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SYNTHESIS OF AZA-SURFACTIN AND 3-EPI-AZA-SURFACTIN

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Abstract – Two synthetic approaches to aza-surfactins are reported. In the first, our recently developed synthesis of unnatural β^3-amino acids was used to prepare the requisite monomer, which was used in solid phase peptide synthesis, and followed by macrolactamization of a partially protected precursor. In the second, the corresponding β^3-*N*-hydroxyaminoacid was used to prepare a fully unprotected cyclization precursor that was closed to give 3-epi-aza-surfactin by the first example of cyclization by the α-ketoacid–hydroxylamine amide formation.

Dedicated to Professor Albert Padwa on the occasion of his 75th birthday

INTRODUCTION

Surfactin, the principle member of the lipopeptides, is a powerful antibiotic with a unique mechanism.[1] This growing class of natural products is produced by the genus Bacillus and has found application not only as antibiotics but also as antivirals, fibrin clot inhibitors, and other biological applications.[2] Their mode of action is thought to be membrane permeabilization by a detergent-like disruption of the lipid bilayer along with complexation and transport of ions through the membrane. The precise mechanism, however, remains unknown and several competing theories have been proposed.[3]

Several different surfactin structures are known but they all share a common architecture: seven α-amino acids joined as a depsipeptide by macrolactonization with a β-hydroxy fatty acid. Structural studies with NMR techniques reveal a characteristic saddle-horse shape.[4] The long alkyl hydrocarbon chains and their cyclic cores endow these peptides with an amphipathic nature, which allow them to penetrate the membrane and disturb the target cells. Surfactin has attracted considerable attention for their potential medical applications. Improvements to the bioactivity, particularly to reduced toxicity, may be gained by modification of their cyclic peptide core. To date surfactin has been produced almost exclusively by

bacterial fermentation and only few synthetic approaches have appeared.[5,6] Therefore, synthetic analogues have not been thoroughly studied for either their biological activity or as tools for investigating their mechanism of action.

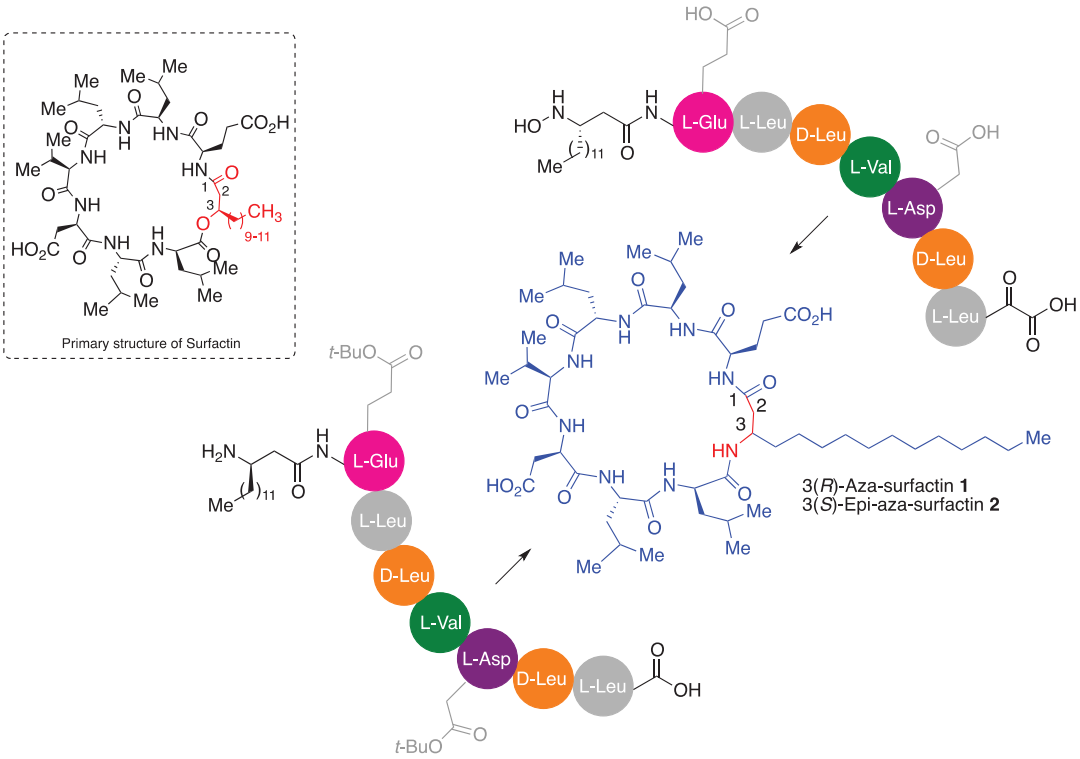
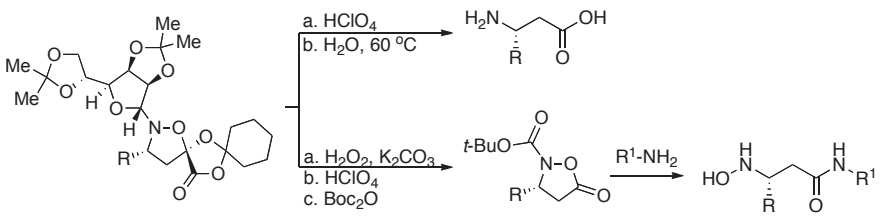


Figure 1. Primary structure of surfactin and the two aza-derivatives prepared in this paper

In our own efforts, we sought to prepare the aza-analogue of surfactin and probe the role of the stereochemistry at the hydrophibic tail (Figure 1). The formation of macrolactams is often easier than the synthesis of macrolactones and numerous research groups have shown that depsi-peptides can often be mutated to their aza-analogues without loss of biological activity.[7] We also believed that these modifications would offer insights into the structure and mechanism of the lipopeptide class of antibiotics and provide a foundation for a more advanced investigation of the structure-activity relationship of these compounds.[8,9] This article documents two synthetic approaches to the requisite β-amino acid and formation of the macrolactam, one in each enantiomeric series.



