ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 328 (2000) 459–466

CARBOHYDRATE RESEARCH

Total synthesis of the natural antigen involved in the hyperacute rejection response to xenotransplants☆

Christian Gege [a], Willy Kinzy [b], Richard R. Schmidt [a,*]

[a] *Faculty of Chemistry, University of Konstanz, Box M725, D-78457 Konstanz, Germany*
[b] *Transplantation Research, NOVARTIS Pharma AG, S-507.701, CH-4002 Basel, Switzerland*

Received 15 February 2000; accepted 4 May 2000

Abstract

The major glycosphingolipid in pig vascular endothelium is the ceramide pentasaccharide Galα(1→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glcβ(1→0)Cer (**1**), which binds specifically to human anti-Gal antibody and is involved in the hyperacute rejection response in xenotransplantation from pig to man. The synthesis of **1** and its methyl glycoside **2** is described.

Keywords: Synthesis; O-Glycosylation; α-Galactosyl epitopes; Xenotransplantation; Glycosphingolipid; Ceramide

1. Introduction

Human allotransplantation is nowadays a generally accepted treatment of choice for several illnesses. The major hindrance towards widened indications of organ transplantation as the preferred treatment, is the shortage of donor organs suitable for such clinical application. Xenotransplantation, i.e., transplantation of tissue between different species, is considered one promising possible solution. The main problem in xenografting between discordant species however is the hyperacute rejection (HAR) of xenotransplants, which results in the destruction of the vascular endothelium of the donor organ within minutes [1]. Man differs from other mammals in the expression of α-galactosyl epitopes. These epitopes are carbohydrate structures bearing an Galα(1→3)Gal terminus (α-Gal epitopes). All human sera contain a large amount of naturally occurring antibody (anti-Gal), which binds to α-Gal epitopes. These antigens are established as major xenoantigens on pig endothelium and are responsible for initiating the HAR of pig organs by humans and are thus of primary interest in the development of xenotransplantation. The major glycosphingolipid in pig vascular endothelium is the ceramide pentasaccharide Galα(1→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glcβ(1→0)Cer (**1**), which binds specifically to human anti-Gal antibody [2].

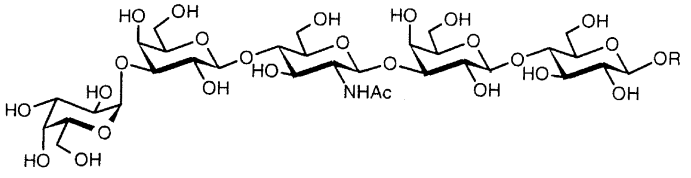
☆ Presented at the 37th IUPAC-Kongress, Berlin/Germany, 14.-19.8.1999, SYN-1-128.

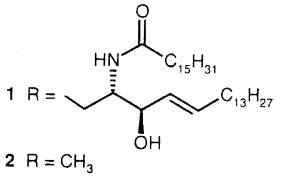
* Corresponding author. Tel.: +49-7531-882538; fax: +49-7531-883135.

E-mail address: rrs@chclu.chemie.uni-konstanz.de (R.R. Schmidt).

0008-6215/00/$ - see front matter
PII: S0008-6215(00)00145-2

For the purpose of elucidating the biological role of the pentasaccharide, we have developed a direct synthesis of its ceramide **1** and the corresponding methyl glycoside **2** [3] based on trichloroacetimidates as glycosyl donors [4].

