ORGANIC LETTERS

2005
Vol. 7, No. 17
3753−3756

Pentaerythritol as the Core of Multivalent Glycolipids: Synthesis of a Glycolipid with Three SO_3Le^a Ligands

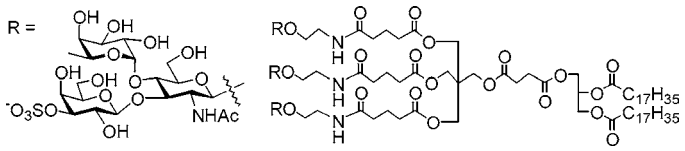
Jie Xue,[†,‡] Junmin Zhu,[§] Roger E. Marchant,*[,§] and Zhongwu Guo*[,†,‡]

Departments of Chemistry and Biomedical Engineering, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106-7078, and Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202

zxg5@case.edu

Received June 18, 2005

ABSTRACT



A glycolipid containing three SO_3Le^a ligands was synthesized with pentaerythritol as the core. The glycolipid was used to prepare glycoliposomes that showed stability similar to that of DSPC liposomes without glycolipid. The easily prepared derivatives of pentaerythritol proved to be useful scaffolds for multivalent displaying of carbohydrates in the form of glycolipids and clustered glycoliposomes.

While carbohydrate−protein interactions shape the basis of glycobiology,[1] the significant enhancement of these interactions by the so-called "multivalent effect" is critical to various biological functions.[2,3] It is believed that nature uses this mechanism to offset the generally low affinity of carbohydrate ligands for their protein receptors. Actually, compelling results have revealed that clustering multiple carbohydrate binding motifs in a glycoconjugate ligand can dramatically increase its protein affinity.[3,4]

Recently, a wide range of multivalent glycoconjugates have been designed, prepared, and studied,[3,4] of which the majority are dendrimers with multiple carbohydrate ligands anchored to peptides, acrylamides, or other multifunctional cores. Another important form of multivalent presentation of carbohydrate ligands is glycoliposomes, i.e., liposomes decorated with carbohydrates,[5−7] such as the display of selectin ligands on liposome surfaces.[8−11] A unique property of this latter approach is that liposomes can offer superb structural versatility in terms of chemical composition and molecular fluidity, allowing self-assembly of glycolipids to form special carbohydrate domains on liposome surfaces. Thus, glycoliposomes have been studied extensively as tools for targeted delivery of drugs,[5−7] which makes use of the specific interactions between carbohydrate ligands on a glycoliposome and the particular receptors on the targeted tissue.

In an effort to develop a useful system for the targeted delivery of drugs to inflammatory tissues, we have recently studied the stability and other properties of glycoliposomes made of a monovalent glycolipid of SO_3Le^a,[12] the natural

[†] Department of Chemistry, Case Western Reserve University.
[‡] Wayne State University.
[§] Department of Biomedical Engineering, Case Western Reserve University.

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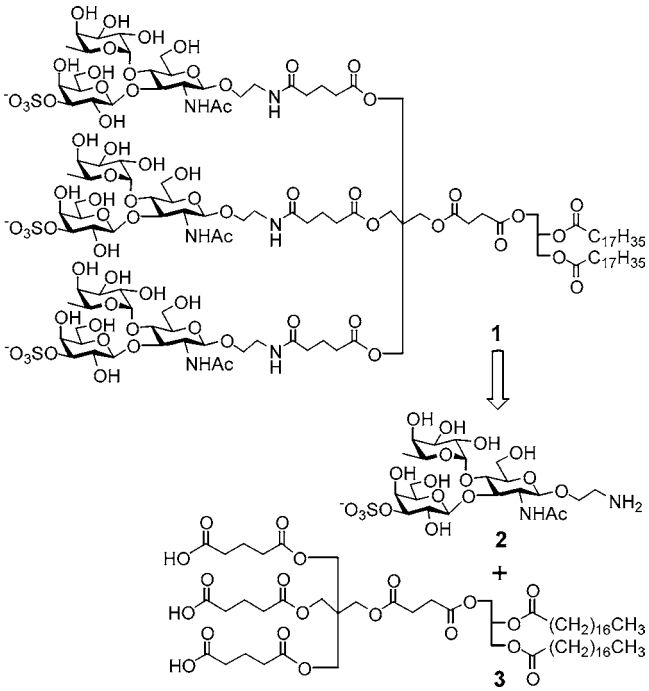
10.1021/ol0514202 CCC: $30.25
Published on Web 07/19/2005

carbohydrate ligand of E-/P-selectins.[13−15] It was found that glycoliposomes consisting of a low concentration (5 mol %) of SO_3Le^a−lipid conjugate were basically as stable as PEG-modified liposomes, while the affinity of glycoliposomes for activated platelets increased drastically. It was also found, however, that there was a limit to the concentration of glycolipids that can be incorporated into the liposomes. If the incorporation concentration of glycolipids was more than 15 mol %, the resultant glycoliposomes were large in size and became unstable.[16]

