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

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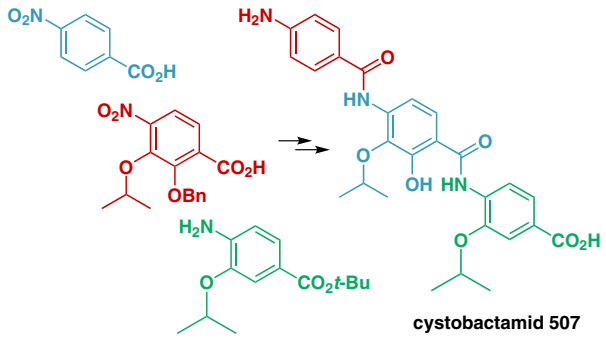

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Abstract The first total synthesis of cystobactamid 507, a member of a class of new natural products with strong inhibitory activity towards bacterial topoisomerases, is reported. Synthetic key challenges are the central tetrasubstituted arene and the low chemical reactivity of anilines and *ortho*-phenolic and isopropoxy-substituted benzoic acids. Biological evaluations demonstrate that cystobactamid 507 inhibits several Gram-positive pathogens but at significantly lower concentrations than described for the larger members of this natural product family.

Key words amides, antibiotics, medicinal chemistry, natural products, total synthesis

Recently, Müller and co-workers reported on an unusal group of nonribosomal peptides called the cystobactamids 919-1, 919-2, and 507 (**1**–**3**, Figure 1), which were isolated in rather small amounts (<100 µg/L) from *Cystobacter* sp. besides several more structurally similar derivatives that could not be fully characterized yet.[1] They are potent antibacterial agents that inhibit several clinically relevant Gram-negative and Gram-positive bacteria such as *Acinetobacter baumannii* (minimum inhibitory concentration, MIC = 7.4 to >59 µg/mL), *Enterococcus faecalis* (MIC = 0.1–7.4 µg/mL), *Staphylococcus aureus* (MIC = 0.1–32.5 µg/mL), *Streptococcus pneumoniae* (MIC = 0.1–14.7 µg/mL) as well as *E. coli* (MIC = 0.9–29.4 µg/mL).

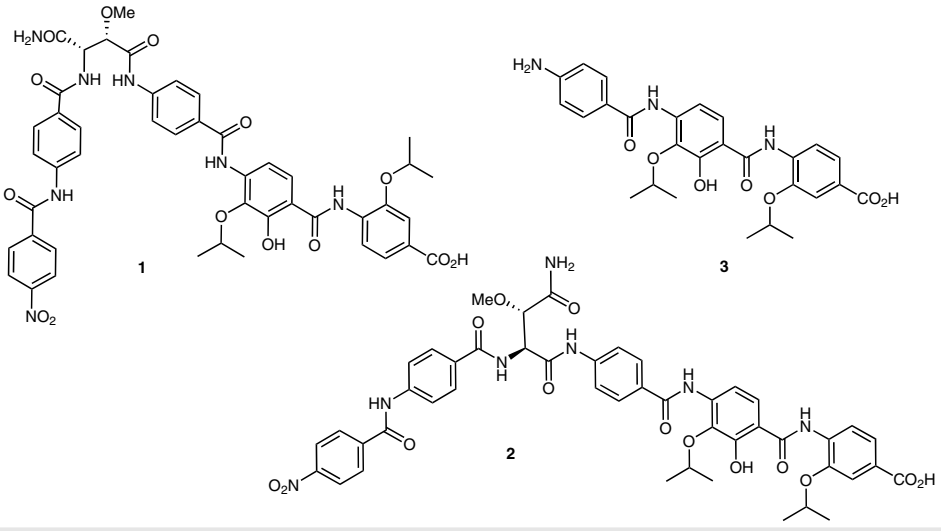


Figure 1 Structures of cystobactamids 919-1, 919-2, 507 (**1**–**3**)

Most of these strains are made responsible for nosocomial infections.[2] Preliminary studies showed[1] that the cystobactamids target bacterial type IIa topoisomerases which are validated antibacterial targets. However, as quinolones are not suited anymore to serve as template for new inhibitors the cystobactamids offer new opportunities in search for new anti-infectives,[3] especially as this novel structural scaffold and the limited cross-resistance found make the cystobactamids promising lead structures. Structurally, cystobactamid 507 (**3**) is the simplest member. It was reported to exert similar but lower inhibitory activity than cystobactamids **1** and **2**.

