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A Versatile Synthesis of the *lactoneo*-Series Antigens - Synthesis of Sialyl Dimer Lewis X and of Dimer Lewis Y[1]

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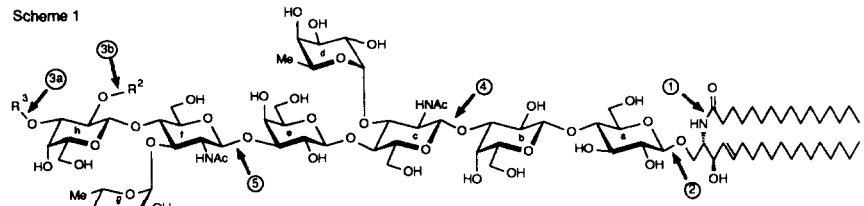
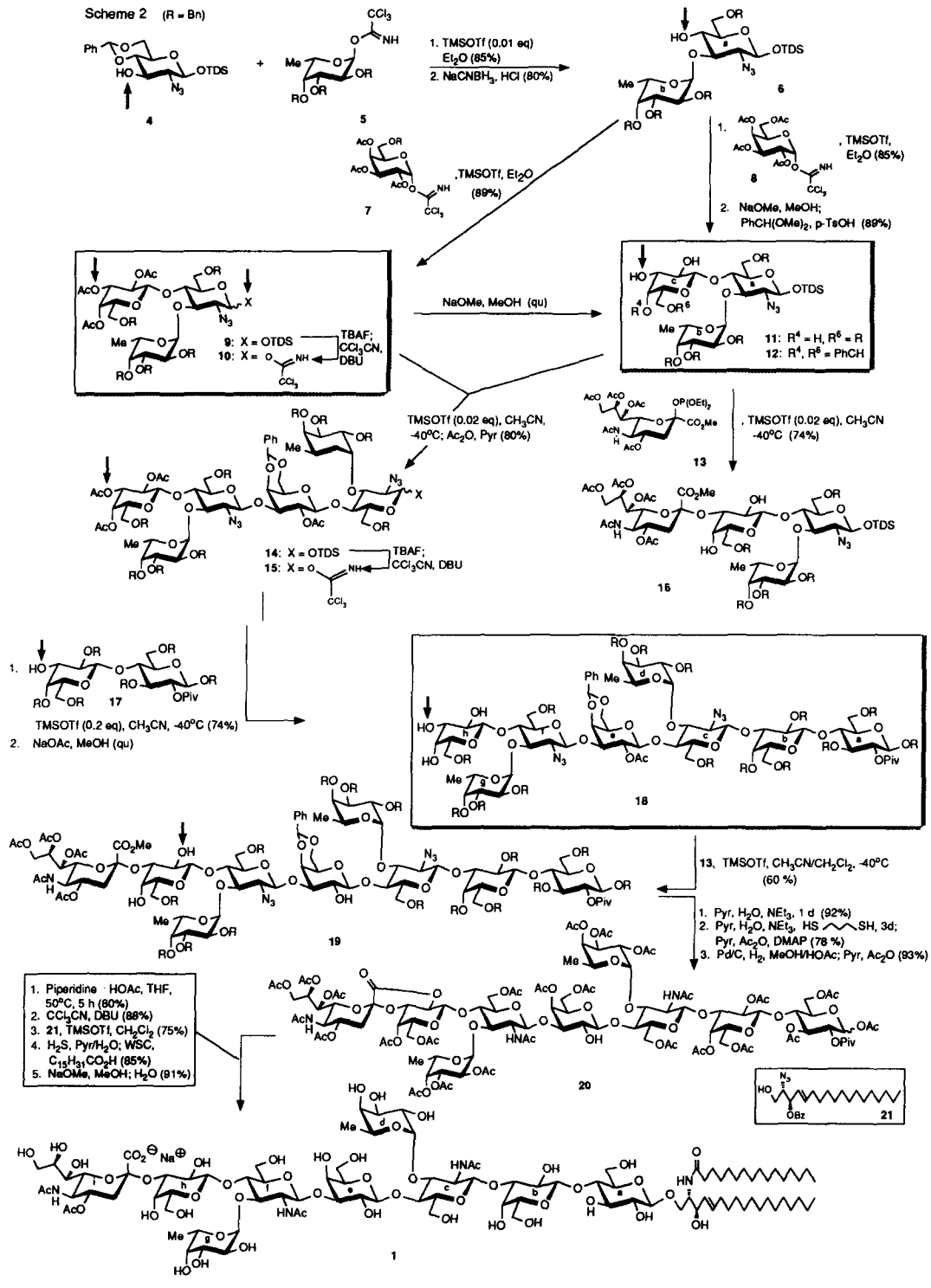
Abstract: Readily available fucosyl α(1-3)azidoglucose disaccharide **6** is transformed with galactosyl donors **7** and **8** into Lewis X trisaccharide building blocks **9** and **12**, respectively, in high yield. Their suitability for the construction of the *lactoneo*-series glycosphingolipids is demonstrated. For instance, **9** readily affords glycosyl donor **10** and acceptor **11**, which permits the required regioselective 3-O-sialylation of the galactose moiety. Thus, from **11** and **12** hexasaccharide **14** is generated which afforded with lactose acceptor **17** the decisive octasaccharide intermediate **18** which is then transformed either into sialyl dimer Lewis X (**1**) or into dimeric Lewis Y (**2**).

