Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 595–606 **595**
DOI: 10.1021/jm901052r

Antimicrobial Activity of Small β-Peptidomimetics Based on the Pharmacophore Model of Short Cationic Antimicrobial Peptides

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Received July 16, 2009

We have synthesized a series of small β-peptidomimetics (M_w < 650) that were based on the minimal pharmacophore model for anti-*Staphylococcal* activity of short cationic antimicrobial peptides. All β-peptidomimetics had a net charge of +2 and formed an amphipathic scaffold consisting of an achiral lipophilic $\beta^{2,2}$-amino acid coupled to a C-terminal L-arginine amide residue. By varying the lipophilic side-chains of the $\beta^{2,2}$-amino acids, we obtained a series of highly potent β-peptidomimetics with high enzymatic stability against α-chymotrypsin and a general low toxicity against human erythrocytes. The most potent β-peptidomimetics displayed minimal inhibitory concentrations of 2.1−7.2 μM against *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), methicillin resistant *Staphylococcus epidermidis* (MRSE), and *Escherichia coli*. Small amphipathic β-peptidomimetics may be a promising class of antimicrobial agents by means of having a similar range of potency and selectivity as larger cationic antimicrobial peptides in addition to improved enzymatic stability and lower costs of production.

Introduction

Infections caused by multiresistant bacteria have become a major concern to society over the past 20−25 years.[1,2] This is especially a problem in hospitals where infections caused by methicillin resistant *Staphylococcus aureus* (MRSA[a]), vancomycin resistant *Staphylococcus aureus* (VRSA), and methicillin resistant *Staphylococcus epidermidis* (MRSE) can result in severe injury, prolonged hospitalization, and death, especially among immune compromised patients.[3,4] Vancomycin was once a drug of last resort, but reports from hospitals around the world shows that infrequent use of vancomycin is no longer the case.[2,5,6] At the same time, there has been a drought among the largest pharmaceutical companies for developing novel classes of antimicrobial compounds, basically stating it to be a high-risk investment due to huge costs of development and relatively limited duration of patient treatment as opposed to treatment of chronic diseases.[7−9] However, the need for novel antimicrobial agents is urgent, and in the U.S., deaths caused by hospital acquired infections are currently superseding HIV-related mortality.[10]

A promising class of antimicrobial agents is cationic antimicrobial peptides (AMP's), also known as host defense peptides, which have been heavily investigated over the past 20 years.[11−13] AMP's have a unique mode of action by targeting the inner and/or outer membranes of bacteria in a nonreceptor specific manner.[14,15] The detailed mechanism of membrane disruption by AMP's is still not fully understood, and various models have been proposed to explain the observed effects such as the carpet, barrel-stave, and micelles-aggregate models (see Giuliani et al. for an excellent review).[14] However, the use of peptides as drugs is challenged by deficiency in patient-friendly delivery technologies, low bioavailability, low metabolic and proteolytic stability, high costs of manufacture, and potential danger of immunological responses.[16] Currently there are only two peptide-based antimicrobial drugs on the market, polymyxin B, indicated for skin infections by Gram-negative bacteria and Daptomycin, indicated for skin infections by Gram-positive bacteria.[14] However, there is hope for the future, and three AMP's are currently in phase III clinical trials and yet another 12 compounds from 10 different companies are in the early stages of clinical trials.[14]

One method to overcome the obstacles of transforming natural AMP's into peptide-drugs has been to prepare shorter derivatives with, e.g., a high content of basic and aromatic amino acids. The minimal pharmacophore model of short AMP's ranging in size from two to six amino acid residues has been established by the research group of Svendsen.[17] The pharmacophore model for such short AMP's can be described relatively simply in which activity against the Gram-negative bacterium *Escherichia coli* (*E. coli*) necessitates an amphipathic peptide with three bulky/lipophilic groups and two positively charged groups.[17] For activity against the Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), MRSA and MRSE, the pharmacophore is even simpler by only necessitating an amphipathic peptide with two bulky/lipophilic groups and two positively charged groups.[17] Further studies of the pharmacophore model for anti-staphylococcal

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[a] Abbreviations: AMP, cationic antimicrobial peptide; DIPEA, diisopropyethylamine; MBC, minimal bactericidal concentration; MRSA, methicillin resistant *Staphylococcus aureus*; MRSE, methicillin resistant *Staphylococcus epidermidis*; Ra/Ni, Raney nickel catalyst; RBC, red blood cells; TEA, triethylamine; TFFH, fluoro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate; TIS, triisopropylsilane.

Published on Web 12/08/2009

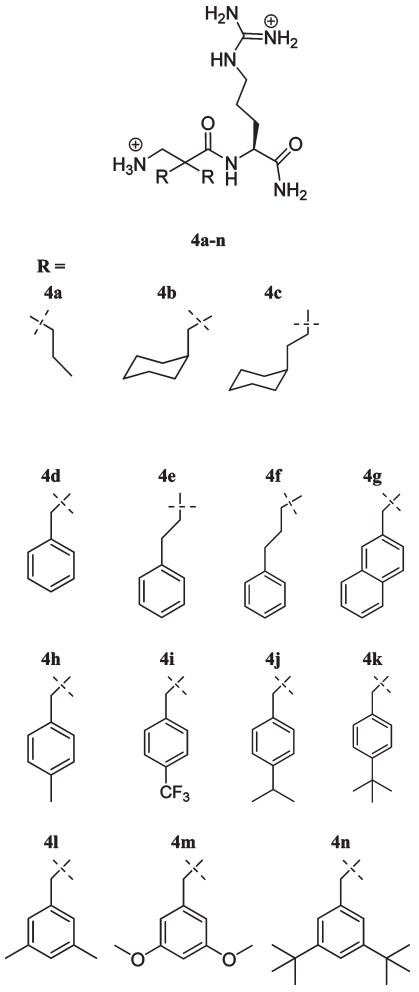


Figure 1. β-Peptidomimetics prepared for investigations of antimicrobial activity. All compounds were designed to fulfill the pharmacophore model of short AMP's with anti-staphylococcal activity and were based on a scaffold consisting of a lipophilic $\beta^{2,2}$-amino acid coupled to a C-terminal L-arginine amide residue.

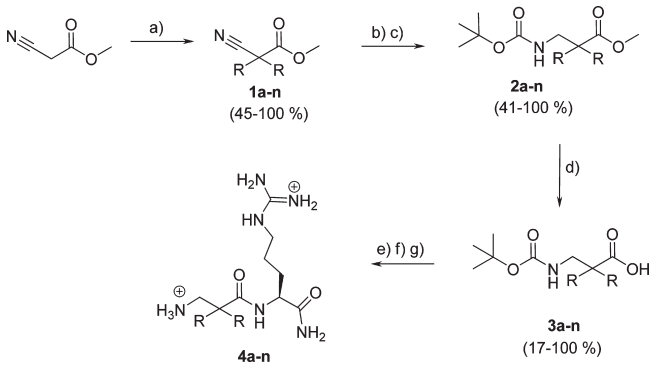
activity have shown that the bulky groups should be aromatic and have a size larger than a benzyl group.[18]

The purpose of the current project was to address some of the general problems associated with peptide drugs by designing novel antimicrobial compounds based on the pharmacophore model for anti-staphylococcal activity. The result of our investigations was a novel class of highly potent antimicrobial β-peptidomimetics based on the scaffold of an achiral lipophilic 3-amino-2,2-disubstituted propionic acid ($\beta^{2,2}$-amino acid) coupled to a C-terminal L-arginine amide residue (Figure 1). The overall size of the resulting β-peptidomimetics resembled the size of a dipeptide while having the side-chain functionalities of a tripeptide due to the $\beta^{2,2}$-amino acid derivative. A wide range of lipophilic substituents of the $\beta^{2,2}$-amino acid derivative was explored as shown in Figure 1. The results demonstrated that the scaffold was highly efficient for preparing novel antimicrobial compounds based on the pharmacophore model of short AMP's and with particularly high potency against the Gram-positive bacteria *S. aureus*, MRSA, and MRSE.

Results

Synthesis. The β-peptidomimetics were prepared in a seven-step synthesis in which only the three intermediates

Scheme 1. Overview of Synthesis of the β-Peptidomimetics **4a**−**n**[a]



[a] (a) NaOMe, R-Br, performed twice, 78 °C s: MeOH. (b) Ra/Ni, H_2 (g), 45 °C, 5 days, s: MeOH. (c) TEA, pH 8, Boc_2O, rt, 18 h, s: H_2O:dioxane (1:5). (d) LiOH, 18 h, 100 °C, s: H_2O:dioxane (1:3). (e) DIPEA, TFFH, rt, s: DMF. (f) H-Arg−NH_2 × 2 HCl, DIPEA, rt, 7 days, s: DMF. (g) TFA: TIS: H_2O (95:2.5:2.5), rt 2 h, s: DCM.

shown in Scheme 1 were isolated (side-chain structures are shown in Figure 1). Synthesis of the $\beta^{2,2}$-amino acid methyl esters (steps a and b) were based on the studies by Cronin et al.,[19] and the chosen strategy made it possible to use parallel-synthesis of all compounds on a 6- or 12-position Radleys reaction carousel. The critical step in our synthesis was the hydrolysis of the most lipophilic $\beta^{2,2}$-amino acid methyl esters due to their low solubility. The problem was partly solved by individually adjusting the ratio between water and 1,4-dioxane for each compound, as described in the Experimental Section. The total yields for synthesis of the crude Boc-$\beta^{2,2}$-amino acids (**3a**−**n**) ranged therefore from 30 to 71%, with the exception of compound **3k**, which gave a total yield of 15%, and compound **3b**, which gave a total yield of only 5%. To improve the overall yields of the Boc-$\beta^{2,2}$-amino acids (**3a**−**n**), we also tried benzyl and allyl esters that could be removed together with the reduction of the nitrile. However, we experienced that the following Boc protection did not work satisfactorily. The reason for this was not investigated in detail, but we suspected that it may have been caused by complexation between nickel ions (from the Ra/Ni catalyst) with the free amino- and carboxylic acid groups of the $\beta^{2,2}$-amino acids.[20,21]

Because of sterical hindrance of the carboxyl group of the Boc-protected $\beta^{2,2}$-amino acids, we performed coupling of the C-terminal arginine amide residue through an acid fluoride intermediate according to Carpino and El-Faham.[22] However, we found it mandatory to increase the reaction time for preactivation with TFFH from 15 min to 2 hours and to increase the coupling time from 1 h to up to 7 days for the most sterically hindered derivatives, as determined by studying the progress of the reaction by MS-analysis. After acidic cleavage of the Boc-protecting group, the crude β-peptidomimetic was purified by RP-HPLC to a final purity of >95% as determined by analysis with an analytical RP-HPLC C_{18}-column and UV detection at 214 and 254 nm.

Antimicrobial Activity. The results from screening of antimicrobial activity showed that the β-peptidomimetics were in general more potent against the Gram-positive bacteria than against the Gram-negative bacterium *E. coli* (Figure 1 and Table 1). A closer examination of the results revealed that the overall most potent β-peptidomimetic was compound **4n**, which contained two *super*-bulky 3,5-di-*tert*-butyl

Table 1. Minimal Inhibitory Concentration (MIC) against *S. aureus*, MRSA, MRSE, and *E. coli* and EC_{20} Values against Human RBC for Antimicrobial β-Peptidomimetics Prepared in the Study

	MIC[a] (μM)					
entry	*S. aureus*[c]	MRSA[d]	MRSE[e]	*E. coli*[f]	EC_{20}[b] (μM) RBC[g]	therapeutic index[h] MRSA
4a	-	-	-	-	-	-
4b	150	150	75	-	-	10[h]
4c	7.2	7.2	7.2	144	-	200[h]
4d	-	-	-	-	nt	-
4e	147	147	74	-	-	10[h]
4f	49	49	35	-	-	29[h]
4g	6.6	5.0	6.6	266	259	52
4h	147	74	74	-	-	20[h]
4i	12.7	3.2	12.7	254	756	236
4j	10.2	6.8	6.8	136	254	37
4k	6.5	3.3	5.0	46	578	175
4l	60	49	35	-	-	29[h]
4m	259	259	129	-	-	5[h]
4n	2.9	2.1	10.1	5.7	51	24

[a] Highest concentration tested was 200 μg/mL. [b] Highest concentration tested was 1000 μg/mL. [c] *Staphylococcus aureus* (ATCC 25923). [d] Methicillin resistant *Staphylococcus aureus* (ATCC 33591). [e] Methicillin resistant *Staphylococcus epidermidis* (ATCC 27626). [f] *Escherichia coli* (ATCC 25922). [g] Human red blood cells. [h] Therapeutic index was calculated as the hemolytic activities (EC_{20}) divided by the MIC values against MRSA. For nonhemolytic compounds, the EC_{20} value was set to an arbitrary value of 1000 μg/mL in order to calculate a therapeutic index. The notation "-" denotes no detectable activity (MIC or EC_{20}) within the concentration range tested. nt: not tested.

benzylic side-chains in the $\beta^{2,2}$-amino acid derivative. It is noteworthy that this was the only β-peptidomimetic that showed the same high potency against both Gram-positive and Gram-negative bacteria. The somewhat less bulky β-peptidomimetic compound **4k**, which contained two *para*-*tert*-butyl benzylic side-chains, showed the second highest antimicrobial potency against all Gram-positive bacteria and the Gram-negative bacterium *E. coli*. The naphthalene derivative compound **4g** was also among the most potent anti-staphylococcal β-peptidomimetics prepared but much less potent against *E. coli*.

