JOURNAL OF combinatorial CHEMISTRY

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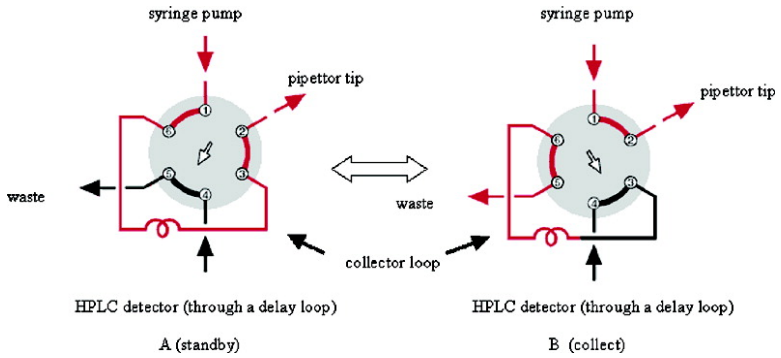
J. Comb. Chem., **2005**, 7 (1), 58-62• DOI: 10.1021/cc049861k • Publication Date (Web): 17 November 2004



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UV-Triggered Main-Component Fraction Collection Method and Its Application for High-Throughput Chromatographic Purification of Combinatorial Libraries

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Received August 27, 2004

A maximum-seeking, algorithm-driven fraction collection method was developed to support high-throughput chromatographic purification, which provides new possibilities for off-line high-performance liquid chromatography mass spectroscopy (HPLC/MS) quality control experiments. The method is based on manipulation of a six-port valve that is installed downstream from the UV detector and equipped with a fraction collector loop. The detector signal is monitored by a programmable microcontroller that controls the state of the fraction collector valve. After detecting a chromatographic peak, the appropriate fraction is stored in the collector loop. The height of the next peak is compared to the previous one (using a maximum-seeking algorithm) and, depending on the result, the collected fraction is or is not exchanged with the new one. At the end of the run, the stored UV main component is pumped into the external fraction vial. This configuration was used for chromatographic purification of large compound libraries (the results of the purification of 5324 compounds are reported here), as well as for high-throughput off-line HPLC quality control experiments, where the collected main component fractions of an analytical-scale separation were subjected to further mass spectrometric molecular weight verification.

Introduction

As the performance of current high-throughput screening (HTS) instrumentation increases, an increasing need for large-sized compound repositories can be observed. To maintain the reliability of biological screening results, analytical characterization of the compound libraries produced by automated, high-throughput synthesis techniques is essential. The present quality control protocols for combinatorial libraries generally demand high-performance liquid chromatography (HPLC) measurements to evaluate the purity of the compounds and mass spectrometric experiments to verify their identity.[1−3] Because the high-throughput preparation environment is not beneficial, with respect to individual optimization of the reaction conditions, the products are often not suitably pure for biological screening. To fulfill the purity criteria of the compounds, the application of high-throughput purification methods is often indispensable.

Large compound numbers require rapid techniques, which are capable of being fully automated. Liquid−liquid extraction methods[4,5] and the application of functionalized polymers (scavenger resins) have been used for purification of crude reaction mixtures.[6,7] However, these methods can only be applied effectively when the character of the impurity reasonably differs from the desired end product.

HPLC is a well-established separation technique, and it has a significant role both in compound purification and quality determination. The fraction collection method is an important element of the chromatographic purification process. The most simple and cost-effective method is UV-triggered fractionation.[8−12] The usual adjustable triggering parameters are the retention time window, the slope detection, and the intensity threshold. Because the samples that are subjected to purification definitely contain impurities, the exact number of the expected chromatographic peaks—and, therefore, the collection vial consumption—cannot be predicted. Thus, an excessively large fraction collection bed, post-purification analysis, and fraction sortation is needed. To avoid these time-consuming processes, the application of mass spectrometric detectors have been reported.[13−22] In this case, the fraction is collected using a selected ion chromatogram trace of the expected molecular ion as a trigger sign. With this configuration, in most cases, one injection outputs one fraction, resulting in the radical simplification of post-purification processes. However, this technique can only be applied for compounds that have proper mass spectrometric sensitivity. Samples that contain more than one component of the targeted molecular mass can also be problematic.

