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Synthesis of a ^{15}N, ^{13}C-Labeled Lactam Analog of a G_{M4}-Lactone Cell-Surface Glycolipid

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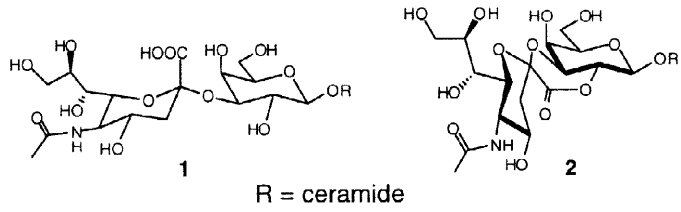
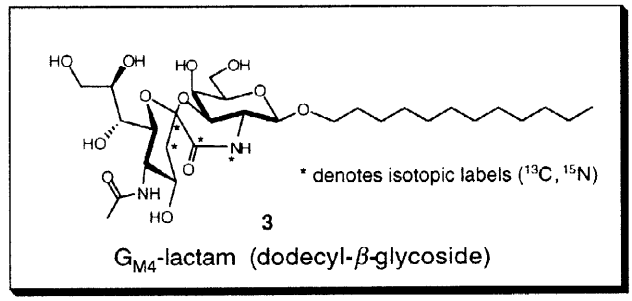
Abstract A ^{13}C and ^{15}N-labeled lactam analog of a naturally occurring G_{M4} lactone glycolipid was synthesized in a convergent manner. The ^{13}C-labels in the sialic acid portion of the molecule were derived from uniformly ^{13}C-labeled pyruvate, and the ^{15}N was introduced using a sulfonylamidoglycosylation reaction with labeled benzenesulfonamide.

Keywords: carbohydrates; sialic acid; glycosidation; lactam; labelling

We are engaged in the development of NMR methods for the study of the carbohydrate portion of membrane-anchored glycolipids. The study of these molecules in an oriented micelle bilayer system often requires the incorporation of isotopic labels through synthesis. Unfortunately, this is not always an easy task. Ideally, three or four isotopic labels need to be introduced in contiguous, rigidly-defined positions. Also, the introduction of labels at specific sites is often limited by the availability of suitable methods and economical starting materials. Thus, we have made an effort to identify, adapt, and develop practical synthetic methods for the incorporation of ^{13}C and ^{15}N labels into carbohydrates. Previously, we incorporated three contiguous ^{13}C-sites in sialic acid and sialic acid-containing molecules using pyruvate as a ^{13}C source and the enzyme-assisted chemistry of Whitesides *et al.*[1] Here we report the total synthesis of a ^{15}N, ^{13}C-labeled G_{M4} lactam, a stable analog of a naturally occurring lactone.

Figure 1





Gangliosides are one of the major classes of membrane-anchored glycolipids in mammalian cells. These all contain one or more sialic acid residues attached to a ceramide group by one or more intervening sugars. Evidence suggests that these molecules are in involved in the regulation of cell growth, differentiation, and the immune response.[2] They are also known to display varying degrees of immunosuppression. When subjected to mildly acidic conditions, they will form lactones, internal esters involving the sialic acid carboxyl group. Generally, such lactones are hydrolytically labile. Yu *et al.* previously reported solution NMR studies on a lactone of the ganglioside, G_{M3}, in which the rigidity of the lactone structure was established.[3]

