Surface Plasmon Resonance Imaging Measurements of the Inhibition of Shiga-Like Toxin by Synthetic Multivalent Inhibitors

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Supplementary Information

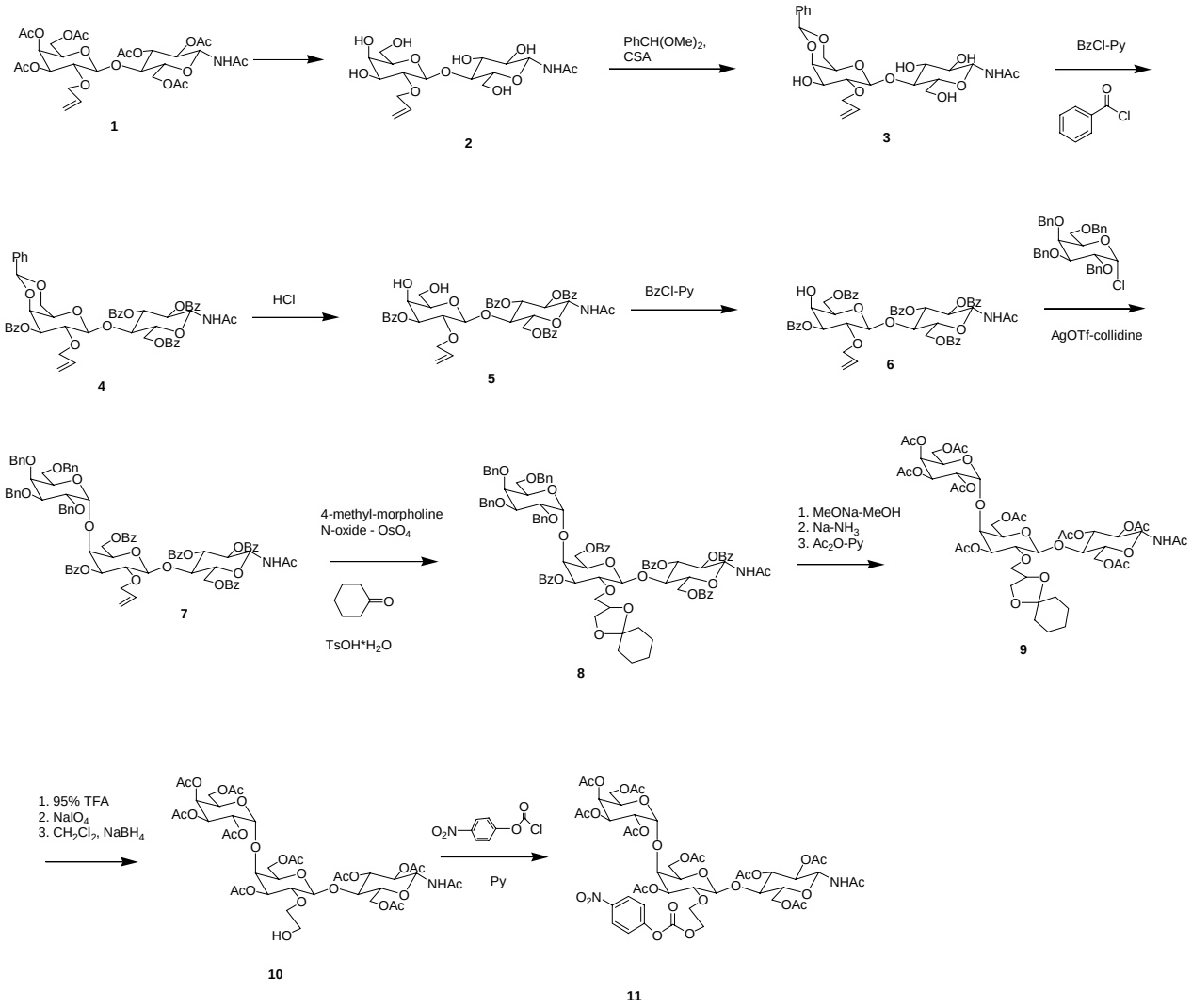
Synthesis of STARFISH-2

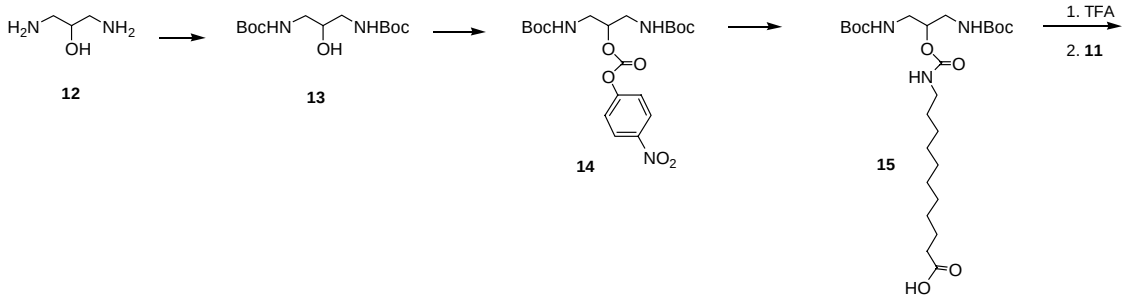
Previously described lactose derivative **1** was deacetylated to give compound **2**, which was derivatized as 4',6'-benzylidene cyclic acetal and benzoylated to give **3** and **4**. Removal of acetal afforded diol **5**, which was selectively benzoylated to provide glycosyl acceptor **6**. Glycosylation of 6 with tetra-benzyl galactosyl chloride gave trisaccharide **7**.

Benzyl protective groups are generally stable at most conditions used in glycoside chemistry. However, removal of multiple O-benzyl groups is a problem and it was desirable to replace them with more convenient ester protecting groups on the early stages of the scheme. Because of incompatibility of double bond functionality with most methods of O-benzyl removal, allylic double bond was partially processed (dihydroxylated) and protected with cyclic ketal **8** prior to trisaccharide deprotection. This maneuver has allowed to efficiently remove both benzyl and benzoyl groups by Na in liquid