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Synthesis and absolute configuration of hormone α1

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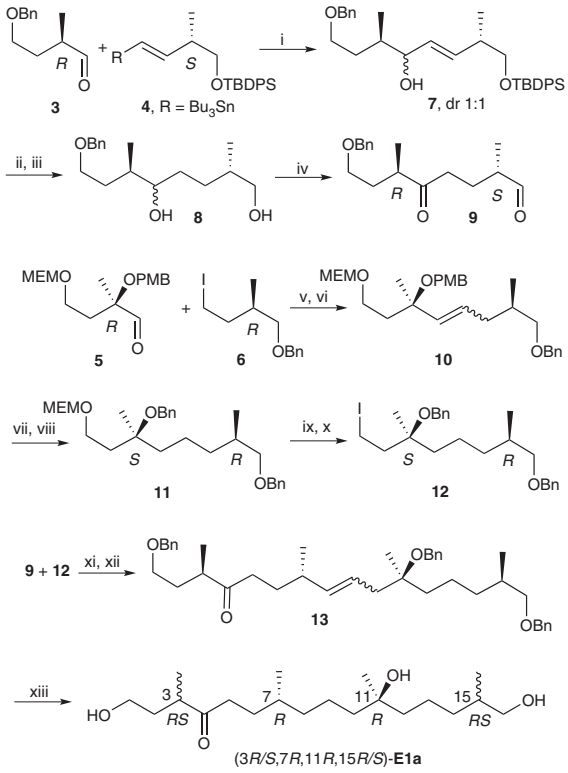
An important biological event in phytopathogens of the genus *Phytophthora* is sexual reproduction, which is conducted by two mating types, A1 and A2. A factor known as hormone α1 is secreted by the A1 mating type and induces the formation of sexual spores (oospores) in the A2 mating type. Here we describe the asymmetric synthesis and assignment of the absolute configuration of hormone α1 by oospore-inducing assays of the synthesized isomers.

Molds in the genus *Phytophthora* are some of the most destructive phytopathogens in the world. In the mid 1840s, late blight, the plant disease caused by *Phytophthora*, devastated potato crops in Europe and the United States and caused the Irish potato famine[1]. Oospores from *Phytophthora* have a doubly thick-walled structure that allows them (in the absence of a living host plant) to survive for months or years under harsh conditions, such as drying or freezing. Sexual reproduction results in increased genetic diversity, which allows the rapid spread of fungicide-resistant species[2]. In 1929, the sexual reproduction in *Phytophthora* regulated by a hormone-like compound was reported[3]. Although extensive studies have been conducted, isolation of this hormone has been difficult. Recently, the isolation of 1.2 mg of hormone α1 from 1,830 l of culture broth of *Phytophthora nicotianae* was reported, but the absolute configuration of four chiral carbons was uncertain[4]. Surprisingly, hormone α1 was found to induce oospore formation in the A2 mating types of several other species (*Phytophthora capsici*, *Phytophthora cambivora* and *Phytophthora infestans*)[4]. This indicates that hormone α1 is a universal mating hormone in the heterothallic species of *Phytophthora*.

The distinction of natural hormone α1 from its 16 diastereomers[5,6] became possible when the recent NMR study on the corresponding bis-α-methoxy-α-trifluoromethylphenylacetates (MTPAs, **2**) (**Supplementary Fig. 1** online) elucidated the C15 configuration as (*R*) and the C3 configuration as a 3:2 mixture of (*R*) and (*S*), which limited the number of possible diastereomers to four[7]. Although naturally occurring compounds are not always optically pure[8,9], we speculated that the natural product of hormone α1 might originally have a (3*R*,15*R*) configuration. Because C3 is adjacent to the carbonyl group, epimerization readily occurs during isolation of the natural product or the fermentation of *Phytophthora*.

