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Total Synthesis and Initial Structure–Activity Relationships of Longicatenamycin A

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Dedicated to Professor Wolfgang Steglich on the occasion of his 75th birthday

Natural products have provided the majority of lead structures for marketed antibacterials. In addition, they are biological guide principles to new therapies. Nevertheless, numerous "old" classes of antibiotics such as the longicatenamycins have never been explored by chemical postevolution. Longicatenamycin A is the first defined longicatenamycin congener that has been totally synthesized and tested in pure form. This venture required the de novo syntheses of the non-proteinogenic amino acids (2S,3R)-β-hydroxyglutamic acid (HyGlu), 5-chloro-D-tryptophan (D-ClTrp), and (S)-2-amino-6-methylheptanoic acid (hhLeu). In the key step, the sensitive HyGlu building block was coupled as a pentafluorophenyl active ester to the unprotected H-D-ClTrp-Glu-hhLeu-D-Val-D-(Cbz)Orn-OH fragment. This first total synthesis of longicatenamycin A provided new congeners of the natural product (deacetyllongicatenamycin, dechlorolongicatenamycin, and longicatenamycin-A-amide).

Introduction

In the course of the coevolution of antibacterial metabolites from microorganisms and their bacterial competitors, nature has developed efficient strategies to control bacterial growth.[1] Today, the emergence of bacterial resistance to current antibiotics is recognized as a major challenge. New antibacterial drugs with new modes of action are urgently needed to ensure future therapeutic efficacy (*bad bugs, no drugs*).[2] In this critical situation, it seems wise to search for novel natural products[3] as well as to seriously re-explore known antibacterial structures. Both ways can induce new and valid therapies. Many known antibiotics have never profoundly been assessed by core variations in terms of *chemical postevolution*.[1b] Therefore, known non-ribosomal peptides and cyclopeptides have been a rich source for our medicinal chemistry programs during the past years.[4–6]

By a systematic analysis of published databases we "re-discovered" the unappreciated longicatenamycins (S-520 antibiotic complex). These non-ribosomal peptides were isolated from *Streptomyces diastaticus* by Shoji and Sakazaki in 1970 (Figure 1).[7] They showed that "longicatenamycin" was a mixture of various peptidic congeners (S-520 antibiotic complex, **1**). In a subsequent Edman degradation study, Shiba et al. proposed the hexacyclopeptide **1a** as the major component of the "longicatenamycin" mixture **1**.[8] However, the isolation and spectroscopic characterization of defined longicatenamycin congeners had never been undertaken. It remained unclear which component from the peptide mixture **1** was ultimately responsible for the reported in vitro *and* in vivo activity against Gram-positive bacteria.

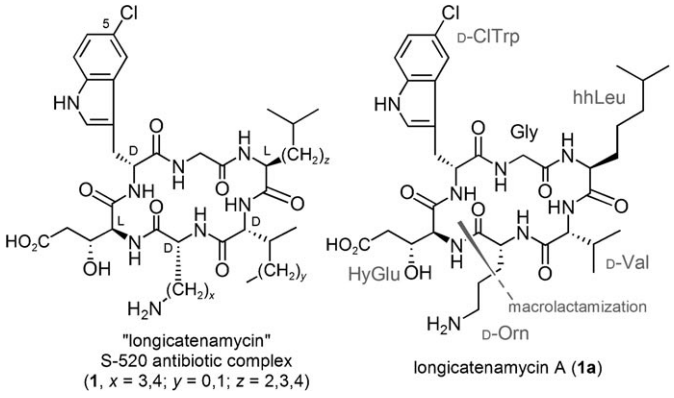

Figure 1. The S-520 "longicatenamycin" antibiotic complex from *Streptomyces diastaticus* contains various intriguing non-proteinogenic amino acids: D-ClTrp = 5-chloro-D-tryptophan; HyGlu = (2*S*,3*R*)-β-hydroxyglutamate; hhLeu = (*S*)-2-amino-6-methylheptanoic acid ("*homohomo*leucine").

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Results and Discussion

Which is the active component from "longicatenamycin"?

With the help of a flexible and efficient synthesis we wanted to identify the active chemical entity from the S-520 complex **1**. A first de novo synthesis of pure longicatenamycin congeners should demonstrate the swift chemical feasibility in this modular class of natural products and should facilitate structural variations and initial structure–activity relationships (SAR). Undoubtedly, the in vitro potency of "longicatenamycin" **1** was only moderate. However, the in vivo efficacy reported in a mouse sepsis model attracted our attention and was seen as a valid foundation for starting a project. We selected structure **1a** ("longicatenamycin A") as initial synthetic target.

Molecular modeling studies

The simulated molecular structure of longicatenamycin A **1a** adopts a stable "bicyclic" conformation (Figure 2). Computational studies suggested a direct "inner-ring" hydrogen bond between D-Orn and Gly and an "outer-ring" salt bridge between the protonated amino group of D-Orn and the carboxylate of HyGlu that contributes 71 kJ mol^{-1} to the stabilization of the ring conformation.

Synthetic strategy

For tackling target structure **1a**, we chose a "classical" linear liquid-phase synthesis. This assessment was based on the predominance of non-proteinogenic amino acids, Gly being the sole proteinogenic amino acid in the cyclic hexapeptide complex **1** (Figure 1). Moreover, natural **1** contains the unusual and acid-sensitive 5-chloro-D-tryptophan (D-ClTrp) as well as a set of lipophilic "homoleucines", such as hhLeu.[8] Clearly, β-hydroxyglutamic acid (HyGlu)[9] is the structural hot spot in all longicatenamycins. Although β-hydroxy-α-amino acids represent a common structural element in peptide antibiotics, and the closely related β-hydroxyglutamine (HyGln)[10] has been found in non-ribosomal peptides and depsipeptides, to our knowledge, the corresponding acid HyGlu has not been detected in other peptide antibiotics. The challenging structure of this tetrafunctional amino acid could explain why no synthetic efforts toward the longicatenamycins have been reported so far. In the area of non-ribosomal peptides, the synthetic availability[11] of a single complex amino acid building block can be decisive for the success and timelines of the whole project.