A somewhat surprising high antimicrobial potency against the Gram-positive strains was displayed by compound **4c**, although the activity against *E. coli* was poor. In contrast to the β-peptidomimetics described above, compound **4c** was not aromatic but belonged to the group of aliphatic β-peptidomimetics by containing two cyclohexyl-ethyl side-chains in the lipophilic part of the molecule. The related aliphatic compound **4b** showed, however, poor anti-staphylococcal activity and no antimicrobial activity was detected against *E. coli*. The least bulky derivative within the group of aliphatic β-peptidomimetics, compound **4a**, was inactive against all test bacteria within the concentration range tested.

A similar increase in potency as observed for compounds **4b** and **4c** by increasing the length of the lipophilic side-chains was observed for the aromatic compounds **4e** and **4f**. Although compound **4d** was inactive against all test strains, the longer β-peptidomimetic compound **4e** displayed anti-staphylococcal activity, with highest potency obtained against MRSE. Further side-chain elongation resulted in a more than 2-fold improvement in antimicrobial potency as observed for compound **4f**. However, none of these β-peptidomimetics were active against the Gram-negative bacterium *E. coli* within the concentration range tested.

When comparing the *para*-substituted benzylic β-peptidomimetics, compounds **4h**, **4i**, **4j**, and **4k**, the potency of the β-peptidomimetics increased by increasing the size and lipophilicity of the *para*-substituents. As described above, compound **4k** with two *para*-*tert*-butyl benzylic side-chains was the overall second most potent β-peptidomimetic against both the Gram-positive and Gram-negative bacteria. The less bulky compound **4j** with two *para*-*iso*propyl benzylic side-chains showed similar potency as compound **4k** against the Gram-positive bacteria but was much less potent against *E. coli* than compound **4k**.

The least potent β-peptidomimetic among the *para*-substituted benzylic derivatives was compound **4h**, which contained two *para*-methyl benzylic side-chains in the $\beta^{2,2}$-amino acid derivative. It was noteworthy that the potency of compound **4h** was comparable to the potency of compound **4e**, in which the total lengths of the side-chains were approximately the same but differed structurally (Figure 1). A small difference in potency against MRSA may have been influenced by this structural difference because compound **4h** was a little more potent against MRSA, but both of these β-peptidomimetics were inactive against *E. coli* within the concentration range tested.

A 6−23-fold increase in potency against the Gram-positive strains was obtained by replacing the *para*-methyl benzylic side-chains in compound **4h** with two *para*-trifluoromethyl benzylic side-chains. The resulting compound **4i** displayed also measurable activity against *E. coli*.

The final three β-peptidomimetics shown in Figure 1 contained two 3,5-disubstituted benzylic side-chains in the $\beta^{2,2}$-amino acid derivatives, and as described above, the largest β-peptidomimetic compound **4n** was also the overall most potent β-peptidomimetic prepared in the study. Compound **4l**, containing two 3,5-dimethyl benzylic side-chains, had the same molecular mass as the elongated aromatic compound **4f** and showed similar potency against the Gram-positive bacteria. However, neither compounds **4l** nor **4f** showed antimicrobial activity against *E. coli*.

Despite its large size and high molecular mass, compound **4m** containing two 3,5-dimethoxy benzylic side-chains showed very poor Gram-positive activity and no antimicrobial activity against *E. coli* within the concentration range tested. Seemingly, the polar methoxy substituents in the benzylic side-chains of the $\beta^{2,2}$-amino acid constituting the lipophilic part of the scaffold had a negative influence on antimicrobial potency.

Hemolytic Activity. Hemolytic activity against human red blood cells (RBC) was used as a measurement of toxicity, but due to a general low hemolytic activity of the majority of the β-peptidomimetics, the threshold of detection was changed from the standard 50% hemolytic activity (EC_{50}) to measurement of 20% hemolytic activity (EC_{20}). Furthermore, a therapeutic index was calculated based on the EC_{20} results against human RBC and the antimicrobial activity against MRSA (Table 1).

The results showed that only compounds **4g**, **4i**, **4j**, **4k**, and **4n** displayed measurable EC_{20} values, whereas all the other β-peptidomimetics prepared showed no measurable hemolytic activity within the concentration range tested, i.e., up to 1000 μg/mL. A comparison of the hemolytic profile of these five β-peptidomimetics is shown in Figure 2. Highest hemolytic activity was displayed by the *super*-bulky compound **4n**,

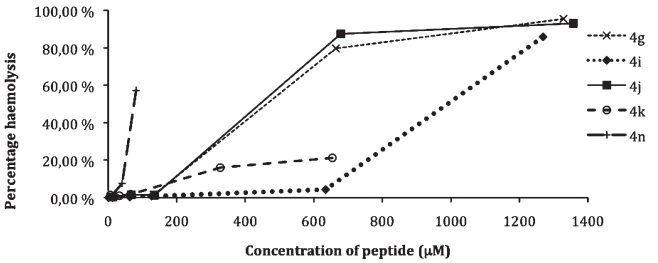


Figure 2. Hemolytic activity of the five β-peptidomimetics that displayed hemolytic activity below 1000 μg/mL. No hemolytic activity at all was detected for any of the other β-peptidomimetics. All β-peptidomimetics were tested for hemolytic activity up to a concentration of 1000 μg/mL, except for compounds **4k** (tested up to 500 μg/mL) and **4n** (tested up to 71.5 μg/mL) due to limited solubility in the test media.

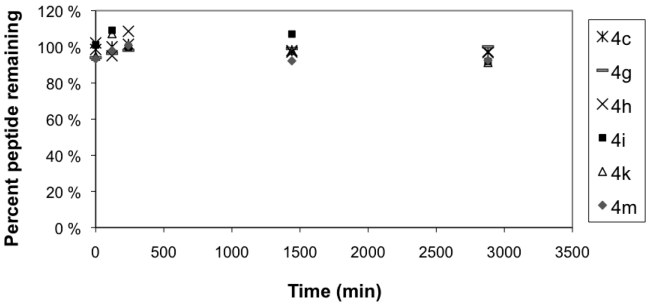


Figure 3. Degradation of six selected β-peptidomimetics by α-chymotrypsin. The ratio was calculated as remaining substance in samples with enzyme divided by samples without enzyme; 100% equals no enzymatic degradation. Only samples collected at 0, 60, 120 min and 24 and 48 h are shown.

but the therapeutic index revealed nevertheless 24-fold selectivity for MRSA compared to human RBC.

To calculate a therapeutic index for all the β-peptidomimetics, the EC_{20} value of the nonhemolytic compounds was set to an arbitrary EC_{20} value of 1000 μg/mL (Table 1). When comparing the β-peptidomimetics **4c**, **4g**, **4i**, **4j**, **4k**, and **4n** that all showed MIC values below 10 μM against MRSA, the highest therapeutic index was displayed by compound **4i** containing two *para*-trifluoromethyl benzylic side-chains followed by compound **4c**, which contained two cyclohexyl-ethyl side-chains, and compound **4k** finishing third with two *para-tert*-butyl benzylic side-chains in the β-amino acid derivative.

Stability. A selection of six β-peptidomimetics (**4c**, **4g**, **4h**, **4i**, **4k**, and **4m**) was investigated for proteolytic degradation by α-chymotrypsin (Figure 3). The results from evaluation of proteolytic stability against α-chymotrypsin revealed that no degradation could be detected for any of the compounds over a period of 48 h. We also found that all test compounds were chemically stable in aqueous solutions at pH 7.4 for at least 48 h.

Discussion

The research field on antimicrobial β-peptides or β-peptidomimetics is relatively new, and only a limited number of research groups have reported design of novel antimicrobial β-peptides.[23−27] Tests in vivo and in vitro have shown that β-peptides are highly stable against proteolytic degradation by human enzymes such as trypsin, chymotrypsin, and elastase.[28−30] Incorporation of β-amino acids can thereby be used as bioisosteric replacements to improve the pharmacokinetic properties of biological active peptides such as AMP's.

In the current study, we chose to investigate a scaffold that would take advantage of the profound enzymatic stability reported for β-peptides and at the same time fulfill the pharmacophore model of short AMP's with anti-staphylococcal activity.[17] The scaffold was based on an achiral lipophilic $\beta^{2,2}$-amino acid coupled to a C-terminal L-arginine amide residue, which ensured that all the β-peptidomimetics consisted of two lipophilic groups and had a net charge of +2. The resulting β-peptidomimetics were thereby bioisosteres of dipeptides but having the side-chain functionalities of tripeptides due to the $\beta^{2,2}$-amino acid derivatives.

The selection of arginine and not lysine as cationic residue was based on the presumption that the guanidine group of arginine would interact more strongly with the negatively charged phospholipids of the bacterial cell membrane by forming both electrostatic and hydrogen-bonding interactions.[31] Furthermore, the choice of a C-terminal arginine residue and not an N-terminal arginine residue was to further protect the β-peptidomimetics against proteolytic degradation by, e.g., trypsin. Trypsin has an anionic specificity-pocket favoring binding to cationic amino acids and hydrolysis of the amide bond between the cationic residue and the next amino acid in the sequence.[32] In this way, the β-peptidomimetics prepared would form poor substrates for trypsin. However, the N-terminal lipophilic $\beta^{2,2}$-amino acids could render the β-peptidomimetics susceptible to degradation by other proteases, e.g., chymotrypsin. Chymotrypsin has a lipophilic specificity pocket and normally hydrolyzes amide bonds between lipophilic/aromatic amino acids such as tyrosine, phenylalanine, tryptophan, or leucine, and the following amino acid in the sequence.[33] Thus, the resemblance of some of the $\beta^{2,2}$-peptidomimetics to naturally occurring lipophilic/aromatic amino acids could render them substrates for chymotrypsin. To investigate this, we selected six different β-peptidomimetics (**4c**, **4g**, **4h**, **4i**, **4k**, and **4m**) and treated them with α-chymotrypsin. The results revealed that no proteolytic digestion could be detected for any of the compounds within the experimental time frame of 48 h (Figure 3). The results were in agreement with other studies on pure β-peptides, and in our case the protection against proteolytic degradation could be explained by the sterical hindrance offered by the disubstituted α-carbon of the $\beta^{2,2}$-amino acids, the increased distance between the N-terminal amino group, and the carboxyl group of the $\beta^{2,2}$-amino acids, or that the β-peptidomimetics simply were too small to bind to the active site of the enzyme.

On the basis of the results of the β-peptidomimetics, the close spatial localization of the two lipophilic side-chains of the $\beta^{2,2}$-amino acid residues seemed to be an efficient motif for preparing highly potent antimicrobial β-peptidomimetics. Wessolowski et al. have recently shown that three adjacent aromatic residues in short arginine- and tryptophan-rich AMP's is an efficient structural motif for ensuring high antimicrobial potency.[34] The scaffold we have developed enabled an even more closely localization of the lipophilic groups by bringing together two lipophilic side-chains on the same α-carbon in a single $\beta^{2,2}$-amino acid residue.

