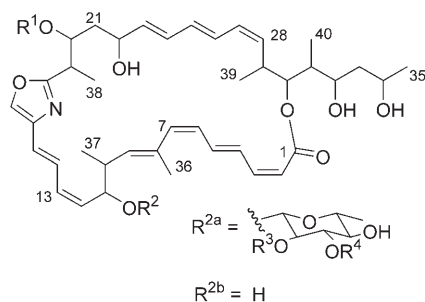


Chivosazole A—Elucidation of the Absolute and Relative Configuration

Dominic Janssen, Dieter Albert, Rolf Jansen, Rolf Müller, and Markus Kalesse*

Myxobacteria are a valuable source of structurally diverse biologically active natural products. Among the various strains isolated from *Sorangium cellulosum*, strain Soce12 produces several important antibiotic or cytotoxic compounds such as soranigicin A,^[1] sorangiolides,^[2] disorazoles,^[3] and chivosazoles. The chivosazoles (Table 1) form a family of 31-

Table 1:



	Chivosazole	R ¹	R ²	R ³	R ⁴
1	A	Me	2a	Me	Me
2	A ₁ (6,7-E)	Me	2a	Me	Me
3	B	H	2a	Me	Me
4	C	Me	2a	H	H
5	D	H	2a	H	Me
6	E	H	2a	H	H
7	F	Me	2b	—	—

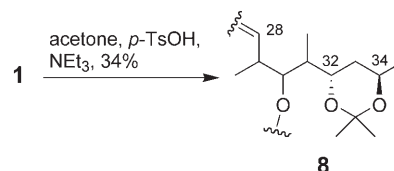
membered macrolides which were isolated from *S. cellulosum* Soce12 at the Helmholtz Centre for Infection Research (HZI, formerly GBF) in 1997.^[4] They are active against yeasts and filamentous fungi and they are highly cytotoxic against mammalian cell cultures (IC₅₀ 9 ng mL⁻¹ for L 929 and HeLa). Structurally, the 31-membered macrolactone can be

dissected into three polyene segments (C2–C9, C12–C15, and C23–C28), hydroxylated ketide segments (C20–C23 and C29–C35), and an oxazole moiety. Except for chivosazole F (7), all the natural variants possess a 6-deoxyglucopyranose unit (chinovose) at C11.

The structure of chivosazole A (1) was established by mass spectrometry and NMR studies.^[4] However, chemical synthesis of this very potent natural product was obstructed by the lack of stereochemical information on the ten stereocenters. Nevertheless, its remarkable biological activity and potency combined with its complex structure prompted us to determine the configuration of chivosazole A (1) as a prerequisite for its chemical synthesis.

Herein we describe how the absolute and relative configuration of chivosazole has been assigned through a combination of chemical degradation, partial synthesis, NMR spectroscopy, and genetic analysis.^[5]

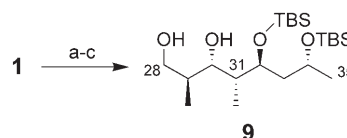
At the start of our investigations we analyzed the relative configuration of C32 and C34. An acetonide was generated in the side chain and examined with the aid of the method described by Rychnovsky and Evans;^[6] three new ¹³C NMR signals were found at $\delta = 24.8$, 25.2, and 101.8 ppm, thereby indicating a 1,3-*anti*-diol relationship between C32 and C34 (Scheme 1). To determine the configuration of the adjacent



Scheme 1. Formation of acetonide 8. Ts = toluene-4-sulfonyl.

stereocenters, chivosazole A (1) was protected as a TBS ether and then degraded by reductive ozonolysis to produce fragment 9 (Scheme 2).

NOESY experiments (Figure 1) were used to assign the relative configuration of the four contiguous stereocenters in fragment 9. Since the relationship of the C32–C34 diol was



Scheme 2. a) TBSOTf, 2,6-lutidine, CH₂Cl₂, 30% fourfold TBS-protected 1; b) O₃, CH₂Cl₂, –78 °C; c) NaBH₄, CH₂Cl₂, 46% over 2 steps. TBS = *tert*-butyldimethylsilyl.

