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Synthesis and spectroscopic analysis of a stereoisomer library of the phytophthora mating hormone α1 and derived *bis*-Mosher esters

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Abstract

Fluorous mixture synthesis provided all eight diastereomers of the *phytophthora* hormone α1 with the *R* configuration at C11 as individual samples after demixing and detagging. The library of all possible *bis*-Mosher esters (16) was then made by esterification. Complete sets of ^{1}H, ^{13}C and (for the Mosher esters) ^{19}F NMR spectra were recorded, assigned, and compared with each other and with published spectra. Not all of the spectra are unique, and the ^{1}H NMR spectra of the Mosher esters provided the most information. The previous assignment of the natural sample as "all-*R*" stereoisomer mixed with its 3*S*-epimer was confirmed.

Introduction

The power of modern NMR spectroscopy to characterize small organic molecules has increased to such a level that Saielli and Bagno dared to ask the provocative question in a 2009 article entitled: "Can two molecules have the same NMR spectrum?"[1] Their short answer was "no".[2] Saielli and Bagno were comparing isomers, and perhaps they were thinking primarily about constitutional isomers. Because there are plenty of diastereoisomers that have the same NMR spectra.

We have been making libraries of natural product stereoisomers by fluorous mixture synthesis[3] with the immediate goals of structure assignment and SAR. In the larger picture, the various libraries of spectra pose questions of how to decide when spectra are the same. They also begin to give a feeling for when spectra of diastereomers are likely to be the same and when they are not. At one extreme, natural products like murisolins (Figure 1) with remote stereocenters pose big problems because many diastereomers have substantially identical spectra.[4] For example, each of the 32 diastereomers of murisolin exhibits one of only six different ^{1}H NMR spectra. There are 64 Mosher ester diastereomers derived from the murisolins, but even here only 16 of these exhibit a unique spectrum. The other 48 diastereomers come in 24 pairs with identical spectra. With murisolins, having the same spectrum is the rule, not the exception.

At the other extreme, natural products like passifloricin with nearby stereocenters can be analyzed straightforwardly. Each of the eight diastereomers of passifloricin has a unique ^{1}H and ^{13}C NMR spectrum.[5] This means that spectra of synthetic and natural samples, for example, can be compared directly to give an unambiguous yes/no answer about identity.

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Supporting Information: Contains complete experimental details, tabular NMR data, supplemental Figures and copies of NMR spectra of the hormone and Mosher ester libraries.

Here we describe the synthesis and analysis of the complete stereoisomer library of the *phytophthora* α1 mating hormone **1** (Figure 2) and the derived Mosher esters. With four stereocenters separated by three carbon atoms each, the disposition in **1** falls roughly in between the remote stereocenters of murisolin and the nearby ones of passifloricin. We challenge readers with the question of Saielli and Bagno: can any two of the eight diastereomers of **1** have the same ^{1}H or ^{13}C NMR spectrum? Further, a *bis*-Mosher ester forms at C1 and C16 of **1**. Can any of the resulting sixteen diastereomers have the same ^{1}H, ^{13}C or ^{19}F NMR spectrum? Pause to make your assessment, then read the rest of the paper.

Hormone α1 is the universal mating hormone of heterothalic (sexually reproducing) species of *Phytophthora*.[6] These hardy, fungi-like species are among the most destructive plant parasites, causing diseases of worldwide importance.[7] For example, the late blight of potato, caused by *Phytophthora infestans*, resulted in the Irish potato famine during the mid-19th century.

In 2005, Ojika and coworkers first isolated 1.2 mg of hormone α1 containing “unknown impurities” from 1830 L of cultural broth of the A1 mating type of *P. nicotianae*.[6] The two-dimensional structure of the hormone α1 was shown to be 1,11,16-trihydroxy-3,7,11,15-tetramethylhexadecan-4-one (**1**, Figure 2).

Yajima and coworkers quickly confirmed the 2D-structure of **1** by intentionally synthesizing a mixture containing all 16 possible stereoisomers.[8] Yajima commented that this mixture looked like a single compound by ^{1}H and ^{13}C NMR spectroscopy. In turn, this spectrum matched that of the natural sample. This suggests that all eight isomers have the same spectra, which turns out not to be the case.

In 2007, we made isomers of **1** and learned that the extraneous peaks in the spectra of the natural sample derived not from impurities *per se*, but from minor hemiacetal forms in equilibrium with **1**.[9] Also, we and Ojika both showed by different means that the isolated sample was a mixture of two epimers at C3. By using Mosher esters, Ojika proposed that hormone α1 was (15*R*) and a 3/2 mixture of (3*R*/3*S*).[10]

By testing a series of biased stereoisomer mixtures on *phytophthora* oospores (spores) in 2008, Yajima was able to show that the natural product had the (11*R*) configuration.[11] The first phase of this work was complicated because epimerization occurred at several stereocenters during the synthesis, so the stereoisomer composition of the tested samples could only be estimated. However, Yajima then made all four possible isomers with the (11*R*) configuration and showed that only one, (3*R*,7*R*,11*R*,15*R*)-**1**, was active in the oospore assay. Loh and coworkers have also recently reported a synthesis of the “all-*R*” isomer of **1**.[12]

At the same time as Yajima, Feringa made two stereoisomers of **1**, (3*S*,7*S*,11*S*,15*S*)-**1** (“all-*S*”-**1**), and its C7 epimer, (3*S*,7*R*,11*S*,15*S*)-**1**, by using his asymmetric conjugate addition method, and observed that both samples were active in carefully controlled oospores assays.[13] According to Yajima’s work, these isomers should not have been active. However, it is difficult to compare the results of such functional assays across laboratories.

Because of the discrepancies in assays and problems with epimerization, the stereostructure of **1** should be confirmed by spectroscopic or analytical means. Samples of the natural hormone are highly precious, but copies of its ^{1}H and ^{13}C NMR spectra are available along with ^{1}H NMR spectra of its *bis*-(*R*)- and *bis*-(*S*)-Mosher esters.[6] These are the basis for comparison and assignment.

Here we report the fluorous mixture synthesis of all diastereomers of **1** with the *R* configuration at C11. In turn, each of these was converted to its *bis*-(*R*)- and *bis*-(*S*)-Mosher esters.[14] The comparison of the spectra of these two complete stereoisomer libraries (eight isomers of **1** and 16 *bis*-Mosher esters) is an interesting exercise in deciding whether similar spectra are the same or different. We confirm that the natural sample of **1** is a 3/2 mixture of (3*R*,7*R*,11*R*,15*R*)-**1** and its (3*S*) epimer (along with some hemiacetals). Taking into account Ojika's reasonable assertion that isomerization occurred at C3 during isolation,[10] the hormone α1 is (3*R*,7*R*,11*R*,15*R*)-**1** as proposed by Yajima.

Results and Discussion

Synthetic plan

Our first generation synthesis of isomers of **1** capitalized on the latent symmetry of the fragment spanning carbons C6 to C16.[9] Strategically, this led to problems in controlling the configuration at C11. Tactically, we learned that epimerization at C3 was facile. We designed the revised synthetic plan shown in Figure 3 both to address these issues and to accommodate the needed fluorous tags. Yajima's testing of stereoisomer mixtures and individual samples pointed firmly to the 11*R* configuration,[11] so we fixed that stereocenter and made all eight possible diastereomers by varying the other three stereocenters.

We expected that reduction, demixing and detagging of **M2**[15] would provide the eight target isomers. A Kocienski-Julia olefination[16] couples **M3** and **M4** to provide **M2**. In the tagging plan, left fragment **M3** will be made as two mixtures of two quasiisomers[17] with either the 7*S* or 7*R* configuration fixed. The configuration at C3 is encoded by the fluorous PMB (^{F}PMB) group on O1, as indicated at the bottom of Figure 3. Right fragment **M4** will be made as one mixture of two quasiisomers with a fluorous PMB group encoding the configuration at C15 and with the *R* configuration at C11. The pairwise coupling of (7*S*)-**M3** and (7*R*)-**M3** with **M4** then provides two corresponding mixtures of **M2**, now of four quasiisomers each, ready for reduction, demixing and detagging.

