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Supporting Information

The Solid Phase Supported Peptide Synthesis of Analogues of the Lantibiotic Lactocin S

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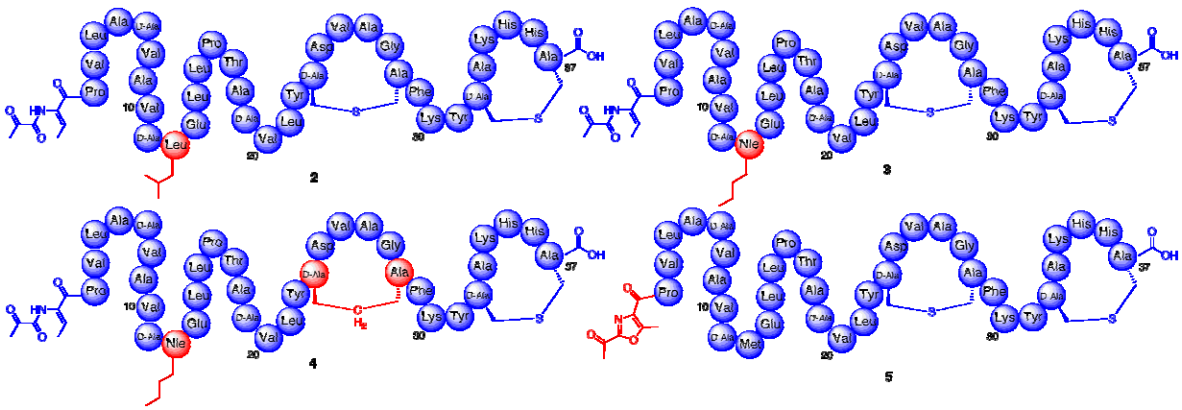

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Supporting Information Contents:

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List of Abbreviations

$[\alpha]_D^{26}$	specific rotation
Ac_2O	acetic anhydride
All	allyl
Aloc	allyloxycarbonyl
Boc	*tert*-butoxycarbonyl
br	broad
^{t}Bu	*tert*-butyl
c	concentration
C-terminal	carboxy terminal
d	doublet
DAP	diaminopimelate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
equiv	equivalents
ES	electrospray
ESI	electrospray ionization
Et_3N	triethylamine
EtOAc	ethyl acetate

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Et_2O	diethyl ether
Fmoc	9*H*-fluorenylmethoxycarbonyl
FTICR	Fourier transform ion cyclotron resonance
HCCA	4-hydroxy-α-cyanocinnamic acid
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IR	infrared
J	coupling constant
m	multiplet
MALDI-TOF	matrix-assisted laser desorption ionization – time of flight
MeOH	methanol
MQ	milli-Q
MRS	de Man, Rogosa and Sharpe
Ms	methanesulfonyl
MS	mass spectrometry
MW	molecular weight
N-terminal	amino terminal
N-terminus	amino terminus
Nle	norleucine
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

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q	quartet
R_f	retention factor
RP	reverse phase
s	singlet
SPPS	solid phase peptide synthesis
t	triplet
TBAF	tetra-*N*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography
Trt	triphenylmethyl
UV-Vis	ultraviolet-visible

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General Information

Characterization: NMR spectra were recorded on a Varian Inova 600, Inova 500, Inova 400, Inova 300 or Unity 500 spectrometer. For ^{1}H (300, 400, 500 or 600 MHz) spectra, δ values were referenced to $CDCl_3$ (7.26 ppm) or CD_3OD (3.30 ppm), and for ^{13}C (75, 100, 125 or 150 MHz) spectra, δ values were referenced to $CDCl_3$ (77.0 ppm) or CD_3OD (49.0 ppm), as the solvents. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on a Kratos AEIMS-50, Bruker 9.4T Apex-Qe FTICR (high resolution, HRMS) or on a Perspective Biosystems Voyager™ Elite MALDI-TOF MS using either 4-hydroxy-α-cyanocinnamic acid (HCCA) or 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) as matrices. MS/MS was performed on a Bruker Ultraflextreme MALDI/TOF/TOF. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at ambient temperature.

Reagents and solvents: All commercially available reagents and protected amino acids were purchased and used without further purification. All solvents used for anhydrous reaction were distilled prior to use. Dichloromethane and 1,2-dichloroethane were distilled over calcium hydride, tetrahydrofuran was distilled over sodium with benzophenone as an indicator and ethyl acetate was distilled over potassium carbonate. HPLC grade dimethylformamide and methanol were used without further purification. Commercially available ACS grade solvents (> 99.0% purity) were used for column chromatography without any further purification.

Reactions and Purifications: All reactions were performed under an atmosphere of argon. All reactions and fractions from column chromatography were monitored by thin

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layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; Staining with Phosphomolybdic acid in ethanol (10 g/100 mL) or staining with Ninhydrin (Ninhydrin : acetic acid : n-butanol/ 0.6 g : 6 mL : 200 mL) spray. Flash chromatography was performed using Merck type 60, 230-400 mesh silica gel.

High performance liquid chromatography (HPLC) was performed on a Varian Prostar chromatograph equipped with a model 325 variable wavelenth UV detector and a Rheodyne 7725i injector fitted with a 100 µL sample loop. The column used was a GE Healthcare stainless steel walled µRPC C2/C18 (3 µm, 4.6 × 100 mm). All HPLC solvents were filtered with a Millipore filtration system under vacuum before use.

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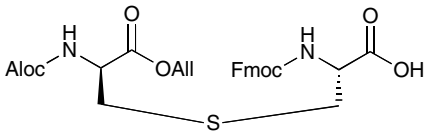
Experimental Details

Characterization of previously synthesized literature compounds

(*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(((*S*)-3-(allyloxy)-2-(((allyloxy)carbonyl)amino)-3-oxopropyl)thio)propanoic acid (1a)



1a

Compound **1a** was synthesized according to literature procedure.[1,2] $[\alpha]_D^{26}$ -3.30 (*c* 1.0, MeOH); IR (DCM cast) 3325, 3068, 2946, 1720, 1522, 1248, 1210 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz, *2 diastereomers in a ~ 9:1 ratio*): δ 7.78 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.67 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.38 (t, 2H, *J* = 7.5 Hz, Ar-H), 7.30 (t, 2H, *J* = 7.5 Hz, Ar-H), 5.95-5.84 (m, 2H, $-OCH_2CH=CH_2$), 5.30 (dd, 1H, *J* = 17.0, 1.5 Hz, $-OCH_2CH=CH_2$), 5.29 (d, 1H, *J* = 17.0 Hz, $-OCH_2CH=CH_2$), 5.19 (d, 1H, *J* = 10.5 Hz, $-OCH_2CH=CH_2$), 5.15 (d, 1H, *J* = 11.0 Hz, $-OCH_2CH=CH_2$), 4.60 (d, 2H, *J* = 5.5 Hz, $-OCH_2$), 4.53 (d, 2H, *J* = 5.0 Hz, $-OCH_2$), 4.47-4.38 (m, 2H, 2 × $-CH_\alpha$), 4.37-4.29 (m, 2H, Fmoc-CH_2), 4.23 (t, 1H, *J* = 7.0 Hz, Fmoc-CH), 3.11-3.02 (m, 2H, $-CH_\beta$), 2.98-2.88 (m, 2H, $-CH_\beta$); ^{13}C NMR (CD_3OD, 125 MHz): δ 173.8, 172.0, 158.5, 158.3, 145.29, 145.25, 142.6, 134.2, 133.1, 128.8, 128.2, 126.4, 120.9, 118.9, 117.7, 68.3, 67.1, 66.8, 55.7 (major), 55.5 (minor), 55.4 (major), 55.3 (minor), 48.4, 35.5, 35.2; HRMS (ES) Calculated for $C_{28}H_{30}N_2O_8S$ 553.1650, found 553.1654 $(M+H)^+$. The diastereomeric ratio (~ 9:1) could be determined by integrating the ^{13}C NMR α-carbon signal as described previously.[1,2]

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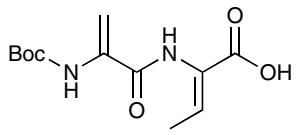
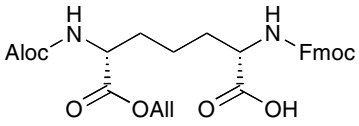
(*Z*)-2-(2-(*tert*-butoxycarbonylamino)acrylamido)but-2-enoic acid (6)



6

Compound **6** was synthesized according to literature procedure.[3] IR ($CHCl_3$ cast) 3354, 2980, 2934, 1706, 1506 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): 6.90 (q, 1H, J = 7.2 Hz, C=C<u>H</u>CH_3), 5.83 (s, 1H, C=C<u>H</u>H), 5.47(s, 1H, C=CH<u>H</u>), 1.77 (d, 3H, J = 7.2 Hz, -C<u>H</u>$_3$), 1.47 (s, 9H, -C(C<u>H</u>$_3$)$_3$); ^{13}C NMR (CD_3OD, 100 MHz): δ 167.3, 165.7, 154.7, 137.2, 136.9, 128.7, 102.8, 81.5, 28.3, 14.0; HRMS (ES) Calculated for $C_{12}H_{18}N_2NaO_5$ 293.1107, found 293.1106 $(M+H)^+$.

(2*S*,6*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-7-(allyloxy)-6-(((allyloxy)carbonyl)amino)-7-oxoheptanoic acid (4b)



4b

Compound **4b** was synthesized according to literature procedure.[4] $[\alpha]_D^{26}$ 3.3 (*c* 0.25, DCM); IR (DCM cast) 3327, 3067, 2951, 1722, 1528, 1210 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz): δ 7.76 (d, 2H, J = 8 Hz, Ar-<u>H</u>) 7.65 (t, 2H, J = 8 Hz, Ar-<u>H</u>) 7.36 (t, 2H, J = 8 Hz, Ar-<u>H</u>) 7.29 (t, 2H, J = 8 Hz, Ar-<u>H</u>) 5.94-5.85 (m, 2H, -OCH_2C<u>H</u>=CH_2) 5.29 (d, 1H, J = 17.0 Hz, -OCH_2CH=C<u>H</u>$_2$) 5.28 (d, 1H, J = 17.0 Hz, -OCH_2CH=C<u>H</u>$_2$) 5.18 (d, 1H, J = 11.0 Hz, -OCH_2CH=C<u>H</u>$_2$) 5.15 (d, 1H, J = 10.5 Hz, -OCH_2CH=C<u>H</u>$_2$) 4.58 (m, 2H, -

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OC$\underline{H}_2$) 4.52 (m, 2H, -OC$\underline{H}_2$) 4.33 (d, 2H, J = 7.0 Hz, Fmoc-C$\underline{H}_2$) 4.19 (t, 1H, J = 7.0 Hz, Fmoc-C$\underline{H}$) 4.17-4.12 (m, 2H, 2 × -C$\underline{H}_\alpha$) 1.90-1.80 (m, 2H, -C$\underline{H}_\beta$), 1.74-1.62 (m, 2H, -C$\underline{H}_\beta$) 1.58-1.49 (m, 1H, -C$\underline{H}_{2\gamma}$) 1.49-1.41 (m, 1H, -C$\underline{H}_{2\gamma}$); ^{13}C NMR (CD_3OD, 150 MHz): δ 173.7, 158.7, 158.5, 145.4, 145.2, 142.6, 134.3, 133.3, 128.8, 128.2, 126.3, 121.0, 118.7, 117.7, 68.0, 66.7, 66.6, 55.5, 55.4, 48.4, 32.2, 32.2, 23.4; HRMS (ES) Calculated for $C_{29}H_{32}N_2NaO_8$ 559.2051, found 559.2047 $(M+Na)^+$.

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General procedure for elongation using Fmoc Solid Phase Peptide Synthesis (SPPS)

NMM (6 equiv) was added to a solution of Fmoc protected amino acid (5.0 equiv to resin loading), HOBt (5.0 equiv) and PyBOP (4.9 equiv) in DMF (10 mL). The solution was allowed to pre-activate for 5 min. The solution was transferred to the reaction vessel containing pre-swelled resin and was bubbled with argon for 3 h. A small sample of the peptide was cleaved from the resin (by treatment with 95% TFA/2.5% TIPS or anisole[1]/2.5% H_2O for 2 h) and the completion of the reaction was determined by MALDI-TOF analysis. Resin was washed with DMF (3 × 10 mL), then 20% Ac_2O in DMF (10 mL) was added to the resin for 10 min to effect end capping. Resin was again washed with DMF (3 × 10 mL). Then 20% piperidine in DMF (3 × 10 mL) was added to remove the N-terminal Fmoc protecting group, this reaction was monitored by UV-Vis spectroscopy, observing the dibenzofulvene-piperidine adduct at λ = 301 nm.

General procedure for cleavage and purification of lactocin S analogue peptides

To simultaneously cleave the peptide from 2-chlorotrityl resin, remove side chain protecting groups, and generate the α-ketoamide functionality at the N-terminus of the peptides, a solution of 95:2.5:2.5 TFA:anisole:H_2O: was added to the resin-bound peptide for 2 hours. The resin beads were removed via filtration through glass wool and the filtrate was concentrated *in vacuo*. The crude peptide was obtained by precipitation with cold Et_2O. All cleavage and precipitation steps were done under an atmosphere of argon gas to reduce the oxidation of remaining sulfur atoms in methionine or lanthionine residues. The crude peptide was dissolved in a 1:1 water:methanol (0.1% TFA) and

[1] TIPS was used as a scavenger for peptide cleavage up until residue 13. Afterwards, anisole was used instead as it produced cleaner MALDI spectra

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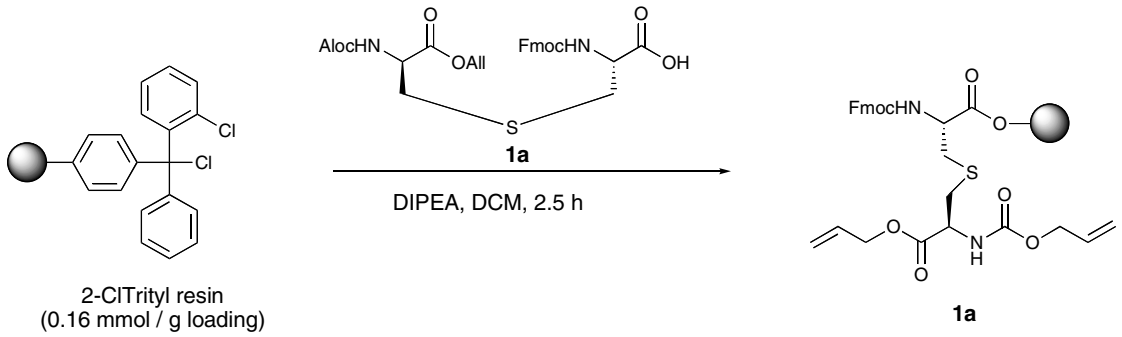
injected onto a C_2/C_{18} RP-HPLC column (GE Healthcare, 4.6 x 100 mm, 3 μm) using water (0.1% TFA) and methanol (0.1% TFA) as eluents. TCEP (1.5 mg/L) was added to the HPLC solvents and the product was collected under a stream of argon gas to minimize oxidation of the sulfur atoms in methionine and lanthionine residues. The HPLC method followed was: gradient beginning at 5% methanol for 1 min, climb to 60% over 4 min, then climb to 100% over 8 min, remain at 100% for 2 min, return to 5% over 2 min and remain at 5% for 8 min (flow rate 1 mL/min, UV detection at 220 nm). The peptide was collected as a broad peak at ~13 min and solvent was removed *in vacuo*. The residue was then dissolved in approximately 100 μL methanol and 900 μL water (both with 0.1% TFA) and lyophilized to give the final product.

Scheme 1. Coupling of protected lanthionine to resin



2-Chlorotrityl chloride resin (2.0 g, 2.6 mmol) was pre-swelled in DCM (20 mL) without stirring for 30 min. A solution of orthogonally protected lanthionine **1a** (0.177 g, 0.32 mmol) and DIPEA (0.28 mL, 1.6 mmol) in DCM (20 mL) was added and the mixture stirred very gently for 2.5 h. The slurry was transferred to a peptide reaction vessel and the resin washed with DCM (2 × 20 mL). To cap the remaining reactive sites on the resin a solution of acetic acid (0.114 mL, 2.0 mmol) and DIPEA (1.394 mL, 8.0 mmol) in

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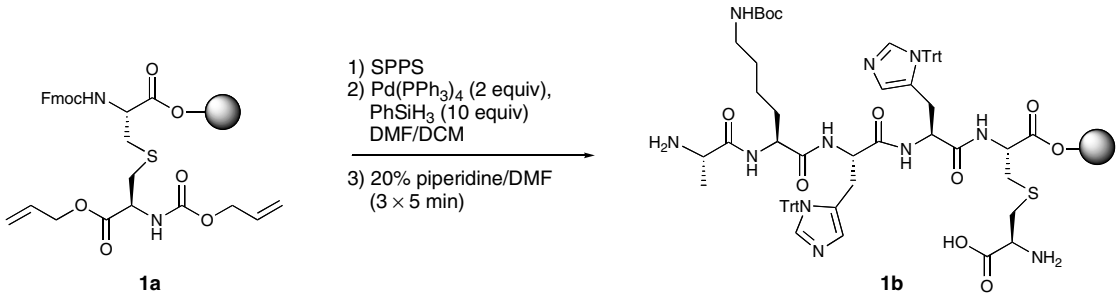
DCM (20 mL) was added to the resin and the reaction mixture was bubbled with argon for 2 h. The resin was then washed with DCM (2 × 20 mL). This yielded a resin with a reduced loading (0.16 mmol/g) of reactive sites functionalized with the C-terminal amino acid of lactocin S ready for SPPS.