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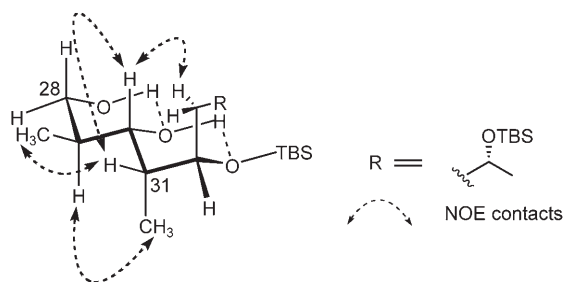
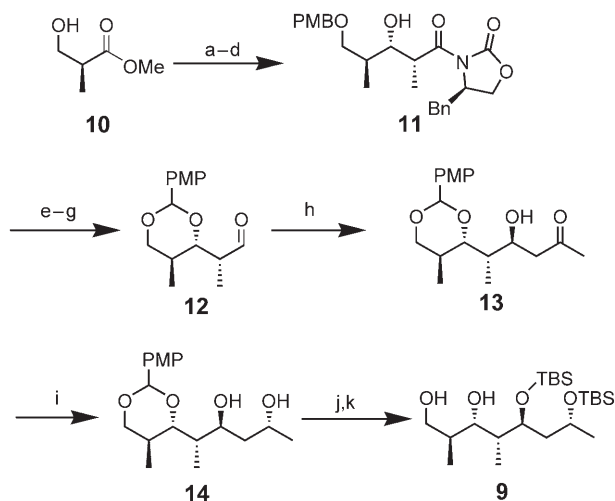


Figure 1. NOE contacts in fragment **9**. (relevant coupling constants: H29-H30: 9.7 Hz, H30-H31: 1.0 Hz, H31-H32: 1.8 Hz).

already established as *anti*, the relative configuration of the whole fragment could be determined.

To confirm our proposed configurations and to determine the absolute configuration we synthesized segment **9** by an independent experiment (Scheme 3). A *syn*-selective Evans



Scheme 3. a) PMBO(NH)CCl₃, CSA, CH₂Cl₂, RT, 91%; b) DIBAL-H, CH₂Cl₂, −78 °C, 76%; c) (COCl)₂, DMSO, NEt₃, CH₂Cl₂, −78 °C→0 °C, 95%; d) (*R*)-(+)-4-benzyl-3-propionyloxazolidin-2-one, TfOBBu₂, NEt₃, CH₂Cl₂, 85%; e) DDQ, MS (4 Å), CH₂Cl₂, 0 °C→RT, 73%; f) LiBH₄, EtOH, THF, 0 °C→RT, 96%; g) (COCl)₂, DMSO, NEt₃, CH₂Cl₂, −78 °C→0 °C, 99%; h) 2-(trimethylsilyloxy)propene, BF₃·Et₂O, toluene, −78 °C, 86%, d.r. 97:3; i) Me₄NHB(OAc)₃, CH₃CN/AcOH (1:1), −40 °C, 57%; j) TBSOTf, 2,6-lutidine CH₂Cl₂, 72%; k) Pd(OH)₂/C, H₂, *i*PrOH, 74%. PMB = *para*-methoxybenzyl, CSA = camphorsulfonic acid, DIBAL-H = diisobutylaluminum hydride, Tf = trifluoromethanesulfonyl, DDQ = 2,3-dichloro-5,6-dicyan-1,4-benzoquinone, MS = molecular sieves, PMP = *para*-methoxyphenyl, Bn = benzyl.

aldol reaction on the aldehyde derived from Roche ester (**10**), followed by an *anti*-Felkin-selective Mukaiyama aldol reaction afforded hydroxy ketone **13**. Reduction of **13** with Me₄NHB(OAc)₃ selectively generated *anti*-diol **14**. Protection of the diol as TBS ethers and removal of the acetal established segment **9**. The spectroscopic data was identical to those of the segment derived by degradation. Comparison of the [α]_D values enabled the absolute configuration to be determined as 29*S*, 30*S*, 31*S*, 32*S*, and 34*R*.

We used a combination of NMR data and molecular modeling studies to determine the configuration of the remaining five stereocenters of the macrocycle. The decisive NMR signal in the conformational analysis was a transannular NOE interaction between H7 and one of the diastereotopic protons at C21.^[7] On the basis of this NOE interaction, a conformational search was initiated in which all the substituents were omitted. Only conformations that were consistent with the observed transannular NOE interaction were considered further.

