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Syntheses of glycosylamides as glycolipid analogs

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Abstract

In search of a simple synthetic access to analogs of naturally occurring glycolipids, glycosylamides have been synthesised in a two-step procedure from unprotected sugars, long-chain amines, and fatty acids. The N-glycosylation proceeded stereospecifically yielding crystalline β-glycopyranosylamines. ^{13}C NMR spectroscopy of the glycosylamines in organic solvents revealed partial anomerisation, leading to α-glycosylamines and in part to corresponding *N*-furanosides. Selective *N*-acylation of either pure β-glycosylamines or anomeric mixtures thereof with activated fatty acid led to formation of β-glycosylamides exclusively. As evidenced by NMR spectroscopy, the glycosylamides exhibited rotameric isomerism. The glycosylamides were found to be strong stimulators of the specific immune response against antigens. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Glycolipid analog; Glycosylamine; Glycosylamide; Anomerisation; Immunomodulator

1. Introduction

Numerous studies in recent years have revealed some of the functions for mammalian glycoproteins and glycolipids. Glycoconjugates play essential roles in biological processes, including cell development, cell adhesion, differentiation, regulation, infection, metastasis, immune reactions and other signalling events [1,2]. Beyond these, unique carbohydrate or glycopeptide epitopes on cellular surfaces are of great importance for the development of vaccines for the prevention or treatment of infectious [3–5] or malignant [6–9] diseases.

We were interested in the specific immunisation of mammals against selected carbohydrate antigens. In order to induce a protective immune response, it is essential to convert an epitope of low molecular weight into an antigen of high molecular weight. This is usually accomplished by covalently coupling the epitope to a protein carrier molecule [10], e.g., bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). In contrast to this well-established and widely used approach, we favoured investigating a less elaborated but more simple one based on self-assembling epitope–carrier complexes as alternative antigen delivery systems. It is well known that, due to their amphiphilic character, glycolipid molecules in aqueous systems form vesicles such as micelles and liposomes. Their polar carbohydrate head-groups are assembled and clustered on the surface of cell-like globular bodies [11]. After absorption onto the surface of macrophages, the vesicles are taken up, processed, and presented to other lymphocytes [12,13], thereby priming the immune system against non-self surface structures of bacterial, fungal, or viral pathogens, or malignant cells.

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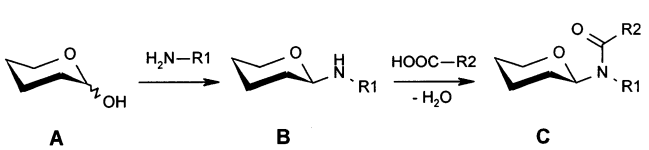
Using a flexible synthetic approach, it was our goal to prepare glycolipids with defined mono- or oligosaccharide epitopes obtained either through isolation from natural sources or from synthesis. The chemical syntheses of glycosphingolipids [14,15] and glycoglycerolipids [16] are well developed and take advantage of various efficient O-glycosylation protocols [17–20]. Nevertheless, O-glycosylation requires some preceding blocking and subsequent deblocking steps which are hard to tolerate if expensive and/or rare natural sugars are selected. We therefore favoured a more efficient and simple alternative.

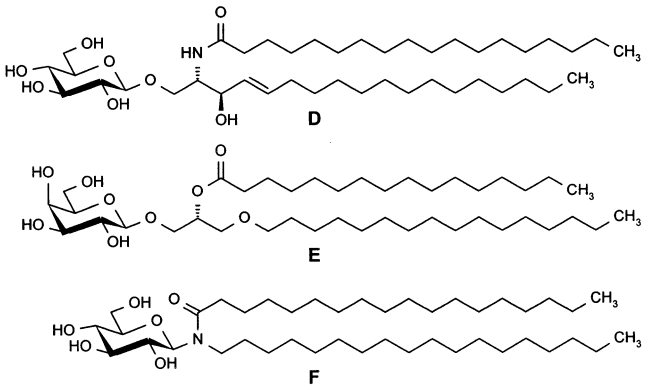
2. Results and discussion

To be flexible with respect to various carbohydrate sources we sought selective conversions of unprotected mono- or oligosaccharides at their anomeric centres. A suitable reaction seemed to be the synthesis of glycosylamines. Glycosylamines (**B**) are formed regio- and stereoselectively from reducing sugars (**A**) and primary and secondary amines in polar solvents [21] (see Scheme 1). The pyranoid structure is favoured, as well as the equatorial orientation of the amino group. The resulting glycosylamine **B** is labile towards hydrolysis. It can, however, be acylated regioselectively at the anomeric nitrogen atom, giving rise to a stable glycosylamide **C** [22]. We assumed that, if we could exploit this two-step sequence for the conversion of unprotected sugars or oligosaccharides with primary fatty amines in the first step and with activated fatty acids in the second step, we should be able to construct glycolipid-like structures **F** (glycolipid analogs, GLA) [23,24] which should mimic the naturally occurring glycosphingolipids **D** or glycerolipids **E** (see Scheme 2). Several other types of neoglycolipids have been described in the meantime [15,25].

The reaction of D-glucose and long-chain amines is known to proceed in hot alcoholic



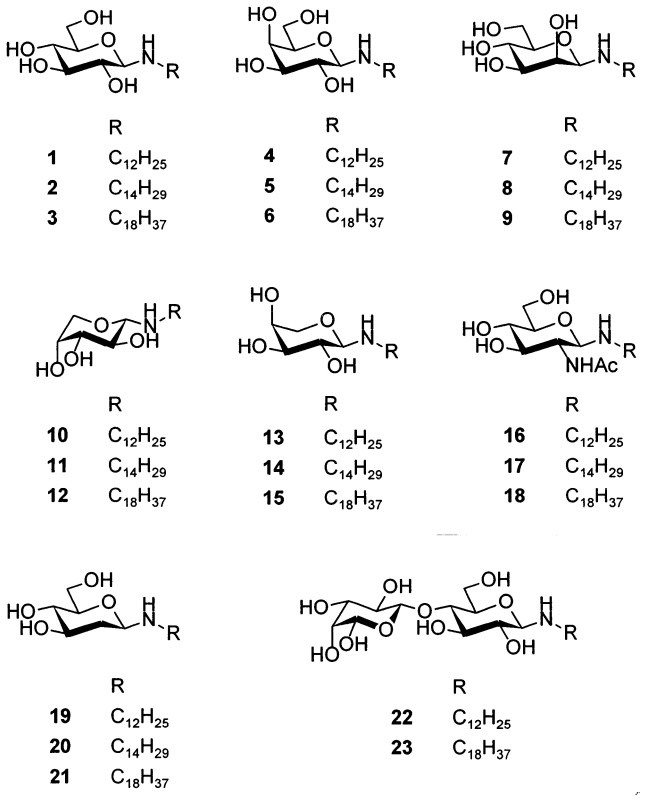
Scheme 1. Two-step synthesis of glycosylamides.



