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Published on Web 08/08/2002

Solution-Phase Preparation of a 560-Compound Library of Individual Pure Mappicine Analogues by Fluorous Mixture Synthesis

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Received May 17, 2002

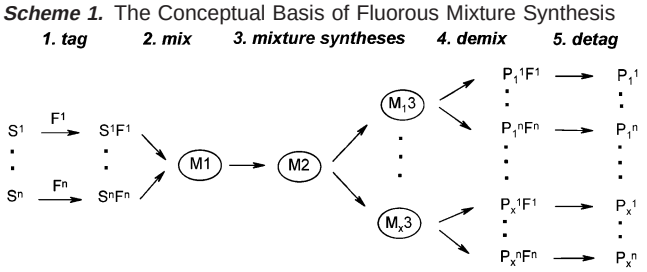
Abstract: Solution-phase mixture synthesis has efficiency advantages and favorable reaction kinetics. Applications of this technique, however, have been discouraged by the difficulty in obtaining individual, pure final products by using conventional separation and identification processes. Introduced here is a new strategy for mixture synthesis that addresses the separation and identification problems. Members of a series of organic substrates are paired with a series of fluorous tags of different chain lengths. The tagged starting materials are then mixed and taken through a multistep reaction process. Fluorous chromatography is used to demix the tagged product mixtures on the basis of the fluorine content of the tags to provide the individual pure components of the mixture, which are detagged to release the final products. The utility of fluorous mixture synthesis is demonstrated by the preparation of a 560-membered library of analogues of the natural product mappicine. A seven-component mixture is carried through a four-step mixture synthesis (two one-pot and two parallel steps) to incorporate two additional points of diversity onto the tetracyclic core. Methods for analysis and purification of the intermediates are established for the quality control of the mixture synthesis.

Introduction

Mixture synthesis techniques increase efficiency in direct proportion to the number of compounds mixed. Despite this inherent efficiency, solution-phase mixture synthesis is not considered to be a viable route to individual, pure target molecules. Solid-phase mixture techniques (split-pool) are now well established, but they mix beads (or containers), not compounds.[1] Solution-phase mixture techniques usually do not target the isolation of the individual components and instead use deconvolution methods to identify only the most active compounds.[2] These are then resynthesized individually.

Conducting reactions on mixtures of organic molecules is straightforward. However, analyzing, identifying, and ultimately separating the mixture components have until now seemed liked insurmountable barriers. We suggest that these barriers can be surmounted by a strategy based on separation tags.[3] Substrates are tagged with a series of different tags, and the tagged substrates are mixed and taken through a series of reactions. Central to this conceptual approach is the availability of at least one separation technique that is targeted toward tag differentiation. This technique can then be used at any time to demix (separate based on tag) the mixture for analysis, identification, and purification.

Scheme 1. The Conceptual Basis of Fluorous Mixture Synthesis



Fluorous mixture synthesis is the first solution-phase mixture technique that allows the isolation of the individual pure components at the end of the mixture exercise. Scheme 1 outlines the conceptual basis of fluorous steps. A series of *n* initial substrates ($S^1 - S^n$) are tagged with *different* fluorous tags ($F^1 - F^n$). The fluorous separation tags bear a homologous series of fluoroalkyl groups. The tagged substrates ($S^1F^1 - S^nF^n$) are mixed to give a single mixture (M1) that is then taken through a series of mixture steps. The mixture steps can be either one-pot reactions to give new mixtures (for example, M1 → M2) or split-parallel reactions with a set of *x* building blocks

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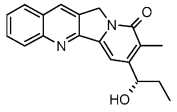
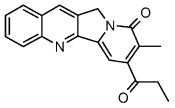
to generate x numbers of new mixtures (for example, M2 → $M_1$3 − M_x3). The one-pot mixture reaction is an "internal parallel" approach with no change of library size, while the split-parallel mixture reaction proceeds in "double parallel" fashion that has powerful library amplification capability. At the end of the synthesis, all of the mixtures are separated chromatographically over fluorous silica gel.[4] This process is called "demixing", and each mixture containing n tagged products ($P_x^1F^1$ − $P_x^nF^n$) elutes in order of increasing tag size. The chromatography serves both to separate the mixture components (from each other and from other impurities) and to identify the products. Detagging provides a total of nx number of final products (P_1^1 − P_x^n).

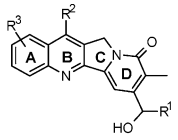
The natural product (*S*)-mappicine **1** was originally isolated from *Mappa foetida*.[5] Its analogue mappicine ketone **2**, also known as nothapodytine B,[6] was isolated from *Nothapodytes foetida* and is active on herpes viruses (HSV) and human cytomegalovirus (HCMV) at a range of 3−13 μM.[7] One of the most efficient synthetic routes to the construction of the mappicine skeleton is radical tandem cyclization of isonitriles developed by Curran and co-workers.[8] Incorporation of three diversity points (R^1, R^2, R^3) into the parallel solution-phase synthesis of 64 mappicine analogues **3** has also been accomplished in the same group.[9] Subsequent attempts to transfer the synthesis to the solid phase, however, were abandoned after extensive effort because the yields of the key reactions could not be optimized to the levels needed for practical utility.[10] Now the problems associated with the solid-phase synthesis have been successfully bypassed by a homogeneous fluorous solution-phase synthesis. We report herein the first example of the use of fluorous mixture synthesis to make a challenging natural product library.



