Euclidean shape-encoded combinatorial chemical libraries

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A method for the encoding of split/mix combinatorial chemical libraries based on Euclidean shapes is described. The shapes are fashioned from a polymeric matrix designed to swell in common organic solvents while retaining their unique forms, and exhibit good mechanical strength. The lightly crosslinked gel-type polymer was processed into an array of Euclidean forms that serve as encoding elements in the synthesis of combinatorial chemical libraries by using the split/pool methodology. To assess the viability of this approach, a library of compounds based on a urea scaffold was prepared. The validity of this methodology was demonstrated through correct deconvolution of the library mixture by shape discrimination. Furthermore, because the shapes used have a large surface area to volume ratio, each monolith can act as an independent chemical reactor. This simplifies the analytical identification process because each compound can be prepared in significant quantities and isolated as single entities. Given the high loading capacity of the monoliths and the conceptually simple encoding strategy, it is envisioned that these Euclidean forms will find significant application in combinatorial and high-throughput synthetic chemistry.

Combinatorial synthesis, a format in which the number of chemical reactions performed is less than the number of compounds synthesized, provides a means of rapidly generating libraries of compounds (1, 2). Indeed, synthetic chemical libraries produced by combinatorial synthesis techniques have rapidly become important tools for drug discovery in the pharmaceutical industry. One of the more elegant and popular combinatorial techniques is the split/pool method, which allows the assembly of a statistical sampling of all possible combinations of a set of chemical building blocks (3). Hand-in-glove with combinatorial library synthesis is a need for rapid determination of the structural identity of the active library members. This, in fact, has represented a major hurdle because each library member is usually prepared in subanalytical quantities. To overcome this limitation, many ingenious methods for library member identification have been devised, including deconvolution strategies (4, 5) and encoding with molecular (6–10), radiofrequency (11, 12), or isotopic tags (13).

Although there have been many strategies used for the preparation of chemical libraries, a key element to successful library synthesis is the support on which it is prepared and how the support allows for easy compound isolation and purification. As such, library synthesis has typically been achieved by using solid- (14, 15) or liquid-phase techniques (16). With regards to solid-phase supports, emphasis has been devoted to adapting synthetic regimens to accommodate the polymeric support, rather than the converse (17). Only recently have reports appeared describing the development and use of new polymeric platforms for library synthesis. Paramagnetic (18), dendrictic (19), and "large" resins (20) as well as monolithic disks (21, 22) have been introduced to meet the complicated demands of high-throughput synthesis.

To increase the potential applicability of combinatorial chemistry, we set out to design a new strategy for the synthesis of combinatorial libraries in which the polymeric support itself serves as an encoding element. Our goal was to devise a straightforward means of encoding based on simple Euclidean shapes. With judicious size considerations, a variety of monolithic shapes can be produced having identical surface areas/volumes while having different minimum diameters. Having the monoliths conform to identical surface area/volume ratios is crucial for uniform mass-transfer of reagents through the range of shapes and therefore reaction reproducibility from monolith to monolith. The criterion of different minimum diameters is of benefit in terms of separation by mechanical sorting.

It should be noted that the same level of encoding could be accomplished by using different diameter polymeric spherical beads. However, drawbacks to this approach include the facts that that sorting by size of small spherical particles is inherently inefficient (23) and that, as the attached molecules become larger, the diameter of the beads change and the size discrimination ranges are blurred. Furthermore, the use of simple sized spheres results in different surface areas, volumes, and loading capacities per bead, all of which create undesirable heterogeneity in the reaction mixture.

In the present study, five Euclidean forms were selected, and monoliths 2 mm in thickness were prepared. The shapes chosen were circles, triangles, squares, pentagons, and hexagons. By tailoring their dimensions [(*i*) circle diameter of 5.0 mm; (*ii*) triangle of 5.9 mm/side; (*iii*) square of 3.3 mm/side; (*iv*) pentagon of 4.7 mm/side; and (*v*) hexagon of 3.3 mm/side], monoliths having the same surface area and volume but different minimum diameters were obtained. For successful application of a Euclidean approach, the crosslinked polymer must be resilient enough to accommodate agitation such as magnetic stirring, manipulation with tweezers, or sieving. In addition, the shapes need to be able to swell sufficiently so reactions could ensue within the matrix while maintaining their form over the course of several reactions. We therefore prepared the monoliths from polystyrene incorporating a flexible crosslinking agent, 1,4-bis(vinylphenoxy)butane, that has been reported to impart such properties (24).

