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Fluorous Mixture Synthesis of Four Stereoisomers of the C21–C40 Fragment of Tetrafibrin

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Abstract

Four stereoisomers of the C21–C40 fragment are synthesized in a single exercise with the aid of fluororous tagging to encode configurations at C37 and C33. After demixing and detagging, the isomers were found to have substantially identical ¹H NMR spectra. However, there were some small but reliable differences in their ¹³C NMR spectra.

Keywords

tetrafibrin; fibrinogen; fluororous mixture synthesis; quasiisomers; Kocienski-Julia reaction

In 1993, Kamiyama and co-workers described the isolation and two-dimensional structure (constitution) of the interesting natural product tetrafibrin (**1**, Figure 1).¹ The compound is an ω-amino acid whose terminal groups are separated by 39 carbon atoms. Adding the carboxylate, the total length of the carbon backbone is 40 atoms. The chain features seven *E*-alkenes, ten hydroxy groups, a ketone, and a lone methyl group.

Tetrafibrin strongly inhibited the binding of fibrinogen to its glycoprotein receptor (IC₅₀ = 46 nM). It also inhibited ADP-, collagen-, and thrombin-induced aggregation of human platelets.¹ Accordingly, it has potential as a therapeutic agent for arterial thrombotic diseases.

Assignment of the three-dimensional structure (configuration) of tetrafibrin is a problem because the stereocenters or groups of stereocenters are insulated from each other by 2–3 chain carbon atoms. Kishi and co-workers addressed the problem without using either classical derivatization or degradation by comparing NMR data of the natural product and its ketone reduction products collected in both chiral and achiral solvents to values in a database built from spectra of suitable model compounds.²

It would be valuable to confirm the structure of tetrafibrin by total synthesis. Syntheses of various fragments and assorted couplings have been described by Cossy^{3a} and Roush^{3b} as well as by our group.^{3c,d} We are also interested in making stereoisomers of tetrafibrin both to flesh out SAR and to learn whether and how the isomers can be differentiated. Towards

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Dedicated to Prof. Gerry Pattenden in celebration of his 70th birthday

Supporting Information for this article is available online at <http://www.thieme-connect.com/ejournals/toc/synlett>. The file contains full experimental details and compound characterizations along with copies of NMR spectra of quasiisomers **21** and diastereomers **2** and a comparison table of ¹³C NMR chemical shifts of **2**.

these ends, we describe herein the synthesis of four stereoisomers of a large bottom fragment of tetrafibricin. We use the technique of fluororous mixture synthesis⁴ to make all four isomers together in a single synthetic sequence.

The retrosynthetic analysis of tetrafibricin breaks the molecule at the C20–C21 alkene into large top and bottom fragments of comparable size and complexity. Further analysis of the bottom fragment **2** is shown in Scheme 1. This has two stereocenters (C33 and C37) that are isolated from others by three atoms along with a group of four stereocenters (C23–C29) on alternating carbon atoms.

Accordingly, coupling of **3**, **4**, and **5** by Kocienski–Julia reactions⁵ should provide the bottom fragment **2** with flexible access to various isomers. To make the set of four possible diastereomers that these three isomer groups engender, we decided to fix the absolute configuration of the large fragment **5** while making small fragments **3** and **4** as quasiisomer mixtures^{4c} with component configurations encoded by fluororous tags (mixture samples are denoted by the prefix ‘M’). This fluororous mixture synthesis was patterned after a single isomer synthesis by Dr. V. Gudipati.^{3c}

The synthesis of quasiracemate M-**3** is summarized in Scheme 2. Epoxide (*R*)-**6** was prepared by a Jacobsen hydrolytic kinetic resolution (HKR),⁶ then opened with lithiodithiane to give (*R*)-**7** in 70% yield.⁷ Likewise, (*S*)-**7** was prepared from (*S*)-**6** (not shown). The configurations at C37 were encoded by silylation with fluororous silyl tri-flates; (*R*)-**7** was reacted with **8a** bearing a C₄F₉ substituent while (*S*)-**7** was reacted with **8b** bearing a C₃F₇ substituent.⁸ The resulting quasienantiomers (*R*)-**9a**/*(S)*-**9b** (88% and 90% yields) were mixed in equal amounts to make a quasiracemate M-**9a,b**, then the dithiane was carefully hydrolyzed with mercuric perchlorate to provide M-**10a,b** (90%).⁹