The first synthetic effort for a nonlinear synthetic approach to the four diastereomers via Wittig condensation as a key step is summarized in **Scheme 1**, which shows the synthesis of (3*R*,7*R*,11*R*,15*R*)-hormone α1 (**E1a**) starting with chiral synthons **3**, **4**, **5** and **6** (**Supplementary Methods** online). Addition of lithium salts of **4** generated by tin/lithium exchange to **3** provided intermediate **7** as a



Scheme 1 Synthesis of (3*R*/*S*,7*R*,11*R*,15*R*/*S*)-**E1a**. Reagents and conditions: (i) *n*-BuLi, THF, –78 °C, 82%; (ii) TBAF, aq. THF, 88%; (iii) H_2, 5% Pd/C, Na_2CO_3, MeOH, 85%; (iv) $(COCl)_2$, Et_3N, DMSO, CH_2Cl_2, –60 °C, 88%; (v) Ph_3P, toluene, 110 °C, 24 h; (vi) LiHMDS, THF, –78 °C to 0 °C, then **5**, THF, –78 °C, 70% for two steps; (vii) H_2 (100 psi), 5% Pd/C, MeOH, 77%; (viii) BnBr, NaH, THF, 95%; (ix) PPTS, *t*-BuOH, 75%; (x) Ph_3P, I_2, imidazole, CH_2Cl_2, 95%; (xi) neat Ph_3P, 90 °C, 5 days; (xii) LiHMDS, THF, –78 °C to 0 °C then **9**, THF, –78 °C, 68% for two steps; (xiii) H_2, 5% $Pd(OH)_2$/C, EtOH, 72%. THF, tetrahydrofuran; Bn, benzyl; TBDPS, *t*-butyldiphenylsilyl; TBAF, tetrabutylammonium fluoride; MEM, methoxyethoxymethyl; PMB, *p*-methoxybenzyl; HMDS, hexamethyldisilazide; PPTS, pyridinium *p*-toluenesulfonate.

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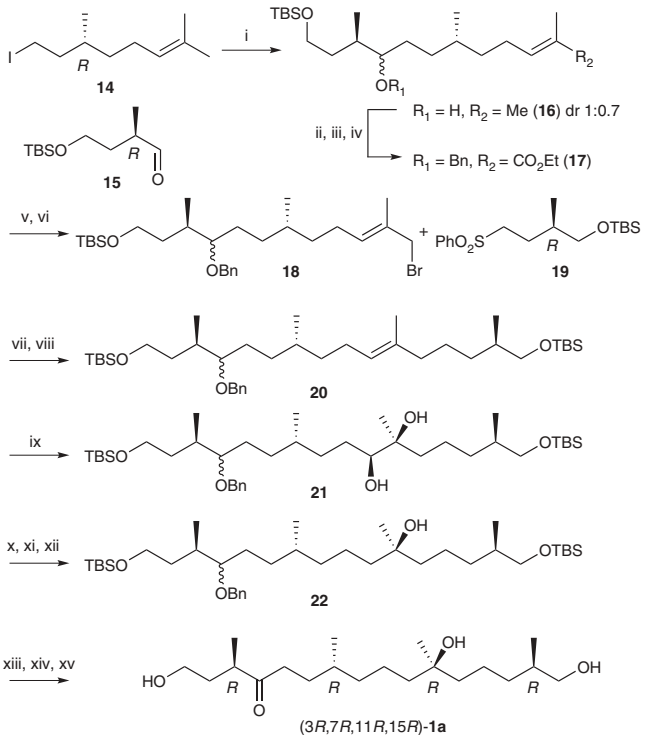

Scheme 2 Synthesis of (3*R*,7*R*,11*R*,15*R*)-**1a**. Reagents and conditions: (i) *t*-BuLi, ethyl ether, –78 °C, then **15**, ether (43% based on **15**); (ii) NaH, BnBr, Bu_4NI, DMF; (iii) O_3, CH_2Cl_2, –78 °C, then Me_2S; (iv) (carbethoxyethylidene)triphenylphosphorane, benzene, reflux (39% in three steps); (v) DIBAL, CH_2Cl_2 (92%); (vi) *n*-BuLi, MsCl, LiBr, –78 °C to 20 °C; (vii) **19**, *n*-BuLi, –78 °C, then **18**, THF; (viii) 5% Na/Hg, Na_2HPO_4, MeOH, –15 °C (72% in three steps); (ix) AD-mix-α, $MeSO_2NH_2$, *t*-BuOH, H_2O, 0 °C (88%); (x) Ms_2O, Et_3N, CH_2Cl_2, 0 °C; (xi) K_2CO_3, MeOH, (95%); (xii) DIBAL, CH_2Cl_2, hexane, –80 °C (83%); (xiii) Li, liquid NH_3, THF, –70 °C (quant.); (xiv) DMP, $NaHCO_3$, CH_2Cl_2, 0 °C (88%); (xv) TBAF, AcOH, THF (87%). DMF, *N*,*N*-dimethylformamide; DIBAL, diisobutylaluminium hydride; Ms, methanesulfonyl; DMP, Dess-Martin periodinane.

