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Article

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Trehalose- and glucose-derived glycoamphiphiles: small-molecule and nanoparticle Toll-like Receptor 4 (TLR4) modulators

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ABSTRACT. An increasing number of pathologies have been linked to Toll-like Receptor 4 (TLR4) activation and signaling, therefore new hit and lead compounds targeting this receptor

activation process are urgently needed. We report on the synthesis and biological properties of glycolipids based on glucose and trehalose scaffolds which potently inhibit TLR4 activation and signaling *in vitro* and *in vivo*. Structure-activity relationship studies on these compounds indicate that the presence of fatty ester chains in the molecule is a primary prerequisite for biological activity and points to facial amphiphilicity as a preferred architecture for TLR4 antagonism.

The cationic glycolipids here presented can be considered as new lead compounds for the development of drugs targeting TLR4 activation and signaling in infectious, inflammatory and autoimmune diseases. Interestingly, the biological activity of the best drug candidate was retained after adsorption at the surface of colloidal gold nanoparticles, broadening the options for clinical development.

INTRODUCTION

Toll-like receptors (TLRs) play a critical role in the recognition of conserved pathogen-associated molecular patterns (PAMPs) derived from various microbial pathogens including viruses, bacteria, protozoa and fungi, and in the subsequent initiation of innate immune response.[1] Among TLRs, TLR4 selectively responds to bacterial endotoxin (E), composed by bacterial lipopolysaccharides (LPS) or part of it (lipooligosaccharides, LOS, lipid A).[2, 3] LPS is the main chemical component of the Gram negative bacteria outer membrane, and the lipid A, a negatively charged phosphorylated lipodisaccharide represents the LPS moiety that is responsible for TLR4 activation through specific molecular recognition processes (Figure 1).

TLR4 is also activated by endogenous molecules, generally known as danger-associated molecular patterns (DAMPs).[4] Typical DAMPs acting as TLR4 agonists are released as a consequence of injury and inflammation. Most of the reported DAMPs, are proteins, which are

very different from lipid A, and the molecular details of DAMP interaction with the TLR4 receptorial system are still unknown, although in some cases endotoxin contamination seems to be responsible for TLR4 activity of DAMPs. Chemical entities that block TLR4 activation by bacterial endotoxin (LPS) thus acting as antagonists, are hit compounds for developing drugs active against acute sepsis and septic shock derived from excessive and deregulated TLR4 activation and signaling.[5] On the other hand, the inhibition of TLR4 stimulation by DAMPs could be used to contrast a wide range of inflammatory and autoimmune disorders associated to the release of inflammatory cytokines. In this context, TLR4 is an emerging molecular target related to an impressively broad spectrum of modern day disorders including autoimmune disorders, chronic inflammations, allergies, asthma, atherosclerosis, aortic aneurysm, CNS diseases such as neuropathic pain, Amyotrophic Lateral Sclerosis (ALS), Alzheimer's Disease (AD), and some types of cancer.[6] As the majority of these pathologies still lack specific pharmacological treatment, small molecules active in inhibiting TLR4 activation have attracted increasing interest in a wide range of possible clinical settings.[7]

The molecular mechanism by which endotoxin activate TLR4 is a complex process[8] and depends on LPS binding protein (LBP)[9]-catalyzed extraction and transfer of individual LPS molecules from aggregated LPS to the CD14 (cluster of differentiation 14) receptor,[10] and then from CD14 to myeloid differentiation protein 2 (MD-2).[11, 12] This process is followed by engagement and dimerization of TLR4 thus forming the cell surface complex $(LPS{\cdot}MD\text{-}2{\cdot}TLR4)_2$,[13] which initiates the intracellular signaling by recruiting specific adaptor proteins and activating downstream signaling pathways.

Several natural and synthetic small molecules are known to modulate TLR4 activation and subsequent intracellular signaling acting as agonists (activators) or antagonists (inhibitors).[6] The

majority of these molecules are lipid A variants and synthetic lipid A mimetics that reproduce the structural motif of the anionic disaccharide. Monophosphoryl lipid A (MPL) and some aminoalkyl glucosaminide phosphates (AGPs) are agonists in use as vaccine adjuvants,[14] while underacylated variants such as natural lipid IVa[15] and synthetic Eritoran[16] are antagonists (Figure 1). In general Lipid A variants are anionic lipids, bearing one or two negatively charged phosphate groups and a hydrophobic domain (lipid chains). Although counterintuitive, several cationic lipids made of positively charged headgroups (usually tertiary or quaternary ammonium salts or polyamines) and a hydrophobic domain (alkyl chains or steroids) have been found to be active in modulating TLR4 activity,[17] acting either as agonists or antagonists of TLR4. Thus, some positively charged liposomes formed by cationic amphiphiles induce the expression of pro-inflammatory mediators. For instance, diC14-amidine (Figure 1) liposomes trigger the secretion of a cytokine pattern reminiscent of the TLR4-dependent LPS secretion pattern by activating both MyD88/NF-κB/JNK and TRAM/TRIF pathways.[18] Other cationic lipids activate cytokine production through NF-κB-independent, TRIF-dependent pathways, which requires the presence of CD14.[19, 20] Structural changes make cationic lipids switch from agonism to antagonism, as in the case of dioleoyl trimethylammonium propane (DOTAP), that inhibits TLR4 signal by competing with LPS for interaction with LBP or/and CD14.[20] Complexes of the commercial cationic lipid formulation Lipofectamine with LPS reduce its TLR4 activity. Interestingly LPS complexed with Lipofectamine co-localizes with CD14 at the cell surface and inside cells, but does not co-localizes with TLR4·MD-2 complex, suggesting that the mechanism of inhibition may result from the uncoupling of CD14 from TLR4·MD-2.[21]

Specific binding of amino glycolipids and aromatic ammonium salts to CD14 (compounds IAXO-101, -102, -103, Figure 1), was recently shown by our group.[22, 23] These compounds are

active in inhibiting LPS-stimulated TLR4-dependent cytokine production in cells and in animals.[24]

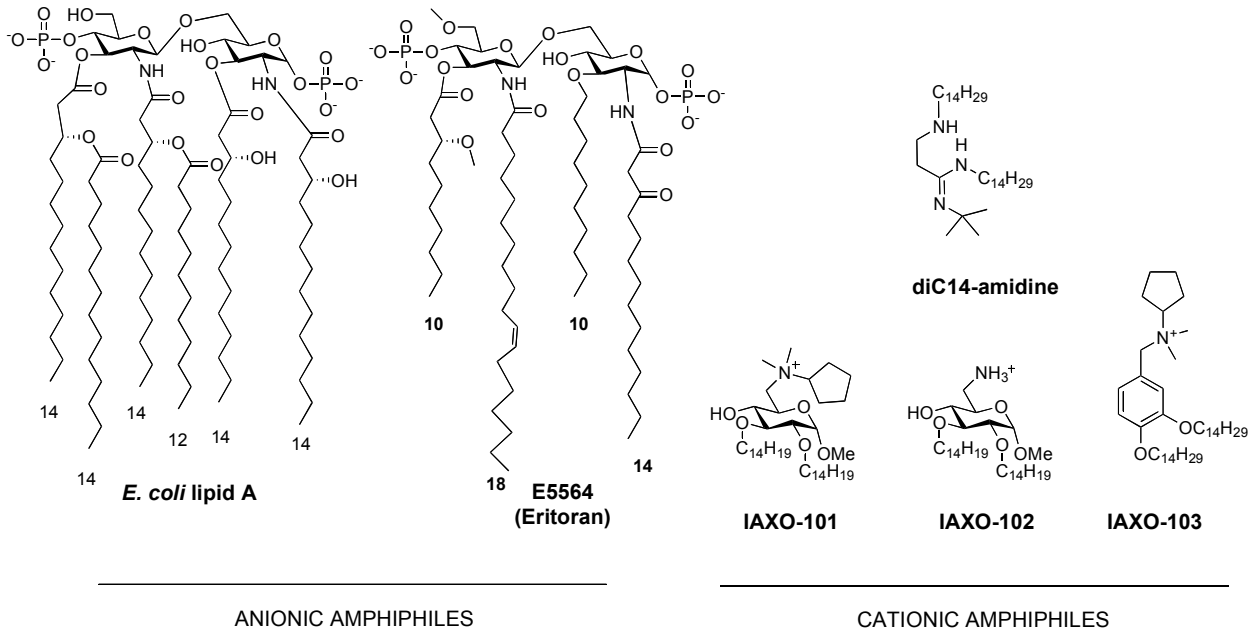


Figure 1. Anionic and cationic TLR4 modulators. From the left: lipid A from *E. coli*, the natural TLR4 agonist, synthetic anionic (the antagonist Eritoran) and cationic amphiphiles (diC14-amidine, IAXO compounds).

Evaluation of transfer of LOS from the monomeric soluble form of CD14 (sCD14) to His_6-tagged CD14 or MD-2 by co-capture to a metal chelating resin clearly showed that the cationic lipids derived from D-glucose or benzylamine inhibit the transfer of LOS from sCD14 to CD14-His_6, but not the transfer of LOS from sCD14 to MD-2.[22] Finally, saturation transfer difference (STD) NMR data demonstrated direct binding of the cationic lipids to CD14, through alkyl chains mainly.[22] Altogether, these data suggest that the lipid tails of cationic amphiphiles interact with the hydrophobic binding site of CD14[25] and compete with LPS or LOS chains. The carbohydrate scaffold in amino glycolipids probably acts by preventing random conformations and providing a favorable orientation of the lipid chains that is reminiscent of that found in lipid

A. Most interestingly, through the interplay of regioselective functionalization methodologies and conformational bias, the installation of differentiated cationic and hydrophobic domains in carbohydrate platforms can be made compatible with molecular diversity-oriented strategies and structure-activity relationship (SAR) studies. As a proof of concept, we have now prepared a series of new cationic glycoamphiphiles using the monosaccharide methyl α-D-glucopyranoside and the disaccharide α,α'-trehalose as the sugar cores. Systematic modification of the cationic heads and the lipophilic tails and evaluation of their capacity to interfere with TLR4 activation and signaling *in vitro* and *in vivo* allowed the identification of a drug lead that has been further incorporated in gold nanoparticles to test the effect of multivalent presentation on its biological activity.

RESULTS AND DISCUSSION

Ligand-based rational design. It is known that the self-assembling capabilities and the ability of cationic amphiphiles to interact with CD14 as liposomes or micellar aggregates have a strong impact on their TLR4 modulatory activity.[17] Yet, very little is still known on the molecular aspects underlying the mechanisms at play, mainly because structural data of cationic compounds bound to MD-2 or CD14 receptors are still lacking. Conducting SAR studies on series of homologous cationic amphiphiles and relating the biological activity to the aggregation properties is expected to provide new insights in this matter. Glucose-derived cationic glycolipids are particularly appealing for this purpose. First, the secondary hydroxyls of the glucopyranose ring are well suited anchoring points to link lipophilic chains in a similar orientation as the fatty acid acyl chains in lipid A. Second, the incorporation of protonatable headgroups at the primary position imparts facial amphiphilicity to the molecule, a biomimetic feature that is associated to improved cell-membrane crossing abilities and proneness to form

supramolecular complexes with complementary biomolecules by either electrostatic or hydrophobic interactions.[26] On these grounds, we have now synthesized amino glycolipids derived from methyl α-D-glucopyranoside (**1-6)** and from α,α'-trehalose (**7-11,** Figure 2). The latter can be formally considered as dimeric homologues of the glucose amphiphiles. We keep in mind that the confluence of two exoanomeric effects at the 1-1 interglycosidic linkage in α,α'-trehalose strongly limits rotation about the glycosidic bonds,[27] preserving a rigid conformation that warrants facial anisotropy after differential functionalization at the primary and secondary positions, even in highly constrained constructs.[28] Both the methyl α-D-glucopyranoside and the α,α'-trehalose scaffolds have previously demonstrated their efficiency in the design of TLR4 modulators with anionic amphiphilic structures.[29,30] Structural modifications have been projected by varying the number, the nature and the length of the lipid chains and the number and disposition of amino groups, in order to evaluate how these structural elements influence the TLR4 activity.

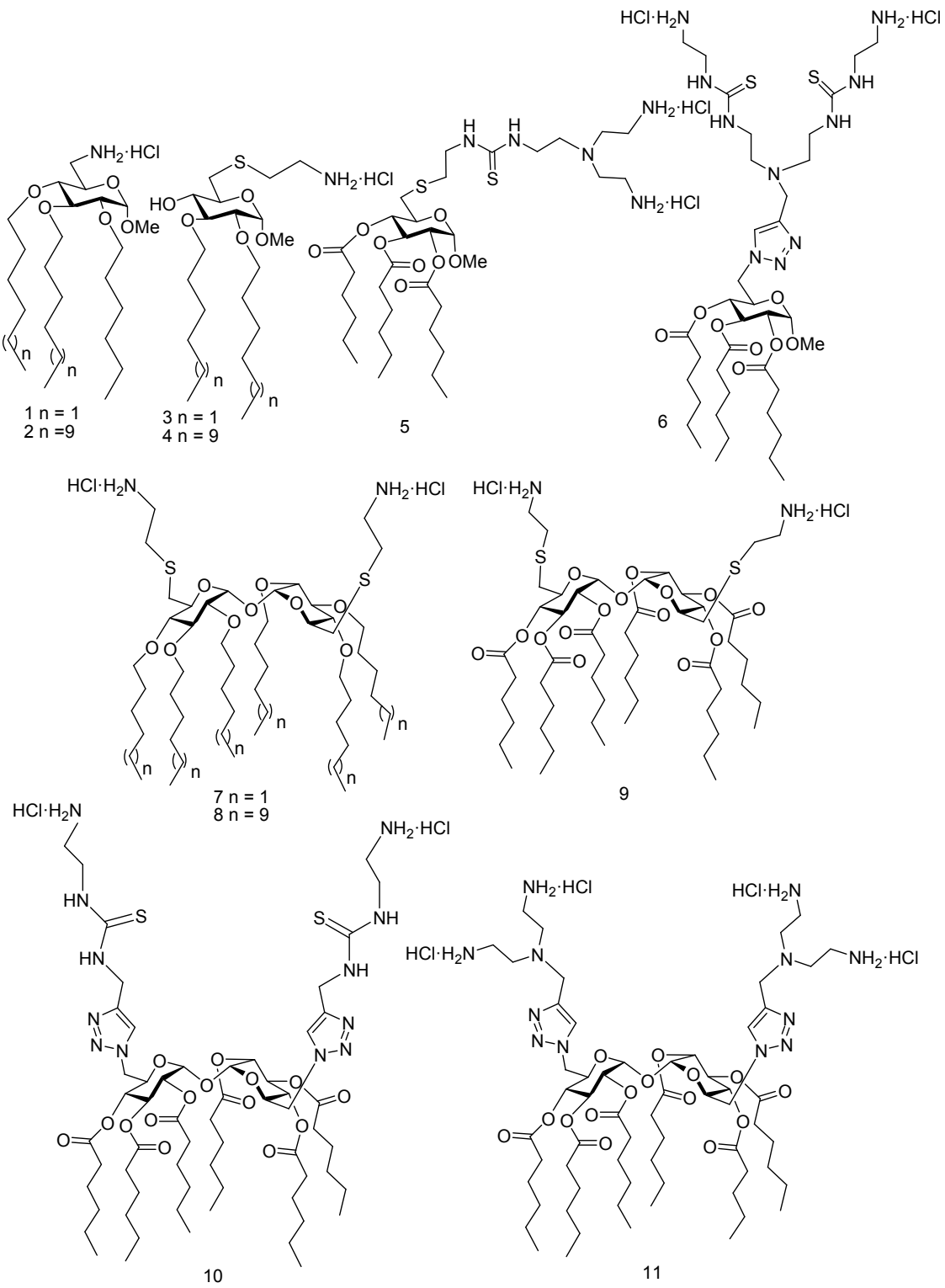
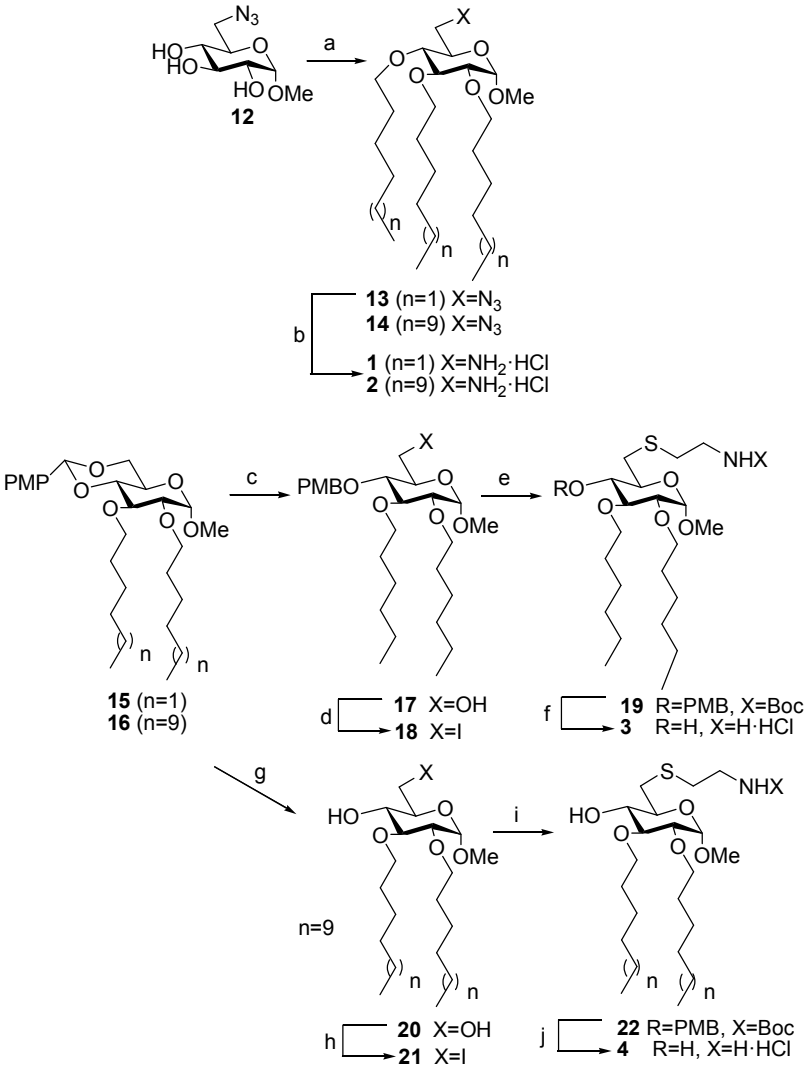


Figure 2. Synthetic monosaccharide and disaccharide protonatable amphiphiles derived, respectively, from D -glucose (**1**-**6**) and α,α'-trehalose (**7**-**11**).

Synthesis of glucose-derived cationic glycolipids. The syntheses of the tri-*O*-alkylated 6-amino-6-deoxyglucoside derivatives **1** and **2** (Scheme 1) were successfully accomplished by reaction of the known methyl 6-azido-6-deoxy-α-D-glucopyranoside **12**[31] with hexyl or tetradecyl bromide and sodium hydride (**13** and **14**) followed by reduction of the azido group by either catalytic hydrogenation or Staudinger reaction with triphenylphosphine and hydrolysis of the corresponding phosphazene intermediate.[32] The target ether-type amino glycolipids **1** and **2** were isolated as the corresponding hydrochloride salts.

Scheme 1. Syntheses of monosaccharides 1-4.



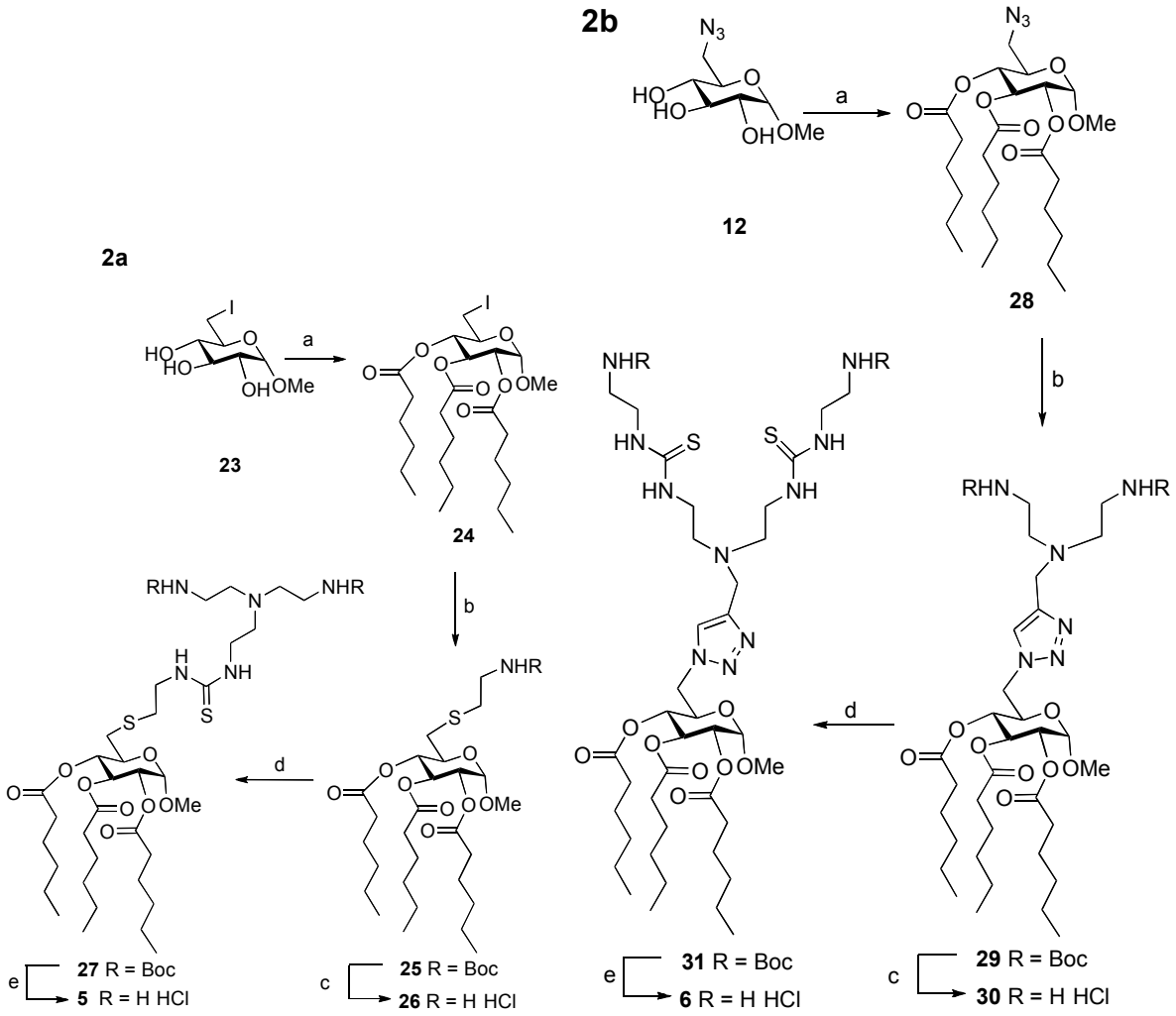
Reagents and conditions: a) 1-bromohexane, NaH, DMF, overnight, 48% or 1-bromotetradecane, NaH, DMF, 55 ºC, 52%; b) H_2, Pd/C, MeOH, 2 h, 87%; or PPh_3, THF; then, NH_4OH, 50 ºC, overnight, 82%;c) 1 M $LiAlH_4$ in THF, $AlCl_3$, DCM, Et_2O, 83%; d) I_2, PPh_3, imidazole, toluene, 94%; e) $HS(CH_2)_2NHBoc$, Cs_2CO_3, DMF, 60 ºC, 99%; f) 1:1 TFA-DCM, 80%; g) $AlCl_3$, DCM, Et_2O, 87%; h) I_2, PPh_3, imidazole, toluene, 91%; i) $HS(CH_2)_2NHBoc$, Cs_2CO_3, DMF, 60 ºC, 95%; j) 1:1 TFA-CH_2Cl_2, quant.

