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ORIGINAL ARTICLE

Isolation and structure elucidation of the nucleoside antibiotic strepturidin from *Streptomyces albus* DSM 40763

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The antibiotic strepturidin (1) was isolated from the microorganism *Streptomyces albus* DSM 40763, and its structure elucidated by spectroscopic methods and chemical degradation studies. The determination of the relative and absolute stereocenters was partially achieved using chiral GC/EI-MS analysis and microderivatization by acetal ring formation and subsequent 2D-NMR analysis of key $^{1}H,^{1}H$-NOESY NMR correlations and extraction of $^{1}H,^{13}C$ coupling constants from $^{1}H,^{13}C$-HMBC NMR spectra. Based on these results, a biosynthesis model was proposed.
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INTRODUCTION

The genus *Streptomyces* (Order *Actinomycetales*, colloquial '*Actinomycetes*') is a widely studied genus of the *Actinomycete* family. Among all *Actinomycetes*, *Streptomycetes* play an important role in drug discovery as from this genus 90% of the more than 3000 known antibiotics have been isolated.[1] In comparison with other Gram-positive bacteria like *Staphylococcus*, *Streptococcus* and *Bacillus*, the GC-content of the DNA of *Streptomyces* is with ~70% comparatively high. Examples of prominent antibiotics produced by these bacteria are the aminoglycoside streptomycin (antituberculosis), the chromopeptide actinomycin (cytostatic) and the polyketide tetracycline (antibacterial).

Here we report on an antibiotic compound produced by *Streptomyces albus* that exhibits a specific antibacterial activity against the Gram-negative nitrogen-fixing soil bacterium *Azotobacter chroococcum*.[2] This antibacterial activity has been initially discovered in 1956 by Kutzner[3] (see also Flaig and Kutzner[4]), and, as shown later by Böttiger,[5] extends to all other members of the family *Azotobacteraceae*. However, this antibacterial effect is independent from the nitrogen status of the medium and therefore the nitrogenase system of *Azotobacter* has been excluded as a molecular target.[6] The first isolation of the active compound, named strepturidin (**1**), and its preliminary structure elucidation has already been described by Steinhaus[7] in 1993. Initial attempts to characterize the new antibiotic substance with the above-mentioned features by test reactions proved positive for guanidine-groups, sugars and amino sugars. However, test reactions yielded negative results for aromatic amines, organic acids, amino acids and peptides.

This report presents in detail the cultivation of the producing strain, as well as the isolation and structure elucidation of the nucleoside antibiotic strepturidin (**1**), by means of HPLC-HR-ESI-(+)-Orbitrap-MS, chiral GC/EI-MS, extensive 2D-NMR spectroscopy including partial assignment of the relative stereochemistry and absolute stereochemistry. Based on the investigations on the structure elucidation and determination of the stereochemistry, we postulated a plausible biosynthesis model for (**1**).

RESULTS

Producing strain and bacterium for antibiotic assay

S. albus has been extensively characterized in the past, notably by Gordon *et al.*,[6] Böttiger[5] and Herrmann.[8] This streptomycete species can be easily isolated from its natural habitat, that is, self-heated hay or compost, and can be rapidly identified by classical properties. The strain DSM 40763 has been selected from the numerous representatives of this species, which are kept in worldwide culture collections. For the antibacterial assay, the strain *A. chroococcum* DSM 281 has been employed.

Cultivation and isolation of strepturidin (1)

S. albus DSM 40763 was cultivated as described by Herrmann.[8] With a seed culture of 200 ml, a 10 l preculture was inoculated to be used subsequently to inoculate the production fermenter containing 80 l of complex medium (mannitol 10 g l^{-1}, soy meal (full-fat) 10 g l^{-1}, meat extract 5 g l^{-1}, sodium chloride 1 g l^{-1}, calcium carbonate 1 g l^{-1}, tegosipon 10 ml). The cultivation was carried out at 37 °C at 300 r.p.m.

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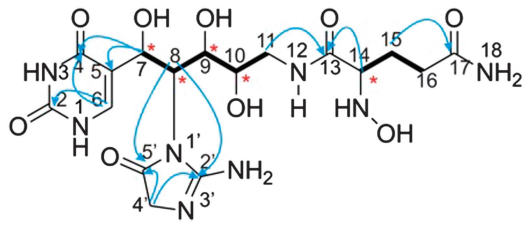

Figure 1 Structure of strepturidin (**1**). Bold lines indicate $^{1}H,^{1}H$-COSY correlations. Blue arrows indicate $^{1}H,^{13}C$-HMBC correlations. Asterisks mark the five stereogenic centers. The list of chemical shifts is summarized in Table 1.

and an aeration of 0.5 v.v.m. Growth and antibiotic production were monitored by wet cell mass weight and HPLC analysis, respectively. After 26 h, 130 g l^{-1} wet cell mass and an antibiotic titer of 92 mg l^{-1} were determined. The culture broth was harvested by centrifugation, sodium heptanesulfonate was added to the supernatant and the crude antibiotic obtained by ion-pair extraction on Amberlite XAD-16 (Sigma-Aldrich, Taufkirchen, Germany). From the eluate of the resin an excess of sodium heptanesulfonate was extracted as an ion-pair with methyltrioctylammonium chloride using dichloromethane as the solvent. Further purification was carried out by preparative RP18 HPLC and desalting with Amberlite XAD-16 followed by CM-Sephadex NH_4^+ chromatography as described below. After purification, 1.76 g of pure strepturidin (**1**) was obtained as a white powder with no defined m.p.