The conformational analysis was performed by a Monte Carlo search using the MacroModel program (version 8.0).^[8] The starting structure was a simplified molecule in which all the side chains (except Me36) and the OH groups of the macrocycle were replaced by hydrogen atoms. Ten torsion angles (see Figure 2) were subjected to random changes

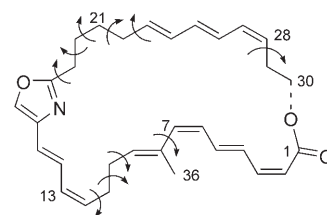


Figure 2. Macrocytic backbone of chivosazole A subjected to a Monte Carlo (MC) conformational search. The dotted line indicates the site of the formal ring opening.

according to the MCMM^[9] (Monte Carlo Multiple Minimum) algorithm. Formal ring opening at bond C30–O1 (dotted bond in Figure 2) was simulated, and a ring closing only accepted if the C30...O1 distance was less than 4.5 Å. The energies of the conformations found were minimized both in a vacuum and in chloroform (GB/SA; generalized Born surface area solvation model^[10]) by using the MMFF force field and the Polak-Ribière (PR) conjugate gradient method. The calculated conformations were accepted if the configuration of the double bonds was retained and if the distance between H7 and one of the diastereotopic protons at C21 was less than 4 Å.

The identified structures were further grouped together and ranked on the basis of the lowest energy representative of each group. The omitted substituents were then added to these representative conformations according to the observed ³*J*(H,H) coupling constants.^[11] In every case only one configuration was in agreement with the coupling information. After further structure optimization, the overall backbone conformations remained unchanged compared to the initial backbone conformation and were in accord with the experimental NOE data. The key structural details are summarized in Table 2. The structure obtained from the lowest energy conformation corresponds to the structure derived from NMR-derived data. The calculated structural parameters which differ from the NMR data are shown in bold.

Finally, the so-generated structure was resubmitted to a Monte Carlo search to find out if the presence of the substituents would result in a new energy minimum. This time only the geometries of the double bonds and the configu-

Table 2: Key atom distances determined by Monte Carlo calculations; stereoisomers which do not satisfy the NOE data are indicated in bold.

Compound	10L-11L-19D- 20D-22L-29D- 30D	10D-11D-19L- 20L-22D-29L- 30L	10L-11L-19L- 20L-22D-29L- 30L	10L-11L-19D- 20L-22D-29L- 30L	10L-11L-19D- 20L-22D-29L- 30L	10D-11D-19L- 20D-22L-29D- 30D	10L-11D-19D- 20L-22D-29L- 30L	10L-11L-19L- 20D-22L-29D- 30D
Energy [kJ mol ⁻¹] ^[a]	579.630	604.198	626.566	637.536	591.998	610.873	603.238	642.117
internuclear distances [Å]								
H7-H9	3.4	3.4	2.4	3.3	3.3	3.3	3.5	3.2
H7-H21a	3.8	3.2	5.8	2.8	4.1	3.8	3.8	3.3
H7-H21b	2.8	2.7	4.2	3.2	3.0	2.6	2.6	3.3
H7-H36	2.6	2.5	3.5	2.5	2.6	2.6	2.7	2.6
H7-H38	4.1	2.9	7.6	3.9	3.6	2.5	3.5	3.0
H9-H10	3.1	3.1	2.3	2.3	3.1	3.1	3.1	2.2
H9-H11	3.5	3.3	2.8	2.7	3.4	3.4	2.7	2.7
H9-H14	3.7	3.1	3.6	3.2	3.5	3.2	4.1	3.3
H9-H37	2.5	2.5	3.6	3.5	2.5	2.5	2.7	3.6
H10-H11	2.4	2.5	2.5	2.6	2.5	2.5	2.7	2.5
H10-H36	2.2	2.1	4.0	4.0	2.2	2.1	2.1	4.0
H11-H36	2.6	3.0	4.1	4.1	2.8	2.7	4.1	4.0
H12-H37	2.5	2.6	2.4	2.6	2.5	2.6	2.8	2.6
H20-H21a	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
H20-H21b	2.6	2.6	2.4	2.5	2.6	2.5	2.5	2.5
H20-H38	3.8	3.8	3.9	2.5	2.4	2.5	2.5	2.5
H21a-H38	2.3	2.4	2.3	3.2	3.5	3.5	3.3	3.1
H21b-H38	2.8	2.8	3.4	2.3	2.3	2.3	2.2	2.3
H22-H23	2.5	2.5	2.6	2.5	2.5	2.5	2.5	2.5
H28-H29	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
H28-H30	2.4	2.5	2.4	2.5	2.5	2.5	2.5	2.6
H28-H39	2.9	2.8	2.9	2.8	2.9	2.7	2.9	3.1
H29-H26	2.2	2.1	2.2	2.1	2.2	2.1	2.2	2.2
H29-H40	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.4

