Supporting Information File 1

for

Synthesis of a leopolic acid-inspired tetramic acid with antimicrobial activity against multidrug-resistant bacteria

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Experimental. General information.

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries by a SMP3 apparatus and are uncorrected. $^1$H spectra were recorded on Varian Mercury 300 MHz and Bruker AV600 spectrometers. TMS was used as an internal standard and the chemical shifts were reported in parts per million. The peak patterns are indicated as follows: $s$, singlet; $d$, doublet; $dd$, doublet of doublet; $t$, triplet; $m$, multiplet; $q$, quartet. The coupling constants, $J$ are reported in Hertz (Hz). $^{13}$C NMR spectra were recorded on Varian 300 MHz and Bruker AV600 spectrometers. Optical rotations were measured with a Perkin Elmer 241 polarimeter. IR spectra were recorded on a Perkin Elmer 1310 spectrophotometer and reported in wave numbers (cm$^{-1}$). The elemental analyses were recorded with a CARLO ERBA EA 1108 instrument. Mass spectra were recorded on a Q-TOF mass spectrometer (Synapt G2-S HDMS, Waters) equipped with an ESI source. Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et$_2$O) were obtained by distillation from sodium-benzophenone ketyl; dry methylene chloride was obtained by distillation from phosphorus pentoxide. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow and all glassware were oven dried and/or flame dried. Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was conducted on TLC plates (silica gel 60 F254, aluminium foil). Compounds on TLC plates were detected under UV light at 254 and 365 nm or were revealed spraying with 10% phosphomolybdic acid (PMA) in ethanol.

Compound 5 was prepared as reported in literature. [1]
Compound 6a was prepared as reported in literature. [2]
Benzyl tosylate was prepared as reported in literature. [3]
Compound 15 was prepared as reported in literature. [4]
Compound 17 was prepared as reported in literature. [5]

$N$-Ethoxycarbonylmethyl-$N$-(4-methoxybenzyl)malonamic acid benzyl ester (6b): mono-benzyl malonate (4.1 g, 21.51 mmol, 2 equiv) was added to a solution of compound 5 (2.4 g, 10.75 mmol, 1 equiv) in CH$_2$Cl$_2$, followed by a solution of DCC (3.32 g, 16.13 mmol, 1.5 equiv) and DMAP (0.065 g, 0.5 mmol, 0.05 equiv) in CH$_2$Cl$_2$ at 0 °C. The solution was stirred 12 h at room temperature. After completion of reaction, the mixture was filtered and the filtrate was washed with water and brine solution. The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/hexane) to afford compound 6b (3.5 g, 83%) as an oil. $R_f$: 0.5 (EtOAc/hexane 1:4).
1H-NMR (300 MHz, CDCl₃) δ: 7.42-7.28 (5H, m); 7.21-7.03 (2H, m); 6.93-6.76 (2H, m); 5.18 (2H, s); 4.55 (2H, s); 4.16 (2H, q, J = 7.3 Hz); 4.05 (2H, s); 3.80 (3H, s); 3.63 (2H, s); 1.23 (3H, t, J = 7.3 Hz). Anal. C 66.15, H 6.31, N 3.51, calcd for C₂₂H₂₅NO₆, Found: C 66.27, H 6.32, N 3.50.

3-Decyl-1-(4-methoxybenzyl)-2,4-dioxopyrrolidine-3-carboxylic acid ethyl ester (7a): to a solution of diester 6a (200 mg, 0.6 mmol, 1 equiv) in anhydrous diethyl ether (3 mL) tetrabutylammonium fluoride (1M in THF, 1.2 mL, 1.2 mmol, 2 equiv) was added under N₂ atmosphere. The reaction was stirred at rt for 2 h, then concentrated in vacuo. The resulting solid was suspended in THF (3 mL), and decyl iodide (0.25 mL, 1.2 mmol, 2 equiv) was added at room temperature. The mixture was stirred for 24 h, then evaporated to dryness. The crude was purified by flash column chromatography (30% EtOAc/Hexane) to afford compound 7a (102 mg, 22%) as a yellow oil. Rf: 0.5 EtOAc: hexane 3:7

