CHEMISTRY
A EUROPEAN JOURNAL

Synthesis and Evaluation of 5-Thio-L-Fucose-Containing Oligosaccharide

Masayuki Izumi,*[a, d] Osamu Tsuruta,[a] Yasuhiro Kajihara,[b] Shin Yazawa,[c] Hideya Yuasa,[a] and Hironobu Hashimoto*[a]

Abstract: 5-Thio-L-fucose-containing trisaccharide H-type II was synthesized. The 3′,4′-*O*-isopropylidene-2-azido-2-deoxylactoside derivative, which was prepared from lactose by azidonitration of lactal, was used as a starting material. By regio- and stereoselective 5-thio-L-fucosylation of the 6,6′-dibenzoate **5** with 5-thiofucosyl trichloroacetimidate **6** and subsequent deprotection gave the 5-thio-L-fucose-containing H-type II **1**. Conformational analysis of the 5-thio-L-fucose-containing H-type II and the native H-type II was carried out through NOESY experiments. The observed NOE values between *N*-acetylglucosamine and galactose, and galactose and fucose were same for these two trisaccharides. However, NOE values between fucose and *N*-acetylglucosamine were significantly different. Binding of the 5-thio-L-fucose-containing H-type II to lectins and antibodies were in some case stronger and in some case weaker than those of the native trisaccharide.

Keywords: biological activity · carbohydrates · conformation analysis · lectins · synthesis design

Introduction

It has recently become apparent that carbohydrates play crucial roles in important biological recognition processes, including: bacterial and viral infections, cell adhesion in inflammation and metastasis, differentiation, development, regulation and other intercellular communication and signal transduction events.[1] For the study of such recognition processes, development of biologically stable oligosaccharide ligand is very important. Since natural oligosaccharides are easily degraded in the blood stream, biologically stable oligosaccharides are also important for the development of carbohydrate-based therapeutics and vaccines. In this context, we have been investigating 5-thiosugar-containing oligosaccharides because glycosidic bond of 5-thiosugars are known to be resistant to enzymatic degradation by glycosidases.[2–5] However, replacing a sugar residue in an oligosaccharide ligand with a 5-thiosugar would alter its affinity to its receptor. One of the known effects, which originates directly from the sulfur atom, is the increase of the affinity by a stronger hydrophobic interaction compared with the oxygen atom. This was observed in the binding of 5-thio-L-fucose to α-L-fucosidase.[6] On the contrary, it could decrease affinity by destroying hydrogen bond to the ring oxygen, which was observed in the binding of the 1,6-linked 5-thio-D-mannose-containing di- and trisaccharide to concanavalin A.[7] We selected the H-type II trisaccharide **1**[8,9] as our target (Figure 1) because there are a lot of reports not only on its synthesis but also on its conformation and biological activity. This trisaccharide was compared with the native oligosaccharide in terms of conformation and affinity with carbohydrate binding proteins to evaluate 5-thiosugar-containing oligosaccharides as molecular probes for biological and medicinal studies.

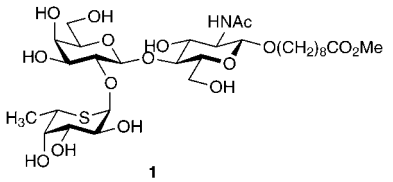
[a] Dr. M. Izumi, Dr. O. Tsuruta, Prof. Dr. H. Yuasa, Prof. Dr. H. Hashimoto
Department of Life Science
Graduate school of Bioscience and Biotechnology
Tokyo Institute of Technology
4259 Nagatsuta, Midori-ku, Yokohama 226-8501 (Japan)
Fax: (+81) 45-924-5704
E-mail: masayuki.izumi@aist.go.jp
hhashimo@bio.titech.ac.jp

[b] Prof. Dr. Y. Kajihara
Graduate school of Integrated Science
Yokohama City University, 22-2 Seto
Kanazawa-ku, Yokohama 236-0027 (Japan)

[c] Dr. S. Yazawa
Japan Immunoresearch Laboratories Co. Ltd. (JIMRO)
351-1 Nishiyokote-cho, Takasaki, Gunma 370-0021 (Japan)

[d] Dr. M. Izumi
Current address:
Research Center of Advanced Bionics
National Institute of Advanced Industrial Science and Technology (AIST)
1-1-1 Higashi, Tsukuba, Ibaraki 305-8565 (Japan)
Fax: (+81) 29-861-4680

DOI: 10.1002/chem.200400831



1

Figure 1. Target 5-thio-L-fucose-containing H-type II **1**.

For the synthesis of 5-thiosugar-containing oligosaccharide, glycoside formation is the most important step. This step has been investigated extensively by using glycosyl trichloroacetimidate of 5-thio-L-fucose[5,10] and 5-thio-D-glucose[11–13] as the donors. In most cases, a 1,2-*cis* axial glycoside was formed predominantly in the presence of an equatorial acyloxy group at the 2-position. Glycosylation of the native (ring oxygen) sugar under similar conditions usually leads to a formation of a 1,2-*trans* equatorial glycoside as a result of the neighboring group participation. Alternative access to construct 5-thioglycoside is the use of glycosyltransferases, although there are only three examples reported to date. Galactosyltransferase could transfer 5-thio-D-galactose[2] and lactose synthetase could transfer *N*-acetyl-5-thio-D-galactosamine from their UDP derivatives.[14] Chemoenzymatic synthesis of the 5-thio-L-fucose-containing Lewis X by using GDP-5-thio-L-fucose and α-1,3-fucosyltransferase has been reported by our group.[15]

