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Total Synthesis of a 28-Member Stereoisomer Library of Murisolins

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Abstract: Total syntheses of two 16-member libraries of murisolin isomers are reported. In the first library, fluorous PMB (*p*-methoxybenzyl) groups encode configurations, and four mixtures of four dihydroxy-tetrahydrofurans are prepared by Shi epoxidation followed (optionally) by Mitsunobu reaction. The mixtures are coupled by Kocienski−Julia reaction with a single hydroxybutenolide followed by hydrogenation. Demixing and detagging provide the 16 pure stereoisomers. In the second synthesis, a single mixture of four fluorous-tagged dihydroxy-tetrahydrofurans is coupled with a four-compound mixture of hydroxy-butenolides that bear derivatives of DMB (dimethoxybenzyl) groups with oligoethylene glycol (OEG) units that encode the configurations at C4 and C34. The 16-compound mixture is subjected to hydrogenation, double demixing, and detagging to provide the 16 isomerically pure murisolins. Twelve of these isomers are new, while four match samples from the first library.

Introduction

A collection of some or all possible stereoisomers of a given constitutional isomer of an organic compound is called a stereoisomer library.[1] In the field of natural products chemistry, stereoisomer libraries are of high interest for studying stereo-structure/activity relationships (SSAR). They can also be useful for structure assignments of natural products whose stereo-isomers can reasonably be expected to share similar or even identical spectra. However, studies of stereoisomer libraries have largely been limited to sugars and other fundamental bio-molecule building blocks.[2] This is because stereoisomer libraries are too difficult to make by using current solution phase synthetic technology.

With hundreds of members, the annonaceous acetogenins comprise a large class of fatty acid derived natural products that are isolated from members of the *Annonaceae* (custard-apple) family.[3] Most acetogenins sort into mono-THF or bis-THF classes depending on whether they have one or two dihydroxyalkyl-substituted tetrahydrofuran rings (Figure 1). The two long chain alkyl substituents (R^{α} or $R^{\alpha'}$) are of variable lengths and are oxidized in assorted ways (alkenes, alcohols, ketones). There is often a butenolide ring terminating one chain, while the other is terminated by a methyl group.

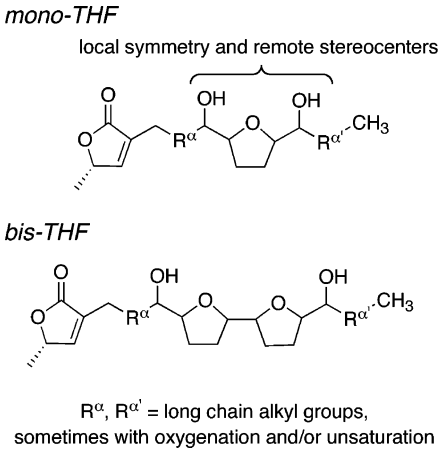

Figure 1. Dihydroxy mono- and bis-tetrahydrofuran classes of acetogenins.

The interesting structures and potent antitumor or pesticidal activities of acetogenins make them appealing synthetic targets.

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(2) For representative serial or solid-phase syntheses of multiple stereoisomers of natural products or related compounds, see: (a) Busch, J.; Grether, Y.; Ochs, D.; Sequin, U. *J. Nat. Prod.* **1998**, *61*, 591−597. (b) Larsson, M.; Nguyen, B.-V.; Högberg, H.-E.; Hedenström, E. *Eur. J. Org. Chem.* **2001**, *2*, 353−363. (c) Takahashi, T.; Kusaka, S.; Doi, T.; Sunazuka, T.; Omura, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 5230−5234. (d) Tietze, L. F.; Rackelmann, N.; Sekar, G. *Angew. Chem., Int. Ed.* **2003**, *42*, 4254−4257. (e) Mulzer, J.; Kaselow, U.; Graske, K.-D.; Kühne, H.; Sieg, A.; Martin, H. J. *Tetrahedron* **2004**, *60*, 9599−9614. (f) Harrison, B. A.; Pasternak, G. W.; Verdine, G. L. *J. Med. Chem.* **2003**, *46*, 677−680. (g) Crossman, J.; Perkins, M. V. *J. Org. Chem.* **2006**, *71*, 177−124.

(3) Reviews: (a) Zafra-Polo, M. C.; González, M. C.; Estornell, E.; Sahpaz, S.; Cortes, D. *Phytochemistry* **1996**, *42*, 253−271. (b) Zafra-Polo, M. C.; Figadère, B.; Gallardo, T.; Tormo, J. R.; Cortes, D. *Phytochemistry* **1998**, *48*, 1087−1117. (c) Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275−306. (d) Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Seglich, W., Tamm, Ch., Eds.; Springer-Verlag: Wien, 1997; Vol. 70, pp 81−288. (e) Alali, F. Q.; Liu, X.-X.; McLaughlin, J. L. *J. Nat. Prod.* **1999**, *62*, 504−540. (f) Tormo, J. R.; Gallardo, T.; González, M. C.; Bermejo, A.; Cabedo, N.; Andreu, I.; Estornell, E. *Curr. Top. Phytochem.* **1999**, *2*, 69−90. (g) Bermejo, A.; Figadère, B.; Zafra-Polo, M. C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269−303. (h) Ramirez, E. A.; Hoye, T. R. In *Studies in Natural Products Chemistry, Vol. 17*; Rahman, A., Ed.; Elsevier: Amsterdam, 1995; pp 251−282.

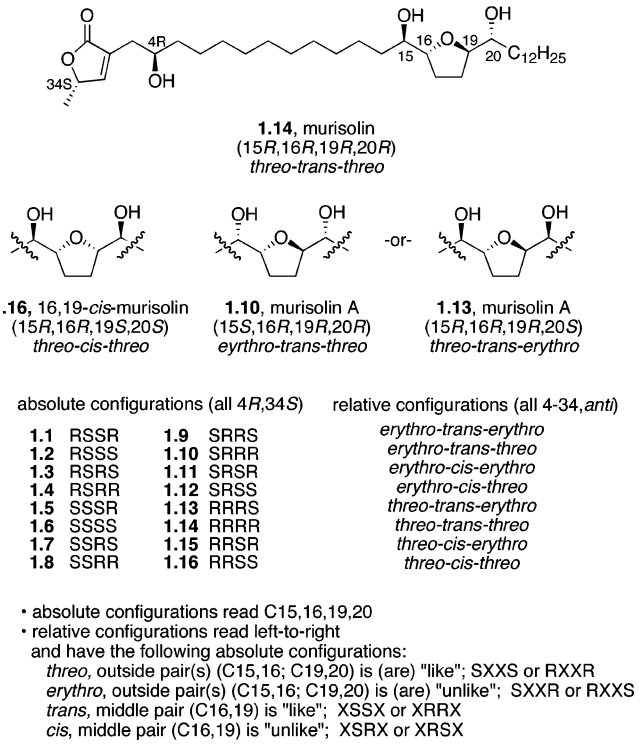

Figure 2. Structures of the (4*R*,34*S*)-murisolin family of acetogenins with CIP designations of configurations for C15,16,19,20.

But assigning stereostructures in acetogenins can be difficult, and to date there are very few X-ray crystal structures reported for these waxy compounds.[3d] While solving connectivities and assigning stereostructures of isolated THF and hydroxybutenolide fragments are problems amendable to modern spectroscopic techniques,[4] it can be very difficult to deduce (1) what the configurations of isolated fragments are relative to each other and (2) which long alkyl chain (R^{α} or $R^{\alpha'}$) is on which hydroxy-bearing carbon of the dihydroxy-THF ring. The elegant assignments of uvaricin, bullaticin, and related bis-THF acetogenins by McLaughlin, Hoye and co-workers show that rigorous structure proofs by appropriate derivatizations are possible, but substantial effort is involved.[5]

The murisolins are an important group of three mono-THF acetogenins (Figure 2) whose structures and the attendant assignment problems are representative of many acetogenins. Murisolin was isolated from the seeds of *Annona muricata* by Cavé and co-workers in 1990.[6] These workers assigned the relative configuration of the dihydroxy-THF ring by ^{1}H NMR spectroscopy studies. Five years later, McLaughlin again isolated murisolin, this time from *Asmina triloba*, along with 16,19-*cis*-murisolin and murisolin A.[7,8] The structure of 16,19-*cis*-murisolin was assigned as **1.16** by a combination of ^{1}H NMR studies on the natural product to establish the relative configuration of the dihydroxy-THF ring and Mosher ester studies to establish the absolute configuration. By using similar methods, the structure of murisolin was refined to **1.14**[7b] and the structure of murisolin A was narrowed to two candidates, **1.10** and **1.13**. These compounds show potent (sometimes extremely potent) cytotoxic activities against several human cancer cell lines and act by inhibition of complex I (NADH/ubiquinone oxidoreductase) in mitochondrial electron transport systems.[3,7,8]

Commensurate with the structural and biological interest, there has been much synthetic work directed toward members of both the mono-THF and bis-THF classes of acetogenins.[3,9] Among others, the approaches of Sinha[10] and Tanaka[11] are well designed for flexibility in stereoisomer synthesis. Through an efficient plan that diverges from common intermediates as late as possible to minimize work, the Sinha group has synthesized 36 isomers of a bis-THF-lactone that are suitably functionalized to make all 64 isomers of the bis-dihydroxy-THF fragment of these acetogenins.[10] But the inexorable consequences of divergence catch up sooner or later; many reactions will be needed to convert the 36 intermediates in a serial or parallel fashion to the 64 possible acetogenin products.

