Supporting Information

Synthesis and Biological Evaluation of Cystobactamid 507: A Bacterial Topoisomerase Inhibitor from Cystobacter sp.

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1. General Information

All reactions were performed in oven dried glassware under an atmosphere of nitrogen gas unless otherwise stated. \(^1\)H-NMR spectra were recorded at 300 MHz with a Bruker Fourier 300 or 400 MHz with a Bruker AVS-400 or at 500 MHz with a Bruker DRX-500. \(^13\)C-NMR spectra were recorded at 75 MHz with a Bruker Fourier 300 or 100 MHz with a Bruker AVS-400 or 125 MHz with a Bruker DRX-500. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, hept = heptuplet, b = broad. Chemical shift values of \(^1\)H and \(^13\)C NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard. The multiplicities refer to the resonances in the off-resonance decoupled spectra. These were elucidated using the distortionless enhancement by polarization transfer (DEPT) spectral editing technique, with secondary pulses at 90° and 135°, and/or Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) 2D-NMR techniques. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), t = triplet (methylene), q = quartet (methyl). Mass spectra (EI) were obtained at 70 eV with a type VG Autospec spectrometer (Micromass), with a type LCT (ESI) (Micromass) or with a type Q-TOF (Micromass) spectrometer in combination with a Waters Aquity Ultraperformance LC system, or by LC/MS Finnigan Surveyor MSQ Plus (Thermo Fisher Scientific, Dreieich, Germany). The system consists of LC pump, autosampler, PDA detector, and single-quadrupole MS detector, as well as the standard software Xcalibur for operation. Analytical thin-layer chromatography was performed using precoated silica gel 60 F\(_{254}\) plates (Merck, Darmstadt), and the spots were visualized with UV light at 254 nm or alternatively by staining with potassium permanganate, phosphomolybdic acid, 2,4-dinitrophenol or \(p\)-anisaldehyde solutions. Tetrahydrofuran (THF) was distilled under nitrogen from sodium/benzophenone. Dichloromethane (CH\(_2\)Cl\(_2\)) was dried using a Solvent Purification System (SPS). Commercially available reagents were used as supplied. Preparative high performance liquid chromatography using a Merck Hitachi LaChrom system [pump L- 7150, interface D-7000, diode array detector L-7450 (\(\lambda = 220-400\) nm, preferred monitoring at \(\lambda = 230\) nm)] with column (abbreviation referred to in the experimental part given in parentheses): Trentec Reprosil-Pur 120 C18 AQ 5 \(\mu\)m, 250 \(\times\) 8 mm, with guard column, 40 \(\times\) 8 mm (C18-SP). Flash column chromatography was
performed on Merck silica gel 60 (230-400 mesh). Eluents used for flash chromatography were distilled prior to use. Melting points were measured using a SRS OptiMelt apparatus or a Stuart Scientific melting point apparatus SMP3 (Bibby Sterlin, UK). Arenes 5, 11 and p-nitrobenzoic acid 15 are commercially available.

2. Synthesis of Cystobactamid 507

Methyl 3-hydroxy-4-nitrobenzoate (S1)

3-Hydroxy-4-nitrobenzoic acid (10) (5.10 g, 27.85 mmol) was dissolved in MeOH (43 mL) and cooled to 0 °C. SOCl₂ (3.20 mL, 43.69 mmol) was slowly added and the reaction mixture was stirred under refluxing conditions for 17 hours. The residual oil was redissolved in MeOH and concentrated (4x in order to remove the excess of SOCl₂), to yield the title compound S1 (5.49 g, 27.85 mmol, quantitative) as a yellow solid.

mp: 91 – 92°C; ¹H NMR (400 MHz, CDCl₃) δ 10.49 (s, 1H, OH), 8.17 (d, J = 8.8 Hz, 1H), 7.83 (d, J = 1.8 Hz, 1H), 7.61 (dd, J = 8.8, 1.8 Hz, 1H), 3.96 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 164.96 (Cq), 154.81 (Cq), 138.14 (Cq), 135.95 (Cq), 125.41 (CH), 121.81 (CH), 120.74 (CH), 53.06 (CH₃) ppm; HRMS (ESI): Calculated for C₇H₆NO₃ (M-H)⁻: 196.0246, found: 196.0249.

Methyl 3-isopropoxy-4-nitrobenzoate (S2)

Methyl 3-hydroxy-4-nitrobenzoate S1 (5.47 g, 27.75 mmol) was dissolved in DMF (32.4 mL). K₂CO₃ (19.17 g, 138.73 mmol) and iPrI (3.90 mL, 38.85 mmol) were added, and the reaction mixture was stirred at 50 °C for 17 hours. The resulting mixture was poured into ethyl acetate (100 mL) and washed with H₂O (2x) and brine (1x). The organic layer was dried over MgSO₄, filtered and the solvent was evaporated in vacuo to give an oily residue, which was purified by flash chromatography (petroleum ether/ethyl acetate= 8:2) to yield the title compound S2 (5.66 g, 23.66 mmol, 85%) as a yellow oil.

¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, J = 8.4 Hz, 2H), 7.64 (dd, J = 8.4, 1.6 Hz, 1H), 4.77 (hept, J = 6.1 Hz, 1H), 3.95 (s, 3H), 1.41 (d, J = 6.1 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 165.48 (Cq), 150.92 (Cq), 143.90 (Cq), 134.57 (Cq), 125.20 (CH), 121.24 (CH), 117.08 (CH), 73.15 (CH), 52.92 (CH₃), 21.93 (CH₃) ppm; HRMS (Qtof): Calculated for C₁₁H₁₃NO₃Na (M+Na)⁺: 262.0691, found: 262.0700.
3-Isopropoxy-4-nitrobenzoic acid (S3)

![Chemical Structure](image)

Methyl 3-isopropoxy-4-nitrobenzoate (S2) (4.75 g, 19.87 mmol) was dissolved in a mixture of THF/H₂O (105 mL/105 mL). Then, solid LiOH (4.76 g, 198.68 mmol) was added and the reaction mixture was stirred at room temperature for 17 hours. The aqueous layer was acidified with 1M HCl until pH~1 was reached and extracted with ethyl acetate (3x). The organic extracts were combined, dried over anhydrous MgSO₄ and filtered. The solvent was concentrated in vacuo to yield the title compound S3 (4.12 g, 18.29 mmol, 92%) as a pale yellow solid.

