I. SOLUTION-PHASE COMBINATORIAL CHEMISTRY: THE ACTIVATED CORE APPROACH

II. PORPHYRIN-BASED SMALL MOLECULE RECEPTORS

by

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ABSTRACT

A solution-phase combinatorial technique which combines the numerical advantages characteristic of a solid-phase split synthesis with the reaction versatility and quantity advantages of the solution-phase was used to create libraries based on a number of rigid core molecules. The strategy is conceptually simple and involves the simultaneous addition of a number of building blocks to an activated core molecule, generating up to tens of thousands of condensation products in a one-pot procedure. The design of its three components— the building blocks, linkages, and core structures— has been optimized to give pure, statistical, small-molecule libraries capable of interacting with biological structures. A number of analytical methods, including CE-MS, LC/MS, and HPLC, have been utilized to examine the reactivity of the individual systems, and the results have supported our claims that the mixtures are statistical ensembles of the desired molecules.

Several libraries have been screened for biological interactions and mixtures active in binding to DNA, in killing cancer cells, and in preventing HIV infection have been found. To ascertain which individual component(s) of the library mixtures were responsible for activity a process known as deconvolution was implemented whereby smaller mixtures of compounds were synthesized iteratively. This process has successfully reduced an active mixture of 2,080 xanthene tetraureas to two unique compounds capable of binding to DNA.

Activated core molecules that have been synthesized to expand this approach to chemical diversity include tetraacid chlorides of 2,4,5,7-xanthene, 1,3,5,7-adamantane, 2,2′,6,6′-biphenyl, 2,2′,4,4′-biphenyl, and the triacid chloride of *trans*-1,3,5-cyclohexane. Isocyanate-based linkages have also been installed on the 2,4,5,7-xanthene and 1,3,5,7-adamantane cores. The acid chloride and isocyanate functionalities provide two types of conformationally rigidified linkages, amides and ureas, upon condensation with amines. Molecular modeling of representative structures has been performed to assess the orientation of the building blocks and estimate the spatial coverage provided by different core molecules.

Porphyrin-based molecules with convergent recognition groups have been synthesized and characterized. Molecular modeling suggested that a number of 5- and 6-membered ring compounds would be complementary to the relatively small cavity. These host molecules were observed to bind guests with a range of association constants from 43 to $>10^5$ M^{-1} in chloroform with concomitant large upfield shifts (up to 4.4 ppm) in certain guest ^{1}H NMR resonances. The synthesis of a porphyrin containing asymmetric functional groups was also completed.

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to Lori

and my Parents

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I. SOLUTION-PHASE COMBINATORIAL CHEMISTRY: THE ACTIVATED CORE APPROACH

A. Simultaneous, Solution-Phase Combinatorial Chemistry: An Overview

A1. Introduction: Combinatorial Chemistry

One of the most rapidly developing areas in science is combinatorial chemistry, and advances in high-throughput screening, automation, analytical techniques, and synthetic methodology have emerged to facilitate its practice. While definitions of combinatorial chemistry differ,[1] Dennis Curran's description seems appropriate: "any method which multiple reaction products are made together or in parallel by a common sequence of reactions."[2] While early combinatorial efforts focused on peptides or oligonucleotides, recent applications have centered on more pharmacologically-relevant[3] small molecules. Although the majority of chemical diversity is still practiced on insoluble solid supports,[4] a number of solution-phase techniques[5,6] have also been devised, including the approach elaborated in this chapter.

The products of combinatorial synthesis are collections of related molecules or chemical libraries, and they have been screened for a wide variety of structure-function relationships, but these screens have, for the most part, focused on biologically-relevant activity. However, several recent studies have demonstrated that libraries can be screened for other purposes, including catalysis,[7-10] metal coordination,[11-13] and even magnetism[14] and superconductivity.[15] Several recent review articles[16-20] have summarized published work; however, many industrial studies remain undisclosed as

proprietary secrets. In this chapter an overview of combinatorial chemistry will be presented, organized according to the phase (solid or solution) in which the synthesis is conducted. In addition, techniques for library component identification (encoding, indexed libraries and deconvolution) will also be discussed. Subsequent sections will highlight the advantages of a solution-phase approach which uses activated core molecules to generate large libraries in a one-pot procedure. An analysis of this approach will expand upon the design principles, analytical techniques, and methodology for identifying active compounds in large mixtures. Finally, recent experimental work related to previous studies will be presented.

A2. Solid Phase Combinatorial Synthesis.

The advantages of using solid supports[4] are derived from the fact that the intended reaction site is appended to something insoluble; solution-phase impurities, excess reagents used to drive the reaction to completion, and solvents can all be eliminated via rinsing and filtration. The products can then be reacted in subsequent steps, or liberated from the resin by chemical or photochemical cleavage. For systems where a number of consecutive reactions need be performed, the solid phase permits use of another technique, known as mixing and splitting (**Figure 1**), which can generate small quantities of relatively large numbers of compounds (over 100,000). As only one type of reagent is generally employed per reaction vessel, one need not worry about the relative reaction rates of two (or more) different reagents. However, there are practical drawbacks to solid-phase chemistry, including the high cost of some resins, limits on the quantity of materials that be synthesized, and the need to optimize attachment and detachment protocols. Other chemical problems have also been observed from the heterogeneous reaction conditions, including nonlinear kinetic behavior, solvation problems, and the inability to use other insoluble reagents (especially catalysts). Finally, while many solution-phase reactions

have been translated to solid supports, the scope of reactions that can be performed has yet to be completely realized.

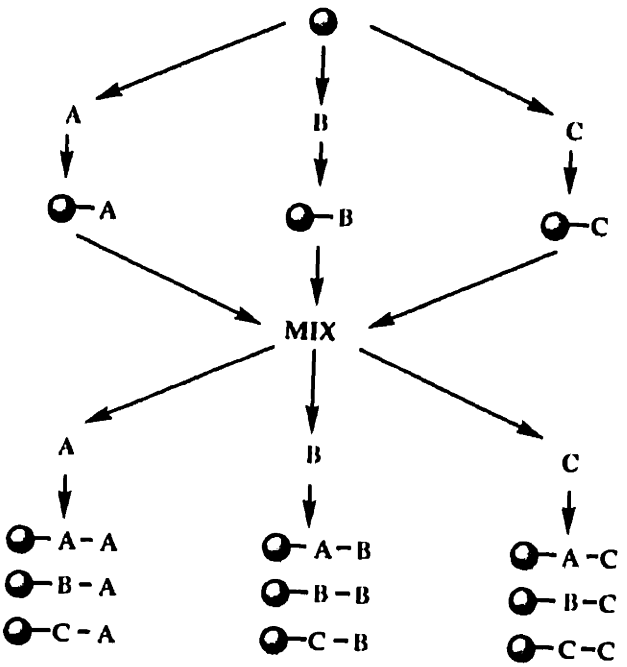


Figure 1: The mix and split method (also known as split synthesis) applied to the solid-phase generation of nine compounds. The resins (beads) are pooled after each step to effect randomization and also to save time in preparing (activating) the beads for the next round of synthesis. The number of compounds generated in this type of linear oligomer synthesis by *m* number of building blocks over n rounds is m^n.

A2-1. Identification of Active Compounds Derived from Solid-Phase Synthesis

The products of split synthesis, resin-bound small molecules, are usually separated (cleaved) from their resin and tested in solution, although on-bead assays have also been performed.[13,21] A typical example of the process begins by sequestering the beads into a 96-well formatted plate (10-15 per well) from the final round of split synthesis, liberating the compound from the resin, then screening mixtures of 10-15 compounds. In this example only the last building block is known, and only small quantities of each molecule are available, too little for standard characterization methods. A number of novel characterization techniques have been devised, including

embedding memory chips with radio frequency transponders into each bead,[22,23] spatially isolating compounds using lithographic techniques and light-directed activation,[24,25] and employing a type of recursive deconvolution known as positional scanning.[26,27] Here we will focus on two other techniques, encoding (tagging) and orthogonal (self-deconvoluting) libraries.

Chemical or biochemical tags can be incorporated onto (or into) the solid support following each step of the split synthesis. Biochemical tags (oligonucleotides) can be amplified using PCR to increase their quantity,[28] however their lability has limited their use. More inert chemical tags containing easily detected functional groups have been more generally applied.[29,30] An example is the polychlorinated aromatic tags used by Pharmacopeia, Inc. (**Figure 2**). Each tag corresponds to a synthetic building block, and a unique tag is inserted into the polymeric resin matrix following each round of split synthesis. The tags are removed selectively using ceric ammonium nitrate (CAN), silylated, and detected at very low concentrations using electron capture gas chromatography (ECGC).[29] The retention times of the peaks in the ECGC trace allow for identification of the compound as the tags correspond not only to particular building blocks, but also to the order they were used (a chemical 'bar code'). When the bead-derived compounds are assayed for activity, the parent beads can be individually isolated and decoded. The active compound can then be made in larger quantity for verification and traditional characterization.

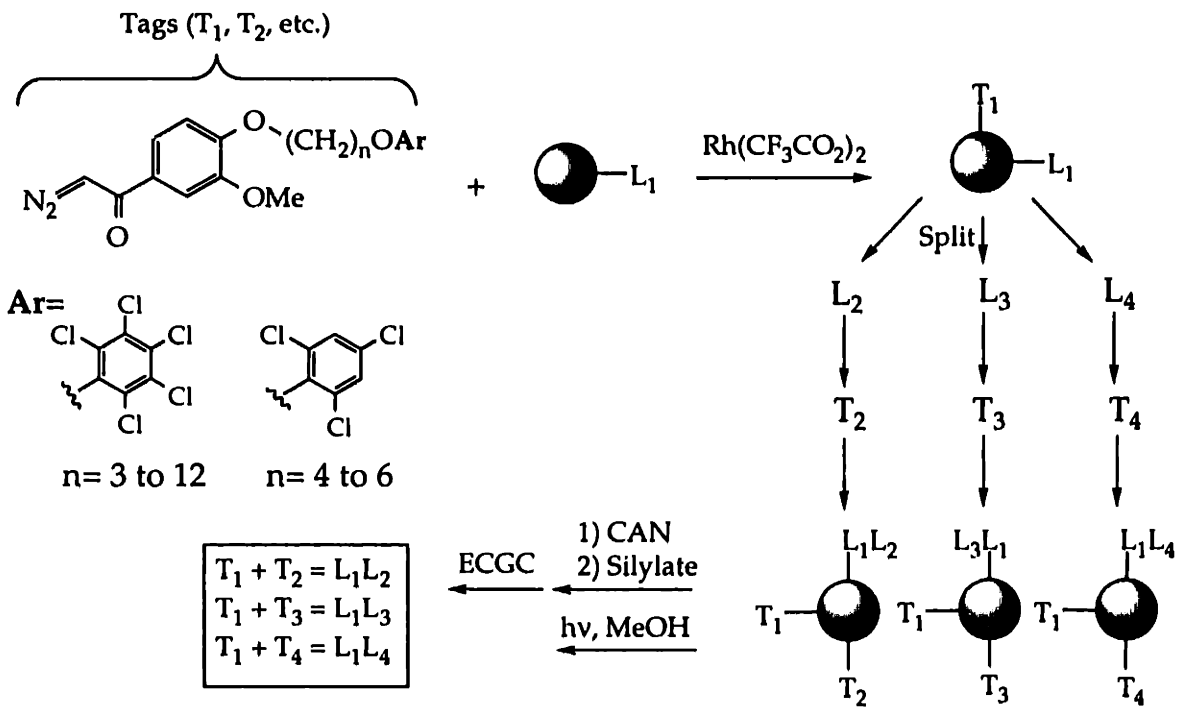


Figure 2: Pharmacopeia's tagging methodology as applied to one round of a split synthesis. L_{1-4} represent the building blocks of the small molecule being synthesized. The final steps indicate the isolation of a particular bead, photochemical cleavage of the small molecule from the resin, oxidative (CAN) liberation of the characteristic tags, and tag analysis using ECGC.[29]

A second method has been devised by André Tartar and coworkers at Glaxo which allows for rapid screening of libraries and identification of actives (**Figure 3**).[31] The Tartar approach is based on a modified split synthesis where instead of pooling (mixing) the beads after every round of reactions they are divided (split) into more vessels (for subsequent reactions) and their locations are noted. Because the sequence of added reagents is known for each bead, one (in principle) knows the identity of the final compounds. The power of this approach is derived from the fact that, in each reaction step, mixtures (rather than one amino acid/well) of five amino acids (subsets A1-A5, **Figure 3**) are used. The implication is that the final tripeptides are obtained as 5^3 or 125 different products, rather than the one-bead-one-compound standard in split synthesis. A second library is made using the same twenty five amino acids (subsets B1-B5), but they are grouped such that only one amino acid from any given 'A' subset is used by only one

'B' subset; resulting in a reagent grid. If one final, bead-derived mixture made with the 'A' subsets (for example A1, A2 then A5) was active and one library from the 'B' subset (B2, B2 then B5) was active, then the active tripeptide would be composed of the three building blocks which intersect on the grid. In this example A1/B2= Arg, A2/B2= Ile, and A5/B5= Phe and the active tripeptide would be Arg-Ile-Phe. While this method can be applied to systems which do not permit chemical encoding, if the amino acids within each set are incorporated into the peptide in a biased manner, the actual number of compounds which are assayed may be less than the theoretical number. These type of approaches have been termed orthogonal or indexed, and have recently been applied to other systems.[32,33]

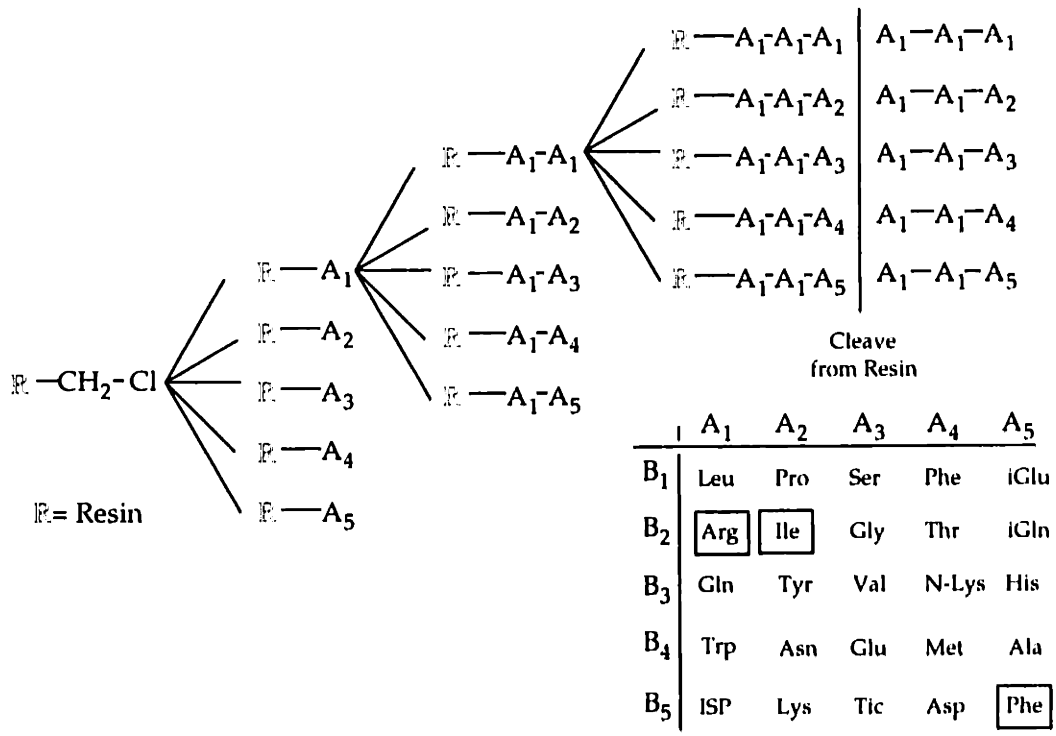


Figure 3: The Tartar method for creating orthogonal chemical libraries.[31] Only one branch of the synthesis is expanded for clarity. A second library (not shown) is made using the B1-B5 subsets, such that two 15,625-compound libraries are created, and any sublibrary from A and any sublibrary from B share only one trimer. The boxed amino acids refer to the in-text example.

A3. Liquid- and Fluorous-Phase Combinatorial Techniques.

Two new methods have been developed which straddle the division between the solid and solution phase. Kim Janda and coworkers at the Scripps Research Institute have developed a liquid-phase combinatorial synthesis that utilizes a polymer (polyethylene glycol monomethyl ether) which is soluble in the reaction medium (a number of organic or aqueous solvent systems), but can be precipitated with ether or ethanol (**Figure 4**).[34,35] The reaction conditions are homogenous, avoiding some pitfalls associated with insoluble resins, and a filtration eliminates unwanted materials from the polymer-bound products.

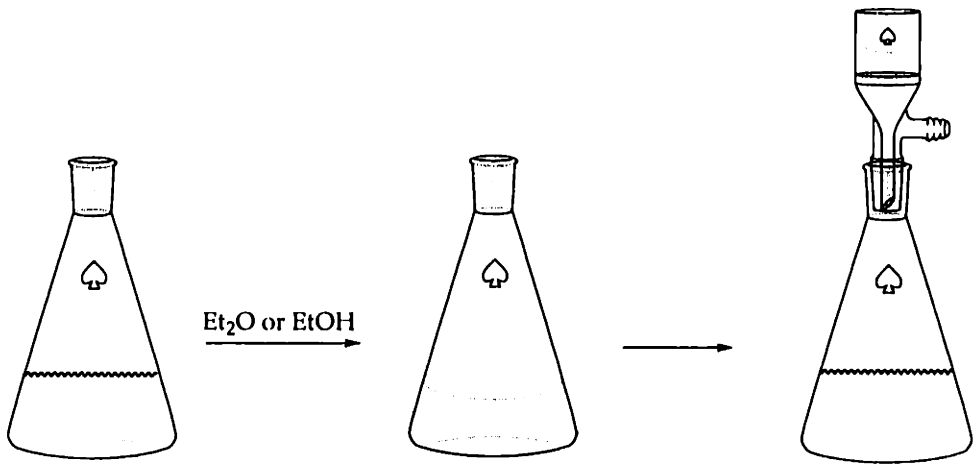


Figure 4: In Janda's liquid-phase approach the substrate is attached to the polymer support and dissolved in (1:1) DCM:DMF (left), permitting coupling-type reactions. Upon the completion of the reaction ether or ethanol is added, causing the polymer to precipitate (middle). After the polymer is filtered, rinsed, and dried (right) it is ready for the next round.[36,37]

The second approach was developed by Dennis Curran at the University of Pittsburgh and utilizes the insolubility of fluorocarbons in either organic or aqueous phases.[2,38] This approach was initially motivated by the need to efficiently eliminate trialkyl tin by-products from reaction mixtures (see **Figure 5**).[38] The success of some initial experiments has led to proposed extensions to combinatorial synthesis (**Figure 5**).[2,39] Unlike Janda's filtration-based approach, an extraction is used as fluorocarbons typically have

high partition coefficients between fluorous and organic media. While these two methods seem likely to contribute to combinatorial synthesis, it remains to be seen how applicable they will be to more diverse reactions that are already practiced on either the solid or solution-phase.

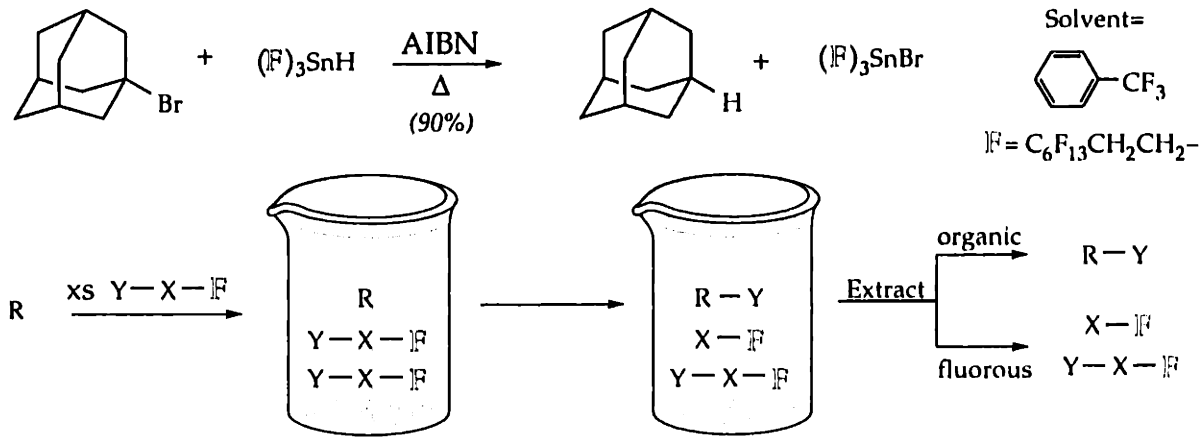


Figure 5: The practice and principle of fluorous-phase chemistry. The above methodology should be applicable to combinatorial chemistry (bottom).[2]

A4. Solution-Phase Combinatorial Chemistry

In theory, solution-phase combinatorial synthesis offers many advantages over the use of solid supports. Limitations of scale and cost are usually minimal and attachment/detachment chemistry is avoided. Since most of the literature has been devoted to solution-phase reactions, a much larger list of reactions is, in principle, available. The disadvantages relative to the solid-phase are isolation of products and the production of fewer numbers of compounds. Methods for the purification of solution-phase libraries are mostly limited to extractions, although solid-phase scavenger reagents have been utilized to covalently bind to unreacted reagents[40,41] and chromatography[42] has been used in certain particular cases. Numerical considerations often determine which approach, solution or solid phase, should be applied. Because many more compounds can be synthesized using split synthesis, this technique is almost always used to construct large libraries

to check for activity ('lead libraries'). When larger quantities of individual compounds are desired solution-phase approaches become more practical.

While a number of solution-phase combinatorial investigations have been performed,[43,44] two general (template-based) strategies will be highlighted here. Dale Boger and coworkers at the Scripps Research Institute have recently synthesized, in parallel, low molecular weight triamides from an anhydride template (**Figure 6**).[5,42,45] The products were separated using simple liquid-liquid or liquid-solid extractions. While the multistep procedure may appear time-consuming if many compounds are needed, it can be automated and the final compounds usually do not require additional purification.

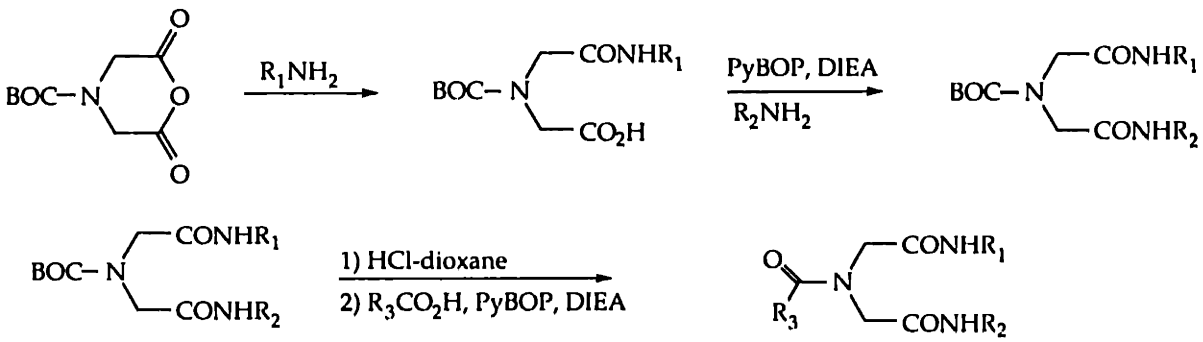


Figure 6: Use of an anhydride template to create triamides in solution. Each step is followed by purification using an acid and/or base rinse.[45]

A5. Introduction: The Solution-Phase Activated Core Approach

A different solution-phase approach was published from this research group which avoids the use of multiple steps or protecting groups by creating the diversity in a one-pot procedure (**Figure 7**).[6,46,47] It combines the numerical advantages of split synthesis on the solid support with the homogenous reaction conditions of the solution-phase. While the condensation of amines with acid chlorides was chosen for the initial studies,[46,47] in principle any reaction which proceeds in high yield is applicable.

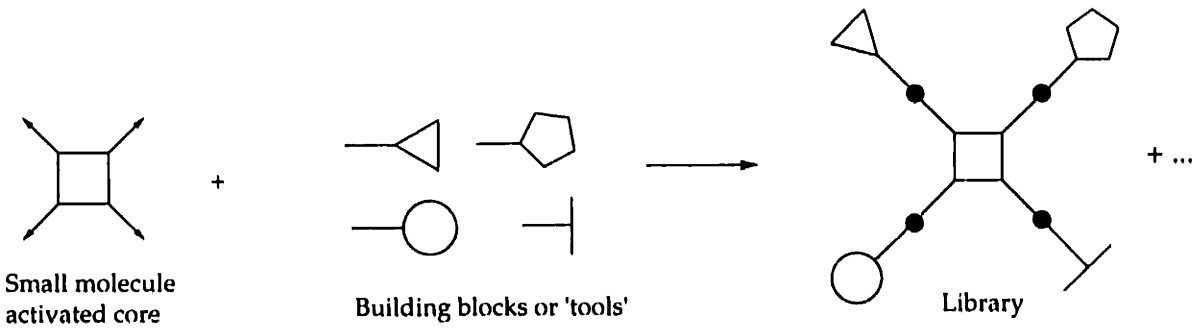


Figure 7: A schematic representation of the solution-phase approach used by Rebek and coworkers. The arrows on the core molecule indicate reactive sites, and •'s indicate the linking functionality used to append the building blocks to the core.

In the initial tetraamide-based studies, xanthene and cubane tetraacid chloride (see **Figure 8**) cores were reacted with sets of amino acid esters (building blocks or 'tools') to form mixtures of tetraamides. Since many of the tools contained protecting groups (i.e. *t*-butyl esters, BOC groups) a subsequent (acid) deprotection step was necessary to make the libraries water soluble. These core molecules allowed for two simple purifications to be performed. Following the diversification step the reaction was partitioned between dichloromethane and citric acid, removing unreacted building blocks and triethylamine. A second purification was performed after deprotection by precipitating the libraries with ether/hexanes, eliminating hydrophobic byproducts generated during deprotection, as well as any remaining TFA.

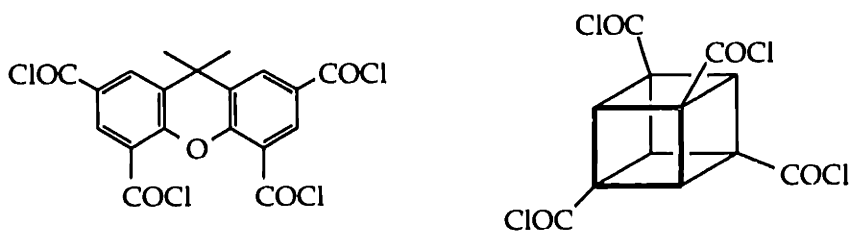


Figure 8: Core molecules (left: 2,4,5,7-xanthene, right: 1,3,5,7-cubane) used by Rebek and coworkers to synthesize libraries of tetraamides.[46,47]

These libraries were subsequently screened for activity against trypsin, and activity was found.[46,47] Since the approach was not amenable to encoding, a

deconvolution routine was implemented to identify the active components. This will be discussed in **Section A5-3**.

A5-1. Design Considerations for the Activated Core Approach

The activated core approach can be divided into three components: the core, linker, and building blocks. Each can be designed to give the desired level of diversity (numbers of compounds) and/or the desired physical property (solubility, shape space, chemical functionality). The symmetry of the core molecule, the number of reaction sites, and the number of building blocks determines the size (numbers of compounds) of the libraries. To maximize the numerical advantage of this approach we have chosen a minimum of three sites for simultaneous functionalization, with four being typical. Symmetrical core molecules are advantageous because they are usually easier to synthesize and equivalent sites facilitate the formation of statistical distributions. However, symmetry limits the numbers of compounds that are formed during library synthesis (more duplicate structures) and can also hinder efforts to obtain multiple address versions for deconvolution (see **Section A5-3**).

The synthesis of new core molecules is often undertaken to provide different orientations of the attached building blocks, thereby increasing structural diversity. The properties of the core molecule have (in several assays) been critical to activity, as libraries made with the same building blocks and linkages have had very different activity levels.[6] Other aspects are also considered to increase chances for interaction with biological targets, including molecular weight (with a weight of <700 Da preferred),[48] conformational rigidity, and the presence of groups capable of recognition (i.e. hydrogen-bond donors or acceptors, heterocyclic nitrogens, etc.). A number of core molecules have been synthesized in this research group (**Figure 9**), with a

range of symmetries and building block orientations. These core molecules will be described in subsequent chapters.

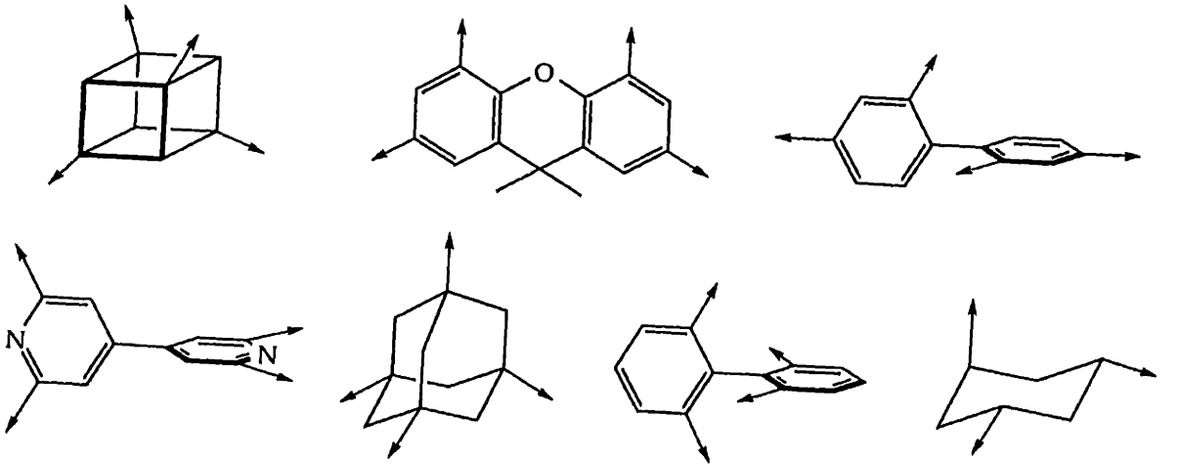


Figure 9: Core molecules that have been developed in the Rebek group, listed in chronological order (from left to right) of their development. This investigator has worked with all but the cubane (top left) and the bipyridyl-based[49] (bottom left) cores.

The manner in which the building blocks are attached to the core, the linking functionality or linkage, is also important. The stability of the group (i.e. amides and ureas) towards a variety of conditions (for example high temperature, tertiary amines, trifluoroacteic acid) is critical to ensure that the building blocks are not cleaved or re-arranged to other species. In the interests of time the linkage should be formed from readily available building blocks (amines, for example). Ideally, the linkage should also enhance water solubility and allow for direct interactions with target molecules by (for example) providing sites for hydrogen bonding. Finally, linkages that conformationally restrict the building blocks are desired to improve the likelihood that active compounds will be formed, since entropic costs associated with binding are minimized for conformationally defined compounds relative to more flexible substrates.[50] Rigid compounds provide the added benefit that if active compounds are found, more structural information about the receptor can be deduced. Two linkages have been successfully applied to this activated core approach, amides and ureas (**Figure 10**).

Figure 10: The amide and urea linkages attached to a generic core molecule (represented by an oval).

The third and most easily varied component of this method is the building blocks (**Figure 11**). Because the libraries are screened for biological activity it is hoped that the attachment of amino acids and their derivatives to the core will increase the chances for biological activity. Many amino acid derivatives are commercially available and their chemistry is well-established. Solubility is an important consideration when choosing building blocks, as the libraries are exposed to organic solvents during synthesis (and are purified using liquid | liquid extractions); but eventually the libraries are dissolved in aqueous-based solutions for biological assays. Although this consideration applies to all three components, it is the building blocks that (in practice) confer this dual solubility. During library synthesis the amino acid tools are protected as esters (usually *t*-butyl), which are subsequently cleaved to give the water soluble carboxylic acids. Protecting groups (i.e. BOC, mtr) are also used for nucleophilic side-chains to prevent side-reactions such as multiple attachments. Finally, non-amino acid based amines (see **Figure 11**) can (and have) been used to expand the diversity of the libraries, although one must be cognizant of the above considerations.

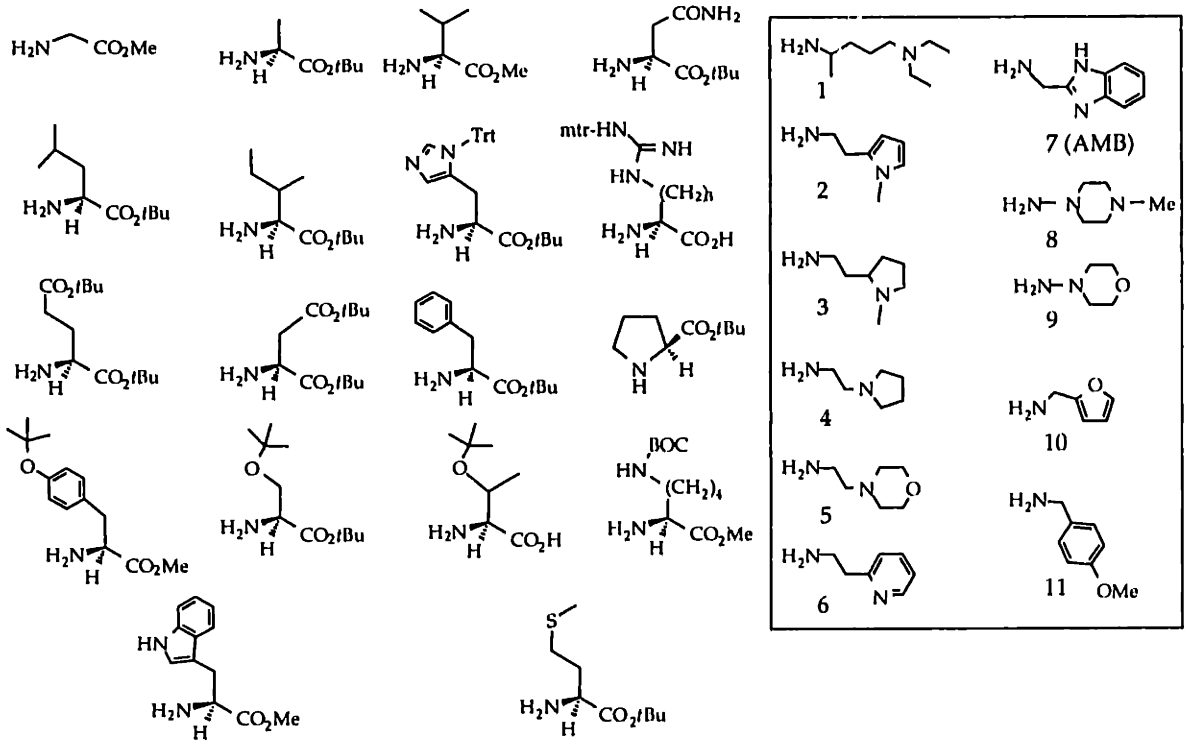


Figure 11: The list of building blocks used to create libraries; the non-amino acid tools are numbered. All (except 2)[51] are commercially available and most of the amino acid derivatives are sold as their hydrochloride salts.

There are two practical reasons why the core molecule is activated, rather than the building blocks. First, one would need to activate the tools prior to condensation and handle them appropriately (i.e. inert atmosphere and dry solvents). Even if some building blocks were available in their activated forms they may not be desirable (most acid chlorides and isocyantes that are commercially available are hydrophobic, abiological, and/or conformationally flexible). Second, while chemistry of the amino acids is well-known, the chemistry of activated amino acids is less developed, and could involve time-consuming optimization trials for the core under consideration. One possible solution would be to prepare a mixture of tools in which the concentrations of the building blocks are proportioned such that slower reacting building blocks are higher in concentration than the faster reacting ones (isokinetic mixtures).[27]

An example of a building block that did not prove to be acceptable was AMB (7 in **Figure 11**). When this tool was used in library synthesis and the ratio of tool:acid chloride was close to 1:1, it was (eventually) determined that it formed multiple attachments (see **Figure 12**). Unfortunately, the same reaction also occurred when coupling reagents (such as PyBOP,[52] **Figure 12**) were used. This scenario was discovered after a complete deconvolution (see **Section A5-3**) routine was applied to a library which inhibited DNA Polymerase, and the isolation of all the possible (expected) compounds failed to produce activity.[53] The conclusion reached after weeks of fruitless synthesis and screening was that the active component was an AMB-based oligomer. The lesson from these findings is that building blocks which are capable of forming side products should be reacted individually with a core molecule and the pure compounds should be fully characterized.

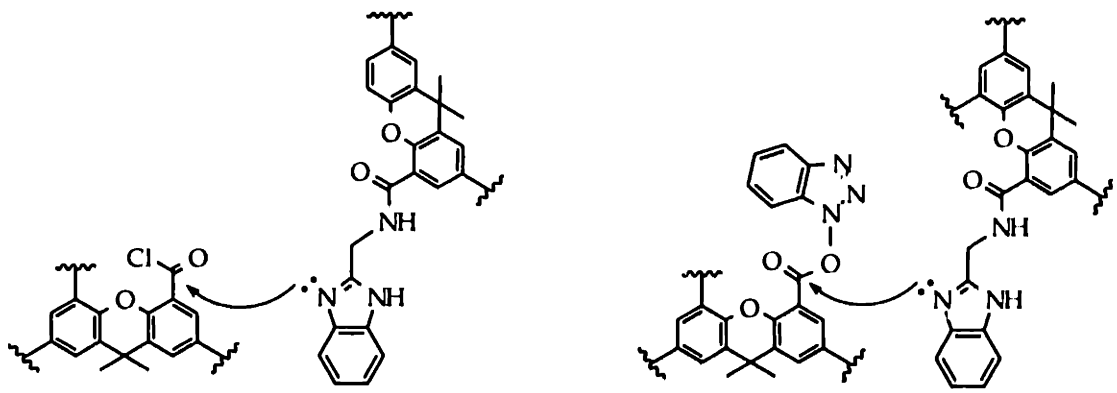


Figure 12: The AMB tool has been observed to form multiple attachments when either acid chlorides (left) or active esters (right) are available.

A5-2. Characterization of Solution-Phase Combinatorial Mixtures

A portion of this section has previously appeared in print.[54]

The simultaneous creation of diversity, while advantageous in many ways, can also result in complications. It is hoped that all building blocks attach randomly to the core, and do not discriminate between different core-

molecule sites. Yet because the building blocks often differ in size and nucleophilicity, biased mixtures can result from the differential reaction rates of the tools. Several analytical tools have been employed to verify the extent (if any) to which these problems exist, including IR, HPLC[47,55] and mass spectrometry.[54,56] The results have indicated, at least for xanthene-based amide libraries, that statistical mixtures were formed when only a slight excess of the building blocks (usually 0.1 equivalent per reactive site) were utilized. These analytical tools also allowed for optimization of the reaction and deprotection protocols.

Infrared spectroscopy (IR), because of its inherently qualitative nature, has been applied in only limited areas. It has proven valuable in studying the progress of reactions, especially when the NMR spectrum of starting material and product are very similar. In addition, IR can be a powerful tool to search mixtures for incomplete or side-reactions, as it can detect functional groups which should not be present. IR proved valuable in studying different isocyanate-generating reactions (as will be discussed in **Chapter B**). However, IR is ill-suited to analyze mixtures of related compounds, and other techniques must be employed.

HPLC has been used in two general ways to characterize libraries obtained from this type of combinatorial synthesis. Early studies utilized HPLC qualitatively to verify that as more building blocks were used, the diversity increased.[47] However, the size of the mixtures analyzed (136 to 97,461) limited the information that could be obtained. More recent HPLC studies have focused on smaller mixtures (7 to 45), which allowed for quantitative analysis of the product distributions. The lower complexity has also permitted detection of side-products and unremoved reagents. The approach that has been generally applied here is to take an activated core molecule and react it with only two or three building blocks, then analyze the distribution. If the chosen tools differ significantly in reactivity but are

incorporated into the libraries with similar frequency, then it is assumed that the system (i.e. disposition of reactive groups on the core, ratio of tools to core, temperature) should not produce statistically-aberrant product distributions. When HPLC is combined with mass spectrometry (LC/MS) the identity of each peak can be assigned, and a number of informative LC/MS analyses have been performed with different core molecules.

While the HPLC studies were useful in examining small libraries, we needed a more powerful technique to study larger mixtures. Mass spectrometry alone was not sufficient to analyze large mixtures as the frequency of isobaric (same molecular weight) compounds is often high. The situation is complicated by the fragmentation of higher molecular weight library components, which can obscure primary ions of the analytes. It is necessary for the library to be partitioned (separated) by some means prior to analysis. While LC/MS worked well for small libraries, separation based on hydrophobicity or polarity (reverse or normal phase LC) was not likely to provide acceptable partitioning of larger libraries (>100 compounds). To provide a better separation method we sought out a collaboration with Paul Vouros and coworkers at Northeastern University, who had devised a way to couple electrophoresis to mass spectrometry. In this technique the sample is passed through a small diameter fused-silica capillary in an electric field (capillary electrophoresis or CE), separating the library constituents based on their electrophoretic mobilities. The analytes then pass into an electrospray mass spectrometer where MS and MS/MS experiments are performed in either positive or negative-ion mode.[54]

To gain the full benefit of CE-MS pure (xanthene-based diamide) compounds and small mixtures were analyzed to identify characteristic fragmentation patterns.[56] For these studies xanthenes with only two reactive sites were used, as the fragmentation patterns were easier to characterize than their tetrasubstituted counterparts. Because the 4 and 5 positions are in close

proximity (see **Figure 13**) several characteristic fragments were observed, which allowed for identification of particular compounds when libraries were analyzed. This was considered essential as libraries often contain many isobaric components. After this phase of the project was completed, we synthesized a 171-component library where the compounds were only functionalized at the 4 and 5 positions.[54]

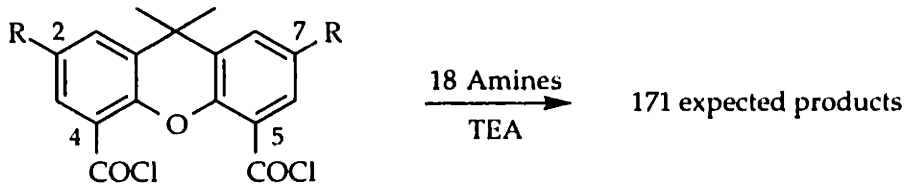


Figure 13: Synthesis of libraries for a CE-MS analysis (R= H or *t*Bu).

The goal was to discern if there were steric problems associated with the proximity of the two groups, which would cause non-statistical mixtures to result, and some components to not form. The results (see **Figure 14**) showed that 160 of the 171 (94% of theoretical) compounds could be detected. Of the 11 compounds that were missing, 10 of them were derivatives of tryptophan methyl ester, which could be explained in part by non-steric effects (it has low solubility in the reaction medium and is unstable to the TFA deprotection conditions).[57] Extrapolating these results to the xanthene tetraamides can be justified as the other two reaction sites (2 and 7 positions) are unhindered and not sterically coupled to any other position. In short, the percentage of compounds that are formed during library synthesis is likely to be very high, well above 90%.

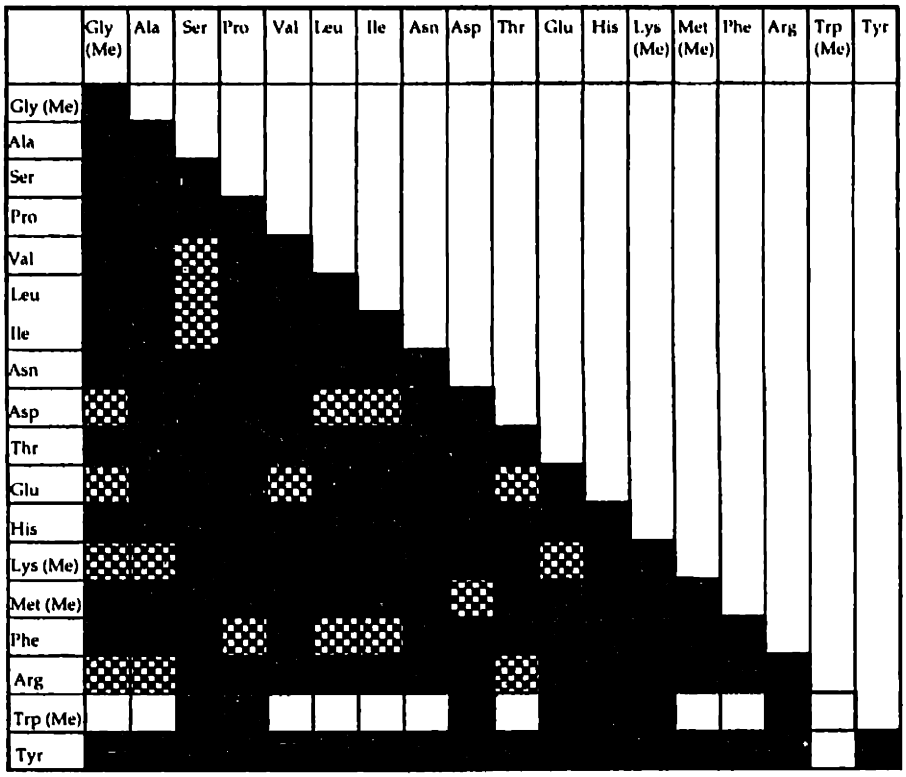


Figure 14: Results of the CE-MS analysis of a library (theoretically) containing 171 components. Gray blocks indicate that the compound was detected by CE-MS, checkered by CE-MS/MS, and white were undetected.[54]

A5-3. Identification of Active Components

As previously mentioned, the libraries derived from solution-phase combinatorial chemistry were (and are being) screened for various types of biological activity. Since the initial libraries are mixtures of thousands of compounds, a method for identifying active compounds was necessary. In the previous trypsin assay[46,58] as well as subsequent assays, an iterative deconvolution/sublibrary approach was implemented. This involved making smaller mixtures derived from subsets of fewer and fewer building blocks (sublibraries) and, based on the activities of each sublibrary, particular building blocks were identified as being either necessary or superfluous to activity (**Figure 15**). Deconvolution has been studied with model systems,[59] with the conclusion that iterative routines are likely to find either the best or close to the best molecule from the initial mixture. In principle, this allows

one to reduce an active library derived from many tools to an active library derived from fewer tools.

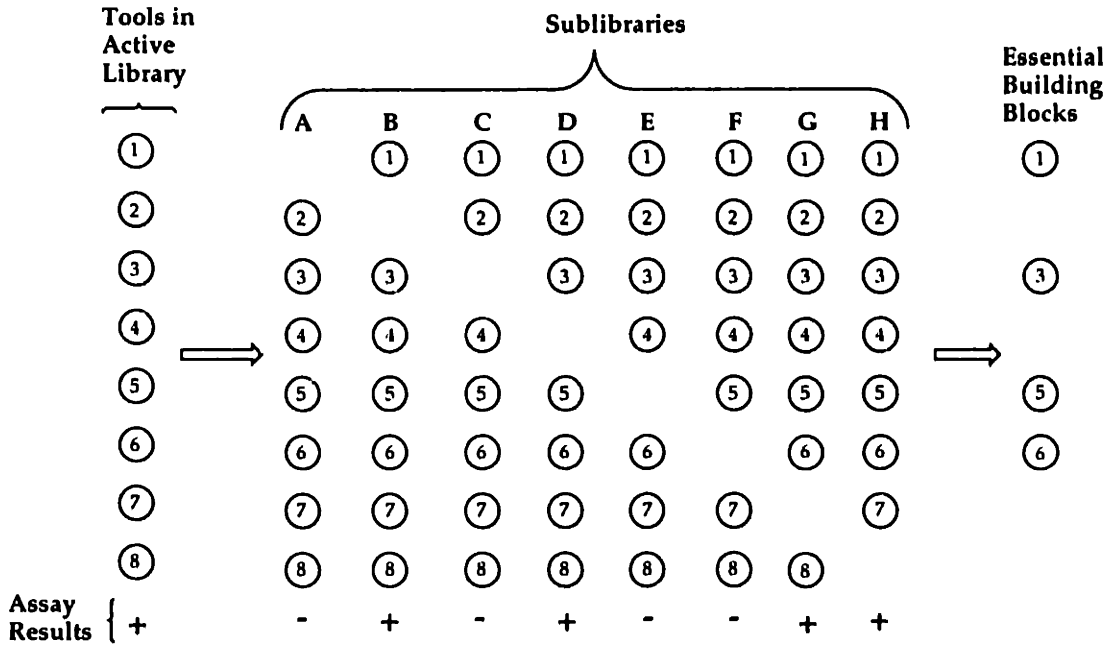


Figure 15: An idealized deconvolution of an active eight building block library. Essential tools are identified by a drop in activity below a set threshold (a '–' assay result) when they are deleted from the original set. In this case, building blocks 1,3,5, and 6 would be essential.

At some point, however, the information gained from mixtures becomes limited and one must begin to move towards pure compounds. This is accomplished by synthesizing derivatives of the core molecule which allow for particular sites to be functionalized, and is referred to as creating multiple addresses. By studying assay results of mixtures where particular building blocks are located at defined positions, structure-activity relationships can begin to be derived, and progress is made towards single compounds. Examples of multiple-address xanthenes will be given in the next section.

A6. Xanthene Tetraamides Revisited

This section was done in collaboration with Edward Wintner

As previously mentioned, libraries of xanthene tetraamides were screened for activity against trypsin. While this assay could be run in the laboratory using equipment familiar to organic chemists, we nevertheless sought out assays which were deemed more interesting. This resulted in a collaboration with the National Cancer Institute (NCI) and five large (14 tools, 19,306 components) libraries were synthesized and screened for two types of activity: *in vivo* inhibition of renal, breast and melanoma cancer cells and a whole-cell HIV prevention assay. While none of the xanthene libraries were active in HIV prevention (however a library based on another core molecule, 2,2',4,4'-biphenyl was active), two out of the five did prove active in the renal and breast cancer cell lines. The fact that the two libraries were not active against melanoma cells and that three other libraries were not active at all, indicated that the samples were not generally cytotoxic, and that there was some specificity for malignant cells. While our collaborators did not reveal the numerical level of activity, they did categorize it as 'very active' and were enthusiastic about continuing the deconvolution process. Their enthusiasm did not extend to an offer of financial compensation for the synthesis of sublibraries, however.

The number of compounds (19,306 per library) presented a problem. The time between submission of samples and results was prolonged (on the order of months). With 14 tools used to make the libraries it was likely that several (at least three) rounds of deconvolution would be required, and it was feared that there was not enough time to complete a rigorous deconvolution procedure. An approximation, or shortcut, was then made in the interest of time and cost. Upon examination of the 14 tools present in each libraries, six were common to both. Since both libraries were active at about the same concentration, it was decided to make a new library based on these six tools

(666 total compounds) together with sublibraries (five tools each, 325 compounds).

Although we were skeptical that this shortcut would be successful, we were pleasantly surprised by the results which implied that our approximation was valid. The data is summarized in **Figure 16** for the two different cell lines; activity is measured by a negative value for cell growth, while a +100 value represents the activity level of the control cells. As one can see, the omission of particular building blocks causes the activity to diminish, indicating that these tools are essential to the active compound. Although two tools (numbers 1 and 10, see **Figure 11** for structures) seem to be essential for both cell assays, a third building block (number 11) appears to be necessary for the breast cancer cell assay. Another round of sublibraries have been submitted to confirm the results. Fortunately, the problematic building block AMB (which can form multiple attachments)[53] did not appear to be essential to activity; in fact it likely degraded the quality of the other libraries through the formation of oligomers. The evidence to support this can be found in the breast cancer result of **Figure 16**, which shows that deletion of this building block increased activity substantially.

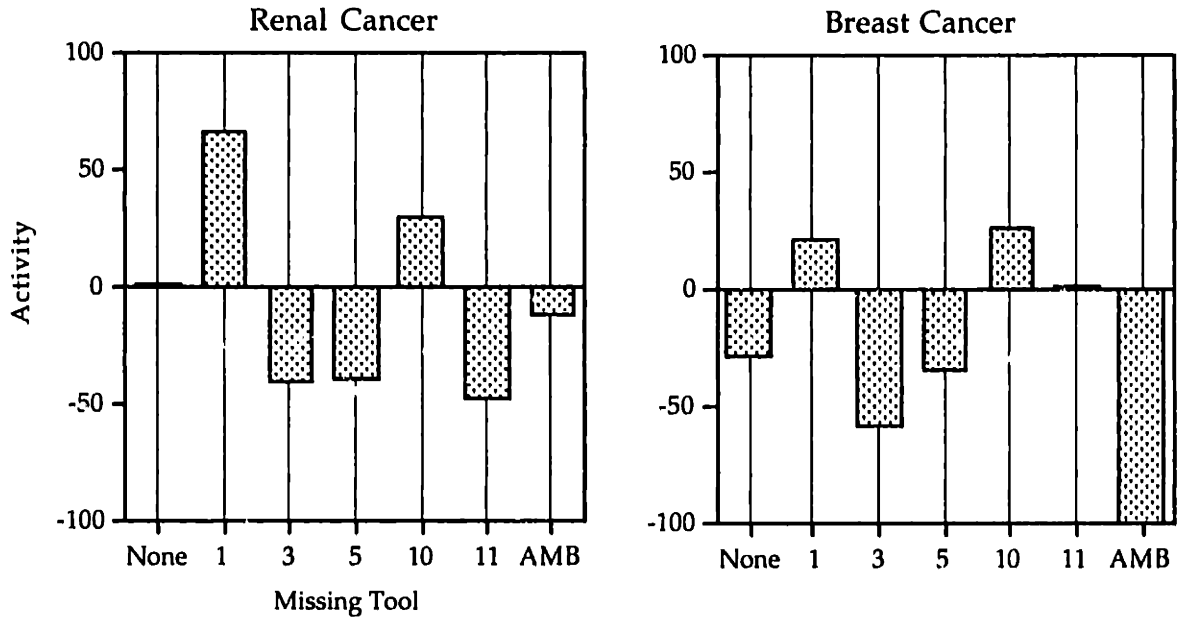
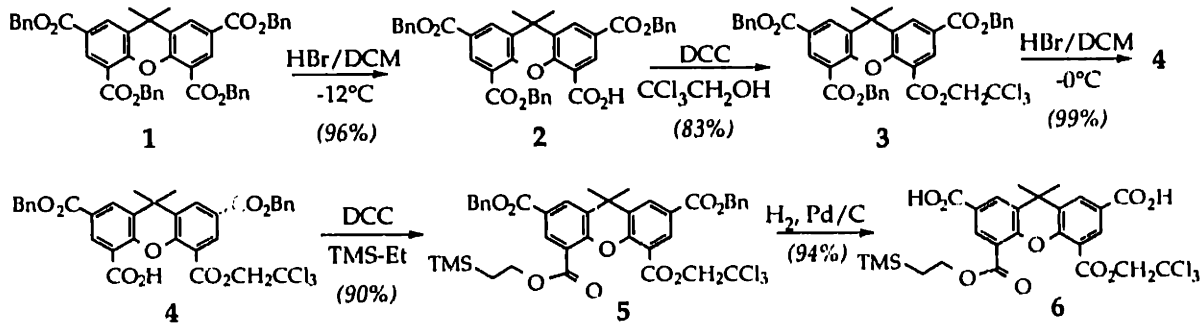


Figure 16: Graphs of the activities of the six tool library (labeled 'none') and its sublibraries (numbered for the building block which was omitted). Note that the sublibraries contained less than one-half of the number of components contained in the six tool library. This helps to explain why some of the activities of the sublibraries were higher than the main libraries. The structure of the tools can be found in **Figure 11**.

A7. Multiple Address Xanthene Compounds

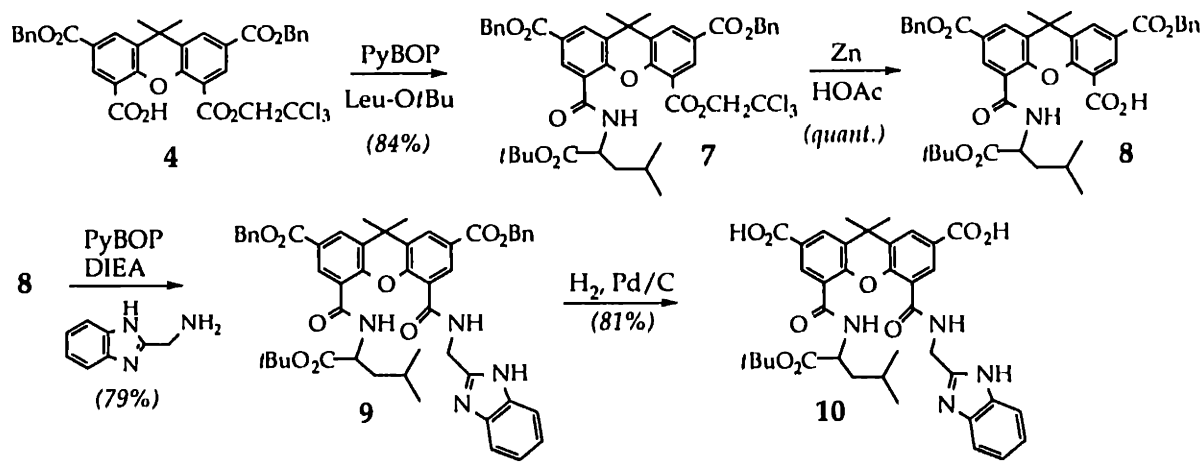
To allow for the further deconvolution of the NCI libraries as well as to synthesize oligomeric xanthenes for a coworker (Ed Wintner, Polymerase I inhibition assay)[53] the synthesis of multiple-address xanthenes was undertaken (**Scheme 1**). The synthesis of the starting tetrabenzyl ester (**1**) will be presented in **Section B8**. Use of the trichloroethyl (TCE) protecting group[60] was advantageous because it could be cleaved selectively using zinc and acetic acid (with sonication), while the more fragile trimethylsilylethyl protecting group[61] could be removed using tetrabutyl ammonium fluoride. The origin of the selective HBr cleavage of the 4-5 positions will be discussed in **Volume II (Section A3)** of this thesis.

Scheme 1: Synthesis of two and three address xanthene core molecules. TMS-Et= 2-trimethylsilyl ethanol.



