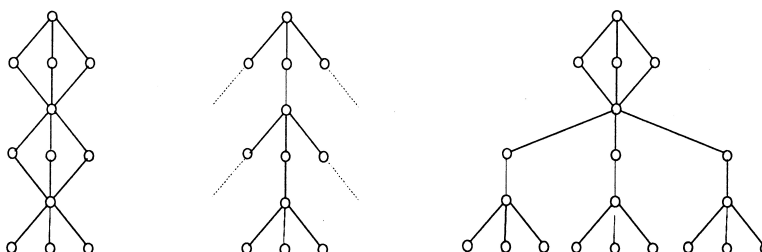Article

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Computer Aided Analysis of Split and Mix Combinatorial Libraries

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Combinatorial chemistry using split and pool synthesis involves making and testing mixtures of compounds in pools which are subsets of the larger compound collection. These subsets are created during the synthesis of the collection through a resin splitting and mixing method. Tests are conducted on each of the final pools of mixtures and the individual compounds within a mixture of interest are then identified through some deconvolution scheme, originally involving selective re-synthesis. It is possible that different schemes for splitting and mixing will have different consequences on the overall effort necessary to deconvolute interesting mixtures. The evaluation of different protocols of splitting and mixing involves consideration of more possibilities than can be exhaustively or optimally determined manually in a realistic time frame for most compound collections. We present herein a computational scheme to aid in this analysis. The approach exhaustively examines possible splitting and mixing strategies for the interrelated values of total library size, number of combinatorial steps, number of reaction vessels, and number of compounds per final pool. Weighting factors may be introduced into the various steps. The resulting complete list of splitting and mixing options is scored based on a variable weighting strategy for the total effort of synthesis and deconvolution. The results indicate the splitting/mixing strategy used has an impact on overall efficiency and should be considered in the design of compound libraries.

Introduction

One of the earliest combinatorial chemistry approaches for drug discovery was the combinatorial synthesis of compound mixtures on resin beads using splitting and mixing approaches, often termed split and pool synthesis.[1] This method produces a pool of beads with a single compound attached to each individual bead and gives a solution mixture of compounds when a mixture of beads is treated to cleavage conditions. Using this strategy, the final pools of beads from the synthesis are not remixed, but are tested in an assay to identify any pools with an interesting level of desirable activity. Once mixtures with the desired activity have been identified, a deconvolution[2] process is needed to identify the specific individual compounds in the pool responsible for the activity. This can involve selective resynthesis and testing of portions of the library to finally deconvolute the mixture. An elegant theoretical analysis evaluating the impact of final pooling strategies showed that active compounds located in various final pool environments can be reliably identified using this approach.[3] Deconvolution by resynthesis is labor intensive, and alternative strategies to identify individual active compounds soon appeared, including orthogonal,[4] indexed,[5] or tagged[6] mixtures, direct identification using mass spectrometry[7] or diffusion-resolved NMR spectroscopy.[8] These methods, and others, are useful alternatives to decon-

volution by selective resynthesis and continue to attract attention.[9]

Split and pool synthesis is a very efficient production strategy for large numbers of compounds, once the underlying reactions have been optimized for solid supported substrates. However, efficiency of library synthesis is an advantage only when the effort of deconvolution is also accounted for. The total effort using deconvolution by selective resynthesis to identify specific individual active compounds from the pool mixtures depends on the sum of the effort of the original synthesis plus the overall effort of resynthesis and testing. We became convinced that different splitting/pooling strategies could lead to different overall efficiencies, especially as the library size increased. A sacrifice in initial synthesis efficiency can lead to a compensating increase in deconvolution efficiency, resulting in an overall improvement. We decided to first test this concept, that the design of splitting/pooling strategy could impact overall efficiency of synthesis and deconvolution, for split and pool synthesis using selective resynthesis for deconvolution in order to determine if an improvement in overall efficiency could be achieved. This concept should also be useful for other deconvolution methods using resynthesis, such as partial tagging.[10]