diastereomeric mixture. After removing the *t*-butyldiphenylsilyl protecting group from **7**, saturation of the double bond in the presence of Na_2CO_3 provided diol **8**. Swern oxidation of the two hydroxyl groups in **8** afforded the C1–C8 fragment **9**. Construction of the C9–C16 fragment **12** was started from **5** and **6**. After conversion of iodide **6** to its phosphonium salts by heating with Ph_3P, the resulting salts were treated with lithium hexamethyldisilazide (LiHMDS) at –78 °C, followed by addition of aldehyde **5**, which yielded olefin **10** predominately in the (*Z*) configuration. Elimination of the (*E*/*Z*) geometric difference and removal of both *p*-methoxybenzyl and benzyl protecting groups from **10** by hydrogenolysis, and reprotection of the C11 and C16 hydroxyl groups with benzyl groups, provided **11**. After removal of the methoxyethoxymethyl protecting group from **11**, iodonation of the resulting alcohol gave the C9−C16 fragment **12**.

With the C1–C8 fragment **9** and the C9–C16 fragment **12** in hand, the final task was to assemble the two fragments together through a Wittig reaction (**Scheme 1**). Reaction of iodide **12** with neat Ph_3P gave its phosphonium salt, which was then subjected to deprotonation with subequivalent LiHMDS at –78 °C, followed by addition of aldehyde **9** to furnish the C1–C16 backbone **13** as a mixture of diastereomeric and geometric isomers. Removal of the three benzyl protecting groups and saturation of the C8–C9 double bond in **13** were simultaneously accomplished by hydrogenolysis to afford the four-isomer mixture (3*R*/*S*,7*R*,11*R*,15*R*/*S*)-**E1a**. About half epimerization at C3 and one-third epimerization at C15 were observed for **E1a** on ^{1}H NMR analysis of its bis-(*S*)-MTPA ester **E2a** (**Supplementary Fig. 1**). Epimerizations probably occurred at C3 due to the use of strong bases during Wittig condensation between fragments **9** and **12** and at C15 during hydrogenolysis in conversion of **10** to **11**[10]. The other three four-isomer mixtures—(3*R*/*S*,7*R*,11*S*,15*R*/*S*)-**E1b**, (3*R*/*S*,7*S*,11*S*,15*R*/*S*)-**E1c** and (3*R*/*S*,7*S*,11*R*,15*R*/*S*)-**E1d**—were consecutively synthesized via the same synthetic route for preparation of **E1a** by changing the chiralities of synthons **4** and **5**. Although there is no direct evidence, the observed epimerization at C15 indicates that partial epimerization at C7 is also possible because similar hydrogenolysis conditions were

