

Article

Synthesis, Antigenicity Against Human Sera and Structure-Activity Relationships of Carbohydrate Moieties from *Toxocara* larvae and Their Analogues

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Abstract: Stereocontrolled syntheses of biotin-labeled oligosaccharide portions containing the Gal β 1-3GalNAc core of the TES-glycoprotein antigen obtained from larvae of the parasite *Toxocara* and their analogues have been accomplished. Trisaccharides Fuc2Me α 1-2Gal4Me β 1-3GalNAc α 1-OR (**A**), Fuc α 1-2Gal4Me β 1-3GalNAc α 1-OR (**B**), Fuc2Me α 1-2Gal β 1-3GalNAc α 1-OR (**C**), Fuc α 1-2Gal β 1-3GalNAc α 1-OR (**D**) and a disaccharide Fuc2Me α 1-2Gal4Me β 1-OR (**E**) (R = biotinylated probe) were synthesized by block synthesis using 5-(methoxycarbonyl)pentyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4-*O*-benzyl-2-deoxy- α -D-galactopyranoside as a common glycosyl acceptor. We examined the antigenicity of these five oligosaccharides by enzyme linked immunosorbent assay (ELISA). Our results demonstrate that the *O*-methyl groups in these oligosaccharides are important for their antigenicity and the biotinylated oligosaccharides **A**, **B**, **C** and **E** have high serodiagnostic potential to detect infections caused by *Toxocara* larvae.

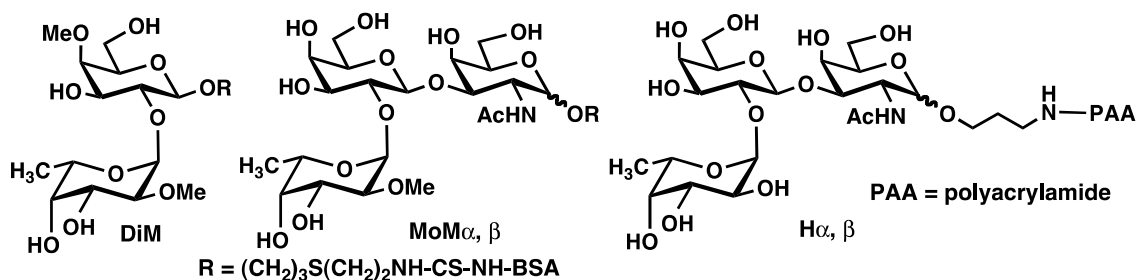
Keywords: glycoprotein; *Toxocara* larvae; host-parasite interaction; stereocontrolled synthesis; antigenicity

1. Introduction

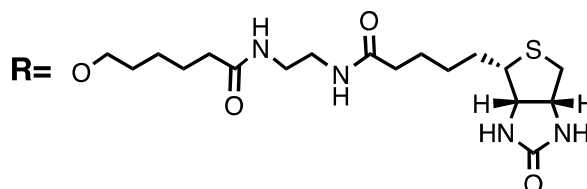
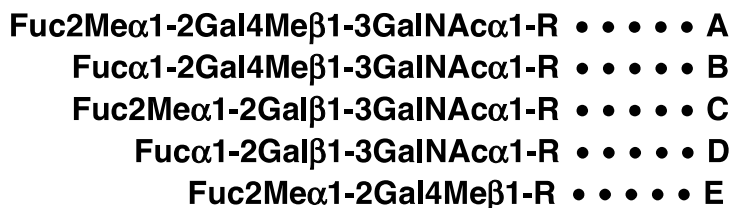
In the course of our studies on unique glycoconjugates found in parasites, we have synthesized various glycosphingolipids and carbohydrate portions of glycoproteins with the aim of elucidating the mechanisms of host-parasite interactions [1–7]. In previous studies we have synthesized unusual carbohydrates from the parasites *Echinococcus multilocularis* [1,3,6], *Schistosoma mansoni* [2] and porcine roundworm (nematode) *Ascaris suum* [7]. In this paper we describe the synthesis of the carbohydrate portion of glycoproteins from *Toxocara canis* and *T. cati*. *T. canis* and *T. cati* are parasitic roundworms and are widely distributed in dogs and cats. Both nematodes cause severe infections in a human host affecting eyes, liver and the central nervous system [8,9].

Khoo *et al.*, isolated *Toxocara* excretory-secretory (TES) antigen, a family of glycoproteins that are heavily *O*-glycosylated from the culture media of *T. canis* and *T. cati* larvae [10]. The TES antigen of *T. canis* is a mixture of mucin-type glycoproteins, containing a Fuc1-2Gal1-3GalNAc structure, and it has been found that the fucose part was *O*-methylated at the 2-position and approximately 50% of the galactose part was *O*-methylated at the 4-position, *i.e.*, it contains the following two sequences: 2-*O*-Me-Fucp(α 1 $\rightarrow$ 2)-4-*O*-MeGalp(β 1 $\rightarrow$ 3)-GalNAcp and 2-*O*-Me-Fucp(α 1 $\rightarrow$ 2)-Galp(β 1 $\rightarrow$ 3)-GalNAcp. Interestingly, although the di-*O*-methylated trisaccharide was found in both *T. canis* and *T. cati* the mono-*O*-methylated sugar was found only in *T. canis*. These structures are similar to the human blood group antigen H, Fucp(α 1 $\rightarrow$ 2)-Galp(β 1 $\rightarrow$ 3)-GalNAcp which does not have any *O*-methyl substitution. Maizels and Kosma *et al.*, synthesized di-*O*-methylated disaccharide (DiM) and mono-*O*-methylated trisaccharide (MoM α,β conjugated to BSA, Figure 1) and studied their antigenicity [11,12]. The results showed that the sera from infected patients recognized the DiM more strongly than MoM α,β [11]. However, the both groups have not studied the effects of the di-*O*-methylated trisaccharide.

Figure 1. Structures of previously prepared DiM, MoM α,β and the human blood group antigen H.



In this paper we report the synthesis of biotin-tagged oligosaccharides A–E (Figure 2), two glycan portions of the glycoprotein antigen of *T. canis* (A and C) and three analogues (B, D and E), to elucidate the antigenicity of the oligosaccharides against sera of *T. canis* infected patients by enzyme-linked immunosorbent assay (ELISA).

Figure 2. Structures of target compounds A–E.

2. Results and Discussion

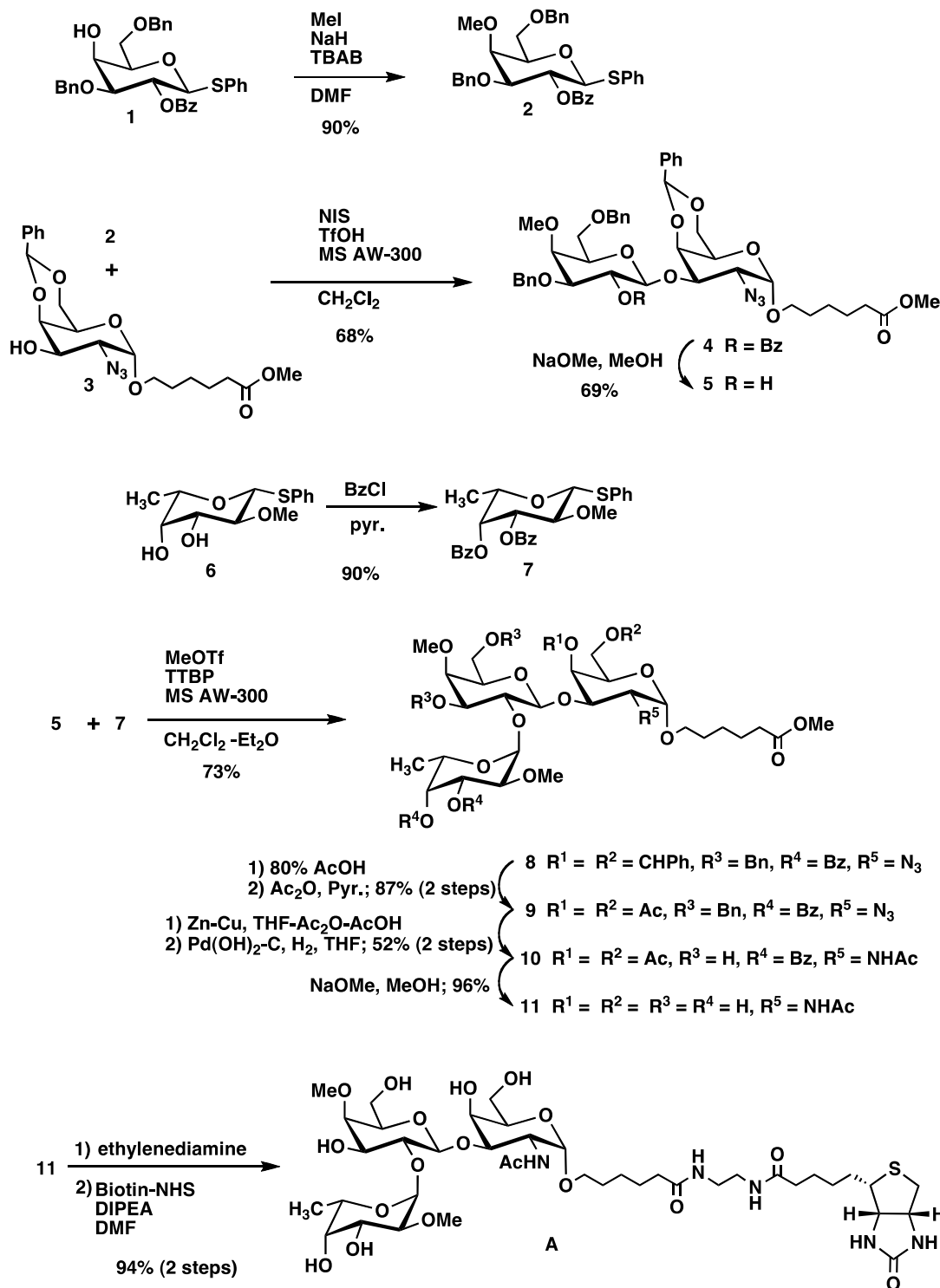
2.1. Chemical Synthesis

Syntheses of the target oligosaccharides **A–E**: In all cases we selected 5-(methoxycarbonyl)pentyl group as the protecting group of reducing end, because this group can be conveniently used for conjugation with biotin for the use in ELISA assay as previously shown by us [1]. The synthetic routes for target compounds **A–E** are outlined in Schemes 1–5.