The 2,3-di-*O*-hexyl and -*O*-tetradecyl glucose derivatives **3** and **4** were synthesized from the corresponding 4,6-*O*-(*p*-methoxybenzylidene) protected precursor **15** and **16**[33] respectively, which at their turn were obtained by standard alkylation of methyl 4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside.[34] The regioselective opening of the acetal ring of **15** using lithium aluminium hydride ($LiAlH_4$) gave the 4-*O*-*p*-methoxybenzyl (PMB) ether **17**. Compound **16** on his side was completely deprotected on C-4 and C-6 positions using aluminium trichloride, affording compound **20**. Iodination of the C-6 hydroxyl groups of **17** and **20** using Garegg's conditions[35] afforded compounds **18** and **21**, which were subjected to cesium carbonate-promoted nucleophilic displacement with *t*-butoxycarbonyl (Boc)-protected cysteamine (**20** and **22**) and final Boc removal in acidic conditions to give compounds **3** and **4**.

The synthetic routes to obtain compounds **5** and **6**, having a tertiary and two primary amino groups in the cationic head (Scheme 2), are based on the thiourea-forming and the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reactions, two "click"-type ligation strategies already proven very efficient to generate polycationic clusters.[36] The preparation of **5** started by hexanoylation of methyl 6-deoxy-6-iodo-α-D-glucopyranoside **23**[35] (**24**; Scheme 2a) followed by nucleophilic displacement of the iodine by Boc-protected cysteamine (**25**) and carbamate hydrolysis, affording the cysteaminyl derivative **26** in 92% overall yield. Condensation of **26**

with 2-[*N,N*-bis(2-(*N*-*tert*-butoxycarbonylamino)ethylamino]ethyl isothiocyanate[37] and final acid-promoted Boc deprotection provided **5**, which was isolated as the dihydrochloride salt.

Scheme 2. Syntheses of monosaccharides **5** (2a) and **6** (2b).



Reagents and conditions (2a). a) Hexanoic anhydride, DMAP, DMF, Ar, rt, 4 h, 55%; b) *tert*-butyl *N*-(2-mercaptoethyl)carbamate, Cs_2CO_3, DMF, Ar, overnight, 85%; c) 1:1 TFA-DCM, quant.; d) 2-[*N,N*-bis(2-(*N*-*tert*-butoxyaminocarbonyl)ethylamino]ethyl isothiocyanate, Et_3N, DCM, Ar, overnight, 50%; e) 1:1 TFA-DCM, rt, 1 h, quant. **Reagents and conditions (2b).** a)

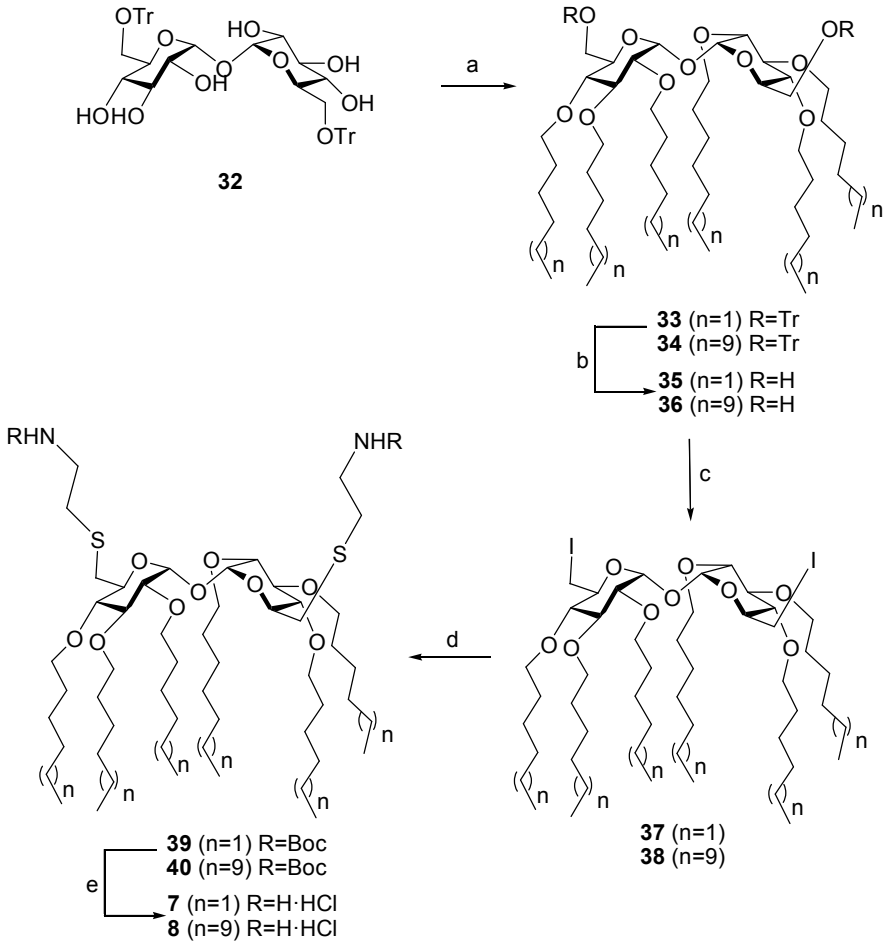
hexanoic anhydride, DMAP, DMF, Ar, 4 h, 55% b) 3-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine, Si-BPA·Cu^{+}, 9:1 $H_2O/^{t}BuOH$, 85 ºC, 36 h. 78%; c) 1:1 TFA/H_2O, rt, 1 h, quant; d) 2-(*N*-*tert*-butoxyaminocarbonyl)ethyl isothiocyanate, Et_3N, DCM, overnight, 52%;e) 1:1 TFA/H_2O, rt, 1 h, quant.

The cationic amphiphile **6** was obtained in good yield from azide **12** following a divergent synthetic strategy in which the hydrophobic and cationic domains are sequentially installed onto the glucopyranoside scaffold. Acylation of **12** with hexanoic anhydride and *N,N*-dimethylaminopyridine (DMAP) in DMF afforded triester **28** (Scheme 2b) that was reacted with 3-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine[38] in the presence of silica based particles incorporating bis(pyridyl)amine (BPA) Cu(I) chelating agent[39] to give the triazol adduct **29** in 78% yield. The use of the solid-supported catalyst has proven advantageous even in multi-CuAAC ligation strategies, highly simplifying the purification step to a simple filtration process.[40] Acid hydrolysis of the Boc protecting groups in **29** provided the corresponding triamine **30**, which was next reacted with 2-(*N*-*tert*-butoxycarbonylamino)ethyl isothiocyanate[41] to give bis(thiourea) **31**. Final hydrolysis of the Boc protecting groups, led to the target compound **6**.

Syntheses of trehalose-derived cationic glycolipids. The strategies implemented for the preparation of the α,α'-trehalose amino glycolipids **7**-**11** parallel those above commented for the corresponding ether- (**1**-**4**) or ester-type (**5**-**7**) methyl α-D-glucopranoside counterparts. Thus, compounds **7** and **8** were obtained in good overall yield in an efficient five-step synthesis starting from 6,6'-di-*O*-trityl-α,α'-trehalose **32**[42] after alkylation of the six secondary hydroxyl groups (**33** and **34**), trityl cleavage with *p*-toluenesulfonic acid in DCM-MeOH (**35** and **36**), Garegg's iodination of the primary hydroxyls (**37** and **38**), nucleophilic displacement of the iodines with

Boc-protected cysteamine (**39** and **40**) and hydrolysis of the carbamate groups (Scheme 3). The hexanoylated analogue **9** was similarly obtained from 6,6'-dideoxy-6,6'-diiodo-α,α'-trehalose **41**[43] by esterification of the secondary hydroxyls (**42**), incorporation of the Boc-protected cysteamine substituents at the primary positions (**43**) and final deprotection (Scheme 4).

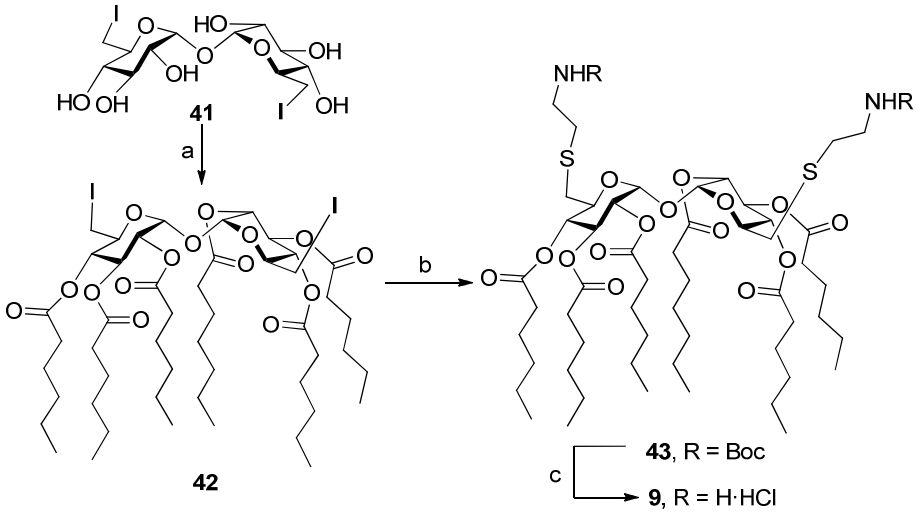
Scheme 3. Syntheses of trehalose derivatives **7** and **8**.



Reagents and conditions. a) Hexyl Bromide, NaH, DMF, overnight, 92% or 1-Bromotetradecane, NaH, DMF, 50 ºC, 77%; b) p-Toluenesulfonic acid,1:1 DCM-MeOH, rt, 4 h, 48% for **35** and 47% for **36**); c) I_2, PPh_3, imidazole, toluene, 70 ºC, 3 h, 94% for **37** and 96% for

38; d) $HS(CH_2)_2NHBoc$, Cs_2CO_3, DMF, 60 ºC, overnight, 85% for **39** and 99% for **40**; e) 1:1 TFA/DCM, rt, 15 min, quant. for **7**, 98% for **8**.

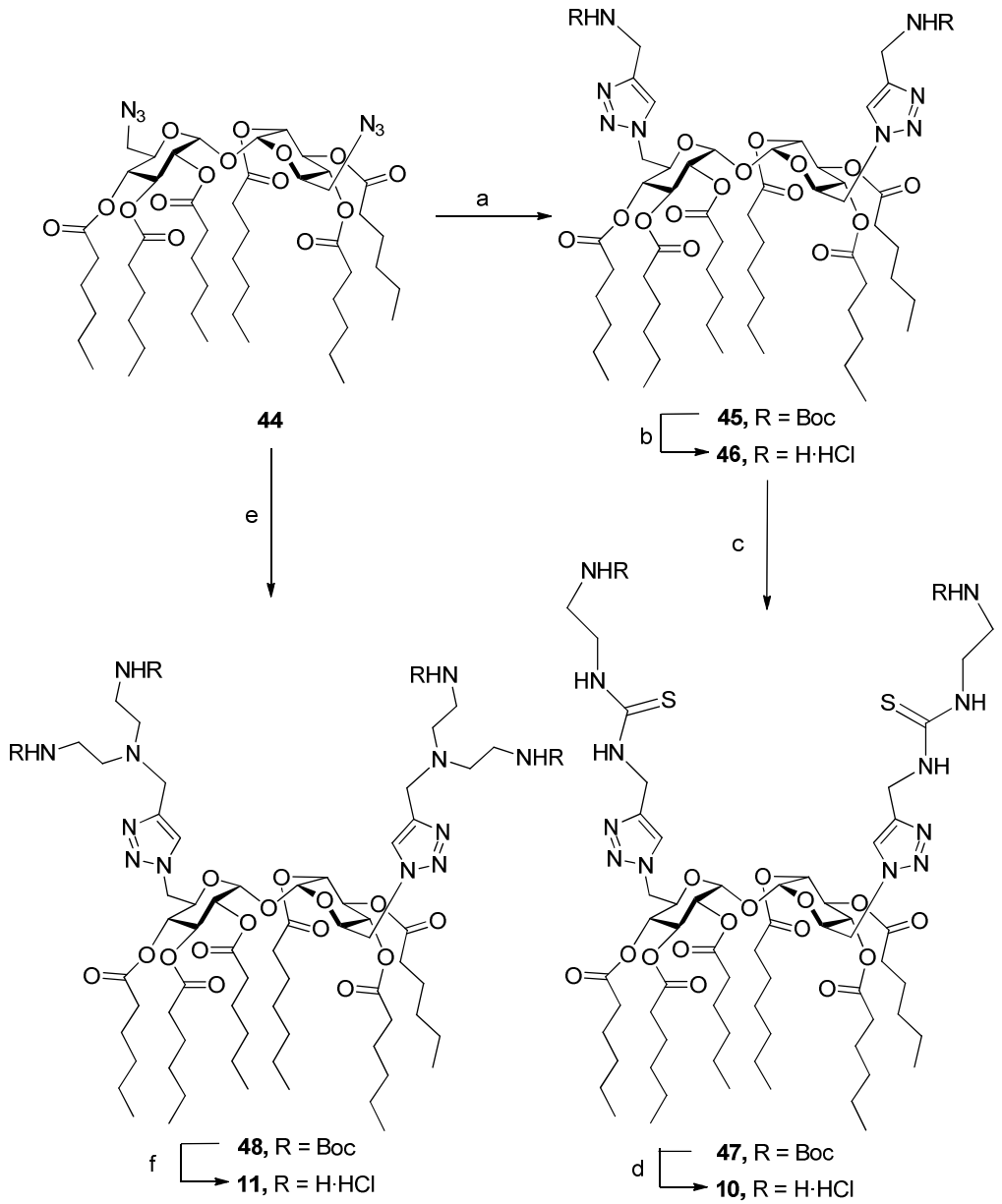
Scheme 4. Synthesis of trehalose derivative **9**.



Reagents and conditions. a) $HS(CH_2)_2NHBoc$, Cs_2CO_3, DMF, 60 ºC, 24 h, 58%; b) 1:1 TFA/DCM, rt, 15 min, quant.

The cationic trehalose amphiphiles **10** and **11** were prepared starting from the common diazide precursor **44**, readily accessed by nucleophilic displacement of the iodine in **42** with sodium azide, by CuAAC ligation using the silica-supported Si-BPA-Cu^+ catalyst. Thus, sequential reaction of **44** with *N*-Boc-propargylamine (**45**), carbamate hydrolysis (**46**), thiourea-coupling with Boc-protected 2-aminoethyl isothiocyanate (**47**) and final Boc removal yielded the diaminoethylthioureido adduct **10**. Alternatively, the CuAAC coupling of **44** with 3-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine (**48**) followed by carbamate hydrolysis afforded compound **11** (Scheme 5)**.**

Scheme 5. Syntheses of trehalose derivatives **10** and **11**.



Reagents and conditions. a) *N*-*tert*-butoxycarbonylpropargylamine, Si-BPA·Cu^{+}, 9:1 H_2O/^{t}BuOH, 24 ºC, 36 h, quant; b) 1:1 TFA/H_2O, rt, 1 h, 97%; c) *tert*-butyl-N-(2-isothiocyanoethyl)carbamate, Et_3N, DCM, overnight, 67%; d) 1:1 TFA/H_2O, rt, 1 h, quant; e) 3-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine, Si-BPA·Cu^{+}, 9:1 H_2O/^{t}BuOH, reflux, 36 h, 91%; f) 1:1 TFA/H_2O, rt, 1 h, quant.

TLR4 modulation in HEK-Blue cells.

Cationic amphiphiles **1**-**11** were first screened for their capacity to interfere with TLR4 activation and signaling on HEK-Blue cells. HEK-Blue cells are stably transfected with TLR4, MD-2, and CD14 genes. In addition, these cells stably express an optimized alkaline phosphatase gene engineered to be secreted (sAP), placed under the control of a promoter inducible by several transcription factors such as NF-κB and AP-1.[29] This reporter gene allows monitoring the activation of TLR4 signal pathway by endotoxin. Compounds **5**, **9**-**11** were inactive in stimulating TLR4 signal when provided alone while inhibited in a dose-dependent way the LPS-stimulated TLR4 signal (Table 1). Compounds **1**-**4** and **6**-**8** resulted inactive or with very low activity both as agonists and antagonists.

Table 1. TLR4 antagonist activity of cationic glycolipids **5**, **9**-**11** on HEK-Blue cells, HEK293 hMD-2/hTLR4 and HEK293 mMD-2/mTLR4 stimulated with *E. coli* O55:B5 LPS (10 nM).

	$IC_{50}(\mu M)$		
Compound	HEK-Blue	HEK293 hMD-2·hTLR4	HEK293 mMD-2·mTLR4
5	3.7±0.4	3.9±1.5	3.3±1.2
9	1.3±0.1	1.4±0.3	0.8±0.2
10	5.0±1.0	0.6±0.02	0.6±0.03
11	0.6±0.05	0.2±0.02	0.2±0.03

The lack of significant activity of all compounds bearing C_6 or C_{14} ether-linked lipophilic chains, namely compounds **1**-**4** and **7**-**8**, strongly suggests that the presence of ester-type linkages at the hydrophobic domain is a primary structural requirement to elicit LPS-antagonist behavior in cationic glycolipids. From the acylated sub-library all compounds are active with the exception of compound **6**, meaning that the cationic head also has an impact on the TLR4 antagonist

activity Among these, the trehalose-based disaccharides **9-11**, bearing more compact cationic headgroups, showed higher potency as TLR4 antagonists than monosaccharide **5**. The observed trend points to a positive relationship between well-ordered facial amphiphilicity and TLR4 antagonist activity of cationic glycolipids. In agreement with this, disaccharide **11** with six hexanoyl chains and six protonable amino groups oriented towards opposite faces in a rather compact arrangement (Figure 3) proved to be the most active TLR4 antagonist. Compounds **5**, **9**, **10**, **11** were further tested for their toxicity by a standard MTT viability test and all resulted non toxic or with very low toxicity in the concentration range used to test their activity (Supp. Info.).

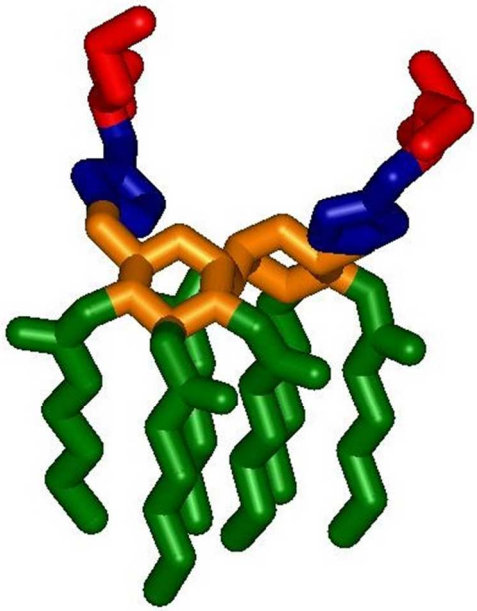


Figure 3. 3D molecular model of compound **11** (cationic headgroups in red, triazol linkers in blue, α,α'-trehalose scaffold in orange, hexanoyl chains in green) evidencing its compact facial amphiphilic character. Hydrogens have been omitted for the sake of clarity.

Activity on HEK293 cells transfected with human and murine MD-2·TLR4.

Biologically active cationic glycolipids **5**, **9**, **10** and **11** were further examined for their capacity to stimulate or to inhibit LPS-induced TLR4 activation and signaling in HEK293 cells

transfected with human or murine TLR4 and MD-2 and a dual luciferase reporter gene (Figure 4).

