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Synthesis of the fungus metabolite cladosin C†

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Cladosin C is one of the few known enaminotetramic acids, isolated from extracts of the deep sea fungus *Cladosporium sphaerospermum*. It was synthesised in ten steps and 14% overall yield by a late-stage amination of the corresponding 3-acyltetramic acid. This was obtained by a Dieckmann condensation of an *N*-β-ketoacylaminoester derived from dehydrovalinate and the thioester-terminated side chain containing the stereogenic centre which stemmed from poly-(*R*)-3-hydroxybutyrate.

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Introduction

The cladosins A–D (**1**–**4**) (Fig. 1) were isolated as mixtures of two geometric isomers from the deep sea derived fungus *Cladosporium sphaerospermum* 2005-01-E3 and structurally characterised by D. Li *et al.* in 2013.[1] They are distinguished by their 3-enamino side chains and their biosynthetic derivation from valine, two features quite rare for naturally occurring tetramic acids.[2] Synthetic 3-enaminotetramic acids were recently shown by Moloney *et al.* to have a different mode of antibacterial action to the more common analogous 3-acyltetramic acids.[3] Also, their bioactivity appears to be less pronounced than that of the far better investigated[4] 3-acyl congeners. Of the cladosins A–D, only cladosin C (**3**) showed moderate bioactivity, namely an anti-influenza A H1N1 virus effect.[1] Here, we report a short first synthesis of cladosin C.

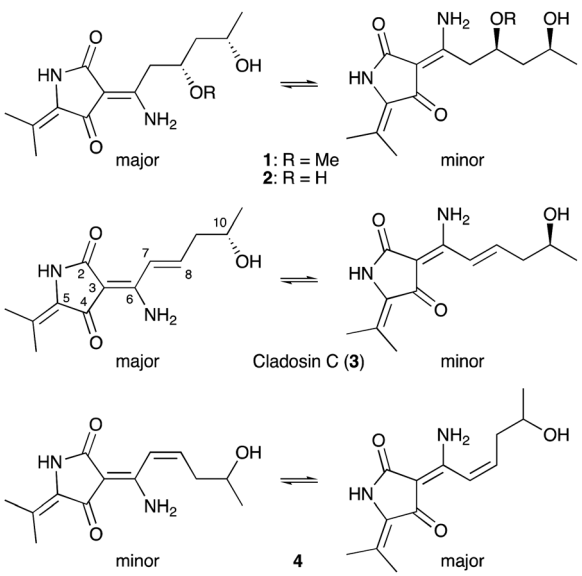

Fig. 1 Structures of cladosins A–D (**1**–**4**).

Results and discussion

Fig. 2 shows our retrosynthetic approach. Cladosin C was to be prepared by a late-stage amination of the corresponding 3-acyltetramic acid **5** which in turn should be obtainable by a Dieckmann condensation of the *N*-(β-ketoacyl)amino ester **6**. The latter was thought to be accessible by aminolysis of the thioester-terminated side chain **7** with amino ester **8** according to Ley *et al.*[5]

The β-ketothioester **7** was prepared in six steps. The cheap, commercially available poly-(*R*)-3-hydroxybutyrate (**9**) was depolymerised to give ethyl ester *R*-**10** in 81% yield (Scheme 1).

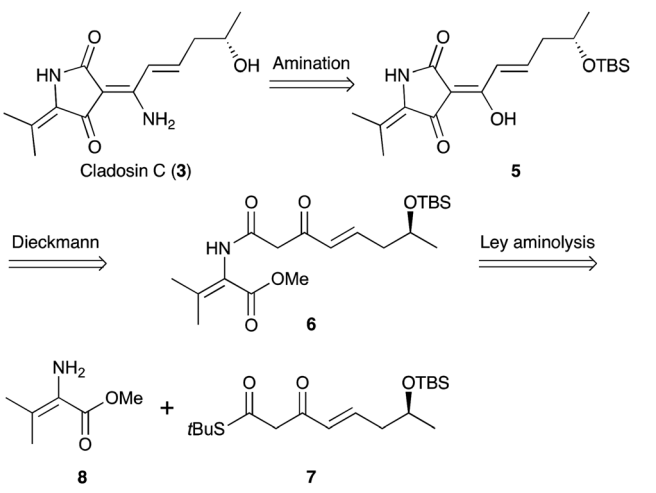

Fig. 2 Retrosynthetic approach to cladosin C (**3**).