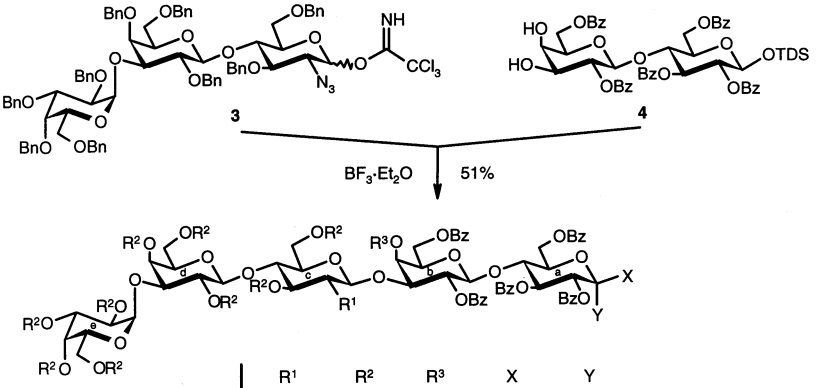




2. Results and discussion

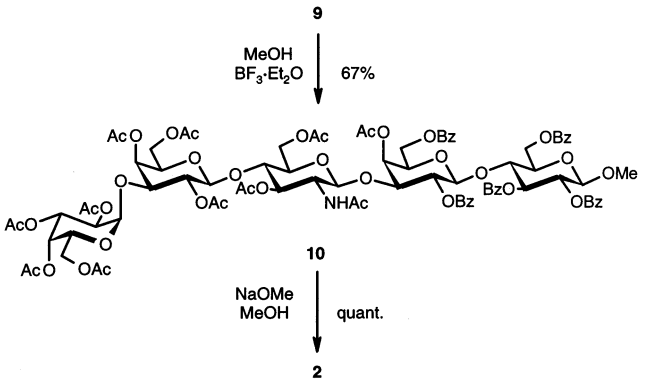
Glycosidation of known trisaccharide donor **3** [5] (Scheme 1) was performed with known disaccharide acceptor **4** [6,7]. The thexyldimethylsilyl (TDS) group for 1-O protection of the acceptor was selected because it turned out to be less acid sensitive under glycosylation conditions than the *tert*-butyldimethysilyl (TBDMS) and *tert*-butyldiphenylsilyl (TBDPS) group. The glycosidation in CH_2Cl_2–*n*-hexane at −20 °C in the presence of $BF_3{\cdot}Et_2O$ as catalyst followed previous related work [7]. The desired pentasaccharide **5** was obtained in 51% yield, in addition to **5**, some α anomer was formed, which was separated by chromatography and not further characterized. The regiochemistry of the newly introduced glycosidic linkage was confirmed by the ^1H NMR data of the acetylated compound **6**. The ^1H NMR spectrum showed the presence of H-4b of the galactose unit at 5.27 ppm compared with 3.92 ppm for compound **5**, indicating the position of the new glycosidic linkage in **5** to be at OH-3 of the acceptor **4**. Its stereochemistry was determined to be β on the basis of the GlcNAc H-1–H-2 coupling constant ($J_{1,2}$ 8.0 Hz). The azido group was reduced with H_2S in pyridine, followed by N,O-acetylation in Ac_2O–pyridine to afford **6** in 76% yield. Hydrogenolytic O-debenzylation and then O-acetylation gave the pentasaccharide **7** in 86% yield. For glycosyl donor formation, removal of the 1-O-TDS group by treatment with tetra-*n*-butyl ammonium fluoride (TBAF) in THF–HOAc was required affording 1a-O-unprotected **8** in 91% yield, which on reaction with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base furnished α-trichloroacetimidate **9** in almost quantitative yield, only trace amounts of the β anomer were present.

With this donor in hand, the methyl glycoside **2** was made by glycosylation of donor **9**



	R1	R2	R3	X	Y
5	N_3	Bn	H	OTDS	H
6	NHAc	Bn	Ac	OTDS	H
7	NHAc	Ac	Ac	OTDS	H
8	NHAc	Ac	Ac	OH, H	
9	NHAc	Ac	Ac	H, $OC(NH)CCl_3$	

Scheme 1.



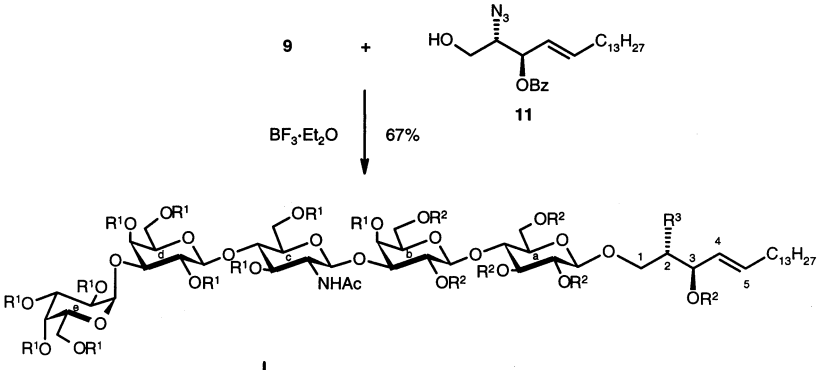
Scheme 2.

with MeOH in CH_2Cl_2 and $BF_3{\cdot}Et_2O$ as catalyst, followed by removal of all *O*-acyl protective groups with NaOMe in MeOH (Scheme 2). The synthesis of this compound has been reported using an alternative route [3].

To obtain the target molecule **1** the standard 'azidosphingosine glycosylation procedure' [8] for glycosphingolipid synthesis was employed (Scheme 3). Thus, reaction of donor **9** with azidosphingosine derivative **11** [8,9] in CH_2Cl_2 with $BF_3{\cdot}Et_2O$ as catalyst afforded intermediate **12** in 67% yield. The azido group was reduced with H_2S in pyridine, followed by coupling of palmitic acid with *N*′-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (water soluble carbodiimide = WSC) to afford **13** in 82% yield. Finally, removal of all *O*-acyl protective groups with NaOMe in Me_2SO–MeOH afforded **1** in almost quantitative yield. The structure was confirmed by ^1H,^{13}C-COSY and HMBC experiments (Table 1). Noteworthy is the fact that the glycosphingolipid **1** exhibits low solubility in $CHCl_3$, MeOH, water and mixtures thereof, a problem also described for the isolation of **1** from natural sources [10]. Work is in progress to evaluate the binding affinities of **1** and **2**.

3. Experimental

General methods.—Solvents were purified according to standard procedures. Flash chromatography was performed on J.T. Baker Silica Gel 60 (40–63 μm) or RP-18 Silica Gel (40 μm) at a pressure of 0.4 bar. Thin-layer chromatography (TLC) was performed on E. Merck Silica Gel plastic plates, $60F_{254}$ or E. Merck Silica Gel glass plates, RP-18 $60F_{254S}$; compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% H_2SO_4 (400 mL) and heating at 150 °C. Optical rotations were measured on a Perkin–Elmer polarimeter 241 in a 1 dm cell at 22 °C. NMR measurements were recorded on a Bruker AC250 Cryospec, Bruker DRX500 or a Bruker DRX600 spectrometer. MALDI-mass spectra were recorded on a Kratos Kompact MALDI I instrument using a 2,5-dihydroxybenzoic acid matrix. FAB-mass spectra were recorded on a Finnigan MAT 312/AMD 5000 spectrometer using a 1:1 (3-nitrobenzyl)alcohol–glycerol matrix.



	R¹	R²	R³
12	Ac	Bz	N_3
13	Ac	Bz	$NHCO(CH_2)_{14}CH_3$
1	H	H	$NHCO(CH_2)_{14}CH_3$

Scheme 3.