1H-NMR (300 MHz, CDCl₃) δ: 7.30-7.18 (2H, m); 6.94-6.79 (2H, m); 4.69 (1H, d, J = 13.9 Hz, AB system); 4.53 (1H, d, J = 13.9 Hz, AB system); 4.17 (2H, q, J = 7.3 Hz); 3.87 (1H, d, J = 17.5 Hz, AB system); 3.80 (3H, s, -OCH₃); 3.60 (1H, d, J = 17.5 Hz); 2.28-2.02 (2H, m); 1.47-1.18 (19H, m); 0.88 (3H, t, J = 7.2 Hz). Anal. C 72.99, H 7.96, N 2.84, calcd for C₃₀H₃₉NO₅, Found: C 72.86, H 7.97, N 2.85.

3-Decyl-1-(4-methoxybenzyl)-2,4-dioxopyrrolidine-3-carboxylic acid benzyl ester (7b): to a solution of compound 6b (1.9 g, 4.7 mmol, 1 equiv) in anhydrous diethyl ether (25 mL) tetrabutylammonium fluoride (1M in THF, 9.5 mL, 9.5 mmol, 2 equiv) was added under N₂ atmosphere. The reaction was stirred 2 h at rt. The colourless solid was removed by filtration and washed with diethyl ether. The solid was suspended in THF, and decyl iodide (2.5 g, 9.5 mmol, 2 equiv) was added at room temperature. The mixture was stirred for 24 h, then evaporated to dryness. The crude was purified by flash column chromatography (10% EtOAc/Hexane) to afford 7b (696 mg, 30%). Rf: 0.5 (EtOAc/hexane 1:4).

1H-NMR (300 MHz, CDCl₃) δ: 7.46-7.28 (3H, m); 7.28-7.15 (2H, m); 7.14-6.99 (2H, m); 6.81-6.63 (2H, m); 5.15 (2H, s); 4.79 (1H, d, J = 14.6 Hz, AB system); 4.44 (1H, d, J = 14.6 Hz, AB system); 3.82 (1H, d, J = 17.8 Hz, AB); 3.77 (3H, s); 3.57 (1H, d, J = 17.8 Hz, AB system); 2.18 (2H, m); 1.48-1.12 (16H, m); 0.88 (3H, t, J = 7.2 Hz). Anal. C 72.99, H 7.96, N 2.84, calcd for C₃₀H₃₉NO₅, Found: C 72.86, H 7.97, N 2.85.

3-decyl-2,4-dioxopyrrolidine-3-carboxylic acid ethyl ester (8a): to a solution of 7a (0.23 g, 0.53 mmol) in acetonitrile/water (3:1) cerium ammonium nitrate (0.87 g, 1.59 mmol, 3 equiv) was added at 0 °C. The solution was stirred 1 hour at room temperature. After completion of the reaction, the
mixture was concentrated in vacuo, diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated. The crude product was purified by flash column chromatography (30% EtOAc/Hexane) to afford 8a (134 mg, 81%) as a yellowish viscous solid. Rf: 0.2 (EtOAC/hexane 3:7)

1H-NMR (300 MHz, CDCl₃) δ: 4.20 (2H, q, J = 7.3 Hz); 4.13 (1H, d, J = 17.9 Hz, AB system); 3.84 (1H, d, J = 17.9 Hz, AB system); 2.22-2.05 (2H, m); 1.48-1.10 (19H, m).


3-Decyl-2,4-dioxopyrrolidine-3-carboxylic acid benzyl ester (8b): to a solution of compound 7b (0.1 g, 0.2 mmol) in acetonitrile : water (3:1), cerium ammonium nitrate (0.3 g, 0.6 mmol, 3 equiv) was added at 0 °C. The solution was stirred 1 h at room temperature. After completion of the reaction, the mixture was diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated. The crude product was purified by flash column chromatography (30% EtOAc/hexane) to afford 8b (49 mg, 66%) as a colourless liquid. Rf: 0.5 (EtOAC/hexane 1:1).

1H-NMR (300 MHz, CDCl₃) δ: 7.43-7.17 (5H, m); 6.95 (1H, brs); 5.16 (2H, s); 4.05 (2H, d, J = 18.1 Hz, AB system); 3.81 (2H, d, J = 18.1 Hz, AB system); 2.16 (2H, m); 1.46-0.96 (16H, m); 0.88 (3H, t, J = 7.3 Hz).