ammonia, and, after per-acetylation, acetyl protected P^k-trisaccharide derivative **9** was obtained. Acid treatment of **9** followed by oxidation of resulting diol with NaIO4 and reduction with NaBH4 provided 2-hydroxyethyl compound **10** which was activated as *p*-nitrophenyl carbonate to give key trisaccharide **11**.

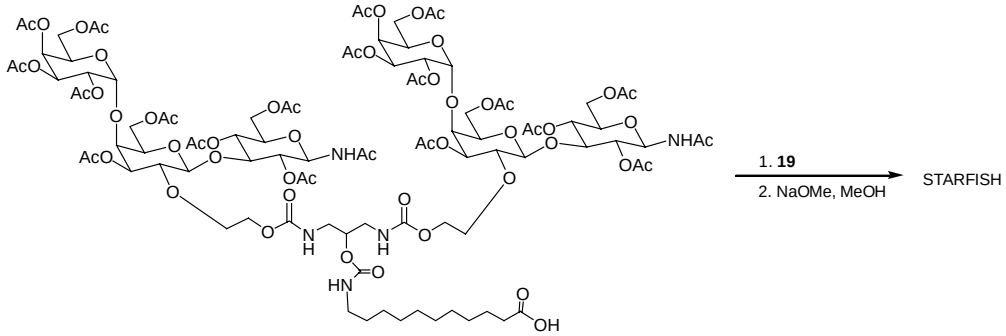
The linker arm containing bifurcation point was obtained by following sequence of reactions. Boc protection of 1,3-diamino-2-hydroxypropane **12** followed by activation of hydroxyl **13** as *p*-nitrophenyl carbonate gave **14**, which reacted with 11-amino-undecanoic acid to give **15**. Removal of Boc protective groups and coupling with trisaccharide **11** afforded branched trisaccharide-terminated linker-arm **16**.

The core structure was assembled starting from penta-allyl glucose derivative **17**. Radical addition of amino ethanethiol resulted in penta-amino derivative **18**, each arm of which was extended by acylation with Boc glycine to give Boc protected derivative of **19**. Treatment of this intermediate with TFA restored **19**, which was acylated with the branched sugar-terminated linker-arm **16**. Deacetylation of the reaction mixture resulted in target **STARFISH** analog.