Scheme 1. Synthesis of β^3-amino acids and β^3-*N*-hydroxyaminoacids from isoxazolidines

We have recently reported synthetic approaches to enantiopure, unnatural β^3-amino acids and β^3-*N*-hydroxyaminoacids suitable for the proposed studies (Scheme 1).[10] First, we have developed a practical and scalable approach to enantiopure β^3-amino acids that is particularly well suited for the synthesis of unnatural derivatives where the corresponding enantiopure α-amino acid is not available. Second, we have prepared β^3-*N*-hydroxyaminoacids from isoxalidinones. The resulting hydroxylamines are substrates for a highly chemoselective amide-formation with an α-ketoacid. This strategy has the advantage that no coupling reagents are needed to introduce the β-amino acid precursor or to effect

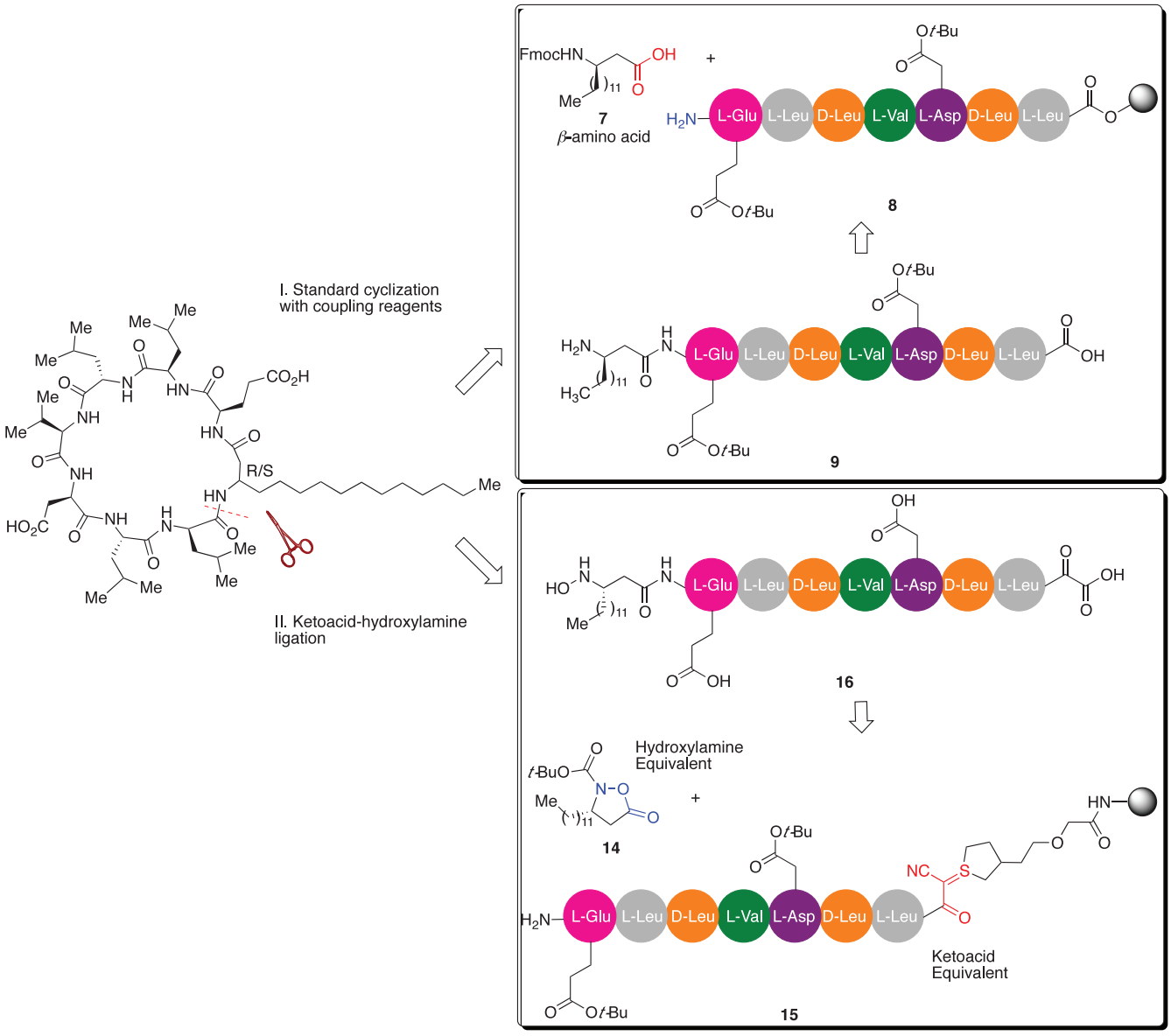
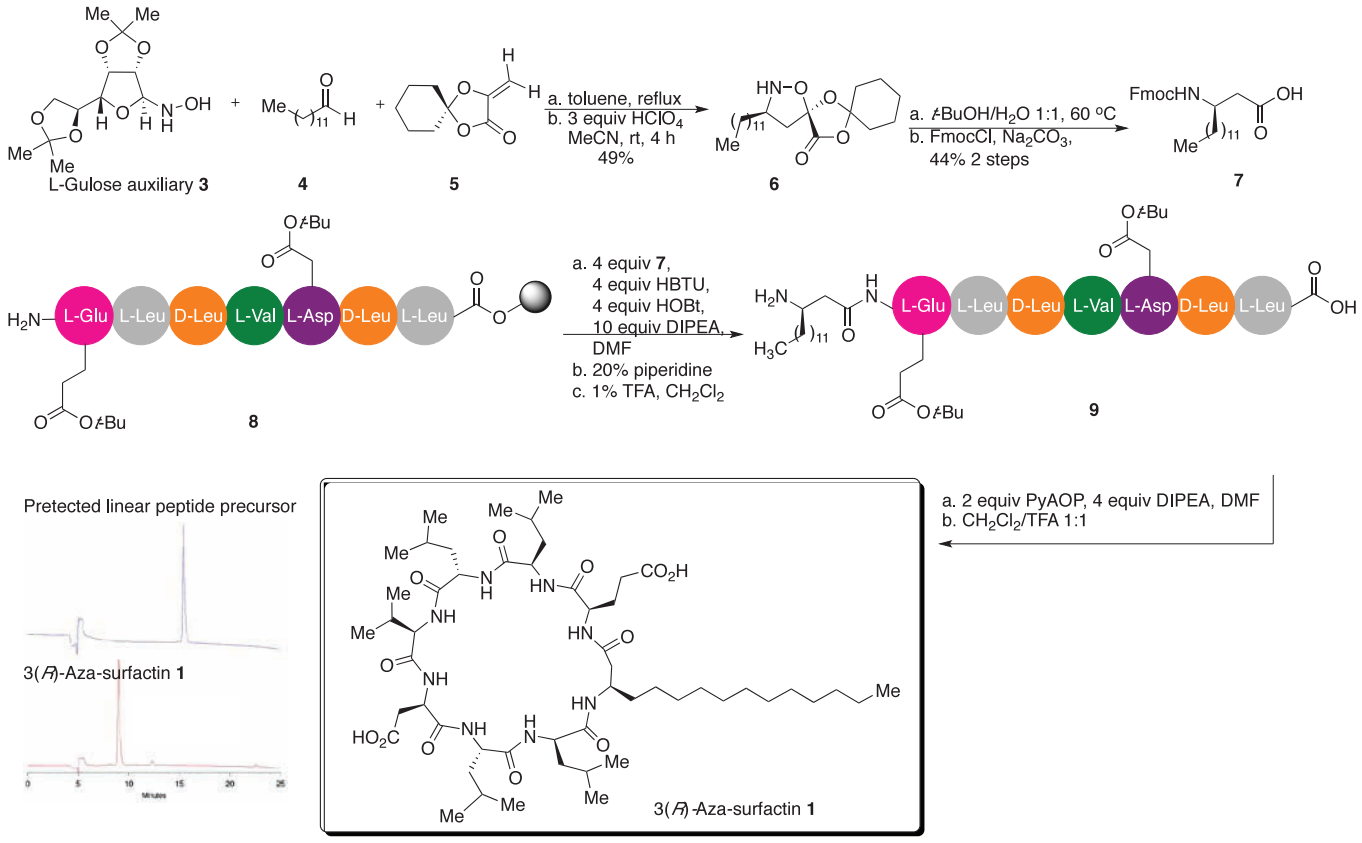


