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Combinatorial Approach to Selective Multivalent Ion Pairing in Mixed Aqueous–Organic Media Using Bead-Supported Libraries of Unnatural Polyamines

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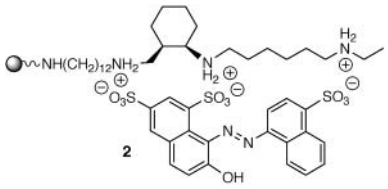
ABSTRACT



Screening of a bead-supported encoded library of unnatural polyamines against model polyanionic targets (1 and 2) demonstrated that a combinatorial approach can highlight structural selectivity in multivalent ion pairing in aqueous solutions. This approach even provided -NH-2Acc^{R}-6Ahx^{R}-Et, a highly target-selective triamine sequence that can discriminate between two trisulfonated dyes displaying subtle structural differences.

Despite their ubiquity and their essential roles in living systems, only a small number of distinct polyamines exist in nature.[1,2] Notable examples of these vital biomolecules include putrescine, spermidine, and spermine. Polyamines are protonated under physiological conditions and are thus predisposed to form strong salt bridges. These cationic molecules were shown to condense with the phosphate groups of DNA and RNA,[3] with anionic oligosaccharides,[4] and with the carboxylate side chains of aspartate and glutamate residues in polypeptides[5] and proteins.[6] Yet, it remains to be seen whether the fine structure of the interammonium spacers in polyamines can be tuned to optimize binding affinity and selectivity in multipoint ion pairing complexes in aqueous media.[7,8] Nature provides relatively few insights to address this question; the main biogenic polyamines, made almost exclusively with 1,3-diaminopropyl and 1,4-diaminobutyl units, display little diversity in their sequence and length as compared to the

(1) Cohen, S. S. *A Guide to the Polyamines*; Oxford University Press: Oxford, New York, 1999.

(2) For recent reviews on the synthesis and biological activity of polyamine analogues, see: (a) Casero, R. A., Jr.; Woster, P. M. *J. Med. Chem.* **2001**, *44*, 1. (b) Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem.* **2000**, 1841. (c) Kuksa, V.; Buchan, R.; Lin, P. K. J. *Synthesis* **2000**, 1189.

(3) For examples, see the following. DNA: (a) Deng, H.; Bloomfield, V. A.; Benevides, S. M.; Thomas, G. J., Jr. *Nucleic Acids Res.* **2001**, *17*, 3379. RNA: (b) Quigley, G. J.; Teeter, M. M.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 64. Inositol-tris(phosphates): (c) Mernissi-Arifi, K.; Zenkouar, M.; Schlewer, G.; Spiess, B. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 3101.

(4) Shilo, M. in *Microbial Toxins*; Kadis, S., Ciegler, A., Ajl, S. J., Eds.; Academic Press: New York, 1971; Vol. VII, pp 67.

(5) (a) Tabet, M.; Labroo, V.; Sheppard, P.; Sasaki, T. *J. Am. Chem. Soc.* **1993**, *115*, 3866. (b) Peczuh, M. W.; Hamilton, A. D.; Sánchez-Quesada, J.; de Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327.

(6) For one example on the calcium-binding protein parvalbumin, see: (a) Sudhakar, K.; Erecinska, M.; Vanderkooi, J. M. *Eur. J. Biochem.* **1995**, *230*, 498. For a review on the interactions of polyamines with ion channel proteins, see: (b) Williams, K. *Biochem. J.* **1997**, *325*, 289.

(7) For seminal examples on the "inverse" approach involving selected cyclic or acyclic polyamines with several linear diacids, see: (a) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1986**, *69*, 587. (b) Hossain, M. A.; Schneider, H.-J. *Chem. Eur. J.* **1999**, *5*, 1284.

(8) The competing effect of water has been known as a notorious frustration in the design of synthetic receptors based on hydrogen bonds. For reviews on receptors for anion recognition, see: (a) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. (b) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486.

