Chemoenzymatic Synthesis of Sialyl-Trimeric-Lewis X

Kathryn M. Koeller and Chi-Huey Wong*[a]

Abstract: The decasaccharide sialyl-trimeric-Lewis x is a component of glycoproteins and glycolipids that serve as E- and P-selectin ligands. The synthesis of this target structure was accomplished by utilizing a combination of chemical and enzymatic methods. Highlights of the chemical synthesis include minimal use of protecting groups and regioselective glycosylations to arrive at a linear tri-lactosamine structure. Glycosyltransferase-catalyzed reactions were then employed for the addition of the terminal sialic acid and branch-point fucose residues. Notably, fucosyltransferases V and VI showed different specificities for the sialyl-tri-lactosamine core structure.

Keywords: chemoenzymatic synthesis · oligosaccharides · regioselective glycosylation · selectin · sialyl Lewis x

Introduction

The sialyl Lewis x tetrasaccharide (sLe^x) has been identified as a minimal ligand for the selectin family of proteins (E-, P-, and L-selectin).[1] However, monovalent sLe^x-selectin binding events are far too weak to account for the intercellular adhesive force observed in biological systems in which selectins function.[2] Attempts to create potent selectin ligands through syntheses of mimetics or multivalent forms of sLe^x have achieved only moderate success.[3] In contrast, the glycoproteins that present sLe^x in the inflammatory response and other disease states act as high affinity selectin ligands.[4]

In nature, sLe^x is rarely present in the form of a simple tetrasaccharide. Sialyl Le^x generally terminates long polylactosamine chains attached to glycoproteins or glycolipids on the cell surface.[5] Specifically, recent studies have revealed the sialyl-trimeric-Lewis x structure (compound **1**, Scheme 1) as a component of the P-selectin glycoprotein ligand-1 (PSGL-1) glycan.[6] This decasaccharide is expected to contribute to high affinity interactions between PSGL-1 and E- or P-selectin. Glycolipids from HL-60 cells also display similar sialylated, multi-fucosylated polylactosamines that function as E-selectin ligands.[7]

In order to generate sialyl-trimeric-Lewis x for biological evaluation, a chemoenzymatic synthesis was developed. This molecule has not previously been synthesized employing chemical means. Syntheses of related polylactosamine structures, such as the VIM-2 epitope, have involved the isolation of natural glycans.[8] In contrast, syntheses of trimeric-Lewis x glycolipids[9] and sialyl-dimeric-Lewis x[10] have relied on complicated chemical synthetic strategies. The chemoenzymatic synthesis described herein takes advantage of the remarkable specificity of glycosyltransferases to simplify the overall route. Minimal use of protecting groups and regioselective glycosylations are highlights of the chemical portion of the synthesis.

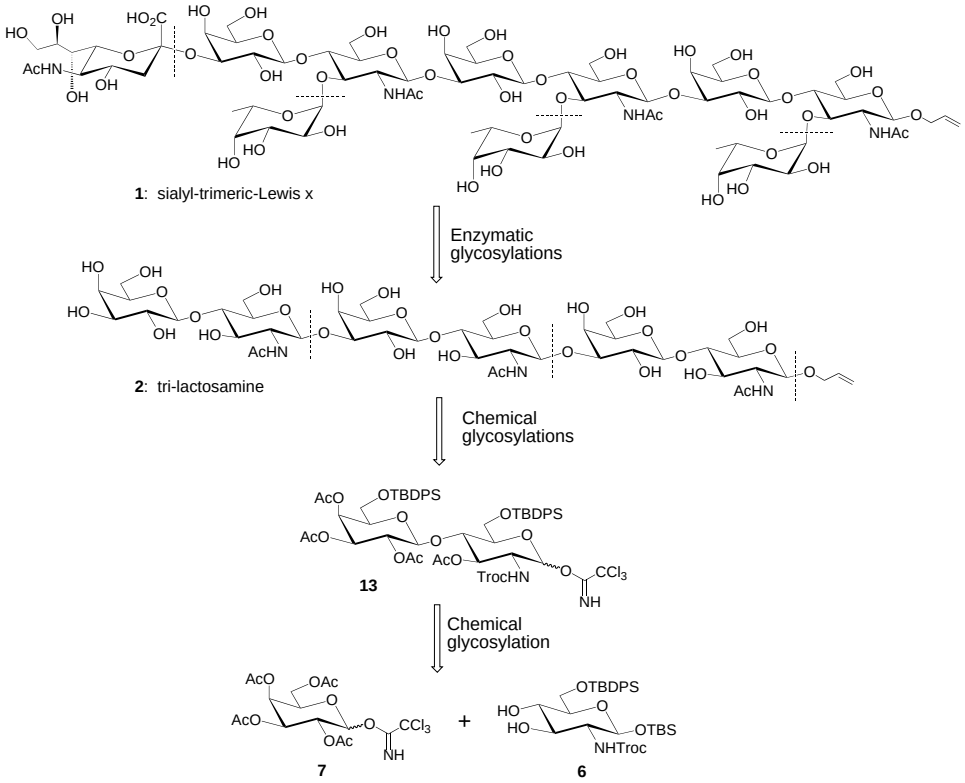
[a] Prof. Dr. C.-H. Wong, K. M. Koeller
Department of Chemistry
The Scripps Research Institute and Skaggs Institute
for Chemical Biology
10550 N. Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2409
E-mail: wong@scripps.edu

Results and Discussion

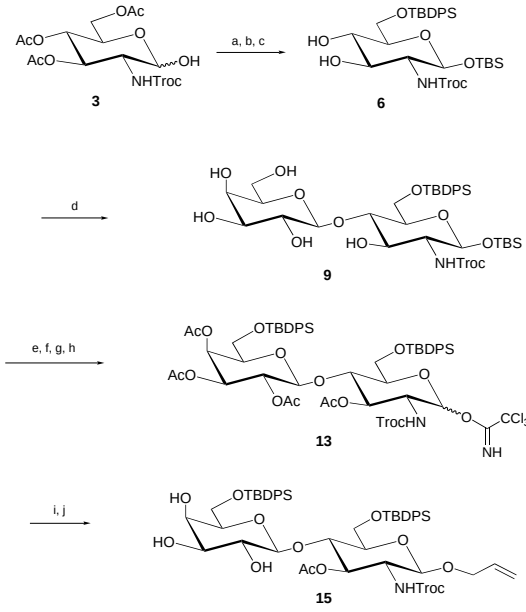
Synthesis began with the chemical construction of tri-lactosamine core **2** (Scheme 1). This required the generation of a suitable lactosamine glycosyl donor for block synthesis. Initially, glucosamine derivative **3** was obtained by previously published methods.[11] Protection of the anomeric position as the *tert*-butyldimethylsilyl ether (TBS) then furnished the β-linked glycoside (Scheme 2). Glycosyl acceptor **6** was then readily accessible through saponification of the acetate esters, followed by selective protection of the primary 6-hydroxyl as the *tert*-butyldiphenylsilyl (TBDPS) ether.

Trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed glycosylation of acceptor **6** with the glycosyl donor imidate **7**[12] then furnished β1,4-linked lactosamine **9** in 50% yield after deprotection. Recovery of unreacted starting material **6** was also possible at this stage. Notably, the β1,3 lactosamine regioisomer was isolated in only 8% yield, and α-linked products were not observed.[13] Compound **9** was then peracetylated for characterization. The position and configuration of the newly formed glycosidic linkage was ascer-

  0947-6539/00/0607-1243 $ 17.50+.50/0



Scheme 1. Retrosynthetic analysis of sialyl-trimeric-Lewis x **1**.



Scheme 2. a) TBDMS-Cl, imidazole, DMF, 100%; b) 1M NaOMe, MeOH, 98%; c) TBDPS-Cl, Et_3N, DMAP, DMF/CH_2Cl_2, 87%; d) 1) **7**, TMSOTf, 4 Å molecular sieves, CH_2Cl_2, $-20\,^{\circ}C$; 2) 1M NaOMe, MeOH, 50%; e) TBDPS-Cl, Et_3N, DMAP, CH_2Cl_2, 99%; f) Ac_2O, DMAP, pyridine, 79%; g) bis(acetonitrile)palladium(II) chloride, acetone, 4 d, 71%; h) Cl_3CCN, Cs_2CO_3, 4 Å molecular sieves, CH_2Cl_2, 87%; i) allyl alcohol, TMSOTf, CH_2Cl_2, 95%; j) 1M NaOMe, MeOH, 93%.

tained to be β1,4 by 2D COSY (correlation spectroscopy) NMR analysis.

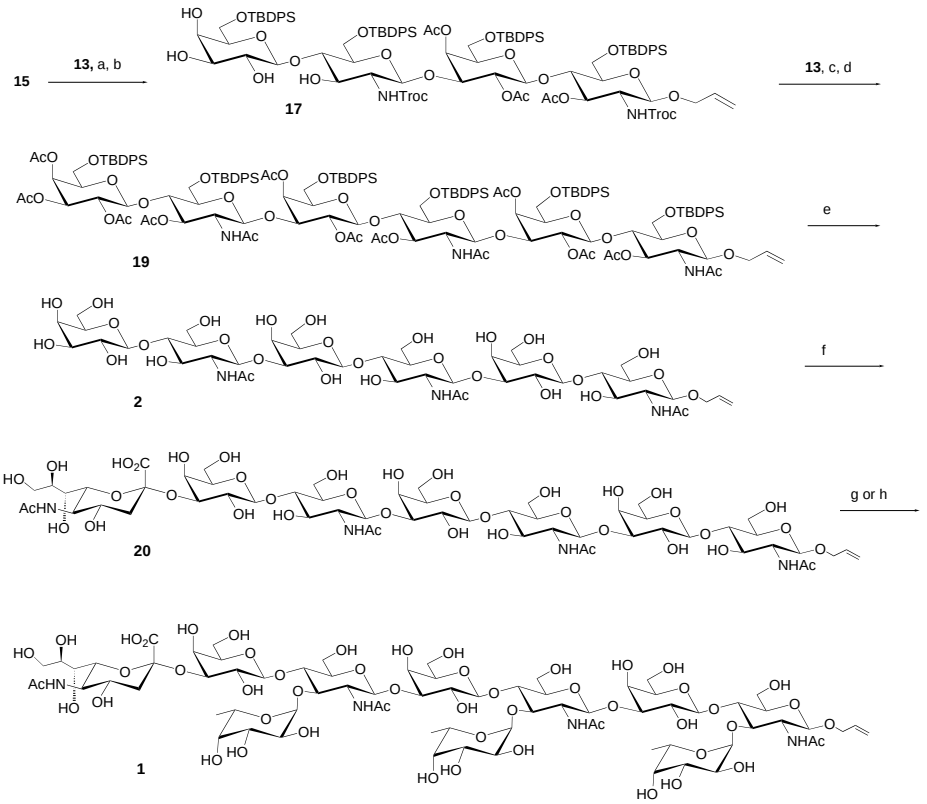
Further protection of **9** was accomplished through formation of the galactose 6-hydroxyl TBDPS ether, followed by peracetylation. The anomeric TBS ether was then removed with bis(acetonitrile)palladium(II) chloride in acetone, a reaction which proceeded without destruction of the TBDPS ethers.[14] Conversion to trichloroacetimidate **13** then gave the appropriate glycosyl donor for block synthesis.

