Lipolanthionine Peptides Act as Inhibitors of TLR2-Mediated IL-8 Secretion. Synthesis and Structure−Activity Relationships

Tobias Seyberth,[†] Söhnke Voss,[‡] Roland Brock,[‡] Karl-Heinz Wiesmüller,[§] and Günther Jung*[,†]

Institute of Organic Chemistry and Institute of Cell Biology, University of Tübingen, 72076 Tübingen, Germany, and EMC microcollections GmbH, Sindelfingerstr. 3, 72070 Tübingen, Germany

Received June 20, 2005

Lipoproteins from Gram-positive and -negative bacteria, mycoplasma, and shorter synthetic lipopeptide analogues activate cells of the innate immune system via the Toll-like receptor TLR2/TLR1 or TLR2/TLR6 heterodimers. For this reason, these compounds constitute highly active adjuvants for vaccines either admixed or covalently linked. The lanthionine scaffold has structural similarity with the *S*-(2,3-dihydroxypropyl)-cysteine core structure of the lipopeptides. Therefore, lanthionine-based lipopeptide amides were synthesized and probed for activity as potential TLR2 agonists or antagonists. A collection of analytically defined lipolanthionine peptide amides exhibited an inhibitory effect of the TLR2-mediated IL-8 secretion when applied in high molar excess to the agonistic synthetic lipopeptide Pam_3Cys-Ser-$(Lys)_4$-OH. Structure−activity relationships revealed the influence of the chirality of the two α-carbon atoms, the chain lengths of the attached fatty acids and fatty amines, and the oxidation level of the sulfur atom on the inhibitory activity of the lipolanthionine peptide amides.

Introduction

Lipoproteins are cell wall components of Gram-positive and -negative bacteria[1,2] and mycoplasma.[3,4] The N-terminal cysteine residue of these bacterial membrane proteins is posttranslationally modified to *S*-(2,3-dihydroxypropyl)cysteine (Dhc[a]), which is acylated by three or two fatty acids. Lipoproteins and synthetic analogues such as the water-soluble lipohexapeptide Pam_3Cys-Ser-$(Lys)_4$-OH[5] stimulate B cell proliferation and possess very favorable adjuvant activities in the generation of T-cell responses to vaccines.[6−10]

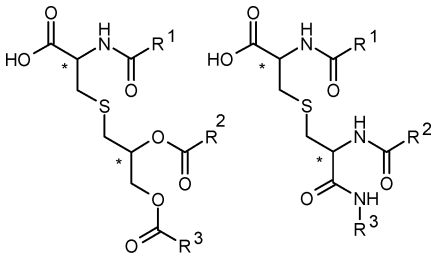
Toll-like receptors (TLRs) are prominent pattern recognition receptors (PRRs) of the innate immune system recognizing various invading microorganisms through conserved motifs termed "pathogen-associated molecular patterns" (PAMPs),[11] which are essential for and unique in microorganisms. In the 1980s, the Toll receptor was found in studies of embryonic development in *Drosophila melanogaster*. To date 11 mammalian TLRs (TLR1−TLR11) have been identified[12] and only for TLR10 no ligands have been described so far. Interaction of PAMPs with TLRs induces a variety of host defense responses, including the production of proinflammatory cytokines and the activation of adaptive immunity.[12−15] Among the TLRs, TLR2 mediates the response to the most diverse set of molecular structures from a variety of microbial organisms, including lipoteichoic acid, lipoarabinomannan, and bacterial lipopeptides/proteins as well as some lipopolysaccharids (LPS).[12] Among these PAMPs lipopeptides are the primary candidates for analyzing the structure−activity relationships of immune modulators. The role of the peptide moiety, the number and structure of the fatty acids, and the stereochemistry of lipopeptides based on Dhc for TLR activation has been analyzed in great detail.[16,17] In contrast, variation of the Dhc core structure has found little attention so far.[18,19] The apparent similarity of the scaffolds *S*-(2,3-dihydroxypropyl)cysteine (**1**) and lanthionine (**2**; Figure 1) led us to the assumption that lanthionine-based lipopeptide amides might show TLR2 agonistic activities similar to those of Pam_3Cys-peptides[5−10] or, due to its structural differences, inhibitory activities.

TLR agonists and antagonists are potential candidates for the treatment of various immune-associated diseases.[20] TLR2 antagonists have been suggested to have beneficial effects in chronic inflammation, acute inflammation, skin diseases such as acne, and treatment of sepsis. To explore these therapeutic options, there is an imminent need for synthetically accessible TLR agonistic and antagonistic compounds. Recently it was demonstrated in a sepsis model that lethal shocklike syndromes could be prevented by treatment of mice with the TLR2 antagonistic antibody mAB T2.5 prior to lipopeptide or *Bacillus*

* Corresponding author. Tel.: +49 (7071) 29-76925. Fax: +49 (07071) 29-5560. E-mail: guenther.jung@uni-tuebingen.de.
† Institute of Organic Chemistry, University of Tübingen.
‡ Institute of Cell Biology, University of Tübingen.
§ EMC microcollections GmbH.

[a] Abbreviations: BEMT, 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate; BMTB, 2-bromo-*N*-methylthiazolium bromide; Boc, *tert*-butoxycarbonyl; CIP, 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIEA, diisopropylamine; Dhc, *S*-(2,3-dihydroxypropyl)cysteine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ES-MS, electrospray ionization mass spectrometry; ELISA, enzyme linked immuno sorbent assay; Fmoc, 9-fluorenylmethoxycarbonyl; FT-ICR-MS, Fourier transform ion-cyclotron resonance mass spectrometry; FCS, fetal calf serum; GC−MS, gas chromatography−mass spectrometry; HATU, 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HONB, *N*-hydroxybicyclo[2.2.1]hept-5-ene-2,3-dicarboximide; HPLC, high performance liquid chromatography; IC_{50}, 50% inhibitory concentration; IL-8, interleukin-8; LPS, lipopolysaccharide; mAB, murine antibody; MALDI, matrix-assisted laser desorption/ionization; mCPBA, 3-chloroperbenzoic acid; MSNT, 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; Myr, myristoyl; Myr_2LanHda-, (2*R*,6*R*)-N^2,N^6-bis(myristoyl)-ϵ-hexadecylamide-lanthioninyl-; NMR, nuclear magnetic resonance; Pam, palmitoyl; Pam_3Cys, *S*-[2,3-bis(palmitoyloxy)-2(*RS*)-propyl]-*N*-palmitoyl-*R*-cysteine; Pam_2LanHda-OH, (2*R*,6*R*)-N^2,N^6-bis(palmitoyl)lanthionine-ϵ-hexadecylamide; Pam_2LanHda-, (2*R*,6*R*)-N^2,N^6-bis(palmitoyl)-ϵ-hexadecylamide-lanthioninyl-; Pam-OH, palmitic acid; PAMP, pathogen-associated molecular pattern; PRR, pattern recognizing receptor; PyBOP, *O*-benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate; rt, room temperature; SDS, sodium dodecyl sulfate; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; tBu, *tert*-butyl; TFA, trifluoroacetic acid; TFA-OPfp, pentafluorophenyl trifluoroacetate; TFFH, fluoro-*N*,*N*,*N*″,*N*″-tetramethylformamidinium hexafluorophosphate; TIPS, triisopropylsilane; THF, tetrahydrofuran; TLC, thin-layer chromatography; TLR, Toll-like receptor.

10.1021/jm050585d CCC: $33.50
Published on Web 02/09/2006



1 **2**

Figure 1. Structures of the natural thioether amino acid scaffolds *S*-(2,3-dihydroxypropyl)cysteine (Dhc) **1** and lanthionine (Lan) **2**. Because of the structural relationship lanthionine is an interesting scaffold for lipopeptides with potential TLR2 agonistic or antagonistic activity. R^1, R^2, and R^3 are long chain alkyl residues.

subtilis challenge.[21] Synthetic low molecular weight compounds with inhibitory activity against TLR2 have not been described so far.

Lanthionine was first detected in alkaline hydrolysates of wool. Moreover, this molecule was found in human hair and in keratin. In human lenses, the photooxidative degradation of cystine residues may also result in the formation of lanthionine, accompanied by protein cross-bridging that leads to increased tissue rigidity and hardening of the lens. Another highly interesting natural occurrence of lanthionine is the class of bioactive lantibiotics.[22−24]

The methods of lanthionine peptide syntheses have been summarized recently.[24] None of the published syntheses can be classified as a routine procedure to yield lanthionines in high enantiomeric purity. One of the main problems is the formation of dehydroalanine during the *S*-alkylation of L-cysteine due to the strongly basic conditions.[25] The desulfurization of cystine also forms large amounts of dehydroamino acid.[26] The use of serine β-lactone for the preparation of triprotected lanthionines may lead to low yields, due to competing *O*-acyl fission.[27,28] Bradley et al.[26] applied a biomimetic synthesis for the preparation of a fully protected lanthionine via Michael addition of Boc-Dha-OMe and Fmoc-Cys-OAll.[29] The resulting mixture of stereoisomers was separated by HPLC. Dugave and Menez[30] followed the original idea of Brown and du Vigneaud[25] using *N*-triphenylmethyl (trityl) protected β-iodoalanine for the *S*-alkylation of cysteine. The trityl group protected the β-iodoalanine derivative from β-elimination. Differently protected β-iodoalanine derivatives have been described by Jobron et al.[31] and Gair et al.[32] However, the application of this method to lanthionine synthesis by Mustapa et al.[33] led to a mixture of lanthionine and norlanthionine in a 1:2 ratio.[34]

This fact and the restricted scope with respect to the protecting groups limited the applicability of the thioalkylation methods for our purposes. In our hands, the synthesis of lanthionine via the β-iodoalanine and the serine β-lactone route was not successful. Therefore, we first elaborated a new preparation method for *N*,*N*-bis(Fmoc)lanthionine with orthogonally protected carboxy groups. Using this building block a representative collection of lipolanthionine peptides was synthesized and tested for biological activity, revealing the ability of these compounds to inhibit the TLR2-mediated IL-8 secretion.

Results

Synthesis of the Lanthionine Scaffold. After several less satisfying test series for the preparation of the desired lanthionine scaffold by reaction of (*R*)-*N*-Fmoc-cysteine-*tert*-butyl ester with (*S*)-*N*-Fmoc-serine β-lactone or (*R*)-*N*-Fmoc-β-iodoalanine-allyl ester, we searched for a new, more efficient access to lanthionines. In particular, we considered the synthesis via β-bromoalanine derivatives as a promising route leading to enantiopure lanthionines, similar to the procedure recently published by Zhu et al.[35] At first we examined a substitution reaction of (*R*)-β-bromo-*N*-Fmoc-alanine-allyl ester with (*R*)-*N*-Fmoc-cysteine-*tert*-butyl ester in 5% DIEA in DMF, analogous to the protocol of the synthesis of *N*-palmitoyl-*S*-[2,3-bis(palmitoyloxy)dihydroxypropyl]cysteine.[36] (*S*)-*N*-Fmoc-serine-allyl ester was obtained in high yields from (*S*)-*N*-Fmoc-serine and allyl bromide. The subsequent conversion to the (*R*)-β-bromo-*N*-Fmoc-alanine-allyl ester with PPh_3 and CBr_4 led to a mixture of *N*-Fmoc-dehydroalanine-allyl ester and (*R*)-*N*-Fmoc-β-bromoalanine-allyl ester in a 4:1 ratio. Comparison of published syntheses of different β-iodoalanine derivatives indicated that bulky protecting groups for either the amino[30] or the carboxy group[31,32] are necessary to hinder the α-hydrogen abstraction. Therefore, we changed the protecting groups and prepared (*R*)-*N*-Fmoc-cysteine-allyl ester (**5a**) and (*R*)-β-bromo-*N*-Fmoc-alanine-*tert*-butyl ester (**8a**; Scheme 1). (*S*)-*N*-Fmoc-serine (**6a**) was esterified with *tert*-butyl alcohol in the presence of DIC and CuCl (**7**, 65% yield).[37] Reaction times longer than 4 h led to increasing amounts of Fmoc-dehydroalanine, consistent with the fact that such conditions are also used for dehydroalanine synthesis.[38]

