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Discovery of Linear Receptors for Multiple Dihydrogen Phosphate Ions Using Dynamic Combinatorial Chemistry

Sophie R. Beeren and Jeremy K. M. Sanders*

University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom

Supporting Information

ABSTRACT: We describe the use of dynamic combinatorial chemistry to discover a new series of linear hydrazone-based receptors that bind multiple dihydrogen phosphate ions. Through the use of a template-driven, selection-based approach to receptor synthesis, dynamic combinatorial chemistry allows for the identification of unexpected host structures and binding motifs. Notably, we observed the unprecedented selection of these linear receptors in preference to competing macrocyclic hosts. Furthermore, linear receptors containing up to nine building blocks and three different building blocks were amplified in the dynamic combinatorial library. The receptors were formed using a dihydrazide building block based on an amino acid-disubstituted ferrocene scaffold. A detailed study of the linear pentamer revealed that it forms a helical ditopic receptor that employs four acylhydrazone hydrogen-bond donor motifs to cooperatively bind two dihydrogen phosphate ions.

We report here the discovery of a series of structurally unprecedented linear receptors that bind multiple dihydrogen phosphate ions; these receptors have been amplified and identified in a hydrazone dynamic combinatorial library (DCL) of ferrocene-based cyclic and linear oligomers. The linear tetrahydrazone oligomer shown in Figure 1, for example, forms a helical receptor that binds two $H_2PO_4^-$ ions with positive cooperativity.

Dynamic combinatorial chemistry[1] provides a means of efficiently synthesizing libraries of receptors whose individual molecular recognition capabilities may be explored through the library's response to the influences of an added template.[2] A DCL is generated when building blocks combine by way of reversible covalent reactions to form a mixture of interconverting library members under thermodynamic control. Stabilization of a particular library member through noncovalent interactions with an added template alters the positions of the equilibria governing the system, ideally resulting in increased production or *amplification* of the most effective hosts. Here we employed reversible hydrazone formation to link building blocks to form a DCL of amino acid-functionalized ferrocene macrocycles and linear oligomers.[2c,3] Ferrocene-based receptors have previously been explored for electrochemical sensing of anions.[4]

Amino acid-functionalized ferrocenes, which have been recently investigated as scaffolds for inducing β-sheets in peptide mimics,[5,6] represent an interesting class of compounds for incorporation into DCLs. The small barrier to rotation[7] and *molecular hinge*-like character[8] of the ferrocene introduces a flexible turn into the building blocks that is conducive to the production of macrocycles of varying sizes. The inter-ring separation of ferrocene is 3.32 Å, which is close to the N···O separation in hydrogen-bonded β-sheets.[9] Strong and well-defined hydrogen bonds are formed between the arms of amino acid-disubstituted ferrocenes in both the solid and solution states.[5] For ferrocene conjugates of the general form Fc−[CO−(AA_1)−OR]$_2$ or Fc−[CO−(AA_1)−(AA_2)−OR]$_2$ [where AA_1 = Val, Phe, Ala, Gly, His, Cys(Me), or Cys(Bz)], a helical "Herrick" conformation has been observed.[9−11] The angle between the cyclopentadienyl (Cp) substituents is ∼72°, and the trans amides are essentially parallel to the Cp rings with the carbonyl groups pointing away from one another, allowing two interstrand hydrogen bonds to form between the NH groups and the carbonyls of AA_1. The helicity is dependent upon the chirality of AA_1: L chirality induces *P* helicity and D chirality induces *M* helicity, which may be detected by, respectively, strong positive and negative circular dichroism (CD) absorbances at ∼485 nm.[11b] We focused on a valine-disubstituted ferrocene dihydrazide building block, Fc−[CO−Val−$NHNH_2$]$_2$ (**V**), which was synthesized in two steps from ferrocene dicarboxylic acid (Figure 2).

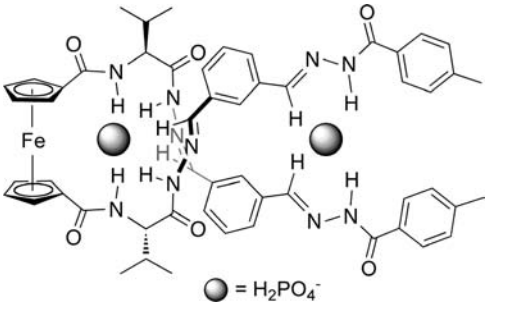

Figure 1. Helical linear hydrazone-based receptor for $H_2PO_4^-$ that binds cooperatively in a 2:1 fashion.

