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First total synthesis of 1,2-dipalmitoyl-3-(*N*-palmitoyl-6′-amino-6′-deoxy-α-D-glucosyl)-*sn*-glycerol—a glycoglycerolipid of a marine alga with a high inhibitor activity against human Myt1-kinase

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ABSTRACT

The first total synthesis of 1,2-dipalmitoyl-3-(*N*-palmitoyl-6′-amino-6′-deoxy-α-D-glucosyl)-*sn*-glycerol, a glycoglycerolipid isolated from a marine alga extract, is described. Starting from α-methylglucopyranoside the multistep strategy allows the stereoselective synthesis of the final compound using various protective group procedures as well as derivatization of partial molecule domains. The latter offers the development of lead structures for inhibitors of human Myt1-kinase.

1. Introduction

Resulting from a systematic search of potential anti-cancer agents from plants and marine organisms a range of constituents were isolated which showed a high activity in a human Myt1-kinase inhibition assay. Complex structure determination of the crude extract led to glycoglycerolipids including the most active compound (IC_{50} of 4 μg/mL) 1,2-dipalmitoyl-3-(*N*-palmitoyl-6′-amino-6′-deoxy-α-D-glucosyl)-*sn*-glycerol (Scheme 1; compound **14**).[1]

The human Myt1-kinase is a Thr-14 and Tyr-15 specific regulator of Cdc2-kinase activity. The inhibitory phosphorylation of Cdc2 is important for timing the entry into mitosis. Various studies have shown that premature activation of Cdc2 would lead to mitotic dysfunction and apoptosis.[2] Inhibition of Myt1-kinase is predicted to cause premature activation of Cdc2.[3,4] Therefore inhibitors of Myt1-kinase are supposed to kill rapidly proliferating cells and interfere with cell cycle checkpoints. Such inhibitors could represent an extension to conventional chemotherapy and could help overcoming resistance.

The rapid development in the field of kinase inhibitors is demonstrated by the commercial launch of imatinib and lapatinib recently. So the current research activity is promoted and accelerated by the development of suitable inhibitors as well as the conception of appropriate biochemical assays.[2,3] Thus, an innovative fluorescence polarization assay for Wee1- and Myt1-kinases was described by Rudolph and Kristjansdottir that was established and expanded in our group for current measurements.[5] All known selective (e.g., imatinib and lapatinib) and non-selective kinase inhibitors (e.g., staurosporine and K-252a) work by binding the ATP binding site of the kinases and therefore lead to inhibition of the enzyme activity of the protein competitively. There are only a few studies that deal with the interaction of glycolipids and kinases. Recently it could be shown by X-ray crystallography and surface plasmon resonance that simple glycolipids and fatty acid derivatives can bind to kinases and act as activators and inhibitors depending on the structural variation.[6] For p38α kinase was shown that three molecules of *n*-octyl-β-D-glucopyranose can bind near the ATP binding site as well as in a lipid binding site. Based on a comparison of p38α crystal structures and our homology model of Myt1-kinase we can find structure analogies in the areas of the binding sites. Therefore we hypothesize that simple glycolipids as well as the marine glycoglycerolipids have a similar binding mode in Myt1-kinase.

Searching for potential Myt1 inhibitors the isolated glycoglycerolipid represents an attractive target for neosynthesis and derivatization of molecule domains. Development of analogues as lead structures is in continuation of our work to glycolipids and is based on our knowledge on neosynthesis of natural products. Here we describe the first total synthesis of the title compound in 13 steps which strategy is shown in Scheme 1.[7–9]

2. Results and discussion

Starting point of our synthesis was the commercially available and inexpensive α-methylglucopyranoside **1** which is characterized by stereoselective protection of the anomeric carbon atom.[10]

