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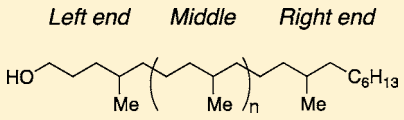
Bare-Minimum Fluorous Mixture Synthesis of a Stereoisomer Library of 4,8,12-Trimethylnonadecanols and Predictions of NMR Spectra of Saturated Oligoisoprenoid Stereoisomers

Edmund A.-H. Yeh, Eveline Kumli, Krishnan Damodaran, and Dennis P. Curran*

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

Supporting Information

ABSTRACT: All four diastereomers of a typical saturated oligoisoprenoid, 4,8,12-trimethylnonadecanol, are made by an iterative three-step cycle with the aid of traceless thionocarbonate fluorous tags to encode configurations. The tags have a minimum number of total fluorine atoms, starting at zero and increasing in increments of one. With suitable acquisition and data processing, each diastereomer exhibits characteristic chemical shifts of methyl resonances in its ^{1}H and ^{13}C NMR spectra. Together, these shifts provide a basis to predict the appearance of the methyl region of the spectrum of every stereoisomer of higher saturated oligoisoprenoids.



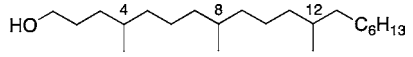
INTRODUCTION

Fluorous mixture synthesis (FMS)[1] is proving to be an especially valuable tool to make natural product stereoisomer libraries. Early work was focused on proving the principle that stereoisomers could be tagged with fluorous tags, mixed, carried through a multistep synthesis, and then reliably separated and detagged to give individual, pure stereoisomers.[2] Recent work has had a dual focus of introducing more efficient tagging strategies and solving structure problems by characterizing the natural product stereoisomer library members.[3] For example, we introduced a new double-tagging method to make macrosphelide stereoisomers that allows more compounds to be tagged with fewer fluorine atoms compared to prior tagging schemes.[4] And by characterizing the library members, we confirmed the structures of macrosphelides E and M and corrected the structure of macrosphelide D.

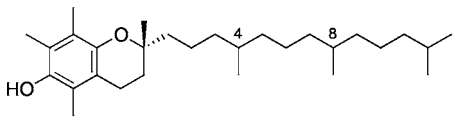
Here we describe an exercise in synthesis of the four diastereomers of 4,8,12-trimethylnonadecanol (**1**, Figure 1) that was undertaken to answer three core questions. First, all prior work in fluorous mixture synthesis has been designed based on the premise that protecting groups can do double duty as fluorous tags. But there are no suitable functional groups to protect in saturated oligoisoprenoids like **1**. How can fluorous mixture synthesis be applied to such compounds? Second, reducing the fluorine content of fluorous tags is an important goal for efficiency, but just how low can you go? Can you have tags with only 0, 1, 2, ... fluorine atoms? And third, are the NMR spectra of the 4,8,12-trimethylnonadecanol isomers the same or different? It turned out that there were small but reliable differences in the spectra of these isomers, and these differences allowed us to pose and answer a fourth question: can the NMR spectra of longer saturated oligoisoprenoid stereoisomers be predicted?

Saturated oligoisoprenoids have long alkyl chains with repeating stereocenters bearing methyl groups every five atoms on the chain. Vitamin E (**2**), for example, has a chain of three isoprene units and two stereocenters. Its diastereomers have been characterized by Ingold and co-workers, who showed that each diastereomer has a unique ^{13}C NMR spectrum.[5] Likewise, other diastereoreomers with two different isoprenoid units can be differentiated.[6] But the differences in ^{13}C NMR spectra are very small (typically 0.05 ppm or less), so it is not clear that these differences will translate to higher oligoisoprenoids as more signals are packed into small areas. With its smaller dispersion of resonances, ^{1}H NMR spectroscopy has not been useful in differentiating such stereoisomers. More typically, direct spectroscopic analysis is abandoned in favor of analysis of derivatives bearing long-range chiral reporter groups.[7]

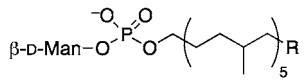
With five stereocenters in their oligoisoprenoid chains, β-D-mannosyl phosphomycoketides like **3a** and **3b** provide a



4,8,12-trimethylnonadecan-1-ol, **1**



Vitamin E, **2**



β-D-mannosyl phosphomycoketides
3a, R = C_4H_9, **3b**, C_6H_{13}

Figure 1. Saturated oligoisoprenoids have repeating stereocenters five atoms apart.

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difficult stereochemical assignment problem.[8] To confirm the two-dimensional structure of **3a**, Crich intentionally synthesized and characterized a stereorandom mixture of all 32 possible side-chain isomers.[9] The NMR spectra of this mixture corresponded well to those of the natural sample. Feringa and Minnaard made the "all-*S*" isomer of **3b**[10] and showed that its biological activity was similar to the natural product.[11] If the stereocenters of **3** are all biosynthesized in the same way, then the natural product is presumably all-*S*. But could there be other stereoisomers that have similar spectra and activity?

We have commented before that, in cases where stereoisomers have identical or very similar spectra, it becomes impossible to prove that a given stereoisomer *is* the natural product.[3b] Instead, it is important to prove that its other stereoisomers *are not* the natural product. Accordingly, there is a need to make and carefully characterize more stereoisomers of higher saturated oligoisoprenoids to see if their spectra reveal stereochemical information.

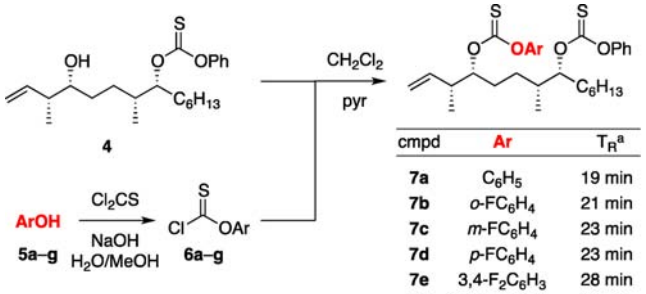
RESULTS AND DISCUSSION

Model Tagging Experiments. We decided to use asymmetric crotylation reactions to introduce the stereocenters because there are several crotylation reagents that reliably produce all stereoisomers with reagent control[12] and because the extra hydroxy group produced in the crotylation could serve as a convenient tag location. After several iterations, we planned to remove the tags by Barton–McCombie deoxygenation,[13] so we selected the aryl ring of the *O*-arylthionocarbonate group[14] (OC(S)OAr) as the location for the fluorine atoms.