Monosaccharide derivative **3** was chosen as a common acceptor for the synthesis of oligosaccharides **A–D** (Scheme 1). Galactopyranosyl donor **2** was obtained from phenyl 3,6-di-*O*-benzyl-2-*O*-benzoyl-1-thio- β -D-galactopyranoside (**1**) [3], by methylation using methyl iodide. Glycosylation of **2** with **3** [1] in the presence of *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) [13] and AW-300 molecular sieves (MS AW-300) in CH_2Cl_2 afforded desired disaccharide (**4**) in 68% yield. The nature of the new glycosidic linkage was determined by the vicinal coupling constant of the anomeric proton (H-1 of Gal, $\delta = 4.79$ ppm, $J = 8.0$ Hz). Removal of the benzoyl groups in **4** under Zemplén conditions gave disaccharide acceptor **5** which was used directly for the next glycosylation step. To prepare the Fuc α 1-2Gal sequence α -stereoselectively, we selected 3,4-di-acyl protected fucosyl donor **7** [14], which was obtained from **6** [11] by benzylation. Previous studies have indicated that 3,4-di-acyl-protected fucopyranosyl donors induce high α -selectivity in fucosylation [2]. Glycosylation of **5** with **7** in the presence of methyl trifluoromethanesulfonate (MeOTf) [15], tri-*tert*-butyl pyridine (TTBP) and MS AW-300 in CH_2Cl_2 afforded desired trisaccharide (**8**) in 73% yield. The nature of the new glycosidic linkage was determined by the coupling constant of anomeric proton (H-1 of Fuc, $\delta = 5.67$ ppm, $J = 3.7$ Hz). Global deprotection was performed by a combination of protection/deprotection steps. At first, the benzylidene acetal of **8** was removed by acidic hydrolysis followed by *O*-acetylation using acetic anhydride in pyridine to afford **9** in 87% yield. Next, the azide group was converted to an acetamide by reduction with Zn/Cu AcOH in the presence of acetic anhydride. The obtained *N*-acylated product **9** was debenzylated by catalytic hydrogenolysis using Pearlman's catalyst followed by *O*-acetylation to give **10** (52%), which was deacetylated to produce deprotected trisaccharide **11** in 96% yield. The 5-(methoxycarbonyl)pentyl glycoside **11** was converted into the ethylenediamine

monoamide by exposure to ethylenediamine and conjugated to biotin to afford trisaccharide-biotin conjugate **A** in 94% yield (Scheme 1).

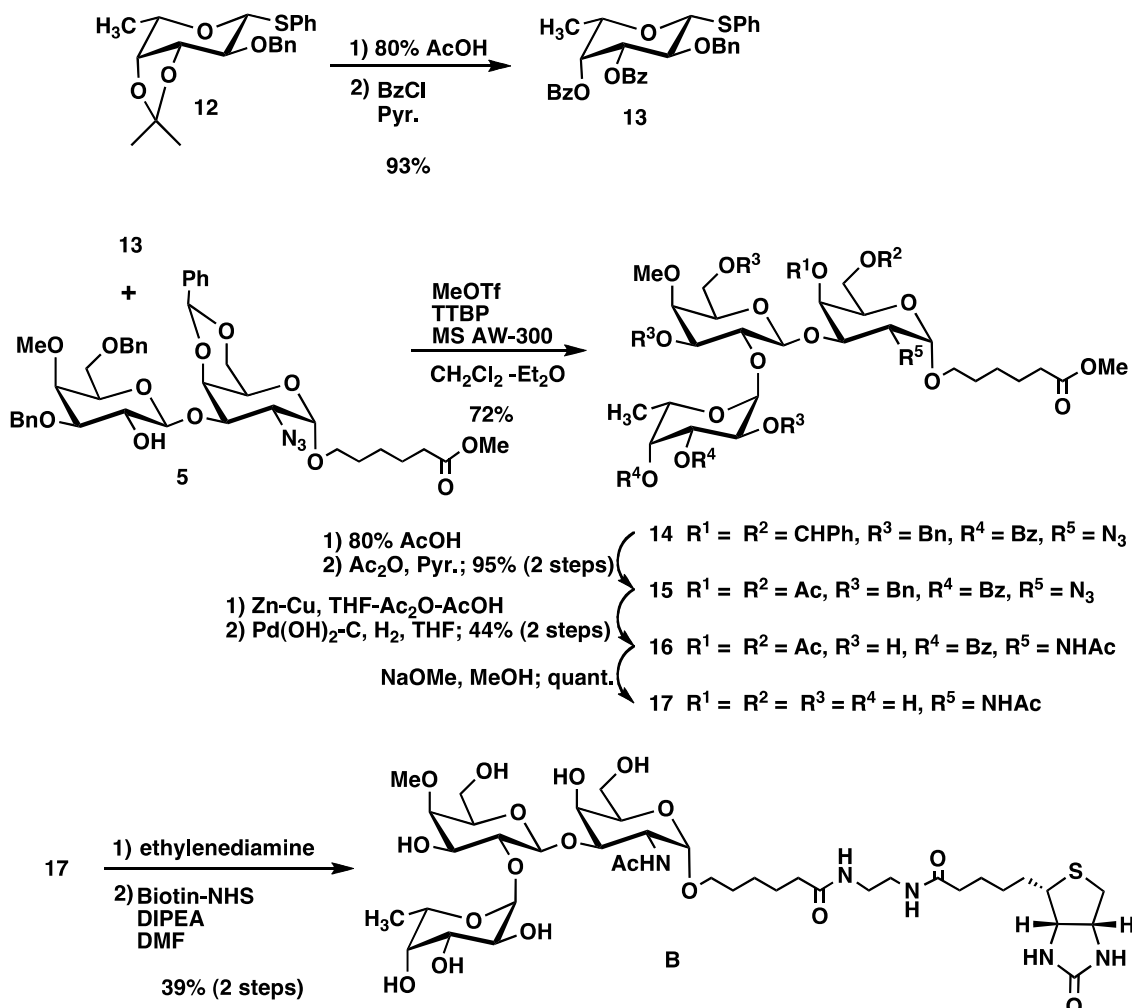
Scheme 1. Synthesis of di-methylated trisaccharide **A**.



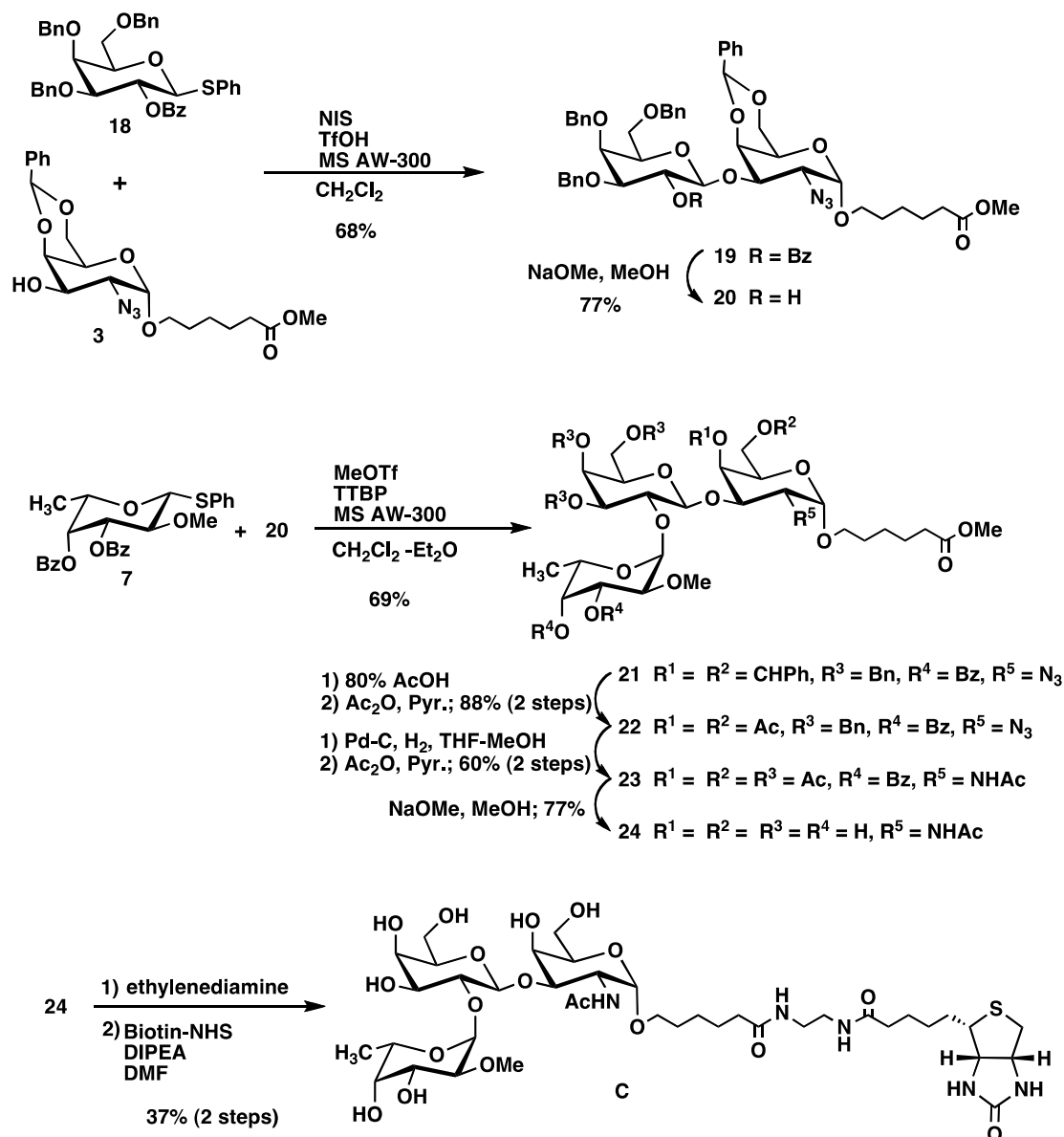
Non-methylated fucopyranosyl donor **13** was synthesized from known donor **12** [16] by two-step procedure (Scheme 2). Hydrolysis of the isopropylidene group in **12** with aqueous AcOH and subsequent benzylation produced fucopyranosyl donor **13**. Trisaccharide **14** was synthesized by a coupling of the fucopyranosyl donor **13** with the disaccharide acceptor **5**. The presence of an α

fucosidic linkage in **14** was indicated by a doublet at δ 5.71 ppm showing small homonuclear coupling constant of 3.3 Hz in the ^1H -NMR spectrum. Deprotection and biotinylation were performed as described for compound **A** to provide target trisaccharide **B** (Scheme 2).

