# Synthesis and Supramolecular Characterization of a Novel Class of Glycopyranosyl-Containing Amphiphiles

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*Received October 27, 1999*

A novel class of glycopeptidolipids is described, which potentially can be used as a novel antigen-delivery system. The compounds have been prepared by a combination of solid-supported and solution-based methods. The use of the orthogonally protected FmocLysDde derivative provided an opportunity to incorporate two different types lipids. It was found that the model compound **1** forms aggregates in aqueous media which can be described as rod or tubelike structures. The aggregates can be stabilized by topotactic photopolymerization. Studies on the structural analogues **2**−**5** revealed the effect of the carbohydrate, peptide, and lipid moiety on the aggregation properties. It is concluded that none of the structure elements can lay claim to be exclusively important for the formation of highly organized aggregates such as tubes, fibers, or helical ribbons from **1**, but the presence of all of these structural elements afforded the most uniformly shaped extended structures.

## Introduction

Conjugates composed of bacterial poly(oligo)saccharides and a carrier protein are successfully used as vaccines against *Haemophilus influenzae* and are under investigation as experimental vaccines against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Streptococcus* group B.[1]

We are developing a new generation of saccharide-containing vaccines based on self-assembling fully synthetic glycopeptidolipids.[2,3] These compounds contain a carbohydrate moiety that will act as a B-epitope, a peptide sequence which is a MHC class II restricted recognition site for human T-cells and a lipid moiety, which confers upon the compound the ability to self-assemble to give macromolecular aggregates. The self-assembly properties of the glycopeptidolipids are very important because small molecules are unable to stimulate a sustainable antibody response.[4] However, highly immunological responses are observed when small haptens are coupled at the surface of macromolecules. Traditionally, antigenic macromolecules were obtained by conjugation of a hapten to a carrier protein; however, it is now recognized that self-assembled aggregates can also function as efficient immunogens. Recently, we established a correlation between molecular structure, aggregation properties, and immunological activities of a range lipopeptides and found that compounds, which arrange in tubular or extended structures, are more immunogenic than compounds that organize in vesicular structures.[3]

Here, we report an efficient synthetic methodology for the preparation of a new class of amphiphiles that contain a carbohydrate, a peptide, and a lipid moiety. These compounds will be used as antigen delivery systems and will have the additional advantage that their aggregates can be stabilized by topotactic photopolymerization of the 10,12-pentacosadiynoic acid moiety. The synthetic methodology was developed by the preparation of model compound **1** (Chart 1). The self-assembly properties of the glycopeptidolipids were studied by transmission electron microscopy (TEM) and low-angle X-ray powder diffraction (XRD). It was discovered that compound **1** organized into fibers or tubular structures, which can be stabilized by polymerization of a diacetylene-containing lipid moiety. The derivatives **2**−**5** (Chart 1) were prepared to reveal the structural motives of **1** that induce tubular or extended aggregate formation.

## Results and Discussion

**Synthesis.** The synthesis of the target compound **1** requires a highly convergent strategy and synthetic procedures that are compatible with carbohydrate, peptide, and lipid chemistry. It was envisaged that tetrapeptide ALFG (**6**) (A = alanine, L = leucine, F = phenyl-

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10.1021/jo991685s CCC: $19.00 
Published on Web 05/04/2000

**Chart 1**

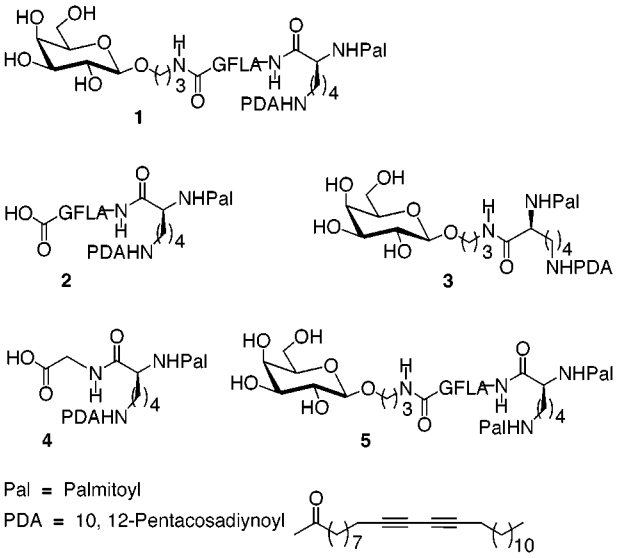
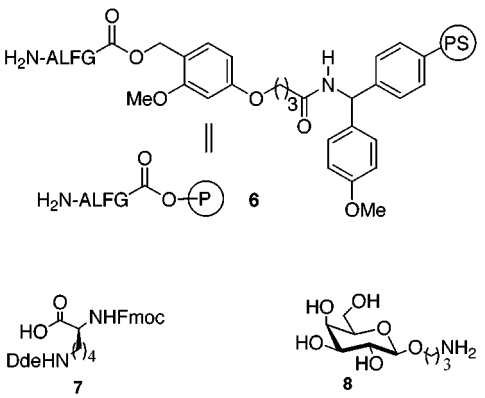


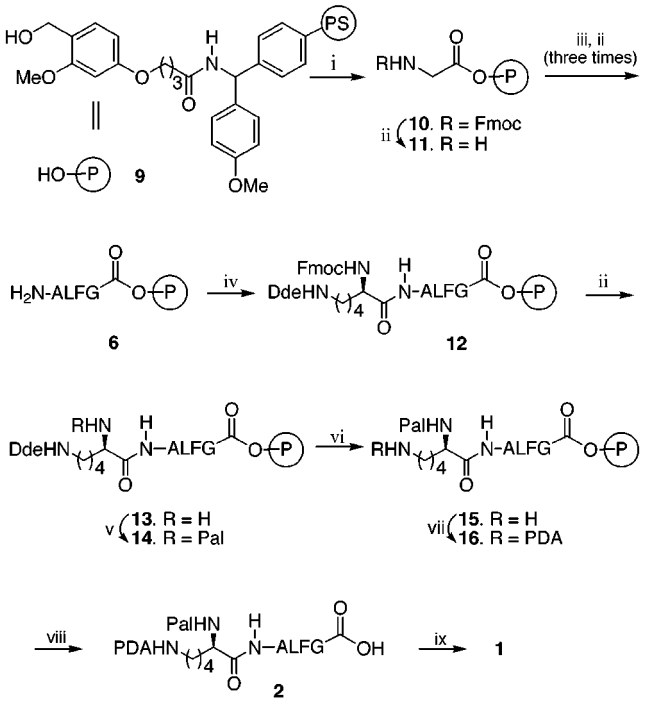

**Chart 2**




alanine, G = glycine) linked to a copolystyrene solid support, containing a hyperacid labile 4-(hydroxymethyl)-3-(methoxyphenyloxy)butyric acid 4-methylbenzhydroxylamine (HMPB-MBHA) linker, the quasi-orthogonally protected lysine derivative FmocLysDdeOH **7** (Dde: 1-(4,4-dimethyl-2,6-dioxycyclohex-1-ylidene)ethyl, Fmoc: fluorenylmethoxycarbonyl) and the aminopropyl derivatized galactoside **8** (Chart 2), would be suitable building blocks for a combined solid- and solution-phase synthesis of **1** (Scheme 1).

The following strategy was devised: in the last cycle of peptide synthesis, the commercially available FmocLysDdeOH derivative **7** will be coupled with polymer-bound tetrapeptide ALFG **6**. The Fmoc and Dde group of the lysine moiety are quasi-orthogonal which enables the selective cleavage of the $N^{\alpha}$-Fmoc group (piperidine/DMF) in the presence of the $N^{\epsilon}$-Dde group.[5] On the other hand, the Dde group can be removed under mild conditions using hydrazine in DMF. Thus, a key aspect of the synthetic approach is that the two amino functionalities of the lysine moiety of the pentapeptide can be selectively derivatized with different lipids. The HMPB-MBHA linker allows cleavage of the lipopeptide from the solid support using mild acidic conditions (1% TFA in $CH_2Cl_2$). Finally, in solution, the revealed carboxylic acid of the released lipopeptide will be condensed with the aminopropyl moiety of saccharide **8** to give the target compound **1**.

**Scheme 1. Preparation of Compound 1**[a]




[a] Reagents and conditions: (i) 10 equiv of FmocGlyOH, 5 equiv of DIPC, DMF/$CH_2Cl_2$ (5/2, v/v), then **9** and 1 equiv of DMAP; (ii) 20% piperidine/DMF; (iii) 5 equiv of FmocAA, 6 equiv of HOBt, 6 equiv of DIPC, $CH_2Cl_2$/DMF (5/2, v/v); (iv) 2 equiv of of **7**, 2 equiv of PyBOP, 2 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v); (v) 4 equiv of PalOH, 4 equiv of PyBOP, 4 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v); (vi) 2 % hydrazine monohydrate/DMF; (vii) 4 equiv of 10,12-pentacosadiynoic acid, 4 equiv of PyBOP, 4 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v); (viii) 1% TFA/$CH_2Cl_2$; (ix) 8, 3 equiv of EDC, 3 equiv of HOBt, 3 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v).