Herein, we have designed and synthesized a multivalent glycolipid **1** (Scheme 1) and used it to construct a new type

Scheme 1



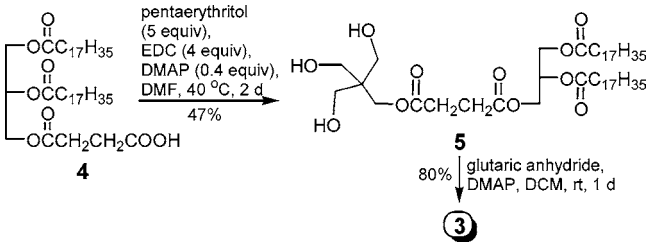
of glycoliposome. It was expected that by this approach we could maximize the number of carbohydrate ligands linked to the liposome surface with minimal impact on liposome stability. Meanwhile, we hoped that these liposomes could fully exploit the multivalent effect of both dendrimeric and liposomal constructs to result in glycoliposomes with the optimum binding affinity for the target tissue.

Our primary design for multivalent glycolipid synthesis was to couple carbohydrate ligands to a lipid that contains several functional groups. In this respect, one of the major issues was the multifunctional core. Although in the past decade many multifunctional scaffolds have been designed for synthesis of glycodendrimers or glycoclusters,[3,4] there have been relatively few reports about their applications to glycolipids. In this work, we planned to use pentaerythritol as the multivalent core, because it is readily available and its four primary hydroxyl groups are symmetric and highly reactive. Thus, pentaerythritol should be easily modified to contain various functionalities suitable for the conjugation with different target structures.

Recently, there have appeared a couple of reports about the selective protection and modification of pentaerythritol as well as its potential applications to glycolipid synthesis.[17,18] However, our design for using pentaerythritol as the multivalent core was especially simple. While one of its arms was linked to the lipid moiety, its remaining three arms were modified by diacids or other bifunctional groups. The resultant scaffold such as **3** that has three free carboxyl groups could then be coupled to the derivatized carbohydrate ligand, e.g., SO_3Le^a derivative **2**, or to other molecules through simple amide linkages (Scheme 1).

Scheme 2 delineates the preparation of the multivalent

Scheme 2



scaffold **3**. For the reaction between pentaerythritol and **4** with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as the condensation reagent, pentaerythritol was employed in large excess (5 equiv) to ensure its monoacylation. The excess pentaerythritol was readily removed from the reaction mixture by flash column chromatography, and the desired product **5** was obtained in a 47% yield. The reaction of **5** with glutaric anhydride was smooth and afforded **3** in a very good yield. Thus, the multivalent scaffold was readily prepared from pentaerythritol in two simple steps.

Scheme 3 shows the preparation of **17**, the key building block for synthesis of SO_3Le^a, and the target glycolipid **1**. A major improvement of this synthetic scheme compared to the one designed for monovalent glycolipid synthesis[12] was the introduction of a 2-azidoethyloxyl group[19,20] to the reducing end of the glycan, instead of a bare azido group. An advantage of the present design is that the free amine resulting from reduction of the azido group should be more stable and less sterically hindered than the glycosylamines involved in previous studies. This should be a very useful feature for subsequent coupling reactions. Another useful

