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## Binary fluorous tagging enables the synthesis and separation of a sixteen-stereoisomer library of macrosphelides

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### Abstract

Fluorous mixture synthesis minimizes the effort to synthesize small molecule libraries by labeling the molecules rather than the reaction vessels. Reactants are labeled with fluorinated tags and products can later be demixed based on fluorine content. A limit in the number of available tags can be overcome by using binary encoding so that a total of four tags can be used to uniquely label a library of sixteen compounds. This strategy, however, means that separation based on fluorine content alone is no longer possible. Here, we solve this problem by selectively removing one tag after an initial demixing step; a second demixing provides each individual compound. The usefulness of this strategy is demonstrated by synthesizing a library containing all sixteen diastereomers of the natural products macrosphelides A and E. Macrosphelide D was not in this library, and so its assigned structure is incorrect. We determined its constitution by using NMR spectroscopy and its configuration by synthesizing four candidate stereoisomers.

### Introduction

Fluorous mixture synthesis (FMS) is a powerful technique for minimizing the effort in making small libraries of complex organic molecules in individual, pure form.<sup>1-3</sup> Whereas the reaction vessels are labeled in traditional parallel synthesis, it is the molecules that are labeled in FMS. For example, stereoisomer libraries of natural products can be made by using the structure of a fluorous tag to encode the configuration of one or more stereocenters of the target. The resulting differentially tagged molecules are called quasiisomers.<sup>4</sup> Syntheses can be conducted on mixtures of quasiisomers (saving effort), then late stage demixing based on the fluorous tags separates the quasiisomers. Finally, detagging gives the true stereoisomers.

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Fluorous tags serve varying purposes in organic synthesis.<sup>5-8</sup> The fluorous tags in FMS serve two roles—they identify the tagged quasiisomers (information) and they provide for their separation by fluorous HPLC (demixing). Both roles are based on fluorine content. The second role is referred to as a “strategic separation”<sup>9</sup> because the separation is dictated primarily by the tag, not the molecule that is tagged.

Fluorous tags usually bear simple perfluoroalkyl groups ( $R_F$ ,  $-(CF_2)_nF$ ) for the encoding, so the number of tags (and therefore tagged components) in a mixture would appear to be large. However, this is not so. Groups from  $R_F = CF_3$  up to about  $C_9F_{19}$  are preferred because larger tags begin to convey undesirable teflon-like properties such as low solubility. Within this already limited tag set, precursors with even numbers of carbon atoms are less expensive and more readily available than those with odd numbers.

So four groups emerge as especially practical,  $C_2F_5$ ,  $C_4F_9$ ,  $C_6F_{13}$ , and  $C_8F_{17}$ , and it is important to leverage these groups as much as possible. In standard single tagging, the four fluorous tags are used to encode and mix four molecules. For example, we made four-compound mixtures of pinesaw fly sex pheromones by using fluorous tags to encode the configurations at C2 and C3 with a fluorous PMB tag on C2 (Figure 1a).<sup>10,11</sup> Each tag has a different number of fluorines, and this forms the basis of demixing by fluorous HPLC.<sup>12</sup>

The approach has been extended to double tagging as illustrated with lagunapyrone.<sup>13</sup> Here a pair of two-compound mixtures was coupled together to make a four-compound mixture of quasiisomers (Figure 16). In this application, the final tagged compounds are demixed based on the sum of two tags. Variants of this approach were used for cytostatins<sup>14</sup> and passifloricins.<sup>15</sup>

Double tagging with fluorous tags and oligoethyleneglycol (OEG) tags<sup>16</sup> allows 16 compounds to be made in one mixture, as illustrated with murisolin stereoisomers (Figure 6).<sup>17,18</sup> Here eight different tags are needed, four fluorous and four OEG, to make the “double mixture”. Both a fluorous and an OEG demixing (double demixing) are needed to separate this mixture. In this approach, the protecting groups bearing the tags are identical or very similar (here DMB and PMB) so that they can be removed together under the same conditions after demixing. For practical reasons, this approach is best applied when two large fragments are made separately by single mixture synthesis (one fluorous-tagged mixture and one OEG-tagged mixture), then coupled very late in the synthesis to make the double mixture. This was the strategy used to make the murisolin stereoisomer library.

Purely from the information standpoint, the most efficient method of tagging is to use simple binary encoding with a single class of tag. In principle, sixteen compounds could be tagged at two sites with only four fluorous groups ( $2^4 = 16$ ), thirty-two with only five groups ( $2^5 = 32$ ), and so on. However, binary fluorous encoding seems impractical at first glance because of redundancy. For example, a binary-tagged mixture with pairs of the four fluorous  $R_F$  groups would have four different tagged compounds containing 22 fluorines ( $C_2F_5/C_8F_{17}$ ,  $C_4F_9/C_6F_{13}$ ,  $C_6F_{13}/C_4F_9$ ,  $C_8F_{17}/C_2F_5$ ). There are other groups of two and three compounds in this mixture that have redundancies as well (see Figure 2 below). In short, binary encoding works for identification but not separation.

We recognized that all the redundancies in a binary-encoded mixture could be lifted to enable separation by sequential removal of the two tags. To prove the principle, we report here synthesis and isolation of sixteen stereoisomers of macrospinelide natural products A<sup>19,20</sup> and E<sup>21-23</sup> (**1-A** and **1-E**, below) from a single mixture of sixteen tagged quasiisomers encoded with only four different R<sub>F</sub> groups. We also expected this library to contain macrospinelide D (**1-D**),<sup>24</sup> but it did not. We deduced the correct structure of **1-D** from spectra, then proved this by synthesis.



This new binary encoding capitalizes on orthogonal protecting groups,<sup>25-27</sup> which are commonly used in synthesis to hide or reveal functional groups at predetermined times. Here we have no need for that application; instead we retool orthogonal protecting groups for the first time for use in strategic separation.

## Results

### Binary tagging with selective tag removal

Figure 2a shows the tag encoding pattern for sixteen compounds that results from a  $4 \times 4$  tagging with two fluororous tags in the “even” perfluoroalkyl series (C<sub>2</sub>F<sub>5</sub>, C<sub>4</sub>F<sub>9</sub>, C<sub>6</sub>F<sub>13</sub>, C<sub>8</sub>F<sub>17</sub>). Only two compounds, the first with 10 fluorines and the last with 34, have unique fluorine contents. The others all share the same number of fluorine atoms with one, two or three other members of the set. This is the redundancy problem.

Imagine an ideal HPLC demixing of this set of compounds, which should produce seven fractions based on fluorine content. The first and last compounds are already individual and can be detagged. Now imagine that the tags are orthogonal, and that either one (but not both) of the two tags is removed from the other five mixtures. This breaks all the remaining redundancies, as illustrated for fraction 4 (22 fluorines) in Figure 2b. Demixing of the mono-detagged fractions followed by detagging now produces all sixteen target compounds as individual samples of unambiguous structure based on the tag pattern.

### FMS of the macrospinelide stereoisomer library

To test this idea of double tagging/double demixing, we selected the stereoisomeric natural products macrospinelides A, D and E, because they and their congeners have high anti-cancer activity,<sup>18-22</sup> because their syntheses are well established,<sup>20,28-31</sup> and especially because they have two identical sub-units (C5–C9 and C11–C15). Accordingly four stereoisomers of this sub-unit (**2**, Figure 3a) will provide a stereoisomer library of macrospinelides containing all 16 diastereomers with the *S* configuration fixed at C3 and all possible configurations at the other four stereocenters. This library should contain macrospinelide A (**1-A**) and the enantiomer of macrospinelide E (ent-**1-E**). One of the

remaining 14 isomers should be macrosphelide D (**1-D**), whose stereostructure was not completely assigned.