An amphipathic structure is often essential for antimicrobial activity, and most natural AMP's obtain this by forming larger secondary structures such as α-helixes or β-sheets.[15,35−37] Irrespective of conformation, the β-peptidomimetics prepared in the present study would be able to form strong

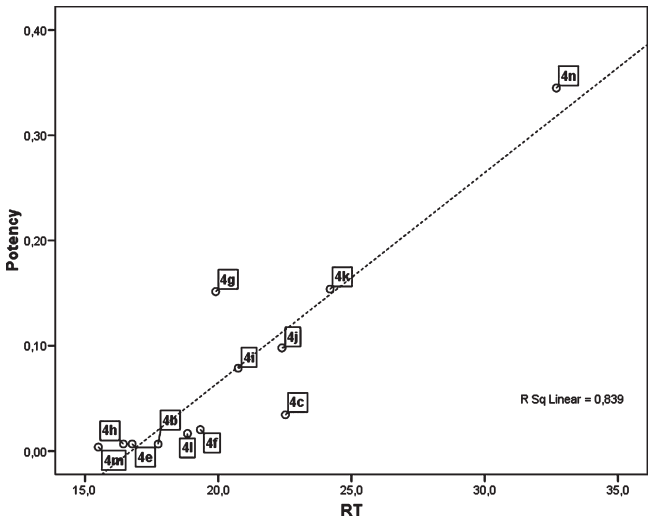


Figure 4. Relationship between antimicrobial potency (1/MIC) against *S. aureus* and the retention time (Rt) of the compounds on an analytical RP-HPLC C18 column as a measurement of overall lipophilicity.

amphipathic structures upon interaction with a bacterial cell membrane by having a cationic N-terminal amino group and a C-terminal arginine amide residue flanking the lipophilic core of the scaffold. The only β-peptidomimetic that deviated from the requirement of a strong amphipathicity was compound **4m**, which contained two relatively polar 3,5-dimethoxy benzylic side-chains. As a consequence, compound **4m** displayed much lower antimicrobial potency compared to other structurally related β-peptidomimetics prepared, such as compound **4l** containing two 3,5-dimethyl benzylic side-chains.

The results demonstrated a strong correlation between antimicrobial potency and overall lipophilicity of the β-peptidomimetics prepared. This can be illustrated by a comparison between the potency of the β-peptidomimetics against *S. aureus* and their retention time (Rt) on an analytical RP-HPLC C_{18} column, which demonstrated the affinity of the β-peptidomimetics for the hydrophobic stationary phase of the column (Figure 4). The synthesized β-peptidomimetics could roughly be grouped into three classes based on their potency and Rt. The least potent β-peptidomimetics compounds **4b**, **4e**, **4f**, **4h**, **4l**, and **4m** had Rt between 15.5 and 19.3 min, the second most potent β-peptidomimetics compounds **4c**, **4g**, **4i**, **4j**, and **4k** had Rt between 19.9 and 24.2 min, whereas the most potent β-peptidomimetic compound **4n** formed a class in solitude by being most lipophilic and having a Rt of 32.7 min. However, the correlation between antimicrobial potency and overall lipophilicity was not straightforward because the relatively long Rt of compound **4c** should indicate a much higher antimicrobial potency against *S. aureus*. Similarly, one would expect a much lower antimicrobial potency for the naphthalene derivative, compound **4g**, based on its relatively short Rt.

In addition to the importance of overall lipophilicity, certain structural alterations of the achiral lipophilic $\beta^{2,2}$-amino acid residues had an important impact on antimicrobial potency. Clearly, the superbulkiness of the *tert*-butyl-groups on the benzylic side-chains of compounds **4k** and **4n** was important to the high potency and the broad range of activity against both Gram-positive and Gram-negative bacteria that these compounds showed. The somewhat less bulky isopropyl group on the benzylic side-chains of compound **4j** ensured sufficient bulkiness for a high potency against the Gram-positive bacteria, although the potency against *E. coli* was reduced compared to compound **4k**. The importance of side-chain structure was also evident for the naphthalene derivative compound **4g**, which was the third most potent β-peptidomimetic against the Gram-positive bacteria but displayed relatively low potency against the Gram-negative bacterium *E. coli*.

The perhaps largest increase in potency by a single side-chain modification was the replacement of the *para*-methyl benzylic side-chains of compound **4h** with the *para*-trifluoromethyl benzylic side-chains, resulting in compound **4i**. The effect of the trifluoromethyl substitution on antimicrobial potency was pronounced and resulted in a 23-fold improvement in antimicrobial potency against MRSA, more than 11-fold improvement against *S. aureus*, a 6-fold improvement against MRSE, and detectable activity against *E. coli*. Substitution of benzylic or aromatic hydrogen atoms with fluorine is often used during drug optimization to increase lipophilicity or modulate basicity, or perhaps more importantly to impede metabolism by human CYP450 enzymes.[38−40] Oxidation by CYP450 enzymes often results in formation of inactive metabolites and increased renal excretion of active metabolites due to increased polarity. In the present case, the trifluoromethyl group of compound **4i** may result in increased metabolic stability compared to compound **4h** by preventing CYP450 oxidation of the side-chains to phenylmethanol, benzaldehyde, or benzoic acid derivatives.[41,42] Furthermore, the latter oxidation to a carboxylic acid group in the bulky part of the β-peptidomimetics would not only disrupt the amphipathicity of the molecule but also reduce the overall net positive charge and result in metabolites that no longer fulfilled the pharmacophore model for anti-staphylococcal activity. Thus, compound **4i** is a promising compound for further investigations by being highly potent against bacteria as a result of high lipophilicity and by possessing improved pharmacokinetic properties with respect to likely increased metabolic stability and bioavailability in vivo.[43,44]

Further structural alterations, which resulted in increased antimicrobial potency, involved elongation of the side-chains of the $\beta^{2,2}$-amino acid derivatives, as observed for the increasingly elongated and more potent aromatic β-peptidomimetics **4e** and **4f**. It was rather surprising that such structural alterations also applied to the aliphatic β-peptidomimetics compounds **4b** and **4c** and that the effect of the side-chain elongation of the aliphatic β-peptidomimetics was even more evident than for the aromatic β-peptidomimetics. The aliphatic compound **4c** had two side-chains consisting of elongated ethyl-cyclohexyl groups and was the fourth to fifth most potent β-peptidomimetic against both the Gram-positive and Gram-negative bacteria. Thus, compound **4c** was 10- to 20-fold more potent against the Gram-positive bacteria than its aromatic counterpart compound **4e**. A similar effect was observed against *S. aureus* and MRSA for the aliphatic compound **4b**, which was more potent than its aromatic counterpart compound **4d**. Previous studies on AMP's have mainly focused on aromatic side-chains due to their preference for localizing in the interface region of the phospholipid bilayer of the bacterial cell membrane.[15,35,45] However, the results of the present study showed that aliphatic side-chains containing cyclohexyl groups can give even more potent derivatives although possibly not having a preference for the membrane interface region. With regard to interaction with

the bacterial cell membrane, the aliphatic compounds will probably be more prone to interact with the aliphatic acyl-chain region in contrast to the membrane interface region and thereby cause a more efficient distortion of the intramolecular organization of the phospholipids in the cell membrane. The highly potent β-peptidomimetics containing *tert*-butyl groups (**4k** and **4n**) may combine the effects of the aliphatic compounds and the less bulky aromatic compounds by being partly aromatic and partly aliphatic.

We used hemolytic activity as a measurement of toxicity against mammalian cells, but due to a general low hemolytic activity, we chose to measure 20% hemolysis (EC_{20}) in place of the standard measurement of 50% hemolysis (EC_{50}). Only compounds **4g**, **4i**, **4j**, **4k**, and **4n** displayed measurable hemolytic activity, whereas no hemolytic activity at all could be detected for the other β-peptidomimetics prepared up to 1000 μg/mL. Highest hemolytic activity was displayed by compound **4n** that, as described above, was the overall most potent β-peptidomimetic against all bacterial strains and also the most lipophilic compound prepared. It was noteworthy that compounds **4j** and **4k**, which displayed comparable antimicrobial activities against the Gram-positive bacteria, showed dissimilar hemolytic activity. Haug et al.[46] have recently reported a series of synthetic di- and tripeptides displaying MIC values of 3.2−28 μM against MRSA and shown that the hemolytic activity (EC_{50}) varies from 97 μM to above the detection limit of 1000 μg/mL, i.e., nonhemolytic. Thus, on the basis of the results from Haug et al. and the present study, minor structural modifications of the lipophilic groups of small AMP-based molecules can have a large effect on hemolytic activity, whereas antimicrobial activity is much less affected.

The EC_{20} measurements were also used to calculate a therapeutic index with respect to antimicrobial activity against MRSA and in which case the hemolytic activity of the nonhemolytic compounds was set to an arbitrary EC_{20} value of 1000 μg/mL. On the basis of a total evaluation of antimicrobial activity against MRSA, metabolic stability, and the therapeutic index, compound **4i** containing two *para*-trifluoromethyl benzylic side-chains, and with a therapeutic index of 236, appeared as the most promising β-peptidomimetic. In the case of antimicrobial activity against MRSE, compound **4k** emerged as the most promising candidate, with a therapeutic index of 116 (calculation not shown). With respect to the Gram-negative bacterium *E. coli*, a therapeutic index of 12.5 was displayed by compound **4k** while the much more potent β-peptidomimetic **4n** had a therapeutic index of 8.9 (calculations not shown). Thus, depending on the pathogenic bacteria in question, different β-peptidomimetics should be used in further studies based on a total evaluation of antimicrobial potency, therapeutic index, and pharmacokinetic properties.

Although determination of the precise mechanism of action of the β-peptidomimetics was not a subject of our study, the β-peptidomimetics reported herein share many similarities with observations from short AMP's and may therefore indicate a resembling mechanism of action. All β-peptidomimetics prepared in the present study fulfilled the pharmacophore model of short AMP's with anti-staphylococcal activity and the general requirements of larger AMP's by having a net charge of +2 and an amphipathic structure.[34,47] As shown in Table 1, all β-peptidomimetics prepared except for compound **4n** were more potent against the Gram-positive bacteria *S. aureus*, MRSA, and MRSE than against the Gram-negative bacteria *E. coli*, which is consistent with what has been reported for many short AMP's.[17] We experienced very small differences in MIC and MBC values, i.e., one titer-step or less, which is not uncommon to short AMP's and may indicate that killing is the main bacteriostatic mode of action (MBC results are shown in the Supporting Information). Another important observation shared by short AMP's was that compounds of similar molecular mass were almost equally potent.[18] This was observed when comparing the potencies of compounds **4e** and **4h** (M_w's of 453.3) and compounds **4f** and **4l** (M_w's of 481.3), which were constitutional isomers and displayed similar MIC values. A similar mass-balance relationship has been observed for short AMP's, showing that there are small differences in potency with respect to constitutional isomers as long as the requirements of a net positive charge and ability to form amphipathic structures are fulfilled.[48]

Tew et al.[49] have recently reported a study on AMP-mimics based on polymeric phenyl acetylenes and have shown that the smallest phenyl acetylene derivative containing three aromatic rings and having a net charge of +2 displays the highest antimicrobial potency. It is noteworthy that this compound was slightly more potent against *E. coli* than against *S. aureus*, although polymeric derivatives of the same series showed the opposite effect. The present study and the study by Tew et al. demonstrate that different types of amphipathic scaffolds can be used to design highly potent mimics of short AMP's but with improved pharmacokinetic properties.

Conclusion

We have prepared a series of highly potent β-peptidomimetics that were based on the pharmacophore model of short AMP's with anti-staphylococcal activity.[17] The β-peptidomimetics consisted of a lipophilic $\beta^{2,2}$-amino acid coupled to a C-terminal L-arginine amide residue, ensuring a net positive charge of +2. The β-peptidomimetics were amphipathic as a consequence of the close association of the lipophilic side-chains on the same α-carbon of the $\beta^{2,2}$-amino acid residue and by having the lipophilic side-chains positioned between two cationic groups. Amphipathicity is a crucial feature for efficient membrane interaction by naturally occurring AMP's and also for selectivity for bacterial cells compared to human cells.[14,35] We observed a strong correlation between antimicrobial potency and overall lipophilicity of the β-peptidomimetics, but structural properties of the $\beta^{2,2}$-amino acid side-chains were also highly decisive for the antimicrobial potency. Side-chain modifications that gave the most potent derivatives included *tert*-butyl benzyl groups (**4k** and **4n**), naphthalene groups (**4g**), aliphatic cyclohexyl groups (**4c**), and trifluoromethyl benzyl groups (**4i**).

On the basis of an evaluation of the therapeutic index for β-peptidomimetics displaying MIC values below 10 μM against the Gram-positive test-bacteria, compound **4i** containing two *para*-trifluoromethyl benzylic side-chains emerged as the most promising β-peptidomimetic against MRSA infections, with a therapeutic index of 236. However, compound **4k** containing two *para-tert*-butyl benzylic side-chains appeared as the most promising candidate against MRSE infections, with a therapeutic index of 116. Compound **4n** was the most potent β-peptidomimetic against the Gram-negative bacterium *E. coli* and had a therapeutic index of 8.9 compared to human RBC. Although compound **4n** had a lower therapeutic index against *E. coli* than compounds **4i** and

4k against the Gram-positive bacteria, compound **4n** is a promising lead compound for further development of small β-peptidomimetics against pathogenic Gram-negative bacteria. Further studies to address this issue are in progress at our laboratory.