Reduction of M-**10a,b** with DIBAL-H (73%), Mitsunobu reaction¹⁰ of the resulting alcohol with 1-phenyl-1*H*-tetra-zole-5-thiol (PTSH, 92%), and finally mCPBA oxidation (80%) provided the target sulfone component M-**3a,b** for first Kocienski–Julia coupling.

The synthesis of the aldehyde component for first coupling is shown in Scheme 3. Coupling of alcohol (*S*)-**11** with PTSH as above (79%) followed by hydrolysis (93%) and selective silylation of the primary alcohol with TBSCl (91%) provided (*S*)-**12**. Likewise, (*R*)-**12** was made by the same sequence of reactions starting from (*R*)-**11**.⁸

The *S*-enantiomer was tagged with silyl triflate bearing a C₆F₁₃ group to give (*S*)-**13c**. In turn quasienantiomer (*R*)-**13a** with a C₄F₉ group was made from (*R*)-**12** and **8a**. Both silylations occurred in 90% yield.

These quasienantiomers were mixed in equal portions to give quasiracemate M-**13a,c**. The TBS group was selectively removed by careful hydrolysis with HCl in methanol (60%), and the resulting alcohol was oxidized with Dess–Martin reagent to give M-**4a,c** (81%, Scheme 3).¹¹

The synthesis of the single enantiomer of the C21–C30 fragment **5** is summarized in Scheme 4. Epoxide (*S,S*)-**14** was prepared by Jacobsen HKR,⁶ then opened with lithiodithiane (83%).⁷ Hydrolysis of the acetal followed by protection of the resulting triol with TBSOTf gave trissilyl ether **15** (88% over two steps).

Deprotonation of **15** with *t*-BuLi and reaction of the derived dithiane anion with epoxide (*R*)-**16** provided **17** in 90% yield. Dithiane hydrolysis (84%) followed by directed reduction provided a 1,3-*anti*-diol trissilyl ether¹² that was further protected with TBSOTf to provide pentakissilyl ether **18** (73% for two steps). Selective desilylation of the primary TBS ether

with HF·pyridine¹³ occurred in 49% yield, then Dess–Martin oxidation¹¹ provided aldehyde **5** as a single isomer.

The fragment couplings and completion of the synthesis of the four isomers of **2** were all conducted in fluoros mixture mode, as summarized in Scheme 5. Kocienski–Julia coupling of quasiracemates M-**3a,b** and M-**4a,c** with KHMDs in DME provided a mixture of four quasiisomers M-**19** with an *E/Z* selectivity of about 9:1. Furthermore, we discovered that we could separate the minor *Z*-isomers without demixing any of the quasiisomers by preparative HPLC on a Whelk-O1 column. Thus, even though the crude Kocienski–Julia product contained eight true isomers (*E/Z*) and quasiisomers combined, the chromatogram exhibited only two peaks; a major one consisting of the four *E*-quasiisomers (M-**19**) and a minor one of the four *Z*-quasiisomers.

Oxidation of the purified M-**19** provided sulfone M-**20**,¹⁴ which was then coupled with **5** as above to provide alkene M-**21**. Again this was a mixture of four quasiisomers, each which was present as about a 9:1 mixture of *E/Z* isomers. Yet again preparative HPLC separated the four minor *Z*-quasiisomers without demixing the major *E*-isomers.

