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Synthesis of *N*-oxyamide analogues of protein kinase B (Akt) targeting anionic glycoglycerolipids and their antiproliferative activity on human ovarian carcinoma cells†

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N-Oxyamides of bioactive anionic glycoglycerolipids based on 2-*O*-β-D-glucosylglycerol were efficiently prepared. However, the oxidation step of the primary hydroxyl group of the glucose moiety in the presence of the *N*-oxyamide function appeared to be a difficult task that was nevertheless conveniently achieved for the first time by employing a chemoenzymatic laccase/TEMPO procedure. The obtained *N*-oxyamides exhibited a higher inhibition of proliferation of ovarian carcinoma IGROV-1 cells in serum-free medium than in complete medium, similarly to the corresponding bioactive esters. Stability and serum binding studies indicated that the observed reduced activity of the compounds in complete medium could be mainly due to a binding effect of serum proteins rather than the hydrolytic degradation of glycoglycerolipid acyl chains. Furthermore, the results of the cellular studies under serum-free conditions suggested that the *N*-oxyamide group could increase the antiproliferative activity of a glycoglycerolipid independently of the presence of the anionic carboxylic group. Cellular studies in other cell lines besides IGROV-1 also support a certain degree of selectivity of this series of compounds for tumor cells with Akt hyperactivation.

Introduction

Protein kinase B (Akt) is a component of the PI3K–Akt–mTOR signaling pathway which is part of the cellular network involved in cell survival. Because Akt activation has been linked to crucial events in cancer development and progression, such as resistance to anti-cancer drugs and metastatic spread, this kinase family represents an important target for cancer treatment.[1,2] Based on the presence of a regulatory Pleckstrin Homology (PH) lipid binding domain in the Akt structure, characterized by positively charged amino acids,[3] anionic glycoglycerolipids based on 2-*O*-β-D-glucosylglycerol (a natural compound named Lilioside B in which glucose is β-linked to the C-2 of glycerol)[4] were recently synthesized and tested as Akt inhibitors targeting the PH domain.[5,6] Their structures (**1** and **2**, Fig. 1) were inspired by the natural anionic lipids sulfoquinovosyldiacylglycerols (SQDG)[7] and glucuronosyldiacylglycerols (GLCADG)[8] mimicking phosphatidylinositol-3-phosphate (PI3P) (Fig. 1), a plasma membrane lipid derived from the action of phosphatidylinositol-3-kinase (PI3K) that is similar to phosphatidylinositol-3,4,5-triphosphate (PIP_3), the natural ligand of the Akt lipid-binding PH domain.[9]

An antiproliferative activity in the human ovarian carcinoma IGROV-1 cell line was observed especially for compounds **1a**,[5] **2a** and **2b**.[6] Actually, a more marked growth inhibitory effect was produced when the drug incubation occurred in serum-free medium as compared to complete medium, thereby suggesting that serum affects the bioavailability of the compounds. Indeed, in serum-free medium, the IC_{50} value of compound **2a** changed from 156.7 to 9.40 μM and that of compound **2b** decreased from 49.0 to 3.35 μM (Fig. 2).

In this work, we prepared *N*-oxyamide analogues of the anionic glucuronosylglycerolipid **2a** and **2b**. Namely, *N*-oxyamides **3a** and **4a**, together with the non-anionic *N*-oxyamides **3b** and **4b** and the known ester **5** (see the Experimental section) used as reference compounds, were syn-

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† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR spectra of all the compounds, Fig. S1–S5. See DOI: https://doi.org/10.1039/d3ob00891f

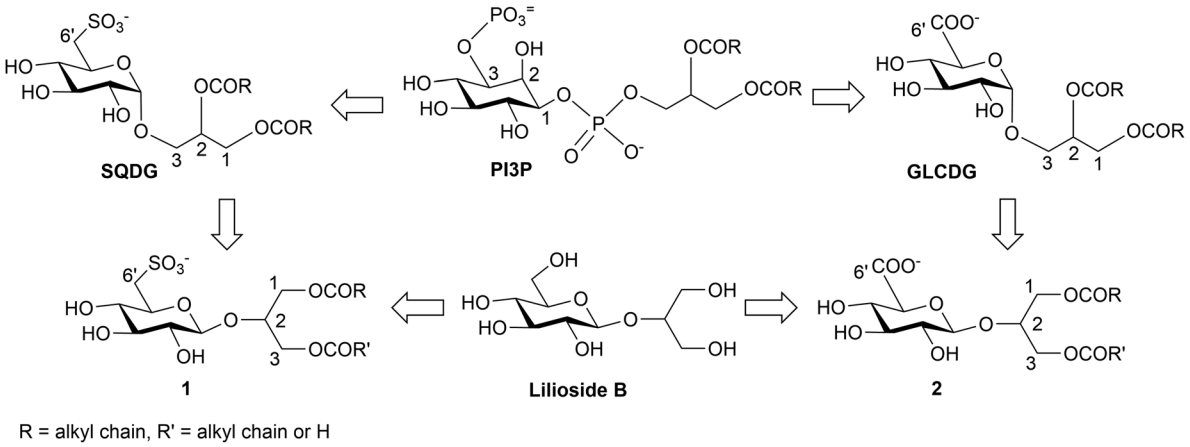


Fig. 1 Structure of the synthetic anionic glycoglycerolipids **1** and **2** based on the merge of the natural compounds, Lilioside B, SQDG and GLCDG, mimicking PI3P.

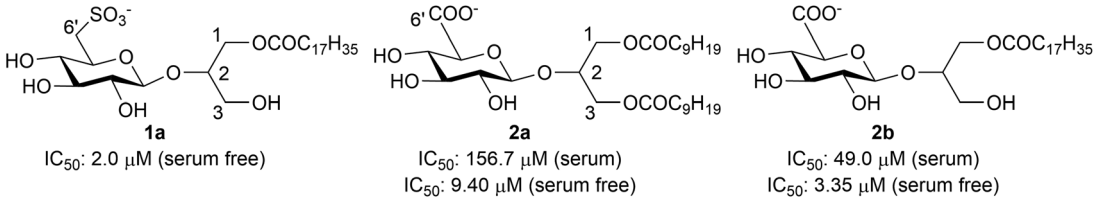


Fig. 2 Structures of anionic glycoglycerolipids and their antiproliferative activity in the human ovarian carcinoma IGROV-1 cell line. Whilst **2a** and **2b** were assayed both in complete and serum-free medium, compound **1a** was tested in serum-free medium only.

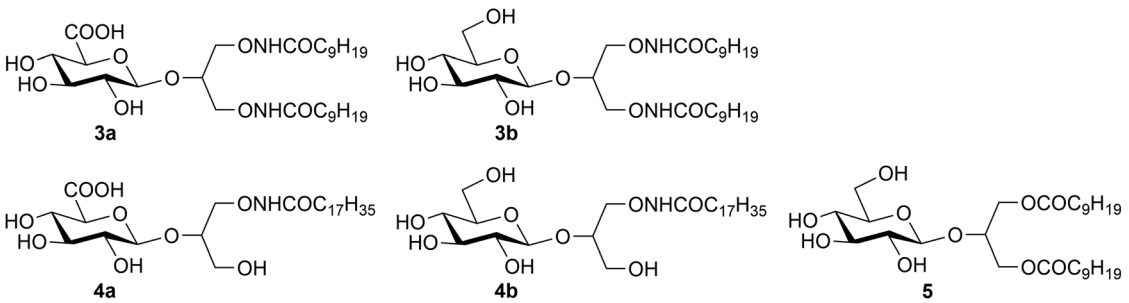


Fig. 3 Structures of the tested compounds.

thesized in order to obtain anionic glycoglycerolipids with improved antiproliferative activity (Fig. 3). Actually, the conversion into *N*-oxyamide is an eligible modification to improve the metabolic stability of carboxylic derivatives as in the case of oligonucleotides,[10] sugars,[11] glycolipids[12,13] and peptides[14] which are reported to be very stable against enzymatic hydrolysis. Thus, stability and serum binding studies were also performed on selected compounds. Antiproliferative activity was assayed in the human ovarian carcinoma IGROV-1 cell line, in which Akt is hyperactivated,[15] in complete or serum-free medium and the obtained results are discussed. In addition, the IGROV-1/Pt1 cisplatin-resistant cell lines, with increased Akt activation as compared to IGROV-1 cells and the TOV112D cell line in which Akt and other cell survival pathways are not activated, were used to prove the selectivity of our approach.

Results and discussion

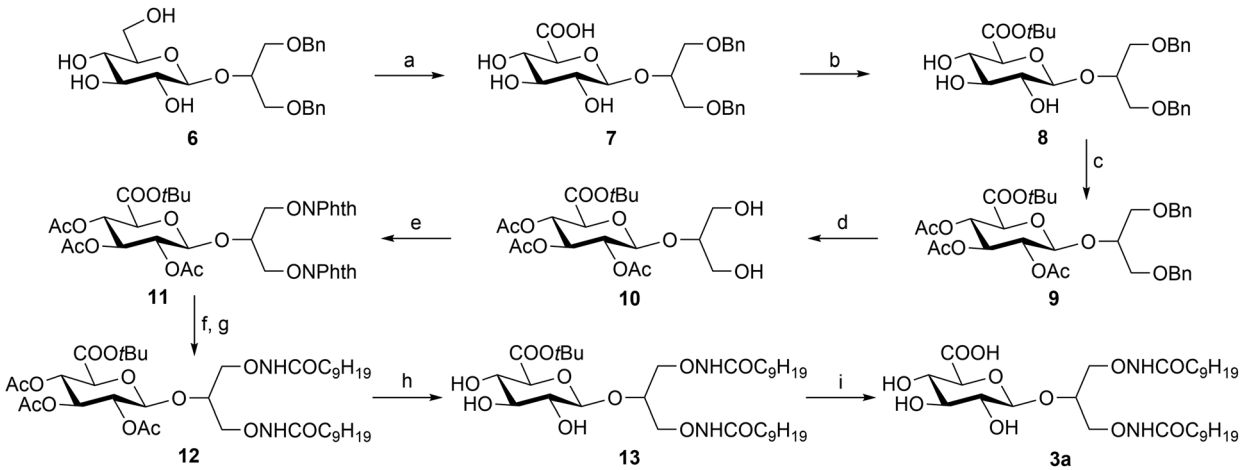
Chemistry

Synthesis of diacyl *N*-oxyamide 3a. Although the synthesis of *N*-oxyamide glycolipids was previously described by Chen *et al.*,[12,13] in the case of our target anionic lipids, the concomitant presence of carboxylic and oxyamide groups made the task quite challenging. In fact, to the best of our knowledge, very few examples of alcohol oxidation in the presence of an

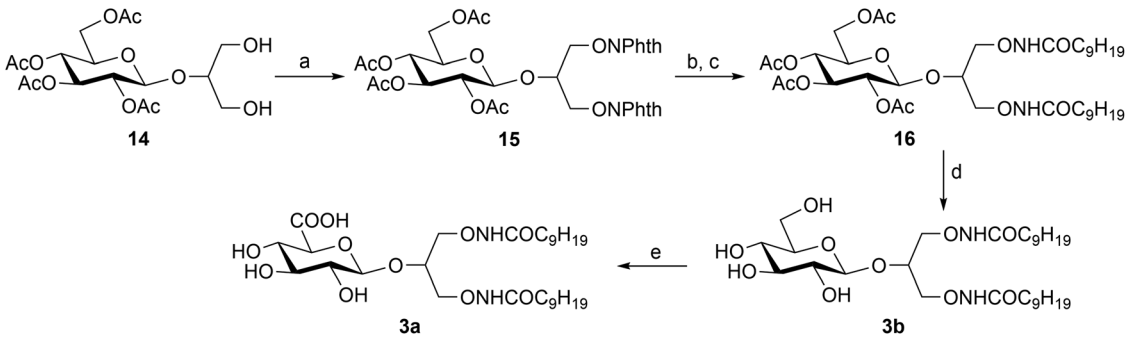
N-oxyamide linkage have been reported and, actually, only a low yield TEMPO-mediated oxidation of an *O*-methyl hydroxamate primary alcohol to give an unstable aldehyde for the synthesis of castanospermine has been reported.[16] Thus, we initially planned to introduce a properly protected carboxylic function into the sugar moiety of the 2-*O*-β-D-glucosylglycerol skeleton at the beginning of the synthetic pathway. Then, we planned to elaborate the glycerol moiety to generate the O–N linkage through the Mitsunobu reaction[17] by using an *N*-hydroxyphtalimide method followed by *N*-oxyamide formation and the final acidic deprotection to generate the free carboxylic function.

Following this synthetic pathway, compound **3a** was prepared (Scheme 1) starting from the known 1,3-di-*O*-benzyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**6**)[18] which was selectively oxidized with $NaClO/NaClO_2$ at the C-6′ of the sugar moiety by a TEMPO-mediated procedure[6] yielding glucuronide (**7**). After several attempts with different common reagents (*e.g. tert*-butanol with DCC), carboxyl esterification to *tert*-butyl ester **8** was achieved by using *tert*-butyl 2,2,2-trichloroacetimidate[19] albeit in a quite low yield, due to the formation of various by-products in some cases identified by NMR as mixed *tert*-butyl ethers (data not shown). The obtained **8** was fully acetylated to **9** and then debenzylated by palladium-catalyzed hydrogenolysis yielding **10**. The Mitsunobu reaction between this compound and *N*-hydroxyphthalimide yielded the di-*O*-phthalimido derivative **11** which was converted into a di-*O*-amino compound by hydrazine treatment. This compound was directly acylated, without previous purification, by treatment with decanoyl chloride and pyridine affording the fully protected *N*-oxyamide **12**. The Zemplén deacetylation reaction yielded **13** which was finally converted into **3a** by trifluoroacetic acid treatment at room temperature.