Fluorous tags have typically been fashioned by adding spacers[15] followed by perfluoroalkyl groups of various lengths.[16] Here we instead replaced hydrogen atoms on the aryl ring of the tag with fluorine atoms (Ar = phenyl, fluorophenyl, difluorophenyl, ...). In addition to evaluating tags with 0, 1, 2, 3, and 5 fluorine atoms, we also evaluated regioisomeric tags with one fluorine atom in the *ortho*, *meta*, and *para* positions.

The performance of seven potential tags was evaluated on a model compound **7** as shown in Scheme 1. Alcohol **4** was synthesized by two Brown–Ramachandran crotylations[17,18] and the standard *O*-phenylthionocarbonate group was added after the first crotylation. After the second crotylation to give **4**, a mixture of the seven phenols **5a–g** was reacted with thiophosgene under standard conditions[14] to presumably produce a mixture of seven *O*-arylphenyl chlorothionoformates **6a–g**. This was added to the alcohol **4** and pyridine in dichloromethane, and the resulting product mixture **7** was analyzed by HPLC on a FluoroFlash PF-8 column.

Scheme 1. Evaluation of Fluorinated Tags with Model Mixture 7



cmpd	Ar	T_R[a]
7a	C_6H_5	19 min
7b	*o*-FC_6H_4	21 min
7c	*m*-FC_6H_4	23 min
7d	*p*-FC_6H_4	23 min
7e	3,4-$F_2C_6H_3$	28 min
7f	3,4,5-$F_3C_6H_2$	37 min
7g	C_6F_5	–[b]

a) retention time on FluoroFlash PF-8 column, 75/25 MeCN/H_2O; b) not detected

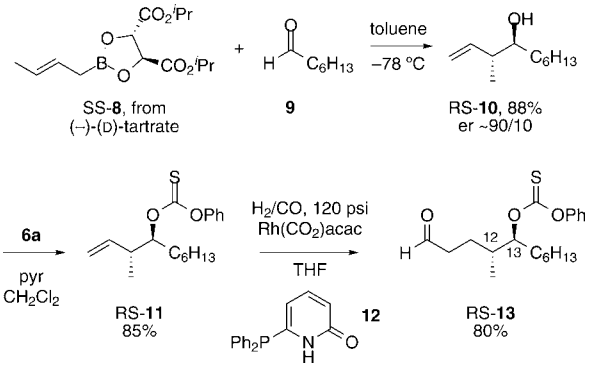
Although seven phenols **5a–g** were added to the starting mixture, only five peaks were observed on the chromatogram of **7**. A preparative separation provided the five fractions, which were identified by ^1H and ^{19}F NMR experiments. These were (in order of elution) (1) phenylthionocarbonate **7a** (19 min); (2) *o*-fluorophenyl **7b** (21 min); (3) a mixture of *m*- and *p*-fluorophenyl **7c,d** (23 min); (4) 3,4-difluorophenyl **7e** (28 min); and (5) 3,4,5-trifluorophenyl **7f** (37 min). The expected product **7g** from the pentafluorophenylchlorothionocarbonate **6g** was not detected. Perhaps **6g** is not stable under the conditions of formation (NaOH and water present). Whatever the case, we simply rejected this tag from further consideration.

Thus, provided that stereoisomers to be tagged all have the same polarity, it should be possible to use tags that start with zero fluorine atoms and differ by only one fluorine atom. Further, it might be possible to use the *o*-fluorophenylthionocarbonate as a kind of "fractional tag" (0.5) because it comes out in between the unfluorinated thionocarbonate (0) and the *m*- and *p*-monofluorinated thionocarbonates (1). This small effect might be because the fluorine atom in the *ortho* isomer is partially shielded from the fluorous silica gel by its neighboring substituent.[19]

Iterative Cycle. The experiments in Scheme 1 and other pilot studies[18] were conducted by Brown–Ramachandran crotylation; however, for the iterative cycles we opted for a Roush crotylation[20] because Roush reagents are convenient to make and store, and their reactions are easy to conduct and work up. The iterative cycle to make the isomers of **1** consists of (1) Roush crotylation of an aldehyde, (2) tagging of the resulting alcohol with a suitable chlorothionoformate to encode configuration, and (3) hydroformylation of the terminal alkene to give a chain-extended aldehyde ready for the next cycle.

In the first cycle, Scheme 2, heptanal **9** was reacted with the Roush reagent *SS*-**8** derived from the (−)-D-diisopropyl tartrate

Scheme 2. First Iteration by Traditional Synthesis of Single Compounds Provides Aldehyde 13



to give *RS*-**10** in 88% yield. This reaction installs the C12 stereocenter[21] of the target product. It is highly diastereoselective (>98% anti), but not completely enantioselective (see below). Reaction of **10** with *O*-phenylchlorothionoformate **6a** and pyridine as above gave *RS*-**11**. Hydroformylation of **11** with syngas (H_2 and CO) and $Rh(CO)_2acac$ (7%) and 6-(diphenylphospino)-2-pyridone **12** (30%) occurred smoothly in THF at 60 °C/120 psi to give *RS*-**13** in 80% yield.[22]

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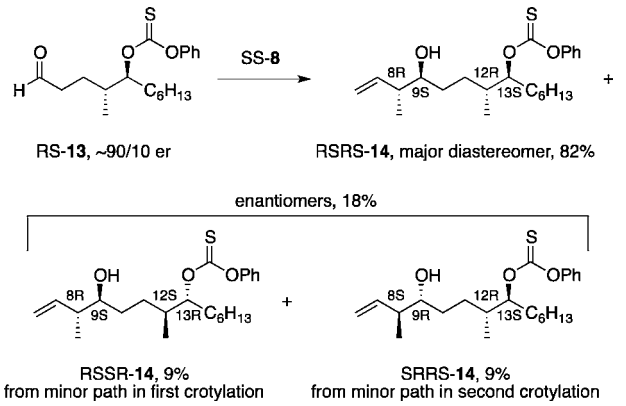
Evidently, the thionocarbonate group does not disturb the catalyst for this reaction. The overall isolated yield of 60% from the three-step cycle was deemed suitable for three iterations.

