to inhibit β-glucuronidase (GUSB) and thus prevent unwanted enzymatic cleavage of IDUA substrates by GUSB and has been included in assay buffers of previously developed methods.6-9

Preparation of IDUA assay cocktail

A 2 mg/mL substrate stock solution in assay buffer and a 13.2 µg/mL IS stock solution in assay buffer were freshly prepared and mixed in a 50:50 ratio to result in an assay cocktail containing 1 mg/mL substrate and 6.6 µg/mL internal standard (IS).

Sample preparation using recombinant human IDUA

10UG enzyme formulation was diluted in 200 µL assay buffer. 16 µL of the resulting solution were added to 1984 µL assay buffer and vortexed for 2 minutes to reach a 400 ng/mL stock solution. This stock was further diluted in assay buffer to reach 200 ng/mL (500 µL + 500 µL buffer), 100 ng/mL (250 µL + 750 µL buffer) and 50 ng/mL (125 µL + 875 µL buffer) concentrations. The respective assay cocktail (see above) was mixed in equal amounts with enzyme solutions (13.5 µL enzyme solution + 13.5 µL assay cocktail) in duplicates and incubated in a well plate for 20 min at 22 °C and 250 rpm orbital shake. The well plate was centrifuged for 3 minutes at 3000 rpm and then quenched by adding 75 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was then centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. Finally, 25 µL of supernatant were transferred into a new plate and diluted with 175 µL water for analysis.

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**Sample preparation – DBS assay (IDUA)**

DBS cards were punched as 3.2 mm spots into a well plate and each spot was extracted with 70 µL of PBS buffer for 1 h at 37 °C. DBS cards without blood were used as controls (blank). 15 µL of the assay cocktail and 10 µL of the sample extracts were mixed in a new well plate, covered with adhesive aluminum foil and incubated for 22 h at 37 °C at 500 rpm orbital shake. The plate was centrifuged for 3 minutes at 3000 rpm and then quenched by addition of 75 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. Finally, 25 µL of supernatant were transferred into a new plate and diluted with 175 µL water for analysis.

**Sample preparation – Triplex DBS assay (IDUA)**

DBS cards were punched as 3.2 mm spots into a well plate and each spot was extracted with 70 µL of PBS Buffer for 1 h at 37 °C. DBS cards without blood were used as controls (blank). 15 µL of assay cocktail and 10 µL of the sample extracts were mixed in a new well plate, covered with adhesive aluminum foil and incubated for 22 h at 37 °C at 500 rpm orbital shake. The plate was centrifuged for 3 minutes at 3000 rpm and then quenched by addition of 75 µL MeCN to each well. The plate was covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. For multiplex measurements 25 µL supernatant of three individually incubated plates that contain different assay cocktail sets (3, 4 and 5) were combined into the same position on a new well plate resulting in 75 µL of mixed sets that were subsequently diluted with 125 µL water for analysis.

**Liquid chromatography, mass spectrometry, data analysis (IDUA)**

Chromatography was performed on a Shimadzu Nexera X2 system (Japan). Samples (1 µL) were injected onto a Waters (ACQUITY UPLC Peptide BEH C18 Column, 130 Å, 1.7 µm, 2.1 mm x 50 mm) column maintained at 60 °C in reversed phase mode. Solvent A contained 0.1 % formic acid in water. Solvent B contained 0.1 % formic acid in MeCN/MeOH = 1:1 (v/v). The flow rate was set to 0.7 mL/min with the following gradient conditions: 0–2.5 min, 19 % B (isocratic); 2.5–2.53 min, 19–95% B (linear gradient); 2.53–3.5 min, 95 % B (isocratic); 3.5–3.53 min, 95–19 % B (linear gradient); 3.53–4.9 min, 19 % B (isocratic). The total run time was 4.9 min with 1 µL injection. Autosampler temperature was set to 5 °C. Mass spectrometric detection was performed using a Shimadzu 8050 spectrometer (Japan) operated in positive ion electrospray ionization (ESI) mode. The source parameters of the mass spectrometer were optimized and maintained as follows: nebulizing gas flow, 3 L/min; heating gas flow, 10 L/min; interface temperature, 400 °C; DL temperature, 200 °C; ion spray voltage, 4000 V; heating block temperature, 300 °C; drying gas flow, 10 L/min; collision pressure (argon), 270 kPa. Nitrogen was used as nebulizer, auxiliary and curtain gas. Data was processed using LabSolutions 5.8 (Shimadzu) and analyzed in Prism 7 (GraphPad). Results are summarized in Table S1. Means of different groups of patients were compared using the Mann-Whitney U test. For MRM see Table S2.
Table S1. Results of DBS assays using IDUA sets 3-5; shown as blank-corrected “mean values (SD)” [µM/h]

| IDUA                        | Set 3               | Set 4               | Set 5               |
|-----------------------------|---------------------|---------------------|---------------------|
| CDC QCL                    | 0.151 (0.037)       | 0.152 (0.019)       | 0.169 (0.008)       |
| CDC QCM                    | 0.277 (0.076)       | 0.298 (0.052)       | 0.305 (0.029)       |
| CDC QCH                    | 0.473 (0.065)       | 0.468 (0.095)       | 0.481 (0.031)       |
| DBS of confirmed MPS I     | 0.170 (0.032)       | 0.116 (0.053)       | 0.084 (0.039)       |
| patients (n=9, anonymized) |                     |                     |                     |
| DBS of random patients      | 0.404 (0.056)       | 0.431 (0.069)       | 0.368 (0.091)       |
| (n=88, anonymized)          |                     |                     |                     |

Table S2. IDUA Multiple reaction monitoring (MRM).

