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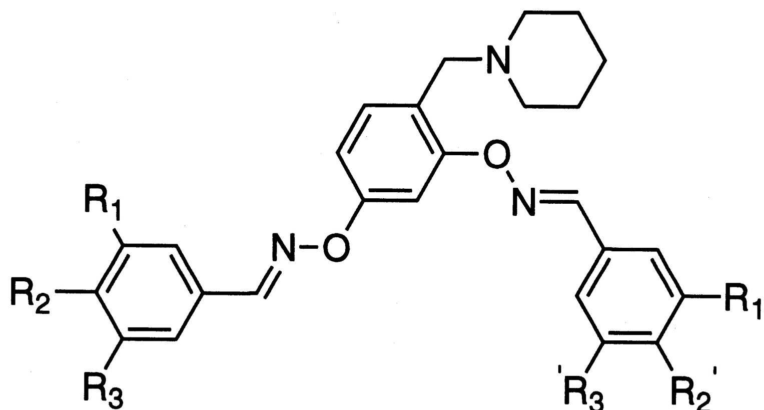

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Synthesis and Characterization of a Mixture-Based Library of Oxime Ethers Based on a Common Aromatic Scaffold

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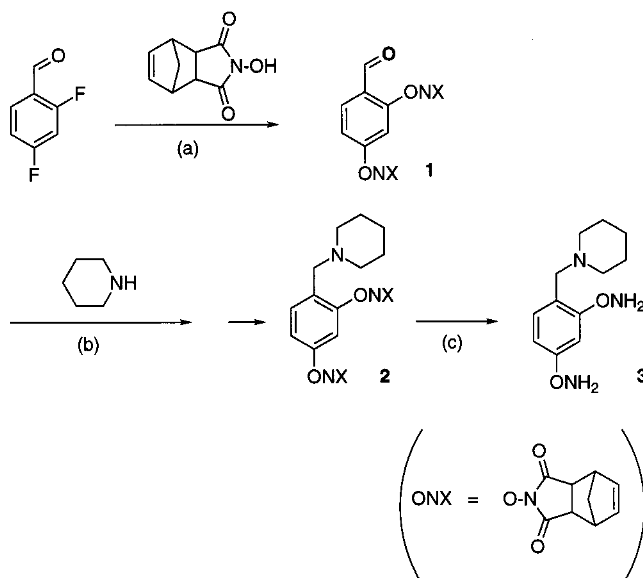
A mixture-based library of oxime ethers of general structure **4** (Scheme 2) has been prepared and characterized. Synthesis of the aromatic scaffold **3** (Scheme 1) containing two aromatic aminooxy groups followed by its reaction with 55 pairwise combinations of 11 aromatic aldehydes **A**−**K** in separate wells results in the formation of a library of sublibraries each containing four major oxime ether components. Extensive characterization of the sublibraries by LC−MS and NMR shows formation of all of the expected products in comparable amounts. The library compositions show little sensitivity of the oximation reaction to the aldehyde structure. This synthetically simple procedure provides a rapid approach to covering diversity space for this class of compounds and has implications for their use in dynamic combinatorial libraries.

Introduction

Technologies based on combinatorial synthesis and high-throughput screening have had an enormous impact on the development of the pharmaceutical industry over the past few years.[1−6] While the diversity and throughput of chemical compounds have been vastly increased, a majority of the existing technologies operate with libraries of individual compounds. As a consequence, development of synthetic techniques for combinatorial chemistry is primarily focused on the synthesis and isolation of each library member and the screening of one compound at a time. Working with mixture-based libraries, one could greatly simplify the diversity generation step.[7−12] However, the mixture-based strategies face serious problems, such as uneven representation of components in the mixtures and difficulties with their identification.

Several groups have recently shown that generation and screening of the mixture-based libraries can be enhanced by the formation of "dynamic" or equilibrating mixtures that can be reorganized by the target binder.[13] One of the basic reactions that has been used to equilibrate the dynamic libraries is the imine exchange in Schiff bases.[14] However, Schiff bases possess low stability in aqueous solutions. To generate stable compounds by the dynamic approach, it may be advantageous to separate the selection and equilibration processes.[15] This requires a reaction in which the equilibration can be "turned on" only upon applying special conditions, such as heating or catalysis. We have recently shown that oxime ethers represent a class of compounds that can be subjected to exchange in aqueous solutions at elevated temperatures, but are quite stable under ambient conditions,[16] and therefore can be isolated after selection on biological targets. In the ensuing work we report the synthesis and characterization of a small library of oxime ethers that can be potentially used in the "dynamic" mode and at the same time serves as an example of a well-sampled mixture-based library.

Scheme 1[a]



[a] Reagents and conditions: (a) DMF, K_2CO_3, 50 °C, 64%; (b) (1) $CHCl_3$, $Ti(iso\text{-}PrO)_4$, 42%, (2) MeOH, $NaBH_3CN$; (c) MeOH/$CHCl_3$, N_2H_4, 56%.