Syntheses of the fragments M3 and M4

The preparation of phenyltetrazolyl sulfone quasiisomers (7*S*)/(7*R*)-**M3** is shown in Scheme 1. Myers reagent (*R*,*R*)-**5**[18] was alkylated with iodo PMB ether **6a** bearing a CF_3 group to give (3*S*)-**7a**, while its quasienantiomer (3*R*)-**7b** (not shown) was made from (*S*,*S*)-**5** and the reagent **6b** bearing a C_4F_9 group.[19] Mixing of **7a** and **7b** and reduction with lithium amidoborohydride[20] gave alcohol **M8a/b** in 87% yield, then Swern oxidation[21] provided aldehyde **M9a/b** (72%). As expected,[22] these and other quasienantiomers behaved like true enantiomers on silica gel and chromatography by ^{1}H or ^{13}C NMR analyses. But they were readily separated or analyzed by fluorous HPLC or ^{19}F NMR spectroscopy.

To assess stereopurity prior to the upcoming addition reaction, a small sample **M9a/b** was reduced back to the corresponding alcohol. The resulting quasienantiomers **M8a/b** were easily demixed, then converted to the Mosher esters (see Supporting Information). Analysis of the ^{1}H NMR spectra as usual[14] showed that each sample had about a 94/6 enantiomer ratio. This ratio presumably reflects the diastereoselectivity in the Myers alkylation.

Next, dibromides (*R*)-**10** and (*S*)-**10**[23] were prepared in three steps from the Roche ester (see Supporting Information). That these fragments were enantiopure was later confirmed by analysis of the final products. Metalation of (*R*)-**10** and addition of fragment **M9a/b** provided alcohol (7*R*)-**M11a/b** in 100% crude yield.[24] The sample appeared to be a 1/1 mixture of isomers at C4 (by ^{1}H NMR analysis), but this is inconsequential since this center will be oxidized at the end. Removal of the TBS group and installation of the phenylsulfonyl tetrazole under standard conditions[25] gave (7*R*)-**M3a/b** in three steps. Likewise the complementary quasiisomer mixture (7*S*)-**M3a/b** was made starting from (*S*)-**10** in comparable yields.

Scheme 2 shows the synthesis of tagged quasienantiomers **M13b/c** bearing the C15 stereocenter. (2*S*) Methyl 3-hydroxy-2-methyl propanoate (Roche ester) was tagged with a fluorous PMB group bearing a C_4F_9 label to provide (*S*)-**12b**. Its quasienantiomer (*R*)-**12c** was prepared analogously from (*S*)-Roche ester and the C_6F_{13}-labeled PMB reagent (see Experimental section). Mixing to make a quasiracemate, then reduction with DiBAL provided a primary alcohol in 98% yield. Standard Mitsunobu coupling with 1-phenyl-5-thiotetrazole (91% yield) followed by molybdate-catalyzed peroxide oxidation provided phenyl sulfonyltetrazole **M13b/c** in 90% yield.

The synthesis of fragment **M4b/c** is summarized in Scheme 3. Alkene **14**, readily available in four steps from butyn-4-ol (see Supplementary Information), was subjected to Sharpless asymmetric epoxidation[26] to give (*S*,*R*)-**15** in an enantiomer ratio of about 92/8 according to Mosher ester analysis. Reduction of **15** by $LiAlH_4$ provided a diol in 65% yield. This was *bis*-silylated with TESOTf (90%), then direct Swern oxidation provided aldehyde (*R*)-**16** in 77% yield.

To complete the right fragment **M4b/c**, Kocienski-Julia coupling of the quasienantiomers **M13b/c** with the single enantiomer (*R*)-**16** provided alkene **M17b/c** as an 80/20 mixture of *E*/*Z* isomers in 83% yield after flash chromatography. The TBS and TES groups were removed with TBAF, then resilylation with TESOTf provided a *bis*-silyl ether that was directly oxidized by the Swern method (77%) to give aldehyde **M4b/c**.

Synthesis and separation of the stereoisomer library

The completion of the synthesis is illustrated in Scheme 4 starting with the 7*S* series quasiisomers **M3a/b**. Kocienski-Julia coupling of this free alcohol and **M4b/c** mediated by 2 equiv NaHMDS provided the full carbon skeleton **M18a/b,b/c** but only in an unacceptable 35% isolated yield. In contrast, silylation of **M3a/b** with TESOTf followed by coupling with **M4b/c** and 1 equiv of NaHMDS provided **M19a/b,b/c** in 87% yield. The C8–C9 alkene is again presumably formed as a mixture of isomers, but overlapping in the alkene region of the ^{1}H NMR spectrum of **M19** prevented assessment of the ratio. It is again remarkable that **M19** exhibits a single spot on TLC analysis and can be readily purified by flash chromatography. Consider that this sample is probably a mixture of 32 compounds: four quasiisomers, plus two stereoisomers at C4, plus *E*/*Z* isomers at both alkenes.

Following desilylation with 2N HCl (98%), all of the true-isomer features of the mixture were removed by diimide reduction to saturate the alkenes and alkyne (89% yield), then DMP oxidation of the C4 alcohol to give ketone **M20** (92%). The ^{1}H NMR spectrum of this mixture was now easily interpretable, and the ^{19}F spectrum showed resonances characteristic of the expected 1/1/1/1 mixture of the four quasiisomers. Likewise, starting from (7*R*)-**M3a/b**, the mixture of the four quasiisomers **M20** bearing the (*R*) configuration at C7 was produced.

Demixing of the quasiisomer mixtures by preparative HPLC over FluoroFlash silica gel proceeded smoothly. About 200 mg of each quasiisomer was produced, and each of these samples was demixed in 40 mg injections. A typical preparative chromatogram is shown in Figure 4. The quasiisomer eluted in order of increasing fluorine content was confirmed by MS and ^{19}F NMR spectroscopic analysis of the pure samples. The overall mass recovery of the preparative HPLC purifications exceeded 80%.

Based on the enantiomer purities of the precursors, quasiisomers **20** should be >80% isomerically pure, with small amounts of epimers at C3, C11 and C15. Careful inspection of the ^{1}H NMR spectra of the eight pure fluorous-tagged samples yielded only one tidbit of information about isomeric purity. Expansions of the ^{1}H NMR spectra of the four quasiisomers **20** in the (7*S*) series are overlaid in Figure 5. Like those of the natural product isomers (see below), these spectra reveal the C3–C7 *syn*/*anti* ratio. The diastereotopic methylene group at C5 appears as a 2H triplet at about 2.45 ppm for the *syn* isomers (top two spectra), but as two doublets of doublets of doublets at about 2.48 and 2.70 ppm (bottom two spectra) for the *anti* isomers. Careful expansion and integration suggested each sample contained 10–12% of its epimer at this pair of centers. Only about 5% was expected based on the enantiomeric purity of the relevant precursors. This suggests that despite our best efforts, some epimerization still occurred at C3. This suggestion was soon confirmed by the Mosher analysis.

After surveying several conditions, we settled on hydrogenolysis for the removal of the PMB groups of **20**. In a typical experiment (Scheme 5), (3*R*,7*S*,11*R*,15*S*)-**20b,c** was dissolved in EtOAc and exposed to palladium on carbon under a balloon of hydrogen. After 2 days, the sample was filtered through Celite, then the product was carefully purified by automated flash chromatography to give 8 mg of the corresponding stereoisomer of **1** in 62% yield.

Likewise, the other seven samples were processed to give 4–9 mg of the corresponding final samples of 1 (62–85% yields after careful purification). These results are summarized in Table 1, which is also a handy reference for the tag-encoding pattern of the final products before detagging. The complete stereostructures of all eight diastereomers of **1** are shown in Supplementary Figure S1.

Spectral comparison of samples of 1 and derived Mosher esters

With the eight isomers of **1** in hand, we first compared the spectra of the samples to each other, then to the natural sample. In this way, we know which spectra are different and which are the same, and therefore what firm conclusions can be drawn about structure.

The complete set of ^{1}H (700 MHz) and ^{13}C (175 MHz) spectra of all eight isomers of **1** in CD_3OD tabulated in the Supporting Information, Tables S1–S3, and copies are also provided. The ^{1}H NMR spectra (Supporting Figure S4) group into two pairs of four compounds with substantially identical spectra. The only difference between these groups is the resonances for H5. Figure 6 shows the H5 resonances for representative isomers with C3,C7-*anti* and C3,C7-*syn* configurations. In the *anti* isomer, the two protons resonate together as a triplet at 2.55 ppm, while the *syn* isomer exhibits a broader, more complex multiplet (presumably two ddd's) from 2.60–2.48 ppm. Again, epimerization at C3 is evident and was estimated at 15–20% for the *anti* isomers. Presumably it is similar for the *syn* isomers, but overlapping prevents estimation in that series.