A DCL of hydrazone-linked, ferrocene-based macrocycles and linear oligomers was generated by reacting **V** (0.5 mM), isophthalaldehyde (**I**) (0.5 mM), and 4-methylbenzhydrazide (**H**) (1 mM) in the presence of 1-naphthoic acid (20 mM) in 96:4 $CHCl_3$/MeOH. Identification of the species present in the solution after 10 days was achieved using LC−MS (Figure 3). In the library were detected the macrocycles (**VI**), (**VI**)$_2$, (**VI**)$_3$, and (**VI**)$_4$ and the linear oligomers **HIH**, **HIVIH**, **HIVIVIH**, and

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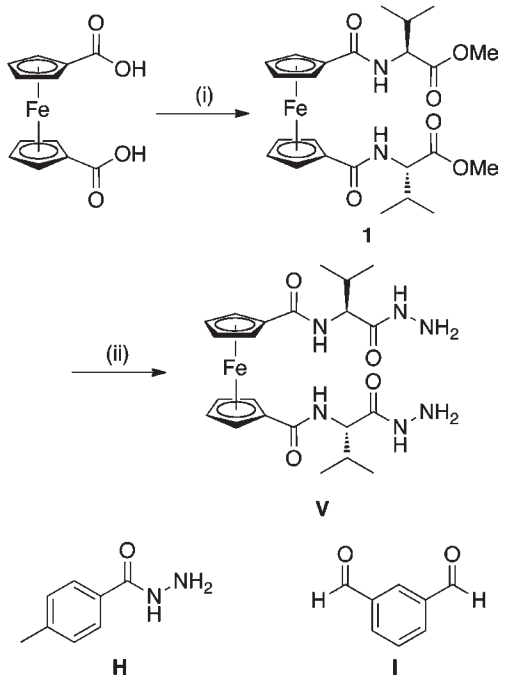


Figure 2. Building blocks Fc−[CO−Val−$NHNH_2$]$_2$ (**V**), isophthaldehyde (**I**), and 4-methylbenzhydrazide (**H**). Reagents and conditions for the synthesis of **V**: (i) H−Val−OMe, HOBt, EDC, Et_3N, DMF, overnight, 72%; (ii) $N_2H_2 \cdot H_2O$, MeOH, 2 days, 77%.

HIVIVIVIH. To test whether the equilibrium was under thermodynamic control, an isolated sample of macrocycle **(VI)$_2$** (0.25 mM) was combined with 4-methylbenzhydrazide (**H**) (1 mM) and 1-naphthoic acid (20 mM) in 96:4 $CHCl_3$/MeOH; after 10 days, a fully re-equilibrated library was established.

When the DCL was allowed to equilibrate in the presence of $Bu_4NH_2PO_4$ (0.25 mM), the concentrations of **HIVIH**, **HIVIVIH**, and **HIVIVIVIH** as well as **(VI)** and **(VI)$_3$** increased at the expense of macrocycle **(VI)$_2$**, indicating that $Bu_4NH_2PO_4$ interacts favorably with these oligomers (Figure 3). The amplification of complex linear oligomers at the expense of macrocycles, and of library members formed from as many as seven and nine building blocks, is unprecedented; the latter is associated with a significant entropic cost to the system.[12]

The linear oligomer **HIVIH** was prepared in two steps from its three components (Scheme 1).

The UV−vis titration of **HIVIH** with $Bu_4NH_2PO_4$ generated a binding isotherm suggestive of multiple cooperative binding.[13] A Job plot showed a maximum at 0.45, which does not indicate a clear 2:1 binding stoichiometry but suggests a binding mode other than 1:1 (Figures S3 and S4 in the Supporting Information).