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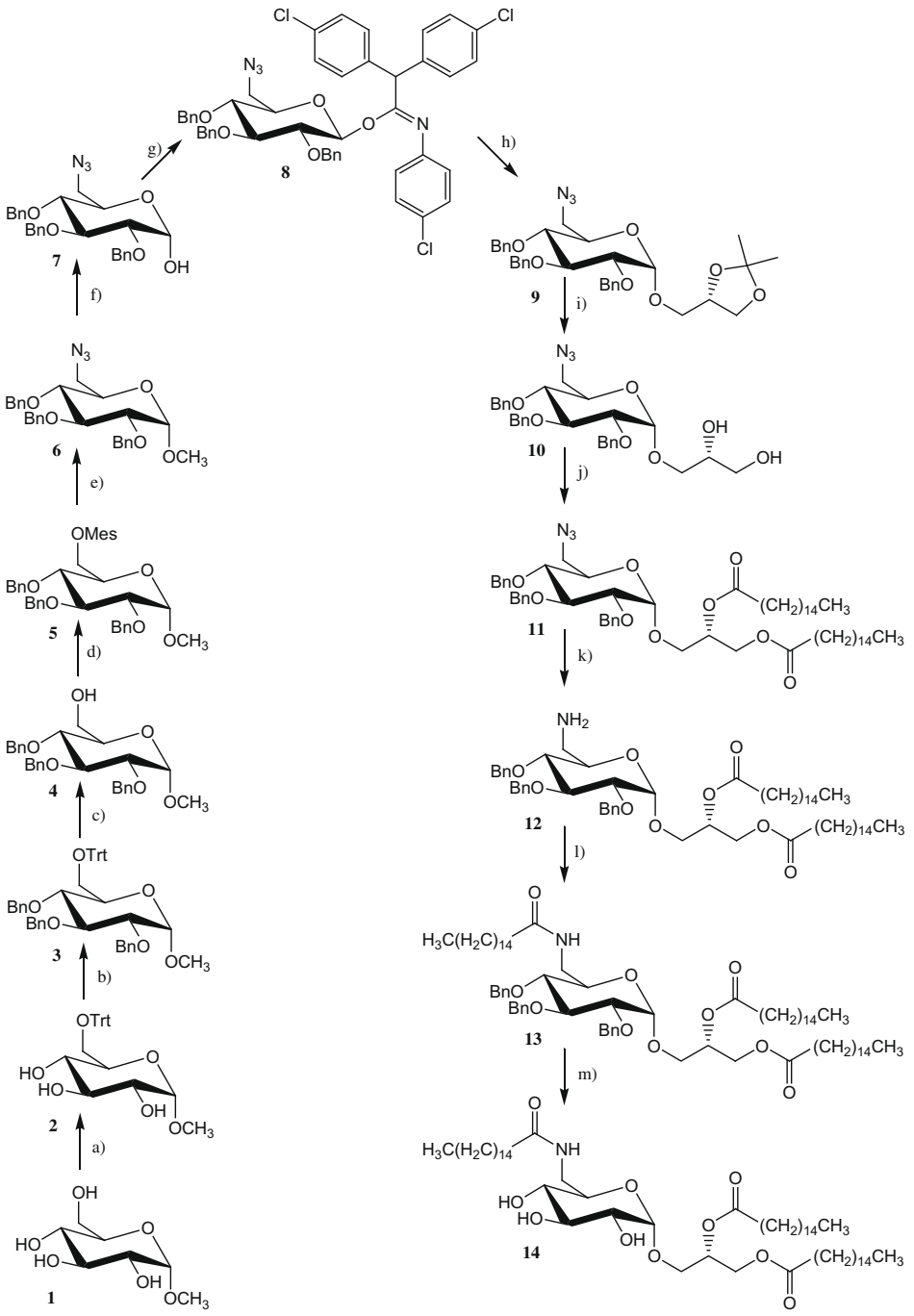

Scheme 1. 13-step synthesis of the compound. Reagents and conditions: (a) TrCl, py, DMAP, 80 °C/4 h, 77%; (b) BnCl, NaH, 123 °C/2 h, 95%; (c) BF_3–Et_2O, CH_2Cl_2, rt/1 h, 92%; (d) MsCl; TEA, CH_2Cl_2, −5 °C/1 h, 71%; (e) NaN_3, DMF, 90 °C/8 h, 95%; (f) H_2SO_4/AcOH, 80 °C/4 h, 65%; (g) NaH, *N*,2,2-tris-(4-chlorphenyl)ketenimine, CH_2Cl_2, rt/5 h, 51%; (h) (*S*)-1,2-isopropylideneglycerol, CH_2Cl_2, TMSOTf, −20 °C/0.5 h, 46%; (i) PPTS, MeOH, 65 °C/5 h, 82%; (j) DCC/palmitic acid, toluene, 50 °C/4 h, 67%; (k) triphenylphosphine, THF, rt/24 h, 95%; (l) PyBOP/palmitic acid, CH_2Cl_2, rt/40 h, 87%; (m) PdOH, THF/IPA, 20 bar/24 h, 95%.

