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Minimal Fluorous Tagging Strategy that Enables the Synthesis of the Complete Stereoisomer Library of SCH725674 Macrolactones

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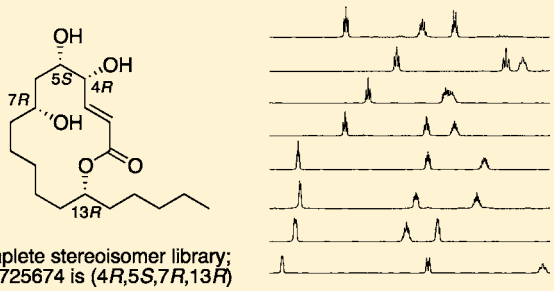
Supporting Information

ABSTRACT: Four mixtures of four fluorous-tagged quasiisomers have been synthesized, demixed, and detagged to make all 16 stereoisomers of the macrocyclic lactone natural product Sch725674. A new bare-minimum tagging pattern needs only two tags—one fluorous and one nonfluorous—to encode four isomers. The structure of Sch725674 is assigned as (5*R*,6*S*,8*R*,14*R*,*E*)-5,6,8-trihydroxy-14-pentyloxacyclotetradec-3-en-2-one. Various comparisons of spectra of 32 lactones (16 with tags, 16 without) and 16 ester precursors (8 with tags, 8 without) provide insights into when and why related compounds have the same or different spectra.



INTRODUCTION

Fluorous mixture synthesis (FMS) is proving to be a powerful tool to make stereoisomer libraries of natural products.[1] In a simple view of this process, molecules are labeled instead of vials or flasks. Different fluorous tags are attached to true isomers to make quasiisomers[2] (the labeled molecules) with encoded stereochemical information.[3] The quasiisomers can then be mixed and carried through multiple steps until a final demixing, which is also controlled by the fluorous tags.

Three of the most important themes in fluorous mixture synthesis have been tagging strategies,[4] expedited natural product structure assignment,[5] and comparison of spectra and properties of stereoisomer libraries.[6] Here we report a total synthesis of the complete stereoisomer library of the macrolactone Sch725674 that advances all three of these themes. We introduce a new tagging strategy that uses fewer total fluorine atoms than any other to date; we confirm the two-dimensional structure of Sch725674 and assign the three-dimensional structure; and we derive interesting information from comparison of spectra of true stereoisomers and quasiisomers both before and after macrolactonization. This is the first time that a natural product stereostructure has been assigned from scratch by making a complete stereoisomer library by FMS.

Macrolactones and related macrolactams are large and important classes of natural products that are often thought to strike a balance between preorganization and conformational flexibility in binding to their biological targets.[7] Because of these features, diversity-oriented synthesis based on macrolactone templates is a lively area of research.[8]

Sch725674 is a macrolactone that was isolated by Yang and co-workers from a culture of *Aspergillus* sp.,[9] and it displayed antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans*. The two-dimensional structure was assigned as (*E*)-5,6,8-trihydroxy-14-pentyloxacyclotetradec-3-en-2-one **1** based on a battery of 2D NMR experiments. We follow the numbering system of the isolation paper, which puts stereocenters at C4, C5, C7, and C13, as shown in Figure 1.

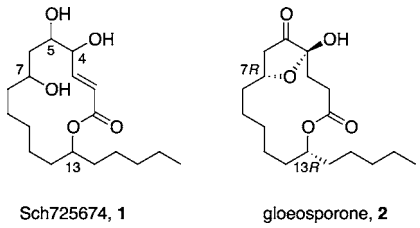


Figure 1. Two-dimensional structure of Sch725674 and complete structure of gloeosporone.

14-Membered macrolactone templates like **1** that lack methyl groups on the backbone are rare, with the popular synthetic target[10] gloeosporone[11] **2** being the closest relative of **1**. All known 14-membered (and related 18-membered ring) macrolactone natural products have the (13*R*)-configuration as established by Celmer[12] and Seebach and Schreiber.[11a] Those that have a hydroxyl group at C7 are also usually (7*R*), again like gloeosporone.

RESULTS AND DISCUSSION

We describe here a second-generation synthesis of the Sch725674 stereoisomer library. The first-generation synthesis, described in the thesis of Dr. X. Wang,[13] produced eight isomers (including Sch725674) on a submilligram scale. Improvements to make the synthesis robust enough to produce all 16 isomers on a multimilligram scale and model tagging experiments are discussed in the thesis of Dr. J. Moretti.[14] Here

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we focus exclusively on the successful second-generation fluorous mixture synthesis.

Tagging Plan. We set out to make all 16 stereoisomers of Sch725674 **1**, and the plan that evolved for the FMS is shown in Figure 2. Late-stage coupling of a pair of enantiopure

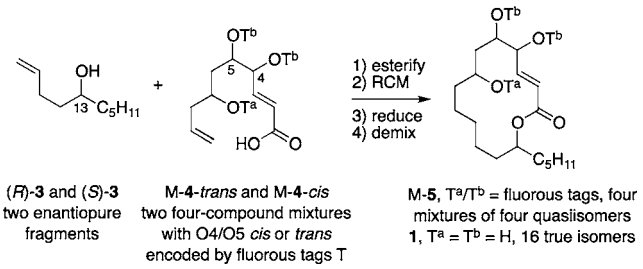
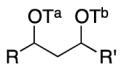