The structures of subunits **2** and <sup>F</sup>PMB- and <sup>F</sup>TIPS-tagged pairs of quasiisomers **3** and **4** are summarized in Figure 3a,b. The four individual stereoisomeric precursors **2** are readily available in 2–5 steps by modifying the asymmetric dihydroxylation route of Omura and Smith.<sup>20</sup> These were each tagged with a fluorine PMB group (<sup>F</sup>PMB) and the TES group was removed in one step to give a first set of quasiisomers **3a-d**.

Tagging of the isomers of **2** with a fluorine silyl group (<sup>F</sup>TIPS) followed by mixing and removal of the trichloroethyl ester with zinc dust gave a second set of quasiisomers **4a-d**. The same encoding was used for both groups. In other words, the (*R,R*)-isomer was encoded with both C<sub>2</sub>F<sub>5</sub><sup>F</sup>PMB and <sup>F</sup>TIPS groups (“a” series), the (*S,S*) with C<sub>4</sub>F<sub>9</sub> groups (“b” series), and so on (“c” and “d” series).

The reaction steps of the fluorine mixture stage of the synthesis are summarized in Figure 3c. The four quasiisomers **3a-d** bearing the <sup>F</sup>PMB groups were mixed and then coupled with the TBS ether of (*S*)-3-hydroxybutanoic acid (**S-5**) to give **M-6a-d**. The TBS group was removed and the resulting mixture was coupled with the four-component quasiisomer mixture of acids **4a-d**. This gave the first 16-quasiisomer mixture **M-7a-d/a-d**. The TES group was removed with HCl and the 2,2,2-trichloroethyl ester was cleaved with zinc dust. The resulting hydroxy acid mixture was subjected to Yamaguchi lactonization<sup>32</sup> to give macrolactone **M-8a-d/a-d**. This 16-quasiisomer mixture exhibited a single spot on standard silica TLC analysis, so it was purified by flash chromatography to provide the sample for demixings.

Table 1 summarizes the relevant information on the final mixture of tagged quasiisomers **8**, including tagging pattern, configuration and HPLC fraction. After macrolactonization, the fluorine analytical HPLC trace (see Supporting Information) was more complex than the seven peaks implied by Figure 2a. In addition to the primary separation based on fluorine content, some secondary separation based on the tag structures or the structure of the macrolactone component evidently occurred. Nonetheless, this secondary separation was small relative to the tag-based separation, leaving obvious gaps between the quasiisomers of different fluorine content. In the preparative demixing of **8** we collected the first five fractions based on the fluorine content, deliberately combining any partially resolved peaks with same fluorine content. The two components of fraction 6 were well resolved and were collected separately. Like fraction 1, fraction 7 was a single compound.

A set of <sup>19</sup>F NMR spectra confirmed that the fractions contained the expected tags. Reading the spectra of fractions 6a and 6b also identified these samples. The four single component fractions (1, 6a, 6b, 7) were directly detagged to give the first four pure isomers. The remaining four fractions had 2, 3 or 4 quasiisomers with the same fluorine content. The <sup>F</sup>TIPS group was removed from these fractions with TBAF, leaving only the <sup>F</sup>PMB group. Then these fractions were demixed into the individual components, which now have different fluorine content.

This process is exemplified with the second fraction in Figure 4. The two components of fraction 2 after the first demixing each have 14 fluorine atoms. The (3*S*,8*S*,9*S*,14*R*,15*R*) component **8ab** with the C<sub>2</sub>F<sub>5</sub> PMB tag and the C<sub>4</sub>F<sub>9</sub> silyl tag is mixed with its (3*S*,8*R*,9*R*,14*S*,15*S*)-isomer **8ba** bearing the C<sub>4</sub>F<sub>9</sub> PMB tag and the C<sub>2</sub>F<sub>5</sub> silyl tag.

The silyl tag was removed from this two-component mixture to provide the (3*S*,8*S*,9*S*,14*R*,15*R*) isomer **9a** with the C<sub>2</sub>F<sub>5</sub> PMB mixed with the (3*S*,8*R*,9*R*,14*S*,15*S*)-isomer **9b** bearing the C<sub>4</sub>F<sub>9</sub> PMB tag (84%). These two compounds were readily demixed by fluororous HPLC. The PMB tags were removed to give the true macrospheptide isomers in individual, pure form. The yields for PMB removal were 76% and 65%, respectively, after flash chromatography. These yields are typical of those obtained from the other 14 quasiisomers.

Likewise, the other three mixed fractions were processed to give all sixteen true macrospheptide isomers (see Supplementary Figure S2). Each isomer was purified by preparative reverse phase HPLC to provide the final samples (1–2 mg each), which were fully characterized by the usual means. Copies of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of all the isomers are contained in the Supporting Information.

Despite the structural similarities, each of the 16-macrospheptide stereoisomers exhibited unique <sup>1</sup>H (700 MHz) and <sup>13</sup>C (175 MHz) NMR spectra. As expected, the spectra for the 3*S*,8*R*,9*S*,14*R*,15*S* isomer (**1-A**) matched those of macrospheptide A while the spectra for the 3*S*,8*S*,9*R*,14*S*,15*R* (ent-**1-E**) isomer matched those of macrospheptide E. These matches show that the double mixture synthesis succeeded, and accordingly provide proof of the principle of binary fluororous mixture synthesis with sequential tag removal. Though the principal goal was now accomplished, an unexpected problem presented itself.

### Correction of the structure of macrospheptide D and confirmation of the structure of macrospheptide M

To our surprise, the spectral data reported for macrospheptide D<sup>20</sup> did not match any of the 16 sets of spectra from the stereoisomer library. This proves that macrospheptide D is not a stereoisomer of macrospheptides A and E. While the <sup>1</sup>H and <sup>13</sup>C resonances for the C1–C9 region of macrospheptide D fall within the range of those of the stereoisomer library members, there are significant differences in the C10–C15 region.

The resonances H14 and H15 in the <sup>1</sup>H NMR spectrum were especially informative (Figure 5a). In all 16 macrospheptide isomers, these fall into narrow ranges: 4.17–4.49 ppm for H14 and 4.85–5.27 ppm for H15. In contrast, at 5.05 ppm, H14 of macrospheptide D is too far downfield. And at 4.06 ppm, H15 is too far upfield. A similar problem was evident in the <sup>13</sup>C NMR spectra. Based on these values, we hypothesized that macrospheptide D was a ring-contracted constitutional isomer of A and E with the acyl group of C1 bonded to O14 rather than O15.

Candidate structures for macrospheptide D are shown in Figure 5a with the same carbon numbering system as the A/E series. If macrospheptide D has the same configurations at the pairs of adjacent stereocenters as macrospheptides A and E, then it could be either **10** (C3 S) or **11** (C3 R). Structure **11** is already a known natural product called macrospheptide M.<sup>33</sup>

Unfortunately, the spectra of macrosphelide D are reported in chloroform- $d_1$  and those of macrosphelide M are in acetone- $d_6$ , so we could not assess at this point whether the two natural samples are the same or different. However, since macrosphelides A and D were isolated from the same broth, the structure **10** seems more likely than **11**.

We also considered two other similar structures, **12** and **13**, for macrosphelide D. Here it is C11 that is contracted down from the oxygen on C9 to the adjacent oxygen on C8. This seemed prudent since two of the hydroxy ester sub-units of these tris-lactones are so similar.

The four candidate isomers **10-13** were synthesized individually from suitably protected fragments bearing standard protecting groups: TBS (*t*-butyldimethylsilyl) to block hydroxy groups needed for esterification, and MEM (methoxyethoxy methyl) to block free hydroxy groups. Isomer **10** proved to be macrosphelide D, and its synthesis is summarized in Figure 5b. Alcohol (*S*)-**14** was coupled with acid **15** bearing MEM and TBS protecting groups on the oxygens of C8 and C9, respectively. Removal of the TBS group produced **16**, which in turn was coupled with **17** (a constitutional isomer of **15** with the protecting groups reversed) to make the second ester at C11.

