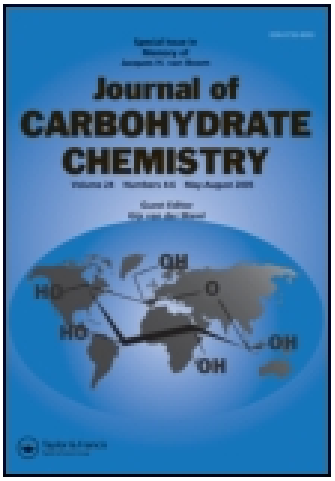

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SYNTHESES OF PEPTIDOGLYCOLIPID ANALOGS WITH DISTINCT IMMUNOMODULATING ACTIVITIES[1,2]

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ABSTRACT

Amphiphilic 2-amino-2-deoxy-β-D-glucopyranosylamides were synthesized from *N*-protected glucosamine derivatives via *N*-glycosidation with fatty amines, subsequent *N*-acylation with fatty acid derivatives and deprotection. They could further be modified with amino acids to give peptido-glycopyranosyl amides, some of which exhibited strong immunostimulatory (BAY Q8939, **8**) or immunoadjuvant (BAY R1005, **9**) activities.

INTRODUCTION

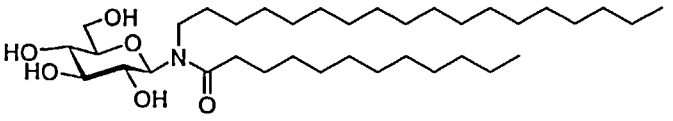
Infection control can be managed by chemotherapy and by agents able to increase the immunity of the host. Vaccination induces a specific immune response against an infectious microorganism or a defined toxin and often leads to formation of immunity in the host organism which is sufficient for sustained or even lifelong protection. In order to achieve a sufficient protective effect, however, many antigens require adjuvants which potentiate the immune response in the immunized species.[3] Biological response modifiers or nonspecific immunostimulants can induce a broader immune response against a variety of pathogens by generally enhancing the alert of the immune system.[4]

The growing threat of antibiotic resistance[5] as well as the increasing number of immunocompromised hosts nowadays demand alternatives to chemotherapeutic treatments. This resulted in an increased interest in the pharmacological modulation of the immune system.

Some years ago we discovered a new class of immunomodulating agents.[6] Glycosylamides carrying long aliphatic residues were synthesized as glycolipid analogs (GLA) and identified as immunostimulating agents.[7] Interestingly, these compounds differed from known immunomodulators like muramyl peptides,[8] lipopeptides[9] and lipid A[10] by their mode of action. GLA acted on an early stage of B cell differentiation and did not behave as mitogens. These and other results stimulated us to further explore the potential of immunomodulating GLA. A review on other glycolipid derivatives as therapeutic agents has appeared recently.[11]

RESULTS AND DISCUSSION

The first generation of immunomodulating glycosylamides consisted of neutral glycosyl moieties.[12] Although several compounds related to the prototype BAY P4581

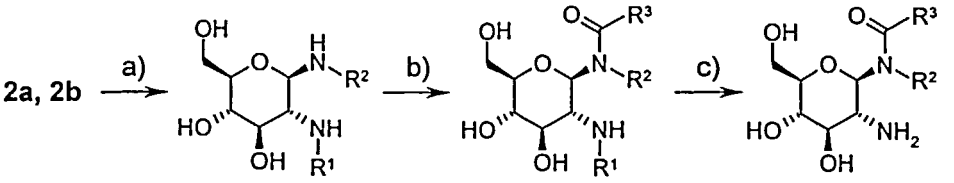


BAY P4581 (1)

(1) exhibited adjuvant effects *in vitro*, we strived to improve their overall therapeutic index and solubility in aqueous systems. This should be accomplished by introducing additional polar substituents or charged functional groups into the head group of the GLA as well as by introducing double bonds into their lipid moieties.

2-Amino-2-deoxysugars were considered as suitable building blocks. The reaction of glucosamine hydrochloride with long chain alkyl amines yielded 2-amino-2-deoxy-ß-D-glucopyranosylamines. Acylation reactions with acetic anhydride in methanol revealed comparable reactivity of both amino groups. A selective conversion of only one of them could not be accomplished. Therefore, *N*-protecting groups had to be employed in order to differentiate between the anomeric and the *C*-2 nitrogen atom.

The *N*-glycosidation of *N*-benzyloxycarbonyl-D-glucosamine[13] (**2a**) with long chain aliphatic amines provided glycosylamines **3a** – **3c** in good yields (Scheme 1). In comparison with the corresponding reactions of neutral aldopyranoses[12] these *N*-glycosidations required higher reaction temperatures and times for complete turnover. The glycosylamines were isolated as solids contaminated with variable amounts of alkyl



3a - e **4a - f** **5a - f**

	R^1	R^2
a	Z	$C_{12}H_{25}$
b	Z	$C_{14}H_{29}$
c	Z	$C_{18}H_{37}$
d	Boc	$C_{14}H_{29}$
e	Boc	$C_{18}H_{37}$

	R^1	R^2	R^3
a	Z	$C_{12}H_{25}$	$C_{11}H_{23}$
b	Z	$C_{12}H_{25}$	$C_{17}H_{35}$
c	Z	$C_{14}H_{29}$	$C_{11}H_{23}$
d	Z	$C_{18}H_{37}$	$C_{11}H_{23}$
e	Boc	$C_{14}H_{29}$	$C_{17}H_{33}$
f	Boc	$C_{18}H_{37}$	$C_{17}H_{33}$

