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Isolation and Synthesis of Chivotriene, a Chivosazole Shunt Product from *Sorangium cellulosum*

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A shunt product of chivosazole biosynthesis was isolated from the fermentation broth of *Sorangium cellulosum*, strain So ce 12. Its structure was elucidated by NMR spectroscopy and exhibits six chiral centers, a triene, and a labile β-hydroxy ketone. The synthesis of chivotriene confirms the structure and in particular the proposed configuration at C22, which was originally deduced for chivosazole from the combination of its genetic and spectroscopic analyses. Remarkably, chivotriene exhibits biological activity usually not observed for segments of complex natural products. However, this activity is not connected to interference with the actin cytoskeleton, the intrinsic target of the chivosazoles.

Introduction

Chivosazoles are in the focus of chemical research due to their complex chemical structure and remarkable biological profile.[1] Their structures contain a 31-membered macrolide constructed from three polyene, one oxazole, and a hydroxylated ketide segment. Additionally, chivosazoles A–E include a 6-deoxyglucose derivative at C11. Chivosazoles exhibit antifungal and cytotoxicity activity against mouse fibroblasts (L-929) and HeLa cells.[1a] Recently, Sasse et al. reported the reduction of actin polymerization by chivosazoles.[2] These studies illustrate that chivosazoles act on F-actin differently than that proposed for cytochalasin D, chondramide, or rhizopodin.[3]

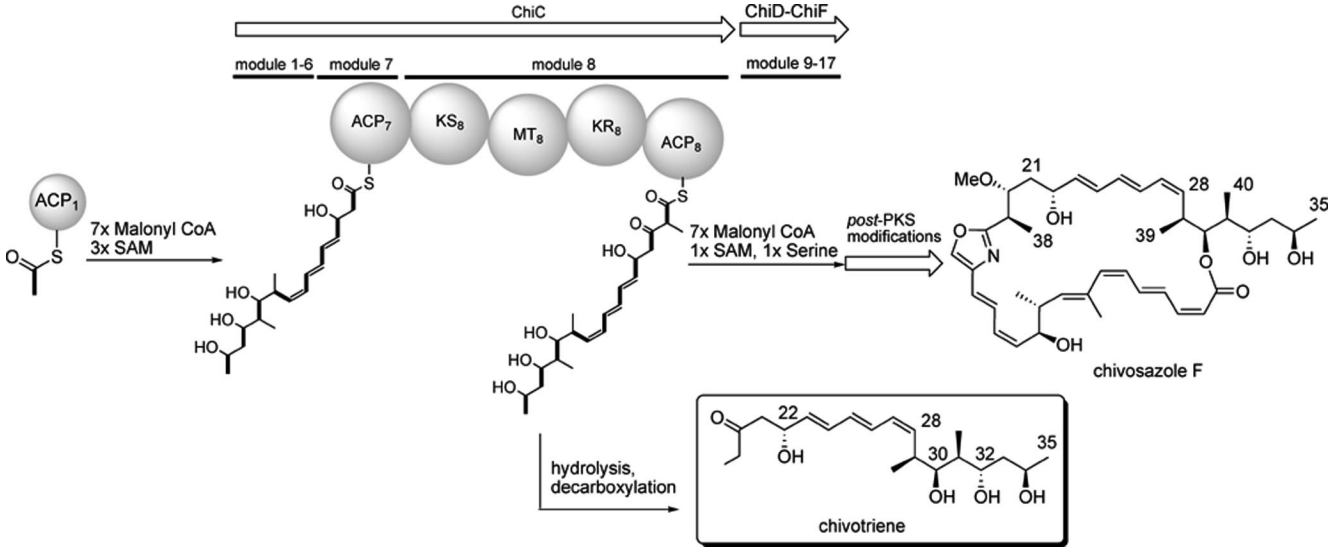

Figure 1. Proposed chivotriene biosynthesis.

Biosynthetic studies revealed that chivosazole is biosynthesized by a trans-AT polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS). The shunt product reported here is consistent with the biosynthetic hypothesis put forward by Perlova et al. and can be rationalized by hydrolysis and decarboxylation of the intermediate polyketide bound to ACP8 (Figure 1).[4]

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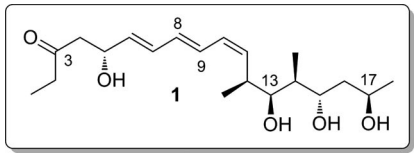
Herein we report the isolation, structure elucidation, and synthesis of the chivosazole shunt product chivotriene (**1**) together with its biological data.