Here, we describe a simple and cost-effective solution for preparative HPLC that follows the one-sample/one-fraction approach. Using a maximum-seeking, algorithm-driven fraction collection method, the system is capable of selecting the largest chromatographic peak during the purification process. Thus, the system provides a unique and safe solution for the high-throughput purification of large-sized compound libraries that have the desired products as chromatographic main components. The collection also can be parametrized

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10.1021/cc049861k CCC: $30.25
Published on Web 11/17/2004

using the usual intensity threshold and retention time window parameters as well, which means that the maximum-seeking procedure will be performed only within the predefined parameter window. This features provide the possibility for purification of samples, where the desired compound is only a minor component but can be separated easily from the major component; thus, a targeted retention time window can be defined within the desired compound that would be the main component. This data-dependent fraction collection method provides relative ordering of the detected peaks and guarantees collection of the main component of the sample, independent of its retention time and absolute intensity. Using this approach, the classical debate of UV-triggered methods can be avoided, whereby the use of low thresholds and/or a broad retention time window might result in the collection of multiple fractions; however, sharpening the criteria (to decrease the number of fractions) risks possible loss of the desired compound.

Using the same configuration but on an analytical scale, the off-line FIA MS measurement of the collected fractions, together with the UV chromatogram, allows us to gain almost all the information about sample purity that an on-line HPLC/MS experiment could provide. Because the cycle time of an FIA MS experiment is approximately one magnitude shorter than that of the chromatographic experiment, a single mass spectrometer can serve as a shared, off-line detector for several HPLC systems.

Experimental Section

1. HPLC Instrumentation. The HPLC experiments were performed using Merck LaChrom gradient systems, each containing an L-7150 pump, a D-7000 interface module, and an L-7400 UV detector (Merck−Hitachi).

2. Chromatographic Conditions. The analytical HPLC separations were performed using 3-μm 4 × 30 Purospher STAR RP-18 end-capped columns (Merck, Darmstadt, Germany). The gradient was constructed by mixing eluent A (5% acetonitrile, 95% water) and eluent B (95% acetonitrile, 5% water), both buffered to pH 7.4 with 0.05 M ammonium formiate. The gradient program was the following: 0% B−100% B in 3 min, hold for 0.4 min, then 0.4 min of equilibration at 0% B. The flow rate was 2 mL/min, and the detection wavelength was 220 nm. A preparative HPLC experiments were performed on a 5-μm 25 × 100 Purospher STAR RP-18 end-capped columns (Merck, Darmstadt, Germany). The ternary gradient was build up from 5% acetonitrile containing water (eluent A), acetonitrile (eluent B), and column washing solvent that is 70% acetonitrile, 30% 2-propanol:0.1% formic acid (eluent C). The gradient program was as follows: 0% B−100% B in 6 min, hold for 3 min, then turn to 100% C for 1 min, followed by 3 min of equilibration at 0% B. The flow rate was 15 mL/min, and the detection wavelength was 220 nm. Data acquisition was controlled, in both cases, by the Merck HSM software.

3. System Configuration and Main Component Collection. A Cavro RSP 9651 liquid handler served as both a fraction collector and an autosampler. The sample injections were performed using an electronically actuated, six-port Valco high-pressure injector valve (C6W for the analytical- and C6UW for the preparative-scale applications) equipped with 5-μL or 3-mL sample loops. The sample capacity of the liquid handler is six pieces of 96-wellplate (3 samples, 3 fractions, each 1.2 mL) for the analytical scale and 96 sample vials (4 mL total) and 96 fraction vials (22 mL total) for the preparative scale. The fraction collector valve was also an electronically actuated, six-port Valco injector (C22Z-3186) that was equipped with a collector loop that had a volume of 0.3 mL or 15 mL for analytic and preparative applications, respectively.

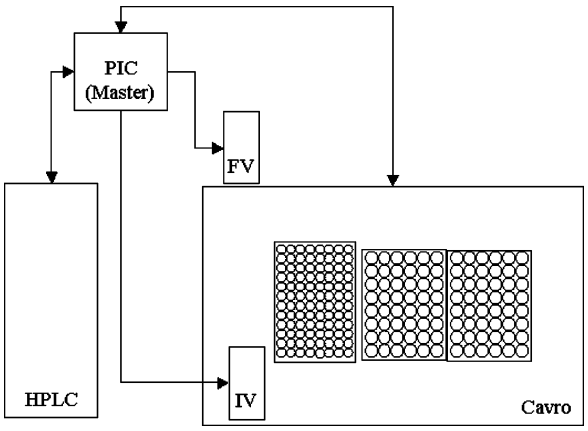

Figure 1. Block diagram of the preparative high-performance liquid chromatography (HPLC) configuration; the arrows represent the communication channels and directions. "IV" represents injector valve, "FV" is the fraction collector valve, and "PIC" is the electronic board with an embedded microcontroller (PIC16F877A) that is responsible for system synchronization and performance of the main-component collection.