As the library size increases, the number of possible split and mix experimental designs grows rapidly and evaluation of alternate strategies by manual methods quickly becomes unwieldy. As a consequence, for large libraries it is difficult to determine if different splitting and mixing schemes offer any advantages in a particular circumstance. We present
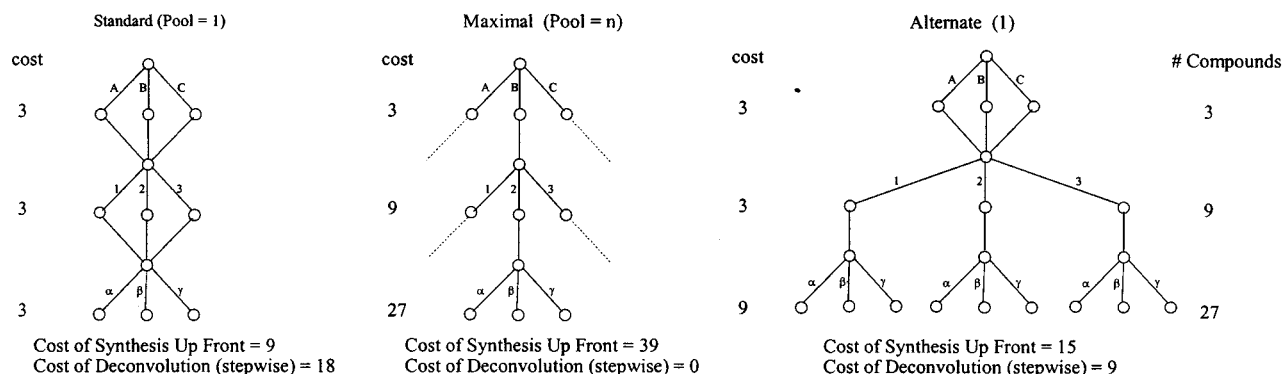
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a)

b)

	CUF	SWD	TOT		CUF	SWD	TOT		CUF	SWD	TOT
1 hit	9	18	27		39	0	39		15	9	24
2 hits	9	36	45		39	0	39		15	18	33

Figure 1. Schematic representation of the scope of splitting and mixing strategies possible for a library made with three compounds per step and having three steps. The example on the left illustrates the mixing of all compounds at each step. The example in the center illustrates library construction without any mixing. The example on the right illustrates an intermediate example.

herein a preliminary report of software that has been developed in an effort to facilitate the a priori analysis of splitting and mixing strategies intended to aid in the selection of an experimental design for libraries to minimize the overall effort for synthesis and deconvolution by selective resynthesis. The software system will quickly provide a summary of all possible solutions for a given library design, subject to desired constraints. **By virtue of an exhaustive search mechanism over all permutations and combinations, it is designed to provide a complete analysis and a global minimum solution for each set of constraints.** The system determines all possible splitting and mixing strategies for a given library size, number of starting materials, number of combinatorial steps, number of reaction vessels, and number of compounds in a final pool. In addition, similar computations can be made allowing for variation in the maximum number of reaction vessels and in the size of the library. The output provides an accounting of the number of synthetic steps, deconvolution steps, assay steps, etc., which can be weighted by user defined weights and searched for optimal solutions. However, the "optimal" of a given "selection criteria" that leads to the choice of one design over others would be sensitive to the scoring system used to represent the difficulty and/or practical feasibility of all the steps involved in library synthesis, assay, and deconvolution. The simulation system is layered and hence will allow the user to directly interrogate the system for an answer at various stages in the design process.

