Chemical and Enzymatic Synthesis of Modified Sialyl Lewis X Tetrasaccharides with High Affinity for E and P-Selectin

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This Paper is Dedicated to Prof. Horst Kunz on the Occasion of his 60th Birthday

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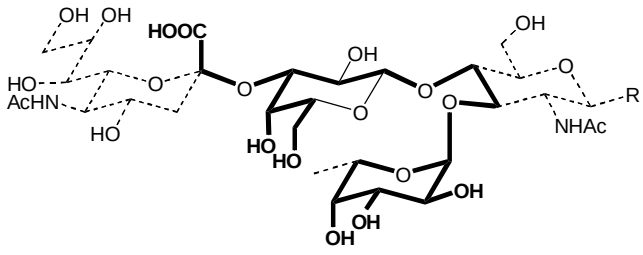
Abstract. A series of sialyl-Lewis X tetrasaccharide analogs **1a**–**d** was prepared using a combined chemical and enzymatic approach. Sialic acid analogs **5b**,**c** were obtained from 2-azido mannose **4c** or 2-deoxy mannose **4b** and pyruvate by an aldolase reaction and converted to the protected thioglycosides **3b**,**c** that served as sialyl donors for the Lewis X acceptor trisaccharide **2**. The resulting sialyl-Lewis X tetrasaccharides **8a**–**c** were deprotected by deacylation and saponification of the methyl ester. Debenzylation was achieved by careful transfer hydrogenation in the presence of formic acid or ammonium formate as a hydrogen source. Three sialyl-Lewis X derivatives **1a**–**c** were thus obtained and the parent compound **1a** was further modified by alkaline hydrolysis of the two acetamides to give the *lyso* sialyl-Lewis X derivative **1d**. The four sialyl-Lewis X tetrasaccharides **1a**–**d** were tested for their binding affinity to *E* and *P*-selectin with the *lyso* sialyl-Lewis X derivative **1d** showing the highest inhibitory potency for both lectins.

The binding of sialyl Lewis X (sLe^X) epitopes and related structures found on the termini of glycolipids and glycoproteins is considered to mediate the initial adhesion of several groups of leukocytes to areas of inflammation in the vascular system [1]. The selectins *E, P*, and *L* are cell surface lectins with distinct carbohydrate recognition domains (CRD) involved in this process [2]. For instance, *P*-selectin plays a role in acute as well as in long-term responses, as shown by the contact hypersensitivity response in P-selectin-deficient mice [3], and in delayed-type hypersensitivity reactions in *L*-selectin-deficient mice [4] to mention only two out of a wealth of studies. SLe^X and derived antagonists (glycomimetics) which are targeted against the selectin CRDs are therefore potential agents to prevent leukocyte adhesion and their subsequent migration into the diseased tissue in several acute and chronic inflammatory diseases.

However, clinical trials directed against ischemia/reperfusion using the native sLe^X-oligosaccharide failed, although this substance was an effective selectin blocker in a variety of mammalian species at quite high doses up to 20 mg/kg [5].

Therefore, considerable effort has been spent during recent years on improved anti-inflammatory drugs based on the sLe^X lead structure [6]. These were mostly designed to replace the complex tetrasaccharide sLe^X, based on the knowledge about structure-activity relationships that have been obtained by variation of functional groups of sLe^X [7].



In summary, all the ensuing attempts to replace the sugars more rigorously by different chemical moieties were either of limited success or provided rather inconsistent results.

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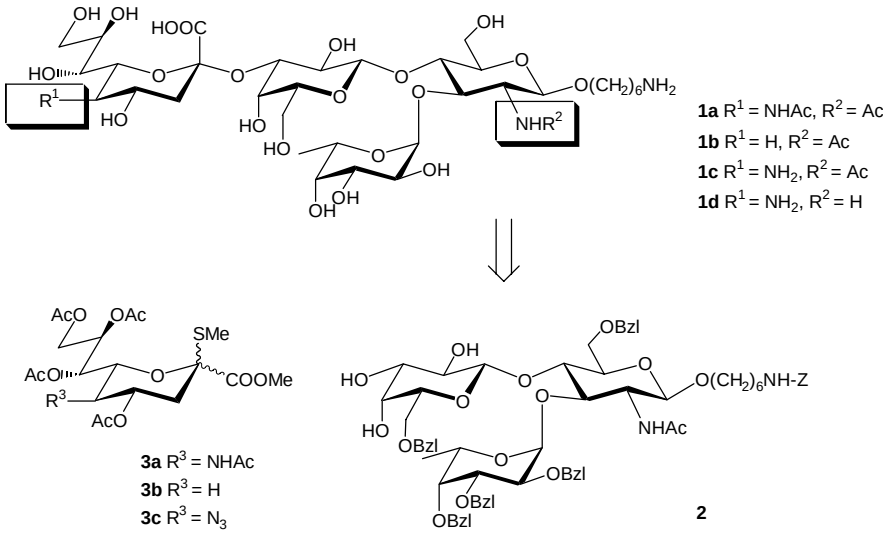
Herein we describe another concise approach leading to slightly modified glycomimetics having specific activities towards *P*- and *E*- selectins. Our approach involves the introduction, respectively the unmasking of functional groups in sLe^X in order to gain potential binding affinity via additional ionic interactions. Interestingly, our synthetic concept pursued here, namely the replacement of the acetamido group present in the sialic acid moiety of sLe^X by hydrogen or an amino group [8] has also been successfully exploited by other groups to improve the binding of some sLeX-glycolipids to *L*-selectin after *N*-5 deacetylation [9].

The four sLe^x tetrasaccharides **1a**–**d** were synthesized from the Le^x trisaccharide acceptor **2** that was sialylated using the modified donors **3** [8]. We have established different routes to acceptor **2** either following the large-scale synthesis protocol [6c] starting from *N*-acetylglucosamine or an alternative sequence that was utilizing a phthalimido protected glucosamine at the reducing end [8] (Scheme 1). The synthetic scheme is of general applicability with respect to the derivatization of the different sialic acids and their latter glycosylation.

awa [11]. Starting with an acid catalyzed esterification the resulting methyl esters **6b**,**c** were acetylated (**7b**,**c**) and transformed to the thiomethyl derivatives **3b**,**c** by reaction with TMS-SMe and TMS-triflate. Despite the increased acid sensitivity of the 5-deoxy and 5-azido sialic acids the protection sequence gave yields similar to sialic acid.