Results and Discussion

Isolation

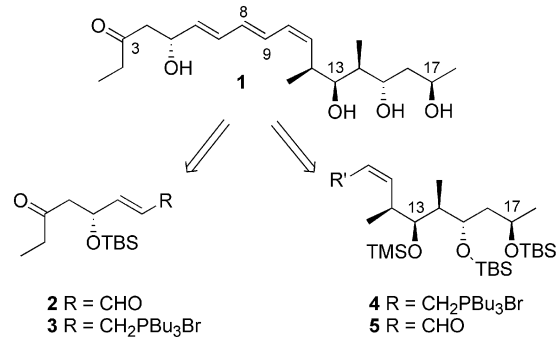
Chivotriene (**1**) was isolated by silica gel chromatography as a byproduct (1 mg) from the purification of disorazole B_2 (fraction 1.1).[5] Comparison of the NMR spectroscopic data of **1** with those of chivosazole A identified the structure from C5 to C20 including the double bond configurations of the *E*,*E*,*Z*-triene (Table 1). However, the ^{1}H NMR signal of the oxymethine at C13 no longer appeared at δ = 5.30 ppm but at δ = 3.61 ppm due to the missing acyl shift of the former lactone. The remaining ketone part (C1–C4) was assigned by ^{1}H–^{1}H COSY correlations and chemical shifts.

Table 1. NMR spectroscopic data of chivotriene (**1**) in CD_3OD [^{1}H/^{13}C: 400/100 MHz].



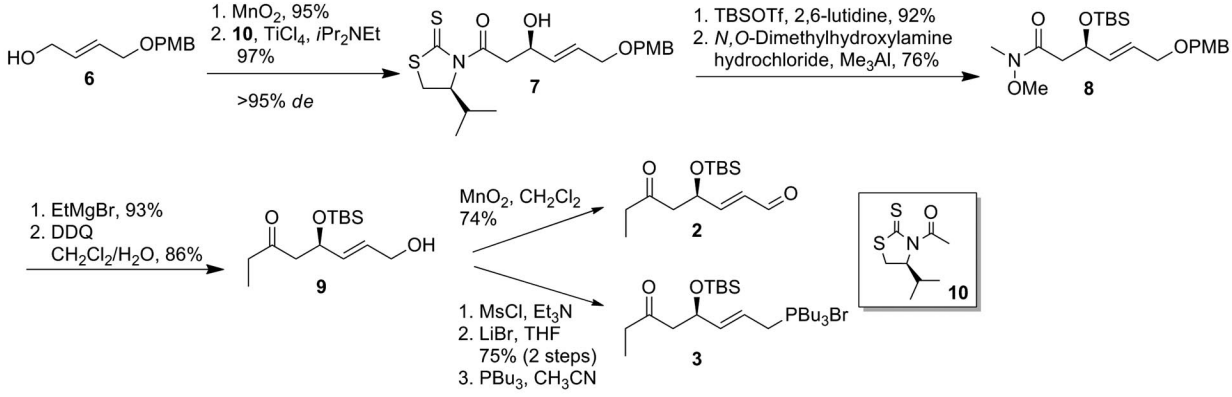
Position	Δ_C [ppm]	δ_H [ppm]
1	7.7	1.01 (t, *J* = 7.3 Hz)
2	37.6	2.52 (q, *J* = 7.3 Hz)
3	212.1	
4	50.6	2.67 (dd, *J* = 15.6, 8.2 Hz) 2.58 (dd, *J* = 15.6, 4.9 Hz)
5	69.5	4.60 (ddd, *J* = 8.2, 6.4, 4.9 Hz)
6	136.1	5.71 (dd, *J* = 15.2, 6.4 Hz)
7	131.8	6.33 (dd, *J* = 10.5, 15.2 Hz)
8	133.4	6.19 (dd, *J* = 10.5, 14.7 Hz)
9	130.1	6.58 (dd, *J* = 11.4, 14.7 Hz)
10	129.9	6.07 (dd, *J* = 11.4, 11.4 Hz)
11	136.7	5.45 (m)
12	37.1	2.92–2.82 (m)
13	76.5	3.61 (dd, *J* = 7.0, 4.1 Hz)
14	42.2	1.72–1.62 (m, 1 H)
15	71.3	3.87 (ddd, *J* = 9.0, 5.6, 4.5 Hz)
16	43.8	1.54–1.47 (m, 2 H)
17	65.5	4.01 (m)
18	24.6	1.21 (d, *J* = 6.3 Hz)
19	10.3	0.96 (d, *J* = 7.0 Hz)
20	18.5	0.95 (d, *J* = 6.8 Hz)

