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Synthesis and High Performance Liquid Chromatography/Electrospray Mass Spectrometry Single-Bead Decoding of Split-Pool Structural Libraries of Polyamines Supported on Polystyrene and Polystyrene/Ethylene Glycol Resins

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Natural polyamines are ubiquitous biomolecules present in all living cells. These cationic compounds play essential roles in both cell growth and differentiation and are known to interact in complex ways with polyanionic biomolecules. Consequently, there is significant interest in expanding nature's polyamine diversity using combinatorial synthesis and screening strategies. This article describes an efficient split-pool solid-phase synthetic strategy toward the generation of encoded libraries of polyamines via the exhaustive borane-promoted reduction of trityl-linked, resin-bound polyamides. Model structural libraries of tetra- and pentaamines were designed from a set of geometrically diverse amino acid building blocks. To encode the libraries, a partial termination synthesis approach was employed at the polyamide stage, allowing each library to be analyzed from single beads by HPLC/ESMS under two sets of conditions featuring both pH extremes. Determination of the sequence of polyamine residues was simply achieved by the mass differences observed between the full oligomers and the terminated ones. Both polystyrene- and TentaGel-supported libraries, including a library of 4913 pentaamines, were prepared and successfully decoded. For the TentaGel-supported libraries, suitable for on-bead aqueous screening of biomolecules, a novel trityl-derivatized resin was prepared in which the trityl group is anchored to the poly(ethylene glycol) chains via a methylene group. The resulting resin is much more resistant than other commercially available polystyrene-poly(ethylene glycol) trityl resins to the harsh borane reduction conditions required. Two workup conditions for the cleavage of the resultant borane-amine adducts were evaluated on the TentaGel bound polyamide **14**. Although the two methods showed a comparable efficiency when using the polystyrene support, with **14** it was found that the piperidine-exchange method afforded polyamines of higher purity than the iodine-based oxidative method previously developed in our laboratory.

Introduction

Natural polyamines are important in biology and medicine because they conduct essential roles in cell growth and differentiation.[1] They are also known to interact with ion channel proteins within the central nervous system and, thus, are the subject of much investigation in neurochemistry.[1−4] The rich biological properties of natural and synthetic polyamines have also been exploited in the development of potential antitumor[5] and antiinfective agents[6,7] and carriers for drug and gene delivery.[8] Since high concentrations of natural polyamines seem to be vital in the growth of cancer cells, various polyamine derivatives have been synthesized to inhibit polyamine biosynthesis and transport.[5,9,10] Putrescine, spermidine and spermine, shown in Figure 1 with their respective pK_a values, are the most ubiquitous of all the natural polyamines, present in both eukaryotic and prokaryotic cells in millimolar concentrations. The complex roles played by the biogenic polyamines in cell growth are unclear, but it is generally accepted that they are involved in controlling gene expression by increasing the rate and accuracy of transcription and translation.[1] At low concentrations, polyamines increase the rate of DNA polymerase activity, perhaps by inducing the dissociation of the polymerase from its complex with DNA. Polyamines may also help in DNA uncoiling, required for transcription, by interactions with topoisomerases, or by causing structural changes to RNA.[4] Under physiological conditions, polyamines are largely polycationic, giving them high affinity for polyanionic biomolecules, such as certain proteins, oligonucleic acids, and sulfated oligosaccharides.

Modifications to the polyamine chains in nature has led to the evolution of acylpolyamine neurotoxins isolated from the venom of wasps and spiders. Figure 1 depicts two examples: HO-416b, isolated from the funnel web spider *Hololena curta*,[11] and PhTX-433, from the Egyptian solitary digger wasp *Philanthrus triangulum*.[12,13] PhTX-433, for instance, is used by the wasp to paralyze its insect prey by inhibiting ionic conductance through cation channels gated by the nicotinic acetylcholine receptor (nAChR).[12] Interest in this class of polyamine in the area of neurochemistry has led to numerous syntheses of the natural compounds and sets of analogues both in solution and solid support,[14−22] including a recent contribution from our laboratory.[23]

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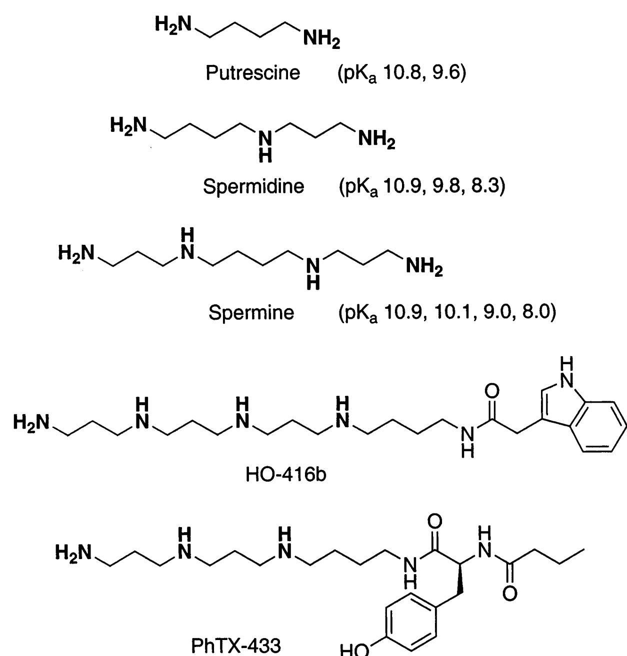


Figure 1. Naturally occurring polyamines with their pK_a's.

Given their biological importance, polyamines can be considered privileged structures in biology and medicinal chemistry, and an expansion of their diversity through combinatorial chemistry can potentially help uncover new biologically active compounds of this class. The synthesis of natural and unnatural polyamines both in solution and on solid support has been extensively reported in the literature and has been the subject of two recent review articles.[24,25] For the synthesis of a large library, solid-phase synthesis is the preferred method, for it allows for easy derivatization of the polyamines without resorting to tedious aqueous isolation procedures complicated by their amphiphilic nature. The majority of reported work done on solid support has basically relied on three methods. The first is the attachment of an advanced natural polyamine building block, like spermidine, to the resin, followed by derivatization and then cleavage.[14,26−28] This method is the simplest and leads to the synthesis of numerous polyamine conjugates via acylation of the amines. However, it is also limited by the diversity that can be incorporated between the internitrogen spacers on the polyamine chain. The second method is a sequence of protection, alkylation, and deprotection steps resembling the traditional methods used in solution-phase polyamine synthesis.[16,18,29−32] In principle, greater structural diversity can be obtained, but overall, this approach lacks convergence and is too laborious for synthesizing a very large library. The final method that has been reported is via the exhaustive reduction of a resin-bound polyamide using borane as the reducing agent.[33−36] This method appears to be the most suitable for library generation, since the peptide library intermediates can be easily prepared and diversified.[37] With these advantages in mind, we chose to employ the solid-phase polyamide reduction approach to generate our polyamine libraries.

Herein, we describe the design, synthesis and single-bead high performance liquid chromatography/electrospray mass spectrometry (HPLC/ESMS) decoding of split-pool structural libraries[38,39] of polyamines toward the aim of performing on-bead screening assays against polyanionic targets.

Results and Discussion

Synthetic Strategy for the Generation of Encoded Libraries. The synthetic plan involves initial production of a split-pool polyamide library assembled via standard Fmoc/peptide coupling chemistry. The amino acid building blocks used to construct the polyamides eventually become reflected in the polyamine library after the exhaustive amide reduction in a so-called "library-to-library" transformation.[40] To encode the libraries, a partial termination synthesis[41] was used whereby Fmoc-protected amino acids were coupled simultaneously with a small amount (∼10%) of the corresponding *N*-acyl amino acid. In applying this method, the two derivatives were assumed to provide a similar coupling rate, and the Fmoc protective group was removed to allow for the continuation of the synthesis while the leftover *N*-acyl group partially terminated the growing sequence. All sequences, completed and terminated, were then transferred from one library to the next. Thus, each bead-supported polyamine was decoded by electrospray mass spectrometry by calculating the mass differences observed between the truncated oligomers to determine the specific sequence of residues in that discrete library member. Electrospray was chosen as the ionization technique because it is a mild and highly sensitive mass-based detection method for charged molecules that provides minimal fragmentation and can be coupled easily with HPLC instruments. The advantages of this decoding technique over other indirect methods (examples: chemical,[42] RF,[43] or optical tags[44]) is that it also allows for the simultaneous evaluation of compound purity while being decoded, alleviating the need to make smaller "test libraries" of individual compounds for characterization. The main disadvantage is the unavoidable "impurities" that are accumulated by continually terminating the sequence, but this can be somewhat lessened by terminating very small amounts during the synthesis. Another disadvantage is in the use of isomeric (or "isobaric") building blocks which, of course, cannot normally be distinguished by mass spectrometry. However, this limitation can be solved in many cases by employing different terminating groups with different masses.