Anal. C 70.75, H 8.37, N 3.75, calcd for C₂₂H₃₁NO₄, Found: C 70.65, H 8.38, N 3.75.

Ethyl 2-(N-(4-methoxybenzyl)dodecanamido)acetate (9): to a solution of compound 5 (1.0 g, 4.4 mmol, 1 equiv) and TEA (1.2 mL, 8.8 mmol, 2 equiv) in CHCl₃ (10 mL) at 0 °C, dodecanoyl chloride (1.1 g, 8.8 mmol, 1.2 equiv) was added dropwise under nitrogen atmosphere. After 10 minutes at 0 °C, the reaction mixture was stirred 3 hours at room temperature. The reaction was monitored by TLC and after completion, the reaction mixture was diluted with CH₂Cl₂ and was washed with water and brine solution. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under vacuo. The crude product was purified by flash column chromatography (diethyl ether/hexane 2:3) to afford the desired product 9 (1.63 g, 90%) as a yellow oil. Rf: 0.3 (diethyl ether/hexane 2:3).

This compound was isolated as a mixture of rotamers in the ratio (2.2:1).

1H-NMR (CDCl₃, 300 MHz) mixture of rotamers δ (major): 7.13 (2H, d, J = 8.8 Hz); 6.90 (2H, d, J = 8.8 Hz); 4.58 (2H, s); 4.18 (2H, q, J = 7.3 Hz); 4.02 (2H, s); 3.82 (3H, s); 2.46 (2H, t, J = 7.3 Hz); 1.75-1.58 (2H, m); 1.41-1.12 (19H, m); 0.89 (3H, t, J = 7.3 Hz).
$^{13}$C-NMR (CDCl$_3$, 75 MHz) mixture of rotamers $\delta$ (major): 174.1, 169.5, 159.3, 128.1 ($\times$2C), 114.3 ($\times$2); 61.0; 55.3; 51.6; 46.8; 33.0; 31.9; 29.6 ($\times$2C); 29.5; 29.4 ($\times$3C); 29.3; 25.2; 22.7; 14.1 ($\times$2C).

Anal. C 71.07, H 9.69, N 3.45, calcd for C$_{24}$H$_{39}$NO$_4$, Found: C 71.18, H 9.67, N 3.44.

3-Decyl-1-(4-methoxybenzyl)pyrrolidine-2,4-dione (10): To a solution of compound 9 (2 mmol, 810 mg) in anhydrous THF, a solution 1M $t$-BuOK in THF (1.1 equiv, 2.2 mmol, 2.2 mL) was added dropwise at room temperature. The resulting mixture was refluxed under nitrogen for 1.5 h. Then it was cooled to room temperature and poured into cooled 5% aqueous HCl (pH 1–2). THF was evaporated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with water, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under vacuum.

The residue was triturated with diethyl ether and hexane to give the tetramic acid 15 as a solid (350 mg). The mother liquor was concentrated and purified by flash column chromatography (3:7 EtOAc/hexane) to give the product (110 mg). Yield: 65%. mp: 67–69 °C. $R_f$: 0.3 (EtOAc : hexane 3:7).

$^1$H-NMR (CDCl$_3$, 600 MHz) $\delta$: 7.19 (2H, d, $J = 8.6$ Hz); 6.87 (2H, d, $J = 8.6$ Hz); 4.63 (1H, AB, $J = 14.5$ Hz); 4.52 (1H, AB system, $J = 14.5$ Hz); 3.80 (3H, s); 3.70 (1H, AB system, $J = 17.8$ Hz); 3.57 (1H, dd, $J = 17.8$ Hz; $J = 1.8$ Hz); 2.85 (1H, td, $J = 5.8$ Hz; $J = 1.8$ Hz); 1.80–1.92 (2H, m); 1.38–1.45 (16H, m); 1.22–1.34 (m); 0.87 (3H, t, $J = 7.0$ Hz).

$^{13}$C-NMR (CDCl$_3$, 150 MHz) $\delta$: 207.3; 171.9; 159.4; 129.7 ($\times$2); 127.2; 114.3 ($\times$2); 55.5; 55.3; 50.3; 46.1; 45.4; 31.9; 29.5; 29.5; 29.4; 29.3; 27.3; 26.1; 22.7; 14.1.