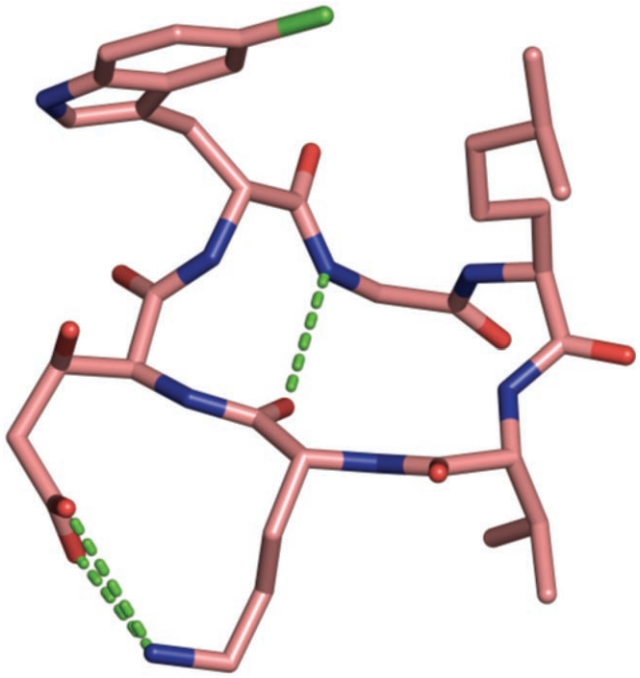


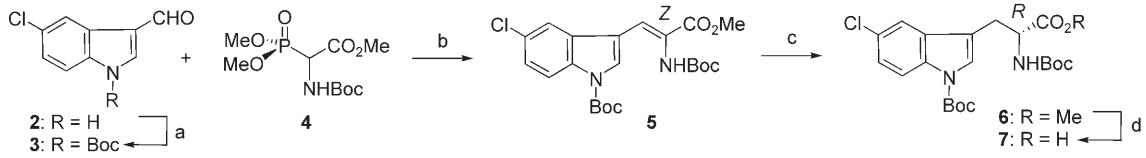
Figure 2. Simulated molecular structure of longicatenamycin A (**1a**). A hydrogen bond between D-Orn and Gly and a salt bridge between D-Orn and HyGlu stabilize the ring conformation. Hydrogen atoms have been removed. D-Orn = D-ornithine.

Although Gly is usually a preferred cyclization site in cyclopeptide synthesis (low steric hindrance, no racemization), we planned to close the ring amide bond between HyGlu and D-Orn. Here, encouraged by our modeling studies, we expected a prominent hydrogen bond to favor conformational preorganization and ring closure (Figure 2). Furthermore, the valuable building blocks D-ClTrp and HyGlu should preferentially be introduced toward the end of the synthesis. Their sensitive functional groups would eventually interfere with standard peptide chemistry protocols and cause an attrition in coupling yields, especially when present early in the synthetic sequence. Most problematic were the easily reducible chloroindole of D-ClTrp and the ω-ester of HyGlu, that turned out to be significantly destabilized by the adjacent nucleophilic β-alcohol, giving rise to unwanted saponification events (vide infra).

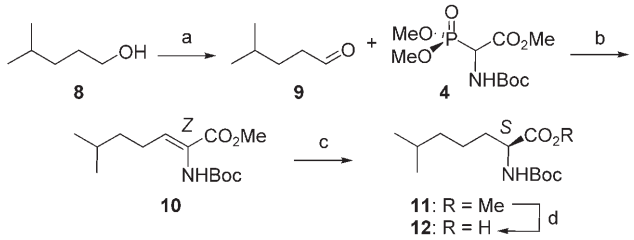
Synthesis of the non-proteinogenic amino acid precursors

For the *N*-Boc protected D-ClTrp[12] and hhLeu[13] derivatives **7** and **12** we employed U. Schmidt's phosphonoglycine chemistry (Scheme 1 and 2).[14] In both cases, good overall yields and excellent enantiomeric excesses were achieved. Notably, the catalytic reduction of **5** did not affect the 5-chloro substituent (Scheme 1).

Obtaining a suitable HyGlu module by enantioselective synthesis was a challenging task: its ω-carboxylic acid function was situated in critical proximity to the α-amine. Moreover, the β-alcohol and the α-carboxylic acid gave rise to various side reactions, causing the undesired formation of γ-lactams, β-lactones, and anhydrides. Although various strategies for the synthesis of enantioselective *threo*-β-hydroxy-α-amino acids, such as asymmetric aminohydroxylations,[16] elaboration of serine aldehydes[17] and ketones,[18] and additions of bislactim ether titanium enolates,[19] aziridines,[20] or glycine anion equivalents to aldehydes[21] have been very successful in other cases, none of these methods appeared to be ideal for a large-scale synthesis of orthogonally protected HyGlu. In particular, the question how to regioselectively differentiate between the α- and ω-carboxylate functions impeded the use of established methodolo-