12 13 14 15



16

17 R_1 = All
18 R_1 = - $(CH_2)_3S(CH_2)_2NH_2$
19 R_1 = - $(CH_2)_3S(CH_2)_2NHC(O)CH_2NH_2$

1-acetamido-1-deoxy-4-O-(2-O-allyl-β-D-galactopyranosyl)-β-D-glucopyranose (**2)**

Compound **1** (10.58 g) was dried, dissolved in 100 mL of dry MeOH and K_2CO_3 (~ 0.5 g) was added. After 16 h at 40^{o}C the mixture was conc. Dissolved in water, neutralized with 2 g of DOWEX (H^+) and concentrated. Crystallization from EtOH afforded **2** (95%).TLC: DCM-MeOH (2:1), R_f ~0.5. ^{1}H-NMR (D_2O): δ 6.05-5.9 (m, 1 H, All), 5.4-5.25 (m, 2 H, All), 5.00 (d, 1 H, $J_{1,2}$ 9.1 Hz, H-1), 4.50 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.36-4.24 (m, 2 H, All), 3.94-3.91 (m, 2 H, H-6a, H-4), 3.82 - 3.66 (m, 8 H, H-3, H-5, H-6b, H-3', H-4', H-5', H-6'a,H-6'b), 3.46-3.42 (m, 2 H, H-2, H-2'), 2.09 (3 H, NAc).

Synthesis of *1-acetamido-1-deoxy-4-O-(2-O-allyl-3-O-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranose* (**5)**

Compound **2** (6.27 g, 14.8 mmol) was suspended in dry MeCN (160 mL), $PhCH(OMe)_2$ (2.66 mL) and D,L-10-camphorsulfonic acid (~200 mg) was added. The mixture was

sonicated with occasional shaking for 1 h. the precipitate has changed in appearance. For monitoring of the reaction sample with precipitate was taken. TLC: DCM-MeOH (2:1), R_f ~0.9. After the reaction was complete dry pyridine (14 mL) and slowly BzCl (15 mL) was added. TLC: hexane-ethyl acetate (1:1), R_f ~0.3. After 16 h the reaction was quenched with MeOH, concentrated, co-evaporated with toluene 3 times and dried. The residue (**4**) was dissolved in 80% AcOH (100 mL) and stirred at 90^{o}C for 4 h then diluted with brine, extracted with DCM, washed with saturated $NaHCO_3$ and concentrated. Chromatography on silica gel with hexane:acetone = 70:30-50:50 gave **5** (9 g, 72%). ^{1}H-NMR ($CDCl_3$): δ 8.10-7.90 and 7.60-7.30 (m, 20 H, Bz), 6.37 (d, 1 H, $J_{1,NH}$ 9.1 Hz, NH), 5.82 (t, 1 H, $J_{2,3}$ ~ $J_{3,4}$ = 9.5 Hz, H-3), 5.76-5.70 (m, 1 H, All), 5.48 (t, 1 H, $J_{1,2}$ ~ $J_{1,NH}$ = 9.3 Hz, H-1), 5.30 (t, 1 H, $J_{1,2}$ ~ $J_{2,3}$ = 9.7 Hz, H-2), 5.14-5.11 (broad d, 1 H, All), 5.00 (broad d, 1 H, All), 4.87 (dd, 1 H, $J_{6a,6b}$ 12.3 Hz, $J_{6a,5}$ 1.8 Hz, H-6a), 4.84 (dd, 1 H, $J_{2',3'}$ 10.1 Hz, $J_{3',4'}$ 3.1 Hz, H-3'), 4.55 (dd, 1 H, $J_{6b,5}$ 4.6 Hz, H-6b), 4.49 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.24-4.21 (m, 1 H, All), 4.20 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 4.15-4.10 (m, 1 H, All), 4.06-4.02 (m, 1 H, H-5), 4.03 (d, 1 H, H-4'), 3.72 (dd, 1 H, H-2'), 3.28 (dd, 1H, $J_{6'a,6'b}$ 11.5 Hz, $J_{6'a,5'}$ 3.5 Hz, H-6'a), 3.23-3.18 (m, 2 H, H-5', H-6'b), 1.90 (3 H, NAc).

1-acetamido-1-deoxy-4-O-(2-O-allyl-3,6-di-O-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranose (**6)**

