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Syntheses and Biofilm Reducing Effects of L-Dopa-Derived Analogues of the Fungal Macrocidins A and Z

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Abstract: Four analogues of the fungal metabolites macrocidin A and Z, featuring [13]*para*- or [13]*meta*cyclophanes, were synthesised from fully and orthogonally protected L-dopa instead of L-tyrosine. They were tested for antibiotic activities and for effects on the growth and persistence of microbial biofilms. Tentative structure-activity relationships and distinct differences when compared with the natural lead compounds were identified.

Introduction

Macrocidins are L-tyrosine-derived polycyclic tetramic acid macrolactams (PTMs) featuring a 1-oxa-[13]paracyclophane, which are produced by the plant pathogenic fungus *Phoma macrostoma.* Macrocidin A (**1**) (Figure 1) was first isolated in 2003 by a Dow AgroSciences group,[1] and was later synthesised on two different ways.[2,3] Macrocidin Z (**2**), which differs from macrocidin A by an *E*-alkene replacing the expoxide, was isolated, unambiguously characterised and synthesised only recently.[4] Macrocidin-rich extracts of *P. macrostoma* fermenter broth were found to induce chlorosis in broadleaf weeds by interfering with essential enzymes of the carotenoid biosynthesis.[5] Syntheses of simplified macrocidin derivatives were sporadically reported, for example by Graupner's group,[6] or SYNGENTA,[7] albeit without disclosing synthetic or biological details. Lately, our group published the synthesis of derivatives of macrocidins A and Z that exhibit not only structure-dependent herbicidal but also hitherto unknown bacterial biofilm reducing effects.[8] Due to the significance of this antibiofilm effect, we now made and studied macrocidin analogues **3**–**6** derived from L-dopa rather than tyrosine, including PTMs featuring a 1-oxa-[13]metacyclophane. To this end, we had to develop new efficient routes to both mono-*O*-protected L-dopa precursors.

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Results and Discussion

Syntheses

The new macrocidin Z analogues **4** and **6** were planned to be built up by first reacting an *N*-Boc protected L-dopa, carrying an allyl group and a second orthogonal protecting group on the catechol oxygen atoms, with Meldrum's acid to afford the corresponding tetramic acid. This was then to be 4-*O*-acylated with hept-6-enoic acid to give the corresponding tetramate which should then be rearranged to the 3-acyltetramic acid under Yoshii conditions.[9] By means of a Grubbs' catalyst, the macrocycle would then be closed via a ring-closing metathesis (RCM) reaction between the terminal alkenes of the allyl "protecting" group, located either in *meta*- or in *para*-position, and that of the 3-acyl residue. Removal of the protecting groups on the second catechol oxygen and the nitrogen atom of the pyrrolidin-2,4-dione core should eventually afford the corresponding macrocidin Z analogues. The synthesis of the required

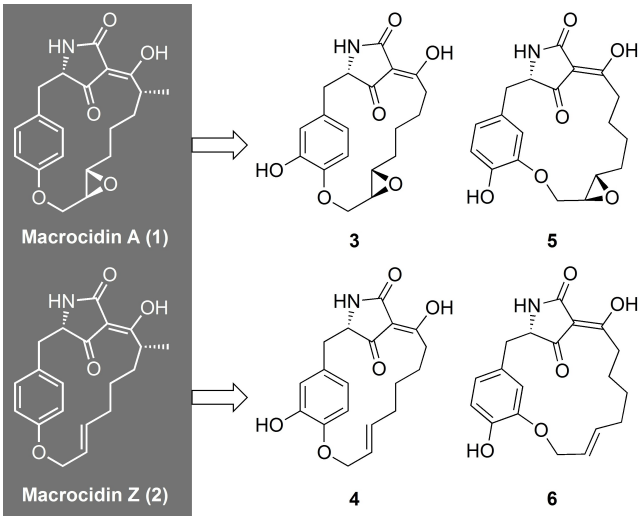
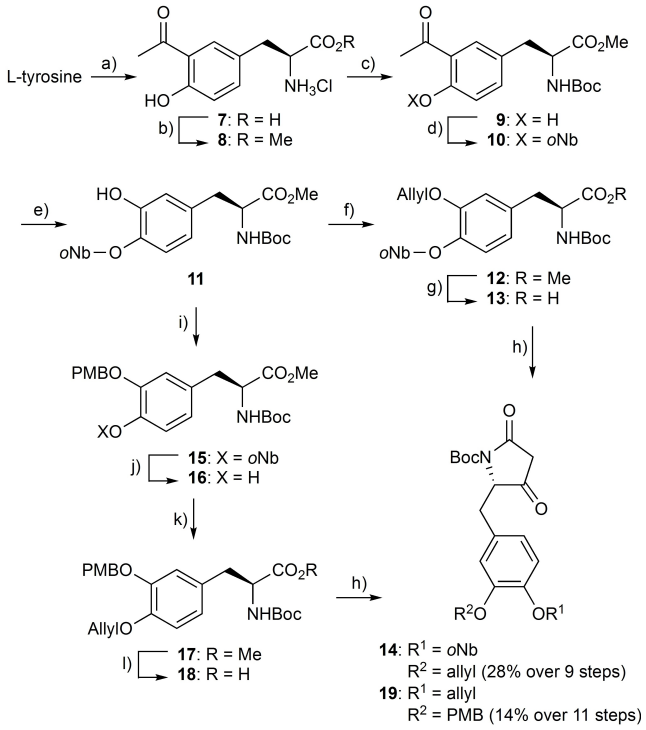


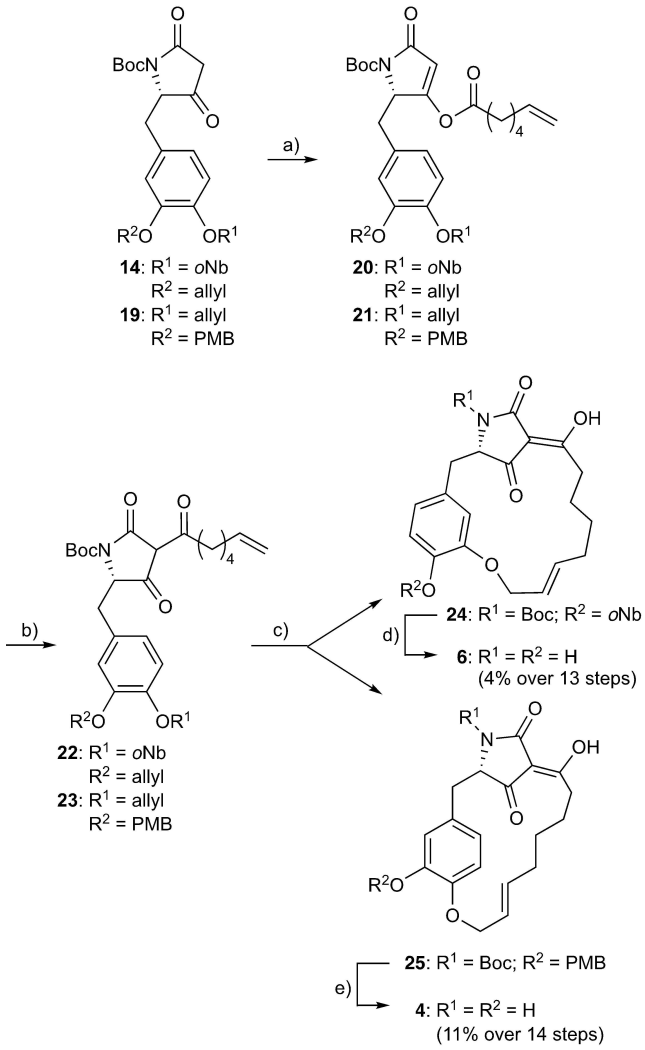
Figure 1. Structures of natural products macrocidin A (**1**) and Z (**2**), as well as of the analogous L-dopa-derived paracyclophanes **3**/**4** and metacyclophanes **5**/**6**.

selectively mono-protected and allylated L-dopa precursors took some experimentation (Scheme 1). According to Boger's protocol,[10] L-tyrosine was acetylated exclusively to ketone **7**, which was converted to ester **8** and *N*-Boc protected to compound **9**. The remaining phenolic OH-group was protected with the photo-cleavable *ortho*-nitrobenzyl (*o*Nb) group to give ketone **10** which was submitted to a Baeyer-Villiger oxidation with mCPBA affording *para*-protected catechol **11**. Depending on which oxygen atom we wanted to carry the allyl group, **11** was either directly allylated to *meta*-allyloxy derivative **12** which was saponificated to carboxylic acid **13** and treated with Meldrum's acid and EDC×HCl according to Jouin's protocol,[11] to afford metacyclophane precursor **14**. Alternatively, **11** was protected with a *para*-methoxybenzyl (PMB) group to give completely protected compound **15**. This was then photolytically converted to *para*-phenol **16** which was allylated to compound **17**. Its saponification furnished the trisprotected carboxylic acid **18**, which was converted to the corresponding tetramic acid **19**, again by using Jouin's protocol.