Functionality

1. Fixed Library Size, Number of Reaction Vessels, and Number of Starting Materials. The most basic aspect of the design of splitting and pooling strategies is the determination of the various possibilities for a given library size, number of reaction vessels, and number of starting materials. As an illustration, consider the generation of a library of 27 compounds using a three-step synthesis with three starting materials at each step and 50 as the maximum number of reaction vessels at each step. Figure 1a illustrates the scope of possible solutions (experimental designs). To compare the synthetic expense of various schemes, we define the terms CUF (cost-up-front) and SWD (cost of stepwise deconvolution) as follows (see Figure 2). CUF is the weighted sum, over all synthetic steps, of all the starting materials (= number of splittings) for the initial library synthesis. SWD is defined similarly to CUF, for the synthetic steps required for the deconvolution process, after the initial library synthesis. The computation of SWD assumes only one final vessel shows activity at each cycle, and that each cycle begins with the first step (i.e., intermediates are not saved). However, the present approach can easily be extended to schemes wherein multiple vessels show activity or other schemes, such as resin archiving, are used for the deconvolution cycles (vida infra). The first scheme, "Standard", pools all solutions together at the end of each step into a single reaction vessel. This results in a scheme which requires the least number of synthetic steps (i.e., least costly) in the initial synthesis of the library (CUF = 9) and is the most costly
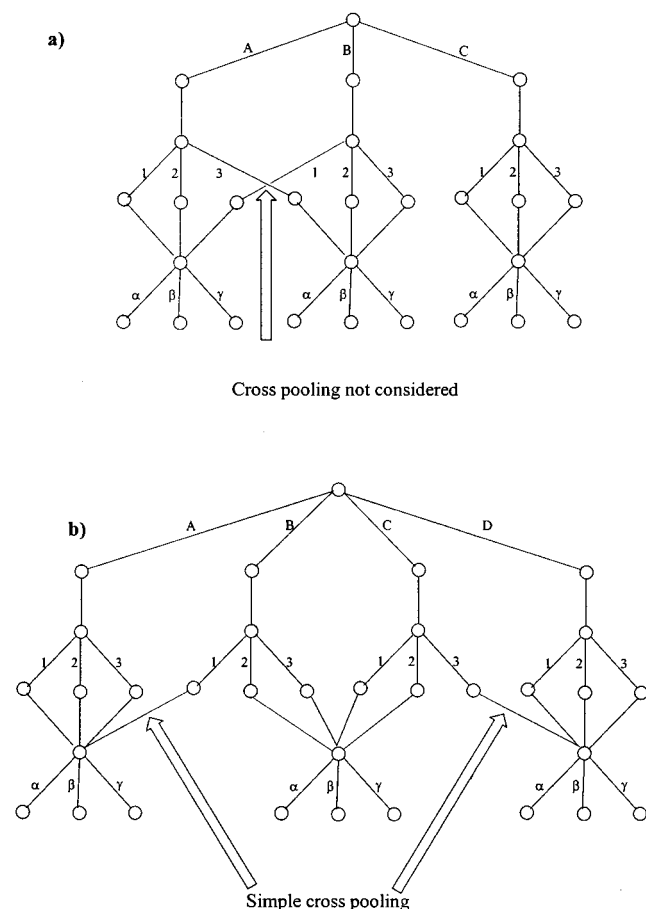
Standard (Pool = 1)

Figure 2. Schematic representation of the determination of the values of CUF (cost-up-front) and SWD (cost of stepwise deconvolution). In the example shown, it is assumed that only the vessel containing "β" is active after the initial synthesis of the library, and that after the first deconvolution cycle, only the vessel containing "2" is active.

