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Synthesis of pseudo-disaccharide analogues of lipid A: haptens for the generation of antibodies with glycosidase activity towards lipid A

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Citation

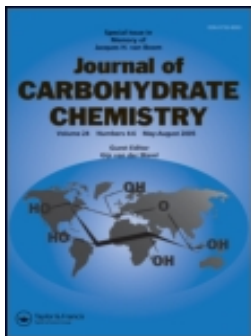
Berg, R. J. B. H. N. van den, Noort, D., Marel, G. A. van der, Boom, J. H. van, & Benschop, H. P. (2002). Synthesis of pseudo-disaccharide analogues of lipid A: haptens for the generation of antibodies with glycosidase activity towards lipid A. *Journal Of Carbohydrate Chemistry*, 21(3), 167-188. doi:10.1081/CAR-120004331

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To cite this article: Richard J.B.H.N. van den Berg , Daan Noort , Gijs A. van der Marel , Jacques H. van Boom & Hendrik P. Benschop (2002) SYNTHESIS OF PSEUDO-DISACCHARIDE ANALOGUES OF LIPID A: HAPTENS FOR THE GENERATION OF ANTIBODIES WITH GLYCOSIDASE ACTIVITY TOWARDS LIPID A, Journal of Carbohydrate Chemistry, 21:3, 167-188, DOI: [10.1081/CAR-120004331](https://doi.org/10.1081/CAR-120004331)

To link to this article: <https://doi.org/10.1081/CAR-120004331>



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J. CARBOHYDRATE CHEMISTRY, 21(3), 167–188 (2002)

SYNTHESIS OF PSEUDO-DISACCHARIDE ANALOGUES OF LIPID A: HAPTENS FOR THE GENERATION OF ANTIBODIES WITH GLYCOSIDASE ACTIVITY TOWARDS LIPID A

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ABSTRACT

In order to develop a generic treatment of sepsis caused by infections with Gram-negative bacteria, a series of pseudo-disaccharide analogues of lipid A (**1–5**) was synthesized. These adducts not only harbor a 2-acylamino-dideoxynojirimycin unit mimicking the transition state of the glycosidic hydrolysis, but also a 2-*N*, 3-*O*-diacylated glucosamine moiety capable of generating catalytic antibodies with more selective glycosidase properties towards lipid A.

INTRODUCTION

Endotoxins, the complex lipopolysaccharides (LPS) situated in the outer membrane of Gram-negative bacteria, are extremely potent toxins.^[1] Most of the biological activities of LPS reside in the small terminal disaccharide phospholipid moiety known

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as lipid A.^[2] Therapeutic strategies under development aim at either preventing endotoxin interaction with host effector cells or interrupting endotoxin mediated signal transduction pathways. This objective can be attained in blocking the synthesis and binding of endotoxin, thus neutralizing its activity.^[3–5]

Our approach to suppress sepsis caused by Gram-negative bacteria is based on catalytic antibodies capable of degrading lipid A via hydrolysis of the interglycosidic bond resulting in the formation of non-toxic monosaccharides. Since catalytic antibodies are supposed to have catalytic activities with tailor-made specificities, these abzymes have many potential therapeutically applications. For example, Landry et al. succeeded in the generation of a catalytic antibody that was effective in detoxifying cocaine from the blood stream via hydrolysis of the benzoyl ester function.^[6–9] Based on this result it was envisioned that a generic treatment of sepsis caused by Gram-negative bacteria might be feasible using specific and selective glycosidase antibodies capable of degrading lipid A.

Several groups have reported the design and generation of antibodies with glycosidase activity.^[10–16] In general, haptens are based on iminocyclitol glycosidase inhibitors such as deoxynojirimycin and isofagomine, which in terms of polarity and shape resemble the transition state of the glycosidic cleavage reaction. The protonated endocyclic nitrogen atom of iminocyclitols mimics the electronic charge developing in the transition state formed during cleavage of the interglycosidic bond. In a previous paper^[17] we reported the preparation of 2-acylaminodideoxynojirimycin derivatives mimicking the transition state of the hydrolysis of the interglycosidic bond at the non-reducing end of lipid A. It turned out that monoclonal antibodies raised against these haptens showed promising glycosidase activity (results to be published).

It was envisaged, based on the early studies by Dong^[18] as well as Yu,^[14] that conjugation of 2-*N*, 3-*O*-diacylated glucosamine derivatives to 2-acylaminodideoxynojirimycin units would afford haptens suitable to raise specific glycosidase antibodies towards lipid A. The latter can be achieved by anchoring the 2-*N*, 3-*O*-diacylated glucosamine units, which mimic the reducing part of lipid A, to the endocyclic nitrogen atom of the iminoglucitol moieties via a flexible linker.

We here report the synthesis of several pseudo-disaccharide analogues of lipid A (i.e. compounds **1–5**, Figure 1) containing 2-acylaminodideoxynojirimycins as well as 2-*N*, 3-*O*-diacylated glucosamine units.

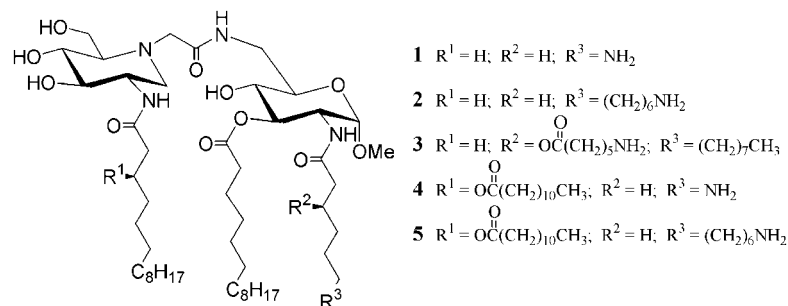
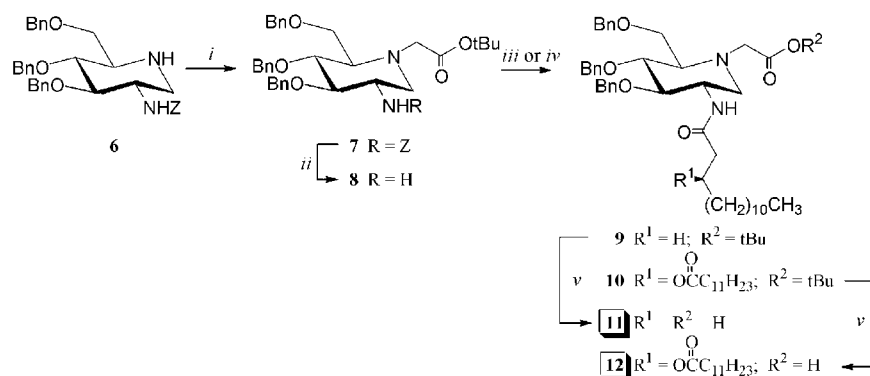


Figure 1.

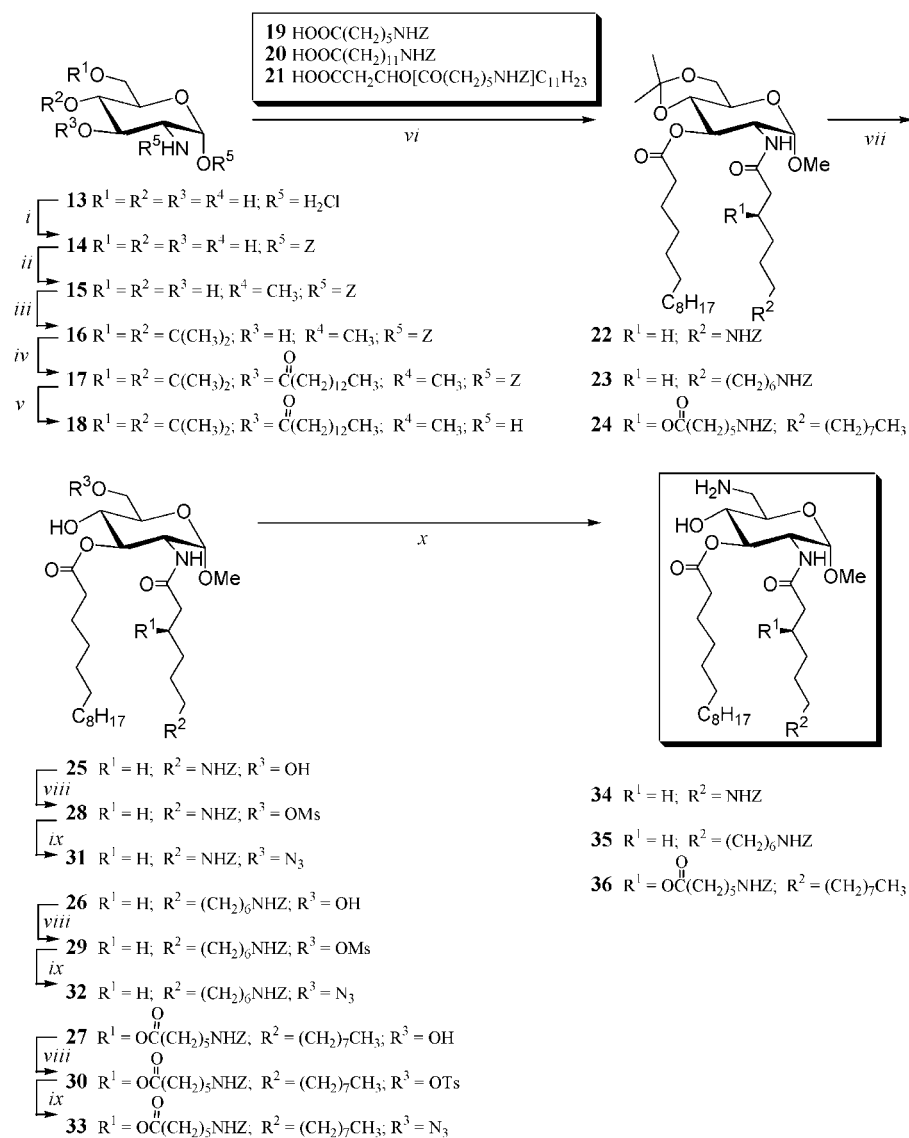
RESULTS AND DISCUSSION

The easily accessible 3,4,6-tri-*O*-benzyl-2-*N*-benzyloxycarbonylamino-1,2,5-tri-deoxy-1,5-iminoglucitol (**6**)^[17] was used as a starting compound for the preparation of iminocyclitols **11** and **12** (Scheme 1) mimicking the non-reducing part of lipid A. In the first step, the carboxymethyl linker in **7** was introduced by alkylation of the endocyclic nitrogen atom of **6** with *tert*-butyl bromoacetate under the agency of cesium carbonate in dimethyl formamide^[19] to give compound **7** in a yield of 95%. Selective removal of the benzyloxycarbonyl (*Z*) protecting group in **7** proceeded smoothly by hydrogenation over Degussa type palladium on carbon to give **8** in a quantitative yield. PyBOP mediated coupling^[20] of amine **8** with myristic acid and (*R*)-3-dodecanoxytetradecanoic acid^[21] gave the *N*-acylated derivatives **9** and **10**, respectively, in good yield. Removal of the *tert*-butyl group in compounds **9** and **10** was readily effected by treatment with neat trifluoroacetic acid, affording building blocks **11** and **12** in 100% and 83% yield, respectively.

The individual hapten units **34**, **35** and **36**, mimicking the reducing part of lipid A, were obtained by subjecting D-glucosamine (**13**) to the sequence of reactions depicted in Scheme 2. Accordingly, D-glucosamine (**13**) was converted in two steps into *N*-benzyloxycarbonyl protected glucosamine **15**.^[22] Acetonation of **15** with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid gave partially protected derivative **16** in a quantitative yield. Condensation of the secondary hydroxyl group in **16** with myristic acid under the agency of DCC^[23] afforded **17** in a yield of 86%. Removal of the *Z*-protecting group in **17** by hydrogenation over palladium on carbon in ethyl acetate gave amine **18** in a quantitative yield. PyBOP mediated condensation of amine **18** with the individual acids **19**–**21**, of which the primary amine function was protected with the *Z*-group, afforded the corresponding derivatives **22**–**24**. At this stage, *N*-acylated compound **22** was transformed into the primary amino derivative **34** by following the four-step process as portrayed in Scheme 2. Thus, de-acetonation of compound **22** with trifluoroacetic acid in aqueous tetrahydrofuran,^[24] followed by



Scheme 1. Conditions: (i) *tert*-butyl bromoacetate, Cs₂CO₃, DMF, 95%; (ii) EtOAc, 5% Pd/C, H₂, 100%; (iii) myristic acid, PyBOP, DiPEA, DCM, 89%; (iv) (*R*)-3-HOOCCH₂CHO (COC₁₁H₂₃)C₁₁H₂₃, PyBOP, DiPEA, DCM, 70%; (v) TFA (**11**, 100%), (**12**, 83%).

