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A new glycosylation method part 3: study of microwave effects at low temperatures to control reaction pathways and reduce byproducts☆

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ABSTRACT

The efficiency of microwave irradiation at low temperature for glycosylations is described. Although oligosaccharide synthesis usually requires reactive donors for glycosylations, which have leaving groups on the anomer positions, i.e., trichloroacetoimidates, halogenates, thioalkyl glycosides, etc., the suitable donors in our microwave supported synthesis of Lewis X oligosaccharide were very stable acetate derivatives. Regarding glycosylation with a fucosyl acetate donor and a glucosamine acceptor, microwave irradiation with simultaneous cooling improved yields. Moreover, further synthesis to Lewis X derivatives was achieved only with microwave irradiation at low temperatures. Without microwave irradiation, we could only obtain byproducts and none of the designed product at any reaction temperature.
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1. Introduction

With the development of carbohydrate research, the biological importance of oligosaccharides has been revealed.[1–3] For example, one of its important roles is cell to cell adhesion[4] and inflammation.[5] These phenomena are the result of molecular binding systems, typically carbohydrate–protein and carbohydrate–carbohydrate. We are interested in studying these carbohydrate recognition systems at the molecular level and believe that these studies will open a door to a new drug discovery system. We have previously reported the verotoxin-oligosaccharide binding conformation[6–9] and the dynamics of carbohydrate binding protein.[10]

Since 1986, microwave irradiation has been applied widely in the synthetic research field,[11,12] especially as an effective heating tool in chemical reactions.[13] Although many glycosylations are performed under Lewis acidic conditions and usually proceed at low temperatures, microwave irradiation has been well studied in for carbohydrate chemistry, including Fischer glycosylation,[14] solvent free glycosylation,[15] Ferrier rearrangement,[16,17] glycosylation with oxazoline donor,[18] glycosylation with pentenyl orthoesters,[19] glycosylation with trichloroacetoimidates and DISAL (3,5-dinitrosalicylate) donors,[20,21] synthesis of glycosyl amino acids,[22] etc. These reactions use microwave irradiation as an effective heating tool, but we revealed another factor, which can control chemical reactions and was very effective for the glycosylation[23,24] and glycopeptides syntheses.[25,26] The technique of using microwave with simultaneous cooling is reported[27] and an established machine is now commercially available.[28] In this report, we demonstrate the effectiveness of microwave irradiation at low temperatures, i.e., non-heating for glycosylations through syntheses of Lewis X derivatives (Fig. 1).

2. Results and discussion

2.1. Strategy

Lewis X (Le^x) antigen (**1**) is the terminal oligosaccharide in numerous cell surface glycolipids and glycoproteins that play a key role in selectin-mediated cell–cell adhesion.[29] On a molecular level, this process is dominated by Le^x–Le^x recognition and we plan to study conformations of this weak homo-recognition by NMR. For this purpose, we set thiomethyl Le^x derivative (**2**) as a synthetic target because the thiomethyl group can behave either as a protecting group at the reducing end or as a leaving group to introduce a variety of ligands. Thus, **2** could be converted to a variety of Le^x derivatives.

☆ Part 1: Y. Yoshimura, H. Shimizu, H. Hinou, S.-I. Nishimura 'A novel glycosylation concept; microwave-assisted acetal-exchange type glycosylations from methyl glycosides as donors', *Tetrahedron Lett.*, **2005**, *46*, 4701–4705.

Part 2: H. Shimizu, M. Sakamoto, N. Nagahori, S.-I. Nishimura 'A new glycosylation method part 2: study of carbohydrate elongation onto the gold nanoparticles in a colloidal phase', *Tetrahedron*, **2007**, *63*, 2418–2425.

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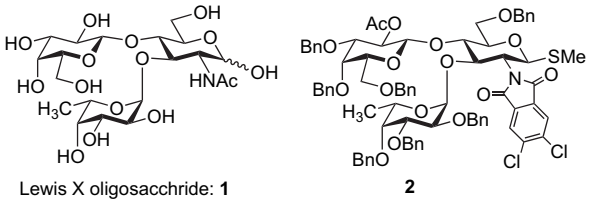


Figure 1. Lewis X oligosaccharide (**1**) and the synthetic target **2**.

