Evidence for a Multivalent Effect in Inhibition of Sulfatases Involved in Lysosomal Storage Disorders (LSDs)

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Synthesis of \((2R,3R,4R)-3,4\text{-bis(benzyloxy)-2-[(benzyloxy)methyl]-1-[6-azido]hexyl}-1\text{H}\text{-pyrrolidine (4)}

To a solution of \(1a\) (125 mg, 0.3 mmol) in 6 ml of ethanol, \(\text{NaBH}_4\) (23 mg, 0.6 mmol) was added and the mixture was stirred at room temperature for 16 h, until a TLC analysis (AcOEt:EP 1:1) showed the disappearance of the starting material (\(R_f = 0.30\)) and the formation of a new product (\(R_f = 0.91\)). After the addition of 0.5 ml of MeOH and 1.5 ml of \(\text{H}_2\text{O}\) the mixture was raised at room temperature and stirred for another 3 h, then it was evaporated under reduced pressure and the crude obtained filtered through Celite® washing the solid several times with ethyl ether. The collected solution was evaporated under reduced pressure affording the desired pure hydroxylamine intermediate (124 mg, 0.3 mmol) that was dissolved in 4 ml of acetic acid. \(\text{Zn}\) powder (351 mg, 6 mmol) and 4 ml of \(\text{H}_2\text{O}\) were added. The reaction mixture was stirred at room temperature for 1.5 h until a TLC analysis (\(\text{CH}_2\text{Cl}_2:\text{MeOH} 30:1\)) showed the disappearance of the starting material (\(R_f = 0.81\)) and the formation of a new product (\(R_f = 0.21\)). After filtration through cotton, the mixture was concentrated at reduced pressure and then saturated aqueous solution of \(\text{Na}_2\text{CO}_3\) was added at 0 °C until basic \(\text{pH}\). After extraction with AcOEt (3 x 30 mL), the organic layers were dried on \(\text{Na}_2\text{SO}_4\), concentrated under reduced pressure and the crude was purified by Flash Column Chromatography (\(\text{CH}_2\text{Cl}_2:\text{MeOH} 30:1\)) affording pure \(2\) (\(R_f = 0.21, 119\) mg, 0.29 mmol, 98% over two steps) as a colorless oil [for an alternative synthesis see of compound \(2\) W.-C. Cheng, C.-Y. Wenga, W.-Y. Yun, S.-Y. Chang, Y.-C. Lin, F.-J. Tsai, F.-Y. Huang and Y.-R. Chen, *Bioorg. Med. Chem*, 2013, 21, 5021].

A solution of \(2\) (175 mg, 0.43 mmol), 1-azido-6-bromohexane (3, 134 mg, 0.65 mmol) and TEA (120 µL, 0.86 mmol) in 4 ml of \(\text{THF}\) was stirred in microwave at 150°C. After 2 h a TLC analysis (\(\text{CH}_2\text{Cl}_2:\text{MeOH} 10:1\)) still showed the presence of the starting material (\(R_f = 0.51\)), then 0.5 equivalents of 1-azido-6-bromohexane (3) were added and the reaction mixture was stirred in microwave at 150°C for another 2 h until complete formation of the desired product (\(R_f = 0.98, \text{CH}_2\text{Cl}_2:\text{MeOH} 10:1\)) was achieved. After evaporation under reduced pressure, the crude was purified by Flash Column Chromatography (EP:AcOEt 4:1) affording pure \(4\) (\(R_f = 0.31, 203\) mg, 0.38 mmol, 88%) as a yellow oil. \([\alpha]_D^{30} = -25.1\) (c
= 1.30, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ = 7.35-7.25 (m, 15H, H-Ar), 4.57-4.42 (m, 6H, H-Bn), 3.92 (d, J = 4.8 Hz, 1H, H-4), 3.88 (d, J = 3.9 Hz, 1H, H-3), 3.62-3.50 (m, 1H, Ha-6), 3.52 (dd, J = 9.8, 6.3 Hz, 1H, Hb-6), 3.24 (t, J = 7.1 Hz, 2H, H-12), 3.24-3.19 (m, 1H, Ha-5), 2.88-2.81 (m, 1H, Ha-7), 2.71 (bs, 1H, H-2), 2.55 (dd, J = 10.3, 4.9 Hz, 1H, Hb-5), 2.38-2.31 (m, 1H, Hb-7), 1.59 (quint, J = 7.1 Hz, 2H, H-11), 1.54-1.48 (m, 2H, H-8), 1.41-1.25 (m, 4H, H-9 and H-10); ¹³C-NMR (50 MHz, CDCl₃): δ = 138.5, 138.4, 138.3 (s, 3C, C-Ar), 128.3-127.6 (d, 15C, C-Ar), 85.5 (d, C-3), 81.7 (d, C-4), 73.2 (t, C-Bn) 71.3 (t, C-Bn), 71.2 (t, C-Bn), 71.0 (t, C-6), 69.4 (d, C-2), 57.3 (t, C-5), 55.5 (t, C-7), 51.4 (t, C-12), 28.8 (t, C-11), 28.0 (t, C-8), 27.0, 26.6 (t, 2C, C-9 and C-10); IR (CDCl₃): ν = 3088, 3066, 3032, 3009, 2937, 2862, 1496, 1453, 1365, 1261, 1096 cm⁻¹; MS (ESI): m/z 529.42 ([M+1]⁺; 100), 551.42 ([M+Na]⁺; 38); elemental analysis calcd (%) for C₃₂H₄₀N₄O₃ (528.68): C 72.70, H 7.63, N 10.60; found: C 72.32, H 7.23, N 10.59.
$\text{H-NMR spectrum of compound 4 (400 MHz, CDCl}_3)$