Roughly concurrent with our initial report[1] of the synthesis of a 16-member library including all the proposed structures of the murisolins, Tanaka reported the synthesis of murisolin.[12a] More recently, he has also described the synthesis of the assigned structure of 16,19-*cis*-murisolin.[12b]

We have recently introduced solution phase mixture synthesis with separation tagging as a means to leverage individual, serial, and parallel syntheses of organic molecules. In the first approach called "fluorous mixture synthesis" (FMS),[13,14] incrementally larger fluorous tags (Rf = $-(CF_2)_nCF_3$) were used to encode either configuration or substituent information.[15] This tagging allows syntheses to be conducted on mixtures yet still provides individual pure compounds of unambiguous structure in the end because the mixtures can be separated (and hence decoded) by

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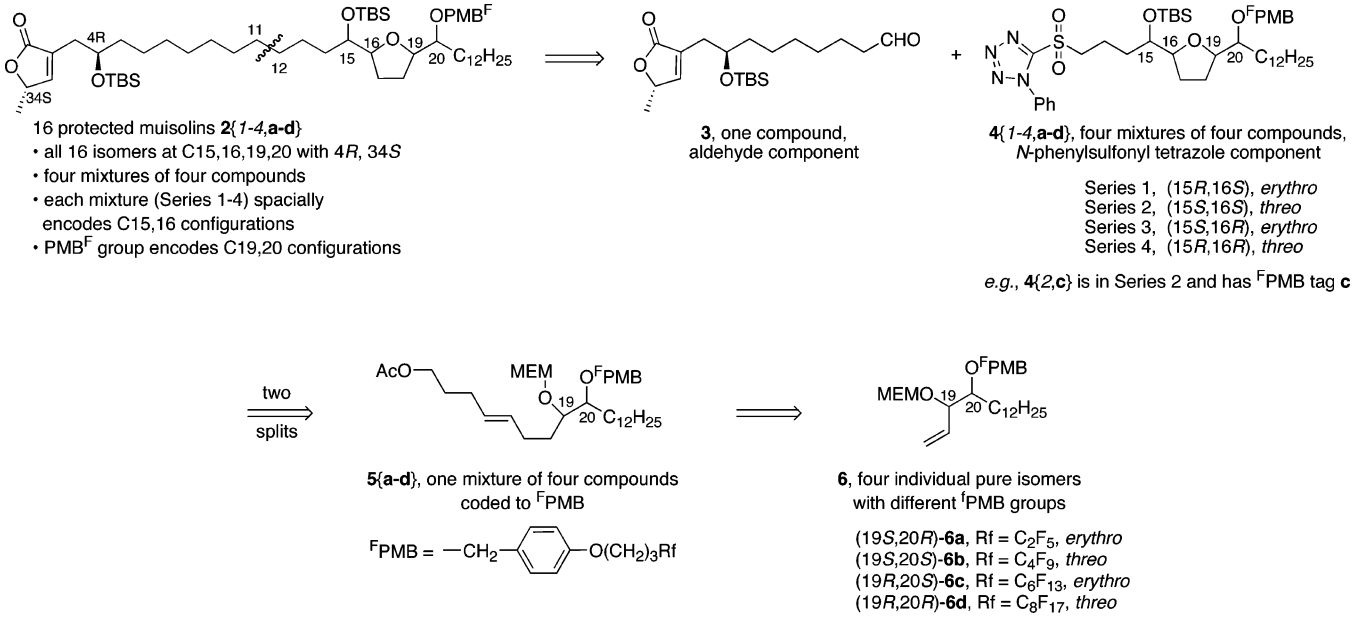


Figure 3. Strategy for murisolin fluorous mixture synthesis.

fluorous chromatography.[16] This type of tag-based separation with decoding is called "demixing" or "sorting". More recently, Wilcox and Turkyilmaz have introduced oligoethylene glycol mixture synthesis (OEGMS).[17] Here, it is the OEG unit (CH_2-CH_2O) that is incrementally varied for coding and separation.

We have previously communicated the synthesis of a 16-member stereoisomer library of murisolins by using fluorous mixture synthesis,[1] and we describe herein the full details of this work. The fluorous mixture synthesis provided four mixtures of four-tagged murisolins, which were demixed and detagged to provide the final target compounds. We have also communicated the union of fluorous mixture synthesis and oligoethylene glycol mixture synthesis to provide a second 16-member library of doubly tagged murisolins, this time as a single mixture.[18] Double demixing and detagging then provided 4 previously made and 12 new murisolin isomers. We describe herein the full details of the fluorous and double mixture components of this work.

Having access to 24 of the 32 possible diastereomers provides a complete picture of spectroscopic similarities and differences among the murisolin isomers. In a forthcoming paper,[19] we will provide a detailed comparison of these spectra and other characterization data. This comparison answers existing questions and raises new ones. Briefly, we rigorously show that the structure of murisolin (**1.14**) is correct but suggest that the structure of 16,19-*cis*-murisolin (**1.16**) is probably wrong and provide a likely alternative (**1.8**). We also suggest structure **1.10** for murisolin A.

Results and Discussion

Fluorous Mixture Synthesis, Strategy: Murisolin has six stereocenters, so its complete stereoisomer library has 64 members grouped as 32 pairs of enantiomers. In the first of two mixture synthesis exercises, we targeted all 16 possible stereoisomers of murisolin at the dihydroxy-THF portion of the molecule with the stereocenters fixed in the hydroxybutenolide fragment as 4*R*,34*S*. This library was prepared by a "mix/split" strategy, as summarized in Figure 3. The long alkyl chain between the two rings of protected murisolins **2**{*1*−*4*,**a**−**d**}[20] was dissected between C11 and C12 to provide a single hydroxybutenolide **3** and 16 protected dihydroxy-THF fragments **4**. The butenolide **3** bears an aldehyde, and the dihydroxy-THF fragments **4** bear phenylsulfonyl tetrazoles for union by a Kocienski−Julia coupling[21] followed by hydrogenation.

The mix/split plan calls for the preparation of four mixtures of protected murisolins **2** (by 2 splits), each mixture with one of the four possible sets of configurations at C15 and C16. In turn, each of these mixture samples comprises four compounds with all possible configurations at C19 and C20 coded to fluorous tags. Compounds that are otherwise stereoisomeric but are not isomers because of differing Rf groups are called quasienantiomers or quasidiastereomers or, more generally, quasi-isomers.[22] The four final mixtures of quasidiastereomers are designated as Series *1*−*4* according to their configurations at C15/C16, as shown in Figure 3. In this plan, the configuration of each isomer is uniquely encoded by the mixture that it is in and by the fluorous tag that it bears. Demixing of the four

(15) (a) Curran, D. P.; Furukawa, T. *Org. Lett.* **2002**, *4*, 2233−2235. (b) Zhang, W.; Luo, Z.; Chen, C. H. T.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 10443−10450. (c) Zhang, Q. Ph.D. Thesis, University of Pittsburgh, 2003. (d) Dandapani, S.; Jeske, M.; Curran, D. P. *Proc. Nat. Acad. Sci. U.S.A.* **2004**, *101*, 12008−12012. (e) Manku, S.; Curran, D. P. *J. Comb. Chem.* **2005**, *7*, 63−68. (f) Manku, S.; Curran, D. P. *J. Org. Chem.* **2005**, *70*, 4470−4473.

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

(17) Wilcox, C. S.; Turkyilmaz, S. *Tetrahedron Lett.* **2005**, *46*, 1827−1829.

(18) Wilcox, C. S.; Gudipati, V.; Lu, H.; Turkyilmaz, S.; Curran, D. P. *Angew. Chem., Int. Ed.* **2005**, *44*, 6938−6940.

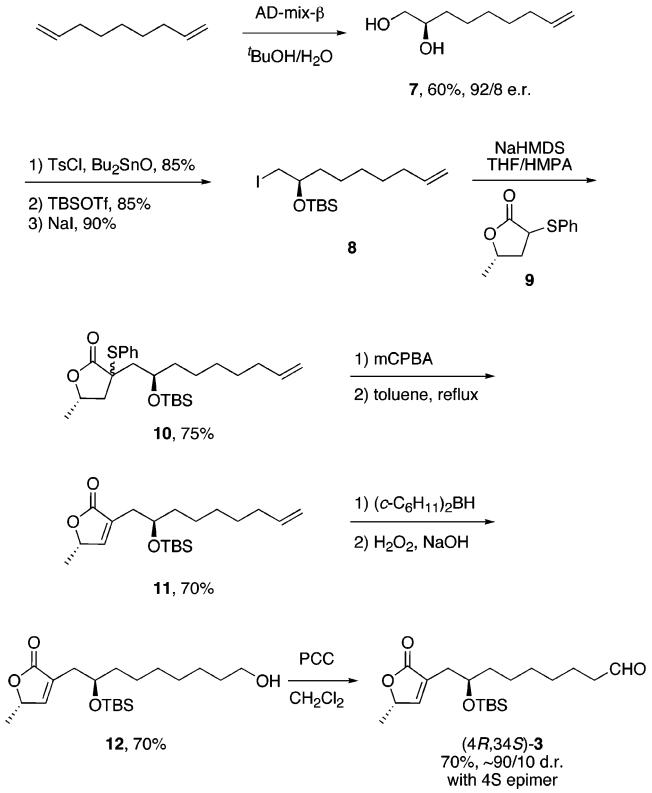
(19) Curran, D. P.; Zhang, Q.; Lu, H.; Gudipati, V. *J. Am. Chem. Soc.* **2006**, in press.

(20) (a) Fluorous- or OEG-tagged compounds are indicated by brackets after numbers. In the fluorous mixture synthesis, the italic number indicates the series (spatial encoding), and the bold letter indicates the fluorous PMB tag. For example, **4**{*2*,**b**} is compound **4** in Series *2* with fluorous tag **b**, whereas **4**{*2*,**a**−**d**} is a mixture of four compounds **4** in Series *2* with all four fluorous tags **a**−**d**. In the double mixture synthesis, the plain text number in the bracket is the OEG tag, and the bold letter is the fluorous tag. (b) Individual stereoisomers of murisolin are indicated by numbers after a decimal point (**1.1**...**1.28**). The order of the numbers after the decimal follows relative configurations and not tags. Table 1 shows the complete numbering and tagging scheme of both libraries of murisolins.

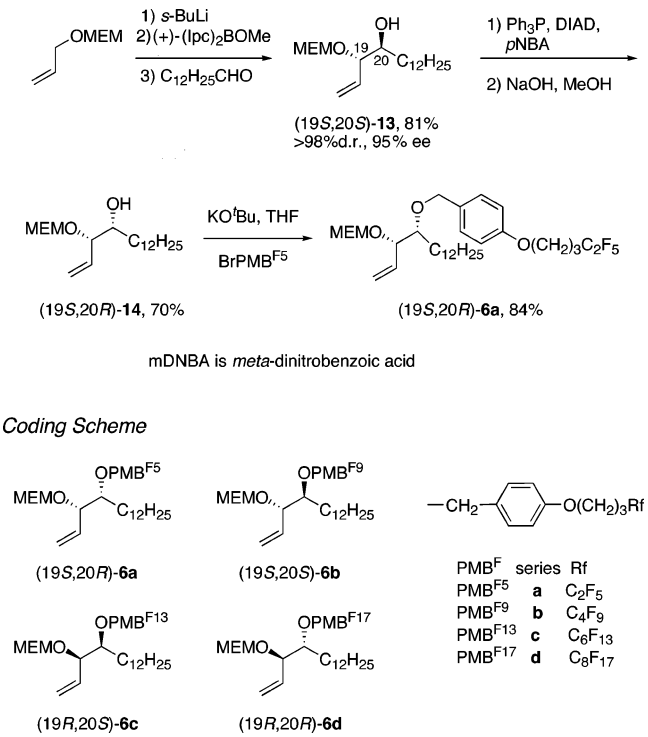
(21) Blakemore, P. R. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2563−2585.

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

Scheme 1. Synthesis of Hydroxybutenolide (4*R*,34*S*)-**3**



Scheme 2. Synthesis of Fluorous-tagged Precursors 6**a**−**d**



mixtures of **2** just prior to deprotection should unambiguously provide all 16 stereoisomers **2**{*1*,**a**} through **2**{*4*,**d**}.