The mixture of four *E,E*-quasiisomers M-**21** was readily demixed into its individual components by preparative HPLC over a Fluoroflash¹⁵ column. A representative HPLC trace of a preparative run is shown in Figure S1 of the Supporting Information, and a summary of the product structures with configurations and tags is shown in Scheme 5. Final detagging to give the true isomers of **2** proved difficult because of the polarity of the product. However, desilylation with TASF in DMF^{4b} followed by solvent removal and direct purification provided each of the four individual isomers in pure form in about 75% yield.

An important goal of this work was to learn whether the four diastereomers of **2** could be differentiated from each other by NMR spectroscopy. Copies of the ¹H NMR and ¹³C NMR spectra of the four pure isomers of **2** are provided in the Supporting Information. The four ¹H NMR spectra in CDCl₃ at 700 MHz were substantially identical. In other words, none of the four isomers of **2** could be differentiated from any other by this means.

The four ¹³C NMR spectra (CDCl₃ at 175 MHz) were very similar, but not identical. In particular, the 33,37-*anti* isomers (*R,R* and *S,S*) could be differentiated from the *syn* isomers (*R,S* and *S,R*) by the chemical shift of alkene carbon 35, which was below $\delta = 128.90$ ppm for the *anti* isomers and above $\delta = 128.90$ ppm for the *syn* isomers. Chemical shift differences for C35 of the *syn/anti* isomers range from 0.12–0.23 ppm. Though small, these differences are probably reliable. Oishi and co-workers have recently identified a similar effect in related substructures of amphidinol 3, which led them to revise the structure of this natural product.¹⁶

Differentiating the pairs of C33/C37 *syn* and *anti* isomers from each other (*R,R* from *S,S* and *R,S* from *S,R*) is more difficult. However, we suggest that this can be achieved by comparing the chemical shifts of C35 (again) and C31. Here, the differences are less, 0.04–0.07 ppm, but the confidence level is increased since there are two values to compare. There are other small differences in the ¹³C NMR spectra, that are tabulated in the Supporting Information along with all possible isomer subtractions to facilitate comparison.

In summary, we have made four stereoisomers of a large bottom fragment of tetrafratricin and compared them to each other by ¹H NMR and ¹³C NMR spectroscopy. Although the stereoclusters are separated from each other by only three carbon atoms, the ¹H NMR spectra of the isomers are substantially identical. The ¹³C NMR spectra are very similar, but

