Carbohydrate Research, 193 (1989) c1–c5
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Total synthesis of sialyl lactotetraosyl ceramide*

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(Received April 18th, 1989; accepted for publication, June 5th, 1989)

Recently, various types of important biological functions of gangliosides in biological systems have been reported by many groups[2–5]. Consequently, a facile, regio- and α-stereo-selective glycoside synthesis of sialic acid is critically important for the synthesis of a variety of gangliosides and their analogs, in order to investigate the structure–function relationship of sialoglycoconjugates at the molecular level. Previously, we demonstrated[6] a new, efficient α-glycosylation of sialic acid by use of dimethyl(methylthio)sulfonium triflate (DMTST)[7] as the glycosyl promoter, and the suitably protected glycosyl acceptors in acetonitrile under kinetically controlled conditions, and accomplished[8] a regio- and stereo-selective synthesis of gangliosides GM_3, GM_4, and their analogs.

In this communication, an application of the method to the first total synthesis of sialyl lactotetraosyl ceramide[9] (IV^3 $NeuAcLc_4Cer$), which has been detected as a ganglioside antigen in human lung carcinoma by a monoclonal antibody in 1985, and found to be widespread as a minor component in many different carcinomas, will be described.

Dibutyltin oxide-mediated, selective etherification[10] of 2-(trimethylsilyl)ethyl *O*-β-D-galactopyranosyl-(1→4)-β-D-glucoyranoside[11] (**1**) to give the 3′-*O*-(4-methoxybenzyl) derivative **2** {m.p. 184.5°, $[\alpha]_D$ −3.2° (1:1 methanol–dichloromethane)} could be achieved in 74% yield. Benzylation of **2** with benzyl bromide in *N,N*-dimethylformamide in the presence of sodium hydride gave **3** (79%), which was treated with 2,3-dichloro-5,6-dicyanobenzoquinone[12] in dichloromethane–water for 1 h at room temperature to afford compound **4** {$[\alpha]_D$ +0.2° (dichloromethane)} in 85% yield.

The glycosylation of **4** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide[13] (**5**) in the presence of silver carbonate, silver perchlorate, and molecular sieves 4A (MS-4A) in dichloromethane for 16 h at room temperature gave the desired β-glycoside **6** {$[\alpha]_D$ −3.7° (chloroform)} in 86% yield; significant

*Synthetic Studies on Sialoglycoconjugates, Part 11. For Part 10, see ref. 1.
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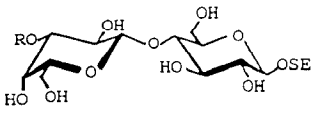
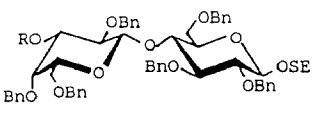
0008-6215/89/$ 03.50



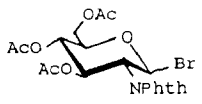
1 R = H
2 R = MPM

SE = 2-(trimethylsilyl)ethyl
MPM = 4-methoxybenzyl



3 R = MPM
4 R = H

Bn = $PhCH_2$



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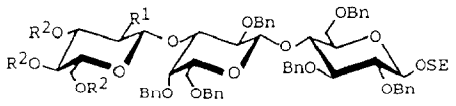
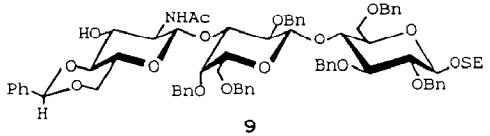
Phth = phthaloyl



6 R^1 = NPhth, R^2 = Ac
7 R^1 = NPhth, R^2 = H
8 R^1 = NHAc, R^2 = H



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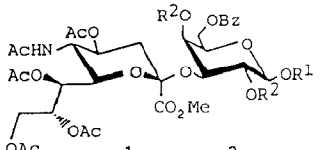
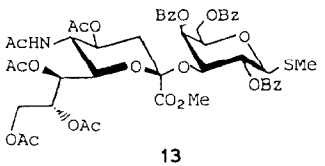


10 R^1 = SE, R^2 = H
11 R^1 = SE, R^2 = Bz
12 R^1 = Ac, R^2 = Bz



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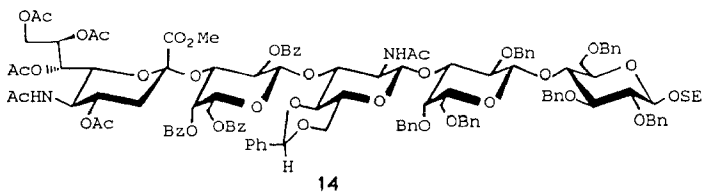