A modified method for tritylation of the primary hydroxyl group with triphenylmethylchloride (TrCl) in absolute pyridine and catalytic amounts of DMAP leads to compound **2** as considerable intermediate for selective derivatization of position C6.[11] Benzylation of the secondary hydroxyl groups by use of a large excess of benzyl chloride (BnCl) in the presence of sodium hydride resulted in **3**.[12] Afterwards the trityl protecting group was removed with trifluoroborane etherate complex yielding compound **4** with a free hydroxyl group in position C6.[13] Conversion into **5** with methanesulfonyl chloride[14] affords the nucleophilic reaction with sodium azide to give **6**.[15]

On the one hand the azido group can be reduced to give a derivative with an amino group, so it is a key intermediate for the synthesis of amino glycosides. Otherwise the azido group can also be used as protective group during the following glycosylation. A requirement for glycosylation is deprotection of the anomeric C1 by acid hydrolysis of the methoxy group to get **7**. This was reached by a modified method with a mixture of sulfuric acid and acetic acid under gentle conditions.[16]

The stereoselective glycosylation of 1-O-unsubstitued hexoses depends on the base which is used. The reaction of derivative **7** as α-D-glucosyl donor with (*S*)-1,2-isopropylideneglycerol as

acceptor substrate was accomplished by activating the remaining free hydroxyl group by base-catalyzed addition of *N*,2,2-tris-(4-chlorphenyl)ketenimine resulting in the ß-glycosyl imidate **8** in good yields with a stereoselectivity of **8α**:**8β** = 26:74.[17,18] This activated intermediate reacted in an acid-catalyzed reaction with (*S*)-1,2-isopropylideneglycerol to form **9** in a mixture of α- and β-anomers at the rate of 78:22 that can be separated by MPLC to obtain both isomers. Thereby stereoselectivity of the glycosylation is influenced by choice of the Lewis acid catalyst. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) as strong Lewis acid apparently affords formation of the α-glycoside as thermodynamic stable form.[19] A better separation of the anomers can be achieved by immediate hydrolysis of the raw isopropylidene protecting group using pyridiniumtosylate (PPTS) in methanol to give **10** caused by the variation of the R_f-values concerning the anomer mixture of **9** and **10**, respectively. Acylation of the remaining free hydroxyl groups was realized using palmitic anhydride which was synthesized from the acid by DCC-method to get the intermediate **11** with two hydrophobic acyl chains. Reduction of the azido group using triphenylphosphine in absolute THF and hydrolysis yielded the appropriate amino derivative **12**. The next step comprised acylation of the remaining amino group with palmitic acid to insert the last chain (**13**). Therefore benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was used as coupling reagent which is suitable as gentle alternative to acylation with long-chain fatty acids. The final step was accomplished by catalytic hydrogenation using PdOH in THF/isopropylalcohol v/v 9:1 mixture under pressure to give the aspired product **14**.

^{1}H NMR studies of the synthetic compounds **14** enable us to establish the absolute structures having the α-linked monosaccharide at the glycerol sn-3 position. The data were completely in accord with the data for the natural product.

3. Conclusions

We describe the first total synthesis of 1,2-dipalmitoyl-3-(*N*-palmitoyl-6′-amino-6′-deoxy-α-D-glucosyl)-*sn*-glycerol in a multistep strategy starting from α-methylglucopyranoside. The strategy also allows the selective modification of defined structure moieties of the synthesized glycoglycerolipid. The aim of our prospective investigations is the systematical derivatization of the carbohydrate domain, variances of length of the fatty acids and substitution of the core-structure. The glycoglycerolipid **14**, first isolated from a marine alga extract, is described to inhibit human Myt1-kinase. So the retrosynthesis of the final compound as well as derivatization of partial molecule domains offers the development of new lead structures for potent and bioavailable inhibitors of Myt1-kinase. Because of their structure they should not show ATP competition contrary to known unspecific kinase inhibitors, for example, staurosporin and describe an innovative class of Myt1-kinase inhibitors.[20]