The polymer-linked tetrapeptide ALFG (**6**) was prepared on a co-polystyrene solid support containing a HMPB-MBHA linker (**9**, Scheme 1). Attachment of the first amino acid was achieved by condensation of the symmetrical anhydride of Fmoc-protected glycine, with co-polystyrene resin **9** in the presence of the acylating reagent DMAP to give glycine-derivatized polymer **10**. The symmetrical anhydride was prepared by dehydration of Fmoc-glycine in the presence of diisopropylcarbodiimide (DIPC). The Fmoc protecting group of **10** was removed under standard conditions (20% piperidine in DMF) to give **11**. The amino acids alanine, leucine, and phenylalanine were subsequently introduced to give **6** by employing Fmoc-protected amino acids (FmocAA), *N*-hydroxybenzotriazole (HOBt)/DIPC as chain elongating reagents, and 20% piperidine in DMF to cleave Fmoc protecting groups.[6] In the last step of the peptide assembly, the commercially available quasi-orthogonally protected lysine derivative FmocLysDdeOH **7** was coupled with the amino functionality of **6** by applying (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP)/diisopropylethylamine (DIPEA) as condensation reagent to give peptide **12**. The condensation reagent PyBOP/DIPEA ensured efficient coupling using low equivalents of the expensive amino acid **7**. The

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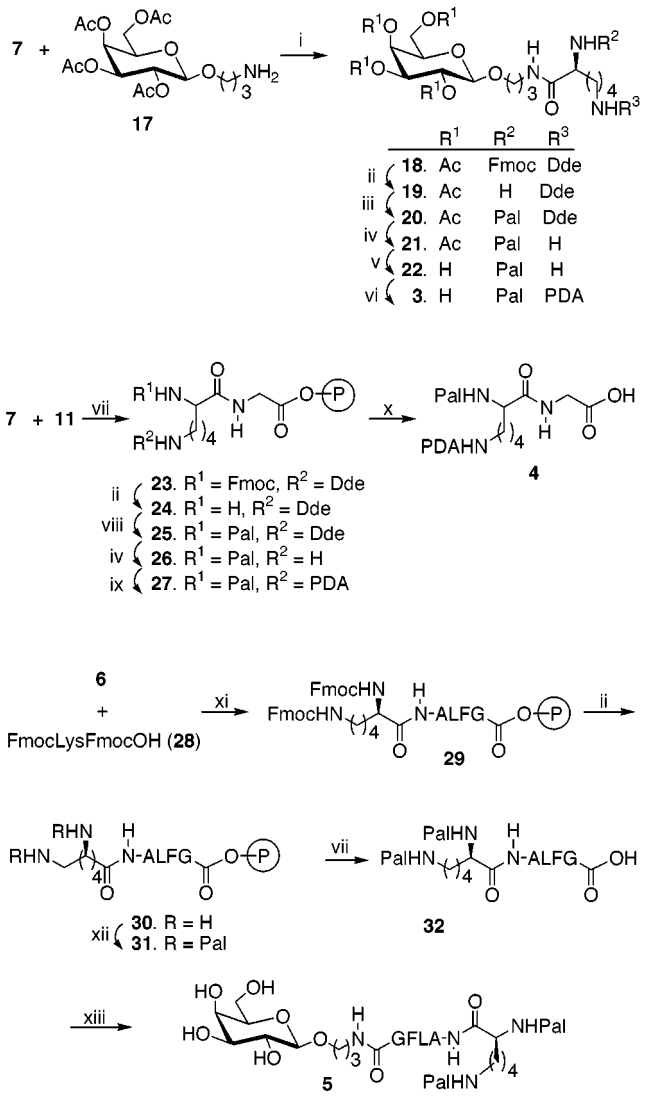
Fmoc protecting group of **12** was selectively cleaved with 20% piperidine in DMF, and palmitoylation of the $N^{\alpha}$-amino functionality of the resulting compound **13** was achieved with palmitic acid in the presence of PyBOP/HOBT to give the lipopeptide **14**. The Dde group of **14** was removed by treatment with hydrazine in DMF to give **15** and the commercially available 10,12-pentacosadiynoic acid (PDA-OH) was coupled with the $N^{\epsilon}$-amino group to afford polymer-bound lipopeptide **16**. Treatment of **16** with 1% TFA/$CH_2Cl_2$ cleaved the lipopetide from the solid support and gave, after purification by Sephadex LH-20 size exclusion column chromatography, compound **2** in an overall yield of 67% (based on initial amino acid loading). Finally, the target compound **1** was obtained by 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDC)/HOBT-mediated coupling of **2** with the aminopropyl spacer of galactoside **8**. Purification of the crude material was accomplished by gel filtration on Sephadex LH-20, and the fully deprotected conjugate was isolated in a yield of 53%. 1D- and 2D-NMR spectroscopy and mass spectrometry confirmed the molecular structure of **1**. FAB mass analysis showed a peak at *m*/*z* 1370.8 corresponding to $[M + Na]^+$, and signals at $\nu = 2375$ and 2325 [$cm^{-1}$] in the IR spectrum confirmed the presence of the diacetylenic unit.

The preparation of compounds **3**−**5** is detailed in Scheme 2. Compound **3** was prepared by a solution-based method. Thus, the *O*-acetylated aminopropyl galactoside **17** was coupled with FmocLysDdeOH (**7**) to give **18**. The $N^{\alpha}$-Pal and $N^{\epsilon}$-PDA were installed by a five-step procedure. Thus, treatment of **18** with piperidine in DMF cleaved the Fmoc group and gave **19** in almost a quantitative yield. The revealed $N^{\alpha}$-amino group of **19** was palmitoylated with palmitic acid in the presence of DIPC to afford **20**. Treatment of **20** with hydrazine in DMF removed the Dde group and gave **21** in a yield of 92%. The *O*-acetyl groups of the sugar moiety of **21** were cleaved by reaction with $Et_3N$ in methanol to give **22**. Finally, the target compound **3** was obtained by coupling the $N^{\epsilon}$-amino group of **22** with 10,12-pentacosadiynoic acid (PDA-OH). The latter reaction exploits the higher reactivity of the amino group compared to the hydroxyls of the sugar moiety.

Compound **4** was easily available by coupling glycine-derivatized polymer **11** (Scheme 1) with FmocLysDdeOH **7** to give **23** which was converted into a $N^{\alpha}$-Pal and $N^{\epsilon}$-PDA functionalized derivative **27** according to a similar procedure used for the preparation of **1**. Thus, the Fmoc protecting group of **23** was cleaved under standard conditions, and the resulting $N^{\alpha}$-amino functionality of **24** was palmitoylated to give **25**. The Dde group of **25** was removed by treatment with hydrazine, and the $N^{\epsilon}$-amino group of **26** was derivatized with a 10,12-pentacosadiynoyl moiety to afford **27**. Target lipopeptide **4** was obtained by treatment of **27** with 1% TFA in $CH_2Cl_2$ followed by purification of the crude product by LH-20 size-exclusion column chromatography.

Finally, compound **5** was obtained according to a similar method used for the preparation of **1**; however, instead of lysine derivative **7**, FmocLysFmocOH **28** was used in the last cycle of peptide assembly to give compound **29**. The two Fmoc groups of **29** were cleaved under standard conditions, and palmitoylation of the $N^{\alpha}$ and $N^{\epsilon}$ moieties of **30** with palmitoyl chloride and pyridine gave lipopeptide **31**. Polymer-bound **31** was cleaved from the solid support, and the revealed carboxylic acid of **32** was coupled with the aminopropyl galactoside **8** to give target compound **5**.

**Scheme 2**[a]




[a] (i) 1.5 equiv of DIPC, 1.5 equiv of HONB, $CH_2Cl_2$/DMF (2/1, v/v); (ii) 20% piperidine/DMF; (iii) 2 equiv of palmitic acid, 2 equiv of HONB, 2 equiv of DIPC, $CH_2Cl_2$/DMF (1/1, v/v); (iv) 2% hydrazine monohydrate/DMF; (v) $Et_3N$/MeOH/$H_2O$ (2/5/5, v/v/v); (vi) 1.5 equiv of 10,12-pentacosaiynoic acid, 2 equiv of EDC, 2 equiv of HOBt, $CH_2Cl_2$/DMF (1/1, v/v); (vii) 2 equiv of PyBOP, 2 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v); (viii) 5 equiv of PalOH, 5 equiv of PyBOP, 5 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v); (ix) 5 equiv of 10,12-pentacosadiynoic acid, 5 equiv of PyBOP; 5 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v/v); (x) 1% TFA/ $CH_2Cl_2$; (xi) 5 equiv of FmocLysFmocOH, 5 equiv of PyBOP, 5 equiv of DIPEA, $CH_2Cl_2$/DMF (5/2, v.v); (xii) 20 equiv of PalCl, pyridine/$CH_2Cl_2$ (1/1, v/v); (xiii) 11 equiv of **8**, 11 equiv of DIPC, 11 equiv ofHONB, $CH_2Cl_2$/DMF (1/1, v/v)

**Aggregation Properties.** The aggregation properties of **1** were studied by transmission electron microscopy (TEM). A water suspension of **1**, obtained by a modified ethanol injection method and prepared for TEM by drying and Pt shadowing, showed the formation of uniformly shaped extended structures (Figure 1a). The structures appear to be cylindrical, and on the basis of the observed diameters (15−20 nm), it is likely that they consist of cylindrical micelles, a few monolayers, or a single bilayer of **1**. On the basis of Figure 1a, it may be considered that tubules, i.e., aggregates with an aqueous inner compart-

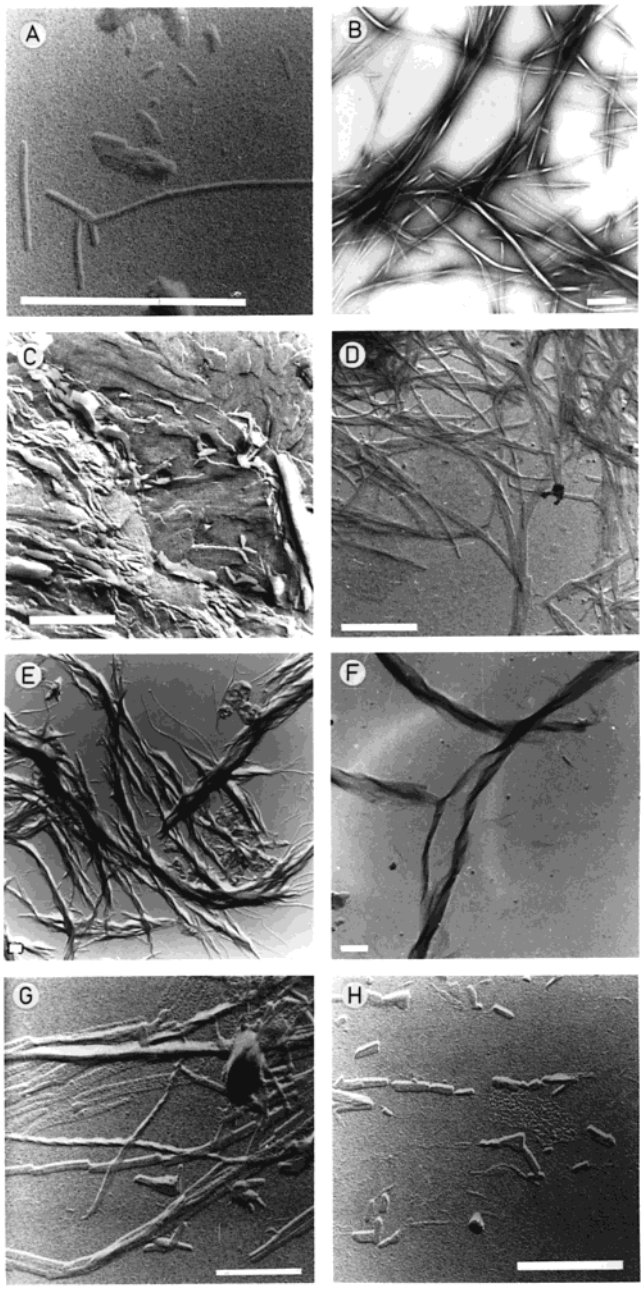



**Figure 1.** Electron micrographs of compounds **1**−**5.** Bar represents 500 nm. For concentrations see Table 3. (A) Pt-shadowing of **1**; (B) negative staining of **1**; (C) freeze fracturing of **1**; (D) Pt-shadowing of polymerized **1**; (E) Pt-shadowing of freshly prepared aggregate of **3**; (F) Pt-shadowing of aged (3 days) aggregate of **3**; (G and H) Pt-shadowing of **4**.

