

Commentary

Peptoid Origins

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ABSTRACT:

Peptoid oligomers were initially developed as part of a larger basic research effort to accelerate the drug-discovery process in the biotech/biopharma industry. Their ease of synthesis, stability, and structural similarity to polypeptides made them ideal candidates for the combinatorial discovery of novel peptidomimetic drug candidates. Diverse libraries of short peptoid oligomers provided one of the first demonstrations in the mid-1990s that high-affinity ligands to pharmaceutically relevant receptors could be discovered from combinatorial libraries of synthetic compounds. The solid-phase submonomer method of peptoid synthesis was so efficient and general that it soon became possible to explore the properties of longer polypeptoid chains in a variety of areas beyond drug discovery (e.g., diagnostics, drug delivery, and materials science). Exploration into protein-mimetic materials soon followed, with the fundamental goal of folding a non-natural sequence-specific heteropolymer into defined secondary or tertiary structures. This effort first yielded the peptoid helix and much later the peptoid sheet, both of which are secondary-structure mimetics that are close relatives to their natural counterparts. These crucial discoveries have brought us closer to building proteinlike structure and function from a non-natural polymer and have provided great

insight into the rules governing polymer and protein folding. The accessibility of peptoid synthesis to chemists and nonchemists alike, along with a lack of information-rich non-natural polymers available to study, has led to a rapid growth in the field of peptoid science by many new investigators. This work provides an overview of the initial discovery and early developments in the peptoid field. © 2010 Wiley Periodicals, Inc. *Biopolymers (Pept Sci)* 96: 545–555, 2011.

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INITIAL DEVELOPMENT OF PEPTOIDS AT PROTOS/CHIRON

Peptoids were originally developed in the late-1980s as part of the nascent small molecule drug-discovery program at a small start-up company called Protos Corp. in Emeryville, CA. It was widely recognized at the time in the biotechnology industry that the revolution in genetic engineering was generating a huge number of potential drug targets at a furious pace. New receptors, viruses, enzymes, and growth factors were being identified, produced, and validated, and yet the relatively young biotech industry did not have the means or the infrastructure to develop small molecule drugs that would bind/inhibit/stimulate these targets. Furthermore, a wealth of protein structural

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information was becoming available from NMR and X-ray crystallography, enabling new large-scale efforts in structure-based drug design. There became an industry-wide opportunity for biotech companies to expand into the pharmaceutical space by embracing chemistry-based technologies. One of the biggest impediments to this shift by the biotech industry, however, was a lack of chemistry expertise and a lack of access to small molecule compound collections from which to identify and develop drug leads.

Chiron Corp., one of the original biotechnology companies, found themselves in exactly this situation and, in 1988, spun out a chemistry-based start-up company called Protos to develop small molecule drugs using Chiron's biopharmaceutical resources. Headed by Prof. Dan Santi, a small group, consisting of synthetic chemists, computational chemists, and assay development/screening scientists were pulled together to take on this challenge.¹ It became clear very quickly that the most significant hurdle to compete in the drug-discovery race was access to large collections of small molecule compounds to screen. Large pharmaceutical companies had highly valued, proprietary compound collections containing hundreds of thousands of small organic molecules amassed over decades, giving them a major advantage in discovering starting points for the discovery of new drugs. This imbalance sets the stage for smaller companies to develop new technologies based on combinatorial chemistry to rapidly and cheaply generate proprietary compound collections.

The original inspiration for combinatorial drug-discovery technologies came, in part, from the observation that in the pharmaceutical industry, random high-throughput screening of large compound collections would often yield excellent starting points for drug development. At a more fundamental science level, chemists and biologists began to examine the molecular diversity found in biological systems, and how it could be understood, harnessed, and engineered. For example, if a population of a billion different antibody sequences circulating in our immune system could almost always produce a tight binder to a foreign molecule, could a large enough collection of synthetic molecules produce a high-affinity ligand to a given protein target for cancer? To see if this could be done, we set out to synthesize large, defined collections of diverse small molecules and screen them for biological activity. Our approach was to mimic the way nature creates molecular diversity in its biopolymers: to combine a small number of biochemical building blocks into all possible oligomeric sequences to generate a very large number of distinct chemical entities. This modular approach is efficient from a synthetic point of view, because it uses the same coupling chemistry to link every building block together. Efficiency was a key driver at the time, as it could

allow a small team of chemists to leverage an investment in a small number of new reactions and yet be able to generate very large numbers of compounds. Thus, we set out to develop new modular synthetic routes to novel (and proprietary) oligomer classes as well as technologies to facilitate the iterative and laborious process of combinatorial library synthesis.

