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Carbohydrate Research 303 (1997) 131–133

CARBOHYDRATE RESEARCH

Rapid Communication

Synthesis of the 3′-*C*-carboxymethyl Lewis X derivative: a novel selectin blocker [1]

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Received 14 April 1997; accepted 23 May 1997

Abstract

The 3′-*C*-carboxymethyl Le^x derivative carrying the 2-(tetradecyl)hexadecyl residue was synthesized by employing 3′-*C*-carboxymethyl galactose as a key intermediate, which was prepared from the suitably protected galactose by Swern oxidation and Wittig–Horner carboxymethylenation, followed by stereoselective reduction of the double bond. The compound obtained showed much more potent activity as a selectin blocker than the sialyl Le^x derivative with 2-(tetradecyl)hexadecyl residue. © 1997 Elsevier Science Ltd.

Keywords: Sialyl-Le^x; Selectin blocker; Ganglioside

Sialyl Lewis X (sLe^x) is found as the terminal tetrasaccharide structure in both cell-membrane glycolipids and glycoproteins. Since it was found that selectin–sLe^x interaction is involved in various inflammatory diseases [2], many mimetics (e.g. ref. [3]) as well as analogues (e.g. ref. [4]) of sLe^x have been designed and synthesized as potential anti-inflammatory agents.

In our continuing efforts to elucidate the structure–function relationship of sLe^x, we have reported the synthesis [5] of sulfo-Le^x analogues containing a ceramide or a 2-(tetradecyl)hexadecyl residue and their strong inhibitory activity [6] against the selectin–sLe^x interaction. In view of these facts, we describe herein the synthesis of 3′-*C*-carboxymethyl Le^x carrying the 2-(tetradecyl)hexadecyl residue, which is expected not only to be a potential substitute of sLe^x ganglioside, but also to be more stable than either sialyl- or sulfo-Le^x for both chemical and enzymatic degradations.

For the synthesis of the required 3-*C*-carboxymethyl galactose, the readily available 2-(trimethylsilyl)ethyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside [7] was chosen as a starting material. Oxidation [8] of the starting material with oxalyl chloride and dimethyl sulfoxide furnished the 3-ulose derivative **1**, which, on treatment [9] with diethyl

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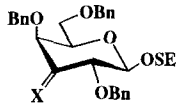
[1] Synthetic studies on sialoglycoconjugates, Part 98. For Part 97, see ref. [1].

[2] Deceased 10 October 1996.

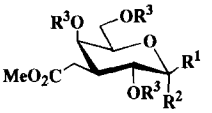
PII S0008-6215(97)00156-0

phosphonoacetate in the presence of sodium methoxide, afforded the methyl ester of the 3-deoxy-3-*C*-carboxymethylene derivative **2** [3] (82%) as a 1:1 mixture of *E*,*Z*-isomers. Transesterification from ethyl to methyl was caused by sodium methoxide used in the reaction (2 h). It is of interest to note that catalytic hydrogenation of **2** over 10% palladium-on-charcoal (10% Pd–C) in methanol (Caution! Severe fire hazard!) at 50 °C stereoselectively gave the desired 3-*C*-carboxymethyl galactoside **3**, while the same reaction conditions at room temperature gave a 1:1 mixture of **3** and the corresponding guloside. Treatment of **3** with benzoyl chloride in pyridine gave 2,4,6-tribenzoate **4** {66% in two steps, $[\alpha]_D$ −43° ($CHCl_3$)}. In the ^1H NMR spectrum of **4**, H-2 (dd, $J_{1,2}$ 7.9, $J_{2,3}$ 11.5 Hz) was observed at δ 5.36, showing the configuration of the substituent at C-3 to be equatorial.