Removal of the TBS group on O14 followed by reductive deesterification provided hydroxy acid **18** ready for macrolactonization. Yamaguchi reaction as above, then removal of the two MEM groups with TFA, provided target structure **10**. All the reactions in this sequence worked well, except for the Yamaguchi reaction sequence, which gave only 26% yield. This yield includes the prior deesterification step; however, the macrolactonization step was clearly the problem. Nonetheless, ample **10** (8 mg) was obtained for complete characterization.

Figure 5c summarizes the syntheses of the other three isomers. These sequences were generally similar to the one in Figure 5b (see Supplementary Schemes S1-S4). However, since all the suitably protected fragments were readily available, we decided to vary the order of the esterification steps. Isomer **11** was made by uniting fragments **17**, **19**, and R-**5**, first by making an ester from C11 to the oxygen on C9, then from C1 to the oxygen on C14. Finally, macrolactonization between C5 and the oxygen on C3 and deprotection provided **11**. Gratifyingly, the two-step sequence of reductive deesterification and Yamaguchi macrolactonization worked in an improved 56% overall yield. Yields of the other steps were comparable to those for **10** (see Supporting Information).

The bond for final macrolactonization was changed again for isomers **12** and **13**. These were made by uniting fragments **17**, **19**, and R- or S-**5**, first by making an ester from C1 to the oxygen on C15, then from C5 to the oxygen on C3. Now macrolactonization between C11 and the oxygen on C8 and deprotection provided **11** and **12**. The final three steps (reductive deesterification, Yamaguchi macrolactonization and removal of the MEM group) were conducted with purification of the intermediates to provide **12** and **13** in 38% and 42% overall isolated yields, respectively.

We recorded  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the four candidate structures in chloroform- $d_1$  (for comparison of the reported data for macrosphelide D) and acetone- $d_6$  (for comparison of the

reported data for macrosphelide M). These are data tabulated in Supplementary Tables S2 and S3. The first step was to compare the spectra of the new samples **10-13** to each other. As expected from the prior library spectra, the four groups of four spectra were similar. Yet each spectrum was unique, exhibiting significant differences with its three comparison spectra.

Finally, by comparing these spectra to those reported, we learned 1) that the assigned structure **11** of macrosphelide M is correct, 2) that macrosphelide M and D are not the same or enantiomers, and 3) that macrosphelide D is indeed **10**. So macrosphelide D and M are ring-contracted constitutional isomers of macrosphelides A and E, respectively.

Translactonization can be induced by acid or base<sup>34</sup> and has been observed with many other hydroxy macrolactone natural products. Recent examples include apoptolidin,<sup>35,36</sup> dolabelide<sup>37</sup> and dictyostatin.<sup>38</sup> This raises the question of whether D/M and A/E are all natural products, or whether trans-lactonization occurs during isolation. The reported conditions for isolation of these natural products do not involve acid or base treatment, so the isomers may occur naturally. If the opportunity arises, then it would be interesting to look for the iso-D and iso-M structures **12** and **13** in extracts with macrosphelides A, D, E, and M. In addition, each constellation of stereocenters has a possible double ring contracted product in which translactonization occurs at both possible hydroxy lactone subunits.

## Discussion

Binary encoding of sixteen compounds with four fluororous tags ( $R_F$ ) provides a unique identity for each compound but does not provide for the complete separation of the mixture based on fluorine content due to redundancies. This problem is solved by selective removal of either one of the tags after the first demixing. A second demixing of the resulting mixed fractions provides each compound individually.

The principle of double tagging and double demixing has been proved by the synthesis of a library of all sixteen diastereomers of the natural products macrosphelides A and E. The core mixture stage of the synthesis (Figure 2c) required only 6 chemical reactions to make all the isomers. Contrast this to an analogous parallel synthesis approach to the stereoisomer library, which would require 72 reactions.

The spectra for the natural product macrosphelide D did not match any of those of the library members. This problem was unexpected, but having the complete stereoisomer library helped us to solve it quickly. First, we knew immediately that macrosphelide D was not a stereoisomer of A and E. Working in the traditional way, if we had made one candidate stereoisomer for D and found that its spectra did not match the natural products, then we probably would have first followed by making other candidate stereoisomers. In a worst case, 14 more isomers would have had to be made serially to learn that it was the constitution and not the stereostructure that was the problem.

Second, having the complete library of macrosphelide A/E spectra, we could quickly see what data fit with macrosphelide D and what did not. This led us directly to a set of four candidate isomers, one of which proved to be the natural product. Finally, since we had

already learned that all the tris-lactone stereoisomers of macrosphelides A and E had unique spectra, it was unnecessary to make the complete library of D/M stereoisomers. There cannot be other stereoisomers in this library that have spectra substantially identical to those of D or M.

So we set out to use the known structures of macrosphelides A and E to help prove the principle of fluororous mixture synthesis with binary tagging, then to use the other members of library to prove the stereostructure of macrosphelide D. It turned out that macrosphelide D was not a stereoisomer of A and E, yet the data from the library members still led us quickly to the correct structure.

## Methods

The Supporting Information contains detailed descriptions of the synthesis and demixings, characterization data for all new individual compounds, and copies of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for the 16-stereoisomer library of **1** and the candidate stereoisomers of macrosphelide D.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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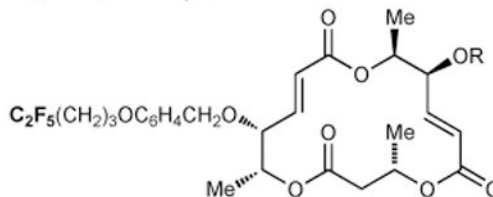
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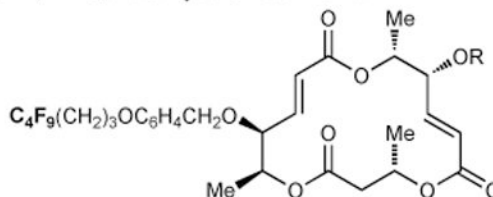
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3*S*,8*S*,9*S*,14*R*,15*R* quasiisomers

double tagged: **8ab**, R = Si(<sup>i</sup>pr)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>4</sub>F<sub>9</sub>

mono detagged: **9a**, R = H

3*S*,8*R*,9*R*,14*S*,15*S* quasiisomers

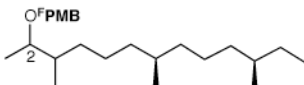
double tagged: **8ba**, R = Si(<sup>i</sup>pr)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>2</sub>F<sub>5</sub>

mono detagged: **9b**, R = H

**Figure 1.**

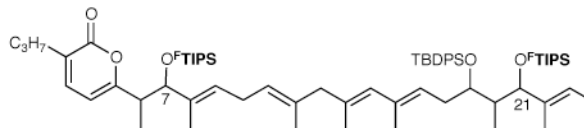
Single and double tagging strategies: (a) four individual fluorous tags provide four pinesaw fly pheromones; (b) two pairs of fluorous tags provide four lagunapyrones, but four different tags are still needed to prevent redundant fluorine content; (c) orthogonal tagging with four fluorous tags and four oligoethylene glycol (OEG) tags provides sixteen murisolins.