ment, are formed. This would be a reasonable expectation on the basis of the known aggregation chemistry of diacetylenic phospholipids.[10] To define in more detail the morphology of **1**, we applied other preparation techniques. With negative staining (Figure 1b), **1** appeared as twisted ribbons. Such structures are distinct from tubules, but they have been reported as intermediates in tubule formation.[7] Alternatively, the morphology can be interpreted as tubules with flat sections. In the freeze-fracturing experiment (Figure 1c), however, **1** appeared as extended structures that are less cylindrical than those in Figure 1a. In view of the fact that our XRD studies (see below) show that **1** preferentially packs as stacks of monolayers, not bilayers, the morphology of **1** in the negative staining (Figure 1b) and freeze fracture (Figure 1c) experiments is best described as ribbons of stacked monolayers, whereas the morphology in the Pt shadowing experiments is best accounted for by a few concentric monolayers, possibly around a micellar fiber and therefore are better described as ribbons or rods. Saccharide amphiphiles generally assemble into spheroidal supramolecular assemblies such as micelles and vesicles[8] and only a very few reports[3,9] deal with other morphologies such as tubules.

Tubular or rodlike structures are starting points for the development of new materials.[10] They are considered to be more ordered than vesicles and several theories of tube formation have been proposed.[11] Also, it has been shown that in some cases extended aggregates are more immunogenic than spherical ones.[3] Some molecular structural features such as chirality and diacetylenic lipids are known to promote tubular or rodlike aggregation, but the limited number of compounds known to organize into these structures makes it difficult to predict this mode of assembly. There is an earlier report[12] of a glycopeptidolipid, based on lysine with a hydrocarbon and fluorocarbon tail and a lactobiose headgroup, which was found to display a rich aggregation chemistry with stacked disklike assemblies, liposomes, helical twisted tapes, and tubuli in a single micrograph although no explanation for the variation in morphology was given.

A successful polymerization, as judged from a color change from transparent to purple, of a water suspension of compound **1** exposed to UV light (254 nm) corroborated the highly ordered nature of the supramolecular assembly. It is well-known[13] that topotactic photopolymerization of diacetylenes is only possible when they are part of highly ordered structures, and the diacetylenic moieties are appropriately positioned relative toward each other. TEM of the precipitated and polymerized material showed fibers at the edge of a polymer drop (Figure 1d) which are much longer than the tubes/rods observed in the unpolymerized water suspension. Therefore, polymerization occurs along the axis of the tubes/rods as depicted in Scheme 3. Probably hydrogen bonding between the amide moieties along the axis of the tubular structure helps to fulfill the structural requirements for polymerization. It is noteworthy that for some reported examples, a tubular aggregation disappeared upon polymerization.[9a]

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**Scheme 3. Topotactic Polymerization of Tubular Diacetylene Aggregates**

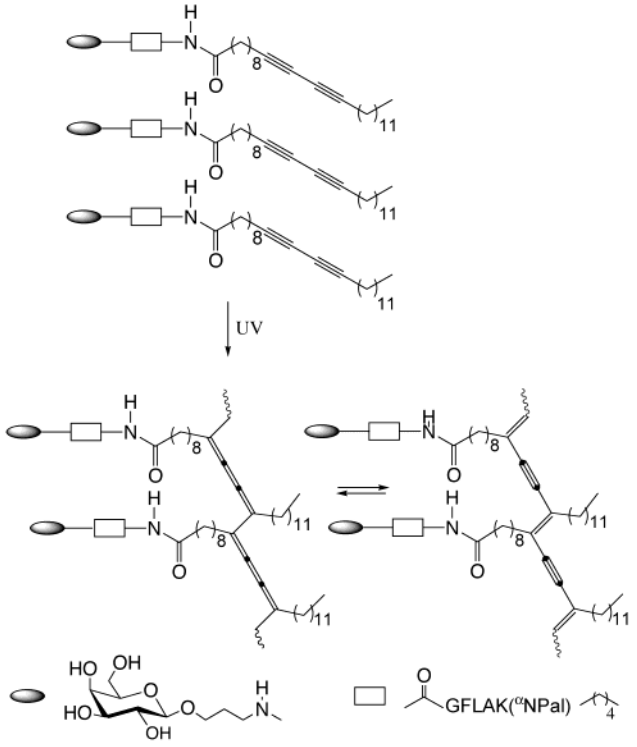


**Table 1. Periodicities Derived from X-ray Diffraction of Lamellar Structures of Nonpolymerized and Polymerized 1**

| rel humidity, % | nonpolymerized, Å | polymerized in suspension, Å | polymerized on Si-wafer, Å |
|---|---|---|---|
| 0 | 84.0 | 82.5 | 82.5 |
| 50 | 86.5 | 91.0 | 84.8 |
| 90 | 91.0 | 94.9 | 88.2 |

The packing of the amphiphile **1** was also investigated by X-ray powder diffraction (XRD). For the study of the self-assembly of molecules of the size described here, it is necessary to use angles smaller than what is typically possible on commercial diffractometers ($2\theta$ value approximately 3°, corresponding to $d = 29$ Å at the wavelength of Cu Kα, 1.54 Å), in particular if detection of the first-order reflection of head-to-tail or head-to-head packing as well as its unambiguous assignment are required. A possible disadvantage of reflection geometry for the study of dispersions is the necessity of drying the sample, with possible introduction of artifacts. In the study presented here, this problem is counteracted in part by the possibility of controlling the relative humidity during the measurements, as well as drying the sample in an environment of controlled relative humidity rather than in a desiccator.[14] In earlier studies using this approach, the reversible binding of one layer of water molecules between gemini bisphosphate monolayers was demonstrated.[15,16] Compound **1** was investigated under a variety of conditions (Table 1 and Figure 2). To avoid polymerization, the dispersion of the amphiphile was kept in the dark during preparation and drying. Also,

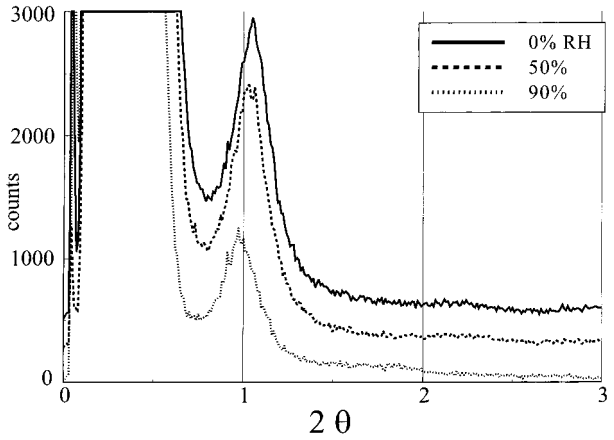


**Figure 2.** X-ray diffractograms of dried suspensions of nonpolymerized **1** at different relative humidities. Solid line, 0%; dashed line, 50%; dotted line, 90% relative humidity.

samples were polymerized by illumination during drying after dispersion in the dark, as well as illuminated while being dispersed. In both cases, polymerization had occurred as judged from the purple color. This is remarkable in view of the topotactic requirements for polymerization.[13] Effects of dehydration of aggregates of diacetylenic amphiphiles have been reported before, for example, a decrease of spacing from 65.5 to 67.5 Å has been reported for a diacetylenic phopholipid.[17] Effects of polymerization have also be noted.[18] All X-ray diffraction results for **1** are in agreement with a lamellar packing with spacing in the range 82.5−94.9 Å. In view of the length of the molecule as estimated from molecular models (approximately 80 Å) this points to a packing in monolayers with varying degrees of hydration. The slightly higher value of periodicity at 0% relative humidity of the nonpolymerized material compared to polymerized material can be explained by a tilt in the molecule upon polymerization (see Scheme 3). The hydration patterns of the nonpolymerized sample and the sample that was polymerized on the wafer are rather similar, with extensions of the periodicity of 7.0 and 5.7 Å, respectively, going from 0 to 90% relative humidity, corresponding to incorporation of five or four water molecules per monolayer. The sample that was polymerized in dispersion expands significantly more upon hydration (12.4 Å), suggesting a more open structure in the packed monolayers. In all cases, the region of the diffractogram where reflections due to the periodicities of alkyl tail packing are expected (approximately 4.2 Å, $2\theta$ range 20−25°) were also studied, but no strong reflections were observed.

To investigate the importance of particular molecular features for tube or rod formation of **1**, the self-assembly properties of compounds **2**−**5** were studied. As expected, the omission of the carbohydrate and peptide parts as in compound **2** and **3**, respectively, or of both as in compound **4** and variation of the nature of the lipid tail as in compound **5** were found to affect the morphology of the supramolecular structures. The water suspension of lipopeptide **2** exhibited mainly the formation of vesicular structures with a typical diameter of 38 nm whereas only a few extended structures were present (not shown).

