Published in final edited form as:

J Comb Chem. 2006 ; 8(5): 687–695.

Fluorous Mixture Synthesis of Two Libraries with Novel Hydantoin- and Benzodiazepinedione-Fused Heterocyclic Scaffolds

Wei Zhang^{a,*}, Yimin Lu^a, Christine Hiu-Tung Chen^a, Lu Zeng^b, and Daniel B. Kassel^b

^a Fluorous Technologies, Inc., 970 William Pitt Way, University of Pittsburgh Applied Research Center, Pittsburgh, PA 15238, USA

^b Takeda San Diego, Analytical and Discovery Technologies, 10410 Science Center Drive, San Diego, California 92121, USA

Abstract

Diversity-oriented synthesis (DOS) and fluorous mixture synthesis (FMS) are two aspects of combinatorial chemistry. DOS generates library scaffolds with skeletal, substitution, and stereochemistry variations, whereas FMS is a highly efficient tool for library production. The combination of these two aspects in solution-phase synthesis of two novel heterocyclic compound libraries is presented in this paper. Mixtures of different fluorous amino acids undergo [3+2] cycloadditions followed by post-condensation reactions. The mixtures are then demixed by fluorous HPLC. Fluorous tags are removed by cyclization to afford hydantoin- and benzodiazepinedione-fused heterocyclic compounds as individual, pure and structurally defined molecules. The application of MS-directed HPLC and parallel four-channel LC/MS analysis further increases the efficiency of FMS.

Introduction

Fluorous synthesis is a highly efficient solution-phase synthetic technology.¹ It employs perfluoroalkyl (Rf) groups as “phase tags” to facilitate reaction mixture separations.^{2,3} This “beadless” combinatorial technology has been applied to parallel and mixture synthesis of small molecules,^{1c-d} peptides,⁴ oligosaccharides,⁵ and biomolecules.⁶ In FMS,⁷ a mixture of substrates paired with distinct homologous fluorous tags is taken through a sequence of reactions to produce mixtures of tagged products (Scheme 1). The mixtures are then demixed by tag-controlled fluorous HPLC³ followed by tag cleavage to give a collection of individual, pure and structurally defined products. The efficiency of FMS has been demonstrated in the synthesis of enantiomers, diastereomers, and analogs of natural products.⁸

DOS is an important aspect of combinatorial chemistry.⁹ Multicomponent reactions (MCRs) and cycloaddition reactions are commonly used in DOS to construct complex library scaffolds with skeletal, substitution, and stereochemistry variations. We report here a new approach which combines DOS and FMS technologies in the synthesis of novel heterocyclic compound libraries.¹⁰ The reaction sequence involves the formation of pyrrolidine (proline) ring **1** by 1,3-dipolar cycloaddition^{11,12} of azomethine ylides followed by post condensation reactions to form tri- and tetracyclic compounds **2** and **3** (Scheme 2). These two heterocyclic skeletons

*To whom correspondence should be addressed. E-mail: w.zhang@fluorous.com.

Supporting Information Available. Spectra of representative compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>

have up to four points of substitution diversity and each has four stereocenters around the central proline ring. Scaffold **2** is structurally related to tricyclic thrombin inhibitors,¹³ whereas scaffold **3** contains a privileged benzodiazepine moiety which has a wide range of pharmaceutical utilities.¹⁴

Results and Discussion

At the method development stage, a three-component reaction involving a fluororous amino acid,¹⁵ a benzaldehyde, and a maleimide was conducted under standard solution-phase conditions using Et₃N as a base and DMF as a solvent.¹⁶ The proline derivative **1** was found to be a single diastereomer;^{12c,17} two ring-fused hydrogen atoms are *cis* to the R¹ and *trans* to the PhR². The stereochemistry was confirmed by X-ray analysis of compound **1a** (R¹ = Me, R² = *p*-MeO, R³ = Et) (Figure 1).

Synthesis of hydantoin-fused tricyclic compound library 2{R¹,R²,R³,R⁴}

A hydantoin-fused tricyclic compound library containing 420 analogs was synthesized by a five-component FMS (Scheme 3). Five α -amino acids bearing different R¹ groups were paired with five perfluoroalkyl alcohols in such: C2F₅/*i*-Bu, C4F₉/Bn, C6F₁₃/*p*-ClBn, C8F₁₇/Me, C9F₁₉/Et. An equal molar mixture of five fluororous amino acids M-4{**1-5**} was split to seven portions for 1,3-dipolar cycloaddition reactions with one of the seven benzaldehydes **5**{**1-7**} and one of the four maleimides **6**{**1-4**}. A slight excess amount of aldehyde (1.2 equiv) and maleimide (1.5 equiv) were used to consume the fluororous amino acids. The resulting seven mixtures of M-7 were each split to twelve portions and reacted with one of the twelve phenylisocyanates **8**{**1-12**} to form 84 mixtures of M-9. The efficiency of FMS was demonstrated in two steps of mixture synthesis (M-4 $\rightarrow$ M-7 $\rightarrow$ M-9). Four hundred twenty ureas M-9 (84 x 5) were prepared by 91 reactions (7 cycloaddition + 84 isocyanate reactions). Conducting similar transformations by individual parallel synthesis would require 455 reactions (35 cycloadditions + 420 isocyanate reactions).

