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Rapid Access to an Orthogonally Protected Lewis X Derivative: An Important Building Block for Synthesis of Lewis Antigens

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A novel Lewis X derivative of **1** with an orthogonal set of protecting groups was systematically prepared. Efficient use of the protecting groups for the amino and the hydroxy groups in lactosamine enabled formation of the diol derivative at C3 and C2′. Glycosidation of 1-thio-L-fucoside donor with the diol derivative progressed preferably at the C3 hydroxy group, resulting in prior production of **1**. Compound **1** is a key building block toward easy assemblies of Lewis X-related carbohydrate epitopes.

Lewis X {Galβ(1 → 4)[Fucα(1 → 3)]GlcNAc; Le^x} trisaccharide is frequently found in the structures of important bioactive oligosaccharides,[1] which play key roles in various biological events such as inflammation, lymphocyte homing, infection by pathogens, and metastasis of tumors.[2–4] For example, Le^x-incorporated oligosaccharides are overexpressed on the surface of tumor cells.[5] Le^x is a promising target for anticancer agents, therefore attracting a lot of glycoscientists to attempt efficient syntheses of Le^x-related glycoconjugates.[6] Various approaches have been reported, however, most of them require lengthy, multistep sequences with complex manipulations despite the development of new synthetic methodologies.[7–10] The Lewis structure is mostly prepared by assembling suitably protected monosaccharide building blocks via conventional chemical methods. A few examples employing a disaccharide of lactose [Galβ(1 → 4)Glc] as a starting material have been reported to avoid problems in the synthesis of Le^x derivatives,[11] where a troublesome glycosylation reaction can be avoided; however, introduction of an acetamido function is often problematic in these strategies. In contrast, 2-aminodeoxy sugars have been successfully prepared from ketoses via the Heyns rearrangement,[12] particularly, this methodology is of great use in employing disaccharidic ketoses. In the present study, we first demonstrate facile access to the Le^x trisaccharide derivative **1** (Figure 1) via the Heyns rearrangement and by rational protecting group strategy. Compound **1** is a key intermediate having a set of orthogonal protecting groups, which are capable of producing a series of important Le^x-related epitopes such as Le^y, Le^x oligomers, sialyl Le^x, sialyl oligomeric Le^x, and sulfated Le^x derivatives.[4] Therefore, this study will open a new access to Lewis antigens, which should be helpful for researchers in many fields even unfamiliar with carbohydrate chemistry.

To obtain the lactosamine derivative **3**, we carried out the Heyns rearrangement of lactulose **2**[12] (Scheme 1). The *N*-phthaloyl (NPhth) group in **3** was introduced because of the selectivity in removal by hydrazine and the capability of forming 1,2-*trans*-glycoside by anchimeric assistance from carbonyl oxygen. Furthermore, it is to be expected that the NPhth group at C2 provides sterically hindered surroundings for the C3 substituent. Compound **3** was isolated at this step (33% yield from **2**). For the anomeric *O*-protection, a 4-methoxyphenyl (MP) group was introduced in a β-manner through glycosylation of MP–OH with trichloroacetimidate **4**,[13] providing **5**[14] in an 86% yield. The MP glycoside is frequently employed in carbohydrate chemistry because of the stability under various conditions except under oxidation using cerium(IV) ammonium nitrate.[15] All of the *O*-acetyl protecting groups in **5** were removed by the Zemplén procedure. The hydroxy groups at C4′ and C6′ of **6** were protected by the benzylidene acetal to give **7** followed by introduction of the *tert*-butyldimethylsilyl (TBDMS) group at C6 to afford **8**. The 4′,6′-*O*-benzylidene and the 6-*O*-TBDMS groups can be removed independently, where the former is cleaved by hydrogenation in the presence of Pd–C and the latter by fluoride anion species. Furthermore, the 4′,6′-*O*-benzylidene acetal can remain protected after removal of *O*-benzyl groups through hydrogenolysis using Pearlman's catalyst.[16] Such orthogonal protection of C6 and C6′ enables facile production of sialylated and/or sulfated Le^x derivatives.

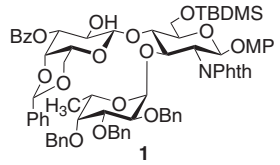
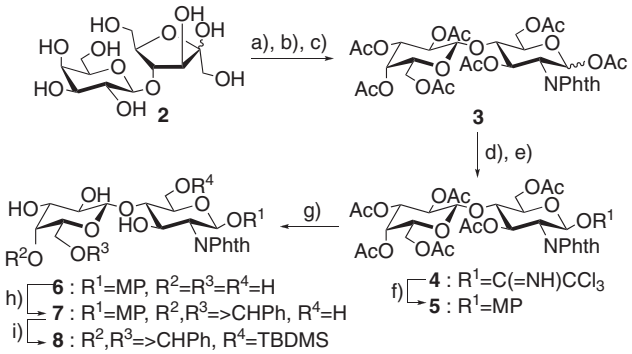
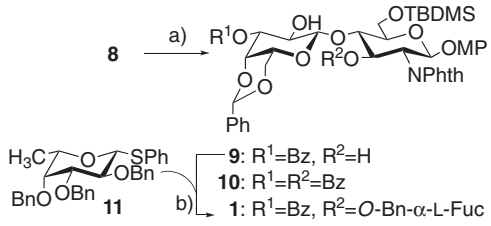


Figure 1. A key building block for synthesis of Lewis antigens.