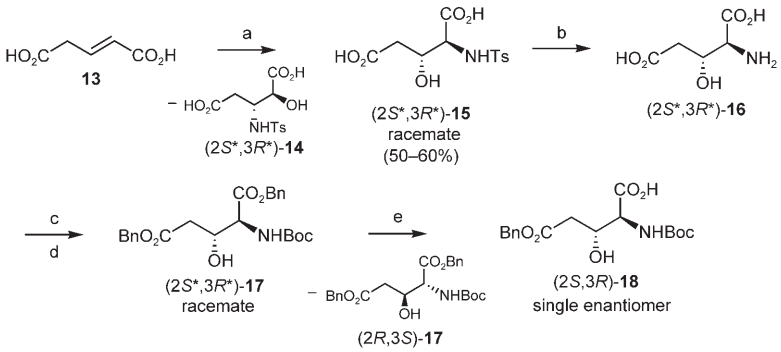
Scheme 1. Synthesis of D-ClTrp. Reagents and conditions: a) Boc_2O (1.1 equiv), DMAP (0.1 equiv), CH_2Cl_2, 1.5 h, 97%; b) **4** (1.3 equiv), TMG (1.3 equiv), THF, −78 °C→RT, 12 h, 79% (crude quant.); c) H_2 (100 kPa), (*R*,*R*)-Et-DuPhos-Rh (0.75 mol%),[24] EtOH, 4 days, quant., 96.7% *ee*; d) LiOH (1.3 equiv), H_2O/THF 2:1, 2 h, 0 °C, quant. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, (*R*,*R*)-Et-DuPhos-Rh = (+)-1,2-bis((2*R*,5*R*)-2,5-diethylphospholano)benzene(cyclooctadiene) rhodium(I)trifluoromethanesulfonate, RT = room temperature, THF = tetrahydrofuran, TMG = 1,1,3,3-tetramethylguanidine.



Scheme 2. Synthesis of hhLeu. Reagents and conditions: a) PCC (1.5 equiv), CH_2Cl_2, 12 h, 40%;[15] b) KO*t*Bu, CH_2Cl_2, −60 °C, 2 h, quant.; c) H_2 (100 kPa), (*S*,*S*)-Et-DuPhos-Rh (1.5 mol%),[24] EtOH, 4 d, 99%, 97.8% *ee*; d) LiOH (1.5 equiv), H_2O/THF 1:2, 0 °C, 1.5 h, quant. (*S*,*S*)-Et-DuPhos-Rh = (+)-1,2-bis-((2*S*,5*S*)-2,5-diethylphospholano)benzene(cyclooctadiene) rhodium(I)trifluoromethanesulfonate, PCC = pyridinium chlorochromate.

gies. Bioorganic considerations in mind, we chose an exclusively ω-protected HyGlu building block **18**. We figured that owing to the planned late-stage introduction of **18**, its free hydroxy function would be tolerated by the requisite reaction conditions.

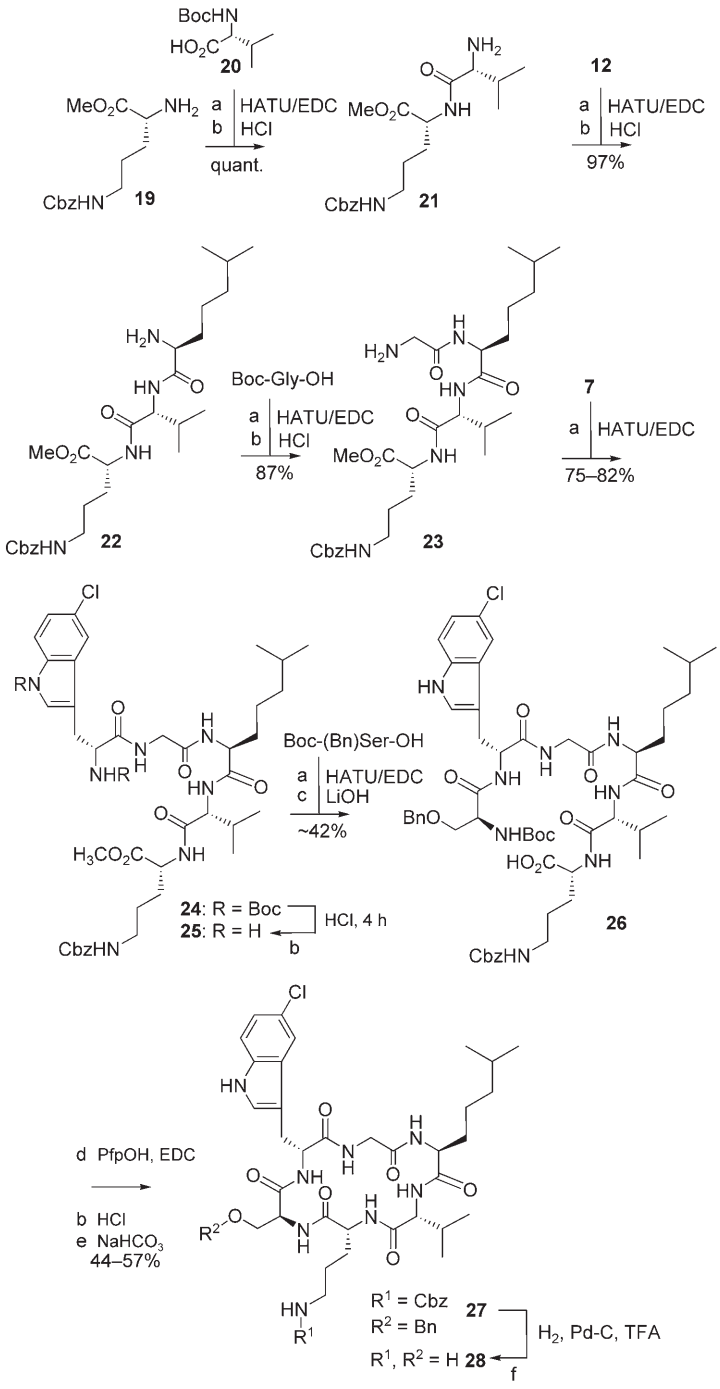
The straightforward and scalable synthesis of the HyGlu key intermediate **18** (Scheme 3) started with an anion-accelerated aminohydroxylation of glutaconic acid (**13**), according to Sharpless.[22] The resulting regioisomers **14** and **15** could be separated in multigram-scale by crystallization from ethyl acetate. Regiochemistry and relative stereochemistry of **15** were unambiguously assigned by an X-ray crystal structure as well as by multidimensional NMR studies (Supporting Information). Attempts to free the amine from sulfonamide **15** by hydrolysis under acidic or basic conditions notoriously yielded complex mixtures. The alcohol function was prone to water elimination under various conditions (e.g. HBr, AcOH or HBr, H_2O, NaOH). However, reductive cleavage of **15** proceeded smoothly with sodium in liquid ammonia to afford polar and highly soluble HyGlu **16**, the purification of which required nonstandard procedures: gel chromatography with Sephadex LH-20 followed by direct *N*-Boc protection and double Steglich esterification[23] yielded dibenzyl ester **17**. The subsequent regioselective cleavage of the α-carboxylic ester again was highly problematic and not possible under smooth saponification conditions (copper ions, cyclic boronic esters). The free β-hydroxy group seemed to severely decrease the stability of the ω-carboxylic ester. Ultimately, regioselective cleavage was achieved enzymatically with subtilisin at pH 8 under semi-denaturing conditions. The presence of DMSO (4%) had a crucial influence on the reaction. Without DMSO no conversion was observed within hours. Subtilisin Carlsberg selectively cleaved the "correct" stereoisomer, yielding the enantiopure monoacid (2*S*,3*R*)-**18** in one step from the racemic diester (2*S**,3*R**)-**17**. The absolute configuration of the "leftover" (2*R*,3*S*)-**17** was determined with X-ray crystallography (Supporting Information).



