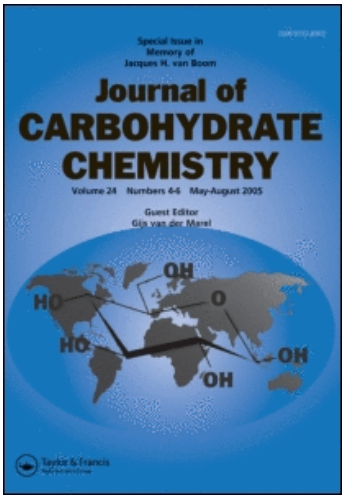

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SYNTHESIS OF TWO TUMOR-ASSOCIATED OLIGOSACCHARIDES: DI- AND TRIFUCOSYLATED PARA-LACTO-*N*-HEXAOSE

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ABSTRACT

Di- and trifucosylated derivatives (21 and 22) of para-lacto-N-hexaose were synthesized. Both structures have been shown to be present in small quantities in human milk, and have also been indicated as tumor-associated antigens. Thioglycoside mono- and disaccharide blocks were used to assemble a properly protected linear hexaose. Di- and trifucosylation at appropriate positions followed by deprotection then gave the desired oligosaccharides.

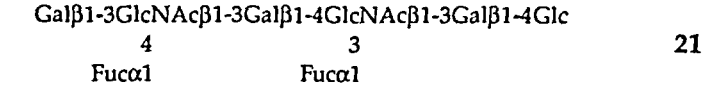
INTRODUCTION

The occurence of complicated oligosaccharides in human milk is well documented. More than 50 different structures,[1] most of them reducing oligosaccharides, have been purified and analyzed. It has become evident, that many milk oligosaccharides have structures similar to the carbohydrate part of cell surface glycoproteins and glycolipids. Interestingly, oligo-

saccharide structures normally associated with tumor cell surfaces occur in milk from healthy donors. For example, the sialyl Le^a and sialyl Le^x epitopes[2,3] are carried by some milk oligosaccharides, as are the dimeric Le^x, trimeric Le^x or Le^a-Le^x epitopes.[4,5] Such polyfucosylated structures occur as the carbohydrate part of glycosphingolipids in the cell membrane of transformed cells, e. g. from adenocarcinoma.[6] The Le^a-Le^x epitope has also been detected on squamous lung carcinoma[7] or testicular carcinoma[8] cells.

If biological studies with tumor-associated oligosaccharides are intended, tumor cells are often an unsatisfactory source of material, because of the small amounts of glycosphingolipids present on each cell, and also because of difficulties in obtaining enough tumor tissue. If the structure is present in human milk this is a better source, even though the abundance here is quite low. For example, approximately 2 mg each of difucosyl-para-lacto-*N*-hexaose (structure **21**,[4] containing the Le^a-Le^x epitope) or the trifucosylated analog (structure **22**,[9] containing the Le^b-Le^x epitope) can be obtained from 1 kg of human milk, and comparable amounts of oligosaccharides in milk carry the dimeric or trimeric Le^x epitope.

An alternative source of tumor-associated oligosaccharide structures is chemical synthesis from the component monosaccharides. We[10,11,12,13] and others[14,15] have previously synthesized derivatives related to the tumor-associated dimeric and trimeric Le^x and Le^y structures. These were synthesized by us in the form of *p*-trifluoroacetamidophenylethyl glycosides. The latter "linker" group was introduced to allow subsequent attachment to proteins or matrixes. We now report the synthesis of di- and trifucosylated para-lacto-*N*-hexaose, **21** and **22**, carrying the Le^a-Le^x and Le^b-Le^x epitope, respectively.



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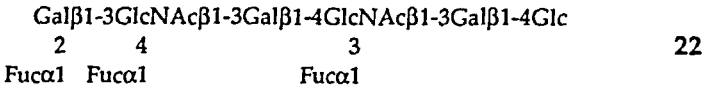

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The reason for synthesizing reducing oligosaccharides (**21** and **22**), instead of linker glycoside derivatives, was that efficient techniques today exist[16,17,18,19]

to convert reducing oligosaccharides into a linkable form, and therefore the linker can be introduced afterwards. Also, these synthetic oligosaccharides could provide synthetic proofs of structure when compared to the isolated structures, something that would be less straightforward with linker glycoside derivatives.

RESULTS AND DISCUSSION

The synthetic strategy chosen for the preparation of **21** and **22** was first to construct, by a stepwise approach starting from the reducing end, the linear type 1/type 2 hexasaccharide benzyl glycoside **16**. This key compound carries appropriate temporary protecting groups at the two intended GlcN fucosylation positions, 3 and 4 respectively, and the terminal Gal 2-position (*p*-methoxybenzyl and allyloxycarbonyl, respectively) and persistent protecting groups (benzyl, *p*-chlorobenzyl, benzoyl, phtalimido or benzylidene) at the other positions. The benzyl glycoside was chosen as protection of the reducing end since the free oligosaccharide was desired after deprotection. Selective removal of the two *p*-methoxybenzyl groups of **16** followed by difucosylation of the resulting diol **17** gave **18**, which was deprotected to give **21**, whereas removal of the allyloxycarbonyl group of **18** and fucosylation of the resulting **19** gave the trifucosylated derivative **20**, which was deprotected to give **22**. The following is an account of the synthetic steps that were performed.

Lactosamine-lactose Tetrasaccharide Synthon

The lactose block, benzyl 2,3,6,2',4',6'-hexa-*O*-benzyl-β-lactoside **2** was synthesized as described[20] from benzyl β-lactoside **1**,[21] which was prepared by debenzoylation of heptabenzoyl benzyl β-lactoside, obtained in turn by reacting benzobromolactose[22] with benzyl alcohol using zinc chloride promotion.[23] Silver imidazolate was not necessary for this reaction. Molecular sieves were sufficient to neutralize the acid produced in the reaction.

Ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[24] was selectively chloroacetylated in the 3-position with chloroacetyl chloride and the

resulting compound was benzoylated with benzoyl chloride to give compound **3** (52% yield). This compound was then converted to the bromide **4** by treatment with bromine (92% yield). Silver triflate promoted condensation of **4** with ethyl 6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside[10] gave the type 2 chain disaccharide block **5** (70% yield).

The obtained blocks, **2** and **5**, were used in a methyl triflate promoted[25] glycosidation reaction which gave the tetrasaccharide glycoside **6** in 83% yield. Removal of the chloroacetyl group of **6** with hydrazine acetate[10] gave the corresponding hydroxy compound **7** (94%).

Type 1 Chain Glucosamine Synthon

Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[25] was treated first with methanolic sodium methoxide, then with *p*-methoxybenzaldehyde dimethyl acetal and *p*-toluenesulfonic acid to give the 4,6-*O*-*p*-methoxybenzylidene acetal **8** (85%). This was acetylated with acetyl chloride/pyridine to give **9** (96%). Opening of the *p*-methoxy-benzylidene acetal of **9** with trimethylsilyl chloride/sodium cyanoborohydride[26] gave the 4-*O*-*p*-methoxybenzyl ether **10** (97%). The regioselectivity of this opening was dependent on the quality of the trimethylsilyl chloride. If used without purification, commercial trimethylsilyl chloride gave predominantly the 6-*O*-benzyl ether, probably because of hydrogen chloride contamination. If the reagent was treated with 3Å molecular sieves before use, the opening with sodium cyanoborohydride gave almost exclusively the 4-*O*-benzyl ether **10**. This compound was benzylated with benzyl bromide and silver oxide in *N*,*N*-dimethylformamide to give **11** (81%), which is a suitable, crystalline type 1 chain glucosamine block with an easily removable acetyl group at the 3-position. It also carries a temporary protecting group at the 4-position, necessary for the intended later fucosylation at this position.