Table 1
^{1}H and ^{13}C NMR assignments for compound **1** in 7:1 $(CD_3)_2SO$–CD_3OD (v/v) at 300 K

Residue		^{1}H or ^{13}C chemical shifts [ppm] (multiplicity, ^{1}H–^{1}H coupling [Hz]) [a]					
		1	2	3	4	5	6
β-Glc (a)	^{1}H	4.20 (d, 7.7)	3.03 (t, 8.2)	3.32	3.28	3.26	3.75/3.58
	^{13}C	103.5	73.3	74.7	80.6	74.9	60.5
β-Gal (b)	^{1}H	4.23 (d, 6.9)	3.43	3.42	3.84	3.48	3.55–3.40
	^{13}C	103.7	69.4	82.0	67.4	75.1	60.4
β-GlcNAc (c)	^{1}H	4.63 (d, 8.0)	3.45	3.55	3.33	3.26	3.76/3.64
	^{13}C	102.3	55.4	72.1	81.4	74.9	60.5
β-Gal (d)	^{1}H	4.28 (d, 7.5)	3.42	3.48	3.84	3.48	3.55–3.40
	^{13}C	103.9	69.3	78.9	64.5	75.1	60.4
α-Gal (e)	^{1}H	4.83 (d, 3.5)	3.60	3.62	3.72	4.00 (t, 6.6)	3.52/3.43
	^{13}C	96.5	68.5	69.4	68.8	70.9	60.2
Aglycon [b]	^{1}H	3.72	3.77	3.88	5.33 (dd, 15.4/7.0)	5.52 (dt, 15.4/7.3)	1.91
	^{13}C	68.8	53.5	70.8	131.2	131.1	31.8

[a] d = doublet, t = triplet, if not otherwise stated: overlapping signals. Chemical shift reference: $(CD_3)_2SO$ (^{1}H, 2.49 ppm; ^{13}C, 39.5 ppm).

[b] Further signals: CH=CHCH$_2$*CH$_2$*: 1.26/28.9, (*CH$_2$*)$_7$: ~1.25/28–30, *CH$_2$*CH$_2$CH$_3$: 1.21/31.4, *CH$_2$*CH$_3$: 1.24/22.2, *CH$_3$*: 0.83/13.7, *C*=O: 172.2, CO*CH$_2$*: 2.03/35.6, COCH$_2$*CH$_2$*: 1.43/25.4.

Thexyldimethylsilyl (2,3,4,6-tetra-O-*benzyl*-α-D-*galactopyranosyl)*-*(1→3)*-*(2,4,6-tri*-O-*benzyl*-β-D-*galactopyranosyl)*-*(1→4)*-*(2-azido*-*3,6-di*-O-*benzyl*-*2-deoxy*-β-D-*glucopyranosyl)*-*(1→3)*-*(2,6-di*-O-*benzoyl*-β-D-*galactopyranosyl)*-*(1→4)*-*2,3,6-tri*-O-*benzoyl*-β-D-*glucopyranoside* (**5**).—A soln of (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranosyl trichloroacetimidate [5] (**3**; 289 mg, 195 μmol, 1.1 equiv) and TDS (2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside [6] (**4**; 178 mg, 177 μmol) in dry 1:1 CH_2Cl_2–*n*-hexane (2 mL) was added $BF_3{\cdot}Et_2O$ (5 μL, 0.2 equiv) under Ar at −20 °C. After 30 min the soln was neutralized with NEt_3 and concd. Flash chromatography (5:1 toluene–EtOAc) of the residue gave **5** (210 mg, 51%) as a colourless foam. $[\alpha]_D + 31.9°$ (*c* 1, $CHCl_3$); R_f 0.41 (4:1 toluene–EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.23–7.06 (m, 70 H, 14 C_6H_5), 5.70 (dd, $J_{2,3} = J_{3,4}$ 9.5 Hz, 1 H, H-3a), 5.45 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8 Hz, 1 H, H-2b), 5.37 (dd, $J_{1,2}$ 7.6, $J_{2,3}$ 10.0 Hz, 1 H, H-2a), 5.19 (d, $J_{1,2}$ 3.4 Hz, 1 H, H-1e), 5.05 (d, J_{gem} 11.5 Hz, 1 H, C*H*HPh), 4.93 (d, J_{gem} 10.3 Hz, 1 H, C*H*HPh), 4.91 (d, $J_{1,2}$ 7.6 Hz, 1 H, H-1a), 4.88 (d, J_{gem} 11.4 Hz, 1 H, C*H*HPh), 4.85 (d, J_{gem} 11.6 Hz, 1 H, C*H*HPh), 4.72 (d, J_{gem} 11.4 Hz, 1 H, C*H*HPh), 4.68 (d, J_{gem} 11.6 Hz, 1 H, C*H*HPh), 4.63, 4.59 (2 d, J_{gem} 12.0 Hz, each 1 H, CH_2Ph), 4.58 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b), 4.54–4.45 [m, 4 H, CH_2Ph, H,H-COSY: 4.52 (H-6′a), 4.46 (H-6a)], 4.34–4.06 [m, 13 H, 4 CH_2Ph, H,H-COSY: 4.31 (H-1d), 4.25 (H-5e), 4.11 (H-2e), 4.09 (H-6′b), 4.08 (H-1c), 4.07 (H-4a)], 3.92–3.89 [m, 3 H, H,H-COSY: 3.92 (H-4b), 3.90 (H-3e), 3.89 (H-4d)], 3.81 (ddd, $J_{4,5}$ 5.6, $J_{5,6}$ 1.9, $J_{5,6'}$ 9.8 Hz, 1 H, H-5a), 3.76 (dd, $J_{3,4} = J_{4,5}$ 9.2 Hz, 1 H, H-4c), 3.72–3.66 [m, 4 H, H,H-COSY: 3.72 (H-2d), 3.69 (H-4e), 3.68 (H-3d), 3.67 (H-3b)], 3.61 (dd, J_{gem} 11.5, $J_{5,6}$ 7.3 Hz, 1 H, H-6b), 3.55 (dd, J_{gem} 10.6, $J_{5,6}$ 4.9 Hz, 1 H, H-6′c), 3.51 (t, 1 H, H-5b), 3.48–3.36 [m, 4 H, H,H-COSY: 3.44 (H-6c), 3.40 (H-6′d), 3.39 (H-6′e), 3.39 (H-6e)], 3.32 (m, 1 H, H-5d), 3.24 (dd, J_{gem} 9.0, $J_{5,6}$ 5.1 Hz, 1 H, H-6d), 3.20–3.12 [m, 3 H, H,H-COSY: 3.18 (H-2c), 3.15 (H-5c), 3.13 (H-3c)], 1.42–1.46 (m, 1 H, CH), 0.67 (m, 12 H, 2 $C(CH_3)_2$), 0.04, −0.02 (2 s, each 3 H, $Si(CH_3)_2$). ^{13}C NMR (151 MHz, $CDCl_3$): δ 139.15–127.34