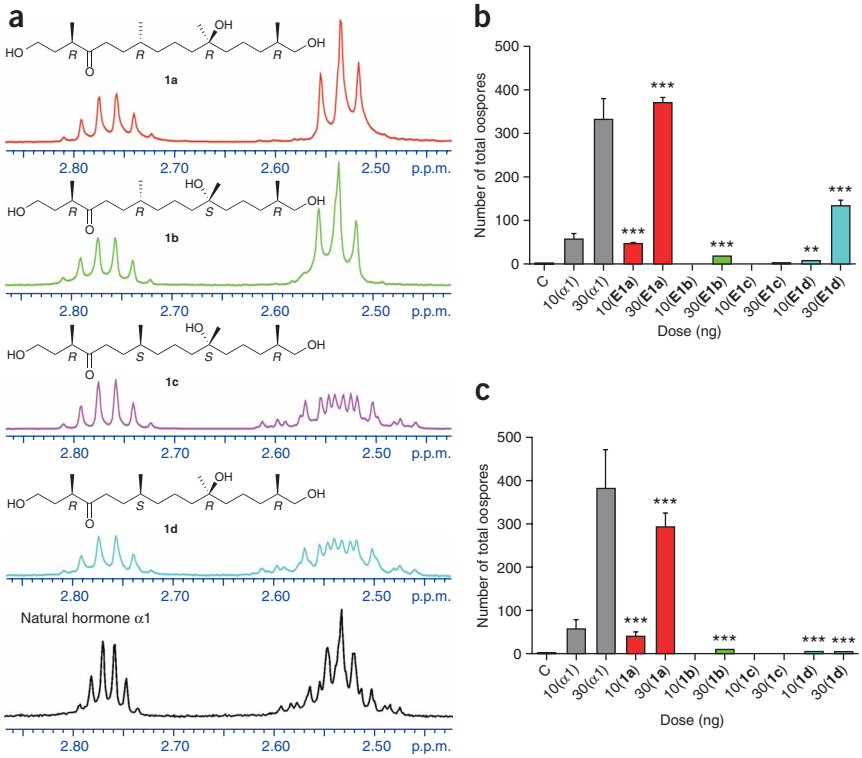

Figure 1 Physical and biological properties of the stereoisomers of hormone α1. (**a**) Key fingerprint resonance in ^{1}H NMR spectra of synthetic **1a**–**1d** recorded at 400 MHz and natural product recorded at 600 MHz. (**b**,**c**) Oospore formation in the A2 mating type of *P. nicotianae* induced by the eight synthesized isomers at doses of 10 and 30 ng disk^{-1}, in comparison with natural hormone α1 at the same doses. Values are means of three replicates ± s.e.m. ** and *** indicate significant difference from the corresponding negative control; $^{**}P < 0.01$ and $^{***}P < 0.001$. C, negative control. Two experiments were carried out in different series; oospore formation was induced by **E1a**–**E1d** (**b**) and **1a**–**1d** (**c**).

used in the conversion of **13** to **E1a**. It is reasonable to infer that the synthesized samples **E1a**–**E1d** contained some C7 epimerized components and were at least four-isomer mixtures.

NMR analysis confirmed that (3*R*/*S*,7*S*,11*S*,15*R*/*S*)-**E1c** readily equilibrates into its hemiacetal isomer (**Supplementary Fig. 2** online). The hemiacetal isomer was suspected to be the unknown trace component accompanying hormone α1 and its diastereomers[4,6]. At higher concentrations (30 mg ml^{-1}), the linear **E1c** gradually cyclized to its hemiacetal isomer until trace amounts of **E1c** were detected after 48 h in methanol-d_4 at 20–25 °C. The hemiacetal isomer was quantitatively converted back to **E1c** by treatment with silica gel in ethyl acetate for 16 h or by treatment with a 1:1 mixture of methanol-d_4 and D_2O for 22 h.

After obtaining the four-isomer mixtures **E1a**–**E1d**, our synthetic efforts were directed to the preparation of four optically pure hormone α1 samples (**1a**–**1d**) with the fixed 3*R* and 15*R* configurations. Because the synthetic route shown in **Scheme 1** exclusively afforded the C3 and C15 epimerized mixture of diastereomers, a new synthetic route had to be developed. A linear synthetic approach to the four optically pure diastereomers **1a**–**1d** using Sharpless asymmetric dihydroxylation as a key reaction is shown in **Scheme 2**, which depicts the synthesis of (3*R*,7*R*,11*R*,15*R*)-**1a**. Using this approach, epimerizations of the final product at C3 and C15 are completely avoided. Halogen-metal exchange of (*R*)-citronellyl iodide (**14**) with *t*-BuLi, followed by coupling with aldehyde **15**, afforded **16** as a diastereomeric mixture (about 1:0.7). After protection of the hydroxyl group in **16** with a benzyl group, oxidative cleavage of the double bond and Wittig condensation of the resulting aldehyde with (carbethoxyethylidene)-triphenylphosphorane afforded **17**. After reduction of **17**, the resulting alcohol was converted to allylic bromide **18**. Coupling of **18** with known sulfone **19**, followed by desulfonation, afforded **20** with the full carbon skeleton of hormone α1. The final task in the total synthesis of **1a** was the stereoselective introduction of a tertiary hydroxyl group at C11 by using a Sharpless asymmetric dihydroxylation-deoxygenation process. Thus, stereoselective dihydroxylation[11] of **20** with AD-mix-α gave diol **21** with a 95:5 diastereomeric ratio (dr). Monomesylation of **21**, demesylation with K_2CO_3 and regioselective reduction of the epoxy ring with diisobutylaluminium hydride gave tertiary alcohol **22**. Removal of the benzyl group, followed by Dess-Martin oxidation of the resulting alcohol and removal of the two *t*-butyldimethylsilyl (TBS) groups under mild conditions afforded optically pure (3*R*,7*R*,11*R*,15*R*)-**1a**. Diastereomers (3*R*,7*R*,11*S*,15*R*)-**1b**, (3*R*,7*S*,11*S*,15*R*)-**1c** and (3*R*,7*S*,11*R*,15*R*)-**1d** were successively synthesized in a similar fashion. The stereochemical purities of **1a**–**1d** at C3 and C15 were confirmed by ^1H NMR analysis of their corresponding bis-(*R*)-MTPA esters **2a**–**2d** (**Supplementary Fig. 3** online).