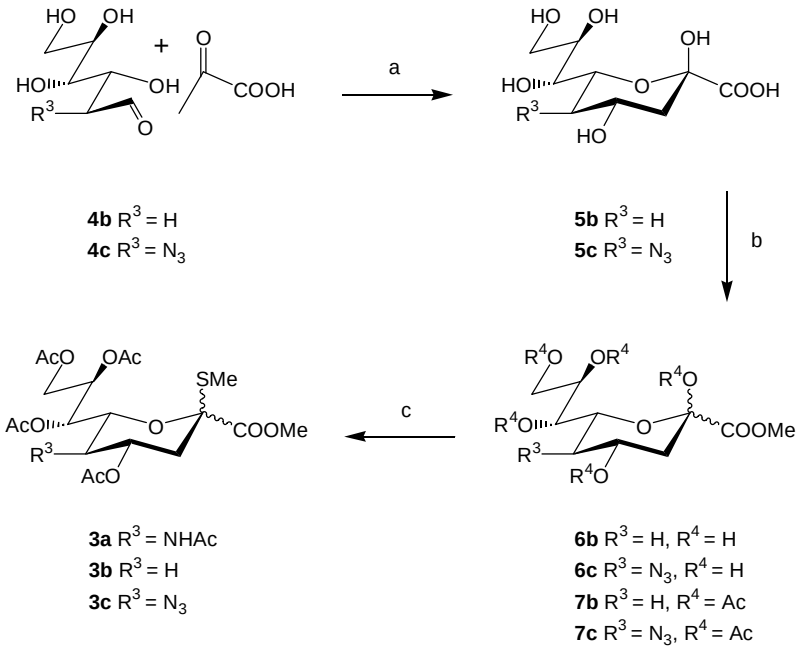
The conditions for regioselective α-(**2**→**3**)-sialylation [12] using *N*-iodosuccinimide and triflic acid in acetonitrile gave the desired sLe^x tetrasaccharides **8a**–**c** (Scheme 3), albeit in significantly lower yields for the modified donors **3b**,**c**. This may be attributed to a higher tendency for elimination upon activation compared to the reference compound **3a**.

Both sLe^x tetrasaccharides **8a** and **b** could be deprotected following a three step procedure [13]. Initially, basic deacetylation gave a mixture of lactones which were debenzylated using formic acid as an activant and a hydrogen source. The final basic step opened the lactone ring formed previously. Despite the increased acid sensitivity of the 5-deoxy sialic acid moiety in compound **1b** the use of formic acid was well tolerated. In

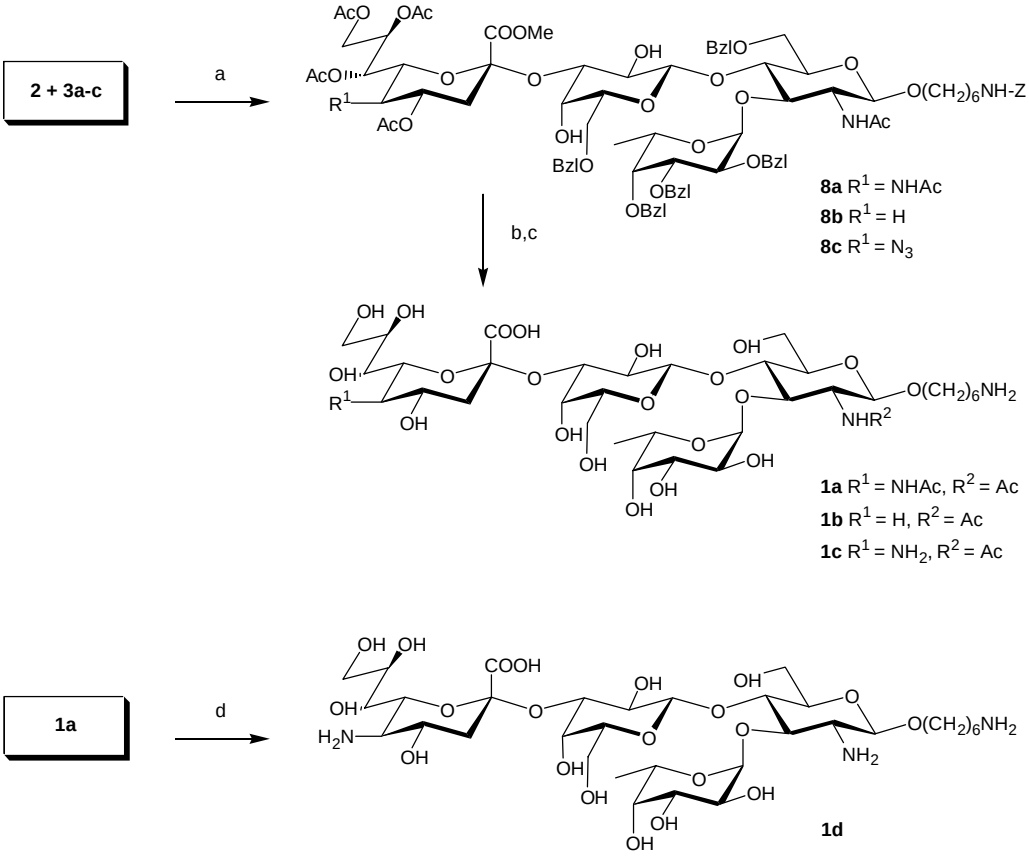


Scheme 1 Retrosynthetic disconnection of the sLe^x derivatives

The modified sialic acids (Scheme 2) were best obtained by enzymatic elongation of the mannose derivatives **4b**,**c** with pyruvate catalyzed by sialic acid aldolase. The substrate tolerance of the aldolase permits the generation of sialic acids where the *N*-acetyl substituent at position 5 is replaced by hydrogen (**5b**) [10a] or an azido group (**5c**) [10b]. An azido function can be selectively reduced to an amine that is easily functionalized further. The modified sialic acids **5b**,**c** were converted to the corresponding donor thioglycosides **3b**,**c** according to a three step sequence introduced by Hasegcontrast, the 5-amino compound derived from **8c** decomposed rapidly after adding formic acid. Thus, the deprotection of **8c** required mild debenzylation conditions to avoid hydrolysis of the highly acid sensitive neuraminyl residue of compound **1c**. The cleavage of the protective groups could be conducted using solely basic or neutral conditions. In the first step the acetates were removed by methanolysis and after the addition of water the newly formed lactones could be hydrolyzed. Subsequent debenzylation was accomplished by a catalytic transfer hydrogenation under neutral conditions



Scheme 2 Synthesis of the sialyl donors. a) sialic acid aldolase; b) 1. MeOH, H^+; 2. Ac_2O-pyridine; **7b** (82%), **7c** (82%); c) TMS-SMe, TMS-OTf; **3b** (91%), **3c** (90%)



Scheme 3 Synthesis and deprotection of sLe^x tetrasaccharides; a) NIS, triflic acid, CH_3CN, –40 °C (**8a**: 54% [8, 6c], **8b**: 21%, **8c**: 26%); b) 1. NaOMe–MeOH 2. H_2-Pd–MeOH, HCOOH 3. NaOH-H_2O (**1a**: 70% [8, 6c]; **1b**: 72%); c) 1. K_2CO_3–MeOH-H_2O; 2. $HCOONH_4$–Pd/C(10%)-MeOH; (**1c**: 81%); d) Bu_4NOH-H_2O, 95 °C, **3d** (53%).

using palladium on charcoal and ammonium formate as a hydrogen source [14]. The desired tetrasaccharide **1c** was isolated in 81% yield over two steps.