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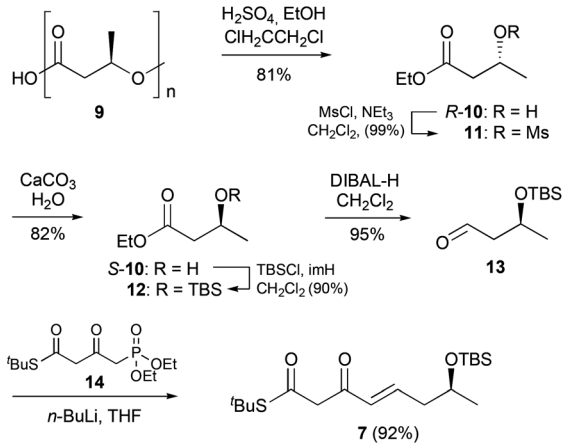
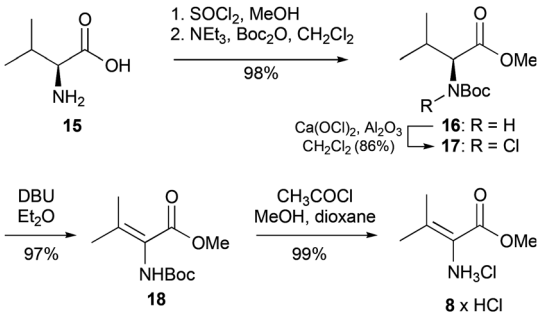
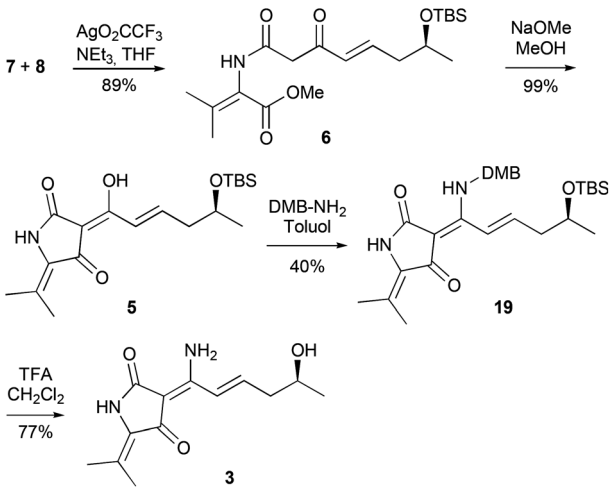


Scheme 1 Synthesis of side chain thioester **7**.



Scheme 2 Synthesis of methyl dehydrovalinate hydrochloride.



Scheme 3 Ley *N*-acylation, Dieckmann cyclisation and amination to give **3**.

Its stereogenic centre was inverted by quantitatively mesylating the OH group to leave **11** which was treated with $CaCO_3$ in water to undergo an S_N2-type hydrolysis furnishing ethyl ester *S*-**10** in 82% yield.[6] TBS protection of its hydroxy group with TBSCl and imidazole afforded ester **12** in 90% yield. The latter was reduced with DIBAL–H to the corresponding aldehyde **13** in 95% yield according to literature.[7] An *E*-selective Horner–Wadsworth–Emmons olefination of aldehyde **13** with Ley's *t*-butyl 4-diethylphosphono-3-oxobutanthioate (**14**),[5] prepared by a modified protocol,[8] afforded β-ketothioester **7** in excellent 92% yield when *n*-BuLi was used as the base. Other bases, such as NaH or KHMDS gave poor yields below 50%.

The required dehydrovaline methyl ester **8** was synthesised in five steps starting from L-valine (**15**), which was converted to the methyl ester hydrochloride with $SOCl_2$ and then Boc-protected with Boc_2O under basic conditions to give methyl *N*-Boc-valinate (**16**) in 98% yield over two steps. In order to oxidise the amino group of **16** to an imine which can tautomerize to the desired dehydrovaline, we chlorinated the nitrogen with $Ca(OCl)_2$ in the presence of Al_2O_3 in 86% yield.[9] Other methods were either cumbersome (*t*-BuOCl) or did not work at all such as the chlorination with NaOCl and Oxon® in the presence of NaCl or trichloroisocyanuric acid. This chlorination only works for N-atoms being part of amides or carbamates.

N-Chloroamino ester **17** was dehydrohalogenated with DBU to afford the protected dehydrovaline methyl ester **18** in 97% yield.[10] Since Ley's aminolysis of thioesters does not work for electron-poor amino groups we had to remove the electron withdrawing Boc-group first. Treatment of **18** with acetyl chloride and MeOH furnished methyl dehydrovalinate hydrochloride **8** × HCl in 99% yield and thus in 81% over all five steps (Scheme 2).

The *N*-acylation of **8** with thioester **7** in the presence of AgO_2CCF_3 according to Ley *et al.*[5] afforded the *N*-(β-ketoacyl) amino ester **6** in 89% yield when carried out under strict exclusion of moisture (Scheme 3). The Dieckmann cyclisation of **6** was carried out with five equivalents of NaOMe in methanol under reflux for one hour to give the corresponding pure 3-acyltetramic acid **5** in 99% yield without further purification. Its conversion into the desired enamine turned out to be far from straightforward. Treatment of **5** with NH_4OH according to Beyer *et al.*[11] gave very little product, while its reaction with HMDS failed completely even after three days refluxing in dichloroethane. However, reaction of **5** with 2,4-dimethoxybenzylamine (DMB-NH_2) afforded the DMB-enamine tetramic acid **19** in 40% yield. Moloney *et al.*[12] also showed that the yield of reactions of 3-acyltetramic acids with alkyl amines may vary from 25% to 95%. A simultaneous deprotection of hydroxy and amino groups of **19** with CH_2Cl_2/TFA 9 : 1 eventually afforded cladosin C (**3**) in 77% yield as a 2 : 1 mixture of 3*E*- and 3*Z*-isomers.

Conclusions

The marine fungus metabolite cladosin C (**3**) was synthesised in ten steps (longest linear sequence) starting from cheap, commercially available compounds. The synthetic approach should be applicable also to other 3-enaminotetramic acids.

Three details of this synthesis are remarkable and probably of a general nature. The *N*-chlorination of methyl valinate was

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possible only after a preceding Boc protection and only with $Ca(OCl)_2$ in the presence of Al_2O_3, however in high yield and under mild conditions. The Dieckmann cyclisation proceeded quantitatively since an excess of base and heating could be applied because of the absence of an acidic H-atom at C5 of the 3-acyltetramic acid **5**. Finally, its amination with DMB-NH_2, although of moderate yield in this particular case, took place under mild and water-free conditions and offers various debenzylation options.

