Synthetic Methods

DOI: 10.1002/ange.200501989

# Solution-Phase Mixture Synthesis with Double-Separation Tagging: Double Demixing of a Single Mixture Provides a Stereoisomer Library of 16 Individual Murisolins**

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Most small organic molecules are synthesized individually, but there is a compelling reason to synthesize molecules as mixtures—the effort conserved in a synthesis is directly proportional to the number of compounds that are mixed. "Split/mix" methods conduct reactions on mixtures of beads yet provide individual products at the end.[1] Recently, a general concept for solution-phase mixture synthesis[2] by separation tagging has been introduced[3] and put into practice with homologous fluorous tags.[4] Chemical reactions are conducted on tagged mixtures and the final reactions mixtures are demixed (sorted) in an orchestrated fashion by a tag-complementary separation technique. Finally, detagging provides the individual pure target products.

Generally, the availability of $n$ tags in a single tagging strategy allows $n$ compounds to be tagged (Figure 1, top). We

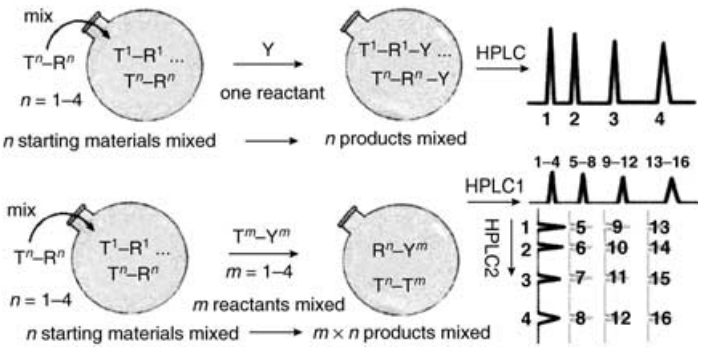


***Figure 1.*** Single (top) and double (bottom) separation tagging.

define a tag "class" as a set of tags that all share a similarly useful response to a given separation process. The addition of $m$ new tags to a given class of $n$ tags increases the maximum possible size of a mixture to $n+m$.

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[**] We thank the US National Institutes of Health for funding this work.

Wilcox and Turkyilmaz recently introduced a new class of oligoethylene glycol (OEG; $(OCH_2CH_2)_nOCH_3$) tags. These were designed for solution-phase mixture synthesis, and a polarity-based demixing process was implemented.[5] The availability of two different classes of tags opens up the possibility for double-tagging strategies. The tagging of $n$ precursors with a first class of tag and $m$ precursors with a complementary second class of tag provides $n+m$ precursors. However, reaction of these two mixtures provides a new mixture of $n \times m$ products, each of which has a unique pair of tags (Figure 1, bottom). Isolation of the final products is achieved by double demixing with a separate demixing process that targets each tag class. We report herein the first example of double tagging with tandem separation. The ability to leverage available tags is shown by the preparation of 16 stereoisomers of the natural product murisolin in a single reaction flask with only eight tags, four fluorous tags and four OEG tags.

A crucial question in any multiple-tagging exercise is tag orthogonality; when combined in a single molecule, will the fluorous tags and OEG tags enable a separation as well as each does alone. To address this question, a mixture of 16 doubly tagged analogues of vanillic acid (4-hydroxy-3-methoxybenzoic acid) M-**1**[6] was created wherein each compound had one of four OEG tags on the phenolic hydroxy group ($n$ = 1–4) and one of four homologous fluorous tags on the carboxylate group ($R^F = C_2F_5$, $C_4F_9$, $C_6F_{13}$, and $C_8F_{17}$). The resulting 16 compounds ($4 \times 4$) are unique and differ by the combination of the OEG and fluorous tags.

The 16-compound mixture was demixed into its individual components according to the tags by using a series of two demixings, one targeted to each class of tag. TLC analysis of the reaction mixture on silica gel showed only four spots (Figure 2a). The mixture was separated by flash chromatography (pentane/EtOAc gradient) into four fractions based on the properties of the OEG tags. The least polar fraction contained all four molecules **1** that bear the OEG1 tag ($n = 1$) and the four different fluorous tags. The successive fractions each contained four molecules with the OEG2, OEG3, and OEG4 tags. Each of these four fractions was further demixed by fluorous HPLC chromatography on a FluoroFlash PF-C8 column.[7] As expected, the products from this chromatographic procedure were eluted in the order of the fluorous tag from $C_2F_5$ up to $C_8F_{17}$, thus providing all 16 individual vanillic esters **1**.