We started a synthetic program on the shunt product to confirm the proposed structure and in particular the configuration at C5. In our retrosynthetic disconnection we took advantage of our established route of the northern fragment of chivosazole A.[6] To achieve a convergent route we decided to perform a Wittig olefination between C8 and C9 (Scheme 1). As a result of our synthetic endeavors we realized that the correct choice of the α,β-unsaturated aldehyde and corresponding phosphonium salt on either the eastern or western fragment was pivotal for obtaining good (*E*) selectivities. Additionally, different reaction parameters were screened to avoid elimination of the TBS-protected β-hydroxy ketone.



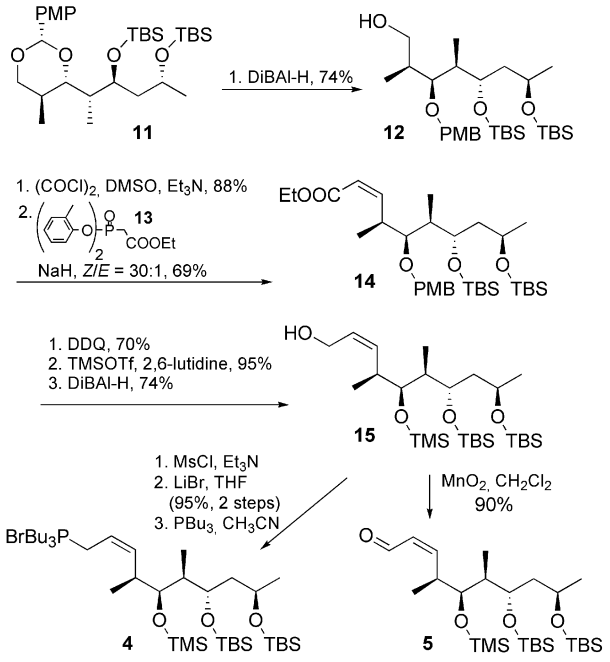
Scheme 1. Retrosynthesis of **1**.

The β-hydroxy ketone group in fragments **2** and **3** was synthesized by a diastereoselective aldol reaction by using the Nagao protocol.[7] Subsequent TBS protection and treatment with *N*,*O*-dimethylhydroxylamine in combination with trimethylaluminum lead to Weinreb amide **8**.[8] Addition of ethylmagnesium bromide and PMB deprotection installed the ethyl ketone moiety and afforded allyl alcohol **9**. Conversion to the corresponding α,β-unsaturated aldehyde **2** or phosphonium bromide **3** provided both possible segments for the Wittig olefination (Scheme 2).



Scheme 2. Synthesis of β-hydroxy ketones **2** and **3**.

Ethyl ester **14** previously synthesized[1d] was obtained after reductive opening of PMB acetal **11**, Swern oxidation, and a (*Z*)-selective olefination by using the Ando protocol.[9] However, PMB deprotection at the end of this route turned out to be problematic. Various reaction conditions led to decomposition of the starting material. Therefore, we changed the PMB protecting group to a TMS ether at an earlier stage of the synthesis. Complementary to the functionalities of ethyl ketones **2** and **3** we generated both phosphonium bromide **4** and α,β-unsaturated aldehyde **5** from alcohol **15** (Scheme 3).



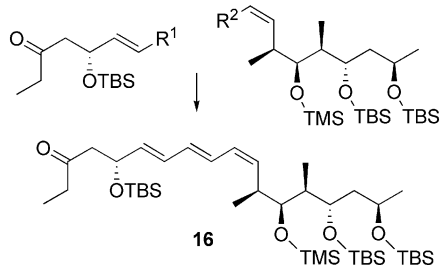
Scheme 3. Synthesis of segments **4** and **5**.

Unfortunately, initial experiments with α,β-unsaturated aldehyde **2** and phosphonium bromide **4** with the use of KO*t*Bu at 0 °C provided a 1:1 mixture of *E*/*Z* isomers and elimination product (36%). Changing the conditions such as performing the reaction at –30 °C prevented elimination but provided only a modest increases in selectivity (*Z*/*E* = 2:1). Also, changing the bases in the olefination reaction did not increase the selectivity either (Table 2).

