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New 6-amino-6-deoxy-glycoglycerolipids derived from 2-*O*-β-D-glucopyranosylglycerol: insights into the structure-activity relationship of glycoglycerolipids as anti-tumor promoters

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Abstract

As part of a project aimed at obtaining compounds capable of inhibiting tumor promotion, new 6-amino-6-deoxyglycoglycerolipids (AGGLs) derived from 2-*O*-β-D-glucopyranosyl-*sn*-glycerol were synthesised and tested for their anti-tumor-promoting activity using a short-term *in vitro* assay of the inhibition of Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The corresponding 6-amino-6-deoxy-β-D-octylglucosides were also prepared as simplified aminoglycolipid models and tested. Comparison with the activity of a series of previously studied glycoglycerolipids showed that replacing the 6-oxygen of the glucose moiety by a nitrogen atom greatly reduced the *in vitro* activity of the compounds. A two-stage mouse skin carcinogenesis test of two representative aminoglycoglycerolipids confirmed their reduced activity also in this *in vivo* model.

Key words: Aminoglycoglycerolipids; cancer chemoprevention; EBV-EA; AGGL

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1. Introduction

Glycoglycerolipids, such as mono- and digalactosyldiacylglycerols (MGDG, Figure 1, and DGDG) and the anionic sulfoquinovosylacylglycerols (SQAG, Figure 1), are widespread in nature especially in plants and bacteria as they are the main lipid components of the thylakoid membranes of chloroplasts and cyanobacteria, in which it is thought they play an important role in a variety of cell functions.[1] Their general structure consists of a carbohydrate moiety that is beta-(MGDG and DGDG) or alpha-linked (SQAG) to the *sn*-3 position of glycerol, which is acylated at the residual hydroxyls by fatty acids of different lengths and degrees of unsaturation.

Given the remarkable biological activities of some MGDGs[2] and particularly their ability to inhibit *in vitro* and *in vivo* tumor-promoting-activity[3,4] induced by some tumor promoters,[5] we have previously prepared a series of analogues based on the easily accessible 2-*O*-β-D-glucosylglycerol skeleton[6] in order to study their structure and anti-tumor-promoting activity relationships, obtaining in some cases more potent compounds than the natural ones.[7] More recently, we have also prepared 2-*O*-β-D-sulfoquinovosylacylglycerol analogues of SQAG, and compared their activity with that of the earlier compounds.[8,9] Interestingly, these compounds were significantly less active suggesting that the negative charge of the sulfonate group had a depressing effect on the activity.

Our continuing interest in the development of novel anti-tumor promoters has now led us to study the activity of nitrogen-containing glycoglycerolipids and, once again, nature inspired us for new compounds as glycoglycerolipids bearing an amino-group at the C-6 of the sugar are reported, namely 6-amino-6-deoxyglycoglycerolipids (AGGLs, Figure 1) although much rarer in nature than MGDGs and SQDGs (to the best of our knowledge only a few recent papers have described their isolation from algae[10-12] or plants[13,14]). All the reported compounds share a common structure in which 6-amino-6-deoxyglucose is alpha-linked to the *sn*-3 position of glycerol (the remaining glycerol hydroxyls are esterified with saturated[10-12,14] or unsaturated[13] fatty acids, and the amino group can be free[10,12-14] or acylated),[11,13] and they have anti-bacterial,[13] anti-tumor,[10,13] anti-stress,[14] free-radical scavenging,[12] and human Myt1 kinase inhibiting activities.[11] Remarkably, the Myt1 kinase inhibitory effect of 1,2-di-*O*-palmitoyl-3-*O*-(*N*-palmitoyl-6-amino-6-deoxy-α-D–glucopyranosyl)-*sn*-glycerol has independently prompted two different research groups to synthesise it because of its potential use in cancer studies and therapy,[15,16] and some analogues have also been prepared.[17]

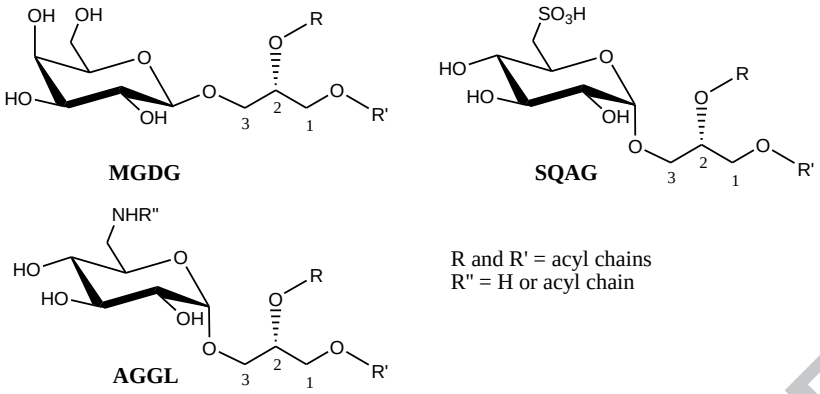


Figure 1 . Structures of natural monogalactosyldiacylglycerols (MGDG), sulfoquinovosylacylglycerols (SQAG) and 6-amino-6-deoxyglycoglycerolipids (AGGL).

In this paper we describe the synthesis and anti-tumor-promoting activity of new 6-amino-6-deoxyglycoglycerolipids derived from 2-*O*-β-D-glucopyranosyl-*sn*-glycerol carrying acyl chains of different lengths (hexanoyl and octadecanoyl) on the glycerol and sugar moieties. We prepared 2-*O*-(*N*-acyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerols **1a**-**b**, the corresponding 1-*O*-esters **2a**-**b**, octyl 6-amino-6-deoxy-β-D-glucopyranoside **3** and the related hexanoyl amide **4** (see Figure 2) as simplified aminoglycolipid models, and their anti-tumor-promoting activity was tested using a short-term *in vitro* assay of the inhibition of Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[7] The most active glycoglycerolipids **1a** and **2a** were also tested using an *in vivo* two-stage mouse skin carcinogenesis test, and the biological data were compared with those of the known compounds **5**-**10**.[9,18,19]

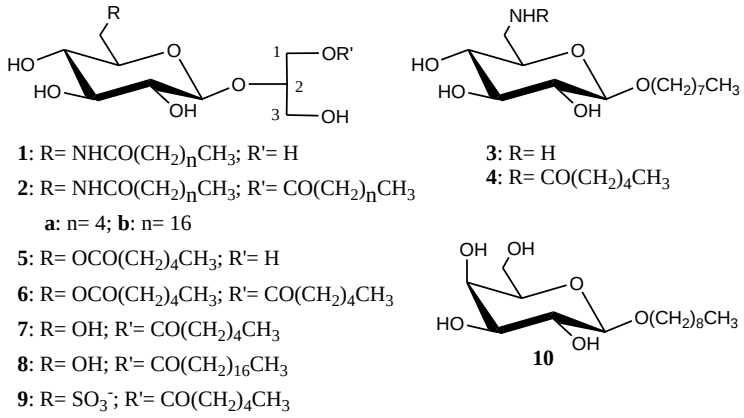


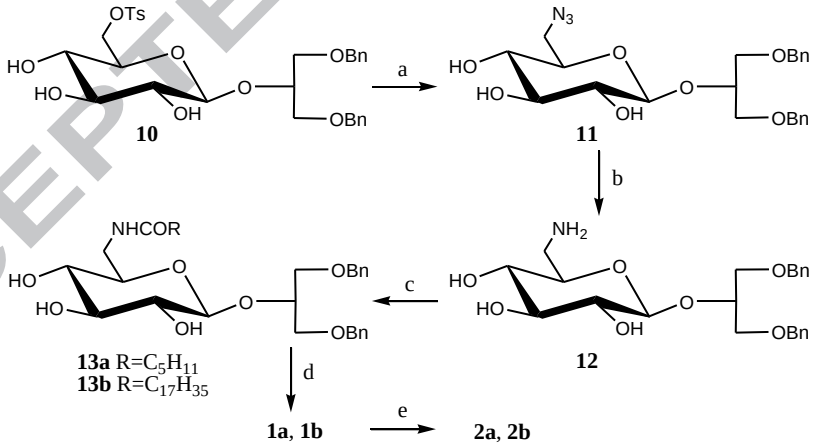
Figure 2. Structures of the studied compounds **1**-**4** and the reference compounds **5**-**10**.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of compounds 1a,b and 2a,b

Compounds **1a**,**b** and **2a**,**b** were efficiently obtained by means of the synthetic pathway shown in Scheme 1, which uses a lipase catalysed acylation to recover the 1-*O*-acylderivatives **2a**,**b** directly from **1a**,**b**. Treatment of the known tosylate **10**[9] with sodium azide in DMF, followed by the Staudinger reduction of the obtained azide **11** allowed the introduction of the amino function on C6 of the glucose moiety, thus affording the amino derivative **12**, which was acylated to the amides **13a**,**b** by means of the proper acyl chloride in pyridine. Debenzylation to the target compounds **1a**,**b** was finally obtained by means of Pd/C catalysed hydrogenolysis. Exploiting the known selectivity of lipase from *Pseudomonas cepacia* (LPS) for 2-*O*-β-D-glucopyranosylglycerol in lipase-mediated transesterification reactions in organic solvent,[6] we directly acylated the *sn*-1 positions of **1a**,**b** simply by treating them with the desired trifluoroethylester in pyridine (in the presence of LPS as catalyst), thus obtaining the *N*,*O*-diacylderivatives **2a**,**b** with good conversion yields (see Experimental).

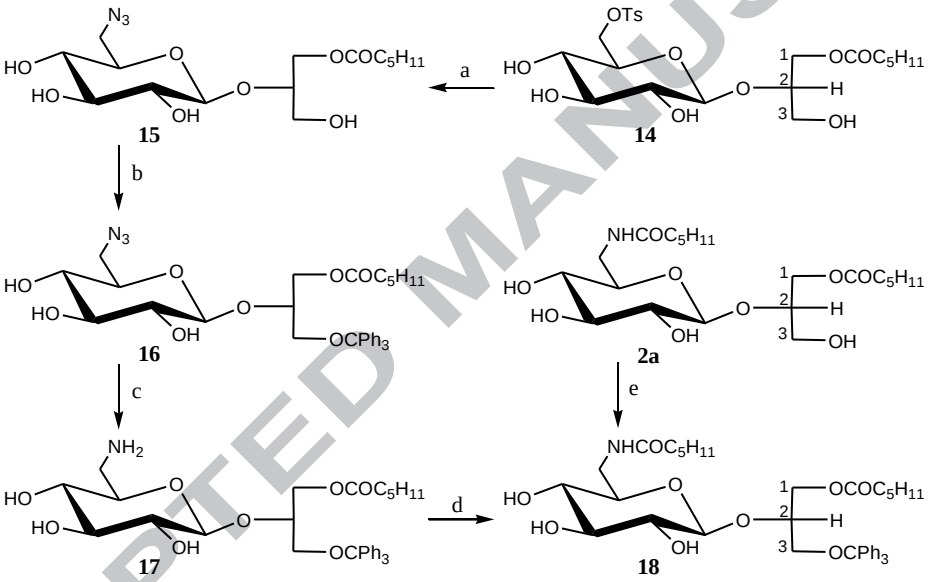


Scheme 1. a) NaN_3, DMF; b) PPh_3 polymer-bound, THF/H_2O (90/10); c) RCOCl, Py; d) H_2, Pd/C, MeOH; e) LPS, Py, CF_3CH_2OCOR.