not completely identical. We have learned from comparison of the spectra which resonances are diagnostic for differentiating the isomers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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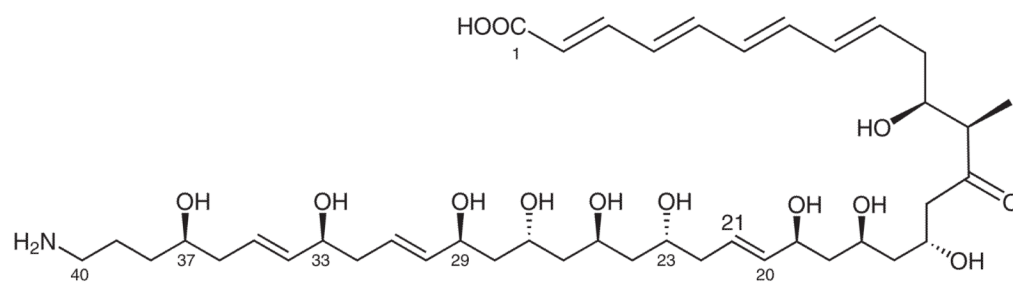
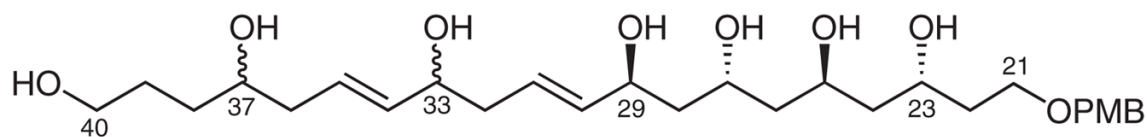
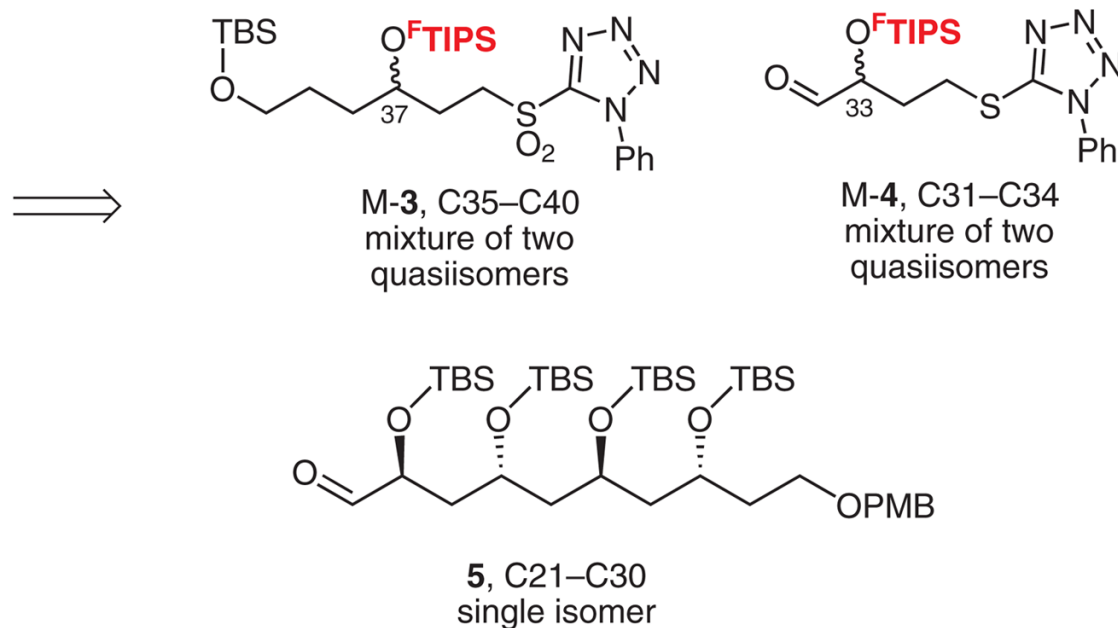


Figure 1.
Proposed structure of tetrafibricin (1)