The plan calls for preparation of the four dihydroxy-THF mixtures **4**{*1*−*4*,**a**−**d**} from one four-compound mixture of alkenes **5**{**a**−**d**}. Splitting of this mixture in half and reagent-controlled Shi epoxidation[23] of **5**{**a**−**d**} of each half with enantiomeric fructose-derived ketones followed by closure of the THF ring should provide the two C15/C16 *erythro* isomer mixtures (Series *1* and *3*). Division of these and Mitsunobu reaction on half of each mixture should then provide the C15/C16 *threo* isomer mixtures (Series *2* and *4*). Finally, the underlying individual component precursors **6a**−**d** of mixture **5**{**a**−**d**} are made by Brown allylboration reactions,[24] followed by fluorous tagging.

Fluorous Mixture Synthesis, Premix Stage: The premix stage involves synthesis of all the individual components of **3** and **6** that are needed for the mixture synthesis plan outlined in Figure 3. Hydroxybutenolide (4*R*,34*S*)-**3** is a known compound[25] that was synthesized as summarized in Scheme 1. Sharpless dihydroxylation[26] of 1,8-nonadiene with AD-mix-β provided the diol **7** in 60% yield with an enantiomer ratio of about 92/8. The primary hydroxy group was selectively activated as a tosylate in 85% yield by treatment with *p*-TsCl/Bu_2SnO.[27] The secondary hydroxy group was protected as the TBS ether (85% yield), and the tosylate was displaced by iodide to provide **8** in 90% yield.

The enolate generated by treating lactone **9**[28] with NaHMDS was alkylated with the iodide **8** to afford **10** in 75% yield. The sulfide **10** was directly converted to butenolide **11** in 70% yield by oxidation with *m*CPBA and thermal elimination. Hydroboration/oxidation of the terminal olefin gave the primary alcohol **12** in 70% yield. Finally, oxidation of **12** with PCC[29] then gave aldehyde (4*R*,34*S*)-**3** in 70% yield.

The minor (4*S*) enantiomer of alcohol **7** translated into a diastereomeric impurity in hydroxylactone **3**, which was an inseparable ~9/1 mixture of epimers as assayed by ^{13}C NMR spectroscopy. The spectra of epimers at C4 for **3** and subsequent intermediates are very similar, so the diastereomeric impurity was carried through the rest of the synthesis. This resulted in minor diastereomeric impurities in each of the 16 final murisolins. These were removed after detagging, as described in the postmix section.

The four fluorous-tagged homoallyl alcohols **6** were made by a Brown allylboration route,[30] as shown in Scheme 2. The MEM (methoxyethoxymethyl) ether of allyl alcohol was deprotonated by *sec*-BuLi to form the corresponding allylic anion, which subsequently reacted with (+)-*B*-methoxydiisopinocampheylborane ((+)-Ipc_2BOMe) in the presence of $BF_3{\bullet}Et_2O$ to generate the intermediate allylborane (not shown). Addition of tridecanal ($C_{12}H_{25}CHO$) then provided the homoallylic alcohol (19*S*,20*S*)-**13** in 81% yield. The reaction was completely

(23) (a) Shi, Y. *Acc. Chem. Res.* **2004**, *37*, 488−496. (b) Frohn, M.; Shi, Y. *Synthesis* **2000**, 1979−2000.
(24) Brown, H. C.; Ramachandran, P. V. In *Advances in Asymmetric Synthesis*; Hassner, A., Ed.; Jai Press: Greenwich, 1995; Vol. 1, pp 147−210.
(25) Sinha and Keinan started from 1,9-decadiene and used a similar series of steps terminating in oxidative cleavage to generate aldehyde **3**. In contrast, we used 1,8-nonadiene and generated the aldehyde by hydroboration and oxidation.
(26) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.
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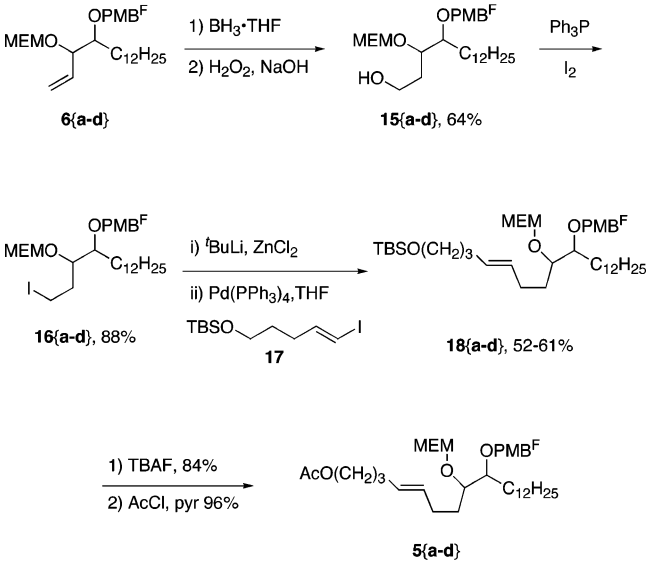
Scheme 3. Synthesis of Shi Epoxidation Precursor Mixture **5**{**a**−**d**}



diastereoselective (>98/2) and the enantiomeric excess of **13** was 95% as assayed by Mosher ester analysis. The configuration of the hydroxyl group in (19*S*,20*S*)-**13** was then inverted by a Mitsunobu reaction followed by the hydrolysis of the intermediate ester to provide the inverted alcohol (19*S*,20*R*)-**14** in 70% yield. Similarly, the reaction of the allylborane derived from (−)-Ipc$_2$BOMe with tridecanal provided the enantiomer (19*R*,20*R*)-**13** (not shown). Another Mitsunobu−hydrolysis sequence provided (19*R*,20*S*)-**14** in 70% yield.

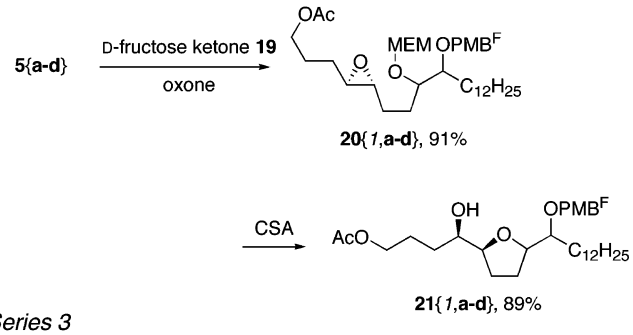
The four stereoisomeric alcohols were then selectively tagged with four fluorous PMB bromides (BrCH$_2$C$_6$H$_4$-*p*-O(CH$_2$)$_3$Rf), which were synthesized from the corresponding alcohols.[31] The perfluorocarbon units (Rf) in the four fluorous tags are C_2F_5, C_4F_9, C_6F_{13}, and C_8F_{17}. The alcohol (19*S*,20*R*)-**14** was deprotonated with potassium *tert*-butoxide (*t*-BuOK) at −30 °C in THF and reacted with fluorous PMB bromide bearing the C_2F_5 group to provide (19*S*,20*R*)-**6a** in 84% yield. Likewise, PMB^F ethers (19*S*,20*S*)-**6b** (Rf = C_4F_9), (19*R*,20*S*)-**6c** (Rf = C_6F_{13}), and (19*R*,20*R*)-**6d** (Rf = C_8F_{17}) were prepared. The structures for these tagged precursors are shown in the lower part of Scheme 2.

Fluorous Mixture Synthesis; Mixture Stage: The four-tagged stereoisomers **6** were weighed and mixed to generate the starting mixture **6**{**a**−**d**} (Scheme 3). The molar ratio of **6a:b:c:d** was approximately 1:1.1:1.2:1.2. Hydroboration of the mixture **6**{**a**−**d**} with BH$_3$/THF in THF for 12 h followed by treatment with hydrogen peroxide in aqueous NaOH for 2 h afforded the desired primary alcohol **15**{**a**−**d**} in 64% yield (Scheme 3). The conversion of **15**{**a**−**d**} to the corresponding iodide **16**{**a**−**d**} was accomplished by treatment with triphenylphosphine and iodine in toluene.

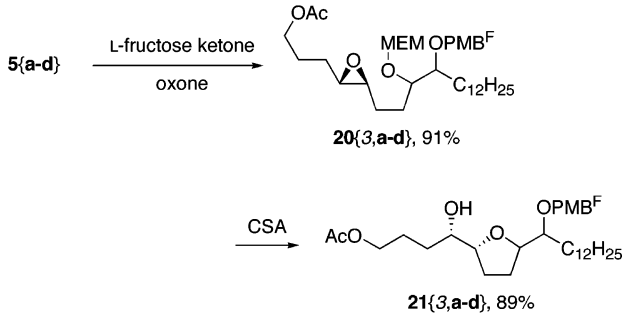
In the key Negishi coupling,[32] the zinc reagent generated by treating the iodide mixture **16**{**a**−**d**} with *t*-BuLi in the presence of dry ZnCl$_2$[33] was transferred to a mixture of vinyl iodide **17** and Pd(PPh$_3$)$_4$ in THF.[34] Following rapid flash chromatography, **18**{**a**−**d**} was isolated in reproducible 52−61% yields. The product of reductive deiodination of **16**{**a**−**d**} (not shown) was also isolated in trace amounts. The TBS protecting group in **18**{**a**−**d**} was removed by TBAF to afford a primary alcohol.

Scheme 4. Shi Epoxidations of Series *1* and Series *3* Mixtures

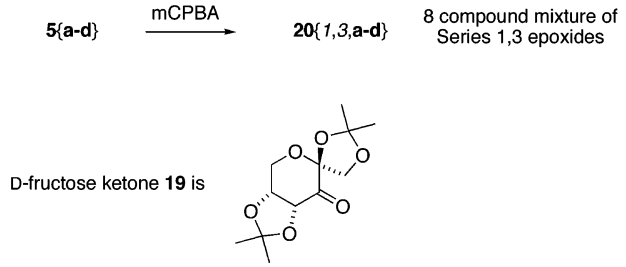
Series 1



Series 3



Series 1,3 mixture



This was reacted with acetyl chloride to provide the key mixture **5**{**a**−**d**} in 96% yield.

The THF ring and the stereocenters at C15 and C16 were introduced along with the first split, as shown in Scheme 4. The epoxidation of **5**{**a**−**d**} by using the ketone **19** derived from D-fructose followed the procedure reported by Shi and coworkers.[35] A stoichiometric amount of ketone **19** was used to maximize the conversion and facial selectivity. The Series *1* epoxide mixture **20**{*1*,**a**−**d**} was obtained in 81% yield together with some recovered starting material **5**{**a**−**d**}. The starting material **5** was separated from the product **20** (81%) by flash chromatography but was only partly separated from the ketone **19**. The recovered alkene **5**{**a**−**d**} containing some residual ketone was subjected to the same reaction conditions to obtain 10% more epoxide **20**{*1*,**a**−**d**} to give an overall yield of 91%.

The removal of the MEM group and the epoxide opening were accomplished together by treating **20**{*1*,**a**−**d**} with cam-

(31) The fluorous PMB alcohols and fluorous silica products were purchased from Fluorous Technologies, Inc. DPC holds an equity interest in this company.
(32) Knochel, P.; Almena Perea, J. J.; Jones, P. *Tetrahedron* **1998**, *54*, 8275−8319.
(33) Smith, A. B.; LaMarche, M. J.; Falcone-Hindley, M. *Org. Lett.* **2001**, *3*, 695−698.
(34) The coupling of the acetate analogue of **17** to directly provide **5**{**a**−**d**} was also successful, but the resulting mixture contained impurities that were difficult to remove by chromatography.
(35) Smith, A. B.; Brandt, B. M. *Org. Lett.* **2001**, *3*, 1685−1688.