mp: 178 – 180°C; ¹H NMR (400 MHz, CDCl₃) δ 7.80 (d, J = 1.5 Hz, 1H), 7.79 (d, J = 9.0 Hz, 1H), 7.74 (dd, J = 9.0, 1.5 Hz, 1H), 4.79 (hept, J = 6.0 Hz, 1H), 1.43 (d, J = 6.0 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 169.91 (Cq), 150.93 (Cq), 144.52 (Cq), 133.44 (Cq), 125.30 (CH), 121.90 (CH), 117.49 (CH), 73.29 (CH), 21.92 (CH₃) ppm; HRMS (ESI): Calculated for C₁₀H₁₀NO₅ (M-H)⁻: 224.0559, found: 224.0557.

 tert-Butyl-3-isopropoxy-4-nitrobenzoate (S4)

![Chemical Structure](image)

3-Isopropoxy-4-nitrobenzoic acid (S3) (0.40 g, 1.77 mmol) was dissolved in toluene (8 mL). Dimethylformamide di-tert-butyl acetal (5.1 ml, 21.32 mmol) was added at room temperature and the resulting reaction mixture was heated up to 80 °C and stirred for 17 hours. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (petroleum ether/ethyl acetate= 95:5) to afford the title compound S4 (0.47 g, 1.65 mmol, 94% yield) as a pale yellow solid.

mp: 68 – 70°C; ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 1.6 Hz, 1H), 7.59 (dd, J = 8.4, 1.6 Hz, 1H), 4.77 (hept, J = 6.0 Hz, 1H), 1.63 (s, 9H), 1.43 (d, J = 6.0 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 164.08 (Cq), 150.92 (Cq), 143.56 (Cq), 136.56 (Cq), 125.06 (CH), 121.06 (CH), 116.99 (CH), 82.59 (Cq), 73.11 (CH), 28.23 (CH₃), 21.97 (CH₃) ppm; HRMS (ESI): Calculated for C₁₁H₁₂NO₅ (M+Na)⁺: 304.1161, found: 304.1161.

 tert-Butyl 4-amino-3-isopropoxybenzoate (11)

![Chemical Structure](image)
**tert-Butyl 3-isopropoxy-4-nitrobenzoate (S4)** (0.43 g, 1.54 mmol) was dissolved in MeOH (13 mL) and degassed. Pd/C (10% wt., 82.0 mg, 0.077 mmol) was added and vacuum was applied under cooling to remove air. The flask was flushed with H\(_2\) gas and the suspension was stirred for 2 days at room temperature. The catalyst was filtered over Celite\(^\oplus\) and washed with MeOH. The solvent was removed under reduced pressure to yield the title compound **11** (0.39 g, 1.54 mmol, quantitative) as a dark oil.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.46\) (dd, \(J = 8.1, 1.8\) Hz, 1H), 7.43 (d, \(J = 1.8\) Hz, 1H), 6.64 (d, \(J = 8.1\) Hz, 1H), 4.61 (hept, \(J = 6.0\) Hz, 1H), 4.16 (s, 2H\(_{\text{NHR}}\)), 1.57 (s, 9H), 1.36 (d, \(J = 6.0\) Hz, 6H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 166.33\) (Cq), 144.35 (Cq), 141.83 (Cq), 123.71 (CH), 121.68 (Cq), 114.19 (CH), 113.45 (CH), 80.19 (Cq), 70.98 (CH), 28.46 (CH\(_3\)), 22.37 (CH\(_3\)) ppm; HRMS (ESI): Calculated for C\(_{14}\)H\(_2\)NO\(_3\) (M+H): 252.1600, found: 252.1597.

### 6-Bromo-2,3-dihydroxybenzaldehyde (S5)

![6-Bromo-2,3-dihydroxybenzaldehyde](image)

To a solution of 6-bromo-2-hydroxy-3-methoxybenzaldehyde (4) (25.0 g, 108.2 mmol) in CH\(_2\)Cl\(_2\) (270 mL) at -30 °C was slowly added BBr\(_3\) (1 M in CH\(_2\)Cl\(_2\), 200.0 mL, 200.0 mmol) via dropping funnel over a period of 45 minutes. The solution was allowed to warm to room temperature and stirred 17 hours. H\(_2\)O was added and the reaction mixture was stirred for additional 30 minutes. The solution was then extracted with ethyl acetate (3x) and washed with H\(_2\)O (1x). The combined, organic layers were dried over anhydrous MgSO\(_4\), filtered and concentrated in vacuo to give the title compound **S5** (22.16 g, 102.11 mmol, 95%) as a yellow solid.

mp: 135 – 136°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 12.13\) (d, \(J = 0.5\) Hz, 1H\(_{\text{OH}}\)), 10.27 (s, 1H\(_{\text{CH}}\)), 7.07 (d, \(J = 8.5\) Hz, 1H), 7.02 (dd, \(J = 8.5, 0.5\) Hz, 1H), 5.67 (b, 1H\(_{\text{OH}}\)) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 198.42\) (Cq), 151.19 (Cq), 144.99 (Cq), 124.40 (CH), 121.96 (CH), 117.45 (Cq), 116.05 (Cq) ppm; HRMS (ESI): Calculated for C\(_{12}\)H\(_9\)Br\(_2\)O\(_3\) (M-H): 214.9344, found: 214.9343.

### 4-Bromo-3-hydroxymethylbenzene-1,2-diol (S6)

![4-Bromo-3-hydroxymethylbenzene-1,2-diol](image)
A solution of 6-bromo-2,3-dihydroxybenzaldehyde (S5) (22.16 g, 102.10 mmol) in THF (650 mL) at -40 °C was added NaBH₄ in three portions (3.86 g, 102.10 mmol). The resulting mixture was stirred for 30 minutes at room temperature. A saturated aqueous solution of NH₄Cl (300 mL) was added and the mixture was stirred for another 10 minutes, before being finally treated with 1M HCl (300 mL). After 10 minutes of additional stirring, the aqueous phase was extracted with ethyl acetate (3x). The combined, organic extracts were dried over anhydrous MgSO₄ and filtered. The solvent was removed under reduced pressure to yield the title compound S6 (20.27 g, 92.53 mmol, 91%) as a colorless solid. mp: 90 – 92°C; ¹H NMR (400 MHz, MeOD) δ 6.88 (d, J = 8.5 Hz, 1H), 6.64 (d, J = 8.5 Hz, 1H), 4.82 (s, 2H) ppm; ¹³C NMR (100 MHz, MeOD) δ 147.06 (Cq), 146.07 (Cq), 126.88 (Cq), 123.86 (CH), 116.55 (CH), 114.41 (Cq), 61.13 (CH₂) ppm; HRMS (ESI): Calculated for C₁₀H₇BrO₃ (M-H): 216.9500, found: 216.9505.