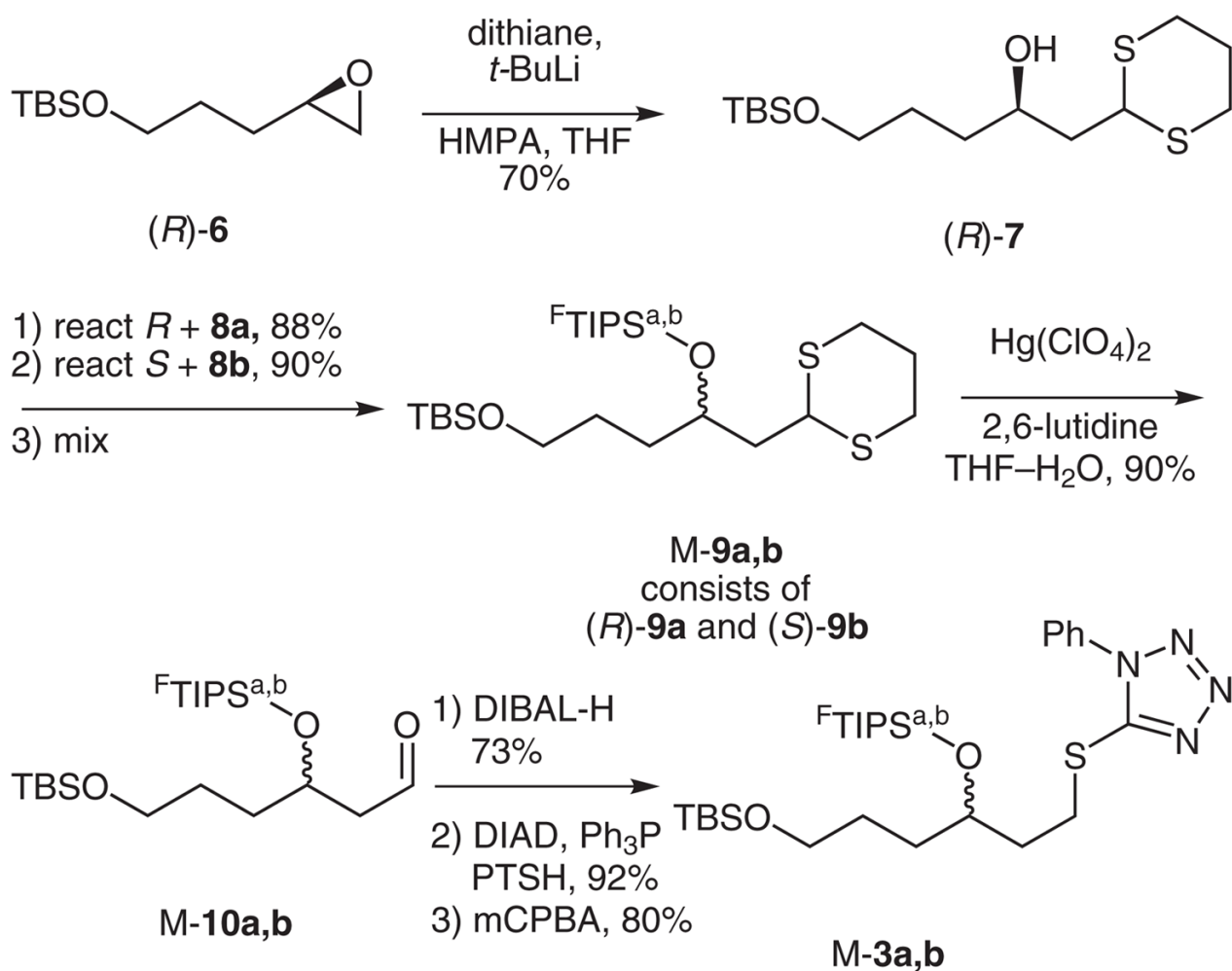
- single epimer at C29–C33
- both epimers at C33 and C37
- OH in place of NH₂ at C40 simplifies
- PMB on O21 for selective protection and UV activity (PMB is *p*-methoxybenzyl)