Materials and Methods

Reagents and General Methods. All starting materials were purchased from Aldrich. 1,4-bis-(vinylphenoxy)butane was prepared as described (25).

Preparation of the Polymeric Monoliths. Argon was bubbled through a mixture of styrene (34 ml, 0.3 mol), 4-vinylbenzyl chloride (6 ml, 0.04 mol), 1,4-bis(vinylphenoxy)butane (3.12 g, 0.01 mol), benzyl peroxide (600 mg, 0.003 mol), and dodecane (20 ml) for 20 min. The mixture was transferred to glass molds, and the molds were sealed with septa and heated at 85°C for 18 h. The glass molds were carefully removed, and the rods of polymer were subjected to soxhalet extraction with acetone for 16 h. The polymer rods were cut into circles, triangles, squares, pentagons,

Abbreviations: DMF, dimethylformamide; TFA, trifluoroacetic acid.

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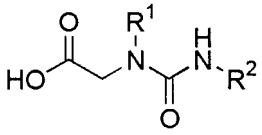
and hexagons by using a razor blade and stencil. The degree of swelling of the monoliths was determined by equilibrating the pieces in the organic solvents for 2 h and then measuring the change in volume with a caliper.

Model Synthesis of a Urea Library Member. The Wang linker (26) was appended to the chloromethyl groups by treatment with 4-hydroxybenzyl alcohol and sodium methoxide in dimethylformamide (DMF) at 85°C for 36 h (27). Five monoliths, one of each shape, were treated with CH_2Cl_2 (2 ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.35 ml) for 15 min. The mixture was cooled with an ice bath, and bromoacetyl bromide (0.4 ml) was added dropwise. The mixture was stirred magnetically for 24 h at room temperature. The liquid was decanted, and the monoliths were washed with CH_2Cl_2 (3×10 ml for 1 h). The monoliths were added to a solution of 1,4-dioxane (5 ml), diisopropylethylamine (0.2 ml), and hexylamine (0.2 ml), and the mixture was heated at 85°C for 24 h. The liquid was decanted, and the shapes were washed with CH_2Cl_2 (3×10 ml for 1 h). The shapes were mixed with CH_2Cl_2 (5 ml) and iso-butylisocyanate (0.2 ml), and the mixture was stirred for 24 h at room temperature. The liquid was decanted, and the monoliths were crushed into a powder. The five crushed monoliths were washed with CH_2Cl_2 (3×10 ml for 1 h), and examination of the last wash material showed no compounds detectable by ^{1}H NMR. The crushed monoliths were treated with 1:1 (vol/vol) trifluoroacetic acid (TFA):CH_2Cl_2 (2 ml) for 3 h. The solid polymeric material was removed by filtration, and the filtrate was concentrated to afford the product as a clear oil (4 mg, 53%): ^{1}H NMR (400 MHz, CD_3OD): δ 1.2–2.3 (multiple signals, 22 H), 3.80 (s, 2 H, CH_2), high resolution mass spectrum calculated for $C_{13}H_{26}N_2O_3$: 258.1938; found: 258.1931.

Library Synthesis. Thirty monoliths of each shape (circles, triangles, squares, pentagons, and hexagons, Wang form) were equilibrated with CH_2Cl_2 (50 ml) and DBU (4.1 ml) for 30 min in an ice bath. Bromoacetyl bromide (4.8 ml) was added dropwise, and the mixture was stirred magnetically at room temperature for 24 h. The solvent was removed, and the shapes were stirred magnetically with CH_2Cl_2 (3×50 ml for 1 h). The monoliths were distributed into separate flasks based on each unique shape. To each flask was added an amine (circle, benzyl amine; triangle, *iso*-butylamine; square, hexylamine; pentagon, *meta*-methoxybenzylamine; hexagon, *para*-fluorobenzylamine) in a mixture of 1,4-dioxane (10 ml) and diisopropylethylamine (0.4 ml). The mixtures were heated at 85°C for 24 h. The liquid was decanted, and the monoliths were washed with CH_2Cl_2 (3×50 ml for 1 h). The monoliths were redistributed to afford five separate flasks, each containing five monoliths of each shape. Each flask was treated with an isocyanate (phenyl isocyanate, cyclohexyl isocyanate, *tert*-butyl isocyanate, *n*-propyl isocyanate, *para*-fluorophenyl isocyanate) in CH_2Cl_2 (10 ml) for 24 h. The monoliths from each flask were regrouped into five sets according to shape to give a total of 25 sets. The shapes were crushed and washed in CH_2Cl_2 (3×50 ml for 1 h). Cleavage of the product urea from the polymer matrix was accomplished by stirring with 1:1 (vol/vol) TFA:CH_2Cl_2 (2 ml) for 3 h. Yields of individual library members are presented in Table 1.