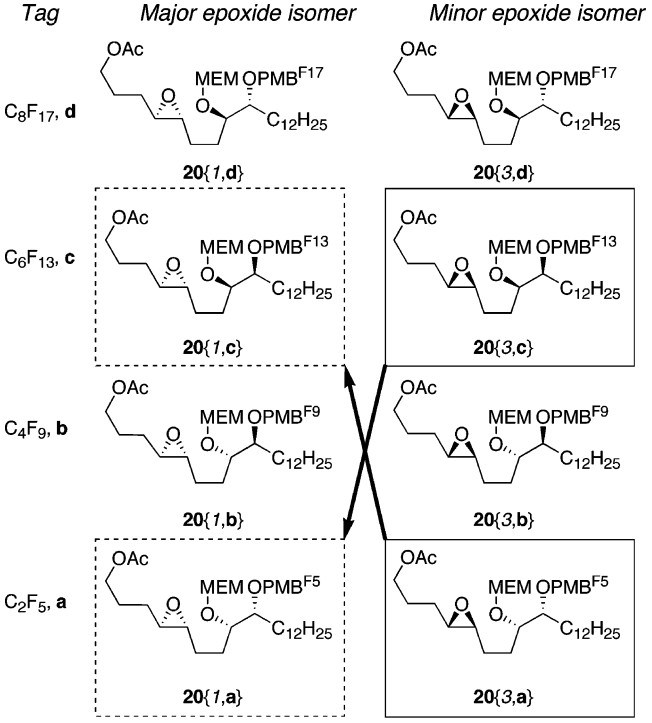


Figure 4. Selected quasienantiomer relationships in the Shi epoxidation product **20**{*1*,**a**−**d**}.

phorsulfonic acid (CSA) in CH_2Cl_2 at room temperature for 2 h to give the substituted-THF mixture **21**{*1*,**a**−**d**} in 81% yield. Concurrently, we used the L-fructose ketone (ent-**19**) for the epoxidation of **5**{**a**−**d**} to generate the Series *3* mixture **20**{*3*,**a**−**d**} in 85% yield. Deprotective cyclization of this mixture by treatment with CSA then afforded **21**{*3*,**a**−**d**} in 83% yield. We also treated **5**{**a**−**d**} with *m*CPBA to obtain a mixture of Series *1* and *3* mixtures **20**{*1*,*3*,**a**−**d**} for reference.

The Shi epoxidation creates two new stereocenters. While good selectivities are observed in related epoxidations of achiral substrates, precedent[23,33] suggests that it is unlikely that the diastereoselectivity of this reaction with any of the four substrates in **5**{**a**−**d**} will approach 100%. Accordingly, both mixtures of **20** are expected to contain four major and four minor diastereomeric epoxides.

To assess the stereoselectivity of the Shi epoxidation, we capitalized on a key "self-indexing" feature of fluorous mixture synthesis. Each mixture is self-indexed because the expected *minor product* of every substrate in the Shi epoxidation is represented by the *quasienantiomer of one of the other major products*. The quasienantiomers differ only in the number of CF_2 groups in the fluorous PMB group, and they are expected to exhibit substantially identical spectra, even under high-resolution conditions.[22]

Representative quasienantiomer relationships in the self-indexed Series *1* **20**{*1*,**a**−**d**} mixture are shown in Figure 4. Shi epoxidation of **5**{**a**} with **19** is expected to give **20**{*1*,**a**} as the major product and **20**{*3*,**a**} as the minor product (C_2F_5 series). Likewise, **5**{**c**} gives **20**{*1*,**c**} and **20**{*3*,**c**} (C_6F_{13} series). The major product **20**{*1*,**a**} of the first epoxidation is the quasienantiomer of the minor product **20**{*3*,**c**} of the second and vice versa. Thus, to identify the minor product in the epoxidation in a spectrum of **5**{**a**}, we need only to look for peaks identical to those of the major product in a spectrum of **5**{**c**}. A parallel relationship holds for the other two compounds in **20**{*1*,**a**−**d**} and for the four compounds in **20**{*3*,**a**−**d**}.

We conducted a thorough analysis of the three epoxide mixtures of **20** by LC NMR spectroscopy. Small samples of each mixture of **20** (∼1 mg) were demixed on a FluoroFlash PF-C8 column[31] by eluting with a gradient of 80% CH_3CN/20% D_2O up to 100% CH_3CN over 30 min. Four peaks were observed in the chromatogram of each sample due to the expected separation based on the fluorous tag. Center cuts from each of the peaks were sequentially pumped into the probe of a 600 MHz NMR spectrometer, and ^1H NMR spectra were recorded by using standard techniques. The whole experiment, including LC separation and subsequent collection of four FIDs, took about 4 h to complete.

Expansions of the resulting 12 spectra in the region 4.20−4.90 ppm are shown in Figure 5. This region of the spectrum proved to be the most diagnostic, even though the resonances arise from protecting group protons: the downfield peak(s) are due to the benzylic methylene group ($ArCH_2O$) of the PMB^F group, while the upfield peaks are due to the acetal methylene group (OCH_2O) of the MEM group. Spectra are listed in order of compound elution from bottom (fastest eluting C_2F_5 tag) to top (slowest eluting C_8F_{17} tag).

These spectra validate the proposed identity of quasi-enantiomers and the indexing in several ways. First, each control spectrum in the (nonselective) *m*CPBA set is the sum of the flanking two spectra in the Shi epoxidation sets. Consider the spectra in line **d** (C_8F_{17} tag). Compounds **20**{*1*,**d**} (left spectrum) and **20**{*3*,**d**} (right spectrum) are diastereomers because they were produced by epoxidation of the same alkene with D-fructose- and L-fructose-derived ketones. Accordingly, their spectra are different. The center spectrum from the nonselective *m*CPBA oxidation should contain resonances from both of these compounds, and indeed it is the sum of its flanking spectra. The same analysis holds for the other three groups of three spectra. So the Series *3* product **20**{*3*,**d**} provides a control for the true minor product of a Series *1* **20**{*1*,**d**} and vice versa. However, one need not look to Series *3* for the controls because the quasienantiomer of the minor product of **20**{*1*,**d**} is indexed in the library as the major product of **20**{*1*,**b**}. Summing left and right spectra **b** and **d** (or **a** and **c**) again provides the control spectra in the center. Finally, the quasienantiomer of each major product of Series *1* is present as one of the major products of Series *3*. For example, the quasienantiomer of **20**{*1*,**d**} is **20**{*3*,**b**}, and the corresponding spectra are identical. This type of analysis holds true for the spectrum of every major product, when compared with the appropriate quasienantiomer from its own mixture or the true enantiomer from the other mixture.

Although these LC NMR experiments validate the principle of self-indexing and prove the success of the early steps of the mixture synthesis, they also leave unresolved the level of stereoselectivity. While all of the epoxidations are clearly stereoselective, the spectral resolution is not sufficient in any case to quantify (or even estimate) the level of stereoselectivity. This may be due to the inherently low resolution of the LC NMR experiment (dilute samples, protic solvent).

Beyond resolution, there are two other problems in quantifying selectivity. First, because the quasi-isomer separations are so large, it is essential to use gradient conditions for the LC. This results in recording successive spectra in different solvents.

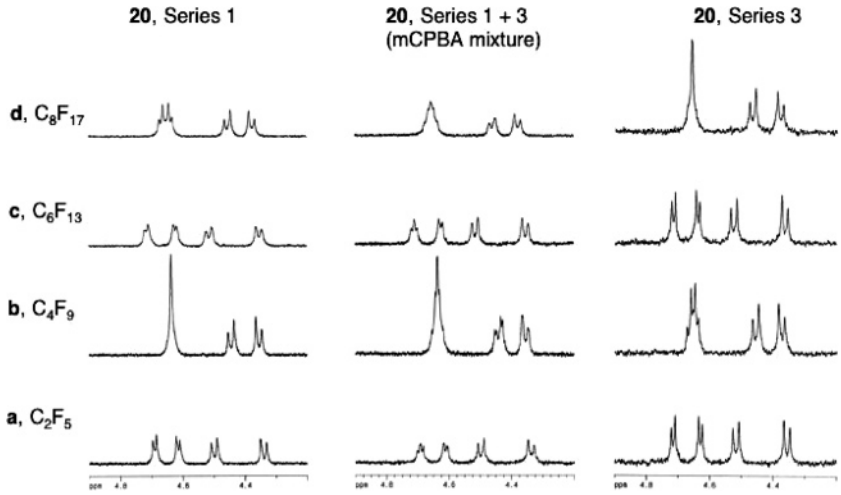


Figure 5. Expansions of the LC NMR spectra (600 MHz, ^{1}H, CH_3CN/D_2O) of epoxide mixtures **20**, Series *1*, Series *3*, and Series *1*,*3* mixture.

So solvent-dependent chemical shifts are of concern, although we did not observe any evidence of this in the spectra in Figure 5. Second, and more seriously, only the sample from the heart of the LC peak is injected into the NMR probe. The broader the peak, the smaller the amount of sample that is injected and the more that is omitted. Because it is conceivable that quasidiastereomers bearing the same fluorous tag could be separated by fluorous HPLC, there is concern that the NMR spectra that are recorded from an LC experiment might not be representative of the bulk sample in that peak. A minor isomer could precede the main component or lag behind it.

To obtain quantitative facial selectivities for the Shi epoxidation and to further support the above analysis, we demixed by fluorous HPLC a small quantity of mixture **21**{*1*,**a**−**d**} following closure of the THF ring. The four individual fractions were concentrated, and 600 MHz ^{1}H NMR spectra of the resulting products were recorded in $CDCl_3$. The whole experiment takes about the same time as an LC NMR experiment (∼4 h) but provides much better quality spectra.

Expansion of these spectra showing the diagnostic resonances of the methylene group of the PMB^F group ($ArCH_2O$) (4.45−4.75 ppm) are reproduced in Figure 6. As expected, the resonances of each minor isomer are identical to those of its corresponding quasienantiomer. For example, compare the boxed minor resonances in **21**{*1*,**d**} with the boxed major resonances in **21**{*1*,**b**}. These are identical because the minor product mixed with **21**{*1*,**d**} is the quasienantiomer of the major product **21**{*1*,**b**}. The reverse is also true, and the same pair of relationships hold with the **a/c** compounds. This proves that these minor resonances arise from the Shi epoxidation reaction and allows integration of the **21**{*1*, **a**−**d**} spectra to provide the following stereoselectivities: **a**, 93/7; **b**, 92/8; **c**, 93/7; **d**, 92/8. A comparable experiment was conducted with the Series *3* mixture **21**{*3*,**a**−**d**}, and similar stereoselectivities were observed. These results show that, for all eight substrates in the two mixtures, the configurations of the precursors are inconsequential; the Shi epoxidation occurs with good reagent control (∼93/7).