Scheme 1: Synthesis of 2-amino-2-deoxy-β-D-glucopyranosylamides **5a** – **5f**. a) alkyl amine, THF, reflux; b) fatty acid, $ClCO_2Et$, NEt_3; c) H_2, Pd-C or CF_3CO_2H/CH_2Cl_2. (Z = CO_2Bzl, Boc = CO_2tBu)

amines. Analytical samples could be purified by crystallization from alcoholic solutions. Glycosylamines **3** showed mutarotation as did the corresponding glycosylamines derived from neutral sugars.[12]

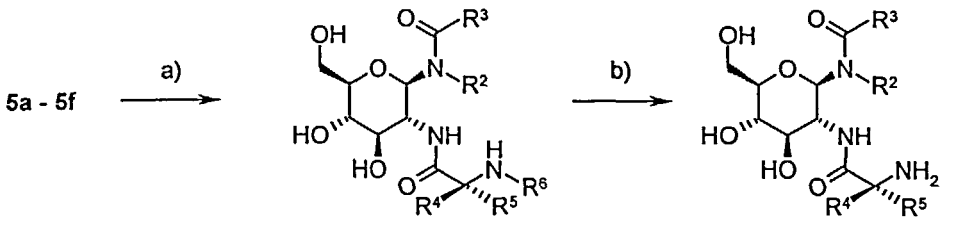
Selective *N*-acylation of **3a** – **3c** was accomplished with mixed anhydrides of saturated fatty acids[12] providing the β-D-glucopyranosylamides **4a** – **4d**. The *N*-benzyloxycarbonyl groups of **4a** – **4d** were removed by catatytic hydrogenation giving 2-amino-2-deoxy-β-D-glucopyranosylamides **5a** – **5d** as crystalline materials.

Unsaturated GLA were obtained from *N-tert*-butyloxycarbonyl-D-glucosamine[14] (**2b**) by selective *N*-acylation of the corresponding glycosylamine intermediates **3d** and **3e** with oleic acid derivatives. The glycosylamide syntheses were performed as described

above providing the glycolipids **4e** and **4f**. They were deblocked by treatment with trifluoroacetic acid providing wax-like **5e** and **5f**.

The 2-amino-2-deoxyglucosylamides **5** were further transformed selectively with a variety of electrophilic substituents. Condensation with activated *N*-protected amino acids gave **6a – 6m** following standard peptide chemistry (Scheme 2). Removal of the *N*-protecting groups was accomplished either by catalytic hydrogenation of **6a – 6f** providing saturated peptidoglycolipids **7a – 7f** or by treatment of **6g – 6m** with trifluoroacetic acid providing unsaturated GLA **7g – 7m**.

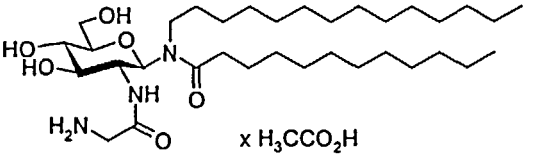
^{13}C NMR spectra of selected glycosylamides were recorded (Table 1). Compounds **4** exhibited amide rotamerism. This phenomenon is known for simple glycosylamides.[12,15] Two distinct sets of signals could be assigned for all carbohydrate



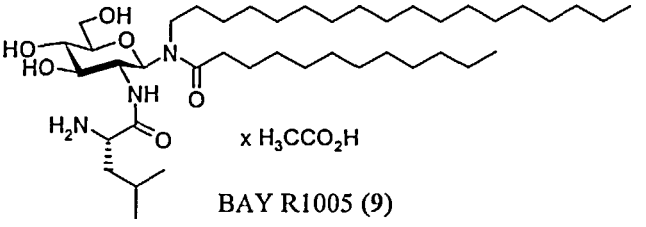
	R^2	R^3	R^4	R^5	R^6
a	$C_{12}H_{25}$	$C_{11}H_{23}$	H	H	Z
b	$C_{12}H_{25}$	$C_{11}H_{23}$	CH_3	H	Z
c	$C_{12}H_{25}$	$C_{17}H_{35}$	CH_2Ph	H	Z
d	$C_{14}H_{29}$	$C_{11}H_{23}$	H	H	Z
e	$C_{18}H_{37}$	$C_{11}H_{23}$	*i*Bu	H	Z
f	$C_{18}H_{37}$	$C_{11}H_{23}$	H	*i*Bu	Z
g	$C_{14}H_{29}$	$C_{17}H_{33}$	H	H	Boc
h	$C_{14}H_{29}$	$C_{17}H_{33}$	CH_3	H	Boc
i	$C_{14}H_{29}$	$C_{17}H_{33}$	*i*Bu	H	Boc
j	$C_{18}H_{37}$	$C_{17}H_{33}$	H	H	Boc
k	$C_{18}H_{37}$	$C_{17}H_{33}$	CH_3	H	Boc
l	$C_{18}H_{37}$	$C_{17}H_{33}$	CH_2Ph	H	Boc
m	$C_{18}H_{37}$	$C_{17}H_{33}$	CH_2OH	H	Boc

Scheme 2: Syntheses of peptidoglycolipids **7a – 7m**. a) *N*-protected amino acid, $ClCO_2Et$, NEt_3; b) H_2, Pd-C or CF_3CO_2H / CH_2Cl_2.

and most other C-atoms. The E-rotamers were the major isomers as deduced from the more intense signals attributed to the anomeric C-atoms at lower field (> 86 ppm). The 2-amino-2-deoxy-β-D-glucopyranosylamides **5**, however, gave only one set of signals indicating that they were present nearly exclusively as E-rotamers. This stands in contrast to the spectra of the corresponding neutral glycosylamides[12] and hints at an influence of the C-2 substituent on glycosylamide conformations. Aminoacid substituted glycosylamides **6** and **7**, however, were present as rotameric mixtures with the E-rotamers as favored isomers. The nature of the investigated lipid or amino acid moieties had no detectable influence on the shift values of the ^{13}C NMR signals except for the *N*-protected D-leucine moiety which significantly influenced the ratio of rotamers favoring the Z-isomer (see Table 1, entries 6e and 6f). This effect was less significant for the unprotected D-leucyl substituent where both sets of the corresponding ^{13}C NMR signals were of equal intensity (Table 1, entry 7e and 7f).