Pentasaccharide Synthon

The lactosamine-lactose tetrasaccharide block **7** was glycosylated with the type 1 chain glucosamine derivative **11**, using dimethyl(methylthio)-sulfonium tetrafluoroborate (DMTSB) promotion,[27] to give the pentasaccharide **12** (83%). Selective alkaline *O*-deacetylation of **12** proved to be troublesome, since **12** contains other alkali-sensitive groups such as benzoyl and phtalimido groups. Among the reagents tried (sodium methoxide, potassium cyanide or magnesium methoxide), magnesium methoxide

showed the best selectivity for acetyl group removal, giving pentasaccharide alcohol **13** in 99% yield, a remarkable yield for this type of reaction. This confirms the usefulness of magnesium methoxide as a selective deacetylation reagent.[28] However, the reason for this superior selectivity remains unclear. It has been suggested,[29] that magnesium methoxide keeps the reaction mixture anhydrous, thereby preventing the formation of hydroxyl ions.

Terminal Galactosyl Synthon

Treatment of ethyl 2-*O*-acetyl-3,4,6-tri-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside[30] with sodium methoxide in methanol/dichloromethane gave **14**, treatment of which with allyl chloroformate in dichloromethane/pyridine containing dimethylaminopyridine gave the glycosyl donor **15** (86%).

Hexasaccharide Synthon

Reaction of **13** with **15**, using methyl triflate promotion, gave the key hexasaccharide **16** in 71% yield. A minor amount (10%) of the α isomer was also obtained.

It should be noted here, that a 2 + 4 thioglycoside block synthesis route to the key hexasaccharide **16** was also tried, starting from tetrasaccharide **7**. This route demands a two-step procedure for the Gal1-3GlcN glycosidation (condensation of the *O*-deacetylated derivative of **11** with **15**) using a non-thiophilic reagent like silver triflate. However, it proved to be difficult to obtain a bromo sugar from **15**, probably because of the reactivity of the allyloxycarbonyl group towards the bromination reagents tried (Br_2/Et_4NBr, DMTSB/Et_4NBr or $CuBr_2$). 2-*O*-Acetylated or 2-*O*-chloroacetylated analogs of **15** gave good yields of glycosyl bromides with bromine, but the subsequent condensation with the *O*-deacetylated derivative of **11** gave poor yields of the desired disaccharide derivatives. The 1 + 1 + 4 route finally chosen for synthesis of **16** gave a greater freedom of choice of glycosylation promotors for this crucial glycosylation, and methyl triflate proved to be the reagent of choice here.

Di- and Trifucosylation

Removal of the two *p*-methoxybenzyl groups in **16** with DDQ[31] provided the diol **17** (60% yield). The low yield was due to oxidative removal of other benzyl ether protective groups as well. Cerium ammonium nitrate[32] gave in

this case an even lower selectivity than DDQ for the removal of the *p*-methoxybenzyl groups.

Mercuric bromide/mercuric cyanide promoted difucosylation of diol **17** with 2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl bromide, prepared in a one-pot operation from ethyl 2,3,4-tri-O-p-chlorobenzyl-1-thio-β-L-fucopyranoside,[30] gave the octasaccharide **18** in 88% yield. The allyloxycarbonyl group of **18** was then removed with tetrakis(triphenylphosphine)-palladium[33] to give the alcohol **19** in 80% yield, fucosylation of which, using the same fucosylation conditions as above, gave **20** in 89% yield. As noted before, for similar reactions,[30] a small amount (10%) of the corresponding β-fucosyl derivative was also formed.

Deprotection

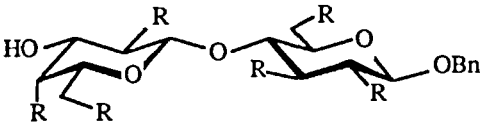
The protected difucosyl octasaccharide **18** was treated successively with hydrazine hydrate (for removal of phthalimido groups), methanolic sodium methoxide (for removal of benzoyl and allyloxycarbonyl groups), acetic anhydride in methanol/dichloromethane (for *N*-acetylation), and hydrogen/palladium on charcoal (for removal of benzyl, benzylidene and *p*-chlorobenzyl groups). In this hydrogenation, the best yields were obtained using athmospheric pressure hydrogen at 60 °C in the presence of a weak anion exchanger (to buffer against liberated acid from hydrogenolysis of *p*-chlorobenzyl groups). The yield of octasaccharide **21** was 81%, the NMR data of which agreed well with those reported[34] for the natural compound.

Similarly, the protected trifucosyl nonasaccharide **20** was subjected to the above deprotection sequence, giving, in 78% yield, nonasaccharide **22**, the NMR data of which agreed well with those reported[9] for the natural compound.

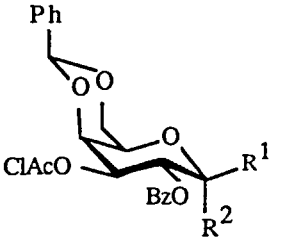
In conclusion, an efficient synthetic route (average yield in each step 84%, all key monosaccharide intermediates crystalline) to oligosaccharides **21** and **22** was developed, providing an independent synthetic source for these important compounds, and verifying the structure of the natural compounds by unambigous synthesis.

EXPERIMENTAL

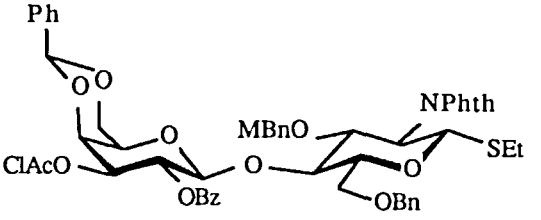
General Methods. Melting points are corrected. Concentrations were performed under reduced pressure at <40 °C bath temperature. Optical



1 R = OH
2 R = OBn



3 R^1 = SEt, R^2 = H
4 R^1 = H, R^2 = Br



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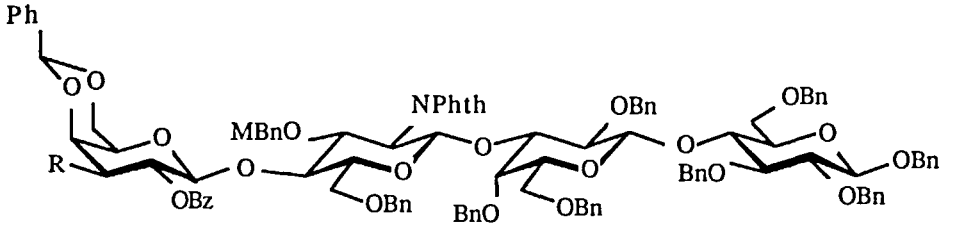
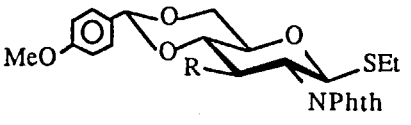
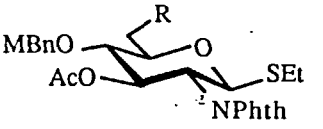


6 R = OClAc
7 R = OH

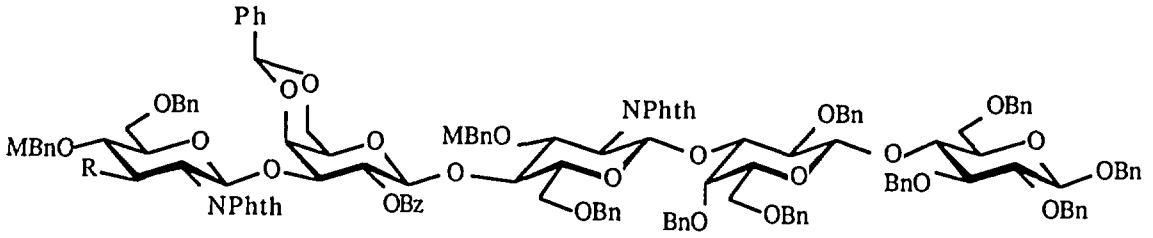


8 R = OH
9 R = OAc



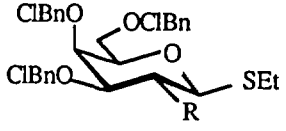
10 R = OH
11 R = OBn

ClAc = chloroacetyl
MBn = p-methoxybenzyl
ClBn = p-chlorobenzyl
AOC = allyloxycarbonyl