(C-phenyl), 102.93 (C-1d), 102.35 (C-1c), 100.78 (C-1b), 96.11 (C-1e), 95.85 (C-1a), 81.82 (C-3b), 81.26 (C-3c), 79.06 (C-2d), 78.98 (C-3e), 78.34 (C-3d), 76.42 (C-2e), 76.17 (C-4a), 75.91 (C-4c), 75.11 (C-5c), 74.87 (C-4e), 73.69 (C-2a), 77.21–73.17 (9 CH_2Ph), 73.09 (C-5d), 72.90 (C-4d), 72.68 (C-3a), 72.39 (C-5b), 72.30 (C-5a), 70.52 (C-2b), 69.30 (C-5e), 68.95 (C-6e), 68.08 (C-6d), 67.88 (C-6c), 67.76 (C-4b), 65.60 (C-2c), 62.95 (C-6a and C-6b), 33.78 ($CHMe_2$), 19.77, 19.71, 18.30, 18.27 (4 CH_3), −2.12, −3.54 ($Si(CH_3)_2$). Anal. Calcd for $C_{136}H_{143}N_3O_{30}Si$ (2327.71): C, 70.18; H, 6.19; N, 1.81. Found: C, 69.89; H, 6.30; N, 1.60.

Thexyldimethylsilyl (2,3,4,6-tetra-O-*benzyl*-α-D-*galactopyranosyl)*-*(1→3)*-*(2,4,6*-*tri*-O-*benzyl*-β-D-*galactopyranosyl)*-*(1→4)*-*(2*-*acetamido*-*3,6*-*di*-O-*benzyl*-*2*-*deoxy*-β-D-*glucopyranosyl)*-*(1→3)*-*(4*-O-*acetyl*-*2,6*-*di*-O-*benzoyl*-β-D-*galactopyranosyl)*-*(1→4)*-*2,3,6*-*tri*-O-*benzoyl*-β-D-*glucopyranoside* (**6**).—A soln of **5** (670 mg, 288 μmol) in 4:1 pyridine–water (25 mL) was saturated with hydrogen sulfide (30 min) and stirred at rt for 10 days, then concd, and co-concd with toluene (3 ×). The residue was stirred 4 h in a mixture of pyridine (3 mL) and Ac_2O (2 mL) and then concd. Flash chromatography (9:1 toluene–acetone) of the residue led to **6** (522 mg, 75%) as a colourless foam. $[\alpha]_D + 38.9°$ (*c* 1, $CHCl_3$); R_f 0.46 (4:1 toluene–EtOAc); ^1H NMR (600 MHz, $CDCl_3$): δ 8.15–6.95 (m, 70 H, 14 C_6H_5), 5.67 (dd, $J_{2,3} = J_{3,4}$ 9.6 Hz, 1 H, H-3a), 5.36–5.31 [m, 2 H, H,H-COSY: 5.33 (H-2a), 5.32 (H-2b)], 5.27 (d, 1 H, H-4b), 5.18 (d, 1 H, NH), 5.15 (d, $J_{1,2}$ 3.4 Hz, 1 H, H-1e), 5.01 (d, J_{gem} 11.6 Hz, 1 H, C*H*HPh), 4.91 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1c), 4.89–4.81 [m, 4 H, H,H-COSY: 4.88 (d, $J_{1,2}$ 7.6 Hz, H-1a), 4.87 (d, J_{gem} 11.0 Hz, C*H*HPh), 4.85 (d, J_{gem} 11.4 Hz, C*H*HPh), 4.82 (d, J_{gem} 11.7 Hz, C*H*HPh)], 4.75 (d, J_{gem} 11.1 Hz, 1 H, C*H*HPh), 4.69–4.57 (m, 4 H, 2 CH_2Ph), 4.52 (d, $J_{1,2}$ 7.9 Hz, 1 H, H-1b), 4.49–4.45 [m, 2 H, H,H-COSY: 4.48 (C*H*HPh), 4.46 (H-6′a)], 4.40–4.33 [m, 3 H, H,H-COSY: 4.39 (d, $J_{1,2}$ 7.3 Hz, H-1d), 4.38 (d, J_{gem} 11.9 Hz, C*H*HPh), 4.34 (H-6a)], 4.31–4.28 (m, 3 H, 3 C*H*HPh), 4.24–4.18 [m, 5 H, 2 CH_2Ph, H,H-COSY: 4.23 (H-5e)], 4.08 (dd, $J_{1,2}$ 3.4, $J_{2,3}$ 10.1 Hz, 1 H, H-2e), 4.02 (dd, $J_{3,4} = J_{4,5}$ 9.5 Hz, 1 H, H-4a), 3.98 ($J_{2,3} = J_{3,4}$ 9.6 Hz, 1 H, H-3c), 3.89 (dd, $J_{2,3} = 10.2$, $J_{3,4}$ 2.8 Hz, 1 H, H-3e), 3.88–3.84 [m, 2 H, H,H-COSY: 3.86 (H-6′b), 3.85 (H-4d)], 3.81 (dd, $J_{3,4} = J_{4,5}$ 8.9 Hz, 1 H, H-4c), 3.76–3.72 [m, 2 H, H,H-COSY: 3.75 (H-3b), 3.74 (H-5a)], 3.69–3.63 [m, 4 H, H,H-COSY: 3.68 (H-4e), 3.67 (H-2d), 3.65 (H-3d), 3.65 (H-6′c)], 3.56–3.51 [m, 2 H, H,H-COSY: 3.55 (H-6c), 3.53 (H-5b)], 3.43–3.24 [m, 7 H, H,H-COSY: 3.40 (H-6′d), 3.36 (H-6′e), 3.32 (H-5c), 3.32 (H-6e), 3.29 (H-5d), 3.27 (H-6d), 3.26 (H-6b)], 2.87 (m, 1 H, H-2c), 1.86 (s, 3 H, $COCH_3$), 1.58 (s, 3 H, $NCOCH_3$), 1.44–1.41 (m, 1 H, CH), 0.67–0.64 (m, 12 H, 2 $C(CH_3)_2$), −0.02, −0.04 (2 s, each 3 H, $Si(CH_3)_2$). Anal. Calcd for $C_{140}H_{149}NO_{32}Si·H_2O$ (2403.81): C, 69.95; H, 6.46; N, 0.58. Found: C, 69.82; H, 6.41; N, 0.48.