Experimental section

General information

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 (300 MHz) or on a Bruker Avance DRX-500 spectrometer (500 MHz, with cryoprobe). Chemical shifts are given in parts per million using the residual solvent peak as an internal standard. Coupling constants (*J*) are quoted in Hz. Abbreviations for the multiplicity used: s singlet, d doublet, t triplet, q quartet, sx sextet, and m multiplet. Mass spectra were measured with a Varian MAT 8500 (EI, 70 eV). High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. Optical rotations were measured at 589 nm (Na-D line) on a PerkinElmer 241 Polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. For chromatography silica gel 60 (230–400 mesh) and RP-silica gel C 18 endcapped polygorep 100-50 were used. All reagents were purchased from commercial sources and were used without further purification. Reactions were routinely carried out under an atmosphere of dry argon unless stated otherwise. All glassware was flame-dried before use. Analytical TLC was carried out using Merck silica gel $60GF_{254}$ pre-coated aluminium-backed plates or Merck 60RP-18 F_{254s} foil plates.

Ethyl (*R*)-3-hydroxybutyrate (*R*-10). A suspension of poly-(*R*)-3-hydroxybutyrate (**9**) (6.0 g, 79.7 mmol, 1 equiv.) in 1,2-dichlorethane (60 mL) was treated with H_2SO_4 (1.3 mL, 23.9 mmol, 0.3 equiv.) and ethanol p.a. (20 mL) and stirred for 75 h at reflux. After adding brine (70 mL) the mixture was stirred for a further 10 min and then extracted with CH_2Cl_2. The combined organic layers were washed with brine, aqueous $NaHCO_3$ solution, and a second time with brine. After drying over $MgSO_4$ the solvent was removed under reduced pressure to give *R*-**10** (8.52 g, 81%) as a light orange oil. R_f = 0.30 (*n*-hexane/ethyl acetate 2 : 1); $[\alpha]_D^{25}$ −36.9 (*c* 1.0 in $CHCl_3$) (lit[6] $[\alpha]_D$ −44.5 (*c* 1.0 in $CHCl_3$)); IR (ν_{max}/cm^{-1}) 3340, 2978, 1732, 1373, 1296, 1178, 1116, 1068, 1027, 947, 845, 593; ^1H NMR (300 MHz, $CDCl_3$) δ 1.14 (3H, d, J = 6.3 Hz, $CHCH_3$), 1.19 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.35 (1H, dd, J = 16.2, 8.3 Hz, OCOCH), 2.40 (1H, dd, J = 16.2, 4.3 Hz, OCOCH), 3.23 (1H, br. s, OH), 4.08 (2H, q, J = 7.2 Hz, CH_2CH_3), 4.02–4.16 (1H, m, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0 (CH_2CH_3), 22.4 ($CHCH_3$), 42.8 (CH_2CH), 60.4 (CH_2CH_3), 64.0 (CH), 172.5 (CO); HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_6H_{13}O_3$ 133.08592, found 133.08596.

Ethyl (*R*)-3-mesyloxybutyrate (11). Ester *R*-**10** (2.0 g, 15.13 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (50 mL), cooled to 0 °C and MsCl (1.41 mL, 18.16 mmol, 1.2 equiv.) and NEt_3 (2.52 mL, 18.16 mmol, 1.2 equiv.) were added dropwise *via* a syringe. The reaction was stirred for 2 h and slowly warmed up to room temperature. The suspension was filtered over Celite and the filtrate was evaporated *in vacuo*. The residue was desolved in Et_2O (50 mL) and filtered a second time over Celite. After removing the solvent under reduced pressure, **11** (3.14 g, 99%) was obtained as a colourless oil. R_f = 0.39 (*n*-hexane/EtOAc 2 : 1); $[\alpha]_D^{25}$ −26.0 (*c* 1.0 in $CHCl_3$) (lit[13] $[\alpha]_D$ −32.1 (*c* 1.0 in $CHCl_3$)); IR (ν_{max}/cm^{-1}) 2984, 1731, 1346, 1300, 1194, 1170, 1105, 1028, 974, 914, 895, 795, 730; ^1H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.46 (3H, d, J = 6.1 Hz, $CHCH_3$), 2.53 (1H, dd, J = 16.4, 4.6 Hz, OCOCH), 2.73 (1H, dd, J = 16.4, 8.7 Hz, OCOCH), 2.99 (3H, s, SCH_3), 4.12 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.15–5.03 (1H, m, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0 (CH_2CH_3), 21.2 ($CHCH_3$), 38.0 (SCH_3), 41.0 (CH_2CH), 60.7 (CH_2CH_3), 75.8 (CH), 169.5 (CO); HRMS (ESI) *m*/*z* $[M + Na]^+$ calcd for $C_7H_{14}O_5SNa$ 233.04542, found 233.04500.