We continued the mixture synthesis with alcohol **21**{*1*,**a**−**d**} emanating from the D-fructose ketone epoxidation and ring closing reaction by first dividing it into two portions (Scheme 5). The "Series *1*" portion was protected as the TBS ether (81%) and the acetate was removed by reduction with DIBAL-H

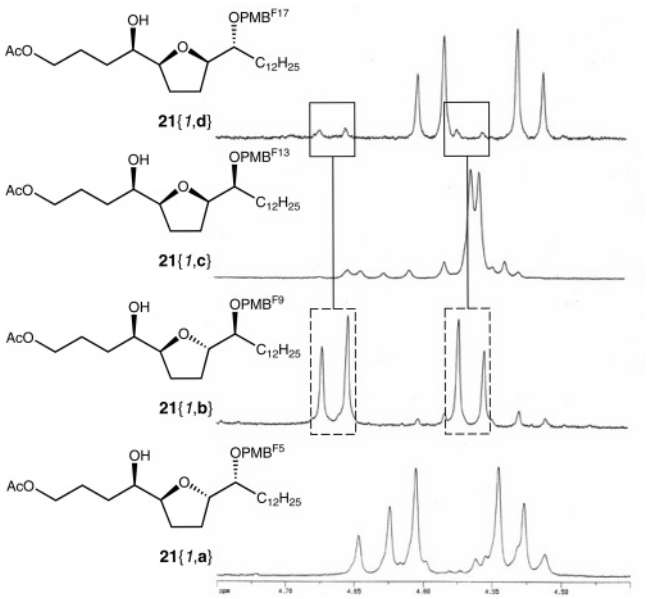


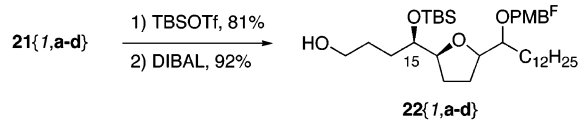
Figure 6. Determining stereoselectivities by quasienantiomer analysis: Expansions of the 600 MHz ^{1}H NMR spectra ($CDCl_3$) of the components of THF mixture **21**{*1*,**a**−**d**}.

(92%). This provides the Series *1* primary alcohol **22**{*1*,**a**−**d**}, ready for the final stage of the synthesis. In turn, the configuration at C15 of the "Series *2*" portion of **21**{*1*,**a**−**d**} was first inverted by a Mitsunobu reaction with *p*-nitrobenzoic acid (*p*NBA), then the acetate and the benzoate were cleaved with NaOH and the resulting diol was protected as a bis-TBS ether (62% overall). Selective cleavage of the primary TBS group with HF/pyridine produced Series *2* alcohol **22**{*2*,**a**−**d**}. Likewise, enantiomeric Series *3* and *4* alcohols **22**{*3*,**a**−**d**} (no inversion at C15) and **22**{*4*,**a**-**d**} (inversion at C15) were prepared by the same set of reactions starting from L-fructose-derived **21**{*3*,**a**−**d**}.

The completion of the mixture reaction sequence is illustrated with Series *1* compounds in Scheme 6. Alcohol **22**{*1*,**a**−**d**} was converted to the corresponding sulfide by a Mitsunobu reaction with 1-phenyl-5-thiotetrazole (PTSH). The so formed sulfide was oxidized to the sulfone mixture **4**{*1*,**a**−**d**} in 88% yield by treatment with *m*CPBA. Deprotonation of **4**{*1*,**a**−**d**} by NaHMDS generated the corresponding anion, which reacted with the aldehyde (4*R*,34*S*)-**3** to afford an alkene in 82% yield.

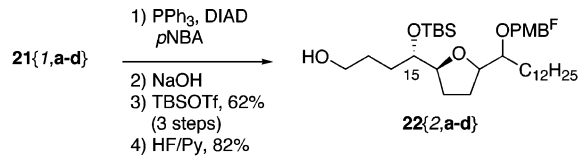
Scheme 5. Synthesis of Series *1*−*4* THF Mixtures **22**

Series 1, 15R,16S, no inversion at C15



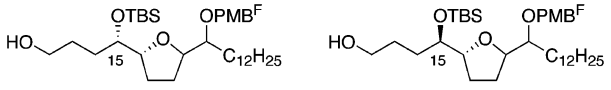
22{*1*,**a-d**}

Series 2, 15S,16S, inversion at C15



22{*2*,**a-d**}

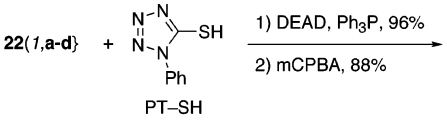
Series 3,15S,16R, no inversion *Series 4,15R,16R, inversion*



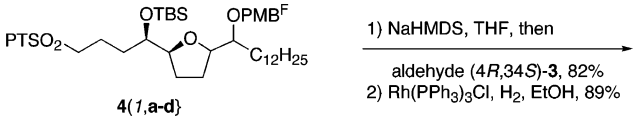
22{*3*,**a-d**}
from **21**{*3*,**a-d**}

22{*4*,**a-d**}
from **21**{*3*,**a-d**}

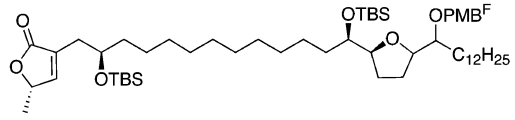
Scheme 6. Synthesis of Final Fluorous-Tagged Mixtures **2**



PT–SH



4(*1*,**a-d**}



2{*1*,**a-d**}

Made analogously, **2**{*2*,**a-d**}, **2**{*3*,**a-d**}, **2**{*4*,**a-d**}

This alkene was then selectively hydrogenated with Wilkinson's catalyst to give the final Series *1* mixture **2**{*1*,**a**−**d**} in 89% yield.

Series *2*−*4* intermediates **22** were converted to the analogous intermediates **2** by the same sequence of steps as Series *1*. Isolated yields were comparable across the four series, and full details are provided in the Supporting Information. Overall, the fluorous mixture synthesis phase (Schemes 3−6) required 39 reaction steps to produce all four final mixtures **2**. Conducting the identical sequence of reactions on individual compounds would have required 156 reaction steps.

Fluorous Mixture Synthesis, Characterization of Intermediate Mixtures: In multistep mixture synthesis, it is not possible to move ahead blindly by assuming that reactions have worked. Instead, characterization of mixtures and their underlying components is a crucial element in identifying and optimizing reaction conditions that provide suitable yields and purities.

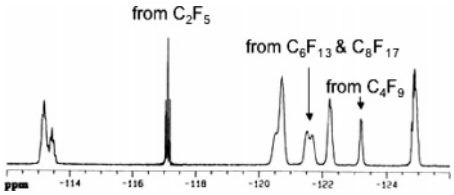


Figure 7. ^{19}F NMR spectrum ($CDCl_3$) of **6**{**a**-**d**}

Likewise, purification of mixtures is important, since it is impractical to take crude products ahead with no purification until the demixing.

To remove reaction byproducts, most of the crude product mixtures were purified by flash chromatography on silica gel. The yields reported were calculated based on the average molecular weight of the mixture. Unlike past work, where we often observed a single spot for four to seven compound mixtures,[15] most of the mixtures in this synthesis showed two closely spaced spots in a TLC on standard silica gel, and some showed three and even four. This indicates that the fluorous-tagged quasi-isomers can be separated on silica gel and, therefore, that selective loss of components in the mixture during the purification process is possible. Either HPLC on an analytical FluoroFlash column (4.6 mm × 250 mm) or ^{19}F NMR spectroscopy were used to analyze chromatography fractions both to ensure that there was no loss of components in the mixture either during the reaction or during the purification and to prevent the remixing of impurities that were separated during the flash chromatographies. Any fractions containing unintentionally separated target products (by LC−MS analysis) were remixed with the main fraction prior to the next reaction step.

In characterizing mixtures either before or after purification, we needed to know whether the underlying components had the expected structures, whether there were impurities present (and what they were, if possible), and whether the mixture components were present in the expected ratios.

LC−MS analysis with a fluorous HPLC column was on the front line for characterization because it potentially answers all three questions by rapidly demixing mixtures, providing component ratios by integration, and providing structural information (molecular weight) all at once. Some crude and all purified product mixtures showed four major peaks with expected masses in an HPLC chromatogram on a FluoroFlash column. Obtaining this kind of "four-peak" chromatogram was the green light for taking a mixture to the next step. Additional minor peaks (for example, unreacted starting materials) were sometimes observed in pairs with the target peaks. In these cases, we either optimized the reaction conditions further or chromatographed the mixture prior to scaling up. In this way, we avoided the problems of accumulated impurities that are a crucial concern in any multistep mixture synthesis.

The approximate ratios of each component in a mixture can quickly be obtained without demixing by ^{19}F NMR spectroscopy. As an example, the CF_2 region of the ^{19}F NMR spectrum ($CDCl_3$) of **6**{**a**−**d**} is shown in Figure 7. The resonance at −123.4 ppm is a CF_2 resonance that is unique to the C_4F_9-tag, while the resonance at −117.3 ppm is a CF_2 group that is characteristic of the C_2F_5-tag. The integration ratio of these two resonances thus represents the ratio of the component with the C_4F_9-tag to that with the C_2F_5-tag. The two closely spaced resonances at −122.4 ppm correspond to one CF_2 group from

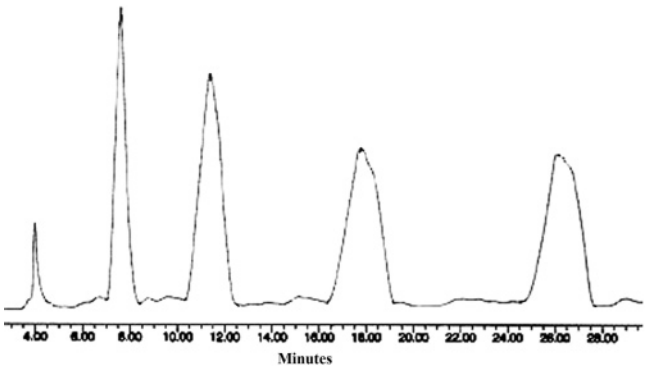


Figure 8. A typical HPLC chromatogram from the preparative demixing of **2**{*1*,**a**−**d**}. FluoroFlash-PF8 column, CH_3CN/THF gradient (100% CH_3CN to 90% CH_3CN/10% THF over 10 min, then to 60% CH_3CN/40% THF over 20 min).

the C_6F_{13}-tag and one from the C_8F_{17}-tag. The integration of this resonance can therefore be used to obtain the ratio of the sum of the C_6F_{13}-tagged component and the C_8F_{17}-tagged component to either the C_2F_5- or C_4F_9-tagged component. The ratio of the C_6F_{13}-tagged component to the C_8F_{17}-tagged one can be analyzed as described previously;[36] however, simple inspection of the two adjacent peak heights usually suffices.