The ^{13}C NMR spectra of all eight isomers are very similar. Most resonances fall in a range of about 0.01–0.02 ppm, though some (notably C10/12 and C18) show somewhat larger differences in some pairs of isomers. However, even though we know that the samples are not isomerically pure, we did not observe the clear doubling of any resonance in any of the spectra. Accordingly, comparison of ^{13}C NMR resonances is not a reliable tool to differentiate isomers.

With these spectra, we can now understand why Yajima's spectra of the sixteen-isomer mixture match the spectra of the natural product. The ^{13}C NMR spectra are so similar that the spectrum of the natural sample with two isomers looks the same as the spectrum of a sample with all the isomers. For the ^{1}H NMR case, the natural sample contains one C3,C7-*syn* isomer and one *anti* isomer. Thus it has one representative of each of the two possible ^{1}H NMR spectra from the stereoisomer mixture, and therefore looks like the spectrum of the complete mixture.

Each of the eight isomers of **1** was converted to both *bis-R* and *bis-S*-Mosher esters to obtain a 16-stereoisomer library of the *bis*-Mosher esters of hormone α1. In the typical esterification reaction (Scheme 5), a solution of (3*S*,7*S*,11*R*,15*R*)-**1** in DCM was treated with DCC and (*S*)-MTPA acid. The product was purified by flash column chromatography to obtain 72% of the *bis*-MTPA ester (3*S*,7*S*,11*R*,15*R*)-**21***S*. The other fifteen *bis*-Mosher esters were made similarly, and their structures and ^{1}H NMR spectra are shown in the Supporting Information.

The ^{1}H NMR spectra of the Mosher esters were much more informative than the spectra of the starting compounds. Regarding isomer purity, the H5 protons are now separate ddd's in each isomer (see Figure 8 below), so both the identity and the amount of isomer contamination could be assessed. Because we have all diastereomers of the Mosher esters, minor resonances in one sample must always match the major resonances in another. Indeed, each Mosher ester had a significant set of minor resonances that matched those of its C3 epimer. The amount of impurity (18–34%) was more than expected (15–20%) for most isomers, suggesting that additional small amounts of epimerization may have occurred during Mosher ester formation. Table 2 summarizes the yields and purities of the Mosher ester library.

Mosher esters are usually used for spectroscopic analysis and not diastereomeric separation. Nonetheless, we subjected the *bis-S*-MTPA ester *(3S,7S,11R,15S)*-**21***S* to purification by semi-prep HPLC with a chiral (*S*,*S*)-Whelk-O column eluting with 97:3 hexanes/2-propanol. The HPLC chromatogram from a 5 mg injection is shown in Figure 7. The major isomer eluted at 52.2 min and the minor C3 epimer eluted at about 52.5 min as a shoulder to the major peak. Because, of the considerable overlap, peak shaving was conducted to obtain several fractions.

The first fraction contained 1.2 mg of essentially pure (3*S*,7*S*,11*R*,15*S*)-**21***S* (>95%) as assessed by ^{1}H NMR spectroscopy. Likewise we subjected the remaining 15 Mosher ester samples to semi-preparative HPLC purification with peak shaving to sacrifice quantity for quality (isomeric purity). The results of these experiments are also summarized in Table 2.

Remarkably, we obtained highly isomerically enriched fractions (>95%) of the major *bis*-Mosher ester from thirteen of the sixteen samples. In two of the three other cases, partial enrichment was observed. These imperfections may have been due to peak shaving problems; however, because we now had the spectrum of the pure minor isomer in each of the three contaminated samples (it was the major isomer of another sample), it was now easy to subtract away the minor resonances and assign the remaining ones. In this way, the resonances of all 16 ^{1}H Mosher NMR spectra (700 MHz) were assigned with the aid of ^{1}H-^{1}H COSY experiments. Likewise, we recorded the complete set of sixteen ^{19}F NMR spectra at 282 MHz.

The full set of ^{19}F NMR spectra are shown in the Supporting Information Figure S7. Each of the sixteen isomers exhibited one of two principal types of ^{19}F NMR spectra. In the eight isomers with the absolute configurations of the Mosher ester at C1 and C3 “matched” (both R or both S), there were two peaks of equal intensity at about −72.47 and −72.53 ppm. In the other eight isomers where these configurations were “mismatched” (one R, the other S), there was a single peak at about −72.53 ppm. The chemical shifts are not identical in all the isomers, but the variations are small (<0.03 ppm).

Apparently then, the chemical shift of the Mosher ester CF_3 group on C16 is about the same (−72.53 ppm) in all isomers. In half of the cases, this resonance overlaps with the resonance of the C1 Mosher ester (one peak is seen) and in the other half it does not (two peaks are seen). Surprisingly, it is the Mosher ester that is further from its stereocenter (C1/C3) that exhibits different ^{19}F chemical shift, not the Mosher ester that is closer to its stereocenter (C16/C15).

The ^{1}H NMR spectra of the Mosher esters provide much information, including the absolute configurations at C3 and C15 and the relative configuration of C3/C7. At 700 MHz, the C5 protons in all the Mosher esters appeared as two well resolved ddd in all cases. The relative configuration of C3/C7 could be read from the difference in chemical shift between the two C5 protons. In the anti isomers these differed by 0.08–0.09 ppm, while in the syn isomers the difference was 0.15–0.16 ppm. Figure 8 shows one representative example of each case. Supplementary Table S4 list chemical shifts of H5 for all the isomers.

The region 4.3–4.1 ppm contains the protons adjacent to the Mosher esters, and is very diagnostic. The configuration at C3 can be read from the protons on C1, while the configuration at C15 can be read from the proton resonances on C16. One spectrum each of the four possibilities here is shown in Figure 9, while the full set of sixteen expansions are in the Supporting Information, Figure S9. When the Mosher ester and C3 configuration are matched, the H1 signals are well resolved as a tdd and a ddd (see spectra 2 and 3). When the configurations are mis-matched, these resonances are coincident and a simple 2H triplet results (see spectra 1 and 4).

The C16 methylene protons always appear as doublets of doublets, but with different $\Delta\delta$. When the Mosher ester configuration and the C15 configuration are matched, the $\Delta\delta$ of the two H16 is 0.17 ppm (spectra 2 and 4), while when the configurations are mismatched, the $\Delta\delta$ of the two H16 is 0.03 ppm (spectra 1 and 3).

With hindsight, we can see that these features (C3 and C15 absolute configuration, C3/C7 relative configuration) are largely mutually independent, meaning that it would have been possible to make as few as four Mosher esters. “Cutting and pasting” of the relevant sections of these four spectra would have made good approximations of the missing twelve spectra.

Unfortunately, the Mosher ester spectra do not provide information about the configuration at C11. In other words, the sixteen ^{1}H NMR spectra of isomers **21** come in eight substantially identical pairs. The members of each pair have the same configurations at the Mosher ester, C3, C7 and C15, but the opposite configuration at C11.

To learn if there were any meaningful differences in the ^{13}C NMR spectra of the *bis*-Mosher esters, we recorded and compared 1D ^{13}C and 2D ^{1}H-^{13}C HMQC spectra of (3*R*,7*R*,11*R*,15*R*)-**21***R* and (3*S*,7*S*,11*R*,15*S*)-**21***S*. These spectra, shown in the Supporting Information, were also substantially identical and no differences were seen in the non-overlapping peaks or cross-peaks.

We also recorded ^{1}H NMR spectra of several pairs of C11 epimeric Mosher esters in C_6D_6, but again could not find clear differences. Thus, it is not currently possible to differentiate C11 epimers of either the natural product or any of its *bis*-Mosher esters by ^{1}H, ^{13}C or ^{19}F NMR spectroscopy.

With the detailed understanding of the Mosher esters provided by the complete library of spectra, we are now in position to assess the published spectra of other synthetic and natural samples, and to confirm the structure of the natural product. Dr. Ojika kindly provided the original FID of his ^{1}H NMR spectrum of the *bis*-(*R*)-Mosher ester of the natural hormone,

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and the overlay of this spectrum with two members of the Mosher ester library is shown in Supplementary Figure S11. The resonances of the major isomer in this spectrum overlay with (3*R*,7*R*,11*R*,15*R*-**21***R* (and the 11*S* epimer), while the minor isomer resonances overlay with the C3 epimer (3*S*,7*R*,11*R*,15*R*)-**21***R* (and the 11*S* epimer).

