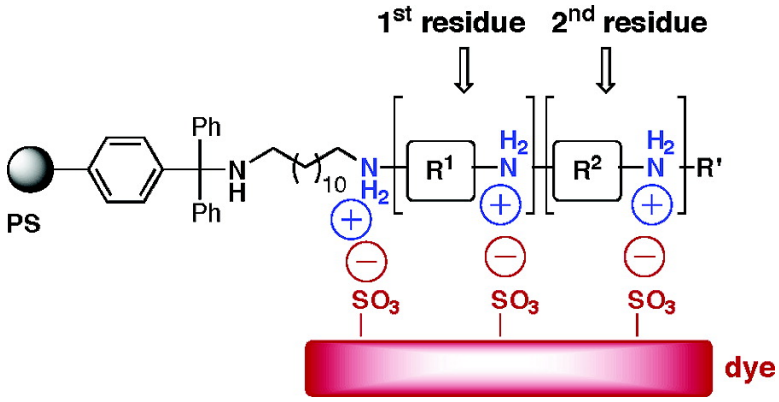
JOURNAL OF combinatorial CHEMISTRY

Article

Screening of a Combinatorial Library of Synthetic Polyamines Displaying Selectivity in Multiple Ion-Pairing Interactions with Model Polyanionic Compounds in Aqueous Organic Solutions

Sukhdev Manku, and Dennis G. Hall

J. Comb. Chem., **2006**, 8 (4), 551-561• DOI: 10.1021/cc0501559 • Publication Date (Web): 05 May 2006



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Screening of a Combinatorial Library of Synthetic Polyamines Displaying Selectivity in Multiple Ion-Pairing Interactions with Model Polyanionic Compounds in Aqueous Organic Solutions

Sukhdev Manku and Dennis G. Hall*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received December 5, 2005

The biological activity of natural polyamines is due in large part to their ability to form ion-pairing interactions with polyanionic biomolecules, such as proteins, oligonucleotides, and sulfated oligosaccharides. Unfortunately, the diversity of biogenic polyamines is compromised by their limitation to only just a few internitrogen spacers. As a proof-of-principle study, a synthetic split−pool library of linear triamines was screened in an on-bead assay against a selection of model trisulfonated azo dyes (**1**, **2**, and **3**) and a short glutamate-rich nonameric peptide (**4**) to demonstrate its use in the discovery of selective ligands via multivalent ion pairing. From screening a 196-membered split−pool library against the dyes in aqueous organic solutions, with or without spermidine as competing ligand, it was found that the most frequent residues possessed internitrogen distances that were very similar to the sulfonate distances on the dyes. The results from these screening assays were used in the design of two polyamine sequences (**8**, $8Aoc^R$-$8Aoc^R$, and **12**, $2Acc^R$-ϵAhx^R) for follow-up studies in solution phase. These triamines demonstrated the same selectively toward dyes **2** and **3** as observed by the solid-phase approach. In addition, resin-supported triamines, synthesized as discrete compounds, were able to selectively extract either dye **2** or **3** from a mixture of the two, further verifying the observations made from the library screening efforts. With peptide **4**, containing three glutamate residues, a preference was found for rather long residues (12 and 8 carbons long), which is suggestive of a linear peptide, rather than a helical motif under the conditions of the screening.

Introduction

Polyamines are ubiquitous biomolecules that are present in prokaryotic and eukaryotic cells in millimolar concentrations. They conduct essential roles in both cell growth and differentiation and have even been implicated in the proliferation of cancer cells.[1] They are also known to interact in complex ways with ion channel proteins and other neuroreceptors.[1−4] The rich biology of natural and synthetic polyamines has also been exploited in the development of potential antitumor agents[5,6]and carriers for drugs[7−9] and in gene delivery.[10,11] Under physiological conditions, polyamines become polycationic, giving them a high affinity toward polyanionic biological molecules, such as proteins, oligonucleotides, and polysulfated oligosaccharides.[12−16] These associations occur largely through the formation of multiple ion pairs (i.e., electrostatic interactions). Unfortunately, the diversity of biogenic polyamines is compromised by their structural limitations to just a few types of internitrogen spacers (up to a maximum of five carbons, and very few with side chains). Thus, given the importance of polyamines and the need to further understand their biological roles, there is great interest in the synthesis of new and diverse polyamine analogues using combinatorial chemistry as well as the development of high-throughput screening methods for discovering new bioactive compounds of this class.

We have previously described the split−pool synthesis of polyamines via the exhaustive reduction of resin-bound polyamides.[17] An efficient encoding method was developed whereby the polyamides were synthesized with a partial termination strategy, allowing each bead-supported library member to be decoded by LC/ES-MS. By this method, the identity of each polyamine "residue" was found by the mass differences between the full and terminated sequences in the electrospray mass-based chromatogram. From our selection of amino acid building blocks, we obtained a series of polyamines with amine residues depicted in Figure 1. With these libraries in hand, we set out to screen model polyanionic targets to investigate the selectivity of polyamine ligands in forming multiple ion-pairing interactions under aqueous conditions.[18−26] Of particular interest is the issue of whether linear and highly flexible polyamines could demonstrate cross-selectivity with polyanionic compounds of slightly different geometry. As an initial foray, we have screened the polyamines against trisulfonated azo dyes as model polyanionic targets.[27−29] These dyes can be considered as charge mimics of polysulfated oligosaccharides, such as heparin. One of their advantages is that they allow the easy detection of the binding event by coloring the beads bearing ligands with which they interact.

Polyanionic dyes were sought that contained a number of anionic sites that is at least equal to that of the ammonium sites on the polyamine library. To this end, a set of trisulfonated dyes were chosen for our 196-membered triamine library. (Note that the amine bound to the trityl linker is inaccessible due to the bulkiness of the surrounding trityl group. Therefore, although it is formally a tetramine,

10.1021/cc0501559 CCC: $33.50
Published on Web 05/05/2006

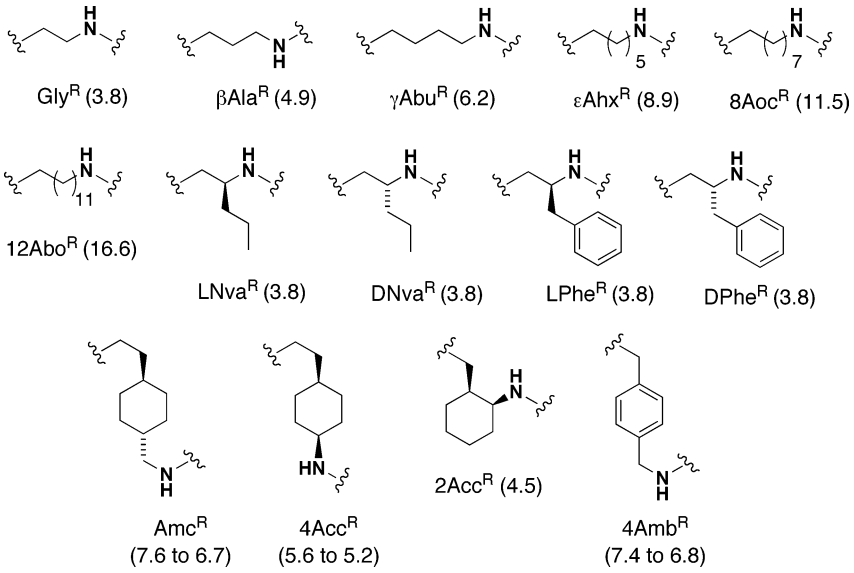


Figure 1. Polyamine residues with calculated (SYBYL) internitrogen distances (Å) with the nitrogen on the adjacent residue.[32] Note: the superscript R alludes to a reduced amide.[17]

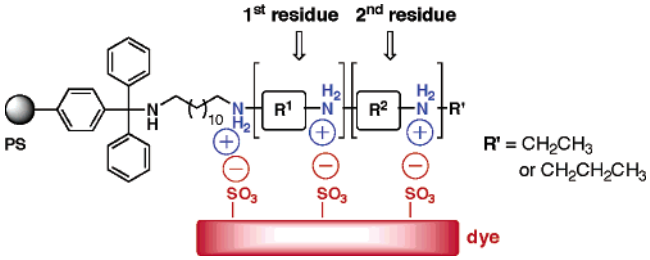


Figure 2. Hypothetical model of a resin-bound triamine complexed to a trisulfonated dye. Note: although it is formally a tetramine, the anchoring amine of this polyamine is not counted.

it effectively behaves as a "triamine" library and is denoted as such in this article (see Figure 2)). Of these trisulfonated dyes, we chose three, **1**, **2**, and **3** (Figure 3). These dyes are not only all dark in color (red and blue) to help their detection on the beads, but they also maintain the same color over the pH range that was studied. These rigid dyes differ mainly in the relative distances and positions of the sulfonate groups, which could possibly lead to different selectivities among the triamines in the library. In other words, could a polyamine, or group of polyamines, target one dye selectively over another (Figure 2)?

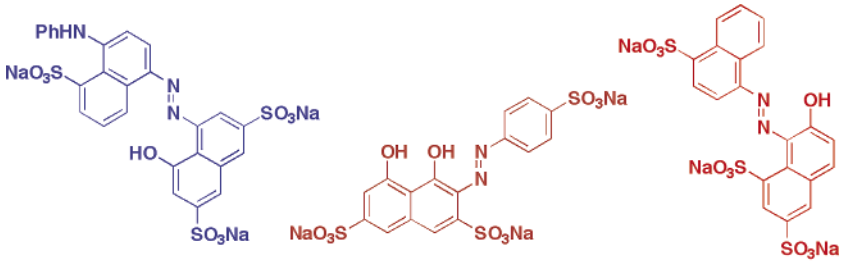
Although rotation about one of the C−N bonds in each dye can occur, it is assumed that the second C−N bond would be locked into position by an intramolecular hydrogen bond between the phenolic hydrogen and the lone pair on the azo nitrogen. Thus, these dyes provide sufficiently rigid targets for our triamine library.

In addition to dyes **1**−**3**, a glutamate-rich nonameric peptide was also tested against the supported triamines. This particular peptide is found within an α-helical motif in the hemoglobin protein. To detect binding to the resin beads, the peptide was labeled with a red-colored dye (Figure 4). By itself, this peptide is too short to exist as an α-helix, but we were interested in whether a distinct triamine sequence could be discovered that can promote and stabilize α-helix formation.

Results and Discussion

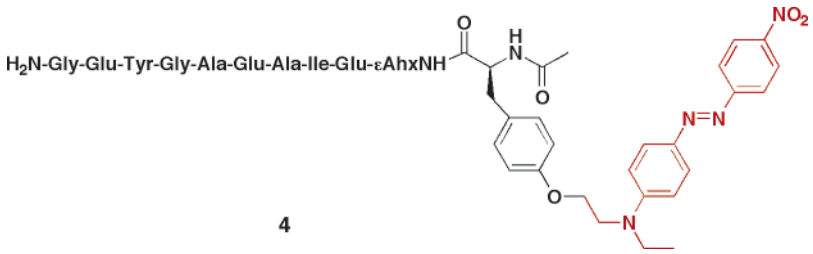
Conditions and Methods for Library Screening. Although both polystyrene and TentaGel-supported libraries were made in our laboratory in the past,[17] we found that most commercial dyes bound nonselectively to the polystyrene/poly(ethylene glycol) matrix of Tentagel.[28] Thus, for these prototypical dye targets, we decided to focus on the polystyrene-supported triamine library. As a result, the dye screening experiments needed to be performed in a mixed organic/aqueous solvent system to ensure that the resin remains adequately swollen during the assays.

It was found that the best solvent system for performing the on-bead assays was a 10% mixture of aqueous buffer in *N*,*N*-dimethylformamide (DMF). A significant proportion of aqueous buffer was added to determine if selective ion-pairing on resin-bound polyamines can occur in the presence of water as a highly competitive solvent and, thus, confer biological relevance to this study.[30] Before each screening experiment, the dyes were tested against a suspension of underivatized polystyrene resin in the same solvent and buffer conditions to confirm that nonselective interactions with the polystyrene matrix would be negligible. A similar control experiment was also performed with the resin-bound tripeptide library[17] precursor to the triamine library. In a typical screening, ∼3 mg of polystyrene-supported triamine library (∼10 000 beads) was added to a small volume (∼0.5 mL) of buffered solution inside a small, shallow Petri dish. Small amounts of the dye were then added to the swirling suspension until a visible change of color was observed in the resin. Only a minimum amount of the dye was added to avoid saturation and reduced selectivity. The darkest of the beads were then removed from the dish using a narrow capillary tube and transferred to a separate dish, where they were washed with 10% water/DMF (containing no buffer) and then dichloromethane. The beads were individually cleaved in a conical microvial using a small volume of 5% TFA in dichloromethane (5.0−7.5 μL) and then analyzed



"Acid Blue-92," **1** **2** "New Coccine," **3**

Figure 3. Three trisulfonate azo dyes used to screen the polystyrene-supported triamine library.



4

Figure 4. Nonameric peptide **4** from hemoglobin protein labeled with Disperse Red 1.

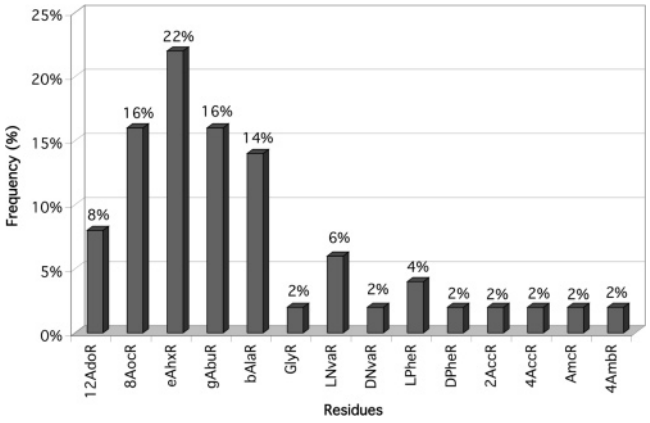


Figure 5. Frequency of triamine residues found in the screening of dye **1** at pH 7. A total of 50 polyamine residues (25 triamines) were decoded.[31] Note: the superscript R alludes to a reduced amide.

by LC/MS as described before.[17] Each screening experiment was performed at least twice to ensure reproducibility.

Screening of Dye 1. The blue dye was screened against the triamine library as a 5.0×10^{-5} M solution in 0.01 M MES−TRIS buffer (pH 7.0) in 10% water/DMF. After ~1 h of swirling the suspension, the dye solution became clear. Under a low-powered microscope, ~50% of the beads appeared as various shades of blue, while the remaining beads stayed colorless. Of all the blue beads, 40% (or 20% overall) were considered very dark and worth isolating. Altogether, 25 beads were isolated, cleaved, and then analyzed for their specific triamine sequence. The results are shown in Figure 5.[31] It became apparent in the screening of dye **1** that the sequences themselves were less important than the individual residues that constitute them. In other words, due to the "pseudosymmetry" of the library members resulting from the long alkyl anchor and the ethyl end group, the dye would not be able to distinguish a triamine sequence R^1-R^2 over the same sequence expressed in the opposite direction, R^2-R^1, from the polymer support. Thus, it was deemed more appropriate to depict the results as the frequency at which individual residues are observed out of the total residues decoded (Figure 5).[31] The most frequent individual residues were the six-carbon ϵAhx^R, appearing a total of 22% of the time (that is, 11 times out of 50 residues decoded), followed by the eight-carbon residue of 8Aoc^R and the four-carbon of γAbu^R, both of which appeared in a 16% frequency. Other linear, flexible residues, such as βAla^R and 12Ado^R (3 and 12 carbons long), also made significant appearances in the screening results.

Examination of the relative positions of the sulfonate groups on dye **1** can provide some rationale into the rather modest selectivity observed in this case. Hand-held molecular models revealed that the most reasonable planar conformation of the dye would be **1-A** in Figure 6, because rotamer **1-B** suffers from steric clashing between the hydroxyl group and the naphthyl ring. From structure **1-A**, the range of distances between oxygens on the sulfonates groups was determined using single-point SYBYL force field calculations. It gave similar values when compared with the maximum nitrogen-to-nitrogen distances (see Figure 1) on the preferred residues selected in the screening assays.[32] From this simple analysis, it would appear that the ϵAhx^R and 8Aoc^R residues would fit ideally between sulfonates I and II on the naphthyl moiety, and even between I and III. It is also conceivable that sulfonates I and II can be accessed by the most distant amines on triamine sequences made of two shorter residues (for example, ϵAhx$^R-\beta$Ala^R), leaving the middle amine uncomplexed to the dye. Indeed, according to Table 1,[31] there are many two-carbon residues (i.e. Nva^R, Phe^R, and Gly^R) paired with βAla^R, which makes a triamine that spans six atoms between the most distant nitrogens (excluding the anchoring nitrogen on the trityl linker), just as in the most favored ϵAhx^R residue. The distance between the most distant sulfonates, II and III, could be reached by the longest residues, 8Aoc^R and 12Ado^R.

Although some consensus was observed in the assay with dye **1**, the overall selectivity was disappointing. This modest selectivity could possibly due to the difficulty the resin-bound triamines have in accessing all three sulfonates with each of

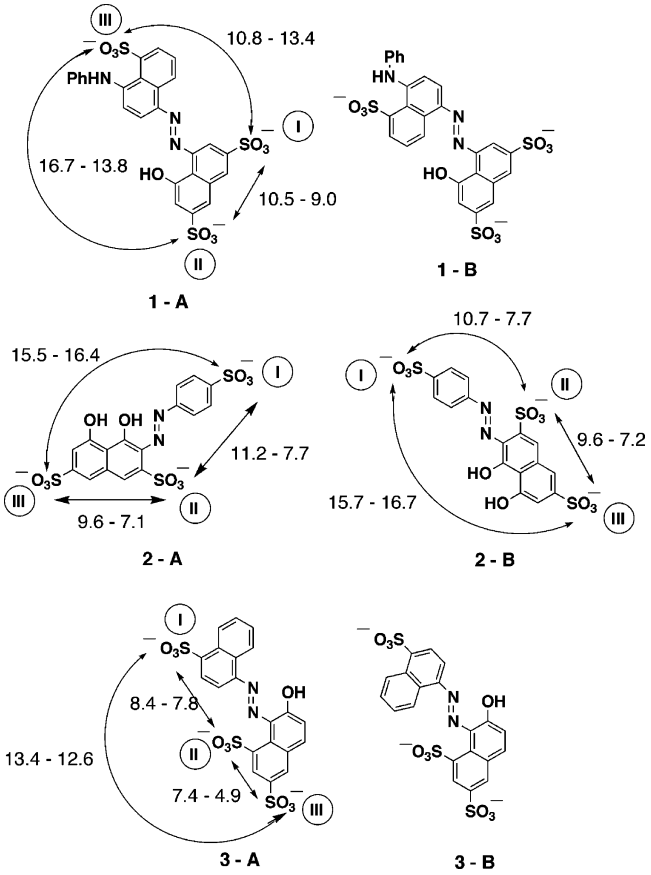


Figure 6. Conformations and range of interoxygen distances in dyes **1**−**3**.[32]

their three available amines. To form three ion pair interactions, many of the triamines will need to "bend" around the dye to properly position their amino groups. The high degree of variability among linear residues may suggest a nondirected, global electrostatic interaction, rather than a highly selective ion pair that is geometrically defined via hydrogen bonding. This observation is supported by a study pointing to a surprisingly small influence of rigidity (preorganization) in similar multiion-pairing systems.[33]

As a negative control, 10 beads that stayed clear (that is, "nonbinding" beads) during the screening were also isolated and sequenced. For the most part, residues identified in the negative controls were rarely observed in the colored beads. The analysis of the clear beads revealed mainly the presence of two-carbon residues, such as Phe^R and Nva^R, as well as the cyclic residues of 4Amb^R, 4Acc^R, and Amc^R. These residues were generally avoided by the dye target, perhaps due to the inappropriate spacing of the amino groups relative to the sulfonates.

The degree of polyprotonation is an additional factor in the ability of some residues to bind to the dye. It has been shown that the extent of complete protonation in a given polyamine at neutral pH depends on the number of carbon units between the nitrogen atoms.[34−36] Long carbon chains will allow a greater degree of protonation, whereas shorter ones will disfavor protonation at two adjacent nitrogens due to greater electronic repulsion felt by the two positively charged centers. Thus, in the dye screening study described herein, the triamines composed of two carbon spacers may

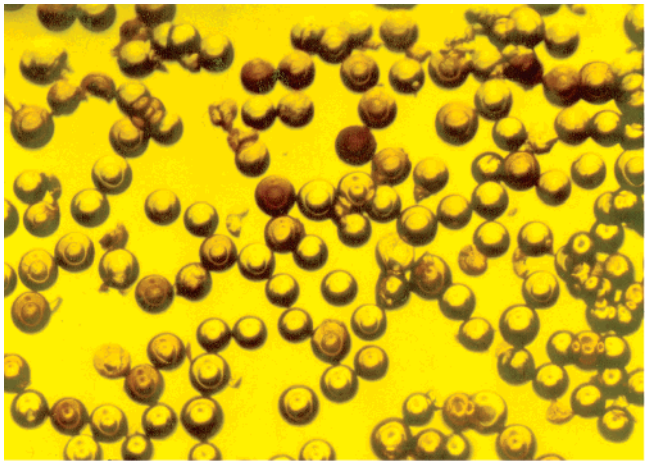


Figure 7. Example of screening assay between the triamine library and trisulfonated dye **2** using an optical microscope.

not be completely protonated at pH 7, resulting in reduced affinity for the trisulfonated dye. The effect of pH on binding selectivity would be further examined with the next series of dyes screened (vide infra).

Screening of Dye 2. To further study the selectivity of the triamine library, a different trisulfonated dye, **2**, was chosen. Dye **2** has its three sulfonate groups positioned almost along the same line with each other, and these groups are easily accessible. The triamines would not need to bend to reach each anionic center or be blocked by any sterically hindering substituent. The screenings with **2**, which is red in color, were performed under three different conditions, and the results are depicted in Figure 8. In the first screening, the library beads were suspended in a solution of the dye in 1:9 water/DMF, buffered at pH 7. In a second experiment, the effect of a competitive inhibitor was tested by the addition of spermidine to the screening mixture. Finally, a third screening assay was performed at a lower pH to observe any possible changes in selectivity upon increasing the extent of polyprotonation on the triamine library.

Under the conditions of the first screening (without spermidine, pH 7.0), ∼5% of the beads appeared dark red , as compared to the bulk of the other beads; 30% were clear (yellowish); and the remaining beads possessed various shades of pink (see Figure 7 for an example). Thirty beads that were among the darkest 5% were isolated and decoded to identify their triamine sequences. The results revealed a good level of selectivity with a strong preference for the 6-, 8-, and 12-carbon linear chains (ϵAhx^R, 8Aoc^R, and 12Ado^R, respectively) with the 8-carbon chain the most favored overall. In addition, two cyclic residues, 4Amc^R and 4Acc^R, appeared frequently in the decoding results. In the presence of spermidine, the selectivity for 12Ado^R increased to 33% frequency from 20% without spermidine as an additive (see Figure 8).[31] Lowering the pH of the screening solution to 5.5 resulted in an unexpected change in the selectivity. Although a slight increase in the appearance of two-carbon residues was somewhat expected due to a higher degree of protonation, there was also a marked increase of the 12Ado^R residue, up to 50% frequency, as well as the cyclic 4Acc^R residue (from 10 to 17%). The increase in these residues

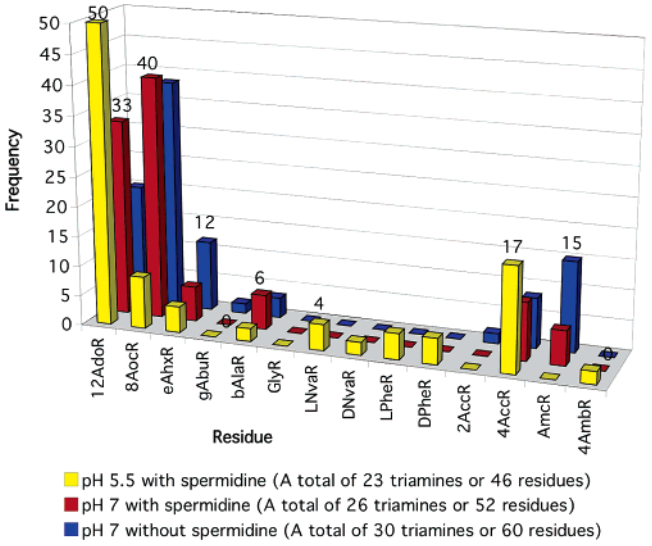


Figure 8. Frequency of triamine residues found in screening of dye **2**. Conditions: ~3 mg of resin suspended in a 3.75×10^{-5} M solution of **2** in 1:9 water/DMF and (A) 0.050 M MES−TRIS (pH 7.0), (B) 3.16×10^{-4} M spermidine with 0.050 M MES−TRIS (pH 7.0), or (C) 3.22×10^{-4} M spermidine with 0.025 M MES (pH 5.5).[31]

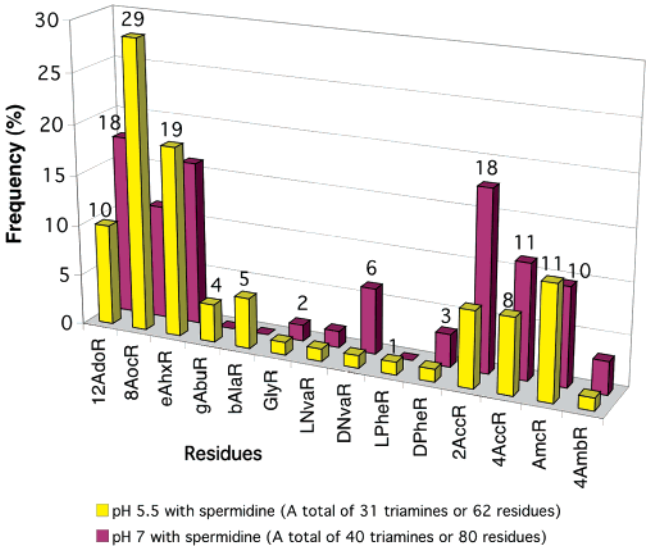


Figure 9. Frequency of triamine residues found in screening of dye **3**. Conditions: ~3 mg of resin suspended in a 3.75×10^{-5} M solution of **3** in 1:9 water/DMF and (A) 3.16×10^{-4}M spermidine with 0.050 M MES−TRIS (pH 7.0) or (B) 3.22×10^{-4} M spermidine with 0.025 M MES (pH 5.5).[31]

seems to come at the expense of $8Aoc^R$, which dropped significantly to 9% frequency from 40%. A possible explanation for the decrease in $8Aoc^R$ is the greater ability at pH 5.5 for spermidine to completely inhibit the eight-carbon residue from binding the dye. Indeed, like $8Aoc^R$, spermidine spans eight atoms between the two terminal amines. Each of these screening assays was repeated at least once, and on each occasion, the same overall selectivity was observed.

Up to 10 clear beads were also randomly isolated from the screening at pH 7 with spermidine. In almost all cases, at least one of the two-carbon residues (Phe^R, Gly^R or Nva^R) or the residue $2Acc^R$ was found in each sequence, with many triamines containing various combinations of these shorter units. As described above, all these residues were generally absent from the darkly colored beads.

From hand-held molecular models, two reasonable planar conformations for the dye have been identified: **2-A** and **2-B**. From these, the range of oxygen-to-oxygen distances between sulfonates was measured by simple force-field calculations (see Figure 6).[32] These calculations revealed only a slight change in the sulfonate distances between the two conformations. Comparison of these distances with the internitrogen distances in $8Aoc^R$ (11.9 Å) and ϵAhx^R (8.9 Å) residues have shown them to be close to sulfonate distances between I and II and between II and III, whereas $12Ado^R$ (16.6 Å) by itself is capable of spanning between the most distant sulfonates, I to III.

Screening of Dye 3. A good way to further demonstrate the selectivity of multivalent ion pairing interactions with the triamine library is to screen another closely related dye with sulfonate groups spaced over distances that are different from those of dye **2**. To this end, we selected dye **3** ("New Coccine"), which has three sulfonate groups in closer proximity, with the expectation that they will attract different residues containing internitrogen distances shorter than those observed with **2**. The screening of the library was done in the presence of spermidine at both pH 7.0 and 5.5. Under conditions identical to those of dye **2**, ~10% of the beads appeared dark red. The results are shown in Figure 9.[31]

The higher proportion of dark red beads in the Petri dish immediately indicated that the overall polyamine residue selectivity for this dye was lower than for dye **2**. Indeed, decoding of isolated beads gave an increased variation in both the triamine sequences and individual residues under both pH conditions. There are some similarities, namely, the preference for long, linear residues, as well as some ring-containing ones over the two-carbon spacers. However, the shorter residues are more frequently encountered with dye **3** at pH 7, as compared to dye **2**. A noticeable difference between the two dyes is the significant reduction in the frequency of $12Ado^R$ binding to **3**. For instance, at pH 7 with spermidine, the frequency of the 12-carbon unit goes from 33% with **2** to only 10% with **3**. Alternatively, the six-carbon unit, ϵAhx^R, is selected more frequently with **3** than with **2** (19% versus 6%). Yet perhaps the most striking difference between the two dyes is the frequent selection of the $2Acc^R$ residue as a binding unit for **3**, which was rarely observed in the screening of **2**. Overall, when compared to the other dyes, this shift toward shorter-length residues reflects the shorter distances between adjacent sulfonate groups on dye **3**.

Again, a comparison was made between the calculated sulfonate distances on dye **3** and internitrogen distances on some of the most favored residues. Two possible conformations, **3-A** and **3-B**, exist for the dye (Figure 6); however, the relative positions of the sulfonates are almost identical in both. The internitrogen distance for the extended conformation of ϵAhx^R is 8.9 Å, which is close to the distance between sulfonates I and II. The internitrogen distance for the optimized conformation of the $2Acc^R$ residue is 4.5 Å, which should be able to fit reasonably well between the meta sulfonate groups II and III. Indeed, molecular docking of dye **2** with a protonated Et-NH−$2Acc^R$−ϵAhx^R-Et triamine,

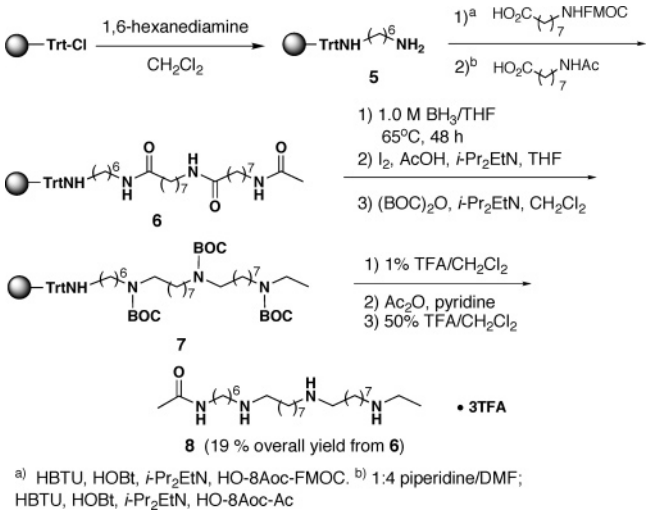
in a highly extended form, showed a high level of overlay among all sulfonates and the ammonium ions (see Supporting Information). In addition, the residues $4Acc^R$ and Amc^R, which are also strong binders according to Figure 9, have extended internitrogen distances of 5.2 to 5.6 and 7.4 Å, respectively, and, therefore, should also be able to interact with the meta sulfonates of dye **3**. Once again, when the screenings were repeated, the same residue consensus was obtained.

Nature of the Triamine−Dye Interaction. To verify that ion pairing is the main interaction responsible for the observed selectivity, a series of control experiments were conducted to determine the nature of the binding between the dyes and the resin-bound triamines. As mentioned earlier, nonspecific binding of the dyes to the underivatized polystyrene support was not observed in the mixed organic/aqueous solvent used herein. The large number of long, hydrophobic residues that were selected in the screenings (for example, $12Ado^R$, $8Aoc^R$) suggested the possibility of hydrophobic interactions as a dominant binding mode in the polar solvent. However, screening of the triamine libraries in the presence of 0.1% Triton X-100, a nonionic detergent known to minimize nonspecific hydrophobic interactions, did not reduce the color intensity of the beads after mixing with the dye targets. Furthermore, no colored beads resulted when we assayed the uncharged, precursor peptide library,[17] which cannot bind via ion-pairing interactions. Likewise, no binding occurred when the neutral triamine library (screened at pH 11) was suspended in the dye solution. If electrostatic interactions are, indeed, important, then increasing the salt concentration of the solution should inhibit the binding of the dyes. Indeed, the presence of 0.5 M tetrabutylammonium bromide maintained the red coloration of the solution and resulted in only clear beads.

Determination of Stability Constants. The screening results suggest that the structure of the polyamine residues determines its affinity for the dye target. These results imply a geometrically defined interaction that depends on the precise positioning of the ammonium ions relative to the sulfonate groups, rather than a general electrostatic attraction. Similar kinds of observations have been reported in the literature for solution-phase guest−host complexes that rely on ion pair interactions. For instance, Dougherty and coworkers have shown, using a carboxylate-based host, that by increasing the length of a dicationic guest by increments of a single methylene group, the binding affinity toward the host reaches a maximum once a specific length of the guest is reached.[30] Although our solid-phase screening observations seem to agree with the solution-phase studies reported in the literature, various factors may influence the outcome of the on-bead binding assays, such as the solvent conditions and the role of the resin matrix. Therefore, to verify that this type of library screening is reliable in identifying high-affinity polyamine ligands, a number of individual triamines were synthesized and examined in solution with both dyes **2** and **3**.

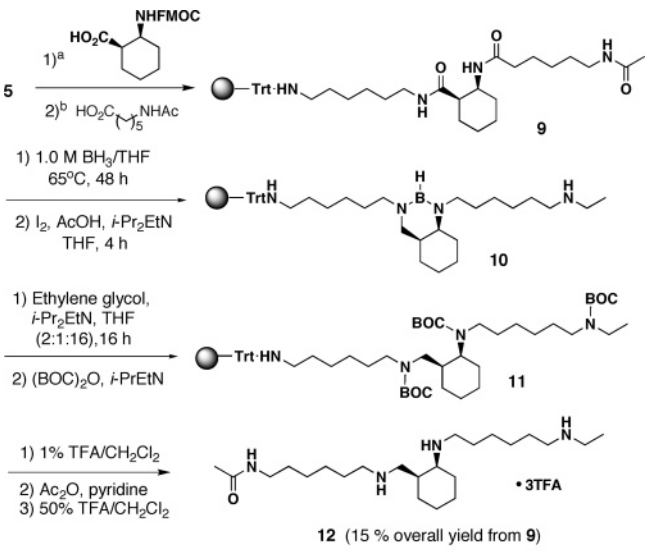
From the screening results given above, a most likely polyamine to have high affinity toward dye **2** in solution is the sequence $8Aoc^R-8Aoc^R$. Synthesis of this triamine was performed on trityl polystyrene resin (Scheme 1) via the exhaustive amide reduction of the corresponding triamide, followed by the iodine-promoted cleavage of the borane−amine adducts.[37] The tritylddiamine linker **5** was employed, which unfortunately required additional synthetic steps to block the terminal amine by acetylation after cleavage. Thus, after reduction of triamide **6**, the resulting resin-supported triamine was treated with $(Boc)_2O$ (di-*tert*-butyl dicarbonate) to provide the Boc-protected triamine derivative **7**. Cleavage of **7** from the resin was effected by exposure to dilute TFA (1%) in dichloromethane. Acetylation of the anchoring primary amine by acetic anhydride was followed by removal of the Boc protective groups with concentrated TFA to give the triamine amide **8** in 19% overall yield from triamide **6**. Compound **8** would be used in subsequent NMR binding assays.

Scheme 1



A solution-phase triamine expected to have a lower affinity for dye **2** than triamine amide **8** would be one combining shorter internitrogen residues. We selected, on the basis of the library screening results, the sequence $2Acc^R-\epsilon Ahx^R$ containing the poorly selected $2Acc^R$ residue. However, although weak for dye **2**, this sequence should be a strong ligand for dye **3**. Our solution-phase model compound for the $2Acc^R-\epsilon Ahx^R$ sequence is triamine amide **12**. The synthesis of **12** on solid support was performed in a manner analogous to that of **8** (Scheme 2). One unexpected complication was the observation by HPLC−UV/ESMS, after cleavage of the resin **10**, of as much as 25−35% of diaminoborohydride originating from the amide reduction of **9** (and iodine-promoted borane−amine cleavage). This type of diaminoborohydride species has been observed previously in this laboratory during the reduction of resin-bound tertiary amides.[37] As in that case, the diaminoborohydride resin **10** was treated with excess ethylene glycol under basic conditions to effect the exchange of the boron, providing the free resin-supported triamine with <5% of diaminoborohydride.[38] BOC protection of the amines proceeded to give **11**, which was cleaved from the resin and subsequently acetylated and deprotected, as described previously for **8**, to give the triamine amide **12** in 15% overall yield from the triamide **9**.

Scheme 2



a) HBTU, HOBt, *i*-Pr_2EtN, HO-2Acc-FMOC.
b) 1:4 piperidine/DMF; HBTU, HOBt, *i*-Pr_2EtN, HO-εAhx-Ac

Initial attempts at determining the stability constants (K_a's) by UV/visible spectroscopy failed as no detectable change in the UV/visible spectra of the dyes were observed upon titration with the triamine amide ligands **8** and **12**. Therefore, ^{1}H NMR was employed[39] using 20% D_2O in DMSO-d_6 as the solvent mixture. This particular solvent system was used due to solubility issues. The stability constants were then obtained from the titration curves using curve-fitting software developed by C. A. Hunter.[40] In the case of dye **2**, the stability constants for **8** and **12** were found to be 6400 and 1100 M^{-1}, respectively (averages of two reproducible titrations; see Supporting Information). Both fit a binding curve that had assumed a 1:1 stoichiometry over other higher-order complexes. In addition, a Job plot of **8** with dye **1** provided further evidence of a 1:1 complex between this pair (see Supporting Information). There are a few important differences between the solution-phase and the solid-phase assays that should be discussed. First, the solvent systems employed were different; the NMR titrations were performed in 20% D_2O in DMSO-d_6, which is a more polar medium than the 10% water/DMF system used for the solid-phase screening work. The higher polarity solvent may reduce the solution-phase binding affinities by increasing the desolvation energy of the uncomplexed ions.[30,41] Different counterions in both cases can also affect the binding affinities. The very high local concentrations of resin-bound triamines within the resin beads may also cause differences in the binding stoichiometries observed between the solution and solid-supported experiments. However, despite these differences, the larger binding constant obtained for triamine **8** adds credence to the library screening results that led toward this preferred ligand.

Stability constant measurements with dye **3** were also performed. Unfortunately, titration of **3** with **8** resulted in precipitation of the complex in the NMR tube, so a K_a value could not be determined for this pair. Between **3** and **12**, the stability constant was 2700 M^{-1}, which is higher than with dye **2**, a result that was predicted from the on-bead screening data.

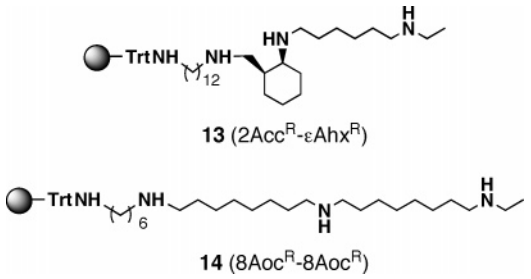


Figure 10. Resin-supported triamines for selective extraction of dyes **2** and **3**.

Cross-Selectivity Experiments between Dyes 2 and 3. The library screening results and the different stability constants between triamine amide **12** and the two dyes suggested the possibility of an alternative test of selectivity that involves the use of resin-supported triamines to selectively extract one trisulfonated dye from another in a mixture of two dyes.

To investigate the feasibility of this type of selective extraction, the resin-bound triamine sequences $2Acc^R-\epsilon Ahx^R$ and $8Aoc^R-8Aoc^R$ (**13** and **14**, Figure 10) were independently prepared by the amide reduction of a triamide precursor on trityl polystyrene.[42] The resin-supported triamine **13** was then suspended in an equimolar mixture of **2** and **3** in 1:9 water/DMF with 0.050 M MES−TRIS buffer at pH 7. The resin, which immediately turned deep red, was filtered and washed three times with a 0.1 M DMF solution of phenylsulfonate sodium salt ($PhSO_3Na$) to remove any weakly bound dye and then three times with a 0.5 M solution to wash away most of the bound dyes. After each wash, the filtrate was collected as a separate fraction. Analysis of the third fraction with 0.5 M $PhSO_3Na$ gave dye **3** in 79% purity by HPLC/UV analysis. Further washes eventually produced the dye in ~97% purity (Figure 11).

The sequence $8Aoc^R-8Aoc^R$ was also tested in the same type of extraction experiment. It was synthesized again via the usual amide reduction/iodine-promoted workup without incident. As expected from the library screening data, resin **14** selectively extracted dye **2** over **3**, for after three washes with 0.5 M $PhSO_3Na$, the resin-supported triamine recovered **2** in 68% purity by HPLC/UV analysis (Figure 11). Thus, from the on-bead library screening, two triamine sequences have been identified that are capable of detecting, quite selectively, the subtle geometrical differences between anionic groups on the two dye targets.

Screening of a Glutamate-Rich Peptide. So far, all the screening experiments described with the triamine library have been with trisulfonated dyes, which were used due to their easy detection on the resin bead. However, alternative targets would be desired in order to expand the applicability of the library to other polyanionic molecules with increased biological relevance. To this end, we chose a nonameric peptide sequence, rich with glutamate residues, that is found within an α-helical motif in hemoglobin protein (Gly-Glu-Tyr-Gly-Ala-Glu-Ala-Leu-Glu).[43,44] To visualize the binding to the resin, the peptide was labeled with a colored label. By itself, this peptide is too short to exist as an α-helix, but we were interested in finding out whether a resin-bound triamine sequence could be discovered that can stabilize and promote α-helix formation.

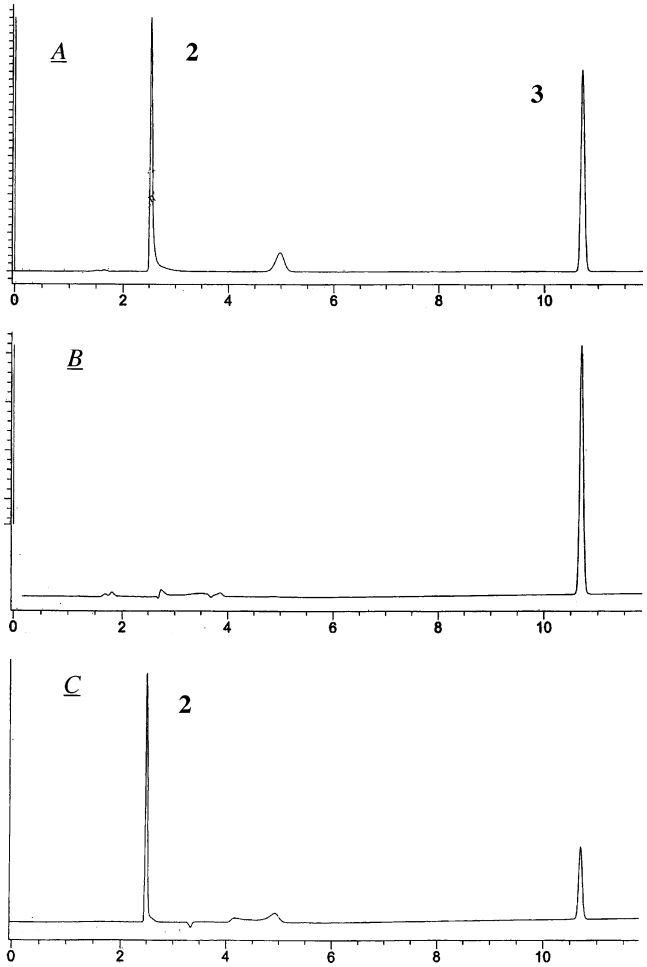
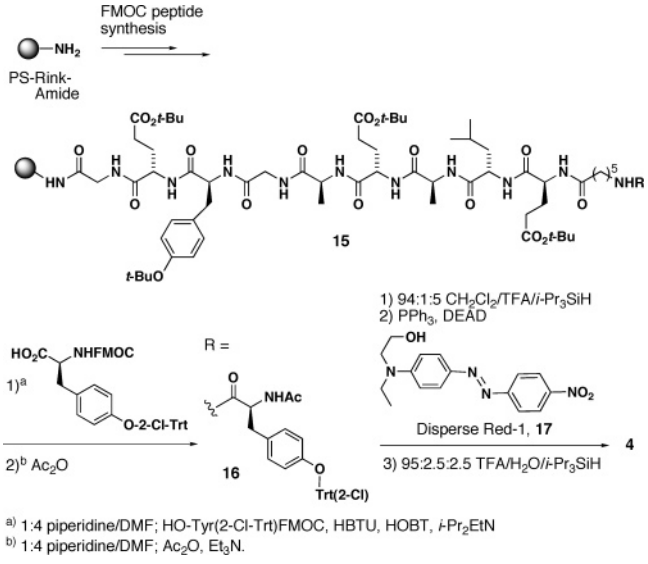


Figure 11. (A) HPLC/UV chromatogram of a 1:1 mixture of dyes **2** and **3**, (B) after washing resin **13** (2Acc^R-ϵAhx^R) with 0.5 M $PhSO_3Na$, and (C) after washing resin **14** (8Aoc^R−8Aoc^R).

Scheme 3



a) 1:4 piperidine/DMF; HO-Tyr(2-Cl-Trt)FMOC, HBTU, HOBT, *i*-Pr_2EtN
b) 1:4 piperidine/DMF; Ac_2O, Et_3N.

The synthesis of the labeled peptide **4** began by preparing the protected peptide **15** by standard FMOC peptide synthesis on Rink amide resin using orthogonally protected glutamate and tyrosine amino acids (Scheme 3). An ϵAhx residue was attached to the *N*-terminal end of the sequence to act as a spacer between the hemoglobin peptide and the Disperse Red-1 dye (**17**). Before linking the dye onto the peptide, the FMOC protective group on the ϵAhx was removed, and an *O*-2-chlorotrityl-protected FMOC-tyrosine was coupled, then *N*-terminated with an acetyl group to provide the peptide intermediate **16**. The 2-chlorotrityl group was then removed from the tyrosine using dilute TFA, liberating the phenolic hydroxyl, which allowed for a Mitsunobu coupling to attach the dye **17**. Concomitant deprotection of the glutamates and the internal tyrosine residues with cleavage of the peptide from the resin was achieved by treatment with 95/2.5/2.5 TFA/water/*i*-Pr_3SiH, affording the labeled peptide **4**.

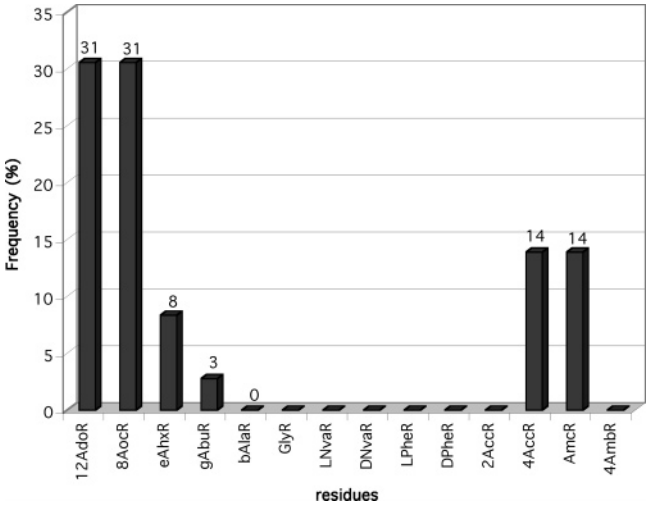


Figure 12. Screening of the triamine library against peptide **4** at pH 7. A total of 36 residues (18 triamines) were decoded. See text for further details.[31]

The on-bead screening experiment was performed with a 2.0×10^{-5} M solution of peptide **4** in 0.010 M MES−TRIS (pH 7.0) in 10% water/DMF. About 5% of the beads were dark red, and a total of 18 of these were isolated and decoded by LC/MS. Figure 12 shows the sequences that were selected.[31]

In previous literature reports, tetracationic ligands, such as spermidine[43] and synthetic tetraguanidinium[44−46] receptors, have induced tetraglutamate and tetraaspartate peptide sequences into forming α-helices. In the report with spermidine,[43] the peptide being studied contained glutamate residues spaced in an i, $i + 4$, $i + 7$, $i + 11$ orientation, which is the same as in peptide **4**. In other words, the distances between the anionic sites along the α-helix of the peptide are complementary to the internitrogen distances in spermidine, which are relatively short; however, the selection of long residues (12Ado^R and 8Aoc^R) from our library screening would seem to indicate that peptide **4** remains as an elongated sequence as it binds to the resin-bound triamines. This suggests that the glutamate residues on the peptide are far apart from each other and are likely not part of an α-helical structure. Presumably, a somewhat longer peptide sequence more prone to display helicity would be required.

Conclusions

In this work, a combinatorial library was used to discover polyamine ligands toward various trisulfonated dyes through the selective formation of multiple ion pair interactions. It appears that the strongest complexes are the result of a

requirement for three geometrically defined ion pairing interactions. This belief is supported by the similar distances between the sulfonate groups on the dye molecules compared to the internitrogen distances on the most favored triamine residues. The results from these on-bead screenings were also used in the design of individual polyamines that exhibit the same selectively toward particular dye targets, both in solution and on solid support during a solid-phase dye extraction experiment. Overall, this study shows that it is possible to achieve reasonably good selectivity based on multiple ion pairing interactions between a resin-supported library of linear and flexible polyamines and a polyanionic target. Using this single-bead approach, it should be possible to screen synthetic polyamine libraries against polyanionic biomolecules, including oligonucleic acids, proteins, and polysulfated oligosaccharides, such as heparin.

Experimental Section

The triamine library was synthesized and decoded as described in ref 17.

General Procedure for the Synthesis of Polyamides on Aminotrityl Resin. The resin was weighed into a polypropylene filter vessel and rinsed three times with DMF. A 0.5 M solution of FMOC-amino acid (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 each). After an additional 2−5 min of vortexing, DIPEA (4 equiv) was added. The suspension was then vortexed for 2 h, filtered, and rinsed with DMF (5×). Ninhydrin[47] and bromophenol blue assays on the resin should be negative. 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 with DMF (5×). Both the amino acid coupling and the FMOC removal were repeated until the final amino acid is attached. The resin was then filtered and rinsed with DMF (3×), methanol (3×), and dichloromethane (5×). It was then dried under high vacuum for >12 h.

General Procedure for the Exhaustive Amide Reduction of Polystyrene-Bound Polyamides. To the resin-bound polyamide, weighed inside a flamed-dried, round-bottom flask equipped with a condenser and a stir bar, was added the 1.0 M BH_3/THF solution (10 equiv per amide) while under argon atmosphere. The suspension was then heated to 65 °C with gentle stirring until the reaction was complete. Upon cooling to room temperature, the suspension was quickly transferred into a polypropylene filter vessel via a pipet using dry THF to rinse the flask. After rinsing the resin with THF (5×), cleavage of the borane−amine adducts was done the following way: to every 100 mg of resin was added 0.5 mL of dry THF, 0.14 mL of DIPEA, and then 0.28 mL of acetic acid in this order with vigorous vortexing between each addition. This was followed by the careful addition (*exothermic!*) of iodine (∼5 equiv per borane−amine adduct) in 0.5 mL of THF. The final ratio of THF/DIPEA/acetic acid should be ∼7:1:2. The vessel was then vortexed for 4 h at room temperature. Afterward, the resin was filtered and rinsed with THF (3×), 1:3 Et_3N/DMF (3×), methanol (3×), and dichloromethane (5×) and dried under high vacuum for >12 h. A clean reaction will usually be indicated by the resin's returning to its original pale color prior to reduction.

Screening of the Triamine Library against Trisulfonated Dyes. Approximately 3 mg of resin-bound triamine library (∼13 000 beads) was added to 400 μL of a 0.050 M solution of MES−TRIS (pH 7.0 in water) in 1:9 water/DMF (solution A) inside a 2.5-cm-diameter, 0.8-cm-deep glass Petri dish. Resin clumps were broken up either physically or by vigorous swirling of the dish. A 12.8-μL portion of a 1.25×10^{-3} M solution of the dye in solution A was then quickly added to the swirling suspension to make a final dye concentration of 3.75×10^{-5} M. For screening in the presence of spermidine, 12.8 μL of a 1.05×10^{-2} M solution of spermidine in solution A was first added and, after 10 min of swirling, was followed by the addition of 12.8 μL of a 1.25×10^{-3} M solution of the dye in solution A. The final concentration of spermidine was 3.16×10^{-4} M. In both cases, the suspension was swirled for 1 h, during which time the dye solution turned clear. While under a microscope, the darkest beads were isolated using a thin glass capillary and transferred onto another Petri dish, where they were washed with 1:9 water/DMF with no buffer (5×) and dichloromethane (5×) using the capillary. The beads were then transferred into a conical microvial and analyzed by LC/MS using the method described in ref 17. Screenings at pH 5.5 were performed the same way as outlined above, except in a solution of 0.025 M MES buffer (pH 5.5 in water) in 1:9 water/DMF (solution B).

Synthesis of Triamine Amide 8. From 1,6-diaminohexane polystyrene−trityl resin **5**,[17] the triamide **6** was prepared using the standard amide coupling protocol described above with the amino acid derivatives HO-8Aoc-FMOC and HO-8Aoc-Ac. Resin **6** (0.418 g, 0.314 mmol, 0.75 mmol/g) was then treated with 1.0 M BH_3 in THF (11.5 mL, 11.5 mmol) as described above. After 2 days at 65 °C, the resin was filtered, rinsed with THF, and then treated with I_2 (1.28 g, 4.95 mmol) in a 7/2/1 mixture of THF, acetic acid, and DIPEA for 4 h according to the general procedure given above. It was then rinsed with THF (4×), 1:3 Et_3N/DMF (4×), methanol (4×), and dichloromethane (5×) to give the supported triamine. The resin was resuspended in 2 mL of dichloromethane, and to this was added DIPEA (0.26 mL, 1.5 mmol), followed by $(Boc)_2O$ (0.69 mL, 3.0 mmol). The suspension was then vortexed for 24 h before it was rinsed with dichloromethane (5×). The BOC-protected triamine **7** was subsequently cleaved from the resin by repeated treatments with 1% solution of TFA in dichloromethane (3 mL each time, 10 times all together). After each treatment, the resin was filtered, and the filtrate was collected as a separate fraction containing 2 mL of 10% pyridine in methanol. The fractions were checked by LC/MS, and those containing pure compound were combined and evaporated. The residue was then dissolved in 2 mL of pyridine and cooled to 0°C before the addition of Ac_2O (50 μL, 0.53 mmol). After stirring for 5 h at room temperature, the reaction was concentrated and then suspended in 50 mL of water. The product was extracted into ether (3 × 30 mL), washed with water (2 × 20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. Flash

column chromatography through silica gel using 5% methanol in dichloromethane yielded the BOC-protected polyamine. Exposure to 1:1 TFA/dichloromethane for 1 h followed by evaporation yielded the final product **8** as a clear oil (42 mg, 19% yield based on theoretical loading of resin-bound triamide **6**). ^{1}H NMR (500 MHz, 20% D_2O in DMSO-d_6): δ 2.98 (t, J = 7.0 Hz, 2H), 2.84 (q, J = 7.5, 2H), 2.82−2.79 (m, 10H), 1.78 (s, 3H), 1.51 (broad s, 8H), 1.35 (quintet, J = 7.0 Hz, 2H), 1.30−1.80 (m, 22H), 1.13 (t, J = 7.5 Hz, 3H). ^{13}C APT NMR (125 MHz, CD_3OD): δ 173.2 (CO), 49.0 (CH_2), 49.0 (CH_2), 48.5 (CH_2), 44.1 (CH_2), 40.2 (CH_2), 30.8 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 27.5 (CH_2), 27.4 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 27.2 (CH_2), 22.7 (CH_3), 11.6 (CH_3); note the large amount of overlap of the aliphatic CH_2 signals in the ^{13}C NMR spectrum. IR (methanol cast) 3300, 2937, 1674 cm^{-1}. HRMS/ESMS for $C_{26}H_{57}N_4O$ (MH^+) calcd, 441.4532; obs, 441.4536.

Synthesis of Triamine Amide 12. The synthesis of the resin-supported triamide **9**, using HO-2Acc-FMOC and HO-ϵAhx-Ac, and the exhaustive amide reduction with the iodine procedure was performed as described above; however, after the iodine-promoted workup, significant amounts (∼25−35%) of triaminoborohydride from cleaved resin **10** (5% TFA/CH_2Cl_2) were detected by HPLC−ESMS. Full conversion to triamine **11** was achieved after vortexing the resin (0.410 g, 0.332 mmol) in 8 mL of THF containing 1.0 mL of ethylene glycol and 0.5 mL of DIPEA for 16 h, followed by rinsing with THF (3×), methanol (3×), and dichloromethane (5×). The entire amount of this resin was subsequently BOC-protected; cleaved from the resin; acetylated; and, finally, deprotected using the same procedure described in the synthesis of triamine amide **6**. Compound **12** was obtained as a clear oil (45 mg, 15% yield based on theoretical loading of resin-bound triamide **9**) ^{1}H NMR (500 MHz, 20% D_2O in DMSO-d_6): δ 3.20 (m, 1H), 3.11 (t, J = 12.5 Hz, 1H), 2.98 (t, J = 7.0 Hz, 2H), 2.95−2.78 (m, 10H), 2.33 (m, 1H), 1.78 (s, 3H), 1.75−1.60 (m, 3H), 1.60−1.55 (m, 6H), 1.43−1.34 (m, 6H), 1.27 (broad s, 9H), 1.31 (t, J = 7.5 Hz, 3H). ^{13}C APT NMR (125 MHz, CD_3OD): δ 173.1 (C), 163.2 (q, *C*OCF_3), 118.1 (q, CO*C*F_3), 60.3 (CH), 47.0 (CH_2), 48.3 (CH_2), 47.1 (CH_2), 45.7 (CH_2), 44.1 (CH_2), 40.2 (CH_2), 35.4 (CH), 30.2 (CH_2), 27.4 (CH_2), 27.2 (CH_2), 27.2 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 27.0 (CH_2), 26.8 (CH_2), 25.4 (CH_2), 24.8 (CH_2), 22.7 (CH_3), 20.8 (CH_2), 11.6 (CH_3). IR (methanol cast) 3425, 2943, 2865, 1676 cm^{-1}. HRMS/ESMS for $C_{23}H_{49}N_4O$ (MH^+) calcd, 397.3906; obs, 397.3901.

NMR Titration of Dyes 2 and 3 with Triamine Amides 8 and 12. To a 2.56 × 10^{-3} M solution of triamine amide in 0.6 mL of 20% D_2O/DMSO-d_6 inside an NMR tube was added increasing amounts of dye (c = 0.066 M), dissolved in the same solvent mixture. Initially, small increments of the dye were added (2.4 μL, 0.1 equiv) but as the change in chemical shift became smaller, larger volumes (12 μL, 0.8 equiv) were added. For **8**, the methylene protons at 1.226 ppm were monitored. For **12**, the methylenes at 1.271 ppm were observed. The K_a's were determined using the curve-fitting software developed by Christopher A. Hunter.[40]

Job Plot of Dye 2 and Triamine Amide 8. Equimolar solutions (c = 0.132 M) of **8** and **2** were prepared in 20% D_2O/DMSO-d_6. In separate NMR tubes, varying proportions of each solution were prepared maintaining a total volume of 20 μL. They were then diluted to 0.7 mL, and the change in the chemical shift of the dye proton at 7.445 ppm was monitored.

Tritylpolystyrene-Bound 2Acc^R−ϵAhx^R (13). The synthesis of the resin-supported triamide precursor to **13** and the exhaustive amide reduction with the iodine procedure was performed as described above in the General Procedure section. The only exception was the additional workup with ethylene glycol that was described for the synthesis of **12**. A portion of the resin (95 mg at 0.74 mmol/g, 0.0705 mmol) was cleaved with 5% TFA in dichloromethane for 1 h and then filtered through glass wool. The resin was washed with methanol, and the filtrate was evaporated, giving a yellow oil (40 mg crude, 63%) corresponding to the tetrakis-(trifluoroacetate) salt corresponding of the cleaved product of **13**. ^{1}H NMR (500 MHz, CD_3OD): δ 3.35 (m, 1H), 3.15−2.96 (m, 6H), 2.98 (m, 2H) 2.91 (m, 4H), 2.55 (m, 1H), 1.94−1.30 (m, 36H), 1.29 (t, J = 7.5 Hz, 3H). ^{13}C APT NMR (125 MHz, CD_3OD): δ 162.6 (q, *C*OCF_3), 118.2 (q, CO*C*F_3), 60.3 (CH), 50.1 (CH_2), 48.3 (CH_2), 47.1 (CH_2), 45.7 (CH_2), 44.1 (CH_2), 40.9 (CH_2), 35.4 (CH), 30.7 (CH_2), 30.7 (CH_2), 30.6 (CH_2), 30.6 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 28.7 (CH_2), 27.7 (CH_2), 27.5 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 25.4 (CH_2), 24.4 (CH_2), 24.5 (CH_2), 11.6 (CH_3). IR (methanol cast) 3300−2700, 2931, 1677 cm^{-1}. HRMS/ESMS for $C_{27}H_{59}N_4O$ (MH^+) calcd, 439.4740; obs, 439.4737.

Tritylpolystyrene-Bound 8Aoc^R−8Aoc^R (14). The synthesis of the resin-supported triamide precursor to **14** and the exhaustive amide reduction with the iodine procedure was performed as described in the general section above. A portion of the resin (175 mg at 0.92 mmol/g, 0.16 mmol) was cleaved with 5% TFA in dichloromethane for 1 h and then filtered through glass wool. The resin was washed with methanol, and the filtrate was evaporated, giving a yellow oil (111 mg crude, 80%) corresponding to the tetrakis-(trifluoroacetate) salt corresponding to **14**. ^{1}H NMR (500 MHz, CD_3OD): δ 3.04 (q, J = 7.5 Hz, 2H), 3.02−2.95 (m, 9H), 2.92 (t, J = 8.0 Hz, 2H), 1.75−1.62 (m, 12H), 1.45−1.42 (m, 8H), 1.42−1.35 (m, 12H), 1.29 (t, J = 7.0 Hz, 3H). ^{13}C APT NMR (125 MHz, CD_3OD): δ 162.9 (q, *C*OCF_3), 118.2 (q, CO*C*F_3), 49.0 (CH_2 broad peak with a shoulder; likely more than one signal), 48.9 (CH_2), 48.5 (CH_2), 44.1 (CH_2), 40.6 (CH_2), 30.0 (CH_2), 28.0 (CH_2), 27.5 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 27.1 (CH_2), 27.0 (CH_2), 11.6 (CH_3); note the large number of overlapping aliphatic CH_2 signals. IR (methanol cast) 3300−2700, 2931, 1677 cm^{-1}. HRMS-ESMS for $C_{27}H_{59}N_4O$ (MH^+) calcd, 439.4740; obs, 439.4737.

Staining of Resin-Bound Triamines with a 1:1 Mixture of 2 and 3. Triamine resin **13** or **14** (∼12 mg, 0.0087 mmol) was vortexed for 1 h in 3 mL of solution A containing 1.7 × 10^{-3} M **2** and 1.7 × 10^{-3} M **3**. The suspension was then filtered. The dark red resin was rinsed four times with 10% water in DMF to remove any unbound dye. Displacement of the dyes from the resin was achieved by sequential washes with $PhSO_3Na$ in DMF starting with a 0.1 M solution (3×), followed by a 0.5 M solution (4×). During each of the $PhSO_3Na$ washings, the eluting solution turned from clear

to red, while the resin progressively became lighter in color. The rinses were collected as separate fractions and then analyzed by HPLC. LC column: Zorbax SB-C18 4.6 × 150 mm, 3.5 μm. Eluent: 5−20% acetonitrile over 10 min in 25 mM phosphate buffer (pH 7.0), then maintained at 20% for 4 min at 0.8 mL/min. UV detection at 507 nm.

Acknowledgment. The authors thank the Natural Science and Engineering Research Council (NSERC) of Canada; Alberta Heritage Foundation for Medical Research (AHFMR); Research Corporation (Tuscon, AZ); and the Department of Chemistry, University of Alberta, for financial support. S.M. is grateful to AHFMR for a Studentship. The assistance of Dr. Mohammed Al Sayah is also acknowledged for his help in the determination of the stability constants.

Supporting Information Available. Tables of selected triamine sequences from the screening of **1**−**4**, titration curves for measurements of association constants, Job's plot between **2** and **8**, docking of dye **2** with a protonated Et-NH−2Acc^R−ϵAhx^R-Et triamine. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Cohen, S. S. *A Guide to Polyamines*; Oxford University Press: New York, 1998.
(2) Williams, K. *Biochem. J.* **1997**, *325*, 289−297.
(3) Scott, R. H.; Sutton, K. G.; Dolphin, A. C. *Trends Neurosci.* **1993**, *16*, 153−160.
(4) Igarashi, K.; Kashiwagi, K. *Biochem. Biophys. Res. Commun.* **2000**, *271*, 559−564.
(5) Casero, R. A.; Woster, P. M. *J. Med. Chem.* **2001**, *44*, 1−26.
(6) Frydman, B.; Valasinas, A. *Expert Opin. Ther. Patents* **1999**, *9*, 1055−1068.
(7) Phanstiel, O.; Price, H. L.; Wang, L.; Juusola, J.; Kline, M.; Shah, S. M. *J. Org. Chem.* **2000**, *65*, 5590−5599.
(8) Klenke, B.; Stewart, M.; Barrett, M. P.; Brun, R.; Gilbert, I. H. *J. Med. Chem.* **2001**, *44*, 3440−3452.
(9) Wang, C.; Delcros, J. G.; Biggerstaff, J.; Phanstiel, O. *J. Med. Chem.* **2003**, *46*, 2663−2671.
(10) Ren, T.; Zhang, G.; Liu, D. *Tetrahedron Lett.* **2001**, *42*, 1007−1010.
(11) Fabio, K.; Gaucheron, J.; DiGiorgio, C.; Vierling, P. *Bioconjugate Chem.* **2003**, *14*, 358−367.
(12) Deng, H.; Bloomfield, V. A.; Benevides, J. M.; Thomas, G. J. *Nucleic Acids Res.* **2000**, *28*, 3379−3385.
(13) Wahl, M. C.; Sundaralingam, M. *Biopolymers* **1997**, *44*, 45.
(14) Tippen, D. B.; Sundaralingam, M. *J. Mol. Biol.* **1997**, *267*, 1171−1185.
(15) Nagamani, D.; Ganesh, K. N. *Org. Lett.* **2001**, *3*, 103−106.
(16) Lawton, G. R.; Appella, D. H. *J. Am. Chem. Soc.* **2004**, *126*, 12762−12763.
(17) Manku, S.; Wang, F.; Hall, D. G. *J. Comb. Chem.* **2003**, *4*, 379−391.
(18) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542−543.
(19) McCleskey, S. C.; Griffin, M. J.; Schneider, S. E.; McDevitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 1114−1115.
(20) Llinares, J. M.; Powell, D.; Bownan-James, K. *Coord. Chem. Rev.* **2003**, *240*, 57−75.
(21) Grawe, T.; Schrader, T.; Finocchiaro, P.; Consiglio, G.; Failla, S. *Org. Lett.* **2001**, *3*, 1597−1600.
(22) Corbellini, F.; Fiammengo, R.; Timmerman, P.; Crego-Calama, M.; Versluis, K.; Heck, A. J. R.; Luyten, I.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2002**, *124*, 6569−6575.
(23) Rekharsky, M.; Inoue, Y.; Tobey, S.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 14959−14967.
(24) Tobey, S. L.; Jones, B. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 4026−4027.
(25) Schmuck, C.; Wienand, W. *J. Am. Chem. Soc.* **2003**, *125*, 452−459.
(26) Miranda, C.; Escartí, F.; Lamarque, L.; Yunta, M. J. R.; Navarro, P.; García-España, E.; Jimeno, M. L. *J. Am. Chem. Soc.* **2004**, *126*, 823−833.
(27) Lam, K. S.; Zhao, Z.-G.; Wade, S.; Krchnak, V.; Lebl, M. *Drug Dev. Res.* **1994**, *33*, 1994.
(28) Wennemers, H.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 6413.
(29) Manku, S.; Hall, D. G. *Org. Lett.* **2002**, *4*, 31−34.
(30) Ngola, S. M.; Kearney, P. C.; Mecozzi, S.; Russell, K.; Dougherty, D. A. *J. Am. Chem. Soc.* **1999**, *121*, 1192−1201.
(31) For tables with frequency of triamine sequences of two residues, refer to the Supporting Information.
(32) SYBYL force field on MacSpartan Plus version 1.1.9 (Wave Function Inc.). For simplicity, all calculations were performed on the neutral molecules in the gas phase and, thus, were used solely as a guide in comparing oxygen-to-oxygen distances with the internitrogen distances.
(33) Hossain, M. A.; Schneider, H.-J. *Chem.—Eur. J.* **1999**, *5*, 1284−1290.
(34) Bergeron, R. J.; Weimar, W. R.; Wu, Q.; Feng, Y.; McManis, J. S. *J. Med. Chem.* **1996**, *39*, 5257−5266.
(35) Alfonso, I.; Dietrich, B.; Rebolledo, F.; Gotor, V.; Lehn, J.-M. *Helv. Chim. Acta* **2001**, *84*, 280.
(36) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1986**, *69*, 587−603.
(37) Manku, S.; Laplante, C.; Kopac, D.; Chan, T.; Hall, D. G. *J. Org. Chem.* **2001**, *66*, 874−885.
(38) It should be noted that the aminoborohydride was never observed in decoding single resin beads expressing the sequence 2Acc^R−ϵAhx^R or any other triamine.
(39) Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.
(40) http://www.shef.ac.uk/uni/projects/smc/soft.html.
(41) Gallivan, J. P.; Dougherty, D. A. *J. Am. Chem. Soc.* **2000**, *122*, 870−874.
(42) In the large scale preparation of **13**, just as for triamine amide **12**, additional treatment with basic ethylene glycol was required following the reduction with borane and workup with iodine.
(43) Tabet, M.; Labroo, V.; Sheppard, P.; Sasaki, T. *J. Am. Chem. Soc.* **1993**, *115*, 3866−3868.
(44) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2494.
(45) Peczuh, M. W.; Hamilton, A. D.; Sanchez-Quesada, J.; deMendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327−9328.
(46) Albert, J. S.; Goodman, M. S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 1143−114.
(47) Kaiser, E.; Colscott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

CC0501559