Short peptide and nucleic acid oligomers were known to bind a wide variety of molecular targets, but they suffered from poor pharmacokinetic properties. We therefore focused efforts on developing peptidomimetics that were as close to polypeptide structure as possible, retaining the chemical diversity and spacing of side chains, as well as a polar backbone, while engineering out protease degradability. We considered many possible oligomer structures that could be accessed by modular synthesis and preserved as much peptidic character as possible.² Retaining an amide-based backbone for ease of synthesis, we focused our efforts on a family of compounds we called "peptoids" (the name was coined by one of our advisory board members, Prof. Paul Bartlett), where we simply moved the side-chain point of connectivity to the backbone (as found in peptides) by one atom: from the alpha carbon to the amide nitrogen.³ The resulting structures are repeating units of *N*-substituted glycine (see Figure 1). Essentially, a peptoid is just a regioisomer of a peptide. A consequence of changing the point of side-chain connectivity is that both a hydrogen bond donor (the NH group) and the chiral center at the α -carbon are lost. It was also expected that peptoids would be more flexible than peptides, in part because the tertiary backbone amides could more easily adopt both *cis* and *trans* conformations. Although these losses were thought to be a potential liability, there were also potential advantages: the installation of a substituent directly on the amide nitrogen was hoped to block the action of proteases. This would address one of the biggest perceived liabilities of peptides as drug candidates. Furthermore, the increased flexibility could be an asset to finding leads in high-throughput screening.

INITIAL SYNTHESIS STRATEGY

We first approached the synthesis of peptoid oligomers in direct analogy to the well-established Merrifield method of solid-phase peptide synthesis (SPPS).⁴ We envisioned that peptoids could be prepared from a set of Fmoc-protected monomers and that these could be coupled to a growing resin-bound chain using conventional activating agents.³ Our first thoughts toward the synthesis of screening libraries was to keep the molecular weight below 500, a value suggested by the size of most known orally bioavailable drugs, which meant that we would need only string three monomers together at the most. With only three couplings, the

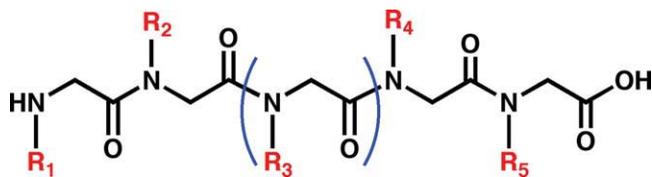


FIGURE 1 Structure of a generic peptoid oligomer. The repeating monomer unit, an *N*-substituted glycine, is enclosed in parentheses. Side-chain substituents, shown in red, can be diverse in structure and arranged in particular sequences.

yield for the addition of each monomer did not have to be absolutely quantitative. This was an important consideration, given that the growing N-terminus of a peptoid chain is a secondary amine, which is more hindered, and should result in slower coupling reactions when compared with the primary amines found in SPPS. Monomer selection was guided by the functional groups found on the side chains of naturally occurring amino acids. To minimize the synthetic burden, we used a reduced monomer set, choosing only 10–12 representative monomers instead of the full 20. Even so, this required the significant synthetic challenge to generate the ~25 g needed of each of the 12 representative peptoid monomers as Fmoc-protected (and side chain protected as needed) *N*-substituted glycine monomers. Enough monomer was needed to cover not just the library synthesis, but also the resynthesis of any hits that would be discovered. This effort, led by Dr. Reyna Simon, took the better part of one year. All the monomers had to be in hand before we could even begin the new work of library synthesis.

Faced with the technical challenge of synthesizing a combinatorial peptoid library, we saw two options. We could either mix all 12 monomers together and couple this mixture to the resin (called the “mixed amino acid” method) or we could keep them separated and perform 12 parallel coupling reactions and then mix all the product resins together (called the “mix and split” or “resin splitting” method). The mixed amino acid method was certainly far easier to perform, as only one reaction was needed per cycle. The downside of the method was that the monomers, typically used in excess, would compete with each other for a limited number of resin sites, and they have inherently and often widely varying coupling rates. The pairwise coupling rates for conventional amino acids were actually determined⁵ with the idea that the differences in coupling rates could be compensated by adjusting the relative concentration of each monomer. My colleague Dr. Verena Huebner and I felt strongly, however, that the increased effort to do separate coupling reactions required by the mix-and-split method⁶ was well worth it, because each individual coupling reaction could be forced to completion,

thus allowing the precise construction of mixtures of equimolar composition. We were among the first groups to demonstrate that by using the mix-and-split method with normal amino acids, one could not only generate precisely equimolar peptide mixtures,⁷ but that affinity selection methods with a purified soluble protein target could be used to identify the highest affinity component within the mixture.^{7–9}

AUTOMATED SYNTHESIS TECHNOLOGIES

Although the mix-and-split method offered clear advantages in the precise control of library composition, we next had to face the fact that it was much more labor-intensive. Because our goal was to feed soluble equimolar peptoid mixtures to panels of high-throughput biochemical screening assays that were already developed, we next focused our efforts on automating the combinatorial synthesis of peptoids. Given the large diversity of compounds that could be generated via simple amide bond formation, we could see early on that the demand for not only combinatorial libraries, but for individual “hit” compounds as well, would only increase with time. Given my training as an organic chemist, and my hobbies of computer programming and auto mechanics, I saw a clear opportunity to enter the space of automated chemical synthesis, where few others had ventured.