In addition, we introduced the fucosyl moiety first to the glucosamine acceptor followed by the introduction of the galactose unit. Although the Le^x analogues are important targets in the history of carbohydrate synthetic research field and have been well studied,[30] the synthesis of thiomethyl Le^x (**2**) in the order described above has several difficulties. The 3-OH and the 4-OH of the glucosamine acceptor are potentially less reactive, the glucosamine acceptor (**3**), which had a fucose unit on the 3-OH produced steric hindrance for the 4-OH, the thiomethyl group had good nucleophilicity, which lead to byproducts, and generally the fucosyl bond was relatively unstable in acidic conditions like glycosylation (Fig. 2). Therefore, for the construction of Le^x building blocks, it has been recommended to first glycosylate the galactose donor to the 4-OH of the glucosamine acceptor followed by the introduction of the fucosyl moiety at 3-OH. There have been some reports of this reverse synthesis strategy.[31] Stahl et al. successfully fucosylated and galactosylated onto the 3-OH and 4-OH of glucosamine acceptor in 95% and 75% yields, respectively,[32] and de la Fuente et al. fucosylated and galactosylated to a phenylthio protected glucosamine acceptor in 83% and 80% yields, respectively.[33] Our synthesis of **2** was more difficult. Because of the thiomethyl groups' nucleophilicity and low reactivity of the 4-OH, the activated oxocarbenium ion from galactosyl donors was more likely to be attacked at S rather than O, and as a result we only achieved byproducts instead of the designed product.

Despite of these findings, we found that microwave irradiation could achieve the synthesis of thiomethyl Le^x (**2**) in high yield in the syntheses order described above. At the same time, we found that microwave irradiation was effective even at low temperatures, which indicates that microwave provides not only a heating effect in chemical reactions but also plays a role at the molecular level.

2.2. Disaccharide synthesis

After syntheses of monosaccharide building blocks by common procedures,[34–36] fucosyl donors, fluoride, trichloroacetoimidate, and acetate have been used for glycosylations to a glucosamine acceptor (**4**) without microwave irradiation. We have tried to synthesize **6** under a variety of conditions, promoters, solvents, and temperatures. (Selected attempts are shown in Scheme S1 in Supplementary data.) Although monitoring by TLC showed that the donors were successfully activated in all cases, only limited attempts resulted in disaccharide **6**. As shown in Scheme 1, glycosylation between **4** and **5** in ether gave **6** in only 27% yield but the

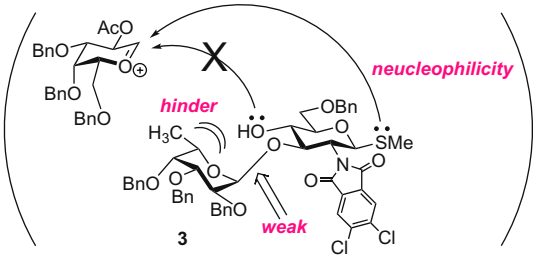
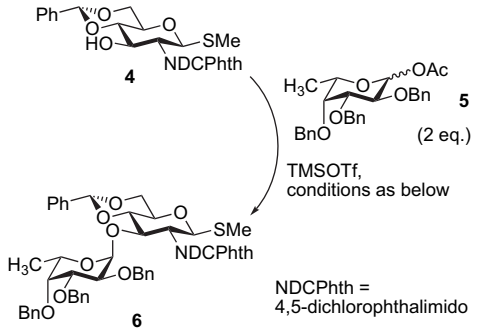


Figure 2. Problems in the glycosylation between oxocarbenium ion from the galactose donor and the fucosyl-glucosamine acceptor **3**.



Conditions:

Entry	Solv.	Microwave	Temp.[a)]	Time[b)]	Yield
1	ether	none	-10 °C	1 h	27 %
2	ether/THF (5/1)	none	-45 °C	6 h	71 %
3	ether	300W	-10 °C	1 h	90 %
4	ether	100W	-18 °C	2.5 h	83 %
5	CH_3CN	300W	-20 °C	1 h	21 %
6	CH_2Cl_2	300W	-20 °C	2.5 h	9 %

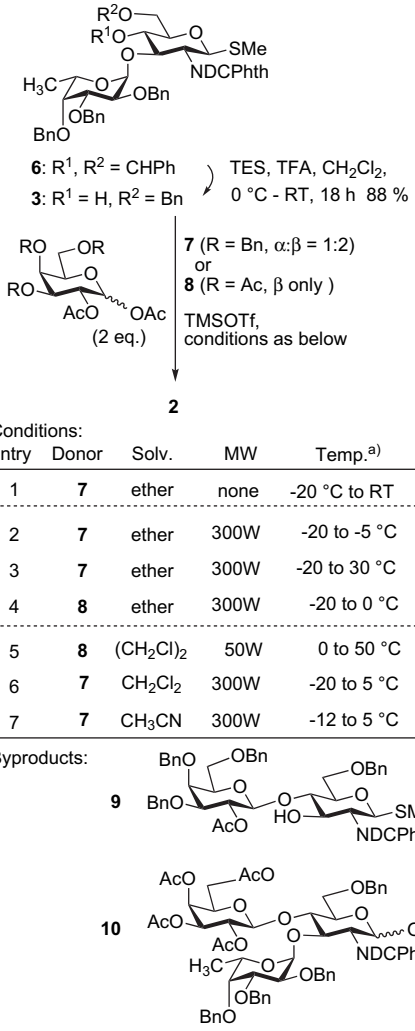
Scheme 1. Synthesis of disaccharide **6**. Microwave irradiation in low temperatures gave fruitful results. (a) Observed temperatures by the CoolMate equipped sensor with the use of microwave irradiation, within ±5 °C. (b) The reactions were monitored by TLC and stopped when acceptor (**6**) was consumed.

