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Synthesis of spacer armed Kdn(2→6') and (2→3')-lactosamines for immunochemical research

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Below is a description of the procedures for glycosylation of acceptors **5a-c** with 2-β-thiopenyl-KDO glycoside **4** in the presence of NIS-TfOH promoting system; deprotection of the formed Kdn-glycosides (debenzylation, Zemplén deacetylation, hydrolysis of methyl ester and *N*-trifluro-acetamide groups), and their purification. For the target products, ^{1}H NMR-spectra are enclosed (see the **Figures S1-S6**).

Unless otherwise stated, all chemicals were purchased from commercial sources and used as received. The methyl ester of per-*O*-acetylated 2-β-thiopenyl glycoside Kdn **4**,[S1] *N*-iodo-succinimide[S2] and acceptors **5a-c**[S3,S4] were synthesized according to the published procedures. All solvents were purified by standard methods; anhydrous DCM was prepared by distillation sequentially over P_2O_5 and CaH_2; anhydrous MeOH was distilled from magnesium turnings.

TLC was carried out on silica gel 60 F_{254}-coated aluminum foil (Merck); spots were visualized by dipping the plates to 10% aqueous H_3PO_4 and subsequent charring. Preparative column chromatography was performed on silica gel (60 Å, 40-63 μm, Merck). Analytical HPLC was carried out at an HP Agilent 1100 chromatographic system equipped with a UV detector (λ 195 nm) and a Phenomenex C18(2) Luna column (4.6×250 mm, 100 Å, 5 μm); flow rate 1 ml/min at 30 °C. Preparative purifications of the target sialooligosaccharides were performed using an HPLC system consisting of a Gilson 305 Pump, Gilson 155 UV/VIS detector (λ 195 nm), Gilson 215 Nebula fraction collector, Rheodyne 7725i manual injection module and Gilson 506C system interface. Preparative purifications were conducted by applying the corresponding analytical methodology on a Phenomenex Luna C18(2) column (21.2×250 mm, 100Å, 5μm); elution with H_2O, flow rate - 10.6 ml/min at 30 °C; injection volume 0.8 ml, maximal amount of analyte taken for separation 10 mg.

^{1}H NMR spectra were recorded at 30 °C with a Bruker Avance 700 instrument. The ^{1}H chemical shifts are referenced to the signals of the residual $CHCl_3$ (δ_H 7.27 ppm) and HDO (δ_H 4.75 ppm). High-resolution mass spectra (HRMS) were obtained on a Bruker microTOF II electrospray mass spectrometer. Specific optical rotation ($[\alpha]_D$) was measured at 20 °C with a PerkinElmer 341LC polarimeter.

Glycosylation. The general approach.

A mixture of donor **4** (59 mg, 100 μmol), acceptor 5**a-c** (120 μmol) and freshly activated 4 Å powdered molecular sieves (~2.0 g/mmol of the donor) in anhydrous CH_2Cl_2 (2 ml) was stirred for 30 min, and then was cooled to -40 °C. After sequential addition of NIS (57 mg, 250 μmol) and TfOH (15 mg, 9.0 μl, 100 μmol), the mixture was stirred at -40 °C for ~2 h until disappearance of **4** (TLC: R_f 0.45, 6:4:2 hexane–$CHCl_3$–Me_2CO), quenched with triethylamine (22.6 μl, 200 μmol) and warmed to room temperature. The mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite, washed with 20% $Na_2S_2O_3$(aq) (10 ml); the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel affording crude anomeric products **6a-c** with purity of 90-93% according to analytical HPLC. The unreacted acceptor was not recovered; the yield of the crude products was calculated on donor taken for glycosylation; after being corrected for purity, the yields meanings were referred in the Table 1 (main text). The resulting α- and β-sialosides **6a-c** were deprotected without additional purification.

3-Trifluoroacetamidopropyl [methyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-glycero-α/β-D-galacto-non-2-ulopyranosyl)onate]-(2→6)-(2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (6a). The reaction mixture residue was applied on a column of silica gel (35 g), and eluted with a gradient 7→12% of *i*-PrOH in 5:1 hexane–$CHCl_3$ to give:

Crude 6a-α: 65.6 mg (53.2%), colorless syrup; TLC: R_f 0.26, 10:4:1 hexane–$CHCl_3$–*i*-PrOH; ^{1}H NMR ($CDCl_3$): 1.70–1.91 (m, 2H, OCH_2CH_2 sp), 1.84 (dd, 1H, $J_{3ax,4}$ 11.4 Hz, H-3ax KDO), 1.95, 1.96, 1.98, 2.03, 2.04, 2.05, 2.10, 2.11, 2.12, 2.13, 2.19 (11s, 11×3H, 11×C(O)CH_3),), 2.51 (dd, 1H, $J_{3eq,3ax}$ 13.1 Hz, $J_{3eq,4}$ 5.1 Hz, H-3eq KDO), 3.19–3.28 (m, 1H, NCH^a sp), 3.53 (dd, 1H, $J_{5,6a}$ 5.8 Hz, H-6a Gal), 3.54-3.60 (m, 2H, OCH^a, NCH^b sp), 3.62 (dd, 1H, $J_{5,6b}$ 7.7 Hz, $J_{6a,6b}$ 10.7 Hz, H-6b Gal), 3.71 (ddd, 1H, $J_{4,5}$ 9.1 Hz, H-5 GlcNAc), 3.81 (t, 1H, $J_{3,4}$~$J_{4,5}$~9.5 Hz, H-4 GlcNAc), 3.83 (s, 3H, CO(O)CH_3), 3.91-3.85 (m, 2H, H-5 Gal, OCH^b sp), 3.98 (dd, 1H, $J_{5,6}$ 10.1 Hz, H-6 Kdn), 3.99 (ddd, 1H, H-2 GlcNAc), 4.02 (dd, 1H, $J_{8,9a}$ 7.7 Hz, H-9a Kdn), 4.17 (dd, 1H, $J_{5,6a}$ 5.3 Hz, H-6a GlcNAc), 4.44 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1 GlcNAc), 4.55 (dd, 1H, $J_{5,6b}$ 2.6 Hz, $J_{6a,6b}$ 12.0 Hz, H-6b GlcNAc), 4.60 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1 Gal), 4.79 (dd, 1H, $J_{8,9b}$ 2.3 Hz, $J_{9a,9b}$ 12.4 Hz, H-9b Kdn), 4.95 (t, 1H, $J_{4,5}$~$J_{5,6}$~10.0 Hz, H-5 Kdn), 5.01 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.4 Hz, H-3 Gal), 5.10 (dd, 1H, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 8.5 Hz, H-3 GlcNAc), 5.12 (dd, 1H, H-2 Gal), 5.17 (dd, 1H, $J_{8,9b}$ 7.9 Hz, H-8 Kdn), 5.26 (ddd, 1H, $J_{4,5}$ 9.7 Hz, H-4 Kdn), 5.36 (dd, 1H, $J_{6,7}$ 2.3 Hz, $J_{7,8}$ 3.9 Hz, H-7 Kdn), 5.45 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1.2 Hz, H-4 Gal), 6,12 (d, 1H, J_{CH} 8.9 Hz, N*H*Ac GlcNAc), 7.55 (t, 1H, J_{CH} 5.8 Hz, N*H*Tfa sp); HRMS: $[M+Na]^+$ calcd. for

