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Versatile synthesis of pathogen specific bacterial cell wall building blocks†

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Full details on the design, strategies and tactics for development of a novel synthetic sequence to farnesyl lipid I and II analogs is reported. The modular route was based on a three coupling strategy involving an efficient solid phase synthesis of the elaborate peptide fragment, which proceeded with excellent yield and stereoselectivity and was efficiently applied for the convergent synthesis of 3-lipid I and II. Furthermore, the generality of this route was demonstrated by synthesis of 3-lipid I congeners that are characteristic for *S. aureus* and *E. faecalis*. All 3-lipid I and II building blocks were obtained in high purity revealing high spectroscopic resolution.

Introduction

Lipid I and biosynthetically derived lipid II represent the key precursors for bacterial cell wall biosynthesis (Fig. 1).[1,2] On a molecular level, they deliver their glycosylated-pentapeptide "building blocks" into the growing peptidoglycan network and play functional roles in the coordination of this process.[1] Furthermore, they regulate cell division and influence various other enzymatic processes.[3] In addition, they present pivotal molecular targets for a broad range of antibiotics and antibacterial agents, adding to their importance for molecular studies.[1,4] Consequently, functional studies with these key building blocks have attracted high interest from the perspective of antibacterial research and medicinal chemistry. However, such studies have been severely hindered by unfavourable physical properties of the original bactoprenol hydrocarbon side chain, leading to unwanted aggregation phenomena, precipitation as well as complex spectroscopic characteristics. Also, such analyses are further hampered by the low natural supply of these key functional compounds,[2,5] which also could not be resolved by synthetic approaches due to the multiple steps required to access their elaborate architectures.[5–7] Furthermore, existing synthetic strategies could not be adapted to pathogen specific modification, such as **5** or **6** (Fig. 1), where the authentic lysine moiety holds an additional peptidic sequence, despite their high structural relevance for cell wall crosslinks.

To resolve the disadvantageous physical properties, a new group of lipid I analogs has been introduced, where the unfavorable authentic undecaprenyl side chain has been shortened, leading to derivatives, such as farnesyl congeners **3** and **4**.[8] In recent years it has become more and more clear, that these truncated analogs represent functional surrogates for cell wall biosynthesis.[9] Consequently, the development of efficient synthetic routes towards these simplified versions is of high interest for functional studies. While several synthetic procedures were published,[10–12] these existing routes still leave ample room for further improvement, with respect to overall yield, synthetic efficiency, experimental documentation, robustness and purity of final compounds, as well as application to strain dependent peptide modifications.

Herein we report in full details our considerations, strategies and tactics for development of a novel synthetic sequence to truncated lipid I and II analogs.[13] The modular route is based on a novel solid phase approach to the pentapeptide chain, which proceeds with full stereochemical control, an improved synthesis of the pyrophosphate fragment and a chemoenzymatic attachment of the second carbohydrate (GlcNAc). Application of these routes allowed for a concise synthesis of farnesyl analogs **3** and **4**, which were obtained with unprecedented purity, revealing excellent spectroscopic resolution. Furthermore, this sequence could be adopted for synthesis of novel pentaglycine derivative **5** specific for *Staphylococcus aureus* (*S. aureus*), as well as new analog **6** that is characteristic for *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus pneumoniae* (*S. pneumoniae*).

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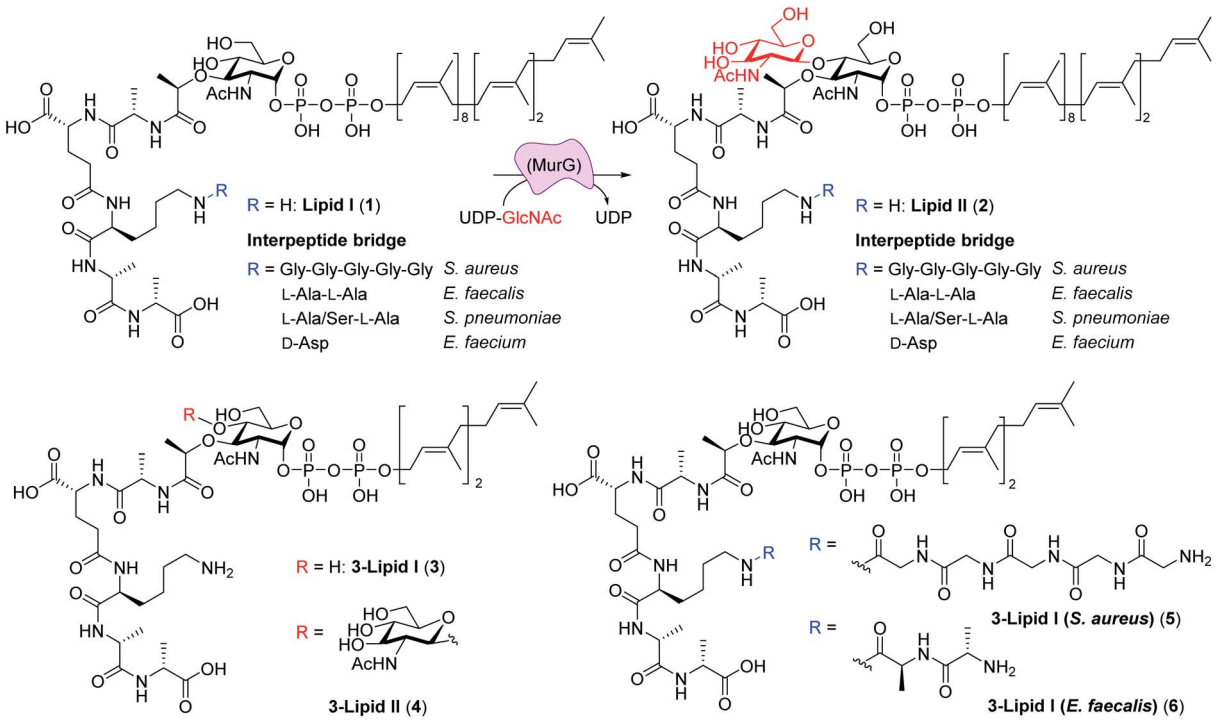


Fig. 1 Natural lipid I (**1**) and biosynthetically derived lipid II (**2**), key building blocks of bacterial cell wall biosynthesis: its farnesyl derivatives **3**, **4** as improved agents for functional studies and interpeptidic analogs **5** and **6**, bearing interpeptidic sequences characteristic for *S. aureus* as well as *E. faecalis* and *S. pneumoniae*.

Results and discussion

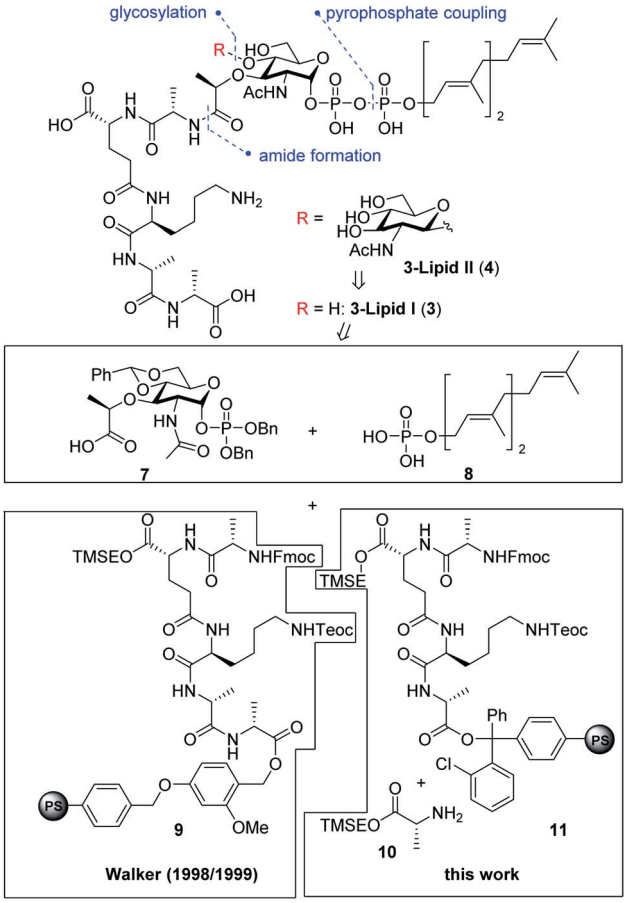
Synthetic strategy

As shown in Fig. 1, the architecture of the targeted lipid analogs is characterized by three structurally distinctively different subunits, *i.e.* a pentapeptide, consisting of L-Ala–γ-D-Glu–L-Lys–D-Ala–D-Ala sequence, in combination with a muramic acid carbohydrate that is linked to a farnesyl chain *via* a pyrophosphate bridge. Consequently, our synthetic approach was based on initial formation of these three components in suitably protected form, revealing monosaccharide **7**, farnesyl subunit **8** and peptide **11**. In a similar fashion to a reported route,[7] these should then be condensed by amide formation and pyrophosphate coupling. Final attachment of the second sugar (GlcNAc) may then be possible, bioenzymatically.[14] Notably, such a strategy would be highly modular and be easily adopted to pathogen specific analogs, such as **5** or **6**.

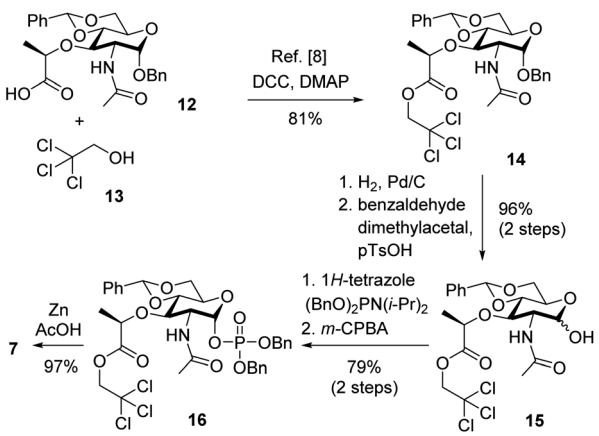
While the monosaccharide **7** (Scheme 1) should be accessible by optimizing a previously reported procedure,[15] synthesis of known pentapeptide **9** was reconsidered. In detail, it became apparent during this study that a more effective approach would involve a solid phase approach of only the tetrapeptide **11** and subsequent attachment of the final D-Ala building block **10** in solution phase, in contrast to a previous solid phase synthesis of full pentapeptide **9**. Since the pentapeptide should include silyl protecting groups allowing for a final global deprotection step, the pentapeptide was initially synthesized *via* the solid phase approach, but it was found that the silyl protection of the D-Ala residue would lead to epimerization at C-4 (Scheme 4). Furthermore, a 2-chlorotrityl chloride (2CTC) resin was chosen to allow for facile peptide cleavage under mild conditions (hexafluoroisopropanol, HFIP), which would also be compatible with the silyl protecting groups selected for the two terminal carboxylates (D-Ala, L-Glu) and the side chain amine of lysine. This identical choice of protecting groups would also enable a joint removal at a late stage of the synthesis. Peptide coupling should then be realized using the respective synthesized silyl and Fmoc protected amino acids. Furthermore, synthesis of the peptide was planned in such a way that only slight modifications would allow access to stem-specific interpeptidic bridges. As in previous procedures and in view of the instability of an allylic pyrophosphate moiety, a late stage carbodiimidazole mediated coupling was envisaged for introduction of farnesyl phosphate **8**.

Synthesis of carbohydrate fragment 7

The final synthesis towards carbohydrate fragment **7** is shown in Scheme 2. While the overall route was adopted from previous described procedures,[7] more technical modifications were implemented to improve the practicability and raise the overall yield. In detail, following a known procedure commercial carbohydrate carboxylate **12** was protected as trichloroethyl ester.[15] Introduction of this ester proved to be more reliable as compared to introduction of a trimethylsilyl ester. Resulting carbohydrate **14** was then exposed to palladium in a hydrogen atmosphere to cleave the anomeric benzyl group, which also led to partial removal of the acetal group. While in principle



Scheme 1 Modular three fragment retrosynthetic approach towards 3-lipid I analogs **3** and **4**: novel solid phase based synthesis of pentapeptide.



Scheme 2 Improved synthesis of protected saccharide building block **7**.[15]

a protocol for reattachment has been reported, which involves treatment with benzaldehyde dimethylacetal and catalytic amounts of *p*-toluenesulfonic acid in DMF,[15] this method proved to be unreliable in our hands and modifications were evaluated. Finally, a solvent exchange to more easily removable MeCN and a slight increase of catalyst loading (0.1 to 0.3 equiv.) gave almost quantitative yields over two steps (96%) and a shortened reaction time (4 h). Additionally, the amount of benzaldehyde dimethylacetal may be dramatically reduced to 1.5 equiv. which further facilitates product isolation. Resulting alcohol **15** resided as a 4 : 1 α : β mixture,[15] while these two anomers may be separated by HPLC giving pure α-anomer and partially enriched β-anomer, which was prone to reequilibration. Fully resolved NMR data for the α-anomer unequivocally confirmed the stereochemical assignment, as shown. The ^3J-coupling constant for the respective proton was found to be 3.6 Hz, which points to a $^3J_{ae}$ coupling to the neighbouring axial proton. Furthermore the signal for the anomeric carbon atom was found to be at 92.3 ppm.

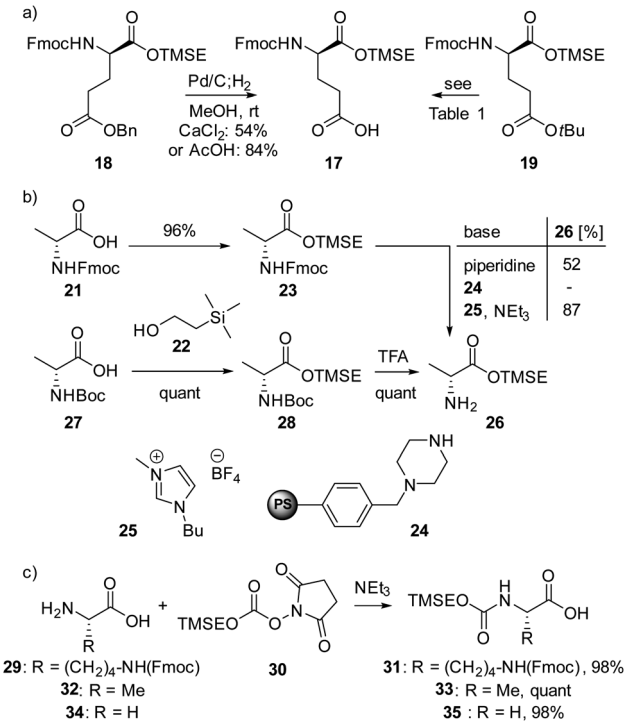
A nucleophilic introduction of the anomeric phosphate was then carried out with the anomeric mixture. Following a reported procedure,[6,15,16] this involved treatment with dibenzyl *N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole, giving the corresponding phosphite (structure not shown). This presumably labile intermediate[5] was then directly oxidized with *m*-CPBA to carbohydrate **16.** In agreement with a previous observation,[6,15,16] the α-anomer was obtained exclusively based on analysis of the fully resolved NMR data. In detail, assignment of the α-anomer was based on a ^3J-coupling constant of 3.2 Hz and the shift of the respective carbon signal, located at 96.6 ppm. Presumably, the high selectivity arises from selective capture of the more reactive α-hydroxyl group.[16] As an alternative to tetrazole, also more readily available 1,2,4-triazole was evaluated.[5,16,17] However, this did not improve the process, presumably due to reduced acidity. Finally, completion of the synthesis involved treatment with zinc powder in acidic condition, rendering the desired monosaccharide scaffold **7** despite the harsh conditions in an excellent yield of 97%. This approach proved superior to likewise evaluated alternatives as it provides comparably good yields without involving an expensive catalyst like $(Cp)_2TiCl$.[18] Overall, carbohydrate fragment **7** was synthesized with a yield of 60% over six steps from commercially available compound **12**, which compared favorably to the previous route.[15] Furthermore, the two step-conversions of **14** to **15** and of **15** to **16** may be carried out in one-pot reactions, which further add to the efficiency of the process.

Synthesis of farnesyl fragment

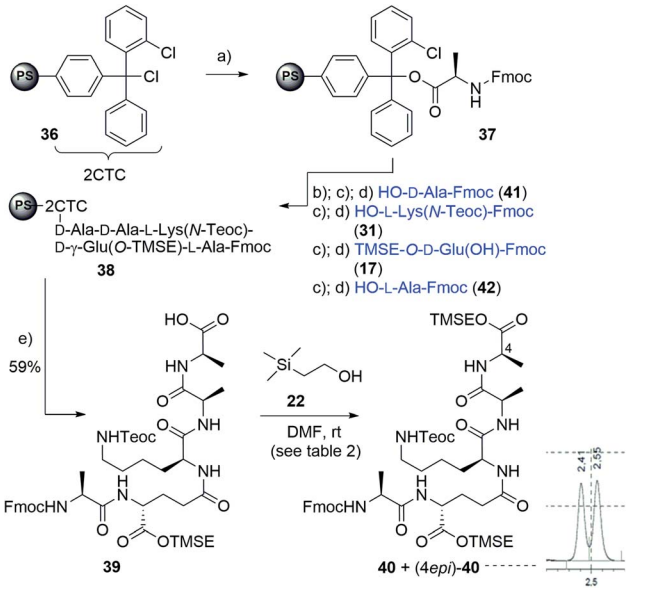
Known farnesyl building block **8** was efficiently prepared by a method published by the group of Wessjohann,[11] that is based on a one-pot conversion of the corresponding alcohol with tetrabutylammonium hydrogen phosphate (TBAP) and trichloroacetonitrile (TCA) giving the required phosphate in a reliable manner with useful yield (71%).

Synthesis of amino acid building blocks

To synthesize the stem peptides of targeted lipid analogs, several amino acid building blocks were required. While some of them were commercially available, glutamic acid derivative **17**, protected alanines **26** and **33** as well as lysine **31** and glycine



Scheme 3 Efficient synthesis of required amino acid building blocks **17** (part a), **26** (part b), **31**, **33** and **35** (part c).



Scheme 4 Solid phase synthesis of pentapeptide **39** and epimerization during TMSE protection of terminal D-Ala. Reagents and conditions: (a) HO–D-Ala–Fmoc (**41**), DIPEA (b) Ac_2O, *N*-methylimidazole, DMF (c), 20% piperidine/DMF (d) HBTU, HOBt, amino acid, DIPEA, DMF (e) 20% HFIP/DCM.

35 had to be synthesized in suitably protected form for the projected sequences.

As shown in Scheme 3a, Fmoc–D-Glu(OH)–TMSE (**17**) was initially targeted by a reported procedure[7] from **18** which in turn was accessible by TMSE protection from the corresponding commercial α-carboxylate.[7] However, selective hydrogenolysis of the benzyl group proved more challenging than expected, leading to various degrees of concomitant removal of also the more stable Fmoc-group under conventional hydrogenation conditions (23% yield of **17**). While this outcome could only be partially remedied (up to 41%) by careful reaction monitoring, it was found that addition of $CaCl_2$ could increase the lifetime of the Fmoc protective group, presumably due to prevention of basic conditions.[19] In detail, addition of $CaCl_2$ to a final concentration of 0.25 M had a positive effect and desired product was isolated in 54% yield. Finally, expected stability of targeted **17** towards a slightly acidic environment and the fact that benzyl protecting groups are acid labile motivated addition of 100 μL (0.38 equiv.) of acetic acid and the yield was further increased to 84%. While cooling and solvent exchange were also evaluated, in the end it turned out that a higher yielding, preparative less laborious and more economical strategy could be implemented, which involved selective cleavage of the *t*Bu-ester of **19**. This ester was readily available by TMSE-protection from the corresponding commercial α-carboxylate by a Steglich esterification (96%, not shown, see ESI section†). As shown in the respective table, a variety of reagents were evaluated to effectuate specific removal of this ester without affecting the TMSE group. Various acidic conditions led to unfavorable or low selectivity (entries 1 and 2).[20] Also, use of triethylsilane previously used as a carbocation scavenger in the deprotection of *tert*-butyl esters,[21] did not circumvent this lack of selectivity (entry 3). A reported procedure using an excess of $ZnBr_2$ furnished only compound **20** (entry 4) and using a previously described aqueous phosphoric acid procedure as much milder alternative showed no reaction at all (entry 5).[22] Finally, an excess of TMSOTf and 2,6-lutidine was able to unmask the side chain carboxylic acid in a selective manner rendering glutamic acid building block **17** in 97% yield (entry 6).[23] Notably, no column chromatography was necessary for this step and simple extractions were adequate to give the desired compound in high purity. This novel two-step procedure compared favorably to the reported method,[7] with respect to yield, cost of starting material and reagent, and does not require a chromatography in the second step (Table 1).[7]

The amino acid building block H_2N–D-Ala–TMSE (**26**) in turn was initially synthesized in a two-step procedure from commercial **21** involving a TMSE-esterification and subsequent Fmoc cleavage (Scheme 3b). While the former conversion could

Table 1 Conditions for the deprotection of *t*Bu-ester **19** to free acid **17** (Scheme 3)

Entry	Conditions	**17**/**20**	Yield	
1	TFA/DCM (1 : 1)	1 : 4	n.d.	
2	TFA/DCM (1 : 9)	~1 : 1	n.d.	
3	Et_3SiH, TFA/DCM (1 : 4)	~1 : 1	n.d.	
4	$ZnBr_2$, DCM	<1 : 20	n.d.	
5	H_3PO_4/DCM (1 : 1)	—	—	
6	TMSOTf, 2,6-lutidine	>20 : 1	97%	FmocHN, OH, OH **20**

be realized in high yield using the Steglich procedure (96%), conventional protocols using piperidine/DMF gave only moderate amounts of desired amine **26**, presumably, due to loss of material during work up. Although various conditions like extractions, NEt_3 addition during chromatography, different solvents or alternative chromatography materials (aluminum oxide, different pH, silica) were employed, the overall yield could not be improved beyond 52%. Also, a recently reported procedure using polymer-bound piperazine **24** (ref. 24) did not show any conversion. Finally, highest yield (87%) was obtained by a unconventional procedure reported by Gioia *et al.*[25] involving NEt_3 in ionic liquid **25** as solvent, which allowed for facile purification. However, elongated reaction times (2 d) required for full conversion was considered as a too disadvantageous leading to the design and implementation of an alternative approach. This involved Boc protected D-Ala **27**, which was esterified as a TMSE ester in quantitative amounts towards **28**,[26] followed by deprotection of the Boc group using a 25% TFA/DCM mixture, which proceeded with excellent selectivity towards carbamate cleavage. After evaporation of all volatiles, amine **26** was synthesized in a quantitative yield over two steps without the need of column chromatography.

Finally, TMSO-carbonyl (=Teoc) protected lysine (**31**) and alanine (**33**), as required for synthesis of interpeptidic analogs (see below), were best prepared from the native amino acids using succinimide reagent **30** with NEt_3 in a solvent mixture of 1,4-dioxane/water.[7]

Synthesis of pentapeptide 44

A reliable, scalable and high yielding synthesis of the required pentapeptides presents one of the most crucial aspects in the development of lipid I/II analogs and it was decided that a solid phase approach would best meet these requirements. Given apparent limitations of existing solid phase approaches,[7] a *de novo* design was pursued within this project.