$\text{C-NMR spectrum of compound 4 (50 MHz, CDCl}_3)$
Synthesis of (2R, 3R, 4R)-1-hexyl[4-(hydroxyethyl)-1H-1,2,3-triazol-1-yl]-3,4-bis(benzyloxy)-2-[(benzyloxy)methyl]-pyrrolidine

To a solution of 4 (64 mg, 0.12 mmol) in 3 ml of 2:1 THF-H_2O CuSO_4 (30 mol%, 6 mg, 0.04 mmol), sodium ascorbate (60 mol%, 14 mg, 0.07 mmol) and 3-buten-1-ol (9 mg, 0.13 mmol) were added. The reaction mixture was stirred in microwave at 80 °C for 45 min, until a TLC analysis (EP:AcOEt 3:1) showed the disappearance of the starting material (R_f = 0.49) and the formation of a new product (R_f = 0.00). After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by Flash Column Chromatography (AcOEt) affording pure 5 (R_f = 0.23, 67 mg, 0.11 mmol, 93%) as a yellow oil. [α]_D^26 = -20.3 (c = 0.94, CHCl_3); ^1H-NMR (400 MHz, CDCl_3): δ = 7.34 (s, 1H, H-Triazole), 7.34-7.25 (m, 15H, H-Ar), 4.56-4.42 (m, 6H, H-Bn), 4.27 (t, J = 7.1 Hz, 2H, H-12), 3.93-3.90 (m, 1H, H-4), 3.91 (t, J = 6.0 Hz, 2H, CH_2OH), 3.88-3.87 (m, 1H, H-3), 3.58 (dd, J = 9.7, 5.3 Hz, 1H, Ha-6), 3.52 (dd, J = 9.7, 6.6 Hz, 1H, Hb-6), 3.22-3.19 (m, 1H, Hb-5), 2.93 (t, J = 6.0 Hz, 2H, CH_2CH_2OH), 2.86-2.79 (m, 1H, Ha-7), 2.74-2.72 (m, 1H, H-2), 2.55 (dd, J = 10.6, 5.1 Hz, 1H, Hb-5), 2.36-2.29 (m, 1H, Hb-7), 1.86 (quint, J = 7.0 Hz, 2H, H-11), 1.54-1.42 (m, 2H, H-8), 1.38-1.22 (m, 4H, H-9 and H-10); ^13C-NMR (50 MHz, CDCl_3): δ = 145.5 (s, C-Triazole), 138.4, 138.1, 138.0 (s, 3C, C-Ar), 128.3-127.5 (d, 15C, C-Ar), 121.4 (d, C-Triazole), 85.4 (d, C-3), 81.6 (d, C-4), 73.2 (t, C-Bn) 71.3 (t, C-Bn), 71.0 (t, 2C, C-Bn and C-6), 69.4 (d, C-2), 61.5 (t, CH_2OH), 57.2 (t, C-5), 55.4 (t, C-7), 50.1 (t, C-12), 30.2 (t, C-11), 28.8 (t, CH_2CH_2OH), 27.8 (t, C-8), 26.8, 26.3 (t, C-9 and C-10); IR (CDCl_3): ν = 3620, 3466, 3088, 3066, 3031, 2936, 2862, 1496, 1453, 1310, 1207, 1098 cm^{-1}; MS (ESI): m/z 599.50 ([M+1]^+; 100), 621.42 ([M+Na]^+; 28); elemental analysis calcd (%) for C_{36}H_{46}N_4O_4 (598.77): C 72.21, H 7.74, N 9.36; found: C 72.06, H 7.52, N 9.27.
$^1$H-NMR spectrum of compound 5 (400 MHz, CDCl$_3$)