Conventional analytical methods such as TLC, LC-MS, NMR, and purification methods such as chromatography and crystallization can be applied to FMS. These tasks are difficult to accomplish in solid-phase synthesis with immobilized resin supports. In the synthesis of M-7, the excess amount of aldehyde, maleimide and other byproducts were removed by flash column chromatography with normal silica gel. Five components in the M-7 mixture have homologous R_f tags, but they share the same parent molecule and thus possess similar polarities. The M-7 mixture was collected as a single fraction without demixing the five components.¹⁸ Mixtures of reaction intermediates M-7 and M-9 were analyzed by HPLC with a FluoroFlash column.¹⁹ Partition of fluororous molecules between a perfluorooctyl stationary phase (-Si(Me)₂CH₂CH₂C8F₁₇) and a gradient MeOH-H₂O mobile phase separated the mixture in an order of increasing fluorine content of the R_f group (Figure 2). The fluororous analytical method was readily scaled up for semi-preparative and even preparative HPLC demixing of M-9 samples. In the preparative HPLC demixing, up to 1 g of a crude sample was loaded onto a 50 x 300 mm FluoroFlash column. Each mixture was separated to five pure compounds in less than 30 min (Figure 3). Only 84 HPLC separations were performed to demix, and at the same time, to purify 420 ureas **9**. The purification efficiency of FMS is obvious.

The formation of the hydantoin ring and removal of the fluororous tag was accomplished by a single-step cyclative cleavage reaction¹⁴ promoted by K₂CO₃ at 110 °C in DMF. The final products were purified by C18 reverse-phase HPLC. A total of 380 out of 420 final products were obtained in >90% purities. The amounts of the final products were in the range of 5–30 mg.

MS-Directed HPLC purification and parallel four-channel LC/MS analysis

Analysis with a 4.6 x 50 mm column and separation with a semi-preparative 2.0 x 30 mm column at higher flow rate have been developed for high-speed HPLC.²⁰ MS-triggered HPLC makes the purification process more efficient.²¹ The incorporation of multiple column HPLC coupled with multiple channel UV or MS interface (MUX) further increase the speed 4 to 8 times faster.²² All these techniques can be applied to fluororous HPLC for sample analysis and demixing of fluororous components in FMS. We developed a 5 min analysis method for M-9 samples; five components in the mixture were separated with baseline resolutions. This method has been successfully extended to four-channel LC/MS/MUX for parallel analysis (Figure 4). We also explored the high-speed and mass-triggered HPLC for demixing of M-9 samples. The run time was only 8 min, much faster than the 30 min run time shown in Figure 3. A representative chromatogram for the separation of M-9{1-5,4,2,12} is shown in Figure 5. The purities of each component after demixing are greater than 95% (Figure 6). It clearly demonstrated that four mixture samples of M-9 could be separated in parallel to give 20 demixed products, which dramatically improves the efficiency of FMS.

Synthesis of benzodiazepine-fused tetracyclic compound library 3{R¹,R²,R³}

The synthesis of benzodiazepine-fused tricyclic prolines **3** was also accomplished by FMS. At the stage of method development, we found that *N*-acylation of M-10 mixtures with 2-nitrobenzoyl chloride was sensitive to the R¹ substitution; only small R¹ groups (H and Me) gave clean acylation products. Thus, a mixture of two fluororous amino acids (R¹ = H and Me) attached to C6F13 and C8F17 was reacted with ten aldehydes and three maleimides to give thirty mixtures of M-10 (Scheme 4). The crude products were purified by fluororous solid-phase extraction (F-SPE) over FluoroFlash cartridges.¹⁹ The *N*-acylation followed by nitro group reduction with zinc dust in acetic acid under sonication gave 30 mixtures of M-12. The preparative HPLC demixing of M-12 gave 60 individual compounds. These compounds then underwent cyclative tag cleavage with 1,8-diazabicyclo[4.3.0]non-5-ene (DBU) to form the corresponding benzodiazepine-fused tetracyclic compounds **3**. Fifty two out of 60 final products were obtained after C18 reverse-phase HPLC purification with >90% purities.

