New Co-metabolites of the Streptazolin Pathway

Carsten Puder,[†] Shoshana Loya,[‡] Amnon Hizi,[‡] and Axel Zeeck*[,†]

Institut für Organische Chemie, Universität Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany, and Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

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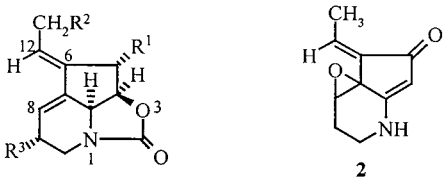
Variation of the culture conditions of *Streptomyces* sp. strain A1, which produces streptazolin (**1**), resulted in the isolation of four new co-metabolites: 5-*O*-(β-D-xylopyranosyl)streptazolin (**3**), 9-hydroxystreptazolin (**4**), 13-hydroxystreptazolin (**5**), and streptenol E (**6**). Their structures were established by spectroscopic and chemical methods. The possible biosynthetic relationship between the streptazolins and the streptenols is discussed.

A practicable approach to gain insight into biosynthetic pathways of microorganisms is based on careful analysis of the secondary metabolite pattern of single strains under varied fermentation conditions. In the case of *Streptomyces* sp. strain A1, application of the OSMAC (one strain/many compounds) approach[1,2] led to the production of various secondary metabolites, depending on the medium. Strain A1 produces rubromycins[3] when grown in an oat bran medium, whereas streptazolin (**1**), streptazone A (**2**), and streptazone B_1 are formed in a soybean meal/mannitol medium.[4] Cultivation of strain A1, using a medium with soil as a supplement, yielded three new congeners of **1**[5−13] named 5-*O*-(β-D-xylopyranosyl)streptazolin (**3**), 9-hydroxystreptazolin (**4**), and 13-hydroxystreptazolin (**5**). In addition, the new streptenol E (**6**) was produced. In this report we describe the isolation and structure elucidation and discuss the biosynthetic relationship of these natural products.



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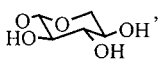
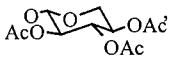
3 R^1 =  , R^2 = R^3 = H

3a R^1 =  , R^2 = R^3 = H

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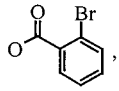
4a R^1 = R^3 =  , R^2 = H

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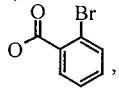
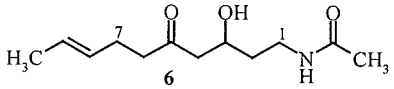
5a R^1 = R^2 =  , R^3 = H



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Results and Discussion

Cultivation of *Streptomyces* sp. strain A1 was carried out in an oat bran/soil medium at 28 °C using 1000-mL Erlenmeyer flasks and a 50-L fermentor as culture vessels. On the basis of TLC analysis, we found that extracts of the culture filtrates obtained from these fermentations showed different metabolite patterns. Thus, the production of secondary metabolites in strain A1 is not only dependent on the medium, but is also dependent on the aeration conditions. In the case of the culture filtrate harvested from shaking cultures, column chromatography of its extract on Si gel and Sephadex LH-20 led to the isolation of compounds **1**−**3**. Purification of the extract of the culture filtrate, obtained from the fermentation in the stirred 50-L fermentor, afforded **1**, **2**, **4**, **5**, and **6**. Compounds **4** and **5** proved difficult to handle because of their tendency to polymerize during the concentration of solutions. In the case of **1**, this property was previously observed by Keller-Schierlein et al.[5]