2.1.2. Configuration assignment of compounds 2a,b

As the procedure used to obtain compounds **2a** and **2b** was the same except for the different acyl carriers used in the final enzymatic step of the synthesis (step e, Scheme 1), only the configuration of compound **2a** was assigned (see Scheme 2). As can be seen, **2a** was correlated to the known 1-*O*-

hexanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol **14**[9] by way of the trityl derivative **18**, which was directly obtained from **2a** by selectively tritylating the glycerol primary hydroxyl with trityl chloride in pyridine (step e). The same compound **18** was obtained in four steps starting from tosylate **14**, which was first converted into the corresponding azide **15** by means of sodium azide treatment in DMF. Subsequent tritylation afforded the trityl derivative **16** and a Staudinger reduction yielded the amine **17**, which was finally transformed (step d) to the target 3-*O*-trityl amide **18** that was identical to that directly obtained from **2a**.



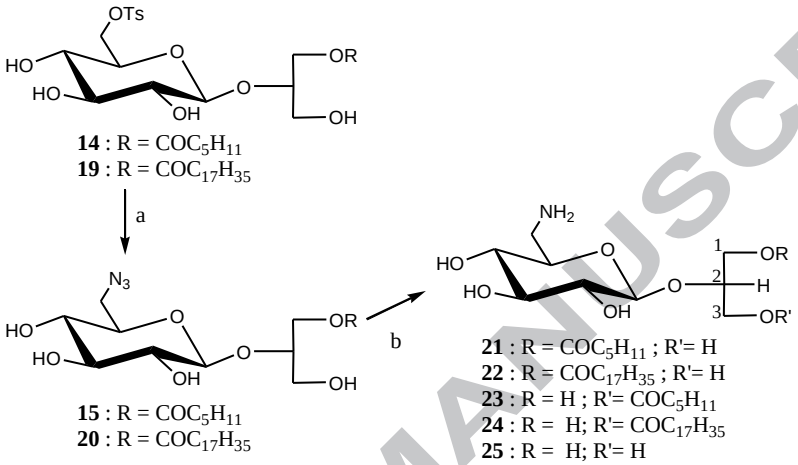
Scheme 2. a) NaN_3, DMF; b) $CClPh_3$, Py 100°C; c) PPh_3 polymer-bound, THF/H_2O (90/10); d) $C_5H_{11}COCl$, Py ; e) $CClPh_3$,Py 100°C

2.1.3. Synthesis of compounds 3 and 4

In addition to the 6'-amides **1a**,**b** and **2a**,**b**, we thought it necessary to obtain the related 6'-amino compounds in order to compare the influence on biological activity of the free *vs* acylated amino group at position 6 of the sugar. We therefore attempted to obtain analogues carrying the free amino group at C6 of the glucose moiety using an alternative route that should have allowed us to obtain both the 6'-NH_2 and the N-acylated derivatives as shown in Scheme 3.

1-*O*-Hexanoyl or octadecanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerols **14** or **19**[9] easily provided the corresponding azides **15** and **20** (see experimental) but, unfortunately, the Staudinger reduction promoted the migration of the acyl chain from the 1-*O* to 3-*O* position of *sn*-glycerol.

Complex mixtures of the amino derivatives **21**, **22** or **23**, **24** were obtained after column chromatography, as shown by their TLC (CH_2Cl_2:MeOH 8:2, NH_3 2%) and ^{1}H-NMR analyses in deuterated methanol at room temperature (see Figure 3 for the hexanoyl derivatives). In particular, the migration and formation of the fully deacylated 6-amino-6-deoxy-2-*O*-β-D-glucopyranoside **25** were clearly observed by collecting ^{1}H-NMR spectra at different times (Figure 3).



Scheme 3. a) NaN_3, DMF; b) PPh_3 polymer-bound, THF/H_2O (90/10)

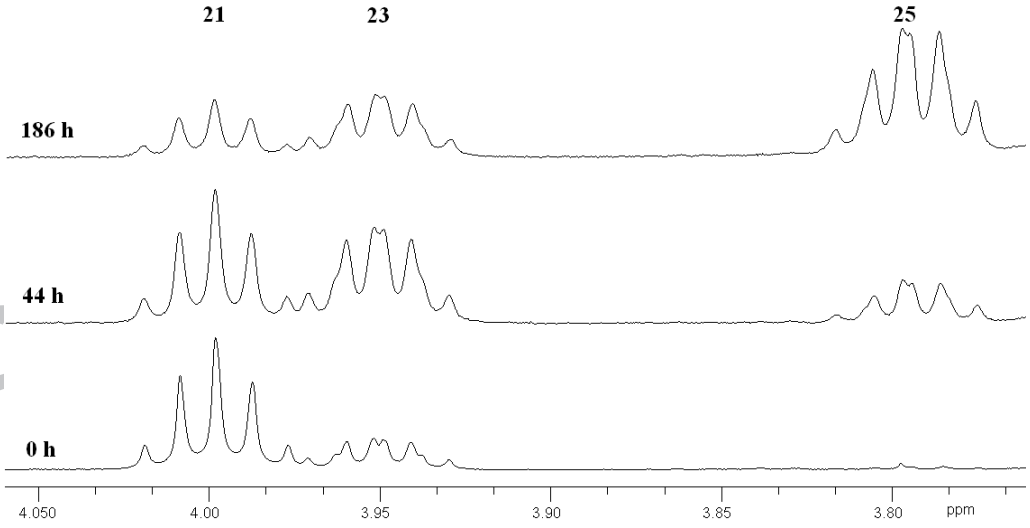
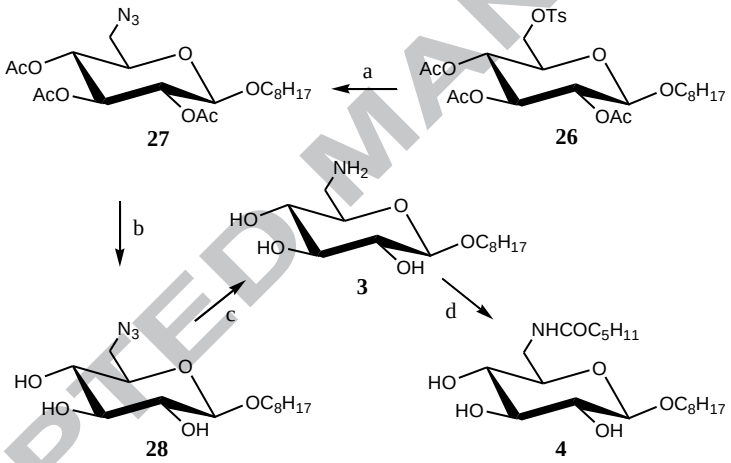


Figure 3. ^{1}H-NMR resonances of glycerol H-2 of compounds **21**, **23** and **25** in deuterated methanol at room temperature. The spectra were collected at 0, 44 and 186 hours.

Given the observed intrinsic instability of the 1-*O*-acyl-6'-aminoglucosylglycerols, we did not explore different means of synthesis (e.g. by removing the trityl group from **17**), but decided to test

the role of the 6'-NH_2 in octyl 6-amino-6-deoxy-β-D-glucopyranoside (**3**) as a simple model that has both a lipophilic aglycone and the 6-amino group on the hydrophilic sugar head. The synthesis (Scheme 4) started from the sodium azide treatment of octyl 2,3,4-tri-*O*-acetyl-6-*O*-tosyl-β-D-glucopyranoside (**26**) (obtained from the one-pot tosylation/acetylation[20] of commercially available octyl β-D-glucopyranoside, see experimental), which afforded octyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside **27**. Deacetylation by means of the Zemplén reaction yielded the azide **28**, which was finally reduced by means of a Staudinger procedure to the amine **3** (direct reduction of **27** yielded the acetyl migration compound octyl 2,3-di-*O*-acetyl-*N*-acetyl-6-amino-6-deoxy-β-D-glucopyranoside, data not shown). Octyl *N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranoside (**4**) was also prepared by treating **3** with hexanoyl chloride in pyridine in order to obtain the reference *N*-acylated derivative (Scheme 4).



Scheme 4. a) NaN_3, DMF; b) MeONa, MeOH; c) PPh_3 polymer-bound, THF/H_2O (90/10); d) $C_5H_{11}COCl$, Py.

2.2. Biological evaluation

2.2.1. EBV-EA assay for antitumor promoters

It is known that Epstein-Barr virus (EBV) is activated by tumor promoters to produce viral early antigens (EAs), and evaluating its inhibition is often used as a primary screening for *in vitro* anti-tumor-promoting activities.[4] The inhibitory effects of AGGLs **1a**,**b** and **2a**,**b** and 6-aminoglucosides **3** and **4** were assayed using a short-term *in vitro* assay of the EBV-EA activation induced in Raji cells by the tumor promoter TPA (as described in Refs. 3 and 21). Table 1 shows the *in vitro* inhibitory activity of compounds **1**-**4** and, for purposes of comparison, the effects of the previously

studied 2-*O*-(6-*O*-hexanoyl-β-D-glucopyranosyl)-*sn*-glycerol (**5**),[18] 1-*O*-hexanoyl-2-*O*-(6-*O*-hexanoyl-β-D-glucopyranosyl)-*sn*-glycerol (**6**),[18] 1-*O*-hexanoyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (**7**),[18] 1-*O*-octadecanoyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (**8**)[9], 1-*O*-hexanoyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerol (**9**),[9] and nonyl β-D-galactopyranoside (**10**)[19] are also reported. Compounds **1**-**4** were only weak cytotoxic against Raji cells (50% viability at 1000 mol ratio/TPA, Table 1) and, in general, all of the compounds were poorly active as indicated by their inhibitory percentages in comparison with control values (400-570 IC_{50}, mol ratio/TPA, Table 1). However, comparison of the short acyl chain derivatives, hexanoyl-amides **1a** and **2a** (IC_{50} 400 and 465, Table 1), with the highly active hexanoates **5** and **6** (IC_{50} 51.8 and 37.3, Table 1, Ref. 18) revealed a remarkable effect. Substituting the 6'-ester oxygen with a nitrogen atom greatly decreased the activity, as can be seen by comparing the corresponding IC_{50} values shown in Table 1 (51.8 *vs* 400 for **5** *vs* **1a** and 37.3 *vs* 465 for **6** *vs* **2a**). The highly negative effect induced by the nitrogen atom was similar to that observed in the case of the SQAG hexanoate **9**,[9] in which an anionic sulfonate head substitutes the primary hydroxyl of glucose (IC_{50} 29.3 *vs* 417 for **7** *vs* **9**, Table 1). However, the same strong depressing effect was not observed when the long chain octadecanoyl-amide **1b** (IC_{50} 502, Table 1) was compared with the slightly active 1-*O*-octadecanoyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (**8**) (IC_{50} 550, Table 1, Ref. 9), thus confirming that, as already reported,[9] the effects of two negative groups such as a long acyl chain[22] and a nitrogen atom are not additive.