Scheme 2. Elongation by SPPS then removal of Aloc/Allyl/Fmoc protecting groups



The N-terminal Fmoc protecting group was removed using a solution of 20% piperidine in DMF. The resin bound peptide was coupled with Fmoc-His(Trt)-OH (residues 36 and 35), Fmoc-Lys(Boc)-OH (res. 34) and Fmoc-Ala-OH (res. 33) in the presence of PyBOP and HOBt as coupling reagents using the general elongation procedure described above. To form the B ring of lactocin S, the orthogonal protecting groups of lanthionine were removed to allow cyclization. The Allyl/Aloc groups were removed by treatment of the resin with a solution of $Pd(PPh_3)_4$ (0.739 g, 0.64 mmol) and $PhSiH_3$ (0.40 mL, 3.2 mmol) in 1:1 DMF/DCM (40 mL) in the absence of light for 2 h. The resin was then washed with DCM (40 mL) until dark brown color was removed. The resin was then washed with 0.5 % sodiumdiethyldithiocarbamate in DMF (3 × 20 mL) and then DMF (2 × 20 mL). This procedure allowed the removal of any remaining palladium from the resin. The N-terminal Fmoc protecting group was removed using the standard protocol of 20 %

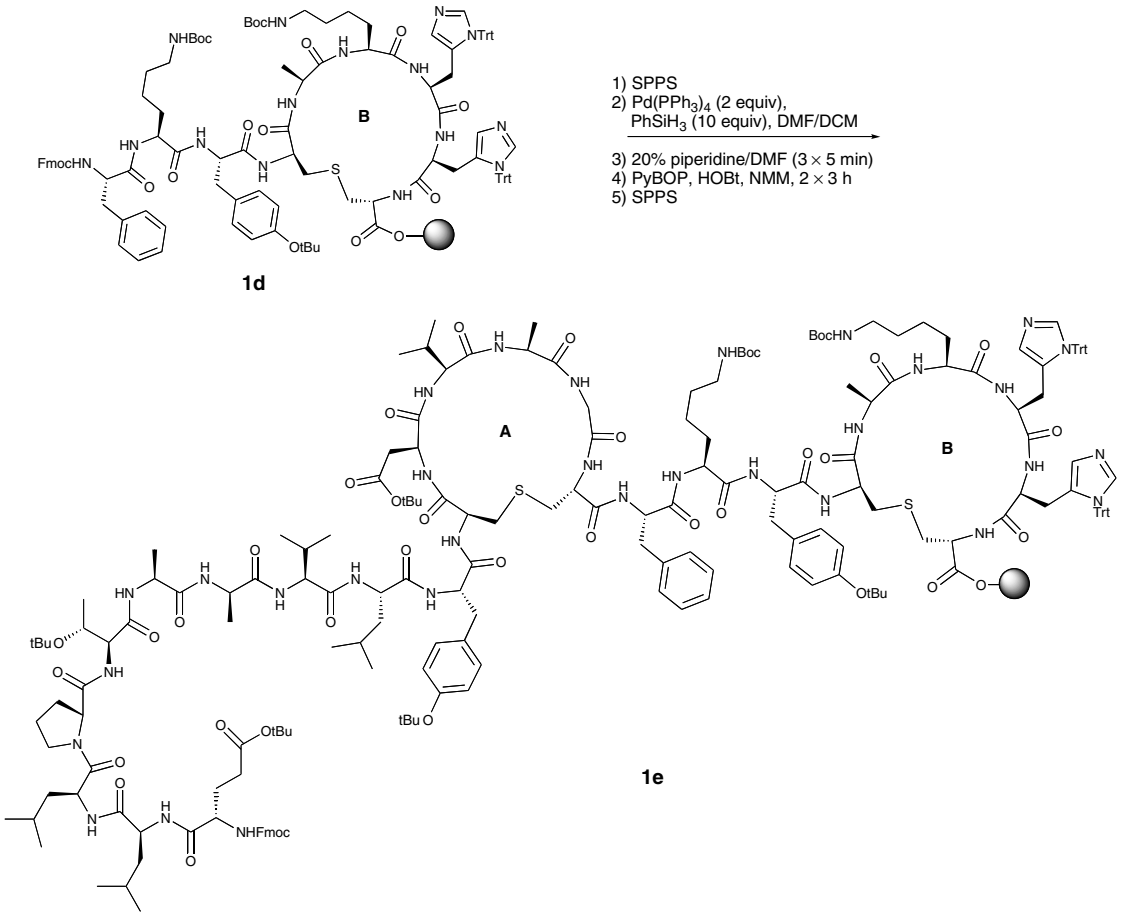
piperidine in DMF. The resin was washed with DCM (3 × 20 mL) and then DMF (3 × 20 mL). Resin-bound peptide **1b** was then ready for cyclization to form ring B.

Scheme 3. Cyclization to form ring B of lactocin S and SPPS elongation

1b **1d**

To cyclize the B ring of lactocin S a solution of PyBOP (0.832 g, 1.6 mmol), HOBt (0.216 g, 1.6 mmol) and NMM (0.35 mL, 3.2 mmol) in DMF (20 mL) was added to **1b** on resin and the reaction mixture was bubbled with argon for 2 h. The resin was washed with DMF (3× 10 mL) and then a second solution of PyBOP (0.832 g, 1.6 mmol), HOBt (0.216 g, 1.6 mmol) and NMM (0.35 mL, 3.2 mmol) in DMF (20 mL) was added to the resin and the reaction mixture was bubbled with argon for 2 h, generating the macrocyclic ring B **1c**. The N-terminus of the peptide was elongated by Fmoc-SPPS with PyBOP and HOBt as coupling reagents to introduce residues 31-29 (Fmoc-Tyr(O^{t}Bu)-OH, Fmoc-Lys(Boc)-OH and Fmoc-Phe-OH). A small sample of the resin-bound peptide was treated with TFA/TIPS/H_2O (95/2.5/2.5) for 2 h to remove the peptide from the solid support. Following filtration the filtrate was concentrated *in vacuo* and the peptide was precipitated by addition of Et_2O at 0 °C. The resulting white solid was analyzed using MALDI-TOF MS: Calculated for $C_{66}H_{92}N_{15}O_{13}S$ 1324.5, found 1324.8 $(M+H)^+$.

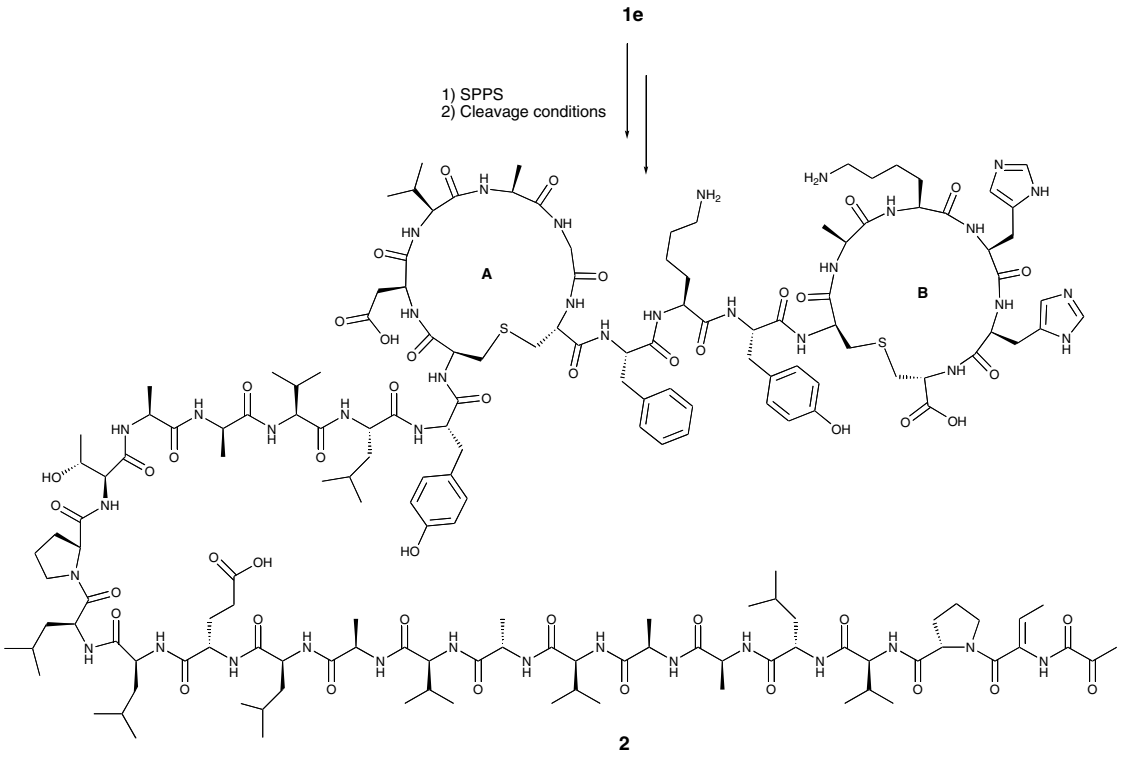
Scheme 4. Formation of ring A of lactocin S and subsequent SPPS elongation



The amino acids of ring A (res. 28-24) of lactocin S were introduced using Fmoc-SPPS in the following order - Fmoc-Aloc/Allyl-lanthionine, Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Asp(O^{t}Bu)-OH. Removal of the Allyl/Aloc groups was carried out in an analogous fashion to that described for ring B. The N-terminal Fmoc was deprotected using 20 % piperidine in DMF and after the resin had been washed with DCM and DMF the cyclization of ring A was effected using PyBOP and HOBt as previously described. To investigate the cyclization reaction by MALDI-TOF MS a small sample of resin (5 mg) was coupled with the next amino acid Fmoc-Tyr(O^{t}Bu)-OH using PyBOP and HOBt. This sample was then cleaved from the resin using TFA/TIPS/H_2O as

described for ring B and analyzed by MALDI-TOF MS: Calculated for $C_{95}H_{121}N_{22}O_{23}S_2$ 2001.8, found 2001.8 $(M+H)^+$. The amino acids corresponding to residues 13-22 were introduced via Fmoc-SPPS with PyBOP and HOBt as coupling reagents in the order – Fmoc-Tyr(O^{t}Bu)-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-D-Ala-OH, Fmoc-Ala-OH, Fmoc-Thr(O^{t}Bu)-OH, Fmoc-Pro-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(O^{t}Bu)-OH. A small sample was then cleaved from the resin using TFA/TIPS/H_2O as described for rings A and B and analyzed by MALDI-TOF MS: Calculated for $C_{138}H_{194}N_{31}O_{35}S_2$ 2908.4, found 2910.5 $(M+H)^+$. Subsequently, the resin was partitioned into 3 equal parts (0.107 mmol each) in order to synthesize novel analogs of lactocin S differing at residue 12.

Scheme 5. Incorporation of Leu-12 and subsequent synthesis of Leu-12 lactocin S (2)



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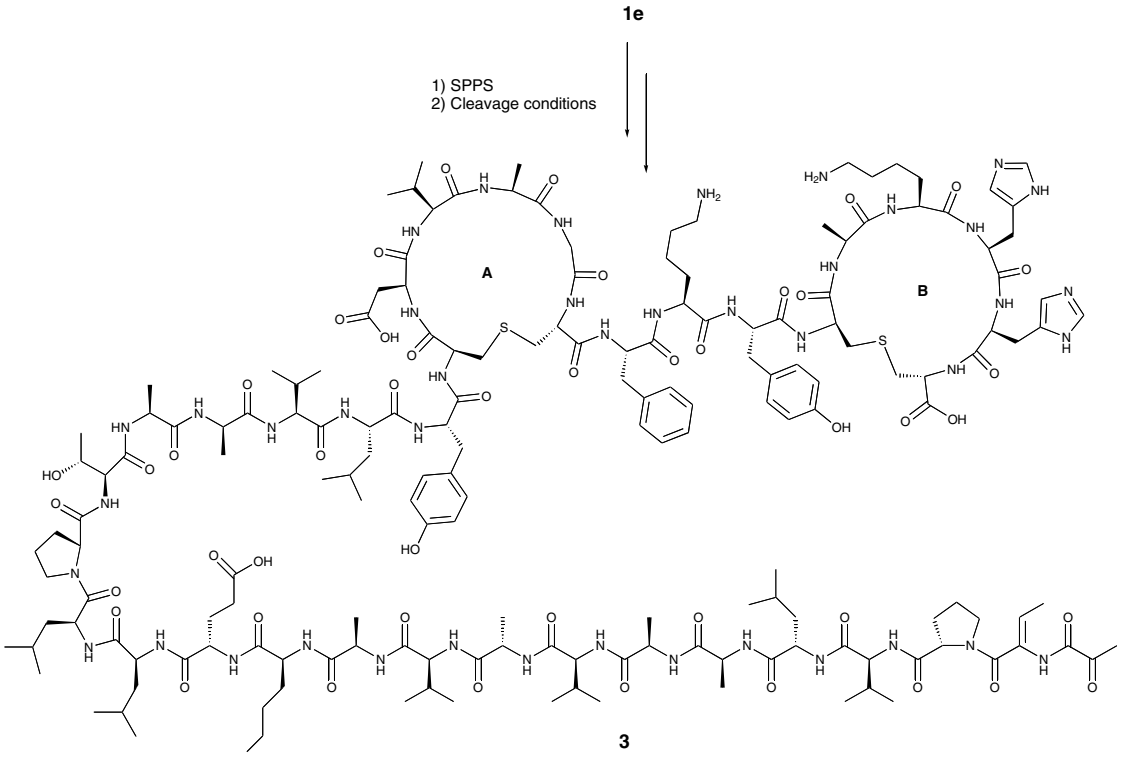
Resin-bound **1e** (0.107 mmol) was transferred to a peptide synthesis vessel and pre-swelled in DMF (10 mL) for 15 min. After endcapping was performed, a pre-activated solution of Fmoc-Leu-OH (94 mg, 0.27 mmol), PyBOP (136 mg, 0.26 mmol), HOBt (36 mg, 0.27 mmol), and NMM (35 μL, 0.32 mmol) in DMF (10 mL) was added and bubbled with argon for 3 hours. The amino acids corresponding to residues 3-11 were also introduced via Fmoc-SPPS with PyBOP and HOBt as coupling reagents in the following order: Fmoc-D-Ala-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-D-Ala-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Pro-OH. A small sample was then cleaved from the resin using TFA/anisole/H_2O as described for rings A and B and analyzed by MALDI-TOF MS: Calculated for $C_{182}H_{270}N_{41}O_{45}S_2$ 3812.9, found 3814.3 $(M+H)^+$. The N-terminal dipeptide **6** was coupled to the Fmoc deprotected 3-37 lactocin fragment using standard solid phase methodology. A pre-activated solution of the dipeptide (61 mg, 0.23 mmol), PyBOP (115 mg, 0.22 mmol), HOBt (31 mg, 0.23 mmol), and NMM (30 μL, 0.27 mmol) in DMF (10 mL) was added and bubbled with argon for 3 hours. After rinsing with DMF, a second 3 hour coupling was subsequently performed with a similarly pre-activated solution of the dipeptide in DMF (10 mL). The peptide was then cleaved from the resin yielding crude peptide (23.1 mg) and was purified as described previously to give 1.0 mg of product (overall yield = 1.5%) as a fluffy white solid. Monoisotopic MW calculated for $C_{174}H_{267}N_{42}O_{46}S_2$ 3744.9286, found *high resolution* (FTICR-ESI-MS) 3744.9269 $(M+H)^+$.

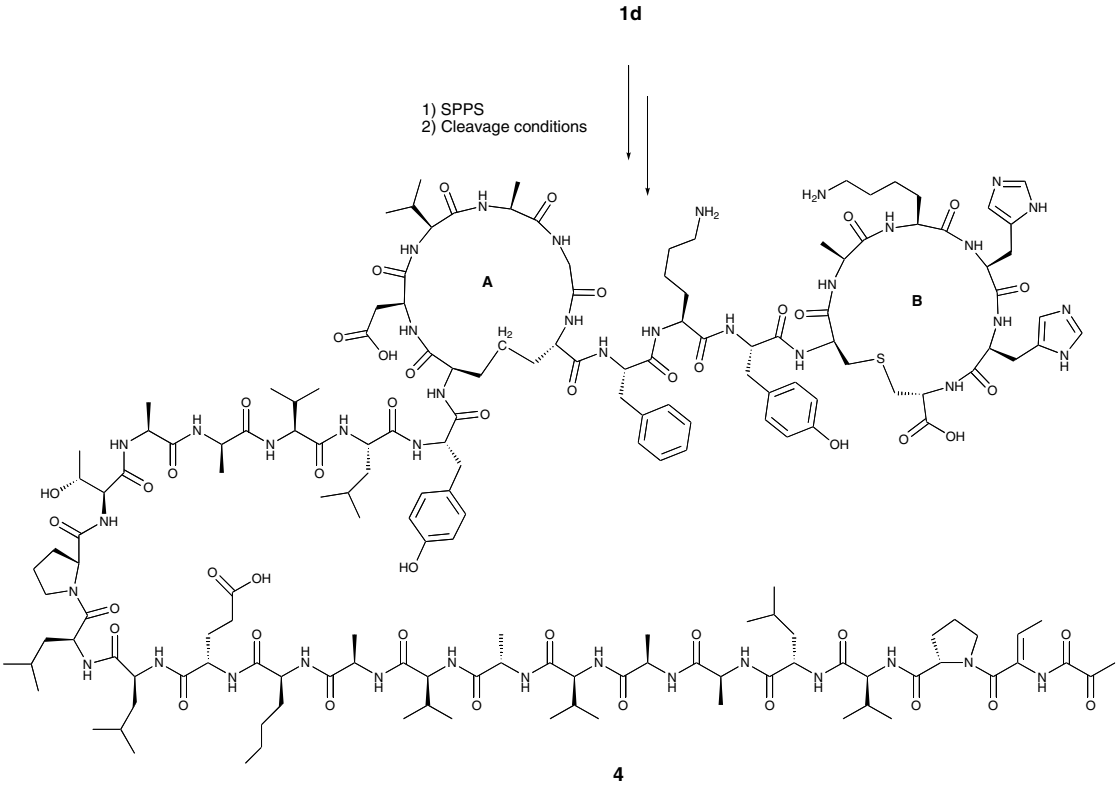
Scheme 6. Incorporation of Nle-12 and subsequent synthesis of Nle-12 lactocin S (3)



Resin-bound **1e** (0.107 mmol) was transferred to a peptide synthesis vessel and pre-swelled in DMF (10 mL) for 15 min. After endcapping was performed, a pre-activated solution of Fmoc-Nle-OH (94 mg, 0.27 mmol), PyBOP (136 mg, 0.26 mmol), HOBt (36 mg, 0.27 mmol), and NMM (35 μL, 0.32 mmol) in DMF (10 mL) was added and bubbled with argon for 3 hours. The amino acids corresponding to residues 3-11 were also introduced via Fmoc-SPPS with PyBOP and HOBt as coupling reagents in the order - Fmoc-D-Ala-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-D-Ala-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Pro-OH. A small sample was then cleaved from the resin using TFA/anisole/H_2O as described for rings A and B and analyzed by MALDI-TOF MS: Calculated for $C_{182}H_{270}N_{41}O_{45}S_2$ 3812.9, found 3814.4 $(M+H)^+$. The N-terminal dipeptide **6** was coupled to the Fmoc deprotected 3-37 lactocin fragment using standard solid phase methodology. Quantitatively, a pre-activated

solution of the dipeptide (61 mg, 0.23 mmol), PyBOP (115 mg, 0.22 mmol), HOBt (31 mg, 0.23 mmol), and NMM (30 µL, 0.27 mmol) in DMF (10 mL) was added and bubbled with argon for 3 hours. After rinsing with DMF, a second 3 hour coupling was subsequently performed with a similarly pre-activated solution of the dipeptide in DMF (10 mL). The peptide was then cleaved from the resin yielding crude peptide (14.2 mg) and was purified as described previously to give 0.8 mg of product (overall yield = 0.8%) as a fluffy white solid. Monoisotopic MW calculated for $C_{174}H_{267}N_{42}O_{46}S_2$ 3744.9286 found *high resolution* (FTICR-ESI-MS) 3744.9281 $(M+H)^+$.