The ^{1}H and ^{13}C NMR spectra of **3** exhibited 21 proton and 16 carbon signals. Comparison of its NMR data with those of **1**[4,11] suggested that this compound contained **1** as the aglycon. The five additional carbon atoms in the ^{13}C and APT NMR spectra of **3** were identified as one O-substituted methylene, three O-substituted aliphatic methines, and one acetalic carbon atom. The DCIMS of **3** (*m*/*z* 357 $[M + NH_4]^+$) confirmed the molecular formula $C_{16}H_{21}NO_7$, as derived from EIMS and HREIMS of its triacetate **3a**. Detailed analysis of ^{1}H−^{1}H COSY, HETCOR, and COLOC experiments and interpretation of the observed $^3J_{H-H}$ coupling constants led to the structures **3** and **3a**, C-5 substituted xylopyranosyl derivatives of **1**. A β-glycosidic linkage was deduced from the ^{13}C chemical shift of C-1′ (δ 101.4)[14] and the value of the coupling constant between H-1′ and H-2′ (J = 7.3 Hz).[15] Xylose was isolated by acid hydrolysis of **3**. The observed optical rotation of the sugar moiety ($[\alpha]^{22}_D$ +16°) was in agreement with that of D-(+)-xylose ($[\alpha]^{22}_D$ +19°).[16] Therefore, **3** was identified as (4*S*,5*S*,11*S*)-5-*O*-(β-D-xylopyranosyl)streptazolin. Tests with four human cancer cell lines [HMO2 (stomach adenocarcinoma), HEP G2 (hepatocellular carcinoma), MCF 7 (breast adenocarcinoma), and Kato III (colon carcinoma)][17] showed **3** to possess significant cytostatic activity (Table 1), whereas **1** showed no inhibition against the first three cell lines at 50 μmol/L.[4]

* To whom correspondence should be adressed. Tel.: (+49) 551-393263. Fax: (+49) 551-399660. E-mail: azeeck@gwdg.de.
[†] Universität Göttingen.
[‡] Tel Aviv University.

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Table 1. Cytotoxic Activity of **3** Against Four Different Tumor Cell Lines

	cell line			
	HMO2	HEP G2	MCF7	Kato III
GI_{50}[a]	0.15	0.3	10	0.7
TGI[b]	>50	5.0	30	1.0

[a] GI_{50} = concentration, which results in a 50% inhibition of the cell growth; μmol/L.[17]. [b] TGI = concentration, which results in a complete inhibition of the cell growth; μmol/L.[17]

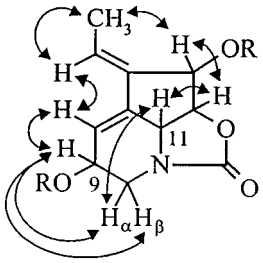

Figure 1. Selected NOE correlations for the compound **4a** (R = *o*-bromobenzoyl).

The EIMS of **4** displayed a molecular ion peak at *m/z* 223, corresponding to a molecular formula of $C_{11}H_{13}NO_4$ (HREIMS). The ^{13}C NMR spectrum of **4** revealed the presence of one methyl, one methylene, four aliphatic methine, two olefinic methine, one carbonyl, and two other quaternary carbons. As the ^1H NMR spectrum of **4** contained some overlapping signals (δ 4.59−4.52), its di-*o*-bromobenzoate (**4a**) was used for structure elucidation. The evaluation of the $^1H-^1H$ COSY and NOESY spectra (Figure 1) led to structure **4a**, from which the structure of the parent compound was identified as 9-hydroxystreptazolin (**4**). The values of the coupling constants between H-9 and H_2-10 in **4a** (J = 1.3 and 5.7 Hz) indicated that OH-9 takes up a pseudoaxial position,[15] in agreement with the fact that H-9 and H-11 did not show a NOE. Analogously to **1**,[5] **4** and **4a** should have the (4*S*,5*S*,9*R*,11*S*)-configuration.

The ^{13}C NMR spectrum of **5** resembled that obtained for **1**,[11] except that there was no signal for a methyl group. Instead, a methylene signal at δ 60.1 was detected. The molecular formula was established as $C_{11}H_{13}NO_4$ (HREIMS), indicating one additional oxygen atom in comparison with **1**. On the basis of these findings, the new metabolite was identified as 13-hydroxystreptazolin (**5**). The absolute configuration of the centers of chirality at C-4, C-5, and C-11 and the configuration of the exocyclic double bond were postulated analogously to **1**.[5,10] Derivatization of **5** with *o*-bromobenzoyl chloride yielded the di-*o*-bromobenzoate **5a**, the MS and NMR data of which confirmed the structure of the parent compound **5**.

