Conference paper

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Synthesis and biological evaluation of *S*-simplexides and other analogues of simplexide

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Abstract: Simplexides are natural glycolipids isolated from the marine sponge *Plakortis simplex*, and contain alkyl 4-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside. Simplexides can release of cytokines (IL-6) and chemokines (CXCL-8) from human monocytes and cause the expansion of natural killer T-cells (iNKTs) *in vitro*, with iNKTs contributing to the sustenance of immune homeostasis. Herein, the stereoselective syntheses of *S*-glycosidic analogues, i.e. *S*-simplexides, are described. The routes included Lewis acid promoted anomerisation of glycosyl thiols and thioglycolipids, as well as anomeric *S*-alkylation. Synthesis of *O*-glycosidic analogues are included. Heptadecanyl *O*- and *S*-glycosides as well as the 17-tritriacontyl 4-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside, a component of the natural simplexide isolate, all induced IL-6 and CXCL-8 production at both 10 and 30 μg/mL concentrations from PBMCs whereas the two *S*-simplexides were inactive. It is speculated that the lack of activity for the *S*-disaccharide analogue could be due to inhibition of cellular α-glucosidase, preventing degradation of the simplex disaccharide to a simpler galactopyranoside, whereas lack of activity for the *S*-galactolipid analogue could be due to increased conformational flexibility of *S*-glycosides. On the other hand, simpler unbranched *O*- and *S*-glycolipid analogues were active. Natural simplexide, and a synthetic simplexide, the 18-pentatriacontanyl 4-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside, were more potent than the new compounds tested.

Keywords: biological activity; biomedical applications; carbohydrates; ICS-29; natural products; synthesis.

Introduction

Simplexides are glycolipids, which were originally isolated from the marine organism *Plakortis simplex* [1]. The lipid component of these marine natural products is distinctive and the disaccharide, where glucopyranoside is α-1→4 linked to β-galactopyranoside, is not found commonly. While five different alkyl chain types were observed in the isolated fraction of simplexide, it has been estimated that up to 25 different molecular species may be produced naturally.

Researchers in Naples have previous demonstrated that both the natural isolated simplexide fraction and synthetic simplexide **1c** (Scheme 1, R=R′ = CH_2CH_3) induced the release of cytokines (IL-6) and chemokines

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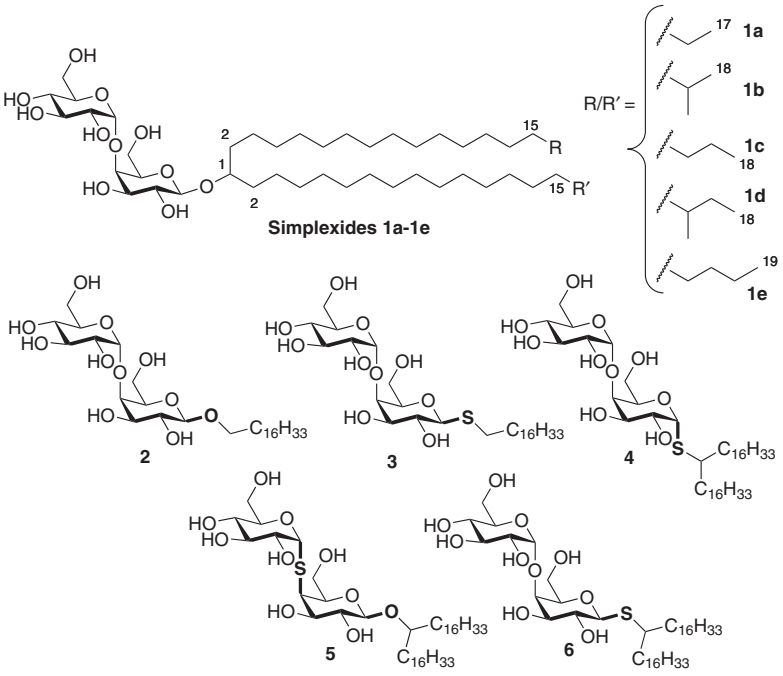


Scheme 1: Structure of natural simplexide glycolipids **1a–1e** and simplexide analogues **2–6**.

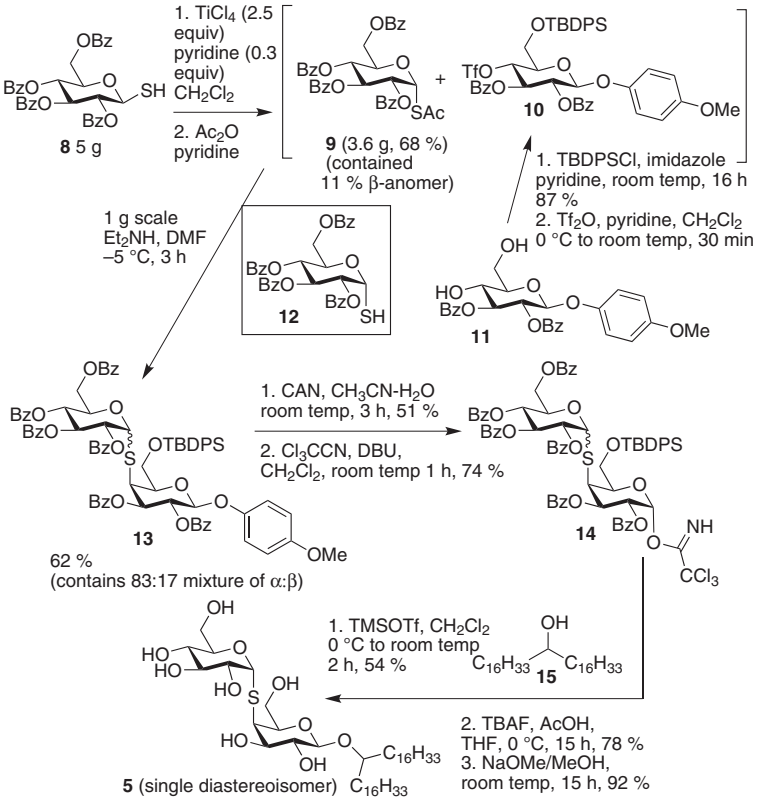
(CXCL-8) from human monocytes and the expansion of natural killer T-cells (iNKT) *in vitro* [2]. These observations suggested that simplexides could exert both proinflammatory and immunoregulatory activities by their interaction with CD1d. The activity of simplexides may, thus, relate to that of the α-GalCer family of glycosphingolipids [3]. α-Galactosyl ceramide (KRN-7000, α-GalCer) enters monocytes and binds to CD1d, an antigen-presenting molecule. CD1d presents α-GalCer to the T cell receptor (TCR), which recognizes it with very high affinity [4]. This leads to the activation of NKT cells, which subsequently release chemokines and cytokines. The latter have an important role influencing both immune and pathogenic processes. While there have been various syntheses and biological studies of α-GalCer and analogues [5–8], there has been limited structure activity studies on simplexides to date. A few syntheses of components of the natural isolate and a fluorescent simplexide have been reported [9–11].

In this manuscript we describe a synthesis of simplexide **1a** (where R=R′ = CH_2CH_3) as well as the syntheses of mimetics of **1a**, *S*-simplexides, where sulfur replaces glycosidic oxygen atoms of the natural product. Glycomimetics [12], including *S*-glycosides, are of interest in drug and development or as probes for basic research. The synthesis of *S*-glycoside analogues [13] of natural bioactive *O*-glycosides is of interest because they are more stable *in vivo* than the corresponding *O*-glycosides, being resistant to hydrolysis by glycosidases [14]. However, there are conformational implications in replacing an oxygen with sulfur as thioglycosides can be more flexible [15]. In addition to *S*-glycosides, analogues which have the α-*O*-anomeric configuration between the galactose and the lipid residue are included in this study. Lewis acid promoted anomerisation has found application during the synthetic work. Biological studies on new compounds generated are included, and some simpler *S* and *O*-glycolipids show ability to stimulate release of IL-6 and CXCL-8 from PBMCs.

Results and discussion

Synthesis of glycolipids and mimetics

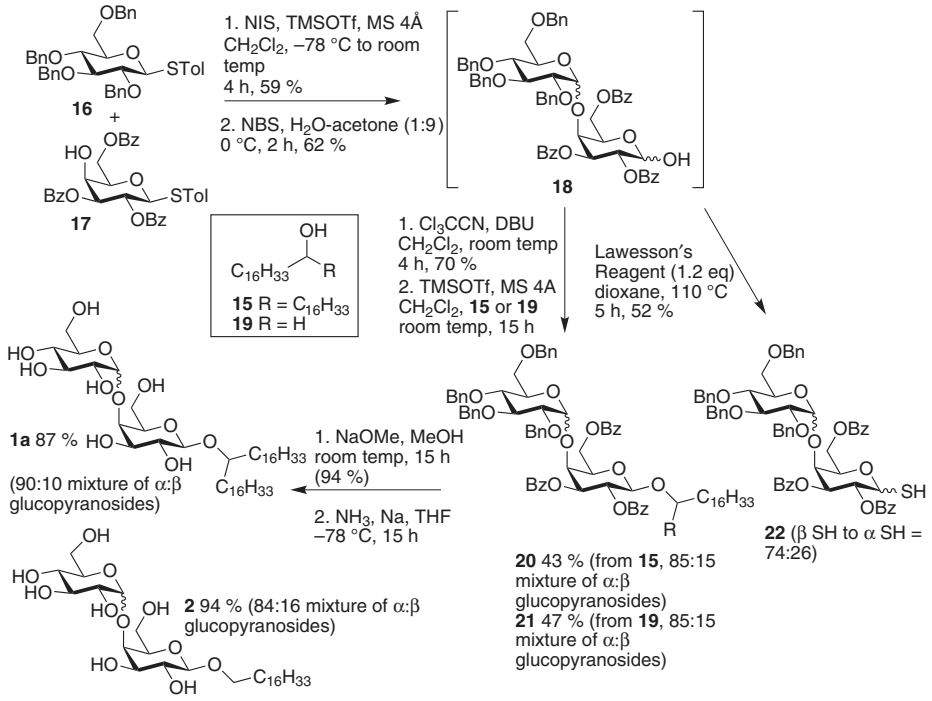
The preparation of **13** (Scheme 2) was achieved via the α-thiol **12** [16] which had been generated *in situ* by $TiCl_4$ induced anomerisation. Thiol **12**, prepared on a multi-gram scale herein, was converted to thioacetate **9**,



Scheme 2: Synthesis of *S*-simplexide.

facilitate long term storage of a more stable precursor to **12**. The thioacetate **9** was obtained as an 89:11 mixture of anomers on a multigram scale, a stereoselectivity lower than previously described for thiol **12** [11]. The unstable triflate **10** was prepared from known glucopyranoside **11** [17, 18] (Scheme 2). De-*S*-acetylation of **9** and reaction of the generated thiolate in the presence of **10**, via anomeric *S*-alkylation, gave the *S*-disaccharide **13**. The thioglycoside **13** was isolated as an 83:17 mixture of α:β anomers after the alkylation and subsequent chromatography. The methoxyphenyl glycoside **13** was then hydrolysed using cermic ammonium nitrate (CAN) in the presence of water-acetonitrile and the hemiacetal that was generated was converted into the trichloroacetimidate donor **14** (83:17 mixture of α-*S* and β-*S*-glucopyranosides) in two steps. Glycosidation with the known alcohol **15** [19] and subsequent protecting group removal gave the *S*-simplexide **5** as a single isomer.