In conclusion, relatively small structural alterations of the β-peptidomimetics had a considerable impact on bacterial strain specificity and an even larger influence on hemolytic activity. Furthermore, the small size of the β-peptidomimetics may provide solutions to many common problems associated with developing AMP's into novel antimicrobial agents, such as being cost-efficient to synthesize, more stable against proteolytic degradation by nature of containing a $\beta^{2,2}$-amino acid derivative, and perhaps more importantly, the small size of the prepared β-peptidomimetics may involve less risk of allergic responses. The study has demonstrated that small β-peptidomimetics can be used to mimic the antimicrobial potency and selectivity of much larger AMP's and that such small amphipathic scaffolds may be promising drug candidates for treatment of serious bacterial infections.

Experimental Section

Chemistry in General. ^{1}H and ^{13}C NMR spectra were recorded on 400 or 600 MHz Varian spectrometers. Chemical shifts are expressed in ppm relative to $CHCl_3$ (^{1}H 7.26 ppm, ^{13}C 77.0 ppm) or methanol (^{1}H 3.31 ppm, ^{13}C 39.0 ppm). The values are given in δ scale. Mass spectra were obtained on a Micromass Quattro LC (Micromass, Manchester, UK). High-resolution mass spectra were obtained on a Waters Micromass LCT Premier mass spectrometer (Micromass, Manchester, UK). Commercially available compounds and solvents were purchased from Sigma-Aldrich and used without further purification. Preparative RP-HPLC was carried out on a Waters system equipped with a RP BondaPak C_{18}, 125 Å, 10 μm, 25 mm × 100 mm column, and eluted with acetonitrile and water, both containing 0.1% TFA. Analytical HPLC was carried out on a Waters 2695 HPLC equipped with an RP-HPLC Delta Pak C_{18}, 100 Å, 5 μm, 3.9 mm × 150 mm column and analyzed at wavelengths 214 and 254 nm with a PDA detector spanning from wavelengths 210 to 310 nm. All compounds were prepared by using parallel reaction carousels from Radleys.

General Procedure for Dialkylation of Methyl Cyanoacetate (GP1) 1a−n. The synthesis was performed in accordance to Cronin et al.[19] In brief, sodium methoxide (20 mmol) was dissolved in methanol (0.2 M) and methyl cyanoacetate (20 mmol) was added. The reaction mixture was stirred for 5 min at rt before the desired benzyl bromide (20 mmol) was added and the solution was heated to reflux. After 15 min, the solution was cooled to rt. A second portion of sodium methoxide (20 mmol) was added, and after 5 min of stirring at rt, the second portion of the desired benzyl bromide (20 mmol) was added followed by 15 min reflux. The volume of the reaction mixture was reduced to about 1/3 under vacuum and extracted with water/ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The product was used in following synthesis without any further purification.

General Procedure for Reduction of Nitriles to Amines with Ra/Ni and Following Boc-Protection (GP2) 2a−n. The synthesis was performed in accordance to Cronin et al.[19] and Bodanzky et al.[50] In brief, Ra/Ni (approximate 2 mL/g methyl cyanoacetate derivative was washed 3 times with methanol under argon before the desired methyl cyanoacetate derivative (3.5 mmol) dissolved in methanol (0.1 M) was added along with acetic acid (approximately 1 mL/g methyl cyanoacetate derivative). The reaction mixture was hydrogenated at 45 °C for 5 days under 1 bar H_2 pressure. Afterward, the reaction mixture was filtered through celite to remove the Ra/Ni before the reaction mixture was evaporated to dryness. The crude $\beta^{2,2}$-amino acid methyl ester (0.35 mmol) was dissolved in a mixture of 1,4-dioxane and water 5:1 (~0.35 M) and the pH adjusted to 8 with TEA. Boc_2O (0.42 mmol) dissolved in as little as possible 1,4-dioxane was added before the solution was stirred at rt for 18 h. The reaction mixture was acidified to pH 2−3 with 10% citric acid and extracted three times with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The product was used in the following synthesis without any further purification.

General Procedure for Ester Hydrolysis (GP3) 3a−n. The synthesis was performed in accordance to Seebach et al.[51] In brief, the Boc-protected $\beta^{2,2}$-amino acid methyl ester (0.35 mmol) was dissolved in a mixture of 1,4-dioxane and water 3:1 (1.17 mM) before lithium hydroxide (2.1 mmol) dissolved in as little water as possible was added. The reaction mixture was stirred at reflux under N_2 for 18 h before the volume was reduced to approximately 1/5 under vacuum. Water (10 mL) was added to the reaction mixture and the pH adjusted to 1−2 with dropwise addition of 0.1 M HCl. The aqueous solution was extracted three times with equal volumes of ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The product was used in the following synthesis without any further purification.

General Procedure for Coupling of Boc-Protected $\beta^{2,2}$-Amino Acids and L-Arginine Amide (GP4) 4a−n. The synthesis was performed in accordance with Chan and White.[52] In brief, the Boc-protected $\beta^{2,2}$-amino acid (0.2 mmol) was dissolved in DMF (0.02 M) and DIPEA (0.6 mmol) was added along with TFFH (0.2 mmol). The $\beta^{2,2}$-amino acid was preactivated for 2 h before H-Arg-NH_2·2HCl (0.3 mmol) was added. The reaction mixture was stirred at rt for 7 days before it was diluted with ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The crude Boc-protected β-peptidomimetic was deprotected by dissolving it in DCM (~0.4 M) and adding an equivalent volume of TFA:TIS:water (95:2.5:2.5). The mixture was stirred at rt for 2 h before it was evaporated to dryness. The crude product was purified by preparative RP-HPLC. The purity of the pure β-peptidomimetics was checked by analytical RP-HPLC before the solution was evaporated to dryness, and the residue was redissolved in water and lyophilized. All compounds possessed purity above 95%.

Synthesis of Cyano-diallyl Acetic Acid Methyl Ester (1a). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 2.54−2.58 (2H, m), 2.63−2.68 (2H, m), 3.80 (3H, s), 5.23 (2H, s), 5.26 (2H, s), 5.78−5.83 (2H, m). ^{13}C NMR ($CDCl_3$): δ 40.6, 49.3, 53.5, 118.3, 121.0, 130.4, 168.5. Brown−yellow oil, yield 71%. MS-ESI+: $[M + H]^+$ calcd180.1, found 179.9

Synthesis of Cyano-dicyclohexylmethyl Acetic Acid Methyl Ester (1b). The synthesis was conducted in accordance to GP1 with the exception of 2 h reflux after each time the bromide was added. ^{1}H NMR ($CDCl_3$): δ 0.79−1.31 (11H, m), 1.45−1.78 (11H, m), 1.80−1.85 (4H, m), 3.77 (3H, s). ^{13}C NMR ($CDCl_3$): δ 25.9, 31.6, 32.8, 33.7, 40.6, 46.1, 53.0, 119.6, 170.4. Red oil, yield: 45%. MS-ESI+: [M + H]+ calcd 292.2, found 292.1

Synthesis of Cyano-di(2-cyclohexyl-eth-1-yl) Acetic Acid Methyl Ester (1c). The synthesis was conducted in accordance to GP1 with the exception of 2 h reflux after each time the bromide was added. ^{1}H NMR ($CDCl_3$): δ 0.83−0.95 (4H, m), 1.08−1.27 (9H, m), 1.32−1.47 (3H, m), 1.59−1.98 (14H, m), 3.69 (3H, s). ^{13}C NMR ($CDCl_3$): δ 26.1, 26.4, 32.7, 32.9, 33.0, 35.1, 37.4, 50.0, 53.1, 119.4, 169.9. Red oil, yield 56%. MS-ESI+: $[M + Na]^+$ calcd 342.2, found 342.1

Synthesis of Cyano-dibenzyl Acetic Acid Methyl Ester (1d). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 3.16 (2H, d, J = 13.6 Hz), 3.38 (2H, d, J = 13.2 Hz), 3.59 (s, 3H), 7.36 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 43.3, 53.1,

53.4, 118.4, 127.9, 128.6, 129.9, 168.6. Yellow viscous oil, yield 96%. MS-ESI+: $[M + H]^+$ calcd 280.1, found 280.0

Synthesis of Cyano-di(2-phenyl-eth-1-yl) Acetic Acid Methyl Ester (1e). The synthesis was conducted in accordance to GP1 with the exception of 2 h reflux after each time the bromide was added. ^{1}H NMR ($CDCl_3$): δ 2.12 (2H, dt, J = 4.8 Hz, J = 13 Hz), 2.28 (2H, m,), 2.45 (2H, m), 2.72 (2H, dt, J = 5.6 Hz, J = 13.2 Hz), 3.78 (3H, s), 7.24−7.40 (10H, m). ^{13}C NMR ($CDCl_3$): δ 32.5, 36.4, 49.5, 53.3, 118.8, 126.6, 128.5, 128.3, 138.8, 169.1. Red oil, yield 98%. MS-ESI+: $[M + H]^+$ calcd 308.2, found 308.1

Synthesis of Cyano-di(3-phenyl-prop-1-yl) Acetic Acid Methyl Ester (1f). The synthesis was conducted in accordance to GP1 with the exception of 2 h reflux, after each time the bromide was added. ^{1}H NMR ($CDCl_3$): δ 1.98 (4H, m), 2.24 (2H, t, J = 7.2 Hz), 2.71 (2H, t, J = 7.2 Hz), 2.85 (2H, t, J = 7.6 Hz), 3.46 (2H, t, J = 6.8 Hz), 3.83 (3H, s), 7.22−7.36 (10H, m). ^{13}C NMR ($CDCl_3$): δ 26.9, 35.1, 36.8, 49.5, 53.2, 119.1, 126.1, 128.2, 128.4, 140.7, 169.5. Red oil, yield 100%. MS-ESI+: $[M + H]^+$ calcd 336.2, found 336.1

Synthesis of Cyano-di(2-methylene-naphthalene) Acetic Acid Methyl Ester (1g). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 3.33 (2H, d, J = 13.5 Hz), 3.51 (3H, s), 3.56 (2H, d, J = 13.5 Hz), 7.44 (2H, d, J = 8.2 Hz), 7.48−7.49 (4H, m), 7.78 (2H, s), 7.82−7.84 (6H, m). ^{13}C NMR ($CDCl_3$): δ 43.4, 53.2, 53.4, 118.4, 126.1, 126.2, 127.6, 127.7, 127.9, 128.3, 129.1, 131.5, 132.8, 133.3, 168.8. Yellow oil, yield 125%. MS-ESI+: $[M + H]^+$ calcd 380.2, found 379.9.

Synthesis of Cyano-di(4-methyl-benzyl) Acetic Acid Methyl Ester (1h). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 2.38 (6H, s), 3.11 (2H, d, J = 13.7 Hz), 3.34 (2H, d, J = 13.3 Hz), 3.61 (3H, s), 7.18 (4H, d, J = 8.2 Hz), 7.23 (4H, d, J = 8.2 Hz). ^{13}C NMR ($CDCl_3$): δ 21.2, 42.9, 53.2, 53.5, 118.6, 129.4, 129.8, 131.1, 137.6, 168.9. Clear oil, yield 103%. MS-ESI+: $[M + H]^+$ calcd 308.2, found 308.1.

Synthesis of Cyano-di(4-trifluoromethyl-benzyl) Acetic Acid Methyl Ester (1i). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 3.17 (2H, d, J = 13.5 Hz), 3.40 (2H, d, J = 13.5 Hz), 3.57 (3H, s), 7.43 (4H, d, J = 7.6 Hz), 7.61 (4H, d, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$): δ 42.8, 52.8, 53.5, 117.7, 123.9 (q, J = 272.2 Hz), 125.7, 129.3, 130.3, 137.7, 168.0. Yellow oil, yield 133%. MS-ESI+: $[M + H]^+$ calcd 416.1, found 416.1

Synthesis of Cyano-di(4-isopropyl-benzyl) Acetic Acid Methyl Ester (1j). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 1.24 (12H, d, J = 6.6 Hz), 2.89 (2H, m), 3.08 (2H, d, J = 13.7 Hz), 3.29 (2H, d, J = 13.7 Hz), 3.56 (3H, s), 7.17−7.23 (8H, m). ^{13}C NMR ($CDCl_3$): δ 23.9, 33.7, 42.8, 53.1, 53.5, 118.7, 126.6, 129.8, 131.3, 148.4, 168.8. White solid product, yield 115%. MS-ESI+: $[M + H]^+$ calcd 364.2, found 364.3

Synthesis of Cyano-di(4-*tert*-butyl-benzyl) Acetic Acid Methyl Ester (1k). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 1.31 (18H, s), 3.28 (2H, d, J = 14 Hz), 3.29 (2H, d, J = 13.6 Hz), 3.56 (3H, s), 7.22 (4H, d, J = 8.4 Hz), 7.34 (4H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 31.3, 34.5, 42.8, 53.1, 53.4, 118.7, 125.5, 129.6, 150.7, 168.8. White viscous, yield 96%. MS-ESI+: $[M + H]^+$ calcd 392.3, found 392.2.

