Antibiotics

Synthetic and Biological Studies on New Urea and Triazole Containing Cystobactamid Derivatives

Therese Planke,[a] Katarina Cirnski,[b, c] Jennifer Herrmann,[b, c] Rolf Müller,[b, c] and Andreas Kirschning*[a]

Abstract: Cystobactamids belong to the group of arene-based oligoamides that effectively inhibit bacterial type IIa topoisomerases. Cystobactamid 861-2 is the most active member of these antibiotics. Most amide bonds present in the cystobactamids link benzoic acids with anilines and it was found that some of these amide bonds undergo chemical and enzymatic hydrolysis, especially the one linking ring C with ring D. This work reports on the chemical synthesis and biological evaluation of thirteen new cystobactamids that still contain the methoxyaspartate hinge. However, we exchanged selected amide bonds either by the urea or the triazole groups and modified ring A in the latter case. While hydrolytic stability could be improved with these structural substitutes, the high antibacterial potency of cystobactamid 861-2 could only be preserved in selected cases. This includes derivatives, in which the urea group is positioned between rings A and B and where the triazole is found between rings C and D.

Introduction

Cystobactamids, an unusual group of oligoamides from *Cystobacter* sp. Cbv34, were first reported in 2014.[1] The extracts inhibited the growth of several Gram-negative and Gram-positive bacteria and HPLC-assisted bioactivity-guided screening provided cystobactamids 919-1 (**1**) and 919-2 (**2**) as active compounds (Figure 1). These oligoamides contain *p*-aminobenzoic acid building blocks and either an *iso*-β-methoxyasparagine or a β-methoxyasparagine unit. Later, additional derivatives such as the cystobactamids 920-1 (**3**), 920-2 (**4**) and 862-1 (**5**) were reported that structurally differ in the E-ring and the aspartate hinge region.[2a]

Cystobactamid 862-1 (**5**) was found to be the most active natural member. It inhibits several clinically relevant Gram-positive and Gram-negative strains (*Acinetobacter baumannii:* MIC = 0.5 μg mL^{-1}, *Citrobacter freundii*: MIC = 0.06 μg mL^{-1}, fluoroquinolone resistant *E. coli* WT-III *marR*Δ74bp: MIC = 0.5 μg mL^{-1}, carbapenem resistant *P. aeruginosa* CRE: MIC = 1.0 μg mL^{-1}, and *Proteus vulgaris:* MIC = 0.25 μg mL^{-1})[2] by in-

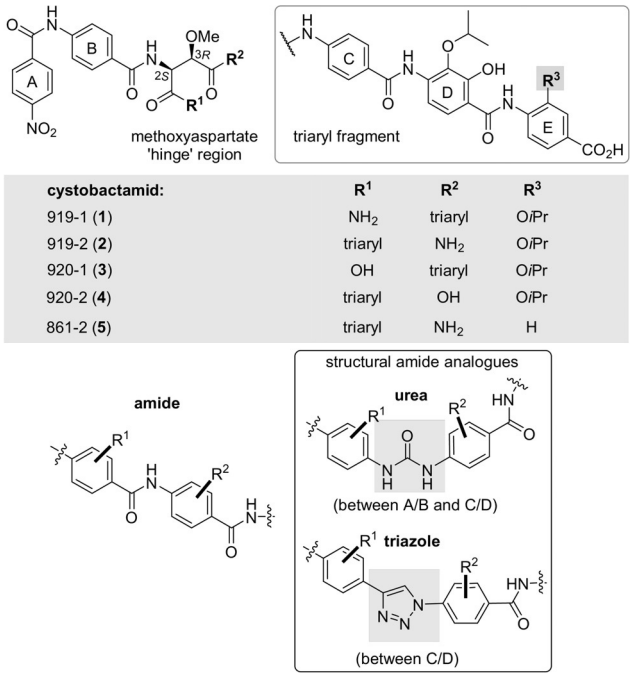


Figure 1. Structures of selected cystobactamids 919-1 (**1**), 919-2 (**2**), 920-1 (**3**), 920-2 (**4**) and 861-2 (**5**) and the urea as well as triazole groups as structural substitutes for amide group (rings are labelled with letters A–E).

[a] *T. Planke, Prof. Dr. A. Kirschning*
Institut für Organische Chemie und
Biomolekulares Wirkstoffzentrum (BMWZ), Leibniz Universität Hannover
Schneiderberg 1B, 30167 Hannover (Germany)
E-mail: andreas.kirschning@oci.uni-hannover.de

[b] *K. Cirnski, Dr. J. Herrmann, Prof. Dr. R. Müller*
Abteilung Mikrobielle Naturstoffe
Helmholtz Institut für Pharmazeutische Forschung Saarland
Helmholtz Zentrum für Infektionsforschung und Universität des Saarlandes
Campus E8.1, 66123 Saarbrücken (Germany)

[c] *K. Cirnski, Dr. J. Herrmann, Prof. Dr. R. Müller*
Deutsches Zentrum für Infektionsforschung (DZIF)
Standort Hannover–Braunschweig, Inhoffenstraße 7
38124 Braunschweig (Germany)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/chem.201904073.

hibiting bacterial type IIa topoisomerases. It has to be stressed, that the cystobactamids are structurally related to albicidin with similar antibacterial properties, which has extensively been studied by Süssmuth et al.[3]

Several total syntheses of cystobactamids 861-2, 919-2 and 920-1 have been published by the Trauner group and by us.[2a,4,5] These endeavours were essential to revise and prove the constitutions as well as the 2*S*,3*R* configurations of cystobactamids.[5] Both, the synthetic programmes and the cystobactamid isolation protocols revealed, that the amide bonds between benzoic acids and anilines are prone to hydrolysis with the amide bond that links rings C and D being the most labile one.

As part of a medicinal chemistry programme on cystobactamids, we therefore envisaged to prepare a library of methoxyaspartate containing cystobactamids. One aspect of this programme covers the substitution of the amide group by supposedly chemically more stable functional elements such as urea and triazole (Figure 1). Besides 1,2,4-triazoles, a *cis* amide bond surrogate,[6] the 1,2,3-triazole ring has recently been found to be a prominent amide bond bioisostere.[7,8]

Here, we report on our recent total synthetic endeavours towards new cystobactamid derivatives with improved chemical stability and the determination of their antibacterial properties. This includes the direct comparison with ciprofloxacin (CP).

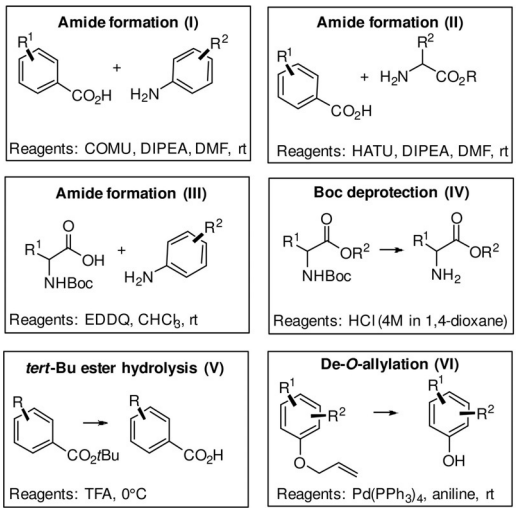
Results and Discussion

Chemical syntheses

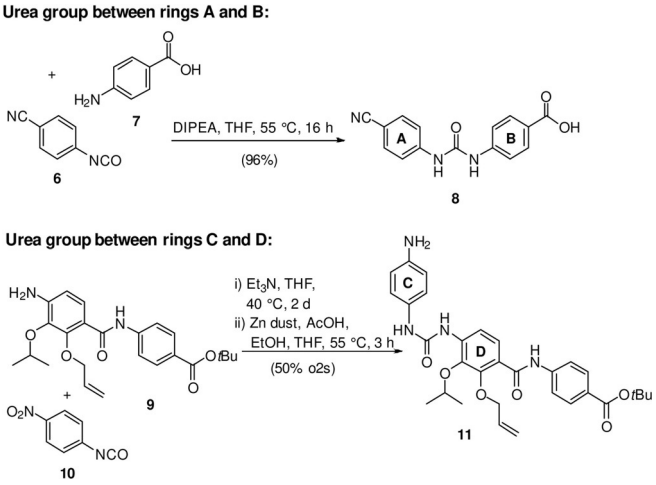
General considerations: Cystobactamids are natural products with a modular architecture. The key structural elements are amide bonds, composed of combinations of benzoic acids, anilines and carboxylate and amino group in the methoxyaspartate unit. In addition, protecting groups for the carboxylates, amines and phenols have to be chosen. Substantial search and optimisation led to a toolbox of reactions listed in Scheme 1 (cases I–VI), that also repeatedly served to prepare the new cystobactamids reported here. Three principal types of amide forming processes have been developed, that combine two aromatic building blocks (case I), a benzoic acid with an amine (case II), and an aniline with a carboxylate (case III).

