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Synthesis of fluorescence labeled sialyl Lewis^X glycosphingolipids

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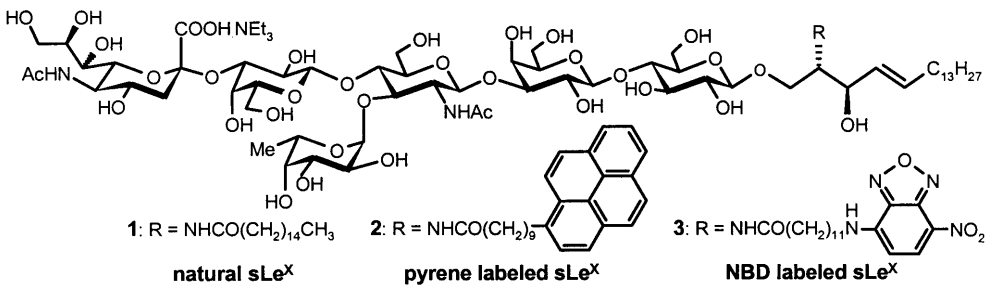
Abstract—The pyrene and nitrobenzoxadiazole (NBD) labeled derivatives of the natural sialyl Lewis^X glycosphingolipid **1** were chemically synthesized as targets for investigating microdomain formation in membranes. The fluorescent analogs **2** and **3** were prepared by replacing the natural amide-linked fatty acid with a fluorescent analog.

The sialyl Lewis^X (sLe^X) epitope Neu5Acα(2→3)Galβ-(1→4)[Fucα(1→3)]GlcNAc has become a prominent target for biological studies because of its role in cell-adhesion and its implication in inflammation through binding to selectins.[1] An important natural occurrence of this epitope is at the terminal end of glycosphingolipids, where a lactose residue serves as spacer to the ceramide moiety (Scheme 1).[2] It was shown that these natural sLe^X-glycosphingolipid **1** mediate a selectin dependent cell rolling when arranged in lateral clusters in a model membrane.[3]

Fluorescent analogs of naturally occurring lipids are widely used in investigations dealing with biophysical aspects of membranes, e.g. lateral mobility or phase separation.[4] When the fluorophores are part of the lipid anchor, they tend to be buried in the hydrophobic interior of the lipid membrane. In this location, they are sensitive to membrane properties such as lipid 'fluidity' and lateral domain formation.

Pyrene-labeled lipids form excimers in a concentration-dependent manner in membranes[5] and thus formation of glycoclusters is exhibited when the carbohydrate is attached to the lipid (such as **2**). Similarly, in low concentrations the NBD derivative **3** should be visible by fluorescence microscopy only when locally concentrated.

Until now, only a few examples exist on the chemical synthesis of pyrene or NBD labeled glycolipids.[6] To maintain close similarity to the natural sLe^X sphingolipid and facile synthesis, we decided to attach the fluorophores via amide linkage to the sphingosine moiety. For the synthesis of the sLe^X sphingolipid **1** some successful approaches have already been reported.[7] We selected here a linear strategy, incorporating the positive results of our previously reported synthesis of the sLe^X tetrasaccharide epitope,[8] namely glycosylation with *N*-trichloroethoxycarbonyl (*N*-Troc) protected glucosamine donor **6**[9] and with 3,4-*O*-acetyl protected



Scheme 1.