Scheme 2 shows the syntheses of both macrocidin Z analogues **4** and **6** on similar routes. 4-*O*-acylation of tetramic acid **14** or **19** with hept-6-enoic acid gave the respective tetramates **20** or **21**. These were rearranged with triethylamine/DMAP to furnish the 3-acyltetramic acids **22** or **23**. Interestingly,



Scheme 1. Syntheses of orthogonally protected L-dopa derivatives **12**–**19**. Reagents and conditions: a) $AlCl_3$, MeCOCl, $PhNO_2$, 100 °C, 17 h. b) $SOCl_2$, MeOH, RT, 17 h. c) NEt_3, Boc_2O, dioxane, H_2O, RT, 20 h; 67% over 3 steps. d) *o*Nb–Br, K_2CO_3, DMF, RT, 18 h; 99%. e) 1. mCPBA, CH_2Cl_2, 60 °C, 20 h; 2. $NaHCO_3$, MeOH, H_2O, RT, 2 d; 64%. f) allyl bromide, K_2CO_3, DMF, 35 °C, 19 h; 92%. g) NaOH, MeOH, H_2O, RT, 48 h; quant. h) Meldrum's acid, EDC×HCl, DMAP, CH_2Cl_2, RT, 12 h; 71% (**14**)/86% (**19**). i) PMBCl, K_2CO_3, DMF, 120 °C, 1 h; 65%. j) hν, CH_2Cl_2, RT, 72 h; 83%. k) allyl bromide, K_2CO_3, DMF, RT, 22 h; quant. l) NaOH, MeOH, RT, 12 h; quant.

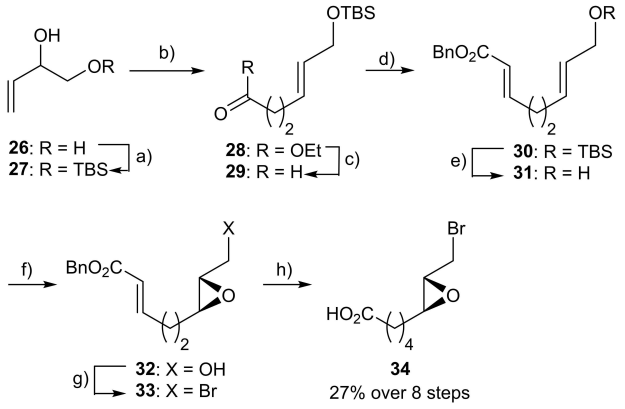


Scheme 2. Syntheses of macrocidin Z analogues **4** and **6**. Reagents and conditions: a) hept-6-enoic acid, EDC×HCl, DMAP, CH_2Cl_2, RT, 3 h; 63% (**20**)/75% (**21**). b) NEt_3, DMAP, CH_2Cl_2, RT, 24 h; 68% (**22**)/75% over 2 steps (**23**). c) Grubb's II catalyst, CH_2Cl_2, 50 °C, 24 h; 82% (**24**)/78% (**25**). d) 1. hν, CH_2Cl_2, RT, 5 d; 2. TFA, CH_2Cl_2, RT, 15 min; 39% over 2 steps. e) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 15 min; 78%.

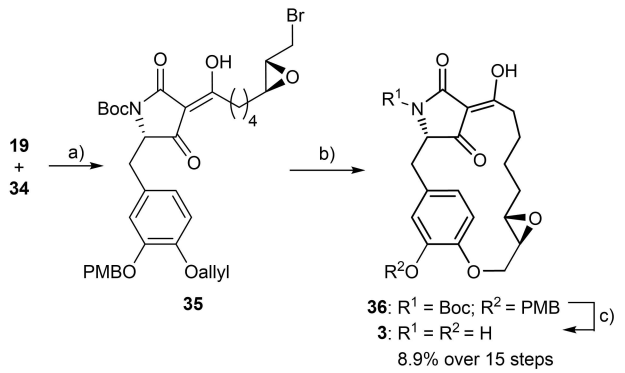
tetramate **21** rearranged in part spontaneously, while analogue **20** was stable enough to be isolated in a pure form. Treatment of tetramic acids **22** or **23** with a 2nd gen. Grubbs' catalyst at 50 °C for a day gave the protected cyclophanes **24** or **25** as products of an RCM reaction. Stepwise deprotection of **24**, by first photolytically removing the *o*Nb group, followed by cleavage of the Boc group with TFA liberated metacyclophane **6** in 32% yield over the last three steps. Paracyclophane **25** could be deprotected globally with TFA to afford macrocidin Z analogue **4** in 61% yield over ring closure and deprotection. So, starting with L-tyrosine, paracyclophane **4** was synthesised in 14 steps and 11% yield, whereas metacyclophane **6** was obtained in 4% yield over 13 steps.

The L-dopa-derived macrocidin A analogues **3** and **5** had to be synthesised on a different route because the oxirane of macrocidins cannot be introduced by a down-stream epoxida-

tion of macrocyclic alkene precursors.[2,3,12,13] We used the strategy we had employed for our synthesis of macrocidin A. Its key steps are the 3-acylation of a suitably protected 5-(*p*-hydroxybenzyl)tetramic acid with an octanoic acid featuring an epibromohydrin end group. The macrocycle is then closed by a Williamson etherification between the bromide and the phenol.[3] The required carboxylic acid **34** was prepared starting from but-1-en-3,4-diol (**26**), whose primary alcohol was TBS-protected affording allyl alcohol **27** (Scheme 3). This was converted to ethyl hex-4-enoate **28** by an *E*-selective Johnson-Claisen rearrangement upon addition of triethyl orthoacetate. Reduction of ester **28** with DIBAL-H gave aldehyde **29** which was alkenated with (benzyloxycarbonyl)methylene triphenyl phosphorane furnishing benzyl enoate **30**. The latter was desilylated under acidic conditions to leave allyl alcohol **31** which was epoxidised under Sharpless conditions[14] to afford alcohol **32**. The latter was converted to bromide **33**. Simultaneous hydrogenation of the double bond and cleavage of the benzyl ester furnished the required ω-bromoacid **34**.



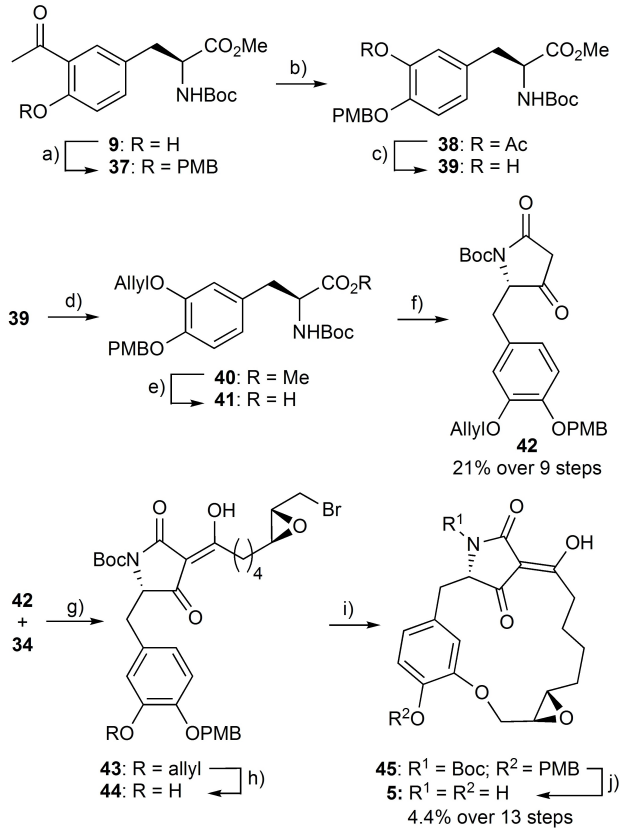
Scheme 3. Synthesis of ω-bromoacid **34**. Reagents and conditions: a) NEt_3, DMAP, TBSCl, CH_2Cl_2, RT, 16 h. b) $(EtO)_3CMe$, $EtCO_2H$, DMF, reflux, 2 h; 63% over 2 steps. c) DIBAL-H, CH_2Cl_2, −78 °C, 30 min; quant. d) Ph_3PCHCO_2Bn, THF, 40 °C, 17 h; 89%. e) AcOH, THF, H_2O, RT, 22 h; 98%. f) L–DET, *t*BuOOH, $Ti(iPrO)_4$, CH_2Cl_2, −25 °C, 19 h; 84%, ee = 97.2%. g) Ph_3PBr_2, CH_2Cl_2, 0 °C to RT, 20 h; 74%. h) H_2, Pd/C, EtOAc, RT, 15 h; 70%.