Alternate Split and Mix (2)

Figure 3. Schematic representation of a splitting and mixing strategy for a library made with three compounds per step and having three steps and invoking branching.

scheme at the deconvolution stage (SWD = 18). At the other extreme, the second scheme, "Maximal", does no pooling at any step. This results in a scheme which is the most expensive in the initial cost (CUF = 39) and the least expensive at the deconvolution stage (SWD = 0) because this scheme results in no mixtures. The last scheme in Figure 1a illustrates one of the many intermediate solutions. The spectrum of various alternatives to this problem is at the core of the system. This may be used directly (or in the context of the more general solutions described below). In the direct-use mode, the user specifies the number of starting materials at each step in the synthesis and the maximum number of reaction vessels for each step. The program also considers

possible "branching" strategies which, for the present case, is illustrated in Figure 3. To take into account the possibility that different steps may have different relative difficulties associated with them, weight factors may be associated with each of the individual synthetic steps (a difficult reaction weighted above one) and assay evaluations (a costly assay weighted above one). For simplification, the examples discussed herein use all weightings equal to one. With regard to branching, the following simplifying conditions are invoked. After a branching split, only the simplest pooling scheme for each possible value of pools in the following pool-step is considered. Thus, all possible permutations of the reaction vessels into different (newly) pooled vessels are

a)

Cross pooling not considered

b)

Simple cross pooling

Figure 4. Illustration of the cross pooling alternatives considered. The example in the upper panel involves an exchange between branches and is not considered. The example in the lower panel involves a transfer from one branch to another which is taken as an allowed solution.

not considered (see Figure 4a). In addition, pooling of vessels from one branch with vessels in a different branch, and which continue in a different branch, are only allowed when necessary to satisfy the required pooling (e.g., if 4 pools are split by 3 giving 12 pools which are then recombined to 3 pools; see Figure 4b).

Figure 5 illustrates a sample run of this software. The user supplies the number of steps, maximum number of reaction vessels, and number of starting materials at each step, along with weighting factors for "synthetic cost" for each step and weights for assaying and deconvolution. Each record (line) of output specifies a possible solution. **All possible solutions within the defined parameters are determined.** (The accompanying diagram, which is not part of the program's output, illustrates the first solution printed). Note that the number of starting materials at each step defines the splitting for that step, and the output therefore contains only the pooling values (P) for each step. The P values (P1, P2, and P3 in this case) specify the number of pools at each step. CUF and SWD are as defined earlier. ASY is the cost for doing the assay, and the last column gives the total cost. In this way the user can evaluate which of the solutions are most appropriate. Table 1 contains an accounting of the solutions obtained for libraries of 1000, 5000, 10 000, and 50 000 compounds where the maximum number of vessels is 100 (see columns labeled "user specified starting materi-

Table 1. Summary of Results for Four Library Sizes

	user specified starting materials[a]		variable starting materials		variable library size[b]	
	time[c]	solutions	time[c]	solutions	time[c]	solutions
1000	≤0.6	20	0.6	789	61.2	21 920
5000	≤0.6	10	0.8	794	329.6	97 806
10 000	≤10.6	8	1.3	1224	721.2	161 535
50 000	≤10.6	4	3.1	567	4986.4	351 887

[a] The number of starting materials used in the three steps were 10, 10, and 10 (for a library of 1000 compounds); 10, 50, and 10 (for a library of 5000 compounds); 10, 10, and 100 (for a library of 10 000 compounds); and 10, 50, and 100 (for a library of 50 000 compounds). [b] For each library size entry, libraries within 5% of that size were evaluated. [c] Times are in seconds on a Silicon Graphics Indigo2 Impact, R10000 workstation. The present version of the program is considered a prototype, i.e., it has not been fully optimized for computing efficiency.

Sample Run

```
>> sandp_layout
 Num_Step, Max_pot ?
3, 12
 Starting materials & synthetic cost for step        1
3, 1
 Starting materials & synthetic cost for step        2
3, 1
 Starting materials & synthetic cost for step        3
3, 1
 Enter weights for assaying and deconvolution
0,1

>> more scores

    P1      P2      P3      CUF     SWD     ASY     TOTAL
    1       3       3       21      7       0       28
    1       1       3       15      9       0       24
    1       1       1       9       18      0       27
```
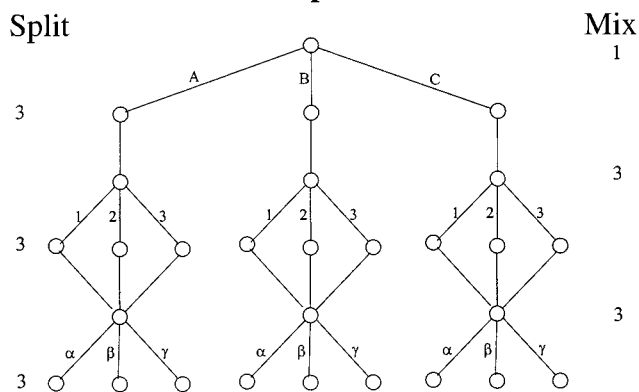
Schematic Representation