Glycosylation of **13** with allyl alcohol as acceptor furnished the β-linked reducing terminal lactosamine block. Unexpectedly, normal saponification conditions then reproducibly furnished mono-acetate **15** in high yield. Reaction of glycosyl acceptor **15** with donor **13** under TMSOTf catalysis then afforded the tetrasaccharide product, which could be isolated only following peracetylation (Scheme 3). 2D COSY NMR analysis confirmed that the desired glycosidic linkage had been formed at the galactose 3-hydroxyl.[15] This result is in accord with the reactivity difference in the galactose hydroxyl groups observed in previous studies.[10, 16] As was the case previously, deprotection of the di-lactosamine construct under Zemplen conditions proceeded incompletely, in this instance giving tri-acetate **17** as the major product.[17]

Construction of the protected tri-lactosamine hexasaccharide was then accomplished by TMSOTf-catalyzed glycosylation of acceptor **17** with donor **13**. After peracetylation, COSY NMR analysis of the product was unclear due to severe overlap of the diagnostic Gal H-4 resonances. However, upon conversion of the NHTroc groups to NHAc by reaction of activated Zn dust in acetic anhydride,[18] clear assignment of **19** was possible. Deprotection of the hydroxyl groups was then accomplished with TBAF in THF, followed by saponification of the acetate esters, yielding tri-lactosamine **2**.

With the chemical portion of the synthesis completed, the remaining synthetic steps involved the enzymatic transfer of a

0947-6539/00/0607-1244 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 7



Scheme 3. a) 1) **13**, TMSOTf, CH_2Cl_2; 2) Ac_2O, DMAP, pyridine, 67%; b) 1M NaOMe, MeOH, 72%; c) 1. **13**, TMSOTf, CH_2Cl_2; 2) Ac_2O, DMAP, pyridine, 35%; d) Zn dust, Ac_2O, 36%; e) 1) 1M TBAF in THF; 2) 1M NaOMe, MeOH, 49%; f) CMP-NeuAc, 1M $MnCl_2$, α2,3-SiaT, alkaline phosphatase, 100 mM HEPES, pH 7.4+0.25% Triton X-100, 2 d, 85%; g) 1) GDP-fucose, 1M $MnCl_2$, α1,3-FucT V, alkaline phosphatase, 100 mM MES, pH 6.0+ 0.25% Triton X-100, 5 d; 2) GDP-fucose, 1M $MnCl_2$, α1,3-FucT VI, alkaline phosphatase, 100 mM MES, pH 6.0+0.25% Triton X-100, 5 d, 30%; h) GDP-fucose, 1M $MnCl_2$, α1,3-FucT VI, alkaline phosphatase, 100 mM MES, pH 6.0+ 0.25% Triton X-100, 6 d, 41%.

single sialic acid and three fucose residues. Transfer of sialic acid was accomplished utilizing CMP-NeuAc as glycosyl donor in the presence of recombinant human α2,3-sialyltransferase as the catalyst. The reaction catalyzed the addition of sialic acid to the non-reducing terminus of the polylactosamine chain to give **20**. In initial fucosylation attempts, **20** was treated with GDP-fucose in the presence of recombinant human α1,3-fucosyltransferase V (FucT V). FucT V catalyzed the addition of two fucose residues to the polylactosamine chain.[19] Further incubation of the difucosylated molecule with GDP-fucose and α1,3-FucT V for an extended period of time failed to furnish tri-fucosylated structure **1**. However, incubation of the di-fucosylated intermediate with GDP-fucose in the presence of recombinant human α1,3-FucT VI successfully appended the third fucose to the polylactosamine chain to give sialyl-trimeric-Lewis x **1**. Alternatively, it was discovered that **1** could be obtained directly from **20** by incubation with 3 equivalents of GDP-fucose in the presence of α1,3-FucT VI. This result clarifies previous biological studies of milk fucosyltransferase specificity for polylactosamine acceptors, in which activity could not be pinpointed to a single fucosyltransferase.[20] The results reported herein clearly show distinct substrate specificity for FucT V and FucT VI.

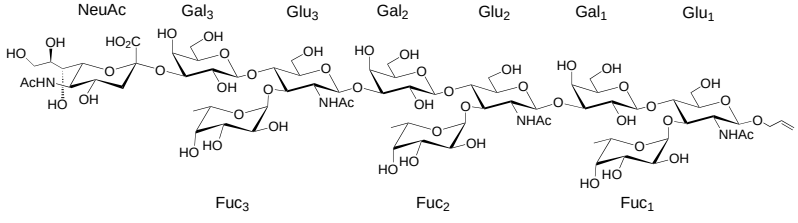


Figure 1. NMR assignment designations.

Conclusion

In summary, glycosyltransferases have the potential to greatly simplify complex carbohydrate synthesis. In the present study, a branched decasaccharide was constructed employing only six glycosylation reactions, two of which were enzymatic. Minimal synthetic manipulations, involving only four protecting groups, were sufficient to arrive at the desired target structure. Thus, the first chemoenzymatic synthesis of sialyl-trimeric-Lewis x **1** has been accomplished. Evaluation of biological activity will be undertaken in the near future.

Experimental Section

All non-aqueous reactions were run in oven-dried glassware under an inert Ar atmosphere. Reactions were monitored by thin-layer chromatography (TLC) utilizing *p*-anisaldehyde or cerium molybdate stain as the developing reagent. Unless otherwise noted, reagents, and materials were obtained from commercial sources and used as provided. All non-aqueous solvents were distilled prior to use. 1D ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AMX-400, AMX-500, DRX-500, or DRX-600 MHz spectrometers, and were referenced to residual solvent peaks ($CDCl_3$: ^{1}H $\delta = 7.24$, ^{13}C $\delta = 77.0$; CD_3OD: ^{1}H $\delta = 3.30$, ^{13}C $\delta = 49.0$; D_2O: ^{1}H $\delta = 4.76$). 2D COSY and HMQC NMR spectra were recorded on a Bruker DRX-600 spectrometer equipped with either a broadband or inverse probe for greater sensitivity. CMP-NeuAc, GDP-fucose, α2,3-sialyltransferase, α1,3-fucosyltransferase V, and α1,3-fucosyltransferase VI were purchased from Calbiochem. Where applicable, NMR assignments refer to the designations given in Figure 1.

Compound 4: Compound **3** (9.77 g, 20.32 mmol), imidazole (1.80 g, 26.42 mmol), and *tert*-butylchlorodimethylsilane (3.98 g, 26.42 mmol) were dissolved in dry *N*,*N*-dimethylformamide (101.6 mL). The reaction was stirred at ambient temperature for 48 h. The reaction was diluted with ethyl acetate (200 mL), washed with water (3×100 mL) and brine (1×100 mL). The aqueous extracts were combined and re-extracted with ethyl acetate (2×100 mL). The combined organic extracts were dried ($MgSO_4$), filtered,

and concentrated. Purification by flash chromatography (silica gel, gradient 2:1 to 3:2 hexane/EtOAc) then afforded **4** (12.50 g, 100%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.22$ (t, 1H, $J = 10$ Hz, H-3), 5.03–4.99 (m, 2H, H-4, N*H*), 4.79 (d, 1H, $J = 8$ Hz, H-1), 4.72 (d, 1H, $J = 12$ Hz, -CH_2-CCl$_3$), 4.62 (d, 1H, $J = 12$ Hz, -CH_2-CCl$_3$), 4.18 (dd, 1H, $J = 12$, 6 Hz, H-6), 4.10 (d, 1H, $J = 12$ Hz, H-6), 3.68–3.62 (m, 1H, H-5), 3.59 (dd, 1H, $J = 20$, 9 Hz, H-2), 2.05 (s, 3H, -COCH_3), 2.01 (s, 6H, -COCH_3), 0.85 (s, 9H, -C(CH_3)$_3$), 0.10 (s, 3H, -CH_3), 0.07 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 170.74, 170.63, 169.50, 153.92, 96.09, 74.56, 71.82, 68.96, 62.43, 58.24, 25.51, 20.71, 20.65, 17.90, −0.02, −4.27, −5.30; HR-MS (FAB) calcd for $C_{21}H_{34}NO_{10}SiCl_3Cs$ $[M+Cs]^+$ 726.0072, found 726.0054.

Compound 5: Compound **4** (3.16 g, 5.31 mmol) was dissolved in methanol (54.6 mL). A 1M solution of sodium methoxide in water (0.5 mL) was added to the stirred solution. After stirring a total of 3 h at ambient temperature, solvent was removed by a rotary evaporator. Purification by flash chromatography (silica gel, 10% methanol/chloroform) then yielded **5** (2.43 g, 98%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 4.70$ (dd, 2H, -CH_2-CCl$_3$), 4.63 (d, 1H, $J = 8$ Hz, H-1), 3.83 (dd, 1H, $J = 12$, 2 Hz, H-6), 3.69 (dd, 1H, $J = 12$, 5 Hz, H-6), 3.42–3.33 (m, 3H), 3.30–3.22 (m, 1H), 0.89 (s, 9H, -C(CH_3)$_3$), 0.13 (s, 3H, -CH_3), 0.12 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 156.89$, 129.90, 129.19, 126.29, 97.86, 77.83, 75.75, 75.59, 72.11, 62.71, 61.13, 26.18, 18.80, −3.96, −5.21; HR-MS (FAB) calcd for $C_{15}H_{28}NO_7SiCl_3Na$ $[M+Na]^+$ 490.0598, found 490.0612.