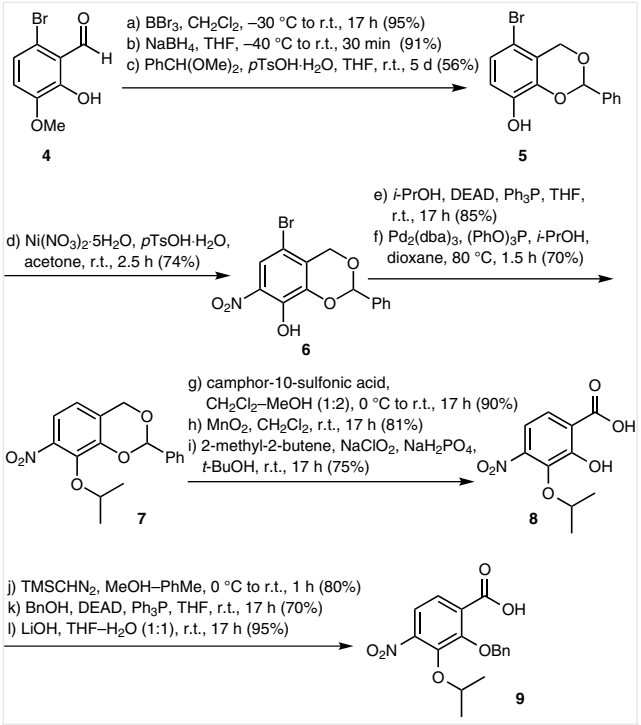
En route to **1** and **2** we synthesized cystobactamid 507 (**3**) which additionally would allow us to further test its biological properties. These tests would show whether the trisaryl unit is an essential element for all cystobactamids and furthermore would clarify if minor impurities present in the natural sample did alter the assay read-outs.

Although cystobactamids only contain *p*-aminobenzoic acids we experienced two major synthetic challenges: a) accessing the tetrasubstituted arene unit and b) the lack of reactivity of anilines and the lack for reactivity of *ortho*-substituted phenolic and isopropoxy benzoic acids in amide formations. This amide formation can only proceed under conditions that are different from those established in peptide synthesis.

The synthesis of the tetrasubstituted arene **9** commenced with *o*-bromobenzaldehyde **4**. The bromo substituent served as 'dummy' group which can be removed after the selective introduction of the nitrogen functionality at C4. In addition, this starting material allows for differentiating between the two phenolic groups and thus enables selective introduction of the isopropyl group at C3 (Scheme 1).

O-Demethylation of **4**, aldehyde reduction and protection of the benzylic alcohol and one phenolic group as benzylidene acetal yielded phenol **5**. This protection paved the way for introducing a) the nitro group to yield nitroarene **6**[4] and b) the isopropyl group. Palladium-catalyzed debromination[5] yielded nitroarene **7**. After removal of the benzylidene protection, the benzylic alcohol was transformed into the carboxylate under standard conditions. The resulting benzoic acid **8** was temporarily methylated in order to protect the *o*-phenolic group, which finally furnished the desired *p*-nitrobenzoic acid **9**. These final steps were crucial as successful amide formation could only be achieved if the *o*-phenolic group is protected.

With this key building block in hand, we could finalize the synthesis of cystobactamid 507 (**3**) by coupling three *p*-aminobenzoic acid units. However, we had to search for conditions that allow for creating an amide bond between two arene moieties. Common reagent systems such as HOAt, EDC, or a mixture of HOBt and EDC that are well established in peptide chemistry gave poor coupling yields. We made the bulky isopropoxy substituents with *ortho* orientation to the amino groups as well as the reduced reactivity of the aromatic amino groups responsible for the difficulties to achieve amide formation.

