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Synthesis of an *O*-sulfo Lewis^X analog as glycolipid antigen

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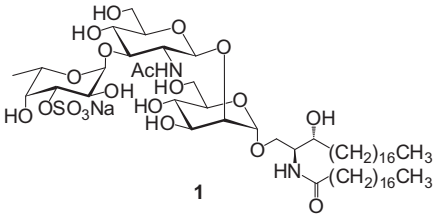
ABSTRACT

The title compound containing dihydroceramide as a ligand for CD1d was accomplished using the mannosyl, glucosaminyl, and fucosyl donors, and a sphinganine analogue, as suitable building blocks. The 2-O-unprotected mannosyl donor was coupled effectively with the sphinganine analog to afford the mannnosyl sphinganine derivative. The coupling of the glucosaminyl donor with the mannnosyl sphinganine acceptor required triflic acid as a promoter and the promoter change to silver triflate led to the undesired glycal production. The reduction of azide group using Zn powder was the key process, in which the amount of acetic acid was restricted to avoid the benzoyl migration and *N*-trichloroacetyl deprotection. The trisaccharide glycolipid was sulfonated at the 3-position of fucose moiety.

1. Introduction

Cancer-associated glycoproteins and glycolipids have been found by characterization of cancers.[1–4] These cancer-cell glycans were associated with malignant transformation, hematogenous metastasis and angiogenesis.[5] Levels of fucosyl-, β-galactosyl-, β-*N*-acetylgalactosaminyl-, sialyl-, and sulfotransferase activities of various cancer cell lines were examined and the existence of unique carbohydrate moieties in cancer-associated glycolipids was predicted.[6] Whereas, the CD1 family of antigen presenting glycoproteins has been well studied[7] and the crystal structure of CD1d revealed that two deep hydrophobic pockets were able to accommodate two hydrocarbon chains.[8]



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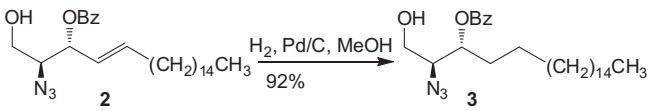
In these contexts, target molecule **1** as a cancer antigen candidate was designed to mimic a partial structure of Lewis^X, which can be constructed by the cancer specific activation of corresponding transferases in N-glycan biosynthesis. A glycolipid-immunized antibody production against glycoproteins is a challenging study because glycolipids are much readily obtainable than glycoproteins.

2. Results and discussion

Three sugar and one lipid components were required for a chemical synthesis of target compound **1**. A saturated sphinganine analog was selected as the ligand for CD1d because of synthetic process truncation. Therefore, known sphingosine analog **2**[9] was hydrogenated using H_2 and Pd/C to afford saturated lipid component **3** (92%) as depicted in Scheme 1.

The glucosaminyl donor was prepared in the following way (Scheme 2). Glucosamine derivative **4**[10] was protected by a *p*-methoxybenzyl (MB) group to afford **5** in 76% yield. The benzylidene group of **5** was hydrolyzed in 90% aqueous acetic acid, followed by benzylation, to provide glucosaminyl donor **6** in a 72% yield over two steps.

The fucosyl donor was obtained in the following manner (Scheme 3). Isopropylidenation of known thiofucoside **7**[11] afforded **8** (65%), of which the protection with an allyl group afforded **9** (90%). The isopropylidene group of **9** was hydrolyzed in 90% aqueous acetic acid to yield deprotected compound **10** (90%). Partial protection of **10** using dibutyltin oxide and *p*-methoxybenzyl



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Scheme 1.

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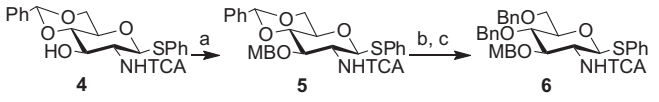
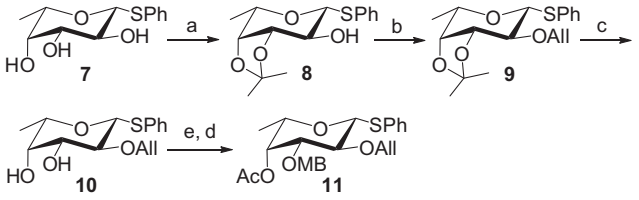


Scheme 2. Reagents and conditions: (a) NaH, MBCl, DMF, 0 °C, 76%; (b) 90% HOAc; (c) NaH, BnBr, DMF, 0 °C, 72% (two steps).



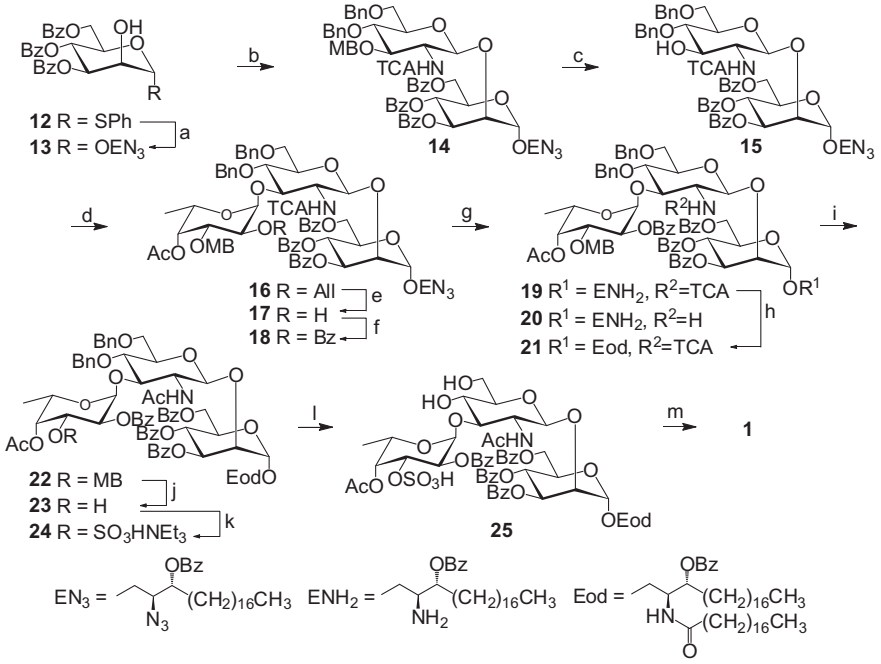
Scheme 3. Reagents and conditions: (a) 2-methoxy propene, *p*-toluenesulfonic acid, acetone, 65%; (b)AllBr, NaH, DMF, 0 °C, 90%; (c) 90% HOAc, 60 °C, 90%; (d) Bu_2SnO, TBAI, NaH, MBCl, 120 °C; (e) Ac_2O, pyridine, 60% (two steps).