Conclusions

We have developed a fluororous protocol for synthesis of two discovery libraries with novel heterocyclic scaffolds. Amino acids attached to different fluororous tags are used as starting materials for FMS. The stereochemistry of the proline derivatives is accomplished by [3+2] cycloadditions. Two sets of post-condensation reactions lead to formation of hydantoin-fused tricyclic and benzodiazepine-fused tetracyclic ring systems. The scaffold *diversity* and *complexity* generated from DOS are integrated with the reaction and separation *efficiency* resulted from FMS. The application of MS-directed HPLC techniques to demixing the reaction mixtures and using parallel four-channel LC/MS for analysis dramatically increases the efficiency of FMS. Results shown in this paper demonstrate that FMS as a new high-throughput synthetic technology has great potential in solution-phase library synthesis.

Experimental Section

Materials and Methods

All starting materials, solvents, and reagents were commercially available and used without further purification. FluoroFlash SPE cartridges were obtained from Fluorous Technologies, Inc. SPE purification was conducted in parallel on a 2 x 12 SPE manifold available from Supelco and Fisher. Microwave reactions were conducted sequentially on a CEM Explorer single mode microwave reactor with a 10 mL cap-sealed tube. NMR spectra were obtained on a Bruker AC-270 spectrometer (270 MHz). CDCl₃ was used as the solvent unless otherwise

specified. ^1H and ^{13}C NMR spectra were recorded at 270 and 67.5 MHz, respectively. Chemical shifts are in ppm relative to TMS (0 ppm). LC-MS spectra were obtained on an Agilent 1100 system. Purities were calculated based on UV254 absorption. APCI was used as ion source for all MS. Analytical HPLCs were performed under the following conditions. Method 1: Column: XTerra MS C18 3.5 μm 4.6 x 100 mm. Eluent A: water; B: MeOH. Flow rate: 1 mL/min. Detection: UV (254 nm). 65–100% B in A over 5 min, 100% B for 5 min. Method 2: Column: XTerra MS C18 3.5 μm 4.6 x 100 mm. Eluent A: water; B: MeOH. Flow rate: 1 mL/min. Detection: UV (254 nm). 50–100% B in A over 7 min, 100% B for 5 min. Method 3: Column: FluoroFlash 5 μm 4.6 x 150 mm. Flow rate: 1 mL/min. Eluent A: water. B: MeOH. Detection: UV (254 nm). 80%–100% B in A over 5 min, 100% B for 5 min. Preparative liquid chromatographies were performed on a Waters PrepLC2100 system. Method 4: Column: FluoroFlash 5 μm 20 x 250 mm. Flow rate: 20 mL/min. Eluent A: water. B: MeOH. C: THF. Detection: UV (254 nm). 80%–100% B in A over 23 min, 100% C for 4 min. Method 5: Column: FluoroFlash 10 μm 50 x 300 mm. Flow rate: 100 mL/min. Eluent A: water. B: MeOH. C: THF. Detection: UV (254 nm). 80%–100% B in A over 23 min, 100% C for 7 min. Method 6: Column: Luna C18 5 μm 21.2 x 250 mm. Eluent A: water; D: acetonitrile. Flow rate: 20 mL/min. Detection: UV (254 nm). 40–100% D in A over 10 min, 100% D for 5 min.

General procedure for mixture synthesis of M-7 by [3+2] cycloadditions

A mixture of five amino esters (M-4; 26.3 mmol), an aldehyde (31.5 mmol), a maleimide (39.4 mmol), and Et₃N (78.8 mmol) in DMF (75 mL) was heated at 130 °C for 2 h. The reaction mixture was cooled to room temperature and diluted with EtOAc (500 mL). The solution was washed with brine (2 x 400 mL) and the aqueous layer was extracted with EtOAc (100 mL). The combined organic layer was dried over MgSO₄ and concentrated under vacuum. The crude product was purified by flash column chromatography (1000 g silica gel) eluted with EtOAc-hexane (20:80 to 50:50) to afford M-7 in 70–85% yields.

General procedure for M-9 and HPLC demixing

A mixture of M-7 (1.3 mmol), an isocyanate (6.5 mmol) and DMAP (0.65 mmol) in toluene was heated at 130°C for 2 h. The concentrated crude product was injected to a preparative FluoroFlash HPLC column (Method 4 or 5) to afford 420 demixed ureas **9** in 45–60% yields.

Representative NMR 9{4,2,1,10}

^1H 0.87(t, J = 7.1 Hz, 3H), 1.91 (s, 3H), 1.97–2.33 (m, 4H), 2.38 (s, 3H), 3.08–3.42 (m, 3H), 4.00 (dd, J = 10.7, 9.2 Hz, 1 H), 4.24–4.52 (m, 2H), 5.27 (d, J = 10.7 Hz, 1H), 6.12 (s, 1H), 6.65 (d, J = 8.0 Hz, 1H), 6.94 (t, J = 8.0 Hz, 1H), 7.03, (d, J = 8.0 Hz, 1H), 7.10–7.33 (m, 3H), 7.34–7.60 (m, 2H); ^{13}C 12.4, 19.8, 21.2, 24.1, 27.8 (t, J = 89 Hz), 34.0, 49.5, 54.3, 62.7, 64.4, 69.0, 117.7, 105–130 (m), 122.1, 122.4, 126.3, 129.9, 130.4, 132.9, 139.2, 140.2, 152.6, 170.1, 172.9, 173.6.