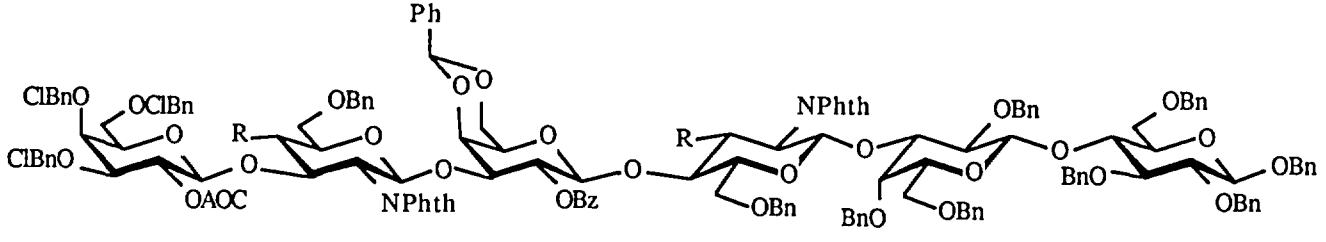
12 R = OAc

13 R = OH



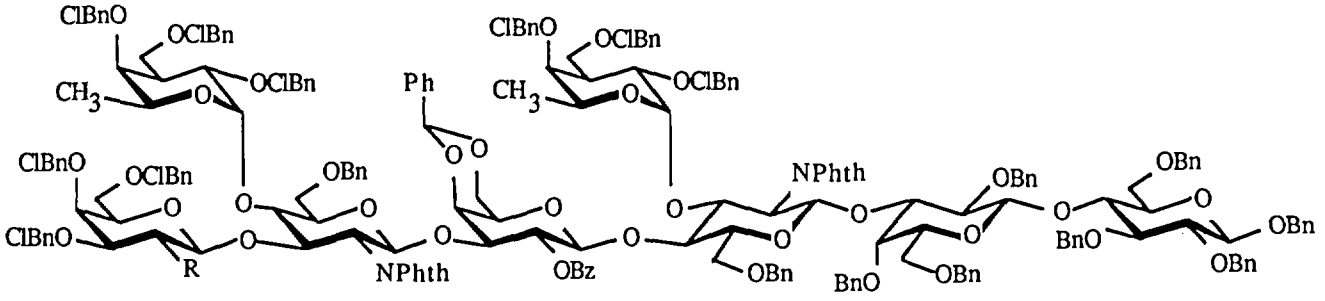
14 R = OH

15 R = OAOC



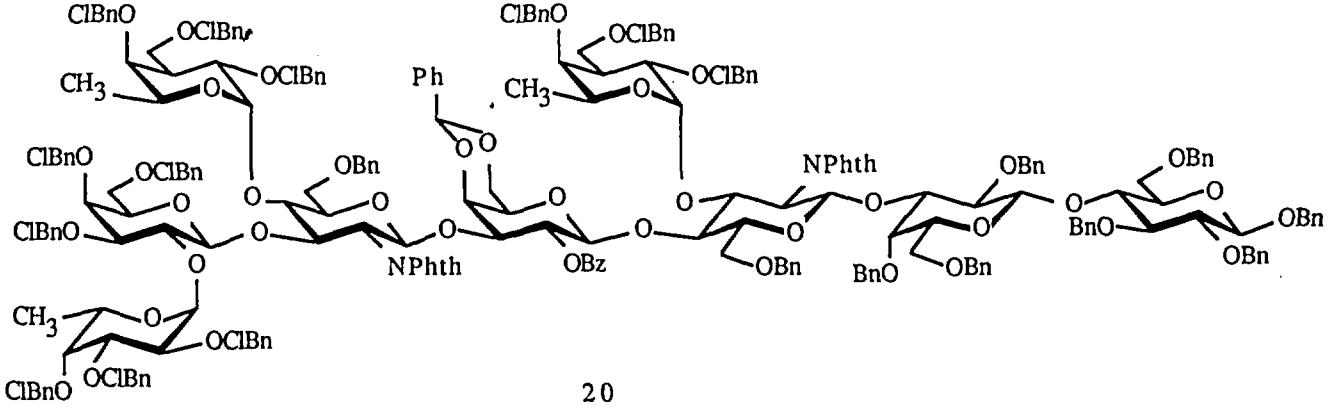
16 R = OMBn

17 R = OH



18 R=OAOC

19 R=OH



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rotations were measured at 25 °C (c=0.5, chloroform) unless otherwise stated, using a Perkin-Elmer 241 Polarimeter. NMR spectra were recorded at 300 K with a Bruker AM 500 instrument. The following reference signals were used: Me_4Si, δ 0.0 (^1H in $CDCl_3$); $CHCl_3$, δ 77.0 (^{13}C in $CDCl_3$); Me_2CO, δ 2.225 (^1H in D_2O); and external dioxan, δ 67.4 (^{13}C in D_2O). Only selected NMR data are reported. Assignments were based on 2 D COSY, J resolved, decoupling, DEPT, NOE and proton-carbon correlation experiments. From the tetrasaccharide **6** on, the sugar residues are named GalA, GlcNA, GalB etc., where A, B and C designate increasing distance from the reducing end. For fucose residues, however, A, B and C designations are arbitrary. For some compounds ^1H shift values and coupling constants (values in parentheses) are given in tabular form. The accuracy of these values are ±0.01 ppm and ±0.2 Hz respectively.The FAB-MS spectra were recorded with a VG ZAB-SE mass spectrometer. The primary beam consisted of xenon atoms with a maximum energy of 8 keV. The samples were dissolved in thioglycerol and the positive ions were extracted and accelerated over a potential of 10 kV. TLC was performed on Silica Gel F_{254} (Merck, Darmstadt, Germany) with detection by UV light and/or by charring with 5% aqueous sulfuric acid. Column chromatography was performed on silica gel (Matrex, 60Å, 20-45 μm or 35-70 μm; Grace, Worms, Germany). Elemental analyses were not obtained for amorphous compounds. These were purified by column chromatography and the purity was ascertained by TLC in at least two different solvent systems and by NMR spectroscopy. Organic solutions were dried over magnesium sulfate. Powdered molecular sieves (3Å or 4Å; Fluka, Buchs, Switzerland) were heated to 300 °C under vaccum overnight. Dichloromethane, DMF, THF, acetonitrile and pyridine were bought puriss.; absolute, over molecular sieve (Fluka, Buchs, Switzerland). Toluene and diethyl ether were dried over sodium wire, and methanol over 3Å molecular sieves. *p*-Methoxybenzaldehyde dimethyl acetal was bought from Lancaster Synthesis, Morecambe, England.

Benzyl *O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (1). Zinc chloride (about 26 g, ≈ 0.19 mol), treated as described,[23] was added to a mixture of *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl bromide[22] (25.8 g, 21.6 mmol), benzyl alcohol (2.92 mL, 28.2 mmol) and 4Å molecular sieves (22 g) in dichloromethane (220 mL). The mixture was refluxed for 15 h, then cooled and filtered through a layer of Celite. The filtrate was washed with cold saturated sodium hydrogen

carbonate. The precipitate formed was filtered off, and the organic phase was washed with water, dried and evaporated to give amorphous material (26.1 g). According to NMR only the β-benzyl glycoside was formed. NMR data ($CDCl_3$): ^{13}C, δ 70.5 (OCH_2Ph), 99.1, 101.0 (C-1,1'); ^1H, δ 3.79 (m, $J_{4,5}$ 9.5 Hz, $J_{5,6a}$ 4.4 Hz, $J_{5,6b}$ 1.8 Hz, H-5), 4.27 (t, $J_{3,4}$ 9.5 Hz, H-4), 4.50 (dd, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.71 (d, $J_{1,2}$ 7.8 Hz, H-1), 4.86 (d, $J_{1',2'}$ 7.9 Hz, H-1'), 5.36 (dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 3.4 Hz, H-3'), 5.53 (dd, $J_{2,3}$ 9.7 Hz, H-2), 5.70 (dd, H-2'), 5.72 (d, H-4'), 5.73 (t, H-3).