Ethyl (*S*)-3-hydroxybutyrate (*S*-10). Mesylate **11** (12.61 g, 60.0 mmol, 1 equiv.) was dissolved in H_2O (120 mL) and $CaCO_3$ (3.30 g, 33.0 mmol, 0.55 equiv.) was added. After stirring at 80 °C for 3 h the mixture was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over $MgSO_4$. After removal of the volatiles under reduced pressure *S*-**10** (6.51 g, 82%) was obtained as a colourless oil. R_f = 0.34 (*n*-hexane/EtOAc 2 : 1); $[\alpha]_D^{25}$ +33.1 (*c* 3.0 in $CHCl_3$) (lit[14] $[\alpha]_D^{25}$ +32.8 (*c* 3.0 in $CHCl_3$)); IR (ν_{max}/cm^{-1}) 3428, 2979, 1731, 1373, 1296, 1176, 1116, 1067, 1028, 948, 845, 594, 563; ^1H NMR (500 MHz, $CDCl_3$) δ 1.14 (3H, d, J = 6.2 Hz, $CHCH_3$), 1.18 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.35 (1H, d, J = 4.1 Hz, CH_2CH), 2.37 (1H, d, J = 1.3, CH_2CH), 3.22 (1H, br. s, OH), 4.08 (2H, q, J = 7.2 Hz, CH_2CH_3), 4.01–4.17 (1H, m, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH_2CH_3), 22.4 ($CHCH_3$), 42.8 (CH_2CH), 60.5 (CH_2CH_3), 64.1 (CH), 172.5 (CO); HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_6H_{13}O_3$ 133.08592, found 133.08589.

Ethyl (*S*)-3-(*t*-butyldimethylsilyloxy)butyrate (12). A solution of *S*-**10** (6.13 g, 46.4 mmol, 1 equiv.) in CH_2Cl_2 (120 mL) was cooled to 0 °C and treated with imidazole (3.79 g, 55.7 mmol, 1.2 equiv.) and TBSCl (7.69 g, 51.0 mmol, 1.1 equiv.). The reaction mixture was stirred at room temperature for 15 h and then quenched with $NaHCO_3$ and washed with H_2O and brine. The aqueous layer was reextracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography, eluting with *n*-hexane/ethyl acetate 8 : 1, to give **12** (10.28 g, 90%) as a colourless oil. R_f = 0.49 (*n*-hexane/ethyl acetate 19 : 1); $[\alpha]_D^{25}$ +24.6 (*c* 1.0 in $CHCl_3$) (lit[15] $[\alpha]_D^{25}$ +24.1 (*c* 1.0 in $CHCl_3$)); IR (ν_{max}/cm^{-1}) 2931, 2858, 1737, 1473, 1376, 1254, 1182, 1081, 1001, 831, 809, 774, 659; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (3H, s, $SiCH_3$), 0.06 (3H, s, $SiCH_3$), 0.86 (9H, s, $SiC(CH_3)_3$), 1.19 (3H, d, J = 6.2 Hz, $CHCH_3$), 1.26 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.36 (1H, dd, J = 14.4, 5.3 Hz, OCOH), 2.46 (1H, dd, J = 14.4, 7.2 Hz, OCOH), 4.17–4.06 (2H, m, CH_2CH_3),

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4.31–4.24 (1H, m, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.0 ($SiCH_3$), −4.4 ($SiCH_3$), 14.3 (CH_2CH_3), 18.1 ($SiC(CH_3)_3$), 24.0 ($CHCH_3$), 25.8 ($SiC(CH_3)_3$), 45.1 (CH_2CH), 60.4 (CH_2CH_3), 65.9 (CH), 171.8 (CO); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{12}H_{27}O_3Si$ 247.17240, found 247.17202.

(*S*)-3-(*t*-Butyldimethylsilyloxy)butanal (13). A solution of **12** (4.0 g, 16.23 mmol, 1 equiv.) in CH_2Cl_2 was cooled to −78 °C and slowly treated with DIBAL-H (17.86 mL, 1 M in Hexan, 1.1 equiv.) by means of a syringe pump over a 90 min period. After this time the reaction was stirred for a further 30 min, treated with methanol (5 mL) and stirred another 15 min. After warming up to room temperature Rochelle salt (40 mg) was added and the mixture was allowed to stir another 20 min. The turbid reaction mixture was extracted with CH_2Cl_2 and the combined organic layers were washed with H_2O and brine and dried over $MgSO_4$. After the solvent had been removed under reduced pressure product **13** (3.12 g, 95%) was obtained as a colourless oil. R_f = 0.70 (*n*-hexane/ethyl acetate 9 : 1); $[\alpha]_D^{25}$ +16.7 (*c* 1.0 in $CHCl_3$) (lit[16] $[\alpha]_D^{25}$ +14 (*c* 1.0 in $CHCl_3$)); IR (ν_{max}/cm^{-1}) 2931, 2858, 1728, 1264, 1098, 1026, 833, 774, 680, 560; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06 (3H, s, $SiCH_3$), 0.07 (3H, s, $SiCH_3$), 0.87 (9H, s, $C(CH_3)_3$), 1.24 (3H, d, *J* = 6.0 Hz, $CHCH_3$), 2.46 (1H, ddd, *J* = 15.8, 5.1, 2.2 Hz, OHCC*H*), 2.55 (1H, ddd, *J* = 15.8, 7.1, 2.8 Hz, OHCC*H*), 4.39–4.32 (1H, m, CH), 9.80 (1H, dd, *J* = 2.8, 2.2 Hz, CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.1 ($SiCH_3$), −4.5 ($SiCH_3$), 17.9 ($C(CH_3)_3$), 24.1 ($CHCH_3$), 25.7 ($C(CH_3)_3$), 52.9 ($OHCCH_2$), 64.6 (CH), 202.3 (C=O); MS: m/z (%) = 202 (2) [M^+], 185 (9), 159 (100) [$C_8H_{19}OSi$], 145 (79) [$C_6H_{13}O_2Si$], 119 (51), 101 (47), 75 (94) [C_2H_7OSi], 73 (55) [$C_3H_5O_2$], 57 (40), 41 (20).