Several possible effects are known to have influence on the conformation of 5-thiosugar-containing oligosaccharide. The conformational preferences and associated geometrical variations have been rationalized by anomeric effects.[16] The puckering distortion of the 5-thiopyranose ring is suggested as the result of longer C–S bond length (1.78 Å, 1.42 Å for C–O) and exact C-S-C bond angle (99°, 114° in C-O-C).[17] Conformational analysis of the 5-thioglucose-containing disaccharide kojibiose[12] and maltose[18] were reported by Pinto group. Conformations found by grid search supported by nuclear Overhauser enhancement (NOE) data coincide well with their oxygen counterpart. Theoretical study of 5-thio-α-D-glucopyranosyl-(1→3)-deoxymannojirimycin by Izumi et al.[19] also suggested good similarity of its three-dimensional structure with that of its oxygen counterpart. For H-type II trisaccharide, Barker and co-workers[20] reported the solution conformation using the ^{13}C enriched trisaccharide synthesized by using glycosyltransferases. $^{3}J_{C,C}$ and $^{3}J_{C,H}$ couplings of interglycosidic bonds were used to estimate their torsion angles. The Φ and Ψ torsion angles of the most abundant conformer were estimated to be $\Phi' \approx 55°$, $\Psi' \approx 0°$ (Φ' = H1″-C1″-O2′-C2′, Ψ' = C1″-O2′-C2′-H2′) in the Fucα1→2Gal and $\Phi \approx 60°$ and $\Psi \approx 15°$ (Φ = H1′-C1′-O4-C4, Ψ = C1′-O4-C4-H4) in the Galβ1→4GlcNAc linkage. However, this approach requires a ^{13}C enriched compound and is not generally applicable. NOE data are generally used for a conformational analysis of oligosaccharides. Bush and co-workers[21] used NOE data and CD data with conformational energy calculations to estimate the torsion angles to be $\Phi' = 30°$, $\Psi' = 30°$ and $\Phi = 60°$, $\Psi = -10°$. For a computer simulation of the H-type II trisaccharide, Lemieux and co-workers[22] used HSEA calculations and predicted the torsion angles to be $\Phi' = 55°$, $\Psi' = 0°$ and $\Phi = 50°$, $\Psi = 15°$. Imberty and co-workers[23] restudied 14 histo-blood group oligosaccharides through a combination of molecular mechanics (MM3) and conformational search (CICADA) methods. For H-type II, they reported that 95 % of conformers have the torsion angles of $\Phi' \approx 34$–$47°$, $\Psi' \approx 17$–$33°$ and $\Phi \approx 41$–$53°$, $\Psi \approx 8$–$17°$, and 4 % of them have $\Phi' \approx 16$–$28°$, $\Psi' \approx -45$ to $-44°$ and $\Phi \approx 29$–$34°$, $\Psi \approx -65$ to $-57°$.

Several biological activities of 5-thiosugar-containing oligosaccharides have been reported to date. Methyl 5′-thioisomaltoside binds to glucoamylase, contrary to methyl isomaltoside which binds to a different subsite.[4] Methyl 5′-thiomaltoside also binds to glucoamylase.[3] Four disaccharides having 5-thio-L-fucose glycosidically linked to 3-, 4- or 6-position of *N*-acetylglucosamine or 2-position of galactose were synthesized and tested as inhibitors of α-1,2-fucosidase.[5] It turned out that only the α(1→2)Gal-linked disaccharide was a competitive inhibitor and the other three did not have any inhibitory activity. The 5-thio-D-mannose-containing oligosaccharides were tested as concanavalin A ligands.[7] Five of the structures had a decreased affinity. Several lectins and antibodies are known to recognize H-type II, though each protein has a different mode of recognition. For example, key hydroxyl groups of H-type II involved in the binding with *Ulex europaeus* agglutinin-I are H2, 3, 4 of fucose, whereas those with *Galactia tenuiflora* lectin are H3, 4 of galactose and H3 of *N*-acetylglucosamine, and those with *Psophocarpus tetragonolobus* lectin II are H3, 4 of galactose and H2 of fucose.[24]

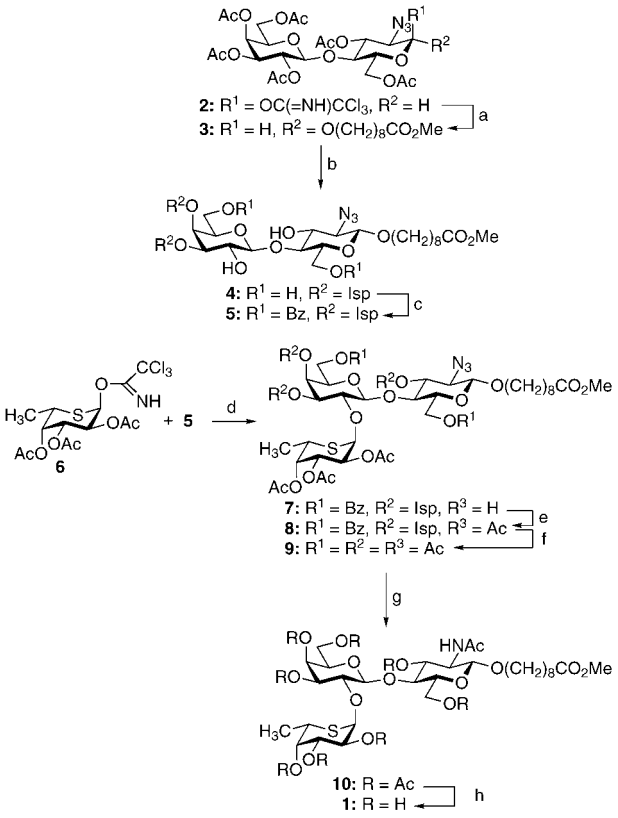
Herein we report the chemical synthesis of the 5-thio-L-fucose-containing H-type II trisaccharide from a disaccharide 2-azido-2-deoxy-lactose derivative. Conformational analysis of the 5-thio-L-fucose-containing H-type II trisaccharide was carried out through NMR experiments, and the result was discussed with the aid of molecular mechanics conformational search. Inhibition study of antibody and lectin binding to native saccharides by 5-thio-L-fucose-containing oligosaccharides is also reported.

Results and Discussion

Synthesis of 5-thio-L-fucose-containing H-type II: We have previously reported the convergent synthesis of 5-thio-L-fucose-containing H-type II and Lewis X.[8] H-type II and Lewis X (Le^x) trisaccharide both contains *N*-acetyllactosamine. The difference between them is the fucosylated position, which is the 2′-OH group in H-type II, whereas it is 3-OH in Le^x. We used *O*-(3,4,6-tri-*O*-acetyl-2-*O*-levulinoyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-glucopyranose as a common intermediate for an efficient synthesis of both trisaccharides. The reducing end was protected in a 1,6-anhydro ring because it can easily be converted into the glycosyl donor so that diverse aglycons can be introduced.

During our first synthesis, Windmüller and Schmidt[25] reported the efficient synthesis of *lactoneo* series oligosaccharides including H-type II and Le^x. Their synthetic strategy of these trisaccharides is based on the regioselective benzoylation and fucosylation of the 2-azido-2-deoxy-3′,4′-*O*-isopropylidene-lactose derivative. The lactose derivative was synthesized from disaccharide lactose so that the glycosylation step was avoided. Since we had some difficulties with the glycosylation of 1,6-anhydro-2-azido-3-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-glucopyranose with 3,4,6-tri-*O*-acetyl-2-*O*-levulinoyl-α-D-galactopyranosyl bromide in our first synthetic route, we examined their synthetic route for the syntheses of the 5-thio-fucose-containing H-type II **1**.