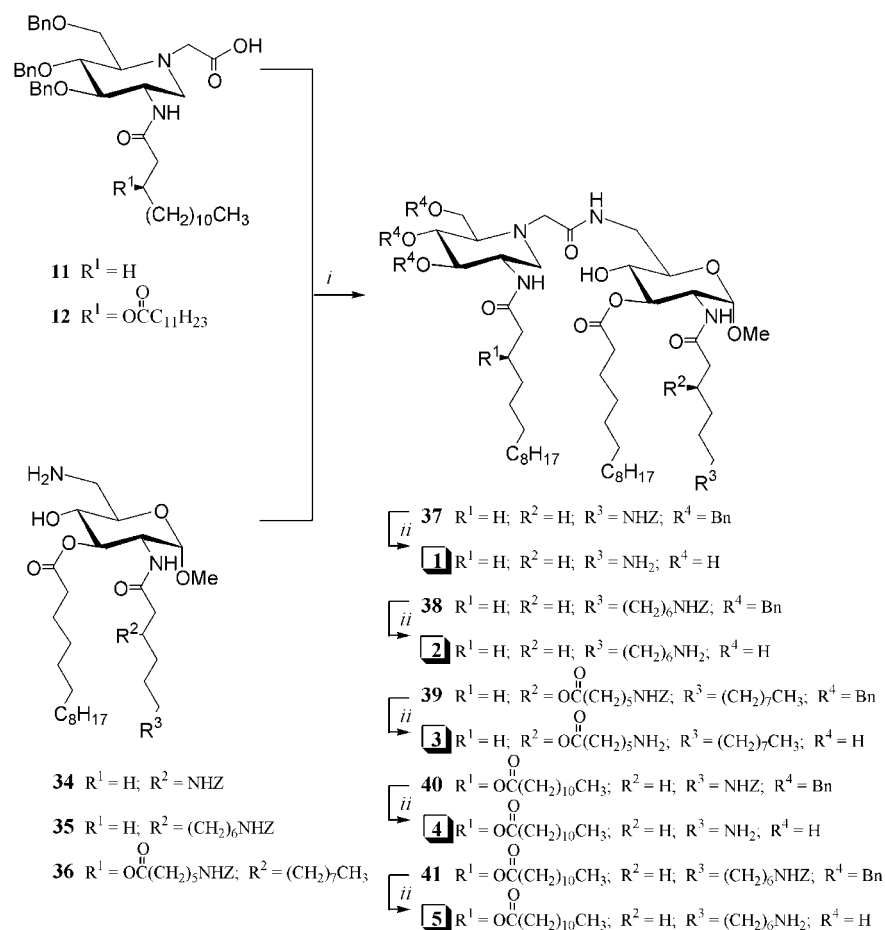


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regio-selective sulfonylation of the primary hydroxyl group of diol **25** gave sulfonylate **28** in an overall yield of 80%. Treatment of the latter derivative with sodium azide in dimethyl formamide at elevated temperature afforded the 6-azidoglucosamine compound **31**. Subsequent reduction of the resulting azido function in **31** under the agency of triphenylphosphine in aqueous tetrahydrofuran^[25] gave the requisite 6-amino derivative **34** in yield of 84% based on sulfonylate **28**. In a similar way compounds **35** and **36** were attained by subjecting acetonides **23** and **24** to the same four-step process as described for the synthesis of **34**.

Having the requisite building blocks **11**, **12**, **34**–**36** at hand, introduction of the required amide bond of the fully protected compounds **37**–**41** could be readily accomplished (see Scheme 3) using PyBOP as the condensation agent. For example, PyBOP-mediated condensation of acid **11** and amine **34** proceeded smoothly to afford the fully protected haptin **37** in a yield of 96%. The identity and homogeneity of compound **37**



Scheme 3. Conditions: (i) PyBOP, DiPEA, DCM, (**37**, 96%), (**38**, 96%), (**39**, 51%), (**40**, 88%), (**41**, 96%); (ii) 10% Pd/C, DMF (**1**, 100%), (**2**, 72%), (**3**, 54%), (**4**, 88%), (**5**, 65%).



was fully ascertained by NMR spectroscopy and mass spectrometry. Similar yields were obtained by condensation of **11** with **35** and **12** with **34** as well as **35** to yield the fully protected haptens **38**, **40** and **41**, respectively. In contrast, the coupling of acid **11** with amine **36** to give amide **39** was in terms of yield not fully satisfactory, and may be ascribed to the increased lipophilicity of amine **36**. ^1H , ^{13}C NMR and mass spectrometric data of the four conjugates **38–41** were in complete accordance with the proposed structures. In the final stage, haptens **37–41** were deprotected by hydrogenolysis over palladium on carbon in dimethyl formamide. Hydrogenolysis of compound **37** proceeded in near quantitative yield to give hapten **1**, the identity and homogeneity of which was fully ascertained by NMR spectroscopy and mass spectrometry. The same results were obtained by hydrogenolysis of compounds **38** and **40** to yield the haptens **2** and **4**, respectively. Unfortunately, unmasking of compounds **39** and **41** was rather sluggish and led to the isolation of haptens **3** and **5** in moderate yields. The disappointing outcome of the latter hydrogenolysis may also be due to the intrinsically high lipophilic nature of compounds **39** and **41**. The identity of compounds **2–5** could be readily ascertained by mass spectrometry. Unfortunately, it turned out that the structure assignment of haptens **2–5** was seriously hampered by the fact that NMR spectra^[26] could not be interpreted due to extensive line broadening.

CONCLUSION

In summary, we have synthesized five pseudo-lipid A analogues as potential haptens for the generation of catalytic antibodies with glycosidase activity towards lipid A. These haptens contain a primary amino function in the *N*-acyl chain of the 2-*N*, 3-*O*-diacylated glucosamine units which will serve as a handle of anchoring haptens **1–5** to a carboxylic acid terminus of a carrier protein. The immunochemical evaluation of the haptens will be reported in due course.

EXPERIMENTAL

General Methods. Toluene (Merck) was distilled from P_2O_5 and stored over sodium wire. Dichloromethane and *N,N*-dimethylformamide were purchased from Biosolve Ltd. and freshly distilled from CaH_2 . *N,N*-Diisopropylethylamine (Acros Chimica) was distilled from *p*-toluenesulfonyl chloride (60 g/L) and redistilled from potassium hydroxide pellets (40 g/L). Benzyl chloroformate, *tert*-butyl bromoacetate, cesium carbonate, *N,N*-dicyclohexylcarbodiimide, 4-(dimethylamino)pyridine, 2,2-dimethoxypropane, D-glucosamine hydrochloride, methanesulfonyl chloride, palladium on carbon (5%, Degussa E101 NO/W), sodium azide, tetrahydrofuran, toluene-*p*-sulfonic acid, toluene-*p*-sulfonyl chloride and triphenylphosphine were purchased from Fluka. PyBOP was purchased from NovaBiochem. Trifluoroacetic acid was purchased from Acros Chimica. ^1H NMR and ^{13}C NMR data were recorded with a Varian VXR-400S (399.9/100.6 MHz). ^1H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane ($\delta=0.00$), DMSO- d_5 ($\delta=2.525$), DMSO- d_6 ($\delta=39.6$) and CDCl_3 ($\delta=77.00$) as internal standard. The purity of the compounds was established by ^1H NMR spectroscopy: >95% in all cases. Mass spectra were recorded with a VG Quattro II triple



quadropole mass spectrometer (Fisons Instruments, Altrincham, UK). Column chromatography was performed on Silica gel 60 (220–440 mesh ASTM, Fluka). TLC analysis was performed with silica gel TLC plates (Fluka) with detection by UV absorption (254 nm) where applicable and charring with 20% H₂SO₄ in MeOH or ammonium molybdate (25 g/L) and ceric ammonium sulfate (10 g/L) in 20% H₂SO₄. Prior to reactions that require anhydrous conditions, traces of water were removed by co-evaporation with dry toluene. These reactions were conducted under dry argon atmosphere. Hydrogenations were executed at atmospheric pressure under an atmosphere of hydrogen gas maintained by an inflated balloon. Polytetrafluoroethylene (PTFE) filters were purchased from Alltech (Breda, The Netherlands).

3,4,6-Tri-*O*-benzyl-2-[(benzyloxycarbonyl)amino]-1,5-*N*-[(*O*-*tert*-butyl-carboxymethyl)imino]-1,2,5-trideoxy-D-glucitol (7). To a solution of **6** (1.20 g, 2.12 mmol) in DMF (20 mL) cesium carbonate (700 mg, 2.14 mmol) and *tert*-butyl bromoacetate (1.15 mL, 7.81 mmol) were added. The reaction mixture was stirred for 16 h at ambient temperature, after which TLC analysis indicated complete conversion of starting material into a compound with $R_f=0.92$ (ethyl acetate/hexane, 1:1, v/v). The mixture was diluted with DCM (100 mL) and washed with aqueous NaOH (1 M, 50 mL). After drying over MgSO₄, the organic layer was concentrated in vacuo. The crude product was purified by silica gel column chromatography. Elution was performed with DCM/MeOH (100:0 → 96.5:3.5, v/v). Yield 1.37 g (95%). ¹H NMR (CDCl₃): δ = 1.37 (s, 9H, CH₃, *t*Bu), 2.71 (dd, 1H, H-1ax, $J_{1ax,1eq}=11.6$ Hz, $J_{1ax,2}=8.9$ Hz), 3.04 (br. s, 1H, H-5), 3.10 (br. d, 1H, H-1eq), 3.27 (d, 1H, Ha-acetyl, $J=17.7$ Hz), 3.35 (br. t, 1H, H-3), 3.52 (dd, 1H, H-6, $J_{6,6'}=10.5$ Hz, $J_{5,6}=2.8$ Hz), 3.57 (d, 1H, Hb-acetyl, $J=17.7$ Hz), 3.59 (t, 1H, H-4), 3.72 (dd, 1H, H-6', $J_{6,6'}=10.5$ Hz, $J_{5,6'}=3.9$ Hz), 3.79 (m, 1H, H-2), 4.40–4.76 (m, 6H, 3 × CH₂ Bn), 4.89 (s, 1H, NH), 5.05 (dd, 2H, CH₂ Z), 7.18–7.39 (m, 20H, CH-arom Bn/Z). ¹³C{¹H} NMR (CDCl₃): δ = 28.16 (CH₃ *t*Bu), 50.88 (C-2), 53.38 (C-1), 55.08 (CH₂ *tert*-butyl acetate), 61.61 (C-5), 66.02 (C-6), 66.51 (CH₂ Z), 73.39, 73.66, 73.97 (3 × CH₂ Bn), 78.39 (C-4), 80.90 (Cq *t*Bu), 81.43 (C-3), 127.58–128.50 (CH-arom Bn/Z), 156.00 (C=O Z), 170.63 (C=O *tert*-butyl acetyl). ES-MS; m/z : 681.5, [M+H]⁺; monoisotopic MW calculated for C₄₁H₄₈N₂O₇ = 680.35.

2-Amino-3,4,6-tri-*O*-benzyl-1,5-*N*-[(*O*-*tert*-butyl-carboxymethyl)imino]-1,2,5-trideoxy-D-glucitol (8). Pd/C (5%, Degussa type E101 NO/W, 100 mg) was added to a solution of **7** (109 mg, 0.160 mmol) in ethyl acetate (5 mL). Hydrogen was passed through the stirred mixture for 1 h, after which TLC analysis indicated the complete conversion of starting material into a compound with $R_f=0.20$ (MeOH/DCM, 5:95, v/v). The mixture was passed over a short column containing a layer of glass wool and a layer of hyflo[®] and, finally, over a PTFE filter. Concentration of the filtrate in vacuo yielded **8** as a white solid (94 mg; 100%). ¹H NMR (CDCl₃): δ = 1.48 (s, 9H, CH₃, *t*Bu), 1.77 (br. s, 2H, NH₂), 2.72 (t, 1H, H-1ax), 2.92 (m, 3H, H-1eq, H-2, H-5), 3.16 (t, 1H, H-3 $J=8.9$ Hz), 3.30 (d, 1H, Ha-acetyl, $J=17.6$ Hz), 3.51 (t, 1H, H-4, $J=9.1$ Hz), 3.55 (dd, 1H, H-6), 3.59 (d, 1H, Hb-acetyl, $J=17.6$ Hz), 3.72 (dd, 1H, H-6', $J_{6,6'}=10.6$ Hz, $J_{5,6'}=3.2$ Hz), 4.44–4.94 (m, 6H, 3 × CH₂ Bn), 7.18–7.35 (m, 15H, CH-arom Bn). ¹³C{¹H} NMR (CDCl₃): δ = 28.22 (CH₃ *t*Bu), 52.64 (C-2), 54.43 (CH₂ *tert*-butyl acetate), 57.78 (C-1), 62.35 (C-5), 65.90 (C-6), 73.50, 74.71, 75.10 (3 × CH₂ Bn), 79.67 (C-4), 81.00 (Cq *t*Bu), 88.52 (C-3), 127.59–128.52 (CH-arom Bn), 137.70, 138.50,

138.78 ($3 \times \text{Cq Bn}$), 170.55 (C=O *tert*-butyl acetyl). ES-MS; m/z : 547.5, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{33}\text{H}_{42}\text{N}_2\text{O}_5 = 546.31$.