Structure elucidation by means of MS and NMR

The exact molecular mass of (**1**) (*m/z* found 486.19193 $[M+H]^+$) derived from the HR-ESI-(+)-Orbitrap-MS analysis (*m/z* calcd 486.18227 $[M+H]^+$, Δm 4.883 p.p.m.) gave a molecular formula of $C_{17}H_{27}O_9N_8$ $[M+H]^+$. The IR spectrum showed absorptions for amine and hydroxyl groups (3341, 2928 cm^{-1}) and amide carbonyls (1662 cm^{-1}) but no characteristic bands for aromatic hydrocarbons were detected below 900 cm^{-1} (C-H deformation vibration). The UV spectrum showed a maximum at $\lambda = 262$ nm (ε 3.89) and displayed characteristic absorptions for heterocycles such as nucleobases. Subsequently to the determination of these parameters, the structure elucidation of (**1**) (Figure 1) was established from extensive use of ^{1}H- and 2D-NMR experiments ($^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-HMBC, $^{1}H,^{13}C$-HSQC, ^{13}C-NMR and ^{13}C-DEPT) in d_6-DMSO that revealed a composition of the molecule from four distinct building blocks. The chemical shifts are summarized in Table 1. In the following a more detailed analysis of the NMR data is given.

The structure elucidation of (**1**) commenced with the interpretation of the ^{1}H-NMR (Table 1) and ^{13}C-DEPT NMR spectra.[7] The ^{1}H-NMR spectrum of (**1**) reveals 14 proton NMR signals, with 8 aliphatic methylene (CH_2) protons, 5 aliphatic protons and 1 olefinic methine (CH) proton. In analogy to the ^{1}H-NMR, the ^{13}C-DEPT NMR spectrum reveals the corresponding signals of four methylene, six methine and, in addition, seven quaternary carbon signals.[7] No primary carbons as represented by methyl groups were detected.

The NMR data of (**1**) can be formally divided into four spin systems. The first spin system was identified as the glutamine (Gln) Gln-N_α-hydroxamate with four methylene protons at $\delta_{H\text{-}16}$ 2.07 p.p.m. (t, $^{3}J_{H\text{-}16,H\text{-}15} = 7.2$ Hz, 2H) and at $\delta_{H\text{-}15}$ 1.9 p.p.m. (m, 2H) and a single aliphatic methine proton at $\delta_{H\text{-}14}$ 4.7 p.p.m. (t, $^{3}J_{H\text{-}14,H\text{-}15} = 3.3$ Hz, $^{3}J_{H\text{-}14,NH} = 8.6$ Hz, 1H). The Gln-N_α-hydroxamate spin system is linked through its α-carbonyl at $\delta_{C\text{-}13}$ 169.8 p.p.m. to form an amide ($\delta_{NH\text{-}12}$ 8.4 p.p.m. (b, 1H)) with the amino group of the subsequent spin system identified as a triol-containing carbon chain, an open-chain aldopentose derivative named diamino aldopentose. The linkage was assigned from the methylene protons of the diamino aldopentose alkyl spin system at $\delta_{H\text{-}11}$ 3.2/3.5 p.p.m. (br, 2H; ABX-part, AB-part with broad lines) correlating to $\delta_{C\text{-}13}$ 169.8 p.p.m. in the $^{1}H,^{13}C$-HMBC NMR spectrum.[7] From the remaining four aliphatic proton signals present in this spin system, three methine protons (CH) at $\delta_{H\text{-}7}$ 4.3 p.p.m. (d, $^{3}J_{H\text{-}7,H\text{-}8} = 5.5$ Hz, 1H), $\delta_{H\text{-}9}$ 3.6 p.p.m. (dd, $^{3}J = 5$ Hz, 1H) and $\delta_{H\text{-}10}$ 3.7 p.p.m. (ddd, $^{3}J = 4$ Hz, 1H) are bound to three carbons bearing hydroxyl groups. One additional aliphatic methine proton at $\delta_{H\text{-}8}$ 3.8 p.p.m. (dd, $^{3}J = 5.4$ Hz, 1H) is connecting a third spin system identified as an 2-aminoimidazol-5-one moiety through $^{1}H,^{13}C$-HMBC correlations to the quaternary carbons at $\delta_{C\text{-}2'}$ 157.6 p.p.m. and $\delta_{C\text{-}5'}$ 168.1 p.p.m., respectively. In addition, the 2-aminoimidazol-5-one spin system accommodates the remaining two methylene protons of structure (**1**) at $\delta_{H\text{-}4'}$ 4.2 p.p.m. (s, 2H). The final spin system constitutes the nucleobase uracil that is attached via a C-C-bond to the open-chain

Table 1 NMR spectroscopic data for strepturidin (1) and monoacetal-strepturidin (3) (500 MHz) in d_6-DMSO