For solid phase a 2-chlorotrityl-chloride (2CTC) **36** resin was chosen in order to enable a facile cleavage under mild conditions (hexafluoroisopropanol, HFIP),[27] which was also expected to be compatible with the silyl protecting groups that had been chosen for the carboxylates of D-alanine **26**, D-glutamic acid **17** as well as the side chain amine of L-lysine **31**. After some experimentation, protocols for resin functionalization, deprotection, coupling, washing and cleavage were developed and then strictly followed. Optimal conditions involved loading with 0.5 equiv. of the first amino acid in presence of DIPEA in DMF (1 h, rt), capping of the unreacted CTC-functionalities with acetic anhydride and *N*-methylimidazole in DMF and stepwise elaboration of the peptide involving Fmoc cleavage of the solid bound material with 20% piperidine/DMF and HBTU/HOBt mediated attachment of the next amino acid and final cleavage of the fully elaborated peptide from the resin with 20% HFIP in DCM.

Following this sequence of repetitive deprotection and coupling with amino acid building blocks **41**, **31**, **17** and **42** gave resin bound pentapeptide **38**. Final treatment with HFIP and precipitation in Et_2O liberated pentapeptide **39** in high yield over these 11 steps (59%). The overall process may be carried out in less than 15 h, which adds to the efficiency of this solid phase approach.

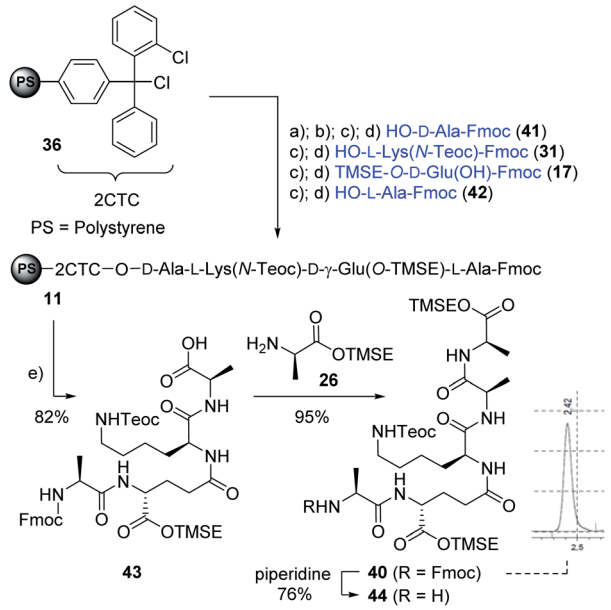
At this stage, the terminal carboxylate had to be protected as a TMSE ester towards **40** to allow for selective attachment of the sugar fragment at a later stage of the synthesis (see below). However, in contrast to previous observations with amino acid building blocks, Steglich esterification of **39** with 2-(trimethylsilyl)ethanol (**22**), suffered from long reaction times, incomplete conversions and epimerization (see Table 2, entry 1). Possibly, this may arise from stabilization of the generated *O*-acylisourea by formation of various hydrogen bonds with parts of the peptide, which was supported by mass spectrometric analysis. Alternatively, also an *N*-acylurea byproduct may be involved. Also, other esterification methods were considered.[28,29] However, they likewise resulted in epimerization (entries 2 and 3) or did not lead to any conversion at all (entry 4).[28,29]

Separate analysis of the two products, which may be separated by HPLC (see chromatogram), revealed identical molecular ion MS data, but small NMR differences, especially in the region of the terminal stereogenic center. Also, optical rotation values were different (see Experimental section for details), which supports an epimerization. Presumably, this may result from decreased acidity of the respective activated ester intermediate, leading to deprotonation and subsequent scrambling of the α-center. Alternatively, also an oxazole intermediate may be possible.[30]

At this stage it was rationalized that this unfavourable issue may be resolved by stronger nucleophilicity of amines as compared to alcohols and therefore coupling of free amino acid building block H_2N–D-Ala–TMSE (**26**) in solution was evaluated. Consequently, instead of the full pentapeptide **39** only tetrapeptide **43** was targeted. As shown in Scheme 5, it was prepared on solid phase in an analogous fashion as before. However, in contrast to the previous sequence, the terminal D-alanine was attached after liberation from solid support by coupling in solution with already TMSE-protected D-alanine **26**. In detail, after cleavage from the solid phase, tetrapeptide **43** was obtained in excellent 82% yield over nine steps starting from resin **36**. Subsequent attachment of final amino acid D-Ala **26**, already incorporating the desired silyl protection, was carried out with PyBOP/HOBt. Gratifyingly, this coupling proceeded not only in high yield (95%) but also without loss of stereochemical purity, as unambiguously proven by HPLC analysis. Finally, Fmoc removal gave desired pentapeptide **40** in 76% yield. In total, this novel route giving key stem peptide **40** in high purity and yield

Table 2 Reaction conditions for the silyl protection of pentapeptide **40** (Scheme 4)

Entry	Conditions	**40**/(4*epi*)-**40**
1	**22** (1.3 equiv.), DCC, [DMAP]	~1 : 1
2	**22** (2.0 equiv.), PyBOP/HOBt, DIPEA	~1 : 1 (42%)
3	**22** (10.0 equiv.), TFFH, DIPEA	~1 : 1
4	**22** (2.0 equiv.), MNBA, [DMAP], DIPEA	—

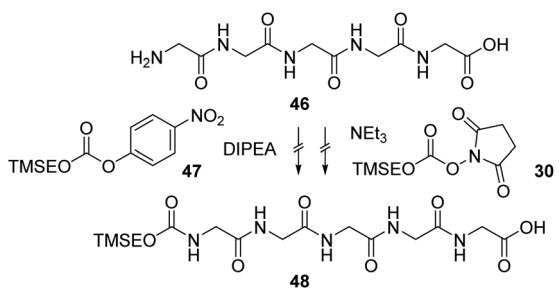


Scheme 5 Stereoselective preparation of protected pentapeptide **40** by solid phase synthesis of tetrapeptide **43** and attachment of the final D-Ala residue in solution. Reagents and conditions: (a) HO–D-Ala–Fmoc (**41**), DIPEA (b) Ac_2O, *N*-methylimidazole, DMF (c), 20% piperidine/DMF (d) HBTU, HOBt, amino acid, DIPEA, DMF (e) 20% HFIP/DCM.

(59%) over eleven steps, proved reliable, scalable, fast and compares favorably to previous lengthy solution phase sequences[10,16] or solid phase procedures giving low yield (15%) and limited purity.[7]

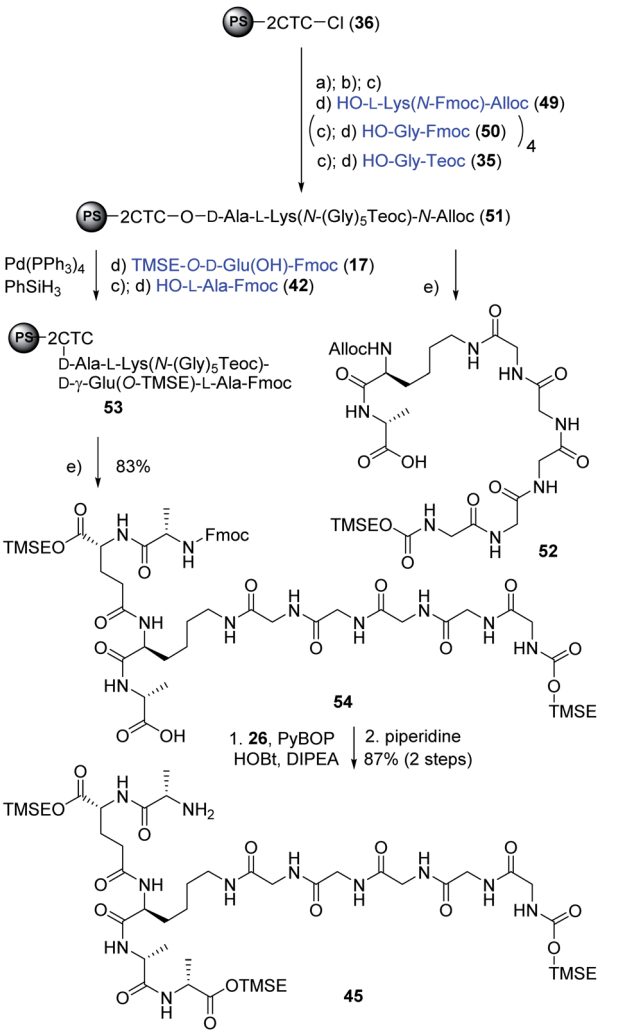
Synthesis of decapeptide 45

At this stage our efforts were directed to evaluate the generality and adaptability of this solid phase peptide approach also to pathogen specific analogs. As a first target we chose decapeptide **45** which is characteristic for *S. aureus*. Synthesis of **45** which required access to the characteristic pentaglycine side chain in suitably protected form (**48**). As shown in Scheme 6, our first approach was to introduce a Teoc protecting group to commercially available pentaglycine **46**, as this silyl protecting group would allow for a late stage attachment of the interpeptidic side chain (*vide infra*).



Scheme 6 Unsuccessful direct protection of pentaglycine **46**.

However, desired product **48** could not be generated under a variety of reaction conditions, presumably due to the very poor solubility of **46** in a variety of solvents. In contrast, Teoc protection of glycine proceeded uneventfully (Scheme 3). Given the reliability and robustness of our novel solid phase approach, we therefore planned to attach the pentaglycine moiety in a stepwise fashion on the resin. Gratifyingly, this could be realized. Following the sequence shown in Scheme 7 this involved again a final attachment of the terminal D-Ala in solution phase. In detail, the successful solid-phase route relied on initial attachment of the penultimate D-Ala amino acid to the chlorotrityl resin and stepwise elaboration using Fmoc protected amino acids. However, in contrast to the previous sequence towards pentapeptide **40**, a modified lysine building block **49** was utilized, now bearing the Fmoc group at the ε-amine and an orthogonal allyloxycarbonyl (alloc) moiety for the α-amine. After coupling of this lysine derivative, the Fmoc group in the side chain was then removed first. Four glycine residues (**50**) were then attached in an iterative fashion using standard

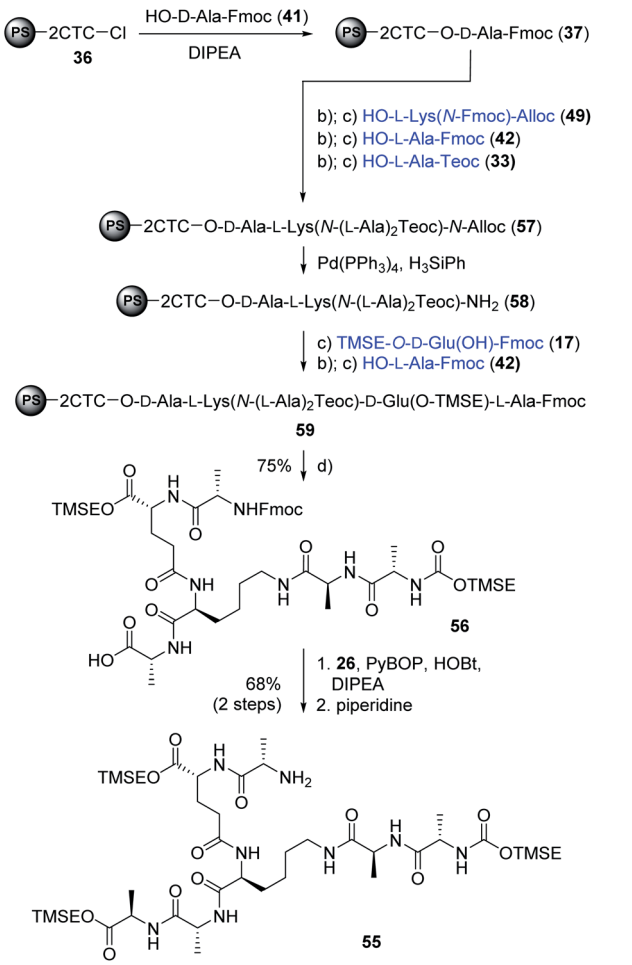


Scheme 7 Solid phase synthesis of decapeptide **45** characteristic for *S. aureus*. Reagents and conditions: (a) HO–D-Ala–Fmoc (**41**), DIPEA (b) Ac_2O, *N*-methylimidazole, DMF (c), 20% piperidine/DMF (d) HBTU, HOBt, amino acid, DIPEA, DMF (e) 20% HFIP/DCM.

coupling and deprotection protocols, before the final glycine was introduced as its silyl protected building block **35**. At this stage, the success of this procedure was verified, by a test cleavage revealing heptapeptide **52**, as expected. After this confirmation of complete incorporation of the full pentaglycine residue, solid bound peptide **51** was further elaborated. This involved cleavage of the alloc protecting group with $Pd(PPh)_4$ and phenylsilane ($PhSiH_3$), and attachment of amino acids **17** and **42**. Finally, the resulting nonapeptide was cleaved from the resin giving peptide **54** in excellent yield (83%) over these nineteen steps on the solid phase support. In the end, remaining (TMSE) protected D-alanine (**26**) was coupled in solution, before the Fmoc group was cleaved using piperidine. In total, desired decapeptide **45** was obtained in high overall yield (40%) over 21 steps starting from commercial resin **36**.

Synthesis of heptapeptide 55

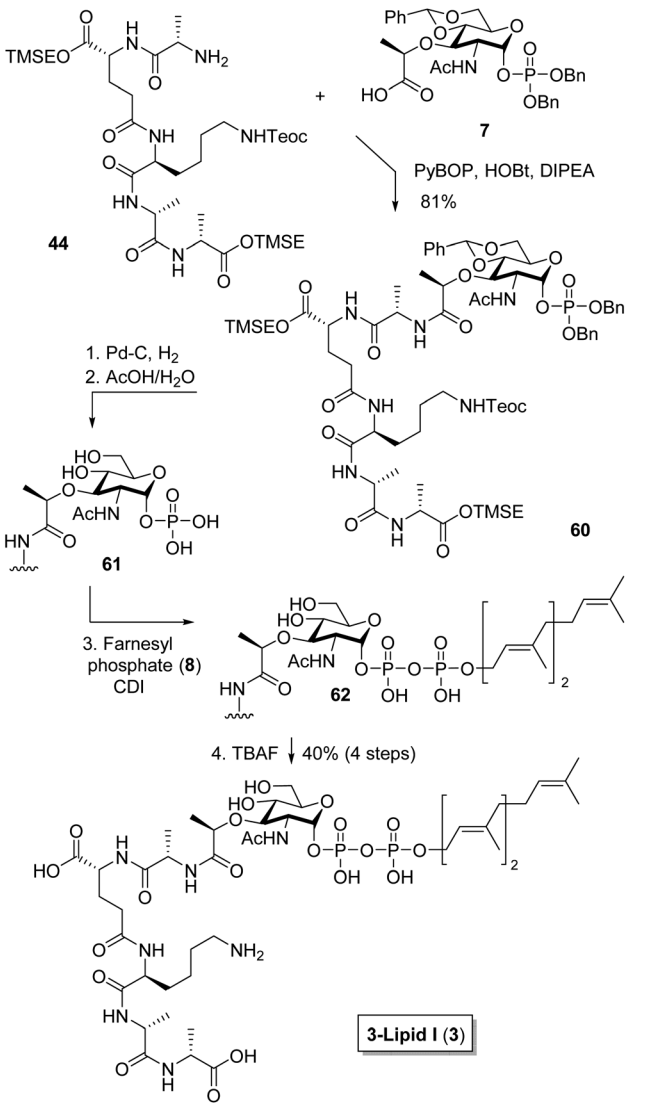
In order to further evaluate the generality of this solid phase sequence, we then opted for synthesis of heptapeptide **55**, incorporating an L-Ala–L-Ala side chain that is characteristic for *E. faecalis* and *S. pneumoniae*. Accordingly, our general solid phase approach was modified as shown in Scheme 8. In detail, this involved again modified lysine building block **49**, bearing a terminal Fmoc group and an orthogonal alloc moiety at the α-amine. After selective Fmoc cleavage, the two L-Ala fragments could be successfully introduced, before the α-amine group was liberated in an orthogonal fashion [$Pd(PPh)_4$; ($PhSiH_3$)]. Amino acids **17** and **42** were then attached to the peptidic backbone. After removal from the solid phase, desired hexapeptide **56** was obtained in high overall yield (75%), demonstrating the generality of this solid phase sequence. Finally, completion of the synthesis of **55** involved attachment of the terminal D-Ala motif and Fmoc removal giving desired branched heptapeptide in high yield (51%) over 15 steps.[8]



Scheme 8 Solid phase synthesis of heptapeptide **55** characteristic for *E. faecalis* and *S. pneumoniae*. Reagents and conditions: (b) 20% piperidine/DMF, (c) HBTU, HOBt, amino acid, DIPEA, DMF, (d) 20% HFIP/DCM.

Fragment coupling and completion of the syntheses of 3-lipid analogs

For fragment union, our strategy relied on first coupling the peptide fragments with the monosaccharide building block and



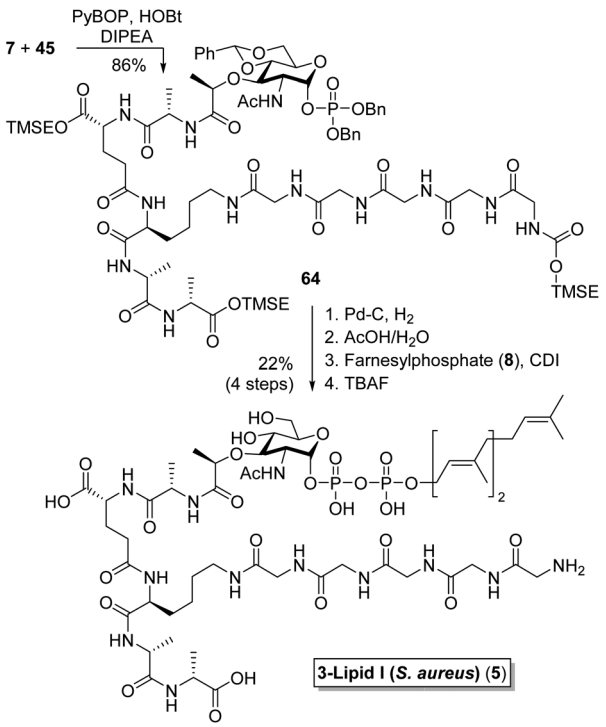
Scheme 9 Completion of the total synthesis of 3-lipid I.

subsequently attaching the farnesyl phosphate subunit. As shown in Scheme 9, we first targeted original lipid I analog **3**.[6,14,31,32] Accordingly, respective peptide **44** was connected to carbohydrate **7** with PyBOP/HOBt giving glycopeptide precursor **60** in high yield (81%). In contrast to a previous report,[15] a joint removal of the benzyl groups and the acetal moiety using hydrogenolysis could not be realized. Even after increase of the reaction time from reported 30 min to 5 h, only the free phosphate was observed, without cleavage of the acetal group. Apparently, such a joint removal appears challenging, as also Arimoto *et al.* reported a two-step procedure for their depsi-lipid I analog.[10] In an analogous fashion for **60**, the benzyl group was first removed by hydrogenolysis, before the acetal was cleaved using acidic conditions giving desired deprotected **61**. Pyrophosphate linkage of farnesyl phosphate **8** and phosphate **61** was then achieved using carbonyldiimidazole coupling.

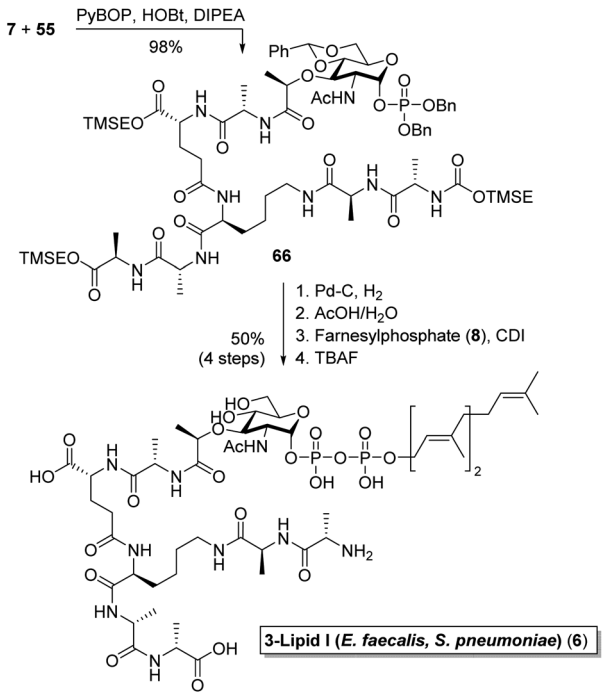
Finally, all silyl protecting groups could be cleaved in a joint fashion using tetrabutylammonium fluoride, proving the success of our synthetic design. For full purification of farnesyl lipid I analog **3**, a three step method was developed. First, gel filtration with Sephadex® LH-20 was applied, followed by ion-exchange chromatography (Dowex® 50WX8) to substitute residual tetrabutylammonium counterions with ammonium, which otherwise proved difficult to remove by HPLC. Final HPLC separation yielded highly pure farnesyl lipid I analog **3** in 40% yield over four steps starting from glycopeptide precursor **60**. In total, farnesyl lipid I **3** was obtained in pure form in 16 steps in the longest linear sequence from commercially available HO–D-Ala–Fmoc (**41**) with an excellent overall yield of 19%, which compared favorably to the previous synthetic route starting from Sasrin resin–D-Ala–Fmoc **63**.[7,15,31] The sequence proved well scalable and highly pure 3-lipid I (**3**) was obtained.

Encouraged by the successful final steps towards farnesyl lipid I analog **3**, an analogous three component sequence was applied to complete the total synthesis of *S. aureus* lipid I analog **5**. As shown in Scheme 10, characteristic decapeptide **45** was coupled to carbohydrate component **7** using a previously established PyBOP/HOBt procedure to give glycopeptide precursor **64** in 86% yield, which was even slightly higher than the yield after coupling with the stem pentapeptide **44** (81%). Caused by the longer peptide chain, solubility in solvents like EtOAc and Et_2O decreased, which can cause problems in handling and reactions but turned out to be advantageous here, since precipitation led to more facile purification. Removal of the phosphate benzyl groups by hydrogenolysis, acidic cleavage of the acetal and CDI mediated pyrophosphate coupling with farnesyl phosphate **8** gave silyl protected **65**, which was semi-purified using Sephadex® LH-20. Again, final global deprotection using TBAF freed the peptide from its silyl groups and farnesyl lipid I analog **5**, containing the *S. aureus* pentaglycine, was gained in pure form after utilization of the three step purification developed above, consisting out of gel filtration with Sephadex® LH-20, ion-exchange chromatography (Dowex® 50WX8) and a final HPLC purification. Overall, lipid I analog **5** was obtained in an overall yield of 8% in its longest linear sequence over 26 steps starting from HO–D-Ala–Fmoc (**41**).