To test whether the thionocarbonate survives the Roush crotylation and to better understand the stereoselectivity of the crotylation, we looked first at one of the products of the second iterative cycle (Scheme 3). Reaction of **13** with Roush reagent

Scheme 3. Synthesis of 14 as a Single Compound in 82% Isomer Purity Shows the Stereoselectivity in Two Successive Roush Crotylations

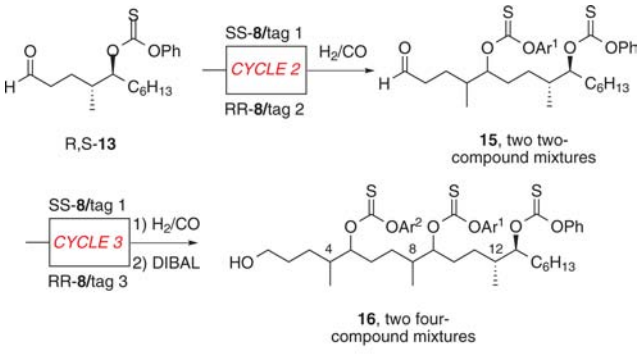


SS-**8** produced *RSRS*-**14** in 87% isolated yield. The sample generally looked like a single compound by ^{13}C NMR analysis, except that the carbinol carbon exhibited two peaks at 74.83 and 74.57 ppm in a ratio of about 82/18. The major peak is *RSRS*-**14** (configuration shown in Scheme 3), and we assign the minor peak as a mixture of SRRS-**14** and its enantiomer *RSSR*-**14**. The *RSSR* enantiomer arises from the minor path in the first Roush crotylation and the major path in the second crotylation, while its enantiomer results from the major path in the first and minor in the second.

Assuming that the two crotylations give about the same selectivity, this means that each gives a 90/10 ratio of isomers (enantiomers after the first crotylation and diastereomers after the second). The product of the minor path in both crotylations is the enantiomer of the major product. Present to the extent of about 1%, this product (*SRSR*-**14**, not shown) can be neglected. The enrichment of the enantiomeric ratio of the major diastereomer of **14** is similar to that observed in sequential asymmetric transformations like dynamic kinetic resolutions.[23] Keep in mind, however, that the diastereomers of **1** cannot be directly separated at the end of this exercise because the stereocenters are so far removed. Nonetheless, the presence of minor isomers proved to be vitally important as controls in the spectroscopic analysis of the final samples.

Second and Third Iterations in Mixture Mode. Because of the ease of the cycle, we were able to conduct two pilot runs in mixture mode, as summarized in Scheme 4. The cycles were essentially the same; only the tags were changed. A sample of aldehyde *RS*-**13** was divided in two, and each half was treated with one enantiomer of the Roush reagent. The products were tagged with appropriate chlorothionocarbonates. For example, in the first cycle of pilot mixture 1, the product from *SS*-**8** was tagged with the thionocarbonate-bearing phenyl ($Ar^1 = C_6H_5$), while that from *RR*-**8** was tagged with the thionocarbonate-bearing *o*-fluorophenyl ($Ar^1 = o\text{-}FC_6H_4$). The tagged

Scheme 4. Two Pilot Mixtures with Different Tagging Patterns Form the Second and Third Iterations



Pilot Mixture 1
tags 1,2,3 are
C_6H_5, *o*-FC_6H_4, *p*-FC_6H_4

center	Ar^1; Ar^2	F's
4R,8R	C_6H_5;C_6H_5	0
4R,8S	C_6H_5;*o*-FC_6H_4	1[a]
4S,8R	*p*-FC_6H_4;C_6H_5	1
4S,8S	*p*-FC_6H_4;*o*-FC_6H_4	2

Pilot Mixture 2
tags 1,2,3 are
C_6H_5, *p*-FC_6H_4, 3,4-$F_2C_6H_4$

center	Ar^1; Ar^2	F's
4R,8R	C_6H_5;C_6H_5	0
4R,8S	C_6H_5;*p*-FC_6H_4	1
4S,8R	3,4-$F_2C_6H_4$;C_6H_5	2
4S,8S	3,4-$F_2C_6H_4$;*p*-FC_6H_4	3

a) *o*-FC_6H_4 used as "fractional tag"

quasiisomers[24] were then mixed back together for the subsequent steps.

Hydroformylation in cycle 2 gave a new aldehyde sample **15** of predominately two quasiisomers that was again divided in half. Each half was reacted with one enantiomer of the Roush reagent, then the samples were tagged appropriately and remixed. Another hydroformylation completed the third cycle, giving a mixture of four aldehydes ready in principle for a fourth cycle. However, for this study we reduced the aldehyde mixture with DIBAL-H to give alcohols **16** ready for demixing.

The tables in the lower part of Scheme 4 show how the mixture members are encoded and provide the total number of fluorine atoms (F's) in each member. All the pairs of adjacent stereocenters in all members have anti configurations. Because the hydroxy groups will be removed, we focus now on the configurations of the methyl-bearing stereocenters at C4, C8, and C12.

Both pilot mixtures have the (12*R*) configuration from the starting aldehyde **13** (to the extent of 90%), and the C13-OH group bears a standard phenylthionocarbonate (C_6H_5). In pilot mixture 1, we used the regioisomeric mono-fluorophenyl tags. After the first pair of Roush crotylations (cycle 2), we tagged the (8*R*)-isomer with the phenyl group (C_6H_5) and the (8*S*)-isomer with the "fractional" *o*-fluorophenyl group (*o*-FC_6H_4), as indicated above. After the second pair of Roush crotylations (cycle 3), we tagged the (4*R*)-isomers again with the phenyl group (C_6H_5). Now the (4*S*)-isomers were tagged with the *p*-fluorophenyl group (*p*-C_6H_4). Notice that only three tags are used to encode four samples of **16** in pilot mixture 1, and that the middle two samples have the same number of fluorines (1).

In pilot mixture 2, we stepped up the tags by one fluorine atom each. After the first pair of Roush crotylations, we tagged the (8*R*)-isomer with the phenyl group (C_6H_5) and the (8*S*)-isomer with the *p*-fluorophenyl group (*p*-FC_6H_4). After the second pair of Roush crotylation, we tagged the (4*R*)-isomer again with the phenyl group, while the (4*S*)-isomer was tagged with the 3,4-difluorophenyl group (3,4-$F_2C_6H_3$). Again three

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tags are used to encode four samples in pilot mixture 2, but now there is no redundancy of total fluorine content.