The three-address xanthene **4** allows for selective functionalization of the 4 position using the PyBOP[52] coupling reagent (**Scheme 2**). Removal of the TCE group with zinc did not affect the Leu residue, and a second PyBOP coupling gave the AMB/Leu **7**. Cleavage of the TCE group of **7** afforded **8**, which could be coupled to an additional amine to give **10**.

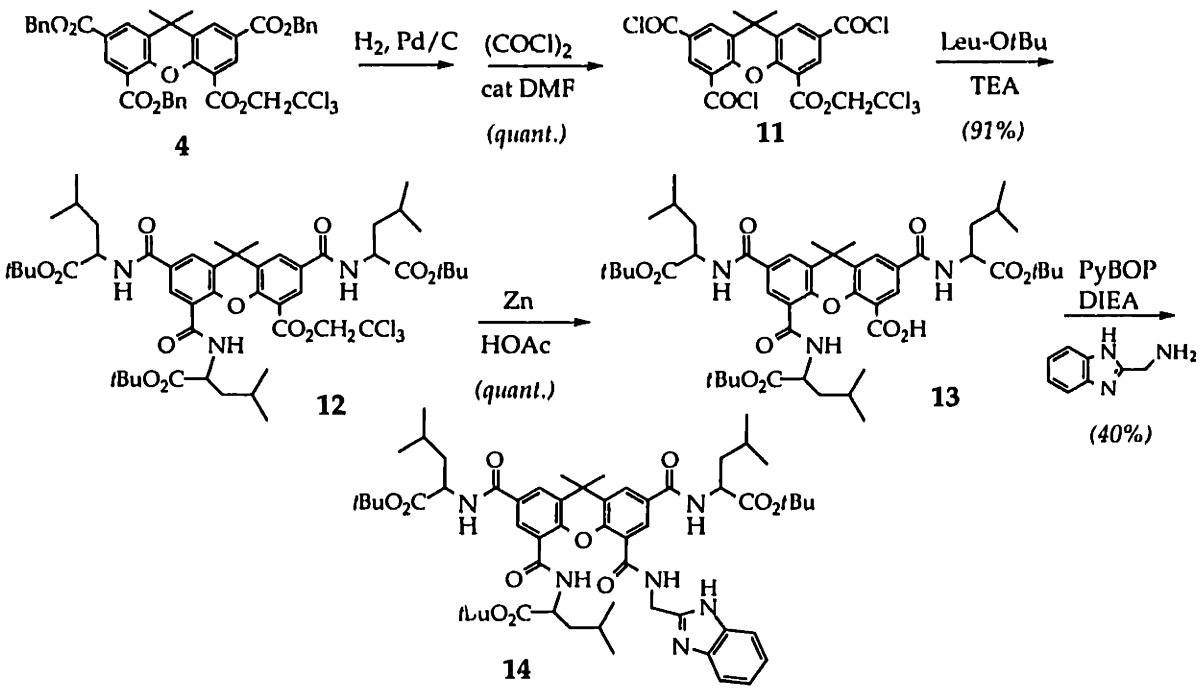
Scheme 2: Application of a three address core to the selective synthesis of a trifunctionalized xanthene.



To further demonstrate the versatility of multiple-address xanthenes, another scheme was used to synthesize the trileucine mono-AMB tetraamide **14** (**Scheme 3**). This compound was not accessible directly from **10** as the imidazole-like nitrogen of the AMB building block was competitive with added amines, and oligomers resulted using a number of different coupling conditions.[53] The triacid derived from **4** became insoluble over the course of

the hydrogenolysis reaction, and could not be removed from the Pd/C catalyst. It was therefore transformed into the dichloromethane-soluble triacid chloride **11**, which condensed in quantitative yield to give the trileucine **12**.

Scheme 3: Synthesis of the trileucine/mono AMB tetraamide.



A8. Conclusion to This Chapter and Introduction to the Next Three

A solution-phase combinatorial technique has been developed which combines the numerical advantage of the solid phase with the quantity advantage of the solution phase. Structurally diverse libraries can be designed and synthesized because the approach contains three easily varied components. Analytical methods have been applied to examine the reactivity of the activated cores and have verified that, in the case of xanthene polyamides libraries, the mixtures contained high percentages of the expected compounds. The high purity of these libraries has allowed for a recursive deconvolution protocol to be successfully implemented to identify active components. A simplified deconvolution route has also been performed

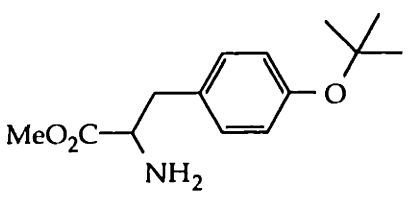
with encouraging initial results; and we expect further sublibrary assay data shortly. In addition, unsymmetrical (multiple address) xanthenes have been prepared to permit synthesis of individual tetraamides to be screened as pure compounds. Over the course of the next three chapters the aforementioned techniques will be applied to new linkages, core molecules, and biological targets.

A9. Experimental Section

Note: Definitions of abbreviations used in this thesis are given in **Appendix B**

A9-1. General

All reagents were purchased from Aldrich Chemical Company and used without further purification except as noted. Amino acid esters and PyBOP were acquired from Novabiochem (San Diego, California). Deuterated solvents were obtained from Cambridge Isotope Laboratories and deuterated chloroform was dried over 4 Å molecular sieves. THF was distilled from sodium/benzophenone ketyl, DCM was distilled from P_2O_5 and triethylamine was distilled from (and stored over) KOH. Acetone was dried over 4 Å molecular sieves for 24 hours. Citric acid refers to a 1 N stock solution. Drying tubes were packed with anhydrous $CaCl_2$. NMR spectra were recorded on either a Bruker AC-250 or a Bruker AM-300 and all peaks were referenced to residual solvent. Either a Finnegan Mat 8200 (for HRMS/EI) or a VG ZAB-VSE (for HRMS/FAB) mass spectrometer was used to ascertain exact masses. Silica gel chromatography was performed with Silica Gel 60 (EM Science or Bodman, 230-400 mesh). TLC analysis was performed using glass-bound Silica Gel 60 (F254) plates. As all xanthene derivatives were 9,9-dimethylated, this portion has been omitted from the naming schemes. An example of the nomenclature used to describe the amino acid residues is given below.



α-carboxyl substituent

Tyr(O*t*Bu)-OMe

side-chain protecting group
(omitted if none)

A9-2. Synthesis and Characterization

2,4,5,7-Xanthene tetrabenzyl ester (**1**). This compound will be characterized in the experimental section of **Chapter B**.

2,4,7-Tribenzyl ester-5-xanthene carboxylic acid (**2**). HBr was bubbled through DCM (500 mL) at rt for 15 min, the solution was cooled to -12°C (dry ice in ethylene glycol), xanthene tetrabenzyl ester (**1**, 10.0 g, 13.4 mmol) was added as a solid and the flask was stoppered. After 40 min the reaction was complete and the solution was sparged with nitrogen for 15 min. Concentration of the reaction mixture afforded an oil, which solidified upon addition of hexanes and sonication. The brown oily solid was filtered and rinsed thoroughly with hexanes to give a brown powder (8.50 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.93 (d, *J*= 2.0 Hz, 1 H), 8.70 (d, *J*= 2.1 Hz, 1 H), 8.40 (d, *J*= 2.1 Hz, 1 H), 8.38 (d, *J*= 2.1 Hz, 1 H), 7.48-7.37 (m, 15 H), 5.46 (s, 2 H), 5.40 (s, 2 H), 5.39 (s, 2 H), 1.72 (s, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{40}H_{32}O_9Cs$ $[M + Cs]^+$ 789.1101, found 789.1129.

2,4,7-Tribenzyl ester-5-xanthene (2,2,2-trichloroethyl) ester (**3**). Tribenzyl ester monoacid **2** (498 mg, 0.76 mmol), 2,2,2-trichloroethanol (90 μL, 0.93 mmol), and DMAP[62] (50 mg, 0.40 mmol) were dissolved in DCM (8 mL). DCC[63] (190 mg, 0.93 mmol) in DCM (5 mL) was then added dropwise and the solution was stirred. After 10 min a hazy suspension developed and the reaction was allowed to run overnight. The solids were filtered off and rinsed with DCM. The filtrate was added to a separatory funnel with more DCM and and the organic layer was rinsed with citric acid (25 mL). Drying over Na_2SO_4 and evaporation gave a yellow foam. Purification using SiO_2 (hexanes:EtOAc 6:1→4:1) yielded a white solid (500 mg, 83%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.54 (d, *J*= 2.0 Hz, 1 H), 8.37 (m, 2 H), 8.30 (d, *J*= 2 Hz, 1 H), 7.49-7.36 (m, 15 H), 5.48 (s, 2 H), 5.40 (s, 2 H), 5.39 (s, 2 H), 4.90 (s, 2 H), 1.72 (s, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{42}H_{33}O_9ClCs$ $[M + Cs]^+$ 919.0244, found 919.0284.

2,7-Dibenzyl ester-4-(2,2,2-trichloroethyl) ester-5-xanthene carboxylic acid (**4**). HBr was bubbled through DCM (25 mL) for 10 min at rt. The flask was then capped and cooled in an ice bath. The starting material **3** (0.80 g, 0.96 mmol) was dissolved in DCM (10 mL) and was added to the HBr solution dropwise via pipette and the flask was stoppered. After 35 min the reaction was complete, and argon was bubbled through the reaction for 10 min. The solution was then concentrated to a white solid and was rinsed with hexanes to remove residual benzyl bromide. Following a SiO_2 column (2%

MeOH/DCM) a white solid was isolated (700 mg, 99%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 11.50 (br s, 1 H), 8.93 (d, *J*= 2.1 Hz, 1 H), 8.85 (d, *J*= 2.1 Hz, 1 H), 8.47 (d, *J*= 2.1 Hz, 1 H), 8.39 (d, *J*= 2.1 Hz, 1 H), 7.49-7.36 (m, 10 H), 5.42 (s, 2 H), 5.40 (s, 2 H), 5.06 (s, 2 H), 1.74 (s, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{35}H_{27}O_9Cl_3Cs$ $[M + Cs]^+$ 828.9775, found 828.9744.

2,7-Dibenzyl ester-4-(2,2,2-trichloroethyl) ester-5-xanthene (2-trimethylsilyl) ethyl ester (**5**). The triester/acid **4** (55 mg, 0.077 mmol), trimethylsilylethanol (14 μL, 0.09 mmol), and DMAP (5 mg, 0.04 mmol) were dissolved in dry DCM (2 mL). DCC (19 mg, 0.09 mmol) in DCM (2 mL) was then added dropwise and the solution was stirred under a drying tube. After a few minutes a hazy suspension developed and the reaction was allowed to run overnight. The solids were filtered off and rinsed with DCM. The filtrate was transferred to a separatory funnel, where it was rinsed with citric acid. The product was isolated as a clear, colorless oil (69 mg, 90%) following SiO_2 chromatography (10:1 hexanes:EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.51 (d, *J*= 2.0 Hz, 1 H), 8.34 (d, *J*= 2.0 Hz, 1 H), 8.30 (d, *J*= 2.0 Hz, 1 H), 8.27 (d, *J*= 2.0 Hz, 1 H), 7.48-7.37 (m, 10 H), 5.39 (br s, 4 H), 5.04 (s, 2 H), 4.48 (m, 2 H), 1.71 (s, 6 H), 1.17 (m 2 H), 0.092 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{40}H_{39}O_9SiCl_3Cs$ $[M + Cs]^+$ 929.0483, found 929.0489.

4-(2,2,2-Trichloroethyl) ester-5-(2-trimethylsilyl)ethyl ester xanthene-2,7-dicarboxylic acid (**6**). The tetraester **5** (287 mg, 0.35 mmol) was dissolved in 4:1 EtOAc:EtOH (10 mL), and 10% Pd/C added (5 mg, cat). The flask was evacuated and back-filled with hydrogen three times, then stirred for 7 h. The suspension was then filtered through celite using EtOAc and concentrated to a white solid (208 mg, 94%). ^{1}H NMR (250 MHz, DMSO-d_6): δ 13.3 (br s, 2 H), 8.35 (d, *J*= 2.0 Hz, 1 H), 8.29 (d, *J*= 2 Hz, 1 H), 8.26 (d, *J*= 2.0 Hz, 1 H), 8.08 (d, *J*= 2.1 Hz, 1 H), 5.15 (s, 2 H), 4.41 (m, 2 H), 1.69 (s, 6 H), 1.11 (m, 2 H), 0.06 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{26}H_{27}O_9SiCl_3Cs$ $[M + Cs]^+$ 748.9544, found 748.9576.

2,7-Dibenzyl ester-4-(2,2,2-trichloroethyl) ester-5-xanthene (leucine-OtBu) amide (**7**). Xanthene acid **4** (1.00 g, 1.4 mmol) and PyBOP (1.5 g, 3.22 mmol) were dissolved in DMF (20 mL) and were stirred for 10 min before Leu-O*t*Bu·HCl (380 mg, 1.7 mmol) and DIEA (0.8 mL, 4.6 mmol) in DMF (10 mL) were added. After 3 h the reaction was complete and the mixture was poured into a separatory funnel with DCM (150 mL) and was rinsed with citric acid (25 mL) and water (2x 50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a yellow oil. Preloading (with DCM) of this material onto a silica gel column and elution with 7:1→5:1 hexanes:EtOAc

afforded the pure material as a white foam (1.03 g, 84%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.96 (d, *J*= 2.2 Hz, 1 H), 8.94 (m, 1 H), 8.72 (d, *J*= 2.2 Hz, 1 H), 8.42 (d, *J*= 2.2 Hz, 1 H), 8.30 (d, *J*= 2.3 Hz, 1 H), 7.48-7.33 (m, 10 H), 5.41 (s, 2 H), 5.37 (s, 2 H), 5.05 (d, *J*= 11.8 Hz, 1 H), 4.93 (m, 1 H), 4.83 (d, *J*= 11.8 Hz, 1 H), 1.85-1.70 (m, 3 H), 1.75 (s, 3 H), 1.70 (s, 3 H), 1.47 (s, 9 H), 0.99 (m, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{45}H_{46}O_{10}NCl_3Cs$ [M + Cs]$^+$ 998.1242, found 998.1288.

2,7-Dibenzyl ester-5-(leucine-OtBu) amide-xanthene-4-carboxylic acid (**8**). Xanthene trichloroethyl ester **7** (0.700 g, 0.791 mmol) was dissolved in HOAc (10 mL) and zinc dust (500 mg, 7.6 mmol) was added. After 1.5 h of vigorous stirring, the reaction was incomplete and was sonicated for 30 min to complete the transformation. The mixture was then filtered and the solution was concentrated to afford a white solid in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6, broad): δ 12 (br s, 1 H), 8.56 (br s, 1 H), 8.46 (br s, 1 H), 8.28 (appar d, *J*= 1.9 Hz, 2 H), 7.49-7.35 (m, 11 H), 5.38 (s, 4 H), 4.41 (m, 1 H), 1.67 (s, 6 H), 1.33 (s, 9 H), 0.86 (m, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{43}H_{45}O_{10}NCs$ [M + Cs]$^+$ 868.2098, found 868.2074.

2,7-Dibenzyl ester-4-AMB amide-5-xanthene (leucine-OtBu) amide (**9**). Xanthene **8** (580 mg, 0.79 mmol) and PyBOP (515 mg, 0.99 mmol) were stirred in DMF (10 mL) for 5 min before AMB (391 mg, 1.8 mmol) and DIEA (0.31 mL, 1.8 mmol) were added in DMF (7 mL). After 3 h the reaction was complete and was poured into a separatory funnel with DCM (150 mL) and was rinsed with water (3x 25 mL). Drying over $MgSO_4$, filtration, and concentration produced an oil. This was preloaded onto SiO_2 using DCM and purified using column chromatography (2:1→1:1 hexanes:EtOAc) to give a yellow solid (543 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.2 (br s, 1 H), 9.47 (t, *J*= 5.5 Hz, 1 H), 9.16 (d, *J*= 7.6 Hz, 1 H), 7.51 (d, *J*= 1.9 Hz, 1 H), 8.35 (d, *J*= 2.0 Hz, 1 H), 8.34 (d, *J*= 2.2 Hz, 1 H), 8.19 (d, *J*= 1.8 Hz, 1 H), 7.51-7.34 (m, 12 H), 7.11 (m, 2 H), 5.41 (s, 2 H), 5.38 (s, 2 H), 4.78 (dd, *J*= 16, 5.3 Hz), 4.71 (dd, *J*= 16, 5.7 Hz), 4.36 (m, 1 H), 1.73 (s, 3 H), 1.71 (s, 3 H), 1.70-1.57 (m, 3 H), 1.34 (s, 9 H), 0.76 (d, *J*= 10.5 Hz, 3 H), 0.74 (d, *J*= 10.3 Hz, 3 H). HRMS (FAB in NBA/CsI) calcd for $C_{51}H_{52}O_9N_4Cs$ [M + Cs]$^+$ 997.2789, found 997.2754.

4-AMB amide-5-(leucine-OtBu) amide xanthene-2,7-dicarboxylic acid (**10**). Dibenzyl ester **9** (88 mg, 0.10 mmol) was dissolved in 4:1 EtOAc:EtOH (5 mL) and 10% Pd/C (cat) was added. The system was evacuated and back-filled with H_2 (atm) three times. After 12 h the reaction was complete and was filtered through celite. Concentration afforded a white solid (55 mg, 81%). ^{1}H NMR (250 MHz, DMSO-d_6): δ 13.1 (br s, 2 H), 12.2 (br s, 1 H), 9.51 (t, *J*= 5.6 Hz, 1 H),

9.14 (d, *J*= 7.2 Hz, 1 H), 8.49 (d, *J*= 2.1 Hz, 1 H), 8.29 (m, 2 H), 8.17 (d, *J*= 2.0 Hz, 1 H), 7.5-7.4 (m, 2 H), 7.14-7.09 (m, 2 H), 4.81 (dd, *J*= 16.3, 5.9 Hz, 1 H), 4.69 (dd, *J*= 16.0, 5.8 Hz, 1 H), 4.36 (m, 1 H), 1.72 (s, 6 H), 1.70 (s, 6 H), 1.63-1.55 (m, 3 H), 1.37 (s, 9 H), 0.77 (d, *J*= 10.2 Hz, 3 H), 0.75 (d, *J*= 10.2 Hz, 3 H). LRMS (FAB in NBA/CsI) calcd for $C_{33}H_{33}N_4O_9$ $[M + H]^+$ 629, found 629.

5-(2,2,2-trichloroethyl) ester xanthene-2,4,7-tricarboxylic acid chloride (**11**). To the tribenzyl ester xanthene **4** (1.00 g, 1.2 mmol) was added EtOAc (9 mL), EtOH (3 mL) and 5% Pd/C (30 mg, cat). The system was evacuated and back-filled three times with H_2 (atm). After stirring for a few hours a white precipitate was observed, and the reaction was allowed to run for a total of 14 h. After the addition of a number of solvents (MeOH, THF, acetone) failed to dissolve the white precipitate the reaction was concentrated to a residue presumably containing the triacid derivative and Pd/C.
The resulting mixture was suspended in DCM (25 mL) and oxalyl chloride (0.50 mL, 6.0 mmol) was added along with DMF (1 μL, cat). After 2 h at rt, the reaction was warmed to reflux for 12 h under a drying tube, at which time only the Pd/C catalyst remained insoluble. Filtration through celite followed by concentration afforded a yellow foam in quantitative yield (over two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (d, *J*= 2.2 Hz, 1 H), 8.56 (d, *J*= 2.2 Hz, 1 H), 8.41 (d, *J*= 2.3 Hz, 1 H), 8.36 (d, *J*= 2.2 Hz, 1 H), 5.14 (s, 2 H), 1.78 (s, 6 H).

2,4,7-Tri(leucine-OtBu) triamide-5-xanthene (2,2,2-trichloroethyl) ester (**12**). Leu-O*t*Bu·HCl (1.00 g, 4.80 mmol) was dissolved in DCM (15 mL), free-based with TEA (1.0 mL, 7.2 mmol), and added to the triacid chloride **11** (688 mg, 1.2 mmol) dissolved in DCM (25 mL). After 6 h DCM (50 mL) was added; the mixture was added to a separatory funnel, and was rinsed with citric acid (2x 25 mL). Concentration gave a white solid (1.12 g, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.90-8.75 (m, 3 H), 8.44 (d, *J*= 1.9 Hz, 1 H), 8.38 (d, *J*= 2.0 Hz, 1 H), 8.29 (d, *J*= 2.1 Hz, 1 H), 8.24 (d, *J*= 1.9 Hz, 1 H), 5.20 (d, *J*= 12.2 Hz, 1 H), 5.05 (d, *J*= 12.1 Hz, 1 H), 4.55-4.45 (m, 1 H), 4.45-4.35 (m, 2 H), 1.9-1.5 (m, 12 H), 1.48-1.38 (m, 30 H), 0.95-0.88 (m, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{51}H_{72}N_3O_{12}Cl_3Cs$ $[M + Cs]^+$ 1156.3236, found 1156.3287.

2,5,7-Tri(leucine-OtBu) triamide xanthene-4-carboxylic acid (**13**). The trileucine mono TCE ester **12** (530 mg, 0.52 mmol) was dissolved in HOAc (15 mL) and zinc dust (1 g, excess) was added. The solution was sonicated at rt for 5 h, then was filtered and concentrated to give a white foam in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6, broad): δ 12.0 (br s, 1 H), 8.82 (m, 2 H, CONH), 8.54 (d, *J*= 2 Hz, 1 H), 8.40 (d, *J*= 2 Hz, 1 H), 8.23 (m, 2 H), 4.43-4.38 (m, 3 H), 1.71 (s, 6 H), 1.65-1.46 (m, 9 H), 1.41 (appar s, 18 H), 1.36 (s, 9 H), 0.94-0.87 (m,

18 H). HRMS (FAB in NBA/CsI) calcd for $C_{49}H_{71}N_3O_{12}Cs$ $[M + Cs]^+$ 1026.4092, found 1026.4105.

2,5,7-Tri(leucine-OtBu) triamide-4-xanthene AMB amide (**14**). Synthesized in an analagous fashion to **9** in 40% isolated yield following silica gel chromatography (4:1→2:1 hexanes:EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$, broad): δ 9.98 (br s, 1 H), 8.56 (d, *J*= 2 Hz, 1 H), 8.19 (m, 2 H), 8.02 (d, *J*= 2 Hz, 1 H), 7.75 (br s, 2 H), 7.29 (br s, 1 H), 7.17 (br s, 1 H), 5.10 (dd, *J*= 16, 6 Hz, 1 H), 5.06 (dd, *J*= 16, 6 Hz, 1 H), 5.06-4.80 (m, 3 H), 1.87-1.59 (m, 9 H), 1.58 (s, 6 H), 1.53 (s, 9 H), 1.52 (s, 9 H), 1.47 (s, 9 H), 1.05-0.93 (m, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{57}H_{78}N_6O_{11}$ $[M + H]^+$ 1023.5807, found 1023.5856.

A10. References

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A portion of this chapter has previously appeared in print.[1]

B. Isocyanate-Based Linkages: Di and Tetraureas on a Xanthene Core

B1. Introduction

One approach to expanding the diversity of our solution-phase combinatorial approach is to introduce a different way to attach building blocks to a core molecule. While amides are easily synthesized, they are known to be unstable *in vivo*. The pharmaceutical industry has responded by moving away from amide-based drug design and toward peptidomimetics.[2] Replacement of amide groups with isocyanate-derived functionality (mostly ureas) has been investigated as the urea moiety is more biologically stable.[3,4] A logical method for generating urea libraries using solution-phase combinatorial chemistry would be to synthesize a polyisocyanate core and condense it with readily-available amines. In comparison to the previously made polyamide-linked libraries,[5-7] the insertion of an additional -NH group between the core and building blocks would provide more extended structures as the building blocks would be more distant from the core. Also, the addition of four heteroatoms should increase the water solubility of the library components.

Ureas are well-known for their hydrogen-bonding capabilities, providing both donor and acceptor sites. These donor-acceptor properties have recently been utilized in a number of fields, including molecular recognition,[8,9] artificial β-sheet formation,[10,11] self-assembly,[12-14] and drug design.[15-17] Recent advances in the syntheses of unsymmetrical ureas[18-21] have assisted a number of investigations. The generation of ureas traditionally involved the transformation of amines to isocyanates using phosgene at elevated temperatures,[22] a second amine was subsequently added

to afford the unsymmetrical urea. While recent improvements have been made in the phosgene reaction,[23] less toxic alternatives have also been developed (triphosgene[19] and $(BOC)_2O$/DMAP[21]) to complete the amine to isocyanate transformation. A second approach is also commonly used; carboxylic acids are transformed into acyl azides, which thermally rearrange to give isocyanates (the Curtius reaction, see **Figure 1**).

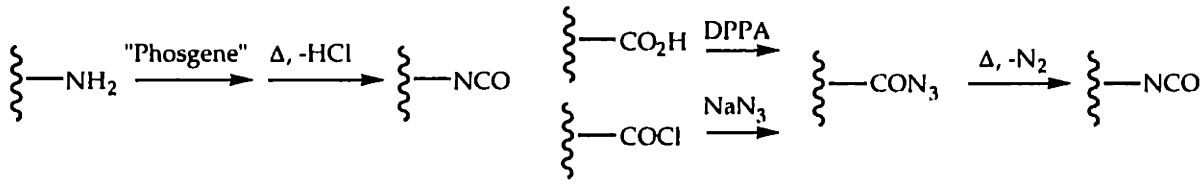


Figure 1: Two approaches to isocyanates. (Left) From the amine either phosgene or a phosgene equivalent can be used. (Right) From either a carboxylic acid or its acid chloride an acyl azide can be formed, which then rearranges to the isocyanate (the Curtius rearrangement).

For a number of reasons the first core chosen for urea-based libraries was the tetrasubstituted xanthene (**Figure 2**). First, this heterocycle has proven to be a versatile scaffold for both molecular recognition[24,25] and tetraamide-based diversity.[7] Second, it has no known toxicities and in fact can be found in an entire class of drugs used to treat psychological disorders (known as tricyclic pharmaceuticals).[26,27] Third, the physical properties of xanthene derivatives facilitate manipulation. It has a strong UV chromophore, is usually soluble in chlorinated solvents and THF, but is nearly insoluble in hexanes/ether mixtures. This differential solubility allows for crude purifications during library synthesis (liquid/liquid extractions) and after deprotection (precipitation from ether/hexane) when applied to the tetraamide mixtures. Finally, the symmetry of xanthene allows for a large number of compounds using only a limited number of building blocks.[7]

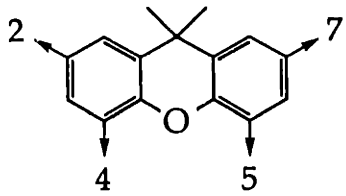
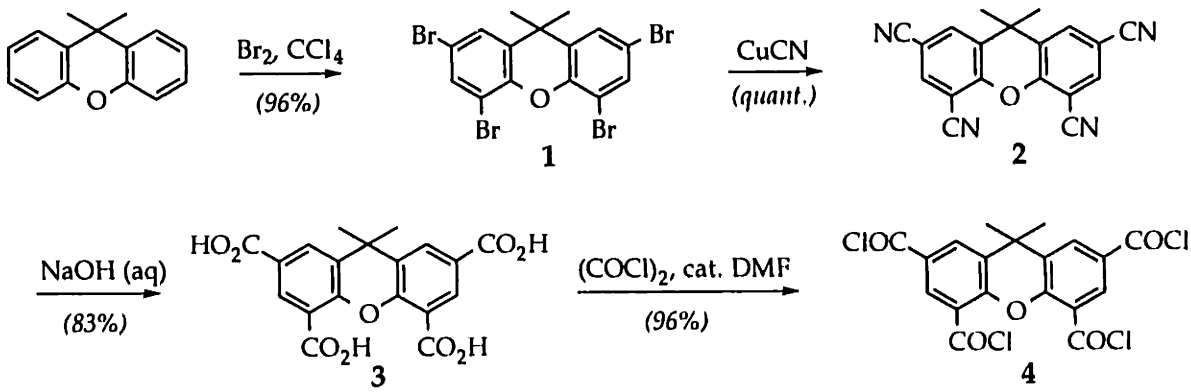


Figure 2: The numbering scheme and substitution pattern for the xanthene tetra core.

B2. Synthesis

The primary synthetic challenge we confronted was to find a reaction that would cleanly (>90% overall, >97% per site) transform a suitably tetra-functionalized xanthene to the tetraisocyanate. In reviewing isocyanate-generating procedures for application to the xanthene core, the acid or acid halide functionality was preferred to the amino group, as the xanthene tetraamine was known to be unstable.[28] To test the suitability of a number of possible reactions the tetraacid of xanthene (**3**) had to be prepared (**Scheme 1**). The synthesis of this core was unexpectedly complicated by the lack of a complete or reproducible synthesis.[29] The experimental section of this chapter details the isolation and characterization of **1-4**.

Scheme 1: Synthesis of the xanthene tetraacid and its tetrachloro derivative.



Starting from the tetraacid **3** several attempts were made to generate the tetraisocyanate. As the tetraacid was insoluble in most solvents (chloroform, toluene), the scope of reactions was limited. For example, the

tetraacid **3** did not react with DPPA in the presence of triethylamine (TEA) as the TEA-based salts were insoluble in both toluene and 1,4-dioxane. The tetraacid **3** was converted to the tetraacid chloride **4** (**Scheme 1**), which served as a precursor to the acyl azide. Using infrared (IR) spectroscopy it was determined that the tetraacyl azide (C=O stretch at 1694 cm^{-1}) could be efficiently formed from the tetraacid chloride **4** using TMS-N_3 [30] in dioxane. However, not all the acyl azide groups rearranged to the isocyanate (-N=C=O stretch at 2252 cm^{-1}) before other, uncharacterized carbonyl-based compounds (new, broad peak at 1710 cm^{-1}) began to form. These carbonyl compounds were likely due to polymerization of the isocyanate, a well-known but poorly understood process. As Curtius-type reactions did not seem to be applicable to this system, the search for a viable method continued.

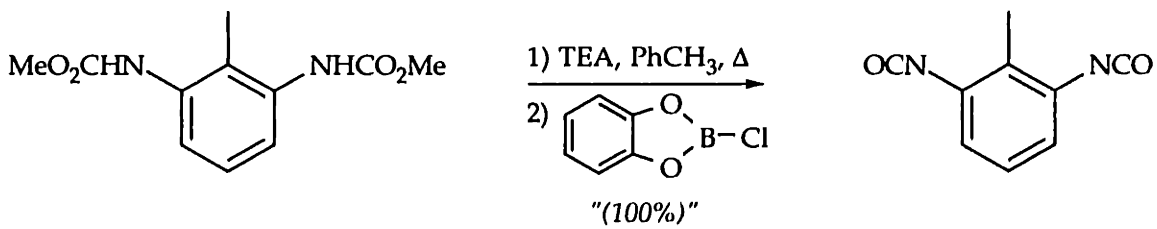
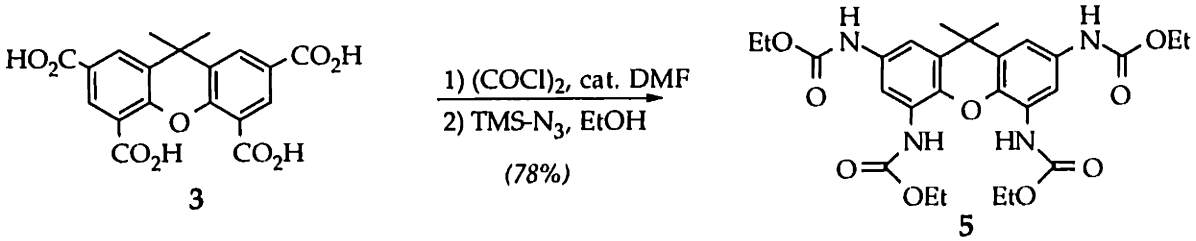


Figure 2: An example of the Valli and Alper method for isocyanate generation.[31]

At about the same time that the above studies were being conducted an article[31] appeared which claimed very high yields for converting carbamates into isocyanates. The study found that when carbamates (urethanes) were heated in hydrocarbon solvents with TEA for five minutes, followed by addition of chlorocatecholborane (CB-Cl), an alkoxycatecholborane formed and isocyanates were generated (see **Figure 2**). Amines were then added to form ureas and the overall yields were high (>90%) for a diverse set of carbamates. While we were initially skeptical of a library synthetic scheme requiring eight transformations, we nevertheless attempted to apply it to the xanthene core. If this was successful it would be a good demonstration of the principle that virtually any high-yielding, solution-phase reaction could be

incorporated into our combinatorial approach to generate libraries. We identified the tetraethyl carbamate **5** as an initial synthetic target. While the acid chloride to acyl azide to isocyanate conversions were not facile enough for library synthesis, they were efficient enough for formation of the stable tetraethyl carbamate **5**, which could be purified using silica gel chromatography (**Scheme 2**).

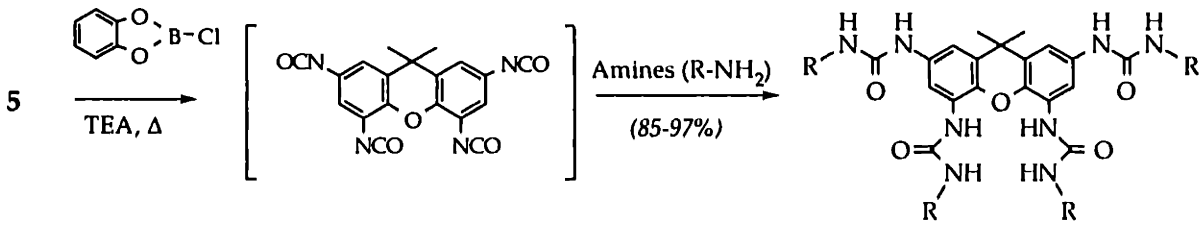
Scheme 2: One-pot synthesis of the tetraethyl carbamate of xanthene.



Initial trials of the chlorocatecholborane method on **5** were stymied by its poor solubility in the non-polar hydrocarbon solvents given in the publication.[31] However, upon switching to 1,4-dioxane the solubility problem was solved and the relatively high boiling point (102°C) enabled the reaction to go to completion, generating the tetraisocyanate *in situ*. After a brief cooling period, amines could be added in DMF or DMF-DCM mixtures,[32] depending upon the solubility properties of the amines, to give tetraureas (**Scheme 3**). Initial tetraurea adducts formed from *p*-methoxybenzylamine and various amino acid esters were found (by HPLC and NMR) to be >80% pure. While encouraging, some optimization was still necessary to raise the purities above the required 90%. A number of different parameters, including equivalents of amines and CB-Cl, concentrations, and work-up conditions were all varied. These studies revealed that an excess of building block amines and CB-Cl (1.5 equivalents/reactive site, each), a concentration of 0.02 M in dioxane, and an aqueous workup allowed for consistent yields and purities of ≥90%. An excess of amines was essential as some would react with the CB-Cl. The workup entailed evaporation of the reaction to dryness and dissolution of the residue in 5% methanol in dichloromethane, rinsing

with 1 N citric acid to eliminate excess amines, then treating with base to eliminate catechol-based compounds. The base requirement could be satisfied by either rinsing with NaOH (0.2 N) or shaking the organic layer with basic alumina and filtering (twice was usually sufficient).

Scheme 3: Synthesis of tetraurea xanthenes using chlorocatecholborane (CB-Cl).



B3. Mixture Analysis: HPLC

The finding that excess amines were required for high yields and purities was potentially troublesome. In the past,[7] excess amines were avoided to diminish the possibility that one amine would react faster than another, resulting in biased libraries. This possibility had to be investigated. To verify that statistical mixtures would be generated under the reaction conditions, a small library containing only two building blocks, glycine methyl ester and leucine *t*-butyl ester, was synthesized. Since the two amines differed[33] significantly in size and nucleophilicity and both were present in excess (3 equivalents each for four isocyanate sites) any biases should be reflected in the product distribution. This experiment revealed that all ten of the possible compounds were formed, and in close to a statistical distribution (**Figure 3**). When the two building blocks were added to the *in situ* tetraisocyanate in a 3:1 (Leu: Gly) ratio, the distribution changed accordingly (**Figure 3**).

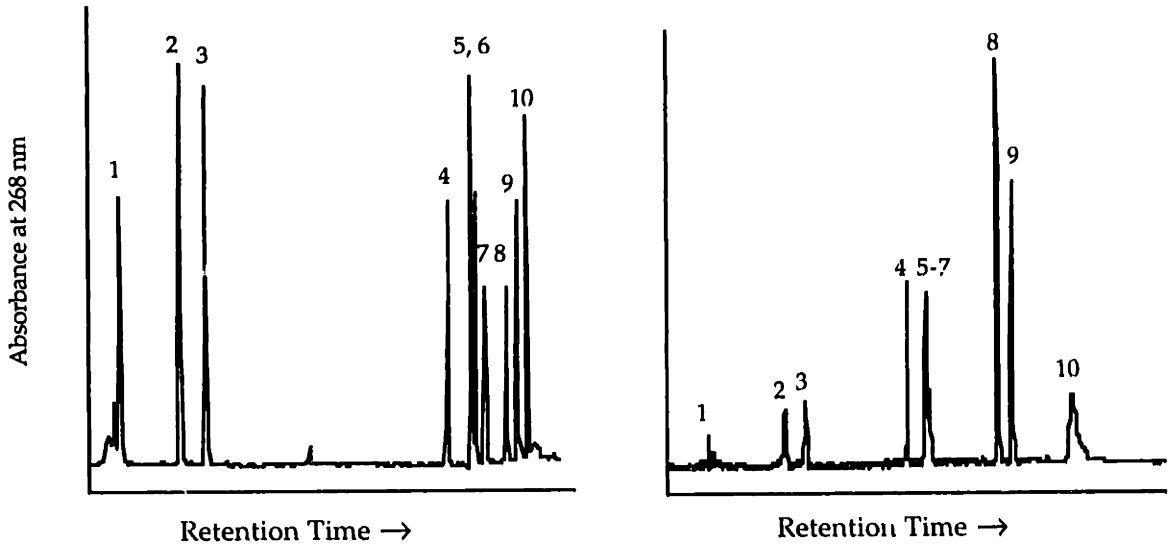


Figure 3: Reverse-phase HPLC traces of libraries made by reacting the xanthene tetraethyl carbamate (**5**, 1 equiv) with CB-Cl (6 equiv), then adding a 1:1 (left) or 3:1 (right) ratio of Leu-O*t*Bu and Gly-OMe (for a total of 6 equiv of amines) and performing an aqueous workup. The HPLC conditions differed slightly, accounting for the variations in retention times. Peaks 1 and 10 were characterized by synthesizing the pure compounds and matching the retention times; the numbered peaks were assumed to be 1: Gly_4, 2-3: Gly_3Leu, 4-7: Gly_2Leu_2, 8-9: $GlyLeu_3$, 10: Leu_4.

Generation of the tetraisocyanate was successful using CB-Cl and the reaction appeared to tolerate a number of different amines. However, before synthesizing libraries, suspect amines had to be checked individually and the compounds had to be analyzed. Derivatives of Arg, Gly, His, Ile, Leu, Lys (*t*Boc), Phe, Ser, Trp, Tyr and Val were synthesized and their purity verified by HPLC and NMR. The experimental section details the synthesis and characterization of a number of tetraureas (not shown) derived from the following amines: *p*-methoxybenzyl amine (**6**), Leu-OMe (**7**), Ser(O*t*Bu)-OMe (**8**), and Tyr-OMe (**9**). Although yields and purities did vary, no impurities over 10% were detected in any of these (homo) tetraureas. While the HPLC studies worked well for pure compounds and small mixtures, the limitations became apparent when three building blocks were used. This generated a theoretical mixture of 45 compounds, and even when the building blocks were chosen to maximize differences in retention time, not all the peaks

could be resolved (**Figure 4**). A more powerful analytical tool was necessary to detect individual compounds in larger mixtures.

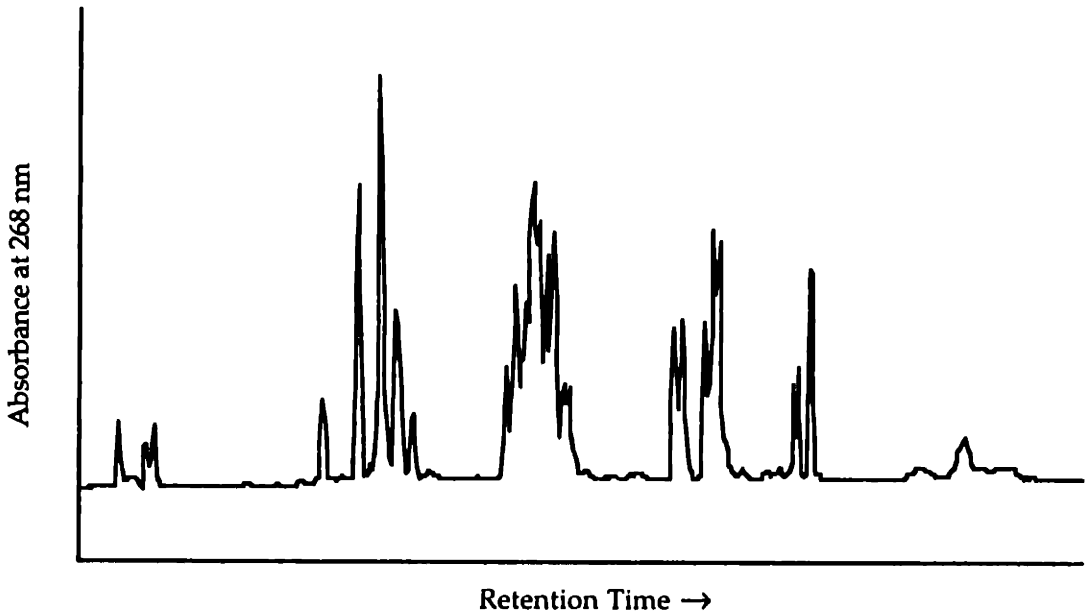


Figure 4: A reverse-phase HPLC trace of a library which theoretically contained 45 compounds made from Gly-OMe, Leu-O*t*Bu and Asn-O*t*Bu.

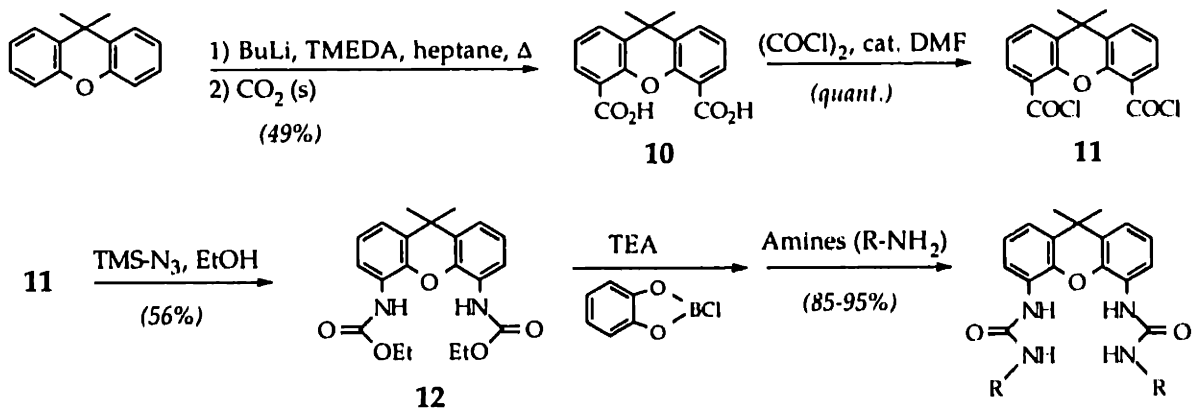
B4. Mixture Analysis: CE-MS and Synthesis of Diureas

As previously mentioned, a number of analytical techniques have been used to quantify the number of compounds in a library. One of the newer techniques is capillary electrophoresis coupled to mass spectrometry (CE-MS). An electric field is applied across a capillary tube, and the components in the mixture are separated according to their mobility in this electrophoretic field. This leads to separation based first on charge, then on the dynamics of the charge in the electric field. The compounds are then passed into a mass spectrometer where both MS and MS/MS experiments can be performed. This technique has been successfully applied to several xanthene polyamide libraries (see **Chapter A**).[34] After discussions with our collaborators in the Vouros group at Northeastern University, it was decided that initial urea-based libraries should contain only two urea groups, and that they should be at the sterically coupled 4 and 5 positions. This would allow for characteristic

fragmentation patterns to be identified, which was necessary to distinguish compounds of the same molecular weight.

A 4,5-disubstituted xanthene compound was desired, however suitable derivatives were not available. Xanthenes with alkyl groups at the 2 and 7 positions were not acceptable, as these compounds were less soluble under the analytical conditions and tended to non-productively fragment. In short, the 2 and 7 positions needed to be unsubstituted. However, synthesis of suitable precursors was not known. To obtain the desired 4,5-diethyl carbamate we planned to dilithiate 9,9-dimethyl xanthene (**Scheme 4**), a procedure which had been published only weeks before,[35] then quench with dry ice. Together with a fellow graduate student, Kent Pryor, we obtained the diacid **10** and converted it into the diethyl carbamate **12** using TMS-N_3,[30] as was done to obtain the tetraethyl carbamate **5**.

Scheme 4: Synthesis of diurea model compounds for CE-MS analysis.



Several pure diureas and small mixtures were analyzed using CE-MS. Characteristic fragmentation patterns were similar to that of the amide-based libraries and were identified for Pro/Leu (Pro adjacent to Leu), Pro/Asp, Asp/Asp, His/Leu, and His/His. A small amount (~ 5%) of incompletely deprotected His was also found, so the deprotection time was adjusted from four to six hours (no further His-trityl groups detected). Concurrently,

libraries of tetraureas were submitted for analysis. Their analyses proved more difficult, as the tetraures based on amino acids ('free acids') were found to aggregate in the capillary, resulting in poor separation. On the other hand, tetraureas of amino acid esters were separable under the CE-MS conditions. This technical limitation restricted the use of CE-MS, as it was more desirable to examine actual, ready-to-screen libraries, rather than pre-deprotection mixtures of esters (which were nearly insoluble in aqueous solvents). Nevertheless, the results that were obtained supported the HPLC analysis, all expected compounds were formed in an approximately statistical distribution.

B5. Deprotection Studies

Cleavage of the *t*-butyl esters as well as side chain protecting groups (BOC, mtr, trityl) could be accomplished under acidic conditions using TFA. Deprotection of the individual polyureas was explored and unanticipated results were observed. Previous studies using protected amino acids on xanthene[7] found that Reagent K (a cocktail of TFA, water, ethane dithiol, and thioanisole)[36] was preferred to neat TFA for deprotection, as the thiols scavenged the cations before they could add to activated aromatic residues (i.e. Trp and Tyr). When Reagent K was used on pure tetraureas, however, intractable mixtures resulted. The products of these reactions were only sparingly soluble in DMSO, and NMR analysis indicated that three or more compounds existed and a reaction had occurred involving the urea group(s) (urea -NH peaks were absent or diminished in intensity). Although uncharacterized, these impurities likely originated from reactions with the thiols (ethane dithiol in particular); formation of hydantoins (a known product of amino acid-based ureas, acid and water)[37] also could not be excluded. Fortunately, when neat TFA was used the products were free of any detectable impurities and deprotection was complete within six hours.

B6. Syntheses of Libraries and Their Physical Properties

Library synthesis commenced following the optimization of the reaction, workup, and deprotection conditions. A conscious decision was made to keep the libraries smaller in magnitude (less than 5,100 components using 10 building blocks) than the previous tetraamide libraries (up to 65,341 using 19). This decision was made for a number of reasons. First, smaller libraries allow for a more rapid deconvolution, as fewer sublibraries are necessary. Second, generation of larger mixtures increases the likelihood that impurities in the solvents (for example, water in DCM or DMF) or in the tertiary amine (diethylamine in TEA, for example)[38] used to "free base" the amino acid hydrochlorides will react with the activated core, preventing the intended building blocks from reacting. These background reactions are always possible and become particularly worrisome when concentrations of individual tools are very low. Identification of these side-reactions also becomes more difficult as the mixture's complexity grows. Third, the concentration of each component is very low in these large mixtures (and is exacerbated if the mixture is not statistical), increasing the chance that active compounds will be overlooked in less sensitive assays. Of course a balance must be struck between generating enough compounds in the mixture to justify synthesis (and screening), yet still create a high enough concentration of individual components to detect activity. As a compromise, mixtures of 2,000-5,100 components (8 or 10 building blocks) were chosen for the largest libraries.

Initially the choice of building blocks included methyl and *t*-butyl esters of amino acids. However, the methyl ester-containing libraries proved to be far less soluble in aqueous solutions than the free acid-containing libraries obtained from the deprotection of the *t*-butyl esters. Therefore, only methyl esters of hydrophilic amino acids (i.e. Gly, Ser) were used to synthesize libraries. In general, the mixtures were less soluble (in water and water-

DMSO mixtures) than their tetraamide counterparts. At first this was puzzling, as the addition of four -NH groups to a structure should provide enhanced water solubility. However, ureas are known to exhibit unique solubility properties in a number of solvents, and aggregation has been observed in solution as well as in the solid state.[12] Log P calculations (see **Figure 5**) on a number of homo tetra compounds support the experimental observation that xanthene tetraamides are more soluble in water than tetraureas. While not a direct indication of water solubility, the Log P values indicate a greater hydrophobicity for the ureas (about ten times more than the tetraamides). Nevertheless, this low solubility only proved troublesome at low pH (3-4); when the pH was raised (to ≥8) most libraries were completely soluble in aqueous solutions.

$(Building\ Block)_4$	Tetraamide Log P	Tetraurea Log P
Ala	0.15	1.59
Asn	-3.16	-1.71
Asp	0.19	1.63
Gly	-1.24	0.20
Leu	5.79	7.23
Phe	7.60	9.04
Ser	-3.39	-1.94

$$P = \frac{[compound]_{oct}}{[compound]_{aq}\,(1 - \alpha)}$$

Figure 5: A comparison of calculated Log P values (see experimental section) for a series of tetraamides and tetraureas. Experimental P values are determined by partitioning the compound between octanol (oct) and water (aq) and applying the equation to the right, where α is the degree of dissociation in water.[39]

B7. Limitations of the CB-Cl Method and Attempts to Isolate the tetraisocyanate

During library synthesis the limitations of using the CB-Cl approach to tetraureas became apparent. First, the use of a moisture-sensitive reagent

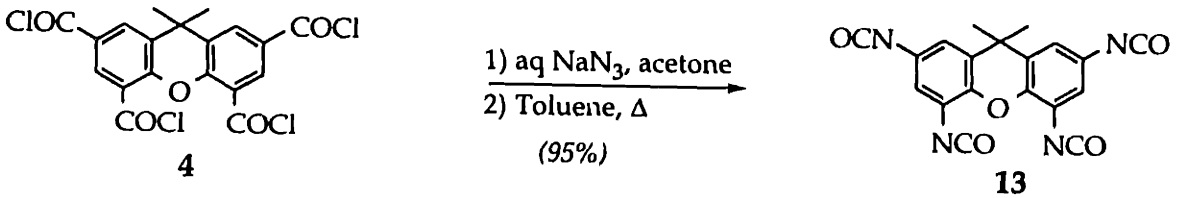
(CB-Cl) necessitated special reaction conditions such as dry solvents and an inert atmosphere. Since stock solutions of chlorocatecholborane were observed to decompose within hours, the solid had to be weighed out into every flask, for every reaction [Personal note: the cloud of HCl that accompanied the weighing of this reagent made this a favorite reaction of my coworkers who worked near the balance]. Second, its elimination required either rinsing with base (not recommended for amino acid derivatives)[40] or repeated use of basic alumina (messy and time consuming for multiple library synthesis). Third, despite our best efforts we were unable to isolate the tetraisocyanate from the reaction mixture.

Isolation of the tetraisocyanate, hopefully as a solid, was advantageous for a number of reasons. First, the overall condensation reaction would be greatly simplified— just dissolve the activated core in a solvent and add free-based amines. A simple rinse with citric acid solution and drying would give the tetraurea. This would save time when doing multiple parallel library syntheses. Second, because the core could be weighed out, the exact amount would be known and (near) stoichiometric amounts of amines could be used. Third, characterization (and possible purification) of the core would allow for a more thorough analysis of the reaction, since the purity of the core would be known. These reasons motivated a review of some of our previous efforts to isolate the activated core.

Initial studies indicated that the xanthene tetraacyl azide did not completely rearrange before side-reactions began to occur. However, the reaction was carried out in dioxane, a solvent known to be hygroscopic.[27] Perhaps the carbonyl-based impurities detected using IR were derived from traces of water. This would lead to hydrolysis of the isocyanate, which would ultimately generate the amine. The amine then could condense with other isocyantes to form dixanthene ureas. We decided that another system should be investigated. The xanthene tetraacid chloride **4** was dissolved in dry

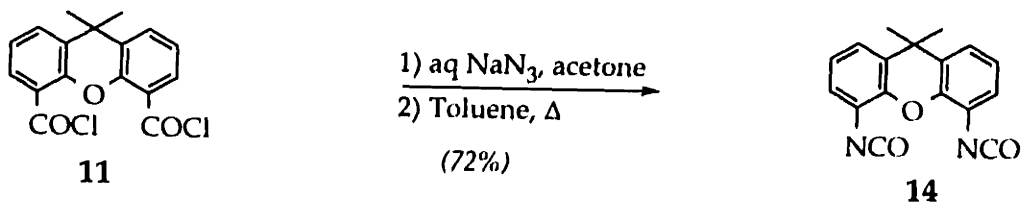
acetone and aqueous sodium azide was added.[41] Subsequent analysis by IR indicated that the tetraacyl azide was completely formed. Dichloromethane was added to the solution, and the layers were separated. This time a non-hygroscopic solvent (toluene) was chosen for the rearrangement. The mixture was partially concentrated to evaporate the dichloromethane, and the toluene solution was heated to reflux. After one hour gas evolution ceased, and the reaction was evaporated to give a beige solid (see **Scheme 5**). NMR indicated that the solid tetraisocyanate **13** was greater than 90% pure. We were pleasantly surprised to learn that the solid was stable enough for routine handling, and could be stored for months in the freezer.

Scheme 5: Preparation of the tetraisocyanate



As might be expected, the isolated core **13** reacted as well as the tetraethyl carbamate did using CB-Cl, giving high yields and statistical distributions of tetraureas. As predicted, the new synthesis and workup were simplified, and enabled facile library production. We also synthesized the xanthene-4,5-diisocyanate (**14**, *via* **Scheme 6**), which was used to synthesize diureas for CE-MS analysis. This diisocyanate was also used by another coworker, Blake Hamann, to synthesize diureas for molecular recognition.[42]

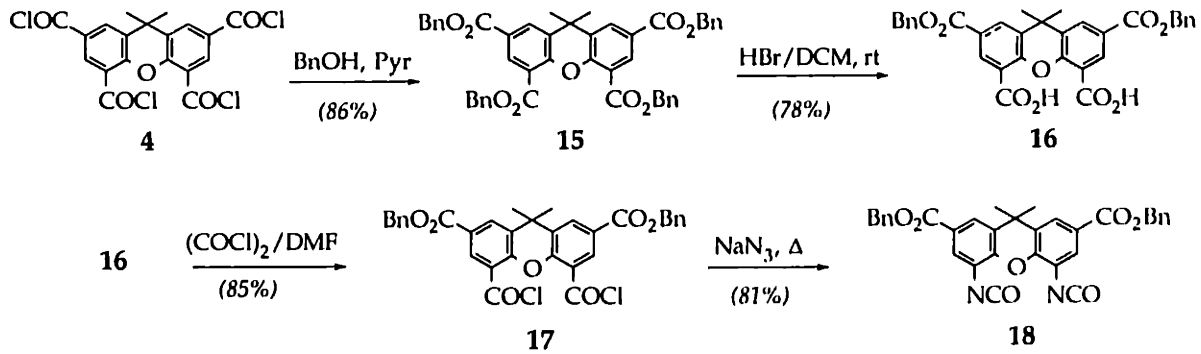
Scheme 6: Synthesis of the 2,7-unsubstituted-4,5-diisocyanate.



B8. Deconvolution: Syntheses of Multiply Addressable Cores

After synthesizing the main libraries we turned our efforts towards deconvolution (**Scheme 7**). While a number of schemes were investigated, including some using CB-Cl, the most practical is presented below. The dibenzylester diacid **16** provided a precursor to a two-address urea core. The benzyl ester protecting groups were convenient as they are easily removed using hydrogenolysis,[43] which is compatible with most peptide protecting groups. To generate ureas at the 4 and 5 positions the diacid chloride **17** was converted to the diisocyanate using sodium azide in acetone,[41] which rearranged to the diisocyanate dibenzyl ester **18**.

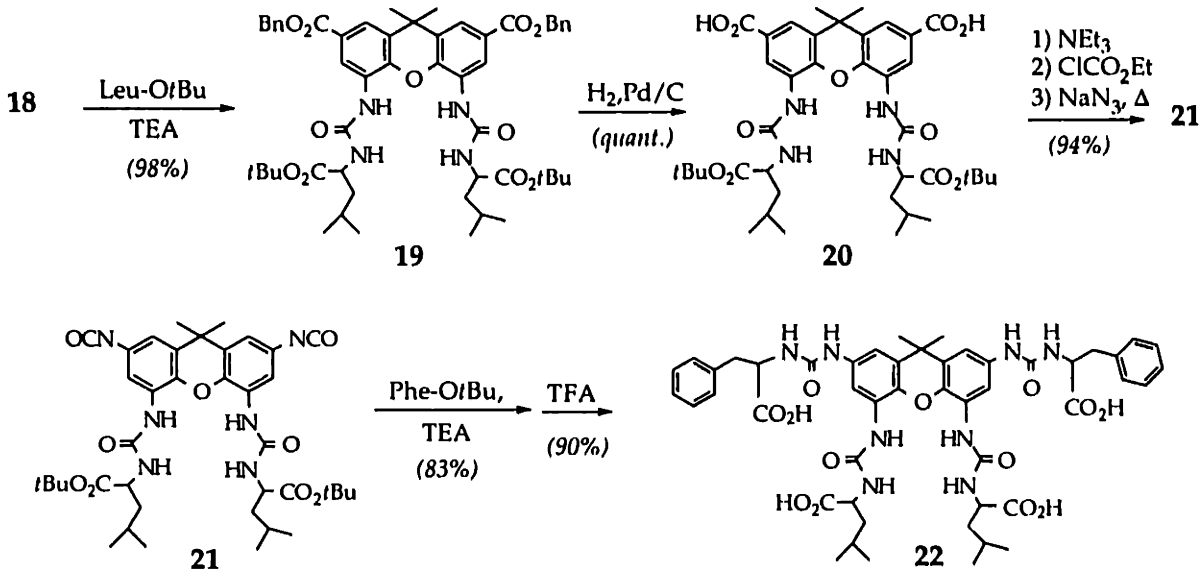
Scheme 7



Xanthene **18** could then be reacted with one or more amines, depending upon the stage of the deconvolution (**Scheme 8**). If one (in this case Leu-O*t*Bu) or two amines were condensed with **18** a separation could be performed to give purified diureas. Hydrogenolysis cleaved the benzyl ester groups in high yield to give the diacid diurea **20**. Activation of the acid groups as acid chlorides proved to be incompatible with acid-sensitive functionality on the amino acid ureas (such as *t*-butyl esters). After a number of different methods were attempted, we decided to convert the diacid into its dicarboxylate salt using triethylamine in acetone (**Scheme 8**). Ethyl chloroformate was then added to form the mixed anhydride, which was subsequently cleaved by sodium azide to give the acyl azide.[44] Following an extraction, dissolution in

toluene, and rearrangement, the diisocyanate di(Leu)urea **21** was isolated. One (Phe-O*t*Bu) or more amines could be reacted with **21** to give the desired tetraurea(s) (**22**, Leu-Leu-Phe-Phe).