Scheme 4. Synthesis of compound **3**. Reagents and conditions: a) EDC×HCl, DMAP, then NEt_3, DMAP, CH_2Cl_2, RT, 16 h; 49%. b) 1. $Pd(PPh_3)_4$, K_2CO_3, MeOH, RT, 18 h; 2. 18-c-6, K_2CO_3, TBAI, DMF, 100 °C, 18 h; 50% over 2 steps. c) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 15 min; 75%.

Acid **34** was then used to *O*-acylate the *m*-OPMB protected tetramic acid **19** to give the corresponding tetramate which was directly rearranged to the 3-acyltetramic acid **35** by adding NEt_3 and DMAP to the reaction mixture.[9,15] After de-allylation of compound **35** a ring-closing Williamson etherification under those special conditions we had optimised for the macrocyclisation leading to macrocidin A took place.[3] The crude product **36** was immediately deprotected with TFA in the presence of triethylsilane to leave (*S*,*S*,*S*)-(hydroxy)normacrocidin A (**3**) in an overall yield of 9% over 15 steps starting from L-tyrosine (Scheme 4).

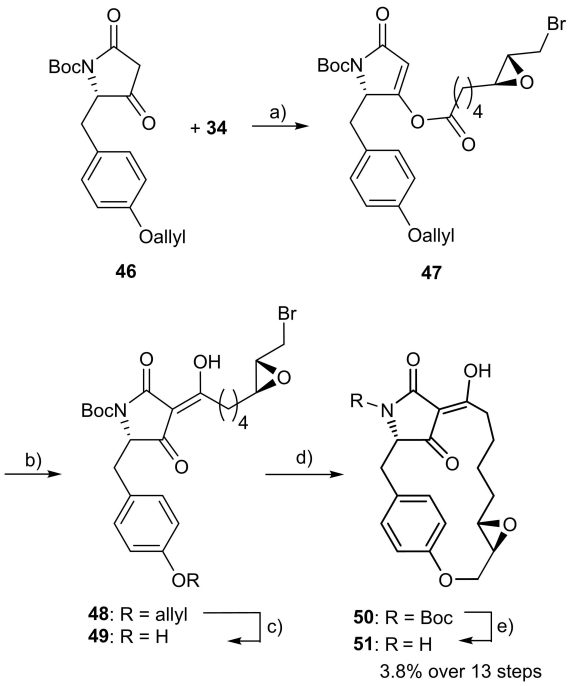
An analogous synthesis of metacyclophane **5** from either 3-acyltetramic acid **35** (by removal of the PMB group instead of the allyl group) or from *o*Nb-allyl-protected tetramic acid **14** and acid **34** foundered on the poor yield of the macrocyclisation step. Thus, the protecting group on the *para*-hydroxy group had to be replaced. As PMB proved viable during the cyclisation of allyl deprotected **35** to give **36**, the amino ester **9** was converted to PMB ether **37** (Scheme 5). Baeyer-Villiger oxidation of the latter gave the acetate **38**, which was selectively saponificated to phenol **39** by stirring it in a mixture of aq. $NaHCO_3$ and MeOH. Its *O*-allylation led to a fully



Scheme 5. Synthesis of compound **5**. Reagents and conditions: a) PMBCl, K_2CO_3, DMF, 120 °C, 2 h; 96%. b) mCPBA, CH_2Cl_2, 60 °C, 20 h; 74%. c) $NaHCO_3$, MeOH, H_2O, 40 °C, 17 h; 70%. d) allyl bromide, K_2CO_3, DMF, RT, 17 h; 92%. e) NaOH, MeOH, H_2O, RT, 17 h. f) Meldrum's acid, EDC×HCl, DMAP, CH_2Cl_2, RT, 22 h; 70%. g) EDC×HCl, DMAP, then NEt_3, DMAP, CH_2Cl_2, RT, 16 h; 51%. h) $Pd(PPh_3)_4$, K_2CO_3, MeOH, RT, 15 h. i) 18-c-6, K_2CO_3, TBAI, DMF, 100 °C, 24 h. j) Et_3SiH, TFA, CH_2Cl_2, 0 °C, 15 min; 42% over 3 steps.

protected L-dopa derivative **40** which was saponificated with sodium hydroxide to the corresponding acid **41**. This, in turn, was converted with Meldrum's acid to tetramic acid **42**. In analogy to the synthesis of the paracyclophane **3**, 3-acyltetramic acid **43** was then prepared from tetramic acid **42** and carboxylic acid **34**. Pd-mediated de-allylation of compound **43** afforded the phenol **44**. Its macrocyclisation via intramolecular Williamson etherification according to the protocol used for the synthesis of **3** gave metacyclophane **45** in mixture with its congener already lacking the *N*-Boc group. Treatment of this mixture with Et_3SiH/TFA afforded *meta*cyclophane **5** in an overall yield of 4.4% over 13 steps starting from L-tyrosine.

The new compounds **3**–**6** are analogues or derivatives of the non-natural normacrocidins, which lack the methyl group of the natural lead compounds. In order to complete the collection of structurally modified macrocidinoids for a meaningful evaluation of structure-antibiofilm activity relations, we now also synthesised normacrocidin A (**51**) with the correct "natural" *S*-configurations of the stereogenic centres (Scheme 6). So far, only a diastereoisomer of **51** with an *R,R*-configured epoxide was reported by our group.[8] To this end, tetramic acid **46**, which was also used for the synthesis of macrocidin A (**1**),[3] was 4-*O*-acylated with acid **34** to give tetramate **47**. The latter was rearranged to 3-acyltetramic acid **48**. Its de-allylation afforded phenol **49**, which was submitted to our standard macrocyclisation protocol to furnish the *N*-Boc-protected macrocycle **50**. Final deprotection with TFA left normacrocidin A (**51**) in just about 4% overall yield.



Scheme 6. Synthesis of (*S,S,S*)-normacrocidin A (**51**). Reagents and conditions: a) EDC×HCl, DMAP, CH_2Cl_2, RT, 3 h; 83%. b) NEt_3, DMAP, CH_2Cl_2, RT, 19 h; 52%. c) $Pd(PPh_3)_4$, K_2CO_3, MeOH, RT, 22 h. d) 18-c-6, K_2CO_3, TBAI, DMF, 100 °C, 24 h. e) TFA, CH_2Cl_2, RT, 10 min; 33% over 3 steps.

Biofilm interference

The macrocidinoids **2**–**6** and **51** were tested[16,17] for inhibitory effects on the formation of biofilms by *Staphylococcus aureus* (DSM 1104) bacteria as well as for dispersive effects on preformed biofilms of *S. aureus* with microporenic acid A (MAA) used as a positive control (cf. Supporting Information for data Table S3). While macrocidin Z (**2**) inhibited the formation of *S. aureus* biofilms by at least 30% relative to untreated controls at concentrations as low as 3.9 μg/mL, which was comparable to our previous studies,[8] only the related metacyclophane **6** showed a significant growth inhibition of 20% odd when applied at a concentration of 15.6 μg/mL (Figure 2A). Its paracyclophane isomer **4** led also to an inhibition of ca. 20% but only at a concentration of 31.3 μg/mL. The epoxide-carrying normacrocidin A analogues **3** (a paracyclophane) and **5** (a metacyclophane) were distinctly less active growth inhibitors with at least 50% inhibition only when applied at a concentration of 250 μg/mL. The (*S,S,S*)-normacrocidin A (**51**) proved a more active inhibitor of biofilm formation than its previously published (*S,R,R*)-diastereomer,[8] and than its more polar, hydroxy derivative **3**. Down to a concentration of 62.5 μg/mL compound **51** caused an inhibition of ca. 30% (cf. Supporting Information), whereas its (*S,R,R*)-diastereomer had given rise to only 17% growth inhibition. While macrocidin Z (**2**) also showed a distinct dispersive effect on preformed biofilms of *S. aureus*,