At the time, automated chemical synthesizers were commercially available for peptide and oligonucleotide synthesis.¹⁰ Multiple peptide synthesizers were only just beginning to appear on the market, but none of them provided a means to perform the operations of the mixing and splitting of resin beads. I actually began to work on the automation of combinatorial synthesis on the very first day of my job at Protos, only a week after graduating from grad school. I built the first versions of the synthesizer (Figures 2a and 2b) with a jigsaw, hand drill, and a series of Teflon valves and tubing. We soon became the first group to build fully automated synthesizers that could generate combinatorial libraries of peptides and peptoids by the mix-and-split method.¹¹ Initial designs consisted of semiautomated workstations, where reagent addition was performed manually (Figures 2a and 2c). But before long, we used a Zymark robot to perform all steps including reagent addition, resin washes, resin mixing, and splitting and even cleavage/deprotection reactions (Figures 2b and 2d). We wrote our own software on a Macintosh IIfx computer equipped with a digital I/O card using Microsoft QuickBasic software at first and was later rewritten in C++ by Steve Banville and Michael Siani. We developed a fully automated method to split a batch of resin beads into equimolar portions via the volumetric distribution of

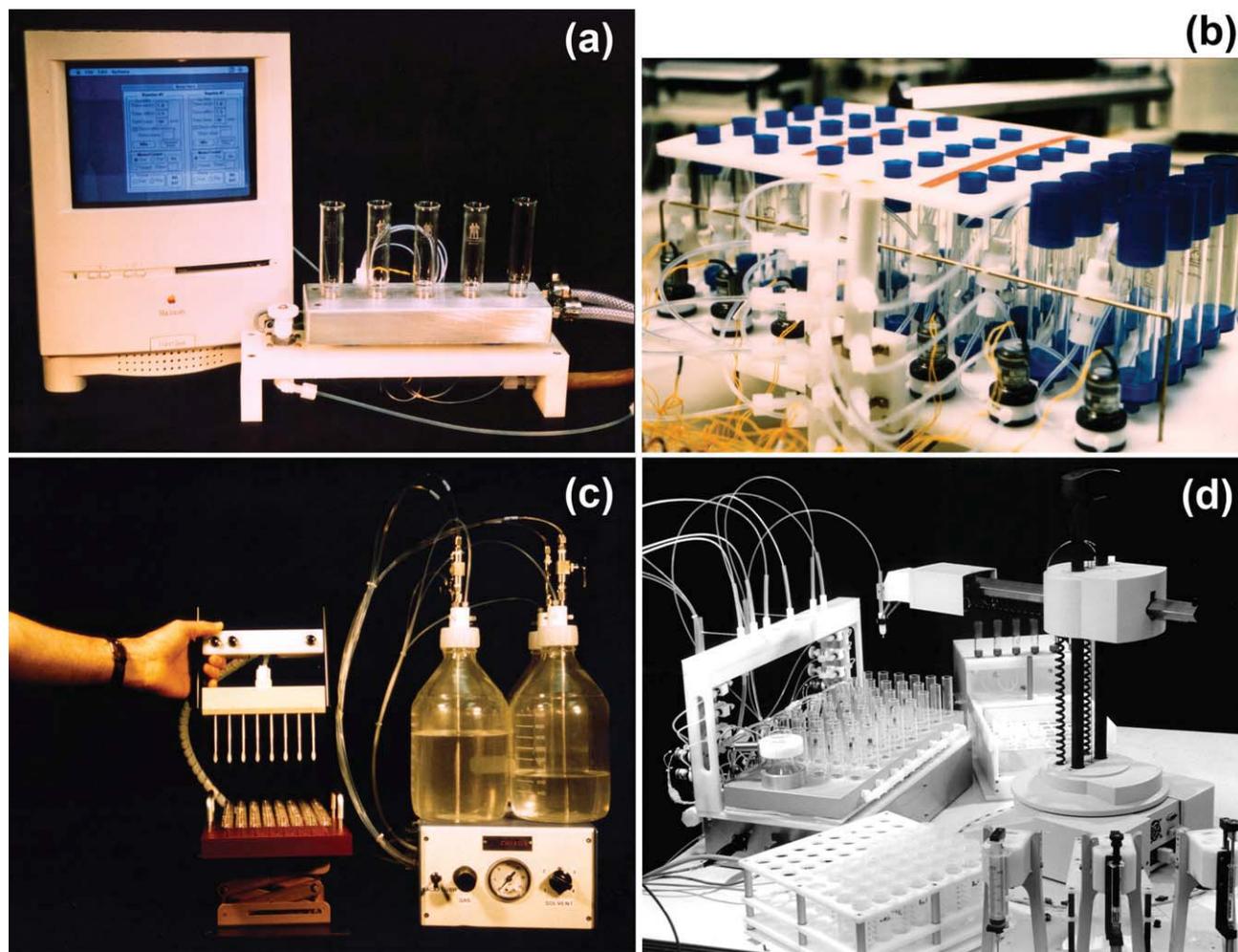


FIGURE 2 Automated and semiautomated tools for combinatorial peptoid synthesis were developed in the early 1990s to make synthetic oligomers easily accessible. (a) An early semiautomated reaction vessel station that heated and bubbled solid-phase synthesis reactions in fritted glass vessels. (b) The first working combinatorial synthesizer reaction block was custom built from fritted chromatography columns arranged in an array with a series of teflon solenoid valves under control by computer. Reagent and solvent addition were achieved using a Zymark Zymate II XP robot. (c) An eight-position solvent washing tool to facilitate solid-phase organic synthesis in vial array plates. (d) Second generation, re-engineered fully automated robotic synthesizers played a key role in producing combinatorial, equimolar peptoid mixtures, and macrobead libraries for Chiron's drug-discovery program.

