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A concise chemical synthesis of a fluorescent βGal-(1,4)-*S*-βGlc-Cer derivative and its enzymatic elongation by glycosyltransferases

Hidenori Tanaka [a], Yayoi Yoshimura [a], Norman J. Dovichi [b], Monica M. Palcic [a], Ole Hindsgaul [a,*]

[a] *Carlsberg Laboratory, Gamle Carlsberg Vej 10, 1799 Copenhagen V, Denmark*
[b] *Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA*

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ABSTRACT

A straightforward chemical synthesis of *lyso*-lactosylceramide with the terminal galactose linked to glucose through a β-*S*-glycosidic bond is reported. The product is labeled on the amino-group with tetramethylrhodamine enabling its ultrasensitive detection in capillary electrophoresis using laser-induced fluorescence. The fluorescent product disaccharide is resistant to hydrolysis by glycosidases but is shown to remain as an acceptor substrate for glycosyltransferases for the conversion into the trisaccharides GM3 and Gb3.

Glycolipids are found on the surface of the cell membrane where oligosaccharides are located outside the cell and can play an important role in biological events such as cellular recognition, cell adhesion, and infection.[1] It is well-known that changes in the structure of such glycolipids accompany embryonic development, tumor progression,[2,3] and many inborn errors of metabolism, including lysosomal storage diseases.[4–6] There has been a long-standing interest in understanding the metabolic pathways for both glycolipid biosynthesis and catabolism.[7] To study the biosynthetic (anabolic) pathways, glycosidase-resistant glycolipid analogs can be a valuable tool.[8–10]

Lactosylceramide (**1**) is the common precursor for the biosynthesis of both the ganglio- and globo-series of animal glycolipids via the addition of sugars in the Golgi apparatus by the appropriate glycosyltransferases (anabolism). It is also a substrate for lysosome-localized glycosidases resulting in its degradation to ceramide and beyond (catabolism). Wishing to selectively investigate the anabolic pathways, we decided to prepare an analog of lactosylceramide (**1**) that would be stable to glycosidases and that was fluorescently tagged to enable the ultrasensitive detection and quantitation of larger oligosaccharides at the single-cell level (ca. 200 molecule detection).[11,12] Herein, we report a facile route to a tetramethylrhodamine (TMR)-labeled analog **2** of lactosylceramide in which the terminal galactose is linked to glucose through a β-*S*-glycosidic bond, and demonstrate its enzymatic elongation to fluorescent-labeled trisaccharides.[13]

Labeling of natural glycolipids can be conveniently carried out via the enzymatic cleavage of the long-chain fatty acids of the ceramide and installation of a fluorescent tag on the resulting free amine.[13] This led us to select thiolactosylsphingolipid **3** as our primary synthetic target, with a sulfur atom in the glycosidic linkage: a class of compound known to be stable to glycosidase enzymes.[8–10] Our retrosynthetic analysis of **3** is shown in Scheme 1. To facilitate the final deprotection and retain the double bond, acyl protecting groups are used in both thiolactosyl donor **4** and sphingolipid acceptor **5**.[14] Formation of the thio-linkage in **4** is achieved by coupling between galactosyl isothiouronium salt **6** and galactosyl triflate **7** via an S_N2 reaction (Scheme 1).[8]

The synthesis began with the formation of the thiolactoside. Compounds **6**[15] and **8**[16] were prepared starting from galactose pentaacetate **9**. After conversion into triflate **7**, condensation with isothiouronium salt **6** in *N*,*N*′-dimethylformamide/dichloromethane using triethylamine was carried out to give thiolactoside **10** in a 44% yield.[17] The new *S*-glycosidic bond was confirmed as a Gal-β(1,4)-Glc linkage from coupling constants ($J^{Gal}_{1,2}$ = 10 Hz, $J^{Glc}_{3,4}$ = 10 Hz and $J^{Glc}_{4,5}$ = 10 Hz). Hydrolysis of phenyl thioglycoside **10** was effected using *N*-bromosuccinimide (NBS)/H_2O. Surprisingly, although an excess of NBS (5.0 equiv) was used, the inner *S*-glycosidic linkage was not affected which was likely due to steric hindrance. The resultant hemiacetal **11** was reacted with trichloroacetonitrile and 1,8-diazabicycloundec-7-ene (DBU) to give imidate donor **4** in a 58% yield over two steps from **10** (Scheme 2).

Next, the *N*-TFA protected sphingosine acceptor **5** was obtained by olefin cross-metathesis. The addition of vinylmagnesium bromide to (*S*)-Garner's aldehyde **12** afforded a mixture of

* Corresponding author. Tel.: +45 3327 5382; fax: +45 3327 4708.
E-mail address: Ole.Hindsgaul@carlsberglab.dk (O. Hindsgaul).