The activity of octylglucosides **3** and **4** (IC_{50} 489 and 474 respectively, Table 1) also seemed to be much less than that of compounds **5** and **6** (IC_{50} 51.8 and 37.3, Table 1, Ref. 18), although in principle the reduction could be due to both the presence of the nitrogen atom and the lack of the glycerol backbone, as previously observed in the case of nonyl β-D-galactopyranoside (**10**) (IC_{50} 410, Table 1, Ref. 19) (the nature of the glycosyl moiety, *e.g* galactose *vs* glucose, plays a secondary role in the EBV inhibition of glycoglycerolipids)[22]. Nevertheless, the data relating to these simple glycosides allowed us to compare the effect of the amino *vs* hydroxyl group: the presence of the amino group in **3** reduced its activity in comparison with **10** (IC_{50} 489 *vs* 410, Table 1). Furthermore, the fact that there was no difference in the inhibitory effects of 6-aminoglucoside **3** and its amide **4** (IC_{50} 489 *vs* 474, Table 1) suggests that nitrogen (acylated or not) also plays a role in the lower level of activity of glycoglycerolipids.

Table 1

Inhibitory effects of **1a-b, 2a-b**, **3**, **4** and **5-9** on TPA-induced EBV-EA activation.

	Concentration (mol ratio/TPA)				
	1000	500	100	10	
	% to control ± SD (n = 3)[a]				IC_{50}
1a	11.5±1.0(50)	53.7±2.0(60)	80.6±1.5(80)	100±0.3(80)	400
2a	14.3±1.2(50)	55.2±2.1(60)	81.3±1.7(80)	100±0.3(80)	465
1b	19.8±1.6(50)	58.4±2.0(60)	84.1±1.9(80)	100±0.5(80)	502
2b	23.5±1.9(50)	62.5±2.3(60)	87.0±1.9(80)	100±0.3(80)	570
3	17.3±1.5(50)	57.4±2.3(60)	83.9±1.8(80)	100±0.5(80)	489
4	15.2±1.3(50)	56.3±2.0(60)	83.4±1.6(80)	100±0.5(80)	474
5[b]	0.0±0.0(70)	19.3±0.3(>80)	37.6±1.5(>80)	78.1±1.8(>80)	51.8
6[b]	0.0±0.0(70)	15.1±0.5 (80)	34.6±1.2(80)	71.5±1.9(80)	37.3
7[b]	0.0±0.0(70)	10.6±0.6 (70)	30.9±1.8(80)	68.2±2.5(80)	29.3
8[c]	19.6±0.7(60)	57.4±2.2(70)	90.6±0.5(80)	100±0.0(80)	550
9[c]	14.3±1.2(50)	53.6±2.0(70)	80.8±1.9(80)	100±0.5(80)	417
10[d]	12.4±0.3(70)	50.6±1.7(>80)	80.5±2.3(>80)	100±0.3(>80)	410

[a] Values are EBV-EA activation (%) in the presence of the test compound relative to the control (100%). Activation was attained by treatment with TPA (32 pmol/mL). IC_{50} represents the mol ratio of compound, relative to TPA, required to inhibit 50% of the positive control activated with TPA (32 pmol/mL). [b]See ref.18. [c]See ref.9. [d]See ref.19.

2.2.2. Two-stage mouse skin carcinogenesis test

Compounds **1a** and **2a**, as representatives of N-containing glycoglycerolipids, were also submitted to an *in vivo* two-stage mouse skin carcinogenesis test of mouse skin papillomas using DMBA as an initiator and TPA as a promoter. After 20 weeks of promotion, there was no statistically significant difference in body weight between the control and treatment groups. Activity was estimated on the basis of incidence (percentage of mice bearing papillomas, Fig. 4A) and multiplicity (average number of papillomas per mouse, Fig. 4B). After ten weeks of promotion, 80% of the mice in the control group (TPA treatment alone) bore papillomas (Fig. 4A) and, after 10 and 20 weeks, the number of papillomas per mouse was respectively two and eight (Fig. 4B). Both of the tested compounds poorly inhibited tumor promotion, and did not reduce the percentage of mice bearing papillomas after 20 weeks of treatment, but only made the process slightly slower. After ten weeks of promotion 40% of the mice treated with **1a** bore papillomas, and 50% of those treated with **2a**; after 20 weeks of promotion, all of the mice in both groups were affected (Fig. 4A). The decrease in the number of papillomas per mouse was also poor: after ten weeks of promotion, the affected mice treated with **1a** had 1.6 papillomas (76% with respect to the control group) and those treated with **1b** had 1.7 (81%), (Fig. 4B); the corresponding figures after 20 weeks were 5.8 (73%) and 6.2 (78%) (Fig. 4B). In comparison, mice treated with 1-*O*-hexanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**7**) (a similar glycoglycerolipid whose *in vivo* activity was tested)[23] for 20 weeks showed an incidence of 80% and a multiplicity of 4.0 papillomas per mouse (44% of the number in the control group) (Figs. 4A and 4B). Overall, the results of the *in vivo* experiments are in line with the *in vitro* findings, and confirmed that the protective effect of **1a** and **2a** against TPA tumor-promoting activity was markedly reduced.

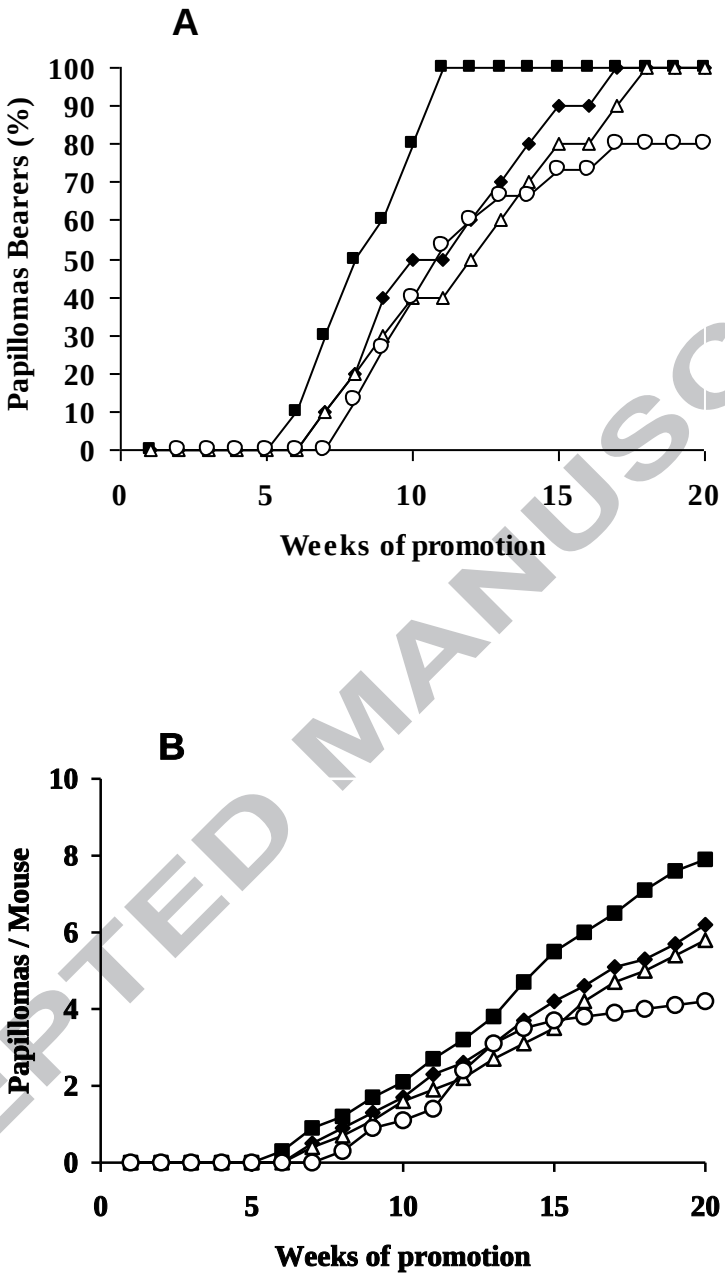


Figure 4. Inhibitory effects of compounds **1a** and **2a** (85 nmol) on DMBA-TPA mouse skin carcinogenesis. All mice were initiated with DMBA (390 nmol) and promoted with TPA (1.7 nmol) twice a week starting one week after initiation. A: percentage of mice with papillomas (papilloma incidence); B: averaged number of papillomas per mouse (papilloma multiplicity). (■, TPA alone; Δ, TPA + **1a**; ◆, TPA + **2a**, ○ TPA + **7**). At 20 weeks of promotion the averaged number of papillomas per mouse was reduced, with respect to the control group (7.9 ± 1.8), to 5.8 ± 1.1 for **1a** ($P < 0.1$), 6.2 ± 1.3 for **2a** ($P < 0.1$) and 4.0 ± 0.9 ($P < 0.001$) for **7** (control group 9.1 ± 1.6) [23].

3. Conclusion

It is not yet known what molecular target is responsible for the anti-tumor-promoting activity of glycolglycerolipids in the EBV-EA assay. However, as some are able to inhibit PKC translocation to the plasma membrane in TPA-treated fibroblasts,[24] it is possible that this kinase (the most important target of phorbol esters and the main signalling molecule involved in their carcinogenic effects)[25,26] could be targeted by this class of compounds. As a part of a project aimed at modifying bioactive glycoglycerolipids in order to obtain compounds capable of inhibiting tumor promotion, in this work the AGGL **1a-b** and **2a-b**, based on 2-*O*-β-D-glucopyranosylglycerol, were efficiently prepared and their ability to inhibit TPA induced tumor-promotion was studied. Although they were less active than previously studied MGDG analogues, they provided further interesting information concerning the structure/anti-tumor-promoting activity of glycoglycerolipids. In particular, the *in vitro* and *in vivo* biological assays showed that the *N*-hexanoyl-6'-amino derivatives **1a** and **2a** were much less active than the corresponding potent 6'-*O*-acylated glucoglycerolipids **5** and **6**, even though the only structural difference was the replacement of an oxygen atom by a nitrogen atom. As in the case of the anionic 6'-sulfonate **9**, which was scarcely active in comparison with **5** and **6**,[9] the reduced activity of the newly prepared **1a** and **2a** was clearly related to the manipulation of the 6-position of the sugar, which therefore seems to play a crucial role in the anti-tumor promoting activity of glycoglycerolipids. Finally, 2-*O*-β-D-glucopyranosylglycerol-based AGGLs represent new and easily accessible analogues of rare natural bioactive AGGLs, thus offering new tools for the study of AGGL-related biological activities.

