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Synthesis and NMR assignments of galactosylgloboside and its β-D-GalNAc-(1→4)-α-D-Gal-linked positional isomer in a conjugatable form☆

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Abstract

Two pentasaccharides suitable for conjugation, namely 3-aminopropyl glactosylgloboside and its β-D-GalNAc-(1→4)-α-D-Gal-linked positional isomer, were synthesized from $3^{III},4^{III}$-di-*O*-unprotected globotrioside and the trichloroacetimidate of β-D-Gal-(1→3)-β-D-GalNPhth derivative. Glycosylation at both positions led to the formation of β-D-GalNPhth-(1→4)-α-D-Gal and β-D-GalNPhth-(1→3)-α-D-Gal-linked products in a ratio of 1:1 without selectivity. Complete NMR spectral assignments are also described.

Keywords: Synthesis; Galactosylgloboside; β-D-Gal-(1→3)-β-D-GalNPhth synthon; β-D-GalNAc-(1→3/4)-α-D-Gal; 3-Aminopropyl spacer

1. Introduction

Galactosylgloboside, also called stage-specific embryonic antigen-3 (SSEA-3), was found as glycosphingolipid (globopentaosyl ceramide) in human [1] and green monkey [2] kidney tissues, as well as in human teratocarcinoma [3] and seminoma cells [4]. Part of its structure, globotrioside and globotetraoside, are also epitopes recognized by antibodies of the P blood-group system and by various bacterial adhesin proteins [5–7]. Furthermore, galactosylgloboside also has the structure of the defucosylated Globo-H antigen, which has been clinically related to breast, pancreatic and stomach cancers, and therefore is of interest for therapeutic applications [8,9].

The syntheses of the glactosylgloboside [10,11] and corresponding sialylated and sulfated forms [12] have been previously reported. Here, we describe the synthesis of galactosylgloboside and its β-D-GalNAc-(1→4)-α-D-Gal-linked positional isomer with an aminopropyl spacer. These oligosaccharides will eventually be conjugated to protein carriers to investigate their immunological properties, such as antibody production and specificity and/or their cross-reactivity with other globosides, and as screening antigens to find monoclonal antibodies useful as either therapeutic or diagnosis reagents.

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2. Results and discussion

The benzylidene derivative [$PhCH(OMe)_2$/*p*-TsOH/CH_3CN] of 3-azidopropyl lactoside (**1**) [13] was conventionally benzylated (BnBr/NaH/DMF) to give **2** in 87% yield (two steps). Removal of the benzylidene protecting group (60% HOAc) to give **3**, followed by selective benzoylation at the 6-OH (BzCl/Py/CH_2Cl_2), afforded **4** in 76% yield. Glycosylation of **4** with thiogalactoside **5** [14] (NIS/TfOH) provided the globotrioside derivative **6** in a moderate yield (47%). The α-(1→4) glycosidic linkage thus formed was confirmed by NMR spectroscopy (δ_H 4.968 ppm, $J_{1,2}$ 3.2 Hz and δ_C 100.07 ppm). Removal of the isopropylidene group (1:9 90% TFA–CH_2Cl_2) furnished **7**, an acceptor with 3^{III},4^{III}-di-*O*-unprotected hydroxyl groups, in 90% yield.

β-D-Gal-(1→3)-D-GalNPhth is among the most difficult glycosidic linkages to be constructed [15]. The β-galactosylation at the 3-*O*-position of GalNPhth resulted in an extremely poor yield (< 5%) of product [16], likely due to the steric hindrance caused by the neighboring phthaloyl group. As an alternative, 2-azido-2-deoxy-galactose derivatives have commonly been used to avoid this problem [15]; however, unlike the phthaloyl group, the 2-azido group is not good for neighboring participation. As a result, further manipulation, such as reduction and phthaloyl protection [15], was required to elaborate the formation of β-linked GalNPhth glycosides through thioglycoside or trichloroacetimidate as glycosyl donors. Moreover, the synthesis of the 2-azido derivative itself on a large-scale was problematic [17]. In summary, the synthesis of β-D-Gal(1→3)-D-GalNPhth takes many steps and is very time consuming (see Scheme 1).