Scheme 1



The sialyl Lewis X (sLe^x) epitope Neu5Acα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAc has become a prominent target for isolation, synthesis, and biological studies because of its role in cell-adhesion and its implication in inflammation through binding to selectins[2]. The sialyl dimer Lewis X form (dimer sLe^x, **1** in Scheme 1) exhibits high potency among the natural epitopes of E-selectin[3]; **1** is also found as tumor-associated antigen[4]. Therefore, the synthesis of glycosphingolipid **1** is of interest; two different synthetic approaches have been reported[5,6]; also structural analogues have been synthesized[7,8]. Another important tumor-associated antigen is dimer Lewis Y (dimer Le^y, **2**)[9,10]. Both compounds, **1** and **2**, are derived from dimer Lewis X (**3**): **1** has an α-linked N-acetyl-neuraminic acid (Neu5Ac) residue at 3-O and **2** has an α-linked fucosyl (Fuc) residue at 2-O of the **h**-Gal moiety. Therefore, a versatile building block based strategy for the convenient synthesis of various *lactoneo*- (and also *lacto*-) series glycosphingolipids was envisaged, in order to make these important epitopes readily available[11]. Our approach is based on disconnections ① to ⑤.

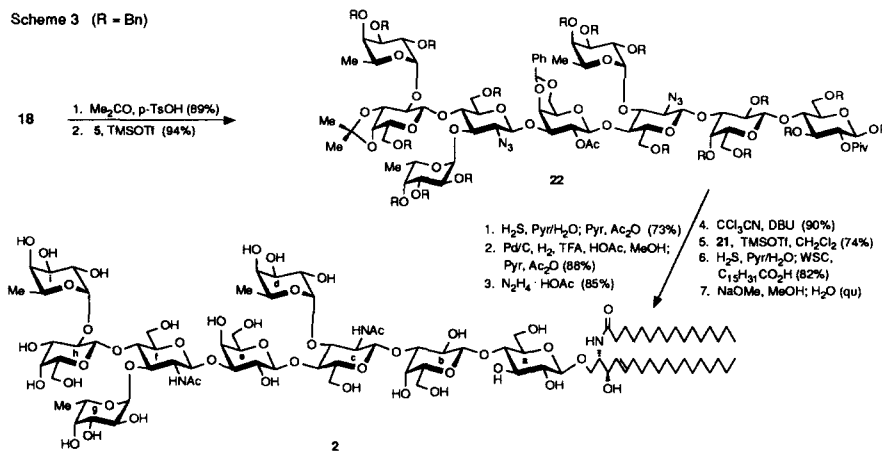
Based on our previous successful synthesis of the tetramer Le^x antigen[12], it was obvious that for the GlcNAc moiety 1-O-silyl-protected azidoglucose derivative **4**[11,13] and for the Fuc moiety fucosyl donor **5**[14] are ideal building blocks (Scheme 2); they could be readily transformed into the corresponding disaccharide **6**[12]. For the required galactosyl moiety regioselective reactions at its 2-O- and 3-O-position, respectively, had to be taken into account. Therefore, 6-O-benzyl-protected galactosyl donor **7**[11] was chosen which gave with acceptor


Scheme 2 (R = Bn)
1. TMSOTf (0.01 eq)
Et2O (85%)
2. NaCNBH3, HCl (80%)
,TMSOTf, Et2O (89%)
1. , TMSOTf, Et2O (85%)
2. NaOMe, MeOH; PhCH(OMe)2, p-TsOH (89%)
NaOMe, MeOH (qu)
9: X = OTDS
10: X =
TBAF; CCl3CN, DBU
11: R4 = H, R6 = R
12: R4, R6 = PhCH
TMSOTf (0.02 eq), CH3CN, -40°C; Ac2O, Pyr (80%)
, TMSOTf (0.02 eq), CH3CN -40°C (74%)
14: X = OTDS
15: X =
TBAF; CCl3CN, DBU
TMSOTf (0.2 eq), CH3CN, -40°C (74%)
2. NaOAc, MeOH (qu)
13, TMSOTf, CH3CN/CH2Cl2, -40°C (60 %)
1. Pyr, H2O, NEt3, 1 d (92%)
2. Pyr, H2O, NEt3, HS SH, 3d; Pyr, Ac2O, DMAP (78 %)
3. Pd/C, H2, MeOH/HOAc; Pyr, Ac2O (93%)
1. Piperidine · HOAc, THF, 50°C, 5 h (80%)
2. CCl3CN, DBU (88%)
3. 21, TMSOTf, CH2Cl2 (75%)
4. H2S, Pyr/H2O; WSC, C15H31CO2H (85%)
5. NaOMe, MeOH; H2O (91%)

6 the protected Le^x trisaccharide **9** in high yield; **9** could be transformed under standard conditions into trichloroacetimidate **10**. Deacetylation of **9** with NaOMe/MeOH (Zemplén conditions[15]) gave **11** which – based on previous experience[16,17] – should exhibit a distinct reactivity order as glycosyl acceptor, namely 3c-OH > 2c-OH > 4c-OH, thus providing the desired regioselectivity for the attachment of a Neu5Ac or a Fuc residue, respectively, at the terminal Le^x unit. For the central Le^x building block, **6** was transformed with known galactosyl donor **8**[18] into the trisaccharide which was de-O-acetylated and then treated with benzaldehyde dimethylacetal in the presence of *p*-TsOH to afford 4,6-O-benzylidene-protected trisaccharide **12**.