The main-component selection is achieved using a maximum-seeking algorithm-driven control of the fraction collector valve (FV). A custom-designed electronic unit (PIC) performs all control functions with an embedded programmable microcontroller (PIC16F877A) in a synchronized manner (Figure 1). The working logic of the system is presented in Figure 2.

To start a purification series, the HPLC system and the liquid handler should be programmed and started independently. The liquid handler first fills the injection loop with the appropriate sample solution and notifies the PIC that this action has been completed. A cycle begins if both the HPLC and the liquid handler send a contact closure signal to the PIC (actions 1 and 2 in Figure 2). Given that both relays are closed, the PIC sends transistor transistor logic (TTL) high signals toward the injector and collector valves, making them turn to the injection and collection positions, respectively (action 3). At the same time, a contact closure signal starts the HPLC program and a TTL signal notifies the liquid handler that the chromatographic cycle has begun, so it starts to wash the pipettor tip and waits until action 5. To specify the interesting chromatographic window, within which the compound is expected to elute, event signals can be programmed in the HPLC method (peak watch start and stop). When the "peak watch start" event signal is detected by the PIC, it makes the FV turn to the standby position (Figure 3), and a continuous monitoring of the UV detector signal starts (action 4). The detected UV peaks (recognized

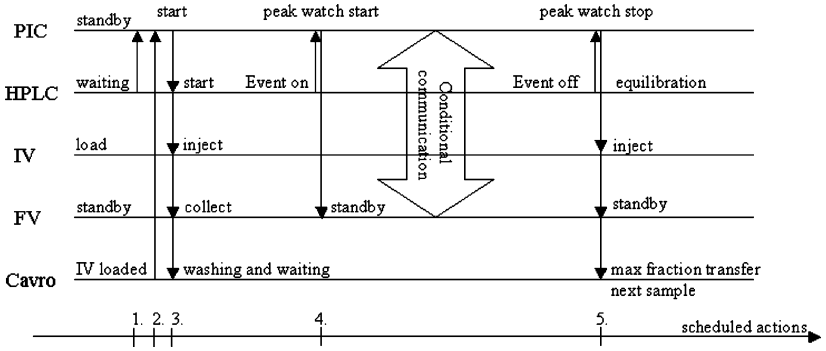

Figure 2. Communication scheme of the system, showing the working logic of a duty cycle.

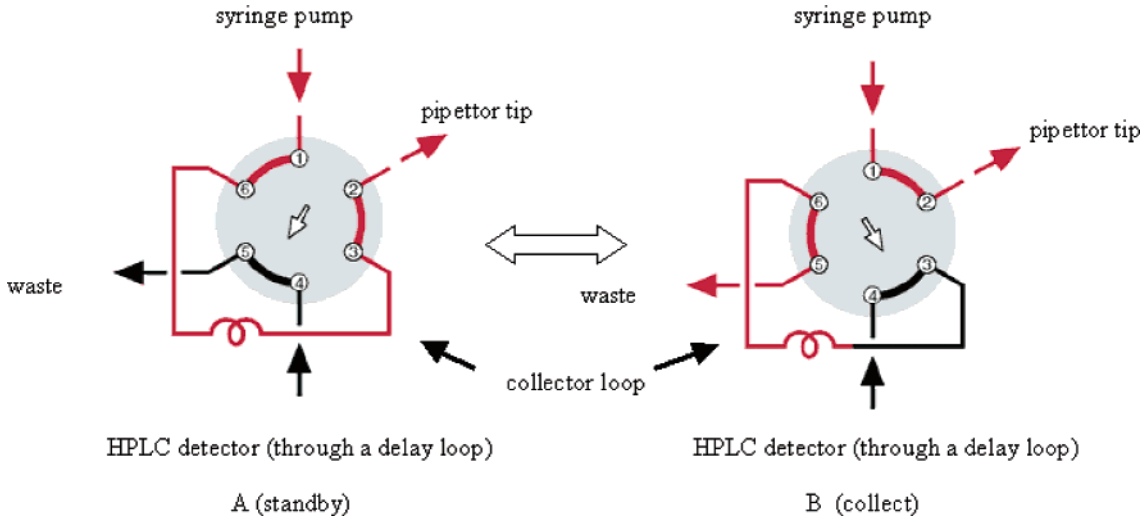

Figure 3. Working scheme of the fraction collector valve (FV) ((A) standby mode and (B) collection mode).