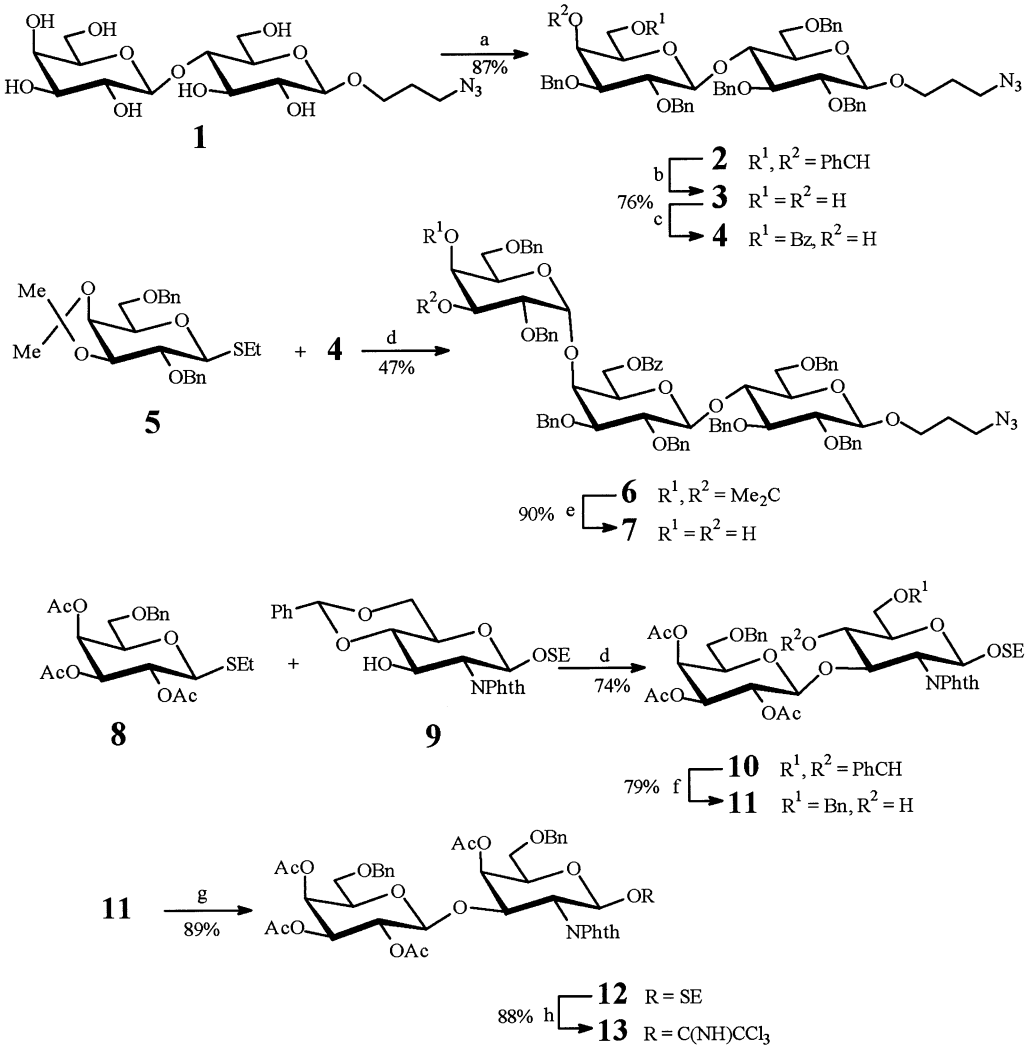
By contrast, β-D-Gal-(1→3)-D-GlcNPhth can be relatively easily synthesized. This disaccharide is also a very important building block in the synthesis of Lewis antigens (Le^a and Le^b). The chemistry of converting Glc (GlcN) to Gal (GalN) by an S_N2 reaction has been investigated [18]. Therefore, it should be possible to efficiently transform β-D-Gal-(1→3)-β-D-GlcNPhth (**11**) into β-D-Gal-(1→3)-β-D-GalNPhth (**12**). We began by synthesizing disaccharide **10** from thiogalactoside **8** as donor [19] and GlcNPhth **9** as acceptor [20] (NIS/TfOH) in excellent yield (70–80%). The reason we chose 6-*O*-benzyl-protected **8** instead of peracetylated thiogalactoside was because we wished eventually to synthesize dimeric Lewis antigens (Le^a–Le^a, Le^b–Le^a) by selective glycosylation at the 3-*O*-position of the terminal galactosyl residue. Reductive ring-opening of the benzylidene acetal of **10** ($NaCNBH_3$/HCl) afforded **11** with a 4-OH in 79% yield. The transformation of **11** into β-D-Gal-(1→3)-β-D-GalNPhth derivative **12** (89%) was then performed in two steps: (1) treatment with triflic anhydride in CH_2Cl_2/pyridine followed by (2) an S_N2 reaction with Me_4NOAc in DMF. The chemical shift of the equatorial H-4^b in **12** was assigned at δ_H 5.48 ($J_{3,4}$ 3.0 Hz). Removal of the 2-(trimethylsilyl)ethyl group in **12** (TFA/CH_2Cl_2) was quantitative, and without purification the 1-OH derivative was transformed ($CNCCl_3$/DBU) into trichloroacetimidate **13** in 88% yield (two steps).

The glycosylation reaction with *N*-phthaloyl-protected trichloroacetimidate donors have been systematically investigated and reviewed by Schmidt and Kinzy [21]. In the reaction with 3,4-di-*O*-unprotected galactose acceptors, higher reactivity was generally observed at the 3-*O*-position. As a result highly selective glycosylation was achieved by taking advantage of the differences in reactivity. However, lower regioselectivity in the glycosylation was also observed. The reaction of the 3,4-di-*O*-unprotected *O*-benzyl-galactose derivative with GlcNPhth trichloroacetimidate donor led to reaction at both positions [22]; thus β-(1→3)- and β-(1→4)-linked products were formed in a ratio of 2–3:1.

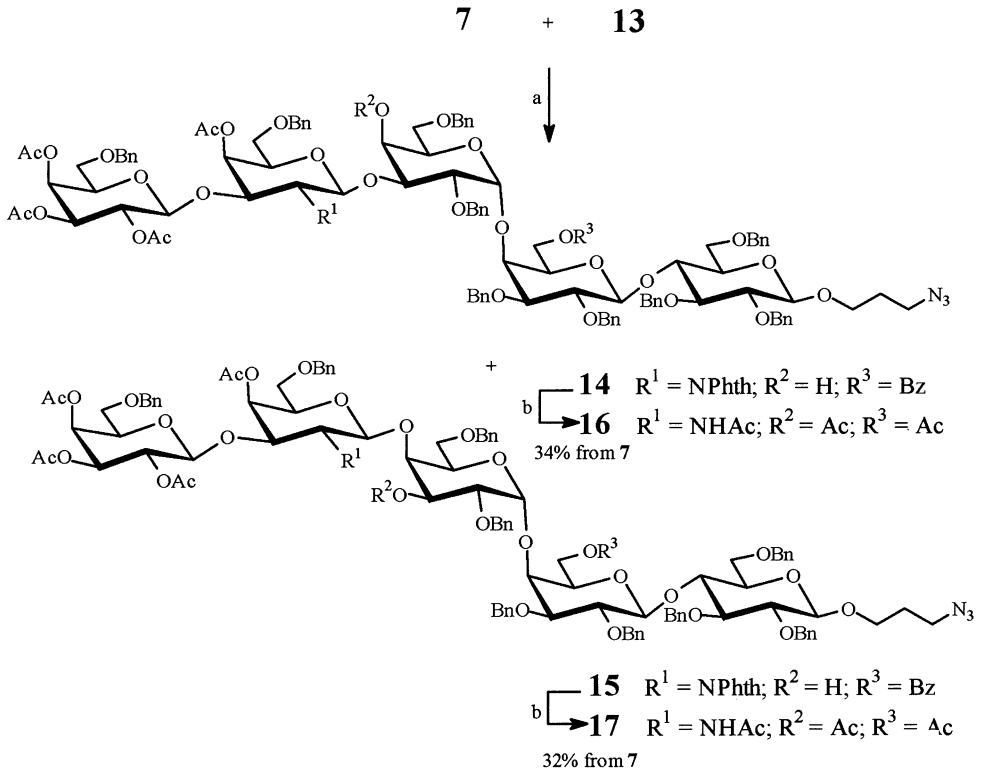
Following the standard glycosylation procedure [23], the reaction of **7** with **13** (Me_3SiOTf/CH_2Cl_2) afforded two pentasaccharide derivatives, namely a β-(1→3)-linked **14** and a β-(1→4)-linked **15** (see Scheme 2). The structures were established by FABMS and NMR spectroscopy. The two positional isomers were formed in a ratio of ca. 1:1 as estimated by TLC. However, the complete separation of the two isomers by chromatog-