General procedure for cyclization reactions of **9**

Urea **9** (0.05–0.1 mmol) and K₂CO₃ (10–20 mg) in DMF (0.25 mL) was heated at 100 °C for 5 min in an open vial. The crude product was purified by a C18 HPLC (method 6) to afford **2**.

Representative NMR 2{1,3,2,12}

^1H 0.95(d, J = 6.5 Hz, 3H), 1.06(d, J = 6.5 Hz, 3H), 1.49 (s, 9H), 1.65–1.90 (m, 2H), 2.24 (dd, J = 13.6, 5.3 Hz, 1H), 3.39 (d, J = 8.0 Hz, 1H), 3.77 (dd, J = 9.5, 8.0 Hz, 1 H), 5.01 (d, J = 9.5 Hz, 1H), 7.04 (d, J = 6.8 Hz, 2H), 7.30–7.44 (m, 4H), 7.47 (d, J = 6.8 Hz, 2H); ^{13}C 23.7, 24.5, 28.1, 29.8, 43.7, 53.5, 53.8, 59.8, 62.8, 72.1, 123.8, 124.2, 126.2, 128.5, 130.1, 131.1, 131.3, 132.6, 134.7, 151.6, 169.3, 173.2, 174.6.

General procedure for parallel LC/MS analysis

The parallel four channel LC/MS analyses were conducted on a Waters ZQ 2000 single quadrupole mass spectrometer equipped with a four-way multiple index ESI source (MUX) and a Waters 2525 binary gradient pumps, a Waters 2488 MUX dual wavelength four channel UV detector (220 and 254 nm), a four-channel CTC autosampler. MassLynx 4.0 was used for data acquisition. The chromatographic separations were carried on Fluorous Technologies Inc. FluoroFlash PFC-8 HPLC analytical columns (4.6 x 50 mm, 5 μ m). The flow rate was set at 12 mL per minute through a four-way manifold to split the flow to four individual columns and then to a four channel UV detector. Following the initial hold at 70% buffer B (B: MeCN with 0.035% TFA and A: water with 0.05% TFA) for 0.5 min, the separations were performed by using a gradient of 70% to 95% buffer B in 3 min with a hold at 95% buffer B for 1 min. The equilibration time between the analyses was 1 min. A flow splitter was situated at the outlet of each column and a small portion of effluent (about 100 μ L) was diverted to each inlet of a four-way MUX interface for mass spectrometric analysis.

General procedure for separations of fluoruous mixtures by mass-directed HPLC

The mass-directed purifications were conducted on a Waters ZQ 2000 single quadrupole mass spectrometer equipped with an electrospray ion (ESI) source and a Waters 2767 sample manager for autosampling and fraction collection, a Waters 2487 dual wavelength UV detector (220 and 254 nm), a Waters 2525 binary gradient pump for the separation and a Waters 515 pump as the makeup flow for mass triggered fractionation. MassLynx 4.0 was used for data acquisition. The HPLC separations were carried on FluoroFlash preparative column (20 x 50 mm, 5 μ m). The flow rate was set at 60 mL per minute for the 2525 pump. Following the initial hold at 70% buffer B (B: MeCN with 0.035% TFA and A: water with 0.05% TFA) for 0.5 min, the separations were conducted with a gradient of 70% to 95% buffer B for 5.5 min with a hold at 95% buffer B for 1 minute. The equilibration time between the analyses was 1 min. A flow splitter was situated at the outlet of the column and a small portion of effluent (about 100 μ L) was mixed with makeup flow (515 pump) at 1.0 mL per minute of MeOH and water (80/20) with 0.1 % formic acid which was further split by a tee to diverted about 100 μ L to ESI interface for mass spectrometric triggered fractionation.

General procedure for *N*-acylation of M-10

A mixture of M-10 (2.4 mmol), 2-nitrobenzoylchloride (7.3 mmol), and Et₃N (2.4 mmol) in DMF (1 mL) was stirred at 80 °C for 2 h. Upon reaction completion, the crude mixture was loaded directly onto a 5g FluoroFlashTM cartridge. The cartridge was eluted with 80:20 MeOH-H₂O (2 x 8 mL) and acetone (2 x 8 mL). The acetone fractions were collected and concentrated to give the *N*-acylated product M-11 in 50–65% yields.