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G_{M_4} is the smallest ganglioside, having just one sialic acid and lacking the glucose of the usual lactose core. It is known to occur in the brain, on myelin, and on certain types of malignant cells. Two isomeric G_{M_4} lactones have been shown to occur naturally in whale brain.[4] Nilsson *et al.* demonstrated the suitability of lactams as mimics of ganglioside lactones.[5] The lactam functionality appealed to us, since the introduction of nitrogen not only leads to a more stable cyclic structure, but permits the addition another NMR active nucleus (^{15}N) to the NMR spin system. Figure 1 illustrates G_{M_4} (**1**), a naturally occurring G_{M_4} lactone (**2**), and our isotopically labeled G_{M_4} lactam target (**3**), which represents a stable lactam mimic of **2**. We have replaced the natural ceramide anchor with a dodecyl chain in **3**. This has served as an adequate membrane anchor in conformational studies of labeled gangliosides at bilayer micelle (*i.e.*, membrane) interfaces.[6] In future work, the dodecyl chain can be replaced with ceramide, using similar chemistry. Herein we report the synthesis of G_{M4} lactam glycolipid, in which we introduced four contiguous ^{13}C and ^{15}N isotopic labels.

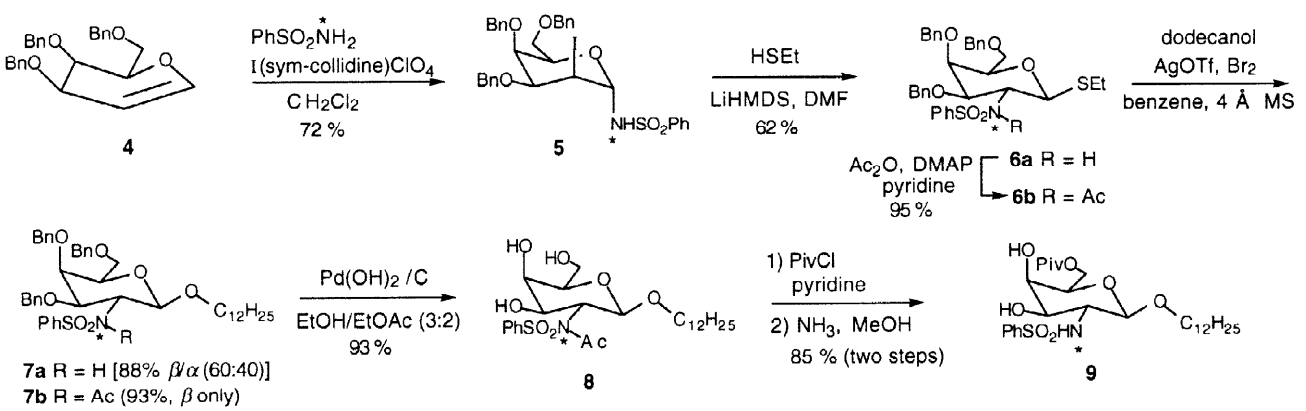
Introduction of ^{15}N into a carbohydrate required an efficient synthetic method. After a lack of success exploring S_N2 reactions[7] and oxime reductions[8] at C(2) in pyranoses, we turned to benzenesulfonamide as a nitrogen source, since it can serve as a starting material in sulfonylamidoglycosylation reactions.[9] We found that ^{15}N-labeled benzenesulfonamide can be readily prepared from commercially-available $^{15}NH_4Cl$ (benzenesulfonyl chloride, triethylamine, DMSO, 25 °C, 20 h), as shown in Scheme 1.

Scheme 1

$$PhSO_2Cl + {}^{*}NH_4Cl \xrightarrow[88\ \%\ (\text{based on } {}^{15}NH_4Cl)]{DMSO,\ Et_3N} PhSO_2{}^{*}NH_2$$

Preparation of the protected ^{15}N-labeled dodecyl-β-glucosamine pyranoside (**9**) from the tribenzylglucal (**4**)[10] is illustrated in Scheme 2. Sulfonylamidoglycosylation of **4** proved effective (benzenesulfonamide, iodonium di-*sym*-collidine perchlorate, 4Å mol. sieves, CH_2Cl_2, 0 °C, 15 min), and provided the iodosulfonamide (**5**) in 72% yield. Treatment of this sulfonamide with base (LiHMDS, DMF, -40 → 25 °C, 4 h) in the presence of ethanethiol facilitated iodide displacement with concomitant aziridine formation. Subsequent aziridine ring-opening at C(1) by the thiolate anion is accompanied by the desired migration of the sulfonamide group to C(2), providing **6a**. Dodecyl-β-glycoside formation was accomplished through *in situ* activation of the thioglycoside (bromine, silver triflate, benzene, 25°C, 1 h).[11] Preliminary experiments showed that glycosidation of **6a** with dodecanol provides a 60:40 β:α mixture of the dodecylglycoside (**7a**).