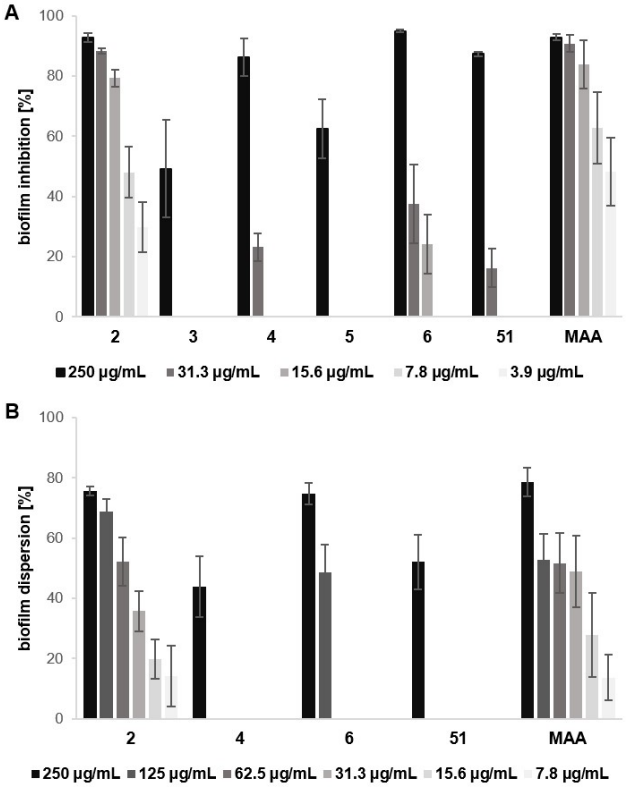


Figure 2. Effects of various concentrations of compounds **2**–**6** and **51** on the formation (A) and dispersion (B) of biofilms of *S. aureus*; positive control: microporenic acid A (MAA); error bars indicate SD of two repeats with duplicates.

the dispersive effects of the new macrocidinoids were generally less pronounced than their growth inhibitory effects, with compounds **3** and **5** being virtually inactive at all concentrations (Figure 2B). The most distinct effects were observed for the normacrocidin Z analogues with paracyclophane **4** leading to a dispersion of ca. 44%, and the metacyclophane **6** to one of ca. 75%, when applied at the highest tested concentration of 250 μg/mL. Interestingly, preliminary studies with combinations of macrocidin Z (**2**) and gentamycin even indicated a potential additive dispersive effect on preformed biofilms of *S. aureus*.

As a tentative SAR, and in line with results of earlier studies with macrocidinoid paracyclophanes,[8] we conclude that strong effects against *S. aureus* were exerted not by macrocidin A analogues but by the more lipophilic macrocidin Z analogues **4** and **6**, and that the activity decreases when hydroxy groups are present.

We also evaluated the inhibitory effects of the test compounds on biofilm formation of the fungus *Candida albicans* (DSM 11225) and *Pseudomonas aeruginosa* bacteria (PA 14). While none of the macrocidinoids **3**–**6** and **51** exhibited growth inhibition of biofilms of *P. aeruginosa*, the epoxide-carrying normacrocidin A analogues **3**, **5**, and **51** showed inhibitory effects of ca. 50% against *C. albicans*, when applied at the highest concentration of 250 μg/mL. It is also worthy of note, that no cytotoxic or antimicrobial effects were observed for the macrocidinoids **3**–**6** and **51** (cf. Supporting Information for data Tables S1, S2).

Conclusion

We developed the first expeditious route to fully and orthogonally protected L-dopa derivatives **12**, **15**, **17**, and **40** which allow selective reactions on any of the four functional groups. We used them to synthesise paracyclophane and metacyclophane analogues of the fungal macrocidin A (**1**) via Williamson-type ring-closure, and of the fungal macrocidin Z (**2**) via ring-closing metathesis reactions.

Together with (*S*,*S*,*S*)-normacrocidin A (**51**), prepared for the first time, they were tested for growth inhibitory and dispersive effects on biofilms of *S. aureus* bacteria. None of the L-dopa-derived compounds **3**–**6** reached the activity of macrocidin Z (**2**) in the growth inhibition and dispersion assays. This might have to do with their reduced lipophilicity due to the missing methyl group and the additional phenolic OH-group. In keeping with this rationale, the macrocidin Z analogues **4** and **6** displayed greater antibiofilm effects than the macrocidin A analogues **3** and **5** which carry a polar epoxide in lieu of the alkene. There was little difference between the antibiofilm activities of the paracyclophanes and their respective metacyclophane counterparts. When compared with the previously reported[8] normacrocidin A and Z derivates, and with the new (*S*,*S*,*S*)-normacrocidin A (**51**), all derived from L-tyrosine, the antibiofilm activities of the L-dopa-derived analogues were less distinct. However, as they showed no toxicity against bacteria, their application as biofilm inhibitors should not elicit bacterial resistance.

Experimental Section

General methods: *IR spectra* were recorded using a Spectrum One FTIR spectrometer from PerkinElmer with an ATR unit. – *^{1}H and ^{13}C NMR spectra* were recorded on a Bruker Avance DRX-500 spectrometer (500 MHz, with cryoprobe). – High resolution mass spectra (*HRMS*) were recorded with a ThermoFisher UPLC/Orbitrap MS system in ESI mode. – **IR spectral data, NMR data and spectra, as well as mass spectrometric data of all synthesised compounds are presented in the Supporting Information**. *Specific rotations* were recorded on a PerkinElmer 241 Polarimeter at 589 nm (Na–D line) using solutions in chloroform and methanol and reported as $[a]_D$ in units of 10^{-1} deg cm^2 g^{-1}. – *Melting points* were measured with a Büchi Melting Point H-565 apparatus and are uncorrected. – *Thin-layer chromatography* (*TLC*) was performed on aluminium plates pre-coated with silica gel 60 F_{254} or aluminum plates pre-coated with aluminium oxide 60 F_{254}, both from Merck. The compounds were detected with UV light (254 nm and/or 360 nm) and/or ceric ammonium molybdate (CAM) and/or potassium permanganate. – *Column chromatography* for purification was performed on normal or reverse phase columns. Normal phase chromatography employed MN silica gel 60 (40–60 μm) from Macherey-Nagel or aluminium oxide (neutral, Brockmann II, 50–200 μm, 60 A) from Acros. Reversed phase chromatography employed LiChroprep RP18 silica gel (40–63 μm) from Merck. – *Analytical High-Performance Liquid Chromatography (HPLC)* was carried out on a Shimadzu Nexera XR with autosampler SIL-20A and a diode array detector SPD-M20A using the column Eurosphere II 100-3C18 (150×4 mm) from Knauer.

Chemicals and procedures: all experiments were routinely carried out under argon atmosphere in heat-dried glassware. All reagents were purchased from commercial sources and used without further purification. All solvents used were of purity level 'p.a.' or were distilled prior to use. They were dried and stored according to standard protocols. All synthesised compounds were stored in the dark under argon atmosphere in a fridge at −20 °C. PMBCl, Ph_3PCHCO_2Bn and compounds **27**–**29** were prepared according to literature.

Syntheses

Methyl (S)-3-(3-Acetyl-4-hydroxyphenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (9):[18] A solution of L-tyrosine (3.00 g, 16.56 mmol, 1.0 equiv.) in nitrobenzene (66 mL) was treated with $AlCl_3$ (8.83 g, 66.23 mmol, 4.0 equiv.) and AcCl (1.4 mL, 19.87 mmol, 1.2 equiv.). After 17 h stirring at 100 °C and cooling to r.t. the mixture was added to ice (90 g), conc. HCl (15 mL) and EtOAc (100 mL). The aqueous phase was washed with EtOAc (3× 50 mL). The combined organic phases were extracted with H_2O (1× 100 mL) and this aqueous phase was washed with EtOAc (1× 50 mL) again. The combined aqueous phases were concentrated to a volume of ca. 30 mL and the resulting precipitate was isolated by filtration. The obtained hydrochloride **7**, dissolved in MeOH (ca. 80 mL), was treated dropwise with SO_2Cl (9.5 mL, 15.56 mmol, 7.9 equiv.) at 0 °C. After stirring for 17 h at r.t. the solvent was evaporated in vacuo to afford methyl ester **8**, which was used without further purification. A solution of **8** in dioxane/H_2O (1:1; 66 mL) was treated with NEt_3 (9.0 mL, 64.57 mmol, 3.9 eq) and Boc_2O (3.9 mL, 18.21 mmol, 1.1 equiv.) at 0 °C. After stirring for 20 h at r.t., HCl (150 mL, 1 M) was added and the resulting mixture was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 100:0→80:20) to afford the Boc-protected amino acid **9**. Yield: 3.742 g (11.09 mmol, 67% over 3

steps), colourless solid. R_f (SiO_2) 0.54 (*c*-hexane:EtOAc 80:20); m.p. 102–103 °C; $[\alpha]^{25}_D + 5.1°$ (c = 1, MeOH).

