Tetrahedron Letters 51 (2010) 2529–2532

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthetic construction of a fucosyl chitobiose as an allergen-associated carbohydrate epitope and the glycopolymer involving highly clustered trisaccharidic sequences

Koji Matsuoka *, Hiroki Yamaguchi, Tetsuo Koyama, Ken Hatano, Daiyo Terunuma

Area for Molecular Function, Division of Material Science, Graduate School of Science and Engineering, Saitama University, Sakura, Saitama 338-8570, Japan

ARTICLE INFO

Article history:
Received 17 February 2010
Revised 24 February 2010
Accepted 1 March 2010
Available online 3 March 2010

Keywords:
Plant carbohydrate chains
Fucosyl chitobiose
Glycosides
Glycopolymers
Glycoclusters

ABSTRACT

Synthetic construction of fucosyl chitobiose [GlcNAcβ1→4(Fucα1→3)GlcNAc] as an allergy-associated carbohydrate epitope was accomplished from three building blocks. The trisaccharidic unit was further transformed into a carbohydrate monomer and polymerization of the glycomonomer proceeded smoothly to provide a series of glycopolymers having various carbohydrate densities. In addition to the organic syntheses, biological evaluations of the glycomonomer and the polymers were carried out and sugar-clustering effects were observed.

Fucosyl chitobiose [GlcNAcβ1→4(Fucα1→3)GlcNAc] is known as an extremely valuable core structure of glycoconjugates such as N-linked glycoproteins in plants,[1] and the trisaccharide structure is suspected to be one of epitopes of allergy.[2] A regioisomeric structure of the trisaccharide is also a fucosyl chitobiose having [GlcNAcβ1→4(Fucα1→6)GlcNAc], and this trisaccharidic structure is a well-known core structure ubiquitously found as N-linked glycoproteins in mammalians.[3] Synthetic assembly of the trisaccharide of Fucα1→6-linked-type was efficiently accomplished by Nishimura et al. using a chitobiosyl derivative as the key starting material.[4] Synthetic assembly of the trisaccharide of Fucα1→3-linked-type was, however, achieved more than 30 years ago by Tejima and co-workers using a convergent strategy.[5] The chitobiose moiety was prepared by means of acid-promoted glycosidation of the oxazoline derived from GlcNAc and the 1,6-anhydro GlcNAc derivative in 41% yield. Further introduction of Fuc into the chitobiose moiety was attempted by using Lemieux's conditions,[6] such as a halide ion-catalyzed glycosidation in 47% yield. It seems that since suitable amounts of the trisaccharide and the derivatives were not obtained, the results of biological evaluations of the trisaccharide and derivatives have not been reported. In this Letter, we describe efficient preparation of trisaccharidic glycomonomer **1** from three building blocks **2**, **3**, and **4** and chemical conversion into a highly clustered glycopolymer. In addition to the syntheses, biological evaluations of the glycomonomer and the glycopolymers were performed.

In our previous study, synthetically efficient construction of an Le^x determinant [Galβ1→4(Fucα1→3)GlcNAc] was accomplished using a convergent synthetic strategy from D-galactose (Gal), L-fucose (Fuc), and D-mannose (Man) as key starting materials, and