Nevertheless, a more concise synthetic route was also planned to increase the overall yield of the desired *N*-oxyamide **3a**. This alternative synthetic strategy relies on the introduction of the carboxylic function into the last synthetic step after the generation of *N*-oxyamides. So, in this way, it should be possible to reduce the number of protection and deprotection steps required (see Scheme 2). The Mitsunobu reaction between the known 2-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (**14**)[20,21] and *N*-hydroxyphthalimide yielded the di-*O*-phthalimido derivative **15** which was converted into the unstable related di-*O*-amino derivative by treatment with hydrazine hydrate. The reaction time of this synthetic step became a very critical factor. We found that a short stirring time and rapid quenching of the reaction are mandatory for the success of the reaction. Actually the cleavage of the phthalimide occurred very rapidly within a few minutes after the addition of hydrazine hydrate, but migration of the acetyl groups from sugar to the free *O*-amino groups on the glycerol moiety also occurred. Because of this, the di-*O*-amino compound was directly acylated without isolation by treatment with decanoyl chloride and pyridine providing the fully protected *N*-oxyamide **16**. Finally, this compound was successfully converted into compound **3b** by the Zemplén transesterification. The first attempt of **3b** oxidation (see Table 1) was performed using a catalytic amount (0.3 equivalent) of TEMPO and $NaClO/NaClO_2$ as terminal oxidants according to Zhao's modification of Anelli's procedure.[22] A similar oxidation procedure was already used in ref. 16 for the synthesis of castanospermine, indicating the possibility of performing TEMPO-mediated oxidation in the presence of the *N*-oxyamide. However, all attempts of oxidation under these conditions were unsuccessful leading to a mixture of degradation products. *N*-Halogenation might be involved in the degradation of the substrate. Indeed, the formation of *N*-alkoxy-*N*-chloroamides from the parent *N*-oxyamide in the presence of *tert*-butyl hypochlorite as a positive chlorine source has been described.[23] In this context, we decided to perform the oxidation in the absence of the terminal oxidants. Indeed, in the TEMPO-mediated oxidation reactions, the active oxidant

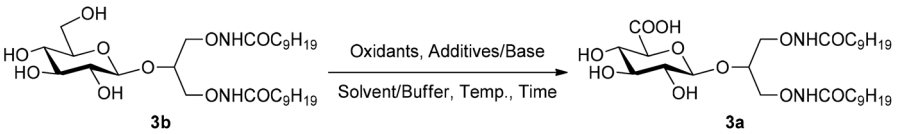


Scheme 1 a. TEMPO, $NaClO/NaClO_2$, CH_3CN, 0.67 M phosphate buffer (pH 6.7), RT, (89%); b. *tert*-butyl 2,2,2-trichloroacetimidate, CH_2Cl_2/cyclohexane, RT, (33%); c. Ac_2O, Py, RT, (80%); d. H_2, 10% Pd/C, CH_3OH, RT, (98%); *e*. PPh_3, DIAD, *N*-hydroxyphthalimide, toluene/CH_2Cl_2, (86%); f. hydrazine hydrate, CH_3OH; g. $C_9H_{19}COCl$, Py, CH_2Cl_2, RT, (45%, two step); h. CH_3ONa, CH_3OH, RT, (50%); i. TFA, CH_2Cl_2, RT, (67%).



Scheme 2 a. PPh_3, DIAD, *N*-hydroxyphthalimide, toluene/CH_2Cl_2, RT, (79%); b. $NH_2NH_2{\cdot}H_2O$, CH_3OH/THF 5 : 2, 0 °C; c. $C_9H_{19}COCl$, Py, CH_2Cl_2, −10 °C (73%, over two step); d. CH_3ONa, CH_3OH, RT, (81%); e. *Trametes versicolor* laccase, TEMPO, air, acetate buffer pH 4.5, 30 °C, (49%).

Table 1 Screening of the reaction conditions[a]



Entry	Oxidants (eq.)	Additives/bases (eq.)	Solvent/buffer	Temp. (°C)	Time (h)	Yield[b] (%)
1	TEMPO (0.3)/ $NaClO$ (0.2)/$NaClO_2$ (3)	—	CH_3CN/phosphate buff. (1.8 M, pH 6.8) 55 : 45	25	6	Dec.
2	**$TEMPO^+BF_4^-$ (4)**	**TBAB (0.06)/$NaHCO_3$ (3)**	**DCM/H_2O 5 : 3**	**0**	**3**	**38**
3	$TEMPO^+BF_4^-$ (6)	TBAB (0.06)/$NaHCO_3$ (3)	DCM/H_2O 5 : 3	0	3	Dec.
4	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)/$NaHCO_3$ (3)	DCM/H_2O 5 : 3	25	3	Dec.
5	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)/$NaHCO_3$ (3)	DCM/H_2O 5 : 3	−10	3	30
6	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)	DCM/H_2O 5 : 3	0	3	Dec.
7	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)	CH_3CN/H_2O 9 : 1	0	24	n.d.[c]
8	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)	DCM/phosphate buffer (1.8 M, pH 6.8) 5 : 3	0	24	n.d.[c]
9	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)	DCM/phosphate buffer (1.8 M, pH 6.8) 5 : 3	25	3	Dec.
10	$TEMPO^+BF_4^-$ (4)	—	CH_3CN/phosphate buffer (1.8 M, pH 6.8) 55 : 45	0	24	n.d.[c]
11	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)	DCM/carbonate buffer (1.8 M, pH 10) 5 : 3	0	3	Dec.
11	$TEMPO^+BF_4^-$ (4)	TBAB (0.06)/pyridine (3)	DCM/H_2O 5 : 3	0	3	Dec.
12[d]	**TEMPO (0.8)/*T. versicolor* laccase (11 U)/air**	**—**	**Acetate buffer (20 mM, pH 4.5)**	**30**	**10 days**	**49**

[a] Reactions were performed using **3b** (0.17 mmol) in 2.25 mL of solvents in the presence of oxidants, bases, and additives (as indicated in the table). [b] Isolated yields. [c] **3b** recovered by flash chromatography (recovery >90%). [d] Further 11 U of *T. versicolor* laccase and 0.8 eq. of TEMPO were added after 3 and 8 days of the reaction.

species is the oxoammonium generated *in situ* by oxidation of TEMPO with a suitable terminal oxidant.[22] Thus, a second attempt of oxidation was performed using the stable TEMPO oxoammonium tetrafluoroborate salt ($TEMPO^+BF_4^-$) as a non-catalytic oxidant.[24] The oxidation of **3b** was performed by adopting the reaction conditions reported by Davis and Flitsch for the oxidation of monosaccharide derivatives to uronic acids[25] in a biphasic dichloromethane water system at 0 °C in the presence of tetrabutylammonium bromide and sodium bicarbonate as bases. Four equivalents of $TEMPO^+BF_4^-$ were used because of the competitive comproportionation between the unreacted oxoammonium salt and the reduced hydroxylamine formed as the by-product of the reaction.[26] Under these conditions, it was not possible to obtain the complete conversion of the starting material, and product **3a** was obtained in 30% yield. The incomplete conversion was attributed to the decomposition of $TEMPO^+BF_4^-$. Indeed, it is known that in diluted basic solution, the oxoammonium salt can be converted in TEMPO with the formation of hydrogen peroxide as the by-product.[27] Thus, in order to improve the conversion of the starting material, the reaction was performed using an excess of the oxidant reagent (6 eq.), but complete decomposition of the product was observed. Therefore, a screening was performed to find the reaction conditions favoring the desired oxidation of **3b** (Table 1). The reaction was performed using different solvent mixtures, at different temperatures and pH values using various buffer systems, or pyridine as an organic base,[28,29] but no significant improvements were obtained. In particular, we observed that the pH has a great influence on the reaction outcome. The best performances were observed using sodium bicarbonate solution and pH ranging from 8 to 9. The use of carbonate buffer with pH around 10 led to com-

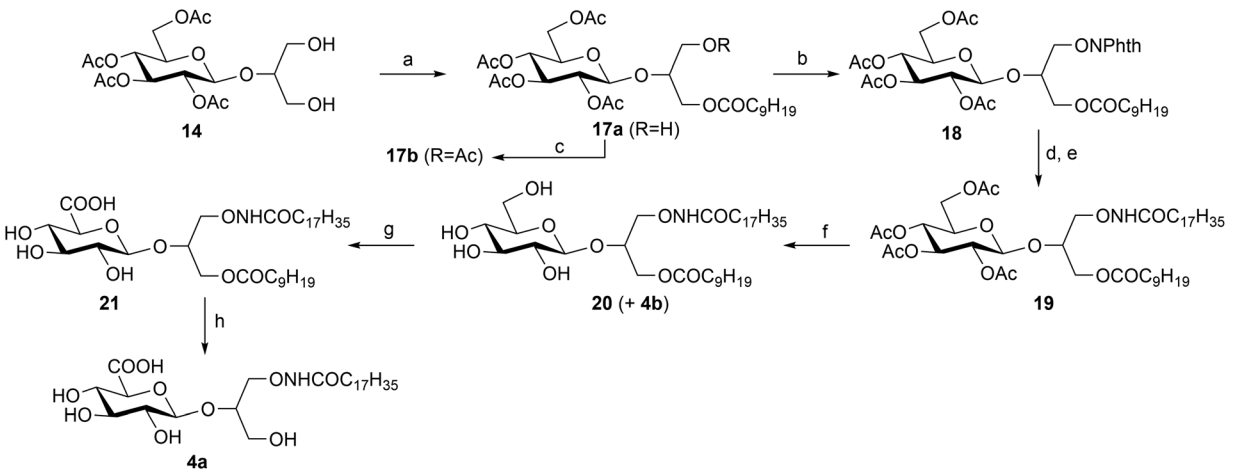
plete decomposition, while no reaction was observed using phosphate buffer at pH 7. This last result was not unexpected as primary alcohols carrying an oxygen atom at the β-position are known to exhibit very poor reactivity towards oxoammonium-mediated oxidations conducted in neutral and slightly acidic media.[30] These substrates are generally oxidized when the reaction is performed in the presence of a base, leading to the formation of the corresponding dimeric esters.[31,32] Moreover, we observed that the substrate and the product of the reaction were also sensitive to the amount of the oxidant, and an excess of $TEMPO^{+}BF_4^{-}$ invariably induced extensive decomposition.

Hence, we reconsidered the use of the catalytic TEMPO-mediated oxidation procedure. In particular, we selected a laccase–TEMPO system that was reported as a strategy for the mild regioselective oxidation of sugar primary hydroxy groups.[33,34] Compound **3b** was suspended in acetate buffer and stirred for ten days in the presence of a catalytic amount of TEMPO and commercially available *Trametes versicolor* laccase. Under these conditions (see the Experimental section), complete conversion of the starting material and reduced formation of by-products were observed allowing a significant increase of the product yield. However, the amphiphilic nature of **3a** made its isolation and purification difficult, limiting its recovery to about 50%. Moreover, to reduce the reaction time, we considered to improve the low solubility of **3b** in acetate buffer by using acetonitrile as a co-solvent as it has been reported that a concentration up to 10% in volume is well tolerated by this laccase without a detrimental effect on its activity,[35] but, unfortunately, compound **3b** exhibited a very low solubility even in pure acetonitrile.

Synthesis of monoacyl *N*-oxyamide 4a. Based on the positive result reported above for the preparation of diacyl derivative **3a**, a similar synthetic strategy was employed for the synthesis of monoacyl derivative **4a** (see Scheme 3). The common intermediate **14** [20,21] was protected as decanoate at the 3-*O* position employing the known selectivity of the lipase from *Candida antarctica* catalyzed transesterification in the presence of trifluoroethyl decanoate as an acyl donor.[36] This reaction proceeded with good regioselectivity affording the desired compound **17a** and its 1-*O* isomer in a 90 : 10 ratio (by NMR). The configuration assignment of **17a** was obtained by chemical correlation of **17b** (Scheme 3) as reported in the Experimental section. The following Mitsunobu reaction yielded the 1-*O*-phthalimido derivative **18** which was converted into the unstable 1-*O*-amino derivative by treatment with hydrazine hydrate. This was directly acylated without isolation by treatment with octadecanoyl chloride and pyridine, providing the fully protected *N*-oxyamide **19**. The mild removal of the protective acetyl groups of **19** was performed with triethylamine adopting the reaction conditions reported by Meier *et al.*[37] in a methanol/triethylamine/water mixture, affording the deacetylated compound **20** in 38% yield. Unfortunately, it was not possible to obtain the complete conversion of the starting material as the prolonged stirring time led to the cleavage of the decanoyl protecting group and the formation of the fully deprotected compound **4b**. Nevertheless, this compound was isolated in 18% yield and was employed as a non-anionic derivative of **4a** for biological assays. The glucuronic derivative **21** was obtained in 66% yield through the same laccase-mediated oxidation previously used for the oxidation of the diacyl derivative. Finally, the desired derivative **4a** was successfully obtained by the Zemplén transesterification.

Stability study

As the hydrolytic activity of serum components, including albumin, *vs.* ester compounds such as drugs or lipids is known,[38–40] in order to compare the hydrolytic resistance of *N*-oxyamide *vs.* ester glycoglycerolipids, a stability study was conducted. Compounds **3b** and **5** were selected as representatives of oxyamides and esters, respectively, and were subjected to conditions similar to those used for cellular studies in com-



Scheme 3 a. $C_9H_{19}COOCH_2CF_3$, *Candida antarctica* lipase, pyridine, 30 °C (76%); b. PPh_3, DIAD, *N*-hydroxyphthalimide, toluene/CH_2Cl_2, RT, (92%); c. Ac_2O, Py, RT. d. $NH_2NH_2{\cdot}H_2O$, CH_3OH/THF 5 : 2, 0 °C; e. $C_{17}H_{35}COCl$, Py, CH_2Cl_2, –10 °C, (86%, over two step); f. Triethylamine/H_2O/CH_3OH, THF, RT, (38%); g. *T. versicolor* laccase, TEMPO, air, acetate buffer pH 4.5, 30 °C, (66%); h. CH_3ONa, CH_3OH, RT, (94%).

plete serum and checked by TLC and ^{1}H-NMR analysis (see the Experimental section). The results indicated that, different from **5**, **3b** was detectable again after 24 h, confirming the greater stability of *N*-oxyamides *vs*. esters for this class of glycoglycerolipids.