Methyl (S)-3-[3-Acetyl-4-(2-nitrobenzyloxy)phenyl]-2-[(tert-butoxycarbo-nyl)amino]propanoate (10):[18] A solution of amino acid **9** (5.32 g, 15.77 mmol, 1.0 equiv.) in DMF (79 mL) was treated with *ortho*-nitrobenzyl bromide (3.75 g, 17.35 mmol, 1.1 equiv.), freshly dried K_2CO_3 (4.58 g, 33.12 mmol, 2.1 equiv.) and TBAI (0.583 g, 1.577 mmol, 0.1 equiv.). The solution was stirred for 18 h at r.t. and then filtered through Celite. After addition of H_2O (ca. 500 mL) the resulting mixture was extracted with EtOAc (3×200 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The remainder was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10→40:60) to leave compound **10** as a colourless solid of m.p. 102 °C. Yield: 7.38 g (15.61 mmol, 99%); R_f (SiO_2) 0.44 (*n*-hexane:E-EtOAc 60:40); $[\alpha]^{25}_D + 5.1°$ (c = 1, MeOH).

Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3-hydroxy-4-((2-nitrobenzyl)-oxy)phenyl)propanoate (11):[18,19] A solution of **10** (4.81 g, 10.18 mmol, 1.0 equiv.) in CH_2Cl_2 (20 mL) was treated with mCPBA (7.53 g, 30.53 mmol, 3.0 equiv. 70%) and stirred for 20 h at 60 °C. After addition of $KHCO_3$ solution (1×20 mL, 5 wt-%) the mixture was extracted with EtOAC (3×15 mL). The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to afford an intermediate aryl acetate, which was used without further purification. A solution of this crude intermediate in MeOH (100 mL) was treated with sat. $NaHCO_3$ solution (25 mL, 0.4 M) and stirred for 6 d at 40 °C. After completion of the ester hydrolysis, H_2O (100 mL) was added. The aqueous phase was acidified with HCl (1 M) and then extracted with EtOAc (3×75 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10→70:30) to afford compound **11** as a beige foam of m.p. 37–39 °C. Yield: 2.93 g (6.56 mmol, 64.4% over 2 steps); R_f (SiO_2) 0.43 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D + 3.4°$ (c = 1, MeOH).

Methyl (S)-3-(3-(Allyloxy)-4-((2-nitrobenzyl)oxy)phenyl)-2-((tert-butoxy-carbonyl)amino)propanoate (12): A solution of **11** (2.85 g, 6.38 mmol, 1.0 equiv.) in DMF (21.5 mL) was treated with freshly dried K_2CO_3 (1.76 g, 12.76 mmol, 2.0 equiv.). After 20 min stirring at r.t., allyl bromide (610 µL, 7.02 mmol, 1.1 equiv.) was added. The mixture was stirred for 19 h at 35 °C and then filtered through Celite. After addition of H_2O (50 mL) the aqueous phase was extracted with EtOAc (3×30 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10→75:25) to afford **12** as colourless crystals of m.p. 107–109 °C. Yield: 2.86 g (5.89 mmol, 92.3%); R_f (SiO_2) 0.62 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D + 33.1°$ (c = 1, $CHCl_3$).

tert-Butyl (S)-2-(3-(Allyloxy)-4-((2-nitrobenzyl)oxy)benzyl)-3,5-dioxopyrrolidin-1-carboxylate (14):[3] A solution of methyl ester **12** (2.35 g, 4.84 mmol, 1.0 equiv.) in MeOH (24 mL) was treated with aqueous NaOH (7.5 mL, 1.5 equiv., 1 M) and stirred for 48 h at r.t. After addition of aqueous HCl (25 mL, 1 M) the mixture was extracted with EtOAc (3×25 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting acid was treated with Meldrum's acid (0.77 g, 5.32 mmol, 1.1 equiv.), DMAP (0.83 g, 6.77 mmol, 1.4 equiv.) and EDC·HCl (1.11 g, 5.80 mmol, 1.2 equiv.) in dry CH_2Cl_2 (16 mL). The mixture was stirred for 20 h at r.t., treated with EtOAc (50 mL) and then washed with H_2SO_4 (2×25 mL, 0.5 M). The aqueous phase was extracted with EtOAc (50 mL), the combined organic phases were washed with brine and dried over Na_2SO_4. After stirring for 2 h under reflux the solvent was evaporated under reduced pressure. The remainder was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→20:80) to afford 1.71 g (3.44 mmol, 71% over 2 steps) of tetramic acid **14** as a beige foam with m.p. 57–59 °C; $[\alpha]^{25}_D + 86.5°$ (c = 1, $CHCl_3$).

Methyl (S)-2-((tert-Butoxycarbonyl)amino)-3-(4-hydroxy-3-((4-methoxybenzyl)oxy)phenyl)propanoate (16): A solution of **11** (3.64 g, 8.15 mmol, 1.0 equiv.) in DMF (20 mL) was treated with PMBCl (1.60 g, 10.19 mmol, 1.25 equiv.) and freshly dried K_2CO_3 (2.82 g, 20.38 mmol, 2.5 equiv.). The suspension was stirred for 1 h at 120 °C and then filtered through Celite. After addition of H_2O (500 mL) the mixture was extracted with EtOAc (3×200 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10→75:25) to afford 3.02 g (5.33 mmol, 65.4%) of the PMB-protected intermediate **15**. A solution of **15** (1.14 g, 3.43 mmol) in dry CH_2Cl_2 (35 mL) was irradiated with a blacklight lamp (4 W, 366 nm) for 3 d. The solvent was evaporated and the residue was purified by column chromatography (aluminium oxide, neutral, *c*-hexane:EtOAc:MeOH 100:0:0→0:100:0→0:95:5) to afford 1.29 g (2.84 mmol, 83%) of **16** as an orange ropy oil; R_f (SiO_2) 0.54 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D + 3.0°$ (c = 1, MeOH).

Methyl (S)-3-(4-(Allyloxy)-3-((4-methoxybenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino) propanoate (17): A solution of **16** (1.22 g, 2.83 mmol, 1.0 equiv.) in DMF (9.5 mL) was treated with K_2CO_3 (1.17 g, 8.48 mmol, 3.0 equiv.), stirred for 20 min at r.t., and treated with allyl bromide (540 µl, 6.22 mmol, 1.1 equiv.). The mixture was stirred for 22 h at r.t. and then filtered through Celite. H_2O (300 mL) was added to the filtrate and the aqueous phase was extracted with EtOAc (3×75 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 100:0→85:15) to afford 1.33 g (2.83 mmol, quant.) of **17** as a beige solid with m.p. 75–77 °C; R_f (SiO_2) 0.62 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D + 3.51°$ (c = 1, $CHCl_3$).

tert-Butyl (S)-2-(4-(Allyloxy)-3-((4-methoxybenzyl)oxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate (19):[3] A solution of **17** (4.45 g, 9.44 mmol, 1.0 equiv.) in MeOH (46 mL) was treated with aq. NaOH (14 mL, 1.5 equiv., 1 M) and stirred for 25 h at r.t. HCl (50 mL, 1 M) was added and the mixture was extracted with EtOAc (3×35 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting acid **18** was treated with Meldrum's acid (1.45 g, 10.38 mmol, 1.1 equiv.), DMAP (1.61 g, 13.21 mmol, 1.4 equiv.) and EDC·HCl (2.17 g, 11.32 mmol, 1.2 equiv.) in CH_2Cl_2 (30 mL). After 18 h stirring at r.t., EtOAc (200 mL) was added and the resulting mixture was washed with H_2SO_4 (2×150 mL, 0.5 M). The aqueous phase was extracted with EtOAc (2×75 mL). The combined organic phases were washed with brine and dried over Na_2SO_4. After stirring for 2 h under reflux the solvent was evaporated under reduced pressure. The remainder was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→30:70) to afford 3.91 g (8.11 mmol, 85.9% over 2 steps) of tetramic acid **19** as a beige foam with m.p. 47–49 °C; $[\alpha]^{25}_D + 71.7°$ (c = 1, $CHCl_3$).