raphy was very difficult, and as repeated chromatography was required, the yield was greatly sacrificed. To overcome this difficulty the mixture was treated with hydrazine hydrate to remove the *N*-phthaloyl group and the *O*-acetyl groups, and the products obtained were then fully acetylated (Ac_2O/Py) to give a mixture of **16** and **17**. The separation of **16** (34%) from **17** (32%) was then achieved by chromatography on silica gel in 66% overall yield (two steps). The structures of **16** and **17** were unambiguously assigned by 2D NMR spectroscopic analysis. A characteristic chemical shift of H-4^{III}, a broad singlet at 5.35 ppm, was observed in **16** and that of H-3^{III}, a broad doublet at 5.24 ppm, in **17**. The partial 2D ^{1}H COSY spectrum of **17** is shown in Fig. 1. Removal of protecting groups (*O*-acetyl groups and *O*-benzyl groups) was then achieved by the treatment of **16** or **17** with 0.1% NaOMe/MeOH followed by debenzylation (H_2–Pd/C) to obtain pentasaccharides **18** (79%) and **19** (75%), respectively (see Scheme 3).

Complete NMR assignments of galactosylgloboside **18** and its positional isomer **19** were achieved based on various 2D NMR spectra (COSY, NOESY, ROESY, TOCSY, HSQC and HSQC–TOCSY); the data are listed in Table 1. A comparison of both ^{1}H and ^{13}C anomeric resonances shows that only slight differences in chemical shifts are observed for GalNAc-(1 → 3/4)-α-D-Gal.



Scheme 1. Reagents and conditions: (a) i. PhCH(OMe)2/*p*-TsOH/$CNCH_3$, ii. NaH/BnBr/DMF; (b) 60% HOAc; (c) $BzCl/CH_2Cl_2$/Py at 0 °C; (d) NIS/TfOH/CH_2Cl_2 at −40 °C; (e) 10% TFA (aq 90%)/CH_2Cl_2 at 0 °C; (f) $NaCNBH_3$/HCl/THF at 0 °C; (g) i. $(TfO)_2O$/Py at 0 °C, ii. Me_4NOAc/DMF at rt; (h) i. 33% TFA/CH_2Cl_2, ii. $CNCCl_3$/DBU at 0 °C.



Scheme 2. Reagents and conditions: (a) Me_3SiOTf/CH_2Cl_2 at −40 °C; (b) i. $NH_2NH_2H_2O$/95% EtOH reflux 16 h, ii. Ac_2O/Py.

We also observed repeatedly the partial reduction of azido groups in azidopropyl glycoside derivatives to corresponding amines during the FABMS analysis. The same observation has been previously reported by Peltier et al. [24].

3. Experimental

General methods.—Optical rotations were measured at room temperature (rt) with a Perkin–Elmer 243 polarimeter, using a 10 cm, 1 mL cell. ^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, with an INOVA-500 instrument at 300 K unless otherwise noted. Chemical shifts are given in ppm relative to the signal of internal Me_4Si or indirectly to solvent signals 7.25 ($CDCl_3$) or 2.225 (acetone in D_2O) for ^1H NMR spectra, and to the solvent signals 76.9 ($CDCl_3$) or 31.07 (internal acetone) for ^{13}C NMR spectra. The ^1H NMR resonances of oligosaccharides were assigned on the basis of 2D ^1H COSY and ^1H–^{13}C chemical shift correlated experiments. FABMS analyses were performed with a Jeol JMS-AX505H mass spectrometer.

Column chromatography was performed on Silica Gel 60 (E. Merck, 230–400 mesh), and fractions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) unless otherwise noted. Detection was performed by examination under UV light and by charring with 5% H_2SO_4 in EtOH. Solutions were concentrated at or below 40 °C and dried with anhydrous Na_2SO_4.

3-Azidopropyl 2,3-di-O-*benzyl-4,6-di*-O-*benzylidene-β*-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**2**).—To a solution of 3-azidopropyl lactoside (**1**) (2.0 g, 4.51 mmol) in dry MeCN (20 mL), Ph-CH(OMe)$_2$ (1.5 mL) and *p*-toluenesulfonic acid (50 mg) were added. The mixture was stirred at rt for 6 h, neutralized by the addition of triethylamine (0.5 mL), and concentrated. The residue obtained above was dissolved in EtOAc–MeOH, and precipitated with the addition of hexane. The solid was collected and dried (2.2 g). To a solution of the above product in DMF (20 mL), NaH (50%, 1.5 g) was added. The mixture was stirred at rt for 0.5 h. Benzyl bromide (5 mL) was added to the mixture and the stirring was continued for 3 h. Methanol (2 mL) was added to the mixture to destroy excess

NaH, and then cold water (100 mL) was added. The emulsion was extracted with EtOAc (150 mL). The organic phase was subsequently washed with water, aq $NaHCO_3$, and water, dried and concentrated to a residue. Purification by chromatography (1:3 EtOAc–hexane) gave **2** (3.8 g, 87%) as a wax: $[\alpha]_D$ +10.4° (*c* 2.2, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.869 (m, 2 H, $CH_2CH_2N_3$), 4.011 (d, 1 H, H-4′, $J_{3,4}$ 3.1 Hz), 4.364 (d, 1 H, H-1, $J_{1,2}$ 8.8 Hz), 4.438 (d, 1 H, H-1′, $J_{1,2}$ 7.9 Hz), 5.443 (s, 1 H, PhC*H*), 7.152–7.495 (m, 30 H, 6 Ph) ppm. FABMS Calcd for $C_{57}H_{61}N_3O_{11}$: 964.1. Found: 963.5 (M). Anal. Calcd for $C_{57}H_{61}N_3O_{11}$ (964.12): C, 71.0; H, 6.4; N, 4.4. Found: C, 70.9; H, 6.2; N, 4.6.