Scheme 2. Prototypes of glycosphingolipids (**D**), glycoglycerolipids (**E**), and glycosylamides (**F**).

solvents within several minutes [26–28] or at room temperature in aqueous ethanol within one to several days [29]. At higher temperatures and extended reaction times, decreased yields and browning reactions may be observed. Both versions were investigated comparatively in our study. Since acid-labile glycosylamines are partly hydrolysed on silica gel, the course of the reactions was monitored by TLC after peracetylation of a sample of the reaction mixture and comparison of the products with the corresponding peracetates of the starting monosaccharides. With D-glucose, as well as D-galactose and D-mannose, both process versions gave comparable results in our hands. The corresponding D-glucosylamines were obtained in good yields after recrystallisation, giving melting points in accordance with literature data [27,29]. The products obtained, however, gave unsatisfactory elementary analyses exhibiting higher nitrogen values than calculated. We assumed that this was probably caused by fractional coprecipitation of fatty amines—especially octadecylamine—which are only slightly soluble in ethanol or aqueous alcohol solutions. Therefore we diluted the alcoholic reaction mixtures with hexane (which is a good solvent for long-chain alkylamines) or recrystallised the crude products in the presence of hexane. The glycosylamines formed were collected as colourless crystals having melting points slightly higher than reported and giving acceptable elementary analyses.

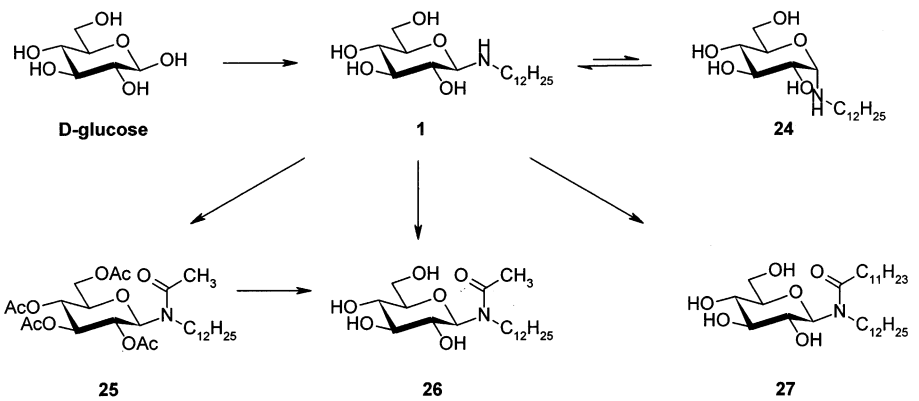
Condensation reactions of a series of simple reducing saccharides with dodecyl-, tetradecyl-, or octadecylamine were achieved and gave the expected glycosylamines **1–23** (see Scheme



Scheme 3. Structure of glycosylamines **1**–**23**.

3 and Table 1). Neutral hexoses, *N*-acetylglucosamine, and lactose were advantageously reacted at elevated temperatures according to Refs. [26,27] (method B) within several minutes. The (more-reactive) pentoses as well as 2-deoxy-D-*arabino*-hexose were transformed at room temperature [29] (methods A and C). All glycosylamines were isolated by crystallisation in good yields and with sufficient purity for further reactions. Recrystallisations from alcohol–hexane mixtures removed coprecipitated alkylamine and led to pure glycosylamines in slightly decreased yields. As may be seen from Table 1, the melting points of a series of glycosylamines were influenced more significantly by the nature of the polar headgroup than by the length of the alkyl chain. Optical rotation values within series of homologs reflected the increase in molecular weight. Irregular behaviour was observed, however, in the case of the *N*-dodecyl-D- and -L-arabinosylamines (**10** and **13**).

N-Dodecyl-β-D-glucopyranosylamine **1** is a crystalline and homogeneous compound. In various solvents, however, NMR spectra of **1** showed the existence of two complete sets of resonance signals having different intensities. In the ^{1}H NMR spectrum in CD_3OD, a significant doublet was detected at δ 3.82 with an 8.6 Hz coupling constant, indicating the predominance of the β-glycosylamine. Another significant doublet with 1/10 of intensity downfield at δ 4.49 with a 4.8 Hz coupling constant was assigned to the α-glycosylamine **24** (see Scheme 4). Comparable ^{1}H NMR data sets were also obtained from spectra recorded in pyridine-d_5. All data are in good accordance with those determined from the smaller homolog *N*-methyl-D-glucopyranosylamine [30]. ^{13}C NMR spectra of **1** after equilibration, as well as those of all other glycosylamines in either pyridine-d_5 or Me_2SO-d_6 also showed two sets of signals with different intensities (see Table 2). In all cases the β-glycopyranosylamines were the major components. In the *galacto* series, additional signals of both anomeric furanosylamines were observed. This was also noticed with D- and L-arabinosylamines, where signals could not easily



Scheme 4. Anomerisation and acylation of *N*-dodecyl-β-D-glucopyranosylamine (**1**).