Figure 2. Tagging plan calls for one principal fragment **3** as two enantiomers and the other principal fragment **4** as two mixtures of four quasiisomers. One of these mixtures is the 4,5-*trans* series, and the other is the 4,5-*cis* series. Coupling of the two enantiomers of **3** with the two mixtures of **4** gives four mixtures of four tagged quasiisomers M-**5** as penultimate precursors of the 16 true isomers of **1**.

fragments (*R*)-**3** and (*S*)-**3** with mixtures M-**4**[15] followed by ring-closing metathesis, hydrogenation, demixing, and detagging would give the final products. In turn, the preparation of the 4,5-*trans* series (4*R*,5*R* and 4*S*,5*S*) and 4,5-*cis* series (4*R*,5*S* and 4*S*,5*R*) isomers needed different chemistry, so two four-compound mixtures of **4** were prepared.

The tagging pattern for each of the four-compound mixtures needs to encode two pieces of information (configurations at C4,5 and at C7) to allow for orderly demixing of the four components. Information and separation (demixing) aspects of several possible tagging strategies are shown in Figure 3. Here the numbers (#) on the tags $T^{\#}$ serve as proxies for fluorine content, so the prospect for demixing of a mixture can be assessed simply by summing the tag numbers of each component. After the tagging is complete, each component of the mixture should be a quasiisomer of all the other

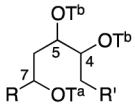
two stereocenters, tags introduced at two stages (a,b), four isomers targeted



6

two tags		three tags		four tags	
T^a/T^b	#F's	T^a/T^b	#F's	T^a/T^b	#F's
T^1/T^1	2	T^1/T^1	2	T^1/T^3	4
T^1/T^2	3	T^2/T^1	3	T^2/T^3	5
T^2/T^1	3	T^1/T^3	4	T^1/T^5	6
T^2/T^2	4	T^2/T^3	5	T^2/T^5	7

three stereocenters, tags introduced at two stages (a,b), four (not eight) isomers targeted, tag b is used twice



7

two tags	
$T^a/T^b/T^b$	#F's
$T^1/T^1/T^1$	3
$T^2/T^1/T^1$	4
$T^1/T^2/T^2$	5
$T^2/T^2/T^2$	6

Figure 3. Tagging patterns for making four quasiisomers of compounds with two (**6**) and three (**7**) stereocenters. Tags "a" and "b" encode stereochemical information. The superscripted numbers are proxies for the fluorine content. Notice the redundancy when two tags encode two stereocenters that is broken when two tags encode three stereocenters.

components, having different configuration and constitution (specifically, fluorine content) from all the other components.

Consider first compound **6** (Figure 3) with two tagged stereocenters. From the information standpoint, only two tags (T^1 and T^2) are needed to uniquely encode four bits of information, here the four possible configurations of the two stereocenters. However, summing the tag numbers of the four different components that are generated with two tags shows that two of the components will be constitutional isomers (not quasiisomers). These have the same fluorine content ($T^1 + T^2 = T^2 + T^1$), so demixing (which is based primarily on fluorine content) will not be reliable.

The use of four different tags (in the sequence T^1, T^2, T^3, T^5) solves this redundancy problem for **6**. Less obvious but also reliable is the use of only three tags.[4] Here, the first stereocenter gets T^1 and T^2. However, instead of using T^3 and T^5 to tag the second stereocenter (the four-tag method), T^1 is reused along with a T^3. This gives products with different fluorine count; in other words, all are quasiisomers, and none are constitutional isomers.

We recognized that the redundancy problem that is apparently inherent in the two-tag approach could be lifted by putting one set of tags (here "a") on once and the other set of tags ("b") on twice. This is shown in Figure 3 with a triol **7** as used in the Sch725674 setting.

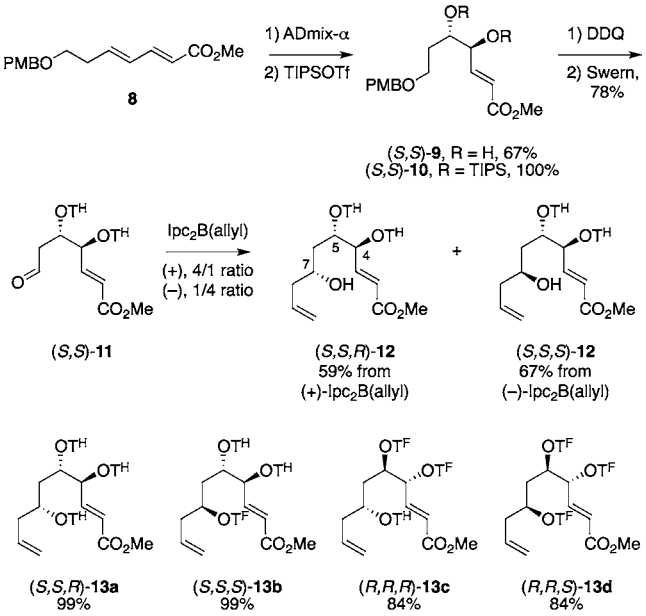
Stereocenter C7 gets one tag that encodes its absolute configuration; for example, (7*R*) gets T^1 and (7*S*) gets T^2. In contrast, stereocenters C4 and C5 receive tags at the same time and accordingly get the same tag. These tags again encode absolute configuration, but of only one of the possible pairs of relative configurations (two *cis* or two *trans*).