(*S*)-mappicine **1**

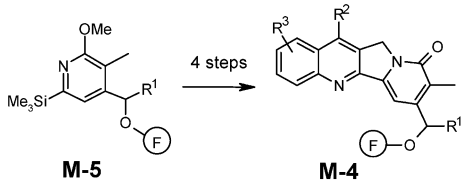


mappicine ketone **2**



mappicine analogs **3**

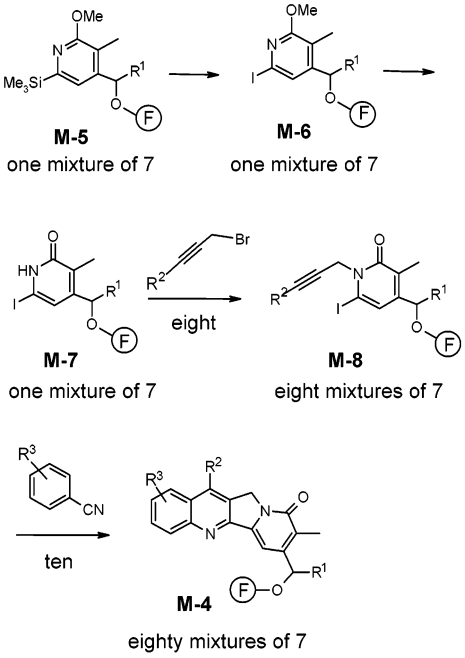
Scheme 2



F represents a fluorous tag

Preliminary work to construct a 100-membered tagged mappicine library has recently been communicated.[11a] Four tagged alcohols **M-5** were mixed and carried through four steps of mixture synthesis to provide tagged mappicines **M-4** (Scheme 2). A total of 25 four-component mixtures were made, and the mixtures were demixed analytically by an HPLC column packed with fluorocarbon-bonded silica gel.[12] Following this validation of fluorous mixture synthesis at the proof-of-principle level, we have now extended both the synthetic scale and the scope to make a 560-membered library to demonstrate the practical utility of fluorous mixture synthesis. The focal points of this work are (1) to conduct a multistep library synthesis starting from a mixture of seven components, (2) to assess the reactivity of each component of the mixture in multistep mixture synthesis, (3) to establish a general protocol to analyze intermediates and to separate impurities in mixture mode, (4) to evaluate the reliability of the semipreparative scale demixing with fluorous HPLC columns, and (5) to produce milligram quantities of the final product for appropriate characterization.

Scheme 3. Plan of Mixture Synthesis



Results and Discussion

Mixture Plan. The plan for the synthesis of a 560-member mappicine library is outlined in Scheme 3. Seven pyridinyl

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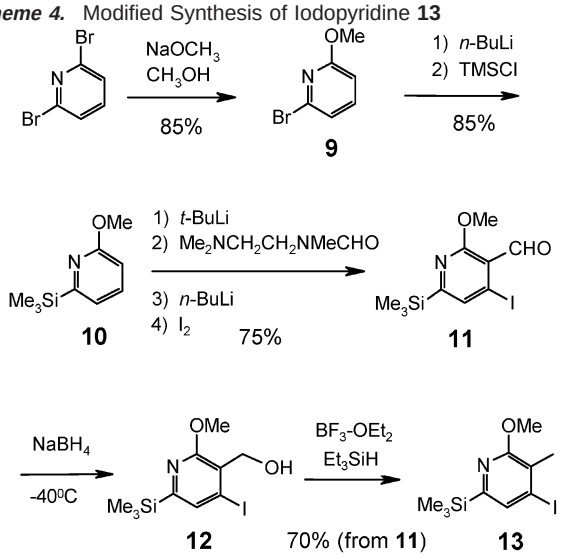
(11) (a) Luo, Z. Y.; Zhang, Q. S.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766. (b) For a full paper describing the quasiracemic synthesis of mappcine and pyridovericin, see: Zhang, Q.; Rivkin, A.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 5774.
(12) At the time of this preliminary work, preparative fluorous HPLC columns were not available, and most of the mixtures were not preparative demixed and detagged.

Scheme 4. Modified Synthesis of Iodopyridine **13**



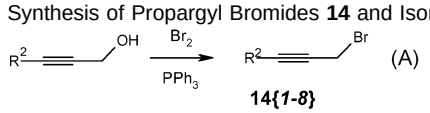
alcohols bearing different R^1 groups are first attached to seven different tags and then mixed together. The mixture **M-5** undergoes two consecutive one-pot reactions to generate a mixture of tagged pyridones **M-7**. The new mixture is then divided to eight portions for *N*-alkylation with eight different propargyl bromides in parallel. The resulting eight mixtures **M-8** are each split into 10 portions for parallel free radical reactions with 10 isonitriles to generate 80 mixtures of tagged mappicines **M-4**. Each mixture containing seven components is then demixed by HPLC to give a total of 560 individual, pure mappicine analogues after detagging. This four-step mixture synthetic process consists of two one-pot and two parallel reactions, and allows combinatorialization of three sets of building blocks. The yield for the mixture synthesis is calculated on the basis of the average molecular weight of a compound mixture.

Synthesis of Starting Materials, Building Blocks, and Tags. The key starting material for the mixture synthesis is iodopyridine **13**. We quickly found that the previous small-scale synthesis[9] was not reliable on a larger scale. A major problem was the deoxygenation of aldehyde **11**, which gave only 30% yield of **13** in gram-scale reactions (Scheme 4). After some experimentation, we found that a two-step sequence involving primary alcohol **12** reliably gave 70% overall yield of iodopyridine **13** from aldehyde **11**. Using the modified procedure, we obtained a total of 65 g (202 mmol) of compound **13** from 2,6-dibromopyridine.

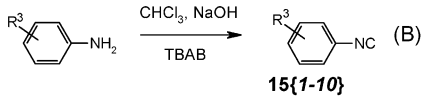
The three sets of building blocks needed for the diversity plan are easily obtained. All seven aldehydes (R^1CHO, 15 mmol each) are commercially available. Eight propargyl bromides **14**-{***1-8***} (3 mmol each) were selected from commercial sources or prepared from the corresponding alcohols (Scheme 5A).[13] A total of 10 aryl isonitriles **15**{***1-10***} (8 mmol each) were prepared from the corresponding anilines (Scheme 5B).[13] Of the 10 isonitriles, nine have substituents at the para position of the aryl ring to avoid potential formation of regioisomers in the radical annulation reaction. The 10th, 2-fluorophenyl isonitrile **15**{***8***}, has an ortho substituent that is expected to direct the final cyclization to the other ortho position.

(13) Bom, D. Ph.D. Dissertation, University of Pittsburgh, 2000.