The synthesis of the H-type II trisaccharide **1** from the hexaacetyl-2-azido-2-deoxy-α-lactosyl trichloroacetimidate **2** was showed in Scheme 1. Glycosidation of the α-imidate **2** with 8-methoxycarbonyloctanol gave β-lactoside **3** in 33% yield. The low yield is due to the difficulty of the separation of the pure β-glycoside from the complex mixture including α-glycoside formed in the glycosylation. Deacetylation of **3** with methanolic sodium methoxide followed by treatment in refluxing acetone in the presence of $FeCl_3$[26] gave the 3′,4′-*O*-isopropylidene derivative **4** in 40% yield along with the 4′,6′-*O*-isopropylidene isomer as a byproduct in 18% yield. The primary hydroxyl groups of **4** were selectively benzoylated[27] with BzCN in DMF at −40 °C to give the diol **5** in 71% yield. The glycosylation of the diol **5** with the 5-thiofucosyl trichloroacetimidate **6** in the presence of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at −20 °C proceeded α-selectively and regioselectively to 2′-OH group, yielding the H-type II precursor **7**. The acceptor **5** could not be separated from **7** at this point. Hydrolysis of isopropylidene group with 50% TFA in CH_2Cl_2 after acetylation of the mixture of **5** and **7**, and subsequent deacylation and acetylation gave the pure octaacetate **9**. The structure of **9** was confirmed at this stage by comparing its ^{1}H NMR spectrum with the spectrum of our previously[8] synthesized sample. Then azido group was converted into acetamido group in 95% yield by treatment with hydrogen sulfide in aqueous pyridine and subsequent acetylation. Finally, *O*-deacetylation with methanolic sodium methoxide gave the 5-thio-L-fucose-containing H-type II trisaccharide **1** in 62% yield after purification by solid-phase extraction with reverse-phase C18 cartridge.



Scheme 1. a) $HO(CH_2)_8CO_2Me$, $BF_3{\cdot}OEt_2$, $(CH_2Cl)_2$/hexane, −40 °C (33%); b) 1. NaOMe, MeOH, 2. acetone, $FeCl_3$ (40%); c) BzCN, Et_3N, DMF, −40 °C (71%); d) $BF_3{\cdot}OEt_2$, MS 4 Å, CH_2Cl_2, −20 °C; e) Ac_2O, pyridine; f) 1. 50% TFA, CH_2Cl_2, 2. NaOMe, MeOH, 3. Ac_2O, pyridine (24% from **5**); g) 1. H_2S, pyridine, H_2O, 2. Ac_2O, pyridine (95%); h) NaOMe, MeOH (62%).

Conformational analysis of 5-thio-L-fucose-containing H-type II: The chemical shift of every proton of the 5-thio-L-fucose-containing H-type II **1** was determined by HOHAHA and HSQC experiments and are summarized in Table 1, along with the native H-type II **11**. Most of the resonances of *N*-acetylglucosamine and galactose of **1** are in good accordance with those of its oxygen counterpart; only the resonance of 5-thio-L-fucosylated site (H2 of Gal) has been shifted about 0.25–0.3 ppm to low field. The conformational analysis of the 5-thio-L-fucose-containing H-type II **1** and the H-type II **11** was carried out with NMR experiments. The NOESY experiments were carried out with several mixing times, and NOESY spectra and observed NOE values are depicted in Figure 2. NOEs between H1 of galactose and H4 and H6a,b of *N*-acetylglucosamine, and H2 of galactose and H1 of fucose were observed in the both compounds. However, we could not determine which proton of galactose had an NOE with H1 of 5-thiofucose since H2, 3 and 4 of galactose overlapped. A significant difference was the NOEs between H5 of fucose and H5, 6a of *N*-acetylglucosamine, which were observed in **11** but were not observed in **1**. ROESY experiments were also carried out for both compounds and they gave similar results.

Molecular modeling studies of the methyl glycoside of the 5-thio-L-fucose-containing H-type II (5″S H-type II, **12**) and the native H-type II **13** (Figure 3) were carried out. Monte Carlo conformational search was performed on MacroModel ver.5.5 software[28] by using AMBER* force field and GB/SA water model and NOESY data were included as constraints. After 15000 Monte Carlo steps, the global minimum structure found for 5″S H-type II **12** had the interglycosidic torsion angles of $\Phi' = 49.8°$, $\Psi' = 20.2°$ (Φ' = H1″-C1″-O2′-C2′, Ψ' = C1″-O2′-C2′-H2′) in the 5SFucα1→2Gal and $\Phi = 50.3°$, $\Psi = -5.6°$ (Φ = H1′-C1′-O4-C4, Ψ = C1′-O4-C4-H4) in the Galβ1→4GlcNAc linkage. The global minimum structure of native H-type II (**13**) had the torsion