For example, the two possible *trans* configurations of **7**, (4*R*,5*R*) and (4*S*,5*S*), are again encoded with T^1 and T^2, respectively. These are the same tags as on C7; however, each isomer needs two tags at this point, so the fluorine content of the second set of tags is doubled and the redundancy problem lifted. Four quasiisomers result. The up side of this strategy is that four quasiisomers can be tagged with two tags, but there is also a down side: there are eight total isomers, so the other four *cis*-isomers are not provided for at all. However, this fits the Sch725674 plan well since the *trans*- and *cis*-series of precursors will be made by different chemistry.

To summarize, the 16-compound library of isomers **1** will be made from four mixtures of four quasiisomers each. The absolute configuration at C13 and the relative configuration at C4/C5 are known by which mixture the sample comes from (in other words, the labels on the flasks), while the identities of each of the four quasiisomers that constitute those mixtures are known by the fluorous tags (in other words, the labels on the molecules). For the first time, the encoding and separation of four quasiisomers is enabled with only two tags, and only one of the two tags needs to be fluorous.

Stereoisomer Library Synthesis. Prior to starting the fluorous mixture synthesis phase, the needed stereoisomeric precursors were made as summarized in Schemes 1 and 2. In the 4,5-*trans* series (Scheme 1), Sharpless asymmetric dihydroxylation[16] of diene **8** with Admix-α produced diol (*S*,*S*)-**9** as a 96/4 ratio of enantiomers as assessed by Mosher ester analysis[17] (see Supporting Information). The (*S*,*S*)-configuration was encoded by attachment of two standard TIPS groups (triisopropylsilyl, $Si(iPr)_3$, hereafter shown as T^H) to give (*S*,*S*)-**10** in 100% yield.

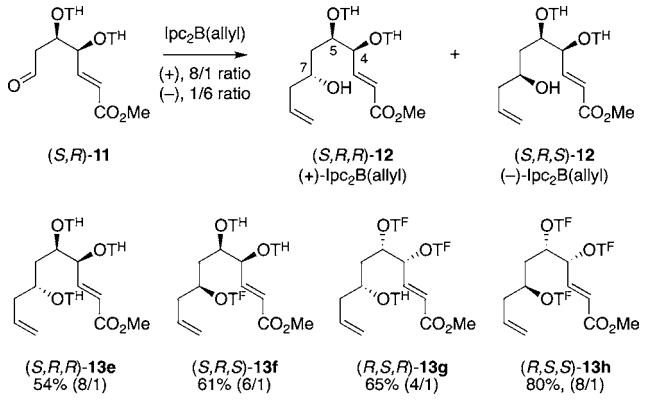
Scheme 1. Synthesis of *trans*-Series Quasiisomers 13a–d[a]



*tagged quasiisomers after silylation with TIPSOTf (T^H) or ^{F}TIPSOTf (T^F): mixed as M-**13a-d**:*
(*S,S,R*)-**13a**, from Admix-α/TIPS and (+)-Ipc$_2$B(allyl)/TIPS
(*S,S,S*)-**13b**, from Admix-α/TIPS and (–)-Ipc$_2$B(allyl)/^{F}TIPS
(*R,R,R*)-**13c**, from Admix-β/^{F}TIPS and (+)-Ipc$_2$B(allyl)/TIPS
(*R,R,S*)-**13d**, from Admix-β/^{F}TIPS and (–)-Ipc$_2$B(allyl)/^{F}TIPS

[a]Minor products **12** from allylboration were separated prior to silylation. T^H is $-Si(iPr)_3$; T^F is $-Si(iPr)_2CH_2CH_2C_2F_5$.

Scheme 2. Synthesis of *cis*-Series Quasiisomers 13e–h[a]



*tagged quasiisomers after silylation with TIPSOTf (T^H) or ^{F}TIPSOTf (T^F); mixed as M-**13e-h**;*
(*S,R,R*)-**13e**, from L-deoxyribose/TIPS and (+)-Ipc$_2$B(allyl)/TIPS
(*S,R,S*)-**13f**, from L-deoxyribose/TIPS and (–)-Ipc$_2$B(allyl)/^{F}TIPS
(*R,S,R*)-**13g**, from D-deoxyribose/^{F}TIPS and (+)-Ipc$_2$B(allyl)/TIPS
(*R,S,S*)-**13h**, from D-deoxyribose/^{F}TIPS and (–)-Ipc$_2$B(allyl)/^{F}TIPS

[a]The minor allylation products could not be separated so the products **12** were silylated directly. Numbers in parentheses are epimer ratios of the tagged quasiisomers at C7.

Standard removal of the PMB group and Swern oxidation provided aldehyde (*S,S*)-**11**, which was divided in half and exposed to both enantiomers of the Brown/Ramachandran reagent,[18] Ipc$_2$B(allyl). Each reaction produced a major diastereomer, (*SSR*)-**12** or (*SSS*)-**12**, in about 4/1 ratio along with the C7 epimer. To ensure maximum stereopurity of the tagged isomers, these mixtures were separated to give the two pure isomers in 59% and 67% yield, respectively. Then the configurations at C7 were encoded with a standard TIPS group (T^H) for (7*R*) and a fluorous TIPS group bearing a pentafluoroethyl substituent ($Si(iPr)_2CH_2CH_2C_2F_5$, hereafter ^{F}TIPS or simply T^F) for (7*S*). This gives (*S,S,R*)-**13a** with zero fluorines and (*S,S,S*)-**13b** with five (suffixes **a–d** or **e–h** indicate the tagging pattern ("**a**" is all three T^H, etc.)).