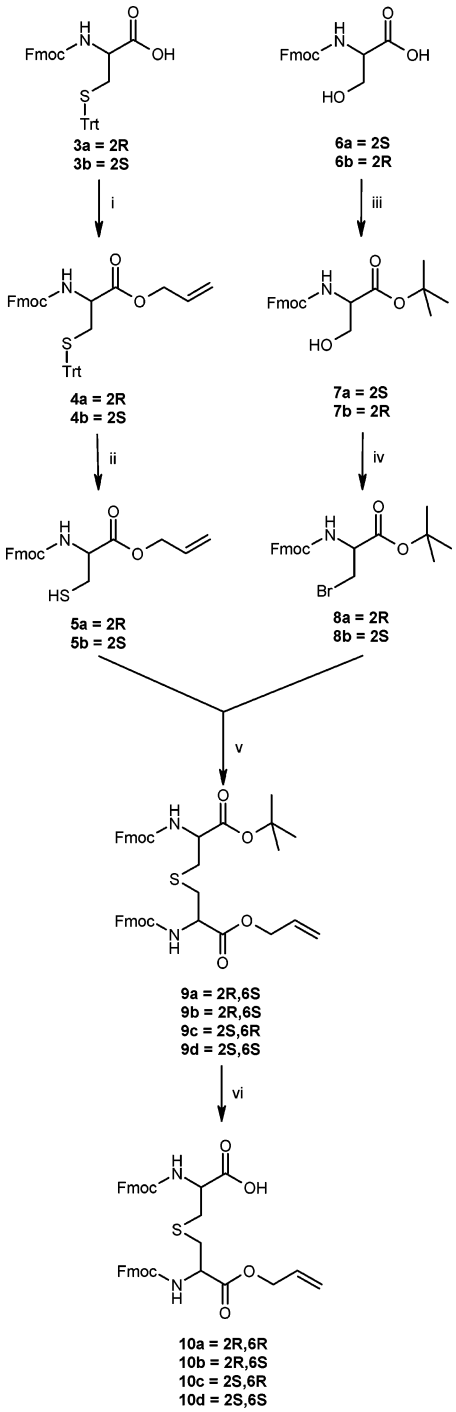
The conversion of **7a** to the bromide **8a** was achieved with PPh_3 and CBr_4 in good yield (85%). The (*R*)-*N*-Fmoc-cysteine-allyl ester (**5a**) was synthesized by esterification of (*R*)-*N*-Fmoc-S-trityl-cysteine (**3a**) with allyl bromide (**4a**, 92% yield) followed by acidic cleavage of the trityl group (**5a**, 95% yield). Synthesis of the fully protected (2*R*,6*R*)-lanthionine derivative was carried out by stirring **5a** and **8a** in 5% DIEA in DMF to obtain compound **9a** in 65% yield. Cleavage of the *tert*-butyl ester of **9a** with neat TFA followed by lyophilization from *tert*-butyl alcohol/water led to the desired (2*R*,6*R*)-N^2,N^6-bis(Fmoc)-lanthionine-ϵ-allyl ester **10a**.

NMR Analysis of Lanthionine. Mustapa et al.[33] described the formation of norlanthionine during the synthesis of lanthionine from β-iodoalanine. The comparison of the observed NMR data of the product **9a** with the simulated spectra of lanthionine and the isomeric norlanthionine (Supporting Information) and with the NMR data published by Mustapa et al.[33] and Zhu et al.[35] showed a high purity of the lanthionine. The identity of the compound **9a** was finally proven by H/H−COSY 2D-NMR (Supporting Information). Since we did not observe further byproducts apart from dehydroalanine, norlanthionine formation is not a problem of our protocol for lanthionine synthesis.

Synthesis and GC−MS Analysis of the Four Stereoisomers of Protected Lanthionine. The efficient synthesis protocol (Scheme 1) was applied to the preparation of the four configurational isomers (**10a**−**d**). Using the appropriate L- or D-amino acid derivatives, the desired isomers of protected lanthionine (**9a**−**d**) were obtained in high enantiomeric purities (>95%; Table 1). The enantiomeric purities were analyzed by chiral GC−MS (Chirasil-Val column) after derivatization.[39] Since the allyl group proved to be stable during the cleavage of the *tert*-butyl ester, we used N^2,N^6-bis(trifluoroacetyl)lanthionine-α-allyl-ϵ-methyl diester as volatile derivative for GC−MS analysis, leading to the fragment-ion $m/z = 228$ u, which was used for single-ion-mode MS-detection (Supporting Information).

(2*R*,6*R*)-N^2,N^6-bis(palmitoyl)lanthionine-ϵ-hexadecylamide. In the next step, we synthesized the (2*R*,6*R*)-N^2,N^6-bis-(Fmoc)lanthionine-ϵ-hexadecylamide (**13**; Scheme 2) from compound **9a** by allyl cleavage with $Pd(PPh_3)_4$ (**11**, 91%) followed by coupling of **11** to hexadecylamine with HOBt/DIC

Scheme 1. Synthesis of N^2,N^6-Bis(Fmoc)lanthionine-α-allyl Ester (**10a**−**d**)[a]



[a] (i) 1.1 equiv of DIEA, allyl bromide/acetonitrile (1:2), rt, 16 h; (ii) 3.3 equiv of TIPS, TFA/DCM (1:1), rt, 1 h; (iii) 4 equiv of *t*BuOH, 3 equiv of DIC, CuCl, rt for 16 h; add **6a/b** in DCM, rt, 4 h; (iv) 1.6 equiv of PPh_3, 1.22 equiv of CBr_4, 0 °C→rt, 3 h; (v) 1 equiv of **5a/b**, 1.1 equiv of **8a/b**, 5% DIEA/DMF, rt, 4 h; (vi) TFA, rt, 1 h.

(**12**, 67%). One-half of compound **12** was treated with neat TFA for 1 h to yield (2*R*,6*R*)-N^2,N^6-bis(Fmoc)lanthionine-ϵ-hexadecylamide (**13**) after lyophilization. The remaining amount of **12** was treated with piperidine/DMF (1:1) and, after removal of the solvents in vacuo, Pam-OH was coupled with DIC/HOBt, resulting in (2*R*,6*R*)-N^2,N^6-bis(palmitoyl)lanthionine-ϵ-hexadecylamide-α-*tert*-butyl ester (**14**) in 63% yield. Treatment with neat TFA followed by lyophilization led to (2*R*,6*R*)-N^2,N^6-bis-(palmitoyl)lanthionine-ϵ-hexadecylamide (Pam_2LanHda-OH, **15**).

Table 1. Summary of the Enantiomeric Purities of Lanthionines **9a**−**d** as Determined by Chiral Phase GC−MS Analysis

	% purity		
lanthionine	(2*R*,6*R*)	(2*S*,6*R*), (2*R*,6*S*)	(2*S*,6*S*)
2*R*,6*R* (**9a**)	97.3	2.7	0.0
2*R*,6*S* (**9b**)	1.3	97.1	1.6
2*S*,6*R* (**9c**)	2.6	96.7	0.7
2*S*,6*S* (**9d**)	0.1	4.1	95.8

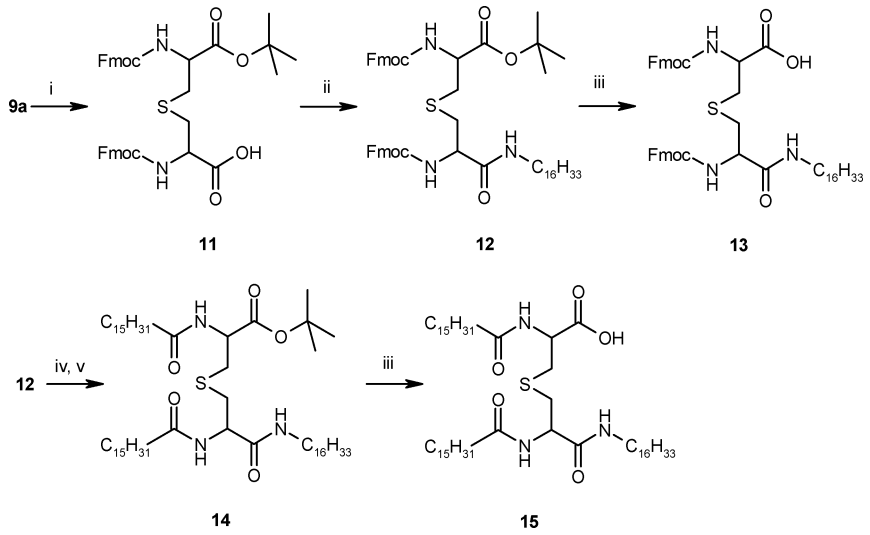
The enantiomeric purity of product **15** was determined after total hydrolysis and derivatization with TFA/methanol by chiral GC−MS. The product contained 91% of the 2*R*,6*R* enantiomer according to the peak ratios.

Lipolanthionine Peptide Amide Syntheses. As test compounds for biological assays (2*R*,6*R*)-Myr_2LanHda- and (2*R*,6*R*)-Pam_2LanHda-Ser-$(Lys)_4$-NH_2 were synthesized. The pentapeptide Ser-$(Lys)_4$ amide moiety[5] was introduced in order to improve water solubility. The peptide was synthesized on Rink-amide resin (0.74 mmol/g loading) by HOBt/DIC coupling using *N*-Fmoc-Lys(Boc)-OH and *N*-Fmoc-Ser(tBu)-OH, leading to resin bound Fmoc-Ser(tBu)-$[Lys(Boc)]_4$ (**16**). The synthesis was monitored by Kaiser test,[40] and the Fmoc-protected peptide amides, after cleavage, were characterized by ES-MS and HPLC (>95%). Following the last coupling step, the resin-loading was determined by cleavage of the Fmoc protecting group with 20% piperidine in DMF, and spectrophotometric analysis of the cleaved fluorene confirmed a quantitative yield of **16** (resin-loading 0.415 mmol/g). Coupling **15** (Scheme 2) to the Fmoc-deprotected resin **16** proved to be difficult, since **15** is insoluble at room temperature. Quantitative coupling was achieved by double couplings with HOBt/DIC at 45 °C in $CHCl_3$/DMF (8:1). Since the lanthionine moiety of the product was strongly racemized (61% 2*R*,6*R*), this lipolanthionine peptide was not used for the biological tests. To circumvent the racemization, the urethane-protected lanthionine monoamide **13** was coupled to the resin-bound pentapeptide **16** with HOBt/DIC repeatedly until a negative Kaiser test was observed. Again, quantitative coupling of **13** was proven by spectrophotometric Fmoc quantification (resin-loading: 0.329 mmol/g). Fmoc deprotection and coupling of myristic acid and palmitic acid respectively with HOBt/DIC yielded the lipolanthionine peptide amides (2*R*,6*R*)-Myr_2LanHda- (**17**) and (2*R*,6*R*)-Pam_2LanHda-Ser-$(Lys)_4$-NH_2 (**18**) after cleavage.

Coupling the Fatty Amine to Resin-Bound Carboxy Functions. The first biological tests of lipolanthionine peptide amides (**17**, **18**) showed a significant inhibition of the TLR2-mediated IL-8 secretion for both of the test compounds. Therefore, we decided to investigate the influence of different fatty acids and fatty amines as well as of different configurations of the compounds and different oxidation levels of the sulfur atom. First, a suitable protocol for coupling the carboxy group of resin-bound lanthionine to the fatty amines had to be developed. The urethane-protected lanthionine derivative **10a** was coupled to Fmoc-deprotected resin **16** (Scheme 3) and the allyl group cleaved quantitatively from the resulting resin-bound compound **19** with phenylsilane and $Pd(PPh_3)_4$ within 2 h (**20**).

For the attachment of the fatty amine, a variety of coupling conditions were tested: activation of the polymer-bound acid component **20** by TFFH, CIP, BEMT, or BMTB and activation as active esters using HOBt/DIC, HONB/DIC, MSNT, TBTU/HOBt, TFA-OPfp, HATU, or PyBOP. Preactivation as pentafluorophenyl ester with pentafluorophenyl trifluoroacetate (TFA-OPfp) in DMF/pyridine followed by treatment with the fatty amine and DIEA in THF proved to be the most effective

Scheme 2. Synthesis of (2*R*,6*R*)-N^2,N^6-Bis(Fmoc)lanthionine-ϵ-hexadecylamide (**13**) and N^2,N^6-Bis(palmitoyl)lanthionine-ϵ-hexadecylamide (**15**)[a]



[a] (i) 1.3 equiv of morpholine, cat. $Pd(PPh_3)_4$, rt, 1 h; (ii) 1 equiv of HOBt, 1 equiv of DIC, 1.1 equiv of hexadecylamine, rt, 6 h; (iii) TFA, rt, 1 h; (iv) piperidine/DMF (1:1), rt, 2 h; (v) 1.8 equiv of Pam-OH, 1.8 equiv of HOBt, 1.8 equiv of DIC, rt, 16 h.

coupling protocol on the solid phase. The drawback of the synthesis is a partial Fmoc cleavage by the primary amine; therefore, the resin-bound lanthionine peptide was subsequently reprotected with a surplus of fluoren-9-ylmethyl chloroformate in pyridine/DMF prior to further usage for analytics and following syntheses.