Very small differences among synthesized samples were observed by ^{13}C NMR (**Supplementary Methods** and **Supplementary Tables 1** and **2** online). However, on ^1H NMR spectra, the signals around 2.5 p.p.m. (H5) showed substantial differences (**Fig. 1a** and **Supplementary Tables 3** and **4** online). The signals of the 3,7-*anti* isomers (**1a** and **1b**) were observed as triplets, whereas those of the 3,7-*syn* isomers (**1c** and **1d**) were observed as multiplets. Thus, the *anti* and *syn* isomers of the C3 and C7 diastereomers are distinguishable on ^1H NMR analysis. The C3 epimerized mixtures **E1a**–**E1d** showed ^1H NMR spectra with mixed *syn* and *anti* isomer signals, which were very similar to that of the natural hormone α1 (**Supplementary Fig. 4** online). Thus, it was difficult to distinguish the natural hormone α1 from the synthetic samples. Consequently, determination of the absolute configurations of natural hormone α1 by NMR analysis alone is impossible for this linear diterpene.

Therefore, the oospore-inducing activities of the eight synthesized isomers **E1a**–**E1d** and **1a**–**1d** were tested in comparison with natural hormone α1. The isomers (3*R*/*S*,7*R*,11*R*,15*R*/*S*)-**E1a** and (3*R*,7*R*,11*R*,15*R*)-**1a** induced significant oospore formation ($P < 0.001$) in the A2 mating type of *P. nicotianae* at a dose of 10 ng, and the number of oospores increased dose dependently, which is very similar to the behavior of the natural hormone α1 (**Fig. 1b**,**c**). However, no noteworthy oospore formation was induced by **E1b**–**E1c** and **1b**–**1c** at a dose of 10 ng (**Fig. 1b**,**c**). Oospore formation induced by (3*R*/*S*,7*S*,11*R*,15*R*/*S*)-**E1d** was observed at a dose of 30 ng (**Fig. 1b**), presumably as a result of partial epimerization of **E1d** at C7 during synthesis. These results indicate that the natural hormone α1 has the (3*R*,7*R*,11*R*,15*R*) absolute configuration.

To our knowledge, we have achieved the first asymmetric total synthesis of four four-isomer mixtures (**E1a**–**E1d**) and four optically pure diastereomers (**1a**–**1d**). The absolute configuration of natural hormone α1 was determined to be (3*R*,7*R*,11*R*,15*R*) by direct comparison of the biological activities of the eight synthesized samples with natural hormone α1. Although it is not clear whether the natural product was biosynthesized with pure stereochemistry at C3, the major product should have (3*R*) stereochemistry. We cannot conclude whether the (3*S*) epimer is bioactive, as the bioassay is not quantitative and the C3 position epimerizes easily. The remaining three stereogenic centers are certainly important in terms of the hormonal activity of *Phytophthora*. This indicates that a stereospecific receptor may exist at the beginning of the signal transduction cascade resulting in oospore formation. More than 70 years after the first proposal of the existence of the hormone-like compound[3], we have succeeded in establishing the complete structure of *Phytophthora* hormone α1.

Note: Supplementary information and chemical compound information is available on the Nature Chemical Biology website.

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AUTHOR CONTRIBUTIONS

X.Z. and N.K. contributed equally to this work. Y.Q., X.Z., X.X., J.W., D.Z. and Y.W. synthesized hormone α1 isomers **E1a**–**E1d**, and A.Y. and N.K. synthesized hormone α1 isomers **1a**–**1d**. J.Q. and T.A. carried out the bioassays. A.Y., Y.Q., T.N., G.Y., J.Q. and Y.S. designed the project and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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