Table 1
Synthesis of glycosylamines **1–23**

Compd	Config.	Method	Yield	Mp (°C)	$[\alpha]_D^{22}$ (Me_2NCHO)	Mol. formula	Anal. Calcd			Anal. Found			ESIMS (m/z)
							C	H	N	C	H	N	
1	β-D-Glc	B	89	112[a]	−14.7° (*c* 0.41)	$C_{18}H_{37}NO_5$	62.22	10.73	4.03	62.0	10.4	4.0	348.4 ($M+H^+$)
2	β-D-Glc	B	90	111[b]	−12.1° (*c* 0.66)	$C_{20}H_{41}NO_5$	63.97	11.00	3.73	63.9	10.6	3.7	
3	β-D-Glc	B	87	110[c]	−8.8° (*c* 0.64)	$C_{24}H_{49}NO_5$	66.78	11.44	3.24	66.7	11.0	3.2	
4	β-D-Gal	B	86	110	−5.7° (*c* 0.87)	$C_{18}H_{37}NO_5$	62.22	10.73	4.03	61.9	10.5	4.0	348.4 ($M+H^+$)
5	β-D-Gal	B	91	112	−4.2° (*c* 0.50)	$C_{20}H_{41}NO_5$	63.97	11.00	3.73	64.0	10.8	3.8	
6	β-D-Gal	B	85	113	−3.4° (*c* 0.63)	$C_{24}H_{49}NO_5$	66.78	11.44	3.24	67.0	11.5	3.4	
7	β-D-Man	B	88	103	−29.2° (*c* 0.67)	$C_{18}H_{37}NO_5$	62.22	10.73	4.03	62.2	10.6	3.9	348.4 ($M+H^+$)
8	β-D-Man	B	80	103	−26.9° (*c* 0.69)	$C_{20}H_{41}NO_5$	63.97	11.00	3.73	63.8	10.9	3.8	
9	β-D-Man	B	87	105	−21.1° (*c* 1.02)	$C_{24}H_{49}NO_5$	66.78	11.44	3.24	66.6	11.2	3.1	
10	α-D-Ara	A	66	65	−12.5° (*c* 0.56)	$C_{17}H_{35}NO_4$	64.32	11.11	4.41	64.6	11.0	4.4	318.4 ($M+H^+$)
11	α-D-Ara	A	72	68	+3.5° (*c* 0.57)	$C_{19}H_{39}NO_4$	66.05	11.38	4.05	66.2	11.6	4.2	
12	α-D-Ara	A	69	73	+3.1° (*c* 0.78)	$C_{23}H_{47}NO_4$	68.78	11.80	3.49	68.7	11.8	3.5	
13	β-L-Ara	A	73	65	+12.1° (*c* 0.54)	$C_{17}H_{35}NO_4$	64.32	11.11	4.41	64.5	11.2	4.5	318.4 ($M+H^+$)
14	β-L-Ara	A	69	68	−3.5° (*c* 0.46)	$C_{19}H_{39}NO_4$	66.05	11.38	4.05	66.2	11.4	4.2	
15	β-L-Ara	A	76	72	−2.7° (*c* 0.37	$C_{23}H_{47}NO_4$	68.78	11.80	3.49	68.5	11.6	3.6	
16	β-D-GlcNAc	B	62	127	+12.8° (*c* 0.68)	$C_{20}H_{40}N_2O_5$	61.83	10.38	7.21	62.0	10.5	7.1	
17	β-D-GlcNAc	B	69	128	+12.5° (*c* 0.47)	$C_{22}H_{44}N_2O_5$	63.43	10.65	6.72	63.6	10.8	6.8	
18	β-D-GlcNAc	B	77	129	+3.9° (*c* 0.55)	$C_{26}H_{52}N_2O_5$	66.06	11.09	5.93	66.1	11.2	5.8	
19	β-D-Glc2H	C	72	97	−10.6° (*c* 0.52)	$C_{18}H_{37}NO_4$	65.22	11.25	4.23	65.6	11.1	4.1	332.4 ($M+H^+$)
20	β-D-Glc2H	C	78	97	−10.1° (*c* 0.51)	$C_{20}H_{41}NO_4$	66.81	11.49	3.90	66.7	11.6	4.0	
21	β-D-Glc2H	C	76	98	−7.9° (*c* 0.52)	$C_{24}H_{49}NO_4$	69.35	11.88	3.37	69.4	12.0	3.4	
22	β-Lacto	B	54	121[d]	+1.2° (*c* 0.84)	$C_{24}H_{47}NO_{10}$	56.56	9.30	2.75	56.8	9.5	2.9	
23	β-Lacto	B	42	123[e]	+0.8° (*c* 0.63)	$C_{30}H_{59}NO_{10}$	60.68	10.02	2.36	61.0	10.3	2.6	

[a] Lit. 107–109 °C [29].
[b] Lit. 103–106 °C [45].
[c] Lit. 106–107 °C [26].
[d] Lit. 117–119 °C [27].
[e] Lit. 119–121.5 °C [29].

Table 2
Selected ^{13}C NMR chemical shifts (δ, ppm) for *N*-dodecylglycosylamines **1**, **4**, **7**, **16**, and **19** (100.6 MHz)[a]

Compd.	Solv.	Isom.	C-1	C-2	C-3	C-4	C-5	C-6	N-CH_2–	CO
1	CD_3OD	β-D-Glc*p*	91.90	75.01	79.00	71.90	79.00	63.00	47.28	
1	Me_2SO-d_6	β-D-Glc*p*	93.95	76.65	80.75	73.65	80.58	64.51	48.73	
	Me_2SO-d_6	α-D-Glc*p*	90.08	74.09	76.87	73.79	74.74	64.26	49.08	
1	Pyr-d_5	β-D-Glc*p*	92.29	75.35	79.34	72.09	79.34	63.13	46.68	
	Pyr-d_5	α-D-Glc*p*	88.70	72.73	75.90	72.65	73.27	63.33	47.17	
4	Pyr-d_5	β-D-Gal*p*	92.84	72.86	76.06	70.65	77.71	62.73	46.66	
	Pyr-d_5	α-D-Gal*p*	88.98	70.11	71.21	71.19	[b]	62.87	[b]	
	Pyr-d_5	β-D-Gal*f*	92.43	78.25	[b]	84.13	[b]	64.65	[b]	
	Pyr-d_5	α-D-Gal*f*	97.35	81.81	78.63	84.74	[b]	64.73	[b]	
7	Pyr-d_5	β-D-Man*p*	89.09	73.23	76.74	68.59	79.38	65.52	46.04	
	Pyr-d_5	α-D-Man*p*	89.85	73.43	73.31	70.02	73.70	63.64	46.04	
16	Pyr-d_5	β-D-GlcNAc*p*	91.36	57.11	77.41	72.94	78.98	63.10	46.10	171.48
	Pyr-d_5	α-D-GlcNAc*p*	86.28	55.39	72.94	72.57	73.62	63.23	46.25	170.74
19	Pyr-d_5	β-D-*arabino*-Hex	87.64	41.65	73.45	74.05	79.18	63.43	46.11	
	Pyr-d_5	α-D-*arabino*-Hex	84.95	39.27	70.00	72.72	74.71	63.56	46.07	

[a] Shifts of sugar *C*-atoms were assigned according to [31]. Methylene and methyl signals are not given. Isomers were detected after mutarotation.
[b] Not resolved.

be assigned to individual C-atoms (data not given). When a ^{13}C NMR spectrum of **1** was recorded immediately after dissolution of the sample, distinct signals of the β-glycosylamine were observed exclusively. All ^{13}C NMR resonances could be completely assigned and are in good accord with data for β- and α-glycosides [31].

Since the formation of glycosylamines is reported to proceed stereospecifically, the occurrence of the α-glycosylamines must have resulted from mutarotation. This isomerisation of glycosylamines is well known [21,27]. In order to prove this assumption also for lipophilic glycosylamines, we recorded the change of optical rotation of **1** as a function of time, reflecting the kinetics of the isomerisation. As may be seen from Table 3, there was a time- and solvent-dependent increase in the optical rotation of **1**, showing a slow interconversion of the β-glycosylamine **1** into the α-glycosylamine **24**.