Synthesis of the Lipolanthionine Peptide Amide Collection. To determine structure−activity relationships, we synthesized a variety of lipolanthionine peptide amides (Figure 2) following Scheme 3. Several *n*-fatty amines R^1-NH_2 (C_nH_{2n+1}-NH_2) were attached to the resin **20**, and the resin-bound N^2,N^6-bis(Fmoc)-ϵ-(C_nH_{2n+1}) amide-lanthionine peptide amides **21**−**27** (n = 6, 8, 10, ..., 18) were obtained in good purities (>88%, HPLC). Fmoc was cleaved from these resin-bound lanthionine derivatives, and myristic acid was coupled to both of the amino functions, yielding the lipolanthionine peptides **17** and **28**−**33** (Chart 1) after cleavage from the resin.

Variation of the fatty acid moiety was carried out by coupling different *n*-fatty acids (C_mH_{2m+1}-COOH; m = 5, 7, ..., 11 and 15, 17, 19) to Fmoc-deprotected resin **26** (carrying $Fmoc_2$-LanHda-Ser(tBu)-[Lys(Boc)]$_4$) to yield compounds **18** and **34**−**39** after cleavage. To determine the influence of different configurations, the stereoisomeric lanthionines **10b**−**d** were coupled to Fmoc-deprotected resin **16** (leading to **40**−**42**). The subsequent steps were carried out as described for (2*R*,6*R*)-Myr_2-LanHda-Ser-$(Lys)_4$-NH_2 **17** in Scheme 3, first cleaving the allyl group (**43**−**45**) and subsequently coupling hexadecylamine (**46**−**48**) and myristic acid, which results in (2*R*,6*S*)-, (2*S*,6*R*)-, and (2*S*,6*S*)-Myr_2LanHda-Ser-$(Lys)_4$-NH_2 (**49**, **50**, and **51**; Chart 1) after cleavage.

We were also interested in the generation of the stereoisomers of Myr_2Lan(OH)-Ser-$(Lys)_4$-NH_2. These lipopeptide amides lack the lipophilic fatty amine moiety in the 7-position of the lanthionine. The stereoisomers **10a**−**d** were coupled to resin **16** (leading to **19**, **40**−**42**) and myristic acid was coupled to the resin-bound lanthionines after Fmoc-deprotection (**52**−**55**). The allyl group was cleaved to yield the Myr_2Lan(OH)-Ser-$(Lys)_4$-NH_2 (**56**−**59**) after cleavage from the resin. The resulting lipolanthionine peptide amides are summarized in Chart 1.

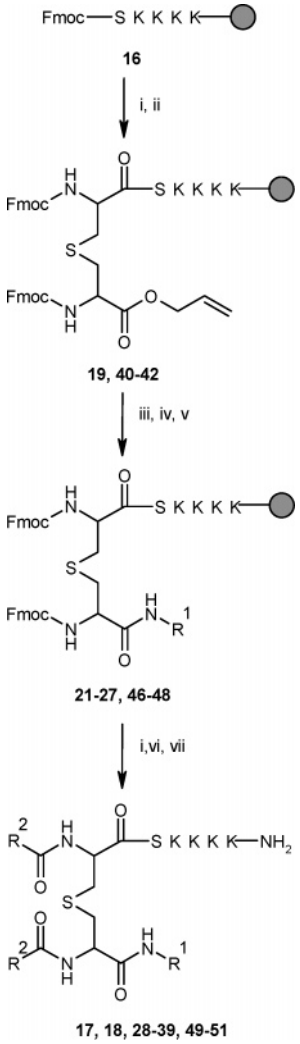
Oxidation of the Lanthionine to Sulfone and Sulfoxide. To investigate whether the oxidation level of the sulfur influences the biological activity, the sulfoxide and sulfone of resin-bound Pam_2LanHda-Ser(tBu)-[Lys(Boc)]$_4$ amide (**60**; Scheme 4) were synthesized and cleaved from the resin. The protocol used by Matteucci et al.[41] to oxidize resin-bound *S*-methylcysteine to its sulfoxide with H_2O_2 and scandium trifluoromethanesulfonate in DCM/methanol (9:1) proved suitable for lanthionine−sulfur oxidation in the lanthionine-containing lipopeptides. The sulfoxide of Pam_2LanHda-Ser-$(Lys)_4$-NH_2 was obtained after cleavage from the resin (**61**; Scheme 4). Oxidation to the sulfone was achieved by treatment of resin-bound Pam_2LanHda-Ser(tBu)-[Lys(Boc)]$_4$ amide with mCPBA (5 equiv) in DCM, a method used by Mata[42] for the oxidation of resin-bound penicillin derivatives. The desired sulfone **62** was obtained after cleavage in high yield and good purity. Mata also described a protocol for sulfone generation (1.4 equiv of mCPBA in DCM, 0 °C), which in our case led to a mixture of non-, mono- and dioxidized lanthionines. The sulfoxide **61** and sulfone **62** of Pam_2LanHda-Ser-$(Lys)_4$-NH_2 (**18**) were characterized by MALDI-MS and used for testing TLR2 inhibition.

Structure−Activity Relationships. The representative collection of analytically defined single lipolanthionine peptide amides (Chart 1) was tested for agonistic activity and for their ability to inhibit the TLR2-mediated IL-8 secretion. To test the inhibitory activity, the well-studied agonist Pam_3Cys-Ser-$(Lys)_4$-OH was used for stimulation of THP-1 cells. The synthesized lipolanthionine compounds enabled the analysis of the structure−activity relationships for the (i) fatty amine chain length, (ii) fatty acid chain length, (iii) stereochemical properties, and (iv) the oxidation level of the sulfur.

First we investigated the influence of the fatty amine chain length using the derivatives based on the bis-myristoylated (2*R*,6*R*)-lanthionine lipopeptide amides (**17**, **28**−**33**). At concentrations of 25 μM only for the hexylamine derivative (**28**) a weak agonistic activity was observed.

Interestingly, except for the hexylamine derivative (**28**), all analogues of lipolanthionine peptide amides exhibited a pronounced and comparable TLR2 inhibitory activity (Figure 3). When applied in a 500-fold excess (25 μM) over the highly potent Pam_3Cys-Ser-$(Lys)_4$-OH TLR2 agonist (50 nM), IL-8 expression was reduced by 48−65%. Therefore, given a minimum length of eight carbon atoms, the length of the fatty

Scheme 3. Protocol for the Solid Phase Synthesis of **17**, **18**, **28**−**39**, and **49**−**51** on Rink-Amide Resin[a]



[a] (i) 20% piperidine/DMF, rt, 20 min; (ii) 3 equiv of **10a**−**d**, 3 equiv of HOBt, 3 equiv of DIC, rt, 8 h; (iii) 0.25 equiv of $Pd(PPh_3)_4$, 24 equiv of phenylsilane, rt, 2 h, argon (**20**, **43**−**45**); (iv) pyridine/DMF/TFA-OPfp (3:2:1); (v) 10 equiv of R^1-NH_2, 30 equiv of DIEA, rt, 6 h; (vi) 3 equiv of R^2COOH, 3 equiv of HOBt, 3 equiv of DIC, rt, 8 h; (vii) TFA, rt, 3 h.

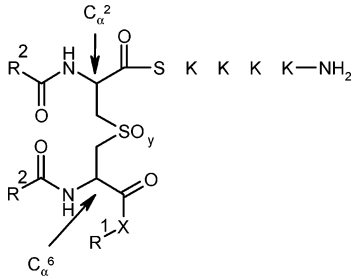


Figure 2. Core structure of the synthesized lipolanthionine peptide amides. (Definition of the residues and abbreviations for the figure and Chart 1: R^1, R^2, alkyl chains; SO_y, $y = 0$, sulfide; $y = 1$, sulfoxide; $y = 2$, sulfone; configurations $C_\alpha{}^2$,$C_\alpha{}^6$, 2*R*,6*R*, 2*R*,6*S*, 2*S*,6*R*, 2*S*,6*S*; X = NH, O).

amine chain seemed to be of minor significance. Except for the octadecylamine derivative (**33**), no cell toxic properties were observed at the tested concentration as determined by the MTT test. The derivative containing the hexadecylamine residue was selected for further tests.

Next we studied the influence of the variation of the amide-bound fatty acid moieties. In this case, only for the caprylic acid derivative (C-8, **35**) was an agonistic activity observed. The derivatives containing lauric (C-12, **37**), myristic (C-14, **17**), and palmitic acid (C-16, **18**) had the highest inhibitory effect on the lipopeptide-induced IL-8 secretion (Figure 4). The analogues with short fatty acids (C-6 to C-10, **34**−**36**) exhibited less inhibitory activities.

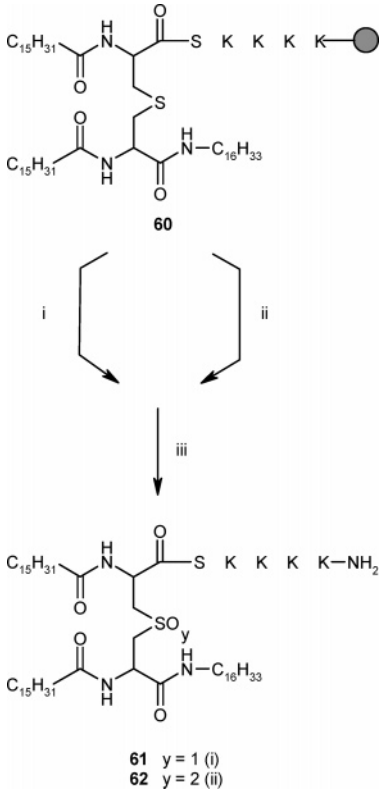
For two of the most active inhibitors (**17**, **18**), the concentration dependence of the inhibitory activity was determined. For both compounds a concentration-dependent decrease in IL-8 expression was observed (Figure 5). For the myristic acid derivative, IL-8 expression was reduced by up to 86% for the palmitic acid derivative by up to 68%. Both compounds exhibited IC_{50} values of about 5 μM. However, the inhibitory activity of compounds **17** and **18** varied between several experiments (38%, 18%, and 14% residual activity for compound **17**, respectively, and 30% and 42% for compound **18**). Since these activities were obtained from independent cellular experiments, such variations represent slight alterations that could not be excluded for cellular assays. Nevertheless, for all experiments, using the compounds **17** and **18**, the inhibitory activity in the presented range was reproducible.

The agonistic activity of Pam_3Cys-Ser-$(Lys)_4$-OH and even of lipopeptide vaccines[43] is dependent on the configuration of the *S*-glyceryl-cysteine scaffold.[43−45] Therefore, we tested the four stereoisomers of the bis-myristoylated hexadecylamide derivative, the most active inhibitor identified so far. The agonistic activity negatively correlated with the inhibitory

Chart 1. The Synthesized Lipolanthionine Peptide Amide Collection: Definition of the Structures of All Tested Compounds

	R^1	X	R^2	SO_y	$C_\alpha{}^2$	$C_\alpha{}^6$
28	C_6H_{13}	NH	$C_{13}H_{27}$	0	R	R
29	C_8H_{17}	NH	$C_{13}H_{27}$	0	R	R
30	$C_{10}H_{21}$	NH	$C_{13}H_{27}$	0	R	R
31	$C_{12}H_{25}$	NH	$C_{13}H_{27}$	0	R	R
32	$C_{14}H_{29}$	NH	$C_{13}H_{27}$	0	R	R
17	$C_{16}H_{33}$	NH	$C_{13}H_{27}$	0	R	R
33	$C_{18}H_{37}$	NH	$C_{13}H_{27}$	0	R	R
34	$C_{16}H_{33}$	NH	C_5H_{11}	0	R	R
35	$C_{16}H_{33}$	NH	C_7H_{15}	0	R	R
36	$C_{16}H_{33}$	NH	C_9H_{19}	0	R	R
37	$C_{16}H_{33}$	NH	$C_{11}H_{23}$	0	R	R
18	$C_{16}H_{33}$	NH	$C_{15}H_{31}$	0	R	R
38	$C_{16}H_{33}$	NH	$C_{17}H_{35}$	0	R	R
39	$C_{16}H_{33}$	NH	$C_{19}H_{39}$	0	R	R
49	$C_{16}H_{33}$	NH	$C_{13}H_{27}$	0	R	S
50	$C_{16}H_{33}$	NH	$C_{13}H_{27}$	0	S	R
51	$C_{16}H_{33}$	NH	$C_{13}H_{27}$	0	S	S
56	H	O	$C_{13}H_{27}$	0	R	R
57	H	O	$C_{13}H_{27}$	0	R	S
58	H	O	$C_{13}H_{27}$	0	S	R
59	H	O	$C_{13}H_{27}$	0	S	S
61	$C_{16}H_{33}$	NH	$C_{15}H_{31}$	1	R	R
62	$C_{16}H_{33}$	NH	$C_{15}H_{31}$	2	R	R

Scheme 4. Synthesis of the Sulfoxide and Sulfone of $Pam_2LanHda$-Ser-$(Lys)_4$ Amide from the Resin-Bound Sulfide[a]



[a] (i) 5 equiv of H_2O_2, 0.2 equiv of $Sc(OTf)_3$, DCM/methanol (9:1), rt, 40 min; (ii) 5 equiv of mCPBA, rt, 24 h; (iii) TFA, rt, 3 h.