In contrast to the high sensitivity of compound **1c** to acidic conditions the stability of glycosides in basic environments is quite high. This includes sialosides as shown previously for the basic deamidation of the 5-acetamido group [15]. Thus, we exposed the tetrasaccharide **1a** to pH 13 at 95 °C for three days using concentrated tetrabutylammonium hydroxide in water. This procedure removed both acetamido groups from **1a** furnishing the *lyso* sLe^x derivative **1d** in 53% yield.

The four sLe^x derivatives were assayed as inhibitors [16] of the sLe^x binding proteins E and P selectin (table 1). Compared to the reference compound **1a** the variations at C-5 of the sialic acid moiety gave only modest improvements in inhibitory potency. Surprisingly, the fully deacetylated *lyso*-tetrasaccharide **1d** showed a significant increase in binding affinity. For *E*-selectin the relative binding strength improved nearly fourfold whereas the potency towards *P*-selectin was enhanced by a factor of 12.5. These findings are in contrast to a recent study [18] where 2-amino and 2-azido sLe^x derivatives were tested for *P*-selectin and the affinities were not elevated compared to the 2-*N*-acetyl compound. However, for *E*-selectin the 2-amino compound showed a sixfold improvement of binding. These data are in agreement with the reduction of the IC_{50} for the diamino compound **1d** compared to the diamide **1a**.

Table 1 Inhibition of HL60 cell adhesion to recombinant *E*- and *P*-selectin-IgG fusion proteins by synthetic sLe^X tetrasaccharides **1a**–**d**. IC_{50} values are concentrations of inhibitors required to block adhesion of 50% of the cells compared to the negative control [17].

	1a	**1b**	**1c**	**1d**
E-selectin IC_{50}	1000 µM	700 µM	900 µM	270 µM
P-selectin IC_{50}	2000 µM	700 µM	1000 µM	160 µM

In summary, the data obtained from the binding studies of the synthetic sLe^x derivatives support our interpretation of the increased binding of the *lyso*-sLe^x compound **1d** to both *E* and *P*-selectin as a synergistic effect caused by the liberated amino groups.

This work was supported by Aventis Research and Technologies. Gratitude is expressed to Prof. Horst Kessler for his generous support.

Experimental

The solvents were dried by standard methods. NMR spectra were recorded on a Bruker AMX-500 instrument. For spectra recorded in D_2O the HOD signal (4.81 ppm) was used as reference. Coupling constants in Hz. FAB-MS: Finnigan MAT 95Q (thioglycerine-HOAc matrix; MB or m-nitrobenzylacohol NBA). ESI-MS: Finnigan TSQ.

Methyl 3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosonate (**6b**)

To a 0.415 g portion of 5-deoxy sialic acid **5b** (1.65 mmol) in 35 ml of absolute methanol were added 1.66 g of Amberlyst 15 ion exchange resin. The mixture was carefully stirred for 12 h at ambient temperature. After complete reaction (TLC: R_f = 0.51 isopropanol/1 M NH_4OAc, 4:1, v:v) the ion exchange resin was filtered off and washed repeatedly. The filtrate was concentrated in a rotary evaporator and the remainder was dried in high vacuo. (0.44 g crude **6b**) $C_{10}H_{18}O_8$ (266.25). – ^{1}H NMR (500 MHz, [D_6]DMSO): δ/ppm = 4.05 (m, 1H, H-4), 3.67 (s, 3H, OCH_3), 3.59 (m, 1H, H-9a), 3.58 (m, 1H, H-6), 3.48 (m, 1H, H-7), 3.34 (m, 1H, H-9b), 3.13 (m, 1H, H-8), 1.89 (dd, J_{gem} = 12.3, J_{vic} = 3.4, 1H, H-3eq), 1.58 (m, 1H, H-5eq), 1.46 (m, 1H, H-5ax), 1.45 (m 1H, H-3ax). – ^{13}C NMR (125 MHz, [D_6]DMSO): δ/ppm = 171.6 C-1, 95.4 C-2, 72.8 C-8, 70.6 C-7, 68.1 C-4, 63.5 C-9, 63.3 C-6, 51.8 OCH_3, 40.3 C-3, 36.5 C-5.

Methyl 5-azido-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosonate (**6c**)

To a 0.478 g portion of 5-azido sialic acid **5c** (1.63 mmol) in 40 ml of absolute methanol were added 1.91 g of Amberlyst 15 ion exchange resin. The mixture was carefully stirred for 4.5 h at ambient temperature. After complete reaction (TLC: R_f = 0.58 isopropanol/1 M NH_4OAc, 4:1, v:v) the ion exchange resin was filtered off and washed repeatedly. The filtrate was concentrated in a rotary evaporator and the remainder was dried in high vacuo. (0.50 g crude **6c**). $C_{10}H_{17}N_3O_8$ (307.26). – ^{1}H NMR (500 MHz, [D_6]DMSO): δ/ppm = 3.84 (ddd, $J_{4,5}$ = 9.9, 1H, H-4), 3.72 (m, 1H, H-6), 3.69 (s, 3H, $OC\underline{H}_3$), 3.63 (m, 1H, H-9a), 3.48 (m, 1H, H-8), 3.47 (m, 1H, H-7), 3.39 (m, 1H, H-5), 3.38 (m, 1H, H-9b), 2.00 (dd, J_{gem} = 12.9, $J_{3eq,4}$ = 5.0, 1H, H-3eq), 1.75 (dd, $J_{3ax,}$ = 11.7, 1H, H-3ax). – ^{13}C NMR (125 MHz, [D_6]DMSO): δ/ppm = 169.8 C-1, 94.8 C-2, 70.0 C-7, 69.4 C-6, 68.8 C-8, 67.9 C-4, 63.4 C-5, 63.4 C-9, 52.3 OCH_3, 39.4 C-3.

Methyl 2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosonate (**7b**)

To a cooled solution (0 °C) of 0.44 g of methylester **6b** (1.65 mmol) in 13 ml of pyridine were added 14 ml of acetic anhydride. The mixture was stirred for 48 h at ambient temperature. After complete reaction (TLC: R_f = 0.50 hexane/acetone, 1:1) the solution was concentrated and codistilled with toluene three times. The residue was further dried in high vacuo and purified by flash chromatography (hexane/

acetone, 2:1). (0.64 g, 82.1%). $[\alpha]^{22}_D = -25.8°$ (1.0, dichloromethane). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ/ppm = 5.14 (m, 1H, H-7), 5.10 (m, 1H, H-4), 5.02 (m, 1H, H-8), 4.30 (dd, $J_{8,9a}$ = 2.4, J_{gem} = 12.6, 1H, H-9a), 4.21 (m, 1H, H-6), 4.11 (dd, $J_{8,9b}$ = 5.2, 1H, H-9b), 3.66 (s, 3H, OCH$_3$), 2.32 (dd, J_{gem} = 12.6, J_{vic} = 8.0, 1H, H-3eq), 2.10, 2.09, 2.00, 1.99, 1.97 (5s, 15H, OAc), 1.91 (m, 1H, H-5eq), 1.65 (dd, J_{vic} = 11.8, 1H, H-3ax), 1.40 (m, 1H, H-5ax). – ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ/ppm = 169.9, 169.7, 169.6, 169.3, 169.1 OAc, 166.0 C-1, 96.5 C-2, 70.3 C-7, 69.0 C-6, 69.0 C-8, 65.7 C-4, 60.1 C-9, 52.7 OCH$_3$, 35.3 C-3, 31.1 C-5, 20.4, 20.3 OAc.