4. Experimental

4.1. Chemical procedures

4.1.1. Materials

Pseudomonas cepacia lipase (LPS, lipase PS, specific activity 30.5 triacetin units/mg solid), from Amano Pharmaceutical Co (Mitsubishi Italia), was supported on celite.[6] All reagents were bought at highest commercial quality and used without further purification except where noted. Air- and moisture-sensitive liquids and solutions were transferred *via* oven-dried syringe or stainless steel cannula through septa. Evaporation under reduced pressure was always effected with a bath temperature below 45°C. Dry solvents and liquid reagents were distilled prior to use. Pyridine (Py) and dichloromethane were distilled from calcium hydride; dimethylformamide (DMF) was dried on

activated 4 Å molecular sieves. The acyl carriers, 2,2,2-trifluoroethyl esters, were synthesized according to reference 27. 1,3-Di-*O*-benzyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol **10**, 1-*O*-hexanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol **14** and 1-*O*-octadecanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol **19** were synthesized according to a literature procedure.[9] Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected. The structures of all the new synthesized compounds were confirmed through full ^{1}H and ^{13}C NMR characterization and mass spectroscopy. ^{1}H NMR analysis were performed at 500 MHz with a Bruker FT-NMR AVANCE™ DRX500 spectrometer using a 5 mm z-PFG (pulsed field gradient) broadband reverse probe at 298 K, and ^{13}C NMR spectra at 125.76 MHz were done of all the new compounds. The signals were unambiguously assigned by 2D COSY and HSQC experiments (standard Bruker pulse program). Chemical shifts are reported as δ (ppm) relative to residual $CHCl_3$, CH_3OH or Py fixed at 7.24, 3.30 and 7.19 (higher field line) ppm, respectively, for ^{1}H NMR spectra and relative $CDCl_3$ fixed at 77.0 (central line), CD_3OD at 47.0 (central line) or Pyd_5 123.0 (higher field line) ppm for ^{13}C NMR spectra; scalar coupling constants are reported in hertz. Mass spectra were recorded in negative or positive-ion electrospray (ESI) mode on a Thermo Quest Finnigan LCQTM DECA ion trap mass spectrometer; the mass spectrometer was equipped with a Finningan ESI interface; sample solutions were injected with a ionization spray voltage of 4.5 kV or 5.0 kV (positive and negative-ion mode, respectively), a capillary voltage of 32 V or -15 V (positive and negative-ion mode, respectively), and capillary temperature of 250 °C. Data were processed by Finnigan Xcalibur software system. All reactions were monitored by TLC on silica gel 60 F-254 plates (Merck), spots being developed with an anysaldehyde-based reagent, and subsequently heated at 110 °C. Flash column chromatography was performed on silica gel 60 (230-400 mesh, Merck). TLC, NMR and MS analysis confirmed purity of all synthesized compounds (see supplementary material for ^{13}C and ^{1}H NMR spectra). High resolution mass spectra (HRMS) of the target compounds (**1a-b**, **2a-b**, **3** and **4**) and of tritylated **18** (the key compound for the configuration assignment) were also recorded at C.I.G.A. (Centro Interdipartimentale Grandi Attrezzature, University of Milan) on a Fourier Transform Ion Ciclotron Resonance (FT-ICR) mass spectrometer APEX II & Xmass software (Bruker Daltonics) – 4.7 T magnet (Magnex), in positive-ion electrospray (ESI) mode. Samples were injected as <1 to 5 μg/mL methanol solutions and eluted with methanol (120 μL/h). FT-IR spectra of the same compounds were registered on a PerkinElmer instrument (mod. FT-IR spectrum one, PerkinElmer, Waltham, MA, USA) equipped with universal attenuated total reflection (ATR) sampling.

4.1.2. Synthesis of compounds 1a and 1b

4.1.2.1. 1,3-di-*O*-benzyl-2-*O*-(6-azido-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (**11**)

1,3-Di-*O*-benzyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol **10**[9] (1.2 g, 2.04 mmol) was dissolved in DMF (15 mL), NaN_3 (0.57 g, 8.77 mmol) was added, the reaction mixture was warmed to 85 °C and stirred for 6 h under Argon atmosphere (TLC, CH_2Cl_2:CH_3OH, 90:10, v/v). The suspension was filtered and evaporated under vacuum, yielding a crude compound, which was purified by flash chromatography (CH_2Cl_2:CH_3OH, 90:10, v/v) to yield azide **11** (0.66 g, 1.44 mmol, 70% yield) as a light yellow oil: $[\alpha]_D^{20} = -2.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.36-3.52 (m, 6H, H-2', H-3', H-4', H-5', H-6'a, H-6'b), 3.54-3.66 (m, 4H, H-1a, H-1b, H-3a and H-3b), 4.05 (m, 1H, H-2), 4.45 (d, $J_{1',2'}$= 7.8 Hz, 1H, H-1'), 4.45-4.53 (m, 4H, 2 CH_2Ph), 7.23-7.34 (m, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$): δ = 51.41 (C6'), 70.18 (C1 or C3), 70.45 (C3 or C1), 70.69, 73.25, 73.42 (O*C*H_2Ph), 73.45 (O*C*H_2Ph), 75.30, 76.10, 77.11 (C2), 102.88 (C1'), 127.75 (Ph), 127.78 (Ph), 127.82 (Ph), 127.85 (Ph), 128.38 (Ph), 128.47(Ph), 137.63 (Ph), 137.76 (Ph). ESI-MS (CH_3OH, positive-ion mode): m/z = 482.2 $[M+Na]^+$. Calcd for $C_{23}H_{29}N_3O_7$, m/z 459.2 [M].

4.1.2.2. 1,3-di-*O*-benzyl-2-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (**12**)

Azide **11** (0.64 g, 1.39 mmol) was dissolved in THF/H_2O (90/10, 14 mL) and polymer-bound PPh_3, (3 mmol/g, 0.95 g, 2.85 mmol), used to efficiently remove triphenylphosphine oxide by-product, was added. The reaction mixture was left under stirring overnight at 40°C (TLC, CH_2Cl_2:CH_3OH, 80:20, v/v), then filtered and the resin repeatedly washed with MeOH. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The obtained crude compound was purified by flash chromatography (CH_2Cl_2:CH_3OH, 85:15, v/v, 2% NH_3) to afford amine **12** (0.51 g, 1.18 mmol, 85%) as an oil: $[\alpha]_D^{20} = -10.0$ (*c* 1, CH_3OH); ^{1}H NMR (MeOD): δ 2.68 (dd, $J_{5',6'a}$=7.0 Hz, $J_{6'a,6'b}$=13.3 Hz, 1H, H-6'a), 2.98 (dd, $J_{5',6'b}$=2.5 Hz, 1H, H-6'b), 3.12-3.18 (m, 2H, H-4' and H-5'), 3.20 (dd, $J_{1',2'}$= 7.8 Hz, $J_{2',3'}$=9.3 Hz, 1H, H-2'), 3.34 (dd, $J_{3',4'}$=9.0 Hz, 1H, H-3'), 3.61-3.72 (m, 4H, H-1a, H-1b, H-3a and H-3b), 4.07 (m, 1H, H-2), 4.47 (d, 1H, H-1'), 4.50-4.56 (m, 4H, 2 CH_2Ph), 7.24-7.35 (m, 10H, 2Ph); ^{13}C-NMR (MeOD): δ = 41.75 (C6'), 69.04 (C1 or C3), 69.38 (C3 or C1), 71.21 (C4'), 72.32 (O*C*H_2Ph), 72.46 (O*C*H_2Ph), 73.20 (C2'), 75.55 (C5'), 75.69 (C3'), 76.19 (C2), 101.98 (C1'), 126.76 (Ph), 126.92 (Ph), 126.99 (Ph), 127.40(Ph), 137.44 (Ph), 137.49 (Ph). ESI-MS (CH_3OH, positive-ion mode): m/z = 434.1 $[M+1]^+$. Calcd for $C_{23}H_{31}NO_7$, m/z 433.21 [M].

4.1.2.3. General procedure for the synthesis of amides 13a and 13b

To a solution of compound **12** (0.23 g, 0.53 mmol) in dry pyridine (3.5 mL) at 0 °C the proper acyl chloride (0.79 mmol) was added and the reaction mixture was stirred at rt for 4 h (hexanoyl chloride) or 7 h (octadecanoyl chloride) (TLC, CH_2Cl_2:CH_3OH, 80:20, v/v). After quenching with CH_3OH, the solvent was evaporated under vacuum by repeated adding of toluene and the obtained crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, from 90:10, to 80:20 v/v).

4.1.2.3.1. 1,3-di-*O*-benzyl-2-*O*-(*N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol 13a: 0.20 g (0.38 mmol, 72% yield); oil: $[\alpha]_D^{20} = -19.8$ (*c* 1, CH_3OH); ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, *J*=7.0 Hz, 3H, CH_3), 1.21-1.34 (m, 4H, $2CH_2$), 1.57 (m, 2H, CH_2), 2.13 (m, 2H, CH_2), 3.04 (ddd, $J_{5',6'a}$=2.7 Hz, $J_{6'a,6'b}$=14.8 Hz, $J_{6'a,NH}$= 4.6, 1H, H-6'a), 3.12 (dd, $J_{3',4'}$=9.6 Hz, $J_{4',5'}$=9.6 Hz, 1H, H-4'), 3.21 (ddd, $J_{5',6'b}$=2.7 Hz, 1H, H-5'), 3.36 (dd, $J_{1',2'}$= 7.8 Hz, $J_{2',3'}$=9.1 Hz, 1H, H-2'), 3.52-3.62 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-3'), 3.92 (ddd, $J_{6'b,NH}$= 8.4 Hz, 1H, H-6'b), 4.04 (m, 1H, H-2), 4.45 (d, 1H, H-1'), 4.52 (br s, 4H, 2 CH_2Ph), 5.83 (dd, 1H, NH), 7.25-7.35 (m, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$): δ = 13.88 (CH_3), 22.30 (CH_2), 25.25 (CH_2), 31.38 (CH_2), 36.31 (CH_2), 39.81 (C6'), 69.74 (C4'), 70.12 (C1 or C3), 70.23 (C3 or C1), 73.26 (O*C*H_2Ph), 73.52 (O*C*H_2Ph), 73.95 (C2'), 74.81 (C3'), 75.13 (C5'), 78.46 (C2), 103.74 (C1'), 127.52 (Ph), 127.74 (Ph), 127.82 (Ph), 127.90 (Ph), 128.36 (Ph), 128.48 (Ph), 137.46 (Ph), 137.96 (Ph), 175.56 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 530.5 $[M-1]^-$. Calcd for $C_{29}H_{41}NO_8$, m/z 531.28 [M].