Anal. C 73.50, H 9.25, N 3.90, calcd for C$_{22}$H$_{33}$NO$_3$, Found: C 73.45, H 9.27, N 3.90.

1-(4-Methoxybenzyl)-4-(benzyllox)-3-decyl-1H-pyrrol-2(5H)-one (11): To a stirred solution of compound 10 (200 mg, 0.56 mmol, 1 equiv) in anhydrous THF (2.8 mL), KHMDS 0.5 M in toluene (1.22 mL, 0.62 mmol, 1.1 equiv) was added dropwise under nitrogen, and the mixture was stirred for 10 minutes at 0 °C. Then, benzyl tosylate (213 mg, 0.83 mmol, 1.5 equiv) and crown ether 18-crown-6 (169 mg, 0.64 mmol, 1.15 equiv) were added and the reaction mixture was slowly warmed up to room temperature. After 3 h, the reaction was completed and THF was evaporated in vacuo. The residue was diluted with dichloromethane and was washed with water and brine. The combined organic phases were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under vacuum. The crude was purified by flash column chromatography (30% EtOAc/hexane) to give the product 11 (86 mg, 35%) as a brownish oil. $R_f$: 0.3 (EtOAc/hexane 3:7).
H-NMR (CDCl$_3$, 300 MHz) δ: 7.41 – 7.27 (5H, m); 7.14 (2H, d, $J = 8.8$ Hz); 6.85 (2H, d, $J = 8.8$ Hz); 4.98 (2H, s); 4.52 (2H, s); 3.79 (3H, s); 3.71 (2H, s); 2.28 (2H, t, $J = 7.5$ Hz); 1.54-1.44 (m, 2H); 1.42 – 1.16 (m, 14H); 0.88 (t, 3H, $J = 6.7$ Hz).

Anal. C 77.47, H 8.74, N 3.12, calcd for C$_{29}$H$_{39}$NO$_3$, Found: C 77.52, H 8.73, N 3.13.

3-Decylpyrrolidine-2,4-dione (12): A solution of 10 (210 mg, 0.58 mmol, 1 equiv) in trifluoroacetic acid (8.4 mL) was refluxed at 60 °C for 2 h, under nitrogen. Then, trifluoroacetic acid was evaporated in vacuo and the residue was re-dissolved in toluene. The toluene was evaporated and the residue was triturated with a mixture of diethyl ether/hexane (1:9) to give a solid, which was filtered and used directly for the next step without any further purification. R$_f$: 0.27 (CH$_2$Cl$_2$/MeOH 19:1).

H-NMR (CDCl$_3$, 300 MHz) δ: 10.40 (1H, s); 7.05 (1H, s); 3.64 (2H, s); 2.01 (2H, t, $J = 6.7$ Hz); 1.40 – 2.15 (16H, m); 0.86 (3H, t, $J = 6.6$ Hz).

C-NMR (CDCl$_3$, 75 MHz) δ: 176.0; 166.9; 105.7; 45.4; 31.8; 29.5 (×2); 29.4; 29.3; 29.2; 28.4; 22.6; 21.3; 14.4.

4-(Benzylxoy)-3-decyl-1H-pyrrol-2(5H)-one (13): to a solution of compound 12 (100 mg, 0.41 mmol, 1 equiv) in anhydrous THF (1.9 mL), KHMDS 0.5 M in toluene (0.86 mL, 0.43 mmol, 1.03 equiv) was added dropwise under nitrogen, and the mixture was stirred for 10 minutes at 0 °C. Then, benzyl tosylate (131 mg, 0.5 mmol, 1.2 equiv) and crown ether 18-crown-6 (119 mg, 0.45 mmol, 1.08 equiv) were added and the reaction mixture was slowly warmed up to room temperature and stirred for 2 hours. The solvent was evaporated in vacuo, the residue was diluted with dichloromethane and washed with water and brine. The combined organic phases were dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under vacuum. Purification of the crude by flash column chromatography (5% MeOH/CH$_2$Cl$_2$) gave the desired product (40 mg) in 30% yield. R$_f$: 0.45 (CH$_2$Cl$_2$/MeOH 19:1).

