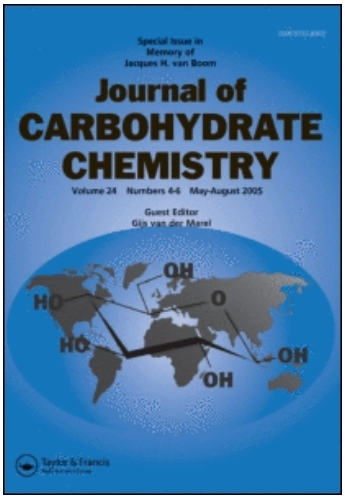

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Regioselective α(2⟶3)-Sialylation of Le^x and Le^a by Sialidase-Catalyzed Transglycosylation

Yutaka Makimura[a]; Hideharu Ishida[a]; Akihiro Kondo[b]; Akira Hasegawa[a]; Makoto Kiso[a]
[a] Department of Applied Bio-organic Chemistry, Gifu University, Gifu, Japan [b] Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., Shiga, Japan

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COMMUNICATION

REGIOSELECTIVE α(2→3)-SIALYLATION OF Le^x and Le^a BY SIALIDASE-CATALYZED TRANSGLYCOSYLATION[1]

Yutaka Makimura,[a] Hideharu Ishida,[a] Akihiro Kondo,[b] Akira Hasegawa[a,2] and Makoto Kiso[a]*

[a] Department of Applied Bio-organic Chemistry, Gifu University, Gifu 501-11, Japan
[b] Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., Seta 3-4-1, Otsu, Shiga 520-21, Japan

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Considerable effort has been devoted to the development of new methods for α-selective sialylation due to the growing importance of the synthetic sialoglycoconjugates in glycobiology.[3] The synthesis of α-sialoside has been establised by chemical routes,[4] which often involve many steps and are complicated. The promising chemoenzymatic procedure through the use of sialyltransferases has already become a preparative technique.[5] However, laborious isolation and the pronounced acceptor specificity of the transferases limit their synthetic potential. Recently, a novel procedure for α-sialylation has been reported, which uses sialosides of synthetic substrate as donors and is catalyzed by sialidase in place of sialyltransferase. Thiem et al.[6] have reported the enzymatic synthesis of α(2→6)-linked sialyl galactose, glucose, lactose and lactosamine in preference to the corresponding α(2→3)-linked derivatives employing sialidase from *vibrio cholerae*, while Ajisaka et al.[7] have synthesized α(2→3)-linked sialyl lactose and lactosamine with sialidase from new castle disease virus.

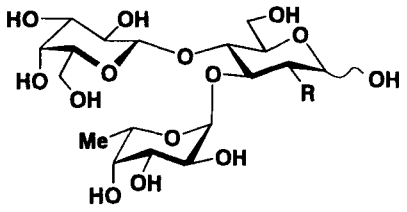
As a part of our continuous effort devoted to the synthesis of sialoglycoconjugates, we describe herein an efficient chemoenzymatic synthesis of sialyl

Le^x (sLe^x) and sialyl Le^a (sLe^a) by applying the sialidase-catalyzed α(2→3)-sialylation as a key step. These tetrasaccharides have been identified[8] as the minimal carbohydrate ligands for selectins.

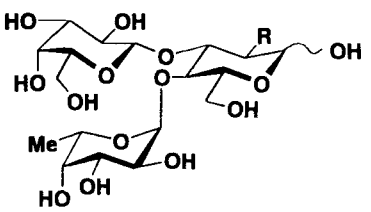
In transglycosylation reactions, selection of enzyme is important in order to improve the regioselectivity of the reaction. Consequently, we employed the sialidase from *Salmonella typhimurium LT2*,[9] which hydrolyzes α(2→3)-sialoside with a high substrate-specificity and is, therefore, expected to give α(2→3)-linked transglycosylation product with high regioselectivity. Selection of solvent, which affects the yield of the reaction, is another important factor in transglycosylation reactions. We have already observed that addition of acetonitrile to the reaction mixture is remarkably effective, in comparison with DMSO or acetone, in the transformation of lactose to sialyl α(2→3) lactose under transglycosylation conditions using sialidase from *Salmonella typhimurium LT2* (data not shown). In the present study, we performed regioselective α(2→3)-sialylation of Le^x (**1**), Le^a (**3**), and their deaminated analogs (**2** and **4**) under transglycosylation conditions (Scheme) based on the results described above. The detailed procedure to transform Le^a (**3**) to sLe^a (**8**) is described as an example, and others are conducted essentially in the same way.

The glycosyl donor, *p*-nitrophenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid (**5**),[10] and the glycosyl acceptor, *O*-(β-D-galactopyranosyl)-(1→3)-*O*-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-D-glucopyranose (**3**),[11] were synthesized by chemical procedures, respectively. The donor **5** (12.5 μmol) and the acceptor **3** (159 μmol) were dissolved in 320 μL of acetate buffer (pH 5.0) containing 1M sodium acetate (32 μL) and acetonitrile (32 μL). Sialidase (200 mU) was added to the solution, which was incubated at 37 °C until the desired compound reached a maximum (2 h). The reaction was stopped by heating in boiling water for 3 min. The solution was applied to an Acell QMA column. Elution with water gave unreacted glycosyl acceptor, and the following elution with 0.1M NaCl gave a mixture of the desired sLe^a, sialic acid and some salts. Further chromatography of the mixture on a column of Sephadex LH-20 with water afforded sLe^a (**8**) as a sole compound. The ^1H NMR spectrum of **8** was identical with that of the authentic sample synthesized by the chemical procedure.[11]

