Regiochemical Tagging: A New Tool for Structural Characterization of Isomeric Components in Combinatorial Mixtures

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Abstract: In this contribution we present a new combined synthetic and analytical strategy (*regiochemical tagging*) that allows facile determination of complete structure, including substituent position and regiochemistry, of mass-redundant components in complex combinatorial mixtures. The libraries of components (oxime ethers) are formed by the reaction of a mixture of substituents (aldehydes) with a scaffold containing several chemically similar attachment points (aminooxy groups). The structure of the resulting library components can then be determined from a combination of single MS and the tandem (MS/MS) spectra. Determination of the unique isomeric motif for each component is made possible via the following features of library design: (1) part of the scafforld moiety, "transferable group" (the nitrogen atom from the oxime group) is transferred to the substituent during fragmentation in the tandem experiment, (2) transferable groups on the scaffold differ from each other by either isotopic labels or fragmentation energies, and (3) mass-redundant substituents are isotopically labeled to create at least a 2 mass unit difference between them. The components of the resulting library thus become labeled with different mass- and energy tags, which allows for precise regiochemical assignment of the functional group positions on the scaffold and substituents by mass spectrometry. The approach has been used to create and analyze a mixture of 27 isomeric compounds, each containing three boronic acid groups. The combination of the MS and MS/MS spectra of the tagged mixture has yielded a unique and structurally definitive signature of each component. Applications of the regiochemical tagging techniques to rapid synthesis and screening of combinatorial mixtures are discussed.

Introduction

The explosive development of combinatorial chemistry over the past decade has successfully addressed the needs of applied and fundamental research for rapid generation and screening of large numbers of compounds.[1]

In designing a combinatorial library, an investigator always faces the choice of working either with arrays of individual compounds prepared via parallel syntheses or with mixtures generated by one or several one-pot protocols.[1e,2] While mixtures are readily accessible and allow one to build up a vast diversity of compounds for a short time, they have apparent drawbacks, such as difficulty with the identification of active components.

The two most common ways to address the identification problem are tagging techniques[3] and deconvolution strategies.[2a,b,e,3a,4] However, complications arise when a library contains multiple components of the same molecular weight and similar structural features.

One of the most convenient and rapid ways to explore functional diversity space of the mixture-based libraries is via reactions of templates (scaffolds) containing two or more chemically similar attachment points with a variety of substituents (see Scheme 1).[4,5] The resulting "branched" library components cover multiple tiny structural variations, such as isomerism of the functional substituents on the scaffold, that play an important role in the recognition of biomolecular targets.

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Scheme 1
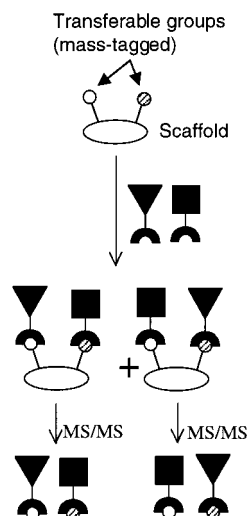
As a tradeoff, these minor differences severely complicate the identification of a specific component regiochemistry.

Combinatorial mixtures are commonly analyzed by mass spectrometry as one of the most sensitive and informative techniques.[2e,6] However, information from the mass spectrum does not generally yield the complete structure of a "branched" library component, such as relative position of substituents on the scaffold, even when coupled to identification of the component fragments via a tandem (MS/MS) experiment.[7]

We introduce here a new combined synthetic/analytical strategy of library design, referred to as *regiochemical tagging*, that allows complete identification of structure, including regiochemistry, of each component by mass spectrometry. Unlike existing tagging techniques, including isotopic mass tagging,[8] our approach is based on labeling building blocks and their position in the resulting components, rather than whole molecules.

Results and Discussion

The basic idea of the regiochemical tagging strategy is outlined in Scheme 1. The library is synthesized by a divergent approach from the central scaffold and substituents attached to it via similar linkages. The main requirement of the linking group is its ability to form fragments that contain one or several atoms transferred from the scaffold to substituent moieties (transferable groups). Such fragmentation may generally result from any chemical reaction, but for analytical purposes, it is

Scheme 2
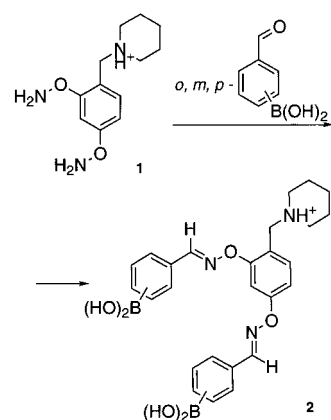
particularly convenient to form the fragments directly in the mass spectrometer by breaking the component molecular ions by the collision-induced dissociation in the tandem MS (MS/MS) experiment.[9] The mass tags, such as isotopes, are introduced into the transferable groups at the stage of the scaffold synthesis. Once the unique tag of each linker is transferred to its substituent upon fragmentation, the substituent fragment will "remember" its attachment point via its specific mass or isotopic distribution. Hence, the substitution motif in the parent compound can be reconstructed from the MS/MS fragment peaks.

This approach has been tested in this work on model mixtures of compounds containing oxime ether linkages. This class of compounds is interesting from several standpoints. Oxime ethers can be easily formed from commercially available or readily synthesized alkoxyamines, in which the ONH_2 groups serve as attachment points and aldehydes serve as substituents. As shown previously, the latter reaction is relatively insensitive to the electronic and steric properties of the building blocks and results in good representation of most of the library components.[10] Finally, oxime ethers may be used in dynamic combinatorial libraries[11] wherein the effective components can be amplified in a combined synthesis−screening process.[12]

As described later, the regiochemical tagging method can be used with scaffolds containing more than two attachment points. However, to test the approach we first synthesized compound **1** containing just two ONH_2 groups. The mixture components were generated by the reaction of scaffold **1** with substituted benzaldehydes. As one of the systems of interest, we used the aldehydes containing boronic acid units in *ortho*, *meta*, and *para* positions (Scheme 2). Boronic acids have recently been a subject of extensive studies in the molecular-recognition community[13] due to their ability to reversibly form cyclic esters with carbohydrate units in aqueous solutions, thereby providing one of the few effective ways to selectively recognize sugars in water.[14] Because the binding strength in such cyclic esters is

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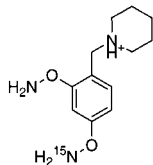
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Scheme 3
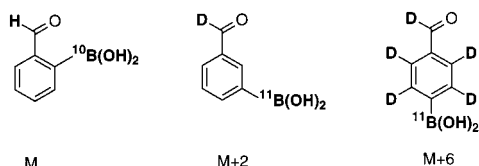
a. **Substituent tagging (MS resolution)**

b. **Scaffold tagging (MS/MS resolution)**

strongly dependent upon relative positions of the boronate moieties, it is likely that in these libraries one can find and isolate by affinity selection new specific binders for certain carbohydrate motifs. This approach may be used for fine-tuning recognition properties of the boronate-based artificial receptors.