(S)-tert-Butyl-2-(3-(allyloxy)-4-((2-nitrobenzyl)oxy)benzyl)-4-(1-hydroxyhept-6-en-1-ylidene)-3,5-dioxo-pyrrolidine-1-carboxylate (22):[3] A solution of 6-heptenoic acid (0.477 g, 3.72 mmol, 1.1 equiv.) in CH_2Cl_2 (17 mL) was treated with EDC·HCl (0.875 g, 4.57 mmol, 1.35 equiv.) and DMAP (0.103 g, 0.845 mmol, 0.25 equiv.) at 0 °C. After 30 min, tetramic acid **14** (5.52 g, 15.99 mmol, 1.3 equiv.) dissolved in CH_2Cl_2 (10 mL) was added. The solution was stirred for 3 h, diluted with EtOAc (250 mL) and washed with sulfuric acid (2×250 mL, 0.5 M). The aqueous phase

was extracted with EtOAc (2×100 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The remaining yellow oil was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10→50:50) to yield 1.29 g (2.12 mmol, 63%) of 4-O-acyltetramic acid **20**. A solution of **20** (1.154 g, 1.90 mmol, 1.0 equiv.) in CH_2Cl_2 (19 mL) was treated with NEt_3 (320 µl, 2.283 mmol, 1.2 equiv.). After 10 min DMAP (0.116 g, 0.95 mmol, 0.5 equiv.) was added and the mixture was stirred for 16 h at r.t. Additional DMAP (0.058 g, 0.48 mmol, 0.25 equiv.) was added and stirring was continued for a further 5 h. The suspension was diluted with EtOAc (200 mL) and washed with H_2SO_4 (2×200 mL, 0.5 M). The combined aqueous phases were extracted with EtOAc (2×100 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The remainder was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→0:100) to afford 835 mg (1.38 mmol, 68%) of 3-acyltetramic acid **22** as an orange oil; $[\alpha]^{25}_D$ – 9.1° (c = 1, $CHCl_3$).

(S)-tert-Butyl-2-(4-(allyloxy)-3-((4-methoxybenzyl)oxy)benzyl)-4-(1-hydroxyhept-6-en-1-ylidene)-3,5-dioxo-pyrrolidine-1-carboxylate (23):[3] Analogously to **22**, tetramic acid **23** (483 mg, 816 µmol, 75%) was obtained as an orange oil from 6-heptenoic acid (181 mg, 1.409 µmol) in CH_2Cl_2 (5.4 mL), EDC·HCl (332 mg, 1.734 µmol), DMAP (2×66 mg, 1.10 µmol), tetramic acid **19** (522 mg, 1.084 µmol), and NEt_3 (181 µl, 1.30 µmol); $[\alpha]^{25}_D$ – 13.7° (c = 1, $CHCl_3$).

(3S,6Z,12E)-4-Aza-7-hydroxy-17-((2-nitrobenzyl)oxy)-15-oxa-5,21-dioxo-tricyclo[14.3.1.13,6]henicosa-1(19),6,12,17,20(16)-pentaene (24):[8] A solution of diene **22** (308 mg, 508 µmol, 1.0 equiv.) in degassed CH_2Cl_2 (100 mL) was treated with 2nd generation Grubbs catalyst (21.6 mg, 2.5 µmol, 0.05 equiv.) and heated at reflux for 18 h. After evaporation of the solvent under reduced pressure the remainder was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→0:100) to afford 241 mg (417 µmol, 82%) of macrocycle **24** as a beige foam with m.p. 59–61 °C; $[\alpha]^{25}_D$ + 50.9° (c = 1, $CHCl_3$).

(3S,6Z,12E)-4-Aza-7,17-dihydroxy-15-oxa-5,21-dioxo-tricyclo[14.3.1.1$_{3,6}$]henicosa-1(19),6,12,17,20(16)-pentaene (6): A solution of macrocycle **24** (206 mg, 356 µmol) in CH_2Cl_2 (100 mL) was irradiated with a blacklight lamp (4 W, 366 nm) for 5 d. After completion, TFA (10 mL) was added and the mixture was stirred for a further 15 min. The volatiles were evaporated under reduced pressure and the residue was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→20:80) to afford *meta*-cyclophane **6** (48 mg, 140 µmol, 39%) as a beige foam; $[\alpha]^{25}_D$ + 58.7° (c = 1, MeOH).

(3S,6Z,12E)-4-Aza-7,17-dihydroxy-15-oxa-5,21-dioxo-tricyclo[14.2.2.1$_{3,6}$]henicosa-1(18),6,12,16(17),19-pentaene (4):[8] A solution of diene **23** (288 mg, 487 µmol, 1.0 equiv.) in degassed CH_2Cl_2 (100 mL) was treated with 2nd generation Grubbs catalyst (20.7 mg, 24 µmol, 0.05 equiv.) and heated at reflux for 18 h. After evaporation of the solvent the residue was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→0:100) to afford macrocycle **25** (215 mg, 381 µmol, 78%). A solution of crude **25** (196 mg, 347 µmol, 1.0 equiv.) in CH_2Cl_2 (18 mL) was treated with Et_3SiH (285 µL, 1783 µmol, 5.0 equiv.) and TFA (2.85 mL) at 0 °C and stirred at this temperature for 1 h. After stirring for 2 h at r.t., toluene (25 mL) was added. The solution was concentrated under reduced pressure, treated with MeOH, and concentrated once more. Purification of the residue by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 90:10→30:70) yielded *para*-cyclophane **4** (92.5 mg, 269 µmol, 78%) as a beige foam; $[\alpha]^{25}_D$ + 22.4° (c = 1, MeOH).

Benzyl-(2E,6E)-8-[[(1,1-dimethylethyl)dimethylsilyl]oxy]octa-2,6-dienoate (30): A mixture of aldehyde **29** (5.48 g, 23.97 mmol, 1.0 equiv.) and Ph_3PCHCO_2Bn (12.79 g, 31.16 mmol, 1.3 equiv.) in THF (120 mL) was stirred at 40 °C for 17 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 99:1→95:5) to afford ester **30** (7.691 g, 21.330 mmol, 89%) as a colourless oil; R_f (SiO_2) 0.46 (*c*-hexane:EtOAc 95:5).

Benzyl (2E,6E)-8-hydroxyocta-2,6-dienoate (31): A mixture of **30** (3.19 g, 8.86 mmol, 1.0 equiv.), THF (44 mL), H_2O (44 mL) and AcOH (132 mL) was stirred for 21 h at r.t., neutralised with aqueous NaOH (4 M, 500 mL), and extracted with EtOAc (3×300 mL). The combined organic phases were concentrated to a volume of 300 mL and washed with sat. $NaHCO_3$ solution (300 mL). The aqueous phase was re-extracted with EtOAc (3×150 mL), the combined organic phases were finally washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:E-EtOAc 75:25) to afford alcohol **31** (2.13 g, 8.64 mmol, 98%) as a colourless oil; R_f (SiO_2) 0.52 (*c*-hexane:EtOAc 50:50).

Benzyl-(E)-5-((2S,3S)-3-(hydroxymethyl)oxiran-2-yl)pent-2-enoate (32): A suspension of ground 3 Å molecular sieve (ca. 12.5 g) in CH_2Cl_2 (165 mL) was treated with titanium isopropoxide (8.7 mL, 29.36 mmol, 0.6 equiv.), (+)-L-diethyltartrate (5.0 mL, 29.36 mmol, 0.6 equiv.) and *tert*-butyl hydroperoxide (5.5 M in *n*-decane, 10.7 mL, 58.73 mmol, 1.2 eq) at r.t. The suspension was cooled to −25 °C and allyl alcohol **31** (12.054 g, 48.94 mmol, 1.0 equiv.) was added. After stirring for 19 h at −25 °C the reaction was quenched with a citric acid solution (30% in H_2O, 150 mL). The resulting mixture was extracted with Et_2O (2×100 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10c→c60:40) to afford compound **32** (10.75 g, 40.98 mmol, 84%) as a colourless oil; R_f (SiO_2) 0.27 (*c*-hexane:EtOAc 50:50); *ee* = 97.2%; $[\alpha]^{25}_D$ −26.8° (c = 1, $CHCl_3$).

Benzyl (E)-5-((2S,3R)-3-(bromomethyl)oxiran-2-yl)pent-2-enoate (33): A solution of PPh_3 (5.66 g, 21.57 mmol, 1.2 equiv.) and imidazole (1.47 g, 21.57 mmol, 1.2 equiv.) in CH_2Cl_2 (30 mL) was treated with Br_2 (1.1 mL, 21.57 mmol, 1.2 equiv.) at 0 °C. After 10 min stirring at 0 °C alcohol **32** (4.72 g, 17.98 mmol, 1.0 equiv.) was added. The mixture was warmed to r.t. over 4 h and stirred at this temperature for a further 16 h. *c*-Hexane was added and the resulting precipitate was removed by filtration. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 97.5:2.5→92.5:7.5) to afford the bromide **33** (4.34 g, 13.35 mmol, 74%) as a colourless oil, which solidified upon storage at −20 °C; R_f (SiO_2) 0.54 (*c*-hexane:EtOAc 75:25); m.p. 39 °C; $[\alpha]^{25}_D$ −5.8° (c = 1, $CHCl_3$).