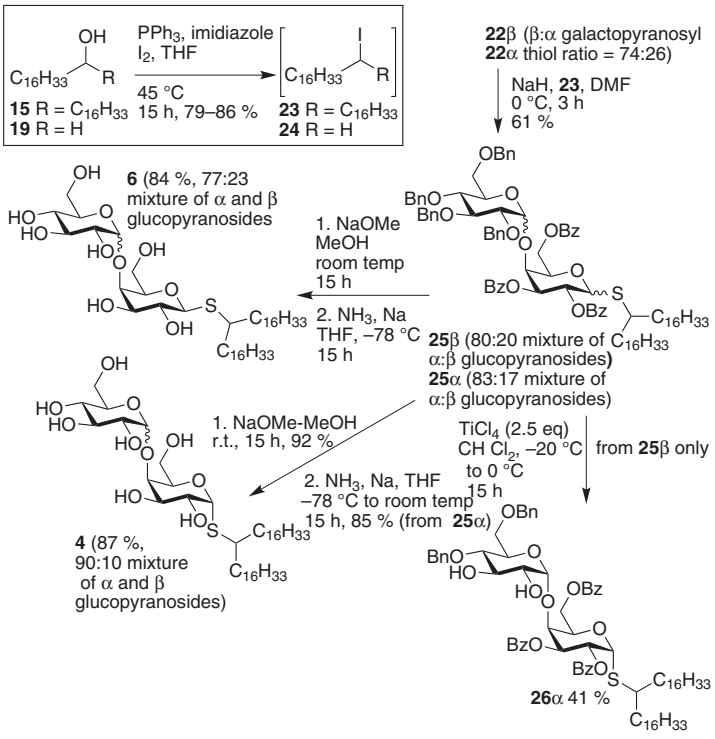
The disaccharide **18** was next used to synthesise naturally occurring **1a**. Thus **18** was obtained from the glycosylation reaction using the thioglycoside **16** and glycosyl acceptor **17** using N-iodosuccinimide and TMSOTf as activator followed by reaction of the disaccharide product with N-bromosuccinimide in the presence of water and acetone. The glycosidation promoted by NIS-TfOH had a selectivity ~70:30 in favour of the α-anomer, with the β-glucopyranoside also formed; these diastereoisomers were not separated. A related glycosidation reaction was reported previously by Li and co-workers,[6c] who used a 6-*O*-TBDPS protected thiogalactopyranoside donor as part of a one pot strategy towards simplexide, in diethyl ether and dichloromethane; the formation of the β-glucopyranoside was not reported in their earlier report. It appears the absence of the TBDPS group and/or the use of dichloromethane only as solvent leads to a reduction in the stereoselectivity of the glycosylation reaction. The thioglycoside of **17** is less reactive than **16** due to its acyl groups, and was not activated with NIS at –78 °C. Hemiacetal **18** (70:30 mixture of glucopyranosides) was converted into its trichloroacetimidate and subsequent glycosylation of alcohols **15** and **19** gave, respectively, β-galactopyranosides **20** and **21**, with each being an 85:15 mixture of α:β D-glucopyranosides. Debenzoylation followed by de-*O*-benzylation using the Birch conditions gave **1a**, which after chromatography was a 90:10 mixture of α and β-D-glucopyranosides (Scheme 3). The ^{1}H- and ^{13}C-NMR data for **1a** (α-glucopyranoside component) showed very good agreement with all the data reported by Li and co-workers, with one exception.



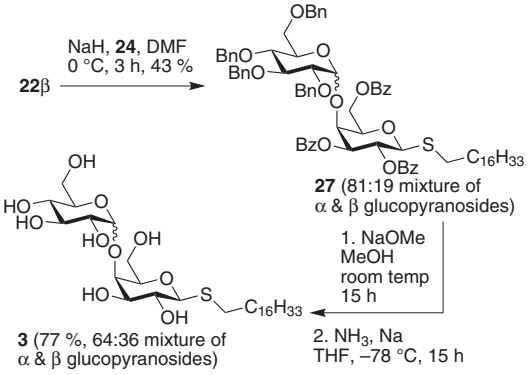
Scheme 3: Synthesis of simplexide **1a** and analogues.

They report a signal at δ 4.61 (dd, J = 11.6, 2.4 Hz, 1H) for one of the glucopyranoside H-6 protons, whereas we recorded a signal for this proton at δ 4.48 (dd, J = 11.5, 2.5 Hz, 1H). Reaction of **21** gave **2** which was isolated as an 84:16 mixture of glucopyranosides with the α-anomer being the major constituent.

The disaccharide **18** was used for the synthesis of thiogalactopyranosides **3**, **4** and **6**. Its reaction with Lawesson's reagent (Scheme 3) gave a mixture of the galactopyranosyl thiols **22**, where the β-anomer was the major component (β:α = 74:26). The reaction of 1-iodoheptadecane **24** prepared from **19** (Scheme 4), with **22β** in the presence of sodium hydride at 0 °C in DMF followed by protecting group removal (NaOMe-MeOH, followed by Na-NH_3) gave **3** via **27** (33 % over 3 steps, shown in Scheme 5); the final product **3** contained a 64:36 mixture of α and β-glucopyranosides. The reaction of tritriacontan-17-ol with triphenylphosphine in the presence of imidazole gave the iodide **23** (Scheme 4). When **23** was reacted with a mixture of thiols **22β** and **22α**, it gave a mixture of thioglycosides **25β** and **25α**. Separation of the thioglycosides and removal of protecting groups from both anomers gave **4** and **6**, both of which contained some β-glucopyranoside as indicated in Scheme 4. The preparation of α-anomer **4** was also shown to be possible via **26α**, which was obtained from **25β** after treatment with $TiCl_4$. Thus, this Lewis acid was able to promote anomerisation of the β-*S*-glycolipid to the α-*S*-glycolipid. In earlier work from our group, glycosphingolipid anomerisation required the presence of a galacturonic acid residue rather than galactopyranoside. Here, this was not the case as the anomerisation proceeded for the *S*-galactopyranoside derivative. Interestingly, the $TiCl_4$ was also found to remove, in a regioselective manner, two of the benzyl protecting groups, at C-2 and C-3, of the glucopyranoside residue. Remarkably, the $TiCl_4$ did not remove the benzyl group at the glucopyranoside C-6, which might have been predicted to be more reactive. In terms of the anomerisation reaction promoted by $TiCl_4$, it is more likely that it involves an endocyclic cleavage pathway as opposed to exocyclic cleavage. In the former, the Lewis acid coordinates to the pyranose ring oxygen atom and isomerisation is facilitated by breaking and reforming the bond between this oxygen and the anomeric carbon atom, with C-1 to C-2 bond rotation enabling the generation of the axial anomer. On the other hand, the exocyclic cleavage pathway involves breaking and reforming the bond between the anomeric carbon and sulfur. An earlier study showed that rates of *S*-galactospyranoside anomerisation to be faster than that of the *O*-galactopyranoside and it was argued that this is consistent with endocyclic cleavage rather than the alternative pathway [20]. Anomerisation of other types of glycosides with



Scheme 4: Synthesis of *S*-simplexides.



Scheme 5: Synthesis of simplexide analogue **3**.

additional cyclic constraints are also believed to go via endocyclic cleavage as discussed by Manabe, Ito and their co-workers [21]. Recent research on this reaction has recently been reviewed [22].

Biological evaluation of the new glycolipids

The effects of simplexide **1a** and mimetics (compounds **2–5**) on cytokine and chemokine production by human PBMCs were examined and these were compared to those of the natural and synthetic simplexide **1c** (R=R′ = $CH_2CH_2CH_3$), prepared in Naples as described previously [2]. Cells were incubated with increasing concentrations (3–30 μg/mL) of compounds and the release of cytokines and chemokines (IL-6, TNF-α and CXCL-8) was determined. The simpler *O*-glycosidic analogue **2** and its *S*-glycoside mimetic **3**, as well as the synthetic **1a**, all induced both IL-6 (Fig. 1) and CXCL-8 (Fig. 2) production at both 10 and 30 μg/mL

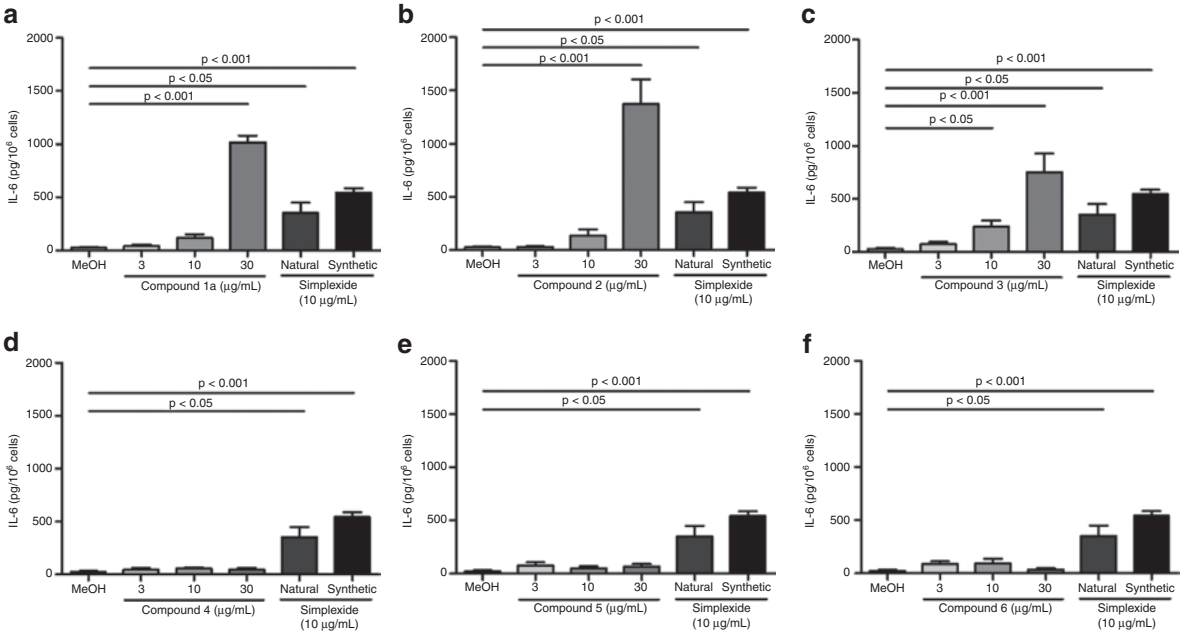


Fig. 1: Effect of increasing concentrations of synthetic compounds on IL-6 release from PBMCs. Polystyrene plates were coated with indicated concentrations of compound **1a** (panel A), **2** (panel B), **3** (panel C), **4** (panel D), **5** (panel E), **6** (panel F), natural and synthetic (panel A–F, compound **1c**) simplexide glycolipids dissolved in methanol. Solvent was dried under nitrogen immediately before the addition of cells. Cells were then added and after 24 h of incubation, the supernatant was collected and centrifuged (1000 × *g*, 4 °C, 5 min). IL-6 concentrations were determined by ELISA. The values are expressed as pg of IL-6 per 10^6 cells. Data are the mean ± SEM of six experiments.

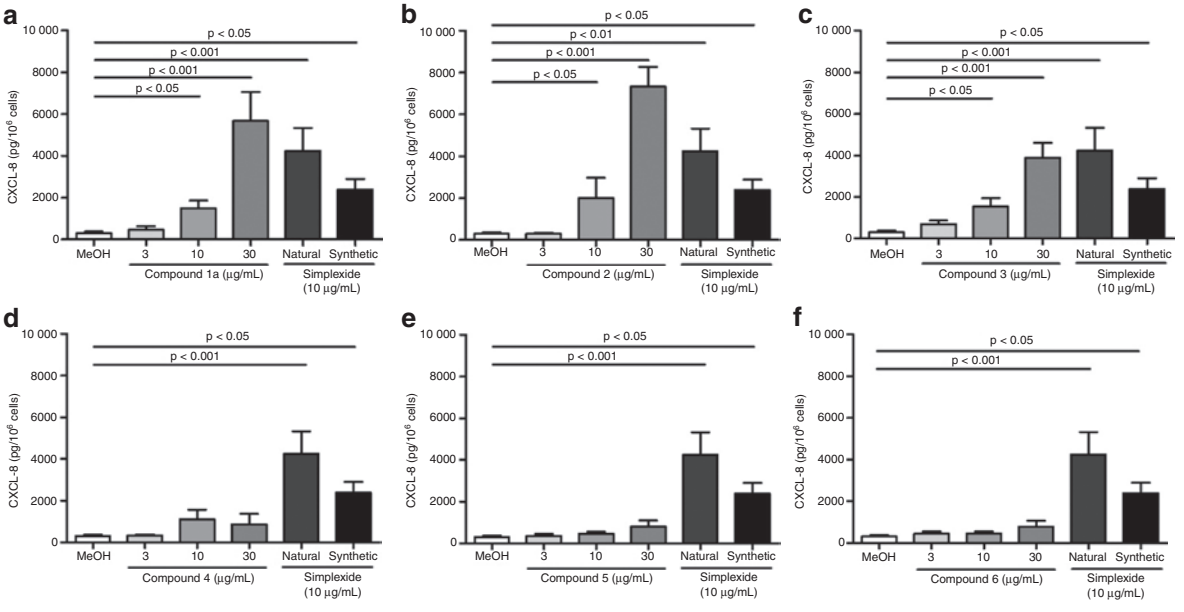


Fig. 2: Effect of increasing concentrations of synthesised compounds on CXCL-8 release from PBMC. Polystyrene plates were coated with indicated concentrations of compound **1a** (panel A), **2** (panel B), **3** (panel C), **4** (panel D), **5** (panel E), **6** (panel F), natural and synthetic (panel A–F, compound **1c**) simplexide glycolipids dissolved in methanol. Solvent was dried under nitrogen immediately before the addition of cells. Cells were then added and after 24 h of incubation, the supernatant was collected and centrifuged (1000 × *g*, 4 °C, 5 min). CXCL-8 levels were determined by ELISA. The values are expressed as pg of CXCL-8 per 10^6 cells. Data are the mean ± SEM of six experiments.

concentrations from PBMCs whereas compounds **4–6** had no effect (Figs. 1–4). By contrast, none of tested new compounds induced the TNF-α release (Fig. 3). Natural and synthetic simplexide **1c**, with the longer lipid chains, were more potent in inducing the release of IL-6 (Fig. 4a) and CXCL-8 (Fig. 4b) compared to the tested sample of **1a**, and the mimetics **2** and **3**.