## (a) Pinesaw fly pheromones



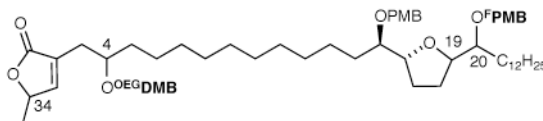
FPMB =  $\text{CH}_2\text{C}_6\text{H}_4\text{O}(\text{CH}_2)_3\text{R}_F$   
 4 fluorous  $\text{R}_F$  FPMB groups encode  
 4 quasiisomers at C2 and C3

## (b) Lagunapyrones



FTIPS =  $\text{Si}(\text{iPr}_2)(\text{CH}_2)_3\text{R}_F$   
 4 fluorous  $\text{R}_F$  FTIPS groups encode 4 quasiisomers,  
 two at C6, C7 and two at C19-C21

## (c) Murisolins



FPMB =  $\text{CH}_2\text{C}_6\text{H}_4\text{O}(\text{CH}_2)_3\text{R}_F$   
 OEGDMB =  $\text{CH}_2\text{C}_6\text{H}_3(\text{OMe})(\text{OCH}_2\text{CH}_2)_n\text{OMe}$ ,  $n = 1-4$   
 4 FPMB groups encode C19, C20  
 4 OEGDMB groups encode C4, C34

(a) First demixing produces seven fractions based on total F content

Fluorous HPLC retention time  $\rightarrow$

				$C_8F_{17}$ $C_2F_5$			
			$C_6F_{13}$ $C_2F_5$	$C_6F_{13}$ $C_4F_9$	$C_8F_{17}$ $C_4F_9$		
	$C_4F_9$ $C_2F_5$	$C_4F_9$ $C_4F_9$	$C_4F_9$ $C_6F_{13}$	$C_6F_{13}$ $C_6F_{13}$	$C_8F_{17}$ $C_6F_{13}$		
$C_2F_5$ $C_2F_5$	$C_2F_5$ $C_4F_9$	$C_2F_5$ $C_6F_{13}$	$C_2F_5$ $C_8F_{17}$	$C_4F_9$ $C_8F_{17}$	$C_6F_{13}$ $C_8F_{17}$	$C_8F_{17}$ $C_8F_{17}$	
fraction #	1	2	3	4	5	6	7
# of F atoms	10	14	18	22	26	30	34
# of compds	1	2	3	4	3	2	1

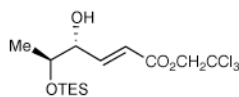
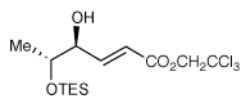
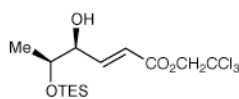
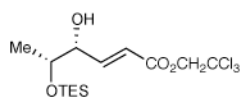
(b) Removal of tag 1 and second demixing, exemplified with fraction 4; four single compounds are produced

Fluorous HPLC retention time  $\rightarrow$

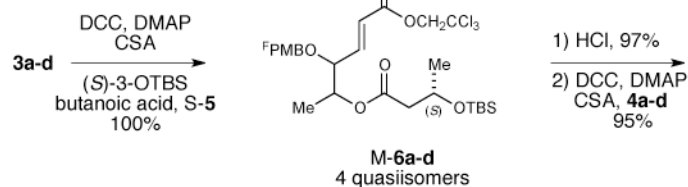
$C_8F_{17}$ $C_2F_5$	$C_6F_{13}$ $C_4F_9$	$C_4F_9$ $C_6F_{13}$	$C_2F_5$ $C_8F_{17}$
-------------------------	-------------------------	-------------------------	-------------------------

tag 1 (struck out) is removed before HPLC injection

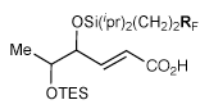
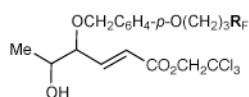
(a) stereoisomeric subunits **2**



(c) fluorous mixture synthesis

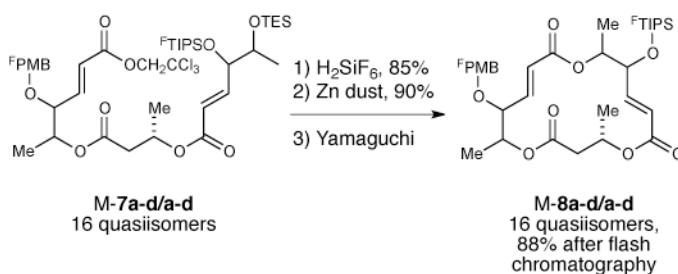


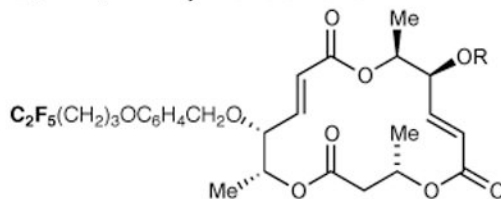
(b) tagged quasiisomers



encoding scheme for  
FPMB and FTIPS

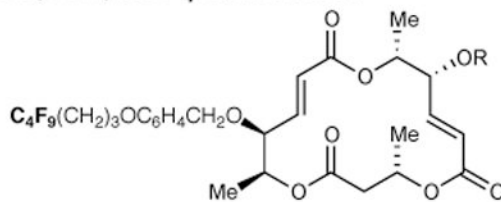
letter	config.	R <sub>F</sub>
a	(R,R)	C <sub>2</sub> F <sub>5</sub>
b	(S,S)	C <sub>4</sub> F <sub>9</sub>
c	(S,R)	C <sub>6</sub> F <sub>13</sub>
d	(R,S)	C <sub>8</sub> F <sub>17</sub>



*3S,8S,9S,14R,15R quasiisomers*

double tagged: **8ab**, R = Si(<sup>i</sup>pr)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>4</sub>F<sub>9</sub>

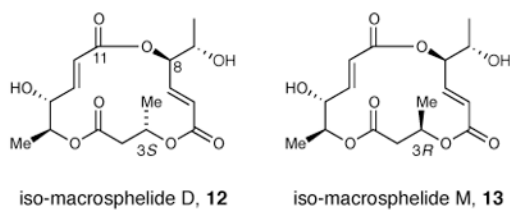
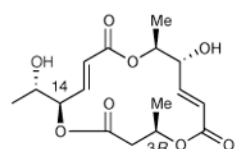
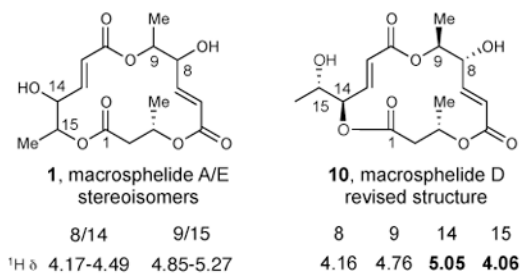
mono detagged: **9a**, R = H

*3S,8R,9R,14S,15S quasiisomers*

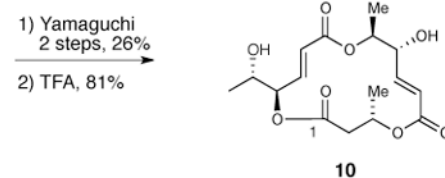
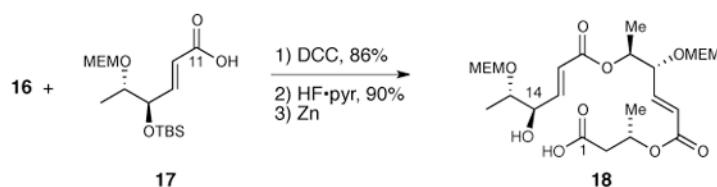
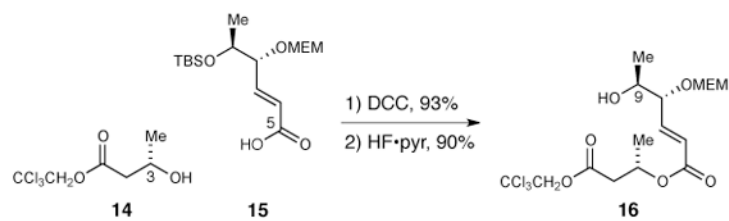
double tagged: **8ba**, R = Si(<sup>i</sup>pr)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>2</sub>F<sub>5</sub>