Boc-deprotection of the methoxyaspartate hinge region was achieved under mildly acidic conditions (case IV), avoiding the hydrolysis of the *tert*-butyl ester in ring E. This ester was commonly cleaved under stronger acidic conditions in neat TFA, routinely the final step of our chemical syntheses (case V). Finally, the allyl deprotection of the phenol function was achieved under Pd^0-catalysed conditions (case VI).

Syntheses of urea-modified cystobactamids: For exchanging the amide group by urea one of the coupling partners was chosen to contain the isocyanate group as in 4-isocyanatobenzonitrile **6** and 1-isocyanato-4-nitrobenzene **10** (Scheme 2). These building blocks were flexibly used to prepare cystobactamid derivatives containing an urea bridge between rings A and B as well as rings C and D. Several coupling partners **9**, **19** and **20** were at hand from our previously published syntheses of cystobactamids 861-2 (**5**) and 920-1 (**3**).[2a,5]



Scheme 1. Reagents employed for principal reaction for constructing cystobactamids (COMU = (1-cyano-2-ethoxy-2-oxoethylidene-aminooxy)dimethylaminomorpholinocarbenium-hexa-fluorophosphate; EEDQ = *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; HATU = [*O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexa-fluorophosphate]; DIPEA = diisopropylethylamine, DMF = dimethylformamide, TFA = trifluoroacetic acid).

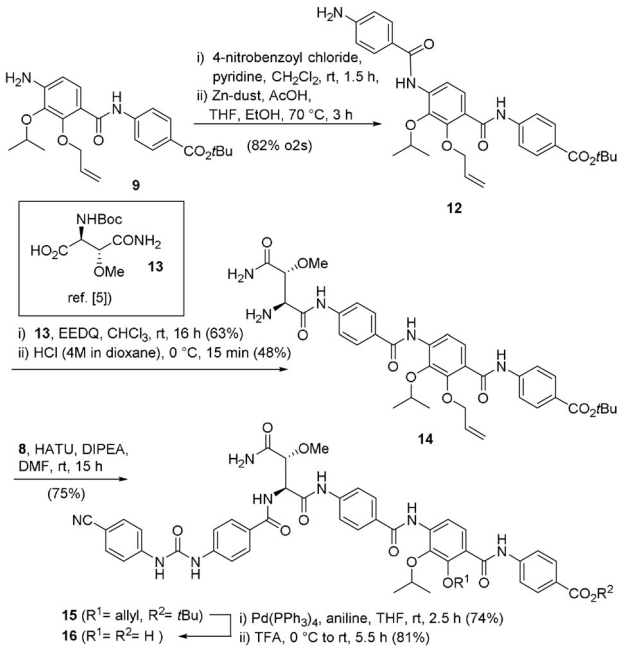


Scheme 2. Synthesis of urea-containing building blocks **8** and **11**.

4-Isocyanato-benzonitrile (**6**) was efficiently coupled with 4-aminobenzoic acid **7** to yield disubstituted urea **8** resembling the linkages between rings A and B.

Diamide **9** was coupled with 1-isocyanato-4-nitrobenzene **10** followed by reduction of the nitro group yielding urea derivative **11** in which the urea group is located between rings C and D. We first prepared cyano derivative **16** in which the urea group is located between the A and B rings (Scheme 3). The cyano derivative was chosen because it has been established that exchange of the nitro group by cyanide leads to improved antibacterial properties.[8]

The synthesis commenced with the coupling of amide **9** with 4-nitrobenzoic acid and reduction of the resulting nitroarene to yield the aniline **12**. Next, the aniline was linked to the (2*S*,3*R*)-methoxyaspartate derivative **13**.[2a] Then, the Boc group



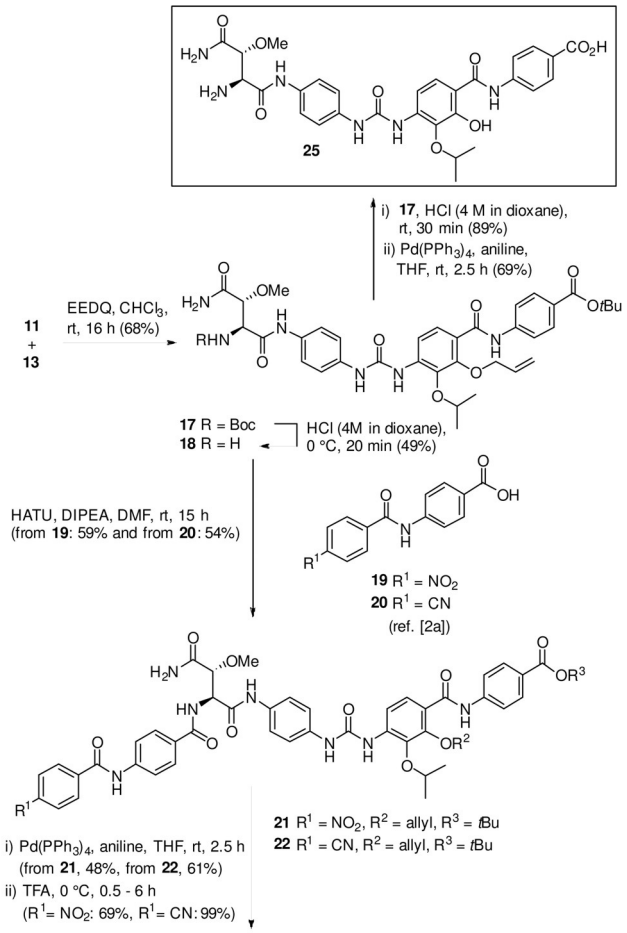
Scheme 3. Synthesis of cystobactamid derivative **16** bearing an urea group between rings A and B.

was hydrolysed under mildly acidic conditions circumventing cleavage of the *tert*-butyl ester. Next, the resulting tetramide **14** was coupled with the urea derivative **8** and the resulting product **15** was transformed into the cystobactamid derivative **16** after *O*-deallylation and ester hydrolysis.

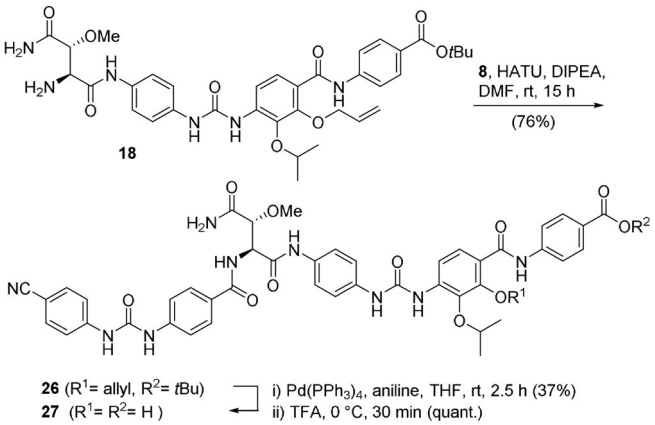
Next, we installed the urea group between rings C and D (Scheme 4). For that, urea derivative **11** was coupled with methoxyaspartate derivative **13** to yield Boc-amide **17**, which was transformed into amine **18** under mildly acidic conditions. Amide formation with diamides **19** and **20**, respectively, yielded cystobactamid precursors **21** and **22**, that underwent the common two-step protocol for removing the allyl- and *tert*-butyl groups. The two urea-bearing cystobactamid derivatives **23** and **24** were purified by RP-HPLC. The truncated derivative **25** was prepared from Boc-protected tetramide **17** under harsher acidic conditions that led to hydrolysis of both, the Boc-group as well as the *tert*-butyl ester. This was followed by cleavage of the allylether group. In order to remove any traces of the transition metal the crude product was also purified by RP-HPLC.

Finally, urea derivative **18** also served as starting point to prepare cystobactamid **27** with two urea groups between rings A/B and C/D (Scheme 5). Coupling with urea derivative **8** yielded compound **26** which was transformed into the cystobactamid derivative **27** in two steps under our standard conditions.