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**Table 2. Suprastructures Observed by TEM**

| | suprastructures | suprastructures after aging* |
|---|---|---|
| 1 | tubular structures | no changes |
| 2 | vesicles, few tubular structures | no changes |
| 3 | intertwined rods | helical ribbons |
| 4 | rods, boomerang shaped structures, tubular structures | no changes |
| 5 | nonuniformly shaped elongated structures | helical ribbons |

*Ageing for 3 and 5 days.

Thus, the sugar moiety of **1** seems to be important for tube formation. On the other hand, glycolipid **3** missing the tetrapeptide unit formed bundles of tubes or rods, which are intertwined and reached a length of up to 20 $\mu$m and a width of around 270 nm (Figure 1e). Upon aging for 3 days, very long ribbons were observed (Figure 1f), and the direction of their winding appeared to be left-handed. It has been suggested that ribbons are precursors and/or side products of tube formation.[7,19] Surprisingly, lipopeptide **4** lacking the carbohydrate and possessing only a short peptide moiety exhibited the formation of long clustered tubes/rods of lengths of up to a few micrometers and a width of about 150 nm (Figure 1g). Furthermore, smaller tubular and "boomerang" shaped structures were observed (Figure 1h). Recently, it was shown[20] that particular histidine surfactants assemble into boomerang shaped structures from bilayers upon addition of copper ions, and it was suggested that they are intermediates for the formation of helical structures. The dipalmitoylated glycopeptidolipid **5** allowed us to assess the importance of the diacetylenic unit. Compound **5** showed formation of extended structures which were not uniformly shaped and had a tendency to cluster. In an aged sample (3 days), ribbon type structures were seen (not shown).

Water suspensions of the compounds **2**−**4** could be polymerized by UV light (254 nm), but TEM showed amorphous and not very dense structures (not shown). Comparison of the different supramolecular behaviors of compounds **1**−**5** gives some information about the importance of the different moieties for self-assembly behavior (Table 2).

X-ray diffraction patterns of compound **2** showed a pattern of reflection that decreased in intensity when the relative humidity was increased but showed no shift in peak position and was best accounted for by assuming a hexagonal packing with an interccolumnar distance $D$ of 98 Å. As the length of the extended molecule is 75 Å, such packing would have to involve intercalation of the alkyl chains, which is likely in view of the expected large diameter of the peptide part. The hexagonal packing is difficult to reconcile, however, with the observation of vesicles in the microscope; apparently, slight variations in the substrates (silicon wafer for the X-ray diffraction, carbon-coated Formvar for the electron microscopy) has a large effect on the result.

The XRD pattern of compound **3** also showed a slight decrease in relative intensity with increasing relative humidity but no change in peak positions. At 0% relative humidity, reflections of a lamellar packing with $d = 76.3$ Å were observed up to $n = 1$, consistent with a lamellar packing with the glycopeptidolipid (extended length 60 Å) in intercalated bilayers with a tilt of 30°. Analysis of the diffraction peak widths using the Scherrer formula[16] gives a value of 560 Å for the diameter of the independent domains at 0% RH, which increases to 760 Å at 90% relative humidity. An alternative and more likely interpretation of the broadening of the diffraction peaks at low relative humidity is an increase of stress in packing upon dehydration.

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(20) Sommerdijk, N. A. J. M.;Lambermon, M. H. L.; Feiters, M. C.; Nolte, R. J. M.; Zwanenburg, B. *Chem. Commun.* **1997**, 455.

## Conclusions

An efficient synthesis of the novel glycopyranosyl-containing amphiphiles **1** is described which is based on a combination of solid-phase and solution-based methods. The use of the orthogonally protected FmocLysDde derivative provided an opportunity to incorporate two different lipids in the amphiphiles. The synthetic methodology allowed the efficient synthesis of the derivatives **2**−**5**.

It was found that compound **1** forms tubular or rodlike structures in aqueous media, which can be stabilized by topotactic photopolymerization. We conclude that none of the structure elements we have omitted in the derivatives **2**−**5** can lay claim to be exclusively important for the formation of highly organized aggregates such as tubes, fibers, or helical ribbons from **1**, but the presence of all of these structural elements afforded the most uniformly shaped structures. The absence of the galactose in **2** appears to have a dramatic influence, as mainly vesicles are formed, which are considered to be a lower form of organization than tubules. A possible explanation for the effect of the galactose residue is that the tetrapeptide actually introduces a mismatch in the intermolecular hydrogen bonding network, leading to vesicle formation for **2**, which is counteracted by the presence of the galactose, leading to tubules for **1**. The X-ray diffraction studies of some of the glycopeptide surfactants described here have shown that lamellar monolayer, lamellar intercalated bilayer, and hexagonal packing can be found depending on the functionalization.

As part of a future project, a derivative of **1** will be prepared which contains an antigenic saccharide and peptide moiety. This compound will be used for the formation of coaggregates with the glycopeptidolipid **1**. Polymerization of a tubular or rodlike aggregate may provide a highly immunogenic material. Results will be compared with the immunological properties of an aggregate, which is composed of one derivative only.

The methodology discussed in the paper will also provide a powerful approach to reveal structural motives which may promote tube/rod formation since a range of compounds can easily be synthesized which differ in saccharide, peptide, and lipid composition.

## Experimental Section

**Synthesis. General Methods.** Gravity column chromatography was performed on silica gel 60 (Merck, 70−230 mesh) and flash column chromatography on silica gel 60 (Merck, 230−240 mesh). Gel filtration was performed on Sephadex LH 20 (Pharmacia) or on Sephadex G15 (Pharmacia). TLC analysis was conducted on Kieselgel 60 F254 (Merck) plates, and compounds were detected by UV light (254 nm), by charring with concentrated sulfuric acid/methanol (1/20, v/v), molybdate reagent (0.02 M solution of ammonium cerium(IV) sulfate

dihydrate, and ammonium molybdate(VI) tetrahydrate in aqueous 10% sulfuric acid) or ninhydrin reagent (3 mL acetic acid, 0.3 g of ninhydrin in 100 mL of *n*-butanol). Dichloromethane and acetonitrile were purchased from Fisher Scientific, distilled from calcium hydride, and stored over 4 Å molecular sieves under argon. *N*,*N*-Dimethylformamide was purchased from Fisher Scientific, distilled from calcium hydride under reduced pressure, and stored over 4 Å molecular sieves under argon. Petroleum ether (60−80 °C) and ethyl acetate were purchased from Fisher Scientific as well as dimethyl sulfoxide (GPC grade) for HPLC. Amino acids, PyBOP, and Fmoc-Gly-HMPB-MBHA resin were purchased from NovaBiochem, piperidine, diisopropylcarbodiimide (DIPC), and DMSO from Fluka, and all other chemicals from Aldrich. Reactions were conducted under anhydrous conditions, under inert gas atmosphere ($N_2$), and at room temperature if not mentioned otherwise. LSI and HR mass spectra were recorded using a VG Zabspec mass spectrometer. All empirical formulas are within the capability of the HRMS operating at a resolution of 10.000.

**(*S*)-Alanyl-(*S*)-leucinyl-(*S*)-phenylalanyl-glycenyl-HMPB-MBHA (6) Attachment of the First Amino Acid.** Fmoc-Gly-OH (832 mg, 2.8 mmol) was suspended in $CH_2Cl_2$ (13 mL), and DMF was added until a clear solution was obtained. The solution was cooled (0 °C) and DIPC (0.22 mL, 1.43 mmol) added. Subsequently, the reaction mixture was stirred at 0 °C for 20 min. The precipitated symmetrical anhydride was redissolved by addition of DMF and stirring continued for 10 min at 0 °C. The mixture was concentrated under reduced pressure and the residue dissolved in a minimal amount of DMF and subsequently added to preswollen (30 min) HMPB-MBHA resin (500 mg) in DMF. After adding DMAP (35 mg, 0.285 mmol), the mixture was agitated for 1 h. About 20 mg of resin was removed to measure the extent of the first amino acid attachment. It was washed with DMF (5 mL) and $CH_2Cl_2$ (5 mL) and dried in vacuo.

**Determination of Amino Acid Attachment.** Dry resin (17.5 mg) was treated with piperidine/DMF (1/4, v/v, 30 mL) for 10 min after which an aliquot of 3 mL was removed and the UV absorbance measured at 290 nm against piperidine/DMF (1/4, v/v, 3 mL). Comparison with a correlation table showed an attachment of >95%.

**General Procedure for Peptide Chain Elongation.** Solid-phase synthesis was performed manually, using a cylindrical reaction vessel equipped with a sintered filter at the bottom to enable the removal of solvents and the agitation of the resin by a nitrogen flow.

**Standard Procedure.** (1) Washing the resin with $CH_2Cl_2$ (39 mL) for 10 min. (2) Cleavage of the Fmoc-group by treatment with piperidine/DMF (1/4, v/v, 10 mL) for 30 min. (3) Kaiser test.[2121] (4) Elongation of the peptide chain by addition of $CH_2Cl_2$/DMF (5/2, v/v, 28 mL), Fmoc amino acid (5 equiv), HOBt (5 equiv), DIPC (5 equiv). Agitation of the mixture for 3−16 h. (5) Kaiser test.[21] (6) Washing with $CH_2Cl_2$/DMF (5/2, v/v, 28 mL) for 3 × 10 min.

**General Procedure for Cleavage from the HMPB-MBHA Resin.** The resin was treated with TFA/$CH_2Cl_2$ (1/99, v/v). After 1 min, the solvent was removed into a flask containing pyridine/MeOH (1/9, v/v, double the volume of the TFA solution). This procedure was repeated several times, and the product containing fractions (TLC analysis) were combined and concentrated in vacuo.