mono detagged: **9b**, R = H

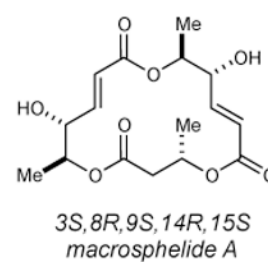
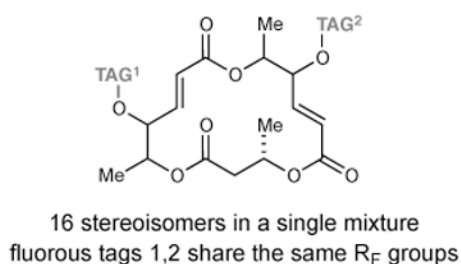
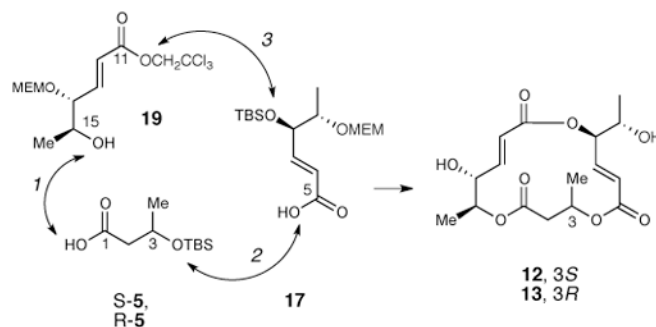
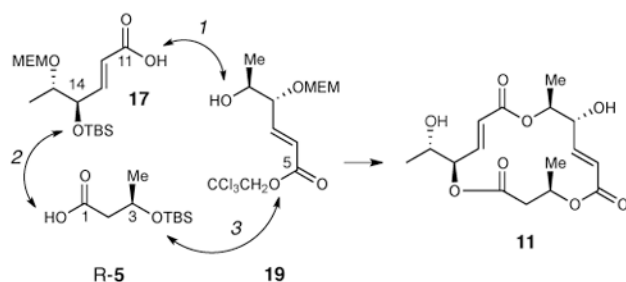
## (a) what is macrophelide D?

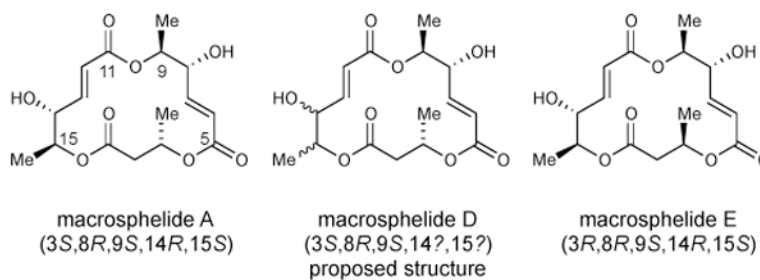


## (b) synthesis of candidate 10



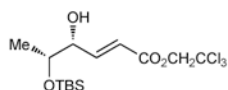
## (c) outlines of the syntheses of 11-13



**Figure 2.**

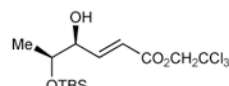
Binary tagging with four fluororous tags. Redundancies are lifted by sequential (rather than simultaneous) removal of the tags: (a) the encoding pattern and HPLC retention order of 16 compounds with four  $R_F$  groups; (b) detagging and second demixing exemplified with fraction 4 (22 fluorine atoms) produces four individual compounds.





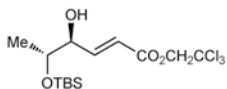
(4R,5R)-2a

(4*R*,5*R*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-hydroxyhex-2-enoate



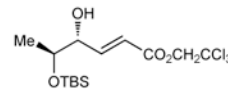
(4S,5S)-2b

(4*S*,5*S*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-hydroxyhex-2-enoate



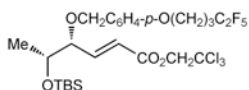
(4S,5R)-2c

(4*S*,5*R*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-hydroxyhex-2-enoate



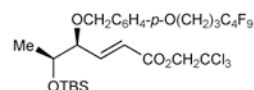
(4R,5S)-2d

(4*R*,5*S*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-hydroxyhex-2-enoate



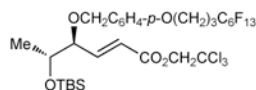
3a

(4*R*,5*R*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-((4-(4,4,5,5,5-pentafluorophenyl)oxy)benzyl)oxy)hex-2-enoate



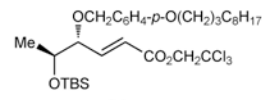
3b

(4*S*,5*S*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-((4-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)benzyl)oxy)hex-2-enoate



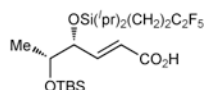
3c

(4*S*,5*R*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-((4-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)benzyl)oxy)hex-2-enoate



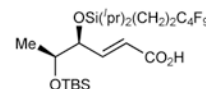
3d

(4*R*,5*S*,*E*)-2,2,2-trichloroethyl 5-((*tert*-butyldimethylsilyl)oxy)-4-((4-((4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptafluoroundecyl)oxy)benzyl)oxy)hex-2-enoate



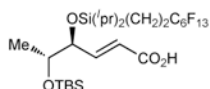
4a

(4*R*,5*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-4-((diisopropyl(3,3,4,4,4-pentafluorobutyl)silyl)oxy)hex-2-enoic acid



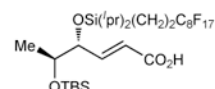
4b

(4*S*,5*S*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-4-((diisopropyl(3,3,4,4,5,5,6,6,6-nonafluorohexyl)silyl)oxy)hex-2-enoic acid



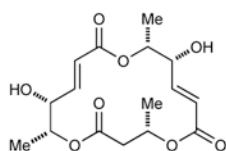
4c

(4*S*,5*R*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-4-((diisopropyl(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)silyl)oxy)hex-2-enoic acid



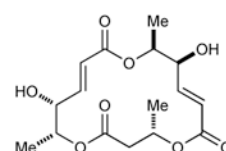
4d

(4*R*,5*S*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-4-(((3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptafluorodecyl)diisopropylsilyl)oxy)hex-2-enoic acid



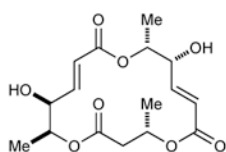
(3S,8R,9R,14R,15R)-1

(4S,7E,9R,10R,13E,15R,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



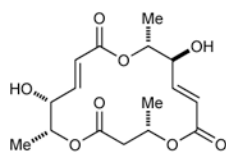
(3S,8S,9S,14R,15R)-1

(4S,7E,9S,10S,13E,15R,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



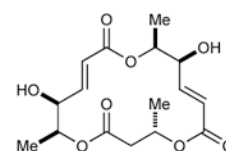
(3S,8R,9R,14S,15S)-1

(4S,7E,9R,10R,13E,15S,16S)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



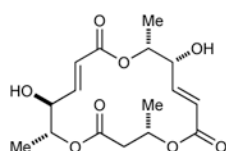
(3S,8S,9R,14R,15R)-1

(4S,7E,9S,10R,13E,15R,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



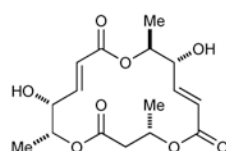
(3S,8S,9S,14S,15S)-1

(4S,7E,9S,10S,13E,15S,16S)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



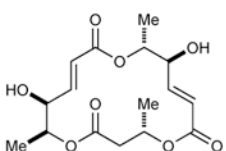
(3S,8R,9R,14S,15R)-1

(4S,7E,9R,10R,13E,15S,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



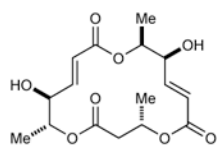
(3S,8R,9S,14R,15R)-1

(4S,7E,9R,10S,13E,15R,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



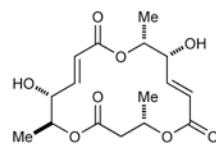
(3S,8S,9R,14S,15S)-1

(4S,7E,9S,10R,13E,15S,16S)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



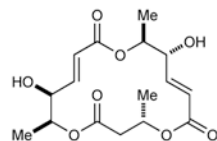
(3S,8S,9S,14S,15R)-1

(4S,7E,9S,10S,13E,15S,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



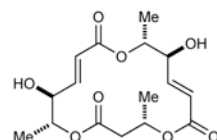
(3S,8R,9R,14R,15S)-1

(4S,7E,9R,10R,13E,15R,16S)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione

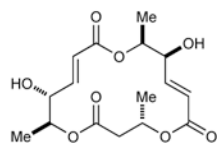


(3S,8R,9S,14S,15S)-1

(4S,7E,9R,10S,13E,15S,16S)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione

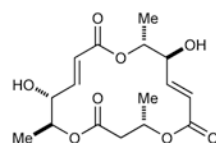
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ent-macrosphelide E

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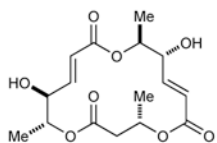
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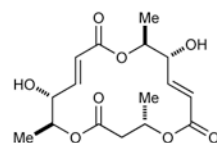
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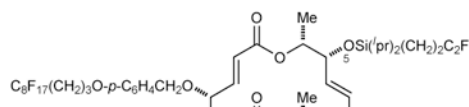
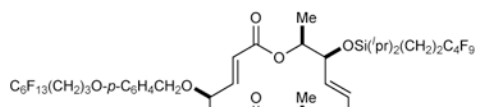


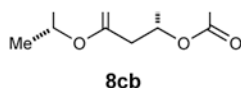
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(4S,7E,9R,10S,13E,15S,16R)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione

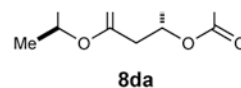
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macrosphelide A

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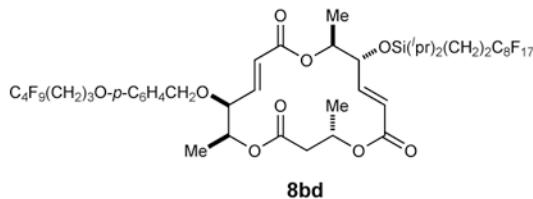




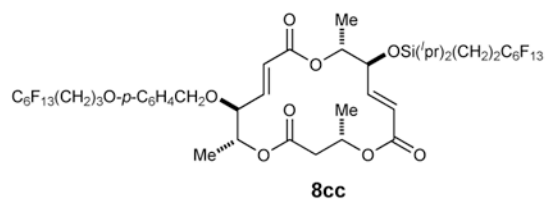
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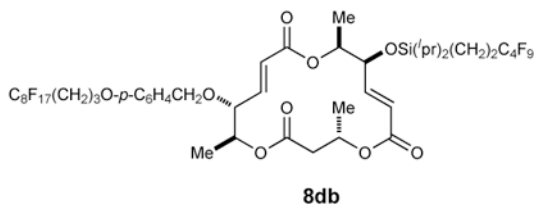
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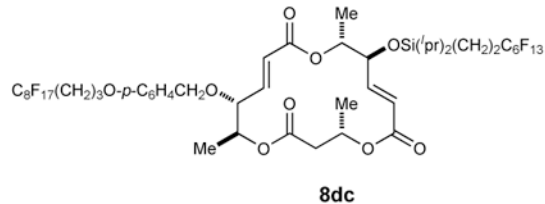
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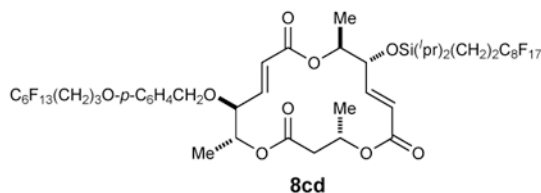
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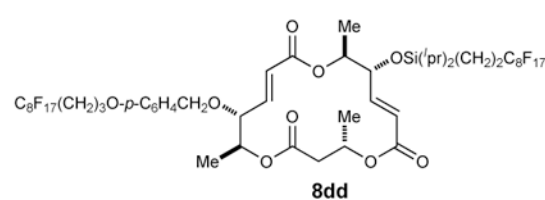
(4*S*,7*E*,9*S*,10*S*,13*E*,15*R*,16*S*)-9-((diisopropyl(3,3,4,4,5,5,6,6,6-nonafluorohexyl)silyloxy)-15-((4-(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)oxy)benzyl)oxy)-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



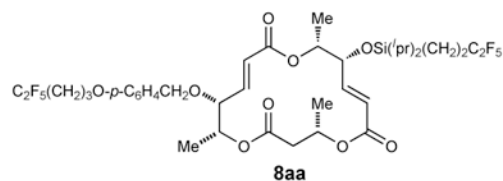
(4*S*,7*E*,9*S*,10*R*,13*E*,15*R*,16*S*)-9-((diisopropyl(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)silyloxy)-15-((4-(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)oxy)benzyl)oxy)-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



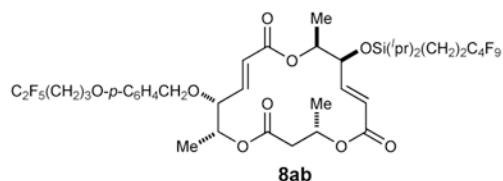
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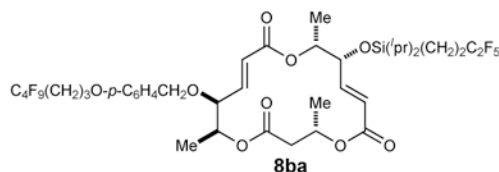
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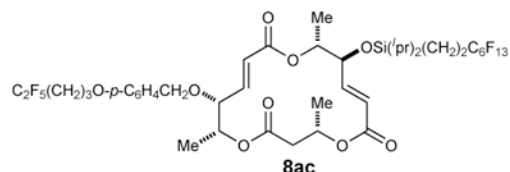
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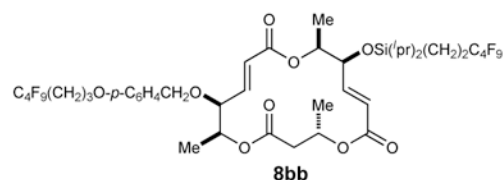
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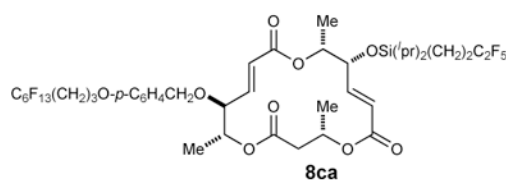
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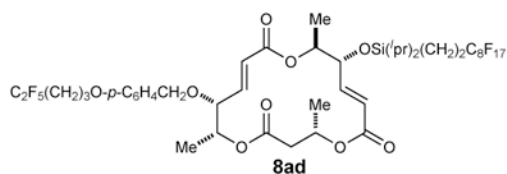
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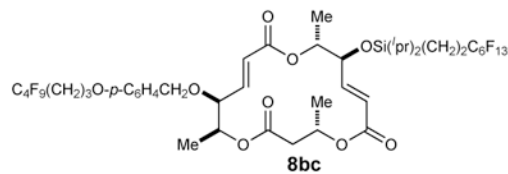
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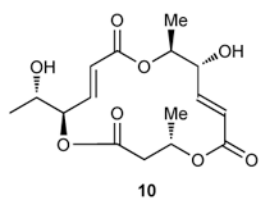
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(4*S*,7*E*,9*R*,10*S*,13*E*,15*R*,16*R*)-9-(((3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hepta-decafluorodecyl)diisopropylsilyloxy)-4,10,16-trimethyl-15-((4-((4,4,5,5,5-pentafluoropentyl)oxy)benzyl)oxy)-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione

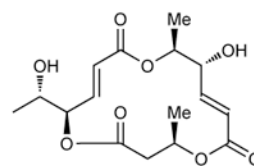


(4*S*,7*E*,9*S*,10*R*,13*E*,15*S*,16*S*)-9-((diisopropyl(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-octyl)silyloxy)-4,10,16-trimethyl-15-((4-((4,4,5,5,6,6,7,7,7-nonafluoroheptyl)oxy)benzyl)oxy)-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione



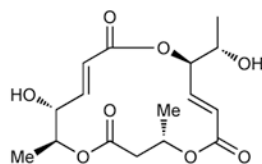
10

(2*S*,6*R*,7*E*,11*S*,12*R*,13*E*)-12-hydroxy-6-((*S*)-1-hydroxyethyl)-2,11-dimethyl-1,5,10-trioxacyclopentadeca-7,13-diene-4,9,15-trione



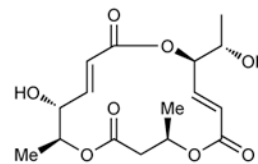
11

(2*R*,6*R*,7*E*,11*S*,12*R*,13*E*)-12-hydroxy-6-((*S*)-1-hydroxyethyl)-2,11-dimethyl-1,5,10-trioxacyclopentadeca-7,13-diene-4,9,15-trione



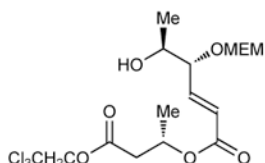
12

(4*S*,7*E*,9*R*,12*E*,14*R*,15*S*)-14-hydroxy-9-((*S*)-1-hydroxyethyl)-4,15-dimethyl-1,5,10-trioxacyclopentadeca-7,12-diene-2,6,11-trione



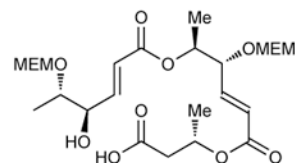
13

(4*R*,7*E*,9*R*,12*E*,14*R*,15*S*)-14-hydroxy-9-((*S*)-1-hydroxyethyl)-4,15-dimethyl-1,5,10-trioxacyclopentadeca-7,12-diene-2,6,11-trione



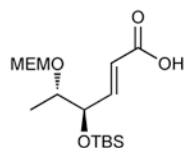
14

(4*R*,5*S*,*E*)-(*S*)-4-oxo-4-(2,2,2-trichloroethoxy)butan-2-yl 5-hydroxy-4-((2-methoxyethoxy)methoxy)hex-2-enoate



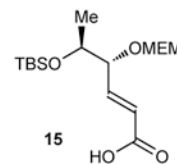
15

(8*S*,9*R*,10*E*,14*S*,15*R*,16*E*,20*S*)-9-hydroxy-15-((2-methoxyethoxy)methoxy)-8,14,20-trimethyl-12,18-dioxo-2,5,7,13,19-pentaoxadocosa-10,16-dien-22-oic acid



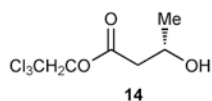
16

(4*R*,5*S*,*E*)-4-((*tert*-butyldimethylsilyloxy)-5-((2-methoxyethoxy)methoxy)hex-2-enoic acid



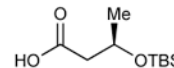
17

(4*R*,5*S*,*E*)-5-((*tert*-butyldimethylsilyloxy)-4-((2-methoxyethoxy)methoxy)hex-2-enoic acid



18

(*S*)-2,2,2-trichloroethyl 3-hydroxybutanoate



19

(*R*)-3-((*tert*-butyldimethylsilyloxy)butanoic acid

### Figure 3.

Fragments, tagged quasiisomers and mixture synthesis: (a) structures of the four single-isomer fragments **2**; (b) tagging scheme for the fluorine mixture synthesis of the 16-quasiisomer mixture shown with the tagged quasiisomers **3a-d** and **4a-d**. Each isomer of **2** gets two tags (<sup>F</sup>PMB and <sup>F</sup>TIPS) with the same number of fluorine atoms; (c) fluorine mixture synthesis of final tagged quasiisomer mixture **M-8a-d/a-d** starting from precursors **3a-d** and **4a-d**. Because the substrates are four- or sixteen-compound mixtures, each of the six reactions was conducted only once. Samples with the “M” prefix are mixtures with the letter suffixes indicating the tags (as <sup>F</sup>PMB/<sup>F</sup>TIPS for the samples with both tags). In turn,

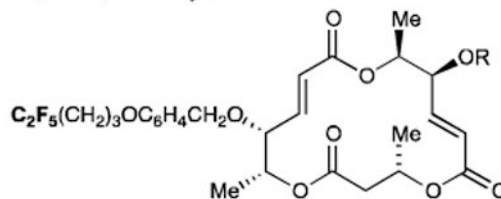
the tags encode the configuration. For example, one of the sixteen components in **M-8a-d/a-d** is **8ab**. Its <sup>F</sup>PMB group **a** bears C<sub>2</sub>F<sub>5</sub> (which encodes 14*R*,15*R*) and its <sup>F</sup>TIPS group **b** bears C<sub>4</sub>F<sub>9</sub> (which encodes 8*S*,9*S*).

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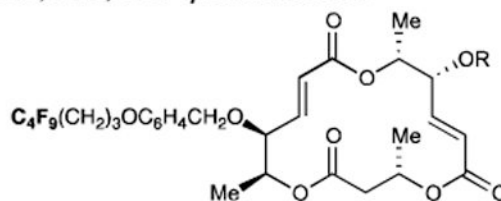
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**3*S*,8*S*,9*S*,14*R*,15*R* quasiisomers**

double tagged: **8a/b**, R = Si(<sup>t</sup>pr)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>4</sub>F<sub>9</sub>

mono detagged: **9a**, R = H

**3*S*,8*R*,9*R*,14*S*,15*S* quasiisomers**

double tagged: **8b/a**, R = Si(<sup>t</sup>pr)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>2</sub>F<sub>5</sub>