Synthesis of Cyano-di(3,5-dimethyl-benzyl) Acetic Acid Methyl Ester (1l). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 2.39 (12H, s), 3.06 (2H, d, J = 13.3 Hz), 3.28 (2H, d, J = 13.8 Hz), 3.67 (3H, s), 6.98 (4H, s), 7.02 (2H, s). ^{13}C NMR ($CDCl_3$): δ 21.1, 42.9, 52.8, 53.2, 118.3, 127.6, 129.2, 133.8, 137.8, 168.8. Yellow oil, yield 80%. MS-ESI+: $[M + H]^+$ calcd 336.2, found 336.1.

Synthesis of Cyano-di(3,5-dimethoxy-benzyl) Acetic Acid Methyl Ester (1m). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 3.06 (2H, d, J = 13.3 Hz), 3.28 (2H, d, J = 13.8 Hz), 3.66 (3H, s), 3.81 (12H, s), 6.44 (2H, s), 6.48 (4H, s). ^{13}C NMR ($CDCl_3$): δ 43.4, 53.0, 53.3, 55.3, 100.0, 107.9, 118.5, 136.0, 160.7, 168.7. Light-brown viscous oil, yield 100%. MS-ESI+: $[M + H]^+$ calcd 400.2, found 400.1.

Synthesis of Cyano-di(3,5-*ditert*-butyl-benzyl) Acetic Acid Methyl Ester (1n). The synthesis was conducted in accordance to GP1. ^{1}H NMR ($CDCl_3$): δ 1.35 (36H, s), 3.15 (2H, d, J = 13.3 Hz), 3.38 (2H, d, J = 13.3 Hz), 3.51 (3H, s), 7.14−7.40 (6H, m). ^{13}C NMR ($CDCl_3$): δ 31.4, 34.8, 44.0, 52.9, 54.1, 118.6, 122.0, 124.3, 133.2, 150.8, 168.9. White solid product, yield 100%. MS-ESI+: $[M + H]^+$ calcd 504.4, found 504.2.

Synthesis of Boc-2,2-dipropyl β-Amino Acid Methyl Ester (2a). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 0.84 (6H, t, J = 7.0 Hz), 1.17−1.27 (4H, m), 1.38−1.48 (13H, m), 3.28 (2H, br d, J = 6.2 Hz), 3.64 (3H, s). ^{13}C NMR ($CDCl_3$): δ 14.5, 17.2, 28.2, 36.1, 43.5, 50.2, 51.6, 79.0, 155.9, 176.8. Yellow oil, yield 53%. MS-ESI+: $[M + H]^+$ calcd 288.2, found 288.1.

Synthesis of Boc-2,2-dicyclohexylmethyl β-Amino Acid Methyl Ester (2b). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 0.82−1.68 (26H, m), 1.48 (9H, s), 3.38 (2H, d, J = 6.3 Hz), 3.63 (3H, s). ^{13}C NMR ($CDCl_3$): δ 26.1, 26.3, 28.3, 33.5, 34.0, 35.2, 43.3, 43.7, 51.4, 79.6, 155.8, 170.0. Yellow oil, yield 62%. MS-ESI+: $[M + H]^+$ calcd 396.3, found 396.2.

Synthesis of Boc-2,2-di(2-cyclohexyl-eth-1-yl) β-Amino Acid Methyl Ester (2c). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 0.20−0.35 (4H, m), 0.95−1.20 (14H, m), 1.35−1.40 (9H, m), 1.50−1.68 (12H, m) 3.22 (2H, br d, J = 6.2 Hz), 3.60 (3H, s). ^{13}C NMR ($CDCl_3$): δ 26.1, 26.4, 28.2, 30.9, 31.1, 33.1, 38.0, 43.4, 49.9, 51.5, 78.7, 155.7, 176.9. Yellow oil, yield 58%. MS-ESI+: $[M + H]^+$ calcd 424.3, found 424.5

Synthesis of Boc-2,2-dibenzyl β-Amino Acid Methyl Ester (2d). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 2.81 (2H, d J = 13.7 Hz), 3.14 (2H, d, J = 14.0 Hz), 3.29 (2H, br d, J = 5.9 Hz), 3.59 (s, 3H), 7.13−7.29 (10H, m). ^{13}C NMR ($CDCl_3$): δ 28.4, 41.3, 43.1, 51.6, 52.2, 79.2, 126.7, 128.2, 130.2, 136.4, 156.0, 175.5. Yellow oil, yield 86%. MS-ESI+: $[M + H]^+$ calcd 384.2, found 384.1.

Synthesis of Boc-2,2-di(2-phenyl-eth-1-yl) β-Amino Acid Methyl Ester (2e). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.55 (9H, s), 1.90−2.00 (4H, m), 2.62−2.71 (4H, m), 3.58 (2H, br d), 3.72 (3H, s), 7.21−7.30 (10H, m). ^{13}C NMR ($CDCl_3$): δ 28.1, 30.4, 36.0, 43.2, 51.6, 51.8, 79.1, 125.8, 128.2, 128.4, 141.7, 155.9, 176.0. Yellow oil, yield 41%. MS-ESI+: $[M + H]^+$ calcd 412.3, found 412.2.

Synthesis of Boc-2,2-di(3-phenyl-prop-1-yl) β-Amino Acid Methyl Ester (2f). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.44 (13H, s), 1.46 (4H, m), 1.54 (4H, m), 2.56 (2H, br t), 3.32 (2H, br d, J = 6.2 Hz), 3.65 (3H, s), 7.14−7.29 (10H, m). ^{13}C NMR ($CDCl_3$): δ 25.6, 28.2, 33.2, 36.1, 43.6, 50.0, 51.6, 79.0, 125.7, 128.2, 128.3, 141.8, 155.8, 176.4. Yellow oil, yield 55%. MS-ESI+: $[M + H]^+$ calcd 440.3, found 440.1.

Synthesis of Boc-2,2-di(2-methylene-naphthalene) β-Amino Acid Methyl Ester (2g). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.53 (9H, s), 2.98−3.39 (6H, m), 3.63 (3H, s), 7.26 −7.80 (14H, m). ^{13}C NMR ($CDCl_3$): δ 27.4, 29.3, 51.6, 52.3, 126.0, 127.2, 127.5, 127.7, 128.9, 129.0, 131.0, 133.3. Yellow oil, yield 89%. MS-ESI+: $[M + H]^+$ calcd 484.2, found 484.0.

Synthesis of Boc-2,2-di(4-methyl-benzyl) β-Amino Acid Methyl Ester (2h). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, s), 2.30 (6H, s), 2.75 (2H, d, J = 14.1 Hz), 3.08 (2H, d, J = 14.1 Hz), 3.25 (2H, br d, J = 5.9 Hz), 3.68 (3H, s), 7.01 (4H, d, J = 8.2 Hz), 7.06 (4H, d, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$): δ 21.0, 28.4, 40.9, 43.0, 51.6, 52.2, 79.1, 128.9, 130.0, 133.3, 136.2, 156.0, 175.7. Yellow oil, yield 64%. MS-ESI+: $[M + H]^+$ calcd 412.3, found 412.1

Synthesis of Boc-2,2-di(4-trifluoromethyl-benzyl) β-Amino Acid Methyl Ester (2i). The synthesis was conducted in accordance to GP2 with the exception of only 24 h hydrogenation with Ra/Ni. ^{1}H NMR ($CDCl_3$): δ 1.35 (9H, s), 3.08 (2H, d, J = 13.3 Hz), 3.20 (2H, br d, J = 5.9 Hz), 3.32 (2H, d, J = 13.5 Hz), 3.47 (3H, s), 7.17 (2H, d, J = 9.7 Hz), 7.33 (2H, d, J = 8.1 Hz), 7.44

(2H, d, J = 7.9 Hz), 7.52 (2H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 28.3, 41.2, 42.8, 51.9, 53.5, 125.4, 125.6, 130.3, 146.7, 168.0. Yellow oil, yield 98%. MS-ESI+: $[M + H]^+$ calcd 520.2, found 520.0.

Synthesis of Boc-2,2-di(4-isopropyl-benzyl) β-Amino Acid Methyl Ester (2j). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.20−1.22 (12H, m), 1.45 (9H, s), 2.76 (2H, d, J = 13.8 Hz), 2.89 (2H, m), 3.09 (2H, d, J = 13.8 Hz), 3.28 (2H, br d, J = 6.4 Hz), 3.71 (3H, s), 7.01−7.12 (8H, m). ^{13}C NMR ($CDCl_3$): δ 23.9, 27.4, 33.6, 41.0, 51.6, 51.9, 126.4, 129.9, 132.8, 146.7, 156.3, 172.0. Yellow oil, yield 111%. MS-ESI+: $[M + H]^+$ calcd 468.3, found 468.2.

Synthesis of Boc-2,2-di(4-*tert*-butyl-benzyl) β-Amino Acid Methyl Ester (2k). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.30 (18H, s), 1.54 (9H, s), 2.77 (2H, d, J = 14.0 Hz), 3.11 (2H, d, J = 13.3 Hz), 3.30 (2H, br s), 3.69 (3H, s), 7.07 (2H, d, J = 7.8 Hz), 7.28 (2H, d, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$): δ 27.8, 31.3, 34.3, 40.9, 43.2, 51.6, 52.2, 79.0, 125.1, 129.8, 133.3, 149.4, 156.0, 175.7. Yellow oil, yield 75%. MS-ESI+: $[M + H]^+$ calcd 496.3, found 496.2.

Synthesis of Boc-2,2-di(3,5-dimethyl-benzyl) β-Amino Acid Methyl Ester (2l). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.47 (9H, s), 2.27 (12H, s), 2.72 (2H, d, J = 13.7 Hz), 3.06 (2H, d, J = 16.6 Hz), 3.24 (2H, br s), 3.69 (3H, s), 6.73 (4H, s), 6.85 (2H, s). ^{13}C NMR ($CDCl_3$): δ 21.2, 28.3, 41.0, 42.8, 51.4, 52.0, 79.0, 128.1, 128.3, 136.1, 137.5, 155.9, 175.9. Yellow oil, yield 79%. MS-ESI+: $[M + H]^+$ calcd 440.3, found: 440.1.

Synthesis of Boc-2,2-di(3,5-dimethoxy-benzyl) β-Amino Acid Methyl Ester (2m). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.43 (9H, s), 2.69 (2H, d, J = 13.7 Hz), 3.07 (2H, d, J = 13.6 Hz), 3.32 (2H, br d, J = 5.5 Hz), 3.70 (3H, s), 3.75 (12 H, s), 6.30−6.49 (6H, m). ^{13}C NMR ($CDCl_3$): δ 27.3, 41.3, 42.6, 51.8, 52.1, 55.2, 79.2, 98.9, 108.2, 138.5, 160.5, 175.6. Brown oil, yield 80%. MS-ESI+: $[M + H]^+$ calcd 504.2, found 504.1.

Synthesis of Boc-2,2-di(3,5-di-*tert*-butyl-benzyl) β-Amino Acid Methyl Ester (2n). The synthesis was conducted in accordance to GP2. ^{1}H NMR ($CDCl_3$): δ 1.30 (36H, s), 1.44 (9H, s), 2.80 (2H, d, J = 13.9 Hz), 3.14 (2H, d, J = 13.8 Hz), 3.25 (2H, br d), 3.42 (3H, s), 7.00−7.36 (6H, m). ^{13}C NMR ($CDCl_3$): δ 27.4, 31.5, 34.7, 44.0, 51.7, 58.2, 122.0, 124.3, 146.7, 150.5, 168.9. Yellow oil, yield 60%. MS-ESI+: $[M + H]^+$ calcd 608.5, found 608.3

Synthesis of Boc-2,2-dipropyl β-Amino Acid (3a). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 0.90 (6H, m), 1.17−1.31 (4H, m), 1.42 (9H, s), 1.51−1.53 (4H, m), 3.32 (2H, br d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 14.5, 17.2, 28.3, 36.0, 43.5, 50.1, 79.2, 156.0, 182.6. Yellow solid product, yield 75%. MS-ESI+: $[M + H]^+$ calcd 274.2, found 274.1.