The nucleophilicity of the N-atom of glycosylamines can be exploited for regioselective acylation to yield a glycosylamide. When an alcoholic solution of **1** was treated with acetic anhydride, the glycosylamide **26** was obtained (Scheme 4). Compound **26** was also obtained after treatment of **1** with acetic anhydride and pyridine, giving **25**, and subsequent O-deacetylation. Both samples of **26** were homogeneous by TLC and gave identical ^1H and ^{13}C NMR spectra indicating mixtures of two compounds. Two doublets with coupling constants >8 Hz (δ 5.41: $J_{1,2}=8.4$ Hz and δ 6.50: $J_{1,2}=9.4$ Hz) were found at low field indicating the equatorial configuration. No other doublet with smaller coupling constants, characteristic of an α-glycosylamide, could be detected. The ^{13}C NMR spectra also showed two sets of resonances. All signals were attributable [24,32–35] to Z/E isomers of β-D-glucosylamides. According to the empirical rules [34] for the configurational assignment of *N*-acylamino sugars, the *E*-*anti*-conformers of **25** and **26** were the major isomers in Me_2SO-d_6 and pyridine-d_5 solution. As expected, the ^{13}C NMR spectrum of **26** in Me_2SO-d_6 at room temperature showed that the characteristic carbohydrate signal sets well separated at 300 K, whereas at 333 K they all coalesce.

Since all of our glycosylamide syntheses were performed prior [23] to the aforementioned high-resolution NMR studies on the Z/E isomerisms, we sought authentic reference samples of the α-anomers of **25** or **26** for comparison purposes. Therefore, we planned to acetylate **1** in pyridine solution after a 24-h equilibration time. After that time, equilibrium of the α,β-glycosylamines was

Table 3
Change of optical rotation $[\alpha]_D$ of **1** (0.05 g/mL) in pyridine and *N*,*N*-dimethylformamide

Time (h)	0	1	3	5	7	24
Pyridine (°)	−19.4	−11.4	−2.1	1.8	2.4	3.5
DMF (°)	−14.7	−14.3	−13.4	−12.1	−11.3	−3.1

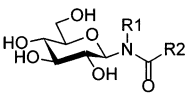
reached, since the optical rotation value did not show any further change (see Table 3). A substantial amount ($\sim 10\%$ as indicated by signal intensities of ^{13}C NMR spectra) of α-D-glucopyranosylamine **24** was formed through mutarotation. We planned to acetylate this anomeric equilibrium mixture and to isolate the α anomer by chromatography, but were unsuccessful in obtaining the α product. Both ^1H and ^{13}C NMR spectra of the peracetylation product obtained from anomeric *N*-glycosylamine mixtures were identical with those obtained previously from anomerically pure β-glycosylamine **1**, and gave no hint for any accompanying diastereomer. This was surprising, since we had expected an α-glycosylamide to be formed from the corresponding α-glycosylamine **24** in solution. Both N-acylation reactions yielding the β-glycosylamide **25** must therefore have proceeded with the same stereospecificity, irrespective of the starting material. It may be assumed that the reanomerisation of the anomeric mixture to give the β-glycosylamine must have proceeded faster than the subsequent N-acylation.

In order to obtain a prototype of a glycosylamide with two long alkyl chains as a mimic of natural glycolipids, compound **1** was treated with activated dodecanoic acid derivatives. The N-acylation could be carried out with dodecanoyl chloride or mixed anhydrides of the dodecanoic acid and alkyl chloroformates. The reaction of **1** with ethyl dodecanoyl carbonate gave the glycosylamide **27** stereospecifically and in good yield (Scheme 4). Both ^1H and ^{13}C NMR spectra established **27** to be a homologue of **26**. No anomeric α-glycosylamide could be detected, but again the existence of two amide rotamers was noted. As expected [34], the *E-anti* conformation was favoured over *Z-anti* in pyridine-d_5 solution.

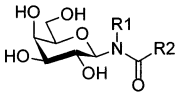
After having elaborated the fundamental chemistry of fatty glycosylamides, we intended to synthesise analogs varying in the carbohydrate moiety as well as in the lipid part. The variation of carbohydrates used should clarify the scope and limitations of the procedures with respect to the polar region. The lipophilic part was also of interest for structural modifications. We considered the lipid part of the molecules as not only being a 'greasy patch' for membrane anchoring, but also of importance for the orientation of the sugar component relative to the lipid surface. On the simple assumption that two alkyl chains of different length aim for maximum overlap of their hydrophobic regions in aqueous systems or for the most dense packing in lipid membranes, this requirement should influence the conformation of the linking amide region in the glycosylamides and thereby the spatial presentation of the carbohydrate. A more profound proof of this hypothesis has recently been described in the glycosphingolipid series, where an aglycone modulation of glycolipid receptor function was demonstrated [36]. We therefore planned to synthesise glycosylamides with two different chain-lengths, both varying from 12 to 18 carbon atoms.

Selective N-acylation of the glycosylamines **1**–**23** with fatty acid chlorides or mixed anhydrides from ethyl chloroformate gave the expected glycosylamides **27**–**80** (see Scheme 5 and Table 4). Reaction yields were generally very good but isolated yields were lower because of incomplete crystallisations. In all cases, the glycosylamides with equatorial anomeric amide substituent were obtained directly by crystallisation from the reaction mixtures. In the D-*manno* series, however, we could only isolate the corresponding α-mannosylamides, such as **46**, in low amounts by chromatography of the mother liquors.

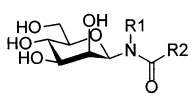
^{13}C NMR spectra of the β-glycosylamides were in accordance with their structures (see Table 5). Two signal sets were observed for each compound indicating *E*/*Z* isomers [34].



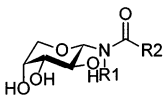
	R1	R2
27	$C_{12}H_{25}$	$C_{11}H_{23}$
28	$C_{12}H_{25}$	$C_{17}H_{35}$
29	$C_{14}H_{29}$	$C_{11}H_{23}$
30	$C_{14}H_{29}$	$C_{17}H_{35}$
31	$C_{18}H_{37}$	$C_{11}H_{23}$
32	$C_{18}H_{37}$	$C_{13}H_{27}$
33	$C_{18}H_{37}$	$C_{17}H_{35}$



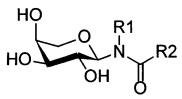
	R1	R2
34	$C_{12}H_{25}$	$C_{11}H_{23}$
35	$C_{12}H_{25}$	$C_{17}H_{35}$
36	$C_{14}H_{29}$	$C_{11}H_{23}$
37	$C_{14}H_{29}$	$C_{17}H_{35}$
38	$C_{18}H_{37}$	$C_{11}H_{23}$
39	$C_{18}H_{37}$	$C_{13}H_{27}$
40	$C_{18}H_{37}$	$C_{17}H_{35}$



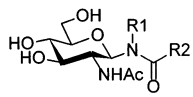
	R1	R2
41	$C_{12}H_{25}$	$C_{11}H_{23}$
42	$C_{12}H_{25}$	$C_{17}H_{35}$
43	$C_{14}H_{29}$	$C_{11}H_{23}$
44	$C_{14}H_{29}$	$C_{17}H_{35}$
45	$C_{18}H_{37}$	$C_{11}H_{23}$
47	$C_{18}H_{37}$	$C_{13}H_{27}$
48	$C_{18}H_{37}$	$C_{17}H_{35}$