Table 1. Yields of ureas formed using Euclidean shapes



Entry	R^1	R^2	Yield* mg	Yield* %
1	Bn	Ph	3.4	4.5
2	Bn	Cy	4.2	5.6
3	Bn	*t*-Bu	4.9	6.5
4	Bn	*n*-Pr	4.1	5.5
5	Bn	4-F-Ph	3.8	5.1
6	*i*-Bu	Ph	4.5	6.1
7	*i*-Bu	Cy	4.6	64
8	*i*-Bu	*t*-Bu	3.5	47
9	*i*-Bu	*n*-Pr	3.9	56
10	*i*-Bu	4-F-Ph	4.2	55
11	*n*-Hexyl	Ph	4.6	50
12	*n*-Hexyl	Cy	4.8	51
13	*n*-Hexyl	*t*-Bu	3.5	54
14	*n*-Hexyl	*n*-Pr	4.2	56
15	*n*-Hexyl	4-F-Ph	4.1	60
16	3-MeOBn	Ph	3.7	60
17	3-MeOBn	Cy	3.8	61
18	3-MeOBn	*t*-Bu	0.0	0
19	3-MeOBn	*n*-Pr	4.2	52
20	3-MeOBn	4-F-Ph	4.5	56
21	4-F-Bn	Ph	5.0	67
22	4-F-Bn	Cy	3.7	49
23	4-F-Bn	*t*-Bu	4.4	59
24	4-F-Bn	*n*-Pr	4.7	63
25	4-F-Bn	4-*F-Ph*	4.8	64

*Yields based on loading of 0.15 μmol per shape. Purity in all cases was >90% as determined by ^{1}H NMR.

Results and Discussion

In assembling the monoliths, we needed a polymeric matrix that would meet the requirements described (*vide supra*). As a starting point, we prepared a polymer comprised of 1,4-bis(4-vinylphenoxy)butane as a crosslinking agent and styrene as the monomer. This combination affords a gel-type resin that swells to a great extent in a variety of solvents (24). In an attempt to optimize this property, various combinations of the degree of crosslinking, the amount of initiator, and the diluent were examined. The optimized combination was found to be 3 mol% 1,4-bis(4-vinylphenoxy)butane, 12 mol% 4-vinylbenzyl chloride, styrene, benzoyl peroxide (wt 1%), and dodecane. This mixture was found to afford a material that exhibited the requisite swelling properties and also had the necessary mechanical strength and was used to prepare the monoliths. Polymeric rods were prepared by polymerization in sealed glass molds, and these rods were subjected to extraction with acetone to remove unreacted monomer. The acetone swollen polymer rods could be cut into the desired shapes by using a razor blade and a stencil guide. The shapes were found to swell up to 5 times their original volume in solvents commonly used for solid phase organic synthesis, including dichloromethane, dioxane, DMF, toluene, and THF, while retaining their form. Importantly, the Euclidean monoliths prepared in this way were stable to mechanical stirring and to manipulation with metal tweezers. Fig. 1 depicts the monoliths in their dry and 1,4-dioxane-swollen states.

It is well known that diffusion of a solvent into small, spherical gel-type resin beads, with their high surface area to volume ratio, is rapid. Diffusion into a much larger monolith, with reduced surface area to volume ratio can be retarded. Thus, to ensure complete reaction and later removal of unreacted reagents and byproducts from the monoliths, an extended time period for all chemical processes and washings was to be expected. To determine what length of time would be required, the pre-swollen rods were immersed in a solution of bromophenol blue in either dichloromethane, DMF, or THF. The diffusion of bromophenol blue sodium salt was studied because it is a relatively large (molecular weight: 691.97) charged species and should approx-