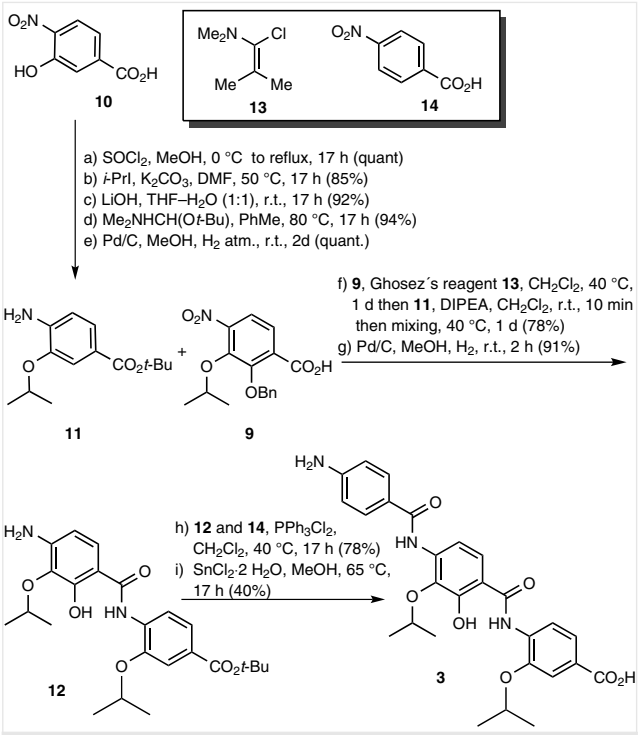


Scheme 1 Synthesis of tetrasubstituted benzoic acid **9**

We found that Ghosez's reagent **13**[6] is best suited to couple benzoates with anilines (Scheme 2). First, aniline **11**, which straightforwardly is accessible from benzoic acid **10**, was coupled[7] with tetrasubstituted benzoic acid **9** to yield amide **12** after hydrogenation of the nitro group and with concomitant removal of the benzyl protecting group. Next, the second amide coupling[8] between **12** and *p*-nitrobenzoic acid **14** yielded cystobactamid 507 (**3**) after simultaneous reduction of the nitro group[9] and removal of the *tert*-butyl ester. It has to be noted that it became necessary to switch from a methyl to a *tert*-butyl ester (**10** → **11**) because the final ester hydrolysis with the corresponding methyl ester (step under basic conditions) led to simultaneous amide hydrolysis of the *p*-aminobenzoic acid moiety.

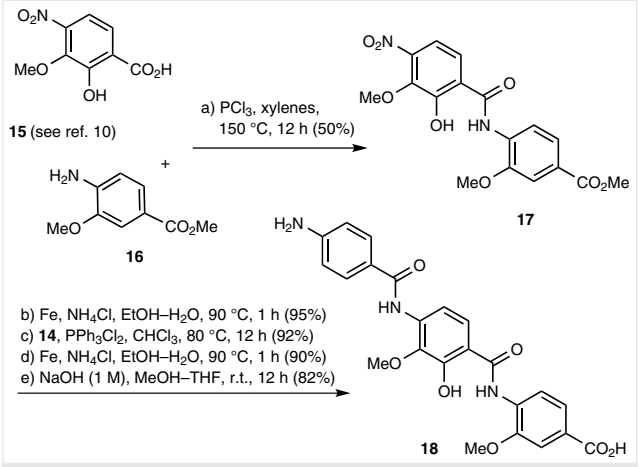
The NMR spectra and chromatographic parameter (HPLC) for the synthetic material were identical to those collected for natural cystobactamid 507 (**3**).

The synthetic hurdles that we encountered in this synthesis stemmed us to prepare a structurally simplified derivate **18** in which both isopropoxy groups of cystobactamid 507 are replaced by the smaller methoxy groups. Starting from *o*-vanillin the tetrasubstituted central arene unit **15**



Scheme 2 Finalization of the synthesis of cystobactamid 507 (**3**)

(Scheme 3 and Supporting Information) was straightforwardly prepared in three steps and coupled with arenes **14** and **16**.[10]



Scheme 3 Synthesis of cystobactamid derivative **18**[10]

The antibacterial profile and the gyrase inhibitory properties of synthetic cystobactamid 507 (**3**) and derivative **18** were assessed. We found that both compounds showed some inhibitory activity against certain Gram-positive bacteria and an efflux-deficient *E. coli* strain with MIC values between 16 and 128 µg/mL (Table 1). Likewise, the IC_{50} values (half-inhibitory concentration) for *E. coli* gyrase were in the high µM range (ca. 300–500 µM).

It has to be stressed that *Cystobacter* sp. generates only very small amounts (<100 µg/L) of cystobactamids and their isolation is further hampered by the fact that the fermentation broth contains at least a dozen structurally closely related cystobactamids.[1] It has already been pointed out that the natural product **3** might be contaminated with trace amounts of cystobactamid hexapeptides, which are responsible for the sample's moderate antibacterial activity.

Here we could demonstrate that synthetic **3** still shows some antibacterial activity mainly against Gram-positive bacteria, which is, however, by 1 to 2 orders of magnitudes less pronounced than initially described for natural cystobactamid 507. Interestingly, also the simpler methyl derivative **18** exhibits a comparable activity spectrum to that of synthetic **3**.

We conclude that the western part of larger cystobactamids including the β-methoxyasparagine linker is mandatory for full biological activity in cell-based studies as well as in vitro topoisomerase inhibition experiments. One reason for the lack of antibacterial activity against Gram-negative pathogens might be explained by insufficient penetration of **3** and **18** through the outer bacterial membrane as demonstrated by the finding that the trisaryl compounds are only active against *E. coli* with increased permeability (Table 1). However, since both tested synthetic compounds **3** and **18** exhibit some residual antibacterial and gyrase inhibitory activity modification of the trisaryl unit might be a promising starting point for the optimization of the larger cystobactamid scaffold.