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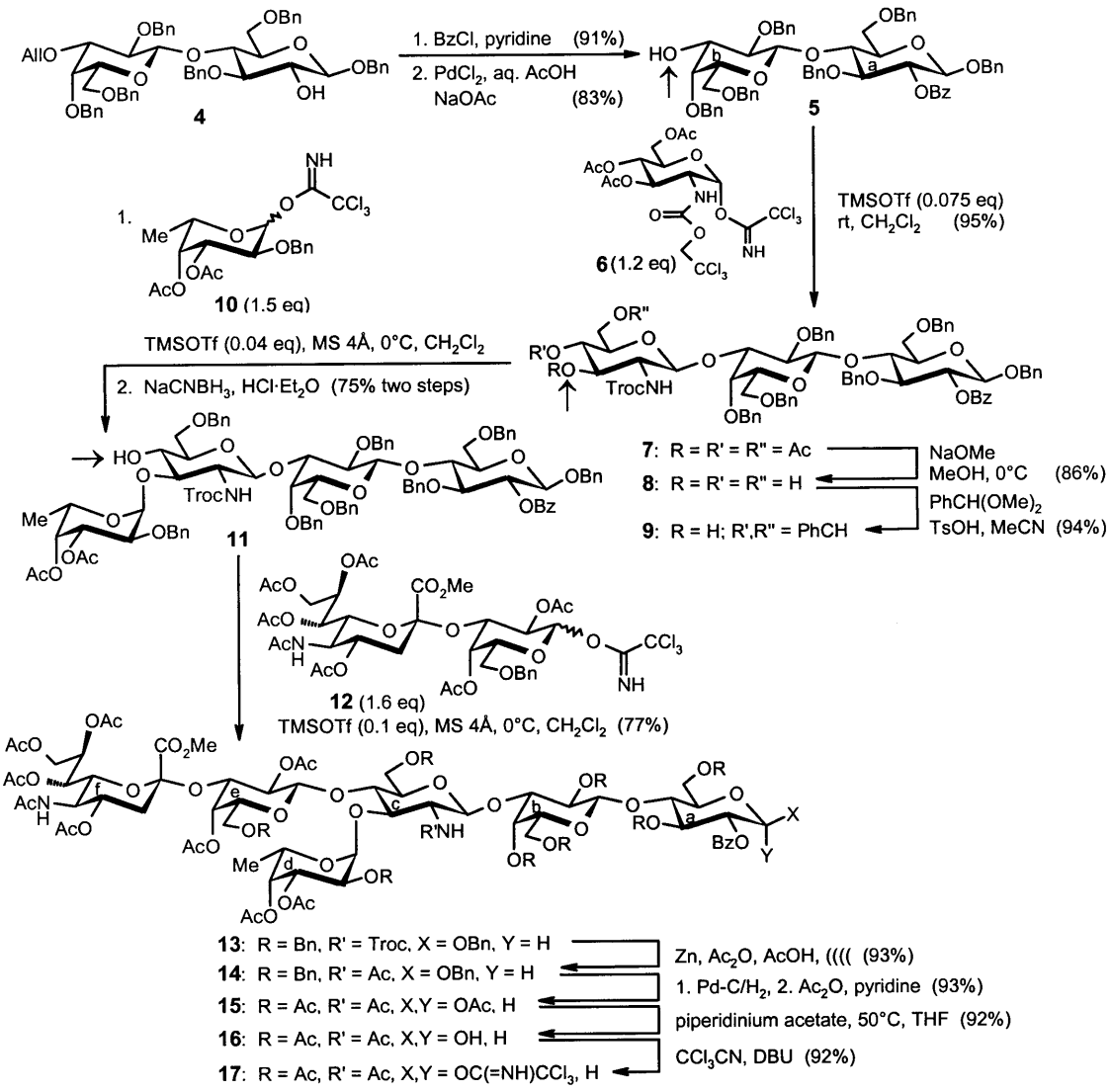
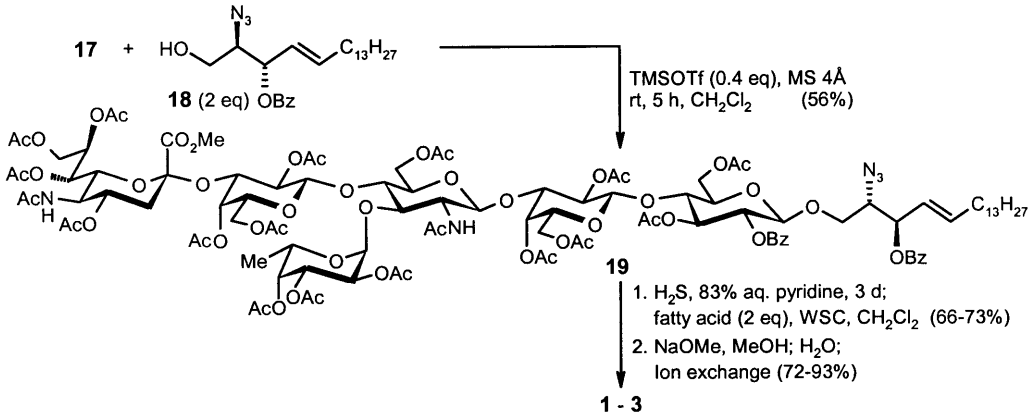
fucosyl donor **10**,[10] respectively, as well as direct introduction of the Neu5Acα(2→3)Gal disaccharide moiety via donor **12**[8,11] (Scheme 2).