Analytical HPLC traces of the two pilot mixtures are shown in Figure 2. In mixture 2, the total fluorine content of six

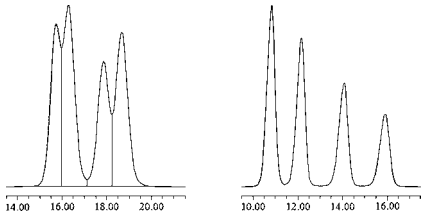


Figure 2. Analytical HPLC traces of pilot mixtures 1 (left, 65/35 MeCN/H_2O) and 2 (right, 70/30 MeCN/H_2O); FluoroFlash PF-8 column.

fluorine atoms spread over the four products is 0, 1, 2, and 3. In pilot mixture 1, the total fluorine content is four fluorine atoms, spread 0, 1, 1, and 2. Both provided four peaks, as expected. But the separation of pilot mixture 2 (right trace) was clearly superior, with baseline resolution being observed between every pair of peaks.

Interestingly, in the inferior pilot mixture 1 (left trace), the two compounds with one fluorine atom and regioisomeric tags (the two middle peaks of the chromatogram) separate rather well, with the *o*-fluoro-tagged compound emerging before the *p*-fluoro compoud. The problem is that the *o*-tag compound (peak 2) is shifted toward the zero-tag compound (peak 1) and the *p*-tag compound (peak 3) is shifted toward the difluoro-tag compound (peak 4). In essence, the two samples with regioisomeric tags can be separated from each other, but it is difficult to "fit" two tags with the same fluorine content in the small space between the higher and lower tags.

As a control, pilot mixture 2 was injected onto a standard reverse-phase HPLC column (RP-C18, comparable retention times observed with 80/20 MeCN/H_2O). However, only a single, very broad peak was observed. Thus, despite the low fluorine content of the tags, the use of a fluorous HPLC column is crucial for demixing.

Based on these results, we scaled up the mixture synthesis by using the tagging pattern in pilot mixture 2. Yields of the cycles on preparative scale were comparable: 60% for cycle 1 (Scheme 1), 59% for cycle 2, and 56% for cycle 3 (including the extra step of DIBAL-H reduction). Preparative separation of the final mixture of four quasiisomers of **16** was conducted on a larger FluoroFlash column, and a trace of a typical run is shown in Figure S1 of the Supporting Information (SI). The recovery of pure samples from the HPLC separation ranged from 68 to 74% for the four combined samples, and the combined weights of each of the four quasiisomers **16** ranged from 16−20 mg.

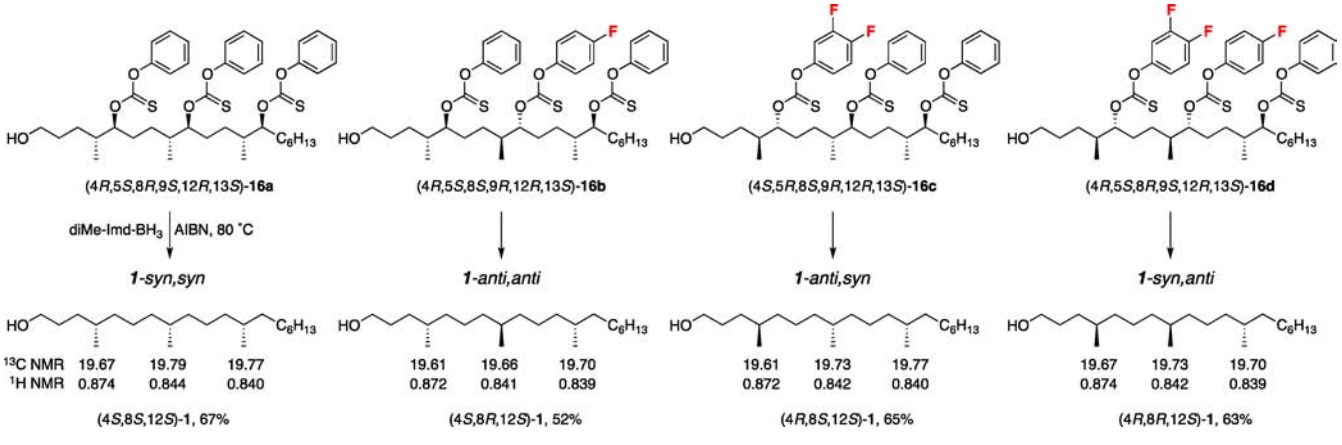
The complete structures of the final purified quasiisomers **16a−d** with both configurations and tags are shown in Scheme 5. Notice how the different aryl tags encode the configurations at C4/5 and C8/9. The four quasiisomers **16** were characterized as usual by NMR and MS experiments. There are small differences in their NMR spectra, but these differences may not be related to configuration and are simply because the tags are chemically different. The purity of each sample was assessed by analytical fluorous HPLC. The first eluting sample **16a** was a single peak, but the subsequent samples **16b−d** were contaminated with 1−3% of the prior eluting quasiisomers. These contaminants translate to stereoisomers after detagging. Because the amounts are small relative to the already known stereoisomer contaminants from the Roush crotylation, we did not repurify the three slightly impure samples.

Each of the *O*-arylthionocarbonates was reduced by exposure to 1,3-dimethylimidazol-2-ylidene borane (di-Imd-BH_3, 5 equiv) and AIBN in refluxing benzene.[25] Simple solvent evaporation and flash chromatography provided the final 4,8,12-trimethylnonadecanol stereoisomer samples **1** in 52−67% yields. This reduction with the carbene-borane reagent is superior to the usual tin hydride reduction because complete separation of tin byproducts from nonpolar samples like **3** can be tedious.

Notice the CIP priority switch that occurs at all the remaining stereocenters on deoxygenation of the quasiisomers **16** to give true isomers **1**. This is of little importance because we are concerned with relative stereochemistry, and going forward we indicate this in the usual way as *anti*/*syn*. For example, (4*S*,8*S*,12*S*)-**1** has the *syn* relative configuration between both C4/C8 and C8/C12 so is called **1**-*syn*,*syn*.

Each sample of **1** can contain up to eight stereoisomers. In round numbers, each sample should consist of about 70% of the stereoisomer from the favored product of each of the three Roush crotylations. This is the structure shown in Scheme 5. Each major product should contain about 9% each of three significant minor isomers (one from the minor product being formed in each of the three Roush crotylations). There are also three double-minor diastereomers, which are enantiomers of

Scheme 5. Structures of Final Tagged Products 16a−d and Reduced Nonadecanol Stereoisomers 1, All in Individual Form



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the major diastereomer impurities, in ~1% each. Adding these to their major enantiomers gives a total diastereomer ratio of about 70/10/10/10. The eighth isomer in each sample is the triple-minor product. This enantiomer of the major product can be neglected because the projected er of the major product is >100/1.