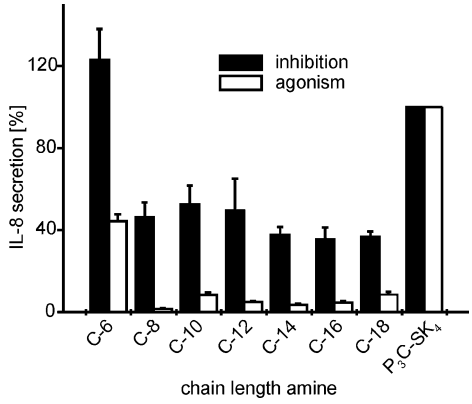


Figure 3. Inhibitory activities of $Myr_2Lan(R^1)$-SK_4-NH_2 with varying fatty amine residue R^1. THP-1 cells were incubated with lipolanthionine peptides (25 μM) in the absence or presence of the agonistic lipohexapeptide Pam_3Cys-SK_4-OH (50 nM) for 5 h. The secretion of IL-8 was detected by ELISA in cell-free supernatants. The Pam_3Cys-SK_4-OH-induced IL-8 secretion is shown in relation to a sample that was incubated with Pam_3Cys-SK_4-OH in the presence of the same amount of solvent as the lipolanthionine-containing samples. Error bars represent the standard deviation of triplicates.

activity. The (2*R*,6*R*)-lanthionine peptide showed the highest inhibitory activity, closely followed by the (2*S*,6*R*) derivative (**17** and **50**; Figure 6). In contrast, a change of the configuration in the C-6 stereocenter led to a loss of the inhibitory activity and to an increase of the agonistic activity. The agonistic activity of the (2*R*,6*S*) derivative was higher than that of the (2*S*,6*S*) one. Therefore, only the configuration of C-6 strongly influences the TLR2 inhibitory activity of the compounds, whereas the configuration at C-2 seems to be of minor significance.

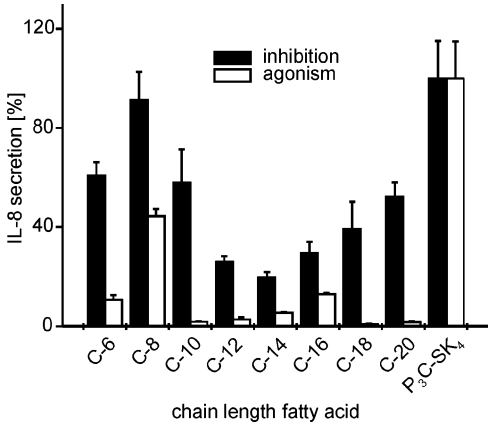


Figure 4. Inhibitory activities of $(R^2)_2Lan(Hda)$-SK_4-NH_2 with varying fatty acid residues R^2. THP-1 cells were incubated with lipolanthionine peptides (25 μM) in the absence or presence of Pam_3Cys-SK_4-OH (50 nM) for 5 h. The secretion of IL-8 was detected by ELISA in cell-free supernatants.

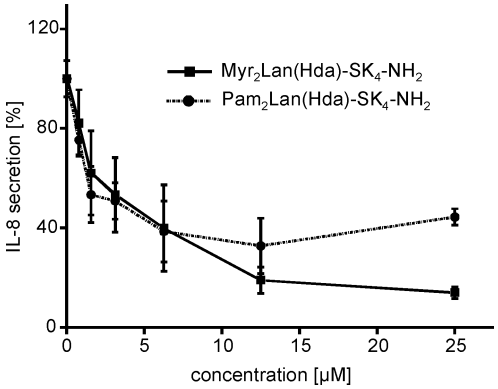


Figure 5. Concentration dependence of the inhibitory activity of Myr_2-$Lan(Hda)$-SK_4-NH_2 (**17**) and $Pam_2Lan(Hda)$-SK_4-NH_2 (**18**). THP-1 cells were incubated with lipolanthionine peptides (25, 12.5, 6.25, 3.13, 1.56, 0.78 μM) in the presence of Pam_3Cys-SK_4-OH (50 nM) for 5 h. The secretion of IL-8 was detected by ELISA in cell-free supernatants. Error bars represent the standard deviation of triplicates.

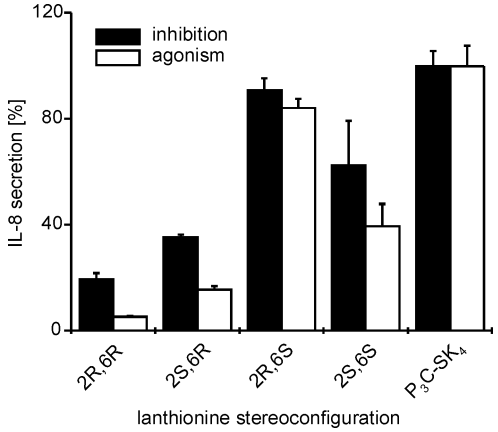


Figure 6. Dependence of the inhibitory activities of $Myr_2Lan(Hda)$-SK_4-NH_2 (**17**) on the configuration of the lanthionine scaffold. THP-1 cells were incubated with lipolanthionine peptides (25 μM) in the absence or presence of Pam_3Cys-SK_4-OH (50 nM) for 5 h. The secretion of IL-8 was detected by ELISA in cell-free supernatants. Error bars represent the standard deviation of triplicates.

Lipolanthionine peptide amides containing free carboxy functions (**56**−**59**) were unable to suppress the Pam_3Cys-Ser-$(Lys)_4$-OH-induced IL-8 secretion (data not shown). Interestingly, the methyl ester derivatives possessed cell toxic properties (MTT test, data not shown). The $Pam_2LanHda$-Ser-$(Lys)_4$-NH_2

oxides (**61** and **62**) showed no significant alteration of the observed inhibitory effect (data not shown).

Discussion

So far, analyses of structure−activity relationships of the well-characterized lipopeptide and highly active TLR2 agonist Pam_3-Cys-Ser-$(Lys)_4$-OH have focused mainly on the periphery of the natural core scaffold (Dhc), i.e., variations of the peptide and/or of the fatty acids. To vary the scaffold, we substituted the Dhc by the structurally related thioether amino acid lanthionine. The lanthionine scaffold possesses the same number of functional groups, suitable for the introduction of structural diversity for lipopeptide synthesis, as the Dhc scaffold. A stereoselective synthesis for a generally applicable protected lanthionine building block was established, the four configurational isomers were synthesized, and the enantiomeric purities were determined by chiral GC−MS analysis. The high enantiomeric purities suggested that the reaction of cysteine with β-bromoalanine follows a substitution mechanism similar to the lanthionine synthesis via β-iodoalanine. Two lipolanthionine peptide amides, derived from the lipopeptide building-block $Fmoc_2$LanHda-OH **13** were tested for their capacity to influence the IL-8 secretion induced by the TLR2 agonist Pam_3Cys-Ser-$(Lys)_4$-OH. These in vitro tests revealed the concentration-dependent and saturable TLR2 inhibitory rather than agonistic activities of the lipolanthionine peptides.

Detailed analyses of the structure−activity relationship for the fatty acid and fatty amine moieties were conducted for the (2*R*,6*R*) configuration. The chain length of the amide-bound fatty acid had a strong influence on the activity, whereby the myristic acid derivative (**17**) was the most effective inhibitor on the lipopeptide-induced IL-8 secretion. In contrast, the chain length of the amide-bound fatty amine residue had no influence on the inhibitory activity for chain lengths from C-8 to C-16 (**17**, **29**−**32**). The C-6 derivative (**34**) exhibited some agonistic activity.

The configuration at the C-6 position of the lanthionine proved to be crucial for inhibitory activity, whereas the C-2 configuration showed little influence. In conclusion, the lipolanthionine peptide amide (2*R*,6*R*)-Myr_2LanHda-Ser-$(Lys)_4$-NH_2 (**17**) exhibited the strongest TLR2 inhibitory activity.

Several possibilities exist in which way the lipolanthionine peptide amides might influence the TLR2-mediated IL-8 secretion. First, the inhibitor may interact with the agonist, preventing receptor recognition and initiation of signal transduction.[46] Second, the inhibitor may influence the TLR2 and/or a coreceptor directly, by interacting with the respective ligand binding site. Third, the lipolanthionine peptides could inhibit the assembly of the TLR2 receptor complex by integrating into cell membrane components, such as lipid rafts. For TLR4 it was demonstrated that LPS induces the coclustering of CD14 and TLR4 in the lipid rafts.[47,48] Recently, it was shown that oxidized phospholipid products inhibited the LPS-induced translocation of TLR4 to lipid rafts (caveolae).[49] It was also reported, that *n*-3 unsaturated fatty acids caused the alteration of fatty acid composition in membrane lipid rafts, resulting in the inhibition of T-cell activation.[50,51]

A direct inhibition by selective blocking of the TLR2 receptor and/or either the TLR1 or TLR6 coreceptor seems to be rather unlikely, since the lipolanthionine peptides also inhibited the LPS-induced, TLR4-mediated IL-8 secretion (data not shown).

On the other hand, the inhibitory potential of the lipolanthionine peptides was strongly dependent on the configuration of the lanthionine C-6 position, an observation that favors a mechanism of inhibition based on the interaction with a receptor molecule. It is moreover remarkable that the agonistic activity of the lanthionine peptides negatively correlated with their inhibitory activity. Therefore, a nonspecific mode of action by insertion of the lipolanthionine peptides into membrane domains such as lipid rafts seems to be unlikely. Instead of directly interacting with any TLR, the lipolanthionine peptides may bind to CD14, a coreceptor that is shared by LPS and lipopeptides for activation of their respective TLR.[52] Still, the high concentrations necessary for the inhibitory activity of the lipolanthionine peptides may suggest a direct interaction with the agonists. For example, the agonist Pam_3Cys-Ser-$(Lys)_4$-OH might be integrated into micelles composed of lipolanthionine peptides. Future studies will have to elucidate whether the lipolanthionine peptide-mediated IL-8 inhibition follows one of the above-described mechanisms.

Conclusion

Lipolanthionine peptide amides have been prepared and evaluated for their ability to inhibit TLR2-mediated IL-8 secretion in myelomonocytic THP-1 cells. Based on a versatile lanthionine building block, a representative collection of lipolanthionine peptide amides with fatty acid and fatty amine moieties of different length as well as with different lanthionine configurations and sulfur oxidation levels were synthesized and analytically characterized. In vitro tests revealed a TLR2 inhibitory activity that was strongly dependent on the chain length of the fatty acid moiety with a maximal inhibitory activity found for the myristic acid derivative **17**. In contrast, the chain length of the amide bound fatty amine residue had no significant influence on the inhibitory activity concerning chain lengths from C-8 to C-16 (**17**, **29**−**32**). Derivatives with free lanthionine carboxy functions were not able to suppress the IL-8 secretion. It was further shown, that the configuration of the lanthionine C-6 position was crucial for the inhibitory activity of the compounds. A change of the configuration at the lanthionine C-2 position had little influence. Compound **17** [(2*R*,6*R*)-Myr_2-LanHda-Ser-$(Lys)_4$-NH_2] was identified as the best inhibitor, expressing an IC_{50} value of about 5 μM. First tests concerning the ability of the lipolanthionine peptides to inhibit the TLR4-mediated IL-8 secretion revealed a similar inhibitory activity as detected for TLR2. Thus, the synthesized lipolanthionine peptide amides do not selectively inhibit the TLR2-mediated IL-8 secretion.