This confirms the assignment of the *R* configuration to the C3, C7, and C15 stereocenters in the natural hormone. The C11 stereocenter cannot be assigned from the spectra because there are no substantive differences. Fortunately, it is clear from Yajima's testing results that C11 has the *R* configuration. Accordingly, we confirm Yajima's assignment of the natural hormone as "all-*R*", (3*S*,7*R*,11*R*,15*R*).

In the process of assigning hormone configuration, Yajima made all four individual epimers of **1** and published copies of their *bis*-(*R*)-Mosher esters as Supporting Information. Two pairs of these compounds differed only in configuration at C11. Like ours, these pairs of spectra are identical. Further, after accounting for differences in spectrometer frequency, we can show by comparison with our spectra that Yajima's stereochemical assignments are all correct, and more importantly, that the Mosher ester samples are all of good quality and relatively free from isomer impurities.[27]

Feringa and coworkers reported the synthesis and testing of two isomers of **1**, and they commented that the NMR spectra of both of their isomers were identical to the spectra of the natural product. One of Feringa's isomers is the enantiomer of the natural product (all-*S*-**1**), while the other is the C7 epimer of the enantiomer. Because these are C3/C7 *syn*/*anti* isomers, their spectra should not match each other, nor should they match the natural sample.

We compared the key H5 resonances in Feringa's two spectra from the Supporting Information,[27] and indeed these regions are very similar. In addition, we agree that both spectra match that of the natural sample reasonably well. This means that neither of Feringa's samples is pure. Furthermore, while estimating ratios from pdf spectra is difficult, it seems clear that the H5 triplet resonance predominates in both spectra. This means that the C3/C7 *anti* isomer is the major component in both samples, even though one of the samples should have been the *syn* isomer. Apparently epimerization at C3 occurred at the late stages of Feringa's synthesis.

Finally, we also reviewed spectrum in the Supporting Information of Loh for synthetic all *R*-1. The H5 resonance here is indeed a clean triplet for the C3/C7 *anti* isomer, with no evidence of contamination of the *syn* isomer. Recall that this *syn*/*anti* ratio is the only information provided by ^{1}H NMR spectra of the hormones. The presence of minor epimers at other stereocenters cannot be assessed because of the identical spectra.

Conclusions

Fluorous mixture synthesis has provided all eight diastereomers of the *phytophthora* hormone α1 with the *R* configuration at C11 as individual samples after demixing and detagging. The samples were not isomerically pure because some epimerization had occurred at C3. This could be assessed by ^{1}H NMR analysis, but that feature (relative configuration between C3 and C7) proved to be the only difference. In other words, each of the eight isomers exhibited one of only two different ^{1}H NMR spectra. The ^{13}C NMR spectra provided no differentiating information; all eight spectra were very similar.

The library of all possible *bis*-Mosher esters (16) was then made by esterification. Surprisingly, in most of the cases it was possible to substantially enrich the major isomer by chromatography with a chiral HPLC column. The complete set of ^{1}H and ^{19}F NMR spectra were recorded and assigned along with a partial set of ^{13}C spectra. Analysis of this data identified several convenient, redundant features to assign the configurations at C3, C7 and C15.

The sixteen ^{1}H NMR spectra of the Mosher ester library fell into eight identical pairs; no information was provided about the C11 configuration. Fortunately, it was clear from Yajima's prior work that C11 must have the *R* configuration. Knowing this and having access to Ojika's Mosher spectra of the natural sample, we confirmed Yajima's assignment of the hormone as "all-*R*".

Did you make predictions about whether the spectra of the hormone and Mosher ester library members would be the same or different? If so, then how well did you do? We find it surprising that the ^{1}H and especially the ^{13}C NMR spectra of the hormone isomers are so similar. The tabulated ^{13}C NMR resonances exhibit small differences for some isomers, but it is not clear that any of these differences is reliable for assignment. For example, we could not uniquely match any of the published ^{13}C NMR spectra of natural or synthetic samples of the hormone to one of the eight spectra in Table S3 of the Supporting Information. Try it yourself.

In assigning natural product stereoisomers, it is common practice to make two or more candidate stereoisomers and then compare them to the natural product. Often the chemical shifts of resonances of the candidates are subtracted from those of the natural product, then the candidate with the smallest differences is said to be the match. This is especially common for ^{13}C NMR spectra, where resonances are easily and accurately tabulated, then compared in a spreadsheet. Our work points out a problem with this approach; direct comparison of the spectra of the candidates to those of the natural product is out of order. First, the spectra of the candidates have to be compared with each other. Unless these can be reliably differentiated, there is no point in comparing them to the natural product. In addition, the results suggest caution in *ad hoc* assumptions that compounds with stereocenters separated by as few as three atoms will reliably have different spectra.

The ^{19}F and ^{13}C NMR spectra of the Mosher esters provide limited information, but the ^{1}H NMR spectra are by far the most informative. Even so, and despite the presence of not one but two Mosher esters (on O1 and O16), the appearance of the ^{1}H Mosher NMR spectra still did not depend on the configuration of at C11. Impressive long range effects of Mosher esters have been observed,[28] but assumptions that such effects will translate to very different kinds of compounds can be perilous.

Here is where the strengths of fluorous mixture synthesis come to the fore. If all of the relevant isomers can be made together, then no assumptions need to be taken at the outset. You may not be able to predict whether spectra will be identical or not, but in the end you will know with certainty which are and which are not. And if you do have to make Mosher or other chiral derivatives to differentiate isomers, then it does not matter whether the advanced Mosher (or any other) rule works or not. You are matching actual candidate spectra; you do not need any models or associated rules or guidelines derived there from. Either the spectra match, or they do not.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Saielli G, Bagno A. Org Lett. 2009; 11:1409–1412. [PubMed: 19215104]
2. The complete answer was "Clearly, it is difficult to generalize on the statement that two molecules cannot have the same NMR spectrum at all. Nevertheless, it seems unlikely that such an occurrence takes place, except perhaps when dealing with extremely simple or extremely crowded spectra."
3. (a) Luo ZY, Zhang QS, Oderaotoshi Y, Curran DP. Science. 2001; 291:1766–1769. [PubMed: 11230688] (b) Dandapani S, Jeske M, Curran DP. Proc Nat Acad Sci. 2004; 101:12008–12012. [PubMed: 15159539] (c) Fukui Y, Brueckner AM, Shin Y, Balachandran R, Day BW, Curran DP. Org Lett. 2006; 8:301–304. [PubMed: 16408900] (d) Yang F, Newsome JJ, Curran DP. J Am Chem Soc. 2006; 128:14200–14205. [PubMed: 17061905] (e) Jung WH, Guyenne S, Riesco-Fagundo C, Mancuso J, Nakamura S, Curran DP. Angew Chem Int Ed. 2008; 47:1130–1133.
4. Curran DP, Zhang QS, Lu HJ, Gudipati V. J Am Chem Soc. 2006; 128:9943–9956. [PubMed: 16866554]
5. (a) Murga J, GarciaFortanet J, Carda M, Marco JA. J Org Chem. 2004; 69:7277–7283. [PubMed: 15471481] (b) Curran DP, Moura-Letts G, Pohlman M. Angew Chem Int Ed. 2006; 45:2423–2426.
6. Qi J, Asano T, Jinno M, Matsui K, Atsumi K, Sakagami Y, Ojika M. Science. 2005; 309:1828. [PubMed: 16166510]
7. (a) Ashby SF. Trans Br Mycol Soc. 1929; 14:18–38.(b) Galloway LD. Report of the Imperial Mycologist. Sci Rep Agr Res Inst Pusa, 1934–1935. 1936:120–130.(c) Kouyeas V. Ann Inst Phytopathol Benaki. 1953; 7:40–52.(d) Erwin, DC.; Bartnicki-Garcia, S.; Tsao, PH. Phytophthora: its biology, taxonomy, ecology, and pathology. American Phytopathological Society; St. Paul, Minn: 1983. (e) Ko WH. Ann Rev Phytopathol. 1988; 25:57–73.(f) Chern LL, Tang CS, Ko WH. Bot Bull Acad Sin. 1999; 40:79–85.(g) Fabritius AL, Cvitanich C, Judelson HS. Mol Microbiol. 2002; 45:1057–1166. [PubMed: 12180924]
8. Yajima A, Kawanishi N, Qi J, Asano T, Sakagami Y, Nukada T, Yabuta G. Tetrahedron Lett. 2007; 48:4601–4603.
9. Curran DP, Bajpai R. Tetrahedron Lett. 2007; 48:7965–7968. [PubMed: 18989356]
10. Ojika M, Qi J, Kito Y, Sakagami Y. Tetrahedron: Asymmetry. 2007; 18:2497.
11. Yajima A, Qin Y, Zhou X, Kawanishi N, Xiao X, Wang J, Zhang D, Wu Y, Nukada T, Yabuta G, Qi J, Asano T, Sakagami Y. Nat Chem Biol. 2008; 4:235–237. [PubMed: 18297064]
12. Wang SY, Song P, Chan LY, Loh TP. Org Lett. 2010; 12:5166–5169. [PubMed: 20973505]
13. Harutyunyan SR, Zhao Z, Hartog Td, Bouwmeester K, Minnaard AJ, Feringa BL, Govers F. Proc Nat Acad Sci. 2008; 105:8507–8512. [PubMed: 18559862]
14. (a) Ohtani I, Kusumi T, Kashman Y, Kakisawa H. J Am Chem Soc. 1991; 113:4092–4096.(b) Hoye TR, Jeffrey CS, Shao F. Nature Protocols. 2007; 2:2451–2458.
15. The prefix "M" indicates a mixture of fluorous-tagged compounds while the suffixes "a", "b", or "c" indicates the tags on the components of the mixture.
16. Blakemore PR. J Chem Soc, Perkin Trans. 2002; 1:2563–2585.
17. Zhang QS, Curran DP. Chem Eur J. 2005; 11:4866–4880. [PubMed: 15915521]
18. Myers A, Yang BY, Chen H, McKinstry L, Kopecky DJ, Gleason J. J Am Chem Soc. 1997; 119:6496–6511.
19. Fluorous reagents and HPLC columns were purchased from Fluorous Technolgies, Inc. DPC owns an equity interest in this company.
20. Myers AG, Yang BH, David KJ. Tetrahedron Lett. 1996; 37:3623–3626.
21. Mancuso AJ, Swern D. Synthesis. 1981:165–185.
22. Zhang QS, Rivkin A, Curran DP. J Am Chem Soc. 2002; 124:5774–5781. [PubMed: 12010052]
23. Romo D, Johnson DD, Plamondon L, Miwa T, Schreiber SL. J Org Chem. 1992; 57:5060–5063.