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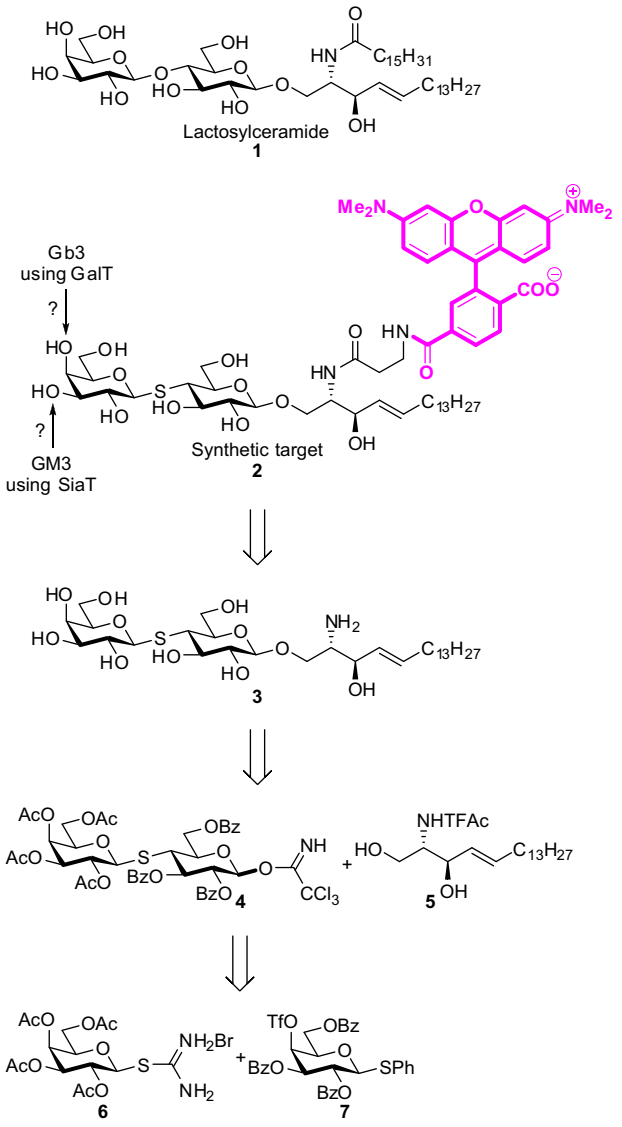

Scheme 1. Retrosynthesis of target **2** having a sulfur linkage between galactose and glucose.

trans- and *syn*-allylic alcohols **13** quantitatively with a dr = 5/1.[18] The diastereomeric mixture was coupled with 1-pentadecene using the 2nd generation Grubbs catalyst,[19] and then protecting group manipulations including benzylidene acetalation to separate the minor isomer which led to the sphingosine derivative **15** in diastereomerically pure form (11% from **12**).[20] Removal of the acetal group gave the target acceptor **5** in a 67% yield (Scheme 3).

With imidate donor **4** and sphingosine acceptor **5** in hand, their coupling could be effected in the presence of catalytic trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give glycosphingolipid **16** in a 22% yield along with di-glycosylated compound **17** in a 23% yield (Scheme 4). Even though the yield of **16** was low, this strategy had the advantage that the final deprotection could be achieved in one-step.

All the acyl groups in **16** were cleaved using sodium methoxide in methanol to afford *S*-lactosylsphingolipid **3** in a quantitative yield, which was easily converted into TMR-labeled glycolipid **2** as previously reported for the natural O-linked compound (Scheme 5).[13]

Disaccharides with sulfur in the glycosidic linkage are known to be resistant to glycosidases. For them to be useful as tools in the study of glycolipid biosynthesis, however, they must remain as substrates for glycosyltransferases which build up larger oligosaccharides. In the biosynthesis of gangliosides in the common GM1 pathway, this would require **2** to be a substrate for an α(2,3)-sialyltransferase, while for the production of glycosphingolipids in the globoside pathway, glycolipid **2** must remain as a substrate for an α(1,4) galactosyltransferase.

Incubation of **2** with CMP-sialic acid in the presence of α(2,3)-sialyltransferase,[21] or with UDP-galactose in the presence of α(1,4)-galactosyltransferase[22] resulted in the complete disappearance of **2** and the production[23] of the desired trisaccharides GM3 **18** and Gb3 **19**, respectively (Scheme 5), as the sole products based on the analysis by capillary electrophoresis with fluorescence detection and MALDI-TOF mass-spectrometry (see Supplementary data).