The reaction between scaffold **1** and three isomeric boronic acids results in the formation of nine isomeric compounds **2** that offer a double challenge from the analysis standpoint. Not only are all components isobaric (i.e., have the same mass), and therefore show up as a single peak in the MS spectrum, but also, due to the isomerism of the starting aldehydes, the molecular fragments that form upon fragmentation of **2** in a collision-induced dissociation are isobaric as well. Thus, the combination of MS and MS/MS analysis is incapable of detecting each individual component in the mixture and identifying structure of an individual component.

To address the problem of the substituent mass-redundancy, we labeled the corresponding aldehydes with the isotopes of hydrogen and boron to introduce the mass-difference of at least 2 units between the isomers (Scheme 3a). The labeled aldehydes were synthesized from commercially available enriched precursors (see Supporting Information). The mass spectrum of the library formed by the reaction of **1** with the labeled aldehydes would thus contain six molecular peaks (three of them for individual components and three for pairs of isomers, see Figure 3). The above labeling scheme resulted in a mass unit difference of at least 2 between the molecular peaks, which allowed us to avoid their interference with the intense M + 1 isotopic peaks to simplify the subsequent MS/MS analysis. The interference with the M + 2 peaks, which are significantly smaller, results only in minor extra signals present in many MS/MS spectra.

The labeling of the substituents yields no information about their position on the scaffold, that is, about isomerism of compounds **2**. To address this issue, we introduced an isotopic label in one of the aminooxy groups (Scheme 3b).

The sequence of reactions used for the scaffold synthesis (Scheme 4) allowed us to introduce the two aminooxy groups in separate steps. The group in the *para* position could thus be labeled with the ^{15}N isotope. Upon propagation of the labeled scaffold **7** to the library components **C1**−**C9**, the substituents in each component become attached to the nitrogen atoms with different masses. Preliminary experiments showed that the collision-induced dissociation of molecular peaks of a variety of oxime ethers in the MS/MS experiment consistently yielded fragments resulting from the breakage of the O−N bond, as one of the weakest bonds in the molecule. Corresponding fragments obtained from each component of the regiochemically

tagged library would yield different masses for the substituents cleaving from the *ortho* and *para* positions.

The overall labeling approach is thus designed to provide double resolution of the library mass spectra: resolution in the single MS spectrum, via the substituent tagging, and MS/MS resolution, via the scaffold regiochemical tagging (Scheme 3). In this way, the combination of MS and MS/MS analysis of the labeled library should show a unique trace of each component in the mixture, from which complete isomeric motif of each component could be determined.
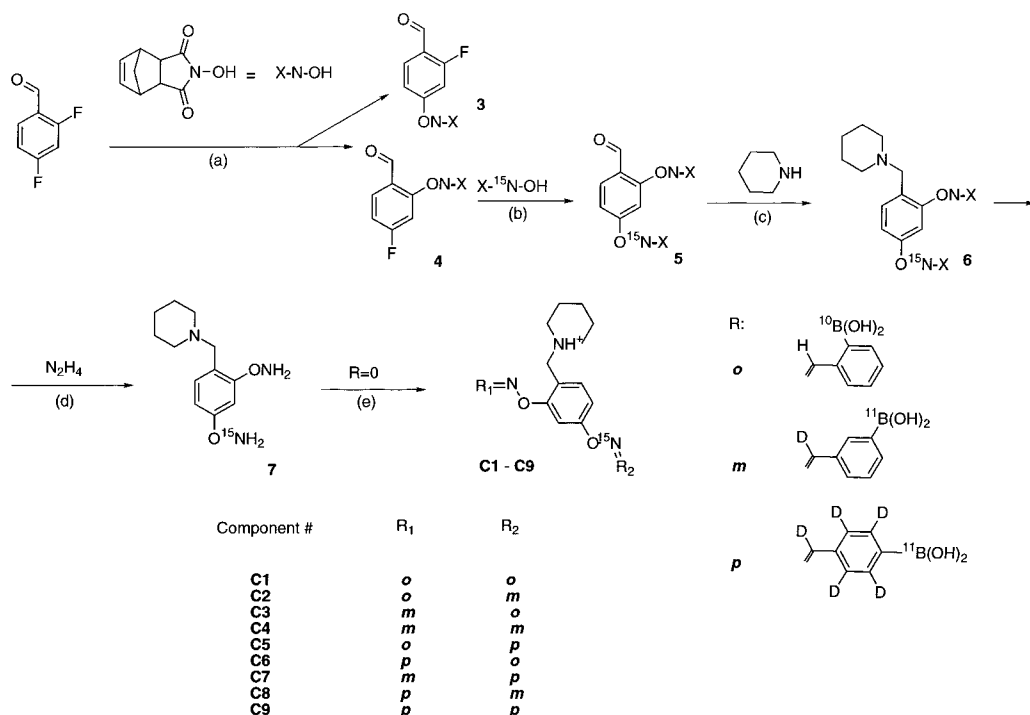
To verify the fragmentation pattern of the library components and provide references for the MS/MS fragments, we first tested a number of individual compounds, such as *o*-[15]N-**8** (Figure 1) and the standards of library components **C1**, **C4**, and **C9** (Scheme 4). As expected, the MS/MS spectrum of the molecular peak of compound *o*-[15]N-**8** showed major fragments at 353.2 and 354.2 atomic mass units (amu) (Figure 1) that one could assign to the cleavage of the ArCHN fragments from the labeled and unlabeled positions on the scaffold, respectively.

However, it came as a surprise that in the MS/MS spectrum of *p*-[15]N-**8**, a similar compound based on the oppositely labeled scaffold, the corresponding fragments were shifted by 1 amu, as if they both had been derived from the cleavage of a lighter substituent. Furthermore, we examined the MS/MS of the residual peak of an unlabeled compound that was present in small amounts in the single MS of *o*-[15]N-**8**. It showed a pattern similar to that of *o*-[15]N-**8**, with major fragment peaks at 353.2 and 354.2. The latter two observations pointed at the possibility of an alternative fragmentation mechanism, perhaps, involving the breakage of the N−O bond only in the *ortho* position to the alkylamino substituent. This mechanism was further elucidated from the spectra of compounds **C4** and **C9** (Figure 2).