With a set of new urea-modified cystobactamids in hand we tested the chemical stability of derivative **24** representing the group of urea cystobactamids prepared here. It was dissolved in $[D_6]$DMSO, triethyl amine (12 equiv.) was added and a series of ^{1}H NMR spectra were recorded over a period of 27 h. Careful inspection revealed no degradation products derived from



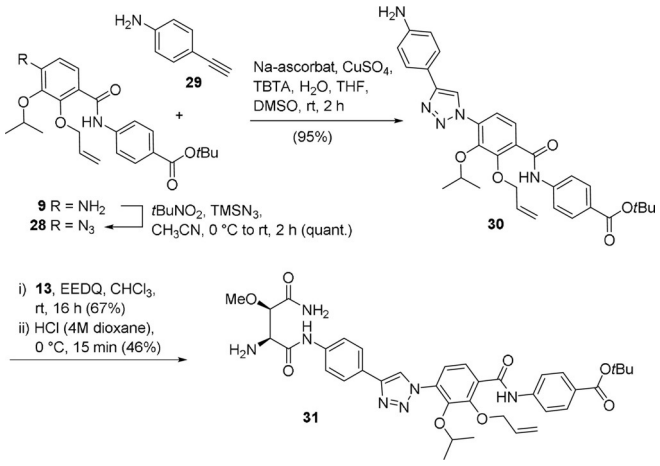
Scheme 4. Synthesis of cystobactamid derivatives **23**–**25** bearing an urea group between rings C and D.



Scheme 5. Synthesis of cystobactamid derivative **27** that contains two urea linkages between rings A/B and C/D.

urea derivative **24**. As expected, exchange of the amide group by urea leads to enhanced stability under basic conditions.

Syntheses of triazole-modified cystobactamids: A second structural element for amide substitution is the triazole ring that is straightforwardly prepared from an alkyne and an azide.[7,8] We pursued to substitute the most labile amide group between rings C and D. Thus, we chose 4-ethynylaniline **29** as alkyne building block and consequently arylazide **28** as second component. The latter was prepared from aniline **9** and reacted under classical Sharpless conditions to yield triazole **30**. Amide coupling with methoxyaspartate **13** provided amine **31** after Boc-deprotection under mildly acidic conditions (Scheme 6).

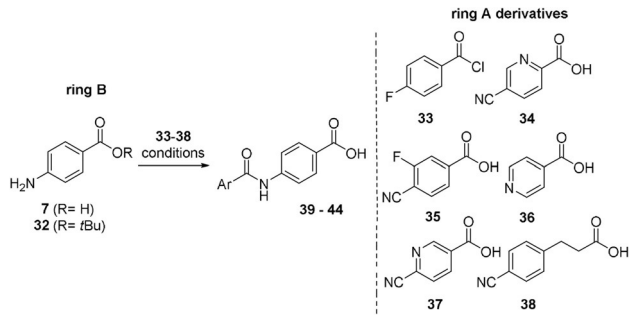


Scheme 6. Synthesis of the central triazole derivative **31**.

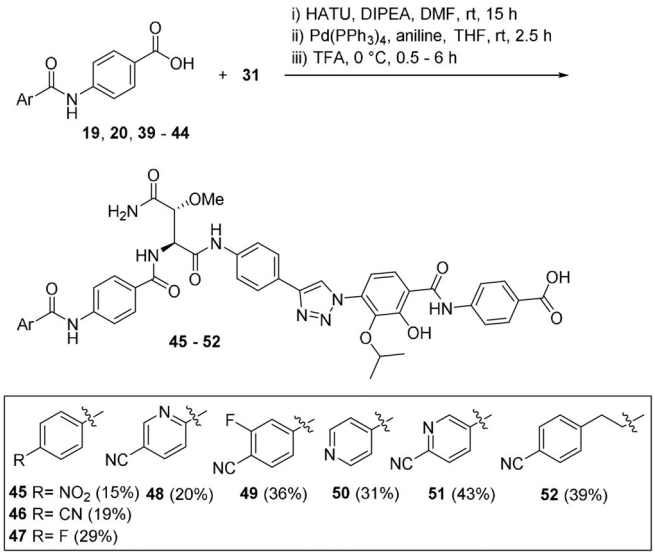
Having installed the triazole ring between rings C and D, we turned our attention to prepare several A/B-ring fragments that vary in ring A (Table 1).

We mainly focused on modifications in the 4-position and exchange of the benzene by the pyridine ring (Table 1; building blocks **33**–**37**). In one case, we used a benzoyl chloride (**33**) which was directly reacted with the aminobenzoic acid (**7**). In most cases, however, the *tert*-butyl ester **32** which was coupled with A-ring derivatives **34**–**37** by in situ activation of their carboxylic function. Finally, also 3-(4-isocyanophenyl)propanoic acid (**38**) was chosen as elongated bishomo analogue of 4-cyanobenzoic acid. Consequently, we had a small library of A/B-ring fragments (**39**–**44**) at hand that was used to access triazole-bearing cystobactamids **45**–**52** in a three step sequence (coupling of A/B fragments to triazole-containing aspartate building block **31**, de-*O*-allylation and *tert*-butyl ester hydrolysis) (Scheme 7).

Table 1. Preparation of diamides **39**–**44** (COMU = 1-cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylamino-morpholino-carbenium-hexafluorophosphate).



Building block ring A	Building block ring B	Conditions	Yields [%]
33	**7**	Na_2CO_3, THF, H_2O, rt, 3 h	**39** (98)
34	**32**	for **34**–**38**: COMU, DIPEA, DMF, rt, 16 h, then	**40** (75)
35	**32**	TFA, rt, 30 min	**41** (66)
36	**32**		**42** (94)
37	**32**		**43** (83)
38	**32**		**44** (79)



Scheme 7. Synthesis of triazole-modified cystobactamids **45**–**52** (yields refer to the three step sequence).

Evaluation of antibacterial properties

The new urea-modified cystobactamids **16**, **23**–**25** and **27** were tested against a panel of different Gram-negative and Gram-positive pathogens, including intrinsically multidrug-resistant *P. aeruginosa* (Table 2) and directly compared with cystobactamid 861-2 (**5**). Incorporation of the urea group between rings C and D as in derivatives **23**–**25** and **27** leads to substantial and even complete loss of antibacterial activity. This also includes truncation that is represented by cystobactamid derivative **25**. In essence, although chemical stabilisation of the cystobactamids can be achieved by exchanging the amide group with urea, this approach fails for connecting rings C and D due to loss of antibacterial activity. However, when the urea group is positioned between rings A and B as in cystobactamid deriv-

Table 2. Biological activity of urea derivatives **16**, **23-25** and **27** compared to cystobactamid 861–2 (**5**) (MIC values in μg mL^{-1}; nd = not determined).[a]

Strain	16	23	24	25	27	5
S. aureus Newman	16	>64	>64	>64	>64	1
E. coli BW25113	≤0.03	nd	1	nd	>64	0.25
E. coli Δ*acrB*	≤0.03	nd	≤0.03	nd	>64	≤0.003
E. coli Δ*tolC*	nd	>64	nd	>64	nd	≤0.003
E. coli DSM-1116	nd	>64	nd	>64	nd	0.2
P. aeruginosa PA14	1	>64	>64	>64	>64	1
PA14Δ*mexAB*	1	>64	>64	>64	>64	0.25

[a] Values for ciprofloxacin (CP) are listed in Table 3.

ative **16**, biological activity is almost completely preserved compared to cystobactamid 861-2 (**5**) and even considerably higher for strain *E. coli* BW25113.

Likewise, antibacterial properties of triazole-modified cystobactamid derivatives **45**–**52** were evaluated (Table 3). Principally, exchange of the amide group by a triazole ring does not lead to loss of antibacterial activity, thus this modification can be regarded as a successful strategy to generate cystobactamid derivatives with enhanced chemical and biological stabilities between rings C and D.[9] Furthermore, this set of new analogues reveals fine-tuning of antibacterial activity by structural changes in ring A. Interestingly, when ring A represents a pyridine ring with N positioned in the *para* and *meta* positions relative to ring B as in compounds **50** and **51** substantial reduction of antibacterial activity against *S. aureus* and *P. aeruginosa* is observed. This is not the case for pyridine derivative **48**, in which the nitrogen atom is located in the *ortho* position. When extending the length between rings A and B by insertion of an ethylene unit as in derivative **52** reduced antibiotic activity is also observed. On the other hand, triazole derivatives **45**–**47** as well as fluoro derivative **49** bearing a cyanide group in the *para* position are still highly active in a comparable range to cystobactamid 861-2 (**5**). Importantly, the activity spans across all four strains tested. The important finding of this study is that the triazole group is a good substitute for the amide linkage between rings C and D.