an isopycnic resin slurry.^{12–15} Coupling reactions could be performed in parallel individual reaction vessels on each aliquot, and then the resin products could be recombined using the slurry method. We were able to demonstrate excellent synthesis performance with polypeptides at first and prove that we could rapidly generate both equimolar mixtures and individual compounds in parallel.¹¹ These robots and associated software have been improved, rebuilt, and rewritten from the ground up several times in subsequent years by my colleagues Joseph Ringgenberg, Michael Connolly, Bill Grikis, and Richard Graham. The current form of the synthesizer, based on a Cavro plat-

form, continues to be the peptoid synthesis workhorses in our laboratories today.

Armed with this synthesis technology, we were able to make combinatorial peptide and peptoid libraries extremely rapidly. In fact, we were able to consume a year's worth of carefully synthesized Fmoc-protected peptoid monomers in only a few days. This rate imbalance between monomer production and library synthesis was quite sobering. Moreover, another serious roadblock also became apparent: once the activity is identified in a combinatorial library, it can be very laborious to identify active compounds in the library (a process called deconvolution). Both of these issues forced us to

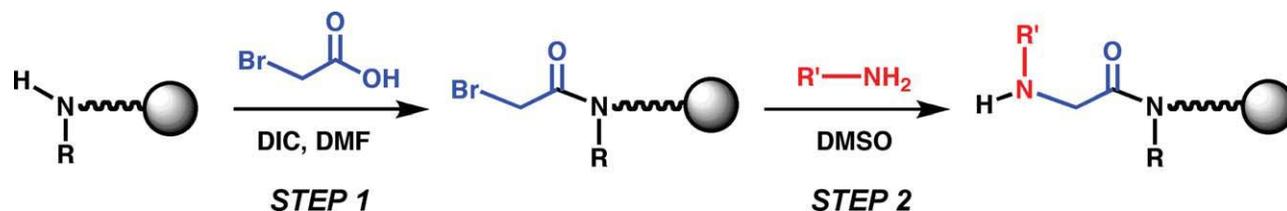


FIGURE 3 The solid-phase submonomer method allows the rapid synthesis of peptoids from cheap and readily available starting materials (submonomers) using a repeating two-step monomer addition cycle of acylation followed by nucleophilic S_N2 displacement. DIC, *N,N'*-diisopropylcarbodiimide; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide.

rethink our method of peptoid synthesis and spurred us to develop a much more efficient synthetic method.

THE SUBMONOMER METHOD OF PEPTOID SYNTHESIS

The aforementioned bottlenecks stimulated a line of work that led to a major breakthrough in 1991 when we developed the solid-phase “submonomer” method of peptoid synthesis.¹⁶ Our thinking was inspired by Prof. Steve Kent’s suggestion to examine methods for the ligation and cyclization of peptide fragments based on coupling nucleophiles to peptidic bromoacetamides.¹⁷ The idea was to elongate the main chain by an acylation reaction with a haloacetic acid and then introduce the side chain with a primary amine (see Figure 3). This approach obviates the need to prepare fully protected monomer units and uses smaller “submonomer” units instead. In fact, the submonomer concept originally dates back to Emil Fischer, who envisioned the synthesis of peptides by using ammonia to displace haloalkylamides.¹⁸ The submonomer method considers the peptoid structure not as a homopolymer of *N*-substituted glycine units, but rather as an alternating copolymer between acetate and amine units. No main-chain protecting groups are required, because each of the two chain-elongation steps are self-limiting.