3,4,6-Tri-*O*-benzyl-1,5-*N*-[(*O*-*tert*-butyl-carboxymethyl)imino]-2-(tetradecanoyl)amino-1,2,5-trideoxy-*D*-glucitol (9). To a stirred mixture of myristic acid (92 mg, 0.403 mmol), PyBOP (231 mg, 0.605 mmol) and DiPEA (76 μL , 0.443 mmol) in DCM (10 mL) a solution of **8** (200 mg, 0.366 mmol) in DCM (10 mL) was added. After 30 min, TLC analysis indicated the complete conversion of starting material into a compound with $R_f = 0.89$ (MeOH/DCM, 5:95, v/v). The reaction mixture was diluted with DCM (100 mL) and washed with water (1×50 mL). After drying over MgSO_4 , the organic layer was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (80:20 \rightarrow 60:40, v/v). Yield 228 mg (89%). ^1H NMR (CDCl_3): $\delta = 0.88$ (t, 3H, CH_3 myristyl), 1.25 (m, 20H, $10 \times \text{CH}_2$ myristyl), 1.48 (s, 9H, $3 \times \text{CH}_3$ *t*Bu), 1.49 (m, 2H, CH_2 myristyl), 1.94 (m, 2H, CH_2 myristyl), 2.57 (dd, 1H, H-1ax, $J_{1\text{ax},1\text{eq}} = 11.8$ Hz, $J_{1\text{ax},2} = 7.0$ Hz), 3.15 (m, 1H, H-5), 3.17 (dd, 1H, H-1eq, $J_{1\text{ax},1\text{eq}} = 11.8$ Hz, $J_{1\text{eq},2} = 3.8$ Hz), 3.29 (d, 1H, Ha-acetyl, $J = 17.7$ Hz), 3.45 (t, 1H, H-3), 3.52 (dd, 1H, H-6, $J_{6,6'} = 10.2$ Hz, $J_{5,6} = 4.9$ Hz), 3.54 (d, 1H, Hb-acetyl, $J = 17.7$ Hz), 3.60 (t, 1H, H-4, $J = 6.1$), 3.77 (dd, 1H, H-6', $J_{6,6'} = 10.3$ Hz, $J_{5,6'} = 4.9$ Hz), 4.00 (m, 1H, H-2), 4.41–4.70 (m, 6H, $3 \times \text{CH}_2$ Bn), 5.85 (d, 1H, NH, $J_{2,\text{NH}} = 7.1$ Hz), 7.24–7.35 (m, 15H, CH-arom Bn). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta = 14.15$ (CH_3 myristyl), 22.74–36.92 (CH_2 myristyl), 28.21 (CH_3 *t*Bu), 48.30 (C-2), 51.15 (C-1), 55.67 (CH_2 acetyl), 61.51 (C-5), 66.28 (C-6), 72.93, 73.31, 73.39 ($3 \times \text{CH}_2$ Bn), 77.73 (C-4), 78.97 (C-3), 80.80 (Cq *t*Bu), 127.67–128.59 (CH-arom Bn), 138.02, 138.41, 138.45 ($3 \times \text{Cq Bn}$), 170.90 (C=O acetyl), 172.96 (C=O myristyl). ES-MS; m/z : 757.5, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{47}\text{H}_{68}\text{N}_2\text{O}_6 = 756.5$.

3,4,6-Tri-*O*-benzyl-1,5-*N*-[(*O*-*tert*-butylcarboxymethyl)imino]-2-[(*R*)-3-(dodecanoyloxytetradecanoyl)]amino-1,2,5-trideoxy-*D*-glucitol (10). (*R*)-3-Dodecanoyloxytetradecanoic acid (73 mg, 0.171 mmol) was coupled with compound **8** (94 mg, 0.172 mmol) as described for the preparation of compound **9**. The crude product was purified by silica gel column chromatography; elution was performed with hexane/ethyl acetate (80:20 \rightarrow 60:40, v/v). $R_f = 0.66$ (hexane/ethyl acetate, 2:1, v/v). Yield 115 mg (70%). ^1H NMR (CDCl_3): $\delta = 0.88$ (m, 6H, $2 \times \text{CH}_3$ acyloxyacyl), 1.25 (m, 34H, CH_2 acyloxyacyl), 1.40 (s, 9H, $3 \times \text{CH}_3$ *t*Bu), 1.55 (m, 4H, $2 \times \text{CH}_2$ acyloxyacyl), 2.21 (m, 4H, $2 \times \text{CH}_2$ acyloxyacyl), 2.56 (dd, 1H, H-1ax, $J_{1\text{ax},\text{eq}} = 11.7$ Hz, $J_{1\text{ax},2} = 7.3$ Hz), 3.12 (m, 2H, H-1eq, H-5), 3.28 (d, 1H, Ha-acetyl, $J = 17.8$ Hz), 3.45 (t, 1H, H-3, $J = 6.5$ Hz), 3.53 (dd, 1H, H-6, $J_{6,6'} = 10.3$ Hz, $J_{5,6} = 3.6$ Hz), 3.55 (d, 1H, Hb-acetyl, $J = 17.8$ Hz), 3.60 (t, 1H, H-4, $J = 6.3$ Hz), 3.76 (dd, 1H, H-6', $J_{6,6'} = 10.3$ Hz, $J_{5,6'} = 5.0$ Hz), 4.00 (m, 1H, H-2), 4.40–4.71 (m, 6H, $3 \times \text{CH}_2$ Bn), 5.11 (m, 1H, CHO acyloxyacyl), 6.06 (d, 1H, NH, $J_{2,\text{NH}} = 7.5$ Hz), 7.23–7.33 (m, 15H, CH-arom Bn). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta = 14.12$ (CH_3 acyloxyacyl), 22.70–41.65 (CH_2 acyloxyacyl), 28.17 (CH_3 *t*Bu), 48.48 (C-2), 51.30 (C-1), 55.52 (CH_2 acetyl), 61.43 (C-5), 66.27 (C-6), 71.21 (CHO acyloxyacyl), 72.92, 73.05, 73.34 ($3 \times \text{CH}_2$ Bn), 77.64 (C-4), 79.33 (C-3), 80.77 (Cq *t*Bu), 127.63–128.54 (CH-arom Bn), 137.98, 138.40, 138.45 ($3 \times \text{Cq Bn}$), 169.43, 170.82, 173.12 ($3 \times \text{C}=\text{O}$ amide, ester). ES-MS; m/z : 954.67 $[\text{M}+\text{H}]^+$, monoisotopic MW calculated for $\text{C}_{59}\text{H}_{90}\text{N}_2\text{O}_8 = 955.63$.



3,4,6-Tri-*O*-benzyl-1,5-*N*-[carboxymethylimino]-2-(tetradecanoyl)amino-1,2,5-trideoxy-*D*-glucitol (11). A solution of **9** (118 mg, 0.156 mmol) in TFA (5 mL) was stirred for 2 h at ambient temperature, after which TLC analysis indicated complete conversion of starting material into a compound with $R_f=0.12$ (MeOH/DCM, 5:95, v/v). After concentration of the reaction mixture, the residue was coevaporated with toluene (3×5 mL). Yield 125 mg (quantitative). ^1H NMR (CDCl_3): $\delta=0.88$ (t, 3H, CH_3 myristyl), 1.25 (m, 20H, CH_2 myristyl), 1.48 (br. s, 2H, CH_2 myristyl), 2.04 (m, 2H, CH_2 myristyl), 2.95 (d, 1H, H-1ax), 3.26 (br. s, 2H, H-1eq, H-5), 3.45 (br. s, 1H, Ha-acetyl), 3.56 (br. s, 2H, H-3, H-6), 3.72 (m, 2H, Hb-acetyl, H-6'), 3.89 (m, 1H, H-4), 4.26 (br. s, 1H, H-2), 4.39–4.62 (m, 6H, $3 \times \text{CH}_2$ Bn), 7.09 (br. s, 1H, NH), 7.14–7.27 (m, 15H, CH-arom Bn), 9.40 (br. s, 1H, COOH). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta=14.15$ (CH_3 myristyl), 22.73–36.68 (CH_2 myristyl), 46.32 (C-2), 50 (C-1), 54.46 (CH_2 acetyl), 61.08 (C-5), 64.88 (C-6), 73.10, 73.33, 73.49 ($3 \times \text{CH}_2$ Bn), 75.38 (C-4), 76.05 (C-3), 127.82–128.71 (CH-arom Bn), 136.42–137.77 (Cq Bn), 165.08 (C=O carboxymethyl), 174.39 (C=O myristyl). ES-MS; m/z : 701.5, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{43}\text{H}_{60}\text{N}_2\text{O}_6=700.45$.

3,4,6-Tri-*O*-benzyl-1,5-*N*-[carboxymethylimino]-2-[(*R*)-3-(dodecanoyloxytetradecanoyl)]amino-1,2,5-trideoxy-*D*-glucitol (12). Compound **10** (115 mg, 0.120 mmol) was treated with TFA as described for the preparation of compound **11**. The crude product was purified by silica gel column chromatography. Elution was performed with MeOH/DCM (0:100 \rightarrow 5:95, v/v). $R_f=0.12$ (hexane/ethyl acetate, 1:2, v/v). Yield 90 mg (83%). ^1H NMR (CDCl_3): $\delta=0.88$ (m, 6H, $2 \times \text{CH}_3$ acyloxyacyl), 1.25 (br. s, 34H, CH_2 acyloxyacyl), 1.56 (m, 4H, CH_2 acyloxyacyl), 2.23 (m, 4H, CH_2 acyloxyacyl), 2.60 (d, 1H, H-1ax), 3.21 (m, 2H, H-1eq, H-5), 3.40 (d, 1H, Ha-acetyl), 3.51 (m, 2H, H-3, H-6), 3.66 (m, 2H, Hb-acetyl, H-6'), 3.86 (m, 1H, H-4), 4.13 (br. s, 1H, H-2), 4.39–4.62 (m, 6H, $3 \times \text{CH}_2$ Bn), 5.05 (m, 1H, CHO acyloxyacyl), 6.70 (br. s, 1H, NH), 7.21–7.35 (m, 15H, CH-arom Bn). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta=14.13$ (CH_3 acyloxyacyl), 22.71–41.86 (CH_2 acyloxyacyl), 47.03 (C-2), 49.49 (C-1), 56.66 (CH_2 acetyl), 61.56 (C-5), 65.94 (C-6), 71.09 (CHO acyloxyacyl), 72.72, 73.12, 73.45 ($3 \times \text{CH}_2$ Bn), 75.83 (C-4), 76.08 (C-3), 127.69–128.70 (CH-arom Bn), 137.27, 137.46, 137.68 ($3 \times \text{Cq}$ Bn), 169.49, 171.96, 173.45 ($3 \times \text{C}=\text{O}$ acid, amide, ester). ES-MS; m/z : 899.7, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{55}\text{H}_{82}\text{N}_2\text{O}_8=898.6$.