Scheme 3. Bioorganic synthesis of (2*S*,3*R*)-Boc-β-hydroxy-ω-benzyl-Glu-OH (**18**). Reagents and conditions: a) 1. chloramine T·3 H_2O, $NaHCO_3$ (2.2 equiv), cat. $K_2[OsO_4(OH)_2]$ (0.007 equiv), 12 h; 2. Na_2SO_3, H_2O, 50–60%, −(2*S**,3*R**)-**14** (~40%), 10 min;[22] b) Na (3.5 equiv), $NH_{3(l)}$, 30 min, 86% (10-g scale); c) 1. Boc_2O (10 equiv), NBu_4I (0.1 equiv), NaOH, H_2O/dioxane 1:5, 12 h, 0 °C→RT; 2. pH 7.5, 84%; d) BnOH (8 equiv), EDC (3 equiv), DMAP (0.5 equiv), NMM (3 equiv), CH_2Cl_2, 12 h, 62%; e) Subtilisin Carlsberg (4 wt%), acetone/DMSO/phosphate buffer (pH 8) 3:1:24, −**12** (40%), 12 h, 32% (64% of theoretical yield). Bn = benzyl, DMSO = dimethyl sulfoxide, EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, NMM = *N*-methylmorpholine.

Synthesis of linear precursors—exploring the cyclization

Consecutive assembly of the amino acid modules by standard protocols proceeded without significant problems and provided the fully protected pentapeptide **24** on a 20-g scale (Scheme 4). At first, the feasibility of the projected cyclization strategy was explored by incorporating Boc-*O*$^{\omega}$-Bn-Ser-OH as a "functional-group-reduced" model of **18.** This provided the simplified linear precursor **26** for initial cyclization studies. However, even in this case, coupling of Boc-(Bn)Ser-OH to the ClTrp N terminus and saponification of the C-terminal methyl ester (**25**→**26**) turned out to be challenging. Fortunately, the pentafluorophenyl ester of hexapeptide **26** readily cyclized under Schmidt's conditions at a concentration of 0.3 mM in good yield. The final step of reductive deprotection was delicate due to the risk of hydrodeha-



Scheme 4. Liquid-phase synthesis of the northeastern fragment **24** by Boc-protection strategy. Synthesis of "functional group reduced" deacetyllongicatenamycin **28**. Reagents and conditions: a) *N*-Boc amino acid as indicated (1.1 equiv), EDC (1.5 equiv), HOBt (4 equiv), NMM (5 equiv), DMF/CH_2Cl_2 (3–10 mL mmol^{-1} of *N*-Boc amino acid), −10 °C→RT, 16 h or *N*-Boc amino acid (1.2 equiv), HATU (1.3 equiv), DIEA (3.3 equiv) DMF/CH_2Cl_2 (3–10 mL mmol^{-1} of *N*-Boc amino acid), −20 °C→RT, 16 h; b) HCl, dioxane, 0 °C→RT, 4 h; c) LiOH (5 equiv), H_2O/THF 1:2, 0 °C, 70%; d) Pfp-OH (10 equiv), DMAP (0.3 equiv), TEA (1 equiv), EDC (4 equiv), CH_2Cl_2; e) aqueous $NaHCO_3$/$CHCl_3$ 15:75 (0.3 M), RT, 44–57%; f) H_2 (100 kPa), 10% Pd-C (100 wt%), TFA (1 equiv), AcOH, 6 h, RT, 52–69%. Cbz = benzyloxycarbonyl, DIEA = diethyl-(isopropyl)amine, DMF = *N*,*N*-dimethylformamide, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, Pfp = pentafluorophenyl, TEA = triethylamine, TFA = trifluoroacetic acid.

logenation of the sensitive 5-chloroindole moiety. Yet, in acetic acid spiked with one equivalent of trifluoroacetic acid, intact deacetyllongicatenamycin A **28** could be obtained.