Scheme 5. Synthesis of Propargyl Bromides **14** and Isonitriles **15**

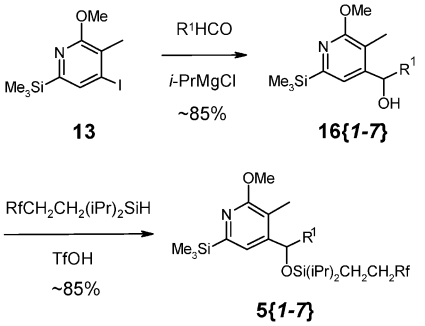


14	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
R^2	H	*m*-OMePh	Me	Et	Pr	Bu	C_5H_{11}	Ph



15	{1}	{2}	{3}	{4}	{5}
R^3	H	*p*-F	*m*-OMe	*p*-CF_3	*p*-Et
15	**{6}**	**{7}**	**{8}**	**{9}**	**{10}**
R^3	*p*-Cl	*p*-OCF_3	*o*-F	*p*-Me	*p*-SMe

Scheme 6. Preparation and Tagging of Alcohols **16**

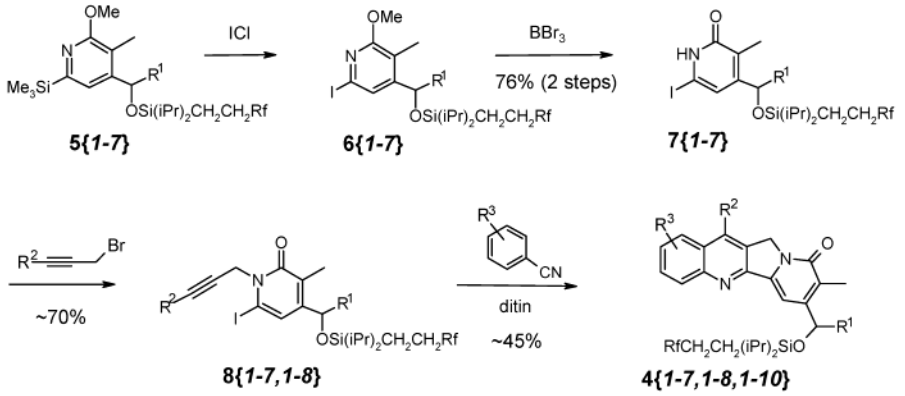


5	{1}	{2}	{3}	{4}
R^1	Me	Pr	Et	*s*-Bu
Rf	C_3F_7	C_4F_9	C_6F_{13}	C_7F_{15}
5	**{5}**	**{6}**	**{7}**	
R^1	*i*-Pr	*c*-C_6H_{11}	CH_2CH_2-*c*-C_6H_{11}	
Rf	C_8F_{17}	C_9F_{19}	$C_{10}F_{21}$	

Fluorous tags (25 mmol each) were prepared by addition of the lithium reagent derived from $RfCH_2CH_2I$ to diisopropylchorosilane to give $RfCH_2CH_2(^iPr)_2SiH$. In earlier work, this silane was converted in situ to the silyl bromide (with dibromine) prior to reaction with the requisite alcohol. Later, an improved procedure involving in situ generation of the silyl triflate (with triflic acid) was developed. Of the seven silanes, the precursors ($RfCH_2CH_2I$) with even number of perfluorocarbons (C_4F_9, C_6F_{13}, C_8F_{17}, and $C_{10}F_{21}$) were purchased with the spacer in place. The odd tags (C_3F_7, C_7F_{15}, and C_9F_{19}) were more readily available without spacers (RfI), but addition to ethylene is readily accomplished to provide the requisite iodides. The C_5F_{11} tag is absent because the precursors ($C_5F_{11}I$ and $C_5F_{11}CH_2CH_2I$) were not available at the time of the synthesis.

Tagging and Mixture Synthesis. Seven alcohols **16**{***1-7***} (13 mmol each) were prepared individually by quenching the Grignard reagent derived from iodopyridine **13** with seven different aliphatic aldehydes (Scheme 6).[13] Silyl triflates were generated from fluorous silanes and used in situ to protect alcohols **16**{***1-7***} to give **5**{***1-7***}. The tags in an order of

Scheme 7. Mixture Synthesis of Tagged Mappicines



Rf	R^1	R^2	R^3
{1} C_3F_7 {5} C_8F_{17}	{1} Me {5} *i*-Pr	{1} H {5} Pr	{1} H {6} *p*-Cl
{2} C_4F_9 {6} C_9F_{19}	{2} Pr {6} *c*-C_6H_{11}	{2} *m*-MeOPh {6} Bu	{2} *p*-F {7} *p*-OCF_3
{3} C_6F_{13} {7} $C_{10}F_{21}$	{3} Et {7} C_2H_4-*c*-C_6H_{11}	{3} Me {7} C_5H_{11}	{3} *p*-OMe {8} *o*-F
{4} C_7F_{15}	{4} *s*-Bu	{4} Et {8} Ph	{4} *p*-CF_3 {9} *p*-Me
			{5} *p*-Et {10} *p*-SMe

increasing fluorine content were matched to the corresponding alcohols in an order of decreasing polarity of the R^1 side chain. In other words, the shortest tag (C_3F_7) was paired with the most polar alcohol side chain (Me), and the longest tag ($C_{10}F_{21}$) was paired with the least polar alcohol side chain (CH_2CH_2-*c*-C_6H_{11}). In this way, the best HPLC separation of the mixture will be achieved by matching the primary separation factor (fluorine content) of fluorous silica gel with a secondary separation factor (polarity).[14] Seven alcohols with different R^1 substituents were coded with silyl tags as follows: Me/C_3F_7, Pr/C_4F_9, Et/C_6F_{13}, *s*-Bu/C_7F_{15}, *i*-Pr/C_8F_{17}, cyclohexyl/C_9F_{19}, cyclohexylethyl/$C_{10}F_{21}$. We deliberately mismatched the tags for the propyl, ethyl, *s*-butyl, and isopropyl groups to test if tag dominance overwhelms side-chain polarity.