To summarize, the mixture synthesis provided ample quantities (5–6 mg) of the four target isomers of **1** for complete characterization. Every major isomer is projected be present in about 70% along with 10% each of three minor diastereomers. This defect from the standpoint of asymmetric synthesis proved to be an asset in the analysis.

Analysis of NMR Spectra. NMR spectra were initially recorded and processed in the usual way for each of the four samples: ^{1}H NMR at 700 MHz and ^{13}C NMR at 175 MHz, all in $CDCl_3$. We could see that the spectra, though very similar, were not identical in places. But we could not assign any specific resonances or determine isomer ratios because the resonances were too close together.

Additional processing of the data by the Traficante algorithm followed by forward linear prediction[26] gave a significant improvement in resolution while maintaining needed sensitivity. Expansions of the region of the ^{13}C NMR spectrum with the resonances of the branched methyl groups (Me-21, 22, 23) are shown in Figure 3 along with simulated spectra whose production will be discussed presently.

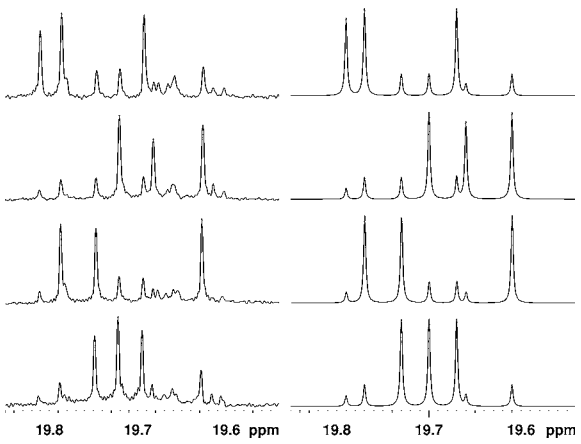
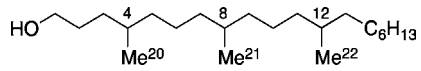


Figure 3. Expansions of the methyl region of the ^{13}C NMR spectra of the four samples of **1**: left, actual spectra with Traficante processing; right, simulated spectra based on predicted isomer ratios. From top to bottom, *syn/syn* (*S,S,S*), *anti/anti* (*S,R,S*), *anti/syn* (*R,S,S*), and *syn/anti* (*R,R,S*).

There are four isomers of **1** with three branched methyl groups each, so twelve signals might be expected in total. However, notice that each experimental spectrum contains the same seven peaks—three larger ones and four smaller ones. The differences between the spectra are which peaks are large and which are small.

With this limited information, it is already possible to assign all of the resonances by a process of elimination.[18] For example, isomers **1**-*syn,syn* and **1**-*syn,anti* share one large resonance at 19.67 ppm while the other large ones are different. The shared resonance has to belong to Me-20 because this is the only methyl group that has the same stereochemical relationship (*syn* to Me-21) in both compounds. Continuing to compare shared large peaks and stereochemical relationships provides a mutually consistent set of assignments[27] that is shown under the structures in Scheme 5 and collected in Table 1.

Table 1. The Seven Resonances of the Branched Methyl Groups in ^{13}C and ^{1}H NMR Spectra of 1



assignment	^{13}C NMR ppm	^{1}H NMR ppm
Me-20, *syn*	19.67	0.874
Me-20, *anti*	19.61	0.872
Me-21, *syn,syn*	19.79	0.844
Me-21, *syn/anti*[a]	19.73	0.842
Me-21, *anti/anti*	19.66	0.841
Me-22, *syn*	19.77	0.840
Me-22, *anti*	19.70	0.839

[a]Or *anti/syn*.

The chemical shift of each kind of methyl group (Me-20, 21, 22) is influenced by the configuration of the nearest stereocenter(s). Thus, there are only two resonances for Me-20 and two for Me-22 because these methyl groups can be *syn* or *anti* to Me-21. In turn, Me-21 has two neighbors, so it can have three relationships, *syn/syn*, *syn/anti* (or *anti/syn*), and *anti/anti*. It does not matter whether Me-21 is *anti* to Me-20 and *syn* to Me-22 or the reverse, so this is why it has three resonances rather than four.

The middle unit is comparable to the repeating monomer in assorted saturated polyisoprenoids $[CH_2CH_2CH(CH_3)CH_2]_n$. The absolute chemical shifts of Me-21 in the stereoisomers of **2** cannot be compared to the polymers because the polymer ^{13}C NMR spectra are acquired at high temperatures.[28] But the various polymer isomer units exhibit the same trends. The polymer terms for the adjacent stereochemical relationships are *meso* and *racemic*, and polymer resonances move slightly upfield in the order: *meso/meso* (*syn/syn*), *meso/rac* or *rac/meso* (*syn/anti* or *anti/syn*), *rac/rac* (*anti/anti*).

The isomer purities of the samples were assessed by ^{13}C NMR analysis with the aid of the simulated spectra shown in Figure 3. These simulations were produced by inputting the measured chemical shifts and the predicted isomer ratios (always 70/10/10/10, but with a different major isomer in each case). The good correspondence of the relative peak heights in the experimental and simulated spectra shows that the actual purities of the samples are similar to the projected purities. This in turn shows that the Roush crotylations on mixture samples (cycle 3) worked as well as those on individual samples (cycles 1, 2).

Though more complicated, the set of ^{1}H NMR spectra yield to the same analysis. There is extensive overlapping in the methyl region because of chemical shift compression, *J* coupling, and addition of a new resonance (the terminal methyl group, C19). Again Traficante processing is crucial, and again spectra were simulated with 70/10/10/10 isomer purities. These pairs of spectra are shown in Figure 4. The simulated spectra are easier to interpret; each sample exhibits three doublets and a triplet in the Me region of the spectrum.