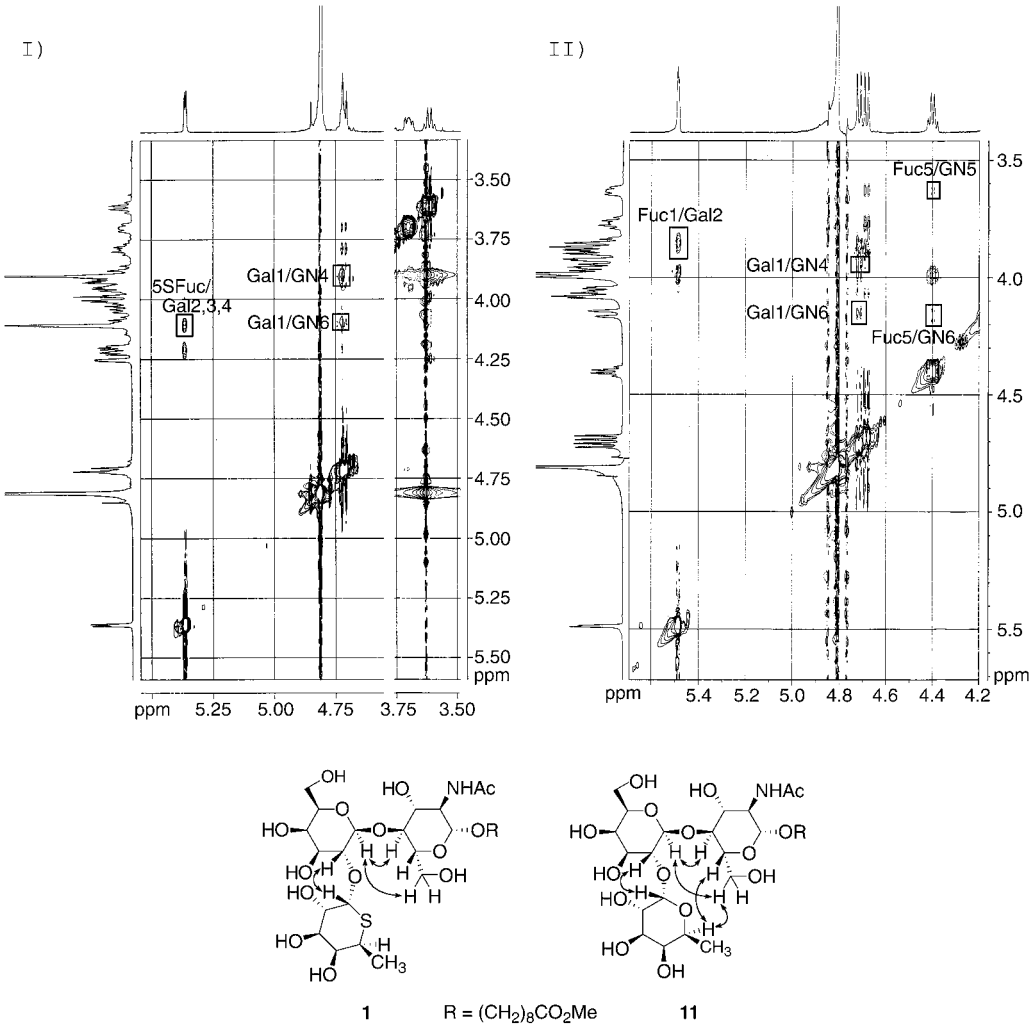


Figure 2. Selected region of NOESY spectrum and observed NOEs of 5″S-H-type II **1**, and native H-type II **11**.

angles of $\Phi' = 45.9°$, $\Psi' = 20.4°$ and $\Phi = 52.2°$, $\Psi = -2.3°$, which was in good accordance with those values reported previously. The conformation of the global minimum of 5″S H-type II (**12**) is very similar to that of the native trisaccharide **13**. Further extensive computational studies are required to explain the difference of the observed NOE between **1** and **11**. CPK models of the global minimum of **12** and **13** are illustrated in Figure 4. It is noteworthy that the sulfur atom sits in the upper surface of the trisaccharide, which consists of the β face of the galactose and the fucose and the α face of the *N*-acetylglucosamine. The existence of the sulfur atom at the surface of the molecule may affect its binding affinity to lectins and antibodies.

Biological activities of 5-thio-L-fucose-containing oligosaccharides: For the preliminary screening of the affinity of the 5-thio-L-fucose-containing saccharides with variety of fucose-binding proteins, inhibition studies of hemagglutination reactions were performed. 5-Thio-L-fucose-containing H-type II **1**, allyl *O*-(5-thio-α-L-fucopyranosyl)-(1→2)-β-D-galactopyranoside (**14**)[5] and 5-thio-L-fucose[29,30] were examined as inhibitors along with their oxygen counterparts (Figure 5). Three fucose-binding lectins, that is *aleuria aurantia* (AAL) isolated from a mushroom,[31] *Angiulla anguilla* agglutinin (AAA) isolated from an eel,[32] and *Ulex europaeus* agglutinin I (UEA-I) isolated from a fern, and two anti-H monoclonal antibodies (MoAb) against H-type II trisaccharide and disaccharide (Fucα1→2Gal), were used as agglutinins. Minimal concentrations required for an inhibition of hemagglutination reactions are summarized in Table 2. No inhibition activities of 5-thio-L-fucose derivatives were observed against AAL. Only weak inhibition activities were observed against AAA by 5-thio-L-fucose and the disaccharide **14** but not by the trisaccharide **1**. The disaccharide **14** and trisaccharide **1** showed good inhibition activities against UEA-I. The trisaccharide **1** showed very strong inhibition activity against both monoclonal antibodies compared to its oxygen counterpart. The disaccharide **14** showed weak activity against anti-H disaccharide MoAb but no activity against anti-H trisaccharide MoAb. No inhibition activities of 5-thio-L-fucose-containing saccharides against AAL and AAA are presumably because of the involvement

Table 1. ^{1}H NMR chemical shifts of 5″S-H-type II **1** and native H-type II **11**.

	1	**11**
GlcNAc		
H1	4.55	4.54
H2	3.78	3.77
H3	3.71	3.70
H4	3.79	3.81
H5	3.54	3.50
H6a	4.06	4.02
H6b	3.87	3.84
NAc	2.09	
Gal		
H1	4.57	4.58
H2	3.96	3.71
H3	3.95	3.92
H4	3.95	3.94
H5	3.75	3.74
H6a	3.85	3.83
H6b	3.80	3.77
Fuc		
H1	5.21	5.33
H2	4.05	3.86
H3	3.83	3.84
H4	4.10	3.85
H5	3.45	4.27
H6	1.27	1.29

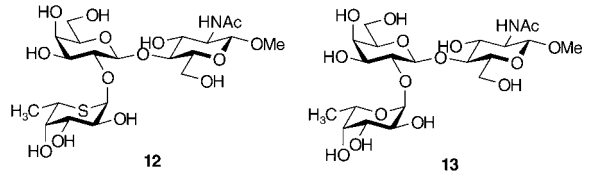


Figure 3. Structures of **12** and **13** used in Monte Carlo conformational search.