Scheme 7. Synthesis of NleDAP lactocin S analogue (4)



Orthogonally protected DAP **4b** was coupled to resin-bound peptide **1d** using Fmoc-SPPS methodology. The remainder of the amino acids of ring A (res. 27-24) of lactocin

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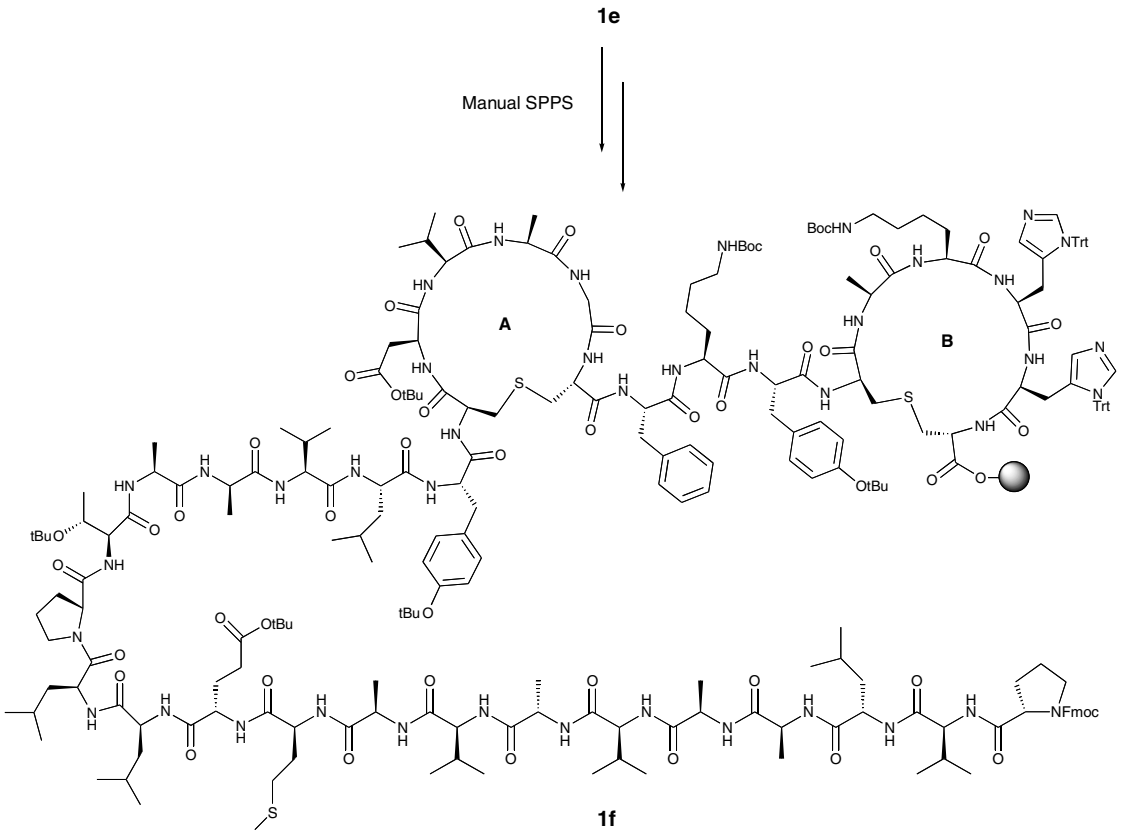
S were introduced using Fmoc-SPPS in the following order - Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Asp(O^{t}Bu)-OH. Removal of the Allyl/Aloc groups was carried out in an analogous fashion to that described for ring B. The N-terminal Fmoc was deprotected using 20 % piperidine in DMF and after the resin had been washed with DCM and DMF the cyclization of ring A was effected using PyBOP and HOBt as previously described. To determine the success of the cyclization reaction by MALDI-TOF MS a small sample of resin (5 mg) was coupled with the next amino acid Fmoc-Tyr(O^{t}Bu)-OH using PyBOP and HOBt. This sample was then cleaved from the resin using TFA/TIPS/H_2O as described for ring B and analyzed by MALDI-TOF MS: Calculated for $C_{96}H_{123}N_{22}O_{23}S$ 1982.9, found 1983.2 $(M+H)^+$. The amino acids corresponding to residues 3-22 were introduced via Fmoc-SPPS with PyBOP and HOBt as coupling reagents in the order – Fmoc-Tyr(O^{t}Bu)-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-D-Ala-OH, Fmoc-Ala-OH, Fmoc-Thr(O^{t}Bu)-OH, Fmoc-Pro-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Nle-OH, Fmoc-D-Ala-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-D-Ala-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Val-OH and Fmoc-Pro-OH. A small sample was then cleaved from the resin using TFA/anisole/H_2O as described for rings A and B and analyzed by MALDI-TOF MS: Calculated for $C_{183}H_{272}N_{41}O_{45}S$ 3796.0, found 3798.3 $(M+H)^+$. The N-terminal dipeptide **6** was coupled to the Fmoc deprotected 3-37 lactocin S fragment using standard solid phase methodology. Quantitatively, a pre-activated solution of the dipeptide (147 mg, 0.54 mmol), PyBOP (230 mg, 0.53 mmol), HOBt (61 mg, 0.54 mmol), and NMM (60 µL, 0.65 mmol) in DMF (10 mL) was added and bubbled with argon for 3 hours. After rinsing with DMF, a second 3 hour coupling was subsequently performed with a similarly

pre-activated solution of the dipeptide in DMF (10 mL). The peptide was then cleaved from the resin yielding crude peptide (39.5 mg) and was purified as described previously to give 1.0 mg of product (overall yield = 1.8%) as a fluffy white solid. Monoisotopic MW calculated for $C_{175}H_{269}N_{42}O_{46}S$ 3726.9722, found *high resolution* (FTICR-ESI-MS) 3726.9716 $(M+H)^+$.

Scheme 8. SPPS elongation to form res 3-37 of lactocin S (1f)



Resin-bound **1e** (0.107 mmol) was transferred to a peptide synthesis vessel and pre-swelled in DMF (10 mL) for 15 min. The amino acids corresponding to residues 3-12 of natural lactocin S were also introduced via Fmoc-SPPS with PyBOP and HOBt as coupling reagents in the order: Fmoc-Met-OH, Fmoc-D-Ala-OH, Fmoc-Val-OH, Fmoc-

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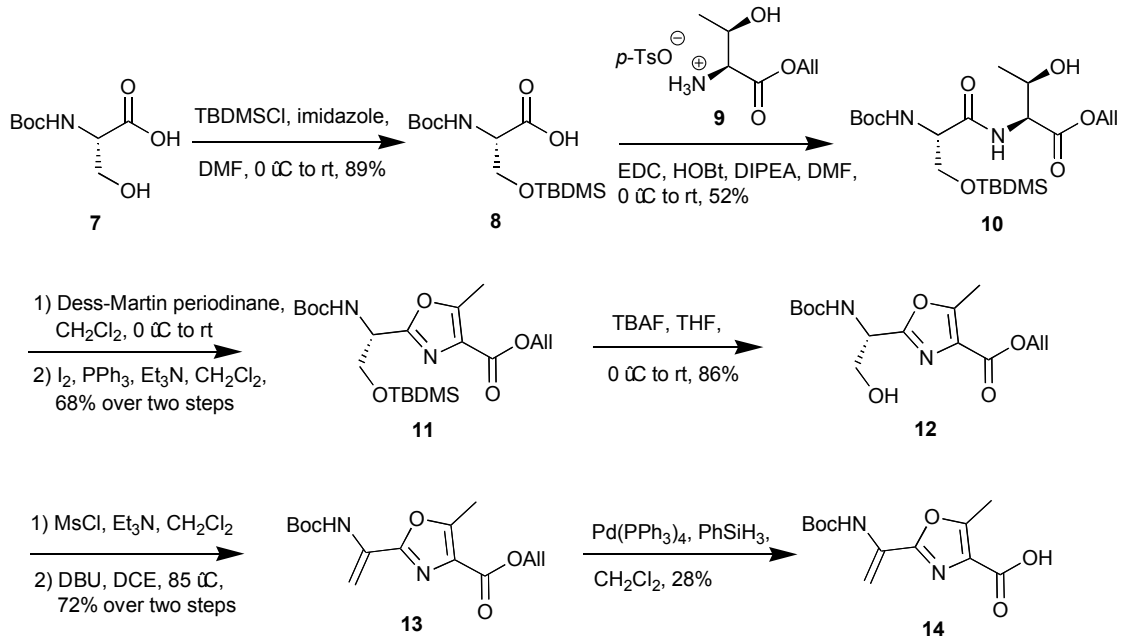
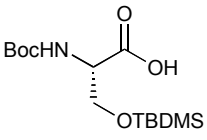
Ala-OH, Fmoc-Val-OH, Fmoc-D-Ala-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Pro-OH. A small sample was then cleaved from the resin using TFA/anisole/H_2O as described for rings A and B and analyzed by MALDI-TOF MS: Calculated for $C_{181}H_{268}N_{41}O_{45}S_3$ 3831.9, found 3832.6 $(M+H)^+$.

Scheme 9. Synthesis of 2-(1-(*tert*-butoxycarbonylamino)vinyl)-5-methyloxazole-4-carboxylic acid



(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)propanoic acid (8)



8

This known compound was prepared according to literature procedure.[5] Imidazole (15.1 g, 244 mmol) was added to a solution of Boc-Ser-OH (**7**) (20.0 g, 97.5 mmol) and

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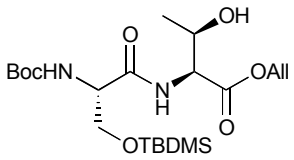
TBDMS-Cl (16.2 g, 107 mmol) in DMF (200 mL) at 0 °C. Reaction was allowed to warm to rt and was stirred for 16 h, then the solvent was removed *in vacuo*. The residue was re-dissolved in EtOAc:MeOH (95 mL:5 mL), washed with H_2O and dried over Na_2SO_4. The solvent was removed *in vacuo* to yield **8** (27.7 g, 89.1 %) as a pale yellow oil. The crude product was carried through to the next reaction without further purification. (R_f 0.83 on SiO_2, 1:1 hexanes:EtOAc); $[\alpha]_D^{26}$ 5.96 (*c* 1.0, MeOH); IR (MeOH cast) 3329, 3148, 2978, 2935, 2880, 2646, 1708, 1519, 1167 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz): δ 4.14 (m, 1H, -C$\underline{H}_\alpha$), 3.96 (dd, 1H, *J* = 10.2, 4.2 Hz, -C$\underline{H}_\beta$), 3.88 (dd, 1H, *J* = 10.2, 3.9 Hz, -C$\underline{H}_\beta$), 1.44 (s, 9H, -C(C$\underline{H}_3$)$_3$), 0.88 (s, 9H, -C(C$\underline{H}_3$)$_3$), 0.06 (s, 3H, -C$\underline{H}_3$), 0.05 (s, 3H, -C$\underline{H}_3$); ^{13}C NMR (CD_3OD, 125 MHz): δ 175.5, 157.5, 80.4, 65.1, 58. 1, 28.8, 26.4, -5.2; HRMS (ES) Calculated for $C_{14}H_{29}NO_5SiNa$ 342.1707, found 342.1712 $(M+Na)^+$.

(2*S*,3*R*)-allyl 2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)propanamido)-3-hydroxybutanoate (10)



10

Allyl protected threonine **9** (6.84 g, 20.7 mmol) and **8** (6.00 g, 18.8 mmol) were dissolved in DMF (200 mL) and EDC (4.32 g, 23.0 mmol) and HOBt (3.04 g, 23.0 mmol) were added. The reaction was cooled to 0 °C and DIPEA (8.17 mL, 47.0 mmol) was added. The reaction was allowed to warm to rt and was left to stir for 18 h. Solvent was removed *in vacuo* and the resulting residue was re-dissolved in EtOAc (400 mL) then washed with

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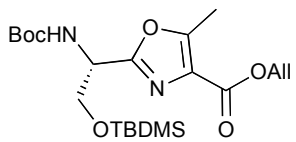
10% citric acid (100 mL), 10% $NaHCO_3$ (100 mL) H_2O (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and then concentrated *in vacuo*. The resulting crude product was purified using flash chromatography (SiO_2, 3:1 hexanes:EtOAc) to yield **10** (4.47 g, 51.7%) as a pale yellow oil. (R_f 0.31 on SiO_2, 3:1 hexanes:EtOAc); $[\alpha]_D^{26}$ 12.54 (*c* 1.0, EtOAc); IR (EtOAc cast) 3356, 2977, 2955, 2931, 2885, 2858, 1745, 1720, 1672, 1523, 1502, 1255, 1171 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.36-7.18 (m, 1H, -NH), 5.92 (ddt, 1H, *J* = 6.0, 10.5, 17.0 Hz ,-$OCH_2CH=CH_2$), 5.42-5.35 (m, 1H, -NH), 5.35 (dd, 1H, *J* = 17.0, 1.5 Hz, -$OCH_2CH=CH_2$), 5.27 (dd, 1H, *J* = 10.5, 1.5 Hz, -$OCH_2CH=CH_2$), 4.69-4.66 (m, 2H, -$OCH_2CH=CH_2$), 4.64 (dd, 1H, *J* = 9.0, 3.0 Hz, Thr-CH_α), 4.39-4.33 (m, 1H, Thr-CH_β), 4.27-4.20 (m, 1H, Ser-CH_α), 4.06 (dd, 1H, *J* = 10.0, 4.0 Hz, Ser-CH_β), 3.73 (dd, 1H, *J* = 10.0, 6.5 Hz, Ser-CH_β), 2.38-2.32 (br s, 1H, -OH), 1.48 (s, 9H, -$C(CH_3)_3$), 1.22 (d, 3H, *J* = 6.0 Hz, Thr-CH_3), 0.91 (s, 9H, Si-$C(CH_3)_3$), 0.11 (s, 6H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.2, 170.2, 155.5, 131.5, 118.9, 80.2, 68.1, 66.1, 63.2, 57.5, 55.8, 28.3, 25.9, 19.9, 18.3, -5.4, -5.5; HRMS (ES) Calculated for $C_{21}H_{40}N_2O_7SiNa$ 483.2497, found 483.2496 $(M+Na)^+$.

(*S*)-allyl 2-(2,2,3,3,10,10-hexamethyl-8-oxo-4,9-dioxa-7-aza-3-silaundecan-6-yl)-5-methyloxazole-4-carboxylate (11)



11

This product was synthesized adapting protocols from a literature source.[6] Dess-Martin periodinane (4.20 g, 9.80 mmol) was added to a solution of **10** (4.30 g, 9.30 mmol) in

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CH_2Cl_2 (100 mL) at 0 °C. After 15 min the reaction was warmed to rt and left to stir for a further 1 h. Et_2O (50 mL) was added and the solvent was removed *in vacuo*. The residue was re-dissolved in Et_2O (200 mL) and washed with a solution of 10% $Na_2S_2O_3$ /10% $NaHCO_3$ (100 mL) followed by H_2O (100 mL) and brine (100 mL). The aqueous layers were back extracted into Et_2O (3 × 75 mL) and then the organic layer was washed with H_2O (50 mL) and brine (50 mL). The organic layers were combined and dried over Na_2SO_4. Solvent was removed *in vacuo* and the residue was dissolved in CH_2Cl_2 (75 mL) and was added drop-wise to a solution of triphenylphosphine (4.90 g, 18.7 mmol), I_2 (4.74 g, 18.7 mmol) and Et_3N (5.20 mL, 37.4 mmol) in CH_2Cl_2 (75 mL). The reaction was stirred for 30 min and was then concentrated *in vacuo*. The resulting residue was dissolved in diethyl ether (250 mL) and was washed with 10% $Na_2S_2O_3$ (100 mL), 10% $NaHCO_3$ (100 mL), H_2O (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting crude product was purified using flash chromatography (SiO_2, 4:1 hexanes:EtOAc) to yield **11** (2.80 g, 68.3%) as a pale yellow oil. (R_f 0.67 on SiO_2, 3:1 hexanes:EtOAc); $[\alpha]_D^{26}$ -15.46 (*c* 0.45, CH_2Cl_2); IR (CH_2Cl_2 cast) 3446, 3356, 2955, 2931, 2885, 2858, 1719, 1502, 1254, 1175, 1099 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.99 (ddt, 1H, *J* = 17.2, 10.4, 5.6 Hz, -$OCH_2C\underline{H}=CH_2$), 5.49 (d, 1H, *J* = 7.6 Hz, -N$\underline{H}$), 5.36 (dq, 1H, *J* = 17.2, 1.6 Hz, -$OCH_2CH=C\underline{H}_2$), 5.26 (dq, 1H, *J* = 10.4, 1.6 Hz, -$OCH_2CH=C\underline{H}_2$), 4.96-4.88 (m, 1H, -$C\underline{H}_\alpha$), 4.83-4.78 (m, 2H, -$OC\underline{H}_2CH=CH_2$), 4.00 (dd, 1H, *J* = 10.4, 4.0 Hz, -$C\underline{H}_\beta$), 3.38 (dd, 1H, J = 10.4, 4.4 Hz, -$C\underline{H}_\beta$), 2.58 (s, 3H, -$C\underline{H}_3$), 1.43 (s, 9H, -$C(C\underline{H}_3)_3$), 0.80 (s, 9H, Si-$C(C\underline{H}_3)_3$), -0.03 (s, 3H, Si-$C\underline{H}_3$), -0.05 (s, 3H, Si-$C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.9, 160.8, 156.5,

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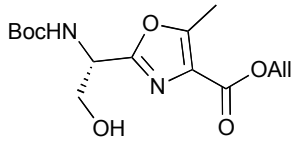
155.1, 132.0, 127.5, 118.7, 80.1, 65.5, 64.5, 51.0, 28.3, 25.6, 18.1, 12.0, -5.6; HRMS (ES) Calculated for $C_{21}H_{37}N_2O_6Si$ 441.2415, found 441.2414 $(M+H)^+$.