Scheme 8: Synthesis of a tetraurea using the deconvolution protocol.



B9. Structures of the Xanthene Tetraureas

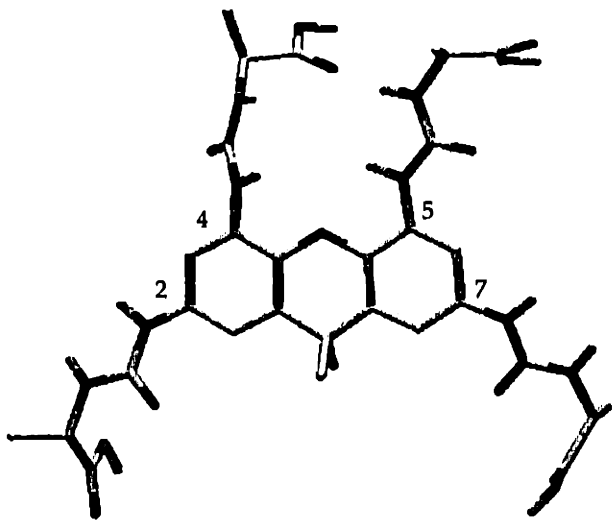


Figure 6: An MM2* minimized tetraalanine tetraurea. Several contact surfaces are available, including the 2/4, 4/5, 5/7, and 2/7.

Xanthene tetraureas have three levels of information built into their structure, allowing for many possible modes of interaction with biological

targets (**Figure 6**). The electron-rich aromatic scaffold could intercalate into DNA[45,46] or form π-π stacks[47] with aromatic amino acids in proteins, although the 9,9-dimethyl group may preclude some binding modes. The urea functional groups provide a second scheme for recognition of biological structures. The ureas at the 2 and 7 positions provide an array of hydrogen-bond donors and acceptors, while the ureas at the 4 and 5 positions provide a cavity (see **Figure 7**) which is known to strongly bind carboxylates.[9] In addition, the 2 and 4 (as well as 5 and 7) ureas provide yet another hydrogen bonding recognition surface. Additionally, the attached building blocks were expected to enhance interactions with biological targets; as most were based on amino acids, obvious biologically-relevant moieties. Molecular modeling calculations (MM2*) were used to minimize a few tetraurea structures and revealed a planar, disc-shaped orientation of the building blocks, similar to the tetraamides (see **Figure 6**). Finally, the use of the urea linkage conformationally limits the building blocks, as rotation about the urea N-C bond is restricted. This should reduce the entropic cost of binding to target molecules.

Structural knowledge of related compounds exists. For example, the solution and solid-state structure of a 4,5-diurea xanthene has been determined (**Figure 7**).[42] These studies showed that the urea -NH groups are directed inward towards the xanthene oxygen, creating a binding pocket between them and leaving the carbonyl oxygen accessible for intermolecular hydrogen bonding.

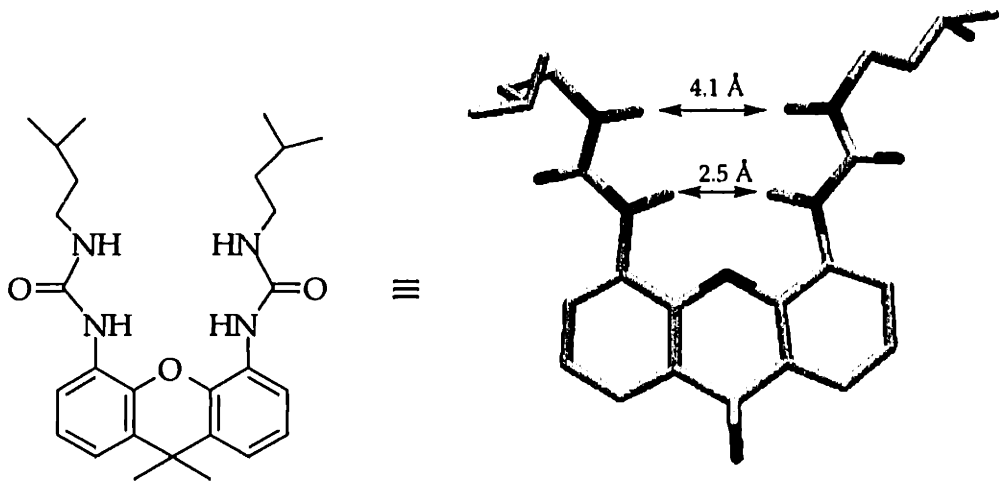


Figure 7: A polytube rendering of the x-ray crystal structure of a xanthene diurea (right).[42] Note the orientation of the ureas. NOE NMR studies suggest a similar solution-phase structure.[9]

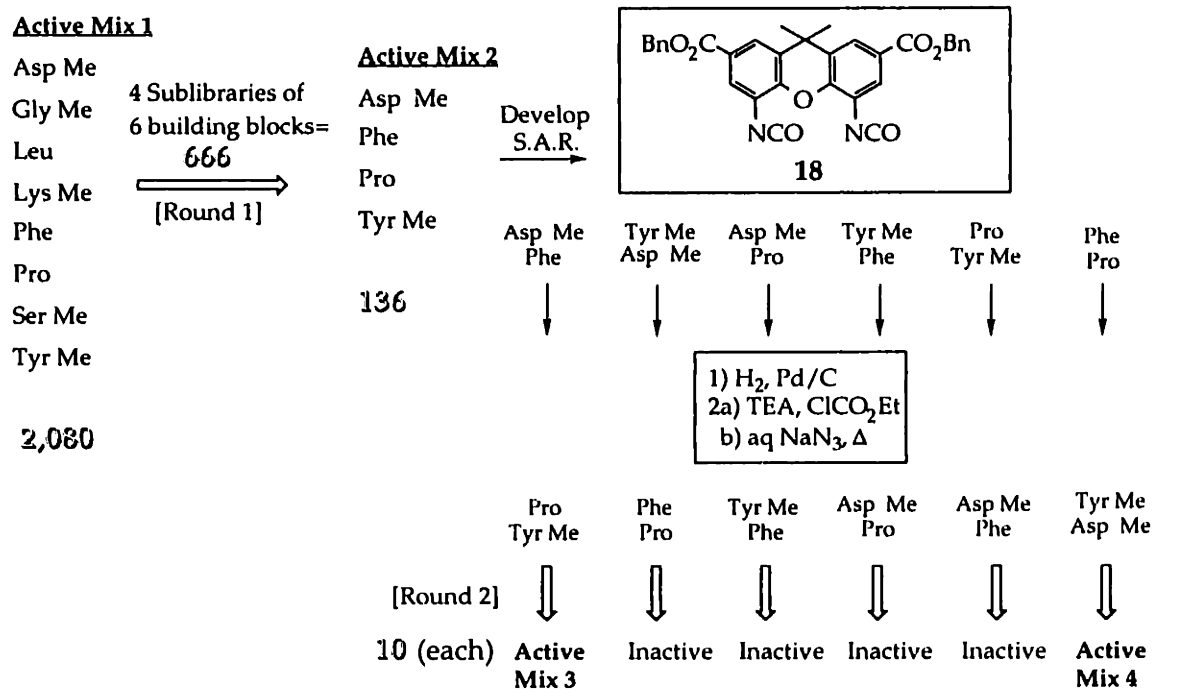
B10. Screening of Tetraurea Xanthene Libraries

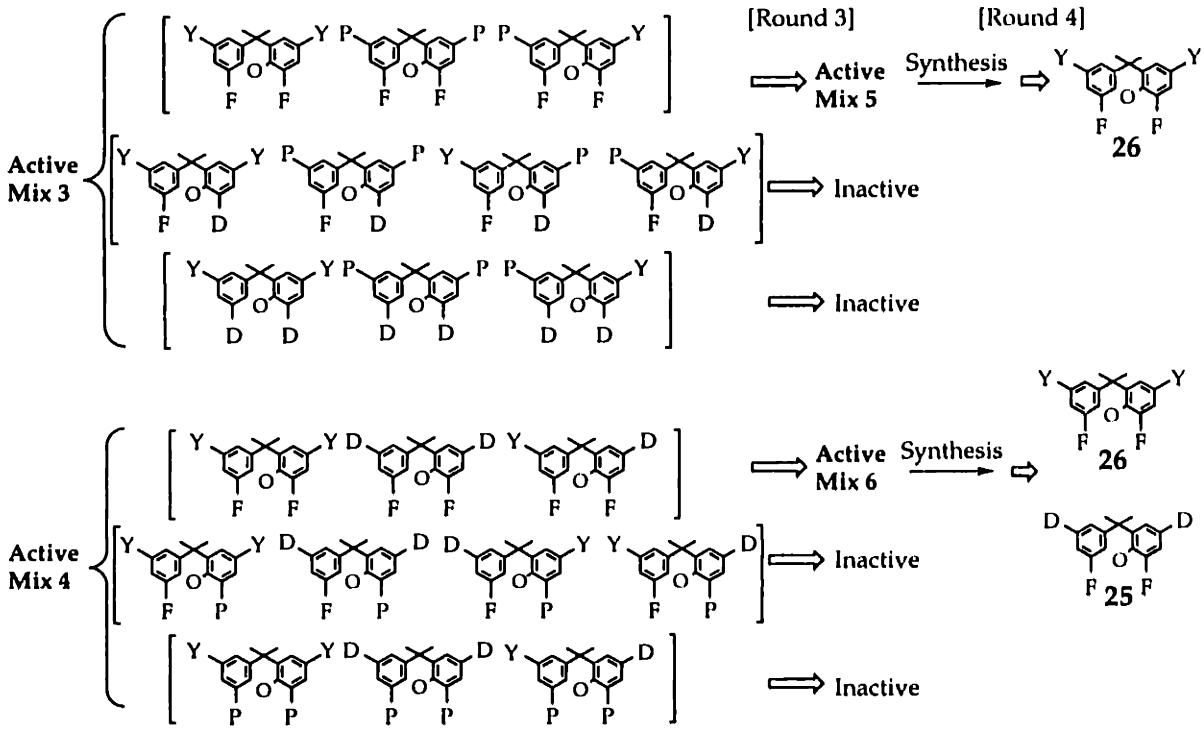
With the synthesis and deprotection methodologies established and libraries in hand, we focused our attention on screening. As previously mentioned, the solubility of the tetraureas in aqueous-based solvents at acidic pH was low. In addition, some buffers proved to be incompatible with the libraries. In particular the libraries had unexpectedly low solubility in tris(hydroxymethyl)aminomethane-based (tris) buffers, where precipitation was observed from water-DMSO mixtures. This precluded trypsin and chymotrypsin assays,[48] as both use tris buffers. In choosing an assay we were obviously limited to conditions that would dissolve our libraries. Also, we were looking for assays which were novel enough to justify our synthetic efforts. To this end, we began a collaboration with Professor Eric Davidson at the California Institute of Technology, who was looking for compounds capable of inhibiting binding of DNA to transcription factors.

The Davidson group prepared two dissimilar oligonucleotides to screen our libraries. The two were derived from transcription factors SpP3A2[49] and SpZ12-1 (a Zn-finger cDNA clone), respectively.[50] A gel-shift assay[51] was used to quantify the apparent inhibition of the transcription factor-DNA binding event. While identification of compounds capable of binding to transcription factors (and thereby causing inhibition) was the ultimate objective of this experiment, we (in the Rebek group) also wanted to demonstrate that our deconvolution strategy could be applied to tetraureas and successfully identify active components from large mixtures containing thousands of related molecules.

Of the five large libraries containing (theoretically) 2,080 tetraureas that were assayed, one was active in inhibiting transcription factor binding. The level of its activity (nanomolar) prompted us to synthesize sublibraries (**Scheme 9**). For the first round of sublibraries it was decided to omit two out of the six building blocks, resulting in four sublibraries of 666 compounds each. This amounted to a modification in the traditional routine, which specified eight sublibraries of seven building blocks containing 1,225 compounds each. Nevertheless, screening of these four sublibraries produced positive results; two were active at the same concentrations as the initially active library, while two others were inactive (round 1, **Scheme 9**). It is worth noting that these first sublibraries were made using the new, isolated tetraisocyanate method; since the activities of the sublibraries were comparable to the initial library it was unlikely that impurities in the CB-Cl method were responsible. These results implied that four out of the original eight building blocks were not essential, as their omission did not cause these sublibraries to lose activity. Since the deletion of the other four building blocks (Phe, Tyr-OMe, Asp-OMe, and Pro) did result in the loss of activity, it was assumed these (two or more of them) were essential for inhibition.

Scheme 9: Deconvolution of an active tetraurea library. Solid arrows represent synthetic or separation steps, hollow arrows indicate screening steps. The outlined numbers represent the number of compounds in each library at that stage in the screening. D= Asp (α) Me ester, F= Phe, P= Pro, Y= Tyr Me ester.

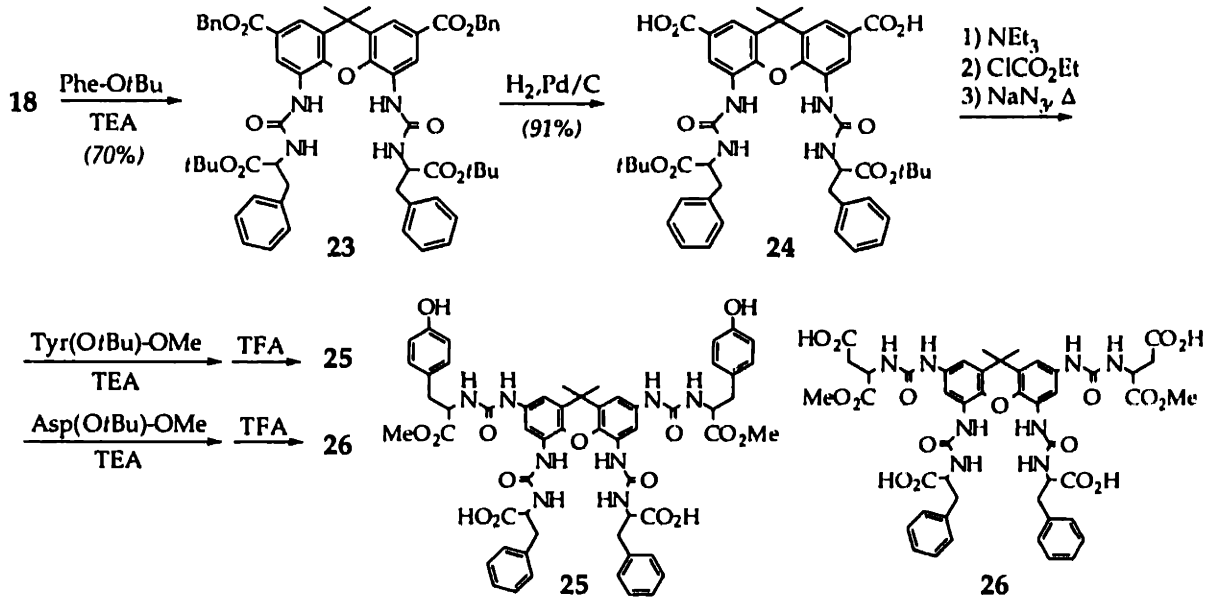




With only 136 theoretical combinations of four building blocks it was decided to attempt to develop structure-activity relationships (S.A.R.) by sequestering particular building blocks at particular positions. Since two or more building blocks were important, 4 of the 136 could be excluded (those of the type AAAA), leaving 132 unique compounds. At this point another assumption was made, that no building block occupied three positions (no AAAB's), this eliminated 48 more structures, leaving only 84 structures. In developing a synthetic pathway to these remaining molecules it was decided to employ the established deconvolution method (**Schemes 7** and **8**), which addressed the 4 and 5 positions first, and the 2 and 7 positions second. This method accessed 56 of the 84 (two-thirds). If activity was not found in these 56 compounds those which were excluded by the above assumptions would have to be synthesized. Fortunately, two of these sublibraries retained the previous (nanomolar) level of activity (round 2, **Scheme 9**).

This narrowed the number of compounds to only 19 (one structure was common to both). The remaining 19 could be conveniently split into six more sublibraries by separating out the 4,5-hetero compounds after the first condensation, then reacting the pure compounds with the other two building blocks. This resulted in mixtures of three or four compounds, or six sets of sublibraries (see mix 3 and 4 in **Scheme 9**). The screening of these six (round 3, **Scheme 9**) revealed that the two sets of four tetraureas were inactive, as well as two others (4,5-Asp,Asp and 4,5-Pro,Pro). Activity was found in two groups (active mix 5 and 6, **Scheme 9**) containing Phe at both the 4 and 5 positions. Synthesis and isolation of pure compounds (**Scheme 10**), followed by a fourth and final round of screening, indicated that two compounds (**25** and **26, Figure 9**) were active at a level of about 50 nM.

Scheme 10: Synthesis of the pure tetraurea inhibitors **25** and **26**.



B10-1. Discussion of Assay Results

During the above described procedures our collaborators disclosed to us that the proper control experiments were not performed. When these control experiments were executed it was revealed that the libraries were inhibiting two entirely different DNA-transcription factor complexes in a similar manner. The hypothesis was that the binding was not to the transcription factor, but instead to the DNA. The most likely mode for binding, which was suggested by our collaborators, was nonspecific intercalation[46] into the DNA bases. Given the structure of the two active compounds this seemed plausible. The core itself could intercalate, and both of the actives contain Phe residues in close proximity. In fact, **25** has four building blocks capable of intercalation. In addition, two families of intercalators are known which have skeletons resembling xanthene (acridines and actinomycins, **Figure 8**).[39] Studies of these systems indicated that seemingly small structural changes can lead to large differences in potency,[52] similar to observations made here in the case of xanthene tetraureas.

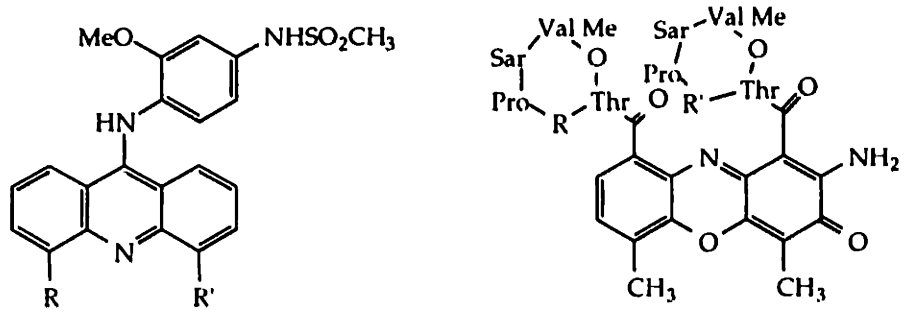


Figure 8: Two families of known intercalators: the acridines (left) and the actinomycins (right).

The fact that only two residues ended up being essential was surprising, but was consistent with the screening results of the initial large libraries, as none of the inactive libraries contained Phe, Tyr Me ester, and/or Asp Me ester. Although assumptions were made during the deconvolution, the activity remained roughly consistent throughout the process. There was, however, one troubling result from this deconvolution. The activity of mixture 6 (and 5, to a lesser extent) was about ten times greater than the purified, isolated compounds. This could be rationalized to a certain extent by the fact that mixture 6 contained two inhibitors, and that the assay might not have been precise enough to distinguish between activities of the same order of magnitude. The uncertainty in concentrations was about ±25% (due to errors in weighing, dilution, evaporation of sample), which also helps to explain the discrepancy. Two alternative explanations were considered but seemed less probable. First, perhaps two different compounds were necessary for the higher level of activity and when the compounds were separated from each other, the activity dropped. This result would be missed in single compound screening. However, entropy argues against such a scenario, especially given that the binding did not seem to be to a particular DNA sequence. Second, it was thought that impurities in the mixture might be responsible for the activity and when the mixture was purified the activity diminished. This is also unlikely as the active concentration remained roughly constant throughout the overall screening process, whereas the ratio of active library components to active impurities should have increased as the number of compounds per library decreased. In addition, three (CB-Cl,

isolated tetraisocyanate, and deconvolution) different synthetic methods were used; therefore, a common synthetically-derived impurity is unlikely. To respond to the impurity hypothesis, active mixtures 5 and 6 were analyzed by HPLC and found to be >85% pure, with no single impurity over 5%. The "pure" compounds **25** and **26** were re-purified using HPLC (reversed-phase) to give analytically pure compounds for re-screening. Testing of these samples has yet to be performed and (due to time constraints within the Davidson group) the screening of these samples has been indefinitely postponed.

B11. Summary of Xanthene tetraureas

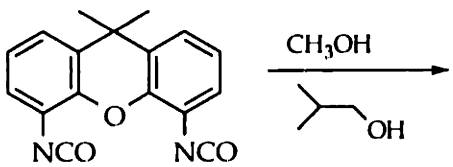
Pure and statistical ensembles of tetraureas were created in solution by using two different methods. Application of the chlorocatecholborane method proved that even reactions requiring several overall transformations (eight in this case) may be applied to our simultaneous, solution-phase approach. Isolating the tetraisocyanate facilitated library synthesis by reducing the complexity of the overall process. A successful synthetic protocol was devised to allow access to individual library components. The design of the tetraureas was tailored to produce compounds which were likely to interact with biomolecules and activity was found in an assay measuring binding of DNA to a transcription factor. While the net result of our screening and deconvolution efforts was the discovery of two DNA intercalators, rather than more novel transcription factor binders, our efforts nevertheless demonstrated that we could use this synthetic and numerical deconvolution strategy to identify single, active components in a library of over 2,000 tetraureas.

B12. Attempts to Extend Isocyanate-based Linkages to Urethanes

An obvious application of an isocyanate core is to produce urethanes by condensation with alcohols. However, alcohols are far less reactive than

amines, and the reaction timescale is often on the order of hours rather than seconds.[53] This invites two complications: biased libraries caused by discrimination of different alcohols and hydrolysis of the isocyanates by ambient water. To address the first pitfall a competition study was conducted using isobutyl alcohol and methanol. The results (as measured by HPLC peak areas) are presented in **Scheme 11** and reveal a strong bias towards the less hindered dimethyl carbamate, even at elevated temperatures. What was omitted from the scheme was the number of uncharacterized peaks, which were likely to be hydrolysis products. It seemed that mixing a polyisocyanate core with alcohols and expecting clean and predictable carbamate libraries was wishful thinking.

Scheme 11: Analysis of the product distributions from the uncatalyzed reaction of a diisocyanate with two different alcohols at three different temperatures.



14

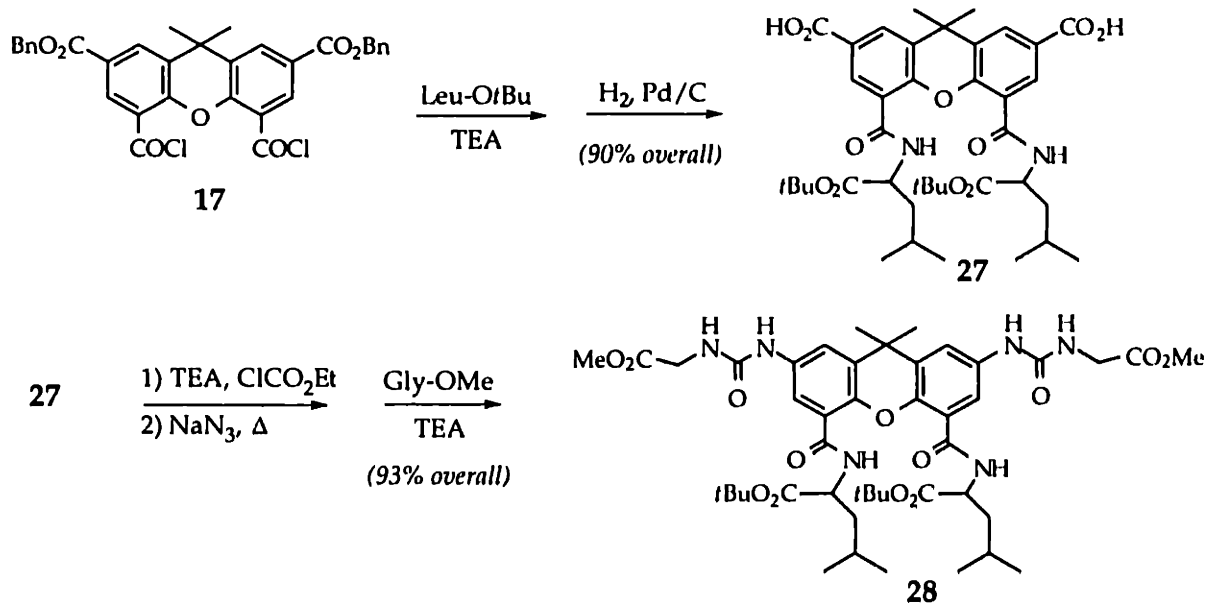
Temp (°C)	dimethyl	methyl/*i*but	di*i*but
25	50%	42	8
50	52	43	6
110	31	58	11

One way to diminish discrimination originating from the slow reaction rates of alcohols would be to catalyze the condensation reaction. As a test, the catalyst dibutyl tin diacetate[54] was used to catalyze the reaction of benzyl alcohol with **14**. However 23% of a much more polar compound was also detected– likely the monobenzyl carbamate monoamine. It seemed as though the catalyst also accelerated the background reaction with water. The implication was that meticulous drying of the solvent, core, building blocks, and catalyst would be necessary. Given that this could be accomplished for a number of different hygroscopic alcoholic building blocks, it still was not clear if the catalyst system would provide statistical distributions. For these reasons we were disinclined to further pursue carbamate linkages.

B13. Synthesis of a Core with Mixed Linkages

While one type of linkage per core molecule is more convenient from a synthetic standpoint, situations may arise (i.e. for optimization of a lead compound) where two different types of linkages are desired. Methodology to obtain a xanthene core molecule with both amide and urea linkages is presented below (**Scheme 12**). Although the building blocks were installed over four steps, the overall yield was high (84%), and (at least for **28**) purification was unnecessary. As the transformations to give **28** have proven to be tolerant of a number of functional groups, **Scheme 12** should be applicable to a variety of amide/urea derivatives.

Scheme 12: Synthesis of a tetrasubstituted xanthene with two different types of linkages.



B14. Experimental Section

B14-1. General

All reagents were purchased from Aldrich Chemical Company and used without further purification except as noted. Amino acid esters and PyBOP were acquired from Novabiochem (San Diego, California). Deuterated solvents were obtained from Cambridge Isotope Laboratories. Deuterated chloroform was dried over 4 Å molecular sieves. THF and 1,4-dioxane were distilled from sodium/benzophenone ketyl, DCM was distilled from P_2O_5 and triethylamine was distilled from (and stored over) KOH. Acetone was dried over 4 Å molecular sieves for 24 hours. Citric acid refers to a 1 N stock solution. Drying tubes were packed with anhydrous $CaCl_2$. NMR spectra were recorded on either a Bruker AC-250 or Varian Unity 300. All peaks were referenced to residual solvent. Either a Finnegan Mat 8200 (for HRMS/EI) or a VG ZAB-VSE (for HRMS/FAB) mass spectrometer was used to ascertain exact masses. IR spectra were obtained on a Mattson Sygus 100 FT IR spectrometer. Silica gel chromatography was performed with Silica Gel 60 (EM Science or Bodman, 230-400 mesh). TLC analysis was performed using glass-bound Silica Gel 60 (F254) plates.

B14-2. HPLC

For tetraester tetraureas (before deprotection) a C18 reverse-phase column (Rainin Microsorb-MV, 4.6 x 250 mm, 5 μm, 100 Å) was used for HPLC analysis with a flow rate of 1 mL/min. A linear gradient of 100% water to 100% MeCN over 30 min with a total run time of 45-50 min was used for most runs. The detection wavelength was 268 nm (λ_{max} for the xanthene tetraurea fragment), and the extinction coefficients of the tetraureas were assumed to be equal. After deprotection a C8 reverse-phase column (Waters Symmetry, 3.9 x 150 mm, 5 μm, 100 Å) was used employing similar gradients and a 1 mL/min flow rate.

B14-3. Molecular Modeling and Log P Calculations

All molecular modeling energy minimizations in this section were done with Macromodel *v*5.5 and the MM2* force field, without solvent parameters. Log P values were calculated using Log P *v*1.0 from Advanced Chemistry Development (Toronto, Canada). This program checks for tautomeric forms, then sums up the hydrophobic contributions of the fragments within each molecule (based on experimental values) to obtain Log P values.

B14-4. Synthesis and Characterization

2,4,5,7-Tetrabromoxanthene (**1**). Bromine (19 mL, 380 mmol, 4 equiv) was dissolved in CCl_4 (75 mL) and cooled in an ice bath. A bubbler was attached to monitor HBr evolution and 9,9-dimethylxanthene (20 g, 95.1 mmol, mixed with 10 mL of CCl_4) was added dropwise. After addition was complete, the reaction was allowed to warm to rt and Fe powder (100 mg) was added. Slow warming of the reaction mixture resulted in significant gas evolution. After 1 h at reflux the reaction was monitored by NMR, and was incomplete. Bromine and Fe powder were added in 1 mL and 20 mg portions respectively until the reaction was complete by NMR. Aqueous sodium sulfite and CCl_4 was added, the layers partitioned, and the organic layer evaporated to give a white solid (47.95 g, 96%). A large excess of bromine should be avoided as pentabromination could result. Note: DCM was not a suitable replacement for CCl_4 as the reaction time was significantly longer and the product was contaminated with a dark impurity. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.63 (d, *J*= 2.0 Hz, 2 H), 7.44 (d, *J*= 2.3 Hz, 2 H), 1.60 (s, 6 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 146.50, 133.90, 132.85, 127.92, 116.24, 112.02, 35.78, 31.75. HRMS (EI) calcd for $C_{15}H_{10}Br_4O$ 521.7465, found 521.7458.

2,4,5,7-Tetracyanoxanthene (**2**). Tetrabromoxanthene **1** (25.0 g, 47.5 mmol) and CuCN (18.7 g, 209 mmol) were mixed with N-methylpyrrolidinone (75 mL) and refluxed for 6 h under a drying tube. After cooling, the reaction was poured into water (200 mL), stirred, and filtered. The light green paste was rinsed with water and dried. The solid was transferred to a 2 L Erlenmeyer flask, and concentrated nitric acid (120 mL) and water (240 mL) were added (caution NO_2 and HCN are evolved). The contents were stirred for 2 h at rt, then slowly warmed to 80°C for 16 h. The cooled suspension was filtered and washed thoroughly with water. The yield was quantitative. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.56 (d, *J*= 1.9 Hz, 2 H), 8.48 (d, *J*= 1.8 Hz, 2 H), 1.67 (s, 6 H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 151.60, 136.85, 136.54, 131.69, 116.02, 113.14, 108.28, 101.65, 34.27, 32.23. HRMS (EI) calcd for $C_{19}H_{10}N_4O$ 310.0855, found 310.0859.

Xanthene-2,4,5,7-tetracarboxylic acid (**3**). Tetracyano xanthene **2** (23.61 g, 76 mmol) was added to aq NaOH (240 mL of 1.7 N) and refluxed for 14 h. After cooling, concentrated HCl was slowly added until pH= 1 and a fine precipitate formed. This was filtered off (slow), redissolved in 0.01 N HCl, and filtered again. Finally the collected solid was refluxed for 1 h with 1:1 water:ethanol

(200 mL), cooled, and filtered. Drying under high vacuum (at 40°C) in a dessicator containing P_2O_5 for 48 h was necessary to free the product from ethanol. A slightly yellow solid (24.4 g, 83%) was isolated. ^1H NMR (250 MHz, DMSO-d_6) δ 13.22 (br s, 4 H), 8.26 (d, J= 1.8 Hz, 2 H), 8.16 (d, J= 1.8 Hz, 2 H), 1.67 (s, 6 H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 165.96, 165.57, 150.16, 130.76, 130.45, 130.13, 126.33, 121.52, 34.12, 31.79. HRMS (FAB in NBA) calcd for $C_{19}H_{15}O_9$ $[M + H]^+$ 387.0716, found 387.0718.

Xanthene-2,4,5,7-tetracarboxylic acid chloride (**4**). Xanthene tetraacid (**3**, 0.50 g, 1.29 mmol) was suspended in ethanol-free chloroform (30 mL); oxalyl chloride (1.2 mL, 13.8 mmol) and DMF (50 mL) were added, and the mixture was stirred for 30 min at rt. After heating to reflux for 6 h under a drying tube, the reaction was concentrated to dryness, toluene was added and the suspension was evaporated. Drying afforded a yellow solid (0.57 g, 96%). mp 144-146°C. NMR (250 MHz, $CDCl_3$) δ 8.70 (d, J= 2.1 Hz, 2 H), 8.40 (d, J= 2.2 Hz, 2 H), 1.7 (s, 6 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.55, 163.34, 131.57, 131.22, 131.11, 123.65, 34.41, 31.99. LRMS (EI) calcd for $C_{19}H_{10}O_5Cl_4$ $[M]^{+\cdot}$ 458, found 458.

2,4,5,7-Tetraaminocarboethoxy xanthene (**5**). Xanthene tetraacid chloride (**4**, 1.50 g, 3.4 mmol) was dissolved in dioxane (35 mL); azidotrimethylsilane (TMS-N_3, 3.2 mL, 24.1 mmol) was added and the solution was stirred for 10 min at rt. Dry ethanol (3.2 mL, 55 mmol) was added and the solution was heated to reflux under a drying tube for 12 h. Upon cooling the reaction mixture was poured onto sat $NaHCO_3$ (100 mL) and EtOAc (200 mL) and the layers were partitioned. The aqueous phase was extracted (2x 150 mL ethyl acetate), rinsed with brine, and dried over Na_2SO_4. Concentration gave a brown foam which was dissolved in 15:1 toluene:ethanol and chromatographed in this eluent on a silica gel column. Weight of the slightly yellow solid was 1.48 g (78%). mp 118-120°C (dec.). ^1H NMR (250 MHz, DMSO-d_6) δ 9.51 (br s, 4 H), 7.82 (d, J= 2.1 Hz, 2 H), 7.34 (d, J= 2.1 Hz, 2 H), 4.19 (q, J=7.0 Hz, 4 H), 4.10 (q, J= 7.1 Hz, 4 H), 1.49 (s, 6 H), 1.30 (t, J= 7.1 Hz, 6 H), 1.23 (t, J=7.0 Hz, 6 H). ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 153.77, 153.54, 135.53, 134.05, 129.16, 125.94, 110.96, 110.53, 60.39, 59.92, 34.08, 31.90, 14.44. HRMS (EI) calcd for $C_{27}H_{34}N_4O_9$ $[M]^{+\cdot}$ 558.2326, found 558.2325.

General synthetic procedure for xanthene tetraureas (**6-9**). Tetraethylcarbamate (1 equiv) was dissolved in dry dioxane (~0.02 M), triethylamine (7 equiv) was added, and the reaction was heated to reflux for 10 min under argon. While still hot, chlorocatecholborane (6 molar equiv in

~0.1 M dioxane) was added (a white precipitate forms), and the mixture was refluxed for 15 min. After cooling to rt, the desired amino acid ester (6 equiv) was dissolved in a minimum amount of dry DMF with an equimolar amount of TEA. The reaction was stirred for 30 min at rt, then evaporated to dryness. The semisolid was dissolved in 5% methanol in DCM and subjected to either an aqueous workup (method A: rinsing with 1 M citric acid, then twice with 0.2 N NaOH); or (method B) shaking with basic alumina, then filtering.

2,4,5,7-Tetraurea of p-methoxy benzylamine (**6**). Worked up using method A, isolated yield of 94%, analytically pure by HPLC. NMR (250 MHz, DMSO-d_6) δ 8.47 (brs, 2 H), 8.40 (brs, 2 H), 7.91 (d, *J*= 2.3 Hz, 2 H), 7.37 (d, *J*= 2.3 Hz, 2 H), 7.22 (dd, *J*= 8.7, 2.7 Hz, 8 H), 7.08 (br t, 2 H), 6.87 (t, *J*= 8.3 Hz, 8 H), 6.35 (br t, 2 H), 4.22 (dd, *J*= 9.1, 5.9 Hz, 8 H), 3.71 (s, 6 H), 3.70 (s, 6 H), 1.47 (s, 6 H). HRMS (FAB in NBA) calcd for $C_{51}H_{55}N_8O_9$ $[M + H]^+$ 923.4092, found 923.4073.

2,4,5,7-Tetraurea of leucine-OMe (**7**). Worked up using method A, isolated yield of 88%, 94% pure by HPLC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.54 (br s, 2 H), 8.40 (br s, 2 H), 7.97 (d, *J*= 2.2 Hz, 2 H), 7.29 (d, *J*=2.3 Hz, 2 H), 6.89 (d, *J*= 7.7 Hz, 2 H), 6.33 (d, *J*= 7.9 Hz, 2 H), 4.26 (m, 4 H), 3.65 (s, 6 H), 3.64 (s, 6 H), 1.78-1.61 (m, 4 H), 1.57-1.51 (m, 8 H), 1.48 (s, 6 H), 0.93-0.87 (m, 14 H). ^{13}C (75.4 MHz, DMSO-d_6) δ 173.84 (br), 154.82, 154.36, 135.36, 133.56, 129.90, 127.39, 107.17, 107.03, 51.87, 51.81, 50.94, 50.69, 40.64, 34.54, 30.72, 24.34, 24.27, 22.69, 21.58, 21.54. HRMS (FAB in NBA) calcd for $C_{47}H_{71}O_{13}N_8$ $[M + H]^+$ 955.5141, found 955.5115.

2,4,5,7-Tetraurea of serine(OtBu)-OMe (**8**). Worked up using method A, 95% yield, >95% pure by NMR, ≥85% pure by HPLC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.87 (brs, 2 H), 8.59 (brs, 2 H), 8.05 (d, *J*=2.3 Hz, 2 H), 7.35 (d, *J*= 2.4 Hz, 2 H), 6.85 (d, *J*= 7.9 Hz, 2 H), 6.32 (d, *J*= 8.5 Hz, 2 H), 4.5-4.45 (m, 2 H), 4.44-4.40 (m, 2 H), 3.7-3.49 (m, 8 H), 3.66 (s, 6 H), 3.64 (s, 6 H), 1.48 (s, 6 H), 1.12 (s, 18 H), 1.11 (s, 18 H). ^{13}C (75.4 MHz, DMSO-d_6) δ 171.65, 171.36, 154.78, 154.21, 135.38, 133.67, 129.82, 127.48, 107.11 (br), 73.04, 72.92, 62.17, 61.97, 54.89, 53.59, 53.01, 51.86, 34.47, 30.84, 27.09. HRMS (FAB in NBA) calcd for $C_{51}H_{79}O_{17}N_8$ $[M + H]^+$ 1075.5563, found 1075.5552.

2,4,5,7-Tetraurea of tyrosine-OMe (**9**). Isolated in 86% yield according to method B, 93% pure by HPLC. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.27 (brs, 4 H), 8.69 (brs, 2 H), 8.42 (brs, 2 H), 7.93 (d, *J*=2.1 Hz, 2 H), 7.29 (d, *J*= 2.1 Hz, 2 H), 7.00-

6.94 (m, 8 H), 6.75 (d, *J*= 6.8 Hz, 2 H), 6.69-6.64 (m, 8 H), 6.18 (d, *J*= 7.9 Hz, 2 H), 4.42 (m, 4 H), 3.62 (s, 6 H), 3.60 (s, 6 H), 3.04-2.82 (m, 8 H), 1.44 (s, 6 H). HRMS (FAB in NBA) calcd for $C_{59}H_{63}N_8O_{17}$ $[M + H]^+$ 1155.4311, found 1155.4305.

4,5-Xanthene dicarboxylic acid (**10**). 9,9-Dimethylxanthene (2.00 g, 9.5 mmol), dry heptane (100 mL), and TMEDA (3.5 mL, 24 mmol) were combined and degassed with Ar for 15 min before *n*BuLi (1.6 M in hexane, 15 mL, 24 mmol) was added. The solution was warmed to reflux under Ar for 15 min, then allowed to cool to rt. The reddish solution was canulated onto a large excess of dry ice, allowed to stand for 1 h, then EtOAc and 2 M HCl were added until most of the solids dissolved and the pH= 1. The layers were separated and the aqueous layer was extracted twice with EtOAc:THF (20:1). The combined organic layers were rinsed once with brine and dried over Na_2SO_4. Upon concentration a white solid precipitated. The flask was then cooled to 0°C, and the product filtered. A slightly yellow powder (1.39 g, 49%) was isolated which was pure by NMR. Additional material (~0.2 g) could be recovered from the orange filtrate but contained about 15% of an impurity. mp 248-249°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.9 (brs, 2 H), 7.80 (dd, *J*= 7.8 and 1.7 Hz, 2 H), 7.69 (dd, *J*= 7.7 and 1.5 Hz, 2 H), 7.23 (t, *J*= 7.8 Hz, 2 H), 1.61 (s, 6 H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 166.19, 147.72, 130.72, 130.40, 129.45, 123.57, 119.93, 33.85, 31.94. HRMS (EI) calcd. for $C_{17}H_{14}O_5$ $[M]^{+\cdot}$ 298.0841, found 298.0839.

4,5-Xanthene dicarboxylic acid chloride (**11**). To the suspended diacid **10** (0.75 g, 0.25 mmol) at 0°C in ethanol-free chloroform (100 mL) was added oxalyl chloride (4.0 mL, 46 mmol) and DMF (5 μL). Within 15 min a clear solution was evident and the contents were warmed to reflux under a drying tube for 1 h. The solution was evaporated to dryness, 5 mL dry toluene was added, and the mixture was again evaporated. The pale yellow solid was pure by NMR and the yield quantitative. mp 144-146°C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.92 (dd, *J*= 7.7 and 1.5 Hz, 2 H), 7.68 (dd, *J*= 7.9 and 1.5 Hz, 2 H), 7.25 (t, *J*= 7.8, 2 H), 1.66 (s, 6 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.55, 163.34, 131.57, 131.22, 131.11, 123.65, 34.41, 31.99. HRMS (EI) calcd for $C_{17}H_{12}Cl_2O_3$ $[M]^{+\cdot}$ 334.0163, found 334.0160.

4,5-Diaminocarboethoxy xanthene (**12**). Diacid chloride **11** (0.50 g, 1.5 mmol) was dissolved in dry dioxane (35 mL) and treated with TMS-azide (1.0 mL, 11.3 mmol, a few bubbles noted). After 5 min at rt absolute ethanol (1.5 mL) was added and the flask was warmed slowly to reflux under a drying tube, during which time nitrogen evolution was noticeable. After 2 h of heating

the contents were cooled to rt, poured onto sat $NaHCO_3$, extracted (3x 50 mL) with EtOAc, and dried over Na_2SO_4. After concentration, the brown foam was dissolved in DCM and loaded onto a silica gel column (5:1 hexanes:EtOAc). The product was isolated as a white, fluffy solid (0.32 g, 56%). ^1H NMR (250 MHz, DMSO-d_6) δ 9.52 (s, 2 H), 7.63 (d, *J*= 7.8 Hz, 2 H), 7.24 (dd, *J*= 7.8 and 1.3 Hz, 2 H), 7.07 (t, *J*= 8.0 Hz, 2 H), 4.18 (q, *J*= 7.1 Hz, 4 H), 1.55 (s, 6 H), 1.29 (t, *J*= 7.1 Hz, 6 H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 153.93, 139.88, 129.29, 125.94, 122.80, 120.74, 119.89, 60.39, 33.72, 32.14, 14.40. HRMS (EI) calcd for $C_{21}H_{23}N_2O_5$ $[M]^{+\cdot}$ 383.1607, found 383.1606.

2,4,5,7-Tetraisocyanato xanthene (**13**). To a solution of **4** (0.40 g, 0.76 mmol, 1 equiv) in dry acetone (20 mL) at 0°C was added a solution of sodium azide (0.81 g, 12.5 mmol, 16.3 equiv) in water (2 mL). A precipitate immediately formed, and the mixture was stirred for 30 min. The resulting slurry was extracted with DCM (2x 100 mL), and the organic layer was dried over $MgSO_4$, filtered, and concentrated to about one-tenth its original volume. Toluene (20 mL) was added, and the remaining DCM was evaporated. The toluene solution was refluxed for 1.5 h under argon, cooled to rt, then filtered through a fine frit to remove traces of suspended solids. Concentration of the solution afforded a beige solid (0.270 g, 95%). mp 173-176°C. ^1H NMR (250 MHz, $CDCl_3$) δ 6.93 (d, *J*= 2.4 Hz, 2 H), 6.79 (d, *J*= 2.4 Hz, 2 H), 1.59 (s, 6 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 141.54, 131.25, 129.16, 126.98, 124.72, 123.26, 119.82, 119.46, 35.13, 31.04. HRMS (EI) calcd for $C_{19}H_{10}N_4O_5$ $[M]^{+\cdot}$ 374.0651, found 374.0643.

4,5-Diisocyanato xanthene (**14**). To a solution of **11** (0.594 g, 1.77 mmol, 1 equiv) in dry acetone (25 mL) at 0°C was added aqueous sodium azide (0.61 g, 9.38 mmol in 1.5 mL water). A fine precipitate formed immediately, and the mixture was stirred for 30 min. The resulting slurry was extracted with DCM (2x 100 mL) and the organic layer was dried over $MgSO_4$, filtered, and concentrated. Toluene (15 mL) was added, and the remaining DCM was removed *in vacuo*. The toluene solution was refluxed for 1 h under argon before the solvent was removed. The residual oil was taken up in DCM, and the solvent was again removed to yield a crystalline product (0.374 g, 72%). mp 83-86°C ^1H NMR (250 MHz, DMSO-d_6, unstable for more than 1 h) δ 7.48 (dd, *J*= 7.8, 1.7 Hz, 2 H), 7.23 (dd, *J*= 7.8, 1.7 Hz, 2 H), 7.14 (t, *J*= 7.8 Hz, 2 H), 1.60 (s, 6 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.85, 130.95, 126.72, 123.67, 123.30, 123.30, 122.13, 34.63, 33.29. HRMS (EI) calcd for $C_{17}H_{12}N_2O_3$ $[M]^{+\cdot}$ 292.0848, found 292.0852.

Tetrabenzyl 2,4,5,7-xanthene tetracarboxylate (**15**). Xanthene tetraacid chloride **4** (3.00 g, 6.56 mmol) was dissolved in DCM (50 mL) and the solution was cooled to 0°C. Benzyl alcohol (4.7 mL, 45.4 mmol) and pyridine (3.9 mL, 48.2 mmol) were added dropwise, along with DMAP (2 mg, cat). After 10 min at 0°C the solution was allowed to warm to rt over 3 h. More DCM (100 mL) was added, the solution was rinsed (2x 50 mL 2 N HCl) and dried over Na_2SO_4. Concentration gave an oil which precipitated upon addition of MeOH (20 mL) and sonication. Filtration afforded a tan solid (4.19 g, 86%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.35 (d, *J*= 2.0 Hz, 2 H), 8.28 (d, *J*= 2.0 Hz, 2 H), 7.45-7.26 (m, 20 H), 5.44 (s, 4 H), 5.37 (s, 4 H), 1.69 (s, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{47}H_{38}O_9Cs$ $[M + Cs]^+$ 879.1571, found 879.1538.

Dibenzyl 4,5-dicarboxylic acid-2,7-xanthene dicarboxylate (**16**). HBr was bubbled through DCM (100 mL) for 15 min. After cooling the system to 0°C, a solution of the tetraester **15** (3.00 g, 4.02 mmol) in DCM (5 mL) was added and the contents were allowed to warm to rt. After 5 h the solution was sparged with nitrogen for 20 min, then concetrated to a brown oily solid. Trituration with hexanes (100 mL), then warming and filtering eliminated the BnBr. SiO_2 chromatography (25:1→10:1 DCM:MeOH) afforded the product **16** (1.50 g, 2.64 mmol, 66%) as well as tribenzyl mono acid (0.65 g, 25%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 11.91 (s, 2 H), 8.64 (d, *J*= 2.0 Hz, 2 H), 8.39 (d, *J*= 2.0 Hz, 2 H), 7.5-7.3 (m, 10 H), 5.41 (s, 4 H), 1.73 (s, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{33}H_{26}O_9Cs$ $[M + Cs]^+$ 699.0631, found 699.0662.

Dibenzyl 4,5-diacid chloride-2,7-xanthene dicarboxylate (**17**). Dibenzyl ester diacid **16** (350 mg, 0.617 mmol) was suspended in DCM (15 mL), oxalyl chloride (0.230 mL, 2.47 mmol) was added and the flask was placed in a water bath (~15°C). DMF (2 μL, cat) was then added and gas evolution was soon observed. The mixture was stirred under a drying tube for 3 h at rt, concentrated to a solid, triturated with toluene, and concentrated again to yield a slightly yellow solid (349 mg, 94 %). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.58 (d, *J*= 2.1 Hz, 2 H), 8.37 (d, *J*= 2.1 Hz, 2 H), 7.46-7.39 (m, 10 H), 5.42 (s, 4 H), 1.71 (s, 6 H).

Dibenzyl 4,5-diisocyanato-2,7-xanthene dicarboxylate (**18**). Dibenzyl ester diacid chloride **17** (325 mg, 0.539 mmol) was added to acetone (20 mL) and sonicated for 2 min. After cooling to 0°C, sodium azide (285 mg, 4.39 mmol, in 0.8 mL water) was added dropwise with brisk stirring. A ppt formed immediately and the suspension was stirred for 30 min before being poured into a separatory funnel containing DCM (125 mL) and water (25 mL). The layers were separated, the water layer extracted with DCM, and the hazy

organic layer rinsed once with brine, dried over $MgSO_4$, and partially concentrated. Toluene was added and the remaining DCM was evaporated. The solution was then warmed to near reflux for 1 h. The solution was concentrated to a yellow foam (375 mg, 81%). ^1H NMR (250 MHz, $CDCl_3$) δ 8.00 (d, *J*= 1.8 Hz, 2 H), 7.75 (d, *J*= 1.7 Hz, 2 H), 7.44-7.39 (m, 10 H), 5.37 (s, 4 H), 1.68 (s, 6 H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.91, 146.51, 135.71, 130.62, 128.66, 128.42, 128.31, 126.88, 126.37, 125.29, 125.05, 122.68, 67.10, 34.88, 32.65. HRMS (EI) calcd for $C_{33}H_{24}N_2O_7$ $[M]^{+\cdot}$ 560.15835, found 560.15822.

Dibenzyl 4,5-dileucine-OtBu diurea-2,7-xanthene dicarboxylate (**19**). To a solution of dibenzyl ester diisocyanate **18** (0.302 g, 0.539 mmol) in DCM (10 mL) was added Leu-O*t*Bu·HCl (0.265 g, 1.19 mmol) in DCM (5 mL) containing TEA (0.20 mL, 1.4 mmol). After 1.5 h the reaction was diluted with DCM (25 mL) and rinsed with citric acid. Concentration of the DCM solution produced a yellow solid (0.481 g, 98%). ^1H NMR (250 MHz, $CDCl_3$) δ 8.15 (s, 2 H), 7.97 (d, *J*= 2 Hz, 2 H), 7.47-7.27 (m, 12 H), 6.45 (d, *J*= 7 Hz, 2 H), 5.34 (appar q, *J*= 12 Hz, 4 H), 4.47 (m, 2 H), 1.75-1.65 (m, 4 H), 1.62 (s, 6 H), 1.43 (s, 18 H), 1.42-1.33 (m, 2 H), 0.93 (m, 12 H).

4,5-Dileucine-OtBu diurea-2,7-xanthene dicarboxylic acid (**20**). The dibenzyl ester diLeu **19** (350 mg, 0.47 mmol) was taken up in 4:1 EtOAc: ethanol (20 mL) and stirred under a H_2 (atm) for 12 h in the presence of 10% Pd/C (10 mg, cat). The solution was filtered through celite using ethyl acetate and concentrated to give the product in quantitative yield as a yellow solid. ^1H NMR (250 MHz, $CDCl_3$) δ 8.65 (d, *J*=1.8 Hz, 2 H), 8.63 (br s, 2 H), 7.71 (d, *J*= 1.8 Hz, 2 H), 6.92 (d, *J*= 6.8 Hz, 2 H), 4.14 (m, 2 H), 1.8-1.44 (m, 6 H), 1.66 (s, 6 H), 0.95-0.89 (m, 12 H). HRMS (FAB in NBA/CsI) calcd for $C_{39}H_{54}O_{11}N_4Cs$ $[M + Cs]^+$ 887.2843, found 887.2853.

2,7-Diisocyanato-4,5-dileucine-OtBu diurea xanthene (**21**). The diacid diLeu urea **20** (130 mg, 0.17 mmol) was dissolved in acetone (0.8 mL) and cooled to 0°C; TEA (54 μL, 0.381 mmol) was added, followed by ethylchloroformate (40 μL, 0.38 mmol). After 20 min, sodium azide (50 mg, 0.76 mmol, in 0.2 mL water) was added and the emulsion was stirred for 25 min. DCM (20 mL) and water (10 mL) were added and the layers were separated. The organic layer was extracted (2x 20 mL DCM), rinsed with brine, and dried over $MgSO_4$. The organic layer was concentrated to near dryness, toluene (5 mL) was added, the remaining DCM stripped off, and the solution refluxed for 1.5 h, with gas evolution beginning at 72°C. Concentration of the solution gave a brown solid (122 mg, 0.16 mmol, 94%). ^1H NMR (250 MHz, $CDCl_3$) δ 7.59 (d, *J*= 2 Hz,

2 H), 7.50 (br s, 2 H), 6.79 (d, *J*= 2 Hz, 2 H), 6.43 (d, *J*= 8.5 Hz, 2 H), 4.44 (appar q, *J*= 5.5 Hz, 2 H), 1.54 (s, 6 H), 1.44 (s, 18 H), 1.41-1.2 (m, 6 H), 0.93, (d, *J*= 6.4 Hz, 12 H).

2,7-Diphenylalanine-4,5-dileucine tetraurea xanthene (**22**). To the diisocyanate **21** (30 mg, 0.040 mmol) in DCM (5 mL) was added Phe-O*t*Bu·HCl (26 mg, 0.10 mmol) in DCM (3 mL) with TEA (15 μL, 0.11 mmol). After 1 h at rt the clear solution was diluted with DCM and rinsed with citric acid; concentration gave a white solid (40 mg, 83%). ^{1}H NMR (broad, 250 MHz, DMSO-d_6) δ 8.69 (s, 2 H), 8.50 (br s, 2 H), 7.80 (d, *J*= 2 Hz, 2 H), 7.31-7.19 (m, 12 H), 6.85 (br s, 2 H), 6.21 (d, *J*= 8.4 Hz, 2 H), 4.41 (appar q, *J*= 6 Hz, 2 H), 4.15 (m, 2 H), 2.96 (d, *J*= 6.3 Hz, 4 H), 1.51-1.45 (m, 10 H), 1.39 (s, 18 H), 1.35 (s, 18 H), 1.28-1.22 (m, 2 H), 0.91 (appar t, *J*= 6.5 Hz, 12 H).
To the above tetra *t*butyl ester (35 mg, 0.029 mmol) was added TFA (2 mL) and small bubbles evolved immediately. After stirring for 6 h under a drying tube the TFA was evaporated to yield a yellow oil. Addition of a 1:1 solution of Et_2O:hexanes caused immediate precipitation. Drying of this solid *in vacuo* yielded a slightly yellow residue (25 mg, 90%). ^{1}H NMR (broad, 250 MHz, DMSO-d_6) δ 8.67 (br d, 2 H), 8.38-8.33 (m, 2 H) 7.93 (br d, 2 H), 7.31-7.19 (m, 10 H) 6.73 (m, 4 H), 6.15 (d, *J*= 7.2 Hz, 2 H), 4.44-4.42 (m, 2 H), 4.18 (m, 2 H), 2.95 (m, 2 H), 1.75-1.51 (m, 6 H), 1.47 (s, 6 H), 0.92-0.85 (m, 12 H). HRMS (EI) calcd for $C_{49}H_{58}N_8O_{13}$ 966.4123, found 966.4114.

2,7-Dibenzyl ester-4,5-diphenylalanine-OtBu diurea xanthene (**23**). Synthesized from **18** and Phe-O*t*Bu·HCl in the same way as **19**. The compound was purified using SiO_2 (3:1 hexanes:EtOAc, preloaded using DCM) to give a white solid in 68% yield. ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.71 (d, *J*=1.8 Hz, 2 H), 8.68 (br s, 2 H), 7.76 (d, *J*= 1.9 Hz, 2 H), 7.47-7.18 (m, 10 H), 6.84 (d, *J*= 7.4 Hz, 2 H), 5.36 (s, 4 H), 4.45 (q, *J*= 7.1 Hz, 2 H), 3.04 (m, 4 H), 1.60 (s, 6 H), 1.29 (s, 18 H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 171.12, 170.30, 165.26, 153.96, 141.66, 136.77, 136.27, 130.06, 129.29, 128.50, 128.19, 128.04, 127.85, 126.60, 125.13, 119.81, 118.45, 81.15, 66.10, 54.53, 37.92, 34.35, 31.20, 27.48. HRMS (FAB in NBA/CsI) calcd for $C_{59}H_{62}N_4O_{11}Cs$ $[M + Cs]^+$ 1135.3469, found 1135.3483.