Serum binding study

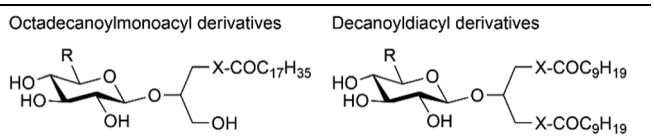
Plasma protein binding is known to affect the concentration of a number of drugs;[41] thus the binding of the oxyamide compounds to serum proteins was evaluated. In particular, compound **3a**, as a representative of the oxyamides, was used for a test mimicking the cellular experimental conditions (see the Experimental section). Among the most commonly reported methods (equilibrium dialysis – ED, rapid equilibrium dialysis – RED, ultrafiltration – UF, and ultracentrifugation – UC),[42,43] UF was used to recover the unbound **3a**. As non-specific binding could interfere with the analysis,[43] we prepared samples with the same concentration of **3a** but with an increasing amount of serum in order to check the trend of binding. Actually, we observed the diminishing of unbound **3a** with the increase of the serum amount by ^{1}H-NMR analysis (see the Experimental section). This result could support the reduced activity of the tested compounds in the cellular experiments performed in the presence of serum.

Cellular studies

The antiproliferative activity of the synthesized *N*-oxyamides was examined using the IGROV-1 ovarian carcinoma cell line known to harbor hyperactivated Akt[15] either in complete medium or serum-free medium. The percentages of the inhibition of IGROV-1 cell growth in drug-treated samples *versus* solvent-treated samples, after 24 h of exposure to compounds **3a**, **3b**, **4a**, **4b**, and **5** either in complete medium or serum-free medium (see the Experimental section), are reported in the representative dose–response curves (Fig. 4).

IC_{50} values of compounds **3–5** and the previously tested compounds **2a** and **2b** are shown in Table 2 in order to compare their effects on cell growth inhibition under both experimental conditions.

Table 2 Cell sensitivity of human ovarian carcinoma cells (IGROV-1) was evaluated by growth inhibition assays after treatment with compounds **2–4a**, **b** and **5**



Cpd	Functional group (R)[c]	Linkage (X)[b]	IC_{50} μM ± SD[a] Serum (+)	IC_{50} μM ± SD[a] Serum (–)
Octadecanoylmonoacyl derivatives				
2b[d]	COOH	O	49.0 ± 7.60	3.35 ± 0.35
4a	COOH	ONH	41.2 ± 20.1	5.85 ± 1.43
4b	CH_2OH	ONH	31.5 ± 15.9	10.4 ± 8.26
Decanoyldiacyl derivatives				
2a[d]	COOH	O	156.7 ± 23.0	9.40 ± 5.00
3a	COOH	ONH	71.5 ± 34.3	6.98 ± 4.94
3b	CH_2OH	ONH	n.d.	13.7 ± 7.13
5	CH_2OH	O	63.4 ± 4.65	33.2 ± 21.2

[a] IC_{50} values represent the compound concentration producing 50% inhibition of cell growth (±SD) in the presence (+) or in the absence (–) of fetal bovine serum. [b] Type of linkage between fatty acid and glycerol. [c] Functional group at the C-6 of glucose. [d] Data from ref. 6.

All the new oxyamides **3a**, **3b**, **4a**, and **4b** were less active in the complete medium experiments (Table 2), as already reported for esters **2a** and **2b**,[6] indicating that the observed negative serum influence could mainly be due to a serum protein binding effect rather than the hydrolytic degradation of glycoglycerolipid acyl chains. In the serum-free medium, oxyamides **3a** and **4a** were as active as the corresponding esters **2a** and **2b** showing similar IC_{50} values (6.98 and 5.85 *vs*. 9.40 and 3.35, respectively, Table 2). Of note, the new monooxyamide **4a** and dioxyamide **3a** showed similar IC_{50} values (6.98 and 5.85, respectively, Table 2), whereas monoester **2b** seemed more active than diester **2a** (3.35 and 9.40, respectively, Table 2).

Interestingly, even oxyamides **3b** and **4b** lacking the carboxylic function were active even though they showed twice the IC_{50} values of the corresponding anionic glycoglycerolipids **3a**

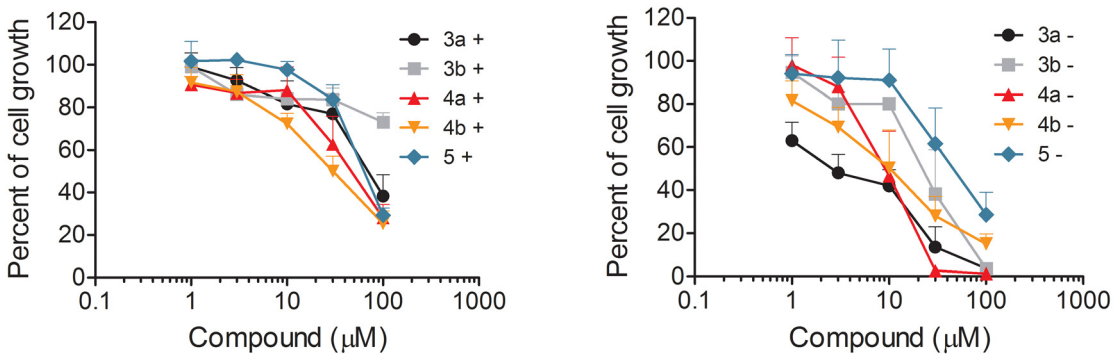


Fig. 4 Cell sensitivity to the studied compounds. IGROV-1 cells were seeded and 24 h later, they were exposed to the compounds in the presence (+) or the absence (–) of fetal bovine serum. Forty-eight hours later, the cells were counted with an automatic cell counter. The graphs report the mean (±SEM) of at least 3 independent experiments.

and **4a** (13.7 and 10.4 *vs.* 6.98 and 5.85, respectively, Table 2). In contrast, the non-anionic ester **5**, lacking both the carboxylic and oxyamide functions, was the less active compound showing the worst IC_{50} value (33.2, Table 2), which was about 4–5 fold higher than that of **3a** (6.98, Table 2).

Based on these results, it seems that the *N*-oxyamide group exerts a generally positive effect on the IGROV-1 anti-proliferative activity of glycoglycerolipids that is similar to that caused by the carboxylic (anionic) function, even if the effect of the two groups seems to be not additive (see **4a** *vs.* **2b** and **3a** *vs.* **2a**, Table 2).

The antiproliferative effect of compounds **4a** and **4b** was examined in two additional ovarian carcinoma cell lines. Specifically, we used the cisplatin-resistant variant IGROV-1/Pt1 showing increased activation of the Akt pathway as compared to the IGROV-1 parental cell line[44] and the TOV112D cell line known to be characterized by the lack of alterations of the PI3K/Akt pathway.[45] When the cells were exposed to the compounds in the absence of serum, we observed a higher activity of both in the IGROV-1/Pt1 cells (IC_{50} was 2.00 ± 0.6 μM and 3.78 ± 1.4 μM for **4a** and **4b**, respectively; $P < 0.05$ by Student's *t* test for comparison *vs.* IGROV-1 values), consistent with a higher activation of the Akt pathway (Fig. 5). Conversely (Fig. 6), the activity of both compounds was reduced in TOV112D cells, and the IC_{50} values were 20.51 ± 4.6 μM and 55.85 ± 10.3 μM for **4a** and **4b**, respectively ($P < 0.05$ by Student's *t* test for comparison *vs.* IGROV-1/Pt1 values). Such findings are in agreement with the lack of Akt-dependence of TOV112D cells and highlight the interest in our compounds as inhibitors of tumor cells with deregulated survival pathways.

As the aminoxy amide NH function is a very good hydrogen bond donor,[12,46] the insertion of an *N*-oxyamide function into the glycoglycerolipid structure could modify the mode of interaction of these compounds with the cell membrane or the possible molecular target. Thus, we subjected oxyamides **3a**, **3b**, **4a**, and **4b** to inhibition cell-free assays (ELISA test, Fig. 5) to check their inhibitory effect on Akt.

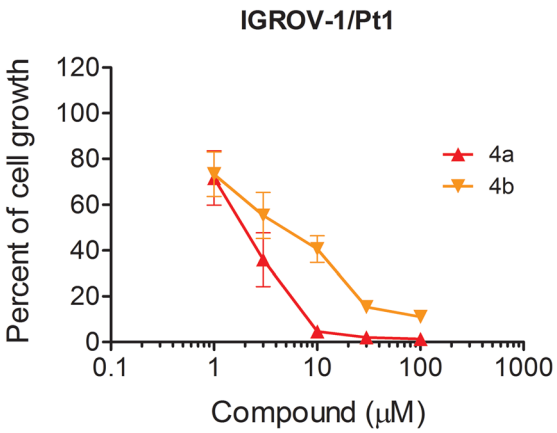


Fig. 5 Cell sensitivity to **4a** and **4b** of IGROV-1/Pt1 cells with increased activation of the Akt pathway in the absence of fetal bovine serum. The graphs report the mean (±SEM) of triplicate experiments.

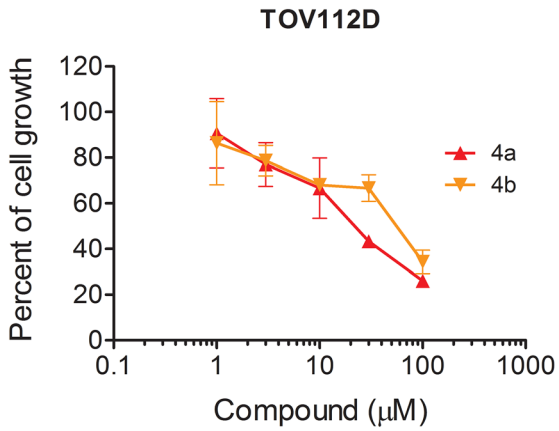


Fig. 6 Cell sensitivity to **4a** and **4b** of TOV112D cells with lack of alterations of the PI3K/Akt pathway in the absence of fetal bovine serum. The graphs report the mean (±SEM) of triplicate experiments.

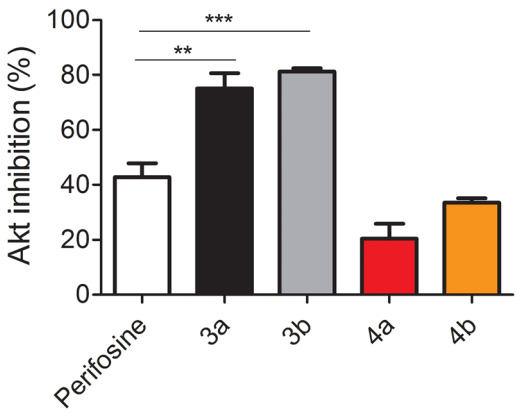


Fig. 7 Akt inhibition in cell-free assays Akt inhibition was assayed by ELISA by measuring the O.D. at 450 nm. Oxyamides **3a**, **3b**, **4a**, and **4b**, were examined besides perifosine, used as a reference compound, at 10 μM concentration. The data represent the mean (±SEM) of three values. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's correction for multiple comparisons. **, $P < 0.01$; ***, $P < 0.001$.

Cell-free evaluation of Akt inhibition

Oxyamides **3a**, **3b**, **4a** and **4b** were tested for inhibitory activity against Akt (Akt1) using an *in vitro* ELISA to measure kinase activity (see the Experimental section). The obtained results at 10 μM sample concentration as reported in Fig. 7 show that diacyloxyamides **3a** and **3b** were more active than the monoacyl derivatives **4a** and **4b**. Comparing the results of oxyamides **3a** *vs.* **3b** and **4a** *vs.* **4b**, it seems that, in both cases, the presence or the absence of the anionic carboxylic group does not modify significantly the inhibition of Akt (75% *vs.* 81% and 21% *vs.* 34%, respectively, Fig. 7), suggesting that the lack of anionic carboxylate was compensated by the presence of the oxyamidic function.

Conclusions

In this work, we aimed to prepare anionic glycoglycerolipids having acyl chains linked to the glycerol moiety with an *N*-oxyamide linkage, and the oxidation of a primary hydroxyl group in the presence of the *N*-oxyamide function, rarely reported in the literature to the best of our knowledge, turned out to be quite a difficult task. However, a careful optimization of the experimental conditions allowed us to obtain the desired new *N*-oxyamide glucuronides **3a** and **4a** in satisfactory yields using a laccase/TEMPO-mediated chemoenzymatic approach. These compounds together with the corresponding non-anionic *N*-oxyamides, namely the newly obtained glycoglycerolipids **3b** and **4b**, were tested for their antiproliferative activity in the human ovarian carcinoma IGROV-1 cell line in complete and serum-free medium. The cellular experiments also revealed that *N*-oxyamides (**3a** and **4a**) were less active when tested in the complete serum medium as already observed in the case of the corresponding esters (**2a** and **2b**), suggesting that binding with serum components could be mainly responsible for their reduced overall activity as suggested also by the stability and serum binding studies. The results in the absence of serum showed that the new anionic oxyamides (**3a** and **4a**) were almost as active as the ester counterparts (**2a** and **2b**), and also that the compounds having only the oxyamide acyl chain(s) but not the carboxylic group (**3b** and **4b**) were just a little bit less active than the *N*-oxyamide glucuronides **3a** and **4a**, suggesting a role of the oxyamide function in maintaining the anti-proliferative activity that conversely was significantly reduced for compound **5** lacking both the groups. An ELISA assay showed an inhibitory effect of all the new compounds against Akt, especially for diacyl derivatives, suggesting that this crucial kinase could be their target as already demonstrated for the ester-based anionic glycoglycerolipid.[6] In conclusion, in this paper, an efficient way for the oxidation of primary hydroxyl in the presence of *N*-oxyamide was demonstrated in the case of a glycoglycerolipid scaffold, affording in this way new anionic *N*-oxyamide glycoglycerolipids. The biological data showed that also the non-anionic *N*-oxyamide glycoglycerolipids were able to inhibit the growth of the human ovarian carcinoma cells IGROV-1 under the experimental conditions used, confirming their already explored potentiality and applications.[12,13,47] In this regard, the analysis of the antiproliferative activity of compound **4a** suggests a certain degree of selectivity of this series, because the best activity was observed in the cells with increased activation of the Akt pathway (*i.e.*, IGROV-1/Pt1) as compared to the corresponding cell line with a lower extent of activation (IGROV-1) and to a cell line lacking Akt activation (*i.e.* TOV112D).

