The Cystobactamids 920-1 and 920-2: Assignment of the Constitution and Relative Configuration by Total Synthesis

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Abstract: Total synthesis of cystobactamid 920-1 and its epimer has allowed an unambiguous assignment of the relative and absolute configuration of the natural product. A careful structural analysis of each isomer using both NMR and computational techniques also prompted a constitutional revision of the structures originally reported for cystobactamids 920-1 and 920-2, and has provided further insight into the unique conformational preferences of the cystobactamid family.

In 2014, a group of nonribosomal peptides, cystobactamids 919-1 (1) and 919-2 (2), were isolated in small amounts (< 100 µg/L) through cultivation of the myxobacterium Cystobacter sp. (Figure 1). Compounds 1 and 2 are closely related to albicidin, whose structure and absolute configuration was determined unequivocally by Süssmuth and coworkers through total synthesis. Only recently, nine new cystobactamid derivatives were isolated from Cystobacter sp., including cystobactamids 920-1 (3), 920-2 (4) and 861-2 (5); the latter being the most potent antibiotic of all known congeners.

Indeed, the cystobactamids and albicidin show strong antibacterial activity, inhibiting several clinically relevant Gram-positive and Gram-negative strains, such as Acinetobacter baumannii (MIC = 0.5 µg/mL for 5), Citrobacter freundii (MIC = 0.06 µg/mL for 5), carbapenem-resistant E. coli WT-III (marKΔ74bp) (MIC = 0.5 µg/mL for 5), carbapenem-resistant P. aeruginosa (CRE) (MIC = 1.0 µg/mL for 5) and Proteus vulgaris (MIC = 0.25 µg/mL for 5). It was also found that the cystobactamids inhibit bacterial type Ila topoisomerases, which are known to be targeted by the clinically established group of quinolone antibiotics.

**Figure 1.** The cystobactamid natural products, synthetic epimer 3a and the structural revision of cystobactamids 920-1 and 920-2 as presented in this work (the related peptide albicidin is also shown).
Total syntheses of the cystobactamids 861-2 (5) and 919-2 (4) were accomplished independently through our earlier efforts and that of the Trauner group, respectively. However, correct assignment of the two stereogenic centers in the central methoxyspartate 'hinge' region has been a matter of debate since the original isolation paper. The relative configuration of 1 and 2 was originally interpreted as anti (relative to the orientation drawn in Figure 1; either 2S,3S or 2R,3R configuration) through comparing the homonuclear vicinal coupling constants (J_HH = 7–8 Hz) and observed ROESY-NMR data to that of other β-oxyasparagine derivatives reported in literature. The absolute configuration of each stereocenter was similarly inferred through direct comparison of the measured optical rotation of 1 and 2 with literature compounds. Consequently, the configuration at C2 and C3 was assigned as 2S,3S.

Later, Kim and coworkers also isolated cystobactamid 919-2 (2) along with two other derivatives, which they named coralloycins A and B, from cultures of Coralloccus coralloides myxobacteria. In contrast to the original stereochemical assignment, Kim proposed that the configuration of the methoxyspartate hinge region of 2 should be revised to 2S,3R. This alternative structural assessment also resulted from detailed NMR analysis of the natural compound, especially concerning the values of J_2H,3H and J_3H,4H. Kim argued that the value of 6.7 Hz (in DMSO-d6/CD2OD = 4:1) is consistent with a gauche conformation of 2-H and the methoxy group at C3. Additionally, it was contested that 2-H and C4, as well as 3-H and C1, are gauche oriented; implying that the methoxyspartate hinge is fixed with syn configuration (relative to the orientation drawn in Figure 1; either 2S,3R or 2R,3S configuration). Despite these differences in interpretation, it is notable that in both accounts the chemical shift values and coupling constant J_2H,3H of compound 2 were identical.

In view of this ongoing stereochemical uncertainty, we endeavored to synthesize both possible epimers of the reported structure of cystobactamid 920-2, 3a and 3b; and to perform a detailed conformational analysis of each isomer, using NMR techniques and computer-aided molecular modeling, to unambiguously assign the relative and absolute configuration of the natural cystobactamid framework. To date, neither the synthesis of a carboxylic acid bearing cystobactamid, nor that of any epimeric cystobactamid pair, have been reported.