Therefore, known lactose derivative **4**[12] was *O*-benzoylated in the 2a-position (Scheme 2) and the 3b-*O*-allyl protecting group was removed using $PdCl_2$ in aqueous acetic acid/sodium acetate to give acceptor **5** in high overall yield. Glycosylation of **5** with glucosamine trichloroacetimidate **6**[9] was performed in the presence of TMSOTf as catalyst in excellent yield. Contrary to previous reports,[13] selective deacetylation with NaOMe/MeOH at 0°C gave **8** without affecting the Troc group. Ensuing 4,6-*O*-benzylidenation gave 3-*O*-unprotected derivative **9** whose fucosylation with **10**[10] in the presence of TMSOTf as catalyst afforded exclusively the α-linked tetrasaccharide, which could be readily transformed into the 4-*O*-unprotected acceptor **11** by regioselective opening of the 4,6-*O*-benzylidene group.[14] Glycosylation with disaccharide donor **12**[8,11] was performed at 0°C; again, TMSOTf served as the catalyst, affording the desired sLe^X intermediate **13** in 77% yield. Treatment with activated zinc in acetic anhydride[9] led to replacement of the *N*-Troc group by an *N*-acetyl group (→**14**) whereby sonification at room temperature as well as use of the solvent mixture THF/Ac_2O/AcOH (6:2:1) improved the yield. Hydrogenolytic *O*-debenzylation and then *O*-acetylation furnished **15**, which was selectively de-*O*-acetylated in the anomeric position by piperidinium acetate at 50°C in THF. Treatment of **16** with trichloroacetonitrile in the presence of DBU as base furnished trichloroacetimidate **17**.

With this donor in hand, the standard 'azidosphingosine glycosylation procedure'[15] for glycosphingo lipid synthesis was employed (Scheme 3). Thus, reaction of **17** with azidosphingosine derivative **18**[16] with TMSOTf as catalyst afforded derivative **19** after 5 h. Interestingly, with a 2a-*O*-benzoyl group, first the corresponding orthoester was formed, which could be isolated almost exclusively after 1 h. This intermediate then rearranged in 4 h to the desired glycoside **19** in good yield. Hence, the use of 2a-*O*-pivaloyl group for anchimeric assistance seems to be preferable in the azi-



Scheme 2.



Scheme 3.

dosphingosine glycosylation procedure,[15] because generally shorter reaction times, higher yields, and no orthoester formation were found for such donors.[17] The azido group of **19** was then reduced with H_2S in aqueous pyridine, followed by coupling with palmitic acid, 1-pyrenedecanoic acid (Fluka) and 12-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoic acid (Molecular Probes),[18] respectively, with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC). Finally, removal of all *O*-acyl protective groups and saponification furnished the target molecules **1**–**3**[19] (Scheme 1); they were isolated as triethylammonium salts after chromatography with $CHCl_3$/MeOH/H_2O/NEt_3 as eluent.

In conclusion, an efficient synthesis of labeled sLe^X glycosphingolipids **1**–**3** was performed which is based on trichloroacetimidate donors and the azidosphingosine glycosylation procedure for the attachment of the labeled fatty acids. Biophysical and biological studies with these compounds will be reported in due course.