To a solution of **5** (3.57 g, 4.25 mmol) in DCM (20 mL) and dry pyridine (1.03 mL) BzCl (1eq., 0.566 mL) was slowly added at 0^{o}C. After 1 h the reaction was quenched with MeOH, concentrated and co-evaporated with pyridine 3 times. Chromatography with hexane:ethyl acetate = 55:45-50:50 gave **6** (3.3 g, 82%). ^{1}H-NMR ($CDCl_3$): δ 8.10-7.90 and 7.60-7.30 (m, 25 H, Bz), 6.40 (d, 1 H, $J_{1,NH}$ 9.1 Hz, NH), 5.86 (t, 1 H, $J_{2,3}$ ~ $J_{3,4}$ = 9.5 Hz, H-3), 5.77-5.70 (m, 1 H, All), 5.48 (t, 1 H, $J_{1,2}$ ~ $J_{1,NH}$ = 9.3 Hz, H-1), 5.27 (t, 1 H, H-2), 5.18-5.14 (m, 1 H, All), 5.04-5.01 (m, 1 H, All), 4.91 (dd, 1 H, $J_{2',3'}$ 10.1, Hz, $J_{3',4'}$ 3.3 Hz, H-3'), 4.86 (dd, 1 H, $J_{6a,6b}$ 12.3 Hz, $J_{6a,5}$ 1.8 Hz, H-6a), 4.56 (dd, 1 H, $J_{6b,5}$ 4.2 Hz, H-6b), 4.51 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.26-4.22 (m, 2 H, H-4, All), 4.15-4.13 (m, 1 H, All), 4.05 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 3.97 (dd, 1H, $J_{6'a,6'b}$ 11.3 Hz, $J_{6'a,5'}$ 7.1 Hz, H-6'a), 3.95 (d, 1 H, H-4'), 3.71-3.66 (m, 2 H, H-2', H-6'b), 3.55 (broad t, 1 H, H-5'), 1.90 (s, 3 H, NAc).

1-acetamido-1-deoxy-4-O-[2-O-allyl-3,6-di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzoyl-β-D-glucopyranose **(7)**

A mixture of **6** (3.3 g, 3.49 mmol) and AgOTf (1.97 g, 2.2 eq.) was suspended in 15 mL of dry toluene, then 2,4,6-collidine (1.03 mL, 2.2 eq.) was added and the mixture was cooled to 0^{o}C. A solution of 2,3,4,6-tetra-O-benzyl-α,β-D-galactopyranosides (5.2 g) in dry toluene (10 mL) was added slowly and the reaction was stirred for 1 h. TLC: a sample was taken by pipette, diluted with ~0.2 mL of ethyl acetate, washed with 1N HCl, TLC performed in hexane:ethyl acetate=1:1, Rf=0.5.The mixture was diluted with toluene, washed with saturated $Na_2S_2O_3$, 10% HCl, water, and $NaHCO_3$ then concentrated. Chromatography with hexane : ethyl acetate = 65:35-50:50 gave **7** (3.7 g, 72%). ^{1}H-NMR ($CDCl_3$): δ 8.10-7.00 (m, 45 H, arom), 6.40 (d, 1 H, $J_{NH,1}$ 9.2 Hz, NH), 5.89 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.52 Hz, H-3), 5.70-5.64 (m, 1 H, All), 5.48 (t, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 5.20 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 5.04 (dd, 1 H, J 17.2 Hz, J 1.3 Hz, All), 4.93-4.91 (m, 1 H, All), 4.88 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6a}$ 1.6 Hz, H-6a), 4.84 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, $J_{3',4'}$ 2.7 Hz, H-3'), 4.79 (d, 1 H, J_{gem} 11.3 Hz, Bn), 4.65 (d, 1 H, J_{gem} 11.7 Hz, Bn), 4.65 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.58 (dd, 1 H, $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.57 (d, 1 H, J_{gem} 11.7 Hz, Bn), 4.54 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.46 (d, 1 H, $J_{5',6'a}$ 6.4 Hz, $J_{6'a,6'b}$ 11.3 Hz, H-6'a), 4.43 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.43 (s, 2 H, Bn), 4.25-4.10 (m, 6 H, H-4, H-4', H-6'b, Bn, All), 4.06-4.02 (m, 2 H, H-5, All), 3.95 (broad t, 1 H, J~6.8 Hz, H-5''), 3.84 (broad s, 1 H, H-4''), 3.79 (dd, 1 H, $J_{2'',3''}$ 10.3 Hz, H-2''), 3.67-3.62 (m, 3 H, H-2', H-3'', H-5'), 3.28 (t, 1 H, J~8.6 Hz, H-6''a), 3.07 (dd, $J_{5'',6''b}$ 5.6 Hz, $J_{6''a,6''b}$ 8.8 Hz, H-6''b), 1.9 (s, 3 H, NHAc).