The molecular peaks of both **C4** and **C9** (505.2 and 513.2, respectively) fragmented at 10 eV to yield two major peaks in the MS/MS spectrum. As with compounds *p*-[15]N-**8** and *o*-[15]N-**8**, the lower-mass fragment corresponded in each case to the cleavage of the O−N bond in the *ortho* position. However, the mass unit difference between the lower- and higher-mass fragments was 2 amu, as opposed to 1 amu in the non-deuterated compounds. The QqTOF instrument used for the mass measurement yielded the precise mass difference of 2.014 between the two fragments. This value matches with the mass of one deuterium atom and most probably corresponds to partial transfer of the imine hydrogen to the scaffold during the fragmentation at the *ortho*-N−O bond. The precise mass measurement also allowed us to eliminate other fragment combinations that would yield similar mass differences (such as the (D + N15) − (H + N14) = 2.003 that might emerge from some cleavage pattern from both *ortho* and *para* positions).

All of the above observations indicate that the O−N bond in the ortho position to the aminoalkyl group undergoes fragmentation at significantly lower energy than the one in *para* position *and* that its fragmentation is accompanied by an unusual hydrogen transfer process from the imine group of the substituent to the scaffold. Because the probability of any intermolecular assistance to the fragmentation reaction in the gas phase is low, the only difference in the environment of both groups is the presence of the alkylammonium group next to the N−O bond that breaks more readily. It is therefore reasonable to assume that the cleavage is assisted by the ammonium group, for example via the six-membered transition state shown in Scheme 5. This mechanism also accounts for the partial transfer of the imine proton/deuterium. The degree of this transfer decreases at higher fragmentation energies (see Figure 1). The possibility

Scheme 4

Component #	R_1	R_2
C1	*o*	*o*
C2	*o*	*m*
C3	*m*	*o*
C4	*m*	*m*
C5	*o*	*p*
C6	*p*	*o*
C7	*m*	*p*
C8	*p*	*m*
C9	*p*	*p*

(a) K_2CO_3, DMF, rt, 86% total; (b) K_2CO_3, DMF, rt, 55%; (c) Ti(O-i-Pr)$_4$, NaBH$_3$CN, CHCl$_3$/MeOH, 46%; (d) N$_2$H$_4$·H$_2$O, MeOH/CHCl$_3$, 64%; (e) 1% TFA in DMSO.

also exists that the preferred fragmentation in the *ortho* position is attributable to steric effects of the neighboring group. We are currently performing control experiments with some structural analogues to verify the transfer mechanism.

The relative stability of the *para*-substituent indicates that the unassisted collision-induced dissociation of the N—O bond requires intrinsically higher energies. This is indirectly supported by the MS/MS of the protected scaffold **6** in which noticeable fragments have been detected only at the energies of 30 eV and above. It is also probable that the breakage of the *p*-O—N bond is cooperative with the *ortho*, because the peak at 208.1, corresponding to cleavage of both substituents, was always observed in the MS/MS spectrum.

Overall, the experiments with the individual compounds revealed an unexpected fragmentation mechanism and considerable difference in the stability of the *ortho*- and *para*-substituent—scaffold linkages. As a result, introduction of the labeled nitrogen into scaffold **7** did not yield the required regiochemical information. Importantly, however, the originally stated goal to detect and identify every single component in the mixture by the MS analysis could be achieved, due to the different stability of the N—O linkers. The molecular peak cluster of the library of nine compounds (Figure 3) showed all six expected peaks, three of which represented pairs of two different components. The MS/MS of these molecular ions yielded distinct traces of each individual component, identifiable as the fragments resulting from the *ortho* cleavage.

The unexpected observation of different O—N bond fragmentation energies prompted us to design a new regiochemical tagging scheme that was implemented in a trisubstituted scaffold **12** (Scheme 6). Compound **12** contains two aminooxy groups in the *ortho* position to the central nitrogen atom, which were expected to fragment at similar energies, and one aminooxy group in the *para* position, which should not show significant fragmentation. To distinguish between the fragments resulting from different *ortho* groups, one of them was labeled with the

[15]N isotope. Thus, one of the attachment points on the scaffold bore the "energy" tag, while the other one was labeled with a mass tag that made it different from the third, untagged point.

The MS/MS spectrum of an individual standard compound **CT8** (see Table 1) based on the new scaffold indeed showed the expected fragmentation pattern (Figure 4a). Its comparison with the unlabeled analogue (Figure 4b) proved that one of the fragments resulted from the cleavage of the ^{15}N-labeled position.

The reaction of scaffold **12** with the three labeled formyl boronic acids resulted in the formation of a mixture library of 27 components listed in Table 1. Because of the molecular mass redundancy, the library includes nine different masses, all of which were detected in the molecular cluster in the single MS spectrum (Figure 5). Again, the component molecular peaks here differ by at least 2 mass units, which helps avoid their interference with the M + 1 molecular peaks of the closest neighbors.

Each of the molecular ions was characterized by its MS/MS spectrum. We chose to perform fragmentation at 20 eV to minimize the peaks resulting from the proton transfer to the scaffold, as discussed above. The results shown in Figure 5 represent an error-free proof of the tagging strategy design in that all 36 actually observed MS/MS peaks were predicted on the basis of library structure. Combination of the MS and MS/MS spectra obtained from a single injection has thus provided unique signature of each of the 27 components that, unlabeled, are all isomers and yield nearly identical spectra!

The most complex peak in the molecular cluster of the **CT** library corresponds to six isomeric components. In the MS/MS spectrum of that and some other peaks, the signals originating from different components are sometimes overlapped. However, if each component were injected individually, its unique signature would unambiguously determine its isomeric motif and distinguish it from other 26 isomers.

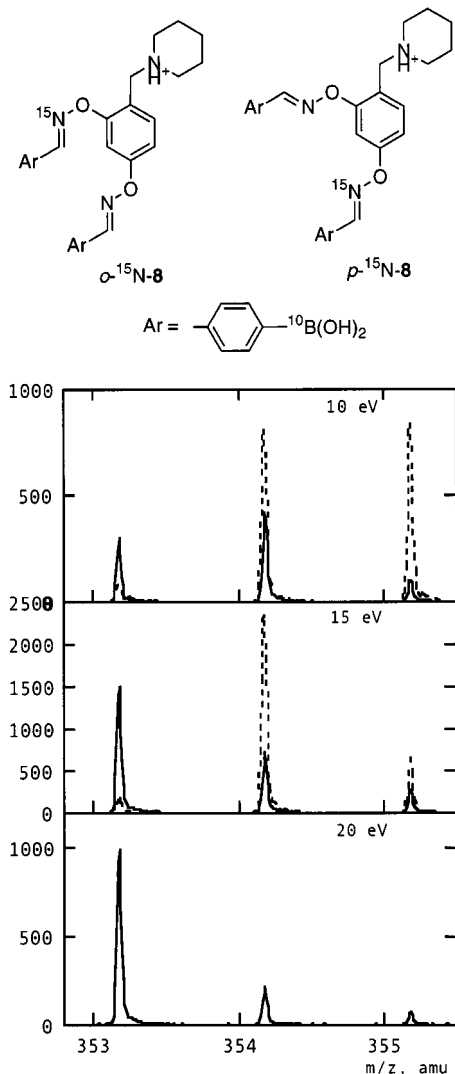This study concentrated primarily on the boronic acid-containing substituents, as those that allowed us to push the

Figure 1. Segments of the MS/MS spectra corresponding to the N−O bond cleavage in the molecular ions of *o*-[15]N-**8** (solid lines) and *p*-[15]N-**8** (dashed lines) at various energies.