Compound 6: Triethylamine (8.00 mL, 57.08 mmol), *N*,*N*-dimethylaminopyridine (DMAP) (0.47 g, 3.81 mmol), and *tert*-butylchlorodiphenylsilane (7.42 mL, 28.54 mmol) were added to a solution of **5** (8.92 g, 19.03 mmol) in a dichloromethane/*N*,*N*-dimethylformamide mixture (95 mL/5 mL). After stirring 5 h at ambient temperature, the reaction was diluted with ethyl acetate (80 mL), washed with water (2 × 50 mL) and brine (2 × 50 mL). Aqueous extracts were combined and re-extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (silica gel, gradient 6:1 to 1:1 hexane/EtOAc) then gave **6** (11.77 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.70$–7.67 (m, 4H, *Ph*), 7.42–7.36 (m, 6H, *Ph*), 5.38 (d, 1H, $J =$ 7 Hz, NH), 4.75–4.64 (m, 3H, H-1, -CH_2-CCl$_3$), 3.91 (dd, 2H, $J = 11$, 4 Hz), 3.85 (dd, 1H, $J = 11$, 5 Hz), 3.70 (br t, 1H), 3.57 (t, 1H, $J = 9$ Hz), 3.47 (br s, 1H), 3.44–3.40 (m, 1H, H-5), 3.39–3.34 (m, 1H, H-2), 1.04 (s, 9H, -C(CH_3)$_3$), 0.88 (s, 9H, -C(CH_3)$_3$), 0.12 (s, 3H, -CH_3), 0.10 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 135.56$, 135.54, 132.89, 132.82, 129.82, 127.78, 95.92, 75.30, 74.78, 74.35, 72.47, 64.36, 59.93, 26.75, 25.61, 19.14, 17.89, −4.01, −5.33; HR-MS (FAB) calcd for $C_{31}H_{46}NO_7Si_2Cl_3Cs$ $[M+Cs]^+$ 840.0911, found 840.0948.

Compound 9: Compound **6** (11.22 g, 15.87 mmol), compound **7** (7.87 g, 15.90 mmol), and 4 Å molecular sieves (3.2 g) were placed in dry dichloromethane (320 mL). The solution was allowed to stir for 1.5 h at ambient temperature, then was cooled to −20 °C. TMSOTf (12.7 mL, 0.01M) was transferred dropwise by cannula into the reaction flask. Following transfer, the reaction was stirred an additional 15 min at room temperature, then quenched by addition of solid sodium bicarbonate. The solution was washed with saturated sodium bicarbonate (2 × 5 mL) and brine (1 × 5 mL). The aqueous extracts were re-extracted with dichloromethane (2 × 5 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (silica gel, gradient 2:1 to 1:2 hexane/EtOAc) yielded a mixture of inseparable disaccharide products, as well as some recovered starting material **1**. After all solvent had been removed in vacuo, the product mixture was dissolved in methanol (320 mL). A 1M solution of sodium methoxide in water (1.6 mL) was added, and the reaction stirred for 4 h at room temperature. The reaction was neutralized with Amberlyst-15 H^+ resin, filtered, and concentrated. Purification by flash chromatography (silica gel, gradient 3–10% methanol/chloroform) then yielded the desired disaccharide **9** (6.70 g, 50%) as well as the undesired β1,3-linked regioisomer (1.20 g, 8%), and recovered starting material **6** (3.30 g). (Yields based on recovered starting material **6** are 72% for compound **9** and 9% for the β1,3 linked isomer). Compound **9**: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.76$–7.72 (m, 4H, *Ph*), 7.41–7.37 (m, 6H, *Ph*), 4.72 (dd, 2H, $J = 18$, 12 Hz, -CH_2-CCl$_3$), 4.66 (d, 1H, $J = 8$ Hz, Glu H-1), 4.53 (1d, H, $J = 8$ Hz, Gal H-1), 4.20 (dd, 1H, $J = 11$, 3 Hz), 3.99 (dd, 1H, $J = 11$, 1 Hz), 3.94 (t, 1H, $J = 9$ Hz, H-4), 3.77–3.72 (m, 2H), 3.65–3.60 (m, 2H), 3.53 (dd, 1H, $J = 10$, 8 Hz), 3.49–3.42 (m, 3H), 3.37 (dd, 1H, $J =$ 10, 3 Hz), 1.06 (s, 9H, -C(CH_3)$_3$), 0.90 (s, 9H, -C(CH_3)$_3$), 0.12 (s, 3H, -CH_3), 0.11 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 156.86$, 137.02, 136.79, 135.06, 134.26, 130.73, 128.81, 128.73, 104.40, 97.80, 79.04, 77.24, 76.80, 75.76, 74.85, 73.89, 72.50, 70.32, 63.55, 62.62, 60.72, 27.51, 26.22, 20.34, 18.85, −3.82, −5.12; HR-MS (ESI-MS) calcd for $C_{37}H_{56}NO_{12}Si_2Cl_3H$ $[M+H]^+$ 868.2485, found 868.2452.

Peracetylated 9: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.72$–7.70 (m, 4H, *Ph*), 7.44–7.39 (m, 6H, *Ph*), 5.29 (d, 1H, $J = 4$ Hz, Gal H-4), 5.19 (d, 1H, $J =$ 9 Hz, N*H*), 5.04 (t, 1H, $J = 9$ Hz, Glu H-3), 4.99 (dd, 1H, $J = 10$, 8 Hz, Gal H-2), 4.80 (dd, 1H, $J = 10$, 3 Hz, Gal H-3), 4.76 (d, 1H, $J = 13$ Hz, -CH_2-CCl$_3$), 4.68–4.63 (m, 3H, Glu H-1, Gal H-1, -CH_2-CCl$_3$), 4.15 (t, 1H, $J =$ 10 Hz, Glu H-4), 4.09 (d, 2H, $J = 6$ Hz, Gal H-6), 3.99 (d, 1H, $J = 11$ Hz, Glu H-6), 3.85 (d, 1H, $J = 12$ Hz, Gal H-6), 3.73 (t, 1H, $J = 10$ Hz, Glu H-2), 3.68 (t, 1H, $J = 7$ Hz, Gal H-5), 3.34 (d, 1H, $J = 9$ Hz, Glu H-5), 2.13 (s, 3H, -COCH_3), 2.08 (s, 3H, -COCH_3), 2.06 (s, 3H, -COCH_3), 1.97 (s, 3H, -COCH_3), 1.73 (s, 3H, -COCH_3), 1.12 (s, 9H, -C(CH_3)$_3$), 0.90 (s, 9H, -C(CH_3)$_3$), 0.15 (s, 3H, -CH_3), 0.10 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.10$, 170.34, 170.15, 168.69, 154.29, 135.88, 135.39, 133.36, 130.05, 129.90, 128.22, 127.99, 127.78, 125.25, 99.84, 96.69, 95.35, 75.30, 74.61, 73.73, 72.17, 70.96, 70.51, 69.17, 66.90, 61.35, 61.17, 57.95, 26.89, 25.49, 20.82, 20.67, 20.61, 20.48, 19.43, 17.90, −4.18, −5.39; ESI-MS (pos) calcd for $C_{47}H_{66}NO_{17}Si_2Cl_3$ $[M+H]^+$ 1078, found 1078.

Compound 10: Compound **9** (0.289 g, 0.333 mmol), triethylamine (0.120 mL, 0.432 mmol), *N*,*N*-dimethylaminopyridine (8.0 mg, 0.067 mmol), and *tert*-butylchlorodiphenylsilane (0.112 mL, 0.432 mmol) were dissolved in dry dichloromethane. After stirring for 24 h at room temperature, the reaction was diluted with ethyl acetate (10 mL), washed with water (2 × 5 mL) and brine (1 × 5 mL). The aqueous extracts were re-extracted with ethyl acetate (2 × 5 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (silica gel, 5% methanol/chloroform) then provided **10** (0.355 g, 99%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.72$–7.63 (m, 8H, *Ph*), 7.45–7.33 (m, 12H, *Ph*), 4.97 (br s, 1H, N*H*), 4.74 (d, 1H, $J = 7$ Hz), 4.64 (dd, 2H, $J = 26$, 12 Hz, -CH_2-CCl$_3$), 4.30–4.28 (m, 2H), 3.97–3.96 (m, 1H), 3.94–3.92 (m, 2H), 3.89 (dd, 1H, $J = 10$, 5 Hz), 3.80 (dd, 1H, $J = 10$, 6 Hz), 3.77–3.76 (m, 1H), 3.58–3.50 (m, 3H), 3.46–3.41 (m, 2H), 3.32 (dd, 1H, $J = 18$, 8 Hz), 1.07 (s, 9H, -C(CH_3)$_3$), 1.06 (s, 9H, -COCH_3), 1.05 (s, 9H, -C(CH_3)$_3$), 0.10 (s, 3H, -CH_3), 0.07 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 135.78$, 135.53, 134.76, 133.48, 132.87, 132.67, 129.87, 129.74, 129.60, 128.99, 128.24, 127.82, 127.75, 127.69, 125.27, 103.12, 95.73, 95.32, 80.10, 75.06, 74.96, 74.68, 73.60, 71.47, 71.41, 68.31, 62.98, 62.24, 59.80, 26.79, 26.74, 26.53, 25.58, 21.43, 19.29, 19.02, 17.87, −4.15, −5.39; HR-MS (FAB) calcd for $C_{53}H_{74}NO_{12}Si_2Cl_3Cs$ $[M+Cs]^+$ 1240.2637, found 1240.2688.