***N*$^{\alpha}$-Palmitoyl-*N*$^{\epsilon}$-10,12-pentacosadiynoyl-(*S*)-lysinyl-(*S*)-alanyl-(*S*)-leucinyl-(*S*)-phenylalanyl-glycine (2).** Resin **6** (152 mg, 0.076 mmol of attached tetrapeptide) was suspended in $CH_2Cl_2$/DMF (5/2, v/v, 28 mL), and *N*$^{\alpha}$-Fmoc-*N*$^{\epsilon}$-Dde-(S)-Lys (**7**) (121 mg, 0.228 mmol), PyBOP (119 mg, 0.228 mmol), and DIPEA (30 mg, 0.228 mmol) were added. The reaction mixture was agitated for 4 h after which a negative Kaiser test indicated completion of the coupling reaction and formation of **12**. The solvents were removed by filtration and the resin washed with $CH_2Cl_2$/DMF (5/2, v/v, 3 × 28 mL, 10 min). The resin **12** was treated with piperidine/DMF (2/8, v/v, 5 mL) for 30 min. A positive Kaiser test indicated removal of the Fmoc-protecting group and formation of **1**3. The solvents were removed by filtration, the resin was washed with DMF (4 × 20 mL, 10 min) and, subsequently, suspended in $CH_2Cl_2$/DMF (5/2, v/v, 28 mL). Palmitic acid (97 mg, 0.38 mmol), PyBOP (198 mg, 0.38 mmol), and DIPEA (49 mg, 0.38 mmol) were added, and the mixture was agitated for 5 h. A negative Kaiser test indicated the completion of the coupling reaction and formation of **14**. The solvent were removed by filtration, and the resin was washed with $CH_2Cl_2$/DMF (5/2, v/v, 3 × 28 mL, 10 min) and treated with hydrazine monohydrate/DMF (2/98, v/v, 5 mL) for 30 min. A positive Kaiser test indicated cleavage of the Dde-protecting group and formation of **15**. After filtration, the resin was washed with DMF (4 × 10 mL, 10 min) and suspended in $CH_2Cl_2$/DMF (5/2, v/v, 28 mL). 10,12-Pentacosadiynoic acid (142 mg, 0.38 mmol), PyBOP (198 mg, 0.38 mmol), and DIPEA (49 mg, 0.38 mmol) were added, and the mixture was agitated in the dark for 6 h. A negative Kaiser test indicated the complete formation of **16**. After filtration and washing with $CH_2Cl_2$/DMF (5/2, v/v, 3 × 28 mL, 10 min), polymer-bound lipopeptide **16** was released from the solid support following the general procedure for cleavage of the HMPB-MBHA linker. The compound containing fractions were combined and concentrated in vacuo, and the residue was purified by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1, v/v) to afford **2** (58 mg, 67%) as a colorless sticky foam. $[\alpha]^{21.5}_D = -9.7°$ ($c$ = 2.2 mg/mL, $CH_2Cl_2$/MeOH, 2/1, v/v). IR (KBr): $\nu$ 2924, 2854 ($CH_2$), 2375, 2325 (C/C-triple bond), 1636, 1548, 1461 (C(O)NH). UV ($CH_2Cl_2$/MeOH, 2/1, v/v): $\lambda_{max}$ 235.6 ($\epsilon$ 4674). $^1$H NMR ($CDCl_3$/$CD_3OD$, 2/1, v/v, 500 MHz): $\delta$ 7.22−7.08 (m, 5H), 4.60 (brs, 1H), 4.14 (brs, 2H), 3.99 (brs, 1H), 3.97 (s, 2H), 3.29−3.16 (m, 2H), 3.07−3.00 (m, 1H), 2.93−2.83 (m, 1H), 2.23 (t, 2H, $J$ = 7.7 Hz), 2.18 (t, 4H, $J$ = 7.0 Hz), 2.13 (t, 2H, $J$ = 7.7 Hz), 1.72−1.61 (m, 2H), 1.61−1.50 (m, 6H), 1.50−1.35 (m, 9H), 1.35−1.15 (m, 55H), 0.84−0.79 (m, 9H), 0.74 (d, 3H, $J$ = 6.2 Hz). $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 2/1, v/v, 125 MHz) $\delta$ 175.7, 175.1, 174.6, 174.4, 174.1, 173.2, 172.2 (CO), 137.3, 129.3, 128.4, 126.7, 120.0 (Phe-aromat), 65.3, 65.2 (C/C-triple), 55.3 (Leu-CH$^{\alpha}$), 54.8 (Phe-CH$^{\alpha}$), 53.2, 52.1, 50.3 (Gly-$CH_2$, Lys-CH$^{\alpha}$, Ala-CH$^{\alpha}$), 46.2, 36.5, 36.4, 35.9 (alkyl-$CH_2$, Lys-$NCH_2$), 31.9, 29.7, 29.5, 29.3, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.4, 25.9, 25.7, 25.6, 24.8 (Alkyl-$CH_2$, Leu-CH, Lys-$CH_2$, Leu-$CH_2$), 22.7 (Leu-$CH_3$), 21.3 (Leu-$CH_3$), 19.2 (alkyl-$CH_3$), 14.0 (alkyl-$CH_3$), 8.5 (Ala-$CH_3$). MALDI TOF MS: $m/z$ = 1152 ([M + Na]$^+$, retinoic acid), 1151 ([M + Na]$^+$, indolacrylic acid). LSIMS: $m/z$ = 1151 (M + Na)$^+$. HRMS (LSIMS): calcd for $C_{67}H_{112}N_6NaO_8$ (M + Na)$^+$ 1151.8439, found 1151.8444.

***N*$^{\alpha}$-Palmitoyl-*N*$^{\epsilon}$-10,12-pentacosadiynoyl-(*S*)-lysinyl-(*S*)-alanyl-(*S*)-leucinyl-(*S*)-phenylalanyl-glycine[3-(β-D-galactopyranosyloxy)propyl]amide (1).** Lipopeptide **2** (56 mg, 0.049 mmol) was dissolved in $CH_2Cl_2$/DMF (3/1, v/v, 4 mL), and HOBt (40 mg, 0.298 mmol) in $CH_2Cl_2$/DMF (1/1, v/v, 1 mL) and DIPC (38 mg, 0.298 mmol) in $CH_2Cl_2$ (0.5 mL) added. Galactoside **8** (59 mg, 0.248 mmol) in DMF (2.5 mL) was added dropwise to the stirred solution, in a manner that all compounds remained in solution. After the reaction mixture was stirred for 5 h in the dark, it was concentrated in vacuo, and the residue purified by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1, v/v) to afford **1** (35 mg, 53%) as a colorless gum. $[\alpha]^{22}_D$: −2.1° ($c$ = 3.2 mg/mL, $CH_2Cl_2$). IR (KBr): $\nu$ 2923, 2851 ($CH_2$), 2471, 2426 (C/C-triple bond), 1746, 1632, 1548, 1467 (C(O)NH). UV ($CH_2Cl_2$/MeOH, 1/1, v/v): $\lambda_{max}$ 269.4 nm ($\epsilon$ 986). $^1$H NMR ($CDCl_3$/$CD_3OD$, 2/1, v/v, 400 MHz): $\delta$ 7.05−6.74 (m, 5H), 4.31 (dd, 1H, $J$ = 10.1 Hz, $J$ = 4.8 Hz), 3.99 (d, 1H, $J$ = 7.0 Hz), 3.97−3.88 (m, 2H), 3.77 (t, 1H, $J$ = 7.1 Hz), 3.74−3.49 (m, 6H), 3.45−3.23 (m, 4H), 3.45−3.23 (m, 4H), 2.97−2.82 (m, 1H), 2.72 (dd, 1H, $J$ = 14.0 Hz, $J$ = 10.2 Hz), 2.13−1.90 (m, 8H), 1.62−0.98 (m, 72H), 0.88 (d, 3H, $J$ = 6.5 Hz), 0.70−0.61 (m, 6H), 0.59 (d, 3H, $J$ = 6.5 Hz). $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 2/1, v/v, 100 MHz): $\delta$ 175.5, 174.2, 174.2, 173.6, 172.2, 169.7 (CO), 136.8, 128.8, 128.0, 126.4, 126.3, 116.7 (Phe-aromat), 103.0 (C-1), 74.6 (C-3), 73.3 (C-5), 71.1 (C-2), 68.8 (C-4), 66.7 (C-6), 65.0, 64.8 (C/C-triple), 61.2 (Sp-$OCH_2$), 55.2, 55.0

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(Phe-$CH^{\alpha}$, Leu-$CH^{\alpha}$), 52.9 (Ala-$CH^{\alpha}$), 50.4 (Lys-$CH^{\alpha}$), 42.6 (Gly-$CH_2$), 39.3 (alkyl-$CH_2$), 37.7 (Lys-$NCH_2$), 36.6 (Phe-$CH_2$), 36.2 (Sp-$NCH_2$), 36.0, 35.4, 31.5, 31.2, 31.0 29.8, 29.2, 29.1, 28.9, 28.6, 28.5, 28.4, 27.9, 25.5, 25.1, 24.4, 22.6, 22.2 (alkyl-$CH_2$, Leu-CH, Leu-$CH_2$, Lys-$CH_2$, Sp-$CH_2$), 20.7 (Leu-$CH_3$), 18.6 (alkyl-$CH_2$), 16.1 (Ala-$CH_3$), 13.4 (alkyl-$CH_3$). MALDI TOF MS: 1373 ($[M + Na]^+$, retinoic acid). LSIMS: $m/z = 1370.8$ $(M + Na)^+$. HRMS (LSIMS): calcd for $C_{76}H_{129}N_7O_{13}Na$ $(M + Na)^+$ 1370.9546, found 1370.9559.