Figure 2. Single MS spectrum of the molecular ion clusters and MS/MS spectra (10 eV) of individually injected **C4** (a, b) and **C9** (c, d).

limits of our approach applicability for structural characterization of very similar compounds in their mixtures. Apparently, the regiochemical tagging schemes can be used for a variety of substituents. We briefly tested several libraries formed from scaffold **7** and *p*-hydroxy and *p*-nitrobenzaldehydes, as well as the formyl boronic acids, and observed all component peaks and their easily identifiable fragments in the MS/MS spectra. This indicates that the method can be used in libraries formed from aldehydes with varying electronic properties. It is essential for unambiguous structure determination of a particular component based on scaffold **12** that the mass difference between closest substituents be at least 2 amu, as it was implemented in the labeled formyl boronic acids. In this case, the combination of the MS and MS/MS spectra is unique for each component.

Concluding Remarks

The method of regiochemical tagging offers a unique approach to complete structural characterization of specially designed combinatorial mixture components, which would be difficult or impossible to achieve through other techniques. The combination of the tagging strategy with the facile mixture generation from the scaffold and substituent building blocks can be very useful for rapid screening of potentially very diverse libraries for binding to a particular target. A complete isomeric
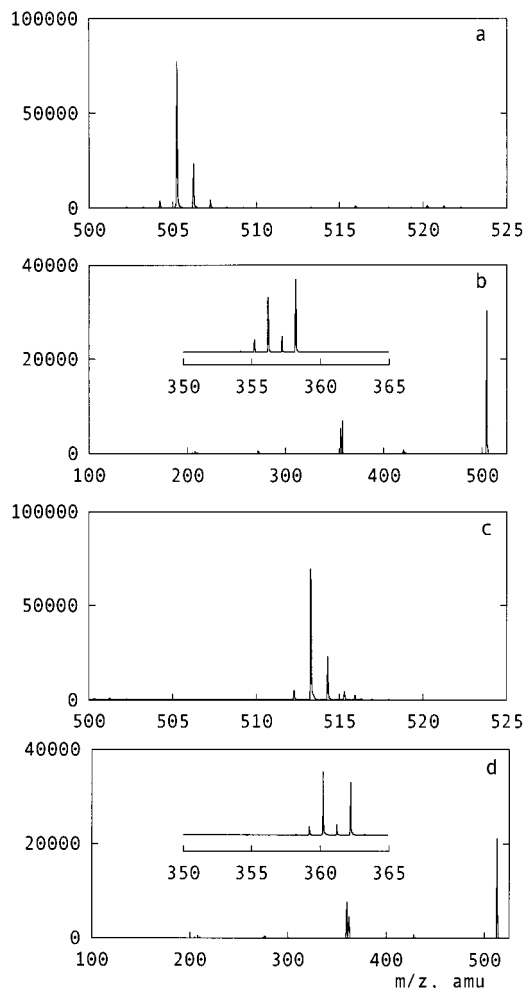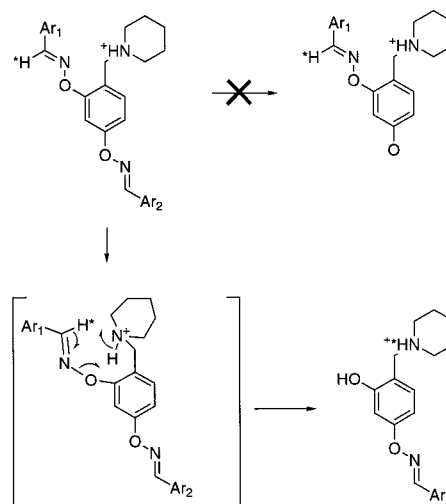
Scheme 5

motif of the strong binders isolated, for example, by affinity selection[15] can be determined by single injection in the mass spectrometer. The method could thus be used for rapid geometric and functional mapping of the target binding sites.

The fact that only isotopes are used as labels simplifies the synthetic part of the technique, which essentially only requires

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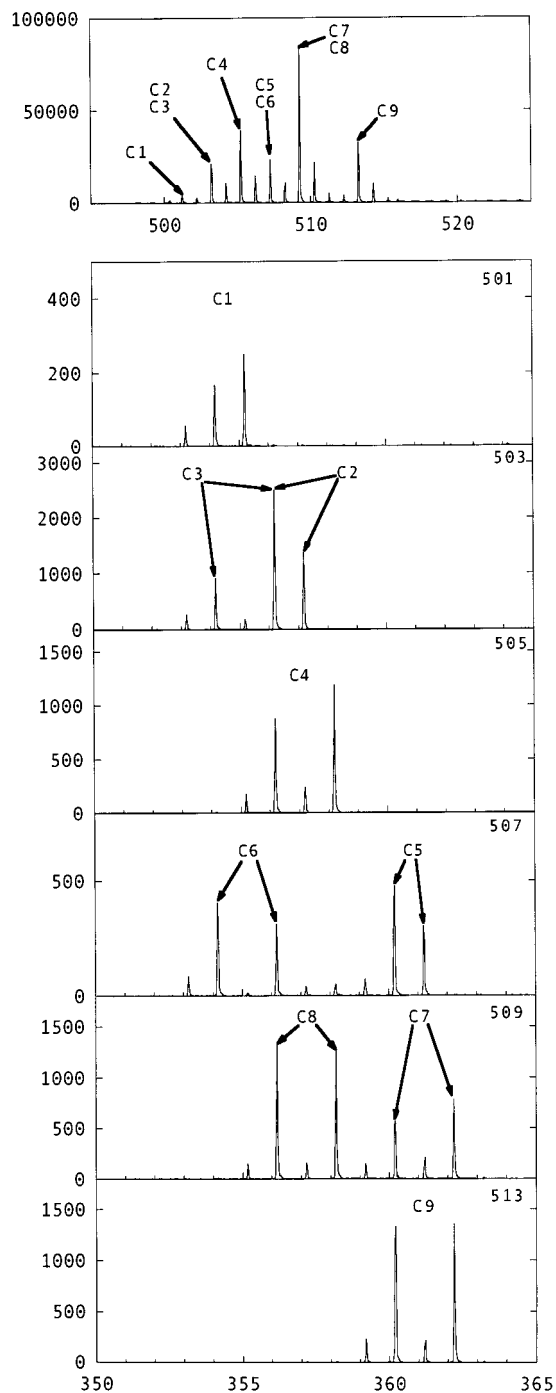
Figure 3. Single MS spectrum of the molecular ion cluster (top) and MS/MS spectra (10 eV) of the molecular ions (marked in the right top corners) of the mixture of components **C1**−**C9**.

repetition of known synthesis with the labeled units. It is important that the tagged components fully retain all chemical properties of their untagged analogues.