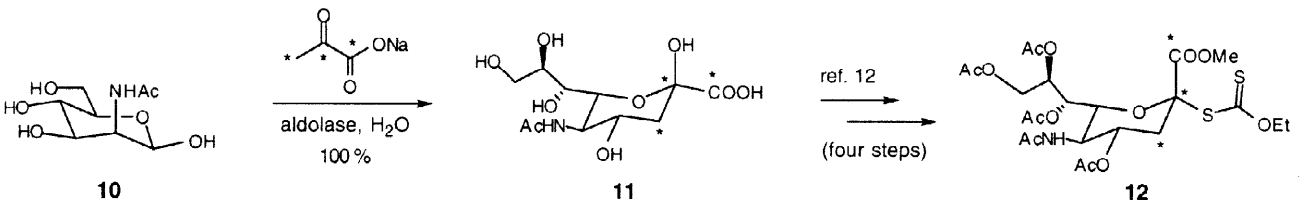
Scheme 2



However, prior acetylation of sulfonamide **6a** (acetic anhydride, dimethylaminopyridine, pyridine, 25 °C, 14 h) gave **6b**, which, in contrast to the above result, underwent glycosidation to give **7b** with complete β-selectivity. This was apparently due to neighboring acetate participation during glycosidation of the *N*-acetylsulfonamide. Hydrogenolysis of **7b** [palladium hydroxide on carbon, EtOH/EtOAc (3:2), 25 °C, 10 h] to remove the benzyl ether protecting groups then provided triol **8.** Selective protection of the primary alcohol in **8** as its pivaloate ester (pivaloyl chloride, pyridine, 0 → 25 °C, 12 h), followed by selective *N*-deacylation of the sulfonamide (ammonia, MeOH, 25 °C, 30 min.), furnished diol **9**.

An outline of the synthesis of 1,2,3-^{13}C-labeled sialosyl donor (**12**) is illustrated in Scheme 3. The enzymatic condensation of *N*-acetyl mannosamine **10** and 1,2,3-^{13}C-sodium pyruvate (*N*-acetylneuraminic acid aldolase, bovine albumin, sodium azide, 25°C, 5 days) provided 1,2,3-^{13}C-sialic acid **11** in quantitative yield after anion exchange