To the best of our knowledge, the lipolanthionine peptides are the first lipopeptidic compounds exhibiting inhibitory activity for TLR2. They may provide a basis for the design of further compounds with enhanced TLR2 inhibitory activity to counteract the lethal effects of septic shock.

Experimental Section

Water-free solvents were from Fluka (Neu-Ulm, Germany). Acetonitrile, methanol, trifluoroacetic acid (Uvasol), and chloroform-d_1 were from Merck (Darmstadt, Germany). Protected amino acids were from Novabiochem (Bad Soden, Germany) or Bachem (Heidelberg, Germany). Rink-amide resin was obtained from Rapp-Polymere (Tübingen, Germany). The reference TLR2 agonist Pam_3-Cys-Ser-$(Lys)_4$-OH was from EMC microcollections GmbH (Tübingen, Germany). All further chemicals were purchased from Aldrich (Taufkirchen, Germany) and Fluka (Neu-Ulm, Germany), respectively.

For HPLC analysis we used a Waters LC System with Waters 712 WISP autosampler, a Waters 626 pump combined with a Waters 600S controller, and a Waters 486 UV-detector (λ = 214 nm). A Nucleosil 300 C_{18} RP-column and a linear gradient of

acetonitrile/0.1% TFA (A) and water/0.1% TFA (B) (10 vol % A to 100 vol % A in 35 min; flow 0.3 mL/min) were used.

ES-MS spectra were recorded on a Bruker Esquire 3000plus LC−ES-MS^n (Bruker Daltonics, Bremen, Germany). High-resolution FT-ICR-MS spectra were recorded using a Bruker Daltonics APEX II system with a 4.7 T magnet and ES ionization. As internal standard for high-resolution analysis, PEG or Ultramark were used. GC−MS analyses were performed on a Agilent (formerly HP) system consisting of a 6890 GC unit and a 5973 MS detector using EI ionization. Helium was used as carrier gas and a Chirasil-Val column as chiral stationary phase. The following temperature program was used: 55 °C for 3 min; 25 °C/min to 145 °C for 10 min; 2.5 °C/min to 180 °C. MALDI-MS analysis was carried out on a Hewlett-Packard G2025A matrix-assisted-laser-desorption time-of-flight system using a DHAP matrix (20 mg of DHAP, 5 mg of ammonium citrate in 1 mL of 80% isopropyl alcohol) on a gold target. For signal generation 20−50 laser shots were added up in the single shot mode.

NMR data were collected on Bruker Avance 400 MHz or Bruker AMX 600 MHz spectrometers using solutions of the samples in chloroform-d_1 (30 mg/mL). The NMR data are given in the Supporting Information.

For TLC, silica gel plates 60 F_{254}, 5 × 10 cm (Merck, Darmstadt, Germany) and the following solvent systems (v/v) were used in solvent saturated glass chambers for determination of R_f values at room temperature: I, hexane/ethyl acetate 3:1; II, hexane/ethyl acetate 2:1; III, hexane/ethyl acetate 7:1; IV, hexane/ethyl acetate/acetic acid 8:4:1; V, chloroform/methanol/acetic acid 90:10:1; VI, ethyl acetate/chloroform/25% ammonia 130:8:1.

Allyl Protection of the Carboxy Group (4; General Procedure). The *N*-Fmoc protected amino acid was dissolved in a mixture of 1.1 equiv of DIEA and acetonitrile (0.66 mL/g). Allyl bromide (0.33 mL/g) was added and the solution stirred for 16 h at room temperature. The reaction mixture was diluted with a 5-fold excess of ethyl acetate, washed three times with 10% Na_2CO_3 and brine, and dried over $MgSO_4$. The solvent was evaporated and the product purified by flash column chromatography (silica gel, hexane/ethyl acetate 3:1) to yield the allyl ester of the Fmoc-amino acid as a colorless amorphous powder.

(*R*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-*S*-tritylcysteine-allyl ester (4a) was synthesized with (*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)-*S*-tritylcysteine (7.50 g, 12.81 mmol), DIEA (2.41 mL), acetonitrile (50 mL), and allyl bromide (25 mL). Yield: 7.37 g, 92.0%. TLC: R_f (I) = 0.23. ES-MS: m/z = 648.4 [M + Na]$^+$.

(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-*S*-tritylcysteine-allyl ester (4b). (S)-*N*-(Fluoren-9-ylmethoxycarbonyl)-*S*-tritylcysteine (2,00 g, 3.42 mmol); DIEA (643.03 μL; 3.75 mmol); acetonitrile (13.33 mL); allyl bromide (6.67 mL). Yield: 1.92 g, 89.6%. TLC: R_f (I) = 0.23. ES-MS: m/z = 648.4 [M + Na]$^+$.

Cleavage of the *S*-Trityl Protecting Group (5; General Procedure). The *S*-tritylated cysteine derivative was dissolved in TFA/DCM (1:1) and 3.3 equiv of TIPS was added. After stirring for 1 h at room temperature, the solvent was removed in vacuo and the residual product **5** recrystallized from DCM/diethyl ether (1:20) at −20 °C.

(*R*)-*N*-(Fluoren-9-ylmethoxycarbonyl)cysteine-allyl ester (5a) was synthesized with **4a** (7.00 g, 11.19 mmol), TFA/DCM (1:1, 40 mL), TIPS (7.58 mL, 36.93 mmol), and DCM/diethyl ether (1:20, 315 mL). Yield: 4.08 g, 95.1% of a colorless amorphous powder. TLC: R_f (I) = 0.34. ES-MS: m/z = 406.1 [M + Na]$^+$ 422.0 [M + K]$^+$. GC−MS: 99.5% ee.

(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)cysteine-allyl ester (5b) was synthesized with **4b** (1.80 g, 2.88 mmol), TFA/DCM (1:1, 20 mL), TIPS (1.95 mL; 9.50 mmol), and DCM/diethyl ether (1:20, 105 mL). Yield: 1.05 g, 95.2% of a colorless amorphous powder. TLC: R_f (I) = 0.34. ES-MS: m/z = 406.1 [M + Na]$^+$, 422.0 [M + K]$^+$. GC−MS: 93.4% ee.

Introduction of the *tert*-Butyl Carboxy Protecting Group (7; General Procedure). A mixture of 3 equiv of DIC, 4 equiv of *tert*-butyl alcohol, and 0.02 equiv of CuCl was stirred under argon for 5 d. After dilution with DCM (7.0 mL/g), the mixture was added dropwise to the *N*-Fmoc amino acid in DCM/DMF (14:1, 7.5 mL/g). After 4 h of stirring at room temperature, the mixture was filtered and the filtrate was washed three times each with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. Purification was by flash column chromatography (silica gel, hexane/ethyl acetate 3:1→1:1).

(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)serine-*tert*-butyl ester (7a) was synthesized with DIC (14.19 mL, 91.66 mmol), *tert*-butyl alcohol (11.61 mL, 122.21 mmol), CuCl (60.00 mg, 0.61 mmol), DCM (70 mL), (*S*)-*N*-(fluoren-9-ylmethoxycarbonyl)serine (10.00 g, 30.55 mmol), and DCM/DMF (14:1, 75 mL). Yield: 7.62 g, 65.0% of a colorless product. TLC: R_f (II) = 0.15. ES-MS: m/z = 406.2 [M + Na]$^+$, 422.1 [M + K]$^+$. GC−MS: 99.7% ee.

(*R*)-*N*-(Fluoren-9-ylmethoxycarbonyl)serine-*tert*-butyl ester-(7b) was synthesized with DIC (2.84 mL, 18.33 mmol), *tert*-butyl alcohol (2.32 mL, 24.42 mmol), CuCl (20.00 mg, 0.20 mmol), DCM (14 mL), (*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)serine (2.00 g, 6.11 mmol), and DCM/DMF (14:1, 15 mL). Yield: 1.30 g, 55.5% of a colorless product. TLC: R_f (II) = 0.15. ES-MS: m/z = 350.0 [M − tBu + Na]$^+$ 406.1 [M + Na]$^+$, 422.0 [M + K]$^+$. GC−MS: 98.7% ee.

Conversion of the Hydroxy Group to the Bromide (8; General Procedure). The serine derivative **7a** or **7b** and CBr_4 (1.22 equiv) were dissolved under argon in dry DCM (10 mL/g) and cooled to 0 °C. To the stirred solution was added PPh_3 (1.6 equiv) in several portions within 1 h. The solution was allowed to warm to room temperature and stirred for an additional 2 h. The solvent was removed in vacuo, and the residue was dissolved in a small amount of DCM and poured in ethyl acetate. The precipitate was removed by filtration and the filtrate cooled to −20 °C and filtered again. The solvent was removed and the residual product purified by flash column chromatography (silica gel; hexane/ethyl acetate 7:1).

(*R*)-β-Bromo-*N*-(Fluoren-9-ylmethoxycarbonyl)alanine-*tert*-butyl ester (8a) was synthesized with **7a** (7.00 g, 18.26 mmol), CBr_4 (7.39 g, 22.27 mmol), DCM (70 mL), PPh_3 (7.66 g, 29.21 mmol), and ethyl acetate (100 mL).Yield: 6.91 g, 84.8% of a colorless compound. TLC: R_f (III) = 0.15. ES-MS: m/z = 412.2, 414.2 [M − tBu + Na]$^+$, 468.0, 470.0 [M + Na]$^+$.

(*S*)-β-Bromo-*N*-(Fluoren-9-ylmethoxycarbonyl)alanine-*tert*-butyl ester (8b) was synthesized with **7b** (500 mg, 1.30 mmol), CBr_4 (527 mg, 1.59 mmol), DCM (5 mL), PPh_3 (547 mg, 2.09 mmol), and ethyl acetate (20 mL). Yield: 451.0 mg, 77.49% of a colorless compound. TLC: R_f (III) = 0.15. ES-MS: m/z = 412.2, 414.2 [M − tBu + Na]$^+$, 468.0, 470.0 [M + Na]$^+$.

General Procedure for the Synthesis of the Lanthionines 9a−d. The appropriate cysteine derivative was dissolved in 5% DIEA in DMF under argon. A solution of the corresponding bromide (1.1 equiv) in DMF was added dropwise. After stirring for 4 h at room temperature the mixture was diluted with a 5-fold excess of diethyl ether and washed five times with 10% citric acid and three times with water. The combined water phases were washed three times with diethyl ether and the combined organic phases again three times with 10% citric acid and brine. After removal of the solvent, the residual product was purified by flash column chromatography (hexane/ethyl acetate 4.5:1→2:1), yielding **9a−d** as colorless compounds.

(2*R*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-ε-allyl-α-*tert*-butyl diester (9a) was synthesized with **5a** (5.00 g, 13.04 mmol), **8a** (6.40 g, 14.34 mmol), and 5% DIEA/DMF (60 mL). Yield: 6.30 g, 64.5%. TLC: R_f (I) = 0.14. ES-MS: m/z = 771.2 [M + Na]$^+$, 787.1 [M + K]$^+$. FT-ICR-MS: m/z = 771.2710250 [M + Na]$^+$ ($C_{43}H_{44}N_2O_8SNa$ = 771.2710582) Δ = 0.04 ppm. GC−MS: 97.3% *R*,*R*; 2.7% *R*,*S*/*S*,*R*; 0.0% *S*,*S*.

(2*R*,6*S*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-ε-allyl-α-*tert*-butyl diester (9b) was synthesized with **5b** (350 mg, 0.91 mmol), **8a** (448 mg, 1.00 mmol), and 5% DIEA/DMF (8 mL). Yield: 420 mg, 61.5%. TLC: R_f (I) = 0.14. ES-MS: m/z = 771.2 [M + Na]$^+$, 787.1 [M + K]$^+$. FT-ICR-MS: m/z = 771.2707890 [M + Na]$^+$ ($C_{43}H_{44}N_2O_8SNa$ = 771.2710582) Δ = 0.3 ppm. GC−MS: 1.3% *R*,*R*; 97.0% *R*,*S*/*S*,*R*; 1.7% *S*,*S*.

(2*S*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-ε-allyl-α-*tert*-butyl diester (9c) was synthesized with **5a** (350 mg, 0.91 mmol), **8b** (448 mg, 1.00 mmol), and 5% DIEA/DMF (8 mL). Yield: 394 mg, 57.6%. TLC: R_f (I) = 0.14. ES-MS: m/z = 771.2 $[M + Na]^+$, 787.1 $[M + K]^+$. FT-ICR-MS: m/z = 771.27107380 $[M + Na]^+$ ($C_{43}H_{44}N_2O_8SNa$ = 771.2710582) Δ = 0.4 ppm. GC−MS: 2.6% *R*,*R*; 96.7% *R*,*S*/*S*,*R*; 0.7% *S*,*S*.