Figure 2. Retrosynthetic analysis

macrocyclization. Prior to these studies, however, the utility of the ketoacid–hydroxylamine ligation (KAHA ligation) for macrolactam formation had not been demonstrated.[11] In this work, we explored both strategies, one as a route to aza-surfactin **1** that uses standard cyclization condition after incorporating the unnatural β^3-amino acid and one to epi-aza-surfactin **2** by KAHA cyclization after the β^3-*N*-hydroxy residue has been introduced into the heptapeptide (Figure 2).

RESULTS AND DISCUSSION

Our studies began with the synthesis of aza-surfactin with the naturally occurring (*R*) configuration at position C-3. The combination of chiral auxiliary **3**, aldehyde **4**, and acrylate **5** in toluene at reflux gave the expected cycloadduct from which the sugar auxiliary was cleaved with perchloric acid to give isoxazolidine **6** in 49% overall yield (two steps) as a single enantiomer and diastereomer after recrystallization. Simply warming this compound in water resulted in concomitant decarboxylation and N–O bond cleavage to give the amino acid, which was protected as its Fmoc-protected amine in moderate yield. The α-heptapeptide **8** was prepared separately without difficultly by Fmoc-solid phase peptide synthesis on 2-chlorotrityl resin (See experimental section for details). Coupling of β-amino acid monomer **7** to the peptide chain procedure proceeded without difficulty using standard HBTU coupling condition.