	*Strepturidin (**1**)*[a]		*Monoacetal-strepturidin (**3**)*	
Pos.	*δ_H in p.p.m. (multiplicity, integral, J in Hz)*	*δ_C in p.p.m., multiplicity*	*δ_H in p.p.m. (multiplicity, integral, J in Hz)*	*δ_C in p.p.m., multiplicity*
1	/	–	/	–
2	–	155.9, qC	–	152.3, qC
3	/	–	/	–
4	–	165.4, qC	–	164.0, qC
5	–	109.1, qC	–	110.4, qC
6	7.34 (s, 6.4, –)	145.4, CH	7.50 (s, 1, –)	140.4, CH
7	4.37 (d, 1, 5.5)	79.9, CH	4.60 (d, 1, 3.2)	79.4, CH
8	3.85 (dd, 1, 5.4)	71.8, CH	4.83 (dd, 1, 6.3; 3.3)	84.2, CH
9	3.64 (dd, 1, 5.0)	73.8, CH	4.68 (/, m, overlap)[b]	82.8, CH
10	3.77 (ddd, 1, 4.0)	81.3, CH	3.70 (dd, 1, 5.2)	81.2 CH
11	3.16; 3.52 (/, 2, /)[c]	40.7, CH_2	3.18 (/, /, /); 3.25 (/, /, /)	40.6 CH_2
12	8.44 (s, b, 1.0)	–	/	–
13	–	169.8, qC	–	169.3, qC
14	4.77 (t, 1, 3.3, 8.6)	59.0, CH	4.70 (m, overlap, /)	59.4, CH
15	1.99 (m, 2, /)	23.9, CH_2	2.11/1.94 (m, overlap, /)	23.3, CH_2
16	2.07 (t, 2, 7.2)	31.8, CH_2	2.08 (m, overlap, /)	31.6, CH_2
17	–	174.1, qC	–	174.7, qC
18	6.77 (s, 1, –); 7.3 (s, 1, –)	–	6.8 (br, 2, –);	–
1′	–	–	–	–
2′	–	157.6, qC	–	157.9, qC
3′	–	–	–	–
4′	4.20 (s, 2, –)	42.1, CH_2	4.18 (m, 2, /)	42.8 CH_2
5′	–	168.1, qC	–	169.2, qC
1″	–	–	1.55 (s, 3, –)	28.4, CH_3
2″	–	–	–	113.5, qC
3″	–	–	–	144.1, qC
4″	–	–	7.35 (/, /, /)	127,7 CH
5″	–	–	7.52 (d, 2, 7.7)	124,0 CH
6″	–	–	7.37 (/, /, /)	124,0 CH

The symbol '/' indicates not assigned and '–' indicates not available.
[a]As reported by Steinhaus.[7]
[b]Overlap in ^{1}H-NMR, coupling constants could not be determined.
[c]ABX-System, AB-Part with broad lines.

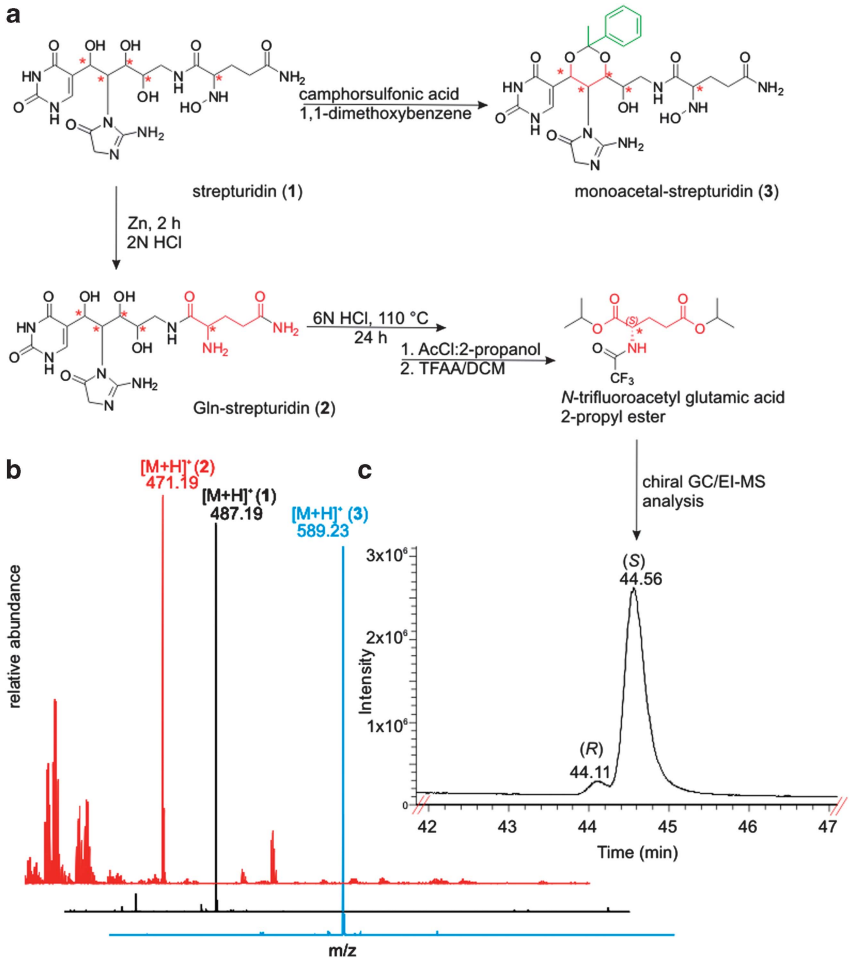

Figure 2 (**a**) Scheme for preparative microreactions performed with strepturidin (**1**). Formation of monoacetal-strepturidin (**3**) with 1,1-dimethoxybenzene and camphorsulfonic acid for 2D-NMR experiments. Reduction of strepturidin (**1**) to Gln-strepturidin (**2**) for chiral GC/EI-MS analysis. (**b**) HR-HPLC-ESI-(+)-Orbitrap-MS data of the compounds (**1**–**3**). (**c**) chiral GC-chromatogram on a LIPPDEX E column. Enantiomer analytics of (*R/S*)-*N*-trifluoroacetyl Glu 2-propyl esters (Rt-(*S*) = 44.56 min, Rt-(*R*) = 44.11 min) after hydrolysis and derivatization of Gln-strepturidin (**2**). The EI-MS spectrum of *N*-trifluoroacetyl (*S*) Glu 2-propyl ester at Rt = 44.56 min is shown in Supplementary Figure S4B. Asterisks mark the stereocenters in strepturidin (**1**).