(2*S*,6*S*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-ε-allyl-α-*tert*-butyl diester (9d) was synthesized with **5b** (350 mg, 0.91 mmol), **8b** (448 mg, 1.00 mmol), and 5% DIEA/DMF (8 mL). Yield: 383 mg, 56.0%. TLC: R_f (I) = 0.14. ES-MS: m/z = 771.3 $[M + Na]^+$, 787.2 $[M + K]^+$. FT-ICR-MS: m/z = 771.2704620 $[M + Na]^+$ ($C_{43}H_{44}N_2O_8SNa$ = 771.2710582) Δ = 0.8 ppm. GC−MS: 0.12% R,R; 4.05% R,S/S,R; 95.83% S,S.

***tert*-Butyl Deprotection of Lanthionines 9a−d (10a−d; General Procedure).** The corresponding fully protected lanthionine was dissolved in neat TFA and stirred for 1 h at room temperature. The TFA was removed in vacuo and the residue lyophilized from acetonitrile/H_2O 4:1 to yield the pure lanthionines in quantitative yield.

(2*R*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-α-allyl ester (10a) was synthesized with **9a** (3.00 g, 4.01 mmol) and TFA (15 mL). Yield: 2.78 g of a colorless amorphous powder. ES-MS: m/z = 715.1 $[M + Na]^+$.

(2*R*,6*S*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-α-allyl ester (10b) was synthesized with **9b** (420 mg, 0.56 mmol) and TFA (5 mL). Yield: 389 mg of a colorless amorphous powder. ES-MS: m/z = 715.1 $[M + Na]^+$.

(2*S*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-α-allyl ester (10c) was synthesized with **9c** (394 mg, 0.53 mmol) and TFA (5 mL). Yield: 365 mg of a colorless amorphous powder. ES-MS: m/z = 715.1 $[M + Na]^+$.

(2*S*,6*S*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-α-allyl ester (10d) was synthesized with **9d** (383 mg, 0.51 mmol) and TFA (5 mL). Yield: 354 mg of a colorless amorphous powder. ES-MS: m/z = 715.1 $[M + Na]^+$.

(2*R*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-α-*tert*-butyl Ester (11). 9a (3.00 g, 4.01 mmol) was dissolved in DCM (50 mL) under argon. Morpholine (454 μL, 5.21 mmol) and $Pd(PPh_3)_4$ (100 mg, 0.09 mmol) were added, and the mixture was protected from light and stirred at room temperature. After 1 h the reaction was complete (TLC) and the solvent removed in vacuo. **11** was purified by flash column chromatography (silica gel; hexane/ethyl acetate/acetic acid 8:4:1) to give a colorless amorphous powder (2.58 g, 90.8%). TLC: R_f (IV) = 0.11. ES-MS: m/z = 731.4 $[M + Na]^+$, 1439.5 $[2M + Na]^+$, 707.1 $[M - H]^-$, 1415.2 $[2M - H]^-$.

(2*R*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-ε-hexadecylamide-α-*tert*-butyl Ester (12). 11 (2.60 g, 3.67 mmol), HOBt (562 mg, 3.67 mmol), and DIC (568 μL, 3.67 mmol) were dissolved in DCM/DMF (5:2, 20 mL) and stirred for 1 h at room temperature. A solution of hexadecylamine (974 mg, 4.03 mmol) in DCM/DMF (5:2, 20 mL) was added dropwise and stirring was continued for 6 h at room temperature. The solvent was removed in vacuo and the residual product purified by flash column chromatography (silica gel; hexane/ethyl acetate 2:1) to yield **12** as a colorless compound (2.35 g, 68.72%). TLC: R_f (II) = 0.23. ES-MS: m/z = 467.2 $[(M + 2H)/2]^+$, 954.6 $[M + Na]^+$.

(2*R*,6*R*)-N^2,N^6-Bis(fluoren-9-ylmethoxycarbonyl)lanthionine-ε-hexadecylamide (13). 12 (1.15 g, 1.23 mmol) was dissolved in neat TFA (11.5 mL) and stirred at room temperature for 1 h. The TFA was removed in vacuo and the product lyophilized from *tert*-butyl alcohol/H_2O. **13** was obtained as an amorphous colorless powder in quantitative yield (1.08 g, 100.0%). TLC: R_f (II) = 0.00. ES-MS: m/z = 898.2 $[M + Na]^+$.

(2*R*,6*R*)-N^2,N^6-Bis(palmitoyl)lanthionine-ε-hexadecylamide-α-*tert*-butyl Ester (14). A solution of palmitic acid (1.11 g, 4.34 mmol), HOBt (664 mg, 4.3 mmol), and DIC (672 μL, 4.3 mmol) in DCM/DMF (5:2, 70 mL) was stirred for 2 h at room temperature. Meanwhile, **12** (1.15 g, 1.23 mmol) was stirred for 2 h at room temperature in piperidine/DMF (1:1, 30 mL), and the solvents were removed in vacuo. The residual mixture containing **12** was dissolved in DCM/DMF (5:2, 49 mL) and added dropwise to the solution of activated Pam-OH. After stirring for 16 h at room temperature and removal of the solvent in vacuo, the residual product was dissolved in $CHCl_3$ and washed three times with 10% Na_2CO_3 and brine. The solution was dried over $MgSO_4$ and the solvent removed in vacuo. **14** was precipitated from $CHCl_3$/methanol (1:6) at −20 °C as a colorless crystalline compound (752 mg, 63.2%). TLC: R_f (V) = 0.83, R_f (VI) = 0.72. ES-MS: m/z = 986.7 $[M + Na]^+$, 998.7 $[M + Cl]^-$.

(2*R*,6*R*)-N^2,N^6-Bis(palmitoyl)lanthionine-ε-hexadecylamide (15). 14 (752 mg, 0.78 mmol) was deprotected in neat TFA (10 mL) for 1 h at room temperature. The TFA was removed in vacuo and the residual product **15** was lyophilized from *tert*-butyl alcohol/H_2O (4:1), giving **15** as a colorless amorphous powder (708 mg, 100%). TLC: R_f (VI) = 0.00. ES-MS: m/z = 906.8 $[M - H]^-$, 930.7 $[M + Na]^+$. GC−MS: 90.68% *R*,*R*; 8.23% *R*,*S*/*S*,*R*; 0.89% *S*,*S*. $C_{54}H_{105}N_3O_5S$ (MW = 908.52): calcd C 71.39, H 11.65, N 4.63, S 3.53; found C 71.29, H 11.40, N 4.55, S 3.50.

Peptide Synthesis (General Procedure). The Fmoc-amino acids were coupled by activation of 3 equiv of Fmoc-amino acid with 3 equiv of HOBt and 3 equiv of DIC in DCM/DMF (8:1) for 1 h. After adding the solution to the dry, Fmoc-deprotected Rink-amide-resin or the corresponding Fmoc-deprotected resin-bound amino acids the mixture was agitated for 8 h at room temperature. The resin was filtered off, washed three times each with DMF/DCM/methanol, and dried in vacuo. If the Kaiser test showed incomplete coupling, the above protocol was repeated. Fmoc was cleaved by agitating the resin in a surplus of 20% piperidine in DMF for 20 min. The resin was filtered off and the procedure repeated. The solvent was removed by filtration, and the resin was washed three times each with DMF/DCM/methanol and dried in vacuo. For cleavage of the final products, the resin-bound peptides were treated with TFA/H_2O (95:5) for 3 h at room temperature. The resin was removed by filtration and the TFA removed in vacuo. The residue was lyophilized from *tert*-butyl alcohol/H_2O (4:1). After each coupling step peptide samples were cleaved from the resin and analyzed by HPLC and ES-MS. All products showed at least 95% purity and the expected $[M + H]^+$ signals.

Immobilization of (*S*)-N^2-(fluoren-9-ylmethoxycarbonyl)-N^6-(*tert*-butoxycarbonyl)lysine (*N*-Fmoc-L-Lys(Boc)-OH) to Rink-amide resin used Rink-amide resin (3.00 g, 2.22 mmol), *N*-Fmoc-L-Lys(Boc)-OH (3120 mg, 6.66 mmol), HOBt (1020 mg, 6.66 mmol), and DIC (1031 μL, 6.66 mmol).

Coupling of *N*-Fmoc-L-Lys(Boc)-OH used *N*-Fmoc-L-Lys(Boc)-OH (3120 mg, 6.66 mmol), HOBt (1020 mg, 6.66 mmol), and DIC (1031 μL, 6.66 mmol).

Coupling of *N*-Fmoc-L-Ser(tBu)-OH (16) used *N*-Fmoc-L-Ser-(tBu)-OH (2553 mg, 6.66 mmol), HOBt (1020 mg, 6.66 mmol), and DIC (1031 μL, 6.66 mmol).

Coupling of 13 to 16 used resin **16** (100.00 mg, 41.60 μmol), **13** (109.35 mg, 124.80 μmol), HOBt (19.11 mg, 124.80 μmol), and DIC (19.32 μL, 124.80 μmol). The coupling was repeated until a negative Kaiser test was observed. HPLC: 98.0%. MALDI-MS: m/z = 1474.2 $[M + H]^+$.

Coupling of the Lanthionines 10a−d to Resin 16. All four stereoisomers of lanthionine (**10a−d**) were coupled to the Fmoc-deprotected resin **16** using the DIC/HOBt protocol. Subsequently, the reaction mixture was filtered off and 1 equiv each of the appropriate lanthionine, HOBt, and DIC was added to the mixture and the above protocol was repeated.

Coupling of 10a to 16 (19) used resin **16** (500.00 mg, 208.00 μmol), **10a** (432.30 mg, 624.00 μmol), HOBt (95.55 mg, 624 μmol), and DIC (96.62 μL, 624 μmol) and **10a** (144.10 mg, 208.00 μmol), HOBt (31.9 mg, 208 μmol), and DIC (32.2 μL, 208 μmol). HPLC: 96.9%. ES-MS: m/z = 1313.3 $[M + Na]^+$, MALDI-MS: m/z = 1291.8 $[M + H]^+$.

Coupling of 10b to 16 (40) used resin **16** (40.00 mg, 16.64 μmol), **10b** (34.59 mg, 49.92 μmol), HOBt (7.64 mg, 49.92 μmol), and DIC (7.73 μL, 49.92 μmol) and **10b** (11.53 mg, 16.64 μmol),

Table 2. Summary of the Fatty Amine Couplings to the Resin Bound, Allyl Deprotected Lanthionines [P/D/T = pyridine/DMF/TFA-OPfp (1:1:1)]

resin used	amount of resin (mg, μmol)	P/D/T (μL)	fatty amine	amount of amine	DIEA (μL)	HPLC (%)	resulting resin
20	20, 7.12	300	hexylamine	9.42 μL, 71.20 μmol	36.57	98.4	**21**
20	20, 7.12	300	octylamine	14.14 μL, 71.20 μmol	36.57	96.5	**22**
20	20, 7.12	300	decylamine	14.14 μL, 71.20 μmol	36.57	96.0	**23**
20	20, 7.12	300	dodecylamine	13.20 mg, 71.20 μmol	36.57	95.7	**24**
20	20, 7.12	300	tetradecylamine	15.19 mg, 71.20 μmol	36.57	92.4	**25**
20	300, 106.80	1200	hexadecylamine	257.88 mg, 1.07 mmol	548.50	93.9	**26**
43	20, 7.12	300	hexadecylamine	17.19 mg, 71.20 μmol	36.57	88.7	**46**
44	20, 7.12	300	hexadecylamine	17.19 mg, 71.20 μmol	36.57	90.0	**47**
45	20, 7.12	300	hexadecylamine	17.19 mg, 71.20 μmol	36.57	92.5	**48**
20	30, 10.68	300	octadecylamine	28.78 mg, 106.80 μmol	54.85	97.9	**27**

Table 3. Summary of the Fatty Acid Couplings to Different Fmoc-Deprotected, Resin-Bound Lanthionine-ε-hexadecylamide Peptides

resin used	amount resin (mg, μmol)	fatty acid	amount fatty acid	DIC (μL)	HOBt (mg)	MALDI-MS $[M + H]^+$	resulting compd
26	10.82, 3.56	caproic	2.68 μL, 21.36 μmol	3.31	3.27	1225.9	**34**
26	10.82, 3.56	caprylic	3.39 μL, 21.36 μmol	3.31	3.27	1282.0	**35**
26	10.82, 3.56	capric	3.68 mg, 21.36 μmol	3.31	3.27	1338.4	**36**
26	10.82, 3.56	lauric	4.28 mg, 21.36 μmol	3.31	3.27	1394.0	**37**
26	10.82, 3.56	myristic	4.88 mg, 21.36 μmol	3.31	3.27	a	**17**
46	10.82, 3.56	myristic	4.88 mg, 21.36 μmol	3.31	3.27	1450.4	**49**
47	10.82, 3.56	myristic	4.88 mg, 21.36 μmol	3.31	3.27	1450.5	**50**
48	10.82, 3.56	myristic	4.88 mg, 21.36 μmol	3.31	3.27	1450.7	**51**
26	10.82, 3.56	palmitic	5.48 mg, 21.36 μmol	3.31	3.27	1506.2	**18**
26	10.82, 3.56	stearic	6.08 mg, 21.36 μmol	3.31	3.27	1561.9	**38**
26	10.82, 3.56	arachidic	6.68 mg, 21.36 μmol	3.31	3.27	1617.9	**39**

[a] MALDI- and FT-ICR-MS data are given in the Experimental Section.