yield improved to 71% in mixed solvents such as ether/THF (5:1). On the other hand, in the same conditions with additional microwave irradiation, the yield increased to 90%. In addition, byproducts, which were hardly purified by column chromatography, were not observed by NMR (Fig. S1 in Supplementary data). We would like to note here that the reaction temperature was −10 °C, which meant that microwave was effective even at low temperatures and played a more significant role than heating. It was also interesting to note that microwave supported glycosylation in a solvent dependent manner as the yields became 21% and 9% in cases using acetonitrile or dichrolomethane, respectively, although we do not know the underlying reason for this yet.

2.3. Trisaccharide synthesis

After selective reduction of the 4,6-benzylidene group of **6**,[37] the galactose unit was introduced for the synthesis of Le^x analogue **2**. As we already discussed, galactosylation to **3** required 'paradoxical' conditions, namely strong glycosylation conditions, i.e., high temperatures, due to the poor reactivity of the hydroxyl group of **3**, but the fucosyl bond was generally weak in acidic conditions. Thus, carrying out the reaction in low temperatures was favorable. Moreover, as a potential nucleophilic group, the thiomethyl group would be more reactive than the hydroxyl group on **3**, which meant the reaction of the oxocarbenium ion would be more favored with 1-SMe than 4-OH, and this synthetic strategy may not result in the production of **2**. In fact, we could not obtain trisaccharide **2** despite many attempts in any temperatures between −45 °C to room temperature (we have shown one typical example in Scheme 2).

Despite these attempts and unlikely results, microwave irradiation turned the 'impossible' into a 'possible'. Microwave irradiation with Lewis acid, trimethylsilyl trifluoromethanesulfonate (TMSOTf), to glycosyl donor **7** gave successfully designed Le^x analogues (**2**) in 70% and 82% yield when 15% of recovered disaccharide acceptor **3** was considered (entry 2 in Scheme 2). This reaction was performed at less than zero degrees for 5 h but when the reaction temperature was raised to 30 °C, the yield became 37% and 43% considered 14% of recovered **3** (entry 3 in Scheme 2). In this case, we isolated the lactosamine derivative (**9**)[38] as a byproduct, which would result in the



Entry	Donor	Solv.	MW	Temp.[a)]	Time[b)]	Yield
1	**7**	ether	none	-20 °C to RT	24 h	c)
2	**7**	ether	300W	-20 to -5 °C	5 h	82%
3	**7**	ether	300W	-20 to 30 °C	6 h	43%[d)]
4	**8**	ether	300W	-20 to 0 °C	6 h	none
5	**8**	$(CH_2Cl)_2$	50W	0 to 50 °C	6 h	e)
6	**7**	CH_2Cl_2	300W	-20 to 5 °C	2 h	f)
7	**7**	CH_3CN	300W	-12 to 5 °C	6 h	f)

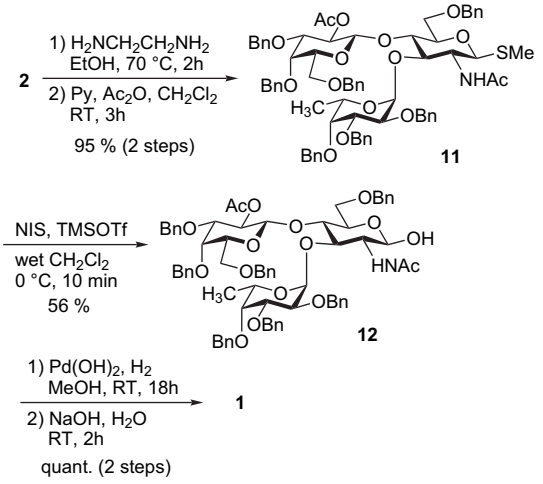
Byproducts:

Scheme 2. Synthesis of trisaccharide **2**. Microwave irradiation at low temperatures made this synthesis possible. (a) Observed temperatures by CoolMate equipped sensor with the use of microwave irradiation. (b) The reactions were monitored by TLC and stopped when acceptor (**3**) was consumed. (c) TLC monitoring showed that **7** was activated but **2** was not observed. (d) Byproduct **9** was also observed. (e) Only 30% of de-thiomethyl trisaccharide (**10**) was isolated. (f) We could not detect **2**.