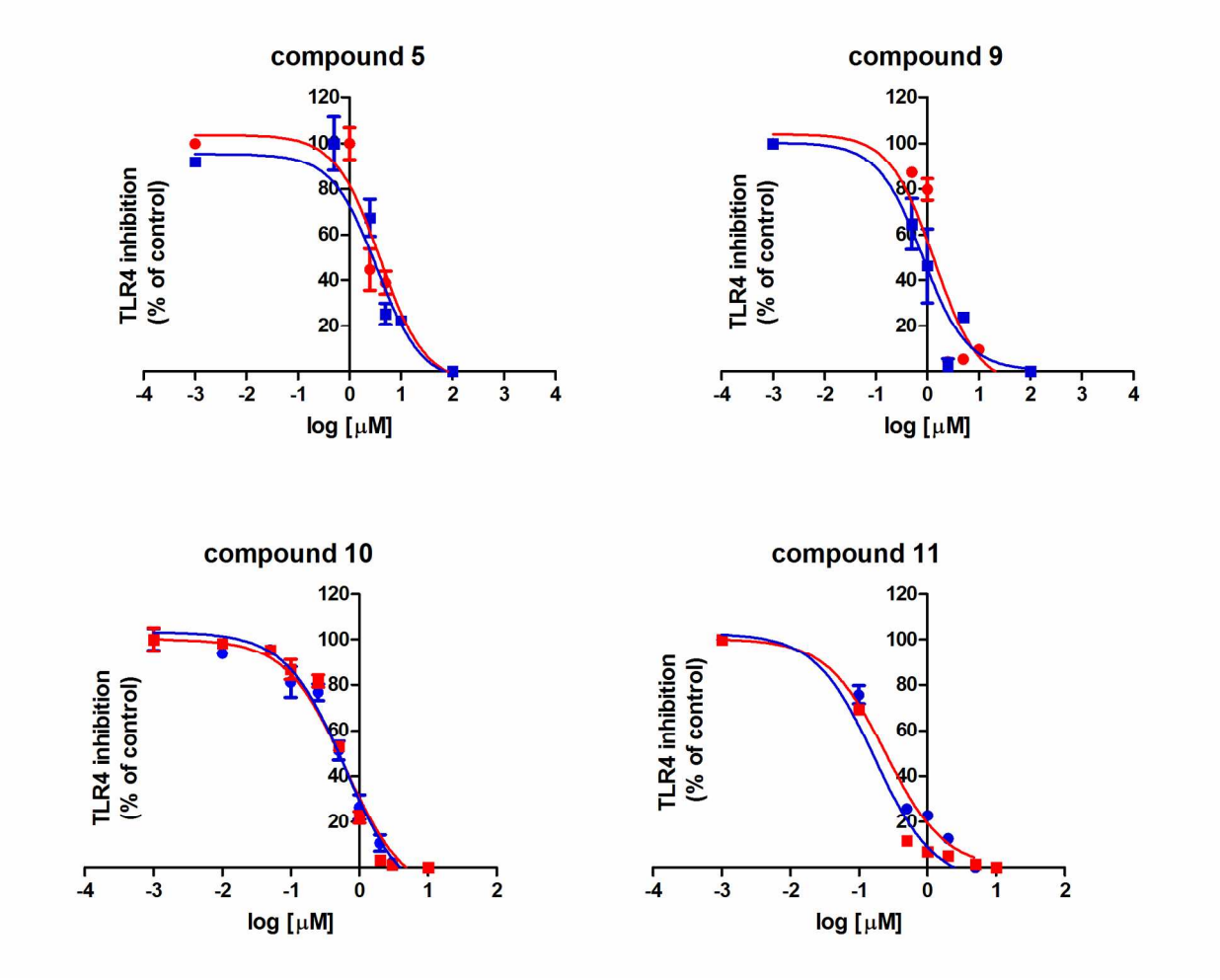


Figure 4. Dose-dependent inhibition of LPS-stimulated TLR4 activation by synthetic glycolipids. HEK293 cells transfected with human MD-2·TLR4 (red line) or murine MD-2/TLR4 (blue line), were treated with increasing concentrations of compounds and stimulated with LPS (5 nM). Normalized data are representative of three independent experiments.

In the absence of LPS, none of the cationic glycolipids stimulate TLR4 signaling (no agonist activity) in cells transfected with hMD-2·hTLR4 or mMD-2·mTLR4. Conversely, in the presence of LPS all compounds were able to inhibit human and murine MD-2·TLR4 activation in a

concentration-dependent manner. The antagonist potency (IC_{50} values, Table 1) of **5**, **9**, **10** and **11** was similar in cells transfected with human and mouse receptors and also very similar to the activity found in HEK-Blue cells (Table 1). In cells transfected with hMD-2·hTLR4 or mMD-2·mTLR4, triacylated monosaccharide **5** and hexacylated disaccharide **9** were less active (IC_{50} = 3.3-3.9 μM and 0.8-1.4 μM, respectively) than disaccharides **10** and **11** (IC_{50} = 0.6 μM and 0.2 μM, respectively). The high potency of trehalose-derived glycolipids **9** and **11** in inhibiting both mouse and human MD-2·TLR4 signals is reminiscent of the activity of synthetic Eritoran that has potent TLR4 antagonist activity in all species.[16, 44] In contrast, natural lipid IVa is agonist on murine and antagonist on human TLR4.[45]

Experiments on murine macrophages.

The activity of compounds **5**, **9** and **11** on LPS-induced TLR4 signaling in bone marrow-derived murine macrophages (BMDM) was subsequently tested. Compounds **5** and **9** showed very low/no activity in activating TLR4 or in inhibiting LPS-stimulated TLR4 signal (Supp. Info.), while compound **11 (**Figure 5**)** gave a concentration-dependent inhibition of IL-6 and TNF-α production at concentrations of 1 and 2 μM (Figure 5), while at 0.1 and 0.5 μM concentrations had no effect or a slightly potentiating effect.

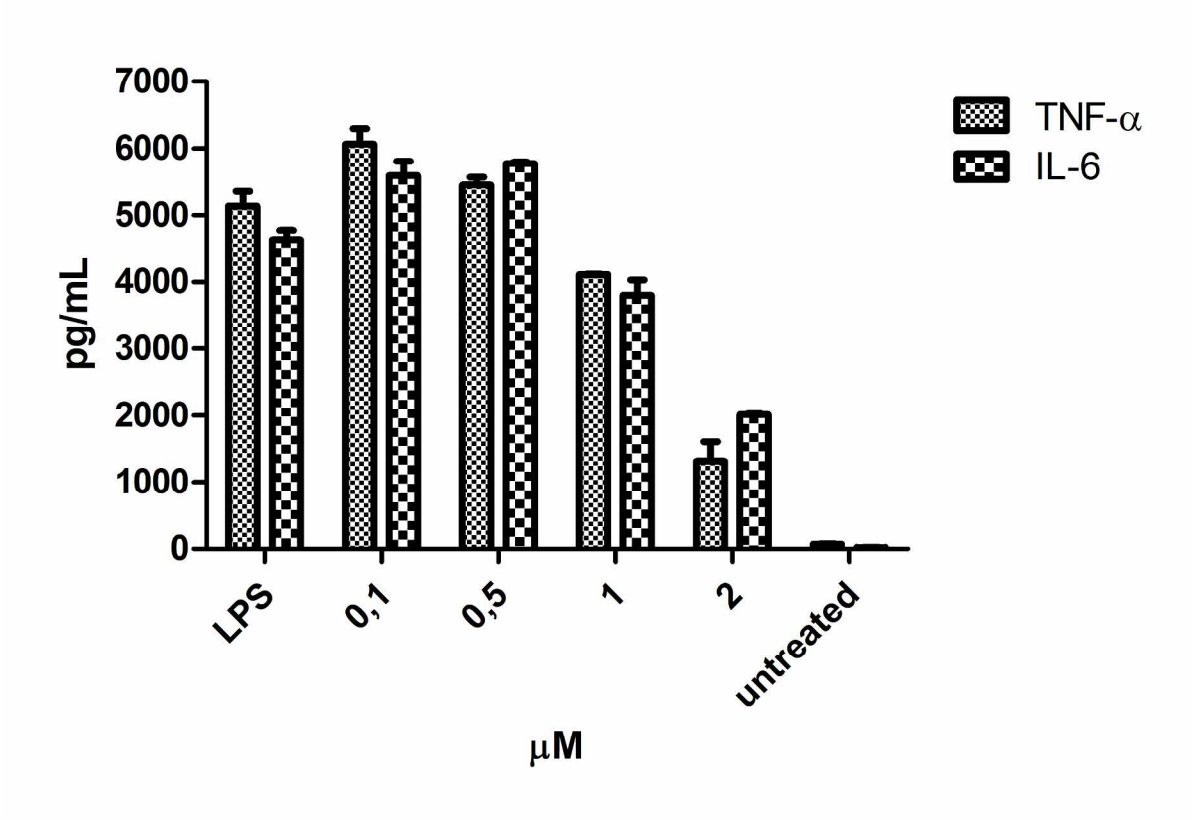


Figure 5. BMDM were treated with increasing concentrations (0-2 μM) of compound **11** in RPMI+FBS 10% in the presence of LPS, administered 1 hour after the treatment with synthetic compound. The ELISA assay, performed after overnight incubation, revealed a dose dependent decrease of LPS-induced IL-6 and TNF-α production. Cytokines productions in cells not treated with LPS are reported as negative controls.

Aggregation properties of cationic glycolipids

Cationic lipids can spontaneously assembly into liposomal structures. Some cationic liposomes induce the expression of pro-apoptotic and pro-inflammatory mediators through the activation of cellular pathways.[17] However, it is still controversial if the initiation of inflammatory and apoptotic response is due to specific interaction with receptors at the cell surface or to the internalization of liposomes into cells through endocytosis and endocytosis-like mechanisms followed by interaction of charged compounds with downstream effectors. In the particular case

of cationic lipids modulating TLR4 activity, there is no information available on whether aggregated species or single molecules are the active species. To have an insight in this question, we have determined the critical micelle concentrations (CMC) of cationic glycolipids **5**, **9**, **10** and **11** using an established technique based on the polarity-induced change in the fluorescence spectra of pyrene when incorporated in micelle formed by synthetic compounds (Table 2).[46] In all cases the CMC values of active compounds are higher than the corresponding IC_{50} values as TLR4 antagonists, suggesting that the biologically active species are prevalently single molecules in solution.

Table 2. Critical micelle concentrations (CMC) in aqueous environment of TLR4 antagonists.

Compound	CMC (μM)
5	59.7 ± 7.4
9	97.7 ± 10.0
10	10.9 ± 2.1
11	350.5 ± 70.5

Synthesis and biological activity of gold nanoparticles coated with glycolipid 11

LPS is an amphiphilic molecule and it is mainly present in the form of micellar aggregates in a concentration range that is relevant for its biological activity. It has been recently proposed that the multiple presentation of LPS or other TLR4 ligands on nanoparticles could be a way to potentiate the agonist or antagonist action of chemicals by mimicking the 3D-structure of LPS aggregates.[47, 48] Moreover, the possibility of *in vitro* and/or *in vivo* delivery based on NP is considered advantageous for clinical development, as it can maximize the effectiveness of drugs, minimize the invasiveness and toxic side effects and speed up the clinical development program.

To test the suitability of this approach in the case of cationic glycolipids, the preparation and biological evaluation of gold nanoparticles coated with the most active compound **11** has been undertaken. Colloidal gold nanoparticles were synthesized by a variation of the Brust-Schiffrin method[49] and coated by surface adsorption with the α,α'-trehalose derivative **11**. The resulting cationic glycolipid-modified nanoparticles (**11**-NP) kept a small size with low-polydispersity. Most interestingly, they retained the biological activity without apparent increase in cytotoxicity. These results represent a proof of concept of the possibility of developing nanoparticulate systems based on cationic glycolipids as modulators of TLR4 signaling pathway, an approach previously demonstrated only for LPS itself.[47] Based on these data, the prepared **11**-NP nanoparticles were engaged in an *in vivo* assay. The inhibitory activity of the nanoparticles coated with glycolipid **11** was tested on HEK293 cells. They exhibited strong LPS-inhibitory activity already at very low concentrations (Figure 6 A, B) on human as well as murine MD-2·TLR4 receptor complex.

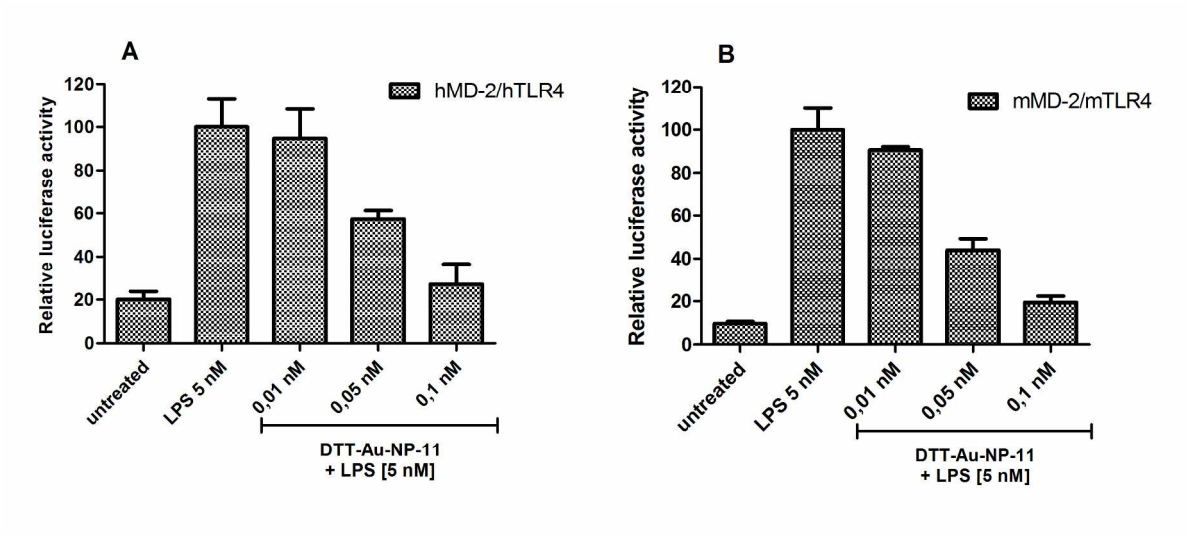


Figure 6. Dose-dependent TLR4 antagonism in HEK293 cells treated with DTT-Au-NP-11. HEK293 cells were transfected with NF-κB-dependent luciferase and constitutive Renilla luciferase reporter plasmids as well as with (A) human or (B) murine MD-2 and TLR4 plasmids.

The indicated amount of the DTT-Au-NP-11 was added to the cells, followed one hour later by stimulation with LPS. Luciferase activity was measured 16 hours later.

***In vivo* activity**

Since the synthetized compounds exhibited strong inhibition of the LPS-induced MD-2·TLR4 activation *in vitro*, we next wished to determine their inhibitory potential *in vivo*. All four selected candidates (**5**, **9**, **10** and **11**) potently inhibited the LPS-induced immune activation in C57/Bl6 mice (Figure 7). The strongest inhibition was exhibited by compound **11**, which totally abolished LPS-induced immune activation. Results in Figure 8 show that compounds **5**, **9**, **10** and **11** are strong MD-2·TLR4 inhibitors not only *in vitro* but also *in vivo*.

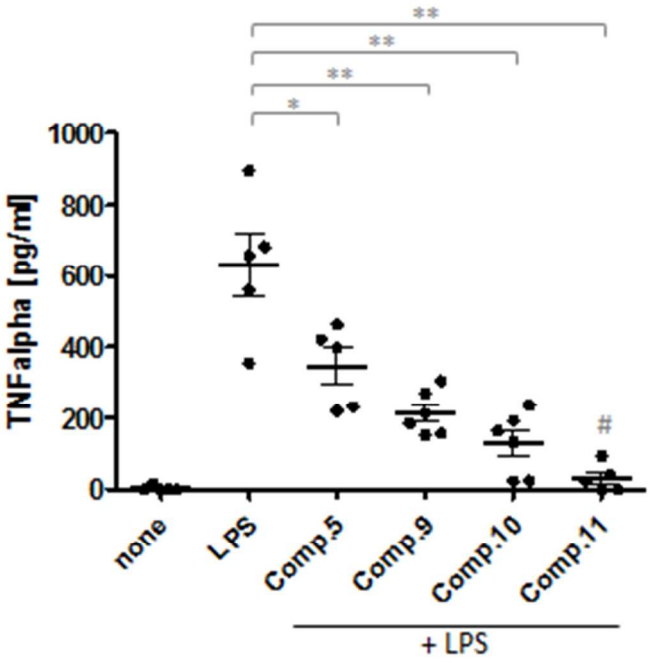


Figure 7. *in vivo* activity of cationic amphiphiles. C57/Bl6 mice were injected i.p. with the indicated compounds ($2 \cdot 10^{-7}$ mol/mouse), followed 1 hour later by i.p. injection of LPS ($1 \cdot 10^{-9}$ mol/mouse). Three hours later sera were collected and TNF-α concentration was determined by

ELISA. (data shown with mean and standard error, N=5-6) two-tailed t-test (* p<0,1; ** p<0,01 – compared to »LPS«) (# not significant – compared to »none«)

Conclusions and perspectives

New cationic amphiphiles **1-11** based on monosaccharide and disaccharide glycolipid scaffolds have been designed, synthesized and their capacity to modulate TLR4 activation and signaling evaluated. Glucose-based compound **5** and trehalose-based compounds **9-11** were active in inhibiting the LPS-triggered TLR4 activation and signaling in HEK cells with IC_{50} values ranging from about 5 to 0.2 μM. The cell toxicity of these molecules is low, and the potency of TLR4 antagonism is in the same order of magnitude of the best synthetic TLR4 antagonists so far tested by us[29] and other groups.[30] The active molecules inhibited the TLR4 signal in HEK cells transfected with human and murine MD-2·TLR4 complexes with very similar potency, similarly to the very efficient TLR4 antagonist Eritoran and differently from the natural TLR4 antagonist lipid IVa that has species-specific activity (antagonist on human and agonist on mice MD-2·TLR4). Compounds **5**, **9**, **10** and **11** significantly inhibited LPS-triggered IL-6 production in mice, with compound **11** showing the most evident effect. Since these compounds are active *in vitro* and *in vivo*, and show low toxicity, they represent good leads for the development of drugs targeting TLR4 signaling.

The biological evaluation of active compounds compared to inactive, structurally related monosaccharides (compounds **1**-**4**, **6**) and disaccharides (compounds **7** and **8**), suggests some general structure-activity relationships in this type of compounds: i) the presence of acyl lipophilic chains at the hydrophobic domain seems to be a primary requisite since all compounds with ether bonds are inactive, ii) the higher *in vitro* and *in vivo* activity of compound **11** suggests that the trehalose scaffold favors the biological activity, probably by providing a well-ordered

facial amphiphilic character. Compound **11** adsorbed on gold nanoparticles (**11**-NP) is still active as TLR4 antagonist in cells, but the high toxicity of these functionalized nanoparticles could prevent their use *in vivo* as carriers for TLR4 antagonists.

Finally, the experimentally determined CMC values for cationic glycolipids **5**, **9**-**11** are one order of magnitude higher than the corresponding IC_{50} as TLR4 antagonists, suggesting that they are active as single monomers in solution. This very likely means that specific molecular interaction with CD14 and MD-2 receptors regulate the TLR4 activity of these compounds. It will be important in the future to define more precisely the molecular determinants of the interaction with CD14 and MD-2 receptors to allow a structure-based rational design of cationic TLR4 modulators.

EXPERIMENTAL SECTION

General Synthetic Methods. Optical rotations were measured at 20 ± 2 °C in 1-dm tubes on a Jasco P-2000 polarimeter. Ultraviolet-visible (UV) spectra were recorded in 1-cm tubes on a Jasco V-630 spectrophotometer. Infrared (IR) spectra were recorded on a Jasco ATR MIRacle™ spectrophotometer. ^{1}H (and ^{13}C NMR) spectra were recorded at 300 (75.5), 500 (125.7) MHz with Bruker 300 AMX, 500 AMX and 500 DRX. 1D TOCSY, 2D COSY, HMQC and HSQC experiments were used to assist on NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with *Sílica Gel 60 F_{254} Merck* with visualization by UV light and by charring with 10% H_2SO_4. With preparative purposes, column chromatography was carried out on Silica Gel 60 F_{254} Merck. ESI mass spectra were recorded in the positive mode on Bruker Daltonics *esquire6000*™ ion-trap mass spectrometer. Typically, samples were dissolved in appropriate volumes of deionised water to give samples concentration of 50 mg/L. Aliquots were mixed with 25:25:1 deionised water-methanol-trifluoroacetic acid, generally in a ratio of

1:10, to give a total volume of 200 μL. Samples were introduced by direct infusion, using a Cole-Parmer syringe at a flow rate of 2 μL/min. Ions were scanned between 300 and 3000 Da with a scan speed of 13000 Da/s at unit resolution using resonance ejection at the multipole resonance of one-third of the radio frequency (Ω = 781.25 kHz). Calibration of the mass spectrometer was performed using ES tuning mix (Hewlett Packard). Recorded data were processed using Bruker Daltonics Esquire 5.0 software (Bruker). Elemental analyses were carried out at the Instituto de Investigaciones Químicas (Sevilla, Spain) using an elemental analyser *Leco CHNS-932* or *Leco TruSpec CHN*.

Methyl 6-azido-6-deoxy-α-D-glucopyranoside,[28] methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside,[34] methyl 6-deoxy-6-iodo-α-D-glucopyranoside,[35] 2-[*N,N*-bis(2-(*N*-*tert*-butoxyaminocarbonyl)ethylamino]ethyl isothiocyanate,[37] 6,6'-dideoxy-6,6'-diiodo-α,α'-trehalose 41,[43] 6,6'-di-*O*-trityl-α,α'-trehalose 32,[42] *N*-(2-isothiocyanoethyl) *tert*-butylcarbamate,[41] and 3-bis[2-*tert*-butoxycarbonylamino)ethyl]propargylamine[38] were obtained according to described procedures. Purity of all final compounds was confirmed to be ≥95% by ^{1}H NMR and combustion microanalysis.