$C_{20}H_{28}O_{13}$ Calcd.: C 50.42 H 5.92
(476.43) Found: C 50.41 H 5.76.

Methyl 2,4,7,8,9-penta-O-acetyl-5-azido-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulopyranosonate (**7c**)

To a cooled solution (0 °C) of 0.50 g of methylester **6c** (1.63 mmol) in 6.8 ml of pyridine were added 7.4 ml of acetic anhydride. The mixture was stirred for 48 h at ambient temperature. After complete reaction (TLC: R_f = 0.57 hexane/acetone, 1:1) the solution was concentrated and codistilled with toluene three times. The residue was further dried in high vacuo and purified by flash chromatography (hexane/acetone, 2:1). (0.69 g, 81.8%). – ^{1}H NMR (500 MHz, [D$_6$]DMSO) *β-anomer* (major product): δ/ppm = 5.41 (dd, $J_{7,8}$ = 7.7, 1H, H-7), 5.13 (ddd, $J_{4,5}$ = 9.7, 1H, H-4), 4.97 (ddd, $J_{8,9a}$ = 5.1, $J_{8,9b}$ = 2.5, 1H, H-8), 4.30 (dd, J_{gem} = 12.5, 1H, H-9a), 4.11 (dd, 1H, H-9b), 3.89 (dd, $J_{6,7}$ = 1.5, 1H, H-6), 3.76 (ddd, $J_{5,6}$ = 10.6, 1H, H-5), 3.67 (s, 3H, OC$\underline{H}_3$), 2.44 (dd, J_{gem} = 13.3, J_{vic} = 4.9, 1H, H-3eq), 2.17, 2.12, 2.07, 1.99, 1.98 (5s, 15H, OAc), 1.92 (m, 1H, H-3ax). *α-anomer*: δ/ppm = 5.35 (dd, $J_{6,7}$ = 1.4, $J_{7,8}$ = 8.2, 1H, H-7), 5.13 (m, 1H, H-4), 5.04 (m, 1H, H-8), 4.23 (m, 1H, H-9a), 4.06 (m, 1H, H-9b), 3.99 (dd, $J_{5,6}$ = 10.4, 1H, H-6), 3.76 (m,1H, H-5), 3.68 (s, 3H, OCH$_3$). – ^{13}C-NMR (125 MHz, [D$_6$]DMSO) *β-anomer*: δ/ppm = 170.0, 169.8, 169.4, 169.3, 169.1 OAc, 165.9 C-1, 96.2 C-2, 70.6 C-6, 69.7 C-4, 69.0 C-8, 67.0 C-7, 60.9 C-9, 58.3 C-5, 52.9 OCH$_3$, 35.1 C-3, 20.5, 20.4 OAc. *α-anomer*: δ/ppm = 165.9 C-1, 90.5 C-2, 68.6 C-8, 35.1 C-3.

$C_{20}H_{27}N_3O_{13}$ Calcd.: C 46.42 H 5.26 N 8.12
(517.45) Found: C 46.46 H 5.32 N 8.13.

Methyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranosid)onate (**3b**)

A suspension of 0.62 g of pentaacetate **7b** (1.30 mmol) and 1.5 g of ground and freshly dried molecular sieves 4 Å and 0.68 ml of trimethylsilyl-methylmercaptane (4.81 mmol) in 10 ml of absolute dichloromethane was stirred for 10 minutes. After addition of 0.25 ml of trimethylsilyl-trifluoromethanesulfonate (1.30 mmol) the reaction was stirred for 16 h (TLC: R_f = 0.59 hexane/acetone, 1:1, v:v). The mixture was filtered through celite, washed twice with 1 M $KHCO_3$ and dried over $MgSO_4$. Filtration and concentration of the organic phase gave a remainder that was purified by flash chromatography (hexane/acetone, 4:1, v:v). (0.58 g, 90.6%). – ^{1}H NMR (500 MHz, [D$_6$]DMSO) *α-anomer*: δ/ppm = 5.25 (m, 1H, H-8), 5.08 (dd, $J_{6,7}$ = 2.4, $J_{7,8}$ = 8.2, 1H, H-7), 4.75 (m, 1H, H-4), 4.28 (dd, $J_{8,9a}$ = 2.5, J_{gem} = 12.4, 1H, H-9a), 4.12 (dd, $J_{8,9b}$ = 5.0, 1H, H-9b), 3.85 (m, 1H, H-6), 3.73 (s, 3H, OCH$_3$), 2.59 (dd, J_{gem} = 12.2, J_{vic} = 3.3, 1H, H-3eq), 2.11, 2.08 (2s, 6H, OAc), 2.03 (s, 3H, SCH$_3$), 1.99, 1.97 (2s, 6H, OAc), 1.79 (m, 1H, H-5eq), 1.54 (m, 1H, H-3ax), 1.28 (m, 1H, H-5ax). *β-anomer*: δ/ppm = 5.21–5.06 (m, 3H, H-8, H-7, H-4), 4.48 (dd, $J_{8,9a}$ = 2.5, J_{gem} = 12.2, 1H, H-9a), 4.27 (m, 1H, H-6), 4.13 (dd, $J_{8,9b}$ = 5.6, 1H, H-9b), 3.70 (s, 3H, OCH$_3$), 2.33 (dd, J_{gem} = 13.3, J_{vic} = 3.0, 1H, H-3eq), 2.10 (s, 3H, OAc), 2.01 (s, 3H, SCH$_3$), 1.99, 1.98, 1.92 (3s, 9H, OAc), 2.04 (m, 1H, H-5eq), 1.83 (dd, J_{vic} = 11.3, 1H, H-3ax), 1.34 (m, 1H, H-5ax). – ^{13}C NMR (125 MHz, [D$_6$]DMSO) *α-anomer*: δ/ppm = 170.0, 169.8, 169.4, 169.3 OAc, 168.1 C-1, 82.4 C-2, 70.7 C-6, 70.4 C-8, 67.9 C-7, 66.8 C-4, 61.5 C-9, 52.7 OCH$_3$, 37.4 C-3, 31.4 C-5, 20.8, 20.4 OAc, 11.0 SCH$_3$. *β-anomer*: δ/ppm = 167.7 C-1, 85.2 C-2, 69.6 C-6, 68.2 C-8, 67.5 C-7, 66.5 C-4, 61.3 C-9, 52.5 OCH$_3$, 36.2 C-3, 31.6 C-5, 20.8, 20.6, 20.4 OAc, 10.3 SCH$_3$.

$C_{19}H_{28}O_{11}S$ Calcd.: C 49.13 H 6.08
(464.48) Found: C 49.13 H 6.10.