Thexyldimethylsilyl (2,3,4,6-tetra-O-*acetyl*-α-D-*galactopyranosyl)*-*(1→3)*-*(2,4,6*-*tri*-O-*acetyl*-β-D-*galactopyranosyl)*-*(1→4)*-*(2*-*acetamido*-*3,6*-*di*-O-*acetyl*-*2*-*deoxy*-β-D-*glucopyranosyl)*-*(1→3)*-*(4*-O-*acetyl*-*2,6*-*di*-O-*benzoyl*-β-D-*galactopyranosyl)*-*(1→4)*-*2,3,6*-*tri*-O-*benzoyl*-β-D-*glucopyranoside* (**7**).—To a soln of **6** (2.23 g, 928 μmol) in 18:6:1 MeOH–EtOAc–HOAc (50 mL) was added 10% Pd–C (450 mg). Hydrogenolysis was performed at atmospheric pressure overnight and then the mixture was filtered through Celite and concd. The residue was dissolved in a mixture of pyridine (25 mL) and Ac_2O (20 mL) and *N*,*N*-dimethylaminopyridine (20 mg) was added. After 14 h the soln was concd and co-concd with toluene (3 ×). Flash chromatography (2:1 toluene–acetone) of the residue led to **7** (1.57 g, 86%) as a colourless foam. $[\alpha]_D + 44.2°$ (*c* 1, $CHCl_3$); R_f 0.43 (3:2 toluene–acetone); ^1H NMR (600 MHz, $CDCl_3$): δ 8.08–7.27 (m, 25 H, 5 C_6H_5), 5.69 (dd, $J_{2,3} = J_{3,4}$ 9.7 Hz, 1 H, H-3a), 5.43–3.39 [m, 35 H, H,H-COSY: 5.43 (H-4e), 5.36 (H-2a), 5.33 (H-2b), 5.30 (H-4b), 5.28 (H-4d), 5.24 (H-2e), 5.21 (H-1e), 5.09 (H-3d), 5.07 (H-3e), 4.91 (d, $J_{1,2}$ 7.5 Hz, H-1a), 4.84 (H-3c, NH), 4.59 (H-1b), 4.56 (H-6′c), 4.51 (H-6′a), 4.38 (H-1c, H-1d), 4.37 (H-6a), 4.17 (H-5e), 4.16 (H-6′e), 4.05 (H-4a), 4.02 (H-6′d, H-6c, H-6e), 3.94 (H-6d), 3.81 (H-6′b), 3.80 (H-2d), 3.79 (H-3b), 3.78 (H-5a), 3.73 (H-5d), 3.73

(H-2c), 3.67 (H-4c), 3.66 (H-5b), 3.42 (H-6b), 3.40 (H-5c)], 2.13–1.94 (10 s, each 3 H, 10 $COCH_3$), 1.57 (s, 3 H, $NCOCH_3$), 1.44–1.41 (m, 1 H, CH), 0.67–0.64 (m, 12 H, 2 $C(CH_3)_2$), 0.04, −0.02 (2 s, each 3 H, $Si(CH_3)_2$). Anal. Calcd for $C_{95}H_{113}NO_{41}Si\cdot H_2O$ (1971.02): C, 57.89; H, 5.88; N, 0.71. Found: C, 57.78; H, 5.92; N, 0.79.