4,5-Diphenylalanine-OtBu diurea-2,7-xanthene dicarboxylic acid (**24**). The dibenzyl diPhe diurea **23** (540 mg, 0.516 mmol) was dissolved in EtOAc:EtOH (4:1, 10 mL), 10% Pd/C was added (20 mg, cat), and the suspension was evacuated and back-filled three times with a H_2 (atm). After stirring for 12 h the reaction was complete and was filtered through celite. Concentration gave a white solid (416 mg, 91%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.9 (br s, 2 H), 8.66 (d, *J*= 1.6 Hz, 2 H), 8.65 (br s, 2 H), 7.71 (d, *J*= 1.8 Hz, 2 H), 7.31-7.21 (m,

10 H), 6.9 (br s, 2 H), 4.45 (q, *J*= 7.1 Hz, 2 H), 3.09-3.01 (m, 4 H), 1.59 (s, 6 H), 1.30 (s, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{45}H_{50}N_4O_{11}Cs$ $[M + Cs]^+$ 955.2530, found 955.2575.

2,7-Dityrosine-OMe-4,5-diphenylalanine xanthene tetraurea (**25**). The tetraester was synthesized from **24** and Tyr(O*t*Bu)-OMe·HCl using the conditions given for **21** and **22**. The resulting thin film was purified using SiO_2 chromatography (30:1 DCM:MeOH). The purified residue was taken up in TFA (2 mL) for 4 h, concentrated, and precipitated with Et_2O:hexanes (1:1) to give white flakes (35% of theoretical). ^1H NMR (250 MHz, DMSO-d_6) δ 8.69 (br s, 2 H), 8.46 (br s, 2 H), 7.91 (d, *J*= 2.1 Hz, 2 H), 7.31 (d, *J*= 2.2 Hz, 2 H), 7.28-7.20 (m, 12 H), 7.09 (d, *J*= 8.4 Hz, 4 H), 6.89 (d, *J*= 8.4 Hz, 4 H), 6.74 (d, *J*= 7.2 Hz, 2 H), 6.27 (d, *J*= 7.7 Hz, 2 H), 4.5-4.4 (m, 4 H), 3.61 (s, 6 H), 3.2-2.9 (m, 8 H), 1.45 (s, 6 H), 1.30 (s, 18 H), 1.25 (s, 18 H). LRMS (ESI +) calcd for $C_{57}H_{59}N_8O_{15}$ $[M + H]^+$ 1095, found 1095. LRMS (ESI –) calcd for $C_{57}H_{57}N_8O_{15}$ $[M - H]^-$ 1093, found 1093.

2,7-Diaspartate-OMe-4,5-diphenylalanine xanthene tetraurea (**26**). The tetraester was synthesized in an analogous way to **25**. Purification using SiO_2 (25:1 DCM:MeOH) allowed the tetraester tetraurea intermediate to be isolated in 49% yield. ^1H NMR (250 MHz, DMSO-d_6) δ 8.87 (s, 2 H), 8.47 (s, 2 H), 7.94 (d, *J*= 2.3 Hz, 2 H), 7.36 (d, *J*= 2.3 Hz, 2 H), 7.28-7.20 (m, 10 H), 6.77 (d, *J*= 7.0 Hz, 2 H), 6.42 (d, *J*= 8.3 Hz, 2 H), 4.59 (m, 2 H), 4.42 (appar q, *J*= 7.1 Hz, 2 H), 3.65 (s, 6 H), 3.07-2.97 (m, 4 H), 2.8-2.6 (m, 4 H), 1.47 (s, 6 H), 1.39 (s, 18 H), 1.30 (s, 18 H). The tetraester was deprotected in TFA for 4 h, concentration followed by precipitation using Et_2O:hexanes gave a quantitative yield of the product tetraacid tetraurea as a pale yellow solid. ^1H NMR (250 MHz, DMSO-d_6) δ 12.8 (br s, 4 H), 8.82 (br s, 2 H), 8.44 (br s, 2 H), 7.96 (d, *J*= 2.2 Hz, 2 H), 7.32 (d, *J*= 2 Hz, 2 H), 7.27-7.21 (m, 10 H), 6.71 (d, *J*= 7.4 Hz, 2 H), 6.49 (d, *J*= 8.5 Hz, 2 H), 4.59 (m, 2 H), 4.48 (m, 2 H), 3.64 (s, 6 H), 3.23-3.16 (m, 2 H), 3.0-2.7 (m, 6 H), 1.46 (s, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{47}H_{51}O_{17}N_8$ $[M + H]^+$ 999.3372, found 999.3421.

4,5-Dileucine-OtBu diamide-2,7-xanthene dicarboxylic acid (**27**). The dibenzyl ester diacid chloride **17** (30 mg, 0.050 mmol) was dissolved in DCM (3 mL), and Leu-O*t*Bu·HCl (28 mg, 0.125 mmol) was added in DCM (2 mL) containing TEA (40 μL, 0.28 mmol). After 2 h the solution was rinsed with citric acid, the organic layer was dried and concentrated to a yellow semisolid. Hydrogenation of the semisolid was performed using 10% Pd/C (5 mg, cat) in 4:1EtOAc:EtOH (3 mL) for 16 h at rt. Filtration through celite and

concentration gave a yellow, oily solid (41 mg, 90% overall). ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.2 (br s, 2 H), 8.90 (d, *J*= 7.0 Hz, 2 H), 8.25 (m, 4 H), 4.45 (m, 2 H), 1.8-1.0 (m, 6 H), 1.68 (s, 6 H), 1.39 (s, 18 H), 0.94-0.89 (m, 12 H).

2,7-Diglycine-OMe diurea-4,5-xanthene dileucine-OtBu diamide (**28**). To the diLeu amide/diacid (**27**, 42 mg, 0.059 mmol) in acetone (2 mL) was added TEA (20 μL, 0.14 mmol) and the solution was cooled in an ice bath. After 10 min, ethyl chloroformate (17 μL, 0.154 mmol) was added dropwise and the solution was stirred for 30 min. Sodium azide (20 mg, 0.34 mmol, in 100 μL water) was added, and the emulsion was stirred for 30 min before it was poured into a separatory funnel containing DCM (12 mL) and water (1 mL). The layers were separated and the organic phase was dried over $MgSO_4$. Toluene was added and the mixture was concentrated until no DCM remained. The toluene-containing flask was then warmed to reflux for 1.5 h, cooled to rt, and concentrated to give an oil. The oil was taken up in DCM (10 mL) and added to a solution of Gly-OMe·HCl (16 mg, 0.13 mmol) in DCM (10 mL) containing TEA (20 μL, 0.14 mmol). After 2 h the DCM solution was rinsed with citric acid, dried over $MgSO_4$, and concentrated to afford a yellow solid (45 mg, 93% overall). ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.94 (s, 2 H), 8.84 (d, *J*= 7.0 Hz, 2 H), 7.90 (d, *J*= 2.5 Hz, 2 H), 7.62 (d, *J*= 2.5 Hz, 2 H), 6.49 (t, *J*= 5.6 Hz, 2 H), 4.40-4.36 (m, 2 H), 3.88 (d, *J*= 5.5 Hz, 4 H), 3.64 (s, 6 H), 1.85-1.55 (m, 6 H), 1.54 (s, 6 H), 1.38 (s, 18 H), 0.94-0.90 (m, 12 H).

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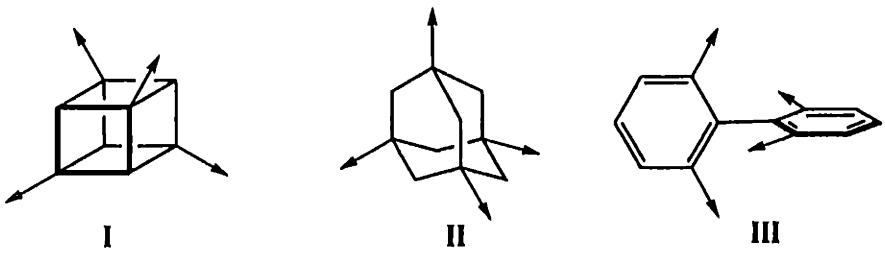
C. Cores with Tetrahedral Orientations: 1,3,5,7-Adamantane and 2,2′,6,6′-Biphenyl

C1. Introduction



I II III

In choosing a scaffold that enhances diversity an important consideration is how it directs the attached building blocks. In our pursuit of different geometric orientations, the tetrahedral display is represented by three tetrasubstituted core molecules: 1,3,5,7-cubane (**I**),[1,2] 1,3,5,7-adamantane (**II**), and 2,2′,6,6′-biphenyl (**III**). All three provide a rigid framework, a low molecular weight and enough hydrophobicity to allow for liquid-liquid extractions. No inherent toxicities of these cores are known. In fact, derivatives of these molecules with different substitution patterns can be found in medicinal agents. Cubanes are under investigation for their anti-HIV activity.[3] Adamantanes are known to have antiviral and antiparkinsonian (as in Amazolon and Mantadan), as well as use as antiseptic activity (Amantol).[4] Biphenyls are found in a number of pharmacologically active compounds, angiotension antagonists in particular.[5] Studies have indicated that the hydrophobicity inherent to these cores facilitates transport across membranes, increasing their bioavailability.[3] Application of our solution-phase combinatorial approach to these core molecules is presented in this chapter.

C2. The 1,3,5,7-Cubane Core

The symmetry, 'compactness,' and low molecular weight of cubane make it a desirable core molecule. The irony of using a molecule which is under active investigation for its explosive properties as a potential therapeutic agent has not escaped attention.[6] Cubane has, in fact, already been used to synthesize libraries;[7] however, these libraries proved to be inactive in a trypsin assay.[8] The deconvolution was not attempted and would likely be quite challenging, as no suitable cubane derivatives of lower symmetry are readily available.[2] With this in mind a decision was made to not pursue this core further.

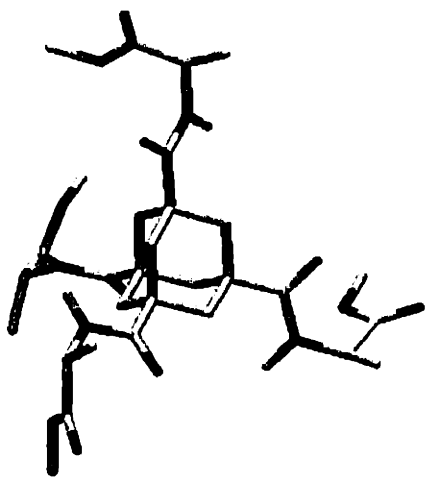


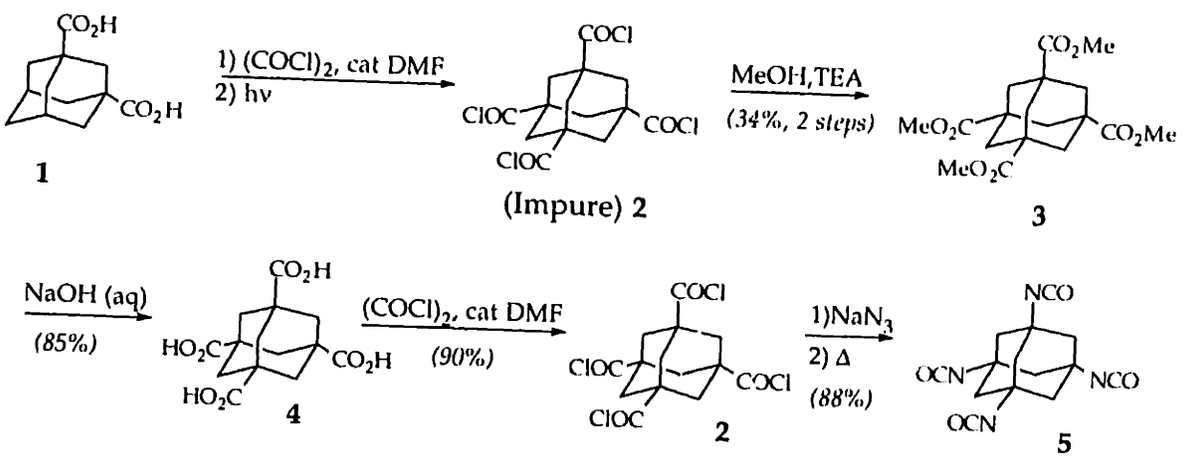
Figure 1: An MM2*-minimized structure of 1,3,5,7- adamantane tetraalanine tetramethyl ester.

C3. The 1,3,5,7-Adamantane Core

The adamantane framework is particularly attractive as its core precursor is commercially available. The molecular weight of the core is low (132 g/mol) and the synthesis of the activated core molecule is straightforward. There are, however, a few disadvantages that must be addressed. First, attached building blocks diverge and the methylene units on the adamantane hinder cooperative interactions (see **Figure 1**). This increases

the likelihood that libraries will behave like detached sets of amino acids (or other tools), rather than core/building block ensembles. Secondly, the volume of the tetrafunctionalized core is large, precluding binding in small active sites. Thirdly, a computational analysis of cubane and adamantane-based tetraamide libraries found a significant overlap in shape-space coverage between the two core structures.[9] Nevertheless, some exploratory synthesis on this core was performed.

Scheme 1: Synthesis of the adamantane tetraacid chloride (**2**) and the tetraisocyanate (**5**) activated core molecules.



The tetraacid of adamantane was isolated after a sequence of reactions (**Scheme 1**), starting from the commercially available 1,3-diacid[10] (**1**) using a photochemical chlorocarbonylation[11] reaction. The crude tetraacid chloride (**2**) derivative formed was impure but could be readily esterified to give the tetramethyl ester (**3**) which was then purified. Saponification of the four methyl ester groups gave the tetraacid **4** in high yield and purity. Conversion to the pure tetraacid chloride **2** was accomplished with oxalyl chloride and catalytic DMF. This versatile intermediate was used to make amide-based molecules directly upon reaction with suitable amines. In addition, urea derivatives can also be made via the tetraisocyanate (**5**), which was made (by a coworker of mine, Kent Pryor) from the tetraacid chloride via a Curtius rearrangement. While the synthesis of the tetrasubstituted compounds proceeded in good yield, unsymmetric isomers applicable to deconvolution

proved synthetically inaccessible. A number of approaches were attempted, including successive chlorocarbonylations (the reaction conditions were too severe for the protecting groups employed) and reacting the tetraacid chloride with fewer than four equivalents of benzyl alcohol (no separation of compounds evident by TLC).

C4. The 2,2′,6,6′-tetrasubstituted Biphenyl Core

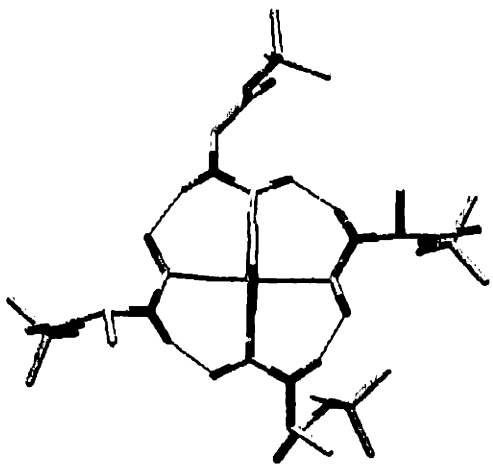


Figure 2: An MM2*-minimized structure of 2,2′,6,6′-biphenyl tetraalanine (*t*-butyl ester); hydrogen bonds are indicated by dashed red lines.

Another core which provides a tetrahedral array is based on the all *ortho* isomer of biphenyl (**III**). It has a compact shape and a low (150 g/mol) molecular weight. These properties have not escaped the eye of medicinal and combinatorial chemists; several recent publications[5,12] have featured biphenyl-based molecules with a variety of substituents and substitution patterns. The 2,2′,6,6′ substitution pattern of the present work has been neglected thus far.

Interactions (especially hydrogen-bonding) between adjacent groups are possible with this substitution pattern, exemplifying one of its inherent advantages. In fact, molecular modeling (MM2*/MacroModel) predicts hydrogen bonds between the adjacent amide groups of a tetraalanine

derivative (see **Figure 2**), concomitantly increasing the rigidity of the system. Also, despite its high symmetry, the lack of rotation[13] about the biaryl bond allows a large number of compounds to be formed with only a few building blocks (see **Figure 3**). Finally, a synthesis of the tetraacid (from pyrene using ozonolysis) is known in the literature,[14] and derivatives suitable for deconvolution have been prepared and isolated.[15]

Number of Building Blocks	Number of Compounds Predicted
4	76
8	1,072
16	16,576

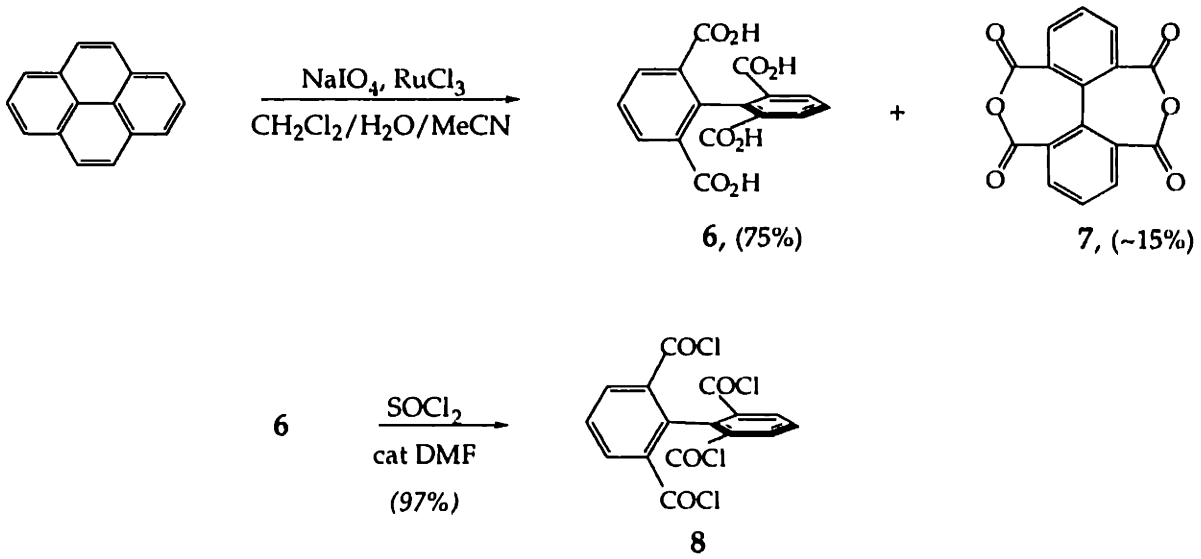
Figure 3: Correlation between the number of added building blocks and the number of library components for the 2,2',6,6'-biphenyl core.

C5. Synthesis of the 2,2',6,6'-Biphenyl tetraacid Chloride Activated Core

The remainder of the experimental work in this chapter was done in collaboration with David Skyler.

In our hands the exisiting tetraacid synthesis[14] was not reproducible. Specifically, we needed to find another oxidation method for pyrene. A recent oxidation technique[16] using $RuCl_3$ and $NaIO_4$ was found to generate the desired tetraacid. When the aforementioned oxidants were used to analyze particular types of coal, traces of 2,2',6,6'-biphenyl tetraacid were found, which were attributed to the presence of pyrene.[16] This $NaIO_4/RuCl_3$ oxidation reaction, once optimized for pure pyrene, proceeded smoothly in one pot to give the tetraacid in 75% yield (**Scheme 2**). Although the reaction also formed the very insoluble dianhydride (**7**), this compound could be eliminated using a hot filtration. The tetraacid chloride (**8**) was formed with phosphorus pentachloride (neat) or thionyl chloride (excess in DCM). The latter method has the advantage that involatile phosphorus-based byproducts are avoided.

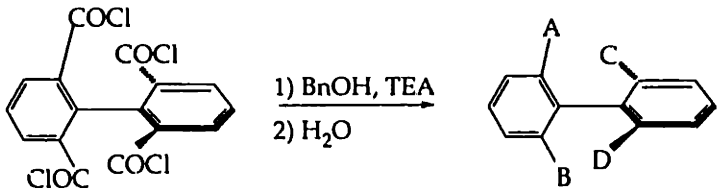
Scheme 2: Preparation of the all *ortho* biphenyl tetraacid core



C6. Synthesis and Isolation of Deconvolution Compounds

For compounds applicable to deconvolution we first attempted to reproduce a literature procedure for the synthesis and isolation of all the possible methyl ester/carboxylic acid combinations of biphenyl.[15] However, difficulties in separation were encountered. While the mono or dianhydride would seem to be logical deconvolution compounds, their insolubility limited their use. When this limitation was overcome by using higher temperatures or more compatible solvents (THF), it was found that intractable mixtures resulted. We ultimately decided to explore benzyl ester/acid compounds, whose deprotection conditions were more compatible with amino acid derivatives.

Scheme 3: Synthesis of biphenyl deconvolution compounds.

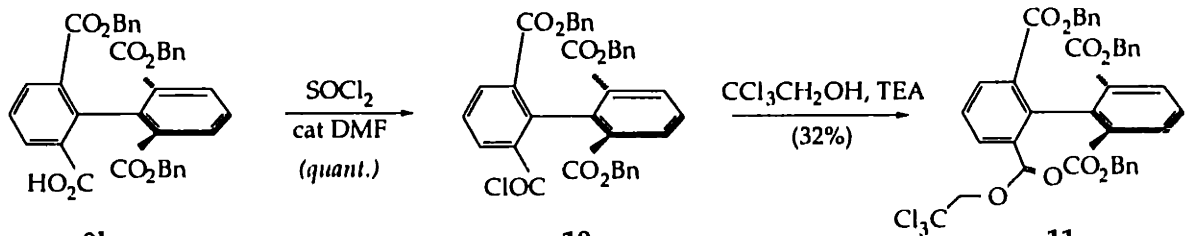


8

9a: A=B=C=D= CO_2Bn
9b: A=C=D= CO_2Bn, B= CO_2H
9c: A=B= CO_2H, C=D= CO_2Bn
9d: A=C= CO_2Bn, B=D= CO_2H
and
A=D= CO_2Bn, B=C= CO_2H
9e: A= CO_2Bn, B=C=D= CO_2H

The tetraacid chloride (**8**) was reacted with 2.5 equivalents of benzyl alcohol in the presence of triethylamine (**Scheme 3**). The resulting mixture was then separated on a silica gel column using ethyl acetate, hexanes and acetic acid (see experimental for gradient conditions). Fortunately this proved successful and all of the compounds were isolated; although only a small amount of the monobenzyl ester (**9e**) was isolated. To compensate for its low yield a substitute compound was synthesized. The tribenzyl ester/mono acid (**9b**) was converted to the tribenzyl ester/mono acid chloride (**10**), then esterified to form the tribenzyl ester/monotrichloroethyl ester (**11**). This can be hydrogenated to give a triacid/ester.

Scheme 4

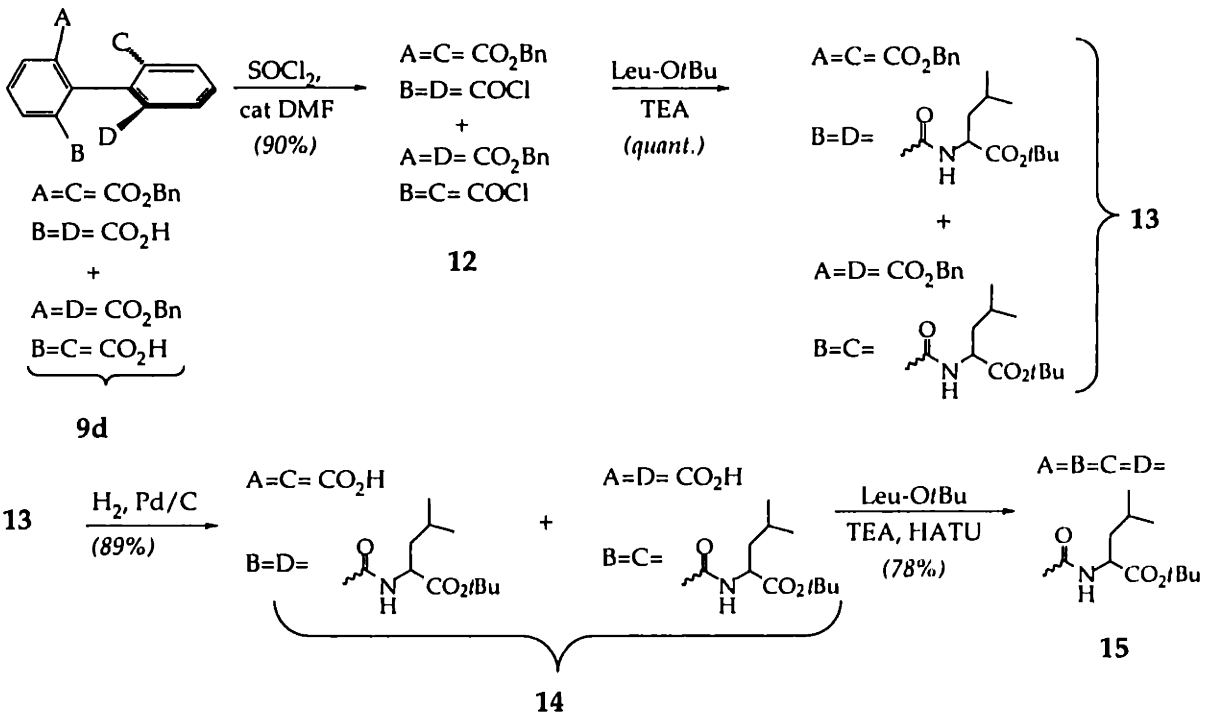


9b **10** **11**

To test the validity of the deconvolution approach a trial synthesis was conducted (**Scheme 5**). The objective was to synthesize a known compound (the tetraleucine tetraamide **15**) using a stepwise approach, beginning with the dibenzyl ester/diacid (**9d**). After two leucines were attached a diastereomeric mixture (**13**) resulted (which could be partially resolved using preparative TLC). Hydrogenolysis afforded the diacid/dileucines **14** in high yield. The

final step of the synthesis proved to be troublesome, however. With the more common coupling reagents (BOP-Cl, PyBOP) the reaction failed to go to completion. Fortunately, the same reaction in the presence of the very reactive (and expensive) coupling reagent HATU[17] proceeded in acceptable yield. While this deconvolution Scheme was used to synthesize a pure compound, different amines could easily be substituted, allowing for a range of isomers and enantiomers to be generated.

Scheme 5: A deconvolution trial synthesis.



C7. Reactivity of the 2,2'6,6'-Biphenyl tetraacid Chloride

A number of different activating (tetraacid to tetraacid chloride) methods were investigated. When oxalyl chloride was used (with catalytic DMF), anhydrides formed nonspecifically, resulting in products of low purity. When a seven component Leu-O*t*Bu/Ala-O*t*Bu mixture was prepared using a PCl_5-activated core, HPLC analysis (**Figure 4**) revealed a number of side products that could not be explained. An LC/MS analysis (similar to the

one described in the experimental section) was performed on this mixture and revealed that all the expected products were formed. Unwanted compounds were likely phosphorus-based, as they had much lower molecular weights and shorter retention times. We decided to avoid PCl_5 and $COCl_2$ in future syntheses.

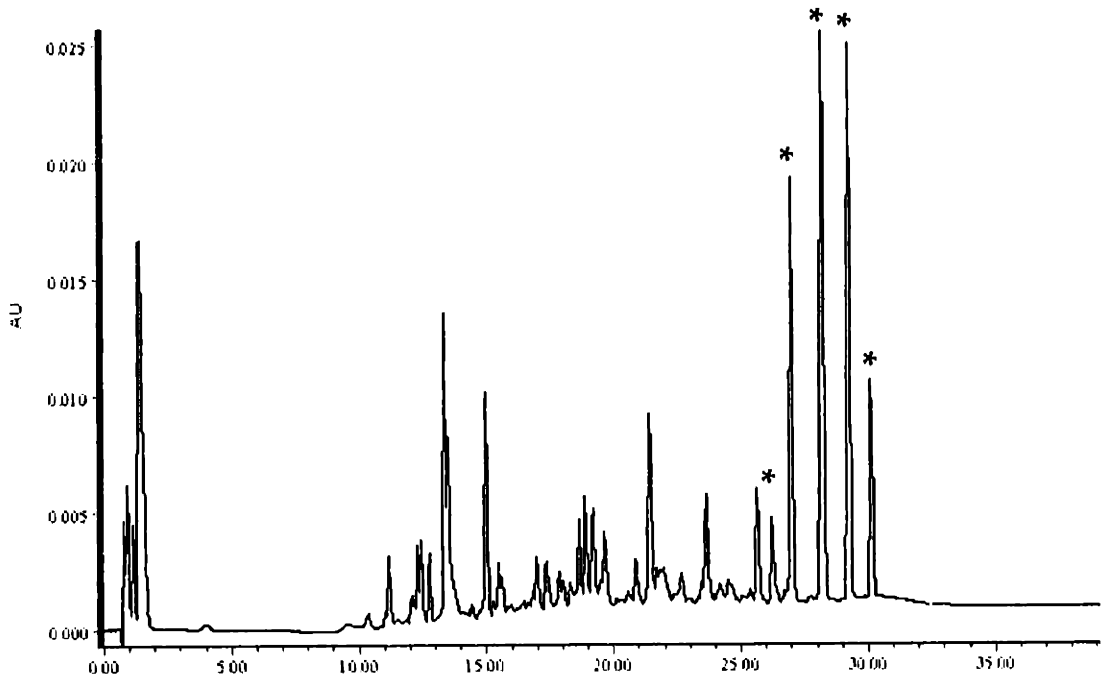


Figure 4: A reverse-phase HPLC trace of a library derived from a PCl_5 generated tetraacid chloride, Ala and Leu *t*-butyl esters. The * compounds represent desired components, the other peaks are likely by-products of unremoved phosphorus chlorides. Product peaks were verified using LC/MS.

An equivolume mixture of thionyl chloride and DCM was found to produce the biphenyl tetraacid chloride in high yield and purity. This was determined by reacting it with a number of single amines, then analyzing the tetraamides by both HPLC and NMR. These products were found to be >90% pure. Synthetic details and characterization for a number of tetraamides can be found in the experimental section (not shown: Leu_4= **15**, Ala_4= **16**, Ser_4= **18**). Next a Leu-O*t*Bu/Ala-O*t*Bu library was produced; it was found (by HPLC, see **Figure 5**) to be of much higher purity than the library made using the PCl_5-derived tetraacid chloride. As a test, both the tetraalanine (**16**) and the

tetraleucine (**15**) derivatives were independently prepared, and their retention times were found to match those in the mixture.

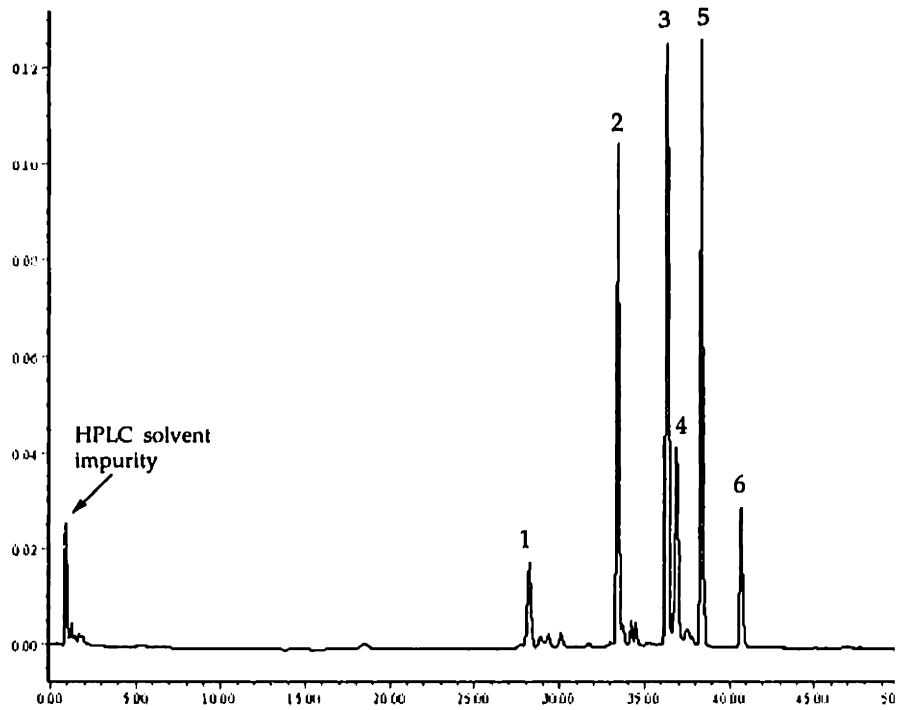


Figure 5: An HPLC trace of a seven component library synthesized from Ala and Leu *t*-butyl esters and the $SOCl_2$-derived 2,2'6,6'-biphenyl tetraacid chloride. Peaks were assigned using an LC/MS analysis. 1: Ala_4 (5%), 2: Ala_3Leu (19%), 3: Ala_2Leu_2 (31%, presumed to be both 2,6'-diLeu-2',6-diAla atropisomers), 4: Ala_2Leu_2 (10%, presumed to be the 2,6-diLeu-2',6'-diAla), 5: $AlaLeu_3$ (22%), 6: Leu_4 (6%), maximum total impurities: 6%.

Deprotection of the attached *t*-butyl amino acid building blocks (Ala_4= **17**, Ser_4= **19**) was performed with neat TFA. However, the reaction time had to be significantly extended (from 3-4 hours to 24 hours) to ensure complete deprotection. Cleavage of *t*-butyl ethers with TFA also produced unexpected results. When tetraserine *t*-butyl ether (**18**) was stirred in TFA, mass spectrometry showed several higher molecular weight compounds had been produced which differed by multiples of 96 mass units, corresponding to replacement of hydrogen by trifluoroacetate. When the sample was taken up in water it was observed that the presumed trifluoro acetate esters slowly convert to free alcohols over a two hour time period (see **19**). These results suggest that whenever alcoholic building blocks (serine, threonine, etc.) are

subjected to TFA deprotection conditions, the libraries must be treated with water to ensure that the desired compounds are isolated.

C8. Conclusion

Activated cores based on adamantane and 2,2',6,6'-biphenyl have been prepared. Both the tetraacid chloride and tetraisocyanate of adamantane reacted smoothly with amines to give amides and isocyanates, respectively. Complexities in deconvolution as well as concerns regarding the disposition of attached building blocks disinclined us to pursue library synthesis on the adamantane core. After the problem of core molecule activation was solved, the tetraacid chloride of 2,2',6,6'-biphenyl reacted cleanly with single building blocks. Optimization of the reaction and deprotection conditions resulted in statistical ensembles of tetraamides when more than one building block was used. A trial deconvolution demonstrated a synthetic pathway to individual compounds. Libraries based on this biphenyl isomer can now be prepared. When these libraries are subjected to screening in biological assays the benefits of having a low molecular weight and a compact, rigid display of building blocks should increase the probability that the mixtures will contain active components.

C9. Experimental

C9-1. General

All reagents were purchased from Aldrich Chemical Company and used without further purification except as noted. Amino acid esters, PyBOP and HATU were acquired from Novabiochem (San Diego, California). Triethylamine was distilled from, and stored over, KOH. Acetone was dried over 4 Å molecular sieves for 24 hours. Citric acid refers to a 1 N stock solution. NMR spectra were recorded on either a Bruker AC-250 or AM-300. All peaks were referenced to residual solvent. Deuterated solvents were obtained from Cambridge Isotope Laboratories. Deuterated chloroform was dried over 4 Å molecular sieves. A Waters Symmetry C8 reverse-phase

column (3.9 x 150 mm) was used for HPLC analysis with a flow rate of 1 mL/min. Either a Finnegan Mat 8200 (for HRMS/EI) or a VG ZAB-VSE (for HRMS/FAB) mass spectrometer was used to ascertain exact masses. Silica gel chromatography was performed with Silica Gel 60 (EM Science or Bodman, 230-400 mesh). TLC analysis was performed using glass-bound Silica Gel 60 (F254) plates.

C9-2. Synthesis and LC/MS Analysis of a Library Containing Ala and Leu

The library was synthesized as follows: Ala-O*t*Bu·HCl (29 mg, 0.16 mmol, 2.1 equiv) and Leu-O*t*Bu·HCl (36 mg, 0.16 mmol, 2.1 equiv) were dissolved in DCM (2 mL) and "free-based" with TEA (126 μL, 0.90 mmol, 12 equiv). This solution was mixed with 1 equiv of tetraacid chloride core (**8**, 25 mg, 0.075 mmol) in DCM (1 mL). The reaction was poured into a separatory funnel with more DCM (25 mL) and rinsed once with citric acid (10 mL). The organic layer was then evaporated, taken up in methanol, and injected into the HPLC.

The separation was accomplished using a Waters Symmetry C8 (3.9 x 150 mm) column, running a linear gradient of 100% water to 100% MeCN over 25 min (total run time= 50 min), and analyzing at a wavelength of 254 nm. The eluent from the HPLC column was then passed into a low-resolution positive ion electrospray mass spectrometer (Michrom UMA microprobe coupled with a single quadrupole Perkin Elmer API 100 Sciex). Ala_4 (retention time of 24.9 min) calcd for $C_{44}H_{63}O_{12}N_4$ $[M + H]^+$ 839.4, found 840; Ala_3Leu (26.5 min) calcd for $C_{47}H_{69}O_{12}N_4$ $[M + H]^+$ 881.5, found 882; Ala_2Leu_2 (isomers unresolved by HPLC, 27.7 min) calcd for $C_{50}H_{75}O_{12}N_4$ $[M + H]^+$ 923.5, found 924; $AlaLeu_3$ (29.3 min) calcd for $C_{53}H_{81}O_{12}N_4$ $[M + H]^+$ 965.6, found 966; Leu_4 (31.6 min) calcd for $C_{56}H_{87}O_{12}N_4$ $[M + H]^+$ 1007.6, found 1008.

C9-3. Synthesis and Characterization of Adamantanes

Adamantane 1,3,5,7-tetracarboxylic acid chloride (**2**). Oxalyl chloride (3.5 mL, 38 mmol) was added to the tetraacid (**4**, 0.50 g, 1.6 mmol) suspended in DCM (30 mL) containing DMF (10 μL). The mixture was warmed to reflux for 16 h, filtered hot, and concentrated to a white solid (560 mg, 90%). ^1H NMR ($CDCl_3$, 250 MHz): δ 2.25 (s).

Adamantane 1,3,5,7-tetramethyl ester (**3**). Adamantane 1,3-dicarboxylic acid (**1**, 1.04 g, 4.64 mmol) was dissolved in DCM (30 mL), oxalyl chloride (2 mL), and DMF (2 μL). The suspension was heated to reflux until a clear solution

appeared (3 h). After concentration, the resulting diacid chloride was irradiated in a Rayonet apparatus (vycor tube, 254 nm, 3 h, 25°C) with oxalyl chloride (55 mL) as the solvent/reactant. The yellow solution was concentrated to an oil, toluene was added, and the solution was evaporated. DCM (35 mL, dry) was added and the solution was cooled to 0°C. Methanol (2.8 mL, 70 mmol) and triethylamine (9.8 mL, 70 mmol, dropwise!) were introduced and the solution was stirred at rt for 2 h before rinsing with citric acid (2x 30 mL). The organic layer was dried over Na_2SO_4 and concentrated to an oil. Purification using silica gel (10:1 DCM:EtOAc) afforded a white solid (0.51 g, 34%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.69 (s, 12 H), 1.99 (s, 12 H). HRMS (FAB in NBA/NaI) calcd for $C_{18}H_{25}O_8$ $[M + H]^+$ 369.1549, found 369.1555.

Adamantane 1,3,5,7-tetracarboxylic acid (**4**). Tetramethyl ester **3** (0.300 g, 0.818 mmol) was pulverized and suspended in 4 N NaOH (15 mL) and warmed to 60°C for 12 h, until a solution formed with only a trace amount of solids. The reaction was filtered hot, cooled to 0°C, and acidified using conc. HCl. The resulting white solid was filtered off and the filtrate was concentrated to dryness. After rinsing with a few milliliters of cold water the liquid was removed and the solid was dried. Both of the resultant solids (total of 216 mg, 85%) were pure by NMR and were combined. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.45 (br s, 4 H), 1.78 (s, 12 H). LRMS (ESI + /NaI) calcd for $C_{14}H_{16}O_8Na$ $[M + Na]^+$ 335, found 335.

1,3,5,7-Tetraisocyanato adamantane (**5**). Acetone (7 mL) was used to dissolve adamantane tetraacid chloride (**2**, 120 mg, 0.31 mmol), and the flask was cooled to 0°C. Sodium azide (400 mg in 1 mL water, 6.2 mmol) was added dropwise and the suspension was stirred for 30 min at 0°C. DCM (25 mL) and water were added, the layers were separated and the aqueous layer was extracted with more DCM (3x 25 mL). The organic layer was dried over $MgSO_4$ and concentrated to near dryness. Toluene (10 mL) was added and the solution was concentrated until the toluene began to evaporate. The solution was warmed to reflux for 1 h and bubbling was observed at 60°C. Evaporation yielded a solid which was taken up in DCM, filtered through glass wool, and concentrated to yield a slightly yellow powder (83 mg, 88%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.95 (s). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 49.98, 55.30, 124.22. HRMS (EI) calcd for $C_{14}H_{12}N_4O_4$, 300.0859; found 300.0858.

C9-4. Synthesis and Characterization of 2,2',6,6'-Biphenyls

Biphenyl 2,2',6,6'-tetraacid (**6**). To pyrene (6.00 g, 29.7 mmol) in DCM (120 mL) was added MeCN (120 mL) and water (180 mL). To the resulting biphasic solution was added $NaIO_4$ (60 g, 281 mmol) followed by $Ru(III)Cl_3$ (240 mg, 1.16 mmol). The solution warmed somewhat as the reaction began but was not vigorous. The reaction was run overnight (~16 h) with stirring and was filtered to give a yellow solid. The mixed solid (tetraacid/$NaIO_4$) was extracted with acetone (750 mL) and the acetone was refiltered to yield a yellow solution. Upon evaporation the product was identified as a mixture of the desired tetraacid and the corresponding dianhydride. The crude product was ground to a fine powder and was refluxed for 1 h in DCM before being filtered hot. The tetraacid was collected as a white powder (7.4 g, 75% yield). ^1H NMR (DMSO-d_6, 300 MHz) δ 12.4 (br s, 4 H), 7.96 (d, *J*= 7.6 Hz, 4 H), 7.45 (t, *J*= 7.8 Hz, 2 H). ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 167.49, 142.07, 132.33, 131.82, 126.49. HRMS (FAB in NBA/NaI) calculated for $C_{16}H_{10}O_8Na$ $[M + Na]^+$ 353.0273, found 353.0264.

Biphenyl 2,2',6,6'-tetraacid chloride (**8**). The tetraacid **6** (25 mg, 0.075 mmol) was suspended in DCM (1.5 mL) and cooled prior to the addition of thionyl chloride (1 mL, excess) and DMF (2 μL, catalytic). The suspension was allowed to warm to rt under a $CaCl_2$ drying tube. After 1 h the suspension was warmed to reflux until a homogenous solution developed (about 4 h). After another hour at reflux the orange solution was concentrated to dryness, dry toluene was added, and the solution was concentrated again to yield a yellow solid. After evacuation on a high vacuum for 1 h the compound (29 mg, 97%) appeared pure by NMR. ^1H NMR ($CDCl_3$, 250 MHz) δ 8.57 (d, *J*= 8.1 Hz, 4 H), 7.81 (t, *J*= 8.1 Hz, 2 H).

Synthesis and isolation of benzylation products (**9a-e**). To a solution of the tetraacid chloride **8** (3.0 g, 7.46 mmol) in DCM (250 mL) was added anhydrous benzyl alcohol (2.5 equiv, 2 mL, 19 mmol) followed by TEA (2.59 mL, 19 mmol). After stirring 12 h water (2 mL) were added and the solution was stirred vigorously for 2 h. After washing with 1 N HCl, the organic layer was evaporated to an orange oil. A similar procedure was carried out using 2 equiv of benzyl alcohol and the two samples of orange oil were pooled for separation (**9**). Column chromatography using an acetic acid gradient (outlined below) provided good separation of most of the expected products.

Biphenyl 2,2',6,6'-tetrabenzyl ester (**9a**). The pooled crude reaction mixture **9** was loaded onto SiO_2 in a small volume of DCM and flushed through with

2:1 hexane:EtOAc until the first product appeared in fractions 5-49. The light yellow product was dried and washed with MeOH to yield a white powder. Recrystallization from a small volume of EtOAc by addition of hexane gave large silver flakes (220 mg, 0.32 mmol). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.94 (d, *J*= 7.8 Hz, 4 H), 7.41 (t, *J*= 7.8 Hz, 2 H), 7.27 (m, 6 H), 7.12 (m, 4 H), 4.90 (s, 8 H). HRMS (FAB in NBA/CsI) calcd for $C_{44}H_{34}O_8Cs$ $[M + Cs]^+$ 823.1308, found 823.1323.

Biphenyl 2,2',6-tribenzyl ester-6'-carboxylic acid (**9b**). After **9a** was flushed from the column, the solvent was changed to 2:1 hexane:EtOAc + 0.25% AcOH. Fractions 65-145 were pooled, washed with water and saturated $NaHCO_3$, and dried with Na_2SO_4. Evaporation gave a brownish solid. The spectral evidence showed this product to be very similar to the desired tribenzyl product. No further products appeared for several fractions, so the solvent was changed to 2:1 hexane:EtOAc + 1% AcOH. Fractions 236-375 were pooled, washed with 1N HCl, dried with Na_2SO_4, and concentrated to yield the tribenzyl (**9b**) as a gummy orange solid (1.40 g, 2.33 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13 (d, *J*= 7.9 Hz, 2 H), 8.05 (dd, *J*= 7.8, 1.4 Hz, 1 H), 8.02 (dd, *J*= 7.8, 1.5 Hz, 1 H), 7.42 (t, *J*= 7.9 Hz, 1 H), 7.32 (m, 13 H), 7.18 (m, 8 H), 5.00 (br s, 4 H), 4.98 (s, 2 H). HRMS (FAB in NBA/CsI) calcd for $C_{37}H_{28}O_8Cs$ $[M + Cs]^+$ 733.0839, found 733.0816.

Biphenyl 2,6-dibenzyl ester-2',6'-dicarboxylic acid (**9c**). Very little material appeared following **9b** so the solvent was changed to 2:1 hexane:EtOAc + 2% AcOH. Fractions 473-530 were pooled and a solid precipitated from solution opon addition of hexane. The product **9c** was filtered, dried, and isolated as a faint yellow powder (140 mg, 0.27 mmol). ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.11 (d, *J*= 7.9 Hz, 2 H), 8.03 (d, *J*= 7.7 Hz, 2 H), 7.51 (t, *J*= 7.7 Hz, 1 H), 7.37 (t, *J*= 7.9 Hz, 1 H), 7.27 (m, 6 H), 7.19 (m, 4 H), 4.96 (s, 4 H). HRMS (FAB in NBA/CsI) calcd for $C_{30}H_{22}O_8Cs$ $[M + Cs]^+$ 643.0369, found 643.0390.

Biphenyl 2,2'-dibenzyl ester-6,6'-dicarboxylic acid (**9d**). After the isolation of **9c**, chromatography was resumed with 2:1 hexane:EtOAc + 2% AcOH. Fractions 571-628 were pooled, concentrated to *ca.* one-third its original volume, and precipitated with hexane. After filtration, the dibenzyl ester **9d** was recovered as a white powder (603 mg, 1.18 mmol). ^{1}H NMR (acetone-d_6, 300 MHz): δ 8.09 (dd, *J*= 7.7, 1.6 Hz, 2 H), 8.04 (dd, *J*= 8.0, 1.5 Hz, 2 H), 7.44 (t, *J*= 7.8 Hz, 2 H), 7.3-7.25 (m, 6 H), 7.20-7.16 (m, 4 H), 4.97 (d, *J*= 12.5 Hz, 2 H), 4.91 (d, *J*= 12.3 Hz, 2 H). ^{13}C NMR (75.4 MHz, acetone-d_6) δ 167.18, 166.33, 149.36,

143.02, 136.58, 133.73, 133.41, 131.87, 128.84, 128.67, 128.47, 127.31, 66.66. HRMS (FAB in NBA/CsI) calcd for $C_{30}H_{22}O_8Cs$ $[M + Cs]^+$ 643.0369, found 643.0381.

Biphenyl 2-benzyl ester-2',6,6'-tricarboxylic acid (**9e**). Following recovery of the product **9d** the acid concentration of the solvent was increased to 3% AcOH. Fractions 666-720 were collected, washed with 1 N HCl and dried to give a yellow solid (56 mg). The mono benzyl product was contaminated by residual dibenzyl **9d** which could not be removed.

Biphenyl 2,2',6-tribenzyl ester-6'-acid chloride (**10**). The tribenzyl ester **9b** (200 mg, 0.33 mmol) was dissolved in DCM (20 mL). An excess of $SOCl_2$ (57 μL, 0.48 mmol) was added, followed by DMF (3 μL, cat). After 2 h at reflux the solution was concentrated to give a yellow oil (215 mg, 0.35 mmol, quantitative). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.11 (d, *J*= 8 Hz, 2 H), 8.11 (dd, *J*= 8, 1 Hz, 1 H), 8.02 (dd, *J*= 8, 1 Hz, 1 H), 7.39 (t, *J*= 8 Hz, 1 H), 7.32-7.2 (m, 9 H), 7.11 (m, 7 H), 4.96 (d, *J*= 1 Hz, 4 H), 4.92 (s, 2 H).

Biphenyl 2,2',6-tribenzyl ester-6'-(2,2,2-trichloroethyl) ester (**11**). The acid chloride **10** (232 mg, 0.38 mmol) from a similar preparation was dissolved in dry DCM (20 mL) and a large excess of trichloroethanol (283 μL, 2.95 mmol) was added under N_2. A slight excess of TEA (60.1 μL, 0.42 mmol) was added and the solution was stirred for 16 h at rt. After washing the organics (2x 1 N HCl) the solution was concentrated via rotary evaporation and loaded onto a silica gel column (1:1 hexane:EtOAc). Evaporation of the pure fractions followed by drying gave a white solid (88 mg, 32%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.06 (d, *J*= 7.9 Hz, 2 H), 8.01 (dd, *J*= 7.9, 1 Hz, 1 H), 7.77 (dd, *J*= 7.8, 1 Hz, 1 H), 7.30 (t, *J*= 7.8 Hz, 1 H), 7.27-7.23 (m, 9 H), 7.20 (t, *J*= 7.9 Hz, 1 H), 7.11-7.07 (m, 6 H), 4.93 (m, 8 H).

Biphenyl 2,2'-dibenzyl ester-6,6'-diacid chloride (**12**). To a solution of **9d** (50 mg, 0.10 mmol) in DCM (25 mL) was added a slight excess of thionyl chloride (35 μL, 0.30 mmol) at rt. Catalytic DMF (0.25 μL) was added and the solution was refluxed for 2 h under N_2. After drying under reduced pressure the product acid chloride **12** was recovered as a light yellow solid (50 mg, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (d, *J*= 7.7 Hz, 2 H), 8.18 (d, *J*= 7.6 Hz, 2 H), 7.47 (t, *J*= 7.9 Hz, 2 H), 7.31 (m, 6 H), 7.14 (m, 4 H), 4.98 (s, 4 H).

Biphenyl 2,2'-dibenzyl ester-6,6'-dileucine-OtBu diamide (**13**). The diacid chloride **12** (50 mg, 0.09 mmol) was dissolved in DCM (20 mL) and a small

excess of Leu-O*t*Bu·HCl (48 mg, 0.22 mmol) was added followed by an excess of TEA (22 μL, 0.30 mmol). The reaction mixture was stirred at rt for 1 h, at which time the reaction did not appear complete by TLC (4:1 hexane:EtOAc). An additional equivalent of leucine and triethylamine were added, immediately following which the TLC showed one major product. After washing with 0.5 N HCl and 0.5 N KOH the product diamide was recovered as a yellow semisolid. The mixture of diastereomers was isolated as a light yellow solid (75 mg, 0.09 mmol). ^{1}H NMR and ^{13}C of the diastereomeric pair were consistent with the proposed structures. The diastereomers were partially separated by preparative TLC (4:1 hexane:EtOAc) to allow peak assignment. The ^{1}H NMR of the lower R_f product appears as follows and the spectra of the isomer may be extrapolated based on comparison to the crude mixture. Integrations are approximate due to overlap with the residual diastereomer. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.38 (d, *J*= 9 Hz, 2 H), 7.99 (dd, *J*= 8, 1 Hz, 1 H), 7.49 (dd, *J*= 7.7, 1 Hz, 1 H), 7.33 (t, *J*= 7.6 Hz, 1 H), 7.29-7.19 (m, 13 H), 5.03 (s, 4 H), 4.30 (m, 2 H), 1.31 (s, 18 H), 1.21 (m, 6 H), 0.79 (d, *J*= 8.0 Hz, 6 H), 0.75 (d, *J*= 8.0 Hz, 6 H). LRMS of diastereomeric mixture (FAB in NBA/CsI) calcd for $C_{50}H_{61}O_{10}N_2$ $[M + H]^+$ 849.4, found 849.

Biphenyl 2,2'-diacid-6,6'-dileucine-OtBu diamide (**14**). To the dileucine diester **13** (70 mg, 0.084 mmol) in 4:1 EtOAc:EtOH (10 mL) was added 5% Pd/C (25 mg, cat) and the suspension was stirred at rt under a H_2 balloon for 2 h. The (3:2 ratio of) diastereomeric diacid products was then filtered through Celite to remove the Pd/C and dried to a fine white powder (50 mg, 89%). ^{1}H NMR of the diastereomeric mixture was consistent with the proposed structures. The amide protons had very different shifts due to hydrogen-bonding microenvironments (δ 8.94 versus δ 8.04). HRMS of the mixture (FAB in NBA/CsI) calcd for $C_{36}H_{48}O_{10}N_2Cs$ $[M + Cs]^+$ 801.2363, found 801.2341.

Biphenyl 2,2',6,6'-tetraleucine-OtBu tetraamide (**15**). To a solution of **14** in dry DMF (5 mL) was added leucine-O*t*Bu·HCl (26 mg, 0.12 mmol, 2.2 equiv) and TEA (23 μL, 0.16 mmol, 3 equiv). The solution was chilled to 0°C and treated with HATU (43.7 mg, 0.12 mmol, 2.2 equiv) to give a bright yellow solution. After stirring at 0°C the yellow color had faded and the reaction appeared incomplete by TLC (4:1 hexane:EtOAc). An excess of TEA was added to drive the reaction to completion and after 1 h at rt only one major product was observed. The reaction mixture was diluted with EtOAc (25 mL), washed (2x 1 N HCl, 2x sat $NaHCO_3$), dried with Na_2SO_4, and evaporated to provide the pure tetraleucine tetraamide as a white powder (95 mg, 78%). This compound was identical to the tetraleucine synthesized using 4.4 equiv of leucine and 1 equiv of biphenyl tetraacid chloride. HPLC analysis: >90% pure (λ= 254 nm).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.67 (d, *J*= 8 Hz, 4 H), 7.47 (d, *J*= 8 Hz, 4 H), 7.30 (t, *J*= 8 Hz, 2 H), 4.30 (m, 4 H), 1.31 (s, 36 H), 1.29 (m, 12 H), 0.79 (d, *J*= 6 Hz, 24 H). HRMS (FAB in NBA/CsI) calcd for $C_{56}H_{87}O_{12}N_4$ $[M + H]^+$ 1007.6320, found 1007.6349.

Biphenyl 2,2',6,6'-tetraalanine-OtBu tetraamide (**16**). To the tetraacid chloride (**8**, 0.075 mmol) dissolved in DCM (1 mL) was added a mixture of Ala-OtBu·HCl (60 mg, 0.33 mmol, 4.4 equiv), TEA (0.13 mL, 0.93 mmol, 12 equiv) and DCM (1.5 mL) at rt. The yellow solution was stirred for 1.5 h loosely capped. The reaction was poured into a separatory funnel with DCM (25 mL), rinsed with citric acid (1x 10 mL), and water (1x 5 mL). Upon evaporation white crystals formed (59 mg, 94%). HPLC analysis: >90% pure (λ= 254 nm, retention time of 27.5 min with a linear gradient of 100% water to 100% MeCN over 25 min and a total run time of 45 min). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.62 (d, *J*= 7.2 Hz, 4 H), 7.51-7.45 (m, 6 H), 4.03 (quintet, *J*= 7.2 Hz, 4 H), 1.32 (s, 36 H), 0.93 (d, *J*= 7.2 Hz, 12 H). HRMS (FAB in NBA/CsI) calcd for $C_{44}H_{62}O_{12}N_4Cs$ $[M + Cs]^+$ 971.3419, found 971.3447.

Biphenyl 2,2',6,6'-tetraalanine tetraamide (**17**). The biphenyl tetraalanine-O*t*Bu (**16**, 50 mg, 0.060 mmol) was dissolved in TFA and stirred under a drying tube. After 3 h the reaction was incomplete, so stirring was continued for 24 h. Upon evaporation a white powder (35 mg, 97%) precipitated. No trace of any partially deprotected compounds was evident by NMR or HRMS. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.0 (br s, 4 H), 8.74 (d, *J*= 7.7 Hz, 4 H), 7.5-7.47 (m, 6 H), 4.13 (quintet, *J*= 7.5 Hz, 4 H), 0.93 (d, *J*= 7.3 Hz, 12 H). HRMS (FAB in NBA/CsI) calcd for $C_{28}H_{31}N_4O_{12}$ $[M + H]^+$ 615.1938, found 615.1948.

Biphenyl 2,2',6,6'-tetraserine(OtBu)-OMe tetraamide (**18**). Biphenyl tetraacid chloride (**6**, 0.075 mmol) was dissolved in DCM (2 mL). Ser(O*t*Bu)-OMe·HCl (70 mg, 0.33 mmol, 4.4 equiv) in DCM (2 mL) with TEA (125 μL, excess) was added to the acid chloride dropwise rapidly. After 3 h the reaction was poured into a separatory funnel with DCM (20 mL), rinsed with citric acid (10 mL) and water (10 mL). The organic layer was then concentrated to a white foam (69 mg, 96%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.10 (d, *J*= 8.1 Hz, 4 H), 7.55-7.48 (m, 6 H), 4.29 (m, 4 H), 3.57 (s, 12 H), 3.35 (dd, *J*= 9, 5 Hz, 4 H), 3.00 (dd, *J*= 9, 5 Hz, 4 H), 1.02 (s, 36 H). HRMS (FAB in NBA/CsI) calcd for $C_{48}H_{70}N_4O_{16}Cs$ $[M + Cs]^+$ 1091.3841, found 1091.3892.