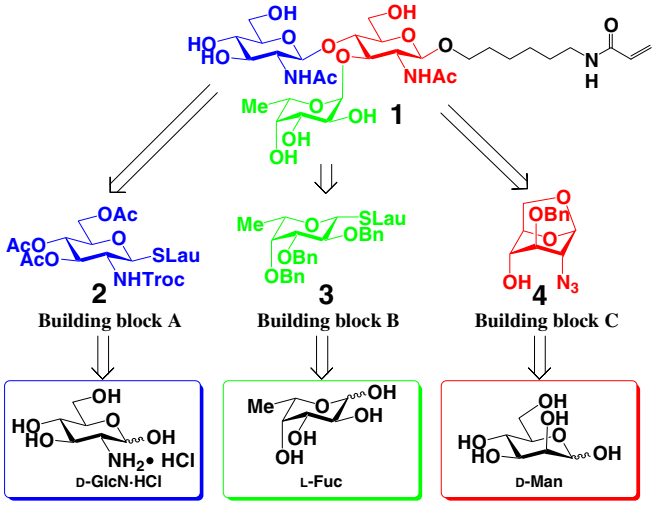
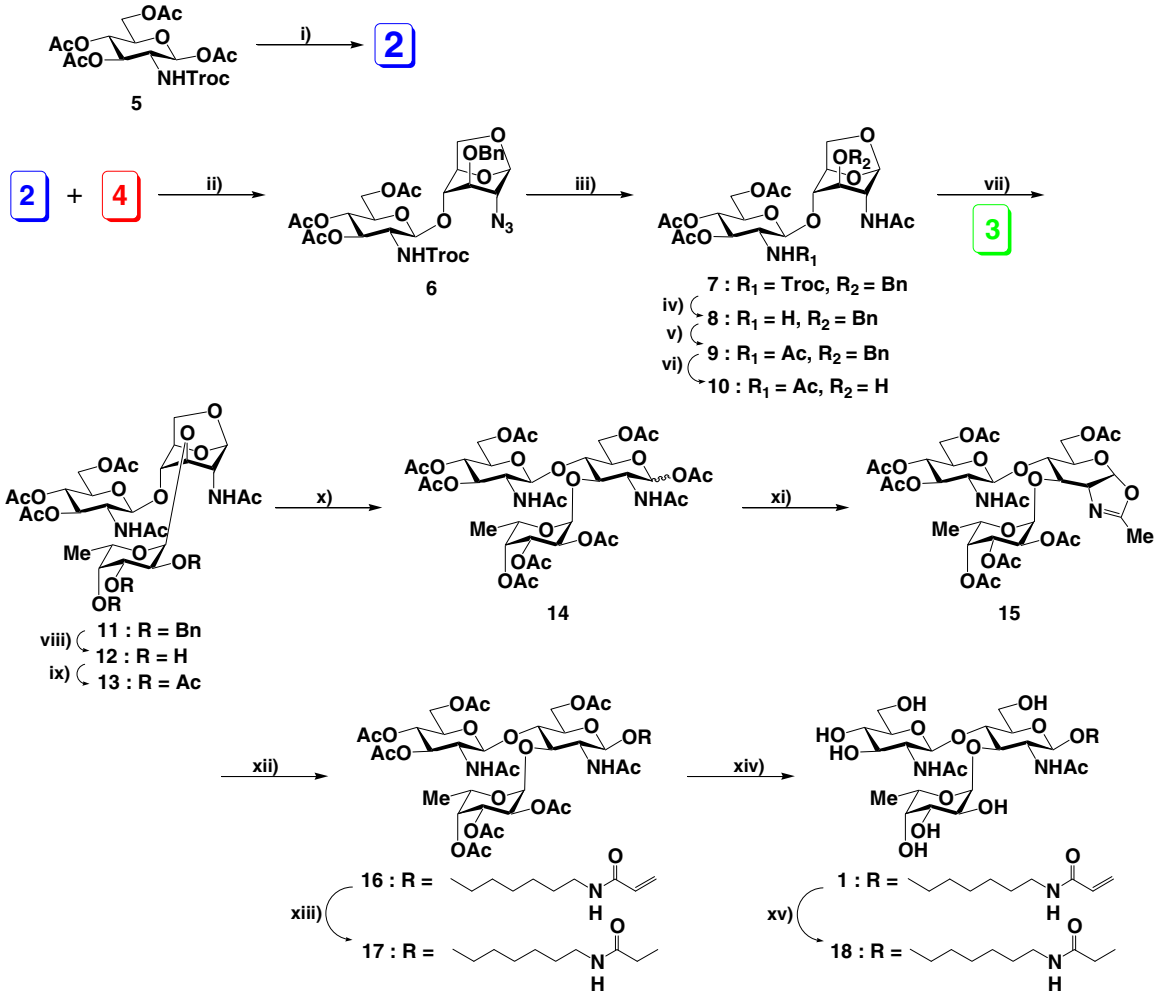


Figure 1. Synthetic plan for the construction of fucosyl chitobiose [GlcNAcβ1→4(Fucα1→3)GlcNAc] derivative.