Methyl 2-[(benzyloxycarbonyl)amino]-2-deoxy- α -*D*-glucopyranoside (15). To a cooled (0°C) solution of glucosamine hydrochloride (**13**) (20 g, 93 mmol) in water (400 mL) NaHCO_3 (14.4 g, 171 mmol) and benzyl chloroformate (17.4 mL, 104 mmol) were added. The mixture was stirred at ambient temperature for 16 h, after which the white crystalline residue (**14**) was filtered off, washed with cold acetone (-20°C) and dried. The white crystals were dissolved in acidic methanol (2% HCl, w/w) and refluxed for 7 h after which the reaction mixture was concentrated. The resulting residue was purified by silica gel column chromatography. Elution was performed with MeOH/DCM (10:90 \rightarrow 15:85, v/v). Yield 16 g (53%). $R_f=0.70$ (MeOH/DCM, 15:85, v/v). ^1H NMR ($\text{DMSO}-d_6$): $\delta=3.16$ (m, 2H, H-4, H-5), 3.27 (s, 3H, OMe), 3.46 (m, 3H, H-2, H₂-6), 3.67 (m, 1H, H-3), 4.51 (t, 1H, OH-6), 4.61 (d, 1H, H-1, $J_{1,2}=3.2$ Hz), 4.76 (d, 1H, OH-3), 4.98 (d, 1H, OH-4), 5.04 (dd, 2H, CH_2 Z), 7.07 (d, 1H, NH, $J_{2,\text{NH}}=7.7$ Hz), 7.31–7.42 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR ($\text{DMSO}-d_6$): $\delta=54.37$



(OMe), 55.95 (C-2), 60.89 (C-6), 65.34 (CH₂ Z), 70.65 (C-5), 70.81 (C-3), 72.72 (C-4), 98.09 (C-1), 127.78, 128.35 (CH-arom Z), 137.16 (Cq Z), 156.17 (C=O Z).

Methyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-4,6-O-isopropylidene- α -D-glucopyranoside (16). To a mixture of **15** (16 g, 49 mmol) in dry acetone (200 mL) and DCM (150 mL) was added 2,2-dimethoxypropane (25 mL, 204 mmol) and *p*-toluene sulfonic acid (0.4 g, 2.1 mmol). The resulting mixture was stirred at ambient temperature and after 16 h, TLC analysis showed complete conversion of starting material into a compound with $R_f=0.80$ (MeOH/DCM, 5:95, v/v). TEA (5 mL) and DCM (100 mL) were added and the mixture was washed with water (50 mL). The organic layer was dried (MgSO₄) and concentrated. Purification of the crude product by silica gel column chromatography (elution with MeOH/DCM (0:100 \rightarrow 5:95, v/v)), yielded 18 g (quantitative) of a yellow oil. ¹H NMR (CDCl₃): δ = 1.42 (s, 3H, CH₃ isopropylidene), 1.51 (s, 3H, CH₃ isopropylidene), 2.84 (s, 1H, OH-3), 3.33 (s, 3H, OMe), 3.59 (m, 2H, H-4, H-5), 3.74 (m, 2H, H-3, H-6), 3.87 (m, 2H, H-2, H-6'), 4.68 (d, 1H, H-1, $J_{1,2}=3.4$ Hz), 5.11 (s, 2H, CH₂ Z), 5.19 (d, 1H, NH, $J_{2,NH}=8.7$ Hz), 7.32–7.36 (m, 5H, CH-arom Z). ¹³C{¹H} NMR (CDCl₃): δ = 19.17, 29.14 (2 \times CH₃ isopropylidene), 55.28 (OMe), 55.92 (C-2), 62.36 (C-6), 63.36 (C-5), 67.30 (CH₂ Z), 70.88 (C-3), 74.63 (C-4), 99.23 (C-1), 99.90 (Cq isopropylidene), 128.09–128.60 (CH-arom Z), 136.22 (Cq Z), 156.87 (C=O Z).

Methyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl- α -D-glucopyranoside (17). To a solution of **16** (5.545 g, 15.10 mmol) in DCM was added DMAP (2.03 g, 16.6 mmol), myristic acid (3.79 g, 16.6 mmol) and DCC (3.43 g, 16.6 mmol). The reaction mixture was stirred for 18 h at ambient temperature. TLC analysis (MeOH/DCM, 1:99, v/v) showed complete conversion of starting material into a compound with $R_f=0.91$. DCU was filtered off and the filter was washed with DCM (3 \times 25 mL). DCM was concentrated and the crude product was purified by silica gel column chromatography. Elution was performed with MeOH/DCM (0:100 \rightarrow 3:97, v/v). Yield: 7.49 g (86%) of a colorless oil. ¹H NMR (CDCl₃): δ = 0.88 (t, 3H, CH₃ myristyl), 1.25 (br. s, 20H, CH₂ myristyl), 1.36 (s, 3H, CH₃ isopropylidene), 1.46 (s, 3H, CH₃ isopropylidene), 1.54 (m, 2H, CH₂ myristyl), 2.22 (m, 2H, CH₂ myristyl), 3.36 (s, 3H, OMe), 3.69 (m, 2H, H-4, H-5), 3.76 (t, 1H, H-6), 3.87 (dd, 1H, H-6', $J_{5,6'}=4.7$ Hz, $J_{6,6'}=10.2$ Hz), 3.97 (m, 1H, H-2, $J_{1,2}=3.7$ Hz, $J=10.2$ Hz), 4.69 (d, 1H, H-1, $J_{1,2}=3.7$ Hz), 5.06 (s, 2H, CH₂ Z), 5.12 (m, 2H, H-3, NH), 7.26–7.36 (m, 5H, CH-arom Z). ¹³C{¹H} NMR (CDCl₃): δ = 14.15 (CH₃ myristyl), 19.11, 29.11 (2 \times CH₃ isopropylidene), 22.74–34.38 (CH₂ myristyl), 54.60 (C-2), 55.32 (OMe), 62.51 (C-6), 63.86 (C-5), 66.93 (CH₂ Z), 70.36 (C-3), 72.15 (C-4), 99.43 (C-1), 99.73 (Cq isopropylidene), 128.03, 128.18, 128.55 (CH-arom Z), 136.40 (Cq Z), 156.01 (C=O Z), 173.83 (C=O ester).

Methyl 2-amino-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl- α -D-glucopyranoside (18). To a solution of **17** (6.69 g, 11.6 mmol) in EtOAc (100 mL) was added Pd/C (10%, 1.0 g). Hydrogen was passed through the stirred mixture for 66 h. TLC analysis showed complete conversion of starting material into a new product with $R_f=0.26$ (MeOH/DCM, 1:99, v/v). The mixture was filtered over a PTFE filter. The filtrate was concentrated under reduced pressure. Yield: 5.19 g (quantitative) of a



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colorless oil. ^1H NMR (CDCl_3): δ =0.88 (s, 3H, t, CH_3 myristyl), 1.25 (br. s, 20H, CH_2 myristyl), 1.36 (s, 3H, CH_3 isopropylidene), 1.45 (s, 3H, CH_3 isopropylidene), 1.64 (m, 2H, CH_2 myristyl), 1.76 (m, 2H, NH_2), 2.34 (q, 2H, CH_2 myristyl), 2.86 (dd, 1H, H-2, $J_{1,2}$ =3.6 Hz, $J_{2,3}$ =10.0 Hz), 3.39 (s, 3H, OMe), 3.56 (d, 1H, H-4), 3.72 (m, 2H, H-5, H-6), 3.87 (m, 1H, H-6'), 4.71 (d, 1H, H-1, $J_{1,2}$ =3.6 Hz), 5.05 (t, 1H, H-3). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.11 (CH_3 myristyl), 19.09, 29.13 ($2 \times \text{CH}_3$ isopropylidene), 22.70–34.61 (CH_2 myristyl), 55.29 (OMe), 55.54 (C-2), 62.64 (C-6), 63.83 (C-5), 72.50 (C-4), 73.76 (C-3), 99.52 (Cq isopropylidene), 101.37 (C-1), 173.63 (C=O ester).

Methyl 2-[(6-benzyloxycarbonylamino)hexanoylamino]-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl- α -D-glucopyranoside (22). To a stirred mixture of 6-benzyloxycarbonylamino-hexanoic acid (**19**; 516 mg, 1.95 mmol), PyBOP (1.522 g, 2.93 mmol) and DiPEA (367 μL , 2.15 mmol) in DCM (50 mL) was added **18** (0.913 g, 2.06 mmol) in DCM (10 mL). After 1 h, TLC analysis indicated the complete conversion of starting material into a product with R_f =0.57 (hexane/ethyl acetate, 1:2, v/v). The reaction mixture was diluted with DCM (100 mL) and washed with water (3×50 mL). The organic layer was dried (MgSO_4) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (60:40 \rightarrow 40:60, v/v). Yield 1.172 g (87%). ^1H NMR (CDCl_3): δ =0.88 (t, 3H, CH_3 myristyl), 1.23–1.64 (m, 28H, CH_2 hexanoyl, myristyl), 1.37 (s, 3H, CH_3 isopropylidene), 1.47 (s, 3H, CH_3 isopropylidene), 2.11–2.35 (m, 4H, hexanoyl, myristyl), 3.17 (br. q, 2H, CH_2 hexanoyl), 3.35 (s, 3H, OMe), 3.66–3.79 (m, 3H, H-4, H-5, H-6), 3.87 (dd, 1H, H-6', $J_{5,6'}$ =5.0 Hz, $J_{6,6'}$ =10.5 Hz), 4.27 (m, 1H, H-2, $J_{1,2}$ =3.7 Hz, $J_{2,3}$ =9.5 Hz), 4.66 (d, 1H, H-1 $J_{1,2}$ =3.7 Hz), 4.95 (s, 1H, NH aminohexanoyl), 5.09 (s, 2H, CH_2 Z), 5.12 (t, 1H, H-3, $J_{2,3}$ =9.5 Hz, $J_{3,4}$ =9.5 Hz), 5.88 (s, 1H, NH), 7.28–7.35 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.08 (CH_3 myristyl), 22.66–40.82 (CH_2 hexanoyl, myristyl), 19.06, 26.23 ($2 \times \text{CH}_3$ isopropylidene), 52.51 (C-2), 55.23 (OMe), 62.43 (C-6), 63.73 (C-5), 66.55 (CH_2 Z), 70.38 (C-3), 71.94 (C-4), 99.07 (C-1), 99.73 (Cq isopropylidene), 128.03, 128.48 (CH-arom Z), 136.72 (Cq Z), 156.21 (C=O Z), 172.82, 174.26 ($2 \times \text{C}=\text{O}$ amide, ester).

Methyl 2-[(12-benzyloxycarbonylamino)dodecanoylamino]-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl- α -D-glucopyranoside (23). 12-Benzyloxycarbonylamino-dodecanoic acid **20** (0.642 g, 1.84 mmol) was coupled with compound **18** (0.815 g, 1.84 mmol) as described for the preparation of compound **22**. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (90:10 \rightarrow 40:60, v/v). Yield 1.368 g (96%). ^1H NMR (CDCl_3): δ =0.88 (t, 3H, CH_3 myristyl), 1.25 (m, 34H, CH_2 dodecanoyl, myristyl), 1.37 (s, 3H, CH_3 isopropylidene), 1.46 (s, 3H, CH_3 isopropylidene), 1.57 (m, 6H, CH_2 dodecanoyl, myristyl), 2.10–2.33 (m, 4H, dodecanoyl, myristyl), 3.17 (q, 2H, CH_2 dodecanoyl), 3.36 (s, 3H, OMe), 3.69–3.79 (m, 3H, H-4, H-5, H-6), 3.87 (dd, 1H, H-6'), 4.27 (m, 1H, H-2), 4.67 (d, 1H, H-1, $J_{1,2}$ =3.6 Hz), 4.82 (s, 1H, NH aminododecanoyl), 5.09 (s, 2H, CH_2 Z), 5.13 (q, 1H, H-3), 5.82 (d, 1H, NH), 7.27–7.35 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.11 (CH_3 myristyl), 22.68–41.15 (CH_2 dodecanoyl, myristyl), 19.07, 29.07 ($2 \times \text{CH}_3$ isopropylidene), 52.49 (C-2), 55.25 (OMe), 62.46 (C-6), 63.78 (C-5), 66.55 (CH_2 Z), 70.28 (C-3), 71.97 (C-4), 99.13 (C-1), 99.74 (Cq

isopropylidene), 128.04, 128.49 (CH-arom Z), 136.5 (Cq Z), 156.5 (C=O Z), 173.14, 174.23 (2 × C=O amide, ester).