chloride and ensuing acetylation furnished the fucosyl donor **11** (60%).

The construction of the target compound **1** is outlined in Scheme 4. Condensation of sphinganine acceptor **3** with mannnosyl donor **12**[12] having a hydroxyl group at C-2, which was designed in this research as a donor with moderate activity afforded α-mannopyranoside **13** (75%), of which the α-configuration was ascertained by ^{13}C NMR spectroscopy ($J_{C\text{-}1,H\text{-}1}$ = 173 Hz).[13] Although it might be expected that 2-O-chloroacetylated **12** would improve a condensation yield, a similar mannosylation using 2-O-chloroacetylated **12** never afforded the desired product because the condensation was preceded by acyl migration of the 2-*O*-chloroacetyl group to the acceptor. A second condensation of acceptor **13** with glucosaminyl donor **7** under NIS–TfOH promotion yielded disaccharide **14**, of which the newly constructed β-configuration was confirmed by ^1H NMR spectroscopy ($J_{1,2}$ = 7.9 Hz). Glycal production was promoted by use of silver triflate instead of triflic acid as the promoter and no desired product was generated. The *p*-methoxybenzyl group of **14** was deprotected using ammonium cerium(IV) nitrate (CAN) to yield acceptor **15** (72%). Condensation of **15** and fucosyl donor **11** under NIS–TfOH promotion afforded trisaccharide **16** (67%), of which newly constructed α-configuration was confirmed by ^1H NMR spectroscopy ($J_{1,2}$ = 3.4 Hz).

Deprotected compound **17** (63%) was prepared by $PdCl_2$-catalized deallylation of **16** and again protected by benzoyl group to afford benzoate **18** in a 92% yield. We attempted to carry out trichloroacetyl (TCA) and azido group reduction using tributyltin hydride; however, benzoyl migration to the newly produced amino group was inevitable. The problem was resolved by an unexpected reaction. Azido group reduction of **18** was accomplished using Zn powder without the removal of the *N*-trichloroacetyl group in spite of its removal conditions. Ratios of HOAc and CH_2Cl_2 were critical during the treatment with zinc: azide group reduction and de-N-trichloroacetylation were normally observed in a high concentration (0.06:1 HOAc–CH_2Cl_2) to afford derivative **20** (60%); the de-N-trichloroacetylation was prevented in a middle concentration (0.05:1 HOAc–CH_2Cl_2) to afford desired compound **19** (61%); starting material **18** was completely recovered in a low concentration (0.02:1 HOAc–CH_2Cl_2). Subsequent coupling of the amino group of **19** with octadecanoic acid using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (WSC) afforded glycolipid derivative **21** (55% two steps) and the trichloroacetyl group was converted to acetyl group by radical reduction using tributyltin hydride to yield **22** (98%).

The *p*-methoxybenzyl group of **22** was deprotected using CAN to afford **23** (69%), which was sulfonated using sulfur trioxide–pyridine complex to yield **24** (79%). Removal of the benzyl group with sodium bromate and sodium dithionite afforded **25** in a 33% yield.[14] The removal of acyl groups with NaOMe in MeOH gave **1** in an 86% yield. The formation of 3-O-sulfonated derivatives was proven by ^1H NMR spectroscopy. The 3^3-protons of the sulfonated **24** and **25** showed the remarkable down-field shifts of 1.29–1.45 ppm compared with that of the non-sulfonated **23**. Moreover, the sulfonated 3^3-proton of **1** also showed the down-field shifts of 0.50 and 0.42 ppm compared with the non-sulfonated 2^3- and 4^3-



Scheme 4. Reagents and conditions: (a) **3**, NIS, AgOTf, MS 4 Å, CH_2Cl_2, 0 °C, 75%; (b) **6**, NIS, HOTf, MS 4 Å, CH_2Cl_2, −15 °C; 70%; (c) CAN, 10:1 CH_3CN/H_2O, 72%; (d) **11**, NIS, HOTf, MS 4 Å, CH_2Cl_2, 0 °C, 67%; (e) $PdCl_2$, 2:1 MeOH/CH_2Cl_2, 63%; (f) BzCl, Pyr, CH_2Cl_2, 92%; (g) Zn, HOAc, CH_2Cl_2, 0 °C, 61%; (h) octadecanoic acid, WSC, CH_2Cl_2, 75%; (i) Bu_3SnH, DMA, AIBN, C_6H_6, 120 °C, 98%; (j) CAN, 10:1 CH_3CN/H_2O, 69%; (k) SO_3/pyridine, DMF, 79%; (l) $NaBrO_3$, $Na_2S_2O_4$, 1:2 AcOEt/H_2O, 33%; (m) NaOMe, MeOH, H_2O, 86%.