Members of the (4*R*,5*R*) series isomers were made from **8** and Admix-*β*, then encoded with the same two fluorous TIPS groups (T^F).[19] Brown/Ramachandran allylation and isomer purification as before provided the *RRR* and *RRS* isomers of **12** (not shown), which were again encoded with TIPS for (7*R*) and ^{F}TIPS for (7*S*) to give (*R,R,R*)-**13c** (10 F's) and (*R,R,S*)-**13d** (15 F's). The resulting four quasiisomers were then mixed in equimolar amounts to make the first *trans*-series mixture M-**13a–d**.

The 4,5-*cis*-series quasiisomers were made by a similar series of reactions starting from both enantiomers of 2-deoxyribose, as detailed fully in the Supporting Information. The key allylation results are summarized in Scheme 2.

While better diastereoselectivities (up to 8/1) were achieved in the Brown/Ramachandran allylations, the minor C7 diastereomers of **12** could not be separated from the major ones in any case, either before or after silylation. Contrast this to the *trans*-series where the minor products for the allylation were conveniently separated before silylation in every case. Lacking a better option, we tagged these mixtures to give **13e–h** and moved ahead. The same tagging pattern was used in the *cis*-series as in the *trans*-series (**a**/**e**, 0 F; **b**/**f**, 5 F; **c**/**g**, 10 F; **d**/**h** 15 F). The four quasiisomers were mixed in equimolar amounts to make the first *cis*-series mixture M-**13e–h**.

Prior to starting the fluorous mixture syntheses in earnest, we tested the demixing of M-**13a–d** and M-**13e–h** by analytical fluorous HPLC. The chromatogram resulting from the *trans*-series isomers is representative and is shown in Figure 4. The quasiisomers eluted in order of fluorine content and four well-separated peaks were observed.

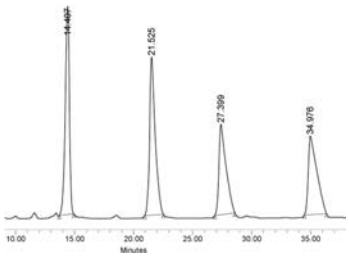
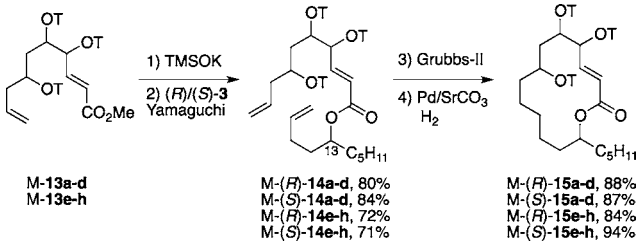


Figure 4. Trial demixing of M-**13a–d**. Compounds elute in order of fluorine content: **13a** (0 F), **13b** (5 F), **13c** (10 F), **13d** (15 F). Fluoro*Flash* PF-C8 column, 90/10 MeCN/water for 10 min, then 100% MeCN.

The mixture synthesis phase of the work is summarized in Scheme 3. Again taking the 4,5-*trans*-series as representative, the methyl ester of M-**13a–d** was cleaved with TMSOK,[20] and then the acid was divided in half and each portion coupled with either (*R*)-**3** or (*S*)-**3** under Yamaguchi conditions[21] to give esters M-(*R*)-**14a–d** and M-(*S*)-**14a–d** in good yields (80% and 84%). Ring-closing metathesis with the second-generation Grubbs catalyst[22] followed by hydrogenation with a poisoned Pd catalyst gave the final tagged quasiisomer mixtures M-(*R*)-**15a–d** and M-(*S*)-**15a–d** (88% and 87%). The $SrCO_3$ poison

Scheme 3. Fluorous Mixture Synthesis Provides the Penultimate Precursors 15 of the Final 16 Stereoisomers of 1 as Four Mixtures of Four Quasiisomers[a]



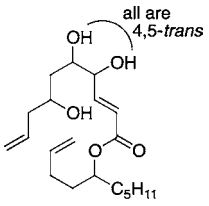
[a](*R*)/(*S*) represents the configuration at C13; **a**–**d** (4,5-*trans* series) or **e**–**h** (4,5-*cis* series) represents the various quasiisomers at C4/C5 and C7. Conditions: (1) TMSOK, Et_2O, rt, 16 h; (2) 2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N, toluene, rt, 3 h; (3) 2nd generation Grubbs catalyst, CH_2Cl_2, reflux, 48 h; (4) Pd/$SrCO_3$, hydrogen balloon, EtOH, rt, 1 h.

was important since other conditions often gave side products in which both double bonds were saturated.

The same reactions were conducted on the *cis*-series M-**13e**–**h** with similar yields to provide the other two mixtures of four quasiisomers, M-(*R*)-**15e**–**h** and M-(*S*)-**15e**–**h**. The final four mixtures of four quasiisomers were produced in substantial quantities (>200 mg each).

