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Fluorous Mixture Synthesis of (−)-Dictyostatin and Three Stereoisomers

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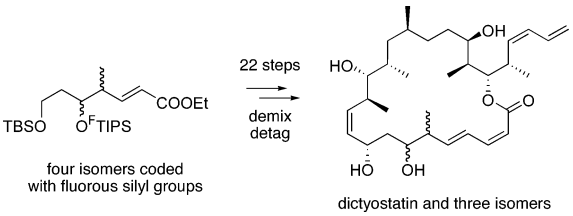
ABSTRACT



A mixture of four stereoisomers whose configurations are encoded by fluorous silyl protecting groups has been prepared and converted over 22 steps to a mixture of protected dictyostatins. Demixing by fluorous HPLC followed by removal of the fluorous protecting groups (detagging) provides dictyostatin and three C6,C7 stereoisomers. Biological evaluation showed that the monoepimers of the natural product retained highly potent activity.

(−)-Dictyostatin is a sponge-derived macrolactone that exhibits potent anticancer activity (Figure 1).[1] For a decade, research moved slowly because of an incomplete (and ultimately incorrect) stereostructure proposal and because only tiny quantities were available. Recent total syntheses[2] have confirmed the revised structure **1**[3] and provided samples for further biological testing.

In vitro testing on synthetic samples shows that dictyostatin exhibits anti-proliferative potencies, comparable or superior to its open-chain cousin discodermolide **2**.[4] It is active against paclitaxel-resistant cell lines and is one of the best microtubule stabilizers known, potently competing with radiolabeled paclitaxel, discodermolide, and epothilone B for the taxoid binding site. With the recent withdrawal of discodermolide from clinical development,[5] the importance of the dictyostatin family increases further.

We have recently made several analogues of dictyostatin including the moderately active C15,16 (*Z*)-alkene **3**[6a] and the highly active 16-normethyl analogue **4**.[6b] We have also made a number of stereoisomers of dictyostatin during our work toward its synthesis and structure confirmation.[6c]

With the structure of **1** secured, we hypothesized that stereoisomers along the bottom chain, especially at C6 and C7, would be among the most interesting to make. This is because discodermolide[7] (and, by inference, dictyostatin) is tolerant to changes in that region of the molecule.

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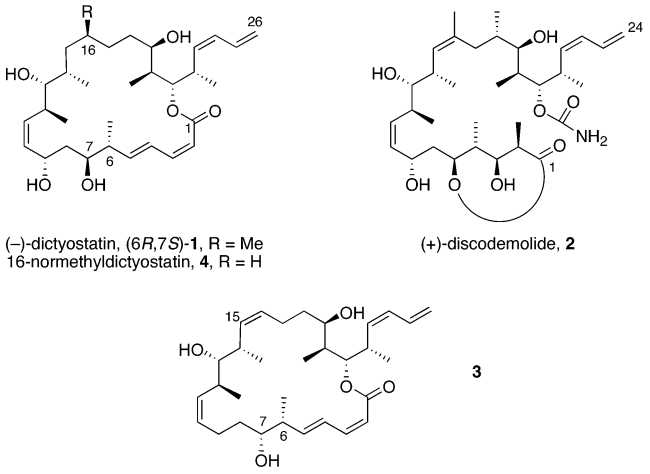


Figure 1. Structures of dictyostatin, discodermolide, and selected analogues.

Faced with a 20−25-step synthesis to make each stereoisomer, we decided to use fluorous mixture synthesis[8] to prepare all four C6,C7 isomers together in a single set of operations. The natural product dictyostatin is one of these isomers and it serves as a control in the fluorous mixture synthesis. At the same time, additional quantities of dictyostatin were needed for further testing, and these were provided by the synthesis. We communicate herein an overview of this synthesis, which is by far the most ambitious undertaking yet in the nascent field of solution phase mixture synthesis with separation tagging.