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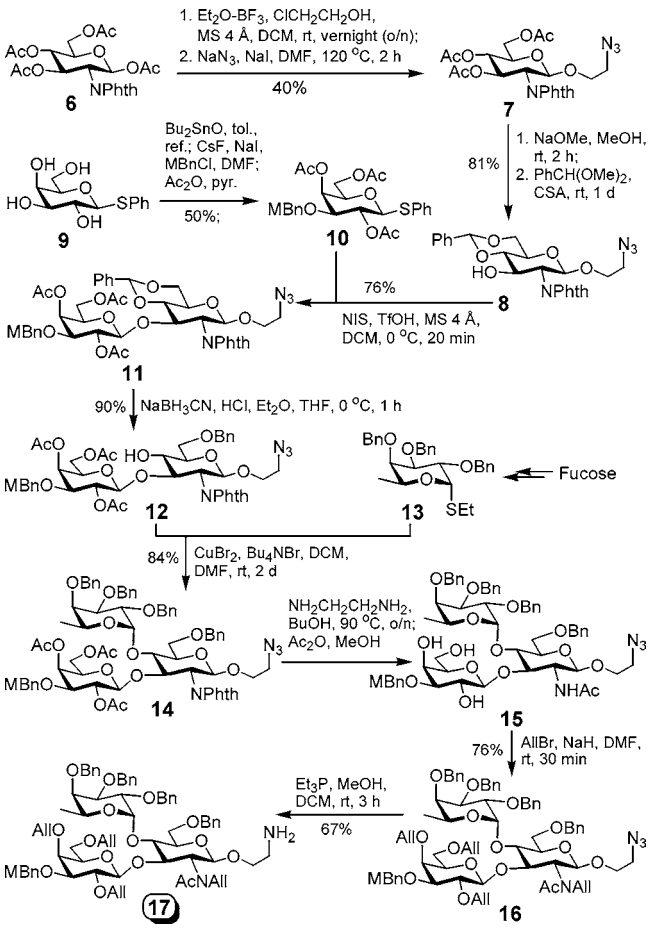
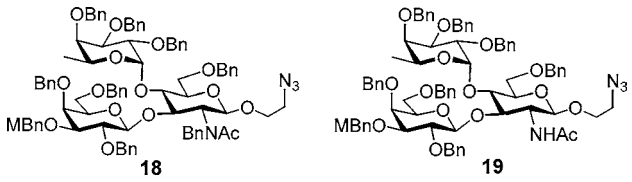
Scheme 3



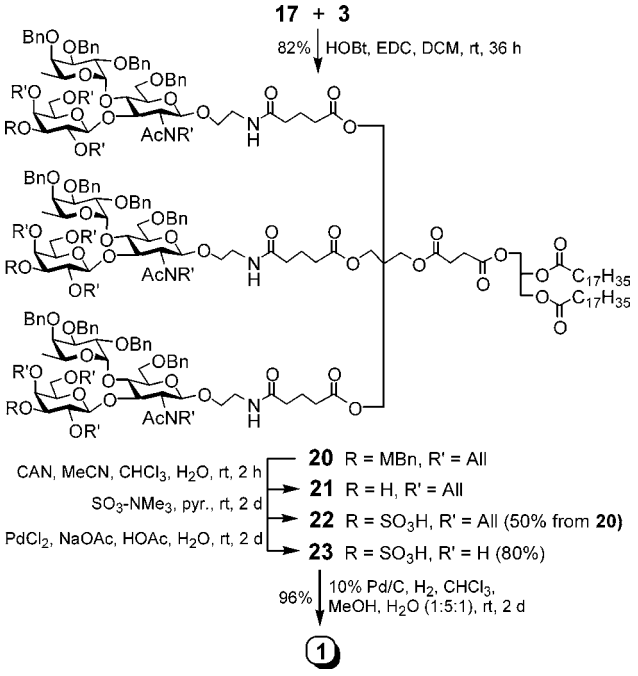
improvement in this synthetic scheme was the use of a *p*-methoxybenzyl (MBn) group to protect the 3-OH group of the galactosyl residue. Because MBn is stable to bases, it was possible to remove the acyl groups and manipulate other functional groups before the oxidative removal of MBn. Thus, the acetyl migration problem observed[12] in the deprotection of galactose could be avoided.