In a very exciting period of only a couple of weeks, with the help of my research assistant Janice Kerr, we were able to reduce the submonomer idea to practice. With our investment in robotic synthesis hardware recently completed, we were in a position to rapidly explore a variety automated synthesis conditions. It required only a simple modification to the robotic synthesizer software, because it required the exact same hardware as SPPS (i.e., used the same reaction vessels, used the same number of reagents, the same solvents, and had a similar two-step monomer addition cycle). Thus, a variety of synthesis conditions could be tested in parallel. What’s more, we imposed a high stringency test for each set of conditions by making entire peptoid pentamers on the

robot. This way, instead of needing to determine the exact yield of each individual synthetic step, we could simply look at the final product purity to see if it worked. A pentapeptoid is the product of 10 chemical steps, and so the only way to get a clean full length final product at say 90% purity would be for each of those 10 steps to have a 99% yield. Because the primary analytical tool available in those days was analytical reverse-phase HPLC, we set up a variety of automated synthesis runs and looked for reaction products with high purity and relatively long retention times. Initial tests were performed with iodoacetic acid, bromoacetic acid, chloroacetic acid, and the standard amino acid activation conditions using *N,N'*-diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBt) in DMF. Simple well-behaved amines like benzylamine and cyclohexylamine were chosen as the initial amine submonomers. We chose to use haloacetic acids instead of their more reactive acid halide derivatives because of the automation requirement to store reagents on the instrument for long periods without degradation. A survey of conditions found dramatic differences in product yield. In fact, once the HOBt was omitted from the in situ activation reaction, exceptionally pure product yields of peptoid pentamer were obtained (see Figure 4)! Dozens of amines were then systematically put through test reactions, in which homopentamers were synthesized to understand the scope and limitations of the submonomer synthesis method. In an early attempt to estimate the coupling efficiency, we prepared a 25 mer, which was obtained with a 65% crude purity, indicating a >98% yield per monomer addition cycle¹⁶!

The submonomer synthesis monomer addition cycle consists of two chemical steps starting from a resin-bound amine: an acylation step using a haloacetic acid, followed by a displacement step using a primary amine (see Figure 3). The first step requires the acylation of a secondary amine, which can be sluggish to acylate (as compared to a primary amine in peptide synthesis). However, the submonomer method counters this, because once the haloacetic acid is activated with an activating agent, such as DIC to form an