diamino aldopentose moiety similar to the *C*-linkage of the ring ribose to uracil in pseudouridine (ψ). This finding is corroborated by the presence of only one single characteristic olefinic methine proton at $\delta_{H\text{-}6}$ 7.60 p.p.m. (s, 1H) as a part of the uracil structure. These data are complemented by the quaternary carbon signals at $\delta_{C\text{-}2}$ 155.9 p.p.m., $\delta_{C\text{-}4}$ 165.4 p.p.m., $\delta_{C\text{-}5}$ 109.1 p.p.m. and the olefinic methine signal at $\delta_{C\text{-}6}$ 145.4 p.p.m. as part of the nucleobase uracil. The complete structure elucidation is further complemented by $^{1}H,^{13}C$-HSQC NMR data[7] yielding the assigned protons together with their corresponding carbon atoms and the correlations within the spin parts by the $^{1}H,^{1}H$-COSY experiment.[7] Figure 1 shows the structure of strepturidin (**1**) with the key $^{1}H,^{13}C$-HMBC contacts with blue arrows and the $^{1}H,^{1}H$-COSY correlations in bold lines.

Assignment of the absolute stereochemistry in the Gln-N_{α}-hydroxamate moiety

The above structure elucidation of (**1**) revealed the constitutional formula and left the relative and absolute stereochemistry of the five stereocenters at C-7, C-8, C-9, C-10 and C-14 unassigned (Figure 1). Various attempts to crystalize the substance failed, most likely because of the high polarity, structural flexibility and also instability of the molecule (**1**). Therefore, the subsequent experiments focused on derivatization reactions to assign a maximum number of stereocenters.

Our efforts started with the carbon C-14 that is the stereocenter of the Gln-N_{α}-hydroxamate. The stereochemistry at C-14 was determined by reduction of the N_{α}-hydroxyl amine group with 2N HCl/Zn (2 h, room temperature) and transformation into the corresponding amino acid Gln (Figure 2a), generating Gln-strepturidin (**2**). The reduction was followed by HPLC-HR-ESI-(+)-Orbitrap-MS (Figure 2b) and HPLC-LR-ESI-(+)-MS^2 (Supplementary Figure S3). Subsequently, reduced Gln-strepturidin (**2**) (Figure 2a) was hydrolyzed (6N, HCl, 24 h, 110 °C, under vacuum) to obtain the glutamic acid (Glu) that was derivatized to the *N*-trifluoroacetyl 2-propyl ester. Amino acid analysis by chiral GC/EI-MS on a LIPODEX E (Macherey-Nagel, Düren, Germany) column (Figure 2c) revealed that the *N*-trifluoroacetyl Glu 2-propyl ester has (*S*)-configuration. Based on these results, the stereocenter at C-14 in (**1**) is also (*S*)-configured.

Assignment of the relative stereochemistry in the aldopentose moiety

The relative configuration of the remaining four stereocenters in the central part of the molecule C-7 to C-10 could not be derived by the