(*S*)-allyl 2-(1-((*tert*-butoxycarbonyl)amino)-2-hydroxyethyl)-5-methyloxazole-4-carboxylate (12)



12

1 M Tetra-*n*-butylammonium fluoride in THF (14.5 mL, 14.5 mmol) was added to a solution of **11** (2.13 g, 4.80 mmol) in THF (200 mL) at 0 °C. The reaction was left to stir for 2.5 h whilst slowly warming to rt. Solvent was removed *in vacuo* and the resulting crude product was purified using flash chromatography (SiO_2, 1:1 hexanes:EtOAc) to yield **12** (1.36 g, 86.2%) as a colourless oil. (R_f 0.37 on SiO_2, 1:1 hexanes:EtOAc); $[\alpha]_D^{26}$ -36.9 (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2 cast) 3449, 2978, 2934, 1718, 1620, 1518, 1171 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.99 (ddt, 1H, *J* = 17.2, 10.4, 6.0 Hz, -$OCH_2C\underline{H}$=CH_2), 5.72 (d, 1H, *J* = 7.6 Hz, -N$\underline{H}$), 5.38 (dq, 1H, *J* = 16.8, 1.2 Hz, -OCH_2CH=$C\underline{H}_2$), 5.28 (dq, 1H, *J* = 10.4, 1.2 Hz, -OCH_2CH=$C\underline{H}_2$), 4.99-4.88 (m, 1H, -$C\underline{H}_\alpha$), 4.82-4.77 (m, 2H, -$OC\underline{H}_2CH$=CH_2), 4.07 (dd, 1H, *J* = 11.2, 4.0 Hz, -$C\underline{H}_\beta$), 3.93 (dd, 1H, *J* = 11.2, 4.0 Hz, -$C\underline{H}_\beta$), 3.41-3.01 (br s, 1H, -O$\underline{H}$), 2.60 (s, 3H, -$C\underline{H}_3$), 1.43 (s, 9H, -$C(C\underline{H}_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.9, 160.9, 157.1, 155.7, 132.0, 127.5, 119.2, 80.6, 65.9, 63.9, 50.8, 28.5, 20.7, 12.4; HRMS (ES) Calculated for $C_{15}H_{22}N_2O_6Na$ 349.1370, found 349.1364 $(M+Na)^+$.

allyl 2-(1-((*tert*-butoxycarbonyl)amino)vinyl)-5-methyloxazole-4-carboxylate (13)

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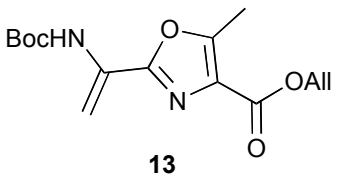
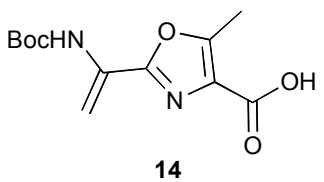


13

Et_3N (0.69 mL, 4.96 mmol) was added to a solution of **12** (0.62 g, 1.99 mmol) in CH_2Cl_2 (50 mL) at 0 °C. After 10 min, methanesulfonyl chloride (0.31 mL, 3.97 mmol) was added and after a further 30 min it was allowed to warm to rt. Solvent was removed *in vacuo* after 1 h and the resulting residue was dissolved in DCE (50 mL). DBU (0.89 mL, 5.95 mmol) was added and the reaction was heated to reflux for 18 h. Solvent was removed *in vacuo* and the resulting crude product was purified using flash chromatography (SiO_2, 5:1 hexanes:EtOAc) to yield **13** (0.42 g, 72.2%) as a yellow oil. (R_f 0.94 on SiO_2, 2:1 hexanes:EtOAc); IR (CH_2Cl_2 cast) 3411, 2980, 2934, 1785, 1723, 1616, 1510, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.25 (s, 1H, -C=C<u>H</u>H), 6.07 (s, 1H, -N<u>H</u>), 6.03 (ddt, 1H, J = 17.2, 10.8, 6.0 Hz, -OCH_2C<u>H</u>=CH_2), 5.53-5.50 (m, 1H, -C=CH<u>H</u>), 5.41 (dq, 1H, J = 17.2, 1.6 Hz, -OCH_2CH=C<u>H_2</u>), 5.31 (dd, 1H, J = 10.4, 1.2 Hz, -OCH_2CH=C<u>H_2</u>), 4.87-4.82 (m, 2H, -OC<u>H_2</u>CH=CH_2), 2.65 (s, 3H, -C<u>H_3</u>), 1.49 (s, 9H, -C(C<u>H_3</u>)$_3$); 13C NMR ($CDCl_3$, 100 MHz): δ 161.9, 157.5, 156.7, 152.9, 132.0, 128.6, 128.3, 119.4, 100.0, 81.1, 66.1, 28.5, 12.6; HRMS (ES) Calculated for $C_{15}H_{21}N_2O_5$ 309.1445, found 309.1443 $(M+H)^+$.

2-(1-((*tert*-butoxycarbonyl)amino)vinyl)-5-methyloxazole-4-carboxylic acid (14)



14

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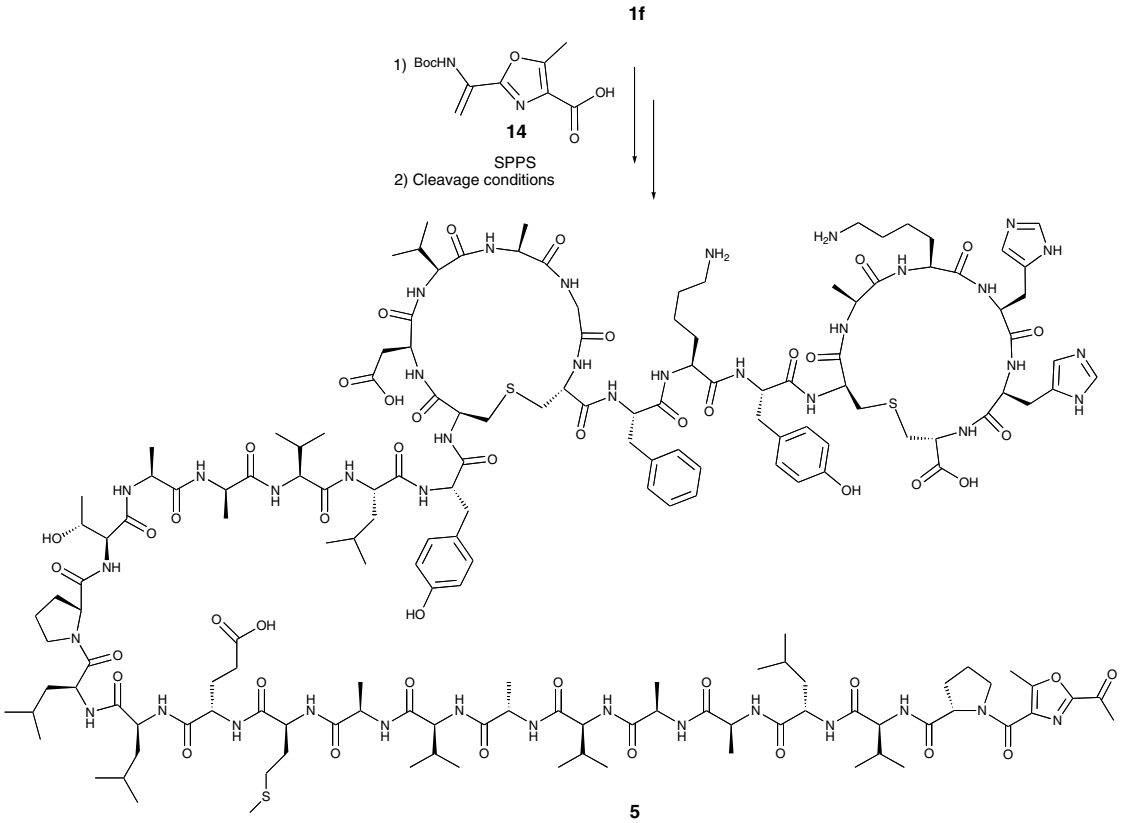
$PhSiH_3$ (0.20 mL, 1.60 mmol) was added to a stirred solution of **13** (0.25 g, 0.82 mmol) in degassed CH_2Cl_2 (100 mL). $Pd(PPh_3)_4$ (0.100 g, 0.080 mmol) was added to the mixture and the reaction was stirred in the absence of light for 1.5 h. The solvent was removed *in vacuo* and the resulting crude product was purified using flash chromatography (SiO_2, 40:1:0.1 CH_2Cl_2:MeOH:Acetic acid) to yield **14** (0.60 g, 27.5%) as a pale orange solid. (R_f 0.13 on SiO_2, 2:1 hexanes:EtOAc); IR (CH_2Cl_2 cast) 3395, 3058, 2979, 2930, 2852, 1781, 1724, 1504, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 10.25-9.47 (br s, 1H, -OH), 7.30 (s, 1H, -NH), 6.03 (s, 1H, -C=CHH), 5.48 (s, 1H, -C=CHH), 2.62 (s, 3H, $-CH_3$), 1.46 (s, 9H, $-C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.9, 157.7, 155.1, 151.2, 126.7, 126.0, 98.8, 79.4, 26.7, 10.9; HRMS (ES) Calculated for $C_{12}H_{16}N_2O_5Na$ 291.0951, found 291.0951 $(M+Na)^+$.

Scheme 10. Synthesis of oxazole lactocin S analogue (5)



The N-terminal oxazole 'dipeptide' derivative **14** was coupled to the Fmoc deprotected 3-37 lactocin S fragment using standard solid phase methodology. Quantitatively, a pre-activated solution of the oxazole (25 mg, 0.093 mmol), PyBOP (48 mg, 0.091 mmol), HOBt (13 mg, 0.093 mmol), and NMM (12.3 µL, 0.11 mmol) in DMF (10 mL) was added and bubbled with argon for 3 hours. After rinsing with DMF, a second 3 hour coupling was subsequently performed with a similarly pre-activated solution of the oxazole in DMF (10 mL). The peptide was then cleaved from the resin yielding crude peptide (5.0 mg) and was purified as described previously to give 0.33 mg of product (overall yield = 0.3%) as a fluffy white solid. Monoisotopic MW calculated for $C_{173}H_{263}N_{42}O_{46}S_3$ 3760.8694, found *high resolution* (FTICR-ESI-MS) 3760.8648 $(M+H)^+$.

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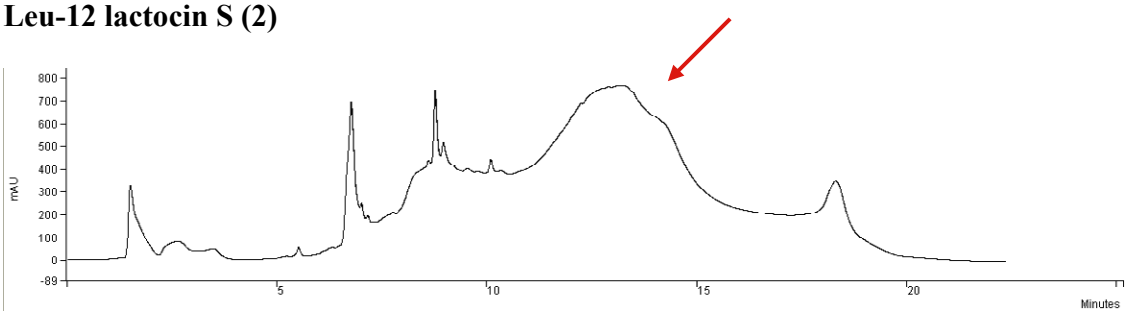
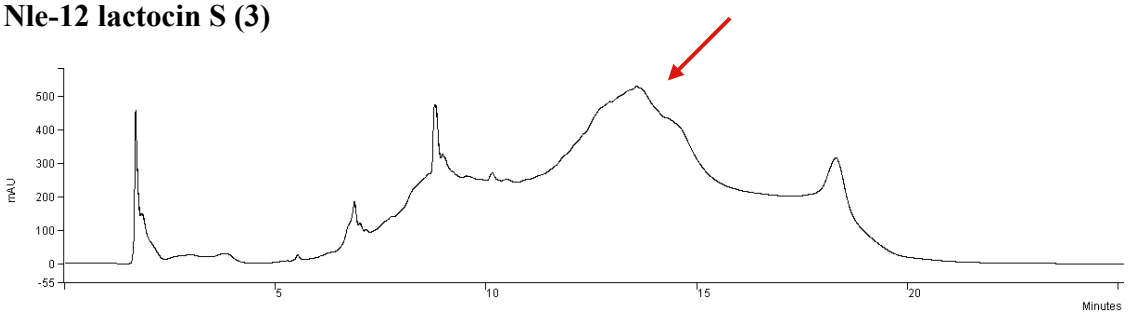
Crude HPLC purification traces of Lactocin S analogues (arrows indicate analogue elution peaks, see S11-S12 for HPLC method information)

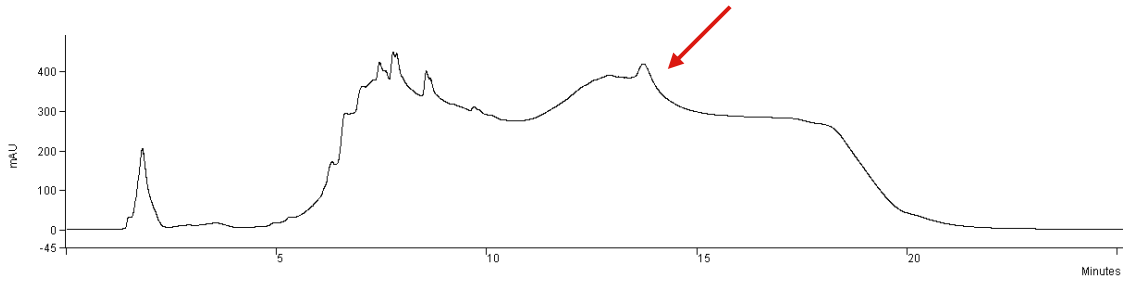
Leu-12 lactocin S (2)



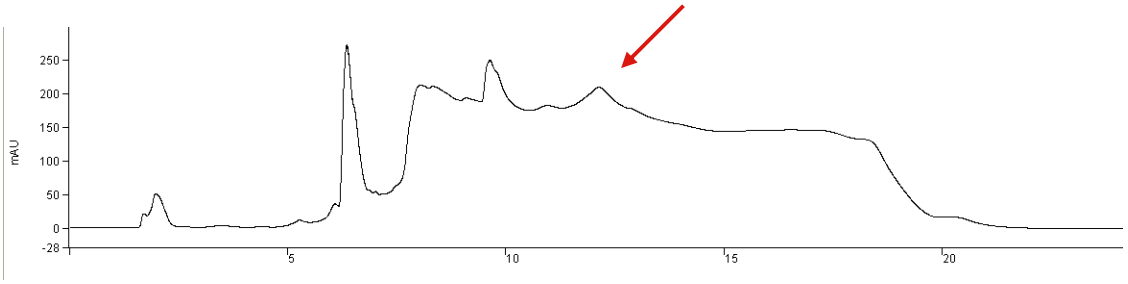
Nle-12 lactocin S (3)



NleDAP lactocin S (4)



Oxazole lactocin S (5)



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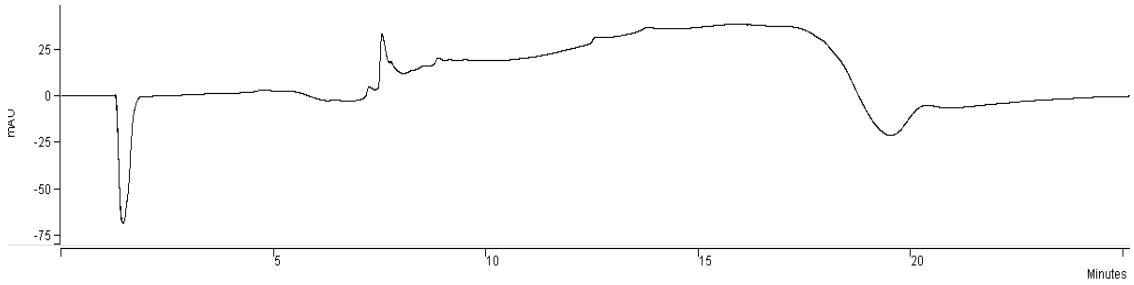
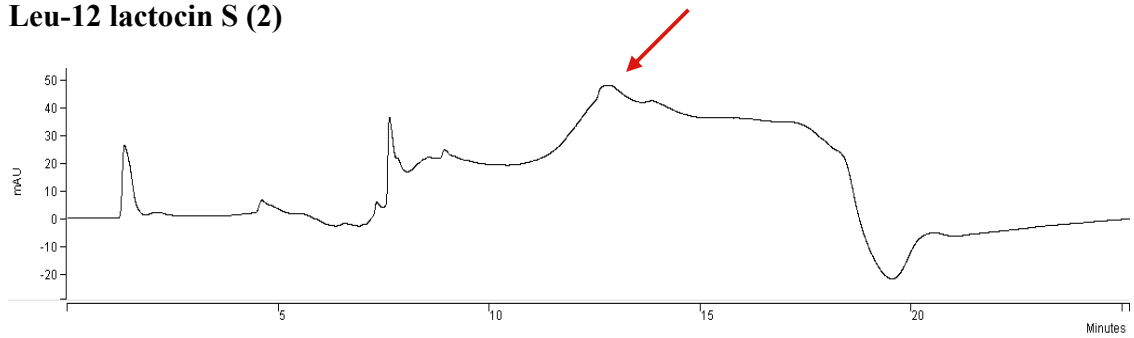
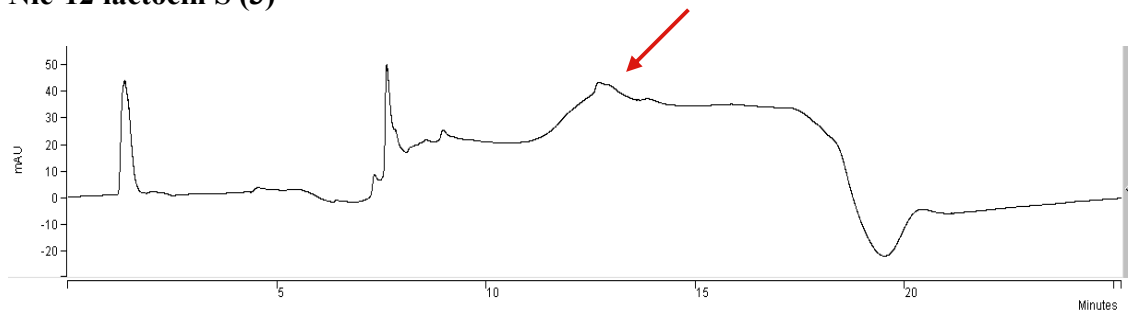
Reinjected HPLC traces of 100 µL of purified lactocin S analogue solutions (200 µM concentrations)
Milli-Q water injection (blank)