4. Experimental

4.1. General methods

Mass spectra (MS) were recorded off-line with nano-ESI (*Proxeon* emitters, Odense, Denmark) on a *LTQ-Orbitrap XL (Thermo Fisher Scientific, San Jose, USA*). ^{1}H NMR spectra were obtained with a *Varian Inova 500* spectrometer operating at 500 MHz; all values are reported in ppm (δ) downfield from solvent. Polarimetric measurements were accomplished by an *Eloptron/Polartronic E* (*Schmid+Haensch GmbH & Co.*). Chromatography was performed on silica gel (*Merck Silica Gel 60, 40–63 mesh*) by MPLC. Therefore columns were prepared with a *Cartriger C-670 (Büchi)*. Fractions were sampled with a *Fraction Collector C-660* (*Büchi*) by discontinuous enhancement of polarity, for pressure we used a *Pump Module C-601 a*nd a *Pump Manager C-615* (*Büchi*). TLC was carried out on silica gel plates (*E. Merck 60 F_{254}*); zones were detected visually by ultraviolet irradiation (254 nm) or by detection with spray (solution of 0.5 g thymol and 5 ml concd sulfuric acid in 100 ml ethanol) and heating at 130 °C for 10 min. All reagents were used as purchased unless otherwise stated. Solvents were dried, according to standard procedures. All reactions were carried out under an atmosphere of dry argon. All chemicals were purchased from *Sigma–Aldrich Chemie GmbH (Germany)*.

4.2. Chemistry

Synthesis of the compounds **2**–**7** was achieved by the modified published methods. Experimental data correspond with the literature.[11–16]

4.2.1. (6′-Azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-β-D-glucosyl)-*N*,2,2-tri(p-chlorophenyl)ethanimidoate (8)

To a solution of **7** (5 g/8.7 mmol) in CH_2Cl_2 (50 ml) was added NaH (3.48 g (60%)/87 mmol) and stirred for 0.5 h. To the reaction mixture was added *N*-2,2-tris-(4-chlorphenyl)ketenimine[17,18] (3.24 g/8.7 mmol) and stirred for 5 h at rt. The mixture was filtered, concentrated and purified by MPLC (heptanes/diethylether) to give 3.78 g of **8** (51%) as a yellow syrup.

4.2.2. 3-*O*-(6′-Azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucosyl)-1,2-isopropylidene-*sn*-glycerol (9)

The intermediate **8** (4 g/4.7 mmol) and (*S*)-1,2-isopropylideneglycerol (2.48 g/18.8 mmol) in CH_2Cl_2 (20 ml) were cooled at −20 °C. To the reaction mixture was added trimethylsilyl trifluormethansulfonate as catalyst (1.8 mmol) and stirred for 0.5 h. For neutralization eq. TEA was added and washed with a saturated $NaHCO_3$ solution and water. The organic layer was dried over Na_2SO_4, filtered and concentrated. Purification was accomplished by MPLC ($CHCl_3$/MeOH) to give 1.32 g of **9** in yields of 46% as a white waxy substance.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.35 (s, 3H, −C**H$_3$**), 1.41 (s, 3H, −C**H$_3$**), 3.31 (dd, 1H, **H-6**, $J_{5,6}$ 5.5 Hz, $J_{6,6'}$ 12.8 Hz), 3.39–3.47 (m, 2H, **H-4**, **H-6′**), 3.51–3.56 (m, 2H, **H-2**, **H*sn*-1**), 3.62 (dd, 1H, **H*sn*-1′**, $J_{1,2}$ 5.5 Hz, $J_{1,1'}$ 10.7 Hz), 3.73 (dd, 1H, **H*sn*-3**, $J_{2,3}$ 6.0 Hz, $J_{3,3'}$ 8.5 Hz), 3.78–3.82 (m, 1H, **H-5**), 3.94 (t, 1H, **H-3**, $J_{2,3} = J_{3,4}$ 9.0 Hz), 4.06 (dd, 1H, **H*sn*-3′**, $J_{2,3'}$ 6.4 Hz, $J_{3,3'}$ 8.2 Hz), 4.34 (m, 1H, **H*sn*-2**), 4.86 (d, 1H, **H-1**, $J_{1,2}$ 3.4 Hz), 4.55–4.98 (m, 6H, –C**H$_2$**–Ph), 7.21–7.35 (m, 15H, –C$_6$**H$_5$**); MALDI TOF mass spectrum: *m*/*z* 612 (M+Na$^+$). HRFABMS *m*/*z* 612.2663 [M+Na$^+$]; (calcd for $C_{33}H_{39}N_3O_7$ 612.2680). $[\alpha]_D^{22}$ +58.50 (*c* 11.96, $CHCl_3$).