The plan for the fluorous mixture synthesis, summarized in Figure 2, is similar to that used for dictyostatin and its normethyl analogue with one significant change. In the earlier work,[2b,6b] we opted for a late introduction of the C23−C26 diene, but here we introduced it prior to fragment coupling to increase convergence.[2a,6c]

Accordingly, the key intermediate is M-**5**,[9] and this is built from a top phosphonate **6**,[2a] a middle alkyne **7**, and a bottom Weinreb amide M-**8**. Fragments **6** and **7** are single compounds, while M-**8** is provided as a mixture of all four isomers coded by fluorous tags.

The premix stage of the synthesis is summarized in Scheme 1 and entails the synthesis of the four tagged esters

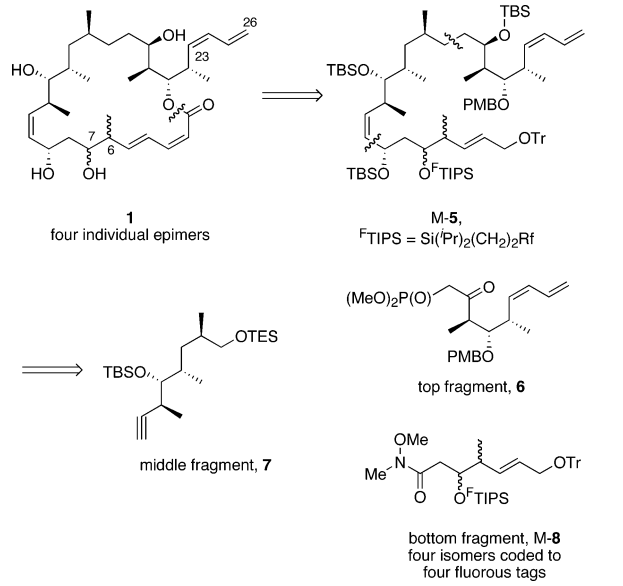
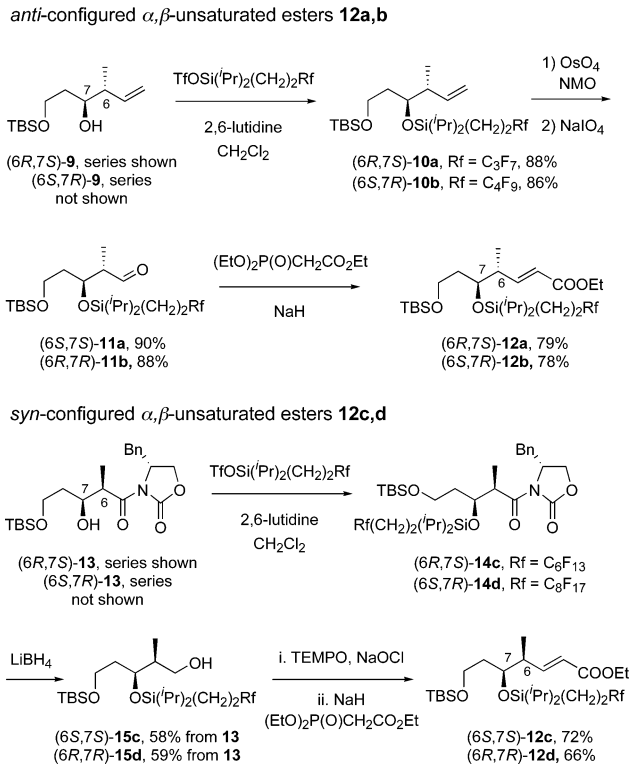


Figure 2. Key bond dissections in the synthetic plan.