Synthesis of Boc-2,2-dicyclohexylmethyl β-Amino Acid (3b). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 0.84−1.63 (26H, m), 1.42 (9H, s), 3.38 (2H, d, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$): δ 26.1, 26.4, 28.3, 33.6, 35.3, 43.4, 48.4, 51.4, 79.2, 156.0, 182.5. Yellow solid product, yield 17%. MS-ESI+: $[M + H]^+$ calcd 382.3, found 382.2.

Synthesis of Boc-2,2-di(2-cyclohexyl-eth-1-yl) β-Amino Acid (3c). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 0.80−0.90 (4H, m), 1.08−1.25 (14H, m), 1.42 (9H, s), 1.50−1.72 (12H, m), 3.32 (2H, br d). ^{13}C NMR ($CDCl_3$): δ 26.2, 26.3, 28.3, 31.1, 33.1, 33.2, 38.2, 43.5, 49.8, 79.2, 155.9, 182.4. Yellow solid product, yield 100%. MS-ESI+: $[M + H]^+$ calcd 410.3, found 410.3.

Synthesis of Boc-2,2-dibenzyl β-Amino Acid (3d). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 2.83 (2H, d J = 14.1 Hz), 3.17 (2H, d, J = 13.5 Hz), 3.29 (2H, br s), 7.20−7.28 (10H, m). ^{13}C NMR ($CDCl_3$): δ 28.4, 37.7, 41.3, 51.9, 79.4, 126.8, 128.2, 128.5, 136.2, 156.2, 180.9. Off-white solid product, yield 76%. MS-ESI+: $[M + H]^+$ calcd 370.2, found 370.2.

Synthesis of Boc-2,2-di(2-phenyl-eth-1-yl) β-Amino Acid (3e). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 1.90−2.05 (4H, m), 2.60−2.75 (4H, m), 3.54 (2H, d, J = 6.3 Hz), 7.18−7.26 (10H, m). ^{13}C NMR ($CDCl_3$): δ 28.1, 30.4, 35.9, 43.2, 50.3, 79.6, 125.9, 128.2, 128.4, 141.7, 156.1, 181.7. Yellow solid product, yield 92%. MS-ESI+: $[M + H]^+$ calcd 398.2, found 398.2.

Synthesis of Boc-2,2-di(3-phenyl-prop-1-yl) β-Amino Acid (3f). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, s), 1.59 (8H, m), 2.57 (4H, br t), 3.33 (2H, br d, J = 6.4 Hz), 7.14−7.29 (10H, m). ^{13}C NMR ($CDCl_3$): δ 25.6, 28.3, 33.1, 36.2, 43.6, 49.8, 79.3, 125.8, 128.2, 128.3, 141.8, 156.0, 182.0. White solid product, yield 58%. MS-ESI+: $[M + H]^+$ calcd 426.3, found 426.5.

Synthesis of Boc-2,2-di(2-methylene-naphthalene) β-Amino Acid (3g). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.43 (9H, s), 2.96−3.53 (6H, m), 7.31 −8.07 (14H, m). ^{13}C NMR ($CDCl_3$): δ 28.3, 34.4, 37.9, 49.0, 79.7, 126.0, 127.2, 127.5, 127.5, 127.6, 128.1, 132.2, 133.4, 136.2, 156.0, 180.4. Gray solid product, yield 80%. MS-ESI+: $[M + H]^+$ calcd 470.2, found 470.1.

Synthesis of Boc-2,2-di(4-methyl-benzyl) β-Amino Acid (3h). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.47 (9H, s), 2.31 (6H, s), 2.80 (2H, d, J = 13.9 Hz), 3.14 (2H, d, J = 13.9 Hz), 3.28 (2H, br d, J = 6.4 Hz), 7.06−7.12 (8H, m). ^{13}C NMR ($CDCl_3$): δ 21.0, 28.3, 40.8, 42.9, 51.9, 79.3, 128.9, 130.2, 136.2, 156.1, 181.0. Yellow solid product, yield 62%. MS-ESI+: $[M + H]^+$ calcd 398.2, found 398.1.

Synthesis of Boc-2,2-di(4-trifluoromethyl-benzyl) β-Amino Acid (3i). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 3.17 (2H, d, J = 13.5 Hz), 3.28 (2H, br d), 3.42 (2H, d, J = 13.5 Hz), 7.39 (2H, d, J = 8.1 Hz), 7.52 (2H, d, J = 8.1 Hz), 7.57 (2H, d, J = 8.1 Hz), 7.63 (2H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 28.2, 37.6, 42.4, 51.7, 80.2, 125.2, 125.8, 129.4, 140.3, 156.4, 179.4. Yellow viscous oil, yield 52%. MS-ESI+: $[M + H]^+$ calcd 506.2, found 506.0.

Synthesis of Boc-2,2-di(4-isopropyl-benzyl) β-Amino Acid (3j). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.25 (12H, t, J = 5.7 Hz), 1.47 (9H, s), 2.81 (2H, d, J = 13.0 Hz), 2.88 (2H, m), 3.17 (2H, d, J = 13.9 Hz), 3.31 (2H, br d, J = 5.4 Hz), 7.14−7.16 (8H, m). ^{13}C NMR ($CDCl_3$): δ 23.9, 28.4, 33.6, 40.8, 49.0, 51.9, 79.8, 126.4, 129.9, 132.8, 146.7, 156.3, 172.0. Yellow solid product, yield 62%. MS-ESI+: $[M + H]^+$ calcd 454.3, found 454.1.

Synthesis of Boc-2,2-di(4-*tert*-butyl-benzyl) β-Amino Acid (3k). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.25 (27H, s), 2.78 (2H, br s), 3.13 (2H, br s), 7.13 (4H, br s), 7.23 (4H, br s). ^{13}C NMR ($CDCl_3$): δ 28.3, 31.3, 34.3, 40.9, 51.5, 79.3, 125.0, 130.1, 133.3, 149.2, 156.3, 181.7. White solid product, yield 56%. MS-ESI+: $[M + H]^+$ calcd 482.3, found 482.1.

Synthesis of Boc-2,2-di(3,5-dimethyl-benzyl) β-Amino Acid (3l). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 2.25 (12 H, s), 2.74 (2H, d, J = 12.9 Hz), 3.09 (2H, d, J = 12.3 Hz), 3.24 (2H, br s), 6.80 (2H, s), 6.85 (1H, s). ^{13}C NMR ($CDCl_3$): δ 21.2, 28.4, 40.9, 42.8, 51.9, 79.3, 126.7, 128.3, 135.9, 137.5, 156.1, 181.6. White solid product, yield 70%. MS-ESI+: $[M + H]^+$ calcd 426.3, found 426.2.

Synthesis of Boc-2,2-di(3,5-dimethoxy-benzyl) β-Amino Acid (3m). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, s), 2.71 (2H, d, J = 13.7 Hz), 3.11 (2H, d, J = 13.6 Hz), 3.32 (2H, br d, J = 5.5 Hz), 3.73 (12 H, s), 6.31−6.49 (6H, m). ^{13}C NMR ($CDCl_3$): δ 28.3, 41.2, 42.6, 55.2, 55.3, 58.0, 79.4, 99.7, 105.2, 108.3, 140.9, 160.4, 180.7. Brown solid product, yield 55%. MS-ESI+: $[M + H]^+$ calcd 490.2, found 490.1.

Synthesis of Boc-2,2-di(3,5-di-*tert*-butyl-benzyl) β-Amino Acid (3n). The synthesis was conducted in accordance to GP3. ^{1}H NMR ($CDCl_3$): δ 1.30−1.40 (45 H, m), 3.12 (2H, d, J = 13.5 Hz), 3.38 (2H, d, J = 13.5 Hz), 3.31 (2H, br d), 7.03−7.41 (6H, m).

^{13}C NMR ($CDCl_3$): δ 28.4, 31.5, 34.7, 53.8, 58.1, 79.0, 120.5, 122.0, 124.6, 136.9, 150.5, 156.0, 182.6. Yellow solid product, yield 23%. MS-ESI+: $[M + H]^+$ calcd 594.5, found 594.3.

Synthesis of 2,2-Dipropyl β-Ala-Arg-NH_2 (4a). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 0.93 (6H, dt, J = 2.83 Hz, J = 7.21), 1.22−1.33 (4H, m), 1.60−1.71 (6H, m), 1.77−1.81 (1H, m), 1.87−1.90 (1H, m), 3.10 (2H, s), 3.22 (2H, dt, J=2.3 Hz, J=7.1 Hz), 4.40 (1H, dd, J= 5.4 Hz, J=9.2 Hz). ^{13}C NMR (CD_3OD): δ 14.7, 18.0, 18.2, 26.7, 29.9, 36.8, 37.0, 41.9, 43.8, 54.2, 158.7, 176.6, 177.3. HRMS-ESI+: $[M + H]^+$ calcd 329.2665, found 329.2658.

Synthesis of 2,2-Di(2-cyclohexyl-eth-1-yl) β-Ala-Arg-NH_2 (4b). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 0.98−1.14 (4H, m), 1.16−1.20 (2H, m), 1.23−1.35 (6H, m), 1.57−1.78 (18H, m), 1.85−1.89 (1H, m), 3.17 (2H, s), 3.21 (2H, dt, J=2.3 Hz, J=7.1 Hz), 4.39 (1H, dd, J= 5.9 Hz, J=8.3 Hz). ^{13}C NMR (CD_3OD): δ 26.7, 27.1, 27.4, 30.1, 34.9, 35.7, 36.0, 36.3, 41.9, 42.2, 42.8, 44.7, 48.4, 54.2, 158.6, 176.3, 177.7. HRMS-ESI+: $[M + H]^+$ calcd 437.3604, found 437.3608.

Synthesis of 2,2-Di(2-cyclohexyl-eth-1-yl) β-Ala-Arg-NH_2 (4c). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 0.92−1.00 (4H, m), 1.12−1.32 (12H, m), 1.64−1.84 (17H, m), 1.89−1.94 (1H, m), 3.12 (2H, s), 3.25 (2H, dt, J=3.1 Hz, J=7.1 Hz), 4.44 (1H, dd, J=5.3 Hz, J=9.2 Hz). ^{13}C NMR (CD_3OD): δ 26.8, 27.4, 27.7, 29.9, 31.8, 31.8, 32.1, 32.3, 34.3, 34.4, 34.6, 39.4, 39.5, 41.9, 44.0, 54.2. 158.6, 176.6, 177.3. HRMS-ESI+: $[M + H]^+$ calcd 465.3604, found 465.3608.

Synthesis of 2,2-Dibenzyl β-Ala-Arg-NH_2 (4d). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.56−1.72 (2H, m), 1.76−1.84 (1H, m), 1.86−1.94 (1H, m), 2.94−3.03 (4H, m), 3.20−3.25 (4H, m), 4.41 (1H, t, J=7.0 Hz), 7.22−7.35 (10H, m). ^{13}C NMR (CD_3OD): δ 26.7, 30.1, 41.0, 41.9, 43.8, 50.4, 54.4, 54.6, 128.4, 129.6, 131.5, 136.5, 158.7, 175.7, 176.3. HRMS-ESI+: $[M + H]^+$ calcd 425.2665, found 425.2652.

Synthesis of 2,2-Di(2-phenyl-eth-1-yl) β-Ala-Arg-NH_2 (4e). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.68−1.86 (3H, m), 1.91−1.97 (1H, m), 2.07−2.16 (4H, m), 2.60−2.76 (4H, m), 3.25 (2H, t, J=7.1 Hz), 4.50 (1H, q, J = 5.6 Hz, J = 9.0 Hz), 7.21−7.33 (10H, m). ^{13}C NMR (CD_3OD): δ 26.9, 29.8, 36.5, 36.9, 41.9, 43.8, 54.4, 127.3, 129.5, 129.6, 142.5, 176.6. HRMS-ESI+: $[M + H]^+$ calcd 453.2978, found 453.2986.

Synthesis of 2,2-Di(3-phenyl-prop-1-yl) β-Ala-Arg-NH_2 (4f). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.46−1.78 (11H, m), 1.84−1.89 (1H, m), 2.57−2.65 (4H, m), 3.09 (2H, s), 3.20 (2H, t, J = 7.0 Hz), 4.38 (1H, m), 7.14−7.18(6H, m), 7.24−7.28 (4H, m). ^{13}C NMR (CD_3OD): δ 26.8, 29.9, 33.8, 36.9, 42.0, 44.1, 46.3, 54.3, 127.1, 129.3, 129.5, 143.0, 158.7, 176.6, 177.0. HRMS-ESI+: $[M + H]^+$ calcd 481.3291, found 481.3297.