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signals of the glucosamine unit in the ^{1}H-n.m.r. spectrum were a one-proton doublet at δ 5.63 ($J_{1,2}$ 8.4 Hz, H-1), a one-proton triplet at δ 5.15 ($J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), and a one-proton triplet at δ 5.87 ($J_{4,5}$ 10.6 Hz, H-4), indicating the structure assigned. *O*-Deacetylation of **6**, followed by heating with hydrazine hydrate in 95% aqueous ethanol, and *N*-acetylation of the product afforded 2-(trimethylsilyl)ethyl *O*-(2-acetamido-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 {**8**; $[\alpha]_D$ −7.3° (dichloromethane)} in 77% yield; characteristic signals in the ^{1}H-n.m.r. spectrum were a three-proton singlet at δ 1.63 (*N*-acetyl) and thirty protons at δ 7.10–7.34 (6 Ph). Treatment of **8** with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid monohydrate gave the benzylidene derivative **9** in 83% yield. Compound **9** was used as the glycosyl acceptor for the synthesis of the oligosaccharide unit of the target ganglioside.

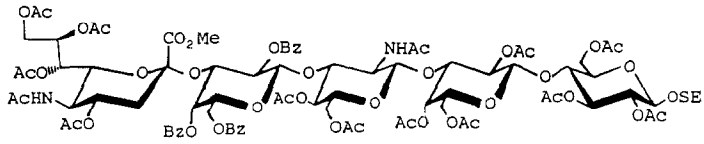
O-Benzoylation of 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-6-*O*-benzoyl-β-D-galactopyranoside[6] (**10**), prepared by coupling of the methyl α-thioglycoside of Neu5Ac and 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside[8b,14] according to the method[6,8] described previously, gave the 2,4,6-tri-*O*-benzoyl derivative **11**, which, on treatment[15] with boron trifluoride etherate in toluene–acetic anhydride, gave the β-1-*O*-acetyl derivative **12** in 93% yield; $[\alpha]_D$ +40.9° (chloroform). The observed chemical shifts and coupling constants of the galactose unit in **12** for H-1 (δ 6.25, $J_{1,2}$ 8.3 Hz), H-2 (δ 5.66, $J_{2,3}$ 10.1 Hz), and H-4 (δ 5.52, $J_{3,4} = J_{4,5} = 3.3$ Hz) are characteristic of the structure assigned.

Direct conversion of the β-1-*O*-acetyl derivative **12** into methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside {**13**; $[\alpha]_D$ +34.4° (chloroform)} was effectively achieved in 82% yield by treatment with methylthiotrimethylsilane and boron trifluoride etherate in dichloromethane, according to the method of Pozsgay and Jennings[16]. The glycosylation of **9** with **13** (1.5 equiv. relative to the acceptor **9**) in dichloromethane for 12 h at 0°, in the presence of DMTST (4.0 equiv. relative to the glycosyl donor **13**) and MS-4A, afforded the corresponding β-glycoside **14** {$[\alpha]_D$ +0.37° (chloroform)} in 87% yield; significant signals in the ^{1}H-n.m.r. spectrum of **14** (Neu5Ac-Gal unit) were a one-proton doublet at δ 5.12 ($J_{1,2}$ 8.0 Hz, H-1), three one-proton doublets of doublets at δ 4.91 (H-3), 5.29 (H-4), and 5.39 (H-2), ($J_{2,3}$ 10.0 Hz, $J_{3,4} = J_{4,5} = 2.9$ Hz), showing the newly formed β-glycosidic linkage. Other ^{1}H-n.m.r. data are consistent with structure **14**.

Removal of the benzyl groups in compound **14** by catalytic hydrogenolysis over 10% Pd–C in ethanol–formic acid at room temperature, and subsequent hydrolysis of the benzylidene group by heating with 80% aqueous acetic acid at 45°, followed by acetylation with acetic anhydride–pyridine, gave the paracylated pentasaccharide **15** {$[\alpha]_D$ +10.3° (chloroform)} in 61% yield. The structure was unambiguously proved by ^{1}H-n.m.r. spectroscopy. The chemical shifts observed were two three-proton singlets at δ 1.53 and 1.77 (*N*-acetyl), signals for twelve *O*-acetyl groups at δ 1.90–2.15, and three benzoyl groups at δ 7.40–8.19, and a three-proton singlet at δ 3.81 (CO_2Me).