12a−**d**. *Anti*-configured alcohol (6*R*,7*S*)-**9** and its (6*S*,7*R*) enantiomer are known compounds[10] that were prepared and silylated with the indicated fluorous TIPS groups (^{F}TIPS = $Si(^iPr)_2(CH_2)_2Rf$, where Rf is perfluoroalkyl).[11] This establishes the stereochemical code in **10a** (Rf = C_3F_7) and its quasienantiomer[12] **10b** (Rf = C_4F_9). Standard oxidative cleavage to give aldehydes **11a**,**b** followed by HWE olefi-

Scheme 1. Premix Stage of the Synthesis



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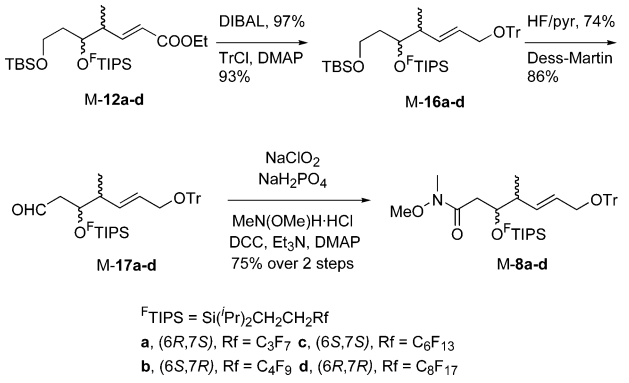
(9) The prefix "M" denotes a four-compound mixture.

nation provided (6*R*,7*S*)-**12a** and (6*S*,7*R*)-**12b**.[13] The *syn*-configured silyl ethers were made through an Evans aldol reaction to give both enantiomers of **13**[14] followed by silylation as above to provide **14c** (Rf = C_6F_{13}) and **14d** (Rf = C_8F_{17}). Cleavage of crude **14c**,**d** with $LiBH_4$ gave alcohols **15c**,**d**.[13] These were purified and then subjected to TEMPO/bleach oxidation and olefination to provide (6*S*,7*S*)-**12c** and (6*R*,7*R*)-**12d**.

The synthesis of the bottom fragment was then completed in a mixture mode, as summarized in Scheme 2. The four

Scheme 2. Synthesis of the Bottom Fragment Mixture



esters **12** were blended in a ratio of 1.5:1:1:1.5, and the resulting mixture M-**12a**−**d** was reduced with DIBAL and then tritylated to provide M-**16a**−**d**. Selective desilylation of the TBS group was accomplished with HF/pyr, and the resulting alcohol was oxidized with the Dess−Martin reagent to furnish aldehyde M-**17a**−**d**. Further oxidation with $NaClO_2$ provided an acid, which was coupled with *N*,*O*-dimethylhydroxylamine to provide M-**8a**−**d**. The products of these mixture reactions were purified by standard flash chromatography without demixing. At key points, intermediates were isolated by small scale demixing (1−5 mg) and characterized by NMR spectroscopic analysis.

The fragment coupling and completion of the synthesis are summarized in Scheme 3. The middle **7** and bottom M-**8a**−**d** fragments were coupled via alkynyllithium addition.

The resulting alkynyl ketone was then reduced by (*S*,*S*)-Noyori catalyst[15] to give M-**18a**−**d** in 94% yield. To determine the stereoselectivity of this catalyst-controlled reduction, we also reduced a small sample of the ketone mixture with $NaBH_4$ as a nonselective control reagent. The fluorous HPLC traces of these two reaction mixtures are compared in Figure 3.[16] The chromatogram of M-**18** from

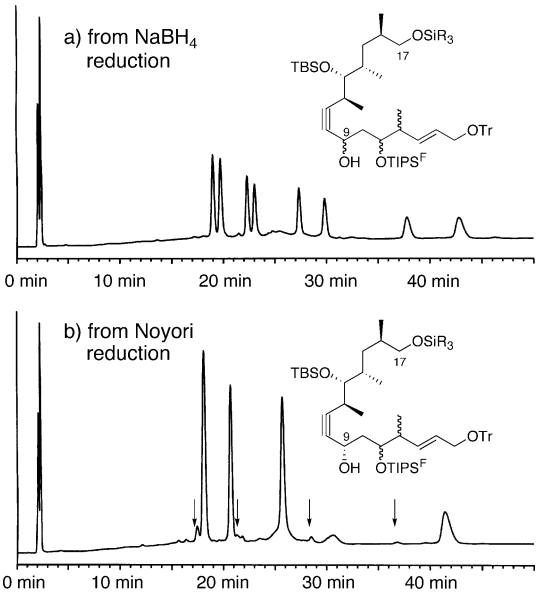


Figure 3. HPLC analyses of the mixtures M-**18a**−**d** (ref 16) from the nonselective (a) and selective (b) reductions at C9. Arrows show the minor (β) epimers in the Noyori reduction mixture.

the $NaBH_4$ reduction showed four pairs of peaks of about equal area with the larger separation between pairs corresponding to the demixing based on fluorous tag and the smaller separation among pairs corresponding to the epimeric alcohols at C9.

In contrast, the chromatogram of the sample of M-**18** from Noyori reduction showed that the four C9-α epimers were formed with excellent selectivity (>15:1), with only very small peaks for the corresponding C9-β epimers (see arrows in Figure 3b). Thus, with only two reactions and without a preparative demixing, we learned that the Noyori reduction exhibited high stereoselectivity that was not affected by the substrates with assorted configurations at nearby C6 and C7.

The alkyne M-**18** was reduced to the *cis*-alkene by Lindlar hydrogenation and the resulting secondary hydroxy group was protected as a TBS ether. The primary TES group was then selectively cleaved with dichloroacetic acid to give M-**19** in 90% yield. Dess−Martin oxidation of the primary alcohol to the aldehyde was followed by HWE coupling with the top fragment **6** to give the α,β-unsaturated ketone in 82% yield for 2 steps. The C17−C18 alkene was selectively reduced with Stryker's reagent.[2a,6c,17] The reduction of the C19 ketone was performed by $LiAl(Ot\text{-}Bu)_3H$, giving the β-alcohol M-**20**β as the major product. The α-isomer was removed by silica gel chromatography of the mixture.

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(11) The silane precursors of the FTIPS triflates and the fluorous silica products were purchased from Fluorous Technologies, Inc. DPC holds an equity interest in this company.

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

(13) Note the changes in CIP priorities between **10**/**11**, **11**/**12**, and **14**/**15**.

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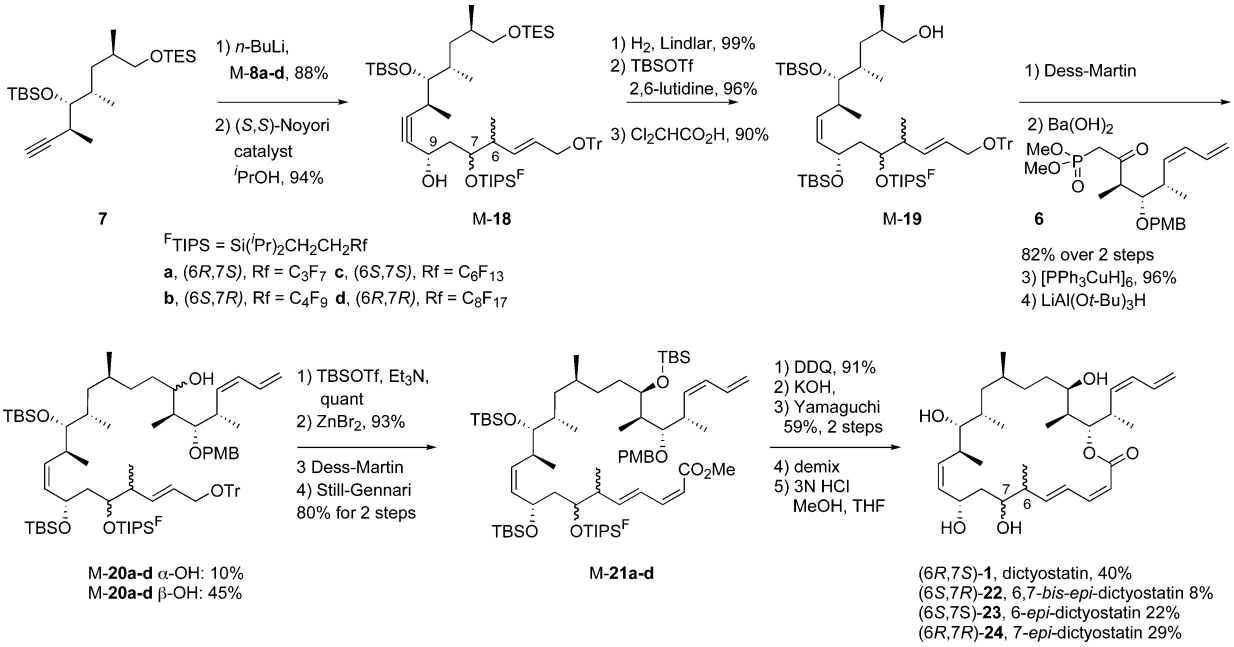
(16) Early work was done with a TBS ether on C17 and traces for these reactions are shown in Figure 3. Similar results were obtained with the TES ether.

(17) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc.* **1988**, *110*, 291−293.

Scheme 3. Fragment Coupling and Completion of the Synthesis



The C19 hydroxy group was protected with TBSOTf, the trityl group was selectively removed by $ZnBr_2$, and the resulting allylic alcohol was oxidized with the Dess−Martin reagent. Still−Gennari reaction[18] then provided (*E*),(*Z*)-diene M-**21** with good yield and selectivity. PMB removal with DDQ, hydrolysis of the conjugated ester by 1N KOH, then macrolactonization under Yamaguchi conditions[19] gave a mixture of major (2*Z*),(4*E*) and minor (2*E*),(4*E*) macrolactones in 59% yield for the 2 steps.

Demixing of the final mixture was accomplished by preparative chromatography over FluoroFlash-PF8.[11] This provided the four individual components, which were desilylated with 3N HCl in MeOH to give dictyostatin (6*R*,7*S*)-**1** and the other three C6,C7-*epi*-dictyostatin diastereomers in the indicated yields after HPLC purification.[20] The sample of dictyostatin (6*R*,7*S*)-**1** was identical to previously synthesized dictyostatin,[2b] thereby confirming the success of the fluorous mixture synthesis.

The antiproliferative effects of these compounds were assayed against human ovarian carcinoma cells (Table 1). The bis-*epi* diastereomer, (6*S*,7*R*)-**22** was less active than the other isomers, but still exhibited a mid-nanomolar potency. In contrast, the monoepimers (6*S*,7*S*)-**23** and (6*R*,7*R*)-**24** showed potent antiproliferative effects; (6*R*,7*R*)-**24** was about equipotent to dictyostatin (6*R*,7*S*)-**1**, and (6*S*,7*S*)-**23** was four times more potent. More detailed biological characterization of the monoepimers is clearly warranted by these exciting preliminary results.

This work shows that fluorous mixture synthesis can be used to leverage effort in a complex total synthesis by providing more compounds without a proportional increase in work. Here, 22 steps were conducted on fluorous mixtures, whereas conducting the same syntheses in parallel to provide the four target products would have required 88 steps. The use of fluorous HPLC was critical to the success of the project since it provided the opportunity to analyze intermediate mixtures and characterize their underlying components, as well as to demix the final mixture to provide the pure target products.

Table 1. 50% Growth Inhibitory Concentrations (GI_{50}) of (C6,C7) Dictytostatin Diastereomers in 1A9/Ptx22 Human Ovarian Carcinoma Cells

compd	GI_{50} (nM)
(6*R*,7*S*)-**1** (dictyostatin)	3.4 ± 0.7
(6S,7*R*)-**22**	123 ± 25
(6S,7*S*)-**23**	0.81 ± 0.17
(6*R*,7*R*)-**24**	4.7 ± 0.6

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

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(19) Inanaga, J.; Kuniko, H.; Hiroko, S.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989−1993.
(20) Small, variable amounts of the C2-(*E*), C4-(*E*) isomers formed during the macrolactonization were removed at this stage. The variable yields of the final products may reflect differences in amounts of (*E*,*E*)-isomers, but may also reflect unequal material losses in purifications at the stage of reduction of the C19 ketone or at other stages.