Equimolar amounts of the seven tagged compounds **5**{***1-7***} (6.5 mmol each) were mixed and subjected to iodonative desilylation with ICl (Scheme 7). The reaction was closely followed by F-HPLC equipped with a Fluofix column to optimize the reaction conditions. We found that slow addition of ICl to a solution of **5**{***1-7***} at 15 °C under sonication increased the conversion of the starting materials from the original 50% to more than 90%.[9] The crude product **6**{***1-7***} was demethylated with boron tribromide, and the resulting mixture of seven pyridones **7**{***1-7***} was purified by standard silica gel column chromatography (see below for a discussion of this type of purification). The purified mixtures were then split into eight portions, and each portion (2.1 mmol) was subjected to N-alkylation with 1.25 equiv of propargyl bromides **14**{***1-8***}. After flash chromatographic purification on silica gel, each of the eight mixtures of N-propargyl pyridones **8**{***1-7,1-8***} was split into 10 portions (0.15 mmol each) and irradiated under a sunlamp with 3.0 equiv of aryl isonitriles **15**{***1-10***} and a catalytic amount of hexamethylditin. These 80 crude mixtures were purified by rapid solid-phase extraction (SPE) with normal silica gel. Unreacted N-propargyl pyridones **8**{***1-7,1-8***} and isonitriles **15**{***1-10***} and other byproducts were washed off with 10% EtOAc/hexanes, and fluorous-tagged mappicine mixtures **4**{***1-7,1-8,1-10***} were eluted with 15% MeOH/EtOAc. All 80 mixtures were analyzed by automated LC-MS before loading onto a semipreparative HPLC column for demixing.

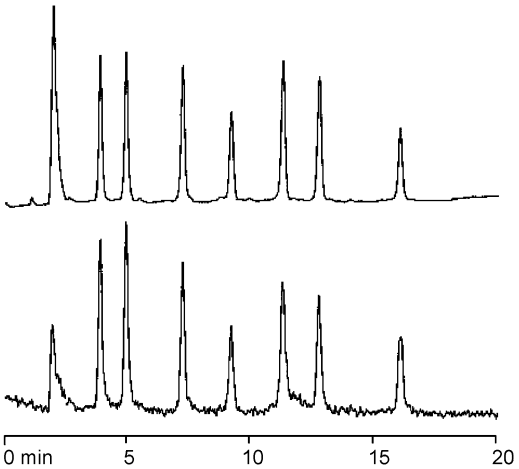


Figure 1. HPLC analysis of tagged mappicine **4**{***1-7,4,3***}. Fluofix column (4.6 × 250 mm, 5 μm), gradient 90% MeOH−H_2O to 100% MeOH in 15 min and then 100% MeOH. Top trace: UV detection at 254 nm. Bottom trace: mass spectrometer detection with a positive APCI ionization source. The first peak is the solvent front.

A typical LC trace for the analytical demixing of **4**{***1-7,4,3***} is shown in Figure 1 (UV detection, upper trace; MS detection, lower trace). Besides the solvent front, seven well-resolved peaks were detected in the UV channel in this and each of the other 79 mixtures. As revealed by mass spectroscopy, the seven compounds are the expected tagged mappicines, which eluted in the order of increasing fluorine content of the tag despite the deliberate reverse of four of the side chains. The molecular ions of all of the expected 560 fluorous-tagged mappicines were detected by LC-MS. This is a powerful demonstration that the elution order of the products can be predicted from the initial fluorous tagging of the starting substrates. To illustrate the analytical separation of the mixture of seven compounds, the retention times of 56 out of the 560 tagged mappicines are listed in Table 1. We noticed that all of the seven peaks of mixtures **4**{***1-7,1-8,4***} and **4**{***1-7,1-8,7***} eluted about 2 min slower than the rest of the library components. This is due to the presence

(14) Curran, D. P.; Oderaotoshi, Y. *Tetrahedron* **2001**, *57*, 5243.

Table 1. Retention Times (min) of 56 of the 560 Tagged Mappicines **4**[a]

4{***1-7,y,z***}	C_3F_7	C_4F_9	C_6F_{13}	C_7F_{15}	C_8F_{17}	C_9F_{19}	$C_{10}F_{21}$
y,z = {***1,1***}	3.5	4.5	6.7	8.7	10.7	12.3	15.7
{***2,2***}	4.0	5.3	7.8	10.0	11.8	13.3	16.4
{***3,3***}	3.6	4.6	6.7	8.6	10.7	12.2	15.5
{***4,4***}[b]	5.3	7.0	9.8	12.0	13.9	15.2	18.0
{***5,5***}	4.6	6.0	8.8	11.0	13.3	14.9	19.7
{***6,6***}	4.4	5.7	8.3	10.4	12.4	13.8	16.8
{***7,7***}[b]	6.9	8.7	11.7	13.8	15.6	16.8	19.8
{***8,8***}	3.7	4.8	7.1	9.1	11.2	12.7	15.9

[a] Fluofix column (4.6 × 250 mm, 5 μm), gradient 90% MeOH−H_2O to 100% MeOH in 15 min and then maintain 100% MeOH for 5 min, flow rate 1 mL/min. [b] Note longer retention times due to the CF_3 group.

Table 2. Fluorous HPLC of Tagged Intermediate Mixtures[a]

R^1	Rf	**5**{***1-7***} (min)	**6**{***1-7***} (min)	**7**{***1-7***} (min)
{**1**} Me	C_3F_7	8.4	4.6	3.6
{**2**} Pr	C_4F_9	12.5	6.8	5.1
{**3**} Et	C_6F_{13}	20.0	11.7	8.6
{**4**} *s*-Bu	C_7F_{15}	25.3	17.1	12.8
{**5**} *i*-Pr	C_8F_{17}	30.8	21.9	17.5
{**6**} *c*-C_6H_{11}	C_9F_{19}	33.3	25.3	20.8
{**7**} C_2H_4-*c*-C_6H_{11}	$C_{10}F_{21}$	39.4	32.4	28.5

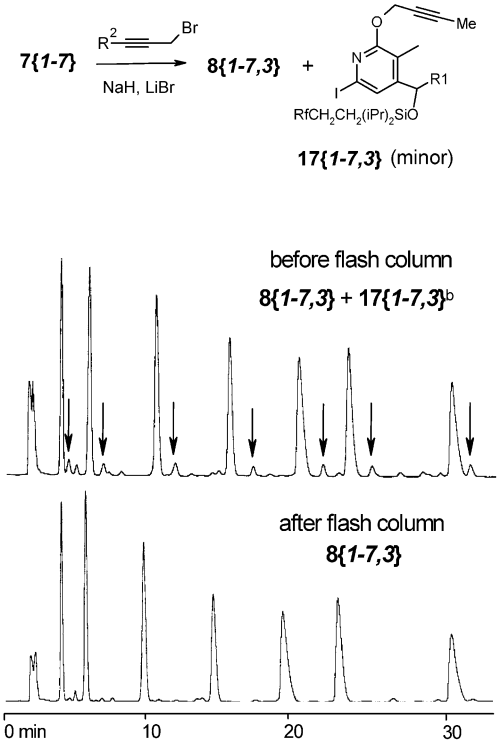
[a] Fluofix column (4.6 × 250 mm, 5 μm), gradient 90% MeOH−H_2O to 100% MeOH in 15 min and then maintain 100% MeOH, flow rate 1 mL/min.

of the extra CF_3 group in the A-ring of mappicine in these mixtures. This body of structure-retention information is a valuable aid for the design of fluorous mixture synthesis in the future.