$C_{49}H_{67}F_3N_2O_{30}Na$, 1243.3628; found: 1243.3623; Anal. HPLC: t_R 12.8 min (purity - 94 %); eluent - 50:50 % (v/v) MeCN–H_2O;

Crude 6a-β: 27.4 mg (22.2%), colorless syrup; TLC: R_f 0.22, 10:4:1 hexane–$CHCl_3$–*i*-PrOH; ^{1}H NMR ($CDCl_3$): 1.75–1.92 (m, 2H, OCH_2CH_2 sp); 1.96, 1.97, 2.06, 2.07, 2.08, 2.09, 2.10, 2.11, 2.14, 2.15, 2.16 (11s, 11×3H, 11×C(O)CH_3), 2.21 (dd, 1H, $J_{3ax,4}$ 1.3 Hz, H-3ax Kdn), 2.45 (dd, 1H, $J_{3eq,3ax}$ 14.8 Hz, , $J_{3eq,4}$ 7.5 Hz, H-3eq Kdn), 3.60-3.68 (m, 5H, H-6 G, H-5GN, OCHb, NCH^a sp), 3.30-3.21 (м, 1H, NCH^b sp), 3.79 (s, 3H, CO(O)CH_3), 3.83 (t, 1H, $J_{3,4}$~$J_{4,5}$~9.8 Hz, H-4 GlcNAc), 3.88-3.92 (m, 1H, OCH^a sp), 3.95 (ddd, 1H, $J_{5,6a}$ 8.1 Hz, $J_{5,6b}$ 6.7 Hz, H-5 Gal), 4.02 (ddd, 1H, H-2 GlcNAc), 4.13 (dd, 1H, $J_{5,6b}$ 6.2 Hz, H-6b GlcNAc), 4.22 (dd, 1H, $J_{8,9a}$ 6.8 Hz, H-9a Kdn), 4.40 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1 GlcNAc), 4.44 (dd, 1H, $J_{8,9b}$ 2.8 Hz, $J_{9a,9b}$ 12.3 Hz, H-9b Kdn), 4.50 (dd, 1H, $J_{5,6b}$ 2.4 Hz, $J_{6a,6b}$ 11.8 Hz, H-6b GlcNAc), 4.55 (dd, 1H, $J_{4,5}$ 2.5 Hz, H-5 Kdn), 4.66 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1 Gal), 5.05 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 8.2 Hz, H-3 GlcNAc), 5.13 (dd, 1H, H-2 Gal), 5.17 (ddd, 1H, H-8 Kdn), 5.18 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.3 Hz, H-3 Gal), 5.26 (dd, 1H, $J_{5,6}$ 5.4 Hz, $J_{6,7}$ 4.4 Hz, H-6 Kdn), 5.33 (ddd, 1H, $J_{4,5}$ 2.5 Hz, H-4 Kdn), 5.42 (dd, 1H, $J_{6,7}$ 4.4 Hz, $J_{7,8}$ 5.4 Hz, H-7 Kdn), 5.58 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1.3 Hz, H-4 Gal), 5.85 (d, 1H, J_{CH} 8.9 Hz, N*H*Ac GlcNAc), 7.49 (t, 1H, J_{CH} 5.8 Hz, N*H*Tfa sp); HRMS: $[M+Na]^+$ calcd. for $C_{49}H_{67}F_3N_2O_{30}Na$, 1243.3628; found: 1241.3619; Anal. HPLC: t_R 11.9 min (purity - 90 %); eluent - 50:50 % (v/v) MeCN–H_2O.

3-Trifluoroacetamidopropyl [methyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-glycero-α/β-D-galacto-non-2-ulopyranosyl)onate]-(2→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (6b). The reaction mixture residue applied on a column of silica gel (35 g), and eluted with a gradient 7→12% of *i*-PrOH in 5:1 hexane–$CHCl_3$ to give:

Crude 6b-α: 49.1 mg (38.4%), colorless syrup; TLC: R_f 0.23, 10:4:1 hexane–$CHCl_3$–*i*-PrOH; ^{1}H NMR ($CDCl_3$): 1.65–1.92 (m, 2H, OCH_2CH_2 sp); 1.86 (dd, 1H, $J_{3ax,4}$ 11.8 Hz, H-3ax Kdn), 1.96, 1.97, 1.98, 2.02, 2.04, 2.06, 2.07, 2.08, 2.11, 2.15 (10s, 10×3H, 10×C(O)CH_3), 2.53 (dd, 1H, $J_{3eq,3ax}$ 13.8 Hz, $J_{3eq,4}$ 4.8 Hz, H-3eq Kdn), 3.24-3.30 (m, 1H, NCH^a sp), 3.52–3.66 (m, 3H, OCH^a, NCH^b sp, H-5 GlcNAc), 3.68–3.76 (m, 2H, H-6 GlcNAc), 3.78–3.86 (m, 4H, H-9a Kdn, C(O)OCH_3), 3.87–3.95 (m, 2H, OCH^b sp, H-4 GlcNAc), 4.05 (dd, 1H, $J_{5,6a}$ 4.6 Hz, H-6a Gal), 4.02–4.05 (m, 1H, H-2 GlcNAc), 4.10 (ddd, 1H, H-5 Gal), 4.12 (dd, 1H, $J_{5,6b}$ 6.3 Hz, $J_{6a,6b}$ 10.0 Hz, H-6b Gal), 4.37 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1 GlcNAc), 4.55 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1 Gal), 4.58–4.63 (m, 3H, H-6 Kdn, CH_2Ar), 4.71 (dd, 1H, $J_{2,3}$ 10.3 Hz, H-3 Gal), 4.79 (t, 1H, $J_{4,5}$~$J_{5,6}$~9.8 Hz, H-5 Kdn), 5.02 (dd, 1H, $J_{2,3}$ 7.9 Hz, $J_{3,4}$ 9.0 Hz, H-3 GlcNAc), 5.11 (dd, 1H, H-2 Gal), 5.12–5.16 (m, 1H, H-4 Kdn), 5.23 (dd, 1H, $J_{8,9b}$ 2.5 Hz, $J_{9a,9b}$ 12.2 Hz, H-9b Kdn), 5.30 (dd, 1H, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.8 Hz, H-4 Gal), 5.31 (dt, 1H, $J_{7,8}$~$J_{8,9a}$~2.5 Hz, $J_{8.9b}$ 9.6 Hz, H-8 Kdn), 5.40 (t, 1H,

$J_{6,7}$~$J_{7,8}$~2.5 Hz, H-7 Kdn), 5.97 (d, 1H, J_{CH} 9.0 Hz, N*H*Ac), 7.30–7.40 (m, 5H, 5×CH Ar), 7.55 (t, 1H, J_{CH} 5.8 Hz, N*H*Tfa sp); HRMS: $[M+Na]^+$ calcd. for $C_{54}H_{71}F_3N_2O_{29}Na$, 1291.3992; found: 1291.3987; Anal. HPLC: t_R 9.8 min (purity - 93 %); eluent - 50:40 % (v/v) MeCN–H_2O;