The HREIMS of **6** gave $[M]^+$ at 227.1521, consistent with a molecular formula $C_{12}H_{21}NO_3$. The ^{13}C NMR spectrum indicated the presence of two methyl, five methylene, one O-substituted aliphatic methine, two olefinic methine, and two carbonyl carbons. $^1H-^1H$ COSY correlations verified the presence of $CH_3CH{=}CHCH_2CH_2$ and $CH_2CH(OH)CH_2$-CH_2NH chains and a methyl group. Linkage of these partial structures by the two remaining carbonyl groups (δ 211.3 and 170.8) led to 1-acetylamino-3-hydroxy-8-decen-5-one (= streptenol E, **6**). The configuration of the double bond was postulated as (*E*) following the closely related metabolite streptenol A[18,19] because of the identity of the ^{13}C NMR data of streptenol A and **6** from C-4 to C-10. The absolute stereochemistry of **6** at C-3 is still unknown.

To obtain a better understanding of the secondary metabolism of *Streptomyces* sp. strain A1, it was necessary to evaluate the biosynthetic relationship between the obtained compounds. We assume that **1**−**6** and some previously described metabolites[4] arise from a common biosynthetic pathway. Considering further structurally related compounds,[18−22] isolated from different streptomycetes by several groups, a conceivable route for the biosynthesis of **1** was established (see Supporting Information), reflecting the hypothesis that streptenol C and streptazone D are direct precursors of the streptazolins. In the case of streptazolin (**1**),[11] [2*S*(1*E*,3*E*),4*S*]-2-(1,3-pentadienyl)-4-piperidinol,[21] and dihydrolatumcidin,[22] the polyketide origin of their carbon skeletons was verified by feeding [^{13}C]-labeled acetate. In all three cases, the feeding experiments resulted in a corresponding incorporation of five acetate units, which confirms a close biosynthetic relationship. The existence of **6** indicates that the nitrogen atom in **1** derives from a conventional nitrogen source (e.g., glutamic acid) and is transferred in an early step, whereas the origin of the carbon atom of the carbamoyl moiety of **1** is still unclear.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert hot-stage microscope and are uncorrected. All homonuclear and heteronuclear 1D and 2D NMR spectra were recorded on Bruker AMX 300, Varian Unity 300, and Varian Inova 500 instruments. Chemical shifts are expressed in δ values with solvents as internal standards. EIMS and DCIMS data were collected on a Finnigan MAT 95 mass spectrometer at 70 eV (high resolution with perfluorkerosine as internal standard) and 200 eV (reaction gas: NH_3), respectively. IR spectra (KBr) were recorded on a Perkin-Elmer FT IR-1600 spectrometer and the UV spectra on a Kontron Uvikon 860 spectrophotometer. Optical rotation values were measured with a Perkin-Elmer 241 polarimeter and CD spectra with a JASCO J 500 A spectrometer. TLC was carried out on Si gel 60 F_{254} plates (Merck, 0.25 mm) and column chromatography on Si gel (ICN, 32−63 μm) or Sephadex LH-20 (Pharmacia). Compounds were viewed under UV and sprayed with *p*-anisaldehyde−H_2SO_4[23] or orcinol−$FeCl_3$ followed by heating.

Organisms and Fermentation. *Streptomyces* sp. strain A1 was maintained and cultivated as previously described[3] and is deposited at the Sackler School of Medicine in Tel Aviv University. Fermentations were carried out in 1000-mL Erlenmeyer flasks with three baffles. Caps of foamed plastic material were used as closures. Each flask was filled with 165 mL of medium composed of oat bran 3% and soil 3% in deionized water (pH 7.0), sterilized 30 min at 121 °C, and then inoculated at room temperature with a 9-cm^2 piece of agar from 5-day-old cultures grown on S/M−agar (degreased soybean meal 2%, mannitol 2%, and agar 2%). The soil used was collected from a hill (Kartoffelstein) near Göttingen, dried for 1 day at 80 °C, passed through a sieve, and sterilized 30 min at 121 °C on three successive days. The flasks were cultivated at 28 °C on a rotary shaker (250 rpm). Flasks of 2-day-old cultures (18 × 160 mL of culture broth) were used for the inoculation of a 50-L fermentor (Biostat U from Braun-Diessel) containing 47 L of sterilized medium (oat bran 3%, soil 3%, and antifoam 0.05%, pH 7.0). The fermentation in the stirred batch fermentor was carried out at 28 °C for 4 days with aeration (35 L/min) under constant agitation (200 rpm). A further 24 flasks were also harvested after 4 days and worked up separately.