This material (26.1 g) was dissolved in methanol (140 mL) and treated with sodium methoxide in methanol (4 mL, 1 M) for 3 h at 40 °C. The mixture was heated and more methanol was added to dissolve all material. The solution was then neutralized with Dowex 50 (H^+) resin, filtered hot and the filtrate was concentrated. The residue was partitioned between water and diethyl ether. Lyophilization of the water phase gave **1** as an amorphous material (9.15 g, 98%). Crystals could be obtained from hot absolute ethanol, mp 176-178 °C, lit.[21] mp 180 °C. NMR data (D_2O): ^1H, δ 3.36 (broad t, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 8.9 Hz, H-2), 3.54 (dd, $J_{1',2'}$ 7.6 Hz, $J_{2',3'}$ 9.8 Hz, H-2'), 3.58 (m, $J_{4,5}$ 9.5 Hz, $J_{5,6a}$ 5.2 Hz, $J_{5,6b}$ 2.1 Hz, H-5), 3.62 (t, $J_{3,4}$ 8.9 Hz, H-3), 3.66 (dd, $J_{3',4'}$ 3.4 Hz, H-3'), 3.66 (broad t, H-4), 3.82 (dd, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.92 (d, H-4'), 3.99 (dd, H-6b), 4.45 (d, H-1'), 4.55 (d, H-1).

Benzyl *O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2). This was synthesized from compound **1** as described.[20]

Ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (3). Chloroacetyl chloride (3.50 mL, 44.0 mmol) in dichloromethane (40 mL) was added dropwise during 1 h to a solution of ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[24] (12.5 g, 40.0 mmol) and 2,4,6-collidine (7.9 mL, 60 mmol) in dichloromethane (200 mL) at -70 °C under a nitrogen atmosphere. The temperature was raised to -40 °C and ice-water was added. The organic phase was washed with cold 2 M sulfuric acid and saturated sodium hydrogen carbonate, dried and concentrated. The residue was precipitated from dichloromethane/light petroleum to give ethyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (9.63 g, 62%).

Benzoyl chloride (10 mL, 86 mmol) was added dropwise to a solution of this material (9.00 g, 23.1 mmol), *N*,*N*-dimethyl-4-aminopyridine (0.35 g, 2.9 mmol) and pyridine (17 mL) in dichloromethane (60 mL) at 0 °C. After 2 h at 0 °C ice-water was added. The organic phase was quickly separated and

washed with cold 1 M sulfuric acid, cold water and cold saturated sodium hydrogen carbonate, dried and concentrated. Crystallization from dichloromethane/light petroleum gave 3 (9.59 g, 84%). Column chromatography (toluene/ethyl acetate, 5/1 by vol) of the mother liquors and crystallization gave more of 3 (0.90 g, 8%), mp 167-168 °C, $[\alpha]_D$ +47°. NMR data ($CDCl_3$): ^{13}C, δ 14.8, 23.0 (SEt), 40.6 (CH_2Cl),67.0, 69.7, 73.5, 74.8 (C-2,3,4,5), 69.1 (C-6), 82.9 (C-1), 101.2 (CHPh), 126.4-137.4 (aromatic C), 165.2, 167.2 (C=O); ^1H, δ 3.65 (broad s, H-5), 4.06 (dd, $J_{5,6a}$ 1.6 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.39 (dd, $J_{5,6b}$ 1.6 Hz, H-6b), 4.50 (broad d, $J_{3,4}$ 3.5 Hz, H-4), 4.65 (d, $J_{1,2}$ 9.8 Hz, H-1), 5.25 (dd, $J_{2,3}$ 9.8 Hz, H-3), 5.76 (t, H-2).

Anal. Calcd for $C_{24}H_{25}ClO_7S$: C, 58.5; H, 5.1; S, 6.5. Found: C, 58.3; H, 5.0; S, 6.4.

2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-galactopyranosyl bromide (4). Bromine (0.196 mL, 4.40 mmol) in dichloromethane (2 mL) was added dropwise during 10 min to a solution of 3 (1.97 g, 4.00 mmol) in dichloromethane (15 mL) at 0 °C. After 15 min tetraethylammonium bromide (0.42 g, 2.0 mmol) was added. The solution was stirred at room temperature for 3 h. Excess bromine was destroyed with cyclohexene (1.0 mL). The solution was washed with cold saturated sodium hydrogen carbonate and water, dried and concentrated. Crystallization from dichloromethane/light petroleum gave 4 (1.88 g, 92%), mp 202-203 °C with decomposition, $[\alpha]_D$ +247°. NMR data ($CDCl_3$): ^{13}C, δ 40.5 (CH_2Cl), 66.9, 67.8, 71.0, 72.8 (C-2,3,4,5), 68.5 (C-6), 89.7 (C-1), 101.0 (CHPh), 126.1-136.9 (aromatic C), 165.2, 167.0 (C=O); ^1H, δ 4.16 (broad s, H-5), 4.35 (dd, $J_{5,6b}$ 2.1 Hz, $J_{6a,6b}$ 13.4 Hz , H-6b), 4.63 (broad d, $J_{3,4}$ 3.5 Hz, H-4), 5.56 (dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.4 Hz, H-2), 5.70 (dd, H-3), 6.93 (d, H-1).

Anal. Calcd for $C_{22}H_{20}BrClO_7$: C, 51.6; H, 3.9. Found: C, 50.6; H, 3.7.

Ethyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (5). A solution of silver triflate (1.04 g, 4.06 mmol) in toluene (13 mL) was added during 15 min to a mixture of 4 (1.80 g, 3.52 mmol), ethyl 6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside[10] (1.53 g, 2.71 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.556 g, 2.71 mmol) and 4Å molecular sieves (4.5 g) in dichloromethane (32 mL) at -40 °C under nitrogen. After 30 min at -40 °C pyridine (1.3 mL) followed by 0.5 M sodium thiosulfate (10 mL) was added. The mixture was filtered through a layer of Celite. The organic phase was washed with

water, cold 1 M sulfuric acid, water and saturated sodium hydrogen carbonate, dried and concentrated. Column chromatography (light petroleum/ethyl acetate, 1/1 by vol) gave **5** as an amorphous material (1.89 g, 70%), $[\alpha]_D$ +20°. NMR data ($CDCl_3$): ^{13}C, δ 14.8, 23.8 (SEt), 40.6 (CH_2Cl), 54.7, 54.8 (C-2, OMe), 66.1, 69.5, 73.2, 73.7, 77.4, 78.0, 78.8, 81.0 (C-1,3,4,5,2',3',4',5'), 67.8, 68.7, 73.4, 74.7 (C-6,6',OCH_2Ph) 100.3, 101.2 (C-1', CHPh), 113.1, 158.5 (aromatic MBn), 123.0-138.3 (aromatic C), 164.7, 167.2 (C=O OBz, OClAc), 167.5, 167.7 (C=O NPhth); ^1H, δ 3.34 (broad s, H-5'), 3.37 (m, $J_{4,5}$ 9.8 Hz, $J_{5,6a}$ 1.5 Hz, $J_{5,6b}$ 3.0 Hz, H-5), 4.16 (dd, $J_{3,4}$ 8.5 Hz, H-4), 4.18 (t, $J_{1,2} = J_{2,3}$ 10.4 Hz, H-2), 4.27 (dd, H-3), 4.81 (d, $J_{1',2'}$ 7.9 Hz, H-1'), 5.04 (dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 3.7 Hz, H-3'), 5.13 (d, H-1), 5.66 (dd, H-2').

Benzyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galacto-pyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galacto-pyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). Methyl triflate (0.73 mL, 6.7 mmol) was added to a stirred mixture of **5** (2.07 g, 2.08 mmol), **2** (1.62 g, 1.67 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.291 g, 1.42 mmol) and 4 Å molecular sieves (6.2 g) in diethyl ether (46 mL) at room temperature. After 13 h triethylamine (3.5 mL) was added and the mixture was stirred for 2 h. The mixture was filtered through Celite. Concentration and column chromatography (toluene/ethyl acetate, 6/1 by vol) of the filtrate gave the tetrasaccharide **6** (2.63 g, 83%), $[\alpha]_D$ -11°. NMR data ($CDCl_3$): ^{13}C, δ 40.6 (CH_2Cl), 54.7 (OMe), 56.4 (C-2 GlcNA), 67.7, 68.2 (C-6 Glc, C-6 GalA), 67.9 (C-6 GlcNA), 68.7 (C-6 GalB), 99.6 (C-1 GlcNA), 100.5 (C-1 GalB), 101.2 (CHPh), 102.4 (C-1 Glc, C-1 GalA), 113.0, 158.4 (aromatic MBn), 164.7 (C=O OBz), 167.2 (C=O OClAc), 167.4, 167.7 (C=O NPhth); ^1H NMR data are shown in Table 1.

Benzyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-*D*-gluco-pyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7). A solution of **6** (2.70 g, 1.42 mmol) and hydrazine acetate[10] (0.39 g, 4.3 mmol) in ethyl acetate/methanol (50 mL, 1/1 by vol) was stirred at room temperature overnight and then concentrated. The residue was partitioned between toluene and water. The organic phase was dried and concentrated. Column chromatography (toluene/ethyl acetate, 3/1 by vol) gave **7** (2.44 g, 94%), $[\alpha]_D$ -28°. NMR data ($CDCl_3$): ^{13}C, δ 54.7 (OMe), 56.5 (C-2 GlcNA), 99.6 (C-1 GlcNA), 100.4 (C-1 GalB), 101.5 (CHPh), 102.4 (C-1

Table 1. ^{1}H NMR Spectral Data for Compound 6.

	H-1 (J1,2)	H-2 (J2,3)	H-3 (J3,4)	H-4 (J4,5)	H-5 (J5,6a)	H-6a (J5,6b)	H-6b (J6a,6b)
Glc	4.29 (7.6)	3.35	3.32 (8.5)	3.81 (9.8)	2.90	3.30	3.46
GalA	4.21 (7.3)	3.40 (9.7)	3.44	3.93	3.29	3.30	3.46
GlcNA	5.28 (8.5)	4.21 (10.7)	4.35 (8.5)	4.17 (10.1)	3.39 (1.4)	3.50 (3.3)	3.74 (11.3)
GalB	4.84 (8.0)	5.67 (10.4)	5.07 (3.7)	4.38 (1.8)	3.37	3.99	4.36

Glc, C-1 GalA), 113.1, 158.4 (aromatic MBn), 165.8 (C=O OBz); ^{1}H, δ 3.74 (m, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.9 Hz, $J_{3,OH}$ 11.3 Hz, H-3 GalB), 5.38 (dd, $J_{1,2}$ 8.0 Hz, H-2 GalB).

Ethyl 2-deoxy-4,6-*O*-*p*-methoxybenzylidene-2-phthalimido-1-thio-β-D-glucopyranoside (8). Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[25] (20.0 g, 41.7 mmol) in dichloromethane/methanol (100 mL, 3/2 by vol) was treated with sodium methoxide in methanol (20 mL, 0.2 M). After 2 h the solution was neutralized with Dowex 50 (H^+) resin, filtered and concentrated.

The residue was dissolved together with *p*-methoxybenzaldehyde dimethyl acetal (15.2 g, 83.4 mmol) and p-toluenesulfonic acid monohydrate (1.59 g, 8.34 mmol) in DMF (75 mL). The solution was stirred at 40 °C and a pressure of 30 mm Hg for 4 h. The solution was partitioned between dichloromethane and saturated sodium hydrogen carbonate. The organic layer was washed with water, dried, concentrated and co-evaporated with xylene. Column chromatography (toluene/ethyl acetate, 5/1 by vol) of the residue gave 8 as an amorphous powder (16.7 g, 85%), $[\alpha]_D$ -12°. NMR data ($CDCl_3$): ^{13}C, δ 14.8, 24.2 (SEt), 55.3, 55.4 (C-2, OMe), 68.6 (C-6), 69.6, 70.4, 81.8, 82.1 (C-1,3,4,5), 101.9 (CHPh), 113.7, 160.3 (aromatic MBn), 123.3-134.2 (aromatic C), 167.7, 168.2 (C=O); ^{1}H, δ 3.58 (broad t, $J_{3,4}$ 9.5 Hz, $J_{4,5}$ 9.2 Hz, H-4), 3.68 (m, $J_{5,6a}$ 9.8Hz, $J_{5,6b}$ 4.9 Hz, H-5), 3.79 (broad t, $J_{6a,6b}$ 10.4 Hz, H-6a), 4.31 (broad t, $J_{1,2}$ 10.4 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.37 (dd, H-6b), 4.64 (m, $J_{3,OH}$ 3.4 Hz, H-3), 5.40 (d, H-1).

Ethyl 3-*O*-acetyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-2-phthalimido-1-thio-β-D-glucopyranoside (9). A solution of acetyl chloride (4.55 mL, 63.6

mmol) in dichloromethane (50 mL) was added dropwise to a solution of **8** (15.0 g, 31.8 mmol) and pyridine (5.0 mL) in dichloromethane (50 mL) at 0 °C. After 1 h at 0 °C ice-water was added. The organic phase was washed with cold 1 M sulfuric acid and saturated sodium hydrogen carbonate, dried and concentrated. Crystallization from ethyl acetate/light petroleum gave **9** (15.7 g, 96%), mp 186-187 °C, $[\alpha]_D$ -11°. NMR data ($CDCl_3$): ^{13}C, δ 14.9, 24.4 (SEt), 20.6 (CH_3CO), 54.3, 55.3 (C-2, OMe), 68.6 (C-6), 70.5, 70.6, 79.2, 81.7 (C-1,3,4,5), 101.6 (CHPh), 113.6, 160.2 (aromatic MBn), 123.6-134.4 (aromatic C), 167.4, 167.8 (C=O NPhth), 170.1 (C=O OAc); ^1H, δ 3.80 (m, H-4,5,6a), 4.36 (broad t, $J_{1,2}$ 10.7 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.40 (m, H-6b), 5.58 (d, H-1), 5.91 (broad t, $J_{3,4}$ 8.9 Hz, H-3).

Anal. Calcd for $C_{26}H_{27}NO_8S$: C, 60.8; H, 5.3; N, 2.7; S, 6.2. Found: C, 60.6; H, 5.2; N,2.8; S, 6.2.

Ethyl 3-*O*-acetyl-2-deoxy-4-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (10). A solution of **9** (2.75 g, 5.36 mmol) and sodium cyanoborohydride (2.0 g, 32 mmol) in acetonitrile (75 mL) was stirred with 3 Å molecular sieves (12.5 g) at room temperature for 45 min and then cooled to 0 °C. Trimethylsilyl chloride (8.10 mL, 63.8 mmol) in acetonitrile (60 mL) was stirred for 15 min with 3Å molecular sieves (6.25 g). The molecular sieves were then allowed to sediment and half of the solution was added dropwise to the reaction solution at 0 °C during 30 min. The cooling bath was removed 15 min later and after 20 h at room temperature the mixture was filtered through a layer of Celite. The filtrate was neutralized with cold saturated sodium hydrogen carbonate and concentrated to about half its volume. Dichloromethane was added and the organic phase was washed with water, dried and evaporated. The residue was purified by column chromatography (dichloromethane/acetone, 10/1 by vol) and crystallization from dichloromethane/light petroleum to give **10** (2.68 g, 97%), mp 164-165 °C, $[\alpha]_D$ +26°. NMR data ($CDCl_3$): ^{13}C, δ 14.9, 24.4 (SEt), 20.6 (CH_3CO), 54.2, 55.2 (C-2, OMe), 61.8 (C-6), 73.9, 75.8, 79.4, 81.0 (C-1,3,4,5), 74.3 (OCH_2Ph), 113.9, 159.4 (aromatic MBn), 123.5-134.3 (aromatic C), 167.5, 167.7 (C=O NPhth), 170.0 (C=O OAc); ^1H, δ 3.63 (m, $J_{4,5}$ 9.8 Hz, $J_{5,6a}$ 4.0 Hz, $J_{5,6b}$ 2.4 Hz, H-5), 3.77 (m, H-4,6a), 3.95 (m, $J_{6b,OH}$ 4.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 4.25 (t, $J_{1,2} = J_{2,3}$ 10.4 Hz, H-2), 5.52 (d, H-1), 5.82 (dd, $J_{3,4}$ 9.0 Hz, H-3).