1-acetamido-1-deoxy-4-O-[2-O-(R,S-2,3-cyclohexylidendioxypropyl)-3,6-di-O-benzoyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzoyl-β-D-glucopyranose **(8)**

To a solution of **7** (1.28 g, 0.87 mmol) and 4-methyl-morpholine N-oxide (205 mg) in 10 mL of acetone 1 mL of OsO_4 solution in t-BuOH was added. The reaction was stirred at 40 ^{o}C for 1 h then diluted with DCM and washed with water, concentrated. The product was used for the next step without further purification. The residue was dried in vacuum overnight then dissolved in MeCN, cyclohexanon (4 mL) and TsOH (cat.) were added and the reaction mixture was heated at 50^{o}C for 0.5 h, then neutralized by Et_3N,

concentrated and chromatographed on silica gel with hexane:ethyl acetate = 70:30-60:40 gave **8** (1.17 g, 85%).

1-acetamido-1-deoxy-2,3,6-tri-O-acetyl-4-O-[2-O-(R,S-2,3-cyclohexylidendioxypropyl)-3,6-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranose **(9)**

To a solution of **8** (3.35 g, 2.11 mmol) in dry MeOH (10 mL) and dry K_2CO_3 (~50 mg) was added the mixture was stirred for 2 days then neutralized by DOWEX, filtered and concentrated. The product was briefly columned on silica gel (10% MeOH-DCM at the end) to remove BzOMe, concentrated and dried. The residue was dissolved in NH_3 (~30 mL) and appropriate amount of Na (~0.4 g) was added to achieve blue colour of the solution. The mixture was stirred for 0.5 h then NH3 was allowed to escape. The residue was dissolved in MeOH, neutralized with DOWEX to pH~8, filtered and concentrated. The product was dried and acetylated with Ac_2O (20 mL) in pyridine (20 mL). After 16 h the reaction was quenched with MeOH, concentrated, co-evaporated with toluene 3 times and dried. Chromatography of the residue on silica gel with hexane : acetone = 55:45 gave **9** (1.66 g, 72%).

1-acetamido-1-deoxy-2,3,6-tri-O-acetyl-4-O-[2-O-(2-hydroxyethyl)-3,6-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranose **(10)**

A solution of **9** (1.66 g, 1.54 mmol) in 80% AcOH was stirred for 1 h at 90^{o}C then concentrated, diluted with DCM, washed with saturated solution of $NaHCO_3$ and concentrated. The residue was dissolved in DCM (10 mL) and a solution of $NaiO_4$ (0.7 g) in water (3 mL) was added. After TLC indicated complete conversion into an aldehyde, NaBH4 (0.5 g) was carefully added under controlled pH~7-8, which was adjusted by addition of portions of AcOH. The mixture was diluted with brine, extracted with DCM 3 times and concentrated. Chromatography of the residue on silica gel in toluene - acetone (75 : 35 - 60 : 40) to gave **10** (1.3 g, 87%). ^{1}H-NMR ($CDCl_3$): δ 6.14 (d, 1 H, $J_{1,NH}$ 9.3 Hz, NH), 5.50 (dd, 1 H, $J_{3'',4''}$ 3.3 Hz, $J_{4'',5''}$ 0.9 Hz, H-4''), 5.34 (dd, 1 H, $J_{2'',3''}$ 11.2 Hz, $J_{3'',4''}$ 3.3 Hz, H-3''), 5.29 (t, 1 H, $J_{2,3} \sim J_{3,4}$ = 9.0 Hz, H-3), 5.22-5.17 (m, 2 H, H-1, H-2''), 4.97 (d, 1 H, $J_{1'',2''}$ 3.7 Hz, H-1''), 4.82 (t, 1 H, $J_{1,2} \sim J_{2,3}$ = 9.7 Hz, H-2), 4.71 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 2.7 Hz, H-3'), 4.48 (dd, 1 H, $J_{6a,6b}$ 11.9 Hz, $J_{6a,5}$ 0.9 Hz, H-6a),

4.41-4.37 (m, 2 H, H-6'a, H-5''), 4.30-4.26 (m, 2 H, H-1', H-6b), 4.15 (dd, $J_{6'',6''b}$ 11.0 Hz, $J_{6'',5''}$ 5.7 Hz, H-6''a), 4.10-4.06 (m, 2 H, H-6'a, H-6''b), 4.00 (d, 1 H, H-4'), 3.83-3.61 (m, 7 H, H-4, H-5, H-5', CH_2), 3.46 (dd, 1 H, H-2'), 2.12, 2.11, 2.09, 2.07, 2.06, 2.05, 2.038, 2.035 (8s, 24 H, Ac), 1.96 (s, 6 H, Ac).