4.2.3. 3-*O*-(6′-Azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucosyl)-*sn*-glycerol (10)

The glycoside **9** (1 g/1.7 mmol) was dissolved in MeOH (50 ml) and pyridiniumtosylate (0.85 g/3.4 mmol) was added. The reaction mixture was stirred for 5 h at 65 °C. Then the solution was concentrated and dissolved in CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, filtered and concentrated. Purification was achieved by MPLC (heptane/diethylether) to give 797 mg of **10** (82%) as a colourless waxy substance.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.31 (dd, 1H, **H-6**, $J_{5,6}$ 5.7 Hz, $J_{6,6'}$ 13.1 Hz), 3.39–3.46 (m, 3H, **H-4**, **H-6′**, **H*sn*-1**), 3.53 (dd, 1H, **H-2**, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.7 Hz), 3.57–3.77 (m, 2H, **H*sn*-3**), 3.79–3.87 (m, 1H, **H-5**), 3.84–3.87 (m, 2H, **H*sn*-1′**, **H*sn*-2**), 3.93 (t, 1H, **H-3**, $J_{2,3} = J_{3,4}$ 9.0 Hz), 4.72 (d, 1H, **H-1**, $J_{1,2}$ 3.7 Hz), 4.55–4.95 (m, 6H, –C**H$_2$**–Ph), 7.23–7.33 (m, 15H, –C$_6$**H$_5$**); MALDI TOF mass spectrum: *m*/*z* 572 (M+Na$^+$). HRFABMS *m*/*z* 572.23673 [M+Na$^+$]; (calcd for $C_{30}H_{35}N_3O_7$ 572.23672). $[\alpha]_D^{22}$ +35.71 (*c* 0.56, $CHCl_3$).

4.2.4. 1,2-Dipalmitoyl-3-*O*-(6′-azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucosyl)-*sn*-glycerol (11)

To a solution of **10** (440 mg/0.78 mmol) in toluene (10 mL) were added palmitic acid anhydride (1.56 mmol) in toluene (10 ml), that was prepared from palmitic acid by DCC-method in CCl_4, and catalytical amounts of DMAP. The reaction mixture was stirred at 50 °C. After 4 h DCC (160 mg/0.78 mmol) was added again and the reaction mixture was stirred for 12 h at rt. The mixture was filtered, washed with water and satd NH_4Cl solution, dried over Na_2SO_4 filtered and concentrated under diminished pressure. The residue was purified by MPLC (heptane/diethylether) to give 548 mg of **11** (67%) as a white paste-like substance. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86 (t, 6H, –C**H$_3$**, J 7.0 Hz), 1.10–1.30 (m, 48H, 2×–CH_2–CH_2–(C**H$_2$**)$_{12}$–CH_3), 1.55–1.61 (m, 4H, 2×–CO–CH_2–C**H$_2$**–),2.25–2.30 (m, 4H, 2×–CO–C**H$_2$**–), 3.29 (dd, 1H, **H-6**, $J_{5,6}$ 5.5 Hz, $J_{6,6'}$ 13.0 Hz), 3.39–3.44 (m, 2H, **H-4**, **H-6′**), 3.49–3.59 (m, 2H, **H-2**, **H$_{sn\text{-}1}$**), 3.73–3.79 (m, 2H, **H-5**, **H$_{sn\text{-}1'}$**), 3.92 (t, 1H, **H-3**, $J_{2,3} = J_{3,4}$ 9.3 Hz), 4.18 (dd, 1H, **H$_{sn\text{-}3}$**, $J_{2,3}$ 6.0 Hz, $J_{3,3'}$ 12.0 Hz), 4.35–4.41 (m, 1H, **H$_{sn\text{-}3'}$**), 4.72 (d, 1H, **H-1**, $J_{1,2}$ 3.3 Hz), 4.53–4.96 (m, 6H, –C**H$_2$**–Ph), 5.21–5.25 (m, 1H, **H$_{sn\text{-}2}$**), 7.22–7.33 (m, 15H, –C_6**H$_5$**); MALDI TOF mass spectrum: m/z 1049 (M+Na^+). HRFABMS m/z 1048.69763 [M+Na^+]; (calcd for $C_{62}H_{95}N_3O_9$ 1048.69605). $[\alpha]_D^{22}$ +46.08 (c 4.34, $CHCl_3$).