Table 1 Biological Activity of **3** and **18**[a]

	3	**18**	CP[b]
E. coli ATCC-25922	>128	>128	0.005
E. coli DSM-1116	>128	>128	0.01
E. coli DSM-26863[c]	>128	>128	0.003
E. coli DSM-26863/PMBN[d]	32–64	64	0.003
B. subtilis DSM-10	32	128	0.1
E. faecalis ATCC-29212	64–128	>128	0.8
M. luteus DSM-1790	128	64	0.8
S. aureus ATCC-29213	128	64	0.1
S. pneumoniae DSM-20566	64	16	0.8

[a] MIC values in µg/mL.
[b] Ciprofloxacin.
[c] *tolC3* genotype.
[d] Cotreatment with 3 µg/mL polymyxin B nonapeptide.

In summary, we reported on the first synthesis of the new antibiotic cystobactamid **3**. We were able to access the synthetically challenging tetrasubstituted benzoic acid **9** and establish coupling conditions for anilines with bulky isopropxy groups positioned in the *ortho* position. This work paves the way for preparing libraries of cystobact-

amid derivatives. Additonally, this work provided a first insight into structure–activity relationships. Clearly, all structural elements present in the larger cystobactamids are essential for their potent antibacterial properties. This work is important for initiating a medicinal chemistry program for further improving the biological profile of the cystobactamids.

Final Synthetic Step and Analytical Data for Cystobactamid C (3)

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). $SnCl_2 \cdot 2H_2O$ (9.2 mg, 0.041 mmol) was added, and the reaction mixture was stirred under refluxing conditions for 17 h. 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 (1×). The aqueous layer was acidified with 1 M HCl until pH = ca. 1 and extracted with EtOAc (3×). The combined organic layers were washed with brine (1×), dried over anhydrous $MgSO_4$, and filtered. The crude product was purified by preparative HPLC (RP-18; run time 100 min; H_2O–MeCN = 100: 0 to 0: 100; t_R = 47 min) providing the title compound **3** (2.8 mg, 5.5 mmol, 40%) as a semisolid material.

^{1}H NMR (400 MHz, MeOD): δ = 8.46 (d, *J* = 8.6 Hz, 1 H), 7.80 (d, *J* = 8.6 Hz, 1 H), 7.75 (d, *J* = 8.6 Hz, 1 H), 7.72 (d, *J* = 8.6 Hz, 2 H), 7.71–7.64 (m, 2 H), 6.74 (d, *J* = 8.6 Hz, 2 H), 4.78 (hept, *J* = 6.1 Hz, 1 H), 4.55 (hept, *J* = 6.1 Hz, 1 H), 1.46 (d, *J* = 6.1 Hz, 6 H), 1.35 (d, *J* = 6.1 Hz, 6 H) ppm. ^{13}C NMR (125 MHz, MeOD): δ = 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. ESI-HRMS: *m/z* calcd for $C_{27}H_{30}N_3O_7$ $[M + H]^+$: 508.2084; found: 508.2085.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1380509. Included are experimental procedures, spectral data and copies of ^{1}H and ^{13}C NMR spectra of all new compounds and intermediates.

References

(1) Baumann, S.; Herrmann, J.; Raju, R.; Steinmetz, H.; Mohr, K. I.; Hüttel, S.; Harmrolfs, K.; Stadler, M.; Müller, R. *Angew. Chem. Int. Ed.* **2014**, *53*, 14605; a range of new cystobactamid derivatives will be reported elsewhere.
(2) Weinstein, R. A.; Gaynes, R.; Edwards, J. R. *Clin. Infect. Dis.* **2005**, *41*, 848.
(3) Hooper, D. C. *Emerg. Infect. Dis.* **2001**, *7*, 337.
(4) Anuradha, V.; Srinivas, P. V.; Aparna, P.; Rao, J. M. *Tetrahedron Lett.* **2006**, *47*, 4933.
(5) Moon, J.; Lee, S. *J. Organomet. Chem.* **2009**, *694*, 473.
(6) Devos, A.; Remion, J.; Frisque-Hesbain, A.-M.; Colens, A.; Ghosez, L. *J. Chem. Soc., Chem. Commun.* **1979**, 1180.
(7) Prabhakaran, P.; Barnard, A.; Murphy, N. S.; Kilner, C. A.; Edwards, T. A.; Wilson, A. J. *Eur. J. Org. Chem.* **2013**, 3504.
(8) Yap, J. L.; Cao, X.; Vanommeslaeghe, K.; Jung, K.-Y.; Peddaboina, C.; Wilder, P. T.; Nan, A.; MacKerell, A. D. Jr.; Smytheb, W. R. Jr.; Fletcher, S. *Org. Biomol. Chem.* **2012**, *10*, 2928.
(9) Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. *Tetrahedron Lett.* **1969**, 4555.
(10) The synthesis of albicidin, a natural product closely related to the cystobactamids, was recently reported: Kretz, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem.* **2015**, *126*, 1992.