Library Synthesis

The structural design of the library of oxime ethers (**4**, Scheme 2) was guided by their potential use as estrogen mimics. The scaffold and substituent structure incorporated a number of units, such as piperidine moiety, several aromatic rings, and hydroxylated aromatics, that have been shown to be effective in synthetic estrogen receptor binders.[17,18]

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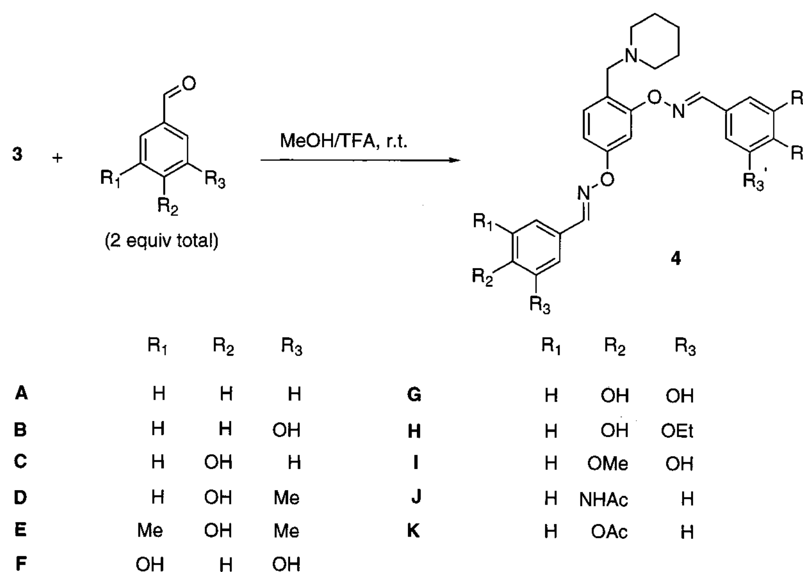
Scheme 2



	R_1	R_2	R_3		R_1	R_2	R_3
A	H	H	H	**G**	H	OH	OH
B	H	H	OH	**H**	H	OH	OEt
C	H	OH	H	**I**	H	OMe	OH
D	H	OH	Me	**J**	H	NHAc	H
E	Me	OH	Me	**K**	H	OAc	H
F	OH	H	OH				

The strategy used involved synthesis of the aromatic scaffold **3** containing two aminooxy groups (Scheme 1) followed by its reaction with various combinations of substituted benzaldehydes (Scheme 2). The scaffold synthesis was accomplished via the aromatic nucleophilic substitution of 2,4-difluorobenzaldehyde with the N-protected hydroxylamine to form compound **1.** It should be noted that reactivity of the 4-position was noticeably higher than that of the 2-position; therefore, the monosubstituted intermediate could be isolated and identified. This fact has implications for the future scale-up synthesis of the specific library members.

The aldehyde group was further coupled with piperidine by reductive amination to form amine **2**. Other amines (e.g., isopropylamine) have been shown to yield similar products. Finally, scaffold **3** was obtained via deprotection of the aminoxy groups of **2** with hydrazine.

The library was then formed by the reaction of **3** with the mixtures of different benzaldehydes in the presence of TFA as the acidic catalyst (Scheme 2). The yield determined by NMR (see Supporting Information) was nearly quantitative. The product composition showed little variability toward the solvents used for the reaction. Similar compositions were obtained in $CHCl_3$, methanol, acetonitrile, and DMSO.

One of the important questions we intended to answer in this study was how sensitive would the mixture composition be to the reactivity of the aldehydes used (i.e., whether the aldehydes containing electron-donating and -withdrawing groups would yield comparable fractions of corresponding library members) as well as to the position of the aminooxy group on the scaffold. To simplify the library analysis and quantify the library composition to the maximum possible extent, the "sublibrary" strategy was used. The eleven aldehydes were reacted with the scaffold pairwise in separate vials to form 55 sublibraries (see Supporting Information for complete description and analytical data). Each sublibrary was thus expected to contain up to four oxime ethers of the general structure **4**. For example, in the case of aldehydes **A** and **C** (Scheme 2) the corresponding substitution products can be denoted as **A-3-A**, **C-3-C**, **A-3-C**, and **C-3-A** (see Figure 5).

Library Characterization

The sublibraries were first characterized by the high-throughput fast HPLC analysis[19,20] with ESI MS detection.[21,22] The fast analysis of each sample in the library showed a range from complete separation of each of the three different molecular weight compounds to no separation at all. Besides the peaks of major products, in every sublibrary sample there was detected a *m/z* 208, which corresponds to the cleavage product of both scaffold aminoxy groups to the hydroxyls, as well as the peaks for the partial cleavage products.[§] The scaffold molecular ion at *m/z* 238 was not detected in the samples, which is indicative of complete transformation of the aminoxy groups.

The complete list of the product ions detected and their integrated extracted ion chromatographic peaks is given in the Supporting Information. Overall, each sample showed presence of major peaks corresponding to the formation of all oxime ethers **4** with equivalent or with different substituents (isobaric peaks). Because all these molecules are similar, it is assumed that the relative response observed with mass spectrometric detection is indicative of the relative amounts in the sample. The relative area of the peak corresponding to the two different substituents (e.g., **A-3-C** and/or **C-3-A**) was the highest in almost all the samples. This indirectly supports the assumption that both regioisomers are being synthesized in the mixture. However, because the complete HPLC separation of the regioisomers was never achieved, we performed additional NMR characterization of sample compositions, as described later.