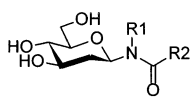
	R1	R2
49	$C_{12}H_{25}$	$C_{11}H_{23}$
50	$C_{12}H_{25}$	$C_{17}H_{35}$
51	$C_{14}H_{29}$	$C_{11}H_{23}$
52	$C_{14}H_{29}$	$C_{17}H_{35}$
53	$C_{18}H_{37}$	$C_{11}H_{23}$
54	$C_{18}H_{37}$	$C_{13}H_{27}$
55	$C_{18}H_{37}$	$C_{17}H_{35}$



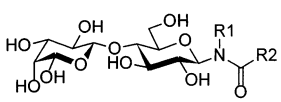
	R1	R2
56	$C_{12}H_{25}$	$C_{11}H_{23}$
57	$C_{12}H_{25}$	$C_{17}H_{35}$
58	$C_{14}H_{29}$	$C_{11}H_{23}$
59	$C_{14}H_{29}$	$C_{17}H_{35}$
60	$C_{18}H_{37}$	$C_{11}H_{23}$
61	$C_{18}H_{37}$	$C_{13}H_{27}$
62	$C_{18}H_{37}$	$C_{17}H_{35}$



	R1	R2
63	$C_{12}H_{25}$	$C_{11}H_{23}$
64	$C_{12}H_{25}$	$C_{17}H_{35}$
65	$C_{14}H_{29}$	$C_{11}H_{23}$
66	$C_{14}H_{29}$	$C_{17}H_{35}$
67	$C_{18}H_{37}$	$C_{11}H_{23}$
68	$C_{18}H_{37}$	$C_{13}H_{27}$
69	$C_{18}H_{37}$	$C_{17}H_{35}$



	R1	R2
70	$C_{12}H_{25}$	$C_{11}H_{23}$
71	$C_{12}H_{25}$	$C_{17}H_{35}$
72	$C_{14}H_{29}$	$C_{11}H_{23}$
73	$C_{14}H_{29}$	$C_{17}H_{35}$
74	$C_{18}H_{37}$	$C_{11}H_{23}$
75	$C_{18}H_{37}$	$C_{13}H_{27}$
76	$C_{18}H_{37}$	$C_{17}H_{35}$



	R1	R2
77	$C_{12}H_{25}$	$C_{11}H_{23}$
78	$C_{12}H_{25}$	$C_{17}H_{35}$
79	$C_{18}H_{37}$	$C_{11}H_{23}$
80	$C_{18}H_{37}$	$C_{17}H_{35}$

Scheme 5. Structure of glycosylamides **27–45** and **47–80**.

The *E*-conformers were the most frequently encountered isomers in pyridine-d_6. It is noteworthy that the β-mannosylamides exist as two amide rotamers, whereas in the α-mannosyl series only one isomer was observed.

The physicochemical properties of selected glycosylamides have been studied. They form liposomes in aqueous systems quite readily [37]. Their aggregation properties have been studied in detail [38,39], as well as their biological activities. In contrast to our initial assumptions, the GLA did not behave as antigens but as immunomodulators. In various in vitro and in vivo models, candidates could be identified which stimulated antibody production against various soluble or particulate antigens, such as ovalbumin or sheep red blood cells (SRBC) in mice experiments (adjuvant activity) [40,41]. Interestingly, GLA of the aforementioned type have a stimulating effect on lymphocytes in vitro, while natural glycolipids from the glycosphingolipid class suppress the response of lymphocytes to antigens in vitro [42]. Unlike other immunomodulators such as lipid A, lipopeptides or muramyl peptides [43,44], GLA exhibit no mitogenic activity by themselves, but act on B lymphocytes only in conjunction with a specific secondary antigenic stimulus. This effect can be induced independently of the presence of T cells. GLA trigger a still unknown mechanism that is essential for the differentiation and proliferation of activated B lymphocytes. These results were encouraging enough to start a medicinal chemistry program for further optimisation of these mentioned lead compounds, and results will be reported elsewhere.