Methyl L-*N*-(*t*-butyloxocarbonyl)valinate (16). To a suspension of L-valine (15.0 g, 0.128 mol, 1 equiv.) in methanol $SOCl_2$ (9.29 mL, 0.13 mmol, 1 equiv.) was slowly added at 0 °C. Stirring was continued for 1 h at room temperature and then for another 22 h at boiling temperature. The solvent was reduced under vacuum to afford the methyl valinate hydrochloride as a white solid which was taken up in CH_2Cl_2 (120 mL) and treated at 0 °C with NEt_3 (34.9 mL, 0.25 mmol, 2 equiv.) and Boc_2O (30.3 g, 0.14 mmol, 1.1 equiv.) and stirred for 17 h at room temperature. Water (100 mL) was added and the resulting mixture was extracted with CH_2Cl_2. To remove residual NEt_3 the organic layers were washed with 1 M citric acid and dried over $MgSO_4$. All volatiles were removed under vacuum to obtain product **16** (29.0 g, 98%) as a colourless oil. R_f = 0.57 (*n*-hexane/ethyl acetate 4 : 1); $[\alpha]_D^{25}$ +10.6 (*c* 1.0 in $CHCl_3$) (lit[17] $[\alpha]_D^{25}$ +15.9 (*c* 1.0 in $CHCl_3$)); IR (ν_{max}/cm^{-1}) 3377, 2967, 1744, 1710, 1499, 1366, 1156, 1015, 779; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (3H, d, *J* = 6.6 Hz, $CHCH_3$), 0.86 (3H, d, *J* = 6.6 Hz, $CHCH_3$), 1.35 (9H, s, $C(CH_3)_3$), 2.12–1.92 (1H, m, $(CH_3)_2CH$), 3.64 (3H, s, OCH_3), 4.17–4.08 (1H, m, NC*H*), 5.03 (1H, br. d, *J* = 7.7 Hz, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.7 ($CHCH_3$), 18.9 ($CHCH_3$), 28.3 ($C(CH_3)_3$), 31.2 ($(CH_3)_2CH$), 51.8 (OCH_3), 58.4 (NHCH), 79.7 ($C(CH_3)_3$), 155.7 (NCO), 172.9 (CO_2CH_3); HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{11}H_{21}NO_4Na$ 254.13594, found 254.13628.

Methyl L-*N*-chloro-*N*-(*t*-butyloxocarbonyl)valinate (17). $Ca(OCl)_2$ (12.56 g, 87.8 mmol, 3 equiv.) and Al_2O_3 (87.9 g, Brockmann V) were suspended in CH_2Cl_2 and **16** (6.77 g, 29.3 mmol, 1 equiv.) was added. The suspension was stirred for 16 h at 40 °C. The reaction mixture was filtered over Celite and the filtrate was evaporated under reduced pressure to afford product **17** (66.4 g, 86%) as a colourless oil. R_f = 0.66 (*n*-hexane/ethyl acetate 4 : 1); $[\alpha]_D^{25}$ −71.5 (*c* 1.0 in $CHCl_3$); IR (ν_{max}/cm^{-1}) 2978, 1746, 1705, 1369, 1280, 1254, 1203, 1157, 1131, 1008, 848, 750; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (3H, d, *J* = 6.8 Hz, $CHCH_3$), 0.98 (3H, d, *J* = 6.8 Hz, $CHCH_3$), 1.44 (9H, s, $C(CH_3)_3$), 2.38–2.24 (1H, m, $(CH_3)_2CH$), 3.69 (3H, s, OCH_3), 4.40 (1H, d, *J* = 10.0 Hz, NHC*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.0 ($CHCH_3$), 19.6 ($CHCH_3$), 27.5 ($CH(CH_3)_2$), 27.9 ($C(CH_3)_3$), 52.0 (OCH_3), 68.0 (NCH), 83.4 ($C(CH_3)_3$), 154.7 (NCO), 169.9 (CO_2CH_3); HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{11}H_{20}ClNO_4Na$ 288.09731, found 288.09672.

Methyl *N*-(*t*-butyloxocarbonyl)dehydrovalinate (18). A solution of **17** (885 mg, 3.33 mmol, 1 equiv.) in Et_2O (30 mL) was treated with DBU (547 μL, 3.66 mmoL, 1.1 equiv.) and stirred at room temperature for 3 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography, eluting with *n*-hexane/ethyl acetate 2 : 1, to give **18** (742 mg, 97%) as a white solid of m.p. 81 °C. R_f = 0.37 (*n*-hexane/ethyl acetate 5 : 1); IR (ν_{max}/cm^{-1}) 3351, 1705, 1497, 1315, 1258, 1226, 1160, 1092, 1054, 756, 574; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (9H, s, $C(CH_3)_3$), 1.85 (3H, s, $=CCH_3$), 2.10 (3H, s, $=CCH_3$), 3.73 (3H, s, OCH_3), 5.77 (1H, br. s, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 ($=CCH_3$), 22.4 ($=CCH_3$), 28.2 ($C(CH_3)_3$), 51.8 (OCH_3), 80.2 ($C(CH_3)_3$), 121.5 (N*C*=C), 145.0 (NC=*C*), 154.1 (NCO), 165.6 (CO_2CH_3); HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{11}H_{19}NO_4Na$ 252.12063, found 252.12028.