4.1.2.3.2. 1,3-di-*O*-benzyl-2-*O*-(*N*-octadecanoyl-6-amino-deoxy-6-β-D-glucopyranosyl)-*sn*-glycerol 13b: 0.25 g (0.36 mmol, 68% yield): oil; $[\alpha]_D^{20} = -62.0$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, *J*=7.0 Hz, 3H, CH_3), 1.13-1.37 (m, 28H, $14CH_2$), 1.58 (m, 2H, CH_2), 2.14 (m, 2H, CH_2), 3.02 (ddd, $J_{5',6'a}$=2.6 Hz, $J_{6'a,6'b}$=14.9 Hz, $J_{6'a,NH}$= 4.7, 1H, H-6'a), 3.12 (dd, $J_{3',4'}$=9.3 Hz, $J_{4',5'}$=9.3 Hz, 1H, H-4'), 3.22 (ddd, $J_{5',6'b}$=2.6 Hz, 1H, H-5'), 3.37 (dd, $J_{1',2'}$= 7.8 Hz, $J_{2',3'}$=9.1 Hz, 1H, H-2'), 3.51-3.62 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-3'), 3.95 (ddd, $J_{6'b,NH}$= 8.5 Hz, 1H, H-6'b), 4.05 (m, 1H, H-2), 4.45 (d, 1H, H-1'), 4.53 (br s, 4H, 2 CH_2Ph), 5.85 (dd, 1H, NH), 7.25-7.34 (m, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$): δ = 14.12 (CH_3), 22.69 (CH_2), 25.61 (CH_2), 29.85-29.20 (12 CH_2), 31.92 (CH_2), 36.39 (CH_2), 39.75 (C6'), 69.51 (C4'), 70.10 (C1 or C3), 70.28 (C3 or C1), 73.29

(O*C*H$_2$Ph), 73.57 (O*C*H$_2$Ph), 74.03 (C2'), 74.65 (C3'), 75.25 (C5'), 78.70 (C2), 103.97 (C1'), 127.55 (Ph), 127.79 (Ph), 127.87 (Ph), 127.98 (Ph), 128.40 (Ph), 128.54 (Ph), 137.38 (Ph), 137.95 (Ph), 175.80 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 698.5 $[M-1]^-$. Calcd for $C_{41}H_{65}NO_8$, m/z 699.47 [M].

4.1.2.4. General procedure for benzylic groups removal (preparation of 1a and 1b)

To a solution of compound **13a** or **13b** (0.31 mmol) in CH_3OH (10 mL) 10% Pd/C (0.15 g) was added under Argon atmosphere and the reaction mixture was stirred under H_2 (1 atm) for 8 h. Dilution with CH_3OH, filtering through a celite pad, washing with pyridine followed by CH_3OH and evaporation of the solvent to dryness afforded, after purification by flash chromatography (CH_2Cl_2:CH_3OH, 90:10, v/v), pure compounds **1a** and **1b**.

4.1.2.4.1. 2-*O*-(*N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (1a): 0.098 g (0.28 mmol, 90% yield); white solid, m.p.: 144-145 °C; $[\alpha]_D^{20}$ = – 24.4 (*c* 1, CH_3OH); ^{1}H NMR (MeOD): δ = 0.91 (t, *J*=7.0 Hz, 3H, CH_3), 1.26-1.39 (m, 4H, 2CH_2), 1.61 (m, 2H, CH_2), 2.21 (m, 2H, CH_2), 3.12 (dd, $J_{3',4'}$=9.1 Hz, $J_{4',5'}$=9.5 Hz, 1H, H-4'), 3.21 (dd, $J_{1',2'}$=7.8 Hz, $J_{2',3'}$=9.1 Hz, 1H, H-2'), 3.3 (ddd, 1H, H-5'), 3.36 (dd, 1H, H-3'), 3.39 (dd, $J_{5',6'a}$=6.6 Hz, $J_{6'a,6'b}$=14.0 Hz, 1H, H-6'a), 3.57 (dd, $J_{5',6'b}$=2.8 Hz, 1H, H-6'b), 3.61-3.70 (m, 4H, H-1a, H-1b, H-3a and H-3b), 3.74 (m, 1H, H-2), 4.39 (d, 1H, H-1'); ^{13}C-NMR (MeOD): δ = 12.27 (CH_3), 21.43 (CH_2), 24.74 (CH_2), 30.58 (CH_2), 35.00 (CH_2), 39.50 (C6'), 60.75 (C1 or C3), 61.17 (C3 or C1), 70.84 (C4'), 73.31 (C2'), 74.02 (C5'), 75.31 (C3'), 81.06 (C2), 102.36 (C1'), 175.01 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 350.3 $[M-1]^-$. Calcd for $C_{15}H_{29}NO_8$, m/z 351.19 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 374.17843 $[M+Na]^+$ (error, 0.3 ppm). Calcd for $C_{15}H_{29}NO_8Na$, m/z = 374.17854 (monoisotopic mass). FT-IR (ATR): ν_{max} 3312, 2918, 1650, 1534 cm^{-1}.

4.1.2.4.2. 2-*O*-(*N*-octadecanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (1b): 0.083 g (0.16 mmol, 51% yield): white solid, m.p.: 138 -141°C; $[\alpha]_D^{20}$ = – 16.6 (*c* 0.6, Py); ^{1}H NMR (Pyd_5): δ = 0.85 (t, *J*=7.0 Hz, 3H, CH_3), 1.15-1.36 (m, 28H, 14CH_2), 1.78 (m, 2H, CH_2), 2.40 (m, 2H,

CH_2), 3.85 (ddd, 1H, H-5'), 3.91 (dd, $J_{3',4'}$=9.4 Hz, $J_{4',5'}$=9.4 Hz, 1H, H-4'), 4.00-4.11 (m, 3H, H-6'a, H-6'b and H-2'), 4.15-4.26 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-3'), 4.37 (m, 1H, H-2), 5.11 (d, $J_{1',2'}$= 7.8 Hz, 1H, H-1'), 8.85 (dd, $J_{6'a,NH}$= 5.7 Hz, $J_{6'b,\ NH}$= 5.7 Hz, 1H, NH); ^{13}C-NMR (Pyd_5): δ = 13.77 (CH_3), 22.43 (CH_2), 25.79 (CH_2), 29.05-29.58 (12 CH_2), 31.62 (CH_2), 36.04 (CH_2), 40.85 (C6'), 62.20 (C1 or C3), 62.55 (C3 or C1), 72.10 (C4'), 75.02 (C2'), 75.91 (C5'), 77.04 (C3'), 83.02 (C2), 104.48 (C1'), 174.09 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 518.5 $[M-1]^-$. Calcd for $C_{27}H_{53}NO_8$, m/z 519.38 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 542.36595 $[M+Na]^+$ (error, 0.7 ppm). Calcd for $C_{27}H_{53}NO_8Na$, m/z = 542.36634 (monoisotopic mass). FT-IR (ATR): ν_{max} 3290, 2916, 2848, 1641, 1554 cm^{-1}.

4.1.3. Enzymatic synthesis of 1-*O*-esters 2a and 2b

4.1.3.1. 1-*O*-hexanoyl-2-*O*-(*N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-sn-glycerol (2a)

Compound **1a** (0.05 g, 0.14 mmol) was dissolved in dry pyridine (2.5 mL) and trifluoroethyl hexanoate (0.35 g, 1.77 mmol) and LPS (0.5 g) were added in the order. The suspension was stirred at 45°C overnight (TLC, CH_2Cl_2:CH_3OH, 90:10 v/v). After 24 h (about 90% conversion by TLC) the reaction was stopped by filtering off the enzyme which was washed with pyridine. After removing the solvent under vacuum, the crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, from 95:5 to 80:20, v/v) yielding pure **2a**: 0.051 g (0.114 mmol, 80% yield) as an oil; $[\alpha]_D^{20}$ = – 26.0 (*c* 1, CH_3OH); ^{1}H NMR (MeOD): δ = 0.91 (m, 6H, $2CH_3$), 1.27-1.38 (m, 8H, $4CH_2$), 1.57-1.65 (m, 4H, $2CH_2$), 2.21 (m, 2H, CH_2), 2.34 (m, 2H, CH_2), 3.12 (dd, $J_{3',4'}$=9.2 Hz, $J_{4',5'}$=9.2 Hz, 1H, H-4'), 3.17 (dd, $J_{1',2'}$= 7.8 Hz, $J_{2',3'}$=9.2 Hz, 1H, H-2'), 3.32 (ddd, 1H, H-5'), 3.35 (dd, 1H, H-3'), 3.38 (dd, $J_{6'a,5'}$= 6.7 Hz, $J_{6'a,6'b}$=14.0 Hz, 2H, H-6'a), 3.58 (dd, $J_{6'b,5'}$= 2.7 Hz, 1H, H-6'b), 3.65 (dd, $J_{3a,2}$= 5.3 Hz, $J_{3a,3b}$=11.7 Hz, 1H, H-3a), 3.67 (dd, $J_{3b,2}$= 4.7 Hz, 1H, H-3b), 3.95 (m, 1H, H-2), 4.19 (dd, $J_{1a,2}$= 5.0 Hz, $J_{1a,1b}$= 11.6 Hz, 1H, H-1a), (dd, $J_{1b,2}$= 5.8 Hz, 1H, H-1b), 4.40 (d, 1H, H-1'); ^{13}C-NMR (MeOD): δ 12.26 (CH_3), 12.30 (CH_3), 21.39 (CH_2), 21.44 (CH_2), 23.67 (CH_2), 24.77 (CH_2), 30.43 (CH_2), 30.59 (CH_2), 32.93 (CH_2), 35.00 (CH_2), 39.50 (C6'), 61.14 (C3), 62.54 (C1), 70.83 (C4'), 73.00 (C2'), 74.04 (C5'), 75.34 (C3'), 76.95 (C2), 102.10 (C1'), 173.40 (CO), 174.97 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 448.3 $[M-1]^-$. Calcd for

$C_{21}H_{39}NO_9$, m/z 449.26 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 472.25112 $[M+Na]^+$ (error, 1.2 ppm). Calcd for $C_{21}H_{39}NO_9Na$, m/z = 472.25170 (monoisotopic mass). FT-IR (ATR): ν_{max} 3301, 2931, 1741, 1644, 1547 cm^{-1}.

4.1.3.2. 1-*O*-octadecanoyl-2-*O*-(*N*-octadecanoyl-6-deoxy-6-amino-β-D-glucopyranosyl)-*sn*-glycerol (2b)

With the same procedure, apart from repeated flash chromatographies to remove stearic acid, starting from compound **1b** (0.05 g, 0.096 mmol), 0.029 g (0.037 mmol, 39% yield) of pure **2b** were obtained as a white solid: m.p.: 151-152°C; $[\alpha]_D^{20}$ = – 20.0 (c 1, Py). ^{1}H NMR (Pyd_5): δ = 0.87 (m, 6H, $2CH_3$), 1.18-1.33 (m, 56H, $28CH_2$), 1.65 (m, 2H, CH_2), 1.79 (m, 2H, CH_2), 2.35-3.43 (m, 4H, $2CH_2$), 3.86 (ddd, 1H, H-5'), 3.90 (dd, $J_{3',4'}$=8.5 Hz, $J_{4',5'}$=9.4 Hz, 1H, H-4'), 3.96 (dd, $J_{1',2'}$= 7.8 Hz, $J_{2',3'}$=8.5 Hz, 1H, H-2'), 4.04-4.09 (m, 2H, H-6'a, H-6'b), 4.09-4.14 (m, 2H, H-3a and H-3b), 4.18 (dd, 1H, H-3'), 4.44 (m, 1H, H-2), 4.67 (dd, $J_{1a,2}$= 4.8 Hz, $J_{1a,1b}$= 11.6 Hz, 1H, H-1a), 4.70 (dd, $J_{1b,2}$= 5.6 Hz, 1H, H-1b), 5.02 (d, 1H, H-1'), 8.60 (dd, $J_{6'a,NH}$= 6.0 Hz, $J_{6'b,NH}$= 6.0 Hz, 1H, NH); ^{13}C-NMR (Pyd_5): δ = 13.77 ($2CH_3$), 22.44 ($2CH_2$), 24.75 (CH_2), 25.81 (CH_2), 28.8-29.8 ($24CH_2$), 31.63 ($2CH_2$), 33.88 (CH_2), 36.07 (CH_2), 40.93 (C6'), 62.20 (C3), 63.82 (C1), 72.15 (C4'), 74.53 (C2'), 75.84 (C5'), 77.07 (C3'), 78.55 (C2), 104.23 (C1'), 173.15 (CO), 173.96 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 784.5 $[M-1]^-$. Calcd for $C_{45}H_{87}NO_9$, m/z 785.64 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 808.62704 $[M+Na]^+$ (error, 0.3 ppm). Calcd for $C_{45}H_{87}NO_9Na$, m/z = 808.62730 (monoisotopic mass). FT-IR (ATR): ν_{max} 3320, 2919, 2850, 1738, 1651, 1550 cm^{-1}.