*(2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α/β-*D*-glucopyranose* (**8**).—A 1 M soln of TBAF (1 mL) was added to a soln of **7** (1.73 g, 878 μmol) in dry THF (30 mL) at −15 °C. The mixture was stirred for 4 h before diluted with EtOAc (150 mL) and washed with a satd soln of $NaHCO_3$ (50 mL). The aq layer was extracted with EtOAc (2 × 150 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concd. The residue, purified by flash chromatography (3:2 toluene–acetone), led to **8** (1.46 g, 91%) as a colourless foam. R_f 0.55 (1:1 toluene–acetone); ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.09–7.29 (m, 25 H, 5 C_6H_5), 6.11–3.38 (m, 36 H, H-1a, H-2a, H-3a, H-4a, H-5a, 2 H-6a, H-1b, H-2b, H-3b, H-4b, H-5b, 2 H-6b, H-1c, H-2c, H-3c, H-4c, H-5c, 2 H-6c, H-1d, H-2d, H-3d, H-4d, H-5d, 2 H-6d, H-1e, H-2e, H-3e, H-4e, H-5e, 2 H-6e, NH), 3.00 (bs, 1 H, OH), 2.13, 2.11, 2.09, 2.04, 2.04, 2.03, 1.97, 1.96, 1.95, 1.94 (10 s, each 3 H, 10 $COCH_3$), 1.55 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{87}H_{95}NO_{41}\cdot 1.5H_2O$ (1837.71): C, 56.86; H, 5.37; N, 0.76. Found: C, 56.97; H, 5.74; N, 0.59.

*(2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α/β-*D*-glucopyranosyl trichloroacetimidate* (**9**).—To a soln of **8** (352 mg, 192 μmol) in dry CH_2Cl_2 (10 mL) were added trichloroacetonitrile (200 μL, 1.9 mmol) and DBU (3 drops). The reaction mixture was stirred for 3 h and concd. Flash chromatography (66:33:1:1 toluene–acetone–MeOH–NEt_3) furnished **9** (334 mg, 88%) as a colourless foam. R_f 0.33 (2:1 toluene–acetone); ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.54 (s, 1 H, =NH), 8.08–7.29 (m, 25 H, 5 C_6H_5), 6.68 (d, $J_{1,2}$ 3.8 Hz, 1 H, H-1a), 6.11–3.38 (m, 35 H, H-2a, H-3a, H-4a, H-5a, 2 H-6a, H-1b, H-2b, H-3b, H-4b, H-5b, 2 H-6b, H-1c, H-2c, H-3c, H-4c, H-5c, 2 H-6c, H-1d, H-2d, H-3d, H-4d, H-5d, 2 H-6d, H-1e, H-2e, H-3e, H-4e, H-5e, 2 H-6e, NH), 2.13–1.94 (10 s, each 3 H, 10 $COCH_3$), 1.57 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{89}H_{95}Cl_3N_2O_{41}\cdot 2H_2O$ (1991.11): C, 53.69; H, 5.01; N, 1.41. Found: C, 53.64; H, 5.01; N, 1.37.

*Methyl (2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**10**).—To a soln of $BF_3\cdot Et_2O$ (2 μL, 16 μmol) in dry CH_2Cl_2 (0.5 mL) and dry MeOH (100 μL) was added dropwise a soln of **9** (52.0 mg, 26.6 μmol) in dry CH_2Cl_2 (0.4 mL) at rt. After 3 h the mixture was neutralized with NEt_3 and concd. Flash chromatography (66:33:1 toluene–acetone–MeOH) furnished **10** (32.6 mg, 68%) as a colourless powder after lyophilization from dioxane. $[\alpha]_D$ +47.6° (*c* 1, $CHCl_3$); R_f 0.33 (66:33:1 toluene–acetone–MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.10–7.28 (m, 25 H, 5 C_6H_5), 5.76–3.37 (m, 39 H, H-1a, H-2a, H-3a, H-4a, H-5a, 2 H-6a, H-1b, H-2b, H-3b, H-4b, H-5b, 2 H-6b, H-1c, H-2c, H-3c, H-4c, H-5c, 2 H-6c, H-1d, H-2d, H-3d, H-4d, H-5d, 2 H-6d, H-1e, H-2e, H-3e, H-4e, H-5e, 2 H-6e, NH, OCH_3), 2.13–1.94 (10 s, each 3 H, 10 $COCH_3$), 1.56 (s, 3 H, $NCOCH_3$). $C_{88}H_{97}NO_{41}$ (1824.71): MALDI-MS (positive Mode, THF): 1848 $[M + Na]^+$, 1864 $[M + K]^+$.

*Methyl (α-*D*-galactopyranosyl)-(1→3)-(β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**2**).—To a soln of **9** (20 mg, 11 μmol) in dry MeOH (3 mL) was added a catalytic amount of NaOMe. After 3 days the mixture was neutralized with Amberlite IR-120 (H^+), filtered and concd. Lyophilization from H_2O furnished **2** (10.4 mg, quant) as colourless powder. R_f 0.25 (1:1:1 EtOAc–*i*PrOH–water);

For NMR data, see Ref. [3]. $C_{33}H_{57}NO_{26}$ (883.80): MALDI-MS (positive Mode, MeOH): 904 $[M + Na]^+$.