Table 4
Synthesis of glycosylamides **27–45** and **47–80**

Compd	Start. mater.	Yield (%)	Mp (°C)	$[\alpha]_D^{22}$ (THF)	Mol. formula	Anal. Calcd			Anal. Found		
						C	H	N	C	H	N
27	**1**	75	Syrup	+8.9° (*c* 0.85)	$C_{30}H_{59}NO_6$	68.01	11.22	2.64	68.2	11.3	2.6
28	**1**	69	Syrup	+8.1° (*c* 1.49)	$C_{36}H_{71}NO_6$	70.43	11.66	2.28	70.4	11.5	2.3
29	**2**	65	51	+8.4° (*c* 0.81)	$C_{32}H_{63}NO_6$	68.90	11.38	2.51	69.0	11.4	2.4
30	**2**	74	63	+8.2° (*c* 0.91)	$C_{38}H_{75}NO_6$	71.09	11.77	2.18	71.2	11.9	2.1
31	**3**	72	72	+8.1° (*c* 1.02	$C_{36}H_{71}NO_6$	70.43	11.66	2.28	70.7	11.6	2.4
32	**3**	77	75	+7.3° (*c* 1.01)	$C_{38}H_{75}NO_6$	71.09	11.77	2.18	71.2	11.8	2.2
33	**3**	78	83	+6.0° (*c* 0.89)	$C_{42}H_{83}NO_6$	72.26	11.98	2.01	72.1	11.9	2.0
34	**4**	55	75	+12.4° (*c* 0.54)	$C_{30}H_{59}NO_6$	68.01	11.22	2.64	68.2	11.1	2.5
35	**4**	69	78	+12.3° (*c* 0.79)	$C_{36}H_{71}NO_6$	70.43	11.66	2.28	70.5	11.8	2.1
36	**5**	61	82	+12.2° (*c* 0.71)	$C_{32}H_{63}NO_6$	68.90	11.38	2.51	68.9	11.5	2.3
37	**5**	63	88	+9.8° (*c* 0.66)	$C_{38}H_{75}NO_6$	71.09	11.77	2.18	71.0	11.9	2.3
38	**6**	68	78	+6.3° (*c* 0.80)	$C_{36}H_{71}NO_6$	70.43	11.66	2.28	70.6	11.5	2.3
39	**6**	72	92	+8.7° (*c* 0.47)	$C_{38}H_{75}NO_6$	71.09	11.77	2.18	71.3	11.6	2.3
40	**6**	81	92	+7.9° (*c* 0.53)	$C_{42}H_{83}NO_6$	72.26	11.98	2.01	72.1	12.0	2.1
41	**7**	62	79	+12.1° (*c* 0.94)	$C_{30}H_{59}NO_6$	68.01	11.22	2.64	68.2	11.1	2.7
42	**7**	79	75	+10.5° (*c* 0.44)	$C_{36}H_{71}NO_6$	70.43	11.66	2.28	70.3	11.7	2.1
43	**8**	72	82	+13.4° (*c* 0.44)	$C_{32}H_{63}NO_6$	68.90	11.38	2.51	69.1	11.4	2.4
44	**8**	82	77	+9.5° (*c* 0.59)	$C_{38}H_{75}NO_6$	71.09	11.77	2.18	71.3	11.6	2.3
45	**9**	64	80	+11.7° (*c* 0.57)	$C_{36}H_{71}NO_6$	70.43	11.66	2.28	70.7	11.6	2.4
47	**9**	56	91	+10.9° (*c* 0.33)	$C_{38}H_{75}NO_6$	71.09	11.77	2.18	71.3	11.9	2.2
48	**9**	91	81	+6.0° (*c* 0.69)	$C_{42}H_{83}NO_6$	72.26	11.98	2.01	72.2	11.9	2.2
49	**10**	55	64	−26.4° (*c* 0.49)	$C_{29}H_{57}NO_5$	69.70	11.50	2.80	69.4	11.7	2.6
50	**10**	66	72	−25.0° (*c* 0.58)	$C_{35}H_{69}NO_5$	71.99	11.91	2.40	72.2	12.0	2.5
51	**11**	58	68	−24.8° (*c* 0.58)	$C_{31}H_{61}NO_5$	70.54	11.65	2.65	70.8	11.5	2.6
52	**11**	77	89	−22.6° (*c* 0.48)	$C_{37}H_{73}NO_5$	72,62	12.02	2.29	72.5	12.2	2.5
53	**12**	69	69	−27.1° (*c* 0.63)	$C_{35}H_{69}NO_5$	71.99	11.91	2.40	72.1	12.2	2.6
54	**12**	72	78	−24.9° (*c* 0.50)	$C_{37}H_{73}NO_5$	72.62	12.02	2.29	72.5	12.2	2.4
55	**12**	81	89	−11.7° (*c* 0.54)	$C_{41}H_{81}NO_5$	73.71	12.22	2.10	73.6	12.4	2.4
56	**13**	49	64	+27.4° (*c* 0.54)	$C_{29}H_{57}NO_5$	69.70	11.50	2.80	70.0	11.8	2.6
57	**13**	69	70	+25.2° (*c* 0.69)	$C_{35}H_{69}NO_5$	71.99	11.91	2.40	71.7	11.8	2.5
58	**14**	52	67	+24.8° (*c* 0.50)	$C_{31}H_{61}NO_5$	70.54	11.65	2.65	70.4	11.4	2.8
59	**14**	75	88	+22.5° (*c* 0.50)	$C_{37}H_{73}NO_5$	72.62	12.02	2.29	72.8	12.2	2.2
60	**15**	58	68	+26.7° (*c* 0.66)	$C_{35}H_{69}NO_5$	71.99	11.91	2.40	72.2	12.1	2.3
61	**15**	66	77	+25.1° (*c* 0.46)	$C_{37}H_{73}NO_5$	72.62	12.02	2.29	72.5	11.8	2.5
62	**15**	78	88	+12.8° (*c* 0.48)	$C_{41}H_{81}NO_5$	73.71	12.22	2.10	73.6	12.4	2.0
63	**16**	65	112	+20.9° (*c* 0.58)	$C_{32}H_{62}N_2O_6$	67.33	10.95	4.91	67.5	11.0	5.0
64	**16**	78	94	+20.5° (*c* 0.86)	$C_{38}H_{74}N_2O_6$	69.68	11.39	4.28	69.5	11.6	4.1
65	**17**	67	110	+19.5° (*c* 0.82)	$C_{34}H_{66}N_2O_6$	68.19	11.11	4.68	68.3	11.0	4.8
66	**17**	72	108	+18.5° (*c* 0.48)	$C_{40}H_{78}N_2O_6$	70.34	11.51	4.10	70.6	11.4	4.0
67	**18**	78	98	+14.5° (*c* 0.72)	$C_{38}H_{74}N_2O_6$	69.68	11.39	4.28	69.9	11.3	4.1
68	**18**	66	100	+12.6° (*c* 0.78)	$C_{40}H_{78}N_2O_6$	70.34	11.51	4.10	70.4	11.4	4.2
69	**18**	85	113	+14.1° (*c* 0.72)	$C_{44}H_{86}N_2O_6$	71.50	11.73	3.79	71.3	11.5	3.6
70	**19**	39	73	+0.4° (*c* 0.47)	$C_{30}H_{59}NO_5$	70.13	11.57	2.73	70.2	11.4	2.8
71	**19**	65	69	+1.6° (*c* 0.49)	$C_{36}H_{71}NO_5$	72.31	11.97	2.34	72.1	12.1	2.3
72	**20**	40	67	+1.4° (*c* 0.56)	$C_{32}H_{63}NO_5$	70.93	11.72	2.58	71.1	11.9	2.7
73	**20**	70	65	+1.1° (*c* 0.46)	$C_{38}H_{75}NO_5$	72.91	12.08	2.24	73.1	12.0	2.4
74	**21**	68	60	+3.3° (*c* 0.72)	$C_{36}H_{71}NO_5$	72.31	11.97	2.34	72.3	11.8	2.4
75	**21**	65	83	+1.7° (*c* 0.65)	$C_{38}H_{75}NO_5$	72.91	12.08	2.24	72.8	12.0	2.1
76	**21**	80	88	+1.0° (*c* 0.40)	$C_{42}H_{83}NO_5$	73.95	12.26	2.05	74.1	12.1	2.2
77	**22**	42	112	+8.3° (*c* 1.03)[a]	$C_{36}H_{69}NO_{11}$	62.49	10.05	2.02	62.7	10.3	2.4
78	**22**	58	79	+4.7° (*c* 1.00)[b]	$C_{42}H_{81}NO_{11}$	65.00	10.52	1.80	65.3	10.8	2.0
79	**23**	52	138	+5.1° (*c* 0.41)	$C_{42}H_{81}NO_{11}$	65.00	10.52	1.80	65.1	10.7	2.0
80	**23**	49	98	+3.2° (*c* 0.63	$C_{48}H_{93}NO_{11}$	67.02	10.90	1.63	67.2	11.1	1.7

[a] From MeOH.
[b] From EtOH.