4.1.4. Configuration assignment of 1-*O*-hexanoylamide 2a

Converging synthesis of 1-*O*-hexanoyl-3-*O*-trityl-2-*O*-(*N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol **18** was obtained starting from known tosylate **14**[9] or from **2a** as here reported. In this section the synthesis of octadecanoyl azide **20** starting from tosylate **19**[9] is also reported.

4.1.4.1. From 1-*O*-hexanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-sn-glycerol (14)

4.1.4.1.1. 1-*O*-hexanoyl-2-*O*-(6-azido-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (15)

Tosylate **14** (1.00 g, 1.97 mmol) was dissolved in DMF (10 mL), NaN_3 (0.55 g, 8.46 mmol) was added under stirring, the reaction mixture was warmed to 85 °C and stirred for 5 h under Argon atmosphere (TLC, CH_2Cl_2:CH_3OH, 90:10 v/v). The mixture was filtered, the DMF was co-evaporated with cyclohexane at reduced pressure and the crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, 95:5, v/v), yielding **15** (0.52 g, 1.38 mmol, 70% yield) as an oil; $[\alpha]_D^{20}$ = –25.6 (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J=7.0 Hz, 3H, CH_3), 1.21-1.35 (m, 4H, $2CH_2$), 1.59 (m, 2H, CH_2), 2.31 (m, 2H, CH_2), 3.37-3.58 (m, 6H, H-2', H-3', H-4', H-5', H-6'a and H-6'b), 3.66 (dd, $J_{3a,2}$=5.3 Hz, $J_{3a,3b}$=12.0 Hz, 1H, H-3a), 3.75 (dd, $J_{3b,2}$=2.0 Hz, 1H, H-3b), 3.96 (m, 1H, H-2), 4.19 (dd, $J_{1a,2}$=6.2 Hz, $J_{1a,1b}$=11.6 Hz, 1H, H-1a), 4.24 (dd, $J_{1b,2}$=5.5 Hz, 1H, H-1b), 4.46 (d, $J_{1',2'}$=7.7 Hz, 1H, H-1'); ^{13}C-NMR ($CDCl_3$): δ = 13.86 (CH_3), 22.26 (CH_2), 24.49 (CH_2), 31.23 (CH_2), 34.11 (CH_2), 51.33 (C6'), 62.51 (C1 or C3), 62.54 (C3 or C1), 70.59 (C4'), 73.03 (C2'), 75.16 (C5'), 75.98 (C3'), 77.77 (C2), 102.16 (C1'), 174.30 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z =376.0 $[M–1]^-$. Calcd for $C_{15}H_{27}N_3O_8$, m/z 377.18 [M].

4.1.4.1.2. 1-*O*-octadecanoyl-2-*O*-(6-azido-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (20)

With the same procedure, starting from tosylate **19** (0.10 g, 0.148 mmol), 0.06 g of **20** (0.11 mmol, 74% yield) were obtained as white solid (m.p.: 70-71 °C); $[\alpha]_D^{20}$ = –18.7 (c 1, CH_3OH). ^{1}H NMR (CD_3OD): δ = 0.89 (t, J=7.0 Hz, 3H, CH_3), 1.20-1.38 (m, 28H, $14CH_2$), 1.61 (m, 2H, CH_2), 2.35 (m, 2H, CH_2), 3.20 (dd, $J_{1',2'}$=7.8 Hz, $J_{2',3'}$=9.0 Hz, 1H, H-2'), 3.25 (dd, $J_{4',3'}$=9.0 Hz, $J_{4',5'}$=9.0 Hz, 1H, H-4'), 3.34 (dd, 1H, H-3'), 3.41 (dd, $J_{6'a,6'b}$=12.6 Hz, $J_{6'a,5'}$=6.6 Hz, 1H, H-6'a), 3.43 (ddd, $J_{5',6'b}$=1.8 Hz, 1H, H-5'), 3.51 (dd, 1H, H-6'b), 3.65 (dd, $J_{3a,2}$=5.3 Hz, $J_{3a,3b}$=11.6 Hz, 1H, H-3a), 3.70 (dd, $J_{3b,2}$=4.7 Hz, 1H, H-3b), 3.97 (m, 1H, H-2), 4.23 (dd, $J_{1a,2}$=5.9 Hz, $J_{1a,1b}$=11.7 Hz, 1H, H-1a), 4.27 (dd, $J_{1b,2}$=4.7 Hz, 1H, H-1b), 4.47 (d, $J_{1',2'}$=7.8 Hz, 1H, H-1'); ^{13}C-NMR (CD_3OD): δ = 12.45 (CH_3), 21.72 (CH_2), 23.97 (CH_2), 28.15-28.90 ($12CH_2$) 31.06 (CH_2), 32.97 (CH_2), 50.72 (C6'), 61.09 (C3), 62.65 (C1), 70.29 (C4'), 72.87 (C2'), 74.86 (C5'), 75.53 (C3'), 76.48 (C2), 101.95 (C1'), 173.46 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 544.3 $[M–1]^-$. Calcd for $C_{27}H_{51}N_3O_8$, m/z 545.37 [M].

4.1.4.1.3. 1-*O*-hexanoyl-3-*O*-trityl-2-*O*-(6-azido-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (16)

Azide **15** (0.29 g, 0.77 mmol) was dissolved in dry pyridine (5.0 mL), trityl chloride (0.43 g, 1.54 mmol) was added under stirring, the reaction mixture was warmed to 100 °C and stirred for 5 h (TLC, CH_2Cl_2:CH_3OH, 90:10 v/v). The solvent was evaporated under reduced pressure and the

crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, from 95:5 to 90:10, v/v) to afford **16** (0.322 g, 0.52 mmol, 68% yield) as an oil: $[\alpha]_D^{20}$ = + 10.8 (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.91 (t, J=7.0 Hz, 3H, CH_3), 1.25-1.37 (m, 4H, $2CH_2$), 1.59 (m, 2H, CH_2), 2.28 (m, 2H, CH_2), 3.27 (dd, $J_{3a,2}$= 7.5 Hz, $J_{3a,3b}$=9.5 Hz, 1H, H-3a), 3.32 (dd, $J_{1',2'}$= 7.7 Hz, $J_{2',3'}$=8.9 Hz, 1H, H-2'), 3.35-3.50 (m, 6H, H-3b, H-4', H-5', H-6'a and H-6'b), 3.53 (dd, $J_{3',4'}$= 8.9 Hz, 1H, H-3'), 3.97 (m, 1H, H-2), 4.26 (dd, $J_{1a,2}$= 5.7 Hz, $J_{1a,1b}$= 11.8 Hz, 1H, H-1a), 4.36 (d, 1H, H-1'), 4.55 (dd, $J_{1b,2}$= 3.0 Hz, 1H, H-1b), 7.23-7.48 (m, 15H, 3Ph); ^{13}C-NMR ($CDCl_3$): δ = 13.88 (CH_3), 22.25 (CH_2), 24.52 (CH_2), 31.23 (CH_2), 34.24 (CH_2), 51.27 (C6'), 62.84 (C3), 63.78 (C1), 70.53 (C4'), 73.56 (C2'), 74.99 (C5'), 76.21 (C3'), 77.83 (C2), 86.97 (O*C*Ph_3), 103.18 (C1'), 127.07 (3C, Ph), 127.80 (6C, Ph), 128.71 (6C, Ph), 143.70 (3C, Ph), 174.39 (CO). ESI-MS (CH_3OH, positive-ion mode): m/z = 642.3 $[M+Na]^+$. Calcd for $C_{34}H_{41}N_3O_9$, m/z 619.29 [M].

4.1.4.1.4. 1-*O*-hexanoyl-3-*O*-trityl-2-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (17)

Azide **16** (0.10 g, 0.16 mmol) was dissolved in THF/H_2O (90/10, 1.5 mL) and polymer-bound PPh_3 (3 mmol/g, 0.12 g, 0.36 mmol) was added. The reaction mixture was left under stirring overnight at 40 °C (TLC, CH_2Cl_2:CH_3OH, 90:10 v/v), then filtered washing with CH_3OH. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The obtained crude compound was purified by flash chromatography (CH_2Cl_2:CH_3OH, 90:10, v/v) to afford **17** (0.074 g, 0.125 mmol, 72%) as a solid; m.p.: 53-54 °C; $[\alpha]_D^{20}$ = +4.3 (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.85 (t, J=7.0 Hz, 3H, CH_3), 1.18-1.31 (m, 4H, $2CH_2$), 1.53 (m, 2H, CH_2), 2.23 (m, 2H, CH_2), 2.84-2.91 (m, 2H, H-6'a and H-6'b), 3.16-3.22 (m, 2H, H-5' and H-3a), 3.25 (dd, $J_{1',2'}$= 7.8 Hz, $J_{2',3'}$=9.0 Hz, 1H, H-2'), 3.31 (dd, $J_{3b,2}$= 5.2 Hz, $J_{3b,3a}$= 9.6 Hz, 1H, H-3b), 3.42 (dd, $J_{3',4'}$= 9.0 Hz, $J_{4',5'}$= 9.0 Hz, 1H, H-4'), 3.49 (dd, 1H, H-3'), 3.89 (m, 1H, H-2), 4.18 (dd, $J_{1a,2}$= 5.9 Hz, $J_{1a,1b}$= 11.8 Hz, 1H, H-1a), 4.30 (d, 1H, H-1'), 4.43 (dd, $J_{1b,2}$= 3.3 Hz, 1H, H-1b), 7.17-7.43 (m, 15H, 3Ph); ^{13}C-NMR ($CDCl_3$): δ = 13.90 (CH_3), 22.25 (CH_2), 24.50 (CH_2), 31.23 (CH_2), 34.19 (CH_2), 43.25 (C6'), 63.18 (C3), 63.88 (C1), 72.13 (C4'), 73.64 (C2'), 75.05 (C5'), 76.12 (C3'), 77.95 (C2), 86.88 (O*C*Ph_3), 103.61 (C1'), 127.11 (3C, Ph), 127.81 (6C, Ph), 128.66 (6C, Ph), 143.71 (3C, Ph), 174.17 (CO). ESI-MS (CH_3OH, positive-ion mode): m/z = 616.3 $[M+Na]^+$. Calcd for $C_{34}H_{43}NO_8$, m/z 593.3 [M].