*(2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-benzoyl-4-octadecen-1,3-diol* (**12**).—To a soln of (2*S*,3*R*,4*E*)-2-azido-3-benzoyloxy-4-octadecen-1-ol [9] (**11**; 192 mg, 447 μmol, 2 equiv) and $BF_3{\cdot}Et_2O$ (12 μL, 95 μmol, 0.4 equiv) was added dropwise a soln of **9** (437 mg, 219 μmol) in CH_2Cl_2 (1 mL), dried over 4 Å molecular sieves (140 mg), at rt. After 90 min the mixture was neutralized with NEt_3 and concd. Flash chromatography (2:1 toluene–acetone) furnished **12** (333 mg, 68%) as a colourless foam. $[\alpha]_D$ +41.1° (*c* 1, $CHCl_3$); R_f 0.40 (2:1 toluene–acetone); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.08–7.27 (m, 30 H, 6 C_6H_5), 5.77–5.71 [m, 2 H, H,H-COSY: 5.72 (dd, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3a), 5.75 (H-5)], 5.45–5.39 [m, 4 H, H,H-COSY: 5.39 (H-3), 5.41 (H-4), 5.43 (H-2a), 5.43 (H-4e)], 5.32 (dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.0 Hz, 1 H, H-2b), 5.29 (dd, $J_{3,4}$ 7.9, $J_{4,5}$ 10.0 Hz, 1 H, H-4d), 5.27 (dd, $J_{3,4}$ 3.6, $J_{4,5}$ 7.9 Hz, 1 H, H-4b), 5.24 (dd, $J_{1,2}$ 3.4, $J_{2,3}$ 11.0 Hz, 1 H, H-2e), 5.21 (d, $J_{1,2}$ 3.5 Hz, 1 H, H-1e), 5.11–5.06 [m, 2 H, H,H-COSY: 5.09 (H-2d), 5.06 (H-3e)], 4.85–4.82 [m, 2 H, H,H-COSY: 4.84 (NH), 4.83 (H-3c)], 4.73 (d, $J_{1,2}$ 7.8 Hz, 1 H, H-1a), 4.60–4.53 [m, 3 H, H,H-COSY: 4.59 (d, $J_{1,2}$ 8.0 Hz, H-1b), 4.56 (H-6′c), 4.54 (H-6′a)], 4.40 (dd, $J_{5,6}$ 4.1, J_{gem} 12.1 Hz, 1 H, H-6a), 4.36 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1d), 4.36 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1c), 4.18–4.14 [m, 3 H, H,H-COSY: 4.17 (H-5e), 4.16 (H-4a), 4.16 (H-6′e)], 4.04–3.99 [m, 3 H, H,H-COSY: 4.02 (H-6d), 4.02 (H-6′d), 4.02 (H-6e)], 3.95–3.69 [m, 9 H, H,H-COSY: 3.93 (H-6c), 3.91 (H-1′), 3.85 (H-2), 3.85 (H-6′b), 3.79 (H-5a), 3.79 (H-3d), 3.76 (H-3b), 3.73 (H-5d), 3.71 (H-2c)], 3.67 (dd, $J_{3,4} = J_{4,5}$ 6.7 Hz, 1 H, H-4c), 3.61 (t, 1 H, H-5b), 3.45–3.38 [m, 3 H, H,H-COSY: 3.43 (H-1), 3.42 (H-6b), 3.39 (H-5c)], 2.13, 2.11, 2.09, 2.04, 2.03, 1.97, 1.95, 1.95, 1.94, 1.92 (10 s, each 3 H, 10 $COCH_3$), 1.56 (s, 3 H, $NCOCH_3$), 1.30–1.18 (m, 24 H, 12 CH_2), 0.88 (t, 3 H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 138.56 (C-5), 122.66 (C-4), 101.28 (C-1a), 101.14 and 101.08 (C-1c, C-1d), 100.39 (C-1b), 93.32 (C-1e), 76.73 (C-3b), 75.34 (C-4c), 75.15 (C-4a), 74.42 (C-3), 72.92 (C-5a), 72.69 (C-3d), 72.47 (C-5c), 72.38 (C-3a), 72.24 (C-3c), 71.53 (C-5b), 71.51 (C-2a), 71.47 (C-2b), 70.64 (C-5d), 69.63 (C-2d), 69.03 (C-1), 68.80 (C-4b), 67.63 (C-4e), 67.09 (C-3e), 66.81 (C-5e), 66.36 (C-2e), 64.49 (C-4d), 64.08 (C-2), 62.21 (C-6a), 61.62 (C-6b), 61.29 (C-6e), 60.83 (C-6d), 60.72 (C-6c), 53.81 (C-2c). Anal. Calcd for $C_{112}H_{132}N_4O_{43}{\cdot}H_2O$ (2240.29): C, 60.05; H, 6.03; N, 2.50. Found: C, 60.03; H, 6.07; N, 2.55.

*(2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzoyl-β-*D*-galac-topyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-benzoyl-2-hexadecanamido-4-octadecen-1,3-diol* (**13**).—A soln of **12** (291 mg, 130 μmol) in 4:1 pyridine–water (13 mL) was saturated with hydrogen sulfide (30 min) and stirred at rt for 3 days, then concd and dried in vacuo (10^{-3} mbar, 2 h). The residue was diluted in dry CH_2Cl_2 (10 mL) and palmitic acid (67 mg, 262 μmol, 2 equiv) and *N*′-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (126 mg, 655 μmol, 5 equiv) was added. After 16 h the mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered and concd. Flash chromatography (72:28:1 toluene–acetone–MeOH) of the residue, followed by lyophilization from dioxane, afforded **13** (263 mg, 81%) as a colourless amorphous solid. $[\alpha]_D$ +29.5° (*c* 1, $CHCl_3$); R_f 0.41 (80:20:1 toluene–acetone–MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01–7.30 (m, 30 H, 6 C_6H_5), 5.75–5.71 [m, 2 H, H,H-COSY: 5.74 (H-5), 5.72 (H-3a)], 5.43 (m, 1 H, H-4e), 5.40 (dd, $J_{1,2}$ 7.8, $J_{2,3}$ 9.9 Hz, 1 H, H-2a), 5.33–5.21 [m, 8 H, H,H-COSY: 5.33 (H-4), 5.31 (H-2b), 5.28 (H-4b), 5.28 (H-4d), 5.27 (H-3), 5.24 (H-2e), 5.21 (H-1e), NH], 5.11–5.06 [m, 2 H, H,H-COSY: 5.09 (H-2d), 5.07 (H-3e)], 4.85–4.81 (m, 2 H, H-3c, NH), 4.62 (d, $J_{1,2}$ 7.8 Hz, 1 H, H-1a),