Methyl 2-[(R)-3-(6-benzyloxycarbonylamino)hexanoyloxytetradecanoylamino]-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl- α -D-glucopyranoside (24). (R)-3-(6-Benzyloxycarbonylamino)hexanoyloxytetradecanoic acid **21** (0.516 g, 1.95 mmol) was coupled with compound **18** (0.913 g, 2.06 mmol) as described for the preparation of compound **22**. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (80:20 → 30:70, v/v). Yield 1.172 g (87%). ¹H NMR (CDCl₃): δ =0.88 (t, 6H, CH₃ acyloxyacyl, myristyl), 1.25–1.67 (m, 48H, CH₂ acyloxyacyl, myristyl), 1.36 (s, 3H, CH₃ isopropylidene), 1.46 (s, 3H, CH₃ isopropylidene), 2.24–2.45 (m, 6H, acyloxyacyl, myristyl), 3.17 (q, 2H, CH₂ hexanoyl), 3.34 (s, 3H, OMe), 3.66–3.78 (m, 3H, H-4, H-5, H-6), 3.87 (dd, 1H, H-6'), 4.24 (m, 1H, H-2), 4.66 (d, 1H, H-1, J_{1,2}=3.7 Hz), 4.92 (s, 1H, NH aminohexanoyl), 5.09 (m, 4H, CH₂ Z, H-3, CHO acyloxyacyl), 5.97 (d, 1H, NH), 7.27–7.35 (m, 5H, CH-arom Z). ¹³C{¹H} NMR (CDCl₃): δ =14.12 (CH₃ myristyl), 22.71–41.29 (CH₂ acyloxyacyl, myristyl), 19.10, 29.09 (2 × CH₃ isopropylidene), 52.59 (C-2), 55.22 (OMe), 62.47 (C-6), 63.79 (C-5), 66.59 (CH₂ Z), 70.27 (C-3), 71.09 (CHO acyloxyacyl), 72.05 (C-4), 98.98 (C-1), 99.77 (Cq isopropylidene), 128.05, 128.52 (CH-arom Z), 156.45 (C=O Z), 169.62, 172.82, 174.26 (3 × C=O amide, ester).

Methyl 2-[(6-benzyloxycarbonylamino)hexanoylamino]-2-deoxy-3-O-tetradecanoyl- α -D-glucopyranoside (25). To a stirred solution of **22** (0.801 g, 1.16 mmol) in THF/water (4:1, v/v; 25 mL) at 0°C was added TFA (1 mL). The resulting solution was allowed to warm to room temperature and left overnight. TLC analysis (ethyl acetate/hexane, 2:1, v/v) showed complete conversion of starting material into a compound with R_f=0.13. The reaction mixture was concentrated under reduced pressure. The residue was diluted with diethyl ether (100 mL) and washed with water (3 × 50 mL). The organic layer was dried over Na₂SO₄, and the solvent was removed under reduced pressure. Purification of the residue by silica gel column chromatography with DCM/ethanol (100:0 → 93:7, v/v) yielded the desired diol **25** as a colorless oil (0.747 g; 99%). ¹H NMR (CDCl₃): δ =0.88 (t, 3H, CH₃ myristyl), 1.24 (m, 22H, CH₂ hexanoyl, myristyl), 1.54 (m, 6H, CH₂ hexanoyl, myristyl), 2.13 (t, 2H, CH₂ hexanoyl, myristyl), 2.30 (m, 2H, CH₂ hexanoyl, myristyl), 3.17 (q, 2H, CH₂ hexanoyl), 3.37 (s, 3H, OMe), 3.65 (m, 1H, H-5, J_{4,5}=9.7 Hz), 3.78 (t, 1H, H-4, J_{3,4}=9.5 Hz, J_{4,5}=9.5 Hz), 3.86 (s, 2H, H₂-6), 4.20 (m, 1H, H-2, J_{1,2}=3.4 Hz, J_{2,NH}=9.5 Hz, J_{2,3}=10.5 Hz), 4.70 (d, 1H, H-1, J_{1,2}=3.4 Hz), 4.99 (br. s, 1H, NH aminohexanoyl), 5.08 (br. s, 2H, CH₂ Z), 5.10 (t, 1H, H-3, J_{2,3}=10.5 Hz, J_{3,4}=9.5 Hz), 5.99 (d, 1H, NH, J_{2,NH}=9.2 Hz), 7.27–7.36 (m, 5H, CH-arom Z). ¹³C{¹H} NMR (CDCl₃): δ =14.11 (CH₃ myristyl), 22.71–40.83 (CH₂ hexanoyl, myristyl), 52.04 (C-2), 55.27 (OMe), 61.98 (C-6), 66.66 (CH₂ Z), 68.93 (C-4), 71.52 (C-5), 73.78 (C-3), 98.49 (C-1), 128.11, 128.53 (CH-arom Z), 136.68 (Cq Z), 156.55 (C=O Z), 173.14, 175.22 (2 × C=O amide, ester).

Methyl 2-[(12-benzyloxycarbonylamino)dodecanoylamino]-2-deoxy-3-O-tetradecanoyl- α -D-glucopyranoside (26). Acetonide **23** (1.368 g, 1.77 mmol) was treated with TFA as was described for the preparation of compound **25**. Silica gel column chromatography with DCM/ethanol (100:0 → 94:6, v/v) of the residue yielded the desired



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diol as a white solid (0.816 g; 63%). ^1H NMR (CDCl_3): δ =0.88 (t, 3H, CH_3 myristyl), 1.25 (m, 34H, CH_2 dodecanoyl, myristyl), 1.48 (br. t, 2H, CH_2 dodecanoyl, myristyl), 1.56 (m, 4H, CH_2 dodecanoyl, myristyl), 2.12 (m, 2H, CH_2 dodecanoyl, myristyl), 2.31 (m, 2H, CH_2 dodecanoyl, myristyl), 2.95 (br. s, 2H, $2 \times \text{OH}$) 3.17 (q, 2H, CH_2 dodecanoyl), 3.38 (s, 3H, OMe), 3.67 (m, 1H, H-5, $J_{4,5}=9.7$ Hz), 3.77 (t, 1H, H-4, $J_{3,4}=9.3$, $J_{4,5}=9.5$ Hz), 3.86 (d, 2H, H₂-6), 4.21 (m, 1H, H-2, $J_{1,2}=3.6$ Hz, $J_{2,\text{NH}}=9.4$ Hz, $J_{2,3}=9.4$ Hz), 4.69 (d, 1H, H-1, $J_{1,2}=3.6$ Hz), 4.78 (br. s, 1H, NH aminohexanoyl), 5.09 (br. s, 3H, CH_2 Z, H-3), 5.83 (d, 1H, NH, $J_{2,\text{NH}}=9.3$ Hz), 7.27–7.36 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.17 (CH_3 myristyl), 22.75–36.78 (CH_2 dodecanoyl, myristyl), 41.20 (NCH₂) 51.92 (C-2), 55.31 (OMe), 62.22 (C-6), 66.66 (CH_2 Z), 69.21 (C-4), 71.45 (C-5), 73.82 (C-3), 98.53 (C-1), 126.81, 128.13, 128.72 (CH-arom Z), 136.72 (Cq Z), 156.49 (C=O Z), 173.34, 175.23 ($2 \times \text{C}=\text{O}$ amide, ester).

Methyl 2-[(R)-3-(6-benzyloxycarbonylamino)hexanoyloxytetradecanoylamino]-2-deoxy-3-O-tetradecanoyl- α -D-glucopyranoside (27). To a cooled (0°C) solution of **24** (138 mg, 0.151 mmol) in DCM (1 mL) was added TFA (0.5 mL). After stirring for 2 h, TLC analysis indicated the complete conversion of starting material into a product with $R_f=0.27$ (ethyl acetate/hexane, 2:1, v/v). The reaction mixture was concentrated and coevaporated with toluene (2×2 mL). Silica gel column chromatography with hexane/ethyl acetate (50:50 \rightarrow 85:15, v/v) of the residue yielded the desired diol as a white solid (115 mg; 87%). ^1H NMR (CDCl_3): δ =0.88 (t, 6H, CH_3 acyloxyacyl, myristyl), 1.25–1.66 (m, 40H, CH_2 acyloxyacyl, myristyl), 1.51–1.66 (m, 8H, CH_2 acyloxyacyl, myristyl), 2.28–2.44 (m, 6H, CH_2 , acyloxyacyl, myristyl), 2.68 (br. s, 1H, OH-6), 3.18 (q, 2H, CH_2 hexanoyl), 3.35 (s, 3H, OMe), 3.49 (br. s, 1H, OH-4), 3.64 (m, 1H, H-5), 3.74 (t, 1H, H-4), 3.84 (d, 2H, H₂-6), 4.16 (m, 1H, H-2), 4.67 (d, 1H, H-1, $J_{1,2}=3.5$ Hz), 5.02 (s, 1H, NH aminohexanoyl), 5.09 (m, 4H, CH_2 Z, H-3, CHO acyloxyacyl), 6.12 (d, 1H, NH), 7.28–7.35 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.09 (CH_3 myristyl), 22.69–41.31 (CH_2 acyloxyacyl, myristyl), 52.06 (C-2), 55.20 (OMe), 62.08 (C-6), 66.63 (CH_2 Z), 69.14 (C-4), 71.14 (CHO acyloxyacyl), 71.55 (C-5), 73.66 (C-3), 98.35 (C-1), 128.03, 128.06, 128.51 (CH-arom Z), 136.55 (Cq Z), 156.55 (C=O Z), 169.80, 172.81, 175.13 ($3 \times \text{C}=\text{O}$ amide, ester).

Methyl 2-[(6-benzyloxycarbonylamino)hexanoylamino]-2,6-dideoxy-6-O-mesyl-3-O-tetradecanoyl- α -D-glucopyranoside (28). To a stirred solution of **25** (301 mg, 0.463 mmol) in dry pyridine (10 mL) was added mesyl chloride (1.5 equivalents, 60 μL). After 24 h, methanol (2 mL) was added and the mixture was stirred for 0.5 h, after which the mixture was concentrated. The residue was dissolved in DCM (50 mL) and washed with water (1×25 mL). The organic layer was dried (MgSO_4) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (70:30 \rightarrow 20:80, v/v). $R_f=0.43$ (hexane/ethyl acetate, 1:2, v/v). Yield 269 mg (80%). ^1H NMR (CDCl_3): δ =0.88 (t, 3H, CH_3 myristyl), 1.25 (m, 24H, CH_2 hexanoyl, myristyl), 1.45–1.60 (m, 4H, CH_2 hexanoyl, myristyl), 2.13 (t, 2H, CH_2 hexanoyl, myristyl), 2.31 (m, 2H, CH_2 hexanoyl, myristyl), 3.05 (s, 3H, CH_3 Ms), 3.15 (q, 2H, NCH₂ hexanoyl), 3.39 (s, 3H, OMe), 3.69 (m, 1H, H-4), 3.81 (br. d, 1H, OH-4), 3.86 (m, 1H, H-5), 4.23 (m, 1H, H-2), 4.50 (q, 2H, H₂-6), 4.70 (d, 1H, H-1, $J_{1,2}=3.6$ Hz), 5.08 (m, 4H, H-3, CH_2 Z, NH aminohexanoyl), 5.99 (d, 1H, NH), 7.28–7.36 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR

(CDCl₃): δ = 14.02 (CH₃ myristyl), 22.58–36.16 (CH₂ hexanoyl, myristyl), 37.41 (CH₃ Ms), 40.70 (CH₂N), 51.60 (C-2), 55.40 (OMe), 66.45 (CH₂ Z), 68.14 (C-4), 68.59 (C-6), 69.77 (C-5), 73.38 (C-3), 98.43 (C-1), 127.92–128.43 (CH-arom Z), 136.58 (Cq Z), 156.42 (C=O Z), 172.87, 174.92 (2 × C=O amide, ester).

Methyl 2-[(12-benzoyloxycarbonylamino)dodecanoylamino]-2-deoxy-6-O-mesyl-3-O-tetradecanoyl- α -D-glucopyranoside (29). Compound **29** (1.175 g, 1.60 mmol) was prepared as described for compound **28**. R_f = 0.68 (hexane/ethyl acetate, 1:2, v/v). Yield 1.01 g (78%). ¹H NMR (CDCl₃): δ = 0.88 (t, 3H, CH₃ myristyl), 1.25 (m, 34H, CH₂ dodecanoyl, myristyl), 1.42–1.65 (m, 6H, CH₂ dodecanoyl, myristyl), 2.12 (t, 2H, CH₂ dodecanoyl, myristyl), 2.32 (m, 2H, CH₂ dodecanoyl, myristyl), 3.07 (s, 3H, CH₃ Ms), 3.17 (q, 2H, NCH₂ dodecanoyl), 3.31 (br. d, 1H, OH-4), 3.39 (s, 3H, OMe), 3.70 (m, 1H, H-4, $J_{3,4}$ = 9.3 Hz, $J_{4,5}$ = 9.7 Hz), 3.86 (m, 1H, H-5, $J_{4,5}$ = 9.9 Hz, $J_{5,6}$ = 6.7 Hz, $J_{5,6}$ = 3.3 Hz), 4.23 (m, 1H, H-2, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.4 Hz), 4.51 (d, 2H, H₂-6), 4.70 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 4.82 (br. s, 1H, NH aminohexanoyl), 5.09 (dd, 3H, H-3, CH₂ Z), 5.81 (d, 1H, NH, $J_{2,NH}$ = 8.9 Hz), 7.27–7.35 (m, 5H, CH-arom Z). ¹³C{¹H} NMR (CDCl₃): δ = 14.12 (CH₃ myristyl), 22.69–36.67 (CH₂ dodecanoyl, myristyl), 37.59 (CH₃ Ms), 41.14 (CH₂N), 51.63 (C-2), 55.52 (OMe), 66.57 (CH₂ Z), 68.29 (C-4), 68.45 (C-6), 69.87 (C-5), 73.44 (C-3), 98.60 (C-1), 128.06, 128.50 (CH-arom Z), 136.70 (Cq Z), 156.43 (C=O Z), 173.12, 175.07 (2 × C=O amide, ester).