***N*$^{\alpha}$-(Fluorenylmethoxycarbonyl)-*N*$^{\epsilon}$-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl-(*S*)-lysine[3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)propyl]amide (18).** Fmoc-LysDdeOH (**7**) (166 mg, 0.312 mmol) was dissolved in $CH_2Cl_2$/DMF (2/1, v/v, 3 mL), and HONB (84 mg, 0.468 mmol) in $CH_2Cl_2$/DMF (1/1, v/v, 0.5 mL), DIPC (59 mg, 0.468 mmol) in $CH_2Cl_2$ (0.5 mL), and compound **17** (189 mg, 0.468 mmol) in $CH_2Cl_2$ (1 mL) were added. After stirring for 18 h, the reaction mixture was diluted with $CH_2Cl_2$ (15 mL) and washed with water (3 × 5 mL). The combined water layers were extracted with $CH_2Cl_2$ (5 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (8 g of silica, 0−3% MeOH/$CH_2Cl_2$) to afford **18** (280 mg, 97%) as a colorless gum. $R_f$: 0.15 (MeOH/$CH_2Cl_2$, 3/97, v/v). $[\alpha]^{17.5}{}_D = -6.25°$ ($c = 18.3$ mg/mL, $CH_2Cl_2$). $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 13.48−13.36 (m, 1H), 7.74 (d, 2H, $J = 7.7$ Hz), 7.65−7.50 (m, 2H), 7.38 (t, 2H, $J = 7.4$ Hz), 7.29 (t, 2H), 6.50 (brs, 1H), 5.75 (d, 1H, $J = 8.1$ Hz), 5.37 (d, 1H, $J = 2.9$ Hz), 5.18 (dd, 1H, $J = 10.3$ Hz, $J = 8.1$ Hz), 5.01 (dd, 1H), 4.50−3.94 (m, 8H), 3.90−3.80 (m, 1H), 3.60−3.48 (m, 1H), 3.44−3.31 (m, 4H), 2.51 (s, 3H), 2.31 (s, 4H), 2.07, 2.03, 2.02, 1.95 (s, 12H), 1.84−1.60 (m, 6H), 1.57−1.40 (m, 2H), 0.99 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 173.5, 171.5, 170.3, 170.1, 170.0 (CO), 156.2 (Fmoc-CO), 143.8, 143.8, 141.3, 127.7, 127.1, 126.8, 125.1, 120.0 (Fmoc-aromat), 107.8 (C/C-double), 101.4 (C-1), 70.8, 70.6 (C-3, C-5), 69.2 (C-2), 68.9 (Sp-$OCH_2$), 67.0 (Fmoc-$OCH_2$), 66.9 (C-4), 61.2 (C-6), 54.5 (Lys-$CH^{\alpha}$), 52.9 (brs, Dde-$CH_2$), 47.2 (Fmoc-CH), 42.8, 37.6 (Sp-$NCH_2$, Lys-$NCH_2$), 32.5 (Dde-$C_q$), 32.0, 29.2, 28.6 (Sp-$CH_2$, Lys-$CH_2$), 28.2 (Dde-$CH_3$), 22.8 (Lys-$CH_2$), 20.8, 20.6, 20.5, (Ac−$CH_3$), 17.8 (C/C-double-$CH_3$). LSIMS: $m/z = 942$ $(M + Na)^+$. HRMS (LSIMS): calcd for $C_{48}H_{61}N_3NaO_{15}$ $(M + Na)^+$ 942.4000, found 942.4019.

***N*$^{\epsilon}$-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl-(*S*)-lysine[3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)propyl]amide (19).** Compound **19** (100 mg, 0.11 mmol) was dissolved in piperidine/DMF (2/8, v/v, 3 mL) and the reaction mixture stirred for 30 min. The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (8 g of silica, MeOH/$CH_2Cl_2$, 7.5/92.5, v/v) to afford **19** (67 mg, 95%) as a colorless sticky foam. $R_f$: 0.35 (MeOH/$CH_2Cl_2$, 1/9, v/v), $[\alpha]^{20.5}{}_D$: −14.4° ($c = 9.44$ mg/mL, $CH_2Cl_2$). $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 13.42 (brs, 1H), 7.44−7.35 (m, 1H), 5.38 (d, 1H, $J = 3$ Hz), 5.18 (dd, 1H, $J = 10$ Hz, $J = 8.0$ Hz), 5.01 (dd, 1H), 4.45 (d, 1H), 4.19 (dd, 1H, $J = 11$ Hz, $J = 7$ Hz), 4.10 (dd, 1H, $J = 6.7$ Hz), 4.00−3.85 (m, 2H), 3.61−3.51 (m, 1H), 3.45−3.20 (m, 5H), 2.54 (s, 3H), 2.35 (s, 4H), 2.16, 2.15, 2.06, 2.04 (s, 12H), 1.90−1.64 (m, 8H), 1.60−1.44 (m, 2H), 1.00 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 174.8, 173.5, 173.4, 170.3, 170.1, 170.0, 169.6 (CO), 107.8 (C/C-double), 101.3 (C-1), 70.8, 70.7 (C-3, C-5), 68.9 (C-2), 68.3 (Sp-$OCH_2$), 67.2 (C-4), 61.2 (C-6), 54.9 (Lys-$CH^{\alpha}$), 52.9 (brs, Dde-$CH_2$), 43.1, 36.6 (Lys-$NCH_2$, Sp-$NCH_2$), 34.5 (Dde-$C_q$), 30.0, 29.6, 29.4, 28.8 (Sp-$CH_2$, Lys-$CH_2$), 28.2 (Dde-$CH_3$), 23.2 (Lys-$CH_2$), 20.8, 20.6, 20.5 (Ac-$CH_3$), 17.8 (C/C-double-$CH_3$). MALDI TOF MS: $m/z = 698$ ($[M + H]^+$, retinoic acid); $m/z = 698$ ($[M + H]^+$, indolacrylic acid). LSIMS: $m/z = 720.3$ $(M + Na)^+$.

***N*$^{\alpha}$-Palmitoyl-*N*$^{\epsilon}$-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl-(*S*)-lysine[3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxy)propyl]amide (20).** Compound **19** (112 mg, 0.16 mmol) was dissolved in $CH_2Cl_2$ (3 mL), and palmitic acid (82 mg, 0.32 mmol) in $CH_2Cl_2$ (0.5 mL), HONB (57 mg, 0.32 mmol) in $CH_2Cl_2$/DMF (1/1, v/v, 0.2 mL) and DIPC (40.3 mg, 0.32 mmol) in $CH_2Cl_2$ (0.1 mL) were added. The reaction mixture was stirred for 18 h. MALDI TOF analysis (retinoic acid) indicated formation of product and absence of starting material. The mixture was concentrated in vacuo. The residue was dissolved in $CH_2Cl_2$ (15 mL) and washed with water (3 × 5 mL), and the combined water layers extracted with $CH_2Cl_2$ (3 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (5 g of silica, 1−3% MeOH/$CH_2Cl_2$) to afford **20** (137.6 mg, 92%) as a colorless gum. $R_f$: 0.41 (MeOH/$CH_2Cl_2$, 5/95, v/v). $[\alpha]^{22}{}_D = -8.0°$ ($c = 10.3$ mg/mL, $CH_2Cl_2$). $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 13.4 (brs, 1H), 6.52 (t, 1H, $J = 5.7$ Hz), 6.41 (d, 1H, $J = 8.5$ Hz), 5.37 (d, 1H, $J = 2.9$ Hz), 5.14 (dd, 1H, $J = 10.5$ Hz, $J = 7.9$ Hz), 5.00 (dd, 1H), 4.54−4.44 (m, 1H), 4.41 (d, 1H), 4.15 (dd, 1H, $J = 11.2$ Hz, $J = 6.4$ Hz), 4.08 (dd, 1H, $J = 6.8$ Hz), 4.00−3.85 (m, 2H), 3.56−3.46 (m, 1H), 3.41−3.27 (m, 4H), 2.50 (s, 3H), 2.32 (s, 4H), 2.20 (t, 2H, $J = 7.5$ Hz), 2.12, 2.06, 2.01, 1.96 (s, 12H), 1.92−1.52 (m, 8H), 1.50−1.39 (m, 2H), 1.21 (s, 24H), 0.99 (s, 6H), 0.84 (t, 3H, $J = 6.6$ Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta$ 173.4, 171.1, 170.1 (CO), 108.9 (C/C-double), 101.4 (C-1), 70.8, 70.6 (C-3, C-5), 69.0 (C-2), 68.9 (Sp-$OCH_2$), 67.0 (C-4), 61.1 (C-6), 52.5 (Lys-$CH^{\alpha}$), 43.1, 37.5 (Sp-$NCH_2$, Lys-$NCH_2$), 36.6 (Dde-$C_q$), 28.3 (C/C-double-$CH_3$), 25.7 (Pal-$CH_2$), 20.7 (Ac-$CH_3$), 17.9 (Dde-$CH_3$), 14.1 (Pal-$CH_3$). MALDI TOF MS: $m/z = 959$ ($[M + Na]^+$, retinoic acid). LSIMS: $m/z = 958.5$ $(M + Na)^+$. HRMS (LSIMS): calcd for $C_{49}H_{82}N_3O_{14}$ $(M + H)^+$ 936.5797, found 936.5751.

***N*$^{\alpha}$-Palmitoyl-(*S*)-lysine[3-(*β*-D-galactopyranosyloxy)propyl]amide (22).** Compound **20** (28 mg, 0.03 mmol) was dissolved in hydrazine monohydrate/DMF (2/98, v/v, 2 mL) and the reaction mixture stirred for 30 min. The reaction mixture was concentrated in vacuo to obtain **21** which was subsequently treated with a mixture of $NEt_3$/MeOH/water (2/5/5, v/v/v, 5 mL). After stirring for 3 h, the reaction mixture was concentrated in vacuo and the residue coevaporated with toluene (3 × 3 mL). The residue was purified by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1, v/v) to afford **22** (11.9 mg, 67%) as a colorless gum. $[\alpha]^{22}{}_D$: −20.4° ($CH_2Cl_2$/MeOH, 2/1, v/v, $c = 10.3$ mg/mL). $^1$H NMR ($CDCl_3$/MeOD, 3/1, v/v, 500 MHz): $\delta$ 4.29 (dd, 1H, $J = 7.9$ Hz, $J = 6.1$ Hz), 4.21 (d, 1H, $J = 7.2$ Hz), 4.15−3.83 (m, 5H), 3.79−3.69 (m, 3H), 3.64−3.57 (m, 1H), 3.53−3.29 (m, 2H), 2.93−2.80 (m, 2H), 2.17 (t, 2H, $J = 7.8$ Hz), 1.81−1.17 (m, 34H), 0.82 (t, 3H, $J = 7.0$ Hz). $^{13}$C NMR ($CDCl_3$/MeOD, 3/1, v/v 125 MHz): $\delta$ 174.9, 172.4 (CO), 103.6 (C-1), 75.0, 73.7, 71.7 (C-2, C-3, C-5), 69.3 (C-4), 68.9 (Sp-$OCH_2$), 61.7 (C-6), 53.7 (Lys-$CH^{\alpha}$), 39.7 (Lys-$NCH_2$), 37.9 (Sp-$NCH_2$), 36.5, 32.1, 31.9, 29.9, 29.7, 29.5, 28.8, 26.7, 25.9, 23.3, 22.8, 22.4 (Pal-$CH_2$, Sp-$CH_2$, Lys-$CH_2$), 14.1 (Pal-$CH_3$). MALDI TOF MS: $m/z = 602$ ($[M + H]^+$, retinoic acid), 602 ($[M + H]^+$, indolacrylic acid). LSIMS: $m/z = 626$ $(M + Na)^+$. HRMS (LSIMS): calcd for $C_{31}H_{61}N_3O_8Na$ $(M + Na)^+$ 626.4356, found 626.4350.