4.58 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b), 4.56 (dd, $J_{5,6}$ 2.4, J_{gem} 11.9 Hz, 1 H, H-6′c), 4.49 (d, J_{gem} 10.8 Hz, 1 H, H-6′a), 4.37–4.30 [m, 4 H, H,H-COSY: 4.36 (H-1d), 4.36 (H-1c), 4.35 (H-6a), 4.34 (H-2)], 4.18–4.13 [m, 3 H, H,H-COSY: 4.17 (H-5e), 4.15 (H-4a), 4.15 (H-6′e)], 4.05–3.98 [m, 3 H, H,H-COSY: 4.02 (H-6c), 4.02 (H-6′d), 4.01 (H-6e)], 3.93 (dd, $J_{5,6}$ 4.2, J_{gem} 11.8 Hz, 1 H, H-6d), 3.83–3.60 [m, 10 H, H,H-COSY: 3.82 (H-1′), 3.81 (H-6′b), 3.79 (H-3d), 3.76 (H-3b), 3.73 (H-5d), 3.71 (H-2c), 3.70 (H-5a), 3.66 (H-4c), 3.63 (H-1), 3.61 (H-5b)], 3.47 (dd, $J_{5,6}$ 7.0, J_{gem} 11.3 Hz, 1 H, H-6b), 3.39 (m, 1 H, H-5c), 2.12–1.35 (m, 39 H, H-6, H-6′, $NCOCH_2CH_2$, 11 $COCH_3$), 1.25–1.12 (m, 46 H, 23 CH_2), 0.89–0.86 (m, 6 H, 2 CH_3). Anal. Calcd for $C_{128}H_{164}N_2O_{44}\cdot 3H_2O$ (2488.74): C, 61.77; H, 6.88; N, 1.13. Found: C, 61.79; H, 6.63; N, 1.00.

(α-D-Galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-2-hexadecanamido-4-octadecen-1,3-diol (**1**).—To a soln of **13** (242 mg, 97.2 μmol) in dry 4:1 Me_2SO–MeOH (60 mL) was added NaOMe (210 mg, 3.89 mmol, 40 equiv). After 14 h the mixture was neutralized with Amberlite IR-120, filtered, and concd. Chromatography of the residue on RP-18 silica (1:0 to 1:1 Me_2SO–MeOH) furnished **1** (133 mg, 98%) as a colourless amorphous solid. R_f 0.30 (1:3 Me_2SO–MeOH; RP-18); For ^1H and ^{13}C NMR see Table 1. $C_{66}H_{120}N_2O_{28}$ (1389.67): FAB-MS (positive Mode, Me_2SO): 1390 $[M+H]^+$

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank Anke Friemel (Faculty of Chemistry, Konstanz) and Dr Josef Schneider (NMR Spectroscopy department of Novartis AG, Core Technology) for the structural assignments.

References

[1] D.K.C. Cooper, R. Oriol, Glycobiology in Xenotransplantation Research, in H.-J. Gabius, S. Gabius (Eds.), *Glycosciences*, Chapman & Hall, Weinheim, Germany, 1997, pp. 531–545.
[2] E.C. Hallberg, V. Strokan, T.D.H. Cairns, M.E. Breimer, B.E. Samuelsson, *Xenotransplantation*, 5 (1998) 246–256.
[3] T. Zhu, G.-J. Boons, *J. Chem. Soc., Perkin Trans. 1*, (1998) 857–861.
[4] (a) R.R. Schmidt, *Angew. Chem.*, 98 (1986) 213–236; *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235. (b) R.R. Schmidt, K.-H. Jung, Oligosaccharide Synthesis via Trichloroacetimidates, in S. Hanessian (Ed.), *Preparative Carbohydrate Chemistry*, Marcel Dekker, 1997, pp. 283–312.
[5] R. Schaubach, J. Hemberger, W. Kinzy, *Liebigs Ann. Chem.*, (1991) 607–614.
[6] L. Lay, R. Windmüller, S. Reinhardt, R.R. Schmidt, *Carbohydr. Res.*, 303 (1997) 39–49.
[7] (a) R. Windmüller, R.R. Schmidt, *Tetrahedron Lett.*, 35 (1994) 7927–7930. (b) R. Windmüller, *Dissertation*, Universität Konstanz, 1995.
[8] (a) R.R. Schmidt, P. Zimmermann, *Angew. Chem.*, 98 (1986) 722–723; *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 725–726. (b) P. Zimmermann, R. Bommer, T. Bär, R.R. Schmidt, *J. Carbohydr. Chem.*, 7 (1988) 43–452.
[9] P. Zimmermann, R.R. Schmidt, *Liebigs Ann. Chem.*, (1988) 663–667.
[10] A.E. Bäcker, S. Thorbert, O. Rakotonirainy, E.C. Hallberg, A. Olling, M. Gustavsson, B.E. Samuelsson, B. Soussi, *Glycoconjugate J.*, 16 (1999) 45–58.