FTIPS groups are Si(*i*-Pr)₂CH₂CH₂R_f where R_f is C₃F₇, C₄F₉ or C₆F₁₃

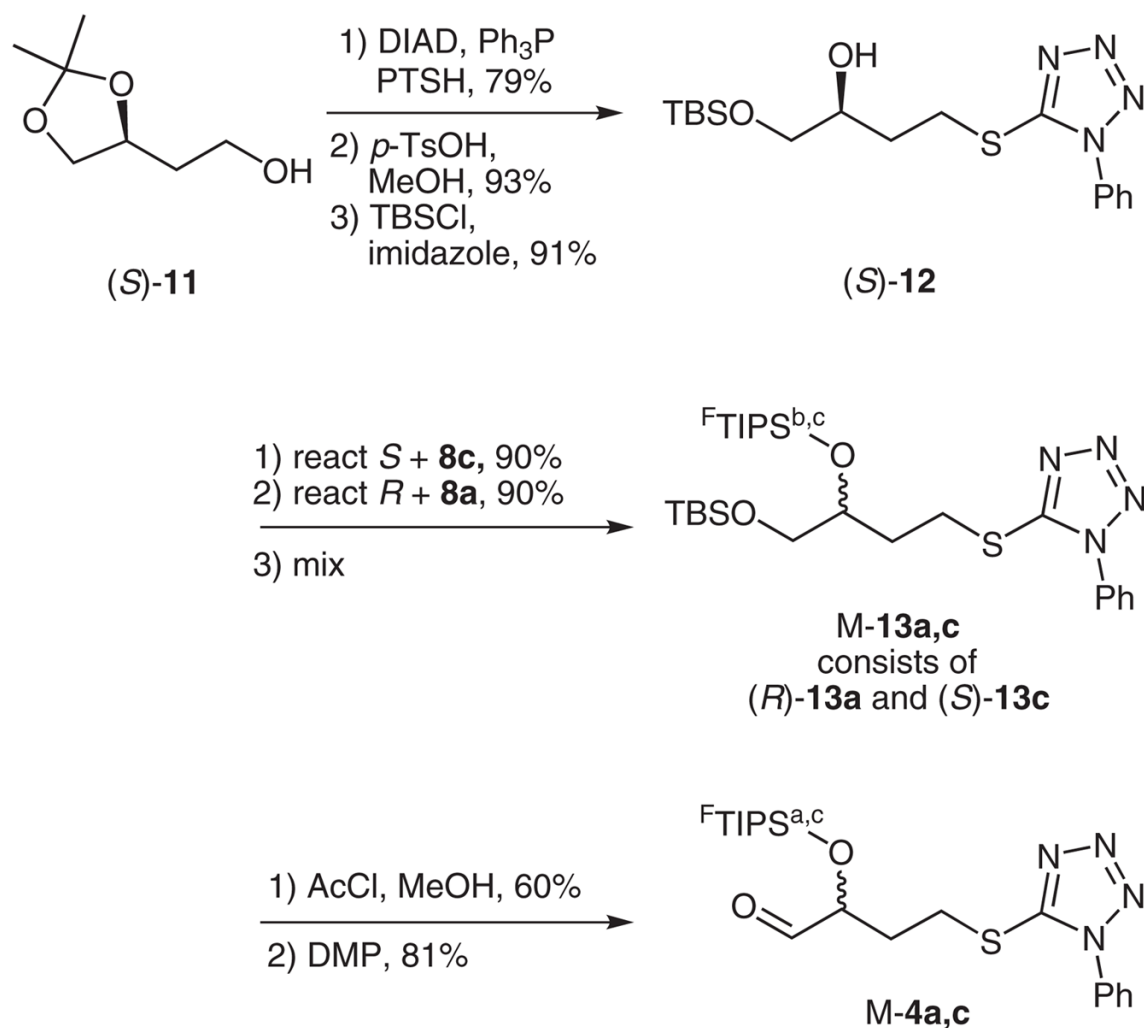
the quasiisomer coding is shown in the synthesis schemes

Scheme 1.
Retrosynthetic analysis of bottom fragment 2



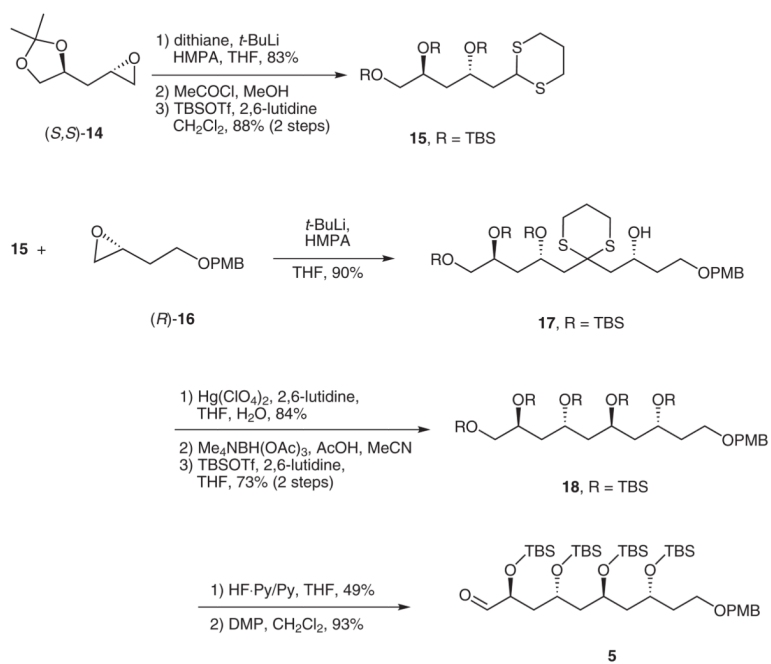
"a" series tags, **8a** = $\text{F}^{\text{TIPS}}\text{OTf}^{\text{a}}$ where $\text{F}^{\text{TIPS}^{\text{a}}}$ is $\text{Si}(i\text{-Pr})_2\text{CH}_2\text{CH}_2\text{C}_4\text{F}_9$
 "b" series tags, **8b** = $\text{F}^{\text{TIPS}}\text{OTf}^{\text{b}}$ where $\text{F}^{\text{TIPS}^{\text{b}}}$ is $\text{Si}(i\text{-Pr})_2\text{CH}_2\text{CH}_2\text{C}_3\text{F}_7$