Scheme 2. Synthesis of mono-methylated trisaccharide **B**.

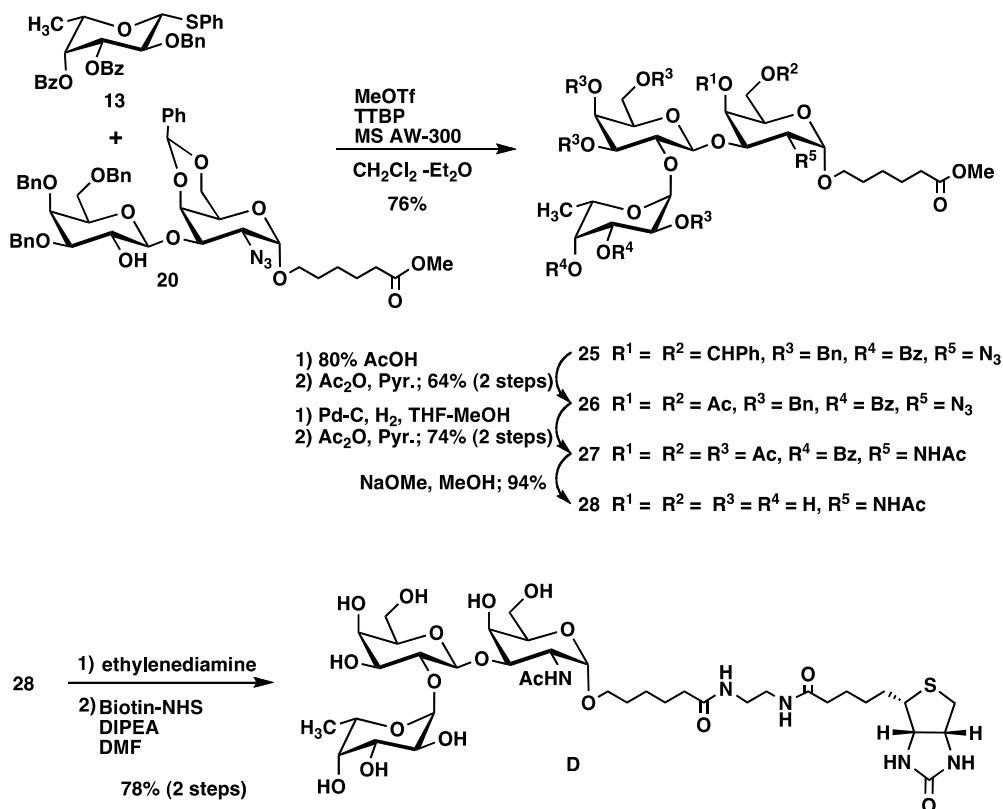
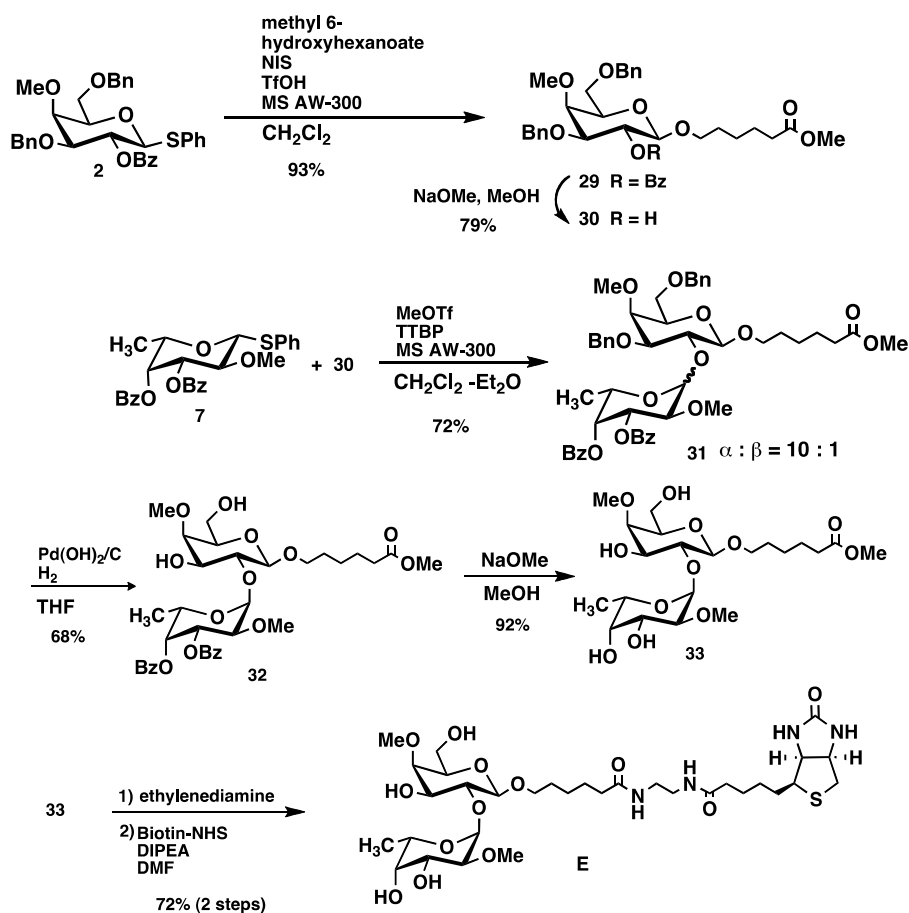


The synthesis of the trisaccharide **C** is outlined in Scheme 3. As **C** does not contain the *O*-methyl substitution in the galactose residue, we chose phenyl 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-1-thio- β -D-galactopyranoside (**18**) [17] as a galactosyl donor. Glycosylation of acceptor **3** with donor **18** in the presence of NIS/TfOH provided disaccharide **19** in 68% yield. Selective removal of the benzoyl group in **19** with NaOMe produced disaccharide acceptor **20**. Fucosylation of **20** with the donor **7** afforded trisaccharide **21**, whose benzylidene acetal was removed as described for **8** and **14** to give **22** in 88% yield. Reduction of the azide group together with removal of benzyl groups of **22** was initially performed as described for compounds **9** and **15** using Zn/Cu and $\text{Pd}(\text{OH})_2$. However, this resulted only in a 24% yield of **23**. In contrast, significantly improved yield was obtained by catalytic hydrogenation of **22** with Pd-C followed by acetylation to produce the desired trisaccharide **23** in 60% yield. Deacetylation and biotinylation were performed as described for compound **A** to give target trisaccharide **C** (Scheme 3).

Scheme 3. Synthesis of mono-methylated trisaccharide **C**.

Non-methylated trisaccharide **D** was synthesized from the glycosyl donor **13** and acceptor **20** as described for compound **C** in a good yield (Scheme 4).

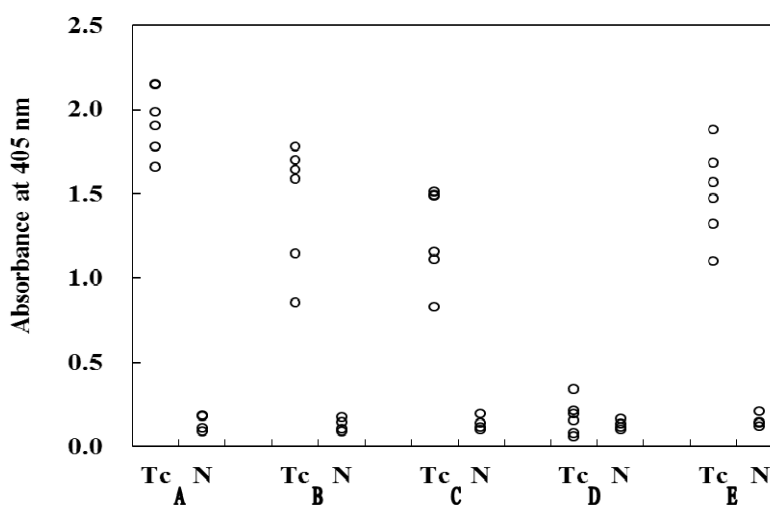
Dimethylated disaccharide **E**, which does not contain a GalNAc residue was synthesized from galactosyl acceptor **30** with the fucosyl donor **7** as outlined in Scheme 5. Compound **30** was obtained by coupling of galactose-based thiophenyl glycoside **2** to methyl 6-hydroxyhexanoate and subsequent debenzoylation using standard conditions. Glycosylation of the acceptor **30** with **7** in the presence of MeOTf and TTBP afforded desired disaccharide **31** in 72% yield. Unfortunately, the anomeric ratio of the fucopyranosyl linkage was 10:1 (α : β , from ¹H-NMR). Although we cannot properly explain the reason of it, we presume the influence by the absence of a GalNAc derivative in monosaccharide acceptor **30**. Deprotection of benzyl groups by catalytic hydrogenolysis using Pearlman's catalyst provided **32**. It was possible to separate the major α -anomer from the minor β -glycoside at this stage. Deacylation and biotinylation were performed as described for compound **A** to provide target trisaccharide **E** in a good yield (Scheme 5).

Scheme 4. Synthesis trisaccharide **D** without *O*-methyl substitution.**Scheme 5.** Synthesis of di-methylated disaccharide **E**.

2.2. Antigenicity of Oligosaccharides by ELISA

The reactivity of the five biotin-labeled oligosaccharides **A** (natural), **B**, **C** (natural), **D** and **E** to patients' sera was examined using streptavidin-coated microplates. In four of the five oligosaccharides, except for **D**, the antibody response of the *T. canis*-infected patient group (Tc) was significantly high compared with that of the normal healthy (N) group (Figure 3). However, the non-methylated trisaccharide **D** did not show any antigenicity to the patients' sera. This is in contrast to previous findings by Kosma and Maizels, who have reported marked differences in the antigenicity between dimethylated disaccharide (DiM) and monomethylated trisaccharide (MoM α,β in the form of BSA conjugates [11]. Our study indicates that not only the natural monomethylated trisaccharide **C** containing a mono-Fuc2Me moiety but also non-natural trisaccharide **B** containing a mono-Gal4Me moiety have the antigenicity. Moreover, the antigenicity of di-methylated trisaccharide **A** was stronger than that of di-methylated disaccharide **E** indicating that the GalNAc portion contributes to the antigenicity. Significant differences of **A**–**C** and **E** were observed between Tc patient group and N group ($p < 0.05$, Student's *t* test).