In summary, we have demonstrated a convergent synthesis of *S*-lactosylceramide analog **2** and shown that, despite the incorporation of a sulfur atom in the glycosidic linkage and a large polyaromatic fluorescent tag, this compound remains as a substrate for two key enzymes involved in the biosynthesis of larger common glycolipids. This compound is being further evaluated as a substrate in the investigation of glycolipid biosynthesis in single cells.[11,12]

Acknowledgment

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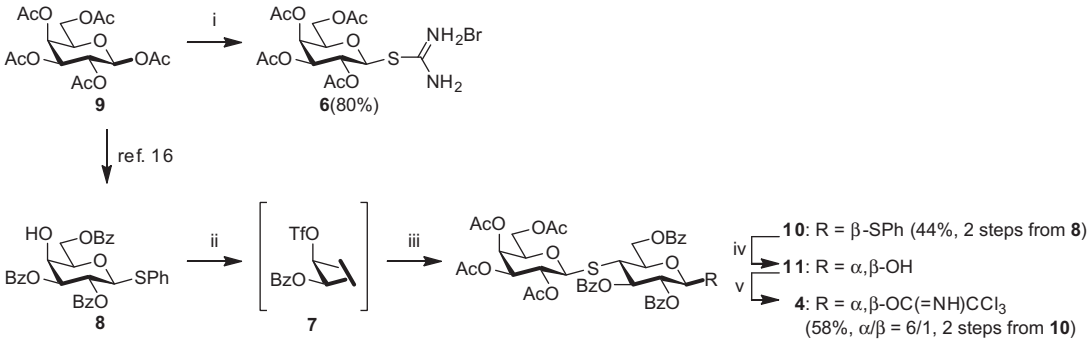
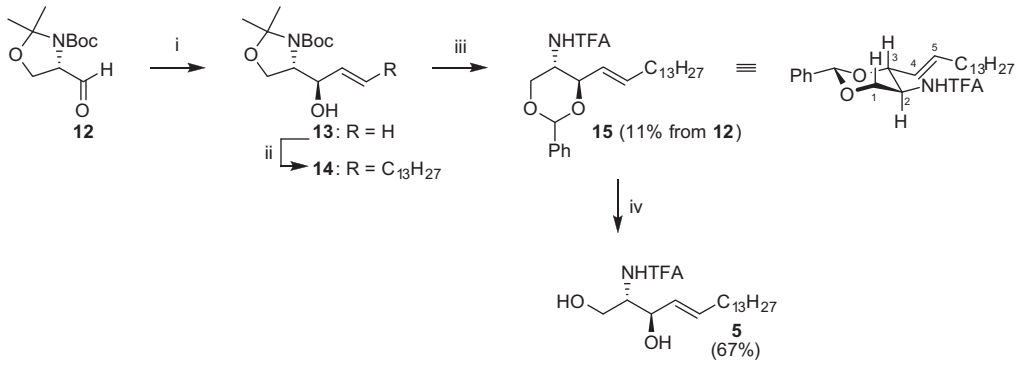
Supplementary data

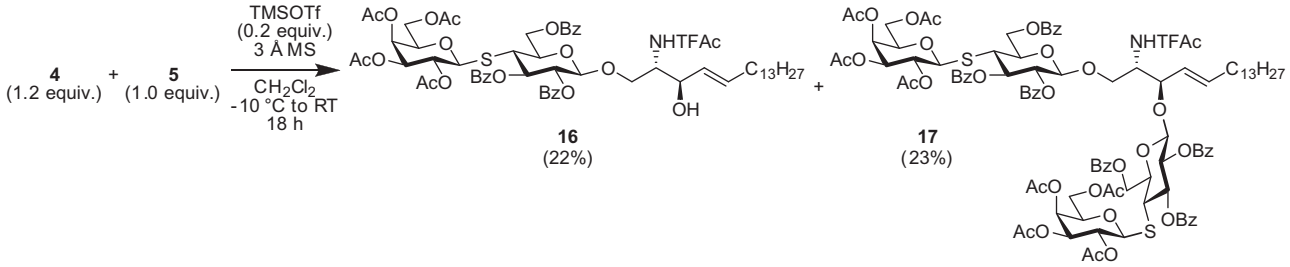
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.01.119.