Table 5
Selected ^{13}C NMR chemical shifts (δ, ppm) of *N*-glycosyl-*N*-tetradecyloctadecanamides (100.6 MHz, pyridine-d_5)[a]

Compd	Config.	C-1	C-2	C-3	C-4	C-5	C-6	N-CH_2–	CO
30*E*	β-D-Glc*p*	88.05	72.05	81.07	71.52	79.88	62.90	42.19	174.26
30*Z*	β-D-Glc*p*	83.75	71.85	80.71	71.75	79.97	62.90	43.77	174.46
37*E*	β-D-Gal*p*	88.61	70.30	76.57	70.30	79.24	62.57	43.85	174.12
37*Z*	β-D-Gal*p*	84.23	69.42	76.69	69.14	78.83	62.32	42.39	174.46
44*E*	β-D-Man*p*	82.25	72.53	76.03	68.91	81.89	63.47	45.25	173.26
44*Z*	β-D-Man*p*	85.67	72.93	76.70	68.76	82.32	63.26	44.11	173.81
46[b]	α-D-Man*p*	83.19	71.10	74.27	66.46	79.04	61.61	45.26	174.45
52*E*	α-D-Ara*p*	84.44	69.98	75.90	69.60	69.33		42.49	173.82
52*Z*	α-D-Ara*p*	88.88	70.15	76.18	69.68	69.03		43.71	174.41
59*E*	α-L-Ara*p*	84.45	69.99	75.92	69.61	69.34		42.50	173.84
59*Z*	α-L-Ara*p*	88.90	70.16	76.19	69.70	69.05		43.72	174.43
66*E*	β-D-GlcNAc*p*	86.10	55.40	81.04	72.48	76.78	62.83	42.47	174.27; 170.64
66*Z*	β-D-GlcNAc*p*	82.87	53.81	81.21	72.28	77.21	63.10	44.31	174.14; 170.41
73*E*	β-D-*arabino*-Hex*p*	83.88	38.77	73.03	73.19	81.05	62.88	42,25	174.02
73*Z*	β-D-*arabino*-Hex*p*	80.74	34.10	71.44	72.03	80.05	63.02	42.60	176.15

[a] Shifts of sugar C-atoms were assigned according to [31]. Methylene and methyl signals are not given. *E* and *Z* depict *E-anti* and *Z-anti* conformers.
[b] Spectrum was recorded at 125 MHz in $CDCl_3$.

3. Experimental

General methods.—Solvents were p.a. grade and were used as received. Column chromatography was performed on Silica Gel 60, 230–400 mesh (E. Merck). Thin-layer chromatography (TLC) was performed on Silica Gel 60 F_{254} (E. Merck). Melting points were determined on a Gallenkamp melting-point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Optical rotation values of glycosylamines were determined immediately after dissolution of samples. ^1H and ^{13}C NMR were recorded on Bruker DPX-400 and DRX-500 spectrometers in the solvents noted (Me_4Si, 0.00 ppm). FAB and ESI mass spectra were obtained on Finnigan MAT 95 and MAT 900 mass spectrometers, and MALDI mass spectra were obtained on a MALDI-Tof Bruker Reflex II mass spectrometer.

General procedure for the preparation of N-*alkylglycosylamines (Method A).*—The alkylamine (0.10 mol) was dissolved in 2-propanol (100 mL) if necessary under warming, and added to a solution of the sugar (0.10 mol) in water (100 mL). After 1–3 days at room temperature the precipitate was filtered by suction and washed with 2:1 2-propanol–water. The residue was dissolved in 2-propanol (400 mL) under slight warming and diluted with *n*-hexane (400 mL). The crystals were collected, washed with hexane, and dried under diminished pressure.

General procedure for the preparation of N-*alkylglycosylamines (Method B).*—The solution of the fatty amine (0.11 mol) in EtOH (175 mL) was heated to 70 °C. The sugar (0.10 mol) was added under stirring. After complete dissolution of the sugar, stirring was continued for further 15 min. The mixture was cooled to ~40 °C and diluted with *n*-hexane (350 mL). After being kept at room temperature the crystals were collected, washed with *n*-hexane (150 mL), and dried under diminished pressure.

*General procedure for the preparation of 2-deoxy-*N*-alkyl-β-*D*-glucopyranosylamines (Method C).*—The fatty amine (0.10 mol) and 2-deoxy-D-*arabino*-hexose (0.10 mol) were dissolved in *N*,*N*-dimethylformamide (200 mL). After 2 h the crystals were collected, washed with tetrahydrofurane (30 mL), and dried under diminished pressure.

N-*Dodecyl-β-*D*-glucopyranosylamine* (**1**) *and* N-*dodecyl-α-*D*-glucopyranosylamine* (**24**).—^1H NMR (400 MHz, CD_3OD): **1**: δ 3.84 (dd, 1 H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 11.9 Hz, H-6a), 3.82 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 3.65 (dd, 1 H, $J_{5,6b}$ 5.3 Hz, H-6b), 3.34 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 8.4 Hz, H-3),

3.27 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.22 (ddd, 1 H, H-5), 3.06 (dd, 1 H, H-2), 2.90 and 2.62 (2 m, each 1 H, 2 NCH_2), 1.50 (m, 2 H, 2 NCH_2CH_2, 1.34–1.26 (m, 18 H, $(CH_2)_9$), 0.90 (dd, 3 H, CH_3); **24**: δ 4.49 (d, 0.1 H, $J_{1,2}$ 4.8 Hz, H-1). ^{1}H NMR (400 MHz, C_5D_3N): **1**: δ 4.42 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1); **24**: δ 5.07 (d, 0.1 Hz, $J_{1,2}$ 4.9 Hz, H-1). MALDI-MS: m/z 348.3 ($M + H^+$), 370.3 ($M + Na^+$).

2,3,4,6-Tetra-O-acetyl-N-dodecyl-N-(β-D-glucopyranosyl)acetamide **(25)**.—*Method a*. Acetic anhydride (5 mL) was added to a solution of **1** (1.0 g, 2.88 mmol) in pyridine (20 mL). After 3 h the mixture was poured on ice, stirred for 30 min and diluted with CH_2Cl_2. The organic phase was washed with 1 M HCl, aqueous $NaHCO_3$, and water, dried ($MgSO_4$) and evaporated to give **25** as a syrup (1.63 g, 100%).