Compound 11: Acetic anhydride (0.248 mL, 2.63 mmol) and *N*,*N*-dimethylaminopyridine (8.0 mg, 0.066 mmol) were added to a solution of **10** (0.355 g, 0.329 mmol) in dry pyridine (0.51 mL). After stirring for 24 h at room temperature, the reaction was diluted with water (0.10 mL) and stirred an additional hour. The reaction was then diluted with ethyl acetate (2 mL), washed with saturated sodium bicarbonate (4 × 1 mL) and brine (1 × 1 mL). The aqueous extracts were re-extracted with ethyl acetate (2 × 2 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (silica gel, 4:1 hexane/EtOAc) then afforded **11** (0.331 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.73–7.69 (m, 4H, *Ph*), 7.60–7.57 (m, 4H, *Ph*), 7.45–7.35 (m, 12H, *Ph*), 5.53 (d, 1H, $J = 3$ Hz, Gal H-4), 5.34 (d, 1H, $J = 9$ Hz, N*H*), 4.98 (t, 1H, $J =$ 10 Hz, Glu H-3), 4.95–4.87 (m, 2H, Gal H-2, Gal H-3), 4.74 (d, 1H, $J =$ 11 Hz, -CH_2-CCl$_3$), 4.64–4.60 (m, 3H, Glu H-1, Gal H-1, -CH_2-CCl$_3$), 4.07 (t, 1H, $J = 9$ Hz, Glu H-4), 3.95 (d, 1H, $J = 10$ Hz, Glu H-6$_a$), 3.81 (d, 1H, $J = 10$ Hz, Glu H-6$_b$), 3.72–3.66 (m, 2H), 3.60 (dd, 1H, $J = 9$, 6 Hz), 3.50 (t, 1H, $J = 9$ Hz, Gal H-5), 3.31 (d, 1H, $J = 9$ Hz, Glu H-5), 1.98 (s, 6H, -COCH_3), 1.77 (s, 3H, -COCH_3), 1.68 (s, 3H, -COCH_3), 1.10 (s, 9H, -C(CH_3)$_3$), 1.04 (s, 9H, -C(CH_3)$_3$), 0.89 (s, 9H, -C(CH_3)$_3$), 0.13 (s, 3H, -CH_3), 0.08 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.91$, 169.89, 169.78, 154.26, 135.84, 135.54, 135.37, 133.32, 132.60, 132.56, 132.27, 130.03, 129.94, 129.80, 128.97, 128.14, 127.93, 127.81, 127.72, 125.24, 99.71, 99.60, 95.42, 75.21, 74.53, 73.55, 72.57, 72.30, 71.20, 69.60, 66.66, 61.38, 60.61, 57.76, 29.58, 29.29, 26.83, 26.67, 25.46, 22.61, 20.55, 20.41, 19.32, 18.94, 17.83, 14.05, −4.26, −5.42; HR-MS (FAB) calcd for $C_{61}H_{82}NO_{16}Si_3Cl_3Cs$ $[M+Cs]^+$ 1408.3054, found 1408.3127.

Compound 12: Bis(acetonitrile)palladium(II) chloride (0.034 g, 0.130 mmol) was added to a foil-covered flask containing **11** (0.331 g, 0.259 mmol) dissolved in acetone (5.2 mL). After stirring 4 d at room temperature, solvent was removed by rotary evaporation. The resulting

0947-6539/00/0607-1246 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 7

residue was purified by flash chromatography (silica gel, 2:1 hexane/EtOAc) to yield **12** (0.215 g, 71%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.70-7.56$ (m, 8H, *Ph*), 7.44–7.34 (m, 12H, *Ph*), 6.41 (d, 1H, $J = 10$ Hz, N*H*), 5.56 (d, 1H, $J = 2$ Hz, Gal H-4), 5.49 (t, 1H, $J = 10$ Hz, Glu H-3), 5.28 (t, 1H, $J = 3$ Hz), 4.99–4.96 (m, 2H), 4.90 (d, 1H, $J = 12$ Hz, -C*H*$_2$-CCl_3), 4.65 (d, 1H, $J = 7$ Hz, Gal H-1), 4.56 (d, 1H, $J = 12$ Hz, -C*H*$_2$-CCl_3), 4.03 (t, 1H, $J = 10$ Hz, Glu H-4), 3.92 (dt, 1H, $J = 11$, 3 Hz), 3.83–3.62 (m, 8H), 3.50 (t, 1H, $J = 9$ Hz, Gal H-5), 2.03 (s, 3H, -COC*H*$_3$), 2.01 (s, 3H, -COC*H*$_3$), 1.76 (s, 3H, -COC*H*$_3$), 1.64 (s, 3H, -COC*H*$_3$), 1.04 (s, 9H, -C(C*H*$_3$)$_3$), 1.03 (s, 9H, -C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.03$, 170.70, 169.88, 169.83, 154.89, 136.01, 135.90, 135.53, 135.43, 135.21, 133.24, 132.54, 131.98, 130.01, 129.92, 129.74, 127.96, 127.84, 127.72, 127.58, 104.96, 99.87, 95.76, 91.66, 74.40, 73.85, 72.64, 70.66, 70.53, 70.04, 66.55, 61.01, 60.48, 54.40, 26.62, 20.62, 20.50, 19.22, 18.95; HR-MS (FAB) calcd for $C_{55}H_{68}NO_{16}Si_2Cl_3Cs$ $[M+Cs]^+$ 1294.2186, found 1294.2121.

Compound 13: Freshly distilled trichloroacetonitrile (0.242 mL, 2.411 mmol) and 4 Å molecular sieves (0.078 g) were added to a solution of **12** (0.560 g, 0.482 mmol) in dry dichloromethane (2.1 mL). The mixture was allowed to stir for one hour at ambient temperature. Dry cesium carbonate (0.039 g, 0.121 mmol) was then added to the reaction flask, and stirring continued for an additional 1.5 h. Triethylamine (2 mL) was then added to the flask and the reaction mixture filtered through a short plug of silica gel. Follwing concentration, purification by flash chromatography (silica gel, 2:1 hexane/EtOAc + 0.05% Et_3N) provided **13** (0.547 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.78$ (s, 1H, N*H*CCl_3), 7.74–7.72 (m, 4H, *Ph*), 7.61–7.58 (m, 4H, *Ph*), 7.46–7.37 (m, 12H, *Ph*), 6.48 (d, 1H, $J = 4$ Hz, N*H*), 5.56 (d, 1H, $J = 3$ Hz, Gal H-4), 5.26 (dd, 1H, $J = 9$, 4 Hz), 5.23 (d, 1H, $J = 10$ Hz), 5.03 (dd, 1H, $J = 10$, 8 Hz), 4.95 (dd, 1H, $J = 10$, 4 Hz, Gal H-3), 4.75–4.69 (m, 3H), 4.19–4.12 (m, 2H), 3.94–3.87 (m, 2H), 3.79–3.73 (m, 2H), 3.68 (1t, H, $J = 7$ Hz, Glu H-2), 3.54 (t, 1H, $J = 9$ Hz, Glu H-5), 2.02 (s, 3H, -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$), 1.80 (s, 3H, -COC*H*$_3$), 1.73 (s, 3H, -COC*H*$_3$), 1.09 (s, 9H, -C(C*H*$_3$)$_3$), 1.05 (s, 9H, -C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.68$, 169.93, 169.76, 168.78, 160.45, 154.15, 135.83, 135.76, 135.55, 135.49, 135.37, 133.11, 132.56, 132.45, 131.93, 130.18, 130.00, 129.90, 129.83, 128.91, 128.14, 128.11, 128.07, 127.91, 127.84, 127.78, 127.70, 125.19, 100.16, 95.24, 94.74, 90.72, 74.39, 73.57, 73.48, 72.84, 72.73, 71.38, 70.07, 69.53, 66.60, 60.67, 54.11, 26.81, 26.72, 26.62, 26.46, 21.36, 20.60, 20.50, 20.47, 20.41, 19.27, 18.90; HR-MS (FAB) calcd for $C_{57}H_{68}N_2O_{16}Si_2Cl_6Cs$ $[M+Cs]^+$ 1437.1275, found 1437.1351.

Compound 14: Allyl alcohol (0.019 mL, 0.280 mmol) and **13** (0.091 g, 0.070 mmol) were dissolved in dry dichloromethane (0.70 mL). The solution was cooled to $-20\,^\circ$C, and TMSOTf (220 μL, 0.05 M) in dichloromethane (0.014 mL) was added dropwise. The reaction was stirred for 15 min at $-20\,^\circ$C, and an additional 15 min at room temperature. A few drops of dry triethylamine were added to quench the reaction. The reaction mixture was diluted with ethyl acetate (2 mL), washed with saturated sodium bicarbonate (3 × 2 mL), and brine (1 × 2 mL). The aqueous extracts were then re-extracted with ethyl acetate (2 × 1 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (silica gel, 3:1 hexane/EtOAc) then gave **14** (0.080 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.78-7.73$ (m, 4H, *Ph*), 7.59–7.57 (m, 4H, *Ph*), 7.45–7.35 (m, 12H, *Ph*), 5.86 (m, 1H, -CH$_2$-C*H*=CH$_2$), 5.55 (s, 1H, Gal H-4), 5.25 (dd, 1H, $J = 16$, 2 Hz, -CH$_2$-CH=C*H*$_2$), 5.20 (br s, 1H, N*H*), 5.17 (dd, 1H, $J = 10$, 2 Hz, -CH$_2$-CH=C*H*$_2$), 5.04–4.98 (m, 3H, Glu H-3, Gal H-2, Gal H-3), 4.77 (d, 1H, $J = 7$ Hz, Gal H-1), 4.72 (dd, 2H, $J = 15$, 12 Hz, -C*H*$_2$-CCl_3), 4.42 (1H, d, $J = 8$ Hz, Glu H-1), 4.34 (dd, 1H, $J = 13$, 5 Hz, -C*H*$_2$-CH=CH$_2$), 4.09–4.04 (m, 2H, Glu H-4, -C*H*$_2$-CH=CH$_2$), 3.92–3.86 (m, 2H, Glu H-6$_a$, Glu H-6$_b$), 3.78–3.67 (m, 3H, Glu H-2, Gal H-6$_a$, Gal H-6$_b$′), 3.52 (t, 1H, $J = 9$ Hz, Gal H-5), 3.29 (d, 1H, $J = 10$ Hz, Glu H-5), 2.02 (s, 3H, -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$), 1.78 (s, 3H, -COC*H*$_3$), 1.77 (s, 3H, -COC*H*$_3$), 1.07 (s, 9H, -C(C*H*$_3$)$_3$), 1.03 (s, 9H, -C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.85$, 170.03, 169.91, 168.94, 163.41, 154.38, 135.96, 135.56, 135.37, 133.61, 132.64, 132.57, 132.08, 129.96, 129.87, 127.96, 127.83, 127.77, 127.66, 117.52, 100.11, 100.06, 75.18, 74.39, 74.03, 72.82, 72.24, 71.31, 69.72, 69.38, 66.72, 61.13, 60.74, 55.89, 26.76, 26.71, 26.63, 20.64, 20.56, 19.33, 19.03; HR-MS (FAB) calcd for $C_{58}H_{72}NO_{16}Si_2Cl_3Cs$ $[M+Cs]^+$ 1334.2500, found 1334.2569.