Conclusion

In summary, we report on the chemical synthesis of thirteen new analogues derived from cystobactamid 861-2 (**5**). These derivatives differ from the most potent natural member of the cystobactamids by exchange of the amide groups by either the urea group or by a triazole ring. These first structure activity studies (SAR) for natural methoxyaspartate bearing cystobactamids reveal that the urea function can be introduced between rings A and B but not between rings D and E for preserving antibacterial activity. We also show that the triazole ring can serve as a substitute for the amide group in cystobactamids. Finally, ring A can be modified in various manners without substantial loss of activity. This work provides a first information on future directions of medicinal chemistry programmes on the cystobactamids and matches recent findings on the albicidins.[10]

Experimental Section

The experimental section below covers general procedures for the synthesis and analytic data of all new cystobactamid derivatives **16**, **23**–**25**, **27** and **45**–**52**. All other synthetic procedures, general information and analytical data including copies of NMR spectra are found in the supporting information.

General procedure V for *tert*-Bu ester hydrolysis (according to Scheme 1)

Precooled TFA (0.02 M) was added slowly to ester (1.0 equiv) at 0 °C. The mixture was warmed up to rt over a period 30 min, then it was stirred between 30 min and 6 h. The reaction was terminated by addition of Et_2O. The precipitate was filtered, washed with an excess of Et_2O and dried under high vacuum.

4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(3-(4-cyanophenyl)ureido)benzamido)-3-methoxy-4-oxo-butanamido)benzamido)-2-hydroxy-3-isopropoxybenz-amido)benzoic acid (16): Following to the general procedure mentioned above phenol **S4** (see SI; 29.8 mg, 32.6 μmol, 1.0 equiv) was stirred for 5.5 h. Acid **16** was obtained as a colourless amorphous solid (22.5 mg, 26.3 μmol, 81 %). $[\alpha]_D^{22} = +23.1^\circ$ (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 12.3$ (1 H, br. s, O*H*), 10.6 (1 H, br. s, N*H*), 10.6 (1 H, s, N*H*), 9.40 (1 H, s, N*H*), 9.38 (1 H, s, NH_{Urea}), 9.26 (1 H, s, NH_{Urea}), 8.37 (1 H, d, $J = 8.1$ Hz, N*H*CH), 7.98–7.95 (4 H, m, Ar*H*), 7.88–7.82 (7 H, m, Ar*H*), 7.76–7.70 (3 H, m, Ar*H*), 7.66 (2 H, m, Ar*H*), 7.58 (2 H, m, Ar*H*), 7.51 (2 H, d, $J = 26.7$ Hz, CONH_2), 4.90 (1 H, t, $J = 8.1$ Hz, C*H*NH), 4.55 (1 H, hept, $J = 6.1$ Hz, C*H*Me$_2$), 4.09 (1 H, d, $J = 8.1$ Hz, C*H*OMe), 3.31 (3 H, s, OCH_3), 1.27 ppm (6 H, d, $J = 6.1$ Hz, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 170.9$ (q, CONH$_2$), 168.8 (q, *C*ONH), 168.5 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.5 (q, *C*ONH), 164.2 (q, *C*ONH), 154.1 (q, C-Ar), 151.9 (q, NH*C*ONH), 144.0 (q, C-Ar), 142.4 (q, C-Ar), 142.3 (q, C-Ar), 142.0 (q, C-Ar), 137.0 (q, C-Ar), 136.3 (q, C-Ar), 133.3 (2C, t, C-Ar), 130.2 (2C, t, C-Ar), 128.5 (2C, t, C-Ar), 128.5 (q, C-Ar), 128.3 (2C, t, C-Ar), 127.1 (q, C-Ar), 126.3 (q, C-Ar), 122.8 (t, C-Ar), 120.7 (2C, t, C-Ar), 119.3 (q, CN), 119.0 (2C, t, C-Ar), 118.2 (2C, t, C-Ar), 117.5 (2C, t, C-Ar), 112.4 (t, C-Ar), 112.2 (q, C-Ar), 103.5 (q, C-Ar), 80.0 (t, *C*HOMe), 74.9 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH_3), 55.7 (t, *C*HNH), 22.3 ppm (2C, p, CH(CH_3)$_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{41}N_8O_{11}$ $[M+H]^+$: 857.2895; found 857.2896.

Table 3. Biological activity of triazole derivatives **45**–**52** compared to cystobactamid 861-2 (**5**) and CP (MIC values in μg mL^{-1}).

Strain	45	46	47	48	49	50	51	52	5	CP
E. coli BW25113	≤0.03	≤0.03	≤0.03	0.125	≤0.03	1	≤0.03	0.25	≤0.03	0.03
E. coli Δ*acrB*	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.25	0.25	0.125	0.06	≤0.003
S. aureus Newman	1	1	8	4	1	>64	64	>64	0.125	0.2
P. aeruginosa PA14	4	4	8	8	4	>64	32	>64	1	0.2
PA14Δ*mexAB*	4	4	8	4	4	>64	32	>64	0.125	0.01

4-(4-(3-(4-((2*S*,3*R*)-4-Amino-3-methoxy-2-(4-(4-nitrobenzamido)-benzamido)-4-oxobutan-amido)phenyl)ureido)-2-hydroxy-3-iso-propoxybenzamido)-benzoic acid (23): Following the general procedure mentioned above phenol **S6** (see SI; 9.0 mg, 9.64 μmol, 1.0 equiv) was stirred for 6 h. Acid **23** was obtained as a colourless amorphous solid (5.8 mg, 6.62 μmol, 69%). $[\alpha]_D^{24} = +38.7^\circ$ (*c* 0.6, DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO +2 μL DCO$_2$D): $\delta = 10.8$ (1H, s, N*H*), 10.6 (1H, br. s, N*H*), 10.2 (1H, s, N*H*), 9.61 (1H, s, NH_{Urea}), 8.40–8.37 (3H, m, Ar*H*, N*H*CH), 8.33 (1H, s, NH_{Urea}), 8.20 (2H, m, Ar*H*), 7.96 (2H, m, Ar*H*), 7.92–7.84 (5H, m, Ar*H*), 7.84 (2H, m, Ar*H*), 7.80 (1H, d, $J = 8.5$ Hz, Ar*H*), 7.60 (2H, m, Ar*H*), 7.49 (2H, d, $J = 46.1$ Hz, CONH_2), 7.42 (2H, m, Ar*H*), 4.89 (1H, t, $J = 8.5$ Hz, C*H*NH), 4.63 (1H, hept, $J = 6.0$ Hz, C*H*Me$_2$), 4.07 (1H, d, $J = 7.3$ Hz, C*H*OMe), 3.30 (3H, s, OCH_3), 1.30 ppm (6H, d, $J = 6.0$ Hz, CH(CH_3)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO +2 μL DCO$_2$D): $\delta = 171.1$ (q, CONH$_2$), 169.0 (q, *C*ONH), 167.6 (q, *C*ONH), 167.0 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 164.3 (q, *C*ONH), 154.5 (q, C-Ar), 152.0 (q, NH*C*ONH), 149.3 (q, C-Ar), 142.1 (q, C-Ar), 141.7 (q, C-Ar), 140.4 (q, C-Ar), 139.1 (q, C-Ar), 135.0 (q, C-Ar), 133.7 (q, C-Ar), 132.9 (q, C-Ar), 130.2 (2C, t, C-Ar), 129.4 (2C, t, C-Ar), 129.2 (q, C-Ar), 128.3 (2C, t, C-Ar), 126.2 (q, C-Ar), 123.6 (2C, t, C-Ar), 123.1 (t, C-Ar), 120.8 (2C, t, C-Ar), 120.2 (2C, t, C-Ar), 119.7 (2C, t, C-Ar), 118.7 (2C, t, C-Ar), 109.3 (q, C-Ar), 108.2 (t, C-Ar), 80.2 (t, *C*HOMe), 74.2 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH_3), 55.6 (t, *C*HNH), 22.0 ppm (2C, p, CH(CH_3)$_2$); HRMS (ESI): *m/z* calc. for $C_{43}H_{40}N_8O_{13}Na$ $[M+Na]^+$: 899.2613; found 899.2613.