Scheme 2.
 Synthesis of sulfone quasiracemate **M-3**

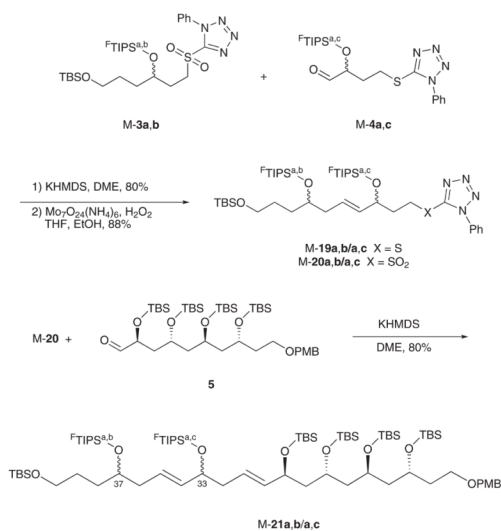


"a" series tags, **8a** = ${}^{\text{F}}\text{TIPSOTf}^{\text{a}}$ where ${}^{\text{F}}\text{TIPS}^{\text{a}}$ is $\text{Si}(\textit{i}\text{-Pr})_2\text{CH}_2\text{CH}_2\text{C}_4\text{F}_9$
 "c" series tags, **8c** = ${}^{\text{F}}\text{TIPSOTf}^{\text{c}}$ where ${}^{\text{F}}\text{TIPS}^{\text{c}}$ is $\text{Si}(\textit{i}\text{-Pr})_2\text{CH}_2\text{CH}_2\text{C}_6\text{F}_{13}$

Scheme 3.
 Synthesis of aldehyde quasiracemate M-4

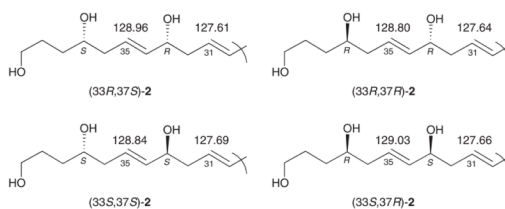


Scheme 4.
Synthesis of single isomer fragment **5**



demixing summary

quasisomer	t _R	O33 tag	O37 tag	total #F's
(33 <i>R</i> ,37 <i>S</i>)- 21a/b	19 min	TIPS ^{F9}	TIPS ^{F7}	16
(33 <i>R</i> ,37 <i>R</i>)- 21a/a	23 min	TIPS ^{F9}	TIPS ^{F9}	18
(33 <i>S</i> ,37 <i>S</i>)- 21b/c	29 min	TIPS ^{F13}	TIPS ^{F7}	20
(33 <i>S</i> ,37 <i>R</i>)- 21a/c	36 min	TIPS ^{F13}	TIPS ^{F9}	22

 final true diastereomers **2**
 with diagnostic ¹³C NMR chemical shifts (ppm)


Scheme 5.
 Fragment coupling, demixing, and detagging