It is known that *S*-glycosides can act as glycosidase inhibitors. A reviewer of this manuscript has pointed out that most glycosylceramides, composed of oligosaccharides rather than a monosaccharide, require lysosomal processing to monoglycosylceramides, before they can cause NKT cell stimulation. In this scenario, cellular glycosidases degrade the lipids presenting oligosaccharides to simpler lipids presenting

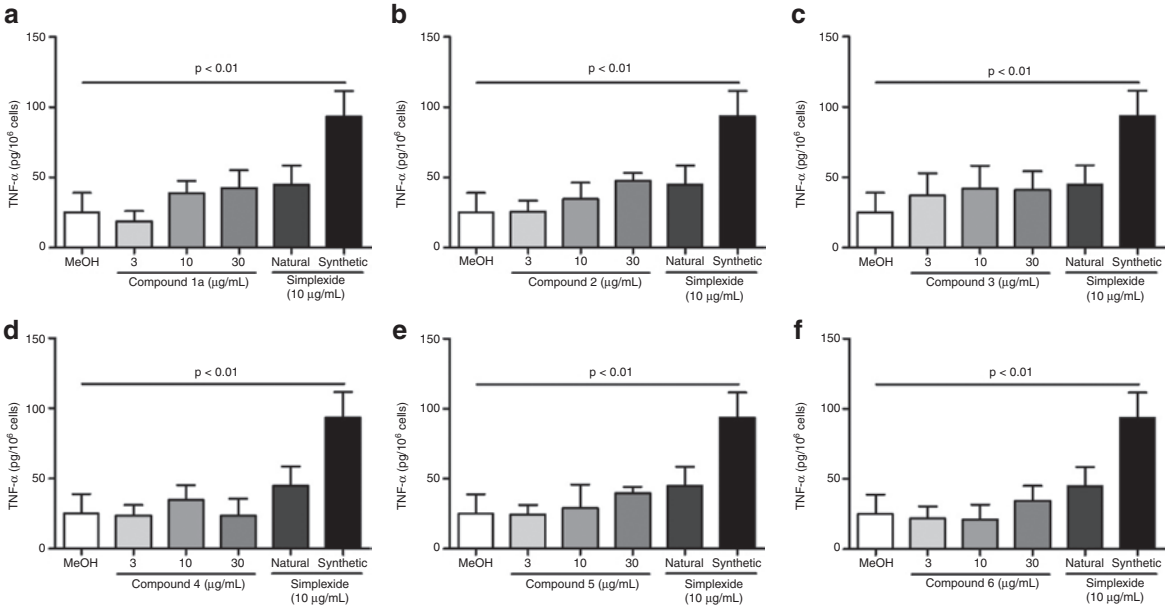


Fig. 3: Effect of increasing concentrations of synthetic compounds on TNF-α release from PBMC. Polystyrene plates were coated with indicated concentrations of compound **1a** (panel A), **2** (panel B), **3** (panel C), **4** (panel D), **5** (panel E), **6** (panel F), natural and synthetic (panel A–F) simplexide glycolipids dissolved in methanol. Solvent was dried under nitrogen immediately before the addiction of cells. Cells were then added and after 24 h of incubation, the supernatant was collected and centrifuged (1000 × *g*, 4 °C, 5 min). TNF-α were determined by ELISA. The values are expressed as pg of TNF-α per 10^6 cells. Data are the mean ± SEM of six experiments.

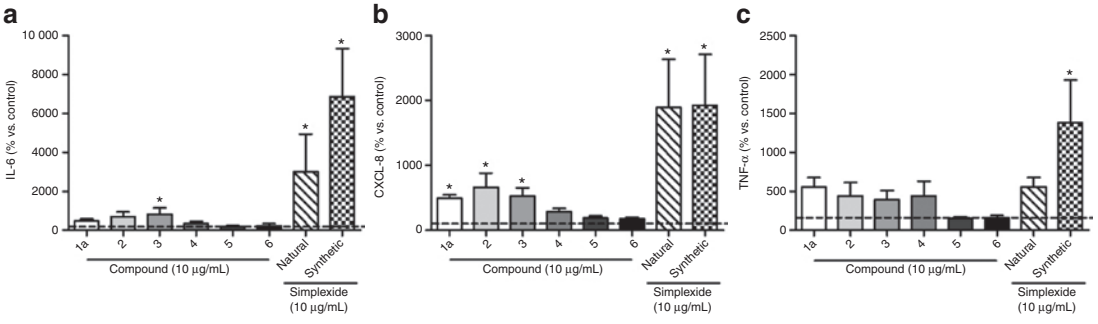


Fig. 4: Effect of **1a** and mimetics **2–6** on IL-6 (panel A), CXCL-8 (panel B) and TNF-α (panel C) production from PBMC. Positive controls were natural and synthetic simplexide. Polystyrene plates were coated with indicated concentrations of glycolipids dissolved in methanol. Solvent was dried under nitrogen immediately before the addiction of cells. Cells were then added and after 24 h of incubation, the supernatant was collected and centrifuged (1000 × *g*, 4 °C, 5 min). Cytokines and chemokines were determined by ELISA. The values are expressed as percent increase versus MeOH control. Data are the mean ± SEM of six experiments. *$p < 0.05$ vs. control.

monosaccharides, with the latter being required to stimulate cytokine release [23]. It is therefore possible that the *S*-glycosidic linkage in **5** prevents degradation to the simpler active galactolipid, and that this would explain the lack of activity found for **5** in this study. This suggests that it would be relevant to synthesise and evaluate the simpler galactopyranoside derivatives of simplexides, work which as far as we are aware, has not been carried out to date. The lack of activity displayed by **6**, on the other hand, could be explained by different conformational preferences between it and the simplexides, as *S*-glycosides are known to have increased flexibility compared to the *O*-glycosides. There is no clear proposal as to why **4** may not be active and synthesis of the α–*O*-simplexide analogue would clarify the relationship between anomeric configuration and bioactivity for the simplexides. It is interesting that the simpler unbranched glycolipids **2** and **3** were active and could be investigated more in future research.

Summary and conclusions

The synthesis of *S*-simplexides were achieved herein, enabling a contribution to be made to the structure activity relationship of glycolipids of immunological interest. The synthesis of *S*-simplexides include application of Lewis acid promoted anomerisation of glycosyl thiols as well as anomerisation of a thioglycolipid. In previous work, successful anomerisation of glycosphingolipids, to give the axial glycosides, with $TiCl_4$ has required the saccharide to be a galacturonic acid residue, with the anomerisation of galactosphingolipids being unsuccessful [24–28]. On the other hand, the thiogalactopyranoside **25β** glycolipid is structurally the most complex glycolipid amenable to anomerisation to date, indicating the reaction may have other applications in this field. From a biological perspective, unbranched heptadecanyl *O*- and *S*-glycosidic analogues as well as the 17-tritriacontyl 4-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside, a component of the natural simplexide isolate, all induced IL-6 and CXCL-8 production at both 10 and 30 μg/mL concentrations from PBMCs whereas the *S*-simplexides were inactive. Natural simplexide, and a synthetic analogue, the 18-pentatriacontanyl 4-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside, were most potent of those samples tested. Hence it would appear that the conformational flexibility induced by having an *S*-glycoside as opposed to an *O*-glycoside leads to a loss of bioactivity or that inhibition of cellular α-glucosidase promoted degradation of the simplexide to simpler galactopyranosides could be a contributing factor. Simpler lipids such as that found in **2** and even the *S*-glycoside **3** displayed activity. The research suggests that simpler galactopyranoside analogues or of more components of the simplexide isolate and analogues may be worthwhile for the provision of new improved immunomodulatory glycolipids in due course.

Experimental section

Reagents and general synthesis and analytical methods

NMR spectra (see Supplementary information section) were recorded with a 500 MHz or 600 MHz Varian spectrometer. Chemical shifts are referenced to the internal solvent signal. All NMR assignments were assigned with the aid of COSY, HSQC and HMBC experiments. Coupling constants *J* are reported in Hertz (Hz). Unless otherwise stated ^{1}H NMR and/or ^{13}C NMR spectroscopic data for previously reported compounds, cited herein, were in good agreement with previously published data. The IR spectra were recorded as thin films using a PerkinElmer Spectrum 100 FT-IR Spectrometer with an ATR attachment. High resolution mass spectra were recorded using a Waters LCT Premier XE (ESI-TOF instrument). Silica gel (pore size 60 Å, particle size 40–60 μm 230–400 mesh particle size) was purchased from Sigma-Aldrich. Dichloromethane, THF and DMF reaction solvents were used as obtained from a Pure Solv™ Solvent Purification System. The following were purchased for biological experiments: L-glutamine, antibiotic-antimycotic solution (10 000 IU/mL penicillin, 10 mg/mL streptomycin, and 25 μg/mL amphotericin B), MEM non-essential amino acids solutions and His-

topaque®-1077 (Sigma-Aldrich, St. Louis, MO, USA); RPMI and fetal calf serum (FCS) (MP Biomedicals Europe, Illkirch, France). All other reagents for bioassays were purchased from Carlo Erba (Milan, Italy).

Benzoylation

The reactant was dissolved in pyridine (10 mL per 1 mmol) and the solution was cooled down to 0 °C. DMAP (0.05 equiv. per OH group) and BzCl (1.2 equiv. per OH group) were then added, and the solution was stirred at room temperature overnight. The mixture was then diluted with EtOAc. The organic phase was washed with HCl until the aqueous phase was acidic, then water and brine. It was then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. Chromatography gave the product.

Debenzoylation

The reactant was dissolved in methanol (10 mL per mmol). Solid sodium prewashed with petroleum ether (0.2 equiv.) was then added to the solution. The mixture was stirred at room temperature overnight. It was then acidified to pH = 6 with Amberlite (IR-120, strongly acidic, hydrogen form), filtered, and the solvent was removed under reduced pressure to give the product.

De-*O*-benzylation

The reactant was dissolved in THF (20 mL per mmol) and solid sodium prewashed with petroleum ether (20 equiv.) was added. The round bottom flask was cooled to –78 °C and gaseous NH_3 (excess) was bubbled into the mixture via a needle. The reaction stopped bubbling and was stirred vigorously for 16 h, while being allowed to attain room temperature. Water was then added, and the mixture was extracted with EtOAc three times. The organic phase was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give the product after chromatography.

p-Methoxyphenyl 2,3-di-*O*-benzoyl-4-*S*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-4-thio-6-tert-butyldiphenylsilyl-β-D-galactopyranoside (13)

Compound **11** [29] (13.2 g, 27 mmol) was dissolved in pyridine (100 mL) and the solution was cooled to 0 °C. Imidazole (5.4 g, 3 equiv.) and TBDPSCl (10 mL, 1.5 equiv.) were then added, and the mixture was stirred at 0 °C for 3 h. It was then diluted with CH_2Cl_2, washed with HCl (5 %), a satd $NaHCO_3$ and water, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 4:1) gave the intermediate (17.0 g, 87 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (overlapping signals, 4H, aromatic H), 7.71 (overlapping signals, 4H, aromatic H), 7.52 (overlapping signals, 2H, aromatic H), 7.40 (overlapping signals, 10H, aromatic H of TBDPS), 6.94 (overlapping signals, 2H, aromatic H), 6.72 (overlapping signals, 2H, aromatic H), 5.65 (dd, *J* = 9.8, 7.9 Hz, 1H, H-2), 5.49 (t, *J* = 9.5 Hz, 1H, H-3), 5.14 (d, *J* = 7.9 Hz, 1H, H-1), 4.06 (overlapping signals, 2H, H-4, H-6a), 4.01 (dd, *J* = 11.0, 5.3 Hz, 1H, H-6b), 3.73 (overlapping signals, 4H, H-5, OC*H*$_3$), 3.20 (s, 1H, OH), 1.08 (s, 9H, Si-C(C*H*$_3$)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.4 (C=O), 165.3 (C=O), 155.5 (C), 151.3 (C), 136.3 (C), 135.7 (C), 135.6 (C), 133.5 (C), 133.2 (C), 132.7 (C), 130.0 (CH), 129.8 (CH), 129.7 (CH), 128.4 (CH), 128.4 (CH), 127.8 (CH), 126.4 (CH), 126.3 (CH), 118.8 (CH), 114.5 (CH), 110.0 (CH), 100.7 (C-1), 77.2 (C-2), 76.1 (C-5), 71.4 (C-3), 70.7 (C-4), 64.0 (C-6), 55.6 (O-CH_3), 26.8 (TBDPS), 19.2 (TBDPS). IR (film) cm^{-1}: 2982, 1722, 1601, 1451, 1263, 1178, 1090, 1069, 1026, 707, 687. This intermediate (5 g, 6.8 mmol) was dissolved in CH_2Cl_2 (43 mL) and the solution was cooled to 0 °C. Pyridine (1.7 mL, 3 equiv.) and triflic anhydride (1.6 mL, 1.4 equiv.) were then added, and the mixture was stirred at room temperature for 1 h. It was then diluted with CH_2Cl_2, washed with HCl 1 mol/L, water and brine, dried over Na_2SO_4, filtered and the solvent was removed under