4-(4-(3-(4-((2*S*,3*R*)-4-Amino-2-(4-(4-cyanobenzamido)benzamido)-3-methoxy-4-oxo-butanamido)phenyl)ureido)-2-hydroxy-3-iso-propoxybenz-amido)benzoic acid (24): Following the general procedure mentioned above phenol **S7** (see SI; 14.0 mg, 15.3 μmol, 1.0 equiv) was stirred for 30 min. Acid **24** was obtained as a yellow amorphous solid (13.0 mg, 15.2 μmol, 99%). $[\alpha]_D^{23} = +37.2^\circ$ (*c* 0.3, DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO +2 μL DCO$_2$D): $\delta = 12.5$ (1H, s, O*H*), 10.7 (1H, s, N*H*), 10.5 (1H, s, N*H*), 10.1 (1H, s, N*H*), 9.61 (1H, s, NH_{Urea}), 8.38 (1H, d, $J = 8.1$ Hz, N*H*CH), 8.34 (1H, s, NH_{Urea}), 8.13 (2H, m, Ar*H*), 8.04 (2H, m, Ar*H*), 7.96 (2H, m, Ar*H*), 7.91–7.83 (7H, m, Ar*H*), 7.81 (1H, d, $J = 9.2$ Hz, Ar*H*), 7.60 (2H, m, Ar*H*), 7.52–7.41 (4H, m, Ar*H*, CONH_2), 4.88 (1H, t, $J = 8.1$ Hz, C*H*NH), 4.62 (1H, hept, $J = 6.1$ Hz, C*H*Me$_2$), 4.06 (1H, d, $J = 8.1$ Hz, C*H*OMe), 3.30 (3H, s, OCH_3), 1.30 ppm (6H, d, $J = 6.1$ Hz, CH(CH_3)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO +2 μL DCO$_2$D): $\delta = 171.1$ (q, CONH$_2$), 169.0 (q, *C*ONH), 167.6 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.3 (q, *C*ONH), 164.5 (q, *C*ONH), 154.3 (q, C-Ar), 151.9 (q, NH*C*ONH), 142.0 (q, C-Ar), 141.7 (q, C-Ar), 139.1 (q, C-Ar), 138.7 (q, C-Ar), 134.9 (q, C-Ar), 133.7 (q, C-Ar), 132.5 (2C, t, C-Ar), 130.2 (2C, t, C-Ar), 129.0 (q, C-Ar), 128.6 (2C, t, C-Ar), 128.3 (2C, t, C-Ar), 126.1 (q, C-Ar), 123.0 (t, C-Ar), 120.8 (2C, t, C-Ar), 120.6 (q, C-Ar), 120.1 (2C, t, C-Ar), 119.6 (2C, t, C-Ar), 118.7 (2C, t, C-Ar), 118.3 (q, CN), 114.1 (q, C-Ar), 109.2 (q, C-Ar), 108.2 (t, C-Ar), 80.1 (t, *C*HOMe), 74.3 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH_3), 55.5 (t, *C*HNH), 21.9 ppm (2C, p, CH(CH_3)$_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{40}N_8O_{11}Na$ $[M+Na]^+$: 879.2714; found 879.2714.