Split   Mix

Figure 5. Upper section illustrates a sample output for the determination of possible libraries with three compounds per step and having three steps. Lower section shows a schematic representation of the first of the solutions shown in the upper section.

als"). The computation times are clearly negligible, and the number of solutions is within the scope of what could readily be generated manually (≤20). The decrease in the number of solutions with increasing library sizes is due to the constraints imposed by limiting the number of vessels to 100.

To illustrate how the present scheme would be extended to multiple active vessels, we reconsider the cost of the schemes shown in Figure 1a for two wells containing an active compound, but all other assumptions being the same.

The initial cost (CUF) remains the same in all three cases, whereas the SWD costs are doubled (see Figure 1b). The total cost for "Standard" would become 45 while the total cost for "Maximal" would remain unchanged at 39. The "Alternate (1)" example would now have a total cost of 33. Thus, in this instance, "Maximal" now has a lower cost than "Standard" but both have a higher cost than "Alternate". Analogous to this extension, the framework described herein can be expanded to more than two wells with active compounds, individual wells with multiple active compounds, etc.

As a simple example of the use of alternate deconvolution protocols, the example in Figure 1a can be evaluated for an assumed resin archiving protocol wherein samples from the first step (A, B, and C) are saved for the deconvolution stages. This would eliminate their generation in the first step of deconvolution cycles 1 and 2 (see Figure 2), thereby reducing the cost of each of these steps by 3, and the overall cost would now be 21.

The illustrations herein include the assumption that each cycle begins with the first step and uses the same library structure. These assumptions can be modified. One can, for example, consider the various parameters as a criterion for modifying or selecting the SWD strategy. For instance, one can introduce a penalty-weighting factor for the number of cycles required to deconvolute a given strategy (e.g., favoring "Maximal" over "Standard" pooling because fewer cycles are required for "Maximal" pooling). Similarly, one could recompute all possible strategies for an upcoming cycle based on the hit profile of the given cycle, i.e., relaxing the constraint of maintaining a common library structure through the deconvolution cycles.

2. User Specified Starting Materials with Fixed Library Size and Number of Reaction Vessels. The above illustration is for situations where the number of starting materials at each step is specified. (This implicitly defines the size of the library as well.) As the starting materials at each step (their number and order) can generally have many values, an automated procedure has been developed for exploring these. Specifically, for a given library size, the procedure can be used to determine all possible values of the number of starting materials at each step (incorporating the constraints above) which would yield libraries of the appropriate size. Each of these solutions can then be used to evaluate their splitting and pooling possibilities. An accounting of such results for libraries of 1000, 5000, 10 000, and 50 000 compounds are shown in Table 1 (see "variable starting materials"). In comparison to the corresponding examples with user specified staring materials, the computation times are still fast enough to be calculated interactively, but the number of possible solutions are well beyond what could readily be generated manually. Again (see previous section) the decrease in the number of solutions with increasing library size is related to the constraints.