Extraction and Isolation. The culture broth of the harvested shaking cultures (4 L, pH 6.5) was separated by filtration into mycelium and culture filtrate. The culture filtrate was extracted twice with Amberlite XAD-2 (0.4 L of resin). The resin was thoroughly washed with H_2O, and the bound compounds were eluted with MeOH (2 L). The methanolic extract was evaporated to dryness under reduced pres-

sure and the residue (2.72 g) was subjected to Si gel chromatography and eluted with CH_2Cl_2−MeOH (gradient from 14:1 to 3:1) into three fractions. Final purification of the single fractions by gel filtration chromatography (Sephadex LH-20, MeOH) led to 224 mg of **1**, 4 mg of **2**, and 47 mg of **3**. The culture broth obtained from the fermentation in the Biostat U-fermentor (50 L, pH 6.8) was analogously worked up as described above. The resulting crude product (19.2 g) was chromatographed over Si gel using a CH_2Cl_2−MeOH gradient (14:1 to 4:1) to give four fractions. The first fraction consisted of 2.2 g of pure **1**. The second fraction was further purified by repeated gel filtration chromatography (Sephadex LH-20, MeOH and Me_2CO) to afford 26 mg of **2** and 7 mg of **6**. Chromatography of the third fraction on a column of Sephadex LH-20 with MeOH furnished 110 mg of **5**. The fourth fraction was passed over Sephadex LH-20 (MeOH) and Si gel (CH_2Cl_2−MeOH, 9:1) yielding 29 mg of **4**. The mycelium obtained from the shaking cultures and the stirred 50-L fermentor, respectively, was extracted with Me_2CO and analyzed by TLC. Only small amounts of **1**[4] and *β*-rubromycin[3] were detectable, for this reason both extracts were discarded.

Streptazone A (2). The spectroscopic data of **2** were published previously.[4]

5-*O*-(*β*-D-Xylopyranosyl)streptazolin (3): white solid, mp 118 °C; R_f 0.06 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde−H_2SO_4, brown; $[\alpha]^{22}_D$ +1.3° (*c* 0.83, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (4.23) nm; CD (MeOH) λ ($\Delta\epsilon$) 233 (+5.93), 269 (−2.08) nm; IR (KBr) ν_{max} 3426 (br), 2923, 1750, 1636, 1377, 1223, 1045 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.21 (1H, q, J = 7.0 Hz, H-12), 6.09 (1H, ddd, J = 7.0, 3.5, 3.5 Hz, H-8), 5.04 (1H, d, J = 5.0 Hz, OH-2′), 4.92 (1H, d, J = 4.5 Hz, OH), 4.91 (1H, d, J = 5.0 Hz, OH), 4.81 (1H, d, J = 6.7 Hz, H-4), 4.76 (1H, s, H-5), 4.33 (1H, dm, J = 6.7 Hz, H-11), 4.29 (1H, d, J = 7.5 Hz, H-1′), 3.73 (1H, dd, J = 11.0, 5.0 Hz, H-5′eq), 3.37−3.03 (5H, m, H_2-10, H-3′, H-4′, H-5′ax), 2.95 (1H, ddd, J = 8.5, 7.5, 5.0 Hz, H-2′), 2.40 (1H, dddd, J = 16.5, 7.0, 7.0, 3.5 Hz, H-9*β*), 2.25−2.10 (1H, m, 9-H-9α), 1.81 (3H, d, J = 7.0 Hz, H_3-13); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 158.4 (s, C-2), 142.8 (s, C-7), 135.2 (s, C-6), 124.4 (d, C-12), 118.4 (d, C-8), 101.4 (d, C-1′), 79.1 (d, C-5), 78.9 (d, C-4), 76.4 (d, C-3′), 72.9 (d, C-2′), 69.4 (d, C-4′), 65.9 (t, C-5′), 58.4 (d, C-11), 39.8 (t, C-10), 22.0 (t, C-9), 14.3 (q, C-13); DCIMS *m/z* 357 $[M + NH_4]^+$ (11), 259 (44), 209 (43), 168 (100), 148 (72).