The triplet for the terminal Me group resonates at 0.882 ppm in all of the isomers. The seven branched Me resonances are again listed with full assignments in Scheme 5 and Table 1. Five doublets (three for Me-21 and two for Me-22) are packed between 0.839 and 0.844 ppm, while the other two doublets

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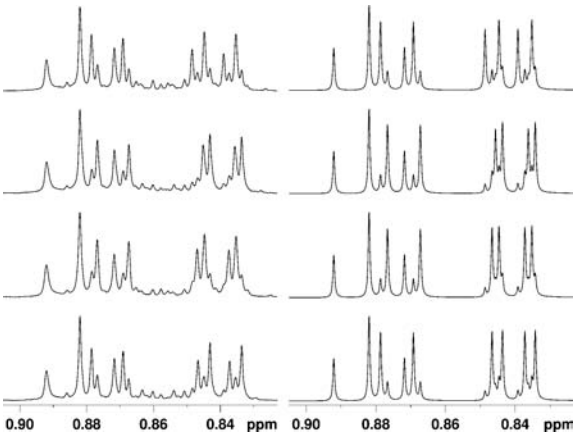
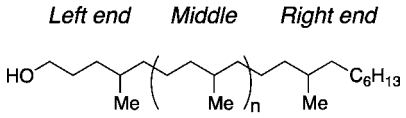


Figure 4. Expansions of the methyl region of the ^{1}H NMR spectra of the four samples of **1**: left, actual spectra with Traficante processing; right, simulated spectra based on predicted isomer ratios. From top to bottom, *syn/syn* (*S,S,S*), *anti/anti* (*S,R,S*), *anti/syn* (*R,S,S*), and *syn/anti* (*R,R,S*).

(Me-20) at 0.874 and 0.872 ppm overlap with each other and with the triplet of the terminal methyl group. Each peak is clear even though the entire range of peaks is less than 35 ppb (parts per billion).

The deduced assignments of the methyl resonances in the ^{1}H NMR spectra were confirmed by 1D TOCSY experiments that are shown in the SI. Finally, 2D COSY experiments correlated each ^{1}H methyl resonance with its ^{13}C counterpart to complete the assignments. These experiments prove that the deduced assignments are correct.

Predictions of NMR Spectra of Higher Oligomers. Nonadecanol **1** is a model for higher saturated oligoisoprenoid side chains like **17** and **18** (Figure 5). All these oligomers have



1, n = 1
17, n = 2
18, n = 3

Figure 5. The basis for prediction of spectra: oligomers **17** and **18** have the same left and right ends as **1**, and the middle unit simply repeats.

the same left and right chain ends. The repeating middle unit appears once in nonadecanol **1**, twice in **17**, and three times in **18** (which is the side chain of phosphomycoketide **3b** in Figure 1). The methyl regions of the spectra for all the isomers of **17**, **18**, and higher oligomers are readily predicted simply by assigning each carbon in an oligomer to one of seven chemical shifts of **1** and then plotting the results.

We simulated the ^{1}H and ^{13}C NMR spectra of the diagnostic methyl regions of the 8 diastereomers of **17** and the 16 diastereomers of **18**. These spectra (48 in total) are displayed in the SI.

The number of branched methyl resonances in the spectrum of each isomer of **17** depends on whether the relative configurations of the pair of middle fragments are the same (one peak, twice as large) or different (two peaks). Add these to the two different ends, and the spectra can have three resonances in a 2/1/1 ratio (present in four isomers) or four resonances in 1/1/1/1 ratio (present in the other four isomers). Although there are only two patterns of peak ratios, the chemical shifts vary in each. So every one of the eight diastereomers of **17** has a unique predicted ^{1}H and ^{13}C NMR spectrum.

Compound **18** has the same seven chemical shifts as **17** and **1**, but now the patterns of peak heights vary more because the three signals of the middle carbons can be present with relative intensities of 0, 1, 2, or 3 in a given spectrum. Globally now, there are only 14 (not 16) different predicted spectra with four spectra having three resonances (3/1/1), eight spectra having four resonances (2/1/1/1) and two spectra having the maximum five resonances (1/1/1/1/1). Twelve of the isomers have a unique spectrum, while two pairs of isomers have identical predicted spectra (both in the "four peak" class). The redundancies arise because the spectra do not differentiate middle carbons with *syn/anti* relationships from those with *anti/syn* relationships.[29]

Predicted spectra of higher oligomers are easily generated as well by the same method. They will never have more than five different peaks (for a pure isomer) because each compound can only have one kind of carbon on each end (*syn* or *anti*) and up to three kinds of carbons in the middle (*syn/syn*, *anti/anti*, *syn/anti* same as *anti/syn*). But the intensities of the peaks continue to vary with the number and configuration of the middle units.

Simulated pairs of ^{1}H and ^{13}C spectra of three variants of **18** are shown in Figure 6. These illustrate how the predictions are

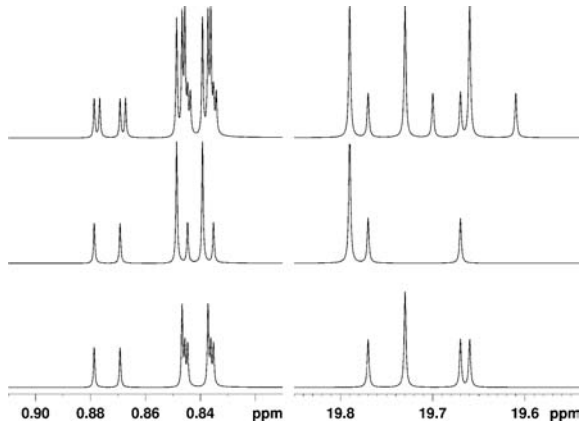


Figure 6. Predicted ^{1}H (left) and ^{13}C (right) NMR spectra for representative samples of **18**: top, an equimolar mixture of all 16 diastereomers; middle, the "all-*S*" (or "all-*R*") isomer with all four relationships *syn*; and bottom, the isomer with the relationship *syn/anti/anti/syn*.

made and what some typical differences are. The top pair of spectra are simulations of an equimolar mixture of all sixteen diastereomers of **18**. This is what the spectra of Crich's side-chain sample[9] might look like under our recording and processing conditions (though he made **3a** with a C_4H_9 tail, see below). There are only seven peaks in the model, so there are seven peaks in the simulated spectra of the all-isomer mixture. This is most easily seen in the ^{13}C NMR spectrum (upper right). The relative intensities of the peaks total to 3 for each of the three middle Me resonances and 1 for each of the four end Me resonances.

The spectra of the "all-*S*" isomer of **18** made by Feringa and Minnaard[10] might look the middle spectrum of Figure 6. In the

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^{13}C spectrum, the smaller peaks at 19.67 and 19.77 ppm are resonances for the two end carbons, both with adjacent *syn* relationships. All three middle carbons have *syn/syn* relationships, so they are predicted to come as one resonance at 19.79 ppm with a relative intensity of three.