Incorporation of HyGlu and HATU cyclization—tackling the natural backbone

Due to the sensitive benzyl ester of **18**, the cyclization conditions worked out for the model series (**25**→**26**) could not simply be transferred to the more complex natural structure **1a.**[25] Therefore, both the C terminus and N terminus had to be deprotected (**24**→**29**) prior to the final chain extension. Notably, chemoselective coupling with the "unprotected" amino acid **29** could be achieved with pentafluorophenyl active ester **30** under strictly controlled conditions ($T = 0 \rightarrow 4$ °C).[26] With all amino acids in place, Schmidt's cyclization sequence could successfully be carried out at a concentration of 0.3 mM (**31**→**34**, method d). However, significantly higher cyclization yields were obtained even at higher concentration (2 mM, method e) by HATU activation.[4] Cautious hydrogenolytic double deprotection of the cyclopeptide **34** yielded longicatenamycin A (**1a**) along with minor amounts of dechlorolongicatenamycin A (**33**) and residual benzyl ester **34** (Scheme 5). All spectroscopic data (NMR: ^{1}H, ^{13}C, COSY, HSQC, HMBC) and HR-FTICR-MS data support the originally proposed structure of longicatenamycin A (**1a**).[7,8]

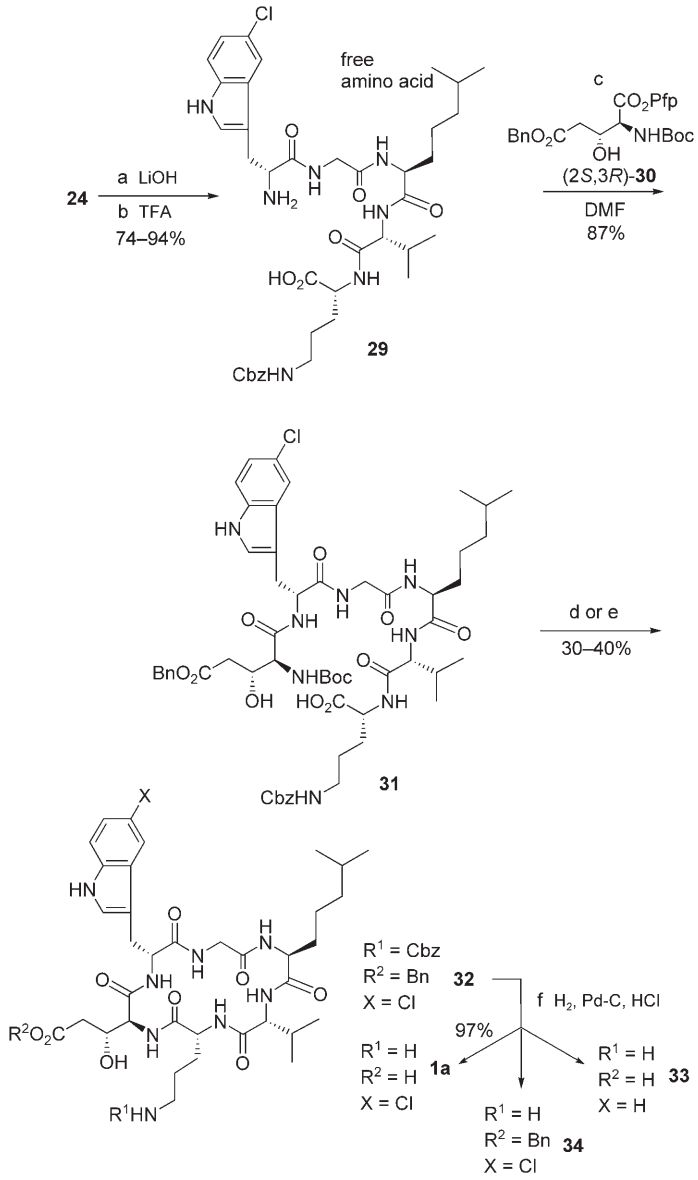
Biological activity and initial structure–activity relationships

In our hands, the antibacterial potency of synthetic longicatenamycin A (**1a**) matched well with the reported[7] data for the natural S-520 antibiotic complex ("longicatenamycin", Table 1). According to Shoji et al.,[7] Gram-negative bacteria should not be susceptible to this antibiotic. Indeed, **1a** exhibited only borderline activity (MIC = 64 μg mL^{-1}) against an efflux pump (AcrA/B)-deficient *E. coli* strain[27] in the presence of a membrane permeabilizer.

The chloro substituent of D-ClTrp turned out to be crucial for antibacterial activity. Dechlorolongicatenamycin A (**33**) was inactive. Likewise, derivatives such as **35** that lack a free D-Orn amino group did not show antibacterial activity. However, derivatization at HyGlu was possible without a total loss in activity. The truncated deacetyl derivative **28** and the benzyl ester **34** were moderately active. Furthermore, a congener carrying the more common HyGln,[10] "longicatenamycin-A-amide" **36,**[28] was obtained as an active compound from **35** in two steps (Scheme 6).

Conclusions

In summary, we have described the total synthesis of longicatenamycin A (**1a**) in 21 linear steps. Compound **1a** is the first defined longicatenamycin congener that has been synthesized and tested in pure form. A successful total synthesis of **1a** required the de novo syntheses of the non-proteinogenic amino acids HyGlu, D-ClTrp, and hhLeu. Our efficient 5-step synthesis of enantiopure *threo*-β-hydroxy-L-glutamate building blocks



Scheme 5. Total synthesis of longicatenamycin A (**1 a**) from fragment **24**. Reagents and conditions: a) LiOH (2 equiv), H_2O/THF/DMF 1:2:0.3, 0 °C, 79–100% (Sephadex LH-20); b) TFA/CH_2Cl_2 1:3, 30 min, 94%; c) (2*S*,3*R*)-**30** (1 equiv), DIEA (3 equiv), DMF, 0 °C→4 °C, 87%; d) 1. Pfp-OH (10 equiv), DMAP (0.3 equiv), TEA (1 equiv), EDC (4 equiv), CH_2Cl_2, −30 °C→RT°C, 16 h, no purification; 2. HCl, dioxane, 0 °C→RT, 4 h, no purification, 3. aqueous $NaHCO_3$/$CHCl_3$ 15:75 (0.3 mM), RT, 25% from **31** or CH_2Cl_2/pyridine 1:30, RT, 36% from **31**; e) 1. TFA/CH_2Cl_2 1:3, 30 min, 93%; 2. HATU (3 equiv), NMM (6 equiv), DMF, 2 mM, 80%; f) H_2 (100 kPa), 10% Pd-C (30 wt%), 4 N HCl (12 vol%), dioxane, 1.4 h, RT, 97%.