We observed that the order of the demixings can also be reversed, with the fluorous demixing being conducted before the OEG demixings. Figure 2b shows an HPLC trace of the 16-compound mixture M-**1** on a FluoroFlash PF-C8 column. The compounds emerge as four groups of four peaks. The larger separations correspond to the fluorous tags, with the four compounds bearing the $C_2F_5$ tag eluting well before the four compounds bearing the $C_4F_9$ tag, and so forth. The smaller separations within the groups of peaks correspond to OEG-tagged compounds, from the more polar $n = 4$ tag to the less polar $n = 1$ tag. The mixture was separated by semi-preparative fluorous HPLC. In this case, only four fractions that correspond to the four different groups of fluorous-tagged compounds were collected. Each of these fractions,

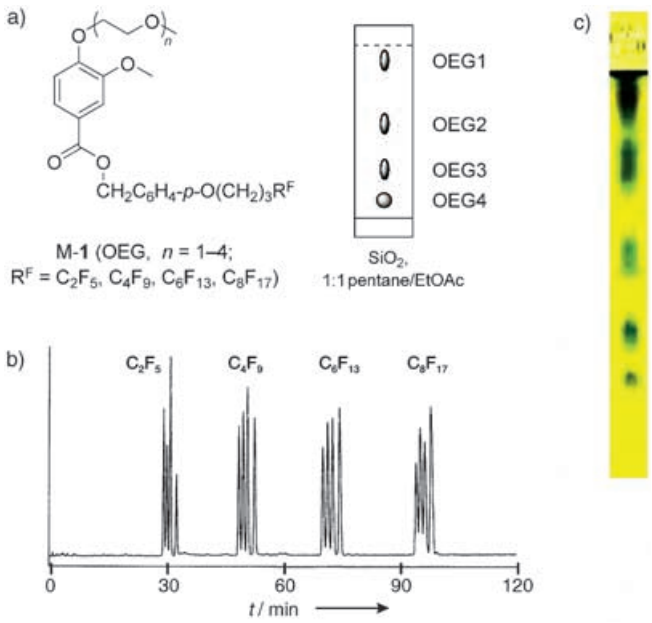


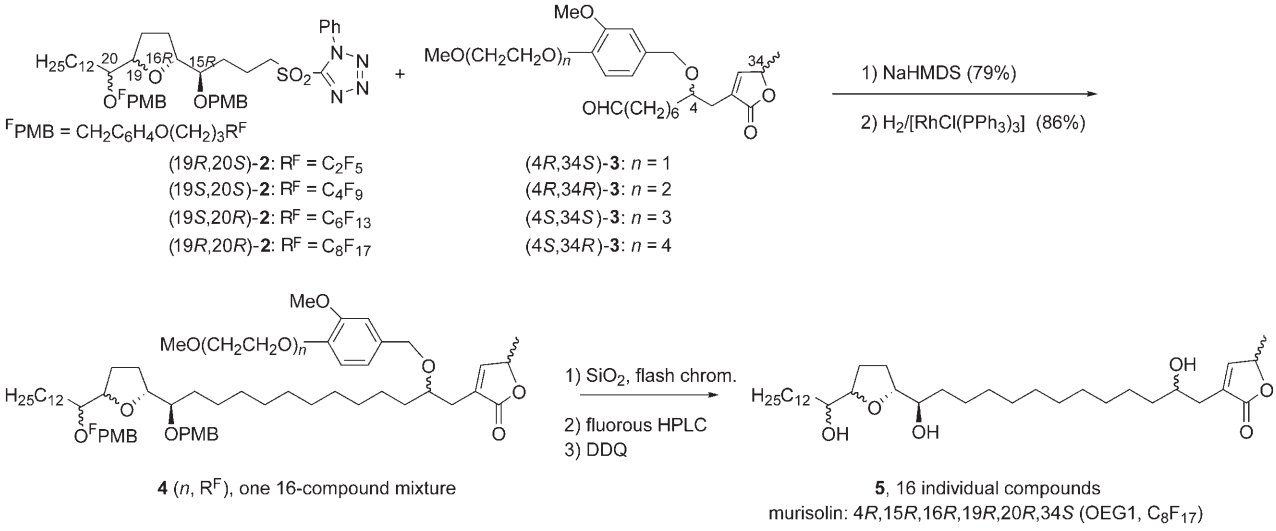

***Figure 2.*** Demixing of 16 fluorous/OEG-tagged vanillic acid esters M-**1** and murisolin quasi-diastereomers **4**. a) TLC plate of the demixing of M-**1** on silica gel based on the OEG tags (pentane/EtOAc, 1:1). b) HPLC trace of the demixing of M-**1** on a FluoroFlash PF-C8 column based on the fluorous tags ($CH_3CN/H_2O$, 40:60 to 90:10). c) Photograph of a developed TLC plate of the mixture of 16 murisolin quasi-isomers **4** (silica gel; EtOAc/hexanes, 80:20); a small spot at the origin lies below the four spots that elute as groups of four fluorous-tagged compounds in order of increasing polarity for OEG $n = 1$–4.