Preparation of 5-*O*-(2′,3′,4′-Tri-*O*-acetyl-*β*-D-xylopyranosyl)streptazolin (3a). An excess of acetanhydride (0.5 mL) was added to a solution of **3** (21 mg, 0.062 mmol) in pyridine (1 mL) at 0 °C. After stirring for 1 h at 0 °C and for an additional 3.5 h at room temperature, the solution was poured into a mixture of CH_2Cl_2 (100 mL) and ice (5 g). The organic layer was washed with H_2O (2 × 25 mL) and evaporated under reduced pressure. Purification of the residue by Si gel chromatography with CH_2Cl_2−MeOH (99:1) and gel filtration chromatography (Sephadex LH-20, MeOH) led to 20 mg of **3a** (0.043 mmol, 69%) as a colorless oil: R_f 0.57 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde−H_2SO_4, green-brown; $[\alpha]^{22}_D$ −22° (*c* 1.73, $CHCl_3$); IR (KBr) ν_{max} 2960, 1758, 1374, 1224, 1046 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.26 (1H, qm, J = 7.3 Hz, H-12), 6.03 (1H, ddd, J = 7.0, 3.5, 3.5 Hz, H-8), 5.17 (1H, dd, J = 9.0, 9.0 Hz, H-3′), 4.97 (1H, d, J = 1.5 Hz, H-5), 4.95 (1H, ddd, J = 9.0, 9.0, 5.3 Hz, H-4′), 4.94 (1H, dd, J = 9.0, 7.3 Hz, H-2′), 4.77 (1H, d, J = 7.0 Hz, H-4), 4.61 (1H, d, J = 7.3 Hz, H-1′), 4.16 (1H, dm, J = 7.0 Hz, H-11), 4.11 (1H, dd, J = 11.5, 5.3 Hz, H-5′eq), 3.45−3.34 (2H, m, H_2-10), 3.29 (1H, dd, J = 11.5, 9.0 Hz, H-5′ax), 2.49 (1H, dddd, J = 16.5, 7.0, 7.0, 3.5 Hz, H-9*β*), 2.23−2.13 (1H, m, H-9α), 2.05, 2.04 and 2.03 (9H, s, 3 × $COCH_3$), 1.83 (3H, d, J = 7.3 Hz, H_3-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1, 169.8, and 169.2 (s, 3 × $COCH_3$), 158.9 (s, C-2), 142.8 (s, C-7), 133.6 (s, C-6), 125.8 (d, C-12), 118.7 (d, C-8), 97.2 (d, C-1′), 80.4 (d, C-5), 80.1 (d, C-4), 71.8 (d, C-3′), 71.0 (d, C-2′), 68.9 (d, C-4′), 62.3 (t, C-5′), 59.0 (d, C-11), 39.7 (t, C-10), 22.7 (t, C-9), 20.6 (q, 3 × $COCH_3$), 14.8 (q, C-13); EIMS *m/z* 465 $[M]^+$ (2), 421 $[M - CO_2]^+$ (12), 259 $[C_{11}H_{15}O_7]^+$ (15), 189 (78), 145 (54), 43 $[C_2H_3O]^+$ (100); HREIMS *m/z* 465.1634 (calcd for $C_{22}H_{27}NO_{10}$, 465.1634).

Acid Hydrolysis of 3. Compound **3** (22 mg, 0.065 mmol) was hydrolyzed with 5 mL of 2 mol/L HCl−THF (1:1) at 60 °C for 24 h. The reaction mixture was neutralized with Ag_2CO_3 (0.9 g), the precipitates were filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by Si gel column chromatography with CH_2Cl_2−MeOH (7:3) to yield 3.7 mg of D-xylose (0.025 mmol, 38%) as a colorless syrup. D-Xylose was identified by high pressure TLC by comparison with an authentic sample: R_f 0.48 (CH_2Cl_2−MeOH, 7:3); color reaction with orcinol−$FeCl_3$, blue-violet; $[\alpha]^{22}_D$ +16° (*c* 0.26, H_2O, equilib.).