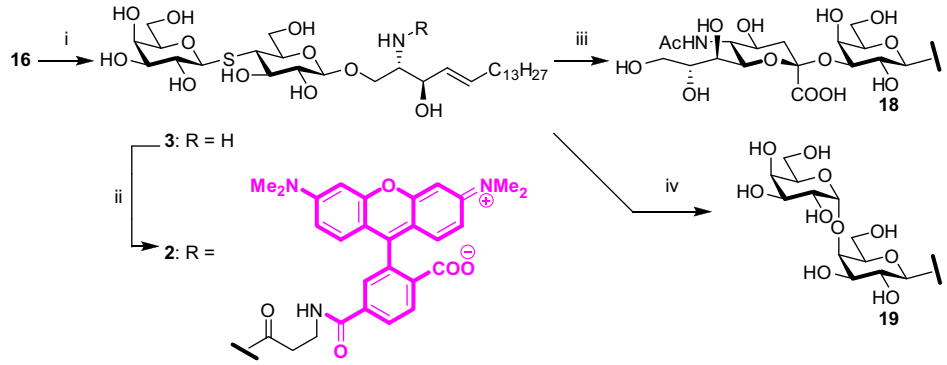
Scheme 2. Synthesis of disaccharide donor **4**. (i) (a) 33% wt. HBr in AcOH/CH_2Cl_2, rt, 2 h; (b) thiourea/acetone, reflux, 20 h; (ii) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 1 h; (iii) **6**, Et_3N, DMF/CH_2Cl_2, rt, 24 h; (iv) NBS/wet acetone, rt, 2 h; (v) trichloroacetonitrile, DBU/CH_2Cl_2, rt, 1 h.



Scheme 3. Synthesis of sphingosine acceptor **5**. (i) vinylmagnesium bromide/THF, −78 °C, 2 h; (ii) 1-pentadecene, Grubbs 2nd generation catalyst/CH_2Cl_2, reflux, 4 h; (iii) (a) aq. AcOH, 50 °C, 13 h; (b) TFA/CH_2Cl_2, rt, 2 h; (c) TFA_2O, Et_3N, MeOH/CH_2Cl_2, rt, 48 h; (d) benzaldehyde dimethyl acetal, TsOH monohydrate/MeCN, rt, 1 h; (iv) aq. AcOH, rt to 50 °C, 38 h.



Scheme 4. Coupling of disaccharide donor **4** with sphingosine acceptor **5**.



Scheme 5. Deprotection, labeling with 6-TMR-β-Ala, and enzymatic reactions. (i) NaOMe/MeOH, rt, 4 days; (ii) 6-TMR-β-Ala NHS ester, DIEA/DMF, rt, 24 h; (iii) CMP-Neu5Ac, α(2,3)-sialyltransferase (MalE fusion protein from *Campylobacter jejuni*), MOPS buffer (pH 7.5) containing $MnCl_2$ and 5% (v/v) DMSO; (iv) UDP-Gal, α(1,4)-galactosyltransferase (*Neisseria meningitidis*), MOPS buffer (pH 7.0) containing $MnCl_2$, BSA, DTT and 5% (v/v) DMSO.