Methyl 2-[(R)-3-(6-benzoyloxycarbonylamino)hexanoyloxytetradecanoylamino]-2-deoxy-3-O-tetradecanoyl-6-O-tosyl- α -D-glucopyranoside (30). Compound **27** (115 mg, 0.131 mmol) was treated with tosyl chloride (138 mg, 0.724 mmol) in pyridine (5 mL). After 96 h, methanol (2 mL) was added and the mixture was concentrated. Further work-up as described for compound **28**. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (60:40 → 35:65, v/v). R_f = 0.84 (hexane/ethyl acetate, 1:2, v/v). Yield 107 mg (79%). ¹H NMR (CDCl₃): δ = 0.88 (t, 6H, CH₃ acyloxyacyl, myristyl), 1.25–1.49 (m, 40H, CH₂ acyloxyacyl, myristyl), 1.53 (m, 8H, CH₂ acyloxyacyl, myristyl), 2.34 (m, 6H, acyloxyacyl, myristyl), 2.44 (s, 3H, CH₃ Ts), 3.13 (s, 1H, OH-4), 3.18 (q, 2H, CH₂ hexanoyl), 3.29 (s, 3H, OMe), 3.59 (m, 1H, H-4), 3.79 (m, 1H, H-5), 4.14 (m, 1H, H-2), 4.30 (d, 2H, H₂-6), 4.59 (d, 1H, H-1, $J_{1,2}$ = 3.6 Hz), 4.94 (t, 1H, NH aminohexanoyl), 5.04 (dd, 1H, H-3), 5.07 (s, 2H, CH₂ Z), 5.09 (m, 1H, CHO acyloxyacyl), 5.99 (d, 1H, NH), 7.27–7.80 (m, 9H, CH-arom Ts/Z). ¹³C{¹H} NMR (CDCl₃): δ = 14.11 (CH₃ acyloxyacyl), 21.65 (CH₃ Ts), 22.69–41.30 (CH₂ acyloxyacyl, myristyl), 51.72 (C-2), 55.35 (OMe), 66.63 (CH₂ Z), 68.60 (C-4), 68.80 (C-6), 69.84 (C-5), 71.13 (CHO acyloxyacyl), 73.43 (C-3), 98.22 (C-1), 127.76–129.84 (CH-arom Z), 133.01, 136.68, 144.98 (Cq Ts, Z), 156.52 (C=O Z), 169.62, 172.76, 175.07 (3 × C=O amide, ester).

Methyl 6-azido-2-[(6-benzoyloxycarbonylamino)hexanoylamino]-2,6-dideoxy-3-O-tetradecanoyl- α -D-glucopyranoside (31). To a solution of **28** (269 mg, 0.369 mmol) in DMF (10 mL) was added sodium azide (36 mg, 0.55 mmol). After stirring for 4 h at 70°C, TLC analysis indicated the complete conversion of starting material. The mixture was concentrated, diluted with DCM (50 mL) and washed with water (20 mL). The organic layer was dried (MgSO₄) and concentrated under reduced pressure. The crude



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product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (55:45 → 30:70, v/v). $R_f=0.76$ (hexane/ethyl acetate, 1:2, v/v). Yield 211 mg (84%). ^1H NMR (CDCl_3): $\delta=0.88$ (t, 3H, CH_3 myristyl), 1.25 (m, 22H, CH_2 hexanoyl, myristyl), 1.54 (m, 6H, CH_2 hexanoyl, myristyl), 2.12 (t, 2H, CH_2 hexanoyl, myristyl), 2.29 (m, 2H, CH_2 hexanoyl, myristyl), 3.17 (q, 2H, CH_2 hexanoyl), 3.41 (s, 3H, OMe), 3.46 (dd, 1H, H-6, $J_{5,6}=6.2$ Hz, $J_{6,6'}=13.2$ Hz), 3.57 (dd, 1H, H-6', $J_{5,6'}=2.5$ Hz, $J_{6,6'}=13.2$ Hz), 3.63 (t, 1H, H-4, $J_{3,4}=9.3$ Hz, $J_{4,5}=9.4$ Hz), 3.79 (m, 1H, H-5, $J_{4,5}=9.4$ Hz, $J_{5,6}=6.2$ Hz, $J_{5,6'}=2.5$ Hz), 4.25 (m, 1H, H-2, $J_{1,2}=3.6$ Hz, $J_{2,3}=10.7$ Hz, $J_{2,\text{NH}}=9.5$ Hz), 4.70 (d, 1H, H-1 $J_{1,2}=3.6$ Hz), 4.92 (br. s, 1H, NH aminohexanoyl), 5.04 (dd, 1H, H-3, $J_{2,3}=10.8$ Hz, $J_{3,4}=9.1$ Hz), 5.08 (s, 2H, CH_2 Z), 5.87 (d, 1H, NH, $J_{2,\text{NH}}=9.4$ Hz), 7.27–7.35 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta=14.10$ (CH_3 myristyl), 22.68–40.83 (CH_2 hexanoyl, myristyl), 51.42 (C-6), 51.64 (C-2), 55.40 (OMe), 66.63 (CH_2 Z), 69.76 (C-4), 71.15 (C-5), 73.97 (C-3), 98.44 (C-1), 128.05, 128.09, 128.52 (CH-arom Z), 136.67 (Cq Z), 156.49 (C=O Z), 172.73, 175.28 ($2 \times \text{C}=\text{O}$ amide, ester).

Methyl 6-azido-2-[(12-benzyloxycarbonylamino)dodecanoylamino]-2,6-dideoxy-3-O-tetradecanoyl- α -D-glucopyranoside (32). Compound **29** (601 mg, 0.738 mmol) was treated with sodium azide as described for the preparation of compound **31**. $R_f=0.60$ (hexane/ethyl acetate, 1:2, v/v). Yield 404 mg (72%). ^1H NMR (CDCl_3): $\delta=0.88$ (t, 3H, CH_3 myristyl), 1.25 (m, 34H, CH_2 dodecanoyl, myristyl), 1.51 (m, 6H, CH_2 dodecanoyl, myristyl), 2.12 (m, 2H, CH_2 dodecanoyl, myristyl), 2.30 (m, 2H, CH_2 dodecanoyl, myristyl), 3.16 (q, 2H, NCH_2 dodecanoyl), 3.42 (s, 3H, OMe), 3.46 (m, 2H, H-6, OH-4), 3.57 (dd, 1H, H-6'), 3.62 (m, 1H, H-4), 3.80 (m, 1H, H-5), 4.25 (m, 1H, H-2), 4.71 (d, 1H, H-1, $J_{1,2}=3.7$ Hz), 4.89 (br. s, 1H, NH dodecanoylamino), 5.05 (dd, 1H, H-3), 5.08 (br. s, 2H, CH_2 Z), 5.87 (d, 1H, NH $J_{2,\text{NH}}=9.4$ Hz), 7.28–7.35 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta=14.07$ (CH_3), 22.64–41.08 (CH_2 dodecanoyl, myristyl), 51.37 (C-6), 51.57 (C-2), 55.32 (OMe), 66.51 (CH_2 Z), 69.62 (C-4), 71.16 (C-5), 73.75 (C-3), 98.40 (C-1), 127.99, 128.44 (CH-arom Z), 136.64 (Cq Z), 156.42 (C=O Z), 173.06, 175.21 ($2 \times \text{C}=\text{O}$ amide, ester).

Methyl 6-azido-2-[(R)-3-(6-benzyloxycarbonylamino)hexanoyloxytetradecanoylamino]-2,6-dideoxy-3-O-tetradecanoyl- α -D-glucopyranoside (33). Compound **30** (107 mg, 0.103 mmol) was treated with sodium azide as described for the preparation of compound **31**. $R_f=0.26$ (hexane/ethyl acetate, 2:1, v/v). Yield 68 mg (73%). ^1H NMR (CDCl_3): $\delta=0.88$ (t, 6H, CH_3 acyloxyacyl, myristyl), 1.25 (m, 40H, CH_2 acyloxyacyl, myristyl), 1.56 (m, 8H, CH_2 acyloxyacyl, myristyl), 2.35 (m, 6H, CH_2 acyloxyacyl, myristyl), 3.05 (d, 1H, OH-4), 3.20 (t, 2H, hexanoyl), 3.39 (s, 3H, OMe), 3.45 (dd, 1H, H-6), 3.56 (dd, 1H, H-6'), 3.61 (t, 1H, H-4), 3.79 (m, 1H, H-5), 4.22 (m, 1H, H-2), 4.70 (d, 1H, H-1, $J_{1,2}=3.6$ Hz), 4.90 (br. s, 1H, NH aminohexanoyl), 5.08 (m, 4H, CH_2 Z, H-3, CHO acyloxyacyl), 6.02 (d, 1H, NH), 7.27–7.35 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): $\delta=14.10$ (CH_3 acyloxyacyl), 22.71–41.31 (CH_2 acyloxyacyl, myristyl), 51.40 (C-6), 51.72 (C-2), 55.39 (OMe), 66.66 (CH_2 Z), 69.78 (C-4), 69.89 (C-5), 71.15 (CHO acyloxyacyl), 73.75 (C-3), 98.28 (C-1), 128.06, 128.12, 128.54 (CH-arom Z), 136.62 (Cq Z), 156.5 (C=O Z), 169.66, 172.82, 175.26 ($3 \times \text{C}=\text{O}$ amide, ester).

Methyl 6-amino-2-[(6-benzyloxycarbonylamino)hexanoylamino]-2,6-dideoxy-3-O-tetradecanoyl- α -D-glucopyranoside (34). To a solution of **31** (491 mg, 0.727 mmol) in THF (73 mL) was added triphenylphosphine (286 mg, 1.09 mmol) and water (16 μ L, 0.871 mmol). The mixture was refluxed for 3.5 h, after which TLC analysis indicated the complete conversion of starting material into baseline material (hexane/ethyl acetate, 1:2, v/v). The reaction mixture was concentrated and the crude product was purified by silica gel column chromatography. Elution was performed with MeOH/DCM/TEA (10:89:1 \rightarrow 14:85:1, v/v/v). Yield 497 mg (100%). ^1H NMR (CDCl_3): δ =0.88 (t, 3H, CH_3 myristyl), 1.25 (m, 22H, CH_2 hexanoyl, myristyl), 1.54 (m, 6H, CH_2 hexanoyl, myristyl), 2.12 (t, 2H, CH_2 hexanoyl, myristyl), 2.30 (m, 2H, CH_2 hexanoyl, myristyl), 2.77 (br. s, 3H, NH_2 -6, OH-4), 3.03 (br. s, 2H, H_2 -6), 3.17 (q, 2H, CH_2 hexanoyl), 3.36 (s, 3H, OMe), 3.60 (m, 1H, H-5), 3.64 (t, 1H, H-4, $J_{3,4}$ =8.6 Hz, $J_{4,5}$ =8.6 Hz), 4.18 (m, 1H, H-2, $J_{1,2}$ =3.6 Hz, $J_{2,3}$ =10.8 Hz, $J_{2,\text{NH}}$ =9.3 Hz), 4.65 (d, 1H, H-1 $J_{1,2}$ =3.6 Hz), 4.95 (br. s, 1H, NH aminohexanoyl), 5.08 (m, 3H, CH_2 Z, H-3, $J_{2,3}$ =9.7 Hz, $J_{3,4}$ =9.7 Hz), 5.87 (d, 1H, NH, $J_{2,\text{NH}}$ =9.3 Hz), 7.28–7.36 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.11 (CH_3 myristyl), 22.69–40.86 (CH_2 hexanoyl, myristyl), 43.74 (C-6), 51.93 (C-2), 55.17 (OMe), 66.59 (CH_2 Z), 71.09 (C-4), 71.34 (C-5), 73.71 (C-3), 98.43 (C-1), 128.06–128.60 (CH-arom Z), 136.74 (Cq Z), 156.45 (C=O Z), 172.69, 174.99 (2 \times C=O amide, ester).