Experimental

Chemistry

General methods. All reagents and solvents used were of reagent grade and purchased from Sigma-Aldrich and were purified before use by standard methods. Dry solvents and liquid reagents were distilled prior to use or dried on 4 Å molecular sieves. Air and moisture sensitive liquids and solutions were transferred using an oven-dried syringe through septa. *Candida antarctica* lipase was purchased from Novo Nordisk. *Trametes versicolor* laccase was purchased from Sigma-Aldrich. The acidic DOWEX 50 × 8 H^+ was prepared from a sodium form resin by washing it with 1 M HCl and distilled water prior to use. Column chromatography was carried out on flash silica gel (Aldrich 230–400 mesh) or using a Biotage Isolera Prime™ flash purification system (Biotage-Uppsala, Sweden). Thin-layer chromatography (TLC) analysis was carried out on silica gel plates (Merck 60F254) with visualization under UV (254 nm) and/or developing with an anisaldehyde-based reagent or ceric ammonium molybdate solution (Hanessian's stain). Evaporation under reduced pressure was always performed at a bath temperature below 40 °C. 1,3-Di-*O*-decanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**)[6] was prepared from **14** as described later in this section. 1,3-Di-*O*-benzyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**6**) was prepared according to ref. 18. Trifluoroethyl decanoate was prepared according to ref. 36. The TEMPO oxoammonium tetrafluoroborate salt ($TEMPO^+BF_4^-$) was prepared according to ref. 24. The structures of all the newly synthesized compounds were confirmed through full ^1H and ^{13}C NMR characterization and mass spectrometry. ^1H NMR analysis was 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 unless otherwise stated, and ^{13}C NMR spectra at 125.76 MHz were obtained for 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 $CHCl_3$ fixed at 7.27 ppm, CH_3OH at 3.30 ppm (also when a $CDCl_3/CD_3OD$ mixture was used) respectively for ^1H-NMR spectra, and relative to $CDCl_3$ fixed at 77.0 ppm (central line), CD_3OD at 49.0 ppm (central line) for ^{13}C-NMR spectra, unless otherwise stated. Scalar coupling constants (J) are reported in hertz (Hz). Splitting patterns are described using the following abbreviations: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublet; ddd, doublet of doublet of doublet. High resolution mass spectra were recorded in negative or positive-ion electrospray (ESI) mode on a QTof (Synapt G2-Si HDMS – Waters) spectrometer by COSPECT (comprehensive substance characterization *via* advanced spectroscopy) utility of Università degli Studi di Milano. ^1H- and ^{13}C-NMR and MS analyses confirmed the purity and identity of all the synthesized compounds. Optical rotations were determined on an Anton Paar MCP 100 polarimeter (Anton Paar Italia S.r.l, Rivoli-To) at 20 °C, in a 1 or 0.1 dm cell. Melting points were recorded on Büchi 510 capillary melting point apparatus and were uncorrected. Amicon® Ultra-0.5 30 K centrifugal filter device and an Eppendorf 5804 R centrifuge were used for serum binding studies.

Synthesis of diacyl derivative 3a according to Scheme 1

1,3-Di-O-benzyl-2-O-β-D-glucuronopyranosyl-sn-glycerol (7). To a suspension of 1,3-di-*O*-benzyl-2-*O*-β-D-glucopyranosyl-*sn*-gly-

cerol (**6**)[18] (2.5 g, 5.7 mmol) in a 55 : 45 mixture of CH_3CN/0.67 M sodium phosphate buffer (56 mL, pH 6–7), TEMPO (0.266 g, 1.71 mmol) and 20% aqueous $NaClO_2$ (7.6 mL, 16.8 mmol) were added. Then, 15% aqueous NaClO (0.7 mL, 1.14 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 5 h, then 2.5 mL of $NaClO_2$ solution and 1.8 mL of NaClO solution were further added and the reaction mixture was stirred overnight. Furthermore, 2 mL of $NaClO_2$ solution and 0.4 mL of NaClO solution were added after 15 h. 0.5 M $Na_2S_2O_3$ (25 mL) was added and the reaction mixture was concentrated under vacuum to reduce the volume. The reaction mixture was basified to pH 10 with 1 M NaOH (25 mL) and extracted with Et_2O (3 × 20 mL) to remove TEMPO by-products. The aqueous phase was then acidified by dropping conc. HCl and extracted with CH_2Cl_2 (4 × 40 mL). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed under vacuum. The crude product (2.3 g, 89%) obtained as a white foam was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 3.46–3.67 (m, 5H, H-2′, H-1a, H-1b, H-3a and H-3b), 3.62 (dd, 1H, $J_{3',2'}$ = 8.7 Hz, $J_{3',4'}$ = 8.7 Hz, H-3′), 3.76 (dd, 1H, $J_{4',5'}$ = 8.7 Hz, H-4′), 3.88 (d, 1H, H-5′), 4.00 (m, 1H, H-2), 4.36–4.45 (m, 4H, 2CH_2Ph), 4.52 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1′), 7.14–7.27 (m, 10H, 2Ph). ^{13}C-NMR ($CDCl_3$): δ = 69.7 (C1 or C3), 69.9 (C3 or C1), 71.4 (C4′), 72.9 (C2′), 73.2 and 72.3 (2 CH_2Ph), 74.3 (C5′), 75.5 (C3′), 77.5 (C2), 127.7, 127.8, 127.9, 128.4, 137.6 and 137.7 (12C, 2Ph), 171.2 (C═O). HRMS (negative-ion mode): *m*/*z* 447.1654 [M − H]$^-$, calcd for $C_{23}H_{28}O_9$, *m*/*z* 448.1733 [M].

*1,3-Di-O-benzyl-2-O-β-D-glucuronopyranosyl-sn-glycerol tert-butylester (**8**).* A solution of *tert*-butyl 2,2,2-trichloroacetamidate (0.320 mL, 1.72 mmol) in dry cyclohexane (5.5 mL) was added dropwise at 0 °C to a solution of **7** (0.7 g, 1.56 mmol) in dry CH_2Cl_2 (50 mL). The reaction mixture was stirred at room temperature. Further amounts of *tert*-butyl 2,2,2-trichloroacetamidate (0.087 mL, 0.46 mmol) in dry cyclohexane (1.5 mL) were added dropwise at 0 °C after 5 and 23 h. Pure *tert*-butyl 2,2,2-trichloroacetamidate (0.100 mL, 0.53 mmol) was finally added after 27 h. After 48 h, the solvent was removed under vacuum and the crude product was purified by flash chromatography (CH_2Cl_2/CH_3OH from 98 : 2 to 95 : 5) affording **8** (0.257 g, 33%) as a colorless oil. $[\alpha]_D^{20}$: −14° ($CHCl_3$, *c* 1.0); ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 1.49 (s, 9H, 3C*H*$_3$–*t*Bu), 3.45 (dd, 1H $J_{2',1'}$ = 7.9 Hz, $J_{2',3'}$ = 9.0 Hz, H-2′), 3.58 (dd, 1H, $J_{3',4'}$ = 8.4 Hz, H-3′), 3.61–3.69 (m, 4H, H-1a, H-1b, H-3a and H-3b), 3.69 (d, 1H, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.73 (dd, 1H, H-4′), 4.09 (m, 1H, H-2), 4.50 (d, 1H, H-1′), 4.50–4.57 (m, 4H, 2C*H*$_2$Ph), 7.24–7.37 (m, 10H, 2Ph). ^{13}C-NMR ($CDCl_3$): δ = 28.0 (3CH_3–*t*Bu), 70.3 (C1 or C3), 70.6 (C3 or C1), 71.4 (C4′), 72.9 (C2′), 73.6 (2 *C*H_2Ph), 74.7 (C5′), 75.4 (C3′), 77.7 (C2), 83.2 (*C*–*t*Bu), 103.2 (C1′), 127.8, 127.9, 128.4, 128.5, 137.7 and 137.8 (12C, 2Ph), 168.5 (C═O). HRMS (positive-ion mode): *m*/*z* 527.2264 [M + Na]$^+$, calcd for $C_{27}H_{36}O_9$, *m*/*z* 504.2359 [M], *m*/*z* 527.2252 [M + Na]$^+$.

*1,3-Di-O-benzyl-2-O-(2′,3′,4′-tri-O-acetyl-β-D-glucuronopyranosyl)-sn-glycerol tert-butylester (**9**).* To a solution of **8** (0.250 g, 0.49 mmol) in dry pyridine (1.87 mL), Ac_2O (1.87 mL) was added dropwise at room temperature. The reaction mixture was stirred for 3 h until complete consumption of the starting material (TLC, CH_2Cl_2/MeOH 95 : 5). The solvent was removed under vacuum and the crude **9** (0.252 g, 81%), obtained as a white waxy solid, was used for the next step without further purification. ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 1.43 (s, 9H, 3CH_3, *t*Bu), 1.93, 2.00 and 2.01 (3s, 9H, 3CH_3CO), 3.55 (dd, 2H, $J_{1(3)a,1(3)b}$ = 10.5 Hz, $J_{1(3)a,2}$ = 6.5 Hz, H-1a and H-3a), 3.68 (dd, 2H, $J_{1(3)b,2}$ = 4.0 Hz, H-1b and H-3b), 3.88 (d, 1H, $J_{5',4'}$ = 9.5 Hz, H-5′), 4.06 (m, 1H, H-2), 4.46–4.54 (m, 4H, 2C*H*$_2$Ph), 4.83 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 5.01 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.19 (dd, 1H, $J_{3',4'}$ = 9.5 Hz, H-3′), 5.25 (dd, 1H, H-4′), 7.23–7.38 (m, 10H, 2Ph). ^{13}C-NMR ($CDCl_3$): δ = 20.6 (3CH_3CO), 27.8 (3CH_3–*t*Bu), 69.5 (C4′), 70.1 (C1 or C3), 71.1 (C3 or C1), 71.4 (C2′), 72.6 (C3′), 73.3 (C5′), 73.5 (2 *C*H_2Ph), 78.3 (C2), 82.9 (*C*–*t*Bu), 100.7 (C1′), 127.5, 127.6, 127.7, 128.4, 138.1 and 138.2 (12C, 2Ph), 165.6 (C═O), 169.0, 169.3, 170.3 (3CH_3*C*═O). HRMS (positive-ion mode): *m*/*z* 653.2570 [M + Na]$^+$, calcd for $C_{33}H_{42}O_{12}$, *m*/*z* 630.2676 [M], *m*/*z* 653.2568 [M + Na]$^+$.

*2-O-(2′,3′,4′-Tri-O-acetyl-β-D-glucuronopyranosyl)-sn-glycerol tert-butylester (**10**).* To a solution of **9** (0.239 g, 0.38 mmol) in dry MeOH, 10% Pd/C (0.119 g) was added under an inert atmosphere. The flask was evacuated and backfilled with H_2 three times and then stirred at room temperature until complete consumption of the starting material (TLC, hexane/acetate 50 : 50, CH_2Cl_2/MeOH 95 : 5). The mixture was filtered on a pad of Celite and the solvent was removed under vacuum. The crude **10**, obtained as a white waxy solid (0.175 g, quantitative yield), was used for the next step without further purification. ^{1}H-NMR (CD_3OD): δ = 1.45 (s, 9H, 3CH_3–*t*Bu), 1.97, 2.00 and 2.02 (3s, 9H, 3CH_3CO), 3.53–3.66 (m, 4H, H-1a, H-1b, H-3a and H-3b), 3.73 (m, 1H, H-2), 4.16 (d, 1H, $J_{5',4'}$ = 10.0 Hz, H-5′), 4.88–4.94 (m, 2H, H-1′ and H-2′), 5.12 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.27 (dd, 1H, $J_{3',2'}$ = 10.0 Hz, H-3′). ^{13}C-NMR (CD_3OD): δ = 20.5 and 20.6 (3CH_3CO), 28.1 (3CH_3–*t*Bu), 62.6 (C1 or C3), 63.1 (C3 or C1), 70.8 (C4′), 72.8 (C2′), 73.8 (C5′), 73.9 (C3′), 83.8 (C2), 84.4 (*C*–*t*Bu), 101.8 (C1′), 167.9 (C═O), 170.8, 171.3, 171.5 (3CH_3*C*═O). HRMS (positive-ion mode): *m*/*z* 473.1631 [M + Na]$^+$, calcd for $C_{19}H_{30}O_{12}$, *m*/*z* 450.1737 [M], *m*/*z* 473.1629 [M + Na]$^+$.