One can easily visualize further various modifications and extensions of the principal regiochemical tagging scheme. For example, it may first appear that the number of "mass-taggable" positions in the scaffold is limited by the number of available stable isotopes. However, mixtures of isotopes can be entered in the tagged positions (e.g., as 50:50 ^{14}N:^{15}N, or in different ratios) to label multiple attachment points with unique *fractions* of the mass tags. Likewise, different scaffold-substituent linkers can be explored that follow the transfer-fragmentation rule depicted in Scheme 1.

Scheme 6
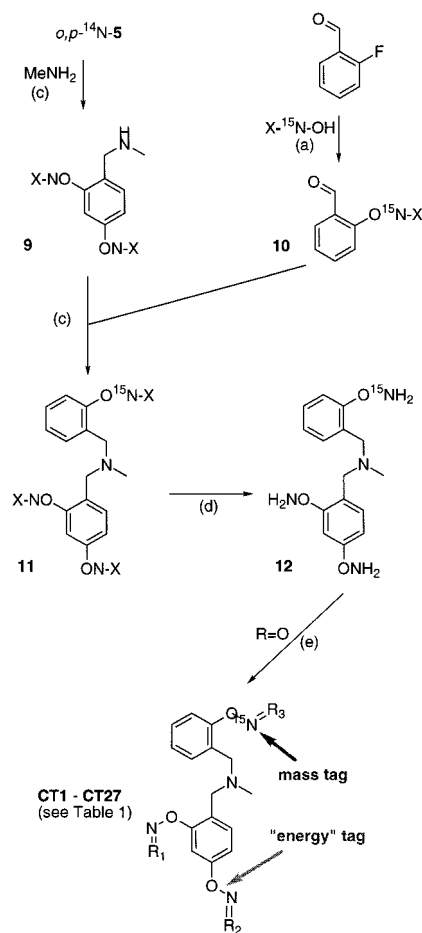
(a) K_2CO_3, DMF, rt, 86% total; (b) K_2CO_3, DMF, rt, 55%; (c) Ti(O-i-Pr)$_4$, NaBH$_3$CN, CHCl$_3$/MeOH, 46%; (d) N$_2$H$_4$·H$_2$O, MeOH/CHCl$_3$, 64%; (e) 1% TFA in DMSO.

Experimental Section

Mass Spectrometry. Most of the experiments were performed on a prototype of a tandem quadrupole time-of-flight mass spectrometer (QqTOF) built at SCIEX.[16] The high resolution and mass accuracy of the QqTOF instrument makes it possible to remove many ambiguities in isotopic labeling. In brief, the instrument consists of four main parts: an electrospray ion source, a quadrupole mass filter Q1, a collision cell Q2 (with argon as collision gas), and a time-of-flight (TOF) mass analyzer with orthogonal injection of ions. In single MS mode of operation, Q1 is operated in radio frequency (rf)-only (non-analyzing) mode, and precursor ions are recorded by TOF. In MS/MS mode, precursors of interest are mass-selected with unit resolution in Q1 and fragmented in Q2, and fragment ions are recorded by TOF. Mass resolution of TOF is ∼10 000 (full width at half-maximum definition). Typical mass accuracy is ∼20 ppm with external calibration (when calibrated in a separate experiment), and 3 ppm with internal calibration (when calibrated with the known peaks in the same spectrum).

Solutions were prepared in water/acetonitrile (75:25) with sample concentration ranging from 1 to 5 μM, and injected into the mass spectrometer at a flow rate of 1 μL/min. Typically, a single MS spectrum was recorded in 30 s, and the MS/MS spectrum was recorded in 1−3 min.

Some spectra of precursors and individual compounds were acquired on an API III Plus triple quadrupole mass spectrometer (PE Sciex, Thornhill, Canada) fitted with an articulated pneumatically assisted nebulization probe. The spectrometer was operated at unit resolution

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Table 1. Molecular Weights of Library Components Based on Scaffold **12** and Their Key Fragments

component no.	$R_1{}^a$	$R_2{}^a$	$R_3{}^a$	M^b	$M-R_1N$	$M-R_3{}^{15}N$
CT1	o	o	o	699.2	552.2	551.2
CT2	o	o	m	701.2	554.2	551.2
CT3	m	o	o	701.2	552.2	553.2
CT4	o	m	o	701.2	554.2	553.2
CT5	m	o	m	703.2	554.2	553.2
CT6	o	m	m	703.2	556.2	553.2
CT7	m	m	o	703.2	554.2	555.2
CT8	m	m	m	705.2	556.2	555.2
CT9	o	o	p	705.2	558.2	551.2
CT10	p	o	o	705.2	552.2	557.2
CT11	o	p	o	705.2	558.2	557.2
CT12	o	m	p	707.2	560.2	553.2
CT13	o	p	m	707.2	560.2	557.2
CT14	m	o	p	707.2	558.2	553.2
CT15	m	p	o	707.2	558.2	559.2
CT16	p	o	m	707.2	554.2	557.2
CT17	p	m	o	707.2	554.2	559.2
CT18	m	m	p	709.2	560.2	555.2
CT19	m	p	m	709.2	560.2	559.2
CT20	p	m	m	709.2	556.2	559.2
CT21	o	p	p	711.2	564.2	557.2
CT22	p	o	p	711.2	558.2	557.2
CT23	p	p	o	711.2	558.2	563.2
CT24	m	p	p	713.2	564.2	559.2
CT25	p	m	p	713.2	560.2	559.2
CT26	p	p	m	713.2	560.2	563.2
CT27	p	p	p	717.2	564.2	563.2

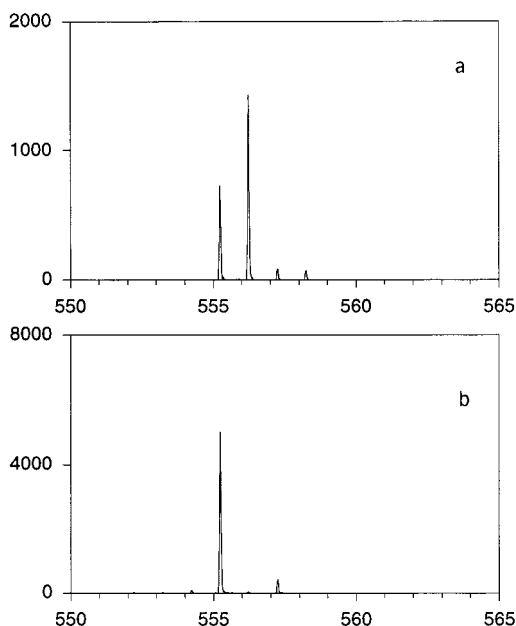*a* See notation in Scheme 3. *b* Protonated ion.