[a] In chloroform (GB/SA) as solvent; force field: MMFF.

rations of the stereocenters were fixed during the calculations, while no distance restraint between H7 and H21b was applied. Gratifyingly, the global minimum found in this Monte Carlo search had the same rigid carbon backbone as found in the first conformational search (Figure 3). There is, however, one small deviation (about 2 Hz) between the calculated coupling constant $^3J_{\text{H10,H11}}$ obtained by applying the empirical Karplus equation proposed by Haasnoot et al.^[11] and the observed coupling constant. This difference indicates that some conformational equilibrium is probable in this region.

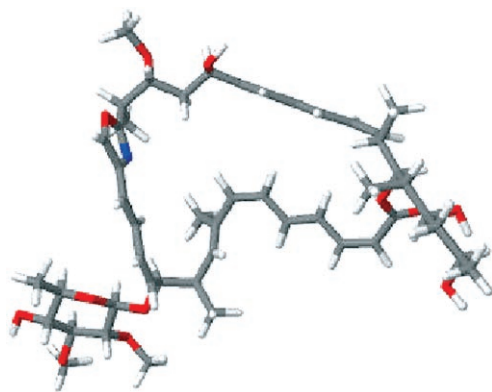


Figure 3. Calculated structure of chivosazole A (1).

Analysis of the two conformations found directly above the global minima indicate there is some flexibility in the C7–C11 and the C19–C22 regions. In the two structures, a hydrogen bond is present between HO22 and either N16 or O20 (see the Supporting Information). A fast equilibrium between the global minimum as the predominant conformation (about 90 %) and the local minima found would explain the above discrepancy of the coupling constant. By combining this result with the absolute configurations of 29D and 30D determined by degradation studies, the NMR experiments and molecular modeling studies give an absolute configuration of 10L-11L-19D-20D-22L-29D-30D.

Nevertheless, the limited availability and stability of natural chivosazole A meant that we were not able to obtain further structural assignment by chemical degradation. To circumvent this drawback, we turned our attention to the information available about chivosazole biosynthesis.^[14] The respective biosynthetic gene cluster encoding a complex hybrid of polyketide synthases (PKS) and a nonribosomal peptide synthetase was described recently by Müller and co-workers.^[12] By using the analysis of the central region of the keto reductase (KR) described by Reid et al.^[13] and Caffrey^[14] we have been able to determine the configuration at C30, C32, and C34. Additionally, with the configuration of these three secondary alcohols already established by degradation, an internal standard was available which confirmed the validity of the amino acid analysis for the assignment of the

remaining secondary alcohols. The presence of an aspartate residue in the keto reductase is indicative of the formation of an alcohol with a *D* configuration. Correspondingly, the lack of this aspartic acid leads to the *L* configuration. Application of this method to chivosazole A (**1**) confirmed the known configurations of C30, C32, and C34 in the side chain (Table 3). Furthermore, the genetic analysis is in agreement with the spectroscopically derived configurational assignment for C11, C20, and C22.

Table 3: Keto reductase regions of the polyketide synthases (amino acids 146–155) involved in chivosazole biosynthesis.

KR	Central region ^[a]	KR product ^[b]
KR1	AGVLRDGLCL	C34 (<i>D</i>)
KR2	ALSYQGAPLA	C32 (<i>L</i>)
KR3	ALRLEDRITD	C30 (<i>D</i>)
KR4	AGLAPSSNVA	DB
KR5	AGVLRDGLAV	DB
KR6	AIVMRDRSLV	DB
KR7	AGGTDATRIG	C22 (<i>L</i>)
KR8	AITLADGLLA	C20 (<i>D</i>)
KR10	AGEMRTSTPA	DB
KR11	AGLIRDALIP	DB
KR12	AFLFASEPLA	C11 (<i>L</i>)
KR13	AMVLADRTLM	DB
KR14	AGLADHERPA	DB
KR15	AGVLRDALIP	DB
KR16	ALVLHQSLA	DB

[a] Aspartate is highlighted in gray. [b] Absolute configuration of the KR products 1,2,3,7,8,12 in parentheses. The other KR domains lead, after elimination of water, to double bonds (DB).^[12]

We have reported here for the first time a combination of classical chemical methods and genetic analysis to assign the configuration of a complex natural product. For parts of the molecule three independent methods were used for the analysis: chemical degradation and synthesis, analysis of NMR data in combination with computational methods, and amino acid assignments. All three methods gave the same configurations for the individual centers. Only the combination of these various methods provides a structure with reliable confidence for chivosazole A (**1**) (Figure 4).