***N*$^{\alpha}$-Palmitoyl-*N*$^{\epsilon}$-10,12-pentacosadiynoyl-(*S*)-lysine[3-(*β*-D-galacto-pyranosyloxy)propyl]amide (3).** Compound **22** (11.9 mg, 002 mmol) was dissolved in $CH_2Cl_2$ (2 mL), and 10,12-pentacosadiynoic acid (11.2 mg, 0.03 mmol) in $CH_2Cl_2$ (0.2 mL), EDC (8 mg, 0.04 mmol) in $CH_2Cl_2$/DMF (1/1, v/v, 0.2 mL), and HOBt (5 mg, 0.04 mmol) in $CH_2Cl_2$/DMF (1/1, v/v, 0.2 mL) were added. The reaction mixture was stirred in the dark for 18 h. MALDI TOF MS analysis (indolacrylic acid) indicated completion of the coupling reaction. Subsequently, the reaction mixture was concentrated in vacuo and the residue purified by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1, v/v) to afford **3** (9.4 mg, 50%) as a sticky foam. $[\alpha]^{22}{}_D$: −7.8° ($CH_2Cl_2$, $c = 5.7$ mg/mL). IR (KBr): $\nu$ 3422 (OH), 2921, 2849 ($CH_2$), 2361, 2343 (C/C-triple bond), 1646, 1550, 1466 (C(O)NH). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 7.39 (brs, 1H), 6.70 (brs, 1H), 5.94 (brs, 1H), 4.40−4.35 (m, 2H), 4.10−3.99 (m, 2H), 3.98−3.78 (m, 3H), 3.76−3.58 (m, 3H), 3.57−3.52 (m, 1H), 3.51−3.31 (m, 2H), 3.29−3.15 (m, 2H), 2.28−2.12 (m, 8H), 1.85−1.75 (m, 2H), 1.74−1.47 (m, 8H), 1.45−1.19 (m, 52H), 0.91−0.85 (m, 6H). $^{13}$C NMR ($CDCl_3$, 125 MHz): $\delta$ not seen (CO), 103.2 (C-1), 77.6 (C/C-triple), 74.4, 73.6, 71.5 (C-5, C-3, C-2), 69.3 (C-4), 69.0 (Sp-$OCH_2$), 65.2 (C/C-triple), 62.2 (C-6), 52.9 (Lys-$CH^{\alpha}$), 38.8 (Lys-$NCH_2$), 37.6, (Sp-$NCH_2$), 36.8, 36.5, 31.9, 31.6, 29.7, 29.4, 29.3, 29.2, 29.1, 29.1, 28.9, 28.8, 28.6, 28.4, 28.3, 25.7, 22.5, 19.2 (alkyl-$CH_2$, Lys-$CH_2$, Sp-$CH_2$, Alkyl-

$CH_3$), 14.1 (alkyl-$CH_3$). MALDI TOF MS: $m/z$ = 983 ([M + Na]$^+$, indolacrylic acid), 983 ([M + Na]$^+$, gentisic acid). LSIMS: $m/z$ = 982.6 (M + Na)$^+$. HRMS (LSIMS): calcd for $C_{56}H_{101}N_3O_9Na$ (M + Na)$^+$ 982.7436, found 982.7443.

***N*$^{\alpha}$-Palmitoyl-*N*$^{\epsilon}$-(10,12-pentacosadiynoyl)-(*S*)-lysinyl-(*S*)-glycine (4).** Fmoc-Gly-HMPB-MBHA resin (**10**) (120 mg, 0.06 mmol of attached amino acid) was preswollen in DMF (5 mL) for 30 min. After removal of the solvent, the Fmoc-group was cleaved by treatment with piperidine/DMF (2/8, v/v, 10 mL) for 30 min. The solvents was removed by filtration and the resin washed with DMF (4 × 20 mL, 10 min) and $CH_2Cl_2$-$Cl_2$/DMF (5/2, v/v, 28 mL). The resulting resin **11** was suspended in $CH_2Cl_2$/DMF (5/2, v/v, 28 mL), and FmocLysDde-OH (**7**) (65 mg, 0.12 mmol), PyBOP (63 mg, 0.12 mmol), and DIPEA (41 $\mu$L, 0.24 mmol) were added. The mixture was agitated for 4 h, when a negative Kaiser test indicated completion of the coupling reaction to give **23**. The solvents were removed by filtration, and the resin was washed with $CH_2Cl_2$/DMF (5/2, v/v, 3 × 28 mL, 10 min). The Fmoc-group of **23** was cleaved by treatment with piperidine/DMF (2/8, v/v, 10 mL) for 30 min. A positive Kaiser test indicated cleavage of the protecting group and formation of **24**. The solvents were removed by filtration, and the resin washed with DMF (4 × 20 mL, 10 min). The resin **24** was resuspended in $CH_2Cl_2$/DMF (5/2, v/v, 28 mL), palmitic acid (78 mg, 0.3 mmol), PyBOP (157 mg, 0.3 mmol), and DIPEA (101 $\mu$L, 0.6 mmol) were added, and the mixture was agitated for 5 h. A negative Kaiser test indicated complete formation of **25**. After removal of the solvent by filtration, the resin **25** was washed with $CH_2Cl_2$/DMF (5/2, v/v, 3 × 28 mL, 10 min) and treated with hydrazine monohydrate/DMF (2/98, v/v, 10 mL) for 30 min. A positive Kaiser test indicated successful cleavage of the Dde-protecting group and formation of **26**. After filtration and washing with DMF (4 × 20 mL, 10 min), the resin **26** was suspended in $CH_2$-$Cl_2$/DMF (5/2, v/v, 28 mL), and 10,12-pentacosadiynoic acid (114 mg, 0.3 mmol), PyBOP (157 mg, 0.3 mmol), and DIPEA (101 $\mu$L, 0.6 mmol) were added. The mixture was agitated in the dark for 6 h when a negative Kaiser test indicated the completion of the reaction and formation of **27**. After filtration and washing with $CH_2Cl_2$/DMF (5/2, v/v, 3 × 28 mL, 10 min), the immobilized lipodipeptide **27** was released from the solid support following the general procedure for cleavage of the HMPB-MBHA linker. Purification of the residue by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1, v/v) afforded **4** (33.1 mg, 68%) as a colorless gum $[\alpha]^{22}_D$: −4.1° ($CH_2Cl_2$/MeOH, 2/1, v/v, $c$ = 5.85 mg/mL). IR (KBr): $\nu$ 3309 (OH), 2921, 2848 ($CH_2$), 2358, 2337 (C/C-triple bond), 1645, 1558, 1467 (C(O)NH). UV ($CH_2Cl_2$/MeOH, 2/1, v/v): $\lambda_{max}$ 233.2 ($\epsilon$ 902). $^1$H NMR ($CDCl_3$, 500 MHz): $\delta$ 7.37 (brs, 1H), 6.84 (brs, 1H), 6.03 (brs, 1H), 4.60−4.50 (m, 1H), 4.00 (s, 2H), 3.32−3.10 (m, 2H), 2.26−2.13 (m, 8H), 1.88−1.76 (m, 1H), 1.75−1.64 (m, 1H), 1.64−1.55 (m, 4H), 1.55−1.64 (m, 6H), 1.41−1.32 (m, 6H), 1.32−1.20 (m, 46H), 0.88 (t, 6H, $J$ = 7 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz): $\delta$ 174.4, 174.0, 172.7 (CO), 65.4, 65.3 (C/C-triple bond), 53.4 (Lys-$CH^{\alpha}$), 50.7 (Gly-$CH_2$), 38.9 (Lys-$NCH_2$), 36.7, 36.4, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.6, 26.7, 24.5 (alkyl-$CH_2$, Lys-$CH_2$), 19.7 (alkyl-$CH_3$), 14.1 (alkyl-$CH_3$). MALDI TOF MS: $m/z$ = 820 ([M + Na]$^+$, retinoic acid). LSIMS: $m/z$ = 820.6 (M + Na)$^+$. HRMS (LSIMS): calcd for $C_{49}H_{87}N_3NaO_5$ (M + Na)$^+$ 820.6543, found 820.6541.