4-(4-(3-(4-((2*S*,3*R*)-4-Amino-2-(4-(3-(4-cyanophenyl)ureido)benz-amido)-3-methoxy-4-oxobutanamido)phenyl)ureido)-2-hydroxy-3-isopropoxybenz-amido)benzoic acid (27): Following the general procedure mentioned above phenol **S8** (see SI; 9.0 mg, 9.70 μmol, 1.0 equiv) was stirred for 30 min. Acid **27** was obtained as a colourless amorphous solid (8.4 mg, 9.70 μmol, 99%). $[\alpha]_D^{23} = +12.7^\circ$ (*c* 1.1, MeOH); ^{1}H NMR (600 MHz, [D$_6$]DMSO +2 μL DCO$_2$D): $\delta = 12.5$ (1H, br. s, O*H*), 10.5 (1H, br. s, N*H*), 10.1 (1H, s, N*H*), 9.61 (1H, s, NH_{Urea}), 9.37 (1H, s, NH_{Urea}), 9.25 (1H, s, NH_{Urea}), 8.34 (1H, s, NH_{Urea}),8.30 (1H, d, $J = 8.1$ Hz, N*H*CH), 7.96 (2H, m, Ar*H*), 7.89 (1H, d, $J = 9.0$ Hz, Ar*H*), 7.85–7.80 (5H, m, Ar*H*), 7.74 (2H, m, Ar*H*), 7.65 (2H, m, Ar*H*), 7.60–7.55 (4H, m, Ar*H*), 7.52–7.41 (4H, m, Ar*H*, CONH_2), 4.86 (1H, t, $J = 8.1$ Hz, C*H*NH), 4.62 (1H, hept, $J = 6.2$ Hz, C*H*Me$_2$), 4.06 (1H, d, $J = 8.1$ Hz, C*H*OMe), 3.30 (3H, s, OCH_3), 1.30 ppm (6H, d, $J = 6.2$ Hz, CH(CH_3)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO +2 μL DCO$_2$D): $\delta = 171.1$ (q, CONH$_2$), 169.0 (q, *C*ONH), 167.7 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 154.3 (q, C-Ar), 152.0 (q, NH*C*ONH), 151.9 (q, NH*C*ONH), 144.0 (q, C-Ar), 142.3 (q, C-Ar), 142.0 (q, C-Ar), 139.1 (q, C-Ar), 134.9 (q, C-Ar), 133.7 (q, C-Ar), 133.3 (2C, t, C-Ar), 132.8 (q, C-Ar), 130.2 (2C, t, C-Ar), 128.5 (2C, t, C-Ar), 127.3 (q, C-Ar), 126.2 (q, C-Ar), 123.0 (t, C-Ar), 120.8 (2C, t, C-Ar), 120.1 (2C, t, C-Ar), 119.3 (q, CN), 118.7 (2C, t, C-Ar), 118.2 (2C, t, C-Ar), 117.5 (2C, t, C-Ar), 109.2 (q, C-Ar), 108.2 (t, C-Ar), 103.5 (q, C-Ar), 80.1 (t, *C*HOMe), 74.3 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH_3), 55.5 (t, *C*HNH), 21.9 ppm (2C, p, CH(CH_3)$_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{42}N_9O_{11}$ $[M+H]^+$: 872.3004; found 872.3004.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-3-methoxy-2-(4-(4-nitrobenzamido)-benzamido)-4-oxo-butanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-isopropoxy-benzamido)-benzoic acid (45): Following to the general procedure mentioned above phenol **S18** (see SI; 8.5 mg, 9.03 μmol, 1.0 equiv) was stirred for 6 h. Acid **45** was obtained as a colourless amorphous solid (6.2 mg, 7.00 μmol, 78%). $[\alpha]_D^{24} = +37.1^\circ$ (*c* 0.1, DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 12.9 (1H, br. s, CO$_2$*H*), 12.2 (1H, br. s, O*H*), 10.8 (1H, br. s, N*H*), 10.8 (1H, s, N*H*), 10.4 (1H, s, N*H*), 8.93 (1H, s, C$H_{Triazol}$), 8.44 (1H, d, $J =$ 8.1 Hz, N*H*CH), 8.39 (2H, m, Ar*H*), 8.21 (2H, m, Ar*H*), 8.00–7.87 (11H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.50 (2H, d, $J = 39.2$ Hz, CONH_2), 7.37 (1H, d, $J = 8.6$ Hz, Ar*H*), 4.92 (1H, t, $J = 8.1$ Hz, C*H*NH), 4.24 (1H, hept, $J = 6.1$ Hz, C*H*Me$_2$), 4.09 (1H, d, $J = 8.1$ Hz, C*H*OMe), 3.32 (3H, s, OCH_3), 1.02 ppm (6H, d, $J = 6.1$ Hz, CH(CH_3)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 171.0$ (q, CONH$_2$), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 164.2 (q, *C*ONH), 154.5 (q, C-Ar), 149.3 (q, C-Ar), 146.3 (q, C-Ar$_{Triazol}$), 141.9 (q, C-Ar), 141.7 (q, C-Ar), 140.3 (q, C-Ar), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 134.4 (q, C-Ar), 130.7 (2C, t, C-Ar), 130.3 (q, C-Ar), 129.4 (2C, t, C-Ar), 128.7 (2C, t, C-Ar), 128.3 (q, C-Ar), 126.5 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (t, C-Ar), 123.6 (2C, t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.7 (2C, t, C-Ar), 118.4 (q, C-Ar), 114.8 (t, C-Ar), 80.1 (t, *C*HOMe), 75.7 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH_3), 55.7 (t, *C*HNH), 21.8 ppm (2C, p, CH(CH_3)$_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{40}N_9O_{12}$ $[M+H]^+$: 886.2796; found 886.2798.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(4-cyanobenzamido)benzamido)-3-methoxy-4-oxo-butanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-iso-propoxybenzamido)-benzoic acid (46): Following the general procedure mentioned above phenol **S19** (see SI; 10.1 mg, 11.0 μmol, 1.0 equiv) was stirred for 3 h. Acid **46** was obtained as a beige amorphous solid (7.4 mg, 8.55 μmol, 78%). $[\alpha]_D^{25} = +55.9^\circ$ (*c* 0.2, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta =$ 12.8 (1H, br. s, CO$_2$*H*), 12.2 (1H, br. s, O*H*), 10.8 (1H, br. s, N*H*), 10.7 (1H, s, N*H*), 10.4 (1H, s, N*H*), 8.93 (1H, s, C$H_{Triazol}$), 8.43 (1H, d, $J =$ 8.1 Hz, N*H*CH), 8.13 (2H, m, Ar*H*), 8.05 (2H, m, Ar*H*), 7.99–7.87 (11H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.50 (2H, d, $J = 45.0$ Hz, CONH_2), 7.34 (1H, d, $J = 7.0$ Hz, Ar*H*), 4.92 (1H, t, $J = 8.1$ Hz, C*H*NH), 4.26 (1H, hept, $J = 6.1$ Hz, C*H*Me$_2$), 4.09 (1H, d, $J = 8.1$ Hz, C*H*OMe), 3.32 (3H, s, OCH_3), 1.02 ppm (6H, d, $J = 6.1$ Hz, CH(CH_3)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 171.0$ (q, CONH$_2$), 168.2 (q, *C*ONH), 167.4 (q, *C*ONH), 166.8 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 164.5 (q, *C*ONH), 154.7 (q, C-Ar), 146.2 (q, C-Ar$_{Triazol}$), 141.9 (q, C-Ar), 141.7 (q, C-Ar), 138.9 (q, C-Ar), 138.8 (q, C-Ar), 138.7 (q, C-Ar), 134.3 (q, C-Ar), 132.5 (2C, t, C-Ar), 130.3 (2C, t, C-Ar), 129.0 (q, C-Ar), 128.6 (2C, t, C-Ar), 128.3 (2C, t, C-Ar), 126.4 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 123.5 (t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.6 (2C, t, C-Ar), 118.4 (q, C-Ar), 118.3 (q, CN), 114.6 (q, C-Ar), 114.0 (t, C-Ar), 80.1 (t, *C*HOMe), 75.6 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH_3), 55.6 (t, *C*HNH), 21.8 ppm (2C, p, CH(CH_3)$_2$); HRMS (ESI): *m/z* calc. for $C_{45}H_{40}N_9O_{10}$ $[M+H]^+$: 866.2898; found 866.2897.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(4-fluorobenzamido)benzamido)-3-methoxy-4-oxo-butanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-iso-propoxybenzamido)-benzoic acid (47): Following the general procedure mentioned above phenol **S20** (see SI; 20.1 mg, 22.0 μmol, 1.0 equiv) was stirred for 3.5 h. Acid **47** was obtained as an orange amorphous solid (19.1 mg, 19.6 μmol, 89%). $[\alpha]_D^{24} = +27.6^\circ$ (*c* 0.9, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta =$ 12.2 (1H, br. s, O*H*), 10.8 (1H, s, N*H*), 10.5 (1H, s, N*H*), 10.4 (1H, s, N*H*), 8.93 (1H, s, C*H*$_{Triazol}$), 8.41 (1H, d, *J* = 8.1 Hz, N*H*CH), 8.07–8.05 (2H, m, Ar*H*), 8.00–7.87 (11H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.50 (2H, d, *J* = 47.2 Hz, CON*H*$_2$), 7.40–7.36 (3H, m, Ar*H*), 4.92 (1H, t, *J* = 8.1 Hz, C*H*NH), 4.25 (1H, hept, *J* = 6.1 Hz, C*H*Me$_2$), 4.09 (1H, d, *J* = 8.1 Hz, C*H*OMe), 3.32 (3H, s, OC*H*$_3$), 1.03 ppm (6H, d, *J* = 6.1 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 171.0 (q, CONH$_2$), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.0 (q, *C*ONH), 164.7 (q, *C*ONH), 164.4 (d, *J* = 311.0 Hz, *C*-F), 158.2 (TFA), 154.5 (q, C-Ar), 146.3 (q, C-Ar$_{Triazol}$), 142.1 (q, C-Ar), 141.9 (q, C-Ar), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 134.4 (q, C-Ar), 131.1 (d, *J* = 2.8 Hz, C-Ar), 130.5 (2C, d, *J* = 9.1 Hz, C-Ar), 130.3 (2C, t, C-Ar), 129.8 (TFA), 128.6 (q, C-Ar), 128.2 (2C, t, C-Ar), 126.5 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 123.5 (t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.5 (2C, t, C-Ar), 118.4 (q, C-Ar), 115.4 (2C, d, *J* = 21.8 Hz, C-Ar), 114.8 (t, C-Ar), 80.1 (t, *C*HOMe), 75.7 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH$_3$), 55.6 (t, *C*HNH), 21.8 ppm (2C, p, CH(*C*H$_3$)$_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{40}N_8O_{10}F$ $[M+H]^+$: 859.2851; found 859.2851.