Figure 4. Segments of the MS/MS spectra corresponding to the N–O bond cleavage in the molecular ions of individually injected **CT8** (**a**, parent ion 705.2) and its unlabeled analogue (**b**, parent ion 704.2) at 20 eV.

(50% valley definition) over the mass range m/z 50–2400. Samples were introduced into the electrospray ionization source at a flow rate of 5 μL/min with a Harvard syringe pump. The electrospray needle was operated at 4.8 kV, the orifice voltage was set at 55 V, and nitrogen was used as the nebulization gas. Full scan mass spectra were acquired over the mass range m/z 200–1500 by scanning the thirst mass spectrometer, Q1, using a m/z 0.2 step size and a 1 ms dwell time. Product ion mass spectra (MS/MS) were acquired by colliding the Q1 selected precursor ion with argon gas (99.999%) at a collision target gas thickness of 9×10^{14} molecules/cm² in Q2 operated in rf-only mode and scanning the second mass spectrometer, Q3, from m/z 15 to just above the mass of the precursor ion.

Synthesis. All commercial reagents were purchased from Aldrich, except for the ¹⁵N-labeled hydroxylamine that was obtained from Cambridge Isotope Laboratories. NMR spectra were recorded on Varian Unity 300, 400, and 500 MHz instruments and processed using the original manufacturer software or the SwanMR shareware for Macintosh.[17] FAB MS spectra were recorded on a VG Analytical 70-SE instrument.

***endo*-¹⁵N-Hydroxy-5-norbornene-2,3-dicarboximide (X-¹⁵N-OH, Scheme 4).** The compound was synthesized in a modification of previously described procedure.[18] *cis*-5-Norbornene-*endo*-2,3-dicarboxylic anhydride (1.307 g, 7.72 mmol) was added to a solution of 0.495 g (7.02 mmol) of hydroxylamine-¹⁵N·HCl (98% ¹⁵N) in 5 mL of water, and the mixture was heated at 60 °C for 3 h. After the mixture cooled to room temperature, the pH was adjusted to 4.0 with glacial acetic acid. After removal of the solvent, purification on a silica gel column (10% MeOH/CH₂Cl₂) yielded 1.153 g (91%) of the product. ¹H NMR (δ ppm, 500 MHz, DMSO-d_6) 10.76 (br s, 1H), 6.05 (t, J = 1.5 Hz, 2H), 3.25(m, 4H), 1.58 (d, J = 10 Hz, 1H), 1.5 (d, J = 10 Hz, 1H). ¹³C NMR (δ ppm, 125 MHz, DMSO-d_6) 172.9 (d, J = 11.9 Hz), 134.4, 51.0 (d, J = 1.4 Hz), 43.8, 42.1 (d, J = 8.3 Hz). FAB MS: 181.2 ([M + H]⁺).

Aldehydes 3 and 4. A solution of *endo-N*-hydroxy-5-norbornene-2,3-dicarboximide (1.69 g, 9.14 mmol) and anhydrous potassium carbonate (1.27 g, 9.14 mmol) in anhydrous DMF (25 mL) was stirred for 30 min at room temperature. Then 2,4-difluorobenzaldehyde (1.00 mL, 9.14 mmol) was added. The reaction progress was monitored by TLC (silica gel, 25% acetone in petroleum ether) performed after express workup of the reaction mixture aliquots (dilution with water and extraction with CH₂Cl₂). After 48 h, TLC showed completion of the reaction and formation of two spots having very close R_f values (0.27 and 0.20, 20% acetone/petroleum ether). Crude NMR showed the presence of **3** and **4** in the ratio of 78/22. The residue was partitioned between saturated aqueous NaCl and CH₂Cl₂. The water layer was additionally extracted with CH₂Cl₂, and the combined organic layers were washed with brine and dried over Na₂SO₄. After the solvent was removed in vacuo, the two isomers were separated on silica gel (20% acetone in petroleum ether) to give 86% overall yield. Minor isomer (**4**): ¹H NMR (δ ppm, 300 MHz, CDCl₃) 10.44 (s, 1H), 7.91 (dd, J_1 = 6.6 Hz, J_2 = 8.7 Hz, 1H), 6.91 (td, J_1 = 2.4 Hz, J_2 = 8.2 Hz, 1H), 6.66 (dd, J_1 = 2.4 Hz, J_2 = 9.6 Hz, 1H), 6.32 (t, J = 1.8 Hz, 2H), 3.52 (br s, 2H), 3.40(m, 2H), 1.85 (dt, J_1 = 1.5 Hz, J_2 = 9.0 Hz, 1H), 1.58 (d, J = 9.0 Hz, 1H). ¹³C NMR (δ ppm, 75 MHz, CDCl₃) 186.20, 170.54, 168.60, 165.17, 161.0, 135.37, 131.08, 130.94, 121.2, 112.53, 112.24, 102.49, 102.12, 51.66, 44.93, 43.16). Major isomer (**3**): ¹H NMR (δ ppm, 500 MHz, CDCl₃) 10.22 (s, 1H), 7.85 (dd, J_1 = 7.5 Hz, J_2 = 8.5 Hz, 1H), 6.89 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H), 6.78 (dd, J_1 = 2.5 Hz, J_2 = 11 Hz, 1H), 6.33(t, J = 2.0 Hz), 3.52 (br, s, 2H), 3.42 (m, 2H), 1.86(d, J = 9.0 Hz, 1H), 1.60 (d, J = 9.0 Hz). ¹³C NMR(δ ppm, 125 MHz, CDCl₃) 185.46, 185.41, 170.33, 166.44, 164.37, 163.12, 163.03, 135.26, 130.48, 130.45, 120.63, 120.56, 110.24, 110.21, 102.25, 102.04, 51.58, 44.88, 43.15).