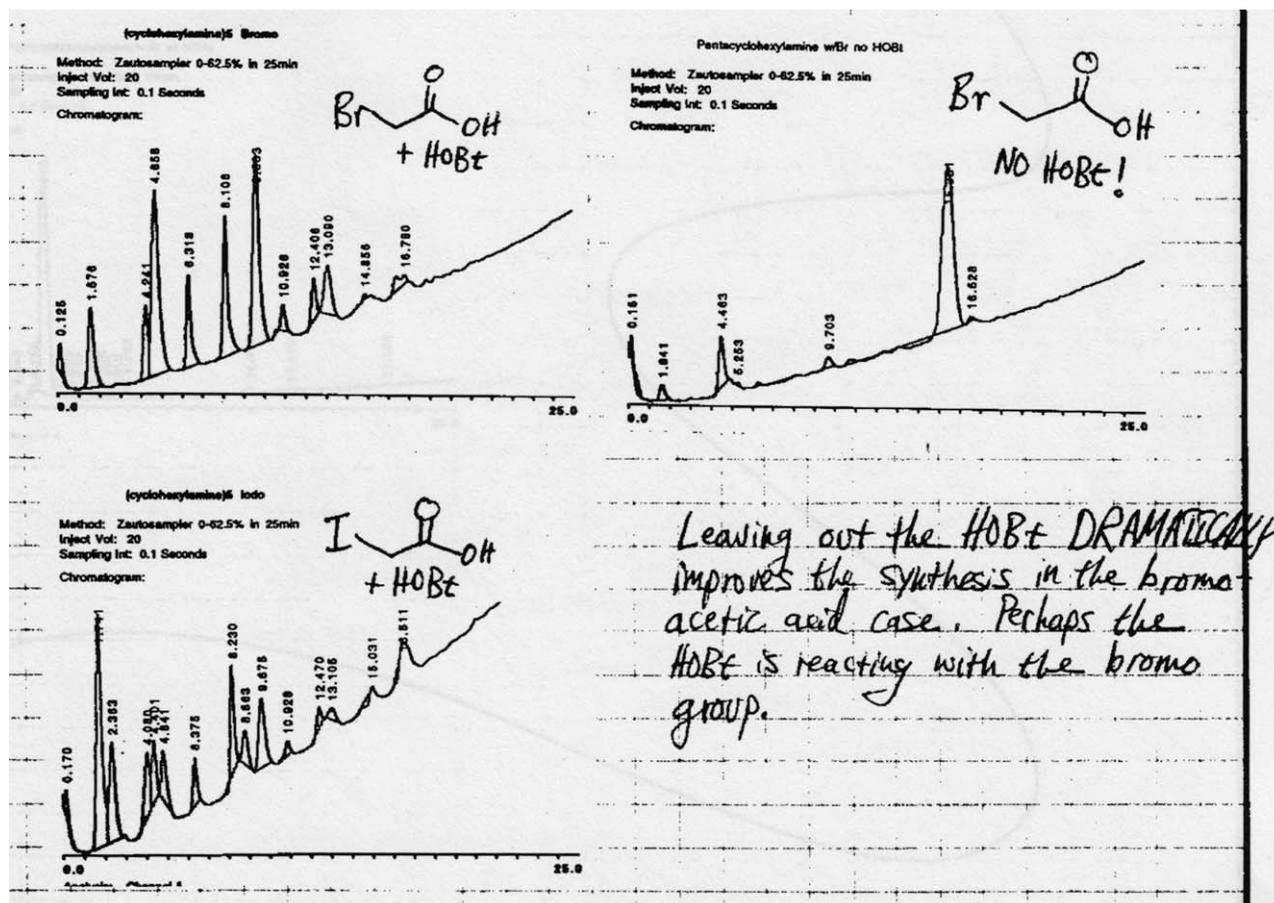


FIGURE 4 Laboratory notebook page showing the first successful synthesis of a peptoid pentamer H-(N-cyclohexylglycine)₅-NH₂ by automated submonomer synthesis in early 1992 (RZ-3926-155).

isourea, the electron withdrawing effect of the halogen makes the haloacetic acid a much hotter acylating reagent than a normal carboxylic acid or amino acid. Furthermore, the activated haloacetic acid is relatively unhindered and, in most cases, couples to secondary amines within seconds at room temperature. The desired reactivity can be tuned by choosing the appropriate halide. For general use, the bromide is preferred; however, in cases where there are side-chain functionalities that contain unprotected heteroatoms, especially those found in many heterocycles (e.g., imidazoles, pyridines, pyrazines, and indoles) the use of bromoacetic acid results in undesired side reactions due to alkylation of the heteroatoms by the bromide. In these cases, we later found that chloroacetic acid can be used,¹⁹ avoiding these unwanted alkylation reactions owing to the reduced leaving group ability of the chloride. An undesirable consequence of this is that the chloride also slows down the subsequent displacement step, but the rate of the displacement reaction can be accelerated by the addition of iodide ion to generate the iodoacetamide via in situ Finkelstein halide exchange.²⁰

The displacement reaction is a simple S_N2 reaction that results in the incorporation of the peptoid side-chain functionality. The beauty of this is that side chains of interest need only have a reactive primary amine in order to be incorporated into the peptoid structure. The significance of this was that it completely solved the problem of monomer availability. Now, we could build peptoids from all readily available materials!²¹ Not every amine can be incorporated in high yield; very poor nucleophiles or hindered amines are sluggish to incorporate, as are amines with poor solubility. Amines with competing pendant nucleophilic centers must be protected. The displacement reactions are typically slower than the acylation step, requiring reasonably high concentrations of amine (~1M) and reaction times of 20 min to 2 h at room temperature. Because the only by-product of the displacement reaction is one equivalent of HBr, the excess amine in the solution-phase can be recovered and reused. In fact, with the help of a team of undergraduates from Harvey Mudd College, we demonstrated that the amine in the reaction mixture can be recycled by pumping it back into its

original reagent reservoir and could be reused in multiple subsequent coupling reactions.²² As in SPPS, acid-removable side-chain protecting groups are typically used, so that global deprotection and resin cleavage can be affected simultaneously using a trifluoroacetic acid cocktail. There are literally many hundreds of amines commercially available in gram quantities, spanning a tremendous range of chemical diversity space, that can be used as submonomers.^{19,21,23–25}

The submonomer method is among the most efficient methods known to access synthetic oligomers of defined sequence, in terms of coupling yields and monomer diversity/availability. The reactions are not air-sensitive or particularly moisture sensitive. Importantly, the two-step submonomer cycle involves only the simple pipeting of stable reagent solutions at room temperature. Thus, the entire process was readily automated and even adapted to most any commercial peptide synthesizer, because peptide couplings also use a two-step monomer addition cycle.²¹ The longest peptoids reported in reasonable yield (that were synthesized in one shot) are about 50 monomers long,^{26,27} suggesting that the yield for one coupling cycle (for well-behaved amines) is in excess of 99%. The submonomer synthesis is also unique in that it requires no main-chain protecting groups. It takes full advantage of the “pseudo-dilution” effect,²⁸ where reactive intermediates are attached to the resin and cannot easily cross react with each other. Monoalkylation of an amine with an alkyl halide, for example, is difficult to control in solution, but on the resin the reaction can be pushed to completion. The reactive haloacetamide intermediate can also be used to conjugate peptoids to other things,²⁹ or as a starting point for more complex heterocycle syntheses.^{30–33} The submonomer method is still used today in very near its original form, despite 20 years of intensive use.²⁵

PEPTOID DRUG DISCOVERY EFFORTS AT CHIRON CORP

With the automation tools and the submonomer method in place, the next challenge in demonstrating the utility of peptoids was to produce a large collection of compounds and to screen them for biological activities. With the promise of this technology platform approaching fruition, Chiron Corp. purchased Protos in 1992 and morphed it into the Small Molecule Drug Discovery division under the direction of Dr. Walter Moos. In this way, all the biological resources of Chiron could be combined with the chemistry and combinatorial technology of Protos.