Nevertheless, when we exchanged the functional groups of both coupling partners by employing **3** and **5** we observed an improved *E*/*Z* selectivity of 7:1 in 73% yield (Scheme 4). With the optimized reaction conditions in hand, we were able to proceed to the global deprotection of the silyl ethers. The spectroscopic data (^{1}H NMR, ^{13}C NMR, HRMS) were in all respect identical to the data of the isolated compound. As the relative and absolute stereochemistry of fragment **5** was already known from comparison of chemical degradation of chivosazole A, this also confirms the stereochemistry of C22.

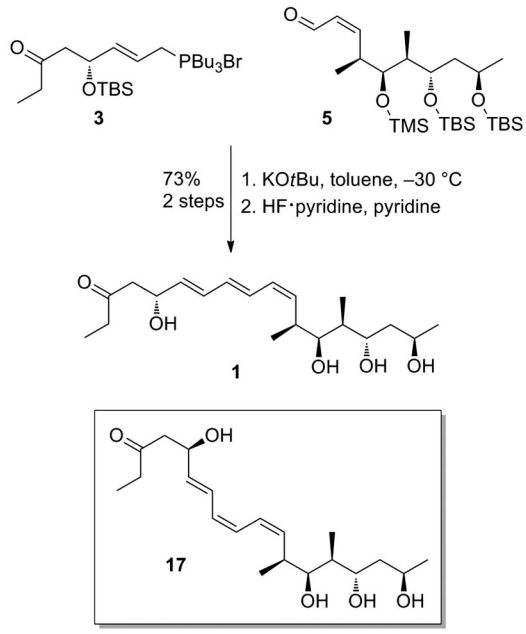
In a subsequent set of experiments, chivotriene (**1**) and its *E*,*Z*,*Z*-isomer **17** were tested with an array of different mammalian cell lines. Surprisingly, both compounds showed at least weak activities in reducing cell proliferation (Table 3). Interestingly, isomer **17** was more active than the shunt product itself. By staining F-actin of treated PtK2 potoroo and L-929 mouse cells we looked for a possible

Table 2. Wittig olefination.



Entry	Base	R^1	R^2	*E*/*Z*	*T* [°C]	Yield[a] [%]
1	KO*t*Bu	CHO	CH_2PBu_3Br	1:1	0	57, 36[b]
2	KO*t*Bu	CHO	CH_2PBu_3Br	2:1	–30	78
3	LiO*t*Bu	CHO	CH_2PBu_3Br	2:1	–30	71
4	LiHMDS	CHO	CH_2PBu_3Br	2:1	–30	80
5	KO*t*Bu	CH_2PBu_3Br	CHO	7:1	–30	73

[a] Calculated over two steps from the corresponding allyl bromide. [b] Elimination product.



Scheme 4. Endgame to shunt product **1**.

Table 3. Antiproliferative activity IC_{50} [μg/mL] of **1** and **17**. Values are means of two determinations in parallel.

Cell line	Origin	**1**	**17**
L-929	murine connective tissue	50	23
KB-3–1	human cervix carcinoma	>100	>100
PC-3	human prostate carcinoma	100	>100
U-937	human lymphoma	n.d.	25
HUVEC	human umbilical vein endothelial cells	100	25

disintegration of the actin cytoskeleton of the cells, but we could not identify any specific interference with actin filament stability.

Conclusions

We have presented the isolation and total synthesis of chivotriene, a chivosazole shunt product from *Sorangium cellulosum*. The convergent synthesis provides substantial material, which enables detailed analysis of the antiproliferative activity. The fact that this shunt product is liberated at the transition from polyketide to nonribosomal biosynthesis is noteworthy from an evolutionary point of view. In particular, the observation that the mode of action of chivotriene differs fundamentally from that of chivosazole adds additional aspects on the interpretation of the evolutionary origin of natural products.

Experimental Section

General Procedure for the Wittig Olefination followed by Global Deprotection: Base (35 μL, 35 mmol, 1 M in THF) was added to a solution of phosphonium bromide **3** or **4** (0.034 mmol, 1 equiv.) and α,β-unsaturated aldehyde **2** or **5** (0.04 mmol, 1.2 equiv.) in toluene (1.5 mL) at –30 °C. The reaction mixture was stirred for 60 min at –30 °C. Water (1 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic layer was washed with brine (5 mL) and dried with Na_2SO_4. The solvents were removed in vacuo, and flash column chromatography provided protected chivotriene **16**. The resulting mixture of *E*/*Z* isomers was directly used for global deprotection of the silyl protecting groups. HF·pyridine (2 mL) was added to a solution of the crude triene (49 mg) in THF (3 mL) and pyridine (3 mL) at room temperature. The solution was stirred for 24 h before quenching with a saturated solution of $NaHCO_3$. It was taken up in ethyl acetate and washed with phosphate buffer (pH 7) solution. The organic layer was dried with $MgSO_4$. The solvents were removed in vacuo, and the crude product was purified by flash column chromatography and HPLC (Detection at 230 nm; column: Merck 50853, LiChroCART® 250–10 LiChrospher® 100, RP-18, 10 μm; eluent A MeOH/eluent B H_2O = 55:45) to obtain chivotriene (**1**) and its isomer **17** (16 mg, 73%).

