Carbohydrate Research, 209 (1991) c1–c4
Elsevier Science Publishers B.V., Amsterdam

Preliminary communication

Total synthesis of sialyl Lewis X*

Akihiko Kameyama, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa†
Department of Applied Bioorganic Chemistry, Gifu University, Gifu 501-11 (Japan)

(Received September 11th, 1990; accepted for publication October 3rd, 1990)

Various important biological functions have been attributed to sialoglycoconjugates, found as gangliosides and glycoproteins in the plasma membranes of animal cells[2–7]. Consequently, a facile, regio- and α-stereo-selective route to glycosides of sialic acid has been of critical importance for the synthesis of a variety of gangliosides and their analogs needed for investigations of the functions of sialoglycoconjugates at the molecular level.

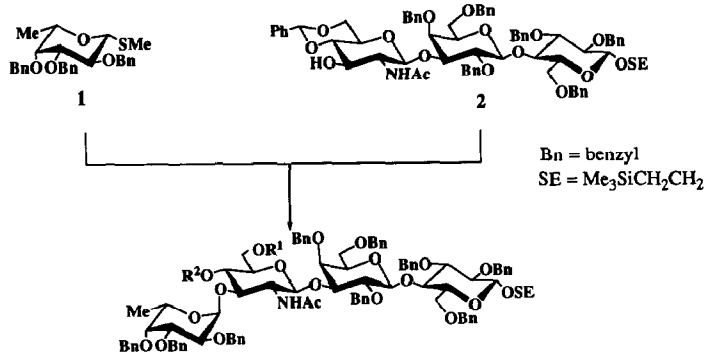
Recently, we demonstrated[8,9] a new, efficient α-glycosidation of sialic acid by the use of dimethyl(methylthio)sulfonium triflate (DMTST)[10,11] as the promoter, the methyl 2-thioglycoside of sialic acid as the glycosyl donor, and suitably protected galactose and lactose derivatives as glycosyl acceptors. This procedure, run in acetonitrile under kinetically controlled conditions, enabled us to accomplish the synthesis of gangliosides GM_3 (ref. 12), GM_4 (ref. 13), sialyl lacto- and neolacto-tetraosyl ceramides[14,15], and other analogs[16,17]. As a part of our continuing efforts toward the synthesis and elucidation of the functions of sialoglycoconjugates, we describe here the first total synthesis of sialyl Lewis X, which has been isolated from human kidney[18], and found[19] to be a widespread, tumor-associated, ganglioside antigen.

Glycosylation of 2-(trimethylsilyl)ethyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[20] (**2**) was effected with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[21] (**1**), newly synthesized from tetra-*O*-acetyl-L-fucose *via* replacement of the anomeric acetoxy group with a methylthio group [(methylthio)trimethylsilane], *O*-deacetylation, and *O*-benzylation. Reaction in the presence of DMTST and molecular sieves 4A (MS-4A) in benzene for 4 h at 6° gave an 86% yield of the desired tetrasaccharide **3** {$[\alpha]_D$ −37° ($CHCl_3$)}, showing in its ^{1}H-n.m.r. spectrum a three-proton doublet at δ 0.84 ($J_{5,6}$ 6.4 Hz, CH_3CH) and a one-proton doublet at δ 5.07 ($J_{1,2}$ 3.6 Hz, H-1), characteristic of the α-fucopyranosyl unit. Reductive ring-opening of the benzylidene group in **3** with sodium cyanoborohydride–hydrogen chloride in dry ether, according to the method of Garegg *et al.*[22], afforded compound **4** in 75% yield.

* Synthetic Studies on Sialoglycoconjugates, Part 21. For Part 20, see ref. 1.
† To whom correspondence should be addressed.