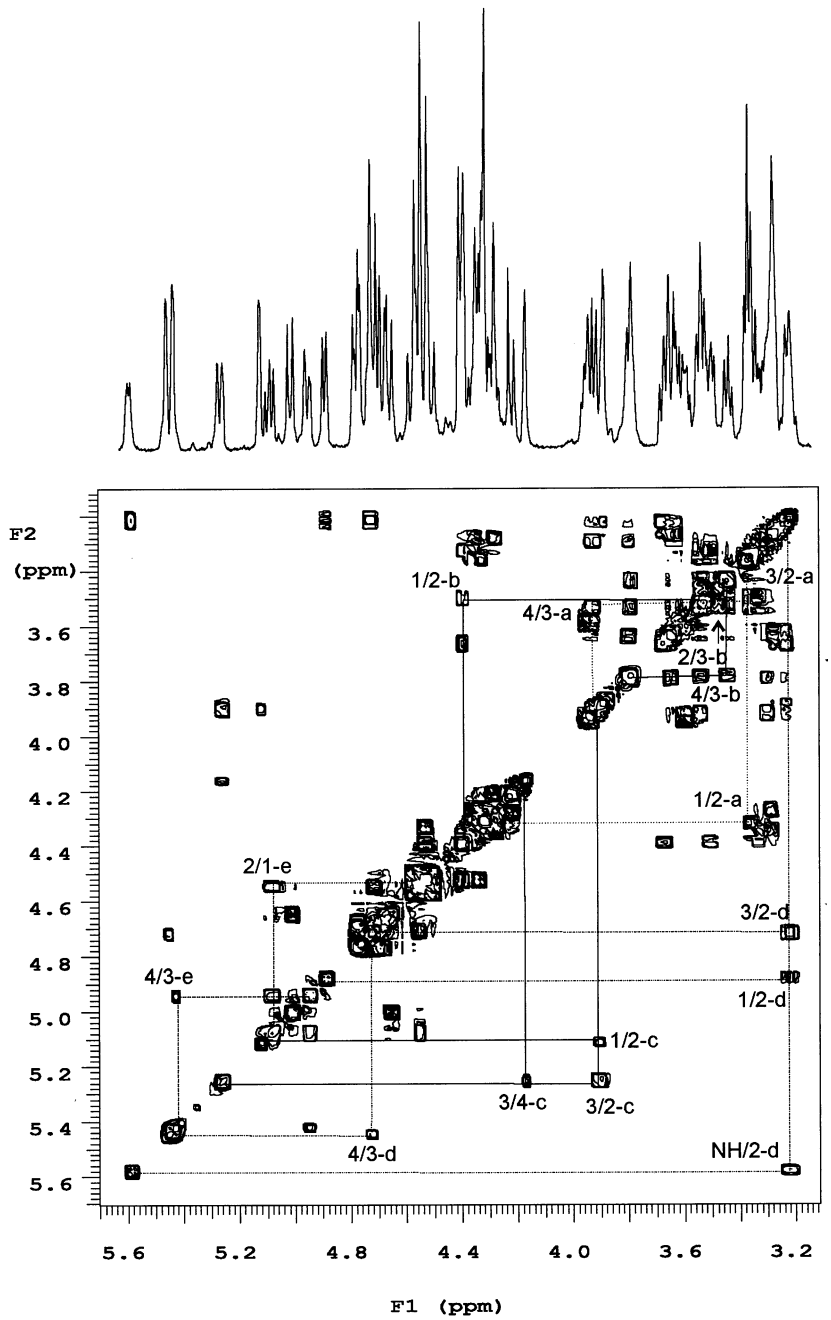
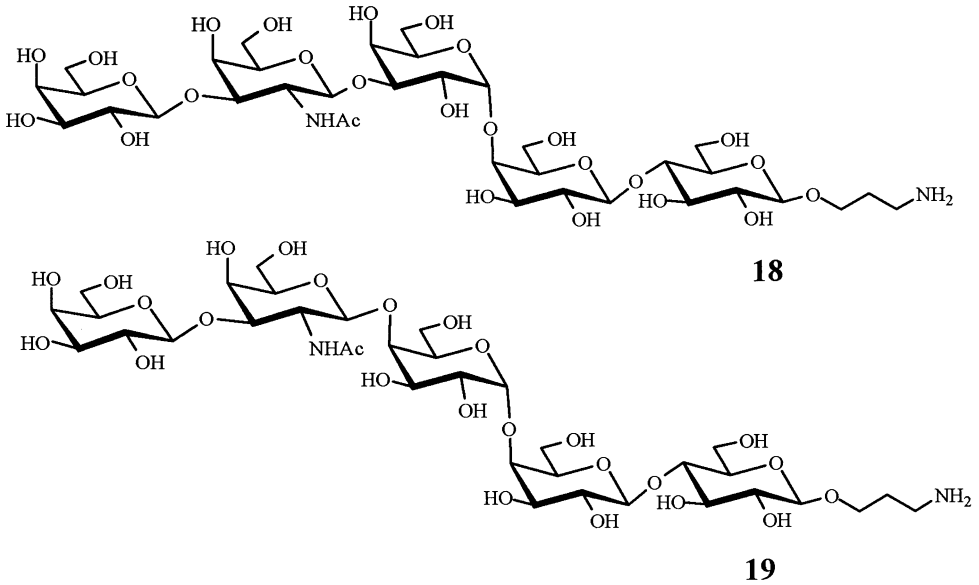


Fig. 1. Partial ^{1}H-COSY spectrum of pentasaccharide **17** recorded in $CDCl_3$ at 300 K.



18

19

Scheme 3. Galactosyl globoside **18** and its positional isomer **19**.