galactosylation of **3** but the fucose unit was detached. MALDI-TOF MS monitoring showed that the byproduct was observed when the reaction temperature increased to over −5 °C (Fig. S2 in Supplementary data). In the case of using galactose pentaacetate (**8**) as a donor, the reaction did not proceed as **8** was not activated under 0 °C even with microwave irradiation (entry 4 in Scheme 2), then we treated **8** at higher temperatures. In this case, we changed the solvent to dichloroethane due to the low boiling point of ether. Although entry 5 in Scheme 2 gave a trisaccharide, it was **10**,[38] the thiomethyl group detached from the Le^x analogue. As we have described in the synthesis of disaccharide (**6**), solvents' dependency was remarkable in this case as acetonitrile and dichrolomethane led to low yields even in similar conditions, entry 2 in Scheme 2.

2.4. Further conversion to Lewis X oligosaccharide (1)

Le^x analogues (**2**) will be a key compound for further research as it can be converted to a variety of Le^x derivatives. At the same time, **2** was deprotected to become a Le^x oligosaccharide (**1**). Treatment with ethylenediamine, followed by acetylation for **2** gave **11**. The thiomethyl group on anomer was removed by NIS/TMSOTf in wet dichloromethane, and resulting **12** was only β-hemiacetal although it is. Deprotection of the benzyl groups by $Pd(OH)_2$ under H_2 and of the acetyl groups by saponification yielded **1**. NMR and MS data were compared with commercially available one to confirm its structure (Scheme 3).



Scheme 3. Further conversion to Lewis X oligosaccharide (**1**).

3. Conclusion

Although there are some reports of chemical reactions using microwave irradiation with simultaneous cooling, many succeeded only in the improvement of product yields,[39–41] although Horikoshi et al. found a case of changing reaction pathways.[42] Earlier this year, Kappe et al. reported inaccuracies in the case of heating by microwave irradiation. They found that the reaction temperature might not be homogeneous in the reaction vessel despite stirring, resulting in partial heating areas, heat-spots or self-heating of the vessels. They were not convinced of the existence of microwave nonthermal effects.[43] However, our results indicated that microwave irradiation worked not only to improve glycosylation yield (Scheme 1) but also to allow for the completion of previously impossible reactions (Scheme 2). Temperature control without microwave irradiation gave only byproducts and microwave irradiation at low temperatures successfully produced the designed product **2** in the reaction showed in Scheme 2. It would be hard to explain our results based on temperature inhomogeneities.

It is also hard to imagine that a microwave can activate molecules directly based on potential energy. We conjecture that one of the efficiencies of microwave irradiation at low temperatures may be the destruction of a cluster. It is reasonable to think that synthons, especially the glycosyl acceptor, might exist as clusters, such as micelles, and the reaction points, hydroxyl groups, may be hidden inside the cluster. As a result, under normal conditions, the hydroxyl groups could not be attacked by the oxocarbenium ion, but microwave irradiation will be able to break the cluster, thus freeing the hydroxyl groups to react. Next, we are planning to study not only the utility of microwave irradiation but also the microwave effect itself in chemical reactions. By revealing the reason for the dependency of the solvents in Schemes 1 and 2, we will determine the true microwave effect itself.

4. Experimental

4.1. General

Microwave supported syntheses at low temperatures were performed in the CEM Discover microwave system and the

CoolMate cooling unit. The CoolMate equipped reaction flasks were jacked by a cooling medium, perfluorinated fluid GALDEN HT55. Reaction temperatures were monitored by an equipped fiber optic probe, which was inserted into the reaction medium. A silicone cap with two holes was placed on the reaction flask. One hole was for the temperature probe and another was for a nitrogen balloon. All reagents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), Tokyo Kasei Kogyo Co. (Tokyo, Japan), or Aldrich Chemical Co. (Milwaukee, WI, USA) and used as purchased without further purification. All dry solvents were purchased from Wako Pure Chemical Industries Ltd. and stored with activated molecular sieves 4A. Reactions were monitored by TLC, which was performed with 0.25 mm precoated silica gel $60F_{254}$ on glass from Merck (Darmstadt, Germany). Compounds were detected by dipping the TLC plates in an ethanolic solution of sulfuric acid (5% v/v) and heating. Silica gel N60 (40–50 nm) from Kanto Chemical (Tokyo, Japan) was used for silica gel chromatography. Optical rotations were recorded on a Perkin Elmer Polarimeter 343 at room temperature (approximately 18–23 °C). All NMR data are reported in parts per million downfield shift from tetramethylsilane. ^{1}H NMR spectra were routinely recorded at 400 MHz on a BRUKER AVANCE 400 spectrometer and 500 MHz on a VARIAN Unity INOVA 500 spectrometer at 300 K, and chemical shifts were expressed relative to methyl proton of tetramethylsilane (δ 0.00 ppm). In disaccharide or trisaccharide cases, proton positions of fucose and galactose parts were shown by using single prime (′) and double prime (″), respectively. ^{13}C NMR spectra were routinely recorded at 100 MHz on a BRUKER AVANCE 400 spectrometer at 300 K and chemical shifts were expressed relative to that of the deuterated solvents (δ 77.0 ppm for $CDCl_3$). MALDI-TOF MS analyses were performed on a BRUKER REFLEX III with DHB (2,5-dihydroxybenzoic acid). Ions generated by a pulsed UV laser beam (nitrogen laser, λ=337 nm, 5 Hz) were accelerated to a kinetic energy of 23.5 kV. High-resolution FAB-mass data were recorded by the in-house mass spectrometry services at the Center for Instrumental Analysis, Hokkaido University.