24. Corey EJ, Fuchs PL. Tetrahedron Lett. 1972; 36:3769–3772.
25. (a) Jasper C, Wittenberg Rd, Quitschalle M, Jakupovic J, Kirschning A. Org Lett. 2005; 7:479–482. [PubMed: 15673269] (b) Schultz HS, Freyermuth HB, Buc SR. J Org Chem. 1963; 28:1140–1142.
26. (a) Katsuki T, Sharpless KB. J Am Chem Soc. 1980; 102:5974–5976.(b) Bonadies F, Rossi G, Bonini C. Tetrahedron Lett. 1984; 25:5431–5434.
27. Keep in mind that we did not have FID's of these spectra and could only inspect the baselines of relatively low resolution pdf files.
28. Hoye TR, Erickson SE, Erickson-Birkedahl SL, Hale CRH, Izgu EC, Mayer MJ, Notz PK, Renner MK. Org Lett. 2010; 12:1768–1771. [PubMed: 20337423]

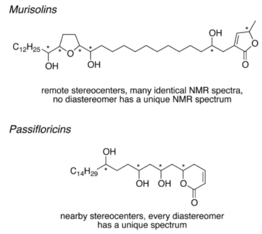

Figure 1.

Examples of natural product stereoisomer libraries with remote (murisolin) and nearby (passifloricin) stereocenters.

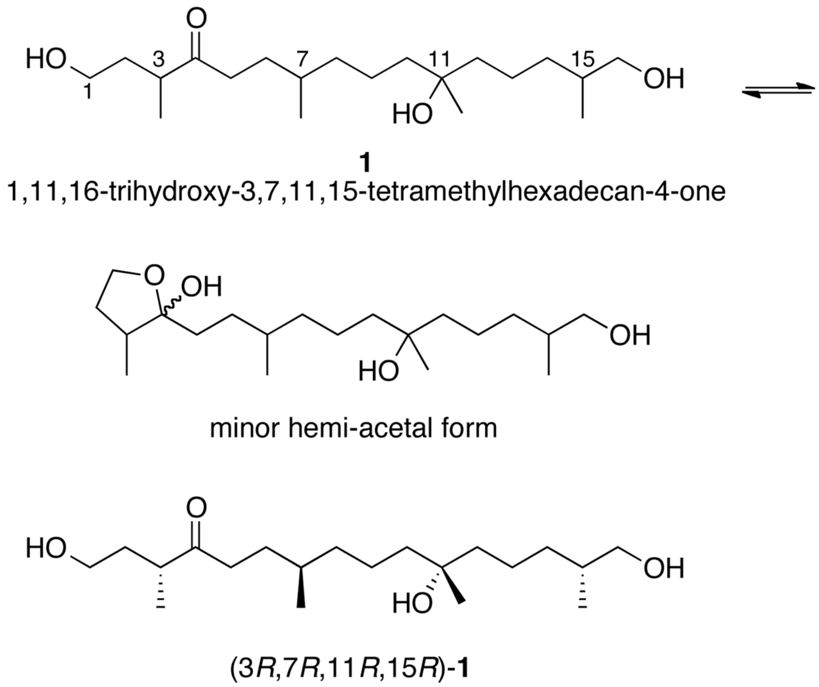

Figure 2.

Structures of the *Phytophthora* α1 mating hormone **1**, open form (major) and hemi-acetal form (minor, two stereoisomers).

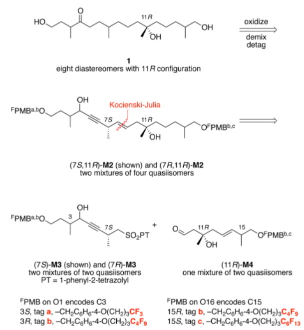

Figure 3.
Retrosynthetic plan and tagging strategy.

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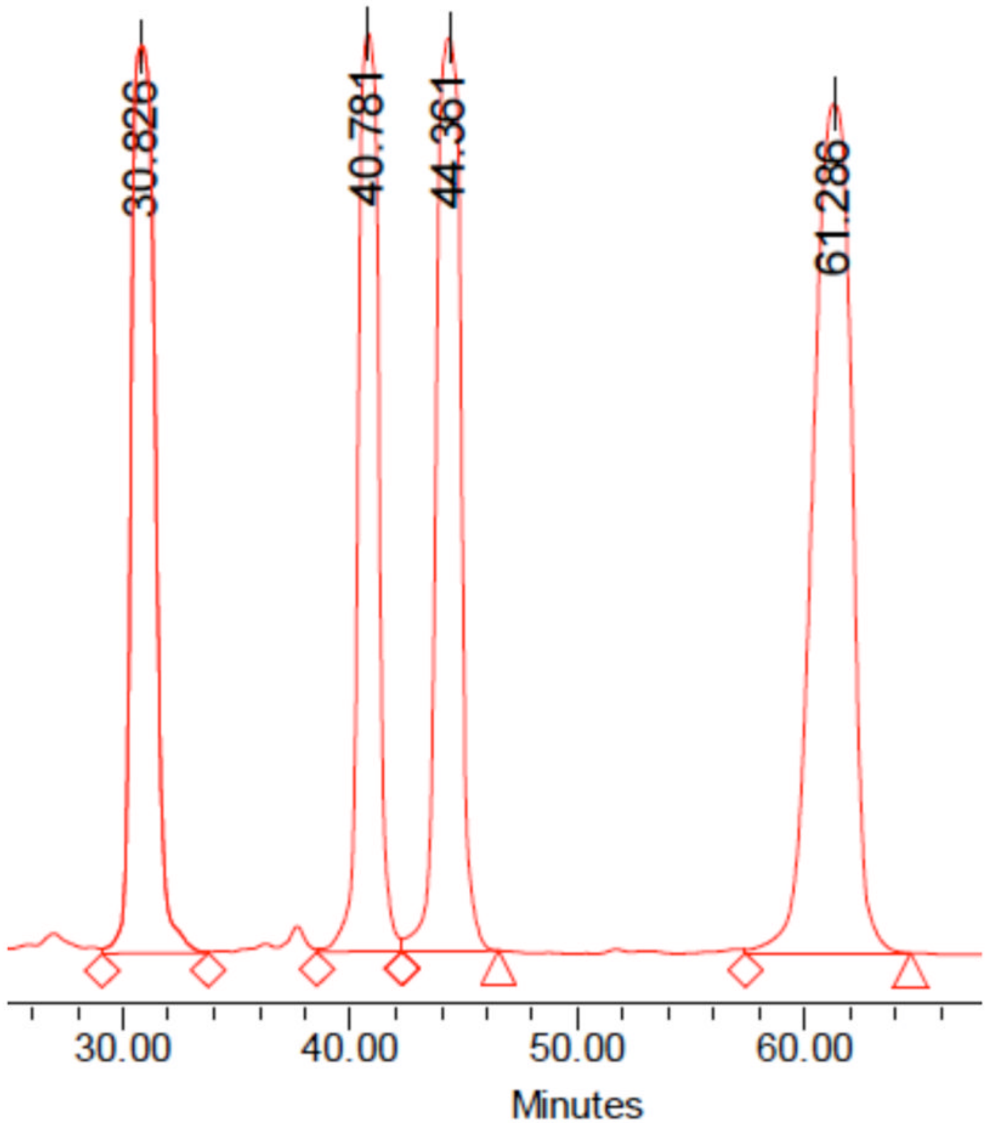