5-Bromo-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (5)

![structure of 5-Bromo-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (5)]

A solution of 4-bromo-3-hydroxymethylbenzene-1,2-diol (S6) (20.27 g, 92.53 mmol) in THF (550 mL) was treated with PhCH(OMe)₂ (20.8 mL, 138.8 mmol) and ρTsOH.H₂O (0.19 g, 1.02 mmol). The mixture was stirred at room temperature for 5 days. CH₂Cl₂ was added and then washed successively with 5% aqueous NaHCO₃ (1x) and brine (1x). The aqueous phase was extracted with ethyl acetate (3x). The combined, organic extracts were dried over anhydrous MgSO₄, filtered and the solvent was removed under reduced pressure. Purification by flash chromatography (petroleum ether/ethyl acetate= 95/5) afforded 5-bromo-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (5) (16.02 g, 52.16 mmol, 56%) as a colorless solid. mp: 89 – 91°C; ¹H NMR (400 MHz, CDCl₃) δ 7.62 – 7.55 (m, 2H), 7.50 – 7.43 (m, 3H), 7.07 (d, J = 8.6 Hz, 1H), 6.78 (d, J = 8.6 Hz, 1H), 5.97 (s, 1H), 5.40 (s, 1H,OH), 4.99 (s, 2H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 143.99 (Cq), 141.77 (Cq), 136.14 (Cq), 130.13 (CH), 128.79 (CH), 126.68 (CH), 124.90 (CH), 120.95 (Cq), 115.00 (CH), 109.40 (Cq), 99.98 (CH), 67.8 (CH₂) ppm; HRMS (ESI): Calculated for C₁₄H₁₀BrO₃ (M-H): 304.9813, found: 304.9813.

5-Bromo-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (6)

![structure of 5-Bromo-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (6)]
5-Bromo-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (5) (6.00 g, 19.54 mmol; max. amount) was dissolved in acetone (250 mL). Then, Ni(NO₃)₂·5H₂O (5.68 g, 19.54 mmol) and pTsOH·H₂O (3.72 g, 19.54 mmol) were added. The mixture was stirred at room temperature for 2.5 hours. The reaction mixture was filtered through a pad of Celite®, washed with CH₂Cl₂ and concentrated in vacuo. Purification by flash chromatography (dry load: SiO₂ + CH₂Cl₂; petroleum ether/ethyl acetate= 9:1) yielded the title compound 6 (5.08 g, 14.43 mmol, 74%) as a bright yellow solid.

mp: 154 – 156°C; ¹H NMR (400 MHz, CDCl₃) δ 10.60 (b, 1H, OH), 7.96 (s, 1H), 7.65 – 7.57 (m, 2H), 7.48 – 7.42 (m, 3H), 6.02 (s, 1H), 4.99 (s, 2H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 144.88 (Cq), 135.45 (Cq), 133.17 (Cq), 130.16 (CH), 128.95 (Cq), 128.86 (CH), 126.65 (CH), 119.17 (CH), 109.16 (Cq), 99.87 (CH), 67.37 (CH₂) ppm; HRMS (ESI): Calculated for C₁₄H₉BrNO₅ (M-H): 349.9664, found: 349.9660.

5-Bromo-8-isopropoxy-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin (S7)

5-Bromo-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin-8-ol (6) (13.79 g, 39.16 mmol) was dissolved in THF (429 mL). iPrOH (4.00 mL, 50.91 mmol) and PPh₃ (13.87 g, 52.87 mmol) were added, and the mixture was stirred until all components were dissolved. DEAD (2.2 M in toluene, 23.1 mL, 50.91 mmol) was slowly added via syringe pump and the mixture was stirred at room temperature 17 hours. The solvent was evaporated in vacuo to give an oily residue, which was purified by flash chromatography (petroleum ether/ethyl acetate= 96:4) to yield the title compound S7 (13.08 g, 33.18 mmol, 85%) as a colorless solid.

mp: 87 – 89°C; ¹H NMR (400 MHz, CDCl₃) δ 7.59 (s, 1H), 7.59 – 7.54 (m, 2H), 7.50 – 7.43 (m, 3H), 5.97 (s, 1H), 5.00 (s, 2H), 4.69 (hept, J = 6.2 Hz, 1H), 1.31 (d, J = 6.2 Hz, 3H), 1.28 (d, J = 6.2 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 149.04 (Cq), 144.46 (Cq), 139.87 (Cq), 135.71 (Cq), 130.05 (CH), 128.80 (CH), 126.36 (CH), 126.15 (Cq), 119.80 (CH), 112.67 (Cq), 99.66 (CH), 78.10 (CH), 67.63 (CH₂), 22.63 (CH₃), 22.37 (CH₃) ppm; HRMS (Qtof): Calculated for C₁₇H₁₆BrNO₅Na (M+Na)⁺: 416.0110, found: 416.0101.

8-Isopropoxy-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin (7)
5-Bromo-8-isopropoxy-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin (S7) (4.00 g, 10.15 mmol), Pd₂(dba)₃ (0.93 g, 1.01 mmol), (PhO)₂P (0.53 mL, 2.03 mmol), Cs₂CO₃ (4.30 g, 13.19 mmol) and iPrOH (4.7 mL, 60.88 mmol) were dissolved in 1,4-dioxane (28 mL). The oil bath was preheated to 60°C and the mixture was stirred at 80°C for 1.5 hours. The reaction mixture was filtered through a pad of Celite® and washed with ethyl acetate. The combined, organic extracts were dried over anhydrous MgSO₄, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (petroleum ether/ethyl acetate= 96:4) to yield the title compound 7 (2.24 g, 7.10 mmol, 70%) as a pale yellow solid.

mp: 80 – 82°C; ¹H NMR (400 MHz, CDCl₃) δ 7.65 – 7.55 (m, 2H), 7.51 – 7.41 (m, 3H), 7.37 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.01 (s, 1H), 5.19 (d, J = 15.5 Hz, 1H), 5.03 (d, J = 15.5 Hz, 1H), 4.71 (hept, J = 6.2 Hz, 1H), 1.32 (d, J = 6.2 Hz, 3H), 1.28 (d, J = 6.2 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 147.67 (Cq), 144.27 (Cq), 140.55 (Cq), 136.26 (Cq), 129.85 (CH), 128.72 (CH), 126.54 (Cq), 126.34 (CH), 118.82 (CH), 116.69 (CH), 99.61 (CH), 77.71 (CH), 66.44 (CH₂), 22.65 (CH₃), 22.41 (CH₃) ppm; HRMS (QToF): Calculated for C₁₃H₁₇NO₃Na (M+Na)⁺: 338.1004. Found: 338.1003.