1-acetamido-1-deoxy-2,3,6-tri-O-acetyl-4-O-{2-O-[2-(p-nitrophenyloxycarbonyloxy)ethyl]-3,6-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl}-β-D-glucopyranose **(11)**

To a solution of **10** (2.4 g, 2.48 mmol) and 4-nitrophenyl chloroformate (0.75 g, 1.5 eq.) in dry DCM (10 mL) pyridine (05 mL) was added After 20 min at RT the reaction was quenched with MeOH, diluted with DCM and washed with brine. Chromatography of the residue on silica gel with pentane - ethyl acetate (60:40-40:60) gave **11** (2.4 g, 85%) ^{1}H-NMR ($CDCl_3$): δ 6.12 (d, 1 H, $J_{1,NH}$ 9.3 Hz, NH), 5.50 (dd, 1 H, $J_{4'',3''}$ 3.3 Hz, $J_{4'',5''}$ 1.3 Hz, H-4''), 5.34 (dd, 1 H, $J_{2'',3''}$ 11.0 Hz, H-3''), 5.28 (dd, 1 H, $J_{2,3}$ 8.8 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.21-5.16 (m, 2 H, H-1, H-2''), 4.98 (d, 1 H, $J_{1'',2''}$ 3.7 Hz, H-1''), 4.82 (t, 1 H, $J_{1,2} \sim J_{2,3}$ = 9.5 Hz, H-2), 4.76 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 6.9 Hz, H-3'), 4.56 (dd, 1 H, $J_{6a,6b}$ 13.3 Hz, $J_{6a,5}$ 1.8 Hz, H-6a), 4.42-4.36 (m, 3 H, H-5'', H-6'a, H-6'b), 4.33 (ddd, 1 H, J 2.8 Hz, J 6.4 Hz, J 9.0 Hz, CH_2), 4.30 (s, 1 H, H-1'), 4.26 (dd, 1 H, $J_{6b,5}$ 4.6 Hz, H-6b), 4.20 (dd, 1 H, $J_{6'',6''b}$ 11.0 Hz, $J_{5'',6''a}$ 6.5 Hz, H-6''a), 4.09-4.00 (m, 4 H, H-4', H-6''b, CH_2), 3.92 (ddd, 1 H, J 2.6 Hz, J 6.2 Hz, J 11.5 Hz, CH_2), 3.80 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 3.77 (t, 1 H, H-4), 3.67 (t, 1 H, $J_{5',6'a} \sim J_{5',6'b}$ 7.0 Hz, H-5'), 3.42 (dd, 1 H, H-2'), 2.14, 2.13, 2.07, 2.06, 2.05, 2.05, 2.035, 2.029, 1.97, 1.96 (10 s, 30 H, Ac).

1,3-di-(t-butoxycarbonylamino)-2-hydroxypropane (**13**).

To a solution of 1,3-di-amino-2-hydroxypropane **12** (2.13 g, 23.64 mmol) in DCM (20 ml) and MeOH (20 ml) di-t-butyl dicarbonate (10.31 g, 47.26 mmol) was added. The mixture was stirred for 1 h then concentrated. Chromatography of the residue on silica gel with DCM-MeOH (5%) gave the title product (6.55 g, 95 %).^{1}H-NMR ($CDCl_3$): δ 5.13 (broad s, 2 H, NH), 3.71 (p, 1 H, ^{3}J 4.6 Hz, CH), 3.4-3.2 (m, 1 H, OH), 3.22 (dd, 2 H, ^{2}J 14.2 Hz, CH_2), 3.14 (dd, 2 H, CH_2), 1.41 (s, 18 H, CH_3).

1,3-di-(t-butoxycarbonylamino)-2-(4-nitrophenyl-carbonyloxy)-propane (**14**).

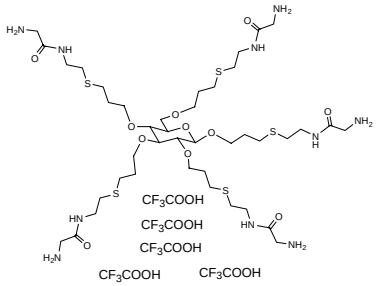
To a solution of **13** (3.57 g, 12.29 mmol) in dry Py a solution of 4-nitrophenyl-chloroformate (3 g, 14.7 mmol) in DCM (5 ml) was added. The mixture was stirred at

60oC for 1 h then concentrated, co-evaporated with toluene. Chromatography of the residue on silica gel with hexane-acetone (60:40) gave the title product (4.36 g, 78 %). ^{1}H-NMR