We recorded ^{1}H NMR spectra of every mixture, and these "mixture spectra" sometimes provided useful information. For example, it is very easy to follow the appearance or disappearance of functional groups such as aldehydes with characteristic resonances. But the mixture spectra do not yield the usual level of information since they are the sum of four overlapping spectra of the components. An LC NMR experiment, however, provides the spectrum of individual components and is thus a powerful tool that we routinely used in this work. The use of LC NMR to validate quasienantiomer analysis is described above in Figure 6. More typically, we used this experiment to simply prove that each underlying precursor in a mixture had been converted to the target product. This use is illustrated in more detail in the Supporting Information.

In short, when used judiciously, existing spectroscopic and chromatographic techniques are capable of filling all separation and characterization requirements for multistep fluorous mixture synthesis.

Fluorous Mixture Synthesis, Demixing: Each of the four final mixtures **2** of fluorous-tagged compounds was then preparatively demixed to provide its four underlying components in pure form. The demixing of Series *1* mixture **2**{*1*,**a**−**d**} is representative. The mixture (302 mg) was preparatively separated over a FluoroFlash PF-8 column (20 mm × 250 mm) under gradient conditions. A typical chromatogram for a preparative run is shown in Figure 8. About 40 mg of the mixture was injected each time. After eight injections, the amounts of pure **2**{*1*,**a**}, **2**{*1*,**b**}, **2**{*1*,**c**}, and **2**{*1*,**d**} obtained were 31.0, 49.9, 54.5, and 60.2 mg, respectively. The molar ratio of the pure components is about 1:1.5:1.5:1.5, and the overall recovery of the preparative separation was 65%.

The moderate (65%) recovery from the preparative demixing is not unexpected because the underlying tagged components at this point are not likely to be diastereomerically pure. Figure 9 summarizes the reactions that were used to introduce the

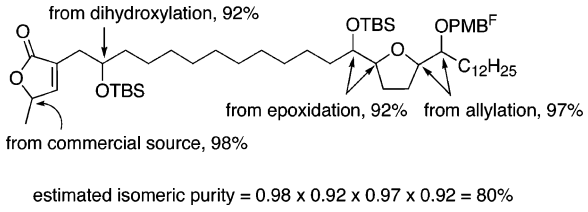
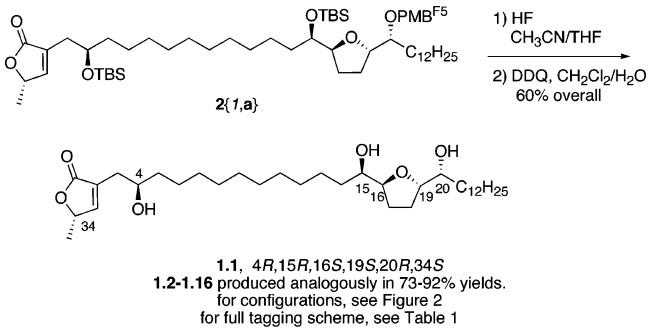


Figure 9. Estimated isomeric purity of the final tagged compounds **2**.

Scheme 7. A Representative Deprotection



stereocenters in **2** along with the approximate stereoselectivities. If no diastereoisomer separation has occurred during the synthesis, then the expected diastereopurity of each of the 16 products is about 80%. In practice, the expected levels of each minor isomer are small enough that they are not easy to quantify in a sample; but the potential cumulative effect is considerable. The only point at which we carefully analyzed for minor products during the mixture synthesis was the Shi epoxidation, and we know that preparative flash chromatography of epoxide **20** or THF **21** did not remove the minor diastereomers from this reaction.

The preparative demixing provided the first clear opportunity to increase the diastereopurity by taking advantage of secondary separations on fluorous chromatography. We used the tagging scheme based on perfluoroethylene (CF_2CF_2) increments to provide wide separation on the fluorous HPLC such that a minor diastereomer that managed to separate from its major diastereomer with the same tag would not run with the prior or subsequent tagged isomer. In the preparative demixing, each main peak was preceded by a very small peak (~3%). In addition to cutting out this unknown impurity and other obvious minor peaks during collection in preparative runs, we were conservative in shaving off both the early and late portions of each main HPLC peak in the hopes of further removing minor isomers.

Series *2*−*4* mixtures **2** were preparatively demixed in a similar fashion to provide the other 12 tagged murisolin isomers. These 16 compounds were then characterized by the standard battery of small molecule techniques (see Supporting Information) to ensure identity. We postponed further assessment of diastereopurity until after the detagging.

Fluorous Mixture Synthesis, Postmix: The demixing concluded the mixture synthesis phase by producing 16 individual tagged murisolins **2**, ready for detagging and final purification. Samples were detagged in four groups of four by initial exposure to aqueous HF in acetonitrile/THF (Scheme 7). The resulting 16 products were not characterized, though TLC analysis showed two spots that were suggestive of the expected product resulting from desilylation and the product resulting from further

(36) Zhang, Q. S.; Rivkin, A.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 5774−5781.

cleavage of the PMB^F group. To complete the PMB^F removal, the crude products were taken up in wet dichloromethane, and DDQ was added. After 1 h, the reaction mixture was worked up, and the crude products were purified by flash chromatography to give the 16 target murisolins as white waxes in yields ranging from 62 to 71%.

At this stage, all 16 isomers of the murisolins **1** were carefully characterized by a set of 1D (^1H and ^{13}C) and 2D ($^1H/^1H$ COSY, HMQC, HMBC) experiments. While the ^1H NMR spectra were clean, each ^{13}C NMR spectrum exhibited two minor peaks very close to the major peaks at about 152 and 70 ppm. Integrations of appropriate resonances in each ^{13}C NMR spectrum showed that the ratio of major to minor products was about 15/1. This minor product cannot be another diastereomer in the library (that is, isomers at C15, C16, C19, or C20), since none of the 16 members of the stereoisomer library has these resonances.

In carefully checking intermediate purity, we located the impurity in the starting TBS-protected hydroxybutenolide (4*R*,34*S*)-**12**. This exhibits major resonances at 151.7 and 70.1 ppm, while there is a small impurity with resonances at 151.9 and 69.8 ppm. The chemical shifts and ratios (~15/1) of these resonances correspond well to the minor resonances in the final product. Accordingly, the impurity is the epimer at C4 that resulted from the Sharpless asymmetric dihydroxylation.

We conducted LC−MS analyses of each sample on a standard reversed-phase column (Xterra C18), and apparent purities ranged from 90 to 97%. Minor peaks were present in each chromatogram, and all peaks exhibited the same molecular ion ($m/e = 581$), suggesting that all the compounds detected in this analysis are diastereomers. However, the analytical LC−MS traces did not reflect our expectations from the ^{13}C NMR spectra. Instead of exhibiting one major impurity in a ratio to the major product of 15/1, each chromatogram exhibited two or more trace products whose amounts summed to 3−9%.

To remove these trace products, each sample was purified by preparative reversed-phase HPLC on a Symmetry C18 column. However, ^{13}C NMR spectra of all these samples still showed the two extraneous resonances at 151.9 and 69.8 ppm, so the samples were not yet pure. Analyses of these samples on a Chiralcel OD column showed purities of 90−96%. This time, each major peak in the chromatogram was accompanied by a single minor peak. Thus, the reversed-phase purification succeeded in removing small amounts of diastereomers that we could not detect by ^1H or ^{13}C NMR analysis, but it did not remove the major impurity (C4 epimer). This was finally removed when each sample was purified by semipreparative HPLC over a Chiralcel OD column to give the 16 pure murisolin stereoisomers. These products did not exhibit the minor peaks adjacent to the major ones at 151.7 and 70.1 ppm in their ^{13}C NMR spectra, and there were no significant impurities in either the reversed-phase or Chiralcel OD phase analytical HPLC chromatograms.

Overall, the HPLC results are qualitatively consistent with the stereochemical analysis in Figure 9. Even though intermediate mixtures were purified by flash chromatography at several stages during the fluorous mixture synthesis, these purifications did not significantly enrich the diastereopurity of the intermediates. Crude samples after detagging were probably 80−90% isomerically pure. Some minor diastereomers were removed in the initial chromatography over reversed-phase silica gel, and

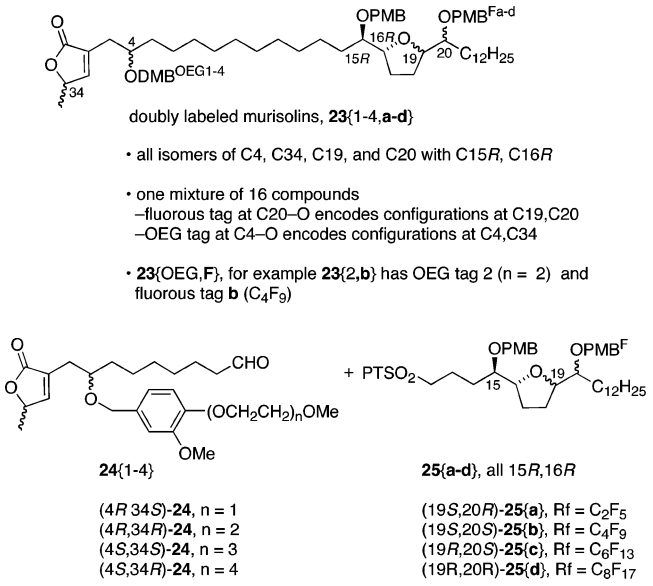


Figure 10. Strategy for the double mixture synthesis with oligoethylene glycol tags and fluorous tags.

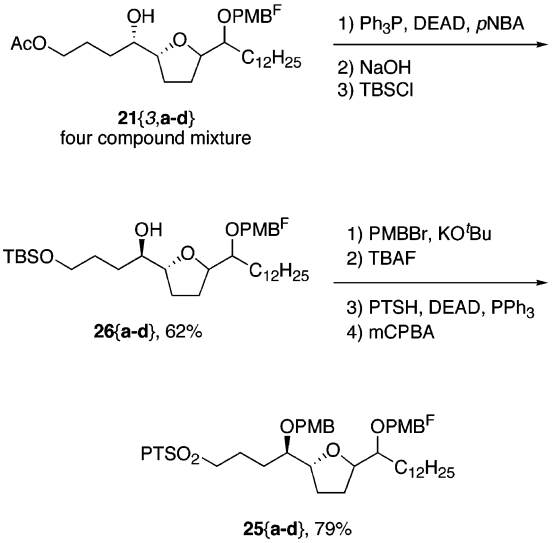
the single largest impurity (C4 epimer) was removed by Chiralcel chromatography. Each of the 16 murisolins **1** has a different retention time on the Chiralcel OD column, so hindsight suggests that the reversed-phase purification was not needed and that a single Chiralcel purification would have given similar results.

We conclude from the HPLC studies that each of the 16 samples in the first-generation murisolin library is substantially (≥98%) isomerically pure. This conclusion is important not only because each isomer was accompanied by three other quasi-isomers during the fluorous mixture synthesis but also because a number of the isomers have identical NMR spectra, even at very high magnetic field.

