MASP−A Program Predicting Mass Spectra of Combinatorial Libraries

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MASP, a program predicting fragmentation-free mass spectra of libraries prepared by combinatorial synthesis, is presented. MASP combines user-defined building blocks with a nonvariable core molecule and calculates isotopically resolved mass spectra. Peak overlap and the abundance of major and minor isotope peaks can be examined interactively and read from a color display of the spectrum. Further, MASP exhaustively screens a Meta-Library Space to identify libraries with minimum peak overlap and maximum diversity. Diversity can be defined by the user, e.g., as size, hydrophobicity, or hydrogen bonding capability. The usefulness of such a screen for the design of libraries is demonstrated for meso-substituted tetraphenylporphyrins and peptides with a meta-library space of up to 3080 libraries and 625 compounds per library. The program operates on a PC platform under MS Windows 95. It may be useful for drug discovery and optimization studies that employ methods of combinatorial synthesis and mass spectrometry-guided *in vitro* selection.

1. INTRODUCTION

Combinatorial synthesis and *in vitro* selection have recently become important for the identification of lead compounds in the pharmaceutical industry.[1] Typically, selection experiments involving large libraries are performed in a "black box" set up, where only a few successful compounds are identified at the end of an assay, with the vast majority of compounds escaping direct measurement of their activity. Traditional, "hand-crafted" medicinal chemistry, on the other hand, focuses on the synthesis of single target compounds and the measurement of their biological activity in a rigorous way but with a low success rate in terms of approved drugs per number of synthesized compounds. Presently, techniques are beginning to emerge that combine the rigorous approach of traditional medicinal chemistry with the efficient approach of combinatorial synthesis and *in vitro* selection. Typically, a small collection of compounds, i.e., a small library, is directly monitored in a selection experiment such that the activity of every compound is known at the end of the assay. Mass spectrometry seems to be best suited for this approach as modern fragmentation-free desorption methods such as electrospray ionization (ESI) and matrix-assisted laser desorption (MALDI) often produce only one peak per compound in a sufficiently large window of a mass spectrum.

It has been shown that electrospray ionization can be useful for identifying inhibitors from libraries in selection experiments involving the *in vacuo* isolation of protein-ligand complexes and gas phase dissociation of the ligands.[2] In this elegant work, peptoide ligands could be selectively identified whose *m*/*z* of the main isotope peak of the molecular ions differed by as little as 0.1 mass units. Others have shown that comparison of predicted and measured ionspray mass spectra can be useful for monitoring combinatorial synthesis.[3] Sequence-related peptide mixtures, so-called peptide ladders, have been used as tools to study protein−protein interactions in affinity selection experiments monitored by MALDI-TOF MS.[4] Our own work in this field has been focused on selecting active compounds from libraries of tetraphenylporphyrins, porphyrin acids, and peptide-DNA hybrids, in assays monitored by quantitative MALDI-TOF mass spectrometry.[5] MALDI-TOF mass spectrometers are commercially available at an affordable cost.

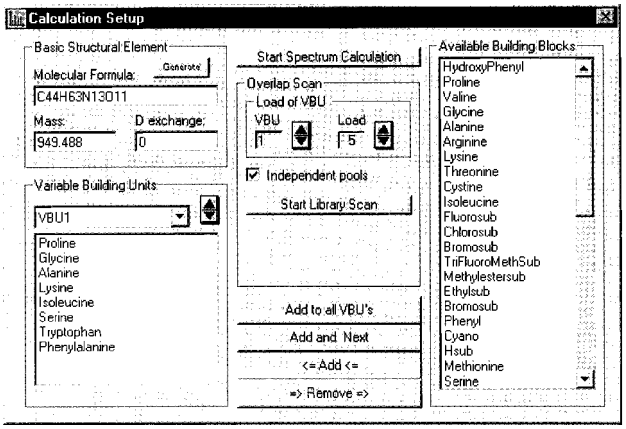

Figure 1. Screenshot of the calculation setup window.

The efficiency of a mass spectrometry-monitored selection assay depends on the number of compounds that can be detected simultaneously, i.e., on minimizing the peak overlap by choosing the "*right*" compounds. The seemingly straightforward problem of choosing the composition of libraries such that they "fill" a region of a mass spectrum efficiently can become challenging when larger libraries are involved. MASP, the program presented here, provides a computational tool to address this problem. It is expected that MASP will be useful for both medicinal chemists and biochemists, as combinatorial libraries monitored by mass spectrometry offer an opportunity to *systematically* study molecular recognition and reactivity in solution.[6,7]

2. RESULTS AND DISCUSSION

MASP runs calculations based on two database tables: (i) a table of isotopes, that can be edited and extended by the user, featuring rows containing the element name, mass

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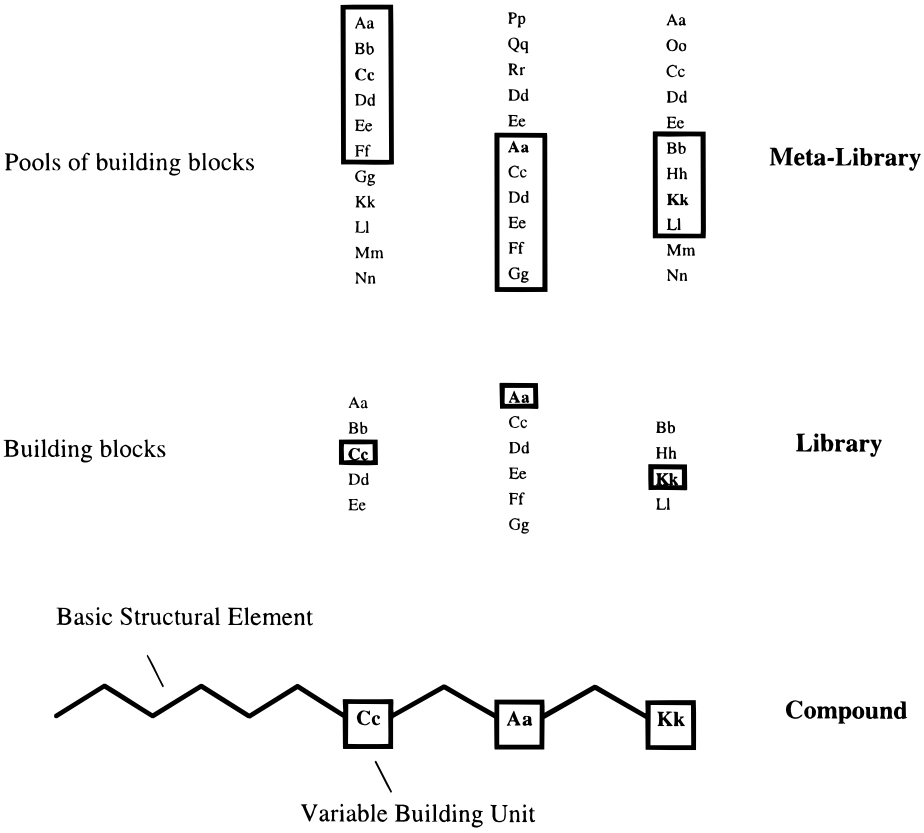

Figure 2. Schematic representation of the definition of the terms used.

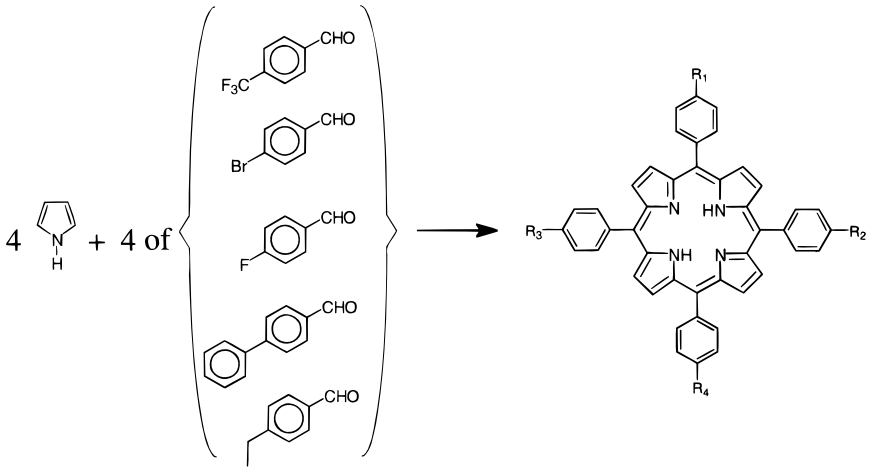

Figure 3. Schematic representation of the combinatorial synthesis of a tetraphenylporphyrin library involving pyrrole and a mixture of para-substituted benzaldehydes. This reaction underlies the examples discussed in sections 4.1 and 4.2.

number, and natural abundance for each isotope, respectively, and (ii) a fragment table containing building blocks for combinatorial synthesis. In this latter table, a name (e.g., alanine) and an abbreviation (e.g., "Ala" or "A") as well as the molecular formula of a building block are given. The formula is calculated according to how the building block appears in the final compound, e.g., C_3H_5ON for an alanine residue in a peptide, where the H_2O lost upon peptide synthesis has already been subtracted. Additionally, MASP features a column entitled "Desorption Factor" with a default value of 1. Desorption Factor refers to MALDI desorption efficiency, and this can be set to values < 1 for building blocks that lower the desorption efficiency of a molecule, leading to a decrease in peak intensity in the mass spectrum. Further, a column containing a "diversity parameter" has been included in the setup of MASP. This column entitled "General Purpose" can be used to define a criterion for ranking libraries according to the diversity of their compounds. A "Calculation Setup" window (Figure 1) has been implemented for conveniently entering the parameters for a MASP calculation.

Combinatorial synthesis often involves one molecular scaffold that is invariable and present in all compounds of a library. In our terminology (Figure 2), this part of the molecule is referred to as "Basic Structural Element" (BSE). In the example shown in Figure 3, the porphyrin ring is the BSE. A variable position in the BSE that is used for a combinatorial step in the synthesis is referred to as "Variable Building Unit" (VBU). MASP has been written such that the user can assign a number of VBUs and then "fill" the pool for each of these VBUs independently with a given number of building blocks from the building block database.

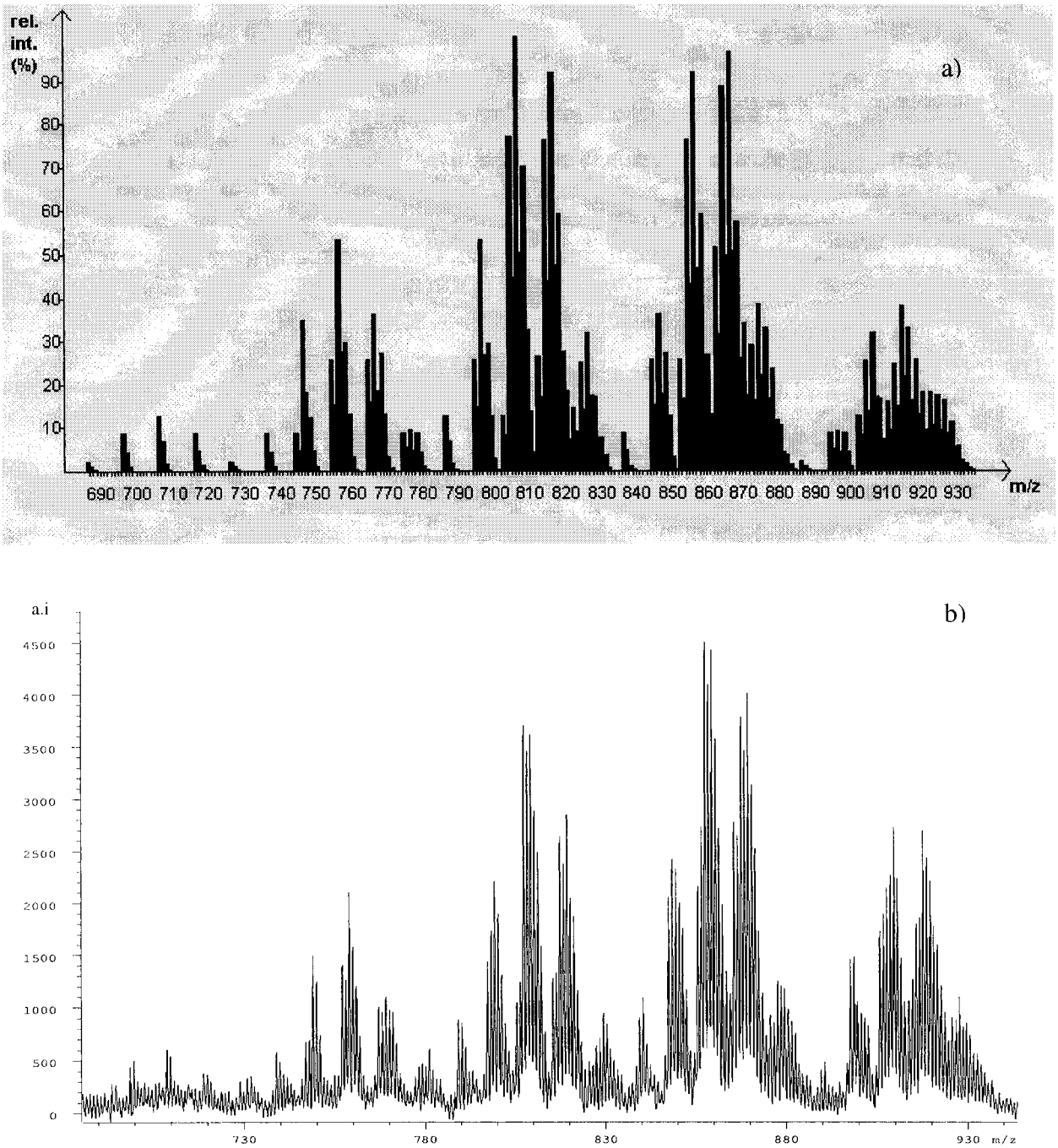

Figure 4. (a) Predicted mass spectrum of the porphyrin library whose synthesis is shown in Figure 3. (b) Experimental laser desorption mass spectrometer of the porphyrin library. Bruker BIFLEX mass spectrometer, 10 kV, positive reflectron mode, 150 ns delayed extraction, matrix-free porphyrin film.

During the library calculation process, the program performs an exhaustive calculation of all possible combinations of the assigned building blocks and the BSE to create a library of compounds. The isotopic mass pattern is then calculated for every compound in this library, and, finally, all patterns are superimposed to give the predicted mass spectrum of the library in question.

MASP has been useful for evaluating the degree of success of combinatorial syntheses. An example of a predicted spectrum and an experimental MALDI-TOF spectrum is shown in Figure 4. It is important to mention, that such MALDI spectra should be acquired under conditions that obey quantitative peak intensity-concentration relationships.[5,8−12]

If severe peak overlap is observed in the mass spectrum of a library of interest, some peaks may be shifted by exchanging acidic protons with deuterium ions. This has been demonstrated for ESI spectra.[13] MASP provides a capability to assign a "deuterium exchange number" to the building blocks and to calculate the deuterium exchanged MALDI-TOF spectrum.

If the predicted mass spectrum shows too severe a peak overlap, a change in the composition of the library becomes mandatory. MASP offers a feature referred to as "meta library scan" to address this problem. A calculation routine was written that produces a *set of libraries* and their corresponding spectra. This routine generates data that allows for the individual libraries to be rated with the most favorable building block mixture for combinatorial synthesis then being selected. The algorithm was designed such that it starts from an assigned number of VBUs, a "load" of l building blocks per VBU, and a pool of p building blocks ($p > l$) to fill the VBU (compare Figure 2). The size of the

Chart 1. Schematic Structure of the Library Calculation Subroutine

```
Subroutine CalculateLibrary(VBU_Number)
  For each building block in VBU "VBU_Number" do
    add current building block to Compound
    if VBU_Number is smaller than number of assigned VBUs then
      call CalculateLibrary(VBU_Number + 1)
    else
    store created compound in the compound database
    end if
    delete recently added building block from compound
  next building block
end of subroutine
```

individual libraries is determined by the number of possible building blocks to be used for filling a given VBU. Given the size of the libraries, the size of the "meta-library space" depends on the number of building blocks in the building block pools. MASP calculates the spectrum of every library in the meta-library and computes the spectral overlap, i.e., the number of peaks that are caused by more than a single compound. Minor isotopic peaks that contribute less than an assigned percentage to the height of a peak can be treated as negligible. After and during a run of MASP, the ten libraries with the lowest mass spectrometric overlap are displayed as a bar chart. These libraries are best suited for mass spectrometrically monitored selection experiments.

An additional level of ranking libraries can be achieved by rating individual libraries according to the diversity among their components. To accomplish this ranking, building blocks receive a number in the "General Purpose" (GP) parameter listing. This number may be the lipophilicity or the size of a building block. For each compound in a library, an average lipophilicity parameter can be computed by calculating the mean value of the GP parameters of its building blocks. The diversity of a library can then be determined from the standard deviation of these average lipophilicity values of its components. Other diversity parameters and ranking methods could be implemented. Often, libraries with the same number of overlapping peaks can be ranked successfully by this diversity criterion.

The meta-library scan may be carried out in one of two different ways, depending on how the combinatorial synthesis is being performed. If all combinatorial steps are performed with the same pool of substances, a "coupled pools case" calculation is appropriate. Here, only one pool of building blocks has to be defined for all VBUs. An example for this case is the synthesis of tetraphenylporphyrins from aldehydes and pyrrole in a single step (Figure 3). In the other case, referred to as the "independent pools case", each VBU is assigned an individual pool of building blocks. This situation is typically encountered in a solid phase synthesis, e.g., of linear oligomers, where randomization is achieved in several of the sequential coupling reactions. A combinatorial peptide synthesis is given as an example for this case (Section 4.3).

3. ALGORITHMS

The algorithms presented in Charts 1-3 above and below are shown in structured English to improve readability by nonprogrammers. A name of a variable or program code is printed in italics.

3.1. Calculation of the Library Composition. The library composition is calculated by a simple recursive routine *CalculateLibrary* (Chart 1), which exhaustively calculates all possible combinations of b building blocks per v VBUs to yield $c = b^v$ compounds (some of which may be indistinguishable). The routine is called up with $VBU = 1$ as its argument. While recursively calling itself, *CalculateLibrary()* adds building blocks to the variable *Compound*, thereby "synthesizing" a new compound. Once the synthesis of a compound is completed it is stored in a temporarily created compound database. Having completed a run through the compound generation, the spectrum is generated by calculating the isotopic pattern of every compound in the library and superimposing the individual patterns to produce the spectrum (listing not shown). The algorithm used for the isotopic pattern calculation was published by Kubinyi in 1991.[14]

Several parameters affect the peak intensity in predicted mass spectra. One such parameter is the abundance of indistinguishable compounds, i.e., isomers. During internal storage of compounds in the compound database, the program recognizes indistinguishable compounds and does not store them separately. A counter maintained for each dataset is incremented instead. This leads to a significant improvement of speed in the subsequent spectrum calculation, as the isotopic pattern calculation has to be carried out only once for all compounds with the same molecular formula. The intensity of the resulting peak pattern is then multiplied by the number of compounds causing it. The intensity of a set of isotopic mass peaks is also determined by the "desorption factor" defined for the building blocks incorporated in the compound causing it. A mean desorption factor is computed for any given compound based on the desorption factors of the building blocks contained. The peak intensity generated by the above algorithm is multiplied by this factor. Desorption factors may be determined experimentally by comparing the peak heights of those compounds in a library that bear only one kind of building block. This is a linear approximation, and we are presently evaluating possible improvements to this algorithm. Finally, a "Molar Amount" value can be assigned to each building block in a VBU listing, resulting in a third factor that modulates peak intensity. This factor is useful for predicting the spectra of products of combinatorial syntheses involving non-equimolar amounts of building blocks or building blocks with different reactivities. MASP multiplies the molar amount values to get a "yield" of the compound in question, which is used as a factor for calculating the peak intensity obtained by the algorithms described above. The default value for the "molar amount value" is 1. We have performed combinatorial syntheses that produce a peak pattern resem-

Chart 2. Scheme of the Library Scan Routine (Independent Pools)

```
Subroutine LibraryScanIndependent(VBU_Number, Position, StartChoice)
  PresentChoice = StartChoice
  Do
    add building unit number "Presentchoice" to VBU "VBU_number"
    if Position < MaxLoad(VBU_Number) and PresentChoice < NumberOfAvailableChoices(VBU_Number) - (MaxLoad(VBU_Number) - Position) then
      call LibraryScanIndependent(VBU_Number, Position + 1, PresentChoice + 1)
    else if VBU_Number < NumberOfAvailablVBUs and Position = MaxLoad(VBU_Number) then
      call LibraryScanIndependent(VBU_Number, 1, 1)
    else if VBU_Number = NumberOfAvailablVBUs and Position = MaxLoad(VBU_Number) then
      evaluate current configuration, i. e. calculate library, count spectral overlap, etc.
    endif
    remove recently added item Number "PresentChoice" from VBU number "VBU_Number"
    PresentChoice = PresentChoice + 1
  loop until PresentChoice > NumberOfAvailableChoices(VBU_Number)
end of subroutine
```

bling the peak pattern predicted by this algorithm with the default values of both the desorption factor and the molar amount value (Figure 4).[15]

3.2. Meta Library Scan. The "Independent Pools" Case. The recursive subroutine *LibraryScanIndependent()* (Chart 2) is called with the number of the *VBU (VBU_Number)* to be processed, the number of positions within this VBU to be filled *(Position)* and the number of the building unit from the pool to choose from *(StartChoice)*. Thus, the first call to this routine will be *LibraryScanIndependent(1, 1, 1)*. Finally the runtime behavior of this subroutine is determined by the maximum load for each VBU *(MaxLoad-(VBU_Number))*, giving the actual size of the resulting libraries, and the size of the pool to choose from *(NumberOfAvailableChoices(VBU_Number))* giving the number of resulting libraries under consideration of *MaxLoad(VBU_Number)*.

The "Coupled Pools" Case. The recursive subroutine *LibraryScanCoupled()* (Chart 3) is a more narrowly defined version of *LibraryScanIndependent()*. It does not require the parameter *VBU_Number*, since it adds the recent choice from the building unit pool to each of the available VBUs.

In the following, working with MASP will be demonstrated in two examples. One is an example for the "independent pools" case. We have chosen an oligopeptide with two randomized residues (VBUs) for this example (Figure 8). The other is an example for the "coupled pools" case. Here, substituents at the four meso positions of a porphyrin are incorporated simultaneously in a single synthetic step: a cyclization reaction involving pyrrole and a set of different benzaldehydes (Figure 4).

4. SELECTED EXAMPLES

4.1. Example 1: Calculation of a Spectrum of a Tetraphenylporphyrin Library. A Rothemund-type porphyrin synthesis is the experimental counterpart to this calculation. In this particular case, the five aldehydes depicted in Figure 3 are being used (R = Ph, Br, F, CF_3, and Et).

First, the isotope database may be supplemented in case a building block contains elements that are not among those commonly used in organic chemistry. The user then continues by entering the data for the required building blocks into the building block database table. This involves entering the name, e.g., *p*-phenylbenzaldehyde, an abbreviation ("shortcut"), and the molecular formula minus the atoms "lost" upon incorporation in the compound to be synthesized. Entering values into the "Deuterium Exchange Number" table and "General Purpose" list are optional.

The calculation is started from the Calculation Setup window. First, the user assigns a basic structural element, i.e., the core molecule that remains unchanged during the library synthesis. MASP also operates without any entry in this category.

In our example, the molecular composition of the porphyrin skeleton ($C_{20}H_{10}N_4$) without the hydrogen atoms at the attachment points of the substituted phenyl rings is entered. The next step is to assign the number of VBUs to be used in the synthesis (four in our example). The five desired aldehydes are being selected from the rightmost listbox using standard Windows selection techniques. This involves transferring the five building blocks into all four listboxes representing the four VBUs by pressing the "Add to all VBUs" button. The "Start Calculation" button then

Chart 3. Scheme of the Library Scan Routine (Coupled Pools)

```
Subroutine LibraryScanCoupled(Position, StartChoice)
  PresentChoice = StartChoice
  Do
    add building unit number "Presentchoice" to all available VBUs
    if Position = MaxLoad then
      evaluate current configuration, i. e. calculate library, count spectral overlap, etc.
    else
      call LibraryScanCoupled(Position + 1, PresentChoice + 1)
    end if
    remove recently added item Number "PresentChoice" from all available VBUs
    PresentChoice = PresentChoice + 1
  loop until PresentChoice > NumberOfAvailableChoices
end of subroutine
```

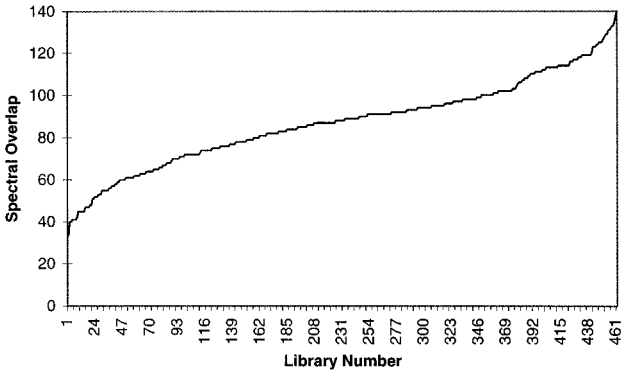

Figure 5. Histogram of the results of the library scan outlined in section 4.2. One number on the *x*-axis corresponds to one library created. The *y*-axis gives the number of peaks in the mass spectrum that cannot be assigned unambiguously because they originate in more than one compound. Peaks whose intensity originates more than 95% in a single compound are counted as assignable. The cutoff for defining an elevation in the baseline as a peak was set to 0.1% of the most intense peak in the spectrum.

invokes the combinatorial synthesis subroutine. The Spectrum Display calculated for the porphyrin library is shown in Figure 4a. The display allows annotations, expansions of spectral regions, assignment of titles, and printouts of the spectrum. Annotations are made by clicking on a peak. Every annotation lists the compounds leading to the peak in question in alphabetical order. Compounds are reported as the sequence of the building blocks they contain. Next to this sequence, one finds the number of compounds having this composition and the fraction that these compounds contribute to the peak height. This fraction is not normalized to a particular value (Figure 7). Expansion of a spectral region is easily accomplished by clicking on the mass values to define the boundaries of the expanded window with the right mouse button. In Figure 4, the spectrum resulting from the MASP calculation is compared with the experimental spectrum of the library.

4.2. Example 2: A Library Scan (Coupled Pools). This example is based on the same chemical reaction as that used in section 4.1. Here, libraries synthesized from five aldehyde building blocks are scanned in a meta library structure space defined by a pool of 11 different aldehydes. The possible combinations of these sets of five aldehydes in the pool of 11 leads to 462 possible libraries. The goal of this scan is to search these 462 libraries for those with the lowest number of peaks caused by more than one compound. One starts with the same setup as described in 4.1, assigning a core skeleton $C_{20}H_{10}N_4$ and a number of 4 VBUs. Next, 11 aldehyde building blocks from the database table are transferred into VBU number 1. Then, the desired "load" of five building blocks per VBU is assigned using the spin button in the "Overlap Scan" frame of the window depicted in Figure 1. Finally, the scan is initiated by pressing the "Start" button. This particular library scan took approximately 4 h on our 100 MHz Pentium PC. The result of this can be visualized as a bar chart during and after the scanning process. The 10 libraries with the lowest mass spectrometric overlap are shown together with their building block composition. Figure 5 gives an overview of the distribution of spectral overlap data of the complete meta library, which spans a range of 33 to 140 overlapping peaks per library. Further, the two calculated spectra with the lowest and the highest amount of spectral overlap are shown in Figure 6.

4.3. Example 3: A Library Scan (Independent Pools). This example shows a typical library scan for a combinatorial synthesis, where several positions of the target molecule are varied in several individual combinatorial steps, allowing for the use of independent pools of building units in each of these steps. Chosen here is an oligopeptide with ten amino acid residues (Figure 8). Two of the ten residues are combinatorially varied to achieve diversity, e.g., to study the molecular interaction with a biological target molecule. The "basic structural element" is most easily generated using the "building unit calculator". This calculator allows for the quick assembly of building units from the building block database table. Here, the following invariable residues are being used: Ala, Val, Arg, Gly, His, Thr, Phe, Tyr, and "H_2O" from the two termini. Next, two VBUs are being assigned. Then, each of the VBUs is filled with a number of eight amino acids as shown in Figure 8. This is done by clicking on the "independent pools" checkbox (Figure 1), leading to a second spin button control that allows for the individual assignment of a fragment load for each VBU. In this example, the same "load" of five building blocks is assigned to both VBUs. The amino acids pools used to define the meta library structure space are [Ala, Gly, Ile, Lys, Phe, Pro, Ser, Try] for pool 1 and [Arg, Cys, His, Leu, Met, Thr, Tyr, Val] for pool 2. The lipophilicity of these amino acid residues was entered as the general purpose parameter according to the lipophilicity values reported by Kyte and Doolittle.[16] The calculation of the meta library scan took ~6 h on our 100 MHz Pentium PC using Windows 95. The result is shown in Figure 9. Libraries with an equal

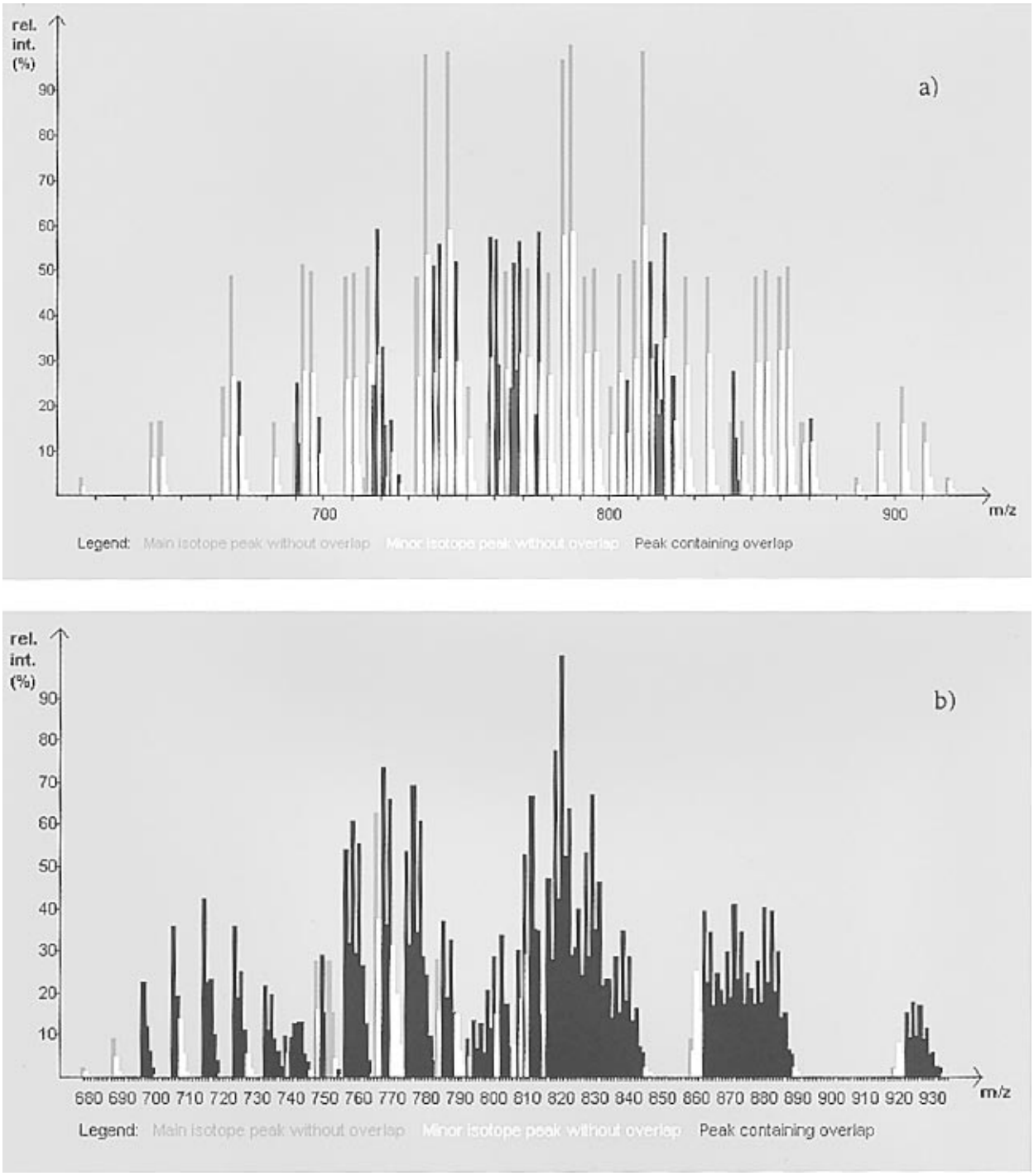

Figure 6. Comparison of two predicted mass spectra generated during the meta-library scan described in section 4.2. (a) Spectrum of the library with the smallest number of overlapping peaks (33). (b) Spectrum of the library with the largest number of overlapping peaks (140). Legend: main isotope peak without overlap (grey), minor isotope peak without overlap (white), and peak containing overlap (black).

amount of spectral overlap are ranked by their diversity in terms of hydrophobicity as described above.

5. CONCLUSION

Described here is MASP, a computer program supporting the design of combinatorial libraries of moderate size to be exhaustively analyzed by mass spectrometry. This program is useful for a broad range of combinatorial synthesis schemes and target molecules, provided that the size of the libraries does not exceed computational power. MASP has been implemented in Visual Basic, using a sophisticated database access tool, leading to a comparatively slow execution for complex meta-library scans. The same algorithms may be implemented under C, C++, or probably Java to improve performance. For experiments involving a set of inhibitors, affinity probes, or derivatives of a pharmaceutical lead compound, MASP produces results within minutes. These classes of compounds are routinely used by biochemists, medicinal chemists, and bioorganic chemists.

6. MATERIALS AND METHODS

Data produced or maintained by the program are stored in MS Access database files that can be read and transformed by many other programs. MASP was written in Microsoft Visual Basic 4.0 and compiled for 32-bit platforms. Thus it is available for Windows 95 and Windows NT operating

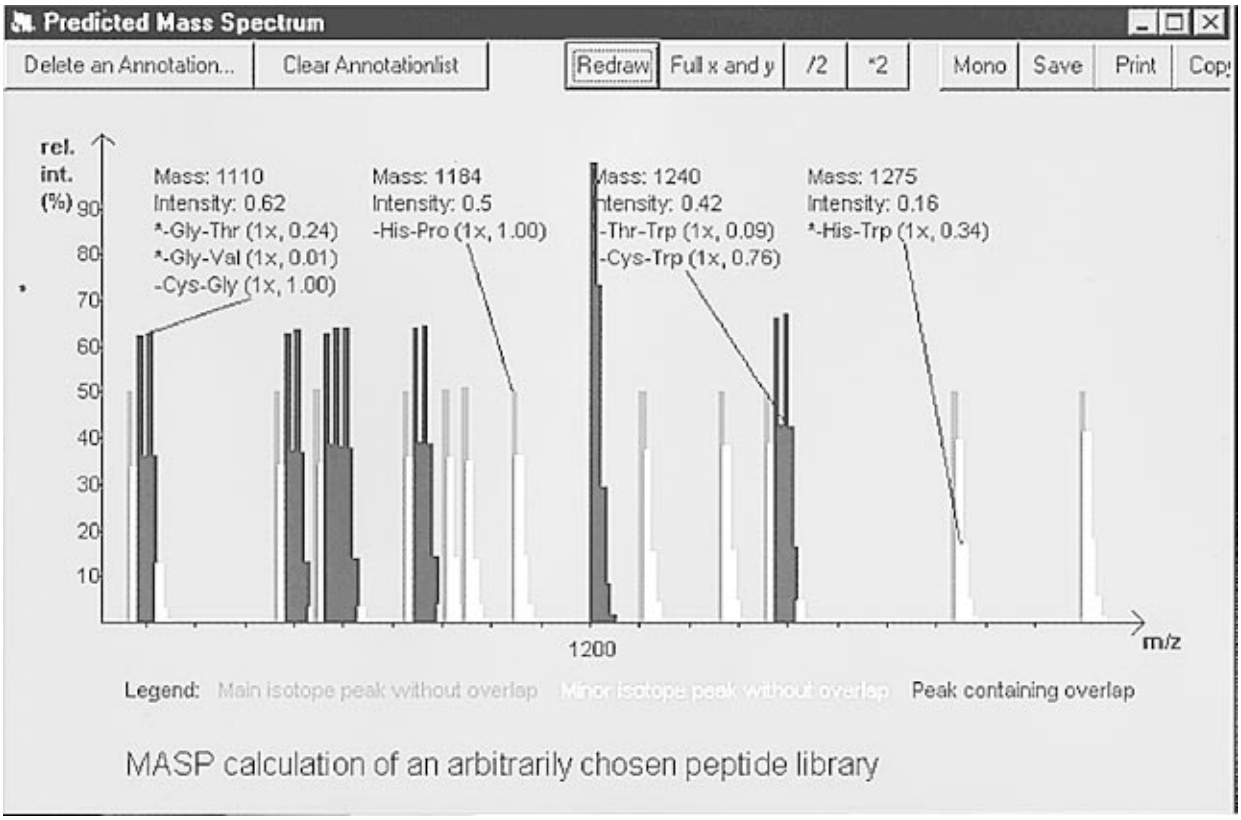

Figure 7. Screenshot of the spectrum display window of MASP showing the result of an arbitrarily chosen peptide library chosen from the meta library described in section 4.3 (See Figure 8). Legend: main isotope peak without overlap (grey), minor isotope peak without overlap (white), and peak containing overlap (black).

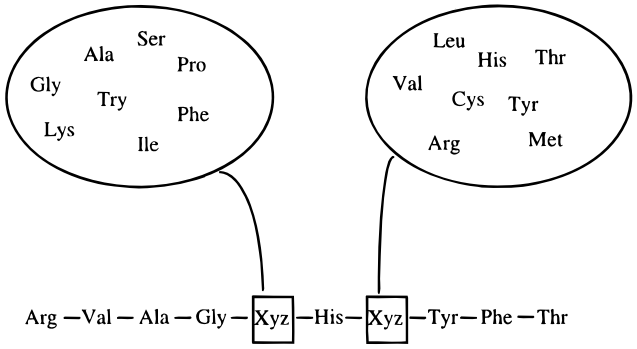

Figure 8. Schematic representation of the structure of compounds contained in an oligopeptide library. This structure underlies the example discussed in section 4.3. Five building blocks out of the pool of eight were chosen for each library.

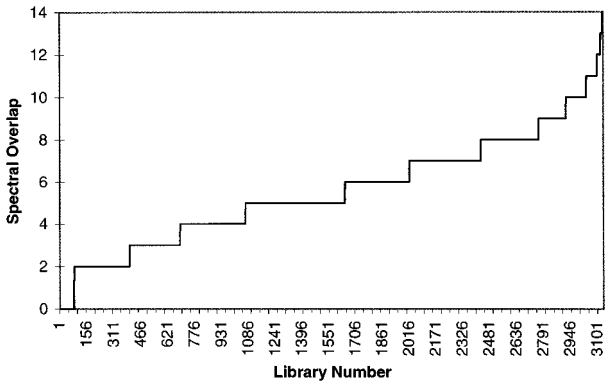

Figure 9. Histogram of the results of the library scan outlined in section 4.3. *y*-Axis values are the number of peaks caused by more than one compound. An oligopeptide 10-mer with two variable residues. Peaks that are caused ≥95% by one compound are treated as nonoverlapped.

systems. A 100 MHz Pentium PC with 16 MB RAM was used for development and testing.

Supporting Information Available: The executables of the MASP program as well as the full source code. The latter is just a copy of our MASP development directory. To work on the MASP source code Microsoft Visual Basic v. 3.0 or higher is needed. For a regular installation procedure a standard Windows 95 setup program is provided in the MASP installation directory. The lastest version of MASP can be downloaded from our web server (http://microvirus.chem.tufts.edu). MASP has been made available under the GNU General Public Licence described by the file LICENCE.TXT included with the distribution. See any current masthead page for Internet access instructions.

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