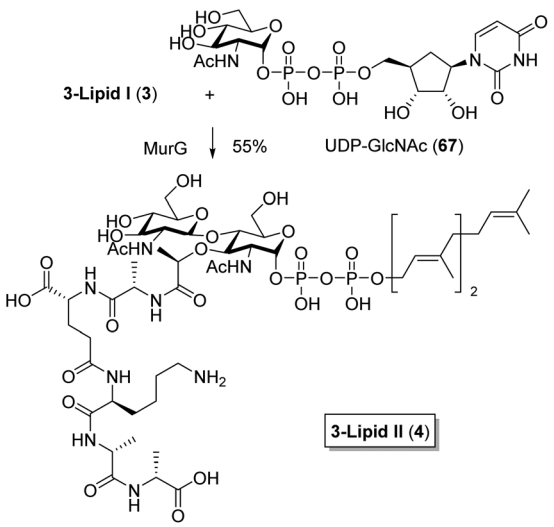
The generality of this final coupling strategy was then further confirmed by synthesis of lipid I analog **6** containing the interpeptidic sequence characteristic for pathogen *E. faecalis* and *S. pneumoniae*. As shown in Scheme 11, the PyBOP/HOBt



Scheme 10 Completion of the total synthesis of 3-lipid I (*S. aureus*).



Scheme 11 Completion of the total synthesis of 3-lipid I (*E. faecalis, S. pneumoniae*).



Scheme 12 Chemoenzymatic conversion of 3-lipid I (**3**) to 3-lipid II (**4**) using UDP–GlcNAc (**66**).

mediated amide coupling between the respective peptidic fragment and the sugar moiety proceeded again with high efficiency. In detail, glycopeptide precursor **66** was obtained in essentially quantitative yield, following a slightly improved procedure. This involved cold diethyl ether as solvent, which does dissolve the reactants but may not solubilize the product. Therefore, simple filtration gave glycopeptide precursor **66** in high purity after washing. Finally, farnesyl lipid I analog **6** was obtained in a straightforward fashion using the four-step sequence established above, demonstrating the general utility of this approach.[8] In total, this novel lipid analog was obtained in 20 steps and likewise very high overall yield (25%) and high purity.

Finally we confirmed the biochemical compatibility of the shortened farnesyl group by conversion of 3-lipid I (**3**) to 3-lipid II (**4**). As shown in Scheme 12, this previously reported conversion,[31] involves MurG catalyzed chemoenzymatic attachment of β-1-4-linked *N*-acetylglucosamine. It was carried out with 1 mg of **3** and lipid II analog **4** was obtained in pure form with a useful yield of 55%, which represented a sufficient amount for characterization including the assignment of NMR signals. The generality of this approach was further confirmed by also converting an analytic sample of **5** to the corresponding lipid II analog (not shown).

Initial NMR studies

Finally, the NMR resolution of all new analogs **3–6** and usefulness for solution NMR measurements were analysed. Gratifyingly, optimum ^{1}H signal dispersion was realised in D_2O for all shortened lipid compounds at the highest available field strengths (500 and 700 MHz). As shown for the NMR spectra in the ESI section† excellent resolution was obtained for all lipid-analogs, allowing for complete assignment of all ^{1}H and ^{13}C resonances based on two dimensional techniques (see ESI section: Table S1†). Furthermore, farnesyl lipid I analog **3** was analyzed in more detail, including temperature dependent NMR measurements, which may give insights into effects like hydrogen bonding, stability of the compound and conformational changes.[33] As shown in Fig. S1 (see ESI section),† there are detectable differences in the chemical shifts at various temperatures. With increasing temperature, a downfield shift was observed. Additionally, broadening of the signals was seen especially for the temperature shift from 278 K to 282 K. If the temperature was increased further, the signals got sharp again. ^{31}P NMR spectra were recorded at the same temperatures as well (Fig. S2†). These measurements showed an even more interesting effect. While the signals for the pyrophosphate were not visible at 278 K, sharp signals appeared at 282 K, which broadened again and vanished completely if the temperature was increased further. Surprisingly, this observation is the opposite of the ^{1}H spectra in which the signals at 282 K were the most broadened. The changes in the signals can be caused by various effects like conformational changes, exchange processes or temperature dependent hydrogen bonding. Possibly, the phosphate counter ions also have an effect of causing the disappearing signals, especially at lower temperatures. During these measurements it was also noted that synthesized farnesyl lipid I analog **3** was stable at elevated temperatures, as the ^{1}H spectra at 363 K did not showed significant degradation. These initial NMR studies make it now even more promising to apply these analogs as chemical tools for structural as well as functional studies.

Conclusion

In summary, an optimized, efficient, scalable and reproducible route to farnesyl lipid I analogs **3**, **5** and **6** was developed. For this purpose, syntheses of the three components being carbohydrate **7**, peptide **44** and farnesyl phosphate **8** were closely examined. Regarding carbohydrate **7**, various technical modifications and optimizations, for example by exchange of the reaction solvent or by adjusting the reagent equivalents, led to a considerable increase of the overall yield from literature reported 41% to 60% over six steps (Scheme 2).[15] Application of a recently reported one-pot procedure for the conversion of alcohols to their phosphates[11] allowed to further simplify the route towards the precious cell wall precursors, giving required farnesyl phosphate **8** from farnesol in 71% yield using only one step.

Considerable efforts were then invested before a novel, efficient and scalable solid phase synthesis of the characteristic stem pentapeptide was developed, which compares favorably to previous lengthy solution phase sequences as well as low reported yield (15%) for a solid phase approach.[6,7,10,15,16,36] This optimized route relied on preparation of the tetrapeptide HO–D-Ala–L-Lys(*N*-Teoc)–D-γ-Glu(*O*-TMSE)–L-Ala–Fmoc (**43**) using 2-chlorotrityl chloride (2CTC) solid phase support and subsequent attachment of the remaining silyl protected D-Ala amino acid (**26**) in solution phase, which turned out to be crucial to avoid an unfavorable epimerization. Following this novel sequence synthesis of pentapeptide **44**, was obtained in an excellent yield of 59% over 11 steps. This route was then further

elaborated and applied for synthesis of pathogen specific interpeptidic analogs, *i.e.* the pentaglycine variation found in *S. aureus* and the L-Ala–L-Ala modification observed in *E. faecalis* and *S. pneumoniae*, were targeted, which are considered most important among the interpeptidic variations described for specific pathogens.[37] In detail, a novel solid phase sequence was designed to allow for the introduction of interpeptidic modifications, which involved an alternative lysine building bearing an ε-Fmoc and an α-alloc group to allowing for an orthogonal strategy. Overall this route enabled the synthesis of required decapeptide **45** in 40% yield over 21 steps and necessary heptapeptide **55** in 51% yield over 15 steps, demonstrating the modularity and true applicability of this sequence to various pathogen specific analogs.

After coupling of the fragments and deprotection, farnesyl lipid I analog **3** was purified with a newly developed three step purification including gel filtration (Sephadex® LH-20), ion exchange chromatography (Dowex® 50WX8) and HPLC separation giving the desired product **3** in high purity over 16 steps (longest linear sequence, 19% yield). This sequence proved well scalable and a batch of 11 mg lipid I analog **3** was readily obtained in high purity. Following analogous three component coupling sequences then enabled completion of the first total syntheses of *S. aureus* interpeptidic analog **5** (8% over 26 steps longest linear sequence) and of *E. faecalis* and *S. pneumoniae* analog **6** (25% over 20 steps longest linear sequence).

Furthermore, shortened lipid I analogs were accepted as substrates by the natural glycosyltransferase MurG, which catalyses attachment of the β-1-4-linked *N*-acetylglucosamine, demonstrating the biochemical compatibility of the shortened side chain, which also allowed preparative synthesis of 3-lipid II analog **4**.

Finally, detailed NMR analyses of all lipid I and lipid II analogs revealed high spectroscopic resolution in D_2O and useful stability at various temperatures, demonstrating that they present valuable tool compounds for structural studies and may now be used to address biochemical questions and to gain new structural insights into bacterial cell wall biosynthesis. Present studies along these lines will be reported in due course.

Experimental

General synthetic procedures

All reactions were performed under an atmosphere of argon in flame-dried glassware which had been cooled under argon unless stated otherwise. All flasks were equipped with rubber septa and reactants were handled using standard Schlenk techniques. Temperatures above rt (23 °C) refer to oil bath temperatures which were controlled by a temperature modulator. For cooling, the following baths were used: acetone/dry ice (−78 °C), ACN/dry ice (−40 °C), water/ice (0 °C). All reagents were purchased from commercial suppliers (Sigma-Aldrich, Alfa Aesar, TCI, Iris Biotech, Acros) in the highest purity grade available and used without further purification unless otherwise stated. Anhydrous solvents (THF, Et_2O, DCM, MeCN and toluene) were freshly obtained from a solvent drying system MB SPS-800 (MBraun) and stored over molecular sieves (3 or 4 Å). Reactions were monitored *via* TLC on silica gel 60 F_{254} precoated plates (0.2 mm SiO_2, Macherey-Nagel) and visualized using UV light and/or staining with a solution of CAM (1 g $Ce(SO_4)_2$, 2.5 g $(NH_4)_6Mo_7O_{24}$, 8 mL conc. H_2SO_4 in 100 mL H_2O) and subsequent heating. For column chromatography, silica gel (pore size 60 Å, 40–63 μm) obtained from Merck or Aldrich was used. Compounds were eluated using the stated mixtures under positive pressure of nitrogen or air. Solvents for column chromatography were distilled prior to use. For ion exchange chromatography Dowex® 50WX8, 100–200 mesh by Acros was used. Gel permeation chromatography was performed using Sephadex® LH-20 purchased from GE Healthcare.

Optical rotations were measured with a PerkinElmer 341 or a Anton Paar MCP 150 polarimeter in a 10 mm cuvette and are uncorrected. All NMR spectra were recorded on Bruker spectrometers at the University of Bonn under supervision of Dr Senada Nozinovic with operating frequencies of 125 (^{13}C), 150 (^{13}C), 175 (^{13}C), 400 (^{1}H), 500 (^{1}H) and 700 MHz (^{1}H) in deuterated solvents obtained from Deutero, Carl Roth, VWR and Sigma-Aldrich. Spectra were measured at room temperature unless otherwise stated and chemical shifts are reported in ppm relative to $(Me)_4Si$ ($\delta = 0$ ppm) and were calibrated to the residual signal of undeuterated solvents. Data for ^{1}H NMR spectra are reported as follows: chemical shift (multiplicity, coupling constants in hertz, number of hydrogens, assignments; atom numbering for ^{1}H and ^{13}C signals can be found in the respective drawings in the ESI section†). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br (broad), pt (pseudo triplet). Mass spectra (MS) and high resolution-mass spectra (HR-MS) were recorded on the documented systems in Table S1† at the University of Bonn under supervision of Dr Marianne Engeser. Ionization processes and mol peaks were given (Tables 3 and 4).

Semi-preparative and analytical HPLC analyses were performed on Knauer Wissenschaftliche Geräte GmbH systems by

Table 3 Used MS systems for MS and HR-MS

Name	Manufacturer	Ionization type
MAT 95 XL	Thermo Finnigan (Bremen)	EI
MAT 90	Thermo Finnigan (Bremen)	EI, LIFDI, CI, FAB
MALDI autoflex II TOF/TOF	Bruker Daltonik (Bremen)	MALDI
micrOTOF-Q	Bruker Daltonik (Bremen)	ESI, APCI, nano-ESI, MS/MS, LC-MS, DC-MS
Orbitrap XL	Thermo Fisher Scientific (Bremen)	ESI, APCI, APPI, nano-ESI
Apex IV FT-ICR	Bruker Daltonik (Bremen)	ESI, nano-ESI, MALDI, EI, CI

Table 4 HPLC configuration for analytical and preparative HPLC

	System A (analytical)	System A (preparative)
Series	PLATINblue	Smartline
Pumps	Binary, HPG P1 system, 5 mL	Binary, S-1800, 100 mL
Pressure	1000 bar	400 bar
Autosampler	AS1 with 10 μL injection loop	Assistant 6000 with a feed pump S-100
Mixing chamber	Static, SmartMix 100	Static, SmartMix 350
Column heater	T1	
Detection type	PDA UV/Vis detection PDA1, D2/Hg halogen lamps, 190–1000 nm	UV-detector S-2550, 190–600 nm
Degasser	Analytical 2-channel-online-degasser	Preparative 2-channel-online-degasser

Andreas Schneider. The solvents for HPLC were purchased in HPLC grade. The chromatograms were recorded by UV-detection at 240, 215, 210 and 205 nm.

Synthesis of compound 14

To a solution of acid **12** (1.00 g, 2.12 mmol) and 4-(dimethylamino)-pyridine (DMAP) (25.9 mg, 212 μmol) in THF (16 mL) was added 2,2,2-trichloroethanol (**13**) (0.48 mL, 4.24 mmol) followed by *N*,*N*′-dicyclohexylcarbodiimide (656 mg, 3.18 mmol). After stirring for 5 h at rt the mixture was filtered through a cotton plug and the precipitate was washed with EtOAc (2 × 20 mL). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (15% EtOAc/CH_2Cl_2) to yield 1.03 g (1.71 mmol, 81%) of a white, crystalline solid.

R_f 0.44 (15% EtOAc/CH_2Cl_2). $[\alpha]_D^{25}$ = +93° (*c* = 0.90 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.46–7.28 (m, 10H, $H_{arom.}$), 7.07 (d, *J* = 5.5 Hz, 1H, NH), 5.58 (s, 1H, H-7), 5.34 (d, *J* = 3.4 Hz, 1H, H-1), 4.97 (d, *J* = 11.9 Hz, 1H, H-11), 4.68 (d, *J* = 11.8 Hz, 1H, H-15), 4.66 (q, *J* = 7.1 Hz, 1H, H-8), 4.60 (d, *J* = 11.9 Hz, 1H, H-11′), 4.52 (d, *J* = 11.9 Hz, 1H, H-15′), 4.21 (dd, *J* = 10.1, 4.6 Hz, 1H, H-6), 4.01–3.98 (m, 2H, H-3, H-5), 3.76 (dd, *J* = 10.3 Hz, 10.3 Hz, 1H, H-6′), 3.73 (dd, *J* = 9.2 Hz, 9.2 Hz, 1H, H-4), 2.03 (s, 3H, CH_3-14), 1.50 (d, *J* = 7.0 Hz, 3H, CH_3-9). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 173.7 (C-10), 170.9 (C-13), 137.5 ($C_{arom.}$), 137.3 ($C_{arom.}$), 129.2 ($C_{arom.}$), 128.6 ($C_{arom.}$), 128.5 ($C_{arom.}$), 128.1 ($C_{arom.}$), 128.0 ($C_{arom.}$), 126.0 ($C_{arom.}$), 101.6 (C-7), 97.5 (C-1), 94.6 (C-12), 83.4 (C-4), 75.2 (C-8), 75.1 (C-3), 74.3 (C-11), 70.5 (C-15), 69.1 (C-6), 63.1 (C-5), 54.2 (C-2), 23.3 (C-14), 18.8 (C-9). HRMS (ESI) *m*/*z*: calcd for $C_{27}H_{30}Cl_3NO_8Na$ [M + Na]$^+$: 624.0929, found: 624.0932. The spectroscopic data were in agreement with those previously reported.[13,15]

Synthesis of compound 15

To a solution of carbohydrate **14** (500 mg, 829 μmol) in EtOAc (40 mL) was added Pd–C (650 mg, 10% Pd). The reaction vessel was filled with hydrogen. After stirring for 20 min at rt the suspension was filtered through Celite and the precipitate was washed with methanol (2 × 20 mL). The solvent was removed under reduced pressure and acetonitrile (30 mL) was added followed by benzaldehyde dimethyl acetal (187 μL, 1.24 mmol) and a solution of *p*-TsOH in acetonitrile (1 mL of a 232 mM solution, 232 μmol) dried over 3 Å MS. After stirring for 4 h at rt the reaction was neutralized with Net_3 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (90% EtOAc/cyclohexane) to yield 409 mg (798 μmol, 96%) of a white, crystalline solid as a mixture of α and β anomers (ratio α : β = 4 : 1). The mixture was used for the next reactions without separation. For analytical purpose, separation of the α and β anomer was achieved with HPLC (50% ACN/water, retention time 11.5 min (β anomer), 16.1 min (α anomer), using a KNAUER Eurospher II 100-5 C18; 5 μm; 250 × 16 mm + precolumn 30 × 16 mm, 210 nm).

R_f (α anomer) 0.33, R_f (β anomer) 0.26 (90% EtOAc/cyclohexane). α anomer: $[\alpha]_D^{25}$ = +59° (*c* = 0.73 in CH_2Cl_2). ^{1}H NMR (700 MHz, CD_2Cl_2) δ = 7.47–7.45 (m, 2H, $H_{arom.}$), 7.40–7.36 (m, 3H, $H_{arom.}$), 7.19 (d, *J* = 4.5 Hz, 1H, NH), 5.60 (s, 1H, H-7), 5.58 (dd, *J* = 3.9 Hz, 3.6 Hz, 1H, H-1), 5.01 (d, *J* = 12.0 Hz, 1H, H-11), 4.68 (q, *J* = 7.1 Hz, 1H, H-8), 4.65 (d, *J* = 12.0 Hz, 1H, H-11′), 4.24 (dd, *J* = 10.3, 5.0 Hz, 1H, H-6), 4.03 (td, *J* = 10.3 Hz, 5.0 Hz, 1H, H-5), 3.94–3.86 (m, 1H, H-3), 3.87–3.83 (m, 1H, H-2), 3.75 (dd, *J* = 10.3 Hz, 10.3 Hz, 1H, H-6′), 3.73 (dd, *J* = 10.3 Hz, 10.3 Hz, 1H, H-4), 3.68 (dd, *J* = 3.9 Hz, 1.0 Hz, 1H, OH), 2.01 (s, 3H, CH_3-14), 1.51 (d, *J* = 7.1 Hz, 3H, CH_3-9). ^{13}C NMR (176 MHz, CD_2Cl_2) δ = 174.3 (C-10), 171.5 (C-13), 138.1 ($C_{arom.}$), 129.5 ($C_{arom.}$), 128.8 ($C_{arom.}$), 126.5 ($C_{arom.}$), 101.9 (C-7), 95.1 (C-12), 92.3 (C-1), 83.8 (C-4), 75.7 (C-8), 75.2 (C-3), 74.7 (C-11), 69.6 (C-6), 63.3 (C-5), 55.2 (C-2), 23.5 (C-14), 19.1 (C-9). HRMS (ESI) *m*/*z*: calcd for $C_{20}H_{24}Cl_3NO_8Na$ [M + Na]$^+$: 534.0460, found: 534.0463. The spectroscopic data were in agreement with those previously reported.[15]

Synthesis of compound 16

Alcohol **15** (100 mg, 195 μmol) was dissolved in dry CH_2Cl_2 (5 mL) and a 0.45 M solution of 1*H*-tetrazole in acetonitrile (1.63 mL, 731 μmol) was added. The reaction was cooled to −40 °C and dibenzyl(*N*,*N*-diisopropyl)phosphoramidite (164 μL, 488 μmol) was added. After 1 h warming to rt the reaction was stirred for another hour. Then, *m*CPBA (101 mg, 585 μmol) was added at −60 °C and the reaction was stirred for 30 min at 0 °C followed by 30 min stirring at rt. The mixture was diluted with 5 mL CH_2Cl_2 and washed two times with aq. Na_2SO_3 (10 mL, 10%), two times with aq. sat. $NaHCO_3$ (10 mL) and two times with water (10 mL). After drying over $MgSO_4$ the mixture was filtered, concentrated and purified by flash chromatography (65% EtOAc/cyclohexane) to yield 101 mg of a white, crystalline solid (79%, 585 μmol).

R_f 0.22 (70% EtOAc/cyclohexane). $[\alpha]_D^{25}$ = +67° (*c* = 0.66 in CH_2Cl_2). ^{1}H NMR (700 MHz, CD_2Cl_2) δ = 7.45–7.44 (m, 2H,

$H_{arom.}$), 7.42–7.33 (m, 13H, $H_{arom.}$), 7.15 (d, J = 4.9 Hz, 1H, NH), 6.04 (dd, J = 6.1, 3.2 Hz, 1H, H-1), 5.57 (s, 1H, H-7), 5.06 (d, J = 8.3 Hz, 4H, 2× CH_2–Ph), 5.01 (d, J = 12.0 Hz, 1H, H-11), 4.65 (q, J = 7.0 Hz, 1H, H-8), 4.62 (d, J = 12.0 Hz, 1H, H-11′), 4.08 (dd, J = 10.3, 4.9 Hz, 1H, H-6), 4.00–3.96 (m, 1H, H-2), 3.92 (ddd, J = 10.3, 10.3, 4.9 Hz, 1H, H-5), 3.82 (dd, J = 9.9, 9.9 Hz, 1H, H-3), 3.77 (dd, J = 10.3 Hz, 9.9 Hz, 1H, H-4), 3.72 (dd, J = 10.3, 10.3 Hz, 1H, H-6′), 1.87 (s, 3H, H-14), 1.51 (d, J = 7.1 Hz, 3H, H-9). ^{13}C NMR (176 MHz, CD_2Cl_2) δ = 174.2 (C-10), 171.3 (C-13), 137.8 ($C_{arom.,quart.}$–Ph), 136.4 (d, J = 7.2 Hz, $C_{arom.,quart.}$–Bn), 136.4 (d, J = 7.2 Hz, $C_{arom.,quart.}$–Bn), 129.6 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.1 ($C_{arom.}$), 128.8 ($C_{arom.}$), 128.4 ($C_{arom.}$), 128.4 ($C_{arom.}$), 126.5 ($C_{arom.}$), 102.1 (C-7), 96.6 (d, J = 6.7 HZ, C-1) 95.0 (C-12), 82.9 (C-4), 75.8 (C-8), 74.8 (C-11), 74.7 (C-3), 70.0 (d, J = 4.1, CH_2–Ph), 70.0 (d, J = 4.1, CH_2–Ph), 69.0 (C-6), 65.1 (C-5), 54.5 (C-2), 23.2 (C-14), 19.0 (C-9). ^{31}P NMR (284 MHz, CD_2Cl_2) δ = −2.4. HRMS (ESI) m/z: calcd for $C_{34}H_{37}Cl_3NO_{11}PH$ $[M + H]^+$: 772.1243, found: 772.1236. The spectroscopic data were in agreement with those previously reported.[15]

Synthesis of compound 7

Carbohydrate **16** (160 mg, 207 μmol) was dissolved in a mixture of 90% AcOH/water (15 mL) and zinc powder (120 mg, 1.84 mmol) was added. The suspension was stirred vigorously for 3 h at rt. After filtering and washing with MeOH the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (0.1% AcOH/10% MeOH/CH_2Cl_2) to yield 129 mg of a white, crystalline solid (97%, 201 μmol).