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(5-cyanopicolinamido)benzamido)-3-methoxy-4-oxo-butanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-iso-propoxybenzamido)benzoic acid (48): Following the general procedure mentioned above phenol **S21** (see SI; 21.8 mg, 23.6 μmol, 1.0 equiv) was stirred for 6 h. Acid **48** was obtained as a yellow amorphous solid (8.2 mg, 9.47 μmol, 40%). $[\alpha]_D^{24} = +27.2^\circ$ (*c* 0.8, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta =$ 12.9 (1H, br. s, CO$_2$*H*), 12.2 (1H, br. s, O*H*), 11.0 (1H, s, N*H*), 10.8 (1H, s, N*H*), 10.4 (1H, s, N*H*), 9.22 (1H, dd, *J* = 0.7, 2.0 Hz, Ar*H*), 8.93 (1H, s, C*H*$_{Triazol}$), 8.61 (1H, dd, *J* = 2.0, 8.2 Hz, Ar*H*), 8.43 (1H, d, *J* = 8.1 Hz, N*H*CH), 8.31 (1H, dd, *J* = 0.7, 8.2 Hz, Ar*H*), 8.06 (2H, m, Ar*H*), 7.99–7.87 (9H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.50 (2H, d, *J* = 43.0 Hz, CON*H*$_2$), 7.37 (1H, d, *J* = 8.6 Hz, Ar*H*), 4.91 (1H, t, *J* = 8.1 Hz, C*H*NH), 4.24 (1H, hept, *J* = 6.1 Hz, C*H*Me$_2$), 4.09 (1H, d, *J* = 8.1 Hz, C*H*OMe), 3.32 (3H, s, OC*H*$_3$), 1.02 ppm (6H, d, *J* = 6.1 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 171.0 (q, CONH$_2$), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.8 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 161.7 (q, *C*ONH), 154.5 (q, C-Ar), 152.3 (q, C-Ar), 151.5 (t, C-Ar), 146.3 (q, C-Ar$_{Triazol}$), 142.3 (t, C-Ar), 141.9 (q, C-Ar), 141.0 (q, C-Ar), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 134.4 (q, C-Ar), 130.3 (2C, t, C-Ar), 129.3 (q, C-Ar), 128.2 (2C, t, C-Ar), 126.5 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 123.5 (t, C-Ar), 122.6 (t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 120.6 (2C, t, C-Ar), 119.9 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 118.4 (q, C-Ar), 116.7 (q, CN), 114.8 (t, C-Ar), 111.8 (q, C-Ar), 80.1 (t, *C*HOMe), 75.7 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH$_3$), 55.6 (t, *C*HNH), 21.8 ppm (2C, p, CH(*C*H$_3$)$_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{39}N_{10}O_{10}$ $[M+H]^+$: 867.2851; found 867.2857.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(4-cyano-3-fluorobenzamido)-benzamido)-3-methoxy-4-oxobutanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-isopropoxybenzamido)-benzoic acid (49): Following the general procedure mentioned above phenol S22 (see SI; 22.4 mg, 23.8 μmol, 1.0 equiv) was stirred for 2 h. Acid **49** was obtained as a beige amorphous solid (21.0 mg, 23.8 μmol, quant.). $[\alpha]_D^{24} = +39.5^\circ$ (*c* 1.3, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta =$ 12.8 (1H, br. s, CO$_2$*H*), 12.2 (1H, br. s, O*H*), 10.8 (1H, s, N*H*), 10.8 (1H, s, N*H*), 10.4 (1H, s, N*H*), 8.93 (1H, s, C*H*$_{Triazol}$), 8.45 (1H, d, *J* = 8.1 Hz, N*H*CH), 8.16–8.13 (1H, m, Ar*H*), 8.09–8.07 (1H, m, Ar*H*), 8.00–7.96 (4H, m, Ar*H*), 7.93–7.87 (8H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.51 (2H, d, *J* = 47.9 Hz, CON*H*$_2$), 7.37 (1H, d, *J* = 8.6 Hz, Ar*H*), 4.92 (1H, t, *J* = 8.1 Hz, C*H*NH), 4.25 (1H, hept, *J* = 6.1 Hz, C*H*Me$_2$), 4.09 (1H, d, *J* = 8.1 Hz, C*H*OMe), 3.32 (3H, s, OC*H*$_3$), 1.03 ppm (6H, d, *J* = 6.1 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 171.0 (q, CONH$_2$), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 163.2 (q, *C*ONH), 162.2 (d, *J* = 256.3 Hz, *C*-F), 154.5 (q, C-Ar), 146.3 (q, C-Ar$_{Triazol}$), 141.9 (q, C-Ar), 141.5 (d, *J* = 6.1 Hz, *C*CONH), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 134.4 (d, *J* = 7.0 Hz, *C*HCCN), 130.7 (q, C-Ar), 130.3 (2C, t, C-Ar), 129.2 (q, C-Ar), 128.8 (q, C-Ar), 128.3 (2C, t, C-Ar), 126.5 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 124.7 (d, *J* = 3.4 Hz, CH*C*HCCONH), 123.5 (t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.7 (2C, t, C-Ar), 118.4 (q, CN), 115.8 (d, *J* = 21.3 Hz, *C*HCF), 114.8 (t, C-Ar), 113.6 (q, C-Ar), 102.9 (d, *J* = 15.3 Hz, *C*CN), 80.1 (t, *C*HOMe), 75.7 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH$_3$), 55.7 (t, *C*HNH), 21.8 ppm (2C, p, CH(*C*H$_3$)$_2$); HRMS (ESI): *m/z* calc. for $C_{45}H_{39}N_9O_{10}F$ $[M+H]^+$: 884.2804; found 884.2808.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(isonicotinamido)benzamido)-3-methoxy-4-oxo-butanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-isopropoxy-benzamido)-benzoic acid (50): Following the general procedure mentioned above phenol **S23** (see SI; 16.5 mg, 18.4 μmol, 1.0 equiv) was stirred for 4 h. Acid **50** was obtained as a yellow amorphous solid (13.7 mg, 16.3 μmol, 89%). $[\alpha]_D^{24} = +22.6^\circ$ (*c* 1.4, DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 12.2 (1H, br. s, O*H*), 10.8 (1H, s, N*H*), 10.8 (1H, s, N*H*), 10.4 (1H, s, N*H*), 8.93 (1H, s, C*H*$_{Triazol}$), 8.85 (2H, m, Ar*H*), 8.44 (1H, d, *J* = 8.0 Hz, N*H*CH), 8.13–7.87 (13H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.50 (2H, d, *J* = 31.9 Hz, CON*H*$_2$), 7.37 (1H, d, *J* = 8.6 Hz, Ar*H*), 4.92 (1H, t, *J* = 8.0 Hz, C*H*NH), 4.25 (1H, hept, *J* = 6.1 Hz, C*H*Me$_2$), 4.09 (1H, d, *J* = 8.0 Hz, C*H*OMe), 3.32 (3H, s, OC*H*$_3$), 1.03 ppm (6H, d, *J* = 6.1 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 171.0 (q, CONH$_2$), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.9 (q, *C*O$_2$H), 165.4 (q, *C*ONH), 164.1 (q, *C*ONH), 154.5 (q, C-Ar), 149.8 (2C, t, C-Ar), 146.3 (q, C-Ar$_{Triazol}$), 141.9 (q, C-Ar), 141.5 (q, C-Ar), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 134.4 (q, C-Ar), 130.3 (2C, t, C-Ar), 129.2 (q, C-Ar), 128.3 (2C, t, C-Ar), 126.5 (q, C-Ar), 125.9 (q, C-Ar),125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 123.5 (t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 122.0 (2C, t, C-Ar), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.7 (2C, t, C-Ar), 118.4 (q, C-Ar), 114.8 (t, C-Ar), 80.1 (t, *C*HOMe), 75.7 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH$_3$), 55.7 (t, *C*HNH), 21.8 ppm (2C, p, CH(*C*H$_3$)$_2$); HRMS (ESI): *m/z* calc. for $C_{43}H_{40}N_9O_{10}$ $[M+H]^+$: 842.2898; found 842.2903.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(6-cyanonicotinamido)benzamido)-3-methoxy-4-oxo-butanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-iso-propoxybenzamido)benzoic acid (51): Following the general procedure mentioned above phenol **S24** (see SI; 24.4 mg, 26.5 μmol, 1.0 equiv) was stirred for 5 h. Acid **51** was obtained as a beige amorphous solid (15.6 mg, 18.0 μmol, 68%). $[\alpha]_D^{22} = +25.1^\circ$ (*c* 1.5, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta =$ 12.8 (1H, br. s, CO$_2$*H*), 12.2 (1H, br. s, O*H*), 10.9 (1H, br. s, N*H*), 10.8 (1H, s, N*H*), 10.4 (1H, s, N*H*), 9.24–9.23 (1H, m, Ar*H*), 8.93 (1H, s, C*H*$_{Triazol}$), 8.55–8.53 (1H, m, Ar*H*), 8.45 (1H, d, *J* = 8.1 Hz, N*H*CH), 8.26–8.25 (1H, m, Ar*H*), 8.00–7.87 (11H, m, Ar*H*), 7.79 (2H, m, Ar*H*), 7.50 (2H, d, *J* = 46.3 Hz, CON*H*$_2$), 7.37 (1H, d, *J* = 8.6 Hz, Ar*H*), 4.92 (1H, t, *J* = 8.1 Hz, C*H*NH), 4.25 (1H, hept, *J* = 6.0 Hz, C*H*Me$_2$), 4.09 (1H, d, *J* = 8.1 Hz, C*H*OMe), 3.32 (3H, s, OC*H*$_3$), 1.03 ppm (6H, d, *J* = 6.0 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta =$ 171.0 (q, CONH$_2$), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.8 (q, *C*O$_2$H), 165.3 (q, *C*ONH), 163.0 (q, *C*ONH), 154.5 (q, C-Ar), 150.2 (t, C-Ar), 146.3 (q, C-Ar$_{Triazol}$), 141.9 (q, C-Ar), 141.5 (q, C-Ar), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 137.3 (t, C-Ar), 134.5 (q, C-Ar), 134.4 (q, C-Ar), 133.4 (q, C-Ar), 130.3 (2C, t, C-Ar), 129.2 (q, C-Ar), 128.8 (t, C-Ar), 128.3 (2C, t, C-Ar), 126.5 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 123.5 (t, C-Ar), 122.6 (t, C-Ar$_{Triazol}$), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.6 (2C, t, C-Ar), 118.4 (q, C-Ar), 117.1 (q, CN), 114.8 (t, C-Ar), 80.0 (t, *C*HOMe), 75.7 (t, *C*H(CH$_3$)$_2$), 57.7 (p, OCH$_3$), 55.6 (t, *C*HNH), 21.8 ppm (2C, p,