Biphenyl 2,2',6,6'-tetraserine-OMe tetraamide (**19**). The biphenyl tetraserine tetraether (**18**) was taken up in TFA (3 mL) and stirred for 8 h under a drying tube. Evaporation yielded a yellow oil. Mass spectrometric analysis revealed peaks at M + 96, M + 192, and M + 288; indicating the presence of trifluoroacetate esters. The residue was then taken up in water and stirred for 2 h at rt. Evaporation yielded a white solid (47 mg, 89%). NMR and mass spectrometry showed that the TFA-ester hydrolysis was complete. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.61 (d, *J*= 7.5 Hz, 4 H), 7.51-7.48 (m, 6 H), 6.0-5.2 (br s, 4 H), 4.25 (m, 4 H), 3.45 (s, 12 H), 3.44 (dd, *J*= 11, 5 Hz, 4 H), 3.30 (dd, *J*= 11, 5 Hz, 4 H). HRMS (FAB in NBA/CsI) calcd for $C_{32}H_{38}N_4O_{16}Cs$ $[M + Cs]^+$ 867.1337, found 867.1369.

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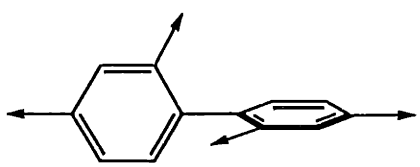
D. FLEXIBLE DIVERSITY SCAFFOLDS: THE 2,2',4,4'-BIPHENYL AND THE *trans*-1,3,5-CYCLOHEXANE CORES.

D1. Introduction to the Biphenyl and Cyclohexane Core Molecules

As mentioned in the previous chapters, synthesis of new core structures is one of several ways to promote molecular diversity. In **Chapter C** the advantages of using a biphenyl core molecule were elaborated; this chapter will introduce a second biphenyl core molecule which directs its building blocks in very different orientations (**I**). In addition, an aliphatic, unsymmetrical, low molecular weight, cyclohexane-based core molecule will be presented (**II**). Finally, this chapter will conclude with a brief comparison of the core molecules and an outlook to future applications.



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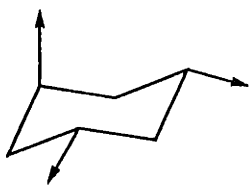


II

D2. 2,2',4,4'-Biphenyl tetraamides

The tetrasubstituted biphenyl **I** is advantageous for a number of reasons. The relatively low (C2) symmetry, for example allows for a number of compounds to be formed with only a few building blocks. While its symmetry is identical to that of the tetrasubstituted xanthene (see **Chapter A**), biphenyl (**i**) has a lower molecular weight and a different display of building blocks. As can be seen in **Figure 1**, two building blocks (at the 4 and 4' positions) diverge in opposite directions, while the other two (2 and 2') can assume a range of conformations, depending upon the torsion angle between the aryl moieties. Additionally, although the building blocks are attached to a

relatively rigid structure, the molecule is able to access more shape-space than the xanthenes because of rotation about the biaryl bond.[1]

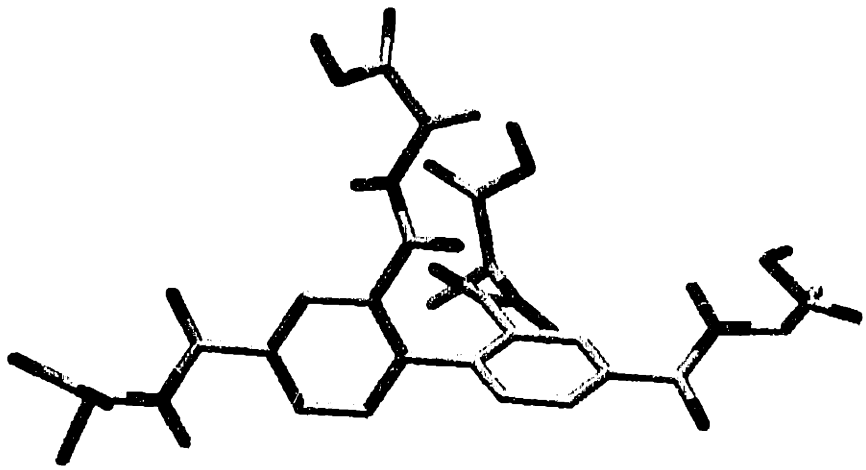


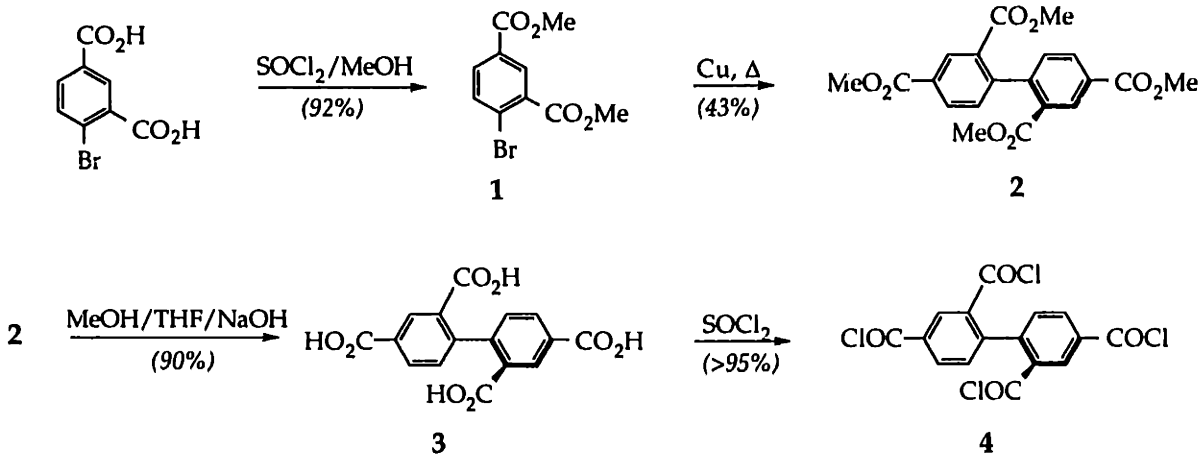
Figure 1: A polytube rendering of an MM2*-minimized 2,2'4,4'-biphenyl tetraalanine tetraamide. While the 4 and 4' groups diverge, the 2 and 2' amides are capable of interacting with each other or with the same region of a target structure.

D3. Synthesis of the 2,2',4,4'-Biphenyl Activated Core

While the original synthesis of a biphenyl tetraacid was completed by a fellow graduate student in our group, Robert Grotzfeld, several subsequent improvements have been made. The synthesis used to obtain such biphenyl derivatives is given in **Scheme 1**. The commercially available 4-bromoisophthalic acid was dissolved in methanol and esterified by slow addition of thionyl chloride[2] to give the dimethyl ester **1**. An Ullmann coupling[3] formed the biaryl bond to give **2**, and saponification of the methyl ester groups using standard procedures gave the tetraacid **3**. Activation with thionyl chloride (catalytic DMF) gave the tetraacid chloride core (**4**) in pure form. Earlier procedures[4] used phosphorus pentachloride to activate the tetraacid **3**; however, in the case of the 2,2',6,6'-biphenyl tetraacid chloride-based libraries (see **Chapter C**) residual phosphorus compounds could not be removed and resulted in library impurities. Activation with thionyl chloride

was preferable because it was easier to handle and could be removed completely *in vacuo*.

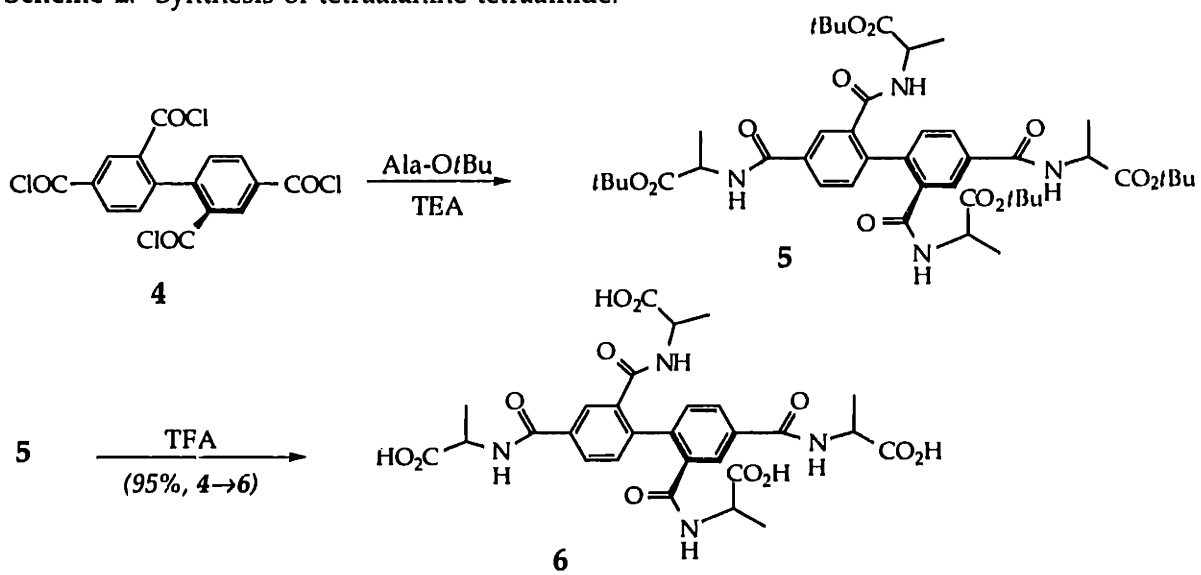
Scheme 1: Synthesis of the 2,2',4,4'-biphenyl tetraacid chloride activated core.



D4. Reactivity of the 2,2',4,4'-Biphenyl tetraacid Chloride

Tetraamides were formed from the tetraacid chloride **4** and amines in a similar manner to that of the previous core molecules. Pure compounds (an example is given in **Scheme 2**) were obtained from this condensation when single amines were used, and deprotection with neat TFA was complete in six hours (compared to 24 hours for the 2,2',6,6'-biphenyl tetraamides). When

Scheme 2: Synthesis of tetraalanine tetraamide.



two different amines were reacted with the tetraacid chloride, close to statistical distributions were observed (**Figure 2**). This result was expected given that the more hindered 2,2',6,6'-biphenyl tetraacid chloride gave high yields and statistical distributions under the same conditions. As with the 2,4,5,7-tetrasubstituted xanthenes, the number of compounds predicted from the condensation of **4** with two different amines was ten. HPLC and LC/MS (see experimental) analysis of the reaction of Ala-O*t*Bu (**5**) and Leu-O*t*Bu confirmed that ten compounds were indeed formed, with only trace impurities.

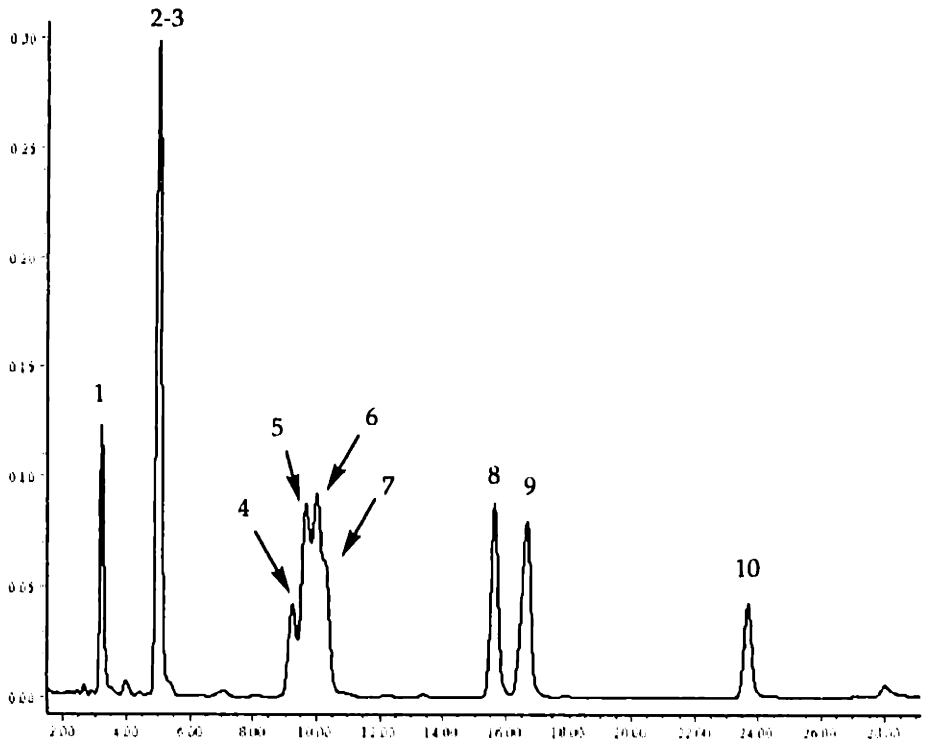


Figure 2: HPLC analysis of a ten component mixture obtained from **4** (1 equiv), Ala-O*t*Bu (2.1 equiv) and Leu-O*t*Bu (2.1 equiv). The identity of the peaks was assigned using the retention time of pure Ala_4 as well as LC/MS data (see experimental). 1: Ala_4 (8%), 2-3: Ala_3Leu (unresolved, 26%), 4: Ala_2Leu_2 (5%), 5: Ala_2Leu_2 (12%), 6: Ala_2Leu_2 (16%), 7: Ala_2Leu_2 (4%), 8: $AlaLeu_3$ (11%), 9: $AlaLeu_3$ (13%), 10: Leu_4 (5%). Areas were based on integral areas and were uncorrected for any differences in extinction coefficients.

D5. Screening of Biphenyl Libraries

This section was done in collaboration with Edward Wintner and David Skyler.

A number of biphenyl libraries have been synthesized and screened for biological activity. In particular, five biphenyl libraries were screened for activity against three cancer cell lines. Although none of these libraries were active against the cancer cell lines, one library (made with 14 tools, 19,306 compounds) did show activity in another assay measuring the prevention of HIV (human immunodeficiency virus) infection. The HIV assay,[5] which was performed by our collaborators at the NCI (National Cancer Institute), involved adding the libraries to samples containing HIV and T4 lymphocytes; if the library interfered with an important virus process then the T4 cells were spared from HIV-induced cytolysis. The graph below (**Figure 3**) illustrates the effect of the active library. The prevention level indicates that the activity of a single component could be as low as nanomolar (20 nM), assuming that all 19,306 components were formed. A rigorous deconvolution of this mixture has been initiated; 14 sublibraries (each omitting one building block from the original library) have been synthesized, and results from the next round of testing are pending.

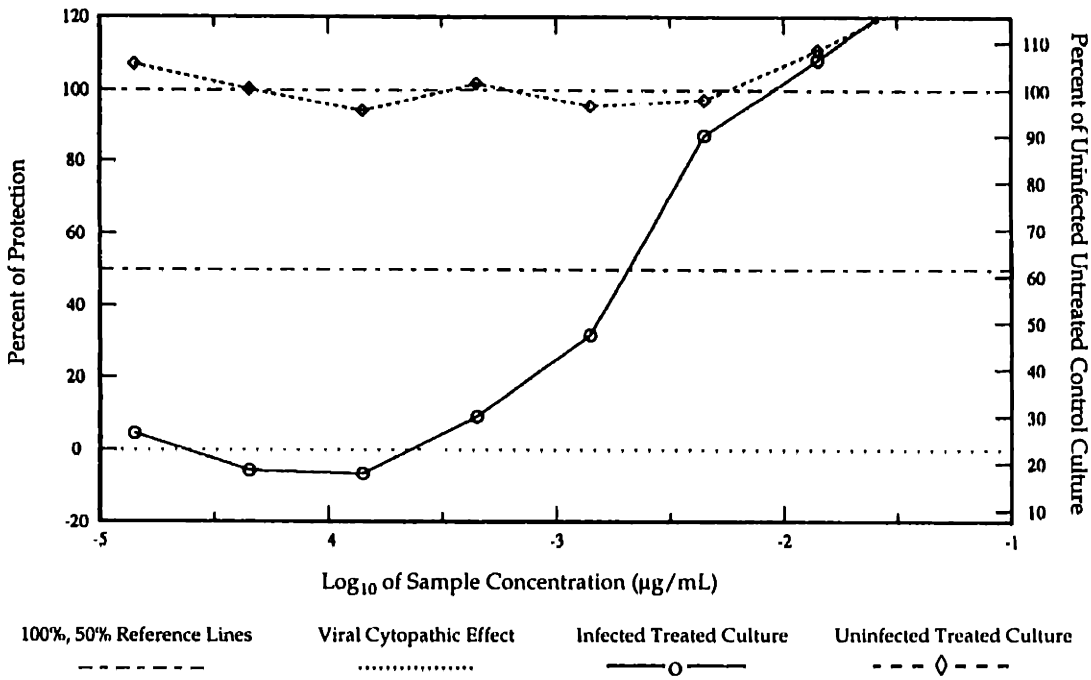


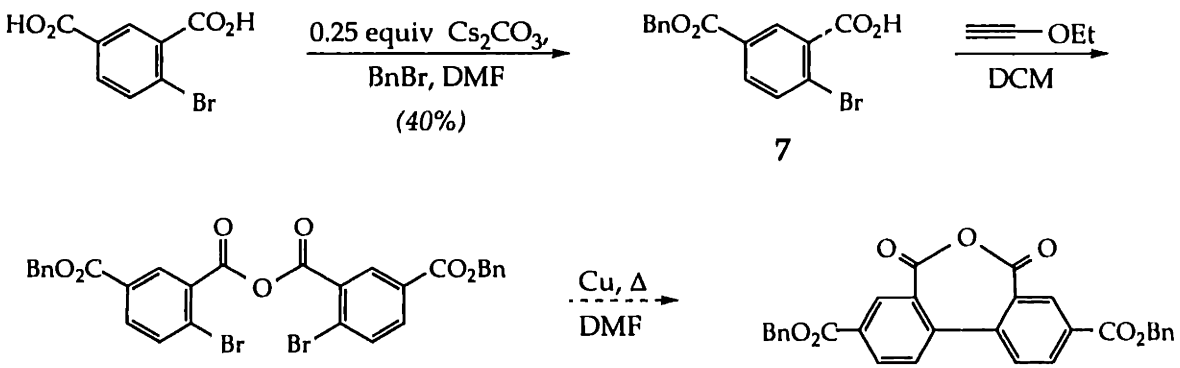
Figure 3: *In vitro* anti-HIV drug screening results. The results indicated that a library concentration of 0.014 μg/mL provided 100% protection.

D6. Synthesis of Biphenyl Derivatives Applicable to Deconvolution

Confronted with the prospect of eventually having to produce individual library components for screening, we endeavored to synthesize compounds suitable for deconvolution. Initial efforts focused on generating the symmetrical anhydride at the 2 and 2′ positions, resulting in a two-address core molecule (as in **Scheme 7**). However, there were two impediments to this approach. First, when standard conditions (using various dehydrating agents such as acetic anhydride or DCC) were applied to generate the anhydride, intractable mixtures of mixed anhydrides formed (including acetic/biphenyl and biphenyl/biphenyl anhydrides). Second, the tetraacid was insoluble in many solvents (including toluene, benzene, and DCM), which precluded thermal formation of the anhydride via dehydration. Alternative approaches which relied on differential reactivity of the 4 position versus the 2 position, such as heating the tetraacid **3** with diphenylmethanol in toluene,[6] also failed due to the tetraacid's insolubility.

Another effort involved monoalkylation of 4-bromoisophthalic acid using cesium carbonate[7] (to give 7), formation of the symmetrical anhydride (uncharacterized, see **Scheme 3**) using ethoxyacetlyene,[8] and an intramolecular Ullmann reaction[3] to form the biaryl bond. This would have given (at least) a two-address system. However, the Ullmann coupling in refluxing dry DMF gave only a mixture of side products, with no evidence of the biphenyl diester anhydride or the biphenyl diester diacid. It is worth noting that the extreme temperatures (typically ≥200°C) of the Ullmann coupling are incompatible with many functionalities; however, the reaction had been performed with similar substrates (anhydrides) in refluxing DMF.[8]

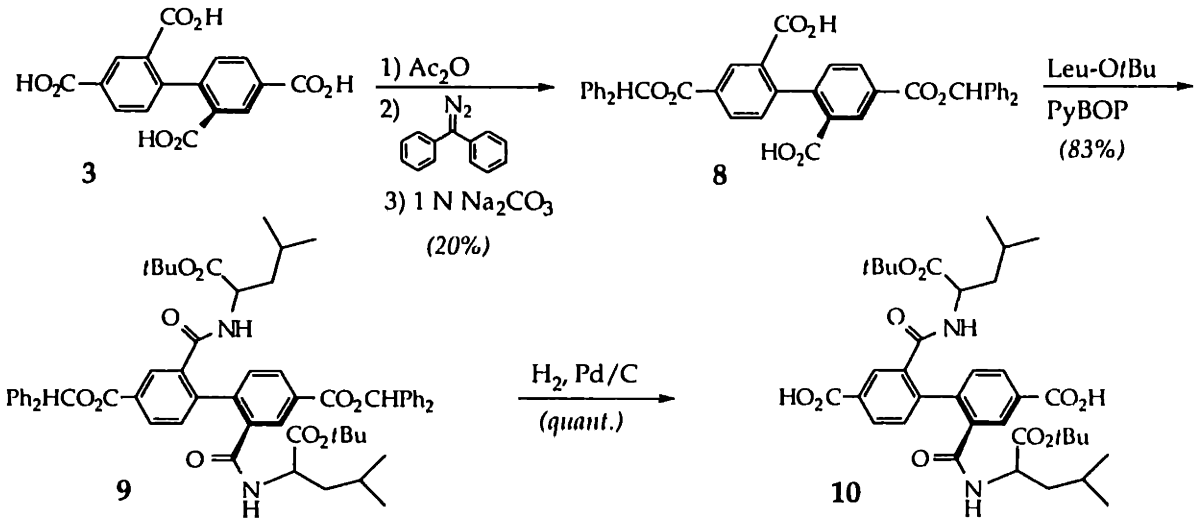
Scheme 3: Unsuccessful route to a multiple address biphenyl derivative.



The breakthrough came when the anhydride was formed in THF (which solubilized the tetraacid) using acetic anhydride. Although the anhydride was not purified, a small aliquot from the reaction mixture was analyzed by NMR and found to be about 80% of the desired 2,2'-symmetrical anhydride. Following evaporation of the residual acetic anhydride, the impure 2,2'-biphenyl anhydride was dissolved in acetone and reacted with diphenyldiazomethane[9] to give the 4,4'-(diphenylmethyl) diester, which was subsequently hydrolyzed by aqueous Na_2CO_3 to give the diester diacid **8**. To demonstrate the feasibility of this deconvolution protocol the diester diacid **8**

was coupled to Leu-O*t*Bu using PyBOP[10] to give the dileucine diester (**9**). The diphenylmethyl protecting groups were then cleaved by hydrogenolysis to give the dileucine diamide diacid **10**, which could then be coupled to additional amines to give the desired tetraamides.

Scheme 4: A route to differentially functionalized 2,2'4,4'-biphenyl tetraamides.



D7. *trans*-1,3,5-Cyclohexane triamides

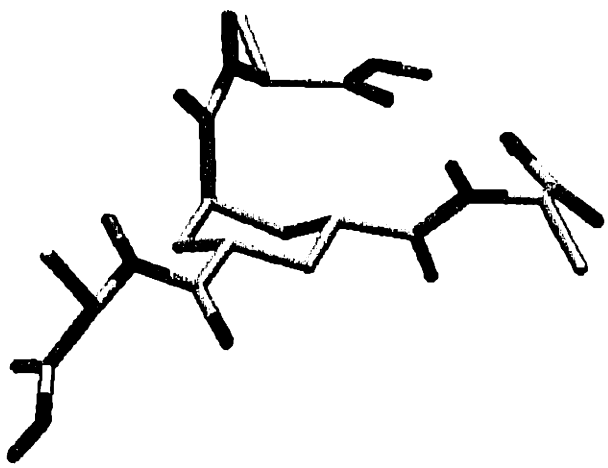


Figure 4: A polytube rendering of an MM2*-minimized *trans*-1,3,5-cyclohexane trialanine triamide.

A core molecule which combines a low molecular weight with conformational flexibility is provided by *trans*-1,3,5-cyclohexane (**Figure 4**). In

this case, the source of the conformational flexibility is derived from two possible chair conformations (see **Figure 5**), which have been calculated (see experimental section) to be 2 kcal/mol different in energy. The use of only three sites for diversity is mitigated by the three prochiral tertiary carbons. This causes the two β carboxyl sites to be inequivalent upon substitution with a chiral building block (such as an amino acid) and increases the number of compounds formed during library synthesis. Although it is structurally similar to Kemp's triacid[11,12] (**IV**, **Figure 5**), the triacid derivative of **II** offers several distinct advantages. It has a lower molecular weight (42 g/mol less than **IV**) and its reactive sites diverge enough to diminish steric biases and allow greater access to the functionalities of the building blocks. Another reason to use **II** rather than **IV** is that **II** has not been used for combinatorial chemistry, while several research groups have already utilized Kemp's triacid-based molecules for molecular diversity.[13,14]

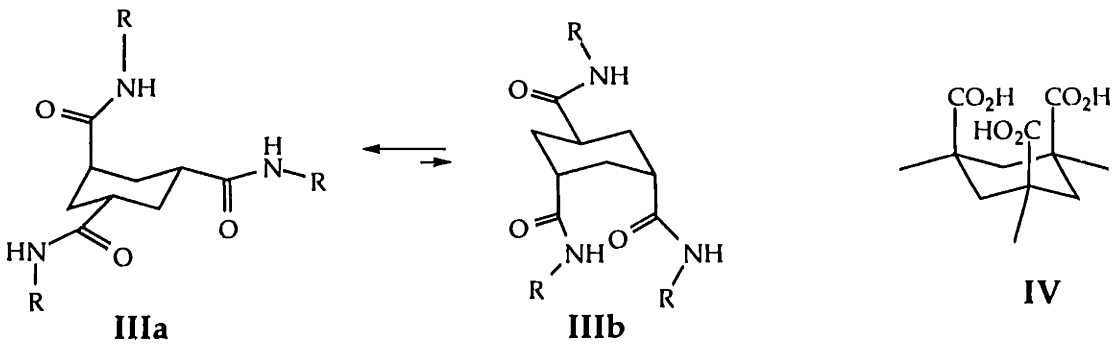


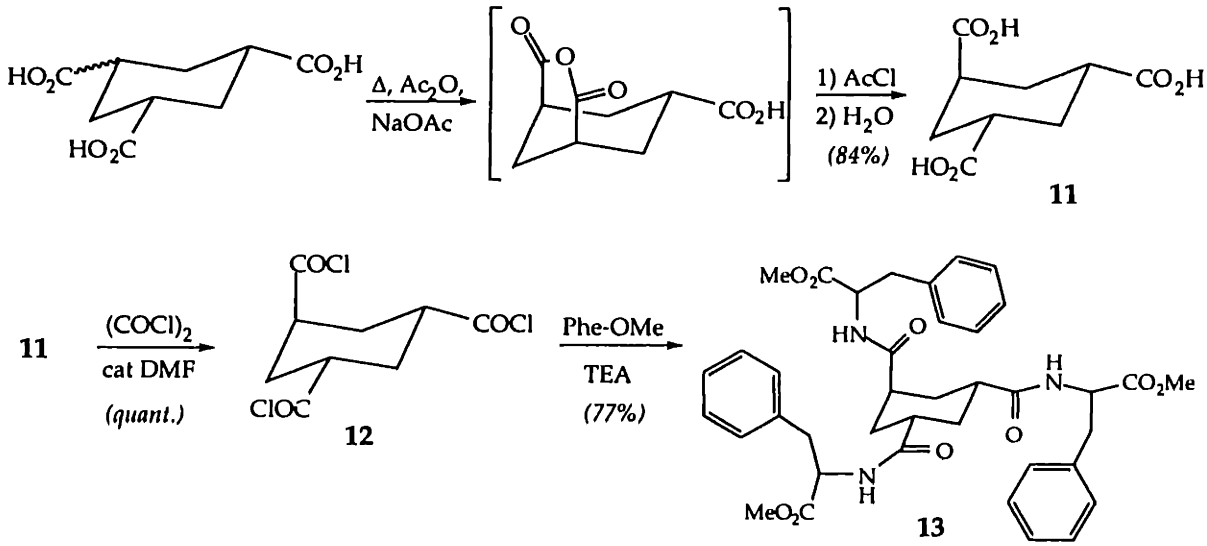
Figure 5: Molecular modeling (MM2*) indicated that the equatorial-equatorial conformation (**IIIa**) is 2 kcal/mol more stable (see experimental section) than the axial-axial conformation (**IIIb**), where R= benzyl. The structure of Kemp's triacid (**IV**) is given for comparison.

D8. Synthesis of the Cyclohexane Activated Core

The synthesis of the activated core molecule was straightforward (**Scheme 5**). The hydrogenation product of trimesic acid (a mixture of 85:15 *cis*:*trans*) was converted into its anhydride (not isolated, see **Scheme 5**) and heated in the presence of NaOAc, which epimerized the α-carbon of the (non

anhydride) carboxyl position to the more thermodynamically stable *trans* isomer.[15] The anhydride was hydrolyzed in the same pot to give the *trans*-triacid **11**. Activation using standard conditions afforded the triacid chloride **12**, which was reacted with amines to form triamides (**13**, for example).

Scheme 5: Synthesis of the triacid chloride activated core.



D9. Reactivity of the *trans*-1,3,5-Cyclohexane tricarboxylic Acid Chloride

Aromatic compounds were selected as building blocks to facilitate HPLC analysis since the core lacked a UV-active chromophore. The two amines chosen were Phe-OMe (smaller) and Tyr(O*t*Bu)-O*t*Bu (larger). Although their extinction coefficients were unequal at the wavelength of analysis, such an analysis provided a qualitative check for reaction bias as well as for impurities. The results indicated that when the ratio of acid chloride:tool was close to 1:1, little bias existed in the product distribution (see **Figure 6**); however, when larger excesses of tools were employed there was some evidence for a bias towards the smaller building blocks.

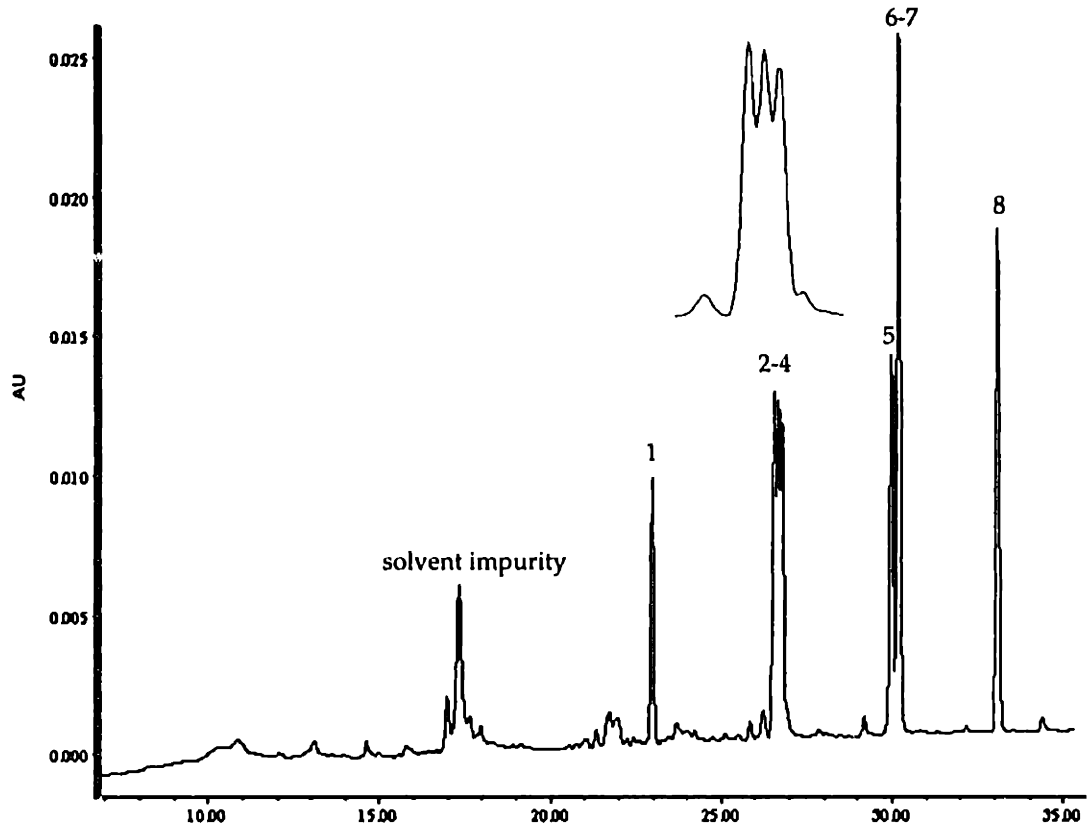


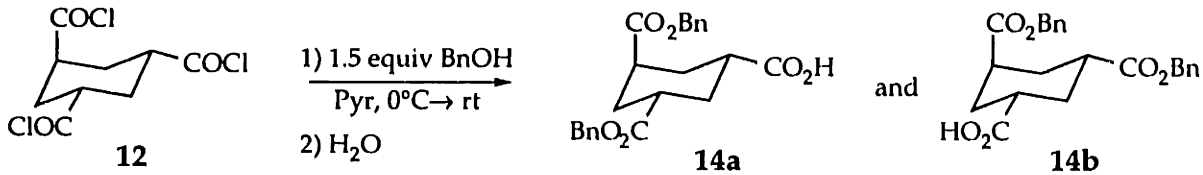
Figure 6: A reverse-phase HPLC trace of a mixture made with the triacid chloride **12** (1 equiv), Phe-OMe (1.65 equiv) and Tyr(O*t*Bu)-O*t*Bu (1.65 equiv). The peaks were identified by matching Phe_3 to a pure sample and using LC/MS data (see experimental). The areas are uncorrected for the slightly larger extinction coefficient of the Tyr residue at the wavelength of analysis (see text). 1: Phe_3 (10%), 2-4: Phe_2Tyr (3 peaks, total area of 34%), 5: $PheTyr_2$ (14%), 6-7 (unresolved, 27%), 8: Tyr_3 (15%).

D10. Deconvolution of the *trans*-1,3,5-Cyclohexane Core, Preliminary Work

Exploratory work has begun on a viable synthetic deconvolution protocol, and thus far has provided a two-address system (**Scheme 6**). This "brute-force" approach is similar to that of the 2,2',4,4'-biphenyl deconvolution, and involves reacting the triacid chloride **12** with 1.5 equivalents of benzyl alcohol, and separating the compounds. Other attempts involving formation of the symmetrical anhydride have been unsuccessful due to the insolubility of the triacid in most organic solvents. While a two address system has been isolated, it is likely a racemate of the enantiomers **14a** and **14b**, as the symmetrical β,β-diester compound is not consistent with the

spectroscopic data. It is hoped that the enantiomers can be resolved using fractional crystallization and an optically active base (brucine, strychnine).[16] The resulting optically pure compounds would then be subjected to x-ray crystallographic analysis, if possible. Nevertheless, this compound could serve as a starting point for a synthetic deconvolution.

Scheme 6: Synthesis of a two-address core molecule.



D11. Summary of Core Molecules and Their Physical Properties

Seven core molecules and two linkages have been developed for this simultaneous, solution-phase, activated core approach to chemical diversity. The core molecules cover a range of symmetries, orientations, chemical functionalities, and molecular weights[17] (see **Figure 7**). In addition, two linkages (amide and urea) have been utilized to further enhance the diversity available to this approach. Analyses of pure compounds and small libraries have verified that close to statistical distributions of compounds are formed when the reaction conditions are optimized, whether the building block amines react with a core-based acid chloride or an isocyanate. Thus far, almost every type of library that has been synthesized has shown activity in at least one assay; including trypsin (xanthene tetraamides),[18] renal and breast cancer (xanthene tetraamides), binding to DNA (xanthene tetraureas), and preventing HIV infection of T4 lymphocytes (2,2'4,4'-biphenyl tetraamides). Libraries based on other core molecules (adamantane, bipyridine, 2,2'6,6'-biphenyl and 1,3,5-cyclohexane) have yet to be tested. If any libraries do prove to be active, a recursive methodology will be used to ascertain the active component(s), as was done in identifying single compounds capable of

inhibiting trypsin and binding to DNA. While the choice of targets for our libraries has been limited in number, in principle these libraries can be screened for numerous types of biological activities as the core molecules can be found in many pharmaceuticals and most of the building blocks are based on simple amino acids.

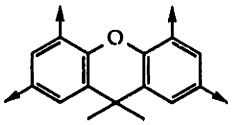
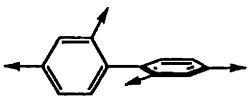
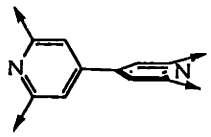
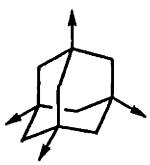
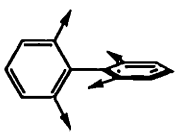
Core Structure	Molecular Weight Range (polyamides)	Numbers of Compounds from 2, 8, and 16 tools	Deconvolution Status
	508-1021	5 400 5696	Not Attempted
	614-1127 674-1187 (ureas)	10 2080 32896	Two and Three Addresses
	558-1071	10 2080 32896	Two Addresses
	560-1073	6 666 9316	Work in Progress (Kent Pryor)
	540-1053	5 400 5696	Not Completed
	558-1071	7 1072 16576	Two Addresses
	387-771	8 512 4096	Two Addresses

Figure 7: Summary of the core molecules and two of their physical properties: the number of compounds formed from 2, 8 or 16 tools, and the library molecular weight range (poly Gly to poly Trp).

D12. Experimental

D12-1. General

All reagents were purchased from Aldrich Chemical Company and used without further purification except as noted. Amino acid esters and PyBOP were acquired from Novabiochem (San Diego, California). 4-Bromoisophthalic acid was purchased from Acros Chemical Company. Diphenyldiazomethane was prepared according to literature procedures.[9] Deuterated solvents were obtained from Cambridge Isotope Laboratories and deuterated chloroform was dried over 4 Å molecular sieves. THF was distilled from sodium/benzophenone ketyl, DCM was distilled from P_2O_5 and triethylamine was distilled from (and stored over) KOH. Acetone was dried over 4 Å molecular sieves for 24 hours. Citric acid refers to a 1 N stock solution. Drying tubes were packed with anhydrous $CaCl_2$. NMR spectra were recorded on either a Bruker AM-300 or a Bruker DRX-600 and all peaks were referenced to residual solvent. A VG ZAB-VSE mass spectrometer was used to ascertain exact masses. Silica gel chromatography was performed with Silica Gel 60 (EM Science or Bodman, 230-400 mesh). TLC analysis was performed using glass-bound Silica Gel 60 (F254) plates.

D12-2. HPLC and LC/MS

Synthesis and LC/MS Analysis of a (Leu/Ala) 2,2',4,4'-Biphenyl Library.

To the 2,2',4,4'-biphenyl tetraacid chloride **4** (30 mg, 0.075 mmol) in DCM (2 mL) was added a mixture of Ala-O*t*Bu·HCl (29 mg, 0.16 mmol), Leu-O*t*Bu·HCl (36 mg, 0.16 mmol) and TEA (0.13 mL, 0.93 mmol) in DCM (2 mL). After stirring for 3 h loosely capped, more DCM (25 mL) was added, the solution was rinsed with citric acid (20 mL), and concentrated to give a white film. A portion was transferred to a HPLC vial containing MeOH.

The separation was accomplished using a Waters Symmetry C8 (3.9 x 150 mm) column, running a linear gradient of 85% water/15% MeCN to 100% MeCN over 30 min (total run time of 45 min), with analysis at a wavelength of 254 nm. The eluate from the HPLC column was then passed into a low-resolution positive ion electrospray mass spectrometer (Michrom UMA microprobe coupled with a single quadrupole Perkin Elmer API 100 Sciex). Ala_4 (retention time of 22.9 min) calcd for $C_{44}H_{63}O_{12}N_4$ $[M + H]^+$ 839.4, found 840; Ala_3Leu (25.6 min) calcd for $C_{47}H_{69}O_{12}N_4$ $[M + H]^+$ 881.5, found 882; Ala_2Leu_2 (isomers unresolved, two fractions analyzed at 27.7 and 28.2 min) calcd for $C_{50}H_{75}O_{12}N_4$ $[M + H]^+$ 923.5, found 924; $AlaLeu_3$ (29.9 min) calcd for

$C_{53}H_{81}O_{12}N_4$ $[M + H]^+$ 965.6, found 966; Leu_4 (31.4 min) calcd for $C_{56}H_{87}O_{12}N_4$ $[M + H]^+$ 1007.6, found 1008.

Synthesis and LC/MS Analysis of a (Phe/Tyr) *trans*-1,3,5-Cyclohexane Library.

To 1α,3β,5β-cyclohexane tricarboxylic acid chloride **12** (100 mg, 0.37 mmol) in DCM (2 mL) was added a mixture of Tyr(O*t*Bu)-O*t*Bu·HCl (201 mg, 0.61 mmol), Phe-OMe·HCl (132 mg, 0.61 mmol) and TEA (0.35 mL, 2.5 mmol) in DCM (3 mL). A mild exotherm was noticed during the addition and the mixture was stirred for 4 h loosely capped. More DCM (25 mL) was added, the solution was rinsed with citric acid (20 mL), and was concentrated to a white solid. A portion of the library was dissolved in MeOH and was used as the HPLC sample.

The separation was performed using a Waters Symmetry C8 (3.9 x 150 mm) column, running a linear gradient of 100% water (initial flow rate= 0.9 mL/min) to 100% MeCN (final flow rate= 1.3 min) over 30 min (total run time of 40 min), with analysis at a wavelength of 258 nm (λ_{max} of library). The eluate from the HPLC column was then passed into a low-resolution positive ion electrospray mass spectrometer (Michrom UMA microprobe coupled with a single quadrupole Perkin Elmer API 100 Sciex). Phe_3 (retention time of 20.2 min) calcd for $C_{39}H_{46}O_9N_3$ $[M + H]^+$ 700.3, found 701; Phe_2Tyr (isomers unresolved, one fraction taken at 23.8 min) calcd for $C_{46}H_{60}O_{10}N_3$ $[M + H]^+$ 814.5, found 815; $PheTyr_2$ (isomers unresolved, one fraction analyzed at 27.0 min) calcd for $C_{53}H_{73}O_{11}N_3$ $[M + H]^+$ 928.6, found 929; Tyr_3 (29.5 min) calcd for $C_{60}H_{87}O_{12}N_3$ $[M + H]^+$ 1042.7, found 1043.

D12-3. Molecular Modeling

Molecular modeling was performed using Macromodel *v*5.5 and the MM2* force field, without solvent parameters. For the calculation of the energy difference between the two chair conformations (**Figure 5**) of *trans*-1,3,5-cyclohexane tribenzylamide, the two conformers were minimized independently and their total energy values subtracted to give 2.1 kcal/mol. This is roughly equivalent to the energy cost of placing two ethyl esters in a 1,3-diaxial relationship (2.2 kcal/mol), as computed by tabulated A values.[19] It is worth noting that molecular modeling did not recognize a hydrogen bond between the carbonyl oxygen of one building block and the amide -NH of the other building block when the 3,5-amides were both in axial positions. The presence of such a hydrogen bond would lower the energy of the diaxial conformer and increase its population relative to the diequatorial conformer.

D12-4. Synthesis and Characterization

1,3-Dimethyl-4-bromobenzene dicarboxylate (**1**). To a suspension of 4-bromoisophthalic acid (10.0 g, 0.0410 mol) in MeOH (150 mL) at 0°C was added thionyl chloride (10.0 mL, 0.140 mol) dropwise. The reaction was allowed to stir for 24 h before it was filtered and concentrated. The resulting oil was taken up in DCM (250 mL) and was rinsed with sat $NaHCO_3$ (2x 35 mL). Upon concentration and standing, a white, oily solid formed. Trituration with hexanes gave a white powder (10.1 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (d, *J*= 2.0 Hz, 1 H), 7.94 (dd, *J*= 8.3, 2.1 Hz, 1 H), 7.75 (d, *J*= 8.2 Hz, 1 H), 3.96 (s, 3 H), 3.93 (s, 3 H). HRMS (FAB in NBA/NaI) calcd for $C_{10}H_{10}O_4Br$ $[M + H]^+$ 272.9762, found 272.9772.

2,2',4,4'-Biphenyl tetramethyl ester (**2**). The dimethyl ester **1** (6.00 g, 22.0 mmol) was pulverized with Cu powder (4.2 g, 94 mmol) and the mixture was added to a 100 mL pressure tube sealed with a teflon cap. This was heated to 200°C for 4 h, cooled to rt, and the brown solid was extracted (4x 100 mL 5% MeOH in $CHCl_3$, with sonication). Filtration and concentration gave a solid, which was preloaded onto silica gel with neat DCM. Elution (DCM→10:1 DCM:MeOH), followed by concentration of the pure fractions resulted in a white powder (2.49 g, 44%). R_f= 0.3 (20:1 DCM:MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.70 (d, *J*= 1.9 Hz, 2 H), 8.20 (dd, *J*= 8.0 Hz, 1.9 Hz, 2 H), 7.26 (d, *J*= 7.8 Hz, 2 H), 3.95 (s, 6 H), 3.66 (s, 6 H). HRMS (FAB in NBA/NaI) calcd for $C_{20}H_{19}O_8$ $[M + H]^+$ 387.1080, found 387.1089.

2,2',4,4'-Biphenyl tetraacid (**3**). The tetraester **2** was suspended in a solution of THF (75 mL), methanol (7.5 mL), and 2 N KOH (28 mL). After 2 h at rt the solution was warmed to reflux for 3 h. The solvents were evaporated and water (50 mL) was added. Acidification to pH= 1 with conc HCl produced a precipitate, which was filtered (after standing overnight at 0°C) to afford a white solid (1.09 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.1 (br s, 4 H), 8.45 (d, *J*= 1.4 Hz, 2 H), 8.09 (dd, *J*= 8.0, 1.7 Hz, 2 H), 7.31 (d, *J*= 8.0 Hz, 2 H). ^{13}C (75.4 MHz, DMSO-d_6) δ 166.89, 166.68, 146.98, 131.96, 130.65, 130.34, 129.95. HRMS (FAB in NBA/NaI) calcd for $C_{16}H_{10}O_8Na$ $[M + Na]^+$ 353.0273, found 353.0257.

2,2',4,4'-Biphenyl tetraacid chloride (**4**). To the biphenyl tetraacid **3** (250 mg, 0.75 mmol) suspended in DCM (15 mL) was added $SOCl_2$ (10 mL, excess), followed by DMF (5 μL, cat). After stirring for 30 min at rt the suspension was warmed to reflux for 2.5 h, at which point the solution became clear.

Concentration of the solution gave a white solid, toluene was added, and the suspension was again evaporated to give a white solid (296 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (d, *J*= 2.0 Hz, 2 H), 8.42 (dd, *J*= 8.5, 2.1 Hz, 2 H), 7.25 (d, *J*= 8.4 Hz, 2 H).

2,2',4,4'-Biphenyl tetraalanine-OtBu tetraamide (**5**). To the biphenyl tetraacid **3** (25 mg, 0.075 mmol) was added DCM (1.5 mL), thionyl chloride (1.0 mL, excess) and DMF (1 μL, cat). After stirring for 30 min at rt the flask was warmed to reflux for 2.5 h under a drying tube, cooled to rt, and was concentrated to a white film. Toluene (0.2 mL) was added and the suspension was concentrated to a thin white film.
To the white film in DCM (1 mL) was added a solution of Ala-O*t*Bu·HCl (60 mg, 0.33 mmol), TEA (125 μL, 0.89 mmol), and DCM (2 mL). The solution was stirred for 3 h before more DCM was added and the mixture was transferred to a separatory funnel. After rinsing with citric acid, the organic layer was concentrated to give a white powder (60 mg, 95% overall). HPLC (see conditions for separating the Ala/Leu biphenyl library) indicated this compound was ≥95% pure. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.84 (d, *J*=7.3 Hz, 2 H), 8.81 (d, *J*= 7.4 Hz, 2 H), 8.04 (s, 2 H), 7.94 (dd, *J*= 8.0, 1.6 Hz, 2 H), 7.18 (d, *J*= 7.9 Hz, 2 H), 4.35 (quintet, *J*= 7.1 Hz, 2 H), 4.08 (qunitet, *J*= 7.2 Hz, 2 H), 1.40 (s, 18 H), 1.37 (d, *J*= 7.4 Hz, 6 H), 1.32 (s, 18 H), 1.00 (d, *J*= 6 Hz, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{44}H_{62}O_{12}N_4Cs$ $[M + Cs]^+$ 971.3419, found 971.3459.

2,2',4,4'-Biphenyl tetraalanine tetraamide (**6**). The tetraaalanine-O*t*Bu **5** (60 mg, 0.071 mmol) was dissolved in TFA (3 mL) and stirred for 6 h under a drying tube. Concentration gave a white film in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.87 (d, *J*= 7.2 Hz, 2 H), 8.80 (d, *J*= 7.6 Hz, 2 H), 8.06 (d, *J*= 1 Hz, 2 H), 7.92 (d, *J*= 7.9 Hz, 2 H), 7.17 (d, *J*= 8.0 Hz, 2 H), 4.43 (quintet, *J*= 7.2 Hz, 2 H), 4.14 (quintet, *J*= 7.2 Hz, 2 H), 1.40 (d, *J*= 7.3 Hz, 6 H), 1.02 (d, *J*= 6 Hz, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{28}H_{30}O_{12}N_4Cs$ $[M + Cs]^+$ 747.0915, found 747.0924.

Benzyl 6-bromobenzoic acid-3-carboxylate (**7**). To a suspension of 4-bromoisophthalic acid (100 mg, 0.41 mmol) in DMF (3 mL) was added Cs_2CO_3 (34 mg, 0.11 mmol) and the mixture was stirred for 5 min. Benzyl bromide (50 μL, 0.47 mmol, 1.2 equiv) was added and the suspension was warmed to 70°C under N_2 for 7 h. After cooling, the mixture was transferred to a separatory funnel with DCM (30 mL), rinsed with 1 N HCl (10 mL); the layers were separated, and the aqueous phase was extracted with DCM (1x 20 mL). Upon concentration a white solid formed, which was dissolved in 3:1 DCM:MeOH and preloaded onto SiO_2. Elution (15:1 DCM:MeOH) through

silica gel, followed by concentration of the fractions with an R_f= 0.4 (15:1 DCM:MeOH), gave a white solid (55 mg, 40%) ^{1}H NMR (300 MHz, acetone-d_6): δ 8.41(br s, 1 H), 8.05 (d, *J*= 7.6 Hz, 1 H), 7.90 (d, *J*= 8.2 Hz, 1 H), 7.55 (d, *J*= 7.8 Hz, 2 H), 7.45-7.37 (m, 3 H), 5.43 (s, 2 H). HRMS (FAB in NBA/NaI) calcd for $C_{15}H_{11}O_4BrNa$ $[M + Na]^+$ 356.9738, found 356.9751.

4,4'-Diphenylmethyl biphenyl dicarboxylate-2,2'-dicarboxylic acid (**8**). Acetic anhydride (0.15 mL, 1.6 mmol) was added to the biphenyl tetraacid **3** (0.40 g, 1.21 mmol) in THF (8 mL). The turbid solution was warmed to reflux under a drying tube for 8 h, more Ac_2O (0.05 mL, 0.50 mmol) was added, and the solution was stirred for 12 h at rt. Concentration afforded a white solid, which appeared by ^{1}H NMR to be 80% product. The crude anhydride was suspended in acetone (10 mL), and diphenyldiazomethane (0.65 g in 10 mL acetone, 3.35 mmol) was added dropwise. The flask was protected from light and was stirred under N_2 for 48 h, during which time the red suspension was observed to almost completely decolorize. To hydrolyze the anhydride Na_2CO_3 (1 N, 30 mL) was added and the suspension was stirred for 1 h. Acidification to pH=2 using 2 N HCl followed by extraction (3x 30 mL $CHCl_3$) and drying over $MgSO_4$ produced a viscous oil. Silica gel chromatography (20:1→4:1 DCM:MeOH) was used to isolate a polar compound (R_f= 0.2, 10:1 DCM:MeOH). Partial concentration, followed by cooling of the product fractions, gave white fluffy crystals (160 mg, 20%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.01 (br s, 2 H), 7.90 (d, *J*= 6.9 Hz, 2 H), 7.50 (d, *J*= 7.5 Hz, 8 H), 7.38 (t, *J*= 7.7 Hz, 8 H), 7.30 (t, *J*=7.4 Hz, 4 H), 7.11 (d, *J*= 7.6 Hz, 2 H), 7.07 (s, 2 H). LRMS (ESI +) calcd for $C_{42}H_{30}O_8Na$ $[M + Na]^+$ 685, found 685. LRMS (ESI –) calcd for $C_{42}H_{29}O_8Na$ $[M - H]^-$ 661, found 661. This compound was not stable to FAB conditions; therefore HRMS could not be obtained.

Biphenyl 2,2'-dileucine diamide-4,4'-diphenylmethyl dicarboxylate (**9**). PyBOP (42 mg, 0.080 mmol) and **8** (20 mg, 0.030 mmol) were dissolved in DMF (2 mL). After 5 min DIEA (0.028 mL, 0.16 mmol) and Leu-O*t*Bu·HCl (18 mg, 0.080 mmol) in DMF (1 mL) were added to the biphenyl-PyBOP flask under a drying tube. After 12 h, water (10 mL) and DCM (25 mL) were added; the organic layer was rinsed (1x 10 mL water, 1x 10 mL citric acid) and dried ($MgSO_4$). Concentration gave an oil which was preloaded onto SiO_2 and chromatographed (5:1 hexanes:EtOAc, R_f= 0.9) to give a white solid (25 mg, 83%). ^{1}H NMR (300 MHz, acetone-d_6): δ 8.42 (d, *J*= 8.0 Hz, 2 H), 8.30 (s, 2 H), 8.22 (dd, *J*= 8.0, 1.6 Hz, 2 H), 7.55 (d, *J*= 7.7 Hz, 8 H), 7.41 (t, *J*= 8.0 Hz, 8 H), 7.33 (t, *J*= 7.6 Hz, 4 H), 7.16 (s, 2 H), 4.26 (q, *J*= 7.3 Hz, 2 H), 1.43-1.3 (m, 24 H), 0.74 (m, 12

H). HRMS (FAB in NBA/CsI) calcd for $C_{62}H_{68}N_2O_{10}Cs$ $[M + Cs]^+$ 1133.3928, found 1133.3923.

Biphenyl 2,2'-dileucine diamide-4,4'-dicarboxylic acid (**10**). A solvent mixture of 4:1 EtOAc:EtOH (5 mL) was used to dissolve **9** (20 mg, 0.020 mmol). 10% Pd/C (5 mg, cat) was added and the flask was evacuated and back-filled three times with H_2 (atm). Stirring for 3 h, filtration through celite, and concentration gave an oily white solid. Trituration with hexanes gave the product as a white solid in quantitative yield. ^{1}H NMR (300 MHz, acetone-d_6): δ 11.5 (br s, 2 H), 8.39 (d, *J*= 8.2 Hz, 2 H), 8.22 (d, *J*= 1.1 Hz, 2 H), 7.29 (d, *J*= 7.9 Hz, 2 H), 4.29 (q, *J*= 7.4 Hz, 2 H), 1.5-1.1 (m, 24 H), 1.16 (m, 2 H), 0.79 (m, 12 H). HRMS (FAB in NBA/CsI) calcd for $C_{36}H_{47}O_{10}N_2Cs_2$ $[M - H^+ + 2Cs^+]^+$ 933.1339, found 933.1301.

trans-1,3,5-Cyclohexane tricarboxylic acid[15] (**11**). To a mixture of *cis* and *trans*-1,3,5-cyclohexane tricarboxylic acid (10.87 g, 50.3 mmol) was added NaOAc (1.00 g, 12.2 mmol) and Ac_2O (20 mL, 210 mmol). After refluxing for 4 h under a drying tube the solution was cooled to rt and acetyl chloride (10.0 mL 141 mmol) was added. The light green solution was refluxed for 2 h. Upon cooling a white precipitate formed and the mixture was filtered. The solid was discarded and the filtrate was poured into water (100 mL) and stirred for 16 h at rt. Concentration of the resulting solution to one-tenth of its original volume gave a fluffy white solid, which was filtered and recrystallized from water to give a white solid (9.14 g, 84%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.28 (s, 3 H), 2.81 (t, *J*= 2.2 Hz, 1H), 2.35 (tt, *J*= 12.4, 3.3 Hz, 2 H), 2.16 (d, *J*= 13 Hz, 2 H), 2.04 (d, *J*= 13 Hz, 1 H), 1.41 (td, *J*= 13.2, 5 Hz, 2 H), 1.33 (q, *J*= 12.7 Hz, 1 H). LRMS (ESI –) calcd for $C_9H_{11}O_6$ $[M - H]^-$ 215, found 215.

trans-1,3,5-Cyclohexane tricarboxylic acid chloride (**12**). The cyclohexane tricarboxylic acid **11** (0.50 g, 2.8 mmol) was suspended in DCM (50 mL), oxalyl chloride (1.5 mL, 17 mmol) was added, followed by DMF (2 μL, cat). After stirring for 1 h at rt the system was heated to reflux for 3 h to give a clear solution. Evaporation of the mixture gave a slightly yellow oil in quantitative yield. ^{1}H NMR (300 MHz, acetone-d_6): δ 2.85 (m, 1 H), 2.47 (tt, *J*= 12.5, 3.5, 2 H), 2.25 (d, *J*= 13.5 Hz, 2 H), 2.14 (d, *J*= 13.1 Hz, 1 H), 1.47 (td, *J*= 13.1, 5.0 Hz, 2 H), 1.38 (q, *J*= 12.7 Hz, 1 H).

trans-1,3,5-Cyclohexane triphenylalanine-OMe triamide (**13**). To the cyclohexane tricarboxylic acid chloride **12** (50 mg, 0.19 mmol) in DCM (2 mL) was added Phe-OMe·HCl (136 mg, 0.627 mmol) and TEA (175 μL, 1.3 mmol) in

DCM (6 mL). After 2 h at rt the mixture was transferred to a separatory funnel with DCM (20 mL); the solution was rinsed with citric acid, dried over $MgSO_4$, filtered, and concentrated to a white foam (102 mg, 77%). HPLC analysis (λ= 258 nm) indicated this compound to be ≥87% pure. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.72-7.67 (m, 2 H), 7.61 (d, *J*= 7.7 Hz, 1 H), 6.73-6.55 (m, 15 H), 3.89-3.81 (m, 3 H), 3.03 (s, 3 H), 3.02 (s, 3 H), 3.01 (s, 3 H), 2.85 (m, 1 H), 2.50-2.30 (m, 6 H), 2.07 (d, *J*= 12 Hz, 2 H), 1.92 (d, *J*= 12 Hz, 1 H), 1.15 (d, *J*= 12 Hz, 1 H), 0.95 (appar t, *J*= 12 Hz, 1 H), 0.83-0.75 (m, 2 H), 0.56 (q, *J*= 12 Hz, 1 H). HRMS (FAB in NBA/CsI) calcd for $C_{39}H_{45}N_3O_9Cs$ $[M + Cs]^+$ 832.2210, found 832.2231.

trans-1,3-Dibenzyl dicarboxylate cyclohexane-5-carboxylic acid (**14a** and **14 b**). To the triacid chloride **12** (270 mg, 0.99 mmol) was added DCM (10 mL), and the flask was placed under N_2 and cooled to 0°C. Benzyl alcohol (160 μL, 1.5 mmol) was added dropwise, followed by pyridine (0.25 mL, 3.1 mmol). The system was allowed to warm to rt and stirred for 16 h under inert atmosphere. Water (1 mL, excess) was added and the emulsion was stirred for 1.5 h. DCM (20 mL) was added and the biphasic mixture transferred to a separatory funnel. The layers were separated, the organic layer was rinsed with HCl (1 N, 10 mL), and concentrated to give an oil. The oil was taken up in DCM, preloaded onto SiO_2, and chromatographed (20:1→3:1 DCM:MeOH) to give a clear oil (98 mg, 25%). Reverse-phase HPLC analysis (see conditions for separating the 2,2',4,4'-biphenyl library in **Section D12-2**) showed this compound to be ≥87% pure (retention time of 36.6 min). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10 (br s, 1 H), 7.4-7.3 (m, 10 H), 5.16 (s, 2 H), 5.15 (s, 2 H), 2.98 (m, 1 H), 2.63 (m, 2 H), 2.42-2.35 (m, 3 H), 1.7-1.5 (m, 3 H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 174.94, 174.25, 173.7, 135.71, 128.53, 128.47, 128.21, 128.15, 128.01, 127.95, 66.40, 66.34, 38.64, 38.39, 37.92, 30.41, 28.77, 28.60. HRMS (FAB in NBA/CsI) calcd for $C_{23}H_{25}O_6$ $[M + H]^+$ 397.1651, found 397.1659.