1 X = O
2 X = $CHCO_2Me$ (*E*, *Z*)

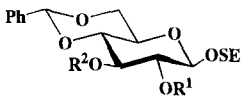
SE = 2-(trimethylsilyl)ethyl



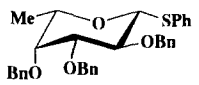
	R^1	R^2	R^3
3	OSE	H	H
4	OSE	H	Bz
5	H , OH		Bz
6	H	OC(=NH)CCl_3	Bz

Compound **4** was converted into the corresponding α-trichloroacetimidate **6** in good yield by selective removal of the 2-(trimethylsilyl)ethyl group with trifluoroacetic acid and subsequent imidate formation [10,11].

The glycosyl acceptor **12** was prepared as follows. Selective 3-*O*-benzoylation of 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-β-D-galactopyranoside [10] with benzoyl chloride in pyridine–dichloromethane at −50 °C (86%), and subsequent 2-*O*-benzylation with benzyl bromide in the presence of silver oxide (80%) afforded the selectively protected galactoside derivative **8** {$[\alpha]_D$ −18° ($CHCl_3$)}. Debenzoylation of **8**, followed by glycosylation of **9** with phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**10**) [12] in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) [13] and 4 Å molecular sieves (MS-4A) in benzene for 10 h at 6 °C, gave the desired disaccharide **11** {86%, $[\alpha]_D$ −49° ($CHCl_3$)}, showing in the ^1H NMR spectrum a signal at δ 5.22 (d, $J_{1,2}$ 3.6 Hz) that is characteristic of an α-L-fucopyranosyl unit. It was then converted into the glycosyl acceptor **12** in an 86% yield by reductive ring-opening of the benzylidene group [14].



7 R^1 = H, R^2 = Bz
8 R^1 = Bn, R^2 = Bz
9 R^1 = Bn, R^2 = H



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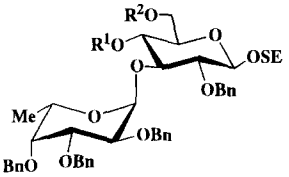
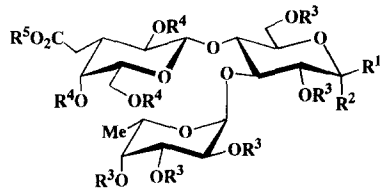


11 R^1 : R^2 = benzylidene
12 R^1 = H, R^2 = Bn

Glycosylation [15] of **12** with **6** in acetonitrile in the presence of DMTST for 2 days at −10 °C afforded the desired trisaccharide **13** {67%, $[\alpha]_D$ −0.9° ($CHCl_3$)}. The β-configuration of **13** was assigned from the ^1H NMR datum that showed the signal at δ 5.19 (dd, $J_{1,2}$ 11.8, $J_{2,3}$ 7.8 Hz) for H-2 of the galactose residue.

Removal of the benzyl groups from **13** by catalytic hydrogenation over 10% Pd–C in ethanol for 6 h at room temperature, and subsequent benzoylation gave the per-*O*-benzoylated trisaccharide **15** {96%, $[\alpha]_D$ −27° ($CHCl_3$)}. Selective removal [10] of the 2-(trimethylsilyl)ethyl group from **15** as described in the preparation of **5** gave the corresponding 1-hydroxy compound **16**. Treatment [11] of **16** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 1 h at −50 °C gave the trichloroacetimidate **17** {quant. $[\alpha]_D$ −8.6° ($CHCl_3$)} in the α-form. Glycosylation of 2-(tetradecyl)hexadecanol with **17** thus obtained in dichloromethane in the presence of boron trifluoride etherate gave only the desired β-glycoside **18** {62%, $[\alpha]_D$ −28° ($CHCl_3$)}.

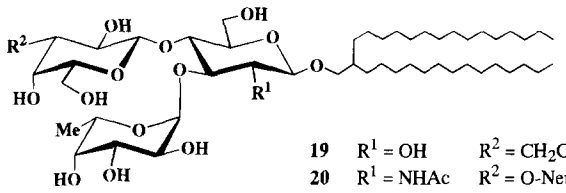
[3] All new compounds were fully characterized by elemental analyses and by IR and ^1H NMR spectroscopy.