* Corresponding author. Tel./fax: +81 48 858 3099.
E-mail address: koji@fms.saitama-u.ac.jp (K. Matsuoka).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.03.004

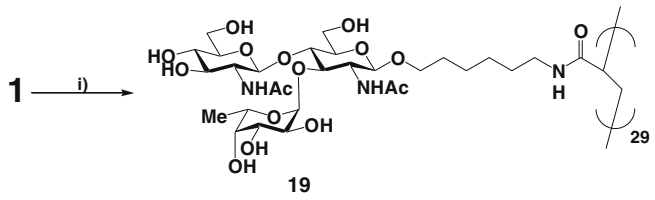


Scheme 1. Reagents and conditions: (i) $BF_3{\cdot}OEt_2$, $CH_3(CH_2)_{11}SH$, $ClCH_2CH_2Cl$, 0 °C to rt, 2.5 h, 97%; (ii) NIS/TMSOTf, CH_2Cl_2, MS 4A, −20 °C, 3 h, 81%; (iii) AcSH, Pyr, 0 °C to rt, on, 100%; (iv) Zn, AcOH, rt, on; (v) Ac_2O, Pyr, rt, 1 day, 98% (two steps); (vi) Pd/C, H_2, EtOAc, rt, 1 day, 100%; (vii) NIS/TMSOTf, CH_2Cl_2, MS 4A, −30 °C, 2.5 h, 74%; (viii) Pd/C, H_2, EtOAc, rt, 1 day; (ix) Ac_2O, Pyr, rt, 94%(two steps); (x) $BF_3{\cdot}OEt_2$, Ac_2O, 0 °C, 4 h, 98%; (xi) TMSOTf, $ClCH_2CH_2Cl$, 50 °C, 5 h, 97%; (xii) 6-acrylamidohexan-1-ol, PPTS, $ClCH_2CH_2Cl$, reflux, 2 h, 68%; (xiii) Pd/C, H_2, MeOH, rt, 5 h, 98%; (xiv) NaOMe, MeOH, rt, on, 96%; (xv) Pd/C, H_2, MeOH, rt, 4 h, 99%.

their biological responses against plant lectin were confirmed.[7] A similar synthetic plan for the construction of a fucosyl chitobiose [GlcNAcβ1→4(Fucα1→3)GlcNAc] structure was selected because of ready access of the key building blocks. Thus, a monosaccharide unit of the reducing end as the pivotal building block C **4** for GlcNAc residue was derived from 1,6-anhydro-β-mannose[8] as shown in Figure 1. Our synthetic plan for the fucosyl chitobiose structure, therefore, was convergent methodology using D-glucosamine hydrochloride (GlcN·HCl), L-fucose (Fuc), and D-mannose (Man) as key starting materials. Building block B **3**[9] was derived by stepwise syntheses from Fuc according to Lewis acid-mediated glycosidation with 1-dodecanethiol (lauryl mercaptan).[10] Building block C **4** was derived from 1,6-anhydro-β-Man by the method previously reported.[8] Scheme 1 shows the preparation of building block A **2** from known **5**.[11] Since an *N*-Troc protective group at the C-2 position in glucosamine promises formation of β-glycosidic linkage with high stereoselectivity reported by Magnusson and Ellervik[12] we chose the *N*-Troc-protected donor as the building block in this study. Thus, a β-acetate **5** was treated with 3 molar equivalents of lauryl mercaptan by means of mediation of 2 molar equivalents of BF_3–OEt_2 to afford β-thioglycoside **2**† in 97% yield after chromatographic purification, which was recrystallized from EtOH, $[\alpha]_D^{30}$ −18.3° (*c* 1.00, $CHCl_3$), mp 108.8–109.5 °C, ^{1}H NMR ($CDCl_3$) δ 5.16 (d, 1H, $J_{2,NH}$ = 9.3 Hz, N*H*), 4.62 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1).