Scheme 3

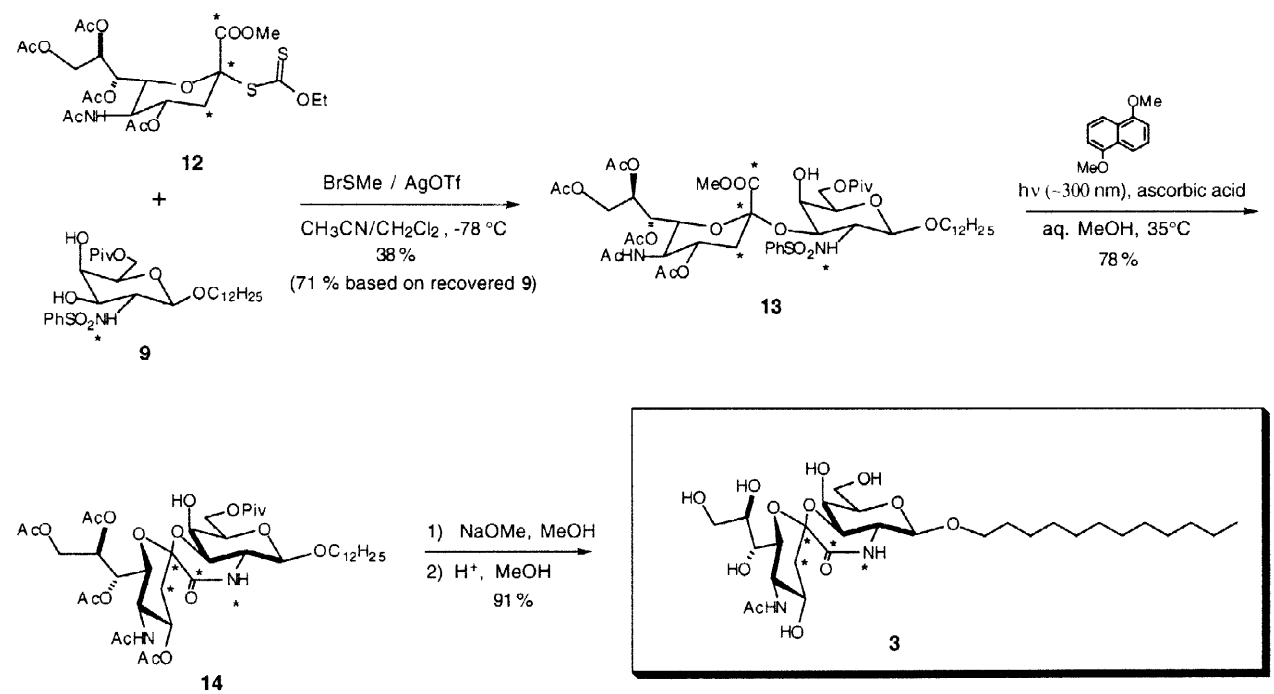


10 **11** **12**

purification (Bio-Rad Laboratories, AG 1-X8).[1b] Conversion of **11** to the protected xanthate (**12**) was then accomplished in four steps, as described by Sinay and Marra.[12] We were thus positioned to carry out a glycosidation between glycosyl donor **12** and glycosyl acceptor **9**.

Completion of the synthesis of the labeled dodecyl glycolipid target (**3**) is illustrated in Scheme 4. Glycosidation of sialic acid was most effectively accomplished via the xanthate [(**12**), silver triflate, methylsulfenyl bromide, 4 Å mol. sieves, CH_3CN/CH_2Cl_2 (9:4), -78 °C, 2.5 h, followed by addition of *i*-Pr_2NH].[13] This reaction provided exclusive β-glycosidation at the

Scheme 4



12 **9** **13** **14** **3**

C(3) hydroxyl group of dodecyl-β-galactoside (**9**). Although numerous attempts were made to improve the yield of **13**, it was modest, because **13** was always accompanied by the α,β-unsaturated ester, which resulted from elimination of the xanthate group from the glycosyl donor (**12**). Completion of the synthesis involved photochemical removal of the benzenesulfonyl group [*Rayonet* photoreactor, *hv* (300 nm), ascorbic acid, 1,5-dimethoxynaphthalene, MeOH/H_2O (95:5), 35 °C, 5 days],[14] followed by closure of the lactam ring to give **14**. Deprotection of the pivaloyl and acetate esters (sodium methoxide, MeOH, 0 → 25 °C, 10 h) was accompanied by lactam opening. Acidification of the solvent with anhydrous hydrogen chloride (generated from acetyl chloride in anhydrous MeOH, 25 °C, 12 h) facilitated reclosure of the lactam ring to provide the targeted G_{M4} lactam dodecyl-β-glycoside (**3**) in excellent yield.[15]

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15. This compound was characterized by 300-MHz ^1H NMR, 75-MHz ^{13}C NMR and high resolution mass spectrometry, as well as 125-MHz ^{13}C-liquid crystal NMR studies in oriented bilayer micelle media (ref. 6).