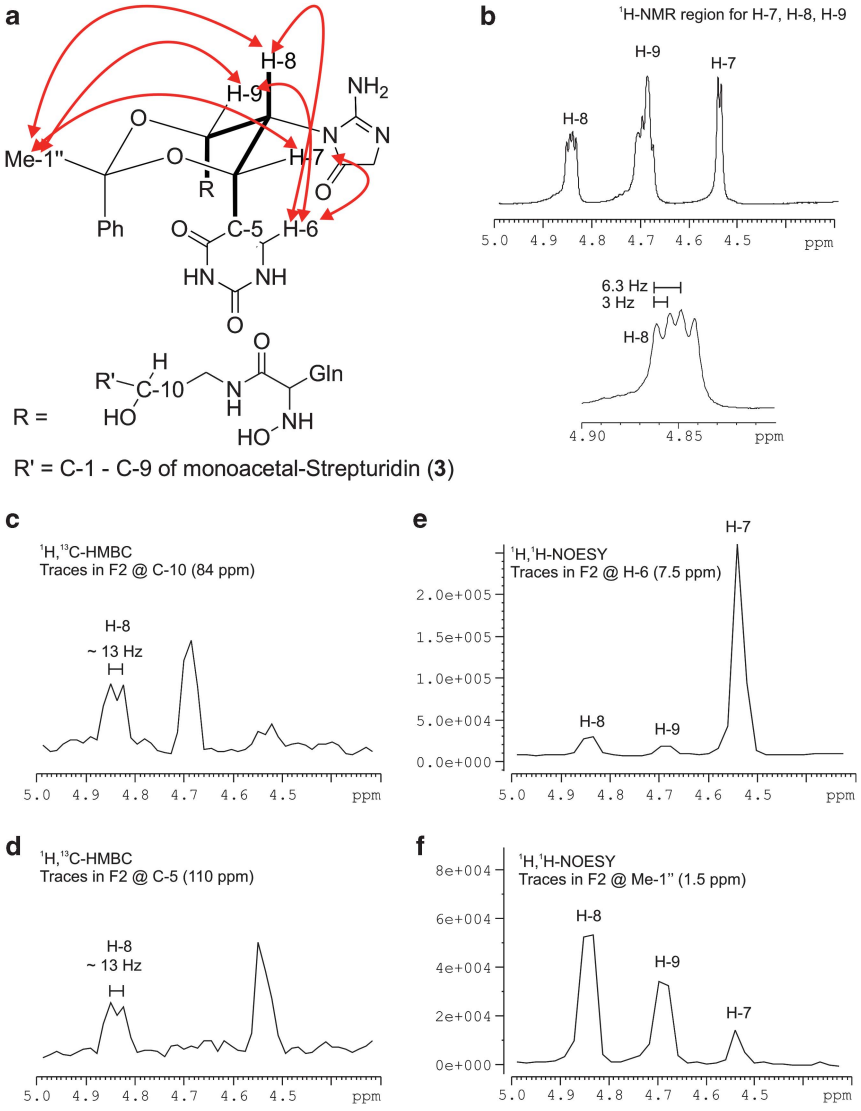

Figure 3 NMR analysis of monoacetal-strepturidin (**3**). (**a**) Plausible model for 1,3 acetal based on NMR analysis. Red arrows indicate ^{1}H,^{1}H-NOESY contacts. Bold lines indicate $^{3}J_{\text{H-8,C-10}}$ and $^{3}J_{\text{H-8,C-5}}$ correlations. (**b**) ^{1}H-NMR region for the three key protons H-7, H-8 and H-9 for compound (**3**) and expanded ^{1}H-NMR region for H-8. (**c**) Traces in F2 extracted from ^{1}H,^{13}C-HMBC spectra for C-10 and C-5 (**d**). The coupling constants $^{3}J_{\text{H-8,C-10}} \approx 10$–13 Hz and $^{3}J_{\text{H-8,C-5}} \approx 10$–13 Hz suggest an axial orientation. (**e**) Traces in F2 extracted from ^{1}H,^{1}H-NOESY spectra for H-6. (**f**) Traces in F2 extracted from ^{1}H,^{1}H-NOESY spectra for the methyl group Me-1″. The weak NOE between H-6 and H-8 in (**e**) cannot be explained with the conformation shown in (**a**). Probably, a second conformer with H-8 and uracil in equatorial position exists.

^{13}C-NMR spectroscopic database approach developed by Kishi and coworkers[9] as these databases are only applicable for 1,3-acetals with a methylene group (CH_2) connecting the diol groups. In order to reduce the polarity and to increase the rigidity of the molecule for additional NMR spectroscopy experiments of (**1**), we considered transformation of the 1,3-diol unit into the corresponding 1,3-acetal using 1,1-dimethoxyethylbenzene and camphorsulfonic acid[10] (Figure 2a) to give the monoacetal-strepturidin (**3**) (*m/z* 589.2 $[M+H]^+$). The monoacetal-strepturidin (**3**) was isolated by preparative reversed-phase C18-HPLC (Supplementary Figure S1).

The formation of the six-membered 1,3-acetal of compound (**3**) was proven by ^{1}H-NMR (Supplementary Figure S5) and 2D-NMR experiments (Supplementary Figures S7–S10; ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC and ^{1}H,^{1}H-NOESY). Application of Mosher's method for assignment of the absolute configuration determination on the free hydroxyl group at C-10 on the acetal-protected compound (**3**) has been unsuccessful and no product formation could be detected by HPLC-HR-ESI-(+)-Orbitrap-MS (data not shown).

The formation of the six-membered 1,3-acetal ring present in (**3**) allowed the analysis of the relative configuration of the triol moiety, using proton–proton coupling constants directly from ^{1}H-NMR data. In addition, selTOCSY-NMR data (Supplementary Figure S9) and long-range couplings from ^{1}H,^{1}H-NOESY data (Figures 3e and f) were used. Because of a lack of strong NOE contacts between the methyl-group protons of $\delta_{\text{Me-1''}}$ 1.55 p.p.m. and the protons H-6, H-7 and H-9 (Figure 3b and Table 1), an axial position of this group is not plausible and we assume a chair conformation model for the six-membered 1,3-acetal ring with the methyl group in equatorial and the phenyl group in axial position (Figure 3a and Table 1). Furthermore, the small homo-nuclear proton–proton coupling constant for $^{3}J_{\text{H-7,H-8}} = 3.3$ Hz and $^{3}J_{\text{H-8,H-9}} = 6.3$ Hz (48–62° and 30–50° on the basis of the equation of Karplus[11] excludes an axial–axial ($^{3}J_{\text{aa}}$)

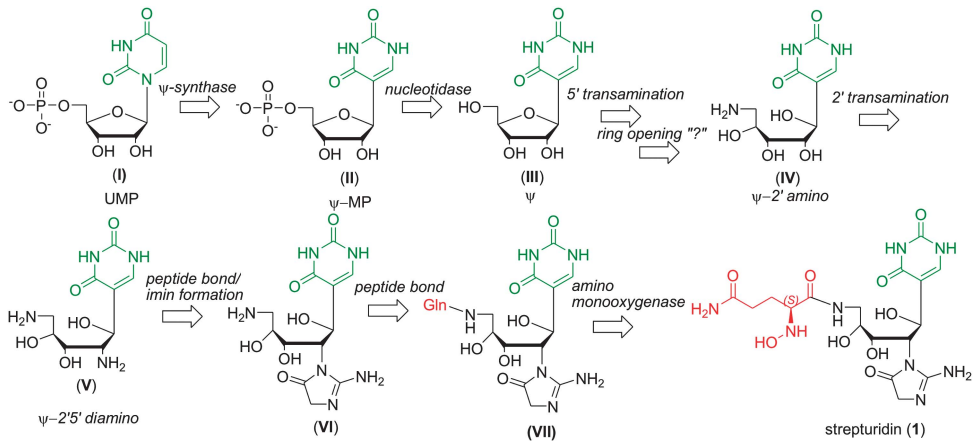

Figure 4 Proposed biosynthesis pathway of strepturidin (**1**).