Demixing and Detagging. The results of the demixing and detagging are summarized globally in Tables 1 and 2. In total, 24 individual, pure samples were produced after detagging. Eight ester stereoisomers **16** in the *trans*-series were isolated for comparison of spectra of pairs of ring-open and ring-closed analogues with the same configurations, and all 16 Sch725674 stereoisomers **1** were isolated to compare with each other and with the natural product. This necessitated six preparative demixings (two ester mixtures and four macrolactone mixtures), which are detailed in the Supporting Information.

Table 1. Summary of Demixing and Detagging of Open Esters M-(*R*)-14a–d and M-(*S*)-14a–d to Give Eight Open Esters 16 in the 4,5-*trans* Series



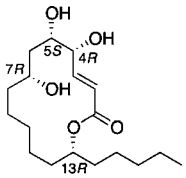
16

isomer of **16**	precursor	amount	yield[a]
(4*S*,5*S*,7*R*,13*R*)	(*R*)-**14a**	17 mg	65%
(4*S*,5*S*,7*S*,13*R*)	(*R*)-**14b**	16 mg	60%
(4*R*,5*R*,7*R*,13*R*)	(*R*)-**14c**	26 mg	69%
(4*R*,5*R*,7*S*,13*R*)	(*R*)-**14d**	11 mg	63%
(4*S*,5*S*,7*R*,13*S*)	(*S*)-**14a**	24 mg	67%
(4*S*,5*S*,7*S*,13*S*)	(*S*)-**14b**	30 mg	85%
(4*R*,5*R*,7*R*,13*S*)	(*S*)-**14c**	24 mg	77%
(4*R*,5*R*,7*S*,13*S*)	(*S*)-**14d**	29 mg	88%

[a]Includes demixing, detagging, and flash chromatography.

Briefly, the preparative demixings of two *trans*-series ester mixtures M-(*R*)-**14a**–**d** and M-(*S*)-**14a**–**d** were straightforward (see Figures S1 and S2 in the SI). Four well-separated peaks were observed in each case, and no cross-contamination of quasiisomers occurred. The pure quasiisomers were characterized (see below) then desilylated with TBAF in THF. The crude products were purified by flash chromatography to give the eight stereoisomeric trihydroxy esters **16** shown in Table 1 in overall yields of 60–88%.

Table 2. Summary of Demixing and Detagging of Lactones M-(*R*)-15a–h and M-(*S*)-15a–h to Give the 16 Final Isomers of 1[a]



(4*R*,5*S*,7*R*,13*R*)-**1** is Sch725674

isomer of **1**	precursor	amount	yield[b]
(4*S*,5*S*,7*R*,13*R*)	(*R*)-**15a**	6 mg	19%[c]
(4*S*,5*S*,7*S*,13*R*)	(*R*)-**15b**	15 mg	51%
(4*R*,5*R*,7*R*,13*R*)	(*R*)-**15c**	13 mg	60%
(4*R*,5*R*,7*S*,13*R*)	(*R*)-**15d**	8 mg	51%
(4*S*,5*S*,7*R*,13*S*)	(*S*)-**15a**	5 mg	15%[c]
(4*S*,5*S*,7*S*,13*S*)	(*S*)-**15b**	17 mg	67%
(4*R*,5*R*,7*R*,13*S*)	(*S*)-**15c**	3 mg	14%[c]
(4*R*,5*R*,7*S*,13*S*)	(*S*)-**15d**	11 mg	29%
(4*S*,5*R*,7*R*,13*R*)	(*R*)-**15e**	11 mg	29%[c]
(4*S*,5*R*,7*S*,13*R*)	(*R*)-**15f**	12 mg	37%[c,d]
(4*R*,5*S*,7*R*,13*R*)	(*R*)-**15g**	11 mg	32%[c]
(4*R*,5*S*,7*S*,13*R*)	(*R*)-**15h**	5 mg	40%[c]
(4*S*,5*R*,7*R*,13*S*)	(*S*)-**15e**	13 mg	45%[c]
(4*S*,5*R*,7*S*,13*S*)	(*S*)-**15f**	6 mg	17%[c]
(4*R*,5*S*,7*R*,13*S*)	(*S*)-**15g**	14 mg	73%[c]
(4*R*,5*S*,7*S*,13*S*)	(*S*)-**15h**	7 mg	36%[c]

[a]The assigned structure of Sch725674 is shown as an example. [b]Includes demixing, detagging, and flash chromatography. [c]Repurified by chiral chromatography. [d]Contains 6% of the C7 epimer.

The demixings of the four lactone quasiisomer mixtures M-**15** (~90 mg injections) are summarized in Table 2. These separations presented solvable problems. Demixing of the two *trans*-series isomers M-(*R*)-**15a**–**d** and M-(*S*)-**15a**–**d** and detagging provided the eight *trans* triol lactone isomers after chromatographic purification, but only five of these were pure (see Figures S3 and S4 in the SI). The two samples containing three TIPS groups and therefore zero fluorine atoms ((*R*)-**15a** and (*S*)-**15a**) were poorly retained by the column. They contained nonisomeric impurities that we think resulted from inadequate separation of this first peak from accumulated nonfluorinated impurities in the solvent front. With 10 fluorines, quasiisomer (*S*)-**15c** was the third-eluting component in its mixture, and it was contaminated with about 25% of the prior, second-eluting quasiisomer (*S*)-**15b** of the mixture.

Of the several ways to solve these problems, we found it most expeditious just to detag the three mixtures and purify the final products by preparative chiral chromatography. An (*S*,*S*)-Whelk-*O*-1 column was used for the triols derived from detagging of (*R*)-**15a** and (*S*)-**15a**, and a Chiralcel OD column was used for the triol from (*S*)-**15c**.