Since three building blocks were prepared, we turned our attention to the construction of each building block. A synthetic assembly of the trisaccharide is illustrated in Scheme 2. TMSOTf-mediated glycosidation of thioglycoside in the presence of appropriate amounts of NIS[13] in CH_2Cl_2 at −20 °C allowed condensation of *N*-Troc donor **2** and an azido acceptor **4** to give the precursor **6** of the chitobiose moiety involving a newly formed β-glycosidic linkage in 81% yield, in which the azide moiety at C-2 was converted into acetamide by means of chemoselective reduction using thioacetic acid—pyridine[14] at rt to furnish the corresponding acetamide **7** in a quantitative yield, $[\alpha]_D^{32}$ −75.2° (*c* 1.00, $CHCl_3$), IR (KBr) 1751 ($\nu_{C=O}$), 1654 ($\nu_{C=O}$, amide I), 1530 (δ_{N-H}, amide II) cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 6.59 (d, 1H, $J_{2',NH'}$ = 8.0 Hz, N*H*), 5.28 (s, 1H, H-1), 4.53 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 101.03 (C-1), 100.10 (C-1′). Selective removal of the *N*-Troc moiety was performed in AcOH in the presence of Zn dust at rt overnight to afford the amine **8**, which was successively acetylated in the usual manner. Chromatographic purification of the reactant gave the diacetamide **9**[5] in 97% yield in two steps. Reductive removal of the benzyl group in **9** was carried out using typical hydrogenolysis with Pd/C in EtOAc under hydrogen atmosphere and the reaction proceeded

† All new compounds with specific rotation data gave satisfactory results of elemental analyses.



Scheme 2. Reagents and conditions: (i) APS, TEMED, H_2O/EtOH (1:1, v/v), rt, 6 h, 86%.

smoothly to afford the corresponding mono alcohol **10** in a quantitative yield, $[\alpha]_D^{31}$ −99.2° (*c* 1.00, $CHCl_3$), IR (neat) 3306 (ν_{O-H}), 1747 ($\nu_{C=O}$), 1659 ($\nu_{C=O}$, amide I), 1537 (δ_{N-H}, amide II) cm^{-1}, ^1H NMR ($CDCl_3$) δ 6.90 (d, 1H, $J_{2',NH'}$ = 10.0 Hz, N*H*′), 6.18 (d, 1H, $J_{2,NH}$ = 9.2 Hz, N*H*), 5.32 (s, 1H, H-1), 4.55 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 101.59 (C-1), 99.76 (C-1′). Since the glycosyl donor **3** and the acceptor **10** were prepared, α-stereoselective glycosidation of Fuc for constructing trisaccharide **14** was performed using the fucosyl donor **3** and the acceptor **10** by means of conditions similar to those used for the production of disaccharide **6** to give crystalline **11** having an α-fucosyl linkage in 74% yield, $[\alpha]_D^{28}$ −89.0° (*c* 1.00, $CHCl_3$), mp 160.2–164.7 °C, ^1H NMR ($CDCl_3$) δ 5.30 (s, 1H, H-1), 5.14 (d, 1H, $J_{1'',2''}$ = 4.0 Hz, H-1″), 4.52 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 101.18 (C-1), 99.76 (C-1′), 95.98 (C-1″). Exchange of the protections in the fucose moiety in **11** from the benzyl group to acetyl group proceeded in EtOAc in the presence of Pd/C under hydrogen atmosphere, followed by the usual acetylation to yield acetate **13** in 94% yield in two steps, $[\alpha]_D^{27}$ −144.5° (*c* 0.67, $CHCl_3$), mp 298.4–300.6 °C (dec.), ^1H NMR ($CDCl_3$) δ 5.38 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1″), 5.23 (s, 1H, H-1), 4.54 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1′), ^{13}C NMR ($CDCl_3$) δ 101.08 (C-1), 99.80 (C-1′), 93.39 (C-1″). The next step was the fission of the 1,6-anhydro ring in **13** involving a highly acid-labile fucosidic bond. In order to avoid such trouble, we used BF_3–OEt_2 as a Lewis acid source for fission of the 1,6-anhydro ring.[15] Thus, acetolysis of **13** gave the corresponding acetate **14** as an anomeric mixture, in which anomeric configuration was estimated to be α/β = 3:2 on the basis of the results of ^1H NMR. Treatment of the anomeric mixture **14** with TMSOTf[16] produced the unstable oxazoline derivative **15** in 97% yield, which has a characteristic coupling constant in ^1H NMR at δ 5.93 (d, 1H, $J_{1,2}$ = 6.42 Hz, H-1). Because of the accessibility and appropriate methylene chain length, 6-acrylamidohexan-1-ol[17] was selected as an alcoholic candidate for the glycosidation. Thus, PPTS-catalyzed glycosidation[18] of the oxazoline **13** with the alcohol proceeded in dichloroethane to afford the corresponding β-glycoside **16** in 68% yield, ^1H NMR ($CDCl_3$) δ 6.33 (dd, 1H, J_{trans} = 17.0 Hz and J_{gem} = 1.4 Hz, –CH=CH*H*$_{trans}$), 6.18 (dd, 1H, J_{cis} = 10.2 Hz, –CH=CH*H*$_{cis}$), 5.66 (dd, 1H, –C*H*=), ^{13}C NMR ($CDCl_3$) δ 130.99 (=*C*H_2), 126.28 (–*C*H=), 100.98 (C-1), 100.09 (C-1′), 95.15 (C-1″). Since the aglycon of **16** was highly polymerizable by itself, elemental analysis was performed after reductive treatment of the terminal C=C double bond to yield the corresponding propanamide **17**, $[\alpha]_D^{28}$ −105.6° (*c* 0.37, $CHCl_3$). Transesterification of **16** by the Zemplén manner[19] was performed and gave the acrylamide alcohol **1** as a white powder after lyophilization in 96% yield, which was further transformed into the corresponding propanamide **18** in the same manner as that described for **16** to give **18** in 99% yield, $[\alpha]_D^{30}$ −77.3° (*c* 0.41, CH_3OH), ^{13}C NMR (CD_3OD) δ 102.93 (C-1), 102.42 (C-1′), 100.13 (C-1″).