configuration between the neighboring protons (Figure 3a). The measurements of proton–carbon coupling constants by extraction of $^{1}H,^{13}C$-HMBC traces in F2 dimension for the correlations $^{3}J_{H\text{-}8,C\text{-}5}$ and for $^{3}J_{H\text{-}8,C\text{-}10}$ (Figures 3c and d) yield, in both cases, high coupling constants of $^{3}J_{H,C} \approx 10$–13 Hz. Although these measurements are only guidelines, they are in line with above data suggesting an axial orientation of H-8/C-5 and of H-8/C-10, respectively (Figure 3a). Further support for the suggested conformation (Figure 3a) is given by the analysis of $^{1}H,^{1}H$-NOESY NMR data (Supplementary Figure S10) with a mixing time of 600 ms. The data showed similarly weak NOE effects from the equatorial methyl protons at $\delta_{Me\text{-}1''}$ 1.55 p.p.m. to the axial H-8 proton and to the equatorial H-7/H-9 protons (Figure 3f). In addition, a strong NOE effect from the uracil proton $\delta_{H\text{-}6}$ 7.60 p.p.m. to H-7 and a weak NOE to H-8 and H-9 only occur in the case of the postulated arrangement (Figure 3e). Based on these results, we can deduce the relative configuration for the two hydroxyl groups at C-7 and C-9 and the 2-aminoimidazol-5-one group at C-8. Hence, both hydroxyl groups at C-7 and C-9 are *syn* to each other and *anti* to the 2-aminoimidazol-5-one substituent at C-8 (Figure 4). For the interpretation of the stereochemistry see the discussion part below.

Biological activity

Bioactivity testing of pure strepturidin (**1**) showed the expected activity against *A. chroococcum* but no activity against the Gram-negative Escherichia *coli* and the Gram-positive *Staphylococcus aureus* or *Streptomyces glaucescens*.[6] In an agar diffusion assay on *A. chroococcum* plates, 40 μg/paper disc produced a 13 mm inhibition zone. Cytotoxicity against the mouse fibroblast cell line L929 was $>40\,\mu g\,ml^{-1}$, indicating very low toxicity.

DISCUSSION

With regard to its chemical structure, strepturidin (**1**) belongs to the group of nucleoside antibiotics. The latter commonly target bacterial peptidoglycan cell wall biosynthesis and fungal chitin wall biosynthesis.[12] The group of nucleoside natural products contain a vast number of representatives including well-known compounds, such as tunicamycin, mureidomycin A, liposidomycin B, pacidamycin and nikkomycin Z.[12] The common core unit of such nucleoside antibiotics is a uridine nucleotide consisting of a 1′-*N*-glycosylation of uridine with a (3′-deoxy)-ribose unit.[12] Unlike the above-mentioned nucleoside antibiotics, strepturidin (**1**) is a *C*-nucleoside with the *C*-glycosylation at the 5′ position of uracil linked to a linear diamino aldopentose of which we partially assigned the relative stereochemistry. Based on building blocks available from primary metabolism and biosynthetic analogies to other nucleoside antibiotics, we propose a biosynthesis model for (**1**) as outlined in Figure 4, of which the sequence of intermediate biosynthetic steps may differ: the biosynthetic logic suggests uridine monophosphate (**I**) with ribose as precursor of the aldopentose-moiety of strepturidin (**1**). This is in line with the NMR data (Figure 3) that suggest a *syn*-configuration of C-7 and C-9 and an *anti*-configuration of C-8 in (**1**). Therefore, from the four aldopentoses, arabinose and xylose are excluded as likely precursors. Considering that lyxose is a very rare aldopentose in nature,[13] this strongly favors ribose as the most likely precursor. As a subsequent step, isomerization of uridine monophosphate (UMP) (**I**) to pseudouridine (ψ-MP) (**II**) occurs that is catalyzed by a ψ synthase-type enzyme.[14] Such a biosynthetic step has been described for one of the first reported *C*-nucleosides, 5-β-*D*-ribofuranosyluracil known as pseudouridine (ψ). Interestingly, *C*-nucleosides are known to inhibit important enzyme processes, as is shown by the recent discovery of the first *C*-nucleoside hepatitis C virus polymerase inhibitor GS-6620.[15] Further important *C*-nucleosides are ezomycin B2 (antifungal),[16] showdomycin (antibiotic, cytotoxic),[17] formycin (antitumor antibiotic)[18] and pyrazofurin (antiviral).[19] In this context, it is worth mentioning that (**1**) shows antibiotic activity exclusively against *A. chroococcum*. In subsequent steps, a nucleotidase could catalyze the hydrolysis of ψ-MP (**II**) to ψ (**III**), which finds its analogy in the biosynthesis of caprazamycin and tunicamycin:[12] The proceeding oxidation of the terminal 5′OH-group to the 5′aldehyde is followed by a transamination to ψ-5′-amino (**IV**). Subsequently, ring opening at C-2 by formal addition of H_2O occurs while the stereocenter at C-10 is maintained.[20] The attachment of the unusual 2-aminoimidazol-5-one building block in (**VI**) could be derived from condensation of glycine (Gly) and carbamoyl phosphate to the 2′-position of the 2′,5′-diamino aldopentose in (**V**). Therefore, we hypothesize an enzyme with a transferase-like action to form the 2′-amino group in the aldopentose of (**IV**) to the intermediate ψ-2′,5′-diamino (**V**), in analogy to the described 3′-amino formation in the proposed biosynthetic pathway of puromycin.[21] Interestingly, the 2-amino pentose formation in *Streptomyces alboniger* by enzymatic amination is known and described to occur in *cis*-configuration to the 3′-hydroxyl group that may also define its stereochemistry.[22] Also, a similar derivative of 2-aminoimidazol-5-one, the 4-formyl-4-imidazolin-2-one, is found in the nikkomycin biosynthesis, derived

from histidine.[12] Finally, the amino acid Gln, the precursor of the Gln-N_α-hydroxamate, which forms a peptide bond between its carboxylic group and the primary 5′amine group of the aldopentose building block in (**VI**). This is assumed to be performed by a non-ribosomal peptide synthetase (NRPS) to form (**VII**) before or after constituting the N_α-hydroxamate through hydroxylation with an putative amino-monoxoygenase to strepturidin (**1**). Interestingly, N_α-hydroxamate is rare in nature, whereas most hydroxamate group modifications were found in the side chain of amino acids.[23,24] Although hydroxamates in general display siderophore-like functions,[25] common also among other natural products like catecholate and carboxylate siderophores, the role of this moiety in strepturidin is still elusive.