*1,3-Di-O-phthalimido-2-O-(2′,3′,4′-tri-O-acetyl-β-D-glucuronopyranosyl)-sn-glycerol tert-butyl ester (**11**).* To a solution of **10** (0.171 g, 0.38 mmol) in a mixture of dry CH_2Cl_2 (1.5 mL) and dry toluene (3 mL), PPh_3 (0.398 g, 1.52 mmol) was added at room temperature. The reaction mixture was cooled to 0 °C before DIAD (0.307 g, 1.52 mmol) and *N*-hydroxyphthalimide (0.247 g, 1.52 mmol) were added. The reaction mixture was slowly warmed to room temperature and then stirred at room temperature 2 h until complete consumption of the starting material (TLC, CH_2Cl_2/MeOH 95 : 5). The solvent was removed under vacuum and the crude product was purified twice by flash chromatography (petroleum ether/AcOEt, from 40 : 60 to 30 : 70), affording **11** as a white/yellow waxy solid (0.240 g, 85%). $[\alpha]_D^{20}$: +24.2 ($CHCl_3$, *c* 1.0); ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 1.40 (s, 9H, 3CH_3, *t*Bu), 1.98, 2.00 and 2.09 (3s, 9H, 3CH_3CO), 3.99 (d, 1H, $J_{5',4'}$ = 9.9 Hz,

H-5′), 4.29 (dd, 1H, $J_{1(3)a,1(3)b}$ = 11.1 Hz, $J_{1(3)a,2}$ = 7.0 Hz, H-1a or H-3a), 4.48 (dd, 1H, $J_{1(3)b,2}$ = 4.0 Hz, H-1b or H-3b), 4.44 (m, 1H, H-2), 4.55 (dd, 1H, $J_{3(1)a,3(1)b}$ = 11.5 Hz, $J_{3(1)a,2}$ = 7.4 Hz, H-3a or H-1a), 4.74 (dd, 1H, $J_{3(1)b,2}$ = 2.5 Hz, H-3b or H-1b), 4.93 (dd, 1H, $J_{2',1'}$ = 7.9 Hz, $J_{2',3'}$ = 9.3 Hz, H-2′), 5.14 (d, 1H, H-1′), 5.17 (dd, 1H, $J_{4',3'}$ = 9.3 Hz, H-4′), 5.24 (dd, 1H, H-3′), 7.70–7.88 (m, 8H, 2Phth). ^{13}C-NMR ($CDCl_3$): δ = 20.6 (3*C*H$_3$CO), 27.7 (3*C*H$_3$, *t*Bu), 69.4 (C4′), 70.9 (C2′), 72.5 (C3′), 73.1 (C5′), 75.1 (C2), 76.7 (C1 or C3), 79.0 (C3 or C1), 82.9 (*C*–*t*Bu), 100.7 (C1′), 123.6, 128.8, 129.0, 134.5 (12C, 2Phth), 163.2 and 163.5 (4 *C*=O Phth), 165.5 (C=O), 169.1, 169.7, 170.1 (3CH$_3$*C*=O). HRMS (positive-ion mode): *m*/*z* 763.1965 [M + Na]$^+$, calcd for $C_{35}H_{36}N_2O_{16}$, *m*/*z* 740.2065 [M], *m*/*z* 763.1957 [M + Na]$^+$.

*1,3-Di-O-decanoylamino-2-O-(2′,3′,4′-tri-O-acetyl-β-D-glucuronopyranosyl)-sn-glycerol tert-butylester (**12**).* To a suspension of **11** (0.235 g, 0.32 mmol) in dry MeOH (5.7 mL), hydrazine hydrate (0.038 g, 0.76 mmol) was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and then 3 h at room temperature until complete consumption of the starting material (TLC CH_2Cl_2/MeOH 95 : 5). The solvent was removed under vacuum and the crude 1,3-di-*O*-amino-2-*O*-(2′,3′,4′-tri-*O*-acetyl-β-D-glucuronopyranosyl)-*sn*-glycerol *tert*-butylester was used for the next step without further purification. To a solution of the crude diamino derivative in dry CH_2Cl_2 (2.8 mL), a 10% (v/v) solution of pyridine in CH_2Cl_2 (1.4 mL) and a 15% (v/v) solution of decanoyl chloride in CH_2Cl_2 (1.16 mL, 0.86 mmol) was added at −10 °C. The reaction mixture was stirred at room temperature for 30 minutes. The reaction mixture was diluted with 30 mL of CH_2Cl_2 and washed with 1 M HCl (1 × 20 mL), water (1 × 20 mL), $NaHCO_3$ saturated solution (1 × 20 mL) and finally with water again (2 × 20 mL). Aqueous layers were re-extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered and the solvent removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 98 : 2 to 95 : 5), affording **12** as a white solid (0.106 g, 42%). $[\alpha]_D^{20}$: −22.4 ($CHCl_3$, *c* 1.0); ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 0.87 (m, 6H, 2 CH_3), 1.19–1.35 (m, 24H, 12 CH_2), 1.45 (s, 9H, 3CH_3, –*t*Bu), 1.56–1.72 (m, 4H, 2 CH_2), 2.02, 2.04 and 2.06 (3s, 9H, 3CH_3CO), 2.05–2.17 (m, 4H, 2 CH_2), 3.86–4.13 (m, 4H, H-1a, H-1b, H-3a and H-3b), 4.03 (d, 1H, $J_{5',4'}$ = 9.6 Hz, H-5′), 4.27 (m, 1H, H-2), 4.89 (br d, 1H $J_{1',2'}$ = 7.4 Hz, H-1′), 5.01 (dd, 1H, $J_{2',3'}$ = 9.2 Hz, H-2′), 5.23 (dd, 1H, $J_{4',3'}$ = 9.2 Hz, H-4′), 5.26 (dd, 1H, H-3′), 9.18 and 9.82 (2br s, 2H, 2NH). ^{13}C-NMR ($CDCl_3$): δ = 14.0 (2 *C*H$_3$), 20.5, 20.6 and 20.7 (3*C*H$_3$CO), 22.6 (2 *C*H$_2$), 25.3–25.6 (br, 2 *C*H$_2$), 27.8 (3*C*H$_3$–*t*Bu), 29.0–29.5 (8 *C*H$_2$), 31.8 (2 *C*H$_2$), 33.0 (br, 2 *C*H$_2$), 68.9 (C4′), 71.2 (C2′), 71.9 (br, C5′), 72.4 (C3′), 75.1 (br, C2), 75.2 (br, C1 or C3), 75.8 (br, C3 or C1), 84.1 (*C*–*t*Bu), 101.1 (C1′), 166.9 (C=O), 169.0, 169.8 (br), 170.0 (3 CH$_3$*C*=O), 171.6 (br, 2 NHC=O). HRMS (positive-ion mode): *m*/*z* 811.4568 [M + Na]$^+$, calcd for $C_{39}H_{68}N_2O_{14}$, *m*/*z* 788.4671 [M], *m*/*z* 811.4563 [M + Na]$^+$.

*1,3-Di-O-decanoylamino-2-O-β-D-glucuronopyranosyl-sn-glycerol tert-butylester (**13**).* To a suspension of **12** (0.086 g, 0.11 mmol) in dry MeOH (4.4 mL), 1 M MeONa (1.2 mL) was added dropwise at room temperature. The reaction mixture was stirred at room temperature until complete consumption of the starting material (TLC, CH_2Cl_2/MeOH 95 : 5). The reaction mixture was neutralized with DOWEX 50 × 8 H$^+$, filtered and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 95 : 5 to 90 : 10), affording compound **13** as a white solid (0.036 g, 50%). $[\alpha]_D^{20}$: −23.7 (MeOH, *c* 1.0); ^{1}H-NMR (CD_3OD): δ = 0.89 (m, 6H, 2 CH_3), 1.22–1.37 (m, 24H, 12 CH_2), 1.50 (s, 9H, 3CH_3–*t*Bu), 1.56–1.66 (m, 4H, 2 CH_2), 2.04–2.14 (m, 4H, 2 CH_2), 3.26 (dd, 1H, $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 9.1 Hz, H-2′), 3.38 (dd, 1H, $J_{3',4'}$ = 9.1 Hz, H-3′), 3.47 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4′), 3.77 (d, 1H, H-5′), 3.91–4.12 (m, 4H, H-1a, H-1b, H-3a and H-3b), 4.18 (m, 1H, H-2), 4.58 (d, 1H, H-1′). ^{13}C-NMR (CD_3OD): δ = 14.4 (2 *C*H$_3$), 23.7 (2 *C*H$_2$), 26.6 (2 *C*H$_2$), 28.3 (3*C*H$_3$–*t*Bu), 30.1–30.7 (8 *C*H$_2$), 33.0 (2 *C*H$_2$), 33.7 (*C*H$_2$), 33.8 (*C*H$_2$), 73.2 (C4′), 74.8 (C2′), 76.7 (br, C1 or C3), 76.9 (C2), 77.0 (C5′), 77.3 (br, C3 or C1), 77.5 (C3′), 83.6 (*C*–*t*Bu), 104.6 (C1′), 170.4 (C=O), 172.9 and 173.0 (2 NHC=O). HRMS (positive-ion mode): *m*/*z* 685.4250 [M + Na]$^+$, calcd for $C_{33}H_{62}N_2O_{11}$, *m*/*z* 662.4354 [M], *m*/*z* 685.4246 [M + Na]$^+$.

*1,3-Di-O-decanoylamino-2-O-β-D-glucuronopyranosyl-sn-glycerol (**3a**).* Compound **13** (0.020 g, 0.030 mmol) was stirred with 15% (v/v) trifluoroacetic acid in CH_2Cl_2 (0.5 mL) at room temperature until complete consumption of the starting material. The solvent was removed under vacuum and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 85 : 15 to 80 : 20). The obtained pure compound was dissolved in CH_2Cl_2, stirred with DOWEX 50 × 8 H$^+$ (previously washed only with deionized water) and filtered. The resin was washed with a 1 : 1 mixture of EtOAc/i-PrOH for a complete recovery of the desired compound. The solvent was removed under vacuum affording **3a** as a white solid (0.012 g, 66%). $[\alpha]_D^{20}$: −5.15° (MeOH, *c* 0.66); ^{1}H-NMR (CD_3OD): δ = 0.89 (m, 6H, 2 CH_3), 1.21–1.38 (m, 24H, 12 CH_2), 1.54–1.64 (m, 4H, 2 CH_2), 2.04–2.13 (m, 4H, 2 CH_2), 3.29 (dd, 1H, $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 9.2 Hz, H-2′), 3.42 (dd, 1H, $J_{3',4'}$ = 9.2 Hz, H-3′), 3.51 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4′), 3.88 (d, 1H, H-5′), 3.90–4.11 (m, 4H, H-1a, H-1b, H-3a and H-3b), 4.21 (m, 1H, H-2), 4.61 (d, 1H, H-1′). ^{13}C-NMR (CD_3OD): δ = 14.4 (2 *C*H$_3$), 23.7 (2 *C*H$_2$), 26.6 (2 *C*H$_2$), 30.1–30.6 (8 *C*H$_2$), 33.0 (2 *C*H$_2$), 33.7 (2 *C*H$_2$), 73.1 (C4′), 74.7 (C2′), 76.2 (br, C5′), 76.6 (C1 or C3), 76.8 (C2), 77.3 (C3 or C1), 77.5 (C3′), 104.5 (C1′), 172.6 (br, C=O), 172.9 and 173.0 (2 NHC=O). HRMS (negative-ion mode): *m*/*z* 605.3653 [M − H]$^-$, calcd for $C_{29}H_{54}N_2O_{11}$, *m*/*z* 606.3728 [M].

Synthesis of diacyl derivative 3a according to Scheme 2

*1,3-Di-O-phthalimido-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (**15**).* To a solution of **14**[20,21] (2 g, 4.73 mmol) in a mixture of dry CH_2Cl_2 (15 mL) and dry toluene (30 mL), PPh_3 (5.16 g, 19.67 mmol) was added at room temperature. The reaction mixture was cooled to 0 °C before DIAD (3.87 mL, 19.67 mmol) and *N*-hydroxyphthalimide (3.21 g, 19.67 mmol) were added. The reaction mixture was slowly warmed and then stirred at room temperature for 2 h, until complete consumption of the starting material (TLC, CH_2Cl_2/MeOH 95 : 5, EtOAc/petroleum ether 70 : 30). The reac-

tion mixture was concentrated under vacuum. The resulting dark red viscous oil was dissolved in about 15 mL of Et_2O and allowed to crystallize at 5 °C overnight. The crystalline solid was collected by filtration using a Büchner filter and further purified by flash chromatography (petroleum ether/EtOAc 30 : 70) affording **15** as a white solid (2.67 g, 79%). $[\alpha]_D^{20}$: −22° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 1.98, 2.00, 2.05 and 2.08 (4s, 12H, 4CH_3CO), 3.80 (ddd, 1H, $J_{5',4'}$ = 9.8 Hz, $J_{5',6a'}$ = 2.3 Hz, $J_{5',6b'}$ = 5.0 Hz, H-5′), 4.09 (dd, 1H, $J_{6'a,6'b}$ = 12.3 Hz, H-6′a), 4.18 (dd, 1H, H-6′b), 4.31 (dd, 1H, $J_{1(3)a,1(3)b}$ = 11.0 Hz, $J_{1(3)a,2}$ = 6.6 Hz, H-1a or H-3a), 4.43 (m, 1H, H-2), 4.46 (dd, 1H, $J_{1(3)b,2}$ = 4.1 Hz, H-1b or H-3b), 4.53 (dd, 1H, $J_{3(1)a,3(1)b}$ = 11.6 Hz, $J_{3(1)a,2}$ = 7.4 Hz, H-3a or H-1a), 4.69 (dd, 1H, $J_{3(1)b,2}$ = 2.8 Hz, H-3b or H-1b), 4.91 (dd, 1H, $J_{2',1'}$ = 8.0 Hz, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.00 (dd, 1H, $J_{4',3'}$ = 9.5 Hz, H-4′), 5.12 (d, 1H, H-1′), 5.24 (dd, 1H, H-3′), 7.72–7.87 (m, 8H, 2Phth). ^{13}C-NMR ($CDCl_3$): δ = 20.6 (4*C*H_3CO), 61.9 (C6′), 68.5 (C4′), 71.2 (C2′), 71.7 (C5′), 72.9 (C3′), 75.2 (C2), 76.8 (C1 or C3), 79.0 (C3 or C1), 100.8 (C1′), 123.6, 128.9, 129.0, 134.5 (12C, 2Phth), 163.2 and 163.5 (4 *C*=O Phth), 169.4, 169.6, 170.1, 170.7 (4 CH_3C=O). HRMS (positive-ion mode): *m*/*z* 735.1646 $[M + Na]^+$, calcd for $C_{33}H_{32}N_2O_{16}$, *m*/*z* 712.1752 [M], *m*/*z* 735.1644 $[M + Na]^+$.