4.2.5. 1,2-Dipalmitoyl-3-*O*-(6′-amino-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucosyl)-*sn*-glycerol (12)

To a solution of **11** (320 mg/0.312 mmol) in THF (20 mL) was added triphenylphosphine (327 mg/1.25 mmol). The reaction mixture was stirred at rt for 24 h. To the reaction mixture was added water and stirred again for another 24 h at rt. The mixture was evaporated, dissolved in heptane to remove triphenyloxide by filtration, washed with brine, dried over Na_2SO_4 filtered and concentrated under diminished pressure. The residue was purified by MPLC ($CHCl_3$/MeOH) to give 297 mg of **12** (95%) as a white paste-like substance. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86 (t, 6H, –C**H$_3$**, J 7.0 Hz), 1.10–1.40 (m, 48H, 2×–CH_2–CH_2–(C**H$_2$**)$_{12}$–CH_3), 1.65–1.83 (m, 4H, 2×–CO–CH_2–C**H$_2$**–), 2.25–2.40 (m, 4H, 2×–CO–C**H$_2$**–), 2.71 (dd, 1H, **H-6**, $J_{5,6}$ 6.0 Hz, $J_{6,6'}$ 13.0 Hz), 2.95 (dd, 1H, **H-6′**, $J_{5,6'}$ 3.0 Hz, $J_{6,6'}$ 13.0 Hz), 3.33 (t, 1H, **H-4**, $J_{3,4} = J_{4,5}$ 9.2 Hz), 3.47 (dd, 1H, **H-2**, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.0 Hz), 3.53–3.58 (m, 1H, **H-5**), 3.53 (dd, 1H, **H$_{sn\text{-}1}$**, $J_{1,2}$ 6.0 Hz, $J_{1,1'}$ 11.0 Hz), 3.72 (dd, 1H, **H$_{sn\text{-}1'}$**, $J_{1',2}$ 6.0 Hz, $J_{1,1'}$ 11.0 Hz), 3.93 (t, 1H, **H-3**, $J_{2,3} = J_{3,4}$ 9.2 Hz), 4.18 (dd, 1H, **H$_{sn\text{-}3}$**, $J_{2,3}$ 6.0 Hz, $J_{3,3'}$ 12.0 Hz), 4.39 (dd, 1H, **H$_{sn\text{-}3'}$**, $J_{2,3'}$ 3.0 Hz, J3,3′ 12.0 Hz), 4.69 (d, 1H, **H-1**, $J_{1,2}$ 3.7 Hz), 4.59–4.97 (m, 6H, –C**H$_2$**–Ph), 5.20–5.25 (m, 1H, **H$_{sn\text{-}2}$**), 7.24–7.67 (m, 15H, –C_6**H$_5$**); MALDI TOF mass spectrum: m/z 1001 (M+H^+). HRFABMS m/z 1000.72382 [M+H^+]; (calcd for $C_{62}H_{97}NO_9$ 1000.72361). $[\alpha]_D^{22}$ +37.62 (c 3.19, $CHCl_3$).

4.2.6. 1,2-Dipalmitoyl-3-(*N*-palmitoyl-6′-amino-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucosyl)-*sn*-glycerol (13)