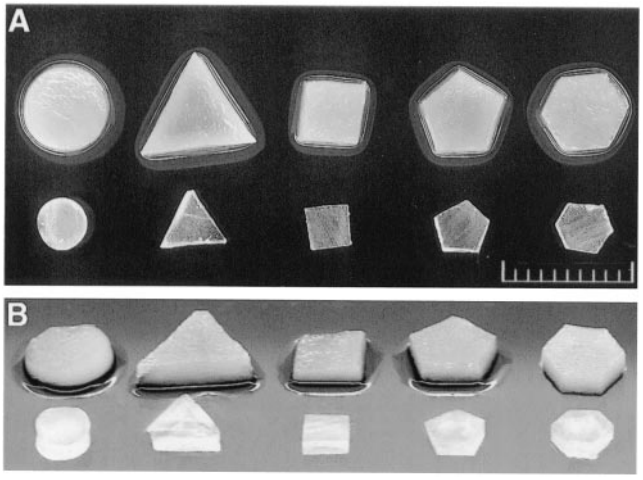


Fig. 1. Photograph of Euclidean shapes in both dry and swollen states. (*A*) Top view of the shapes. (*B*) Photograph of shapes at a 45° angle. In both photographs, the shapes swollen in 1,4-dioxane are on top, with the dry shapes below. The scale is in millimeters

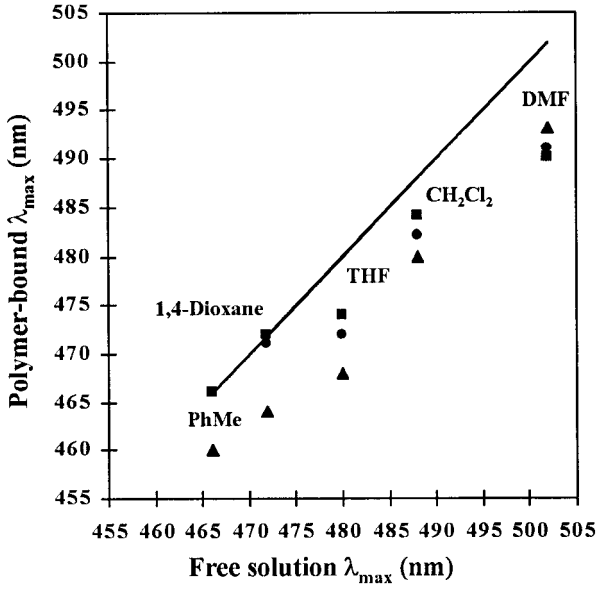


Fig. 2. Emission maxima of free dansyl monomer vs. emission maxima of dansyl monomer polymerized in the form of a shaped disc: ▲, 2% CL Merrifield resin; ●, 2% CL *J*anda*J*el; ■, Lucky Charms.

imate a minimum rate of diffusion. The permeation of the dye into the rod was performed so that dye entered the rods only from the sides. By using this approach, it was determined that complete coloration of the disk (5 mm in diameter) occurred after 4 h in DMF, after 5 h in dichloromethane, and was not complete after 6 h in THF. Because the monoliths planned for use in this study had a greater available surface area than the rods, these times were considered benchmarks with regards to chemical process reaction time to be used in our synthetic procedures.

To complement the diffusion studies, a second investigation was initiated to examine the microenvironment of the monoliths. Monoliths doped with a dansyl fluorophore were synthesized, and the emission maxima of the bound dansyl probe was compared with that of the dansyl probe free in solution. This methodology has been previously used in the study of polymer/solvent interactions (28) and is of particular interest because it directly measures interactions between the polymer backbone and solvent. Furthermore, this measurement is not complicated by the porosity of the material, a substantial concern when measuring swelling simply by changes in volume. Comparison of the freely solvated probe to the bound probe is presented graphically in Fig. 2. What can be gleaned from this plot is that the closer the observed emission maxima is to the free solution emission maxima, the closer the approximation to free solution behavior. Values for spherical beads of 2% crosslinked Merrifield resin and 2% crosslinked *J*anda*J*el are included for comparison. Interaction between the fluorescent probe and the solvent was as good as, if not better than, the interaction between solvent and resin beads. However, it should be noted that equilibration between the monoliths and the solvent was performed over several hours to negate difficulties in mass-transfer.