Figure 3. ELISA reaction between human sera and synthesized oligosaccharides **A**–**E**. Tc: toxocariasis patient group; N: normal healthy group.



3. Experimental

3.1. General Procedures

Optical rotations were measured with a Jasco P-1020 digital polarimeter (Tokyo, Japan). ^1H (500 MHz) and ^{13}C -NMR (125 MHz) spectra were recorded with a Varian 500 FT NMR spectrometer (Palo Alto, CA, USA). Me_4Si and acetone were used as internal standards for CDCl_3 and D_2O , respectively. MALDI-TOFMS was recorded on an AB SCIEX Voyager RP mass spectrometer (Framingham, MS, USA). High-resolution mass spectra were recorded on a JEOL JMS-700 (Tokyo, Japan) under FAB conditions. TLC was performed on Silica Gel 60 F254 (E. Merck, Darmstadt, Germany) with detection by quenching of UV fluorescence and by charring with 10% H_2SO_4 . Column chromatography was carried out on Silica Gel 60. Phenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio- β -D-

galactopyranoside (**1**) [3], 5-(methoxycarbonyl)pentyl 2-azide-4,6-*O*-benzylidene-2-deoxy- β -D-galactopyranoside (**3**) [1], phenyl 2-*O*-methyl-1-thio- β -L-fucopyranoside (**6**) [11], were prepared as reported.

Phenyl 2-O-benzoyl-3,6-di-O-benzyl-4-O-methyl-1-thio- β -D-galactopyranoside (**2**). Sodium hydride was added portionwise to a stirred mixture of compound **1** (253 mg, 0.45 mmol), MeI (56.5 μ L, 0.91 mmol) and *n*-Bu₄NBr (176 mg, 0.56 mmol) in DMF (5.0 mL) at -15°C . The mixture was stirred for 3 h at -15°C , and MeOH was added dropwise to destroy excess NaH. The mixture was diluted with EtOAc, washed with water, dried (MgSO₄), and concentrated *in vacuo*. The product was purified by silica gel column chromatography (6:1 hexane-ethyl acetate) to give **2** (234 mg, 90%). $[\alpha]_{\text{D}}^{+50.6}$ (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 8.02–7.12 (m, 20H, Ar), 5.61 (t, 1H, $J_{1,2} = J_{2,3} = 9.8$ Hz, H-2), 4.76 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1), 4.67 and 4.53 (each d, 2H, $J_{\text{gem}} = 12.8$ Hz, PhCH₂), 4.58 and 4.53 (each d, 2H, $J_{\text{gem}} = 11.6$ Hz, PhCH₂), 3.79–3.67 (m, 5H, H-3, H-4, H-5, H-6a, H-6b), 3.58 (s, 1H, OCH₃). ¹³C-NMR (CDCl₃): δ 165.2, 137.8, 137.5, 134.0, 133.0, 129.8, 128.7, 128.5, 128.3, 127.9, 127.8, 127.6, 127.3, 87.2 (C-1), 80.8 (C-4), 77.5 (C-3), 75.2 (C-5), 73.6 (PhCH₂), 71.6 (PhCH₂), 70.3 (C-2), 68.5 (C-6), 61.3 (OCH₃). MALDI-TOFMS: calcd for C₃₄H₃₄O₆SNa, *m/z* 593.2; found, *m/z* 593.8 [M+Na]⁺. HR-FABMS: calcd for C₃₄H₃₄O₆SNa, *m/z* 593.1974; found, *m/z* 593.1958 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 2-O-benzoyl-3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (**4**). A mixture of **2** (203 mg, 0.37 mmol), **3** (125 mg, 0.30 mmol) and powdered MS AW300 (300 mg) in dry CH₂Cl₂ (3 mL) was stirred under Ar atmosphere for 2 h at room temperature, then cooled to -40°C . NIS (160 mg, 0.71 mmol) and TfOH (6.3 μ L, 71.2 μ mol) were added to the mixture, which was stirred for 1 h at -40°C , then neutralized with Et₃N. The precipitates were filtered off and washed with CHCl₃. The combined filtrate and washings were successively washed with saturated aqueous Na₂S₂O₃ and water, dried (MgSO₄), and concentrated. The product was purified by silica gel column chromatography (5:2 hexane-ethyl acetate) to give **4** (178 mg, 68%). $[\alpha]_{\text{D}}^{+94.6}$ (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 8.05–7.11 (m, 20H, Ar), 5.59 (dd, 1H, $J_{1',2'} = 8.0$ Hz, $J_{2',3'} = 9.8$ Hz, H-2'), 5.45 (s, 1H, PhCH), 4.92 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.79 (d, 1H, $J_{1',2'} = 8.0$ Hz H-1'), 4.68 and 4.52 (each d, 2H, $J_{\text{gem}} = 12.2$ Hz, PhCH₂), 4.57 (s, 2H, PhCH₂), 4.37 (d, 1H, $J_{3',4'} = 3.1$ Hz H-4), 4.15 (dd, 1H, $J_{5,6a} = 12.4$ Hz, $J_{6a,6b} = 1.4$ Hz, H-6a), 4.09 (dd, 1H, $J_{2,3} = 11.0$ Hz, $J_{3,4} = 3.1$ Hz, H-3), 3.92 (dd, 1H, $J_{5,6b} = 12.4$ Hz, $J_{6a,6b} = 1.2$ Hz, H-6b), 3.73–3.48 (m, 14H, H-2, H-5, H-3', H-4', H-5', H-6'a, H-6'b, OCH₃×2, OCH₂CH₂ a), 3.47–3.42 (m, 1H, OCH₂CH₂ b), 2.33–2.28 (m, 2H), 1.67–1.59 (m, 4H), 1.41–1.35 (m, 2H). ¹³C-NMR (CDCl₃): δ 174.0, 165.3, 137.9, 137.7, 137.6, 132.8, 129.8, 128.6, 128.5, 128.3, 128.2, 127.94, 127.85, 127.69, 127.65, 126.2, 102.5 (C-1'), 100.4 (CHPh) 98.7 (C-1), 79.9 (C-3'), 75.8 (C-4), 75.1 (C-4'), 74.1 (C-3), 73.69 (C-5'), 73.65 (PhCH₂), 71.7 (PhCH₂), 71.5 (C-2'), 69.0 (C-6), 68.6 (C-6'), 68.2 (OCH₂CH₂), 63.2 (C-5), 61.5 (OCH₃), 58.8 (C-2), 33.9, 29.0, 25.6, 24.7. MALDI-TOFMS: calcd for C₄₈H₅₅N₃O₁₃Na, *m/z* 904.4; found, *m/z* 905.0 [M+Na]⁺. HR-FABMS: calcd for C₄₈H₅₅N₃O₁₃Na, *m/z* 904.3633; found, *m/z* 904.3594 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (**5**). A solution of **4** (301 mg, 0.34 mmol) and NaOMe (55 mg, 1.02 mmol) in MeOH (10 mL) was stirred at 50°C for 48 h, then neutralized with Amberlite

IR 120 [H⁺]. The mixture was filtered off and concentrated. The product was purified by silica gel column chromatography (2:1 hexane-ethyl acetate) to give **5** (182 mg, 69%). [α]_D +88.5 (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 7.51–7.25 (m, 15H, Ar), 5.48 (s, 1H, PhCH), 5.04 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.81 and 4.76 (each d, 2H, J_{gem} = 12.0 Hz, PhCH₂), 4.55 (s, 2H, PhCH₂), 4.47 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.35 (d, 1H, $J_{3',4'}$ = 3.2 Hz, H-4), 4.19 (dd, 1H, $J_{5,6a}$ = 11.4 Hz, $J_{6a,6b}$ = 1.2 Hz, H-6a), 4.11 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 3.95–3.88 (m, 3H, H-2, H-2', H-6b), 3.73–3.60 (m, 9H, H-5, H-4', H-5', H-6'a, H-6'b, OCH₂CH₂ a, OCH₃), 3.56 (s, 3H, OCH₃), 3.52–3.48 (m, 1H, OCH₂CH₂ b), 3.45 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, $J_{3',4'}$ = 3.0 Hz, H-3'), 2.32 (t, 2H, J' = 7.7 Hz), 1.69–1.62 (m, 4H), 1.43–1.39 (m, 2H). ¹³C-NMR (CDCl₃): δ 174.1, 138.3, 137.9, 137.7, 128.9, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 127.6, 126.3, 105.1 (C-1'), 100.8 (CHPh) 98.5 (C-1), 81.6 (C-3'), 76.07 (C-4'), 76.06 (C-4), 75.3 (C-3), 73.8 (C-5'), 73.6 (PhCH₂), 72.9 (PhCH₂), 71.4 (C-2'), 69.1 (C-6), 68.6 (C-6'), 68.4 (OCH₂CH₂), 63.2 (C-5), 61.5 (OCH₃), 59.1 (C-2), 51.5 (OCH₃), 33.9, 29.1, 25.6, 24.6. MALDI-TOFMS: calcd for C₄₁H₅₁N₃O₁₂Na, *m/z* 800.3; found, *m/z* 800.2 [M+Na]⁺. HR-FABMS: calcd for C₄₁H₅₁N₃O₁₂Na, *m/z* 800.3370; found, *m/z* 800.3384 [M+Na]⁺.