BAY Q8939 (**8**)



BAY R1005 (**9**)

All glycolipid analogs were evaluated relating to their immunopharmacological profiles. To improve their solubility, they were transformed into their corresponding hydroacetate or hydrochloride salts. Prophylactic application of BAY Q8939 (**8**) in mice (dose 1 – 10 mg/kg bodyweight) gave rise to protective effects in various bacterial and fungal infection models and significantly enhanced both the survival rate and the survival time of lethally infected animals.[16] As **8** was also effective in neutropenic (SCID) mice it might be valuable as stimulator of host defence for the therapy of immunocompromised

hosts. In contrast, BAY R1005 (**9**) had only minor improving effects in these models, but exhibited strong immunoadjuvant effects in mice when given together with particulate (sheep red blood cells), soluble (bovine serum albumin), or viral envelope antigens.[17] Persistent protective antibody titers could also be raised in cattle after immunization with recombinant viral marker antigens.[18] This biological differentiation between two major branches of the immune system is remarkable because both **8** and **9** structurally belong to the same subclass of glycosylamides and indicates some specificities in signal transduction during immunomodulation. Pharmacological details will be presented elsewhere.

EXPERIMENTAL

General methods. Solvents were p.a. grade and used as received. Column chromatography was performed on Silica Gel 60, 230-400 mesh (E. Merck). Thin-layer chromatography (TLC) was performed on aluminium plates precoated with silica gel $60F_{254}$ (E. Merck). Melting points were determined with a Gallenkamp melting-point apparatus and are uncorrected. Optical rotation values were determined on a Perkin-Elmer 241 polarimeter at 22 °C. ^{13}C NMR spectra were recorded on a Bruker DPX-400 spectrometer in pyridine-d5 (Me_4Si 0.00 ppm). FAB and ESI mass spectra were obtained on Finnigan MAT 95 and MAT 900 mass spectrometers.

A. General procedure for the synthesis of glycosylamines 3a – 3e: A mixture of D-glucosamine derivative **2** (20.0 mmol) and the *n*-alkylamine (40.0 mmol) in tetrahydrofuran (120 mL) was heated to reflux. The reaction was monitored by TLC (dichloromethane/methanol 10:1 v/v). After 2.5 h the mixture was cooled to 25 °C and diluted with *N,N*-dimethylformamide (75 mL). Tetrahydrofuran was removed under reduced pressure. Excess alkyl amine was removed by repeated washing with *n*-hexane (5 x 50 mL). The residual mixture was used in the following *N*-acylation step without further purification. Estimated yields of **3a – 3e** according to TLC were approximately 70 – 80 % based on **2**.

2-Benzyloxycarbonylamino-2-deoxy-*N*-tetradecyl-β-D-glucopyranosylamine (3b). A sample of the solution was concentrated under high vacuum. The residue was crystallized from methanol: mp 95-96 °C; $[\alpha]_D$ –4.0° (1 min) → +1.7° (24 h) (*c* 1.0, pyridine); ^{13}C NMR see Table 1; MS (ESI): *m/z* 509.6 $(M+H)^+$.

Anal. Calcd for $C_{28}H_{48}N_2O_6$ (508.70): C, 66.11; H, 9.51; N, 5.51. Found: C, 65.8; H, 9.8; N, 5.7.

2-Benzyloxycarbonylamino-2-deoxy-*N*-octadecyl-β-D-glucopyranosylamine (3c). A sample was crystallized from methanol: mp 105-106 °C; $[\alpha]_D$ −4.0° (1 min) → −1.0° (20 h) (*c* 1.1, dimethylformamide); MS (ESI): *m/z* 565.3 $(M+H)^+$.

Anal. Calcd for $C_{32}H_{56}N_2O_6$ (564.81): C, 68.05; H, 9.99; N, 4.96. Found: C, 68.1; H, 10.1; N, 4.9.

B. General procedure for the synthesis of glycosylamides 4a – 4f: To a solution of the fatty acid (20 mmol) and ethyl chloroformate (1.92 mL, 2.18 g, 20.0 mmol) in tetrahydrofuran (30 mL) was added dropwise triethylamine (2.78 mL, 2.02 g, 20.0 mmol) at 0 °C. The mixture was stirred for 2 h at 25 °C and filtered under argon. The filtrate was added to a solution of the crude glycosylamine **3** (maximal 20 mmol) in *N,N*-dimethylformamide (100 mL). Stirring was maintained for 16 h. Tetrahydrofuran was removed under diminished pressure at 40 °C. The mixture was cooled to 20 °C, washed with hexane (3 x 50 ml) and concentrated under high vacuum (≅ 5 mmHg) at 55 °C. The residue was dissolved in *tert*-butyl methyl ether (100 mL), washed with 1 M aq sodium hydroxide (20 mL), 1 M hydrochloric acid (20 mL), saturated aq sodium hydrogen carbonate (20 mL), brine (20 mL), and dried over magnesium sulfate. The residue was purified by flash chromatography (dichloromethane/methanol 20:1 v/v) providing compounds which were not analytically pure.