Selective removal of the 2-(trimethylsilyl)ethyl group in **15** by treatment[15] with boron trifluoride etherate in dichloromethane for 24 h at 0° gave compound **16** {$[\alpha]_D$ +30.1° (chloroform)} in 87% yield after column chromatography. Treatment[17,18] of **16** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) for 4 h at 0° gave the corresponding α-trichloroacetimidate **17** {$[\alpha]_D$ +38.7° (chloroform)} in 88% yield. Significant signals in the ^{1}H-n.m.r. spectrum were a one-proton doublet at δ 6.48 ($J_{1,2}$ 3.8 Hz, H-1) and a one-proton singlet at δ 8.65 (C=NH), indicating α-trichloroacetimidate formation.

The final glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[19] (**18**; 2.0 equiv.) with **17** thus obtained, in the presence of boron trifluoride



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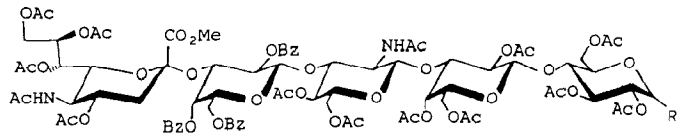
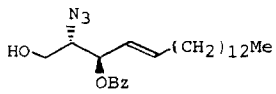


16 R = H
17 R = αOC(=NH)CCl$_3$



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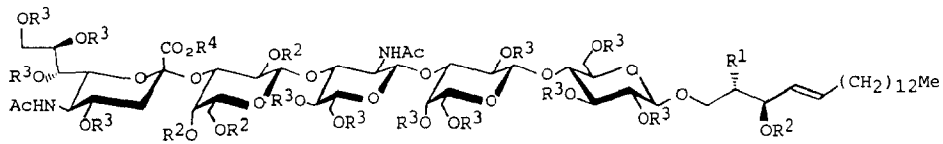

19 R^1 = N_3, R^2 = Bz, R^3 = Ac, R^4 = Me
20 R^1 = NH_2, R^2 = Bz, R^3 = Ac, R^4 = Me
21 R^1 = NHCO$(CH_2)_{16}$Me, R^2 = Bz, R^3 = Ac, R^4 = Me
22 R^1 = NHCO$(CH_2)_{16}$Me, R^2 = R^3 = R^4 = H

etherate[19b] and MS-4A for 5 h at room temperature, afforded only the expected *β*-glycoside **19** {$[\alpha]_D$ +6.5° (chloroform)} in 55% yield after column chromatography.

Selective reduction[20] of the azide group in compound **19** with hydrogen sulfide in 83% aqueous pyridine gave the amine **20**, which, on condensation with octadecanoic acid using 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (WSC) in dichloromethane for 16 h at room temperature, gave the sialyl lactotetraosyl ceramide **21** {$[\alpha]_D$ +13.7° (chloroform)} in 79% yield. Finally, *O*-deacylation of **21** with sodium methoxide in methanol, and saponification of the methyl ester group, yielded the end product **22** {$[\alpha]_D$ −6.2° (water)} in almost quantitative yield after chromatography on a column of Sephadex LH-20.

In conclusion, by using compounds **5, 13,** and **17** as the glycosyl donors, and compounds **4, 9,** and **18** as the glycosyl acceptors, a regio- and stereo-controlled synthesis of sialyl lactotetraosyl ceramide, a complex type of ganglioside, was

achieved. The 2-(trimethylsilyl)ethyl *O*-(*N*-acetyl-α-neuraminyl)-(2→3)-β-D-galactopyranoside derivative **10** was a useful building-block for the sialoglycoconjugate synthesis. Furthermore, the 2-(trimethylsilyl)ethyl group employed here was an efficient protecting group for the anomeric hydroxyl group because of the easy and selective deprotection with boron trifluoride etherate, and of the sufficient stability towards many of the reagents used herein.

New compounds obtained gave elemental analyses and i.r. and ^{1}H-n.m.r. (270 and 400 MHz) data in agreement with the structures assigned.

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