H-NMR (CDCl$_3$, 300 MHz) δ: 7.47-7.31 (5H, m); 5.09 (2H, s); 3.94 (2H, s); 2.32-2.19 (2H, m); 1.60-1.43 (2H, m); 1.42-1.15 (14H, m); 0.90 (3H, t, $J = 6.6$ Hz).

C-NMR (CDCl$_3$, 75 MHz) δ: 176.2; 166.9; 105.7; 45.4; 31.8; 29.5 (×2); 29.4; 29.3; 28.6; 22.7; 21.9; 14.1.


2-[3-(1-Benzylxoycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyric acid tert-butyler ester (18): To a stirred solution of triphosgene (180 mg, 0.61 mmol, 0.35 equiv) in dry CH$_2$Cl$_2$ (3 mL) a
mixture of 15 (735 mg, 1.73 mmol, 1 equiv) and N,N-diisopropylethylamine (0.66 mL, 3.81 mmol, 2.2 equiv) in dry CH₂Cl₂ (7.5 mL) was slowly added dropwise at room temperature. The mixture was stirred for five minutes. Then a solution of L-valine tert-butyl ester 17 (300 mg, 1.73 mmol, 1 equiv) and N,N-diisopropylethylamine (0.66 mL, 3.81 mmol, 2.2 equiv) in dry CH₂Cl₂ (7.5 mL) was added in one portion. The reaction was stirred for 3 h under nitrogen, then evaporated to dryness, diluted with ethyl acetate, washed with 10% aqueous KHSO₄, 5% aqueous NaHCO₃, brine, dried over Na₂SO₄. After filtration and concentration to dryness, the crude was purified by flash column chromatography (25% EtOAc/hexane) to give 350 mg (50%) of the product 18 as a grey solid. m.p.: 110-112 °C. [α]D²⁵: +4.6. (c , 0.5, CH₂Cl₂). Rf: 0.4 (EtOAc : hexane 1:3)

1H-NMR (CDCl₃, 300 MHz) mixture of rotamers δ (major): 7.38-7.24 (5H, m); 7.23-717 (3H, m); 7.06-6.97 (2H, m); 5.15 (1H, d, J = 12.2 Hz); 5.08 (1H, brs ); 5.06 (1H, d, J = 12.2 Hz); 4.96 (1H, brs); 4.87-4.77 (1H, m); 4.28 (1H, dd, J = 4.3); 3.14-3.01 (2H, m); 2.12-2.03 (1H, m); 1.44 (9H, s); 0.93 (3H, d, J = 6.9); 0.82 (3H, d, J = 6.9).

13C-NMR (CDCl₃, 75 MHz) mixture of rotamers δ (major): 172.5; 172.3; 156.8; 136.0; 135.2; 129.4 (× 2); 128.5 (× 2); 128.46 (× 2); 128.43 (× 2); 128.3; 126.8; 81.7, 67.0; 58.3; 54.1; 38.6; 31.6; 28.0 (× 3); 18.9; 17.5.


2-[3-(1-Benzzyloxycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyric acid (19): to a solution of compound 18 (120 mg, 0.26 mmol) in dry CH₂Cl₂ (7.9 mL), trifluoroacetic acid (2.6 mL) was added at 0 °C under nitrogen and the mixture was stirred at room temperature for 3 h. Trifluoroacetic acid was removed in vacuo and the residue was re-dissolved in toluene (5 mL). The toluene was evaporated and the operation was repeated twice. The crude was treated with hexane to give the product 19 as a grey solid (100 mg, 95%). m.p.: 178-180 °C. [α]D²⁵: -2.4. (c , 0.5, MeOH). Rf: 0.2 (CH₂Cl₂: MeOH: AcOH 19: 1: 0.1)

1H-NMR (CD₃OD, 300 MHz) mixture of rotamers δ (major): 7.38-7.25 (5H, m); 7.24-7.17 (3H, m); 7.14-7.07 (2H, m); 5.12 (1H, d, AB system, -O-CH-H-Ph, J = 12.3 Hz); 5.08 (1H, d, AB system, -O-CH-H-Ph, J = 12.3 Hz); 4.64-4.56 (1H, m); 4.20-4-14 (1H, m); 3.12–2.94 (2H, m); 2.19–2.03 (1H, m); 0.98-0.85 (6H, m).