Methyl 6-Azido-6-deoxy-2,3,4-tri-*O*-hexyl-α-D-glucopyranoside (13). To a solution of methyl 6-azido-6-deoxy-α-D-glucopyranoside (0.40 m, 1.82 mmol) in dry DMF (9 mL), NaH (0.65 g, 16.42 mmol) was added, under Ar atmosphere, at 0 ºC. Then 1-bromohexane (2.3 mL, 16.42 mmol) was added dropwise and the reaction mixture was stirred overnight at rt. The solvent was evaporated and the residue diluted in DCM (10 mL) and washed with H_2O (2 x 10 mL). The organic layer was dried ($MgSO_4$), concentrated and purified by column chromatography (1:30 EtOAc-cyclohexane). Yield 48% (0.50 m, 1.06 mmol). R_f = 0.34 (1:18 EtOAc-cyclohexane); $[\alpha]_D$ = +90.0 (*c* 1.0, DCM); IR: ν_{max} = 2099, 1094 cm^{-1} ; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.74

(d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 3.79, 3.64, 3.58 (m, 6 H, OCH_2), 3.67 (m, 1 H, H-5), 3.64-3.24 (m, 2 H, H-3, H-6a), 3.39 (s, 3 H, OCH_3), 3.35 (m, 1 H, H-6b), 3.23 (dd, 1 H, $J_{2,3}$ = 9.6 Hz, H-2), 3.12 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 1.55 (m, 6 H, CH_2), 1.27 (m, 18 H, CH_2), 0.86 (m, 9 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 98.0 (C-1), 81.3 (C-3), 80.7 (C-2), 78.8 (C-4), 73.7, 73.6, 71.8 (OCH_2), 70.2 (C-5), 55.2 (OCH_3), 51.5 (C-6), 31.7-22.6 (CH_2), 14.3 (CH_3); ESI MS: *m/z* = 965.8 $[2M + Na]^+$, 494.6 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{49}N_3O_5$: C, 63.66; H, 10.47; N, 8.91. Found: C, 63.72; H, 10.97; N, 8.33.

Methyl 6-Amino-6-deoxy-2,3,4-tri-*O*-hexyl-α-D-glucopyranoside Hydrochloride (1). To a solution of **13** (0.15 g, 0.32 mmol) in degassed MeOH (12 mL), Pd/C (10%, 0.06 g) was added and the mixture was stirred under H_2 atmosphere (1 bar) at rt until complete consumption of the starting compound. The catalyst was filtered off, the solution concentrated, and the resulting residue purified by column chromatography (1:9 EtOAc-cyclohexane → 45:5:3 EtOAc-EtOH-H_2O) and freeze-dried from 0.1 N HCl solution. Yield 87% (0.12 g, 0.26 mmol) ; $[\alpha]_D$ = +83.0 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, CD_3OD): δ = 4.82 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1), 3.81, 3.64, 3.56 (m, 6 H, OCH_2), 3.65 (m, 1 H, H-5), 3.53 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 3.41 (s, 3 H, OCH_3), 3.25 (dd, 1 H, H-2), 3.11 (dd, 1 H, $J_{6a,6b}$ = 13.1 Hz, $J_{5,6a}$ = 2.7 Hz, H-6a), 3.01 (t, 1 H, $J_{4,5}$ = 9.3 Hz, H-4), 2.84 (dd, 1 H, $J_{5,6b}$ = 8.5 Hz, H-6b), 1.56 (m, 6 H, CH_2), 1.33 (m, 18 H, CH_2), 0.91 (m, 9 H, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 99.1 (C-1), 82.6 (C-3), 81.8 (C-2), 81.1 (C-4), 74.5, 74.3, 72.2 (OCH_2), 70.7 (C-5), 55.8 (OCH_3), 42.9 (C-6), 33.0-24.2 (CH_2), 14.4 (CH_3); ESIMS: *m/z* = 891.7 $[2 M + H]^+$, 446.5 $[M + H]^+$. Anal. Calcd for $C_{25}H_{51}NO_5 \cdot HCl$: calcd. C, 62.28; H, 10.87; N, 2.91. found: C, 62.33; H, 10.69; N, 2.70.

Methyl 6-Azido-6-deoxy-2,3,4-tri-*O*-tetradecyl-α-D-glucopyranoside (14). To a solution of methyl 6-azido-6-deoxy-α-D-glucopyranoside (0.28 g, 1.30 mmol) in dry DMF (4 mL), NaH

(0.55 mg, 13.60 mmol) was added, under Ar atmosphere, at 0 ºC. Then 1-bromotetradecane (4.1 mL, 13.60 mmol) was added dropwise, and the reaction mixture was stirred overnight at 55 ºC. The solvent was evaporated and the residue diluted in DCM (10 mL) and washed with H_2O (2 x 10 mL). The organic layer was dried ($MgSO_4$), concentrated and purified by column chromatography (cyclohexane → 1:40 EtOAc-cyclohexane). Yield 42% (0.45 g, 0.26 mmol) . R_f= 0.25 (1:15 EtOAc-cyclohexane); $[\alpha]_D$ = +52.1 (*c* 1.0, DCM); IR: ν_{max} = 2100, 1096 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.76 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1), 3.79, 3.64, 3,58 (m, 6 H, OCH_2), 3.69 (m, 1 H, H-5), 3.66-3.44 (m, 2 H, H-3, H-6a), 3.39 (s, 3 H, OCH_3), 3.37 (dd, 1 H, $J_{6a,6b}$ = 13.3 Hz, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.25 (dd, 1 H, $J_{2,3}$ = 9.4 Hz, H-2), 3.13 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 1.57 (m, 6 H, CH_2), 1.27 (m, 66 H, CH_2), 0.86 (m, 9 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 98.0 (C-1), 81.3 (C-3), 80.7 (C-2), 78.8 (C-4), 73.7, 73.6, 71.8 (OCH_2), 70.2 (C-5), 55.2 (OCH_3), 51.5 (C-6), 31.9-22.7 (CH_2), 14.1 (CH_3); ESI MS: *m/z* = 830.8 $[M + Na]^+$. Anal. Calcd for $C_{49}H_{97}N_3O_5$: C, 72.81; H, 12.10; N, 5.20. Found: C, 72.89; H, 11.87; N, 4.88.

Methyl 6-Amino-6-deoxy-2,3,4-tri-*O*-tetradecyl-α-D-glucopyranoside Hydrochloride (2). To a solution of **14** (0.15 g, 0.19 mmol) in THF (16.4 mL), TPP (0.10 g, 0.39 mmol) was added and the mixture was stirred at rt for 15 min. Then NH_4OH (1.6 mL) was added, the solution was stirred overnight at 50 ºC and then concentrated. The resulting residue was purified by column chromatography (EtOAc→9:1 DCM-MeOH) and freeze-dried from 10:1 H_2O-HCl 0.1 N solution. Yield 82% (0.13 mg, 0.16 mmol); $[\alpha]_D$ = +43.2 (*c* 1.0, DCM); IR: ν_{max} = 1092 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.79 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1), 3.78, 3.60, 3.55 (m, 7 H, OCH_2, H-3), 3.73 (m, 1 H, H-5), 3.46 (s, 3 H, OCH_3), 3.27 (m, 1 H, H-6^a), 3.22 (dd, 1 H, $J_{1,2}$ = 9.5 Hz, H-2), 2.95 (m, 2 H, H-4, H-6b), 1.55 (m, 6 H, CH_2), 1.25 (m, 66 H, CH_2), 0.87 (t, 9 H, $^3J_{H,H}$ = 6.6 Hz, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ =98.0 (C-1), 81.0 (C-3), 80.4 (C-2), 79.9 (C-4), 77.2

(C-5), 73.7, 73.5, 71.8 (OCH_2), 67.1 (C-6), 55.9 (OCH_3), 31.9-22.7 (CH_2), 14.0 (CH_3); ESI MS: *m/z* = 783.0 $[M + H]^+$. Anal. Calcd for $C_{49}H_{99}NO_5 \cdot HCl$: calcd. C, 71.88; H, 12.31; N, 1.71; found: C, 71.64; H, 12.26; N, 1.49.

Methyl 4,6-*O*-(4-Methoxybenzylidene)-2,3-di-*O*-hexyl-α-D-glucopyranoside (15). To a solution of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside (0.80 g, 2.57 mmol) in DMF (8 mL), NaH (0.62 g, 15.42 mmol) was added. Then, 1-bromohexane (1.8 mL, 12.85 mmol) was added dropwise and the resulting mixture was stirred at 60 ºC overnight. After cooling to rt, the reaction was quenched with MeOH (2 mL) and the solution was stirred for 20 min. Solvents were then evaporated and the residue was diluted with EtOAc (50 mL) and citric acid (satd aq soln, 40 mL). The layers were separated and the organic phase was washed with H_2O (3 x 40 mL), dried ($MgSO_4$), evaporated, and purified by column chromatography (1:9 EtOAc-cyclohexane). Yield 57% (0.71 g, 0.15 mmol); R_f = 0.44 (1:9 EtOAc-cyclohexane); $[\alpha]_D$ = +37.2 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39, 6.87 (2 d, 4 H, A_2X_2, aromatics), 5.49 (s, 1 H, PhC*H*), 4.78 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.25 (dd, 1 H, $J_{6a,6b}$ = 9.6 Hz, $J_{5,6a}$ = 4.5 Hz, H-6a), 3.80 (s, 3 H, $PhOCH_3$), 3.76 (m, 1 H, H-5), 3.72 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3) 3.72-3.57 (m, 5 H, H-6b, OCH_2), 3.47 (t, 1 H, $J_{4,5}$ = 9.2 Hz, H-4), 3.42 (s, 3 H, OCH_3), 3.34 (dd, 1 H, H-2), 1.66-1.49 (m, 4 H, CH_2), 1.41-1.18 (m, 12 H, CH_2), 0.88, 0.84 (2 t, 6 H, $^3J_{H,H}$ = 6.5 Hz, CH_3);^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.9-113.5 (Ph), 101.2 (Ph*C*H), 99.1 (C-1), 81.9 (C-4), 80.4 (C-2), 78.2 (C-3), 73.4, 72.2 (OCH_2), 69.0 (C-6), 62.4 (C-5), 55.6 (OCH_3), 31.7-22.6 (CH_2), 14.0 (CH_3); ESI MS: *m/z*: 519.5 $[M + K]^+$, 503.6 $[M + Na]^+$. Anal. Calcd for $C_{27}H_{44}O_7$: C, 67.47; H, 9.23. Found: C, 67.54; H, 9.30.

Methyl 4,6-*O*-(4-Methoxybenzylidene)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (16).[31] To a solution of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside (0.80 g, 2.57 mmol) in

DMF (8 mL), NaH (60% suspension in mineral oil, 0.62 g, 15.42 mmol) was carefully added in small portions. Tetradecylbromide (3.8 mL, 12.85 mmol) was added dropwise and the resulting mixture was stirred at 60 ºC overnight. After cooling to rt, the mixture was quenched with methanol (2 mL) then the solution was stirred for 20 min. Solvents were then evaporated and the residue was diluted with EtOAc (50 mL). Citric acid (satd aq soln, 40 mL) was added, the layers were separated, the organic layer was washed with water (3 x 40 mL), dried (Na_2SO_4) and evaporated. Flash column chromatography on silica gel of the residue (1:9 EtOAc-cyclohexane) afforded **16.** Yeld 74% (1.33 g, 1.89 mmol). R_f = 0.65 (1:9 EtOAc-cyclohexane); $[\alpha]_D$ = +23.3 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40, 6.87 (2 d, 4 H, A_2X_2, aromatics), 5.49 (s, 1 H, PhC*H*), 4.78 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.25 (dd, 1 H, $J_{6a,6b}$ = 9.6, $J_{5,6a}$ = 4.6 Hz, H-6a), 3.80 (s, 3 H, OCH_3), 3.76 (m, 1 H, H-5), 3.75-3.59 (m, 6 H, H-3, H6b, OCH_2), 3.47 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.42 (s, 3 H, OCH_3), 3.34 (dd, 1 H, $J_{2,3}$ = 9.3, H-2), 1.70-1.49 (m, 4 H, CH_2), 1.22 (bs, 44 H, CH_2), 0.87 (t, 6 H, $^3J_{H,H}$ = 5.8 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.1, 130.0, 127.3, 113.5 (Ph), 101.2 (Ph*C*H), 99.1 (C-1), 81.9 (C-4), 80.4 (C-2), 78.2 (C-3), 73.4, 72.3 (OCH_2), 69.0 (C-6), 62.4 (C-5), 55.2 (OCH_3), 31.9-22.7 (CH_2), 14.1 (CH_3). ESI MS: *m/z*: 503.6 $[M + Na]^+$, 519.5 $[M + K]^+$.

Methyl 2,3-Di-*O*-hexyl-4-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (17). To a solution of 15 (0.71 g, 1.48 mmol) in a mixture of Et_2O-DCM (2:1, 75 mL), under Ar atmosphere, 1 M $LiAlH_4$ in THF (7.4 mL, 7.40 mmol) and $AlCl_3$ (0.81 g, 6.06 mmol) in Et_2O (25 mL) were added dropwise, and the resulting mixture was refluxed for 4 h. After cooling to rt, EtOAc (250 mL) and H_2O (250 mL) were added. The organic layer was washed with brine (2 x 200 mL), dried ($MgSO_4$), evaporated and purified by column chromatography (1:2 EtOAc-cyclohexane). Yield 83% (0.60 g, 1.22 mmol); R_f = 0.26 (1:2 EtOAc-cyclohexane); $[\alpha]_D$ = +76.2 (*c* 1.0, DCM); IR:

ν_{max}= 1076, 1035cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ =7.26, 6.87 (A_2X_2 system, 4 H, aromatics), 4.81 (d, 1 H, $^2J_{Ha,Hb}$ = 10.8 Hz, PhC*Ha*), 4.75 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.57 (d, 1 H, PhC*Hb*), 3.86 (m, 1 H, OCH_2), 3.79 (s, 3 H, $PhOCH_3$), 3.77-3.64 (m, 3 H, H-6a, H-6b, OCH_2), 3.68 (m, 1 H, H-3), 3.67-3.53 (m, 3 H, OCH_2, H-5), 3.42 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.37 (s, 3 H, OCH_3), 3.26 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, H-2), 1.75 (bs, 1 H, OH), 1.64-1.57 (m, 4 H, CH_2), 1.37-1.28 (m, 12 H, CH_2), 0.88 (t, 6 H, $^3J_{H,H}$ = 7.0 Hz, CH_3), 0.87 (t, 6 H, $^3J_{H,H}$ = 6.8 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ =159.3-113.8 (Ph), 98.0 (C-1), 81.6 (C-3), 80.8 (C-2), 77.1 (C-4), 74.5 ($PhCH_2$), 73.7, 71.7 (OCH_2), 70.6 (C-5), 62.0 (C-6), 55.2, 55.0 (OCH_3), 31.8-22.6 (CH_2), 14.0 (CH_3); ESI MS: *m/z*: 505.6 $[M + Na]^+$, 521.5 $[M + K]^+$. Anal. Calcd for $C_{27}H_{46}O_7$: C, 67.19; H, 9.61; found: C, 66.92; H, 9.67.

Methyl 6-Deoxy-2,3-di-*O*-hexyl-6-iodo-4-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (19). To a solution of **17** (0.60 g, 1.23 mmol) in toluene (25 mL) TPP (0.49 g, 1.85 mmol) and imidazole (0.25 g, 3.70 mmol) were added. Iodine (0.49 g, 1.73 mmol) was added in portions and the resulting solution was stirred at 70 ºC for 5 h. After cooling at rt, $NaHCO_3$ sat. (25 mL) was added and the mixture was stirred for 5 min. Additional iodine was added and the mixture was stirred for 10 min. Then $Na_2S_2O_3$ aq. 10% was added to remove the iodine excess. The organic layer was separated, washed with H_2O (3 x 25 mL), dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (1:4 EtOAc-cyclohexane). Yield 94% (0.69 g, 1.16 mmol) . R_f= 0.52 (1:4 EtOAc-cyclohexane); $[\alpha]_D$ = +84.3 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26, 6.88 (A_2X_2 system, 4 H, , aromatics), 4.87 (d, 1 H, $^2J_{Ha,Hb}$ = 10.6 Hz, PhC*Ha*), 4.77 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1), 4.62 (d, 1 H, PhC*Hb*), 3.87 (m, 1 H, CH_2), 3.80 (s, 3 H, $PhOCH_3$), 3.75-3.64 (m, 2 H, H-3, OCH_2), 3.63-3.50 (m, 2 H, OCH_2), 3.46 (dd, 1 H, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6a}$ = 2.4 Hz, H-6a), 3.42 (s, 3 H, OCH_3), 3.37 (m, 1 H, H-5), 3.35-3.26 (m, 2

H, H-2, H-6b), 3.24 (t, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 1.66-1.55 (m, 4 H, CH_2), 1.39-1.25 (m, 12 H, CH_2), 0.88 (t, 6 H, $^3J_{H,H} = 6.5$ Hz, CH_3), 0.87 (t, 6 H, $^3J_{H,H} = 6.8$ Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ =159.4-113.9 (Ph), 98.0 (C-1), 81.2 (C-3), 81.1 (C-2), 80.8 (C-4), 74.9 ($PhCH_2$), 73.7, 71.7 (OCH_2), 69.2 (C-5), 55.4, 55.2 (OCH_3), 31.7-22.6 (CH_2), 14.0 (CH_3), 8.1 (C-6); ESI MS: *m/z*: 631.3 $[M + K]^+$, 615.4 $[M + Na]^+$. Anal. Calcd for $C_{27}H_{45}IO_6$: C, 54.73; H, 7.65; found: C, 54.88; H, 7.71.

Methyl 6-(2-*tert*-Butoxycarbonylaminoethylthio)-2,3-di-*O*-hexyl-4-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (21). To a suspension of **19** (0.69 g, 1.16 mmol) and Cs_2CO_3 (0.53 g, 1.62 mmol) in dry DMF (10 mL), *tert*-butyl (2-mercaptoethyl)carbamate (0.27 mL, 1.62 mmol,) was added and the reaction mixture was stirred at 60 ºC, under Ar atmosphere, for 24 hours. The mixture was concentrated, EtOAc (25 mL) and water (25 mL) were added then the organic layer was separated, washed with H_2O (3 x 25 mL), dried ($MgSO_4$), filtered, concentrated and the residue was purified by column chromatography (1:6→1:4 EtOAc-cyclohexane). Yield 99% (0.74 g, 1.15 mmol). R_f = 0.17 (1:6 EtOAc-cyclohexane); $[\alpha]_D$ = +64.2 (*c* 1.0, DCM); IR: ν_{max}= 1714 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.24, 6.87 (2 d, 4 H, $^3J_{H,H} = 8.7$ Hz, aromatics), 4.98 (bs, 1 H, NH), 4.84 (d, 1 H, $^2J_{Ha,Hb} = 10.8$ Hz, PhC*Ha*), 4.74 (d, 1 H, $J_{1,2} = 3.4$ Hz, H-1), 4.54 (d, 1 H, PhC*Hb*), 3.85 (m, 1 H, OCH_2), 3.79 (s, 3 H, $PhOCH_3$), 3.74-3.52 (m, 3 H, OCH_2), 3.71 (m, 1 H, H-5), 3.64 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 3.40 (s, 3 H, OCH_3), 3.31-3.25 (m, 4 H, H-2, H-4, CH_2N), 2.84 (dd, 1 H, $J_{6a,6b} = 13.9$ Hz, $J_{5,6a} = 2.6$ Hz, H-6a), 2.71 (t, 2 H, $^3J_{H,H} = 6.4$ Hz, CH_2S), 2.57 (dd, 1 H, $J_{5,6b} = 7.5$ Hz, H6b), 1.65-1.55 (m, 4 H, CH_2), 1.44 (s, 9 H, CMe_3), 1.36-1.24 (m, 12 H, CH_2), 0.88, 0.87 (2 t, 6 H, $^3J_{H,H} = 6.6$ Hz, CH_3);^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.3 (CO), 130.5-113.9 (Ph), 97.8 (C-1), 81.6 (C-3), 80.9 (C-2), 80.1 (C-4), 79.3 (*C*Me_3), 74.7 (CH_2Ph), 73.7, 71.7 (2 CH_2), 70.7 (C-5), 55.3, 55.0 (2 OCH_3), 39.7 (CH_2N), 33.6

(C-6), 33.5 (CH_2S), 31.7-29.6 (CH_2), 28.4 (CMe_3), 26.9-22.6 (CH_2), 14.0 (CH_3); ESI MS: *m/z*: 664 $[M + Na]^+$, 680 $[M + K]^+$. Anal. Calcd for $C_{34}H_{59}NO_8S$: C, 63.62; H, 9.26; N, 2.18; S, 5.00; found: C, 63.73; H, 9.21; N, 1.98; S, 4.86.

Methyl 6-(2-Aminoethylthio)-2,3-di-*O*-hexyl-α-D-glucopyranoside Hydrochloride (3). Treatment of **21** (0.35 g, 0.55 mmol) with 1:1 TFA-DCM (2 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **3.**. Yield quant. (0.20 g, 0.54 mmol). R_f= 0.45 (45:5:3 EtOAc-EtOH-H_2O); $[\alpha]_D$ = +74.4 (*c* 1.0, MeOH); IR: ν_{max}= 3404, 1109 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ = 4.80 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1), 3.74 (t, 1 H, $^3J_{H,H}$ = 6.8 Hz, OCH_2), 3.67-3.51 (m, 4 H, H-5, OCH_2), 3.42 (s, 3 H, OCH_3), 3.40 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 3.29 (t, 1 H, $J_{4,5}$ = 9.3 Hz, H-4), 3.23 (dd, 1 H, H-2), 3.16 (t, 2 H, $^3J_{H,H}$ = 6.8 Hz, CH_2N), 2.99 (dd, 1 H, $J_{6a,6b}$ = 14.4 Hz, $J_{5,6a}$ = 2.3 Hz, H-6a), 2.90 (m, 2 H, CH_2S), 2.72 (dd, 1H, $J_{5,6a}$ = 8.0 Hz, H6b), 1.60-1.52 (m, 4 H, CH_2), 1.42-1.31 (bs, 12 H, CH_2), 0.90, 0.89 (2 t, 6 H, $^3J_{H,H}$ = 6.6 Hz, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 99.1 (C-1), 82.6 (C-3), 81.6 (C-2), 74.6 (OCH_2), 74.0 (C-4), 73.4 (C-5), 72.2 (OCH_2), 55.5 (OCH_3), 40.0 (CH_2N), 34.2 (C-6), 33.0-31.1 (CH_2), 31.1 (CH_2S), 26.9, 26.8, 23.7 (CH_2), 14.4 (CH_3); ESI MS: *m/z*: 422.5 $[M - Cl]^+$. Anal. Calcd for $C_{21}H_{43}NO_5S$·HCl: calcd. C, 55.06; H, 9.68; N, 3.06; S, 7.00; found: C, 54.87; H, 9.45; N, 2.79; S, 6.78.