Received: December 22, 2006
Published online: May 15, 2007

Keywords: configuration determination · keto reductase · molecular modeling · natural products · NMR spectroscopy

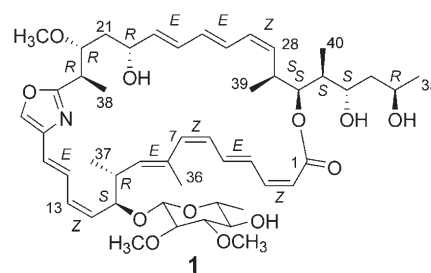


Figure 4. Absolute configuration of chivosazole A (**1**).

- [1] a) R. Jansen, H. Irschik, H. Reichenbach, D. Schomburg, V. Wray, G. Höfle, *Liebigs Ann. Chem.* **1989**, 111–119; b) R. Jansen, H. Irschik, H. Reichenbach, D. Schomburg, V. Wray, G. Höfle, *Tetrahedron Lett.* **1985**, 26, 6031–6034; c) H. Irschik, R. Jansen, K. Gerth, G. Höfle, H. Reichenbach, *J. Antibiot.* **1987**, 40, 7–13.
- [2] R. Jansen, H. Irschik, H. Meyer, H. Reichenbach, V. Wray, D. Schomburg, G. Höfle, *Liebigs Ann.* **1995**, 867–872.
- [3] a) R. Jansen, H. Irschik, H. Reichenbach, V. Wray, G. Höfle, *Liebigs Ann. Chem.* **1994**, 759–773; b) H. Irschik, R. Jansen, K. Gerth, G. Höfle, H. Reichenbach, *J. Antibiot.* **1995**, 48, 31–35.
- [4] R. Jansen, H. Irschik, H. Reichenbach, G. Höfle, *Liebigs Ann.* **1997**, 1725–1732.
- [5] For related approaches, see a) R. K. Kondru, P. Wipf, D. N. Berantan, *J. Am. Chem. Soc.* **1998**, 120, 2204–2205; b) S. Kiren, L. J. Williams, *Org. Lett.* **2005**, 7, 2905–2908; c) N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana, *J. Org. Chem.* **1999**, 64, 866–876; d) J. Hassfeld, F. Christophe, H. Steinmetz, T. Carlomagno, D. Menche, *Org. Lett.* **2006**, 8, 4751–4754.
- [6] a) S. D. Rychnovsky, B. N. Rogers, T. I. Richardson, *Acc. Chem. Res.* **1998**, 31, 9–17; b) D. A. Evans, D. L. Rieger, J. R. Gage, *Tetrahedron Lett.* **1990**, 31, 7099–7100.
- [7] C. R. Landis, L. L. Luck, J. M. Wright, *J. Magn. Reson. Ser. B* **1995**, 109, 44–59.
- [8] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, 11, 440–467.
- [9] a) G. Chang, C. Wayne, W. C. Guida, W. C. Still, *J. Am. Chem. Soc.* **1989**, 111, 4379–4386; b) M. Saunders, K. N. Houk, Y. D. Wu, W. C. Still, M. Lipton, G. Chang, W. C. Guida, *J. Am. Chem. Soc.* **1990**, 112, 1419–1427.
- [10] W. C. Still, A. Tempzyk, R. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, 112, 6127–6129.
- [11] C. A. G. Haasnoot, F. A. A. M. De Leeuw, C. Altona, *Tetrahedron* **1980**, 36, 2783–2792.
- [12] O. Perlova, K. Gerth, O. Kaiser, A. Hans, R. Müller, *J. Biotechnol.* **2006**, 121, 174–191.
- [13] R. Reid, M. Piagentini, E. Rodriguez, G. Ashley, N. Viswanathan, J. Carney, D. V. Santi, C. R. Hutchinson, R. McDaniel, *Biochemistry* **2003**, 42, 72–79.
- [14] P. Caffrey, *ChemBioChem* **2003**, 4, 654–657.