4.1.4.1.5. 1-*O*-hexanoyl-3-*O*-trityl-2-*O*-(*N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (18)

To a solution of compound **17** (0.06 g, 0.10 mmol) in dry pyridine (1.0 mL) at 0 °C hexanoyl chloride (0.02 mL, 0.14 mmol) was added and the reaction mixture was stirred at rt for 5 h (TLC, CH_2Cl_2:CH_3OH, 85:15 v/v). After quenching with MeOH, the solvent was evaporated under vacuum by repeated adding of toluene and the obtained crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, from 95:5 to 90:10, v/v) yielding **18** (0.04 g, 0.058 mmol, 58% yield) as an oil. $[\alpha]_D^{20} = -59.5$ (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.82-0.88 (m, 6H, 2 CH_3), 1.14-1.33 (m, 8H, 4CH_2), 1.50 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 2.00 (m, 2H, CH_2), 2.25 (m, 2H, CH_2), 2.93 (ddd, $J_{5',6'a}$= 2.8 Hz, $J_{6a',6'b}$= 14.8 Hz, $J_{6a',NH}$= 4.5 Hz, 1H, H-6'a), 3.09 (dd, $J_{3',4'}$= 9.4 Hz, $J_{4',5'}$= 9.6 Hz, 1H, H-4'), 3.18 (dd, $J_{2,3a}$= 5.5 Hz, $J_{3a,3b}$= 9.7 Hz, 1H, H-3a), 3.21 (ddd, 1H, H-5'), 3.28-3.34 (m, 2H, H-2' and H-3b), 3.57 (dd, $J_{2',3'}$= 9.2 Hz, 1H, H-3'), 3.91-4.00 (m, 2H, H-2 and H-6'b), 4.17 (dd, $J_{1a,2}$= 6.4 Hz, $J_{1a,1b}$= 11.8 Hz, 1H, H-1a), 4.33 (dd, $J_{1b,2}$= 3.8 Hz, 1H, H-1b), 4.37 (d, 1H, H-1'), 5.58 (dd, $J_{6b,NH}$= 8.4 Hz, 1H, NH), 7.20-7.47 (m, 15H, 3Ph); ^{13}C-NMR ($CDCl_3$): δ = 13.87 (2CH_3), 22.24 (2CH_2), 24.47 (CH_2), 25.16 (CH_2), 31.20 (CH_2), 31.35 (CH_2), 34.13 (CH_2), 36.20 (CH_2), 39.53 (C6'), 63.76 (C3), 63.99 (C1), 69.56 (C4'), 73.80 (C2'), 74.53 (C3'), 74.87 (C5'), 77.82 (C2), 86.85 (O*C*Ph$_3$), 103.73 (C1'), 127.20 (3C, Ph), 127.80 (6C, Ph), 128.70 (6C, Ph), 143.75 (3C, Ph), 174.00 (CO), 175.65 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 690.4 $[M-1]^-$. Calcd for $C_{40}H_{53}NO_9$, m/z 691.37 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 714.36023 $[M+Na]^+$ (error, 1.4 ppm). Calcd for $C_{40}H_{53}NO_9Na$, m/z = 714.36125 (monoisotopic mass). FT-IR (ATR): ν_{max} 3426, 3058, 2927, 1737, 1657, 1524 cm^{-1}**.**

4.1.4.2. From 1-*O*-hexanoyl-2-*O*-(N-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-sn-glycerol (2a)

Compound **2a** (0.02 g, 0.044 mmol) was dissolved in dry pyridine (0.6 mL), trityl chloride (0.024 g, 0.086 mmol) was added under stirring, the reaction mixture was warmed to 100 °C and stirred for 6 h (TLC, CH_2Cl_2:CH_3OH, 90:10, v/v). The solvent was evaporated under reduced pressure and the crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, 95:5, v/v) yielding pure 1-*O*-hexanoyl-3-*O*-trityl-2-*O*-(*N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol **18** (0.024 g, 0.035 mmol, 79% yield), identical in its physico-chemical characteristics, NMR and MS spectra to compound **18** previously obtained from the known tosylate **14**.

4.1.5. Synthesis of compounds 3 and 4

4.1.5.1. Octyl 2,3,4-tri-*O*-acetyl-6-*O*-tosyl-β-D-glucopyranoside (**26**)

Tosyl chloride (0.76 g, 4.0 mmol) was added to a solution of octyl β-D-glucopyranoside (1g, 3.42 mmol) in pyridine (22 mL) at -5°C and left overnight at this temperature. After about 90% conversion of the starting material by TLC (ethyl acetate 100%), acetic anhydride (4.85 mL, 51.3 mmol) was added at 0°C under stirring. After 1 h the reaction mixture was left to gradually reach the room temperature (23°C). After 4 h the reaction was completed (TLC, petroleum ether:ethyl acetate 60:40 v/v), poured into ice/water 100 mL and extracted with CH_2Cl_2 (30 mL x 5). The collected organic layers were then washed with 5M hydrochloric acid (35 mL x 2), sodium bicarbonate (50 mL) and water (50 mL x 2), dried with sodium sulphate and concentrated under reduced pressure. Crystallization of the crude material (1.87 g) with a mixture of ethanol (18 mL) and CH_2Cl_2 (1 mL) yielded 1.332 g (2.33 mmol, 68% yield) of pure **26**. Mp: 109-110°C; $[\alpha]_D^{20}$ = -4.6 ($CHCl_3$, c=1.0); ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, J= 6.7, 3H, CH_3), 1.18-1.33 (m, 10H, 5 CH_2), 1.50 (m, 2H, CH_2), 1.96, 1.97 and 1.99 (3s, 9H, 3 CH_3CO), 2.43 (s, 3H, CH_3), 3.39 (m, 1H, OCHa), 3.71 (ddd, 1H, H-5), 3.75 (m, 1H, OCHb), 4.04 (dd, $J_{5,6a}$= 5.9 Hz, $J_{6a,6b}$= 10.9 Hz, 1H, H-6a), 4.09 (dd, $J_{5,6b}$= 3.0 Hz, 1H, H-6b), 4.41 (d, $J_{1,2}$= 7.9 Hz, 1H, H-1), 4.86 (dd, $J_{2,3}$= 9.5 Hz, 1H, H-2), 4.87 (dd, $J_{3,4}$= 9.5 Hz, $J_{4,5}$= 9.7 Hz, 1H, H-4), 5.14 (dd, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ = 14.03 (CH_3), 20.50, 20.54 and 20.55 (3 *C*H_3CO), 21.62 (*C*H_3Ph), 22.61 (CH_2), 25.80 (CH_2), 29.22 (CH_2), 29.24 (CH_2), 29.35 (CH_2), 31.77 (CH_2), 67.91 (C6), 68.87 (C4), 70.15 (OCH_2), 71.23 (C2), 71.58 (C5), 72.62 (C3), 100.60 (C1), 128.05 (2C Ph), 129.86 (2C Ph), 132.59 (1C Ph), 145.08 (1C Ph), 169.14, 169.46 and 170.16 (3 CO). ESI-MS (CH_3OH, positive-ion mode): m/z = 595.0 $[M+Na]^+$. Calcd for $C_{27}H_{40}O_{11}S$, m/z 572.23 [M].

4.1.5.2. Octyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (27)

Tosylate **26** (0.50 g, 0.87 mmol) was dissolved in DMF (5.0 mL), NaN_3 (0.25 g, 3.80 mmol) was added under stirring, the reaction mixture was warmed to 85°C and stirred for 3 h under Argon atmosphere (TLC, petroleum ether:AcOEt 70:30 v/v). The solution was diluted with water, extracted with CH_2Cl_2 (3 x 30 mL), the organic layer washed with water, dried over Na_2SO_4 and DMF was then co-evaporated with cyclohexane at reduced pressure, yielding a crude compound, which was purified by flash chromatography (petroleum ether:AcOEt, 80:20, v/v) to yield azide **27** (0.37 g, 0.84 mmol, 97% yield) as a wax: m.p.: 55-56°C; $[\alpha]_D^{20}$ = – 31.4 (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.85 (t, J=6.8, 3H, CH_3), 1.17-1.34 (m, 10H, 5CH_2), 1.54 (m, 2H, CH_2), 1.98, 2.00 and 2.01 (3s, 9H, 3 CH_3CO), 3.15 (dd, $J_{5,6a}$= 2.5 Hz, $J_{6a,6b}$= 13.3 Hz, 1H, H-6a), 3.39 (dd, $J_{5,6b}$= 7.6

Hz, 1H, H-6b), 3.46 (m, 1H, OCHa), 3.65 (ddd, 1H, H-5), 3.86 (m, 1H, OCHb), 4.50 (d, $J_{1,2}$= 8.0 Hz, 1H, H-1), 4.93 (dd, $J_{3,4}$= 9.6 Hz, $J_{4,5}$= 9.6 Hz, 1H, H-4), 4.96 (dd, $J_{2,3}$= 9.6 Hz, 1H, H-2), 5.18 (dd, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ = 14.04 (CH_3), 20.57 (3 *C*H_3CO), 22.61 (CH_2), 25.80 (CH_2), 29.21 (2 CH_2), 29.34 (CH_2), 31.77 (CH_2), 51.20 (C6), 69.83 (C4), 70.11 (OCH_2), 71.37 (C2), 72.64 (C3), 73.62 (C5), 100.60 (C1), 169.21, 169.50 and 170.24 (3 CO). ESI-MS (CH_3OH, positive-ion mode): m/z = 466.0 $[M+Na]^+$. Calcd for $C_{20}H_{33}N_3O_8$, m/z 443.23 [M].

4.1.5.3. Octyl 6-azido-6-deoxy-β-D-glucopyranoside (28)

CH_3ONa (3 mL, 1 M solution in CH_3OH) was added to a solution of compound **27** (0.35 g, 0.79 mmol) in dry CH_3OH (1.5 mL). The reaction was stirred overnight at room temperature (TLC CH_2Cl_2:CH_3OH 90:10 v/v), then it was neutralized with an ion-exchange resin (Dowex–50, H^+ form). The resin was filtered-off and the solution was concentrated under reduced pressure. The crude was purified by flash-chromatography (CH_2Cl_2:CH_3OH 90:10 v/v) to give the de-acetylated product **28** (0.215 g, 0.68 mmol, 86% yield) as a solid: m.p.: 71-72°C; $[\alpha]_D^{20}$ = – 48.7 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, *J*=6.8, 3H, CH_3), 1.18-1.35 (m, 10H, 5 CH_2), 1.61 (m, 2H, CH_2), 3.33-3,56 (m, 7H, H-2, H-4, H-5, H6a, H-6b, H-3 and OCHa), 3.85 (m, 1H, OCHb), 4.27 (d, $J_{1,2}$= 8.0 Hz, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ = 14.06 (CH_3), 22.63, 25.89, 29.26, 29.38, 29.61 and 31.82 (6 CH_2), 51.42 (C6), 70.36 (OCH_2), 70.89 (C4), 73.47 (C2), 75.22 (C5), 76.20 (C3), 102.37 (C1). ESI-MS (CH_3OH, negative-ion mode): m/z = 315.9 $[M-1]^-$. Calcd for $C_{14}H_{27}N_3O_5$, m/z 317.2 [M].