(**16**–**18**) from glutaconic acid represents an efficient approach to protected β-hydroxy-Glu and β-hydroxy-Gln[29,9d] cyclopeptide modules. These synthetically demanding non-proteinogenic amino acids are also key components of other bioactive peptides and depsipeptides.[10] The biological activity and synthetic availability of longicatenamycin A (**1 a**) along with our rapid access to key cyclopeptide modules will trigger further investigations with natural longicatenamycins and their related congeners. Initial studies on the mode of action are underway.

Table 1. Antibacterial activity in vitro.

Compound	MIC [μg mL^{-1}] *S. aureus*[a]	*B. subtilis*[b]
1 a·TFA (synthetic longicatenamycin A)	8	8
1 (natural S-520 antibiotic complex)[c]	6.25[c]	6.25[c]
28·TFA (deacetyllongicatenamycin A)	32	64
33·TFA (dechlorolongicatenamycin A)	128	128
34·TFA (benzyl ester)	32	32
35 (Cbz protected)	>128	>128
36·TFA (longicatenamycin-A-amide)	16	16
linezolid	4	0.5
ampicillin	0.5	0.1

[a] *Staphylococcus aureus* 133 (DSMZ No. 11823, DSMZ, Braunschweig, Germany). [b] *Bacillus subtilis* 168 (BGSCID 1A1, BGSC, Columbus, OH, USA). [c] Reported MICs for the natural S-520 "longicatenamycin" complex with *S. aureus* 60658 and *B. subtilis* (not specified);[7] MIC = minimal inhibitory concentration.

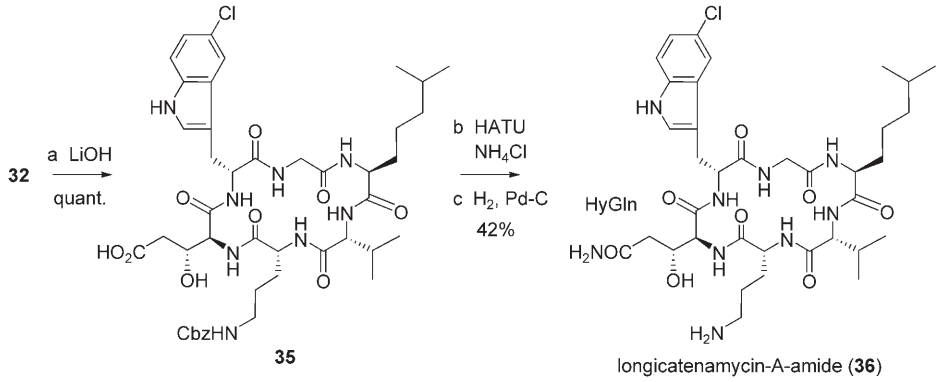
The door is now open for exploring the potential of this class by chemical postevolution.[1b]

Experimental Section

General methods: Reactions were carried out under argon atmosphere if not indicated otherwise. Reactions were monitored by HPLC. Crude products were immediately purified with preparative HPLC or gel chromatography. The fractions obtained were concentrated in vacuo to remove organic solvents. All products were freeze-dried, yielding colorless lyophilisates. Chemicals were obtained in analytical grade from Bachem (Swiss), Merck KGaA (Germany) or Sigma–Aldrich (Germany). Gel chromatography was carried out with Sephadex LH-20 (Pharmacia). Structural representation and nomenclature: longicatenamycins are salts. Counter-ions were omitted in the figures and schemes.

Mass spectrometry: The FT-ICR instrument (HR-FT-ICR-MS) was an APEX II mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with a room-temperature, actively shielded magnet (160 mm, 7 T), electrostatic ion-transfer optics, octupole ion-storage device, and external off-axis electrospray ion source. HPLC-FT-ICR-MS was performed on an HP 1100 HPLC system synchronized to the FT-ICR spectrometer via contact closure. The whole effluent (250 μL min^{-1}) was directed to the external electrospray source. A heated nitrogen flow of 8 L min^{-1} each with a temperature of 250 °C was used as nebulizing and drying gas. Accurate mass TOF-ESI-MS data were obtained on a Micromass-LCT mass spectrometer (capillary 3.2 kV, cone 42 V, source 120 °C). Samples were injected with a syringe pump (Harvard Apparatus). Leucine–enkephalin was used as standard (resolution 5500). HR-ESI-MS date were collected using an LTQ-Orbitrap mass spectrometer (ThermoFisher Scientific). All experimental data were acquired using external calibration prior to data acquisition and leucine–enkephalin as standard lock-mass. Mass spectrometry analyses were carried out on full-scan MS using a resolution of 30000.

NMR spectroscopy: Spectra were recorded on a Bruker Avance DRX400 or a Bruker Avance DRX500 instrument with a $^1H/^{13}C$-Dual Cryoprobe. Microsamples were recorded using a 400 MHz 1 mm TXI-Probe (active volume 5 μL). ^1H and ^{13}C chemical shifts are given with respect to TMS or the solvent as internal standard ([D_6]DMSO: $\delta_H = 2.49$ ppm, $\delta_C = 39.5$ ppm; $CDCl_3$: $\delta_H = 7.25$ ppm, $\delta_C = 77.0$ ppm; [D_5]pyridine: $\delta_H = 7.19$ ppm, $\delta_C = 123.5$ ppm). The data were pro-



Scheme 6. Synthesis of longicatenamycin-A-amide (**36**). Reagents and conditions: a) LiOH (2 equiv), H_2O/THF 1:2, 0 °C, quant.; HATU (5 equiv), TEA (35 equiv), NH_4Cl (30 equiv), DMF, 75%; c) H_2 (100 kPa), 10% Pd-C (30 wt%), 4 N HCl (30 vol%), dioxane, 0.5 h, RT, 56%.

cessed using Bruker XWINNMR 3.5 and Topspin 1.3. Several NMR spectra were visualized with NPNMR (NPNMR 2.0, NMR processing and visualization software, **2006**: http://www.npnmr.com).