reduced pressure. Chromatography (cyclohexane-EtOAc, 8:1) gave unstable triflate **10** (3.1 g, 53 %). Thiol **8**[11] (5 g, 8.2 mmol) was dissolved in CH_2Cl_2 (75 mL) and pyridine (330 µL, 0.5 equiv., 4.1 mmol) was then added, followed by $TiCl_4$ (1 M in CH_2Cl_2, 24.5 mL, 3 equiv.). The mixture was stirred at room temperature for 16 h and then diluted in EtOAc and washed with a saturated solution of NH_4Cl. The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with satd $NaHCO_3$, brine, and then dried over Na_2SO_4 and filtered. After removal of the solvent under reduced pressure, the unpurified α-thiol **12** (5 g, 8.2 mmol) was suspended in pyridine (50 mL) and acetic anhydride (7.7 mL, 10 equiv.), and the resulting mixture was stirred at room temperature for 16 h. The mixture was then cooled to 0 °C and methanol was then added followed by EtOAc. The organic phase was washed with water, then 1 M HCl until the pH of the aqueous phase was acidic, water and brine. It was then dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Chromatography (cyclohexane-EtOAc, 7:2) gave **9** (3.63 g, 68 %) as a white solid, which was an 89:11 mixture of anomers. Analytical data for α-anomer **9α**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (overlapping signals, 2H, aromatic H), 7.93 (overlapping signals, 4H, aromatic H), 7.87 (overlapping signals, 2H, aromatic H), 7.52 (overlapping signals, 4H, aromatic H), 7.39 (overlapping signals, 6H, aromatic H), 7.30 (overlapping signals, 2H, aromatic H), 6.55 (d, *J* = 5.5 Hz, 1H, H-1), 5.87 (t, *J* = 9.8 Hz, 1H, H-3), 5.71 (overlapping signals, 2H, H-2, H-4), 4.59 (dd, *J* = 11.9, 2.6 Hz, 1H, H-6a), 4.47 (overlapping signals, 2H, H-5, H-6b), 2.37 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.2 (S-C=O), 166.1 (C=O), 165.7 (C=O), 165.1 (C=O), 165.0 (C=O), 133.6 (C), 133.5 (C), 133.3 (C), 133.1 (C), 129.9 (CH), 129.8 (CH), 129.8 (CH), 129.8 (CH), 129.7 (CH), 129.7 (CH), 129.6 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 80.8 (C-1), 71.7 (C-5), 71.6 (C-3), 70.1 (C-4), 68.9 (C-2), 62.7 (C-6), 31.5 (CH_3). IR (film) cm^{-1}: 2982, 1722, 1601, 1451, 1263, 1178, 1090, 1069, 1026, 707, 687. Thioacetate **9** (1 g, 1.5 mmol) and freshly prepared triflate **10** (5.28 g, 4 equiv.) were then dissolved in DMF (66 mL) and the solution was cooled to 0 °C. Diethylamine (3.2 mL, 20 equiv.) was then added and the mixture was stirred at 0 °C for 5 h. It was then washed with water and extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 5:1) gave **13** (1.26 g, 62 %) as an 83:17 mixture of the α-*S*- and β-*S*-glucopyranoside (white solid). Analytical data for the α-anomer **13α**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (overlapping signals, 2H, aromatic H), 8.00 (overlapping signals, 6H, aromatic H), 7.89 (overlapping signals, 2H, aromatic H), 7.80 (overlapping signals, 2H, aromatic H), 7.69 (overlapping signals, 5H, aromatic H), 7.44 (overlapping signals, 3H, aromatic H), 7.41 (overlapping signals, 11H, aromatic H), 7.21 (overlapping signals, 5H, aromatic H), 6.81 (overlapping signals, 2H, aromatic H), 6.67 (overlapping signals, 2H, aromatic H), 6.31 (d, *J* = 5.6 Hz, 1H, H-1′), 6.11 (t, *J* = 9.9 Hz, 1H, H-3′), 5.71 (overlapping signals, 2H, H-2, H-4′), 5.60 (dd, *J* = 10.5, 4.7 Hz, 1H, H-3), 5.53 (dd, *J* = 10.3, 5.9 Hz, 1H, H-2′), 4.95 (d, *J* = 7.8 Hz, 1H, H-1), 4.60 (dt, *J* = 10.1, 2.9 Hz, 1H, H-5′), 4.11 (m, 1H, H-4), 4.05 (dd, *J* = 10.7, 7.8 Hz, 1H, H-6a), 3.98 (dd, *J* = 12.6, 2.7 Hz, 1H, H-6′a), 3.88 (dd, *J* = 10.7, 5.7 Hz, 1H, H-6b), 3.76 (t, *J* = 6.9 Hz, 1H, H-5), 3.69 (s, 3H, OCH_3), 3.57 (dd, *J* = 12.7, 3.0 Hz, 1H, H-6′b), 1.07 (s, 9H, Si-C(CH_3)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8 (C=O), 165.6 (C=O), 165.5 (C=O), 165.3 (C=O), 165.2 (C=O), 164.8 (C=O), 155.4 (C), 151.1 (C), 135.6 (C), 135.6 (C), 133.7 (C), 133.6 (C), 133.3 (C), 133.2 (C), 133.2 (C), 133.1 (C), 133.0 (CH), 132.7 (CH), 130.1 (CH), 130.0 (CH), 129.9 (CH), 129.9 (CH), 129.9 (CH), 129.7 (CH), 129.7 (CH), 129.3 (CH), 129.1 (CH), 128.8 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.6 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 118.4 (CH), 114.4 (CH), 101.1 (C-1), 82.3 (C-1′), 74.6 (C-5), 72.7 (C-3), 71.5 (C-2′), 70.8 (C-3′), 70.6 (C-2), 68.7 (C-4′), 68.0 (C-5′), 62.6 (C-6), 61.7 (C-6′), 55.6 (O-CH3), 46.3 (C-4), 26.8 (TBDPS), 19.0 (TBDPS). IR (film) cm^{-1}: 2935, 2858, 1725, 1602, 1507, 1451, 1259, 1178, 1090, 1067, 1026, 825, 801, 743, 703, 687. ESI-HRMS calcd for $C_{77}H_{70}O_{17}SSiNa$ 1349.4001, found *m/z* 1349.4005 $[M + Na]^+$.

2,3-Di-*O*-benzoyl-4-*S*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-4-thio-6-tert-butyldiphenylsilyl-β-D-galactopyranosyl trichloroacetimidate (14)

Compound **13** (1.26 g, 0.95 mmol) was dissolved in a mixture of H_2O-CH_3CN (3:17, 20 mL) and ammonium cerium nitrate (2.6 g, 4.75 mmol) was added. The mixture was stirred at room temperature for 1 h. The solution was diluted in EtOAc, washed with water, a saturated solution of $NaHCO_3$, dried over Na_2SO_4, filtered and

the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 4:1) gave the hemiacetal (0.59 g, 51 %) as a white solid. The hemiacetal precursor (0.59 g, 0.48 mmol) was then dissolved in CH_2Cl_2 (15 mL). Trichloroacetonitrile (1 mL, 20 equiv.) and DBU (0.1 mL, 1.2 equiv.) were then added to the solution, which was stirred at room temperature for 1 h. The solvent was then removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 6:1) gave **14** (0.49 g, 74 %) as a mixture of glucopyranosides (83:17 α-*S* anomer: β-*S* anomer, white solid); ^{1}H NMR (α-*S* anomer, 500 MHz, $CDCl_3$) δ 8.47 (s, 1H, NH), 8.18 (overlapping signals, 2H, aromatic H), 8.07 (overlapping signals, 2H, aromatic H), 8.02 (overlapping signals, 3H, aromatic H), 8.98 (overlapping signals, 3H, aromatic H), 7.91 (overlapping signals, 2H, aromatic H), 7.79 (overlapping signals, 3H, aromatic H), 7.66 (overlapping signals, 5H, aromatic H), 7.41 (overlapping signals, 31H, aromatic H), 6.63 (d, *J* = 3.8 Hz, 1H, H-1), 6.58 (d, *J* = 5.7 Hz, 1H, H-1′), 6.17 (t, *J* = 9.9 Hz, 1H, H-3′), 6.10 (dd, *J* = 10.9, 4.5 Hz, 1H, H-3), 5.70 (overlapping signals, 2H, H-2, H-4′), 5.60 (m, 1H, H-2′), 4.65 (dt, *J* = 10.1, 3.0 Hz, 1H, H-5′), 4.39 (overlapping signals, 2H, H-4, H-5), 4.04 (m, 1H, H-6′a), 4.00 (m, 1H, H-6a), 3.78 (dd, *J* = 10.6, 5.3 Hz, 1H, H-6b), 3.64 (dd, *J* = 12.6, 3.4 Hz, 1H, H-6′b), 1.04 (s, 9H, Si-C(C*H*$_3$)$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8 (C=O), 165.6 (C=O), 165.4 (C=O), 165.4 (C=O), 165.3 (C=O), 164.8 (C=O), 160.5 (*C* = NH), 135.7 (C), 135.6 (C), 133.6 (C), 133.5 (C), 133.4 (C), 133.2 (C), 133.0 (C), 132.9 (CH), 132.6 (CH), 130.0 (CH), 130.0 (CH), 129.9 (CH), 129.8 (CH), 129.8 (CH), 129.7 (CH), 129.7 (CH), 129.1 (CH), 129.0 (CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.7 (CH), 128.6 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 93.5 (C-1), 90.7 (C*Cl*$_3$), 81.5 (C-1′), 71.8 (C-5), 71.5 (C-2′), 70.8 (C-3′), 69.4 (C-3), 69.0 (C-2), 68.8 (C-4′), 68.0 (C-5′), 62.0 (C-6), 61.7 (C-6′), 45.9 (C-4), 26.7 (TBDPS), 19.1 (TBDPS). IR (film) cm^{-1}: 2931, 1726, 1452, 1264, 1106, 1093, 1070, 796, 735, 707. ESI-HRMS calcd for $C_{72}H_{64}Cl_3NO_{16}SSiNa$ 1386.2678, found *m/z* 1386.2642 [M + Na]$^+$. Selected ^{1}H-NMR data for β-*S*-anomer: δ 5.12 (d, *J* = 9.9 Hz, 1H, H-1′).

17-Tritriacontyl 4-*S*-(α-D-glucopyranosyl)-4-thio-β-D-galactopyranoside (5)