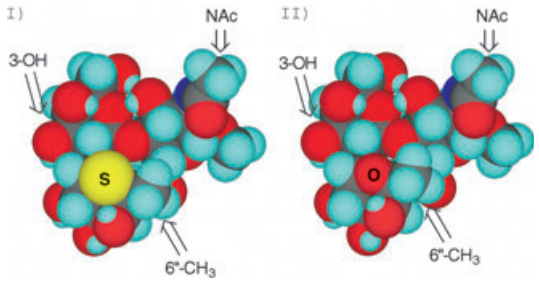


Figure 4. CPK model of the global minimum of (I) **12** and (II) **13**.

of the ring oxygen in recognition by the hydrogen-bonding network. It is known that key hydroxyl groups for binding to UEA-I are the 2,3,4-OH groups of fucose and involvement of 3-OH of galactosyl is also suggested.[24] The fact that UEA-I recognized the 5-thio-L-fucose-containing di- and trisaccharide **14** and **1**, respectively, suggested that these oligosaccharides possess those key hydroxyl groups in proper position, which is consistent with the result of conformational analysis. The inhibition activity of the trisaccharide **1** against antibodies also supports this assumption. Strong inhibition activity of the trisaccharide **1** against antibodies compared with its oxygen counterpart might be the result of additional hydrophobic interaction of the ring sulfur atom at the surface of the molecule.

In conclusion, the 5-thio-L-fucose-containing H-type II trisaccharide **1** was synthesized from 2-azido-2-deoxy-lactose that can be derived from lactose by azidonitration of lactal. The 5-thio-L-fucosylation of the diol **5** proceeded α-selectively and regioselectively to 2′-OH. The resulting fully functionalized trisaccharide was converted to **1** in good yield. Conformational analysis of the 5-thio-L-fucose-containing H-type II and the native H-type II was carried out through NOESY experiments with the aid of molecular modeling conformation search. The observed NOEs between *N*-acetylglucosamine and galactose, and galactose and fucose were same in those two trisaccharides. However, NOEs between fucose and *N*-acetylglucosamine were significantly different. Comparing the inhibition activity of oligosaccharide–protein binding by the 5-thio-L-fucose-containing oligosaccharides with their oxygen counterparts suggested that simply replacing one ring oxygen in oligosaccharide to sulfur resulted in making a compound with the different inhibition activity spectrum. Taking into consideration that each protein has the different way of recognition, it is very difficult to predict the effect of using 5-thiosugar in oligosaccharide to its biological activity, as has already been pointed out by Yuasa et al.[33] Still, stability of 5-thiosugar-containing oligosaccharide to degradation enzymes and the cross-reactivity against antibodies suggest its potential usefulness in carbohydrate-based therapeutics and vaccines.

Experimental Section

General: Melting points are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter using 0.5 dm cell. NMR spectra were recorded on JEOL EX-270 or Varian Unity-400 or Bruker AM-500 instruments. ^{1}H NMR spectra recorded in $CDCl_3$ or in D_2O were referenced to tetramethylsilane at $\delta = 0$ ppm or to acetone at $\delta = 2.225$ ppm, respectively. ^{13}C NMR spectra recorded in $CDCl_3$ or in D_2O were referenced to the central peak of $CDCl_3$ at $\delta = 77.0$ ppm or to 1,4-dioxane at $\delta = 67.4$ ppm, respectively. Column chromatography was performed with Kieselgel 60 (E. Merck) or Wakogel C-300 (Wako Pure Chem.). TLC was carried out on plates precoated with silica gel 60 F254 (E. Merck), with detection by UV light (254 nm) and/or by charring with 5% H_2SO_4 in MeOH or 1% $Ce(SO_4)_2$ and 1.5% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 10% H_2SO_4. SepPak C18 cartridge was purchased from Waters Inc. Dichloromethane was distilled twice from P_2O_5 and stored over MS4 A. Crushed molecular sieves were activated by heating at 250 °C overnight and cooled in vacuo prior to use.

8-Methoxycarbonyloctyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-azido-deoxy-β-D-glucopyranoside (3): A suspension of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl trichloroacetimidate (**2**, 1.484 g, 1.94 mmol), $HO(CH_2)_8CO_2Me$ (0.549 g, 2.92 mmol) and crushed activated MS 4 Å (0.94 g) in $(CH_2Cl)_2$/hexane (1:1 v/v, 16.6 mL) was stirred for 1 h under Ar atmosphere. The suspension was cooled to −40 °C, and to the suspension was added dropwise a solution of $BF_3\cdot OEt_2$ (0.13 mL, 1.06 mmol) in $(CH_2Cl)_2$/hexane (1:1 v/v, 7.0 mL). After being stirred for 30 min at −40 °C, the reaction mixture was warmed to room

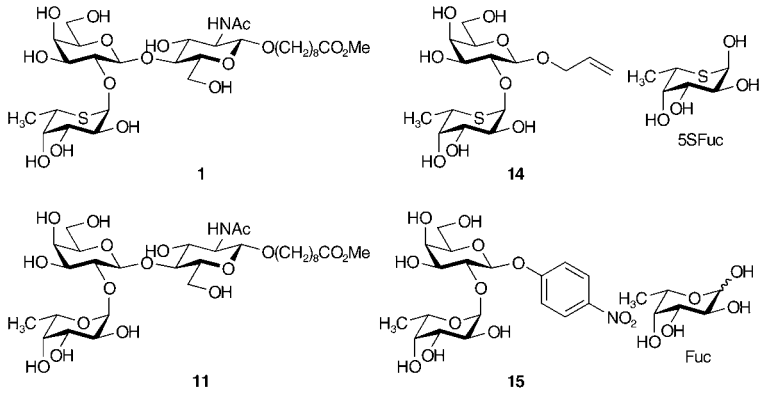


Figure 5. H-type II derivatives used in hemagglutination inhibition assay.

Table 2. Minimum concentration (mM) required for inhibition of hemagglutination reaction.[a]

	1	11	14	15	5SFuc	Fuc
AAL	$>10^{[a]}$	1.25	$>10^{[a]}$	n.d.	$>10^{[a]}$	2.5
AAA	$>10^{[a]}$	0.313	5.0	n.d.	5.0	1.25
UEA I	1.25	0.313	0.625	n.d.	$>10^{[b]}$	$>10^{[b]}$
anti-H di	0.625	5.0	5.0	2.5	$>10^{[b]}$	$>10^{[b]}$
anti-H tri	0.154	1.25	$>10^{[b]}$	5.0	$>10^{[b]}$	$>10^{[b]}$

[a] See Figure 5 for structures of inhibitors; AAL: *Aleuria aurantia* lectin; AAA: *Anguilla anguilla* agglutinin; UEA I: *Ulex europaeus* agglutinin I; anti-H di: anti H disaccharide monoclonal antibody; anti-H tri: anti H-type II trisaccharide monoclonal antibody; n.d. not determined. [b] No inhibition at 10 mM.