General procedure for preparation of M-12 and HPLC demixing

A mixture of M-11 (0.64 mmol) and zinc dust (6.4 mmol) in acetic acid (3 ml) was sonicated for 2 h. The crude mixture was filtered through a plug of celite and the solid was washed with EtOAc (2 mL). The filtrate was washed with aq. NaHCO₃ (2 x 2 mL) and concentrated. The residue was demixed by preparative fluoruous HPLC (Method 5) to give demixed ureas 12 in 55–70% yields.

General procedure for cyclization reactions of 12

A mixture of 12 (0.1 mmol) and DBU (0.2 mmol) in dioxane (0.5 mL) was irradiated in a microwave reactor (150 w, 130 °C, 5 min). The reaction mixture was loaded onto a 2 g FluoroFlash cartridge and eluted with MeOH:H₂O (2 x 8 mL). Concentrated MeOH-H₂O fraction was further purified by C18 HPLC (Method 6) to afford 3.

Representative NMR 3{1,4,2}

¹H 1.14 (t, *J* = 7.2 Hz, 3H), 1.65 (s, 3H), 3.52 (q, *J* = 7.2 Hz, 2H), 3.60 (d, *J* = 10.2 Hz, 1H), 3.72 (s, 3H), 3.86 (t, *J* = 10.5 Hz, 1H), 5.88 (d, *J* = 11.1 Hz, 1H), 6.78 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.48 Hz, 2H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.95 (dd, *J* = 7.8, 1.1 Hz, 1H), 8.26 (s, 1H); ¹³C 12.4, 25.7, 34.2, 46.1, 55.1, 56.7, 66.0, 67.9, 113.9, 120.0, 125.3, 128.5, 129.1, 131.5, 133.6, 135.7, 159.1, 164.3, 170.5, 173.2, 173.9.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by National Institute of General Medical SBIR Grants (1R43GM062717-01 and 2R44GM062717-02). We thank Professors Dennis P. Curran, Peter Wipf, and Dr. John (Jack) Hodges for helpful suggestions and discussions, Dr. Steven Geib for conducting x-ray crystal structure analysis of compound **1a**, and Mr. Jeffery Irwin for preparation of perfluoroalkylpropyl alcohols.

References and Notes

- Selected reviews: a) Curran DP. *Aldrichmica Acta* 2006;39:3–9. b) Gladysz JA, Curran DP, Horvath IT. *Handbook of Fluorous Chemistry* Wiley-VCH, Weinheim, 2004. c) Zhang W. *Chem Rev* 2004;104:2531–2556. [PubMed: 15137799] d) Zhang W. *Tetrahedron* 2003;59:4475–4489. e) Pozzi G, Shepperson I. *Coord Chem Rev* 2003;242:115–124. f) Dobbs AP, Kimberley MR. *J Fluorine Chem* 2002;118:3–17. g) Tzschucke CC, Markert C, Bannwarth W, Roller S, Hebel A, Haag R. *Angew Chem Int Ed* 2002;41:3964–4000. h) Barthel-Rosa LP, Gladysz JA. *Coordination Chem Rev* 1999;190–192:587. i) de Wolf E, van Kotena G, Deelman B-J. *Chem Soc Rev* 1999;28:37–41. Horvath IT. *Acc Chem Res* 1998;31:641–650. j) Studer A, Hadida S, Ferritto SY, Kim PY, Jeger P, Wipf P, Curran DP. *Science* 1997;275:823–826. [PubMed: 9012347] k) Horvath IT, Rabai T. *Science* 1994;266:72–76. [PubMed: 17814001]
- a) Zhang W. *Curr Opin Drug Dis Dev* 2004;7:784–797. Zhang, W. *Handbook of Fluorous Chemistry*. Gladysz, JA.; Curran, DP.; Horvath, IT., editors. Wiley-VCH; 2004. p. 222–236.
- Curran, DP. *Handbook of Fluorous Chemistry*. Gladysz, JA.; Curran, DP.; Horvath, TI., editors. Wiley-VCH; Weinheim: 2004. p. 101–1127. b) Curran DP. *Synlett* 2001:1488–1496. c) Curran DP, Oderaotoshi Y. *Tetrahedron* 2001;57:5243.
- a) Montanari V, Kumar K. *Eur J Org Chem* 2006:874–877. b) Goto K, Miura T, Hosaka D, Matsumoto H, Mizuno M, Ishida H-k, Inazu T. *Tetrahedron* 2004;60:8845–8854. c) Montanari V, Kumar K. *J Am Chem Soc* 2004;126:9528–9529. [PubMed: 15291542] d) Mizuno M, Goto K, Miura T, Hosaka D, Inazu T. *Chem Commun* 2003:972–973. e) de Visser PC, van Helden M, Filippov DV, van der Marel GA, Drijfhout JW, van Boom JH, Noort D, Overkleeft HS. *Tetrahedron Lett* 2003;44:9013–9016. f) Filippov DV, van Zoelen DJ, Oldfield SP, van der Marel GA, Overkleeft HS, Drijfhout JW, van Boom JH. *Tetrahedron Lett* 2002;43:7809–7812.
- a) Miura T, Goto K, Waragai H, Matsumoto H, Hirose Y, Ohmae M, Ishida H-k, Satoh A, Inazu T. *J Org Chem* 2004;69:5348–5353. [PubMed: 15287781] b) Jing Y, Huang X. *Tetrahedron Lett* 2004;45:4615–4618. c) Miura T, Goto K, Hosaka D, Inazu T. *Angew Chem Int Ed* 2003;42:2047–2051. d) Palmacci ER, Hewitt MC, Seeberger PH. *Angew Chem Int Ed* 2001;40:4433–4437. e) Curran DP, Ferritto R, Hua Y. *Tetrahedron Lett* 1998;39:4937–4940.
- a) Pearson WH, Berry DA, Stoy P, Jung KY, Sercel AD. *J Org Chem* 2005;70:7114–7122. [PubMed: 16122230] b) Beller C, Bannwarth W. *Helv Chim Acta* 2005;88:171–179. c) Tripathi S, Misra K, Sanghvi YS. *Org Prep Proc* 2005;37:257–263. d) Mizuno M, Goto K, Miura T. *Chem Lett* 2005;34:426–427.
- Reviews on FMS: a) Zhang Q, Curran DP. *Chem Eur J* 2005;11:4866–4880. b) Zhang W. *Arkivoc* 2004;(i):101–109. c) Luo ZY, Zhang QS, Oderaotoshi Y, Curran DP. *Science* 2001;291:1766–1769. [PubMed: 11230688]
- a) Gudipati V, Curran DP, Wilcox CS. *J Org Chem* 2006;71:asap b) Curran DP, Moura-Letts G, Pohlman M. *Angew Chem Int Ed* 2006;45:2423–2426. c) Wilcox CS, Gudipati V, Lu H, Turkyilmaz S, Curran