Figure 1 shows an example of the extracted ion chromatograms and major ions detected in the fast LC–MS analysis. This demonstrates that for this sample the byproducts of the reaction and the desired compounds are separated

[§] This and other impurities were present in the original scaffold in the total amount not exceeding 15% of the main compound, as detected by NMR.

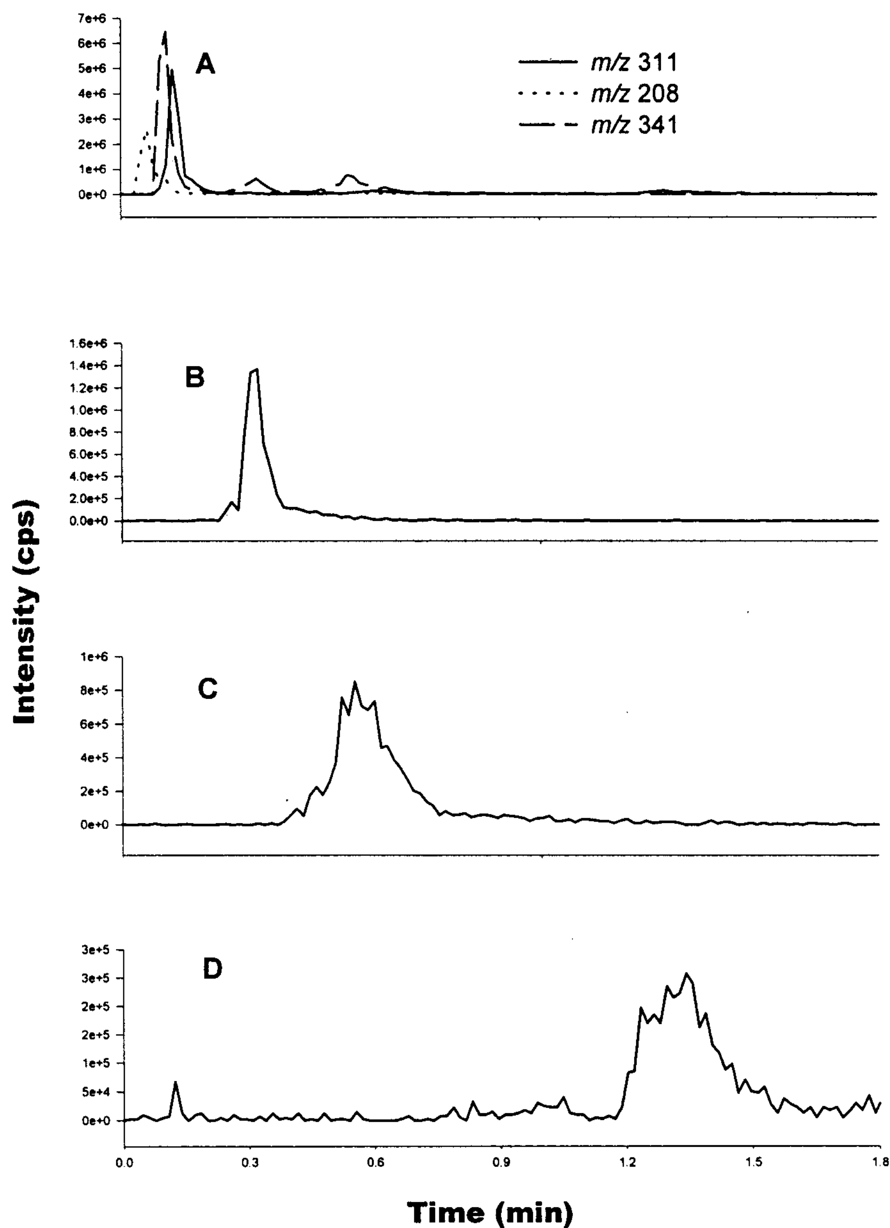


Figure 1. Extracted ion chromatograms (XIC) of sublibrary 3 by fast LC−MS. The panels show extracted ion chromatograms of (A) *m*/*z* 208, 311, and 341; (B) *m*/*z* 474; (C) *m*/*z* 444; and (D) *m*/*z* 414.

with this methodology. If indeed two regioisomers for the expected compounds at *m*/*z* 444.4 are present, they are not being separated. Those compounds would have the structures **A-3-D** and **D-3-A**.

The separation of the compounds in the sublibraries range from being well separated as in the sample shown to not being separated. Figure 2 shows sample 15 (formed from the aldehydes **B** and **G**) where very little separation is accomplished. At the scan rate used, 0.93 scans per second, to collect full mass spectra, it can be seen from the extracted ion chromatograms of Figure 2 that there are only a few data points to represent each peak. This makes it all the more difficult to demonstrate a separation even though one may be present. At the same time, the spectra collected are of good quality. Figure 3 represents the cumulative spectra of all peaks detected in sample 15. Additionally, integration of the extracted ion chromatogram, because of the high selectivity of the mass spectrometer, is not affected by poor chromatographic separation. However, it may be affected by lack of representation of the chromatographic profile because of the relatively slow scan rate.