by the increasing first-order derivative) are characterized with a start and end time and a peak height. To provide enough time for this process, a delay loop is inserted between the detector and the collector valve. A further loop—a collector loop—is installed on the FV, the size of which is dependent on the overall chromatographic peak parameters and the applied flow rate. In the experiments presented here (optimized for a scale of 100 mg), we expected peaks with a base width of 1 min; therefore, we have applied collector and delay loops of 15 and 18 mL, respectively. When the detected peak is the first, or higher than the previous one, the collector valve, after a predefined delay time, will turn to the collection position and change the content of the loop to the appropriate fraction. Otherwise, the given fraction streams directly to the waste (conditional communication; see Figure 2) from the delay loop. A similar procedure is repeated for every detected peak. At the end of the interesting chromatographic window (an event that is often defined in the HPLC method), the PIC stops the peak watch process, turns the collector valve to the standby position (in case if it is not there), turns the injector valve to the load position, and sends the liquid handler to the proper fraction vial to empty the collector loop into it, using the syringe pump (action 5). After this procedure, the injection of the next sample will be processed while the column is equilibrating.

4. Mass Spectrometry. Flow injection mass spectrometric measurements for the analytical applications were performed using a ZQ single-quad (Waters) mass spectrometer that was equipped with an electrospray interface. Centroid spectra were acquired over a range of 100−800 amu, with a scan time of 100 ms. The capillary and cone voltages were 4 kV and 9 V, respectively. The source temperature was set to 120 °C.

Results and Discussion

The maximum-seeking, algorithm-driven main-component fraction collection method provides a unique possibility for purification of the compound repositories. Realizing the one-injection/one-fraction approach, even with application of the nonselective UV detection method, results in simplified and easily traceable post-purification processes. The described configuration was used for high-throughput purification of combinatorial libraries. The chromatographic methods were optimized on each library, with respect to the specific criteria of the fraction collection method (the flow-rate-dependent delay time and the peak-width-dependent collection loop). The selection of the sample set for the purification was performed with respect to net weight and actual purity. Compound registration requires a sample weight above a given threshold; these two pieces of data are used to estimate the possible weight recovery. After the data-handling steps (sample table and data reorganization in the database), the samples were dissolved and injected into the chromatographic system. To minimize sample loss and decrease the possibility of the sample precipitation in the loop, we use the following