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other natural biopolymers (peptides, nucleic acids, and oligosaccharides). By way of multivalent ion pairing, we hypothesize that large combinatorial libraries of unnatural polyamines could reveal highly potent and selective ligands for polyanionic biomolecules (Figure 1). As a first step

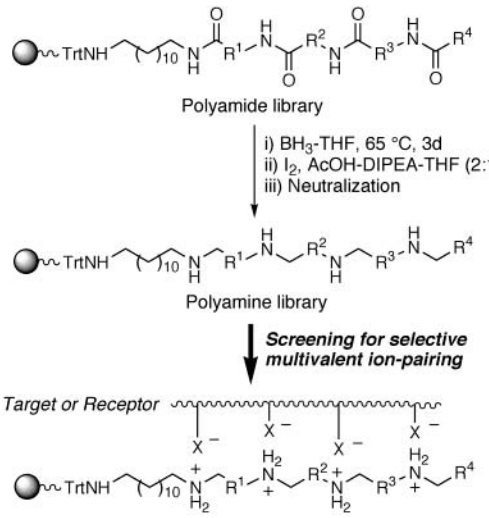

Figure 1. Synthesis and screening of bead-supported unnatural polyamine libraries for multivalent ion pairing. Note: R^1-R^3 represent structurally diverse spacers.

toward this goal, we describe the first demonstration of on-bead screening in mixed aqueous−organic media, using an encoded structural library of unnatural polyamines synthesized by a split-pool approach.[9]

As a proof of principle our initial objective was to synthesize and screen a relatively small prototypical library of polyamines against model polyanionic molecules. The trisulfonated dyes **1** and **2** were selected as examples of rigid targets for multivalent ion pairing (Figure 2).[10] They are also reminescent of the polysulfated heparin oligosaccharides and thus can serve as models for the eventual development of ligands and molecular sensors for these biologically important carbohydrates.[11] From a practical standpoint, these red-colored dyes also provide a direct means of visually detecting bead hits in the screening experiments. In addition, being rather amphiphilic, they are soluble in mixed aqueous organic

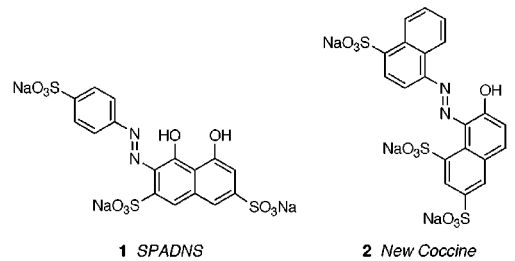

Figure 2. Model trisulfonated targets **1** and **2**.

solvents and thus are appropriate for screening against a library of polyamines supported onto polystyrene-based supports.

To access the library, our synthetic plan was centered on a "library from library" approach[12] whereby a split-pool library of polyamide precursors is exhaustively reduced to polyamines using our borane-promoted procedure for peptides supported onto a trityl linker (Figure 1).[13] Library design was based on optimizing structural diversity with a limited but representative set of 14 amino acid building blocks offering a variety of geometrical features (length and flexibility) (Figure 3). Split-pool libraries of end-acetylated

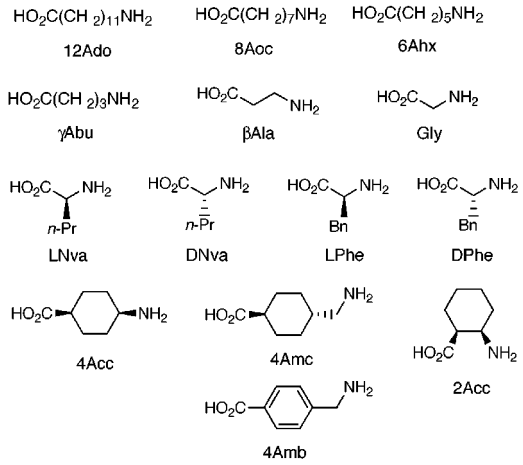

Figure 3. Set of structurally diverse amino acid building blocks for the generation of polyamine libraries. Note: 2Acc is employed as racemate, 4Acc and 2Acc are exclusively *cis*, and 4Amc is exclusively of *trans* configuration.

di- and tripeptides were assembled using Fmoc-amino acid coupling methods from a trityl bound dodecamine spacer.[14] Such a long spacer was selected in order to avoid steric interference from the triphenyl moiety. The library was encoded by termination synthesis whereby 10% of the corresponding *N*-acetyl or *N*-butyryl amino acids were employed in each coupling step.[15] This method, which eventually allows unambiguous identification of the oligo-

(9) For reviews on "one bead-one molecule" libraries made by split-pool synthesis, see: (a) Lam, K. M.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411. (b) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155.

(10) For other examples on dye screening, see: (a) Lam, K. S.; Zhao, Z.-G.; Wade, S.; Krchnák, V.; Lebl, M. *Drug Dev. Res.* **1994**, *33*, 157. (b) Wennemers, H.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 6413.

(11) van Boeckel, C. A. A.; Petitou, M. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1671.

(12) Ostresh, J. M.; Husar, G. M.; Blondelle, S. E.; Dörner, B.; Weber, P. A.; Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11138.

(13) (a) Manku, S.; Laplante, C.; Kopac, D.; Chan, T.; Hall, D. G. *J. Org. Chem.* **2001**, *66*, 874. (b) Wang, F.; Manku, S.; Hall, D. G. *Org. Lett.* **2000**, *2*, 1581.

(14) See details in Supporting Information. The final resin loading of the polyamine libraries is relatively high (ca. 0.7 mmol g^{-1}). Because of the hydrophobic nature of the polymer matrix it is possible that polyamine protonation is uniform mainly at the surface. This should not affect binding selectivity. Similarly, the hindered tritylamine anchor is not expected to interfere.

(15) Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900.

amines by electrospray mass spectrometry (ES-MS), can even distinguish between isobaric sequences by "ladder" decoding of truncated fragments.[16]

Exhaustive reduction of the tripeptide library provided the 2744-member tetraamine library. The corresponding 196-member library of triamines was also made. Over 20 beads from the larger tetraamine library were picked at random in order to test the validity of the synthetic approach and the encoding method. The beads were cleaved individually in microvials with 5% TFA/CH_2Cl_2, and the resulting material was analyzed by LC-ES-MS. From these test runs and other subsequent ones, decoding efficiencies in the 85−95% range are routinely obtained (see examples in Supporting Information).

The *N*-ethyl-terminated triamine library {-$(CH_2)_{12}$NHCH$_2$R^1-NHCH$_2$R^2-NHCH$_2$CH$_3$} was employed for this study because the model sulfonated targets are triply anionic species. The triamines are not quite symmetrical, the two end groups presenting slightly different degrees of size and hydrophobicity. Control experiments (with and without 0.1% Triton X-100) with the uncharged tripeptide library confirmed the absence of nonspecific interactions between the dyes and the polymer matrix. Thus the triamine library (3 mg, approximately 10^4 beads), in a large stoichiometric excess, was screened against 37.5 μM dye **1** in 0.4 mL of 10% aqueous 50 mM TRIS-MES buffer (pH 7.0) in DMF. These conditions provide a huge excess of water molecules vis-à-vis the target dye. A proportion of about 50−70% of all beads became distinctly pink, of which approximately 5−6% had a deeply red-colored appearance. Decoding results for the darkest beads are shown in Table 1 as a function of single residue frequency with respect to their position within the triamine backbone (R^1, R^2).

Out of 30 of the darkest beads picked under a microscope (first column), most had triamines with either the long 12- and 8-carbon interammonium spacers 12Abo^R and 8Aoc^R regardless of the position. For instance, some of the most recurrent sequences were 12Abo^R-8Aoc^R and 8Aoc^R-8Aoc^R (found four times each). A small number of beads also had the relatively long but more rigid spacers 6Ahx^R and 4Acc^R. The assay was repeated with 0.32 mM spermidine (second column) as a competitive ligand to minimize nonspecific ionic interactions and also at pH 5.5 (third column), a precautionary measure to ensure that even short triamines with the two-carbon interammonium spacers from α-amino acids were largely protonated.[17] Binding did not seem affected, and the overall selectivity was conserved.[18] Screening of dye **2** provided similar residue consensus (fourth and fifth columns), however, reduced in 12Abo^R to include more 6Ahx^R and also 2Acc^R as a frequent residue that was essentially absent from the screening of dye **1**. Aside from the remarkable degree of selectivity observed, an interesting feature of these results is the almost total absence of βAla^R and γAbu^R, the respective 3- and 4-carbon interammonium spacers found in the natural polyamines. The presence of 1:1 binding stoichiometry in this system is assumed on the basis of a Job's plot obtained from NMR titration[19] performed between model triamine MeCONH$(CH_2)_6$-NH-8Aoc^R-8Aoc^R-Et and dye **1**.[20] This allows a tentative rationalization of sequence selectivity based on triply ion paired complexes involving a combination of electrostatic and hydrogen bonding interactions. Hydrophobic interactions do not seem dominant since exposure of the same dye to the precursor peptide library and to the triamine library in its neutral form (at pH 11) provided only faint beads. Except for rotation along the *N*-naphthyl bonds, the framework of dyes **1** and **2** is almost entirely rigid. From inspecting hand-held models we have identified two reasonable planar conformers for each dye (Figure 4).

As shown for dye **1**, in both conformers A and B the sulfonate groups are relatively far apart. The *meta* sulfonates of dye **2**, however, are much closer spatially. The relative

Table 1. Screening Results between Triamine Library and Dye Targets **1** and **2** Expressed in Residue Occurrence Per Position[a]

	dye				
	1	**1**	**1**	**2**	**2**
pH	7.0	7.0	5.5	7.0	5.5
additive[b]		SP	SP	SP	SP
no. of beads	30	26	23	40	31
position[c]	1,2	1,2	1,2	1,2	1,2
12Abo^R	8,4	9,8	14,9	3,5	5,6
8Aoc^R	10,13	7,14	1,3	18,5	5,2
6Ahx^R	2,5	3,0	1,1	9,6	7,3
γAbu^R	0,1	0,0	0,0	0,3	0,0
βAla^R	1,1	1,2	0,1	1,3	0,0
Gly^R	0,0	0,0	0,0	0,1	1,0
LNva^R	0,0	0,0	0,2	0,1	0,1
DNva^R	0,0	0,0	0,1	0,1	1,3
LPhe^R	0,0	0,0	0,2	0,1	0,0
DPhe^R	0,0	0,0	2,0	0,1	0,2
2Acc^R	0,1	0,0	0,0	4,2	8,3
4Acc^R	2,3	5,0	4,4	2,4	4,3
4Amc^R	7,2	1,2	0,0	3,6	0,6
4Amb^R	0,0	0,0	1,0	0,1	0,2
	Most Frequent Residues[d]				
	8Aoc^R	8Aoc^R	12Abo^R	8Aoc^R	12Abo^R
	12Abo^R	12Abo^R	4Acc^R	6Ahx^R	2Acc^R
	4Amc^R			4Amc^R	6Ahx^R
	6Ahx^R			12Abo^R	8Aoc^R
				2Acc^R	4Acc^R

[a] The R superscript indicates a reduced amino acid residue. Each assay was repeated once and was shown reproducible. [b] SP = spermidine. [c] Positions 1 and 2 refer to the interammonium spacers CH$_2$R^1 and CH$_2$R^2, respectively (Figure 1), originating from the reduced residues of Figure 3. [d] See Supporting Information for full sequence results.

(16) *N*-Butyryl derivatives of D-α-amino acids were employed in order to distinguish them from the L-enantiomer and also to distinguish 4Acc from 2Acc by mass spectrometry.

(17) Bergeron, R. J.; Weimar, W. R.; Wu, Q.; Feng, Y.; McManis, J. S. *J. Med. Chem.* **1996**, *39*, 5257.

(18) A notable exception is the lower occurrence of 8Aoc^R at lower pH. Spermidine, with distant nitrogens spaced by 8 atoms, might be a more effective competitor of this spacer under these conditions.

(19) Fielding, L. *Tetrahedron* **2000**, *56*, 6151.

(20) Performed in 20% D_2O in DMSO-d_6 using standard methods described in ref 19 (see Supporting Information for more details). It should be noted that different binding stoichiometries may be possible on solid support as a result of the high local concentration of triamines within the beads.

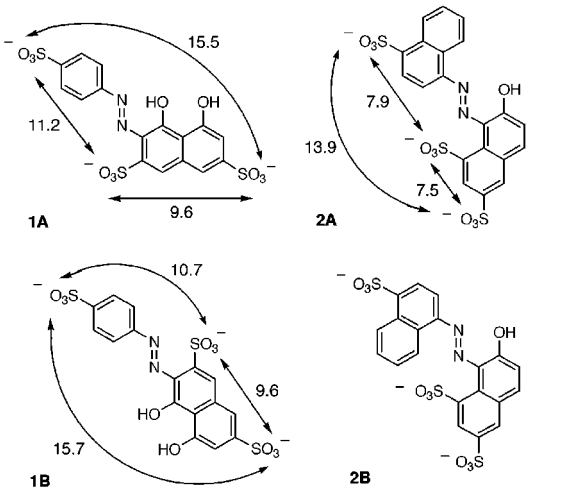

Figure 4. Possible conformations of dye targets **1** and **2** with indicated oxygen-to-oxygen distances (Å) measured between remote sulfonate oxygens.

positioning for the three sulfonate groups in **2** is identical for both conformers. As a basis for guiding comparisons, all indicated distances were measured between farthest oxygens of these groups on minimized structures.[21] Similarly, internitrogen distances were calculated for the most frequent residues in their extended conformation: 12Abo^R (16.6 Å), 8Aoc^R (11.5 Å), 6Ahx^R, (8.9 Å), 4Amc (7.2 Å), 4Acc^R (5.4 Å), and 2Acc^R (4.5 Å). This simple analysis accounts especially well for the high preference for 8Aoc^R, 6Ahx^R, and 4Acc^R in the case of both dyes and for 2Acc^R as a shorter, geometrically suitable spacer for bridging the *meta* sulfonates in dye **2**. The presence of 12Abo^R as one of the preferred residues, in particular with **1**, could be accounted by two-point binding across farthest sulfonate groups. Overall, the most favored dye−triamine complexes appeared to be tightly bound, as shown by the K_a value of 6400 M^{-1} measured between model compound MeCONH$(CH_2)_6$-NH-8Aoc^R-8Aoc^R-Et and dye **1**.[19,22]

Most interestingly, the results of Table 1 should prove useful in the context of designing target-selective triamine ligands. This was demonstrated by the design of 2Acc^R-6Ahx^R, a sequence foreseen as a specific ligand for dye **2** after comparing triamine residue consensus for **1** and **2**,[23] and from the measurement of K_a values using MeCONH-$(CH_2)_6$-NH-2Acc^R-6Ahx^R-Et as model (K_a with dye **1** = 1100 M^{-1}; K_a with dye **2** = 2700 M^{-1}).[19,22] In a "fishing-out" experiment, a mixture of these two dyes (1:1 ratio) was incubated with resin-bound -$(CH_2)_{12}$-NH-2Acc^R-6Ahx^R-Et triamine as described above (pH 7.0, 50 mM MES-TRIS H_2O/DMF 1:9). The beads, which became red instantaneously, were rinsed several times with a 0.1 M DMF solution of $PhSO_3Na$ and then with a 0.5 M solution in order to elute off the bound dye. As shown in the HPLC chromatogram of the final fraction (Figure 5), dye **2** was

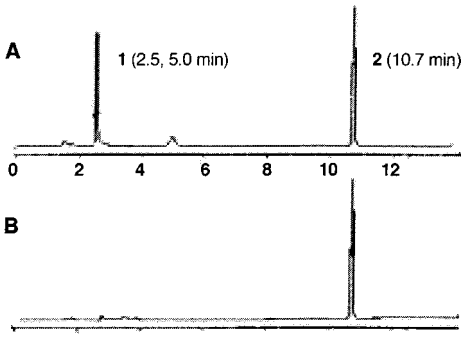

Figure 5. HPLC chromatogram of a 1:1 mixture of dyes **1** and **2** (A), and after elution of bound material from resin-supported -$(CH_2)_{12}$-NH-2Acc^R-6Ahx^R-Et (B). Conditions: 5−20% (10 min) acetonitrile in water containing 25 mM phosphate buffer (pH 7.0), UV detection at 350 nm. Note: dye **1** tends to elute under two forms using these conditions, although it is homogeneous by NMR.

detected with approximately 97% purity, thereby confirming that highly target-selective polyamines can be designed from a library approach. This result is particularly significant given the subtle structural differences between **1** and **2**. All other triamines tested, including 8Aoc^R-8Aoc^R, were less efficient.

This work on bead-supported encoded libraries of unnatural polyamines shows that a combinatorial approach, even with a relatively small library of linear flexible polyamines, can provide structural selectivity in multivalent ion pairing in mixed aqueous−organic media and even turn in target-selective ligands. We expect that the elaboration of larger polyamine libraries on fully hydrophilic supports will help in the discovery of ligands and sensors for polyanionic biomolecules.

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Supporting Information Available: Experimental procedures for library synthesis and decoding with several examples of single bead analyses by LC-ES-MS, full sequence results from the screening, synthesis and characterization data for model triamines, Job's plot for dye **1**, and titration curves for the measurement of K_a's. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Geometrical optimization was performed using the SYBYL force field on MacSpartan Plus version 1.1.9 (Wavefunction Inc.).

(22) Performed in 20% D_2O in DMSO-d_6. K_a values were determined using the 1:1 complexation model program developed by C. A. Hunter (see Supporting Information for more details).

(23) See graphics in Abstract. Molecular docking of dye **2** with the protonated EtNH-2Acc^R-6Ahx^R-Et triamine, in a highly extended form, showed a high level of overlay between the *meta* sulfonates and the 2Acc^R unit, with the 6-carbon interammonium spacer of 6Ahx^R reaching the leftover sulfonate.