Scheme 1. *Reagents and conditions*: a) 1) benzylamine, 40 °C, 2 days, 2) MeOH–AcOH (8:1, v/v), rt, overnight; b) $Pd(OH)_2$–C, H_2, HCl(aq), rt, 3 days; c) 1) MeONa then phthalic anhydride/MeOH, rt, 26 h, 2) Ac_2O/pyridine, rt, 18 h, 33% (5 steps); d) benzylamine/THF, rt, 20 h, 72%; e) CCl_3CN, DBU/CH_2Cl_2, 0 °C, 2 h, 57%; f) 4-methoxyphenol, $BF_3 \cdot OEt_2/CH_2Cl_2$, −50 °C, 3 h, 86%; g) MeONa/MeOH, rt, 15 h, 80%; h) $PhCH(OMe)_2$, 10-camphorsulfonic acid/DMF, 30 °C, 10 h, 68%; i) TBDMS–Cl/pyridine, rt, 24 h, 82%.



Scheme 2. *Reagents and conditions*: a) BzCl/pyridine–CH_2Cl_2 (1:1, v/v), −50 °C, 6 h, **9**, 64%, **10**, 14%; b) NIS–TfOH/CH_2Cl_2–Et_2O (1:2, v/v), −78 °C, 1 h, 68%.

In carbohydrate chemistry, *O*-benzoyl protection, which is selectively removed by the Zemplén procedure, is frequently adopted for regioselective protection of hydroxy groups to reduce multiple protection–deprotection procedures.[17,18] Particularly on some lactoside derivatives, regioselective *O*-benzoylation has been successfully carried out, leading to easy access to Le^x analogs.[19,20] However, to the best of our knowledge, the regioselective *O*-benzoylation of lactosamine derivatives has not been reported so far. Thus, we tried regioselective *O*-benzoylation of **8** to prepare a mono-*O*-benzoylated derivative of **9** (Scheme 2). It is to be pointed out that 3-OH of 2-azidolactose is highly reactive similar to the primary 6- and 6′-OH;[21] however, the nucleophilicity of 3-OH in **8** will be appropriately reduced by the neighboring NPhth group at C2. Therefore, we anticipated the reactivity order of the hydroxy groups to be 3′-OH[22] > 3-OH > 2′-OH. Indeed, compound **9** was obtained in a satisfactory yield of 64% by addition of 1.5 mol equivalents of benzoyl chloride (BzCl) to a solution of **8** in pyridine–dichloromethane (1:1, v/v) mixture at −50 °C under Ar atmosphere. In addition, 3,3′-di-*O*-benzoylated derivative **10** was also formed in a lower yield of 14%. These results clearly indicate that 3′-OH is most easily benzoylated among three hydroxy groups in **8**, resulting in predominant production of **9**. In the case employing 2.1 mol equivalents of BzCl, compounds **9** and **10** were obtained in 62% and 17% yields, respectively. Therefore, it seems difficult to improve the yield of **9** and to completely suppress formation of **10** by controlling the amount of BzCl. Taken together all these results, we tried regioselective *O*-fucosylation at C3 of the diol **9**.

O-Benzyl-protected 1-thio-L-fucoside derivatives are preferred as a glycosyl donor for α-L-fucoside formation via 1,2-*cis*-glycoside to C3 or C4 of glucosamine derivatives in the synthesis of Lewis antigens.[7–10,16,20] Furthermore, some 1-thio-L-fucoside derivatives are commercially available. Therefore, we employed phenyl 1-thio-L-fucoside derivative **11**[23] as a glycosyl donor. Glycosylation of **9** with equimolar amounts of **11** was performed in CH_2Cl_2–Et_2O (1:2, v/v) mixture at −78 °C with *N*-iodosuccinimide–triflic acid as a promoter system.[23] The glycosylation reaction proceeded smoothly, generating the target trisaccharide **1** in a reasonable yield of 68% within 1 h. A trace amount of the Lewis Y tetrasaccharide derivative was formed, which could easily be removed by chromatographic procedures. The Le^y derivative was obtained as a main product when 1.5 mol equivalents of **11** was used at −40 °C within 2 h (Supporting Information).[24] Notably, a trisaccharide derivative bearing *O*-fucoside at only C2′ was not formed at all. Therefore, compound **1** was first formed followed by production of the Le^y derivative during the glycosylation reaction, which can be controlled by the amounts of **11** and reaction temperature. Thus, the Le^x derivative **1** was successfully prepared from **2** via 13 steps.

In conclusion, rapid assembly of the Le^x derivative **1** was first demonstrated by utilizing the Heyns rearrangement and by rational protecting group manipulation on the basis of reactivity order prediction of hydroxy groups. Compound **1** has several orthogonal protecting groups, each of which can be removed without any damage to other protecting groups. Furthermore, all of the reactions in this study were carried out in several gram scales (see SI);[24] this synthetic strategy is applicable for large scale synthesis of Le^x-related carbohydrate antigens. Thus, the present study will accelerate preparation of various Le^x derivatives, which will stimulate research in many fields such as glycoscience, medicine, pharmaceutics, and biology.

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24 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