In the demixing of the two *cis*-series quasiisomer mixtures (SI, Figures S5 and S6), we were more careful to separate the

quasiisomers with zero fluorines from solvent front, and this time seven of the eight products were obtained in good quality. One of the third eluting quasiisomers had some cross contamination of the prior quasiisomer, and this was simply demixed a second time to remove the contaminant.

Recall that the eight quasiisomers in the *cis*-series (second group of eight) are not stereoisomerically pure because we could not separate the minor products of C7 after the allylation. However, because the minor product of one sample is the major product of another sample, it proved easy to analyze all eight samples by ^{1}H NMR spectroscopy. The identity and amount (10−25%) of each minor isomer was as expected from the allylboration results in Scheme 2 (see the SI for the actual ratios).

In preliminary experiments, one of the *cis*-series isomers was desilylated slowly with TBAF in THF, so we switched to HF/CH_3CN/H_2O for preparative detagging. All eight samples were reliably desilylated, and chiral HPLC analyses showed one major and one minor peak for each sample. Finally, preparative chiral HPLC purification provided the eight final samples, seven of which were diastereomerically pure. The eighth sample, (4*S*,5*R*,7*S*,13*R*)-**1**, had the misfortune of arising from quasiisomer (*R*)-**15f** with the lowest starting isomer ratio (75/25) and the tightest separation on chiral HPLC. This was obtained in an enriched 94/6 ratio of diastereomers and as such is the only one of the 24 final samples with a detectable isomeric impurity. Substantial amounts of all 16 of the final lactone isomers (3−17 mg) were obtained, as shown in Table 2.

Isomer Characterization and Structure Assignment of Sch725674. The fluorous mixture synthesis and demixing produced in total 48 individual samples grouped as 24 with tags and 24 without tags. Within each grouping of 24, there are 8 open chain esters (**14** or **16**, all isomers in the *trans*-series) and 16 closed lactones (**15** or **1**, all isomers in both *trans*- and *cis*-series).

The members of the tagged set of 24 (**14** and **15**) were characterized by the usual means with 1D ^{1}H and ^{13}C NMR spectra and HRMS. Characterization of the final 24 detagged compounds (**16** and **1**) depended on enantiomeric series. Members of one enantiomeric series were characterized by IR, HRMS, chiral HPLC analysis, optical rotation, 1D NMR spectra, and a set of full 2D NMR experiments (^{1}H−^{1}H-COSY, ^{1}H−^{13}C-HMQC, and ^{1}H−^{13}C-HMBC). In this way, substantially all the protons and carbon resonances were assigned in all the final isomers.[23]

Members of the other enantiomeric series were characterized by chiral HPLC analysis, ^{1}H NMR spectroscopy, and optical rotation. This produced a substantial data set (presented as Tables S1−S3, S5, S6 and copies of spectra in the SI), which could have multiple uses. Here we use selected data for comparison with each other and for structure assignment of Sch725674.

The values of the optical rotations of **1** are shown in the SI, Table S4 (the concentrations (*c*) in MeOH were all about 1 g/100 mL). The magnitudes of the optical rotations of the final isomers **1** can be loosely characterized as low to moderate, ranging from about ±3 up to ±40. The values are spread out enough to be informative in many cases. In other words, the structure of an unknown isomer could be reduced to two or three candidates (sometimes even one) based on optical rotation alone. The optical rotation of the natural sample of Sch725674 was not recorded, so comparison is not possible.

Fuller discussions of spectra comparisons can be found in the theses of Drs. X. Wang[13] and J. Moretti.[14] Here we focus on selected NMR spectral data that are especially informative. Comparison of the NMR spectra of the tagged isomers **14** and **15** is limited because these spectra depend on the structure of the tag. Unlike most prior work, the two tags here are not homologues. However, we can still compare spectra of ester **14** (open) and lactone **15** (closed) analogues that have the same tags.

The CF_2 regions of the ^{19}F spectra of two related pairs of lactones and esters are shown in Figure 5. The compounds

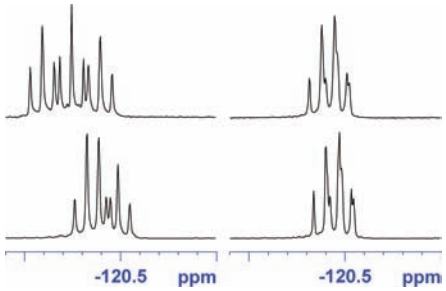


Figure 5. Comparison of the CF_2 resonances of two lactones **15** (left) and esters **14** (right) with three fluorous silyl tags (−Si-(iPr)$_2$CH$_2$CH$_2$C$_2$F$_5$). The top spectra are (13*R*) isomers; the bottom spectra are (13*S*). The other stereocenters are each (4*R*,5*R*,7*S*).

have the same tags (three ^{F}TIPS groups) and the same configurations at C4, C5, and C7, differing only in the configuration at C13. In theory, there should be three triplets because there are three heterotopic CF_2 groups (which couple to the adjacent CH_2 groups but not to the adjacent CF_3 groups). In the two esters (right-side pair of spectra), the resonances look like broad quartets and are identical. In the two lactones (left-side pair), one set of resonances is three clear triplets, and the other is three overlapping but easily assigned triplets. The difference in chemical shifts between the two lactones is remarkable given how remote the CF_2 groups are from the stereocenter at C13 (12 atoms, 14 atoms, and 16 atoms at the shortest counts).