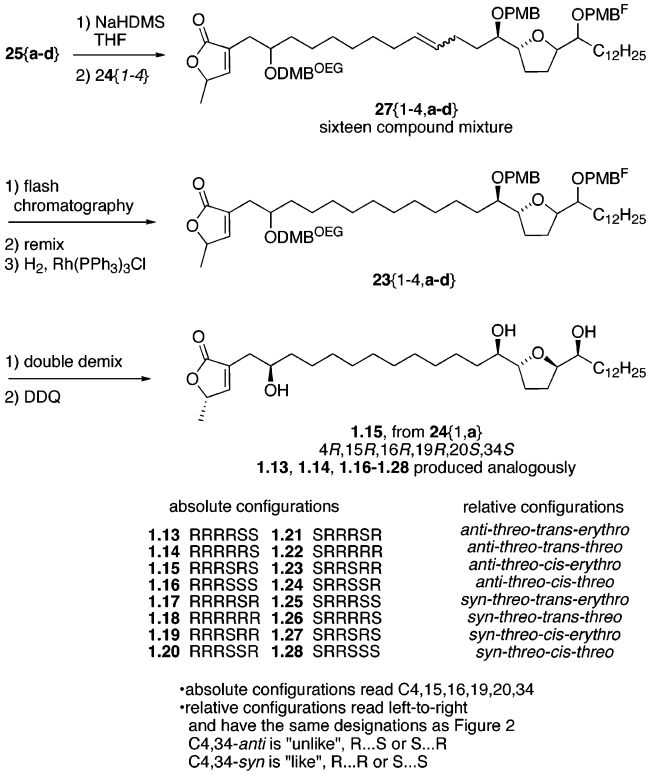
Double Mixture Synthesis: One of the limitations with the fluorous mixture synthesis is that 16 fluorous tags are not currently available, so the 16 isomers were made in four mixtures of four compounds. Although this results in significant savings in effort, we still sought a means to make 16 isomers in a single reaction vessel. Toward this end, we have recently introduced "double fluorous/oligoethylene glycol mixture synthesis", which is in essence the product of the two single mixture synthesis methods.[18] In other words, reaction of one mixture of four fluorous-tagged isomers with another mixture of four oligoethylene glycol-tagged isomers will give a new 16-compound mixture in which each of the 16 isomers is separable and identifiable by its unique combination of the two tags.

The plan for the double mixture synthesis is shown in Figure 10. The tagging strategy calls for the coupling of a four-compound fluorous-tagged dihydroxy-THF fragment **25**{**a**−**d**}[20] with a four-compound OEG-tagged hydroxybutenolide fragment **24**{1−4}. After one hydrogenation reaction to saturate the alkene, the resulting 16-component mixture of doubly tagged murisolins **23**{1−4,**a**−**d**} is ready for double demixing and detagging. Oligoethylene glycol homologues of the popular dimethoxybenzyl group were selected for protection of the C4 hydroxy group of **24** because the needed reagents are readily available and because we expected that the DMB^{OEG} group could be removed under the same conditions as those for the PMB^F group. Minimizing protecting group removal steps is

Scheme 8. Synthesis of Fluorous-Tagged Mixture **25**{**a**−**d**}



Scheme 9. Double Mixture Synthesis and Demixing



absolute configurations		relative configurations
1.13 RRRRSS	**1.21** SRRRSR	*anti-threo-trans-erythro*
1.14 RRRRRS	**1.22** SRRRRR	*anti-threo-trans-threo*
1.15 RRRSRS	**1.23** SRRSRR	*anti-threo-cis-erythro*
1.16 RRRSSS	**1.24** SRRSSR	*anti-threo-cis-threo*
1.17 RRRRSR	**1.25** SRRRSS	*syn-threo-trans-erythro*
1.18 RRRRRR	**1.26** SRRRRS	*syn-threo-trans-threo*
1.19 RRRSRR	**1.27** SRRSRS	*syn-threo-cis-erythro*
1.20 RRRSSR	**1.28** SRRSSS	*syn-threo-cis-threo*

•absolute configurations read C4,15,16,19,20,34
•relative configurations read left-to-right
and have the same designations as Figure 2
C4,34-*anti* is "unlike", R...S or S...R
C4,34-*syn* is "like", R...R or S...S

important because these occur during the postmix stage and therefore must be conducted on 16 samples.

For the fluorous mixture **25** we choose to work in Series *4* (15*R*,16*R*), which by then we knew contained the natural product murisolin **1.14**. The OEG mixture **24** contains all four possible isomers of the hydroxybutenolide, so the final stereoisomer sublibrary will contain murisolin and three of the isomers that are already in hand, along with 12 new isomers. The new set of 12 isomers includes all possible stereoisomers of the natural product murisolin at C4 and C34. Taken together, the two libraries provide a total of 28 of the 64 possible isomers of murisolin, with 24 of the 32 possible diastereomers.

The double mixture reaction sequence follows the established route, but we made one important improvement in each fragment. In the dihydroxy-THF fragment **25**, we replaced the TBS group on O15 with a standard PMB group, again to minimize protecting group removal steps. In the synthesis of the hydroxybutenolide fragment **24**, we exchanged the Sharpless AD reaction with a Jacobsen hydrolytic kinetic resolution[37] of an epoxide. This provided **24** in isomerically pure form and accordingly eliminated the set of minor isomers at C4 that needed to be removed during the postmix stage of the fluorous mixture synthesis. The synthesis of aldehyde **24**{1−4} was the first example of an OEG mixture synthesis, and this work is described elsewhere in full detail.[38]

The synthesis of the needed fluorous mixture **25**{**a**−**d**} is summarized in Scheme 8. Intermediate **21**{*3*,**a**−**d**} (Scheme 4) was inverted by Mitsunobu reaction (inverts Series *3* to Series *4*) and the diol resulting after hydrolysis of both esters was monosilylated with TBSCl to give **26**{**a**−**d**}. The resulting alcohol was reacted with PMBBr by a standard procedure, and the TBS ether then was converted to the *N*-phenyltetrazolyl sulfone **25**{**a**−**d**} by the same sequence of steps used before.

The double mixture steps are summarized in Scheme 9. Kocienski−Julia coupling of four-component fluorous-tagged mixture **25**{**a**−**d**} (0.28 mmol) with four-component OEG mixture **24**{1−4} (0.28 mmol) as usual provided a crude alkene product **27**{1−4,**a**−**d**}.

Fluorous HPLC analysis of this mixture occurred with fluorous demixing but not OEG demixing to provide four pairs of peaks, each pair with a ratio of about 85/15. Both fluorous HPLC analysis and standard TLC analysis showed that the minor peaks belonged to the starting sulfones in **25**{**a**−**d**}. Accordingly, the crude mixture **27** was purified by silica flash chromatography by using a step gradient to sequentially elute groups of molecules based on the OEG tags.[38] First step elution with 25% hexane/ethyl acetate provided unreacted sulfone **25**{**a**−**d**} followed by a fraction of the four coupled products bearing the $n = 1$ OEG tag and all four possible fluorous tags **27**{1,**a**−**d**}. The succeeding steps of the gradient provided the other three four-compound mixtures **27** with OEG = 2, 3, and 4. These four mixtures were then remixed to give a pure 16-compound mixture **27**{1−4,**a**−**d**}, which was analyzed by fluorous HPLC. As expected, the set of four major peaks resulting from the Julia products **27** remained, but the set of four minor peaks resulting from the starting sulfone **25** were absent. Figure 11 shows the HPLC chromatograms of **27**{1−4,**a**−**d**} before and after purification. The total yield of the purified 16-compound mixture **27** obtained by a single flash chromatography was 71%.

Thus for unknown reasons, the large scale, double mixture coupling reaction did not result in complete consumption of the sulfone **25**{**a**−**d**}. Nonetheless, it was still possible to readily remove the four unreacted sulfones from the 16-products **27** prior to the hydrogenation reaction. This reaction proceeded smoothly on a 320 mg sample of **27**{1−4,**a**−**d**} to provide **23**{1−4,**a**−**d**} in crude quantitative yield, ready for double demixing.

Double demixing of a 16-compound mixture requires five separations. A first separation against one class of tags provides

(37) (a) Schaus, S. E.; Brandes, B. D.; Larrow, J. F.; Tokunaga, M.; Hansen, K. B.; Gould, A. E.; Furrow, M. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2002**, *124*, 1307−1315. (b) Liu, P.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2001**, *123*, 10772−10773.

(38) Gudipati, V.; Curran, D. P.; Wilcox, C. S. *J. Org. Chem.* **2006**, ASAP article.

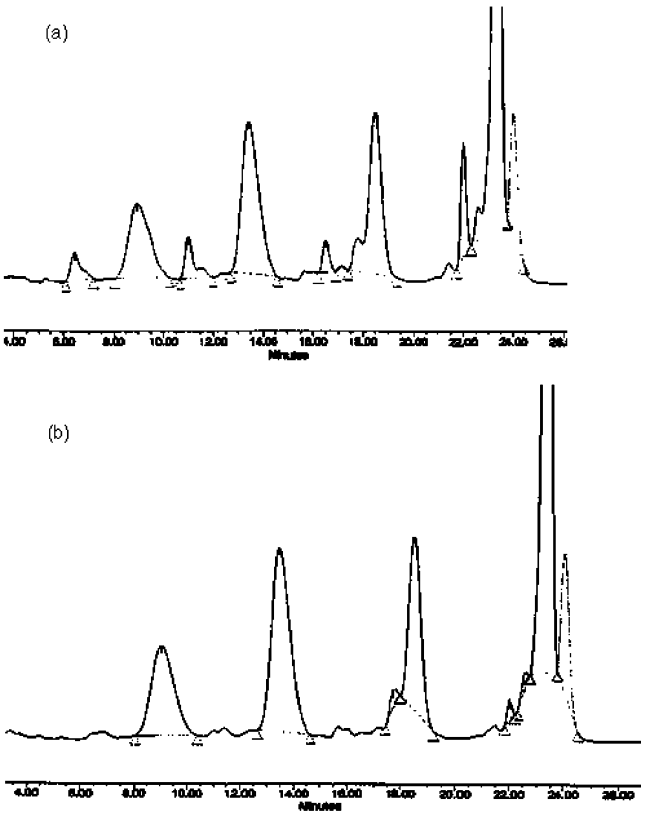


Figure 11. Fluorous HPLC traces of crude (a) and purified (b) **31**{1,**a**−**d**} show that the unreacted sulfones **25**{**a**−**d**} (minor peak set in (a) to the left of major peak set) have been removed.

four mixtures of four compounds. In turn, these four mixtures are each separated against the second class of tags to provide the 16 pure products. While double demixings can be conducted in either order,[18] we decided here to conduct the OEG demixing first because it is a simple flash chromatography that is easier to accomplish than preparative HPLC on the larger initial sample.