Anal. Calcd for $C_{26}H_{29}NO_8S$: C, 60.6; H, 5.7; N, 2.7; S, 6.2. Found: C, 60.0; H, 5.5; N, 2.6; S, 6.2.

Ethyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (11). Freshly prepared silver oxide[35] (17 g, 73

mmol) was added in 5 portions at 10 minute intervals to a solution of **10** (6.75 g, 13.1 mmol) and benzyl bromide (17 mL, 143 mmol) in dry DMF (39 mL). The mixture was stirred in the dark for 24 h and then filtered through a layer of Celite. Pyridine (20 mL) was added to the filtrate. After stirring for 2 h the filtrate was diluted with dichloromethane and 0.5 M aqueous sodium thiosulfate. A small amount of precipitated material was filtered off, and the organic layer was washed with water, cold 1 M sulfuric acid, water and saturated sodium hydrogen carbonate. Drying and concentration gave a residue which was purified by column chromatography (toluene/ethyl acetate, 10/1 by vol) and crystallization from dichloromethane/light petroleum to give **11** (6.42 g, 81%), mp 121-122 °C, $[\alpha]_D$ +21°. NMR data ($CDCl_3$): ^{13}C, δ 15.0, 24.2 (SEt), 20.6 (CH_3C=O), 54.3, 55.3 (C-2, OMe), 68.7, 73.5, 74.1 (C-6, OCH_2Ph), 74.1, 76.1, 79.2 (C-3,4,5), 80.8 (C-1), 113.8, 159.3 (aromatic MBn), 123.5-138.2 (aromatic C), 167.5, 167.7 (C=O NPhth), 170.0 (C=O OAc); ^1H, δ 3.71 (m, H-5), 3.79 (m, H-6a,6b), 4.30 (t, $J_{1,2} = J_{2,3}$ 10.4 Hz, H-2), 5.47 (d, H-1), 5.80 (dd, $J_{3,4}$ 8.9 Hz, H-3).

Anal. Calcd for $C_{33}H_{35}NO_8S$: C, 65.4; H, 5.8;.N, 2.3; S, 5.3. Found: C, 65.0; H, 5.7;.N, 2.2; S, 5.2.

Benzyl *O*-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galacto-pyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12). Dimethyl-(methylthio)sulfonium tetrafluoroborate[36] (0.71 g, 3.6 mmol) was added to a stirred mixture of **7** (3.30 g, 1.80 mmol), **11** (1.37 g, 2.25 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (334 mg, 1.62 mmol) and 4Å molecular sieves (6.7 g) in dichloromethane (30 mL). After stirring overnight at room temperature saturated sodium hydrogen carbonate and ethyl acetate was added. The mixture was filtered through a layer of Celite. The organic phase was dried and concentrated. Column chromatography (toluene/dichloromethane/ethyl acetate, 4/2/1.25 by vol) of the residue gave pentasaccharide **12** (3.57 g, 83%), $[\alpha]_D$ -23°. NMR data ($CDCl_3$): ^{13}C, δ 20.5 (CH_3CO), 54.7, 55.2 (OMe), 54.8 (C-2 GlcNB), 56.3 (C-2 GlcNA), 67.6, 68.3 (C-6 Glc, C-6 GalA), 67.6 (C-6 GlcNA), 68.6 (C-6 GalB), 69.6 (C-6 GlcNB), 99.5 (C-1 GlcNB), 99.6 (C-1 GlcNA), 100.4 (C-1 GalB), 101.0 (CHPh), 102.3 (C-1 GalA), 102.4 (C-1 Glc), 112.9, 113.9, 158.2, 159.4 (aromatic MBn), 164.1 (C=O OBz), 167.3-167.6 (C=O NPhth), 170.0 (C=O OAc); ^1H NMR data are shown in Table 2.

Table 2. ^{1}H NMR Spectral Data for Compound 12.

	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)
Glc	4.27 (7.6)	3.34	3.30	3.78 (9.9)	2.87	3.26	3.43
GalA	4.16 (7.7)	3.35	3.34	3.87	3.25	3.26	3.43
GlcNA	5.15 (8.2)	4.13 (10.7)	4.18 (8.0)	4.04 (9.9)	3.13	3.28 (3.0)	3.55 (11.0)
GalB	4.58 (8.0)	5.38 (10.1)	3.68 (3.7)	4.39	3.21	3.74	4.25
GlcNB	5.53 (8.4)	4.24 (10.6)	5.67 (8.9)	3.66 (9.7)	3.82	3.71	3.83

Benzyl *O*-(6-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galacto-pyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galacto-pyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13). A freshly prepared solution of magnesium methoxide in methanol[37] (9.0 mL, 10% w/v) was added to a solution of compound **12** (1.49 g, 0.628 mmol) in THF (4.5 mL). After 17 h at room temperature the mixture was ice-cold and neutralized with acetic acid (1.5 mL). The mixture was partitioned between toluene and water. The organic phase was washed with saturated sodium hydrogen carbonate, dried and concentrated to give **13** (1.45 g, 99%), $[\alpha]_D$ -27°. NMR data ($CDCl_3$): ^{13}C, δ 54.7, 55.2 (OMe), 56.3 (C-2 GlcNA, C-2 GlcNB), 99.6 (C-1 GlcNA), 99.8 (C-1 GlcNB), 100.5 (C-1 GalB), 100.9 (CHPh), 102.3 (C-1 GalA), 102.4 (C-1 Glc), 112.9, 114.0, 158.2, 159.5 (aromatic MBn), 164.0 (C=O OBz), 167.4-167.7 (C=O NPhth); ^{1}H, δ 4.31 (m, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 8.5 Hz, $J_{3,OH}$ 5.2 Hz, H-3 GlcNB), 5.39 (dd, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.1 Hz, H-2 GalB).

Ethyl 3,4,6-tri-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside (14). A solution of ethyl 2-*O*-acetyl-3,4,6-tri-*O*-*p*-chlorobenzyl-1-thio-β-D-galacto-pyranoside[30] (4.00 g, 6.25 mmol) in methanol/dichloromethane (25 mL, 3/2 by vol) was treated with sodium methoxide in methanol (8 mL, 0.2 M) over-night, neutralized with Dowex 50 (H^+) resin, filtered and concentrated. The residue was crystallized from diethyl ether/light petroleum to give **14** (3.46 g, 93%), mp 114-116 °C, $[\alpha]_D$ -21°. NMR data ($CDCl_3$): ^{13}C, δ 15.3, 24.2 (SEt), 68.4,

71.9, 72.7, 73.8 (C-6, OCH_2Ph), 69.9, 73.9, 77.4, 83.1, 86.6 (C-1,2,3,4,5), 128.3-137.0 (aromatic C); ^1H, δ 3.44 (dd, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 2.9 Hz, H-3), 3.58 (m, H-6a, H-6b), 3.62 (m, H-5), 3.90 (d, H-4), 3.95 (m, $J_{1,2}$ 9.4 Hz, $J_{2,OH}$ 1.6 Hz, H-2), 4.30 (d, H-1).

Anal. Calcd for $C_{29}H_{31}Cl_3O_5S$: C, 58.2; H, 5.2; S, 5.4. Found: C, 57.8; H, 5.2; S, 5.7.