To a solution of **12** (200 mg/0.2 mmol) in CH_2Cl_2 (10 ml) were added PyBOP (100 mg/0.2 mmol) and palmitic acid (50 mg/0.2 mmol). The reaction mixture was stirred for 21 h at rt. CH_2Cl_2 was evaporated under diminished pressure and the residue was purified by MPLC (heptanes/diethylether) to give 216 mg of **13** (87%) as a white waxy substance. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86 (t, 9H, –C**H$_3$**, J 7.0 Hz), 1.12–1.42 (m, 72H, 3×–CH_2–CH_2–(C**H$_2$**)$_{12}$–CH_3), 1.51–1.64 (m, 4H, 3×–CO–CH_2–C**H$_2$**–), 2.07–2.12 (m, 2H, –NH–CO–C**H$_2$**–), 2.25–2.30 (m, 4H, 2×–C**H$_2$**–COO–), 3.26 (t, 1H, **H-4**, $J_{3,4} = J_{4,5}$ 9.4 Hz), 3.31–3.35 (m, 1H, **H-6**), 3.45 (dd, 1H, **H-2**, $J_{1,2}$ 3.4 Hz, $J_{2,3}$ 9.7 Hz), 3.55 (dd, 1H, **H$_{sn\text{-}1}$**, $J_{1,2}$ 5.4 Hz, $J_{1,1'}$ 11.2 Hz), 3.62–3.77 (m, 3H, **H-6′**, **H-5**, **H$_{sn\text{-}1'}$**), 3.93 (t, 1H, **H-3**, $J_{2,3} = J_{3,4}$ 9.0 Hz), 4.17 (dd, 1H, **H$_{sn\text{-}3}$**, $J_{2,3}$ 6.1 Hz, $J_{3,3'}$ 12.0 Hz), 4.38 (dd, 1H, **H$_{sn\text{-}3'}$**, $J_{2,3'}$ 3.6 Hz, $J_{3,3'}$ 11.9 Hz), 4.68 (d, 1H, **H-1**, $J_{1,2}$ 3.6 Hz), 4.60–4.95 (m, 6H, –C**H$_2$**–Ph), 5.15–5.25 (m, 1H, **H$_{sn\text{-}2}$**), 5.59–5.64 (m, 1H, –N**H**–CO–), 7.17–7.32 (m, 15H, –C_6**H$_5$**); MALDI TOF mass spectrum: m/z 1239 (M+H^+). HRFABMS m/z 1238.95190 [M+H^+]; (calcd for $C_{78}H_{127}NO_{10}$ 1238.95328). $[\alpha]_D^{22}$ +13.57 (c 10.32, $CHCl_3$).

4.2.7. 1,2-Dipalmitoyl-3-(*N*-palmitoyl-6′-amino-6′-deoxy-α-D-glucosyl)-*sn*-glycerol (14)

The intermediate **13** (124 mg/0.1 mmol) was dissolved in 50 ml THF/IPA (90:10) and removal of the *O*-benzyl groups was achieved by palladium hydroxide-catalyzed hydrogenolysis (PdOH, 20 bar/24 h). After filtration the solvents were evaporated and the residue was purified by MPLC ($CHCl_3$/MeOH) to provide 92 mg of the final compound **14** (95%) as a white paste-like substance. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86 (t, 9H, –C**H$_3$**, J 7.1 Hz), 1.21–1.29 (m, 72H, 36×–C**H$_2$**–),1.60 (m, 6H, –C**H$_2$**–CH_2–CO–), 2.18–2.27 (m, 6H, –C**H$_2$**CO–), 3.01 (dd, 1H, **H-6**, $J_{5,6}$ 7.4 Hz, $J_{6,6'}$ 14.9 Hz), 3.07 (dd, 1H, **H-4**, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 3.46 (dd, 1H, **H-6′**, $J_{5,6'}$ 3.9 Hz, $J_{6,6'}$ 14.8 Hz), 3.56 (dd, 1H, **H-2**, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 9.5 Hz), 3.61 (dd, 1H, **H$_{sn\text{-}3}$**, $J_{2,3}$ 6.0 Hz, $J_{3,3'}$ 11.0 Hz), 3.71 (dd, 1H, **H-3**, $J_{2,3}$ 9.4, Hz, $J_{3,4}$ 9.7 Hz), 3.77 (dd, 1H, **H$_{sn\text{-}3'}$**, $J_{2,3'}$ 4.6 Hz, $J_{3,3'}$ 11.0 Hz), 4.02 (ddd, 1H, **H-5**, $J_{5,6'}$ 3.8 Hz, $J_{5,6}$ 7.4 Hz, $J_{4,5}$ 9.5 Hz), 4.11 (dd, 1H, **H$_{sn\text{-}1}$**, $J_{1,2}$ 5.9 Hz, $J_{1,1'}$ 11.9 Hz), 4.36 (dd, 1H, **H$_{sn\text{-}1'}$**, $J_{1',2}$ 4.2 Hz, $J_{1,1'}$ 11.8 Hz), 4.78 (d, 1H, **H-1**, $J_{1,2}$ 4.1 Hz), 5.22 (m, 1H, **H$_{sn\text{-}2}$**), 5.83 (m, 1H, –N**H**–CO–); MALDI TOF mass spectrum: m/z 990 (M+Na^+). HRFABMS m/z 968.8121 [M+H^+]; (calcd for $C_{57}H_{110}NO_{10}$ 968.8130). $[\alpha]_D^{22}$ +12.38 (c 0.32, $CHCl_3$).

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