4.2. Methyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(4,5-dichlorophthalimido)-1-thio-β-D-glucopyranoside (6)

4.2.1. Typical method with microwave irradiation: entry 3 in Scheme 1

A solution of TMSOTf (73 μl, 0.403 mmol) in diethyl ether (1 ml) was added dropwise to a stirred −30 °C solution, cooled by the CoolMate, of pre-dried glucosamine acceptor (**4**) (100 mg, 0.201 mmol) and acetate donor (**5**) (190 mg, 0.399 mmol) in diethyl ether (2 ml) in a CEM equipped vessel under nitrogen. The reaction mixture was stirred for 1 h with microwave irradiation (300 W) and the reaction temperature was monitored every second automatically. The mixture was then diluted with chloroform and neutralized with satd $NaHCO_3$ aq. The aqueous layer was extracted with chloroform three times, combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (4:1)] to give **6** (165 mg, 90%) as a white solid.

$[\alpha]_D^{16}$ −45.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (1H, s, Aromatic-*H*), 7.43 (1H, s, Aromatic-*H*), 7.37–6.99 (20H, m, Aromatic-*H*), 5.57 (1H, s, PhC*H*–), 5.32 (1H, d, *J*=10.5 Hz, H-1), 4.94 (1H, d, *J*=3.5 Hz, H-1′), 4.86 (1H, d, *J*=11.8 Hz, PhCH_2–), 4.64 (1H, t, *J*=10.5 Hz, H-3), 4.55 (1H, d, *J*=11.8 Hz, PhCH_2–), 4.51 (1H, d, *J*=11.8 Hz, PhCH_2–), 4.45 (1H, dd *J*=4.5, 9.5 Hz, H-5), 4.40 (1H, d, *J*=11.8 Hz, PhCH_2–), 4.40 (1H, t, *J*=10.5 Hz, H-2), 4.33 (1H, d, *J*=12.0 Hz, PhCH_2–), 4.13 (1H, d, *J*=12.0 Hz, PhCH_2–), 4.08 (1H, q, *J*=6.5 Hz, H-5′), 3.82 (1H, dd, *J*=9.5, 10.5 Hz, H-4), 3.79 (1H, dd, *J*=3.5, 10.5, H-2′), 3.75 (1H, dd, *J*=2.5, 10.0 Hz, H-3′), 3.74–3.69 (2H, m, H-6), 3.56 (1H, br, H-4′), 2.17 (3H, s, CH_3S–), 0.90 (3H, d, *J*=6.5 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.5, 138.5, 138.2, 137.0, 128.9, 128.4, 128.2, 128.2, 128.1, 127.6, 127.5, 127.5, 127.3, 127.3, 126.1, 125.4, 125.1, 101.3, 99.3, 82.1, 81.3, 79.7, 77.9, 77.9, 73.4, 73.2, 70.7, 68.6, 67.5, 54.4, 16.6, 14.1; HRMS FAB *m*/*z* for $C_{49}H_{47}O_{10}NCl_2S$+H $(M+H)^+$ calcd 912.2376, found 912.2382.

4.3. Methyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzyl-2-deoxy-2-(4,5-dichlorophthalimido)-1-thio-β-D-glucopyranoside (3)

Trifluoroacetic acid (282 μl, 3.71 mmol) was added dropwise to a stirred solution of **6** (562 mg, 616 μmol) and triethylsilane (617 μl, 3.87 mmol) in dichloromethane (5 ml) at 0 °C. The mixture was stirred for 4 h at 0 °C and additional triethylsilane (617 μl, 3.87 mmol) and trifluoroacetic acid (282 μl, 3.71 mmol) were added. The reaction mixture was stirred overnight as it was allowed to slowly warm to room temperature. The mixture was then diluted with EtOAc and neutralized with satd $NaHCO_3$ aq. The aqueous layer was extracted with EtOAc three times, combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (3:1)] to give **3** (496 mg, 88%) as a white solid.