*3-Azidopropyl 6-*O*-benzoyl-2,3-di-*O*-benzyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**4**).—A solution of **2** (2.0 g, 2.3 mmol) in 60% HOAc was stirred at 60 °C for 1 h. The solvent was evaporated under diminished pressure to yield compound **3** as a solid. To a solution of the above residue in CH_2Cl_2 (20 mL), 2,6-lutidine (4 mL) and benzoyl chloride (0.35 mL, 2.6 mmol) were added at 0 °C. The solution was stirred for 6 h when the starting material was completely consumed, diluted with CH_2Cl_2 (100 mL), washed subsequently with water, N HCl, and water, dried and concentrated. Purification by chromatography (1:4 EtOAc–hexane) gave **4** (1.7 g, 76%) as a solid: $[\alpha]_D$ +25.6° (*c* 0.50, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.861 (m, 2 H, $CH_2CH_2N_3$), 4.268 (dd, 1 H, H-6′a, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 11.0 Hz), 4.344 (d, 1 H, H-1, $J_{1,2}$ 8.8 Hz), 4.421 (d, 1 H, H-1′, $J_{1,2}$ 7.9 Hz), 4.510 (dd, 1 H, H-6′b, $J_{5,6b}$ 6.0 Hz), 7.150–7.690 (m, 30H, 6 Ph) ppm. HRFABMS Calcd for $C_{57}H_{64}NO_{12}$ [M − N_2 + 3H]: 954.4429. Found: 954.4514. Anal. Calcd for $C_{57}H_{61}N_3O_{12}$ (980.12): C, 69.9; H, 6.3; N, 4.3. Found: C, 69.4; H, 6.5; N, 4.8.

*3-Azidopropyl 2,6-di-*O*-benzyl-3,4-di-*O*-isopropylidene-α-D-galactopyranosyl-(1→4)-6-O-benzoyl-2,3-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside* (**6**).—To a solution of **4** (1.0 g, 1.0 mmol) and **5** (1.0 g, 2.3 mmol) in CH_2Cl_2 (30 mL), powdered 4 Å molecular sieves (3.0 g) and NIS (0.6 g) were added. The mixture was stirred at rt for 1 h, then cooled to −40 °C. To the mixture TfOH (100 μL) was added and the stirring was continued at −40 °C for 1.5 h. The mixture was neutralized by the addition of 1:20 Et_3N–CH_2Cl_2 (50 mL), washed subsequently with water, N HCl, aq $Na_2S_2O_3$, and water, dried and concentrated to a residue. Purification by chromatography (1:3 EtOAc–hexane) afforded **6** (0.65 g, 47%) as a glassy mass: $[\alpha]_D$ +21.6° (*c* 0.58, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.270 and 1.277 (2s, 3 H each, CMe_2), 1.858 (m, 2 H, $CH_2CH_2N_3$), 4.317 (d, 1 H, H-1^I, $J_{1,2}$ 8.7 Hz), 4.415 (d, 1 H, H-1^{II}, $J_{1,2}$ 7.8 Hz), 4.968 (d, 1 H, H-1^{III}, $J_{1,2}$ 3.2 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 26.37 and 28.08 (CMe_2), 29.31 ($CH_2CH_2N_3$), 48.38 ($CH_2CH_2N_3$), 100.07 (C-1^{III}), 102.67 (C-1^{II}), 103.58 (C-1^I), 108.66 (CMe_2), 166.15 (Ph*C*O) ppm. FABMS Calcd for $C_{80}H_{87}N_3O_{17}$: 1362.6. Found: 1385.5 [M + Na], 1337.5 [M − N_2 + 3H].

Table 1
NMR chemical shifts[a] for galactosylgloboside **18** and its positional isomer **19**

Residue	Glycose unit	Atom	Chemical shifts (δ) in ppm[b]			
			18		**19**	
			^{1}H	^{13}C	^{1}H	^{13}C
Glc^{I}	→4)-β-D-Glc(1→	1	4.507	102.93	4.507	102.91
		$J_{1,2}$	(8.1)		(8.1)	
		2	3.34	73.70	3.34	73.72
		3	3.65	75.30	3.64	75.31
		4	3.66	79.64	3.65	79.64
		5	3.62	75.69	3.61	75.73
		6	3.83	60.92	3.82	60.93
		6′	4.00		3.99	
Glc^{II}	→4)-β-D-Gal(1→	1	4.507	104.18	4.507	104.14
		$J_{1,2}$	(8.1)		(8.1)	
		2	3.58	71.75	3.57	71.64
		3	3.75	72.99	3.75	72.94
		4	4.04	78.08	4.01	77.87
		5	3.79	76.32	3.78	76.33
		6	3.86	61.20	3.84	61.12
		6′	3.92		3.91	
Glc^{III}	→3/4)-α-D-Gal(1→	1	4.919	101.29	4.895	101.13
		$J_{1,2}$	(3.2)		(3.2)	
		2	3.92	68.46	3.71	69.70
		3	3.96	79.52	4.01	69.90
		4	4.26	69.79	4.19	77.68
		5	4.38	71.17	4.38	70.98
		6	3.70	61.27	3.66	60.81
		6′	3.70		3.80	
Glc^{IV}	→3)-β-D-GalNAc(1→	1	4.699	103.75	4.679	103.40
		$J_{1,2}$	(9.3)		(9.3)	
		2	4.05	52.36	4.05	52.48
		3	3.92	80.47	3.90	80.46
		4	4.19	68.86	4.16	68.87
		5	3.70	75.46	3.69	75.38
		6	3.74	61.86	3.74	61.90
		6′	3.78		3.79	
Glc^{V}	β-D-Gal(1→	1	4.455	105.66	4.460	105.66
		$J_{1,2}$	(8.1)		(8.1)	
		2	3.54	71.48	3.54	71.53
		3	3.63	73.35	3.63	73.31
		4	3.91	69.44	3.91	69.47
		5	3.65	75.85	3.65	75.89
		6	3.74	61.86	3.74	61.87
		6′	3.77		3.76	
$OCH_2CH_2CH_2NH_2$		OCH_2	3.82, 4.06	68.68	3.82, 4.05	68.70
		CH_2	2.03	27.55	2.01	27.65
		CH_2N	3.16	38.41	3.15	38.48
		NHAc	2.03	23.27	2.05	23.25

[a] Recorded at 500 MHz at 300 K in D_2O.
[b] First-order data.