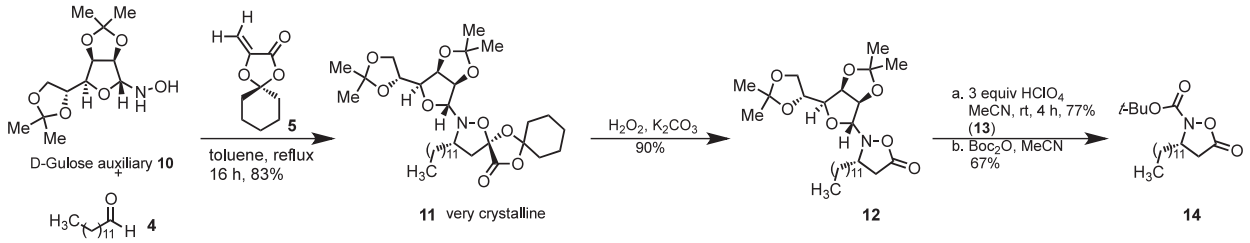


Scheme 2. Synthesis of 3(*R*)-aza-surfactin **1**

Removal of the terminal Fmoc-protecting group and resin cleavage with 1% TFA afforded peptide **9** with the aspartic and glutamic acid side chain *t*-Bu protecting groups intact. Precipitation of this material provided side-chain protected linear peptide that proved to be sufficiently pure for macrolactamization. Treatment of **9** (1 equiv) with PyAOP followed by DIPEA at rt afforded the protected macrolactam in good yield, which was further processed by side chain removal using 50% TFA in CH_2Cl_2. Purification of the final product by reverse phase preparative HPLC afforded aza-surfactin **1**.

For our approach to 3-epi-aza-surfactin **2**, we sought to take advantage of recent developments in our laboratory on amide-forming reactions without coupling reagents. In particular, we reported the highly chemoselective ligation of α-ketoacids and hydroxylamines and introduced reagents for the preparation of peptides with the requisite C-terminal α-ketoacids and N-terminal hydroxylamines.[12] As part of our interest in the synthesis of β^3-oligopeptides, we have shown that isoxazolidinone monomers can be coupled directly to a growing peptide chain. We therefore sought to combine these methods to prepare macrocycles containing β^3-peptide residue such as aza-surfactin.

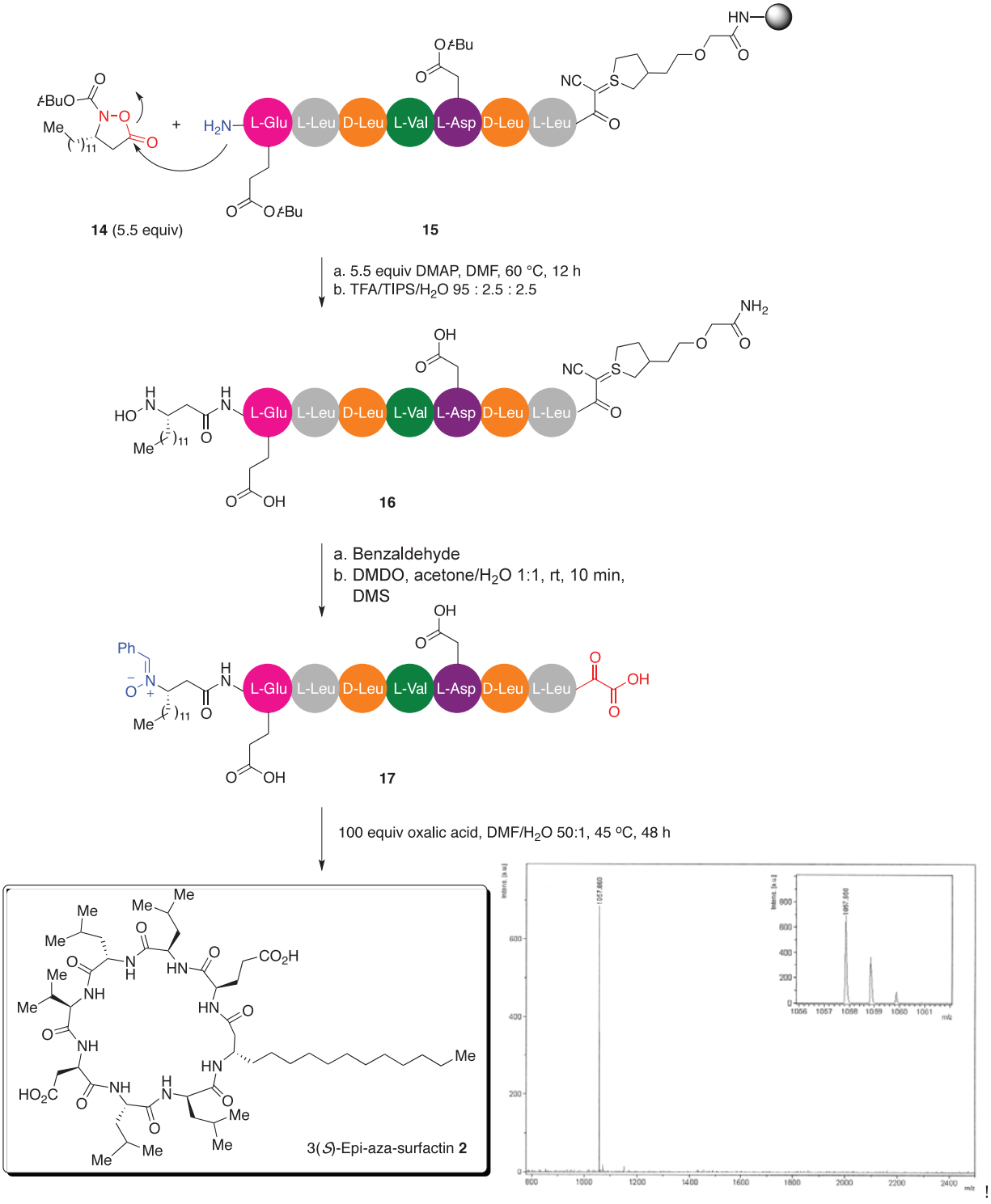
Our efforts began with the preparation of the requisite isoxazolidinone **14**, for which two approaches were considered. In our early work, we employed diastereoselective cyclization of the nitrone formed from tridecanal **4** and D-gulose-derived hydroxylamine **10** with excess vinyl acetate to give isoxazolidine, which was converted to isoxazolidinone **12** by acetate hydrolysis and oxidation. Although effective, we found that isoxazolidine intermediate was reluctant to crystalize, which limited our ability to prepare enantiomerically pure product on scale. As an alterative, we returned to cycloadditions with acrylate **5** to give **11**, which was both highly crystalline and produced with high stereoselectivity. It was easily converted to **12** by oxidative decarboxylation with hydrogen peroxide. Removal of the sugar auxiliary and Boc-protection proceeded smoothly to give **14**.