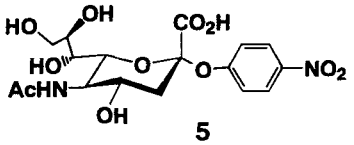
Monitoring of the reaction was performed by the following procedure. After incubation for appropriate periods, a 1 μL portion of the solution was withdrawn and



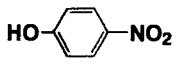
	R
1	NHAc (Le^x)
2	OH



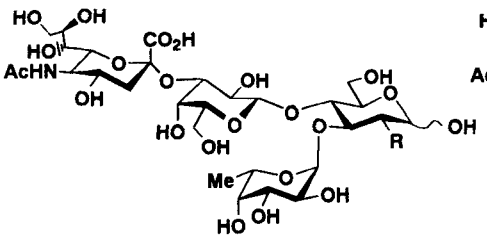
	R
3	NHAc (Le^a)
4	OH



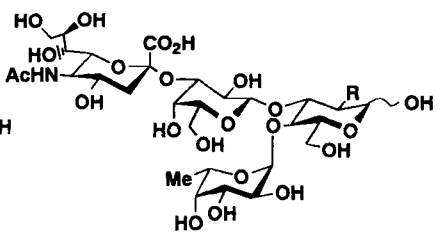
5



α(2→3) Sialidase from *Salmonella typhimurium LT2*



	R
1 → 6	NHAc (sLe^x)
2 → 7	OH



	R
3 → 8	NHAc (sLe^a)
4 → 9	OH

Scheme

Table. Synthesis of sLe^x, sLe^a and their analogs by transsialylation with sialidase

Acceptor	Product	Yield (%)
1 (Le^x)	**6**	9.3 (sLe^x)
2	**7**	12.9
3 (Le^a)	**8**	12.0 (sLe^a)
4	**9**	14.8

heated to quench the reaction. The residue obtained was labeled with a pyridylamino (PA) group[12] and applied for HPLC analysis using a Takara Palpak Type N (NH_2) column. Elution with a mixture of triethylamine-acetic acid buffer and acetonitrile (linear gradient from 1:9 to 1:1) gave a peak corresponding to **8** at 31.28 min, which is compatible with that of authentic material.[11] The Table shows the yield from each transglycosylation reaction. The amount of the product was calculated from the peak area of the product compared with the sum of the peak areas of the acceptor and the product, which was taken as the amount of the acceptor employed in the reaction. The yields were calculated based on the glycosyl donors.

As shown in the table, the procedure employed in the present study transformed all of the acceptors **1-4** to the corresponding $\alpha(2\rightarrow3)$-sialylated derivatives **6-9**, respectively, in yields which are acceptable for the preparation of sialylglycoconjugates under transglycosylation conditions. The sialyltransferases cannot catalyze the sialylation of Le^x and Le^a because of their pronounced acceptor specificity. Therefore the transformation of Le^x and Le^a to sialyl Le^x and sialyl Le^a under the transglycosylation conditions, as described in this paper, gives promising access to a practical synthesis of these biologically important sialoglycoconjugates.

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REFERENCES AND NOTES

1. Synthetic studies on sialoglycoconjugates, Part 101. For Part 100, see H. Ito, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **306**, 1 (1998).
2. Deceased 10 October 1996.
3. (a) W. Reutter, E. Köttgen, C. Bauer, and W. Gerok in *Sialic Acids*; *Chemistry, Metabolism and Function, Cell Biology Monographs*, vol.10; R. Schauer, Ed., Springer-Verlag, Wien, New York, 1982, p 263; (b) H. Wiegandt in *Glycolipids, New Comprehensive Biochemistry*, Vol.10; H. Wiegandt, Ed., Elsevier, Amsterdam, New York, Oxford, 1985, p 199; (c) Singhal, and S. Hakomori in *BioEssays 12*: 1990, p 223; (d) K. Furukawa and A. Kobata in *Carbohydrates; Synthetic Methods and Applications in Medicinal Chemistry*; H. Ogura, A. Hasegawa and T. Suami, Eds., Kodansha/VCH, Tokyo, Weinheim, 1992, p 369; (e) S. Kelm, R. Schauer and P. R. Crocker, *Glycoconjugate J.*, **13**, 913 (1996).
4. A. Hasegawa and M. Kiso in *Preparative Carbohydrate Chemistry*, S. Hanessian, Ed.; Marcel Dekker: New York, 1997, p 357, and references cited therein.
5. H.J.M. Gijen, L. Qiao, W. Fitz and C.-H. Wong, *Chem. Rev.*, **96**, 443 (1996).
6. J. Theim and B. Saverbrei, *Angew. Chem. Int. Ed. Engl.*, **30**, 1503 (1991).
7. K. Ajisaka, H. Fujimoto and M. Isomura, *Carbohydr. Res.*, **259**, 103 (1994).
8. a) L. A. Lasky, *Science*, **258**, 964 (1992); b) T. Feizi, *Curr. Opin. Struct. Biol.*, **3**, 701 (1993); c) A. Varki, *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 7390 (1994). Also see references cited in these articles.
9. L. L. Hoyer, P. Roggentin, R. Schauer and E. R. Vimr, *J. Biochem.*, **110**, 462 (1991).
10. V. Eschenfelder and R. Brossmer, *Carbohydr. Res.*, **162**, 294 (1987).
11. Y. Makimura, H. Ishida, M. Kiso and A. Hasegawa, *J. Carbohydr. Chem.*, **15**, 1097 (1996).
12. A. Kondo, M. Kiso, A. Hasegawa and I. Kato, *Anal. Biochem.*, **219**, 21 (1994).