Compound 15: A solution of 1M sodium methoxide in water (0.017 mL, 0.017 mmol) was added to a solution of **14** (0.199 g, 0.166 mmol) in methanol (1.7 mL). The reaction was stirred for 4 h at ambient temperature, then solvent was removed by rotary evaporation. Purification by flash chromatography (silica gel, 5% methanol/chloroform) then yielded **15** (0.166 g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.74-7.62$ (m, 8H, *Ph*), 7.43–7.26 (m, 12H, *Ph*), 5.85 (1H, m, -CH$_2$-C*H*=CH$_2$), 5.24 (dd, 1H, $J = 17$, 1 Hz, -CH$_2$-CH=C*H*$_2$), 5.16 (dd, 1H, $J = 10$, 1 Hz, -CH$_2$-CH=C*H*$_2$), 5.09 (t, 1H, $J = 10$ Hz, Glu H-3), 4.72 (dd, 2H, $J = 32$, 12 Hz, -C*H*$_2$-CCl_3), 4.52 (d, 1H, $J = 7$ Hz, Gal H-1), 4.44 (1H, d, $J = 8$ Hz, Glu H-1), 4.31 (dd, 1H, $J = 14$, 5 Hz, -C*H*$_2$-CH=CH$_2$), 4.23 (br s, 1H), 4.14 (d, 1H, $J = 10$ Hz, -C*H*$_2$-CH=CH$_2$), 4.08 (t, 1H, $J = 10$ Hz, Glu H-4), 4.04–4.00 (m, 2H), 3.91 (d, 2H, $J = 10$ Hz), 3.86–3.79 (m, 5H), 3.76–3.70 (m, 1H), 3.58–3.43 (m, 4H), 3.37 (d, 1H, $J = 9$ Hz, Glu H-5), 1.83 (s, 3H, -COC*H*$_3$), 1.05 (s, 9H, -C(C*H*$_3$)$_3$), 1.04 (s, 9H, -C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.09$, 163.69, 154.44, 135.94, 135.88, 135.44, 135.39, 133.73, 132.96, 132.84, 132.42, 129.90, 129.75, 129.02, 128.22, 127.79, 127.55, 125.27, 117.27, 102.61, 99.81, 95.68, 75.15, 74.97, 74.84, 74.54, 74.29, 73.95, 72.90, 71.63, 69.26, 68.35, 62.24, 61.42, 55.78, 26.80, 26.71, 21.44, 20.95, 19.33, 19.26, 19.15, 19.07; HR-MS (FAB) calcd for $C_{52}H_{66}NO_{13}Si_2Cl_3Cs$ $[M+Cs]^+$ 1208.2180, found 1208.2118.

Compound 16: A solution of TMSOTf (172 µL, 0.05 M) in dry dichloromethane (0.172 mL, 0.009 mmol) was added dropwise to a stirred solution of **13** (1.183 g, 0.860 mmol) and **15** (0.921 g, 0.856 mmol) in dry dichloromethane (8.6 mL). The reaction was stirred 15 min at room temperature, and then quenched by addition of solid sodium bicarbonate. The reaction was diluted with ethyl acetate (20 mL), washed with saturated sodium bicarbonate (3 × 10 mL) and brine (1 × 10 mL). Aqueous extracts were re-extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (silica gel, gradient hexane to 2:1 hexane/EtOAc, then 10% methanol/chloroform) allowed recovery of unreacted starting material **15** (0.097 g), but the other reaction products were inseparable.

The mixture was dissolved in dry pyridine (1.3 mL). Acetic anhydride (0.400 mL, 4.246 mmol) and *N*,*N*-dimethylaminopyridine (20.8 mg, 0.170 mmol) was added to the reaction flask, and the reaction stirred for 2 h. The mixture was diluted with ethyl acetate (10 mL), washed with saturated sodium bicarbonate (3 × 10 mL) and brine (1 × 10 mL). The aqueous extracts were re-extracted with ethyl acetate (2 × 5 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (silica gel, gradient 4:1 to 2:1 hexane/EtOAc) then yielded **16** (1.321 g, 67%, two steps) (Considering recovery of starting material **15**, the yield is 75% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.74-7.30$ (m, 40H, *Ph*), 5.90–5.82 (m, 1H, -CH$_2$-C*H*=CH$_2$), 5.60 (br s, 1H, Gal$_1$ H-4), 5.53 (d, 1H, $J = 3$ Hz, Gal$_2$ H-4), 5.25 (dd, 1H, $J = 17$, 2 Hz, -CH$_2$-CH=C*H*$_2$), 5.19–5.11 (m, 2H, Glu$_2$ H-3, -CH$_2$-CH=C*H*$_2$), 5.06 (d, 1H, $J = 9$ Hz, Glu$_1$ N*H*), 5.02–4.83 (m, 5H, Glu$_1$ H-3, Glu$_2$ N*H*, Gal$_1$ H-2, Gal$_2$ H-2, Gal$_2$ H-3), 4.75–4.70 (m, 3H, Gal$_2$ H-1, -C*H*$_2$-CCl_3), 4.65–4.58 (m, 4H, Gal$_1$ H-1, Glu$_2$ H-1, -C*H*$_2$-CCl_3), 4.39 (d, 1H, $J = 8$ Hz, Glu$_1$ H-1), 4.33 (dd, 1H, $J = 13$, 4 Hz, -C*H*$_2$-CH=CH$_2$), 4.08–4.02 (m, 2H, Glu$_1$ H-4, -C*H*$_2$-CH=CH$_2$), 3.97–3.84 (m, 6H, Glu$_2$ H-4, Gal$_1$ H-3), 3.78–3.57 (m, 6H, Glu$_1$ H-2), 3.49 (t, 1H, $J = 9$ Hz, Gal$_2$ H-5), 3.41 (d, 1H, $J = 10$ Hz, Glu$_2$ H-5), 3.35 (q, 1H, $J = 9$ Hz, Glu$_2$ H-2), 3.28 (1H, d, $J = 9$ Hz, Glu$_1$ H-5), 2.01 (s, 3H, -COC*H*$_3$), 2.00 (s, 3H, -COC*H*$_3$), 1.99 (s, 3H, -COC*H*$_3$), 1.85 (s, 3H, -COC*H*$_3$), 1.75 (s, 3H, -COC*H*$_3$), 1.71 (s, 3H, -COC*H*$_3$), 1.68 (s, 3H, -COC*H*$_3$), 1.07 (s, 9H, -C(C*H*$_3$)$_3$), 1.06 (s, 9H, -C(C*H*$_3$)$_3$), 1.03 (s, 9H, -C(C*H*$_3$)$_3$), 0.98 (s, 9H, -C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.84$, 170.26, 169.97, 169.84, 169.28, 168.91, 168.76, 154.24, 153.70, 135.88, 135.79, 135.49, 135.42, 135.37, 133.60, 133.53, 133.15, 132.78, 132.70, 132.58, 132.51, 132.27, 132.00, 130.05, 129.94, 129.84, 129.77, 129.63, 129.04, 128.19, 128.03, 127.84, 127.75, 127.65, 117.47, 100.42, 100.20, 100.06, 100.00, 95.56, 95.45, 75.55, 75.28, 74.52, 74.31, 73.93, 73.68, 72.69, 72.19, 71.91, 71.27, 69.69, 69.38, 68.69, 66.57, 62.10, 61.41, 61.15, 60.50, 56.65, 55.85, 26.77, 26.65, 21.01, 20.60, 20.54, 20.41, 19.36, 19.25, 18.96; HR-MS (FAB) calcd for $C_{111}H_{136}N_2O_{30}Si_4Cl_6Cs$ $[M+Cs]^+$ 2435.5437, found 2435.5585.

Compound 17: A 1M sodium methoxide solution in water (54 µL, 0.054 mmol) was added to a stirred solution of **16** (1.24 g, 0.538 mmol). The solution was stirred at ambient temperature for 4 h, and then solvent was removed by rotary evaporation. Flash chromatography (silica gel, gradient chloroform to 3% methanol/chloroform) afforded **17** (0.833 g, 72%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.76-7.54$ (m, 16H, *Ph*), 7.42–7.29 (24H, m, *Ph*), 5.96–5.88 (m, 1H, -CH$_2$-C*H*=CH$_2$), 5.62 (d, 1H, $J = 3$ Hz, Gal$_2$ H-4), 5.29 (dd, 1H, $J = 18$, 2 Hz, -CH$_2$-CH=C*H*$_2$), 5.15 (d, 1H, $J = 11$ Hz, -CH$_2$-CH=C*H*$_2$), 5.09 (t, 1H, $J = 10$ Hz, Glu$_1$ H-3), 4.97 (t, 1H, $J = 10$ Hz, Gal$_1$ H-2), 4.83–4.65 (m, 4H, Gal$_1$ H-1, -C*H*$_2$-CCl_3), 4.58–4.54 (m, 3H, Glu$_1$ H-1, Glu$_2$ H-1, -C*H*$_2$-CCl_3), 4.38–4.34 (m, 2H, -C*H*$_2$-

CH=CH_2), 4.18 (t, 1H, $J=11$ Hz, Glu_1 H-4), 4.13–4.07 (m, 2H, -CH_2-CH=CH_2), 3.97–3.34 (m, 19H), 2.05 s, (3H, -COCH_3), 1.94 (s, 3H, -COCH_3), 1.67 (s, 3H, -COCH_3), 1.07 (s, 9H, -C(CH_3)$_3$), 1.01 (s, 9H, -C(CH_3)$_3$), 0.98 (s, 9H, -C(CH_3)$_3$), 0.96 (s, 9H, -C(CH_3)$_3$); ^{13}C NMR (100 MHz, CD_3OD): $\delta=137.09$, 136.85, 136.79, 130.70, 136.64, 136.61, 135.34, 134.74, 134.49, 134.39, 133.99, 133.39, 131.13, 131.04, 130.91, 130.84, 129.91, 129.12, 129.07, 128.99, 128.89, 128.84, 117.27, 104.84, 101.60, 101.47, 97.27, 80.76, 77.03, 76.80, 76.57, 76.27, 75.71, 75.30, 74.83, 73.93, 72.90, 72.70, 72.34, 70.78, 69.76, 64.06, 63.78, 62.29, 58.95, 58.29, 57.25, 27.68, 27.61, 27.52, 27.42, 27.39, 21.17, 20.81, 20.33, 20.24, 20.03, 19.97, 18.37; HR-MS (FAB) calcd for $C_{103}H_{128}N_2O_{26}Si_4Cl_6Cs$ $[M+Cs]^+$ 2268.1176, found 2268.1062.