Ethyl 2-*O*-allyloxycarbonyl-3,4,6-tri-*O*-*p*-chlorobenzyl-1-thio-β-D-galactopyranoside (15). Allyl chloroformate (9.6 mL, 90 mmol) was added dropwise during 40 min to a stirred solution of **14** (3.6 g, 6.0 mmol) and *N,N*-dimethyl-4-aminopyridine (72 mg, 0.60 mmol) in pyridine/dichloromethane (22 mL, 2/1 by vol) at 0 °C. The mixture was allowed to attain room temperature slowly overnight. The mixture was diluted with dichloromethane, and ice-water was added. The organic phase was washed with water, cold 1 M sulfuric acid, water and saturated sodium hydrogen carbonate solution, dried and concentrated. Column chromatography (toluene/ethyl acetate, 9/1 by vol) gave **15** (3.82 g, 93%) and unreacted **14** (0.22 g, 6%). Crystals (3.78 g) of **15** were obtained from methanol, mp 56-57 °C, $[\alpha]_D$ -4°. NMR data ($CDCl_3$): ^{13}C, δ 14.8, 23.9 (SEt), 68.2, 68,7, 71.6, 72.7, 73.7 (C-6, OCH_2Ph, $OCH_2CH{=}CH_2$), 73.4, 73.9, 77.1, 81.6, 83.5 (C-1,2,3,4,5), 118.8 ($OCH_2CH{=}CH_2$),128.3-136.8 (aromatic C), 131.4 ($OCH_2CH{=}CH_2$), 154.2 (C=O); ^1H, δ 3.58 (m, H-3,5,6a,6b), 3.94 (d, $J_{3,4}$ 2.8 Hz, H-4), 4.40 (d, $J_{1,2}$ 9.9 Hz, H-1), 5.17 (t, $J_{2,3}$ 9.9 Hz, H-2).

Anal. Calcd for $C_{33}H_{35}Cl_3O_7S$: C, 58.1; H, 5.2; S, 4.7. Found: C, 57.9; H, 5.1; S, 4.8.

Benzyl *O*-(2-*O*-allyloxycarbonyl-3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-galactopyranosyl)-(1→3)-*O*-(6-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16). Methyl triflate (0.13 mL, 1.2 mmol) was added to a stirred mixture of **15** (0.342 g, 0.501 mmol), **13** (0.900 g, 0.386 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (71 mg, 0.35 mmol) and 4 Å molecular sieves (2.0 g) in dichloromethane (10 mL) at room temperature. After 13 h piperidine (0.35 mL) was added and the mixture was stirred for 30 min. The mixture was filtered through Celite. The organic layer was washed with water, dried and concentrated. Column chromatography (toluene/dichloromethane/ethyl acetate, 4/2/1 by vol) gave hexasaccharide **16** (814 mg, 71%), $[\alpha]_D$ -15°. NMR data ($CDCl_3$): ^{13}C, δ 54.7, 55.3 (OMe), 55.3 (C-2 GlcNB), 56.3 (C-2 GlcNA), 99.4 (C-1 GlcNB), 99.6 (C-1 GlcNA),

Table 3. ^{1}H NMR Spectral Data for Compound **16**.

	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)
Glc	4.28	3.34	3.30	3.78	2.88	3.26	3.43
	(7.5)	(9.2)	(8.2)	(9.8)	(1.8)	(3.8)	(10.9)
GalA	4.15	3.35	3.32	3.86	3.25	3.25	3.37
	(7.1)	(9.9)	(3.7)				
GlcNA	5.14	4.12	4.16	4.01	3.10	3.23	3.50
	(8.2)	(10.7)	(8.2)	(9.8)			
GalB	4.51	5.30	3.65	4.34	3.19	3.75	4.23
	(7.9)	(10.1)	(3.5)	(1.6)			
GlcNB	5.12	4.23	4.57	3.47	3.69	3.69	3.84
	(8.3)	(10.9)	(8.8)				
GalC	3.93	4.93	2.94	3.74			
	(7.9)	(10.0)	(2.9)				

100.5 (C-1 GalB), 100.7 (CHPh), 100.8 (C-1 GalC), 102.3 (C-1 GalA), 102.4 (C-1 Glc), 112.9, 113.4, 158.2, 159.1 (aromatic MBn), 118.8 ($OCH_2CH{=}CH_2$), 154.2 (C=O OAOC), 164.0 (C=O OBz), 167.3, 167.6 (C=O NPhth); ^{1}H NMR data are shown in Table 3.

Also isolated (118 mg, 10%) from the column chromatography was a substance, which according to its NMR data was the α-isomer of **16**.

Benzyl *O*-(2-*O*-allyloxycarbonyl-3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-galactopyranosyl)-(1→3)-*O*-(6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (17). A mixture of **16** (2.68 g, 0.910 mmol), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.488 g, 2.15 mmol) in dichloromethane/water (54 mL, 18/1 by vol) was stirred at room temperature for 22 h. The mixture was diluted with dichloromethane and saturated sodium hydrogen carbonate. The organic phase was washed with water, dried and concentrated. Column chromatography (toluene/dichloromethane/ethyl acetate, 4/2/1.6 by vol) gave **17** (1.47 g, 60%), $[\alpha]_D$ -16°. NMR data ($CDCl_3$): ^{13}C, δ 54.5, 56.5 (C-2 GlcNA, C-2 GlcNB), 99.4, 99.4, 100.4, 101.1, 101.4, 102.3, 102.4 (6 C-1 and CHPh), 118.5 ($OCH_2CH{=}CH_2$), 153.7 (C=O OAOC), 164.0 (C=O OBz), 166.5, 167.7, 167.8, 168.8 (C=O NPhth).

Benzyl *O*-(2-*O*-allyloxycarbonyl-3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-galactopyranosyl)-(1→3)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (18). Bromine (0.16 mL, 3.5 mmol) was added to a stirred solution of ethyl 2,3,4-tri-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside[30] (1.87 g, 3.21 mmol) in dichloromethane (13 mL) at room temperature. After 15 min cyclohexene (0.45 mL) was added, and after additional stirring for 10 min the solution was added to a mixture of **17** (1.45 g, 0.535 mmol), mercury(II) cyanide (507 mg, 2.01 mmol), mercury(II) bromide (434 mg, 1.20 mmol) and 4Å molecular sieves (8.0 g) in dichloromethane (8 mL). After 2 h pyridine (0.60 mL) was added and after 30 min additional stirring the mixture was filtered through a layer of Celite. The filtrate was diluted with toluene and washed with 0.5 M sodium iodide, 2 M sulfuric acid, water and saturated sodium hydrogen carbonate, dried and concentrated. The residue was chromatographed (toluene/dichloromethane/ethyl acetate, 4/2/0.75 by vol) to give octasaccharide **18** (1.78 g, 88%), $[\alpha]_D$ -62°. NMR data ($CDCl_3$): ^{13}C, δ 15.8, 16.2 (C-6 FucA, C-6 FucB), 55.7, 56.9 (C-2 GlcNA, C-2 GlcNB), 97.0, 97.5 (C-1 FucA, C-1 FucB), 99.3, 99.6, 99.7, 99.8, 100.7, 102.4, 102.5 (6 C-1 and CHPh), 118.7 ($OCH_2CH{=}CH_2$), 154.0 (C=O OAOC), 163.5 (C=O OBz); ^1H NMR data are shown in Table 4.

Benzyl *O*-(3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-galactopyranosyl)-(1→3)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (19). A solution of **18** (500 mg, 0.133 mmol) and tetrakis(triphenylphosphine)palladium (46 mg, 40 μmol) in THF/water (11 mL, 10/1 by vol) was refluxed for 1 h. The mixture was filtered and the filtrate was concentrated and co-evaporated with ethanol and toluene. Column chromatography (light petroleum/ethyl acetate, 1.5/1 by vol) of the residue gave amorphous **19** (431 mg, 80%), $[\alpha]_D$ -76°. NMR data ($CDCl_3$): ^{13}C, δ 15.8, 16.4 (C-6 FucA, C-6 FucB), 55.9, 56.9 (C-2 GlcNA, C-2 GlcNB), 97.0, 97.4 (C-1 FucA, C-1

Table 4. ^{1}H NMR Spectral Data for Compound 18.