Dehydrovaline methyl ester hydrochloride (8 × HCl). Acetyl chloride (9.3 mL, 0.13 mol, 30 equiv.) and dry methanol (5.6 mL, 0.13 mol, 30 equiv.) were carefully added to dioxane at 0 °C and stirred for 5 min. Then carbamate **18** (1.00 g, 4.36 mmol, 1 equiv.) was added in one portion and the resulting mixture was stirred for a further 90 min. The solvent was evaporated under reduced pressure to afford **8** × HCl (715 mg, 99%) as colourless crystals of m.p. 115–120 °C. IR (ν_{max}/cm^{-1}) 2980, 2785, 2594, 1986, 1729, 1622, 1436, 1304, 1228, 1146, 1126, 1043, 877, 770, 617; ^{1}H NMR (300 MHz, D_2O) δ 2.02 (3H, s, CCH_3), 2.25 (3H, s, CCH_3), 3.84 (3H, s, OCH_3); ^{13}C NMR (75 MHz, D_2O) δ 21.6 (CCH_3), 22.0 (CCH_3), 53.0 (OCH_3), 114.8 (N*C*=C), 152.8 (NC=*C*), 164.1 (CO_2CH_3); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_6H_{12}NO_2$ 130.08626, found 130.08632.

***S*-*t*-Butyl (*S*,*E*)-7-(*t*-butyldimethylsilyloxy)-3-oxo-oct-4-enthioate (7).** A solution of *S*-*t*-butyl 4-diethylphosphonio-3-oxobutanthioate **14**[5] (2.87 g, 9.3 mmol, 1.4 equiv.) in THF (90 mL) was cooled to −78 °C and slowly treated with *n*-butyl lithium (7.41 mL, 18.5 mmol, 2.5 M in hexane, 2.8 equiv.). The reaction mixture was stirred for 30 min and then treated with aldehyde **13** (1.34 g, 6.6 mmol, 1 equiv.). After 3 h the reaction was quenched with NH_4Cl, extracted with Et_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure to

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leave **7** (2.18 g, 92%) as a red oil and as a 4 : 1 mixture of enol and keto tautomers. R_f = 0.26 (*ketone*), 0.41 (*enol*) (*n*-hexane/Et_2O 98 : 2); $[\alpha]_D^{25}$ +7.4 (*c* 1.0 in $CHCl_3$); IR (ν_{max}/cm^{-1}) 2957, 2928, 2858, 1656, 1583, 1364, 1255, 1075, 1002, 968, 832, 773; ^{1}H NMR (300 MHz, $CDCl_3$) δ *enol*: 0.03 (3H, s, $SiCH_3$), 0.04 (3H, s, $SiCH_3$), 0.88 (9H, s, $SiC(CH_3)_3$), 1.15 (3H, d, *J* = 6.1 Hz, $CHCH_3$), 1.52 (9H, s, $SC(CH_3)_3$), 2.24–2.35 (2H, m, C=CHCH_2), 3.89 (1H, sx, *J* = 6.1 Hz, CHOSi), 5.35 (1H, s, COC*H*COH), 5.70 (1H, dd, *J* = 15.4, 1.5 Hz, C*H*=CH), 6.68 (1H, dt, *J* = 15.4, 7.6 Hz, CH=C*H*), 12.56 (1H, d, *J* = 1.5 Hz, OH); *ketone*: 0.04 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.88 (9H, s, $SiC(CH_3)_3$), 1.16 (3H, d, *J* = 6.1 Hz, $CHCH_3$), 1.55 (9H, s, $SC(CH_3)_3$), 2.31–2.38 (2H, m, C=CHCH_2), 3.92–3.98 (1H, m, CHOSi), 5.31 (2H, s, $COCH_2CO$), 5.62 (1H, dd, *J* = 12.1, 1.8 Hz, C*H*=CH), 6.91 (1H, dt, *J* = 15.9, 7.5 Hz, CH=C*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ *enol*: −4.8 ($SiCH_3$), −4.6 ($SiCH_3$), 24.0 ($CHCH_3$), 26.0 ($SiC(CH_3)_3$), 29.9 ($SiC(CH_3)_3$), 30.3 ($SC(CH_3)_3$), 43.2 (CH=CHCH_2), 48.4 ($SC(CH_3)_3$), 68.1 (CHOSi), 100.6 (CO*C*HCOH), 126.3 (*C*H=CHCH$_2$), 139.4 (CH=*C*HCH$_2$), 166.6 (SCO), 196.6 (CH*C*OH); *ketone*: −4.8 ($SiCH_3$), −4.6 ($SiCH_3$), 24.0 ($CHCH_3$), 26.0 ($SiC(CH_3)_3$), 29.8 ($SC(CH_3)_3$), 29.9 ($SiC(CH_3)_3$), 42.9 (CH=CHCH$_2$), 48.9, ($SC(CH_3)_3$), 56.0 (CO*C*HCOH), 67.6 (CHOSi), 131.6 (*C*H=CHCH$_2$), 147.3 (CH=*C*HCH$_2$), 191.6 (CH*C*OH), 192.6 (SCO); HRMS (ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{18}H_{34}O_3NaSSi$ 381.18901, found 381.18832.