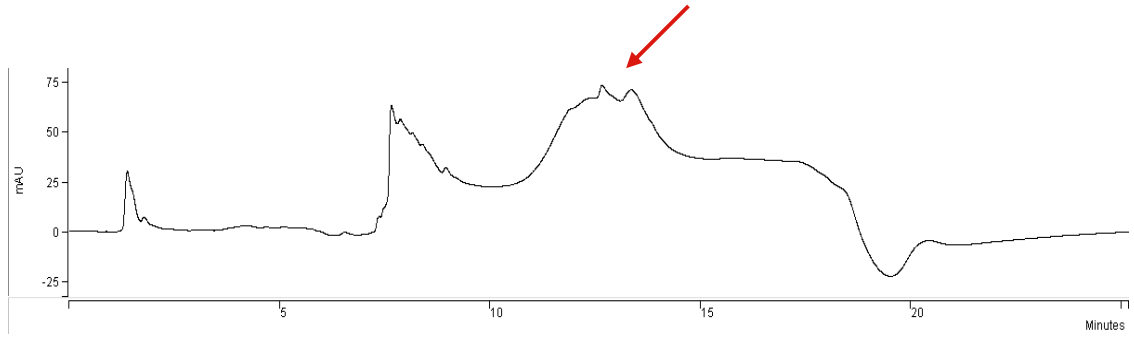
Leu-12 lactocin S (2)



Nle-12 lactocin S (3)



NleDAP lactocin S (4)



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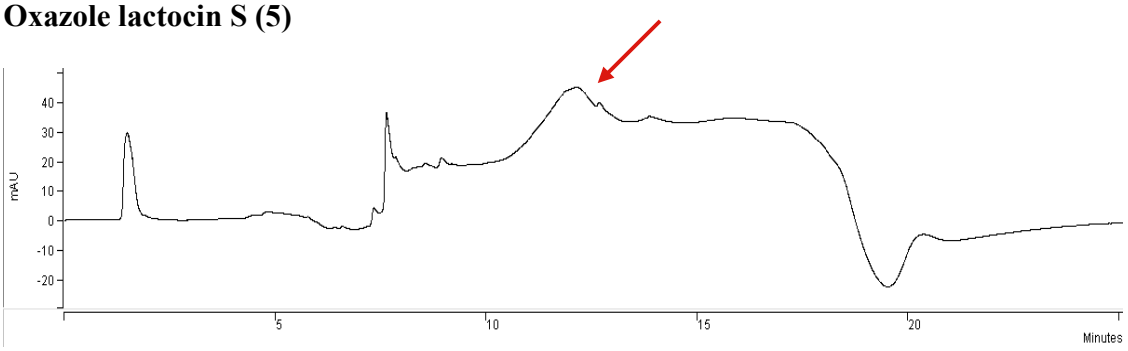
Oxazole lactocin S (5)



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Biological Testing

Spot-on-lawn Activity Monitoring

Anti-bacterial activity was assayed using spot-on-lawn activity assays. Plates containing 10 mL of MRS hard agar (1.5% agar, Difco) were used for spot-on-lawn assays. MRS soft agar overlays (0.75% agar) were prepared by adding 27.5 g/L MRS broth (Difco) with 35 g/L MRS agar (Difco). Lyophilized peptide samples were dissolved in 9:1 MQ-H_2O/MeOH (0.1% TFA) for activity testing. 10 µL aliquots of samples being analyzed for activity were spotted onto a hard MRS agar plate and permitted to dry. 10 mL of molten soft MRS agar was inoculated with 100 µL of the desired organism (1% inoculation) and poured over the hard agar plate and allowed to solidify. Plates were sealed with Parafilm and incubated overnight at the optimal temperature for the organism being examined (30 or 37 °C). Activity was measured as zones of inhibited growth.

Serial Dilution Assay

Solutions of peptide analogues were made in 9:1 MQ-H_2O/MeOH (0.1 % TFA) to yield initial concentrations of 200 µM. Serial dilutions were performed with 9:1 MQ-H_2O/MeOH (0.1 % TFA) to make 100 µM, 50 µM, 20 or 25 µM, 10 µM and 5 µM solutions. All solutions had 1.5 mg/L TCEP added to them to minimize oxidation of peptides due to atmospheric oxygen. Spot on lawn assays were used to test the biological activity of the diluted peptides as described above. Solutions of natural lactocin S and 9:1 MQ-H_2O/MeOH (0.1 % TFA with 1.5 mg/L TCEP added) were spotted on activity plates to serve as positive and negative controls respectively. Activity was measured as zones of inhibited growth.

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Table 1. Organisms used for activity testing

Indicator species	Strain	Growth medium	Growth temperature
Lactobacillus bulgaricus	11842	MRS	37 °C
Lactobacillus helveticus	18009	MRS	37 °C
Lactobacillus sakei (producer organism)	L45	MRS	30 °C
Pediococcus acidilactici	Pac 1.0	MRS	30 °C
Pediococcus pentosaceus	FBB63	MRS	37 °C

Figure 1. *Pediococcus acidilactici* Pac 1.0

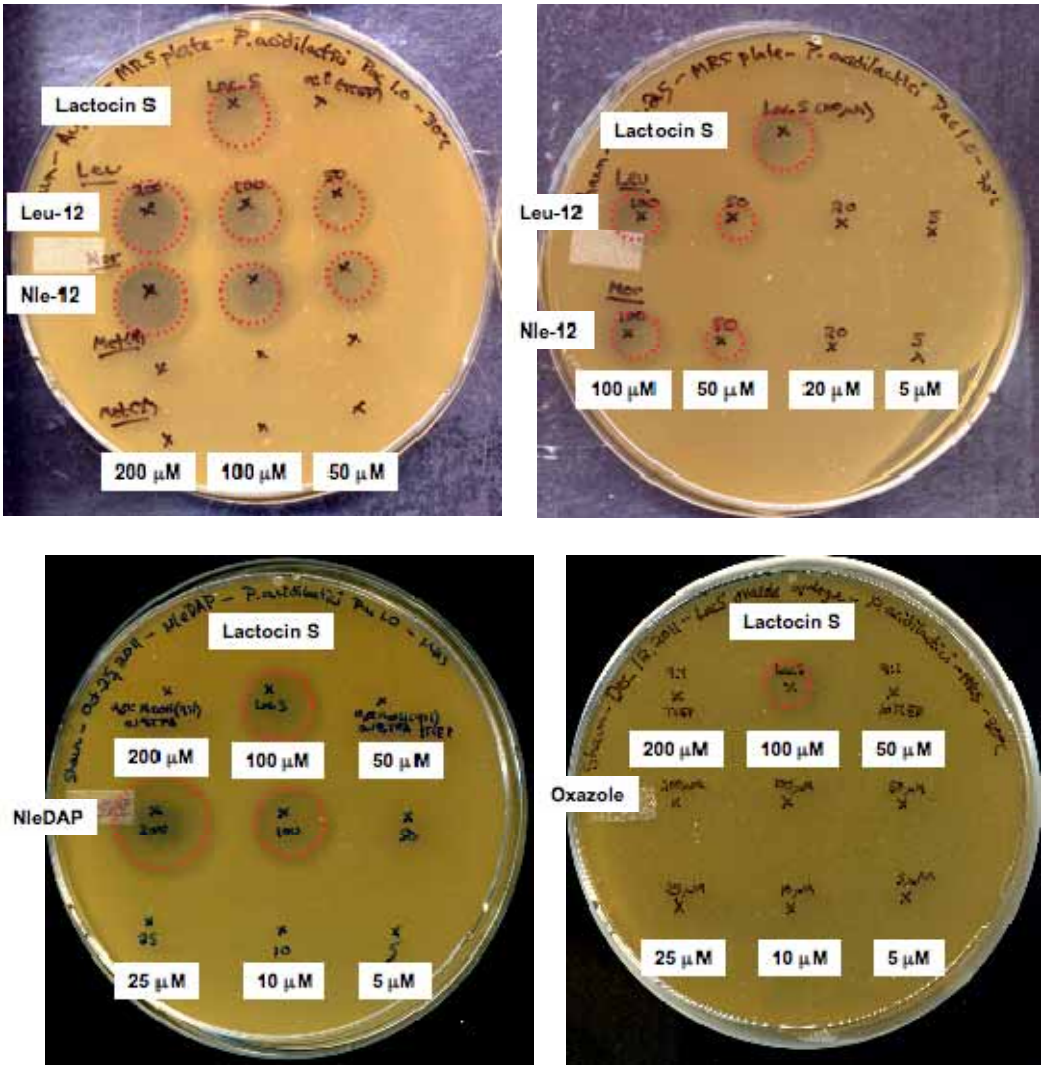

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Figure 2. ***Lactobacillus bulgaricus*** **11842**

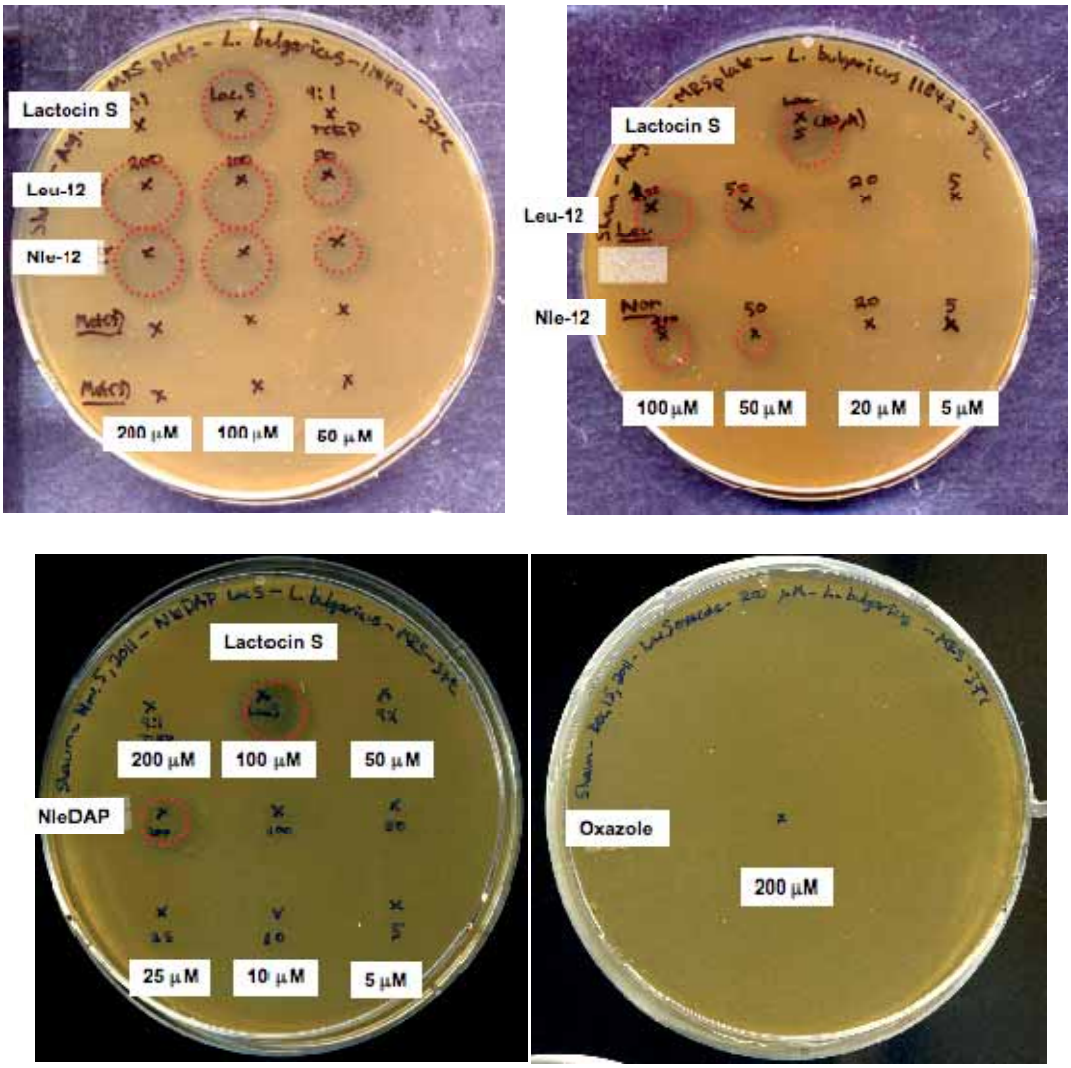

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Figure 3. ***Lactobacillus sakei*** **L45**

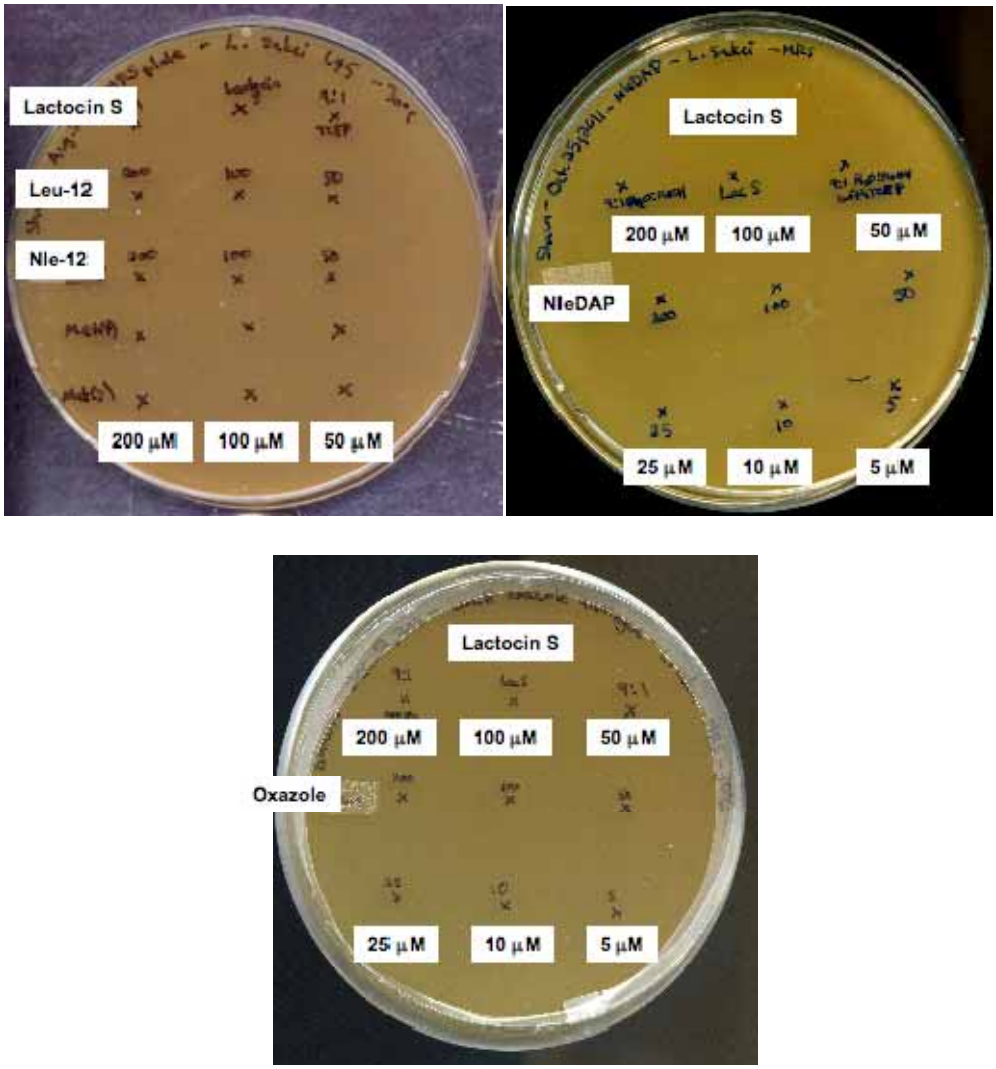

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Figure 4. ***Pediococcus pentosaceus*** **FBB63**