This trend (ester spectra identical, lactone spectra different) held for the other three pairs of tagged C13 epimers, as summarized in Figure 6. Also, the ^{1}H and ^{13}C NMR spectra of the pairs of (*R*/*S*)-epimers of C13 of tagged lactones **15** were different, but the pairs of tagged esters **14** were the same. In

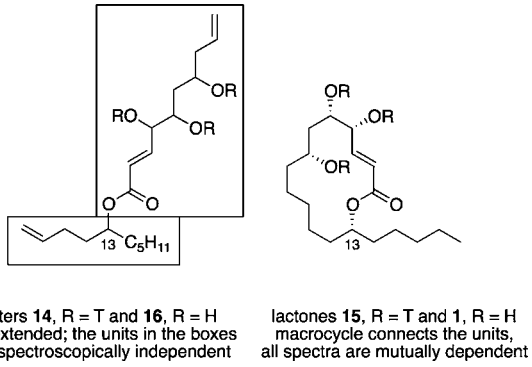


Figure 6. Groups of stereocenters (C13 and C4/C5/C7) behave independently in the spectra of the esters **14** and **16** but are mutually dependent in the spectra of the lactones **15** and **1**.

addition, the same trend held for all of the compounds after detagging to make the free alcohols. In other words, all eight diastereomers of the lactones **1** gave clearly different ^{1}H and ^{13}C NMR spectra, while the four diastereomeric esters **16** (*trans*-series only) gave two pairs of identical ^{1}H and ^{13}C NMR spectra. The pairs differed only in the configuration at C13.

In the open chain esters **14** and **16**, the conformation of the ester C−O bond is *trans*, so C13 in the ester oxygen substituent is remote from the other three stereocenters in the carbonyl substituent. In no case can any spectrum (^{19}F, ^{1}H, or ^{13}C) sense this difference. In the lactones **15** and **1**, the comparable C−O bond is probably also *trans*, but the two parts of the molecule are now connected through the carbon ring backbone as well. A change in configuration in one part of the 14-membered ring can alter conformational populations of the ring and is therefore sensed in the spectral features of the other part of the ring.

This principle—that spectra of large rings with remote stereocenters are more sensitive to configuration changes than spectra of comparable acyclic molecules—seems intuitively sensible. However, it is nonetheless impressive how the effect plays out in these libraries. In the open systems, every one of the 11 pairs of spectra compared was identical to its partner. In the closed systems, not one of the 22 pairs of spectra compared was identical.

Accordingly, each one of the final eight lactone diastereomers **1** exhibited a unique ^{1}H and ^{13}C NMR spectrum; no spectrum was substantially identical to any other spectrum. This makes assignment of the relative configuration of Sch725674 unambiguous; its published spectra were clearly different from seven of the diastereomers and matched only those of (4*R*,5*S*,7*R*,13*R*)-**1** and its enantiomer. Since all such lactone natural products have the (13*R*) configuration,[11] we can confidently assign the former structure to **1** as shown in Table 2.

Figure 7 illustrates some of the most obvious differences in the ^{1}H NMR spectra of **1** with expansions of the regions of the three carbinol protons, H4, H5, and H7. Not one of these spectra has one single resonance that overlaps. This can be seen in every case by eyeball except perhaps that of H5 in spectra 4 and 5. In other words, from the chemical shift of any one resonance selected from any one of these three H's, the stereostructure can be uniquely assigned in every case! Likewise, the alkene protons (H2, H3) exhibited large differences from isomer to isomer (see SI). In previous stereoisomer libraries, we have been surprised by the similarities in the spectra. In this library, we are surprised by the differences.

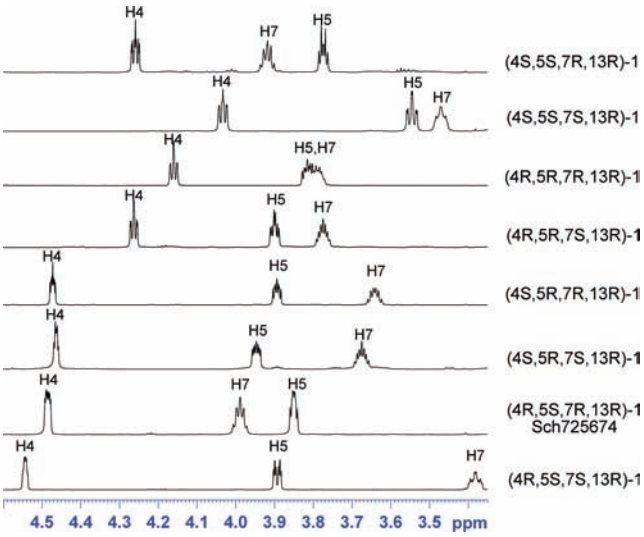


Figure 7. Expansions of the carbinol regions of the ^{1}H NMR spectra (700 MHz, CD_3OD) of the (13*R*) series of lactones **1**.