Figure 4.
Typical chromatogram from a semi-preparative demixing run with 40 mg of (7*S*)-**M20a/b,b/c**. Conditions: 80:20 CH_3CN:H_2O to 100% CH_3CN in 30 min, then 100% CH_3CN for 70 min at a flow rate of 7 mL/min; FluoroFlash PF8 column.

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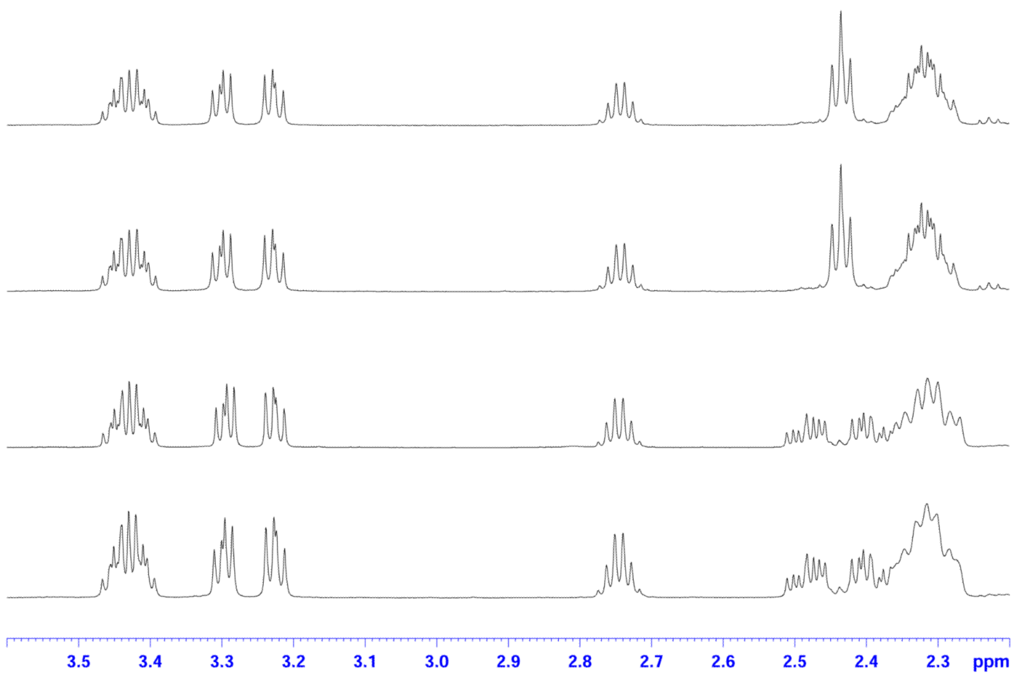


Figure 5.

Partial ^{1}H NMR spectra (600 MHz, $CDCl_3$) of (3*S*,7*S*,11*R*,15*R*)-**20a,b**, (3*S*,7*S*,11*R*, 15*S*)-**20a,c**, (3*R*,7*S*,11*R*,15*R*)-**20b,b**, and (3*R*,7*S*,11*R*,15*S*)-**20b,c** (top to bottom). Most parts of the spectra are very similar, but notice the differences in the region 2.4–2.5 ppm.

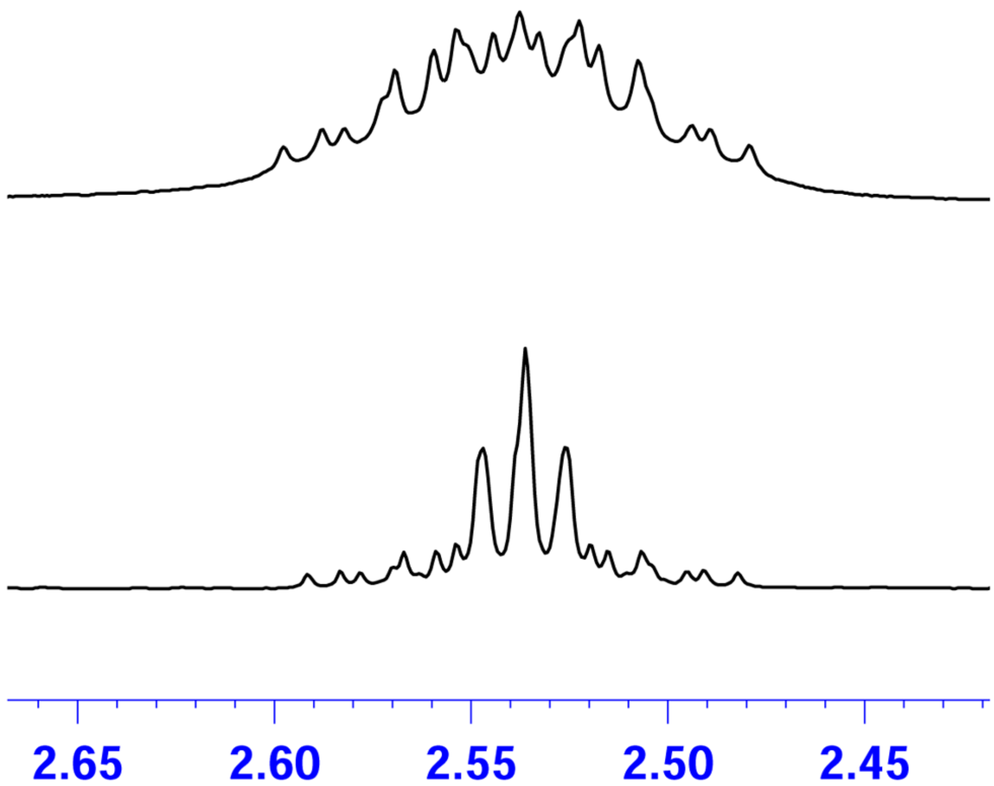


Figure 6.
Expansion of the H5 region of the ^{1}H NMR spectra (700 MHz, CD_3OD) of two representative isomers of **1**. Top spectrum, (3*S*,7*R*,11*R*,15*S*)-**1**; C3/C7 *syn*; bottom spectrum (3*S*,7*S*,11*R*,15*S*)-**1**, C3/C7-*anti*. Note the small resonances from the *syn* contaminant in the bottom spectrum of the *anti* isomer on either side of the triplet at 2.54 ppm.