To examine the synthetic utility of the Euclidean monoliths with the ultimate goal of generation of combinatorial libraries, we synthesized a urea library (Table 1) via a linear synthetic sequence. Urea moieties represent a common motif in pharmacologically active compounds (29, 30), and their synthesis would require several synthetic manipulations on the monoliths. Addition of the Wang linker to the chloromethyl group of each of the differently shaped monoliths was achieved by treatment with 4-hydroxybenzyl alcohol and sodium methoxide in DMF at 80°C (27). Loading was measured by the Fmoc displacement method (31) and was found to be 0.5 mmol/g. Because the average weight of each monolith was 30 mg, this represents a loading of 15 μmol/piece. By comparison, a single Merrifield resin bead (loading capacity 1.0 mmol/g) of diameter 200 μm has the potential to load 40 nmol of material [a recent report concerning dendritic resin bead claims a loading capacity of 200 nmol/bead (32)]. The high loading on an individual monolith is of great relevance as each piece can be considered an individual chemical reactor. Furthermore, because of the relatively large quantity of compound prepared per monolith, structure identification can be accomplished by standard analytical techniques. It should be noted that the loading level is a measure of the effective loading of the monolith and will clearly be influenced by permeation through the polymer matrix.

Treatment of the Wang form of each shape with bromoacetyl bromide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene in CH_2Cl_2 for 24 h effected attachment of a bromoacetate group (Fig. 3). As mentioned previously, permeation through the polymer can be slow; thus, after every reaction, each shape was extensively washed with dichloromethane. The monoliths were then treated with hexylamine in 1,4-dioxane and diisopropylethylamine at 85°C for 24 h and then were treated with iso-butyl isocyanate in CH_2Cl_2 for 24 h. The shapes were crushed into pieces and allowed to equilibrate in dichloromethane overnight.

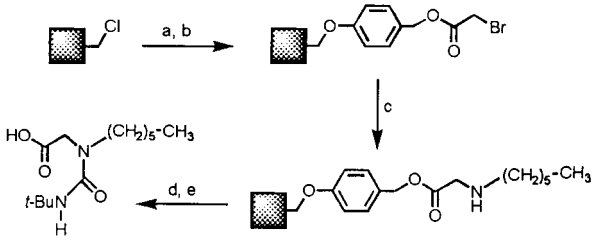


Fig. 3. Reaction sequence for preparation of the urea library. a, 4-hydroxybenzyl alcohol, NaOMe, DMF; b, bromoacetyl bromide, DBU, CH_2Cl_2; c, hexylamine, diisopropylethylamine, 1,4-dioxane; d, iso-butyl isocyanate, CH_2Cl_2; e, 1:1 (vol/vol) TFA:CH_2Cl_2.