Methyl 2,3-di-*O*-Tetradecyl-α-D-glucopyranoside (18). Methyl 4,6-*O*-(4-methoxybenzylidene)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (0.70 g, 0.98 mmol) was dissolved in a mixture of Et_2O-DCM (2:1, 15 mL), under Ar atmosphere. $AlCl_3$ (0.81 g, 6.06 mmol) in Et_2O (15 mL) were added dropwise and the resulting mixture was refluxed for 4 h. After cooling to rt, EtOAc (150 mL) and H_2O (150 mL) were added and the layers separated. The organic layer was washed with brine (3 × 100 mL), dried (Mg_2SO_4) and evaporated. Column chromatography of the residue (1:1 EtOAc-cyclohexane) afforded **18**. Yield 87% (0.50 mg, 0.85

mmol); R_f = 0.47 (1:1 EtOAc-cyclohexane); $[\alpha]_D$ = +24.7 (*c* 1.0, DCM); IR: ν_{max} 3362, 2953, 1468 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.78 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 3.94-3.43 (m, 9 H, H-3, H,4 H-5, H-6a, H-6b, 2 OCH_2), 3.41 (s, 3 H, OCH_3), 3.26 (dd, 1 H, $J_{2,3}$ = 9.2 Hz, H-2), 1.69-1.48 (m, 4 H, CH_2), 1.25 (bs, 44 H, CH_2), 0.87 (t, 6 H, $^3J_{H,H}$ = 6.9 Hz, CH_3);^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 98.1 (C-1), 81.0, 80.6 (C-2, C-3), 73.6 (C-4), 71.3, 70.7 (OCH_2), 70.5 (C-5), 62.5 (C-6), 55.2 (OCH_3), 31.9-22.6 (CH_2), 14.1 (CH_3); ESI MS: *m/z*: 625.6 $[M + K]^+$, 609.8 $[M + Na]^+$. Anal. Calcd for $C_{35}H_{70}O_6$: C, 71.62; H, 12.02; found: C, 71.38; H, 11.76.

Methyl 2,3-di-*O*-Tetradecyl-6-deoxy-6-iodo-α-D-glucopyranoside (20). To a solution of **18** (0.49 g, 0.84 mmol) in toluene (17 mL) triphenylphosphine (0.33 g, 1.26 mmol) and imidazole (0.17 g, 2.52 mmol) were added. Iodine (0.33 g, 1.17 mmol) was added in portions and the resulting solution was stirred at 70 ºC for 3 h. After cooling at rt, $NaHCO_3$ sat. (20 mL) was added and the mixture was stirred for 5 min. Additional iodine was added up to turn brown the organic phase and the mixture was stirred for 10 min. Then, aqueous 10% $Na_2S_2O_3$ was added to remove the iodine excess. The organic layer was separated, washed with H_2O (3 x 20 mL), dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography (1:9 EtOAc-cyclohexane). Yield 91% (0.03 g, 0.76 mmol; R_f = 0.30 (1:9 EtOAc-cyclohexane); $[\alpha]_D$ = +44.1 (*c* 1.0, DCM); IR :ν_{max}= 1041, 722 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.81 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 3.91 (m, 1 H, OCH_2), 3.66-3.49 (m, 5 H, H-6a, OCH_2, H-3), 3.47 (s, 3 H, OCH_3), 3.45 (ddd, 1 H, $J_{4,5}$ = 9.2 Hz, $J_{5,6a}$ = 6.8 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 3.31 (m, 1 H, H-4) 3.29 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, H-2), 3.26 (dd, 1 H, $J_{6a,6b}$ = 11.2 Hz, H-6b), 2.41 (d, 1 H, $J_{4,OH}$ = 2.3 Hz, OH-4), 1.61-1.54 (m, 4 H, CH_2), 1.25 (bs, 44 H, CH_2), 0.88 (t, 6 H, $^3J_{H,H}$ = 6.9 Hz, CH_3);^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 98.2 (C-1), 80.7, 80.5 (C-2, C-3), 73.8 (C-4), 73.6, 71.2 (OCH_2), 69.7 (C-5),

55.5 (OCH_3), 31.9-22.7 (CH_2), 14.1 (CH_3), 7.2 (C-6); ESI MS: *m/z*: 735.6 $[M + K]^+$, 719.7 $[M + Na]^+$. Anal. Calcd for $C_{35}H_{69}IO_5$: C, 60.33; H, 9.98. Found: C, 59.89; H, 9.72.

Methyl 6-(2-*tert*-Butoxycarbonylaminoethylthio)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside (22). To a suspension of **20** (0.53 g, 0.77 mmol) and Cs_2CO_3 (0.35 g, 1.07 mmol) in DMF (7 mL), *tert*-butyl (2-mercaptoethyl)carbamate (0.18 mL, 1.07 mmol) was added and the reaction mixture was stirred at 60 ºC, under Ar atmosphere for 24 h. The mixture was concentrated then EtOAc (20 mL) and H_2O (20 mL) were added and the organic layer was separated, washed with H_2O (3 x 20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (1:3 EtOAc-cyclohexane) affording **22**. Yield 95% (0.54 g,0.73 mmol); R_f = 0.40 (1:3 EtOAc-cyclohexane); $[\alpha]_D$ = +50.4 (*c* 1.0, DCM); IR: ν_{max}= 3631, 1698cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.99 (bs, 1 H, NH), 4.77 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 3.90 (m, 1 H, OCH_2), 3.72 (ddd, 1 H, $J_{4,5}$ = 9.3 Hz, $J_{5,6a}$ = 7.5 Hz, $J_{5,6b}$ = 2.6 Hz, H-5), 3.64-3.46 (m, 3 H, OCH_2), 3.48 (t, 1 H, $J_{2,3} = J_{3,4} =$ 9.3 Hz, H-3), 3.43 (s, 3 H, OCH_3), 3.36 (t, 1 H, H-4), 3.31 (m, 2 H, CH_2N), 3.27 (dd, 1 H, H-2), 2.98 (dd, 1 H, $J_{6a,6b}$ = 14.1 Hz, H-6a), 2.71 (m, 3 H, H6b, CH_2S), 2.63 (bs, 1 H, OH-4), 1.65-1.50 (m, 4 H, CH_2), 1.43 (s, 9 H, CMe_3), 1.24 (bs, 44 H, CH_2), 0.87 (t, 6 H, $^3J_{H,H}$ = 7.0 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 156 (CO), 97.9 (C-1), 80.9 (C-3), 80.6 (C-2), 79.4 (CMe_3), 73.5 (OCH_2), 72.4 (C-4), 71.2 (OCH_2), 71.0 (C-5), 55.2 (OCH_3), 39.6 (CH_2N), 33.5 (C-6), 33.4 (CH_2S), 31.8-29.3 (CH_2), 28.4 (CMe_3), 26.1-22.6 (CH_2), 14.0 (CH_3); ESI MS: *m/z*: 784.7 $[M + K]^+$, 768.8 $[M + Na]^+$. Anal. Calcd for $C_{42}H_{83}NO_7S$: C, 67.60; H, 11.21; N, 1.88; S, 4.30. Found: C, 67.45; H, 10.90; N, 1.62; S, 4.24.

Methyl 6-(2-Aminoethylthio)-2,3-di-*O*-tetradecyl-α-D-glucopyranoside Hydrochloride (4). Treatment of **22** (0.25 g, 0.33 mmol) with 1:1 TFA-DCM (2 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **4**. Yield quant (0.22 g, , 0.33 mmol). Column chromatography

of the residue (4:1 EtOAc-cyclohexane→EtOAc→45:5:3 EtOAc-EtOH-H_2O). R_f = 0.25 (45:5:3 EtOAc-EtOH-H_2O); $[\alpha]_D$ = +41.3 (*c* 0.9, 9:1 DCM-MeOH); IR: ν_{max}= 3406, 722 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.11 (bs, 2 H, NH_2HCl), 4.78 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 3.87 (m, 1 H, OCH_2), 3.74 (ddd, 1 H, $J_{4,5}$ = 9.4 Hz, $J_{5,6b}$ = 6.7 Hz, $J_{5,6a}$ = 2.7 Hz, H-5), 3.66-3.54 (m, 3 H, OCH_2), 3.49 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.9 Hz, H-3), 3.42 (s, 3 H, OCH_3), 3.38 (t, 1 H, H-4), 3.27 (dd, 1 H, H-2), 3.18 (t, 2 H, $^3J_{H,H}$ = 6.2 Hz, CH_2N), 2.97 (dd, 1 H, $J_{6a,6b}$ = 14.2 Hz, H-6a), 2.90 (m, 2 H, CH_2S), 2.74 (dd, 1H, H6b), 1.60-1.54 (m, 4 H, CH_2), 1.25 (bs, 44 H, CH_2), 0.87 (t, 6 H, $^3J_{H,H}$ = 6.9.0 Hz, CH_3);^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 97.9 (C-1), 80.9 (C-3), 80.5 (C-2), 73.7 (OCH_2), 72.1 (C-4), 71.3 (OCH_2), 70.7 (C-5), 55.2 (OCH_3), 38.7 (CH_2N), 33.2 (C-6), 31.9 (CH_2), 30.3 (CH_2S), 30.3-22.7 (CH_2), 14.1 (CH_3); ESI MS: *m/z*: 646.7 $[M - HCl]^+$. Anal. Calcd for $C_{37}H_{75}NO_5S{\cdot}HCl{\cdot}2H_2O$: C, 61.85; H, 11.22; N, 1.95; S, 4.46. Found: C, 61.79; H, 11.03; N, 2.01; S, 4.43.

Methyl 6-(2-*tert*-Butoxycarbonylaminoethylthio)-2,3,4-tri-*O*-hexanoyl-α-D-glucopyranoside (24). To a solution of **23** (0.49 g, 0.83 mmol) in dry DMF (7.5 mL), Cs_2CO_3 (0.38 g, 0.16 mmol) and *tert*-butyl *N*-(2-mercaptoethyl)carbamate (196 μL, 1.16 mmol) were added, under Ar atmosphere, and the reaction mixture was stirred at 60 ºC for 48 h. The reaction mixture was concentrated and the crude product was dissolved in DCM (10 mL) and washed with H_2O (2 x 15 mL). The organic phase was dried ($MgSO_4$), filtered, concentrated, and the residue was purified by flash column chromatography (1:4 EtOAc-cyclohexane). Yield 85% (0.45 mg, 0.70 mmol). R_f= 0.38 (1:3 EtOAc-cyclohexane); $[\alpha]_D$ = +62.1 (*c* 1.0, DCM); IR: ν_{max} = 2958, 1747, 1701cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 5.46 (t, 1 H, $J_{2,3} = J_{3,4}$= 10 Hz, H-3), 4.95 (t, 1 H, $J_{4,5}$= 10.0 Hz, H-4), 3.41 (s, 3 H, OCH_3), 4.90 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.83 (dd, 1 H, H-2), 3.90 (dt, 1 H, $J_{5,6b}$ = 8.0 Hz, H-5), 3.27 (q, 2 H, $J_{H,H}$ = 6.0 Hz, C*H*$_2$N), 2.8-2.5 (m, 4 H, C*H*$_2$S, H-

6[a], H-6b), 2.52 (m, 2 H, H-6[a], H-6b), 2.31 (m, 6 H, H-2_{Hex},), 1.54 (m, 6 H, H-3_{Hex}), 1.41 (s, 9 H, CMe_3), 1.25 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.86 (t, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.9-172.0 (CO ester), 155.7 (CO carbamate) 96.7 (C-1), 71.5 (C-4), 70.9 (C-2), 69.3 (C-3), 69.9 (C-5), 55.1 (OMe), 39.58 (*C*H_2N), 33.7 (*C*H_2S), 33.0 (C-6), 34.0 (C-2_{Hex}), 24.5 (C-3_{Hex}), 28.2 (C*Me*$_3$), 22.1 (C-5_{Hex}), 13.6 (C-6_{Hex}); ESI MS: *m/z* = 670.4 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{57}NO_{10}S$: C, 59.32; H, 8.87; N, 2.16; S, 4.95 found: C, 59.45; H, 8.93; N, 2.31; S, 4.71.

Methyl 6-(2-Aminoethylthio)-2,3,4-tri-*O*-hexanoyl-α-D-glucopyranoside Hydrochloride (25). Treatment of **24** (0.29 g, 0.44 mmol) with 1:1 TFA-DCM (6 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **25**. Yield quant. (0.27 mg, 0.44 mmol) ; $[\alpha]_D$ =+72.0 (*c* 0.7, EtOAc); IR: ν_{max} = 2962, 1749 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.05 (bs, 3 H, N*H*$_3$), 5.28 (t, 1 H $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 4.94 (t, 1 H, $J_{4,5}$ = 9.7 Hz, H-4), 4.88 (m, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.82 (dd, 1 H, H-2), 3.8 (m, 1 H, H-5), 3.34 (s, 1 H, OCH_3), 2.93 (bs, 2 H, C*H*$_2$N), 2.78 (m, 2 H, C*H*$_2$S), 2.73 (dd, 1 H, $J_{6a,6b}$ = 14.0 Hz, $J_{5,6a}$ = 2.7, H-6[a]), 2.62 (dd, 1 H, $J_{5,6b}$ = 7.7 Hz, H-6b), 2.26 (m, 6 H, H-2_{Hex}), 1.44 (m, 6 H, H-3_{Hex}), 1.21 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.82 (t, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 172-171 (CO), 95.98 (C-1), 71.2 (C-4), 70.6 (C-2), 69.4 (C-3), 69.1 (C-5), 55.1 (OMe), 39.1 (*C*H_2N), 32.5 (C-6), 30.5 (*C*H_2S), 33.1 (C-2_{Hex}), 31.1 (C-4_{Hex}), 24 (C-3_{Hex}), 22.1(C-2_{Hex}), 14.4 (C-6_{Hex}); ESIMS: *m/z* = 548.3 $[M + H]^+$. Anal. Calcd for $C_{27}H_{50}ClNO_8S$·HCl: C, 55.51; H, 8.63; N, 2.40; S, 5.49; found: C, 55.37; H, 8.62; N, 2.26; S, 5.27.

Methyl 6-(2-(*N*'-(2-(*N,N*-di-(2-(*N*-*tert*-Butoxycarbonylamino)ethyl)amino)ethyl)thioureido)-ethylthio)-2,3,4-tri-*O*-hexanoyl-α-D-glucopyranoside (27). To a solution of **25** (0.12 g, 0.20 mmol) and Et_3N (56 μL, 0.4 mmol) in DCM (6 mL), 2-[*N,N*-bis(2-(*N*-*tert*-butoxyaminocarbonyl)ethylamino]ethyl isothiocyanate (0.09 g, 0.24 mmol) was added and the

reaction mixture was stirred, under Ar atmosphere, at rt for 48 h. The reaction mixture was washed with aqueous diluted HCl (2 x 20 mL), dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (1:1→ 3:1 EtOAc-cyclohexane). Yield 50% (0.08 g, 0.10 mmol). R_f = 0.2 (1:1 EtOAc-cyclohexane); $[\alpha]_D$ = + 71.6 (*c* 1.0, DCM); IR: ν_{max} = 2959, 1748, 1685 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.46, 6.96 (2 bs, 2 H, N*H*CS), 5.46 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3), 4.96 (t, 1 H, $J_{4,5}$ = 9.8 Hz, H-4), 4.90 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.88 (bs, 2 H, N*H*Boc), 4.84 (dd, 1 H, H-2), 3.93 (bd, 1 H, H-5), 3.77 (q, 2 H, NHC$H_2$$CH_2$N), 3.52 (bs, 2 H, SCH_2CH_2N), 3.42 (s, 3 H, OCH_3), 3.11 (q, 4 H, NCH_2CH_2NHBoc), 2.82 (t, 2 H, $J_{H,H}$ = 7.0 Hz, $NHCH_2CH_2N$), 2.77-2.56 (m, 4 H, H-6a, H-6b, SCH_2), 2.50 (bs, 4 H, NCH_2CH_2NHBoc), 2.37-2.1 (m, 6 H, $H\text{-}2_{Hex}$), 1.57 (m, 6 H, $J_{H,H}$ = 7 Hz, $H\text{-}3_{Hex}$), 1.43 (s, 9 H, CMe_3), 1.27 (m, 12 H, $H\text{-}4_{Hex}$, $H\text{-}5_{Hex}$), 0.87 (t, 9 H, $H\text{-}6_{Hex}$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 182.1 (CS), 172.0-171.0 (CO ester), 155.2 (CO carbamate), 96.6 (C-1), 70.9 (C-4), 70.1 (C-2), 69.9 (C-5), 69.6 (C-3), 55.8 (NCH_2CH_2NHBoc), 55.5 (OMe), 54.0 (SCH_2), 44.1 ($NHCH_2CH_2N$), 42.4 (SCH_2CH_2N), 39.4 (NCH_2CH_2NHBoc), 33.1 (C-6), 32.6 ($NHCH_2CH_2N$), 34.1 ($C\text{-}2_{Hex}$), 31.2 ($C\text{-}5_{Hex}$), 28.4 (CMe_3) 24.5 ($C\text{-}3_{Hex}$), 22.1 ($C\text{-}5_{Hex}$), 13.9 ($C\text{-}6_{Hex}$); ESI MS: *m/z* = 958.6 $[M + Na]^+$ Anal. Calcd for $C_{44}H_{81}N_5O_{12}S_2$: C, 56.44; H, 8.72; N, 7.48; S, 6.85; found: C, 56.61; H, 8.89; N, 7.21; S, 6.60.

Methyl 6-(2-(N'-(2-(*N*,*N*-Bis-(2-aminoethyl)amino)ethyl)thioureido)ethylthio)-2,3,4-tri-*O*-hexanoyl-α-D-glucopyranoside Trihydrochloride (5). Treatment of **27** (0.15 g, 0.16 mmol) with 1:1 TFA-DCM (2 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **5**. Yield quant. (0.13 g, 0.16 mmol). $[\alpha]_D$ = + 46.8 (*c* 0.85, MeOH); IR: ν_{max} = 2958, 1747, 1675 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.98 (bs, 4 H, NHCS, NH_2HCl), 7.60 (bs, 1 H, NHCS), 5.47 (t, $J_{3,4} = J_{2,3}$= 9.5 Hz, 1 H, H-3), 4.99 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 4.91 (dd, 1 H, $J_{1,2}$ = 4 Hz,

H-1), 4.85 (dd, 1 H, H-2), 3.96 (td, 1 H, $J_{5,6b}$ = 7.3 Hz, $J_{5,6a}$ = 2.4 Hz, H-5), 3.73 (bs, 2 H, SCH_2CH_2NHCS), 3.68 (bs, 2 H, $NHCSCH_2CH_2N$), 3.41 (s, 3 H, OCH_3), 3.11 (bs, 4 H, CH_2NH_3Cl), 2.84 (bs, 6 H, $CH_2CH_2NH_3Cl$, SCH_2CH_2NHCS), 2.75 (m, 3 H, H-6a, $NHCSCH_2CH_2N$), 2.66 (dd, 1 H, $J_{6a,6b}$ = 13.8 Hz, H-6b), 2.32-2.19 (m, 6 H, H-2_{Hex}), 1.55 (m, 6 H, H-3_{Hex}), 1.27 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.87 (m, 9 H, H-6_{Hex}); ^{13}C NMR (75 MHz, $CDCl_3$): 173.1, 173.0, 172.6 (CO), 96.6 (C-1), 71.2 (C-4), 70.8 (C-2), 69.6 (C-3), 69.1 (C-5), 55.4 (OCH_3), 53.4 ($NHCSCH_2CH_2N$), 52.0 ($CH_2CH_2NH_2HCl$), 43.1 (SCH_2CH_2NHCS), 41.7 ($NHCSCH_2CH_2N$), 37.6 (CH_2NH_3Cl), 34.1, 34.0 (C-2_{Hex}), 33.0 (C-6), 32.7 (SCH_2CH_2NHCS), 31.2, 31.1 (C-4_{Hex}), 24.5, 24.4 (C-3_{Hex}), 22.2 (C-5_{Hex}), 13.8 (C-6_{Hex}); ESI MS: *m/z* = 736.4 $[M]^+$. Anal. Calcd for $C_{34}H_{65}N_5O_8S_2 \cdot 3HCl$: C, 48.30; H, 8.11; N, 8.28; S, 7.58; found: C, 48.24; H, 8.39; N, 8.15; S, 7.41.