R_f 0.29 (0.1% AcOH/10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +61° (c = 0.46 in MeOH). ^{1}H NMR (700 MHz, CD_3OD) δ = 7.48–7.45 (m, 2H, $H_{arom.}$), 7.41–7.35 (m, 13H, $H_{arom.}$), 6.06 (dd, J = 6.0, 3.3 Hz, 1H, H-1), 5.62 (s, 1H, H-7), 5.11–5.08 (m, 4H, 2× CH_2–Ph), 4.42 (q, J = 7.1 Hz, 1H, H-8), 4.03–4.01 (m, 1H, H-6), 3.87–3.84 (m, 1H, H-2), 3.79–3.71 (m, 4H, H-3, H-4, H-5, H-6′), 1.87 (s, 3H, H-12), 1.37 (d, J = 7.1 Hz, 3H, H-9). ^{13}C NMR (176 MHz, CD_3OD) δ = 178.4 (C-10, not resolved but HMBC correlation), 173.9 (C-11), 138.9 ($C_{arom.,quart.}$–Ph), 137.0 (d, J = 2.4 Hz, $C_{arom.,quart.}$–Bn), 137.0 (d, 2.4 Hz, $C_{arom.,quart.}$–Bn), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 128.4 ($C_{arom.}$), 127.2 ($C_{arom.}$), 102.7 (C-7), 97.3 (d, J = 6.7, C-1), 83.0 (C-4), 77.0 (C-8), 75.1 (C-3), 71.2 (d, J = 2.2 Hz, CH_2–Ph), 71.1 (d, J = 2.3 Hz, CH_2–Ph), 69.1 (C-6), 66.1 (C-5), 55.8 (C-2), 22.6 (C-12), 19.2 (C-9). ^{31}P NMR (284 MHz, CD_2Cl_2) δ = −3.0. HRMS (ESI) m/z: calcd for $C_{32}H_{36}NO_{11}Pna$ $[M + Na]^+$: 664.1918, found: 664.1914. The spectroscopic data were in agreement with those previously reported.[15]

Synthesis of compound 19

Fmoc–D-Glu(*O*-*t*Bu)–OH (1.50 g, 3.53 mmol) and DMAP (86.1 mg, 705 μmol) were dissolved in 20 mL EtOAc and cooled to 0 °C. 2-(Trimethylsilyl)ethanol (758 μL, 5.29 mmol) and DCC (1 M in CH_2Cl_2, 4.23 mL, 4.23 mmol) were added. After stirring for 2 h at rt, the reaction was filtered over Celite and the residue was washed with EtOAc (2 × 20 mL). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (15% EtOAc/cyclohexane) to yield 1.77 g (3.37 mmol, 96%) of a white, amorphous solid.

R_f 0.32 (15% EtOAc/cyclohexane). $[\alpha]_D^{25}$ = +14° (c = 1.98 in MeOH). ^{1}H NMR (700 MHz, CD_3OD) δ = 7.79 (d, J = 7.5 Hz, 2H, $H_{arom.}$), 7.66 (t, J = 8.3 Hz, 2H, $H_{arom.}$), 7.38 (t, J = 7.2 Hz, 2H, $H_{arom.}$), 7.30 (t, J = 8.0 Hz, 2H, $H_{arom.}$), 4.38–4.32 (m, 2H, H-10), 4.23–4.19 (m, 4H, H-2, H-4, H-11), 2.33 (t, J = 7.0 Hz, 2H, H-6), 2.15–2.07 (m, 1H, H-5), 1.92–1.83 (m, 1H, H-5), 1.44 (s, 9H, 3× CH_3–Boc), 1.04–0.96 (m, 2H, H-1), 0.04 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, CD_3OD) δ = 173.8 (C-7), 173.7 (C-3), 158.6 (C-9), 145.3 ($C_{arom.}$), 145.2 ($C_{arom.}$), 142.6 ($C_{arom.}$), 128.8 ($C_{arom.}$), 128.2 ($C_{arom.}$), 128.1 ($C_{arom.}$), 126.3 ($C_{arom.}$), 126.2 ($C_{arom.}$), 120.9 ($C_{arom.}$), 81.8 (C-8), 68.0 (C-10), 64.6 (C-2), 54.8 (C-4), 48.4 (C-11), 32.6 (C-6), 28.4 (3× CH_3–Boc), 27.8 (C-5), 18.2 (C-1), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{29}H_{40}NO_6Si$ $[M + H]^+$: 526.2619, found: 526.2598. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 17

Fmoc–D-Glu(*O*-*t*Bu)–OTMSE (**19**) (1.00 g, 1.90 mmol) was dissolved in CH_2Cl_2 (30 mL). 2,6-Lutidine (4.42 mL, 38.0 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 3.44 mL, 19.0 mmol) were added at 0 °C and stirred for 30 min. The reaction was stirred for further 30 min at rt followed by slowly addition of aq. sat. NH_4Cl (30 mL). The aqueous phase was extracted with EtOAc (3 × 40 mL). The organic phase was washed with 30% AcOH/water, dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield 866 mg (1.84 mmol, 97%) of a white, amorphous solid.

R_f 0.44 (10% EtOAc/CH_2Cl_2). $[\alpha]_D^{25}$ = +19° (c = 1.04 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.79 (d, J = 7.5 Hz, 2H, $H_{arom.}$), 7.67 (t, J = 6.3 Hz, 2H, $H_{arom.}$), 7.39 (t, J = 7.5 Hz, 2H, $H_{arom.}$), 7.31 (td, J = 7.5, 1.1 Hz, 2H, $H_{arom.}$), 4.39–4.32 (m, 2H, H-9), 4.24–4.19 (m, 4H, H-2, H-4, H-10), 2.40 (t, J = 7.4 Hz, 2H, H-6), 2.19–2.12 (m, 1H, H-5), 1.95–1.88 (m, 1H, H-5), 1.02–0.99 (m, 2H, H-1), 0.04 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (126 MHz, CD_3OD) δ = 176.3 (C-7), 173.8 (C-3), 158.6 (C-8), 145.3 ($C_{arom.}$), 145.2 ($C_{arom.}$), 142.6 ($C_{arom.}$), 128.2 ($C_{arom.}$), 128.1 ($C_{arom.}$), 126.3 ($C_{arom.}$), 126.2 ($C_{arom.}$), 120.9 ($C_{arom.}$), 68.0 (C-9), 64.7 (C-2), 55.0 (C-4), 48.4 (C-10), 31.1 (C-6), 27.7 (C-5), 18.2 (C-1), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{25}H_{31}NO_6SiNa$ $[M + Na]^+$: 492.1813, found: 492.1817. The spectroscopic data were in agreement with those previously reported.

Synthesis of compound 28

Boc–D-Ala–OH (**27**) (1.50 g, 7.93 mmol) and DMAP (194 mg, 1.59 mmol) were dissolved in EtOAc (40 mL) and cooled to 0 °C. 2-(Trimethylsilyl)ethanol (**22**) (1.70 mL, 11.9 mmol) and DCC (1 M in CH_2Cl_2, 9.51 mL, 9.51 mmol) were added. After stirring for 2 h at rt, the reaction was filtered through Celite and the residue was washed with EtOAc (2 × 20 mL). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (10% EtOAc/cyclohexane) to yield 2.27 g (7.84 mmol, 99%) of a white, amorphous solid.

R_f 0.17 (10% EtOAc/cyclohexane). $[\alpha]_D^{25} = +31°$ (c = 2.00 in MeOH). ^{1}H NMR (700 MHz, CD_3OD) δ = 4.25–4.18 (m, 2H, H-2), 4.09 (q, J = 7.2 Hz, 1H, H-4), 1.44 (s, 9H, 3× CH_3–Boc), 1.33 (d, J = 7.2 Hz, 3H, H-5), 1.04–1.00 (m, 2H, H-1), 0.06 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, CD_3OD) δ = 175.2 (C-3), 157.9 (C-6), 80.5 (C-7), 64.4 (C-2), 50.8 (C-4), 28.7 (3× CH_3–Boc), 18.2 (C-1), 17.7 (C-5), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{13}H_{27}NO_4SiNa$ [M + Na]$^+$: 312.1597, found: 312.1602. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 26

Boc–D-Ala–TMSE **28** (220 mg, 760 μmol) was dissolved in 20% TFA/CH_2Cl_2 (4.5 mL). After stirring for 1 h at rt, toluene (15 mL) was added and the solvent was removed under reduced pressure to yield 144 mg (760 μmol, quant.) of a white, amorphous solid.

R_f 0.16 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = +1.2°$ (c = 0.82 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 4.35–4.31 (m, 2H, H-2), 4.00 (q, J = 7.2 Hz, 1H, H-4), 1.51 (d, J = 7.2 Hz, 3H, H-5), 1.09–1.06 (m, 2H, H-1), 0.07 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (126 MHz, CD_3OD) δ = 171.7 (C-3), 65.8 (C-2), 50.0 (C-4), 18.2 (C-1), 16.6 (C-5), −1.6 (3× CH_3). HRMS (ESI) m/z: calcd for $C_8H_{20}NO_2Si$ [M + H]$^+$: 190.1258, found: 190.1263. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 31

Fmoc–L-Lys(*N*-Boc)–OH (**29**) (1.50 g, 3.20 mmol) was dissolved in CH_2Cl_2 (20 mL) and TFA (20 mL) was added. The mixture was stirred for 20 min at rt. After removing the solvent under reduced pressure DMF (20 mL) and diisopropylethylamine (2.72 mL, 16.0 mmol) were added. 2-(Trimethylsilyl)ethyl *p*-nitrophenyl carbonate (**47**) (1.09 g, 3.84 mmol) was dissolved in DMF (6 mL) and transferred to the reaction solution. After stirring for 2 h at rt the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (eluting first with EtOAc, then with 10% MeOH/CH_2Cl_2/0.1% AcOH) to yield 1.79 g (3.81 mmol, 99%) of a colorless, amorphous solid.

R_f 0.42 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = -1.1°$ (c = 2.42 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.80 (d, J = 7.5 Hz, 2H, $H_{arom.}$), 7.67 (t, J = 7.4 Hz, 2H, $H_{arom.}$), 7.39 (t, J = 7.3 Hz, 2H, $H_{arom.}$), 7.31 (td, J = 7.5, 1.1 Hz, 2H, $H_{arom.}$), 4.37–4.34 (m, 2H, H-8), 4.23 (t, J = 6.9 Hz, 1H, H-9), 4.18–4.08 (m, 3H, H-11, H-2), 3.09 (t, J = 6.9 Hz, 2H, H-6), 1.89–1.82 (m, 1H, H-3), 1.73–1.65 (m, 1H, H-3), 1.55–1.48 (m, 2H, H-5), 1.45–1.39 (m, 2H, H-4), 0.95 (t, J = 8.6 Hz, 2H, H-12), 0.02 (s, 9H, TMS). ^{13}C NMR (126 MHz, CD_3OD) δ = 159.3 (C-10), 158.7 (C-7), 145.4 ($C_{arom.}$), 142.6 ($C_{arom.}$), 128.8 ($C_{arom.}$), 128.2 ($C_{arom.}$), 126.3 ($C_{arom.}$), 120.9 ($C_{arom.}$), 67.9 (C-8), 63.7 (C-11), 55.9 (C-2), 48.5 (C-9), 41.4 (C-6), 32.7 (C-3), 30.6 (C-5), 24.1 (C-4), 18.7 (C-12), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{27}H_{36}N_2O_6SiNa$ [M + Na]$^+$: 535.2235, found: 535.2231. The spectroscopic data were in agreement with those previously reported.[15]

Synthesis of compound 43

(a) Functionalization of the resin. A frit-containing syringe was loaded with 2-chlorotrityl chloride resin (**36**) (280 mg, initial loading 2.1 mmol g^{-1}). The resin was swelled for 20 min in 3 mL CH_2Cl_2. The solvent was removed and a solution of Fmoc–D-Ala–OH (91.5 mg, 0.29 mmol) and DIPEA (201 μL, 1.16 mmol) in 2 mL CH_2Cl_2 was added. The syringe was shaken for 1 h at rt. After draining the solvent, the resin was washed with DMF (5 × 1 min shaking with 2 mL). The initial resin loading 2.1 mmol g^{-1} was reduced in this step to 1.0 mmol g^{-1}. The new loading was determined by UV-metric Fmoc analysis after the first deprotection. The used amounts for the coupling solutions used in (c) were calculated with the new reduced loading of 1.0 mmol g^{-1}, which was determined as described in (c2).

(b) Capping of free reaction sites. A solution of DMF (900 μL), *N*-methylimidazole (600 μL) and acetic anhydride (300 μL) was added to the resin and the syringe was shaken for 45 min at rt. The reaction solution was removed and the resin was washed with DMF (5 × 1 min shaking with 2 mL).

(c) Deprotection (removal of Fmoc). The resin was shaken 5 min at rt with 20% piperidine/DMF (2 mL). The deprotection solution was removed and the resin was loaded again with 20% piperidine/DMF (2 mL) and was shaken for 15 min at rt. After removal of the deprotection solution, the resin was washed with DMF (4 × 1 min shaking with 2 mL), CH_2Cl_2 (3 × 1 min shaking with 2 mL) and again with DMF (3 × 1 min shaking with 2 mL).

(c2) Deprotection analysis. To get the new loading after (a) or to get predictions about the couplings from (d) the deprotection solutions after (a) or couplings (d) were collected in a 5 mL volumetric flask and filled up with 20% piperidine/DMF. A dilution series was prepared and the loadings were determined using a UV/Vis-spectrometer and the following formula:

$$\text{Loading}\left[\text{mmol g}^{-1}\right] = \frac{E \times V_{\text{stock}} \times D}{\varepsilon \times m \times l}$$

with: E = absorption of the sample solution at 301 nm, V_{stock} = volume of the stock solution [mL], D = dilution factor, ε = molar absorption coefficient at 301 nm = 6054 [L mol^{-1} cm^{-1}], m = sample weight of the resin [mg], l = optical path length of the cell = 1 [cm].

(d) Coupling. The resin was loaded with a solution of amino acid (for Fmoc–L-Lys(*N*-Teoc)–OH, Fmoc–D-Glu(OH)–TMSE 1.5 equiv. and Fmoc–L-Ala–OH 4 equiv.), HBTU (same equiv. as the used amino acid), HOBt (same equiv. as the used amino acid) and DIPEA (twice the equiv. of the used amino acid) in DMF (2 mL). The resin was shaken 40 min at rt, washed with DMF (3 × 1 min shaking with 2 mL) and the resin was loaded again with a fresh reaction solution of the same amino acid and was shaken for 40 min. After this double coupling procedure the resin was washed with DMF (5 × 1 min shaking with 2 mL).

(e) Cleavage from the solid phase. A solution of 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in CH_2Cl_2 (3 mL) was added to the dry resin and shaken for 3 h at rt. The solvent was collected and the resin was washed further 2× with 20% HFIP/CH_2Cl_2. The solvent was removed under reduced pressure and the residue dissolved in *t*BuOH/water and lyophilized to yield 219 mg (82%, 229 μmol) of the tetrapeptide as a white, amorphous solid.

R_f 0.19 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = -9.0°$ (c = 0.44 in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) δ = 12.48 (br, s, 1H,

COOH), 8.25 (d, J = 7.7 Hz, 1H, NH(Glu)), 8.17–8.13 (m, 1H, NH(Ala)), 7.90–7.88 (m, 3H, 2× $H_{arom.}$, NH(Lys)), 7.75–7.69 (m, 2H, $H_{arom.}$), 7.47 (d, J = 7.9 Hz, 1H, NH(Ala)), 7.41 (t, J = 7.4 Hz, 2H, $H_{arom.}$), 7.32 (t, J = 7.4 Hz, 2H, $H_{arom.}$), 6.95–6.93 (m, 1H, NH(Teoc)), 4.28–4.24 (m, 3H, H-4, H-12), 4.22–4.16 (m, 4H, H-8, H-13, H-10, H-2), 4.14–4.10 (m, 2H, H-23), 4.01–3.98 (m, 2H, H-20), 2.93–2.89 (m, 2H, H-18), 2.22–2.13 (m, 2H, H-6), 1.99–1.92 (m, 1H, H-7), 1.81–1.73 (m, 1H, H-7), 1.61–1.55 (m, 1H, H-15), 1.47–1.43 (m, 1H, H-15), 1.36–1.32 (m, 2H, H-17), 1.24 (d, J = 7.2 Hz, 3H, H-14), 1.24 (d, J = 7.2 Hz, 3H, H-25), 1.22–1.17 (m, 2H, H-16), 0.95–0.93 (m, 2H, H-24), 0.91–0.88 (m, 2H, H-21), 0.01 (s, 9H, $Si(CH_3)_3$), −0.00 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) δ = 174.0 (C-1), 172.4 (C-9), 171.5 (C-22), 171.1 (C-5), 170.8 (C-3), 156.0 (C-19), 155.3 (C-11), 149.3 ($C_{arom.}$), 140.4 ($C_{arom.}$), 127.3 ($C_{arom.}$H), 126.8 ($C_{arom.}$H), 125.0 ($C_{arom.}$H), 119.8 ($C_{arom.}$H), 65.4 (C-12), 62.3 (C-23), 61.0 (C-20), 51.9 (C-4), 51.5 (C-8), 49.6 (C-10), 47.2 (C-2), 46.4 (C-13), 39.8 (C-18), 31.8 (C-15), 31.0 (C-6), 28.9 (C-17), 26.9 (C-7), 22.3 (C-16), 18.4 (C-14), 17.1 (C-25), 16.9 (C-21), 16.5 (C-24), −1.4 ((Lys)$Si(CH_3)_3$), −1.5 ((Glu)$Si(CH_3)_3$). HRMS (ESI) m/z: calcd for $C_{43}H_{66}N_5O_{11}Si_2$ $[M + H]^+$: 884.4279, found: 884.4279. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 40

To a solution of Fmoc–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys(*N*-Teoc)–D-Ala–COOH (**43**) (25.0 mg, 28.7 μmol), PyBOB (22.1 mg, 42.4 μmol) and HOBt (5.73 mg, 42.4 μmol) in DMF (0.5 mL) was added dropwise a solution of H_2N–D-Ala-*O*–TMSE **26** (7.49 mg, 39.6 μmol), DIPEA (24.0 μL, 141 μmol) in DMF (0.5 mL). After stirring for 25 min at rt the solvent was removed under reduced pressure and the crude product was purified by HPLC (85% can/water, retention time 14.6 min, using a KNAUER Eurospher II 100-5 C18; 5 μm; 250 × 16 mm + precolumn 30 × 16 mm, 265 nm) to yield 28.3 mg (26.8 μmol, 95%) of a white, amorphous solid.

R_f 0.34 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +10° (c = 0.60 in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) δ = 8.24 (d, J = 7.7 Hz, 1H, NH(Glu)), 8.16 (d, J = 7.5 Hz, 1H, NH(D-Ala)), 8.14 (d, J = 7.5 Hz, 1H, NH(D-Ala)), 8.00 (d, J = 7.3 Hz, 1H, NH(Lys)), 7.89 (d, J = 7.4 Hz, 2H, $H_{arom.}$), 7.75–7.69 (m, 2H, $H_{arom.}$), 7.47 (d, J = 7.5 Hz, 1H, NH(L-Ala)), 7.41 (t, J = 7.4 Hz, 2H, $H_{arom.}$), 7.32 (t, 2H, J = 7.4 Hz, $H_{arom.}$), 6.94 (t, J = 5.4 Hz, 1H, NH(Teoc)), 4.30 (q, J = 7.5 Hz, 1H, H-6), 4.24 (d, J = 7.0 Hz, 2H, H-16), 4.21–4.07 (m, 12H, H-12, H-8, H-4, H-14, H-2, H-17, H-28), 4.01–3.99 (m, 2H, H-25), 2.92–2.90 (m, 2H, H-23), 2.21–2.12 (m, 2H, H-10), 1.95–1.90 (m, 1H, H-11), 1.80–1.75 (m, 1H, H-11), 1.59–1.54 (m, 1H, H-20), 1.47–1.44 (m, 1H, H-20), 1.35 (p, J = 6.8 Hz, 2H, H-22), 1.28 (d, J = 7.3 Hz, 3H, H-18), 1.23 (d, J = 7.5 Hz, 3H, H-30), 1.19 (d, J = 7.0 Hz, 3H, H-19), 1.26–1.15 (m, 2H, H-21), 0.94–0.88 (m, 6H, H-1, H-26, H-28), 0.01 (s, 9H, $Si(CH_3)_3$), 0.01 (s, 9H, $Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) δ = 172.2 (C-14), 171.9 (C-3), 171.3 (C-5), 171.0 (C-27), 171.0 (C-7), 171.0 (C-9), 156.2 (C-24), 155.4 (C-15), 143.6 ($C_{arom.}$), 140.6 ($C_{arom.}$), 127.5 ($C_{arom.}$H), 126.9 ($C_{arom.}$H), 125.2 ($C_{arom.}$H), 120.0 ($C_{arom.}$H), 65.5 (C-16), 62.4 (C-28), 62.3 (C-2), 61.2 (C-25), 52.7 (C-8), 51.6 (C-12), 49.7 (C-14), 47.6 (C-4), 47.4 (C-6), 46.5 (C-17), 39.7 (C-23), 31.3 (C-20), 31.1 (C-11), 29.1 (C-22), 26.9 (C-11), 22.5 (C-21), 18.5 (C-30), 17.9 (C-19), 17.3 (C-26), 16.7 (C-29), 16.6 (C-1), 16.6 (C-18), −1.6 ($Si(CH_3)_3$), −1.6 ($Si(CH_3)_3$), −1.7 ($Si(CH_3)_3$). HRMS (ESI) m/z: calcd for $C_{51}H_{81}N_6O_{12}Si_3Na$ $[M + Na]^+$: 1077.5191, found: 1077.5195. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 44

Fmoc–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys(*N*-Teoc)–D-Ala–D-Ala–*O*-TMSE **40** (100 mg, 94.7 μmol) was dissolved in 20% piperidine/DMF (1 mL). After stirring for 30 min at rt, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (10% MeOH/CH_2Cl_2) to yield 60.3 mg (72.4 μmol, 76%) of a white, amorphous solid.

R_f 0.23 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +23° (c = 0.44 in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) δ = 8.15 (d, J = 7.9 Hz, 1H, NH(D-Ala)), 8.14 (d, J = 7.9 Hz, 1H, NH(D-Ala)) 8.08 (d, J = 6.8 Hz, 1H, NH(Glu)), 8.00 (d, J = 7.3 Hz, 1H, NH(Lys)), 6.94 (t, J = 5.5 Hz, 1H, NH(Teoc)), 4.31–4.27 (m, 1H, H-6), 4.21–4.17 (m, 2H, H-4, H-12), 4.17–4.08 (m, 5H, H-8, H-2, H-25), 4.02–3.97 (m, 2H, H-22), 3.29 (q, J = 6.9 Hz, 1H, H-14) 2.91 (dt, J = 6.8 Hz, 2H, H-20), 2.52 (m, 2H, NH_2), 2.21–2.13 (m, 2H, H-10), 1.97–1.92 (m, 1H, H-11), 1.81–1.76 (m, 1H, H-11), 1.59–1.54 (m, 1H, H-17), 1.49–1.44 (m, 1H, H-17), 1.35 (p, J = 7.5 Hz, 2H, H-19), 1.28 (d, J = 7.3 Hz, 3H, H-15), 1.26–1.22 (m, 2H, H-18), 1.19 (d, J = 7.1 Hz, 3H, H-16), 1.11 (d, J = 6.9 Hz, 3H, H-27), 0.96–0.88 (m, 6H, H-1, H-23, H-26), 0.02 (s, 18H, 2× $Si(CH_3)_3$), 0.01 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) δ = 175.7 (C-13), 172.1 (C-3), 171.7 (C-5), 171.6 (C-24), 171.2 (C-9), 171.1 (C-7), 156.0 (C-21) 62.3 (C-25), 62.2 (C-2), 61.0 (C-22), 52.6 (C-8), 51.1 (C-12), 49.8 (C-14), 47.4 (C-4), 47.2 (C-6), 39.7 (C-20), 31.2 (C-17), 31.0 (C-10), 28.9 (C-19), 26.9 (C-11), 22.3 (C-18), 21.3 (C-27), 17.7 (C-16), 17.1 (C-23), 16.5 (C-26), 16.5 (C-1), 16.4 (C-15), −1.7 (2× $Si(CH_3)_3$), −1.8 ($Si(CH_3)_3$), −1.8 ($Si(CH_3)_3$). HRMS (ESI) m/z: calcd for $C_{36}H_{73}N_6O_{10}Si_3$ $[M + H]^+$: 833.4690, found: 833.4690. The spectroscopic data were in agreement with those previously reported.[34]

Synthesis of compound 35

Glycine (**34**) (250 mg, 3.33 mmol) was dissolved in 50% dioxane/water (8 mL) and NEt_3 (1.38 mL, 9.99 mmol) and *N*-(2-(trimethylsilyl)ethoxycarbonyloxy)succinimide (950 mg, 3.66 mmol) were added. The solution was stirred at rt and diluted with water (20 mL). Citric acid 0.5 M (aq.) was added until the pH turned 4. The aqueous phase was extracted with diethylether (3 × 40 mL) and the combined ether layer was washed twice with water (15 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield 714 mg (3.26 mmol, 98%) of a white, amorphous solid.