Compound **14** (490 mg, 0.36 mmol) and **15** (345 mg, 2 equiv.) were dissolved in CH_2Cl_2 (18 mL) in a round bottom flask containing molecular sieves 4 Å. A 0.1 mol/L solution of TMSOTf in CH_2Cl_2 (0.720 mL, 0.2 equiv.) was then added, and the mixture was stirred at room temperature for 3 h. The reaction was quenched with triethylamine, filtered, and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 8:1) gave the fully protected glycolipid (326 mg, 54 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (overlapping signals, 2H aromatic H), 8.02 (overlapping signals, 2H aromatic H), 7.95 (overlapping signals, 4H aromatic H), 7.85 (overlapping signals, 2H aromatic H), 7.77 (overlapping signals, 2H aromatic H), 7.66 (overlapping signals, 2H aromatic H), 7.60 (overlapping signals, 2H aromatic H), 7.53 (overlapping signals, 3H aromatic H), 7.37 (overlapping signals, 16H aromatic H), 7.25 (overlapping signals, 6H aromatic H), 6.25 (d, *J* = 5.7 Hz, 1H, H-1′), 6.04 (t, *J* = 9.9 Hz, 1H, H-3′), 5.62 (t, *J* = 9.8 Hz, 1H, H-4′), 5.47 (overlapping signals, 3H, H-2′, H-2, H-3), 4.56 (dt, *J* = 10.1, 2.9 Hz, 1H, H-5′), 4.44 (d, *J* = 7.4 Hz, 1H, H-1), 4.02 (dd, *J* = 4.5, 1.3 Hz, 1H, H-4), 3.94 (dd, *J* = 10.6, 8.4 Hz, 1H, H-6a), 3.89 (dd, *J* = 12.6, 2.8 Hz, 1H, H-6′a), 3.72 (dd, *J* = 10.6, 5.4 Hz, 1H, H-6b), 3.58 (dd, *J* = 8.1, 6.0 Hz, 1H, H-5), 3.50 (dd, *J* = 12.6, 3.0 Hz, 1H, H-6′b), 3.40 (m, 1H, C*H*-($C_{16}H_{33}$)$_2$), 1.60 (s, 4H, lipid), 1.47–1.04 (overlapping signals, 54H, lipid), 1.00 (s, 9H, Si-C(C*H*$_3$)$_3$), 0.89 (m, 6H, lipid). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8 (C=O), 165.6 (C=O), 165.4 (C=O), 165.3 (C=O), 165.0 (C=O), 164.8 (C=O), 135.6 (C), 135.5 (C), 133.5 (C), 133.4 (C), 133.3 (C), 133.2 (C), 133.0 (C), 133.0 (C), 132.9 (CH), 132.8 (CH), 130.1 (CH), 129.9 (CH), 129.9 (CH), 129.8 (CH), 129.7 (CH), 129.7 (CH), 129.7 (CH), 129.6 (CH), 129.1 (CH), 129.0 (CH), 128.9 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.2 (CH), 128.2 (CH), 128.2 (CH), 127.8 (CH), 127.7 (CH), 101.2 (C-1), 82.1 (C-1′), 81.2 (*C*H-($C_{16}H_{33}$)$_2$), 73.9 (C-5), 73.0 (C-3), 71.3 (C-2′), 71.2 (C-2), 70.8 (C-3′), 68.7 (C-4′), 67.8 (C-5′), 62.4 (C-6), 61.7 (C-6′), 46.5 (C-4), 34.6, 33.9, 31.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.4, 29.4 (all lipid carbons), 26.7 (CH_3, TBDPS), 25.0 (lipid), 24.8 (lipid), 22.7 (lipid), 19.0 (C, TBDPS), 14.1 (lipid). IR (film) cm^{-1}: 3057, 2964, 1697, 1597, 1573, 1528, 1395, 1293, 1260, 1234, 1088, 992, 875, 794, 742, 695. ESI-HRMS calcd for $C_{103}H_{130}O_{16}SSiNa$ 1705.8747, found *m/z* 1705.8724 [M + Na]$^+$. The disaccharide (326 mg, 0.19 mmol) was then dissolved in THF (5 mL) and acetic acid (0.05 mL) and the solution was cooled to 0 °C. TBAF (1 mol/L in THF, 0.4 mL, 2 equiv.) was then added and the mixture was stirred at room temperature overnight. The solution

was diluted in CH_2Cl_2 and the organic phase was washed with water, a saturated solution of $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. After chromatography, debenzoylation gave **5** (114 mg, 39 % over three steps) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 5.33 (d, *J* = 5.4 Hz, 1H, H-1′), 4.23 (d, *J* = 7.5 Hz, 1H, H-1), 4.14 (ddd, *J* = 10.0, 6.0, 2.4 Hz, 1H, H-5′), 3.95 (dd, *J* = 10.6, 7.8 Hz, 1H, H-6a), 3.84 (dd, *J* = 11.8, 2.4 Hz, 1H, H-6′a), 3.74 (overlapping signals, 2H, H-3, H-6b), 3.68 (overlapping signals, 3H, H-5, H-2′, H-6′b), 3.61 (m, 1H, C*H*-$(C_{16}H_{33})_2$), 3.55 (dd, *J* = 9.8, 8.8 Hz, 1H, H-3′), 3.38 (dd, *J* = 4.6, 1.4 Hz, 1H, H-4), 3.26 (overlapping signals, 2H, H-2, H-4′), 1.49 (overlapping signals, 4H, lipid), 1.26 (overlapping signals, 56H, lipid), 0.88 (t, *J* = 6.9 Hz, 6H, lipid). ^{13}C NMR (126 MHz, CD_3OD) δ 102.9 (C-1), 87.3 (C-1′), 79.3 (*C*H-$(C_{16}H_{33})_2$), 74.2 (C-3′), 74.2 (C-5), 73.3 (C-3), 73.1 (C-5′), 72.8 (C-2), 72.1 (C-2′), 70.4 (C-4′), 61.3 (C-6′), 61.0 (C-6), 51.7 (C-4), 34.4, 33.7, 31.7, 29.6, 29.4, 29.4, 29.3, 29.1, 24.8, 24.7, 22.4, 13.3 (all lipid carbons). IR (film) cm^{-1}: 3351, 2981, 2915, 2850, 1737.5, 1471, 1462, 1381, 1251, 1152, 1070, 955, 828, 717. ESI-HRMS calcd for $C_{45}H_{88}O_{10}SCl$ 855.5787, found *m/z* 855.5795 $[M + Cl]^-$.

17-Tritriacontyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (20)

Compound **17** (4.5 g, 7.5 mmol) and **16** (14.6 g, 22 mmol) were dissolved in CH_2Cl_2 (100 mL) in a round bottom flask containing molecular sieves 4 Å. This solution was stirred at room temperature for 15 min before being cooled to −78 °C. N-Iodosuccinimide (1.7 g, 1.3 equiv.) TMSOTf (340 µL, 0.25 equiv.) were then added and the temperature was let to go to room temperature and stirred for 4 h. It was then diluted with CH_2Cl_2 and the organic phase was washed with a saturated solution of $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 4:1) gave a disaccharide (4.97 g, 59 %; 5:2 mixture of α-and β-anomers). ESI-HRMS calcd for $C_{68}H_{64}O_{13}SNa$ 1143.3965, found *m/z* 1143.4008 $[M + Na]^+$. The mixture of anomers (3.20 g, 2.8 mmol) was dissolved in acetone-H_2O (9:1, 12 mL). *N*-Bromosuccinimide (1.5 g, 3 equiv.) was then added and the solution was stirred at room temperature for 4 h. The mixture was then diluted with EtOAc, and the organic phase was washed with a saturated solution of $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 3:1) gave intermediate **18** (1.80 g, 62 %) as a yellow oil. This intermediate **18** (1 g, 0.98 mmol) was dissolved in CH_2Cl_2 (30 mL) and Cl_3CCN (2 mL, 20 equiv.) and DBU (0.2 mL, 1.2 equiv.) were then added and the mixture was stirred for 1 h. The solvent was then removed under reduced pressure and chromatography (cyclohexane-EtOAc, 5:1) gave the trichloroacetimidate intermediate (0.80 g, 70 %) as a white solid. This precursor (400 mg, 0.34 mmol) and **15** (664 mg, 4 equiv.) were then dissolved in CH_2Cl_2 (17 mL) in a round bottom flask containing molecular sieves 4 Å, and the solution was cooled to 0 °C. TMSOTf (0.1 M in CH_2Cl_2, 0.9 mL, 0.09 mmol) was added, and the mixture was stirred at room temperature for 3 h. Triethylamine was added and after filtration, the solvent was removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 9:1) gave **20** (219 mg, 43 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (overlapping signals, 2H, aromatic H), 7.94 (overlapping signals, 4H, aromatic H), 7.57 (overlapping signals, 2H, aromatic H), 7.45 (overlapping signals, 6H, aromatic H), 7.34 (overlapping signals, 4H, aromatic H), 7.27 (overlapping signals, 12H, aromatic H), 7.15 (overlapping signals, 5H, aromatic H), 5.69 (t, *J* = 9.3 Hz, 1H, H-2), 5.25 (d, *J* = 10.6 Hz, 1H, H-3), 5.00 (d, *J* = 11.0 Hz, 1H, benzyl CH), 4.92 (m, 1H, benzyl CH), 4.79 (overlapping signals, 2H, benzyl CH x2), 4.71 (overlapping signals, 3H, H-6a, H-6b, benzyl CH), 4.41 (overlapping signals, 2H, H-4, benzyl CH), 4.34 (d, *J* = 12.0 Hz, 1H, benzyl CH), 4.17 (t, *J* = 9.6 Hz, 1H, H-3′), 4.09 (t, *J* = 11.7 Hz, 1H, H-5′), 4.03 (overlapping signals, 2H, H-5, benzyl CH), 3.71 (t, *J* = 9.6 Hz, 1H, H-4′), 3.55 (overlapping signals, 3H, H-2′, O-C*H*), 3.34 (d, *J* = 11.0 Hz, 1H, H-6′a), 2.94 (d, *J* = 10.9 Hz, 1H, H-6′b), 1.66 (overlapping signals, 4H, lipid), 1.23 (overlapping signals, 56H, lipid), 0.88 (t, *J* = 7.0 Hz, 6H, lipid); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 166.2, 165.3 (each C=O), 139.0 (C), 138.7 (C), 138.2 (C), 138.0 (C), 133.4 (C), 133.3 (C), 133.1 (C), 130.1 (CH), 130.0 (CH), 129.9 (CH), 129.8 (CH), 129.8 (CH), 129.5 (CH), 129.0 (CH), 128.6 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 127.8 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.6 (CH), 127.6 (CH), 127.5 (CH), 127.5 (CH), 101.8 (C-1), 100.6 (C-1′), 82.4 (*C*H-O), 81.8 (C-3′),

79.9 (C-2′), 77.7 (C-4′), 76.2 (C-4), 75.7 (benzyl CH_2), 74.9 (benzyl CH_2), 74.3 (C-3), 73.9 (benzyl CH_2), 73.4 (benzyl CH_2), 72.8 (C-5), 71.5 (C-5′), 70.2 (C-2), 67.9 (C-6′), 63.0 (C-6), 35.1, 34.3, 32.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.5, 29.5, 25.5, 25.2, 22.8, 14.3 (all lipid carbons). IR (film) cm^{-1}: 2923, 2852, 1725, 1651, 1601, 1452, 1271, 1095, 1070, 1028, 709. ESI-HRMS calcd for $C_{94}H_{128}O_{14}N$ 1494.9335, found *m/z* 1494.9321 $[M+NH_4]^+$.

1-Heptadecanyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (21)

The trichloroacetimidate precursor (400 mg, 0.34 mmol), prepared from **18** as described above in the preparation of **20**, and **19** (0.35 g, 4 equiv.) were dissolved in CH_2Cl_2 (17 mL) in a round bottom flask containing 4 Å molecular sieves, and the solution was cooled to 0 °C. TMSOTf (0.1 M in CH_2Cl_2, 0.9 mL, 0.09 mmol) was then added, and the mixture was stirred at room temperature for 3 h. Triethylamine was added to quench the reaction. After filtration, the solvent was removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 9:1) gave **21** (203 mg, 47 %) as a white solid and which was a mixture of glucosides (85α:15β). ^{1}H NMR (α-glucopyranoside, 500 MHz, $CDCl_3$): δ 8.03 (overlapping signals, 2H, aromatic H), 7.95 (overlapping signals, 4H, aromatic H), 7.57 (m, 1H, aromatic H), 7.45 (overlapping signals, 5H, aromatic H), 7.35 (overlapping signals, 4H, aromatic H), 7.27 (overlapping signals, 13H, aromatic H), 7.15 (overlapping signals, 6H, aromatic H), 5.70 (dd, *J* = 10.6, 7.7 Hz, 1H, H-2), 5.28 (dd, *J* = 10.6, 3.1 Hz, 1H, H-3), 5.00 (d, *J* = 10.9 Hz, 1H, benzyl CH), 4.93 (dd, *J* = 7.2, 3.8 Hz, 1H, benzyl CH), 4.79 (overlapping signals, 4H, H-6a, H-6b, Bn C*H* x2), 4.69 (m, 1H, benzyl CH), 4.42 (overlapping signals, 2H, H-4, benzyl CH), 4.35 (d, *J* = 12.1 Hz, 1H, benzyl CH), 4.16 (t, *J* = 9.5 Hz, 1H, H-3′), 4.10 (m, 1H, H-5′), 4.05 (overlapping signals, 2H, H-5, benzyl CH), 3.94 (dt, *J* = 9.9, 6.2 Hz, 1H, butyl C*H*(H)O), 3.72 (t, *J* = 9.6 Hz, 1H, H-4′), 3.55 (m, 1H, H-2′), 3.51 (m, 1H, butyl C*H*(H)O), 3.34 (dd, *J* = 11.0, 2.1 Hz, 1H, H-6′a), 2.93 (dd, *J* = 11.0, 2.0 Hz, 1H, H-6′b), 1.53 (m, 2H, lipid), 1.34 (m, 28H, lipid), 0.88 (m, 3H, lipid). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 166.1, 165.3 (each C=O), 138.9 (C), 138.6 (C), 138.0 (C), 137.9 (C), 133.3 (C), 133.2 (C), 133.0 (C), 130.2 (CH), 130.0 (CH), 129.8 (CH), 129.7 (CH), 129.7 (CH), 129.6 (CH), 129.3 (CH), 128.7 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.2 (CH), 128.2 (CH), 128.0 (CH), 127.9 (CH), 127.9 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 127.4 (CH), 101.6 (C-1), 100.6 (C-1′), 81.7 (C-3′), 79.7 (C-2′), 77.6 (C-4′), 75.9 (C-4), 75.6 (benzyl CH_2), 74.7 (benzyl CH_2), 73.9 (C-3), 73.9 (benzyl CH_2), 73.3 (benzyl CH_2), 72.7 (C-5), 71.4 (C-5′), 70.2(*C*H_2-O), 69.9 (C-2), 67.7 (C-6′), 62.5 (C-6), 32.8, 31.9, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 26.9, 25.8, 25.8, 22.7, 14.1 (all lipid). ESI-HRMS calcd for $C_{78}H_{96}O_{14}N$ 1270.6831, found *m/z* 1270.6843 $[M+NH_4]^+$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 5.55 (dd, *J* = 10.4, 3.3 Hz, H-3), 5.86 (dd, *J* = 10.5, 8.0 Hz, H-2).