Methyl 6-(4-(2,2-Bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl-6-deoxy)-2,3,4-tri-*O*-hexanoyl-α-D-glucopyranoside (29). To a solution of **28** (0.20 g, 0.39 mmol) and 3-bis[2-*tert*-butoxycarbonylamino)ethyl]propargylamine (0.29 g, 0.85 mmol) in H_2O-^{t}BuOH 9:1 (15 mL), the Cu-supported catalyst Si-BPA·Cu^+ (0.02g) was added and the reaction mixture was refluxed for 36 h at 85 ºC. The catalyst was filtered and the solvent was removed. The residue was purified by column chromatography (1:1→2:1 EtOAc-cyclohexane). Yield 78% (0.25 g, 0.30 mmol). R_f = 0.61 (9:1 DCM-MeOH); $[\alpha]_D$ = +50.5 (*c* 1.0, DCM); IR: ν_{max} = 2957, 2359, 1748, 1703, 734 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (s, 1 H, =CH), 5.49 (t, 1 H, $J_{2,3} = J_{3,4}$ = 10.3 Hz , H-3), 4.89 (bs, 2 H, N*H*Boc), 4.87 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.85 (t, 1 H, $J_{4,5}$ = 10.3 Hz, H-4), 4.81 (dd, 1 H, H-2), 4.53 (dd, 1 H, $J_{6a,6b}$ = 14.0 Hz, $J_{5,6a}$ = 2.6 Hz, H-6a), 4.29 (dd, 1 H, $J_{5,6b}$ = 9.0 Hz, H-6b), 4.17 (ddd, 1 H, H-5), 3.80 (m, 2 H, CH_2-triazole), 3.18 (bs, 4 H, CH_2NHBoc), 3.07 (s, 3 H, OCH_3), 2.55 (t, 4 H, CH_2CH_2NHBoc), 2.27 (m, 6 H, H-2_{Hex}), 1.58

(s, 9 H, CMe_3), 1.44 (m, 6 H, H-3_{Hex}), 1.30 (m, 12 H, H-4_{Hex},H-5_{Hex}), 0.89 (m, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 173.9, 173.5, 173,1 (CO ester), 156.2 (CO carbamate), 144.1 (C-4 triazole), 124.3 (C-5 triazole), 91.4 (C-1), 78.1 (*C*Me_3), 70.6 (C-2), 69.8 (C-4), 69.3 (C-3), 68.0 (C-5), 53.1 (*C*H_2CH_2NHBoc), 50.6 (C-6), 48.3 (CH_2 triazole), 38.4 (*C*H_2NHBoc), 34.1 (C-2_{Hex}), 31.7 (C-4_{Hex}), 28.5 (C*Me*$_3$), 24.6 (C-3_{Hex}), 22.6 (C-5_{Hex}), 13.8 (C-6_{Hex}); ESI MS: *m/z* = 877.5 [M + Na]$^+$. Anal. Calcd for $C_{42}H_{74}N_6O_{12}$: calcd. C, 59.00; H, 8.72; N, 9.83; found: C, 59.09; H, 8.77; N, 9.64.

Methyl 6-Deoxy-6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-2,3,4-tri-*O*-hexanoyl-α-D-glucopyranoside Dihydrochloride (30). Treatment of **29** (0.42 g, 0.49 mmol) with 1:1 TFA-DCM (5 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **30**. Yield quant.(0.35 g, 0.49 mmol). $[\alpha]_D$ = +38.1 (*c* 1.0, MeOH); IR: ν_{max} = 2957, 1748, 1675 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.07 (s, 1 H, =CH), 5.42 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 4.93 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.82 (dd, 1 H, H-2), 4.76 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 4.64 (m, 2 H, H-6a, H-6b), 4.27 (ddd, 1 H, $J_{5,6a}$ = 3.7 Hz, $J_{5,6b}$ = 6.0 Hz, H-5), 3.92 (s, 2 H, CH_2 triazole), 3.26 (s, 3 H, OMe), 3.16 (t, 4 H, $J_{H,H}$ = 6.4 Hz, $CH_2CH_2NH_2$), 2.82 (t, 4 H, $CH_2CH_2NH_2$), 2.48-2.15 (m, 6 H, H-2_{Hex}), 1.58 (m, 6 H, H-3_{Hex}), 1.33 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.92 (m, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 174.2-173.9 (CO), 143.8 (C-4 triazole), 126.9 (C-5 triazole), 98.6 (C-1), 71.8 (C-2), 70.7 (C-3), 70.3 (C-4), 68.9 (C-5), 56.0 (OCH_3), 52.0 ($CH_2CH_2NH_2$), 51.6 (C-6), 47.4 (CH_2 triazole), 38.2 ($CH_2CH_2NH_2$), 34.9, 34.8, 34.7 (C-2_{Hex}), 32.4 , 32.2 (C-4_{Hex}), 25.6, 25.5 (C-3_{Hex}), 23.4 (C-5_{Hex}), 14.2 (C-6_{Hex}); ESI MS: *m/z* = 831.3 [M + TFA + Cl + Cu]$^+$; 717.3 [M + Cu + Cl]$^+$; Anal. Calcd for $C_{32}H_{59}N_6O_8 \cdot 2HCl$: calcd. C, 52.81; H, 8.31; N, 11.55; found: C, 52.69; H, 8.1; N, 11.72.

Dendritic Boc-protected Diminoethyl-bis(thiourea) Glucopyranoside Derivative 31. To a solution of **30** (0.20 g, 0.27 mmol) and Et_3N (115 μL, 0.82 mmol) in DCM (12 mL), *tert*-butyl *N*-(2-isothiocyanoethyl)carbamate (0.17 mg, 0.82 mmol) was added and the mixture was stirred overnight at rt. The reaction mixture was washed with aqueous diluted HCl (3 x 10 mL) and the organic phase was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (3:1 EtOAc-cyclohexane→20:1 DCM-MeOH). Yield 52% (0.15 g, 0.14 mmol). R_f = 0.44 (9:1 DCM-MeOH); $[\alpha]_D$ = +31.7 (*c* 1.0, DCM); IR: ν_{max} = 2959, 1750, 1698, 736 cm^{-1}; UV (DCM): 248 nm (ε_{mM} 47.8). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.62 (s, 1 H, =CH), 7.18, 6.94 (bs, 4 H, N*H*CS), 5.49 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz , H-3), 5.38 (bs, 2 H, NHBoc), 4.82 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.80 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 4.80 (dd, 1 H, H-2), 4.53 (dd, 1 H, $J_{6a,6b}$ = 14.5 Hz, $J_{5,6a}$ = 2.6 Hz, H-6a), 4.37 (dd, 1 H, $J_{5,6b}$ = 8.0 Hz, H-6b), 4.17 (ddd, 1 H, H-5), 3.79 (s, 2 H, CH_2 triazole), 3.62 (bs, 4 H, C$H_2$$CH_2$NHBoc), 3.54 (bs, 4 H, NCH_2CH_2NHCS), 3.31 (m, 4 H, CH_2NHBoc), 3.12 (s, 1 H, OCH_3), 2.69 (bs, 4 H, NC$H_2$$CH_2$NHCS), 2.42-2.13 (m, 6 H, H-2_{Hex}), 1.55 (m, 6 H, H-3_{Hex}), 1.42 (s, 18 H, CMe_3), 1.26 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.87 (t, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 182.7 (CS), 173.0-172.5 (CO ester), 155.5 (CO carbamate), 144.5 (C-4 triazole), 124.4 (C-5 triazole), 96.7 (C-1), 79.8 (*C*Me_3), 70.2 (C-2), 69.6 (C-4), 69.2 (C-3), 67.8 (C-5), 55.4 (OMe), 52.4 (N*C*$H_2$$CH_2$NHCS), 50.7 (C-6), 48.0 (CH_2 triazole), 44.6 (*C*$H_2$$CH_2$NHBoc), 42.1 ($NCH_2$*C*$H_2$NHCS), 40.1 ($CH_2$NHBoc), 34.3 (C-$2_{Hex}$), 30.8 (C-$4_{Hex}$), 28.1 (C*Me*$_3$), 24.2 (C-$3_{Hex}$), 22.6 (C-$5_{Hex}$), 14.1 (C-$6_{Hex}$); ESI MS: *m/z* = 1081.5 [M + Na]$^+$. Anal. Calcd for $C_{48}H_{86}N_{10}O_{12}S_2$: calcd. C, 54.42; H, 8.18; N, 13.22; S, 6.05; found: C, 54.37; H, 7.98; N, 13.28; S, 5.85

Dendritic Diminoethyl-bis(thiourea) Glucopyranoside Dihydrochloride Derivative (6). Treatment of **3**1 (0.12 g, 0.12 mmol) with 1:1 TFA-DCM (2 mL) and freeze-drying from 10:1

H_2O/0.1 N HCl solution afforded **6**. Yield 91%(0.10 g, 0.11 mmol). $[\alpha]_D$ = +47.7 (*c* 1.0, MeOH); IR: ν_{max} = 2955, 1748, 1676 cm^{-1}; UV (MeOH): 244 nm (ε_{mM} 29.1); ^1H NMR (300 MHz, CD_3OD): δ = 8.43 (s, 1 H, =C*H*), 5.43 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 4.97 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.90 (dd, 1 H, H-2), 4.82 (t, 1 H, $J_{4,5}$ = 9.7 Hz, H-4), 4.77 (m, 2 H, CH_2 triazole), 4.70 (m, 2 H, H-6a, H-6b), 4.27 (ddd, 1 H, $J_{5,6a}$ = 3.7 Hz, $J_{5,6b}$ = 5.6 Hz, H-5), 4.06 (bs, 4 H, C*H*$_2$CH$_2$NH$_2$), 3.87 (t, 4 H, $^3J_{H,H}$ = 5.8 Hz, NCH$_2$C*H*$_2$NHCS), 3.52 (t, 4 H, $^3J_{H,H}$ = 5.8 Hz, C*H*$_2$NH$_2$), 3.25 (s, 3 H, OCH_3), 3.21 (t, 4 H, $J_{H,H}$ = 6.0 Hz, NC*H*$_2$CH$_2$NHCS), 2.50-2.15 (m, 6 H, H-2_{Hex}), 1.56 (m, 6 H, H-3_{Hex}), 1.31 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.91 (m, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 184.1 (CS), 174.3-173.9 (CO), 137.2 (C-4 triazole), 130.1 (C-5 triazole), 98.2 (C-1), 71.8 (C-2), 71.2 (C-3), 70.5 (C-4), 68.7 (C-5), 56.2 (OMe), 54.4 (CH_2NH_2), 51.4 (C-6), 48.4 (*C*H$_2$ triazole), 42.2 ($CH_2CH_2NH_2$), 40.4 (N*C*H$_2$CH$_2$NHCS), 40.0 (CH_2NH_2), 34.4 (C-2_{Hex}), 32.0 (C-4_{Hex}), 25.2 (C-3_{Hex}), 22.8 (C-5_{Hex}), 14.0 (C-6_{Hex}). ESI MS: *m/z* = 859.5 $[M + Na]^+$; 921.4 $[M + Cu]^+$. Anal. Calcd for $C_{38}H_{72}Cl_2N_{10}O_8S_2$: calcd. C, 48.97; H, 7.79; N, 15.03; S, 6.88 found: C, 48.71; H, 77.40; N, 15.23; S, 6.65.

2,3,4,2',3',4'-Hexa-*O*-hexyl-6,6'-di-*O*-trityl-α,α'-trehalose (33). To a solution of **32** (1.00 g, 1.21 mmol) in dry DMF (11 mL), NaH (871 mg, 21.78 mmol) was added and the mixture was stirred at 0 ºC for 10 min. 1-Bromohexane (3.06 mL, 21.78 mmol) was added dropwise under Ar atmosphere and the mixture was stirred overnight at rt. The reaction was quenched with MeOH (5 mL) and stirred for 10 min. The solvents were removed and the resulting residue was suspended in DCM (50 mL), washed with H_2O (3 x 15 mL) and the organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography (1:8→1:6 EtOAc-cyclohexane). Yield 92% (1.50 g, 1.11 mmol). R_f = 0.74 (1:5 EtOAc-cyclohexane); $[\alpha]_D$ = +70.3 (*c* 1.0, DCM); IR: ν_{max} = 2923, 2855 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.53-7.23

(m, 30 H, Ph), 5.34 (d, 2 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.03 (bd, 2 H, $J_{4,5}$ = 9.3 Hz, H-5), 3.80 (m, 2 H, OCH_2), 3.78-3.41 (m, 8 H, OCH_2), 3.57 (t, 2 H, $J_{2,3}$= $J_{3,4}$=9.3 Hz, H-3), 3.51-3.41 (m, 4 H, H-4, H-6a), 3.39 (dd, 2 H, H-2), 3.24 (m, 2 H, OCH_2), 3.13 (dd, 2 H, $J_{6a,6b}$ = 10.0 Hz, $J_{5,6b}$ = 3.3 Hz, H-6b), 1.65-1.56 (m, 12 H, CH_2), 1.38-1.04.89 (m, 36 H, CH_2), 0.93-0.82 (m, 18 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 144.0, 128.8, 127.6, 126.8 (Ph), 93.6 (C-1), 86.1 (Ph_3C), 81.5 (C-3), 80.6 (C-2), 78.1 (C-4), 73.7, 73.0, 71.3 (OCH_2), 70.0 (C-5), 62.0 (C-6), 31.8-22.5 (CH_2), 14.1 (CH_3); ESI MS: *m/z* = 1353.8 $[M + Na]^+$. Anal. Calcd for $C_{86}H_{122}O_{11}$: C, 77.55; H, 9.23. Found: C, 77.67; H, 9.31.

2,3,4,2',3',4-Hexa-*O*-tetradecyl-6,6'-di-*O*-trityl-α,α'-trehalose (34). To a solution of **32** (1.00 g, 1.21 mmol) in dry DMF (11 mL), NaH (0.87 g, 21.78 mmol) was added and the mixture was stirred at 0 ºC for 10 min. 1-Bromotetradecane (6.68 mL, 21.78 mmol) was added dropwise, under Ar atmosphere, and the mixture was stirred overnight at 60 ºC. The reaction was quenched with MeOH (5 mL) and stirred for 10 min. Solvents were removed and the resulting residue was suspended in DCM (50 mL). The suspension was washed with H_2O (3 x 15 mL) and the organic layer was dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (1:50 → 1:30 EtOAc-cyclohexane). Yield 77% (1.50 g, 0.93 mmol). R_f = 0.77 (1:15 EtOAc-cyclohexane); $[\alpha]_D$ = +50.2 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52-7.22 (m, 30 H, Ph), 5.33 (d, 2 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.03 (bd, 2 H, $J_{4,5}$ = 9.4 Hz, H-5), 3.79 (m, 2 H, OCH_2), 3.71-3.52 (m, 8 H, OCH_2), 3.55(t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3),3.44 (t, 2 H, H-4), 3.46-3.35 (m, 6 H, OCH_2, H-6a, H-2), 3.22 (m, 2 H, OCH_2), 3.12 (dd, 2 H, $J_{6a,6b}$ = 10.0 Hz, $J_{5,6b}$ = 3.1 Hz, H-6b), 1.76-156 (m, 12 H, CH_2), 1.28 (m, 132 H, CH_2), 0.91 (t, 18 H, $^3J_{H,H}$ = 6.3 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 144.1, 128.8, 127.6, 126.8 (Ph), 93.7 (C-1), 86.2 (Ph_3C), 81.4 (C-3), 80.6 (C-2), 78.2 (C-4), 73.7, 73.0, 71.3 (OCH_2), 70.5(C-5), 62.1 (C-6), 31.9- 22.7 (CH_2), 14.1

(CH_3); ESI MS: *m/z* = 2027.4 $[M + Na]^+$. Anal. Calcd for $C_{134}H_{218}O_{11}$: C, 80.26; H, 10.96. Found: C, 80.35; H, 11.05.

2,3,4,2',3',4-Hexa-*O*-hexyl-α,α'-trehalose (35). To a solution of **33** (0.68 g, 0.52 mmol) in 1:1 DCM-MeOH (25 mL), *p*-toluenesulphonic acid monohydrate (0.08 g, 0.42 mmol) was added and the solution was stirred at rt for 4 h. The mixture was diluted with DCM, washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography (1:8→1:2 EtOAc-cyclohexane). Yield 48% (0.21 g, 0.24 mmol); R_f = 0.25 (1:2 EtOAc-cyclohexane); $[\alpha]_D$ = +103.0 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.06 (d, 2 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.90 (dt, 2 H, $J_{4,5}$ = 9.2 Hz, $J_{5,6a} = J_{5,6b}$ = 2.9 Hz, H-5), 3.79 (m, 4 H, OCH_2), 3.73-3.63 (m, 8 H, H-6a, H-6b, OCH_2), 3.60-3.43 (m, 4 H, OCH_2), 3.57 (t, 2 H, $J_{2,3} = J_{3,4}$= 9.2 Hz, H-3), 3.23 (t, 1 H, H-4), 3.18 (dd, 1 H, H-2), 2.03 (bs, 2 H, OH), 1.60-1.47 (m, 12 H, CH_2), 1.37-1.27 (m, 36 H, CH_2), 0.87 (m, 18 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 93.6 (C-1), 81.0 (C-3), 80.4 (C-2), 77.9 (C-4), 73.6, 73.3, 71.5 (OCH_2), 71.4 (C-5), 61.8 (C-6), 31.8- 22.6 (CH_2), 14.0 (CH_3); ESI MS: *m/z* = 869.7 $[M + Na]^+$, 885.7 $[M + K]^+$. Anal. Calcd for $C_{48}H_{94}O_{11}$: C, 68.05; H, 11.18. Found: C, 67.89; H, 11.04.

2,3,4,2',3',4-Hexa-*O*-tetradecyl-α,α'-trehalose (36). To a solution of **34** (1.49 g, 0.74 mmol) in 1:1 DCM-MeOH (36 mL), *p*-toluenesulphonic acid monohydrate (0.11 g, 0.50 mmol) was added and the solution was stirred at rt for 4 h. The mixture was diluted with DCM, washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated. Purification by column chromatography (1:9→1:5 EtOAc-cyclohexane) of the residue afforded **37**. Yield 47% (0.53 g, 0.34 mmol); R_f = 0.20 (1:5 EtOAc-cyclohexane); $[\alpha]_D$ = +58.4 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.06 (d, 2 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.90 (dt, 2 H, $J_{4,5}$ = 9.2 Hz, $J_{5,6a} = J_{5,6b}$ = 2.6 Hz, H-5), 3.84-3.64 (m, 4 H, OCH_2), 3.73-3.63 (m, 12 H, H-6a, H-6b, OCH_2), 3.58 (t, 2 H, $J_{2,3}$=

$J_{3,4}$= 9.2 Hz, H-3), 3.24 (t, 2 H, H-4), 3.19 (dd, 2 H, H-2), 1.86 (bs, 2 H, OH), 1.61-1.49 (m, 12 H, CH_2), 1.26 (bs, 132 H, CH_2), 0.88 (t, 18 H, $^3J_{H,H}$ = 6.9 Hz,CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 93.7 (C-1), 81.1 (C-3), 80.5 (C-2), 78.0 (C-4), 73.6, 73.3, 71.6 (OCH_2), 71.1 (C-5), 61.9 (C-6), 31.9- 22.7 (CH_2), 14.1 (CH_3); ESI MS: *m/z* = 1543.2 $[M + Na]^+$. Anal. Calcd for $C_{96}H_{190}O_{11}$: C, 75.83; H, 12.59. Found: C, 75.70; H, 12.41.

6,6'-Dideoxy-2,3,4,2',3',4-hexa-*O*-hexyl-6,6'-diiodo-α,α'-trehalose (37). To a solution of **35** (0.10 g, 0.12 mmol) in toluene (5 mL), triphenylphosphine (0.11 g, 0.43 mmol) and imidazole (0.05 g, 0.81 mmol) were added and the mixture was stirred at rt until complete dissolution. Iodine (0.11 g, 0.40 mmol) was added in portions and the solution was vigorously stirred at 70 ºC for 5 h. Satd. aqueous $NaHCO_3$ solution (10 mL) was added and the mixture was stirred for 5 min. Additional iodine was then added until the aqueous solution turned to a slightly brown color, then aqueous 10% $Na_2S_2O_3$ was added until complete decoloration of both organic and aqueous layer. The organic layer was then separated, dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (1:15 EtOAc-cyclohexane). Yield 94% (0.12 g, 0.12 mmol); R_f = 0.75 (1:8 EtOAc-cyclohexane); $[\alpha]_D$ = +50.1 (*c* 1.0, DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.17 (d, 2 H, $J_{1,2}$ = 3.3 Hz, H-1), 3.83 (m, 4 H, OCH_2), 3.72-3.47 (m, 12 H, H-5, H-3, OCH_2), 3.39 (m, 4 H, H-6a, H-6b), 3.24 (dd, 1 H, Hz, $J_{2,3}$ = 9.1 Hz, H-2), 3.05 (t, 1 H, $J_{3,4}$= $J_{3,4}$= 9.1 Hz, H-4), 1.62-1.49 (m, 12 H, CH_2), 1.30 (m, 36 H, CH_2), 0.89 (m, 18 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 92.9 (C-1), 81.9 (C-4), 80.8 (C-3), 80.2 (C-2), 73.5, 73.4, 71.9 (OCH_2), 69.1 (C-5), 31.8- 22.6 (CH_2), 14.0 (CH_3), 8.7 (C-6); ESI MS: *m/z* = 1089.6 $[M + Na]^+$. Anal. Calcd for $C_{48}H_{92}I_2O_9$: C, 54.03; H, 8.69. Found: C, 53.88; H, 8.77.

6,6'-Dideoxy-2,3,4,2',3',4-hexa-*O*-tetradecyl-6,6'-diiodo-α,α'-trehalose (38). To a solution of **36** (0.44 g, 0.29 mmol) in toluene (13 mL), triphenylphosphine (0.27 g, 1.02 mmol) and

imidazole (0.07 g, 1.89 mmol) were added and the mixture was stirred at rt until complete dissolution. Iodine (0.26 g, 0.93 mmol) was added in portions and the solution was vigorously stirred at 70 ºC for 5 h. Satd. $NaHCO_3$ solution (20 mL) was added and the mixture was stirred for 5 min. Additional iodine was then added until the aqueous solution got slightly brown, and then an aqueous 10% $Na_2S_2O_3$ solution was added until complete decoloration of both organic and aqueous layer. The organic layer was then separated, dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography (1:25 EtOAc-cyclohexane). Yield 96% (0.52 g, 0.28 mmol); R_f = 0.69 (1:20 EtOAc-cyclohexane); $[\alpha]_D$ = +47.2 (*c* 1.0, DCM);,^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.17 (d, 2 H, $J_{1,2}$ = 3.5 Hz, H-1), 3.83 (m, 4 H, OCH_2), 3.70-3.48 (m, 12 H, H-5, H-3, OCH_2), 3.42 (dd, 2 H, $J_{6a,6b}$ = 10.7 Hz, $J_{5,6a}$ = 2.9 Hz, H-6a), 3.36 (dd, 2 H, $J_{5,6a}$ = 5.2 Hz, H-6b), 3.24 (dd, 1 H, Hz, $J_{2,3}$ = 9.4 Hz, H-2), 3.05 (t, 1 H, $J_{3,4}$= $J_{3,4}$= 9.4 Hz, H-4), 1.59-1.51 (m, 12 H, CH_2), 1.26 (bs, 132 H, CH_2), 0.88 (t, 18 H, $^3J_{H,H}$ = 6.9 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 92.9 (C-1), 81.9 (C-4), 80.8 (C-3), 80.3 (C-2), 73.6, 73.5, 71.9 (OCH_2), 69.1 (C-5), 31.9- 22.7 (CH_2), 14.1 (CH_3), 8.7 (C-6); ESI MS: *m/z* = 1763.0 $[M + Na]^+$. Anal. Calcd for $C_{96}H_{188}I_2O_9$: C, 66.25; H, 10.89. Found: C, 66.09; H, 10.74.