- DP. *Angew Chem Int Ed* 2006;454:3938–3940. d) Fukui Y, Brückner AM, Shin Y, Balachandran R, Day BW, Curran DP. *Org Lett* 2006;8:301–304. [PubMed: 16408900] e) Manku S, Curran DP. *J Comb Chem* 2005;7:63–68. [PubMed: 15638481] f) Manku S, Curran DP. *J Org Chem* 2005;70:4470–4473. [PubMed: 15903326] g) Jian H, Tour JM. *J Org Chem* 2005;70:3396–3424. [PubMed: 15844974] h) Zhang Q, Lu H, Richard C, Curran DP. *J Am Chem Soc* 2004;126:36–37. [PubMed: 14709047] i) Dandapani S, Jeske M, Curran DP. *Proc Nat Acad Sciences* 2004;101:12008–12012. j) Zhang W, Luo Z, Chen CHT, Curran DP. *J Am Chem Soc* 2002;124:10443–10450. [PubMed: 12197746] k) Zhang Q, Rivkin A, Curran DP. *J Am Chem Soc* 2002;124:5774–5781. [PubMed: 12010052] l) Curran DP, Furukawa T. *Org Lett* 2002;4:2233–2235. [PubMed: 12074675]
9. Recent articles on DOS: a) Martin DB, Schreiber SL. *Angew Chem, Int Ed* 2004;43:46–58. b) Burkner MD, Berger EM, Schreiber SL. *Science* 2004;302:613–618. c) Nefzi A, Ostresh JM, Yu J, Houghten RA. *J Org Chem* 2004;69:3603–3609. [PubMed: 15152987] d) Martin DB, Berger EM, Schreiber SL. *J Am Chem Soc* 2004;126:14095–14104. [PubMed: 15506774] e) Wipf P, Stephenson CRJ, Walczak MAA. *Org Lett* 2004;6:3009–3012. [PubMed: 15330670] f) Spring DR. *Org Biomol Chem* 2003;1:3867–3870. [PubMed: 14664374] g) Liao Y, Hu Y, Wu J, Zhu Q, Donovan M, Fathi R, Yang Z. *Curr Med Chem* 2003;10:2285–2316. [PubMed: 14529343] h) Toure BB, Hoveyda HR, Tailor J, Ulaczyk-Lesanko A, Hall DG. *Chem Eur J* 2003;9:466–474. i) Schreiber SL. *Science* 2000;287:1964–1968. [PubMed: 10720315]
10. Zhang W, Lu Y, Chen CH-T, Curran D, Geib S. Preliminary communication on chemistry development. *Eur J Org Chem* 2006:2055–2059.
11. Recent reviews and monograph on [3+2] cycloaddition of azomethine ylides: a) Coldham I, Hufton R. *Chem Rev* 2005;105:2765–2809. [PubMed: 16011324] b) Ruck-Braun K, Freysoldt THE, Wierschem F. *Chem Soc Rev* 2005;34:507–516. [PubMed: 16137163] c) Harju K, Yli-Kauhaluoma. *J Mol Diversity* 2005;9:187–207. d) Padwa APearson WHSynthetic Application of 1,3-Dipolar Cycloaddition Chemistry toward Heterocycles and Natural ProductsWileyHoboken2003 e) Najera C, Sansano JM. *Curr Org Chem* 2003;7:1105–1150. f) AlbersMMeyerTNicolaouKCHartwigWHHandbook of Combinatorial ChemistryWiley-VCHWeinheim20021453457 g) Kantorowski EJ, Kurt MJ. *Mol Diversity* 1996;2:207–216.
12. Solid-supported [3+2] cycloaddition reactions of azomethine ylides: a) Komatsu M, Okada H, Akaki T, Oderaotashi Y, Minakata S. *Org Lett* 2002;4:3505–3508. [PubMed: 12323055] b) Ganguly AK, Seah N, Popov V, Wang CH, Kuang R, Saksena AK, Pramanik BN, Chan TM, McPhail AT. *Tetrahedron Lett* 2002;43:8981–8983. c) Hoveyda HR, Hall DG. *Org Lett* 2001;3:3491–3494. [PubMed: 11678690] d) Barrett AGM, Boffey RJ, Frederiksen MU, Newton CG, Roberts RS. *Tetrahedron Lett* 2001;42:5579–5581. e) Dondas HA, Grigg R, MacLachlan WS, MacPherson DT, Markandu J, Sridharan V, Suganthan S. *Tetrahedron Lett* 2000;41:967–970. f) Peng G, Sohn A, Gallop MA. *J Org Chem* 1999;64:8342–8349. [PubMed: 11674757] g) Bicknell A, Hird NW. *Bioorg Med Chem Lett* 1996;6:2441–2444. h) Hamper BC, Dukeshere DR, South MS. *Tetrahedron Lett* 1996;37:3671–3674. i) Hollinshead SP. *Tetrahedron Lett* 1996;37:9157–9160.
13. Olsen J, Seiler P, Wagner B, Fisher H, Tschopp T, Obst-Sander U, Banner DW, Kansy M, Muller K, Diederrich F. *Org Biomol Chem* 2004;2:1339–1352. [PubMed: 15105924]
14. a) Kamal A, Reddy KL, Devaiah V, Shankaraiah N, Reddy DR. *Mini-Rev Med Chem* 2006;6:53–68. [PubMed: 16457632] b) Horton DA, Bourne GT, Smythe ML. *Chem Rev* 2003;103:893–930. [PubMed: 12630855]
15. Grieder A, Thomas AW. *Synthesis* 2003:1707–1711.
15. Zhang W, Lu Y. *Org Lett* 2003;5:2555–2558. [PubMed: 12841779]
16. a) Zhang W, Lu Y, Geib S. *Org Lett* 2005;7:2269–2272. [PubMed: 15901186] b) Zhang W, Chen CHT. *Tetrahedron Lett* 2005;46:1807–1810. [PubMed: 18079977]
17. a) Grigg R, Sridharan V, Suganthan S, Bridge AW. *Tetrahedron* 1995;51:295–306. b) Bicknell AJ, Hird NW. *Bioorg Med Chem Lett* 1996;6:2441–2444. c) Aly MF, Younes MI, Metwally SAM. *Tetrahedron* 1994;50:3159–3168.
18. See ref. 8j for additional example of intermediate purification in FMS with out demixing of the components.
19. FluoroFlash HPLC columns and SPE cartridges are available from Fluorous Technologies, Inc., www.fluorous.com.