Compound 18: A solution of TMSOTf (64 μL, 0.05 M) in dry dichloromethane (0.064 mL, 0.003 mmol) was added dropwise to a stirred solution of **13** (0.423 g, 0.324 mmol) and **17** (0.691 g. 0.324 mmol) in dry dichloromethane (3.2 mL). The reaction was stirred 10 min at room temperature, and then quenched by addition of solid sodium bicarbonate. The reaction was diluted with ethyl acetate (5 mL), washed with saturated sodium bicarbonate (3×5 mL) and brine (1×5 mL). Aqueous extracts were re-extracted with ethyl acetate (2×5 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (silica gel, gradient, chloroform to 10% methanol/chloroform) allowed the recovery of unreacted **17** (0.341 g), but the other reaction products were inseparable.

The mixture was dissolved in dry pyridine (0.36 mL). Acetic anhydride (0.219 mL, 2.322 mmol), and *N,N*-dimethylaminopyridine (5.6 mg, 0.046 mmol) were added to the reaction flask, and the reaction stirred for 4 h. The mixture was diluted with ethyl acetate (3 mL). It was washed with saturated sodium bicarbonate (3×3 mL) and brine (1×3 mL). The aqueous extracts were re-extracted with ethyl acetate (2×3 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (silica gel, gradient chloroform to 1% methanol/chloroform) then yielded **18** (0.386 g, 35%, two steps). (Considering the recovery of **17**, the yield is 69% over two steps.) ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=7.74-7.56$ (m, 24H), 7.46–7.27 (m, 36H), 5.87–5.82 (m, 1H), 5.59 (apps, 2H), 5.53 (d, 1H, $J=3$ Hz), 5.25 (dd, 1H, $J=17$, 1 Hz), 5.23–5.14 (m, 2H), 5.08–4.87 (m, 9H), 4.79–4.68 (m, 9H), 4.43 (d, 1H, $J=7$ Hz), 4.39 (1H, d, $J=8$ Hz), 4.32 (dd, 1H, $J=9$, 2 Hz), 4.08–3.38 (m, 25H), 2.00 (s, 3H), 1.99 (s, 6H), 1.86 (s, 3H), 1.81 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H), 1.70 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.07 (s, 9H), 1.06 (s, 9H), 1.05 (s, 9H), 1.03 (s, 9H), 0.96 (s, 9H), 0.95 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 170.41, 170.19, 169.95, 169.81, 169.29, 168.89, 168.77, 168.68, 154.26, 153.64, 135.94, 135.88, 135.80, 135.74, 135.60, 135.54, 135.51, 135.45, 135.41, 135.33, 133.59, 133.50, 133.27, 133.19, 132.78, 132.71, 132.68, 132.61, 132.56, 132.53, 132.23, 132.03, 130.04, 129.93, 129.87, 129.83, 129.76, 129.68, 128.98, 128.16, 128.03, 127.96, 127.93, 127.83, 127.77, 127.74, 127.65, 127.58, 125.24, 117.43, 100.23, 100.09, 100.01, 99.92, 75.64, 75.55, 75.30, 74.35, 74.18, 74.02, 73.72, 72.72, 72.19, 72.10, 71.90, 71.27, 71.21, 69.70, 69.38, 68.45, 66.59, 61.79, 61.40, 60.52, 56.70, 56.50, 55.86, 26.92, 26.86, 26.79, 26.68, 26.61, 26.58, 21.40, 20.95, 20.58, 20.54, 20.48, 20.44, 20.41, 20.35, 20.22, 19.33, 19.21, 18.96; MALDI-MS calcd for $C_{164}H_{200}N_3O_{44}Si_6Cl_9Na$ $[M+Na]^+$ 3421, found 3421.

Compound 19: Activated zinc dust (2.230 g) was added to a solution of **18** (0.372 g, 0.109 mmol) in acetic anhydride (2.7 mL). The reaction was stirred for 24 h at room temperature, then filtered through a plug of Celite, eluting with ethyl acetate. After concentration, purification by flash chromatography (silica gel, 1:1 EtOAc/hexane, then 10:4:1 hexane/EtOAc/methanol) yielded **19** (0.118 g, 36%). ^{1}H NMR (600 MHz, CD_3OD): $\delta=7.75-7.55$ (m, 24H, *Ph*), 7.47–7.23 (m, 36H, *Ph*), 5.95–5.89 (m, 1H, -CH_2-C*H*=CH_2), 5.67 (d, 1H, $J=3$ Hz, Gal_{int}H-4), 5.56 (d, 1H, $J=3$ Hz, Gal_{int}H-4), 5.50 (d, 1H, $J=3$ Hz, Gal_{ext}H-4), 5.37 (dt, 2H, $J=10$, 4 Hz, 2 Glu H-3), 5.27 (d, 1H, $J=17$ Hz, -CH_2-CH=CH_2), 5.16 (d, 1H, $J=11$ Hz, -CH_2-CH=CH_2), 5.04 (t, 1H, $J=10$ Hz), 4.97–4.87 (m, 5H, 2 Glu H-1), 4.76 (d, 1H, $J=8$ Hz, Gal H-1), 4.67 (d, 1H, $J=8$ Hz, Gal H-1), 4.61 (1H, d, $J=8$ Hz, Gal H-1), 4.56 (1H, d, $J=8$ Hz, Glu H-1), 4.33 (dd, 1H, $J=13$, 4 Hz, -CH_2-CH=CH_2), 4.14–3.82 (m, 14H), 3.72–3.47 (m, 11H), 3.43–3.33 (m, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.89 (s, 3H), 1.81 (6H, s), 1.77 (s, 3H), 1.74 (3H, s), 1.66 (s, 3H), 1.65 (s, 3H), 1.08 (s, 9H), 1.06 (9H, s), 1.01 (s, 9H), 1.00 (s, 9H), 0.94 (s, 9H), 0.93 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=173.47$, 173.42, 173.32, 172.15, 172.07, 171.84, 171.68, 171.51, 171.34, 171.06, 170.85, 170.74, 170.67, 137.08, 137.02, 136.73, 136.67, 136.64, 136.53, 135.40, 134.85, 134.78, 134.58, 134.03, 133.87, 133.81, 133.68, 133.57, 133.29, 131.26, 131.14, 130.98, 130.89, 129.23, 129.19, 129.03, 128.93, 128.81, 117.17, 101.34, 101.18, 101.07, 76.50, 76.37, 76.24, 75.75, 75.42, 75.34, 75.21, 74.25, 74.07, 73.24, 73.14, 72.83, 72.64, 70.98, 70.65, 70.55, 68.24, 63.88, 63.63, 63.01, 62.92, 62.63, 62.48, 62.14, 57.05, 55.20, 27.84, 27.61, 27.29, 23.68, 22.97, 22.81, 22.74, 21.99, 21.46, 21.43, 21.28, 21.04, 20.89, 20.79, 20.75, 20.68, 20.60, 20.49, 20.37, 20.27, 20.10, 20.00, 19.95, 19.90; HR-MS (HR-MALDI) calcd for $C_{161}H_{203}N_3O_{41}Si_6Na$ $[M+Na]^+$ 3025.2405, found 3025.2504. See also the COSY spectrum of compound **19** (Figure 2).

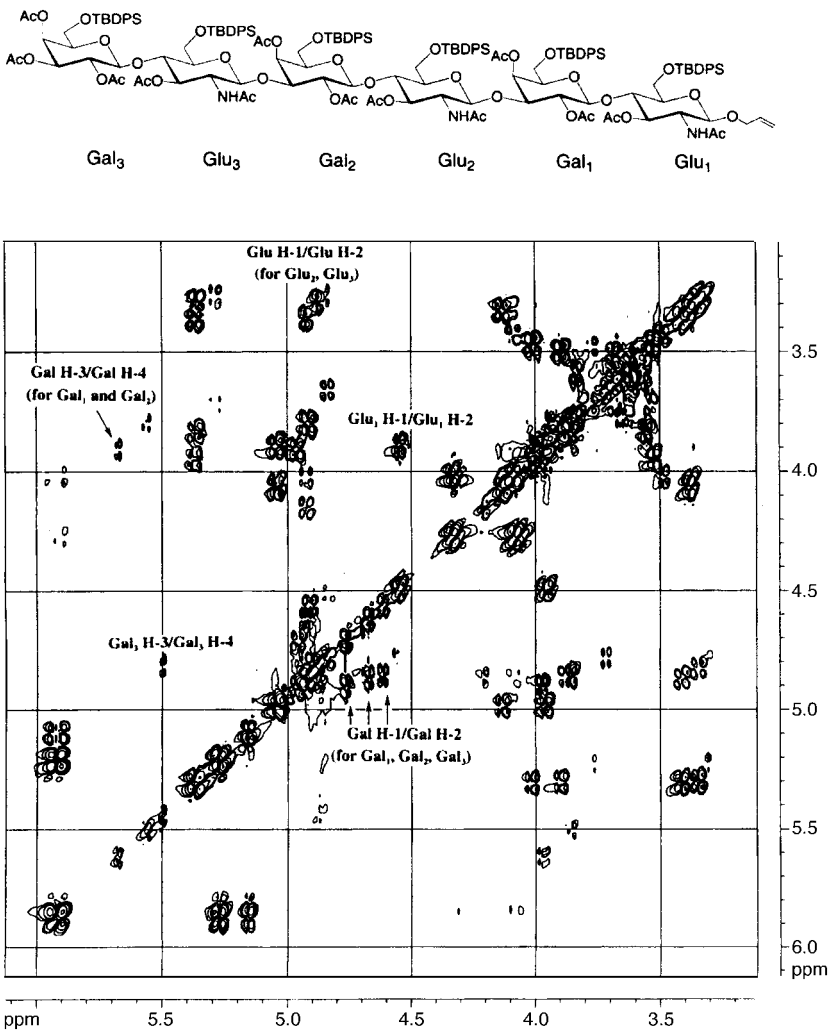


Figure 2. COSY spectrum of compound **19** and partial assignment.