6,6'-Di-(2-*tert*-butoxycarbonylaminoethylthio)-2,3,4,2',3',4'-hexa-*O*-hexyl-α,α'-trehalose (39). To a solution of **37** (0.11 g, 0.10 mmol) in dry DMF (12 mL), Cs_2CO_3 (0.09 g, 0.29 mmol) and *tert*-butyl *N*-(2-mercaptoethyl)carbamate (49μL, 0.29 mmol) were added, under Ar atmosphere, and the reaction mixture was stirred at 60 ºC for 24 h. The reaction mixture was concentrated and the crude product was dissolved in DCM (20 mL) and washed with H_2O (2 x 30 mL). The organic phase was dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (1:8 → 1:6 EtOAc-cyclohexane). Yield 85 %(0.10 g, 0.08 mmol). R_f = 0.26 (1:5 EtOAc-cyclohexane); $[\alpha]_D$ = +86.4 (*c* 1.0, DCM); IR: ν_{max} = 3350, 2928, 2855, 1710 cm^{-1},^{1}H

NMR (300 MHz, $CDCl_3$): δ = 5.12 (d, 2 H, $J_{1,2}$ = 3.3 Hz, H-1), 4.99 (bs, 2 H, NHBoc), 4.01 (ddd, 2 H, $J_{4,5}$= 9.2 Hz, $J_{5,6b}$ = 6.3 Hz, $J_{5,6a}$ = 2.7 Hz, H-5), 3.81 (m, 6 H, H-3, OCH_2), 3.70-3.46 (m, 8 H, OCH_2), 3.58 (t, 2 H, $J_{2,3}$= $J_{3,4}$= 9.2 Hz, H-3), 3.29 (q, 2 H, $^3J_{H,H}$ = $^3J_{H,NH}$ = 6.0 Hz, CH_2N), 3.23 (dd, 1 H, H-2), 3.16 (t, 1 H, H-4), 2.82 (dd, 1 H, $J_{6a,6b}$ = 13.5 Hz, H-6a), 2.72 (dd, 1 H, H-6b), 2.69 (t, 2 H, CH_2S), 1.60-1.47 (m, 12 H, CH_2), 1.43 (s, 18 H, $C(CH_3)_3$), 1.34-1.28 (m, 36 H, CH_2), 0.89 (m, 18 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.7 (CO), 92.3 (C-1), 81.0 (C-3), 80.5 (C-2), 80.4 (C-4), 79.2 (CMe_3), 73.4, 73.2, 71.7 (OCH_2), 71.2 (C-5), 39.8 (CH_2S), 33.8 (C-6), 33.7 (CH_2N), 31.8- 30.2 (CH_2), 28.4 (CMe_3), 25.9, 25.8, 22.6 (CH_2), 14.0 (CH_3); ESI MS: *m/z* = 1187.9 $[M + Na]^+$, 1203.8 $[M + K]^+$. Anal. Calcd for $C_{62}H_{120}N_2O_{13}S_2$: C, 63.88; H, 10.38; N, 2.40 Found: C, 63.69; H, 10.21; N, 5.19.

6,6'-Di-(2-*tert*-butoxycarbonylaminoethylthio)-2,3,4,2',3',4'-hexa-*O*-tetradecyl-α,α'-trehalose (40). To a solution of **38** (0.28 g, 0.13 mmol) in dry DMF (15 mL), Cs_2CO_3 (0.12 g, 0.36 mmol) and *tert*-butyl *N*-(2-mercaptoethyl)carbamate (61μL, 0.36 mmol) were added, under Ar atmosphere, and the reaction mixture was stirred at 60 ºC for 24 h. The solvent was evaporated and the crude product was dissolved in DCM (20 mL) and washed with H_2O (2 x 30 mL). The organic phase was separated, dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (1:8 EtOAc-cyclohexane). Yield 99% (0.24 g, 0.13 mmol). R_f = 0.53 (1:5 EtOAc-cyclohexane); $[\alpha]_D$ = +49.3 (*c* 1.0, DCM); ^1H NMR (300 MHz, $CDCl_3$): δ = 5.12 (d, 2 H, $J_{1,2}$ = 3.4 Hz, H-1), 4.99 (bs, 2 H, NHBoc), 4.01 (ddd, 2 H, $J_{4,5}$= 9.6 Hz, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 6.1 Hz, H-5), 3.81 (m, 4 H, OCH_2), 3.69-3.46 (m, 8 H, OCH_2), 3.58 (t, 2 H, $J_{2,3}$= $J_{3,4}$= 9.6 Hz, H-3), 3.30 (bq, 2 H, $^3J_{H,H}$ = $^3J_{H,NH}$ = 6.0 Hz, CH_2N), 3.23 (dd, 1 H, H-2), 3.17 (t, 1 H, H-4), 2.83 (dd, 1 H, $J_{6a,6b}$ = 13.7 Hz, H-6a), 2.72 (dd, 1 H, H-6b), 2.69 (t, 2 H, CH_2S), 1.60-1.51 (m, 12 H, CH_2), 1.44 (s, 18 H, CMe_3), 1.26 (bs, 132 H, CH_2), 0.88 (t, 18 H, $^3J_{H,H}$ = 6.9 Hz, CH_3); ^{13}C NMR

(75.5 MHz, $CDCl_3$): δ = 155.7 (CO), 92.3 (C-1), 81.0 (C-3), 80.5 (C-2), 80.4 (C-4), 79.3 (*C*Me_3), 73.5, 73.2, 71.6 (OCH_2), 71.2 (C-5), 39.8 (CH_2S), 33.8 (C-6), 33.7 (CH_2N), 31.9, 30.7, 30.5, 30.3, (CH_2), 29.7 (CMe_3), 29.4-22.7 (CH_2), 14.1 (CH_3); ESI MS: *m/z* = 1861.5 $[M + Na]^+$. Anal. Calcd for $C_{110}H_{216}N_2O_{13}S_2$: C, 71.84; H, 11.84; N, 1.52; S, 3.49. Found: C, 71.90; H, 11.72; N, 1.44; S, 3.38.

6,6'-Di-(2-aminoethylthio)-2,3,4,2',3',4'-hexa-*O*-hexyl-α,α'-trehalose Dihydrochloride (7). Treatment of **39** (0.10 g, 0.09 mmol) with 1:1 TFA-DCM (2 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **7**. Yield quant. (0.09 g, 0.09 mmol); R_f = 0.72 (10:1:1CH_3CN-H_2O-NH_4OH); $[\alpha]_D$ = +90.3 (*c* 1.0, DCM); IR: ν_{max} = 3300, 2928, 2859, 1099 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.13 (bs, 6 H, NH_3^+), 5.20 (d, 2 H, $J_{1,2}$ = 3.2 Hz, H-1), 3.98 (dt, 2 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6a} = J_{5,6b}$ = 4.3 Hz, H-5), 3.79 (m, 4 H, OCH_2), 3.69-3.43 (m, 8 H, OCH_2), 3.56 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.25 (dd, 2 H, H-2), 3.18 (bs, 4 H, CH_2N), 3.11 (t, 2H, H-4), 2.97 (bd, 2 H, $J_{6a,6b}$ = 14.0 Hz, H-6a), 2.82 (m, 6 H, CH_2S, H-6b), 1.59-1.50 (m, 12 H, CH_2), 1.28 (m, 36 H, CH_2), 0.88 (m, 18 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 91.9 (C-1), 80.9 (C-3), 80.2 (C-2), 80.0 (C-4), 73.5, 73.4, 71.8 (OCH_2), 71.0 (C-5), 39.4 (CH_2N), 34.4 (C-6), 31.8(CH_2S), 31.7,30.6, 30.4, 30.2, 25.9, 25.8, 22.6, 22.5 (CH_2), 14.0 (CH_3); ESI MS: *m/z* = 965.9 $[M - 2Cl]^+$. Anal. Calcd for $C_{52}H_{102}N_2O_9S_2 \cdot 2HCl$: C, 60.14; H, 10.29; N, 2.70; S, 6.17. Found: C, 59.86 H, 10.02; N, 2.41; S, 5.88.

6,6'-Di-(2-aminoethylthio)-2,3,4,2',3',4'-hexa-*O*-tetradecyl-α,α'-trehalose Dihydrochloride (8). Treatment of **40** (0.09 mg, 0.05 mmol) with 1:1 TFA-DCM (1 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **8**. Yield quant (0.09 g, 0.05 mmol); R_f = 0.21 (EtOAc); $[\alpha]_D$ = +61.2 (*c* 1.0, DCM); ^1H NMR (300 MHz, $CDCl_3$): δ = 6.39 (bs, 6 H, NH_3^+), 5.21 (d, 2 H, $J_{1,2}$ = 3.5 Hz, H-1), 3.97 (dt, 2 H, $J_{4,5}$ = 9.6 Hz, $J_{5,6a} = J_{5,6b}$ = 4.7 Hz, H-5), 3.79 (m, 4 H, OCH_2),

3.69-3.43 (m, 8 H, OCH_2), 3.56 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 3.25 (dd, 2 H, H-2), 3.18 (t, 4 H, $^3J_{H,H}$ = 6.3 Hz,CH_2N), 3.11 (t, 2H, H-4), 3.07-2.94 (m, 4 H, H-6a, H-6b), 2.82 (m, 4 H, CH_2S), 1.58-1.49 (m, 12 H, CH_2), 1.26 (bs, 132 H, CH_2), 0.88 (m, 18 H, $^3J_{H,H}$ = 7.0 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 91.9 (C-1), 80.9 (C-3), 80.2 (C-2), 80.0 (C-4), 73.5, 73.4, 71.8 (OCH_2), 71.0 (C-5), 39.5 (CH_2N), 34.6 (C-6), 31.9(CH_2S), 31.9-22.7 (CH_2), 14.1 (CH_3); ESI MS: *m/z*=1639.4 $[M - 2\ Cl]^+$. Anal. Calcd for $C_{100}H_{200}N_2O_9S_2 \cdot 2HCl$: C, 70.17; H, 11.90; N, 1.64; S, 3.75 Found: C, 69.82; H, 11.77; N, 1.39; S, 3.41.

6,6'-Dideoxy-2,3,4,2',3',4'-hexa-*O*-hexanoyl-6,6'-diiodo-α,α'-trehalose (42). To a solution of 6,6'-dideoxy-6,6'-diiodo-trehalose**41**, (5.78 g, 10.30 mmol) and DMAP (6.37 g, 52.20 mmol) in dry DMF (80 mL), hexanoic anhydride (16 mL, 69.60 mmol) was added dropwise, under Ar atmosphere at 0º C and the reaction mixture was stirred at rt for 6 h. Then MeOH (60 mL) was added and the mixture was stirred ad rt for 2 h. The solution was poured into H_2O-ice mixture (100 mL) and extracted with DCM (50 mL). The organic phase was then washed with 2N H_2SO_4 (2 x 50 mL), H_2O (2 x 50 mL) and saturated aqueous $NaHCO_3$ (2 x 50 mL), dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (1:12 EtOAc-petroleum ether). Yield 76% (1.20 g, 7.82 mmol)R_f = 0.35 (1:6 EtOAc-petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.44 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.35 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.1 (dd, 1 H, H-2), 4.84 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.82 (ddd, 1 H, $J_{5,6a}$ = 2.5 Hz, H-5), 3.11 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, H-6a), 2.97 (dd, 1 H, H-6b), 2.22 (m, 6 H, H-2_{Hex}), 1.58 (m, 6 H, H-3_{Hex}), 1.17 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.84 (m, H-6_{Hex}).^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.6, 172.5, 172.4 (CO), 91.5 (C-1), 72.0 (C-4), 70.0 (C-5), 69.3 (C-3), 69.2 (C-2), 34 (C-2_{Hex}), 31.2 (C-4_{Hex}), 24.4 (C-3_{Hex}), 22.2 (C-5_{Hex}), 14.1 (C-6_{Hex}), 2.6 (C-6_{Hex}). ESI MS: *m/z* = 1173.4 $[M + Na]^+$. Anal. Calcd for $C_{47}H_{78}I_2O_{15}$: calcd. C, 49.65; H, 6.92; found: C, 50.12; H, 7.01.

2,3,4,2',3',4'-Hexa-*O*-hexanoyl-6,6'-bis(2-*tert*-butoxycarbonylaminoethylthio)-α,α'-trehalose (43). To a solution of **42** (0.19 g, 0.17 mmol) in dry DMF (1.5 mL), Cs_2CO_3 (0.15 g, 0.47 mmol) and *tert*-butyl *N*-(2-mercaptoethyl)carbamate (80 μL, 0.47 mmol) were added, under Ar atmosphere, and the reaction mixture was stirred at 60º C for 24 h. The reaction mixture was concentrated and the crude product was dissolved in DCM (10 mL) and washed with water (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (1:3 EtOAc-cyclohexane). Yield 58% (0.12 mg, 0.10 mmol). R_f = 0.52 (1:2 EtOAc-cyclohexane); $[\alpha]_D$ = +84.5 (*c* 1.0, DCM); IR: ν_{max} = 2959, 1749, 1709 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 5.43 (t, 1 H, $J_{3,2} = J_{3,4}$= 9.5 Hz, H-3), 5.25 (d, 1 H, $J_{1,2}$= 4.1 Hz, H-1), 5.00 (dd, 1 H, H-2), 4.94 (t, 1 H, H-4), 3.88 (m, 1 H, H-5), 3.2 (s, 2 H, CH_2N), 2.61 (m, 2 H, CH_2S), 2.52 (m, 2 H, H-6ª, H-6b), 2.23 (m, 6 H, H-2_{Hex}), 1.50 (m, 6 H, H-3_{Hex}),1.41 (s, 9 H, CMe_3), 1.23 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.83 (t, 9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.6, 172.5, 172.4 (C-1_{Hex}), 155.7 (CO carbamate), 91.4 (C-1), 71.2 (C-4), 71.1 (C-5), 69.6 (C-2, C-3), 39.7 (*C*H_2N), 34.1, 34.0 (C-2_{Hex}), 33.8 (*C*H_2S), 24.4 (C-3_{Hex}), 28.4 (C*Me*$_3$), 22.3 (C-4_{Hex}, C-5_{Hex}), 13.8 (C-6_{Hex}); ESI MS: *m/z*=1271.8 $[M + Na]^+$. Anal. Calcd for $C_{62}H_{108}N_2O_{19}S_2$: C, 59.59; H, 8.71; N, 2.24; S, 5.13; found: C, 59.67; H, 8.69; N, 2.32; S, 4.89.

2,3,4,2',3',4'-Hexa-*O*-hexanoyl-6,6'-bis(2-*tert*-aminoethylthio)-α,α'-trehalose dihydrochloride (9). Treatment of **43** (0.07 g, 0.04 mmol) with 1:1 TFA-DCM (2 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **9**. Yield quant (0.07 g, 0.04 mmol). $[\alpha]_D$ = + 66.7 (*c* 0.9, EtOAc) ; IR: ν_{max} = 2957, 1740, 1686 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ = 5.35 (t, $J_{2,3}$ = 9.7 Hz, 1 H, H-3), 5.29 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.08 (m, 2 H, H-2, H-3), 3.95 (m, 1 H, H-5), 2.95 (bs, 2 H, CH_2N), 2.69 (t, 2 H, $J_{H,H}$ = 7.0 Hz, CH_2S), 2.67 (bd, 2 H, H-6ª, H-6b), 2.27 (m, 6 H, H-2_{Hex}), 1.49 (m, 6 H, H-3_{Hex}), 1.24 (m, 12 H, H-4_{Hex}, H-5_{Hex}), 0.85 (t,

9 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 171.8, 171.7 (CO), 90.9 (C-1), 70.9 (C-5), 70.0, 69.2, (C-2, C-4), 69.4 (C-3), 39.2 (*C*H_2N), 31.5 (C-6), 33.9 (C-2_{Hex}), 30.3 (*C*H_2S), 23.9 (C-3_{Hex}), 30.6 (C-4_{Hex}), 21.8 (C-5_{Hex}), 13.3 (C-6_{Hex}); ESI MS: *m/z* = 1049.6 $[M]^+$. Anal. Calcd for $C_{52}H_{94}Cl_2N_2O_{15}S_2$: C, 55.65; H, 8.44; N, 2.50; S, 5.71; found: C, 55.29; H, 8.18; N, 2.14; S, 5.33

6,6'-Diazido-6,6'-dideoxy-2,3,4,2',3',4'-hexa-*O*-hexanoyl-α,α'-trehalose (45). To a solution of **42** (1.58 g, 1.37 mmol) in dry DMF (8 mL), NaN_3 (0.25 g, 3.60 mmol) was added. The reaction mixture was stirred overnight at 40 ºC, under Ar atmosphere. The mixture was poured into H_2O-ice mixture (20 mL), and the product was extracted with DCM (4 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (1:12 EtOAc-cyclohexane) to afford **45**. Yield 72% (0.91 g, 0.98 mmol). R_f = 0.53 (1:6 EtOAc-cyclohexane). $[\alpha]_D$ = +108.7 (*c* 1.0, DCM); IR: ν_{max} = 2958, 2104, 1750, 735 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ = 5.52 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 5.41 (d, 2 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.13 (dd, 2 H, H-2), 5.09 (t, 2 H, $J_{4,5}$ = 9.8 Hz, H-4), 4.08 (ddd, 2 H, $J_{5,6a}$ = 7.0 Hz, $J_{5,6b}$ = 2.6 Hz, H-5), 3.44 (dd, 2 H, $J_{6a,6b}$ = 13.0 Hz, H-6^a), 2.97 (dd, 2 H, H-6b), 2.33 (m, 12 H, H-2_{Hex}), 1.58 (m, 12 H, H-3_{Hex}), 1.33 (m, 24 H, H-4_{Hex},H-5_{Hex}), 0.91 (m, 18 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 175.2-174.2 (CO), 93.7 (C-1), 71.5 (C-3), 71.3 (C-2), 71.6 (C-5), 71 (C-4), 52.2 (C-6), 34.9 (C-2_{Hex}), 33.2 (C-4_{Hex}), 25.8 (C-3_{Hex}), 23.6 (C-5_{Hex}), 14.8 (C-6_{Hex}); ESI MS: *m/z* = 1003.5 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{43}N_8O_{15}$: calcd. C, 58.76; H, 8.22; N, 8.57; found: C, 58.84; H, 8.32; N, 8.60.

6,6'-Di-(4-*tert*-butoxycarbonylaminomethyl-1*H*-1,2,3-triazol-1-yl)-6,6'-dideoxy-2,3,4,2',3',4'-hexa-*O*-hexanoyl-α,α'-trehalose (46). To a solution of **44** (0.25 g, 0.25 mmol) and **45** (0.08 g, 0.56 mmol) in H_2O-^{t}BuOH 9:1 (15 mL) the Cu-supported catalyst Si-BPA·Cu^+ (0.02 g) was added and the reaction mixture was refluxed for 36 h at 85 ºC. The catalyst was

filtered and the solvent was concentrated. The residue was purified by column chromatography (1:1→2:1 EtOAc-cyclohexane). Yield quant. (0.33 g, 0.25 mmol). R_f=0.73 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ = +56.7 (*c* 1.0, DCM); IR: ν_{max} = 2957, 1752, 1714, 735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.52 (s, 2 H, =CH), 5.45 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.8 Hz , H-3), 5.30 (bs, 2 H, N*H*Boc), 4.97 (dd, 2 H, $J_{1,2}$= 4.0 Hz, H-2), 4.87 (t, 2 H, $J_{4,5}$ = 9.8 Hz, H-4), 4.83 (d, 2 H, H-1), 4.48 (bd, 2 H, $J_{6a,6b}$ = 13.7 Hz, H-6a), 4.37 (d, 4 H, $J_{H,H}$ = 6.0 Hz, CH_2 triazole), 4.12 (dd, 2 H, $J_{5,6b}$ = 8.8 Hz, H-6b), 4.07 (m, 2 H, H-5), 2.25 (m, 12 H, H-2_{Hex}), 1.55 (m, 12 H, H-3_{Hex}), 1.46 (s, 18 H, CMe_3), 1.29 (m, 24 H, H-4_{Hex}, H-5_{Hex}), 0.89 (m, 18 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 173.5-173.2 (CO ester), 155.5 (CO carbamate), 146.0 (C-4 triazole), 122.3 (C-5 triazole), 91.7 (C-1), 79.5 (*C*Me_3), 69.9 (C-3, C-5), 69.8 (C-4), 69.3 (C-2), 50.7 (C-6), 36.4 (*C*H_2-triazole), 34.3 (C-2_{Hex}), 31.9 (C-4_{Hex}), 28.7 (C*Me*$_3$) 24.6 (C-3_{Hex}), 22.6 (C-5_{Hex}), 14.1 (C-6_{Hex}); ESI MS: *m/z* = 1313.4 $[M + Na]^+$. Anal. Calcd for $C_{64}H_{106}N_8O_{19}$: calcd. C, 59.52; H, 8.27; N, 8.68; found: C, 59.61; H, 8.33; N, 8.84.