We first demonstrated that peptoids were in fact stable to degradation by a panel of proteases by synthesizing a series

of protease substrate analogs and showed that they were not touched by common proteases.^{34,35} To get an integrated drug-discovery program rolling, we chose to generate combinatorial compound mixtures at first because of the reduced up-front synthesis and screening effort required. Mixture complexity was kept to a modest level (typically 100's of compounds per pool). In the later years, with more people and infrastructure available, we developed macrobead-based methods³⁶ that allowed the screening of much smaller mixtures or even individual compounds. Mixture screening, of course, only identifies active mixtures and to identify an active individual compound requires deconvolution.³⁷ Library deconvolution was initially performed by the iterative resynthesis of successively smaller compound pools until an active single compound was found.³⁸

Under the direction of my assistant Gianine Figliozzi, a series of chemically diverse peptoid libraries were synthesized and used for general screening within Chiron. The libraries were also provided to collaborators as part of technology transfer partnerships. Each library was constructed from a basis set of 15–20 monomers and were typically trimeric to keep the molecular weight down.²¹ Sophisticated computational tools were also developed to help design the libraries. In an effort led by my colleagues, Drs. Eric Martin, Jeff Blaney, and David Spellmeyer, quantitative descriptors of molecular diversity were established that could characterize lipophilicity, shape and branching, chemical functionality, and other specific binding features.³⁹ In this way, one could systematically design maximally diverse libraries or focused libraries, depending on the need. By 1994, synthesis and screening efforts from diverse libraries had yielded several potent (nanomolar) peptoid trimer ligands for G-protein-coupled receptors³⁸ and the urokinase receptor.⁴⁰ Our 1994 report of an α -adrenergic receptor antagonist and a μ -opiate receptor antagonist was the first reported demonstration that a diverse combinatorial library of synthetic compounds could in fact provide high-affinity ligands for pharmaceutically relevant receptors.³⁸ This study helped inspire other groups throughout the biopharmaceutical industry to include combinatorial technologies in their drug-discovery programs. Academic laboratories also began to work on the discovery of potential therapeutics from peptoid libraries, notably the Messeguer⁴¹ and Kodadek⁴² laboratories. There was tremendous excitement in-house and industry-wide about the potential of combinatorial technologies, and it helped establish Chiron as a center of combinatorial technology innovation.^{43–45} Independent laboratories interested in other aspect of peptoids also began to emerge.^{46–48}

PROTEIN MIMETIC POLYMERS

As the development of peptoids as drugs continued^{49,50} and corporate partnerships and alliances were formed to leverage Chiron's leadership in combinatorial drug-discovery technologies, I was faced with growth opportunities in several opposing directions. Deep down, I was completely captivated by the basic science aspects of bioinspired polymer synthesis,⁵¹ and knew I had to follow the road of automated peptoid polymer synthesis to see where it would lead. As a result, I turned down management opportunities within the company in drug development to keep a smaller group focused on basic research.

I reached out to two visionary academic colleagues across the Bay at UC San Francisco, Profs. Fred Cohen and Ken Dill. Both were computational chemists/structural biologists who were developing computational tools to predict and explain the folding of proteins. We began what would be two very long-term collaborations on the fundamental implications raised by the emergence of higher molecular weight polypeptoid materials. We began to ask a whole series of new questions that could be answered by experiment for the first time. For example, how important are hydrogen bonding and chirality in the process of protein folding? Is it possible to synthesize a structurally well-defined protein-like folded tertiary structure from a non-natural sequence specific heteropolymer? Can we discover a new set of rules that govern the folding of a non-natural polymer? Can we create a new class of information-rich materials that bridge the gap between polymers and proteins?

To begin to answer some of these grand questions, we first sought to mimic peptide secondary structure: helices and sheets. A collaborative team was established, consisting of students and postdocs from the Dill and Cohen laboratories who performed their experimental work in my laboratories at Chiron. We convened the 1st Peptoid Summit to brainstorm practical strategies to address the challenge of building peptoids of defined conformation. Helices seemed the more approachable initial target, because they only involve the folding of a single strand. Sheets, by definition, involve the interaction of several strands, making it a more difficult problem to approach.

We first used computational tools to predict that helices could in fact form if we used repeating sequences of chiral, bulky side chains. Chirality was needed to enforce the propagation of the proper handedness, and the side-chain steric bulk was needed to influence the main-chain conformation at each residue. Chiral amines were examined for a balance between steric bulk and good chemical reactivity. Alpha-branched amines were known to be well tolerated in peptoid synthesis as long as the substituent was tied back into a ring