Methyl (*S*,*E*)-*N*-[7-(*t*-butyldimethylsilyloxy)-3-oxo-oct-4-enoyl] dehydrovalinate (6). A suspension of dehydrovaline methyl ester hydrochloride (**8** × HCl) (2.0 g, 12.1 mmol, 2.5 equiv.) and powdered 4 Å molecular sieve in THF (130 mL) was cooled to 0 °C and treated first with NEt_3 (1.74 mL, 12.6 mmol, 2.6 equiv.) and after stirring for 5 min with β-ketothioester **7** (1.73 g, 4.84 mmol, 1 equiv.). The reaction flask was wrapped in light-tight foil and then silver trifluoroacetate (2.14 g, 9.7 mmol, 2.0 equiv.) was added in one portion. After 3 h the reaction mixture was filtered over Celite and the residue was washed with CH_2Cl_2. The solvent was evaporated *in vacuo* and the remainder was purified by column chromatography, eluting with *n*-hexane/ethyl acetate 9 : 1, to leave **6** (1.71 g, 89%) as a white solid of m.p. 81 °C with a ratio ketone/enol of 2 : 1. R_f = 0.46 (*n*-hexane/ethyl acetate 3 : 1); $[\alpha]_D^{25}$ +3.2 (*c* 1.0 in $CHCl_3$); IR (ν_{max}/cm^{-1}) 3243, 2928, 2856, 1721, 1608, 1542, 1307, 1220, 1088, 831, 774; ^{1}H NMR (300 MHz, $CDCl_3$) δ *ketone*: 0.04 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.88 (9H, s, $SiC(CH_3)_3$), 1.17 (3H, d, *J* = 6.2 Hz, $CHCH_3$), 1.85 (3H, s, C=CCH_3), 2.14 (3H, s, C=CCH_3), 2.36–2.40 (2H, m, C=CHCH_2), 3.63 (2H, s, $COCH_2CO$), 3.73 (3H, s, OCH_3), 3.97 (1H, q, *J* = 5.9 Hz, CHOSi), 6.18 (1H, d, *J* = 15.9 Hz, C*H*=CHCH$_2$), 7.01 (1H, dt, *J* = 15.9, 7.6 Hz, CH=C*H*CH$_2$), 8.46 (1H, s, NH); *enol*: 0.03 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.88 (9H, s, $SiC(CH_3)_3$), 1.15 (3H, d, *J* = 6.1 Hz, $CHCH_3$), 1.87 (3H, s, C=CCH_3), 2.18 (3H, s, C=CCH_3), 2.23–2.31 (2H, m, C=CHCH_2), 3.75 (3H, s, OCH_3), 3.90 (1H, q, *J* = 6.4 Hz, CHOSi), 4.94 (1H, s, C*H*COH), 5.78 (1H, d, *J* = 15.4 Hz, C*H*=CHCH$_2$), 6.31 (1H, s, NH), 6.60 (1H, dt, *J* = 15.4, 7.7 Hz, CH=C*H*CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ *ketone*: −4.7 ($SiCH_3$), −4.3 ($SiCH_3$), 18.2 ($SiC(CH_3)_3$), 21.3 (C=*C*H_3), 22.4 (C=*C*H_3), 24.0 ($CHCH_3$); 25.9 ($SiC(CH_3)_3$), 42.9 (CH=CHCH_2), 45.7 ($COCH_2CO$), 52.0 (OCH_3), 67.5 (CHOSi), 121.2 (N*C*=C), 131.9 (*C*H=CHCH$_2$), 144.5 (NC=*C*), 148.1 (CH=*C*HCH$_2$), 164.5 (*C*OOCH$_3$), 165.2 (NCO), 195.7 ($COCH_2CO$); *enol*: −4.7 ($SiCH_3$), −4.3 ($SiCH_3$), 21.3 (C=*C*H_3), 22.4 (C=*C*H_3), 24.0 ($CHCH_3$); 25.9 ($SiC(CH_3)_3$), 39.7 ($SiC(CH_3)_3$), 49.4 (CH=CHCH_2), 52.0 (OCH_3), 67.5 (CHOSi), 91.2 (CO*C*HCOH), 121.2 (N*C*=C), 126.9 (*C*H=CHCH$_2$), 137.0 (CH=*C*HCH$_2$), 144.5 (NC=*C*), 164.5 (*C*OOCH$_3$), 165.2 (NCO), 195.7 ($COCH_2CO$); HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{35}NO_5Si$ 398.23573, found 398.23503.

3-[(*S*,*E*)-5-(*t*-Butyldimethylsilyloxy)hex-2-enoyl]-5-(propan-2-yliden)-pyrrolidin-2,4-dione (5). A solution of β-ketoamide **6** (100 mg, 0.25 mmol, 1 equiv.) in methanol (10 mL) was treated with NaOMe (68 mg, 1.26 mmol, 5 equiv.) and refluxed for 1 h. The reaction was quenched with H_2O and 10% HCl, extracted with Et_2O and washed with brine. After drying with $MgSO_4$ the solvent was removed under reduced pressure to afford **5** (92 mg, 99%) as a yellow oil. R_f = 0.54 (tailing) (CH_2Cl_2/methanol 95 : 5); $[\alpha]_D^{25}$ +9.4 (*c* 1.0 in methanol); IR (ν_{max}/cm^{-1}) 3195, 2955, 2930, 2858, 1694, 1646, 1583, 1374, 1252, 1128, 1088, 991, 833, 806, 773, 662; ^{1}H NMR (500 MHz, MeOD) δ 0.07 (3H, s, $SiCH_3$), 0.08 (3H, s, $SiCH_3$), 0.89 (9H, s, $SiC(CH_3)_3$), 1.20 (3H, d, *J* = 6.1 Hz, $CHCH_3$), 1.89 (3H, s, C=CCH_3), 2.25 (3H, s, C=CH_3), 2.40–2.52 (2H, m, CH=CHCH_2), 4.05 (1H, q, *J* = 6.1 Hz, CHOSi), 7.23 (2H, br. s, C*H*=C*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ *E-isomer*: −4.8 ($SiCH_3$), −4.5 ($SiCH_3$), 17.9 ($SiC(CH_3)_3$), 18.2 (C=*C*CH_3), 21.2 (C=C*C*H_3), 24.0 ($CHCH_3$), 25.8 ($SiC(CH_3)_3$), 42.8 (CH=CH*C*H$_2$), 67.5 (CHOSi), 100.8 (NCO*C*), 122.7 (*C*H=CHCH$_2$), 125.0 (N*C*=C), 129.8 (NC=*C*), 147.0 (CH=*C*HCH$_2$), 164.5 (NCO), 172.3 (*C*OC=CCH$_3$), 175.8 (C=*C*OH); *Z-isomer*: −3.1 ($SiCH_3$), 17.9 ($SiC(CH_3)_3$), 18.9 (C=*C*CH_3), 21.2 (C=C*C*H_3), 24.0 ($CHCH_3$), 25.8 ($SiC(CH_3)_3$), 42.8 (CH=CH*C*H$_2$), 65.3 (CHOSi), 103.1 (NCO*C*), 123.3 (*C*H=CHCH$_2$), 126.2 (N*C*=C), 129.8 (NC=*C*), 147.6 (CH=*C*HCH$_2$), 165.7 (NCO), 171.9 (*C*OC=CCH$_3$), 182.1 (C=*C*OH); HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{19}H_{32}NO_4Si$ 366.20951, found 366.20935.