20. a) Weller HN. *Mol Diversity* 1999;4:47–52. b) Weller HN, Young MG, Michalczyk SJ, Reitnauer GH, Cooley RS, Rahn PC, Loyd DJ, Fiore D, Fischman SJ. *Mol Diversity* 1998;3:61–70.
21. a) Zeng L, Burton L, Yong K, Shushan B, Kassel DB. *J Chromatogr A* 1998;794:3–13. b) Li B, Chan ECY. *J Comb Chem* 2003;5:834–841. [PubMed: 14606813] c) Bolm KF, Glass B, Sparks R, Combs AP. *J Comb Chem* 2004;6:874–883. [PubMed: 15530113]
22. a) Zeng L, Kassel DB. *Anal Chem* 1998;70:4380–4388. b) Wang T, Cohen J, Zeng L, Kassel DB. *Comb Chem High Throughput Screening* 1999;2:327–334. c) de Biasi V, Haskins N, Organ A, Bateman R, Giles K, Jarvis S. *Rapid Commun Mass Spectrom* 1999;13:1165–1168. [PubMed: 10407292] Kassel, DB.; Wang, T.; Zeng, L. US Patent. 60066848. 2000. e) Kassel DB. *Chem Rev* 2001;101:255–267. [PubMed: 11712247]

Figure 1.
X-ray structure of proline derivative **1a**

Figure 2.