$CH(CH_3)_2$); HRMS (ESI): *m/z* calc. for $C_{44}H_{39}N_{10}O_{10}$ $[M+H]^+$: 867.2851; found 867.2858.

4-(4-(4-(4-((2*S*,3*R*)-4-Amino-2-(4-(3-(4-cyanophenyl)-propanamido)-benz-amido)-3-methoxy-4-oxobutanamido)phenyl)-1*H*-1,2,3-triazol-1-yl)-2-hydroxy-3-isopropoxy-benzamido)benzoic acid (52): Following the general procedure mentioned above phenol **S25** (see SI; 19.8 mg, 20.9 μmol, 1.0 equiv) was stirred for 4.5 h. Acid **52** was obtained as a grey amorphous solid (18.6 mg, 20.9 μmol, quant.). $[\alpha]_D^{24} = +20.8^\circ$ (*c* 1.9, DMSO); ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 12.8$ (1 H, br. s, CO_2H), 12.2 (1 H, br. s, O*H*), 10.8 (1 H, s, N*H*), 10.3 (1 H, s, N*H*), 10.2 (1 H, s, N*H*), 8.92 (1 H, s, $CH_{Triazol}$), 8.35 (1 H, d, $J = 8.1$ Hz, N*H*CH), 8.00–7.95 (3 H, m, Ar*H*), 7.93–7.87 (4 H, m, Ar*H*), 7.82–7.75 (6 H, m, Ar*H*), 7.67 (2 H, m, Ar*H*), 7.53–7.45 (4 H, m, Ar*H*, $CONH_2$), 7.37 (1 H, d, $J = 8.6$ Hz, Ar*H*), 4.90 (1 H, t, $J = 8.1$ Hz, *CH*NH), 4.25 (1 H, hept, $J = 6.1$ Hz, *CH*Me_2), 4.07 (1 H, d, $J = 8.1$ Hz, *CH*OMe), 3.31 (3 H, s, OCH_3), 3.01 (2 H, t, $J = 7.5$ Hz, ArCH_2CH_2), 2.71 (2 H, t, $J = 7.5$ Hz, ArCH_2CH_2), 1.02 ppm (6 H, d, $J = 6.1$ Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 171.0$ (q, $CONH_2$), 170.4 (q, *C*ONH), 168.2 (q, *C*ONH), 167.5 (q, *C*ONH), 166.8 (q, CO_2H), 165.4 (q, *C*ONH), 154.5 (q, C-Ar), 147.3 (q, C-Ar), 146.3 (q, C-$Ar_{Triazol}$), 142.1 (q, C-Ar), 141.9 (q, C-Ar), 138.9 (q, C-Ar), 138.7 (q, C-Ar), 134.4 (q, C-Ar), 132.2 (2C, t, C-Ar), 130.3 (2C, t, C-Ar), 129.5 (2C, t, C-Ar), 128.3 (2C, t, C-Ar), 128.0 (q, C-Ar), 126.5 (q, C-Ar), 125.8 (2C, t, C-Ar), 125.4 (q, C-Ar), 123.5 (t, C-Ar), 122.6 (t, C-$Ar_{Triazol}$), 120.6 (2C, t, C-Ar), 119.8 (2C, t, C-Ar), 119.0 (q, C-Ar), 118.4 (q, CN), 118.2 (2C, t, C-Ar), 114.8 (t, C-Ar), 108.9 (q, C-Ar), 80.1 (t, *C*HOMe), 75.7 (t, $CH(CH_3)_2$), 57.7 (p, OCH_3), 55.6 (t, *C*HNH), 37.1 (s, ArCH_2CH_2), 30.6 (s, ArCH_2CH_2), 21.8 ppm (2C, p, $CH(CH_3)_2$); HRMS (ESI): *m/z* calc. for $C_{47}H_{43}N_9O_{10}$ $[M+H]^+$: 916.3031; found 916.3029.

Biological activity

Minimal inhibitory concentrations (MIC). MIC values were determined in standard microbroth dilution assays as described elsewhere.[1,2]

Acknowledgements

This work was supported by the German Center for Infection Research (DZIF) and the Bundesministerium für Bildung und Forschung (BMBF; project OpCyBac).

Conflict of interest

The authors declare no conflict of interest.

Keywords: amides · antibiotics · chemical synthesis · medicinal chemistry · triazoles · urea

[1] S. Baumann, J. Herrmann, R. Raju, H. Steinmetz, K. I. Mohr, S. Hüttel, K. Harmrolfs, M. Stadler, R. Müller, *Angew. Chem. Int. Ed.* **2014**, *53*, 14605 – 14609; *Angew. Chem.* **2014**, *126*, 14835 – 14839.

[2] a) S. Hüttel, G. Testolin, J. Herrmann, T. Planke, F. Gille, M. Moreno, M. Stadler, M. Brönstrup, A. Kirschning, R. Müller, *Angew. Chem. Int. Ed.* **2017**, *56*, 12760 – 12764; *Angew. Chem.* **2017**, *129*, 12934 – 12938. This paper also provides an extended list of new cyctobactamids; b) M. Moreno, W. A. M. Elgaher, J. Herrmann, N. Schläger, M. M. Hamed, S. Baumann, R. Müller, R. W. Hartmann, A. Kirschning, *Synlett* **2015**, *26*, 1175 – 1178.

[3] a) L. von Eckardstein, D. Petras, T. Dang, S. Cociancich, S. Sabri, S. Grätz, D. Kerwat, M. Seidel, A. Pesic, P. C. Dorrestein, M. Royer, J. B. Weston, R. D. Süssmuth, *Chem. Eur. J.* **2017**, *23*, 15316 – 15321; b) D. Kerwat, S. Grätz, J. Kretz, L. von Eckardstein, M. Seidel, M. Kunert, J. B. Weston, R. D. Süssmuth, *ChemMedChem* **2016**, *11*, 1899; c) S. Grätz, D. Kerwat, J. Kretz, L. von Eckardstein, S. Semsary, M. Seidel, M. Kunert, J. B. Weston, R. D. Süssmuth, *ChemMedChem* **2016**, *11*, 1499; d) D. Petras, D. Kerwat, A. Pesic, B. Hempel, L. von Eckardstein, S. Semsary, J. Arasté, M. Marguerettaz, M. Royer, S. Cociancich, R. D. Süssmuth, *ACS Chem. Biol.* **2016**, *11*, 1198; e) L. Vieweg, J. Kretz, A. Pesic, D. Kerwat, S. Graetz, M. Royer, S. Cociancich, A. Mainz, R. D. Süssmuth, *J. Am. Chem. Soc.* **2015**, *137*, 7608; f) J. Kretz, D. Kerwat, V. Schubert, S. Grätz, A. Pesic, S. Semsary, S. Cociancich, M. Royer, R. D. Süssmuth, *Angew. Chem. Int. Ed.* **2015**, *54*, 1969 – 1973; *Angew. Chem.* **2015**, *127*, 1992.

[4] B. Cheng, R. Müller, D. Trauner, *Angew. Chem. Int. Ed.* **2017**, *56*, 12755 – 12759; *Angew. Chem.* **2017**, *129*, 12929 – 12933.

[5] T. Planke, M. Moreno, J. Fohrer, F. Gille, A. Kanakis, M. D. Norris, M. Siebke, L. L. Wang, S. Hüttel, R. Müller, A. Kirschning, *Org. Lett.* **2019**, *21*, 1359 – 1363.

[6] a) Y. Hitotsuyanagi, S. Motegi, H. Fukaya, K. Takeya, *J. Org. Chem.* **2002**, *67*, 3266 – 3271; b) Y. Hitotsuyanagi, S. Motegi, T. Hasuda, K. Takeya, *Org. Lett.* **2004**, *6*, 1111 – 1114.

[7] a) E. Bonandi, M. S. Christodoulou, G. Fumagalli, D. Perdicchia, G. Rastelli, D. Passarella, *Drug Discov. Today* **2017**, *22*, 1572 – 1581; b) I. Mohammed, I. R. Kummetha, G. Singh, N. Sharova, G. Lichinchi, J. Dang, M. Stevenson, T. M. Rana, *J. Med. Chem.* **2016**, *59*, 7677 – 7682.

[8] G. Testolin, K. Cirnski, K. Rox, H. Prochnow, V. Fetz, C. Grandclaudon, T. Mollner, A. Baiyoumy, A. Ritter, C. Leitner, J. Krull, J. van den Heuvel, A. Vassort, S. Sordello, M. M. Hamed, W. A. M. Elgaher, J. Herrmann, R. W. Hartmann, R. Müller, M. Brönstrup, *Chem. Sci.* DOI: https://doi.org/10.1039/c9sc04769g.

[9] V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51 – 68.

[10] I. Behroz, P. Durkin, S. Grätz, M. Seidel, L. Rostock, M. Spinczyk, J. B. Weston, R. D. Süssmuth, *Chem. Eur. J.* **2019**, *25*, 16538 – 16543.

Manuscript received: September 4, 2019
Accepted manuscript online: December 13, 2019
Version of record online: March 11, 2020