$^{13}$C-NMR spectrum of compound 5 (50 MHz, CDCl$_3$)
Synthesis of (2\textit{R}, 3\textit{R}, 4\textit{R})-1-hexyl\[4-(hydroxyethyl)-1\textit{H}\text{-1,2,3-triazol-1-yl}]\text{-3,4-bis(hydroxy)-2-[(hydroxy)methyl]-pyrrolidine}

To a solution of 5 (63 mg, 0.105 mmol) in 15 ml of methanol, 32 mg of 10\% Pd/C and two drops of 37\% HCl were added under nitrogen atmosphere, then the mixture was stirred under hydrogen atmosphere at room temperature for two days, until a NMR control showed the disappearance of the starting material. The mixture was then filtered through Celite\(^\circledR\) and the solvent was removed under reduced pressure affording a crude yellow oil. Free amine was obtained by passing the hydrochloride salt through a Dowex 50WX8 ion-exchange resin. Elution with 6\% NH\(_4\)OH afforded the free base 6 (25 mg, 0.076 mmol, 74\%) as a yellow oil. \([\alpha]_D^{23} = -15.7\ (c = 0.21, \text{MeOH})\); \(^1\text{H}-\text{NMR (400 MHz, D}_2\text{O)}: \delta = 7.69\ (s, 1H, H-\text{Triazole}), 4.26\ (t, J = 6.8 \text{ Hz, 2H, H-12}), 4.04-4.03\ (m, 1H, H-4), 3.85-3.83\ (m, 1H, H-3), 3.72\ (t, J = 6.6 \text{ Hz, 2H, CH}_2\text{OH}), 3.64\ (d, J = 5.4 \text{ Hz, 2H, H-6}), 3.04\ (d, J = 11.2 \text{ Hz, 1H, Ha-5}), 2.86-2.76\ (m, 2H, Hb-5 and Ha-7), 2.80\ (t, J = 6.4 \text{ Hz, 2H, CH}_2\text{CH}_2\text{OH}), 2.68-2.62\ (m, 1H, H-2), 2.45-2.39\ (m, 1H, Hb-7), 1.76\ (quint, J = 6.7 \text{ Hz, 2H, H-11}), 1.46-1.32\ (m, 2H, H-8), 1.24-1.08\ (m, 4H, H-9 and H-10); \(^{13}\text{C}-\text{NMR (50 MHz, D}_2\text{O)}: \delta = 145.2\ (s, \text{C-Triazole}), 123.8\ (d, \text{C-Triazole}), 78.3\ (d, C-3), 75.0\ (d, C-4), 72.8\ (d, C-2), 60.6\ (t, \text{CH}_2\text{OH}), 60.5\ (t, C-6), 58.4\ (t, C-5), 55.6\ (t, C-7), 50.2\ (t, C-12), 29.1\ (t, C-11), 27.8\ (t, \text{CH}_2\text{CH}_2\text{OH}), 25.9, 25.8, 25.2\ (t, 3C, C-8, C-9 and C-10); \text{MS (ESI)}: m/z 329.17 ([M+1]\text{\textsuperscript{+}; 94}), 351.08 ([M+Na]\text{\textsuperscript{+}; 100}); \text{elemental analysis calcd (\% for C}_{15}\text{H}_{28}\text{N}_4\text{O}_4 (328.41): C 54.86, H 8.59, N 17.06; found: C 54.79, H 8.57, N 17.09.}
\[ \text{\(^1\)H-NMR spectrum of compound 6 (400 MHz, D}_2\text{O)} \]

\[ \text{\(^{13}\)C-NMR spectrum of compound 6 (50 MHz, D}_2\text{O)} \]
A solution of 7 (111 mg, 0.83 mmol), 1-azido-6-bromohexane (3, 258 mg, 1.25 mmol) and K₂CO₃ (173 mg, 1.25 mmol) in 6 ml of a mixture CH₃CN/H₂O 5:1 was stirred in microwave at 120°C for 2 h, until a TLC analysis (CH₂Cl₂:MeOH 2:1 + 1% v/v 6% NH₄OH) showed the disappearance of the starting material (Rᶠ = 0.42) and the formation of a new product (Rᶠ = 0.98). After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by Flash Column Chromatography (CH₂Cl₂:MeOH 6:1 + 1% v/v 6% NH₄OH) affording pure 8 (Rᶠ = 0.20, 197 mg, 0.76 mmol, 92%) as a yellow oil. [α]D²⁹ = -23.3 (c = 0.36, MeOH); ¹H-NMR (400 MHz, D₂O): δ = 3.99-3.97 (m, 1H, H-4), 3.81-3.79 (m, 1H, H-3), 3.63-3.55 (m, 2H, H-6), 3.20 (t, J = 6.9 Hz, 2H, H-12), 2.89 (d, J = 10.7 Hz, 1H, Ha-5), 2.71 (td, J = 11.2, 5.8 Hz, 1H, Ha-7), 2.63 (dd, J = 11.2, 5.9, 1H, Hb-5), 2.42 (q, J = 5.3 Hz, 1H, H-2), 2.26 (td, J = 11.2, 5.3 Hz, 1H, Hb-7), 1.53-1.46 (m, 2H, H-11), 1.44-1.34 (m, 2H, H-8), 1.32-1.17 (m, 4H, H-9 and H-10); ¹³C-NMR (50 MHz, D₂O): δ = 79.4 (d, C-3), 75.6 (d, C-4), 72.1 (d, C-2), 61.4 (t, C-6), 58.5 (t, C-5), 55.4 (t, C-7), 51.4 (t, C-12), 28.0 (t, C-11), 26.7 (t, C-8), 26.4, 25.9 (t, 2C, C-9 and C-10); MS (ESI): m/z 281.25 ([M+Na]⁺; 100); elemental analysis calcd (%) for C₁₁H₂₂N₄O₃ (258.32): C 51.15, H 8.58, N 21.69; found: C 51.08, H 8.35, N 21.54.
$^{1}$H-NMR spectrum of compound 8 (400 MHz, D$_2$O)

$^{13}$C-NMR spectrum of compound 8 (50 MHz, D$_2$O)
Synthesis of nonavalent pyrrolidine iminosugars 10

To a solution of 8 (169 mg, 0.65 mmol) in 7.5 ml of 2:1 THF/H₂O CuSO₄ (30 mol%, 3 mg, 0.02 mmol), sodium ascorbate (60 mol%, 8 mg, 0.04 mmol) and 9 (60 mg, 0.07 mmol) were added. The reaction mixture was stirred in microwave at 80 °C for 45 min, until a TLC analysis (EP: AcOEt 1:1) showed the disappearance of the starting material (Rₓ = 0.74) and the formation of a new product (Rₓ = 0.00). After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by Flash Column Chromatography (MeOH:CH₂Cl₂:NH₄OH 2:1:0.5) and Size Exclusion Chromatography Sephadex LH-20 (eluting with H₂O) affording pure 10 (Rₓ = 0.88, 182 mg, 0.057 mmol, 81%) as a yellow oil. [α]D²⁸ = -4.2 (c = 0.9, H₂O); ¹H-NMR (400 MHz, D₂O): δ = 7.85 (s, 3H, H-Ar), 7.75 (s, 9H, H-Triazole), 4.43 (s, 18H, OCH₂Triazole), 4.14-4.13 (m, 9H, H-4), 4.10 (t, J = 7.1 Hz, 18H, H-12), 3.92-3.91 (m, 9H, H-3), 3.79 (dd, J = 12.4, 5.2 Hz, 9H, Ha-6), 3.73 (dd, J = 12.4, 7.6 Hz, 9H, Hb-6), 3.69 (s, 18H, CCH₂O), 3.44-3.41 (m, 9H, Ha-5), 3.27-3.15 (m, 27H, Hb-5, H-2 and Ha-7), 2.94-2.86 (m, 9H, Hb-7), 1.59 (quint, J = 7.2 Hz, 18H, H-11), 1.52-1.44 (m, 18H, H-8), 1.16-1.00 (m, 36H, H-9 and H-10); ¹³C-NMR (50 MHz, D₂O): δ = 167.0 (s, 3C, C=O), 143.0 (s, 9C, C-Triazole), 134.2 (s, 3C, C-Ar), 128.5-
128.1 (d, 3C, C-Ar), 123.9 (d, 9C, C-Triazole), 75.2 (d, 9C, C-3), 74.3 (d, 9C, C-2), 73.0 (d, 9C, C-4), 66.3 (t, 9C, CCH2O), 62.7 (t, 9C, OCH2Triazole), 59.9 (s, 3C, CCH2O), 57.9 (t, 9C, C-6), 57.7 (t, 9C, C-5), 55.7 (t, 9C, C-7), 49.3 (t, 9C, C-12), 28.3 (t, 9C, C-11), 24.4, 24.2 (t, 18C, C-9 and C-10), 23.7 (t, 9C, C-8); MS (ESI): m/z 1063.08 ([M+3]/3; 66); elemental analysis calcd (%) for C147H249N39O39 (3186.79): C 55.40, H 7.88, N 17.14; found: C 55.21, H 7.64, N 17.01.

\(^1\)H-NMR spectrum of compound 10 (400 MHz, D\(_2\)O)

\(^1\)C-NMR spectrum of compound 10 (50 MHz, D\(_2\)O)
Synthesis of (2R,3R,4S)-1-[6-azido]hexyl-3-hydroxymethylhexahydro-1H-pyrrolidine-1,2-diol

A solution of 11 (43 mg, 0.32 mmol), 1-azido-6-bromohexane (3, 98 mg, 0.48 mmol) and K$_2$CO$_3$ (66 mg, 0.48 mmol) in 3 ml of a mixture CH$_3$CN/H$_2$O 5:1 was stirred in microwave at 120°C for 2 h, until a TLC analysis (CH$_2$Cl$_2$:MeOH 2:1 + 1% v/v 6% NH$_4$OH) showed the disappearance of the starting material (R$_f$ = 0.42) and the formation of a new product (R$_f$ = 0.98). After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by Flash Column Chromatography (CH$_2$Cl$_2$:MeOH 5:1 + 1% v/v 6% NH$_4$OH) affording pure 12 (R$_f$ = 0.31, 53 mg, 0.21 mmol, 66%) as a yellow oil. [$\alpha$]$_D^{23}$ = -28.3 (c = 0.70, MeOH); $^1$H-NMR (400 MHz, CD$_3$OD): $\delta$ = 4.06-4.00 (m, 1H, H-4), 3.85 (t, J = 5.1 Hz, 1H, H-3), 3.60-3.55 (m, 2H, H-6), 3.30-3.23 (m, 3H, H-12 and Ha-5), 2.83 (ddd, J = 11.9, 8.8, 7.3 Hz, 1H, Ha-7), 2.58 (q, J = 4.7 Hz, 1H, H-2), 2.46-2.40 (m, 2H, Hb-7 e Hb-5), 1.63-1.56 (m, 2H, H-11), 1.55-1.48 (m, 2H, H-8), 1.44-1.28 (m, 4H, H-9 e H-10); $^{13}$C-NMR (50 MHz, CD$_3$OD): $\delta$ = 72.7 (d, C-3), 71.4 (d, C-2), 69.5 (d, C-4), 61.3 (t, C-6), 57.6 (t, C-5), 55.6 (t, C-7), 51.0 (t, C-12), 28.5 (t, C-11), 27.5 (t, C-8), 26.6, 26.3 (t, 2C, C-9 and C-10); MS (ESI): m/z: 259.17 ([M+1]$^+$; 100); elemental analysis calcd (%) for C$_{11}$H$_{22}$N$_4$O$_3$ (258.32): C 51.15, H 8.58, N 21.69; found: C 51.12, H 8.54, N 21.66.
\[ \text{\(^1\text{H-NMR spectrum of compound 12 (400 MHz, CD}_3\text{OD)}\) } \]

\[ \text{\(^{13}\text{C-NMR spectrum of compound 12 (50 MHz, CD}_3\text{OD)}\) } \]
To a solution of 12 (64 mg, 0.22 mmol) in 6 ml of 2:1 THF/H₂O CuSO₄ (30 mol%, 11 mg, 0.07 mmol), sodium ascorbate (60 mol%, 26 mg, 0.13 mmol) and 3-butyn-1-ol (20 µL, 0.26 mmol) were added. The reaction mixture was stirred in microwave at 80 °C for 45 min, until a TLC analysis (CH₂Cl₂:MeOH 6:1 + 1% v/v 6% NH₄OH) showed the disappearance of the starting material (Rᵣ = 0.22) and the formation of a new product (Rᵣ = 0.00). After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by Flash Column Chromatography (CH₂Cl₂:MeOH 6:1 + 1% v/v 6% NH₄OH) affording pure 13 (Rᵣ = 0.13, 64 mg, 0.19 mmol, 89%) as a yellow oil. [α]D₂₂ = -10.0 (c = 0.67, MeOH); ¹H-NMR (400 MHz, D₂O): 7.65 (s, 1H, H-Triazole), 4.22 (t, J = 6.9 Hz, 2H, H-12), 3.99 (q, J = 5.8 Hz, 1H, H-4), 3.80 (t, J = 5.4 Hz, 1H, H-3), 3.68 (t, J = 6.4 Hz, 2H, CH₂OH), 3.58-3.45 (m, 2H, H-6), 3.19 (dd, J = 10.7, 5.9 Hz, 1H, Ha-5), 2.76 (t, J = 6.4 Hz, 2H, CH₂CH₂OH), 2.76-2.71 (m, 1H, Ha-7), 2.66 (q, J = 5.2 Hz, 1H, H-2), 2.45 (dd, J = 10.7, 6.8 Hz, 1H, Hb-5), 2.39 (td, J = 11.7, 5.4 Hz, 1H, Hb-7), 1.75-1.68 (m, 2H, H-11), 1.39-1.27 (m, 2H, H-8), 1.17-1.05 (m, 4H, H-9 and H-10); ¹³C-NMR (100 MHz, D₂O): δ = 145.2 (s, C-Triazole), 123.8 (d, C-Triazole), 72.1 (d, C-3), 70.8 (d, C-2), 69.0 (d, C-4), 60.7, 59.9 (t, C-6 and CH₂OH), 56.9 (t, C-5), 56.2 (t, C-7), 50.2 (t, C-12), 29.1 (t, C-11), 27.8 (t, CH₂CH₂OH), 25.8, 25.7, 25.2 (t, 3C, C-8, C-9 and C-10); MS (ESI): m/z 329.40 ([M+1]⁺; 32), 351.36 ([M+Na]⁺; 100); elemental analysis calcd (%) for C₁₅H₂₈N₄O₄ (328.41): C 54.86, H 8.59, N 17.06; found: C 54.81, H 8.56, N 17.04.
$^{1}H$-NMR spectrum of compound 13 (400 MHz, D$_2$O)

$^{13}$C-NMR spectrum of compound 13 (100 MHz, D$_2$O)
Synthesis of nonavalent pyrrolidine iminosugars 14

To a solution of 12 (41 mg, 0.158 mmol) in 3 ml of 2:1 THF/H$_2$O CuSO$_4$ (30 mol%, 1 mg, 0.005 mmol), sodium ascorbate (60 mol%, 2 mg, 0.010 mmol) and 9 (15 mg, 0.017 mmol) were added. The reaction mixture was stirred in microwave at 80 °C for 45 min, until a TLC analysis (EP/AcOEt 2:1) showed the disappearance of the starting material ($R_f$ = 0.26) and the formation of a new product ($R_f$ = 0.00). After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by Flash Column Chromatography (MeOH:CH$_2$Cl$_2$:NH$_4$OH 2:1:0.75) and Size Exclusion Chromatography Sephadex LH-20 (eluting with H$_2$O) affording pure 14 ($R_f$ = 0.26, 38 mg, 0.012 mmol, 71%) as a yellow oil. [$\alpha$]$_D^{23}$ = -2.2 (c = 0.75, H$_2$O); $^1$H-NMR (400 MHz, D$_2$O): $\delta$ = 7.86 (s, 3H, H-Ar), 7.76 (s, 9H, H-Triazole), 4.43 (s, 18H, OCH$_2$Triazole), 4.19 (pseudo q, J = 4.1 Hz, 9H, H-4), 4.11 (t, J = 6.9 Hz, 18H, H-12), 4.01 (dd, J = 7.3, 4.4 Hz, 9H, H-3), 3.80 (dd, J = 13.1, 3.4 Hz, 9H, Ha-6), 3.70 (dd, J = 13.1, 4.9 Hz, 9H, Hb-6), 3.69 (s, 18H, CCH$_2$O), 3.60 (dd, J = 12.7, 4.4 Hz, 9H, Ha-5), 3.36-3.33 (m, 9H, H-2), 3.21 (dt, J = 12.2, 8.3 Hz, 9H, Ha-7), 3.08 (dd, J = 12.7, 2.9 Hz, 9H, Hb-5), 2.98 (dt, J = 12.2, 8.1 Hz, 9H, Hb-7), 1.64-1.56 (m,
$^{1}H$-NMR spectrum of compound 14 (400 MHz, D$_2$O)

$^{13}C$-NMR spectrum of compound 14 (100 MHz, D$_2$O)

18H, H-11), 1.56-1.44 (m, 18H, H-8), 1.18-0.98 (m, 36H, H-9 and H-10); $^{13}C$-NMR (100 MHz, D$_2$O): $\delta$ = 167.9 (s, 3C, C=O), 143.9 (s, 9C, C-Triazole), 135.2 (s, 3C, C-Ar), 128.9 (d, 3C, C-Ar), 124.7 (d, 9C, C-Triazole), 70.7 (d, 9C, C-3), 70.5 (d, 9C, C-2), 68.6 (d, 9C, C-4), 67.2 (t, 9C, CCH$_2$O), 63.5 (t, 9C, OCH$_2$Triazole), 60.7 (s, 3C, CCH$_2$O), 57.2, 57.1, 57.0 (t, 27C, C-6, C-5 and C-7), 50.1 (t, 9C, C-12), 29.1 (t, 9C, C-11), 25.0, 24.9 (t, 18C, C-9 and C-10), 24.4 (t, 9C, C-8); MS (ESI): m/z 1070.58 ([M+3]/3$^{+}$; 81); elemental analysis calcd (%) for C$_{147}$H$_{249}$N$_{39}$O$_{39}$ (3186.79): C 55.40, H 7.88, N 17.14; found: C 55.29, H 7.74, N 17.02.
Biochemical tests on human GALNS and IDS

For nonavalent compounds \( \text{10 and 14} \) and their monovalent counterparts \( \text{6 and 13} \) the percentage of inhibition, at \( 1 \text{ mM} \) concentration, towards GALNS and IDS using leukocyte extracts from healthy donors was evaluated.

Leukocyte pellets were disrupted by sonication in water and the micro BCA protein assay kit (Sigma-Aldrich) was used to set up the protein amount for the enzymatic assay, according to the manufacturer’s instructions.

**GALNS enzymatic test:**

Enzyme activity was measured by setting the reaction in \( 0.2 \text{ ml} \) tubes and performing the experiments in triplicates as follows:

**Step 1:**

Iminosugars solution (3 µl), leukocytes homogenate (7 µl) and 20 µl of 4-methylumbelliferyl-β-galactoside-6-sulphate-Na (Moscerdam Substrates) substrate solution in Na-Acetate/acetic acid buffer (0.1 M/0.1 M, pH 4.3) containing 0.1 M NaCl , 0.02% (w/v) NaN₃ and 5 mM Pb-acetate were incubated for 17 h at 37 °C.

**Step 2:**

After step 1 the tubes were placed on an ice cooler and the reaction was stopped by addition of 5 µl of Na-phosphate buffer (0.9 M, pH 4.3) containing 0.02 % of NaN₃ and by efficient mixing with vortex. Then, 10 µl of β-Gal-A-10U were added to each sample and the suspension mixed again in vortex apparatus, then samples were incubated for 2 h at 37°C.

At the end of this period the tubes were placed on an ice cooler and the samples were transferred in a cooled flat–bottomed 96 well plate and the reaction was immediately stopped with 200 µl of NaHCO₃/Na₂CO₃ buffer (0.5M/0.5M pH 10.7) containing 0.025% (w/v) of Triton X-100. Fluorescence was measured in a SpectraMax M2 microplate reader (Molecular-Devices) using a 365 nm excitation wavelength and a 435 nm emission wavelength.
Percentage of GALNS inhibition was given with respect to the control (without iminosugar). Experiments were performed in triplicate, and the mean ± S. D. was calculated.¹

**IDS enzymatic test:**

Enzyme activity was measured by setting the reaction in 0.2 ml tubes and performing the experiments in triplicates as follows:

**Step 1:**

Iminosugars solution (3 µl), leukocytes homogenate (7 µl) and 20 µl of 4-methylumbelliferyl-α-L-Iduronide-2-sulphate 2Na (Moscerdam Substrates) substrate solution were incubated for 4 h at 37 °C.

**Step 2:**

After step 1 the tubes were placed on an ice cooler and the reaction stopped by addition of 20 µl of Na-Phosphate/Citrate buffer (0.2M/ 0.1M pH 4.5) and by efficient mixing with vortex. Then, 10 µl of LEBT (Lysosomal Enzymes purified from Bovine Testis) were added to each sample and the incubation was continued for 24 hours at 37°C.

At the end of this period tubes were placed on an ice cooler and the samples were transferred in a cooled flat–bottomed 96 well plate and the reaction was immediately stopped by addition of 200 µl of NaHCO₃/Na₂CO₃ buffer (0.5M/0.5M pH 10.7) containing 0.025% (w/v) of Triton X-100.

Fluorescence was then measured in a SpectraMax M2 microplate reader (Molecular-Devices) using a 365 nm excitation wavelength and a 435 nm emission wavelength.

Percentage of IDS inhibition was given with respect to the control (without iminosugar). Experiments were performed in triplicate, and the mean ± S. D. was calculated.²

**IC₅₀ determination³**

The IC₅₀ values against the GALNS and IDS inhibitors were determined by measuring the initial hydrolysis rate under fixed 4-methylumbelliferyl-β-galactoside-6-sulphate·Na concentration (6.66 mM). and 4-methylumbelliferyl-α-L-Iduronide-2-sulphate·2Na

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concentration (0.833 mM) respectively. Data obtained were fitted to the following equation using the Origin Microcal program:

\[
\frac{V_i}{V_o} = \frac{\text{Max} - \text{Min}}{1 + \left( \frac{x}{IC_{50}} \right) \text{slope}} + \text{Min}
\]

where \(\frac{V_i}{V_o}\), represents the ratio between the activity measured in the presence of the inhibitor (\(V_i\)) and the activity of the control without the inhibitor (\(V_o\)), “x” the inhibitor concentration, Max and Min, the maximal and minimal enzymatic activity observed, respectively.
GALNS

IC\textsubscript{50} = 0.047 +/- 0.005 mM
GALNS

IC$_{50}$ = 0.085 +/- 0.008
GALNS

\[ IC_{50} = 3.9 \pm 0.2 \text{ mM} \]
IC$_{50} = 5.0 +/- 0.2$ mM
IDS

$IC_{50} = 0.14 \pm 0.005 \text{ mM}$

$V_i / V_0$ vs. [10] mM
IC₅₀ = 0.031 ± 0.002 mM
$\text{IC}_{50} = 3.2 \pm 0.16 \text{ mM}$
IDS

$V_i / V_0$

$IC_{50} = 5.5 \pm 0.3 \text{ mM}$

$[C13] \text{ mM}$