R_f 0.41 (10% MeOH/0.1% AcOH/CH_2Cl_2). ^{1}H NMR (500 MHz, CD_3OD) δ = 4.18–4.14 (m, 2H, H-4), 3.80 (s, 2H, H-2), 1.02–0.99 (m, 2H, H-5), 0.05 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (126 MHz, CD_3OD) δ = 173.7 (C-1), 159.4 (C-3), 64.2 (C-4), 43.0 (C-2), 18.6 (C-5), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_8H_{16}NO_4Si$ $[M - H]^-$: 218.0854, found: 218.0851. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 54

(a) Functionalization of the resin. A frit-containing syringe was loaded with 2-chlorotrityl chloride resin (137 mg, initial loading 2.1 mmol g^{-1}). The resin was swelled for 20 min in CH_2Cl_2 (2 mL). The solvent was removed and a solution of Fmoc–D-Ala–OH (44.7 mg, 142 μmol) and DIPEA (98.3 μL, 0.57 mmol) in CH_2Cl_2 (1 mL) was added. The syringe was shaken for 1 h at rt. After draining the solvent, the resin was washed with DMF (5 × 1 min shaking with 2 mL). The initial resin loading 2.1 mmol g^{-1} was reduced in this step to 1.0 mmol g^{-1}. The new loading was determined by UV-metric Fmoc analysis after the first deprotection. The used amounts for the coupling solutions used in (c) were calculated with the new reduced loading of 1.0 mmol g^{-1}, which was determined as described in (c2).

(b) Capping of free reaction sites. A solution of DMF (450 μL), *N*-methylimidazole (300 μL) and acetic anhydride (150 μL) was added to the resin and the syringe was shaken for 45 min at rt. The reaction solution was removed and the resin was washed with DMF (5 × 1 min shaking with 2 mL).

(c) Deprotection (removal of Fmoc). The resin was shaken 5 min at rt with 20% piperidine/DMF (2 mL). The deprotection solution was removed and the resin was loaded again with 20% piperidine/DMF (2 mL) and was shaken for 15 min at rt. After removal of the deprotection solution, the resin was washed with DMF (4 × 1 min shaking with 2 mL), CH_2Cl_2 (3 × 1 min shaking with 2 mL) and again with DMF (3 × 1 min shaking with 2 mL).

(c2) Deprotection analysis. To get the new loading after (a) or to get predictions about the couplings from (d) the deprotection solutions after (a) or couplings (d) were collected in a 5 mL volumetric flask and filled up with 20% piperidine/DMF. A dilution series was prepared and the loadings were determined using a UV/Vis-spectrometer and the following formula:

$$\text{Loading } \left[\text{mmol g}^{-1}\right] = \frac{E \times V_{\text{stock}} \times D}{\varepsilon \times m \times l}$$

with: E = absorption of the sample solution at 301 nm, V_{stock} = volume of the stock solution [mL], D = dilution factor, ε = molar absorption coefficient at 301 nm = 6054 [L mol^{-1} cm^{-1}], m = sample weight of the resin [mg], l = optical path length of the cell = 1 [cm].

(d) Coupling. The resin was loaded with a solution of amino acid (for Alloc–L-Lys(*N*-Fmoc)–OH, Fmoc–D-Glu(OH)–TMSE 1.5 equiv. and Fmoc–Gly–OH, Teoc–Gly–OH, Fmoc–L-Ala–OH 4 equiv.), HBTU (same equiv. as the used amino acid), HOBt (same equiv. as the used amino acid) and DIPEA (twice the equiv. of the used amino acid) in 2 mL DMF. The resin was shaken 40 min at rt washed with DMF (3 × 1 min shaking with 2 mL) and the resin was loaded again with a fresh reaction solution of the same amino acid and was shaken again for 40 min. After this double coupling procedure the resin was washed with DMF (5 × 1 min shaking with 2 mL).

(e) Cleavage from the solid phase. A solution of 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in CH_2Cl_2 (3 mL) was added to the dry resin and shaken for 3 h at rt. The solvent was collected and the resin was washed further 2 × with 20% HFIP/CH_2Cl_2. The solvent was added slowly to cold ether resulting in precipitation and after centrifugation the supernatant solvent was discarded and the precipitate dissolved in *t*BuOH/water and lyophilized to yield 133 mg (114 μmol, 83%) of the nonapeptide as a white, amorphous solid. After the glycine couplings and before the alloc removal a test cleavage from the resin was performed with 15.0 mg to yield 9.10 mg of the alloc protected heptapeptide.

(f) Deprotection (removal of alloc). The peptide containing resin was dried at HV and flushed with argon. CH_2Cl_2 (0.5 mL) were added and the suspension was stirred for 15 min at rt. Then, phenylsilane (591 μL, 4.79 mmol) in 1 mL CH_2Cl_2 and $Pd(PPh_3)_4$ (57.6 mg, 49.9 μmol) in 3 mL CH_2Cl_2 were added and the reaction was stirred for 2 h at rt. The suspension was filled back to a syringe containing a frit and the solvent was drained. The resin was washed with CH_2Cl_2 (6 × 1 min shaking with 3 mL), DMF (4 × 1 min shaking with 3 mL) and again with CH_2Cl_2 (4 × 1 min shaking with 3 mL). Before the next coupling the resin was swollen again with 2 mL of DMF for 20 min at rt and the solvent was drained.

Characterization data for compound 52 (test cleavage)

R_f 0.30 (20% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = -3.5°$ (c = 0.28 in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) δ = 8.22–8.16 (br, m, 2H, 2× NH(Gly)), 8.10 (t, J = 5.2 Hz, 2H, 2× NH(Gly)), 8.04 (s, 1H, NH(Ala)), 7.73 (t, J = 5.6 Hz, 1H, NH(Lys)), 7.27–7.23 (m, 2H, NH(Lys), NH(Teoc)), 5.93–5.87 (m, 1H, H-24), 5.29 (dd, J = 17.2 Hz (*trans* coupling), 1.5 Hz, 1H, H-25), 5.17 (d, J = 10.5 Hz (*cis* coupling), 1.2 Hz, 1H, H-25), 4.46 (d, J = 5.2 Hz, 2H, H-23), 4.17–4.12 (m, 1H, H-2), 4.05–4.02 (m, 2H, H-20), 3.98–3.96 (m, 1H, H-4), 3.75 (d, J = 5.7 Hz, 2H, H-14), 3.75 (d, J = 5.7 Hz, 2H, H-16), 3.73 (d, J = 5.8 Hz, 2H, H-12), 3.65 (d, J = 5.9 Hz, 2H, H-10), 3.62 (d, J = 6.0 Hz, 2H, H-18), 3.02 (q, J = 6.7 Hz, 2H, H-8), 1.63–1.54 (m, 1H, H-5), 1.52–1.47 (m, 1H, H-5), 1.40–1.34 (m, 2H, H-7), 1.31–1.26 (m, 2H, H-6), 1.24 (d, J = 7.2 Hz, 3H, H-26), 0.93–0.91 (m, 2H, H-21), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) δ = 174.0 (C-1), 171.5 (C-3), 169.7 (C-17), 169.3 (C-15), 169.3 (C-13), 169.0 (C-11), 168.4 (C-9), 156.7 (C-19), 155.7 (C-22), 133.6 (C-24), 116.9 (C-25), 64.4 (23), 61.9 (C-20), 54.4 (C-4), 47.7 (C-2), 43.5 (C-18), 42.1 (C-16), 42.0 (C-14), 42.0 (C-12), 42.0 (C-10), 38.3 (C-8), 31.8 (C-5), 28.6 (C-7), 22.7 (C-6), 17.6 (C-21), 17.3 (C-26), −1.4 ($Si(CH_3)_3$). HRMS (ESI) m/z: calcd for $C_{29}H_{49}N_8O_{12}Si$ $[M + H]^+$: 729.3245, found: 729.3241.

Characterization data for compound 54

R_f 0.21 (15% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = -2.8°$ (c = 0.70 in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) δ = 8.26 (d, J = 7.5 Hz, 1H, NH(Glu)), 8.19 (br, s, 2H, 2× NH(Gly)), 8.10–8.07 (m, 3H, 2× NH(Gly), NH–D-Ala), 7.91 (d, J = 8.2 Hz, 1H, NH(Lys)), 7.89 (d, J = 7.5 Hz, 2H, $H_{arom.}$), 7.74–7.71 (m, 3H, $NH(Lys_{side\ chain})$), 2× $H_{arom.}$), 7.48 (d, J = 7.0 Hz, 1H, NH–L-Ala), 7.41 (t, J = 7.8 Hz, 2H, $H_{arom.}$), 7.32 (t, J = 7.4 Hz, 2H, $H_{arom.}$), 7.23 (t, J = 5.9 Hz, 1H, NH(Teoc)), 4.25–4.10 (m, 9H, H-33, H-12, H-13, H-10, H-2, H-4, H-8), 4.04–4.02 (m, 2H, H-30), 3.75 (d, J = 5.7 Hz, 2H, H-24), 3.75 (d, J = 5.7 Hz, 2H, H-26), 3.73 (d, J = 5.8 Hz, 2H, H-22), 3.65 (d, J = 5.8 Hz, 2H, H-20), 3.62 (d, J = 5.9 Hz, 2H, H-28), 3.01 (q, J = 6.4 Hz, 2H, H-18), 2.22–2.14 (m, 2H, H-6), 1.98–1.93 (m, 1H, H-

7), 1.81–1.75 (m, 1H, H-7), 1.62–1.57 (m, 1H, H-15), 1.47–1.46 (m, 1H, H-15), 1.36 (p, $J = 7.1$ Hz, 2H, H-17), 1.24 (d, $J = 7.0$ Hz, 3H, H-35), 1.23 (d, $J = 7.3$ Hz, 3H, H-14), 1.28–1.19 (m, 2H, H-16), 0.95–0.90 (m, 4H, H-31, H-34), 0.02 (s, 9H, $Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) $\delta = 174.0$ (C-1), 172.7 (C-9), 171.7(C-3), 171.3 (C-32), 171.1 (C-5), 169.7 (C-27), 169.3 (C-25), 169.3 (C-23), 169.0 (C-21), 168.4 (C-19), 156.7 (C-29), 155.6 (C-11), 143.8 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}H$), 127.1 ($C_{arom.}H$), 125.3 ($C_{arom.}H$), 120.1 ($C_{arom.}H$), 65.7 (C-12), 62.6 (C-33), 61.9 (C-30), 52.3 (C-4), 51.7 (C-8), 49.8 (C-10), 47.6 (C-2) 46.6 (C-13), 43.5 (C-28), 42.1 (C-24), 42.1 (C-22), 42.0 (C-26), 42.0 (C-20), 38.4 (C-18), 31.9 (C-15), 31.3 (C-6) 28.7 (C-17), 27.1 (C-7), 22.6 (C-16), 18.6 (C-14), 17.3 (C-35), 16.8 (C-31), 16.8 (C-34), −1.4 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). HRMS (ESI) *m*/*z*: calcd for $C_{53}H_{79}N_{10}O_{16}Si_2$ $[M + H]^+$: 1167.5220, found: 1167.5216. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of alanine derivative of 54 (compound 68)

Fmoc–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys((Gly)$_5$–Teoc)–D-Ala–COOH (**54**) (60.0 mg, 51.3 μmol), PyBOB (40.1 mg, 76.9 μmol), HOBt (10.4 mg, 76.9 μmol) and H_2N–D-Ala–*O*-TMSE **26** (13.6 mg, 71.8 μmol) were dissolved in DMF (2 mL) and DIPEA (34.9 μL, 205 μmol) was added immediately. After stirring for 40 min at rt the solvent was removed under reduced pressure and the crude product was dissolved in a minimum DMF and added to ice cold diethyl ether. The formed precipitate was filtered and washed with cold ether and dichloromethane, redissolved in MeOH and the solvent was removed under reduced pressure to yield to yield 38.5 mg (28.7 μmol, 56%) of a white, amorphous solid.

R_f 0.27 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = +7.4°$ ($c = 0.68$ in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) $\delta = 8.24$ (d, $J = 7.6$ Hz, 1H, NH(Glu)), 8.17–8.12 (m, 4H, 2× NH–D-Ala, 2× NH(Gly)), 8.08–8.04 (m, 2H, 2× NH(Gly)), 8.01 (d, $J = 7.4$ Hz, 1H, NH(Lys)), 7.89 (d, $J = 7.6$ Hz, 2H, $H_{arom.}$), 7.73–7.70 (m, 3H, NH($Lys_{side\ chain}$), 2× $H_{arom.}$), 7.47 (d, $J = 7.0$ Hz, 1H, NH–L-Ala), 7.41 (t, $J = 7.4$ Hz, 2H, $H_{arom.}$), 7.32 (t, $J = 7.3$ Hz, 2H, $H_{arom.}$), 7.22 (t, $J = 6.0$ Hz, 1H, NH(Teoc)), 4.31 (q, $J = 7.6$ Hz, 1H, H-6), 4.25–4.07 (m, 11H, H-2, H-38, H-16, H-17, H-14, H-12, H-8, H-4), 4.04–4.02 (m, 2H, H-35), 3.75 (d, $J = 5.6$ Hz, 2H, H-31), 3.75 (d, $J = 5.6$ Hz, 2H, H-29), 3.73 (d, $J = 5.8$ Hz, 2H, H-27), 3.65 (d, $J = 5.8$ Hz, 2H, H-25), 3.62 (d, $J = 6.0$ Hz, 2H, H-33), 3.02–2.99 (m, 2H, H-23), 2.22–2.12 (m, 2H, H-10), 1.95–1.90 (m, 1H, H-11), 1.80–1.76 (m, 1H, H-11), 1.60–1.56 (m, 1H, H-20), 1.50–1.45 (m, 1H, H-20), 1.39–1.35 (m, 2H, H-22), 1.28 (d, $J = 7.3$ Hz, 3H, H-18), 1.27–1.20 (m, 2H, H-21), 1.23 (d, $J = 7.1$ Hz, 3H, H-40), 1.19 (d, $J = 7.1$ Hz, 3H, H-19), 0.94–0.90 (m, 6H, H-1, H-36, H-39), 0.02 (s, 9H, $Si(CH_3)_3$), 0.01 (s, 9H, $Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) $\delta = 172.7$ (C-13), 172.4 (C-3), 172.0 (C-5), 171.7 (C-7), 171.5 (C-37), 171.4 (C-9), 169.7 (C-32), 169.3 (C-30), 169.3 (C-28), 169.0 (C-26), 168.4 (C-24), 156.7 (C-34), 155.6 (C-15), 143.8 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}$), 127.1 ($C_{arom.}$), 125.3 ($C_{arom.}$), 120.1 ($C_{arom.}$), 65.7 (C-16), 62.6 (C-38), 62.5 (C-2), 61.9 (C-35), 52.8 (C-8), 51.7 (C-12), 49.8 (C-14), 47.7 (C-4), 47.5 (C-6), 46.6 (C-17), 43.5 (C-33), 42.1 (C-29), 42.1 (C-27), 42.0 (C-31), 42.0 (C-25), 38.4 (C-23), 31.4 (C-20), 31.2 (C-10), 28.8 (C-22), 27.0 (C-11), 22.7 (C-21), 18.7 (C-40), 18.1 (C-19), 17.3 (C-18), 16.8 (C-36), 16.7 (C-1), 16.7 (C-39), −1.4 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). HRMS (ESI) *m*/*z*: calcd for $C_{61}H_{97}N_{11}O_{17}Si_3Na$ $[M + Na]^+$: 1362.6264, found: 1362.6238. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 45

Fmoc–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys((Gly)$_5$–Teoc)–D-Ala–D-Ala–*O*-TMSE (**68**) (35.0 mg, 26.1 μmol) was dissolved in 20% piperidine/DMF (2 mL). After stirring for 30 min at rt the solvent was removed under reduced pressure. Cold diethyl ether was added and the resulting precipitate was filtered, washed with cold diethyl ether, dissolved in MeOH and the solvent was removed under reduced pressure to yield 25.5 mg (22.8 μmol, 87%) of a white, amorphous solid.

R_f 0.53 (22% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = +10°$ ($c = 0.99$ in MeOH). ^{1}H NMR (700 MHz, $(CD_3)_2SO$) $\delta = 8.22$–8.11 (m, 5H, NH(Glu), 2× NH–D-Ala, 2× NH(Gly)), 8.07 (dt, $J = 11.3$, 5.7 Hz, 2H, 2× NH(Gly)), 8.01 (d, $J = 7.4$ Hz, 1H, NH(Lys)), 7.73 (t, $J = 5.5$ Hz, 1H, NH($Lys_{side\ chain}$)), 7.22 (t, $J = 5.9$ Hz, 1H, NH(Teoc)), 4.29 (p, $J = 7.1$ Hz, 1H, H-6), 4.20–4.08 (m, 7H, H-2, H-4, H-8, H-12, H-35), 4.06–3.99 (m, 2H, H-32), 3.75 (d, $J = 5.5$ Hz, 2H, H-28), 3.75 (d, $J = 5.5$ Hz, 2H, H-26) 3.73 (d, $J = 5.8$ Hz, 2H, H-24), 3.65 (d, $J = 5.8$ Hz, 2H, H-22), 3.62 (d, $J = 6.0$ Hz, 2H, H-30), 3.29 (q, $J = 6.9$ Hz, 1H, H-14), 3.01 (q, $J = 6.9$ Hz, 2H, H-20), 2.52 (m, 2H, NH_2), 2.20–2.14 (m, 2H, H-10), 1.97–1.95 (m, 1H, H-11), 1.80–1.78 (m, 1H, H-11), 1.61–1.56 (m, 1H, H-17), 1.49–1.46 (m, 1H, H-17), 1.39–1.34 (m, 2H, H-19), 1.28 (d, $J = 7.3$ Hz, 3H, H-15), 1.28–1.19 (m, 2H, H-18), 1.19 (d, $J = 7.1$ Hz, 3H, H-16), 1.16 (d, $J = 6.9$ Hz, 3H, H-37), 0.96–0.91 (m, 6H, H-1, H-26, H-33), 0.02 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, $(CD_3)_2SO$) $\delta = 172.4$ (C-3), 172.0 (C-5), 171.8 (C-7), 171.5 (C-34), 171.4 (C-9), 169.7 (C-29), 169.3 (C-27), 169.3 (C-25), 169.0 (C-23), 168.4 (C-21), 156.7 (C-31), 62.7 (C-35), 62.5 (C-2), 61.9 (C-32), 52.8 (C-8), 51.5 (C-12), 49.8 (C-14), 47.7 (C-4), 47.5 (C-6), 43.5 (C-30), 42.1 (C-26), 42.1 (C-24), 42.0 (C-28), 42.0 (C-22), 38.4 (C-20), 31.4 (C-17), 31.2 (C-10), 28.8 (C-19), 27.1 (C-11), 22.7 (C-18), 20.9 (C-37) 18.0 (C-16), 17.3 (C-15), 16.8 (C-33), 16.8 (C-1), 16.7 (C-39), −1.4 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). HRMS (ESI) *m*/*z*: calcd for $C_{46}H_{88}N_{11}O_{15}Si_3$ $[M + H]^+$: 1118.5764, found: 1118.5750. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 33

L-Ala (250.0 mg, 2.81 mmol, 1.00 equiv.) was dissolved in 50% dioxane/water (6.7 mL) and triethylamine (1.2 mL, 8.43 mmol, 3.00 equiv.) and Teoc–OSu (802 mg, 3.1 mmol, 1.10 equiv.) were added. The reaction mixture was stirred overnight at room temperature and then diluted with water (17 mL). Afterwards a 0.5 M solution of citric acid in water was added until the pH turned 4. The aqueous phase was extracted with diethyl ether (3 × 35 mL) and the organic phase was washed with water (2 × 13 mL). The combined organic phases were dried over magnesium

sulfate and the solvent was removed under reduced pressure to yield 656 mg (2.81 mmol, quant.) of a colorless, amorphous solid. The product was used without further purification.

$R_f = 0.55$ (10% MeOH/0.1% AcOH/CH_2Cl_2). $[\alpha]_D^{25} = +3.4°$ (c = 1.20 in MeOH). ^{1}H-NMR (500 MHz, CD_3OD) δ = 15.93 (br, 1H, COOH), 7.05 (d, J = 7.2 Hz, 1H, NH), 4.17 (t, J = 6.8 Hz, 1H, H-2), 4.14 (t, J = 7.1 Hz, 2H, H-4), 1.37 (d, J = 7.3 Hz, 3H, H-6), 1.02–0.98 (m, 2H, H-5), 0.05 (s, 9H, TMS). ^{13}C-NMR (126 MHz, CD_3OD) δ = 176.6 (C-1), 158.7 (C-3), 64.0 (C-4), 50.7 (C-2), 18.6 (C-5), 17.9 (C-6), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_9H_{20}NO_4Si$ $[M + H]^+$: 323.1633, found: 323.1639.

Synthesis of compound 56

(a) Funtionalization of the resin. The 2-chloro-trityl resin (**36**) (200 mg, initial loading: 1.39 mmol g^{-1}) was filled in a syringe containing a frit. First the resin was swollen in CH_2Cl_2 (2 mL) for 20 min. The solvent was removed and a solution of Fmoc–D-Ala–OH (173 mg, 556 μmol, 2.00 equiv.) and DIPEA (95.2 μL, 556.0 μmol, 2.00 equiv.) in CH_2Cl_2 (2 mL) was added. The syringe was shaken for 1 h at room temperature. Afterwards the solvent was discarded and the resin washed with DMF (5 × 1 min shaking with 2 mL).

(b) Deprotection (removal of Fmoc). To remove the Fmoc-protection group a solution of 20% piperidine/DMF (2 mL) was added to the resin and shaken for 5 min at room temperature. The deprotection solution was discarded and again 2 mL of a 20% piperidine/DMF solution were added. The syringe was shaken for 15 min at room temperature. Afterwards the resin was washed with DMF (4 × 1 min shaking with 2 mL), CH_2Cl_2 (3 × 1 min shaking with 2 mL) and again DMF (3 × 1 min shaking with 2 mL).