***N*-(2-Benzyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyldodecanamide (4c).** Yield 6.69 g (raw material; 48.4 %, based on **2a**). Column chromatography (dichloromethane/methanol 40:1 v/v) of a sample provided pure **4c**: syrup; $[\alpha]_D$ +14.1° (*c* 0.7, methanol); ^{13}C NMR see Table 1; MS (ESI): *m/z* 691.4 $(M+H)^+$; 1381.7 $(2M+H)^+$.

Anal. Calcd for $C_{40}H_{70}N_2O_7$ (691.01): C, 69.53; H, 10.21; N, 4.05. Found: C, 69.4; H, 10.1; N, 3.9.

(Z)-*N*-(2-*tert*-Butyloxycarbonylamino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (4e). Yield 9.19 g (raw material; 62.1 %, based on **2b**). Column chromatography (dichloromethane/methanol 40:1 v/v) of a sample provided pure **4e**: syrup; $[\alpha]_D$ +18.4° (*c* 0.9, tetrahydrofuran); ^{13}C NMR see Table 1; MS (ESI): *m/z* 739.4 $(M+H)^+$.

Table 1. ^{13}C NMR shifts[a] of glycosylamine **3b** and selected glycosylamides **4 – 7**

Compound	Isomer	C-1	C-2	C-3	C-4	C-5	C-6	C-1'	C-2'	C-3'	COAlk	NCH_2Alk	$COCH_2Alk$
3b		91.50	59.14	77.17	72.83	79.05	63.15					46.05	
4c	E	86.59	56.66	76.99	72.19	81.36	62.96				174.05	42.73	33.79
	Z	82.88	56.09	77.46	72.40	81.11	63.13				174.38	42.20	33.95
4d	E	86.59	56.65	76.99	72.17	81.37	62.94				174.04	42.71	33.78
	Z	82.88	56.09	77.44	72.39	81.12	63.12				174.36	44.20	33.94
4e	E	86.74	56.12	76.90	72.27	81.32	63.02				173.78	42.82	33.76
	Z	82.99	55.39	77.57	72.40	81.03	63.16				174.16	44.23	33.92
5c	E	88.86	55.72	79.01	71.42	81.01	62.82				174.30	41.84	34.02
6b	E	86.00	55.43	77.07	72.44	81.13	63.07	170.81	45.06		174.37	42.47	33.88
	Z	82.76	54.03	76.55	72.28	81.28	62.85	170.55	45.30		174.60	44.20	33.78
6c	E	85.87	55.63	76.61	72.31	81.34	62.87	173.20	57.59	39.04	174.46	42.48	33.96
	Z	82.76	53.90	77.17	72.66	81.14	63.09	172.80	57.22	39.26	174.58	44.22	33.78
6d	E	86.05	55.51	76.61	72.52	81.20	63.12	170.83	45.10		174.37	42.51	33.91
	Z	82.82	54.09	77.16	72.36	81.36	62.91	170.54	45.35		174.59	44.25	33.82
6e	E	85.76	55.57	76.62	72.36	81.36	62.90	174.46	54.31	42.46	174.33	42.64	33.69
	Z	82.81	54.31	77.39	72.71	81.10	63.13	174.29	53.70	42.46	174.43	44.19	33.90
6f	Z	82.49	54.44	77.34	72.08	81.09	63.02	173.94	53.67	42.57	174.22	44.03	34.03
	E	85.92	55.57	75.96	72.30	81.25	62.88	174.06	54.62	42.13	173.84	42.68	34.03
6i	E	85.73	55.54	76.60	72.34	81.33	62.88	174.41	54.29	42.44	174.29	42.62	33.68
	Z	82.78	54.28	77.37	72.69	81.07	63.11	174.24	53.68	42.44	174.39	44.17	33.88

Table 1 (continued)

Compound	Isomer	C-1	C-2	C-3	C-4	C-5	C-6	C-1'	C-2'	C-3'	COAlk	NCH_2Alk	$COCH_2Alk$
7b	E	86.17	55.09	77.34	72.45	81.15	63.00	176.85	51.60		174.25	42.60	33.89
	Z	82.76	53.85	76.69	72.45	81.41	63.14	176.89	51.68		174.13	44.11	33.97
7c	E	86.19	55.45	77.25	72.40	81.16	63.16	175.66	57.52	41.82	174.38	42.57	33.95
	Z	82.95	55.11	76.89	72.54	81.35	62.91	175.58	57.52	42.14	174.25	44.12	33.77
7d	E	86.25	55.10	77.25	72.37	81.38	62.98	174.36	45.80		174.24	42.52	33.86
	Z	82.85	54.00	76.88	72.46	81.17	63.16	174.30	45.95		174.21	44.23	33.92
7e	E	86.12	55.09	76.69	72.49	81.36	62.97	177.13	54.39	45.14	174.15	42.57	33.94
	Z	82.78	53.78	77.33	72.49	81.11	63.15	177.05	54.39	45.08	174.22	45.08	33.99
7f	E	86.15	54.76	77.09	71.75	81.03	62.90	173.86	53.83	43.20	174.22	42.55	34.16
	Z	82.27	54.10	77.24	71.75	81.03	62.72	173.52	53.72	43.11	174.17	43.96	33.99
7i	E	86.10	55.11	76.69	72.49	81.37	62.98	177.07	54.38	45.10	174.11	42.57	33.91
	Z	82.79	563.79	77.33	72.49	81.11	63.15	177.92	54.38	45.10	174.17	44.14	33.99

a. Recorded in pyridine-d_5 with TMS as an external standard; C-1 – C-6: C-atoms of carbohydrate moiety; C-1' – C-3': first three C-atoms of aminoacid chain. Signals of major rotameric isomers of each compound (as deduced from signal integrals) are listed in upper rows of entries. Further characteristic ^{13}C NMR signals observed: 130.20 – 130.23 and 130.17 – 130.19 (*C*H=*C*H); 66.11 – 66.55 (O*C*H_2Ph), 78.05 – 78-41 (*C*Me_3). Signals of aromatic residues, lipid alkyl chains of further alkyl signals of aminoacid in accordance with structure.