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References

1. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833–862.
2. Pauvala, H. *J. Chem. Biol.* **1976**, *251*, 7517–7520.
3. Vogel, J.; Bendas, G.; Bakowsky, U.; Hummel, G.; Schmidt, R. R.; Kettmann, U.; Rothe, U. *Biochim. Biophys. Acta* **1998**, *1372*, 205–215.
4. Kok, J. W.; Hoekstra, D. In *Fluorecent and Luminescent Probes for Biological Activity*; Mason, W. T., Ed.; Academic Press: San Diego, 1999; pp. 136–155.
5. (a) Galla, H.-J.; Sackmann, E. *J. Am. Chem. Soc.* **1975**, *97*, 4114–4120; (b) Galla, H.-J.; Hartmann, W. *Chem. Phys. Lipids* **1980**, *27*, 199–219; (c) Barenholz, Y.; Cohen, T.; Haas, E.; Ottolenghi, M. *J. Biol. Chem.* **1996**, *271*, 3085–3090.
6. (a) Marchesini, S.; Preti, A.; Aleo, M. F.; Casella, A.; Dagan, A.; Gatt, S. *Chem. Phys. Lipids* **1990**, *53*, 165–174; (b) Albrecht, B.; Pütz, U.; Schwarzmann, G. *Carbohydr. Res.* **1995**, *276*, 289–308; (c) Sakai, T.; Ehara, H.; Koezuka, Y. *Org. Lett.* **1999**, *1*, 359–361; (d) Mayer, T. G.; Weingart, R.; Münstermann, F.; Kawada, T.; Kurzchalia, T.; Schmidt, R. R. *Eur. J. Org. Chem.* **1999**, 2563–2572.
7. (a) Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, C1–C4; *J. Carbohydr. Chem.* **1991**, *10*, 549–560; (b) Hummel, G.; PhD thesis, University of Konstanz, 1998.
8. Gege, C.; Bendas, G.; Vogel, J.; Rothe, U.; Schmidt, R. R. *Chem. Eur. J.* **2000**, *6*, 111–122.
9. Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135–147.
10. (a) Windmüller, R.; Diploma Thesis, University of Konstanz, 1990; (b) Windmüller, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 7927–7930.
11. Seifert, J.; Ogawa, T.; Ito, Y. *Tetrahedron Lett.* **1999**, *40*, 6803–6807.
12. (a) Sato, S.; Nunomura, S.; Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 4097–4100; (b) Toepfer, A.; PhD Thesis, University of Konstanz, 1992.
13. Ellervik, U.; Magnusson, G. *Tetrahedron Lett.* **1997**, *38*, 1627–1628.
14. Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97–101.
15. (a) Schmidt, R. R.; Zimmermann, P. *Angew. Chem.* **1986**, *98*, 722–723; *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 725–726; (b) Zimmermann, P.; Bommer, R.; Bär, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435–452.
16. Zimmermann, P.; Schmidt, R. R. *Liebigs Ann. Chem.* **1988**, 663–667.
17. (a) Toepfer, A.; Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994**, 449–464; (b) Castro-Palomino, J. C.; Ritter, G.; Fortunato, S. R.; Reinhardt, S.; Old, L. J.; Schmidt,

R. R. *Angew. Chem.* **1997**, *109*, 2081–2085; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1998–2001; (c) Hummel, G.; Schmidt, R. R. *Pol. J. Chem.* **1999**, *73*, 941–954.

18. 12-[*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoic acid can be prepared by reaction of 4-chloro-7-nitrobenzofurazan with 12-aminododecanoic acid in aqueous $NaHCO_3$ at 50°C.[6d]
19. Selected ^1H NMR (600 MHz) data, measured at 303 K in a 320 mmolar solution of [D_{25}]sodium dodecyl sulfate (SDS) in D_2O with [D_4]-3-(trimethylsilyl)propionic acid sodium salt (TSP) as internal standard according: Jiang, Z.-H.; Geyer, A.; Schmidt, R. R. *Angew. Chem.* **1995**, *107*, 2730–2734; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2520–2524. Compound **1**: $\delta = 5.72$ (m, 1H, =C*H*-CH_2), 5.37 (m, 1H, C*H*=CH-CH_2), 5.09 (d, $J_{1,2} = 4.0$ Hz, 1H, H-1d), 4.79 (q, $J_{5,6} = 6.8$ Hz, 1H, H-5d), 4.69 (H-1c in HDO signal), 4.50 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1e), 4.47 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1a), 4.41 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1b); **2**: $\delta = 5.71$ (m, 1H, =C*H*-CH_2), 5.38 (m, 1H, C*H*=CH-CH_2), 5.11 (d, $J_{1,2} = 3.9$ Hz, 1H, H-1d), 4.81 (q, $J_{5,6} = 6.8$ Hz, 1H, H-5d), 4.70 (H-1c in HDO signal), 4.51 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1e), 4.49 (d, $J_{1,2} = 7.7$ Hz, 1H, H-1a), 4.43 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1b); **3**: $\delta = 5.73$ (m, 1H, =C*H*-CH_2), 5.38 (m, 1H, C*H*=CH-CH_2), 5.10 (d, $J_{1,2} = 4.0$ Hz, 1H, H-1d), 4.80 (q, $J_{5,6} = 6.8$ Hz, 1H, H-5d), 4.70 (H-1c in HDO signal), 4.51 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1e), 4.48 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1a), 4.42 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1b).