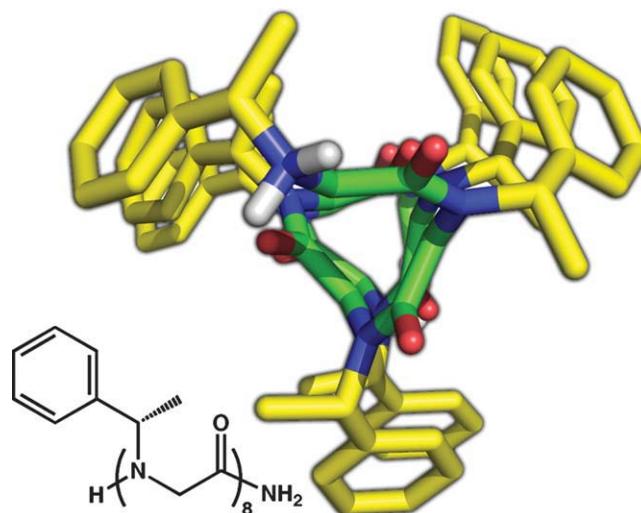


FIGURE 5 Molecular modeling of a peptoid octamer containing bulky, chiral *S*-1-phenylethyl side chains (yellow) predicted that a stable helix could form, despite being devoid of hydrogen bonds.

(e.g., cyclohexylamine or cyclopropylamine). For noncyclic amine substituents, the largest alpha branching group that could be well tolerated in the submonomer synthesis was a methyl group. Thus, the early work on peptoid helices was done with the readily available, chiral α -methylbenzylamine derivatives. An octamer model of (*S*)-*N*-(1-phenylethyl)glycine was built, and molecular dynamics calculations predicted that a stable helix, consisting of all *cis* amide bonds and having three residues per turn, would form⁵² (see Figure 5). Shortly thereafter, an experimental effort headed by Dill joint graduate student Kent Kirshenbaum and postdoc Annelise Barron demonstrated that long peptoids could in fact adopt a helical conformation by circular dichroism⁵³ and 2D NMR.^{54,55} We showed that the helical conformations were exceptionally stable and could persist with even very short main-chain lengths.

The discovery of the peptoid helix made it clear that many future directions were feasible to consider. Fundamental studies on the helix structural requirements and peptoid helix stability were soon addressed by the Barron and Kirshenbaum labs.^{56–60} A long and continuing effort began in my laboratories to study how amphiphilic helices could pack together to make functional protein-mimetic helix bundle tertiary structures.^{36,61,62} The search for practical applications also began toward the development of reagents that could facilitate the cellular delivery of nucleic acids^{26,63,64} and serve as molecular recognition and capture reagents for diagnostic tests.⁶⁵ We also established collaborations with other laboratories to explore the inhibition of protein–protein interactions,^{66,67} the development of vaccines,⁶⁸ antimicrobial

peptoids,⁶⁹ and lung surfactant mimetic⁷⁰ peptoids. Research continues today in these areas as we address fundamental problems in nanoscience, self-assembly, polymer physics, protein mimicry, and molecular recognition.⁷¹

CONCLUSION

Peptoids played a critical role in the development of combinatorial discovery technologies, primarily because of their simplicity and efficiency of synthesis. Peptoids were and still are one of the fastest ways to “prototype” new combinatorial approaches with synthetic molecules to discover new materials properties or biological activities. The structural similarity to polypeptides has been responsible for the impressive diversity of biological activities observed by a growing number of investigators.⁷² The ability to precisely engineer polypeptoid structure is unprecedented for a synthetic polymer and will no doubt open up many fundamental studies in polymer physics and polymer self-assembly. The discovery of peptoids nearly 20 years ago has enabled a rapidly expanding new field of applied and basic research that continues to grow to this day.²⁵

Ultimately, as the understanding of peptoid conformational control, chain folding, and self-assembly continues to grow, we expect to be able to generate a new family of advanced materials that rival the structure and function of proteins. Such protein-mimetic materials would be capable of sophisticated functions like molecular recognition and catalysis and yet would have enhanced stability to biological, chemical, and environmental stresses. Because long peptoid sequences can be made in good yield, and because so many different side chains can be incorporated with ease, there is great current interest in studying peptoids for their drug-delivery properties,⁶³ materials properties,⁷³ and their self-assembly into biomimetic nanostructures.^{62,74,75}

The field is actually in an unusual situation, in that the organic synthesis of polypeptoids is *not* limiting the field. The synthetic tools are well developed and easily adopted. The challenges are rather centered on how to design molecules with the desired properties and activities. Combinatorial screening methods will continue to be important, while the tools for prediction of peptoid structure and function catch up. Because peptoids are synthesized one monomer at a time from readily available building blocks, an almost infinite sequence-space awaits to be explored. It is a very exciting time for the field, as we see the immense gap between biopolymers and synthetic polymers begin to close. Pioneering work by a broad and growing group of investigators is continuing to address these, and many more new fundamental questions made possible by these bioinspired poly-

mers.^{25,49,76–79} Over 130 of these researchers, including professors, students, and postdocs, as well as industrial researchers had the opportunity to share the latest findings with each other in person at the 7th Peptoid Summit (<http://www.peptoids.org>) held at the Molecular Foundry in August 2010. Original research from the meeting has been collected in accompanying articles in this special issue of *Biopolymers: Peptide Science* devoted to work presented at the 7th Peptoid Summit.

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