With trisaccharide building blocks **10**, **11**, and **12** in hand, the *lactoneo*-series glycosphingolipids should be readily accessible. Reaction of **10** and **12** in acetonitrile at -40 °C[19] and in the presence of TMSOTf as catalyst afforded the corresponding hexasaccharide, which after acetylation was isolated as O-acetyl derivative **14** in 80% yield; under standard conditions **14** was transformed into donor **15**. Ensuing glycosylation of known lactose derivative **17**[20] afforded the desired octasaccharide in 74% yield, which on treatment with NaOMe/MeOH led only to removal of O-acetyl groups at the **h**-Gal moiety, thus furnishing acceptor **18**.

In order to study the suitability of **18** as acceptor for regioselective sialylations, known sialyl donor **13**[17] was reacted with acceptor **11**, which encomprises essentially the same structural features at the Gal moiety as **18**; thus, the desired tetrasaccharide **16** was obtained in 74% yield. The same reaction with octasaccharide **18** gave the desired sialyl group containing nonasaccharide **19** in 60% yield.



For the ceramide attachment, **19** was transformed by treatment with aqueous pyridine/NEt_3 into the acid, then the azido group was reduced with propane-1,3-dithiol and N,O-acetylation and intrasaccharidic lactone formation was carried out with Ac_2O/pyridine in the presence of DMAP; hydrogenolytic O-debenzylation and debenzylidenation and then O-acetylation gave fully acylated nonasaccharide **20**. For glycosyl donor formation, selective removal of the 1-O-acetyl group in the presence of the reactive lactone group was required. This could be accomplished with piperidinium acetate in THF at 50 °C; then treatment with CCl_3-CN in the presence of DBU as catalyst afforded the trichloroacetimidate; it was used in the standard "azidosphingosine glycosylation procedure"[21] for glycosphingolipid synthesis, namely, reaction with azidosphingosine derivative **21**, then reduction of the azido group and N-acylation, and finally removal of all O-acyl protective groups to afford **1**, which was identical with material obtained previously[5,6,22].

For the selective introduction of a fucosyl residue at 2-O-position of Gal moiety **h** in **18**, regioselective

3,4-O-isopropylidenation and then fucosylation with **5** affording nonasaccharide **22** could be performed in very high overall yield (Scheme 3). Azido group reduction and then acetylation was performed as described above. The critical de-O-isopropylidenation could be combined with the hydrogenolytic O-debenzylation and debenzylidenation by performing the reaction with palladium on carbon in MeOH in the presence of HOAc and trifluoroacetic acid (TFA), thus giving after treatment with Ac_2O/pyridine the fully acylated nonasaccharide. Application of the "azidosphingosine glycosylation procedure"[21] gave target molecule **2** in high overall yield. It was structurally assigned by the NMR data[10,23].

In conclusion, based on readily accessible Le^x trisaccharide building blocks **9** (available from apropriately protected monosaccharides **4**, **5**, and **7** in three steps) and **12** (available from **4**, **5**, and **8** in four steps) a convergent and very efficient strategy for the synthesis of the *lactoneo-* (and *lacto-*)[11]series glycosphingolipids is available. This is shown for the synthesis of **1** and **2**.

References and Notes

1. This work was supported by the *Deutsche Forschungsgemeinschaft,* the *Fonds der Chemischen Industrie,* and *Fa. E. Merck, Darmstadt.*– Glycosylimidates, Part 79. For Part 78, see T.G. Mayer, R.R. Schmidt, *Liebigs Ann.,* submitted.
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23. ^1H NMR data of **2** (600 MHz, 10 mg in 320 mmol SDS_{d25} in D_2O): δ = 4.35 (d, $J_{1a,2a}$ = 8.0 Hz, 1a-H), 4.30 (d, $J_{1b,2b}$ = 8.1 Hz, 1b-H), 4.58 (d, $J_{1c,2c}$ = 8.4 Hz, 1c-H), 5.00 (d, $J_{1d,2d}$ = 4.5 Hz, 1d-H), 4.30 (d, $J_{1e,2e}$ = 8.7 Hz, 1e-H), 4.57 (d, $J_{1f,2f}$ = 8.4 Hz, 1f-H), 5.00 (d, $J_{1g,2g}$ = 4.0 Hz, 1g-H), 4.39 (d, $J_{1h,2h}$ = 7.7 Hz, 1h-H), 5.15 (d, $J_{1i,2i}$ = 3.5 Hz, 1i-H).

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