4.1.5.4. Octyl 6-amino-6-deoxy-β-D-glucopyranoside (3)

Azide **28** (0.20 g, 0.63 mmol) was dissolved in THF/H_2O (90/10, 10 mL) and polymer-bound PPh_3 (3 mmol/g, 0.42 g, 1.26 mmol) was added. The reaction mixture was left under stirring overnight at 40°C, then filtered (TLC, CH_2Cl_2:CH_3OH 90:10, NH_3 1% v/v). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The obtained crude compound was purified by flash chromatography (CH_2Cl_2:CH_3OH 90:10, NH_3 1% v/v) to afford amine **3** (0.17 g, 0.58 mmol, 92%) as an oil; $[\alpha]_D^{20}$ = – 36.7 (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, *J*=6.9, 3H, CH_3), 1.18-1.36 (m, 10H, 5 CH_2), 1.60 (m, 2H, CH_2), 3.01 (dd, $J_{5,6a}$= 6.4 Hz, $J_{6a,6b}$= 12.8 Hz, 1H, H-6a), 3.09 (dd, $J_{5,6b}$= 5.8 Hz, 1H, H-6b), 3.25 (ddd, 1H, $J_{4,5}$= 8.9 Hz, H-5), 3.35 (dd, 1H, $J_{1,2}$= 7.8 Hz, $J_{2,3}$= 8.9 Hz, H-2), 3.49 (m, 1H, OCHa), 3.50 (dd, $J_{3,4}$= 8.9 Hz, 1H, H-4), 3.55 (dd, 1H, H-3), 3.85 (m, 1H,

OCHb), 4.26 (dd, 1H, H-1). ^{13}C NMR ($CDCl_3$): δ = 14.07 (CH_3), 22.64 (CH_2), 25.97 (CH_2), 29.30 (CH_2), 29.48 (CH_2), 29.78 (CH_2), 31.84 (CH_2), 42.48 (C6), 70.17 (OCH_2), 71.01 (C4), 73.51 (C2), 76.39 (C5), 77.00 (C3), 102.93 (C1). ESI-MS (CH_3OH, negative-ion mode): m/z =290.0 $[M-1]^-$. Calcd for $C_{14}H_{29}NO_5$, m/z 291.2 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 314.19361 $[M+Na]^+$ (error, 0.6 ppm). Calcd for $C_{14}H_{29}NO_5Na$, m/z = 314.19379 (monoisotopic mass). FT-IR (ATR): ν_{max} 3368, 2922, 2854, 1573 cm^{-1}**.**

4.1.5.5. Octyl *N*-hexanoyl-6-amino-6-deoxy-β-D-glucopyranoside (4)

To a solution of compound **3** (0.14 g, 0.48 mmol) in dry pyridine (3 mL) at 0 °C hexanoyl chloride (0.1 mL, 0.72 mmol) was added and the reaction mixture was stirred at rt for 3 h (TLC, CH_2Cl_2:CH_3OH 90:10, v/v). After quenching with CH_3OH, the solvent was evaporated under vacuum by repeated adding of toluene and the obtained crude was submitted to flash column chromatography (CH_2Cl_2:CH_3OH, 95:5, v/v) to afford pure compound **4** (0.12 g, 0.30 mmol, 62% yield), white crystals; m.p.: 142-144°C; $[\alpha]_D^{20}$ = – 13.7 (c 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.83-0.91 (m, 6H, $2CH_3$), 1.19-1.37 (m, 14H, $7CH_2$), 1.56-1.67 (m, 4H, $2CH_2$), 2.17-2.23 (m, 2H, CH_2), 3.12 (ddd, $J_{5,6a}$= 2.6 Hz, $J_{6a,6b}$= 15.0 Hz, $J_{6a,NH}$= 4.6 Hz, 1H, H-6a), 3.14 (dd, $J_{3,4}$= 9.5 Hz, $J_{4,5}$= 9.2 Hz, H-4), 3.26 (ddd, 1H, H-5), 3.34 (dd, $J_{1,2}$= 7.8 Hz, $J_{2,3}$= 9.2 Hz, 1H, H-2), 3.51 (m, 1H, OCHa), 3.59 (dd, 1H, H-3), 3.85 (m, 1H, OCHb), 4.05 (ddd, $J_{5,6b}$= 2.6 Hz, $J_{6b,NH}$= 8.3 Hz, 1H, H-6b), 4.28 (d, 1H, H-1), 5.93 (dd, 1H, NH); ^{13}C NMR ($CDCl_3$): δ = 13.89 (CH_3), 14.06 (CH_3), 22.33 (CH_2), 22.63 (CH_2), 25.30 (CH_2), 25.92 (CH_2), 29.20 (CH_2), 29.36 (CH_2), 29.60 (CH_2), 31.41 (CH_2), 31.79 (CH_2), 36.40 (CH_2), 39.78 (C6), 69.73 (C4), 70.66 (OCH_2), 73.84 (C2), 74.88 (C3), 74.99 (C5), 103.14 (C1), 175.86 (CO). ESI-MS (CH_3OH, negative-ion mode): m/z = 388.4 $[M-1]^-$. Calcd for $C_{20}H_{39}NO_6$, m/z 389.28 [M]. ESI-HRMS (CH_3OH, positive-ion mode): m/z = 412.26656 $[M+Na]^+$ (error, 1.0 ppm). Calcd for $C_{20}H_{39}NO_6Na$, m/z = 412.26696 (monoisotopic mass). FT-IR (ATR): ν_{max} 3313, 2922, 2872, 1642, 1553 cm^{-1}**.**

4.2. Biological methods

4.2.1. Short term *in vitro* bioassay for anti-tumor promoters

Inhibition was tested using a short term *in vitro* assay for EBV activation in Raji cells (obtained first from Prof. G. Klein, Karolinska Institute, Stockholm - Sweden) cultivated in RPMI 1640 medium containing 10% fetal calf serum, and induced by TPA as described previously.[3,21] Raji cells (1×10^6/mL) were incubated at 37°C for 48 h in 1mL of a medium containing n-butyric acid (4mM), 32 pmol of TPA in DMSO, and a known amount of the test compound in DMSO. The cells were stained by high titer EBV-positive sera from nasopharyngeal carcinoma patients and fluorescein-isothiocyanate-labelled anti-human IgG. After staining, they were detected by a conventional indirect immunofluorescence technique. The assays were performed in triplicate for each compound in which at least 500 cells were counted. The average EBV-EA inhibitory activity of the test compounds was compared to that of control experiments (100%) with butyric acid (4 mM) and TPA (32 pmol) in which EBV-EA induction was typically around 30%. The viability of the cells was assayed against treated cells using the Trypan Blue staining method. For an accurate determination of cytotoxicity, the cell viability was required to be more than 60% 3 days after treatment with the compounds.

4.2.2. *In vivo* two-stage mouse skin carcinogenesis test

Female SENECAR mice were obtained at 5-6 weeks of age from SLC Co. Ltd. (Shizuoka, Japan). Groups of animals (15 animals per group) were housed in bunches of five in polycarbonate cages. Mice were permitted free access to MP solid diet (Oriental yeast Co., Ltd. Chiba, Japan) and drinking water at all times during the study. The back of each mouse was shaved with surgical clippers before the first day of initiation. Tumors on the back of the mice were initiated with dimethylbenz[a]anthracene (DMBA; 390 nmol) in acetone (0.1 mL). One week after initiation, they were promoted twice a week by application of TPA (1.7 nmol) in acetone (0.1 mL). For the animals in the test compound treated groups the mice were treated with the test compounds (85 nmol) in acetone (0.1 mL) 1 h before each TPA treatment. The incidence of papillomas was observed weekly for 20 weeks. The differences in mouse skin papillomas between control and experiments were analyzed by means of the Student's t-test at 20 weeks of promotion.

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CAPTIONS TO SCHEMES AND FIGURES

Scheme 1

a) NaN_3, DMF; b) PPh_3 polymer-bound, THF/H_2O (90/10); c) RCOCl, Py; d) H_2, Pd/C, MeOH; e) LPS, Py, CF_3CH_2OCOR.

Scheme 2

a) NaN_3, DMF; b) $CClPh_3$, Py 100°C; c) PPh_3 polymer-bound, THF/H_2O (90/10); d) $C_5H_{11}COCl$, Py ; e) $CClPh_3$,Py 100°C

Scheme 3

a) NaN_3, DMF; b) PPh_3 polymer-bound, THF/H_2O (90/10)

Scheme 4

a) NaN_3, DMF; b) MeONa, MeOH; c) PPh_3 polymer-bound, THF/H_2O (90/10); d) $C_5H_{11}COCl$, Py.

Figure 1

Structures of natural monogalactosyldiacylglycerols (MGDG), sulfoquinovosylacylglycerols (SQAG) and 6-amino-6-deoxyglycoglycerolipids (AGGL).

Figure 2

Structures of the studied compounds **1**-**4** and of the reference compounds **5**-**10**.

Figure 3

^{1}H-NMR resonances of glycerol H-2 of compounds **21**, **23** and **25** in deuterated methanol at room temperature. The spectra were collected at 0, 44 and 186 hours.

Figure 4.

Inhibitory effects of compounds **1a** and **2a** (85 nmol) on DMBA-TPA mouse skin carcinogenesis. All mice were initiated with DMBA (390 nmol) and promoted with TPA (1.7 nmol) twice a week starting one week after initiation. A: percentage of mice with papillomas (papilloma incidence); B: averaged number of papillomas per mouse (papilloma multiplicity). (■, TPA alone; Δ, TPA + **1a**; ◆, TPA + **2a**, ○ TPA + **7**). At 20 weeks of promotion the averaged number of papillomas per mouse was reduced, with respect to the control group (7.9 ± 1.8), to 5.8 ± 1.1 for **1a** ($P < 0.1$), 6.2 ± 1.3 for **2a** ($P < 0.1$) and 4.0 ± 0.9 ($P < 0.001$) for **7** (control group 9.1 ± 1.6) [23].

HIGHLIGHTS

- 6-amino-6-deoxyglycoglycerolipid based on 2-O-β-D-glucosylglycerol were synthesized
- Compounds were tested for their in vitro and in vivo anti-tumor-promoting activity
- Compounds resulted less active than previously studied glycoglycerolipids
- The presence of the nitrogen atom strongly reduced the activity of the compounds
- In general 6-position of the sugar plays a crucial role in glycoglycerolipid activity

New 6-amino-6-deoxy-glycoglycerolipids derived from 2-*O*-β-D-glucopyranosylglycerol: insights into the structure-activity relationship of glycoglycerolipids as anti-tumor promoters

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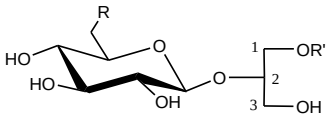
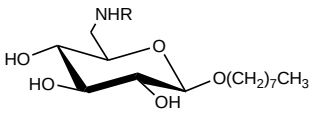


1: R= $NHCO(CH_2)_nCH_3$; R'= H

2: R= $NHCO(CH_2)_nCH_3$; R'= $CO(CH_2)_nCH_3$

a: n= 4; **b**: n= 16



3: R= H

4: R= $CO(CH_2)_4CH_3$

Compounds **1**-**4** were prepared and their anti-tumor-promoting activity tested through a short term in vitro assay for the inhibition of EBV activation induced by TPA.