Protected 2,4-¹⁵N-Diaminooxy Benzaldehyde 5. A solution of *endo*-¹⁵N-hydroxy-5-norbornene-2,3-dicarboximide (180 mg, 1.0 mmol) and anhydrous potassium carbonate (138 mg, 1.0 mmol) in anhydrous DMF (20 mL) was stirred for 30 min at room temperature, and then **4** (603 mg, 2.0 mmol) was added. After the solution stirred for 4 days, the residue was partitioned between saturated aqueous NaCl and CH₂Cl₂. The water layer was extracted two more times with CH₂Cl₂. The combined organic layers were washed with brine and dried over Na₂SO₄. After removal of the solvent in vacuo, purification by silica gel chromatography (step gradient from 1% EtOAc in CH₂Cl₂ to 5% EtOAc in CH₂Cl₂) yielded 253.8 mg (55%) of TLC-pure **5**. ¹H NMR was the same as for the nonlabeled analogue.[10] ¹³C NMR (δ ppm, 125 MHz, DMSO-d_6): 186.27, 171.24, 171.08 (d, J = 8.8 Hz), 162.94, 160.12, 135.12, 134.98, 131.92, 119.65, 108.12, 99.70, 51.17, 44.26, 44.23, 42.89. ¹⁵N NMR (δ ppm, 51 MHz, DMSO-d_6, formamide in DMSO-d_6 = 90.0 ppm) 201.29. FAB MS: 462.1 ([M + H]⁺).

Piperidine Derivative 6. The compound was synthesized as the nonlabeled analog[10] and showed identical ¹H NMR. Yield 48%. ¹³C

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(18) Bauer, L.; Miarka, S. V. *J. Org. Chem.* **1959**, *24*, 1293–1296.
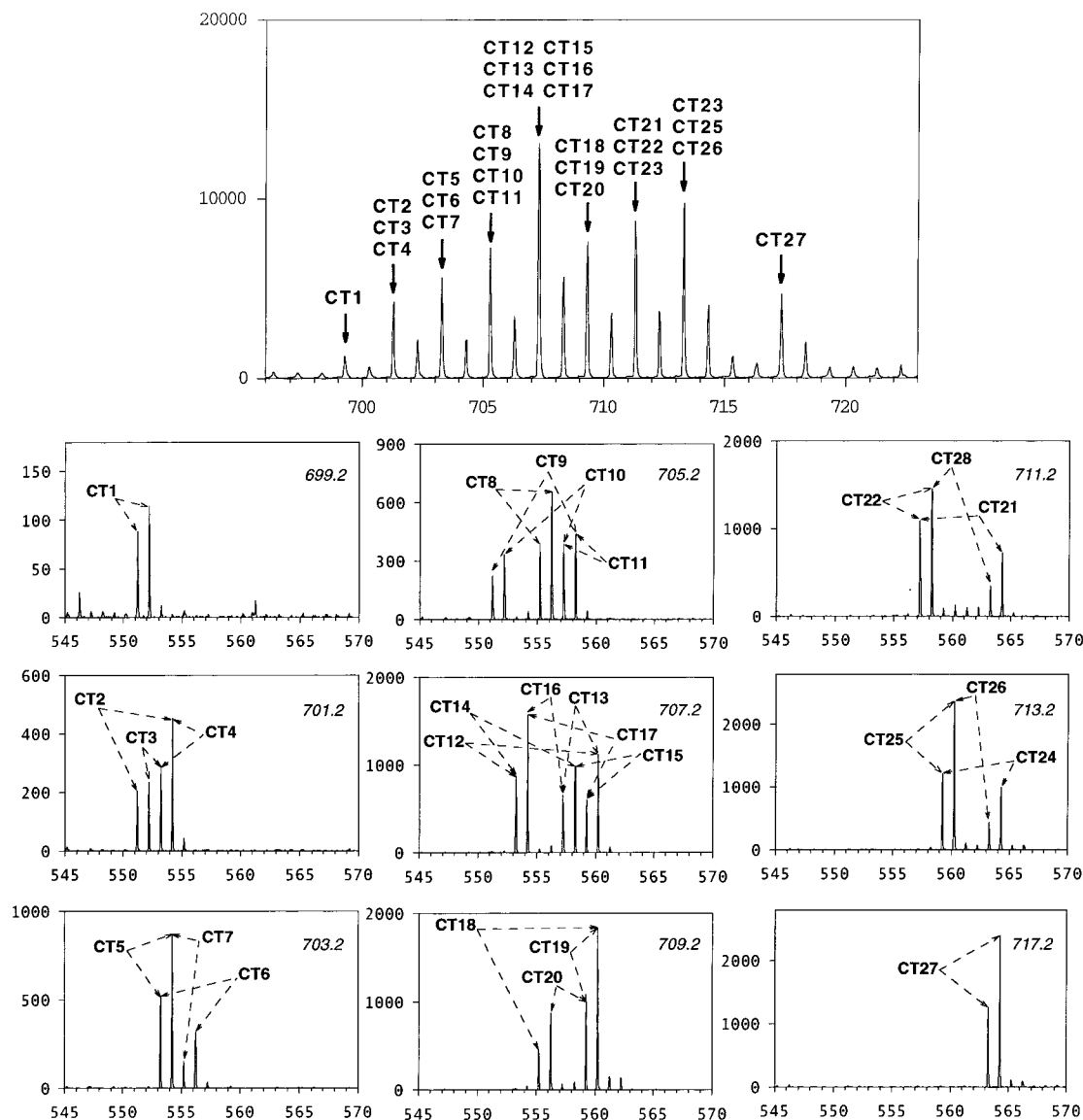
Figure 5. Single MS spectrum of the molecular ion cluster (top) and MS/MS spectra (20 eV) of the molecular ions (marked in the right top corners) of the mixture of components **CT1 − CT27**.

NMR (δ ppm, 125 MHz, CDCl$_3$) 171.13 (d, J = 5 Hz), 170.98, 157.41, 156.41, 135.16, 134.88, 131.30, 123.68, 111.08, 102.75, 55.39, 54.29, 51.44, 51.35, 44.77, 43.00, 42.88, 42.81, 29.17, 25.87, 24.14. ESI MS (CH$_3$CN/water) 531.2 ([M + H]$^+$). ESI MS/MS of the M + H ion: 465.2, 369.2.

Aminooxy Scaffold 7. The compound was synthesized from **6** using the previously described deprotection procedure, and the ^{1}H NMR was identical to that of the nonlabeled analogue.[10] Yield 64%. ESI MS (CH$_3$-CN/water) 239.2 ([M + H]$^+$).