Mixture **23**{1−4,**a**−**d**} was demixed over silica gel by a four-step gradient (EtOAc in hexanes, 25%, 50%, 65%, 80%) to provide four fractions of products bearing increasingly larger OEG tags. Each fraction contained four compounds with the four possible fluorous tags: **23**{1,**a**−**d**, 52 mg; **23**{2,**a**−**d**}, 66 mg; **23**{3,**a**−**d**}, 55 mg; **23**{4,**a**−**d**}, 50 mg 84% overall from alkene **31**). Each of the resulting four fractions was demixed in the fluorous dimension on a FluoroFlash HPLC column by the conditions described above. Sample sizes were about 20 mg per injection, so each fraction required three injections for complete demixing. Minor peaks were not collected, and the leading and trailing edges of the major peaks were shaved off to provide the maximum possible purity. This provided all 16 individual products **2.13**−**2.28** in a total recovery of 72% from the purified mixture **23**.

In the postmix stage, the normal PMB group, the fluorous PMB group, and the OEG DMB group were simultaneously removed by treatment of the compounds with 3.3 equiv of DDQ. Samples were initially purified by preparative TLC to remove DDQ remnants. Careful analysis of one of the products by ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) showed traces of impurities; however, resonances at 151.1 and 69.8 from the C4 epimer that were present in the first library were not observed. This is in line with expectations, since the starting aldehyde **24** was made in diastereopure form by a different route.

Since the prior work suggested that the reversed-phase chromatography was not needed, the products **1.13**−**1.28** were purified by semipreparative HPLC over a Chiralcel OD column to remove minor diastereomeric impurities. The purities of these products under both reversed-phase and chiral phase conditions were assessed. All compounds exhibited single peaks on the chiral column, showing the success of the preparative separation. 13 of the samples also showed excellent purity in the reversed-phase analysis (≥95%). However, unexpectedly three of the samples (see Supporting Information) showed significant impurities (5−15%), and accordingly these three were repurifired by reversed-phase chromatography to provide all 16 pure products.

Four of the products (**1.13**−**1.16**) were compared with the corresponding products from the prior library, and the samples were identical by spectroscopy and (more importantly) by co-injection on the Chiralcel OD column. The other 12 compounds are new and were fully characterized as usual.

Compound Characterization: Table 1 compiles information about all 28 members of the stereoisomer library, including their configurations, their tags in the single and/or double mixture library, their retention times on a Chiralcel OD column, and their optical rotations in MeOH ($c = 0.05-0.27$). The retention times on the chiral column vary over a wide range, and are accordingly very useful for matching or differentiating isomers. In contrast, the optical rotations are of little value because they are very small and may be concentration dependent. All 28 compounds were fully characterized by the usual spectroscopic techniques, and data are compiled in text format in the Supporting Information.

Comparisons of spectra and other data to make murisolin structure assignments are the topics of a forthcoming full paper.[19] Briefly, there is substantial spectral identity among the murisolin isomers. Indeed, all 32 murisolin isomers exhibit one of only six sets of ^{1}H NMR spectra at 600 MHz; no isomer has a unique spectrum. Nonetheless, we have rigorously confirmed that the structure of murisolin **1.14** is correct but suggest that the structure of 16,19-*cis*-murisolin **1.16** is probably incorrect and that it may be **1.8** instead. We also suggest that murisolin A may be **1.10**.

Conclusions

Despite their inherent value, stereoisomer libraries of complex natural products are rarely made because too much effort is involved in traditional "one compound at a time" approaches to total synthesis. Fluorous mixture synthesis, oligoethylene glycol mixture synthesis, and double mixture synthesis use separation tags to leverage the power of traditional solution phase methods by providing more compounds per unit of synthetic effort. Standard small molecule separation and characterization techniques can be used even though they are applied to mixtures rather than single compounds. The separation tags (fluorous or oligoethylene glycol) provide for orchestrated demixing of the mixtures at any time. The syntheses of murisolin isomers show that combining solution phase mixture synthesis techniques with synthetic plans that minimize steps both with efficient routes and with late splits allows for a substantial increase in the number of stereoisomers of complex compounds that can be made.

Table 1. Configurations, Tags, Optical Rotations, and Retention Times of the 28 Murisolin Isomers

number	abs config[a]	rel config[b]	FMS prec[c]	FTag	DMS prec[d]	OEG tag	F tag	α_D[e]	T_R[f]
1.1	RRSSRS	aete	**2**{*1*,**a**}	C_2F_5	−	−	−	+1.1	27.8
1.2	RRSSSS	aett	**2**{*1*,**b**}	C_4F_9	−	−	−	+0.7	20.3
1.3	RRSRSS	aece	**2**{*1*,**c**}	C_6F_{13}	−	−	−	+9.9	20.6
1.4	RRSRRS	aect	**2**{*1*,**d**}	C_8F_{17}	−	−	−	+8.2	18.0
1.5	RSSSRS	atte	**2**{*2*,**a**}	C_2F_5	−	−	−	+1.9	22.6
1.6	RSSSSS	attt	**2**{*2*,**b**}	C_4F_9	−	−	−	+2.1	20.7
1.7	RSSRSS	atce	**2**{*2*,**c**}	C_6F_{13}	−	−	−	+7.5	14.0
1.8	RSSRRS	atct	**2**{*2*,**d**}	C_8F_{17}	−	−	−	+4.5	15.9
1.9	RSRRSS	aete	**2**{*3*,**c**}	C_6F_{13}	−	−	−	+13.5	25.3
1.10	RSRRRS	aett	**2**{*3*,**d**}	C_8F_{17}	−	−	−	+9.5	22.1
1.11	RSRSRS	aect	**2**{*3*,**a**}	C_2F_5	−	−	−	+3.1	21.6
1.12	RSRSSS	aece	**2**{*3*,**b**}	C_4F_9	−	−	−	+6.9	14.3
1.13	RRRRSS	atte	**2**{*4*,**c**}	C_6F_{13}	**23**{1,**c**}	$n = 1$	C_6F_{13}	+14.0/+10.3	21.6/22.0[g]
1.14	RRRRRS	attt	**2**{*4*,**d**}	C_8F_{17}	**23**{1,**d**}	$n = 1$	C_8F_{17}	+16.1/+6.6	23.2/24.6[g]
1.15	RRRSRS	atce	**2**{*4*,**a**}	C_2F_5	**23**{1,**a**}	$n = 1$	C_2F_5	+3.0/+4.1	15.9/16.0[g]
1.16	RRRSSS	atct	**2**{*4*,**b**}	C_4F_9	**23**{1,**b**}	$n = 1$	C_4F_9	+8.1/+9.8	16.4/17.2[g]
1.17	RRRRSR	stte	−	−	**23**{2,**c**}	$n = 2$	C_6F_{13}	−2.5	23.8
1.18	RRRRRR	sttt	−	−	**23**{2,**d**}	$n = 2$	C_8F_{17}	+5.2	26.3
1.19	RRRSRR	stce	−	−	**23**{2,**a**}	$n = 2$	C_2F_5	−5.0	17.5
1.20	RRRSSR	stct	−	−	**23**{2,**b**}	$n = 2$	C_4F_9	+4.1	17.9
1.21[h]	SRRRSR	atte	−	−	**23**{4,**c**}	$n = 4$	C_6F_{13}	+4.3	21.9
1.22[h]	SRRRRR	attt	−	−	**23**{4,**d**}	$n = 4$	C_8F_{17}	−1.2	25.1
1.23[h]	SRRSRR	atce	−	−	**23**{4,**a**}	$n = 4$	C_2F_5	+1.5	15.6
1.24[h]	SRRSSR	atct	−	−	**23**{4,**b**}	$n = 4$	C_4F_9	−0.3	16.3
1.25	SRRRSS	stte	−	−	**23**{3,**c**}	$n = 3$	C_6F_{13}	+3.4	22.1
1.26	SRRRRS	sttt	−	−	**23**{3,**d**}	$n = 3$	C_8F_{17}	−1.9	24.5
1.27	SRRSRS	stce	−	−	**23**{3,**a**}	$n = 3$	C_2F_5	−11.9	15.8
1.28	SRRSSS	stct	−	−	**23**{3,**b**}	$n = 3$	C_4F_9	−1.4	16.6

[a] C4,15,16,19,20,34. [b] [c] s/a, *syn/anti* at C4,34, t/e, *threo/erythro* at C15,16 and C19,20; c/t, *cis/trans* at C16,19; {series number, fluorous tag}. [d] {OEG tag, fluorous tag}. [e] In MeOH, $c = 0.05-0.27$; see Supporting Information for concentrations. [f] Retention time in min on a Chiralcel OD column, 94/6, hexane/2-propanol, 1.5 mL/min; the absolute retention. [g] The retention times correspond to samples run from the fluorous mixture library (first value) and the double mixture library (second value) injected several months apart. The samples were identical on co-injection. [h] **1.21**−**1.24** are enantiomers of **1.5**−**1.8**.

The fluorous mixture synthesis and double mixture synthesis described herein provided 32 murisolins, 28 of which are unique (four compounds are the same in the two libraries). Among these 28, there are four pairs of enantiomers, and therefore we have made 24 of the 32 possible diastereomers of murisolin. The mixture synthesis plans provide for substantial savings in effort over the same plans conducted in parallel or serial on individual compounds; the fluorous mixture synthesis saves well over 100 chemical steps, while the double mixture synthesis saves about 60 steps.

The effect of leveraging of effort can also be seen by posing the question: is it realistically possible to make all 64 isomers of murisolin by using these techniques? The answer is certainly "yes". Indeed, if we had available to us the four-compound mixture of hydroxybutenolides **25**{**a**−**d**} at the end of the first mixture synthesis, then we could have used this instead of the single hydroxybutenolide **3** in the four final couplings to produce four mixtures of 16 compounds instead of four mixtures of four compounds. Double demixing and detagging of these four mixtures would have provided all 64 isomers of murisolin. This "paper synthesis" of the complete murisolin stereoisomer library has the same number of premix and mixture synthesis steps as the actual synthesis that we conducted in making the second 16-compound library; no new intermediates have to be made! It requires only 15 extra demixings (4×5 demixings instead of 1×5) and 48 extra detaggings to provide 64 products instead of 16. The only trade off is sample size, since on the same scale each of the 64 compounds would be produced in one-fourth of the amount as 16. That we could potentially make 48 more compounds with only a few more HPLC injections and detaggings is a dramatic example of how tagging and splitting can combine to provide a large stereoisomer library.

Although we have the capability, we do not currently plan to make all 64 isomers of murisolin because we have already obtained a complete picture of the spectroscopic behavior of this family of compounds from the 28 isomers in hand, along with derivatives. This is the first such complete picture to be obtained for any acetogenin. A forthcoming paper[19] analyzes these data fully and discusses ramifications for stereostructure assignments of murisolins, other acetogenins, and related compounds with local symmetry and remote groups of stereocenters.

Acknowledgment. We thank the National Institutes of Health (National Institute for General Medical Sciences) for funding of this work. We also thank B. Figadère and T. Tanaka for providing samples, spectra, and helpful comments (BF). We also thank Mr. Serhan Turklyilmaz for help preparing OEG tagging reagents and Ms. F.-M. Lin and Dr. F. -T. Lin for running LC NMR experiments. We dedicate this paper to Prof. Barry M. Trost on the occasion of his 65th birthday.

Supporting Information Available: Contains full experimental details and key characterization data of new compounds (65 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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