Finally, the process can of course be conducted in reverse; that is, as an assignment rather than a prediction. For example, what isomer of **18** has the spectra exhibited in the bottom of Figure 6? Again looking at the simpler ^{13}C NMR spectrum, the peaks at 19.67 and 19.77 show that the right and left end methyl group both have *syn* relationships to their neighbors. The peak at 19.73, relative intensity two, shows that two middle carbons have a *syn/anti* relationship while the peak at 19.66 shows that the third is *anti/anti*.

The result is five pieces—*syn*, two times; *syn/anti*, two times; and *anti/anti,* one time—that fit together like a little puzzle. The two end pieces, *syn* and *syn*, have the three middle pieces in between. In the puzzle, each relationship has to match its adjacent relationship, and every *syn/anti* middle piece can be inserted either way (*syn/anti* or *anti/syn*). In this puzzle, for example, you have to put one of the *syn/anti* pieces after the first *syn* piece; you cannot put the *anti/anti* piece there. So these five pieces fit together in only one way (*syn-syn/anti-anti/anti-anti/syn-syn*), and this is the (4*S*,8*S*,12*R*,16*S*,20*R*) isomer of **18**.

Users of the predicted spectra sets need to keep in mind the assumption. We already know from the data set that the chemical shifts of the two end stereocenters are only affected by the configuration of the middle stereocenter, not by each other. This is the basis of the assumption that the chemical shifts can be predicted based only on nearest neighbor configurations. This assumption can only be tested by making higher oligomers. Even if it ultimately proves to be wrong, secondary effects of more remote stereocenters are likely to be smaller than the observed differences in **1**, so the predicted spectra still have value.

Notice also in Table 1 that the resonances of both *syn* ends are downfield from their *anti* counterparts, in both ^{1}H and ^{13}C spectra. And in the middle the chemical shifts have the same trend: *syn/syn*, most downfield; *syn/anti*, in the middle; *anti/anti*, most upfield. Converting this to an assumption (*syn* always downfield from *anti*), the spectra of other saturated oligoisopreniods having different right/left ends from **1** can be analyzed, even as mixtures.

For example, the right chain end of **3a** has a C_4H_9 group, not C_6H_{13}. And assorted compounds with other left end groups (other functional groups or oxidation states, shorter/longer chain lengths) are likewise of interest. A stereoisomer library of compounds with, say, the left end different from **1** will have five of the same resonances (middle and right end) and two new resonances. The two new resonances have to be from the stereocenter nearest to the left end, with the downfield one being *syn*, the upfield, *anti*. A library of isomers with both ends different will have four new resonances (two from each new end). Now one end pair has to be assigned spectroscopically (as we did with the 1D TOCSY experiments to prove the assignment of Me-20). Process of elimination gives the other end pair, then the "*syn* downfield" guideline completes the assignment.

To determine these chemical shifts and guidelines, we needed to make pure stereoisomers of **1** to assign the peaks. However, now that the model is complete, it becomes possible to make true mixtures of isomers in future work on compounds related but not identical to **1**. By comparing the model chemical shifts to those of an actual mixture according to the steps above, the chemical shifts of all the components can be extracted and assigned to their relevant isomers without ever recording spectra of the pure isomers.

■ CONCLUSIONS

Each of the four questions posed at the outset has been answered satisfactorily. First the combination of asymmetric crotylation reactions, thionocarbonate encoding, hydroformylation and Barton−McCombie deoxygenation allows for the reliable synthesis of saturated oligoisoprenoids by mixture methods. The strategy introduces extra hydroxy-bearing stereocenters in each cycle. These provide sites for tags and allow the use of asymmetric crotylation, one of a few reactions that can provide reliable access to all stereoisomers with complete reagent control.

Second, the bare minimum increment in fluorous tags has been reached with the quasiisomers of **16** having 0, 1, 2, or 3 total fluorine atoms. Indeed, these compounds do not even fit the accepted definition of a "fluorous compound".[30] More precisely, the technique here should be called "*fluorinated* mixture synthesis" rather than "*fluorous* mixture synthesis". But it is still "FMS", not just because the abbreviations coincide but because the unique interaction between fluorine atoms and the fluorous stationary phase is crucial to the separation. We even showed in principle that it is possible to go below the bare minimum with a "fractional tag" (*o*-fluorophenyl). However, in practice, the observed separation was tight and the incremental tags were better. Nonetheless, with improved separation, such fractional tags might be useful.

Third, we made the four enriched samples of the stereoisomers of 4,8,12-trimethylnonadecanol **1** and discovered that their ^{1}H and ^{13}C NMR spectra were very similar but not identical. With processing to enhance resolution, characteristic differences were identified in the methyl-group regions of the spectra. The 90/10 selectivity of the Roush crotylation left minor isomer impurities that conveniently functioned as controls in sorting out the NMR spectra of isomers **1** and proving that the Roush crotylation worked on mixtures as well as on single isomers. However, better stereoselectivites are desirable going forward to make higher oligomers like **17** and **18**.

Fourth, the simple correlation of the seven types of stereogenic carbon atoms to the seven methyl resonances in both the ^{1}H and ^{13}C NMR spectra of **1** can be extrapolated to predict the NMR spectra of oligomers, and the SI contains the 48 predicted spectra for all of the isomers of **17** and **18**. Spectra for higher oligomers up to polymers are readily generated.

This work sets the stage for synthesis of higher oligomer stereoisomer libraries like those of **17** and **18**. Such work could validate the predicted spectra or perhaps refine the predictions. This is especially important for mycoketide side chains like **18** because it now appears certain that careful analysis of NMR spectra of such compounds will yield valuable information about relative configuration and isomer purity.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Complete experimental details, additional figures, predicted spectra of the isomers of **17** and **18**, and ^{1}H and ^{13}C NMR spectra of all final products and key intermediate products. This

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material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

curran@pitt.edu

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Luo, Z. Y.; Zhang, Q. S.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766−1769. (b) Zhang, Q. S.; Rivkin, A.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 5774−5781.

(2) (a) Dandapani, S.; Jeske, M.; Curran, D. P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12008−12012. (b) Zhang, Q. S.; Lu, H. J.; Richard, C.; Curran, D. P. *J. Am. Chem. Soc.* **2004**, *126*, 36−37. (c) Dandapani, S.; Jeske, M.; Curran, D. P. *J. Org. Chem.* **2005**, *70*, 9447−9462.