Following our previous work on the synthesis of cystobactamid 861-2 (5), we sought to construct both epimers of the reported structure of cystobactamid 920-2 using a similar approach. Thus, the synthesis of both methoxyspartate epimers, 10 and 12, began with methyl cinnamate (6) (Scheme 1). Similar to our earlier strategy, we envisaged that the phenyl ring in 6 would ultimately act as an effective masked carboxyl group, which may be unmasked at a later stage through chemoselective Kuhn–Roth oxidation.

For synthesis of the 2S,3S-epimer 10, α-acetoxy-β-bromo methylester 7 was first prepared from 6 following a known procedure, which began with Sharpless asymmetric dihydroxylation as the key step (Scheme 1, part A). Nucleophile substitution of the benzylic bromide with NaOMe yielded ester 8 with inversion of configuration, and saponification followed by t-butyl ester formation delivered azide 9. Notably, the latter transformation was sensitive to azido elimination under acidic conditions, necessitating use of the milder reagent Me_3NCH(OtBu). Under optimised conditions, we were pleased that this ester switch proceeded in 87% yield over two steps. With t-butyl ester 9 in hand, selective oxidation of the pendant phenyl ring to a carboxylic acid with RuCl_3 and NaOAc allowed us to simultaneously introduce the second carboxyl group of the methoxyspartate linker, and to maintain its differentiation from the existing carboxyl moiety. This rarely employed transformation proceeded with remarkable chemoselectivity and yielded amine 10 after O-methylation and Staudinger reduction of the azido group.

Scheme 1. Preparation of the two epimetric methoxyspartates.

Synthesis of the 2S,3R-diastereomer 12 was accomplished through extension of our existing route to azide 11 from methyl cinnamate (6). Saponification and t-butyl ester formation paved the way for Kuhn–Roth oxidation, and the resulting carboxylate was advanced to amine 12 through O-methylation and Staudinger reduction (Scheme 1, part B).

With both epimers in hand, we proceeded to couple the eastern and western polyaromatic fragments in a stepwise approach. Best results for the first coupling reaction were realized when acid 13 was activated with ethyl chlorocarbonate, then added to freshly-prepared methoxyspartate amines 10 or 12 in DMF (Scheme 2 and 3). Under these conditions, amides 14 and 17 were accessed in good yield from amines 10 and 12, respectively, after t-butyl ester elimination. Compound 14 was then coupled to the known polyaromatic aniline fragment 15 after activation with PPh_3Cl to give amide 16. However, under these same conditions, we were surprised to find that the epimeric acid 17 reacted exclusively at the phenol oxygen of 15. In order to circumvent this issue, we had to employ the known allyl protected aniline fragment instead. Thus, activation of 17 with POCI_3 in the presence of DIPEA and subsequent addition of 18 afforded the desired amide 19 in 59% yield.

Subsequent hydrolysis of the aspartate methyl ester proved to be challenging. Under conditions typical for base hydrolysis, the amino acid linker was highly sensitive to epimerization, resulting in a mixture of isomers that were difficult to separate on a preparative scale. We found that the exceptionally mild reagent Me_3SnOH was best suited to affect methyl ester hydrolysis, although partial epimerization could not be
completely suppressed. After some optimization, methyl ester 16 was successfully hydrolyzed with Me$_3$SnOH in 1,2-dichloroethane at 80 °C, and t-butyl ester elimination afforded the 2S,3S-isomer of the originally assigned structure of cystobactamid 920-2 (3a).