13C-NMR (CD₃OD, 75 MHz) mixture of rotamers δ (major): 174.4; 172.3; 158.6; 136.4; 135.6; 129.0 (×2); 128.1 (×2); 128.0 (×4); 127.9; 126.4; 66.5; 57.9; 54.4; 37.7; 30.6; 18.2; 16.5.


2-[3-(1-Benzzyloxycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyric acid pentafluorophenyl ester (20): a solution of 19 (90 mg, 0.22 mmol, 1 equiv), pentafluorophenol (46.5 mg, 0.25 mmol,
1.13 equiv), DCC (52 mg, 0.25 mmol, 1.13 equiv) in EtOAc (1.6 mL) was stirred at 0 °C for 1 h, then 3 h at room temperature. The resultant mixture was filtered and the filtrate was concentrated under reduced pressure. The crude was treated with hexane and Et₂O to give the product 20 as a white solid (74 mg, 60%). m.p.: 93-95 °C. [α]D^25 = +6.8 (c, 0.5, CH₂Cl₂). Rf: 0.3 (ETP/EtOAc 85:15)

1H-NMR (CDCl₃, 300 MHz) δ: 7.41–7.27 (5H, m); 7.23–7.15 (3H, m); 7.03–6.95 (2H, m); 5.17 (1H, AB system, J = 12.2 Hz); 5.10 (1H, d, J = 8.1 Hz); 5.09 (1H, AB system, J = 12.2 Hz), 5.04 (1H, d, J = 9.0 Hz); 4.85 (1H, dt, J = 8.1 Hz; J = 5.6 Hz); 4.77 (1H, dd, J = 9.0 Hz, J = 4.8 Hz); 3.09 (2H, d, J = 5.6 Hz); 2.38–2.23 (m, 1H); 1.04 (3H, d, J = 6.9 Hz); 0.97 (3H, d, J= 6.9 Hz).

13C-NMR (CDCl₃, 75 MHz) δ: 172.6; 169.4; 156.4; 142.7; 141.3; 139.5; 138.0; 136.3; 135.8 (×2); 135.0; 129.4 (×2); 128.6 (×2); 128.5 (×3); 128.4 (×2); 126.9; 67.2; 58.1; 54.2; 38.4; 31.2; 18.8; 17.4.

2-{3-[1-(4-Benzoyloxy-2-oxo-3-decyl-2,5-dihydropyrrole-1-carbonyl)-2-methylpropyl]-ureido)-3-phenylpropionic acid benzyl ester (21): to a stirred solution of 13 (53 mg, 0.161 mmol, 1 equiv) in THF (3 mL) at –60 °C, under nitrogen, a solution of n-BuLi 1.6 M in hexane (60 μl) was added dropwise. The mixture was stirred for 20 minutes. Then, a solution of 20 (100 mg, 0.177 mmol, 1.1 equiv) in THF (2 mL) was added dropwise over 20 minutes. The mixture was allowed to stir for further 3 hours. The temperature was allowed to rise to –45 °C and the mixture was quenched with AcOH (60 μl) and then the mixture was concentrated under reduced pressure. The crude was purified by flash column chromatography (acetone/hexane 3:17) to give the product (67 mg, 60%) as a white solid. m.p.: 131-133°C. [α]D^25 +9 (c, 0.5, CH₂Cl₂). Rf: 0.3 (hexane/acetone 4:1).

1H-NMR (CDCl₃, 600 MHz) δ: 7.45–7.30 (8H, m); 7.23-7.14 (4H, m); 7.11-7.06 (1H, m); 7.00-6.90 (2H, m); 6.64 (1H, brs); 6.14 (1H, d, J = 8.4 Hz); 5.90 (1H, d, J = 8.8 Hz); 5.16-5.21 (1H, m); 5.06 (2H, AB system, J = 11.9 Hz); 4.98 (2H, AB system, J = 12.5 Hz); 4.07 (1H, AB system, J = 17.9 Hz); 3.38 (1H, AB system, J = 17.9 Hz); 3.15 (1H, dd, J = 17.9 Hz; J = 3.7 Hz); 2.94 (1H, dd, J = 13.6 Hz; J = 5.3 Hz); 2.17 – 2.12 (3H, m); 1.55 – 1.45 (14H, m); 1.35-1.20 (2H, m); 1.05 (3H, d, J = 6.8 Hz); 0.88 (3H, t, J = 7.2 Hz); 0.78 (3H, d, J = 6.8 Hz).