In comparison to the fast LC−MS analysis, a gradient elution was performed on each of the samples. Figure 4 shows the result of that analysis for sample 15. The group of peaks at the beginning of the total ion chromatogram (TIC) are the $[M + H]^+$ ions of the scaffold cleavage product, *m*/*z* 208, and the products of a partial N−O bond cleavage in **4**, *m*/*z* 327 and 343, respectively. Extracted ion chromatograms are included for *m*/*z* 421 (identity unknown), 493, 478, 462, and 446. The *m*/*z* 493 may be the result of methanol addition at the C=N oxime double bond to the desired product whose *m*/*z* is 462. The peaks at *m*/*z* 478, 462, and 446 correspond to the compounds **B-3-B**, **B-3-G**/**G-3-B**, and **G-3-G**, respectively.

Because the LC−MS analysis did not provide direct evidence for the formation of both of the regioisomers of **4**

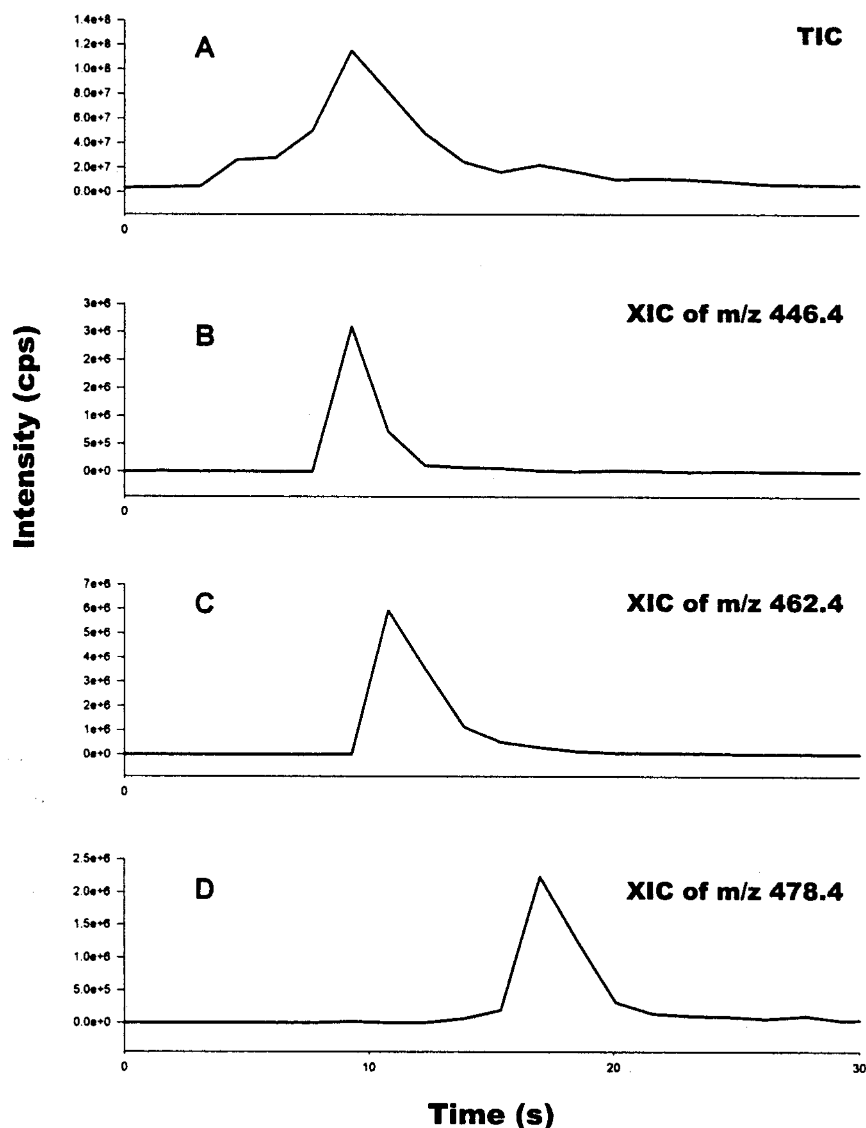


Figure 2. Total ion chromatogram (TIC) and XIC of the major ions detected in the fast HPLC−MS analysis of sublibrary 15. The panels are (A) the TIC, (B) *m/z* 446, (C) *m/z* 462, and (D) *m/z* 478.