Intermediate Analysis, Characterization, and Purification. Standard mixture synthesis without separation tags is difficult because mixture components cannot easily be analyzed, characterized, and purified. In fluorous mixture synthesis, fluorous chromatography provides a powerful tool to monitor the mixture synthesis because the fluorine−fluorine interaction dominates the separation. The polarity and other features of the substrate contribute a secondary effect to the separation.[14] Table 2 lists three sets of HPLC retention times obtained from different mixtures of tagged intermediates. The polarity gradually increases from trimethylsilyl pyridines **5** to iodopyridines **6** and then to pyridones **7**; the retention times of corresponding molecules with the same tag are gradually decreased. However, within each mixture family, the seven components kept the same elution order and were well separated from each other. Those purposely "mismatched" R^1/Rf pairs (Pr/C_4F_9, Et/C_5F_{11}, *s*-Bu/C_6F_{13}, and *i*-Pr/C_7F_{15}) did not cause compound overlapping or crossing in the HPLC analysis.

Fluorous HPLC provides a tool to analyze intermediate mixtures. Purification of tagged intermediates from tagged byproducts, however, requires a nontag-based separation strategy because the mixed components should not be demixed until the end of the mixture synthesis. We discovered that standard silica gel chromatography addressed the purification problem because the separation is based on the polarity of the substrates and is relatively insensitive to the attached tags. Scheme 8 shows an example of purification of propargylation products. Fluorous HPLC of the crude product indicated the presence of about 10% byproduct mixture. On the basis of our previous experience, these byproducts are O-propargylated pyridines **17**{***1-7,3***} (Scheme 8, top HPLC trace, O-alkylation mixtures are pointed

Scheme 8. Purification of **8**{***1-7,3***} by Flash Column Chromatography[a]



[a] Fluofix column (4.6 × 250 mm, 5 μm), gradient 90% MeOH−H_2O to 100% MeOH in 15 min, then 100% MeOH. [b] *O*-Alkylated byproducts **17**{***1-7,3***} are pointed by the arrows.

out by the arrows).[15] This crude mixture showed only two spots on a normal silica gel TLC plate: a high R_f spot of O-alkylated mixture **17**{***1-7,3***} (minor) and a low R_f spot of N-alkylated mixture **8**{***1-7,3***} (major). Silica gel flash column chromatography with hexanes−AcOEt (80:20) thus separated the O-alkylated byproducts as a mixture and gave N-propargylation products **8**{***1-7,3***} as a significantly purer mixture (Scheme 8, bottom HPLC trace). The relative ratio of seven components of **8**{***1-7,3***} in the crude mixture had no significant change after chromatography purification. The technique of silica gel flash column chromatography was also used in the purification of other intermediates (see Experimental Section). In principle, whenever tagged products differ collectively from tagged byproducts and other impurities in polarity, silica gel flash column chromatography can be applied for purification in a mixture mode.

Demixing and Detagging. Selection of an optimum column is critical to the success of HPLC demixing. Three commercially available analytical columns with perfluoroalkyl and perfluoroaryl stationary phases[16] have been evaluated (Fluofix, $SiMe_2C_2H_4C(CF_3)_2CF_2CF_2CF_3$; Fluophase-RP, $SiMe_2C_2H_4C_6F_{13}$; Fluophase-PFP, $SiMe_2C_6F_5$). HPLC retention times for a typical mixture of seven tagged mappicines **4**{***1-7,6,2***} are shown in Figure 2. For the purpose of comparison, the separation result on a normal reverse-phase C_{18} column (Symmetry) is also listed.

The Fluophase-RP and Fluofix columns have demonstrated comparably good separation with a time window of 12 min

(15) Liu, H.; Ko, S. B.; Curran, D. P. *Tetrahedron Lett.* **1995**, *36*, 8917.
(16) New Fluoro*Flash* fluorous HPLC columns ($SiMe_2C_2H_4C_8F_{17}$) are now available for both analytical and preparative scales from Fluorous Technologies, Inc., www.fluorous.com.

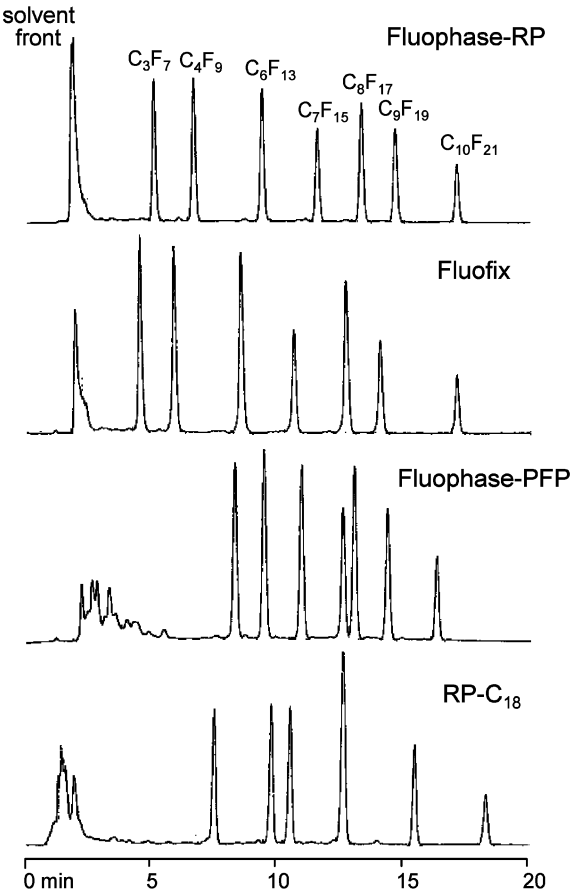


Figure 2. HPLC separation of tagged mappicines **4**{***1-7,6,2***}. Gradient 90% MeOH−H_2O to 100% MeOH in 15 min and then maintain 100% MeOH.