0947-6539/00/0607-1248 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 7

Compound 2: Compound **19** (0.109 g, 0.036 mmol) was dissolved in dry tetrahydrofuran (0.4 mL) and a 1M solution of tetrabutylammonium fluoride (0.726 mL, 0.726 mmol) added. The reaction was stirred 24 h at room temperature. Solvent was removed by rotary evaporation and the residue redissolved in methanol (0.4 mL). A 1M solution of sodium methoxide in water (0.030 mL, 0.030 mmol) was added to the reaction flask, and stirring continued for 24 h. The reaction was concentrated, and the TBAF and NaOMe conditions repeated an additional time. Purification by size-exclusion chromatography (Biogel P-2, water as mobile phase) afforded **2** (20.5 mg, 49%). ^{1}H NMR (400 MHz, D_2O) identifiable resonances: $\delta = 5.89-5.81$ (m, 1H, -CH_2-C*H*=CH_2), 5.39 (app d, 1H, $J =$ 17 Hz, -CH_2-CH=C*H*$_2$), 5.21 (app d, 1H, $J = 10$ Hz, -CH_2-CH=C*H*$_2$), 4.65 (app d, 2H, $J = 8$ Hz, Glu_2 H-1, Glu_3 H-1), 4.53 (d, 1H, $J = 8$ Hz, Glu_1 H-1), 4.44–4.40 (m, 3H, Gal_1H-1, Gal_2 H-1, Gal_3 H-1), 4.29 (dd, 1H, $J = 13$, 4 Hz), 1.98 (s, 9H); ^{13}C NMR (100 MHz, D_2O): $\delta = 172.15$, 171.81, 130.52, 115.43, 100.13, 100.01, 97.21, 79.29, 75.63, 75.35, 72.59, 72.11, 71.99, 71.78, 69.71, 69.41, 68.20, 67.70, 67.20, 65.78, 65.55, 58.27, 58.19, 57.26, 57.06, 52.38, 52.24, 19.41, 19.38; ESI-MS (pos) calcd for $C_{45}H_{75}N_3O_{31}Na$ $[M+Na]^+$ 1176, found 1176.

Compound 20: Compound **2** (11.7 mg, 0.010 mmol) and CMP-sialic acid (7.3 mg, 0.011 mmol) were dissolved in 100mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) buffer, pH 7.4 (2.0 mL) containing 0.25% Triton X-100 and a freshly prepared $MnCl_2$ solution (0.011 mmol). α2,3-Sialyltransferase (200 mU) and alkaline phosphatase (300 mU) were added to the reaction vial, and the solution stirred gently at room temperature for 3 d. After lyophilization, the reaction mixture was dissolved in water and purified on a column of Biogel P-2, eluting with water. The product was further purified on a DEAE Sephadex A-25 anion-exchange column, eluting with a gradient of 0 to 100mM NH_4HCO_3. Final desalting was accomplished on Biogel P-2, eluting with water. Product fractions were combined and lyophilized, yielding **20** (12.3 mg, 85%). ^{1}H NMR (400 MHz, D_2O) identifiable resonances: $\delta =$ 5.91–5.81 (m, 1H, -CH_2-C*H*=CH_2), 5.28–5.20 (m, 2H, -CH_2-CH=C*H*$_2$), 4.65 (app d, 2H, $J = 8$ Hz, Glu_2 H-1, Glu_3 H-1), 4.54–4.50 (m, 2H, Glu_1 H-1, Gal_3 H-1), 4.41 (app d, 2H, $J = 8$ Hz, Gal_1 H-1, Gal_2 H-1), 4.29 (dd, 1H, $J =$ 13, 4 Hz, -C*H*$_2$-CH=CH_2), 2.71 (app d, 1H, $J = 9$ Hz, NeuAc H-3_{eq}), 1.99 (s, 12H), 1.75 (t, 1H, J = 11 Hz, NeuAc H-3_{ax}); ^{13}C NMR (100 MHz, D_2O): $\delta = 130.75$, 115.64, 100.35, 100.27, 100.23, 100.00, 97.44, 82.81, 79.52, 75.88, 75.59, 72.95, 72.63, 72.34, 72.23, 72.01, 70.35, 69.93, 69.63, 69.34, 69.24, 67.94, 67.70, 67.68, 67.42, 67.37, 67.26, 67.21, 67.16, 66.98, 66.91, 66.85, 66.69, 65.80, 65.54, 64.92, 64.61, 60.03, 58.49, 58.42, 57.85, 57.49, 57.29, 55.57, 54.32, 52.61, 52.45, 49.13, 35.16, 29.33, 19.62, 19.59; ESI-MS (neg) calcd for $C_{56}H_{91}N_4O_{39}$ $[M-H]^-$ 1443.5, found 1443.6.

Compound 1

Method A: Compound **20** (7.4 mg, 5.2 μmol) and GDP-fucose (10.0 mg, 16.9 μmol) were dissolved in 100mM 4-morpholine ethanesulfonic acid

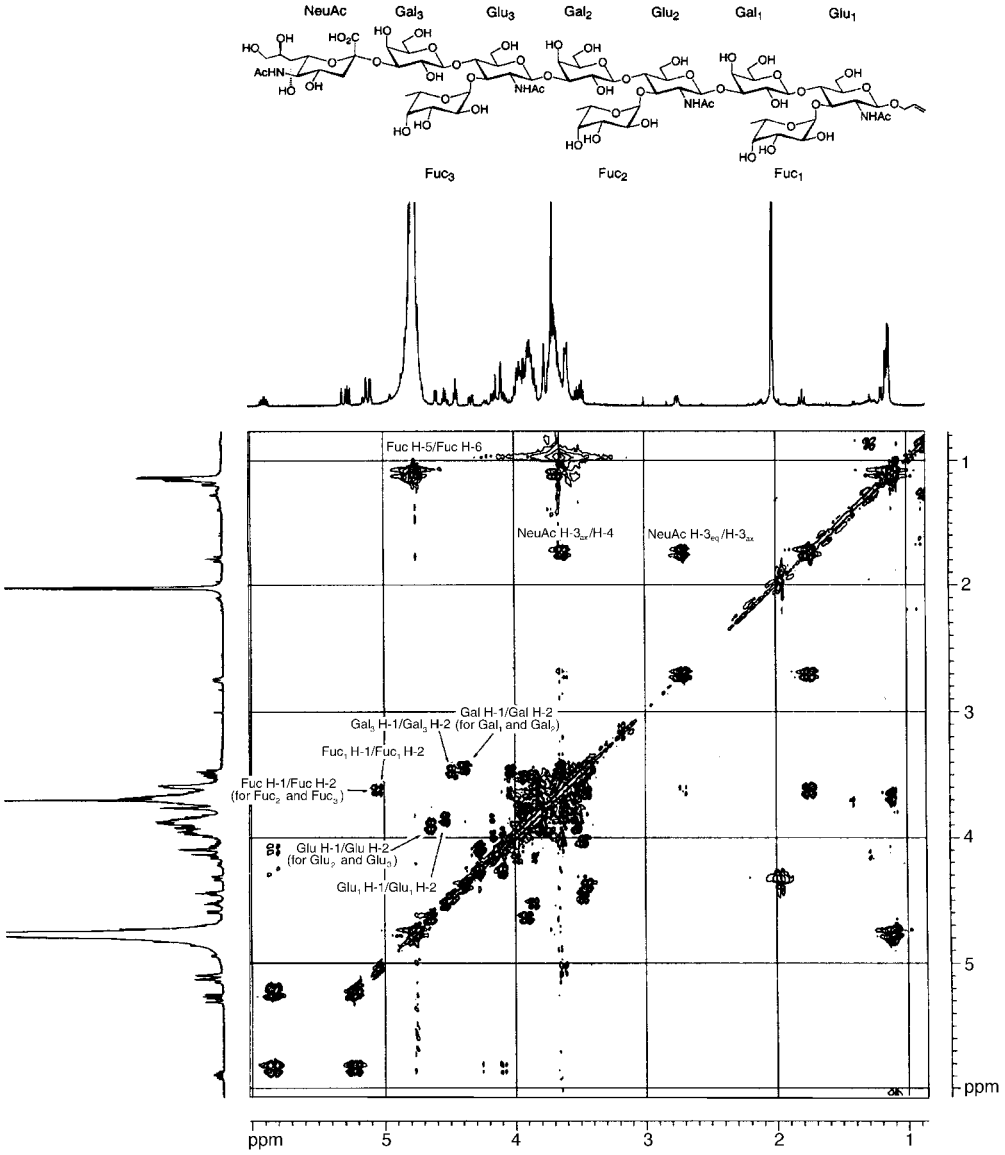


Figure 3. COSY spectrum of compound **1** and partial assignment.

monohydrate (MES) buffer pH 6.0 (1.0 mL) containing 0.25% Triton X-100 and a freshly prepared $MnCl_2$ solution (16.9 μmol). α1,3-Fucosyltransferase V (34.3 mU) and alkaline phosphatase (156 mU) were added to the reaction vial, and the solution stirred gently at room temperature for 5 d. After lyophilization, the reaction mixture was dissolved in water and purified on a column of Biogel P-2, eluting with water. Product fractions were combined and lyophilized, to yield a di-fucosylated molecule. This intermediate structure (7.4 mg, ≈4.0 μmol) and GDP-fucose (2.6 mg, 4.4 μmol) were dissolved in 100 mM MES buffer pH 6.0 (0.8 mL) containing 0.25% Triton X-100 and a freshly prepared $MnCl_2$ solution (4.4 μmol). α1,3-Fucosyltransferase VI (40.0 mU) and alkaline phosphatase (120.0 mU) were added to the reaction vial, and the solution stirred gently at room temperature for 5 d. After lyophilization, the reaction mixture was dissolved in water and purified on a column of Biogel P-2, eluting with water. Product fractions were combined and lyophilized. These were further loaded onto a DEAE Sephadex A-25 column, eluted first with water, and then with 100 mM NH_4HCO_3. This material was purified an additional step on Biogel P-2, eluting with water, to give sialyl-trimeric-Lewis x **1** (3.0 mg, 30%, two steps).

