tagging, this source of interference is avoided. However, this approach is specific to peptide libraries and is not necessarily applicable to other types of combinatorial libraries.

Another approach that eliminates possible interference from the chemical tags, "ratio encoding," has been developed for the mass spectrometric identification of bioactive leads using stable isotopes incorporated into the library compounds (29, 34). Within the ligand itself, the code might be a single-labeled atom that is conveniently inserted whenever a common reagent transfers at least one atom to the target compound or ligand. The code consists of an isotopic mixture having one of the many predetermined ratios of stable isotopes and can be incorporated in the linker or added through a reagent used during the synthesis. The mass spectrum of the compound shows a molecular ion with a unique isotope ratio that codes for a particular library compound. For example, Wagner et al. (29) used isotope ratio encoding during the synthesis of a 1000-compound peptoid library and was able to identify uniquely all the components based on their isotopic patterns and molecular weights. Because isotope ratio codes are contained within each combinatorial compound, a chemical tag is not required. The speed of MS-based decoding outperforms most other decoding technologies, which are time consuming and decode a restricted set of active compounds.

Although combinatorial synthesis provides rapid access to large numbers of compounds for screening during drug discovery and lead optimization, these libraries are usually based on a small number of common structures or scaffolds. There is a constant need for increasing the molecular diversity of combinatorial libraries and finding new scaffolds, and natural products have always been a rich source of chemical diversity for drug discovery. The traditional approach to screening natural products for drug leads uses bioassays to test organic solvent extracts for activity. If strong activity is detected, then activity-guided fractionation of the crude extract is used to isolate the active compound(s), which is identified using mass spectrometry (including tandem mass spectrometry and exact mass measurements), IR, UV/VIS spectrometry, and NMR. Recently, a variety of mass spectrometry–based affinity screening methods have been developed to streamline the tedious process of activity-guided fractionation. These approaches are discussed in Section 2.4.

Whether lead compounds in natural product extracts are isolated using bioassay-guided fractionation or mass spectrometry–based screening, there is a high probability that the structure of the active compound(s) has already been reported in the natural product literature. In such cases, the tedious process of complete structure elucidation using a battery of spectrometric tools should be unnecessary. Instead, mass spectrometry alone may be used to quickly "dereplicate" or identify the known compounds based on molecular weight, fragmentation patterns, and elemental composition in combination with natural product database searching (35–39). Commercially available natural products databases include NAPRALERT (40), Scientific & Technical Information Network (STN) (41), and the Dictionary of Natural Products (42). Because some of these databases also contain UV/VIS absorbance data, it is also advantageous to use a photodiode array detector between the HPLC and mass spectrometer to obtain additional spectrometric data during LC-W-MS dereplication (36, 37).

2.4 Mass Spectrometry–Based Screening

The earliest approaches to combinatorial synthesis used portioning and mixing (26) and enabled the synthesis of combinatorial libraries containing hundreds of thousands to millions of compounds. Today, this approach remains the most efficient method for preparing enormous libraries of compounds. However, until the mid-1990s, efficient screening techniques did not exist to rapidly identify the "hits" within large combinatorial mixtures. Therefore, chemists were motivated to develop ways to prepare large numbers of discreet compounds using massively parallel synthesis, which could be assayed quickly for pharmacological activity using high throughput screening one compound at a time. Recently, several mass spectrometry–based screening assays have been developed that are suitable for screening combinatorial library mixtures, and some are even useful for screening natural product extracts which have always been a