3. Variable Library Size and Number of Reaction Vessel. The library size and maximum number of reaction vessels at each step are often parameters for which one may only have approximate requirements (e.g., library of 10 000 vs 50 000). For each specific library size and maximum

number of reaction vessels, there are a finite number of possible solutions. Because of these limitations on possible solutions, there may be instances where certain library sizes (or number of vessels) are inherently less efficient. In order to examine whether other, possibly more efficient, solutions exist in the neighborhood of the specified parameters, with slightly different constraints, a top level of evaluation was introduced for flexibility in the choice of the library size and maximum number of reaction vessels at each step. This allows the user to specify a range (in percent deviation) for the library size and maximum number of reaction vessels. All possible solutions within these ranges are then determined automatically. The last section of Table 1 ("variable library size") provides an accounting of the results for library sizes within 5% of 1000, 5000, 10 000, and 50 000 compounds. In comparison to the previous examples, the calculations times are now in the range of minutes. The increase in execution times relative to the corresponding execution times with only starting materials variable is simply related to the number of equivalent calculations that must be performed. Thus, for libraries within 5% of a library of 1000 compounds, the equivalent of 10% (i.e., ±5%) of 1000, or 100, additional calculations must be performed. The number of solutions in each of these cases is dramatically outside the scope of manual determination. In contrast to the results cited above for user specified or variable starting materials, the total number of solutions for each entry increases with nominal (e.g., 1000 ± 5%) library size. This is a consequence of the greater number of libraries represented by the common percentages (e.g., 100 additional libraries for a central value of 1000 versus 1000 for a central value of 10 000).

With the extensive data that may be generated in these calculations, the value of analysis tools becomes evident. Future plans include the development of an analysis system to facilitate the use of these tools by chemists.

Examples

To illustrate the approach outlined herein, we summarize in Table 2 the salient features of studies done on two combinatorial libraries described in the literature for libraries of 1000[11] and 30 752[12] compounds.

For a library of 1000 compounds, the number of possible solutions (i.e., strategies) is 552 when the number of vessels is limited to 100, but increases to 789 when 200 vessels are available. The solution with the minimal number of total steps is 74 in either case and is shown in Figure 6. In the case of 200 vessels, there are 20 different solutions which correspond to using values of 10, 10, and 10 ("10/10/10" solutions) for the starting materials for the three steps (see Table 3). These 10/10/10 solutions range in total cost from 90 to 330, i.e., over a 3-fold difference in total cost is possible depending on the strategies used even within the constraints of a specified set of starting materials at each step. Also, none of these intuitive 10/10/10 solutions yield the lowest costing solution (i.e., 74).

For the libraries of 30 752 compounds, there are 25 and 108 solutions possible with 100 and 200 reaction vessels, respectively. The solutions for 100 reaction vessels are shown in Table 4). Comparing the solutions with 100 reaction

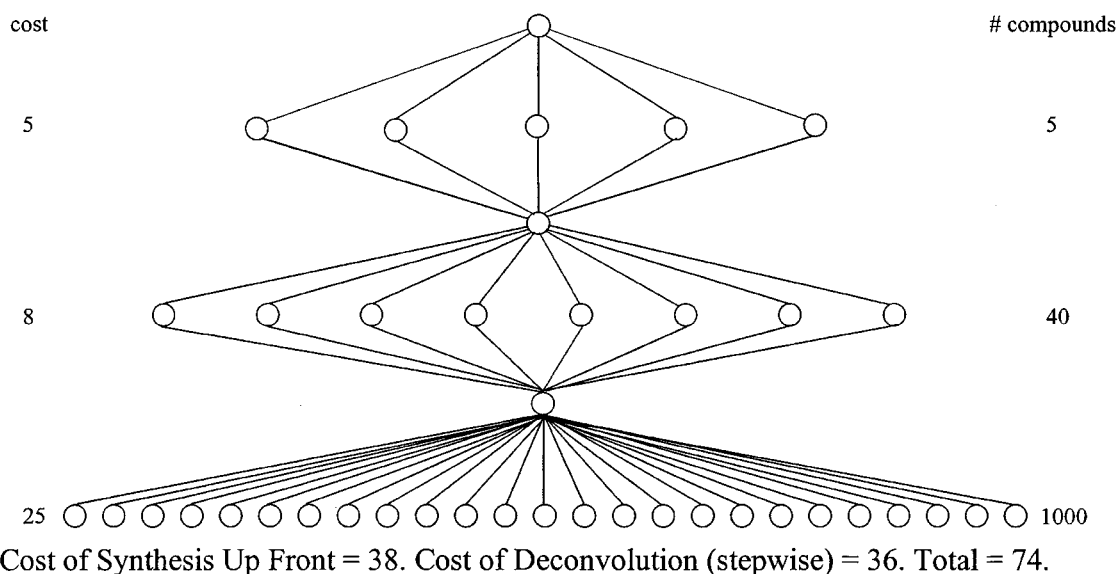## Schematic of the lowest cost solution for 1000 compounds and 3 steps