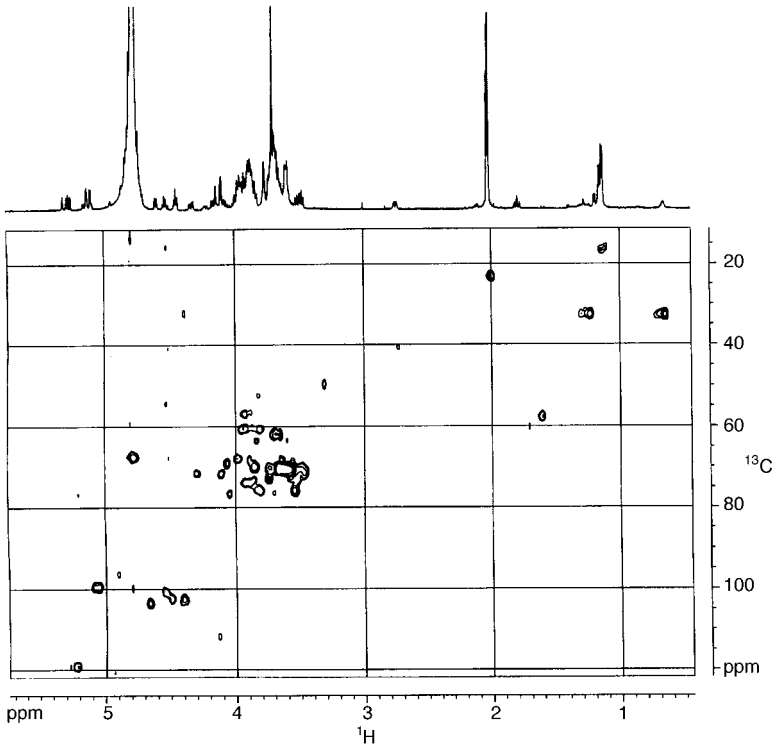


Figure 4. HMQC spectrum of compound **1**.

Method B: Compound **20** (5.0 mg, 3.5 μmol) and GDP-fucose (6.7 mg, 11.6 μmol) were dissolved in 100 mM MES buffer pH 6.0 (0.7 mL) containing 0.25% Triton X-100 and a freshly prepared $MnCl_2$ solution (11.6 μmol). α1,3-Fucosyltransferase VI (35.0 mU) and alkaline phosphatase (105 mU) were added to the reaction vial, and the solution stirred gently at room temperature for 6 d. After lyophilization, the reaction mixture was dissolved in water and loaded onto a column of Biogel P-2, eluting with water. Fractions containing compound were further loaded onto a DEAE Sephadex A-25 column, eluted first with water, and then with 100 mM NH_4HCO_3. This material was purified an additional step on Biogel P-2, eluting with water, to give sialyl-trimeric-Lewis x **1** (2.7 mg, 41%). ^{1}H NMR (750 MHz, D_2O) identifiable resonances: $\delta = 5.91-5.85$ (m, 1H, -CH_2-CH=CH_2), 5.27 (dd, 2H, $J = 18$, 2 Hz, -CH_2-CH=CH_2), 5.11 (2d, 2H, $J =$ 4 Hz, Fuc_2, Fuc_3 H-1), 5.08 (d, 1H, $J = 4$ Hz, Fuc_1 H-1), 4.71 (2d, 2H, $J =$ 8 Hz, Glu_2, Glu_3 H-1), 4.58 (d, 1H, $J = 8$ Hz, Glu_1 H-1), 4.51 (d, 1H, $J =$ 8 Hz, Gal_3 H-1), 4.44 (d, 1H, $J = 8$ Hz, $Gal_{(1\,or\,2)}$ H-1), 4.42 (d, 1H, $J = 8$ Hz, $Gal_{(1\,or\,2)}$ H-1), 4.31 (dd, 1H, $J = 13$, 5 Hz, -CH_2-CH=CH_2) 2.73 (dd, 1H, $J =$ 12, 4 Hz, NeuAc H-3_{eq}), 2.02 (3H, s), 2.01 (s, 3H), 2.00 (s, 6H), 1.78 (t, 1H, $J = 12$ Hz, NeuAc H-3_{ax}), 1.14 (d, 3H, $J = 7$ Hz, Fuc H-6), 1.12 (d, 6H, $J =$ 7 Hz, 2Fuc H-6); ESI-MS (neg) calcd for $C_{74}H_{121}N_4O_{51}$ $[M-H]^-$ 1882, found 1882. See also the COSY spectrum (Figure 3) and HMQC spectrum (Figure 4) for compound **1**.

Acknowledgement

This work was supported by the N. I. H. K.M.K. thanks the American Chemical Society Organic Division for a fellowship and also Dr. Dee-Hua Huang and Dr. Laura Pasternack for aid in NMR experiments and analysis.

[1] a) M. L. Phillips, L. E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S.-I. Hakomori, J. C. Paulson, *Science* **1990**, *250*, 1130; b) J. B. Lowe, L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhend, R. M. Marks, *Cell* **1990**, *63*, 475; c) G. Waltz, A. Aruffo, W. Kolanus, M. Bevilaqua, B. Seed, *Science* **1990**, *250*, 1132; d) M. J. M. Polley, M. L. Phillips, E. Wayner, L. E. Nudelman, A. K. Singhal, S.-I. Hakomori, J. C. Paulson, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 6224; e) Q. Zhou, K. L. Moore, A. Varki, R. McEver, R. D. Cummings, *J. Cell Biol.* **1991**, *115*, 557; f) S. Hemmerich, C. R. Bertozzi, H. Leffler, S. D. Rosen, *Biochemistry* **1994**, *33*, 4820; g) S. Hemmerich, S. D. Rosen, *Biochemistry* **1994**, *33*, 4830.

[2] L. Poppe, G. S. Brown, J. S. Philo, P. V. Nikrad, B. H. Shah, *J. Am. Chem. Soc.* **1997**, *119*, 1727.

[3] a) G. Kretschmar, U. Sprengard, H. Kunz, E. Bartnik, W. Schmidt, A. Toepfer, B. Horsch, M. Krause, D. Seiffge, *Tetrahedron* **1995**, *51*, 13015; b) G. Baisch, R. Öhrlein, *Angew. Chem.* **1996**, *108*, 1949; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1812; c) U. Sprengard, M. Schudok, W. Schmidt, G. Kretschmar, H. Kunz, *Angew. Chem.* **1996**, *108*, 359; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 321; d) S. A. DeFrees, W. Kosch, W. Way, J. C. Paulson, S. Sabesan, R. Halcomb, D.-H. Huang, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1995**, *117*, 66; e) see also:E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C.-H. Wong, *Chem. Rev.* **1998**, *98*, 833, and references therein.

[4] a) S. D. Rosen, C. R. Bertozzi, *Curr. Opin. Cell Biol.* **1994**, *6*, 663; b) T. M. Carlos, J. M. Harlan, *Blood* **1994**, *84*, 2068; c) G. S. Kansas, *Blood* **1996**, *88*, 3259.

[5] a) M. Ujita, J. McAuliffe, T. Schwientek, R. Almeida, O. Hindsgaul, H. Clausen, M. Fukuda, *J. Biol. Chem.* **1998**, *273*, 34843; b) M. Fukuda, in *Molecular Glycobiology*, Ch. 1, pp. 1–52, IRL Press, Oxford, **1994**; c) O.Renkonen, S. Toppila, L. Penttila, H. Salminen, J. Helin, H. Maaheimo, C. E. Costello, J. P. Turunen, R. Renkonen, *Glycobiology* **1997**, *7*, 453; d) R. Niemela, J. Natunen, M.-L. Majuri, H. Maaheimo, J. Helin, J. B. Lowe, O. Renkonen, R. Renkonen, *J. Biol. Chem.* **1998**, *273*, 4021.

[6] P. P. Wilkins, R. P. McEver, R. D. Cummings, *J. Biol. Chem.* **1996**, *271*, 18732.

[7] M. R. Stroud, K. Handa, M. E. K. Salyan, K. Ito, S. B. Levery, S.-I. Hakomori, *Biochemistry* **1996**, *35*, 770.

[8] a) T. de Vries, D. H. van den Eijnden, *Biochemistry* **1994**, *33*, 9937; b) J. Rabina, J. Natunen, R. Niemela, H. Salminen, K. Ilves, O. Aitio, H. Maaheimo, J. Helin, O. Renkonen, *Carbohydr. Res.* **1998**, *305*, 491.

[9] a) K. C. Nicolaou, T. J. Caulfield, H. Kataoka, N. A. Stylianides, *J. Am. Chem. Soc.* **1990**, *112*, 3693; b) A. Toepfer, W. Kinzy, R. R. Schmidt, *Liebigs Ann. Chem.* **1994**, 449.

  0947-6539/00/0607-1250 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 7

[10] K. C. Nicolaou, C. W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.* **1992**, *114*, 3126.
[11] W. Dullenkopf, J. C. Castro-Palomino, L. Manzoni, R. R. Schmidt, *Carbohydr. Res.* **1996**, *296*, 135; trichloroethyl carbamate (Troc) protection of the amino group was chosen for its documented β-directing effect in glycosylation reactions, and its relative stability during protecting group manipulations.
[12] a) R. R. Schmidt, *Angew. Chem.* **1986**, *98*, 213; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212; b) R. L. Halcomb, S. H. Boyer, S. J. Danishefsky, *Angew. Chem.* **1992**, *104*, 314; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 338.
[13] For related examples of regioselective glycosylation, see U. Ellervik, G. Magnusson, *J. Org. Chem.* **1998**, *63*, 9314.
[14] B. B. Lipshutz, D. Pollart, J. Monforte, H. Kotsuki, *Tetrahedron Lett.* **1985**, *26*, 705.
[15] For structural analysis, the galactose H-4 protons were utilized as reporter peaks. When acetylated, these protons are shifted downfield from all other carbohydrate resonances present ($\approx$5.6 ppm), and are readily identified through their small coupling constant. Gal H-4 cross peaks in the COSY spectrum are thus diagnostic for coupling to a neighboring 3-position acetate or glycosidic ether, as the coupling of Gal H-4 to Gal H-5 is essentially non-existent.
[16] G. Hummel, R. R. Schmidt, *Tetrahedron Lett.* **1997**, *38*, 1173.
[17] The positions of the remaining acetate esters were determined by the 2D COSY NMR spectrum.
[18] The low yield for this conversion was unexpected, as yields in monoamide model systems were $>90\,\%$.
[19] Although it is clear that one fucose residue was appended to the reducing terminal GlcNAc, clear assignment of the second fucose equivalent was not possible.
[20] See [8a], [8b], also T. de Vries, T. Norberg, H. Lonn, D. H. van den Eijnden, *Eur. J. Biochem.* **1993**, *216*, 769.

Received: October 8, 1999 [F2178]