9-Hydroxystreptazolin (4): colorless oil; R_f 0.18 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde−H_2SO_4, brown; $[\alpha]^{22}_D$ +38° (*c* 1.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (3.84) nm; CD (MeOH) λ ($\Delta\epsilon$) 238 (+4.51), 274 (−1.54) nm; IR (KBr) ν_{max} 3424 (br), 1732, 1635, 1391, 1225, 1036 cm^{-1}; ^1H NMR (300 MHz, Me_2CO-d_6) δ 6.24 (1H, qd, J = 7.3, 1.5 Hz, H-12), 6.20 (1H, dm, J = 5.5 Hz, H-8), 4.75 (1H, dm, J = 5.5 Hz, H-5), 4.67 (1H, d, J = 6.7 Hz, H-4), 4.59−4.52 (3H, m, OH-5, H-9, H-11), 4.19 (1H, d, J = 3.5 Hz, OH-9), 3.56 (1H, dd, J = 13.5, 5.5 Hz, H-10*β*), 3.30 (1H, ddd, J = 13.5, 1.5, 1.5 Hz, H-10α), 1.88 (3H, d, J = 7.3 Hz, H_3-13); ^{13}C NMR (75.5 MHz, Me_2CO-d_6) δ 159.8 (s, C-2), 147.8 (s, C-7), 140.1 (s, C-6), 125.6 (d, C-12), 120.8 (d, C-8), 82.8 (d, C-4), 74.5 (d, C-5), 62.5 (d, C-9), 59.2 (d, C-11), 50.3 (t, C-10), 15.1 (q, C-13); EIMS *m/z* 223 $[M]^+$ (100), 205 $[M - H_2O]^+$ (3), 195 $[M - CO]^+$ (6), 150 (30), 121 (57); HREIMS *m/z* 223.0844 (calcd for $C_{11}H_{13}NO_4$, 223.0844).

Preparation of 5,9-Di-*O*-(*o*-brombenzoyl)-9-hydroxystreptazolin (4a). A solution of **4** (11 mg, 0.0490 mmol) and *o*-bromobenzoyl chloride (48 mg, 0.22 mmol) in pyridine (2.5 mL) was stirred for 1.5 h at 0 °C. Then CH_2Cl_2 (100 mL) was added, and the mixture was washed with H_2O (50 mL), diluted HCl (50 mL), and H_2O (50 mL). After removal of the solvent under reduced pressure, the crude product was purified by Si gel chromatography with CH_2Cl_2−MeOH (99:1) to yield 21 mg of **4a** (0.036 mmol, 73%) as a colorless, amorphous solid: mp 53 °C; R_f 0.68 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde−H_2SO_4, brown; $[\alpha]^{22}_D$ +4° (*c* 1.25, $CHCl_3$); IR (KBr) ν_{max} 2934, 1766, 1732, 1286, 1246, 1097, 1029, 746 cm^{-1}; ^1H NMR (300 MHz, Me_2CO-d_6) δ 7.87−7.79 (2H, m, 2 × H-6′), 7.77−7.69 (2H, m, 2 × H-3′), 7.53−7.43 (4H, m, 2 × H-4′, 2 × H-5′), 6.60 (1H, qd, J = 7.3, 1.7 Hz, H-12), 6.53 (1H, dd, J = 6.7, 2.0 Hz, H-8), 5.96 (1H, s br, H-5), 5.84 (1H, ddd, J = 6.7, 5.7, 1.3 Hz, H-9), 5.13 (1H, d, J = 7.0 Hz, H-4), 4.92 (1H, ddd, J = 7.0, 1.7, 1.7 Hz, H-11), 3.89 (1H, dd, J = 14.0, 5.7 Hz, H-10*β*), 3.71 (1H, ddd, J = 14.0, 1.3, 1.3 Hz, H-10α), 1.91 (3H, d, J = 7.3 Hz, H_3-13); ^{13}C NMR (75.5 MHz, Me_2CO-d_6), δ 166.2 and 165.7 (s, 2 × C-7′), 159.1 (s, C-2), 150.2 (s, C-7), 135.4 (s, C-6), 135.1 and 135.0 (d, 2 × C-3′), 134.1 and 133.8 (d, 2 × C-4′), 133.3 and 132.6 (s, 2 × C-1′), 132.12 and 132.10 (d, 2 × C-6′), 130.3 (d, C-12), 128.54 and 128.49 (d, 2 × C-5′), 121.6 and 121.4 (s, 2 × C-2′), 117.1 (d, C-8), 80.1 (d, C-4), 77.2 (d, C-5), 67.7 (d, C-9), 59.8 (d, C-11), 47.2 (t, C-10), 15.8 (q, C-13); EIMS *m/z* 591 $[C_{25}H_{19}{}^{81}Br_2NO_6]^+$ (1), 589 $[C_{25}H_{19}{}^{79}Br^{81}BrNO_6]^+$ (2), 587 $[C_{25}H_{19}{}^{79}Br_2NO_6]^+$ (1), 389 (22), 387 (22), 202 (30), 200 (31), 185 (96), 183 (100); HREIMS *m/z* 586.9579 (calcd for $C_{25}H_{19}{}^{79}Br_2NO_6$, 586.9579).