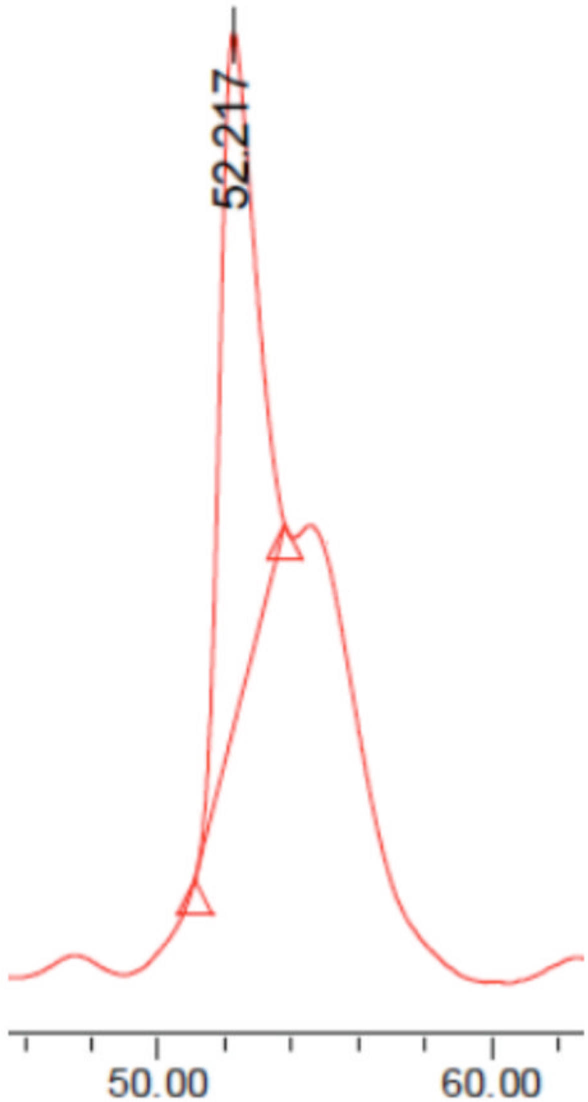


Figure 7.
Typical chromatogram from semi-preparative HPLC separation of the Mosher ester *(3S,7S, 11R,15S)*-**21***S*. The shoulder on the tail of the main peak is the C3 epimer *(3R,7S,11R, 15S)*-**21***S*.

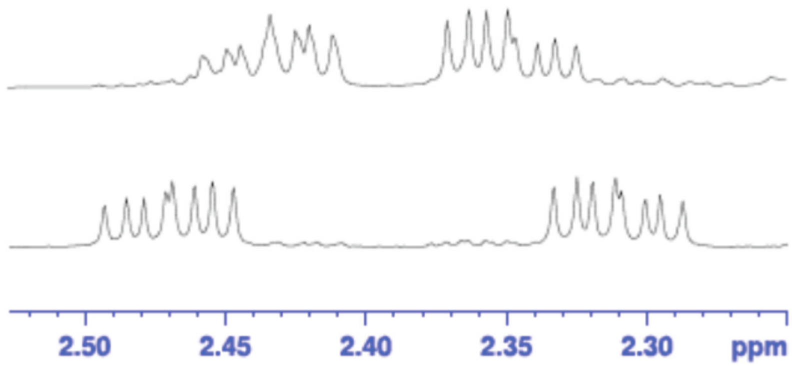


Figure 8.

Two examples of the H5 region of the ^{1}H NMR spectra of the Mosher esters. (3*S*,7*S*,11*R*, 15*S*)-**21***S* (top, C3,C7-*anti*) and (3*S*,7*R*,11*R*,15*S*)-**21***S* (bottom, C3,C7-*syn*).

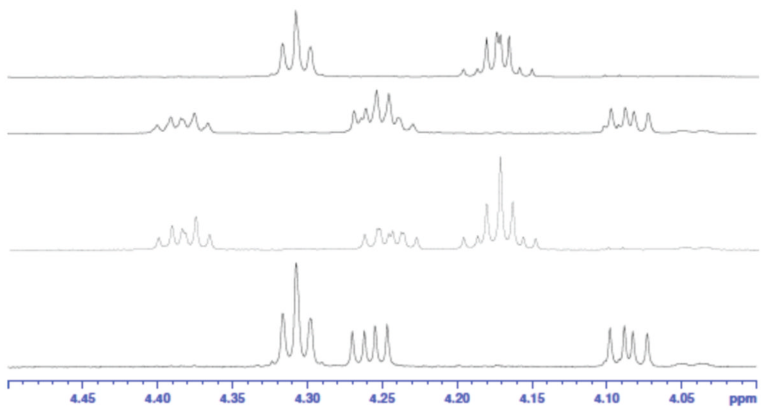
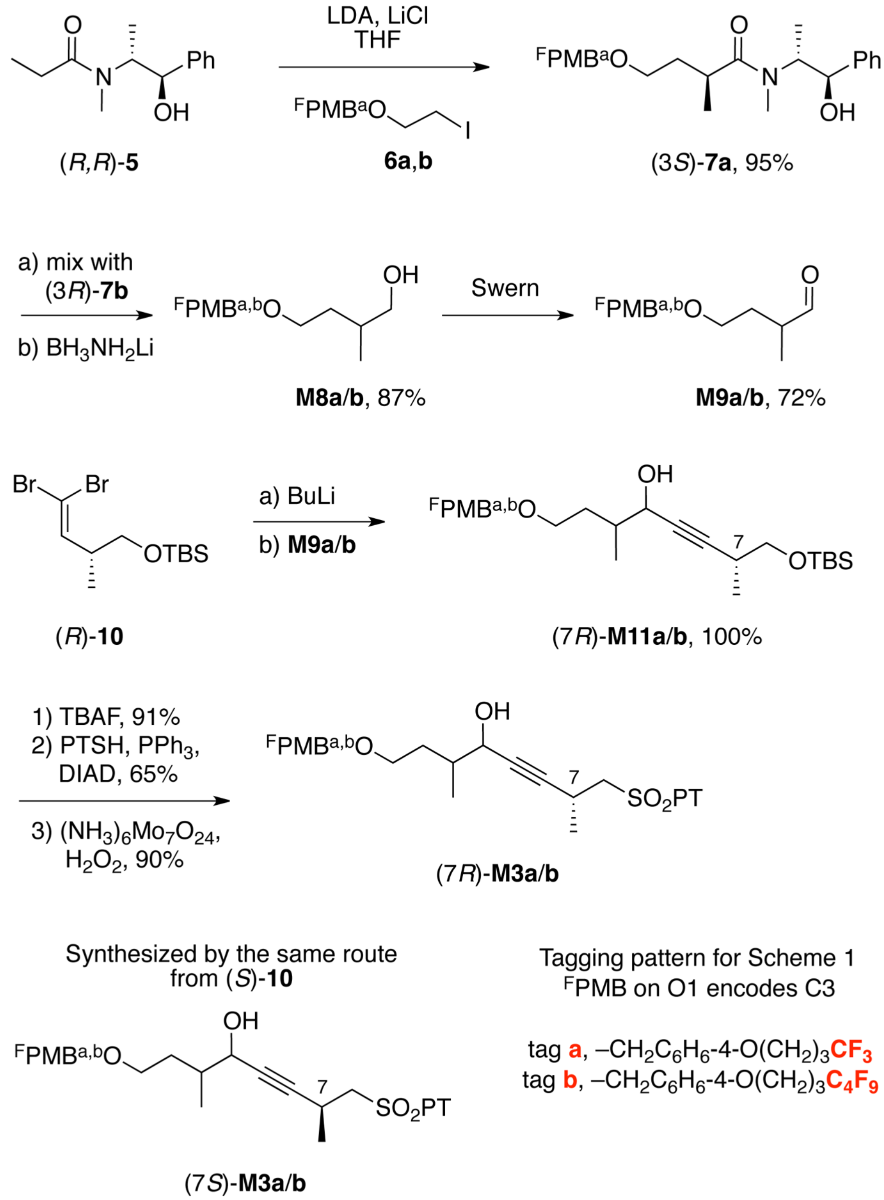
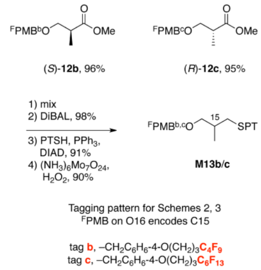


Figure 9.
Four examples of the H1/H16 region of the ^{1}H NMR spectra of the Mosher esters. Protons H1 are downfield from H16 in all spectra. Spectra in order from top to bottom are: 1) (3*S*,7*R*,11*R*,15*S*)-**21***R*; 2) (3*R*,7*R*,11*R*,15*R*)-**21***R*; 3) (3*S*,7*S*,11*R*,15*R*)-**21***S*; 4) (3*R*,7*S*,11*R*,15*S*)-**21***S*.