	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)
Glc	4.28	3.35	3.30	3.81	2.85	3.22	3.42
	(7.5)	(9.4)	(8.6)			(3.8)	(11.0)
GalA	4.17	3.34	3.30	3.89			
	(7.4)	(9.7)					
GlcNA	5.05	4.38	4.57	4.07	3.03	3.27	3.72
	(8.2)	(10.9)	(8.9)	(9.9)			
GalB	4.52	5.17	3.54	4.29	3.14	3.83	4.24
	(8.0)	(10.0)	(3.8)				
GlcNB	5.12	4.29	4.57	3.84	3.66	3.73	3.81
	(8.2)	(10.9)					
GalC	3.89	4.79	2.83	3.81			
	(8.0)	(10.0)	(2.9)				
FucA	4.91	3.89	3.71	3.06	4.54	1.10	
	(4.0)	(10.2)	(2.8)		(6.5)		
FucB	4.51	3.38	3.73	3.01	4.64	0.92	
	(3.7)	(10.3)	(3.2)		(6.5)		

FucB), 99.3, 99.6, 99.7, 99.7, 102.4, 102.5, 103.0 (6 C-1 and CHPh), 163.5 (C=O OBz), 168.2 (C=O NPhth).

Benzyl *O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-galactopyranosyl)-(1→3)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (20). Bromine (15 μL, 0.34 mmol) was added to a stirred solution of ethyl 2,3,4-tri-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside[30] (179 mg, 0.306 mmol) in dichloromethane (3.0 mL) at room temperature. After 15 min cyclohexene (45 μL) was added, and after additional stirring for 10 min the solution was added to a mixture of **19** (375 mg, 0.102 mmol), mercury(II) cyanide (48 mg, 0.19 mmol), mercury(II) bromide (41 mg, 0.11 mmol) and 4Å molecular sieves (2.0 g) in dichloromethane (2 mL). After 2 h pyridine (0.15 mL) was added and after 30 min additional stirring the mixture was filtered through a layer of Celite. The filtrate was diluted with toluene and washed with 0.5 M

Table 5. ^{1}H NMR Spectral Data for Compound 20.

	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)
Glc	4.29	3.35	3.30	3.82	2.86	3.23	3.42
	(7.5)	(9.4)	(8.3)	(10.3)		(3.8)	(11.0)
GalA	4.17	3.34	3.30	3.88			
	(7.2)	(9.7)					
GlcNA	5.05	4.38	4.57	4.08	3.03	3.29	3.70
	(8.1)	(10.6)	(9.1)	(9.7)			
GalB	4.55	5.11	3.73	4.25	3.16	3.90	4.28
	(8.1)	(10.0)					
GlcNB	5.08	4.19	4.98	3.78	3.64	3.78	3.93
	(8.2)	(11.0)	(9.0)				
GalC	4.01	3.85	3.13	3.78			
	(7.7)	(9.9)	(3.1)				
FucA	5.39	3.89	4.03	3.94	4.42	1.27	
	(4.0)	(10.4)	(2.7)		(6.7)		
FucB	4.96	3.92	3.72	2.97	4.45	1.17	
	(3.7)				(6.4)		
FucC	4.51	3.38	3.72	2.92	4.59	0.76	
	(3.8)	(10.4)			(6.4)		

sodium iodide, 2 M sulfuric acid, water and saturated sodium hydrogen carbonate, dried and concentrated. The residue was chromatographed (isooctane/toluene/ethyl acetate, 1/1/1 by vol) to give nonasaccharide **20** (380 mg, 89%), $[\alpha]_D$ -81°. NMR data ($CDCl_3$): ^{13}C, δ 15.4, 16.2, 16.2 (3 C-6 Fuc), 56.6 (C-2 GlcNB), 56.9 (C-2 GlcNA), 97.0 (C-1 FucC), 97.9 (C-1 FucB), 98.5 (C-1 FucA), 98.8 (C-1 GlcNB), 99.6 (C-1 GlcNA, CHPh), 99.7 (C-1 GalB), 100.3 (C-1 GalC), 102.4 (C-1 Glc), 102.5 (C-1 GalA), 163.6 (C=O OBz), 167.3, 168.8 (C=O NPhth); ^{1}H NMR data are shown in Table 5.

Also isolated (43 mg, 10%) from the column chromatography was a substance, which according to its NMR data was the β-isomer of **20**.

***O*-(β-D-Galactopyranosyl)-(1→3)-*O*-[2-acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (21).** A mixture of **18** (355 mg, 0.0947 mmol) and hydrazine monohydrate (4.0 mL) in 90% aqueous ethanol/dioxane (25 mL, 3/1 by vol) was refluxed overnight. After cooling the

mixture was concentrated and co-evaporated three times with ethanol and toluene. The residue was dissolved in methanol/dichloromethane (10 mL, 2/1 by vol) and treated with sodium methoxide in methanol (1.5 mL, 1 M) at reflux overnight. The solution was cooled, neutralized with acetic acid (120 μL) and evaporated. The residue was partitioned between toluene and water, and the organic phase was concentrated and co-evaporated with ethanol and toluene to dispose of water residues. The residue was dissolved in methanol/dichloromethane (10 mL, 1/1 by vol) and treated with acetic anhydride (1.0 mL) at room temperature. After 2 h, the solution was concentrated and co-evaporated three times with ethanol and xylene. Column chromatography (toluene/ethyl acetate/ethanol, 7/5/1 by vol) gave amorphous material (283 mg).

A solution of this material (283 mg) in ethyl acetate/ethanol/water (25 mL, 2/4/1 by vol), containing Amberlite IRA-93 (564 mg dry resin), was hydrogenated over Pd/C (10%, 170 mg) at 60 °C and athmospheric pressure for 20 h. The solution was filtered and the filter mass was washed with hot ethanol and water. The filtrate was concentrated and purified on a Bio-Gel P-4 column, using water as eluent. Concentration and lyophilization gave compound **21** (105 mg, 81%), $[\alpha]_D$ -52° (c=0.5, H_2O). The ^1H NMR spectrum of **21** in D_2O agreed with the spectrum of material isolated from human milk. The identity was also verified by HPLC (column:Nucleosil C-18, eluent:water, detection: refraction index). The purity of compound **21** was more than 95% according to NMR.

The FAB-MS spectrum of **21** showed an $[M+Na]^+$ ion of m/z 1387. (The nuclide mass sum of **21** is 1364.5.)

***O*-(α-L-Fucopyranosyl)-(1→2)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[2-acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (22).** Compound **20** (329 mg, 0.0786 mmol) was successively treated with hydrazine monohydrate (5.0 mL) in 90% aqueous ethanol/dioxane (30 mL, 2/1 by vol), sodium methoxide (2.0 mL, 1 M) in dioxane/methanol (8 mL, 3/2 by vol), neutralized with acetic acid (230 μL) and acetylated with acetic anhydride (1.0 mL) in methanol/dichloromethane (10 mL, 1/1 by vol) as described for compound **21**. Column chromatography (toluene/ethyl acetate/ethanol, 7/5/1 by vol) gave amorphous material (260 mg).

This material (260 mg) was hydrogenated in ethyl acetate/ethanol/water (26 mL, 2/4/1 by vol), containing Amberlite IRA-93 (600 mg dry resin), over Pd/C (10%, 156 mg) and purified as described for compound **21**. Lyophilization gave compound **22** (93 mg, 78%), $[\alpha]_D$ -71° (c=0.5, H_2O). The ^1H NMR spectrum of **22** in D_2O agreed with the spectrum of material isolated from human milk. The identity was also verified by HPLC (column:Nucleosil C-18, eluent:water, detection: refraction index). The purity of compound **22** was more than 95% according to NMR.

The FAB-MS spectrum of **22** showed an $[M+Na]^+$ ion of m/z 1534. (The nuclide mass sum of **22** is 1510.6.)

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