Method b. Compound **1** (1.0 g, 2.88 mmol) was dissolved in pyridine (20 mL). After 24 h, Ac_2O (5 mL) was added. Workup was done as just described. Both methods provided identical products. $[\alpha]_D^{22}$ +10.5° (*c* 0.90, DMF); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.93 (d, 1H, $J_{1,2}$ 9.4 Hz, H-1_Z), 5.19 (d, 0.1 H, $J_{1,2}$ 8.5 Hz, H-1_E); ^{1}H NMR (400 MHz, C_5D_5N): δ 6.53 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1); ^{13}C NMR (100.63 MHz, $CDCl_3$): 171.87, 170.50, 170.44, 170.21, 169.92, 169.88, 169.63, 169.36, 169.04 (CO), 85.91 (C-1_E), 80.45 (C-1_Z), 74.15 (C-3_E), 74.00 (C-3_Z), 73.54 (C-5_E), 73.39 (C-5_Z), 68.95 (C-2_E), 68.75 (C-2_Z), 68.26 (C-4_Z), 67.91 (C-4_E), 61.87 (C-6_Z and C-6_E), 53.46 (NCH_{2E}), 44.43 (NCH_{2Z}); ^{13}C NMR (100.63 MHz, C_5D_5N): δ 171.77, 170.57, 170.43, 170.39, 170.19, 170.06, 169.89, 169.83, 169.61 (CO), 86.05 (C-1_E), 81.16 (C-1_Z), 74.43 (C-5_E), 74.35 (C-3_E), 74.12 (C-5_Z and C-3_Z), 69.91 (C-2_E), 69.50 (C-2_Z), 68.96 (C-4_Z), 68.81 (C-4_E), 62.42 (C-6_E), 62.34 (C-6_Z), 55.06 (NCH_{2E}), 44.72 (NCH_{2Z}). Anal. Calcd for $C_{28}H_{47}NO_{10}$: C, 60.30; H, 8.49; N, 2.51. Found: C, 60.2; H, 8.5; N, 2.6.

N-Dodecyl-N-(β-D-glucopyranosyl)acetamide **(26)**.—*Method a*. A solution of **1** (1.0 g, 2.88 mmol) in MeOH (20 mL) was treated with Ac_2O (5 mL). After 20 min the mixture was evaporated, the residue was coevaporated twice with MeOH and twice with toluene. Compound **26** was obtained as a syrup (yield: 1.70 g, 100%).

Method b. To a solution of **25** (100 mg; 0.18 mmol) in methanol (2 mL) was added 1 M NaOMe (0.01 mL). The mixture was made neutral with the ion-exchanger resin Lewatit SC102 (H^+-form), filtered, and evaporated to give a syrup (70 mg, 100%); $[\alpha]_D^{22}$ −12.6° (*c* 0.65, DMF); ^{1}H NMR (400 MHz, C_5D_5N): δ 6.50 (d, 0.2 H, $J_{1,2}$ 9.4 Hz, H-1_Z), 5.41 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1_E), ^{13}C NMR (100.63 MHz, C_5D_5N): δ 171.90 (CO_Z), 171.70 (CO_E), 89.03 (C-1_E), 83.62 (C-1_Z), 81.10 (C-3_E), 80.87 (C-3_Z), 80.01 (C-5_Z), 79.82 (C-5_E), 72.14 (C-2_E), 71.88 (C-2_Z), 71.79 (C-4_Z), 71.53 (C-4_E), 62.94 (C-6_E and C-6_Z), 44.56 (NCH_{2Z}), 42.20 (NCH_{2E}), 32.13–22.96 (CH_2), 22.45 ($COCH_{3E}$), 22.32 ($COCH_{3Z}$), 14.31 (CH_3). ^{13}C NMR (298 K, 125.77 MHz, Me_2SO-d_6): δ 170.89 (CO_Z), 170.75 (CO_E), 87.07 (C-1_E), 83.70 (C-1_Z), 79.21 (C-3_Z), 79.11 (C-3_E), 77.70 (C-5_Z), 77.60 (C-5_E), 70.47 (C-2_E and C-2_Z), 70.00 (C-4_Z), 69.90 (C-4_E), 61.13 (C-6_E and C-6_Z); ^{13}C NMR (333 K, 125.77 MHz, Me_2SO-d_6): δ 170.40 (CO), 87.02 (C-1), 78.80 (C-3), 77.47 (C-5), 70.31 (C-2), 69.90 (C-4), 61.09 (C-6). Anal. Calcd for $C_{20}H_{39}NO_6$: C, 61.67; H, 10.09; N, 3.60. Found: C, 61.8; H, 10.1; N, 3.6.

General procedure for the preparation of N-alkyl-N-glycopyranosylcarbonamides (**27**–**80**).—To the solution of the fatty acid (10.0 mmol) and ethyl chloroformate (0.96 mL, 1.09 g, 10.0 mmol) in tetrahydrofuran (15 mL) was added dropwise Et_3N (1.39 mL, 1.01 g, 10.0 mmol) at 0 °C. The mixture was stirred for 2 h at 20 °C and filtered under N_2. The filtrate was added to the solution of the glycosylamine **1**–**23** (10.0 mmol) in *N*,*N*-dimethylformamide (50 mL). Stirring was maintained for 16 h. Tetrahydrofuran was removed under diminished pressure at 40 °C. The mixture was cooled to 20 °C and washed with heptane (2 × 20 mL) and concentrated under high vacuum ($\cong$ 7 mmHg) at 55 °C. The residue was dissolved in *tert*-butyl methyl ether (50 mL), washed with 1 M NaOH (10 mL), 1 M HCl (10 mL), saturated $NaHCO_3$ solution (10 mL), brine (10 mL), and dried over $MgSO_4$. Crystallisation was from hot EtOH–water or acetone. Crude products that did not crystallise were purified by column chromatography (silica gel, eluent 30:1 CH_2Cl_2–MeOH). Workup

in the *arabino* series was done by evaporation of the reaction mixture. The residue was taken up in acetone, and the product was precipitated by addition of water. The crude product was recrystallised from hot EtOH after addition of water.

N-*Tetradecyl*-β-D-*glucopyranosylamine* (**2**). —^{1}H NMR (400 MHz, C_5D_5N): δ 4.40 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1); α anomer: 5.04 (d, 0.2 H, $J_{1,2}$ 4.8 Hz, H-1).

N-β-D-*Glucopyranosyl*-N-*octadecyldodecanamide* (**31**).—IR (KBr) *v* 3400 (broad, alcohol), 2920, 2850 and 1470 (methylene), and 1635 cm^{-1} (disubst. amide); ^{1}H NMR (400 MHz, C_5D_5N): δ 6.49 (d, 0.2 H, $J_{1,2}$ 9.6 Hz, H-1_Z), 5.50 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1_E); FAB-MS: m/z 620 ($M + Li^+$).

N-β-D-*Mannopyranosyl*-N-*tetradecyldodecanamide* (**43**).—IR (KBr) *v* 3400 (broad, alcohol), 2920, 2850 and 1470 (methylene), and 1635 cm^{-1} (disubst. amide).

N-α-D-*Mannopyranosyl*-N-*octadecyldodecanamide* (**46**).—Chromatography (20:1 CH_2Cl_2–MeOH) of the mother liquor of **45** and crystallisation from acetone yielded **46** as crystals (0.31 g, 5%), mp 127 °C, $[\alpha]_D^{22}$ + 16.6° (*c* 0.21, tetrahydrofuran). Anal. Calcd for $C_{36}H_{71}NO_6$: C, 70.43; H, 11.66; N, 2.28. Found: C, 70.5; H, 11.7; N, 2.2.

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