$[\alpha]_D^{16}$ +12.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, s, Aromatic-*H*), 7.48 (1H, s, Aromatic-*H*), 7.38–6.97 (20H, m, Aromatic-*H*), 5.27 (1H, d, *J*=10.3 Hz, H-1), 4.90 (1H, d, *J*=11.3 Hz, PhCH_2–), 4.84 (1H, d, *J*=3.3 Hz, H-1′), 4.63 (1H, d, *J*=11.5 Hz, PhCH_2–), 4.61 (1H, d, *J*=12.0 Hz, PhCH_2–), 4.60 (1H, d, *J*=11.3 Hz, PhCH_2–), 4.58 (1H, d, *J*=11.5 Hz, PhCH_2–), 4.56 (1H, d, *J*=12.0 Hz, PhCH_2–), 4.31 (1H, t, *J*=10.3 Hz, H-2), 4.30 (1H, d, *J*=12.0 Hz, PhCH_2–), 4.29 (1H, d, *J*=12.0 Hz, PhCH_2–), 4.26 (1H, t, *J*=10.3 Hz, H-3), 4.21 (1H, dd, *J*=10.3, 7.8 Hz, H-4), 4.12 (1H, q, *J*=6.5 Hz, H-5′), 3.90–3.76 (2H, m, H-6), 3.89–3.85 (1H, m, 4-O*H*), 3.84 (1H, dd, *J*=10.3, 3.3 Hz, H-2′), 3.79 (1H, dd, *J*=2.7, 10.3 Hz, H-3′), 3.62–3.59 (1H, m, H-5), 3.57 (1H, br, H-4′), 2.15 (3H, s, SCH_3), 1.11 (3H, d, *J*=6.5 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.5, 166.0, 138.6, 138.6, 138.5, 138.2, 137.9, 131.1, 130.8, 128.5, 128.3, 128.3, 128.2, 127.7, 127.7, 127.6, 127.6, 127.5, 126.7, 125.2, 124.9, 100.1, 83.8, 80.4, 79.4, 78.8, 77.7, 75.8, 74.9, 73.5, 73.0, 71.0, 69.2, 68.6, 53.5, 16.5, 11.5; HRMS FAB *m*/*z* for $C_{49}H_{49}O_{10}NCl_2S$+H $(M+H)^+$ calcd 914.2533, found 914.2534.

4.4. Methyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-(4,5-dichlorophthalimido)-1-thio-β-D-glucopyranoside (2)

4.4.1. Typical method with microwave irradiation: entry 2 in Scheme 2

A solution of TMSOTf (20 μl, 0.110 mmol) in diethyl ether (1.5 ml) was added dropwise to a stirred −30 °C solution, cooled by the CoolMate, of pre-dried disaccharide acceptor (**3**) (51.5 mg, 0.0563 mmol) and galactosyl donor (**7**) (59.7 mg, 0.112 mmol) in diethyl ether (3 ml) in a CEM equipped vessel under nitrogen atmosphere. The reaction mixture was stirred for 5 h with microwave irradiation (300 W) and the reaction temperature was monitored every second automatically. The mixture was then diluted with chloroform and neutralized with satd $NaHCO_3$ aq. The aqueous layer was extracted with chloroform four times, and combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography [Sephadex LH-20: chloroform/methanol (1:1)] to give **2** (54.4 mg, 70%) as a white solid and recovered as **3** (7.6 mg, with a calculated yield of **2** of 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (1H, s, Aromatic-*H*), 7.38–6.97 (36H, m, Aromatic-*H*), 5.27 (1H, dd, *J*=9.8, 8.3 Hz, H-2″), 4.99 (1H, d, *J*=10.4 Hz, H-1), 4.87 (1H, d, *J*=10.6 Hz, PhCH_2–), 4.76 (1H, d,