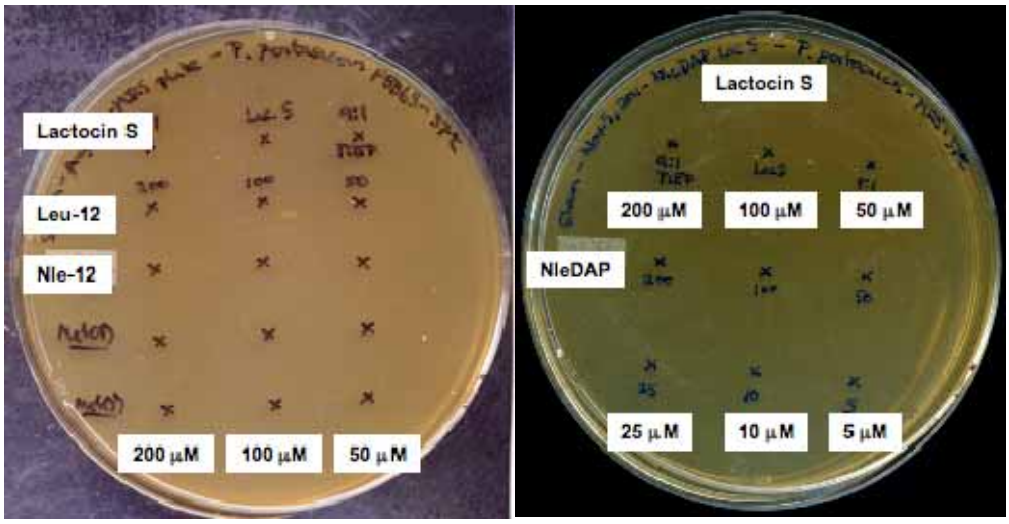
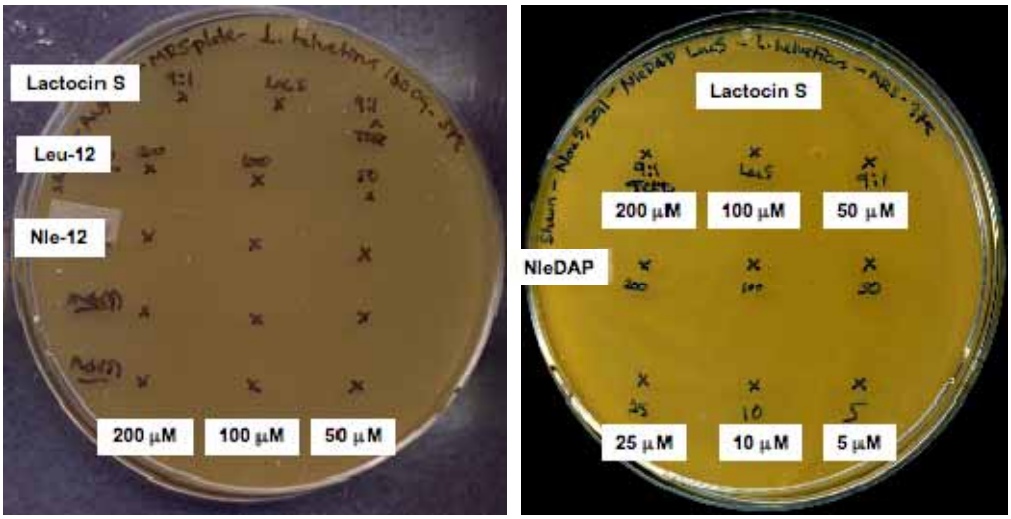


Figure 5. ***Lactobacillus helveticus*** **18009**



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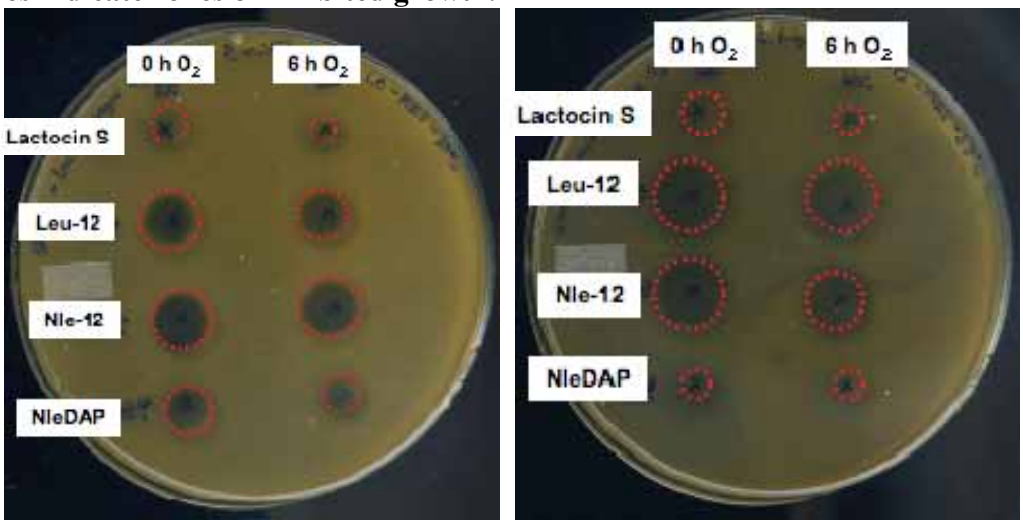
Oxidative Stability Assay of lactocin S analogues versus lactocin S

100 µM solutions (in 9:1 MQ-H_2O:MeOH with 0.1% TFA and 1.5 mg/L TCEP) of lactocin S and the peptide analogues Leu-12, Nle-12, NleDAP, and oxazole lactocin S were prepared and placed under a sealed atmosphere of oxygen for 6 hours at room temperature (the containers were sealed to prevent evaporation of solvent from artificially concentrating samples). The activity of peptide solutions was compared to solutions maintained under an atmosphere of argon (0 h O_2 exposure) by using spot on lawn assays against sensitive bacterial strains *P. acidilactici* Pac 1.0 and *L. bulgaricus* 11842 as described above.

Figure 6. Lactocin S and peptide analogues tested against *P. acidilactici* Pac 1.0 (left) and *L. bulgaricus* 11842 (right) before and after 6 h exposure to oxygen gas. Red circles indicate zones of inhibited growth.



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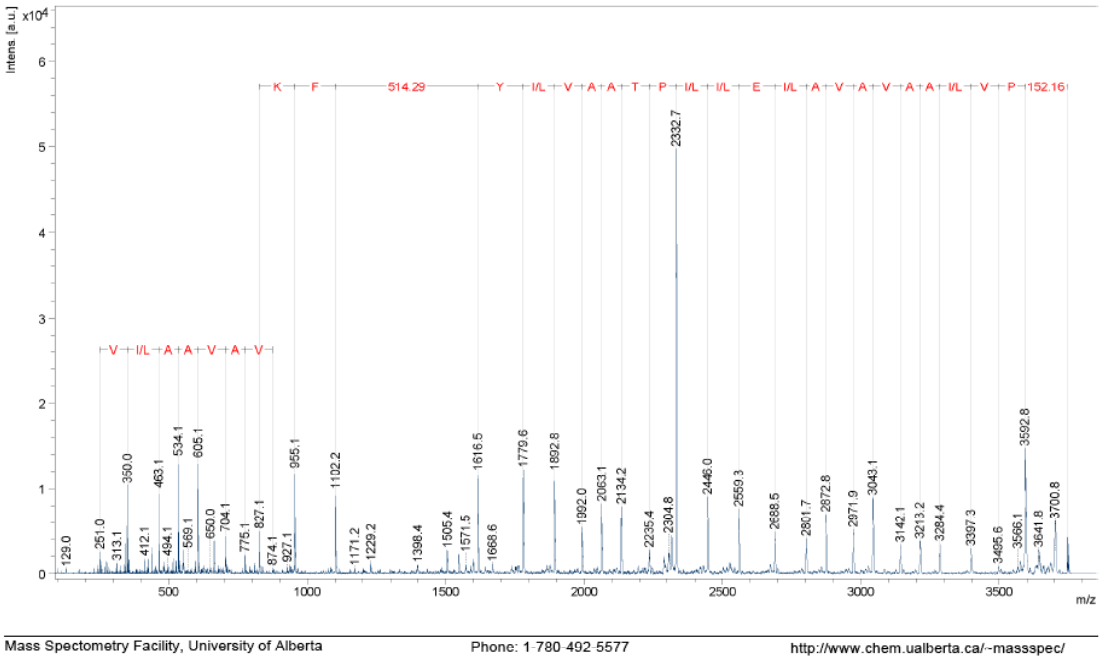
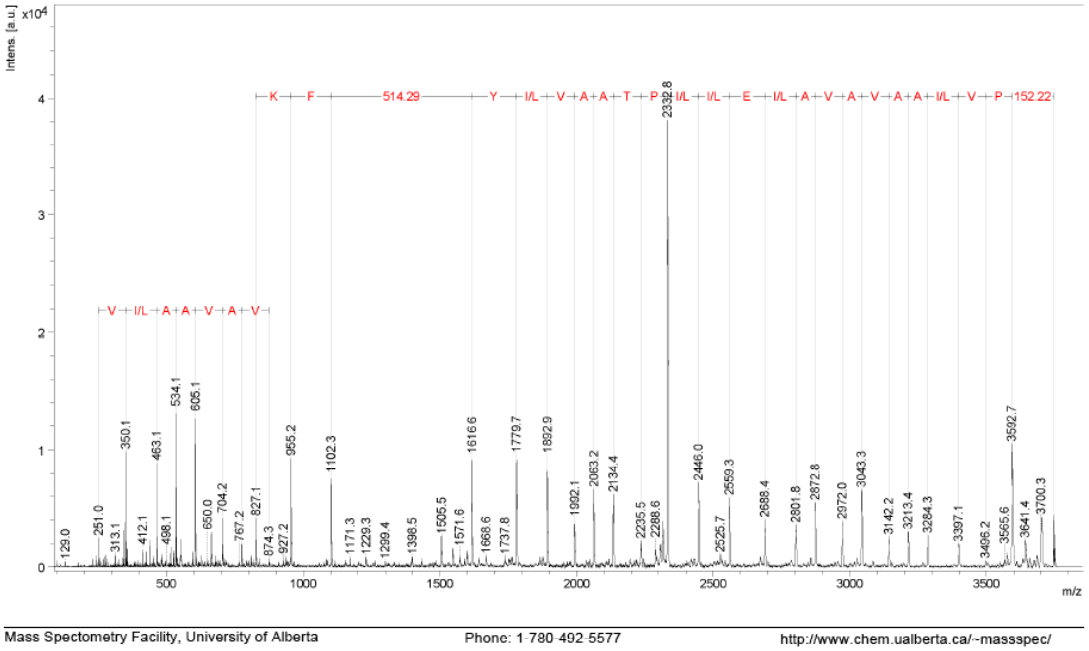
MS/MS Analysis

The sequences of the peptide analogues were confirmed by tandem MS/MS. Individual amino acid residues responsible for the changes in masses are noted in red above the spectra.

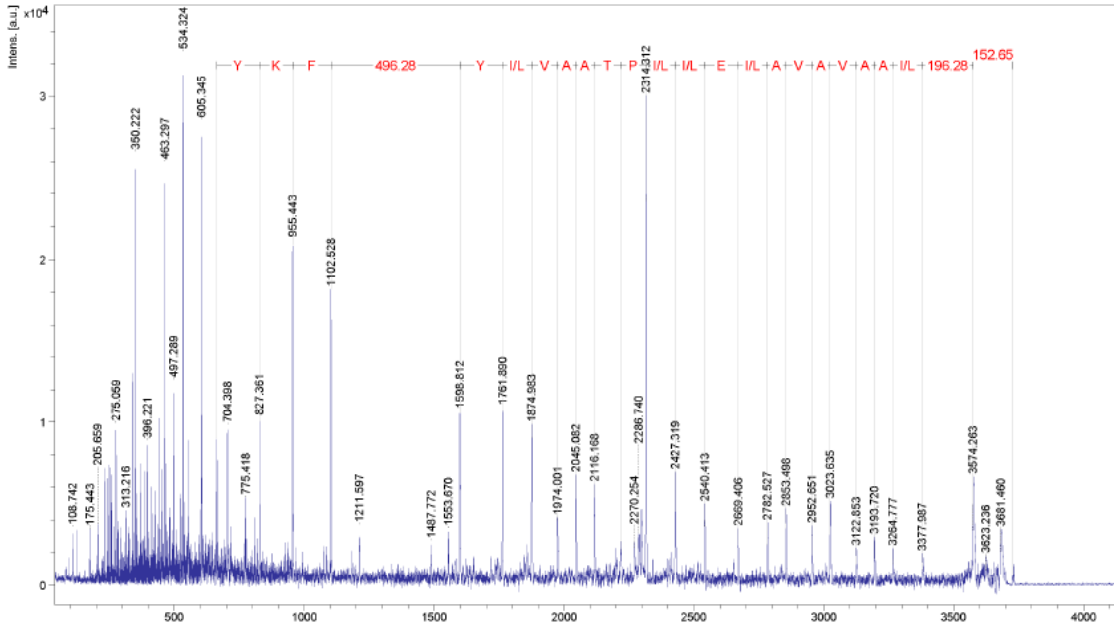
MS/MS Spectrum for Leu-12 lactocin S (2)



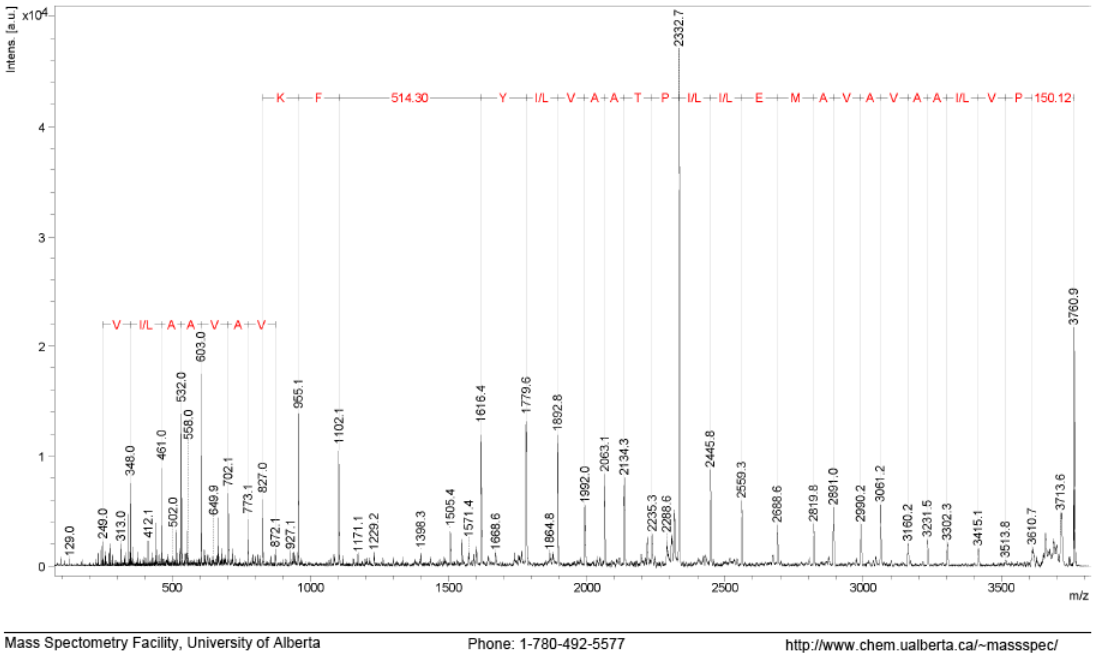
MS/MS Spectrum for Nle-12 lactocin S (3)



MS/MS spectrum for NleDAP lactocin S (4)



MS/MS spectrum for oxazole lactocin S (5)



High Resolution Mass Spectrometry Analysis of Leu-12 Lactocin S (2)

Mass Spectrometry Facility Department of Chemistry

Analysis Info

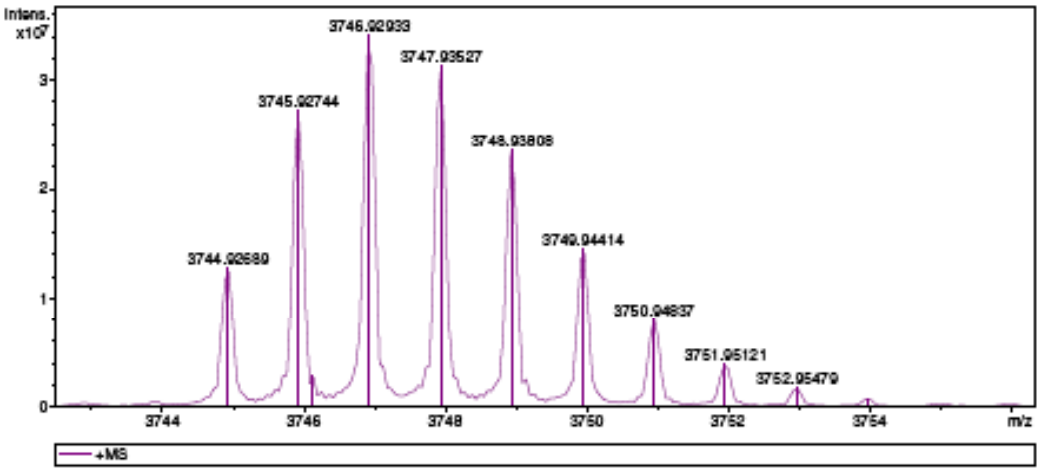
Analysis Name:	X:\Aug11\110830_0_D8_000002.d	Acquisition Date:	8/30/2011 4:17:45 PM
Method:	MALDI_HCCA_2k_new	Instrument:	apex-Qe
Sample Name:	MJL-61-Leu	Operator:	
Comment:	Shaun McKinnie, HCCA as matrix, 9.4T FTICR		

Acquisition Parameter

Ionisation Mode:	Positive	MALDI Mode:	n/a

Generate Molecular Formula Parameter

Formula, min.:	C173						
Formula, max.:	C175H300N43O46S3						
Measured m/z:	3744.93			Charge:	1	Tolerance:	2 ppm
Check Valence:	no	Min.:	0	Max.:	0		
Nitrogen Rule:	no						
Filter H/C Ratio:	no	Min.:	0	Max.:	3		
Estimate # of C:	yes					Electron Configuration:	even



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 174 H 267 N 42 O 46 S 2	0.104	3744.92806	0.31	0.26	1.17	62.50	ok	even

Bruker Daltonics DataAnalysis 3.4 printed: 1/16/2012 1:59:59 PM Page 1 of 1

High Resolution Mass Spectrometry Analysis of Nle-12 Lactocin S (3)

Mass Spectrometry Facility Department of Chemistry

Analysis Info

Analysis Name: X:\Aug11\110830_0_D13_000009.d
Method: MALDI_HCCA_2k_new
Sample Name: MJL-63 Nle Peak 2
Comment: Shaun McKinnie, HCCA as matrix, 9.4T FTICR

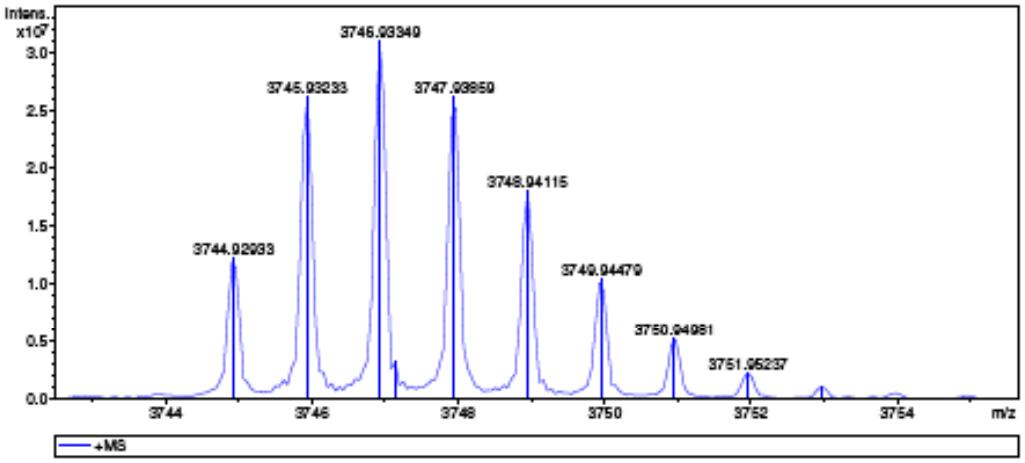
Acquisition Date: 8/30/2011 4:39:18 PM
Instrument: apex-Qe
Operator:

Acquisition Parameter

Ionisation Mode: Positive MALDI Mode: n/a

Generate Molecular Formula Parameter

Formula, min.: C174
Formula, max.: C175H300N43O46S3
Measured m/z: 3744.93 Charge: 1 Tolerance: 2 ppm
Check Valence: no Min.: 0 Max.: 0
Nitrogen Rule: no
Filter H/C Ratio: no Min.: 0 Max.: 3
Estimate # of C: yes Electron Configuration: even



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 174 H 267 N 42 O 46 S 2	0.042	3744.92806	-0.34	-0.60	-1.27	62.50	ok	even

Bruker Daltonics DataAnalysis 3.4 printed: 1/16/2012 1:58:32 PM Page 1 of 1

High Resolution Mass Spectrometry Analysis of NleDAP Lactocin S (4)

Mass Spectrometry Facility Department of Chemistry

Analysis Info

Analysis Name: X:\Oct11\111019_0_H21_000001.d
Method: MALDI_HCCA_2k_new
Sample Name: Shaun LacS NleDAP
Comment: Shaun McKinnie, HCCA as matrix, 9.4T FTICR

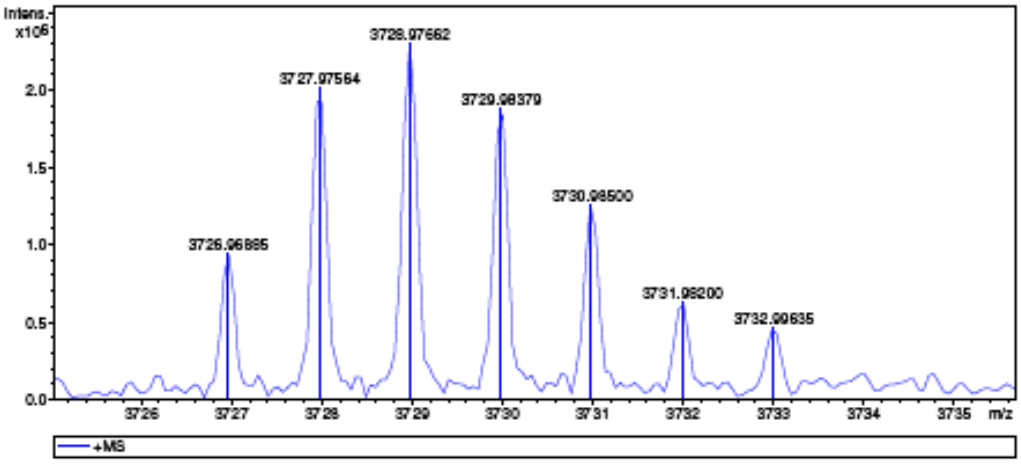
Acquisition Date: 10/19/2011 10:55:13 AM
Instrument: apex-Qe
Operator:

Acquisition Parameter

Ionisation Mode: Positive MALDI Mode: n/a

Generate Molecular Formula Parameter

Formula, min.: C174
Formula, max.: C175H300N42O46S
Measured m/z: 3726.97 Charge: 1 Tolerance: 2 ppm
Check Valence: no Min.: 0 Max.: 0
Nitrogen Rule: no
Filter H/C Ratio: no Min.: 0 Max.: 3
Estimate # of C: yes Electron Configuration: even



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 175 H 299 N 42 O 46 S 1	0.047	3726.97164	0.75	-0.35	2.79	62.50	ok	even

Bruker Daltonics DataAnalysis 3.4 printed: 1/16/2012 1:52:29 PM Page 1 of 1

High Resolution Mass Spectrometry Analysis of Oxazole Lactocin S (5)

Mass Spectrometry Facility Department of Chemistry

Analysis Info

Analysis Name: X:\Aug11\110830_0_D4_000001.d
Method: MALDI_HCCA_2k_new
Sample Name: Lac S oxazole
Comment: Shaun McKinnie, HCCA as matrix, 9.4T FTICR

Acquisition Date: 8/30/2011 4:06:13 PM
Instrument: apex-Qe
Operator:

Acquisition Parameter

Ionisation Mode: Positive MALDI Mode: n/a

Generate Molecular Formula Parameter

Formula, min.: C168
Formula, max.: C175H300N43O46S3
Measured m/z: 3760.86 Charge: 1 Tolerance: 2 ppm
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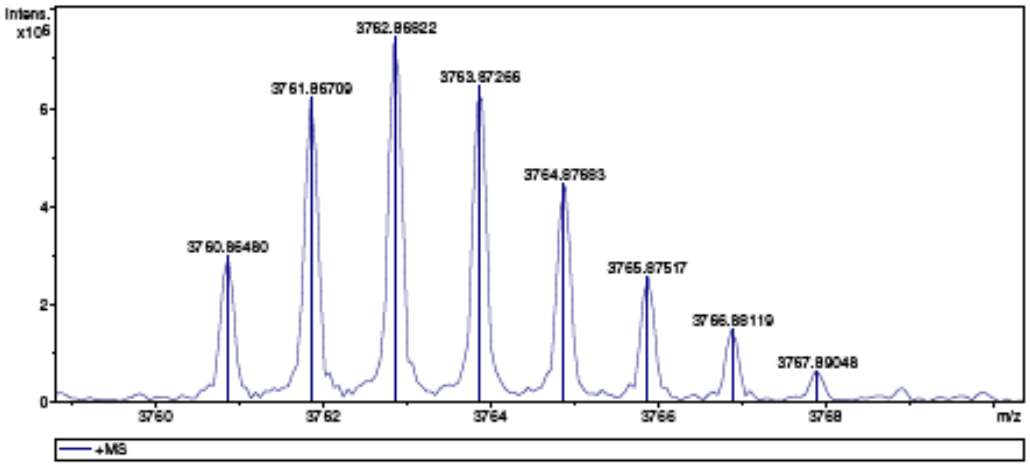
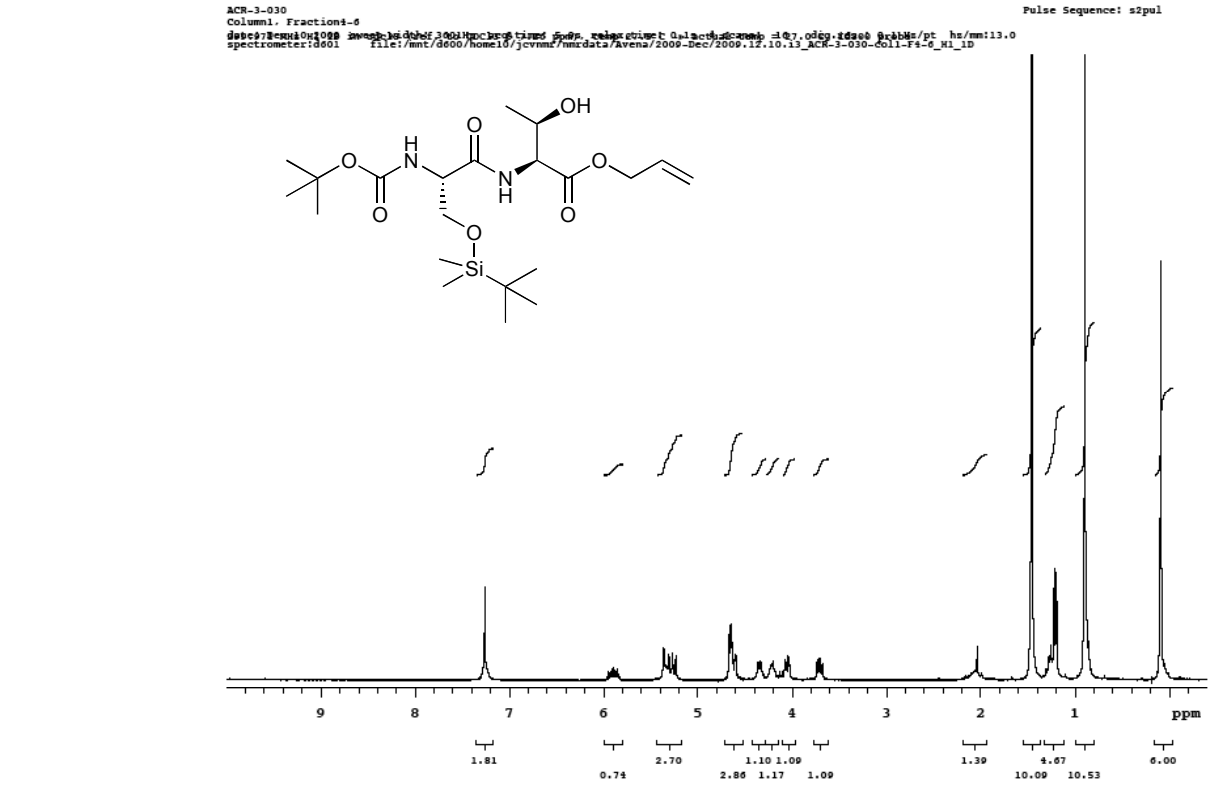
Nitrogen Rule: no
Filter H/C Ratio: no Min.: 0 Max.: 3
Estimate # of C: yes Electron Configuration: even

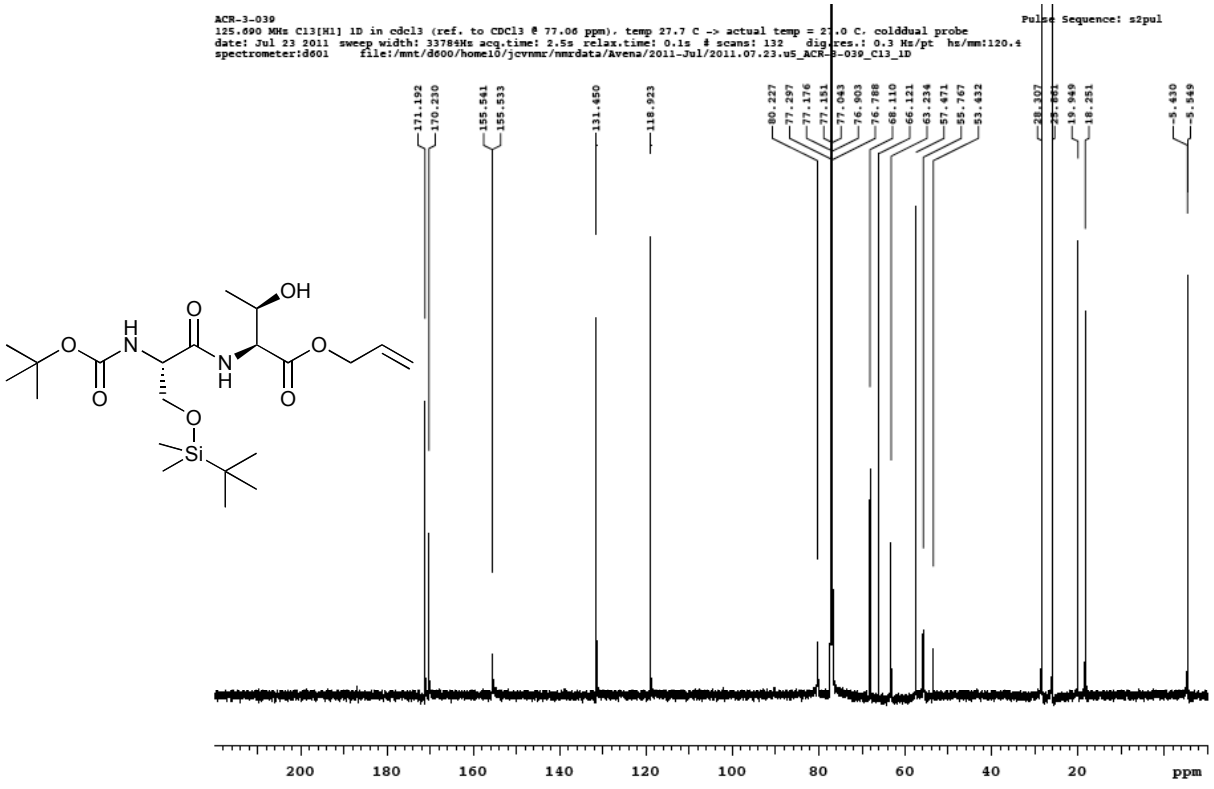


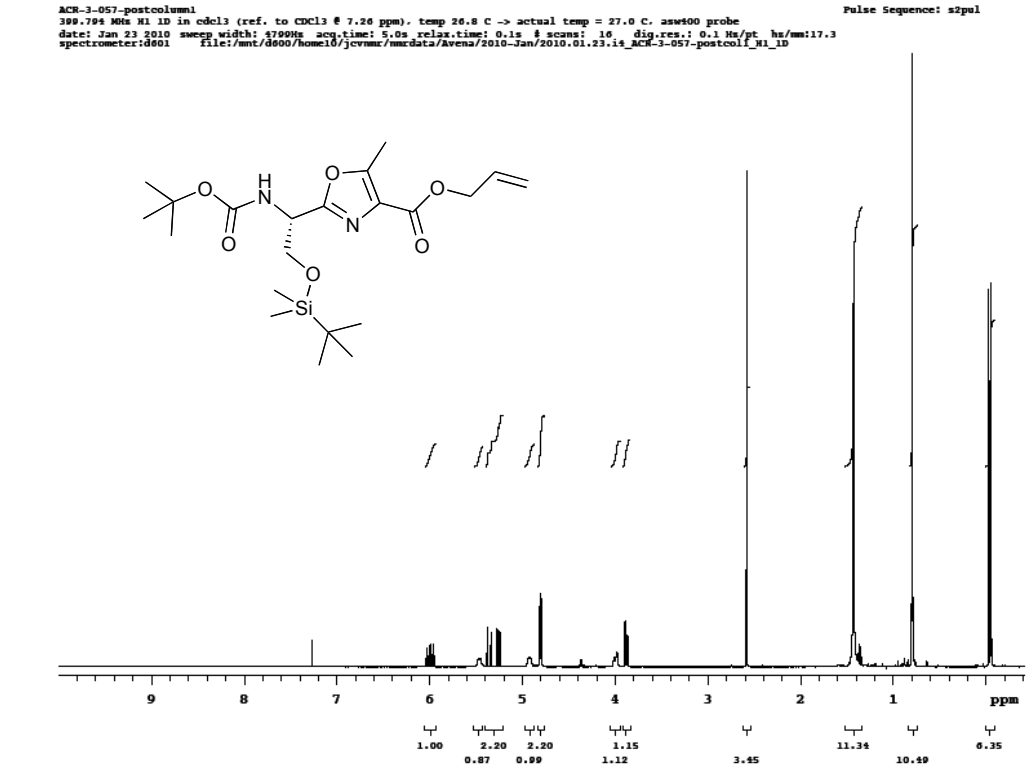
Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 173 H 263 N 42 O 46 S 3	0.098	3760.86883	1.07	0.97	4.02	63.50	ok	even

Bruker Daltonics DataAnalysis 3.4 printed: 1/16/2012 1:55:59 PM Page 1 of 1

^{1}H NMR and ^{13}C NMR Spectra of Intermediates



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Pulse Sequence: s2pul
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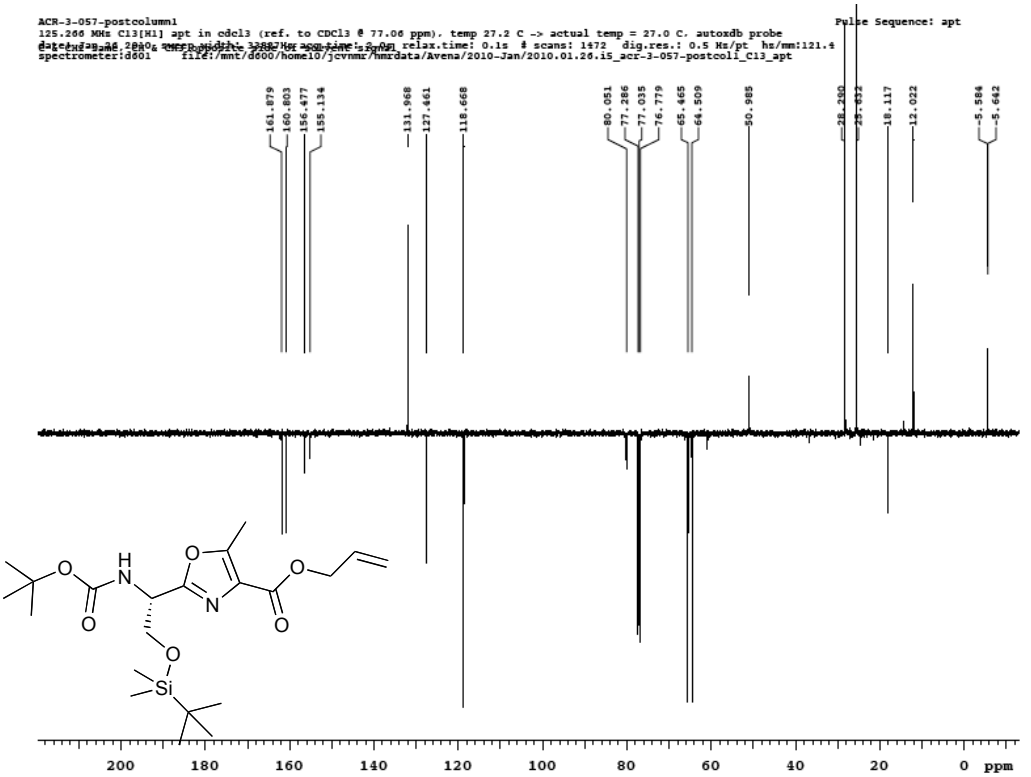
2.20
2.20
1.15
11.34
6.35
0.87
0.99
1.12
3.45
10.49