HOBt (2.54 mg, 16.64 μmol), and DIC (2.57 mg, 16.64 μmol). HPLC: 91.6%. ES-MS: $m/z = 1313.2$ $[M + Na]^+$.

Coupling of 10c to 16 (41) used resin **16** (40.00 mg, 16.64 μmol), **10c** (34.59 mg, 49.92 μmol), HOBt (7.64 mg, 49.92 μmol), and DIC (7.73 μL, 49.92 μmol) and **10c** (11.53 mg, 16.64 μmol), HOBt (2.54 mg, 16.64 μmol), and DIC (2.57 mg, 16.64 μmol). HPLC: 93.9%. ES-MS: $m/z = 1291.3$ $[M + H]^+$, 1313.2 $[M + Na]^+$.

Coupling of 10d to 16 (42) used resin **16** (40.00 mg, 16.64 μmol), **10d** (34.59 mg, 49.92 μmol), HOBt (7.64 mg, 49.92 μmol), and DIC (7.73 μL, 49.92 μmol) and **10d** (11.53 mg, 16.64 μmol), HOBt (2.54 mg, 16.64 μmol), and DIC (2.57 mg, 16.64 μmol). HPLC: 93.0%. ES-MS: $m/z = 1291.3$ $[M + H]^+$, 1313.2 $[M + Na]^+$.

General Procedure for the Allyl Deprotection. A solution of 24 equiv of phenylsilane in DCM and a solution of 0.25 equiv of $Pd(PPh_3)_4$ in DCM was prepared under argon. The phenylsilane solution was added to the dry resin and the mixture agitated for 5 min under argon. After addition of the $Pd(PPh_3)_4$ solution the mixture was protected from light and agitated for 2 h under argon at room temperature. The resin was washed three times with a 0.5% sodium diethyl dithiocarbamate trihydrate solution in DMF for 15 min and afterward thoroughly with DMF/DCM/methanol. The resin was then dried in vacuo.

The resin-bound lanthionine derivatives **19** (590.00 mg, 205.91 μmol →**20**), **40** (30.00 mg, 10.47 μmol →**43**), **41** (30.00 mg, 10.47 μmol →**44**), **42** (30.00 mg, 10.47 μmol →**45**), **52** (7.00 mg, 2.47 μmol →**56**), **53** (7.00 mg, 2.47 μmol →**57**), **54** (7.00 mg, 2.47 μmol →**58**), and **55** (7.00 mg, 2.47 μmol →**59**) were deprotected following this procedure in good purities (HPLC, >89%). In the MALDI- or ES-MS spectra the $[M + H]^+$, $[M + Na]^+$, and/or $[M - H + 2Na]^+$ peaks were observed.

General Procedure for the Amine Coupling to Resin-Bound, Allyl-Deprotected Lanthionine. The appropriate resin (**20** and **43**−**45**) was agitated in a mixture of pyridine/DMF/pentafluorophenyl trifluoroacetate (TFA-OPfp) (3:2:1) for 2 h at room temperature. The solvents were removed by filtration and the resin washed twice each with DCM/diethyl ether. A mixture of 10 equiv of amine and 30 equiv of DIEA in THF was added and the mixture agitated for 6 h at room temperature. The solvents were removed by filtration, and the resin was washed thrice each with DMF/DCM/diethyl ether. For analytical purposes 1 mg of the resin was treated with 10 equiv of fluoren-9-ylmethyl chloroformate in pyridine/DMF (1:4) prior to cleavage. Table 2 gives a summary of the amounts of reagents and the HPLC purities. The MALDI-MS spectra of the products showed the expected $[M + H]^+$ and $[M + Na]^+$ peaks.

General Procedure for the Fmoc-Protection of the Resin Bound Lanthionine with Fluoren-9-ylmethyl Chloroformate. The resin was agitated with a mixture of 10 equiv of fluoren-9-ylmethyl chloroformate (Fmoc-Cl) in pyridine/DMF (1:4). The solvents were removed by filtration, and the resin was washed three times each with DMF/DCM/methanol and dried in vacuo. The resins **21**−**25, 46**−**48** (36.83 mg, 71.20 μmol each), **26** (552.5 mg, 1.07 mmol), and **27** (55.26 mg, 106.80 μmol) were Fmoc protected using this protocol and the appropriate amounts of the reagents.

General Procedure for the Coupling of the Fatty Acids. A mixture of 3 equiv of the fatty acid, 3 equiv of HOBt, and 3 equiv of DIC was stirred in DCM/DMF (8:1) for 1 h. The mixture was then added to the freshly Fmoc-deprotected resin. After 8 h of stirring at room temperature, the solvents were removed by filtration, and the resin was washed three times each with DMF/DCM/methanol and dried in vacuo. Table 3 summarizes the reaction procedures for the coupling of different fatty acids to the resin-bound and Fmoc-deprotected lanthionine N^7-hexadecylamides, and Table 4 summarizes the couplings of myristic acid to the different resin-bound lanthionine amides.

Analytical Data for Coupling of Myristic Acid to Fmoc-Deprotected Resin 26 (17). MALDI-MS: $m/z = 1450.6$ $[M + H]^+$. FT-ICR-MS: $m/z = 726.07824$ $[(M + 2H)/2]^+$ ($[C_{77}H_{151}N_{13}O_{10}S+H]^+ = 1451.15004$; $[(C_{77}H_{151}N_{13}O_{10}S+2H)/2]^+ = 726.07844$) $\Delta = 0.28$ ppm.

Oxidation of the Lanthionine Sulfur to Its Sulfoxide (61). A solution of 0.2 equiv of $Sc(OTf)_3$ (0.32 mg, 0.65 μmol) in DCM/ethanol (9:1, 500 μL) was added to the dry resin **60** (10.00 mg, 3.26 μmol), 5 equiv of H_2O_2 (30%, 1.68 μL, 16.30 μmol) was added, and the mixture was agitated for 40 min at room temperature. The solvents were removed by filtration, the resin was washed three

Table 4. Summary of the Myristic Acid Couplings to Different Resin Bound Lanthionine Peptide Amides[a]

resin used	amount resin (mg, μmol)	MALDI-MS $[M + H]^+$	resulting compd
21	10.32, 3.56	1311.1	**28**
22	10.44, 3.56	1339.0	**29**
23	10.53, 3.56	1366.6	**30**
24	10.63, 3.56	1395.0	**31**
25	10.72, 3.56	1423.1	**32**
26	10.82, 3.56	1450.6	**17**
27	10.92, 3.56	1479.4	**33**
19	10.00, 3.49	1267.3	**52**
40	10.00, 3.49	1267.5	**53**
41	10.00, 3.49	1267.1	**54**
42	10.00, 3.49	1267.2	**55**

[a] Myristic acid (4.88 mg, 21.36 μmol), DIC (3.31 μL, 21.36 μmol), HOBt (3.27 mg, 21.36 μmol).

times each with DMF/DCM/methanol and dried in vacuo. MALDI-MS: $m/z = 1522.6$ $[M + H]^+$.

Oxidation of the Lanthionine Sulfur to Its Sulfone (62). A solution of 5 equiv of mCPBA (2.83 mg, 16.30 μmol) in DCM (500 μL) was added to resin **60** (10.00 mg, 3.26 μmol) and the mixture was agitated for 24 h at room temperature. After removal of the solvents, the resin was washed three times each with DMF/DCM/methanol and dried in vacuo. MALDI-MS: $m/z = 1538.5$ $[M + H]^+$.

Cell Culture. The human myelomonocytic cell line THP-1 (ATCC number TIB-202) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (Braunschweig, Germany). The cells were cultured in RPMI 1640 medium (PAN Biotech, Aidenbach, Germany) supplemented with 10% FCS (PAN Biotech) in a 5% CO_2 humidified atmosphere at 37 °C. Cells were passaged every third or fourth day.

IL-8 Assay. THP-1 cells were dispensed into 96-well culture plates at a cell density of 4×10^4 cells per 200 μL of medium per well. Stock solutions of lipolanthionine peptides and Pam_3Cys-SK_4-OH (EMC microcollections, Tübingen, Germany) in DMSO were prepared at a concentration of 10 mM. Cells were incubated with lipolanthionine peptides at the indicated concentrations for 10 min at 37 °C. Controls with lipolanthionine-free medium containing equal amounts of DMSO were included in each assay. The agonist Pam_3Cys-SK_4-OH was added to the samples at a concentration of 50 nM. After a further incubation for 5 h at 37 °C cell-free supernatants were collected and stored at −80 °C prior to the quantification of IL-8 secretion by matched-pair ELISA (clone G265-5 and clone G265-8, BDPharMingen, San Diego, CA) according to the protocol provided by the manufacturer.

Cell Viability Assay. Cell viability was measured using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay. THP-1 cells were treated with the corresponding lipolanthionine peptides and Pam_3Cys-SK_4-OH for 5 h (see above), and after removing 120 μL of the supernatant for ELISA analysis, cells were incubated with MTT (Sigma) at a concentration of 1 mg/mL for 4 h. The formazan product was solubilized with SDS (10% (m/v) in 10 mM HCl). The fraction of viable cells was determined by measuring the absorbance of each sample at 570 nm relative to the absorbance of untreated control cells using the microplate reader.

Acknowledgment. We thank Graeme Nicholson for chiral GC−MS analysis, and Paul Schuler for NMR (600 MHz) measurements. Tobias Seyberth received a stipend from the DFG Graduiertenkolleg "Analytical Chemistry". This work was supported by the Federal Ministry of Education and Research, project BioChance no. 0312662.