Methyl (methyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranosid)onate (**3c**)

A suspension of 0.63 g of pentaacetate **7c** (1.22 mmol) and 1.5 g of ground and freshly dried molecular sieves 4 Å and 0.64 ml of trimethylsilyl-methylmercaptane (4.50 mmol) in 10 ml of absolute dichloromethane was stirred for 10 minutes. After addition of 0.24 ml of trimethylsilyl-trifluoromethanesulfonate (1.22 mmol) the reaction was stirred for 16 h (TLC: R_f = 0.61 hexane/acetone, 1:1, v:v). The mixture was filtered through celite, washed twice with 1 M $KHCO_3$ and dried over $MgSO_4$. Filtration and concentration of the organic phase gave a remainder that was purified by flash chromatography (hexane/acetone, 4:1, v:v). (0.55g, 89.6%). – ^{1}H NMR (500 MHz, [D$_6$]DMSO) *α-anomer*: δ/ppm = 5.34 (dd, $J_{7,8}$ = 8.8, 1H, H-7), 5.21 (ddd, $J_{8,9a}$ = 2.6, $J_{8,9b}$ = 4.6, 1H, H-8), 4.81 (ddd, $J_{4,5}$ = 9.6, 1H, H-4), 4.28 (dd, J_{gem} = 12.6, 1H, H-9a), 4.11 (dd, 1H, H-9b), 3.74 (s, 3H, OCH$_3$), 3.66 (dd, $J_{5,6}$ = 10.5, 1H, H-5), 3.52 (dd, $J_{6,7}$ = 1.4, 1H, H-6), 2.70 (dd, J_{gem} = 12.5, 1H, H-3eq), 2.17, 2.08, 2.06, 2.04, 1.98 (5s, 15H, OAc SMe), 1.83 (dd, J_{vic} = 12.1, 1H, H-3ax). *β-anomer*: δ/ppm = 5.46 (dd, $J_{7,8}$ = 6.7, 1H, H-7), 5.10 (m, 2H, H-8, H-4), 4.48 (dd, $J_{8,9a}$ = 2.4, J_{gem} = 12.6, 1H, H-9a), 4.13 (dd, $J_{8,9b}$ = 5.6, 1H, H-9b), 3.73 (s, 3H, OCH$_3$), 3.93 (dd, $J_{6,7}$ = 1.2, 1H, H-6), 3.64 (dd, $J_{4,5}$ = 9.8, $J_{5,6}$ = 10.5, 1H, H-5), 2.43 (dd, J_{gem} = 13.8, J_{vic} = 5.0, 1H, H-3eq), 2.17, 2.07 (2s 6H, OAc), 2.03 (s 3H, SCH$_3$), 2.00 (m, 1H, H-3ax), 1.98, 1.91 (2s, 6H, OAc). – ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ/ppm = 169.9, 169.8, 169.5, 169.3 OAc, 168.3 C-1, 82.7 C-2, 71.5 C-6, 70.9 C-4, 67.7 C-8, 67.0 C-7, 61.4 C-9, 58.8 C-5, 53.0 OCH$_3$, 36.4 C-3, 20.8, 20.5, 20.3 OAc, 11.2 SCH$_3$. – FAB-MS (NBA): $M_{ber.}$ = 505.2 $M_{gef.}$ = 528 (M+Na)

$C_{19}H_{27}N_3O_{11}S$ Calcd.: C 45.15 H 5.38 N 8.31
(505.50) Found: C 45.07 H 5.42 N 8.12.

*N^6-(Benzyloxycarbonyl)-6-aminohexyl-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(**2→3**)-O-(6-O-benzyl-β-D-galactopyranosyl)-(**1→4**)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(**1→3**)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside* (**8b**)

A mixture of 50 mg of donor **3b** (0.108 mmol), 207 mg of trisaccharide **2** (0.215 mmol) and 103 mg of ground and freshly

dried molecular sieves 4 Å in 0.8 ml of absolute acetonitrile was stirred in the dark for 2 h at room temperature. The suspension was cooled to −40 °C and a solution of 48.4 mg of *N*-iodosuccinimide (0.215 mmol) in 0.7 ml of absolute acetonitrile was added. After 20 min 1.9 µl of trifluoro-methanesulfonic acid (21.5 µmol) were added. The reaction was complete after 2 h at −40 °C (TLC: R_f = 0.35 (hexane/acetone, 0.8:1, v:v). The solids were filtered off from the diluted reaction mixture and the filtrate was extracted with dilute Na_2CO_3, dried over $MgSO_4$ and concentrated. The remainder was purified repeatedly by flash chromatography (hexane/acetone, 2:1, v:v). In addition to 37.5 mg of tetrasaccharide **8b** (21.3%) 130 mg of the acceptor **2** were isolated. $[\alpha]^{22}_D = -38.9°$ (1.0, dichloromethane). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ/ppm = 8.00 (d, $J_{NH,2}$ = 8.9, 1H, NH-2), 7.33–7.23 (m, 30H, Ar), 7.22 (m, 1H, NH urethane), 5.26 (m, 1H, H-1α'), 5.25 (m, 1H, H-7^N), 5.04 (m, 1H, H-8^N), 5.00 (s, 2H, OCH_2 Z), 4.83 (m, 1H, OH-2''), 4.80 (m, 1H, OCH_2Ph), 4.77 (m, 1H, H-4^N), 4.76 (m, 1H, H-5'), 4.74 (m, 1H, OH-4''), 4.73, 4.68, 4.53, 4.42, 4.40, 4.38 (6m, 9H, OCH_2Ph), 4.38 (m, 1H, H-1β''), 4.37 (m, 1H, H-1β), 4.27 (m, 1H, H-9a^N), 4.01 (m, 1H, H-9b^N), 3.96 (m, 1H, H-3'), 3.95 (m, 1H, H-5), 3.94 (m, 1H, H-3''), 3.92 (m, 1H, H-6a''), 3.81 (m, 1H, H-3), 3.80 (m, 1H, H-4''), 3.78 (m, 1H, H-6b''), 3.75 (m, 1H, H-2'), 3.74 (m, 1H, H-5''), 3.71 (m, 1H, H-4'), 3.70 (m, 1H, H-6a), 3.69 (m, 1H, αCH_2), 3.68 (s, 3H, OCH_3), 3.63 (m, 1H, H-2), 3.60 (m, 1H, H-6b), 3.48 (m, 1H, H-6^N), 3.47 (m, 1H, H-4), 3.38 (m, 1H, H-2''), 3.34 (m, 1H, αCH_2), 2.97 (m, 2H, κCH_2), 2.48 (m, 1H, H-3eq^N), 2.01, 1.91, 1.87 (3s, 12H, OAc), 1.83 (s, 3H, NAc), 1.81 (m, 1H, H-5eq^N), 1.78 (m, 1H, H-3ax^N), 1.42 (m, 2H, βCH_2), 1.37 (m, 2H, εCH_2), 1.28 (m, 1H, H-5ax^N), 1.22 (m, 4H, $\gamma,\delta CH_2$), 1.04 (d, $J_{5,6}$ = 6.4, 3H, H-6'). – ^{13}C NMR (125 MHz, [D$_6$]DMSO, 300 K): δ/ppm = 170.0, 169.6, 169.5, 169.3, 169.1 C=O OAc NAc, 168.4 C-1^N, 156.0 C=O urethane, 139.3, 139.2, 139.0, 138.5, 138.4, 137.3 C-i Ar, 128.3-126.7 Ar, 102.0 C-1β'', 100.8 C-1β, 99.0 C-2^N, 95.4 C-1α', 78.6 C-3, 77.9 C-3', 76.1 C-2´, 76.1 C-4', 74.6 C-4, 74.5 OCH_2Ph, 73.4 C-3'', 73.4 C-4'', 72.5 C-2'', 72.0, 71.9, 71.7, 70.3 OCH_2Ph, 70.3 C-8^N, 69.1 C-5, 68.5 αCH_2, 68.5 C-7^N, 68.4 C-6, 68.1 C-6'', 67.1 C-5'', 66.8 C-4^N, 65.3 C-5', 65.0 OCH_2 Z, 61.2 C-9^N, 60.3 C-6^N, 55.8 C-2, 52.6 OCH_3, 40.2 κCH_2, 35.9 C-3^N, 31.8 C-5^N, 29.4 εCH_2, 29.0 βCH_2, 26.0 γCH_2, 25.1 δCH_2, 23.1 NAc, 20.8, 20.7, 20.4, 20.1 OAc, 16.4 C-6'.