Anal Calcd for $C_{43}H_{82}N_2O_7$ (739.14): C, 69.88; H, 11.18; N, 3.79. Found: C, 70.0: H, 11.2; N, 3.7.

C. General procedure for the synthesis of 2-amino-2-deoxy-β-D-glucopyranosylamides 5a – 5d: To the solution of crude *N*-benzyloxycarbonyl protected glycosylamides **4a – 4d** in methanol/ethanol/1 N hydrochloric acid (15:5:2, v/v/v, 88 mL) was added palladium on charcoal (10 %, 250 mg). The mixture was hydrogenated under atmospheric pressure. After 3 h the mixture was filtered. The residue was washed with methanol/ethanol (3:1, v/v). The liquid phases were combined and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol/concd ammonia, 30:1:0.1 v/v/v). Crystals were obtained in some cases from methanol/concd ammonia (10:1, v/v).

***N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-dodecyldodecanamide (5a).** Yield 4.47 g (42.3 %, based on **2a**): syrup; $[\alpha]_D$ +11.4° (*c* 0.8, tetrahydrofuran); MS (ESI): *m/z* 529.3 $(M+H)^+$.

Anal. Calcd for $C_{30}H_{60}N_2O_5$ (528.82): C, 68.14; H, 11.44; N, 5.30. Found: C, 68.1; H, 11.3; N, 5.4.

***N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-dodecyloctadecanamide (5b).** Yield 4.59 g (37.4 %, based on **2a**): mp 69-71 °C; $[\alpha]_D$ +10.0° (*c* 0.5, tetrahydrofuran); MS (ESI): *m/z* 613.4 $(M+H)^+$.

Anal. Calcd for $C_{36}H_{72}N_2O_5$ (612.99): C, 70.54; H, 11.84; N, 4.57. Found: C, 70.7; H, 12.0; N, 4.8.

***N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyldodecanamide (5c).** Yield 4.62 g (41.5 %, based on **2a**): mp 65-67 °C; $[\alpha]_D$ +9.9° (*c* 0.9, tetrahydrofuran). ^{13}C NMR see Table 1; MS (ESI): *m/z* 557.4 $(M+H)^+$.

Anal. Calcd for $C_{32}H_{64}N_2O_5$ (556.88): C, 69.02; H, 11.58; N, 5.03. Found: C, 69.3; H, 11.3; N, 5.2.

***N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyldodecanamide (5d).** Yield 5.50 g (44.9 %, based on **2a**): mp 63-64 °C; $[\alpha]_D$ +9.6° (*c* 1.0, tetrahydrofuran); MS (ESI): *m/z* 613.3 $(M+H)^+$.

Anal. Calcd for $C_{36}H_{72}N_2O_5$ (612.99): C, 70.54; H, 11.84; N, 4.57. Found: C, 70.8; H, 12.1; N, 4.4.

(Z)-*N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (5e). Crude **4e** was dissolved in dichloromethane/trifluoroacetic acid (4:1 v/v, 75 mL). After 2 h at 25 °C the mixture was concentrated under reduced pressure and coevaporated with toluene (3 x 50 mL). The residue was purified by column chromatography (dichloromethane/methanol/concd ammonia, 30:1:0.1 v/v/v). Yield 4.28 g (33.5 %, based on **2b**): syrup; $[\alpha]_D$ +8.2° (*c* 0.8, tetrahydrofuran); MS (ESI): *m/z* 639.3 $(M+H)^+$.

Anal. Calcd for $C_{38}H_{74}N_2O_5$ (639.02): C, 71.43; H, 11.67; N, 4.38. Found: C, 71.1; H, 11.9; N, 4.2.

(Z)-*N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (5f). Crude **4f** was treated with dichloromethane/trifluoroacetic acid as described above. Yield 5.55 g (39.9 %, based on **2b**): syrup; $[\alpha]_D$ +7.3° (*c* 0.4, tetrahydrofuran); MS (ESI): *m/z* 695.4 $(M+H)^+$.

Anal. Calcd for $C_{42}H_{82}N_2O_5$ (695.13): C, 72.57; H, 11.89; N, 4.03. Found: C, 72.4; H, 12.0; N, 4.0.

D. General procedure for the synthesis of glycosylamides 6a – 6m: To a stirred solution of *N*-protected amino acid (5 mmol) in dry tetrahydrofuran (10 mL) was added at 0 °C ethyl chloroformate (0.54 g, 0.48 mL, 5 mmol) and a solution of triethylamine (0.51 g, 0.70 mL, 5 mmol) in tetrahydrofuran (5 mL). After 2 h at 0 °C, the precipitate was filtered off and washed with cold tetrahydrofuran. The liquid phases were combined and added to a solution of glycosylamine **5** (5 mmol) in tetrahydrofuran (15 mL). After 16 h the mixture was evaporated under reduced pressure. The residue was purified by column chromatography (toluene/ethanol 9:1 v/v).