containing different side chains, we performed additional ^{1}H NMR studies of several sample sublibraries formed in similar conditions but at a higher concentration necessary for NMR. Signals of the oxime protons in **4** were found in all cases to be in the range between 8.1 and 8.7 ppm and isolated from other signals. This range was deemed convenient for determining the ratios of all oximes in the library. Figure 5a shows the oxime proton ^{1}H NMR area of the library formed from aldehydes **A** and **C**. Tentative assignments were made by comparison with the standard compounds **A**-**3**-**A** and **C**-**3**-**C**. Integration of the peaks shows that the four oxime ethers are present in equimolar amounts, within the experimental error. Similar experiments were performed with different aldehyde combinations (i.e., **B**, **C**; **F**, **G**; **E**, **J**; **F**, **J**). All four oximes were present in all combinations, the ratio of the most to the least abundant of them never exceeding the factor of 3. Quantification of these sublibraries by the peak intensities in their MS (see Supporting Information) showed slightly different ratios, perhaps because of different ionization degrees of the components. Notably, the NMR measurements have thus allowed us to characterize even the sublibraries composed of isobaric aldehydes (**B** and **C**, **F** and **G**) that show redundant peaks in the MS analysis.

The structural design of the library presumed the use of the series of aldehydes containing predominantly electron-donating or moderately electron-withdrawing substituents (range of the Hammett σ constants from $-0.37-0.24$).[23] However, to test the reactivity in the oximation reaction of aldehydes with drastically different electronic properties, we made one sample sublibrary with the aldehyde **C** and *p*-nitrobenzaldehyde. The distribution of products, determined by NMR again, showed no less than 18% of each component (Figure 5b).

Conclusion

The strategy of a common scaffold with identical linking units used to form the library of oxime ethers offers a synthetically simple way to quickly generate diverse mixture-based libraries. Compounds of the general structure **4** can be readily generated from scaffold **3** and a large variety of commercially available aromatic aldehydes with various substituents, providing a rapid and simple way to explore

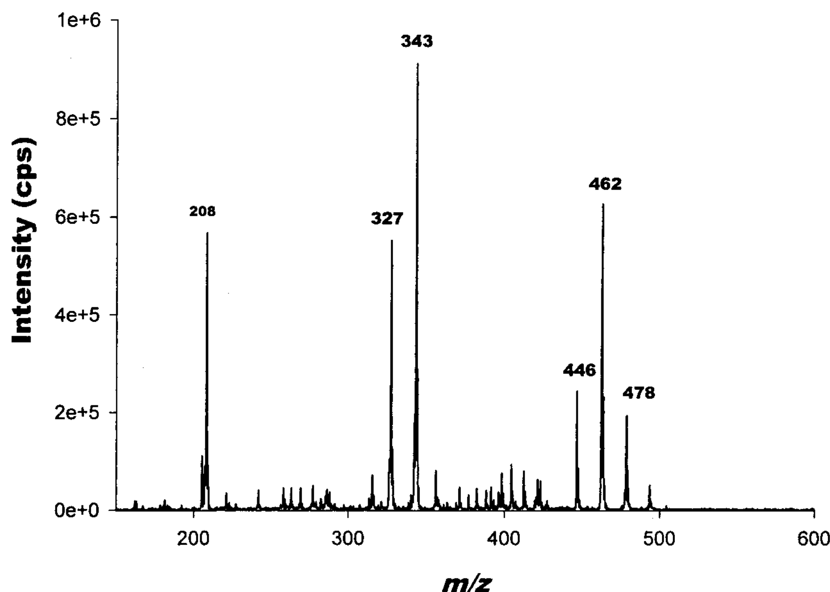


Figure 3. Cumulative mass spectrum of the chromatogram for sublibrary 15 of the fast HPLC−MS analysis. The spectrum represents the sum of 19 scans from time 0 to 0.2 min in the chromatographic file. The peaks at 446, 462, and 478 correspond to compounds **B**-**3**-**B**, **B**-**3**-**G**+**G**-**3**-**B**, and **G**-**3**-**G**, respectively. Other peaks correspond to the products of cleavage of one (327, 343) and two (208) N−O bonds in the major components.

the diversity space of this structure. As demonstrated by the LC−MS and NMR analysis, the composition of the resulting sublibraries reflects a limited variability toward the oximation reaction, with all theoretically possible products present in comparable amounts. The latter fact is essential for the use of the oxime ethers in dynamic combinatorial libraries where the statistically random distribution of components is a key prerequisite.[15]

Experimental Section

General. All compounds for synthesis were purchased from Aldrich (Milwaukee, WI) and used without further purification. NMR spectra were recorded on Varian Unity 300, 400, and 500 MHz instruments and processed using the original manufacturer software or the SwanMR shareware.[24] The methanol, acetonitrile, and 2-propanol was Baker Analyzed HPLC grade (J. T. Baker, Phillipsburg, PA). Formic acid was GFS doubly distilled (GFS Chemicals, Columbus, OH). Water was 18 MΩ from an in-house Barnstead filter system. The HPLC column was a 1 mm id x 30 mm LUNA C_{18} (Phenomenex, Torrence, CA). The HPLC mobile phase was delivered with a Hitachi L-6200A Intelligent pump (Tokyo, Japan). The autosampler was from the Integral Workstation (Perceptive Biosystems, Inc., Framingham, MA). The mass spectrometer was an API-365 (PE Sciex Inc., Ontario, Canada) run with Turboionspray.

Scaffold and Library Synthesis. (a) Protected 2,4-Diaminooxy Benzaldehyde 1. A solution of *N*-hydroxy-5-norbornene-2,3-dicarboximide (3.38 g, 18.3 mmol) and anhydrous potassium carbonate (2.53 g, 18.3 mmol) in anhydrous DMF (100 mL) was stirred vigorously for 1 h at room temperature. Then 2,4-difluorobenzaldehyde (1 mL, 9.14 mmol) was added, and the reaction mixture was continued to stir at room temperature. The reaction progress was checked by TLC (silica, 10% EtOAc in CH_2Cl_2) performed after express workup of the reaction mixture aliquots (dilution with water and extraction with CH_2Cl_2). After 48 h TLC showed formation of one major new spot ($R_f = 0.5$, monosubstituted product). The temperature was then slowly raised to 50 °C, and the reaction continued for another 96 h. After removing the solvent in vacuo, the residue was partitioned between saturated aqueous NaCl and CH_2Cl_2. The water layer was additionally extracted with CH_2Cl_2; combined organic layers were washed with brine and dried over Na_2SO_4. Purification by silica chromatography (step gradient from 2% EtOAc in CH_2Cl_2 to 5% EtOAc in CH_2Cl_2) yielded 2.10 g (50%) of TLC-pure **1**. In repeated syntheses the yield increased to 64%. ^{1}H NMR (δ ppm, 300 MHz, $CDCl_3$): 10.45 (s, 1H), 7.86 (d, $J = 8.4$ Hz, 1H), 6.76 (d, $J = 8.4$ Hz, 1H), 6.73 (s, 1H), 6.31 (t, $J = 1.5$ Hz, 2H), 6.28 (t, $J = 1.8$ Hz, 2H), 3.51 (br s, 4H), 3.36 (m, 4H), 1.84 (d, $J = 9.0$ Hz, 2H), 1.58 (d, $J = 9.0$ Hz, 2H). ^{13}C NMR (δ ppm, 75 MHz, DMSO-d_6): 186.4, 171.4, 171.2, 163.1, 160.2, 135.2, 135.1, 132.0, 119.7, 108.2, 99.8, 51.2, 44.2, 42.9. FAB MS: 461.2 ($[M + H]^+$).

(b) Piperidine Derivative 2. To a solution of aldehyde **1** (1.01 g, 2.2 mmol) in $CHCl_3$ (180 mL) was added titanium(IV) isopropoxide (0.913 mL, 3 mmol). After the mixture was stirred for 20 min, piperidine (0.200 mL, 2.0 mmol) was added, and the mixture was refluxed for 30 min and then kept at room temperature for another 30 min, followed by addition of methanol (60 mL) and $NaBH_3CN$ (172 mg, 2.6 mmol, in two portions, in 4 h). After the mixture was stirred for 15 h at room temperature, the solvent was removed, and the residue was partitioned between 200 mL of CH_2Cl_2 and 20 mL of brine and stirred for 1 h. The mixture was then filtered, the aqueous layer was extracted twice more with CH_2Cl_2, combined organic layers were washed with brine and dried over Na_2SO_4. Removal of the solvent followed by silica chromatography (acetone/petroleum ether 1/2 v/v) resulted in 404 mg (38%, increased to 42% in repeated syntheses; further attempts to increase the

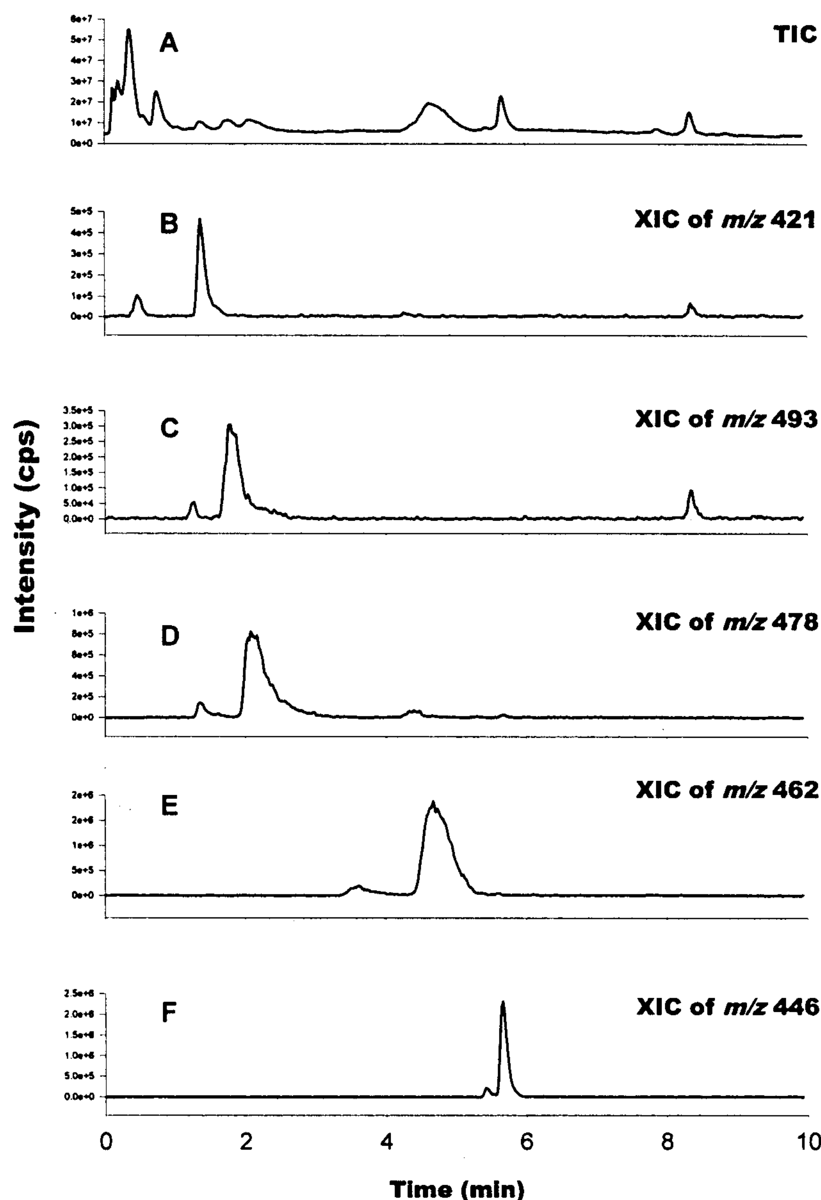


Figure 4. Gradient elution HPLC−MS of sublibrary 15. The panels are (A) the TIC, (B) *m/z* 421, (C) *m/z* 493, (D) *m/z* 478, (E) *m/z* 462, and (F) *m/z* 446.

yield failed because of competing reduction of the aldehyde to alcohol) of **2** (TLC-pure, $R_f = 0.2$, 10% MeOH in CH_2Cl_2). ^{1}H NMR (δ ppm, 300 MHz, $CDCl_3$): 7.43 (d, $J = 8.4$ Hz, 1H), 6.74 (dd, $J = 8.4$ Hz, 2.4 Hz, 1H), 6.65 (d, $J = 2.4$ Hz, 1H), 6.31 (t, $J = 1.8$ Hz, 2H), 6.21 (t, $J = 1.8$ Hz, 2H), 3.70 (s, 2H), 3.46 (br s, 4H), 3.30 (m, 4H), 2.50 (br s, 4H), 1.81−1.77 (m, 2H), 1.63−1.50 (m, 6H), 1.47−1.39 (m, 2H). ^{13}C NMR (δ ppm, 75 MHz, $CDCl_3$): 171.04, 171.02, 158.2, 156.7, 135.3, 135.0, 132.4, 111.0, 102.8, 55.9, 54.0, 51.6, 51.5, 44.9, 43.1, 43.0, 42.9, 29.7, 25.0, 23.5. ESI MS (MeOH): 530.4 ($[M + H]^+$), 562.4 ($[M + MeOH + H]^+$). ESI MS/MS of the M + H ion: 464.2, 368.2, 302.2, 206.2, 162.0, 84.2.

(c) Aminooxy Scaffold 3. Hydrazine monohydrate (50 μL, 1.0 mmol) was added to the solution of **2** (190 mg, 0.36 mmol) in 10% MeOH in $CHCl_3$ (7 mL) and left at room temperature for 12 h. The solvent was then removed, the reaction mixture was then subjected to silica chromatography (2.5% Et_3N in CH_2Cl_2), and 47.5 mg (56%) of product **3** was isolated. ^{1}H NMR (δ ppm, 500 MHz, CD_3OD): 7.38 (d, $J = 2.5$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 1H), 7.74 (dd, $J = 2.5$, 8.5 Hz, 1H), 4.10 (s, 2H), 3.13 (br s, 4H), 1.81 (br s, 4H), 1.62 (br s, 2H). ESI MS (MeOH/water 1/1 v/v): 238.2 ($[M + H]^+$).

Library Formation. The sublibraries were formed in 55 vials, each of which contained 2 out of 11 aldehydes (see detailed description in the Supporting Information) and scaffold **3**. To each of the 55 vials were added two aldehydes (each as 20 μL of 12.7 mM stock solution in methanol), 1% TFA in MeOH (5 μL), and scaffold **3** (10 μL of 25.3 mM stock solution in methanol). The vials were kept for 10 h at room temperature, then the solvent was removed in vacuo, and the mixtures were used for LC−MS analysis.

LC−MS Library Characterization. Samples were kept frozen at −20 °C until prepared. Each sample vial was reconstituted by pipetting 300 μL of methanol with mild shaking to ensure dissolution. The solution was then diluted by pipetting 700 μL of 0.1% of formic acid in water so that

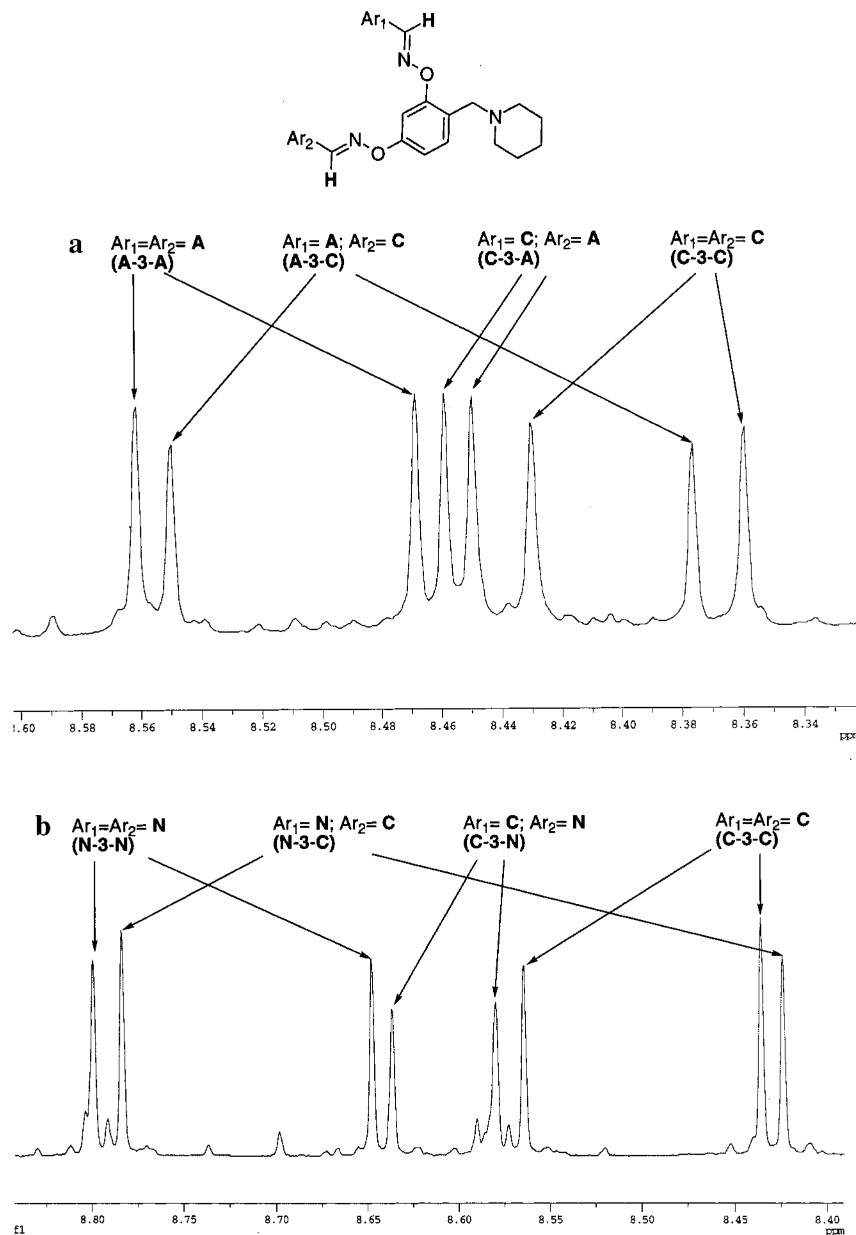


Figure 5. ^{1}H NMR (500 MHz) oxime signal area of the sublibraries formed from (a) **1** (0.0125 M) and aldehydes **A** and **C** (0.0125 M each) in 0.5% TFA/$CDCl_3$; (b) **1** (0.0125 M) and aldehyde **C** and *p*-nitrobenzaldehyde (**N**) (0.0125 M each) in 0.5% TFA/CD_3CN. Peak assignment for the components containing identical two identical substituents was made by comparison with the standard compounds (**A**-**3**-**A** and **C**-**3**-**C**, and **C**-**3**-**C** and **N**-**3**-**N**, for parts a and b, respectively). Assignment for the compounds with two different substituents is tentative.

the sample was in a solution similar to the HPLC mobile phase. Samples were then loaded into the autosampler tray and kept at 4 °C. Each sample was mixed by ultrasonication before injection. The fast HPLC analysis was done with an isocratic mobile phase consisting of 35% acetonitrile:2-propanol (9:1) and 65% of 0.1% formic acid in water. Flow was at 0.4 mL/min with a pressure of 3500 psi. Injection was made with a 5 μL loop filled with 15 μL of sample. The entire eluent was sent to the Turboionspray interface operated at 450 °C with a nitrogen gas flow rate of 7 L/min. The mass spectrometer was operated in positive ion mode with Q1 scan. The analyzer was scanned from 150 to 600 Da at a rate of 0.93 s per scan. All lenses were optimized using sample 3 (molecule AD at *m/z* 444.4). This molecule showed facile fragmentation in the high-pressure region of the API source. To maximize the $[M + H]^+$ signal, orifice, ring, and Q0 were set to low voltages, reducing CID in this region.

Supporting Information Available. Library description, tabulated results of the LC−MS library analysis, and NMR spectra of representative libraries. This material is available free of charge via the Internet at http://pubs.acs.org.

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