Cladosin C (3). Tetramic acid **5** (90 mg, 0.25 mmol, 1 equiv.) was dissolved in toluene (4 mL). 3 Å molecular sieve pellets and 2,4-dimethoxybenyzlamine (37 μL, 0.25 mmol, 1 equiv.) were added and the mixture was refluxed for 80 min. The resulting solution was filtrated and washed with CH_2Cl_2. Upon removal of the solvent, tetramic acid **19** (50 mg, 40%) was obtained as a yellow oil. The crude product was used for the next step without further purification.

Crude compound **19** (40 mg, 0.08 mmol, 1 equiv.) was added to a solution of TFA (0.2 mL) in CH_2Cl_2 (1.8 mL) and the mixture was stirred at room temperature for 1 h. The solvent was removed as an azeotrope with toluene under reduced pressure and the resulting crude product was purified by RP–column chromatography, eluting with methanol/H_2O 40 : 60, to leave cladosin C (**3**) (15 mg, 77%) as a pale yellow oil as a 2 : 1 mixture of 3*E*- and 3*Z*- isomers. R_f = 0.25 (CH_2Cl_2/methanol 95 : 5); $[\alpha]_D^{25}$ +7.9 (*c* 0.1 in methanol) (lit[1] $[\alpha]_D^{25}$ +10.5 (*c* 0.1 in methanol)); IR (ν_{max}/cm^{-1}) 3204, 2973, 1659, 1615,

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1512, 1457, 1381, 1289, 1203, 1137, 1034, 837, 801, 722, 618, 585, 557; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ *E-isomer*: 1.10 (3H, d, J = 6.2 Hz, CHCH_3), 1.74 (3H, s, C=CCH_3), 2.15 (3H, s, C=CCH_3), 2.29–2.34 (2H, m, CH=CHCH_2), 3.72–3.79 (1H, m, CHOH), 4.71 (1H, d, J = 5.0 Hz, OH), 6.87–6.98 (1H, m, CH=CHCH$_2$), 7.42 (1H, d, J = 16.2 Hz, CH=CHCH$_2$), 8.70 (1H, br. s, NH$_2$), 9.21 (1H, s, NH), 9.88 (1H, br. s, NH$_2$); *Z-isomer*: 1.10 (3H, d, J = 6.2 Hz, CHCH_3), 1.73 (3H, s, C=CCH_3), 2.12 (3H, s, C=CCH_3), 2.29–2.34 (2H, m, CH=CHCH_2), 3.72–3.79 (1H, m, CHOH), 4.71 (1H, d, J = 5.0 Hz, OH), 6.87–6.98 (1H, m, CH=CHCH$_2$), 7.42 (1H, d, J = 16.2 Hz, CH=CHCH$_2$), 8.60 (1H, br. s, NH$_2$), 9.25 (1H, s, NH$_2$), 9.39 (1H, br. s, NH); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ *E-isomer*: 18.3 (C=CCH$_3$), 21.3 (C=CCH$_3$), 23.8 (CHCH$_3$), 43.3 (CH=CHCH$_2$), 66.0 (CHOH), 96.8 (C=CNH$_2$), 118.3 (C=CCH$_3$), 123.2 (CH=CHCH$_2$), 130.2 (C=CCH$_3$), 142.1 (CH=CHCH$_2$), 161.2 (C=CNH$_2$), 168.3 (NHCO), 187.2 (COC=CCH$_3$); *Z-isomer*: 18.4 (C=CCH$_3$), 21.2 (C=CCH$_3$), 23.8 (CHCH$_3$), 43.4 (CH=CHCH$_2$), 66.0 (CHOH), 96.5 (C=CNH$_2$), 118.6 (C=CCH$_3$), 123.5 (CH=CHCH$_2$), 130.8 (C=CCH$_3$), 142.1 (CH=CHCH$_2$), 161.4 (C=CNH$_2$), 171.6 (NHCO), 183.7 (COC=CCH$_3$); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{13}H_{19}O_3N_2$ 251.13902, found 251.13831.

Conflicts of interest

There are no conflicts to declare.

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