Table 2
^{1}H NMR chemical shifts[a] (δ) in ppm for oligosaccharides **7**, **12**, **16** and **17**[b]

Saccharide	Glycose unit	H-1[c]	H-2	H-3	H-4	H-5	H-6, 6′
7	α-D-Gal(1→	5.05	3.75	3.88	3.97	4.13	3.31, 3.38
	→4)-β-D-Gal(1→	4.46	3.53	3.24	3.89	3.41	4.60, 4.67
	→4)-β-D-Glc(1→	4.35	3.37	3.58	4.01	3.35	3.70, 3.80
	$OCH_2CH_2CH_2N_3$	3.59	3.96		1.87		3.40
12	β-D-Gal(1→	4.35	4.96	4.74	5.32	3.87	3.56, 3.58
	→3)-β-D-GalNphth(1→	5.09	4.50	4.66	5.48	3.33	3.45, 3.68
	$OCH_2CH_2SiMe_3$	3.46	3.93		0.765		−0.181
16	β-D-Gal(1→	4.44	5.06	4.96	5.42	-	
	→3)-β-D-GalNAc(1→	4.87	3.82	3.92	5.45		
	→3)α-D-Gal(1→	5.14	4.00	3.66	5.35		
	→4)-β-D-Gal(1→	4.42	3.58	3.49	3.81		
	→4)-β-D-Glc(1→	4.32	3.36	3.51	4.00		
	$OCH_2CH_2CH_2N_3$	3.62	3.95		1.85		3.39
17	β-D-Gal(1→	4.55	5.07	4.93	5.43		
	→3)-β-D-GalNAc(1→	4.88	3.22	4.73	5.45		
	→4)α-D-Gal(1→	5.11	3.90	5.24	4.15		
	→4)-β-D-Gal(1→	4.40	3.51	3.44	3.79		
	→4)-β-D-Glc(1→	4.33	3.38	3.54	3.93		
	$OCH_2CH_2CH_2N_3$	3.62	3.95		1.85		3.39

[a] Recorded at 500 MHz at 300 K in $CDCl_3$.
[b] The chemical shifts of protecting groups were not listed.
[c] First-order data.

3-Azidopropyl 2,6-di-O-*benzyl*-α-D-*galacto*-*pyranosyl*-(1→4)-*6*-O-*benzoyl*-*2,3-di*-O-*benz*-*yl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*benzyl*-β-D-*glucopyranoside* (**7**).—To a solution of **6** (0.8 g, 0.59 mmol) in CH_2Cl_2 (45 mL), aq 90% TFA (5 mL) was added at 0 °C. The solution was stirred for 0.5 h, after which time the starting material had completely disappeared. The solution was subsequently washed with cold water, aq $NaHCO_3$, and water, dried and concentrated. Purification by chromatography (1:2 EtOAc–hexane) afforded **7** (0.7 g, 90%) as a syrup: $[\alpha]_D$ +37.0° (*c* 0.63, MeOH); ^{1}H NMR data are listed in Table 2 (BnO and BzO are not included). ^{13}C NMR ($CDCl_3$) δ 99.85 (C-1^{III}), 102.02(C-1^{II}), 103.10 (C-1^{I}) ppm. FABMS Calcd for $C_{77}H_{83}N_3O_{17}$: 1322.5. Found: 1345.5 [M + Na], 1297.5 [M − N_2 + 3H].

2-Trimethylsilylethyl 2,3,4-tri-O-*acetyl*-*6*-O-*benzyl*-β-D-*galactopyranosyl*-(1→3)-*4,6-di*-O-*benzylidene*-*2-deoxy*-*2-phthalimido*-β-D-*glucopyranoside* (**10**).—To a solution of **8** (1.0 g, 2.27 mmol) and **9** (1.0 g, 2.01 mmol) in CH_2Cl_2 (30 mL), powdered 4 Å molecular sieves (2.5 g) and NIS (0.65 g) were added. The mixture was stirred at rt for 1 h, then cooled to −40 °C. To the mixture, TfOH (100 μL) was added and the stirring was continued at −40 °C for 1 h. The mixture was neutralized by the addition of 1:20 Et_3N–CH_2Cl_2 (50 mL), washed subsequently with water, N HCl, aq $Na_2S_2O_3$, and water, dried and concentrated to a residue. Purification by chromatography (1:2 EtOAc–hexane) afforded **10** (1.3 g, 74%) as a solid: $[\alpha]_D$ +31.3° (*c* 0.80, MeOH); ^{1}H NMR ($CDCl_3$) δ −0.159 (s, 9 H, $SiMe_3$), 0.735 (m, 2 H, $CH_2CH_2SiMe_3$), 1.523, 1.814, 1.968 (3 s, 3 H each, 3 OAc), 4.169, 4.365 (2 d, 1 H each, CH_2Ph, *J* 11.9 Hz), 4.276 (dd, 1 H, H-2^{I}, $J_{2,3}$ 9.8 Hz), 4.525 (d, 1 H, H-1^{II}, $J_{1,2}$ 7.9 Hz), 4.684 (dd, 1 H, H-3^{I}, $J_{3,4}$ 9.1 Hz), 4.725 (dd, 1 H, H-3^{II}, $J_{2,3}$ 10.3 Hz), 4.935 (dd, 1 H, H-2^{II}), 5.147 (d, 1 H, H-1^{I}, $J_{1,2}$ 8.5 Hz), 5.257 (d, 1 H,

H-4^{II}, $J_{3,4}$ 2.7 Hz), 5.480 (s, 1 H, PhC*H*), 7.159–7.850 (m, 14 H, 2 Ph and Phth) ppm; ^{13}C NMR ($CDCl_3$) δ −0.846 ($SiMe_3$), 18.53 (CH_2Si), 20.39, 21.13, 21.30 (3 × CH_3CO), 55.56 (C-2^{I}), 63.69, 67.88, 67.96, 68.39, 69.56, 71.50, 71.76, 73.12, 74.46, 76.06, 82.66 (11 C, C-3^{I}, 4^{I}, 5^{I}, 6^{I}, 2^{II}, 3^{II}, 4^{II}, 5^{II}, 6^{II}, CH_2Ph, and OCH_2CH_2Si), 98.42 (C-1^{I}), 101.63 (C-1^{II}), 101.83 (Ph*C*H), 124.20–137.69 (Ph, Phth), 169.43, 170.64, 171.86 (3 OAc) ppm. HRFABMS Calcd for $C_{45}H_{53}NO_{15}SiNa$ [M + Na]: 898.3082. Found: 898.3054. Anal. Calcd for $C_{45}H_{53}NO_{15}Si$ (875.99): C, 61.7; H, 6.1; N, 1.6. Found: C, 61.4; H, 5.9; N, 1.7.