Chivotriene (1): R_f = 0.06 (5% MeOH in CH_2Cl_2). $[\alpha]_D^{23}$ = +10.0 (*c* = 0.10, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.58 (dd, *J* = 14.7, 11.4 Hz, 1 H), 6.33 (dd, *J* = 15.2, 10.5 Hz, 1 H), 6.19 (dd, *J* = 14.7, 10.5 Hz, 1 H), 6.07 (dd, *J* = 11.4, 11.4 Hz, 1 H), 5.71 (dd, *J* = 15.2, 6.4 Hz, 1 H), 5.45 (m, 1 H), 4.60 (ddd, *J* = 8.2, 6.4, 4.9 Hz, 1 H), 4.05–3.95 (m, 1 H), 3.87 (ddd, *J* = 9.0, 5.6, 4.5 Hz, 1 H), 3.61 (dd, *J* = 7.0, 4.1 Hz, 1 H), 2.90–2.79 (m, 1 H), 2.67 (dd, *J* = 15.6, 8.2 Hz, 1 H), 2.58 (dd, *J* = 15.6, 4.8 Hz, 1 H), 2.52 (q, *J* = 7.3 Hz, 2 H), 1.72–1.63 (m, 1 H), 1.57–1.44 (m, 2 H), 1.21 (d, *J* = 6.3 Hz, 3 H), 1.01 (t, *J* = 7.3 Hz, 3 H), 0.96 (d, *J* = 7.0 Hz, 3 H), 0.95 (d, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.1, 136.7, 136.1, 133.4, 131.8, 130.1, 129.9, 76.5, 71.3, 69.5, 65.5, 50.6, 43.8, 42.2, 37.6, 37.1, 24.6, 18.5, 10.3, 7.7 ppm. HRMS (ESI): calcd. for $C_{20}H_{34}O_5Na$ $[M + Na]^+$ 377.2304; found 377.2299.

***E*,*Z*,*Z*-Isomer 17:** R_f = 0.06 (5% MeOH in CH_2Cl_2). $[\alpha]_D^{23}$ = –104.3 (*c* = 0.07, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.77 (dd, *J* = 14.9, 11.1 Hz, 1 H), 6.54 (dd ≈ t, *J* = 11.4 Hz, 1 H), 6.33 (dd ≈ t, *J* = 11.3 Hz, 1 H), 6.00 (dd ≈ t, *J* = 11.1 Hz, 1 H), 5.74 (dd, *J* = 15.0, 6.2 Hz, 1 H), 5.54 (dd ≈ t, *J* = 10.4 Hz, 1 H), 4.67–4.59 (m, 1 H), 4.05–3.95 (m, 1 H), 3.89–3.81 (m, 1 H), 3.63 (dd, *J* = 7.0, 3.9 Hz, 1 H), 2.86 (dt, *J* = 9.4, 6.8 Hz, 1 H), 2.68 (dd, *J* = 15.7, 8.2 Hz, 1 H), 2.59 (dd, *J* = 15.7, 4.8 Hz, 1 H), 2.52 (q, *J* = 7.3 Hz, 2 H), 1.72–1.62 (m, 1 H), 1.57–1.44 (m, 2 H), 1.20 (d, *J* = 6.5 Hz, 3 H), 1.01 (t, *J* = 7.3 Hz, 3 H), 0.95 (d, *J* = 6.8 Hz, 6 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.0, 137.4, 137.3, 129.7, 126.4, 126.2, 124.8, 76.5, 71.4, 69.5, 65.5, 50.6, 44.0, 42.1, 37.6, 36.7, 24.6, 18.4, 10.3, 7.8 ppm. HRMS (ESI): calcd. for $C_{20}H_{35}O_5$ $[M + H]^+$ 355.2484; found 355.2469.

Supporting Information (see also the footnote on the first page of this article): Analytical and spectroscopic data of all new compounds.

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