*1,3-Di-O-decanoylamino-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (**16**).* To a suspension of **15** (1.69 g, 2.37 mmol) in a mixture of dry THF (16.9 mL) and dry MeOH (42.3 mL), a freshly prepared solution of hydrazine hydrate (100 μL mL^{-1}) in dry MeOH (2.3 mL, 4.74 mmol) was added dropwise at 0 °C and the reaction mixture was stirred at the same temperature, under an inert atmosphere, for 5 minutes (TLC CH_2Cl_2/MeOH 95 : 5). The reaction was diluted with CH_2Cl_2 (250 mL) and washed with water (150 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude 1,3-di-*O*-amino-2-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol was used for the next step without further purification. To a solution of the crude diamino derivative in dry CH_2Cl_2 (34 mL), a 10% (v/v) solution of pyridine in dry CH_2Cl_2 (11.5 mL, 14.22 mmol) and a 15% (v/v) solution of decanoyl chloride in dry CH_2Cl_2 (9.8 mL, 7.11 mmol) were added at −10 °C. The reaction mixture was stirred at the same temperature for 15 minutes, until complete consumption of the starting material (TLC CH_2Cl_2/MeOH 95 : 5). The reaction mixture was diluted with 100 mL of CH_2Cl_2 and washed with 1 M HCl (1 × 150 mL), and the aqueous layers were re-extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was washed with hexane (3 × 3 mL) to remove most of the decanoic acid and then purified by flash chromatography (EtOAc/petroleum ether +2% v/v MeOH from 70 : 30 to 80 : 20) affording **16** (1.32 g, 73%) as a white waxy solid. $[\alpha]_D^{20}$: −9.9° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$) (residual $CHCl_3$ fixed at 7.26 ppm): δ = 0.86 (m, 6H, 2 CH_3), 1.17–1.35 (m, 24H, 12 CH_2), 1.56–1.66 (m, 4H, 2 CH_2), 1.99, 2.02, 2.04 and 2.09 (4s, 12H, 4CH_3CO), 2.07–2.14 (m, 4H, 2 CH_2), 3.74 (br ddd, 1H, $J_{5',4'}$ = 9.7 Hz, $J_{5',6'a}$ = 4.2 Hz, $J_{5',6'b}$ < 1.0 Hz, H-5′), 3.93–4.13 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-2), 4.17 (dd, 1H, $J_{6'a,6'b}$ = 12.4 Hz, H-6′a), 4.31 (brdd, 1H, H-6′b), 4.77 (br d, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.94 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.06 (dd, 1H, $J_{4',3'}$ = 9.5 Hz, H-4′), 5.21 (dd, 1H, H-3′), 9.58 and 10.10 (2br s, 2H, 2NH). ^{13}C-NMR ($CDCl_3$): δ = 14.1 (2 *C*H_3), 20.6, 20.7 and 20.8 (4*C*H_3CO), 22.7 (2 *C*H_2), 25.4 (br, 2 *C*H_2), 29.2–29.7 (8 *C*H_2), 31.9 (2 *C*H_2), 33.0 (br, 2 *C*H_2), 61.5 (C6′), 68.3 (C4′), 71.4 (C2′), 72.0 (C5′), 72.5 (C3′), 75.6–75.8 (br, C1, C2 and C3), 100.6 (C1′), 169.5, 169.9, 170.1 and 171.1 (4 CH_3C=O), 171.3 (br, 2 NHC=O). HRMS (positive-ion mode): *m*/*z* 783.4253 $[M + Na]^+$, calcd for $C_{37}H_{64}N_2O_{14}$, *m*/*z* 760.4358 [M], *m*/*z* 783.4250 $[M + Na]^+$.

*1,3-Di-O-decanoylamino-2-O-β-D-glucopyranosyl-sn-glycerol (**3b**).* To a suspension of **16** (0.66 g, 0.87 mmol) in dry MeOH (30 mL), a freshly prepared solution of sodium methoxide 0.075 M (7.0 mL, 0.52 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 4 hours, until complete consumption of the starting material (TLC CH_2Cl_2/MeOH 90 : 10). The reaction mixture was acidified with DOWEX 50 × 8 H^+, filtered and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 90 : 10 to 85 : 15) affording compound **3b** (0.417 g, 81%) as a white waxy solid. $[\alpha]_D^{20}$: −35.3° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$: CD_3OD 70 : 30): δ = 0.83 (m, 6H, 2 CH_3), 1.14–1.32 (m, 24H, 12 CH_2), 1.50–1.60 (m, 4H, 2 CH_2), 1.99–2.07 (m, 4H, 2 CH_2), 3.23–3.34 (m, 3H, H-2′, H-4′, H-5′), 3.38 (dd, 1H, $J_{3',4'}$ = 8.5 Hz, $J_{3',2'}$ = 8.5 Hz, H-3′), 3.63 (dd, 1H, $J_{6'a,6'b}$ = 11.7 Hz, $J_{6'a,5'}$ = 5.9 Hz, H-6′a), 3.84–4.06 (m, 5H, H-1a, H-1b, H-3a, H-3b and H-6′b), 4.11 (m, 1H, H-2), 4.43 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1′). ^{13}C-NMR ($CDCl_3$: CD_3OD 70 : 30): δ = 14.2 (2 *C*H_3), 22.9 (2 *C*H_2), 25.8 (2 *C*H_2), 29.3–30.0 (8 *C*H_2), 32.1 (2 *C*H_2), 33.1 (*C*H_2), 33.2 (*C*H_2), 62.1 (C6′), 70.7 (C4′), 73.9 (C2′), 75.7 (C1 or C3), 76.2 (C2), 76.4 (C3 or C1), 76.8 (C5′), 76.9 (C3′), 103.6 (C1′), 171.9 and 172.3 (2 NHC=O). HRMS (positive-ion mode): *m*/*z* 615.3830 $[M + Na]^+$, calcd for $C_{29}H_{56}N_2O_{10}$, *m*/*z* 592.3935 [M], *m*/*z* 615.3827 $[M + Na]^+$.

*1,3-Di-O-decanoylamino-2-O-β-D-glucuronopyranosyl-sn-glycerol (**3a**).* In an open reaction vial, to a suspension of **3b** (0.05 g, 0.084 mmol) in 20 mM acetate buffer (4 mL, pH 4.5), TEMPO (0.010 g, 0.064 mmol) and *Trametes versicolor* laccase (0.022 g, 11 U) were added at room temperature. The reaction mixture was stirred for 10 days in open air at 28–30 °C until complete consumption of the starting material (TLC, CH_2Cl_2/MeOH 85 : 15). A further amount of TEMPO (0.010 g) and laccase (0.022 g) were added after 3 and 8 days of the reaction. The reaction mixture was diluted with water (10 mL), acidified with 0.1 M HCl up to pH 2, and extracted with diethyl ether (4 × 10 mL) and CH_2Cl_2 (1 × 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 90 : 10 to 80 : 20). The collected fractions were treated with DOWEX 50 × 8 H^+, filtered and the solvent was removed under vacuum affording compound **3a** (0.025 g, 49%).

Synthesis of monoacyl glucuronide 4a

*3-O-Decanoyl-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (**17a**).* To a solution of **14** (1.04 g, 2.46 mmol) in dry THF (27 mL), trifluoroethyl decanoate[36] (1.88 g, 0.71 mmol) and *Candida antarctica* lipase (3.0 g, 0.0005 PLU) were added at room temperature. The reaction mixture was stirred at 45 °C for 24 hours, until complete consumption of the starting material (TLC EtOAc/petroleum ether 70 : 30). The enzyme was removed by filtration using a Büchner filter, and the solvent was removed under vacuum. The crude product, containing 10% of the 1-*O* isomer (by NMR), was purified by flash chromatography (petroleum ether/EtOAc 50 : 50) affording pure **17a** (1.08 g, 76%) as a white waxy solid. $[\alpha]_D^{20}$: −4.4° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, J = 6.8 Hz, 3H, CH_3), 1.24–1.32 (m, 12H, 6 CH_2), 1.57–1.64 (m, 2H, CH_2), 2.00, 2.02, 2.05 and 2.08 (4s, 12H, 4CH_3CO), 2.31 (t, J = 7.7 Hz, 2H, CH_2), 3.58–3.69 (m, 2H, H-1a, H-1b), 3.72 (ddd, $J_{5',4'}$ = 10.0, $J_{5',6'b}$ = 5.3 Hz, $J_{5',6'a}$ = 2.4 Hz, 1H, H-5′), 3.87–3.94 (m, 1H, H-2), 4.06–4.15 (m, 2H, H-3a, H-6′a), 4.24 (dd, $J_{6'a,6'b}$ = 12.3 Hz, 1H, H-6′b), 4.30 (dd, J = $J_{3b,3a}$ = 11.8 Hz, $J_{3b,2}$ = 4.9 Hz, 1H, H-3b), 4.69 (d, $J_{1',2'}$ = 8.0 Hz, 1H, H-1′), 5.00 (dd, $J_{2',3'}$ = 9.6 Hz, 1H, H-2′), 5.06 (dd, $J_{4',3'}$ = 9.4 Hz, 1H, H-4′), 5.21 (dd, 1H, H-3′). ^{13}C-NMR ($CDCl_3$): δ = 14.1 (CH_3), 20.5–20.6 (4 CH_3C═O), 22.6 (CH_2), 24.8 (CH_2), 29.1–29.4 (4 CH_2), 31.8 (CH_2), 34.1 (CH_2), 62.0 (C6′), 62.2 (C1), 62.8 (C3), 68.3 (C4′), 71.6 (C2′), 72.0 (C5′), 72.6 (C3′), 79.4 (C2), 100.9 (C1′), 169.4, 169.6, 170.2 and 170.6 (4 CH_3C═O), 173.6 (CH_2C═O decanoyl). HRMS (positive-ion mode): *m*/*z* 599.2689 $[M + Na]^+$, calcd for $C_{27}H_{44}O_{13}$, *m*/*z* 576.2782 [M], *m*/*z* 599.2674 $[M + Na]^+$.

Configuration assignment of compound 17a. The known 3-*O*-decanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol[18] having 2*R* configuration was treated with acetic anhydride and pyridine. The peracetylation product was identical to compound **17b** which was obtained by acetylation of **17a** at the same time confirming its 2*R* configuration.

*1-O-Acetyl-3-O-decanoyl-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (**17b**).* An enriched chromatographic fraction of **17a** (0.011 g, 0.02 mmol) containing about 23% (by NMR) of the corresponding diastereoisomer was acetylated by acetic anhydride (0.004 mL, 0.04 mmol) and pyridine (0.3 mL) treatment. After 12 hours, the solvent was removed under vacuum and the crude product was subjected to ^{1}H-NMR analysis in deuterated chloroform. Here, we report the resonances of the anomeric protons of the reaction mixture enriched in **17b**. ^{1}H-NMR ($CDCl_3$): δ = 4.64 ppm **17b** (about 77%), 4.63 ppm (about 23%). In the same way, pure 3-*O*-decanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (possessing 2*R* configuration) prepared according to ref. 18 (0.0085 g, 0.02 mmol), was fully acetylated by acetic anhydride (0.004 mL, 0.04 mmol) and pyridine (0.3 mL) treatment, affording, after usual work-up, **17b**. $[\alpha]_D^{20}$: −10.0° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$): δ = 0.87 (t, J = 6.8 Hz, 3H, CH_3), 1.24–1.32 (m, 12H, 6 CH_2), 1.56–1.64 (m, 2H, CH_2), 2.00, 2.02, 2.03, 2.07 and 2.08 (5s, 15H, 5CH_3CO), 2.30 (t, J = 7.6 Hz, 2H, CH_2), 3.70 (ddd, $J_{5',4'}$ = 10.1, $J_{5',6'b}$ = 5.2 Hz, $J_{5',6'a}$ = 2.4 Hz, 1H, H-5′), 4.06 (m, 1H, H-2), 4.08–4.20 (m, 4H, H-1a, H-1b, H-3a, H-6′a), 4.20–4.29 (m, 2H, H-3b, H-6′b), 4.64 (d, $J_{1',2'}$ = 8.0 Hz, 1H, H-1′), 4.97 (dd, $J_{2',3'}$ = 9.6 Hz, 1H, H-2′), 5.06 (dd, $J_{4',3'}$ = 9.7 Hz, 1H, H-4′), 5.19 (dd, 1H, H-3′). ^{13}C-NMR ($CDCl_3$): δ = 14.1 (CH_3), 20.5–20.7 (5 CH_3C═O), 22.6 (CH_2), 24.8 (CH_2), 29.1–29.4 (4 CH_2), 31.8 (CH_2), 34.0 (CH_2), 61.9 (C6′), 63.0 (C3), 63.4 (C1), 68.3 (C4′), 71.3 (C2′), 71.9 (C5′), 72.7 (C3′), 75.7 (C2), 100.8 (C1′), 169.1, 169.4, 170.2, 170.5 and 170.6 (5 CH_3C═O), 173.3 (CH_2C═O decanoyl). HRMS (positive-ion mode): *m*/*z* 641.2795 $[M + Na]^+$, calcd for $C_{29}H_{46}O_{14}$, *m*/*z* 618.2888 [M], *m*/*z* 641.2780 $[M + Na]^+$. The comparison of the two spectra showed that the resonances of the main isomer from **17a** acetylation were superimposable to that of pure **17b**, confirming in this way the 2*R* configuration of **17a** (Fig. S1†).