There are also differences in the coupling constants from isomer to isomer, but these seem to represent primarily local effects of the group of three stereocenters. For example, H4 has vicinal protons on each side and can have a long-range allylic coupling as well. When H4 and H5 are *trans*, these *J*'s play out as a broad triplet or a doublet of triplets (the first four spectra, two larger *J*'s and one smaller *J*). When H4 and H5 are *cis*, then a narrower peak shape results from three relatively small *J*'s (the last four spectra). In other words, the closure of the macrocycle changes the chemical shifts more than the coupling constants.

This conclusion is reinforced by comparing ^{1}H NMR spectra of ester and lactone pairs that have the same configurations at C4, C5, and C7 but differ at C13. There are four sets of these groupings, one of which is shown in Figure 8. As mentioned above, the spectra of the two esters are identical to each other, and the spectra of the two lactones are different from each other.

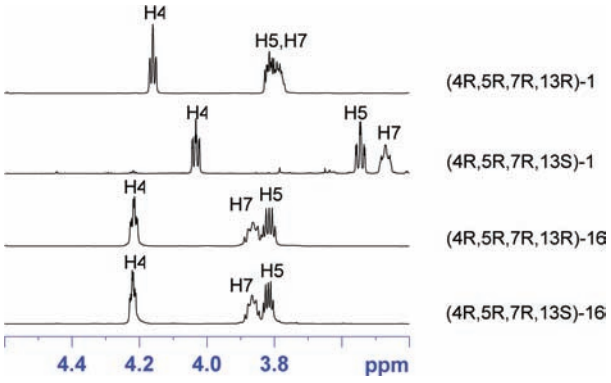


Figure 8. Expansions of the carbinol regions of the ^{1}H NMR spectra of two lactones **1** (top) and two esters (bottom) **16** with the same configurations at C4, C5, and C7, differing only at C13.

In comparing the chemical shifts of these spectra of the esters to the lactones, it might be prudent to ignore H7, which is homoallylic in the esters **16** but not in the lactones **1** (the alkene is removed after RCM and reduction). No resonance of H4 or H5 of the lactone matches its ester counterpart, and while there are some differences in the lactone and ester coupling constants, it is the differences in chemical shifts that are more remarkable.

■ CONCLUSIONS

We have described a fluorous mixture synthesis of all 16 stereoisomers of the macrocyclic lactone natural product Sch725674. The general approach to making four mixtures of four fluorous-tagged quasiisomers each is by now standard; however, the tagging pattern has been advanced significantly in two ways. First, only two tags are used, and only one of these is fluorous. Second, only 30 fluorine atoms are used in total to make all four quasiisomers. With the previous tag sets in comparable libraries, the smallest total number of fluorines was

46.[4b] The new approach is atom economical, and larger, environmentally persistent groups[26] (C_6F_{13}, C_8F_{17}) are not used.

After demixing and detagging, the structure of Sch725674 was assigned as (4*R*,5*S*,7*R*,13*R*)-**1** by comparison of the published spectra to the library spectra. This is the first time that a complete stereoisomer library has been made by FMS to assign a natural product of completely unknown configuration.

A substantial amount of data were produced by characterizing the 16 open esters and the 32 lactones. In each case, half of the compounds had three silyl tags, and half had three free hydroxy groups. These data took significant time to collect but provided substantial information beyond the structure of Sch725674. For example, compared to previous libraries that we have made, we were surprised by the large differences between all of the spectra of the final lactones **1**. In the 28 possible comparisons of pairs of spectra, there is little overlapping at all of the ^{1}H spectra except in the regions of the consecutive methylene groups. In contrast to the lactones, the spectra of the esters came in pairs with two isomers differing at C13 exhibiting substantially identical spectra.

It was also surprising that the differences in chemical shifts in the ^{1}H NMR spectra of the various lactone isomers are more remarkable than the differences in coupling constants. The ^{13}C NMR spectra presented in the SI give only chemical shift information, but again there are many differences in the lactones, and these spectra likewise suffice to assign the structure of Sch725674.

The data provide encouragement and caution for groups using various models of NMR spectra to assign configurations of macrolactones. The caution is for use of NMR spectra of model compounds to assign stereochemistry.[24] The technique has great power that is increasing with the size of the databases. However, at least for 14-membered lactones, a database of acyclic stereoisomers will not be a good model set for predicting chemical shifts. For example, the database chemical shifts of esters **14** or **16** is of no use in assigning the configuration of lactones **15** or **1**.

The encouragement is for groups that are calculating NMR spectra of isomers, and especially the ability to calculate ^{13}C NMR spectra has advanced in recent years.[25] Here the set of ^{13}C NMR spectra of lactones **1** are all significantly different. This provides a present challenge to NMR modelers. Pose yourself the following question: could you have confidently assigned the structure of Sch725674 if it were any one of the eight possible diastereomers? In other words, calculate the eight possible ^{13}C NMR spectra of the diastereomers of **1** and match them to the actual spectra. If there is a unique match in each case, then you could have assigned the structure of Sch725674 only by calculation, no matter which isomer it ultimately proved to be. A structure problem like this would usually involve comparison of eight calculated spectra with one actual spectrum (the natural product). Here is the rare opportunity to compare the eight calculated spectra with all eight actual spectra.[27]

■ ASSOCIATED CONTENT

Supporting Information

Contains complete experimental details, tabular NMR data, supplemental Figures, and copies of NMR spectra of the stereoisomer library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): HPLC columns were purchased from Fluorous Technologies, Inc. DPC owns an equity interest in this company. The other authors declare no competing financial interest.

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