17-Tritriacontyl 4-*O*-(D-glucopyranosyl)-β-D-galactopyranoside (1a)

Compound **20** (219 mg, 0.15 mmol) was reacted as described in the general procedures for debenzoylation and *O*-debenzylation successively to give **1a** (97 mg, 82 % over two steps, 90:10 mixture of α & β-glucopyranosides); ^{1}H NMR (α-glucopyranoside, 600 MHz, pyridine-d_5) δ 5.81 (d, *J* = 3.8 Hz, 1H, H-1′ α), 4.93 (ddd, *J* = 10.1, 5.7, 2.5 Hz, 1H, H-5′), 4.86 (d, *J* = 7.5 Hz, 1H, H-1), 4.75 (d, *J* = 3.3 Hz, 1H, H-4), 4.70 (t, *J* = 9.6 Hz, 1H, H-6a), 4.58 (t, *J* = 9.3 Hz, 1H, H-3′), 4.48 (dd, *J* = 11.5, 2.5 Hz, 1H, H-6′a), 4.38 (dd, *J* = 9.9, 7.6 Hz, 1H, H-2), 4.33 (overlapping signals, 2H, H-6b, H-6′b), 4.20 (overlapping signals, 3H, H-3, H-2′, H-4′), 4.16 (m, 1H, H-5), 3.98 (m, 1H, O-C*H*), 1.76 (overlapping signals, 4H, lipid), 1.52 (overlapping signals, 2H, lipid), 1.29 (overlapping signals, 54H), 0.88 (t, *J* = 6.9 Hz, 6H, lipid). ^{13}C NMR (151 MHz, Pyridine-d_5) δ 105.1 (C-1), 103.1 (C-1′), 80.4 (*C*H-O), 80.1 (C-4), 76.0 (C-5), 75.6 (C-3), 75.4 (C-3′), 75.2 (C-5′), 74.6 (C-2′), 73.4 (C-2), 72.5 (C-4′), 62.9 (C-6′), 60.9 (C-6), 35.9, 35.0, 32.5, 30.7, 30.5, 30.4, 30.4, 30.4, 30.3, 30.3, 30.0, 26.1, 25.8, 23.3, 14.7 (all lipid carbons). IR (film) cm^{-1}: 3400, 2955, 2915, 2850, 1470, 1111, 1042, 1024, 992, 793, 718, 660, 668. ESI-HRMS calcd for $C_{45}H_{88}O_{11}Cl$ 839.6015, found *m/z* 839.6030 $[M+Cl]^-$. Selected ^{1}H-NMR spectroscopic data for the β-glucopyranoside: δ 5.27 (d, *J* = 7.9 Hz, H-1′ β).

1-Heptadecanyl 4-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside (2)

Compound **21** (203 mg, 0.16 mmol) was reacted as described in the general procedures for de-*O*-benzoylation and de-*O*-benzylation successively to give **2** (75 mg, 80 % over two steps) as a mixture of glucopyranosides. ^{1}H NMR (α-D-glucopyranoside, 500 MHz, $CDCl_3$) δ 4.98 (d, *J* = 3.8 Hz, 1H, H-1′), 4.28 (d, *J* = 7.4 Hz, 1H, H-1), 4.03 (overlapping signals, 2H, H-4, H-4′), 3.86 (overlapping signals, 3H, H-6a, H6′a, O-C*H*-lipid), 3.76 (dd, *J* = 11.1, 5.7 Hz, 1H, H-6b), 3.70 (overlapping signals, 2H, H-3′, H-6′b), 3.64 (m, 1H, H-5), 3.56 (overlapping signals, 2H, H-3, O-C*H*-lipid), 3.50 (dd, *J* = 10.1, 7.4 Hz, 1H, H-2), 3.46 (dd, *J* = 9.8, 3.7 Hz, 1H, H-2′), 3.30 (dd, *J* = 10.0, 9.0 Hz, 1H, H-5′), 1.64 (overlapping signals, 2H, lipid), 1.27 (s, 28H, lipid), 0.90 (m, 3H, lipid). ^{13}C NMR (126 MHz, $CDCl_3$) δ 104.2 (C-1), 101.5 (C-1′), 78.9 (C-4), 74.8 (C-5), 74.1 (C-3), 74.0 (C-3′), 73.5 (C-4′), 73.1 (C-2′), 72.1 (C-2), 71.2 (C-5′), 71.0 (O-*C*H_2-lipid), 62.2 (C-6′), 60.1 (C-6), 32.4, 30.2, 30.1, 29.9, 26.4, 23.2, 14.3 (all lipid carbons). IR (film) cm^{-1}: 3358, 2915, 2850, 1653, 1471, 1376, 1262, 1099, 1047, 1020, 794, 717. ESI-HRMS calcd for $C_{29}H_{56}O_{11}Cl$ 615.3511, found *m/z* 615.3499 $[M+Cl]^-$. ESI-HRMS calcd for $C_{29}H_{56}O_{11}Na$ 603.3720, found *m/z* 603.3743 $[M+Na]^+$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 4.52 (d, *J* = 7.7 Hz, H-1′), 4.22 (d, *J* = 7.2 Hz, H-1).

4-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-1-thio-D-galactopyranose (22)

Intermediate **18** (700 mg, 0.69 mmol), prepared as described above in preparation of **20**, was dissolved in dioxane (17 mL). Lawesson's reagent (0.4 g, 1.5 equiv.) was then added, and the mixture was stirred at 100 °C overnight. The solvent was then removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 7:2) gave **22β** (270 mg, 38 %) and **22α** (96 mg, 14 %). Analytical data for **22β** ^{1}H NMR (α-glucopyranoside, 500 MHz, $CDCl_3$) δ 8.08 (overlapping signals, 3H, aromatic H), 8.97 (overlapping signals, 5H, aromatic H), 7.60 (overlapping signals, 16H, aromatic H), 7.49 (overlapping signals, 6H, aromatic H), 7.27 (overlapping signals, 28H, aromatic H), 5.68 (t, *J* = 9.9 Hz, 1H, H-2), 5.32 (dd, *J* = 10.3, 2.9 Hz, 1H, H-3), 5.03 (d, *J* = 11.0 Hz, 1H, benzyl CH), 4.97 (overlapping signals, 2H, H-1′, benzyl CH), 4.83 (overlapping signals, 2H, benzyl CH x2), 4.78 (overlapping signals, 2H, H-1, H6a), 4.71 (overlapping signals, 2H, H-6b, benzyl CH), 4.47 (d, *J* = 3.4 Hz, 1H, H-4), 4.41 (overlapping signals, 2H, benzyl CH x2), 4.16 (t, *J* = 9.6 Hz, 1H, H-3′), 4.10 (overlapping signals, 3H, H-5, H-5′, benzyl CH), 3.76 (t, *J* = 9.5 Hz, 1H, H-4′), 3.57 (dd, *J* = 9.9, 3.5 Hz, 1H, H-2′), 3.43 (dd, *J* = 11.0, 2.1 Hz, 1H, H-6′a), 3.04 (dd, *J* = 11.0, 2.1 Hz, 1H, H-6′b), 2.51 (d, *J* = 14.5 Hz, 1H, S*H*). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1 (C=O), 166.1 (C=O), 165.5 (C=O), 138.8 (C), 138.5 (C), 137.9 (C), 137.9 (C), 133.4 (C), 133.3 (C), 133.2 (C), 130.0 (CH), 129.8 (CH), 129.7 (CH), 129.7 (CH), 129.3 (CH), 129.2 (CH), 128.5 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.1 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.5 (CH), 100.5 (C-1′), 81.7 (C-3′), 79.8 (C-2′), 79.4 (C-1), 77.6 (C-4′), 77.3 (C-5), 76.2 (C-4), 75.6 (benzyl CH_2), 74.9 (benzyl CH_2), 74.7 (C-3), 74.0 (benzyl CH_2), 73.4 (benzyl CH_2), 72.1 (C-2), 71.5 (C-5′), 67.8 (C-6′), 62.9 (C-6). ESI-HRMS calcd for $C_{61}H_{58}O_{13}SNa$ 1053.3496, found *m/z* 1053.3496 $[M+Na]^+$.

Analytical data for **22α:** ^{1}H NMR (α-glucopyranoside, 500 MHz, $CDCl_3$) δ 8.07 (overlapping signals, 3H, aromatic H), 8.95 (overlapping signals, 5H, aromatic H), 7.59 (m, 1H, aromatic H), 7.48 (overlapping signals, 5H, aromatic H), 7.19 (overlapping signals, 28H, aromatic H), 6.22 (t, *J* = 5.3 Hz, 1H, H-1), 5.79 (dd, *J* = 10.8, 5.5 Hz, 1H, H-2), 5.61 (dd, *J* = 10.8, 3.0 Hz, 1H, H-3), 5.02 (m, 1H, benzyl CH), 4.93 (overlapping signals, 2H, benzyl CH, H-1′), 4.73 (overlapping signals, 6H, benzyl CH x3, H5, H6a, H6b), 4.47 (d, *J* = 3.1 Hz, 1H, H-4), 4.39 (overlapping signals, 2H, benzyl CH x2), 4.10 (overlapping signals, 3H, H-3′, H-5′, benzyl CH), 3.70 (t, *J* = 9.5 Hz, 1H, H-4′), 3.55 (dd, *J* = 9.9, 3.4 Hz, 1H, H-2′), 3.28 (dd, *J* = 11.0, 2.5 Hz, 1H, H-6′a), 2.91 (dd, *J* = 11.0, 2.0 Hz, 1H, H-6′b), 1.88 (d, *J* = 4.9 Hz, 1H, S*H*). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0 (C=O), 166.0 (C=O), 165.3 (C=O), 138.8 (C), 138.3 (C), 137.8 (C), 137.7 (C), 133.4 (C), 133.4 (C), 133.2 (C), 129.9 (CH), 129.8 (CH), 129.8 (CH), 129.7 (CH), 129.3 (CH), 129.0 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.2 (CH), 128.2 (CH), 128.2 (CH), 128.1 (CH), 127.9 (CH), 127.9 (CH), 127.9 (CH), 127.9 (CH), 127.8 (CH), 127.8 (CH), 127.7 (CH), 127.7 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.5 (CH), 127.3 (CH) (aromatic carbons), 100.1 (C-1′), 81.8 (C-3′), 79.6 (C-2′), 78.2 (C-1), 77.6 (C-4′), 76.0 (C-4), 75.6 (benzyl CH_2), 74.9 (benzyl CH_2), 74.2 (benzyl CH_2), 73.4 (benzyl CH_2), 71.3 (C-5′), 70.5 (C-3), 69.6 (C-5), 68.4 (C-2), 67.7 (C-6′), 62.5 (C-6). ESI-HRMS calcd for $C_{61}H_{58}O_{13}SNa$ 1053.3496, found *m/z* 1053.3503 $[M+Na]^+$.