$C_{87}H_{108}N_2O_{28}$	Calcd.: C 64.12	H 6.68	N 1.72
(1629.81)	Found: C 64.02	H 6.66	N 1.70.

*N^6-(Benzyloxycarbonyl)-6-aminohexyl-O-(methyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(**2**→**3**)-O-(6-O-benzyl-β-D-galactopyranosyl)-(**1**→**4**)-O-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(**1**→**3**)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside* (**8c**)

A mixture of 50 mg of donor **3c** (0.099 mmol), 190 mg of trisaccharide **2** (0.198 mmol) and 107 mg of ground and freshly dried molecular sieves 4 Å in 0.8 ml of absolute acetonitrile was stirred in the dark for 2 h at room temperature. The suspension was cooled to −40 °C and a solution of 44.5 mg of *N*-iodosuccinimide (0.198 mmol) in 0.7 ml of absolute acetonitrile was added. After 20 min 1.7 µl of trifluoromethanesulfonic acid (19.8 mmol) were added. The reaction was complete after 2 h at −40 °C (TLC: R_f = 0.33 (hexane/acetone, 0.8:1, v:v). The solids were filtered off from the diluted reaction mixture and the filtrate was extracted with dilute Na_2CO_3, dried over $MgSO_4$ and concentrated. The remainder was purified repeatedly by flash chromatography (hexane/acetone, 2:1, v:v). In addition to 42.4 mg of tetrasaccharide **8c** (25.6 %) 110 mg of the acceptor **2** were isolated. $[\alpha]^{22}_D = -40.4°$ (1.0, dichloromethane). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ/ppm = 8.01 (d, $J_{NH,2}$ = 9.6, 1H, NH-2), 7.33–7.21 (m, 30H, Ar), 7.22 (m, 1H, NH urethane), 5.27 (m, 1H, H-7^N), 5.27 (m, 1H, H-1α'), 5.17 (m, 1H, H-8^N), 4.99 (s, 2H, OCH_2 Z), 4.98 (m, 1H, OH-2''), 4.86 (m, 1H, H-4^N), 4.78 (m, 1H, OC$\underline{H}_2$Ph), 4.78 (m, 1H, H-5'), 4.68, 4.63, 4.53 (3m, 5H, OC$\underline{H}_2$Ph), 4.52 (m, 1H, OH-4''), 4.48, 4.42 (2m, 2H, OC$\underline{H}_2$Ph), 4.40 (m, 1H, H-1β''), 4.38 (d, $J_{1,2}$ = 8.0, 1H, H-1β), 4.28 (m, 1H, H-9a^N), 4.00 (m, 1H, H-9b^N), 3.98 (m, 1H, H-3'), 3.93 (m, 1H, H-5), 3.92 (m, 1H, H-3''), 3.87 (m, 1H, H-6a''), 3.82 (m, 1H, H-3), 3.80 (m, 1H, H-5''), 3.79 (m, 1H, H-6b''), 3.78 (m, 1H, H-2'), 3.78 (m, 1H, H-4''), 3.76 (m, 1H, H-4'), 3.70 (s, 3H, OCH_3), 3.70 (m, 1H, H-6a), 3.69 (m, 1H, αCH_2), 3.68 (m, 1H, H-5^N), 3.63 (m, 1H, H-2), 3.62 (m, 1H, H-6b), 3.58 (m, 1H, H-6^N), 3.51 (m, 1H, H-4), 3.43 (m, 1H, H-2''), 3.35 (m, 1H, αCH_2), 2.97 (m, 2H, κCH_2), 2.52 (dd, J_{gem} = 12.5, J_{vic} = 4.9, 1H, H-3eq^N), 2.10 (m, 1H, H-3ax^N), 2.09, 2.03, 1.93, 1.86 (4s, 12H, OAc), 1.84 (s, 3H, NAc), 1.40 (m, 2H, βCH_2), 1.39 (m, 2H, εCH_2), 1.24 (m, 4H, $\gamma,\delta CH_2$), 1.06 (d, $J_{5,6}$ = 6.1, 3H, H-6'). – ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ/ppm = 170.8, 169.9, 169.8, 169.3, 169.1 C=O OAc NAc, 167.8 C-1^N, 156.0 C=O urethane, 102.2 C-1'', 101.8 C-1, 98.9 C-2^N, 95.5 C-1', 78.4 C-6'', 78.1 C-3', 76.4 C-2', 75.9 C-4', 74.9 C-4, 74.8 O$\underline{C}H_2$Ph, 73.6 C-3'', 73.3 C-3, 72.6 C-2'', 72.3, 72.1, 71.9 O$\underline{C}H_2$Ph, 71.0 C-6^N, 70.9 C-4^N, 70.5 O$\underline{C}H_2$Ph, 68.8 C-8^N, 68.5 C-6, 68.5 C-4'', 68.5 αCH_2, 68.4 C-5, 67.6 C-5'', 67.0 C-7^N, 65.4 C-5', 65.0 OCH_2 Z, 61.2 C-9^N, 58.9 C-5^N, 55.8 C-2, 52.8 OCH_3, 40.3 κCH_2, 34.5 C-3^N, 29.4 εCH_2, 29.0 βCH_2, 26.0 γCH_2, 25.1 δCH_2, 23.1 NAc, 20.6, 20.4, 20.3OAc, 16.5 C-6'.

$C_{87}H_{107}N_5O_{28}$	Calcd.: C 62.54	H 6.45	N 4.19
(1670.82)	Found: C 62.43	H 6.41	N 4.17.