Phenyl 3,4-di-O-benzoyl-2-O-methyl-1-thio-β-L-fucopyranoside (7). A solution of **6** (616 mg, 2.28 mmol) and benzoyl chloride (793 μL, 6.84 mmol) in pyridine (8 mL) was stirred for 16 h at room temperature. After completion of the reaction, the mixture was poured into ice H₂O and extracted with CHCl₃. The extract was washed sequentially with 5% HCl, aq NaHCO₃, and brine, dried (MgSO₄), and concentrated. The product was purified by silica gel column chromatography (10:1 hexane-ethyl acetate) to give **7** (976 mg, 90%). [α]_D −63.9 (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 7.96–7.26 (m, 15H, Ar), 5.63 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 5.35 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 4.71 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 4.01 (q, 1H, $J_{5,6}$ = 6.2 Hz, H-5), 3.63 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz, H-2), 3.49 (s, 3H, OCH₃), 1.32 (d, 1H, $J_{5,6}$ = 6.3 Hz, H-6). ¹³C-NMR (CDCl₃): δ 165.7, 165.5, 133.3, 133.13, 133.08, 132.6, 129.9, 129.6, 129.53, 129.49, 128.9, 128.5, 128.3, 127.8, 86.7 (C-1), 76.5 (C-2), 75.5 (C-3), 73.4 (C-5), 71.6 (C-4), 61.0 (OCH₃), 16.7 (C-6). MALDI-TOFMS: calcd for C₂₇H₂₆O₆SNa, *m/z* 501.1; found, *m/z* 501.6 [M+Na]⁺. HR-FABMS: calcd for C₂₇H₂₇O₆S, *m/z* 479.1528; found, *m/z* 479.1532 [M+H]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl-α-L-fucopyranosyl-(1→2)-3,6-di-O-benzyl-4-O-methyl-β-D-galactopyranosyl-(1→3)-2-azide-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (8). A mixture of **5** (98.3 mg, 0.13 mmol), **7** (121 mg, 0.25 mmol) and MS AW300 (200 mg) in dry CH₂Cl₂-Et₂O (1:1, 2.0 mL) was stirred for 2 h at room temperature. MeOTf (120 μL, 1.06 mmol) and TTBP (93.5 mg, 0.38 mmol) were added, and the mixture was stirred for 14 h at room temperature, then neutralized with Et₃N. The precipitates were filtrated off and washed with CHCl₃. The combined filtrate and washings were washed with water, dried (MgSO₄), and concentrated. The product was purified by silica gel column chromatography (3:2 hexane-EtOAc) to give **8** (105 mg, 73%). [α]_D −7.9 (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃) δ 8.02–7.23 (m, 25H, Ar), 5.67 (d, 1H, $J_{1'',2''}$ = 3.7 Hz, H-1''), 5.18 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.74 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'). ¹³C-NMR (CDCl₃): δ 103.3 (C-1'), 99.2 (C-1), 97.4 (C-1''). MALDI-TOFMS: calcd for C₆₂H₇₁N₃O₁₈Na, *m/z* 1168.5; found, *m/z* 1168.9 [M+Na]⁺. HR-FABMS: calcd for C₆₂H₇₁N₃O₁₈Na, *m/z* 1168.4630; found, *m/z* 1168.4591 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-di-O-acetyl-2-azide-2-deoxy- α -D-galacto-pyranoside (**9**). A solution of **8** (167 mg, 0.15 mmol) in 80% AcOH (5.0 mL) was stirred at 70 °C for 2 h, then diluted with toluene and concentrated. The residue was acetylated with acetic anhydride (3.0 mL) in pyridine (5.0 mL). After the reaction was quenched with MeOH, toluene was added and co-evaporated several times. The product was purified by silica gel column chromatography (3:2 hexane-ethyl acetate) to give **9** (145 mg, 87%). $[\alpha]_D -3.8$ (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃). δ 8.05–7.26 (m, 20H, Ar), 5.70 (d, 1H, $J_{1'',2''} = 3.6$ Hz, H-1''), 5.18 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.74 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1'). ¹³C-NMR (CDCl₃): δ 103.3 (C-1'), 98.9 (C-1), 97.4 (C-1''). MALDI-TOFMS: calcd for C₅₉H₇₁N₃O₂₀Na, *m/z* 1164.4; found, *m/z* 1164.7 [M+Na]⁺. HR-FABMS: calcd for C₅₉H₇₁N₃O₂₀Na, *m/z* 1164.4529; found, *m/z* 1164.4508 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-di-O-acetyl-2-acetamide-2-deoxy- α -D-galactopyranoside (**10**). A mixture of **9** (145 mg, 0.13 mmol) and Zn-Cu (600 mg) in THF-AcOH-Ac₂O (3:2:1, 6.0 mL) was stirred for 30 min. at room temperature. After completion of the reaction, the mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel column chromatography (6:1 toluene-acetone) to give an acetamide compound (128 mg). A solution of this compound in THF (2.0 mL) was hydrogenolysed (0.4 MPa) under hydrogen in the presence of 10% Pd/C (100 mg) for 2 h at room temperature. The mixture was filtered and concentrated, and the product was purified by silica gel column chromatography (2:1 toluene-acetone) to give **10** (64.1 mg, 52%). $[\alpha]_D -11.9$ (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃). δ 7.98–7.17 (m, 10H, Ar), 5.36 (d, 1H, $J_{1'',2''} = 3.7$ Hz, H-1''), 5.15 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1), 4.74 (d, 1H, $J_{1',2'} = 6.9$ Hz, H-1'). ¹³C-NMR (CDCl₃): 102.9 (C-1'), 99.1 (C-1''), 96.9 (C-1). MALDI-TOFMS: calcd for C₄₇H₆₃NO₂₁Na, *m/z* 1000.4; found, *m/z* 1000.4 [M+Na]⁺. HR-FABMS: calcd for C₄₇H₆₄NO₂₁, *m/z* 978.3971; found, *m/z* 978.3983 [M+H]⁺.

5-(Methoxycarbonyl)pentyl 2-O-methyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-acetamide-2-deoxy- α -D-galactopyranoside (**11**). A solution of **10** (64.1 mg, 65.5 μ mol) and NaOMe (30 mg) in MeOH (3.0 mL) was stirred for 3 h and then neutralized with Amberlite IR 120 [H⁺]. The mixture was filtered and concentrated. The product was purified by Sephadex LH-20 column chromatography in MeOH to give **11** (43.0 mg, 96%). $[\alpha]_D +17.4$ (*c* 1.0, H₂O). ¹H-NMR (D₂O). δ 5.24 (d, 1H, $J_{1'',2''} = 3.8$ Hz, H-1''), 4.65 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.40 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'). ¹³C-NMR (D₂O): δ 101.6 (C-1'), 96.1 (C-1), 95.9 (C-1''). MALDI-TOFMS: calcd for C₂₉H₅₁NO₁₇Na, *m/z* 708.3; found, *m/z* 708.8 [M+Na]⁺.

Biotinylated trisaccharide (A). Compound **11** (43.0 mg, 62.7 μ mol) was dissolved in anhydrous ethylenediamine (5.0 mL) and heated at 70 °C for 44 h. The mixture was concentrated with toluene and the product was purified by Sephadex LH-20 column chromatography with H₂O to give an amine intermediate. The amine (42.0 mg, 58.8 μ mol) was dissolved in DMF (3.0 mL), and the pH was adjusted to 8–9 with DIPEA. Biotine-NHS (24.0 mg, 70.6 μ mol) was added and the mixture was stirred for 13 h at room temperature. The solvent was removed by repeated co-evaporation with toluene and the product was purified by Sephadex LH-20 column chromatography with MeOH to give

A (55.5 mg, 94%). $[\alpha]_D +30.8$ (*c* 1.0, MeOH). $^1\text{H-NMR}$ (D_2O): δ 5.24 (d, 1H, $J_{1,2} = 3.9$ Hz, H-1"), 4.65 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.41 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1'). $^{13}\text{C-NMR}$ (D_2O): δ 101.6 (C-1'), 96.1 (C-1), 95.9 (C-1"). MALDI-TOFMS: calcd for $\text{C}_{40}\text{H}_{69}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 962.4; found, m/z 963.0 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{40}\text{H}_{69}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 962.4256; found, m/z 962.4271 $[\text{M}+\text{Na}]^+$.

Phenyl 3,4-di-O-benzoyl-2-O-benzyl-1-thio- β -L-fucopyranoside (13). A solution of **12** (985 mg, 2.55 mmol) in 80% AcOH (20 mL) was stirred at 70 °C for 2 h, then diluted with toluene and concentrated. The residue was benzoylated with benzoyl chloride (887 μL , 7.65 mmol) in pyridine (20 mL). After the reaction was quenched with MeOH, the mixture was concentrated by repeated co-evaporation with toluene. The product was purified by silica gel column chromatography (10:1 hexane-ethyl acetate) to give **13** (1.31 g, 93%). $[\alpha]_D -66.2$ (*c* 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 7.97–7.15 (m, 20H, Ar), 5.65 (d, 1H, $J_{3,4} = 3.0$ Hz, H-4), 5.44 (dd, 1H, $J_{2,3} = 9.4$ Hz, $J_{3,4} = 3.3$ Hz, H-3), 4.81 (d, 1H, $J_{1,2} = 9.5$ Hz, H-1), 4.80 and 4.58 (each d, 2H, $J_{\text{gem}} = 10.7$ Hz, PhCH_2), 4.04 (q, 1H, $J_{5,6} = 6.4$ Hz, H-5), 3.97 (t, 1H, $J_{1,2} = J_{2,3} = 9.6$ Hz, H-2), 1.33 (d, 1H, $J_{5,6} = 6.5$ Hz, H-6). $^{13}\text{C-NMR}$ (CDCl_3): δ 165.8, 165.5, 137.5, 133.3, 133.1, 132.90, 132.86, 130.2, 129.9, 129.6, 129.51, 129.46, 129.0, 128.5, 128.3, 128.2, 128.1, 127.81, 127.76, 87.0 (C-1), 75.3 (C-3, PhCH_2), 74.9 (C-2), 73.4 (C-5), 71.7 (C-4), 16.8 (C-6). MALDI-TOFMS: calcd for $\text{C}_{33}\text{H}_{30}\text{O}_6\text{SNa}$, m/z 577.2; found, m/z 577.5 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{33}\text{H}_{31}\text{O}_6\text{S}$, m/z 555.1841; found, m/z 555.1816 $[\text{M}+\text{H}]^+$.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (14). Compound **14** was prepared from **5** (140 mg, 0.18 mmol) and **13** (200 mg, 0.36 mmol) as described for preparation of **8**. The product was purified by silica gel column chromatography (3:1 hexane-ethyl acetate) to give **14** (157 mg, 72%). $[\alpha]_D -14.1$ (*c* 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 7.85–6.83 (m, 30H, Ar), 5.71 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1"), 5.19 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1), 4.77 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1'). $^{13}\text{C-NMR}$ (CDCl_3): δ 103.4 (C-1'), 99.2 (C-1), 97.1 (C-1"). MALDI-TOFMS: calcd for $\text{C}_{68}\text{H}_{75}\text{N}_3\text{O}_{18}\text{Na}$, m/z 1244.5; found, m/z 1244.6 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{68}\text{H}_{75}\text{N}_3\text{O}_{18}\text{Na}$, m/z 1244.4943; found, m/z 1244.4974 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-di-O-acetyl-2-azide-2-deoxy- α -D-galactopyranoside (15). Compound **15** was prepared from **14** (157 mg, 0.13 mmol) as described for preparation of **9**. The product was purified by silica gel column chromatography (2:1 hexane-ethyl acetate) to give **15** (148 mg, 95%). $[\alpha]_D -14.6$ (*c* 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 7.87–6.85 (m, 25H, Ar), 5.74 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1"), 5.06 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1), 4.71 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1'). $^{13}\text{C-NMR}$ (CDCl_3): δ 103.4 (C-1'), 98.9 (C-1), 97.0 (C-1"). MALDI-TOFMS: calcd for $\text{C}_{65}\text{H}_{75}\text{N}_3\text{O}_{20}\text{Na}$, m/z 1240.4; found, m/z 1240.6 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{65}\text{H}_{75}\text{N}_3\text{O}_{20}\text{Na}$, m/z 1240.4842; found, m/z 1240.4852 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-4-O-methyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-di-O-acetyl-2-acetamide-2-deoxy- α -D-galactopyranoside (16). Compound **16** was prepared from **15** (148 mg, 0.12 mmol) as described for preparation of **10**. The product was purified by silica gel column chromatography (3:2 toluene-acetone) to give **16** (50.9 mg, 44%). $[\alpha]_D -0.7$ (*c* 1.0, CHCl_3).