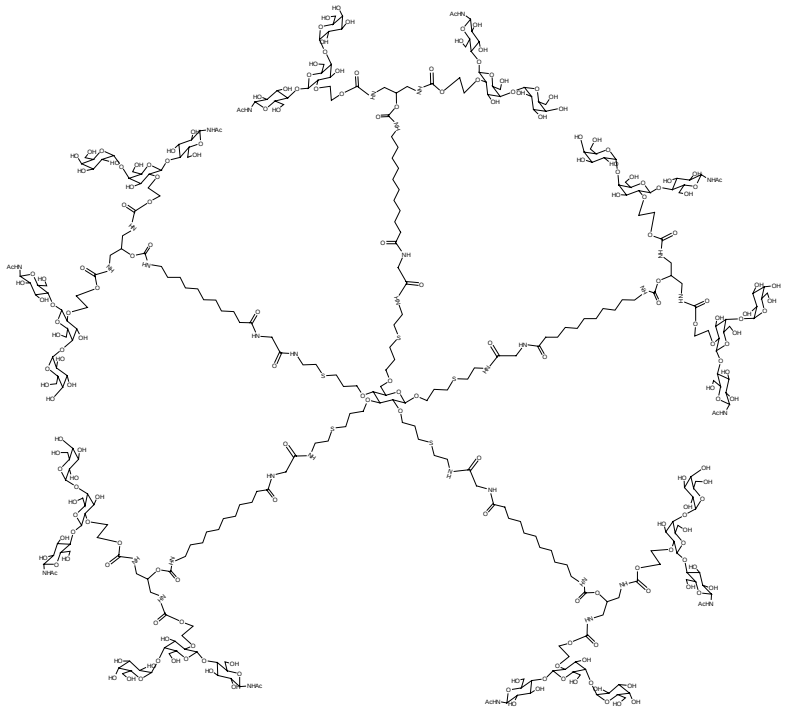
1,3-di-(t-butoxycarbonylamino)-2-[N-(10-carboxydecyl)carbamoyloxy]-propane (**15**).
To a solution of **14** (1.87 g, 4.1 mmol) and 11-amino-undecanoic acid (1.65 g, 8.2 mmol) in THF (20 ml) triethylamine (2 ml) was added. The mixture was refluxed overnight then concentrated. Chromatography of the residue on silica gel with DCM-MeOH (4%) gave the title product (1.0 g, 47%). ^{1}H-NMR (CD_3OD): δ 6.7-6.5 (m, 1 H, NH), 4.7 (broad s, 2 H, NH), 3.3-3.2 (m, 5 H, CH_2, CH), 3.07 (t, 2 H, ^{3}J 6.7 Hz, C$\underline{H_2}$COOH), 2.26 (t, 2 H, ^{3}J 7.5 Hz, CH_2N), 1.59 (m, 2 H, C$\underline{H_2}$ CH_2COOH), 1.5-1.3 (m, 14 H, CH_2), 1.41 (s, 18 H, CH_3).
1,3-Bis-{1-acetamido-1-deoxy-2,3,6-tri-O-acetyl-4-O-[3,6-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranose-2'-yloxyethyl}oxycarbamoyl-2-[N-(10-carboxydecyl)carbamoyloxy]-propane (**16**).
Compound **15** (115 mg, 0.222 mmol) was dissolved in TFA (1 mL). After 1 h the mixture was concentrated, co-evaporated with water, freeze-dried from water to give ~120 mg of TFA salt. To this product a solution of activated carbonate **11** (600 mg, 0.53 mmol) in dry MeCN (4 mL) and triethylamine (0.2 mL) was added. The mixture was sonicated and heated at 80 ^{o}C overnight then concentrated. Chromatography of the residue on silica gel with DCM-MeOH (10%) gave the title product (391 mg, 76%). ^{1}H-NMR (CD_3OD): δ 5.51 (dd, 2 H, $J_{3'',4''}$ 3.3 Hz, $J_{4'',5''}$ 1.1 Hz, H-4''), 5.36 (dd, 2 H, $J_{2'',3''}$ 11.0 Hz, H-3''), 5.29-5.24 (m, 4 H, H-1, H-3), 5.19 (dd, 2 H, $J_{1'',2''}$ 3.5 Hz, H-2''), 5.02 (d, 2 H, H-1''), 4.93 (t, 2 H, $J_{1,2}$ ~ $J_{2,3}$ ~ 9.7 Hz, H-2), 4.83-4.80 (m, 2 H, H-3'), 4.59 (dd, 2 H, $J_{6a,6b}$ 12.2 Hz, $J_{6a,5}$ 1.2 Hz, H-6a), 4.41-4.37 (t, 2 H, H-5''), 4.45-4.42 (m, 4 H, H-1', H-6b), 4.28-4.08 (m, 14 H, H-4', CH_2, H-6'a, H-6'b, H-6''a, H-6''b), 3.94-3.83 (m, 10 H, H-4, H-5, H-5', CH_2), 3.5 (m, 2 H, H-2'), 3.4-3.26 (m, 5 H, CH_2, CH), 3.08 (t, 2 H, J 7.1 Hz, CH_2), 2.26 (t, 2 H, J 7.5 Hz, CH_2), 2.13, 2.13, 2.08, 2.07, 2.07, 2.04, 2.03, 2.00, 1.95, 1.94 (10 s, 60 H, Ac), 1.61-1.56 (m, 2 H, CH_2), 1.50-1.44 (m, 2 H, CH_2),), 1.34-1.28 (m, 12 H, CH_2),.
1,2,3,4,6-penta-O-(6-amino-4-thia-hexyl)-β-D-glycopyranose (**18**)