|                  | Precursor [m/z] | Product 1 [m/z] | Product 2 [m/z] | Product 3 [m/z] |
|------------------|-----------------|-----------------|-----------------|-----------------|
| Set 1 P          | 320.20          | 264.10          | 165.10          | 202.20          |
| Set 1 IS         | 323.20          | 267.10          | 168.10          | 205.10          |
| Set 2 P          | 319.20          | 100.05          | 220.20          | 202.10          |
| Set 2 IS         | 322.20          | 100.15          | 223.15          | 205.20          |
| Set 3 P          | 406.30          | 306.10          | 202.20          | 146.10          |
| Set 3 IS         | 409.30          | 309.20          | 205.15          | 166.25          |
| Set 4 P          | 420.30          | 320.15          | 105.15          | 202.10          |
| Set 4 IS         | 423.30          | 323.20          | 205.15          | 107.15          |
| Set 5 P          | 434.30          | 334.25          | 202.15          | 174.20          |
| Set 5 IS         | 437.30          | 337.25          | 205.15          | 177.15          |

Assaying GALNS

GALNS assay buffer
Sodium formate (1.7 g) was dissolved in water (200 mL). To the stirred solution were added 430 µL formic acid and lead (II) formate (2.23 g) in small portions. The mixture was stirred for approximately 20 minutes, until it formed a clear, homogenous solution. The pH was adjusted to 4.04 by addition of 0.1 M NaOH solution. Water was added to reach an end volume of 250 mL. The buffer was freshly prepared once a month and stored at 0-5 °C.

Preparation of GALNS assay cocktail
A 2 mg/mL substrate stock solution in assay buffer and a 20 µg/mL IS stock solution in assay buffer were freshly prepared and mixed in a 1:1 ratio to obtain an assay cocktail containing 1 mg/mL substrate and 10 µg/mL internal standard.

Sample preparation – DBS assay (GALNS)
DBS cards were punched as 3.2 mm spots (4 spots each for blank, QCL, QCM, QCH; 2 spots each for affected patient DBS, and 1 spot each for random newborn DBS) into a 96 well plate. DBS cards without blood were used as controls (blank). Then 30 µL of assay cocktail were added, the plate was covered with adhesive aluminum foil and incubated for 22 h at 37 °C at 500 rpm orbital shake. The plate was centrifuged for 3 minutes at 3000 rpm and the assays quenched by adding 100 µL MeCN to each well.
The plate was again covered with aluminum foil and subjected to orbital shaking at 350 rpm and room temperature for 2 min. The plate was centrifuged for 15 min at 3200 rpm and subsequently cooled to 4 °C for 10 min to complete precipitation. A volume of 50 µL of supernatant was transferred to a new plate and diluted with 100 µL water for analysis.

Liquid chromatography, mass spectrometry, data analysis (GALNS)

Chromatography was performed on a TLX2 system with Accela pumps and a PAL RTC autosampler (Switzerland). Samples (1 µL) were analyzed using a Waters ACQUITY CSH column (C18, 130 Å, 1.7 µm, 2.1 x 50 mm) and a Turboflow HTC column (Thermo Cyclom P, 0.5 x 50 mm) maintained at 40 °C. Solvent A contained 0.1 % formic acid in water. Solvent B contained 0.1 % formic acid in MeCN/MeOH = 1:1 (v/v). A mixture of MeCN/2-propanol/acetone = 45:45:10 (v/v) was used as solvent C. The autosampler was run at room temperature. The total flow rate during separation was 0.7 mL/min with an injection volume of 1 µL, using a turboflow® online sample pre-treatment according to following gradient conditions:

Mass spectrometric detection was performed with a Thermo Scientific TSQ Quantum Ultra (USA) operated in positive ion electrospray ionization (ESI) mode. The source parameters of the mass spectrometer were optimized and used as follows: ion spray voltage, 4500 V; vaporizer temperature, 289°C; sheath gas pressure, 35 psi; ion sweep gas pressure, 2.0 psi, aux gas pressure, 35 psi; capillary temperature, 289 °C, skimmer offset, 0; argon was used as collision gas; nitrogen was used as nebulizer, auxiliary and curtain gas. Data was processed using LCquan™ 2.7 (Thermo Scientific, USA) and analyzed in Prism 7 (GraphPad). Results are summarized in Table S3. Means of different groups of patients were compared using the Mann-Whitney U test. For MRM see Table S4.
Table S3. Results of DBS assays using GALNS sets 6-8; shown as blank-corrected “mean values (SD)” [μM/h]

| GALNS               | Set 6     | Set 7     | Set 8     |
|---------------------|-----------|-----------|-----------|
| CDC QCL             | 1.26 (0.13) | 1.21 (0.14) | 1.51 (0.09) |
| CDC QCM             | 7.30 (0.03) | 8.27 (0.09) | 7.08 (0.02) |
| CDC QCH             | 12.50 (0.75) | 13.11 (0.06) | 12.11 (0.46) |
| DBS of confirmed MPS IVa patients (n=9, anonymized) | 0.756 (0.722) | - | - |
| Random patients (n=116, anonymized) | 13.50 (5.87) | - | - |

Table S4. GALNS Multiple reaction monitoring.

| Precursor [m/z] | Product 1 [m/z] | Product 2 [m/z] | Product 3 [m/z] |
|-----------------|-----------------|-----------------|-----------------|
| Set 6 P         | 568.3           | 306.15          | 468.1           | 202.15          |
| Set 6 IS        | 571.323         | 471.150         | -               | -               |
| Set 7 P         | 582.3           | 320.2           | 482.2           | 105.05          |
| Set 7 IS        | 585.313         | 485.12          | 323.15          | -               |
| Set 8 P         | 596.3           | 496.15          | 334.2           | 174.1           |
| Set 8 IS        | 599.327         | 499.05          | 327.09          | -               |
4) NMR spectra
5) References

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