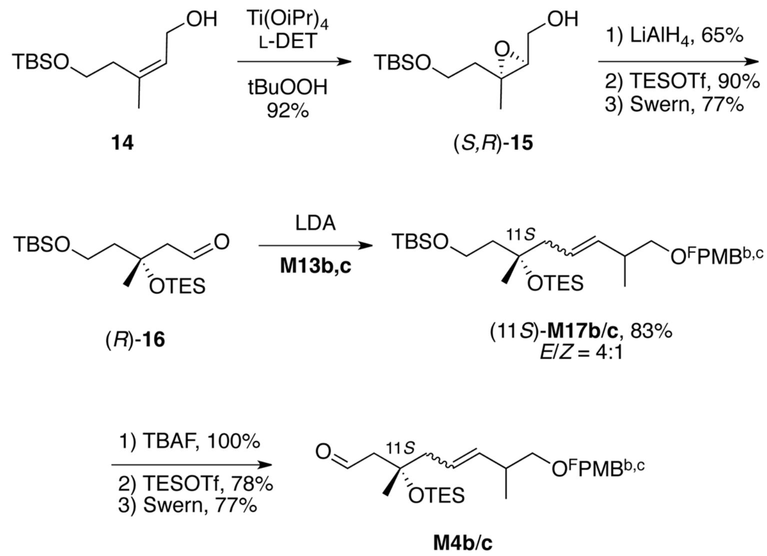
Scheme 1.

Synthesis of the left fragment, a pair of two quasiisomers (7*S*)-**M3a/b** and (7*R*)-**M3a/b**



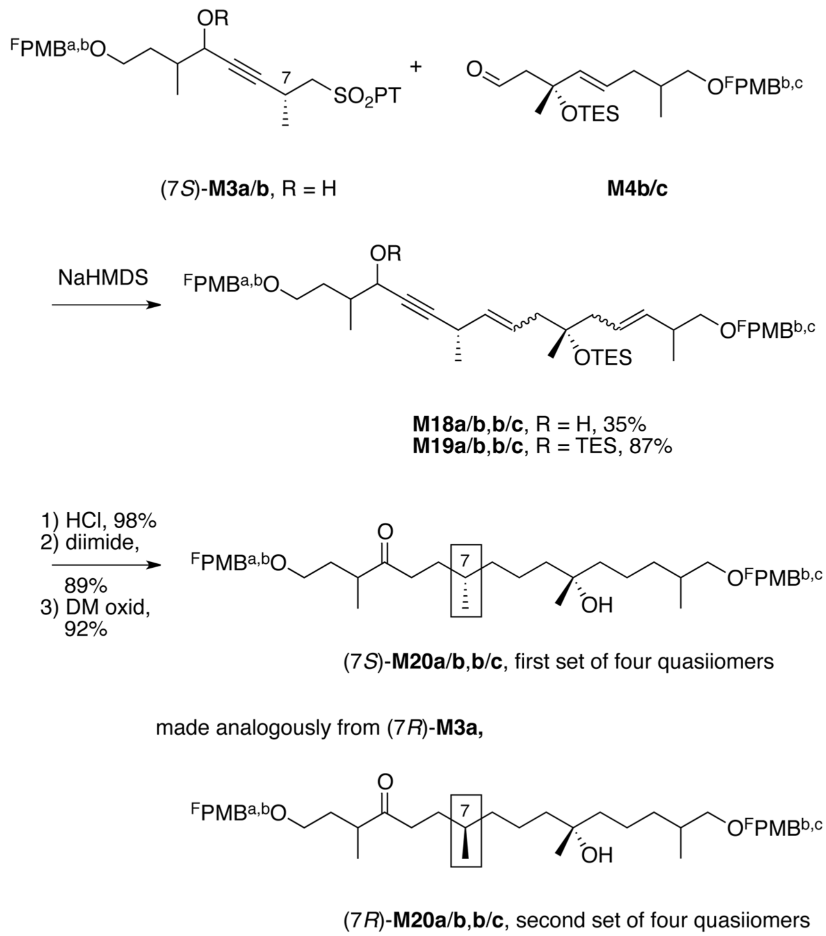
Scheme 2.

Synthesis of tagged quasiracemate **M13b/c** bearing the C15 stereocenter



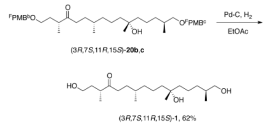
Scheme 3.

Synthesis of the right fragment, quasiracemate **M4b/c**



Scheme 4.

Fragment coupling and completion of the synthesis illustrated in the 7*S* series



Scheme 5.
A representative detagging reaction

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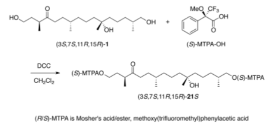

Scheme 6.
Synthesis of one representative of the 16-member Mosher ester stereoisomer library

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Table 1

The results of hydrogenolysis reactions of PMB^F ethers **20**, the amount of products isolated and the percentage yields

Precursor	PMB^F at C1	PMB^F at C16	Product	% Yield
From the mixture (7*S*,11*R*)-**20**				
20a,b	CF_3	C_4F_9	(3*S*,7*S*,11*R*,15*R*)-**1**	62
20a,c	CF_3	C_6F_{13}	(3*S*,7*S*,11*R*,15*S*)-**1**	69
20b,b	C_4F_9	C_4F_9	(3*R*,7*S*,11*R*,15*R*)-**1**	69
20b,c	C_4F_9	C_6F_{13}	(3*R*,7*S*,11*R*,15*S*)-**1**	86
From the mixture (7*R*,11*R*)-**20**				
20a,b	CF_3	C_4F_9	(3*S*,7*R*,11*R*,15*R*)-**1**	64
20a,c	CF_3	C_6F_{13}	(3*S*,7*R*,11*R*,15*S*)-**1**	66
20b,b	C_4F_9	C_4F_9	(3*R*,7*R*,11*R*,15*R*)-**1**	85
20b,c	C_4F_9	C_6F_{13}	(3*R*,7*R*,11*R*,15*S*)-**1**	63

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Table 2

Summary of the synthesis and purity of the Mosher ester library.

starting material	MTPA acid	product	yield	C3 epimer before/after HPLC
(3*S*,7*S*,11*R*,15*R*)-**1**	*R*	(3*S*,7*S*,11*R*,15*R*)-**21***R*	72%	18%/<5%
(3*S*,7*S*,11*R*,15*R*)-**1**	*S*	(3*S*,7*S*,11*R*,15*R*)-**21***S*	73%	19%/<5%
(3*S*,7*S*,11*R*,15*S*)-**1**	*R*	(3*S*,7*S*,11*R*,15*S*)-**21***R*	63%	33%/<5%
(3*S*,7*S*,11*R*,15*S*)-**1**	*S*	(3*S*,7*S*,11*R*,15*S*)-**21***S*	61%	34%/<5%
(3*R*,7*S*,11*R*,15*R*)-**1**	*R*	(3*R*,7*S*,11*R*,15*R*)-**21***R*	82%	23%/<5%
(3*R*,7*S*,11*R*,15*R*)-**1**	*S*	(3*R*,7*S*,11*R*,15*R*)-**21***S*	64%	21%/<5%
(3*R*,7*S*,11*R*,15*S*)-**1**	*R*	(3*R*,7*S*,11*R*,15*S*)-**21***R*	68%	26%/11%
(3*R*,7*S*,11*R*,15*S*)-**1**	*S*	(3*R*,7*S*,11*R*,15*S*)-**21***S*	77%	26%/<5%
(3*S*,7*R*,11*R*,15*R*)-**1**	*R*	(3*S*,7*R*,11*R*,15*R*)-**21***R*	79%	28%/<5%
(3*S*,7*R*,11*R*,15*R*)-**1**	*S*	(3*S*,7*R*,11*R*,15*R*)-**21***S*	78%	28%/28%
(3*S*,7*R*,11*R*,15*S*)-**1**	*R*	(3*S*,7*R*,11*R*,15*S*)-**21***R*	77%	17%/<5%
(3*S*,7*R*,11*R*,15*S*)-**1**	*S*	(3*S*,7*R*,11*R*,15*S*)-**21***S*	73%	16%/<5%
(3*R*,7*R*,11*R*,15*R*)-**1**	*R*	(3*R*,7*R*,11*R*,15*R*)-**21***R*	64%	24%/<5%
(3*R*,7*R*,11*R*,15*R*)-**1**	*S*	(3*R*,7*R*,11*R*,15*R*)-**21***S*	80%	25%/17%
(3*R*,7*R*,11*R*,15*S*)-**1**	*R*	(3*R*,7*R*,11*R*,15*S*)-**21***R*	77%	19%/<5%
(3*R*,7*R*,11*R*,15*S*)-**1**	*S*	(3*R*,7*R*,11*R*,15*S*)-**21***S*	63%	20%/<5%