temperature and filtered through a pad of Celite. The filtrate was washed with saturated aqueous $NaHCO_3$, and the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified on a column of silica gel (hexane/EtOAc 1:1) to give **3** (0.499 g, 33%) as a syrup. $[\alpha]_D^{25} = -6.4$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.35$ (dd, $^3J(H,H) = 0.7$, 3.3 Hz, 1H; H4′), 5.09 (dd, $^3J(H,H) = 7.6$, 10.6 Hz, 1H; H2′), 4.97 (t, $^3J(H,H) = 9.6$ Hz, 1H; H3), 4.94 (dd, $^3J(H,H) = 3.3$, 10.6 Hz, 1H; H3′), 4.46 (dd, $^3J(H,H) = 2.0$, 11.9 Hz, 1H; H6a), 4.45 (d, $^3J(H,H) = 7.6$ Hz, 1H; H1′), 4.34 (d, $^3J(H,H) = 8.1$ Hz, 1H; H1), 4.17 (dd, $^3J(H,H) = 6.6$, 11.2 Hz, 1H; H6′a), 4.14–4.04 (m, 2H; H6b,6′b), 3.93–3.85 (m, 1H; CH_2O), 3.87 (t, $^3J(H,H) = 6.6$ Hz, 1H; H5′), 3.70 (t, $^3J(H,H) = 9.6$ Hz, 1H; H4), 3.66 (s, 3H; OMe), 3.58–3.50 (m, 2H; H5, CH_2O), 3.38 (dd, $^3J(H,H) = 7.6$, 10.2 Hz, 1H; H2), 2.30 (t, $^3J(H,H) = 7.4$ Hz, 2H; CH_2CO), 2.16, 2.12, 2.12, 2.07, 2.03, 1.97 ppm (each s, 6×3H; 6×Ac); elemental analysis calcd (%) for $C_{34}H_{51}N_3O_{18}$: C 51.71, H 6.51, N 5.32; found: C 51.70, H 6.52, N 4.95.

8-Methoxycarbonyloctyl *O*-(6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2-azido-6-O-benzoyl-2-deoxy-β-D-glucopyranoside (5): A 0.5 M methanolic NaOMe solution (0.27 mL) was added to a solution of **3** (0.499 mg, 0.632 mmol) in MeOH (6.2 mL). After being stirred overnight at room temperature, the solution was neutralized with Dowex 50W-X8 (H^+). The resin was filtered off and the filtrate was concentrated. $FeCl_3$ (30 mg, 0.11 mmol) was added to a solution of the residue in acetone (132 mL). After being stirred under reflux for 30 min, the solution was cooled and neutralized with 10% aqueous K_2CO_3 (26 mL), and acetone was evaporated. The aqueous layer was extracted with EtOAc (132 mL), and the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified on a column of silica gel ($CHCl_3$/MeOH 15:1) to give **4** (146 mg, 40%) and its 4′,6′-*O*-isopropylidene isomer (66.5 mg, 18%) each as a syrup. A solution of BzCN (0.15 mL, 0.13 mmol) and Et_3N (0.21 mL) in DMF (1.0 mL) was added under Ar atmosphere at −40 °C to a solution of **4** (0.283 g, 0.490 mmol) in DMF (3.9 mL). After being stirred for 1 h at −40 °C, excess of the reagent was decomposed by adding MeOH and the solution was concentrated. The residue was dissolved in EtOAc, and washed with brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified on a column of silica gel (hexane/EtOAc 6:5) to give a syrup. Recrystallization from EtOAc/Et_2O gave **5** (0.181 g). The mother liquor was concentrated and another silica gel chromatography (hexane/EtOAc 6:5) gave further **5** (93.5 mg, 71%). M.p. 125–126 °C; $[\alpha]_D^{20} = +43.8$ ($c = 0.97$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.17$–7.42 (m, 10H; 2×Ph), 4.89–4.84 (m, 2H; H6a,6′a), 4.65 (br s, 1H; OH3), 4.46–4.37 (m, 2H; H6b,6′b), 4.30 (d, $^3J(H,H) = 8.3$ Hz, 1H; H1′), 4.26 (d, $^3J(H,H) = 8.3$ Hz, 1H; H1), 4.23–4.19 (m, 2H; H4′,5′), 4.13 (dd, $^3J(H,H) = 5.6$, 6.9 Hz, 1H; H3′), 3.93–3.85 (m, 1H; OCH_2), 3.72–3.66 (m, 1H; H2′), 3.66 (s, 3H; OMe), 3.65–3.57 (m, 2H; H3,5), 3.57–3.49 (m, 1H; OCH_2), 3.39 (dd, $^3J(H,H) = 8.3$, 9.7 Hz, 1H; H4), 3.28 (dd, $^3J(H,H) = 8.3$, 9.7 Hz, 1H; H2), 2.29 (t, $^3J(H,H) = 7.4$ Hz, 2H; $COCH_2$), 1.63–1.25 (m, 12H; $(CH_2)_6$), 1.56, 1.37 ppm (each s, 2×3H; CMe_2); elemental analysis calcd (%) for $C_{39}H_{51}N_3O_{14}$: C 59.61, H 6.54, N 5.35; found: C 59.59, H 6.51, N 5.29.