***N*-(2-(*N*-Benzyloxycarbonylglycyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-dodecyldodecanamide (6a).** Crystallization from methanol provided **6a** (2.96 g, 82.3 %): mp 114-116 °C; $[\alpha]_D$ +1.2° (*c* 0.6, dimethylformamide).

Anal. Calcd for $C_{40}H_{69}N_3O_8$ (720.01): C, 66.73; H, 9.66; N, 5.84. Found: C, 66.6; H, 9.7; N, 6.0.

***N*-(2-(*N*-Benzyloxycarbonyl-L-alanyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-dodecyldodecanamide (6b).** Yield 2.66 g (72.4 %): mp 63-66 °C; $[\alpha]_D$ +16.6° (*c* 0.8, tetrahydrofuran); ^{13}C NMR see Table 1.

Anal. Calcd for $C_{41}H_{71}N_3O_8$ (734.04): C, 67.09; H, 9.75; N, 5.72. Found: C, 67.1; H, 9.9; N, 5.6.

***N*-(2-(*N*-Benzyloxycarbonyl-L-phenylalanyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-dodecyloctadecanamide (6c).** Yield 2.75 g (61.5 %): syrup; $[\alpha]_D$ +7.9° (*c* 0.6, tetrahydrofuran); ^{13}C NMR see Table 1.

Anal. Calcd for $C_{53}H_{87}N_3O_8$ (894.30): C, 71.18; H, 9.81; N, 4.70. Found: C, 71.0; H, 10.0; N, 4.7.

***N*-(2-(*N*-Benzyloxycarbonylglycyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyldodecanamide (6d).** Yield 2.91 g (77.7 %): mp 79-81 °C; $[\alpha]_D$ +16.9° (*c* 1.0, dichloromethane); ^{13}C NMR see Table 1.

Anal. Calcd for $C_{42}H_{73}N_3O_8$ (748.07): C, 67.44; H, 9.84; N, 5.62. Found: C, 67.2; H, 9.8; N, 5.8.

***N*-(2-(*N*-Benzyloxycarbonyl-L-leucyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyldodecanamide (6e).** Crystallization from methanol at –20 °C provided **6e** (2.98 g, 69.3 %): mp 90–94 °C ; $[\alpha]_D$ +5.4° (*c* 1.4, tetrahydrofuran); ^{13}C NMR see Table 1.

Anal. Calcd for $C_{50}H_{89}N_3O_8$ (860.28): C, 69.81; H, 10.43; N, 4.88. Found: C, 69.6; H, 10.3; N, 5.0.

***N*-(2-(*N*-Benzyloxycarbonyl-D-leucyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyldodecanamide (6f).** Crystallization from methanol provided **6f** (2.40 g, 55.8 %): mp 103-104 °C; $[\alpha]_D$ +30.2° (*c* 0.6, dichloromethane); ^{13}C NMR see Table 1; MS (FAB): *m/z* 882.6 $(M+Na)^+$.

Anal. Calcd for $C_{50}H_{89}N_3O_8$ (860.28): C, 69.81; H, 10.43; N, 4.88. Found C, 69.9; H, 10.4; N, 4.9.

(Z)-*N*-(2-(*N*-Benzyloxycarbonylglycyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (6g). Yield 2.56 g (64.2 %): syrup; $[\alpha]_D$ +13.6° (*c* 0.6, tetrahydrofuran).

Anal. Calcd for $C_{45}H_{85}N_3O_8$ (796.19): C, 67.89; H, 10.76; N, 5.28. Found: C, 68.1; H, 10.6; N, 5.3.

(Z)-*N*-(2-(*N*-Benzyloxycarbonyl-L-alanyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (6h). Yield 2.59 g (63.9 %): syrup; $[\alpha]_D$ +8.8° (*c* 0.7, tetrahydrofuran).

Anal. Calcd for $C_{46}H_{87}N_3O_8$ (810.22): C, 68.19; H, 10.28; N, 5.19. Found: C, 68.0; H, 10.4; N, 5.4.

(Z)-*N*-(2-(*N*-Benzyloxycarbonyl-L-leucyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (6i). Yield 3.17 g (74.4 %): syrup; $[\alpha]_D$ +4.5° (*c* 0.5, tetrahydrofuran); ^{13}C NMR see Table 1.

Anal. Calcd for $C_{49}H_{93}N_3O_8$ (852.30): C, 69.05; H, 11.0; N, 4.93. Found: C, 69.0; H, 11.1; N, 5.1.

(Z)-*N*-(2-(*N*-Benzyloxycarbonylglycyl)amino-2-deoxy-β-D-glucopyranosyl)-octadecyl-9-octadecenamide (6j). Yield 2.83 g (66.3 %): syrup; $[\alpha]_D$ +11.9° (*c* 0.7, tetrahydrofuran).

Anal. Calcd for $C_{49}H_{93}N_3O_8$ (852.30): C, 69.05; H, 11.00; N, 4.93. Found: C, 68.8; H, 11.1; N, 4.7.

(Z)-*N*-(2-(*N*-Benzyloxycarbonyl-L-alanyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (6k). Yield 3.13 g (72.2 %): syrup; $[\alpha]_D$ +8.5° (*c* 0.7, tetrahydrofuran).

Anal. Calcd for $C_{50}H_{95}N_3O_8$ (866.33): C, 69.32; H, 11.05; N, 4.85. Found: C, 69.2; H, 11.1; N, 4.8.

(Z)-*N*-(2-(*N*-Benzyloxycarbonyl-L-phenylalanyl)amino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (6l). Yield 3.74 g (79.4 %): syrup; $[\alpha]_D$ +15.1° (*c* 0.9, tetrahydrofuran).