Given the success of the preparation of the polymerizable fucosyl chitobiose **1**, our attention turned to polymerization ability of the carbohydrate monomer **1**. In our previous synthetic study of artificial glycoconjugates, bioactive carbohydrate moieties were incorporated into the polymer chain as pendant-type epitopes using linear polymers.[7,20] Therefore, the same strategy as that used for making macromolecules having carbohydrate chains[21] was applied to the glycomonomer **1**. Thus, the homopolymerization of **1** was initially tried and the reaction proceeded efficiently in an aqueous media to afford white powdery glycopolymer **19** in 86% yield after chromatographic purification by Sephadex G-50 followed by lyophilization, $\overline{Mn}$ 13 kDa, $\overline{Mw}$ 21 kDa, $\overline{Mw}/\overline{Mn}$1.6, ^1H NMR δ (D_2O) 5.11 (br s, 1H, H-1″), 4.52 (br s, 1H, H-1), 4.48 (br s, 1H, H-1′), ^{13}C NMR (D_2O) δ 100.87 (C-1), 100.35 (C-1′), 98.48

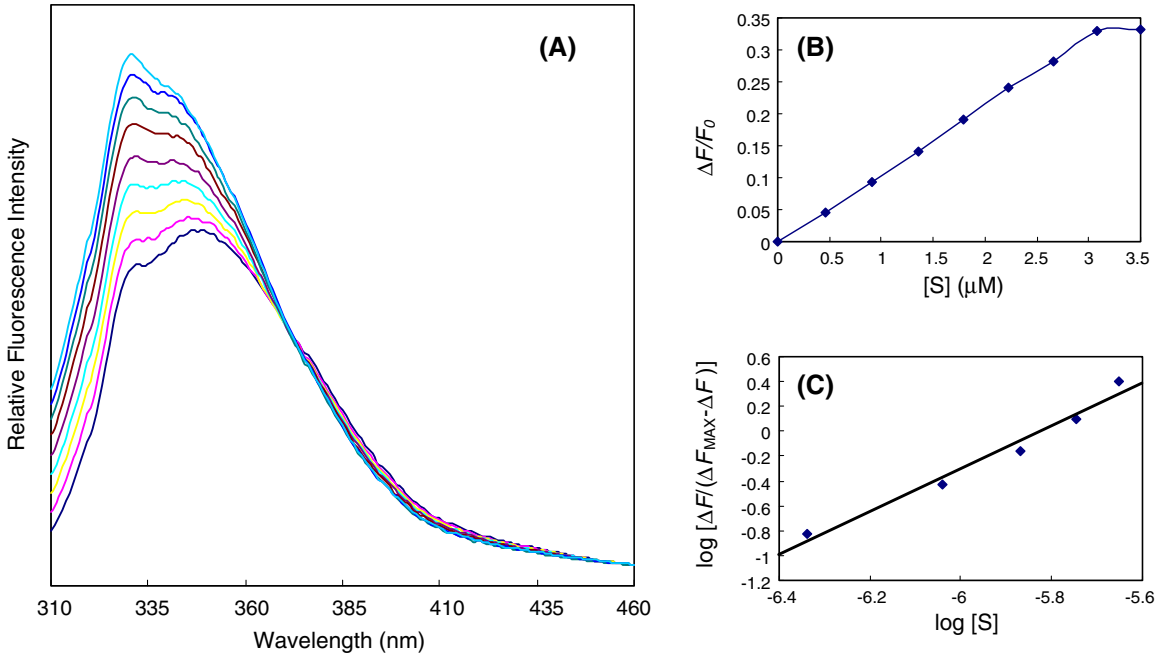


Figure 2. (A) Changes in fluorescence emission spectra of WGA (0.65 μM) upon the addition of 20-μL aliquots of glycopolymer **19** (69.1 μM) at 5 °C. The excitation wavelength (λ_{ex}) was used at 295 nm. (B) Plots of $\Delta F/F_0$ versus [*S*], where ΔF is change in the intensity at 348 nm of WGA with various concentrations of **19**, F_0 is the intensity of WGA alone, and [*S*] is total ligand concentration. (C) Hill plots of log $[\Delta F/(\Delta F_{MAX} - \Delta F)]$ versus log [*S*].

(C-1″). From the weight-average molecular weight ($\overline{Mw}$) of the glycopolymer **19**, the degree of polymerization was estimated to be 29. Biological activity of the glycopolymer **19** against a plant lectin as a model protein was preliminarily examined on the basis of fluorescence measurement using wheat germ agglutinin (WGA), which binds to an *N*-acetyl-D-glucosamine and its oligomers.