Synthesis of 2,2-Di(2-methylene-naphthalene) β-Ala-Arg-NH_2 (4g). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.53−1.64 (2H, m), 1.75−1.82 (1H, m), 1.86−1.94 (1H, m), 3.06−3.15 (4H, m), 3.22−3.29 (2H, m), 3.45−3.51 (2H, m), 4.45 (1H, dd, J = 6.3 Hz, J = 8.3 Hz), 7.36−7.40 (2H, m), 7.45−7.51 (4H, m), 7.75 (1H, s), 7.78 (1H, s), 7.82−7.87 (6H, m). ^{13}C NMR (CD_3OD): δ 26.6, 30.2, 41.1, 41.2, 42.0, 44.1, 50.9, 54.6, 127.1, 127.2, 127.4, 127.5, 128.6, 128.7, 128.9, 129.3, 129.4, 130.3, 130.4, 134.0, 134.1, 134.8, 134.9, 158.6, 175.8, 176.3. HRMS-ESI+: $[M + H]^+$ calcd 525.2978, found 525.3002.

Synthesis of 2,2-Di(4-methyl-benzyl) β-Ala-Arg-NH_2 (4h). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.60−1.74 (2H, m), 1.78−1.86 (1H, m), 1.86−1.94 (1H, m), 2.35 (6H, d, J=0.3 Hz), 2.94−3.01 (4H, m), 3.20 (2H, d, J = 14.4 Hz), 3.25 (2H, t, J = 7.1 Hz), 4.43 (1H, t, J = 7.2 Hz), 7.12−7.18 (8H, m). ^{13}C NMR (CD_3OD): δ 21.1, 26.6, 30.1, 40.5, 42.0, 43.8, 50.4, 54.4, 54.5, 130.3, 131.3, 133.4, 138.3, 158.7, 175.9, 176.2. HRMS-ESI+: $[M + H]^+$ calcd 453.2978, found 453.2984.

Synthesis of 2,2-Di(4-trifluoromethyl-benzyl) β-Ala-Arg-NH_2 (4i). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.65−1.76 (2H, m), 1.80−1.84 (1H, m), 1.91−1.98 (1H, m), 2.96−3.03 (2H, m), 3.11−3.27 (2H, m), 3.26 (2H, t, J=7.2 Hz), 4.43 (1H, t, J=7.3 Hz), 7.46−7.51 (4H, m), 7.66−7.70 (4H, m). ^{13}C NMR (CD_3OD): δ 26.7, 30.0, 40.4, 42.0, 43.6, 50.3, 54.7, 108.8, 126.6, 132.2, 158.7, 174.9, 176.2. HRMS-ESI+: $[M + H]^+$ calcd 561.2413, found 561.2420.

Synthesis of 2,2-Di(4-isopropyl-benzyl) β-Ala-Arg-NH_2 (4j). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.23 (12H, dd, J = 1.5 Hz, J = 6.9 Hz), 1.63−1.74 (2H, m), 1.79−1.84 (1H, m), 1.89−1.94 (1H, m), 2.87−3.00 (6H, m), 3.18−3.25 (4H, m), 4.41 (1H, dd, J = 6.4 Hz, J = 8.1 Hz), 7.13−7.21 (8H, m). ^{13}C NMR (CD_3OD): δ 24.4, 26.6, 30.2, 35.0, 40.6, 42.0, 43.8, 50.5, 54.5, 127.7, 131.5, 133.7, 133.9, 149.3, 158.7, 176.0, 176.2. HRMS-ESI+: $[M + H]^+$ calcd 509.3604, found 509.3610.

Synthesis of 2,2-Di(4-*tert*-butyl-benzyl) β-Ala-Arg-NH_2 (4k). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.31 (18H, s), 1.62−1.77 (2H, m), 1.77−1.86 (1H, m), 1.88−1.95 (1H, m), 2.94−3.00 (4H, m), 3.18−3.32 (4H, m), 4.42 (1H, t, J=7.2 Hz), 7.16 (4H, dd, J=8.2 Hz, J=17.3 Hz) 7.37 (4H, dd J=3.4 Hz, J=8.2 Hz). ^{13}C NMR (CD_3OD): δ 26.7, 30.2, 31.7, 35.3, 42.1, 43.8, 50.5, 54.5, 54.6, 126.6, 131.2, 133.5, 151.5, 158.7, 176.0, 176.3. HRMS-ESI+: $[M + H]^+$ calcd 537.3917, found 539.3907.

Synthesis of 2,2-Di(3,5-dimethyl-benzyl) β-Ala-Arg-NH_2 (4l). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.58−1.73 (2H, m), 1.75−1.82 (1H, m), 1.86−1.95 (1H, m), 2.28 (12H, d, J=6.6 Hz), 2.9−3.00 (4H, m), 3.13 (2H, dd J=3.6 Hz, J=14.3 Hz), 3.22 (2H, dt, J=2.7 Hz, J=7.0 Hz), 4.42 (1H, t J=7.2 Hz), 6.94 (4H, s), 6.92 (1H, s), 6.92 (1H, s). ^{13}C NMR (CD_3OD): δ 21.3, 26.7, 30.4, 40.1, 42.1, 44.1, 50.2, 54.4, 129.1, 129.9, 136.4, 139.4, 158.7, 175.9, 176.1. HRMS-ESI+: $[M + H]^+$ calcd 481.3291, found 481.3302.

Synthesis of 2,2-Di(3,5-dimethoxy-benzyl) β-Ala-Arg-NH_2 (4m). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.60−1.72 (2H, m), 1.77−1.83 (1H, m), 1.87−1.94 (1H, m), 2.94−3.07 (4H, m), 3.14−3.23 (4H, m), 3.75, 3.76 (12H, 2s), 4.44 (1H, dd, J = 5.7 Hz, J = 8.5 Hz), 6.37−6.43 (6H, m). ^{13}C NMR (CD_3OD): δ 26.6, 30.4, 41.1, 41.3, 42.0, 44.3, 50.1, 54.5, 55.8, 99.8, 100.2, 109.4, 109.7, 138.7, 138.8, 158.7, 162.5, 175.9, 176.4. HRMS-ESI+: $[M + H]^+$ calcd 545.3088, found 545.3101.

Synthesis of 2,2-Di(3,5-di-*tert*-butyl-benzyl) β-Ala-Arg-NH_2 (4n). The synthesis was conducted in accordance to GP4. ^{1}H NMR (CD_3OD): δ 1.31 (36H, m), 1.64−1.72 (2H, m), 1.79−1.86 (1H, m), 1.92−1.96 (1H, m), 2.88−3.04 (3H, m), 3.15−3.24 (4H, m), 4.44 (1H, dd, J=5.8 Hz, J=8.4 Hz), 7.05 (2H, br d, J=1.6 Hz), 7.09 (2H, br d, J=1.6 Hz), 7.37 (2H, br d, J=1.9 Hz). ^{13}C NMR (CD_3OD): δ 26.8, 30.4, 31.9, 35.7, 40.4, 40.6, 42.0, 44.4, 49.7, 54.8, 122.2, 122.3, 125.5, 125.7, 135.8, 136.0, 152.3, 153.4, 158.7, 176.5, 176.6. HRMS-ESI+: $[M + H]^+$ calcd 649.5169, found 649.5161.

Antimicrobial Activity. The antimicrobial testing was conducted by TosLab A/S (Tromsø, Norway). Each compound was diluted to 1 mg/mL in water and tested in duplicates at 200, 100, 50, 35, 15, 10, 5, 2.5, 1, and 0.5 μg/mL, except for compound **4n**, which was tested at 50, 35, 15,10, 5, 2.5, 1, and 0.5 μg/mL due to limited water solubility. All tested β-peptidomimetics were di-TFA salts. All compounds displaying MIC values below 50 μg/mL were retested using the earlier stated procedure.

Hemolytic Activity. For the RBC toxicity tests, 8 mL of blood was collected from healthy adult male donors. The blood was divided equally and distributed into a commercial available EDTA containing test tube (BD vacutainer, 7.2 mg K2 EDTA) and into a 10 mL reaction vial containing 40 μL of a heparin

solution (1000 U/mL in 0.9% sodium chloride). After 30 min, the hematocrit of the EDTA treated blood was determined. The heparinized blood was centrifuged for 10 min at 1500 rpm and the supernatant removed. Subsequently, the RBCs were washed with prewarmed PBS three times and diluted to 10% hematocrit. The β-peptidomimetics were dissolved in PBS (concentration 1 μg/mL to 1000 μg/mL) and incubated together with the RBCs under agitation at 37 °C for 1 hour. A positive control with an end concentration of 0.1% Triton X-100 and a negative control containing pure PBS buffer were included. The samples were centrifuged (4000 rpm) for 5 min, and the absorption of the supernatant was measured at 405 nm. The percentage of hemolysis was calculated as the ratio of the peptide treated sample and the Triton X-100 treated sample. Each compound was tested from a concentration of 1000 μg/mL and lower, except for compound **4k**, which was only tested up to 500 μg/mL, and compound **4n**, which was only tested up to 71.5 μg/mL due to limited solubility in the PBS buffer. The values given in Table 1 correspond to 20% hemolysis (EC_{20}).

Stability. The stability testing against α-chymotrypsin was conducted by dissolving the β-peptidomimetics (1 mg/mL) in water. α-Chymotrypsin was dissolved (0.1 mg/mL) in 1 mM HCl containing 2 mM $CaCl_2$. The enzymatic digestion was performed in 100 mM TRIS HCl buffer containing 10 mM $CaCl_2$. Final enzyme concentration was 2 μg/mL, and final β-peptidomimetic concentration was 100 μg/mL in a total volume of 0.5 mL. Then 15 μL samples were collected at 0, 15, 30, 60, 120, and 240 min, in addition to samples collected at 24 and 48 h. An external standard (atenolol hydrochloride) was applied to the sample together with 100 μL 10% acetic acid to terminate enzymatic digestion before the sample was diluted to 1 mL with water. For every test, a negative control without enzyme was incubated to ensure that degradation was due to enzyme activity and not to other factors. Succinyl-Ala-Ala-Pro-Phe-*para*-nitroanhiline was used as positive control. All tests were run in triplicates. Quantitative analyses of remaining β-peptidomimetics were done by HPLC-MS. HPLC separation was done with a Waters 2695 separation module with a Sunfire C_{18} 2.1 mm × 150 mm, 3.5 μm column (100 Å pore diameter, 16% carbon load, 340 m^2/g surface area). The column heater was set to 35 °C and the sample temperature to 8 °C. Solvents used in this system were: solvent A, purified water with 0.1% formic acid, and solvent B, 90% acetonitrile (Merck, HPLC grade) and 10% purified water and 0.1% formic acid. The gradient chosen for separation started with an isocratic elution with 95% A and 5% B for 2 min, then a linear gradient to 40% A and 60% B after 3 min. The gradient was increased linearly to 10% A and 90% B after 10 min and was kept isocratic for 2 min. Flow speed was 0.2 mL/min at all times and needle depth was set to 0.00 mm and draw speed to normal. Quantification was performed on a Micromass quattro LC mass spectrometer operated in positive MRM mode. The mass spectrometer was operated with the following settings: capillary voltage 3.3 kV, extractor voltage 3 V, Rf lens 0.4, source temperature 100 °C, desolvation temperature 250 °C, LM1 and HM1 resolution were 15, LM2 and HM2 resolution were 14, and ion energies 1 and 2 was 1.0. Nebulizer gas was set to 25 L/h, and desolvation gas was set to 1000 L/h (both nitrogen 4.0). Collision gas was argon 4.0, and the pressure of the collision cell was kept at 1.5×10^{-3} Pa. The mother ion for every compound was the doubly charged species $[M + 2H]^{2+}$. Optimized conditions, ion transitions, and retention window are described in Table 2. All samples were run in duplicates. Analysis of positive control (Succinyl-Ala-Ala-Pro-Phe-*para*-nitroanhiline) was done by RP-HPLC PDA due to problems with creating a robust method for HPLC-MS analysis. Separation was done by a Waters 2695 separation module with a Sunfire C_{18} 4.6 mm × 250 mm, 5 μm column (100 Å pore diameter, 16% carbon load, 340 m^2/g surface area). The column heater was set to 25 °C, and the sample temperature was set to 15 °C. Solvents used in this system was solvent A, 95% purified water with 5% acetonitrile and 0.1% trifluoro acetic acid, and solvent B, 95% acetonitrile, 5% purified water, and 0.1% trifluoro acetic acid. The gradient used on this system started with an isocratic elution of 100% A for 3 min and was then increased linearly to 95% B after 33 min. Detection was performed with a Waters 996 PDA detector at 350 nm, which was appropriate for detection of the *para*-nitroaniline moiety. No internal standard was used; the degree of hydrolysation was determined based on relative ratios of the two peaks corresponding to peptide and the *para*-nitroaniline group. The flow rate was 1 mL/min at all times.