Anal. Calcd for $C_{56}H_{99}N_3O_8$ (942.43): C, 71.37; H, 10.59; N, 4.46. Found: C, 71.3; H, 10.8; N, 4.6.

(Z)-*N*-(2-(*N*-Benzyloxycarbonyl-L-seryl)amino-2-deoxy-β-D-glucoyranosyl)-*N*-octadecyl-9-octadecenamide (6m). Yield 3.02 g (68.5 %): syrup; $[\alpha]_D$ +18.5° (*c* 0.5, tetrahydrofuran).

Anal. Calcd for $C_{50}H_{95}N_3O_9$ (882.33): C, 68.07; H, 10.85; N, 4.76. Found: C, 68.1; H, 10.7; N, 4.7.

E. General procedure for the synthesis of saturated peptidoglycolipids 7a – 7f: To a solution of *N*-benzyloxycarbonyl protected **6a – 6f** (2.5 mmol) in methanol (40 mL) and tetrahydrofuran (20 mL) was added 1 N hydrochloric acid (2.5 mL) and palladium on charcoal (10 %, 0.2 g). The mixture was kept in a hydrogen atmosphere for 3 h at 25 °C. The catalyst was filtered off and washed with methanol. The liquid phases

were combined and concentrated under reduced pressure. The residue was dissolved in methanol (25 mL) and treated with concd aq ammonia (2.5 mL). The crystals were collected by filtration and dried under reduced pressure.

***N*-(2-Deoxy-2-glycylamino-β-D-glucopyranosyl)-*N*-dodecyldodecanamide (7a).** Yield 1.30 g (88.9 %): mp 153-155 °C; $[\alpha]_D$ +21.1° (*c* 0.6, tetrahydrofuran); MS (FAB): *m/z* 586 $(M+H)^+$, 584 $(M-H)^-$.

Anal. Calcd for $C_{32}H_{63}N_3O_6$ (585.88): C, 65.60; H, 10.84; N, 7.17. Found: C, 65.5; H, 10.6; N, 7.1.

***N*-(2-L-Alanylamino-2-deoxy-β-D-glucopyranosyl)-*N*-dodecyldodecanamide (7b).** Yield 1.42 g (94.5 %): syrup; $[\alpha]_D$ +24.0° (*c* 0.7, tetrahydrofuran). ^{13}C NMR see Table 1; MS (FAB): *m/z* 600 $(M+H)^+$, 598 $(M-H)^-$.

Anal. Calcd for $C_{33}H_{65}N_3O_6$ (599.90): C, 66.07; H 10.92; N 7.00. Found: C, 66.3; H, 10.8; N, 6.8.

***N*-(2-Deoxy-2-L-phenylalanylamino-β-D-glucopyranosyl)-*N*-dodecyloctadecanamide (7c).** Yield 1.59 g (83.5 %): syrup; $[\alpha]_D$ +9.5° (*c* 1.2, tetrahydrofuran); ^{13}C NMR see Table 1; MS (FAB): *m/z* 760 $(M+H)^+$, 758 $(M-H)^-$.

Anal. Calcd for $C_{45}H_{81}N_3O_6$ (760.16): C, 71.10; H, 10.74; N, 5.53. Found: C, 71.2; H, 10.9 N, 5.4.

***N*-(2-Deoxy-2-glycylamino-β-D-glucopyranosyl)-*N*-tetradecyldodecanamide (7d).** Yield 1.32 g (85.5 %): mp 150-152 °C; $[\alpha]_D$ +20.7° (*c* 1.0, methanol). ^{13}C NMR see Table 1; MS (FAB): *m/z* 614 $(M+H)^+$, 612 $(M-H)^-$.

Anal. Calcd for $C_{34}H_{67}N_3O_6$ (613.93): C, 66.52; H, 11.00; N, 6.84. Found: C, 66.6; H, 10.9; N, 7.0.

***N*-(2-Deoxy-2-L-leucylamino-β-D-glucopyranosyl)-*N*-octadecyldodecanamide (7e).** Yield 1.64 g (90.3 %): mp 57-59 °C; $[\alpha]_D$ +22.5° (*c* 1.0, ethanol). ^{13}C NMR see Table 1; MS (FAB): *m/z* 726 $(M+H)^+$, 724 $(M-H)^-$.

Anal. Calcd for $C_{42}H_{83}N_3O_6$ (726.15): C, 69.47; H, 11.52; N, 5.79. Found: C, 69.4; H, 11.4; N, 5.9.

***N*-(2-Deoxy-2-D-leucylamino-β-D-glucopyranosyl)-*N*-octadecyldodecanamide (7f).** Yield 1.50 g (82.5 %): mp 130-131 °C; $[\alpha]_D$ +14.5° (*c* 0.8, ethanol). ^{13}C NMR see Table 1; MS (FAB): *m/z* 726 $(M+H)^+$, 724 $(M-H)^-$.

Anal. Calcd for $C_{42}H_{83}N_3O_6$ (726.15): C, 69.47; H, 11.52; N, 5.79. Found: C, 69.3; H, 11.5; N, 6.0.

F. General procedure for the synthesis of unsaturated peptidoglycolipids 7g – 7m: The *N*-Boc protected peptidoglycolipids **6g – 6m** (2.5 mmol) were treated with trifluoroacetic acid/dichloromethane (2:5 v/v, 15 mL) at 0 °C. After 3 h the solvents were removed under reduced pressure. The residue was coevaporated with toluene (2 x 5 mL). Purification was achieved by column chromatography (dichloromethane/methanol/concd ammonia, 15:1.5:0.1 v/v/v).