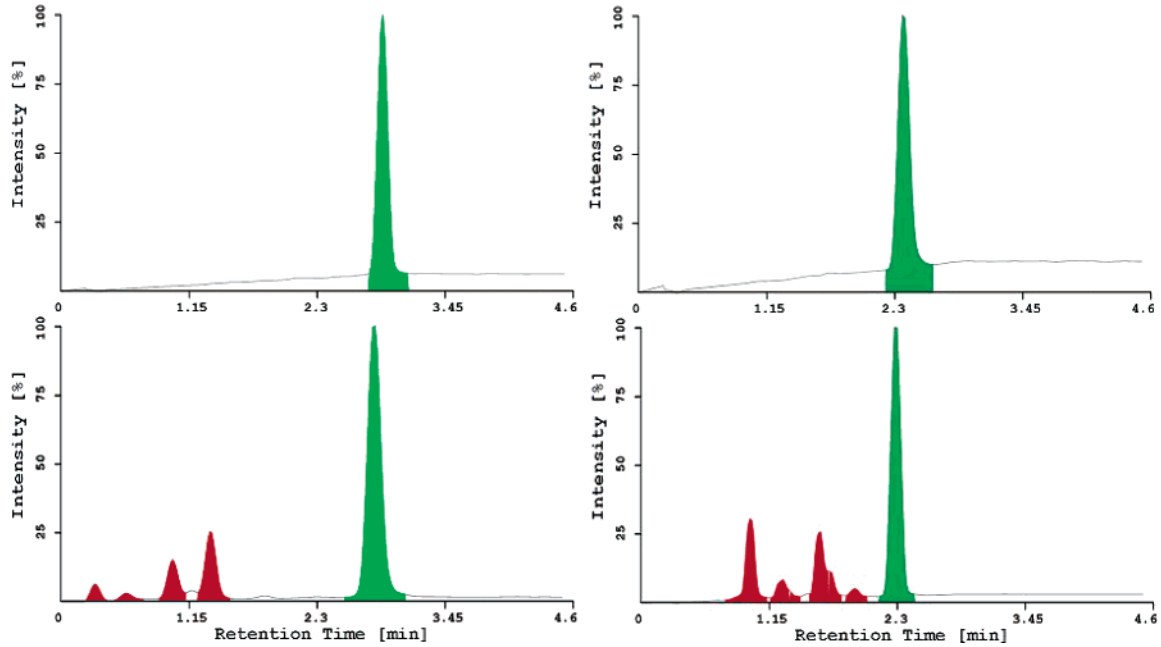

Figure 4. Two representative examples illustrating the efficiency of the purification using main-component fraction collection. The chromatograms on the top and bottom are the on-line HPLC/UV/MS results of compounds after and before the purification process, respectively.

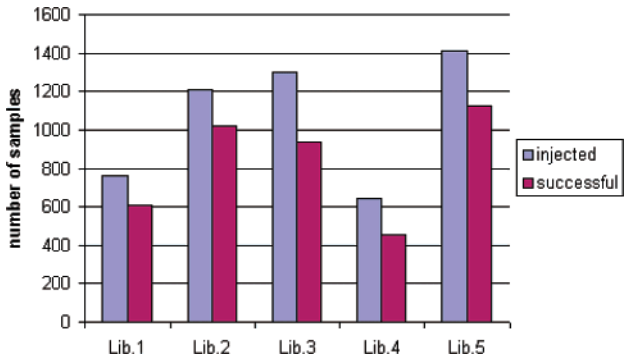

Figure 5. Statistics of the purification of 5324 samples from five different compound libraries.

"sandwich" injection method. The sample loop (3 mL) is first filled with acetonitrile and then with the sample solution (usually dissolved in 2 mL of DMSO), and finally, the sample vial is flushed out with 0.5 mL of acetonitrile that is introduced into the loop after the sample (thus, the sample solution is surrounded by acetonitrile zones). According to our experiences, the initial tube blocking, which is caused by sudden compound precipitation when the concentrated solution meets the eluent of high water content, can be avoided effectively this way. The status of the system was tested periodically by pure test compounds; the overall recovery determined by weighing was within the range of 85%−95%.

Several libraries were purified using this instrumental setup, and they have diverse chemical properties. To illustrate the performance of the purification method, analytical chromatograms before and after the purification process are shown in Figure 4. To provide a more-general view, summarized data of the purification of 5324 compounds of five different libraries can be seen in Figure 5. The initial purity of the selected compounds was in the range of 40%−89%, and the targeted purity was 90% or higher. The success rate of the purification process varies from 71% to 84%, with an average of 77.4% (with respect to the post-purification quality control (QC) results that have been determined using on-line HPLC/UV/MS). We have found 164 cases (3.1%) where a nondesired impurity was collected as the main component during the purification project. This error rate (a measure of the efficiency of the described fraction collection method) could further be minimized using more-rigorous compound selection criteria or application of a narrower fraction collection window optimized based on the prepurification QC results. The majority of the unsuccessful purifications can be attributed to the poor chromatographic separation, even during the analytical-scale HPLC experiments, which results in co-eluted components in the 100-mg-scale preparative runs. Because these compounds can be sorted out based on the analytical results, to increase the overall success rate, we plan to handle them separately, using more-specialized purification methods (focused gradients and buffer application).

The main-component collection provides a unique and cost-effective solution for the high-throughput quality control of combinatorial libraries as well. While the purity determination using fast generic gradient HPLC experiments can be done within 3−5 min, the mass spectrometric molecular weight verification for identity control requires only 20−30 s. Using the main-component collection algorithm, the FIA MS experiments can be conducted on a purified copy of the original sample set. Functionally, the FIA MS measurement of the main-component fraction, together with the HPLC chromatogram, effectively can substitute for the on-line HPLC/MS experiments in the high-throughput library quality control. Using this off-line coupling, a single mass spectrometer act as a shared detector for several independent HPLC systems.