6-Hydroxymethyl-2-isopropoxy-3-nitrophenol (S8)

![Image](image_url)

To a mixture of 8-isopropoxy-7-nitro-2-phenyl-4H-benzo-[1,3]-dioxin (7) (4.24 g, 13.43 mmol) in MeOH (102 mL) and CH₂Cl₂ (42 mL) at 0°C was added camphor sulfonyl acid (3.12 g, 13.43 mmol). The mixture was stirred at room temperature for 17 hours. The reaction mixture was quenched with Et₃N until pH~8, concentrated in vacuo and purified by flash chromatography (petroleum ether/ethyl acetate= 7:3) to yield the title compound S8 (2.75 g, 12.09 mmol, 90%) as a light brown solid.

mp: 39 – 41°C; ¹H NMR (400 MHz, CDCl₃) δ 7.46 (d, J = 7.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 6.61 (s, 1H₂OH), 4.81 (d, J = 3.5 Hz, 2H), 4.39 (hept, J = 7.4 Hz, 1H), 1.36 (d, J = 6.2 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 149.07 (Cq), 138.67 (Cq), 132.55 (Cq), 122.28 (CH), 116.63 (CH), 79.38 (CH), 61.47 (CH₂), 22.65 (CH₃) ppm ; HRMS (ESI): Calculated for C₁₉H₁₂NO₅ (M-H)⁻: 226.0715, found: 226.0717.

2-Hydroxy-3-isopropoxy-4-nitrobenzaldehyde (S9)

![Image](image_url)
6-Hydroxymethyl-2-isopropoxy-3-nitrophenol (S8) (2.97 g, 13.05 mmol) was dissolved in CH₂Cl₂ (58 mL). Then MnO₂ (11.35 g, 130.53 mmol) was added and the mixture was stirred at room temperature for 17 hours. The mixture was filtered through a pad of Celite® and washed with CH₂Cl₂. The solvent was concentrated to give the title compound S9 (2.38 g, 10.57 mmol, 81%) as a brown oil.

¹H NMR (400 MHz, CDCl₃) δ 11.44 (s, 1H, CH=O), 9.97 (s, 1H, OH), 7.39 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 4.88 (hept, J = 6.2 Hz, 1H), 1.33 (d, J = 6.2 Hz, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 196.39 (Cq), 156.53 (Cq), 149.36 (Cq), 139.74 (Cq), 127.28 (CH), 122.57 (Cq), 114.32 (CH), 77.42 (CH), 22.51 (CH₃) ppm; HRMS (ESI): Calculated for C₁₀H₁₀NO₅ (M-H)⁻: 224.0559. Found: 224.0535.

2-Hydroxy-3-isopropoxy-4-nitrobenzoic acid (8)

2-Hydroxy-3-isopropoxy-4-nitrobenzaldehyde (S9) (2.36 g, 10.49 mmol) was dissolved in tert-butanol (71 mL). 2-Methyl-2-butene (2M in THF, 36.7 mL, 73.45 mmol) and a solution of NaClO₂ (2.85 g, 31.48 mmol) and NaH₂PO₄ (6.32 g, 47.22 mmol) in H₂O (51 mL) were added in sequential order. The reaction mixture was stirred at room temperature for 17 hours. 6M NaOH was added until pH–10 and the solvent was concentrated in vacuo. H₂O was added and the organic layer was extracted with petroleum ether (2x). The aqueous layer was acidified with 6M HCl until pH–1 and extracted with ethyl acetate (3x). The organic extracts were combined, dried over MgSO₄ and filtered. The solvent was concentrated in vacuo to yield the title compound 8 (1.90 g, 7.87 mmol, 75%) as a yellow semisolid material.

¹H NMR (400 MHz, MeOD) δ 7.72 (d, J = 8.7 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 4.86 – 4.82 (m, 1H), 1.27 (d, J = 6.2 Hz, 6H) ppm; ¹³C NMR (100 MHz, MeOD) δ 172.74 (Cq), 158.01 (Cq), 149.63 (Cq), 139.97 (Cq), 125.8 (CH), 117.41 (Cq), 113.75 (CH), 77.54 (CH), 22.59 (CH₃) ppm; HRMS (ESI): Calculated for C₁₀H₁₀NO₅ (M-H)⁻: 240.0508, found: 240.0511.

Methyl 2-hydroxy-3-isopropoxy-4-nitrobenzoate (S10)
TMSCHN$_2$ (2.0 M in Et$_2$O, 0.87 mL, 1.75 mmol) was added to a solution of 2-hydroxy-3-isopropoxy-4-nitrobenzoic acid (8) (0.32 g, 1.35 mmol) in a mixture 5/1 of toluene/MeOH (10.4/2 mL) at 0 °C. After stirring at 0 °C for 1 hour, the reaction was terminated by addition of acetic acid. The solvent was evaporated in vacuo to give an oily residue, which was purified by flash chromatography (petroleum ether/ethyl acetate= 95:5) to yield the title compound S10 (0.72 g, 2.82 mmol, 80%) as a yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$) δ 11.29 (s, 1H, OH), 7.63 (d, $J = 8.8$ Hz, 1H), 7.12 (d, $J = 8.8$ Hz, 1H), 4.84 (hept, $J = 6.2$ Hz, 1H), 4.00 (s, 3H), 1.32 (d, $J = 6.2$ Hz, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) δ 169.98 (Cq), 157.03 (Cq), 149.21 (Cq), 139.84 (Cq), 123.92 (CH), 115.68 (Cq), 113.35 (CH), 77.36 (CH), 53.21 (CH$_3$), 22.47 (CH$_3$) ppm; HRMS (ESI): Calculated for C$_{13}$H$_{12}$NO$_5$ (M-H)$^-$: 254.0665, found: 254.0666.

**Methyl 2-benzyloxy-3-isopropoxy-4-nitrobenzoate (S11)**

Methyl 2-hydroxy-3-isopropoxy-4-nitrobenzoate (S10) (0.87 g, 3.47 mmol) was dissolved in THF (38 mL). BnOH (0.47 mL, 4.51 mmol) and PPh$_3$ (1.23 g, 4.68 mmol) were added, and the mixture was stirred until all components are dissolved. DEAD (2.2 M in toluene, 2.05 mL, 4.51 mmol) was slowly added via syringe pump and the mixture was stirred at room temperature 17 hours. The solvent was evaporated in vacuo to give an oily residue, which was purified by flash chromatography (petroleum ether/ethyl acetate= 95:5) to yield the title compound S11 (0.84 g, 2.43 mmol, 70%) as a colorless oil.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.53 (d, $J = 8.6$ Hz, 1H), 7.50 (d, $J = 8.6$ Hz, 1H), 7.48 – 7.44 (m, 2H), 7.42 – 7.35 (m, 3H), 5.14 (s, 2H), 4.74 (hept, $J = 6.2$ Hz, 1H), 3.86 (s, 3H), 1.28 (d, $J = 6.2$ Hz, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.27 (Cq), 153.38 (Cq), 148.37 (Cq), 145.65 (Cq), 136.36 (Cq), 130.85 (Cq), 128.65 (CH), 128.71 (CH), 128.72 (CH), 125.07 (CH), 119.29 (CH), 78.18 (CH), 76.39 (CH$_3$), 52.81 (CH$_3$), 22.45 (CH$_3$) ppm; HRMS (QTof): Calculated for C$_{18}$H$_{19}$NO$_6$Na (M+Na)$^+$: 368.1110, found: 368.1112.