(c) Coupling. To introduce a new amino acid to the growing chain the resin was loaded with a solution of the amino acid (for Fmoc–L-Lys(Teoc)–OH, Fmoc–D-Glu(OH)–OTMSE 1.50 equiv. and Fmoc–L-Ala–OH·H_2O 4.00 equiv.), HBTU (same equivalents as the used amino acid), HOBt (same equivalents as the used amino acid) and DIPEA (twice the equivalents as the used amino acid) in DMF (2 mL). The resin was shaken for 30 min at room temperature, washed with DMF (3 × 1 min shaking with 2 mL) and loaded again with fresh reaction solution. After the double coupling procedure the resin was washed with DMF (5 × 1 min shaking with 2 mL).

(d) Cleavage from the solid phase. The resin was loaded with 2 mL of a 20% HFIP/CH_2Cl_2 solution and shaken for 3 h at room temperature. The solvent was collected and the resin washed with the cleavage solution (2 × 1 min with 2 mL). To the combined organic phases ice cold diethyl ether was added and the suspension centrifuged. The supernatant was discarded and the precipitant dissolved in ^{t}BuOH/H_2O and lyophilised to yield 213.4 mg of a colorless, amorphous solid (207.9 μmol, 75%).

(e) Deprotection (removal of alloc). After the completed synthesis of the side chain the peptide containing resin was dried at HV and flushed with argon. CH_2Cl_2 (0.73 mL) was added and the suspension was stirred 15 min at room temperature. Afterwards phenylsilane (722 μL, 5.85 mmol) in 1.5 mL CH_2Cl_2 and $Pd(PPh_3)_4$ (80.3 mg, 69.49 μmol) in 4.4 mL CH_2Cl_2 were added and the reaction was stirred for 2 h at room temperature. The suspension was filled back to a syringe containing a frit and the solvent was drained. The resin was washed with CH_2Cl_2 (6 × 1 min shaking with 4 mL), DMF (4 × 1 min shaking with 4 mL) and again with CH_2Cl_2 (4 × 1 min shaking with 4 mL). Before the next coupling the resin was swollen again with 2 mL of DMF for 20 min at room temperature and the solvent was discarded.

R_f = 0.24 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = -5.8°$ (c = 0.88 in MeOH). ^{1}H-NMR (700 MHz, $(CD_3)_2SO$) δ = 12.53 (br, 1H, COOH), 8.26 (d, J = 7.6 Hz, 1H, NH–Glu), 8.09 (m, J = 7.6 Hz, 1H, NH–D-Ala), 7.91 (d, J = 8.4 Hz, 1H, NH–Lys), 7.89 (d, J = 7.5 Hz, 2H, $H_{arom.}$), 7.83 (m, 1H, NH–Ala), 7.77 (m, 1H, NH($Lys_{side\ chain}$)), 7.73 (dd, J = 7.8 Hz, 1.8 Hz, 2H, $H_{arom.}$), 7.47 (d, J = 7.8 Hz, 1H, NH–Ala), 7.41 (td, J = 7.4 Hz, 1.1 Hz, 2H, $H_{arom.}$), 7.32 (td, J = 7.4 Hz, 1.2 Hz, 2H, $H_{arom.}$), 7.20 (d, J = 7.5 Hz, 1H, NH(Teoc)), 4.25 (s, 1H, H-4), 4.24 (s, 2H, H-12), 4.22–4.15 (m, 4H, H-2, H-8, H-10, H-13), 4.15–4.09 (m, 2H, H-29), 4.05–3.97 (m, 2H, H24), 3.06–3.00 (m, 1H, H-18), 2.98–2.92 (m, 1H, H-18), 2.23–2.13 (m, 2H, H-6), 1.99–1.92 (m, 1H, H-7), 1.83–1.75 (m, 1H, H-7), 1.63–1.56 (m, 1H, H-15), 1.49–1.42 (m, 1H, H-15), 1.39–1.31 (m, 2H, H-17), 1.24 (d, J = 5.1 Hz, 3H, H-31), 1.23 (d, J = 5.1 Hz, 3H, H-14), 1.25–1.22 (dd, J = 7.2 Hz, 5.1 Hz, 2H, H-20), 1.17 (d, J = 7.2 Hz, 8H, H-16, H-26, H-27), 0.95–0-90 (dt, J = 8.7 Hz, 16.4 Hz, 4H, H-25, H-30), 0.01 (s, 9H, TMS), −0.00 (s, 9H, TMS). ^{13}C-NMR (176 MHz, $(CD_3)_2SO$) δ = 173.9 (C-1), 172.7 (C-9), 171.8 (C-3), 171.4 (C-28), 171.1 (C-5), 169.0 (C-21), 168.4 (C-19), 156.8 (C-23), 155.9 (C-11), 143.8 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}$–H), 127.1 ($C_{arom.}$–H), 125.3 ($C_{arom.}$–H), 120.1 ($C_{arom.}$–H), 65.7 (C-12), 62.6 (C-29), 61.8 (C-24), 52.2 (C-4), 51.7 (C-8), 50.5 (C-20), 50.3 (C-22), 49.8 (C-10), 48.1 (C-2), 46.6 (C-13), 38.3 (C-18), 31.9 (C-15), 31.3 (C-6), 28.6 (C-17), 27.1 (C-7), 22.6 (C-16), 18.5 (C-14), 18.5 (C-26), 17.8 (C-27), 17.3 (C-31), 16.8 (C-25), 16.8 (C-30), −1.5 (3× CH_3), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{49}H_{76}N_7O_{13}Si_2$ $[M + H]^+$: 1026.5034, found: 1026.5034.

Synthesis of alanine derivative of 56 (compound 69)

The free acid **56** (210.3 mg, 204.6 μmol, 1.00 equiv.), PyBOP (160 mg, 307 μmol, 1.50 equiv.), HOBt (41.5 mg, 307 μmol, 1.50 equiv.) and 2-(trimethylsilyl)ethyl–D-alaninate (**26**) (55.7 mg, 287 μmol, 1.40 equiv.) were dissolved in DMF (7.0 mL). DIPEA (139 μL, 818 μmol, 4.00 equiv.) was added directly and the reaction mixture was stirred for 40 min at room temperature. The solvent was removed under reduced pressure and the residue dissolved in minimum DMF. Ice cold diethyl ether was added to the solution and the precipitate was filtered and washed with cold diethyl ether (two times) and CH_2Cl_2 (two times). The precipitate was dissolved in MeOH and the solvent removed under reduced pressure to yield 227 mg of a colorless, amorphous solid (190 μmol, 93%). The product was used for the next reaction without further purification.

R_f = 0.34 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = +12°$ (c = 0.66 in MeOH). ^{1}H-NMR (500 MHz, $(CD_3)_2SO$) δ = 8.24 (d, J = 7.3 Hz, 1H, NH–Glu), 8.16 (d, J = 6.9 Hz, 1H, NH–D-Ala), 8.14 (d, J = 7.9 Hz, 1H, NH–D-Ala), 7.97 (d, J = 8.3 Hz, 1H, NH–Lys), 7.88 (d, J = 7.6 Hz, 2H, $H_{arom.}$), 7.80 (d, J = 7.3 Hz, 1H, NH–Ala), 7.78–7.75

(m, 1H, NH($Lys_{side\ chain}$)), 7.72 (t, J = 7.3 Hz, 2H $H_{arom.}$), 7.46 (d, J = 7.9 Hz, 1H, NH–Ala), 7.41 (t, J = 7.6 Hz, 2H, $H_{arom.}$), 7.32 (t, J = 6.9 Hz, 2H, $H_{arom.}$), 7.19 (d, J = 7.8 Hz, 1H, NH(Teoc)), 4.30 (quint, J = 7.5 Hz, 1H, H-6), 4.26–4.23 (m, 2H, H-16), 4.23–4.15 (m, 4H, H-17, H-12, H-14, H-4), 4.13–4.06 (m, 5H, H-8, H-34, H-2), 4.06–3.96 (m, 2H, H-29), 3.63–3.58 (m, 2H, H-20), 3.17–3.10 (m, 2H, H-22), 3.03–3.00 (m, 2H, H-23), 2.21–2.12 (m, 2H, H-10), 1.97–1.89 (m, 1H, H-11), 1.83–1.76 (m, 1H, H-11), 1.28 (m, 3H, H-18), 1.25 (m, 2H, H-27, H-25), 1.22 (m, 3H, H-36), 1.20 (m, 3H, H-19), 1.17 (m, 8H, H-31, H-32, H-21), 0.95–0.89 (m, 6H, H-30, H-1, H-35), 0.01 (s, 9H, TMS), 0.01 (s 9H, TMS), −0.00 (s, 9H, TMS). ^{13}C-NMR (126 MHz, $(CD_3)_2SO$) δ = 172.3 (C-3), 172.1 (C-13), 172.0 (C-5), 171.7 (C-7), 171.4 (C-9), 169.0 (C-26), 168.5 (C-24), 157.1 (C-28), 156.0 (C-15), 143.9 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}$–H), 127.0 ($C_{arom.}$–H), 125.3 ($C_{arom.}$–H), 120.1 ($C_{arom.}$–H), 65.7 (C-16), 62.6 (C-34), 62.5 (C-2), 61.8 (C-29), 53.6 (C-8), 51.7 (C-12), 50.0 (C-14), 49.8 (C-6), 48.0 (C-25), 47.7 (C-4), 46.6 (C-17), 45.9 (C-27), 38.3 (C-23), 31.7 (C-20), 31.2 (C-10), 28.6 (C-22), 27.1 (C-11), 22.6 (C-21), 18.6 (C-31), 18.5 (C-19), 18.1 (C-32), 18.1 (C-36), 17.4 (C-18), 16.8 (C-30), 16.7 (C-1), 16.7 (C-35), −1.5 (3× CH_3), −1.5 (3× CH_3), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{57}H_{92}N_8O_{14}Si_3Na$ $[M + Na]^+$: 1219.5939, found: 1219.5934. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 55

Fmoc–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys((L-Ala)$_2$–Teoc)–D-Ala–D-Ala–*O*-TMSE (**69**) (223 mg, 186 μmol, 1.00 equiv.) was dissolved in 20% piperidine/DMF (14.4 mL) and stirred for 2 h at room temperature. The solvent was removed under reduced pressure and cold diethyl ether was added. The formed precipitate was filtered and washed with diethyl ether. Afterwards it was dissolved in MeOH and the solvent was removed under reduced pressure to yield 136.1 mg of a colorless, amorphous solid (140 μmol, 75%), which was used in the next reaction without further purification.

R_f = 0.28 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +21° (c = 0.66 in MeOH). ^{1}H-NMR (700 MHz, $(CD_3)_2SO$) δ = 8.18 (d, J = 6.9 Hz, 1H, NH–D-Ala), 8.14 (d, J = 7.9 Hz, 1H, NH–D-Ala), 8.01 (d, J = 7.5 Hz, 1H, NH–Glu), 7.80 (d, J = 7.7 Hz, 1H, NH–Lys), 7.76 (d, J = 5.7 Hz, 1H, NH($Lys_{side\ chain}$)), 7.50 (d, J = 8.2 Hz, 1H, NH–Ala), 7.19 (d, J = 7.4 Hz, 1H, NH(Teoc)), 4.30 (quint, J = 7.6 Hz, 1H, H-6), 4.23–4.16 (m, 2H, H-4, H-12), 4.16–4.11 (dd, J = 9.1 Hz, 7.8 Hz, 2H, H-8, H-14), 4.11–4.08 (td, J = 8.4 Hz, 1.3 Hz, 2H, H-31), 4.06–3.97 (m, 4H, H-2, H-26), 3.07–2.93 (m, 2H, H-20), 2.52 (t, J = 2.0 Hz, 2H, NH_2), 2.23–2.13 (m, 2H, H-10), 1.99–1.93 (m, 1H, H-11), 1.83–1.77 (m, 1H, H-11), 1.77–1.73 (m, 1H, H-22), 1.65–1.61 (m, 1H, H-24), 1.61–1.52 (m, 1H, H-17), 1.50–1.43 (m, 1H, H-17), 1.41–1.33 (m, 2H, H-19), 1.28 (d, J = 7.3 Hz, 3H, H-15), 1.23 (d, J = 6.4 Hz, 3H, H-33), 1.18 (d, J = 7.2 Hz, 3H, H-16), 1.17 (dd, J = 7.1 Hz, 2.6 Hz, 8H, H-28, H-29, H-18), 0.96–0.91 (m, 6H, H-1, H-27, H-32), 0.03 (s, 9H, TMS), 0.02 (s, 9H, TMS), 0.02 (s, 9H, TMS). ^{13}C-NMR (176 MHz, $(CD_3)_2SO$) δ = 172.4 (C-3), 172.1 (C-13), 172.0 (C-5), 171.8 (C-7), 171.6 (C-30), 171.3 (C-9), 169.1 (C-23), 168.5 (C-21), 156.8 (C-25), 62.7 (C-31), 62.5 (C-2), 61.8 (C-26), 53.0 (C-8), 51.6 (C-12), 50.0 (C-14), 48.1 (C-22), 47.7 (C- 4), 47.5 (C-6), 45.9 (C-24), 38.3 (C-20), 31.5 (C-17), 31.2 (C-10), 28.7 (C-19), 27.1 (C-11), 22.6 (C-18), 18.5 (C-28), 18.1 (C-16), 18.1 (C-33), 18.0 (C-29), 17.4 (C-15), 16.9 (C-27), 16.8 (C-1), 16.7 (C-32), −1.4 (3× CH_3), −1.5 (3× CH_3), −1.5 (3× CH_3). HRMS (ESI) m/z: calcd for $C_{42}H_{83}N_8O_{12}Si_3$ $[M + H]^+$: 975.5433, found: 975.5436. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 8

Trichloroacetonitrile (TCA, 433 μL, 4.32 mmol) was added to farnesol (449 μL, 1.80 mmol). To this stirred solution was added tetrabutylammonium phosphate (TBAP, 0.4 M in acetonitrile, 8.99 mL, 3.60 mmol) over 1 h *via* syringe pump. The reaction was stirred for further 7 h at rt and the solvent was removed under reduced pressure. The crude product was first purified by flash chromatography (10% water/20% NH_3 (conc.)/isopropanol) and then percolated through Dowex® 50WX8 with a 0.025 M NH_4HCO_3 solution. Dowex® 50WX8 was washed before usage with 3 : 1 NH_3/water and 0.025 M NH_4HCO_3 solution until pH of the eluent turned 8. The solvent was removed by lyophilisation to yield 425 mg (1.27 mmol, 71%) of a white powder.

R_f 0.14 (10% water/20% NH_3 (conc., aq.)/isopropanol). ^{1}H NMR (500 MHz, D_2O) δ = 5.40 (t, J = 6.4 Hz, 1H, H-2), 5.12 (t, J = 7.5 Hz, 1H, H-6), 5.08 (t, J = 6.8 Hz, 1H, H-10), 4.38 (s, 2H, H-1), 2.14–1.88 (m, 8H, H-4, H-5, H-8, H-9), 1.69 (s, 3H, H-13), 1.64 (s, 3H, H-15), 1.58 (s, 3H, H-14), 1.56 (s, 3H, H-12). ^{13}C NMR (126 MHz, D_2O) δ = 141.4 (C-3), 135.0 (C-7), 130.8 (C-11), 124.5 (C-10), 124.1 (C-6), 120.2 (d, J = 7.3 Hz, C-2), 61.9 (d, J = 4.8 Hz, C-1), 39.6 (C-4), 39.5 (C-5), 26.6 (C-8), 26.4 (C-9), 25.2 (C-15), 17.2 (C-12), 15.9 (C-13), 15.6 (C-14). ^{31}P NMR (202 MHz, D_2O) δ = 0.76. HRMS (ESI) m/z: calcd for $C_{15}H_{26}O_4P$ $[M]^{2-}$: 301.1574, found: 301.1575. The spectroscopic data were in agreement with those previously reported.[11]

Synthesis of compound 60

H_2N–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys(*N*-Teoc)–D-Ala–D-Ala–*O*-TMSE **44** (45.6 mg, 54.7 μmol), PyBOB (32.9 mg, 63.1 μmol), HOBt (8.53 mg, 63.1 μmol) and **7** (27.0 mg, 42.1 μmol) were dissolved in DMF (2 mL) and DIPEA (35.8 μL, 210 μmol) was added immediately. After stirring for 45 min at rt the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (2.5% MeOH/CH_2Cl_2) to yield 49.8 mg (34.2 μmol, 81%) of a white, crystalline solid.

R_f 0.35 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +41° (c = 0.32 in MeOH). ^{1}H NMR (700 MHz, CD_3OD) δ = 7.49–7.48 (m, 2H, $H_{arom.}$), 7.39–7.33 (m, 13H, $H_{arom.}$), 5.85 (dd, J = 5.9, 3.5 Hz, 1H, H-19), 5.63 (s, 1H, H-22), 5.15–5.08 (m, 4H, 2× CH_2–Ph), 4.38 (q, J = 7.1 Hz, 1H, H-16), 4.38 (q, J = 7.1 Hz, 1H, H-6), 4.36–4.31 (m, 3H, H-4, H-12, H-14), 4.20–4.11 (m, 8H, H-2, H-8, H-18, H-31, H-34), 4.04 (dd, J = 9.9, 4.1 Hz, 1H, H-23), 3.85–3.79 (m, 3H, H-17, H-20, H-21), 3.77 (dd, J = 9.9 Hz, J = 9.9 Hz, 1H, H-23), 3.11–3.07 (m, 2H, H-29), 2.26 (t, J = 7.2 Hz, 2H, H-10), 2.22–2.15 (m, 1H, H-11), 1.93–1.89 (m, 1H, H-11′), 1.85 (s, 3H, CH_3–NHAc), 1.79–1.74 (m, 1H, H-26), 1.69–1.63 (m, 1H, H-26), 1.48 (p, J = 7.0 Hz, 2H, H-28), 1.39 (d, J = 7.3 Hz, 3H, CH_3), 1.37 (d, J = 7.1 Hz, 3H, CH_3),

1.35 (d, $J = 7.2$ Hz, 1H, CH_3), 1.36 (d, $J = 6.8$ Hz 3H, CH_3), 1.37–1.32 (m, 2H, H-27), 1.02–0.95 (m, 6H, H-1, H-32, H-35), 0.04 (s, 9H, $Si(CH_3)_3$), 0.04 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, CD_3OD) $\delta = 175.6$ (C=O), 174.9 (C=O), 174.7 (C=O), 174.6 (C=O), 174.4 (C=O), 174.1 (C=O), 173.8 (NHAc–C=O), 172.9 (C=O), 159.5 (not resolved in ^{13}C-cpd but hmbc correlation, C-40), 138.9 ($C_{arom.,quart.}$–Ph), 137.0 (d, $J = 6.5$ Hz, $C_{arom.,quart.}$–Bn), 137.0 (d, $J = 6.5$ Hz, $C_{arom.,quart.}$–Bn), 130.0 ($C_{arom.}$), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.3 ($C_{arom.}$), 129.2($C_{arom.}$), 129.2 ($C_{arom.}$), 127.3 ($C_{arom.}$), 102.8 (C-22), 97.9 (C-19), 82.3 (C-21), 78.3 (C-16), 76.4 (C-17), 71.2 (d, $J = 6.0$ Hz, CH_2–Ph), 71.1 (d, $J = 6.0$ Hz, CH_2–Ph), 69.1 (C-23), 66.0 (C-20), 64.7 (C-2), 64.5 (C-34), 63.7 (C-31), 55.4 (C-18), 55.1 (C-8) 53.1 (C-12), 50.4 (C-14/C-4/C-6), 50.1 (C-4/C-14/C-6), 49.7 (C-6/C-14/C-4), 41.3 (C-29) 32.4 (C-10), 32.2 (C-26), 30.6 (C-28), 28.2 (C-11), 24.1 (C-27), 22.9 (NHAc–CH_3), 19.9 (CH_3), 18.7 (C-32), 18.5 (CH_3), 18.3 (C-35), 18.2 (C-1), 17.9 (CH_3), 17.4 (CH_3), −1.4 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). ^{31}P NMR (202 MHz, CD_3OD) $\delta = -3.0$. HRMS (ESI) *m*/*z*: calcd for $C_{68}H_{106}N_7O_{20}Si_3Pna$ $[M + Na]^+$: 1478.6430, found: 1478.6431. The spectroscopic data were in agreement with those previously reported.[15]

Debenzylation of compound 60: synthesis of compound 70

To a solution of glycopeptide **60** (35.6 mg, 24.4 μmol) in MeOH (2.5 mL) was added Pd–C (40.0 mg, Pd-10%). The reaction vessel was filled with hydrogen. After stirring for 30 min at rt full conversion was detected by mass spectrometry and the suspension was filtered over Celite. The precipitate was washed with methanol and the solvent was removed under reduced pressure to yield 29.0 mg (24.4 μmol, quant.) of a colorless, crystalline solid, which was used for the next reaction without further purification or analysis.

HRMS (ESI) *m*/*z*: calcd for $C_{54}H_{93}N_7O_{20}Si_3P$ $[M - H]^-$: 1274.5526, found: 1274.5628. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 61

Phosphate **70** (27.0 mg, 21.2 μmol) was dissolved in 80% AcOH/water (3 mL) and stirred for 2 d at rt. Mass spectrometry showed full conversion at this point and toluene (20 mL) was added and the solvent was removed under reduced pressure to yield 25.1 mg (21.1 μmol, quant.) of a white, amorphous solid, which was used for the next reaction without further purification or analysis.

HRMS (ESI) *m*/*z*: calcd for $C_{47}H_{89}N_7O_{20}Si_3P$ $[M - H]^-$: 1186.5213, found: 1186.5334. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 62

Farnesol phosphate **8** (60.0 mg, 200 μmol) was coevaporated two times from toluene (1 mL) under argon and dissolved in 50% DMF/THF (1.4 mL). Carbonyldiimidazole (CDI, 130 mg, 799 μmol) was dissolved in 50% DMF/THF (1 mL) and added to the farnesol phosphate solution. The reaction was stirred for 2 h at rt and MeOH (35 μL) was added before stirring for further 45 min. The solvents were removed under reduced pressure and the synthesized phosphoimidazole intermediate **A** was coevaporated twice from toluene (1 mL) under argon before the addition of DMF (1 mL). Mass spectrometry showed the successfully synthesized **A**.

HRMS (ESI) *m*/*z*: calcd for $C_{18}H_{28}N_2O_3P$ $[M - H]^-$: 351.1843, found: 351.1835.

In the meantime **61** (28.0 mg, 23.6 μmol) was coevaporated first from pyridine (200 μL) and then, twice from toluene (1 mL) under argon. DMF (1 mL) and the freshly prepared phosphoimidazole solution were added and the mixture was stirred for 3 d at rt. The solvent was removed under reduced pressure and the crude product was semi-purified by gel permeation chromatography (Sephadex® LH-20, GE Healthcare, 260 × 20 mm, methanol) to yield 26.8 mg (<18.2 μmol, <77%) of a colorless, amorphous solid, which was used for the next reaction without further purification or analysis.