A solution of 1,2,3,4,6-penta-O-allyl-β-D-glycopyranose **17** (500 mg, 1.31 mmol) and 2-aminoethanethiol hydrochloride (1.1 g, 9.68 mmol) in degassed MeOH (5 ml) was irradiated at 254 nm for 20 h. The mixture was concentrated and the residue was purified by size-exclusion chromatography using Sephadex G-10 with water as an eluent to give the title compound (1.01 g, 81%). ^{1}H-NMR (D_2O): δ 4.46 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.02-3.64 (m, 22 H, H-6a, H-6b, CH_2O, CH_2N), 3.51 (ddd, 1 H, $J_{5,4}$ 7.3 Hz, $J_{5,6a}$ 2.0 Hz, $J_{5,6b}$ 5.1 Hz, H-5), 3.43 (t, 1 H, $J_{3,4}$~$J_{3,2}$ 9.5 Hz, H-3), 3.34 (t, 1 H, H-4), 3.26-3.30 (m, 10 H, CH_2N), 3.14 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2) 2.90-2.84 (m, 10 H, CH_2S), 2.72-2.65 (m, 10 H, CH_2S), 1.99-1.89 (m, 10 H, $CH_2C\underline{H}_2CH_2$).

1,2,3,4,6-penta-O-[7-aza-6-(t-butoxycarbonylamino)-8-oxo-4-thia-nonyl]-β-D-glycopyranose (**19**). To a suspension of **18** (759 mg, 0.803 mmol), TBTU (1.91 g, 5.96 mmol), HOBt (974 mg, 5.6 mmol) and N-(t-butoxycarbonyl)-glycine (1.04 g, 5.93) in dry DCM (5 ml) 4-ethyl-morpholine (1.37 g, 11.9 mmol) was added. When all components were dissolved, the reaction mixture was washed with water and concentrated. Chromatography of the residue on silica gel with DCM-MeOH (5-7%) gave the title product (1.02 g, 82%). ^{1}H-NMR ($CDCl_3$): δ 7.1-6.8 (m, 5 H, NH), 5.8-5.2 (m, 5 H, NH), 4.18 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.76 (s, 10 H, CH_2 of Gly), 4.0-3.4 (m, 23 H, H-5, H-6a, H-6b, CH_2O, CH_2N), 3.22-3.18 (m, 2 H, H-3, H-4), 3.00 (t, 1 H, H-2) 2.7-2.5 (m, 20 H, CH_2S), 1.9-1.8 (m, 10 H, $CH_2C\underline{H}_2CH_2$), 1.43 (s, 40 H, CH_3).



$C_{51}H_{87}F_{15}N_{10}O_{21}S_5$
Mol. Wt.: 1621.60



SF-2 (L26)
$C_{346}H_{597}N_{35}O_{206}S_5$
Exact Mass: 8698.5920
Mol. Wt.: 8703.8847

SF-2

Amine salt, obtained from PK-11 (23 mg, 14.2 μmol) was mixed with PK-41 (190 mg, 82 μmol) TBTU (45 mg), HOBt (22 mg) and dissolved in dry DMF (5 mL). The mixture was stirred overnight at 60^{o}C, then concentrated, dissolved in MeOH and solution of NaOH in MeOH was added. The mixture was neutralized with AcOH and the product was purified on HPLC C-18 column. The compound eluted with 60-70% MeOH. to give SF-2 (84 mg, 2.4 μmol, 68%).