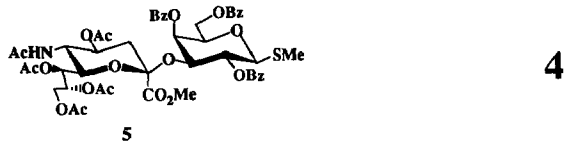
0008-6215/91/$ 03.50

The glycosylation of **4** with methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[20] (**5**), in dichloromethane for 20 h at room temperature in the presence of DMTST and MS-4A, gave a 41% yield of the hexasaccharide **6** {$[\alpha]_D$ −14.5° ($CHCl_3$)}, which had the desired stereochemistry. Significant signals in the ^{1}H-n.m.r. spectrum of **6** were a three-proton doublet at δ 1.06 ($J_{5,6}$ 6.6 Hz, CH_3CH, fucose unit), two three-proton singlets, at δ 1.45 and 1.50 (N-$COCH_3$), four three-proton singlets, at δ 1.78, 1.90, 1.93, and 2.13 (O-$COCH_3$), a three-proton singlet at δ 3.77 (O-CH_3), a complex resonance at δ 7.05–8.19 (13 C_6H_5), and a one-proton doublet of doublets at δ 5.43 ($J_{1,2}$ 8.1, $J_{2,3}$ 9.9 Hz, H-2 of the benzoylated Gal unit), indicating the newly formed glycosidic linkage to be β.



Bn = benzyl
SE = $Me_3SiCH_2CH_2$

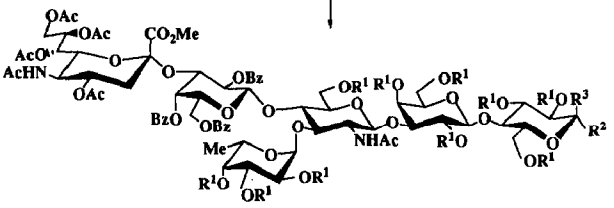
3 R^1,R^2 = benzylidene
4 R^1 = Bn, R^2 = H

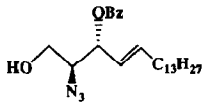


5

Bz = benzoyl

4





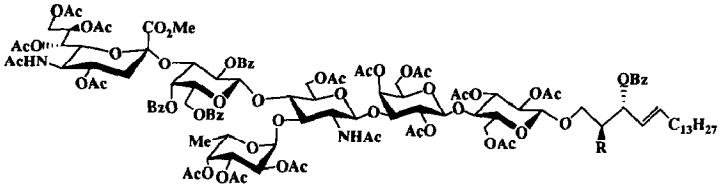
10

6 R^1 = Bn, R^2 = OSE, R^3 = H
7 R^1 = Ac, R^2 = OSE, R^3 = H
8 R^1 = Ac, R^2,R^3 = H, OH
9 R^1 = Ac, R^2 = H, R^3 = OC(=NH)CCl_3

Removal of the benzyl groups from **6** by catalytic hydrogenolysis over 10% Pd–C in 3:1 ethanol–acetic acid for 4 days at 45°, and subsequent acetylation with acetic anhydride–pyridine, gave compound **7** $\{[\alpha]_D$ −20° ($CHCl_3$)$\}$ in 81% yield. Selective removal of the 2-(trimethylsilyl)ethyl group from **7** by treatment[23] with trifluoroacetic acid in dichloromethane for 1 h at room temperature gave compound **8** $\{[\alpha]_D$ −8.5° ($CHCl_3$)$\}$ in 94% yield after column chromatography. Treatment[24] of **8** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 3 h at 0° gave the trichloroacetimidate **9** in 91% yield. Significant signals in the ^{1}H-n.m.r. spectrum were a one-proton doublet at δ 6.47 ($J_{1,2}$ 3.9 Hz, H-1) and a one-proton singlet at δ 8.65 (C=N*H*), indicating α-trichloroacetimidate formation.

The final glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[25] (**10**; 2.0 equiv.) with **9** thus obtained, in the presence of boron trifluoride etherate[24a,25a] and MS-4A AW-300 for 3 h at 0°, afforded only the expected β-glycoside **11** $\{[\alpha]_D$ −23° ($CHCl_3$)$\}$, in 56% yield after column chromatography. The ^{1}H-n.m.r. spectrum of the newly coupled product included a one-proton doublet at δ 4.49 ($J_{1,2}$ 7.7 Hz, H-1 of the Glc unit) and a one-proton doublet of triplets at δ 5.91 ($J_{4,5}$ 13.9, $J_{5,6} = J_{5,6'}$ = 7.0 Hz, H-5, sphingosine unit). Selective reduction[25a,26] of the azido group in **11** with hydrogen sulfide in aqueous pyridine gave the amine **12**, and this on condensation with octadecanoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSC) in dichloromethane for 16 h at room temperature afforded the sialyl Lewis X derivative **13** $\{[\alpha]_D$ −12° ($CHCl_3$)$\}$ in 81% yield. Finally, *O*-deacylation of **13** with sodium methoxide in methanol, and saponification of the methyl ester group, furnished the end product **14** $\{[\alpha]_D$ −17.5° (5:4:0.7 $CHCl_3$–MeOH–H_2O)$\}$ in quantitative yield after chromatography on a column of Sephadex LH-20.