*1-O-Phthalimido-3-O-decanoyl-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (**18**).* To a solution of **17a** (0.925 g, 1.60 mmol) in a mixture of dry CH_2Cl_2 (7 mL) and dry toluene (14 mL), PPh_3 (0.881 g, 3.36 mmol) was added at room temperature. The reaction mixture was cooled to 0 °C before DIAD (0.662 mL, 3.36 mmol) and *N*-hydroxyphthalimide (0.548 g, 3.36 mmol) were added. The reaction mixture was slowly warmed and then stirred at room temperature for 1 hour, until complete consumption of the starting material (TLC, petroleum ether/EtOAc 50 : 50). The solvent was removed under vacuum, and the crude product was purified by flash chromatography (petroleum ether/EtOAc/toluene 50 : 50 : 10) affording **18** as a white waxy solid (1.06 g, 92%). $[\alpha]_D^{20}$: +4.0° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$): δ = 0.87 (t, J = 6.9 Hz, 3H, CH_3), 1.18–1.32 (m, 12H, 6 CH_2), 1.53–1.60 (m, 2H, CH_2), 2.00, 2.02, 2.08 and 2.09 (4s, 12H, 4CH_3CO), 2.29 (brt, 2H, CH_2), 3.79 (ddd, $J_{5',4'}$ = 10.0, $J_{5',6'b}$ = 4.9 Hz, $J_{5',6'a}$ = 2.4 Hz, 1H, H-5′), 4.11–4.21 (m, 2H, H-3a, H-6′a), 4.21–4.36 (m, 5H, H-1a,b, H-3b, H-2, H-6′b), 5.00 (d, $J_{1',2'}$ = 8.0 Hz, 1H, H-1′), 5.04 (dd, $J_{2',3'}$ = 9.2 Hz, 1H, H-2′), 5.08 (dd, $J_{4',3'}$ = 9.3 Hz, 1H, H-4′), 5.25 (dd, 1H, H-3′), 7.74–7.81 (m, 2H, Phth), 7.81–7.88 (m, 2H, Phth). ^{13}C-NMR ($CDCl_3$): δ = 14.1 (CH_3), 20.6–20.7 (4 CH_3C═O), 22.6 (CH_2), 24.8 (CH_2), 29.1 (CH_2), 29.2 (2 CH_2), 29.4 (CH_2), 31.8 (CH_2), 34.0 (CH_2), 62.0 (C6′), 63.1 (C3), 68.5 (C4′), 71.2 (C2′), 71.8 (C5′), 72.9 (C3′), 75.2 (C2), 78.9 (C1), 100.8 (C1′), 123.7, 128.8 and 134.7 (6C, Phth), 163.3 (2 C═O Phth), 169.4, 169.7, 170.2 and 170.7 (4 CH_3C═O), 173.3 (CH_2C═O decanoyl). HRMS (positive-ion mode): *m*/*z* 744.2850 $[M + Na]^+$, calcd for $C_{35}H_{47}NO_{15}$, *m*/*z* 721.2946 [M], *m*/*z* 744.2838 $[M + Na]^+$.

*1-O-Octadecanoylamino-3-O-decanoyl-2-O-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol (**19**).* To a suspension of **18** (1.04 g, 1.44 mmol) in a mixture of dry THF (25 mL) and dry MeOH (10 mL), hydrazine hydrate (0.070 mL, 1.44 mmol) was added dropwise at 0 °C and the reaction mixture was stirred at the same temperature, under an inert atmosphere, for 5 minutes (TLC petroleum ether/EtOAc 50 : 50). The reaction was diluted with CH_2Cl_2 (100 mL) and washed with water (50 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 30 mL), the combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude 1-*O*-amino-3-*O*-decanoyl-2-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol was used for the next step without further purification. To a solution of the crude amine derivative in dry CH_2Cl_2 (20 mL), a 10% (v/v) solution of pyridine in dry CH_2Cl_2

(3.5 mL, 4.32 mmol) and a 15% (v/v) solution of octadecanoyl chloride in dry CH_2Cl_2 (4.9 mL, 2.16 mmol) were added at −10 °C. The reaction mixture was stirred at the same temperature for 5 minutes, until complete consumption of the starting material (TLC petroleum ether/EtOAc 50 : 50). The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed once with a mixture of 1 M HCl (50 mL) and brine (30 mL), and the aqueous layers were re-extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by flash chromatography (petroleum ether/EtOAc/toluene 50 : 50 : 10), affording **19** (1.07 g, 87%) as a white waxy solid. $[\alpha]_D^{20}$: −16.0° ($CHCl_3$, *c* 0.6). ^{1}H-NMR ($CDCl_3$): δ = 0.86 (m, 6H, 2CH_3), 1.07–1.42 (m, 40H, 20 CH_2), 1.51–1.68 (m, 4H, CH_2), 1.99, 2.01, 2.04 and 2.07 (4s, 12H, 4CH_3CO), 2.02–2.11 (m, 2H, CH_2), 2.28 (t, J = 7.6 Hz, 2H, CH_2), 3.73 (ddd, $J_{5',4'}$ = 10.1, $J_{5',6'b}$ = 5.3 Hz, $J_{5',6'a}$ = 2.4 Hz, 1H, H-5′), 3.91 (dd, $J_{1a,1b}$ = 12.1, $J_{1,2}$ = 7.3 Hz, 1H, H-1a), 4.02–4.15 (m, 4H, H-1b, H-2, H-3a, H-6′a), 4.18–4.30 (m, 2H, H-3b, H-6′b), 4.73 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1′), 4.99 (dd, $J_{2',3'}$ = 9.7 Hz, 1H, H-2′), 5.04 (dd, $J_{4',3'}$ = 9.7 Hz, 1H, H-4′), 5.22 (dd, 1H, H-3′), 8.66 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 14.1 (2 *C*H_3), 20.5, 20.6 and 20.7 (4 *C*H_3C═O), 22.6 (2 *C*H_2), 24.8 (*C*H_2), 25.3 (br, *C*H_2) 28.9–29.8 (16 *C*H_2), 31.8 (*C*H_2), 31.9 (*C*H_2), 33.1 (br, *C*H_2) 34.0 (*C*H_2), 61.9 (C6′), 62.8 (C3), 68.3 (C4′), 71.7 (C2′), 71.9 (C5′), 72.4 (C3′), 76.8 (C2), 77.2 (C1), 100.9 (C1′), 169.4, 170.1 and 170.6 (4 CH_3*C*═O), 171.2 (br, NH*C*═O), 173.3 (CH_2*C*═O decanoyl). HRMS (positive-ion mode): *m*/*z* 880.5416 $[M + Na]^+$, calcd for $C_{45}H_{79}NO_{14}$, *m*/*z* 857.5501 [M], *m*/*z* 880.5393 $[M + Na]^+$.

*1-O-Octadecanoylamino-3-O-decanoyl-2-O-β-D-glucopyranosyl-sn-glycerol (**20**).* To a solution of **19** (0.2 g, 0.23 mmol) in a mixture of THF (2 mL) and methanol (0.8 mL), water (1 mL) and freshly distilled Et_3N (0.4 mL) were added at room temperature. The reaction mixture was stirred at 30 °C for 15 hours (TLC CH_2Cl_2/MeOH 95 : 5). Then it was diluted with CH_2Cl_2 (20 mL), washed once with a mixture of 0.1 M HCl (10 mL) and brine (10 mL), and the aqueous layers were re-extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 90 : 10 to 80 : 20) affording **20** (0.061 g, 38%) as a white waxy solid. The unreacted starting material **19** (0.026 g, 13%) and deacylated product **4b** (0.022 g, 18%) were also collected as white waxy solids after chromatographic separation. $[\alpha]_D^{20}$: −16.0° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$: CD_3OD 70 : 30): δ = 0.84 (m, 6H, 2CH_3), 1.15–1.32 (m, 40H, 20 CH_2), 1.51–1.62 (m, 4H, CH_2), 2.03 (t, J = 7.5 Hz, 2H, CH_2), 2.29 (t, J = 7.6 Hz, 2H, CH_2), 3.23–3.34 (m, 3H, H-2′, H-4′, H-5′), 3.38 (dd, $J_{3',2'}$ = 8.7, $J_{3',4'}$ = 8.7 Hz, 1H, H-3′), 3.67 (dd, $J_{6'a,6'b}$ = 12.0 Hz, $J_{6'a,5'}$ = 5.4 Hz, 1H, H-6′a), 3.83 (dd, $J_{6'b,5'}$ = 2.5 Hz, 1H, H-6′b), 3.88 (dd, $J_{1a,1b}$ = 11.6 Hz, $J_{1a,2}$ = 7.8 Hz, 1H, H-1a), 4.06 (dd, $J_{1b,2}$ = 2.6 Hz, 1H, H-1b), 4.08–4.12 (m, 1H, H-2), 4.16 (dd, $J_{3a,3b}$ = 11.5 Hz, $J_{3a,2}$ = 5.7 Hz, 1H, H-3a), 4.23 (dd, $J_{3b,2}$ = 4.3 Hz, 1H, H-3b), 4.46 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1′). ^{13}C-NMR ($CDCl_3$: CD_3OD 70 : 30): δ = 14.2 (2 *C*H_3), 23.0 (2 *C*H_2), 25.2 (*C*H_2), 25.9 (*C*H_2), 29.5 (*C*H_2), 29.6 (*C*H_2), 29.6 (2 *C*H_2), 29.7 (2 *C*H_2), 29.8 (*C*H_2), 29.9 (*C*H_2), 30.0 (2 *C*H_2), 30.1 (6 *C*H_2), 32.2 (*C*H_2), 32.3 (*C*H_2), 33.3 (br, *C*H_2) 34.4 (*C*H_2), 62.3 (C6′), 64.1 (C3), 70.7 (C4′), 74.1 (C2′), 76.8 (C5′), 77.0 (C1), 77.1 (C2), 77.1 (C3′), 104.0 (C1′), 171.9 (NH*C*═O), 174.6 (CH_2*C*═O decanoyl). HRMS (positive-ion mode): *m*/*z* 712.4977 $[M + Na]^+$, calcd for $C_{37}H_{71}NO_{10}$, *m*/*z* 689.5078 [M], *m*/*z* 712.4970 $[M + Na]^+$.

*1-O-Octadecanoylamino-3-O-decanoyl-2-O-β-D-glucuronopyranosyl-sn-glycerol (**21**).* In an open reaction vial, to a suspension of **20** (0.03 g, 0.043 mmol) in 20 mM acetate buffer (1 mL, pH 4.5), TEMPO (0.005 g, 0.032 mmol) and *Trametes versicolor* laccase (0.011 g, 11 U) were added at room temperature. The reaction mixture was stirred for 5 days in open air at 28–30 °C, until complete consumption of the starting material (TLC CH_2Cl_2/MeOH 85 : 15). A further amount of TEMPO (0.005 g) and laccase (0.011 g) was added after 2, 3 and 4 days of the reaction. The reaction mixture was diluted with water (10 mL), acidified with 0.1 M HCl up to pH 2, and extracted with diethyl ether (4 × 10 mL) and CH_2Cl_2 (1 × 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH from 90 : 10 to 80 : 20). The collected fractions were treated with DOWEX 50 × 8 H^+, filtered and the solvent was removed under vacuum affording compound **21** (0.02 g, 66%) as a white waxy solid. $[\alpha]_D^{20}$: −16°: ($CHCl_3$, *c* 0.9). ^{1}H-NMR (CD_3OD): δ = 0.89 (m, 6H, 2 CH_3), 1.23–1.38 (m, 40H, 20 CH_2), 1.59 (m, 4H, CH_2), 2.08 (t, J = 7.4 Hz, 2H, CH_2), 2.32 (t, J = 7.5 Hz, 2H, CH_2), 3.27 (dd, $J_{2',3'}$ = 9.4 Hz, $J_{2',1'}$ = 7.9 Hz, 1H, H-2′), 3.40 (dd, $J_{3',4'}$ = 9.1 Hz, 1H, H-3′), 3.52 (dd, $J_{4',5'}$ = 9.7 Hz, 1H, H-4′), 3.81 (d, 1H, H-5′), 3.92 (dd, $J_{1a,1b}$ = 11.4 Hz, $J_{1a,2}$ = 6.5 Hz, 1H, H-1a), 4.03 (dd, $J_{1b,2}$ = 3.3 Hz, 1H, H-1b), 4.13–4.20 (m, 2H, H-3a, H-2), 4.25 (dd, $J_{3a,3b}$ = 13.1 Hz, $J_{3a,2}$ = 6.9 Hz, 1H, H-3b), 4.56 (d, 1H, H-1′). ^{13}C-NMR (CD_3OD): δ = 14.4 (2 *C*H_3), 23.7 (2 *C*H_2), 25.9 (*C*H_2), 26.6 (*C*H_2), 30.1 (*C*H_2), 30.2 (*C*H_2), 30.4 (4 *C*H_2), 30.6 (2 *C*H_2), 30.7–30.8 (8 *C*H_2), 33.0 (2 *C*H_2), 33.7 (*C*H_2), 34.9 (*C*H_2), 64.9 (C3), 73.1 (C4′), 74.8 (C2′), 76.7 (C5′), 77.3 (C1), 77.5 (C3′), 77.6 (C2), 104.8 (C1′), 172.5 (*C*OOH), 172.9 (br, NH*C*═O), 175.2 (CH_2*C*═O decanoyl). HRMS (negative-ion mode): *m*/*z* 702.4792 $[M - H]^-$, calcd for $C_{37}H_{69}NO_{11}$, *m*/*z* 703.4871 [M].