17-Tritriacontyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (25β)

Compound **15** (2 g, 4.2 mmol) was dissolved in THF (50 mL). To this suspension were added imidazole (850 mg, 3 equiv.), triphenylphosphine (3.3 g, 3 equiv.) and iodine (3.2 g, 3 equiv.), and the resulting mixture was stirred at 45 °C overnight. Methanol was then added to the solution, and the solvent was removed under reduced pressure. Chromatography (100 % of cyclohexane) gave **23** (2.11 g, 86 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.12 (tt, *J* = 8.8, 4.6 Hz, 1H, I-C*H*), 1.85 (overlapping signals, 2H), 1.68 (overlapping signals, 2H), 1.51 (overlapping signals, 2H), 1.26 (overlapping signals, 54H), 0.88 (t, *J* = 6.8 Hz, 6H). Compound **22β** (100 mg, 0.27 mmol) was dissolved in DMF (5 mL) and cooled to 0 °C. NaH (5 mg, 1.2 equiv) was then added and the reaction was stirred for 5 min, and a solution of **23** (115 mg, 2 equiv.) in DMF (5 mL) was added. The mixture was stirred at room temperature for 3 h. It was then diluted with Et_2O, and the organic phase was washed with water and brine, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 15:1) gave **25β** (88 mg, 61 %, 80:20 mixture of α and β-glucopyranosides, white solid; ^{1}H NMR (α-glucopyranoside, 500 MHz, $CDCl_3$): δ 8.02 (overlapping signals, 2H, aromatic H), 7.93 (overlapping signals, 5H, aromatic H), 7.57 (overlapping signals, 2H, aromatic H), 7.45 (overlapping signals, 6H, aromatic H), 7.29 (overlapping signals, 26H, aromatic H), 7.15 (overlapping signals, 8H, aromatic H), 5.70 (t, *J* = 10.0 Hz, 1H, H-2), 5.32 (dd, *J* = 10.2, 3.0 Hz, 1H, H-3), 5.00 (d, *J* = 10.9 Hz, 1H, benzyl CH), 4.92 (d, *J* = 11.0 Hz, 1H, benzyl CH), 4.89 (d, *J* = 3.5 Hz, 1H, H-1′), 4.81 (overlapping signals, 2H, benzyl CH x2), 4.76 (m, 1H, H-1), 4.73 (overlapping signals, 2H, H-6a, H-6b), 4.69 (t, *J* = 11.7 Hz, 1H, benzyl CH), 4.44 (overlapping signals, 2H, H-4, benzyl CH), 4.37 (d, *J* = 12.1 Hz, 1H, benzyl CH), 4.14 (t, *J* = 9.5 Hz, 1H, H-3′), 4.08 (overlapping signals, 3H, H-5, H-5′, benzyl CH), 3.73 (t, *J* = 9.6 Hz, 1H, H-4′), 3.53 (m, 1H, H-2′), 3.39 (dd, *J* = 11.1, 2.1 Hz, 1H, H-6′a), 3.01 (dd, *J* = 11.1, 1.9 Hz, 1H, H-6′b), 2.85 (m, 1H, S-C*H* lipid), 1.53 (overlapping signals, 7H, lipid), 1.20 (overlapping signals, 72H, lipid), 0.88 (t, *J* = 6.8 Hz, 6H, lipid); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.1 (C=O), 165.0 (C=O), 164.2 (C=O), 137.8 (C), 137.6 (C), 137.0 (C), 136.9 (C), 132.2 (C), 132.1 (C), 132.0 (C), 129.2 (CH), 129.0 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.6 (CH), 128.3 (CH), 127.6 (CH), 127.4 (CH), 127.4 (CH), 127.3 (CH), 127.3 (CH), 127.3 (CH), 127.2 (CH), 127.2 (CH), 127.1 (CH), 127.0 (CH), 126.9 (CH), 126.9 (CH), 126.9 (CH), 126.7 (CH), 126.6 (CH), 126.6 (CH), 126.6 (CH), 126.5 (CH), 126.4 (CH), 126.4 (CH), 126.3 (CH), 99.5 (C-1′), 83.3 (C-1), 80.7 (C-3′), 78.7 (C-2′), 76.5 (C-4′), 75.6 (C-5), 75.3 (C-4), 74.6 (benzyl CH_2), 74.2 (C-3), 73.7 (benzyl CH_2), 72.8 (benzyl CH_2), 72.3 (benzyl CH_2), 70.5 (C-5′), 67.6 (C-2), 66.8 (C-6′), 62.1 (C-6), 45.8 (*C*H-S), 34.3, 34.2, 30.9, 28.7, 28.7, 28.6, 28.6, 28.6, 28.6, 28.6, 28.5, 28.5, 28.5, 28.3, 25.8, 25.3, 21.7, 13.1 (all lipid carbons); ESI-HRMS calcd for $C_{94}H_{124}O_{13}SNa$ 1515.8660, found *m/z* 1515.8699 $[M + Na]^+$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 5.85 (t, *J* = 10.0 Hz, H-2), 5.58 (dd, *J* = 10.1, 3.2 Hz, H-3).

17-Tritriacontyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-1-thio-α-D-galactopyranoside (25α)

Compound **22α** (100 mg, 0.097 mmol) was dissolved in DMF (5 mL) and cooled to 0 °C. NaH (5 mg, 1.2 equiv.) was then added and the reaction was stirred for 5 min, and a solution of **24** (115 mg, 2 equiv.) in DMF (5 mL) was then added. The mixture was stirred at room temperature for 3 h. It was then diluted with Et_2O, and the organic phase was washed with water and brine, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 15:1) gave **25α** (88 mg, 61 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (overlapping signals, 2H, aromatic H), 7.95 (overlapping signals, 4H, aromatic H), 7.57 (m, 1H, aromatic H), 7.46 (overlapping signals, 5H, aromatic H), 7.31 (overlapping signals, 19H, aromatic H), 7.20 (overlapping signals, 5H, aromatic H), 7.11 (overlapping signals, 6H, aromatic H), 5.96 (d, *J* = 5.7 Hz, 1H, H-1), 5.75 (dd, *J* = 11.0, 5.7 Hz, 1H, H-2), 5.56 (dd, *J* = 11.0, 2.9 Hz, 1H, H-3), 5.03 (d, *J* = 11.0 Hz, 1H, benzyl CH), 4.92 (d, *J* = 10.9 Hz, 1H, benzyl CH), 4.89 (d, *J* = 3.2 Hz, 1H, H-1′), 4.80 (overlapping signals, 2H, benzyl CH x2), 4.70 (overlapping signals, 4H, H-5, H-6a, H-6b, benzyl CH), 4.46 (d, *J* = 3.0 Hz, 1H, H-4), 4.37 (overlapping signals, 2H, benzyl CH x2), 4.12 (t, *J* = 9.5 Hz, 1H, H-3′), 4.05 (overlapping signals, 2H, H-5′, benzyl CH), 3.69 (t, *J* = 9.7 Hz, 1H, C-4′), 3.54 (dd, *J* = 9.6, 3.4 Hz, 1H, C-2′), 3.22 (dd, *J* = 11.0, 2.3 Hz, 1H, H-6′a),

2.83 (d, *J* = 10.8 Hz, 1H, H-6′b), 2.73 (m, 1H, S-C*H* lipid), 1.46 (overlapping signals, 4H, lipid), 1.36–1.03 (overlapping signals, 56H, lipid), 0.88 (t, *J* = 6.7 Hz, 6H, lipid). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0 (C=O), 165.9 (C=O), 165.6 (C=O), 138.9 (C), 138.4 (C), 137.8 (C), 137.7 (C), 133.2 (C), 133.2 (C), 133.1 (C), 129.9 (CH), 129.8 (CH), 129.8 (CH), 129.8 (CH), 129.7 (CH), 129.6 (CH), 129.4 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 128.2 (CH), 128.0 (CH), 127.9 (CH), 127.9 (CH), 127.9 (CH), 127.8 (CH), 127.8 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 100.2 (C-1′), 81.9 (C-1), 81.8 (C-3′), 79.5 (C-2′), 77.6 (C-4′), 76.4 (C-4), 75.6 (benzyl CH_2), 74.9 (benzyl CH_2), 74.2 (benzyl CH_2), 73.3 (benzyl CH_2), 71.3 (C-3), 71.2 (C-5′), 69.0 (C-2), 69.0 (C-5), 67.7 (C-6′), 62.8 (C-6), 45.2 (*C*H-S), 35.8, 35.1, 34.6, 31.9, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 27.1, 26.4, 22.7, 14.1 (all lipid carbons). ESI-HRMS calcd for $C_{94}H_{124}O_{13}SNa$ 1515.8660, found *m/z* 1515.8619 $[M + Na]^+$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 6.01 (d, *J* = 5.6 Hz, H-1), 5.85 (dd, *J* = 10.8, 5.5 Hz, H-2), 5.79 (dd, *J* = 10.8, 3.2 Hz, H-3).

17-Tritriacontyl 4-*O*-(D-glucopyranosyl)-1-thio-β-D-galactopyranoside (6)

Compound **25β** (25 mg, 0.017 mmol) was de-*O*-benzoylated and de-*O*-benzylated successively. Chromatography (CH_2Cl_2-MeOH 9:1) gave **6** (11 mg, 80 % over two steps, 77:23 mixture of α and β-glucopyranosides, white solid); ^{1}H NMR (α-glucopyranoside, 500 MHz, $CDCl_3$-CD_3OD, 1:1) δ 4.97 (d, *J* = 3.8 Hz, 1H, H-1′), 4.40 (d, *J* = 9.5 Hz, 1H, H-1), 4.08 (d, *J* = 2.9 Hz, 1H, H-4), 4.01 (ddd, *J* = 9.8, 6.6, 2.5 Hz, 1H, H-5′), 3.90 (m, 1H, H-6′a), 3.83 (dd, *J* = 11.0, 8.4 Hz, 1H, H-6a), 3.69 (overlapping signals, 4H, H-5, H-6b, H-3′, H-6′b), 3.55 (dd, *J* = 9.6, 2.8 Hz, 1H, H-3), 3.47 (overlapping signals, 2H, H-2, H-2′), 3.29 (t, *J* = 9.5 Hz, 1H, H-4), 2.90 (m, 1H, S-C*H*), 1.62 (overlapping signals, 4H, lipid), 1.42 (overlapping signals, 4H, lipid), 1.27 (overlapping signals, 52H, lipid), 0.89 (t, *J* = 6.8 Hz, 6H, lipid). ^{13}C NMR (126 MHz, $CDCl_3$-CD_3OD, 1:1) δ 101.5 (C-1′), 86.8 (C-1), 79.2 (C-4), 78.2 (C-5), 75.2 (C-3), 73.9 (C-3′), 73.3 (C-5′), 72.9 (C-2), 71.1 (C-2′), 71.0 (C-4), 62.2 (C-6′), 59.8 (C-6), 46.7 (S-*C*H), 35.3, 35.3, 32.3, 30.0, 29.9, 29.7, 26.8, 23.0, 14.2 (all lipid carbons). IR (film) cm^{-1}: 3338, 2916, 2850, 1470, 1260, 1018, 797, 717. ESI-HRMS calcd for $C_{45}H_{87}O_{10}S$ 819.6020, found *m/z* 819.6049 $[M - H]^-$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 4.51 (d, *J* = 7.4 Hz, H-1′), 4.35 (d, *J* = 9.1 Hz, H-1).

17-Tritriacontyl 4-*O*-(α-D-glucopyranosyl)-1-thio-β-D-galactopyranoside (4)

Compound **25α** (23 mg, 0.015 mmol) was de-*O*-benzoylated and de-*O*-benzylated successively. Chromatography (CH_2Cl_2-MeOH 9:1) gave **4** (11 mg, 83 % over two steps, 90:10 mixture of α and β-glucopyranosides, white solid); ^{1}H-NMR (500 MHz, $CDCl_3$-CD_3OD 1:1): δ 5.41 (d, *J* = 5.4 Hz, 1H, H-1), 4.98 (d, *J* = 3.7 Hz, 1H, H-1′), 4.32 (dd, *J* = 8.0, 5.2 Hz, 1H, H-5), 4.09 (m, 1H, H-4), 4.04 (dd, *J* = 10.5, 5.4 Hz, 1H, H-2), 3.93 (dd, *J* = 10.0, 2.7 Hz, 1H, H-5′), 3.90 (d, *J* = 11.0 Hz, 1H, H-6′a), 3.86 (dd, *J* = 12.1, 8.8 Hz, 1H, H-6a), 3.68 (overlapping signals, 4H, H-3, H-3′, H-6b, H-6′b), 3.50 (dd, *J* = 9.8, 3.8 Hz, 1H, H-2′), 3.29 (t, *J* = 9.5 Hz, 1H, H-4′), 2.76 (p, *J* = 6.5 Hz, 1H, CH-lipid), 1.61 (overlapping signals, 4H, lipid), 1.44 (overlapping signals, 9H), 1.29 (overlapping signals, 60H, lipid), 0.89 (t, *J* = 6.8 Hz, 7H). ^{13}C NMR (126 MHz, $CDCl_3$-CD_3OD 1:1) δ 102.0 (C-1′), 86.5 (C-1), 81.7 (C-4), 74.0 (C-5′), 74.0 (C-3′), 72.8 (C-2′), 71.8 (C-3), 71.1 (C-4′), 70.8 (C-5), 69.6 (C-2), 62.3 (C-6′), 60.7 (C-6), 46.2 (*C*H-lipid), 35.9, 35.3, 32.4, 30.2, 30.1, 30.1, 30.1, 29.8, 27.2, 23.1, 14.3 (all lipid carbons). IR (film) cm^{-1}: 3385, 2913, 2849, 1471, 1260, 1081, 1019, 799, 716. ESI-HRMS calcd for $C_{45}H_{87}O_{10}S$ 819.6020, found *m/z* 819.6038 $[M - H]^-$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 5.38 (d, *J* = 5.9 Hz), 4.50 (d, *J* = 7.6 Hz, 1H).