Scheme 3. Preparation of isoazolidinone **14**

The synthesis of the cyclization precursor began with the preparation of α-heptapeptide **15** using a sulfur ylide linker as a precursor to the C-terminal α-ketoacid.[14] This proceeded uneventfully by Fmoc-based solid-phase synthesis and no complications from the sulfur ylide linker. Although we have previously shown that isoxazolidinone monomers can be coupled to peptides with catalytic DMAP in solution, the

introduction of this highly lipophilic β-peptide precursor onto α-heptapeptide **15** proved problematic. After numerous attempts, we found that 5.5 equiv of **14** and 5.5 equiv of 4-dimethylaminopyridine (DMAP) in DMF at 60 °C for prolonged reaction times were required for effective coupling. Side-chain deprotection and cleavage of the resulting peptide from resin provide terminal peptide hydroxylamine **16**.



Scheme 4. Synthesis of 3(*S*)-epi-aza-surfatin **2**

In order to reveal the α-ketoacid, oxidation of the sulfur ylide was required. Although unprotected amines and acids are readily tolerated by the mild oxidation reagents used, hydroxylamines are especially prone to oxidation and require protection. We therefore first added benzaldehyde to the reaction mixture to form, in situ, the corresponding nitrone that acts as both a protecting group as well as a means of slowly releasing the hydroxylamine during the cyclization. Subsequent treatment of the sulfur ylide peptide with dimethyldioxirane (DMDO) followed by quenching of excess oxidant with dimethylsulfide (DMS) gave the side-chain unprotected cyclization precursor **17**. Warming this peptide in 50:1 DMF/H_2O at 45 °C in the presence of oxalic acid afforded a mixture of products, from which the desired cyclization product could be isolated. Purification by preparative HPLC provided 3(*S*)-epi-aza-surfactin **2**.[13] Epi-aza-surfactin **2** is currently undergoing preliminary biological evaluation to establish the role of the stereochemistry in inducing the antimicrobial properties of surfactin.

In summary, we have employed our recently developed gulose-based chiral auxiliary approach to unnatural β^3-amino acid **7** and β^3-*N*-hydroxyaminoacid **14** to the synthesis of two analogues of surfactin. This work establishes that this metholodogy is an alternative approach to prepare lipophilic β-amino acids and that cyclizations to give aza-lipopeptides are readily achieved. This work is also the first example of the use of the α-ketoacid–hydroxylamine ligation for the synthesis of peptide macrocycles. The unique advantages of this approach, which does not require side chain protecting groups or coupling reagents for the key cyclization step, will be further explored on other natural and unnatural peptide targets.

EXPERIMENTAL

General Methods. All reactions using air- or moisture-sensitive reagents were performed in oven-dried glassware under an atmosphere of dry N_2. CH_2Cl_2 was distilled from CaH_2. THF and Et_2O were distilled from Na/benzophenone. Thin layer chromatography (TLC) was performed on EMD precoated plates (silica gel 60 F_{254}, Art 5715, 0.25 mm) and were visualized by fluorescence quenching under UV light and by staining with phosphomolybdic acid or potassium permanganate. Preparative thin-layer chromatography (PTLC) was performed using plates prepared from silica gel EMD 60 PF_{254} (Art 7749). Column chromatography was performed on EMD Silica Gel 60 (230–400 Mesh) using a forced flow of air at 0.5–1.0 bar. NMR spectrum were measured on Bruker Avance 400 MHz, 101 MHz. Chemical shifts are expressed in parts per million (ppm) and coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded on a JASCO FT/IR-4100 spectrophotometer and are reported as wavenumber (cm^{-1}). Optical rotations were measured in a Jasco P-2000 polarimeter with a 100 mm path length cell operating at the sodium D line and recorded as $[\alpha]_D^T$= (concentration g/100 mL, solvent), T =

temperature (^{o}C). Enantiomeric purity was determined by preparation of both enantiomers of the isoxazolidines followed by analysis on chiral SFC. Melting points were measured on an Electrothermal Mel-Temp melting point apparatus using open glass capillaries and are uncorrected.

SFC (Supercritical Fluid Chromatography) Conditions. Column: Daicel Chiralpak OJ-H (4.6x 250 mm). Eluents: gradient 5%–80% ^{i}PrOH (0.1% TFA v/v) in CO_2, rate 3%/min or 5%/min, flow rate 2.0 ml/min; isocratic ^{i}PrOH (0.1% TFA v/v) in CO_2, Flow rate 2.0 ml/min. Detection: 220 nm.

HPLC (High Performance Liquid Chromatography) Conditions. Column: Shiseido CAPCELL PAK C18 UG120 (S-5 μm, 4.6 mm I.D. × 250 mm); Grace C4 VYDAC (S-5 μm, 4.6 mm I.D. × 250 mm). Eluents: gradient of MeCN (0.1% TFA v/v) and Millipure water (0.1% TFA v/v), flow rate 1.0 ml/min; Detection: 220, 254, 301 nm.