between C_3F_7 and $C_{10}F_{21}$ peaks under a standard HPLC condition. The Fluophase-PFP column has low resolution on C_7F_{15} and C_8F_{17} peaks, and the time window between C_3F_7 and $C_{10}F_{21}$ peaks is only 8 min. The reverse C_{18} column does not separate C_7F_{15} and C_8F_{17} tagged mappicines at all, presumably because of the polarity mismatching of the tag and side chain (*s*-Bu/C_7F_{15} vs *i*-Pr/C_8F_{17}). Fluofix and Fluophase-RP are fluorous columns that separate the mixture mainly on the basis of fluorine content. On these columns, nonfluorous compounds eluted out together with the solvent front. The pentafluorophenyl group does not have a sheath of fluorines such as the perfluoroalkyl groups and is not considered to be fluorous.[17] Indeed, Fluophase-PFP behaved more like a normal reverse-phase C_{18} column than like a fluorous column. Its separation is sensitive to the polarity (or size) of the substrates, and demixing is not guaranteed by the fluorine content. On these reverse-phase columns, nonfluorous byproducts can be separated to some extent from the solvent front.

On the basis of separation results obtained from analytical columns, a semipreparative Fluophase-RP column (20 × 250 mm, 5μm) was selected for demixing of 80 tagged mappicine mixtures. A modified MeOH−H_2O−THF solvent system was used as the mobile phase. The strong fluorophilic solvent THF was introduced at the end of each gradient program to facilitate the elution of the molecules containing $C_{10}F_{21}$ tags and also for column conditioning between injections. Samples containing

(17) Gladysz, J.; Curran, D. P. Special issue on fluorous chemistry, *Tetrahedron* **2002**, *58*, 3823.

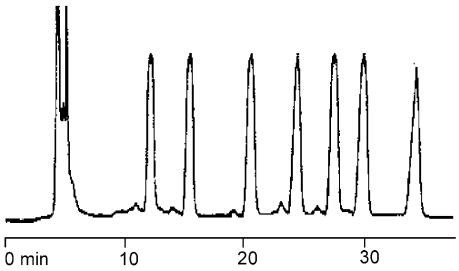
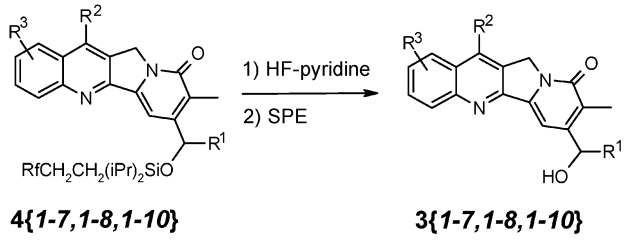


Figure 3. Semipreparative demixing of tagged mappicines **4**{***1-7,6,2***}. Fluophase-RP column (20 × 250 mm, 5 μm), gradient 88% MeOH−12% H_2O to 100% MeOH in 28 min, then to 100% THF in 7 min, flow rate 12 mL/min.

Scheme 9. Detagging and SPE Purification



about 0.07 mmol (45−65 mg) of seven-component mixtures were dissolved in 300−350 μL of THF and were loaded onto the column. One-half of 80 mixture samples were demixed on a Waters HPLC system, and the fractions were collected manually. The other one-half were demixed on a Gilson serial HPLC with an automated fraction collector. The two methods were equally successful, and 560 individually pure tagged mappicines were obtained in an average amount of 0.005−0.01 mmol (3−6 mg) each. A typical chromatogram of demixing of **4**{***1-7,6,2***} is shown in Figure 3.

The fluorous silyl protecting groups were cleaved with HF−pyridine in THF (Scheme 9). The short tags (C_3F_7 and C_4F_9) can be easily detached within 1 h at 60 °C, while longer tags required extended heating times up to 10 h. The crude products were partitioned between AcOEt and H_2O. The concentrated organic layers were loaded onto reverse-phase SPE cartridges and eluted with MeOH/H_2O (80/20) or THF/H_2O (50/50). Mappicines eluted first. Cleaved tags (silanols) followed when the cartridges were washed with MeOH or THF. The 560 SPEs were conducted manually in groups of 24 in a parallel manifold.

Finally, the products were quantified by weighing, and the amount of 560 mappicines was distributed as follows: 315 samples (56%) were between 1 and 2 mg, 180 samples (32%) were less than 1 mg, and 65 samples (12%) were greater than 2 mg.

Product Characterization. The fluorous mixture synthesis provides quality control at each reaction step by F-HPLC analysis and flash column chromatographic purification. The most critical purification occurs at the step of demixing because that separates the mixture components and also offers product purification. Because the structures of all 560 compounds were characterized by LC-MS before demixing, the final analyses were focused on the assessment of product purity. Several ^{19}F NMR analyses were carried out to ensure that there were no tag residues after detagging and SPE. HPLC analysis with UV detection of 112 (20% of the library) randomly selected samples indicated that the product purity was greater than 90%.

Additional product characterizations included MS/LC-MS and ^{1}H NMR/LC NMR analyses (see Supporting Information).

All of the mappicine samples had the expected substituents with one exception. HPLC analysis of those 56 mappicine analogues **4**{***1-7,1-8,10***} with the MeS− substituent at the A ring gave more than one peak. MS analysis revealed that one of the peaks was the expected sulfide, and the others (usually one peak, sometimes two) were the sulfoxides as a mixture of diastereomers. We suspect that detagging with HF−pyridine at high-temperature conditions may have caused the oxidation. Using a different desilylation agent such as CsF under milder conditions might prevent the formation of sulfoxides.