Longicatenamycin A trifluoroacetate (**1a**·TFA):[30] HPLC–UV/Vis (CH_3CN/H_2O/TFA): λ_{max} = 230 (s), 280 (m), 288 (m), 298 nm (sh). $[\alpha]_{Na}^{20} = +9°$ (c = 0.17, MeOH); IR (KBr): $\tilde{\nu}$ = 3268 (br), 1634 (s), 1532 (s), 1465 (w), 1201 (s), 1186 (sh), 1139 (w), 894 (w), 837 (w), 799 (w), 722 cm^{-1} (w); ^1H NMR (500 MHz, [D_5]pyridine): δ = 0.74 (br d, J = 4.7 Hz, 6H, hhLeu CH_3), 1.05, 1.44 (2 br m, 11H, D-Val β-CH_3, hhLeu γ-CH_2, hhLeu ε-CH, and presumably hhLeu δ-CH_2), 1.94 (m, 2H, hhLeu β-CH_2), 2.16 (m, 2H, hhLeu β-CH_2, D-Orn γ-CH_2), 2.26 (m, 2H, D-Orn β-CH_2, D-Orn γ-CH_2), 2.39 (m, 1H, D-Orn β-CH_2), 2.55 (m, 1H, D-Val β-CH), 2.99 (m, 2H, HyGlu γ-CH_2), 3.29 (m, 2H, hhLeu δ-CH_2,), 3.5 (dd, J = 14.8, 8.2 Hz, 1H, ClTrp β-CH_2), 3.8 (dd, J = 14.8, 5.7 Hz, 1H, ClTrp β-CH_2), 4.17 (dd, J = 4.7, 16.4 Hz, 1H, Gly α-CH_2), 4.33 (dd, J = 16.7, 5.0 Hz, 1H, Gly α-CH_2), 4.76 (m, 1H, D-Val α-CH), 4.83 (m, 1H, D-Orn α-CH), 4.97 (m, 1H, hhLeu α-CH), 5.26 (m, 1H, HyGlu α-CH, HyGlu β-CH, hhLeu α-CH), 7.21 (d, 1H, ClTrp ArH, overlap with solvent signal), 7.41 (d, J = 8.5 Hz, 1H, ClTrp ArH), 7.63 (s, 1H, ClTrp ArH), 7.86 (s, 1H, ClTrp ArH), 8.7 (br s, 1H, hhLeu NH, overlap with solvent signal), 8.86 (m, 1H, HyGlu NH), 9.02 (m, 2H, hhLeu NH, D-Orn NH), 9.22 (br s, 1H, Gly NH), 9.39 ppm (m, 1H, ClTrp NH); ^{13}C NMR (126 MHz, [D_5]pyridine): δ = 18.3, 19.5, 22.2, 22.3, 23.8, 24.8, 26.9, 27.7 (2C), 28.8, 29.6, 31.6, 39.2, 39,8, 43.5, 53.6, 54.0, 54.9, 58.6, 60.1, 67.9, 10.5, 112.9, 118.1 (q, J = 297.1 Hz, TFA), 118.3, 119.2, 121.5, 124.3, 125.9, 129.1, 161.6 (q, J = 33.8 Hz, TFA), 169.9, 171.8, 172.0, 172.4, 172.6, 173.3, 173.9 ppm; LC–MS (CH_3CN–HCO_2H): MS (ESI+): m/z (%) = 777 (100) $[M+H]^+$; MS (ESI−): m/z (%) = 775 (100) $[M-H]^-$ (purity 100%); HR-ESI-MS: calcd for $C_{36}H_{53}ClN_8O_9$ $[M+H]^+$ 777.3697, found 777.3703; HR-FT-ICR-MS: calcd for $C_{36}H_{53}ClN_8O_9$ $[M+H]^+$ 777.3697, found 777.3691.

7: HPLC (Chiral AD, *n*-heptane–EtOH–TFA 90:10:0.2): >99% *ee*; $[\alpha]_{Na}^{20} = -18°$ (c = 0.12, EtOH); ^1H NMR (500.1 MHz, [D_6]DMSO): δ = 1.28 (s, 9H, C(CH_3)$_3$), 1.60 (s, 9H, C(CH_3)$_3$), 2.92 (dd, J = 14.2, 10.3 Hz, 1H, β-CH), 3.10 (dd, J = 14.6, 3.4 Hz, 1H, β-CH), 4.17 (m, 1H, α-CH), 7.16 (d, J = 8.3 Hz, 1H, NH), 7.32 (dd, J = 8.8, 1.5 Hz, 1H, ArH), 7.55 (s, 1H, ArH), 7.99 (d, J = 8.8 Hz, 1H, ArH), 12.67 ppm (s, 1H, CO_2H); ^{13}C NMR (126 MHz, [D_5]pyridine): δ = 26.1, 27.5 (3C), 28.0 (3C), 53.4, 78.0, 84.0, 116.0, 116.4, 118.8, 124.1, 125.4, 127.1, 131.6, 133.1, 148.6, 155.2, 173.2 ppm; LC–MS (CH_3CN–HCO_2H): MS (ESI+): m/z (%) = 283 (100), 439 (60) $[M+H]^+$; MS (ESI−): m/z (%) = 363 (60), 437 (100) $[M-H]^-$ (purity 100%); HRMS: calcd for $C_{21}H_{27}ClN_2O_6$ $[M+Na]^+$ 461.1455, found 461.1434.