which contain four molecules with one fluorous tag and all four OEG tags, were then separated by simple flash column chromatography to provide the same 16 individual products.

These proof-of-concept experiments are remarkable. It is well known that the addition of $CF_2$ groups decreases the polarity of molecules,[8] yet this effect is completely overwhelmed on silica gel by the presence of the OEG tag. Conversely, the large polarity effects of the OEG tags are largely masked on the fluorous column, and the fluorine content rules. This complementarity suggests that fluorous tags and OEG tags are compatible partners in double-tagging strategies. To verify this hypothesis, we proceeded to synthesize 16 stereoisomers of the acetogenin murisolin in a single reaction flask. The pathway follows the recently completed fluorous mixture synthesis in which 16 stereoisomers were prepared in groups of four with the aid of four fluorous tags.[4e]

We planned to use a Kocienski–Julia reaction[9] to couple a mixture of four diastereomers of the dihydroxy THF subunit M-**2** with another mixture of four diastereomers of the hydroxybutenolide subunit **3** to give a doubly tagged 16-component mixture, in which the tag pairs encode the configurations and enable double demixing (Scheme 1). The tetrazolylsulfonyl component **2** was synthesized as four stereoisomers coded with a fluorous PMB ($^F$PMB) tag by using methods described recently.[4e] These stereoisomers all had the 15*R*,16*R* configuration, and all four possible isomers at C19 and C20 were present. In the first multistep OEG mixture synthesis, the aldehyde component **3** of the coupling was prepared as all four possible stereoisomers at C4,34 coded with the OEG-modified dimethyloxybenzyl group (hereafter called an OEG tag).

The Kocienski–Julia coupling of the four-compound fluorous-tagged mixture M-**2** and the four-compound OEG-tagged mixture M-**3** provided a 16-compound mixture, which was directly hydrogenated to saturate the new alkene. This sequence provided mixture M-**4** composed of 16 doubly tagged murisolins. A photograph of the TLC analysis of the mixture on silica gel shows the anticipated demixing into four spots (Figure 2c).[10] Flash column chromatography[11] sorted this mixture into four fractions based on the OEG tag from OEG1 (least polar) to OEG4 (most polar). Each of these four-compound mixtures was then demixed on a semipreparative FluoroFlash PF-C8 HPLC column[11] to provide all 16 individual tagged stereoisomers **4**. All pairs of peaks in the fluorous demixings were separated by 6 minutes or more, so quasi-isomer cross-contamination was not a problem. Even




***Scheme 1.*** Preparation of the doubly tagged 16-component mixture and subsequent demixing. PMB = *para*-methoxybenzyl ($CH_2C_6H_4OCH_3$); HMDS = hexamethyldisilazide; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; chrom. = chromatography.

though the tagged compounds **4** are now quasi-diastereomers,[10,12] the double demixing in Scheme 1 proceeded analogously to the model demixing of **1** in Figure 2.

Simultaneous removal of the PMB protecting group, the fluorous tag, and the OEG tag was effected by DDQ oxidation. The 16 stereoisomers of **5** were purified by semi-preparative HPLC to provide the target isomers on a scale of 1–3 mg.

The stereoisomer library was designed with four control compounds (the OEG1-tagged compounds with 4*R*,34*S* configuration), and these compounds were shown to be identical to authentic samples by spectroscopic and chiral HPLC analysis.[4e] The resulting data proved that the double demixing worked as expected and allowed the configurations of the remaining 12 new compounds to be assigned solely on the basis of their tag pairings and the associated demixing order. This identification was crucial because the $^{1}H$ and $^{13}C$ NMR spectra of many of the compounds in the library are identical with other members of this or the prior library. In our previous 16-compound library, we observed only six different $^{1}H$ and $^{13}C$ NMR spectra, and we predicted that six more sets of spectra were possible.[13] This predication was confirmed by observation of the other six spectra. Accordingly, none of the 32 possible diastereomers of murisolin is spectroscopically unique under standard NMR spectroscopic conditions.