5-Thio-L-fucose-containing H-type II 1: A mixture of 2,3,4-tri-*O*-acetyl-5-thio-L-fucopyranosyl trichloroacetimidate **6** (77.1 mg, 0.171 mmol), **5** (133 mg, 0.169 mmol) and crushed activated MS 4 Å (0.53 g) in CH_2Cl_2 (3.6 mL) was stirred under Ar atmosphere for 1 h at room temperature and cooled to −20 °C. A solution of $BF_3{\cdot}OEt_2$ (7.2 μL, 56 μmol) in CH_2Cl_2 (1.4 mL) was added dropwise to the cooled mixture, which was stirred for 30 min. The reaction mixture was neutralized with Et_3N and filtered through a Celite pad. The filtrate was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated. The residue was purified on a column of silica gel (hexane/EtOAc 12:5) to give a mixture of trisaccharide **7** and **5** (184 mg) as a syrup. ^{1}H NMR ($CDCl_3$): $\delta = 5.29$ (s, $^3J(H,H) = 2.6$ Hz, 1H; H1″), 1.15 ppm (d, $^3J(H,H) = 6.9$ Hz, 1H; H6″). The mixture was treated with Ac_2O and pyridine to give **8**. To the solution was added MeOH and concentrated. The residue was treated with 50% TFA (0.5 mL) in CH_2Cl_2 (15 mL). The solution was concentrated, and the residue was treated with methanolic sodium methoxide. The solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was concentrated, and the residue was purified by silica gel chromatography ($CHCl_3$/MeOH 4:1) to give a syrup. The syrup was acetylated with Ac_2O and pyridine to give octaacetate **9** (42 mg, 24%) as a syrup. $[\alpha]_D^{19} = -108$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.48$ (br s, 1H; H4″), 5.30 (br d, $^3J(H,H) = 3.3$ Hz, 1H; H4′), 5.23 (dd, $^3J(H,H) = 2.3$, 10.6 Hz, 1H; H2″), 5.16 (br d, $^3J(H,H) = 10.6$ Hz, 1H; H3″), 5.07 (d, $^3J(H,H) = 2.3$ Hz, 1H; H1″), 5.02 (dd, $^3J(H,H) = 3.3$, 10.2 Hz, 1H; H3′), 4.85 (t, $^3J(H,H) = 9.9$ Hz, 1H; H3), 4.48 (br d, $^3J(H,H) = 12.2$ Hz, 1H; H6a), 4.39 (d, $^3J(H,H) = 7.9$ Hz, 1H; H1′), 4.35 (d, $^3J(H,H) = 7.9$ Hz, 1H; H1), 4.28 (dd, $^3J(H,H) = 5.6$, 12.2 Hz, 1H; H6b), 4.18 (dd, $^3J(H,H) = 6.6$, 10.9 Hz, 1H; H6′a), 4.12–4.04 (m, 2H; H2′,6′b), 3.92 (m, 1H; OCH_2), 3.84 (t, $^3J(H,H) = 6.6$ Hz, 1H; H5′), 3.79 (t, $^3J(H,H) = 9.2$ Hz, 1H; H4), 3.66 (s, 3H; OMe), 3.60–3.54 (m, 2H; H5″,OCH_2), 3.43 (m, 1H; H5), 3.23 (dd, $^3J(H,H) = 7.9$, 9.9 Hz, 1H; H2), 2.30 (t, $^3J(H,H) = 7.3$ Hz, 2H; $COCH_2$), 2.17, 2.14, 2.07, 2.00, 1.97, 1.96 (each s, 3H, 9H, 5×3H; 8×Ac), 1.64–1.26 (m, 12H; $(CH_2)_6$), 1.19 ppm (d, $^3J(H,H) = 6.9$ Hz, 3H; H6″); ^{13}C NMR ($CDCl_3$): $\delta = 170.9$, 170.7, 170.5, 170.3, 170.0, 170.0, 169.8, 169.7, 102.2, 100.1, 80.3, 73.7, 73.4, 73.0, 72.6, 71.8, 71.7, 71.3, 70.9, 70.7, 68.5, 67.3, 63.8, 62.3, 61.0, 51.5, 34.2, 34.2, 29.5, 29.1, 29.0, 25.8, 24.9, 20.9, 20.8, 20.7, 20.6, 15.5 ppm.

A solution of **9** (42 mg 41 μmol) in pyridine/water (1:1, 3.5 mL) was saturated with H_2S at room temperature. After being stirred overnight at room temperature, the reaction mixture was concentrated, and the resi-

due was acetylated with Ac_2O/pyridine, and purified on a column of silica gel (hexane/EtOAc 1:2) to give acetamide **10** (40 mg, 95%) as a syrup. ^{1}H NMR ($CDCl_3$): δ = 5.69 (d, ^3J(H,H) = 8.9 Hz, 1H; NH), 5.50 (brs, 1H; H4″), 5.30 (brd, ^3J(H,H) = 2.6 Hz, 1H; H4′), 5.22 (m, 2H; H2″,3″), 5.11 (t, ^3J(H,H) = 8.6 Hz, 1H; H3), 5.09 (d, ^3J(H,H) = 2.3 Hz, 1H; H1″), 5.04 (dd, ^3J(H,H) = 2.6, 10.2 Hz, 1H; H3′), 4.56–4.53 (m, 1H; H6a), 4.54 (d, ^3J(H,H) = 7.3 Hz, 1H; H1), 4.44 (d, ^3J(H,H) = 7.6 Hz, 1H; H1′), 4.29 (dd, ^3J(H,H) = 5.3, 11.5 Hz, 1H; H6b), 4.19–4.03 (m, 3H; H2′,6′a,6′b), 3.99–3.81 (m, 4H; H2, 4, 5′, OCH_2), 3.67–3.61 (m, 5H; H5, 5′, OMe), 3.47–3.43 (m, 1H; OCH_2), 2.30 (t, ^3J(H,H) = 7.3 Hz, 2H; COCH_2), 2.17, 2.13, 2.13, 2.09, 2.06, 2.00, 1.97, 1.96 (each s, 6×3H, 6H, 3H; 9×Ac), 1.60–1.26 (m, 12H; $(CH_2)_6$), 1.18 ppm (d, ^3J(H,H) = 6.9 Hz, 3H; H6″).

To a solution of **10** (40 mg, 38 μmol) in MeOH (3.0 mL) was added a catalytic amount of NaOMe. After being stirred overnight, the solution was neutralized with Dowex 50W-X8 (H^+), and the resin was filtered off. The filtrate was concentrated, and the residue was dissolved in water and absorbed to the SepPak C18 cartridge. After wash with water, compound **1** (17.2 mg, 62%) was eluted with MeOH. ^{1}H NMR see Table 1; ^{13}C NMR (D_2O): δ = 178.7, 175.2, 101.9, 101.5, 84.8, 77.6, 77.2, 76.1, 76.0, 74.8, 74.5, 73.2, 72.1, 71.5, 71.4, 70.0, 61.9, 61.1, 56.1, 52.9, 37.1, 34.5, 29.3, 29.1, 29.0, 25.8, 25.1, 23.1, 16.4 ppm; HR-MALDI-FTMS: *m/z*: calcd for $C_{30}H_{53}NO_{16}SNa$: 738.2983, found 738.2963 $[M+Na]^+$.

NMR methods for conformational analysis: The NMR experiments were run on a Bruker DMX-500 instrument. The temperature was maintained at 313 K. Two-dimensional ^{1}H–^{13}C HMQC, TOCSY, NOESY, and ROESY spectra were measured by use of pulse programs in the Bruker standard library (invbtp, mlevprtp, noesyprtp, and roesyprtp, respectively). During acquisition, GARP decoupling was performed toward ^{13}C (HMQC). TOCSY spectrum was recorded by using MLEV-17 pulse sequence with a total spin locking time for 100 ms. The mixing times in NOESY pulse sequence were varied from 100 to 1000 ms. Spin locking time in ROESY were varied from 200 to 300 ms and the carrier frequency was placed at the left side of the spectrum at 6 ppm in order to minimize HOHAHA type magnetization transfer.