D-Gulose-(*S*)-3-tridecylisoxazolidin-5-carboxycyclohexan-1,1-acetal (**11**) A 0.300 M solution of D-gulose oxime **10** (2.00 g, 7.26 mmol), tridecanal **4** (3.50 mL, 14.5 mmol), and spiroacrylate **5** (1.46 g, 8.70 mmol) in toluene, was heated at reflux overnight in a round-bottomed flask equipped with a Dean-Stark trap fitted with a reflux condenser. The reaction was monitored by TLC for the disappearance of the UV active nitrone spot. After cooling to room temperature the mixture was concentrated under reduced pressure. The crude product was purified by flash chromatography using hexanes/EtOAc (8:1 v/v) as the eluent affording **11** as a diastereomeric mixture. The resulting solid was recrystallized from heptane to give the major diastereomer (3.76 g, 6.02 mmol, 83% yield). mp 67 – 68 °C; $[\alpha]_D^{25}$ (c 0.510, CH_2Cl_2) = –12.1; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.87 (d, *J* = 6.1 Hz, 1H), 4.70 – 4.63 (m, 2H), 4.35 (dt, *J* = 8.5, 6.7 Hz, 1H), 4.20 (dd, *J* = 8.5, 6.7 Hz, 1H), 4.04 (dd, *J* = 8.5, 3.8 Hz, 1H), 3.82 – 3.74 (m, 1H), 3.71 (dd, *J* = 8.5, 6.7 Hz, 1H), 2.91 (dd, *J* = 13.7, 7.7 Hz, 1H), 2.12 (dd, *J* = 13.7, 1.9 Hz, 1H), 1.96 – 1.60 (m, 9H), 1.43 (dd, *J* = 23.2, 11.4 Hz, 13H), 1.35 – 1.15 (m, 22H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.6, 113.0, 111.8, 109.9, 105.8, 96.9, 84.5, 84.3, 80.4, 75.8, 66.2, 61.1, 41.1, 37.7, 36.5, 33.5, 32.1, 29.82, 29.80, 29.75, 29.7, 29.5, 29.5, 27.3, 26.9, 26.2, 25.5, 25.1, 24.4, 23.1, 23.0, 22.8, 14.3; IR (thin film) ν 2987, 2923,2854, 1803, 1454, 1376, 1236, 1085 cm^{-1}; HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{34}H_{57}NNaO_9$ 646.3926, found 646.3930.

D-Gulose-(*S*)-3-tridecylisoxazolidin-5-one (**12**) A solution of D-gulose-isoxazolidine cyclohexanone acetal **11** (970 mg, 1.55 mmol) in THF/H_2O (6:1 v/v, 15.5 mL, 0.100 M) was cooled to 0 °C and K_2CO_3 (1.07 g, 7.75 mmol) and H_2O_2 (30% w/w, 790 μL, 7.75 mmol) were slowly added. After stirring the reaction for one hour at room temperature, another 7.75 mmol of both K_2CO_3 and H_2O_2 were added. The reaction was monitored by TLC, The resulting mixture was quenched by the addition of saturated

$Na_2S_2O_3$ and extracted with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$, saturated NH_4Cl and dried over Na_2SO_4. The solution was filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography using hexanes/EtOAc (6:1 v/v) as the eluent afforded the isoxazolidinone **15** as a clear oil (0.69 g, 1.39 mmol, 90%). $[\alpha]_D^{25}$ (c 0.710, CH_2Cl_2) = +17.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (d, *J* = 6.1 Hz, 1H), 4.72 (m, *J* = 6.2, 3.8 Hz, 2H), 4.35 (dt, *J* = 8.6, 6.7 Hz, 1H), 4.19 (dd, *J* = 8.6, 6.7 Hz, 1H), 4.04 (dd, *J* = 8.4, 4.0 Hz, 1H), 3.86 – 3.76 (m, 1H), 3.73 (dd, *J* = 8.6, 6.7 Hz, 1H), 2.83 (dd, *J* = 17.6, 8.0 Hz, 1H), 2.46 (dd, *J* = 17.6, 6.5 Hz, 1H), 1.84 – 1.66 (m, 1H), 1.59 – 1.12 (m, 33H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.8, 113.2, 110.0, 98.3, 85.4, 84.1, 80.3, 75.9, 66.1, 61.4, 33.9, 33.7, 32.0, 29.78, 29.76, 29.73, 29.68, 29.6, 29.51, 29.47, 26.9, 26.3, 26.1, 25.5, 24.8, 22.8, 14.2; IR (thin film) ν 2986, 2925, 2854, 1795, 1456, 1372, 1210, 1164, 1084 cm^{-1}; HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{27}H_{47}NNaO_7$ 520.3245, found 520.3250.