J=12.0 Hz, PhCH_2–), 4.67 (1H, d, J=12.2 Hz, PhCH_2–), 4.61 (1H, t, J=9.6 Hz, H-3), 4.61 (1H, m, H-5′), 4.60 (1H, d, J=8.3 Hz, H-1″), 4.59 (1H, d, J=4.6 Hz, H-1′), 4.52 (1H, d, J=12.2 Hz, PhCH_2–), 4.50 (1H, d, J=11.2 Hz, PhCH_2–), 4.45 (1H, d, J=12.0 Hz, PhCH_2–), 4.44 (1H, d, J=12.4 Hz, PhCH_2–), 4.43–4.27 (6H, m, PhCH_2–), 4.40 (1H, m, H-2), 4.29 (1H, d, J=12.4 Hz, PhCH_2–), 4.11 (1H, t, J=9.6 Hz, H-4), 3.99 (1H, d, J=2.6 Hz, H-4″), 3.89–3.79 (2H, m, H-6), 3.85 (1H, dd, J=10.3, 2.6 Hz, H-3′), 3.76–3.67 (2H, m, H-6″), 3.71 (1H, m, H-2′), 3.55 (1H, d, J=9.6 Hz, H-5), 3.34 (1H, dd, J=9.8, 2.6 Hz, H-3″), 3.33 (1H, m, H-5″), 3.18 (1H, br, H-4′), 2.07 (3H, s, SCH_3), 2.02 (3H, s, COCH_3), 1.13 (3H, d, J=6.5 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 166.4, 165.2, 139.2, 138.9, 138.7, 138.3, 138.1, 138.0, 137.8, 128.8, 128.6, 128.4,128.4, 128.3, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.1, 127.0, 125.8, 99.8, 97.7, 80.8, 80.2, 80.0, 79.9, 78.4, 77.2, 75.5, 74.9, 74.4, 73.8, 73.4, 73.3, 73.2, 73.1, 72.8, 72.7, 72.0, 71.8, 71.5, 67.9, 67.7, 66.8, 55.1, 16.2, 10.7; MALDI-TOF MS m/z for $C_{78}H_{79}Cl_{21}NO_{18}S$+Na $[M+Na]^+$ calcd 1410.4, found 1410.5; HRMS ESI m/z for $C_{78}H_{79}Cl_{21}NO_{18}S$+Na $[M+Na]^+$ calcd 1410.4394, found 1410.4374.

4.5. Methyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-acetoamide-2-deoxy-1-thio-β-D-glucopyranoside (11)

Ethylenediamine (30 μl, 0.449 mmol) was added dropwise to a stirred solution of **2** (16.3 mg, 0.0117 mmol) in ethanol (1.5 ml) at room temperature. The reaction mixture was stirred for 2 h at 70 °C and concentrated in vacuo. The resulting residue was solved with dichloromethane (0.5 ml), and pyridine (1 ml) and acetic anhydride (1 ml) were added successively at 0 °C. The reaction mixture was stirred for 3 h at room temperature and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (2:1)] to give **11** (13.8 mg, 95%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.14 (35H, m, Aromatic-*H*), 5.53 (1H, d, J=8.4 Hz, –N*H*Ac), 5.24 (1H, dd, J=10.0, 8.2 Hz, H-2″), 5.06 (1H, br, H-1′), 4.88 (1H, d, J=10.8 Hz, PhCH_2–), 4.73 (1H, d, J=12.7 Hz, PhCH_2–), 4.70 (1H, d, J=12.4 Hz, PhCH_2–), 4.68 (1H, d, J=12.4 Hz, PhCH_2–), 4.66 (1H, d, J=12.4 Hz, PhCH_2–), 4.57 (2H, d, J=11.4 Hz, PhCH_2–), 4.52 (1H, d, J=12.2 Hz, PhCH_2–), 4.50 (1H, d, J=8.2 Hz, H-1″), 4.49 (1H, q, J=6.5 Hz, H-5′), 4.47 (1H, d, J=10.7 Hz, PhCH_2–), 4.42 (1H, d, J=12.2 Hz, PhCH_2–), 4.35 (2H, s, PhCH_2–), 4.15–4.05 (1H, m, H-2 or H-3), 4.09 (2H, d, J=11.4 Hz, PhCH_2–), 4.00 (1H, m, H-4), 3.99 (1H, m, H-2′), 3.98 (1H, m, H-4″), 3.83 (1H, dd, J=7.5, 3.1 Hz, H-3′), 3.78 (1H, dd, J=11.0, 3.3 Hz, H-6), 3.75–3.70 (1H, m, H-5″), 3.75–3.70 (1H, m, H-2 or H-3), 3.73 (1H, dd, J=11.0, 2.8 Hz, H-6), 3.62 (1H, dd, J=8.5, 4.7 Hz, H-6″), 3.45 (1H, m, H-5), 3.37 (1H, dd, J=10.0, 2.7 Hz, H-3″), 3.32 (1H, dd, J=8.5, 4.9 Hz, H-6″), 3.26 (1H, br, H-4′), 2.11 (3H, s, SCH_3), 1.99 (3H, s, COCH_3 or NHCOCH_3), 1.73 (3H, s, COCH_3 or NHCOCH_3), 1.11 (3H, d, J=6.5 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 169.2, 139.2, 139.1, 138.8, 138.6, 138.2, 137.9, 137.8, 128.7, 128.5, 128.5, 128.4, 128.4, 128.3, 126.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 99.8, 97.7, 80.5, 79.6, 79.4, 78.3, 75.3, 74.9, 73.4, 73.3, 73.2, 73.0, 72.9, 72.2, 72.1, 71.6, 68.4, 67.7, 66.7, 23.4, 21.0, 16.3, 11.9; MALDI-TOF MS m/z for $C_{72}H_{81}Cl_{21}NO_{15}S$+Na $[M+Na]^+$ calcd 1254.5, found 1254.8; HRMS ESI m/z for $C_{72}H_{81}Cl_{21}NO_{15}S$+Na $[M+Na]^+$ calcd 1254.5224, found 1254.5222.