Table 2. Optimized MS Conditions for External Standard and the Different β-Peptidomimetics Undergoing Enzymatic Degradation

compd	ion transition	collision energy (eV)	cone voltage (V)	retention window (min)
4c	233.2→174.1	13.00	24.00	4.0−10.0
4d	213.1→174.1	18.00	18.00	5.0−10.0
4g	263.2→141.3	23.00	26.00	5.0−10.0
4i	281.1→174.3	14.00	22.00	5.0−10.0
4k	269.2→174.2	15.00	29.00	5.0−10.0
4m	327.1→174.1	18.00	21.00	5.0−10.0
atenolol (ES)	267.1→145.0	26.00	36.00	2.0−5.0

Acknowledgment. We thank Espen Hansen (MarBio, Tromsø) for invaluable help with HRMS and Lytix Biopharma AS for financial support.

Supporting Information Available: MBC values and purity data for compounds undergoing biological evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rice, L. B. Antimicrobial resistance in Gram-positive bacteria. *Am. J. Med.* **2006**, *119*, S11–S19.
(2) Todd, B. Beyond MRSA: VISA and VRSA: what will ward off these pathogens in health care facilities? *Am. J. Nurs.* **2006**, *106*, 28–30.
(3) Gang, R. K.; Sanyal, S. C.; Bang, R. L.; Mokaddas, E.; Lari, A. R. Staphylococcal septicaemia in burns. *Burns* **2000**, *26*, 359–366.
(4) Klevens, M. R.; Morrison, M. A.; Nadle, J.; Petit, S.; Gersman, K.; Ray, S.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Craig, A. S.; Zell, E. R.; Fosheim, G. F.; McDouglas, L. K.; Carey, R. B.; Fridkin, S. K. Invasive methicillin-resistant *Staphylococcus aureus* infections in the united states. *J. Am. Med. Assoc.* **2007**, *298*, 1763–1771.
(5) Severin, A.; Tabei, K.; Tenover, F.; Chung, M.; Clarke, N.; Tomasz, A. High level oxacillin and vancomycin resistance and altered cell wall composition in *Staphylococcus aureus* carrying the staphylococcal *mecA* and the enterococcal *vanA* gene complex. *J. Biol. Chem.* **2004**, *279*, 3398–3407.
(6) National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1990−May 1999, Issued June 1999. *Am. J. Infect. Control* **1999**, *27*, 520−532.
(7) Projan, S. J.; Bradford, P. A. Late stage antibacterial drugs in the clinical pipeline. *Curr. Opin. Microbiol.* **2007**, *10*, 441–446.
(8) Projan, S. J. Why is big Pharma getting out of antibacterial drug discovery? *Curr. Opin. Microbiol.* **2003**, *6*, 427–430.
(9) Poupard, J. A. Is the pharmaceutical industry responding to the challenge of increasing bacterial resistance? *Clin. Microbiol. Newslett.* **2006**, *28*, 13–15.
(10) Projan, S. J. Whither antibacterial drug discovery? *Drug Discovery Today* **2008**, *13*, 279–280.
(11) Boman, H. G.; Nilsson, I.; Rasmuson, B. Inducible antibacterial defence system in *Drosophila*. *Nature* **1972**, *237*, 232–235.
(12) Zasloff, M. Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5449–5453.
(13) Zasloff, M.; Martin, B.; Chen, H.-C. Antimicrobial activity of synthetic magainin peptides and several analogues. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 910–913.

(14) Giuliani, A.; Pirri, G.; Nicoletto, S. F. Antimicrobial peptides: an overview of a promising class of therapeutics. *Cent. Eur. J. Biol.* **2007**, *2*, 1–33.
(15) Boman, H. G. Antibacterial peptides: basic facts and emerging concepts. *J. Intern. Med.* **2003**, *254*, 197–215.
(16) Latham, P. W. Therapeutic peptides revisited. *Nat. Biotechnol.* **1999**, *17*, 755–757.
(17) Strøm, M. B.; Haug, B. E.; Skar, M. L.; Stensen, W.; Stiberg, T.; Svendsen, J. S. The pharmacophore of short cationic antibacterial peptides. *J. Med. Chem.* **2003**, *46*, 1567–1570.
(18) Haug, B. E.; Stensen, W.; Stiberg, T.; Svendsen, J. S. Bulky nonproteinogenic amino acids permit the design of very small and effective cationic antibacterial peptides. *J. Med. Chem.* **2004**, *47*, 4159–4162.
(19) Cronin, J. R.; Yuen, G. U.; Pizzarello, S. Gas chromatographic−mass spectral analysis of the five-carbon β-, γ-, and δ-amino alkanoic acids. *Anal. Biochem.* **1982**, *124*, 139–149.
(20) Masond, M. S.; Abd El-Hamid, O. H. Structural chemistry of amino acid complexes. *Transition Met. Chem.* **1989**, *14*, 233–234.
(21) Gupta, A.; Loew, G. H.; Lawless, J. Interaction of metal ions and amino acids: possible mechanisms for the adsorption of amino acids on homoionic smectite clays. *Inorg. Chem.* **2002**, *22*, 111–120.
(22) Carpino, L. A.; El-Faham, A. Tetramethylfluoroformamidinium hexafluorophosphate: a rapid-acting peptide coupling reagent for solution and solid phase peptide synthesis. *J. Am. Chem. Soc.* **1995**, *117*, 5401–5402.
(23) Porter, E. A.; Weisblum, B.; Gellman, S. H. Mimicry of host-defense peptides by unnatural oligomers: antimicrobial β-peptides. *J. Am. Chem. Soc.* **2002**, *124*, 7324–7330.
(24) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. De novo design of antibacterial β-peptides. *J. Am. Chem. Soc.* **1999**, *121*, 12200–12201.
(25) Arvidsson, P. I.; Ryder, N. S.; Weiss, H. M.; Gross, G.; Kretz, O.; Woessner, R.; Seebach, D. Antibiotic and hemolytic activity of a beta2/beta3 peptide capable of folding into a 12/10-helical secondary structure. *ChemBioChem* **2003**, *4*, 1345–1347.
(26) Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. Structure−activity studies of 14-helical antimicrobial β-peptides: probing the relationship between conformational stability and antimicrobial potency. *J. Am. Chem. Soc.* **2002**, *124*, 12774–12785.
(27) Arvidsson, P. I.; Frackenpohl, J.; Ryder, N. S.; Liechty, B.; Petersen, F.; Zimmermann, H.; Camenisch, G. P.; Woessner, R.; Seebach, D. On the antimicrobial and hemolytic activities of amphiphilic β-peptides. *ChemBioChem* **2001**, *2*, 771–773.
(28) Seebach, D. D. Biological and pharmacokinetic studies with β-peptides. *Chimia* **1998**, *52*, 734.
(29) Hintermann, T.; Seebach, D. The biological stability of β-peptides. No interactions between α-and β-peptidic structures? *Chimia* **1997**, *51*, 244–247.
(30) Frackenpohl, J.; Arvidsson, P. I.; Schreiber, J. V.; Seebach, D. D. The outstanding biological stability of β- and γ-peptides toward proteolytic enzymes: an in vitro investigation with fifteen peptidases. *ChemBioChem* **2001**, *2*, 445–455.
(31) Kang, J. H.; Lee, M. K.; Kim, K. L.; Hahm, K.-S. Structure−biological activity relationships of 11-residue highly basic peptide segment of bovine lactoferrin. *Int. J. Pept. Protein Res.* **1996**, *48*, 357–363.
(32) Collins, J. M.; Leadbeater, N. E. Microwave energy: a versatile tool for the biosciences. *Org. Biomol. Chem.* **2007**, *5*, 1141–1150.
(33) Burrell, M. M. *Enzymes of Molecular Biology*; Humana Press: Totowa, NJ, 1993; p 370.
(34) Wessolowski, A.; Bienert, M.; Dathe, M. Antimicrobial activity of arginine- and tryptophan-rich hexapeptides: the effects of aromatic clusters, D-amino acid substitution and cyclization. *J. Pept. Res.* **2004**, *64*, 159–169.
(35) Shai, Y. Mode of action of membrane active antimicrobial peptides. *Biopolymers* (*Pept. Sci.*) **2002**, *66*, 236–248.
(36) Hicks, R. P.; Bhonsle, J. B.; Venugopal, D.; Koser, B. W.; Magill, A. J. De novo design of selective antibiotic peptides by incorporation of unnatural amino acids. *J. Med. Chem.* **2007**, *50*, 3026–3036.
(37) Klüver, E.; Adermann, K.; Schulz, A. Synthesis and structure−activity relationship of β-defensins, multifunctional peptides of the immune system. *J. Pept. Sci.* **2006**, *12*, 243–257.
(38) Hagmann, W. K. The many roles for fluorine in medicinal chemistry. *J. Med. Chem.* **2008**, *51*, 4359–4369.
(39) Bohm, H. J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Muller, K.; Obst-Sander, U.; Stahl, M. Fluorine in medicinal chemistry. *ChemBioChem* **2004**, *5*, 637–643.
(40) Gimenez, D.; Andreu, C.; del Olmo, M.-l.; Varea, T.; Diaz, D.; Asensio, G. The introduction of fluorine atoms or trifluoromethyl groups in short cationic peptides enhances their antimicrobial activity. *Bioorg. Med. Chem.* **2006**, *14*, 6971–6978.
(41) Zhao, Z.; Koeplinger, K. A.; Padbury, G. E.; Hauer, M. J.; Bundy, G. L.; Banitt, L. S.; Schwartz, T. M.; Zimmermann, D. C.; Harbach, P. R.; Mayo, J. K.; Aaron, C. S. Bioactivation of 6,7-Dimethyl-2,4-di-1-pyrrolidinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (U-89843) to Reactive Intermediates That Bind Covalently to Macromolecules and Produce Genotoxicity1. *Chem. Res. Toxicol.* **1996**, *9*, 1230–1239.
(42) Diana, G. D.; Rudewicz, P.; Pevear, D. C.; Nitz, T. J.; Aldous, S. C.; Aldous, D. J.; Robinson, D. T.; Draper, T.; Dutko, F. J. Picornavirus Inhibitors: Trifluoromethyl Substitution Provides a Global Protective Effect against Hepatic Metabolism. *J. Med. Chem.* **2002**, *38*, 1355–1371.
(43) Park, K.; Kitteringham, N. R.; O'Neill, P. M. Metabolism of fluorine-containing drugs. *Annu. Rev. Pharmacol.* **2001**, *41*, 443–470.
(44) Hester, J. B.; Gibson, J. K.; Buchanan, L. V.; Cimini, M. G.; Clark, M. A.; Emmert, D. E.; Glavanovich, M. A.; Imbordino, R. J.; LeMay, R. J.; McMillan, M. W.; Perricone, S. C.; Squires, D. M.; Walters, R. R. Progress toward the Development of a Safe and Effective Agent for Treating Reentrant Cardiac Arrhythmias: Synthesis and Evaluation of Ibutilide Analogues with Enhanced Metabolic Stability and Diminished Proarrhythmic Potential. *J. Med. Chem.* **2001**, *44*, 1099–1115.
(45) Chan, D. I. D. Tryptophan- and arginine-rich antimicrobial peptides: structures and mechanisms of action. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758*, 1184–1202.
(46) Haug, B. E.; Stensen, W.; Kalaaji, M.; Rekdal, O.; Svendsen, J. S. Synthetic antimicrobial peptidomimetics with therapeutic potential. *J. Med. Chem.* **2008**, *51*, 4306–4314.
(47) Mookherjee, N.; Hancock, R. E. W. Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. *Cell. Mol. Life Sci.* **2007**, *64*, 922–933.
(48) Haug, B. E.; Stensen, W.; Svendsen, J. S. Application of the Suzuki−Miyaura cross-coupling to increase antimicrobial potency generates promising novel antibacterials. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2361–2364.
(49) Tew, G. N.; Clements, D.; Tang, H.; Arnt, L.; Scott, R. W. Antimicrobial activity of an abiotic host defense peptide mimic. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758*, 1387–1392.
(50) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis.* Springer-Verlag: New York, 1994.
(51) Seebach, D.; Abele, S.; Sifferlen, T.; Hänggi, M.; Gruner, S.; Seiler, P. Preparation and structure of β-peptides consisting of geminally disubstituted $\beta^{2,2}$- and $\beta^{3,3}$-amino acids: a turn motif for β-peptides. *Helv. Chim. Acta* **1998**, *81*, 2218–2243.
(52) Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: Oxford, 2000; 346 p.