**2-Benzylloxy-3-isopropoxy-4-nitrobenzoic acid (9)**
Methyl 2-benzyloxy-3-isopropoxy-4-nitrobenzoate (S11) (0.82 g, 2.38 mmol) was dissolved in a mixture 1/1 of THF/H2O (12.6/12.6 mL). Then, solid LiOH (0.57 g, 23.76 mmol) was added and the reaction mixture was stirred at room temperature for 17 hours. The aqueous layer was acidified with 1M HCl until pH=1 and extracted with ethyl acetate (3x). The organic extracts were combined, dried over anhydrous MgSO4 and filtered. The solvent was concentrated in vacuo to yield the title compound 9 (0.75 g, 2.26 mmol, 95%) as a yellow semisolid material.

1H NMR (400 MHz, CDCl3) δ 7.91 (d, J = 8.7 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.39-7.41 (m, 5H), 5.35 (s, 2H), 4.67 (hept, J = 6.0 Hz, 1H), 1.35 (d, J = 6.0 Hz, 6H) ppm; 13C NMR (100 MHz, CDCl3) δ 163.55 (Cq), 152.60 (Cq), 149.55 (Cq), 144.60 (Cq), 133.91 (Cq), 129.41 (CH), 129.45 (CH), 129.28 (CH), 127.15 (Cq), 127.11 (CH), 120.13 (CH), 79.15 (CH), 77.89 (CH2), 22.52 (CH3) ppm; HRMS (ESI): Calculated for C17H16NO6 (M-H): 330.0978, found: 330.0976.

tert-Butyl 4-[2-(benzyloxy)-3-isopropoxy-4-nitrobenzamido]-3-isopropoxybenzoate (S12)

![Structure S12](image)

2-Benzylxloxy-3-isopropoxy-4-nitrobenzoic acid (9) (23.3 mg, 0.070 mmol) was dissolved in CH2Cl2 (3.5 mL) and preactivated with Ghosez’s reagent (13) (37.2 µL, 0.28 mmol) for 1 day at 40 ºC. tert-Butyl 4-amino-3-isopropoxybenzoate (11) (61.6 mg, 0.25 mmol) was dissolved in CH2Cl2 (3.5 mL) and N,N-diisopropylethylamine (DIPEA) was added (0.10 mL, 0.57 mmol). The solution containing the acyl chloride was then added and the reaction mixture was stirred for 1 day at 40 ºC. The solvent was removed and the crude product was purified by preparative HPLC (RP-18; run time 100 min; H2O/McCN= 100 : 0 →0 : 100; tr = 62 min) providing the title compound S12 (0.32 g, 0.056 mmol, 80%) as a light orange oil.

1H NMR (400 MHz, CDCl3) δ 10.33 (s, 1H, NH), 8.52 (d, J = 8.6 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.63 (dd, J = 8.6, 1.7 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.55 (d, J = 1.7 Hz, 1H), 7.24 – 7.13 (m, 5H), 5.25 (s, 2H), 4.70 (hept, J = 6.1 Hz, 1H), 4.63 (hept, J = 6.1 Hz, 1H), 1.61 (s, 9H), 1.39 (d, J = 6.1 Hz, 6H), 1.27 (d, J = 6.1 Hz, 6H) ppm; 13C NMR (100 MHz, CDCl3) δ 165.72 (Cq), 161.32 (Cq), 151.06 (Cq), 147.88 (Cq), 146.06 (Cq), 145.21 (Cq), 134.13 (Cq), 133.02 (CH), 132.42 (Cq), 130.00 (CH), 129.40 (CH), 128.66 (CH), 127.57 (Cq), 125.77 (CH), 123.06 (CH), 120.03 (CH), 119.34 (CH), 113.18 (CH), 81.19 (Cq), 78.88 (CH), 77.37 (CH2), 71.67 (CH), 28.40 (CH3), 22.57 (CH3), 22.11 (CH3) ppm; HRMS (ESI): Calculated for C31H36N2O8Na (M+Na+) : 587.2369, found: 587.2368.
**tert-Butyl 4-(4-amino-2-hydroxy-3-isoproxybenzamido)-3-isoproxybenzoate (12)**

![Chemical Structure of 12](attachment:image.png)

**tert-Butyl 4-[2-(benzyloxy)-3-isoproxy-4-nitrobenzamido]-3-isoproxybenzoate (S12)** (59.0 mg, 0.10 mmol) was dissolved in MeOH (1.0 mL) and degassed. Pd/C (10% wt., 10.0 mg, 0.0095 mmol) was added and vacuum was applied under cooling to remove air. The flask was flushed with H2 gas and the suspension was stirred for 2 hours at room temperature. The catalyst was filtered off over a pad of Celite®, washed with MeOH and the solvent was removed under reduced pressure. The title compound 12 (42.1 mg, 0.095 mmol, 91%) was obtained as a yellow oil.

1H NMR (400 MHz, CDCl3) δ 12.27 (s, 1H,OH), 8.79 (s, 1H, NH), 8.46 (d, J = 8.6 Hz, 1H), 7.63 (dd, J = 8.6, 1.8 Hz, 1H), 7.55 (d, J = 1.8 Hz, 1H), 7.07 (d, J = 8.6 Hz, 1H), 6.28 (d, J = 8.6 Hz, 1H), 4.74 (hept, J = 6.2 Hz, 1H), 4.68 (hept, J = 6.2 Hz, 1H), 4.27 (b, 2H, NH2), 1.60 (s, 9H), 1.43 (d, J = 6.1 Hz, 6H), 1.34 (d, J = 6.1 Hz, 6H) ppm; 13C NMR (100 MHz, CDCl3) δ 168.49 (Cq), 165.70 (Cq), 156.38 (Cq), 146.38 (Cq), 145.96 (Cq), 132.29 (Cq), 131.98 (Cq), 127.06 (Cq), 123.16 (CH), 121.43 (CH), 118.92 (CH), 113.32 (CH), 106.56 (Cq), 106.25 (CH), 81.09 (Cq), 74.41 (CH), 72.02 (CH), 28.39 (CH3), 22.87 (CH3), 22.40 (CH3) ppm; HRMS (ESI): Calculated for C23H23N2O6 (M+H)+: 445.2339, found: 445.2337.