(2*S*,3*R*)-**12:** HPLC (Chiral OD-H, *n*-heptane–*i*PrOH 85:15): >95% *ee*; $[\alpha]_{Na}^{20} = +6°$ (c = 0.12, EtOH); ^1H NMR (500.1 MHz, [D_6]DMSO): δ = 0.82 (m, 6H, CH_3), 1.11 (m, 2H), 1.27 (m, 2H), 1.36 (s, 9H, C(CH_3)$_3$), 1.41–1.64 (m, 3H), 3.82 (m, 1H, α-CH), 6.99 ppm (d, J = 7.8 Hz, 1H, NH); DCI-MS (NH_3): m/z (%) = 277 (100) $[M+NH_4]^+$, 536 (10) $[2M+NH_4]^+$.

(2*S*,3*R*)-**18:** HPLC (Chiralcel OD-RH, H_2O–MeOH–$HClO_4$): >96% *ee*; $[\alpha]_{Na}^{20} = +27.5°$ (c = 0.51, $CHCl_3$); ^1H NMR (500.1 MHz, [D_6]DMSO): δ = 1.37 (s, 9H, C(CH_3)$_3$), 2.45 (dd, J = 15.5, 8.5 Hz, 1H, γ-CH), 2.26 (dd, J = 15.5, 4.7 Hz, 1H, γ-CH), 4.09 (dd, J = 2.5, 9.2 Hz, 1H, α-CH), 4.37 (m, J = 3.5, 3.2 Hz, 1H, β-CH), 5.09 (s, 2H, η-CH_2), 6.46 (d, J = 9.2 Hz, 1H, NH), 7.28–7.40 ppm (m, 5H, ArH); ^{13}C NMR (126 MHz, [D_6]DMSO): δ = 28.3 (3C, C(CH_3)$_3$, Boc), 39.3 (γ-CH_2, overlap with solvent signal), 58.0 (α-CH), 65.7 (CH_2, Bz), 67.91 (β-CHOH), 78.7 (C(CH_3)$_3$, Boc), 128.0 (2C, Ar*C*, Bn), 128.2 (Ar*C*, Bn) 128.6 (2C, Ar*C*, Bn), 136.3 (Ar*C*, Bn), 155.9 (CO, Boc), 170.7 (δ CO_2Bn), 172.3 ppm (CO_2H); LC–MS (CH_3CN–HCO_2H): MS (ESI+): m/z (%) = 254 (100), 298 (30), 376 (20) $[M+Na]^+$; MS (ESI−): m/z (%) = 352 (100) $[M-H]^-$ (purity 100%); HRMS: calcd for $C_{17}H_{23}NO_7$ $[M+H]^+$ 354.1553, found 354.1529.

Computational methods: Initial structural minimizations used the OPLS2005 force field of Schrödinger's MacroModel Engine.[31] Subsequent optimization was carried out in vacuum and aqueous solution modeled via the analytical Generalized-Born/Surface Area,[32] starting with conformations with and without salt bridge, respectively. Conformations with a salt bridge between the protonated amino group of D-Orn and the HyGlu carboxylate were clearly more stable. The energy of the resulting structures of the force field calculation was more reliably determined using Schrödinger's Jaguar[33] implementation of ab initio DFT methods,[34] namely the Becke B3LYP functionals[35] together with the 6–31G[36] basis. Additional density functional single point calculations were applied to account for solvent effects. Reported stabilization energies were drawn from these solvent-corrected density functional calculations. The treatment of aqueous solution was carried out by using a Poisson–Boltzmann Solvation Model.[37]

X-ray structure determination of (2*S**,3*R**)-**15 and** (2*R*,3*S*)-**17:** CCDC 657972 and 657973 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (UK); fax: (+44) 1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

Antibacterial susceptibility tests were performed in cation-adjusted Mueller–Hinton (MH) medium. The minimal inhibitory concentrations (MICs) were determined according to the CLSI (Clinical and Laboratory Standards Institute) document M7A6 "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically".

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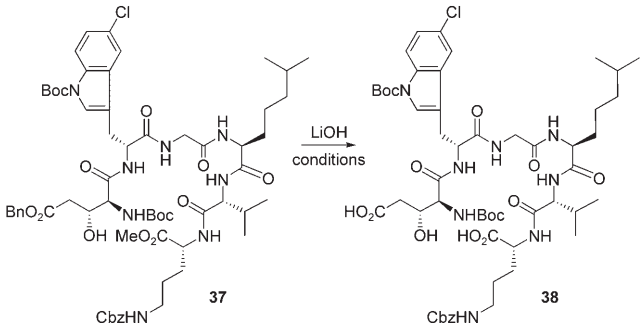

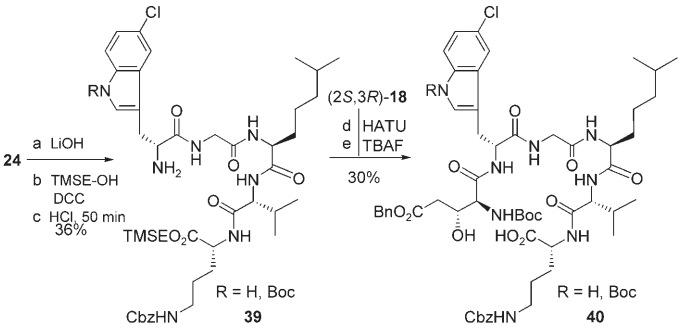

[26] Obtained from (2*S*,3*R*)-**18** [Pfp-OH (5 equiv), EDC (1.5 equiv), CH_2Cl_2, 80%]. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, Pfp = pentafluorophenyl.

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