Through combination of the known fluorous synthesis[4e] and the new double-tagging synthetic technique, we have prepared 28 of the 64 possible stereoisomers of murisolin. Although all the compounds were prepared from mixtures of intermediates, each isomer has been isolated in pure form and characterized by the usual spectroscopic and chromatographic means, including optical-rotation studies.

The ability to prepare 16 stereoisomers of a natural product as complex as murisolin in a single solution-phase synthesis (without splits) demonstrates the potential of these new mixture methods. Central to the potential of this technique is the newly introduced method of double tagging.

Received: June 8, 2005
Published online: October 5, 2005

**Keywords:** asymmetric synthesis · mixture synthesis · natural products · separation · tags

[1] a) A. Furka, L. K. Hamaker, M. L. Peterson in *Combinatorial Chemistry, Vol. 233* (Ed.: H. Fenniri), Oxford University Press, New York, **2000**, pp. 1–31; b) K. S. Lam, M. Lebl, V. Krchnak, *Chem. Rev.* **1997**, *97*, 411–448.

[2] For solution-phase synthesis of mixture libraries without separation tagging, see: a) R. A. Houghten, C. Pinilla, J. R. Appel, S. E. Blondelle, C. T. Dooley, J. Eichler, A. Nefzi, J. M. Ostresh, *J. Med. Chem.* **1999**, *42*, 3743–3778; b) H. Y. An, P. D. Cook, *Chem. Rev.* **2000**, *100*, 3311–3340.

[3] a) First paper: Z. Y. Luo, Q. S. Zhang, Y. Oderaotoshi, D. P. Curran, *Science* **2001**, *291*, 1766–1769; b) Short review: W. Zhang, *ARKIVOC* **2004**, 101–109.

[4] a) D. P. Curran in *The Handbook of Fluorous Chemistry*, J. Gladysz, I. Horváth, D. P. Curran, Wiley-VCH, Weinheim, **2004**, pp. 128–155; b) S. Dandapani, M. Jeske, D. P. Curran, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12008–12012; c) W. Zhang, *Tetrahedron* **2003**, *59*, 4475–4489; d) W. Zhang, Z. Y. Luo, C. H.-T. Chen, D. P. Curran, *J. Am. Chem. Soc.* **2002**, *124*, 10443–10450; e) Q. S. Zhang, H. J. Lu, C. Richard, D. P. Curran, *J. Am. Chem. Soc.* **2004**, *126*, 36–37.

[5] C. S. Wilcox, S. Turkyilmaz, *Tetrahedron Lett.* **2005**, *46*, 1827–1829.

[6] The prefix "M" denotes a tagged mixture of compounds.

[7] The columns and fluorous-tagging reagents were purchased from Fluorous Technologies, Inc. (http://www.fluorous.com); D.P.C. holds an equity interest in this company.

[8] a) P. C. Sadek, P. W. Carr, *J. Chromat.* **1984**, *288*, 25–41; b) D. P. Curran in *The Handbook of Fluorous Chemistry* (Eds.: J. A. Gladysz, D. P. Curran, I. T. Horváth), Wiley-VCH, Weinheim, **2004**, pp. 101–127.

[9] P. R. Blakemore, *J. Chem. Soc. Perkin Trans. 1* **2002**, 2563–2585.

[10] On close inspection, each of the four main spots appears to be a composite of two almost overlapping spots, which are presumably the pairs of quasi-diastereomers with the same OEG tag.

[11] Conditions for preparative demixings: a) flash column chromatography with a step gradient (25, 50, 65, and 80% EtOAc/hexanes) to elute each successive OEG-tagged fraction; b) fluorous HPLC separation of each of the four OEG-tagged demixed fractions with a $20 \times 250\ mm^2$ PF-C8 column, linear gradient of 85% $CH_3CN/H_2O$ to 100% $CH_3CN$ over 25 min (typical retention times: $C_2F_5$ 8 min; $C_4F_9$ 14 min; $C_6F_{13}$ 20 min; $C_8F_{17}$ 28 min).

[12] Q. Zhang, D. P. Curran, *Chem. Eur. J.* **2005**, *11*, 4866–4880

[13] *Syn* and *anti* isomers at C4,34 have slightly different spectra; the compounds are also differentiated by the ratio of *syn*/*anti* isomers at C15,16 and C19,20 and the ratio of *cis*/*trans* isomers at C16,19.