$^1\text{H-NMR}$ (CDCl_3): δ 7.99–7.16 (m, 10H, Ar), 5.26 (d, 1H, $J_{1'',2''} = 3.6$ Hz, H-1''), 5.12 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1), 4.44 (d, 1H, $J_{1',2'} = 7.1$ Hz, H-1'). $^{13}\text{C-NMR}$ (CDCl_3): δ 103.0 (C-1'), 101.2 (C-1''), 96.9 (C-1). MALDI-TOFMS: calcd for $\text{C}_{46}\text{H}_{61}\text{NO}_{21}\text{Na}$, m/z 986.4; found, m/z 986.8 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl α -L-fucopyranosyl-(1 $\rightarrow$ 2)-4-O-methyl- β -D-galacto-pyranosyl-(1 $\rightarrow$ 3)-2-acetamide-2-deoxy- α -D-galactopyranoside (17). Compound **17** was prepared from **16** (50.9 mg, 52.8 μmol) as described for preparation of **11**. The product was purified by Sephadex LH-20 column chromatography in MeOH to give **17** (35.8 mg, quant.). $[\alpha]_{\text{D}} +19.7$ (c 1.0, H_2O). $^1\text{H-NMR}$ (D_2O): δ 5.03 (d, 1H, $J_{1'',2''} = 4.2$ Hz, H-1''), 4.61 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.42 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1'). $^{13}\text{C-NMR}$ (D_2O): δ 101.5 (C-1'), 98.8 (C-1''), 96.1 (C-1). MALDI-TOFMS: calcd for $\text{C}_{28}\text{H}_{49}\text{NO}_{17}\text{Na}$, m/z 694.3; found, m/z 694.7 $[\text{M}+\text{Na}]^+$.

Biotinylated trisaccharide (B). Compound **B** was prepared from **17** (43.0 mg, 62.7 μmol) as described for preparation of **A**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **B** (22.7 mg, 39%). $[\alpha]_{\text{D}} +35.2$ (c 0.5, MeOH). $^1\text{H-NMR}$ (D_2O): δ 5.03 (d, 1H, $J_{1'',2''} = 4.0$ Hz, H-1''), 4.68 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.42 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1'). $^{13}\text{C-NMR}$ (D_2O): δ 101.6 (C-1'), 96.1 (C-1), 95.9 (C-1''). MALDI-TOFMS: calcd for $\text{C}_{39}\text{H}_{67}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 948.4; found, m/z 948.6 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{39}\text{H}_{67}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 948.4100; found, m/z 948.4130 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 2-O-benzoyl-3,4,6-tri-O-benzyl- α -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4,6-O-benzylidene-2-deoxy- β -D-galactopyranoside (19). Compound **19** was prepared from **3** (326 mg, 0.77 mmol) and **18** (600 mg, 0.93 mmol) as described for preparation of **4**. The product was purified by silica gel column chromatography (3:1 hexane-ethyl acetate) to give **19** (566 mg, 79%). $[\alpha]_{\text{D}} +88.1$ (c 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 8.05–7.12 (m, 25H, Ar), 5.71 (dd, 1H, $J_{1',2'} = 8.0$ Hz, $J_{2',3'} = 10.0$ Hz, H-2'), 5.45 (s, 1H, PhCH), 5.01 and 4.63 (each d, 2H, $J_{\text{gem}} = 11.8$ Hz, PhCH₂), 4.91 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.82 (d, 1H, $J_{1',2'} = 8.0$ Hz H-1'), 4.64 and 4.50 (each d, 2H, $J_{\text{gem}} = 12.4$ Hz, PhCH₂), 4.46 and 4.43 (each d, 2H, $J_{\text{gem}} = 11.7$ Hz, PhCH₂), 4.39 (d, 1H, $J_{3',4'} = 3.3$ Hz H-4), 4.14 (dd, 1H, $J_{5,6a} = 12.3$ Hz, $J_{6a,6b} = 1.4$ Hz, H-6a), 4.09 (dd, 1H, $J_{2,3} = 10.8$ Hz, $J_{3,4} = 3.3$ Hz, H-3), 3.99 (d, 1H, $J_{3',4'} = 2.7$ Hz, H-4'), 3.89 (dd, 1H, $J_{5,6b} = 12.3$ Hz, $J_{6a,6b} = 1.4$ Hz, H-6b), 3.72–3.63 (m, 9H, H-2, H-5, H-3', H-6'a, H-6'b, OCH₃, OCH₂CH₂ a), 3.57 (s, 1H, H-5'), 3.45–3.41 (m, 1H, OCH₂CH₂ b), 2.30 (t, 2H, $J' = 7.3$ Hz), 1.66–1.57 (m, 4H), 1.40–1.35 (m, 2H). $^{13}\text{C-NMR}$ (CDCl_3): δ 174.1, 165.3, 138.5, 137.9, 137.7, 137.6, 132.8, 130.3, 129.8, 128.6, 128.5, 128.3, 128.22, 128.20, 127.97, 127.92, 127.85, 127.70, 127.67, 127.65, 126.2, 102.6 (C-1'), 100.5 (CHPh), 98.7 (C-1), 80.1 (C-3'), 75.8 (C-4), 74.5 (C-3), 74.4 (PhCH₂), 73.9 (C-5), 73.6 (PhCH₂), 72.7 (C-4'), 71.9 (PhCH₂), 71.7 (C-2'), 69.12 (C-6'), 69.06 (C-6), 68.2 (OCH₂CH₂), 63.2 (C-5'), 58.6 (C-2), 51.5 (OCH₃), 33.9, 29.0, 25.6, 24.7. MALDI-TOFMS: calcd for $\text{C}_{54}\text{H}_{59}\text{N}_3\text{O}_{13}\text{Na}$, m/z 980.4; found, m/z 981.4 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{54}\text{H}_{59}\text{N}_3\text{O}_{13}\text{Na}$, m/z 980.3946; found, m/z 980.3924 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 3,4,6-tri-O-benzyl- α -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4,6-O-benzylidene-2-deoxy- β -D-galactopyranoside (20). Compound **20** was prepared from **19** (200 mg, 0.22 mmol) as described for preparation of **5**. The product was purified by silica gel column chromatography (5:2 hexane-ethyl acetate) to give **20** (87.3 mg, 41%). $[\alpha]_{\text{D}} +61.0$ (c 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ

7.51–7.24 (m, 20H, Ar), 5.47 (s, 1H, PhCH), 5.02 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.94 and 4.60 (each d, 2H, $J_{\text{gem}} = 11.6$ Hz, PhCH₂), 4.81 and 4.74 (each d, 2H, $J_{\text{gem}} = 11.9$ Hz, PhCH₂), 4.49 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'), 4.45 and 4.41 (each d, 2H, $J_{\text{gem}} = 11.9$ Hz, PhCH₂), 4.38 (d, 1H, $J_{3',4'} = 3.2$ Hz, H-4), 4.18 (dd, 1H, $J_{5,6a} = 12.4$ Hz, $J_{6a,6b} = 1.4$ Hz, H-6a), 4.12 (dd, 1H, $J_{2,3} = 10.8$ Hz, $J_{3,4} = 3.3$ Hz, H-3), 4.05 (dd, 1H, $J_{1',2'} = 8.3$ Hz, $J_{2',3'} = 9.1$ Hz, H-2'), 3.95–3.88 (m, 3H, H-2, H-6b, H-4'), 3.72–3.68 (m, 1H, OCH₂CH₂ a), 3.66–3.55 (m, 7H, H-5, H-5', H-6'a, H-6'b, OCH₃), 3.51–3.47 (m, 2H, H-3', OCH₂CH₂ b), 2.32 (t, 2H, $J' = 7.4$ Hz), 1.69–1.61 (m, 4H), 1.43–1.38 (m, 2H). ¹³C-NMR (CDCl₃): δ 174.1, 138.6, 138.4, 137.9, 137.7, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 126.3, 105.2 (C-1'), 100.8 (CHPh) 98.5 (C-1), 81.8 (C-3'), 76.0 (C-4), 75.7 (C-3), 74.6 (PhCH₂), 73.9 (C-5'), 73.7 (C-4'), 73.5 (PhCH₂), 73.0 (PhCH₂), 71.5 (C-2'), 69.2 (C-6'), 69.1 (C-6), 68.3 (OCH₂CH₂), 63.1 (C-5), 58.9 (C-2), 51.5 (OCH₃), 33.9, 29.1, 25.6, 24.6. MALDI-TOFMS: calcd for C₄₇H₅₅N₃O₁₂Na, m/z 876.4; found, m/z 876.5 [M+Na]⁺. HR-FABMS: calcd for C₄₇H₅₅N₃O₁₂Na, m/z 876.3683; found, m/z 876.3721 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→3)-2-azide-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (21). Compound **21** was prepared from **20** (119 mg, 0.14 mmol) and **7** (133 mg, 0.28 mmol) as described for preparation of **8**. The product was purified by silica gel column chromatography (25:1 toluene-acetone) to give **21** (118 mg, 69%). $[\alpha]_D +1.1$ (c 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 8.03–7.22 (m, 30H, Ar), 5.72 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1''), 5.17 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1), 4.76 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1'). ¹³C-NMR (CDCl₃): δ 103.4 (C-1'), 99.3 (C-1), 97.5 (C-1''). MALDI-TOFMS: calcd for C₆₈H₇₅N₃O₁₈Na, m/z 1244.5; found, m/z 1244.1 [M+Na]⁺. HR-FABMS: calcd for C₆₈H₇₅N₃O₁₈Na, m/z 1244.4943; found, m/z 1244.4974 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-O-acetyl-2-azide-2-deoxy-α-D-galactopyranoside (22). Compound **22** was prepared from **21** (130 mg, 106 μmol) as described for preparation of **9**. The product was purified by silica gel column chromatography (2:1 hexane-ethyl acetate) to give **22** (114 mg, 88.4%). $[\alpha]_D -1.1$ (c 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 8.05–7.26 (m, 25H, Ar), 5.73 (d, 1H, $J_{1'',2''} = 3.8$ Hz, H-1''), 5.05 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.75 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'). ¹³C-NMR (CDCl₃): δ 103.1 (C-1'), 98.9 (C-1), 97.4 (C-1''). MALDI-TOFMS: calcd for C₆₅H₇₅N₃O₂₀Na, m/z 1240.5; found, m/z 1240.6 [M+Na]⁺. HR-FABMS: calcd for C₆₅H₇₅N₃O₂₀Na, m/z 1240.4842; found, m/z 1240.4852 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-O-acetyl-2-acetamide-2-deoxy-α-D-galactopyranoside (23). A solution of **22** (98.1 mg, 80.5 μmol) in MeOH–THF (1:1, 3 mL) was hydrogenolysed (0.4 MPa) under hydrogen in the presence of 10% Pd/C (150 mg) for 18 h at room temperature, then filtered and concentrated. The residue was acetylated with acetic anhydride (2 mL) in pyridine (3 mL). The reaction mixture was poured into ice H₂O and extracted with CHCl₃. The extract was washed sequentially with 5% HCl, aq NaHCO₃, and brine, dried (MgSO₄), and concentrated. The residue was purified by silica gel column chromatography (4:1 toluene-acetone) to give **23** (61.3 mg, 60%). $[\alpha]_D -10.4$ (c 0.4, CHCl₃). ¹H-NMR (CDCl₃): δ 7.97–7.18 (m, 10H, Ar), 5.35 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1''), 5.15