Cost of Synthesis Up Front = 38. Cost of Deconvolution (stepwise) = 36. Total = 74.

Figure 6. Schematic representation of the lowest cost solution for a library of 1000 compounds made with three steps (see text).

Table 2. Solutions for Libraries of 1000 and 30 752 Compounds

library size	number of reaction vessels	number of solutions found	lowest total cost	lowest total cost for libraries within 5% of nominal size
1000	100	552	74	73
1000	200	789	74	73
30 752	100	25	235	228
30 752	200	108	235	228

Table 3. Output Scores (10/10/10 splits only) for a 1000 Compound Library and 200 Reaction Vessels

lib	P1	S1	P2	S2	P3	S3	CUF	SWD	ASY	total
1000	1	10	1	10	1	10	30	60	0	90
1000	1	10	1	10	2	10	40	50	0	90
1000	1	10	1	10	5	10	70	44	0	114
1000	1	10	1	10	10	10	120	30	0	150
1000	1	10	2	10	1	10	40	65	0	105
1000	1	10	2	10	2	10	50	40	0	90
1000	1	10	2	10	4	10	70	30	0	100
1000	1	10	2	10	5	10	80	28	0	108
1000	1	10	2	10	10	10	130	24	0	154
1000	1	10	2	10	20	10	230	15	0	245
1000	1	10	5	10	1	10	70	116	0	186
1000	1	10	5	10	2	10	80	62	0	142
1000	1	10	5	10	5	10	110	28	0	138
1000	1	10	5	10	10	10	160	18	0	178
1000	1	10	10	10	1	10	120	210	0	330
1000	1	10	10	10	2	10	130	105	0	235
1000	1	10	10	10	4	10	150	53	0	203
1000	1	10	10	10	5	10	160	42	0	202
1000	1	10	10	10	10	10	210	21	0	231
1000	1	10	10	10	20	10	310	11	0	321

Table 4. Output Scores for a 30 752 Compound Library and 100 Reaction Vessels

lib	P1	S1	P2	S2	P3	S3	CUF	SWD	ASY	total
30752	1	8	1	62	1	62	132	156	0	288
30752	1	16	1	31	1	62	109	126	0	235
30752	1	16	2	31	1	62	140	164	0	304
30752	1	16	1	62	1	31	109	188	0	297
30752	1	16	1	62	2	31	140	126	0	266
30752	1	31	1	16	1	62	109	156	0	265
30752	1	31	1	31	1	32	94	186	0	280
30752	1	31	1	32	1	31	94	188	0	282
30752	1	31	1	32	2	31	125	156	0	281
30752	1	31	1	62	1	16	109	248	0	357
30752	1	31	1	62	2	16	125	186	0	311
30752	1	32	1	31	1	31	94	190	0	284
30752	1	32	2	31	1	31	125	204	0	329
30752	1	32	2	31	2	31	156	126	0	282
30752	1	62	1	8	1	62	132	264	0	396
30752	1	62	2	8	1	62	140	187	0	327
30752	1	62	1	16	1	31	109	280	0	389
30752	1	62	1	16	2	31	140	264	0	404
30752	1	62	2	16	1	31	125	219	0	344
30752	1	62	2	16	2	31	156	156	0	312
30752	1	62	1	31	1	16	109	310	0	419
30752	1	62	2	31	1	16	140	279	0	419
30752	1	62	2	31	2	16	156	186	0	342
30752	1	62	1	62	1	8	132	372	0	504
30752	1	62	1	62	2	8	140	310	0	450