Crude 6b-β: 26.7 mg (20.9%), colorless syrup; TLC: R_f 0.20, 10:4:1 hexane–$CHCl_3$–*i*-PrOH; ^{1}H NMR ($CDCl_3$): 1.76–1.92 (m, 2H, OCH_2CH_2 sp), 1.96, 1.97, 2.06, 2.07, 2.08, 2.09, 2.10, 2.11, 2.14, 2.15 (10s, 10×3H, 11×C(O)CH_3), 2.21 (dd, 1H, $J_{3ax,4}$ 1.3 Hz, H-3ax Kdn), 2.68 (dd, 1H, $J_{3eq,3ax}$ 15.3 Hz, , $J_{3eq,4}$ 7.9 Hz, H-3eq Kdn), 3.21–3.30 (m, 1H, NCH^a sp), 3.60-3.68 (m, 5H, H-6 Gal, H-5 GlcNAc, OCH^a, NCH^b sp), 3.79 (s, 3H, CO(O)CH_3), 3.83 (t, 1H, $J_{3,4}$~$J_{4,5}$~9.8 Hz, H-4 GlcNAc), 3.88-3.92 (m, 1H, OCH^b sp), 3.95 (ddd, 1H, $J_{5,6a}$ 8.1 Hz, $J_{5,6b}$ 6.7 Hz, H-5 Gal), 4.02 (ddd, 1H, H-2 GlcNAc), 4.13 (dd, 1H, $J_{5,6a}$ 6.2 Hz, H-6a GlcNAc), 4.36 (dd, 1H, $J_{8,9a}$ 3.2 Hz, H-9a Kdn), 4.38 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1 GlcNaAc), 4.42 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1 Gal), 4.44 (dd, 1H, $J_{8,9b}$ 2.8 Hz, $J_{9a,9b}$ 12.3 Hz, H-9b Kdn), 4.50 (dd, 1H, $J_{5,6b}$ 2.4 Hz, $J_{6a,6b}$ 11.8 Hz, H-6b GlcNac), 4.55 (dd, 1H, $J_{4,5}$ 2.5 Hz, H-5 Kdn), 4.64, 4.68 (2d, 2×1H, J_{hem} 12.0 Hz, 2×C*H*Ar), 5.05 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 8.2 Hz, H-3 GlcNAc), 5.13 (dd, 1H, H-2 Gal), 5.18 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.3 Hz, H-3 Gal), 5.26 (dd, 1H, $J_{5,6}$ 5.4 Hz, $J_{6,7}$ 4.4 Hz, H-6 Kdn), 5.33 (ddd, 1H, $J_{4,5}$ 2.5 Hz, H-4 Kdn), 5.42 (dd, 1H, $J_{6,7}$ 4.4 Hz, $J_{7,8}$ 5.4 Hz, H-7 Kdn), 5.47 (ddd, 1H, H-8 Kdn), 5.58 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 1.3 Hz, H-4 Gal), 5.90 (d, 1H, J_{CH} 8.9 Hz, N*H*Ac), 7.40–7.30 (m, 5H, 5×CH Ar), 7.49 (t, 1H, J_{CH} 5.8 Hz, N*H*Tfa sp); HRMS: $[M+Na]^+$ calcd. for $C_{54}H_{71}F_3N_2O_{29}Na$, 1291.3992; found: 1289.3989; Anal. HPLC: t_R 8.8 min (purity - 91 %); eluent - 60:40 % (v/v) MeCN–H_2O.

3-Trifluoroacetamidopropyl [methyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-glycero-α/β-D-galacto-non-2-ulopyranosyl)onate] (6c). The reaction mixture residue was applied on a column of silica gel (35 g), and eluted with a gradient 10→15% of Me_2CO in 3:2 hexane–$CHCl_3$ to give:

Crude 6c-α: 52.8 mg (81.1%), colorless syrup; TLC: R_f 0.30, 6:4:2 hexane–$CHCl_3$–Me_2CO; ^{1}H NMR ($CDCl_3$): 1.80–1.93 (m, 2H, OCH_2CH_2 sp), 1.96 (dd, 1H, $J_{3ax,4}$ 11.7 Hz, H-3ax), 2.00, 2.01, 2.03, 2.10, 2.14 (5s, 5×3H, 5×C(O)CH_3), 2.65 (dd, 1H, $J_{3eq,3ax}$ 13.0 Hz, $J_{3eq,4}$ 4.5 Hz, H-3eq), 3.42–3.52 (m, 3H, OCH^a, CH_2N sp), 3.83 (s, 3H, C(O)OCH_3), 3.85–3.90 (m, 1H, OCH^asp), 4.08 (dd, 1H, $J_{8,9a}$ 5.7 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.23 (dd, 1H, $J_{5,6}$ 10.0 Hz, $J_{6,7}$ 2.3 Hz, H-6), 4.32 (dd, 1H, $J_{8,9a}$ 2.5 Hz, H-9b), 4.89 (t, 1H, $J_{4,5}$~$J_{5,6}$~9.8 Hz, H-5), 4.91 (ddd, 1H, $J_{4,5}$ 9.8 Hz, H-4), 5.35 (dd, 1H, $J_{7,8}$ 9.2 Hz, H-7), 5.41 (ddd, 1H, H-8), 7.49 (t, 1H, J_{CH} 5.8 Hz, N*H*Tfa sp); HRMS: $[M+Na]^+$ calcd. for $C_{25}H_{34}F_3NO_{15}Na$, 668.1778; found: 668.1773; Anal. HPLC: t_R 9.4 min (purity - 93 %); eluent - 70:30 % (v/v) MeCN–H_2O;

Crude 6c-β: 5.4 mg (8.3%), colorless syrup; TLC: R_f 0.23, 6:4:2 hexane–$CHCl_3$–Me_2CO; ^{1}H NMR ($CDCl_3$): 1.78–1.92 (m, 2H, CH_2CH_2Osp), 2.06, 2.08, 2.09, 2.10, 2.11 (5s, 5×3H, 5×C(O)CH_3), 2.18 (dd,1H, $J_{3ax,4}$ 2.0 Hz, H-3ax), 2.56 (дд, 1H, $J_{3eq,3ax}$ 14.7 Hz, $J_{3eq,4}$ 7.9 Hz, H-

3eq), 3.47–3.62 (m, 3H, OCH^a, CH_2N sp), 3.75–3.81 (m, 1H, OCH^{a}sp), 3.83 (s, 3H, C(O)OCH_3), 4.15 (dd, 1H, $J_{8,9a}$ 6.8 Hz, $J_{9a,9b}$ 12.1 Hz, H-9a), 4.39 (dd, 1H, $J_{8,9a}$ 3.0 Hz, H-9b), 4.41 (dd, 1H, $J_{5,6}$ 5.6 Hz, $J_{6,7}$ 3.4 Hz, H-6), 5.33 (t, 1H, $J_{4,5}$~$J_{5,6}$~5.6 Hz, H-5), 5.34–5.45 (m, 3H, H-4,7,8), 7.53 (t, 1H, J_{CH} 5.8 Hz, N*H*Tfa sp); HRMS: $[M+Na]^+$ calcd. for $C_{25}H_{34}F_3NO_{15}Na$, 668.1778; found: 668.1775; Anal. HPLC: t_R 8.8 min (purity - 90 %); eluent - 70:30 % (v/v) MeCN–H_2O.

Deprotection of the target Kdn-glycosides. To a solution of crude *O*-benzyl derivative **6b** (**α** or **β**) in MeOH (20 mg/ml), 10% Pd/C (~5 mg/30 mg of the substrate) was added, and the mixture was stirred under H_2 atmosphere pressure at ambient temperature for ~ 3 h (TLC control for the debenzylation: R_f 0.36 (**6d-α**), R_f 0.32 (**6d-β**) (the resulting products), R_f 0.64 (**6b-α**), R_f 0.60 (**6b-β**), 9:3:2 $CHCl_3$–EtOAc–MeOH). The catalyst was filtered off, washed with MeOH (5 ml), the combined filtrates were concentrated to dryness with co-evaporation with toluene, and the residue was taken for deacetylation.

To a solution of crude acetate **6a**,**c-d** (**α** or **β**) in anhydrous MeOH (20 mg/ml), 2M MeONa in MeOH (20 μl/ml of the soln.) was added, the mixture was kept for 3 h at room temperature and concentrated without heating. A solution of the residue in H_2O (10 mg/ml) was kept for 12 h at room temperature and applied on a Dowex 50X4-400 ion-exchange resin column (H^+-form in H_2O, ~2 ml). The resin was washed sequentially with H_2O (3 ml) and 1 M aqueous Py (5 ml) to give crude **7a-c** (**α** or **β**). The final purification of Kdn-glycosides was performed by preparative HPLC, the fractions containing products (pure according to Anal. HPLC) were collected, combined, and concentrated to dryness to afford the target glycosides as colorless foams in 70-75% yields.

3-Aminopropyl [(3-deoxy-D-glycero-α/β-D-galacto-non-2-ulopyranosyl)onic acid]-(2→6)-(β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7a).

7a-α: $[\alpha]_D^{20}$ +12.3 (*c* 0.1, H_2O), TLC: R_f 0.12, 4:3:2 *i*-PrOH–MeCN–H_2O; ^{1}H NMR (D_2O): 1.60 (t, 1H, $J_{3ax,3eq}$~$J_{3ax,4}$~12.3 Hz, H-3ax), 1.93–2.02 (m, 2H, OCH$_2$CH_2 sp), 2.06 (s, 3H, NC(O)CH_3), 2.34 (dd, 1H, $J_{3ax,3eq}$ 13.2 Hz, $J_{3eq,4}$ 4.7 Hz, H-3eq Kdn), 3.08–3.14 (m, 2H, NCH_2 sp), 3.51–3.59 (m, 3H), 3.61-3.92 (m, 14H), 3.93 (dd, 1H, $J_{3,4}$ 3.6 Hz, $J_{4,5}$<1.0 Hz, H-4 Gal), 3.98–4.08 (m, 3H), 4.48 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1 GlcNAc), 4.55 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1 Gal); HRMS: $[M+H]^+$ calcd. for $C_{26}H_{48}N_2O_{19}$, 691.2773; found: 691.2768; Anal. HPLC: one peak, t_R 3.7 min, eluent - H_2O;

7a-β: $[\alpha]_D^{20}$ -7.8 (*c* 0.5, H_2O), TLC: R_f 0.28, 4:3:2 *i*-PrOH–MeCN–H_2O; ^{1}H NMR (D_2O): 1.94–2.00 (m, 2H, OCH$_2$CH_2 sp), 2.06 (s, 3H, NC(O)CH_3), 2.17 (dd, 1H, $J3_{ax,4}$ 2.0 Hz, H-3ax), 2.36 (dd, 1H, $J_{3ax,3eq}$ 14.6 Hz, $J_{3eq,4}$ 7.8 Hz, H-3eq Kdn), 3.06–3.13 (m, 2H, NCH_2 sp), 3.50 (dd, 1H, $J_{5,6a}$ 4.3 Hz, $J_{5,6a}$ 10.2 Hz, H-6a Gal), 3.57 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 8.0 Hz, H-4 GlcNAc), 3.60–3.65 (m, 1H, H-5 GlcNAc), 3.66–3.78 (m, 7H), 3.79–3.88 (m, 4H), 3.89–3.97 (m, 3H), 3.99–4.07 (m, 3H), 4.30 (t, 1H, $J_{4,5}$~$J_{5,6}$ 4.5Hz, Kdn), 4.48–4.52 (m,2H, H-1 Gal, GlcNAc); HRMS:

$[M+H]^+$ calcd. for $C_{26}H_{48}N_2O_{19}$, 691.2773; found: 691.2768; Anal. HPLC: one peak, t_R 4.2 min, eluent - H_2O.

3-Aminopropyl [(3-deoxy-D-glycero-α/β-D-galacto-non-2-ulopyranosyl)onic acid]-(2→3)-(β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (7b).

7b-α: $[\alpha]_D^{20}$ +18.3 (*c* 0.7, H_2O), TLC: R_f 0.32, 4:3:2 *i*-PrOH–MeCN–H_2O; ^{1}H NMR (D_2O): 1.67 (t, 1H, $J_{3ax,3eq}$~$J_{3ax,4}$~12.3 Hz, H-3ax), 1.93–1.99 (m, 2H, OCH_2CH_2 sp), 2.06 (s, 3H, $NC(O)CH_3$), 2.43 (dd, 1H, $J_{3ax,3eq}$ 13.0 Hz, $J_{3eq,4}$ 4.6 Hz, H-3eq Kdn), 3.05–3.12 (m,2H, NCH_2 sp), 3.59–3.79 (m,12H), 3.81–3.94 (m,4H), 4.00–4.05 (m, 2H), 4.12 (ddd, 1H, $J_{3ax,4}$ 11.7 Hz, $J_{4,5}$ 9.3 Hz, H-4 Kdn), 4.29 (dd, 1H, $J_{3,4}$ 3.6 Hz, $J_{4,5}$<1.0 Hz, H-4 Gal), 4.50–4.55 (m, 2H, H-1 Gal, GlcNac); HRMS: $[M+H]^+$ calcd. for $C_{26}H_{48}N_2O_{19}$, 691.2773; found: 691.2768; Anal. HPLC: one peak, t_R 4.5 min, eluent - H_2O;

7b-β: $[\alpha]_D^{20}$ -12.1 (*c* 0.5, H_2O), TLC: R_f 0.40, 4:3:2 *i*-PrOH–MeCN–H_2O; ^{1}H NMR (D_2O): 1.93-2.00 (m, 2H, OCH_2CH_2 sp), 2.06 (s, 3H, $NC(O)CH_3$), 2.31 (dd, 1H, $J_{3ax,4}$ 1.9 Hz, H-3ax), 2.35 (dd,1H, $J_{3ax,3eq}$ 14.6 Hz, $J_{3eq,4}$ 6.2 Hz, H-3eq Kdn), 3.02–3.13 (m, 2H, NCH_2 sp), 3.59–3.69 (m, 3H), 3.71–3.82 (m, 8H), 3.82-3.90 (m, 3H), 3.93–4.06 (m, 5H), 4.47 (dd, 1H, $J_{5,6}$ 5.9 Hz, , $J_{6,7}$ 2.1 Hz, H-6 Kdn), 4.54 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1 Gal), 4.55–4.59 (m, 2H, H-1 GlcNAc, H-7 Kdn); HRMS: $[M+H]^+$ calcd. for $C_{26}H_{48}N_2O_{19}$, 691.2773; found: 691.2768; Anal. HPLC: one peak, t_R 5.4 min, eluent - H_2O;

3-Aminopropyl 3-deoxy-D-glycero-α/β-D-galacto-non-2-ulopyranosidonic acid (7c).

7c-α: $[\alpha]_D^{20}$ +8.4 (*c* 0.8, H_2O), TLC: R_f 0.43, 4:3:2 *i*-PrOH–EtOAc–H_2O; ^{1}H NMR (D_2O): 1.87–2.00 (m, 2H, OCH_2CH_2 sp), 2.31 (dd, 1H, $J_{3ax,4}$ 7.8 Hz, H-3ax), 2.52 (dd,1H, $J_{3ax,3eq}$ 13.4 Hz, $J_{3eq,4}$ 7.1 Hz, H-3eq Kdn), 3.07–3.20 (m, 2H, NCH_2 sp) 3.37–3.43 (m, 1H, OCH^b sp), 3.69 (dd, 1H, $J_{8,9a}$ 6.8, $J_{9a,9b}$ 11.8 Hz, H-9a), 3.78–3.88 (m, 3H), 3.90–3.97 (m, 2H), 4.27 (t, 1H, $J_{4,5}$~$J_{5.8}$ Hz, H-5), 4.57–4.65 (m,1H, H-8); HRMS: $[M+H]^+$ calcd. for $C_{12}H_{24}NO_9$, 340.1451; found: 691.1446; Anal. HPLC: one peak, t_R 3.1 min, eluent - H_2O;

7c-β: $[\alpha]_D^{20}$ -2.7 (*c* 0.1, H_2O), TLC: R_f 0.52, 4:3:2 *i*-PrOH–EtOAc–H_2O; ^{1}H NMR (D_2O): 1.91-2.03 (m, 2H, OCH_2CH_2 sp), 2.18 (dd, 1H, $J_{3ax,4}$ 1.4 Hz, H-3ax), 2.29 (dd,1H, $J_{3ax,3eq}$ 14.4 Hz, $J_{3eq,4}$ 7.3 Hz, H-3eq Kdn), 3.10–3.23 (m, 2H, NCH_2 sp), 3.44–3.50 (m, 1H, OCH^a sp), 3.68 (dd, 1H, $J_{8,9a}$ 7.2 Hz, $J_{9a,9b}$ 11.8 Hz, H-9a), 3.79–3.84 (m, 2H, H-7 OCH^b sp), 3.85 (dd, 1H, $J_{8,9b}$ 2.9 Hz, H-9b), 3.93 (dd, 1H, $J_{6,7}$ 5.9 Hz, H-6), 3.95 (ddd, 1H, $J_{7,8}$ 6.9 Hz, H-8), 4.32 (dd, 1H, $J_{5,6}$ 5.3 Hz, H-5), 4.58 (ddd, 1H, $J_{4,5}$ 2.8 Hz, H-4); HRMS: $[M+H]^+$ calcd. for $C_{12}H_{24}NO_9$, 340.1451; found: 691.1446; Anal. HPLC: one peak, t_R 3.9 min, eluent - H_2O.

Evaluation of the synthesized Kdn-glycans as antigens with a glychip methodology.

Spacered KDN-glycans and their N-acetylated analogs were printed at 12 replicates each as described [S5] with some modifications: the epoxy-activated slides (Semiotik LLC, Russia) were used, a glycan concentration was 20 μM and blocking was performed by 50 mM ethanolamine in 100 мM borate buffer containing 0.2% of Tween20, pH 8.5 for 1.5 h. The glycochips were incubated with sera of healthy donors and antibodies bound to printed glycans were visualized with the fluorescently labeled secondary antibodies against human IgG and IgM as described [S5]. Fluorescence signal intensities were collected with 10 μm scanning resolution using confocal fluorescent scanner InnoScanAL1100 (Innopsys, France) and quantified with ProScanArray v. 4.0 software (PerkinElmer, USA) using method of "fixed circle" with 100 μm diameter followed by Microsoft Excel. Data are reported as median RFU (relative fluorescence units) of replicates. For each glycan absolute median deviation (MAD) was calculated. A signal, whose fluorescence intensity exceeded the background value by a factor of five, was considered as significant.

The synthesized spacer Kdn glycans **7a-c** (**α** and **β**), as well as their Neu5Ac and NeuGc analogs, were printed at 12 repeats each as previously described[S5] with a few modifications: the epoxy-activated slides (Semiotik LLC, Russia) were used, the glycans concentration was 20 μM, and blocking was performed for 1.5 h with 50 mM ethanolamine in 100 mM borate buffer containing 0.2% of Tween 20, pH 8.5. The glycochips were incubated with sera of healthy donors and antibodies bound to printed glycans were visualized with the fluorescently labeled secondary antibodies against human IgG and IgM as earlier described.[S5] Fluorescence signal intensities were collected with 10 μm scanning resolution using confocal fluorescent scanner InnoScanAL1100 (Innopsys, France) and quantified with ProScanArray v. 4.0 software (PerkinElmer, USA) using method of "fixed circle" with 100 μm diameter followed by Microsoft Excel. Data are reported as median RFU (relative fluorescence units) of replicates. For each glycan absolute median deviation (MAD) was calculated. A signal, whose fluorescence intensity exceeded the background value by a factor of five, was considered as significant.

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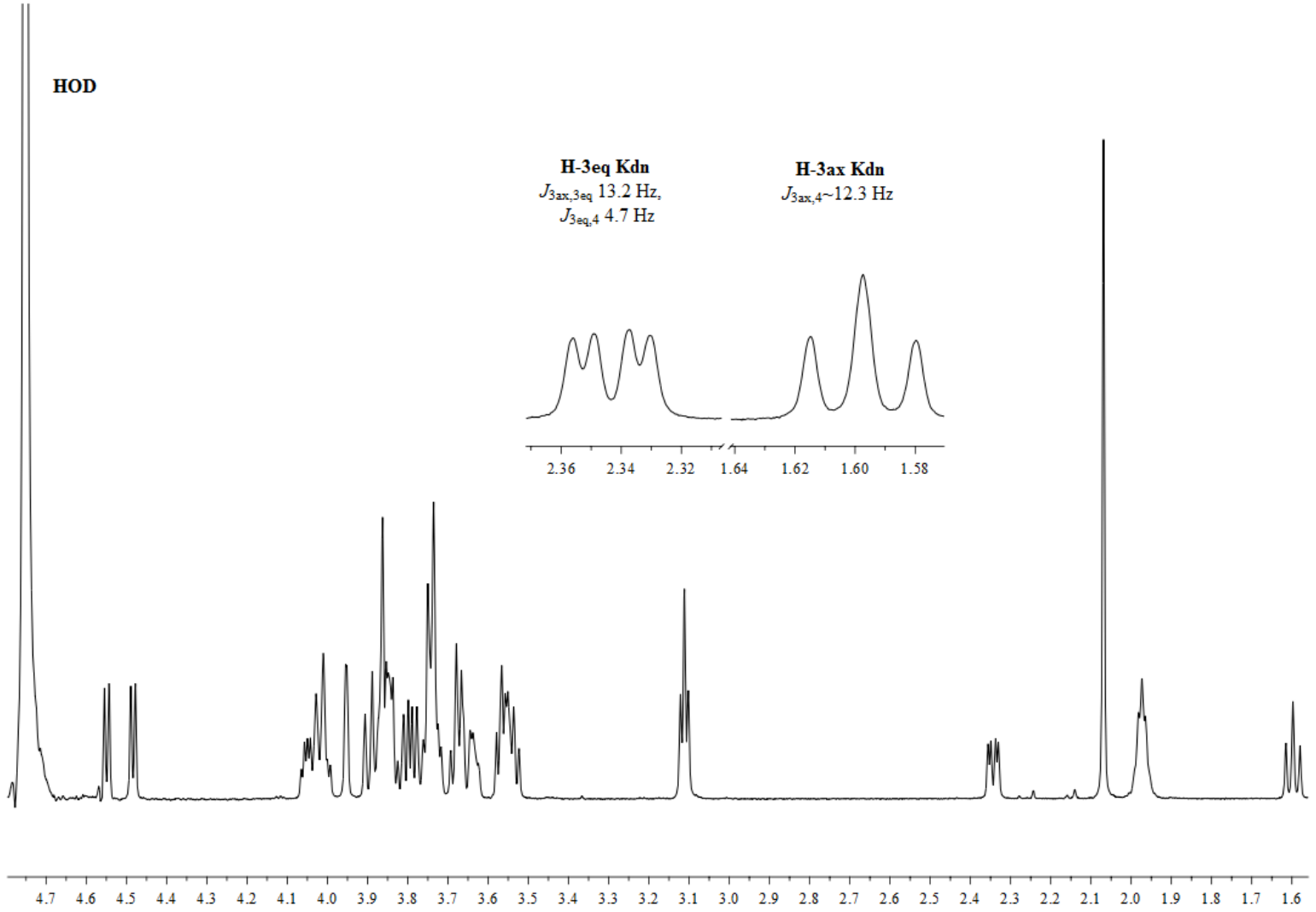

Figure S1 ^{1}H NMR spectrum of Kdnα(2→6)Galβ(1→4)GlcNAcβ—O$(CH_2)_3NH_2$ (**7a-α**)

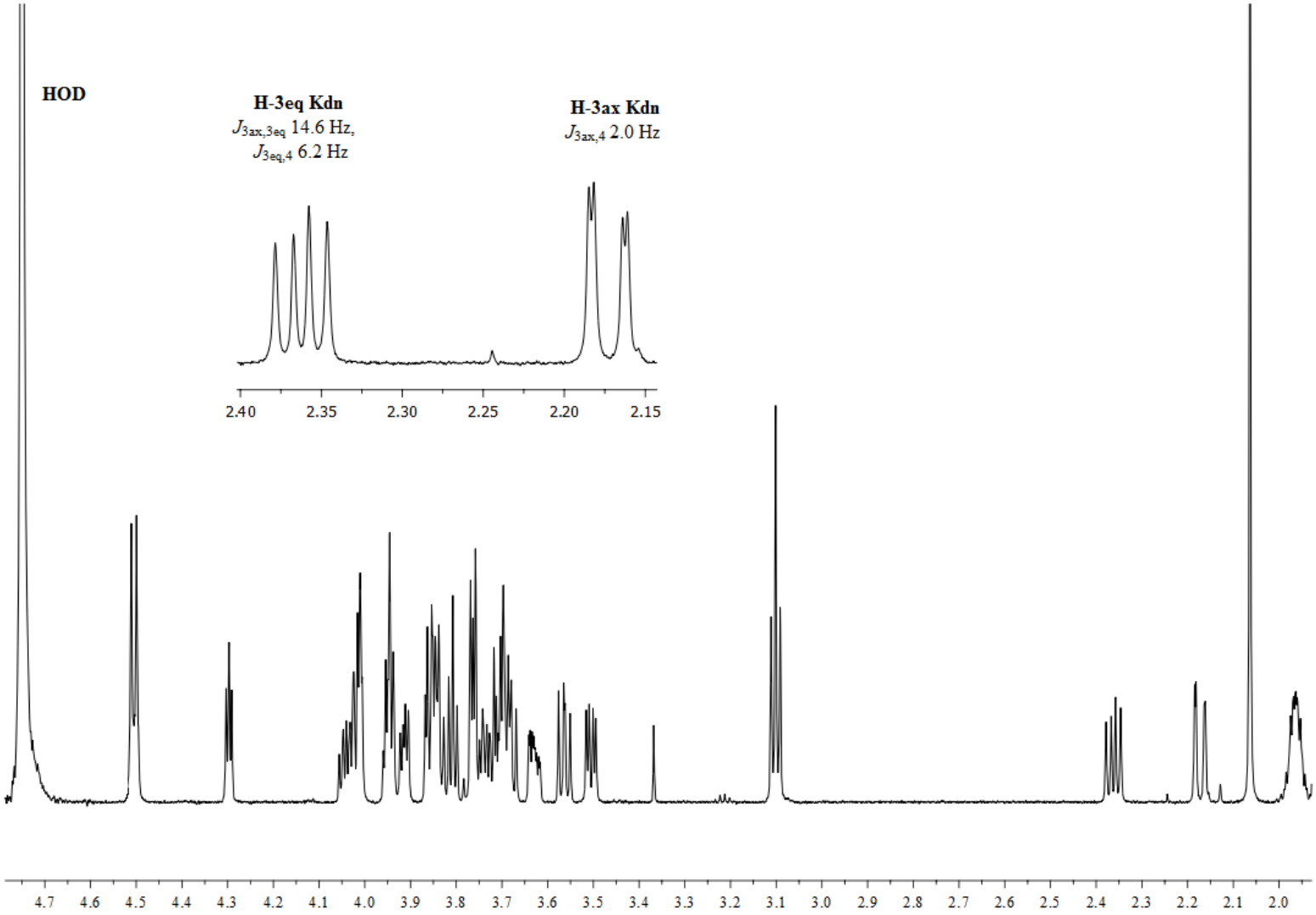

Figure S2 ^{1}H NMR spectrum of Kdnβ(2→6)Galβ(1→4)GlcNAcβ—O$(CH_2)_3NH_2$ (7**a-β**)

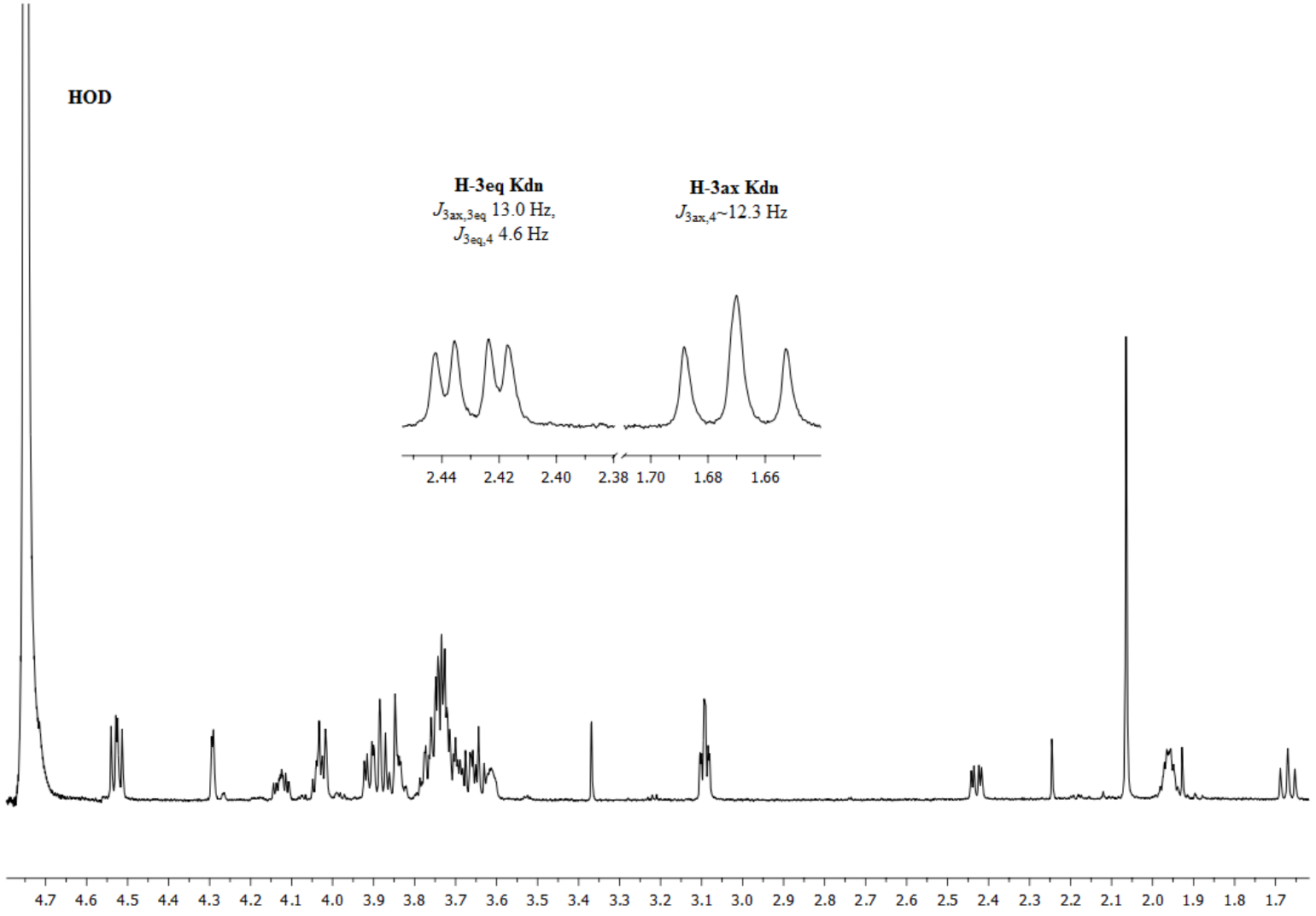

Figure S3 ^{1}H NMR spectrum of Kdnα(2→3)Galβ(1→4)GlcNAcβ—O$(CH_2)_3NH_2$ (**7b-α**)

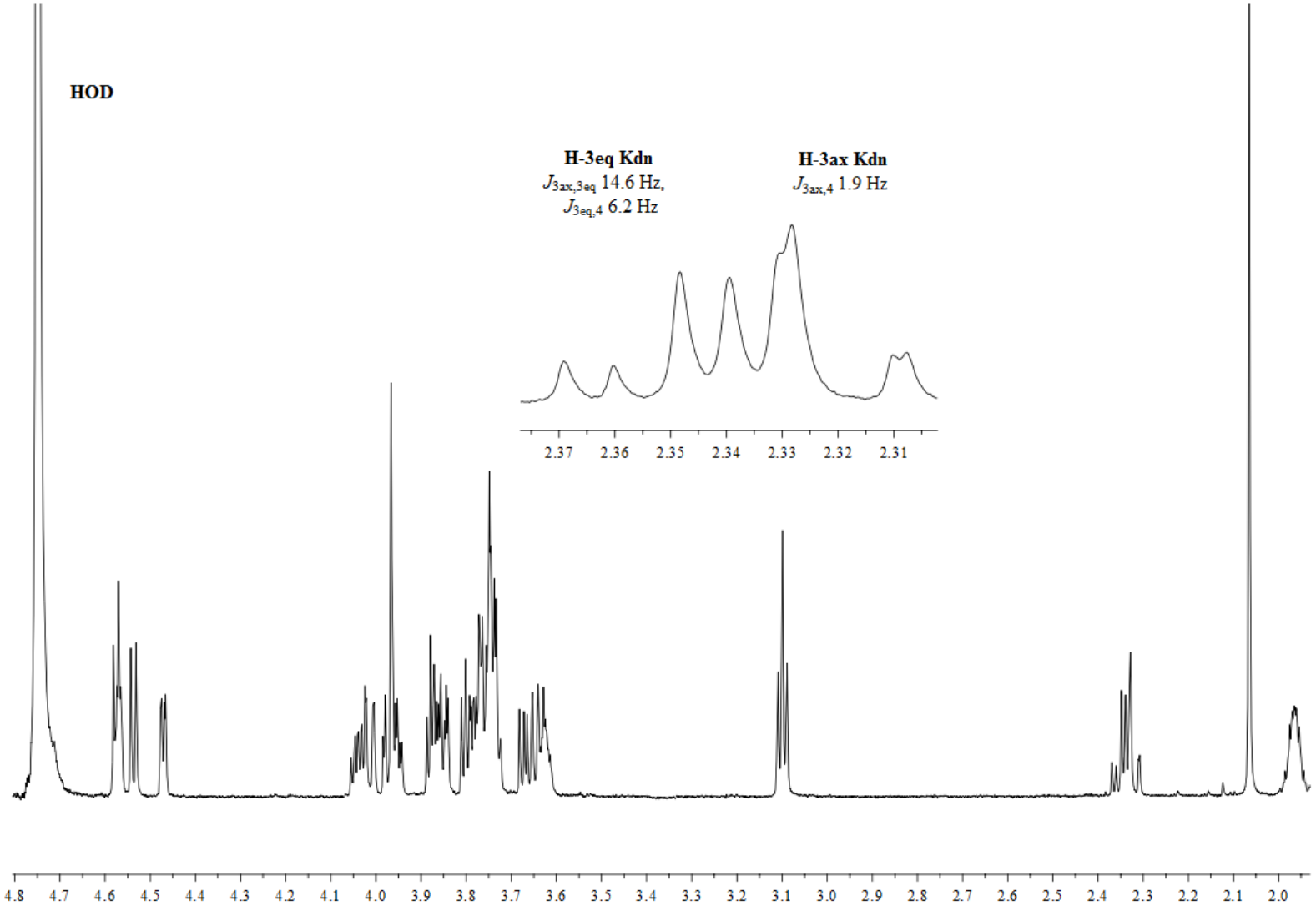

Figure S4 ^{1}H NMR spectrum of Kdnβ(2→3)Galβ(1→4)GlcNAcβ—O$(CH_2)_3NH_2$ (**7b-β**)

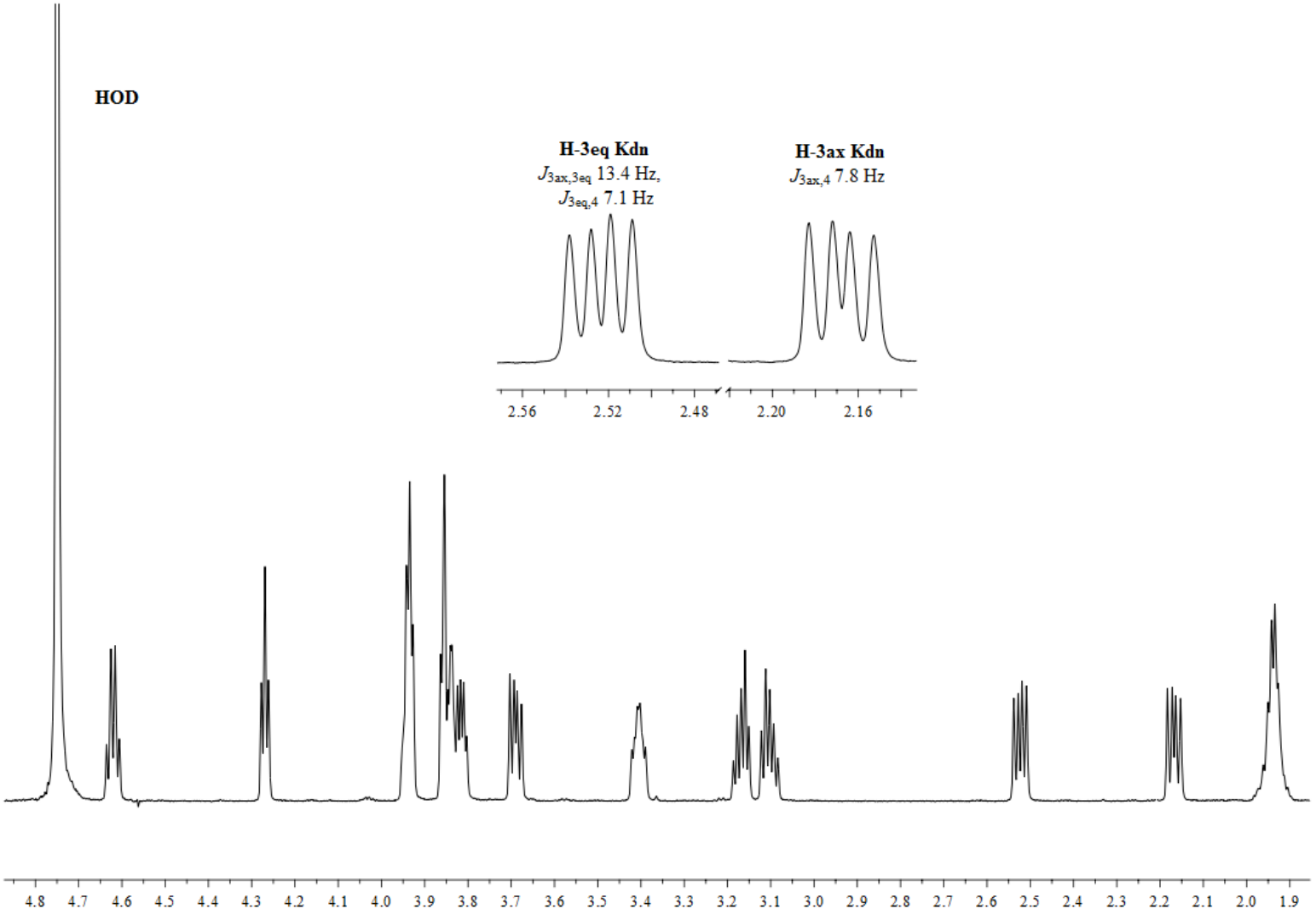

Figure S5 ^{1}H NMR spectrum of Kdnα—$O(CH_2)_3NH_2$ (**7c-α**)

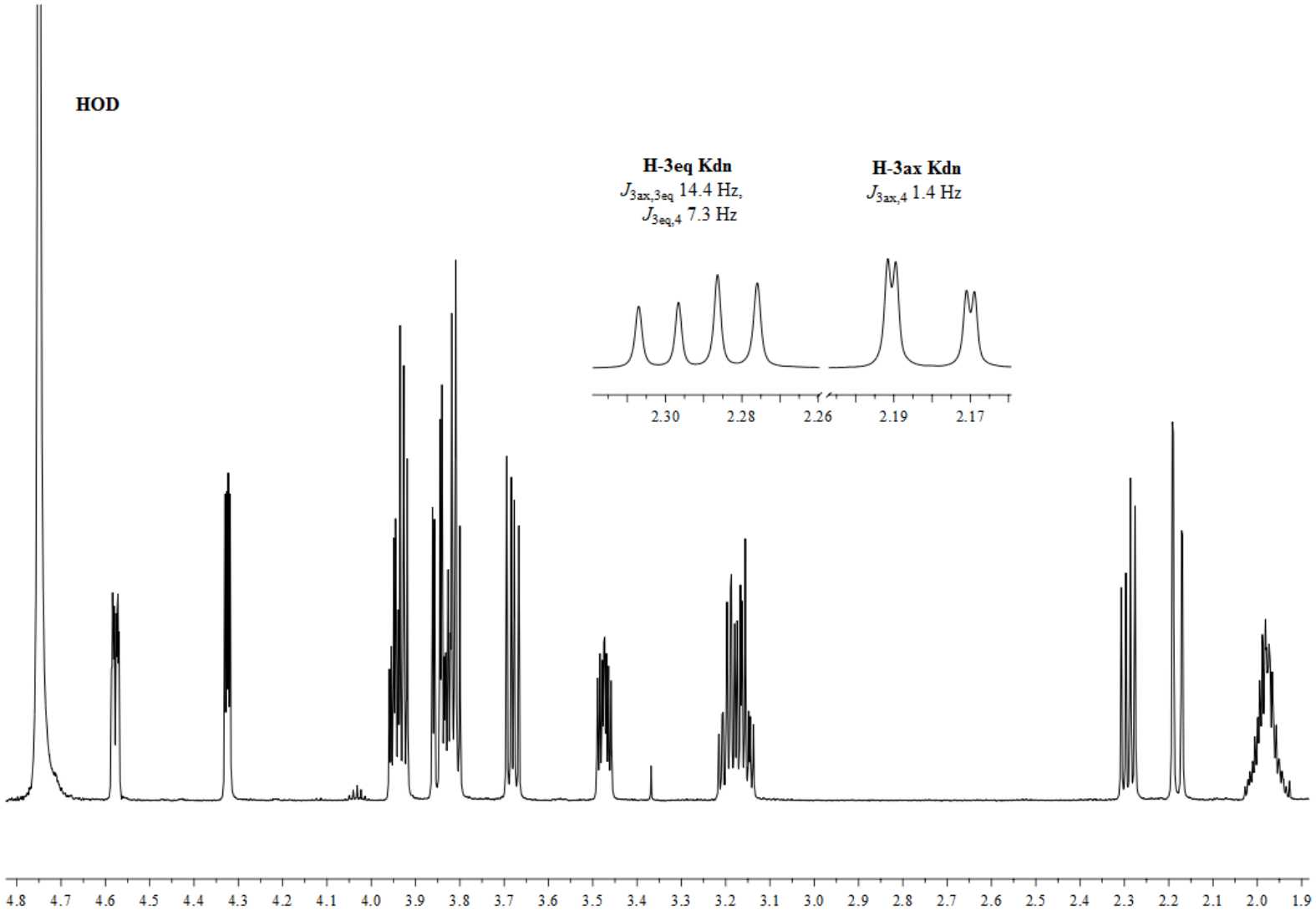

Figure S6 ^{1}H NMR spectrum of Kdnβ—$O(CH_2)_3NH_2$ (**7c-β)**