1-Heptadecanyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (27)

Compound **19** (1 g, 3.9 mmol) was suspended in THF (50 mL) and to this was added imidazole (0.8 g, 3 equiv.), triphenylphosphine (3 g, 3 equiv.) and iodine (3 g, 3 equiv.), and the resulting mixture was stirred at 45 °C

overnight. Methanol was then added to the solution, and the solvent was removed under reduced pressure. Chromatography (cyclohexane) gave intermediate **24** (1.13 g, 79 %) as a white solid. Analytical data for **24** was in agreement with that reported previously [30]. Compound **22β** (100 mg, 0.097 mmol) was dissolved in DMF (5 mL) and cooled to 0 °C. NaH (5 mg, 1.2 equiv.) was then added and the reaction was stirred for 5 min, and **24** (71 mg, 2 equiv.) in DMF (5 mL) was then added. The mixture was stirred at room temperature for 3 h. It was then diluted with Et_2O, and the organic phase was washed with water and brine, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. Chromatography (cyclohexane-EtOAc, 14:1) gave **27** (53 mg, 43 %, 81α:19β mixture of glucopyranosides, white solid); ^{1}H NMR (α-glucopyranoside, 500 MHz, $CDCl_3$): δ 8.03 (overlapping signals, 2H, aromatic H), 7.94 (overlapping signals, 4H, aromatic H), 7.59 (overlapping signals, 2H, aromatic H), 7.47 (overlapping signals, 5H, aromatic H), 7.29 (overlapping signals, 18H, aromatic H), 7.16 (overlapping signals, 4H, aromatic H), 5.80 (t, *J* = 10.0 Hz, 1H, H-2), 5.34 (dd, *J* = 10.2, 2.9 Hz, 1H, H-3), 4.98 (d, *J* = 10.9 Hz, 1H, benzyl CH), 4.93 (overlapping signals, 2H, H-1′, benzyl CH), 4.80 (overlapping signals, 2H, benzyl CH x2), 4.75 (overlapping signals, 2H, H-6a, H-6b), 4.71 (overlapping signals, 2H, H-1, benzyl CH), 4.46 (overlapping signals, 2H, H-4, benzyl CH), 4.37 (d, *J* = 12.1 Hz, 1H, benzyl CH), 4.10 (overlapping signals, 4H, H-5, H-3′, H-5′, benzyl CH), 3.73 (t, *J* = 9.6 Hz, 1H, H-4′), 3.56 (dt, *J* = 9.9, 3.0 Hz, 1H, H-2′), 3.34 (dd, *J* = 10.9, 2.1 Hz, 1H, H-6′a), 2.95 (dd, *J* = 11.0, 1.9 Hz, 1H, H-6′b), 2.82 (ddd, *J* = 12.5, 8.3, 6.3 Hz, 1H, butyl C*H*(H)O), 2.72 (dt, *J* = 12.5, 7.6 Hz, 1H, butyl C*H*(H)O), 1.61 (m, 2H, lipid), 1.25 (overlapping signals, 28H, lipid), 0.89 (m, 3H, lipid); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 166.0, 165.3 (each C=O), 138.8 (C), 138.6 (C), 138.0 (C), 137.9 (C), 133.3 (C), 133.2 (C), 133.1 (C), 130.0 (CH), 129.7 (CH), 129.7 (CH), 129.5 (CH), 129.2 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 128.3 (CH), 128.2 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.9 (CH), 127.7 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.5 (CH), 127.3 (CH), 100.2 (C-1′), 83.9 (C-1), 81.9 (C-3′), 79.7 (C-2′), 77.5 (C-4′), 76.5 (C-5), 75.7 (C-4), 75.6 (benzyl CH_2), 75.0 (C-3), 74.7 (benzyl CH_2), 73.9 (benzyl CH_2), 73.3 (benzyl CH_2), 71.5 (C-5′), 68.2 (C-2), 67.7 (C-6′), 62.7 (C-6), 29.8 (O-*C*H_2), 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.2, 28.9, 26.9, 22.7, 14.1 (all lipid carbons). ESI-HRMS calcd for $C_{78}H_{92}O_{13}SNa$ 1286.6602, found *m/z* 1286.6595 $[M+Na]^+$. Selected ^{1}H-NMR data for β-glucopyranoside: δ 5.90 (t, *J* = 10.1 Hz, H-2), 5.59 (dd, *J* = 10.1, 3.2 Hz, H-3).

1-Heptadecanyl 4-*O*-(α-D-glucopyranosyl)-1-thio-β-D-galactopyranoside (3)

Compound **27** (30 mg, 0.024 mmol) was reacted as described in the general procedures for debenzoylation and *O*-debenzylation successively. Chromatography (CH_2Cl_2-MeOH 9:1) gave **3** (11 mg, 77 % over two steps, 64α:36β mixture of glucopyranosides, white solid); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.86 (d, *J* = 3.9 Hz, 1H, H-1′), 4.28 (d, *J* = 9.3 Hz, 1H, H-1), 3.99 (overlapping signals, 2H, H-2, H-4), 3.91 (ddd, *J* = 12.6, 5.7, 2.5 Hz, 1H, H-5′), 3.80 (m, 1H, H-6a), 3.75 (overlapping signals, 2H, H-6′a, H-6′b), 3.70 (t, *J* = 6.5 Hz, 1H, H-5), 3.58 (overlapping signals, 3H, H-3, H-3′, H-6b), 3.37 (dd, *J* = 9.9, 3.8 Hz, 1H, H-2′), 3.20 (t, *J* = 9.5 Hz, 1H, H-4′), 3.10 (m, 2H, O-C*H*$_2$-lipid), 1.73 (m, 2H, lipid), 1.45 (overlapping signals, 6H, lipid), 1.18 (overlapping signals, 28H, lipid), 0.79 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 100.4 (C-1′), 89.5 (C-1), 78.7 (C-5), 77.6 (C-4), 73.5 (C-3), 72.7 (C-3′), 72.3 (C-5′), 71.8 (C-2), 69.9 (C-4′), 65.9 (C-2), 60.9 (C-6′), 59.2 (C-6), 50.3 (S-CH_2), 31.1, 28.9, 28.8, 28.8, 28.7, 28.6, 28.5, 28.3, 28.0, 27.8, 27.7, 24.1, 21.9, 20.3, 12.9 (all lipid carbons). Selected ^{1}H-NMR data for β-glucopyranoside: δ 4.51 (d, *J* = 7.8 Hz, H-1′), 4.30 (d, *J* = 9.5 Hz, H-1).

17-Tritriacontyl 4-*O*-(4,6-di-*O*-benzyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-1-thio-α-D-galactopyranoside (26α)

Compound **25β** (50 mg, 0.033) was dissolved in CH_2Cl_2 (0.75 mL). $TiCl_4$ (1 M in CH_2Cl_2, 90 µL, 2.5 equiv.) was then added, and the mixture was stirred at room temperature for 15 h. It was then diluted in EtOAc and washed with a saturated solution of NH_4Cl. The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with satd $NaHCO_3$, brine, and then dried over Na_2SO_4 and filtered. After removal

of the solvent under reduced pressure, chromatography (cyclohexane-EtOAc, 6:1) gave **26α** (18 mg, 41 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (overlapping signals, 3H, aromatic H), 7.94 (overlapping signals, 4H, aromatic H), 7.58 (m, 1H, aromatic H), 7.47 (overlapping signals, 5H, aromatic H), 7.29 (overlapping signals, 13H, aromatic H), 7.15 (m 2H, aromatic H), 7.10 (overlapping signals, 3H, aromatic H), 5.94 (d, *J* = 6.0 Hz, 1H, H-1), 5.72 (dd, *J* = 11.1, 5.9 Hz, 1H, H-2), 5.57 (d, *J* = 11.0 Hz, 1H, H-3), 5.02 (d, *J* = 3.9 Hz, 1H, H-1′), 4.79 (m, 1H, H-5), 4.67 (overlapping signals, 3H, H-6a, H-6b, benzyl CH), 4.54 (d, *J* = 2.8 Hz, 1H, H-4), 4.42 (d, *J* = 11.5 Hz, 1H, benzyl CH), 4.35 (d, *J* = 12.1 Hz, 1H, benzyl CH), 4.03 (m, 1H, benzyl CH), 3.97 (overlapping signals, 2H, H-3′, H-5′), 3.60 (s, 1H, H-2′), 3.55 (t, *J* = 9.8 Hz, 1H, H-4′), 2.99 (d, *J* = 11.3 Hz, 1H, H-6′a), 2.74 (m, 1H, S-C*H*), 2.63 (d, *J* = 11.2 Hz, 1H, H-6′b), 2.51 (s, 1H, OH), 2.47 (d, *J* = 8.1 Hz, 1H, OH), 1.63 (overlapping signals, 4H, lipid), 1.23 (overlapping signals, 54H, lipid), 0.88 (t, *J* = 6.9 Hz, 6H, lipid). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 165.8, 165.6 (each C=O), 138.4 (C), 137.5 (C), 133.3 (C), 133.3 (C), 133.3 (C), 129.9 (CH), 129.8 (CH), 129.8 (CH), 129.5 (CH), 129.3 (CH), 129.2 (CH), 128.5 (CH), 128.4 (CH), 128.4 (CH), 128.2 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 127.7 (CH), 99.8 (C-1′), 82.1 (C-1), 76.9 (C-4′), 74.7 (C-4), 74.4 (benzyl *C*H_2), 74.3 (C-3′), 73.4 (benzyl CH_2), 72.6 (C-2), 71.0 (C-3), 70.9 (C-5′), 68.7 (C-2, C-5 (overlapping signals)), 67.3 (C-6′), 62.0 (C-6), 45.4 (*C*H-S), 35.0, 34.6, 31.9, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.4, 27.1, 26.3, 22.7, 14.1 (all lipid carbons). IR (film) cm^{-1}: 3459, 2923, 2853, 1724, 1452, 1269, 1095, 1069, 1046, 1026, 708. ESI-HRMS calcd for $C_{80}H_{112}O_{13}SNa$ 1335.7721, found *m/z* 1335.7678 $[M+Na]^+$.

Isolation and characterization of natural and synthetic simplexide 1c

Simplexide was isolated from *Plakortis simplex* at the Department of Pharmacy, University of Naples Federico II as described previously [1, 31]. The structure and purity of the isolated glycolipids was confirmed by ^{1}H-NMR spectroscopy and mass spectrometry as described earlier [1]. As with most glycolipids from sponges, each of the obtained glycolipids was an inseparable mixture of homologues, which are identical in the polar part of the molecule but slightly different in the length and branching of the lipophilic chains. The relative amounts of branched and unbranched chains were evaluated by ^{1}H-NMR, while their length was determined by mass spectrometry. Both resulted to be very close to those reported earlier [15, 32]. Stock solutions of glycolipid were prepared and stored in MeOH at a concentration of 3 mM unless otherwise specified and diluted to working concentration in RPMI immediately before the experiment.

Synthetic simplexide **1c** was prepared as reported earlier from 1-octadecanol, 1-bromoheptadecane, methyl α-D-glucopyranose, and methyl β-D-galactopyranose and final purification of **1c** was achieved using reverse-phase HPLC using an RP-18 column and MeOH as eluent as previously described.

Cell isolation and purification

The study protocol involving the use of human blood cells was approved by the Ethical Committee of the University of Naples Federico II (N°301/2018), and written informed consent was obtained from blood donors in according to the principles expressed in the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMCs) were purified from buffy coats of healthy donors (HCV^-, $HBsAg^-$, HIV^-) obtained from the Leukapheresis Unit. PBMCs were obtained by dextran stratification and centrifugation over Histopaque®-1077.

Cell incubations

PBMCs were incubated (37 °C, 24 h) in RMPI supplemented with 2 mM L-glutamine and 1 % antibiotic solution and various concentrations of synthesised compounds (from 1a to 6) (3–30 μg/mL), or natural and synthetic simplexide (10 μg/mL). All compounds were routinely checked for LPS contamination (*Limulus amebocyte* test, MP Biomedicals) and discarded if the LPS concentration was above the detection limit of the assay (0.125 EU/mL). The experiments to compare the effects of synthetic and natural compounds were done in

polystyrene plates coated with various concentrations of glycolipids dissolved in methanol. Solvent was dried under nitrogen immediately before the addiction of cells for 24 h at 37 °C. At the end of the experiment, the supernatant was removed, centrifuged (1000 *g*, 4 °C, 5 min) and stored at −80 °C for subsequent determination of IL-6, CXCL-8 and TNF-α release.

Cytokine assay

The release of IL-6, CXCL-8 and TNF-α in the culture supernatant was measured in duplicate determinations by commercially available ELISA kits (R&D, Minneapolis, MN, USA) according to the manufacturer's instructions. The results were normalized as pg/mL of proteins for 10^6 cells.

Statistical analysis

Data were analyzed with the GraphPad Prism 5 software package. The data are expressed as mean values ± SE of the indicated number of experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's test (when comparison was made against a control) or Bonferroni's test (when comparison was made between each pair of groups) A p value of 0.05 or lower was considered to be significant.

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