**tert-Butyl 4-(2-hydroxy-3-isoproxy-4-(4-nitrobenzamidobenzamido)-3-isoproxybenzoate (S13)**

![Chemical Structure of S13](attachment:image.png)

**tert-Butyl 4-(4-amino-2-hydroxy-3-isoproxybenzamido)-3-isoproxybenzoate (12)** (29.0 mg, 0.065 mmol) and p-nitrobenzoic acid (14) (21.8 mg, 0.13 mmol) were dissolved in CH2Cl2 (0.65 mL). PPh3Cl2 (0.13 g, 0.39 mmol) was added and the mixture was stirred under refluxing conditions for 17 hours. The solvent was evaporated in vacuo to give an oily residue, which was purified by flash chromatography (petroleum ether/ethyl acetate= 8:2) to yield the title compound S13 (28.7 mg, 0.048 mmol, 78%) as a yellow semisolid material.

1H NMR (400 MHz, CDCl3) δ 12.49 (s, 1H,OH), 8.96 (s, 1H, NH), 8.93 (s, 1H, NH), 8.47 (d, J = 8.8 Hz, 1H), 8.40 (d, J = 8.8 Hz, 2H), 8.17 (d, J = 8.8 Hz, 1H), 8.07 (d, J = 8.8 Hz, 2H), 7.65 (dd, J = 8.8, 1.6 Hz, 1H), 7.58 (d, J = 1.6 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 4.93 (hept, J = 6.1 Hz,1H), 4.77 (hept, J =
6.1 Hz, 1H), 1.61 (s, 9H), 1.46 (d, $J = 6.1$ Hz, 6H), 1.38 (d, $J = 6.1$ Hz, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.03 (Cq), 165.52 (Cq), 163.22 (Cq), 155.07 (Cq), 150.12 (Cq), 146.18 (Cq), 140.19 (Cq), 136.64 (Cq), 135.05 (Cq), 131.48 (Cq), 128.32 (CH), 127.88 (Cq), 124.41 (CH), 123.06 (CH), 120.67 (CH), 119.12 (CH), 113.30 (CH), 112.23 (Cq), 109.93 (CH), 81.29 (Cq), 75.60 (CH), 72.21 (CH), 28.37 (CH$_3$), 23.09 (CH$_3$), 22.40 (CH$_3$) ppm; HRMS (ESI): Calculated for C$_{31}$H$_{56}$N$_3$O$_9$ (M+H$^+$): 594.2452, found: 594.2454.

Cystobactamid C (3)

\[ \text{3} \]

\text{tert-Butyl 4-(2-hydroxy-3-isopropoxy-4-(4-nitrobenzamido)benzamido)-3-isopropoxybenzoate (S13)}

(8.1 mg, 0.014 mmol) was dissolved in MeOH (1 mL). SnCl$_2$.2H$_2$O (9.2 mg, 0.041 mmol) was added and the reaction mixture was stirred under refluxing conditions for 17 hours. The solvent was evaporated under reduced pressure and the residue diluted with EtOAc. After addition of a saturated solution of NaHCO$_3$ and separation of the phases, the aqueous layer was extracted with EtOAc (1x). The aqueous layer was acidified with 1M HCl until pH~1 and extracted with ethyl acetate (3x). The combined organic layers were washed with brine (1x), dried over anhydrous MgSO$_4$ and filtered. The crude product was purified by preparative HPLC (RP-18; run time 100 min; H$_2$O/MeCN = 100 : 0 $\rightarrow$ 0 : 100; tr = 47 min) providing the title compound 3 (2.8 mg, 5.5 mmol, 40%) as a semisolid material.

$^1$H NMR (400 MHz, MeOD) $\delta$ 8.46 (d, $J = 8.6$ Hz, 1H), 7.80 (d, $J = 8.6$ Hz, 1H), 7.75 (d, $J = 8.6$ Hz, 1H), 7.72 (d, $J = 8.6$ Hz, 2H), 7.71 – 7.64 (m, 2H), 6.74 (d, $J = 8.6$ Hz, 2H), 4.78 (hept, $J = 6.1$ Hz, 1H), 4.55 (hept, $J = 6.1$ Hz, 1H), 1.46 (d, $J = 6.1$ Hz, 6H), 1.35 (d, $J = 6.1$ Hz, 6H) ppm; $^{13}$C NMR (125 MHz, MeOD) $\delta$ 167.80 (Cq), 167.02 (Cq), 154.27 (Cq), 152.92 (Cq), 148.39 (Cq), 138.21 (Cq), 138.16 (Cq), 134.11 (Cq), 130.23 (CH), 125.50 (CH), 124.02 (CH), 122.35 (Cq), 121.26 (CH), 116.22 (CH, Cq), 115.22 (Cq), 114.79 (CH), 114.32 (CH), 77.13 (CH), 73.26 (CH), 22.71 (CH$_3$), 22.32 (CH$_3$) ppm.; HRMS (ESI): Calculated for C$_{25}$H$_{36}$N$_3$O$_7$ (M+H$^+$): 508.2084, found: 508.2085.
3. Synthesis of cystobactamid 507 derivative 18

Scheme S1. Overview on the synthesis of compound 18

**Step 1:** Synthesis of the tetrasubstituted arene

\[
\text{o-vanillin} \xrightarrow{\text{AcCl/pyridine}} \text{S14} \xrightarrow{\text{KNO}_3/\text{TFAA}} \text{S15} \xrightarrow{\text{(1) NaOH, (2) AgNO}_3} \text{15}
\]

**Step 2:** Synthesis of the trisubstituted arene

\[
\text{COOH} \xrightarrow{\text{SO}_2(\text{OMe})_2, \text{K}_2\text{CO}_3/\text{DMF}} \text{COOMe} \xrightarrow{\text{Fe/NH}_4\text{Cl}} \text{16}
\]

**Step 3:** Synthesis of compound 18

\[
\text{COOH} \quad \text{COOMe} \xrightarrow{\text{PPh}_3/\text{xylene}} \text{S17: } X = \text{O} \quad \xrightarrow{\text{Fe/NH}_4\text{Cl}} \text{18}
\]

\[
\text{COOH} \quad \text{S18: } X = \text{O} \quad \xrightarrow{\text{NaOH 1M, MeOH/THF}} \text{18}
\]

\[
\text{COOH} \quad \text{S19: } X = \text{H} \quad \xrightarrow{\text{Fe/NH}_4\text{Cl}} \text{18}
\]
2-Formyl-6-methoxyphenyl acetate (S14)

To a stirred solution of o-vanillin (4.56 g, 30 mmol), and pyridine (2.43 mL, 30 mmol) in CH₂Cl₂ (40 mL), acetyl chloride (2.36 g, 30 mmol) was added drop wise. The reaction was stirred at room temperature overnight then the solvent was removed by vacuum distillation. The obtained material was triturated with cold dil. HCl and collected by filtration, washed with cold water and then with n-hexane.