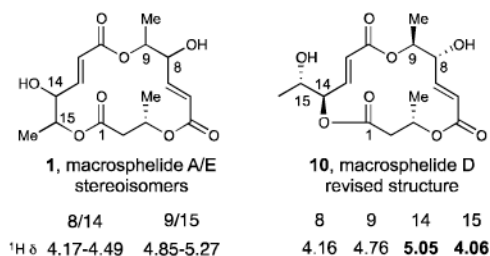
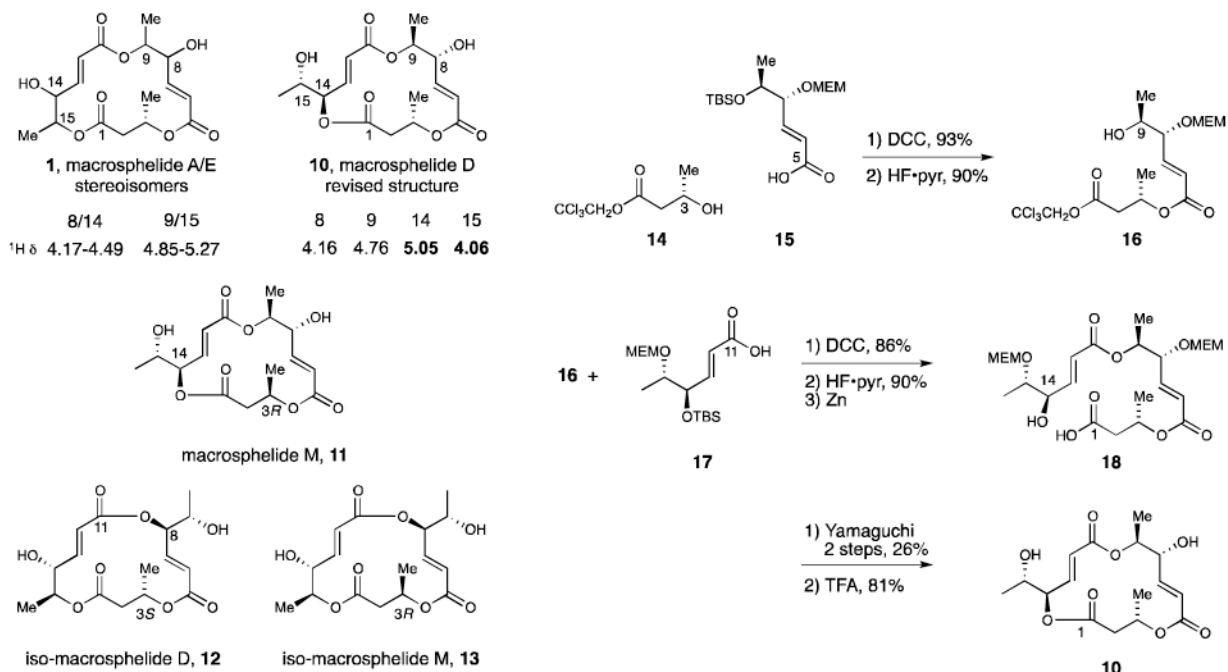
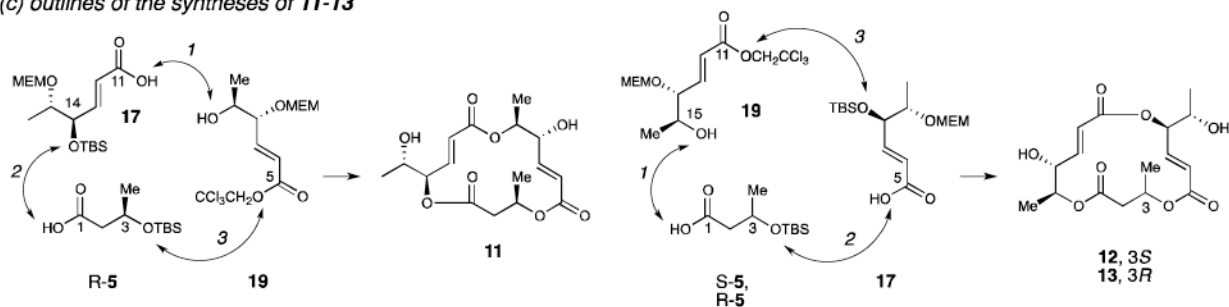
mono detagged: **9b**, R = H

**Figure 4.**

Double demixing illustrated with fraction 2. The first demixing produced four single-compound fractions (1, 6a, 6b (which separated even though they have the same number of fluorines) and 7) and four mixture fractions numbered 2–5. Fraction 2, for example, contains double-tagged quasiisomers **8ab** and **8ba**, each having 14 fluorines. Removal of the <sup>F</sup>TIPS tag provides a mixture of mono detagged adducts **9a** and **9b** that are readily separated in the second demixing because one (**9a**) has five fluorines and the other (**9b**) has nine.



## (a) what is macrophelide D?

(b) synthesis of candidate **10**(c) outlines of the syntheses of **11-13****Figure 5.**

Macrophelide D structure candidates and syntheses: (a) analysis of key chemical shifts in  $^1\text{H}$  NMR spectra of macrophelides A, D and E suggests four candidate structures (**10-13**) for macrophelide D; (b) step-by-step summary of the synthesis of candidate **10**, which proved to be macrophelide D; (c) schematic summary of the precursors and bond connections to make candidates **11-13**.

Table 1

## Tagging pattern for the macrophelide library of quasisomers 8

double tag number	configurations	F <sub>PMB</sub> R <sub>F</sub>	F <sub>TIPS</sub> R <sub>F</sub>	total fluorine #	HPLC fraction
8aa	3 <i>S</i> ,8 <i>R</i> ,9 <i>R</i> ,14 <i>R</i> ,15 <i>R</i>	C <sub>2</sub> F <sub>5</sub>	C <sub>2</sub> F <sub>5</sub>	10	1
8ab	3 <i>S</i> ,8 <i>S</i> ,9 <i>S</i> ,14 <i>R</i> ,15 <i>R</i>	C <sub>2</sub> F <sub>5</sub>	C <sub>4</sub> F <sub>9</sub>	14	2
8ba	3 <i>S</i> ,8 <i>R</i> ,9 <i>R</i> ,14 <i>S</i> ,15 <i>S</i>	C <sub>4</sub> F <sub>9</sub>	C <sub>2</sub> F <sub>5</sub>	14	2
8ac	3 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> ,14 <i>R</i> ,15 <i>R</i>	C <sub>2</sub> F <sub>5</sub>	C <sub>6</sub> F <sub>13</sub>	18	3
8bb	3 <i>S</i> ,8 <i>S</i> ,9 <i>S</i> ,14 <i>S</i> ,15 <i>S</i>	C <sub>4</sub> F <sub>9</sub>	C <sub>4</sub> F <sub>9</sub>	18	3
8ca	3 <i>S</i> ,8 <i>R</i> ,9 <i>R</i> ,14 <i>S</i> ,15 <i>R</i>	C <sub>6</sub> F <sub>13</sub>	C <sub>2</sub> F <sub>5</sub>	18	3
8ad	3 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,14 <i>R</i> ,15 <i>R</i>	C <sub>2</sub> F <sub>5</sub>	C <sub>8</sub> F <sub>17</sub>	22	4
8bc	3 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> ,14 <i>S</i> ,15 <i>S</i>	C <sub>4</sub> F <sub>9</sub>	C <sub>6</sub> F <sub>13</sub>	22	4
8cb	3 <i>S</i> ,8 <i>S</i> ,9 <i>S</i> ,14 <i>S</i> ,15 <i>R</i>	C <sub>6</sub> F <sub>13</sub>	C <sub>4</sub> F <sub>9</sub>	22	4
8da	3 <i>S</i> ,8 <i>R</i> ,9 <i>R</i> ,14 <i>R</i> ,15 <i>S</i>	C <sub>8</sub> F <sub>17</sub>	C <sub>2</sub> F <sub>5</sub>	22	4
8bd	3 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,14 <i>S</i> ,15 <i>S</i>	C <sub>4</sub> F <sub>9</sub>	C <sub>8</sub> F <sub>17</sub>	26	5
8cd <sup>a</sup>	3 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> ,14 <i>S</i> ,15 <i>R</i>	C <sub>6</sub> F <sub>13</sub>	C <sub>6</sub> F <sub>13</sub>	26	5
8db	3 <i>S</i> ,8 <i>S</i> ,9 <i>S</i> ,14 <i>R</i> ,15 <i>S</i>	C <sub>8</sub> F <sub>17</sub>	C <sub>4</sub> F <sub>9</sub>	26	5
8dc	3 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> ,14 <i>R</i> ,15 <i>S</i>	C <sub>8</sub> F <sub>17</sub>	C <sub>6</sub> F <sub>13</sub>	30	6a
8cd	3 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,14 <i>S</i> ,15 <i>R</i>	C <sub>6</sub> F <sub>13</sub>	C <sub>8</sub> F <sub>17</sub>	30	6b
8dd <sup>b</sup>	3 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,14 <i>R</i> ,15 <i>S</i>	C <sub>8</sub> F <sub>17</sub>	C <sub>8</sub> F <sub>17</sub>	34	7

<sup>a</sup> precursor of ent-macrophelide E (ent-1-E)<sup>b</sup> precursor of macrophelide A (1-A)