(Z)-*N*-(2-Deoxy-2-glycylamino-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (7g). Crystallization from methanol (1.50 g, 86.4 %): mp 112–115 °C; $[\alpha]_D$ +11.6° (*c* 1.0, dichloromethane); MS (FAB): *m/z* 696 $(M+H)^+$, 694 $(M-H)^-$.

Anal. Calcd for $C_{40}H_{77}N_3O_6$ (696.08): C, 69.02; H, 11.15; N, 6.04. Found: C, 69.2; H, 11.0; N, 6.2.

(Z)-*N*-(2-L-Alanylamino-2-deoxy-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (7h). Yield 1.38 g (77.6 %): syrup; $[\alpha]_D$ +18.5° (*c* 1.0, dichloromethane); MS (FAB): *m/z* 710 $(M+H)^+$, 708 $(M-H)^-$.

Anal. Calcd for $C_{41}H_{79}N_3O_6$ (710.10): C, 69.35; H, 11.21; N, 5.92. Found: C, 69.3; H, 11.4; N, 6.0.

(Z)-*N*-(2-Deoxy-2-L-leucylamino-β-D-glucopyranosyl)-*N*-tetradecyl-9-octadecenamide (7i). Yield 1.62 g (86.4 %): syrup; $[\alpha]_D$ +11.8° (*c* 1.1, dichloromethane). ^{13}C NMR see Table 1; MS (FAB): *m/z* 752 $(M+H)^+$, 750 $(M-H)^-$.

Anal. Calcd for $C_{44}H_{85}N_3O_6$ (752.18): C, 70.26; H, 11.39; N, 5.59. Found: C, 70.0; H, 11.6; N, 5.8.

(Z)-*N*-(2-Deoxy-2-glycylamino-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (7j). Crystallization from methanol (1.49 g, 79.4 %): mp 117-118 °C; $[\alpha]_D$ +17.2° (*c* 1.0, tetrahydrofuran); MS (FAB): *m/z* 752 $(M+H)^+$, 750 $(M-H)^-$.

Anal. Calcd for $C_{44}H_{85}N_3O_6$ (752.18): C, 70.26; H, 11.39; N, 5.59. Found: C, 70.2; H, 11.4; N, 5.7.

(Z)-*N*-(2-Deoxy-2-L-phenylalanylamino-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (7k). Yield 1.61 g (84.3 %): syrup; $[\alpha]_D$ +17.9° (*c* 1.0, dichloromethane); MS (FAB): *m/z* 766 $(M+H)^+$, 764 $(M-H)^-$.

Anal. Calcd for $C_{45}H_{87}N_3O_6$ (766.21): C, 70.54; H, 11.45; N, 5.48. Found: C, 70.3; H, 11.6; N, 5.7.

(Z)-*N*-(2-L-Alanylamino-2-deoxy-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (7l). Yield 1.62 g (76.8 %): syrup; $[\alpha]_D$ −1.1° (*c* 1.1, tetrahydrofuran); MS (FAB): *m/z* 842 $(M+H)^+$, 840 $(M-H)^-$.

Anal. Calcd for $C_{51}H_{91}N_3O_6$ (842.31): C, 72.72; H, 10.89; N, 4.99. Found: C, 72.6; H, 11.0; N, 5.1.

(Z)-*N*-(2-Deoxy-2-L-serylamino-β-D-glucopyranosyl)-*N*-octadecyl-9-octadecenamide (7m). Yield 1.70 g (87.1 %): mp 127-129 °C; $[\alpha]_D$ +18.7° (*c* 0.95, tetrahydrofuran); MS (FAB): *m/z* 782 $(M+H)^+$, 780 $(M-H)^-$.

Anal. Calcd for $C_{45}H_{87}N_3O_7$ (782.21): C, 69.10; H, 11.21; N, 5.37. Found: C, 68.9; H, 11.2; N, 5.5.

***N*-(2-Deoxy-2-glycylamino-β-D-glucopyranosyl)-*N*-tetradecyldodecanamide hydroacetate (BAY Q8939, 8).** A solution of **7d** (61.3 g, 100 mmol) in dry ethanol (120 mL) and acetic acid (5.9 g, 5.6 mL, 100 mmol) was diluted with distd water (400 mL) and lyophilized to give **8** as a colorless powder (66.05 g, 98 %): sintering from 100 °C, liquid melt from 115 °C; $[\alpha]_D$ +20.7° (*c* 1.0, methanol); MS (FAB): *m/z* 614 $(M+H)^+$, 636 $(M+Na)^+$.

Anal. Calcd for $C_{34}H_{67}N_3O_6 \times C_2H_4O_2$ (613.93 + 60.05): C, 64.16; H, 10.62; N, 6.23. Found: C, 64.3; H, 10.6; N, 6.3.

***N*-(2-Deoxy-2-L-leucylamino-β-D-glucopyranosyl)-*N*-octadecyldodecanamide hydroacetate (BAY R1005, 9).** A solution of **7e** (100 g, 137.7 mmol) in abs ethanol 100 mL) and acetic acid (8.27 g, 7.9 mL, 137.7 mmol) was diluted with distd water (1.9 L) and lyophilized to give **9** as a colorless powder (105.1 g, 97 %): melting range 60-70 °C; $[\alpha]_D$ +21.3° (*c* 1.0, ethanol); MS (FAB): *m/z* 726 $(M+H)^+$, 724 $(M-H)^-$.

Anal. Calcd for $C_{42}H_{83}N_3O_6 \times C_2H_4O_2$ (726.15 + 60.05): C, 67.22; H, 11.15; N, 5.34. Found: C, 66.9; H, 11.0; N, 6.3.

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