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II. PORPHYRIN-BASED SMALL MOLECULE RECEPTORS

A. Synthesis and Binding Studies of Porphyrin Molecular Clefts

A1. Introduction: Convergent Recognition Groups

Small molecule hosts employing convergent recognition elements separated by unsaturated platforms are known as molecular clefts (**Figure 1**).[1-3] These concave molecules direct functional groups into a well-defined space, an essential property of many biomolecules. Activities usually associated with enzymes, such as binding small molecules,[1] metal coordination,[4] and catalysis[5] have been reproduced in a number of synthetic cleft-based systems. They feature a modular, relatively high-yielding synthesis and a high degree of preorganization - improvements over the more flexible and difficult to synthesize macrocycle-based hosts which preceded them.[6]

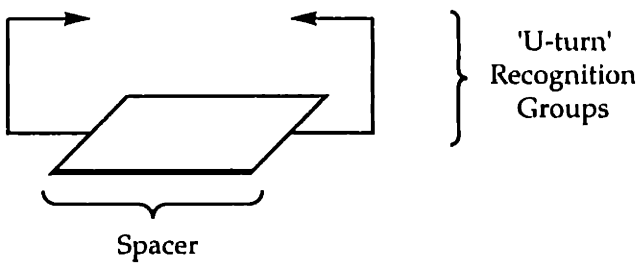


Figure 1: Schematic of a synthetic molecular cleft with convergent functional groups.

Since their introduction in 1985 by Rebek and coworkers, various combinations of spacer and recognition groups have been utilized. Spacers have included 4,4'-biphenyl, acridine, perylene, carbazole, and others.[1,3] While providing differing levels of rigidity, the spacer units must fix the

recognition groups at a distance to maintain a binding pocket and prevent functional group collapse. Two different 'U-turn' recognition moieties deserve mention in this context: Kemp's triacid and a xanthene diacid.

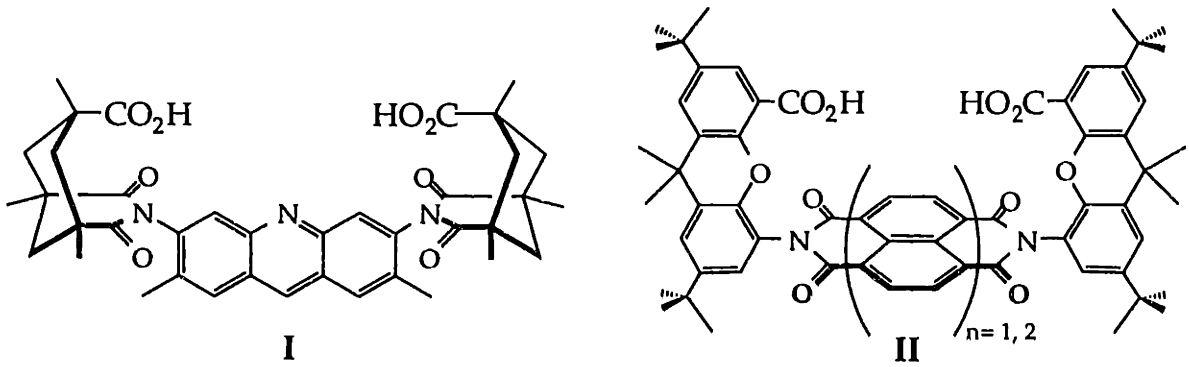


Figure 2: Acridine-Kemp's triacid (**I**) and naphthalene (n= 1)/perylene (n= 2)-xanthene (**II**) molecular clefts.

The acridine-Kemp's triacid cleft (**I**) proved versatile in binding small molecules and facilitating catalysis. What distinguished this cleft from others was the presence of functionalization inside the binding area. This allowed for catalysis within the cleft; both enolization and hemiacetal cleavage were accelerated by this system.[5,7] The second system, based on the naphthalene or perylene aromatic surfaces (**II**), were shown to bind small molecules[8] and a naphthalene-based system coordinated metal ions.[9] Perylene-xanthene derivatives capable of asymmetric guest recognition[10] and intramolecular acyl transfer[11] have also been prepared. The naphthalene and perylene receptors have the advantage that they can be synthesized in a modular fashion and the spacer unit is commercially available. The xanthene-based recognition groups also provide a deeper binding pocket than the Kemp's triacid. However, interior functionalization of the perylene spacer is not possible.

It is no surprise that over the past decade another aromatic surface, the porphyrin (**III**), has been utilized in molecular recognition studies. Some of these studies utilized an included metal to contribute to binding interactions,[12] while others[13-16] relied on recognition groups present on attached tetraaryl moieties. The work[17] of Ogoshi and coworkers has received

the most attention. One such investigation was the binding of quinones (see **Figure 3**) via two hydrogen-bonding contacts and π stacking.[18] They have also constructed other porphyrin receptors, a few of which have catalytic abilities. Finally, as noncovalent porphyrin assemblies are responsible for many critical energy exchange processes (photosynthesis and electron transfer in particular), there has been recent interest in developing self-assembling porphyrin networks.[19-21]

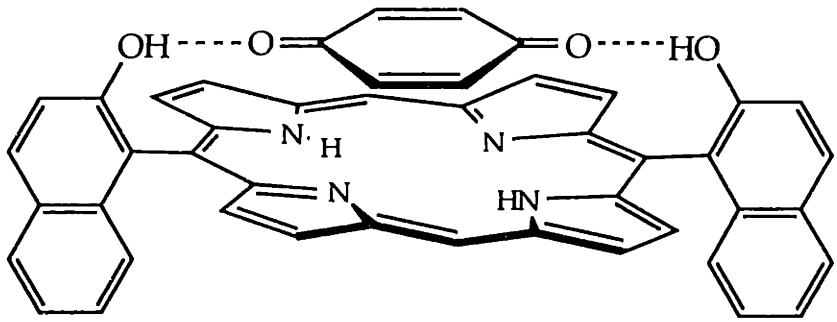
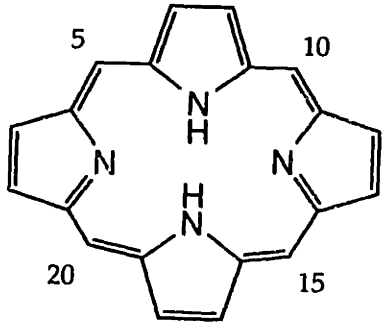


Figure 3: By using two hydrogen bonds and π stacking Ogoshi demonstrated recognition of a number of quinones with association constants of 35-230 M^{-1}.[18]

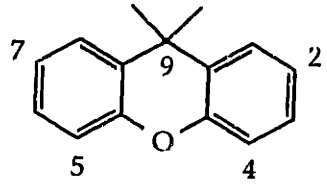
To justify the synthesis of another cleft two criteria had to be satisfied. First, the synthesis had to be modular and allow for preparation of derivatives. Second, the spacer had to be capable of participating in a supramolecular process (binding, catalysis). The porphyrin macrocycle fulfilled both requirements. Porphyrin syntheses were established and methods were available to synthesize most porphyrin isomers. At a minimum the porphyrin framework provided a site for π–π interactions[22] and four sites for (weak) hydrogen bonding via the core nitrogens.[15] Metallating the porphyrin would greatly extend its capabilities, since metalloporphyrins were known to perform a number of reactions, including hydroxylation, epoxidation, and amide-bond cleavage.[23,24]

A2. Design of Porphyrin-based Molecular Clefts

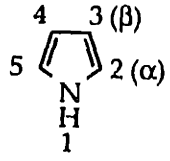
The bulk of this section has previously appeared in print.[25]



III



IV



V

Figure 4: Numbering of relevant positions on the porphyrin, xanthene, and pyrrole heterocycles.

There are a number of parameters which must be considered when designing artificial receptors.[26,27] The dimensions and topology should allow for guest complexation. There should be enough contact points between host and guest to expect an interaction, and the functional groups should be well-matched to maximize the binding energy of the event (ΔH). In addition, the host and guest should be preorganized so that the loss of entropy (ΔS) upon binding is minimized. Finally, the host should be stable to the conditions used to measure binding. These principles were considered in the design of the porphyrin cleft system.

To allow for two binding contacts, yet still leave enough space for guest inclusion, it was decided to place the recognition groups at the 5 and 15 (*meso*) porphyrin positions (see **III**). The remaining *meso* positions (10 and 20) could be either unsubstituted or derivatized easily with other aryl groups. As it was known that *meso* unsubstituted porphyins were susceptible to oxidation,[28] it was decided that phenyl groups should occupy the 10 and 20 positions. A 4,5-disubstituted xanthene subunit (see **IV**) was a logical choice

to provide the covergent functional groups. Not only were the syntheses of necessary xanthene precursors known, but the xanthene group would also be stable toward a variety of conditions, and would enhance solubility. Use of the xanthene group however, lead to questions regarding preorganization, as rotation about the porphyrin-xanthene bond was possible. One way to prohibit this rotation would be to place substituents on the pyrrole units (see **V**). To ensure a well-defined cleft, the synthesis of porphyrins containing β-pyrrole substituents was undertaken.

A3. Syntheses of Porphyrin Precursors

Porphyrin synthesis traditionally involves combination of a pyrrole with an aldehyde under acidic conditions. An oxidant must be present during the condensation (such as ambient dioxygen),[29] or added after the condensation has reached equilibrium (such as *p*-chloroanil or DDQ)[30,31] to oxidize the tetrapyrrole intermediate (the porphyrinogen) to the porphyrin. As water is also generated during the condensation, dehydrating agents (such as triethyl orthoacetate) have been used to drive the equilibrium. Metal ion 'templates' are not usually used, as neither pyrroles nor porphyrinogens are known to complex metals under the reaction conditions.[30]

Unfortunately, when this project was begun the desired 5,15-substitution pattern (**VIII**) was not easily accessible. In the past, multiple step synthesis with relatively unstable intermediates resulted in low overall yield of such a tetraaryl porphyrin isomer. Higher yielding approaches based on covalently linked dipyrrole fragments have given so-called 'strapped' or 'vaulted' porphyrins.[32] Others have condensed two different aldehydes together with pyrrole and separated the mixture. Neither of these methods could easily be applied to the desired system. In particular, the last method

would give ten porphyrin compounds (if the xanthene-porphyrin rotational barrier was >25 kcal/mol), impractical in terms of separation and yield.

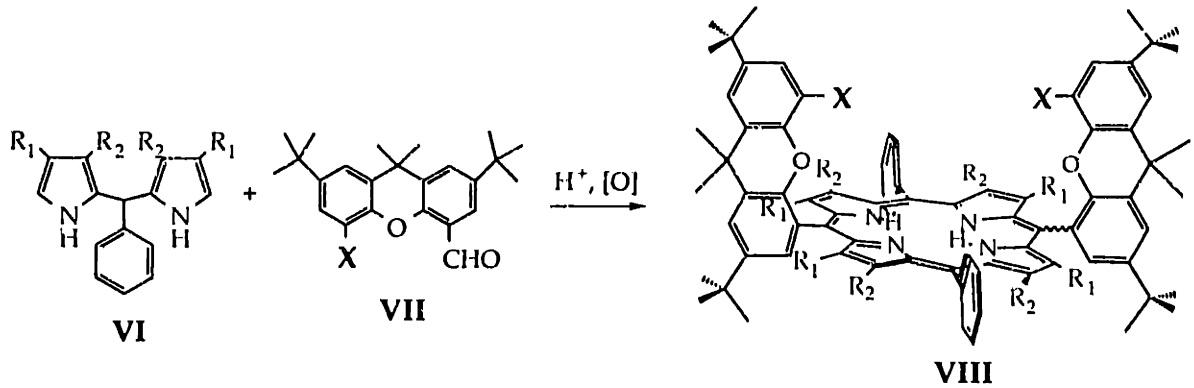


Figure 5: Assembly of the desired porphyrin sustitution pattern. R_1, R_2 = H or alkyl groups.

A phenyl-dipyrrole fragment (**VI**) was chosen as the porphyrin skeleton precursor. This compound was more attractive than the analogous xanthene-dipyrrole unit, as the xanthene aldehyde (**VII**) required a multiple-step synthesis. However there was one known complication of this approach; under certain conditions a side-reaction involving rearrangment of the tetrapyrrole before oxidation was observed. This acid-redistribution of aldehydes has been observed in a few systems[33] and can lead to a statistical distribution of porphyrin products. For example, if re-distribution occured in the system outlined in **Figure 5** mixtures of tetraxanthene, trixanthene-monophenyl, dixanthene-diphenyl, etc. would be observed. Conditions would have to be controlled to avoid this side reaction.

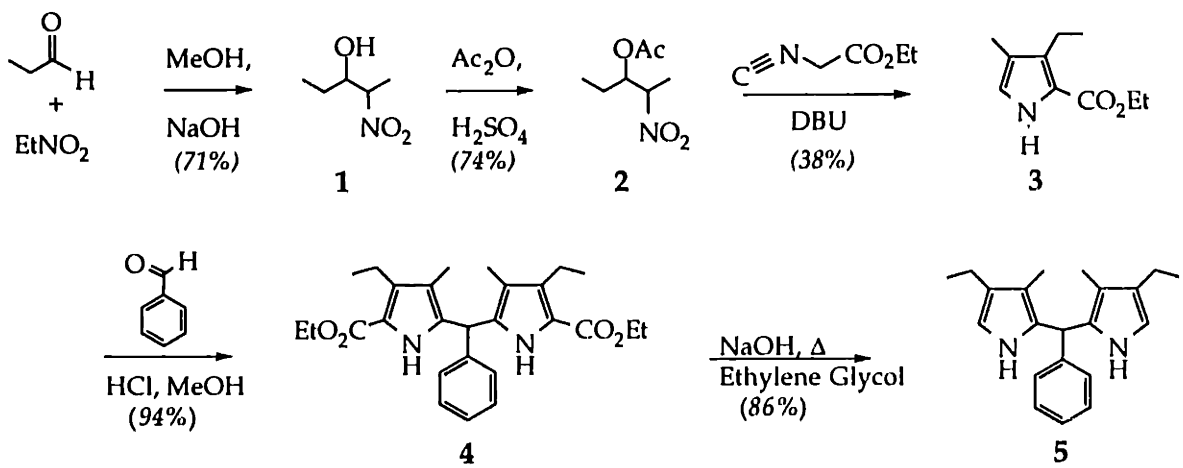
Preparation of a suitable pyrrole required several design considerations. Alkyl groups were desired at the β positions of the pyrrole to inhibit rotation of the xanthene moiety. One α pyrrole position would need to be protected by an electron withdrawing group (EWG), as alkyl-substituted pyrroles are less stable than their unsubstituted counterpart. The presence of an EWG was known to increase its resistance towards air and acid.[34] The 3-ethyl-4-methyl

pyrrole **3** satisfied these requirements. An efficient approach to analogous pyrroles was the recently developed Barton-Zard method.[34,35]

The method of Barton and Zard uses the cyclization of a nitroalkene with an isonitrile in the presence of a hindered base, such as DBU (**Scheme 1**).[34] This general method has recently been used to create many novel porphyrins.[35-37] A Henry condensation[38] was used to make the nitroalcohol **1**. It was then acetylated[39] to give **2**, converted into the nitroalkene *in situ* using DBU,[34] and reacted with ethyl isocyanoacetate.[40] The alpha-free pyrrole **3** condensed with benzaldehyde to give the diester dipyrrole **4**. Saponification followed by spontaneous decarboxylation gave the final dipyrrole **5**.[41]

Removal of the ethyl ester groups of **4** did, however, prove to be challenging. The diester was dissolved in ethylene glycol, alkali added, and the mixture heated to reflux.[41] Upon cooling the product was expected to precipitate. However, in many cases this did not occur, and the product could not be otherwise isolated as the solution contained impurities and the product decomposed on silica gel. Careful optimization of the conditions was necessary to obtain product.

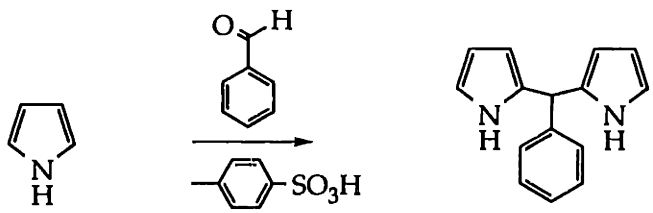
Scheme 1: Synthesis of a dipyrrole with β substituents



It should be noted that an alternate route involving the dibenzyl ester derivative of **4** (not shown), which decarboxylates following hydrogenolysis,[42,43] did not produce better results than the optimized saponification route.

In principle, a readily synthesized dipyrrole compound could be obtained by reacting excess pyrrole with benzaldehyde (**Scheme 2**). No procedure for this transformation could be found in the literature,[44] although a postdoctoral associate in the research group (Thomas Carell) had performed the reaction for his thesis.[45] Surprisingly, the dipyrrole compound **6** was purifiable using silica gel chromatography; although higher yields were achieved when argon, rather than compressed air, was used as the pressure source. While lack of β substituents on the pyrrole units increased the probability of rotation about the xanthene-porphyrin axis, its synthesis could be rapidly completed.

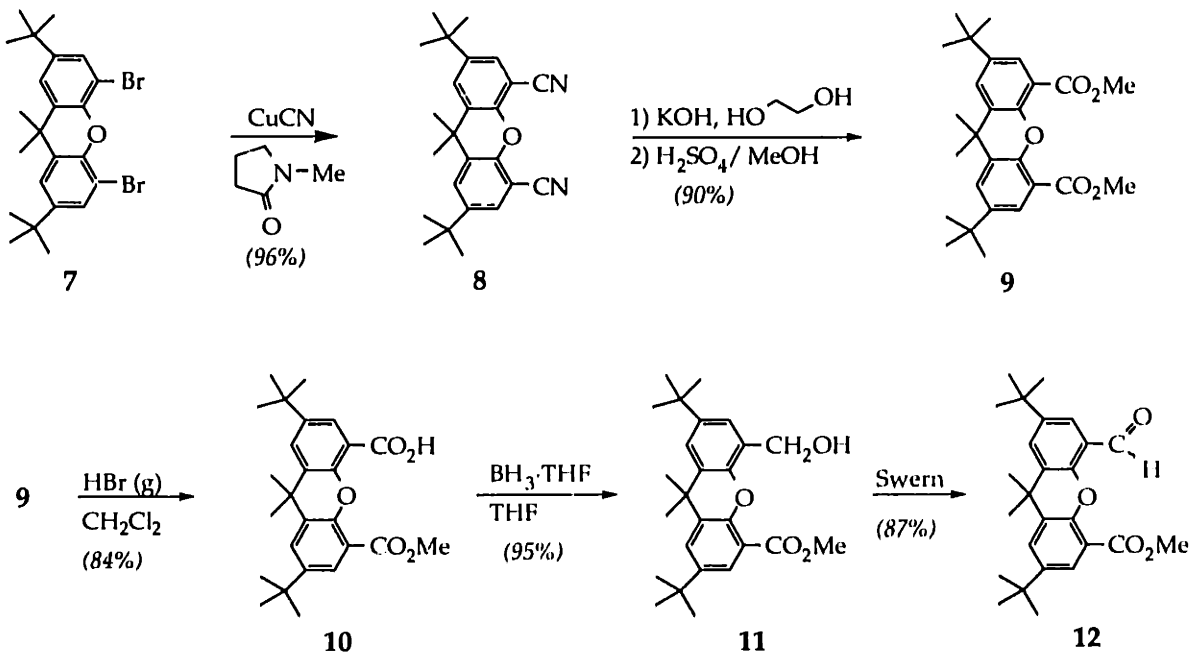
Scheme 2: Synthesis of the unsubstituted dipyrrole



With the pyrrole-containing units in hand, it was necessary to synthesize the xanthene aldehyde. An initial target was the 4-aldehyde-5-methyl ester (**12, Scheme 3**). It was anticipated that, after the porphyrin cyclization, this methyl ester group could be converted into a number of functional groups capable of recognition. *Tert*-butyl groups would substitute the 2 and 7 positions to increase solubility and hinder porphyrin stacking.[22]

The dibromo xanthene **7** (three steps from commercially available xanthone) was subjected to a Rosenmund-von Braun cyanide exchange reaction[46] to give the dinitrile **8** in nearly quantitative yield. Hydrolysis and esterification afforded the diester **9**. Gaseous HBr in dichloromethane (DCM) at 0°C was used to selectively cleave the diester to the acid/ester **10**. This reaction apparantly involves protonation of the xanthene etheral oxygen/adjacent (4 or 5 position-) carbonyl group, activating the methyl ester group for cleavage by bromide. The syntheses of compounds **7-9** were based on known procedures.[2,8] Direct conversion of the acid functionality in **10** (using either DIBAL-H[47] or borane-dimethyl sulfide and pyridinium chlorochromate[48]) or via the acid chloride derivative of **10** (using $LiHAl(O\textit{t}\text{-}Bu)_3$),[49] to the desired aldehyde gave low yields (<50%). Ultimately a two-step conversion of the acid to the aldehyde was implemented. The carboxylic acid group was selectively reduced to the hydroxymethyl compound **11** using borane-THF,[50] and subsequently oxidized to the aldehyde **12** using Swern conditions.[51]

Scheme 3: Synthesis of a xanthene aldehyde



Despite the number of overall steps (nine from commercially available xanthone), the yields of the individual steps were high and most did not require extensive purification.

A4. Synthesis and Identification of Porphyrin Atropisomers

Condensation of the xanthene aldehyde **12** with dipyrrole **6** was attempted using a number of conditions. The crude reaction mixtures were analyzed by UV spectroscopy. Since porphyrins have an intense and unique chromophore, this technique proved quite useful for analyzing and optimizing reaction conditions. The results demonstrated that reactions which used quinones (*p*-chloranil or DDQ) for oxidation gave no porphyrin-based compounds, producing instead unidentifiable black solids. The most successful conditions were based on the method of Adler and Longo. The aldehyde **12** and dipyrrole **6** were dissolved in propionic acid (40-50°C) and the mixture was warmed to 90°C. The flask was opened to the air to effect the necessary oxidation. Reaction yields were low in an absolute sense (4-15%), but typical for porphyrin condensations using this procedure.

TLC analysis (hexanes:ethyl acetate) indicated that only one porphyrin compound was formed. This result indicated that either the xanthene was rotating rapidly about the porphyrin axis, or that the two isomers had identical R_f values. To check the rotation theory a TLC was developed in the same solvents at -10°C, but again only one spot was observed. Proton NMR, which showed two distinct compounds, was not helpful as the rotational barrier was likely high enough that both atropisomers would be observed. A second TLC solvent system (toluene:ethyl acetate) was used which gave very different results than the previous solvents and clearly showed two porphyrin compounds with significantly different R_f's. Silica gel

chromatography in this solvent system allowed isolation of the two porphyrin atropisomers.

The rotational barriers of a number of *ortho*-substituted aryl porphyrins have been studied.[52-54] Halogens and -OH substituents produced a barrier of 22-23 kcal/mol, barriers too low to allow isomer isolation at room temperature, while the methoxy group (24 kcal/mol) did allow for isolation ($t_{1/2}$= 34 h at 67°C).[52] After isolation of the two porphyrins it was determined that the compounds did not interconvert until strongly heated (refluxing toluene, 30 minutes). This allowed for a lower limit of 27 kcal/mol to be established for the barrier to rotation. Analysis using molecular modeling (see experimental section) indicated that the barrier to interconversion was at least 32 kcal/mol (stable for months at room temperature). The calculated value was close to the experimental value as analysis of porphyrins stored at 0°C for two years showed less than 5% interconversion.

After establishing the thermal stability of the porphyrin atropisomers, the next task was to characterize and identify each isomer- the 'C-shaped' *syn* and the 'S-shaped' *anti*. Isomer confirmation can be a tedious and ambiguous process which has often relied on x-ray crystallography or intramolecular reactions.[8] There was some literature precedent that the *syn* isomer would have a lower R_f on silica gel.[55,56] Fortunately, the phenyl groups on the 10 and 20 positions provided a way to distinguish between the two (see **Figure 6**) definitively. Rotation about the phenyl-porphyrin bond was hindered enough (about 18 kcal/mol in similar systems)[54] that the NMR detected two different sets of protons. In the *syn* isomer the two *ortho* -phenyl protons were in different environments (H_1 and H_2 in **Figure 6**); while the same protons in the *anti* isomer were equivalent due to a rotational axis of symmetry. The spectrum of the more polar isomer was found to be consistent with the *syn* atropisomer. The *ortho* protons were found to be at

different chemical shifts and broad at room temperature. A variable temperature NMR experiment (see experimental section) showed coalescence of the two signals at 115°C, consistent with the 18 kcal/mol estimate. On the other hand, the NMR spectrum of the less polar compound was temperature-independent and the *ortho* protons gave a single, well defined resonance.

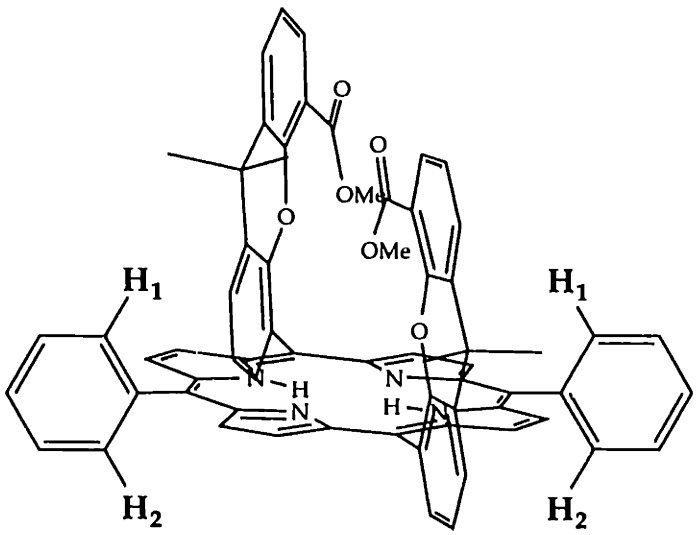


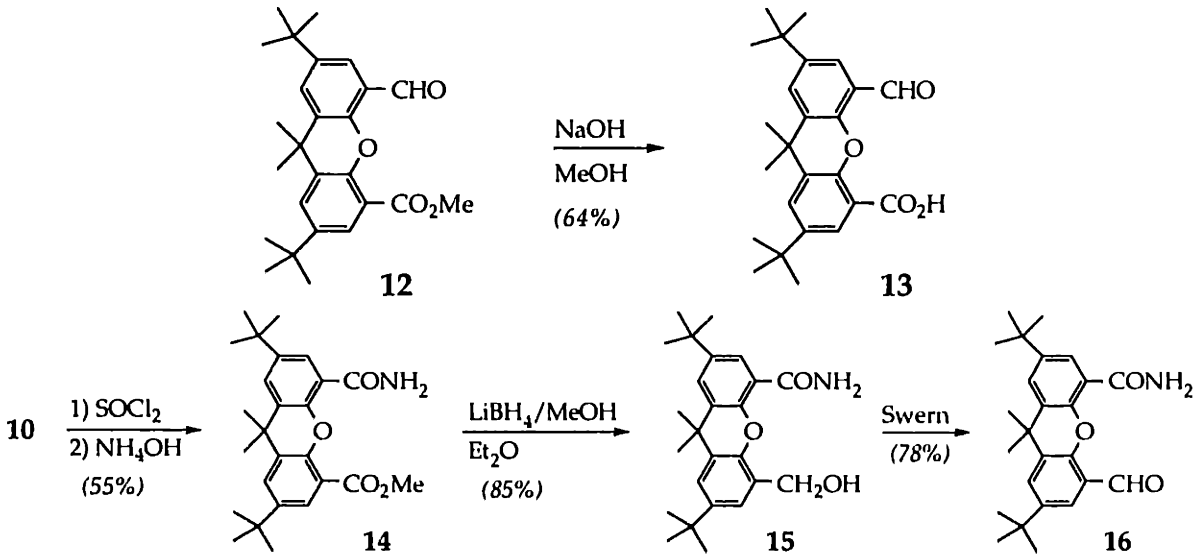
Figure 6: Use of *ortho*-phenyl protons for atropisomer assignment

It should be noted that under the optimized reaction conditions (warming to 90°C in propionic acid, open to air, two hours) only two atropisomeric porphyrins were isolated. However, when the dipyrrole **6** and a xanthene aldehyde were added to refluxing propionic acid (141°C), a number of porphyrin compounds (not characterized) were detected by TLC. Refluxing acetic acid (118°C) also gave a similar distribution. Fortunately, this acid-redistribution side reaction[33] was avoided using the procedure described above.

Chemical modification of the xanthene methyl ester porphyrins proved troublesome. It was anticipated that the methyl ester groups could be cleaved to the corresponding acid. However, two complications became apparent. The first of these was solubility. Despite the presence of the four *tert*-butyl groups on the xanthenes, the porphyrins proved insoluble in all solvents that were neither halogenated nor aromatic. This precluded the

standard saponification ester cleavages. The second was steric crowding within the cleft, which further reduced the scope of reactions that could be applied. Similar synthetic complications due to steric crowding within cleft systems have been reported.[3] A number of cleavage conditions[57] were attempted including LiI/pyridine, TMS-I, hydroxides in alcohols, and HBr/DCM; however, only sodium thiomethoxide in DMSO (80°C, four days) proved successful. Unfortunately, the reaction necessitated purification and the elevated temperature resulted in xanthene rotation. This motivated exploration of a better route to xanthene derivatives capable of recognition.

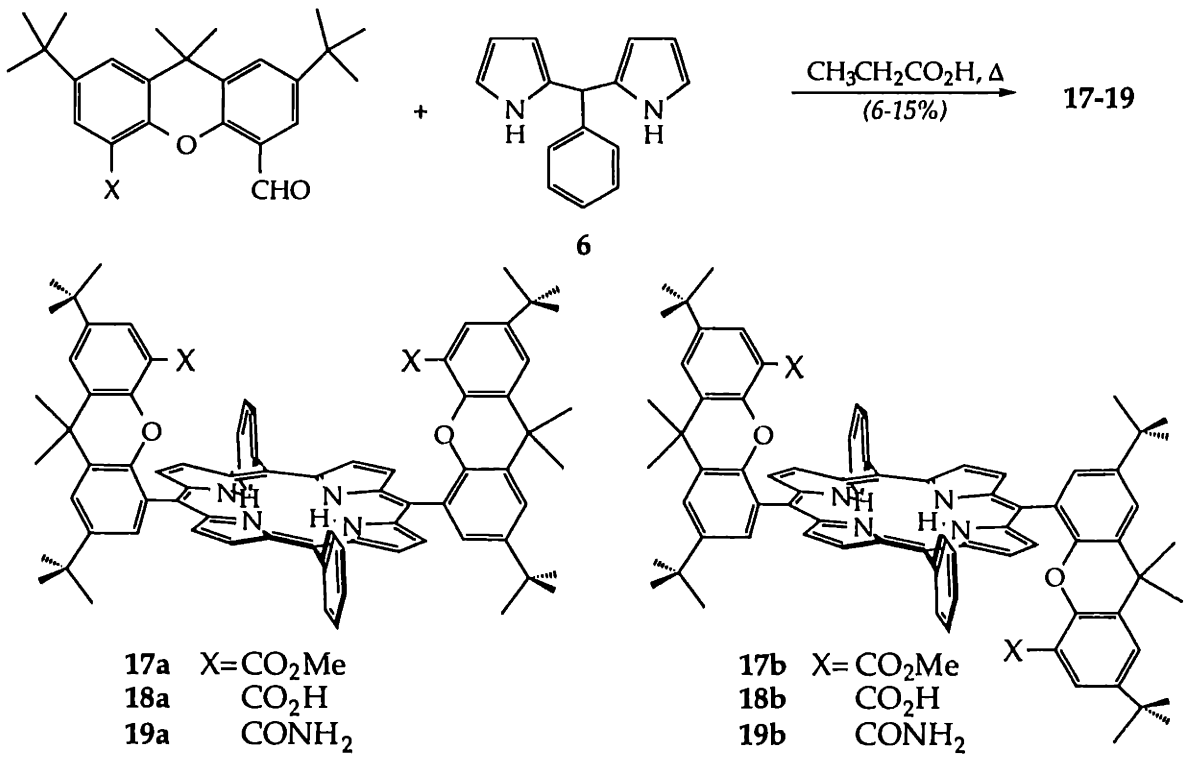
Scheme 4



As the synthetic problems originated after porphyrin cyclization, it was decided to prepare the xanthene aldehyde/acid **13** and xanthene aldehyde/amide **16** prior to porphyrin formation. Saponification of the xanthene aldehyde/ester **12** gave the acid/aldehyde **13** (the isolated yield was diminished by a Cannizzaro-type side reaction). The amide/aldehyde was synthesized in three steps from the ester/acid **10**. First, the acid chloride of **10** was reacted with ammonium hydroxide to give the amide/ester **14**. Second, the ester was then selectively reduced to the hydroxymethyl derivative **15** using $LiBH_4$ and stoichiometric methanol.[58] Finally a Swern oxidation[51] gave

the amide/aldehyde **16**. Both the acid and amide xanthenes condensed with dipyrrole **6** to give porphyrins **18** and **19** in yields comparable to that of the methyl ester. Separation of the atropisomers was also performed using silica gel and toluene:ethyl acetate solvents as the eluents.

Scheme 5: Porphyrin Syntheses



With the porphyrins lacking β substituents in hand, attention was placed on synthesizing their β-substituted counterparts. When the substituted dipyrrole **5** was condensed with the xanthene aldehyde **12** using the Adler-Longo conditions, no product was observed. Methods[30,31] utilizing quinones for oxidation gave results analogous to the previous system (intractable black solids). The inability to form these compounds was attributed to steric crowding on the porphrin periphery. During the equilibrium-driven cyclization process the tetramer must not have been as stable as the linear oligomers, thus no porphyrin compounds were formed. Studies of (completely) peripherally functionalized porphyrins[59] have

recently been performed and further explain the synthetic difficulties. Crystallographic analysis showed these structures to be nonplanar, thus decreasing orbital overlap and aromatic stabilization. This causes the porphyrins to be more susceptible towards oxidation and orders of magnitude more basic than unsubstituted tetraaryl porphyrins.[59,60] In addition, synthetic yields for these compounds were lower. In retrospect, with the yields for the β-unsubstituted porphyrins often in single digits, it was not surprising that no peripherally crowded porphyrins were isolated. This was unfortunate as the increased nitrogen basicity combined with the convergent recognition groups could have produced interesting results.

A5. Guest Complexation and Computational Analysis

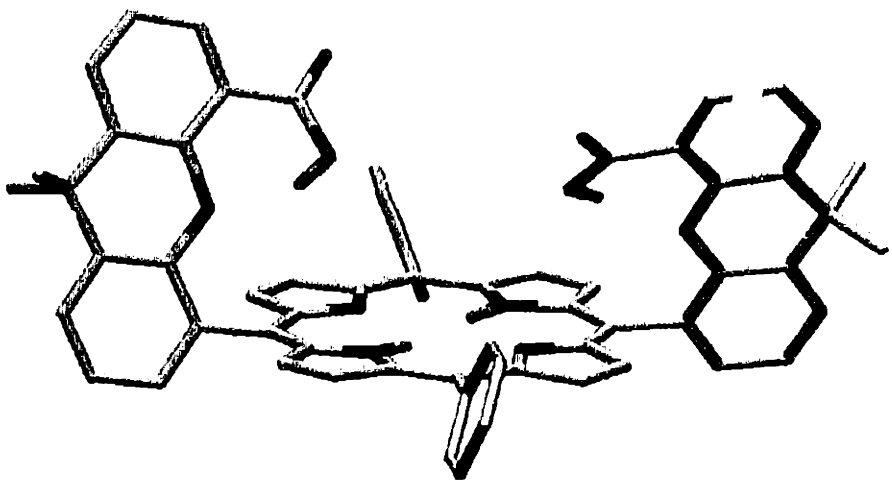


Figure 7: A polytube rendering of an MM2* minimized diacid porphyrin cleft. The carboxyl proton is likely hydrogen bonded to the xanthene oxygen.[2] Oxygen is represented in red, nitrogen in blue, and carbon in gray. The *t*-butyl groups were omitted.

Porphyrins **18a** and **19a** were first analyzed by molecular modeling modeling (see experimental) to ascertain which guests would be appropriate. Computer modeling (see **Figure 7**) calculated a O-O carbonyl distance of 5 Å, assuming that the porphyrin was planar and the xanthene-porphyrin angle was 60-90°. This short heavy atom distance limited the range of guests to relatively small molecules. Bifunctional guest molecules were chosen to take advantage of the convergent functionalities. DABCO (diazabicylcooctane),

pyrazine, imidazole, diethylmalonic acid, and 9-ethyladenine were chosen for analysis. Other guests were envisioned (ureas, oxalic acid, and various malonates), but they were not soluble in the same solvents as the porphyins. Insoluble guests were subjected to liquid-solid extractions, however no extraction was observed. NMR titrations in chloroform were used to assay binding (see experimental). The large ring current of the porphyrin was an effective 'litmus test' for binding, as complexed guests experienced relatively large upfield shifts in the NMR spectrum. This upfield shift roughly correlated to binding affinity. The *anti* atropisomers of these clefts were also analyzed to test for the effectiveness of convergency.

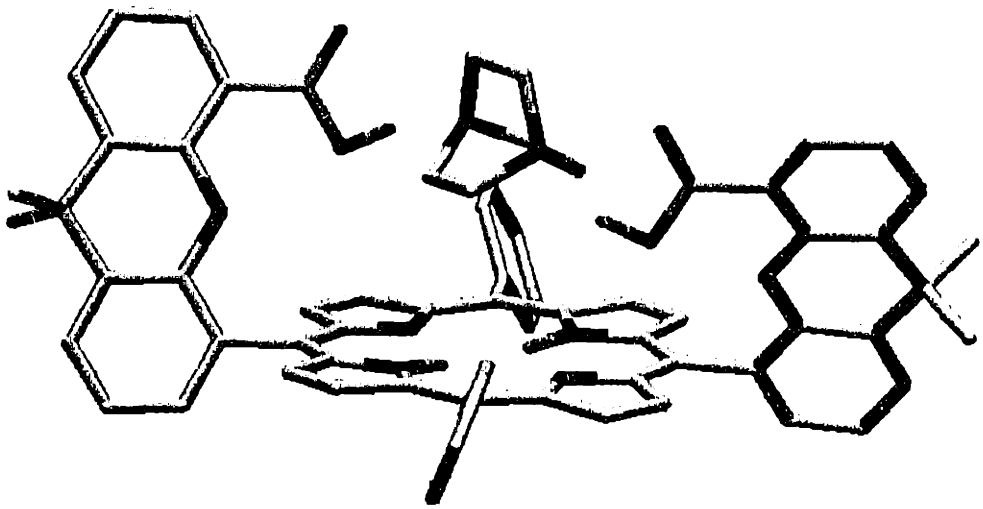


Figure 8: An MM2* minimized complex of **18a** with DABCO (the *t*-butyl groups on xanthene are omitted).

The binding data was then fit to either a 1:1 binding equation (for *syn* isomers), or a 2:1 equation (for *anti* isomers).[61] For all the binding plots the experimental data points are given along with the calculated binding curve as a graphical means to describe the quality of the fit. The y-axis is represented either by the frequency of the host proton, or by the $\Delta\delta$ value, which is the difference between the observed shift and the shift of the uncomplexed (free) host proton (δ_{obs}- δ_f). Since the $\Delta\delta$ value is derived from the shift of the host proton, both scales are equivalent. This nonlinear curve fit method is

applicable to situations where a curve is actually generated; very weak or very strong binding causes this approach to break down.[61]

The diacid **18a** was found to complex DABCO and imidazole. Binding to the weak bases pyrazine, phenazine, and the larger 9-ethyladenine was barely detectable and too weak to allow determination of association constants. The binding plots for the *syn* diacid with DABCO and with imidazole are given in **Figure 7**. The binding was very strong to DABCO. The NMR spectrum was in slow exchange; the guest was detected either as being bound or free, with no free guest detected until more than one equivalent of DABCO was added. This resulted in a lack of curvature in the binding plot and a binding constant could not be accurately determined by NMR. A lower limit of 10^5 was estimated.[61] Molecular modeling of the diacid-DABCO complex (see **Figure 8**) showed good size complementarity between the cleft and the guest, as well as two short hydrogen bonds (-OH...N distance of 2.4 Å and 3.0 Å respectively). Imidazole complexation proved easier to study using NMR, as its binding constant was much lower (1.5×10^3).

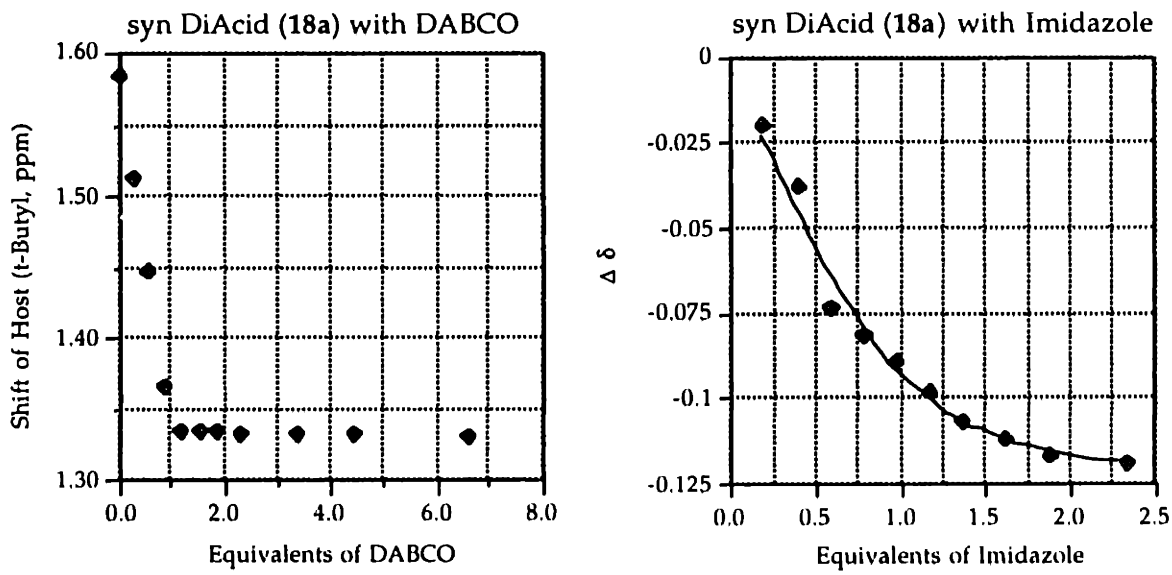


Figure 9: Binding plots for the *syn* diacid host **18a** with DABCO and imidazole. A lack of curvature in the DABCO plot precluded determination of an accurate binding constant. The imidazole data points were plotted along with the calculated binding curve.

The *anti* diacid atropisomer showed weaker binding to both DABCO and imidazole. DABCO was now in intermediate exchange on the NMR timescale and enough curvature was observed in the binding plot to allow for estimation of association constants (K_{a1}= 12,000 M^{-1}, K_{a2}= 3,900 M^{-1}). Imidazole was found to bind only weakly (K_{a1}= 43 M^{-1}, K_{a2}= 37 M^{-1}). The binding plots are given in Figure **10**.

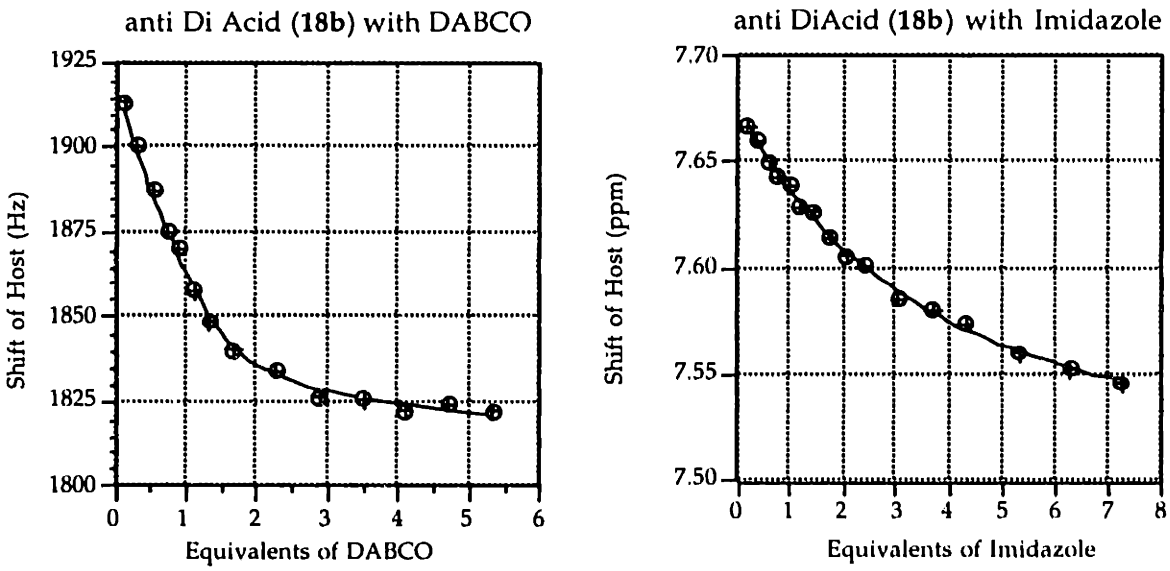


Figure 10: NMR titration curves for binding of the *anti* diacid **18b**. Raw data points plotted along with the calculated binding curve.

The diamide porphyrin cleft (**19a**) was also used for binding studies. Its amide protons were easily followed, shifting downfield over the course of guest addition. Of the guests that were analyzed (same as for the diacid), only diethyl malonic acid was found to bind to a significant extent (**Figure 11**, K_a= 890 M^{-1}). Although DABCO did interact with the cleft (the amide host protons were slightly shifted), it was not substantial enough to calculate a binding constant. No binding to diethyl malonic acid could be detected for the *anti* diamide porphyrin (**19b**).

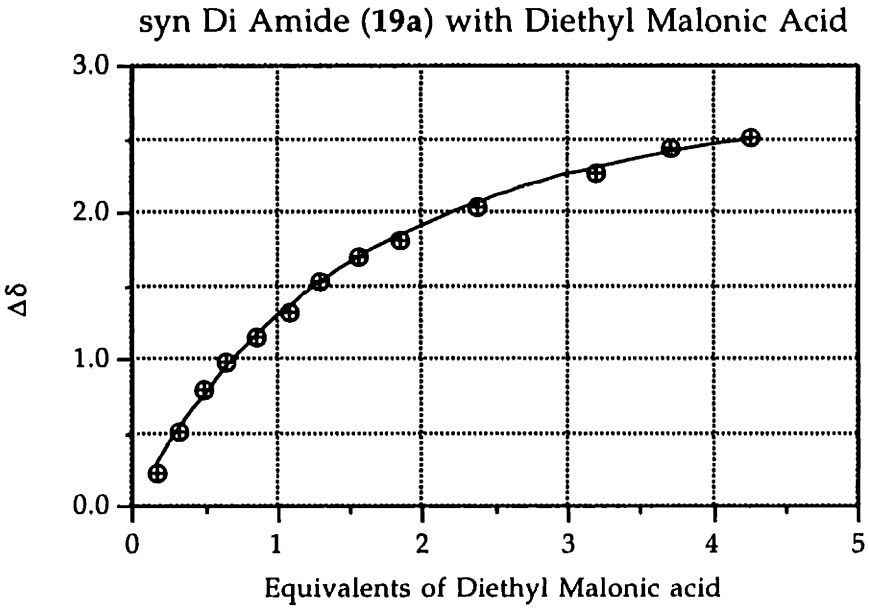


Figure 11

A6. Conclusion

The synthesis and complexation studies revealed a number of facts about these systems. First, they could be assembled in a modular fashion. Yields were low, thus sufficient quantity of the xanthene aldehydes was required to obtain reasonable (50+ mg) amounts of porphyrins. Second, space within the C-shaped porphyrin isomers was too small to permit even simple reactions (such as ester cleavage) from proceeding with facility. These size considerations also limited the scope of guests. Third, from **Figure 12** it can be seen that a number of small molecules were bound, covering a range of binding constants (37 to $>10^5$ M^{-1}). The convergent *syn* clefts had larger binding constants than their divergent *anti* counterparts. It was anticipated that information gained in this investigation would be applicable to further[10] studies of molecular recognition.

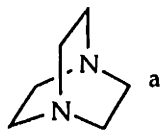
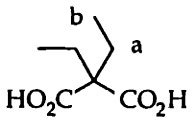
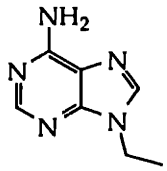
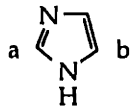
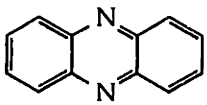
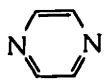
Structure	Common Name	K_a's for syn diacid	K_a's for anti diacid	K_a's for syn diamide
	DABCO	$>10^5$ (a= 3.2)	$K_{a1}= 1.2 \times 10^5$ $K_{a2}= 3.9 \times 10^4$ (a= 3.0)	Very Weak
	Diethylmalonic Acid	Not Observed	Not Observed	$K_a= 890$ (a= 0.4, b= 1.0)
	9-Ethyladenine	Not Observed	Not Observed	Not Observed
	Imidazole	1.5×10^3 (a= 4.4, b= 2.6)	$K_{a1}= 43$ $K_{a2}= 37$ (a= 0.87, b= 0.55)	Not Observed
	Phenazine	Very Weak	Not Observed	Not Observed
	Pyrazine	Very Weak	Not Observed	Not Observed

Figure 12: Summary of Titration Results. Association constants (K_a's) given in M^{-1}. Maximum upfield shifts ($\Delta\delta$) of protons (a, b) given in ppm.

[NOTE: The Experimental Section and References can be found after **Volume II: Chapter B**]

B. Beyond Binding Experiments: Further Studies of Xanthene-Porphyrin Systems

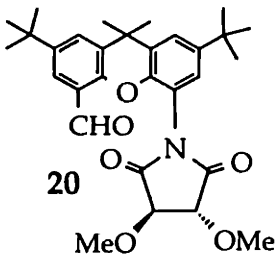
B1. Introduction: Xanthene Metalloporphyrins

Metallation of the porphyrins would allow for a reactive functional group to be added. When combined with directed recognition groups a microenviroment capable of binding to, and reacting with, substrates would be established. However most reactions[24] of metal-bound porphyrins (epoxidations, hydroxylations, sulfoxidations) would be difficult to limit to the 'alpha' face of the *syn* porphyrins (which contains the recognition groups), rather than the less obstructed 'beta' face. The small volume available for binding in the C-shaped isomer would also severely restrict the scope of substrates. However the S-shaped isomer does not have the size constraints nor the facial ambiguity that plagues the C-shaped isomer, and the xanthene functionality could still be used for binding. It could also be transformed into a chiral group, capable of 'directing' substrates into a particular orientation for reaction with a metal-bound oxidant. For these reasons, the synthesis of S-shaped metalloporphyrin systems containing chiral recognition groups was attempted.

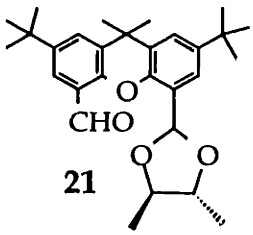
B2. Design and Syntheses of Chiral Porphyrins

Careful consideration of what type of chiral auxiliary to use was necessary. While steric issues were less severe for the S-shaped systems than for the C-shaped ones, small chiral recognition groups were still needed to allow access to the metal center. In addition, the asymmetric group could not contain functional groups that would react with the catalytic species. C_2-symmetric chiral groups were first considered as many were known in various shapes and containing a variety of functional groups. An added

advantage to C_2-symmetric groups was that the chiral field would be better defined and results would be easier to predict and/or rationalize. Three xanthene aldehydes (**20**, **21**, and **22**) containing asymmetric groups were identified as synthetic targets.



20



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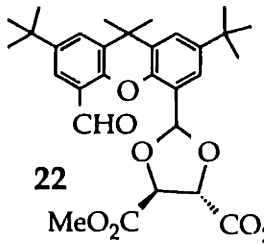
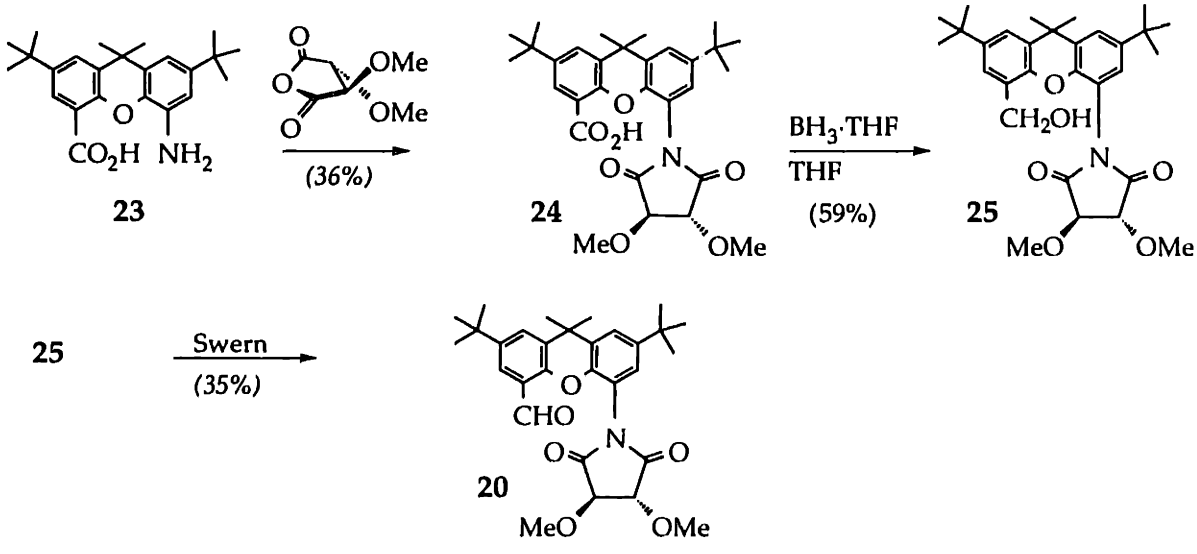


22

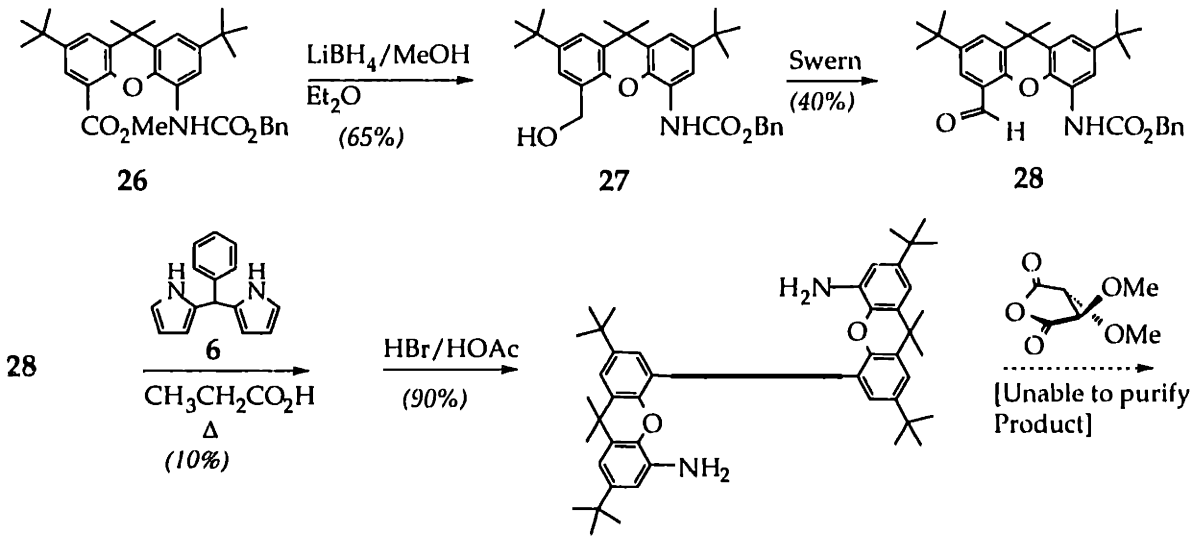
The imide/aldehyde **20** was synthesized in three steps from the xanthene amine/acid **23** (**Scheme 7**). Compound **23** was condensed with the anhydride of di-OMe tartaric acid[62] to form the imide/acid **24**. This was reduced to the hydroxymethyl/imide **25**, then oxidized to the aldehyde **20**. However, the presence of the relatively large and sensitive imide functional group led to reduced yields for every transformation (relative to the previously mentioned aldehyde/ester synthesis). This led to only small quantities of the aldehyde **20**.