**Scheme 2. Preparation of 2S,3S isomer 3a.**

![Scheme 2](image)

Comparison of the $^1$H NMR spectra of natural cystobactamids 920-1 and 920-2 with synthetic 3a revealed significant differences (Figure 2, part A). This included the chemical shift values (δ; designated with * for natural 920-1 and for natural 920-2) of one N–H amide proton, 8.34 (d, $J = 8.9$ Hz, 1H$_{NH}$) compared to 8.72 (d, $J = 8.67$ Hz, 1H$_{NH}$) and 8.47 (d, $J = 8.1$ Hz, 1H$_{NH}$), and one of the C–H protons in the methoxyaspartate hinge region, 4.48 (d, $J = 4.6$ Hz, 1H$_{CH}$) compared to 4.13 (d, $J = 8.05$ Hz, 1H$_{CH}$) and 4.31 (d, $J = 5.0$ Hz, 1H$_{CH}$) (see atoms marked in grey in Fig. 2). From this we ultimately concluded that synthetic 3a is a diastereomer of natural cystobactamid 920-1. This was confirmed when comparing the $^1$H-NMR spectra of synthetic 3b with natural samples of cystobactamids 920-1 and 920-2 (Figure 2, part B). Again, the amide proton and CHOMe group in the methoxyaspartate hinge region were diagnostic elements. Clearly, isomer 3b matches the authentic cystobactamid 920-1 and not its constitutional isomer cystobactamid 920-2, as it was originally proposed. Our side-by-side comparison confirms that: a) the relative configuration of the methoxyaspartate unit in both cystobactamids 920-1 and 920-2 is 2S,3R, and b) the constitutional assignment of each natural product must be reassigned to the structures depicted in Figure 1.

Compound 19 was similarly advanced to the 2S,3R-isomer of the originally assigned structure of cystobactamid 920-2 (3b), although for purification purposes, it was advantageous to perform methyl ester hydrolysis in the final step. Applying stoichiometric PhSiH$_3$ as a scavenger in the palladium catalyzed deallylation of 19 was troublesome, leading to partial reduction of the aryl nitro group. Rather, aniline was found to be superior, resulting in a clean transformation to the desired product on treatment with Pd(PPh)$_3$Cl$_2$. Curiously, hydrolysis of the remaining ester was also complicated by inadvertent alkylation of 1,2-dichloroethane (solvent) to the exposed phenol group under our previously optimized conditions; necessitating the use of toluene as solvent in this reaction. Owing to its poor solubility, the final product 3b was isolated in pure form using a combination of different chromatographic techniques (see Supporting Information).

**Scheme 3. Preparation of 2S,3R-isomer 3b.**

![Scheme 3](image)

To better understand the difficulty of structural elucidation in this family of compounds, we explored a conformational analysis of cystobactamid 920-1 (3) using the Schrödinger suite$^{13}$ maestro 9.8 computational model with the mixed torsional/low-mode sampling conformational search (LMCS) method and force field OPLS2005.$^{14}$ Water was chosen as solvent and energy potentials were optimized for simulation in solution (OPLS2005) to improve the quality of force field parameters.$^{14,15}$ We expected an out-of-plane orientation of the aromatic rings of 3 that might also accommodate a helical conformation. Indeed, our calculations showed that the aromatic rings of cystobactamid 920-1 (3) are not likely to preferentially be oriented in-plane. The calculations also revealed...
that the helical shape is stabilized through intramolecular hydrogen bonds between an NH and the phenolic group of the adjacent chain and another NH group of ring e with the isopropoxy group of ring b (Figure 3, and numbering see figure 1).

Calculating the ten lowest energy conformers of cystobactamid 920-1 (3), we discovered, that the terminal aminoarene unit of the eastern fragment has greater conformational flexibility compared to the benzene rings of the western part. This observation is consistent with the work of Wilson and coworkers, who found similarly enhanced conformational flexibility in the terminal rings of oligobenzamidazoles. Furthermore, the western peptide unit of cystobactamid 920-1 rotates backward in the terminal rings of oligobenzamidazoles. The authors declare no competing financial interest.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website.

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**REFERENCES**


**Figure 3.** a) Lowest energy conformation (lowest energy conformer on the left and higher energy conformer on the right) of cystobactamid 920-1 (3) in the initially published 2S, 3S configuration. The calculations show that both aromatic chains stack on top of each other, forming a loop around the hinge region. This loop is observed in calculations for all possible combinations of absolute configuration of the two stereocenters. b) Magnification of the hinge region with the two stereogenic centers and c) observed ROE of the natural compounds.