Yield 94% (off-white solid); mp: 75–76 °C; ¹H NMR (300 MHz, CDCl₃) δ 10.14 (s, 1H), 7.47 (dd, J = 8.1, 1.6 Hz, 1H), 7.34 (t, J = 8.1 Hz, 1H), 7.22 (dd, J = 8.1, 1.6 Hz, 1H), 3.89 (s, 3H), 2.41 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 188.68 (CH), 168.68 (Cq), 151.76 (Cq), 141.54 (Cq), 129.22 (Cq), 126.77 (CH), 121.29 (CH), 117.83 (CH), 56.35 (CH₃), 20.39 (CH₃); m/z (ESI+) 195 [M + H]⁺.

6-Formyl-2-methoxy-3-nitrophenyl acetate (S15)

To a stirred ice-cold suspension of S14 (1.94 g, 10 mmol), and KNO₃ (1.01 g, 10 mmol) in CHCl₃ (15 mL), trifluoroacetic anhydride (12 mL) was added. The reaction was stirred in an ice bath for 2 h then at room temperature overnight. The reaction was diluted very carefully with water (50 mL) and extracted with CHCl₃ (3 × 30 mL). The combined organic extracts were dried with anhydrous MgSO₄, and the solvent was removed by vacuum distillation. The residue was dissolved in toluene and purified using flash chromatography (SiO₂, n-hexane/EtOAc = 3:1).

Yield 45% (yellow oil); ¹H NMR (300 MHz, CDCl₃) δ 10.10 (s, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 3.99 (s, 3H), 2.48 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 186.95 (CH), 168.14 (Cq), 147.71 (Cq), 146.92 (Cq), 146.74 (Cq), 132.13 (Cq), 124.98 (CH), 122.22 (CH), 62.91 (CH₃), 20.43 (CH₃); m/z (ESI+) 239 [M⁺].

2-Hydroxy-3-methoxy-4-nitrobenzoic acid (15)
To a stirred suspension of S15 (957 mg, 4 mmol) in water (50 mL), NaOH (0.8 g, 20 mmol) was added. The reaction was heated under refluxing conditions for 2 h then cooled to room temperature. AgNO₃ (3.4 g, 20 mmol) was added portionwise and the reaction mixture was heated under refluxing conditions overnight before it was cooled and filtered through a pad of Celite™. The filtrate was cooled in an ice bath and acidified by HCl 37% to pH 3–4. The precipitated solid was collected by filtration, washed with cold water and then with n-hexane.

Yield 65% (beige solid); mp: 203–205 °C; ¹H NMR ⁸² (300 MHz, DMSO-d₆) δ 13.67 (br s, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 8.7 Hz, 1H), 5.70 (br s, 1H), 3.93 (s, 3H); ¹³C NMR (75 MHz, DMSO-d₆) δ 170.81 (Cq), 155.95 (Cq), 147.25 (Cq), 140.53 (Cq), 125.22 (CH), 117.88 (Cq), 112.69 (CH), 61.42 (CH₃); m/z (ESI+) 213 [M⁺].

**Methyl 3-methoxy-4-nitrobenzoate (S16)**

\[
\text{O} \quad \text{O} \\
\text{NO}_2
\]

To a stirred mixture of 3-hydroxy-4-nitrobenzoic acid (9.16 g, 50 mmol) and K₂CO₃ (15.2 g, 110 mmol) in DMF (150 mL), dimethyl sulfate (25.2 g, 200 mmol) was added portionwise. The reaction mixture was heated at 90 °C overnight and then cooled to room temperature. The reaction mixture was poured onto ice-cooled water (400 mL), the precipitate was collected by filtration, washed with cold water and then with n-hexane.

Yield 95% (pale yellow solid); mp: 90–91 °C; ¹H NMR ⁸³ (300 MHz, CDCl₃) δ 7.84 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 1.6 Hz, 1H), 7.70 (dd, J = 8.4, 1.6 Hz, 1H), 4.02 (s, 3H), 3.97 (s, 3H); ¹³C NMR ⁸³ (75 MHz, CDCl₃) δ 165.22 (Cq), 152.46 (Cq), 142.41 (Cq), 134.90 (Cq), 125.34 (CH), 121.40 (CH), 114.63 (CH), 56.76 (CH₃), 52.84 (CH₃); m/z (ESI+) 212 [M + H⁺].

**Methyl 4-amino-3-methoxybenzoate (16)**

\[
\text{O} \quad \text{O} \\
\text{NH}_2
\]

To a stirred solution of S16 (2.11 g, 10 mmol) in EtOH (60 mL), iron powder (2.80 g, 50 mmol) was added at 55 °C followed by an aqueous solution of NH₄Cl (266 mg, 5 mmol) in 30 mL. The reaction mixture was heated at 90 °C for 1 h, then iron was filtered off while the suspension was still hot and the filtrate was concentrated in vacuo. The residue was diluted with water (30 mL) and treated with a
saturated aqueous solution of NaHCO₃ (to pH 7–8). The reaction mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried over anhydrous MgSO₄, and the solvent was removed by vacuum distillation. The obtained material was triturated with n-hexane, and collected by filtration.

Yield 85% (beige crystals); mp: 130–132 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.55 (dd, J = 8.2, 1.6 Hz, 1H), 7.46 (d, J = 1.6 Hz, 1H), 6.67 (d, J = 8.2 Hz, 1H), 4.24 (br s, 2H), 3.90 (s, 3H), 3.87 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 167.29 (Cq), 146.09 (Cq), 140.99 (Cq), 124.02 (CH), 119.49 (Cq), 113.11 (CH), 111.10 (CH), 55.56 (CH₃), 51.68 (CH₃); m/z (ESI+) 182 [M + H]⁺.