(d, 1H, $J_{1,2} = 3.3$ Hz, H-1), 4.51 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1'). ^{13}C -NMR (CDCl_3): δ 102.8 (C-1'), 99.1 (C-1''), 96.9 (C-1). MALDI-TOFMS: calcd for $\text{C}_{46}\text{H}_{61}\text{NO}_{21}\text{Na}$, m/z 986.4; found, m/z 987.2 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{46}\text{H}_{61}\text{NO}_{21}\text{Na}$, m/z 986.3634; found, m/z 986.3618 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 2-O-methyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-acetamide-2-deoxy- α -D-galactopyranoside (24). Compound **24** was prepared from **23** (18.7 mg, 19.4 μmol) as described for preparation of **11**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **24** (10.0 mg, 77%). $[\alpha]_{\text{D}} +24.7$ (c 0.2, H_2O). ^1H -NMR (D_2O): δ 5.27 (d, 1H, $J_{1'',2''} = 4.0$ Hz, H-1''), 4.66 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.44 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'). ^{13}C -NMR (D_2O): δ 101.7 (C-1'), 96.1 (C-1), 95.9 (C-1''). MALDI-TOFMS: calcd for $\text{C}_{28}\text{H}_{49}\text{NO}_{17}\text{Na}$, m/z 694.3; found, m/z 694.9 $[\text{M}+\text{Na}]^+$.

Biotinylated trisaccharide (C). Compound **C** was prepared from **24** (10.0 mg, 14.9 μmol) as described for preparation of **A**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **C** (6.1 mg, 37%). $[\alpha]_{\text{D}} +8.9$ (c 0.2, MeOH). ^1H -NMR (D_2O): δ 5.27 (d, 1H, $J_{1'',2''} = 3.9$ Hz, H-1''), 4.66 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1), 4.44 (d, 1H, $J_{1',2'} = 7.4$ Hz, H-1'). ^{13}C -NMR (D_2O): δ 101.6 (C-1'), 96.1 (C-1), 95.9 (C-1''). MALDI-TOFMS: calcd for $\text{C}_{39}\text{H}_{67}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 948.4; found, m/z 948.8 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{39}\text{H}_{67}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 948.4100; found, m/z 948.4132 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3,4,6-tri-O-benzyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-azide-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (25). Compound **25** was prepared from **20** (152 mg, 0.18 mmol) and **13** (197 mg, 0.356 mmol) as described for preparation of **8**. The product was purified by silica gel column chromatography (2:1 hexane-ethyl acetate) to give **25** (176 mg, 76%). ^1H -NMR (CDCl_3): δ 7.86–6.82 (m, 35H, Ar), 5.76 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1''), 5.18 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1), 4.79 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1'). ^{13}C -NMR (CDCl_3): δ 103.4 (C-1'), 99.3 (C-1), 97.2 (C-1''). MALDI-TOFMS: calcd for $\text{C}_{74}\text{H}_{79}\text{N}_3\text{O}_{18}\text{Na}$, m/z 1320.5; found, m/z 1321.6 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{74}\text{H}_{80}\text{N}_3\text{O}_{18}$, m/z 1298.5437; found, m/z 1298.5442 $[\text{M}+\text{H}]^+$.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-benzyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3,4,6-tri-O-benzyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-di-O-acetyl-2-azide-2-deoxy- α -D-galactopyranoside (26). Compound **26** was prepared from **25** (176 mg, 0.14 mmol) as described for preparation of **9**. The product was purified by silica gel column chromatography (2:1 hexane-ethyl acetate) to give **26** (113 mg, 64%). $[\alpha]_{\text{D}} -11.2$ (c 1.0, CHCl_3). ^1H -NMR (CDCl_3): δ 7.87–6.83 (m, 30H, Ar), 5.77 (d, 1H, $J_{1'',2''} = 3.4$ Hz, H-1''), 5.06 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.77 (d, 1H, $J_{1',2'} = 7.4$ Hz, H-1'). ^{13}C -NMR (CDCl_3): δ 103.2 (C-1'), 98.9 (C-1), 97.1 (C-1''). MALDI-TOFMS: calcd for $\text{C}_{71}\text{H}_{79}\text{N}_3\text{O}_{20}\text{Na}$, m/z 1316.5; found, m/z 1316.9 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{71}\text{H}_{79}\text{N}_3\text{O}_{20}\text{Na}$, m/z 1316.5155; found, m/z 1316.5186 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl- α -L-fucopyranosyl-(1 $\rightarrow$ 2)-3,4,6-tri-O-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-O-acetyl-2-acetamide-2-deoxy- α -D-galactopyranoside (27). Compound **27** was prepared from **26** (176 mg, 135 μmol) as described for preparation of **23**. The product was purified by silica gel column chromatography (5:1 toluene-acetone) to give **27** (110 mg, 74%). $[\alpha]_{\text{D}} -5.1$ (c 0.5,

CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 7.97–7.18 (m, 10H, Ar), 5.35 (br. s, 1H, H-1"), 5.06 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1), 4.46 (d, 1H, $J_{1',2'} = 7.1$ Hz, H-1'). $^{13}\text{C-NMR}$ (CDCl_3): δ 102.9 (C-1'), 101.0 (C-1"), 97.0 (C-1). MALDI-TOFMS: calcd for $\text{C}_{45}\text{H}_{59}\text{NO}_{21}\text{Na}$, m/z 972.3; found, m/z 972.7 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl α -L-fucopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-acetamide-2-deoxy- α -D-galactopyranoside (28). Compound **28** was prepared from **27** (25.8 mg, 27.2 μmol) as described for preparation of **11**. The product was purified by Sephadex LH-20 column chromatography in MeOH to give **28** (16.8 mg, 94%). $[\alpha]_{\text{D}} +22.3$ (c 0.4, H_2O). $^1\text{H-NMR}$ (D_2O): δ 5.05 (d, 1H, $J_{1'',2''} = 4.0$ Hz, H-1"), 4.68 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.45 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'). $^{13}\text{C-NMR}$ (D_2O): δ 101.6 (C-1'), 98.8 (C-1"), 96.1 (C-1). MALDI-TOFMS: calcd for $\text{C}_{27}\text{H}_{47}\text{NO}_{17}\text{Na}$, m/z 680.3; found, m/z 681.2 $[\text{M}+\text{Na}]^+$.

Biotinylated trisaccharide (D). Compound **D** was prepared from **28** (18.6 mg, 25.5 μmol) as described for preparation of **A**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **D** (18.2 mg, 78%). $[\alpha]_{\text{D}} +26.5$ (c 0.5, MeOH). $^1\text{H-NMR}$ (D_2O): δ 5.05 (d, 1H, $J_{1'',2''} = 4.0$ Hz, H-1"), 4.69 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1), 4.46 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'). $^{13}\text{C-NMR}$ (D_2O): δ 101.6 (C-1'), 98.8 (C-1"), 96.1 (C-1). MALDI-TOFMS: calcd for $\text{C}_{38}\text{H}_{65}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 934.4; found, m/z 934.5 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{38}\text{H}_{65}\text{N}_5\text{O}_{18}\text{SNa}$, m/z 934.3943; found, m/z 934.3956 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 2-O-benzoyl-3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranoside (29). Compound **29** was prepared from methyl 6-hydroxyhexanoate (134 mg, 0.918 mmol) and **2** (575 mg, 1.01 mmol) as described for preparation of **4**. The product was purified by silica gel column chromatography (4:1 hexane–ethyl acetate) to give **29** (520 mg, 93%). $[\alpha]_{\text{D}} +7.2$ (c 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 8.02–7.12 (m, 15H, Ar), 5.52 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 4.69 and 4.53 (each d, 2H, $J_{\text{gem}} = 12.4$ Hz, PhCH_2), 4.60 and 4.56 (each d, 2H, $J_{\text{gem}} = 11.7$ Hz, PhCH_2), 4.44 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 3.79–3.67 (m, 3H, H-4, H-6a, OCH_2CH_2 a), 3.67 (dd, 1H, $J_{5,6b} = 9.1$ Hz, $J_{6a,6b} = 5.5$ Hz, H-6b), 3.63–3.59 (m, 8H, H-3, H-5, $\text{OCH}_3 \times 2$), 3.41–3.37 (m, 1H, OCH_2CH_2 b), 2.08–1.95 (m, 2H), 1.51–1.39 (m, 4H), 1.20–1.14 (m, 2H). $^{13}\text{C-NMR}$ (CDCl_3): δ 174.1, 165.2, 137.8, 137.7, 132.9, 130.3, 129.8, 128.5, 128.29, 128.28, 128.0, 127.9, 127.7, 101.4 (C-1), 79.6 (C-3), 74.9 (C-4), 73.7 (PhCH_2), 73.4 (C-5), 71.8 (C-2), 71.5 (PhCH_2), 69.0 (OCH_2CH_2), 68.3 (C-6), 61.3 (OCH_3), 51.3 (OCH_3), 33.7, 29.0, 25.3, 24.4. MALDI-TOFMS: calcd for $\text{C}_{35}\text{H}_{42}\text{O}_9\text{Na}$, m/z 629.3; found, m/z 629.7 $[\text{M}+\text{Na}]^+$. HR-FABMS: calcd for $\text{C}_{35}\text{H}_{42}\text{O}_9\text{Na}$, m/z 629.2727; found, m/z 629.2690 $[\text{M}+\text{Na}]^+$.