13C-NMR (CDCl₃, 125 MHz) δ: 173.8; 172.5; 170.3; 169.8; 157.5; 153.8; 153.4; 153.0; 129.8; 129.1 (× 2); 129.0 (× 2); 128.8 (× 2); 128.6 (× 2); 128.5; 128.2 (× 2); 127.5 (× 2); 127.2; 110.6; 72.0; 67.0; 57.6; 53.6; 45.5; 39.6; 32.1; 31.2; 29.8; 29.7; 29.5; 29.4; 28.1; 22.8; 21.6; 19.9; 16.5; 14.2.
2-[3-[1-(3-Decyl-4-hydroxy-2-oxo-2,5-dihydropyrrrole-1-carbonyl)-2-methylpropyl]-ureido]-3-phenylpropionic acid (1): in a similar manner as described in [6], to a stirred solution of 21 (40 mg, 0.056 mmol) in EtOAc (5 mL) a catalytic amount of Pd/C 10% (8 mg, 20% w/w) was added. The reaction mixture was evacuated and flushed with hydrogen gas (3 times) and stirred at room temperature under hydrogen atmosphere for 100 minutes at rt. After completion, the reaction mixture was filtered through a short pad of celite and the pad was washed with EtOAc (3 × 5 mL). The filtrate was concentrated in vacuo to give the pure product 1 as a white solid (21 mg, 72%). m.p.: 134°C dec. [α]D<sup>25</sup> +36 (c, 0.5, MeOH). R<sub>f</sub> =0.44 (MeOH/DCM 1:9).

<sup>1</sup>H-NMR (CD<sub>3</sub>OD, 600 MHz) δ: 7.30-7.16 (5H, m); 5.51 (1H, brs); 4.54 (1H, t, J = 5.9 Hz); 4.17 (1H, AB system, J = 17.7 Hz); 4.05 (1H, AB system, J = 17.7 Hz); 3.09 (1H, dd, J = 13.7 Hz; J = 5.3 Hz); 2.99 (1H, dd, J = 13.7 Hz; J = 6.9 Hz); 2.16 (3H, m); 1.43-1.49 (2H, m); 1.25 – 1.36 (14H, m); 1.02 (3H, d, J = 6.9 Hz); 0.89 (3H, t, J = 6.9 Hz); 0.85 (3H, d, 6.9 Hz).

<sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz) δ: 175.6; 174.0; 173.3; 171.9, 160.2, 138.2; 130.6 (× 2); 129.3 (× 2); 127.7; 107.6; 58.9; 55.5; 49.4 (overlapped to solvent signal CD<sub>3</sub>OD); 39.2; 33.1; 31.7; 30.7 (× 2); 30.5 (× 2); 30.4; 29.1; 23.7; 21.9; 20.2; 16.7; 14.4.

HDMS: (ES<sup>-</sup>) calculated for C<sub>29</sub>H<sub>42</sub>N<sub>3</sub>O<sub>6</sub> (M-H) - 528.3074, Found: 528.3084.
Biological activity. MIC determination.

In a similar manner as described in [6], MIC were determined for each bacterial strains (*Staphylococcus pseudintermedius* and *Escherichia coli*) using the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) reference methods [7]. The drug solutions were prepared in DMSO to obtain a initial concentration of 1 mg/mL. Overnight cultures were prepared and adjusted to a density of 0.5 Mc Farland scale; 80 strains of *Staphylococcus pseudintermedius* and 25 strains of *Escherichia coli* were used. For the tests 96-wells sterile microtiter plates were used and compounds dilutions (1:2) were performed in Mueller Hinton Broth (Thermo Fisher Scientific, Italy). Negative and positive control were included. After incubation at 37 °C for 24 h, the MIC was determined as the lowest concentration of compound at which there was visible growth.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>μg/disk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin + clavulanic acid</td>
<td>30</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30</td>
</tr>
<tr>
<td>Cefovecin</td>
<td>30</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30</td>
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<tr>
<td>Enrofloxacin</td>
<td>5</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>5</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>5</td>
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</table>
References