Scheme 7



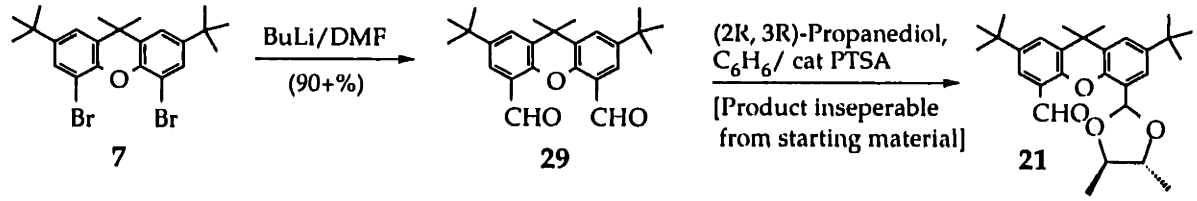
An alternative synthesis (see **Scheme 8**) using the urethane/aldehyde **28** with installation of the imide after porphyrin cyclicization failed due to difficulties with purification of the final product. It should be noted that the diamine porphyrin in **Scheme 8** was synthesized in low yield and was observed to atrop-isomerize to an appreciable extent at 55°C over one hour. Both routes (**Schemes 7 and 8**) were unsatisfactory for producing porphyrins in acceptable quantity.

Scheme 8



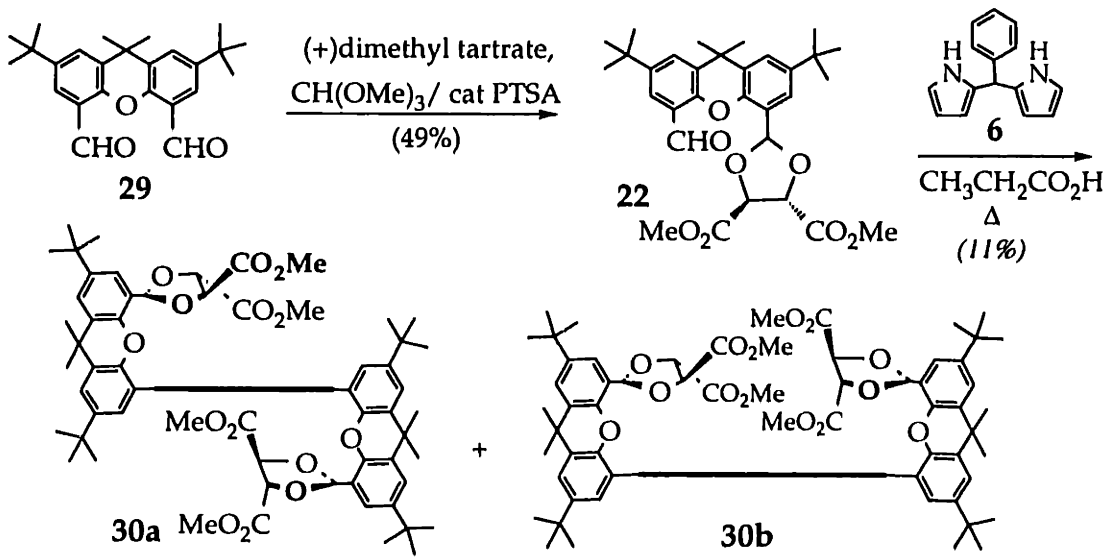
A route which was more amenable to scale-up is presented in **Scheme 9**. The dibromo xanthene **7** and the dialdehyde **29** were produced in multi-gram amounts. The dialdehyde **29** could, in principle, be reacted with a suitable chiral diol to give a chiral ketal, such as **21**. Unfortunately reaction of the dialdehyde **29** with (2R, 3R)-propandiol resulted in an inseparable mixture of starting material, product, and diketal. Since reaction of this impure monoaldehyde to form porphyrins would have undoubtedly given an intractable mixture it was decided that a different (more polar acetal) would be preferable.

Scheme 9



Inexpensive (+)-dimethyl tartrate was chosen this time as the diol (**Scheme 10**). Optically active tartrate diols not only produce C_2 symmetric ketals, but they are well established[63] as chiral auxiliaries. In this case activation of the diols required the use of trimethyl orthoformate. The product Rf differed significantly from the starting material (and diacetal), thus facilitating its isolation using a silica gel column. Condensation of the monoacetal **22** with dipyrrolphenylmethane **6**[44] yielded the two porphyrin atropisomers. Although yields were again low (*ca* 10%), the reaction could be scaled-up. Sterically this chiral auxiliary was the least preferred of the three initial targets. However, it proved suitable for exploratory work on oxidations. Curiously, despite the large size of this ketal the *syn* isomer was also formed, and in almost the same quantity as the *anti* isomer.

Scheme 10: Synthesis of a xanthene-porphyrin system containing a C_2-chiral auxiliary.



B3. X-ray Crystallographic Analysis of the Chiral Porphyrin 30a

The porphyrin **30a** crystallized well enough to allow its structure to be solved (**Figure 13**). This was an ultimate confirmation of our assignment of the *syn* and *anti* isomers. It also revealed that, at least in the solid state, the xanthene moieties are not perpendicular to the porphyrin (actually 68° and 75°); this agreed well with molecular modeling (67° and 65°). More surprisingly was that the xanthene units were not completely planar, which molecular modeling (and intuition), suggested. In fact, the xanthene was distorted 26° from planarity. Previous x-ray analysis of xanthene clefts showed a smaller deviation from planarity (10-12°).[64] The porphyrin surface was planar and undistorted. This agreed well with the NMR data (the -NH pyrrole protons were observed far upfield at -2.6 ppm) as well as the UV-vis profile (sharing similar peak intensities and absorbances to undistorted porphyrins).

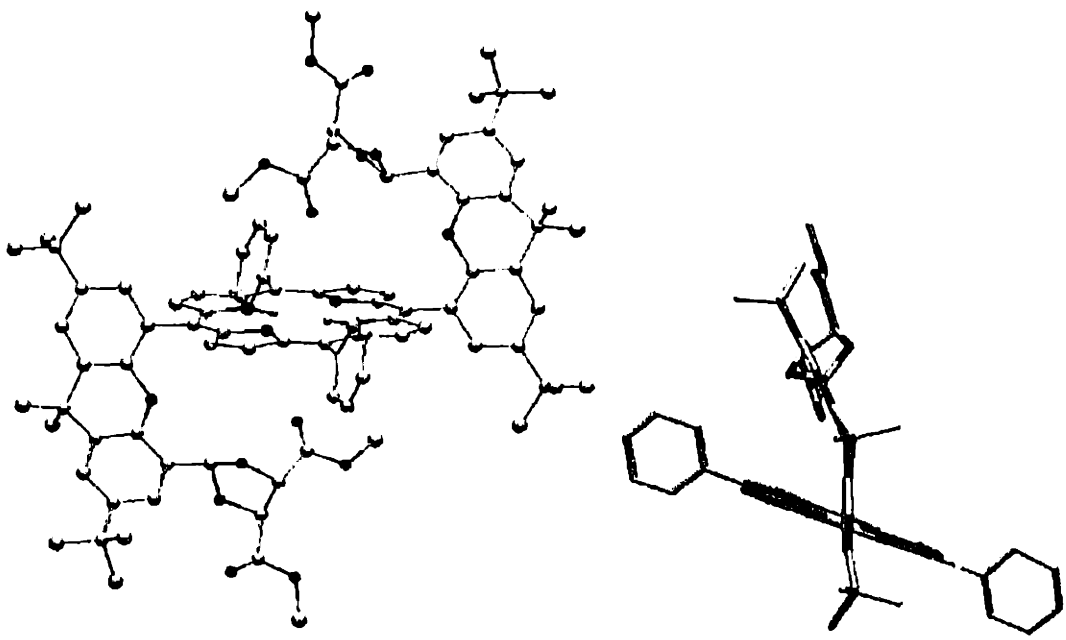


Figure 13: Left: ball-and-stick representation of the x-ray crystal structure of **30a**. Right: a polytube depiction of the deviation from planarity of the xanthene (other xanthene omitted for clarity).

B4. Attempted Metallation of Xanthene Porphyrins

Insertion of a metal ion into a porphyrin is a well-known process. In fact, most metals (and even a few nonmetals) can be inserted into the tetrapyrrole macrocycle. In our systems zinc(II) inserted readily at room temperature. This made us optimistic about inserting redox-active metals suitable for epoxidations such as Fe(III) and Mn(III) into our porphyrins. Porphyrin **30a** was dissolved in DMF and refluxed with $FeCl_2$ according to well-established methods.[65] The reaction color changed from red to brown over the reaction period. Inexplicably TLC showed four distinct compounds of close R_f, and NMR of the paramagnetic mixture was unrevealing. The UV-Vis spectrum of the mixture was very broad and lacked the defined bands present in either the starting material or typical iron porphyrins. In the event that the reaction was incomplete the reaction time was doubled. This resulted in the formation of even more compounds (six defined spots using TLC). Thus a combination of atropisomerization and incomplete metallation was unlikely. In addition, neither the starting material nor the product could be detected by mass spectrometry. Formation of a μ-oxo like species (two iron porphyrins linked by an oxygen bridge) was unlikely as the site for metal insertion was shielded by the large xanthene and phenyl groups. In addition, the UV-Vis spectrum did not match those of other μ-oxo diporphyrins.[66] It was also hypothesized that the ketal functionality was degrading under the conditions. However, degradation of the ketal group as the sole side-reaction was unlikely given that the porphyrin chromophore was also changed. When the xanthene dimethyl ester porphyrin **17a** was subjected to the same conditions, it too produced an unidentifiable distribution of products. These results, combined with the failure of the quinone-based synthetic routes to yield anything but black intractable solids, suggested a more fundamental instability in the xanthene-porphyrin system.

Recall that one design aspect of the porphyin systems was protection against oxidation. Saturation of the *meso* positions with aryl groups was intended to provide this resistance, as oxidation of *meso*-unsubstituted porphyrins was well established.[28] Since both the xanthene ester and xanthene ketal porphyrins proved unstable to the above iron-insertion conditions, it was considered that this particular insertion method was problematic. However when another method was used ($Fe(CO)_5$ and iodine at 100°C in toluene)[32] similar results were obtained. Insertion of manganese (III) was also attempted, as manganese-porphyrin epoxidation systems were also known.[24] However insertion of Mn(III) ($MnCl_3$/refluxing THF/*p*-nitrophenol)[67] also produced disappointing results. A closer review of the literature revealed a possible explanation, which was buried in a footnote:

> "Tetraphenylporphyrins whose *meso*-phenyl moieties are substituted in the *ortho* positions by alkoxy substituents are quite susceptible to destructive oxidation."[68]

Since the oxidant in the above case (*p*-cyano-*N*,*N*-dimethylaniline *N*-oxide) was considered a relative weak oxidant, it was regrettably decided that these systems were too unstable for oxidations.

Two additional steps could have been taken to proceed with these studies. First, halogenated porphyrin analogues could have been prepared. Perhalogenated porphyrins have proven quite resistant to oxidation.[69] However halogenation of the pyrrole units and/or the phenyl groups would have necessitated a re-working of the entire porphyrin synthesis, and the isolated yields of these asymmetric porphyrins was already low. The second step would have been to change the xanthene group for a more robust moiety, such as anthracene or biphenylene. This again would have required extensive synthesis and these modules would likely have been less soluble than the xanthene-based units.

B5. Conclusion

Despite synthetic challenges, porphyrin-based molecular clefts capable of binding small molecules were synthesized and studied. Complexation of a number of guests, computer modeling, NMR, UV-Vis spectroscopy, and x-ray crystallography were all used to characterize these systems. The results support a preorganized, defined microenvironment for supramolecular processes. Applications to oxidative catalysis were precluded by an unforeseen instability in the xanthene-porphyrin systems.

B6. Experimental Section for Chapters A and B

B6-1. General

All commercially available reagents were purchased from Aldrich Chemical Company except as noted below. THF was distilled from Na/Benzophenone ketyl, and DCM was distilled from P_2O_5. Triethylamine was distilled from, and stored over, KOH. Anhydrous grade DMSO was used in all Swern oxidations. Melting points were determined using an Electrothermal 9100 melting point apparatus and were uncorrected. Except where noted NMR spectra were recorded on a Bruker AC-250. All chemical shift values were referenced to residual solvent. Deuterated solvents were obtained from Cambridge Isotope Laboratories. UV-Vis spectra were acquired using a Perkin-Elmer Lamda 12 spectrophotometer. Either a Finnegan Mat 8200 (for HRMS/EI) or a VG ZAB-VSE (for HRMS/FAB) mass spectrometer was used to ascertain exact masses. Silica gel chromatography was performed with Silica Gel 60 (EM Science or Bodman, 230-400 mesh). TLC analysis was achieved using glass-bound Silica Gel 60 (F254) plates.

B6-2. Molecular Modeling

Macromodel (*v*.3.5X or 5.5) was used for molecular dynamics and energy minimizations. The MM2* force field was used in most cases, without any solvent parameters. All torsion angles of the porphyrin macrocycle were fixed for energy minimizations to preclude ruffling of the macrocycle (since all experimental evidence supported porphyrin planarity). For estimates of rotational barriers a short program was written which rotated the bond under investigation by increments of 0.5°, the structure was then re-minimized.

The rotational barrier was calculated as the energy difference between the lowest and highest energy structures. In the case of the phenyl-porphyrin bond this was calculated to be 17 kcal/mol, for the xanthene methyl ester-porphyrin bond it was 32 kcal/mol. This xanthene-porphyrin rotational barrier decreased to 21 kcal/mol (AMBER force field) if the torsion angles were not fixed to prevent distortion of the porphyrin and was an artifact of the non-recognition of its aromatic stabilization.

B6-3. Experimental Determination of the Phenyl-Porphyrin Rotational Barrier

The Zn derivative of the *syn* diacid **18a** was dissolved in toluene-d_8. A variable-temperature NMR experiment was performed using a Varian VXR-500 (500 MHz) spectrometer. Data points were recorded at 25, 40, 50, 60, 85, 90, and 100°C. The two *ortho*-phenyl protons were observed to broaden and nearly coalesce at 100°C. By plotting the difference in chemical shift versus temperature a coalescence temperature of 115°C was determined. From the coalescence temperature (in K) and chemical shift difference ($\Delta\nu$, in Hz) the free energy of activation ($\Delta G_c^{\ddagger}$, in cal/mol) can be calculated using **equation 1**.[70] This equation was derived from the Bloch equation, the solution of which relates the mean life time (τ) to the chemical shift difference (**equations 2** and **3**).[70] Using a T_c of 388 K and a $\Delta\nu$ of 159 Hz, **equation 1** gives a $\Delta G_c^{\ddagger}$= 18.4 kcal/mol.

$$\Delta G_c^{\ddagger} = 4.57 T_c \left(9.97 + \log_{10} \frac{T_c}{\Delta \nu} \right) \text{ cal mol}^{-1} \qquad \textbf{Equation 1}$$

$$\tau = \frac{\sqrt{2}}{2\pi\Delta\upsilon} \qquad \tau = \frac{1}{2}k \qquad \textbf{Equations 2, 3}$$

B6-4. Titrations

All titration data was recorded on a Bruker AC-250 (250 MHz) spectrometer. Dueterated chloroform (dried over 4 Å molecular sieves) was used exclusively as the solvent. Analytical grade volumetric flasks and syringes were used to prepare all solutions. A 4-10 mM porphyrin host solution was prepared, and aliquots of a guest stock solution (0.030-0.050 mM) were added sequentially until saturation was reached. After each addition the sample was shaken, allowed to equilibrate for 10 minutes, and a spectrum recorded. For any given titration a number of protons on the host (and guest) were observed to shift. For binding of DABCO to **18a** the *t*-butyl protons shifted significantly and these protons were used in the calculations. Other

protons shifted to a much smaller extent, resulting in data that was unreliable. In most cases it was the xanthene protons *ortho* to the recognition group that shifted the most and provided the most reliable data. For each data point the total host $[H]_T$ and guest $[G]_T$ concentrations were determined. The points were then fit to an appropriate binding equation (either the 1:1 or 2:1 binding isotherm)[61] using Systat (*v.* 5.2 for the Macintosh) to give association constants. The calculated binding curve was then plotted along with the experimental data points as a graphical display of the fit accuracy.

B6-5. X-ray Crystal Structure

Anti tartrate porphyrin **30a** was crystallized from a 7:1 MeOH:DCM solution over a period of 48 h. A crystal of about 0.5x0.3x0.1 mm was mounted on a fiber embedded in a matrix of Paraton N. Data was collected at 23°C on a Rigaku AFC5R diffractometer equipped with a graphite monochromated Cu-Kα radiation (1.54178 Å) and a 12 kW rotating generator. A total of 6,124 reflections were collected ($2\Theta_{max}$= 120.1°), of which 5,809 were unique (R_{int}= 0.080); equivalent reflections were merged.

The space group was determined to be triclinic P1 (#1). The unit cell dimensions were a= 12.348(7) Å, b= 16.93(1) Å, c= 9.925(5) Å, α= 102.19(4)°, β= 99.09(4)°, γ= 77.11(5)°, V= 1963(4) $Å^3$ with Z= 1. The structure was solved using the direct methods of program Sir92 of the teXan (v. 1.7-1) crystallographic package of Molecular Structure Corporation. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 4,246 reflections (I> 2.80σ (I)) and 908 variables gave a final R= 0.083 ($1/\sigma^2$) and R_w=0.113.

B6-6. Synthesis and Characterization

2-Nitro-3-acetoxy-pentane (**2**). Propionaldehyde (57.0 mL, 0.79 mol) and NaOH (3.3 mL, 10 N) were added to a solution of nitroethane (60.0 mL, 0.83 mol) dissolved in MeOH (150 mL). The solution was warmed to 40°C for 72 h before it was concentrated, quenched with HCl (10 mL of 2.5 N), extracted into ethyl acetate (2x 50 mL), and washed with brine. Concentration of the reaction afforded an oil, which was distilled (bp 68°C at 0.05 torr) to give 2-nitro-3-pentanol **1** (75.0 g, 71%) as a mixture of diastereomers.
The nitroalcohols **1** (51.1 g, 0.38 mol) were then mixed with acetic anhydride (37.0 mL, 0.381 mol) and one drop of concentrated H_2SO_4 was added. The reaction was heated (60°C, 1 h) and directly distilled (bp 85°C at 0.05 torr) to

afford the diastereomeric nitroacetoxy compounds **2** (48.6 g, 74%). ^{1}H NMR of one diasteromer; isolated by column chromatography (250 MHz, $CDCl_3$) δ 5.27 (td, *J*= 7.7, 4.2 Hz, 1 H), 4.75 (quintet, *J*= 6.9 Hz, 1 H), 2.06 (s, 3 H), 1.78 (septet of d, *J*= 7.3, 3.0 Hz, 1 H), 1.67 (q, *J*= 6.1 Hz, 1 H), 1.53 (d, *J*= 6.7 Hz, 3 H), 0.95 (t, *J*= 7.5 Hz, 3 H).

Ethyl 3-ethyl-4-methyl-2-pyrrole carboxylate (**3**). A solution of dry THF:isopropanol (3:1 (w/v), 50 mL) was used to dissolve ethyl isocyanoacetate (7.70 mL, 70 mmol) and DBU (22.0 mL, 147 mmol). 2-Nitro-3-acetoxy-pentane **2** (both diastereomers, 12.3 g, 70.2 mmol) was dissolved in THF (15 mL) and syringed into the DBU solution dropwise at 0°C. After 15 min the ice bath was removed and the reaction was allowed to warm to rt overnight. The solution appeared orange in color and a solid appeared (presumably $DBUH^+$). After 12 h a TLC of the reaction showed no starting material and a new UV active spot (R_f= 0.5 in 5:1 hexanes:ethyl acetate) was observed. Concentration of the solution gave an oil. Aqueous HCl (70 mL, 0.5 N HCl) was added to the oil with hexanes:EtOAc (3:1, 150 mL), the aqueous phase was extracted (2x 50 mL 3:1 hexanes:EtOAc), rinsed (1x brine), and dried ($MgSO_4$). Purification was performed on silica gel (10:1 hexanes:EtOAc, preloaded using DCM). Pure pyrrole (4.83 g, 38%) was isolated as a white solid, more (2 g) remained as impure. mp 69-70°C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (br s, 1 H), 6.65 (d, *J*= 2.5 Hz, 1 H), 4.31 (q, *J*=7.1 Hz, 2 H), 2.75 (q, *J*=7.5 Hz, 2 H), 2.04 (s, 3 H), 1.35 (t, *J*=7.1 Hz, 3 H), 1.12 (t, *J*=7.5 Hz, 3 H). HRMS (FAB in NBA/NaI) calcd for $C_{10}H_{15}NO_2$ $[M]^{+\cdot}$ 181.1103, found 181.1107.

Diethyl 3,3'-diethyl-4,4'-dimethyl-dipyrryl-5,5'-phenylmethane-2,2'-carboxylate (**4**). Argon was bubbled through a solution of absolute ethanol (60 mL) and concentrated HCl (2 mL) for 20 min before benzaldehyde (0.65 mL, 6.3 mmol) and ethyl-3-ethyl-4-methylpyrrole carboxylate **3** (2.30 g, 12.7 mmol) were added. The reaction was refluxed under argon for 3 h and turned a bright pink color. Upon cooling a precipitate formed which was filtered and washed with cold methanol. TLC (3:1 hexanes:EtOAc) showed the white solid to be two products of close R_f. Silica gel chromatography (8:1 hexanes:EtOAc) followed by recrystallization from ethanol afforded pure product (1.37 g, 50%). mp 158°C. R_f= 0.4 (4:1 hexanes: EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.19 (br s, 2 H), 7.37-7.28 (m, 3 H), 7.09 (d, *J*= 7.1 Hz, 2 H), 5.48 (s, 1 H), 4.25 (q, *J*= 7.0 Hz, 4 H), 2.73 (q, *J*= 7.5 Hz, 4 H), 1.79 (s, 6 H), 1.31 (t, *J*= 7.0 Hz, 6 H), 1.11 (t, *J*= 7.4 Hz, 6 H). HRMS (FAB in NBA/CsI) calcd for $C_{27}H_{34}N_2O_4Cs$ $[M + Cs]^+$ 583.1573, found 583.1584.

4,4'-Diethyl-3,3'-dimethyl-dipyrrylphenylmethane (**5**). Dipyrrylphenylmethane dicarboxylate **4** (1.00 g, 2.22 mmol) was suspended in ethylene glycol (20 mL). Argon was bubbled through the mixture for 15 min, KOH (0.55 g) was added and the reaction was warmed to reflux under argon. The solid dissolved at 140°C to form a golden yellow solution and further heating to 190°C for 30 min caused the solution to darken slightly. The solution was cooled to rt over 1 h during which time a red oil was suspended above the solvent while some crystals were observed in solution. The solution was stirred causing the oil to coalesce into an insoluble dark red ball. The solution was cooled to 0°C and filtered to afford the crude product (155 mg, 23%). The compound was ~90% pure by NMR, and further purification using silica gel was precluded by decomposition. R_f= 0.3 (20:1 hexanes:EtOAc, slight streaking). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.4-7.2 (m, 3 H), 7.13 (d, *J*= 6.6 Hz, 2 H), 6.37 (d, *J*= 2.1 Hz, 2 H), 5.49 (s, 1 H), 2.42 (q, *J*= 7.5 Hz, 4 H), 1.79 (s, 6 H), 1.17 (t, *J*= 7.5 Hz, 6 H). HRMS (FAB in NBA/NaI) calcd for $C_{21}H_{26}N_2$ $[M]^{+\cdot}$ 306.2100, found 306.2106.

2,2'-Dipyrrylphenylmethane (**6**). Pyrrole (10.0 g, 149 mmol) was mixed with benzaldehyde (2.50 g, 23.6 mmol) in toluene (100 mL); argon was bubbled through the solution for 20 min and the flask was protected from light. *p*-Toluenesulfonic acid monohydrate (400 mg, cat) was added and the reaction was warmed to reflux. After 90 min no benzaldehyde was detected by TLC; the reaction was cooled to rt and the solvent and excess pyrrole were evaporated to yield a black viscous oil. This mixture was preloaded onto Florisil and purified on a silica gel column (10:1 hexanes:ethyl acetate). Concentration of the pure fractions afforded a tan solid (2.70 g, 51%). This sensitive compound was stored cold and dry. R_f= 0.45 (5:1 hexanes:ethyl acetate). mp 90-92°C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.92 (br s, 2 H), 7.5-7.05 (m, 5 H), 6.7 (dd, *J*= 4.1, 2.7 Hz, 2 H), 6.16 (q, *J*= 2.9, 2 H), 5.92 (m, 2 H), 5.48 (s, 1 H). HRMS (FAB in NBA/NaI) calcd for $C_{15}H_{14}N_2$ $[M]^{+\cdot}$ 222.1157, found 222.1153.

Methyl 4-hydroxymethyl-2,7-bis(1,1-dimethylethyl)-5-xanthene carboxylate (**11**). Xanthene methyl ester/acid **10** (5.00 g, 11.8 mmol) was dissolved in THF (20 mL) and the solution was cooled to 0°C. BH_3·THF (13.5 mL of a 1.0 M THF solution) was added dropwise with hydrogen evolution. The reaction was monitored by TLC and allowed to run for 12 h before being quenched with acetic acid:water (1:1, 7 mL; bubbling noted). Ether (50 mL) was added and the water layer extracted (2x 20 mL ether). The organic layer was washed with sat $NaHCO_3$, brine, and dried over $MgSO_4$. The crude solid was purified using a silica gel column (5:1 hexanes:EtOAc) to give a white solid (4.58 g, 95%). mp 137°C. R_f= 0.4 (3:1 hexanes:ethyl acetate). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.83 (d,

J= 2.4 Hz, 1 H), 7.63 (d, *J*= 2.4 Hz, 1 H), 7.34 (d, *J*= 2.3 Hz, 1 H), 7.16 (d, *J*= 2.2 Hz, 1 H), 4.96 (t, *J*= 7.5 Hz, 1 H), 4.78 (d, *J*= 7.5 Hz, 2 H), 3.97 (s, 3 H), 1.64 (s, 6 H), 1.35 (s, 9 H), 1.32 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{26}H_{34}O_4Cs$ $[M + Cs]^+$ 543.1511, found 543.1525.

Methyl 4-formyl-2,7-bis(1,1-dimethylethyl)-5-xanthene carboxylate (**12**). Oxalyl chloride (1.00 mL, 11.5 mmol) was added to DCM (25 mL) and the flask was cooled (-70°C, dry ice-isopropanol). To this solution was added dry DMSO (1.5 mL, 21 mmol) in DCM (5 mL), dropwise over 5 min. After stirring for 2 min a solution of xanthene alcohol **11** (4.10 g, 9.98 mmol) in DCM (25 mL) was added over five min and stirred for 15 min. TEA (6.8 mL, 48.7 mmol) was added and the reaction was warmed to rt over 1 h. The reaction was then quenched with water (25 mL). The layers were separated and extracted (2x 25 mL DCM), rinsed with brine, and evaporated to a crude yellow solid. Silica gel chromatography (12:1 toluene:ethyl acetate) produced a pure white solid (3.53 g, 87%). R_f= 0.5 (5:1 toluene:ethyl acetate). mp 149°C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.79 (s, 1 H), 7.81 (m, 2 H), 7.67 (d, *J*= 2.3 Hz, 1 H), 7.62 (d, *J*= 2.3 Hz, 1 H), 3.97 (s, 3 H), 1.67 (s, 6 H), 1.35 (s, 9 H), 1.34 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{26}H_{32}O_4Cs$ $[M + Cs]^+$ 541.1355, found 541.1368.

4-Formyl-2,7-bis(1,1-dimethylethyl)-5-xanthene carboxylic acid (**13**). A solution of NaOH (2.4 g in 30 mL methanol) was heated to 60°C and the xanthene aldehyde/ester **12** (1.00 g, 2.45 mmol) was added. TLC verified that the reaction was complete after 30 min. HCl (1 N, 3 mL) was added and the solvent was evaporated. Water (15 mL) dissolved the remaining salts and the solution was acidified to pH 1. The aq. layer was extracted (3x 30 mL $CHCl_3$) and dried over Na_2SO_4. The product was purified using silica gel (15:1 DCM:MeOH) and the first compound which eluted was identified as the product (0.620 g, 64%). R_f= 0.35 (10:1 DCM:MeOH). ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.09 (s, 1 H), 8.24 (d, *J*= 2.7 Hz, 1 H), 7.76 (d, *J*= 2.4 Hz, 1 H), 7.70 (d, *J*=2.5 Hz, 1 H), 7.66 (d, *J*= 2.4 Hz, 1 H), 1.70 (s, 6 H), 1.40 (s, 9 H), 1.36 (s, 9 H). HRMS (FAB in NBA/NaI) calcd for $C_{25}H_{31}O_3$ $[M + H]^+$ 379.2273, found 379.2261.

4-Hydroxymethyl-2,7-bis(1,1-dimethylethyl)-5-aminocarbonyl xanthene (**15**). DCM (75 mL) and $SOCl_2$ (9 mL, excess) were used to dissolve the xanthene methyl ester/acid **10** (2.00 g, 4.75 mmol). The solution was refluxed for 1.5 h. Solvent and excess $SOCl_2$ were removed on a rotary evaporator. The white solid was taken up in DCM (100 mL) and concentrated NH_4OH was added dropwise, with gas evolution. The mixture was poured into a separatory

funnel along with more DCM (100 mL) and water (100 mL). The layers were separated, the organic layer was washed with 1N HCl (75 mL) and dried over Na_2SO_4. Anaylsis by TLC and ^1H NMR showed 2 compounds: the less polar dimethyl ester xanthene (0.324 g, 16% of recovered yield), and the product amide/methyl ester **14** (1.12 g, 55%).
The amide/methyl ester **14** (1.08 g, 2.36 mmol) was dissolved in ether (80 mL). $LiBH_4$ (2 M in THF, 1.9 mL, 3.81 mmol) was added followed by MeOH (0.11 mL, 2.7 mmol) and bubbling was noted. After stirring at rt for 1 h the reaction was shown complete by TLC. Work-up and purification were similar to **11**, giving the product as a white powder (0.890 g, 85%). ^1H NMR (250 MHz, $CDCl_3$) δ 8.04 (br s, 1 H), 8.00 (d, *J*= 2.4Hz, 1 H), 7.57 (d, *J*= 2.4 Hz, 1 H), 7.42 (d, *J*= 2.3 Hz, 1 H), 7.19 (d, *J*=2.3 Hz, 1 H), 5.93 (br s, 1 H), 4.80 (d, *J*=6.2 Hz, 2 H), 2.24 (t, *J*= 6.2 Hz, 1 H), 1.66 (s, 6 H), 1.35 (s, 9 H), 1.34 (s, 9 H). HRMS (FAB in NBA/NaI) calcd for $C_{25}H_{33}NO_3Na$ $[M + Na]^+$ 418.2358, found 418.2375.

4-Formyl-2,7-bis(1,1-dimethylethyl)-5-aminocarbonyl xanthene (**16**). Oxalyl chloride (2 M in DCM, 1.1 mL, 2.2 mmol) was added to dry DCM (70 mL) and the solution was cooled to -70°C before DMSO (0.30 mL, 4.2 mmol) in DCM (5 mL) was added dropwise. After 15 min the xanthene alcohol **15** (0.70 g, 1.77 mmol) in 25 mL of DCM was added dropwise. TEA (1.1 mL, 7.9 mmol) was added after 20 min and the system was warmed to rt over 45 min. Water (50 mL) was added, the layers were separated, and the water layer was extracted (3x 25 mL $CHCl_3$). Purification on a silica gel column (5:1 hexanes:EtOAc) produced a white powder (0.544 g, 78%). R_f= 0.6 (10:1 hexanes:ethyl acetate). ^1H NMR (250 MHz, $CDCl_3$) δ 10.03 (s, 1 H), 9.35 (br s, 1 H), 8.29 (d, *J*= 2.4 Hz, 1 H), 7.75 (d, *J*= 2.4 Hz, 1 H), 7.66 (d, *J*= 2.4 Hz, 1 H), 7.59 (d, *J*= 2.4 Hz, 1 H), 6.05 (br s, 1 H), 1.68 (s, 6 H), 1.39 (s, 9 H), 1.36 (s, 9 H). HRMS (FAB in NBA/NaI) calcd for $C_{25}H_{32}NO_3$ $[M + H]^+$ 394.2382, found 394.2392.

Syn- and anti-5,15-Bis(methyl-2,7-bis(1,1-dimethylethyl)-5-xanthene carboxylate)-10,20-diphenylporphyrin (**17a,b**). 2,2'-Dipyrrophenylmethane **6** (300 mg, 1.35 mmol) and the xanthene aldehyde/methyl ester **12** (550 mg, 1.35 mmol) were dissolved in propionic acid (30 mL, 40 °C) and heated to 90°C to give a dark green solution. After 2 h no starting material remained (by TLC) and the reaction was poured into a separatory funnel. DCM (300 mL) was added and the organic layer was washed (2x 150 mL water, 1x 100 mL saturated $NaHCO_3$, and brine), then dried over $MgSO_4$. Following concentration, a silica gel column (2% ethyl acetate in toluene) was used to purify the mixture. Several collected fractions were deep red while the majority were green. The red fractions, still impure, were crystallized from DCM:MeOH (1:7), then rinsed with MeOH to afford deep violet crystals (90 mg, 11%). By TLC (toluene eluent) two fluorescent compounds were detected, the first at R_f=

0.60 and the second at R_f= 0.27. The two atropisomers were separated and purified using a silica gel column (toluene eluent) to give the *syn* (**17a**, 40 mg, 5% isolated yield) and the *anti* (**17b**, 43 mg, 5%). For **17a** (R_f= 0.27, neat toluene) ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.08 (br s, 8 H), 8.3-8.15 (m, 4 H), 7.96 (d, *J*= 2.3, 2 H), 7.87 (d, *J*= 2.3Hz, 2 H). 7.8-7.65 (m, 6 H), 7.63 (d, *J*= 2.5, 2 H), 7.31 (d, *J*= 2.3, 2 H), 1.91 (s, 12 H), 1.48 (s, 18 H), 1.24 (s, 18 H), -0.12 (s, 6 H), -2.6 (br s, 2 H). HRMS (EI) calcd for $C_{82}H_{82}N_4O_6$ $[M]^{+\cdot}$ 1218.6234, found 1218.6241. UV-Vis (nm): 416, 515, 549, 590, 646.
For **17b** (R_f= 0.60, neat toluene) ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.82 (br s, 8 H), 8.3-8.2 (m, 4 H), 8.08 (d, *J*= 2.2 Hz, 2 H), 7.87 (d, *J*= 2.2 Hz, 2 H), 7.8-7.7 (m, 6 H), 7.62 (d, *J*= 2.3 Hz, 2 H), 7.29 (d, *J*= 2.3 Hz, 2 H), 1.91 (s, 12 H), 1.52 (s, 18 H), 1.24 (s, 18 H), -0.41 (s, 6 H), -2.6 (br s, 2 H). HRMS (FAB in NBA/CsI) calcd for $C_{82}H_{83}N_4O_6$ $[M + H]^+$ 1219.6313, found 1219.6364.

Syn-5,15-Bis(2,7-bis(1,1-dimethylethyl)-5-xanthene acid)-10,20-diphenylporphyrin (**18a**). To DMSO (10 mL) was added **17a** (30 mg, 0.025 mmol) and sodium thiomethoxide (100 mg, 1.4 mmol). The suspension was heated to 80°C under Ar for two days, after which more thiomethoxide (50 mg. 0.7 mmol) was added to complete the reaction. HCl (25 mL of 0.1N) was added to the cooled solution along with DCM (100 mL) and the layers were separated. The green organic layer was then washed (2x 25 mL water, and 25 mL of pH 7 buffer- which restored a deep red color). The product was purified using silica gel chromatography (20:1 to 5:1 toulene:EtOAc) to give the titled compound (20 mg, 66%).
This compound was also synthesized in 7% yield by condensing **16** with **6** under conditions identical to **17**. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.83 (s, 8 H), 8.4 (br s, 2 H), 8.31 (d, 2 H, *J*= 2.1 Hz), 8.1 (br s, 2 H), 7.88 (d, 2 H, *J*= 2.2 Hz), 7.73 (br s, 6 H), 7.60 (d, 2 H, *J*= 2.1 Hz), 7.35 (d, 2 H, *J*= 2.3 Hz), 1.86 (s, 12 H), 1.59 (s, 18 H), 1.22 (s, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{80}H_{78}N_4O_6Cs$ $[M + Cs]^+$ 1323.4976, found 1323.4925. UV-Vis (nm): 418, 517, 552, 591, 647.
18a Was transformed into its Zn derivative by sonicating it with a methanol solution of $Zn(OAc)_2$ in chloroform. LRMS (EI) calcd for Zn derivative, $C_{80}H_{76}N_4O_6Zn$: 1252.5056 and 1254.5025 (for ^{64}Zn and ^{66}Zn respectively), found LRMS 1252.6, 1254.7.

Anti-5,15-Bis(2,7-bis(1,1-dimethylethyl)-5-xanthene acid)-10,20-diphenylporphyrin (**18b**). Isolated in 6% yield from the condensation of **16** with **6** under conditions similar to **17**. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.79 (m, 8 H), 8.26 (m, 4 H), 8.06 (d, *J*= 2.2 Hz, 2 H), 7.92 (d, *J*= 2.2 Hz, 2 H), 7.74-7.7 (m, 6 H), 7.73 (d, *J*= 2 Hz, 2 H), 7.67 (d, *J*= 2.4 Hz, 2 H), 1.96 (s, 12 H), 1.53 (s, 18 H), 1.25

(s, 18 H), -2.6 (br s, 2 H). HRMS (FAB in NBA/CsI) calcd for $C_{80}H_{78}N_4O_6Cs$ [M + Cs]$^+$ 1323.4976, found 1323.5031.

Syn- 5,15-bis-(2,7-bis-(1,1-dimethylethyl)-9,9-dimethyl-5-xanthene carbamyl)-10,20-diphenylporphyrin (**19a,b**). To a warm (45°C) solution of propionic acid was added xanthene aldehyde **16** (0.410 g, 1.08 mmol) and **6** (0.248 g, 1.12 mmol). The system was heated to 80°C for 45 min, then to 95°C for 45 min. The solution was allowed to cool and was poured into a separatory funnel with DCM (200 mL). The dark green mixture was washed (2x 150 mL water, 1x 50 mL of 1 N NaOH, and 1x 100 mL sat. $NaHCO_3$). The organic layer was then concentrated to a dark residue. This was dissolved in toluene and chromatographed using 5% EtOAc in hexanes. The still impure compounds were crystallized from DCM/MeOH (1:10) to yield 25 mg of the less polar isomer and 12 mg of the more polar isomer (combined yield of 6%). For the more polar isomer **19a**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.85 (s, 8 H), 8.26 (br s, 2 H), 8.21 (br s, 2 H), 7.97 (d, 2 H, *J*= 1.8 Hz), 7.92 (d, 2 H, *J*= 7.92 Hz), 7.75 (br s, 6 H), 7.72 (d, 2 H, *J*=2.4 Hz), 7.65 (d, 2 H, *J*= 2.4 Hz), 4.82 (CONH, br s, 2 H), 2.22 (CONH, br s, 2 H), 1.96 (s, 12 H), 1.51 (s, 18 H), 1.26 (s, 18 H), -2.74 (br s, 2 H). HRMS (EI) calculated mass for $C_{80}H_{81}N_6O_4$ [M + H]$^+$ 1189.6319, found 1189.6322. UV-Vis (nm): 418, 515, 549, 589, 645.
For the less polar atropisomer (**19b**): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.86 (s, 8 H), 8.22 (m, 4 H), 8.17 (d, *J*= 2 Hz, 2 H), 7.92 (d, *J*= 2 Hz, 2 H), 7.75-7.6 (m, 10 H), 4.39 (CONH, br s, 2 H), 1.99 (CONH, br s, 2 H), 1.94 (s, 12 H), 1.54 (s, 18 H), 1.22 (s, 18 H), -2.7 (br s, 2 H). HRMS (FAB in NBA/CsI) $C_{80}H_{81}N_4O_6$ [M + H]$^+$ 1189.6320, found 1189.6361.

Xanthene 4-formyl-5-(dimethoxy) tartrate acetal imide (**20**). Standard Swern conditions (oxalyl chloride, DMSO) yielded the product in 35% yield, which was purified on silica gel (5:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.26 (s, 1 H), 7.70 (d, *J*= 2.4 Hz, 1 H), 7.66 (d, *J*= 2.3 Hz, 1 H), 7.51 (d, *J*= 2.2 Hz, 1 H), 7.08 (d, *J*= 2.3 Hz, 1 H), 4.66 (d, *J*= 5.3 Hz, 1 H), 4.39 (d, *J*= 5.2 Hz, 1 H), 3.80 (s, 6 H), 1.65 (s, 6 H), 1.33 (s, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{30}H_{37}NO_6Cs$ [M + Cs]$^+$ 640.1675, found 640.1662.

Xanthene-4-formyl-5-(2R, 3R)-propane diketal (**21**). Xanthene dialdehyde **29** (1.5 g, 3.95 mmol), (2R, 3R)-propane diol (0.40 mL, 4.2 mmol) and *p*-toluenesulfonic acid monohydrate (76 mg, 0.40 mmol) was dissolved in benzene (30 mL). The mixture was heated to reflux under an addition funnel containing 4 Å molecular sieves. After 6 h the reaction appeared to have reached equilibrium (by NMR). DCM was added, and the organic layer rinsed (2x 25 mL sat $NaHCO_3$), and dried (Na_2SO_4). Only one spot (R_f= 0.45) was

observed by TLC (20:1 hexanes:EtOAc). A silica gel column (25:1 hexanes:EtOAc) produced fractions only slightly enriched in the product (estimated yield of 20% with a purity of 80%). HRMS (FAB in NBA/CsI) calcd for $C_{29}H_{38}O_4Cs$ $[M + Cs]^+$ 583.1824, found 583.1851.

4-Formyl-5-dimethyl L-tartrate acetal-2,7-bis(1,1-dimethylethyl)-xanthene (**22**). DCM (45 mL) was used to dissolve trimethyl orthoformate (1.3 mL, 11.9 mmol), *p*-toluenesulfonic acid monohydrate (80 mg, 0.4 mmol), and (+)-dimethyl tartrate (1.97 g, 11.1 mmol) and the solution was warmed to reflux for 45 min under a $CaCl_2$ drying tube. The xanthene dialdehyde **29** (3.00 g, 7.96 mmol) was added and after 24 h at reflux TLC indicated the presence of starting material, product, and diacetal. The solution was poured onto sat. $NaHCO_3$ and stirred vigorously for 15 min. This mixture was extracted (2x 20 mL DCM), then the DCM extracts were rinsed with brine, and dried over $MgSO_4$. The organic layer was concentrated and purified using column chromatography (7:1 hexanes:EtOAc) to afford a white flaky solid (1.66 g, 49%); starting material (0.33 g) was also recovered. ^1H NMR ($CDCl_3$, 250 MHz) δ 10.75 (s, 1 H), 7.82 (d, *J*= 2.3 Hz, 1 H), 7.79 (d, *J*= 2.3 Hz, 1 H), 7.68 (d, *J*= 2.7 Hz, 1 H), 7.49 (d, *J*= 2.3 Hz, 1 H), 6.68 (s, 1 H), 5.06 (d, *J*=3.4 Hz, 1 H), 4.96 (d, *J*= 3.4 Hz, 1 H), 3.97 (s, 3 H), 3.83 (s, 3 H), 1.66 (s, 6 H), 1.36 (s, 9 H), 1.34 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{31}H_{38}O_8Cs$ $[M + Cs]^+$ 671.1621, found 671.1652.

4-Amino-2,7-bis(1,1-dimethylethyl)-xanthene-5-carboxylic acid (**23**). Prepared according to literature procedures.[8] HRMS (FAB in NBA/NaI) calcd for $C_{24}H_{31}NO_3$ $[M]^{+\cdot}$ 381.2304, found 381.2314.

Xanthene 4-carboxylic acid-5-(dimethoxy) tartrate acetal imide (**24**). The dimethoxy tartrate anhydride[62] (1.05 g, 6.6 mmol) was suspended in DCM (60 mL) and pyridine (4.2 mL, 52 mmol). Xanthene amine/acid **23** (2.38 g, 6.24 mmol) was added and the mixture was refluxed for 4 h under a drying tube. After cooling, the mixture was concentrated to a brown solid. This was suspended in acetic anhydride (12 mL); a few drops of TFA were added and the reaction was heated to 50°C for 48 h under Ar. Evaporation gave a brown foamy solid. Column chromatography on silica gel (3:1 hexanes:EtOAc) yielded three compounds, the last of which to elute was the product (830 mg, 16 mmol, 36%). A substantial amount of product remained impure (total weight: 1.41 g). ^1H NMR ($CDCl_3$, 250 MHz) δ 7.88 (d, *J*= 2.4 Hz, 1 H), 7.60 (d, *J*= 2.3 Hz, 1 H), 7.48 (d, *J*= 2.2 Hz, 1 H), 7.05 (d, *J*= 2.0 Hz, 1 H), 4.61 (d, *J*= 5.6 Hz, 1 H), 4.37 (d, *J*= 5.8 HZ, 1 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 1.67 (s, 3 H), 1.62 (s, 3 H) 1.31 (s, 9 H), 1.30 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{30}H_{37}NO_7Cs$ $[M + Cs]^+$ 656.1624, found 656.1640.

Xanthene 4-hydroxymethyl-5-(di-OMe) tartrate acetal imide (**25**). THF (7 mL) was used to dissolve xanthene acid/tartrate **24** (0.40 g, 0.76 mmol) and the flask was placed in a water bath at 15°C. A BH_3·THF solution (1 M, 1.2 mL) was added dropwise. After bubbling subsided the flask was placed under argon. Analysis by TLC revealed that the reaction was incomplete, even after 48 h, so more BH_3·THF (0.5 mL) was added. After another 24 h quenching was performed at 0°C by addition of ice, then acetic acid:water (1:1, 10 mL). The mixture was purified by silica gel chrmoatography using hexanes:ethyl acetate (4:1-3:1) to yield a white foam (230 mg, 59%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.50 (d, *J*= 2.2 Hz, 1 H), 7.36 (d, *J*= 2.3 Hz, 1 H), 7.23 (d, *J*= 2.3 Hz, 1 H), 7.06 (d, *J*= 2.2 HZ, 1 H), 4.8-4.6 (m, 2 H), 4.55 (d, *J*= 5.4 Hz, 1 H), 4.41 (d, *J*= 5 Hz, 1 H), 3.81 (s, 6 H), 2.15 (br s, 1 H, O<u>H</u>), 1.66 (s, 3 H), 1.65 (s, 3 H), 1.33 (s, 18 H), 1.32 (s, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{30}H_{39}NO_6Cs$ $[M + Cs]^+$ 642.1832, found 642.1843.

4-Hydroxymethyl-2,7-bis(1,1-dimethylethyl)-5-aminocarbobenzoxy xanthene (**27**). To ether (10 mL) was added xanthene carbamate/methyl ester **26** (1.00 g, 1.9 mmol) and $LiBH_4$ in THF (2 M, 1.3 mL, 2.6 mmol). Some bubbling was noted and the solution became homogenous. The flask was cooled in an ice bath as methanol (100 μL, 2.5 mmol) was added dropwise (vigorous gas evolution). The reaction was kept under Ar and was complete after 6 h. The reaction was quenched with HCl (15 mL, 0.1 N), which caused gas evolution. The two phases were stirred for 5 min, NaOH (4 mL, 2 N) was added, and the phases again stirred. The mixture was extracted with DCM (2x 50 mL), and the organic layers were dried over Na_2SO_4. Concentration gave an oil which was purified on a silica gel column (12:1-5:1 hexanes:EtOAc) to give the product as a solid (0.614 g, 65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.97 (br s, 1H), 7.5-7.2 (m, 8 H), 7.12 (d, *J*= 2.2 Hz, 1 H), 5.26 (s, 2 H), 4.87 (d, *J*= 5.9 Hz, 2 H), 1.88 (t, *J*= 6.0 Hz, 1 H), 1.63 (s, 6 H), 1.33 (s, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{32}H_{40}NO_4Cs$ $[M + Cs]^+$ 634.1933, found 634.1948.

4-Formyl-2,7-bis(1,1-dimethylethyl)-5-aminocarbobenzoxy xanthene (**28**). Synthesized analagously to **16**. Isolated in 40% yield after silica gel column chromatography (10:1 hexanes:EtOAc). ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.36 (s, 1 H), 8.15 (br s, 1 H), 7.85 (br s, 1 H), 7.69 (s, 2 H), 7.5-7.2 (m, 6 H), 7.09 (d, *J*= 2.2 Hz, 1 H), 5.29 (s, 2 H), 1.65 (s, 6 H), 1.37 (s, 9 H), 1.34 (s, 9 H). HRMS (FAB in NBA/CsI) calcd for $C_{32}H_{38}NO_4Cs$ $[M + Cs]^+$ 632.1777, found 632.1764.

4,5-Diformyl-2,7-bis(1,1-dimethylethyl) xanthene (**29**). To a solution of BuLi (1.6 M in hexanes, 50 mL) and THF (100 mL) at -70°C was added 4,5-dibromo xanthene **7** (10.0 g, 20.8 mmol) in THF (10 mL). The cloudy solution was stirred for 20 min under Ar. DMF (9.0 mL, 116 mmol) was added at -70°C and the solution was warmed to rt over 45 min. HCl (0.1N, 25 mL) was added and the mixture was stirred for 5 min. Extraction with ether (1x 250 mL and 2x 50 mL), followed by a rinse with brine and drying (Na_2SO_4), gave a white solid (7.79 g, 99%) upon concentration. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.68 (s, 2 H), 7.82 (d, *J*= 2.4 Hz, 2 H), 7.71 (d, *J*= 2.3 Hz, 2 H), 1.70 (s, 6 H), 1.36 (s, 18 H). HRMS (FAB in NBA/CsI) calcd for $C_{35}H_{31}O_3$ $[M + H]^+$ 379.2273, found 379.2265.

Anti-5,15-Bis(2,7-bis(1,1-dimethylethyl)-9,9-dimethyl-5-dimethyl L tartrate)-10,20-diphenylporphyrin (**30a**). Prepared by condensing 2,2'-dipyrrylphenylmethane **6** (0.165 g, 0.75 mol) and **22** (0.400 g, 0.74 mmol) in propionic acid (30 mL) at 80°C for 1.75 h. After a basic aq workup and column chromatography (20:1 hexanes:EtOAc) **30a** (65 mg, 5%) was isolated as a purple solid. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.85 (m, 8 H), 8.26 (m, 4 H, *o*-phenyl), 7.98 (d, *J*= 2.3 Hz, 2 H), 7.87 (d, *J*= 2.3 Hz, 2 H), 7.5 (m, 6 H), 7.48 (d, *J*= 2.3 Hz, 2 H), 7.14 (d, *J*= 2.3 Hz, 2 H), 3.91 (s, 2 H), 3.51 (d, *J*= 4.6 Hz, 2 H), 3.30 (s, 3 H), 2.00 (s, 6 H), 1.80 (d, *J*= 4.7 Hz, 2 H), 1.77 (s, 6 H), 1.54 (s, 18 H), 1.22 (s, 18 H), 0.01 (s, 3 H), -2.64 (br s, 2 H). HRMS (FAB in NBA/CsI) calcd for $C_{92}H_{95}N_4O_{14}$ $[M + H]^+$ 1479.6845, found 1479.6780. UV (nm): 420, 516, 551, 591, 647. An x-ray crystal structure confirmed the identity of this compound (see text).

Syn-5,15-Bis(2,7-bis(1,1-dimethylethyl)-9,9-dimethyl-5-dimethyl L-tartrate)-10,20-diphenylporphyrin (**30b**). Synthesized in the same reaction as **30a** and isolated as a dark red oil (67 mg, 6% yield). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.84 (m, 8 H), 8.39 (br s, 2 H, *o*-phenyl), 8.20 (br s, 2 H), 7.74 (m, 6H), 7.73 (d, *J*= 2.4 Hz, 2 H), 7.55 (d, *J*= 2.4 Hz, 2 H), 7.30 (d, *J*= 2.3 Hz, 2 H), 4.50 (s, 2 H), 4.02 (d, *J*= 3.5 Hz, 2 H), 3.50 (s, 3 H), 3.25 (d, *J*= 3.4 Hz, 2 H), 1.99 (s, 6 H,), 1.88 (s, 6 H), 1.47 (s, 18 H), 1.27 (s, 18 H), 0.56 (s, 3H), -2.63 (br s, 2 H). HRMS (FAB in NBA/CsI) calcd for $C_{92}H_{95}N_4O_{14}$ $[M + H]^+$ 1479.6845, found 1479.6778.

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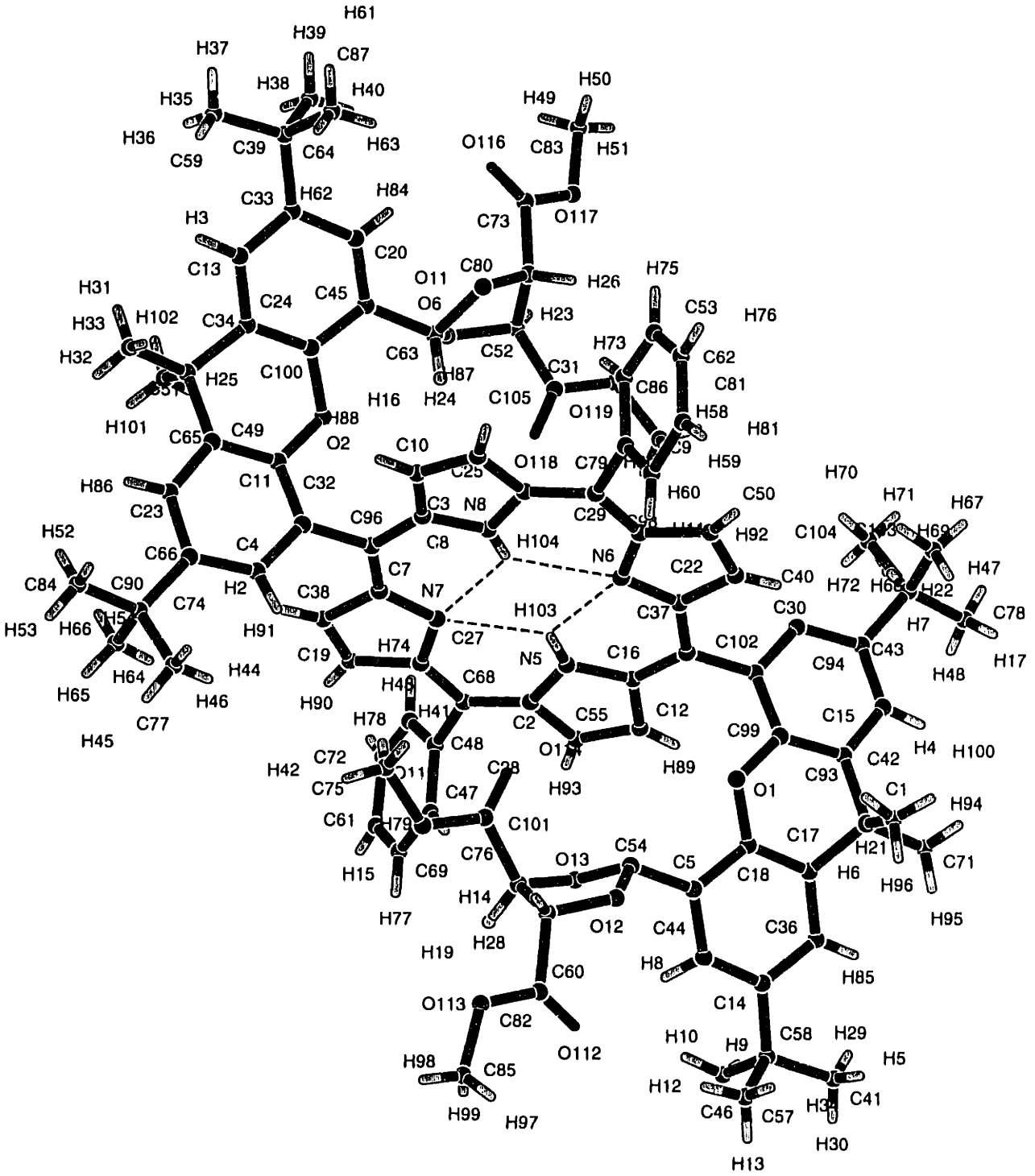
Appendix A: X-ray Crystallographic Parameters for Porphyrin 30a

a (Å)	12.348(7)
b (Å)	16.93(1)
c (Å)	9.925(5)
α (deg)	102.19(4)
β (deg)	99.09(4)
γ (deg)	77.11(5)
Volume ($Å^3$)	1963(4)
space group	P1 (#1)
Z	1
ρ_{calc} (g/cm^3)	1.292
μ (cm^{-1})	6.96
radiation, λ (Å)	1.54178
2Θ limit (deg)	120.1
temperature (°C)	23.0
total/unique reflections	5809/6124
no. of observations (I>2.8σ)	4246
no. of parameters	1308
R	0.083
Rw	0.113

Appendix A: Numbering of Porphyrin Atoms in X-ray Crystal Structure. In this rendering green represents carbon, yellow hydrogen, red oxygen, and blue nitrogen.