5-((2S,3R)-3-(bromomethyl)oxiran-2-yl)pentanoic acid (34): A suspension of **33** (231 mg, 710 µmol, 1.0 equiv.) in EtOAc (140 mL) was treated with 5% palladium on charcoal (46 mg) at r.t., saturated with H_2 gas and stirred under an atmosphere of H_2 for 15 h. The mixture was then filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH 99.5:0.5→99:1) to afford carboxylic acid **34** (106 mg, 447 µmol, 63%) as a colourless wax; R_f (SiO_2) 0.24 (CH_2Cl_2:MeOH 95:9); m.p. 67.5 °C; $[\alpha]^{25}_D$ −9.6° (c = 1, $CHCl_3$).

tert-Butyl-(S)-2-(4-(allyloxy)-3-((4-methoxybenzyl)oxy)benzyl)-4-(5-((2S,3R)-3-(bromomethyl)oxiran-2-yl)-1-hydroxypentylidene)-3,5-dioxopyrrolidine-1-carboxylate (35): A solution of **34** (1.28 g, 5.40 mmol, 1.3 equiv.) in CH_2Cl_2 (21 mL) was treated with EDC·HCl

(1.27 g, 6.64 mmol, 1.6 equiv.) and DMAP (0.254 g, 2.08 mmol, 0.5 equiv.) at 0 °C. After 10 min the tetramic acid **19** (2.00 g, 4.15 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) was added. The resulting solution was stirred for 6 h at r.t. and then treated with NEt_3 (695 µl, 4.98 mmol, 1.2 equiv.). After 10 min more DMAP (0.254 g, 2.08 mmol, 0.5 equiv.) was added and the mixture stirred for a further 16 h at r.t. The suspension was diluted with CH_2Cl_2 (150 mL) and washed with H_2SO_4 (2×150 mL, 0.5 M). The combined aqueous phases were extracted with EtOAc (2×100 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The remainder was purified by column chromatography on reversed phase silica gel (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→20:80) to afford 3-acyltetramic acid **35** (1.415 g, 2.02 mmol, 49%) as an orange oil; $[\alpha]^{25}_D$ – 16.3° (c = 1, $CHCl_3$).

(3S,12S,14S,6E)-4-Aza-7,18-dihydroxy-13,16-dioxa-5,22-dioxo-tetracyclo[15.2.2.1$_{3,6}$.0$_{12,14}$]docosa-1(19),6,17(18),20-tetraene (3): A solution of **35** (230 mg, 328 µmmol, 1.00 equiv.) in MeOH (16.4 mL) was treated with $Pd(PPh_3)_4$ (19 mg, 16 µmol, 0.05 equiv.) and freshly dried K_2CO_3 (91 mg, 657 µmol, 2.00 equiv.). After stirring for 23 h at r.t., CH_2Cl_2 (50 mL) was added. The organic phase was washed with H_2SO_4 (2×100 ml, 0.5 M) and the combined aqueous phases were extracted with CH_2Cl_2 (3×50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The allyl deprotected product was used straight away without purification. A solution of it in DMF (66 mL) was treated with freshly dried K_2CO_3 (91 mg, 657 µmol, 2.00 equiv.), 18-c-6 (43 mg, 164 µmol, 0.50 equiv.) und TBAI (1.2 mg, 52 µmol, 0.01 equiv.) and heated to 100 °C for 22 h. After cooling to r.t., CH_2Cl_2 (50 mL) was added and the organic phase was washed with H_2SO_4 (2×100 mL). The combined aqueous phases were extracted with CH_2Cl_2 (3×50 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on reversed phase silica gel (RP–C18, H_2O:MeCN + 0.1% HCO_2H 90:10→35:65), to afford macrocycle **36** (95 mg, 164 µmol, 50% over 2 steps) as a beige foam. A solution of **36** in CH_2Cl_2 (8 mL) was treated with Et_3SiH (93 µL, 802 µmol, 5.0 equiv.) and TFA (1.3 mL) at 0 °C. After stirring for 15 min at 0 °C, toluene (25 mL) was added. The mixture was concentrated under reduced pressure, toluene was added once more, and the volatiles removed in vacuo. Purification by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 90:10→40:60) yielded the *para*-cyclophane **3** (43 mg, 120 µmol, 75%) as a beige foam; $[\alpha]^{25}_D$ + 45.7 (c = 1, MeOH).

Methyl (S)-3-(3-acetyl-4-((4-methoxybenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (37): A solution of phenol **9** (3.50 g, 10.38 mmol, 1.0 equiv.) in DMF (26 mL) was treated with PMBCl (2.03 g, 12.98 mmol, 1.25 equiv.) and freshly dried K_2CO_3 (3.59 g, 25.96 mmol, 2.5 equiv.). The mixture was stirred for 1 h at 120 °C, then additional PMBCl (1.02 g, 6.49 mmol, 1.13 equiv.) was added, and the solution was stirred for a further 1 h at 120 °C. After addition of H_2O (400 mL) the mixture was extracted with EtOAc (3× 200 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:E-EtOAc 90:10→70:30) to afford **37** (4.56 g, 9.96 mmol, 96%) as a colourless gum; R_f (SiO_2) 0.51 (*n*-hexane:EtOAc 60:40); $[\alpha]^{25}_D$ + 7.3° (c = 1, MeOH).

Methyl (S)-3-(3-acetoxy-4-((4-methoxybenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (38): A solution of **37** (239 mg, 522 µmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) was treated with mCPBA (515 mg, 1.09 mmol, 4.0 equiv. 70%) and stirred for 20 h at 60 °C. After addition of sat. K_2CO_3 solution (20 mL) the mixture was extracted with EtOAC (3×15 mL). The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 80:20→75:25) to leave **38** (182 mg, 384 µmol, 74%) as a pale orange crystalline solid with m.p. 96 °C; R_f (SiO_2) 0.51 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D$ + 4.6° (c = 1, MeOH).

Methyl (S)-2-[(tert-butoxycarbonyl)amino]-3-[3-hydroxy-4-((4-methoxybenzyl)oxy)phenyl]-propanoate (39): A solution of **38** (187 mg, 395 µmol, 1.0 equiv.) in MeOH (4 mL) was treated with sat. $NaHCO_3$ solution (990 µL) and stirred for 17 h at 40 °C. Aqueous HCl (80 mL, 1 M) was added and the aqueous phase was extracted with EtOAc (3×30 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The remainder was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 95:5→80:20) to afford **39** (119 mg, 276 µmol, 70%) as a beige foam with m.p. 64 °C; R_f (SiO_2) 0.52 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D$ + 3.5° (c = 1, MeOH).

Methyl (S)-3-(3-(allyloxy)-4-((4-methoxybenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (40): A solution of phenol **39** (260 mg, 603 µmol, 1.0 equiv.) in DMF (2 mL) was treated with freshly dried K_2CO_3 (250 mg, 1.81 mmol, 3.0 equiv.) and stirred for 20 min at r.t. Allyl bromide (115 µL, 1.33 mmol, 2.2 equiv.) was added and stirring continued for a further 17 h at r.t. After addition of H_2O (100 mL) the aqueous phase was extracted with EtOAc (3× 25 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, *c*-hexane:E-EtOAc 95:5→84:16) to afford **40** (262 mg, 556 µmol, 92%) as a colourless crystalline solid of m.p. 92 °C; R_f (SiO_2) 0.61 (*c*-hexane:E-EtOAc 60:40); $[\alpha]^{25}_D$ + 2.8° (c = 1, MeOH).

tert-Butyl (S)-2-(3-(allyloxy)-4-((4-methoxybenzyl)oxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate (42): A solution of ester **40** (458 mg, 971 µmol, 1.0 equiv.) in MeOH (4.9 mL) was treated with aqueous NaOH (1.5 mL, 1.5 equiv., 1 M) and stirred for 17 h at r.t. After addition of aqueous HCl (25 mL, 1 M) the mixture was extracted with EtOAc (3×15 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting acid **41** was dissolved in CH_2Cl_2 (3.25 mL), treated with Meldrum's acid (210 mg, 1.46 mmol, 1.5 equiv.), DMAP (166 mg, 1.36 mmol, 1.4 equiv.) and EDC·HCl (242 mg, 1.26 mmol, 1.3 equiv.), and stirred for 22 h at r.t. EtOAc (50 mL) was added and the mixture was washed with H_2SO_4 (2× 60 mL). The aqueous phase was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and stirred for 4 h under reflux. The solvent was removed under reduced pressure and the remainder was purified by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→ 40:60) to afford tetramic acid **42** (325 mg, 675 µmol, 70% over 2 steps) as a beige foam; $[\alpha]^{25}_D$ + 155° (c = 1, MeOH).