Supporting Information Available: 1D- and 2D-NMR spectra of the synthesized (2*R*,6*R*)-lanthionine derivatives **9a** and Pam_2-LanHda **15**; exemplary chiral GC−MS chromatograms of the derivatized enantiomers (2*R*,6*R*)-lanthionine **9a** and (2*S*,6*S*)-lanthionine **9d** and of a mixture of D-, L-, and *meso*-lanthionine; fragmentation scheme of the lanthionine derivatives used for chiral GC−MS analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Braun, V. Covalent lipoprotein from the outer membrane of *Escherichia coli*. *Biochim. Biophys. Acta* **1975**, *415*, 335−377.
(2) Braun, V.; Wu, H. C. Lipoproteins, structure, function, biosynthesis and model for protein export. In *Comprehensive Biochemistry, Bacterial Cell Wall*. 27; Ghuysen, J. M., Hakenback, R., Eds.; Elsevier: Amsterdam, 1993; 319−342.
(3) Mühlradt, P. F.; Kiess, M.; Meyer, H.; Süssmuth, R.; Jung, G. Structure and specific activity of macrophage-stimulating lipopeptides from *Mycoplasma hyorhinis*. *Infect. Immunol.* **1998**, *66*, 4804−4810.
(4) Mühlradt, P. F.; Kiess, M.; Meyer, H.; Süssmuth, R.; Jung, G. Isolation, structure elucidation, and synthesis of a macrophage stimulatory lipopeptide from *Mycoplasma fermentans* acting at pico molar concentration. *J. Exp. Med.* **1997**, *185*, 1951−1958.
(5) Metzger, J.; Wiesmüller, K.-H.; Schaude, R.; Bessler, W.; Jung, G. Synthesis of novel immunologically active tripalmitoyl-S-glyceryl-cysteinyl lipopeptides as useful intermediates for immunogen preparations. *Int. J. Pept. Protein Res.* **1991**, *37*, 46−57.
(6) Wiesmüller, K.-H.; Jung, G.; Hess, G. Novel low-molecular weight synthetic vaccine against foot-and-mouth disease containing a potent B-cell and macrophage activator. *Vaccine* **1989**, *7*, 29−33.
(7) Wiesmüller, K.-H.; Bessler, W. G.; Jung, G. Solid-phase peptide synthesis of lipopeptide vaccines eliciting epitope-specific B-, T-helper and T-killer cell response. *Int. J. Pept. Protein Res.* **1992**, *40*, 255−260.
(8) Deres, K.; Schild, H. J.; Wiesmüller, K.-H.; Jung, G.; Rammensee, H.-G. In vivo priming of virus specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. *Nature* **1989**, *342*, 561−564.
(9) Schild, H.; Deres, K.; Wiesmüller, K.-H.; Jung, G.; Rammensee, H.-G. Efficiency of peptides and lipopeptides for in vivo priming of virus-specific cytotoxic T cells. *Eur. J. Immunol.* **1991**, *21*, 2649−2654.
(10) Wiesmüller, K.-H.; Fleckenstein, B.; Jung, G. Peptide vaccines and peptide libraries. *Biol. Chem.* **2001**, *382*, 571−579.
(11) Janeway, C. A., Jr.; Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **2002**, 197−216.
(12) Akira, S.; Takeda, K. Toll-like receptor signaling. *Nature Rev. Immun.* **2004**, *4*, 499−511.
(13) Schnare, M.; Barton, G. M.; Holt, A. C.; Takeda, K.; Akira, S.; Medzhitov, R. Toll-like receptors control activation of adaptive immune responses. *Nature Immunol.* **2001**, *2*, 947−950.
(14) Tsunoda, I.; Sette, A.; Fujinami, R. S.; Oseroff, C.; Ruppert, J.; Dahlberg, C.; Southwood, S.; Arrhenius, T.; Kuang, L. Q.; Kubo, R. T.; Chesnut, R. W.; Ishioka, G. Y. Lipopeptide particles as the immunologically active component of CTL inducing vaccines. *Vaccine* **1999**, *17*, 675−685.
(15) Sieling, P. A.; Chung, W.; Duong, B. T.; Godowski, P. J.; Modlin, R. L. Toll-like receptor 2 ligands as adjuvants for human Th1 responses. *J. Immunol.* **2003**, *170*, 194−200.
(16) Spohn, R.; Buwitt-Beckmann, U.; Brock, R.; Jung, G.; Ulmer, A. J.; Wiesmüller, K.-H. Synthetic lipopeptide adjuvants and Toll-like receptor 2−structure−activity relationships. *Vaccine* **2004**, *22*, 2494−2499.
(17) Müller, S. D. C.; Müller, M. R.; Huber, M.; van der Esche, U.; Kirschning, C. J.; Wagner, H.; Bessler, W. G.; Mittenbühler, K. Triacyl-lipopentapeptide adjuvants: TLR2-dependent activation of macrophages and modulation of receptor-mediated cell activation by altering acyl-moieties. *Int. Immunopharm.* **2004**, *4*, 1287−1300.
(18) Metzger, J.; Jung, G.; Bessler, W. G.; Hoffmann, P.; Strecker, M.; Lieberknecht, A.; Schmidt, U. Lipopeptides containing 2-palmitoyl-amino-6,7-bis(palmitoyloxy)-heptanoic acid: Synthesis, stereospecific stimulation of B-lymphocytes and macrophages, adjuvanticity in vivo and in vitro. *J. Med. Chem.* **1991**, *34*, 1969−1974.
(19) Schmidt, U.; Lieberknecht, A.; Kazmaier, U.; Griesser, H.; Jung, G.; Metzger, J. Amino Acids and Peptides; 73. Synthesis of di- and trihydroxy-amino acids−Construction of lipophilic tripalmitoyldihydroxy-α-amino acids. *Synthesis* **2001**, 49−56.
(20) Zuany-Amorim, C.; Hastewell, J.; Walker, C. Toll-like receptory as potential therapeutic targets for multiple diseases. *Nature Rev. Drug. Discuss.* **2002**, *1*, 797−807.
(21) Meng, G.; Rutz, M.; Schiemann, M.; Metzger, J.; Grabiec, A.; Schwandner, R.; Luppa, P. B.; Ebel, F.; Busch, D. H.; Bauer, S.; Wagner, H.; Kirschning, C. J. Antagonistic antibody prevents toll-like receptor 2-driven lethal shock-like syndromes. *J. Clin. Invest.* **2004**, *113*, 1473−1481.

(22) Jung, G. Lantibiotics—ribosomally synthesized biologically active polypeptides containing sulfide bridges and α,*β*-didehydroamino acids. *Angew. Chem., Int. Ed.* **1991**, *30*, 1051−1068.
(23) Jung, G. Lantibiotics: A survey. In *Nisin and Novel Lantibiotics;* Jung, G., Sahl, H. G., Eds.; ESCOM: Leiden, 1991; pp 1−34.
(24) Süssmuth, R. D.; Jack, R. W.; Jung, G. Lanthionine Peptides. In *Synthesis of Peptides and Peptidomimetics (Houben-Weyl)*; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Thieme: Stuttgart, 2003; Vol. E 22b, pp 184−213.
(25) Brown, G.; du Vigneaud, V. The stereoisomeric forms of lanthionine. *J. Biol. Chem.* **1941**, *140*, 767−771.
(26) Probert, J. M.; Rennex, D.; Bradley, M. Lanthionines for solid-phase synthesis. *Tetrahedron Lett.* **1996**, *37*, 1101−1104.
(27) Shao, H.; Wang, S. H. H.; Lee, C. W.; Ösapay, G.; Goodman, M. A facile synthesis of orthogonally protected stereoisomeric lanthionines by regioselective ring opening of serine *β*-lactones. *J. Org. Chem.* **1995**, *60*, 2956−2957.
(28) Ösapay, G.; Prokai, L.; Kim, H. S.; Medzihradszky, K. F.; Coy, D. H.; Liapakis, G.; Reisine, T.; Melacini, G.; Zhu, Q.; Wang, S. H. H.; Mattern, R. H.; Goodman, M. Lanthionine-somatostatin analogs: Synthesis, characterization, biological activity, and enzymatic stability studies. *J. Med. Chem.* **1997**, *40*, 2241−2251.
(29) Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. New diastereoselective synthesis of protected meso-lanthionine with discrimination of the chiral centers. *Tetrahedron Asymmetry* **1992**, *3*, 85−94.
(30) Dugave, C.; Menez, A. Synthesis of natural and non natural orthogonally protected lanthionines from *N*-tritylserine and allothreonine derivatives. *Tetrahedron Asymmetry* **1997**, *8*, 1453−1465.
(31) Jobron, L.; Hummel, G. Solid-phase synthesis of new *S*-glycoamino acid building blocks. *Org. Lett.* **2000**, *2*, 2265−2267.
(32) Gair, S.; Jackson, R. F. W.; Brown, P. A. Stereoselective synthesis of all stereoisomeric 2-amino-3-hydroxy-4-phenylbutanolides. *Tetrahedron Lett.* **1997**, *38*, 3059−3062.
(33) Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliott, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. Synthesis of orthogonally protected lanthionines. *J. Org. Chem.* **2003**, *68*, 8185−8192.
(34) Mustapa, M. F. M.; Harris, R.; Esposito, D.; Chubb, N. A. L.; Mould, J.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. Synthesis of a cyclic peptide containing norlanthionine: Effect of the thioether bridge on peptide conformation. *J. Org. Chem.* **2003**, *68*, 8193−8198.
(35) Zhu, X. M.; Schmidt, R. R. Efficient synthesis of differently protected lanthionines via beta-bromoalanine derivatives. *Eur. J. Org. Chem.* **2003**, 4069−4072.
(36) Wiesmüller, K.-H.; Bessler, W.; Jung, G. Synthesis of the mitogenic *S*-[2,3-bis(palmitoyloxy)propyl]-*N*-palmitoylpentapeptide from *Escherichia coli* lipoprotein. *Hoppe-Seyler's Z. Physiol. Chem.* **1983**, *364*, 593−606.
(37) Schultz, M.; Kunz, H. Synthetic O-glycopeptides as model substrates for glycosyltransferases. *Tetrahedron: Asymmetry* **1993**, *4*, 1205−1220.
(38) Miller, M. J. Isourea-mediated preparation of dehydro amino acids. *J. Org. Chem.* **1980**, *45*, 3131−3132.
(39) Kuesters, E.; Allgaier, H.; Jung, G.; Bayer, E. Resolution of sulfur-containing amino acids by chiral phase gas chromatography. *Chromatographia* **1984**, *18*, 287−293.
(40) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem.* **1970**, *34*, 595−598.
(41) Matteucci, M.; Bhalay, G.; Bradley, M. Cystine mimetics—Solid-phase lanthionine synthesis. *Tetrahedron Lett.* **2004**, *45*, 1399−1401.
(42) Mata, E. G. *β*-Lactams on solid support: Mild and efficient removal of penicillin derivatives from Merrifield resin using aluminium chloride. *Tetrahedron Lett.* **1997**, *38*, 6335−6338.
(43) Wiesmüller, K.-H.; Hess, G.; Bessler, W. G.; Jung, G. Diastereomers of tripalmitoyl-S-glyceryl-l-cysteinyl carrier adjuvant systems induce different mitogenic and protective immune response. In *Chirality and Biological Activity;* Homstedt, B., Frank, H., Testa, B., Eds.; Alan R. Liss: New York, 1990; pp 267−272.
(44) Reichel, F.; Roelofsen, A. M.; Geurts, H. P. M.; Hämäläinen, T. I.; Feiters, M. C.; Boons, G.-J. Stereochemical dependence of the self-assembly of the immunoadjuvants Pam3Cys and Pam3Cys-Ser. *J. Am. Chem. Soc.* **1999**, *121*, 7989−7997.
(45) Kurimura, M.; Takemoto, M.; Achiwa, K. Synthesis of optically active lipopeptide analogues from the outer membrane of *Escherichia coli*. *Chem. Pharm. Bull.* **1991**, *39*, 1590−2596.
(46) Rustici, A.; Velucchi, M.; Faggioni, R.; Sironi, M.; Ghezzi, P.; Quataert, S.; Green, B.; Porro, M. Molecular mapping and detoxification of the lipid A binding site by synthetic peptides. *Science* **1993**, *259*, 361−365.
(47) Triantafilou, M.; Miyake, K.; Golenbock, D. T.; Triantafilou, K. Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. *J. Cell Science* **2002**, *115*, 2603−2611.
(48) Pfeiffer, A.; Böttcher, A.; Orso, E.; Kapinsky, M.; Nagy, P.; Bodnar, A.; Spreitzer, I.; Liebisch, G.; Drobnik, W.; Gempel, K.; Horn, M.; Holmer, S.; Hartung, T.; Multhoff, G.; Schutz, G.; Schindler, H.; Ulmer, A. J.; Heine, H.; Stelter, F.; Schutt, C.; Rothe, G.; Szollosi, J.; Damjanovich, S.; Schmitz, G. Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts. *Eur. J. Immunol. 31*, 3153−3164.
(49) Walton, K. A.; Cole, A. L.; Yeh, M.; Subbanagounder, G.; Krutzik, S. R.; Modlin, R. L.; Lucas, R. M.; Nakai, J.; Smart, E. J.; Vora, D. K.; Berliner, J. A. Specific phospholipid oxidation products inhibit ligand activation of toll-like receptors 4 and 2. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 1197−1203.
(50) Stulnig, T. M.; Huber, J.; Leitinger, N.; Imre, E. M.; Angelisova, P.; Nowotny, P.; Waldhausl, W. Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. *J. Biol. Chem.* **2001**, *276*, 37335−37340.
(51) Zeyda, M.; Staffler, G.; Horejsi, V.; Waldhausl, W.; Stulnig, T. M. LAT displacement from lipid rafts as a molecular mechanism for the inhibition of T cell signaling by polyunsaturated fatty acids. *J. Biol. Chem.* **2002**, *277*, 28418−28423.
(52) Manukyan, M.; Triantafilou, K.; Triantafilou, M.; Mackie, A.; Nilsen, N.; Espevik, T.; Wiesmueller, K. H.; Ulmer, A. J.; Heine, H. Binding of lipopeptide to CD14 induces physical proximity of CD14, TLR2 and TLR1. *Eur. J. Immunol.* **2005**, *35*, 911−921.

JM050585D