*1-O-Octadecanoylamino-2-O-β-D-glucuronopyranosyl-sn-glycerol (**4a**).* To solid **21** (0.015 g, 0.021 mmol), a freshly prepared solution of sodium methoxide 0.037 M (0.91 mL, 0.034 mmol) was directly added dropwise at room temperature. The reaction mixture was stirred for 3 h, until complete consumption of the starting material (TLC CH_2Cl_2/MeOH 80 : 20). The reaction mixture was acidified with DOWEX 50 × 8 H^+, filtered and the solvent was removed under vacuum. The crude product was purified by precipitation from diethyl ether affording compound **4a** (0.011 g, 95%) as a white waxy solid. $[\alpha]_D^{20}$: −32.0° (CH_3OH, *c* 1.0). ^{1}H-NMR (CD_3OD): δ = 0.89 (t, J = 6.9 Hz, 3H, CH_3), 1.25–1.35 (m, 28H, 14 CH_2), 1.59 (m, 2H, CH_2), 2.08 (t, J = 7.4 Hz, 2H, CH_2), 3.28 (dd, $J_{2',3'}$ = 9.2 Hz, $J_{2',1'}$ = 7.8 Hz, 1H, H-2′), 3.41 (dd, $J_{3',4'}$ = 9.1 Hz, 1H, H-3′), 3.51 (dd, $J_{4',5'}$ = 9.8 Hz, 1H, H-4′), 3.65 (m, 2H, H-3a, H-3b), 3.83 (d, 1H, H-5′), 3.92 (dd, $J_{1a,1b}$ = 10.6 Hz, $J_{1a,2}$ = 7.0 Hz, 1H, H-1a), 3.94–4.00 (m, 1H, H-2), 4.06 (dd, $J_{1b,2}$ = 3.2 Hz, 1H, H-1b), 4.57 (d, 1H, H-1′).

^{13}C-NMR (CD_3OD): δ = 14.4 (CH_3), 23.7 (CH_2), 26.6 (CH_2), 30.2 (CH_2), 30.4 (CH_2), 30.5 (CH_2), 30.6 (2 CH_2), 30.7–30.8 (7 CH_2), 33.1 (CH_2), 33.7 (CH_2), 63.1 (C3), 73.1 (C4′), 74.7 (C2′), 76.4 (C5′), 77.4 (C3′), 77.5 (C1), 80.5 (C2), 104.5 (C1′), 172.7 (*C*OOH), 172.9 (br, NH*C*=O). HRMS (negative-ion mode): *m*/*z* 548.3432 $[M - H]^-$, calcd for $C_{27}H_{51}NO_{10}$, *m*/*z* 549.3513 [M].

Synthesis of monoacyl derivative 4b

*1-O-Octadecanoylamino-2-O-β-D-glucopyranosyl-sn-glycerol (**4b**).* Compound **4b** (0.022 g, 18%) was obtained as a white waxy solid by-product from the synthesis of **20**. $[\alpha]_D^{20}$: −27.7° ($CHCl_3$: CH_3OH = 9 : 1, *c* 0.45). ^{1}H-NMR ($CDCl_3$): δ = 0.83 (t, *J* = 6.9 Hz, 3H, CH_3), 1.11–1.37 (m, 28H, 14 CH_2), 1.56 (m, 2H, CH_2), 2.02 (t, *J* = 7.6 Hz, 2H, CH_2), 3.24–3.35 (m, 3H, H-2′, H-5′, H-4′), 3.39 (dd, $J_{3',2'}$ = 8.7 Hz, $J_{3',4'}$ = 8.7 Hz, 1H, H-3′), 3.58 (dd, $J_{3a,2}$ = 5.6 Hz, $J_{3a,3b}$ = 12.0 Hz, 1H, H-3a), 3.63 (dd, $J_{3b,2}$ = 4.1 Hz, 1H, H-3b), 3.66 (dd, $J_{6'a,5'}$ = 5.4 Hz, $J_{6'a,6'b}$ = 12.0 Hz, 1H, H-6′a), 3.83 (dd, $J_{6'b,5'}$ = 2.3 Hz, 1H, H-6′b), 3.87 (dd, 1H, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 7.3 Hz, H-1a), 3.93 (m, 1H, H-2), 4.01 (dd, $J_{1b,2}$ = 2.9 Hz, 1H, H-1b), 4.43 (d, $J_{1',2'}$ = 7.8 Hz, 1H, H-1′). ^{13}C-NMR ($CDCl_3$): δ = 14.2 (CH_3), 23.0 (CH_2), 25.9 (CH_2), 29.5 (CH_2), 29.7 (2 CH_2), 29.9 (2 CH_2), 30.0 (7 CH_2), 32.3 (CH_2), 33.3 (CH_2), 61.9 (C6′), 62.4 (C3), 70.5 (C4′), 74.1 (C2′), 76.7 (C1), 76.9 (C5′), 77.0 (C3′), 80.1 (C2), 103.3 (C1′), 172.0 (br, NH*C*=O). HRMS (positive-ion mode): *m*/*z* 558.3621 $[M + Na]^+$, calcd for $C_{27}H_{53}NO_9$, *m*/*z* 535.3720 [M], *m*/*z* 558.3613 $[M + Na]^+$.

Synthesis of the diester 5. The known **5** [6] was more efficiently prepared by acylation of **14** to the corresponding didecanoyl derivative and its subsequent mild deacetylation with hydrazine hydrate in aqueous ethanol at room temperature[48] according to the following procedure.

To a solution of **14** (0.5 g, 1.18 mmol) in dry CH_2Cl_2 (10 mL), pyridine (0.57 mL, 7.08 mmol) and decanoyl chloride in dry CH_2Cl_2 (0.74 mL, 3.55 mmol) were added dropwise at −10 °C. The reaction mixture was stirred at the same temperature for 1 hour, until complete consumption of the starting material (TLC CH_2Cl_2/MeOH 95 : 5; petroleum ether/EtOAc 2 : 1). The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed once with a mixture of 1 M HCl (30 mL), water (30 mL), a saturated solution of sodium bicarbonate (30 mL), and brine (30 mL). The organic layer was dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was subjected to flash chromatography (petroleum ether/EtOAc 20 : 10) affording pure 1,3-di-*O*-decanoyl-2-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (0.78 g, 90%) as a white waxy solid. $[\alpha]_D^{20}$: −9.6° ($CHCl_3$, *c* 1.0). ^{1}H-NMR ($CDCl_3$): δ = 0.88 (m, 6H, 2 CH_3), 1.20–1.35 (m, 24H, 12 CH_2), 1.60 (m, 4H, 2 CH_2), 2.00, 2.02, 2.03 and 2.09 (4s, 12H, 4CH_3CO), 2.31 (m, 4H, 2 CH_2), 3.69 (ddd, $J_{5',4'}$ = 10.1, $J_{5',6'b}$ = 5.1 Hz, $J_{5',6'a}$ = 2.4 Hz, 1H, H-5′), 4.03–4.08 (m, 1H, H-2), 4.08–4.16 (m, 3H, H-1a,b or H-3a,b and H-6′a), 4.18 (dd, $J_{1(3)a,2}$ = 11.6 Hz, $J_{1(3)a,1(3)b}$ = 4.0 1H, H-1a or H-3a), 4.21–4.28 (m, 2H, H-1b or H-3b and H-6′b), 4.64 (d, $J_{1',2'}$ = 8.0 Hz, 1H, H-1′), 4.98 (dd, $J_{2',3'}$ = 9.6 Hz, 1H, H-2′), 5.07 (dd, $J_{4'',3'}$ = 9.7 Hz, 1H, H-4′) 5.19 (dd, 1H, H-3′). ^{13}C-NMR ($CDCl_3$): δ = 14.1 (2 CH_3), 20.6 and 20.7 (4 CH_3CO), 22.7 (2 CH_2), 24.8 (2 CH_2), 29.2–29.4 (8 CH_2), 31.8 (2 CH_2), 34.0 (CH_2), 34.1 (CH_2), 61.9 (C6′), 63.0 (C1 or C3), 63.1 (C1 or C3), 68.3 (C4′), 71.2 (C2′), 71.9 (C5′), 72.7 (C3′), 75.7 (C2), 100.8 (C1′), 169.2, 169.4, 170.3, 170.7 (4 CH_3CO), 173.4 (CH_2C=O decanoyl). HRMS (positive-ion mode): *m*/*z* 753.4041 $[M + Na]^+$, calcd for $C_{37}H_{62}O_{14}$, *m*/*z* 730.4140 [M], *m*/*z* 753.4032 $[M + Na]^+$.

To a solution of the previously prepared didecanoyl derivative (0.1 g, 0.14 mmol) in 85% (v/v) aqueous ethanol (5 mL), hydrazine hydrate (0.027 mL, 0.55 mmol) was added at room temperature. The reaction mixture was stirred for 3 hours, until complete consumption of the starting material TLC (CH_2Cl_2/MeOH 95 : 5, petroleum ether/EtOAc 20 : 10). The reaction mixture was concentrated under vacuum, diluted with cold water (10 mL), and extracted with CH_2Cl_2 (6 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 90 : 10) affording 1,3-di-*O*-decanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**) (0.058 g, 74%) as a white waxy solid having the same characteristics as reported in ref. 6.

Stability study of compounds 3b and 5. Compound **3b** or **5** (3.3 mM) in pH 7.4 phosphate buffered saline (PBS) containing 60% of fetal bovine serum (FBS) and DMSO (50 mM) was stirred at 37 °C for 24 h and TLC analysis was performed at 16 and 24 h (CH_2Cl_2/MeOH 85 : 15). Afterwards, the complete disappearing of **5** but not of **3b** was observed (see the ESI, Fig. S2†). Then the mixtures were freeze-dried and 600 μL of $CDCl_3$ and MeOD (1 : 1) were added to the lyophilized residue of each sample. The suspension was filtered and the solution obtained was analysed by ^{1}H-NMR (see the ESI, Fig. S3†). Whilst the spectrum of treated ester **5** showed no more signals related to the starting compound in the **3b** spectrum, the characteristic resonances of this oxyamide were detectable again.

Serum binding study of compound 3a. The binding of the oxyamide compounds to serum proteins was investigated through protein binding experiments, with compound **3a** chosen as a representative of the oxyamides. Three samples of the compound (samples 1–3) were prepared with phosphate-buffered saline (PBS) and serum in order to obtain increasing serum percentages (0%, 10%, and 60%). A 3.3 mM concentration of compound **3a** was maintained in each sample, where, in addition, 2 μL of DMSO were added to improve its solubility. The samples, thus prepared, were incubated for 24 h at 37 °C and then centrifuged to separate the free compound from the protein-bound one, following the same procedure for all samples. 500 μL of the mixture were transferred to an Amicon® Ultra-0.5 30 K centrifugal filter device and centrifuged with an Eppendorf 5804 R centrifuge at 13 500 rpm for 30 min. The filtrates, collected in the filter collection tubes, were then acidified with a 0.1 M HCl solution to a slightly acidic pH, so that our compound, bearing a carboxylic group, could be found in its undissociated form for a clearer NMR analysis. The samples were then freeze-dried and prepared for NMR analysis. 500 μL of a 1 : 1 solution of $CDCl_3$ and MeOD were added to each one of the collection tubes along with 5 μL of 0.14 M DMSO in $CDCl_3$, used as an internal standard. The solutions obtained were then analysed by ^{1}H-NMR (see the ESI, Fig. S4 and S5†).

Biology

Cellular studies

Stock preparation. All compounds were dissolved in DMSO at 50 mM and directly added to the cell culture medium for cell sensitivity assays. For ELISA, the compounds were prepared as 10× solutions and then diluted in kinase buffer.

Cell culture and cell growth inhibition assay. The human ovarian carcinoma IGROV-1, IGROV/Pt1,[15] and TOV112D (ATCC, CRL 11731) cell lines were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C under a 5% CO_2 atmosphere. For cell sensitivity assays, all cell lines (10 000 cells per cm^2) were plated in complete medium using 12-well plates. Twenty-four hours later, the cells were exposed to the solvent (DMSO) or different concentrations of the compounds for 24 h either in complete medium or serum-free medium. After treatment, the cells were washed with saline and complete medium was added and for 48 h. The cells were then harvested using trypsin and counted using a Coulter Counter (Z1, Beckman Coulter). The percentages of inhibition in drug-treated *versus* solvent-treated samples are reported in dose–response curves. IC_{50} represents the drug concentration inhibiting growth by 50%. Each experiment was performed 3 times or with triplicate samples.

Cell-free studies. The screening of novel Akt inhibitors **3a**, **3b**, **4a**, and **4b** and perifosine (SelleckChem, Houston, Texas, USA; used as reference inhibitors) was performed by ELISA (CycLex AKT/PKB Kinase Assay/Inhibitor screening kit, MBL Life Sciences, Japan) according to user's manual for quantitative analysis. The compounds were diluted in kinase buffer. All samples were assayed in triplicate. The absorbance was measured at 450 nm using a spectrophotometric plate reader (iMark Microplate Reader, Biorad).

Author contributions

MZ: conceptualization, methodology, investigation, writing – original draft, and writing – review and editing. GO: conceptualization and investigation. MQ: investigation, writing – original draft, and writing – review and editing. LM: investigation. CC: investigation and visualization. NC: investigation. PP: conceptualization, resources, writing – original draft, writing – review and editing, and funding acquisition. DC: conceptualization, methodology, investigation, resources, writing – original draft, writing – review and editing, supervision, and funding acquisition.

Conflicts of interest

There are no conflicts to declare.

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