Conclusions

Fluorous mixture synthesis is a new solution-phase high-throughput technique that allows the production of more compounds from a synthetic exercise without a proportional increase in effort. The broad scope and favorable reaction kinetics associated with solution-phase synthesis are united with the efficiency advantages of mixture synthesis. The use of fluorous tags and the associated tag-based separation (demixing) allows intermediate mixtures to be analyzed and characterized and produces target products as individual, pure compounds. The features of fluorous mixture synthesis are highly suitable for synthesizing relatively small (100−1000) but high quality optimization libraries for structure−activity relationship (SAR) studies in medicinal chemistry or other chemical discovery settings.

The practical utility of fluorous mixture synthesis has been demonstrated by the synthesis of a mappicine library. A seven-component mixture undergoes one-pot and split-parallel syntheses with two sets of building blocks to reach a size of 560 in four steps of mixture synthesis. The economy of the mixture approach is readily illustrated by counting synthetic steps: 90 reactions (1 + 1 + 8 + 80) were conducted during the mixture synthesis in Scheme 7, whereas 630 (7 + 7 + 56 + 560) would be needed to conduct the same sequence in parallel. The savings increase with the number of compounds mixed, with the length of the sequence, and with the number of splits after mixing. Comparable savings accrue in the separations; only 80 chromatographies were used in the final demixing to produce 560 pure samples.

Fluorous HPLC is a reliable separation method not only for demixing of tagged products, but also for analysis of tagged intermediates. Purification of tagged intermediates as mixtures (that is, without demixing), however, requires a nontag-based separation method. Fluorous tags are relatively nonpolar, and experience is showing that normal silica gel flash column chromatography can sometimes be used to purify tagged mixtures on the basis of the polarity differences of subsets of tagged molecules. This unexpected ability to go from impure mixtures to pure mixtures without demixing adds to the practicality of the technique.

The bounds of fluorous tagging for mixture synthesis are not yet clear. The synthesis of multiple isomers by fluorous mixture approaches promises to be reliable because the tags should easily override the differences of isomers. The mappicine library described herein shows that fluorous mixture synthesis will also be useful for synthesizing related, nonisomeric analogues in a logical series. As the analogues being mixed become more structurally diverse, it will be helpful to give some thought to tag/analogue pairings. This is because large differences in the size and/or polarity of the tagged molecules could upset the tag dominance.[4] To spread rather than contract the fluorous HPLC chromatogram, we tentatively suggest that smaller and/or more polar analogues be given smaller fluorous tags and larger and/or less polar analogues be given larger tags. More sophisticated approaches may become possible as we learn more about structure/retention effects on fluorous HPLC columns, but a simple, qualitative approach may suffice for many applications. As in any mixture synthesis technique, efficiency is maximized by mixing early and demixing late.

The rapid transition of fluorous mixture synthesis techniques from "proof-of-principle" experiments to practical applications in several areas[11b,18] bodes well for the wider adoption of this technique. We suggest that it be considered for mainstream synthesis of natural products and related molecules or for high-throughput synthesis of drug candidates whenever leveraging of a multistep synthetic exercise by making more compounds is beneficial.

Experimental Section

Building blocks, tags, and other starting materials used in the mappicine library synthesis were readily available. All seven aldehydes (R^1CHO) and two propargyl bromides ($HCCCH_2Br$ and $MeCCCH_2B$) were obtained commercially. The other six propargyl bromides (R^2-$CCCH_2Br$), 10 isonitriles **15**{***1-10***}(R^3PhCN), perfluoroalkylsilanes ($RfCH_2CH_2{}^iPr_2SiH$), and alcohols **16**{***1-7***} were prepared by following known literature procedures.[9,13]

General LC-MS Analysis Conditions. A Fluofix column (4.6 × 250 mm, 5 μm, Keystone Scientific, Inc.) was used, with a gradient 90% MeOH−H_2O to 100% MeOH in 15 min, then maintained 100% MeOH for 5−20 min. Mass spectrometer detection was done with a positive APCI ionization source. Similar conditions were applied to F-HPLC analyses of intermediates in the mixture synthesis.

Modified Procedure for the Preparation of 13. To a solution of aldehyde **11** (3.69 g, 11.0 mmol) in ethanol (20 mL) at −40 °C was added $NaBH_4$ (419 mg, 11.0 mmol). The reaction mixture was further stirred for 1 h at −40 °C and then quenched with water. The crude product was purified by silica gel column chromatography (10% EtOAc/hexanes) to give alcohol **12** (2.60 g, 70%) as a colorless oil. To a solution of alcohol **12** (18.40 g, 54.44 mmol) in 1,2-dichloroethane (75 mL) was added triethylsilane (63.15 g, 0.54 mol) followed by slow addition of boron trifluoride etherate (34.5 mL, 0.27 mmol) at room temperature. The reaction mixture was then heated at 75 °C for 2 h before quenching with aqueous $NaHCO_3$. After being extracted with diethyl ether, the organic layer was dried and passed through a silica plug with hexanes to give the pure product **13**[9] (15.8 g, 90%).

General Procedure for Tagging Alcohols 16{***1-7***} **with Perfluoroalkylsilanes ($RfCH_2CH_2({}^iPr)_2SiH$). Preparation of 5**{***3***}**.** To C_6F_{13}-$CH_2CH_2({}^iPr)_2SiH$ (9.88 g, 21.4 mmol) was added trifluoromethanesulfonic acid (2.05 mL, 16.4 mmol) at 0 °C. The reaction mixture was then stirred at room temperature for 15 h. A solution of **16**{***3***} (4.16 g, 16.4 mmol) and 2,6-lutidine (3.8 mL, 32.8 mmol) in dry CH_2Cl_2 (40 mL) was added. After being stirred at room temperature for 2 h, the reaction mixture was quenched with aqueous NH_4Cl and extracted with CH_2Cl_2 and ether. The combined organic layers were dried, and chromatography on silica gel with EtOAc/hexanes (5/95) gave **5**{***3***} (9.97 g, 85%) as a colorless clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.25 (s, 9H), 0.77 (dd, 2H), 0.88 (t, 3H), 0.97−1.08 (m, 14H), 1.67 (m, 2H), 1.72−1.98 (m, 2H), 2.10 (s, 3H), 4.91 (s, 1H), 7.21 (s, 1H).