[22] Figure 2 shows fluorescence emission of WGA and of its complexes with various concentrations of glycopolymer **19**. When the protein was saturated with the glycopolymer **19**, the maximum fluorescence intensity was enhanced by 35% and the emission maximum was downfield-shifted from 347 nm to 341 nm (−6 nm). The results suggested that the environment of tryptophan residues located at or near the binding sites of WGA is altered from hydrophilic to relatively more hydrophobic upon interaction with the glycopolymer **19**. In a plot of $\Delta F/F_0$ versus [*S*] based on sugar unit concentration followed by analyses using the Hill equation,[23] association constant K_a was estimated to be 6.9×10^5 (M^{-1}). These results clearly indicated that the lectin showed a higher affinity for the glycopolymer **19** than that for GlcNAc and oligomeric GlcNAc.[24] In addition, interference of the fucose residue located on the glucosamine unit of the reducing end was not observed in this study.

In summary, we have successfully described an efficient preparation of a fucosyl chitobiose [GlcNAcβ1→4(Fucα1→3)GlcNAc] derivative and its chemical modification to provide a water-soluble glycomonomer. Homopolymerization using the glycomonomer was performed to give a water-soluble glycopolymer having $\overline{Mw}$ 21 KDa in high yield, which displayed highly clustered glycoepitopes. Plant lectin binding activity of the glycopolymer was preliminarily examined on the basis of fluorescence measurement and showed enhancement of the affinity due to a sugar-clustering effect. Further polymerization conditions including copolymerization conditions with acryl amide are now under investigation, and the biological activities of the glycopolymers as the epitope for allergy will also be examined. The results of these experiments will be reported in the near future.