2-Trimethylsilylethyl 2,3,4-tri-O-*acetyl-6*-O-*benzyl-β-*D-*galactopyranosyl-*(1→3)-*6*-O-*benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranoside* (**11**).—To a mixture of sodium cyanoborohydride (0.55 g), powdered 3 Å molecular sieves (2.0 g) and compound **10** (1.0 g, 1.14 mmol) in THF (20 mL), a saturated solution of HCl in ethyl ether was added dropwise at 0 °C until the mixture became acidic (pH 3). The mixture was further stirred for another 2 h, when TLC indicated completion of the reaction. The mixture was diluted with EtOAc (80 mL) and filtered through Celite. The filtrate was subsequently washed with water, aq $NaHCO_3$, and water, dried and concentrated to a residue. Purification by chromatography (2:3 EtOAc–hexane) gave **11** (0.79 g, 79%) as a solid: $[\alpha]_D$ −7.5° (*c* 1.18, MeOH); ^{1}H NMR ($CDCl_3$) δ −0.241 (s, 9 H, $SiMe_3$), 0.747 (m, 2 H, $CH_2CH_2SiMe_3$), 1.398, 1.967, 2.020 (3s, 3 H each, 3 OAc), 4.213 (dd, 1 H, H-2^{I}, $J_{2,3}$ 10.8 Hz), 4.394 (d, 1 H, H-1^{II}, $J_{1,2}$ 8.1 Hz), 4.601 (s, 2 H, CH_2Ph), 4.770 (dd, 1 H, H-3^{II}, $J_{2,3}$ 10.3 Hz), 5.027 (d, 1 H, H-1^{I}, $J_{1,2}$ 8.5 Hz), 5.089 (dd, 1 H, H-2^{II}), 5.292 (d, 1 H, H-4^{II}, $J_{3,4}$ 3.2 Hz), 7.193–7.848 (m, 14 H, 2 Ph and Phth) ppm. HRFABMS Calcd for $C_{45}H_{55}NO_{15}SiNa$ [M + Na]: 900.3237. Found: 900.3265. Anal. Calcd for $C_{45}H_{55}O_{15}Si$ (878.01): C, 61.6; H, 6.3; N, 1.6. Found: C, 61.2; H, 6.0; N, 1.4.

2-Trimethylsilylethyl 2,3,4-tri-O-*acetyl-6*-O-*benzyl-β-*D-*galactopyranosyl-*(1→3)-*4*-O-*acetyl-6*-O-*benzyl-2-deoxy-2-phthalimido-β-*D-*galactopyranoside* (**12**).—To a solution of **11** (1.4 g, 1.6 mmol) in 4:1 CH_2Cl_2–pyridine (20 mL), triflic anhydride (1 mL, 5.9 mmol) was added at 0 °C. The solution was stirred at 0 °C, then at rt for 4 h, after which time TLC indicated the completion of the reaction. The yellowish solution was diluted with CH_2Cl_2 (100 mL) and washed subsequently with ice water, N HCl, and ice water again, dried and concentrated to a residue. To a solution of the above residue in DMF (10 mL), Me_4NOAc (1.1 g, 8.3 mmol) was added at rt. The mixture was stirred for 2 h, diluted with EtOAc (100 mL), washed subsequently with water, aq $NaHCO_3$, and water, dried and concentrated. Purification by chromatography (1:2 EtOAc–hexane) gave **12** (1.3 g, 89%) as a solid: $[\alpha]_D$ +4.3° (*c* 0.37, MeOH); ^{1}H NMR data are listed in Table 2 (BnO and AcO are not included). HRFABMS Calcd for $C_{47}H_{57}NO_{16}SiNa$ [M + Na]: 942.3344. Found: 942.3378. Anal. Calcd for $C_{47}H_{57}NO_{16}Si$ (920.05): C, 61.4; H, 6.2; N, 1.5. Found: C, 61.2; H, 6.3; N, 2.0.

2,3,4-Tri-O-*acetyl-6*-O-*benzyl-β-*D-*galactopyranosyl-*(1→3)-*4*-O-*acetyl-6*-O-*benzyl-2-deoxy-2-phthalimido-β-*D-*galactopyranosyl trichloroacetimidate* (**13**).—To a stirred solution of **12** (1.2 g, 1.3 mmol) in CH_2Cl_2 (20 mL), TFA (8 mL) was added. The stirring was continued at rt for 1 h, and the solution was then diluted with CH_2Cl_2 (50 mL), washed subsequently with water, aq $NaHCO_3$, and water, dried and concentrated to a residue. To a solution of the above residue and trichloroacetonitrile (1.0 mL) in CH_2Cl_2 (15 mL) at 0 °C, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (240 μL, 1.5 mmol) was added. The mixture was stirred for 2 h at 0 °C, and then concentrated. Purification by chromatography (2:3 EtOAc–hexane) gave **13** (1.1 g, 88%) as a solid: $[\alpha]_D$ +11.8° (*c* 0.70, MeOH); ^{1}H NMR ($CDCl_3$) δ 4.371 (d, 1 H, H-1^{II}, $J_{1,2}$ 8.5 Hz), 6.586 (d, 1 H, H-1^{I}, $J_{1,2}$ 9.0 Hz), 8.737 (s, 1 H, C=NH) ppm. FABMS Calcd for $C_{44}H_{45}Cl_3N_2O_{16}$: 964.2. Found: 987.2, 985.2 [M + Na], 802.3 [M − $OCNHCCl_3$].