(*S*)-3-tridecylisoxazolidin-5-one (13) To a 0.1 M solution of the gulose-isoxazolidinone **12** (160 mg, 0.320 mmol) in MeCN was slowly added $HClO_4$ (70% w/w, 60.3 μL, 1.00 mmol), and the solution was stirred at room temperature for 4 h. The resulting mixture was quenched by the slow addition of saturated $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography using hexanes/EtOAc (4:1 v/v) as eluent afforded the isoxazolidinone **13** as a clear oil (63.0 mg, 0.250 mmol, 77%). $[\alpha]_D^{26}$ (c 0.960, CH_2Cl_2) = –4.98; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (br, 1H), 3.79 (m, 1H), 2.76 (dd, *J* = 16.8, 5.9 Hz, 1H), 2.39 (dd, *J* = 16.8, 8.4 Hz, 1H), 1.73 – 1.57 (m, 1H), 1.56 – 1.43 (m, 1H), 1.43 – 1.11 (m, 20H), 0.85 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 59.7, 36.2, 32.6, 32.0, 29.68, 29.66, 29.6, 29.5, 29.43, 29.38, 26.2, 22.7, 14.1; IR (thin film) ν 2920, 2850, 1777, 1468, 1184 cm^{-1}; HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_{30}NO_2$ 256.2271, found 256.2276.

(*S*)-*tert*-Butoxycarbonyl-3-tridecylisoxazolidin-5-one (14) A 0.100 M solution of the free isoxazolidinone **13** (86.7 mg, 0.340 mmol) in dry MeCN was cooled to 0 °C and Boc_2O (110 mg, 0.510 mmol) and DMAP (24.9 mg, 0.204 mmol) were added and the mixture and stirred at room temperature for 1.5 h. After completion as judged by TLC, the mixture was concentrated under reduced pressure and the resulting residue was purified by column chromatography to afford the *N*-Boc protected isoxazolidinone **14**. Purification by column chromatography using hexanes/EtOAc (12:1 v/v) as the eluent afforded the product as a yellow oil (81.0 mg, 0.230 mmol, 67%). $[\alpha]_D^{28}$ (c 0.890, CH_2Cl_2) = +76.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (m, 1H), 2.95 (dd, *J* = 17.7, 8.9 Hz, 1H), 2.44 (dd, *J* = 17.7, 2.7 Hz, 1H), 1.84 – 1.70 (m, 1H), 1.60 – 1.44 (m, 10H), 1.44 – 1.16 (m, 20H), 0.86 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR

(101 MHz, $CDCl_3$) δ 173.4, 156.2, 83.9, 60.3, 34.3, 32.0, 29.8, 29.73, 29.7, 29.6, 29.54, 29.45, 29.2, 28.2, 25.5, 22.8, 14.2; IR (thin film) ν 2925, 2854, 1806, 1747, 1718, 1458, 1336, 1254, 1141 cm^{-1}; HRMS (ESI): *m/z* calcd for $[M+Na]^+ C_{20}H_{37}NNaO_4$ 378.2615, found 378.2621.

(*R*)-3-tridecylisoxazolidin-5-carboxycyclohexan-1,1-acetal (**6**) The requisite isoxazolidine (600 mg, 0.96 mmol), prepared according to the synthesis procedure of **11** but with L-gulose auxiliary instead, was dissolved in MeCN (25.0 mL). $HClO_4$ (250 μL, 2.89 mmol) was added. After 4 h of stirring the solution at room temperature, sat. $NaHCO_3$ (30 mL) was used to quench the reaction. The mixture was extracted with EtOAc, washed with brine and dried over $MgSO_4$. Removing the solvents yielded a yellow viscous oil, which was purified by column chromatography using hexanes/EtOAc (20:1 v/v). Compound **6** (180 mg, 0.472 mmol, 49%) was isolated as a clear oil. $[\alpha]_D^{26}$ (c 1.15, CH_2Cl_2) = –46.5; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.60 (br, 1H), 3.48 (m, 1H), 2.81 (m, 1H), 2.11 – 1.54 (m, 10H), 1.51 – 1.04 (m, 23H), 0.85 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.0, 111.6, 107.2, 61.6, 45.6, 37.2, 36.4, 32.0, 30.8, 29.71, 29.68, 29.59, 29.56, 29.5, 29.4, 27.2, 24.4, 23.0, 22.9, 22.7, 14.2; IR (thin film) ν 2925, 2853, 1803, 1644, 1451, 1266, 1176 cm^{-1}; HRMS (ESI): *m/z* calcd for $[M+H]^+ C_{22}H_{40}NO_4$ 382.2952, found 382.2950.

(*R*)-3-*N*-Fmoc-aminohexadecanoic acid (**7**) Compound **6** (0.12 g, 0.315 mmol) was added to a 1:1 mixture of H_2O/*t*-BuOH (6.00 mL). After stirring at 60 °C overnight, the reaction mixture was cooled down and Na_2CO_3 (67.0 mg, 0.630 mmol) as well as Fmoc-Cl (200 mg, 0.788 mmol) were added. The suspension was stirred at room temperature for another 20 h. 1.00 M HCl was added to adjust the pH of the reaction mixture to < 1. It was then extracted with EtOAc and the combined organic layers were washed with brine and dried over $MgSO_4$. The solvents were removed and the crude was purified by column chromatography using a hexanes/EtOAc (9:1 v/v) mixture to obtain the Fmoc-protected amino acid **7** as a white solid (70.0 mg, 0.150 mmol, 44%). mp 93 – 95 °C; $[\alpha]_D^{27}$ (c 0.600, CH_2Cl_2) = +3.33; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.75 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.30 (td, *J* = 7.5, 1.0 Hz, 2H), 5.18 (d, *J* = 8.3 Hz, 1H), 4.39 (d, *J* = 6.8 Hz, 2H), 4.22 (t, *J* = 6.8 Hz, 1H), 3.97 (m, 1H), 2.59 (m, 2H), 1.55 (s, 1H), 1.49 – 0.98 (m, 21H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 176.8, 156.1, 144.0, 141.5, 127.8, 127.2, 125.2, 120.1, 66.8, 48.2, 47.4, 39.0, 34.5, 32.1, 31.0, 29.81, 29.78, 29.72, 29.67, 29.49, 29.45, 26.3, 22.8, 14.3; IR (thin film) ν 3324, 2920, 2851, 1697, 1539 cm^{-1}; HRMS (ESI): *m/z* calcd for $[M+Na]^+ C_{30}H_{41}NaNO_4$ 502.2928, found 502.3001.