Appendix A: X-ray Crystallographic Data Tables for Porphyrin **30a**

Table 1. Atomic coordinates and B_{iso}/B_{eq}

atom	x	y	z	B*eq*
O(1)	0.4254	0.4745	0.6997	3.9(2)
O(2)	-0.0843(6)	0.0471(4)	0.0305(7)	3.9(2)
O(6)	-0.0365(8)	-0.0138(5)	0.3448(10)	4.4(2)
O(11)	0.1164(9)	-0.1106(6)	0.278(1)	5.0(2)
O(12)	0.3910(9)	0.5708(6)	0.376(1)	4.9(2)
O(13)	0.2212(9)	0.5983(6)	0.450(1)	4.6(2)
O(112)	0.349(1)	0.7275(7)	0.317(1)	8.0464
O(113)	0.279(1)	0.6752(7)	0.102(1)	7.1326
O(114)	0.187(1)	0.4461(7)	0.291(1)	8.5237
O(115)	0.1155(10)	0.5028(7)	0.113(1)	7.1603
O(116)	0.044(1)	-0.2319(6)	0.369(1)	7.8835
O(117)	0.081(1)	-0.1702(6)	0.587(1)	6.1491
O(118)	0.109(1)	0.0961(9)	0.439(1)	9.9081
O(119)	0.1521(10)	0.0477(7)	0.638(1)	5.9795
N(5)	0.1403(9)	0.3694(6)	0.514(1)	3.1(2)
N(6)	0.3141(9)	0.2231(6)	0.473(1)	2.9(2)
N(7)	0.0150(9)	0.3020(6)	0.263(1)	3.2(2)
N(8)	0.1881(10)	0.1531(6)	0.219(1)	3.1(2)
C(1)	0.560(1)	0.5363(7)	0.940(1)	3.8(3)
C(2)	0.049(1)	0.4329(7)	0.511(1)	3.6(3)
C(3)	0.268(1)	0.0323(7)	0.093(1)	3.3(3)
C(4)	-0.071(1)	0.1971(7)	-0.175(1)	3.9(3)
C(5)	0.382(1)	0.6018(6)	0.621(1)	3.4(2)
C(7)	-0.032(1)	0.2605(7)	0.145(1)	3.3(2)

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	Beq
C(8)	0.118(1)	0.1345(7)	0.102(1)	3.3(3)
C(9)	0.467(1)	0.0120(7)	0.305(1)	4.0(3)
C(10)	0.169(1)	0.0621(6)	0.025(1)	3.4(2)
C(11)	-0.101(1)	0.0867(8)	-0.083(1)	3.6(3)
C(12)	0.168(1)	0.4690(8)	0.707(1)	4.4(3)
C(13)	-0.165(1)	-0.1505(7)	-0.131(1)	3.9(3)
C(14)	0.440(1)	0.7247(7)	0.765(1)	4.2(3)
C(15)	0.509(1)	0.4255(7)	1.049(1)	3.7(3)
C(16)	0.216(1)	0.3882(7)	0.627(1)	3.2(3)
C(17)	0.492(1)	0.5923(7)	0.845(1)	3.1(2)
C(18)	0.435(1)	0.5545(7)	0.726(1)	3.2(2)
C(19)	-0.158(1)	0.3787(8)	0.199(1)	4.5(3)
C(20)	-0.049(1)	-0.1636(7)	0.083(1)	3.9(3)
C(22)	0.363(1)	0.2611(7)	0.594(1)	3.1(3)
C(23)	-0.182(1)	0.1058(8)	-0.311(1)	4.2(3)
C(24)	-0.160(1)	-0.0676(7)	-0.112(1)	3.9(3)
C(25)	0.282(1)	0.0895(7)	0.217(1)	3.3(2)
C(27)	-0.060(1)	0.3769(7)	0.302(1)	3.4(2)
C(28)	0.168(1)	0.5020(9)	0.238(1)	5.1523
C(29)	0.387(1)	0.1502(7)	0.442(1)	3.3(3)
C(30)	0.396(1)	0.3319(7)	0.915(1)	3.8(3)
C(31)	0.446(1)	-0.0623(9)	0.321(2)	5.5(4)
C(32)	-0.052(1)	0.1548(7)	-0.062(1)	3.1(2)
C(33)	-0.111(1)	-0.2005(7)	-0.035(1)	3.7(3)
C(34)	-0.227(1)	-0.0120(8)	-0.213(1)	3.7(3)
C(36)	0.496(1)	0.6756(8)	0.863(1)	4.2(3)

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	B*eq*
C(37)	0.320(1)	0.3409(7)	0.669(1)	3.1(3)
C(38)	-0.143(1)	0.3103(8)	0.102(1)	4.4(3)
C(39)	-0.113(1)	-0.2932(8)	-0.057(2)	5.1(4)
C(40)	0.470(1)	0.2130(8)	0.637(1)	3.9(3)
C(41)	0.505(1)	0.8487(9)	0.912(2)	6.1(4)
C(42)	0.672(1)	0.4980(8)	0.885(1)	4.6(3)
C(43)	0.486(1)	0.3068(10)	1.155(2)	5.5(4)
C(44)	0.391(1)	0.6841(8)	0.644(1)	4.3(3)
C(45)	-0.040(1)	-0.0828(9)	0.106(1)	4.2(3)
C(46)	0.324(1)	0.8638(8)	0.787(2)	5.7(4)
C(47)	-0.135(1)	0.5116(8)	0.433(1)	3.6(3)
C(48)	-0.237(1)	0.5099(8)	0.469(2)	5.3(3)
C(49)	-0.167(1)	0.0592(7)	-0.203(1)	3.6(3)
C(50)	0.482(1)	0.1457(8)	0.545(1)	4.3(3)
C(51)	-0.246(1)	-0.0590(8)	-0.358(2)	5.9(4)
C(52)	0.039(1)	-0.0207(7)	0.466(1)	4.2(3)
C(53)	0.530(2)	-0.1322(9)	0.311(2)	5.8(4)
C(54)	0.335(1)	0.5624(7)	0.485(1)	4.1(3)
C(55)	0.066(1)	0.4924(8)	0.632(1)	4.9(3)
C(57)	0.488(2)	0.8317(9)	0.659(2)	8.8(5)
C(58)	0.440(1)	0.8155(7)	0.783(1)	4.0(2)
C(59)	-0.179(1)	-0.3236(8)	-0.193(1)	5.6(3)
C(60)	0.313(1)	0.5859(7)	0.259(1)	4.1(3)
C(61)	-0.309(1)	0.649(1)	0.451(2)	6.8(4)
C(62)	0.628(2)	-0.129(1)	0.277(2)	7.9(5)
C(63)	0.028(1)	-0.0439(9)	0.235(2)	4.3(3)
C(64)	-0.156(1)	-0.3117(8)	0.064(2)	5.9(4)

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	B*eq*
C(65)	-0.340(1)	0.025(1)	-0.155(2)	6.7(5)
C(66)	-0.166(1)	0.2276(8)	-0.412(1)	4.6(3)
C(68)	-0.044(1)	0.4369(7)	0.414(1)	2.9(2)
C(69)	-0.215(2)	0.6537(8)	0.415(2)	7.7(4)
C(71)	0.585(1)	0.5839(8)	1.090(1)	4.9(3)
C(72)	-0.322(2)	0.574(1)	0.485(2)	7.8(5)
C(73)	0.075(1)	-0.1792(8)	0.451(1)	4.8576
C(74)	-0.137(1)	0.1752(7)	-0.299(1)	3.4(2)
C(75)	0.082(2)	0.430(1)	0.039(2)	7.8073
C(76)	-0.121(1)	0.5848(8)	0.403(2)	5.2(3)
C(77)	-0.073(2)	0.273(1)	-0.411(2)	10.6(6)
C(78)	0.518(2)	0.356(1)	1.293(2)	9.6(6)
C(79)	0.566(1)	0.018(1)	0.265(2)	6.3(4)
C(80)	0.113(1)	-0.1049(8)	0.418(1)	5.0(3)
C(81)	0.651(1)	-0.059(1)	0.256(2)	7.0(4)
C(82)	0.316(1)	0.6747(8)	0.235(1)	4.6718
C(83)	0.050(2)	-0.2370(9)	0.640(2)	8.7100
C(84)	-0.200(2)	0.1821(9)	-0.552(2)	7.7(4)
C(85)	0.270(2)	0.755(1)	0.061(2)	9.9155
C(86)	0.221(2)	0.110(1)	0.698(2)	8.6425
C(87)	0.013(1)	-0.3441(9)	-0.059(2)	6.7(4)
C(90)	-0.269(2)	0.2940(10)	-0.369(2)	9.0(4)
C(93)	0.499(1)	0.4682(7)	0.941(1)	3.8(3)
C(94)	0.460(1)	0.3591(8)	1.039(1)	4.3(3)
C(96)	0.015(1)	0.1856(7)	0.066(1)	3.1(3)
C(98)	0.371(1)	0.0899(7)	0.326(1)	3.7(3)

Table 1. Atomic coordinates and B*iso*/B*eq* (continued)

atom	x	y	z	B*eq*
C(99)	0.437(1)	0.4397(7)	0.818(1)	3.2(2)
C(100)	-0.096(1)	-0.0363(7)	0.006(1)	3.6(3)
C(101)	0.200(1)	0.5845(8)	0.303(1)	4.6(3)
C(102)	0.384(1)	0.3729(7)	0.803(1)	3.2(3)
C(103)	0.577(2)	0.234(1)	1.117(2)	11.1(6)
C(104)	0.379(2)	0.269(2)	1.159(2)	14.9(7)
C(105)	0.103(1)	0.0474(9)	0.512(1)	6.0024
H(2)	-0.0260	0.2401	-0.1569	3.0000
H(3)	-0.2015	-0.1781	-0.2172	3.0000
H(4)	0.5521	0.4524	1.1284	3.0000
H(5)	0.5823	0.8261	0.9074	7.3259
H(6)	0.7034	0.5400	0.8650	5.4697
H(7)	0.4102	0.3031	1.1846	3.0000
H(8)	0.3376	0.7118	0.5776	3.0000
H(9)	0.2923	0.8722	0.8757	3.0000
H(10)	0.2765	0.8388	0.7140	3.0000
H(11)	0.5548	0.1050	0.5389	3.0000
H(12)	0.4433	0.8144	0.5755	10.5781
H(13)	0.4869	0.8889	0.6701	10.5781
H(14)	0.3452	0.5479	0.1847	3.0000
H(15)	-0.3683	0.6977	0.4635	3.0000
H(16)	0.0663	-0.0019	0.2087	3.0000
H(17)	0.5937	0.3607	1.2997	11.5562
H(19)	0.1536	0.6335	0.2687	3.0000
H(21)	0.6609	0.4588	0.8027	5.4697
H(22)	0.4104	0.3031	1.1845	3.0000

Table 1. Atomic coordinates and B*iso*/B*eq* (continued)

atom	x	y	z	B*eq*
H(23)	0.0020	-0.0194	0.5493	3.0000
H(24)	0.0663	-0.0020	0.2087	3.0000
H(25)	-0.3274	0.0571	-0.0651	7.9916
H(26)	0.1934	-0.1058	0.4689	3.0000
H(28)	0.1536	0.6335	0.2688	3.0000
H(29)	0.4819	0.8344	0.9891	7.3259
H(30)	0.4930	0.9070	0.9229	7.3259
H(31)	-0.2839	-0.1018	-0.3580	7.0430
H(32)	-0.2893	-0.0228	-0.4164	7.0430
H(33)	-0.1756	-0.0822	-0.3924	7.0430
H(34)	0.5624	0.8017	0.6560	10.5781
H(35)	-0.2553	-0.2971	-0.1919	6.6856
H(36)	-0.1497	-0.3113	-0.2671	6.6856
H(37)	-0.1730	-0.3815	-0.2043	6.6856
H(38)	-0.2322	-0.2855	0.0667	7.1388
H(39)	-0.1493	-0.3696	0.0539	7.1388
H(40)	-0.1132	-0.2918	0.1473	7.1388
H(41)	0.0297	0.4162	0.0866	9.3688
H(42)	0.0475	0.4382	-0.0514	9.3688
H(43)	0.1452	0.3863	0.0306	9.3688
H(44)	-0.0086	0.2345	-0.4402	12.7453
H(45)	-0.0965	0.3114	-0.4718	12.7453
H(46)	-0.0554	0.3013	-0.3193	12.7453
H(47)	0.5092	0.3284	1.3640	11.5562
H(48)	0.4712	0.4089	1.3036	11.5562
H(49)	-0.0245	-0.2415	0.6046	10.4520

Table 1. Atomic coordinates and B*iso*/B*eq* (continued)

atom	x	y	z	B*eq*
H(50)	0.0992	-0.2877	0.6100	10.4520
H(51)	0.0574	-0.2248	0.7382	10.4520
H(52)	-0.2589	0.1555	-0.5474	9.2467
H(53)	-0.2249	0.2197	-0.6147	9.2467
H(54)	-0.1379	0.1421	-0.5844	9.2467
H(58)	0.2508	0.1045	0.7905	10.3710
H(59)	0.2808	0.1030	0.6439	10.3710
H(60)	0.1763	0.1638	0.6981	10.3710
H(61)	0.0138	-0.4012	-0.0676	8.0023
H(62)	0.0434	-0.3357	-0.1364	8.0023
H(63)	0.0569	-0.3256	0.0241	8.0023
H(64)	-0.2489	0.3263	-0.2813	10.7747
H(65)	-0.2921	0.3286	-0.4362	10.7747
H(66)	-0.3278	0.2681	-0.3619	10.7747
H(67)	0.6005	0.2051	1.1924	13.3622
H(68)	0.5504	0.1978	1.0378	13.3622
H(69)	0.6391	0.2521	1.0961	13.3622
H(70)	0.3710	0.2271	1.0797	17.9093
H(71)	0.3890	0.2455	1.2407	17.9093
H(72)	0.3139	0.3110	1.1604	17.9093
H(73)	0.3741	-0.0655	0.3391	6.6113
H(74)	-0.2472	0.4588	0.4851	6.3127
H(75)	0.5171	-0.1822	0.3284	6.9601
H(76)	0.6838	-0.1781	0.2681	9.4343
H(77)	-0.2065	0.7045	0.3956	9.2521

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	B*eq*
H(78)	-0.3886	0.5693	0.5171	9.4070
H(79)	-0.0525	0.5894	0.3750	6.2617
H(80)	0.5788	0.0675	0.2450	7.5644
H(81)	0.7237	-0.0595	0.2347	8.4538
H(84)	-0.0118	-0.1962	0.1504	4.6963
H(85)	0.5379	0.7000	0.9437	5.0394
H(86)	-0.2254	0.0884	-0.3952	5.0893
H(87)	0.3179	-0.0176	0.0636	3.9134
H(88)	0.1394	0.0369	-0.0636	4.0406
H(89)	0.1997	0.4982	0.7910	5.2900
H(90)	-0.2221	0.4217	0.2011	5.3838
H(91)	-0.1936	0.2964	0.0222	5.2486
H(92)	0.5200	0.2276	0.7167	4.7245
H(93)	0.0147	0.5422	0.6574	5.8230
H(94)	0.6398	0.5496	1.1411	5.8750
H(95)	0.6111	0.6319	1.0857	5.8750
H(96)	0.5180	0.5996	1.1335	5.8750
H(97)	0.3421	0.7682	0.0722	11.8986
H(98)	0.2389	0.7524	-0.0334	11.8986
H(99)	0.2228	0.7969	0.1177	11.8986
H(100)	0.7212	0.4714	0.9528	5.4697
H(101)	-0.3861	0.0597	-0.2148	7.9916
H(102)	-0.3762	-0.0177	-0.1500	8.0(3)
H(103)	0.1498	0.3196	0.4479	3.7573
H(104)	0.1753	0.2006	0.2895	3.6794

$$B_{eq} = \frac{8}{3}\pi^2\left[U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha\right]$$

Table 2. Anisotropic Displacement Parameters

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	0.071(6)	0.033(4)	0.048(5)	-0.006(4)	0.007(4)	0.019(3)
O(2)	0.048(5)	0.044(5)	0.048(4)	-0.007(4)	-0.019(4)	0.010(4)
O(6)	0.049(5)	0.055(4)	0.055(4)	0.010(4)	0.009(4)	0.012(3)
O(11)	0.054(5)	0.072(6)	0.055(5)	0.028(4)	0.005(4)	0.035(4)
O(12)	0.050(5)	0.071(5)	0.063(5)	0.004(4)	0.003(4)	0.027(4)
O(13)	0.048(5)	0.075(6)	0.053(5)	-0.003(5)	0.003(4)	0.023(4)
N(5)	0.026(5)	0.036(4)	0.051(6)	0.0H(4)	-0.005(4)	0.018(4)
N(6)	0.034(5)	0.021(4)	0.048(6)	0.007(4)	0.002(4)	0.007(4)
N(7)	0.040(6)	0.034(5)	0.042(5)	0.003(4)	-0.010(4)	0.012(4)
N(8)	0.037(6)	0.032(5)	0.044(6)	0.000(5)	0.002(5)	0.007(4)
C(1)	0.058(8)	0.033(6)	0.057(7)	-0.014(6)	0.000(6)	0.013(5)
C(2)	0.045(7)	0.044(6)	0.045(7)	-0.016(5)	-0.002(6)	-0.001(5)
C(3)	0.047(8)	0.033(6)	0.030(6)	0.014(6)	0.006(5)	-0.003(5)
C(4)	0.050(8)	0.052(7)	0.051(7)	-0.009(6)	-0.001(6)	0.024(6)
C(5)	0.053(7)	0.019(5)	0.056(6)	-0.001(4)	0.001(5)	0.015(4)
C(7)	0.046(6)	0.039(6)	0.036(6)	0.001(5)	0.002(5)	0.005(5)
C(8)	0.047(7)	0.041(7)	0.025(5)	0.008(6)	-0.006(5)	0.004(5)
C(9)	0.058(9)	0.034(5)	0.043(8)	0.018(6)	-0.002(7)	0.001(5)
C(10)	0.055(7)	0.032(5)	0.034(6)	-0.015(5)	0.002(5)	-0.013(4)
C(11)	0.038(6)	0.055(7)	0.034(7)	0.008(6)	-0.012(5)	0.020(6)
C(12)	0.053(8)	0.052(7)	0.047(8)	0.010(6)	-0.005(7)	0.004(6)
C(13)	0.059(9)	0.030(6)	0.052(8)	0.000(6)	0.001(7)	0.003(5)
C(14)	0.043(7)	0.036(6)	0.080(9)	0.005(5)	0.003(7)	0.028(6)
C(15)	0.043(6)	0.045(6)	0.049(8)	0.000(5)	-0.010(5)	0.018(5)

Table 2. Anisotropic Displacement Parameters (continued)

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(16)	0.040(7)	0.026(5)	0.062(7)	-0.014(5)	0.007(6)	0.012(5)
C(17)	0.047(7)	0.032(6)	0.041(6)	-0.006(5)	0.001(5)	0.021(5)
C(18)	0.040(7)	0.038(6)	0.040(6)	-0.005(5)	0.002(5)	0.010(5)
C(19)	0.046(7)	0.039(7)	0.062(7)	0.024(6)	-0.007(6)	0.002(6)
C(20)	0.07(1)	0.020(6)	0.050(7)	0.003(6)	0.006(7)	0.010(5)
C(22)	0.035(7)	0.030(5)	0.057(7)	-0.008(5)	-0.007(6)	0.023(5)
C(23)	0.057(8)	0.050(6)	0.045(7)	-0.002(6)	-0.013(6)	0.013(5)
C(24)	0.034(7)	0.041(7)	0.063(8)	0.003(6)	-0.002(6)	0.007(6)
C(25)	0.028(6)	0.037(6)	0.053(7)	0.025(5)	0.005(5)	0.020(5)
C(27)	0.037(6)	0.032(6)	0.053(7)	0.016(5)	0.013(5)	0.0[illegible](5)
C(29)	0.032(7)	0.044(6)	0.048(7)	-0.011(6)	-0.010(6)	0.015(6)
C(30)	0.045(7)	0.036(6)	0.053(7)	0.008(6)	-0.002(6)	0.006(5)
C(31)	0.08(1)	0.039(8)	0.090(10)	0.007(8)	0.015(9)	0.014(7)
C(32)	0.050(7)	0.023(5)	0.042(6)	-0.002(5)	0.004(6)	0.004(5)
C(33)	0.061(8)	0.039(7)	0.039(6)	-0.006(6)	0.010(6)	0.001(5)
C(34)	0.029(6)	0.049(7)	0.060(9)	0.000(6)	-0.012(6)	0.022(7)
C(36)	0.042(7)	0.057(8)	0.056(7)	-0.007(6)	-0.010(6)	0.015(6)
C(37)	0.028(6)	0.032(6)	0.057(7)	0.001(5)	0.005(5)	0.009(5)
C(38)	0.033(7)	0.062(7)	0.065(8)	0.000(6)	-0.019(6)	0.026(6)
C(39)	0.07(1)	0.036(6)	0.10(1)	-0.011(7)	0.009(10)	0.026(7)
C(40)	0.038(7)	0.052(7)	0.045(7)	0.002(6)	-0.014(5)	0.003(6)
C(41)	0.080(9)	0.056(8)	0.10(1)	-0.017(7)	0.034(8)	0.014(8)
C(42)	0.058(7)	0.036(6)	0.075(8)	0.007(6)	0.011(6)	0.014(6)
C(43)	0.04(1)	0.108(10)	0.07(1)	-0.016(8)	-0.002(9)	0.050(9)
C(44)	0.039(6)	0.065(7)	0.056(7)	0.001(5)	-0.014(5)	0.031(6)

Table 2. Anisotropic Displacement Parameters (continued)

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(45)	0.038(7)	0.067(10)	0.046(8)	0.006(6)	0.001(6)	0.012(7)
C(46)	0.076(10)	0.032(6)	0.09(1)	0.007(6)	0.007(8)	-0.005(7)
C(47)	0.026(6)	0.044(8)	0.059(6)	0.006(5)	-0.008(5)	0.014(6)
C(48)	0.050(8)	0.042(7)	0.10(1)	0.005(6)	0.017(7)	0.016(6)
C(49)	0.026(6)	0.049(7)	0.063(8)	-0.001(6)	-0.013(6)	0.029(6)
C(50)	0.038(8)	0.051(7)	0.064(7)	0.003(6)	-0.006(6)	0.010(6)
C(51)	0.10(1)	0.048(7)	0.071(8)	-0.036(8)	-0.047(8)	0.028(6)
C(52)	0.058(7)	0.056(6)	0.046(6)	-0.013(6)	0.010(6)	0.004(5)
C(53)	0.07(1)	0.065(10)	0.076(9)	0.017(8)	0.014(8)	0.027(7)
C(54)	0.040(7)	0.054(6)	0.064(8)	-0.002(5)	0.001(6)	0.025(6)
C(55)	0.040(7)	0.051(8)	0.083(9)	0.007(6)	-0.011(6)	0.018(7)
C(57)	0.19(2)	0.052(9)	0.12(1)	-0.02(1)	0.07(1)	0.026(9)
C(58)	0.061(7)	0.041(6)	0.034(5)	0.003(5)	-0.012(5)	-0.004(4)
C(59)	0.103(10)	0.037(7)	0.063(7)	-0.016(7)	-0.019(7)	0.009(6)
C(60)	0.052(7)	0.053(6)	0.052(6)	-0.011(6)	0.003(5)	0.012(5)
C(61)	0.039(8)	0.10(1)	0.081(9)	0.046(8)	-0.008(7)	0.013(9)
C(62)	0.13(2)	0.07(1)	0.07(1)	0.05(1)	0.02(1)	-0.002(8)
C(63)	0.030(7)	0.09(1)	0.063(8)	-0.017(6)	-0.012(6)	0.058(7)
C(64)	0.079(10)	0.058(8)	0.089(9)	-0.028(8)	0.011(8)	-0.003(7)
C(65)	0.049(10)	0.09(1)	0.12(1)	-0.006(8)	-0.008(10)	0.07(1)
C(66)	0.075(10)	0.054(7)	0.053(7)	-0.015(7)	-0.003(7)	0.028(6)
C(68)	0.030(6)	0.038(6)	0.040(6)	0.003(5)	-0.002(5)	0.013(5)
C(69)	0.14(1)	0.039(7)	0.08(1)	0.050(9)	0.00(1)	0.016(6)
C(71)	0.065(9)	0.060(8)	0.058(7)	-0.003(7)	-0.001(6)	0.022(6)
C(72)	0.05(1)	0.11(1)	0.11(1)	0.02(1)	0.006(10)	0.00(1)

Table 2. Anisotropic Displacement Parameters (continued)

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(71)	0.065(9)	0.060(8)	0.058(7)	-0.003(7)	-0.001(6)	0.022(6)
C(72)	0.05(1)	0.11(1)	0.11(1)	0.02(1)	0.006(10)	0.00(1)
C(74)	0.053(7)	0.029(5)	0.046(7)	-0.007(5)	0.002(6)	0.006(4)
C(76)	0.056(8)	0.059(7)	0.077(9)	0.023(6)	0.013(7)	0.031(7)
C(77)	0.17(2)	0.15(1)	0.13(2)	-0.06(1)	-0.01(1)	0.10(1)
C(78)	0.22(2)	0.10(1)	0.043(7)	-0.01(1)	-0.01(1)	0.040(7)
C(79)	0.039(8)	0.09(1)	0.09(1)	0.031(8)	0.015(8)	0.020(10)
C(80)	0.045(8)	0.086(8)	0.061(7)	0.005(6)	-0.001(6)	0.042(6)
C(81)	0.037(7)	0.11(1)	0.09(1)	0.023(8)	0.011(7)	0.001(9)
C(84)	0.15(1)	0.063(9)	0.070(9)	0.003(9)	-0.012(10)	0.028(7)
C(87)	0.082(10)	0.064(9)	0.09(1)	0.017(7)	0.005(9)	0.022(9)
C(90)	0.13(1)	0.085(9)	0.11(1)	0.037(9)	0.000(10)	0.050(8)
C(93)	0.052(7)	0.039(7)	0.045(6)	0.002(6)	0.002(5)	0.004(5)
C(94)	0.050(8)	0.044(8)	0.069(8)	0.005(6)	-0.001(6)	0.029(7)
C(96)	0.046(7)	0.037(6)	0.038(6)	-0.014(6)	-0.008(5)	0.018(5)
C(98)	0.034(6)	0.037(6)	0.062(8)	0.022(5)	0.015(6)	0.015(5)
C(99)	0.040(7)	0.025(5)	0.057(7)	0.000(5)	0.013(5)	0.010(5)
C(100)	0.048(8)	0.019(5)	0.062(7)	0.010(5)	0.012(6)	0.006(5)
C(101)	0.057(8)	0.063(8)	0.050(6)	0.007(6)	-0.010(6)	0.032(6)
C(102)	0.025(6)	0.041(6)	0.049(7)	0.004(5)	-0.011(5)	0.013(5)
C(103)	0.17(2)	0.08(1)	0.17(2)	0.06(1)	0.04(2)	0.09(1)
C(104)	0.15(2)	0.32(3)	0.18(2)	-0.15(2)	-0.07(1)	0.20(2)

The general temperature factor expression:

$$\exp\left[-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + C^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl\right]$$

Table 3. Bond Lengths (Å)

atom	atom	distance	atom	atom	distance
O1	C18	1.35(3)	O1	C99	1.42(4)
O2	C11	1.40(4)	O2	C100	1.41(4)
O6	C52	1.43(5)	O6	C63	1.36(5)
O11	C63	1.42(4)	O11	C80	1.40(4)
O12	C54	1.48(6)	O12	C60	1.31(4)
O13	C54	1.46(4)	O13	C101	1.21(6)
O112	C82	1.09(5)*	O113	C82	1.41(5)*
O113	C85	1.59(5)*	O114	C28	1.19(6)*
O115	C28	1.51(5)*	O115	C75	1.26(5)*
O116	C73	1.09(6)*	O117	C73	1.36(6)*
O117	C83	1.5(1)*	O118	C105	1.23(7)*
O119	C86	1.52(7)*	O119	C105	1.29(6)*
N5	C2	1.36(4)	N5	C16	1.44(4)
N6	C22	1.44(4)	N6	C29	1.20(4)
N7	C7	1.28(4)	N7	C27	1.61(4)
N8	C8	1.36(4)	N8	C25	1.42(4)
C1	C17	1.49(5)	C1	C42	1.65(5)
C1	C71	1.29(5)	C1	C93	1.63(6)
C2	C55	1.45(4)	C2	C68	1.45(5)
C3	C10	1.36(5)	C3	C25	1.31(4)
C3	H87	1.09	C4	C32	1.49(5)
C4	C74	1.50(4)	C4	H2	1.00
C5	C18	1.56(6)	C5	C44	1.3(1)
C5	C54	1.28(5)	C7	C38	1.53(4)
C7	C96	1.43(4)	C8	C10	1.47(4)

Table 3. Bond Lengths (Å) (continued)

atom	atom	distance	atom	atom	distance
C8	C96	1.27(5)	C9	C31	1.63(6)
C9	C79	1.46(6)	C9	C98	1.61(4)
C10	H88	0.91	C11	C32	1.33(5)
C11	C49	1.31(4)	C12	C16	1.44(4)
C12	C55	1.41(4)	C12	H89	1.04
C13	C24	1.30(5)	C13	C33	1.32(4)
C13	H3	1.09	C14	C44	0.84(10)
C15	C93	1.34(6)	C15	C94	1.46(5)
C15	H4	0.97	C16	C37	1.57(5)
C17	C18	1.26(5)	C17	C36	1.43(5)
C19	C27	1.65(5)	C19	C38	1.15(5)
C19	H90	1.00	C20	C33	1.45(4)
C20	C45	1.21(4)	C20	H84	0.90
C22	C37	1.44(5)	C22	C40	1.41(4)
C23	C49	1.41(5)	C23	C74	1.21(5)
C23	1186	1.00	C24	C34	1.71(5)
C24	C100	1.51(4)	C25	C98	1.33(4)
C27	C68	1.25(4)	C28	C101	1.52(5)
C29	C50	1.39(4)	C29	C98	1.48(4)
C30	C94	1.27(4)	C30	C102	1.36(6)
C31	C53	1.42(6)	C31	H73	0.75
C32	C96	1.49(4)	C33	C39	1.60(4)
C34	C49	1.43(5)	C34	C51	1.67(4)
C34	C65	1.39(5)	C36	H85	0.96
C37	C102	1.37(5)	C38	H91	0.96

Table 3. Bond Lengths (Å) (continued)

atom	atom	distance	atom	atom	distance
C40	C50	1.41(4)	C40	H92	0.93
C42	H6	0.93	C42	H21	0.99
C42	H100	0.91	C44	H8	1.23
C45	C63	1.55(5)	C45	C100	1.48(5)
C47	C48	1.46(6)	C47	C68	1.44(4)
C47	C76	1.36(6)	C48	C72	1.33(5)
C48	H74	0.82	C50	H11	0.98
C51	H31	0.92	C51	H32	0.93
C51	H33	0.99	C52	C80	1.51(4)
C52	C105	1.56(6)	C52	H23	0.91
C53	C62	1.29(7)	C53	H75	1.06
C55	H93	0.88	C60	C82	1.70(7)
C60	C101	1.51(7)	C60	H14	1.04
C61	C69	1.8(1)	C61	C72	1.46(8)
C61	H15	1.02	C62	C81	0.92(9)
C62	H76	0.99	C63	H16	1.18
C63	H24	1.18	C65	H25	0.84
C65	H101	1.01	C65	H102	1.04
C69	C76	1.43(5)	C69	H77	0.77
C71	H94	0.95	C71	H95	1.06
C71	H96	1.02	C72	H78	1.03
C73	C80	1.63(7)	C74	C66	1.43(8)
C75	H41	1.01	C75	H42	1.10
C75	H43	0.76	C76	H79	1.09
C79	C81	1.80(8)	C79	H80	1.08

Table 3. Bond Lengths (Å) (continued)

atom	atom	distance	atom	atom	distance
C80	H26	0.97	C81	H81	1.25
C83	H40	1.04	C83	H50	0.98
C85	H97	0.92	C85	H08	1.16
C85	H99	0.76	C86	H58	0.59
C86	H59	1.29	C86	H60	1.27
C93	C99	1.30(5)	C94	C43	1.67(5)
C99	C102	1.47(6)	C101	H10	1.15
C101	H28	1.15	C43	C78	1.54(5)
C43	C104	1.54(7)	C43	C103	1.54(5)
C78	H103	0.95	C78	H104	0.95
C78	H47	0.95	C104	H71	0.95
C104	H72	0.95	C104	H70	0.05
C103	H68	0.05	C103	H69	0.95
C103	H67	0.95	C66	C77	1.5(1)
C66	C84	1.5(1)	C66	C90	1.5(2)
C77	H46	0.05	C77	H44	0.95
C77	H45	0.95	C84	H53	0.05
C84	H54	0.05	C84	H52	0.95
C90	H66	0.95	C90	H64	0.95
C90	H65	0.95	C58	C41	1.5(2)
C58	C57	1.5(2)	C58	C46	1.5(2)
C41	H29	0.95	C41	H5	0.95
C41	H30	0.95	C57	H13	0.95
C57	H34	0.05	C57	H12	0.05
C46	H10	0.95	C46	H105	0.95

Table 3. Bond Lengths (Å) (continued)

atom	atom	distance	atom	atom	distance
C46	H106	0.95	C39	C87	1.54(4)
C30	C64	1.54(5)	C39	C59	1.54(4)
C87	H62	0.95	C87	H63	0.95
C87	H61	0.95	C64	H30	0.95
C64	H40	0.95	C64	H38	0.95
C59	H35	0.95	C59	H36	0.95
C59	H37	0.95			

*restrained during refinement

Table 4. Bond Angles(°)

atom	atom	atom	angle	atom	atom	atom	angle
C18	O1	C99	110(2)	C11	O2	C100	120(2)
C52	O6	C63	102(2)	C63	O11	C80	103(2)
C54	O12	C60	111(3)	C54	O13	C101	105(3)
C82	O113	C85	104(2)*	C28	O115	C75	120(3)*
C73	O117	C83	84(9)*	C86	O119	C105	133(4)*
C2	N5	C16	107(2)	C22	N6	C29	105(2)
C7	N7	C27	109(2)	C8	N8	C25	107(2)
C17	C1	C42	104(3)	C17	C1	C71	125(3)
C17	C1	C93	102(2)	C42	C1	C71	107(3)
C42	C1	C93	103(2)	C71	C1	C93	111(3)
N5	C2	C55	109(2)	N5	C2	C68	124(2)
C55	C2	C68	121(2)	C10	C3	C25	114(2)

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
C10	C3	H87	118.6	C25	C3	H87	125.2
C32	C4	C74	115(3)	C32	C4	H2	117.5
C74	C4	H2	126.6	C18	C5	C44	98(3)
C18	C5	C54	128(3)	C44	C5	C54	131(4)
N7	C7	C38	111(2)	N7	C7	C96	124(2)
C38	C7	C96	124(2)	N8	C8	C10	108(3)
N8	C8	C96	126(2)	C10	C8	C96	125(3)
C31	C9	C79	121(3)	C31	C9	C98	119(2)
C79	C9	C98	113(3)	C3	C10	C8	101(2)
C3	C10	H88	129.3	C8	C10	H88	126.3
O2	C11	C32	107(2)	O2	C11	C49	120(3)
C32	C11	C49	131(3)	C16	C12	C55	109(2)
C16	C12	H89	125.4	C55	C12	H89	124.3
C24	C13	C33	127(3)	C24	C13	H3	107.4
C33	C13	H3	119.4	C93	C15	C94	121(3)
C93	C15	H4	108.0	C94	C15	H4	130.6
N5	C16	C12	105(2)	N5	C16	C37	129(2)
C12	C16	C37	124(2)	C1	C17	C18	127(3)
C1	C17	C36	112(3)	C18	C17	C36	120(3)
O1	C18	C5	102(2)	O1	C18	C17	125(3)
C5	C18	C17	130(3)	C27	C19	C38	114(3)
C27	C19	H90	100.3	C38	C19	H90	144.9
C33	C20	C45	120(3)	C33	C20	H84	116.5
C45	C20	H84	122.5	N6	C22	C37	134(2)
N6	C22	C40	108(2)	C37	C22	C40	115(3)
C49	C23	C74	124(3)	C49	C23	H86	119.4
C74	C23	H86	115.8	C13	C24	C34	134(2)

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
C13	C24	C100	114(2)	C34	C24	C100	109(2)
N8	C25	C3	106(2)	N8	C25	C98	119(2)
C3	C25	C98	133(2)	N7	C27	C19	94(2)
N7	C27	C68	128(3)	C19	C27	C68	136(3)
O114	C28	O115	112(3)*	O114	C28	C101	122(3)
O115	C28	C101	125(3)	N6	C29	C50	116(2)
N6	C29	C98	122(2)	C50	C29	C98	121(2)
C94	C30	C102	119(3)	C9	C31	C53	101(3)
C9	C31	H73	105.4	C53	C31	H73	149.0
C4	C32	C11	112(2)	C4	C32	C96	115(2)
C11	C32	C96	132(3)	C13	C33	C20	117(2)
C13	C33	C39	126(2)	C20	C33	C39	115(2)
C24	C34	C49	111(2)	C24	C34	C51	99(2)
C24	C34	C65	105(3)	C49	C34	C51	113(3)
C49	C34	C65	111(2)	C51	C34	C65	114(2)
C17	C36	H85	127.0	C16	C37	C22	114(3)
C16	C37	C102	119(3)	C22	C37	C102	126(3)
C7	C38	C19	109(3)	C7	C38	H91	125.1
C19	C38	H91	124.4	C22	C40	C50	103(2)
C22	C40	H92	127.0	C50	C40	H92	129.0
C1	C42	H6	106.6	C1	C42	H21	110.3
C1	C42	H100	106.7	H6	C42	H21	108.4
H6	C42	H100	115.2	H21	C42	H100	109.6
C5	C44	C14	151(9)	C5	C44	H8	110.6
C14	C44	H8	83.1	C20	C45	C63	122(3)
C20	C45	C100	122(3)	C63	C45	C100	114(2)

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
C48	C47	C68	123(3)	C48	C47	C76	117(3)
C68	C47	C76	117(3)	C47	C48	C72	117(3)
C47	C48	H74	109.5	C72	C48	H74	133.0
C11	C49	C23	114(3)	C11	C49	C34	123(3)
C23	C49	C34	121(2)	C29	C50	C40	105(2)
C29	C50	H11	137.7	C40	C50	H11	114.5
C34	C51	H31	110.9	C34	C51	H32	105.2
C34	C51	H33	108.8	H31	C51	H32	114.5
H31	C51	H33	109.1	H32	C51	H33	108.2
O6	C52	C80	102(2)	O6	C52	C105	114(3)
O6	C52	H23	115.8	C80	C52	C105	109(3)
C80	C52	H23	119.8	C105	C52	H23	95.1
C31	C53	C62	138(4)	C31	C53	H75	100.9
C62	C53	H75	120.1	O12	C54	O13	98(3)
O12	C54	C5	107(3)	O13	C54	C5	111(3)
C2	C55	C12	103(2)	C2	C55	H93	122.0
C12	C55	H93	128.9	O12	C60	C82	109(3)
O12	C60	C101	100(3)	O12	C60	H14	107.0
C82	C60	C101	109(2)	C82	C60	H14	106.4
C101	C60	H14	123.9	C69	C61	C72	124(4)
C69	C61	H15	119.2	C72	C61	H15	114.7
C53	C62	C81	114(5)	C53	C62	H76	120.4
C81	C62	H76	105.5	O6	C63	O11	111(3)
O6	C63	C45	107(3)	O6	C63	H16	115.6
O6	C63	H24	115.6	O11	C63	C45	104(2)
O11	C63	H16	108.9	O11	C63	H24	108.8

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
C45	C63	H16	108.6	C45	C63	H24	108.6
H16	C63	H24	0.0	C34	C65	H25	114.8
C34	C65	H101	107.9	C34	C65	H102	110.9
H25	C65	H101	113.6	H25	C65	H102	110.1
H101	C65	H102	98.4	C2	C68	C27	125(2)
C2	C68	C47	115(2)	C27	C68	C47	118(3)
C61	C69	C76	93(3)	C61	C69	H77	88.9
C76	C69	H77	149.1	C1	C71	H94	123.3
C1	C71	H95	112.5	C1	C71	H96	115.4
H94	C71	H95	101.4	H94	C71	H96	103.8
H95	C71	H96	96.4	C48	C72	C61	122(4)
C48	C72	H78	113.9	C61	C72	H78	123.2
O116	C73	O117	128(5)*	O116	C73	C80	128(4)
O117	C73	C80	101(3)	C4	C74	C23	120(3)
C4	C74	C66	106(3)	C23	C74	C66	99(4)
O115	C75	H41	103.6	O115	C75	H42	98.2
O115	C75	H43	122.3	H41	C75	H42	94.1
H41	C75	H43	121.3	H42	C75	H43	111.5
C47	C76	C69	141(4)	C47	C76	H79	125.6
C69	C76	H79	89.4	C9	C79	C81	102(3)
C9	C79	H80	120.9	C81	C79	H80	134.9
O11	C80	C52	106(2)	O11	C8O	C73	101(2)
O11	C80	H26	111.1	C52	C80	C73	113(3)
C52	C80	H26	115.6	C73	C80	H26	108.2
C62	C81	C79	130(5)	C62	C81	H81	131.2
C79	C81	H81	86.0	O112	C82	O113	133(5)*

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
O112	C82	C60	126(4)	O113	C82	C60	97(3)
O117	C83	H49	101.5	O117	C83	H50	100.0
H49	C83	H50	100.5	O113	C85	H97	106.2
O113	C85	H98	96.9	O113	C85	H99	110.3
H97	C85	H98	95.8	H97	C85	H99	134.4
H98	C85	H99	105.7	O119	C86	H58	150.8
O119	C86	H59	95.7	O119	C86	H60	97.2
H58	C86	H59	105.4	H58	C86	H60	107.6
H59	C86	H60	74.8	C1	C93	C15	122(3)
C1	C93	C99	117(3)	C15	C93	C99	119(3)
C15	C94	C30	121(3)	C15	C94	C43	125(2)
C30	C94	C43	111(3)	C7	C96	C8	126(2)
C7	C96	C32	117(2)	C8	C96	C32	116(2)
C9	C98	C25	119(2)	C9	C98	C29	107(2)
C25	C98	C29	131(2)	O1	C99	C93	123(3)
O1	C99	C102	120(2)	C93	C99	C102	116(3)
O2	C100	C24	119(2)	O2	C100	C45	124(2)
C24	C100	C45	115(2)	O13	C101	C28	118(4)
O13	C101	C60	110(3)	O13	C101	H19	118.2
O13	C101	H28	118.2	C28	C101	C60	105(3)
C28	C101	H19	105.3	C28	C101	H28	105.4
C60	C101	H19	94.6	C60	C101	H28	94.6
H19	C101	H28	0.0	C30	C102	C37	119(3)
C30	C102	C99	122(3)	C37	C102	C99	118(3)
O118	C105	O119	121(4)*	O118	C105	C52	121(4)
O119	C105	C52	113(4)	C94	C43	H7	98.2

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angleatom	atom	atom	angle	
C94	C43	H22	98.2	C94	C43	C78	100(2)
C94	C43	C104	115(3)	C94	C43	C103	111(3)
H7	C43	H22	0.1	H7	C43	C78	81.8
H7	C43	C104	37.6	H7	C43	C103	145.1
H22	C43	C78	81.8	H22	C43	C104	37.7
H22	C43	C103	145.1	C78	C43	C104	109(3)
C78	C43	C103	109(3)	C104	C43	C103	109(3)
H17	C78	H48	95.7	H17	C78	C43	114.9
H17	C78	H103	18.1	H17	C78	H104	120.1
H17	C78	H47	91.6	H48	C78	C43	119.0
H48	C78	H103	83.9	H48	C78	H104	25.6
H48	C78	H47	121.6	C43	C78	H103	109.5
C43	C78	H104	109.5	C43	C78	H47	109.5
H103	C78	H104	109.5	H103	C78	H47	109.5
H104	C78	H47	109.5	H7	C104	H22	0.1
H7	C104	C43	37.5	H7	C104	H71	114.4
H7	C104	H72	73.2	H7	C104	H70	132.1
H22	C104	C43	37.3	H22	C104	H71	114.4
H22	C104	H72	73.3	H22	C104	H70	132.0
C43	C104	H71	109.5	C43	C104	H72	109.5
C43	C104	H70	109.5	H71	C104	H72	109.5
H71	C104	H70	109.5	H72	C104	H70	109.5
C43	C103	H68	109.5	C43	C103	H69	109.5
C43	C103	H67	109.5	H68	C103	H69	109.5
H68	C103	H67	109.5	H69	C103	H67	109.5

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
C74	C66	C77	103(6)	C74	C66	C84	89(9)
C74	C66	C90	133(8)	C77	C66	C84	109(11)
C77	C66	C90	109(9)	C84	C66	C90	109(14)
C66	C77	H46	109.5	C66	C77	H44	109.5
C66	C77	H45	109.5	H46	C77	H44	109.5
H46	C77	H45	109.5	H44	C77	H45	109.5
C66	C84	H53	109.5	C66	C84	H54	109.5
C66	C84	H52	109.5	H53	C84	H54	109.5
H53	C84	H52	109.5	H54	C84	H52	109.5
C66	C90	H66	109.5	C66	C90	H64	109.5
C66	C90	H65	109.5	H66	C90	H64	109.5
H66	C90	H65	109.5	H64	C90	H65	109.5
C41	C58	C57	109(12)	C41	C58	C46	109(14)
C57	C58	C46	109(10)	C58	C41	H29	109.5
C58	C41	H5	109.5	C58	C41	H30	109.5
H29	C41	H5	109.5	H29	C41	H30	109.5
H5	C41	H30	109.5	H9	C57	C58	143.5
H9	C57	H13	34.2	H9	C57	H34	93.3
H9	C57	H12	88.1	C58	C57	H13	109.5
C58	C57	H34	109.5	C58	C57	H12	109.5
H13	C57	H34	109.5	H13	C57	H12	109.5
H34	C57	H12	109.5	C58	C46	H10	109.5
C58	C46	H105	109.5	C58	C46	H106	109.5
H10	C46	H105	109.5	H10	C46	H106	109.5
H105	C46	H106	109.5	C33	C39	C87	116(2)

Table 4. Bond Angles(°) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
C33	C39	C64	106(2)	C33	C39	C59	105(2)
C87	C39	C64	109(2)	C87	C39	C59	109(2)
C64	C39	C59	109(2)	C39	C87	H62	109.5
C39	C87	H63	109.5	C39	C87	H61	109.5
H62	C87	H63	109.5	H62	C87	H61	109.5
H63	C87	H61	109.5	C39	C64	H39	109.5
C39	C64	H40	109.5	C39	C64	H38	109.5
H39	C64	H40	109.5	H39	C64	H38	109.5
H40	C64	H38	109.5	C39	C59	H35	109.5
C39	C59	H36	109.5	C39	C59	H37	109.5
H35	C59	H36	109.5	H35	C59	H37	109.5
H36	C59	H37	109.5				

*restrained during refinement

Table 5. Torsion Angles(°)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
O1	C18	C5	C44	-169(3)	O1	C18	C5	C54	-3(6)
O1	C18	C17	C1	-1(7)	O1	C18	C17	C36	-178(3)
O1	C90	C93	C1	-1(4)	O1	C90	C93	C15	178(3)
O1	C90	C102	C30	179(2)	O1	C90	C102	C37	1(4)
O2	C11	C32	C4	-177(2)	O2	C11	C32	C96	-2(4)

Table 5. Torsion Angles(°) (continued)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
O2	C11	C49	C23	177(2)	O2	C11	C49	C34	0(4)
O2	C100	C24	C13	-177(3)	O2	C100	C24	C34	-9(4)
O2	C100	C45	C20	-171(3)	O2	C100	C45	C63	-2(4)
O6	C52	C80	O11	23(4)	O6	C52	C80	C73	-87(3)
O6	C52	C105	O118	-30(5)	O6	C52	C105	O119	170(3)
O6	C63	O11	C80	-19(3)	O6	C63	C45	C20	-96(4)
O6	C63	C45	C100	95(3)	O11	C63	O6	C52	34(3)
O11	C63	C45	C20	22(5)	O11	C63	C45	C100	-146(3)
O11	C80	C52	C105	-98(3)	O11	C80	C73	O116	-13(5)
O11	C80	C73	O117	179(2)	O12	C54	O13	C101	34(3)
O12	C54	C5	C18	-121(4)	O12	C54	C5	C44	39(6)
O12	C60	C82	O112	-8(6)	O12	C60	C82	O113	155(3)
O12	C60	C101	O13	24(3)	O12	C60	C101	C28	-105(3)
O13	C54	O12	C60	-19(3)	O13	C54	C5	C18	131(4)
O13	C54	C5	C44	-67(7)	O13	C101	C28	O114	-23(6)
O13	C101	C28	O115	151(3)	O13	C101	C60	C82	-90(3)
O112	C82	O113	C85	-14(6)	O112	C82	C60	C101	99(5)
O113	C82	C60	C101	-95(3)	O114	C28	O115	C75	-7(5)
O114	C28	C101	C60	102(5)	O115	C28	C101	C60	-83(4)
O116	C73	O117	C83	13(6)	O116	C73	C80	C52	100(4)
O117	C73	C80	C52	-66(3)	O118	C105	O119	C86	20(7)
O118	C105	C52	C80	84(4)	O119	C105	C52	C80	-75(4)
N5	C2	C55	C12	20(4)	N5	C2	C68	C27	-14(6)
N5	C2	C68	C47	166(3)	N5	C16	C12	C55	8(4)
N5	C16	C37	C22	11(6)	N5	C16	C37	C102	-174(4)
N6	C22	C37	C16	-12(7)	N6	C22	C37	C102	173(4)

Table 5. Torsion Angles(°) (continued)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
N6	C22	C40	C50	3(4)	N6	C29	C50	C40	-4(4)
N6	C29	C98	C9	-171(3)	N6	C29	C98	C25	-4(6)
N7	C7	C38	C19	4(5)	N7	C7	C96	C8	-4(6)
N7	C7	C96	C32	179(3)	N7	C27	C19	C38	-1(5)
N7	C27	C68	C2	8(7)	N7	C27	C68	C47	-173(3)
N8	C8	C10	C3	9(4)	N8	C8	C96	C7	8(6)
N8	C8	C96	C32	-175(3)	N8	C25	C3	C10	11(5)
N8	C25	C98	C9	167(3)	N8	C25	C98	C29	2(6)
C1	C17	C18	C5	-169(3)	C1	C93	C15	C94	-176(2)
C1	C93	C99	C102	177(2)	C2	N5	C16	C12	4(4)
C2	N5	C16	C37	-177(4)	C2	C55	C12	C16	-17(4)
C2	C68	C27	C19	-179(4)	C2	C68	C47	C48	-114(3)
C2	C68	C47	C76	78(4)	C3	C10	C8	C96	-174(4)
C3	C25	N8	C8	-4(4)	C3	C25	C98	C9	-13(7)
C3	C25	C98	C29	-178(4)	C4	C32	C11	C49	-6(5)
C4	C32	C96	C7	64(4)	C4	C32	C96	C8	-112(3)
C4	C74	C23	C49	5(5)	C4	C74	C66	C77	23(8)
C4	C74	C66	C84	133(9)	C4	C74	C66	C90	-110(14)
C5	C18	O1	C99	-158(2)	C5	C18	C17	C36	13(7)
C5	C54	O12	C60	-135(3)	C5	C54	O13	C101	147(4)
C7	N7	C27	C19	3(4)	C7	N7	C27	C68	178(4)
C7	C38	C19	C27	-1(5)	C7	C96	C8	C10	-167(3)
C7	C96	C32	C11	-111(4)	C8	N8	C25	C98	174(3)
C8	C10	C3	C25	-12(5)	C8	C96	C7	C38	179(3)
C8	C96	C32	C11	72(5)	C9	C31	C53	C62	-7(6)
C9	C79	C81	C62	-16(7)	C9	C98	C29	C50	6(4)

Table 5. Torsion Angles(°) (continued)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
C10	C3	C25	C98	-167(4)	C10	C8	N8	C25	-3(4)
C10	C8	C96	C32	8(5)	C11	O2	C100	C24	-21(4)
C11	O2	C100	C45	159(3)	C11	C32	C4	C74	4(4)
C11	C49	C23	C74	-6(4)	C11	C49	C34	C24	-30(4)
C11	C49	C34	C51	-142(3)	C11	C49	C34	C65	86(3)
C12	C16	C37	C22	-171(3)	C12	C16	C37	C102	3(6)
C12	C55	C2	C68	179(3)	C13	C24	C34	C49	-162(3)
C13	C24	C34	C51	-41(5)	C13	C24	C34	C65	77(4)
C13	C24	C100	C45	1(4)	C13	C33	C20	C45	-1(5)
C13	C33	C39	C87	126(3)	C13	C33	C39	C64	-111(3)
C13	C33	C39	C59	4(4)	C14	C44	C5	C18	-44(16)
C14	C44	C5	C54	150(14)	C15	C93	C1	C17	-154(3)
C15	C93	C1	C42	97(3)	C15	C93	C1	C71	-17(4)
C15	C93	C99	C102	-2(5)	C15	C94	C30	C102	0(5)
C15	C94	C43	C78	28(4)	C15	C94	C43	C104	146(3)
C15	C94	C43	C103	-87(3)	C16	N5	C2	C55	-16(4)
C16	N5	C2	C68	-174(3)	C16	C37	C22	C40	-179(3)
C16	C37	C102	C30	113(4)	C16	C37	C102	C99	-68(5)
C17	C1	C93	C99	25(3)	C17	C18	O1	C99	30(5)
C17	C18	C5	C44	0(6)	C17	C18	C5	C54	166(4)
C18	O1	C99	C93	-27(4)	C18	O1	C99	C102	153(3)
C18	C17	C1	C42	81(5)	C18	C17	C1	C71	-153(5)
C18	C17	C1	C93	-26(5)	C19	C27	C68	C47	-1(7)
C10	C38	C7	C96	-179(4)	C20	C33	C13	C24	15(5)
C20	C33	C39	C87	-56(3)	C20	C33	C39	C64	65(3)
C20	C33	C39	C59	-178(2)	C20	C45	C100	C24	10(5)

Table 5. Torsion Angles(°) (continued)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
C22	N6	C29	C50	6(4)	C22	N6	C29	C98	-175(3)
C22	C37	C102	C30	-72(6)	C22	C37	C102	C99	105(5)
C22	C40	C50	C29	0(4)	C23	C49	C11	C32	6(5)
C23	C49	C34	C24	151(2)	C23	C49	C34	C51	40(4)
C23	C49	C34	C65	-91(3)	C23	C74	C4	C32	-4(4)
C23	C74	C66	C77	149(7)	C23	C74	C66	C84	-100(9)
C23	C74	C66	C90	15(15)	C24	C13	C33	C39	-167(3)
C24	C100	C45	C63	178(2)	C25	N8	C8	C96	-179(4)
C25	C98	C9	C31	-52(4)	C25	C98	C9	C79	100(4)
C25	C98	C29	C50	173(4)	C27	N7	C7	C38	-4(4)
C27	N7	C7	C96	178(3)	C27	C68	C2	C55	-170(4)
C27	C68	C47	C48	66(5)	C27	C68	C47	C76	-99(4)
C28	C101	O13	C54	84(4)	C28	C101	C60	C82	139(3)
C29	N6	C22	C37	-174(5)	C29	N6	C22	C40	-6(4)
C29	C98	C9	C31	116(3)	C29	C98	C9	C79	-91(3)
C30	C94	C15	C93	-2(5)	C30	C94	C43	C78	-165(3)
C30	C94	C43	C104	-46(4)	C30	C94	C43	C103	78(4)
C30	C102	C90	C93	0(4)	C31	C9	C79	C81	-18(4)
C31	C53	C62	C81	-22(7)	C32	C4	C74	C66	106(4)
C32	C11	O2	C100	-157(2)	C32	C11	C49	C34	-170(3)
C32	C96	C7	C38	3(5)	C33	C13	C24	C34	-177(3)
C33	C13	C24	C100	-13(5)	C33	C20	C45	C63	-177(3)
C33	C20	C45	C100	-9(5)	C34	C24	C100	C45	169(2)
C34	C49	C23	C74	171(3)	C36	C17	C1	C42	-101(3)
C36	C17	C1	C71	23(6)	C36	C17	C1	C93	151(3)
C37	C16	C12	C55	-169(3)	C37	C22	C40	C50	174(3)

Table 5. Torsion Angles(°) (continued)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
C37	C102	C30	C94	179(3)	C37	C102	C90	C93	-178(3)
C38	C10	C27	C68	-175(5)	C40	C22	C37	C102	6(7)
C40	C50	C29	C98	178(3)	C42	C1	C93	C90	-83(3)
C45	C20	C33	C30	-179(3)	C45	C63	O6	C52	148(2)
C45	C63	O11	C80	-135(3)	C47	C48	C72	C61	-1(5)
C47	C68	C2	C55	11(5)	C47	C76	C69	C61	-21(7)
C48	C47	C76	C69	25(7)	C48	C72	C61	C69	2(7)
C40	C11	O2	C100	29(4)	C40	C11	C32	C96	169(3)
C49	C23	C74	C66	-109(4)	C49	C34	C24	C100	33(3)
C51	C34	C24	C100	153(2)	C52	C80	O11	C63	-3(3)
C52	C105	O119	C86	180.0	C53	C31	C9	C79	25(4)
C53	C31	C9	C98	175(2)	C53	C62	C81	C79	34(8)
C54	O12	C60	C82	115(3)	C54	O12	C60	C101	0(3)
C54	O13	C101	C60	-38(3)	C60	C82	O113	C85	-176(2)
C63	O6	C52	C80	-33(3)	C63	O6	C52	C105	84(3)
C63	O11	C80	C73	115(2)	C65	C34	C24	C100	-87(3)
C68	C47	C48	C72	-174(3)	C68	C47	C76	C69	-167(5)
C71	C1	C93	C99	161(3)	C72	C48	C47	C76	-8(4)
C72	C61	C60	C76	5(6)	C73	C80	C52	C105	150(3)
C74	C4	C32	C96	-172(2)	C75	O115	C28	C101	178(4)
C80	C73	O117	C83	-179(4)	C81	C79	C9	C98	-170(2)
C93	C15	C94	C43	162(3)	C94	C15	C93	C90	3(5)
C94	C30	C102	C99	1(5)	C102	C30	C94	C43	-167(2)

Appendix B: List of Commonly used Abbreviations

AMB	2-(aminomethyl)benzimidazole
appar	apparent
atm	atmospheric pressure
BOC	*tert*-butoxycarbonyl
bp	boiling point
calcd	calculated
cat	catalytic
CE	capillary electrophoresis
CB-Cl	chlorocatecholborane
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DIEA	diisopropylethyl amine
DMAP	4-dimethylamino pyridine
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DPPA	diphenylphosphoryl azide
EI	electron impact
ESI	electrospray ionization
equiv	equivalent(s)
FAB	fast atom bombardment
h	hour(s)
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HRMS	high resolution mass spectrometry
HPLC	high pressure liquid chromatography
LRMS	low resolution mass spectrometry
min	minute(s)
mp	melting point
mtr	4-methoxy-2,3,6-trimethylbenzenesulphonyl
NBA	*m*-Nitrobenzyl alcohol
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
Pyr	pyridine
rt	room temperature
sat	saturated
TCE	trichloroethyl
SiO_2	silica gel
TEA	triethylamine
TFA	trifluoroacetic acid
TLC	thin layer chromatography
THF	tetrahydrofuran
TMEDA	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine