

Fluorous mixture synthesis (FMS) of enantiomers, diastereomers, and compound libraries

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Abstract

Fluorous mixture synthesis (FMS) is a newly developed highly-efficient solution-phase technology. A set of substrates attached to homologous fluorinated tags is mixed and taken through a sequence of organic reactions. The final mixtures are demixed by fluorinated HPLC and detagged to give individually pure final products. Applications of FMS for the preparation of two enantiomers of pyridovericin, 16 diastereomers of murisolin, and a 560-member mappicine library are highlighted in this article.

Keywords: Fluorous mixture synthesis, fluorinated HPLC, pyridovericin, murisoline, mappicine

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Introduction

Traditional solution-phase organic reactions have advantages of favorable reaction kinetics and easy intermediate analysis by TLC, HPLC, and NMR. However, compounds are usually produced “one at a time” followed by a tedious purification process such as chromatography, distillation or crystallization. Recent advances of high-throughput screening technologies have generated a strong demand on production of large number of molecules for biological tests. Many new technologies such as solid-phase synthesis, solution-phase parallel synthesis, and

high-throughput purification have been developed to speed up the synthetic process. Among them, the “split and pool” mixture synthesis on solid support has high efficiency in preparation of large compound libraries. However, the nature of heterogeneous reaction and difficulty in monitoring the reaction process have limited the growth of solid-phase synthesis. Implementation of mixture synthesis strategy to the solution phase was not successful simply because of the lack of general ways to separate, analyze, and identify reaction intermediates and final products. The recent development in fluororous technologies¹ made it possible for the first time to prepare individually pure compounds by solution-phase mixture synthesis. Fluororous synthesis successfully combines the favorable characteristics of solution-phase reactions with solid-phase type separations.

In FMS, perfluoroalkyl groups are employed as the “phase tags” attached to the substrates. The tagged molecules are well soluble in common organic solvents. The capability of fluororous HPLC to separate mixtures of fluororous compounds based on their fluorine content provides a powerful tool to analyze reaction intermediates and demix products.² The fluororous HPLC column containing a stationary phase of $\text{Si}(\text{Me})_2\text{CH}_2\text{CH}_2\text{C}_8\text{F}_{17}$ has a strong and selective retention of fluororous compounds.³ Molecules with longer fluorine chains (Rf) have longer retention times on the column. A typical mobile phase for fluororous HPLC is a gradient of MeOH-H₂O with increasing MeOH up to 100%, similar to reverse-phase HPLC. Other solvents such as MeCN or THF can be used to replace MeOH for the gradient elution. Fluororous HPLC separation of a 9-component mixture of isonipicotic acid derivatives containing different tags (Rf) is demonstrated in Figure 1.⁴ Eight fluororous compounds (Rf = C₃F₇ to C₁₀F₂₁) are well separated in 30 min. The non-fluororous compound (Rf = C₇H₁₅) is eluted out with the solvent front simply because of its low affinity for the fluororous stationary phase.

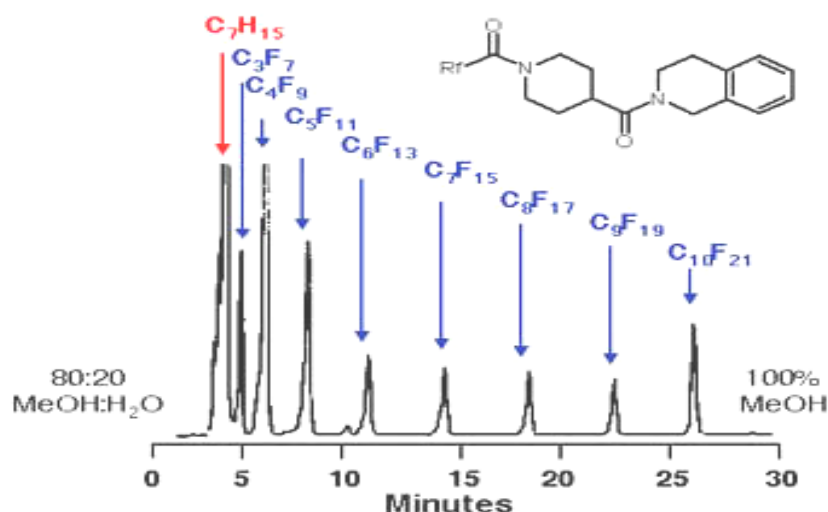
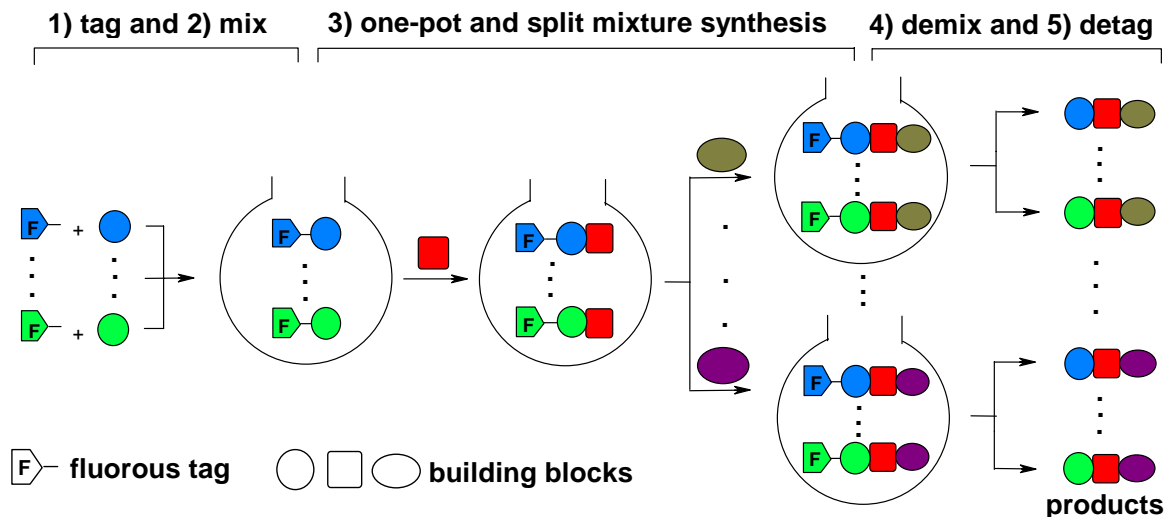


Figure 1. F-HPLC of eight fluororous homologues on FluoroFlash™ column.

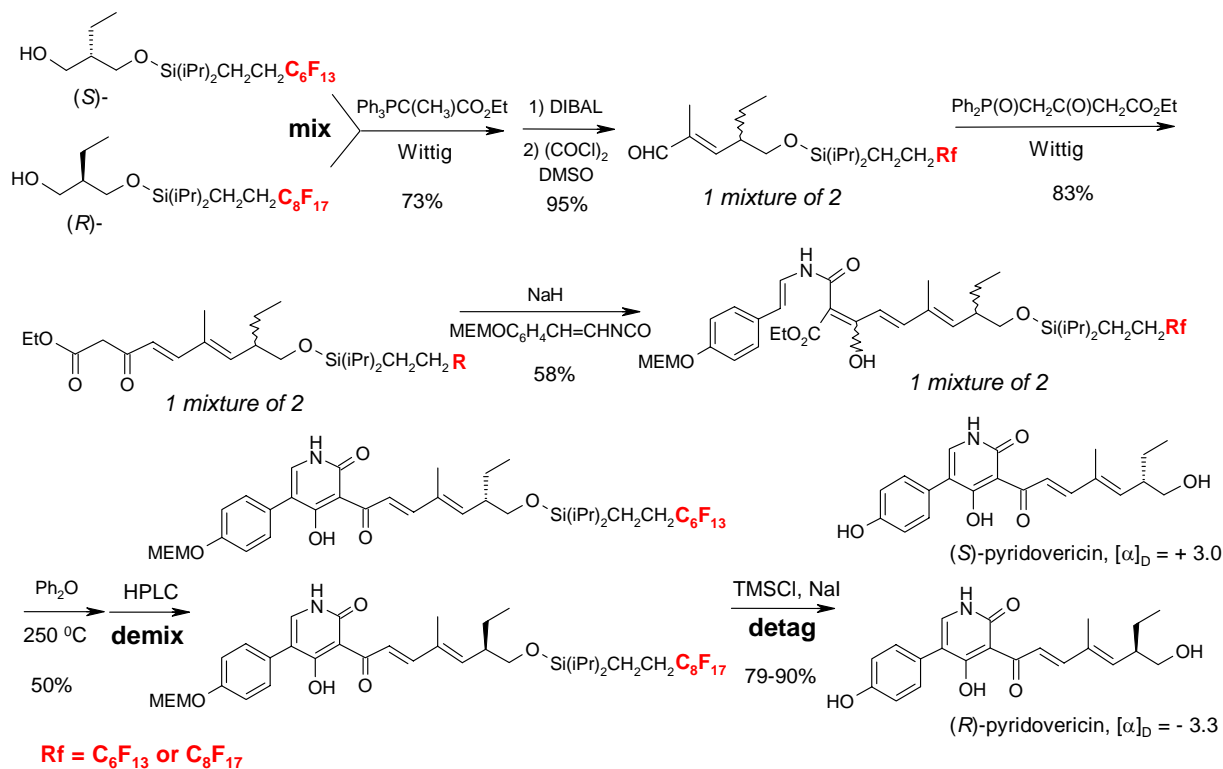
Scheme 1 illustrates the concept and general procedures involved in FMS:⁵ 1) attaching of a set of substrates with a corresponding set of homologous fluororous tags with increasing fluorine content; 2) mixing of the tagged substrates together; 3) conducting multi-step mixture synthesis in one-pot or split-parallel fashion; 4) demixing of mixtures of tagged products by fluororous HPLC; and 5) detagging to release final products. The efficiency of FMS is directly proportional to the number of components mixed (step 2), the length of mixture synthesis (step 3), and the number of split (step 3) as well.



Scheme 1. Schematic diagram of the concept of FMS.

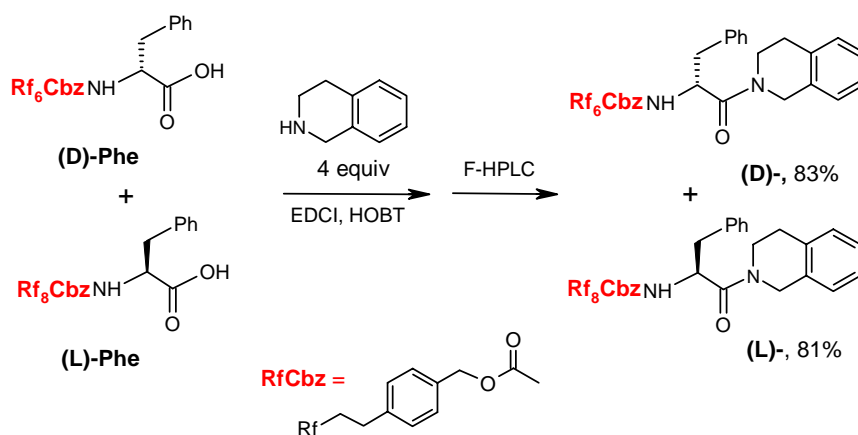
1. Synthesis of enantiomers

Enantiopure or enantioenriched compounds are usually prepared by asymmetric synthesis or by resolution of a racemic synthesis. FMS provides an alternative and efficient way to produce enantiomeric products by FMS. The Curran group reported the first example of one-pot synthesis of two enantiomers of pyridovericin (Scheme 2).⁶ The (*S*)- and (*R*)-enantiomers of the starting material were attached to two different fluororous silanes ($R_f = C_6F_{13}$ and C_8F_{17}) and combined together to make a quasienantiomeric mixture. The mixture was then taken through a multi-step synthesis to make a final tagged product mixture. Two quasienantiomers were demixed by fluororous HPLC. The tags were then removed to release the (*S*)- and (*R*)-enantiomers of pyridovericin. Quasiracemic synthesis is the simplest version of FMS which has only two mixture components. Only one-pot reactions, no split-parallel reactions are conducted in the quasiracemic synthesis.



Scheme 2. Quasiracemic FMS of (*S*)- and (*R*)-pyridovericins.

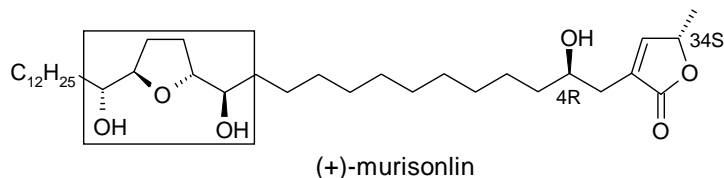
Enantiomeric amino acid attached to different fluorous Cbz groups provides a useful starting point for the FMS of peptides.⁷ The (*D*)- and (*L*)-phenylalanines were tagged with fluorous Cbz with C_6F_{13} and C_8F_{17} , respectively. The mixture of two quasienantiomers was then coupled with tetrahydroisoquinoline under conventional solution-phase reaction conditions (Scheme 3). The crude product was purified and resolved into its quasienantiomeric components by fluorous HPLC.



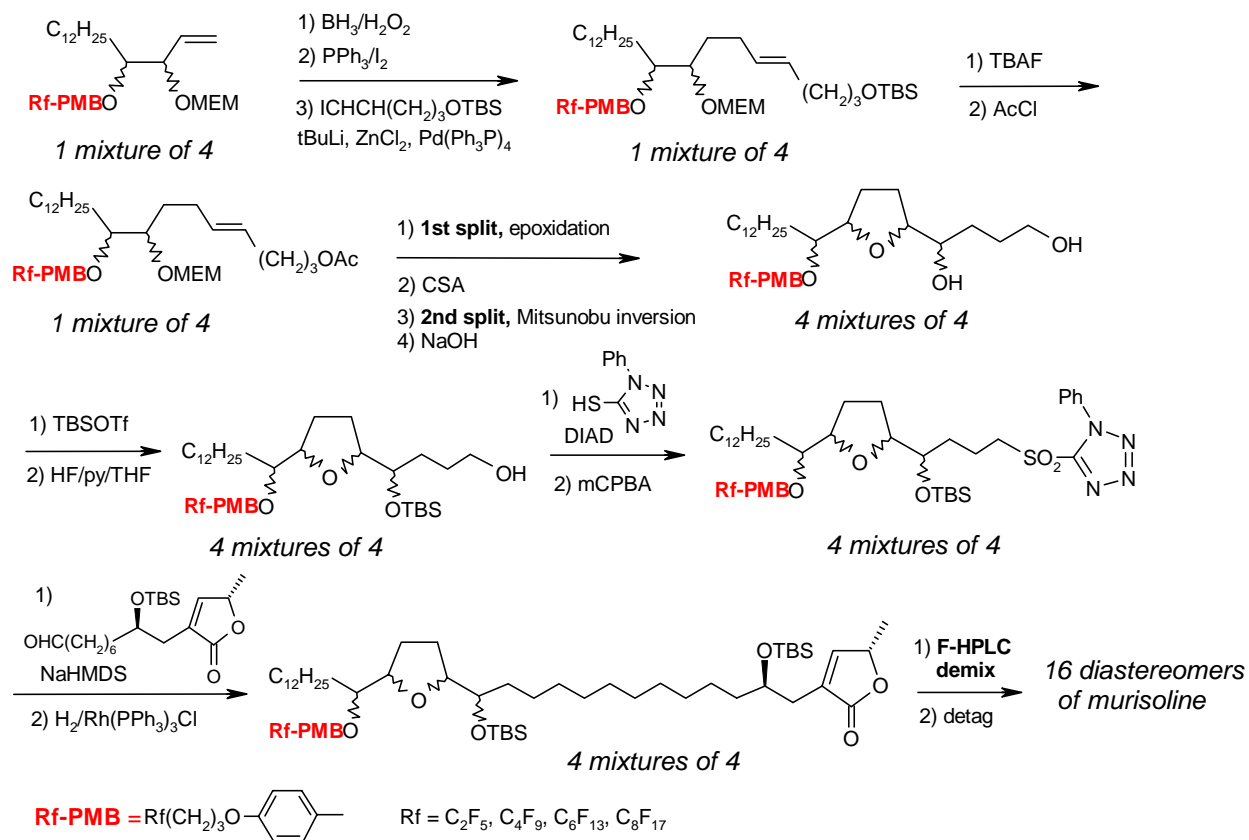
Scheme 3. FMS of (*D*)- and (*L*)-Phe derivatives.

2. Synthesis of stereoisomers

The Curran group recently reported the synthesis of 16 diastereomers of murisolin by FMS.⁸ The murisolin family of acetogenins has 6 stereocenters and this research focused on the synthesis of 16 stereoisomers of the dihydroxy tetrahydrofuran fragment (shown in the box) with the 4(*R*) and 34(*S*) centers fixed.



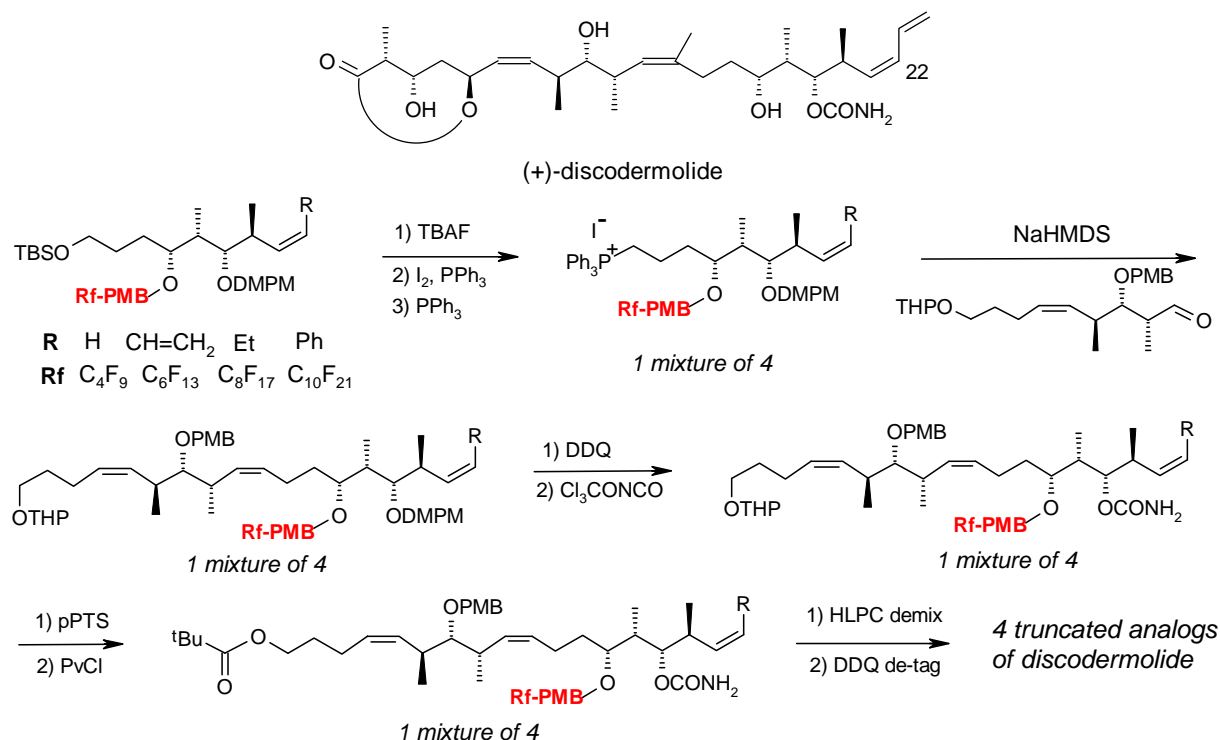
The FMS started with a mixture of four enantiomerically pure compounds, each tagged with a PMB containing differing Rf group (C₂F₅, C₄F₉, C₆F₁₃, and C₈F₁₇). This mixture was then taken through a sequence of organic reactions, including 2 splits and parallel syntheses to give 4 mixtures of four tagged products (Scheme 4). Fluorous HPLC demix of the 4 mixtures followed by detagging provided 16 desired diastereomers of murisoline. Since FMS has a total of 39 steps, compared to 156 steps that would be required for the same transformations using conventional parallel synthesis, the efficiency advantage is obvious.



Scheme 4. FMS of 16 stereoisomers of murisoline.

3. Synthesis of analogs

Synthesis of truncated analogs of the natural product (+)-discodermolide at C22 position is another successful application of FMS.⁹ Four starting materials with different R (H, CH=CH₂, Et, Ph) were protected with the corresponding fluoros PMB (Rf = C₄F₉, C₆F₁₃, C₈F₁₇, C₁₀F₂₁) (Scheme 4) and mixed. Multistep synthesis of this 4-component mixture led to 4 truncated analogs of discodermolide after demix and detag (Scheme 5).

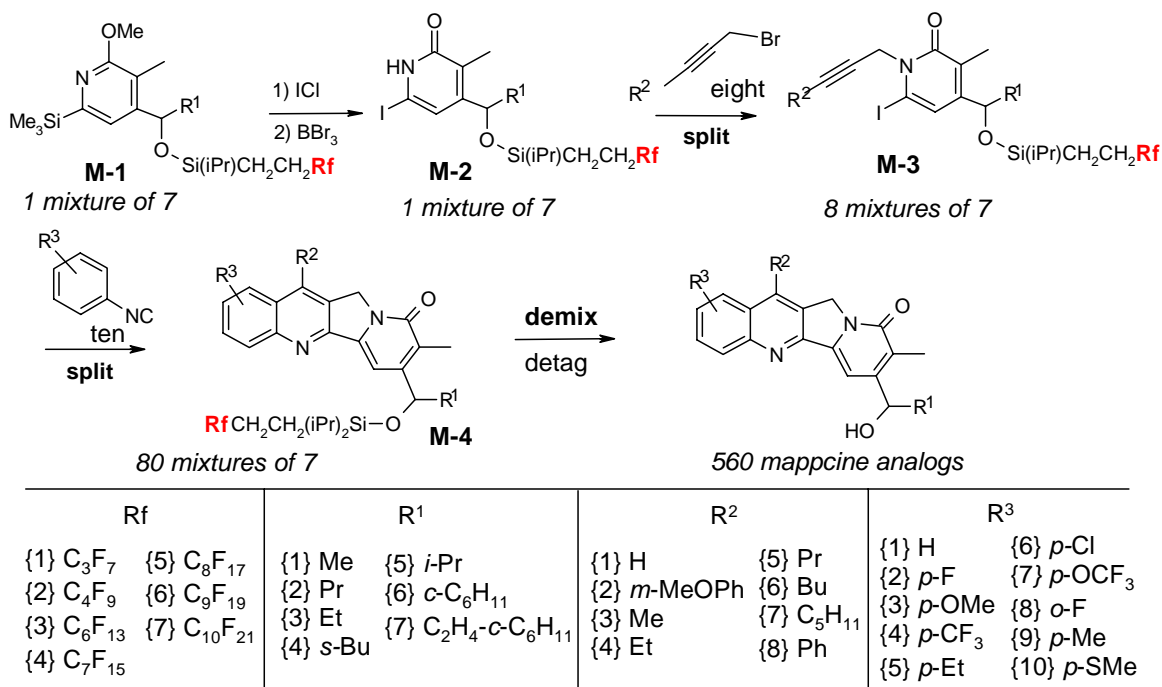


Scheme 5. FMS of 4 truncated discodermolide analogs.

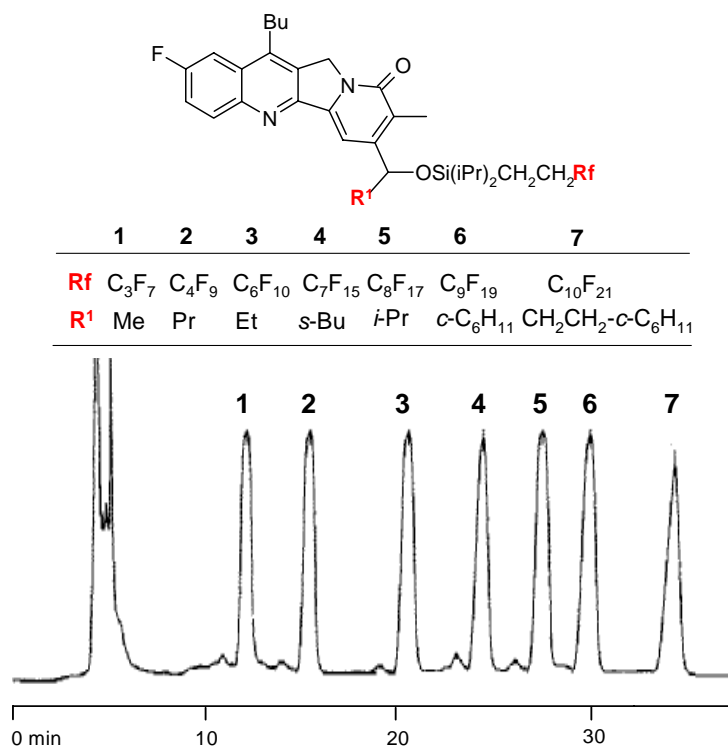
4. Synthesis of compound libraries

The power of FMS has been further demonstrated in the preparation of a 560-member library of mappicine analogs (Scheme 6).¹⁰ A mixture of seven pyridines **M-1** (7 different R¹ groups paired with 7 different Rf) underwent iodo exchange followed by demethylation to give **M-2**. The mixture was then split into 8 portions and subjected to *N*-propargylations with 8 different bromides to give 8 mixtures of **M-3**. Each of the 8 mixtures of **M-3** was further split to 10 portions for radical annulation reaction with isonitriles. The resulting 80 mixtures of **M-4** each containing seven tagged-products were demixed by fluoros HPLC and then detagged by HF-pyridine to give a 560-member mappicine library (Figure 2). After each step, the reaction

mixture could be analyzed by fluoros HPLC and the byproducts or unreacted starting materials were removed by normal-phase silica gel flash column chromatography. The synthesis of this 560-membered library is accomplished in 90 reactions (not including detag), while 630 steps are needed for a corresponding parallel synthesis. The overall separations required only 90 chromatography steps compared to 630 chromatography steps needed for the parallel synthesis.



Scheme 6. 7-Component FMS of a 560-member mappicine library.



Fluorous HPLC column (20 x 250 mm, 5 μ m), gradient 88% MeOH-12% H₂O to 100% MeOH in 28 min, then to 100% THF in 7 min, flow rate 12mL/min.

Figure 2. A typical HPLC demix of a 7-component mixture.

Conclusions

The fluororous tag-based HPLC has made possible the development of highly efficient solution-phase mixture synthesis. Compared to solid-phase synthesis, FMS has advantages of homogeneous reaction environment, easy analysis and purification of reaction intermediates, easy adoption of traditional solution-phase reaction conditions, does not require large excess of reagents for reaction completion, and good chemical and thermostability of fluororous tags. This new technology has been demonstrated for the synthesis of enantiomers, diastereomers, and compound libraries. There is no doubt it will play a more important role in organic, medicinal, and combinatorial chemistries.

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Biography: Dr. Wei Zhang received his B.S from Nanjing University and Ph.D in 1993 from the University of Pittsburgh under the supervision of late Professor Paul Dowd. After a two-year appointment at the same university as a Research Assistant Professor, he joined DuPont in 1995 and moved back to Pittsburgh in early 2001 to become one of the founding members at Fluorous Technologies, Inc. He is now the Director of Discovery Chemistry responsible for research and development of fluororous technologies for solution-phase synthesis and separations. Over the years Dr. Zhang has gained academic and industrial experience. His professional areas of interest include development of free radical reactions for ring-expansion and formation of heterocyclic systems, design and synthesis of drug-like small molecule libraries using the combinatorial technologies of fluororous synthesis, microwave-assisted organic reactions, and multicomponent reactions. He has published over 50 peer-reviewed papers including a recent article entitled "Fluorous synthesis of heterocyclic systems" in *Chemical Reviews* (2004, 104, 2531-2556). He has delivered many invited lectures on the topics of fluororous technologies and combinatorial chemistry.