*6-Aminohexyl-O-(3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(**2**→**3**)-O-(β-D-galactopyranosyl)-(**1**→**4**)-O-[α-L-fucopyranosyl-(**1**→**3**)]-2-acetamido-2-deoxy-β-D-glucopyranoside* (**1b**)

200 mg of sodium metal were dissolved in 100 ml of absolute methanol. A volume of 3 ml of this solution was added to the tetrasaccharide **8b** (61.4 µmol). After 1 h (TLC: R_f = 0.25, hexane/acetone, 1:2, v:v) the mixture was neutralized by adding Amberlyst 15 ion exchange resin. After filtration the concentrated and dried residue was dissolved in 2 ml of absolute methanol. To this solution were added palladium-black (obtained by hydrogenation of 250 mg of $PdCl_2$ in 12.5 ml of absolute methanol) and 50 µl of formic acid in absolute methanol giving a final reaction volume of 4 ml. The solution was stirred under a hydrogen atmosphere for 48 h. After complete debenzylation (TLC: R_f = 0.29 isopropanol/1 M NH_4OAc, 4:1, v:v) the solids were filtered off and the solution was concentrated. The remainder was codestilled with toluene, dried in high vacuo and dissolved in 2.5 ml of water followed by

adjustment of pH 9 by addition of 1 M NaOH. After 16 h at ambient temperature the mixture was purified twice by Sephadex-G25 chromatography (eluent 0.1 M NH_4HCO_3) and lyophilized. (38.2 mg, 72.2%). $[\alpha]^{22}_D = -59.3°$ (0.15, H_2O). – ^{1}H NMR (500 MHz, D_2O): δ/ppm = 5.07 (d, $J_{1,2}$ = 3.9, 1H, H-1α'), 4.80 (q, $J_{5,6}$= 6.5, 1H, H-5'), 4.53 (m, 1H, H-1β), 4.52 (m, 1H, H-1β''), 4.04 (dd, $J_{3,4}$ = 3.2, 1H, H-3''), 3.99 (m, 1H, H-6a), 3.93 (m, 1H, H-4), 3.92 (m, 1H, H-4''), 3.90 (m, 1H, H-8^N), 3.90 (m, 1H, αCH_2), 3.89 (m, 1H, H-3'), 3.88 (m, 1H, H-2), 3.88 (m, 1H, H-9a^N), 3.88 (m, 1H, H-6b), 3.85 (m, 1H, H-4^N), 3.85 (m, 1H, H-6^N), 3.78 (m, 1H, H-4'), 3.70 (m, 2H, H-6a'', H-6b''), 3.68 (m, 1H, H-7^N), 3.68 (m, 1H, H-9b^N), 3.60 (m, 1H, H-5''), 3.59 (m, 1H, H-3), 3.58 (m, 1H, H-2'), 3.58 (m, 1H, H-5), 3.58 (m, 1H, αCH_2), 3.49 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.8, 1H, H-2''), 2.96 (t, J_{gem} = 7.5, 2H, κCH_2), 2.65 (dd, J_{gem} = 11.8, J_{vic} = 2.4, 1H, H-3eq^N), 2.00 (s, 3H, NAc), 1.84 (m, 1H, H-5eq^N), 1.62 (m, 2H, εCH_2), 1.53 (m, 2H, βCH_2), 1.50 (m, 1H, H-5ax^N), 1.47 (dd, J_{vic} = 11.8, 1H, H-3ax^N), 1.34 (m, 2H, δCH_2), 1.33 (m, 2H, γCH_2), 1.14 (d, 3H, H-6'). – ^{13}C NMR (125 MHz, D_2O): δ/ppm = 171.9, 171.6 C=O NAc, C-1^N, 99.3 C-1'', 98.5 C-1, 97.7 C-2^N, 96.0 C-1', 73.0 C-5, 73.0, C-3'', 72.5 C-5'', 72.4 C-6^N, 71.0 C-4, 69.8 C-3', 69.4 C-4', 69.1 C-2', 68.7 C-4^N, 67.9 αCH_2, 66.7 C-2'', 66.7 C-8^N, 65.3 C-7^N, 64.9 C-4'', 64.2 C-5', 60.2 C-9^N, 58.9 C-6'', 57.2 C-6, 53.3 C-2, 38.1 C-3^N, 36.9 κCH_2, 31.6 C-5^N, 25.8 βCH_2, 24.9 εCH_2, 22.7 δCH_2, 22.1 γCH_2, 19.8 NAc, 12.8 C-6'. $C_{35}H_{62}N_2O_{22}$ (862.88).
FAB-MS (NBA): $M_{calcd.}$= 862.4 M_{found} = 863.6 (M+H)

*6-Aminohexyl-O-(5-amino-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(**2→3**)-O-(β-D-galactopyranosyl)-(**1→4**)—O-[α-L-fucopyranosyl-(**1→3**)]-2-acetamido-2-deoxy-3-β-D-glucopyranoside* (**1c**)