Methyl 6-amino-2-[(12-benzyloxycarbonylamino)dodecanoylamino]-2,6-dideoxy-3-O-tetradecanoyl- α -D-glucopyranoside (35). Compound **32** (914 mg, 1.20 mmol) was treated with triphenylphosphine as described for the preparation of compound **34**. R_f =0.43 (MeOH/DCM, 1:9, v/v). The crude product was purified by silica gel column chromatography. Elution was performed with MeOH/DCM/TEA (0:99.5:0.5 \rightarrow 10:89.5:0.5, v/v/v). Yield 698 mg (78%). ^1H NMR (CDCl_3): δ =0.88 (t, 3H, CH_3 myristyl), 1.25 (m, 34H, CH_2 hexanoyl, myristyl), 1.52 (m, 6H, CH_2 hexanoyl, myristyl), 2.09 (m, 2H, CH_2 hexanoyl, myristyl), 2.31 (m, 2H, CH_2 hexanoyl, myristyl), 2.80 (br. s, 3H, NH_2 -6, OH-4), 3.03 (br. d, 2H, H_2 -6), 3.17 (q, 2H, CH_2 hexanoyl), 3.37 (s, 3H, OMe), 3.55–3.67 (m, 2H, H-4, H-5), 4.19 (m, 1H, H-2), 4.66 (d, 1H, H-1, $J_{1,2}$ =3.6 Hz), 4.83 (s, 1H, NH aminohexanoyl), 5.09 (br. t, 3H, H-3, CH_2 Z), 5.81 (d, 1H, NH), 7.28–7.36 (m, 5H, CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ =14.12 (CH_3 myristyl), 22.70–41.14 (CH_2 hexanoyl, myristyl), 43.59 (C-6), 51.86 (C-2), 55.19 (OMe), 66.55 (CH_2 Z), 71.00 (C-4), 71.17 (C-5), 73.55 (C-3), 98.45 (C-1), 128.05, 128.50 (CH-arom Z), 136.74 (Cq Z), 156.43 (C=O Z), 173.05, 174.95 (2 \times C=O amide, ester).

Methyl 6-amino-2-[(R)-3-(6-benzyloxycarbonylamino)hexanoyloxy]-tetradecanoyl-amino-2,6-dideoxy-3-O-tetradecanoyl- α -D-glucopyranoside (36). Compound **36** was prepared as described for compound **34**. The crude product was purified by silica gel column chromatography. Elution was performed with MeOH/DCM/TEA (0:99:1 \rightarrow 9:90:1, v/v/v). Yield 35 mg (52%). ^1H NMR (CDCl_3): δ =0.88 (t, 6H, CH_3 acyloxyacyl, myristyl), 1.25 (m, 40H, CH_2 acyloxyacyl, myristyl), 1.55 (m, 8H, CH_2 acyloxyacyl, myristyl), 2.35 (m, 6H, CH_2 acyloxyacyl, myristyl), 3.04–3.33 (m, 7H, OH-4, NH_2 -6, H_2 -6, NCH_2 hexanoyl), 3.35 (s, 3H, OMe), 3.62–3.66 (m, 2H, H-4, H-5), 4.18 (m, 1H, H-2), 4.65 (d, 1H, H-1, $J_{1,2}$ =3.5 Hz), 4.95 (br. s, 1H, NH aminohexanoyl), 5.08 (m, 4H, CH_2 Z, H-3, CHO acyloxyacyl), 6.00 (d, 1H, NH), 7.27–7.36 (m, 5H,



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CH-arom Z). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ = 14.17 (CH_3 acyloxyacyl, myristyl), 22.74–41.34 (CH_2 acyloxyacyl, myristyl), 43.52 (C-6), 52.00 (C-2), 55.29 (OMe), 66.64 (CH_2 Z), 70.43 (C-4), 71.15 (CHO acyloxyacyl), 71.49 (C-5), 73.32 (C-3), 98.31 (C-1), 128.11, 128.56 (CH-arom Z), 136.74 (Cq Z), 156.5 (C=O Z), 169.71, 172.79, 174.98 ($3 \times \text{C}=\text{O}$ amide, ester).

Compound 37. To a stirred mixture of **11** (30 mg, 0.428 mmol), PyBOP (25 mg, 0.480 mmol) and DiPEA (8.1 μL , 0.480 mmol) in DCM (3.6 mL) a solution of amino sugar **34** (28 mg, 0.431 mmol) in DCM (2.8 mL) was added. After 1 h, TLC analysis indicated the complete conversion of starting material into a compound with R_f = 0.62 (MeOH/DCM, 7:93, v/v). The reaction mixture was diluted with DCM (50 mL) and washed with water (1×20 mL). After drying over MgSO_4 , the organic layer was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography. Elution was performed with hexane/ethyl acetate (40:60 \rightarrow 100:0, v/v). Yield 55 mg (96%). ^1H NMR (CDCl_3): δ = 0.88 (m, 6H, CH_3 myristyl), 1.25 (br. s, 42H, CH_2 hexanoyl, myristyl), 1.52 (m, 8H, CH_2 hexanoyl, myristyl), 1.94 (m, 3H, CH_2 hexanoyl, OH-4), 2.11 (t, 2H, CH_2 hexanoyl), 2.27 (m, 2H, OCOCH_2), 2.41 (dd, 1H, H-1'ax, $J_{1'ax,1'eq}$ = 12.5 Hz, $J_{1'ax,2'}$ = 5.5 Hz), 2.90 (m, 1H, H-6a), 3.04 (br. s, 1H, H-5'), 3.10 (dd, 1H, H-1'eq, $J_{1'ax,1'eq}$ = 12.5 Hz, $J_{1'eq,2'}$ = 3.0 Hz), 3.17 (q, 2H, NCH_2 hexanoyl), 3.29 (s, 3H, OMe), 3.39 (m, 3H, H-4, NCH_2 acetyl), 3.52 (m, 1H, H-6'a), 3.55 (m, 1H, H-3'), 3.59 (m, 1H, H-5), 3.67 (t, 1H, H-4'), 3.83 (dd, 1H, H-6'b), 4.02 (m, 2H, H-2', H-6b), 4.12 (m, 1H, H-2), 4.45–4.67 (m, 7H, $3 \times \text{CH}_2$ Bn, H-1), 4.82 (br. s, 1H, NHCOO), 5.09 (s, 2H, CH_2 Z), 5.11 (t, 1H, H-3), 5.77 (d, 1H, NH-2), 6.35 (br. d, 1H, $\text{NH-2}'$), 7.25–7.35 (m, 20H, CH-arom Bn/Z), 7.82 (m, 1H, NH-6). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ = 14.14 (CH_3 $2 \times$ myristyl), 22.72–38.84 (CH_2 hexanoyl, myristyl), 39.85 (C-6), 40.91 (NCH_2 hexanoyl), 47.56 (C-2'), 50.78 (C-1') 52.55 (C-2), 55.19 (OMe), 57.69 (CH_2 acetyl), 62.22 (C-5'), 66.44 (C-6'), 66.62 (CH_2 Z), 69.23 (C-4), 70.84 (C-5), 72.10 (C-3), 72.82, 73.35, 73.50 ($3 \times \text{CH}_2$ Bn), 76.93 (C-3'), 77.28 (C-4'), 98.62 (C-1), 127.80–128.64 (CH-arom Bn, Z), 136.75, 137.66, 137.83, 137.86 ($4 \times \text{Cq}$ Bn, Z), 156.75 (C=O Z), 172.59, 172.76, 173.18, 174.65 ($4 \times \text{C}=\text{O}$ amide, ester). ES-MS; m/z : 1332.9, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{78}\text{H}_{117}\text{N}_5\text{O}_{13}$ = 1331.86.

Compound 38. Compound **11** (22 mg, 31.4 μmol) was coupled with compound **35** (24 mg, 32.7 μmol) as described for the preparation of compound **37**. R_f = 0.53 (MeOH/DCM, 5:95, v/v). Yield 42.8 mg (96%). ^1H NMR (CDCl_3): δ = 0.88 (m, 6H, CH_3 myristyl), 1.25 (br. s, 52H, CH_2 dodecanoyl, myristyl), 1.46 (m, 4H, CH_2 dodecanoyl, myristyl), 1.55 (m, 8H, CH_2 dodecanoyl, myristyl), 1.94 (q, 1H, OH-4), 2.10 (m, 2H, CH_2 dodecanoyl), 2.28 (m, 2H, OCOCH_2), 2.44 (dd, 1H, H-1'ax, $J_{1'ax,1'eq}$ = 12.4 Hz, $J_{1'ax,2'}$ = 5.2 Hz), 2.90 (m, 1H, H-6a, $J_{6a,6b}$ = 12.4 Hz), 3.05 (br. s, 1H, H-5'), 3.10 (dd, 1H, H-1'eq, $J_{1'ax,1'eq}$ = 12.5 Hz, $J_{1'eq,2'}$ = 3.1 Hz), 3.18 (q, 2H, NCH_2 dodecanoyl), 3.29 (s, 3H, OMe), 3.38 (m, 3H, H-4, NCH_2 acetyl), 3.52 (s, 1H, H-3'), 3.55 (m, 1H, H-6'a, $J_{5',6'a}$ = 3.3 Hz, $J_{6a',6b'}$ = 10.3 Hz), 3.60 (br. d, 1H, H-5, $J_{4,5}$ = 9.6 Hz), 3.68 (t, 1H, H-4'), 3.84 (dd, 1H, H-6'b, $J_{5,6'b}$ = 6.2 Hz, $J_{6'a,6'b}$ = 10.2 Hz), 4.02 (m, 1H, H-6b), 4.07 (m, 1H, H-2'), 4.11 (m, 1H, H-2), 4.45–4.71 (m, 8H, H-1, NHCOO , $3 \times \text{CH}_2$ Bn, H-1), 5.09 (s, 2H, CH_2 Z), 5.12 (t, 1H, H-3, J = 10.4 Hz), 5.73 (d, 1H, NH-2), 6.36 (br. d, 1H, $\text{NH-2}'$), 7.22–7.35 (m, 20H, CH-arom Bn, Z), 7.85 (m, 1H, NH-6). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3):



δ = 14.20 (CH₃ dodecanoyl, myristyl), 22.78–36.88 (CH₂ dodecanoyl, myristyl), 39.85 (C-6), 41.24 (NCH₂ dodecanoyl), 47 (C-2'), 50 (C-1') 52.56 (C-2), 55.25 (OMe), 57.78 (CH₂ acetyl), 62 (C-5'), 66.47 (C-6'), 66.66 (CH₂ Z), 69.20 (C-4), 70.94 (C-5), 71.93 (C-3), 72.86, 73.39, 73.52 (3 × CH₂ Bn), 77.30 (C-3', C-4'), 98.69 (C-1), 127.86–128.71 (CH-arom Bn/Z), 136.80, 137.87 (Cq Bn, Z), 156 (C=O Z), 173.04, 173.30, 174.69, (C=O amide, ester). ES-MS; *m/z*: 1416.85, [M+H]⁺; monoisotopic MW calculated for C₈₄H₁₂₉N₅O₁₃ = 1415.96.

Compound 39. Compound **11** (10 mg, 14.3 μmol) was coupled with compound **36** (13.7 mg, 15.7 μmol) as described for the preparation of compound **37**. *R_f* = 0.86 (MeOH/DCM, 5:95, v/v). Yield 11.4 mg (51%). ¹H NMR (CDCl₃): δ = 0.88 (t, 9H, CH₃ acyloxyacyl, myristyl), 1.25 (m, 60H, CH₂ acyloxyacyl, myristyl), 1.52 (m, 10H, CH₂ acyloxyacyl, myristyl), 1.94 (m, 3H, OH-4, CH₂ myristyl), 2.30 (m, 6H, NCH₂ hexanoyl, OCOCH₂), 2.42 (dd, 1H, H-1'ax), 2.88 (m, 1H, H-6a), 3.06 (br. s, 1H, H-5'), 3.11 (dd, 1H, H-1'eq), 3.18 (q, 2H, NCH₂ hexanoyl), 3.27 (s, 3H, OMe), 3.35 (m, 1H, H-4), 3.40 (s, 2H, NCH₂ acetyl), 3.53 (m, 2H, H-3', H-6'a), 3.58 (m, 1H, H-5), 3.68 (t, 1H, H-4'), 3.83 (dd, 1H, H-6'b), 4.03 (m, 2H, H-2', H-6b), 4.10 (m, 1H, H-2), 4.45–4.67 (m, 7H, 3 × CH₂ Bn, H-1), 4.85 (br. s, 1H, NHCOO), 5.10 (m, 4H, CH₂ Z, H-3, CHO acyloxyacyl), 5.93 (d, 1H, NH-2), 6.32 (br. d, 1H, NH-2'), 7.25–7.35 (m, 20H, CH-arom Bn, Z), 7.86 (m, 1H, NH-6). ¹³C{¹H} NMR (CDCl₃): δ = 14.20 (CH₃ myristyl), 22.78–36.89 (CH₂ acyloxyacyl, myristyl), 39.85 (C-6), 40.94 (C-1'), 41.35 (NCH₂ hexanoyl), 47.47 (C-2'), 52.61 (C-2), 55.19 (OMe), 57.81 (CH₂ acetyl), 62.16 (C-5'), 66.48 (C-6'), 66.67 (CH₂ Z), 69.20 (C-4), 70.87 (C-5), 71.16 (CHO acyloxyacyl), 71.89 (C-3), 72.81, 73.40, 73.48 (3 × CH₂ Bn), 76 (C-3'), 77.30 (C-4'), 98.48 (C-1), 127.85–128.71 (CH-arom Bn, Z), 136.77, 137.64, 137.85 (Cq Bn, Z), 156.49 (C=O Z), 169.64, 172.80, 173.36, 174.67 (4 × C=O amide, ester). ES-MS; *m/z*: 1559.00, [M+H]⁺; monoisotopic MW calculated for C₉₂H₁₄₃N₅O₁₅ = 1558.05.