ACR-3-057-postcolumn1
125.266 MHz C13[H1] apt in cdcl3 (ref. to CDCl3 @ 77.06 ppm), temp 27.2 C -> actual temp = 27.0 C, autoxdb probe
Pulse Sequence: apt
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160.803
156.477
155.134
131.968
127.461
118.668
80.051
77.286
77.035
76.779
65.465
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28.452
18.117
12.022
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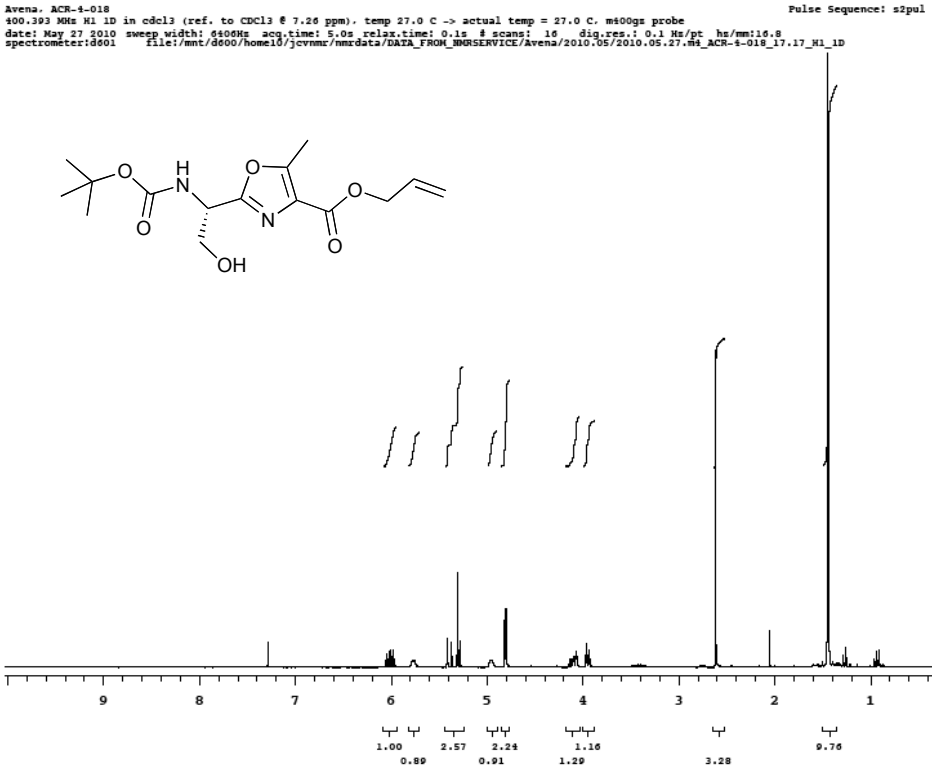
-5.642
ppm


Avena, ACR-4-018
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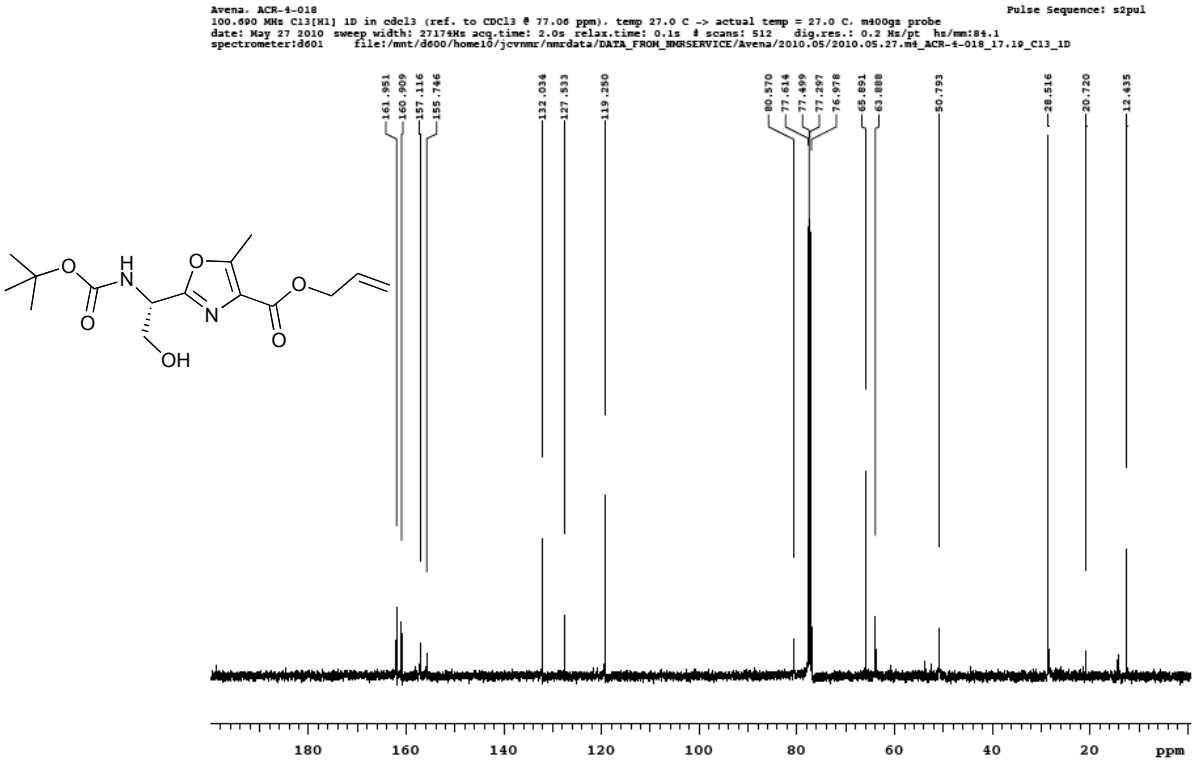
date: May 27 2010 sweep width: 6406Hz acq.time: 5.0s relax.time: 0.1s # scans: 16 dig.res.: 0.1 Hz/pt hs/mm:16.8
spectrometer:d601 file:/mnt/d600/home10/jcvnmr/nmrdata/DATA_FROM_NMRSERVICE/Avena/2010.05/2010.05.27.m4_ACR-4-018_17.17_H1_1D
O
H
N
O
O
O
O
N
OH
9
8
7
6
5
4
3
2
1
ppm
1.00
0.89
2.57
2.24
0.91
1.16
1.29
3.28
9.76


Avena, ACR-4-018
Pulse Sequence: s2pul
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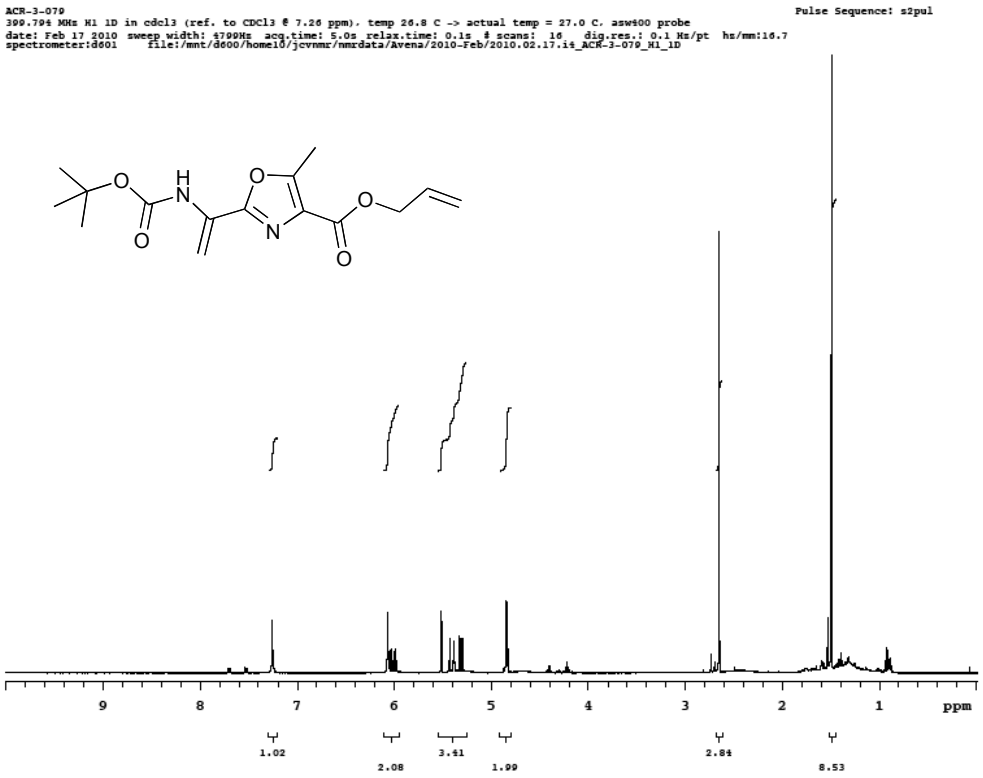
date: May 27 2010 sweep width: 27174Hz acq.time: 2.0s relax.time: 0.1s # scans: 512 dig.res.: 0.2 Hz/pt hs/mm:84.1
spectrometer:d601 file:/mnt/d600/home10/jcvnmr/nmrdata/DATA_FROM_NMRSERVICE/Avena/2010.05/2010.05.27.m4_ACR-4-018_17.19_C13_1D
161.951
160.909
157.116
155.746
132.034
127.533
119.250
80.570
77.614
77.499
77.297
76.978
65.891
63.888
50.793
28.516
20.720
12.435
O
H
N
O
O
O
O
N
OH
180
160
140
120
100
80
60
40
20
ppm


ACR-3-079
Pulse Sequence: s2pul
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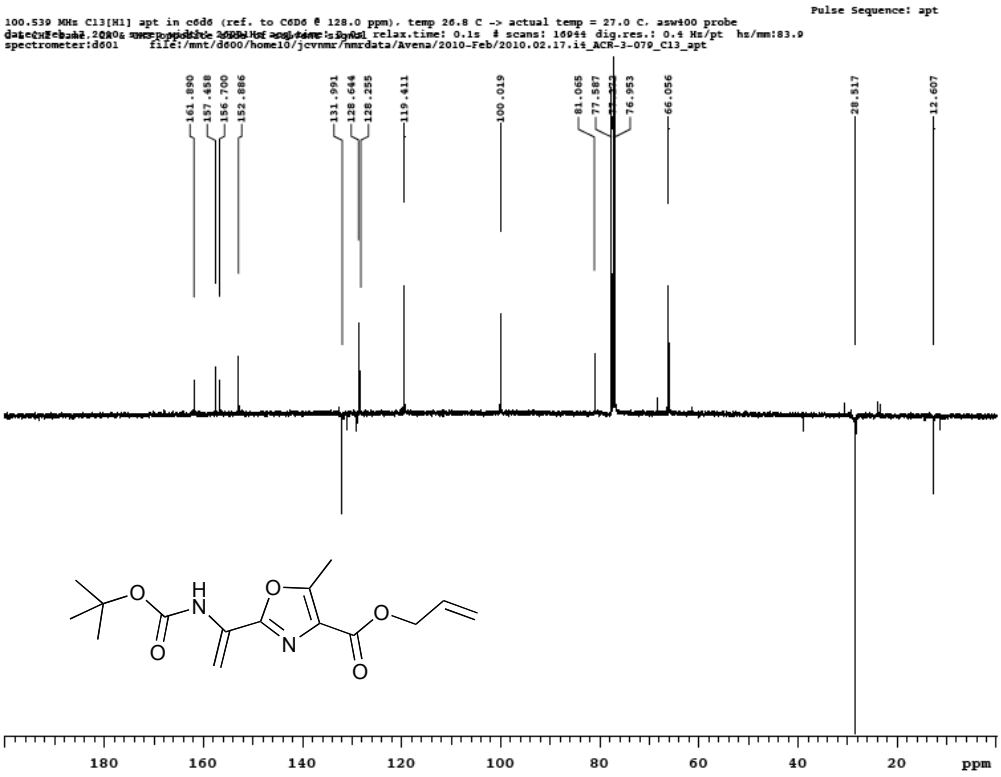
spectrometer:d601 file:/mnt/d600/home10/jcvnmr/nmrdata/Avena/2010-Feb/2010.02.17.i4_ACR-3-079_H1_1D
ppm
1.02
2.08
3.41
1.99
2.84
8.53


Pulse Sequence: apt
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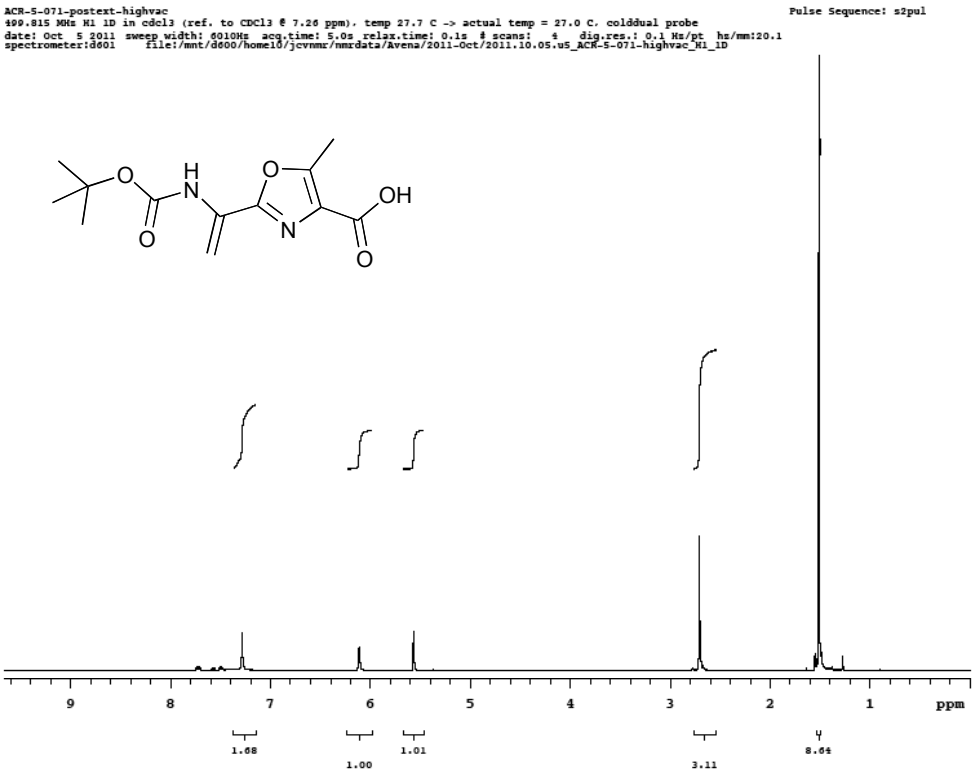
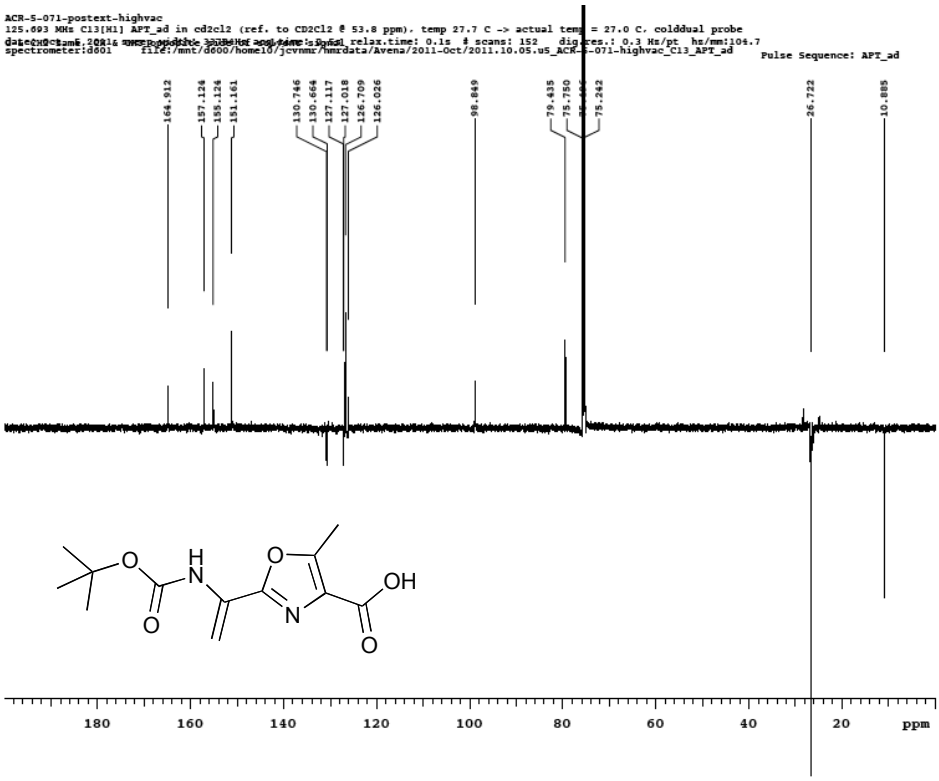
spectrometer:d601 file:/mnt/d600/home10/jcvnmr/nmrdata/Avena/2010-Feb/2010.02.17.i4_ACR-3-079_C13_apt
161.890
157.458
156.700
152.886
131.991
128.644
128.255
119.411
100.019
81.065
77.587
76.953
66.056
28.517
12.607
ppm


ACR-5-071-postext-highvac
Pulse Sequence: s2pul
499.815 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 27.7 C -> actual temp = 27.0 C, colddual probe
date: Oct 5 2011 sweep width: 6010Hz acq.time: 5.0s relax.time: 0.1s # scans: 4 dig.res.: 0.1 Hz/pt hz/mm:20.1
spectrometer:d601 file:/mnt/d600/home10/jcvnmr/nmrdata/Avena/2011-Oct/2011.10.05.us_ACR-5-071-highvac_H1_1D
O
H
N
O
O
N
OH
O
9
8
7
6
5
4
3
2
1
ppm
1.68
1.00
1.01
3.11
8.64


ACR-5-071-postext-highvac
125.693 MHz C13[H1] APT_ad in cd2cl2 (ref. to CD2Cl2 @ 53.8 ppm), temp 27.7 C -> actual temp = 27.0 C, colddual probe
spectrometer:d601 file:/mnt/d600/home10/jcvnmr/nmrdata/Avena/2011-Oct/2011.10.05.us_ACR-5-071-highvac_C13_APT_ad
Pulse Sequence: APT_ad
164.912
157.124
155.124
151.161
130.746
130.664
127.117
127.018
126.709
126.026
98.849
79.435
79.750
75.242
26.722
10.885
O
H
N
O
O
N
OH
O
180
160
140
120
100
80
60
40
20
ppm

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