6,6'-Di-(4-Aminomethyl-1*H*-1,2,3-triazol-1-yl)-6,6'-dideoxy-2,3,4,2',3',4'-hexa-*O*-hexanoyl) -α,α'-trehalose Dihydrochloride (47). Treatment of **46** (0.30 g, 0.23 mmol) with 1:1 TFA-DCM (4 mL) and freeze-drying from 10:1 H_2O/0.1 N HCl solution afforded **47**. Yield 97% (0.26 g, 0.22 mmol). $[\alpha]_D$ = +48.9 (*c* 1.0, MeOH); IR: ν_{max} = 2956, 1751, 1464, 1026 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ = 8.05 (s, 2 H, CH triazole), 5.49 (t, 2 H, $J_{3,4} = J_{2,3}$ = 9.8 Hz, H-3), 5.06 (dd, 2 H, $J_{1,2}$ = 4.0 Hz, H-2), 4.96 (d, 2 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.95 (t, 2 H, $J_{4,5}$ = 9.8 Hz, H-4), 4.63 (dd, 2 H, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 14.6 Hz, H-6), 4.53 (dd, 2 H, $J_{5,6b}$ = 7.9 Hz, H-6b), 4.27 (bs, 4 H, C*H*$_2$$NH_2$), 4.24 (m, 1 H, H-5), 2.32 (m, 12 H, H-2_{Hex}), 1.59 (m, 12 H, H-3_{Hex}), 1.33 (m, 24 H, H-4_{Hex},H-5_{Hex}), 0.93 (m, 18 H, H-6_{Hex}); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 174.5-173.6 (CO), 141.5 (C-4 triazole) 127.1 (C-5 triazole), 92.7 (C-1), 71.2 (C-2), 70.6 (C-3), 70.4 (C-4),

70.3 (C-5), 51.5 (C-6), 35.2 (CH_2NH$_2$), 34.8 (C-2$_{Hex}$), 32.1 (C-4$_{Hex}$), 25.8 (C-3$_{Hex}$), 22.6 (C-5$_{Hex}$), 14.2 (C-6$_{Hex}$); ESI MS: *m/z* = 1091.4 $[M]^+$. Anal. Calcd for $C_{54}H_{92}N_8O_{15}$: calcd. C, 55.71; H, 7.96; N, 9.62; found: C, 55.48; H, 7.77; N, 9.97.

6,6'-Di-[4-(2-N'-(2-(N-*tert*-butoxycarbonyl)aminoethyl)thioureido))methyl-1*H*-1,2,3-triazolyl-]2,3,4,2',3',4'-hexa-*O*-hexanoyl)-6,6'-dideoxy-α,α'-trehalose (48). To a solution of **47** (0.14 g, 0.12 mmol) and Et_3N (37 μL, 0.36 mmol) in DCM (10 mL), *tert*-butyl *N*-(2-isothiocyanoethyl) carbamate (0.07 g, 0.36 mmol) was added and the reaction mixture was stirred overnight at rt. The reaction mixture was washed with aqueous diluted HCl (3 x 10 mL) and the organic phase was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (2:1 → 3:1 EtOAc-cyclohexane). Yield 67% (0.12 g, 0.08 mmol). R_f = 0.40 (3:1 EtOAc-cyclohexane). $[\alpha]_D$ = +59.8 (*c* 1.0, DCM); UV (DCM): 249 nm (ε_{mM} 53.9); IR: ν_{max} = 2959, 1752, 1701, cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (s, 2 H, CH triazole), 7.07 (bs, 4 H, NHCS), 5.39 (t, 2 H, $J_{3,4} = J_{2,3}$ = 9.7 Hz , H-3), 5.29 (bs, 2 H, N*H*Boc), 4.98-4.76 (m, 4 H, H-1, H-4, CH_2 triazole), 4.62 (dd, 2 H, H-2), 4.47 (d, 2 H, $J_{6a,6b}$ = 14.0 Hz, H-6^a), 4.28 (dd, 2 H, $J_{5,6b}$ = 8.0 Hz, H-6b), 3.97 (bt, 2 H, H-5), 3.60 (bs, 4 H, CH_2CH$_2$NHBoc), 3.31 (m, 4 H, $^3J_{H,H}$ = 5.4 Hz, CH_2NHBoc), 2.22 (m, 12 H, H-2$_{Hex}$), 1.53 (m, 12 H, H-3$_{Hex}$), 1.39 (s, 18 H, CMe_3), 1.27 (m, 24 H, H-4$_{Hex}$, H-5$_{Hex}$), 0.86 (m, 18 H, H-6$_{Hex}$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 173.2-172.5 (CO ester), 156.2 (CO carbamate), 141.5 (C-4 triazole), 124.3 (C-5 triazole), 96.7 (C-1), 79.7 (*C*Me$_3$), 69.6-68.7 (C-2, C-3, C-4, C-5), 50.3 (C-6), 40.0 (*C*H$_2$CH$_2$NHBoc), 39.5 (*C*H$_2$NHBoc), 33.9 (C-2$_{Hex}$), 31.2 (C-4$_{Hex}$), 28.3 (C*Me*$_3$), 24.3 (C-3$_{Hex}$), 22.2 (C-5$_{Hex}$), 13.1 (C-6$_{Hex}$). ESI MS: *m/z* = 1517.4 $[M + Na]^+$. Anal. Calcd for $C_{70}H_{118}N_{12}O_{19}S_2$: calcd. C, 56.20; H, 7.95; N, 11.24; O, 20.32; S, 4.29; found: C, 55.95; H, 7.84; N, 11.04; S, 11.04

[(6,6'-Di-4-(2-*N*'-(2-aminoehtyl)thioureido))methyl-1*H*-1,2,3-triazol-1-yl)-6,6'-dideoxy-2,3,4,2',3',4'-hexa-*O*-hexanoyl]-α,α'-trehalose Dihydrochloride (10). Treatment of **48** (0.11 g, 0.07 mmol) with TFA-DCM (2 mL) and freeze-drying from 0.1 N HCl solution afforded **10**. Yield quant (0.10 g, 0.07 mmol). $[\alpha]_D$ = +38.2 (*c* 1.0, MeOH); UV (MeOH): 243 nm (ε_{mM} 16.9); IR: ν_{max} = 2956, 2862, 1752, 1675 721 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ = 7.92 (s, 2 H, CH triazole), 5.44 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 5.08 (dd, 2 H, $J_{1,2}$ = 4.2 Hz, H-2), 4.95 (t, 2 H, H-4), 4.82 (m, 4 H, H-4, H-1, CH_2 triazole), 4.62 (dd, 2 H, $J_{6a,6b}$ = 14.0 Hz, $J_{5,6a}$ = 2.5 Hz, H-6[a]), 4.48 (dd, 2 H, $J_{5,6b}$ = 8.2 Hz, H-6b), 4.19 (ddd, 2 H, H-5), 3.91 (m, 4 H, C*H*$_2$CH$_2$NH$_2$), 3.21 (dt, 4 H, $J_{H,H}$ = 5.8 Hz, C*H*$_2$NH$_2$), 2.31 (m, 12 H, H-2$_{Hex}$), 1.59 (m, 12 H, H-3$_{Hex}$), 1.32 (m, 24 H, H-4$_{Hex}$, H-5$_{Hex}$), 0.93 (m, 18 H, H-6$_{Hex}$); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 182.6 (CS), 176.0-174.5 (CO), 146.1 (C-4 triazole), 125.6 (C-5 triazole), 92.4 (C-1), 71.4 (C-4), 70.6 (C-3), 70.4 (C-5), 70.3 (C-2), 51.5 (C-6), 42.6 (*C*H$_2$CH$_2$NH$_2$), 40.9 (*C*H$_2$NH$_2$), 40.2 (CH$_2$-triazole), 35-34.8 (C-2$_{Hex}$), 32.5-32.4 (C-4$_{Hex}$), 25.7-25.4 (C-3$_{Hex}$), 23.2 (C-5$_{Hex}$), 14.3 (C-6$_{Hex}$); ESI MS: *m/z* = 1357 $[M + Cu]^+$; 711 $[M + Cu]^{2+}$ Anal. Calcd for $C_{60}H_{104}N_{12}O_{15}S_2$: calcd. C, 52.66; H, 7.66; N, 12.28; S, 4.69; found: C, 52.71; H, 7.50; N, 12.55, 4.69.

6,6'[-4-(2,2-Bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]-α,α'-trehalose (49). To a solution of **44** (0.31 g, 0.31 mmol) and **29** (0.24 g, 0.73 mmol) in H_2O-^{t}BuOH 9:1(15 mL), the Cu-supported catalyst Si-BPA·Cu^+ (0.02 g) was added and the reaction mixture was refluxed for 36 h at 85 ºC. The catalyst was filtered and the solvent was removed. The residue was purified by column chromatography (2:1 → 3:1 EtOAc-cyclohexane). Yield 91% (0.54 g, 0.29 mmol). R_f = 0.36 (3:1 EtOAc-cyclohexane). $[\alpha]_D$ = +57.0 (*c* 1.0, DCM); IR: ν_{max} = 2958, 2359, 2341, 1751, 1700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.57 (s, 2 H, =C*H*), 5.47 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.8 Hz , H-3), 5.20 (bs, 4 H, NHBoc),

4.93 (dd, 2 H, $J_{1,2}$ = 3.8 Hz, H-2), 4.86 (t, 2 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.77 (d, 2 H, H-1), 4.46 (bd, 2 H, $J_{6a,6b}$ = 14.0 Hz, H-6[a]), 4.25 (dd, 2 H, $J_{5,6b}$ = 8.7 Hz, H-6b), 4.12 (m, 2 H, H-5), 3.83 (bs, 4 H, CH_2 triazole), 3.19 (bd, 8 H, $^3J_{H,H}$ = 6.0 Hz, C*H*$_2$NHBoc), 2.55 (t, 8 H, $J_{H,H}$ = 6.0 Hz, C*H*$_2$CH$_2$NHBoc), 2.27 (m, 12 H, H-2$_{Hex}$), 1.58 (s, 36 H, CMe$_3$), 1.44 (m, 12 H, H-3$_{Hex}$), 1.30 (m, 24 H, H-4$_{Hex}$, H-5$_{Hex}$), 0.89 (m, 18 H, H-6$_{Hex}$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 174.2-173.0 (CO ester), 156.2 (CO carbamate), 143.9 (C-4 triazole), 124.3 (C-5 triazole), 91.4 (C-1), 79.1 (*C*Me$_3$), 69.5 (C-4), 69.3 (C-3), 69.2 (C-5), 68.8 (C-2), 53.1 (*C*H$_2$CH$_2$NHBoc), 50.6 (C-6), 48.0 (CH_2-triazole), 38.4 (*C*H$_2$NHBoc), 34.1 (C-2$_{Hex}$), 31.7 (C-4$_{Hex}$), 28.5 (C*Me$_3$*), 24.6 (C-3$_{Hex}$), 22.6 (C-5$_{Hex}$), 13.8 (C-6$_{Hex}$); ESI MS: *m/z* = 1686.6 $[M + Na]^+$. Anal. Calcd for $C_{82}H_{142}N_{12}O_{23}$: calcd. C, 59.18; H, 8.60; N, 10.10; found: C, 59.20; H, 8.51; N, 10.15.

6,6'[4-(2,2-Diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6,6'-dideoxy-2,3,4,2',3',4'-tri-*O*-hexanoyl]-α,α'-trehalose Tetrahydrochloride (11). Treatment of **49** (0.56 g, 0.34 mmol) with TFA-DCM 1:1 (6 mL) and freeze-drying from 0.1 N HCl solution afforded **11**. Yield quant. (0.48 g, 0.33 mmol). $[\alpha]_D$ = +22.1 (*c* 1.0, MeOH); IR: ν_{max} = 2956, 2356, 1753, 1676, 721 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.33 (s, 2 H, CH triazole), 5.51 (t, 2 H, $J_{3,4} = J_{2,3}$ = 9.7 Hz, H-3), 5.08 (dd, 2 H, $J_{1,2}$ = 3.9 Hz, H-2), 5.01 (t, 2 H, $J_{4,5}$ = 9.7 Hz, H-4), 4.95 (d, 2 H, H-1), 4.65 (dd, 2 H, $J_{5,6a}$= 3.0 Hz, $J_{6a,6b}$ = 14.5 Hz, H-6a), 4.56 (dd, 2 H, $J_{5,6b}$ = 8.1 Hz, H-6b), 4.28 (bs, 4 H, CH_2 triazole), 4.18 (ddd, 2 H, H-5), 3.41 (bt, 8 H, $^3J_{H,H} = {}^3J_{H,NH}$ = 6.0 Hz, C*H*$_2$NH$_2$), 3.22 (bt, 8 H, C*H*$_2$CH$_2$NH$_2$), 2.31 (m, 12 H, H-2$_{Hex}$), 1.59 (m, 12 H, H-3$_{Hex}$), 1.33 (m, 24 H, H-4$_{Hex}$, H-5$_{Hex}$), 0.93 (m, 18 H, H-6$_{Hex}$); ^{13}C NMR (75.5 MHz, CD_3OD): δ = 174.2-173.2 (CO), 141.1 (C-4 triazole), 128.8 (C-5 triazole), 91.9 (C-1), 71.0 (C-4), 70.6 (C-3), 70.5 (C-5), 70.4 (C-2), 51.8 (C-6), 51.6 (*C*H$_2$CH$_2$NH$_2$), 47.9 (CH_2-triazole), 37.2 (*C*H$_2$NH$_2$), 35.1-34.8 (C-2$_{Hex}$), 32.5-32.4 (C-4$_{Hex}$), 25.6-25.5 (C-3$_{Hex}$), 23.4 (C-5$_{Hex}$), 14.3 (C-6$_{Hex}$); ESI MS: *m/z* = 1263.5 $[M]^+$; 632.0 $[M]^{2+}$.

Anal. Calcd for $C_{62}H_{114}N_{12}O_{15}$: calcd. C, 52.83; H, 8.15; N, 11.93; found: C, 52.87; H, 8.04; N, 11.74.

Determination of CMC *via* Pyrene Fluorescence Measurements. In order to assess the amphiphilicity, the critical micelle concentrations (CMC) of all derivatives have been determined using an established fluorescence technique based on pyrene.[46] This extremely hydrophobic dye is preferentially incorporated in the interior of micelles. The onset of micelle formation can be observed in a shift of the fluorescence excitation spectra of the samples at an emission wavelength of 372 nm. In the concentration range of aqueous micellar solutions, a shift of the excitation band in the 335 nm region toward higher wavelengths confirms the incorporation of pyrene in the hydrophobic interior of micelles. The ratio of the fluorescence intensities at 339 and 335 nm was used to quantify the shift of the broad excitation band. The critical micelle concentrations were determined from the crossover point in the low concentration range. Fluorescence spectra were recorded with an F-2500 Hitachi spectrofluorophotometer and conventional 1-cm quartz cuvettes at 37 ± 0.1 ºC, using 2.5 mm excitation and emission slits.

Synthesis of Dodecanethiol Coated Gold Nanoparticles (DDT-Au NPs). A solution of tetrachloroaurate acid in milli-Q water (25 mL, 0.03 M) was mixed with a solution of tetraoctylammonium bromide in toluene (80 mL, 0.05 M). The two phases mixture was vigorously stirred until all the tetrachloroaurate was transferred into the organic layer, and the aqueous layer was discarded. To the solution was added dropwise a $NaBH_4$ aqueous solution (25 mL, 0.35 M) for 1 minute, then the mixture was stirred for 1 h. The biphasic system was washed with 0.01 M HCl (1 x 25 mL), 0.01 M NaOH (1 x 25 mL) and H_2O milli-Q (3 x 25 mL). Aqueous layers were discarded and the organic phase was stirred overnight at rt. Dodecanethiol (10 mL, 42 mmol) was added and the mixture was refluxed for 3 h. The system was cooled to rt

and spin-dried at 2000 rpm for 10 min. The supernatant was recovered, and MeOH was added to reach 1:1 mixture to precipitate the NPs and eliminate the excess of dodecanethiol, and the system was spin-dried for 10 min at 2000 rpm. Then supernatant was discarded and the precipitate was suspended in 1 mL of $CHCl_3$.

The final concentration of DDT-Au NPs was determined by UV spectrometry. A small aliquot of NPs was 1000-fold diluted and absorbance was measured using a $\varepsilon = 8.63 \cdot 10^6\ M^{-1}cm^{-1}$.[50]

Coating of DDT-Au_NPs with Compound 11. To a solution of DDT-Au NP (120 μL) in $CHCl_3$ (30 mL) was added a solution of compound **11** in MeOH (1 mL, 2 mM). The mixture was concentrated and milli-Q water (300 μL) was added before 1 min sonication. The dark red solution was spin-dried in a ultrafiltration device with a polyethersulfone membrane (Corning® Spin-X® UF) for 5 min at 6000 rpm. The precipitate was recovered by addition of milli-Q water (500 μL). Final concentration was determined measuring the absorbance at 450 nm using an $\varepsilon = 3.07 \cdot 10^7\ M^{-1}cm^{-1}$.

Biological Assays

Reagents and Cell Cultures. Expression plasmid for mouse MD-2 was a gift from Dr. Y. Nagai (University of Tokyo, Japan). Expression plasmid for mouse TLR4 was purchased from InvivoGen (CA, USA). Expression plasmids containing sequences of human TLR4 and MD-2 as well as the pELAM-1 firefly luciferase plasmid were a gift from Dr. C. Kirschning (Technical University of Munich, Germany). The Renilla luciferase phRL-TK plasmid was purchased from Promega (WI, USA).

The human embryonic kidney (HEK) 293 cells were provided by Dr. J. Chow (Eisai Research Institute, Andover, USA). HEK293 cells were grown in DMEM supplemented with 10 % FBS. Compounds were dissolved in 100% DMSO to provide 4 mM stock solutions; further working

dilutions were prepared immediately before stimulation with cell medium (DMEM supplemented with 10 % FBS).

Cell Activation Assay – NF-κB-Luciferase Reporter Assay. HEK 293 cells were seeded in 96-well Costar plates (Corning, NY, USA) at 1.6·104 cells/well and incubated overnight in a humidified atmosphere (5 % CO_2) at 37 °C. The next day, when cells were 40-60 % confluent, they were co-transfected with MD-2 (10 ng), NF-κB-dependent luciferase (70 ng) and constitutive Renilla (15 ng) reporter plasmids and TLR4 plasmid (1 ng) using PEI (7.5 molar polyethylenimine pH 7.5, Polysciences) transfection reagent. Cells were stimulated 4 hours after transfection with the synthetic compounds, then1 h later with LPS (5 nM) that was extensively vortexed immediately prior to stimulation. Cells were lysed after 16 hours of stimulation in 1x reporter assay lysis buffer (Promega, USA) and analyzed for reporter gene activities using a dual-luciferase reporter assay system. Relative luciferase activity (RLA) was calculated by normalizing each sample's firefly luciferase activity for constitutive Renilla activity measured within the same sample. When plotting the data the value of the wild type MD-2·TLR4 sample stimulated with LPS was normalized to 100 and other values were adjusted accordingly.

HEK-Blue™ Assay. HEK-Blue-TLR4 cells (InvivoGen) were cultured according to manufacturer's instructions. Briefly, cells were cultured in DMEM high glucose medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 1x Normocin (InvivoGen), 1x HEK-Blue Selection (InvivoGen). Cells were detached by the use of a cell scraper and the cell concentration was estimated by using Trypan Blue (Sigma Aldrich). The cells were diluted in DMEM high glucose medium supplemented as described before and seeded in multiwell plate at a density of $2x10^4$ cells/well in 200 µL. After overnight incubation (37°C, 5% CO_2, 95% humidity), supernatant was removed, cell monolayers were washed with warm PBS without Ca^{2+}

and Mg^{2+} and treated with increasing concentrations of compounds dissolved in DMSO-ethanol (1:1). After 30 minutes, the cells were stimulated with 10 nM LPS from *E. coli* O55:B5 (Sigma Aldrich) and incubated overnight at 37°C, 5% CO_2 and 95% humidity. As a control, the cells were treated with or without LPS (10 nM) alone. Then, the supernatants were collected and 50 µL of each sample was added to 100 µL PBS, pH 8, 0.84 mM para-Nitrophenylphosphate (pNPP) for a final concentration of 0.8 mM pNPP. Plates were incubated for 2-4 h in the dark at rt and then the plate reading was assessed by using a spectrophotometer at 405 nm (LT 4000, Labtech). The results were normalized with positive control (LPS alone) and expressed as the mean of percentage ± SD of at least three independent experiments.

MTT Cell Viability Assay. HEK-Blue cells were seeded in 100 µL DMEM without Phenol Red at a density of $2x10^4$ cells per well. After overnight incubation, 10 µL compounds were added and the plates were incubated overnight at 37 °C, 5% CO_2, 95% humidity. DMSO and PBS were included as control. Then 10 µl of MTT solution (5 mg/mL in PBS) were added to each well. After 3 h incubation (37 °C, 5% CO_2, 95% humidity), HCl 0.1 N in isopropanol was added (100 µl/well) to dissolve formazan crystals. Formazan concentration in the wells was determined measuring the absorbance at 570 nm (LT 4000, Labtech). The results were normalized with untreated control (PBS) and expressed as the mean of percentage ± SD of three independent experiments.

***In vivo* Endotoxin Inhibition.** C57BL/6J mice (11-13 weeks old) were randomly assigned into groups and injected intraperitoneally with vehicle control (5% DMSO in PBS) (groups none and LPS only) or the inhibitory compound ($2x10^{-7}$ mol compound/mouse for compounds **5-11**, all in 5% DMSO solution). One hour later the mice were injected intraperitoneally with vehicle control (PBS) (group none) or with LPS from *E. coli* 055:B5 ($1x10^{-9}$ mol / mouse ≈ 10 µg LPS / mouse).

Three hours later the blood was collected. Serum was tested with the mouse TNF-α ELISA kit ("ReadySetGo", eBioscience) to determine the levels of mouse TNF-α. The experiment was performed according to the manufacturer's instructions.

SUPPORTING INFORMATION

Supporting Information Available: activity of compounds **5**, **9**, **10** on bone marrow-derived murine macrophages (BMDM); MTT cell toxicity tests for synthetic molecules. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

BMDM, bone marrow derived machophages; CNS, central nervous system; DC, dendritic cell; DCC, dicyclohexylcarbodiimide; DMEM, Dulbecco's modified Eagle's medium; DMAP, 4-dimethylaminopyridine; DMSO, dimethysulfoxide; HEK, human embryonic kidney; PTSA, *p*-toluenesulfonic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TPP, triphenylphosphine;

TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adapter-inducing interferon-β.

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