R_f 0.80 (4 MeOH/2 $CHCl_3$/0.5 water). HRMS (ESI) *m*/*z*: calcd for $C_{62}H_{114}N_7O_{23}Si_3P_2$ $[M - H]^-$: 1470.6755, found: 1470.6750. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 3

Protected, semi-pure **62** (26.8 mg, <18.2 μmol) was coevaporated from toluene (1 mL) twice and dissolved in DMF (0.5 mL). The solution was cooled to 0 °C and a 1 M solution of tetrabutylammonium fluoride in THF (TBAF, 419 μL, 419 μmol) was added. The reaction mixture was allowed to warm to rt and was stirred for 2 d at rt before removing the solvent under reduced pressure. The crude product was purified by gel permeation chromatography (Sephadex® LH-20, GE Healthcare, 260 × 20 mm, methanol) and the tetrabutylammonium counterions were exchanged to ammonium ions using percolation through Dowex® 50WX8 with a 0.02 M NH_4HCO_3 solution. Dowex® 50WX8 was washed before usage with 3 : 1 NH_3/water and 0.02 M NH_4HCO_3 solution until pH of the eluent turned 8. The solvent was removed by lyophilisation to yield 20.3 mg (<18.0 μmol) of the semi-purified **3**. Final purification was achieved by HPLC (25–50% NH_4HCO_3 (0.1% aq.)/methanol, retention time 8.9 min, using a KNAUER Eurospher II 100-5 C8; 5 μm; 250 × 16 mm + precolumn 30 × 16 mm, 205 nm) yielding 10.7 mg (7.28 μmol, 40% over four steps) of a white, amorphous solid.

R_f 0.46 (3 MeOH/3 $CHCl_3$/1 water). $[\alpha]_D^{25} = +59°$ ($c = 0.51$ in MeOH). ^1H NMR (500 MHz, D_2O) $\delta = 5.50–5.47$ (m, 1H, H-17), 5.46 (t, $J = 7.1$ Hz, 1H, H-31), 5.22 (t, 1H, $J = 6.5$ Hz, H-35), 5.20 (t, $J = 7.8$ Hz, 1H, H-39), 4.54–4.49 (m, 2H, H-30), 4.35 (q, $J = 7.2$ Hz, 1H, H-4), 4.29 (q, $J = 7.2$ Hz, 1H, H-12), 4.25–4.22 (m, 2H, H-6, H-14), 4.20–4.10 (m, 3H, H-2, H-10, H-16), 3.98–3.96 (m, 1H, H-18), 3.92–3.84 (m, 2H, H-20), 3.80 (dd, $J = 9.6$ Hz, $J = 9.6$ Hz, 1H, H-15), 3.66 (dd, $J = 9.6$ Hz, $J = 9.6$ Hz, 1H, H-19), 3.02 (t, $J = 7.5$ Hz, 2H, H-26), 2.32 (t, $J = 7.8$ Hz, 2H, H-8), 2.20–2.15 (m, 3H, H-9, H-34), 2.15–2.11 (m, 4H, H-33, H-38), 2.04 (t, $J = 7.1$ Hz, 2H, H-37), 2.02 (s, 3H, CH_3–NHAc), 1.93–1.89 (m, 1H, H-9), 1.87–1.77 (m, 2H, H-23), 1.74 (s, 3H, H-43), 1.72–1.70 (m, 2H, H-25), 1.70 (s, 3H, H-42), 1.64 (s, 6H, H-41, H-44), 1.52–1.47 (m, 2H, H-24), 1.46 (d, $J = 7.3$ Hz, 3H, H-28), 1.43 (d, $J = 6.8$ Hz, 3H,

H-29), 1.39 (d, J = 7.2 Hz, 3H, H-22), 1.35 (d, J = 7.2 Hz, 3H, H-21). ^{13}C NMR (176 MHz, D_2O) δ = 179.8 (C-1), 177.6 (C-27), 175.8 (C-11), 175.7 (C-7), 174.1 (C-5), 174.1 (NHAc–C=O), 174.0 (C-3), 173.6 (C-13), 143.2 (C-32), 136.7 (C-36), 133.4 (C-40), 124.4 (C-39), 124.2 (C-35), 119.3 (C-31), 94.7 (C-17), 79.9 (C-15), 78.0 (C-4), 73.0 (C-18), 68.0 (C-19), 63.1 (C-30), 60.3 (C-20), 54.2 (C-10), 54.2 (C-6) 53.4 (C-16), 51.0 (C-2), 49.9 (C-12), 49.6 (C-14), 39.1 (C-26), 38.8 (C-33), 38.8 (C-37), 31.7 (C-8), 30.5 (C-23), 28.1 (C-9), 26.3 (C-25), 25.8 (C-38), 25.5 (C-34), 24.9 (C-42), 22.2 (NHAc–CH_3), 22.0 (C-24), 18.6 (C-22), 17.4 (C-21), 17.0 (C-41), 16.8 (C-28), 16.5 (C-29), 15.7 (C-43), 15.3 (C-44). ^{31}P NMR (284 MHz, D_2O) δ = −10.9 (d, J = 14.0 Hz), −13.4 (d, J = 14.0 Hz). HRMS (ESI) m/z: calcd for $C_{46}H_{78}N_7O_{21}P_2$ $[M - H]^-$: 1126.4731, found: 1126.4715. The spectroscopic data were in agreement with those previously reported.[31]

Synthesis of compound 4

In vitro synthesis of compound **4** was performed in a total volume of 4.5 mL containing 1.50 mg (1.33 μmol) compound **3**, 2 mM UDP–D-GlcNAc, 50 mM NaP_i and 0.5 mM $MgCl_2$, pH 6.5. The reaction was initiated by the addition of 300 μg of MurG–His_6. After incubation for 4 h at 30 °C the reaction was quenched by the addition of 9 mL MeOH and evaporated to dryness. Dried samples were dissolved in distilled water for mass spectrometric analysis. The crude product was purified by HPLC (35–50% NH_4HCO_3 (0.1% aq.)/methanol, retention time 8.0 min, using a KNAUER Eurospher II 100-5 C8; 5 μm; 250 × 16 mm + precolumn 30 × 16 mm, 205 nm) yielding 0.98 mg (0.73 μmol, 55%) of a white, amorphous solid. Recombinant MurG–His_6 enzyme was overexpressed and purified as described for MurG[35] and dialyzed against 10 mM NaP_i buffer, pH 7.0.

$[\alpha]_D^{25}$ = +11° (c = 0.18 in H_2O). ^{1}H NMR (700 MHz, D_2O) δ = 5.50–5.47 (m, 1H, H-17), 5.46 (t, J = 6.9 Hz, 1H, H-31), 5.23 (t, J = 6.4 Hz, 1H, H-35), 5.20 (t, J = 6.4 Hz, 1H, H-39), 4.63 (d, J = 8.3 Hz, 1H, H-45), 4.51–4.49 (m, 2H, H-30), 4.35 (q, J = 7.1 Hz, 1H, H-4), 4.32–4.27 (m, 2H, H-12, H-14), 4.24–4.20 (m, 2H, H-6, H-10), 4.15–4.12 (m, 3H, H-2, H-16), 3.98–3.89 (m, 4H, H-18, H-19, H-20, H-50), 3.86–3.81 (m (pt), 1H, H-15), 3.79–3.74 (m, 3H, H-20′, H-49, H-50′), 3.58 (pt (dd), J = 8.6 Hz, J = 8.6 Hz, 1H, H-48), 3.46–3.91 (m, 2H, H-46, H-47), 2.97 (t, J = 7.0 Hz, 2H, H-26), 2.34–2.33 (m, 2H, H-8), 2.19–2.17 (m, 3H, H-9, H-34), 2.14–2.11 (m, 4H, H-33, H-38), 2.07 (s, 3H, CH_3–NHAc), 2.05 (t, J = 7.7 Hz, 2H, H-37), 2.01 (s, 3H, CH_3–NHAc), 1.93–1.89 (m, 1H, H-9′), 1.86–1.82 (m, 1H, H-23), 1.81–1.79 (m, 1H, H-23′), 1.74 (s, 3H, H43), 1.71 (s, 3H, H-42), 1.69–1.66 (m, 2H, H-25), 1.64 (s, 6H, H-41, H-44), 1.47 (d, J = 6.9 Hz, H-28), 1.46 (d, J = 6.8 Hz, 3H, H-29), 1.45–1.39 (m, 2H, H-24), 1.40 (d, J = 7.2 Hz, 3H, H-22), 1.35 (d, J = 7.2 Hz, 3H, H-21). ^{13}C NMR (176 MHz, D_2O) δ = 179.8 (C-1), 177.6 (C-27), 175.7 (C-11), 175.7 (C-7), 174.4 (NHAc–C=O), 174.2 (NHAc–C=O), 174.1 (C-5), 174.0 (C-3), 173.6 (C-13), 143.2 (C-32), 136.7 (C-36), 133.5 (C-40), 124.4 (C-39), 124.2 (C-35), 119.3 (C-31), 100.1 (C-45), 94.2 (C-17), 78.4 (C-14), 77.9 (C-15), 75.9 (C-46), 74.0 (C-48), 73.8 (C-18), 72.4 (C-19), 70.3 (C-47), 63.1 (C-30), 61.1 (C-50/C-20), 59.7 (C-50/C-20), 56.1 (C-49), 54.3 (C-6), 53.7 (C-10), 53.5 (C-16), 51.0 (C-2), 50.0 (C-12), 49.6 (C-4), 39.2 (C-26), 38.8 (C-33), 38.8 (C-37), 31.8 (C-8), 30.5 (C-23), 28.2 (C-9), 26.5 (C-25), 25.8 (C-38), 25.6 (C-34), 24.9 (C-42), 22.2 (C-24), 22.2 (NHAc–CH_3), 22.1 (NHAc–CH_3), 18.7 (C-22), 17.4 (C-21), 17.0 (C-41), 16.9 (C-28), 16.5 (C-29), 15.7 (C-43), 15.3 (C-44). ^{31}P NMR (284 MHz, D_2O) δ = −10.9 (d, J = 20.7 Hz), −13.4 (d, J = 19.2 Hz). HRMS (ESI) m/z: calcd for $C_{54}H_{90}N_8O_{26}P_2$ $[M - 2H]^{2-}$: 664.2726, found: 664.2719, calcd for $C_{54}H_{89}N_8O_{26}P_2$ $[M - 3H]^{3-}$: 442.5127, found: 442.5117. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 64

H_2N–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys((Gly)$_5$–Teoc)–D-Ala–D-Ala–*O*-TMSE **45** (21.1 mg, 18.9 μmol), PyBOB (13.4 mg, 25.7 μmol), HOBt (3.47 mg, 25.7 μmol) and acid **7** (11.0 mg, 17.4 μmol) were dissolved in DMF (1.5 mL) and DIPEA (11.7 μL, 68.6 μmol) was added immediately. After stirring for 45 min at rt the solvent was removed under reduced pressure. Cold EtOAc was added and the resulted precipitate was filtered off, washed with cold EtOAc, dissolved in MeOH and the solvent was removed under reduced pressure to yield 26.0 mg (14.9 μmol, 86%) of a colorless, amorphous solid.

R_f 0.27 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +25° (c = 0.36 in MeOH). ^{1}H NMR (700 MHz, CD_3OD) δ = 7.49–7.47 (m, 2H, $H_{arom.}$), 7.43–7.34 (m, 13H, $H_{arom.}$), 5.84 (dd, J = 5.8, 3.6 Hz, 1H, H-19), 5.63 (s, 1H, H-22), 5.15–5.08 (m, 4H, 2× CH_2–Ph), 4.38 (q, J = 7.1 Hz, 1H, H-16), 4.38 (q, J = 7.1 Hz, 1H, H-6), 4.35–4.30 (m, 3H, H-4, H-12, H-14), 4.20–4.12 (m, 8H, H-2, H-8, H-18, H-41, H-44), 4.03 (dd, J = 9.8, 4.0 Hz, 1H, H-23), 3.92–3.77 (m, 15H, H-17, H-20, H-21, H-23, H-31, H-33, H-35, H-37, H-39), 3.21–3.19 (m, 2H, H-29), 2.28 (t, J = 7.2 Hz, 2H, H-10), 2.19–2.12 (m, 1H, H-11), 1.91–1.87 (m, 1H, H-11′), 1.85 (s, 3H, CH_3–NHAc), 1.77–1.72 (m, 1H, H-26), 1.68–1.62 (m, 1H, H-26), 1.56–1.49 (m, 2H, H-28), 1.40 (d, J = 7.2 Hz, 3H, CH_3), 1.37 (d, J = 7.2 Hz, 3H, CH_3), 1.37–1.32 (m, 2H, H-27) 1.35 (d, J = 7.2 Hz, 3H, CH_3), 1.34 (d, J = 6.8 Hz, 3H, CH_3), 1.02–0.94 (m, 6H, H-1, H-42, H-45), 0.05 (s, 9H, $Si(CH_3)_3$), 0.04 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (176 MHz, CD_3OD) δ = 175.6 (C=O), 174.9 (C=O), 174.7 (C=O), 174.6 (C=O), 174.5 (C=O), 174.1 (C=O), 173.8 (NHAc–C=O), 173.4 (C=O), 172.9 (C=O), 172.7 (C=O), 172.7 (C=O), 172.1 (C=O), 171.5 (C=O), 159.4 (C-40), 138.9 ($C_{arom.,quart.}$–Ph), 137.0 (d, J = 6.5 Hz, $C_{arom.,quart.}$–Bn), 137.0 (d, J = 6.5 Hz, $C_{arom.,quart.}$–Bn), 130.1 ($C_{arom.}$), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.3 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 127.3 ($C_{arom.}$), 102.8 (C-22), 97.9 (C-19), 82.3 (C-21), 78.4 (C-16), 76.5 (C-17), 71.2 (d, J = 6.0 Hz, CH_2–Ph), 71.1 (d, J = 6.0 Hz, CH_2–Ph), 69.1 (C-23), 66.0 (C-20), 64.7 (C-2), 64.5 (C-44), 64.5 (C-41), 55.5 (C-18), 55.0 (C-8), 53.2 (C-12), 50.3 (C-14/C-4/C-6), 50.1 (C-4/C-14/C-6), 49.7 (C-6/C-14/C-4), 45.0 (CH_2–Gly), 44.0 (CH_2–Gly), 43.9 (CH_2–Gly), 43.8 (CH_2–Gly), 43.7 (CH_2–Gly), 40.0 (C-29), 32.4 (C-10), 32.2 (C-26), 29.8 (C-28), 28.2 (C-11), 24.1 (C-27), 22.9 (NHAc–CH_3), 19.9 (CH_3), 18.7 (C-42), 18.5 (CH_3), 18.3 (C-45), 18.2 (C-1), 18.0 (CH_3), 17.4 (CH_3), −1.4 ($Si(CH_3)_3$), −1.4 ($Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). ^{31}P NMR (284 MHz, CD_3OD) δ = −2.6. HRMS (ESI) m/z: calcd for $C_{78}H_{121}N_{12}O_{25}Si_3pNa$ $[M + Na]^+$: 1763.7503, found: 1763.7525. The spectroscopic data were in agreement with those previously reported.[8]

Debenzylation of compound 64: synthesis of compound 71

To a solution of glycopeptide **64** (24.2 mg, 13.9 μmol) in MeOH (2 mL) was added Pd–C (25.0 mg, Pd-10%). The reaction vessel was filled with hydrogen. After stirring for 30 min at rt full conversion was detected by mass spectrometry and the suspension was filtered over Celite. The catalyst was washed with methanol and the solvent was removed under reduced pressure to yield 19.5 mg (12.5 μmol, 90%) of a colorless, crystalline solid, which was used for the next reaction without further purification or analysis.

HRMS (ESI) *m*/*z*: calcd for $C_{64}H_{108}N_{12}O_{25}Si_3P$ $[M - H]^-$: 1559.6599, found: 1559.6516. The spectroscopic data were in agreement with those previously reported.[8]

Acetal cleavage of compound 71: synthesis of compound 72

Acetal **71** (16.7 mg, 10.7 μmol) was dissolved in 80% AcOH/water (3 mL) and stirred for 2 d at rt. Mass spectrometry showed full conversion at this point and toluene (30 mL) was added and the solvent was removed under reduced pressure to yield 15.7 mg (10.7 μmol, quant.) of a white, crystalline solid, which was used for the next reaction without further purification or analysis.

HRMS (ESI) *m*/*z*: calcd for $C_{57}H_{104}N_{12}O_{25}Si_3P$ $[M - H]^-$: 1471.6286, found: 1471.6201. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 65

Farnesol phosphate **8** (27.7 mg, 92.1 μmol) was coevaporated from toluene (1 mL) two times and dissolved in 50% DMF/THF (1.4 mL). Carbonyldiimidazole (CDI, 59.7 mg, 368 μmol) was dissolved in 50% DMF/THF (1 mL) and added to the farnesol phosphate solution. The reaction was stirred for 2 h at rt and MeOH (16 μL) was added before stirring further 45 min. The solvents were removed under reduced pressure and the synthesized phosphoimidazole intermediate was coevaporated from toluene (1 mL) twice before the addition of DMF (1 mL). Mass spectrometry showed the successful synthesis.

HRMS (ESI) *m*/*z*: calcd for $C_{18}H_{28}N_2O_3P$ $[M - H]^-$: 351.1843, found: 351.1835.

In the meantime phosphate **72** (15.7 mg, 10.7 μmol) was coevaporated first from 95 μL pyridine and then twice from 1 mL toluene under argon. DMF (0.5 mL) and the freshly prepared phosphoimidazole solution were added and the reaction was stirred for 3 d at rt. The solvent was removed under reduced pressure and the crude product was semi-purified by gel permeation chromatography (Sephadex® LH-20, GE Healthcare, 260 × 20 mm, methanol) to yield 10.9 mg (<6.20 μmol, <58%) of a colorless, amorphous solid, which was used for the next reaction without further purification or analysis.

R_f 0.62 (4 MeOH/2 $CHCl_3$/0.5 water). HRMS (ESI) *m*/*z*: calcd for $C_{72}H_{129}N_{12}O_{28}Si_3P_2$ $[M - H]^-$: 1755.7828, found: 1755.7826, calcd for $C_{72}H_{128}N_{12}O_{28}Si_3P_2$ $[M - 2H]^{2-}$: 877.3877, found: 877.3879. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 5

Protected, semi-pure **65** (10.9 mg, <6.20 μmol) was coevaporated twice from 1 mL toluene under argon and dissolved in DMF (0.7 mL). The solution was cooled to 0 °C and a 1 M solution of tetrabutylammonium fluoride in THF (TBAF, 116 μL, 116 μmol) was added. The reaction mixture was allowed to warm to rt and was stirred for 2 d at rt before removing the solvent under reduced pressure. The crude product was purified by gel permeation chromatography (Sephadex® LH-20, GE Healthcare, 260 × 20 mm, methanol) and the tetrabutylammonium counterions were exchanged to ammonium using percolation through Dowex® 50WX8 with a 0.02 M NH_4HCO_3 solution. Dowex® 50WX8 was washed before usage with 3 : 1 NH_3/water and 0.02 M NH_4HCO_3 solution until pH of the eluent turned 8. The solvent was removed by lyophilisation to yield 8.20 mg (<5.80 μmol) of the semi-purified **5**. Final purification was achieved using HPLC (25–50% NH_4HCO_3 (0.1% aq.)/methanol, retention time 8.1 min, using a KNAUER Eurospher II 100-5 C8; 5 μm; 250 × 16 mm + precolumn 30 × 16 mm, 205 nm) yielding 4.4 mg (3.04 μmol, 22% over four steps) of a white, amorphous solid.[8]

R_f 0.38 (3 MeOH/3 $CHCl_3$/1 water). $[\alpha]_D^{25} = +21°$ ($c = 0.44$ in MeOH). ^{1}H NMR (700 MHz, D_2O) δ = 5.50–5.48 (m, 1H, H-17), 5.46 (t, J = 6.7 Hz, 1H, H-31), 5.22 (t, J = 6.7 Hz, 1H, H-35), 5.20 (t, J = 6.7 Hz, 1H, H-39), 4.54–4.49 (m, 2H, H-30), 4.37 (q, J = 7.2 Hz, 1H, H-14), 4.30 (q, J = 7.2 Hz, 1H, H-4), 4.26–4.17 (m, 3H, H-6, H-10, H-12), 4.16–4.10 (m, 2H, H-2, H-16), 4.08 (s, 2H, H-52), 4.03 (H-48/H-50), 4.01 (H-48/H-50), 3.98–3.96 (m, 1H, H-18), 3.91 (s, 2H, H-46), 3.90–3.83 (m, 2H, H-20), 3.82 (s, 2H, H-45), 3.80 (dd, J = 9.6 Hz, J = 9.6 Hz, 1H, H-15), 3.66 (dd, J = 9.6 Hz, J = 9.6 Hz, 1H, H-19), 3.23 (t, J = 6.7 Hz, 2H, H-26), 2.37–2.28 (m, 2H, H-8), 2.20–2.16 (m, 3H, H-9, H-34), 2.15–2.11 (m, 4H, H-33, H-38), 2.04 (t, J = 7.1 Hz, 2H, H-37), 2.02 (s, 3H, CH_3–NHAc), 1.93–1.88 (m, 1H, H-9), 1.83–1.73 (m, 2H, H-23), 1.74 (s, 3H, H-43), 1.72–1.70 (m, 2H, H-25), 1.71 (s, 3H, H-42), 1.64 (s, 6H, H-41, H-44), 1.56–1.51 (m, 2H, H-24), 1.46 (d, J = 7.2 Hz, 3H, H-22), 1.43 (d, J = 6.8 Hz, 3H, H-28), 1.38 (d, J = 7.2 Hz, 3H, H-29), 1.35 (d, J = 7.2 Hz, 3H, H-21). ^{13}C NMR (176 MHz, D_2O) δ = 179.9 (C-1), 177.7 (C-27), 175.7 (C-11), 175.6 (C-7), 174.2 (C-5), 174.1 (NHAc–C=O), 174.0 (C-3), 173.6 (C-13), 172.2 (C-47/C-49/C-51), 172.1 (C-47/C-49/C-51), 172.0 (C-47/C-49/C-51), 171.0 (C-45), 169.9 (C-53) 143.2 (C-32), 136.7 (C-36), 133.4 (C-40), 124.4 (C-39), 124.2 (C-35), 119.3 (C-31), 94.7 (C-17), 79.9 (C-15), 78.0 (C-12), 73.0 (C-18), 68.0 (C-19), 63.2 (C-30), 60.3 (C-20), 54.3 (C-10), 54.3 (C-6), 53.4 (C-16), 51.0 (C-2), 49.8 (C-4), 49.5 (C-14), 42.6 (C-48/C-50), 42.5 (C-48/C-50), 42.5 (C-46), 42.4 (C-52), 41.2 (C-54), 39.0 (C-26), 38.8 (C-33), 38.8 (C-37), 31.8 (C-8), 30.7 (C-23), 28.1 (C-9), 27.8 (C-25), 25.8 (C-38), 25.5 (C-34), 24.9 (C-42), 22.4 (NHAc–CH_3), 22.2 (C-24), 18.7 (C-28), 17.4 (C-21), 17.0 (C-41), 16.9 (C-22), 16.6 (C-29), 15.7 (C-43), 15.3 (C-44). ^{31}P NMR (162 MHz, D_2O) δ = −10.8 (d, J = 15.4 Hz), −13.3 (d, J = 15.4 Hz). HRMS (ESI) *m*/*z*: calcd for $C_{56}H_{93}N_{12}O_{26}P_2$ $[M - H]^-$: 1411.5805, found: 1411.5810, calcd for $C_{56}H_{92}N_{12}O_{26}P_2$ $[M - 2H]^{2-}$: 705.2866, found: 705.2877. The spectroscopic data were in agreement with those previously reported.[8]

Synthesis of compound 66

H_2N–L-Ala–γ-D-Glu(*O*-TMSE)–L-Lys((L-Ala)$_2$–Teoc)–D-Ala–D-Ala–OTMSE (**55**) (16.0 mg, 16.4 μmol, 1.10 equiv.), PyBOP (12.2 mg, 23.4 μmol, 1.50 equiv.) and the free acid **7** (9.6 mg, 14.9 μmol, 1.00 equiv.) were dissolved in DMF (1.34 mL) and DIPEA (10.5 μL, 61.8 μmol, 4.00 equiv.) was added directly. The reaction was stirred for 45 min at room temperature and then the solvent was removed under reduced pressure. The residue was dissolved in cold ethyl acetate and the formed precipitate filtered and washed with cold ethyl acetate. Afterwards it was dissolved in MeOH and the solvent was removed under reduced pressure to yield 6.5 mg of a colorless, crystalline solid (4.07 μmol, 27%).