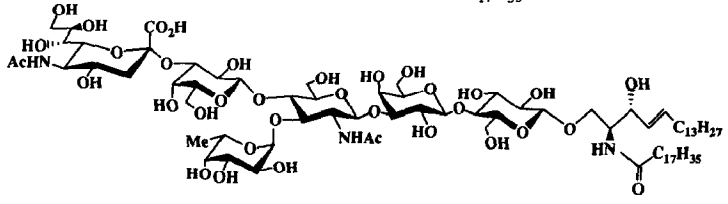
In conclusion, by using compounds **1**, **5**, and **9** as glycosyl donors, and compounds **2**, **4**, and **10** as glycosyl acceptors, a regio- and stereo-controlled synthesis of sialyl Lewis X, a complex-type ganglioside, was achieved.



11 R = N_3

12 R = NH_2

13 R = $NHCOC_{17}H_{35}$



14

New compounds obtained gave elemental analyses and i.r. and ^{1}H-n.m.r. (270 MHz) data in agreement with the structures assigned.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 02259206) for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1 A. Hasegawa, M. Morita, Y. Kojima, H. Ishida, and M. Kiso, *Carbohydr. Res.* in press.
2 H. Wiegandt (Ed.), *Glycolipids, New Comprehensive Biochemistry*, Vol. 10, Elsevier, Amsterdam, 1985, pp. 199–260.
3 S. Tsuji, T. Yamakawa, M. Tanaka, and Y. Nagai, *J. Neurochem.*, 50 (1988) 414–423.
4 D. D. Roberts, L. D. Olson, M. F. Barile, V. Ginsberg, and H. C. Krivan, *J. Biol. Chem.*, 264 (1989) 9289–9293.
5 P. L. Smith, D. Kaetzel, J. Nilson, and J. U. Baenziger, *J. Biol. Chem.*, 265 (1990) 874–881.
6 E. C. Bremor, J. Schlessinger, and S. Hakomori, *J. Biol. Chem.*, 261 (1986) 2434–2440.
7 Y. Suzuki, Y. Nagao, H. Kato, M. Matsumoto, K. Nerome, K. Nakajima, and E. Nobusawa, *J. Biol. Chem.*, 261 (1986) 17 057–17 061.
8 T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 184 (1988) c1–c4.
9 A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, and M. Kiso, *Carbohydr. Res.*, in press.
10 P. Fügedi and P. J. Garegg, *Carbohydr. Res.*, 149 (1986) c9–c12.
11 O. Kanie, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 7 (1988) 501–506.
12 T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 188 (1989) 71–80.
13 T. Murase, A. Kameyama, K. P. R. Kartha, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 265–283.
14 A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 193 (1989) c1–c5.
15 A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 799–804.
16 A. Hasegawa, T. Murase, K. Adachi, M. Morita, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 9 (1990) 181–199.
17 A. Hasegawa, T. Murase, M. Morita, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 9 (1990) 201–214.
18 H. Pauvala, *J. Biol. Chem.*, 251 (1976) 7517–7520.
19 K. Fukushima, M. Hirota, P. I. Terasaki, A. Wakisaka, H. Togashi, D. Chia, N. Suyama, Y. Fukushi, E. Nudelman, and S. Hakomori, *Cancer Res.*, 44 (1984) 5279–5285.
20 A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 200 (1990) 269–285.
21 F. Yamazaki, T. Kitajima, T. Numata, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 15–30.
22 P. J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, 108 (1982) 97–101.
23 K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, and K. Stenvall, *J. Org. Chem.*, 53 (1988) 5629–5641.
24 (a) R. R. Schmidt and G. Grundler, *Synthesis*, (1981) 885–887; (b) M. Numata, M. Sugimoto, K. Koike, and T. Ogawa, *Carbohydr. Res.*, 163 (1987) 209–225.
25 (a) Y. Ito, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 285–294; (b) R. R. Schmidt and P. Zimmermann, *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 725–726.
26 T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Synthesis*, (1977) 45–46.