vessels with the results for a library of 1000 compounds, it is striking that a 30-fold increase in the size of the library is accompanied by a 22-fold decrease in the number of possible solutions. This is primarily due to the fact that there are relatively fewer divisors (reflecting the possible numbers of starting materials) for 30 752 as well as the greater effect of the limitation to 100 vessels on the larger library. The design

of the library with 30 752 was based on a strategy wherein the starting materials were 32, 31, and 31 for the three steps. We note that for just the "32/31/31" strategies, there are six possibilities (assuming 200 reaction vessels), ranging in total cost from 284 to 491 (see Table 5), i.e., almost a 2-fold spread in values. *Thus, even within highly specified libraries, the ability to explore all possible strategies can have a profound impact on the cost alternatives.* There are three "31/32/31" and one "31/31/32" solutions. Of these various permutations, the value of the lowest costing solution is 280. The overall lowest cost solution for a library of exactly 30 752 is 235 (a "16/31/62" solution). In the final column of Table 2, we provide the lowest cost solutions for all

Table 5. Output Scores (32/31/31 splits only) for a 30 752 Compound Library and 200 Reaction Vessels

lib	P1	S1	P2	S2	P3	S3	CUF	SWD	ASY	total
30752	1	32	1	31	1	31	94	190	0	284
30752	1	32	2	31	1	31	125	204	0	329
30752	1	32	2	31	2	31	156	126	0	282
30752	1	32	4	31	1	31	187	304	0	491
30752	1	32	4	31	2	31	218	164	0	382
30752	1	32	4	31	4	31	280	94	0	374

libraries with a ±5% range of the indicated library size. For the case of libraries in the region of 30 752, we see that the lowest cost solution is 228 (for 100 or 200 vessels). While this is not significantly lower than the lowest costing solution for a library of exactly 30 752, we note that this result was obtained from generating all possible strategies, for the range specified. In this case there were 287 989 possible strategies. *The conclusion that there would be minimal gain in total cost (228 vs 235) from using the best solution in the defined range could clearly not be reached through manual examination of all possible (i.e., 287,989) strategies.*

Conclusions

A computational strategy has been developed to aid in the exploration and optimal design of schemes for splitting and pooling libraries of compounds. The present work is aimed at introducing a framework for such studies and has included a number of assumptions which may be modified at will. For some of these, e.g., number of starting materials at each step and number of vessels, we have illustrated the effect of variations of these parameters. By virtue of exhaustively determining all possible solutions and introducing scoring factors for the various steps, it becomes possible to evaluate all alternative schemes. This exhaustive evaluation can be done quickly and far exceeds what would be possible without such a computational approach. Moreover, the results give insight which can guide design strategies. It is clear that different strategies lead to different overall efficiencies. In some cases, efficiency will not be the highest concern, and other constraints, such as the desire to explore particular areas of molecular space, will drive the design of compound collections. Where such additional considerations may be less important, especially for libraries built for general lead finding, overall efficiency should be highly valued. With this first generation method to evaluate efficiency of split and mix library strategies in place, a logical next step would be to compare the overall efficiencies of other approaches commonly in use, such as mix and sort, parallel synthesis, bead tagging, etc. A more sophisticated treatment should evaluate additional cost parameters such as cleavage, registration, inventory, sorting steps, tagging steps, and multiple active compounds.[13]

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(10) Partial tagging can be considered to include any approach where at least one, but not all, of the steps are encoded by some tag, including molecule tags, isotopic tags, radio frequency tags, colored supports, etc.

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