(3) (a) Yang, F.; Newsome, J. J.; Curran, D. P. *J. Am. Chem. Soc.* **2006**, *128*, 14200−14205. (b) Curran, D. P.; Zhang, Q. S.; Lu, H. J.; Gudipati, V. *J. Am. Chem. Soc.* **2006**, *128*, 9943−9956. (c) Curran, D. P.; Zhang, Q. S.; Richard, C.; Lu, H. J.; Gudipati, V.; Wilcox, C. S. *J. Am. Chem. Soc.* **2006**, *128*, 9561−9573. (d) Jung, W.-H.; Guyenne, S.; Riesco-Fagundo, C.; Mancuso, J.; Nakamura, S.; Curran, D. P. *Angew. Chem., Int. Ed.* **2008**, *47*, 1130−1133. (e) Curran, D. P.; Sui, B. *J. Am. Chem. Soc.* **2009**, *131*, 5411−5413. (f) Moretti, J. D.; Wang, X.; Curran, D. P. *J. Am. Chem. Soc.* **2012**, *134*, 7963−7970.

(4) Curran, D. P.; Sinha, M. K.; Zhang, K.; Sabatini, J. J.; Cho, D.-H. *Nat. Chem.* **2012**, *4*, 124−129.

(5) (a) Baker, J. W.; Myers, C. W. *Pharm. Res.* **1991**, *8*, 763−770. (b) Brownstein, S.; Burton, G. W.; Hughes, L.; Ingold, K. U. *J. Org. Chem.* **1989**, *54*, 560−569.

(6) Heathcock, C. H.; Finkelstein, B. L.; Jarvi, E. T.; Radel, P. A.; Hadley, C. R. *J. Org. Chem.* **1988**, *53*, 1922−1942.

(7) Ohrui, H. *Proc. Jpn. Acad., Ser. B* **2007**, 83.

(8) Moody, D. B.; Ulrichs, T.; Muhlecker, W.; Young, D. C.; Gurcha, S. S.; Grant, E.; Rosat, J.-P.; Brenner, M. B.; Costello, C. E.; Besra, G. S.; Porcelli, S. A. *Nature* **2000**, *404*, 884−888.

(9) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2002**, *124*, 2263−2266.

(10) van Summeren, R. P.; Moody, D. B.; Feringa, B. L.; Minnaard, A. J. *J. Am. Chem. Soc.* **2006**, *128*, 4546−4547.

(11) de Jong, A.; Arce, E. C.; Cheng, T.-Y.; van Summeren, R. P.; Feringa, B. L.; Dudkin, V.; Crich, D.; Matsunaga, I.; Minnaard, A. J.; Moody, D. B. *Chem. Biol.* **2007**, *14*, 1232−1242.

(12) (a) Buitrago Santanilla, A.; Leighton, J. L. In *Stereoselective Synthesis 2: Stereoselective Reactions of Carbonyl and Imino Groups*; Molander, G. A., Ed.; Georg Thieme Verlag: Stuttgart-New York, 2010; Vol. 2, pp 401−447. (b) Hoffmann, R. W. In *Asymmetric Synthesis—The Essentials*; Christmann, M., Bräse, S., Eds.; Wiley-VCH: Weinheim, 2007; pp 29−33.

(13) (a) Crich, D.; Quintero, L. *Chem. Rev.* **1989**, *89*, 1413−1432. (b) Saraiva, M. F.; Couri, M. R. C.; Le Hyaric, M.; de Almeida, M. V. *Tetrahedron* **2009**, *65*, 3563−3572. (c) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574−1585.

(14) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932−933.

(15) Gladysz, J. A. In *The Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds.; Wiley-VCH: Weinheim, 2004; pp 41−55.

(16) (a) Zhang, W. *Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds.; Wiley-VCH: Weinheim, 2004; pp 222−236. (b) Curran, D. P. In *The Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds.; Wiley-VCH: Weinheim, 2004; pp 128−156.

(17) Ramachandran, P. V. *Aldrichimica Acta* **2002**, *35*, 23−35.

(18) For extensive additional information about the synthesis, characterization, and spectral predictions, see: Yeh, E. A.-H. Ph.D. Thesis, University of Pittsburgh, 2012.

(19) Curran, D. P. In *The Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds.; Wiley-VCH: Weinheim, 2004; pp 101−127.

(20) Roush, W. R.; Ando, K.; Powers, D. B.; Palkowitz, A. D.; Halterman, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 6339−6348.

(21) The carbon numbers of all the intermediates are based on the numbering of the final product **1**.

(22) Breit, B.; Seiche, W. *J. Am. Chem. Soc.* **2003**, *125*, 6608−6609.

(23) (a) Pellissier, H. *Tetrahedron* **2003**, *59*, 8291−8327. (b) Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475−1490.

(24) Zhang, Q. S.; Curran, D. P. *Chem.—Eur. J.* **2005**, *11*, 4866−4880.

(25) (a) Ueng, S.-H.; Makhlouf Brahmi, M.; Derat, É.; Fensterbank, L.; Lacôte, E.; Malacria, M.; Curran, D. P. *J. Am. Chem. Soc.* **2008**, *130*, 10082−10083. (b) Ueng, S.-H.; Fensterbank, L.; Lacôte, E.; Malacria, M.; Curran, D. P. *Org. Lett.* **2010**, *12*, 3002−3005.

(26) Traficante, D. D.; Nemeth, G. A. *J. Magn. Reson.* **1986**, *71*, 237−245.

(27) "Mutually consistent" means that the same assignments emerge independent of how you conduct the process of elimination.

(28) Depending on how they are made, comparable polymers are called poly(1-methyltetramethylene) or alternating copolymers of ethylene and propylene. (a) Zetta, L.; Gatti, G.; Audisio, G. *Macromolecules* **1978**, *11*, 763−766. (b) Fan, W.; Leclerc, M. K.; Waymouth, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 9555−9563.

(29) For example, the isomer with *syn*/*syn*/*anti*/*syn* relative configuration (two *syn* ends and the middle carbons in the order *syn*/*syn* next to *syn*/*anti* next to *anti*/*syn*) has the same four model carbons in the same ratio (2/1/1/1) as the isomer *syn*/*anti*/*syn*/*syn* (two *syn* ends and the middle carbons in the order *syn*/*anti* next to *anti*/*syn* next to *syn*/*syn*).

(30) Gladysz, J. A.; Curran, D. P. *Tetrahedron* **2002**, *58*, 3823−3825.