Molecular modeling: All calculations were performed on a Silicon Graphics O2 workstation using MacroModel ver.5.5 software. Initial structures of **12** and **13** were built within MacroModel. The Monte Carlo (MC) approach was used for the global conformation search, and the torsion angles of interglycosidic linkages were randomly modified at each MC step. MC steps (15000) were carried out for both compounds and resultant geometry was minimized using gradient conjugate steps with the AMBER* force field and the GB/SA water model with NOE data as constraints.

Inhibition studies of hemagglutination reaction: Lectins (AAA, AAL, UEA-I) and antibodies (anti-H type II antibody, anti-H disaccharide antibody) were diluted to titer 4 with phosphate-buffered saline (PBS). Inhibitors (**1**, **14**, **15**, **16**, 5SFuc, Fuc) were diluted serially with PBS starting from final concentration of 10 mM. Agglutinins were incubated with inhibitors, and then human type O erythrocyte in PBS was added to see the residual hemagglutination activity. The minimum concentration of inhibitors that inhibited the hemagglutination reaction completely was determined.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Area (A) No. 11121208 from the Ministry of Education, Culture, Sports, Science and Technology.

[1] A. Varki, *Glycobiology* **1993**, *3*, 97–130.
[2] H. Yuasa, O. Hindsgaul, M. Palcic, *J. Am. Chem. Soc.* **1992**, *114*, 5891–5892.
[3] S. Mehta, J. S. Andrews, B. D. Johnston, B. Svensson, B. M. Pinto, *J. Am. Chem. Soc.* **1995**, *117*, 9783–9790.
[4] H. Hashimoto, M. Kawanishi, H. Yuasa, *Chem. Eur. J.* **1996**, *2*, 556–560.
[5] M. Izumi, O. Tsuruta, S. Harayama, H. Hashimoto, *J. Org. Chem.* **1997**, *62*, 992–998.
[6] H. Yuasa, Y. Nakano, H. Hashimoto, *Carbohydr. Lett.* **1996**, *2*, 23–26.
[7] H. Yuasa, S. Matsuura, H. Hashimoto, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1297–1300.
[8] M. Izumi, O. Tsuruta, H. Hashimoto, S. Yazawa, *Tetrahedron Lett.* **1996**, *37*, 1809–1812.
[9] O. Tsuruta, H. Yuasa, H. Hashimoto, S. Kurono, S. Yazawa, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1019–1022.
[10] H. Hashimoto, M. Izumi, *Tetrahedron Lett.* **1993**, *34*, 4949–4952.
[11] S. Mehta, B. M. Pinto, *Tetrahedron Lett.* **1992**, *33*, 7675–7678.
[12] S. Mehta, K. L. Jordan, T. Weimar, U. C. Kreis, R. J. Batchelor, F. W. B. Einstein, B. M. Pinto, *Tetrahedron: Asymmetry* **1994**, *5*, 2367–2396.
[13] S. Mehta, J. S. Andrews, B. D. Johnston, B. M. Pinto, *J. Am. Chem. Soc.* **1994**, *116*, 1569–1570.
[14] O. Tsuruta, G. Shinohara, H. Yuasa, H. Hashimoto, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2523–2526.
[15] O. Tsuruta, H. Yuasa, H. Hashimoto, K. Sujino, A. Otter, H. Li, M. M. Palcic, *J. Org. Chem.* **2003**, *68*, 6400–6406.
[16] B. M. Pinto, R. Y. N. Leung in *The anomeric effect and associated stereoelectronic effects, Vol. 539* (Ed.: G. R. J. Thatcher), American Chemical Society, Washington, DC, **1993**, pp. 126–155.
[17] J. B. Lambert, S. M. Wharry, *Carbohydr. Res.* **1983**, *115*, 33–40.
[18] T. Weimar, U. C. Kreis, J. S. Andrews, B. M. Pinto, *Carbohydr. Res.* **1999**, *315*, 222–233.
[19] M. Izumi, Y. Suhara, Y. Ichikawa, *J. Org. Chem.* **1998**, *63*, 4811–4816.
[20] P. R. Rosevear, H. A. Nunez, R. Barker, *Biochemistry* **1982**, *21*, 1421–1431.
[21] B. N. N. Rao, V. K. Dua, C. A. Bush, *Biopolymers* **1985**, *24*, 2207–2229.
[22] H. Thøgersen, R. U. Lemieux, K. Bock, B. Meyer, *Can. J. Chem.* **1982**, *60*, 44–57.
[23] A. Imberty, E. Mikros, J. Koca, R. Mollicone, R. Oriol, S. Pérez, *Glycoconjugate J.* **1995**, *12*, 331–349.
[24] M.-H. Du, U. Spohr, R. U. Lemieux, *Glycoconjugate J.* **1994**, *11*, 443–461.
[25] R. Windmüller, R. R. Schmidt, *Tetrahedron Lett.* **1994**, *35*, 7927–7930.
[26] R. Bommer, W. Kinzy, R. R. Schmidt, *Liebigs Ann. Chem.* **1991**, 425–433.
[27] K. C. Nicolaou, T. Caulfield, H. Kataoka, T. Kumazawa, *J. Am. Chem. Soc.* **1988**, *110*, 7910–7912.
[28] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
[29] H. Hashimoto, T. Fujimori, H. Yuasa, *J. Carbohydr. Chem.* **1990**, *9*, 683–694.
[30] M. Izumi, O. Tsuruta, H. Hashimoto, *Carbohydr. Res.* **1996**, *280*, 287–302.
[31] F. Fukumori, N. Takeuchi, T. Hagiwara, H. Ohbayashi, T. Endo, N. Kochibe, Y. Nagata, A. Kobata, *J. Biochem.* **1990**, *107*, 190–196.
[32] S. Baldus, J. Thiele, Y. O. Park, F. G. Hanisch, J. Bara, R. Fischer, *Glycoconjugate J.* **1996**, *13*, 585–590.
[33] H. Yuasa, H. Hashimoto, *Rev. Heteroat. Chem.* **1999**, *19*, 35–65.

Received: August 12, 2004
Revised: January 24, 2005
Published online: March 15, 2005