To a solution of 20.3 mg of tetrasaccharide **8c** (12.0 mmol) in 1.2 ml methanol were added 24.1 mg of potassium carbonate (156 mmol) and the mixture was stirred for 2h. After addition of 1.2 ml of water the reaction was allowed to proceed for 3 days (TLC: R_f = 0.49 isopropanol/1 M NH_4OAc 4:1, v:v). The reaction mixture was concentrated and lyophilized. The remainder was dissolved in 250 µl of acetonitrile and diluted with 4.25 ml of water. The turbid solution was passed through a SepPak RP 18 cartridge (Waters) followed by six washing steps with water to remove the salts. Subsequently a step gradient (5 ml each: 0%, 20%, 40%, 60% 80% acetonitrile-water) was used to elute the product. The fractions obtained from 40 and 60% acetonitrile-water were lyophilized yielding 18.35 mg of crude tetrasaccharide. A 17.89 mg portion of the crude tetrasaccharide was dissolved in 2 ml of absolute methanol under an argon atmosphere. Ammoniumformate (57 mg) and 43 mg of palladium on charcoal (10%) were added and the mixture was stirred. After three days another portion of 180 mg of ammoniumformate was added. The reaction was complete within 48 h (TLC: R_f = 0.52 isopropanol/1 M NH_4OAc, 1:1, v:v) and the catalyst was filtered off and washed repeatedly. The concentrate (12.4 mg) was purified by size exclusion chromatography (Superdex 16/60, eluent 0.1 M NH_4HCO_3). (8.64 mg, 81.0%). $[\alpha]^{22}_D$ = –53.1° (0.8, H_2O). – ^{1}H NMR (500 MHz, D_2O): δ/ppm = 5.07 (d, $J_{1,2}$ = 3.8, 1H, H-1'), 4.77 (m, 1H, H-5'), 4.50 (m, 1H, H-1), 4.49 (m, 1H, H-1''), 4.04 (m, 1H, H-3''), 3.98 (m, 1H, H-6a), 3.90 (m, 1H, H-4), 3.89 (m, 1H, H-8^N), 3.89 (m, 1H, H-4''), 3.88 (m, 1H, H-9aN), 3.87 (m, 1H, H-3'), 3.87 (m, 1H, ?CH_2), 3.86 (m, 1H, H-6b), 3.85 (m, 1H, H-2), 3.83 (m, 1H, H-3), 3.80 (m, 1H, H-7^N), 3.75 (m, 1H, H-4'), 3.67 (m, 1H, H-9b^N), 3.66 (m, 2H, H-6a'', H-6b''), 3.66 (m, 1H, H-5''), 3.65 (m, 1H, H-2'), 3.56 (m, 1H, H-5), 3.55 (m, 1H, αCH_2), 3.51 (m, 1H, H-6^N), 3.49 (m, 1H, H-2''), 3.45 (m, 1H, H-4^N), 2.97 (t, J_{vic} = 7.6, 2H, κCH_2), 2.77 (dd, $J_{4,5}$ = $J_{5,6}$ = 9.6, 1H, H-5^N), 2.70 (dd, J_{gem} = 13.2, J_{vic} = 4.5, 1H, H-3eq^N), 1.99 (s, 3H, NAc), 1.69 (dd, J_{vic} = 12.1, 1H, H-3ax^N), 1.63 (m, 2H, εCH_2), 1.54 (m, 2H, βCH_2), 1.35 (m, 2H, δCH_2), 1.34 (m, 2H, γCH_2), 1.14 (d, $J_{5,6}$ = 6.5, 3H, H-6'). – ^{13}C NMR (125 MHz, D_2O): δ/ppm = 171.5, 171.5 C=O NAc, C-1^N, 99.1 C-1'', 98.4 C-1, 97.1 C-2^N, 96.0 C-1', 73.0 C-3'', 72.7 C-5, 72.3 C-5'', 72.3 C-6^N, 72.2 C-3, 70.8 C-4, 69.6 C-8^N, 69.3 C-4', 67.8 αCH_2, 67.7 C-4^N, 66.6 C-2'', 66.5 C-3', 65.4 C-7^N, 65.1 C-2', 64.7 C-4'', 64.1 C-5', 60.0 C-9^N, 58.9 C-6'', 57.1 C-6, 53.2 C-2, 49.8 C-5^N, 37.2 C-3^N, 36.8 κCH_2, 25.8 βCH_2, 24.0 εCH_2, 22.6 δCH_2, 22.0 γCH_2, 19.7 NAc, 12.7 C-6'. $C_{35}H_{63}N_3O_{22}$ (877.89) ESI-MS (0.01 M NH_4OAc/MeCN): $M_{calcd.}$ = 877.4 $M_{found.}$ = 878.6 (M+H)

*6-Aminohexyl-O-(5-amino-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(**2→3**)-O-(β-D-galactopyranosyl)-(**1→4**)-O-[α-L-fucopyranosyl-(**1→3**)]-2-amino-2-deoxy-β-D-glucopyranoside* (**1d**)

A solution of 4 ml of 25% tetramethylammoniumhydroxide in methanol was evaporated to dryness and the residue was taken up in 0.5 mL of water. This solution was added to a solution of 21.6 mg of compound **1a** (23.5 µmol) in 0.5 ml of water. After 4 days at 95 °C the reaction was terminated as indicated by thin layer chromatography (TLC R_f = 0.43 isopropanol /1 M NH_4OAc, 4:1, v:v). After cooling to room temperature the solution was diluted with water to a volume of 10 ml and adjusted to pH 7.0–8.0 with 50% acetic acid. Lyophilization gave a crude mixture (751.6 mg) which was purified by size exclusion chromatography on Sephadex G-25 column (3.5 × 25 cm, eluent 0.1 M NH_4HCO_3). The product fraction (60.0 mg) was dissolved in 10 ml of water and applied to a CM Sephadex C25 column (1 × 3 cm; eluent: gradient of ammoniumacetate 0.1 M to 1 M). The product eluted at a concentration of 0.3 M–0.4 M ammoniumacetate (lyophilized material 24.6 mg) which was purified by chromatography on a Sephadex G-25 column (3.5 × 25 cm, eluent 0.1 M NH_4HCO_3). (10.4 mg; 53%). $[\alpha]^{22}_D$ = -33.3° (0.7, H_2O).
$C_{33}H_{61}N_3O_{21}$ (835.85). – ESI-MS (0.01 M NH_4OAc/ACN): $M_{calc.}$ = 835.4 M_{found} = 836.6 (M+H). – ^{1}H NMR (500 MHz, D_2O): δ/ppm = 5.14 (d, $J_{1,2}$ = 3.0, 1H, H-1'), 4.78 (m, 1H, H-5'), 4.45 (d, $J_{1,2}$ = 8.0, 1H, H-1''), 4.37 (d, $J_{1,2}$ = 8.0, 1H, H-1), 4.03 (m, 1H, H-3''), 3.98 (m, 1H, H-6a), 3.89 (m, 1H, H-4''), 3.89 (m, 1H, αCH_2), 3.88 (m, 1H, H-8^N), 3.87 (m, 2H, H-9a^N,H-9b^N), 3.85 (m, 1H, H-6b), 3.84 (m, 1H, H-3'), 3.83 (m, 1H, H-4), 3.79 (m, 1H, H-2'), 3.78 (m, 1H, H-7^N), 3.74 (m, 1H, H-4'), 3.64 (m, 2H, H-6a'', H-6b''), 3.64 (m, 1H, H-5''), 3.63 (m, 1H, αCH_2), 3.59 (m, 1H, H-3), 3.54 (m, 1H, H-6^N), 3.55 (m, 1H, H-5), 3.52 (m, 1H, H-2''), 3.47 (m, 1H, H-4^N), 2.95 (m, 2H, κCH_2), 2.81 (m, 1H, H-5^N), 2.80 (m, 1H, H-2), 2.68 (dd, J_{gem} = 12.8, J_{vic} = 3.5, 1H, H-3eq^N), 1.69 (dd, J_{vic} = 12.1, 1H, H-3ax^N), 1.63 (m, 2H, εCH_2), 1.61 (m, 2H, βCH_2),

1.37 (m, 4H, $\gamma,\delta CH_2$), 1.13 (d, $J_{5,6}$ = 6.1, 3H, H-6'). – ^{13}C NMR (125 MHz, D_2O): δ/ppm = 171.0 C-1^N,100.0 C-1, 99.3 C-1", 97.4 C-1', 97.2 C-2^N, 77.2 C-3, 73.0 C-5, 73.0 C-6^N, 73.0 C-5", 73.0 C-3", 70.9 C-4, 69.9 C-4', 69.9 C-8^N, 67.8 ?CH_2, 67.8 C-4^N, 66.8 C-3', 66.8 C-2", 65.8 C-7^N, 65.8 C-2', 64.8 C-4", 64.7 C-5', 60.5 C-9^N, 59.5 C-6", 57.5 C-6, 55.5 C-2, 50.3 C-5^N, 37.8 C-3^N, 36.8 κCH_2, 26.4 βCH_2, 24.3 εCH_2, 22.2, $\gamma,\delta CH_2$, 13.0 C-6'.

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