References and notes

1. For example, see: Ishihara, H.; Takahashi, N.; Oguri, S.; Tejima, S. *J. Biol. Chem.* **1974**, *254*, 10715–10719.
2. (a) Ogawa, H.; Hikijima, A.; Amano, M.; Kojima, K.; Fukushima, H.; Ishizuka, I.; Kurihara, Y.; Matsumoto, I. *Glycoconjugate J.* **1996**, *13*, 555–566; (b) Ueda, H.; Ogawa, H. *Trend Glycsci. Glycotech.* **1999**, *11*, 413–428. and references cited therein.
3. For example, see: Kornfeld, R.; Kornfeld, S. *Ann. Rev. Biochem.* **1985**, *54*, 631–664. and references cited therein.
4. Nishimura, S.-I.; Kurita, K.; Kuzuhara, H. *Chem. Lett.* **1990**, 1611–1614.
5. Oguri, S.; Ishihara, H.; Tejima, S. *Chem. Pharm. Bull.* **1980**, *28*, 3196–3202.
6. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
7. Matsuoka, K.; Kohzu, T.; Hakumura, T.; Koyama, T.; Hatano, K.; Terunuma, D. *Tetrahedron Lett.* **2009**, *50*, 2593–2596. The Le^x trisaccharide is composed of Galβ1→4(Fucα1→3)GlcNAc and the synthetic construction of the trisaccharidic unit was achieved by means of Gabriel amine synthesis as the key reaction.
8. Sakairi, N.; Takahashi, S.; Wang, F.; Ueno, Y.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1756–1758.
9. Son, S.-H.; Tano, C.; Furuike, T.; Sakairi, N. *Carbohydr. Res.* **2009**, *344*, 285–290.
10. Matsui, H.; Furukawa, J.; Awano, T.; Nishi, N.; Sakairi, N. *Chem. Lett.* **2000**, 326–327.
11. Myszka, H.; Bednarczyk, D.; Najder, M.; Kaca, W. *Carbohydr. Res.* **2003**, *338*, 133–141.
12. Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 251–260.
13. Matsuoka, K.; Onaga, T.; Mori, T.; Sakamoto, J.-I.; Koyama, T.; Sakairi, N.; Hatano, K.; Terunuma, D. *Tetrahedron Lett.* **2004**, *45*, 9383–9386.
14. Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580–1582.
15. Umino, A.; Hinou, H.; Matsuoka, K.; Terunuma, D.; Takahashi, S.; Kuzuhara, H. *J. Carbohydr. Chem.* **1998**, *17*, 231–239.
16. Nakabayashi, S.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1986**, *150*, C7–C8.
17. Sarfati, S. R.; Pochet, S.; Neumann, J.-M.; Igolen, J. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1065–1070.
18. Yoshino, T.; Sato, K.-I.; Wanme, F.; Takai, I.; Ishido, Y. *Glycoconjugate J.* **1992**, *9*, 287–291.
19. Zemplén, G.; Pacsu, E. *Ber.* **1929**, *62*, 1613–1614.
20. (a) Miyagawa, A.; Kurosawa, H.; Watanabe, T.; Koyama, T.; Terunuma, D.; Matsuoka, K. *Carbohydr. Polym.* **2004**, *57*, 441–450; (b) Matsuoka, K.; Goshu, Y.; Takezawa, Y.; Mori, T.; Sakamoto, J.-I.; Yamada, A.; Onaga, T.; Koyama, T.; Hatano, K.; Snyder, P. W.; Toone, E. J.; Terunuma, D. *Carbohydr. Polym.* **2007**, *69*, 326–335; (c) Matsuoka, K.; Takita, C.; Koyama, T.; Miyamoto, D.; Yingsakmongkon, S.; Hidari, K. I. P. J.; Jampangern, W.; Suzuki, T.; Suzuki, Y.; Hatano, K.; Terunuma, D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3826–3830.
21. Nishimura, S.-I.; Matsuoka, K.; Kurita, K. *Macromolecules* **1990**, *23*, 4182–4184.
22. (a) Nagata, Y.; Burger, M. *J. Biol. Chem.* **1972**, *247*, 2248–2250; (b) Nagata, Y.; Burger, M. *J. Biol. Chem.* **1974**, *249*, 3116–3122.
23. (a) Lotan, R.; Sharon, N. *Biochem. Biopys. Res. Commun.* **1973**, *55*, 1340–1346; (b) Privat, J.-P.; Delmotte, F.; Mialonier, G.; Bouchard, P.; Monsigny, M. *Eur. J. Biochem.* **1974**, *47*, 5–14.
24. Nishimura, S.-I.; Furuike, T.; Matsuoka, K.; Maruyama, K.; Nagata, K.; Kurita, K.; Nishi, N.; Tokura, S. *Macromolecules* **1994**, *27*, 4876–4880.