General procedures for the preparation of the linear and cyclic peptide.

I. Standard Cyclization with coupling reagents:

Loading of the resin: 2-chloro-trityl resin (1.00 g, 1.60 mmol/ g) was swelled with CH_2Cl_2 in a plastic

syringe equipped with a filter. A solution of Fmoc-Leu-OH (70.7 mg, 0.200 mmol) and DIPEA (4.00 equiv) in 8.00 mL CH_2Cl_2 was added to the resin and stirred for 1.5 h. Afterwards the resin was capped with CH_2Cl_2/MeOH/DIPEA (17:2:1), and washed with 3x CH_2Cl_2, 3x DMF, 3x CH_2Cl_2.

Coupling of the α-amino acids: The Fmoc groups were deprotected by shaking with a 20% solution of piperidine in DMF for 10 min. Then resin was washed with 5x DMF/CH_2Cl_2/DMF. In a vial, a solution of the Fmoc-protected amino acid (5.00 equiv), HOBt (5.00 equiv), HBTU (5.00 equiv) and DIPEA (10.0 equiv) in a minimum volume of DMF were stirred for 10 min and then added to the resin. The resin was stirred for 1.5–2 h and followed by Kaiser test untill completion of the coupling. After that, the resin was washed with 5x DMF, 5x CH_2Cl_2, 5x DMF.

Coupling of the β-amino acid to the α-heptapeptide: A solution of the β-amino acid **7** (25.0 mg, 52.0 μmol), HOBt (7.00 mg, 52.0 μmol), HBTU (20.0 mg, 52.0 μmol) and DIPEA (22.5 μL, 0.130 mmol) in a minimum volume of DMF was added to the resin bearing the heptapeptide **8** (65.2 mg, loading = 0.200 mmol/g) after 2 h stirring.

Cleavage of the peptide from the resin: A 1% of TFA solution in CH_2Cl_2 was added to the resin and stirred for 1 min. A few drops of DIPEA were added and the solvents were removed under reduced presssure. The crude was washed with Et_2O and dried under vacuum.

Cyclization of the linear peptide: The protected octapeptide **9** (6.20 mg, 5.32 μmol) was dissolved in DMF (5.30 mL, 1.00 mM). PyAOP (5.5 mg, 10.6 μmol) was added and the solution was stirred for 10 min before DIPEA (3.70 μL, 21.2 μmol) was added and the solution was stirred for another 2.5 h, the reaction mixture was analysed by HPLC (Shiseido C18 column 60 to 95% MeCN in 15 min) and MALDI (DHB matrix).

Final global deprotection of the cyclic peptide: A 50% of TFA solution in CH_2Cl_2 was added to the peptide and stirred for 30 min, the solvents removed under reduced pressure, and the crude product purified by HPLC (Shiseido C18 column 60 to 95% MeCN in 15 min) and MALDI (DHB matrix).

II. Ketoacid-Hydroxylamine Cyclization:

Coupling of the α-amino acids: The Rink amide MBHA resin, with the linker and the first amino acid bound (loading = 0.300 mmol/g), was treated with 20% piperidine in DMF.[14] The resin was drained and washed with 3x DMF, 3x CH_2Cl_2, 3x DMF. The subsequent peptide coupling followed the standard

HBTU protocol.

Coupling of the isoxazolidinone to the α-heptapeptide: Isoxazolidinone **14** (35.5 mg, 0.100 mmol) and DMAP (12.2 mg, 0.100 mmol) were added to the resin with the α-heptapeptide **15** (100 mg, loading = 0.180 mmol/g), and the mixture was agitated for 12 h at 60 ºC. The resin was drained and washed with 5x DMF, 5x CH_2Cl_2, 5x DMF.

Cyclization of the linear peptide: The crude peptide was cleaved from the resin by TFA/TIPS/H_2O 95:2.5:2.5 at room temperature for 1 h to afford **16**. The resin was removed by filtration and the clear solution was concentrated to a small volume under reduced pressure. Cold Et_2O was added and the crude peptide was precipitated as a white solid. The crude N-hydroxylamine-sulfur ylide peptide **16** was allowed to react with 1 mL benzaldehyde for 12 h. Cold Et_2O was added to give a white solid of the nitrone-sulfur ylide peptide. To a solution of nitrone-sulfur ylide peptide in acetone/H_2O 1:1 (1.80 mL, 10.0 mM), DMDO (60.0 mM in acetone, 1.20 mL, 72.0 μmol) was added at room temperature and stirred for 10 min. The excess DMDO was quenched by few drops of DMS and the solvent was removed under reduced pressure to give the crude nitrone-ketoacid peptide **17**, which was dissolved in a degassed DMF/H_2O 50:1 solution (18.0 mL, 1.00 mM) with oxalic acid (162 mg, 1.80 mmol) under 45 ºC for 48 h. The solvent was removed under vacuum and surfactin analogue **2** was purified by HPLC (Grace C4 column 10 to 95% MeCN in 40 min). HRMS (ESI): *m/z* calcd for $[M+H]^+ C_{53}H_{95}N_8O_{12}$ 1035.7069, found 1035.7072.

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