R_f = 0.31 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +39° (*c* = 0.28 in MeOH). ^{1}H-NMR (700 MHz, CD_3OD) δ = 7.50–7.44 (m, 2H, $H_{arom.}$), 7.40–7.33 (m, 13H, $H_{arom.}$), 5.85 (dd, *J* = 3.7 Hz, 2.4 Hz, 1H, H-19), 5.63 (m, 1H, H-22), 5.14–5.08 (m, 4H, 2× CH_2–Ph), 4.39 (d, *J* = 7.1 Hz, 1H, H-6), 4.38 (d, *J* = 7.1 Hz, 1H, H-16), 4.35–4.30 (m, 3H, H-4, H-12, H-14), 4.21–4.14 (m 8H, H-2, H-8, H-18, H-35, H-40), 4.03 (dd, *J* = 5.9 Hz, 3.9 Hz, 1H, H-23), 3.86–3.78 (m, 3H, H-17, H-20, H21), 3.78–3.72 (m, 1H, H-23), 3.17–3.14 (m, 2H, H-29), 2.38–2.29 (dt, *J* = 7.2 Hz, 6.7 Hz, 2H, H-10), 2.25–2.17 (m, 1H, H-11), 1.99–1.93 (m, 2H, H-33, H-31), 1.93–1.87 (m, 1H, H-11), 1.85 (s, 3H, CH_3–NHAc), 1.82–1.71 (m, 1H, H-26), 1.69–1.61 (m, 1H, H-26), 1.55–1.48 (m, 2H, H-28), 1.43–1.40 (td, *J* = 7.5 Hz, 4.5 Hz, 3H, CH_3), 1.40–1.38 (dd, *J* = 7.5 Hz, 3.0 Hz, 3H, CH_3), 1.38–1.36 (m, 6H, 2× CH_3), 1.34 (s, 3H, CH_3), 1.33–1.32 (m, 2H, H-27), 1.33 (s, 3H, CH_3), 1.02–0.98 (m, 6H, H-1, H-36, H-41), 0.04 (s, 9H, TMS), 0.04 (s, 9H, TMS), 0.02 (s, 9H, TMS). ^{13}C-NMR (176 MHz, CD_3OD) δ = 175.6 (C=O), 174.9 (C=O), 174.6 (C=O), 174.6 (C=O), 174.4 (C=O), 174.1 (C=O), 174.1 (C=O), 173.8 (C=O), 173.4 (C=O), 172.9 (C=O), 159.3 (C=O), 158.9 (C-34), 138.9 ($C_{arom.,quart.}$–Ph), 137.0 ($C_{arom.,quart.}$–Bn), 137.0 ($C_{arom.,quart.}$–Bn), 130.0 ($C_{arom.}$), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 127.3 ($C_{arom.}$), 102.8 (C-22), 97.9 (C-19), 82.3 (C-21), 78.3 (C-16), 76.4 (C-17), 71.1 (CH_2–Ph), 71.1 (CH_2–Ph), 69.1 (C-23), 66.0 (C-20), 64.7 (C-2), 64.5 (C-35), 64.5 (C-40), 55.5 (C-18), 55.0 (C-8), 52.2 (C-12), 50.6 (C-14/C-4/C-6), 50.1 (C-4/C-14/C-6), 49.5 (C-6/C-14/C-4), 47.4 (C-31), 47.4 (C-33), 40.0 (C-29), 32.5 (C-10), 32.1 (C-26), 29.8 (C-28), 27.4 (C-11), 24.0 (C-27), 22.9 (NHAc–CH_3), 19.9 (CH_3), 18.7 (C-36), 18.5 (CH_3), 18.3 (C-41), 18.2 (C-1), 18.1 (CH_3), 18.0 (CH_3), 18.0 (CH_3), 17.4 (CH_3), −1.4 ((CH_3)$_3$Si), −1.5 ((CH_3)$_3$Si), −1.5 ((CH_3)$_3$Si). HRMS (ESI) *m*/*z*: calcd for $C_{74}H_{116}N_9O_{22}PSi_3Na$ $[M + Na]^+$: 1620.7178, found: 1620.7180.

Debenzylation of compound 66: synthesis of compound 73

Compound **66** (61.6 mg, 38.5 μmol, 1.00 equiv.) was dissolved in MeOH (3.85 mL) and Pd/C (62.8 mg, Pd-10%) was added. The flask was flooded with H_2 and was stirred at rt o.n. until full conversion to **73** was detected by mass spectrometry. The reaction mixture was filtered over Celite and the residue was washed with methanol. The solvent was removed under reduced pressure to yield 44.4 mg (31.3 μmol, 81%) of a colorless, crystalline solid which was used without further purification or analysis.

HRMS (ESI) *m*/*z*: calcd for $C_{60}H_{103}N_9O_{22}PSi_3$ $[M - H]^-$: 1416.6269, found: 1416.6289.

Acetal cleavage of compound 73: synthesis of compound 74

Acetal **73** (44.4 mg, 31.3 μmol) was dissolved in 80% AcOH/water (3.13 mL) and the reaction was stirred 2 d at rt. Mass spectrometry showed full conversion at this point and toluene (21 mL) was added and the solvent was removed under reduced pressure to yield 37.1 mg of a colorless, crystalline solid (27.9 μmol, 89%) which was used without further purification or analysis.

HRMS (ESI) *m*/*z*: calcd for $C_{53}H_{98}N_9O_{22}Psi_3$ $[M - 2H]^{2-}$: 663.2965, found: 663.2962.

Synthesis of silyl-protected compound 75

Farnesol phosphate **8** (78.6 mg, 266.1 μmol) was coevaporated from toluene (2 mL) two times and dissolved in 50% DMF/THF (3.82 mL). Carbonyldiimidazole (CDI, 170.2 mg, 1.06 mmol) was dissolved in 50% DMF/THF (3.82 mL) and added to the farnesol phosphate solution. The reaction was stirred for 2 h at rt and MeOH (76.3 μL) was added before stirring further 45 min at rt. The solvents were removed under reduced pressure and the synthesized phosphoimidazole intermediate was coevaporated from toluene (2 mL) twice before the addition of DMF (3.1 mL). Mass spectrometry showed the successful synthesis.

HRMS (ESI) *m*/*z*: calcd for $C_{18}H_{28}N_2O_3P$ $[M - H]^-$: 351.1843, found: 351.1835.

In the meantime phosphate **74** (37.1 mg, 27.9 μmol) was coevaporated first from 0.4 mL pyridine and then twice from 2 mL toluene under argon. DMF (3.1 mL) and the freshly prepared phosphoimidazole solution were added and the reaction was stirred for 3 d at rt. The solvent was removed under reduced pressure and the crude product was semi-purified by gel permeation chromatography (Sephadex® LH-20, GE Healthcare, 260 × 20 mm, methanol) to yield 53.9 mg (33.4 μmol, 90%) of a colorless, amorphous solid (**75**), which was used for the next reaction without further purification or analysis.

R_f 0.78 (4 MeOH/2 $CHCl_3$/0.5 water). HRMS (ESI) *m*/*z*: calcd for $C_{68}H_{123}N_9O_{24}P_2Si_3$ $[M - 2H]^{2-}$: 791.8737, found: 791.8740.

Synthesis of compound 6

Protected, semi-pure **75** (53.9 mg, 33.4 μmol) was coevaporated twice from 1 mL toluene under argon and dissolved in DMF (0.5 mL). The solution was cooled to 0 °C and a 1 M solution of tetrabutylammonium fluoride in THF (TBAF, 0.70 mL, 701 μmol) was added. The reaction mixture was allowed to warm to rt and was stirred for 2 d at rt before removing the solvent under reduced pressure. The crude product was purified by gel permeation chromatography (Sephadex® LH-20, GE Healthcare, 260 × 20 mm, methanol) and the tetrabutylammonium counterions were exchanged to ammonium using percolation through Dowex® 50WX8 with a 0.02 M NH_4HCO_3 solution. Dowex® 50WX8 was washed before usage with 3 : 1 NH_3/water and 0.02 M NH_4HCO_3 solution until pH of the eluent turned 8. The solvent was removed by lyophilisation to yield 21.9 mg (16.7

μmol, 50% over four steps) of **6** as a colorless, amorphous solid, which may further purified by HPLC (25–50% NH_4HCO_3 (0.1% aq.)/methanol, retention time 8.1 min, using a KNAUER Eurospher II 100-5 C8; 5 μm; 250 × 16 mm + precolumn 30 × 16 mm, 205 nm).

$R_f = 0.87$ (3 MeOH/3 CH_2Cl_2/1 water). $[\alpha]_D^{25} = +33°$ ($c = 0.34$ in MeOH). ^{1}H (500 MHz, D_2O) $\delta = 5.50–5.46$ (m, 1H, H-17), 5.46 (t, $J = 6.8$ Hz, 1H, H-37), 5.23 (t, $J = 6.8$ Hz, 1H, H-41), 5.21–5.16 (m, 1H, H-45), 4.52 4.47 (m, 2H, H-36), 4.36 (q, $J = 7.2$ Hz, 1H, H-14), 4.31–4.29 (m, 1H, H-4), 4.29–4.24 (m, 3H, H-6, H-10, H-12), 4.23–4.21 (m, 11H, H-28), 4.21–4.19 (m, 1H, H-30), 4.19–4.14 (m, 2H, H-2, H-16), 3.99–3.94 (m, 1H, H-18), 3.91–3.85 (m, 2H, H-20), 3.80 (dd, $J = 9.7$ Hz, $J = 9.7$ Hz, 1H, H-15), 3.64 (pt, $J = 9.7$ Hz, 1H, H-19), 3.26–3.23 (m, 2H, H-26), 2.33–2.28 (m, 2H, H-8), 2.20–2.14 (m, 3H, H-9, H-40), 2.15–2.11 (m, 4H, H-39, H-44), 2.02 (t, $J = 7.5$ Hz, 2H, H-43), 2.00 (s, 3H, CH_3–NHAc), 1.93–1.88 (m, 1H, H-9), 1.83–1.76 (m, 2H, H-23), 1.73 (s, 3H, H-50), 1.72–1.70 (m, 2H, H-25), 1.70 (s, 3H, H-48), 1.64 (s, 3H, H-47), 1.63 (s, 3H, H-49), 1.55 (d, $J = 7.1$ Hz, 3H, H-32), 1.53–1.50 (m, 2H, H-24), 1.45 (d, $J = 7.2$ Hz, 3H, H-22), 1.42 (d, $J = 6.9$ Hz, 3H, H-34), 1.38 (d, $J = 7.1$ Hz, 3H, H-35), 1.37 (d, $J = 7.2$ Hz, 3H, H-31), 1.34 (d, $J = 7.1$ Hz, 3H, H-21). ^{13}C (176 MHz, D_2O) $\delta =$ 181.4 (C-1), 177.9 (C-33), 175.9 (C-11), 175.8 (C-7), 174.8 (C-5), 174.1 (NHAc–C=O), 174.1 (C-3), 173.7 (C-13), 172.1 (C-27), 172.0 (C-29), 143.2 (C-38), 136.8 (C-42), 133.4 (C-46), 124.5 (C-45), 124.4 (C-41), 119.3 (C-37), 94.8 (C-17), 79.9 (C-15), 78.2 (C-12), 73.1 (C-18), 68.2 (C-19), 63.2 (C-36), 58.2 (C-20), 54.3 (C-10), 54.3 (C-6), 53.3 (C-16), 52.2 (C-28), 51.1 (C-2), 50.2 (C-4), 49.9 (C-30), 49.5 (C-14), 38.9 (C-26), 38.9 (C-39), 38.9 (C-43), 31.9 (C-8), 30.7 (C-23), 28.2 (C-9), 27.7 (C-25), 25.9 (C-44), 25.5 (C-40), 25.0 (C-48), 22.5 (NHAc–CH_3), 22.2 (C-24), 19.2 (C-32), 18.7 (C-34), 17.5 (C-21), 17.0 (C-47), 17.0 (C-22), 16.7 (C-35), 16.6 (C-31), 15.7 (C-50), 15.3 (C-49). ^{31}P (284 MHz, D_2O) $\delta =$ −10.8 (d, $J = 14.8$ Hz), −13.4 (d, $J = 14.8$ Hz).

HRMS (ESI) *m*/*z*: calcd for $C_{53}H_{87}N_9O_{25}P_2$ $[M - 2H]^{2-}$: 655.7650, found: 655.7651, calcd for $C_{53}H_{88}N_9O_{25}P_2$ $[M - H]^-$: 1312.5372, found: 1312.5381, calcd for $C_{53}H_{87}DN_9O_{25}P_2$ [M − 2H + D]: 1313.5435, found: 1313.5421.

Synthesis of 3-lipid II (*E. faecalis*, *S. pneumoniae*)

In vitro synthesis of 3-lipid II (*E. faecalis*, *S. pneumoniae*) was performed in a total volume of 30 μL containing 10 μg compound **5**, 2 mM UDP–D-GlcNAc, 50 mM NaP_i and 0.5 mM $MgCl_2$, pH 6.5. The reaction was initiated by the addition of 2 μg of MurG–His_6. Afterincubation for 4 h at 30 °C the reaction was quenched by the addition of 60 μL MeOH and evaporated to dryness. Dried samples were dissolved in distilled water for mass spectrometric analysis. Recombinant MurG–His_6 enzyme was overexpressed and purified as described for MurG[35] and dialized against 10 mM NaP_i buffer, pH 7.0.

HRMS (ESI) *m*/*z*: calcd for $C_{64}H_{105}N_{13}O_{31}P_2$ $[M - 2H]^{2-}$: 806.8263, found: 806.8255; calcd for $C_{64}H_{104}N_{13}O_{31}P_2Na$ $[M - 3H + Na]^{2-}$: 817.8173, found: 817.8166; calcd for $C_{64}H_{104}N_{13}O_{31}P_2$ $[M - 3H]^{3-}$: 537.5484, found: 537.5475. The spectroscopic data were in agreement with those previously reported.[8]

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 A. Müller, A. Klöckner and T. Schneider, *Nat. Prod. Rep.*, 2017, **34**, 909–932, https://pubs.rsc.org/en/content/articlehtml/2017/np/c7np00012j.
2 B. de Kruijff, V. van Dam and E. Breukink, *Prostaglandins, Leukotrienes Essent. Fatty Acids*, 2008, **79**, 117–121.
3 P. Hardt, I. Engels, M. Rausch, M. Gajdiss, H. Ulm, P. Sass, K. Ohlsen, H.-G. Sahl, G. Bierbaum, T. Schneider and F. Grein, *Int. J. Med. Microbiol.*, 2017, **307**, 1–10.
4 A. Bouhss, A. E. Trunkfield, T. D. H. Bugg and D. Mengin-Lecreulx, *FEMS Microbiol. Rev.*, 2008, **32**, 208–233.
5 S. A. Hitchcock, C. N. Eid, J. A. Aikins, M. Zia-Ebrahimi and L. C. Blaszczak, *J. Am. Chem. Soc.*, 1998, **120**, 1916–1917.
6 B. Schwartz, J. A. Markwalder and Y. Wang, *J. Am. Chem. Soc.*, 2001, **123**, 11638–11643.
7 H. Men, P. Park, M. Ge and S. Walker, *J. Am. Chem. Soc.*, 1998, **120**, 2484–2485.
8 L. M. Wingen, M. Rausch, T. Schneider and D. Menche, *J. Org. Chem.*, 2020, **85**, 10206–10215.
9 (*a*) C. A. Smith, *J. Mol. Biol.*, 2006, **362**, 640–655; (*b*) H. Barreteau, A. Kovac, A. Boniface, M. Sova, S. Gobec and D. Blanot, *FEMS Microbiol. Rev.*, 2008, **32**, 168–207.
10 J. Nakamura, H. Yamashiro, H. Miya, K. Nishiguchi, H. Maki and H. Arimoto, *Chem.–Eur. J.*, 2013, **19**, 12104–12112.
11 L. M. Lira, D. Vasilev, R. A. Pilli and L. A. Wessjohann, *Tetrahedron Lett.*, 2013, **54**, 1690–1692.
12 (*a*) A. Katsuyama, K. Sato, F. Yakushiji, T. Matsumaru and S. Ichikawa, *Chem. Pharm. Bull.*, 2018, **66**, 84–95, https://www.jstage.jst.go.jp/article/cpb/66/1/66_c17-00828/_article/-char/ja/; (*b*) S. A. Cochrane, B. Findlay, A. Bakhtiary, J. Z. Acedo, E. M. Rodriguez-Lopez, P. Mercier and J. C. Vederas, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 11561–11566.
13 Part of these results have been previously communicated: (8).
14 L. Chen, H. Men, S. Ha, X.-Y. Ye, L. Brunner, Y. Hu and S. Walker, *Biochemistry*, 2002, **41**, 6824–6833.
15 S. Ha, E. Chang, M.-C. Lo, H. Men, P. Park, M. Ge and S. Walker, *J. Am. Chem. Soc.*, 1999, **121**, 8415–8426.
16 M. S. VanNieuwenhze, S. C. Mauldin, M. Zia-Ebrahimi, J. A. Aikins and L. C. Blaszczak, *J. Am. Chem. Soc.*, 2001, **123**, 6983–6988.
17 M. M. Sim, H. Kondo and C. H. Wong, *J. Am. Chem. Soc.*, 1993, **115**, 2260–2267.

18 (*a*) A. Gansäuer and T. Dahmen, *Chimia*, 2012, **66**, 433–434; (*b*) A. J. Pearson and K. Lee, *J. Org. Chem.*, 1994, **59**, 2304–2313.
19 R. Pascal and R. Sola, *Tetrahedron Lett.*, 1998, **39**, 5031–5034.
20 (*a*) J. M. Benito and M. Meldal, *QSAR Comb. Sci.*, 2004, **23**, 117–129; (*b*) L. Sambri, G. Bartoli, G. Bencivenni and R. Dalpozzo, *Curr. Org. Synth.*, 2012, **9**, 137–148.
21 A. Mehta, R. Jaouhari, T. J. Benson and K. T. Douglas, *Tetrahedron Lett.*, 1992, **33**, 5441–5444.
22 (*a*) R. Kaul, Y. Brouillette, Z. Sajjadi, K. A. Hansford and W. D. Lubell, *J. Org. Chem.*, 2004, **69**, 6131–6133; (*b*) B. Li, M. Berliner, R. Buzon, C. K.-F. Chiu, S. T. Colgan, T. Kaneko, N. Keene, W. Kissel, T. Le, K. R. Leeman, B. Marquez, R. Morris, L. Newell, S. Wunderwald, M. Witt, J. Weaver, Z. Zhang and Z. Zhang, *J. Org. Chem.*, 2006, **71**, 9045–9050.
23 A. Trzeciak and W. Bannwarth, *Synthesis*, 1996, **1996**, 1433–1434.
24 K. Lang, L. Davis, S. Wallace, M. Mahesh, D. J. Cox, M. L. Blackman, J. M. Fox and J. W. Chin, *J. Am. Chem. Soc.*, 2012, **134**, 10317–10320.
25 M. L. Di Gioia, P. Costanzo, A. de Nino, L. Maiuolo, M. Nardi, F. Olivito and A. Procopio, *RSC Adv.*, 2017, **7**, 36482–36491.
26 G. W. Liechti, E. Kuru, E. Hall, A. Kalinda, Y. V. Brun, M. VanNieuwenhze and A. T. Maurelli, *Nature*, 2014, **506**, 507–510.
27 R. Bollhagen, M. Schmiedberger, K. Barlos and E. Grell, *J. Chem. Soc., Chem. Commun.*, 1994, 2559.
28 M. Tsakos, E. S. Schaffert, L. L. Clement, N. L. Villadsen and T. B. Poulsen, *Nat. Prod. Rep.*, 2015, **32**, 605–632.
29 I. Shiina, M. Kubota and R. Ibuka, *Tetrahedron Lett.*, 2002, **43**, 7535–7539.
30 T. I. Al-Warhi, H. M. Al-Hazimi and A. El-Faham, *J. Saudi Chem. Soc.*, 2012, **16**, 97–116.
31 X. Y. Ye, M. C. Lo, L. Brunner, D. Walker, D. Kahne and S. Walker, *J. Am. Chem. Soc.*, 2001, **123**, 3155–3156.
32 C.-Y. Liu, C.-W. Guo, Y.-F. Chang, J.-T. Wang, H.-W. Shih, Y.-F. Hsu, C.-W. Chen, S.-K. Chen, Y.-C. Wang, T.-J. R. Cheng, C. Ma, C.-H. Wong, J.-M. Fang and W.-C. Cheng, *Org. Lett.*, 2010, **12**, 1608–1611.
33 K. Trainor, J. A. Palumbo, D. W. S. MacKenzie and E. M. Meiering, *Protein Sci.*, 2020, **29**, 306–314.
34 Y. Zhang, E. J. Fechter, T.-S. A. Wang, D. Barrett, S. Walker and D. E. Kahne, *J. Am. Chem. Soc.*, 2007, **129**, 3080–3081.
35 T. Schneider, T. Kruse, R. Wimmer, I. Wiedemann, V. Sass, U. Pag, A. Jansen, A. K. Nielsen, P. H. Mygind, D. S. Raventós, S. Neve, B. Ravn, A. M. J. J. Bonvin, L. de Maria, A. S. Andersen, L. K. Gammelgaard, H.-G. Sahl and H.-H. Kristensen, *Science*, 2010, **328**, 1168–1172.
36 M. S. VanNieuwenhze, S. C. Mauldin, M. Zia-Ebrahimi, B. E. Winger, W. J. Hornback, S. L. Saha, J. A. Aikins and L. C. Blaszczak, *J. Am. Chem. Soc.*, 2002, **124**, 3656–3660.
37 V. L. Healy, I. A. D. Lessard, D. I. Roper, J. R. Knox and C. T. Walsh, *Chem. Biol.*, 2000, **7**, R109–R119.