	R^1	R^2	R^3	R^4	R^5
13	OSE	H	Bn	Bz	Me
14	OSE	H	H	Bz	Me
15	OSE	H	Bz	Bz	Me
16	H , OH		Bz	Bz	Me
17	$OC(=NH)CCl_3$	H	Bz	Bz	Me
18	B-30	H	Bz	Bz	Me

B-30 = 2-(tetradecyl)hexadecyl

A significant signal in the ^1H NMR spectrum of **18** was a one-proton doublet at δ 4.28 (d, $J_{1,2}$ 8.6 Hz, H-1 of Glc), showing the newly formed glycosidic linkage to be β. *O*-Debenzoylation of **18** with NaOMe in MeOH, with subsequent saponification of the methyl ester group by addition of water, yielded the desired 3′-*C*-carboxymethyl Le^x derivative **19** {quant. $[\alpha]_D$ −20° (1:1 $CHCl_3$–MeOH)}.



19 R^1 = OH R^2 = CH_2COOH
20 R^1 = NHAc R^2 = O-Neu5Ac

Compound **19** was much more potent (IC_{50}, 6 μM for E-selectin, 3 μM for P-selectin, 2 μM for L-selectin) [4] than the corresponding sialyl Le^x derivative **20** (IC_{50}, 330 μM for E-selectin, 250 μM for P-selectin, 40 μM for L-selectin) [6] in the ligand–selectin competitive binding assay.

[4] Detailed studies on the biological activities will be published elsewhere.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (B) (No. 07456162) and Scientific Research on Priority Areas (No. 05274102) from the Ministry of Education, Science and Culture of Japan.

References

[1] T. Ikami, H. Hamajima, T. Usui, T. Mitani, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, (1997) in press.
[2] (a) R.D. Cummings and D.F. Smith, *BioEssays*, 14 (1992) 849–856; (b) R.P. McEver, *Curr. Opin. Immunol.*, 6 (1994) 75–84.
[3] K. Hiruma, T. Kajimoto, G. Weitz-Schmidt, I. Ollmann, and C.-H. Wong, *J. Am. Chem. Soc.*, 118 (1996) 9265–9270, and references therein.
[4] M. Yoshida, Y. Kawakami, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 15 (1996) 399–418, and references therein.
[5] A. Hasegawa, K. Ito, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, 14 (1995) 353–368.
[6] Y. Wada, T. Saito, N. Matsuda, H. Ohmoto, K. Yoshino, M. Ohashi, H. Kondo, H. Ishida, M. Kiso, and A. Hasegawa, *J. Med. Chem.*, 39 (1996) 2055–2059.
[7] A. Hasegawa, T. Ando, A. Kameyama, and M. Kiso, *J. Carbohydr. Chem.*, 11 (1992) 645–658.
[8] A.J. Mancuso, S.-L. Huang, and D. Swern, *J. Org. Chem.*, 43 (1978) 2480–2482.
[9] A.G. Andrews and S. Liaaen-Jensen, *Acta. Chem. Scand.*, 27 (1973) 1401–1409.
[10] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, and K. Stenvall, *J. Org. Chem.*, 53 (1988) 5629–5647.
[11] (a) M. Numata, M. Sugimoto, K. Koike, and T. Ogawa, *Carbohydr. Res.*, 163 (1987) 209–225; (b) R.R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 731–732.
[12] S. Komba, H. Ishida, M. Kiso, and A. Hasegawa, *Bioorg. Med. Chem.*, 4 (1996) 1833–1847.
[13] P. Fügedi and P.J. Garegg, *Carbohydr. Res.*, 149 (1986) C9–C12.
[14] P.J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, 108 (1982) 97–101.
[15] (a) R.R. Schmidt and G. Grundler, *Synthesis*, (1981) 885; (b) T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 188 (1989) 71–80.