Butyl-(S)-2-(3-(allyloxy)-4-((4-methoxybenzyl)oxy)benzyl)-4-(5-((2S,3S)-3-(bromomethyl)oxiran-2-yl)-1-hydroxypentylidene)-3,5-dioxopyrrolidine-1-carboxylate (43): A solution of acid **34** (191 mg, 619 µmol, 1.55 equiv.) in CH_2Cl_2 (3.1 mL) was treated with EDC·HCl (190 mg, 990 µmol, 1.9 equiv.) and DMAP (38 mg, 309 µmol, 0.6 equiv.) at 0 °C. After 10 min, tetramic acid **42** (250 mg, 519 µmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) was added. The solution was stirred for 2 h at r.t. and then treated with NEt_3 (103 µL, 743 µmol, 1.4 equiv.). After 10 min, DMAP (38 mg, 309 µmol, 0.6 equiv.) was added and the mixture was stirred for a further 16 h at r.t. The suspension was diluted with CH_2Cl_2 (30 mL) and washed with H_2SO_4 (2×50 mL, 0.5 M). The combined aqueous phases were extracted with CH_2Cl_2 (2×30 mL). The combined organic phases were dried over Na_2SO_4, washed with brine and concentrated under reduced pressure. The residue was purified by column chromatography on reversed phase

silica gel (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→20:80) to afford 3-acyltetramic acid **43** (184 mg, 263 μmol, 51%) as an orange oil; $[\alpha]^{25}_D$ – 26.2° (c = 1, MeOH).

(3S,12S,14S,6E)-4-Aza-7,18-dihydroxy-13,16-dioxa-5,22-dioxo-tetracyclo[15.3.1.1$_{3,6}$.01$_{2,14}$]docosa-1(20),6,17(21),18-tetraene (5): A solution of **43** (169 mg, 241 μmol, 1.00 equiv.) in MeOH (12 mL) was treated with $Pd(PPh_3)_4$ (14 mg, 12 μmol, 0.05 equiv.) and freshly dried K_2CO_3 (67 mg, 482 μmol, 2.00 equiv.). After stirring for 15 h at r.t. H_2SO_4 (60 mL, 0.5 M) and CH_2Cl_2 (60 mL) were added. The organic phase was washed with H_2SO_4 (60 mL, 0.5 M) and the combined aqueous phases were extracted with CH_2Cl_2 (3×25 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. A solution of the resulting crude tetramic acid **44** in DMF (48 mL) was treated with freshly dried K_2CO_3 (67 mg, 482 μmol, 2.00 equiv.), 18-c-6 (32 mg, 121 μmol, 0.50 equiv.) and TBAI (0.9 mg, 2.4 μmol, 0.01 equiv.). The solution was stirred at 100 °C for 24 h. After cooling to r.t., H_2SO_4 (300 mL, 0.5 M) was added and the aqueous phase was extracted with CH_2Cl_2 (3× 50 mL). The combined organic phases were washed with H_2SO_4 (300 ml, 0.5 M) and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on reversed phase silica gel (RP–C18, H_2O:MeCN + 0.1% HCO_2H 90:10→20:80), which afforded a mixture of Boc-protected and deprotected macrocycle **45**. This mixture was dissolved in CH_2Cl_2 (6.3 mL) and treated with Et_3SiH (101 μl, 632 μmol, 5.0 equiv.) and TFA (1.0 ml) at 0 °C. After stirring for 15 min at 0 °C, toluene (25 mL) was added. The solution was concentrated under reduced pressure, toluene was added once more and the mixture concentrated. Purification by column chromatography (RP–C18, H_2O:MeCN + 0.1% HCO_2H 90:10→40:60) yielded the *meta*-cyclophane **5** (36 mg, 100 μmol, 42% over 3 steps) as a beige foam; $[\alpha]^{25}_D$ + 18° (c = 1, MeOH).

tert-Butyl-(S)-2-(4-(allyloxy)benzyl)-3-((((2S,3R)-3-(bromomethyl)oxiran-2-yl)-pentanoyl)oxy)-5-oxo-2,5-dihydro-1H-pyrrol-1-carboxylate (47): A solution of carboxylic acid **34** (2.92 g, 12.30 mmol, 1.0 equiv.) in CH_2Cl_2 (61.5 mL) was treated with EDC·HCl (2.83 g, 14.76 mmol, 1.2 equiv.) and DMAP (0.30 g, 2.46 mmol, 0.2 equiv.) at 0 °C. After 30 min stirring at r.t. tetramic acid **46** (5.52 g, 15.99 mmol, 1.3 equiv.) was added. The solution was stirred for 2.5 h at r.t. and then diluted with EtOAc (75 mL). The organic phase was washed with sulfuric acid (2×50 mL). The aqueous phase was extracted with EtOAc (3×50 mL), the combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The remaining yellow oil was purified by column chromatography (SiO_2, *c*-hexane:EtOAc 90:10→60:40) to yield **47** (5.73 g, 10.15 mmol, 82.5%) as a red orange oil; R_f (SiO_2) 0.55 (*c*-hexane:EtOAc 60:40); $[\alpha]^{25}_D$ + 86.5° (c = 1, $CHCl_3$).

tert-Butyl-(S)-2-(4-(allyloxy)benzyl)-4-(((2S,3R)-3-(bromomethyl)oxiran-2-yl)-1-hydroxy-2- pentylidene)-3,5-dioxopyrrolidine-1-carboxylate (48): A solution of tetramate **47** (361 mg, 640 μmol, 1.0 equiv.) in CH_2Cl_2 (6.4 mL) was treated with NEt_3 (107 μl, 767 μmol, 1.2 equiv.). After 10 min, DMAP (39 mg, 320 μmol, 0.5 equiv.) was added and the mixture was stirred for 19 h at r.t. The suspension was diluted with EtOAc (10 ml) and washed with H_2SO_4 (2×10 mL, 0.5 m). The combined aqueous phases were extracted with EtOAc (3×10 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The remaining residue was purified by column chromatography on reversed phase silica gel (RP–C18, H_2O:MeCN + 0.1% HCO_2H 60:40→0:100) to afford 3-acyltetramic acid **48** (188 mg, 333 μmol, 52%) as a bright orange oil; $[\alpha]^{25}_D$ – 54.2° (c = 1, $CHCl_3$).

Nor-Macrocidin A (51): A solution of 3-acyltetramic acid **48** (2.912 g, 5.16 mmol, 1.00 equiv.) in MeOH (260 mL) was treated with $Pd(PPh_3)_4$ (299 mg, 258 μmol, 0.05 equiv.) and freshly dried K_2CO_3 (1.426 g, 10.32 mmol, 2.00 equiv.), stirred at r.t. for 22 h, and then treated with H_2SO_4 (250 mL, 0.5 M). The aqueous phase was extracted with EtOAc (3×250 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. A solution of the resulting crude tetramic acid **49** in DMF (350 mL) was treated with K_2CO_3 (1.426 g, 10.32 mmol, 2.00 equiv.), 18-c-6 (681 mg, 2.58 mmol, 0.50 equiv.) and TBAI (19 mg, 52 μmol, 0.01 equiv.). The resulting mixture was heated to 100 °C for 24 h. After cooling to r.t. H_2SO_4 (1.4 L, 0.5 M) was added and the aqueous phase was extracted with EtOAc (3×250 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product **50** was purified by column chromatography on reversed phase silica gel (RP–C18, H_2O:MeCN + 0.1% HCO_2H 70:30→0:100), which afforded a mixture of Boc-protected and deprotected nor-macrocidin A. This mixture was dissolved in CH_2Cl_2 (35 mL) and treated with TFA (3.5 mL, 10 vol.–%). After stirring at r.t. for 10 min, toluene (25 mL) was added. The volatiles were removed under reduced pressure and more toluene (25 mL) was added. Removal of all volatiles yielded nor-macrocidin A (**51**) (588 mg, 1.71 mmol, 33% over 3 steps) as an orange ropy foam of m.p. 97–99 °C; $[\alpha]^{25}_D$ + 91.2° (c = 1, MeOH).

Biological evaluation

Assays for antimicrobial, cytotoxic, and antibiofilm activities are described in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: bacterial biofilms · macrocidin · metacyclophanes · polycyclic tetramate macrolactams

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