(18) For a mixture synthesis of four truncated discodermolide analogues, see: Curran, D. P.; Furukawa, T. *Org. Lett.* **2002**, *4*, 2233.

General Procedure for TMS−Iodo Exchange. Preparation of 6{*1-7*}. A mixture of seven tagged alcohols **5**{*1-7*} (1.5 mmol each, total 10.5 mmol, 8.2 g) in CH_2Cl_2/CCl_4 (3:1, 80 mL) was sonicated at 15 °C. To this mixture was added a solution of ICl (5.1 g, 31.5 mmol) in CH_2Cl_2/CCl_4 (3:1, 55 mL) over 30 min via an addition funnel. The reaction was followed by F-HPLC analysis to completion. The mixture was washed with aqueous $Na_2S_2O_3$, the aqueous layer was extracted with ether, and the organic layer was concentrated to give **6**{*1-7*} (8.9 g) as a clear yellow oil. This crude product **I** was used for the next reaction without further purification.

General Procedure for Demethylation. Preparation of 7{*1-7*}. To a solution of **6**{*1-7*} (8.7 g of crude) in $CHCl_3$ (150 mL) was added BBr_3 (7.9 g, 31.5 mmol) at room temperature. The reaction mixture was then refluxed for 2.5 h. The reaction was followed by F-HPLC analysis to completion. The cooled reaction mixture was slowly poured into aqueous $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted with ether, and the combined organic layers were washed with brine and concentrated. The crude product was purified by chromatography on silica gel with hexanes followed by hexanes/AcOEt (9:1) to give **7**{*1-7*} (6.50 g, 76% from **5**{*1-7*}**)** as a clear brown oil. The purity was checked by F-HPLC.

General Procedure for N-Propargylation. To a mixture of seven pyridones **7**{*1-7*} (0.30 mmol each, total 2.1 mmol) in DME (6 mL) and DMF (2 mL) was added NaH (60% in mineral oil, 0.095 g, 2.40 mmol) at 0 °C followed by LiBr (0.37 g, 4.2 mmol) after 10 min. The reaction mixture was warmed to room temperature. A propargyl bromide **14**{*1-8*} (3.1 mmol) was added. The mixture was heated at 75 °C for 7 h. The cooled reaction mixture was extracted with ether and washed with brine. The concentrated organic layer was purified by chromatography on silica gel with hexanes followed by hexanes/AcOEt (8:2) to give **8**{*1-7,1-8*} in an average yield of 70%. The purity was checked by F-HPLC.

General Procedure for Radical Annulation. To a mixture of seven N-propargyl pyridones **8**{*1-7,1-8*} (0.024 mmol each, total 0.17 mmol) was added hexamethylditin (15 μL, 0.069 mmol) and a solution of aryl isonitrile (1.0 M in benzene, 0.5 mL, 0.5 mmol). The mixture was purged with nitrogen for 5 min and sealed in a vial. The mixture was irradiated with a sunlamp for 5 h and then loaded onto a SPE cartridge packed with 2.5 g of silica. The cartridge was eluted with 10% EtOAc/hexanes (20 mL) followed by 15% MeOH/EtOAc (10 mL). The MeOH/EtOAc fraction was evaporated to dryness to give a mixture of seven tagged mappicines **4**{*1-7,1-8,1-10*}. All 80 mixtures were analyzed by LC-MS before the next step of demixing.

General Procedure for Demixing of Tagged Mappicines 4{*1-7,1-8,1-10*}. Demixing of 80 tagged mappicines mixtures was carried out on a Waters HPLC system (manually) or a Gilson serial HPLC with an automatic fraction collector. The separation conditions were as follows: Fluophase-RP column (20 × 250 mm, 5 μm, Keystone Scientific, Inc.), gradient 88% MeOH−12% H_2O to 100% MeOH in 28 min, then to 100% THF in 7 min with a flow rate of 12 mL/min. A mixture sample dissolved in a minimum amount of THF was injected. The demixed fractions were collected by following the UV detector signal (manually) or by an automatic fraction collector. The desired fractions were concentrated to give 560 individually pure tagged mappicines **4**{*1-7,1-8,1-10*} (3−6 mg of each).

General Procedure for Detagging. Preparation of Individually Pure Mappicines 3{*1-7,1-8,1-10*}. Detagging of 560 **4**{*1-7,1-8,1-10*} was accomplished in parallel. To each vial containing a tagged mappicine (3−6 mg) in THF (0.2 mL) was added 10 drops of HF−pyridine at room temperature, and then it was heated at 60 °C. The reactions were followed by TLC for completion. Reactions of shorter-tagged (C_4F_9 to C_6F_{13}) mappicine analogues took 1 h to complete, whereas longer reaction times (up to 10 h) for long-tagged (C_7F_{15} to $C_{10}F_{21}$) mappicine analogues were necessary for the reaction to go to completion. Upon completion of the reaction, the mixture was diluted with EtOAc, washed with aqueous $NaHCO_3$, and the organic layers were air-dried. Upon solvent evaporation, the residue from each vial was subjected to solid-phase extraction on reverse-phase silica gel (0.5 g) packed into syringe cartridges of 2.5 mL volume. The residue was dissolved in a minimum amount of 80:20 MeOH:H_2O (several drops of THF were sometimes added to aid in dissolving the residue) and loaded onto the prewet (80:20 MeOH:H_2O) SPE cartridge which was set on a 12 × 2 SPE manifold. The first fraction (5−8 mL) eluted with 80:20 MeOH:H_2O was collected, transferred into a vial, and air-dried giving the mappicine analogue **3** in an average amount of 1−2 mg. The purity was assessed by HPLC analysis (Novapak C_{18} column, MeOH−H_2O gradient) of 20% of randomly selected library samples. Additional structure characterizations including MS, LC-MS, ^{1}H NMR, and LC NMR analyses were also carried out.

Acknowledgment. We thank the National Institutes of Health for the SBIR funding (1 R43 GM62717), Dr. Fu-Mei Lin (University of Pittsburgh) for the help of LC-NMR and LC-MS analyses, and Mr. Brian Bucher's effort on making the starting material. We dedicate this paper to the memory of Prof. Jean-Marie Surzur.

Supporting Information Available: Analytical data and spectra of representative intermediates and final products (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA026947D