Because 2-azidoethanol is not commercially available, a 2-azidoethyloxyl group was introduced to the reducing end of glucosamine in two steps from **6**, i.e., glycosylation with commercial 2-chloroethanol followed by azido substitution using sodium azide as a nucleophile, to afford the desired product **7** in an acceptable yield (40% overall). The acetyl groups in **7** were then removed with NaOMe, and the 4,6-hydroxyl groups were protected in the form of benzylidene acetal. On the other hand, the MBn group was attached to the O-3 position via a tin-complex-directed regioselective alkylation of thiogalactoside **9**. Acetylation of its product eventually afforded the galactosyl donor **10**. The reaction of **8** and **10** utilizing NIS/TfOH[21] as promoters was highly stereoselective to form the β-linked disaccharide **11** in an excellent yield. The cyclic benzylidene acetal was thereby regioselectively opened to expose the glucosamine 4-OH. The subsequent fucosylation using **13** as a glycosyl donor gave no or low stereoselectivity under ordinary conditions, e.g., using NIS as the promoter. However, when $CuBr_2$ and Bu_4NBr were used as the promoters, the reaction proved to be stereospecific and afforded the desired trisaccharide **14** in an excellent yield (84%). It is believed that the reaction involves in situ formation of glycosyl bromide donors and an anomerization process.[22,23] This reaction was followed by conventional protecting group manipulations, including base-catalyzed deacylation, regioselective acetylation of the free amine, and then allylation to give **16**. The azido group of **16** was thereby reduced by phosphine to afford the key intermediate **17**, which was ready to be coupled with the multivalent lipid scaffold **3**. On the other hand, benzylation of **15** gave an inseparable mixture of the perbenzylated and partially benzylated products **18** and **19**; thus, this procedure was not adopted here.



The coupling reaction between **17** and **3** using EDC as the condensation reagent was very effective, and the fully protected glycoconjugate **20** was obtained in an 82% yield (Scheme 4). Thereafter, the MBn groups were removed by oxidation using ammonium cerium nitrate (CAN), which was followed by sulfation of the exposed hydroxyl groups and

Scheme 4



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$PdCl_2$-catalyzed deallylation. These reactions were monitored by TLC and was rather clean. The relatively low isolated yields were probably caused by the absorption of products by silica gel during column chromatography, which may be overcome by using reversed-phase chromatography. Finally, **23** was dissolved in a mixture of $CHCl_3$, MeOH, and H_2O (1:5:1) and completely deprotected by catalytic hydrogenation to afford the target glycolipid **1**.

Glycolipid **1** (5 mol %) was used with 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC, 55 mol %) and cholesterol (40 mol %) to prepare glycoliposomes by a freeze−thraw and extrusion method.[12] Liposome vesicles were obtained by extrusion of the mixed lipid suspension through a 100 nm polycarbonate membrane 10 times employing a miniextruder operated at 75 °C. The vesicle size and size distribution of liposomes were determined by laser scattering. The resultant glycoliposomes gave a mean vesicle size of 120 nm and were narrowly distributed with a polydispersity index (PDI) of 0.11, which is comparable to that of DSPC liposome (110 nm, PDI of 0.14) without incorporation of **1**. The slightly larger size (by ca. 9%) of the glycoliposomes may result from the relatively large polar head of the glycolipid **1**. The vesicle stability at room temperature was analyzed by observing the size change for an extended period. The results showed that vesicle size remained unchanged for at least one month. This means that the glycolipid **1** can provide an effective steric barrier on the liposome surface to stabilize the vesicles. The study on the influence of glycolipid concentration on vesicle size and stability of SO_3Le^a−liposomes showed that the trivalent glycolipid could be incorporated in a concentration as high as 15 mol % with the vesicle size being 171 nm. The resultant liposomes had good stability, as shown by the observation of their steady vesicle size for over one month. Increasing the incorporation rate to 20 mol % resulted in a larger vesicle size (192 nm) and liposomal aggregation after 5 days in storage. On the other hand, our preliminary study by flow cytometry indicated an obvious increase of the binding (94%) of activated platelets by liposomes of **1** compared to the binding (82%) by liposomes of monovalent glycolipid containing the same concentrations of the glycolipids. This research, as well as in vivo biological studies of the glycoliposomes, is in progress, and the results will be reported in due course.

In summary, this work has revealed that pentaerythritol and its multifunctional derivatives, e.g., **3**, can be excellent cores or scaffolds for multivalent glycolipids. A glycolipid (**1**) containing three SO_3Le^a ligands was synthesized from scaffold **3**. In this process, a robust route was developed for preparing SO_3Le^a derivatives suitable for conjugation with lipids or other carrier molecules containing carboxyl or carbonyl functionalities. Glycoliposomes with 5−15 mol % incorporation of **1** were stable at room temperature for at least one month.

Acknowledgment. We gratefully acknowledge financial support of this work by a grant from National Institutes of Health (R01 HL70263) and thank Mr. J. Faulk for the MS measurements, and Dr. Anirban Sen Gupta for the flow cytometry analysis.

Supporting Information Available: Experimental data and selected NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0514202

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