HPLC analysis of representative reaction mixtures M-7{1-5,6,3} and M-9{1-5,6,3,3}.

FluoroFlash column (4.6 x 150 mm, 5 μ m), gradient 80:20 MeOH-H₂O to 100% MeOH in 5 min, then 100% MeOH 5 min, flow rate 1 mL/min.

Figure 3. HPLC analysis (top) and demixing (bottom) of M-7{1-5,7,4,3}. (A) FluoroFlash column (4.6 x 150 mm, 5 μ m), gradient 80:20 MeOH-H₂O to 100% MeOH in 5 min, then 100% MeOH 5 min, flow rate 1 mL/min; (B) FluoroFlash column (20 x 250 mm, 5 μ m), gradient 80:20 MeOH-H₂O to 100% MeOH in 23 min, then THF for 4 min, flow rate 20 mL/min.

Figure 4.

Analysis of four M-9 mixtures by four-channel LC/MS with four FluoroFlash columns (4.6 x 50 mm, 5 μ m), gradient 70:30 MeCN-H₂O-TFA to 5:95% in 3 min, then 95% MeCN-TFA 1 min, total flow rate 12 mL/min, 3 mL/min per channel.

Figure 5.

Chromatograms of the mass-triggered purification of M-9{1-5,4,2,12}. FluoroFlash column (20 x 50 mm, 5 μ m), gradient 70:30 MeCN-H₂O-TFA to 5:95% in 5.5 min, then 95% MeCN-TFA 1 min, flow rate at 60 mL/min. (A): UV 220 nm chromatograms; (B): extracted ion chromatograms (XIC) with each targeted mass labeled on each peak; (C): total ion current chromatogram (TIC).

Figure 6.

Purity analysis of five components of M-9{1-5,4,2,12} after demixing. FluoroFlash columns (4.6 x 50 mm, 5 μ m), gradient 70:30 MeCN-H₂O-TFA to 5:95% in 3 min, then 95% MeCN-TFA 1 min, flow rate at 3 mL/min. (A): TIC chromatograms; (B): UV 220 chromatograms.

Scheme 1.
Schematic overview of FMS

Scheme 2.

DOS of hydantoin- and benzodiazepine-fused heterocyclic scaffolds 2 and 3

R ¹ /R ¹ {1-5}	R ² {1-7}	R ³ {1-4}	R ⁴ {1-12}
{1} C ₂ F ₅ / <i>i</i> -Bu	{1} H {5} <i>p</i> -F	{1} Et	{1} H {7} <i>p</i> -CF ₃
{2} C ₄ F ₉ /Bn	{2} <i>p</i> -Me {6} <i>p</i> -Cl	{2} <i>t</i> -Bu	{2} <i>p</i> -Me {8} 3,4-diCl
{3} C ₆ F ₁₃ / <i>p</i> -ClBn	{3} <i>p</i> -Br {7} <i>m</i> -Me	{3} <i>c</i> -C ₆ H ₁₁	{3} <i>p</i> -Br {9} <i>m</i> -Me
{4} C ₈ F ₁₇ /Me	{4} <i>p</i> -OMe	{4} Bn	{4} <i>p</i> -OMe {10} <i>m</i> -Br
{5} C ₉ F ₁₉ /Et			{5} <i>p</i> -F {11} <i>m</i> -F
			{6} <i>p</i> -Cl {12} <i>m</i> -Cl

i) M-4 (1 equiv), 5 (1.2 equiv), 6 (1.5 equiv), Et₃N (3 equiv), DMF, 130 °C, 2 h, flash chromatography, 70-85%. ii) 8 (5.0 equiv), DMAP (0.5 equiv), toluene, 130 °C, 2 h. iii) FluoroFlash semipreparative (20 x 250 mm, 5 μ m) or preparative (50 x 300 mm, 10 μ m) HPLC demixing, 45-60%. iv) K₂CO₃, DMF, 100 °C, 5 min; C₁₈ HPLC.

Scheme 3.

FMS of a 420-member hydantoin-fused tricyclic compound library 2

i) 2-nitrobenzoylchloride (3 equiv), Et₃N (1 equiv), DMF, 80 °C, 2 h, F-SPE, 50-65%. ii) Zn dust (10 equiv), AcOH, sonication, 2 h, F-HPLC demixing, 55-70%. iii) DBU (2 equiv), dioxane, μ w (150 w, 130 °C, 5 min); C₁₈ HPLC.

Scheme 4.

FMS of a 60-member benzodiazepine-fused tetracyclic compound library 3