In summary, the structure elucidation of strepturidin (**1**) renders an unprecedented structure of a novel nucleoside-type antibiotic with an exclusive antibacterial activity against *A. chroococcum*. Although the biosynthesis of strepturidin is still unknown, we propose similarities to biosynthesis pathways of other (*C*)-nucleoside antibiotics as mentioned above.

METHODS/EXPERIMENTAL SECTION

Producing strain

Large-scale cultivation of strain *S. albus* (DSM 40763) was performed by Steinhaus[7] in collaboration with the Bio-Plant of the GBF (Gesellschaft für Biotechnologisches Forschung) in 1993. A preculture was prepared in a 15 l Giovanola bioreactor (Giovanola Freres, Monthey, Switzerland) charged with a complex medium consisting of mannitol 10 g l^{-1}, biopepton 5 g l^{-1}, yeast extract 5 g l^{-1}, meat extract 5 g l^{-1}, calcium chloride 0.7 g l^{-1} (pH 7.2) and tegosipon 5 ml, and inoculated with 200 ml of a seed culture. After 14 h, only 5 l of this preculture was left because of extensive foaming that, however, was sufficient for subsequent inoculation. For the production of strepturidin (**1**), 80 l of a complex medium consisting of mannitol 10 g l^{-1}, soy meal (full-fat) 10 g l^{-1}, meat extract 5 g l^{-1}, sodium chloride 1 g l^{-1}, calcium carbonate 1 g l^{-1} and tegosipon 10 ml in a 100-l bioreactor (MBR, Zurich, Switzerland) was inoculated with the remaining 5 l of the preculture. The cultivation was carried out at 37 °C at 300 r.p.m. and an aeration of 0.5 v.v.m. (volume per volume per min). After 26 h, 130 g l^{-1} wet cell mass and an antibiotic titer of 92 mg l^{-1} were determined. The culture broth was harvested by centrifugation, 180 g of sodium heptanesulfonate was added to the 72 l of supernatant, and the solution passed over a column (10 cm diameter) with 4 l of Amberlite XAD-16 at a constant flow of 8 l h^{-1}. Strepturidin (**1**) was bound as a heptanesulfonate ion-pair, and, after rinsing the column with water (4 l), (**1**) eluted with MeOH/H_2O, 1:1 (v/v). The eluate was concentrated *in vacuo*, and the remaining aqueous phase was lyophilized to give 106.4 g of crude extract containing 5.3 g of (**1**) (yield: 80% by HPLC). To reduce the sodium heptanesulfonate load, ion-pair extraction in two batches was performed. Therefore, each batch was dissolved in 200 ml H_2O with methyltrioctylammonium chloride (76.6 g) and each batch was extracted twice with 200 ml CH_2Cl_2. The aqueous phase was lyophilized to give 28.44 g crude extract with 2.36 g of (**1**) (yield 95%). Preparative HPLC of this material was performed on a Eurosil C-18 column (10 µm, 250 × 50 mm, Knauer GmbH, Berlin, Germany) with a MeOH/67 mM phosphate buffer (pH 5.0) (solvent A) and 10 mM sodium heptanesulfonate (solvent B) gradient starting with 4:96 (solvent A/B), and ending after 5 min with 9:91 (solvent A/B) at a flow of 100 ml min^{-1}. The detection was carried out at the UV detection wavelength $\lambda = 260$ nm. For each preparative separation, 3.0–3.5 g of the crude extract was dissolved in 20 ml of H_2O to obtain in total 110 g of salts, which after lyophilization of the combined peak fractions contained 2.13 g of (**1**) (yield 90%). Desalting was performed with 15–20 g portions dissolved in 350 ml H_2O and loaded on an Amberlite XAD-16 column (bed volume of 370 ml). The column was rinsed with two to three bed volumes of H_2O, and (**1**) eluted with MeOH/H_2O, 1:1 (v:v). From the combined fractions, MeOH was evaporated *in vacuo* to obtain 16.5 g salts containing 1.96 g (**1**) (yield 92%). Final desalting was achieved by CM-Sephadex A-25 NH_4^+ column chromatography (amount of gel 72 g, column size 27.2 × 5 cm, flow 9–10 ml min^{-1}, detection at UV detection wavelength $\lambda = 254$ nm). The two batches (8 g per batch) were dissolved in 250 ml water and applied to the column followed by two to three bed volumes of water with a flow of 9–10 ml min^{-1}. The pure compound (**1**) eluted with 50 mM NH_3 solution as a sharp peak in the chromatogram. The combined fractions were lyophilized to give 1.76 g of pure (**1**) as white powder (overall yield 27%).

Analytical HPLC of (**1**) was performed on a Nucleosil RP-18 (7 µm, 250 × 4 mm, Macherey-Nagel) column with the mobile phase acetonitrile/67 mM phosphate buffer pH 5 with 10 mM sodium heptanesulfonate with a flow of 1.4 ml min^{-1}. Detection was carried out at the UV wavelength $\lambda = 262$ nm and the retention time of (**1**) at 11.7 min.

MS

HPLC-HR-ESI-(+)-Orbitrap-MS mass spectra were recorded using a LTQ-Orbitrap XL (Thermo Scientific, Bremen, Germany) coupled to an Agilent 1260 HPLC system (Agilent Technologies, Waldbronn, Germany). The HPLC system was equipped with a Hypersil-Gold column (5 µm, 50 × 2.1 mm, Thermo Scientific, Bremen, Germany). A H_2O/MeOH (solvent A/solvent B) + 0.1% HCOOH gradient was used starting with 5% B and increasing to 100% B in 6 min, holding 100% B for 4 min, flushing to starting conditions 5% B for 3 min and a flow rate of 0.25 ml min^{-1}.