13-Hydroxystreptazolin (5): colorless oil; R_f 0.26 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde-H_2SO_4, brown; $[\alpha]^{22}_D$ +39° (*c* 1.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (4.04), 315 (2.70) nm; CD (MeOH) λ ($\Delta\epsilon$) 234 (+4.96), 265 (−2.45) nm; IR (KBr) ν_{max} 3427 (br), 1735, 1638, 1403, 1222, 1037 cm^{-1}; ^1H NMR (300 MHz, Me_2CO-d_6) δ 6.21−6.13 (2H, m, H-8, H-12), 4.74 (1H, s br, H-5), 4.69 (1H, s br, OH), 4.66 (1H, d, J = 6.7 Hz, H-4), 4.37−4.30 (3H, m, H-11, H_2-13), 4.16−4.07 (1H, m, OH), 3.35 (2H, dd, J = 8.5, 5.5 Hz, H_2-10), 2.54−2.42 and 2.30−2.13 (2H, m, H_2-9); ^{13}C NMR (75.5 MHz, Me_2CO-d_6) δ 159.5 (s, C-2), 144.1 (s, C-7), 140.5 (s, C-6), 127.1 (d, C-12), 120.7 (d, C-8), 82.4 (d, C-4), 75.1 (d, C-5), 60.1 (t, C-13), 59.3 (d, C-11), 40.4 (t, C-10), 23.1 (t, C-9); EIMS *m/z* 223 $[M]^+$ (18), 205 $[M - H_2O]^+$ (81), 194 (34), 161 $[M - H_2O - CO_2]^+$ (39), 132 (100), 105 (40), 77 (44); HREIMS *m/z* 223.0844 (calcd for $C_{11}H_{13}NO_4$, 223.0844).