Methyl 4-(2-hydroxy-3-methoxy-4-nitrobenzamido)-3-methoxybenzoate (17)

![Chemical Structure of 17](image)

To a stirred solution of arene 15 (213 mg, 1 mmol) in a mixture of xylenes (30 mL) and CH₂Cl₂ (5 mL), arene 16 (181 mg, 1 mmol) was added. The reaction mixture was heated to 60 °C and then PCl₅ (0.05 mL, 0.5 mmol) was added. The reaction mixture was heated at 150 °C for 12 h. The solvent was removed by vacuum distillation. The residue was dissolved in MeOH and mixed with silica gel and the resulting paste was dried in vacuo. The silica adsorbed material was purified using flash chromatography (SiO₂, n-hexane/EtOAc = 1:1).

Yield 50% (yellow solid); mp: 189–191 °C; ¹H NMR (500 MHz, CDCl₃) δ 12.07 (br s, 1H), 9.02 (br s, 1H), 8.51 (d, J = 8.5 Hz, 1H), 7.77 (dd, J = 8.5, 1.9 Hz, 1H), 7.64 (d, J = 1.9 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.30 (d, J = 8.8 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 3.94 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 166.43 (Cq), 166.32 (Cq), 156.64 (Cq), 147.93 (Cq), 146.76 (Cq), 142.87 (Cq), 130.28 (Cq), 126.55 (Cq), 123.35 (CH), 120.54 (CH), 119.47 (CH), 118.99 (Cq), 113.54 (CH), 110.94 (CH), 62.01 (CH₃), 56.34 (CH₃), 52.28 (CH₃); m/z (ESI+) 377 [M + H]⁺.

Methyl 4-(4-amino-2-hydroxy-3-methoxybenzamido)-3-methoxybenzoate (S17)

![Chemical Structure of S17](image)

To a stirred solution of 17 (150 mg, 0.4 mmol) in EtOH (20 mL), iron powder (112 mg, 2 mmol) was added at 55 °C followed by NH₄Cl (11 mg, 0.2 mmol) solution in water (2 mL). The reaction was heated at 90 °C for 1 h, then iron was filtered while hot and the filtrate was concentrated in vacuo. The
residue was diluted with water (20 mL) and treated with a saturated aqueous solution NaHCO₃ (to pH 7–8). The reaction mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous MgSO₄, and the solvent was removed by vacuum distillation. The obtained material was triturated with n-hexane, and collected by filtration. Yield 95% (pale yellow solid); m/z (ESI+) 347 [M + H]+.

**Methyl 4-[2-hydroxy-3-methoxy-4-(4-nitrobenzamido)benzamido]-3-methoxybenzoate (S18)**

![Chemical Structure]

To a stirred solution of S17 (115 mg, 0.33 mmol), 14 (56 mg, 0.33 mmol) in anhydrous CHCl₃ (30 mL) under a nitrogen atmosphere, dichlorotriphenylphosphorane (500 mg, 1.5 mmol) was added. The reaction mixture was heated at 80 °C for 12 h. The solvent was removed by vacuum distillation. The residue was dissolved in toluene and purified by flash chromatography (SiO₂, n-hexane/EtOAc = 1:1). Yield 92% (yellow crystals); m/z (ESI+) 496 [M + H]+.

**Methyl 4-[4-(4-aminobenzamido)-2-hydroxy-3-methoxybenzamido]-3-methoxybenzoate (S19)**

![Chemical Structure]

To a stirred solution of S18 (124 mg, 0.25 mmol) in EtOH (20 mL), iron powder (70 mg, 1.25 mmol) was added at 55 °C followed by an aqueous solution of NH₄Cl (7 mg, 0.12 mmol in 2 mL). The reaction mixture was heated at 90 °C for 1 h, then iron was filtered while hot and the filtrate was concentrated in vacuo. The residue was diluted with water (15 mL) and treated with a saturated aqueous solution of NaHCO₃ (to pH 7–8). The reaction mixture was extracted with EtOAc/THF (1:1, 3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous MgSO₄, and the solvent was removed by vacuum distillation. The obtained material was triturated with n-hexane/EtOAc (6:1, 50 mL), and collected by filtration. Yield 90% (white crystals); m/z (ESI+) 466 [M + H]+.
4-[4-(4-Aminobenzamido)-2-hydroxy-3-methoxybenzamido]-3-methoxybenzoic acid (18)

To a stirred solution of S19 (46 mg, 0.1 mmol) in a mixture of MeOH (3 mL) and THF (1 mL), NaOH 1 M (0.5 mL) was added. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo. The remaining residue was dissolved in water (10 mL), cooled in an ice bath and acidified by a saturated aqueous solution KHSO₄ (to pH 6), then extracted with EtOAc/THF (1:1, 3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous MgSO₄, and the solvent was removed by vacuum distillation. The obtained material was triturated with n-hexane/EtOAc (4:1, 25 mL), and collected by filtration.

Yield 82% (pale yellow crystals); mp: 219–221 °C; ¹H NMR (500 MHz, DMSO-d₆) δ 12.71 (br s, 1H), 11.55 (br s, 1H), 10.96 (br s, 1H), 9.16 (br s, 1H), 8.46 (d, J = 8.2 Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 1H), 7.62 (dd, J = 8.2, 1.6 Hz, 1H), 7.57 (d, J = 1.6 Hz, 1H), 6.63 (d, J = 8.8 Hz, 2H), 5.86 (br s, 2H), 3.97 (s, 3H), 3.78 (s, 3H); ¹³C NMR (126 MHz, DMSO-d₆) δ 166.99 (Cq), 165.03 (Cq), 163.83 (Cq), 152.60 (Cq), 149.96 (Cq), 148.43 (Cq), 139.10 (Cq), 136.47 (Cq), 131.95 (Cq), 129.42 (2CH), 125.91 (Cq), 125.23 (CH), 122.68 (CH), 120.25 (Cq), 119.63 (CH), 115.01 (Cq), 113.63 (CH), 112.77 (2CH), 111.15 (CH), 60.44 (CH₂), 56.17 (CH₃); m/z (ESI+) 452 [M + H]⁺.

**Biological evaluation**

Compounds 3 and 18 have been tested in antimicrobial susceptibility and in vitro gyrase assays as described previously. The IC₅₀ values on E. coli gyrase of synthetic 3 and 18 were 328 μM and 463 μM, respectively.

**Reference supporting information**


Attachment: $^1$H- and $^{13}$C- NMR spectra of the described compounds.
Cystobactamide C (3)
Compound S14
Compound S15
Compound 15
Compound S16
Compound 16
Compound 17

Chemical Shift (ppm)

1H NMR spectrum of Compound 17

Chemical Shift (ppm)

13C NMR spectrum of Compound 17
Compound 18