HPLC-LR-ESI-(+)-MS^2 spectra were measured on a QQQ-MS-6460 mass spectrometer (Agilent Technologies) coupled to an Agilent 1290 UHPLC-system (Agilent Technologies). The HPLC system was equipped with an Eclipse Plus C18 RRHD column (1.8 µm, 2.1 × 50 mm, Agilent Technologies). A H_2O/ACN (solvent A/solvent B) + 0.1% HCOOH linear gradient was used starting with 5% B and increasing to 100% B in 6 min, holding 100% B for 4 min, flushing to starting conditions 5% B for 3 min and a constant flow rate of 0.25 ml min^{-1}.

NMR spectroscopy

The 1D- and 2D-NMR spectra were recorded on a Bruker Avance III 500 spectrometer (Bruker, Karlsruhe, Germany) equipped with a broadband inverse detection probe with Z-Gradient at 500 and 125 MHz for ^{1}H and ^{13}C, respectively. Sample was dissolved in 600 µl d_6-DMSO for NMR experiments. Chemical shifts are given relative to TMS. For the calibration, the residual solvent peaks of d_6-DMSO (2.50 p.p.m. (^{1}H) and 39.5 p.p.m. (^{13}C)) were used. All experiments were performed at T = 298 K.

Synthesis of Gln-strepturidin (2)

To strepturidin (**1**) (5 mg, 0.011 mmol) dissolved in 2 M HCl (5 ml), Zn dust (0.1 mg) was added in small portions. The extent of reduction of the N_α-hydroxamate group of (**1**) was followed by HPLC-MS. After 24 h, the sample was centrifuged. The supernatant was purified by C18 solid-phase extraction), washing with H_2O and eluting with 100% MeOH. The compound was concentrated *in vacuo* and then freeze-dried to obtain a yellow oily material. Compound (**2**) was used for total hydrolysis, derivatization and subsequent chiral GC/EI-MS analytics described in the section below.

Synthesis and purification of monoacetal-strepturidin (3)

The procedure was mainly adopted from the work published by Jundt *et al.*[10] Strepturidin (**1**) (36 mg, 0.07 mmol), 1,1-dimethoxyethylbenzene (1.15 ml, 0.7 mmol) and camphorsulfonic acid (1.2 mg, 0.005 mmol) were dissolved in 1 ml of d_6-DMSO. After stirring overnight at 50 °C, saturated Na_2CO_3 solution was added to quench the reaction. The mixture was freeze-dried and the residue was dissolved in MeOH and separated by C18-HPLC to yield monoacetal-strepturidin (**3**) (5 mg, 0.008 mmol, 11% overall yield). Preparative HPLC was performed on an Agilent HPLC 1100 system (Agilent) equipped with a Grom-Sil-120-ODS column (5 µm, 250 mm × 20 mm). A H_2O/MeOH (solvent A/solvent B) gradient with a constant flow rate of 15 ml min^{-1} was used starting with 20% B and increasing to 80% B in 40 min, holding 100% B for 5 min, flushing to starting conditions 20% B for 3 min. Peak collection was carried out at the UV detection wavelength $\lambda = 254$ nm. The chromatogram is shown in Supplementary Figure S1.

Total hydrolysis of Gln-strepturidin (2) for chiral GC/EI-MS analysis

Total hydrolysis of Gln-strepturidin (**2**) (0.1 mg) was performed at 110 °C in aqueous 6 M hydrochloric acid solution (200 µl, grade for amino acid analysis; Sigma-Aldrich) under vacuum for 24 h in glass ampoules. After 24 h, the hydrochloric acid was removed in a gentle stream of nitrogen. Compound (**2**) was semi-purified by C18 solid-phase extraction before chiral GC/EI-MS analysis.

Chiral GC/EI-MS analysis

Chiral GC/EI-MS analysis from the total hydrolysate of Gln-strepturidin (**2**) was performed on a GC800 Top Voyager (Thermo Finnigan, Waltham, MA, USA) GC-mass spectrometer equipped with a chiral LIPODEX E column (25 m, I.D. 0.25 mm). The scan range was 45–465 amu, detection temperature 210 °C, source temperature 220 °C, ionization energy 70 eV and injection temperature 250 °C. Helium was used as a carrier gas with constant pressure at 60 kPa. Temperature program: 70 °C (2 min isotherm), 90 °C, 3 °C per min (15 min isotherm), 140 °C, 3 °C per min (10 min isotherm), 200 °C, 3 °C per min (1 min isotherm). The injection volume was 1 µl.

Antimicrobial assay

Agar test plates inoculated with *A. chroococcum* were prepared according to Reis.[26] Samples to be tested were applied as 20 µl solutions on paper discs of 6 mm diameter. After incubation for 20 h at 30 °C, the diameter of the inhibition zones was measured. The minimum inhibitory amount of pure strepturidin was 1.7 µg, giving an 8 mm inhibition zone.

Cytotoxicity assay

The inhibitory activity of strepturidin (**1**) was tested with mouse fibroblasts L-929 (DSMZ ACC 2). Next, 60 µl of a serial dilution of the compound were added to 120 µl aliquots of a cell suspension (50 000 cells per ml) in 96-well microplates. The plates were incubated at 37 °C and 10% CO_2 for 6 days. After that time, the MIC was estimated visually using an inverted microscope.

Supporting information

MS data and 2D NMR data are free of charge available via Internet at http://pubs.acs.org.

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