Preparation of 5,13-Di-*O*-(*o*-brombenzoyl)-13-hydroxystreptazolin (5a). A solution of **5** (20 mg, 0.090 mmol) and *o*-bromobenzoyl chloride (79 mg, 0.36 mmol) in 2.5 mL of CH_2Cl_2−pyridine (4:1) was stirred for 1 h at 0 °C, an additional hour at room temperature and then worked up as described for **4a**. Chromatography of the crude product on Si gel by using CH_2Cl_2−MeOH (98:2) as the eluent gave 31 mg of **5a** (0.053 mmol, 59%) as a colorless, amorphous solid: mp 65 °C; R_f 0.64 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde−H_2SO_4, brown; $[\alpha]^{22}_D$ +39° (*c* 1.78, $CHCl_3$); IR (KBr) ν_{max} 2923, 1761, 1733, 1289, 1248, 1103, 1028, 745 cm^{-1}; ^1H NMR (300 MHz, Me_2CO-d_6) δ 7.90−7.82 (2H, m, 2 × H-6′), 7.74−7.63 (2H, m, 2 × H-3′), 7.50−7.37 (4H, m, 2 × H-4′, 2 × H-5′), 6.48−6.38 (2H, m, H-8, H-12), 6.04 (1H, s, H-5), 5.10 (1H, dd, J = 13.5, 7.5 Hz, H-13β), 5.08 (1H, d, J = 7.0 Hz, H-4), 5.00 (1H, dd, J = 13.5, 7.5 Hz, H-13α), 4.60 (1H, dm, J = 7.0 Hz, H-11), 3.45−3.36 (2H, m, H_2-10), 2.65−2.52 and 2.40−2.22 (2H, m, H_2-9); ^{13}C NMR (75.5 MHz, Me_2CO-d_6), δ 166.2 and 165.5 (s, 2 × C-7′), 158.8 (s, C-2), 143.0 (s, C-7) 139.9 (s, C-6), 135.1 and 134.9 (d, 2 × C-3′), 134.2 and 133.7 (d, 2 × C-4′), 133.2 (s, 2 × C-1′), 132.3 and 131.9 (d, 2 × C-6′), 128.4 and 128.3 (d, 2 × C-5′), 124.2 and 122.1 (d, C-8, C-12), 121.8 and 121.6 (s, 2 × C-2′), 80.0 (d, C-4), 77.8 (d, C-5), 63.1 (t, C-13), 59.7 (d, C-11), 40.2 (t, C-10), 23.4 (t, C-9); EIMS *m*/*z* 591 $[C_{25}H_{19}{}^{81}Br_2NO_6]^+$ (1), 589 $[C_{25}H_{19}{}^{79}Br^{81}BrNO_6]^+$ (2), 587 $[C_{25}H_{19}{}^{79}Br_2NO_6]^+$ (1), 389 (11), 387 (10), 202 (15), 200 (16), 185 (97), 183 (100); HREIMS *m*/*z* 586.9579 (calcd for $C_{25}H_{19}{}^{79}Br_2NO_6$, 586.9579).

Streptenol E (6): colorless oil; R_f 0.28 ($CHCl_3$−MeOH, 9:1); color reaction with *p*-anisaldehyde−H_2CO_4, orange-brown; $[\alpha]^{22}_D$ +33° (*c* 0.34, $CHCl_3$); IR (KBr) ν_{max} 3392 (br), 2927, 1709, 1650, 1556, 1438, 1374 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.17 (1H, s br, NH), 5.50−5.29 (2H, m, H-8, H-9), 4.07 (1H, dddd, J = 8.2, 8.2, 4.2, 4.2 Hz, H-3), 3.84 (1H, br, OH-3), 3.58 (1H, m_c, H-1β), 3.17 (1H, dddd, J = 14.0, 5.3, 5.3, 5.3 Hz, H-1α), 2.55 (2H, m, 2H, H_2-4), 2.47 (2H, t, J = 7.0 Hz, H_2-6), 2.22 (2H, dt, J = 7.0, 7.0 Hz, H_2-7), 1.95 (3H, s, $COCH_3$), 1.60 (3H, d, J = 5.5 Hz, H_3-10), 1.69−1.44 (2H, m, H_2-2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 211.3 (s, C-5), 170.8 (s, $NHCOCH_3$), 129.1 (d, C-8), 126.2 (d, C-9), 66.1 (d, C-3), 48.9 (t, C-4), 43.4 (t, C-6), 36.8 (t, C-1), 35.7 (t, C-2), 26.5 (t, C-7), 23.3 (q, $NHCOCH_3$), 17.9 (q, C-10); EIMS *m*/*z* 227 $[M]^+$ (3), 209 $[M - H_2O]^+$ (27), 150 (*56*), 116 (62), 98 (67), 87 (42), 72 (100), 55 (50), 43 (83) $[C^2H_3O]^+$, 41 (36) $[C_3H_5]^+$; HREIMS *m*/*z* 227.1521 (calcd for $C_{12}H_{21}NO_3$, 227.1521).

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Supporting Information Available: Hypothetical streptazolin pathway (scheme). This material is available free of charge via the Internet at http://pubs.acs.org.

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