Conclusion

The discussed main-component fraction collection method provides a simple and inexpensive solution for supporting high-throughput chromatographic purification of compound libraries. The one-injection/one-fraction approach makes the purification process more apparent and radically decreases the required number of post-purification experiments. The method can be applied for performing off-line HPLC/MS measurements as well, where the result of the further mass spectrometric measurement (FIA MS) of the collected main fractions, together with the original HPLC result, can substitute for the on-line experiments.

References and Notes

(1) Yan, B.; Fang, L.; Irving, M.; Zhang, S.; Boldi, A. M.; Woolard, F.; Johnson, C. R.; Kshirsagar, T.; Figliozzi, G. M.; Krueger, C. A.; Collins, N. *J. Comb. Chem.* **2003**, *5*, 547.
(2) Morand, K. L.; Burt, T. M.; Regg, B. T.; Chester, T. L. *Anal. Chem.* **2001**, *73*, 247.
(3) Yang, L.; Mann, T. D.; Little, D.; Clement, R. P.; Wu, N.; Rudzewicz, P. *Anal. Chem.* **2001**, *73*, 1740.
(4) Breitenbucher, J. G.; Arienti, K. L.; McClure, K. J. *J. Comb. Chem.* **2001**, *3*, 528.
(5) Peng, S. X.; Henson, C.; Strojnowsky, M. J.; Golebiowski, A.; Klopfenstein, S. R. *Anal. Chem.* **2000**, *72*, 261.
(6) Kaldor, S. W.; Siegel, M. G.; Fritz, J. E.; Dressman, B. A.; Hahn, P. J. *Tetrahedron Lett.* **1996**, *37*, 7193.
(7) Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 4882.
(8) Elridge, G. R.; Vervoort, H. C.; Lee, C. M.; Cremin, P. A.; Williams, C. T.; Hart, S. M.; Goering, M. G.; O'Neil-Johnson, M.; Zeng, L. *Anal. Chem.* **2002**, *74*, 3963.
(9) Edwards, C.; Hunter, D. J. *J. Comb. Chem.* **2003**, *5*, 61.
(10) Tommasi, R. A.; Whaley, L. W.; Marepalli, H. R. *J. Comb. Chem.* **2000**, *2*, 447.
(11) Yan, B.; Collins, N.; Wheatley, J.; Irving, M.; Leopold, K.; Chan, C.; Shornikov, A.; Fang, L.; Lee, A.; Stock, M.; Zhao, J. *J. Comb. Chem.* **2004**, *6*, 255.
(12) Ripka, W. C.; Barker, G.; Krakover, J. *Drug Discovery Today* **2002**, *7*, 56.
(13) Bauser, M. *J. Chromatogr. Sci.* **2002**, *40*, 292.
(14) Xu, R.; Wang, T.; Isbell, J.; Cai, Z.; Sykes, C.; Brailsford, A.; Kassel, D. B. *Anal. Chem.* **2002**, *74*, 3055.
(15) Blom, K. F.; Sparks, R.; Doughty, J.; Everlof, J. G.; Haque, T.; Combs, A. P. *J. Comb. Chem.* **2003**, *5*, 670.
(16) Zeng, L.; Kassel, D. *Anal. Chem.* **1998**, *70*, 4380.
(17) Zeng, L.; Burton, L.; Yung, K.; Shushan, B.; Kassel, D. B. *J. Chromatogr., A* **1998**, *794*, 3.
(18) Isbell, J.; Xu, R.; Cai, Z.; Kassel, D. B. *J. Comb. Chem.* **2002**, *4*, 600.
(19) Leister, W.; Strauss, K.; Wisnovski, D.; Zhao, Z.; Lindsley, C. *J. Comb. Chem.* **2003**, *5*, 322.
(20) Li, B.; Chan, C. Y. *J. Comb. Chem.* **2003**, *5*, 834.
(21) Kiplinger, J. P.; Cole, R. O.; Robinson, S.; Roskamp, E. J.; Ware, R. S.; O'Connell, H. J.; Brailsford, A.; Batt, J. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 658.
(22) Kassel, D. B. *Chem. Rev.* **2001**, *101*, 255.

CC049861K