Compound 40. Compound **12** (30 mg, 0.334 mmol) was coupled with compound **34** (23 mg, 0.334 mmol) as described for the preparation of compound **37**. *R_f* = 0.48 (hexane/ethyl acetate, 1:4, v/v). Yield 45 mg (88%). ¹H NMR (CDCl₃): δ 0.88 = (dt, 9H, CH₃ acyloxyacyl, myristyl), 1.25 (br. s, 56H, CH₂ acyloxyacyl, hexanoyl, myristyl), 1.52 (m, 10H, CH₂ acyloxyacyl, hexanoyl, myristyl), 1.86 (s, 1H, OH-4), 2.11 (t, 2H, NCH₂ hexanoyl), 2.16–2.33 (m, 6H, CH₂ acyloxyacyl, hexanoyl, myristyl), 2.40 (dd, 1H, H-1'ax, *J*_{1'ax,1'eq} = 12.5 Hz, *J*_{1'ax,2'} = 5.8 Hz), 2.96 (m, 1H, H-6a), 3.05 (br. s, 1H, H-5'), 3.10 (dd, 1H, H-1'eq, *J*_{1'ax,1'eq} = 12.5 Hz, *J*_{1'eq,2'} = 3.4 Hz), 3.17 (q, 2H, CH₂ hexanoyl), 3.29 (s, 3H, OMe), 3.37 (m, 1H, H-4), 3.40 (s, 2H, NCH₂ acetyl), 3.54 (m, 2H, H-6a', H-3'), 3.59 (m, 1H, H-5), 3.67 (t, 1H, H-4'), 3.83 (dd, 1H, H-6b'), 4.03 (m, 2H, H-2', H-6b), 4.12 (m, 1H, H-2), 4.44–4.67 (m, 6H, 3 × CH₂ Bn), 4.50 (d, 1H, H-1), 4.81 (s, 1H, NHZ), 5.09 (m, 4H, CH₂ Z, H-3, CHO acyloxyacyl), 5.79 (d, 1H, NH-2), 6.55 (d, 1H, NH-2'), 7.25–7.35 (m, 20H, CH-arom Bn, Z), 7.87 (m, 1H, NH-6). ¹³C{¹H} NMR (CDCl₃): δ = 14.16 (CH₃, dodecanoyl, myristyl), 22.74–36.49 (CH₂ dodecanoyl, hexanoyl, myristyl), 39.78 (C-6), 40.91, 41.80, 41.92 (CH₂ myristyl, hexanoyl, dodecanoyl), 47.65 (C-2'), 50.93 (C-1'), 52.56 (C-2), 55.19 (OMe), 57.69 (CH₂ acetyl), 62.20 (C-5'), 66.43 (C-6'), 66.66 (CH₂ Z), 69.21 (C-4), 70.95 (C-5), 71.15 (CHO acyloxyacyl), 72.12 (C-3), 72.83, 72.99, 73.35 (3 × CH₂ Bn), 76 (C-3'), 77 (C-4'), 98.61 (C-1), 127.73–128.65 (CH-arom Bn, Z), 136.76, 137.80, 137.86, 137.89



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(4 × Cq Bn, Z), 156.46 (C=O Z), 169.34, 172.64, 173.18, 173.32, 174.68 (5 × C=O amide, ester). ES-MS; *m/z*: 1531.1, [M+H]⁺; monoisotopic MW calculated for C₉₀H₁₃₉N₅O₁₅ = 1530.0.

Compound 41. Compound **12** (20 mg, 22.2 μmol) was coupled with compound **35** (18 mg, 24.5 μmol) as described for the preparation of compound **37**. *R_f* = 0.62 (MeOH/DCM, 5:95, v/v). Yield 34.5 mg (96%). ¹H NMR (CDCl₃): δ = 0.88 (m, 9H, CH₃ dodecanoyl, myristyl), 1.25 (m, 68H, CH₂ acyloxyacyl, dodecanoyl, myristyl), 1.54 (m, 10H, CH₂ acyloxyacyl, dodecanoyl, myristyl), 2.10 (m, 2H, NCH₂ dodecanoyl), 2.23 (m, 6H, CH₂ acyloxyacyl, dodecanoyl, myristyl), 2.43 (dd, 1H, H-1'ax, *J*_{1'ax,1'eq} = 12.4 Hz, *J*_{1'ax,2'} = 5.1 Hz), 2.97 (m, 1H, H-6a, *J*_{6a,6b} = 12.6 Hz), 3.08 (br. s, 1H, H-5'), 3.11 (dd, 1H, H-1'eq, *J*_{1'ax,1'eq} = 12.6 Hz, *J*_{1'eq,2'} = 2.8 Hz), 3.18 (q, 2H, NCH₂ acyl), 3.29 (s, 3H, OMe), 3.39 (m, 3H, H-4, NCH₂ acetyl), 3.55 (m, 2H, H-6a', H-3'), 3.60 (m, 1H, H-5, *J*_{4,5} = 9.8 Hz), 3.67 (t, 1H, H-4'), 3.84 (dd, 1H, H-6b', *J*_{5',6b'} = 6.3 Hz, *J*_{6a',6b'} = 10.5 Hz), 4.03 (m, 1H, H-6b, *J*_{5,6b} = 4.2 Hz, *J*_{6a,6b} = 12.6 Hz), 4.06 (m, 1H, H-2'), 4.12 (m, 1H, H-2, *J*_{1,2} = 3.5 Hz, *J*_{2,3} = 9.8 Hz), 4.42–4.78 (m, 8H, H-1, NHZ, 3 × CH₂ Bn), 5.08 (m, 4H, CH₂ Z, H-3, CHO acyloxyacyl), 5.74 (d, 1H, NH-2, *J*_{2,NH} = 9.1 Hz), 6.59 (d, 1H, NH-2', *J*_{2',NH} = 6.3 Hz), 7.25–7.36 (m, 20H, CH-arom Bn, Z), 7.87 (m, 1H, NH-6). ¹³C{¹H} NMR (CDCl₃): δ = 14.20 (CH₃ 3 × dodecanoyl, myristyl), 22.78–36.87 (CH₂ acyloxyacyl, dodecanoyl, myristyl), 39.78 (C-6), 41.23, 41.79 (CH₂ acyloxyacyl, dodecanoyl, myristyl), 47.79 (C-2'), (50, C-1'), 52.53 (C-2), 55.21 (OMe), 57.69 (CH₂ acetyl), 62.20 (C-5'), 66.41 (C-6'), 66.66 (CH₂ Z), 69.18 (C-4), 71.03 (C-5), 71.13 (CHO acyloxyacyl), 71.95 (C-3), 72.83, 73.30, 73.35 (3 × CH₂ Bn), 77.09 (C-3'), 77.31 (C-4'), 98.61 (C-1), 127.74–128.68 (CH-arom Bn, Z), 136.80, 137.88, (Cq Bn, Z), 156.46 (C=O Z), 169.31, 173.02, 173.18, 173.25, 174.66 (4 × C=O amide, ester). ES-MS; *m/z*: 1615.00, [M+H]⁺; monoisotopic MW calculated for C₉₆H₁₅₁N₅O₁₅ = 1614.12.

Compound 1. To a solution of **37** (10.4 mg, 7.54 μmol) in DMF (0.5 mL), Pd/C (10%, 5 mg) was added. Hydrogen was passed through the stirred mixture for 46 h. After filtration of the mixture over a PTFE filter, the filtrate was concentrated under reduced pressure. Yield 7 mg quantitative. ¹H NMR (pyridine-d₅): δ = 0.87 (m, 6H, 2 × CH₃ acyl), 1.24 (m, 43H, CH₂ acyl), 1.39 (m, 2H, CH₂ acyl), 1.53 (m, 2H, CH₂ acyl), 1.65 (m, 2H, CH₂ acyl), 1.82 (m, 4H, 2 × CH₂ acyl), 2.06 (m, 2H, CH₂ acyl), 2.18 (t, 1H, H-1'ax), 2.57–2.29 (m, 8H, CH₂ acyl), 2.65 (m, 1H, H-5'), 3.05 (d, 1H, H-acetyl), 3.26 (t, 2H, CH₂ acyl), 3.38 (dd, 1H, H-1'eq), 3.41 (s, 3H, OMe), 3.75 (m, 3H, H-acetyl, H-4', H-6a), 4.04 (dd, 1H, H-6b), 4.10 (dd, 1H, H-3'), 4.26 (t, 1H, H-4), 4.35 (t, 1H, H-6'a), 4.57 (m, 1H, H-5), 4.62 (m, 1H, H-2'), 4.86 (m, 1H, H-2), 4.97 (dd, 1H, H-6'b), 5.11 (d, 1H, H-1), 5.84 (dd, 1H, H-3), 8.65 (d, 1H, NH), 8.89 (d, 1H, NH'). ¹³C{¹H} NMR (pyridine-d₅): δ = 13.65 (CH₃ myristyl), 22.29–38.90 (CH₂ acyl), 40.62 (C-1'), 50.75 (C-2'), 51.76 (C-2), 54.69 (C-6), 55.16 (OMe), 57.14 (CH₂ acetyl), 59.73 (C-5'), 69.02 (C-5), 69.96 (C-4), 72.36 (C-6'), 73.07 (C-3), 73.56 (C-4'), 75.78 (C-3'), 99.12 (C-1), 166.63 (C=O ester), 172.71, 173.24, 173.31 (3 × C=O amide). ES-MS; *m/z*: 928.77, [M+H]⁺; monoisotopic MW calculated for C₄₉H₉₃N₅O₁₁ = 927.69.

Compound 2. Compound **38** (12.9 mg, 9.11 μmol) was treated as described for the preparation of compound **1**. Yield 6.6 mg (72%) of a white solid. ES-MS; *m/z*: 1012.54, [M+H]⁺; monoisotopic MW calculated for C₅₅H₁₀₅N₅O₁₁ = 1011.78.



Compound 3. Compound **39** (1.9 mg, 1.2 μmol) was treated as described for the preparation of compound **1**. Yield 0.7 mg (54%) of a white solid. ES-MS; m/z : 1154.98, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{63}\text{H}_{119}\text{N}_5\text{O}_{13}$ = 1153.88.

Compound 4. Compound **40** (6.7 mg, 4.38 μmol) was treated as described for the preparation of compound **1**. Yield 4.3 mg (88%) of a white solid. ES-MS; m/z : 1126.9, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{61}\text{H}_{115}\text{N}_5\text{O}_{13}$ = 1125.85.

Compound 5. Compound **41** (8.2 mg, 5.1 μmol) was treated as described for the preparation of compound **1**. Yield 4.0 mg (65%) of a white solid. ES-MS; m/z : 1211.18, $[\text{M}+\text{H}]^+$; monoisotopic MW calculated for $\text{C}_{67}\text{H}_{127}\text{N}_5\text{O}_{13}$ = 1209.94.

ACKNOWLEDGMENTS

We thank S.H. van Krimpen for recording the NMR spectra and A.G. Hulst for performing electrospray MS analysis.

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26. NMR spectrometry of compounds **2–5** was performed with CDCl_3 , methanol- d_4 and pyridine- d_5 as solvents at ambient temperature.

Received November 5, 2001

Accepted January 28, 2002