Synthesis and analytical data for the oppositely labeled standard compound *o*-[15]N-**8** as well as for the labeled formyl boronic acids are given in the Supporting Information.

Amine 9. To a solution of the unlabeled aldehyde **5** (1.01 g, 2.2 mmol) in CHCl$_3$ (150 mL) was added titanium(IV) isopropoxide (1.24 mL, 4.0 mmol). After the mixture stirred for 20 min, methylamine hydrochloride (135 mg, 2.0 mmol was added. The mixture was refluxed for 30 min and then kept at room temperature for another 30 min, followed by addition of methanol (50 mL) and NaBH$_3$CN (800 mg, 6.0 mmol, in two portions within 1 h). The solvent was then removed, and the residue was partitioned between 200 mL of CH$_2$Cl$_2$ and 50 mL of brine and stirred for 1 h. The mixture was then filtered, the aqueous layer was extracted twice more with CH$_2$Cl$_2$, and the combined organic layers were washed with brine and dried over Na$_2$SO$_4$. Removal of the solvent followed by silica gel chromatography (step gradient

from 1% MeOH in CH$_2$Cl$_2$ to 20% MeOH in CH$_2$Cl$_2$) resulted in 425.4 mg (45%) of **9**. ^{1}H NMR (δ ppm, 500 MHz, CDCl$_3$) 7.37 (d, J = 8.5 Hz, 1H), 6.80 (dd, J_1 = 2.5 Hz, J_2 = 8.0, 1H), 6.74 (d, J = 2.5 Hz, 1H), 6.33 (t, J = 2.0 Hz, 2H), 6.25 (t, J = 1.8 Hz, 2H), 3.98 (s, 2H), 3.50 (br. m, 4H), 3.35 (dm, J = 14.5 Hz, 4H), 2.49 (s, 3H), 1.84 (tm, J = 12.5 Hz, 2H), 1.58 (t, J = 9.0 Hz, 2H). ^{13}C NMR (δ ppm, 75 MHz, CDCl$_3$) 171.54, 170.93, 159.13, 156.87, 135.05, 134.91, 133.19, 117.54, 110.90, 103.26, 51.32, 50.17, 46.42, 44.65, 42.85, 32.65.

Protected 2[15]N−Aminooxy Benzaldehyde 10. A solution of *endo*-^{15}N-hydroxy-5-norbornene-2,3-dicarboximide (360 mg, 2.0 mmol) and anhydrous potassium carbonate (276 mg, 2.0 mmol) in anhydrous DMF (10 mL) was stirred for 30 min at room temperature. Then 2-fluorobenzaldehyde (652 µL, 6.0 mmol) was added, and the reaction mixture was stirred at 65 °C for 48 h. The reaction progress was monitored by TLC (silica gel, 5% EtOAc in CH$_2$Cl$_2$) performed after express workup of the reaction mixture aliquots (dilution with water and extraction with CH$_2$Cl$_2$). The pure product was obtained in 62% yield using the same workup procedure as for **4**. ^{1}H NMR (δ ppm, 500 MHz, CDCl$_3$) 10.57 (s, 1H), 7.86 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 7.52 (dt, J_1 = 1.5 Hz J_2 = 8.0 Hz, 1H), 7.21 (dt, J_1 = 1.0 Hz, J_2 = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H) 6.27 (t, J = 2.0 Hz, 2H), 3.49 (m, 2H), 3.38(m, 2H), 1.81 (dd, J_1 = 1.5 Hz, J_2 = 9.0 Hz, 1H), 1.56 (d, J = 9.5 Hz, 1H). ^{13}C NMR (δ ppm, 125 MHz, CDCl$_3$) 187.68, 170.80 (d, J = 9.1 Hz),

159.59, 135.41, 135.02, 128.30, 124.82,124.60, 114.41, 51.49, 44.80, 43.02, 42.95. FAB MS: 285.3 ([M + H]$^+$).

Protected Triaminooxy Amine 11. The compound was synthesized from **9** and **10** using the same reductive amination procedure as for **9**. Yield 45%. ^{1}H NMR (δ ppm, 500 MHz, CDCl$_3$) 7.51 (d, J = 8.5 Hz, 2H), 7.15 (t, J = 7.8 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.78 (td, J_1 = 1.0 Hz, J_2 = 8.0 Hz, 2H) 6.68 (t, J = 2.0 Hz, 1H), 6.33 (d, J = 1.5 Hz, 2H), 6.27 (d, J = 1.5 Hz, 2H),), 6.22 (d, J = 1.5 Hz, 2H), 3.79 (s, 2H), 3.74 (s, 2H), 3.47 (br, s, 6H), 3.31 (m, 6H), 2.25 (s, 3H), 1.79 (t, J = 8.5 Hz, 3H), 1.53 (t, J = 9.5 Hz, 3H). ^{13}C NMR (δ ppm, 125 MHz, CDCl$_3$) 171.19 (d, J = 9.5 Hz), 171.09, 170.92 157.41, 156.30, 155.95, 135.21, 135.15, 135.02, 134.89, 131.18, 130.49, 127.68, 127.23, 124.30, 113.12, 111.16, 102.76, 54.86, 54.22, 53.42, 51.52, 51.44, 51.38, 44.78, 44.75, 43.04, 43.00, 42.97, 42.87, 42.27. ESI MS (CH$_3$CN/water 1/3 v/v) 744.3 ([M + H]$^+$).

Scaffold 12. The compound was synthesized from **11** by using the previously described deprotection procedure.[10] Yield 55%. ^{1}H NMR (δ ppm, 400 MHz, 1%D$_2$O/CD$_3$CN v/v) 7.39 (dd, J_1 = 1.2 Hz, J_2 = 6.8 Hz, 1H), 7.25−7.30 (m, 2H), 7.21(d, J = 2.8 Hz, 1H), 7.14 (d, J = 8 Hz, 1H), 6.913 (t,d, J_1 = 1.6 Hz, J_2 = 7.6 Hz, 1H), 6.61 (dd J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 3.64 (s, 2H), 3.59(s, 2H), 2.20 (s, 3H). ESI MS (CH$_3$CN/water 1/3 v/v) 306.2 ([M + H]$^+$).

General Procedure for Library Formation. Stock solutions of the aldehydes in DMSO (0.0125 M each) were mixed in equal volumes, and then 1% TFA (v/v) was added. The mixture was then added to a 0.0100 M solution of **7** or **12** to reach the stoichiometry of 1.25 equiv of aldehyde per aminooxy group. The resulting solution was diluted and injected into the mass spectrometer no earlier than 24 h.

Supporting Information Available: Synthetic procedures and analytical data for labeled formyl boronic acids and compound *o*-^{15}N-**8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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