4.6. *O*-(3,4,6-Tri-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-acetoamide-2-deoxy-β-D-glucopyranose (12)

N-Iodosuccinimide (2.6 mg, 11.6 μmol) and TMSOTf (10 μl, 55.5 μmol) were added successively to a solution of **11** (11.8 mg, 95.7 μmol) in aqueous dichloromethane (1 ml) at 0 °C, and the reaction mixture was stirred for 10 min. The mixture was diluted with chloroform and washed with satd $Na_2S_2O_3$ aq and satd $NaHCO_3$ aq successively. The combined aqueous layer was extracted with chloroform three times, and combined extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by column chromatography [silica gel: *n*-hexane/EtOAc (2:1)] to give **β-12** (6.4 mg, 56%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.21 (35H, m, Aromatic-*H*), 5.90 (1H, d, J=7.5 Hz, H-1), 5.22 (1H, dd, J=10.1, 8.0 Hz, H-2″), 5.19 (1H, d, J=3.7 Hz, H-1′), 4.87 (1H, d, J=11.2 Hz, PhCH_2–), 4.77 (2H, d, J=11.5 Hz, PhCH_2–), 4.76 (1H, d, J=11.7 Hz, PhCH_2–), 4.70–4.61 (5H, m, PhCH_2–), 4.50 (1H, d, J=11.2 Hz, PhCH_2–), 4.45 (1H, d, J=12.1 Hz, PhCH_2–), 4.45 (1H, d, J=11.7 Hz, PhCH_2–), 4.44 (1H, d, J=8.0 Hz, H″-1), 4.34 (2H, J=11.5 Hz, PhCH_2–), 4.32 (1H, d, J=12.0 Hz, PhCH_2–), 4.30 (1H, d, J=12.0 Hz, PhCH_2–), 4.19 (1H, q, J=6.4 Hz, H-5′), 4.14 (1H, t, J=4.4 Hz, H-3), 4.05–4.02 (1H, m, H-2), 3.99 (1H, m, H-4), 3.96 (1H, dd, J=10.1, 3.7 Hz, H-2′), 3.94 (1H, m, H-4″), 3.82 (1H, dd, J=10.1, 2.7 Hz, H-3′), 3.66–3.61 (2H, m, H-6), 3.63–3.61 (1H, m, H-5″), 3.55 (1H, dd, J=8.6, 5.0 Hz, H-6″), 3.43–3.38 (1H, m, H-5), 3.41–3.37 (1H, m, H-6″), 3.38–3.36 (1H, m, H-4′), 3.33 (1H, dd, J=10.1, 2.8 Hz, H-3″), 2.75 (1H, s, O*H*), 1.99 (3H, s, NHCOCH_3), 1,93 (3H, s, COCH_3), 1.04 (3H, d, J=6.4 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.1, 165.4, 139.1, 139.0, 138.6, 138.5, 138.0, 137.9, 137.8, 101.3, 102.3, 97.7, 80.3, 79.3, 78.2, 77.8, 76.3, 74.9, 74.7, 73.4, 73.3, 73.0, 72.9, 72.8, 72.6, 71.8, 71.6, 70.9, 68.6, 67.9, 67.16, 66.6, 21.0, 16.4, 14.1; MALDI-TOF MS m/z for $C_{71}H_{79}NO_{16}$+Na $[M+Na]^+$ calcd 1224.5, found 1224.6; HRMS ESI m/z for $C_{71}H_{79}NO_{16}$+Na $[M+Na]^+$ calcd 1224.5297, found 1224.5300.

4.7. *O*-(β-D-Galactopyranosyl)-(1→4)-[*O*-(α-L-fucopyranosyl)-(1→3)]-2-acetoamide-2-deoxy-α,β-D-*gluco*-pyranose; Lewis X oligosaccharide (1)[44]

Pd(OH)$_2$/C (cat.) was added to a stirred solution of **10** (6.4 mg, 5.32 μmol) in methanol (1 ml) at room temperature and the mixture was treated under hydrogen. The reaction mixture was stirred overnight at room temperature. The mixture was filtered through Celite. The filtrate was concentrated in vacuo. The resulting residue was solved with water (1 ml), and 15% NaOH aq (one drop) was added. The reaction mixture was stirred for 2 h at room temperature, neutralized with Dowex 500WX8-400 and filtered. The filtrate was concentrated in vacuo. The resulting residue was purified by column chromatography [Sephadex G-10: water] to give Lewis X oligosaccharide (**1**)[44] (2.8 mg, 99%).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.08.011.

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