Initially, the libraries were prepared on polystyrene resin and then later made on the more hydrophilic TentaGel support, which is more suitable for screening in aqueous media against water-soluble biomolecules. In addition, we describe a "library-from-library" strategy in the derivatization of the polyamines to new polyacylated libraries with potentially unique biological and structural properties.

Amino Acid Building Blocks. A set of 14 straight-chain (long and short), cyclic and sterically natural and unnatural amino acid building blocks were chosen for our first polystyrene-supported model library (Figure 2). A number of factors determined the choice of these amino acids. First, they had to be readily available, either commercially or synthetically. The minimum derivatization required was either the attachment of the Fmoc protective group[45] or the encoding *N*-acyl group[46] onto a free amino acid. Some chiral

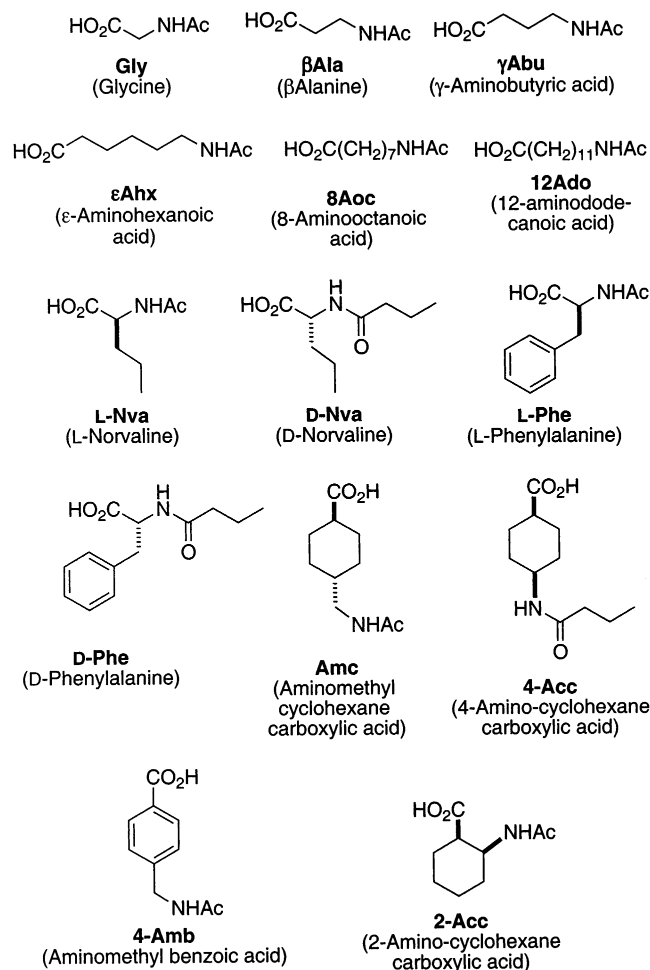
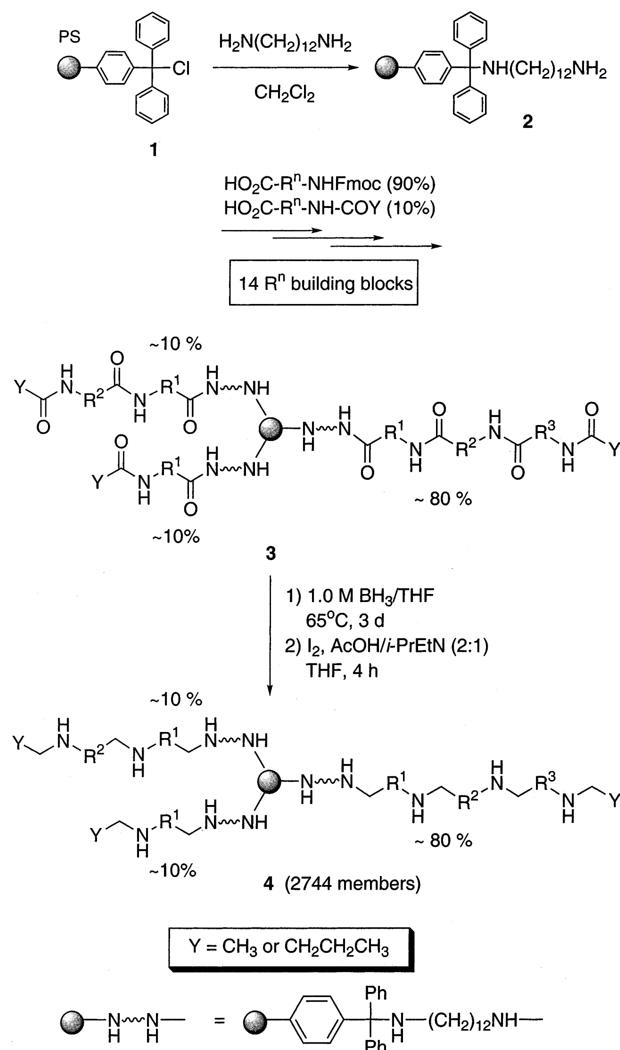


Figure 2. Fourteen amino acid building blocks (represented as their encoding acyl derivatives) used in the polystyrene-supported polyamine library.

α-amino acids were included because the solid-phase reduction methodology was previously shown to preserve their stereochemical integrity.[35,36] Yet, α-amino acids with elaborate side chains were avoided for the time being in order to preserve the backbone amines as the only functionality that can bind to a polyanionic target. In addition, the building blocks should not present any steric bulk that may affect any future derivatization of the polyamine (eg., valine). The building blocks also needed to be as structurally diverse as possible in order to cover a wide range of geometrical variations. Finally, the masses of the amino acids needed to be considered since our decoding method relies on mass spectrometry. By using two different *N*-acyl groups (acetyl and butyryl) we were able to include isomers of phenylalanine, norvaline, and aminocyclohexane carboxylic acid. However, other possible amino acid candidates had to be rejected because of conflicting masses with amino acids that have already been chosen. Using 14 amino acids, each coupled three times in a split-pool synthesis scheme, we assembled a library of 2744 tripeptides that can be converted into an equal number of pentaamines with a subsequent "library-to-library" transformation, exhaustive borane reduction.[40] Coupling each amino acid twice provides a 196-membered dipeptide library (i.e., triamide library) and, subsequently, the tetraamine.[47]

Scheme 1



Polyamine Library Synthesis on Polystyrene Resin.[48] The synthesis of the bead-supported library of 2744 pentaamines was carried out as shown in Scheme 1 with the initial set of 14 amino acid building blocks. The trityl linker was chosen because it allows the mild release of the final polyamines from the resin by exposure to dilute acid, which will facilitate the cleavage of single beads prior to LC/MS analysis. Furthermore, this linker was proven to be ideal in earlier solid-phase polyamide reductions in our laboratory.[35,36] Thus, from chlorotrityl polystyrene resin (**1**) (Rapp-Polymere, 90−150-μm diameter, 1.07 mmol g^{-1} loading), a 12-carbon diamine spacer was conjugated to give resin **2**. Such a long spacer should help minimize any possible interactions between the screening target and the bulky trityl group in on-bead assays. For the synthesis of a pentaamine library, an encoded tetraamide (acyl-capped tripeptide) library **3** was initially prepared. During the first two amino acid couplings, a 9:1 mixture of an Fmoc-protected amino acid and its corresponding *N*-acyl derivative were coupled in NMP (*N*-methylpyrrolidinone) using HBTU and HOBt as the coupling reagents. For the final amino acid coupling, only the *N*-acylamino acid was added. The tetraamide library was then converted to the pentaamine library **4** by exhaustive amide reduction with borane at 65 °C for 3 days followed by iodine-

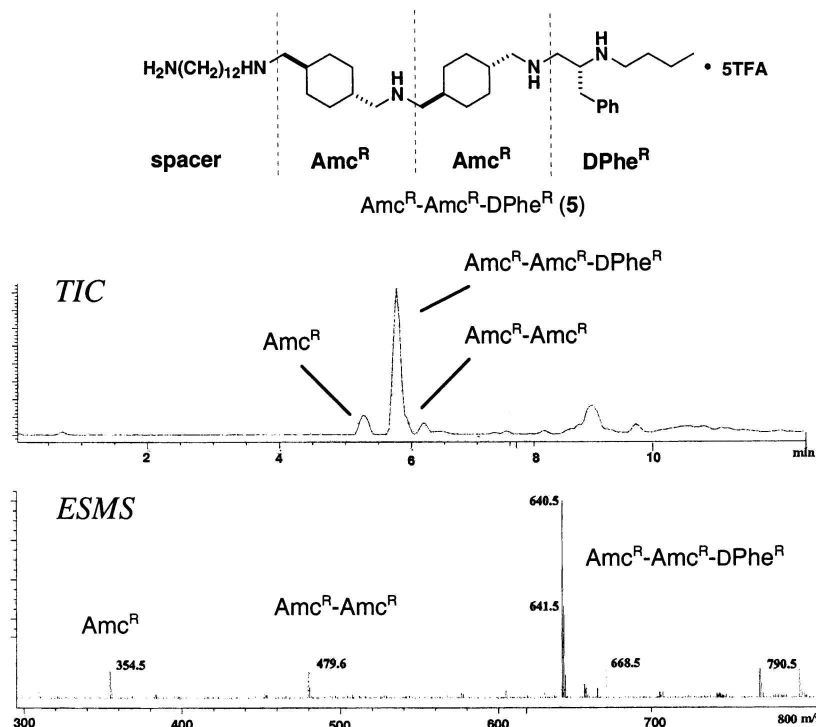


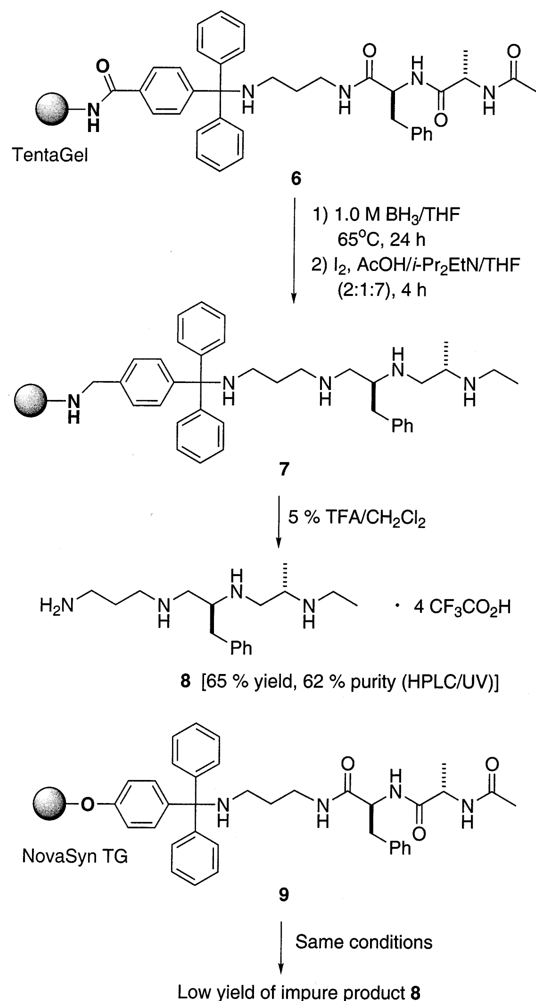
Figure 3. Sample LC/ESMS of pentaamine taken randomly from the split-pool polystyrene-bound library. The total ion chromatogram (TIC) is shown on the top, and the corresponding ESMS spectrum, on the bottom.

promoted cleavage of the resulting borane-amine adducts.[36] The triamide library (196 members) could be similarly obtained by removing a portion of the resin en route to the tetraamides and then converting it to the corresponding tetraamines. Alternatively, it can be made separately by first coupling the Fmoc and *N*-acylamino acid mixture and then the *N*-acyl amino acid by itself.

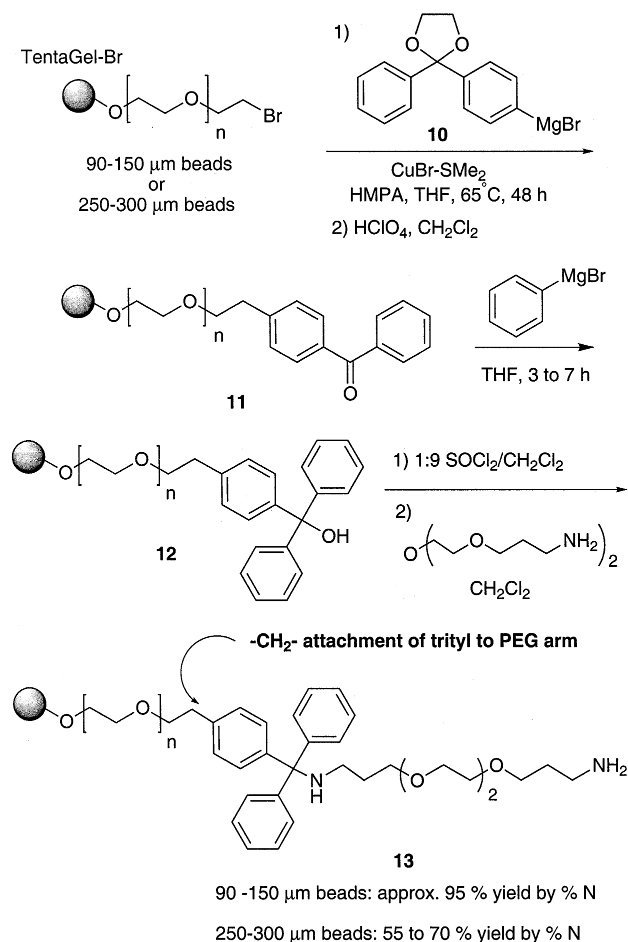
Of all of the individual beads isolated from the pentaamine library (over 50), ~80% of them were successfully decoded by LC/MS using 0.1% TFA in acetonitrile and water as the LC eluent (see Experimental Section for practical details on bead picking and full LC/MS conditions). An example of a decoding LC/MS chromatogram is illustrated in Figure 3 for the pentaamine sequence $Amc^R-Amc^R-DPhe^R$ (**5**) (the superscripted R denotes a reduced amino acid residue). The top section of Figure 3 shows the fully drawn structure of the pentaamine along with its written abbreviation (note that two of the amines arise from the diamine spacer, whereas the other three originate from the reduction of the amino acid building blocks, Amc and DPhe). The middle part of the figure is the total ion current (TIC) chromatogram, while shown on the bottom is the ESMS of the TIC peaks eluting between 5 and 6 min. Clearly observed in the mass spectrum are the two terminated sequences and the full pentaamine, along with a few minor impurities. At ~8.7 min in the TIC is another impurity peak, but an injection of a control sample (that is, everything except the bead) revealed that this component was not originating from the bead but, rather, likely contaminants from an external source (examples: syringe, vial, or solvent). These ubiquitous impurities were found to be unavoidable due to the minute quantities of material being handled. Although the terminated sequences and the presence of external impurities makes it difficult to assess polyamine purity at the single-bead level, larger-scale analyses have confirmed the reliability of this synthetic methodology.[36] Those pentaamines that were only partially decoded (12% in total) were due mainly to the lack of one of the terminated sequences in the mass spectrum, usually the first sequence. A small number of beads (8%) gave absolutely no MS signal, perhaps because of poor handling of the bead or its contents after it was cleaved. In some cases, the decoding was aided by the comparison of LC retention times with known sequences. Overall, although the decoding efficiency is quite acceptable, these results highlight the greater level of difficulty associated with decoding nonpeptidic molecules, especially multiply charged ones, at a single-bead level.

Polyamine Synthesis on TentaGel Resin. Prior to preparing a polyamine library bound to a hydrophilic support, an attempt was made in the exhaustive polyamide reduction and subsequent iodine-promoted workup on commercially available resins prederivatized with the trityl linker.[36] There are two methods used by commercial resin manufacturers to anchor the trityl linker to the PEG chains: via an amide bond or through a phenoxy ether linkage (Scheme 2). Using the amide-linked trityl chloride linker used in TentaGel resin from Rapp Polymere, a model triamide, LPhe-LAla-Ac **6** was prepared and then subjected to the usual borane treatment and iodine-promoted workup to furnish bound tetraamine **7**. The result after cleavage from the resin was crude polyamine **8** in 65% yield and 62% purity by HPLC/UV, which are both noticeably lower than our previous example on polystyrene (95% yield, 91% HPLC/UV purity).[36] An additional cause for concern was the formation of an undesired amine on the opposite side of the trityl group, an artifact from the reduction of the arylamide anchor, which would undoubtedly

Scheme 2



Scheme 3

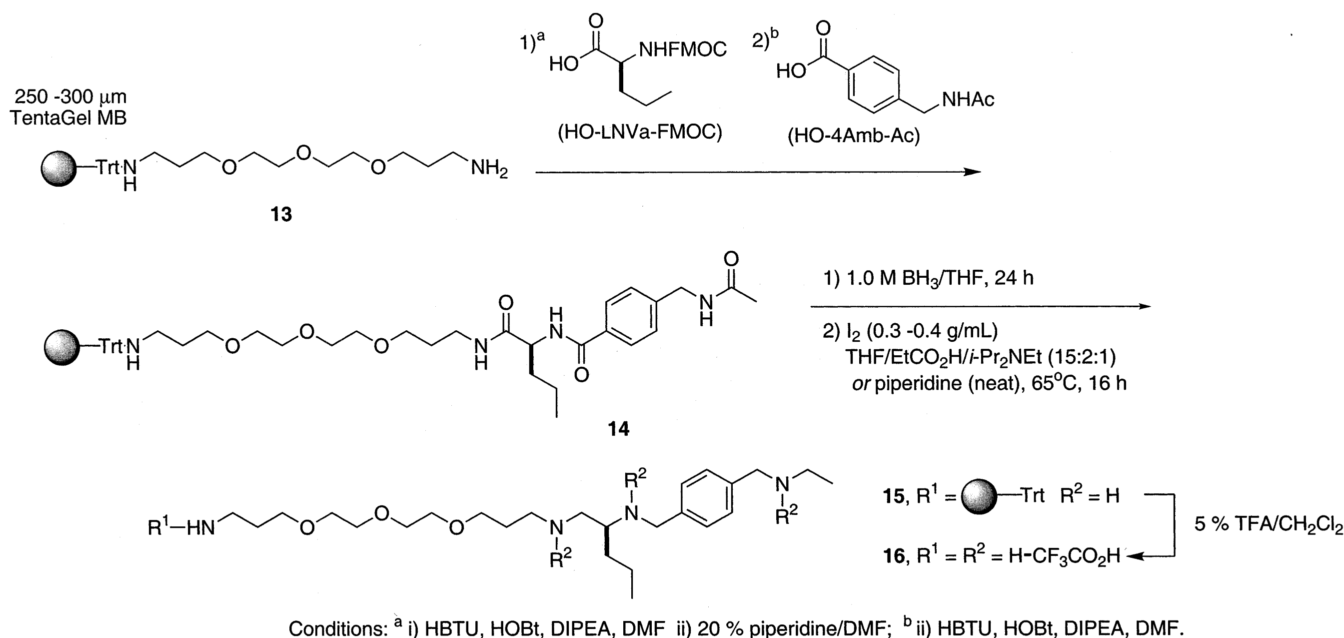


hamper any on-bead screening of a polyamine library. However, the outcome was no better with the phenoxy ether linkage used on NovaSyn TG resin (**9**). In this case, only a low yield of impure product was obtained. The possible occurrence of partial cleavage of the trityl resin anchor is the suspected cause for the lower yields and purity when using resins **6** and **9**.

Development of a Borane-Proof Trityl TentaGel Resin. In view of the disappointing results provided by the commercial resins, an alternative trityl-PS−PEG resin was sought that did not include the amide or phenoxy functionalities. It was anticipated that such a nonfunctionalized trityl resin anchor would be more stable to the borane reduction protocol and the ensuing workup. To this end, we looked at attaching the trityl linker to the PEG spacer through an inert methylene bridge. The synthesis of this resin (Scheme 3) was performed beginning with TentaGel bromide resin using two different bead sizes. Initially, the standard-sized resin (90−150-μm bead diameter) was employed, but the smaller capacity per bead on this resin (~90 pmol) compared to the equivalent sized polystyrene beads (~300 pmol) made single-bead LC/ESMS decoding more challenging in terms of instrument sensitivity. Thus, to aid in decoding of the polyamine, larger (250−300-μm) beads (TentaGel MB, ~2 nanomoles per bead) were also used. With the larger beads, the reaction times employed were slightly longer than the standard sized resin to account for the slower rates of reagent diffusion.

Beginning from TentaGel bromide resin, a copper-catalyzed Grignard reaction with **10** displaced the bromide, and after acid-mediated cleavage of the acetal protective group, the resin bound benzophenone **11** was obtained. Through elemental analysis, the amount of unreacted bromide on resin **11** was found to ranged from 7 to 0.4% on both standard and macrobead resins. Formation of hydroxytrityl resin **12** was achieved by reacting **11** with freshly prepared phenylmagnesium bromide. A successful reaction in this case was indicated by the disappearance of the carbonyl stretch and the appearance of the hydroxyl band in the FTIR spectrum of the resin. Activation of **12** by treatment with thionyl chloride gave the trityl chloride resin, which was subsequently coupled in this case with a PEG-mimicking diamine spacer to give **13**. The yields of the resulting diamine resin of standard bead size, starting from the brominated TentaGel, were consistently up to 95% according to elemental analysis on the nitrogen, while with the macrobeads, the yields ranged from 56 to 70%. The lower yield with the macrobeads may be attributed in part to the incomplete addition of the phenylmagnesium bromide to **11**, perhaps arising from an inability to constantly stir the resin due to its fragility (vide infra). In fact, a tiny amount of carbonyl stretching was observed by IR microscopy on beads analyzed after the reaction. Unfortunately, even longer reaction times did not improve the yields. According to subsequent decoding

Scheme 4



Conditions: [a] i) HBTU, HOBt, DIPEA, DMF ii) 20 % piperidine/DMF; [b] ii) HBTU, HOBt, DIPEA, DMF.

analyses, incomplete resin linker functionalization did not seem to make a significant effect on the purity of the finished polyamine libraries.

It was found by the end of the synthetic sequence that the resin becomes noticeably softer and easily breakable, presumably as a result of some of the harsh reaction conditions it was subjected to. This fragility meant that the use of stir bars and magnetic agitators, often employed in automated synthesizers, were avoided to prevent further damage to the resin beads to the point of making it impossible to conduct an on-bead screening assay. Even the act of weighing out the resin could result in damage to the beads, so extreme care was required at all times when working with it.

A model polyamine (**15**) was prepared in order to evaluate the efficiency of the synthetic sequence on the larger bead sizes. (Scheme 4). The triamide precursor **14** was prepared from resin **13** (250−300 μm) using the standard Fmoc coupling protocol with *N*-Fmoc-norvaline and *N*-acetyl-aminomethylbenzoic acid as the amino acid derivatives, and HBTU/HOBt as the coupling reagents. The choice of amino acid building blocks was based on the reasonable degree of complexity that they would provide to the model tetraamine, testing both the presence of a side chain and an aromatic residue, while keeping its NMR spectra relatively simple for characterization purposes. Exposure of triamide **14** to 1.0 M BH_3 in THF for 24 h at 65 °C was sufficient to afford complete reduction of all the amides. An amended iodine protocol for cleaving borane-amine adducts was employed with propionic acid instead of acetic acid in the buffer, a slightly higher concentration of iodine (0.3−0.4 g/mL), and a greater proportion of THF (THF/propionic acid/DIPEA ratio, 15:2:1). It was found that these conditions provide slightly more pure polyamine products on TentaGel, as compared to the conditions originally used in the case of polystyrene-supported compounds (0.2 g/mL I_2, 7:2:1 THF/acetic acid/DIPEA).[49] The bound tetraamine **15** was cleaved from the support to provide **16** as a tetrakis(trifluoroacetate) salt. LC/MS analysis of **16** gave only 59% purity by MS, 65% by UV (210 nm), with a significant amount of unidentifiable MH + 24 derivative despite the new workup conditions. The use of excess piperidine to cleave the borane-amine adducts led to polyamines of much higher purity (84% by MS, 90% by UV at 210 nm) with little detectable MH + 24 signal present in the mass spectrum.[50] The difference in purity was also noticed in the ^{1}H NMR spectra of **16**. The piperidine-exchange protocol led to a cleaner spectrum, as compared to that of the iodine-promoted treatment. It is suspected that the oxidative conditions of the iodine-based workup may induce partial cleavage of the poly(ethylene glycol) chains.

Synthesis and Decoding of TentaGel-Supported Polyamine Libraries. The relatively large proportion of flexible, linear building blocks in the original polystyrene supported library unfortunately restricted its structural diversity and, therefore, required attention before synthesizing a more advanced library on TentaGel beads. However, most commercially available amino acids are based on natural α-amino acids with elaborate side chains that would not satisfy our requirements for designing structural polyamine libraries. The exception is 3-aminomethylbenzoic acid (3-Amb), which recently became available (Figure 4). Furthermore, what was also needed to optimize structural diversity was building blocks that complement the many long and flexible ones already in use. To this end, we prepared two long and rigid biphenyl amino acids (1,4-Amp and 1,3-Amp), via Suzuki cross-coupling chemistry, both as their Fmoc derivatives for polyamide elongation and in their *N*-acylated form required for the termination synthesis and decoding (Figure 4).[51]

With a new set of 17 building blocks in hand, we were ready to prepare a more advanced 289-membered tetraamine library and a 4913-membered pentaamine library on TentaGel support. As described in Scheme 1 for the polystyrene-supported libraries, but this time using resin **13**, a partial termination synthesis strategy was employed in the initial

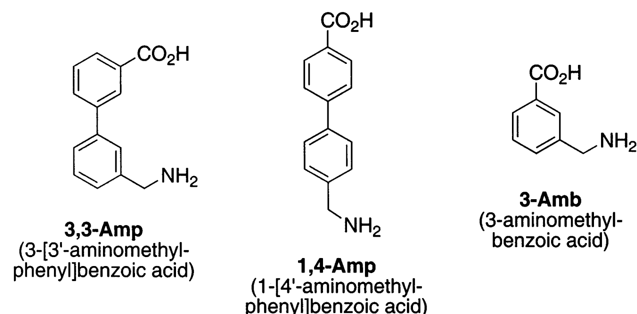


Figure 4. Three additional building blocks used in TentaGel-supported polyamine libraries.

building of the polyamides using a 9:1 mixture of Fmoc protected amino acid and the corresponding *N*-acyl derivative. The exhaustive amide reduction was followed with either the iodine-based workup method adapted for the TentaGel resin or with the piperidine-exchange protocol.

The challenge in decoding the standard sized, 90−150 μm TentaGel beads is the reduced loading (~0.3 mmol/g), as compared to the polystyrene beads used earlier (~1.0 mmol/g). This difference meant that smaller amounts of material would need to be analyzed by LC/MS, especially the minor terminated sequences that may be present in no more than a quantity of 2−3 pmol. To improve LC/MS sensitivity, attention was focused on the 0.1% TFA component used earlier in the eluent (pH ~2) for the polystyrene-bound libraries to ensure that all polyamines elute as their ammonium salts. TFA is known to suppress ionization of the analyte within the mass spectrometer by forming strong ion pairs in the gas phase that are not detected by the mass analyzer.[52] With the polystyrene-bound libraries, the relatively large amounts of compound on a single bead were sufficient to compensate for the ionization suppression, and therefore, the TFA counterion was not a serious problem during decoding. However, with the TentaGel-supported pentaamine library, very little signal was observed by LC/MS from a single bead when using TFA in the eluent. Rather than eluting the polyamines as their salts, it was decided to elute and analyze them as their free bases by using high pH conditions with an appropriate column (see Experimental Section for detailed conditions). When single beads were analyzed with 0.02 M ammonium hydroxide in the eluent (pH ~11), the intensity of the signals was much greater, as compared to the acidic conditions; however, the chromatographic resolution was extremely poor, giving very broad peaks. Fortunately, peracetylation of the secondary amines with acetic anhydride prior to cleavage from the bead greatly improved the resolution. This method provided monoamine derivatives (the primary amine attachment to the linker) that gave nice, sharp and intense peaks in the eluent containing ammonium hydroxide. Single resin beads from both the tetraamine and pentaamine libraries were reliably decoded using these basic chromatographic conditions. Polyacetylation of the entire resin-bound library prior to single bead isolation tended to give better decoding efficiencies (~100%) and cleaner LC traces than that of individually peracetylated single beads. An example of a decoded tetraacetylated pentaamine library member from a single bead is shown in Figure 5 for the sequence βAla^{R-Ac}-3,3-Amp^{R-Ac}-1,4-Amp^{R-Ac} (**17**, the superscripted R-Ac denotes an acetylated

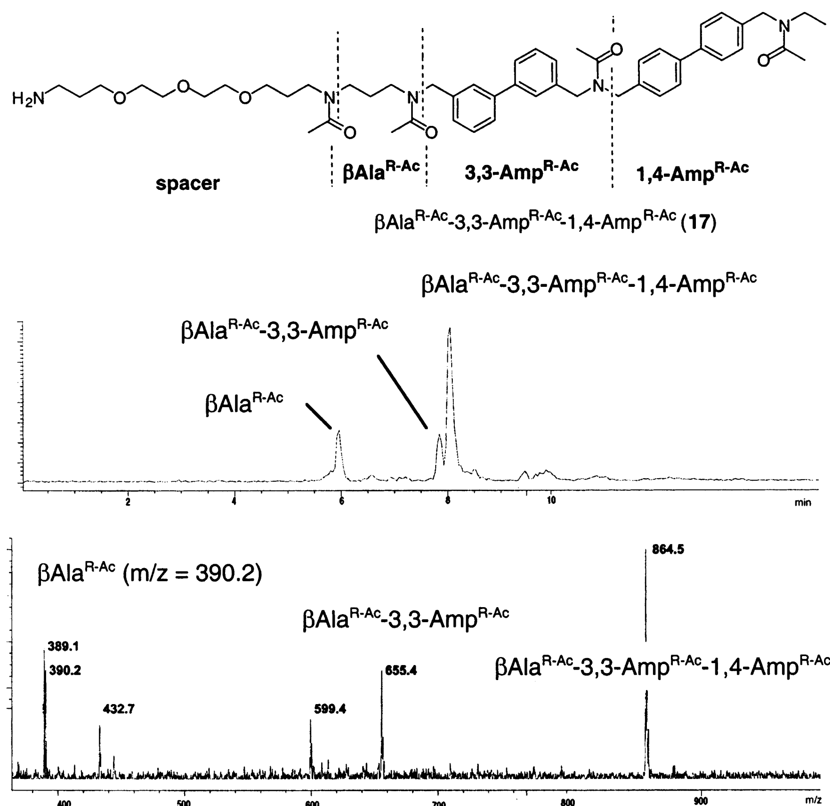


Figure 5. Sample LC/ESMS, run under high-pH conditions, of peracetylated pentaamine taken randomly from the split-pool TentaGel (90−150 μm)-bound library. The total ion chromatogram (TIC) is shown on the top, and the corresponding ESMS spectrum, on the bottom. Note: m/z = 389.1 is the doubly charged ion of **17**; 432.7 and 599.4 are fragmentation signals of **17**.

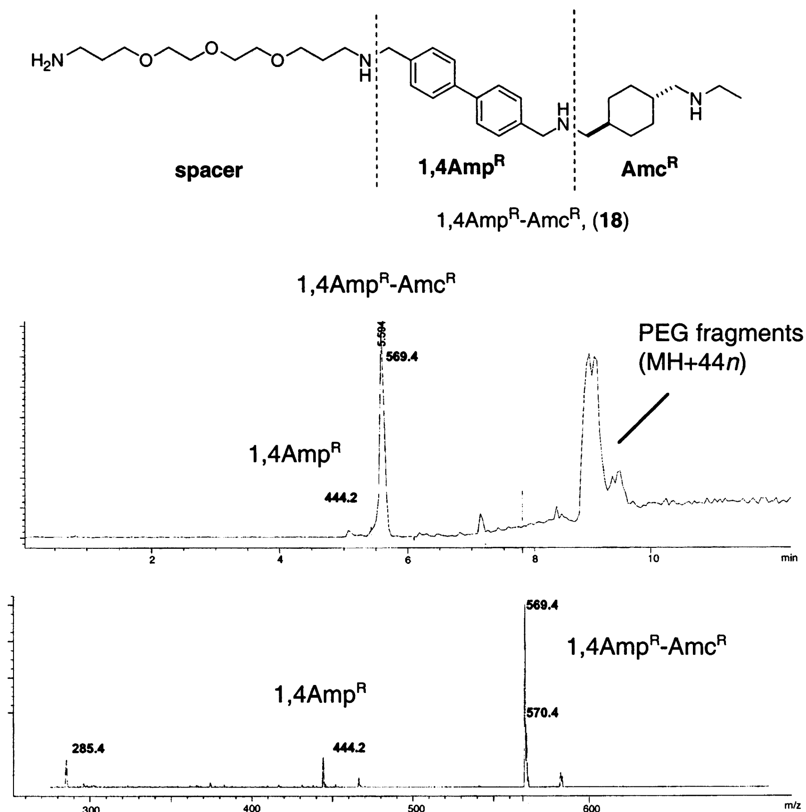


Figure 6. Sample LC/ESMS, run under low-pH conditions, of tetraamine taken randomly from the split-pool TentaGel MB (250−300 μm)-bound library. The total ion chromatogram (TIC) is shown on the top, and the corresponding ESMS spectrum, on the bottom. Note: m/z = 285.4 is the doubly charged ion of **18**.

reduced amine). It was found common to have the full sequences show a doubly charged ion in the mass spectrum ([M + 2H]/2) along with some fragmentation signals. In this particular example, it is noteworthy that chromatographic resolution helped uncover the mass of the first terminated sequence, which was buried underneath the doubly charged ion of the full sequence in the mass spectrum.

Although the standard sized (90−150 μm) TentaGel beads were eventually decoded, it was rather inconvenient to peracetylate polyamines on individual beads. Furthermore, peracetylation would prevent us from making other derivative libraries by alkylation of the secondary amines. Therefore, the switch was made to larger, 250−300-μm beads that have a much larger capacity as compared to the standard-sized beads and, therefore, assist in the direct decoding of polyamines without the need for precolumn derivatization.

With these larger beads, only a tetraamine library was synthesized. The amide reductions were carried out using both the iodine-based method and the piperidine-exchange method as workups. The decoding was performed on underivatized beads using the same LC/MS conditions that were employed with the polystyrene library, that is, 0.1% TFA in the eluent of acetonitrile and water. In general, it was again found that tetraamine beads produced on the PS−PEG resins via the piperidine-based workup gave higher quality LC/MS traces. Although the iodine-based method did provide some good quality traces, there were many that contained unidentifiable signals, including some with the previously mentioned MH + 24 signal. In addition, the iodine tended to stain the beads very slightly after the workup, resulting in an intense background fluorescence when viewed under an epifluorescence microscope. This strong background can be problematic when screening the library against fluorescently tagged targets. The decoding efficiency for tetraamines from both the iodine and piperidine methods was about ∼80%. Occasionally, beads would be encountered that gave absolutely no signal. In these cases, there may have been nothing on the bead to begin with, or a problem may have occurred in the sample handling or injection (example: insolubility in methanol, incomplete cleavage from the bead, etc). As commonly encountered with PEG containing supports, in many LC traces, some PEG polymer (MH + 44n) eluted after the tetraamine sequences, presumably from the breakdown of the resin matrix. An example is shown in Figure 6 for the tetraamine sequence 1,4-Amp^R-Amc^R (**18**) produced using the piperidine exchange workup.

Conclusion

A partial termination encoding split-pool synthesis of polyamide libraries followed by exhaustive amide reduction was employed to generate bead-supported libraries of polyamines. Model structural libraries of tetra- and penta-amines (containing up to 4913 members) were designed from a set of geometrically diverse amino acid building blocks. The use of HPLC/ESMS was successfully demonstrated in the decoding of polyamine libraries supported on both polystyrene and TentaGel resins. In the case of the TentaGel-supported libraries, an alternative approach for anchoring a trityl linker to the poly(ethylene glycol) chains was devel-

oped. The resulting PS−PEG trityl resin better withstood the harsh conditions of the borane-promoted amide reduction and subsequent workup. The decoding efficiencies on the polystyrene-supported polyamine libraries were ∼80% using low-pH chromatographic conditions. With the TentaGel-supported libraries, the lower bead capacity of the standard 90−150-μm beads was overcome by precolumn derivatization through peracetylation and then the use of high-pH chromatographic conditions. With these conditions, the sequence and identity of close to 100% of all beads from a representative sample of a pentaamine library were unambiguously decoded. However, larger sized 250−300-μm TentaGel beads of much higher capacity were also employed in the polyamine library synthesis and were successfully decoded without the need for peracetylation and using the previous low-pH chromatographic conditions. In general, the use of the piperidine-exchange method for the workup of exhaustive borane-promoted amide reductions led to cleaner polyamines after cleavage from the TentaGel support, as compared to the use of an iodine-based oxidative workup protocol. With its successful optimization on hydrophilic supports, this combinatorial synthetic approach to expand Nature's polyamine diversity is suitable for on-bead aqueous screening of water-soluble biomolecules. With these libraries in hand, we project to screen polyanionic targets of biological interest toward the discovery of novel polyamine ligands via selective multivalent ion-pairing interactions. In particular, the sulfated glycosaminoglycan, heparin, and nucleic acids are targets currently under investigation.

Experimental Section

General. Commercially available Fmoc-protected amino acids and *N*-acetyl amino acids were purchased from Novabiochem (La Jolla, California) or Advanced Chemtech (Louisville, Kentucky). Those Fmoc amino acids not commercially available were prepared using the procedure of Lapatsanis et al.[45] Polystyrene resins (90−150 μm) were purchased from Rapp-Polymere (Tübingen, Germany) or Novabiochem, whereas TentaGel resins (90−150 and 250−300 μm) were purchased from Rapp-Polymere exclusively. THF was dried by distillation over sodium/benzophenone; CH_2Cl_2, pyridine, and triethylamine, over calcium hydride. Anhydrous DMF and NMP were obtained commercially from Aldrich (Oakville, Ontario) and stored at 4 °C to minimize decomposition to dimethylamine and carbon dioxide. HPLC-grade solvents (acetonitrile, and methanol) were obtained from Caledon (Edmonton, Alberta) and used without further purification. HPLC-grade deionized water was obtained by passage of distilled water through a Millipore Simplicity water purification system. Borane−tetrahydrofuran complex (1.0 M) was obtained from Aldrich in 100-mL bottles and stored at 4 °C. Peptide coupling reagents, HBTU and HOBt·H_2O, were purchased from Novabiochem and used without further purification. Parallel reactions in some cases were performed using an Argonaut Quest semiautomated synthesizer. Loading of resins was based on the initial loadings provided by the commercial supplier unless stated otherwise. Yields of products cleaved off the resin are crude yields unless stated otherwise. HPLC/ESMS analyses were performed on a Hewlett-Packard/Agilent 1100 MSD using an atmospheric pressure ionization (API) spray chamber. LC and MS conditions for each LC/MS/UV analysis are described separately for each compound or library.

1,2-Diaminodedacane Trityl Polystyrene Resin (2). In a 100 mL silanized round-bottom flask, 1,12-diaminododedacane (12.5 g, 62.6 mmol) was dissolved in 60 mL of dry CH_2Cl_2 (turbid solution). Chlorotrityl polystyrene resin (**1**, 1.07 mmol/g according to the commercial supplier, 1.95 g, 2.09 mmol) was then added slowly to the stirring solution over an hour. After an additional hour of stirring, 10 mL of methanol was added, followed by stirring for another 20 min. The suspension was transferred into a large polypropylene reaction vessel, then rinsed with methanol (3×), CH_2Cl_2 (3×), 1:3 Et_3N/DMF (3×), methanol (3×), and then CH_2Cl_2 (5×). Drying under high vacuum for 16 h led to 1.47 g of resin (theoretical loading is 0.91 mmol/g.)

Partial Termination Synthesis of the Triamide Library on Polystyrene. The synthesis of the split-pool triamide library was done using a semiparallel automated synthesizer. Resin **2** was split into 14 equal portions (102 mg, 0.093 mmol each), placed into 5-mL reaction vessels, and rinsed with dichloromethane (3×) and then NMP (3×). To each vessel was added 0.7 mL of an NMP solution containing a mixture of 0.51 M of the Fmoc amino acid (0.36 mmol) and 0.057 M of the *N*-acyl amino acid (0.04 mmol). The suspensions were mixed for 5 min before the addition of 1.2 mL of an NMP solution containing 0.30 M HBTU (0.36 mmol) and 0.30 M HOBt·H_2O (0.36 mmol). After mixing for 1 min, the DIPEA (0.12 mL 0.7 mmol) was added. The suspensions were then mixed for 2 h before they were drained and rinsed with NMP (3×) and dichloromethane (5×). Ninhydrin assays[53] of all 14 portions were negative, indicating no unreacted free amines. The resin pools were then resuspended in dichloromethane and mixed thoroughly into a single polypropylene reaction vessel. The pooled resin was then dried under high vacuum for 16 h. The average loading, using the average molecular mass of all amino acids that had been coupled, was calculated to be 0.69 mmol/g. The resin was again split into 14 portions (100 mg 0.069 mmol) and placed into 5-mL reaction vessels. The Fmoc protective groups were removed by two treatments with 1:4 piperidine in NMP (5 min, then 30 min), and then the resin was rinsed with NMP (5×). An amide coupling procedure similar to that described above was employed, except using 0.55 mL of a 0.50 M solution of the *N*-acyl amino acids (0.28 mmol) in NMP, 0.92 mL of an NMP solution containing 0.30 M HBTU (0.28 mmol), and 0.30 M HOBt·H_2O (0.28 mmol), and then DIPEA (96 μL, 0.55 mmol) were used. Ninhydrin assays from each vessel were negative. The resin portions were then mixed using the method described above to give the encoded, split-pool library of triamides. The average loading was calculated to be 0.72 mmol/g.

Preparation of the Tetraamide Library (3). The tetraamide library (**3**) was synthesized in a manner similar to that described for the triamide library, except involving a second amide coupling with a 9:1 mixture of Fmoc amino acid and *N*-acyl amino acid before complete sequence-

termination with the *N*-acyl amino acid. Average loading: 0.62 mmol/g.

Preparation of the Tetraamine Library. To the tripeptide library (398 mg, ~0.29 mmol), weighed into a 25-mL round-bottom flask equipped with a condenser and stir bar, was added 1.0 M BH_3/THF (8.6 mL, 8.6 mmol) while under nitrogen atmosphere. The suspension was slowly stirred for 2 days at 65 °C. It was then transferred into a 10-mL polypropylene reaction vessel and rinsed with THF (4×). A 1-mL portion of THF was added, followed by 0.28 mL of DIPEA, 0.56 mL acetic acid, and a 1-mL solution of I_2 (1.11 g, 4.29 mmol) in THF. The suspension was vortexed for 4 h until it was filtered and rinsed with THF (4×), 1:3 Et_3N/DMF (4×), methanol (4×), and dichloromethane (5×) and then dried for 16 h under high vacuum to give the triamine library. Average loading: 0.74 mmol/g.

Preparation of the Pentaamine Library (4). The reduction of the tetraamides **3** toward the pentaamine library was done in a manner similar to that for the tripeptide library, except the reaction time was 3 days. Average loading: 0.64 mmol/g.

Single-Bead Resin Cleavage and LC/MS Analysis. The dried beads were spread out on a glass Petri dish and observed under a microscope. The beads were picked up using the tip of a 25-μL glass microsyringe containing 5 to 7.5 μL of 5% TFA in dichloromethane and then transferred into a 200-μL glass conical microvial. The TFA solution was injected into the microvial, which concomitantly removed the bead from the syringe tip into the microvial with the solution. The microvial was then placed inside a 2-mL-volume LC autosampler vial, which was then capped. After 15 min, the cap was removed from the autosampler vial to allow the TFA solution to evaporate. Methanol (5.0−7.5 μL) was added to the conical microvial, and the solution was injected into an LC/MS. The identity of each polyamine sequence was determined by the mass differences between the partially terminated sequences and the full sequence that eluted through the LC column. LC conditions: column, Zorbax SB-C8 4.6 × 50 mm, 3.5 μm; eluent, 15−85% acetonitrile (0.1% TFA) in water (0.1% TFA) over 5 min, then maintained at 85% for 7 min at 0.7 mL/min; MSD conditions: capillary voltage, 3200 V (positive mode); fragmentor voltage, 120 V; mass scanning range, 250−900 amu; nebulizer pressure, 40 psig; gas temperature, 350 °C; drying gas flow, 10.0 L/min.

Preparation of Grignard Reagent 10. Magnesium turnings (0.328 g, 13.5 mmol) were weighed into a flamed-dried 50-mL, three-necked round-bottom flask fitted with an addition funnel and condenser and containing a small iodine crystal and stir bar. 4-Bromobenzophenone ethylene acetal[54] (4.14 g, 13.6 mmol) was then added to the addition funnel. The reaction apparatus was then evacuated and backfilled with argon three times until it finally was kept under an atmosphere of argon gas. A 2-mL portion of dry THF was added to the magnesium, and 15 mL, to the bromide in the addition funnel. About 2 mL of the bromide solution was added to the magnesium, and within a few minutes of stirring, the Grignard reaction was initiated. The remaining bromide was added to the flask slowly enough to maintain a gentle reaction. After the addition was complete, the reaction was heated to reflux for 1.5 h. It was then cooled and used directly in the subsequent reaction described below.

TentaGel MB (250 μm)-Bound Benzophenone Resin (11). (The procedure is the same for the standard sized beads except as noted.) TentaGel MB bromide resin was dried before the reaction by storage under high vacuum (<1 Torr) over drierite/KOH for 2 days. The resin (2.50 g, 0.675 mmol at 0.27 mmol/g according to elemental analysis on bromine) and CuBr·DMS (0.278 g, 1.35 mmol) were weighed into a flame-dried 250-mL, three-necked, round-bottom flask fitted with a condenser. (Note: no stir bar was used because it was found to damage this type of resin while stirring.) The flask was evacuated and backfilled with argon three times and then maintained under an argon atmosphere. A 35-mL portion of dry THF was then added, followed by 4 mL of HMPA. Using an airtight syringe, the Grignard solution prepared in the above reaction (i.e., **10**) was transferred to the flask. The flask was then kept for 5 days at 70 °C with occasional swirling (3 days was sufficient with the standard sized beads). The reaction was then cooled to room temperature and quenched by the slow addition of 10 mL of saturated NH_4Cl (aq). The suspension was transferred into a large 100-mL glass filter vessel and rinsed with saturated NH_4Cl (aq) (3×), water (3×), methanol (3×), and dichloromethane (5×). The resin was resuspended in 10 mL dichloromethane before adding 1 mL of $HClO_4$ (70% in water), which turned the resin green in color. The suspension was shaken for 24 h until it was drained and rinsed with dichloromethane (3×), 1:3 Et_3N/DMF (3×), methanol (3×), and dichloromethane (5×). The resin was then dried under high vacuum over drierite/KOH for 16 h to give resin **11**. Elemental analysis on bromine gave 0.001% Br or 1.25×10^{-4} mmol/g Br. IR (microscope): 1654 cm^{-1} (C=O stretch).

TentaGel MB Trityl Alcohol (12). The reaction was found to work best when freshly prepared phenylmagnesium bromide was used. Resin **11** was weighed (2.38 g, 0.64 mmol at ~0.27 mmol/g) into a flame-dried 100-mL round-bottom flask and placed under an atmosphere of argon gas. A 20-mL portion of dry THF was added to the resin, followed by 1 M phenylmagnesium bromide solution in THF (7 mL, 7 mmol). The reaction flask was gently shaken on a mini-vortexer with a clamp loosely holding the neck of the flask. After 24 h of shaking at room temperature, the reaction was quenched with 10 mL of 0.5 M HCl (aq), transferred into a 100-mL glass filter vessel, and then rinsed with water (3×), 5% $NaHCO_3$ (aq) (3×), water (3×), THF (3×), methanol (3×), and dichloromethane (5×). The resin was then dried under high vacuum over drierite/KOH for 16 h, affording resin **12**. IR (microscope): 3511 cm^{-1} (OH stretch) and the loss of carbonyl stretch at 1654 cm^{-1}.

TentaGel MB-Bound 1-Trityl-4,7,10-trioxa-1,13-tridecanediamine Resin (13). To resin **12** (2.3 g), inside a glass filter vessel, was added 10 mL of 10% thionyl chloride in dichloromethane. The vessel was sealed and shaken for 45 min at room temperature and then drained. The same thionyl chloride treatment was repeated two more times until it was finally drained and rinsed with dry chloromethane (5×) to

give the trityl chloride. Dichloromethane was then added to resuspend the resin. This suspension was added over 1 h, via a pipet, into a solution of 4,7,10-trioxa-1,13-tridecanediamine (10 mL) in dichloromethane (10 mL) inside a dry 250-mL round-bottom flask shaking on a minivortexer. After the addition was complete, the flask was shaken for an additional 16 h until it was quenched with 2 mL of methanol. The resin was transferred to a 100-mL polypropylene filter vessel and rinsed with methanol (3×), 1:3 Et_3N/DMF (3×), methanol (3×), and dichloromethane (5×) and then dried under high vacuum over drierite/KOH for 48 h to give resin **13**. Elemental analysis on nitrogen: 0.4148% or 0.148 mmol/g (60.3% yield based on theoretical commercial loading; 0.246 mmol/g).

TentaGel MB-Bound Triamide (14). Resin **13** was weighed into a polypropylene filter vessel and rinsed three times with DMF. A 0.5 M solution of HO-LNva-Fmoc (2 equiv with respect to the loading of the resin) in DMF was then added to the resin. The suspension was vortexed for 2−5 min before the addition of a 0.3 M DMF solution of HBTU and HOBt·H_2O (2 equiv). After an additional 2−5 min of vortexing, DIPEA (4 equiv) was added. The suspension was then vortexed for 2 h, drained, and rinsed five times with DMF. The Fmoc protective group was removed by treating the resin two times with 20% piperidine in DMF, first for 5 min, then for 30 min. The resin was then rinsed five times with DMF. The amino acid coupling was then repeated with HO-4Amb-Fmoc before the resin was rinsed with DMF (3×), methanol (3×), and dichloromethane (5×) and then dried under high vacuum for 16 h to give **14**.

Tetraamine (16). Resin **14** was weighed (0.418 g, 0.0502 mmol) into a flame-dried ,25-mL round-bottom flask without a stir bar. The flask was fitted with a condenser and placed under argon atmosphere. A 1.0 M borane−THF complex was then added (6.0 mL, 6.0 mmol) to the resin. The suspension was heated to 65 °C for 30 h until it was cooled and transferred into a 25-mL polypropylene filter vessel and rinsed extensively with dry THF. The resin was then resuspended in 2 mL of THF before adding 0.27 mL of DIPEA. After vortexing for 1 min, 0.53 mL of $EtCO_2H$ was added. The suspension was vortexed for 1 min before the slow addition of a THF solution of iodine (1.2 g in 2 mL). The final ratio of THF/$EtCO_2H$/DIPEA should be 12:2:1. After vortexing for 4 h, the resin was drained and then rinsed with THF (3×), 1:3 Et_2N/DMF (3×), methanol (3×), and dichloromethane (5×). The resin was then dried under high vacuum over drierite/KOH for 48 h to give **15**. On a separate batch of resin on the same scale, another amide reduction was performed, except a piperidine-promoted workup was carried out, as described before (2 mL piperidine/100 mg resin, 65 °C, 16 h). In both reactions, the resins were cleaved with 5% TFA in dichloromethane to give the polyamine **16** as the tetrakis(trifluoroacetate) salt. Iodine-based procedure: crude yield, 42%. Purity by LC/MS/UV: 65% at 210 nm, 59% by ESMS. Piperidine-exchange procedure: crude yield, 47%. HPLC purity: 90% by UV (210 nm), 84% by ESMS. HPLC conditions: column, Zorbax SB-C8 (2.1 × 50 mm, 3.5 μm); eluent, 5−85% acetonitrile (0.1% TFA) in water (0.1% TFA) over 5 min at 0.7 mL/min then maintained at 85% for another 7 min; column temperature, 25 °C. MS conditions: capillary voltage (positive mode), 3200 V; mass scanning range, 100−900 amu; fragmentor voltage, 80 V; drying gas temperature, 350 °C; gas flow 10 L/min; nebulizer pressure, 40 psig. ^{1}H NMR (500 MHz, CD_3OD) δ 7.64 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 4.39 (d, J = 13.0 Hz, 1H), 4.27 (d, J = 13.0 Hz, 1H), 4.22 (s, 2H), 3.68−3.58 (m, 13H), 3.50 (dd, J = 6.5, 14 Hz, 1H), 3.45 (dd, J = 4.5, 14 Hz, 1H), 3.25 (dd, J = 7.5, 12.5 Hz, 1H), 3.20 (dd, J = 7.0, 12.5 Hz, 1H), 3.10 (q, J = 7.3 Hz, 2H), 3.07 (t, J = 6.6 Hz, 2H), 2.01 (q, J = 6.9 Hz, 2H), 1.91 (quintet, J = 5.8 Hz, 2H), 1.94−1.88 (m, 1H), 1.82−1.74 (m, 1H), 1.53−1.42 (m, 2H), 1.32 (t, J = 7.3 Hz, 3H), 1.04 (t, J = 7.2 Hz, 3H). ^{13}C APT NMR (125 MHz, CD_3OD) δ 134.3 (C), 134.0 (C), 131.8 (CH), 131.5 (CH), 71.3 (CH_2), 71.3 (CH_2), 71.2 (CH_2), 71.1 (CH_2), 70.1 (CH_2), 69.2 (CH_2), 57.0 (CH), 51.4 (CH_2), 49.8 (CH_2), 49.0 (CH_2), 48.2 (CH_2), 43.9 (CH_2), 39.8 (CH_2), 32.1 (CH_2), 28.3 (CH_2), 27.4 (CH_2), 19.6 (CH_2), 14.1 (CH_3), 11.5 (CH_3). IR (methanol cast): 3400−2500, 1675, 1202, 1134 cm^{-1}. HRMS−ESMS for $C_{25}H_{49}N_4O$ (MH^+), calcd. 453.37992 obs. 453.37982.

Syntheses of Tetraamide/Triamide Libraries on Standard-Sized (90−150 μm) Beads. Note: TentaGel-supported polyamide libraries were made on a Quest synthesizer, instead of a vortexer, to avoid bead damaging. Resin **13** (0.24 mmol/g, standard-sized) was split into 17 equal portions and placed into 5-mL polypropylene filter vessels (17 × 114 mg, 0.027 mmol each). The resin was rinsed with dichloromethane (3×) and NMP (3×) before the addition of 0.44 mL of an NMP solution containing 0.27 M of the Fmoc amino acid (0.120 mmol) and 0.030 M of the corresponding *N*-acylamino acid (0.013 mmol). The suspensions were vortexed for 5 min before adding to each vessel 0.44 mL of an NMP solution containing 0.30 M HBTU and 0.30 M HOBt·H_2O (0.13 mmol each). After an additional 1 min of vortexing, DIPEA (50 μL, 0.27 mmol) was added to the suspensions. The suspensions were then vortexed for 2 h before they were filtered and rinsed with DMF (4×) and dichloromethane (4×). The resins were then suspended in dichloromethane and mixed together thoroughly in a large 100-mL polypropylene filter vessel and dried under high vacuum for 16 h. The resin was then split again (17 × 112 mg, 0.0256 mmol each) and rinsed with dichloromethane and NMP. The Fmoc protective group was removed by two treatments with 1:4 piperidine/NMP (5 min, then 30 min). After rinsing the resin pools with NMP (5×), they were coupled to their respective amino acid derivatives exactly as described above. They were then rinsed and mixed the same way as described earlier. A portion of the resin was removed and stored as a triamide library. Following Fmoc removal with 1:4 piperidine/DMF, the final coupling was done on 17 portions of the resin (17 × 89 mg, 0.021 mmol each), using 0.34 mL of 0.30 M solutions of the *N*-acyl amino acids (0.10 mmol), 0.34 mL of 0.30 M solution of HBTU and HOBt·H_2O (0.10 mmol each), and DIPEA (36 μL, 0.20 mmol). After 2 h, the resins were rinsed, mixed, and dried as before to give the tetraamide library.

TentaGel-Supported Pentaamine Library. The reduction of the tetraamide library was done as described for

compound **15** using the modified iodine-promoted workup with 0.3 g/mL iodine in a 12:2:1 mixture of THF, propionic acid, and DIPEA to give the pentaamine library (average loading was ~0.12 mmol g^{-1}).

Peracetylation and LC/MS Decoding of the Pentaamine Library. Peracetylation on a milligram scale of resin (10 mg, ~0.0012 mmol) was achieved by first suspending the resin in 0.5 mL of DMF. DIPEA (22 μL, 0.13 mmol) was added, followed by acetic anhydride (50 μL, 0.53 mmol). The suspension was vortexed for 22 h and then rinsed with DMF (4×) methanol (4×), and dichloromethane (5×). Single beads were isolated as described above and then analyzed by LC/MS. LC conditions: column, Zorbax Extend-C18 2.1 × 50 mm, 5.0 μm; eluent, (solution A: 0.020 M NH_4OH in 80% acetonitrile in water; solution B: 0.020 M NH_4OH in water) 10% solution A in solution B up to 85% solution A over 4 min, then maintained at 85% for another 11 min at 0.5 mL/min; MSD conditions: capillary voltage, 3200 V (positive mode); fragmentor voltage, 80 V; mass scanning range, 350−1000 amu; nebulizer pressure, 40 psig; gas temperature, 350 °C; drying gas flow, 10 L/min.

For the peracetylation of single beads, the bead was isolated into a conical microvial, as outlined above, and covered with 5 μL of a solution containing 10% acetic anhydride and 5% triethylamine in dichloromethane. The microvial was placed inside the autosampler vial, which was then capped tightly and stored at 4 °C for 24 h. The cap was then removed from the vial to allow the solution to evaporate. The bead in the microvial was then exposed to 5 μL of 5% TFA in dichloromethane for 15 min. The cleaved contents were dissolved in 7.5 μL of methanol and analyzed by LC/MS as described in the previous paragraph.

Triamide Library Syntheses on Macrobead (250−300 μm) TentaGel Resin. The synthesis of the triamide library supported on macrobead TentaGel resin was done in a manner similar to that of other polyamide libraries. Resin **13** (0.14 mmol/g, macrobeads) was split into 17 equal portions (17 × 130 mg, 0.0182 mmol). Each resin was suspended in 0.30 mL NMP. The resins were then treated sequentially with 0.30 mL of an NMP solution containing 0.27 M of the Fmoc amino acid (0.081 mmol) and 0.030 M of the corresponding *N*-acylamino acid (0.01 mmol), along with 0.30 mL of an NMP solution containing 0.30 M HBTU and 0.30 M HOBt·H_2O (0.091 mmol each) and then DIPEA (32 μL, 0.182 mmol). After 2 h of vortexing, the resin pools were filtered, rinsed, and then mixed together. In the final amide coupling, the resin was split into 17 portions (17 × 130 mg, 0.17 mmol each), deprotected with 1:4 piperidine in DMF, and then suspended in 0.30 mL NMP. The resins were then treated with 0.29 mL of a 0.30 M NMP solution of the *N*-acyl amino acid derivative (0.086 mmol), 0.29 mL of a 0.30 M NMP solution of HBTU and HOBt·H_2O (0.086 mmol each), and then DIPEA (30 μL, 0.17 mmol). The resin was vortexed for 2 h, rinsed, combined, and then dried as usual to give the triamide library.

TentaGel-Supported Tetraamine Library. The reduction of the triamide library was done as described for compound **15** using both the modified iodine-promoted workup and the piperidine based workup to give the tetraamine library. Single beads were isolated and decoded in the same manner and under the same LC/MS conditions as the polystyrene-bound polyamines described above.

Supporting Information Available. Synthetic procedures and characterization data for amino acid derivatives 3,3-Amp, 1,4-Amp, and 3-Amb. Additional examples of polyamines decoded by HPLC/ESMS. This material is available free of charge via the Internet at http://pubs.acs.org.

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