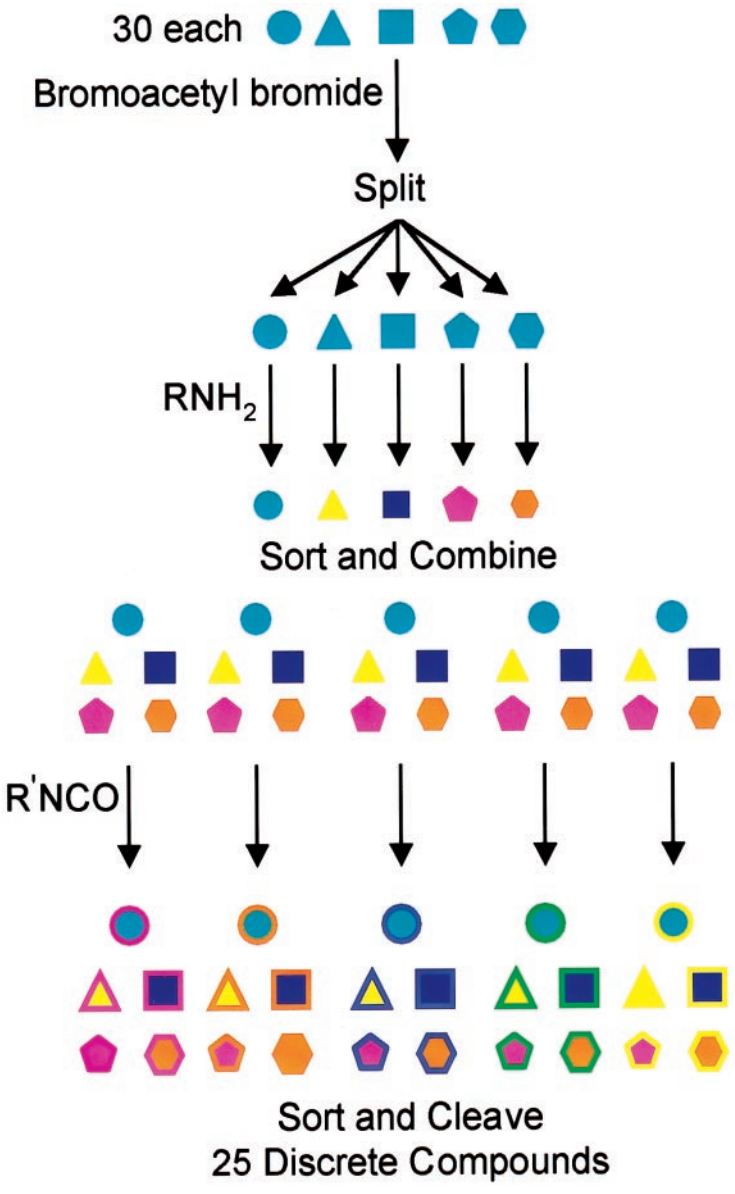


Fig. 4. Split-mix sequence for the formation of ureas on the Euclidean shapes. Amines (R) are depicted as the core color, isocyanates (R′) as the peripheral color.

The solvent was decanted, and fresh CH_2Cl_2 was added. After 2 h, the CH_2Cl_2 was removed by filtration and concentrated. Examination of this residue revealed no detectable product. This confirmed that chemical synthesis was not occurring as a simple trapped species within the polymer matrix. Compound cleavage from the crushed resin was accomplished with 1:1 TFA:CH_2Cl_2. The crushed resin was removed by filtration. The product was judged to be pure by ^{1}H NMR, and the overall yield for the three reactions was 53%.

With the success of the model synthesis, the next step was to prepare a combinatorial library. To begin the split-mix regime, 150 monoliths (30 of each unique shape) were mixed, and bromoacetate groups were attached as described above. After washing, the monoliths were partitioned according to shape (Fig. 4). Separation of the 150-member mixture by shape into five flasks with a pair of tweezers was readily accomplished in about 5 min. Each flask was treated with a different amine (benzyl, *meta*-methoxybenzyl, *iso*-butyl, *n*-hexyl, or *para*-fluorobenzyl) at 85°C in 1,4-dioxane with diisopropylethylamine for 24 h. The monoliths were washed extensively with CH_2Cl_2 and were distributed into five flasks with each flask containing five each of the differently shaped monoliths. The flasks were then treated with an isocyanate (phenyl, cyclohexyl, *tert*-butyl, *n*-propyl, *para*-fluorophenyl) in CH_2Cl_2 for 24 h. The monoliths from each of the five flasks were redistributed a final time such that each flask contained a unique compound. Because encoding was no longer needed after this last step, the monoliths were crushed as described (*vide supra*) to permit faster mass transfer, and compounds were cleaved from the polymer by using 1:1 TFA:CH_2Cl_2. Yields of the isolated compounds are presented in Table 1. Unfortunately, one synthesis failed. In all other cases, the compound predicted from tracking of the encoding element was observed.

In summary, we have developed novel polymeric monoliths based on Euclidean shapes on which combinatorial split/pool synthesis can be performed. By formation of the crosslinked polymer into shapes, encoding and deconvolution of mixtures of products from a single reaction is achieved in a very simple manner. The shapes were applied to a split/pool combinatorial synthesis of a library of ureas. A total of 24 different com-

pounds were prepared by using only 11 reactions. Preparing the same library by conventional parallel synthesis would have required 30 reactions. Furthermore, the loading capacity of one of the monoliths is approximately 4,000 times greater than that of a typical resin bead and therefore allows the individual compounds to be prepared in quantities that permit conventional spectroscopic characterization. While we have demonstrated the validity of our Euclidean approach to the synthesis of a library of 24 compounds, we envision that it will be applicable for much larger libraries. To increase the sophistication of this system, we are currently synthesizing additional shapes, some of which incorporate fluorescent dyes. We believe that the use of approximately 30 shapes in combination with fluorescent dyes will allow for encoding of libraries of approximately 1,000 members. Finally, because of the simplicity and variety of the shapes used, we consider this new encoding technique to be ''lucky charm''-like, in analogy with a popular breakfast cereal.

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