3-Azidopropyl 2,3,4-tri-O-*acetyl-6*-O-*benzyl-β-*D-*galactopyranosyl-*(1→3)-*4*-O-*acetyl-6*-O-*benzyl-2-deoxy-2-phthalimido-β-*D-*galacto-*

pyranosyl-(1→3/4)-*2,6-di*-O-*benzyl*-α-D-*galactopyranosyl*-(1→4)-*6*-O-*benzoyl*-*2,3*-*di*-O-*benzyl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*benzyl*-β-D-*glucopyranoside* (**14**/**15**).—A mixture of **7** (330 mg, 0.25 mmol), **13** (340 mg, 0.35 mmol) and powdered 4 Å molecular sieves (1.3 g) in CH_2Cl_2 (5 mL) was stirred at rt for 1 h. The mixture was cooled to −40 °C and Me_3SiOTf (50 μL) was added. The mixture was stirred at −40 °C for 1.5 h and was then neutralized with a solution of 2,6-lutidine (0.5 mL) in CH_2Cl_2 (20 mL). The filtrate was subsequently washed with water, N HCl, and water, dried and concentrated to a residue. Purification by chromatography (1:1 EtOAc–hexane) gave **14** (10 mg), **15** (12 mg) and a mixture of **14**/**15** (0.5 g). **14**: ^{1}H NMR ($CDCl_3$) δ 4.824 (bs, 1 H, H-1^{III}), 4.960 (dd, 1 H, H-2^V, $J_{2,3}$ 9.0 Hz), 5.224 (d, 1 H, H-1^{IV}, $J_{1,2}$ 8.5 Hz), 5.346 (bs, 1 H, H-4^V), 5.438 (bs, 1 H, H-4^{IV}) ppm. FABMS Calcd for $C_{119}H_{126}N_4O_{32}$: 2124.3. Found: 2146.6 (M + Na). **15**: ^{1}H NMR ($CDCl_3$) δ 4.819 (bs, 1 H, H-1^{III}), 4.947 (dd, 1 H, H-2^V, $J_{2,3}$ 9.0 Hz), 5.152 (d, 1 H, H-1^{IV}, $J_{1,2}$ 8.5 Hz), 5.314 (bs, 1 H, H-4^V), 5.485 (bs, 1 H, H-4^{IV}) ppm. FABMS Calcd for $C_{119}H_{126}N_4O_{32}$: 2124.3. Found: 2146.6 [M + Na].

3-Azidopropyl 2,3,4-tri-O-*acetyl*-*6*-O-*benzyl*-β-D-*galactopyranosyl*-(1→3)-*2-acetamido*-*4*-O-*acetyl*-*6*-O-*benzyl*-*2-deoxy*-β-D-*galactopyranosyl*-(1→3/4)-*4/3*-O-*acetyl*-*2,6*-*di*-O-*benzyl*-α-D-*galactopyranosyl*-(1→4)-*6*-O-*acetyl*-*2,3*-*di*-O-*benzyl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*benzyl*-β-D-*glucopyranoside* (**16** and **17**).—A mixture of **14**/**15** (0.5 g, 0.24 mmol), derived above, and hydrazine hydrate (2.0 mL) in 95% EtOH (18 mL) was refluxed for 16 h. Upon cooling, the solvent was evaporated and the residue was treated with 1:1 Ac_2O–pyridine (10 mL) for 6 h. To the mixture, ice water was added and the precipitate was collected, which was then purified by chromatography (3:2 EtOAc–hexane) to give **16** (170 mg, 34%) and **17** (162 mg, 32%). ^{1}H NMR data for both **16** and **17** are listed in Table 2 (BnO and AcO are not included). **16**: $[\alpha]_D$ +13.6° (*c* 2.0, MeOH); ^{13}C NMR ($CDCl_3$) δ 99.39 (C-1^{IV}), 99.44 (C-1^{III}), 100.45 (C-1^V), 102.05 (C-1^{II}), 103.15 (C-1^I) ppm. FABMS Calcd for $C_{110}H_{126}N_4O_{32}$: 2016.2. Found: 2038.7 [M + Na], 2016.7 [M + H] 1190.7 [M − N_2 + 3H]. **17**: $[\alpha]_D$ +18.7° (*c* 2.0, MeOH); ^{13}C NMR ($CDCl_3$) δ 98.34 (C-1^{IV}), 98.53 (C-1^{III}), 99.63 (C-1^V), 102.13 (C-1^{II}), 103.07 (C-1^I) ppm. FABMS Calcd for $C_{110}H_{126}N_4O_{32}$: 2016.2. Found: 2038.7 [M + Na], 1190.7 [M − N_2 + 3H].

3-Aminopropyl β-D-*galactopyranosyl*-(1→3)-*2-acetamido*-*2-deoxy*-β-D-*galactopyranosyl*-(1→3/4)-α-D-*galactopyranosyl*-(1→4)-β-D-*galactopyranosyl*-(1→4)-β-D-*glucopyranoside* (**18** and **19**).—A solution of **16** (or **17**) (50 mg, 24.8 μmol) in 0.1% NaOMe/MeOH (3 mL) was stirred at rt for 2 h. The solution was neutralized with Dowex 50 (H^+) ion-exchange resin, and the filtrate was concentrated to a residue. A solution of above residue and 10% Pd/C (50% water, 30 mg) in 1:8:1 H_2O–MeOH–AcOH (10 mL) was subjected to hydrogenation (40 psi) for 16 h. The filtrate was concentrated under diminished pressure to remove methanol and then lyophilized to give crude products. Purification by passage through a Sephadex G-10 column using water as eluent afforded, after lyophilization, respectively **18** (18 mg, 79%) and **19** (17 mg, 75%) as solids. Both ^{1}H and ^{13}C NMR data for **18** and **19** are listed in Table 1. **18**: $[\alpha]_D$ +24.0° (*c* 0.78, H_2O); FABMS Calcd for $C_{35}H_{62}N_2O_{26}$: 926.9. Found: 949.3 [M + Na], 927.3 [M + H]. **19**: $[\alpha]_D$ +31.2° (*c* 0.98, H_2O); FABMS Calcd for $C_{35}H_{62}N_2O_{26}$: 926.9. Found: 949.3 [M + Na], 927.3 [M + H].

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