5-(Methoxycarbonyl)pentyl 3,6-di-O-benzyl-4-O-methyl- β -D-galactopyranoside (30). Compound **30** was prepared from **29** (199 mg, 0.328 mmol) as described for preparation of **5**. The product was purified by silica gel column chromatography (4:1 hexane–ethyl acetate) to give **30** (130 mg, 79%). $[\alpha]_{\text{D}} -5.5$ (c 1.0, CHCl_3). $^1\text{H-NMR}$ (CDCl_3): δ 4.0–7.27 (m, 10H, Ar), 4.78 and 4.72 (each d, 2H, $J_{\text{gem}} = 12.0$ Hz, PhCH_2), 4.57 and 4.53 (each d, 2H, $J_{\text{gem}} = 11.7$ Hz, PhCH_2), 4.20 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.89–3.81 (m, 2H, H-2, OCH_2CH_2 a), 3.72 (dd, 1H, $J_{5,6a} = 9.1$ Hz, $J_{6a,6b} = 7.6$ Hz, H-6a), 3.67–3.61 (m, 5H, H-4, H-6b, OCH_3), 3.57–3.55 (m, 4H, H-5, OCH_3), 3.50–3.45 (m, 1H, OCH_2CH_2 b), 3.40 (dd, 1H, $J_{2,3} = 9.8$ Hz, $J_{3,4} = 2.9$ Hz, H-3), 2.30 (t, 2H, $J' = 7.5$ Hz), 1.66–1.59 (m, 4H), 1.40–1.35 (m, 2H). $^{13}\text{C-NMR}$ (CDCl_3): δ 174.2, 138.2, 137.9, 128.50, 128.47, 127.89, 127.85, 127.8, 127.7, 103.1 (C-1),

81.7 (C-3), 75.4 (C-4), 73.6 (PhCH₂), 73.4 (C-5), 72.4 (PhCH₂), 71.3 (C-2), 69.4 (OCH₂CH₂), 68.3 (C-6), 61.2 (OCH₃), 51.5 (OCH₃), 33.9, 29.1, 25.5, 24.6. MALDI-TOFMS: calcd for C₂₈H₃₈O₈Na, *m/z* 525.3; found, *m/z* 526.1 [M+Na]⁺. HR-FABMS: calcd for C₂₈H₃₉O₈, *m/z* 503.2645; found, *m/z* 503.2636 [M+H]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl-L-fucopyranosyl-(1→2)-3,6-di-O-benzyl-4-O-methyl-β-D-galactopyranoside (31). Compound **31** was prepared from **30** (130 mg, 0.26 mmol) and **7** (248 mg, 0.52 mmol) as described for preparation of **8**. The product was purified by silica gel column chromatography (30:1 toluene-acetone) to give **31** (105 mg, 72%). MALDI-TOFMS: calcd for C₄₉H₅₈O₁₄Na, *m/z* 893.4; found, *m/z* 893.8 [M+Na]⁺. HR-FABMS: calcd for C₄₉H₅₈O₁₄Na, *m/z* 893.3724; found, *m/z* 893.3768 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 3,4-di-O-benzoyl-2-O-methyl-α-L-fucopyranosyl-(1→2)-4-O-methyl-β-D-galactopyranoside (32). A solution of **31** (113 mg, 87.3 μmol) in THF (2.0 mL) was hydrogenolysed (0.4 MPa) under hydrogen in the presence of 10% Pd/C (150 mg) for 2 h at room temperature. The mixture was filtered and concentrated, and the product was purified by silica gel column chromatography (4:1 toluene-acetone) to give **32** (88.2 mg, 68%). [α]_D −115 (*c* 1.0, CHCl₃). ¹H-NMR (CDCl₃): δ 8.06–7.31 (m, 10H, Ar), 5.69 (dd, 1H, *J*_{2',3'} = 10.1 Hz, *J*_{3',4'} = 3.2 Hz, H-3'), 5.65 (d, 1H, *J*_{3',4'} = 3.3 Hz, H-4'), 5.41 (d, 1H, *J*_{1',2'} = 3.6 Hz, H-1'), 4.56 (q, 1H, *J*_{5',6'} = 6.3 Hz, H-5'), 4.36 (d, 1H, *J*_{1,2} = 7.5 Hz, H-1), 3.97–3.88 (m, 3H, H-6a, H-2', OCH₂CH₂ a), 3.81–3.71 (m, 3H, H-2, H-3, H-6b), 3.66 (s, 3H, OCH₃), 3.59 (s, 3H, OCH₃), 3.58–3.55 (m, 3H, H-4, H-5, OCH₂CH₂ b), 3.53 (s, 3H, OCH₃), 2.33 (t, 2H, *J'* = 7.5 Hz), 1.70–1.63 (m, 4H), 1.45–1.39 (m, 2H), 1.20 (d, 3H, *J*_{5',6'} = 6.6 Hz, H-6'). ¹³C-NMR (CDCl₃): δ 174.2, 165.9, 165.6, 133.3, 133.1, 129.8, 129.72, 129.68, 129.6, 128.5, 128.3, 101.9 (C-1), 99.7 (C-1'), 81.1 (C-3), 78.6 (C-4), 77.4 (C-2'), 75.23 (C-5), 75.16 (C-2), 72.2 (C-4'), 71.5 (C-3'), 69.6 (C-6), 65.4 (C-5'), 62.3 (OCH₂CH₂), 61.6 (OCH₃), 60.2 (OCH₃), 51.5 (OCH₃), 33.9, 29.5, 25.6, 24.7, 16.1 (C-6'). MALDI-TOFMS: calcd for C₃₅H₄₆O₁₄Na, *m/z* 713.3; found, *m/z* 713.9 [M+Na]⁺. HR-FABMS: calcd for C₃₅H₄₆O₁₄Na, *m/z* 713.2785; found, *m/z* 713.2770 [M+Na]⁺.

5-(Methoxycarbonyl)pentyl 2-O-methyl-α-L-fucopyranosyl-(1→2)-4-O-methyl-β-D-galactopyranoside (33). Compound **33** was prepared from **32** (88.2 mg, 0.13 mmol) as described for preparation of **11**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **33** (56.9 mg, 92%). [α]_D −78.6 (*c* 1.0, MeOH). ¹H-NMR (CDCl₃): δ 5.36 (d, 1H, *J*_{1',2'} = 3.4 Hz, H-1'), 4.29 (d, 1H, *J*_{1',2'} = 7.7 Hz, H-1'). ¹³C-NMR (CDCl₃): δ 102.0 (C-1), 97.7 (C-1'). MALDI-TOFMS: calcd for C₂₁H₃₈O₁₂Na, *m/z* 505.2; found, *m/z* 505.9 [M+Na]⁺. HR-FABMS: calcd for C₂₁H₃₈O₁₂Na, *m/z* 505.2261; found, *m/z* 505.2242 [M+Na]⁺.

Biotinylated trisaccharide (E). Compound **E** was prepared from **33** (56.9 mg, 118 μmol) as described for preparation of **A**. The product was purified by Sephadex LH-20 column chromatography with H₂O to give **E** (51.2 mg, 72%). [α]_D −30.9 (*c* 1.0, MeOH). ¹H-NMR (D₂O): δ 5.29 (d, 1H, *J*_{1',2'} = 3.8 Hz, H-1'), 4.25 (d, 1H, *J*_{1',2'} = 7.9 Hz, H-1). ¹³C-NMR (D₂O): δ 102.5 (C-1), 97.3 (C-1'). MALDI-TOFMS: calcd for C₃₂H₅₆N₄O₁₃SNa, *m/z* 759.3; found, *m/z* 760.1 [M+Na]⁺. HR-FABMS: calcd for C₃₂H₅₆N₄O₁₃SNa, *m/z* 759.3462; found, *m/z* 759.3503 [M+Na]⁺.

3.2. Serum Samples

Serum samples examined by ELISA were obtained from 6 patients who were confirmed to have *Toxocara canis*-visceral larva migrans (Tc) and 4 normal healthy individuals (NH).

3.3. ELISA Protocol

ELISA was performed as previously described [1,18]. Biotin-labeled oligosaccharides A-E in H₂O (13 pmol per well) were added to the wells of flat-bottomed microplates (Streptavidin C96, No. 236001; Nunc, Roskilde, Denmark) coated with streptavidin, and these plates were incubated for 1 h at 37 °C. After the coating solution was discarded, the microplates were washed with 0.05% Tween-PBS (250 µL per well). Serum samples diluted 1:250 with 0.05% Tween-PBS (200 µL per well) were then added to the wells of the microplates and incubated overnight at 4 °C. After being washed with 0.05% Tween-PBS, 200 µL of anti-human IgG/HRP (P0214; DakoCytomation, Glostrup, Denmark; 1:1,000 in 0.05% Tween-PBS) was added, and the microplates were incubated for 1 h at 37 °C. After further washing, bound antibodies were detected by the addition of 2,2'-azino-di(3-ethyl-benzthiazoline-6-sulfonate) (ABTS) peroxidase substrate solution (KPL, Gaithersburg, MD, USA, 200 µL per well). After incubation period of 8 min at 37 °C, the reaction was stopped by the addition of 1% sodium dodecyl sulphate (SDS), and the absorbance (A) values were read at 405 nm on a microplate reader (Model 680; BioRad, Hercules, CA, USA).

4. Conclusions

We have prepared oligosaccharide-biotin conjugates A–E in order to study the antigenicity of putative carbohydrate sequences at the parasite *T. canis* and their analogues. Antigenicity of these compounds was examined by ELISA. Mono- or di-*O*-methylated forms showed good serodiagnostic potential to detect infections caused by *T. canis*. These results demonstrate that biotin-labeled oligosaccharides may serve as a diagnostic tool to detect *T. canis* and *T. cati* infections in humans.

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Sample Availability: Samples of the compounds **A–E** are available from the authors.

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