References and notes

1. Karlsson, K.-A. *Ann. Rev. Biochem.* **1989**, *58*, 309–350.
2. Hakomori, S. *Cancer Res.* **1996**, *56*, 5309–5318.
3. Hakomori, S. *Adv. Exp. Med. Biol.* **2001**, *491*, 369–402.
4. Taylor, M. E.; Drickamer, K. *Introduction to Glycobiology*, second Ed.; Oxford University Press: Oxford, UK, 2003. pp. 74–75.
5. Birkle, S.; Zeng, G.; Gao, L.; Yu, R. K.; Aubry, J. *Biochimie* **2003**, *85*, 455–463.
6. Marquardt, T.; Denecke, J. *Eur. J. Pediatr.* **2003**, *162*, 359–379.
7. Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999**, *8*, 1532–1568.
8. Albrecht, B.; Pütz, U.; Schwarzmann, G. *Carbohydr. Res.* **1995**, *276*, 289–308.
9. Schwarzmann, G.; Hofmann, P.; Pütz, U. *Carbohydr. Res.* **1997**, *304*, 43–52.
10. Xia, C.; Zhou, D.; Liu, C.; Lou, Y.; Yao, Q.; Zhang, W.; Wang, P. G. *Org. Lett.* **2006**, *8*, 5493–5496.
11. (a) Whitmore, C. D.; Hindsgaul, O.; Palcic, M. M.; Schnaar, R. L.; Dovichi, N. J. *Anal. Chem.* **2007**, *79*, 5139–5142; (b) Cohen, D.; Dickerson, J. A.; Whitmore, C. D.; Turner, E. H.; Palcic, M. M.; Hindsgaul, O.; Dovichi, N. J. *Annu. Rev. Anal. Chem.* **2008**, *1*, 165–190.
12. (a) Whitmore, C. D.; Olsson, U.; Larsson, E. A.; Hindsgaul, O.; Palcic, M. M.; Dovichi, N. J. *Electrophoresis* **2007**, *28*, 3100–3104; (b) Dada, O. O.; Essaka, D. C.; Hindsgaul, O.; Palcic, M. M.; Prendergast, J.; Schnaar, R. L.; Dovichi, N. J. *Anal. Chem.* **2011**, *83*, 2748–2753; (c) Krylov, S. N.; Zhang, Z.; Chan, N. W.; Arriaga, E.; Palcic, M. M.; Dovichi, N. J. *Cytometry* **1999**, *37*, 14–20.
13. Larsson, E. A.; Olsson, U.; Whitmore, C. D.; Martins, R.; Tettamanti, G.; Schnaar, R. L.; Dovichi, N. J.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **2007**, *342*, 482–489.
14. Lee, Y. M.; Lee, S.; Jeon, H.; Baek, D. J.; Seo, J. H.; Kim, D.; Kim, S. *Synthesis* **2011**, 867–872.
15. Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 828–833.
16. Wang, P.; Fukuda, M.; Seeberger, P. H. *Chem. Commun.* **2007**, 1963–1965.
17. The coupling yield could be improved using acetonitrile as the solvent, see: Ibatullin, F. M.; Selivanov, S. I.; Shavva, A. G. *Synthesis* **2001**, 419–422.
18. The diastereoselectivity was assigned by ^1H NMR spectroscopy of the crude product: (a) Srivastava, A. K.; Panda, G. *Chem. Eur. J.* **2008**, *14*, 4675–4688; (b) Lankalapalli, R. S.; Ouro, A.; Arana, L.; Gómez-Muñoz, A.; Bittman, R. *J. Org. Chem.* **2009**, *74*, 8844–8847.
19. Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953–956.
20. The configuration of cyclic protected sphingosine **15** was confirmed from the coupling constants ($J_{1ax,2} = J_{2,3}$ = approx. 10 Hz, $J_{4,5}$ = 15.2 Hz).

21. (a) Gilbert, M.; Brisson, J. R.; Karwaski, M. F.; Michniewicz, J.; Cunningham, A. M.; Wu, Y. Y.; Young, N. M.; Wakarchuk, W. W. *J. Biol. Chem.* **2000**, *275*, 3896–3906; (b) Jacques, S.; Rich, J. R.; Ling, C.-C.; Bundle, D. R. *Org. Biomol. Chem.* **2006**, *4*, 142–154.
22. (a) Wakarchuk, W. W.; Cunningham, A.; Watson, D. C.; Young, N. M. *Protein Eng.* **1998**, *11*, 295–302; (b) Adlercreutz, D.; Weadge, J. T.; Peterson, B. O.; Duss, J. Ø.; Dovichi, N. J.; Palcic, M. M. *Carbohydr. Res.* **2010**, *345*, 1384–1388.
23. (a) Enzymatic synthesis of GM3 **18**. Compound **2** was dissolved in DMSO to yield a 250 μM solution; 2 μl (0.5 nmol) of this solution was used so that the final concentration of DMSO was 5% of the reaction volume (40 μl). The reaction mixture (40 μl) consisted of compound **2** (0.5 nmol), CMP-Neu5Ac (0.6 nmol), and α(2,3)-sialyltransferase (1.0 μg) in 50 mM MOPS buffer (pH 7.5) containing 60 mM $MnCl_2$ and 5% (v/v) DMSO. After 5 h, CMP-Neu5Ac (0.5 nmol) was added. The reaction was incubated at room temperature for a total of 14 h. The formation of GM3 was confirmed by mass spectrometry and capillary electrophoresis(CE). *m/z* (MALDI-TOF): found $[M-H]^-$ 1412.72, $C_{69}H_{99}N_5O_{24}S$ calcd for $[M-H]^-$ 1412.63. (b) Enzymatic synthesis of Gb3 **19**. The reaction mixture (40 μl) consisted of compound **2** (0.5 nmol), UDP-Gal (1.5 nmol), and α(1,4)-galactosyltransferase (1.6 μg) in 50 mM MOPS buffer (pH 7.0) containing 20 mM $MnCl_2$, 0.01% BSA, 0.5 mM DTT, and 5% (v/v) DMSO. The reaction mixture was incubated at room temperature for 3 h. The formation of Gb3 was confirmed by mass spectrometry and CE. *m*/*z* (MALDI-TOF): found $[M+H]^+$ 1285.83, $C_{64}H_{93}N_4O_{21}S$ calcd for $[M+H]^+$ 1285.64.