***N*,*N*′-Dipalmitoyl-(*S*)-lysinyl-(*S*)-alanyl-(*S*)-leucinyl-(*S*)-phenylalanyl-glycine (32).** The resin **6** (500 mg, 0.25 mmol) was suspended in $CH_2Cl_2$/DMF (5/2, v/v, 28 mL), *N*,*N*′-Bis-Fmoc-LysOH **28** (738 mg, 1.25 mmol), HOBt (203 mg, 1.5 mmol), and DIPC (189 mg, 1.5 mmol) were added, and the reaction mixture was agitated for 8 h to give resin bound **29**. The solvents were removed by filtration, the resin was washed with $CH_2Cl_2$/DMF (5/2, v/v, 28 mL, 3 × 10 min). The Fmoc protecting groups of **29** were removed by treatment with piperidine/DMF (2/8, v/v, v/v, 15 mL) for 30 min to give **30**. After washing with DMF (30 mL, 3 × 10 min), the resin was suspended in pyridine/$CH_2Cl_2$ (1/1, v/v, 30 mL), palmitoyl chloride (1.374 g, 5 mmol) in $CH_2Cl_2$ (1 mL) was added, and the mixture was agitated for 5 h. A negative Kaiser test indicated completion of the reaction and formation of **31**. The solvent was removed by filtration and the resin washed with $CH_2Cl_2$ (30 mL, 4 × 10 min). Lipopeptide **31** was released from the HMPB-MBHA resin according to the general procedure. The combined product containing fractions were concentrated in vacuo, and the residue was purified by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1, v/v) to afford pure **32** (147 mg, 58%) as a white powder. $[\alpha]^{22}_D$: −19.6° ($CH_2Cl_2$/MeOH, 1/1, v/v, $c$ = 3.73 mg/mL). mp 185−190 °C (decomp). $^1$H NMR ($CD_3OD$/$CDCl_3$, 1/1, v/v, 500 MHz): $\delta$ 7.20−7.05 (m, 5H), 4.59 (dd, 1H, $J$ = 10.1 Hz, $J$ = 4.4 Hz), 4.20−4.09 (m, 2H), 4.01−3.90 (m, 1H), 3.96, 3.80 (AB, 2H), 3.23−3.14 (m, 2H), 3.05−2.98 (m, 1H), 2.84 (dd, 1H, $J$ = 13.9 Hz), 2.23 (t, 2H, $J$ = 7.7 Hz), 2.10 (t, 2H, $J$ = 7.6 Hz), 1.71−1.60 (m, 2H), 1.59−1.48 (m, 6H), 1.47−1.35 (m, 4H), 1.28−1.13 (m, 52H), 0.82−0.77 (m, 9H), 0.71 (d, 3H, $J$ = 6.2 Hz). $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 1/1, v/v, 125 MHz): $\delta$ 175.3, 174.9, 173.4, 172.8, 172.0 (CO), 136.8, 128.8, 127.8, 126.1 (Phe-aromat), 54.7 (Lys-$CH^{\alpha}$), 54.2 (Phe-$CH^{\alpha}$), 52.5, 49.8 (Leu-$CH^{\alpha}$, Ala-$CH^{\alpha}$), 40.7 (Gly-$CH_2$), 39.7, 37.8 (Pal-$CH_2$, Lys-$NCH_2$), 37.1 (Phe-$CH_2$), 29.2, 29.0, 29.0, 28.9, 28.8, 28.5, 28.2, 25.5, 25.3, 25.1, 24.3 (Pal-$CH_2$, Lys-$CH_2$, Leu-CH, Leu-$CH_2$), 22.2 (Leu-$CH_3$), 22.1 (Pal-$CH_2$), 20.6 (Leu-$CH_3$), 16.1 (Ala-$CH_3$), 13.3 (Pal-$CH_3$). MALDI TOF MS: $m/z$ = 1034 ([M + Na]$^+$, retinoic acid), 1034 ([M + Na]$^+$, indolacrylic acid). LSIMS: $m/z$ = 1055.7 ([M + 2Na − H]$^+$). HRMS (LSIMS): calcd for $C_{58}H_{102}N_6NaO_8$ (M + Na)$^+$ 1033.7657, found 1033.7659.

***N*,*N*′-Dipalmitoyl-(*S*)-lysinyl-(*S*)-alanyl-(*S*)-leucinyl-(*S*)-phenylalanyl-glycine[3-(*β*-D-galactopyranosyloxy)-propyl]amide (5).** Lipopeptide **32** (76 mg, 0.075 mmol) was suspended in $CH_2Cl_2$/DMF (2/1, v/v, 9 mL), and DIPC (103 mg, 0.820 mmol) in $CH_2Cl_2$ (1 mL) and HONB (147 mg, 0.820 mmol) in $CH_2Cl_2$/DMF (1/1, v/v, 1 mL) were added. Galactoside **8** (195 mg, 0.820 mmol) in DMF (2 mL) was added dropwise together with DMF in a manner that all compounds remained in solution. The mixture was stirred for 7 days and subsequently concentrated in vacuo. The residue was purified by gel filtration (Sephadex LH-20, MeOH/$CH_2Cl_2$, 1/1) to afford **5** (12.8 mg, 13%) as a sticky gum. $[\alpha]^{21}_D$ = −6.6° ($CH_2Cl_2$/MeOH, 2/1, v/v, $c$ = 0.87 mg/mL). $^1$H NMR ($CDCl_3$/$CD_3OD$, 1/1, v/v, 500 MHz): $\delta$ 7.24−7.10 (m, 5H), 4.49 (dd, 1H, $J$ = 10.1 Hz, $J$ = 4.8 Hz), 4.21−4.01 (m, 4H), 3.97−3.69 (m, 6H), 3.60−3.41 (m, 4H), 3.29−3.02 (m, 5H), 2.90 (dd, 1H, $J$ = 14.6 Hz), 2.28 (t, 2H, $J$ = 7.7 Hz), 2.17−2.11 (m, 2H), 1.82−1.00 (m, 66H), 0.87−0.70 (m, 12H). $^{13}$C NMR ($CDCl_3$/$CD_3OD$, 1/1, v/v, 125 MHz): 176.0, 175.4, 174.8, 174.1, 172.7, 170.1 (CO), 137.2, 129.2, 128.4, 126.7, 126.6 (Phe-aromat), 103.5 (C-1), 75.0, 73.7 (C-3, C-5), 71.5 (C-2), 69.2 (C-4), 67.1 (C-6), 61.6 (Sp-$OCH_2$), 55.8 (Leu-$CH^{\alpha}$), 55.5 (Phe-$CH^{\alpha}$), 53.4, 53.0 (Ala-$CH^{\alpha}$, Lys-$CH^{\alpha}$), 50.7 (Gly-$CH_2$), 39.7, 38.1, 36.6 (Pal-$CH_2$, Sp-$NCH_2$, Lys-$NCH_2$), 37.0 (Phe-$CH_2$), 36.4, 35.8, 31.9, 30.1, 30.0, 29.7, 29.5, 29.4, 29.3, 29.0, 28.5, 26.7, 26.0, 25.5, 24.8 (Pal-$CH_2$, Sp-$CH_2$, Lys-$CH_2$, Leu-$CH_2$, Leu-CH), 22.8, 22.6 (Pal-$CH_2$, Leu-$CH_3$), 21.1 (Leu-$CH_3$), 16.5 (Ala-$CH_3$), 13.8 (Pal-$CH_3$). MALDI TOF MS: $m/z$ = 1254 ([M + Na]+, retinoic acid). LSIMS: $m/z$ = 1253 (M + Na)$^+$. HRMS (LSIMS): calcd for $C_{67}H_{119}N_7NaO_{13}$ 1252.8764 (M + Na)$^+$, found 1252.8720.

**Table 3. Concentrations of Water Suspensions as Used for TEM and of Organic Gels**

| | Pt-shadowing, % (w/v) | negative staining, % (w/v) | freeze fracturing, % (w/v) | solvent (v/v) |
|---|---|---|---|---|
| 1 | 0.59 | 0.53 | 0.66 | THF/EtOH (1/1) |
| 2 | 0.39 | | 0.84 | THF/EtOH (8/2) |
| 3 | 0.26 | | 0.7 | THF/EtOH (1/1) |
| 4 | 0.15 | | 0.7 | THF/EtOH (1/1) |
| 5 | 0.15 | | | THF/EtOH (8/2) |

**Electron Microscopy. General Procedure for Pt-Shadowing.** The dry compound was dissolved in an appropriate mixture of EtOH and THF. An aliquot of this solution was injected into hot (72 °C) vortexed water resulting in the concentration listed in Table 3. One drop of the resulting homogeneous water suspension was applied to a carbon-coated copper grid. After allowing the aggregates to settle for 1 min,

the excess water was drained with filter paper. After drying, the aggregates were shadowed with Pt atoms. Transmission electron microscopy (TEM) was performed on a Philips EM201 instrument using an acceleration voltage of 60 kV. Pt shadowing was performed using an Edwards 306 system.

**General Procedure for Negative Staining.** The water suspension was prepared and applied to a copper grid as described above. Subsequently, a uranyl acetate solution (1/49, v/v) was added for 1 min and then removed by filter paper.

**General Procedure for Freeze Fracturing and Etching.** Aqueous suspensions were prepared as described for Pt-shadowing. A freshly cleaned gold (400 mesh) grid was immersed in the suspension and placed between two freshly cleaned copper plates. The grid and copper plates were rapidly frozen in liquid propane and fractured at $10^{-6}$ Torr and −105 °C. After etching (2 min), Pt-shadowing (2 nm), and carbon deposition (20 nm), the resulting replicas were cleaned ca. 28 h with 70% $H_2SO_4$ and subsequently transferred to water (several times) and finally dried.

General procedure for polymerization of compounds containing a diacetylenic unit: Water suspensions were prepared as described above and transferred to a Quartz cuvette and placed under an UV lamp. Irradiation at 254 nm was continued for 18 h. A color change from colorless to purple/blue indicated a successful polymerization. A drop of the polymer suspension was applied to a carbon-coated copper grid and treated in a way as described for Pt-shadowing. Freeze fracturing experiments were performed in a Balzers Freeze Etching System BAF 400 D, with samples rapidly frozen in a BAL-TEC JFD 030 Jet-Freeze Device.

**X-ray Powder Diffraction.** Samples were prepared by placing a drop of an aggregate dispersion on a silicon wafer. For the studies presented here, the best results were obtained by drying in an environment of controlled relative humidity, and not in a desiccator. The diffraction experiments were carried out on a commercial Philips X-ray powder diffractometer of the Bragg Brentano type that was optimized for measurements at low angle. The X-ray tube was ceramic with a long fine focus and gave Cu Kα radiation (generator 40 kV, 40 mA). The goniometer had a variable divergence and antiscatter slits, with the receiving slits set at 0.1 mm. The detector was of the Peltier-cooled Si/Li type. During the measurements, the sample was mounted in a chamber of which the relative humidity could be controlled by a humidifying instrument flushed with $N_2$ gas.

**Acknowledgment.** The authors thank Dr. Neil Spencer and Mr. Peter Ashton who have performed the NMR experiments and the MS measurements, respectively. We also thank Professor Roeland J. M. Nolte for helpful suggestions. The Medical Research Council (MRC) and the Novartis Foundation have provided financial support.

**Supporting Information Available:** $^1H$ and $^{13}C$ NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO991685S