The NMR spectrum of **HIVIH** in 96:4 $CHCl_3/CD_3OD$ revealed the presence of two conformational isomers in slow exchange on the chemical shift time scale. One isomer (**HIVIH$_A$**) exhibited C_2 symmetry, as deduced from the presence of only one set of resonances for the two substituted Cp ligands of the molecule, while the other isomer (**HIVIH$_B$**) showed C_1 symmetry. When the solution of **HIVIH** was titrated with $Bu_4NH_2PO_4$, a striking change in the relative concentrations of the two isomers was observed over the course of the titration, with **HIVIH$_B$** gradually being converted to **HIVIH$_A$** (Figure 4). After the addition of 5 equiv of $Bu_4NH_2PO_4$, over 95% of the material was present as **HIVIH$_A$**, which is evidence of its superior binding.

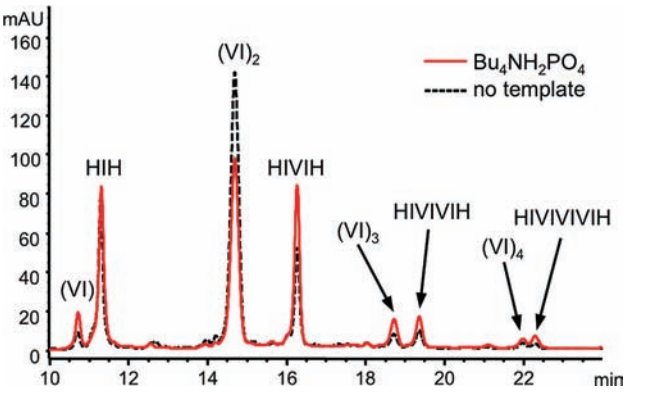
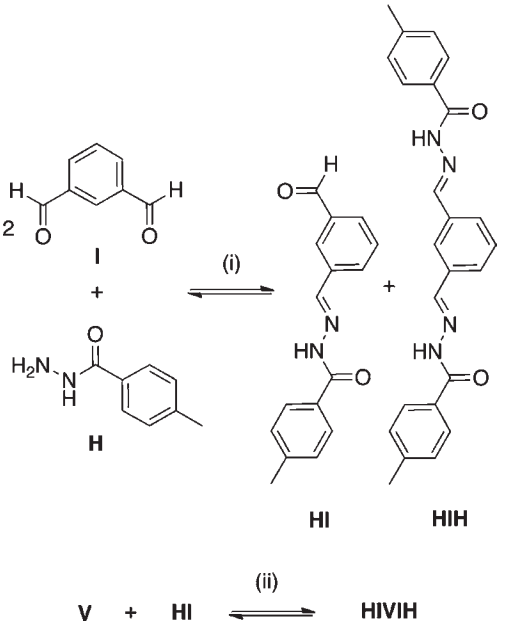


Figure 3. HPLC chromatogram (290 nm) showing the equilibrium distribution of components in a DCL formed from **V** (0.5 mM), **I** (0.5 mM), and **H** (1 mM) in 96:4 $CHCl_3$/MeOH solution containing 1-naphthoic acid (20 mM) in the absence (dashed black curve) and presence (solid red curve) of $Bu_4NH_2PO_4$ (0.25 mM).

Scheme 1. Synthesis of HIVIH[a]



[a] Reagents and conditions: (i) CH_3COOH, $CHCl_3$, 3 Å molecular sieves; (ii) CH_3COOH, $CHCl_3$, MeOH, 3 Å molecular sieves, 55%.

In order to determine the association constants for binding of $H_2PO_4^-$ by the two isomers of **HIVIH**, we used the program DCL*fit*, which is designed to calculate binding constants directly from measurements of the concentrations of the various species in a DCL in the presence of different quantities of template.[14,15] The NMR titration solution containing a mixture of isomers **HIVIH$_A$** and **HIVIH$_B$** may be considered as a two-membered DCL. The concentrations of each isomer in the presence of increasing amounts of $Bu_4NH_2PO_4$ were calculated by integration of the H3 and α-proton resonances of **HIVIH$_A$** and **HIVIH$_B$**, respectively, during the titration. The measured changes in isomer concentration fit poorly to a 1:1 binding model but very well to a 2:1 binding mode with positive cooperativity (Figure 5). The fitting indicated that both isomers bound two $H_2PO_4^-$ anions strongly and cooperatively,

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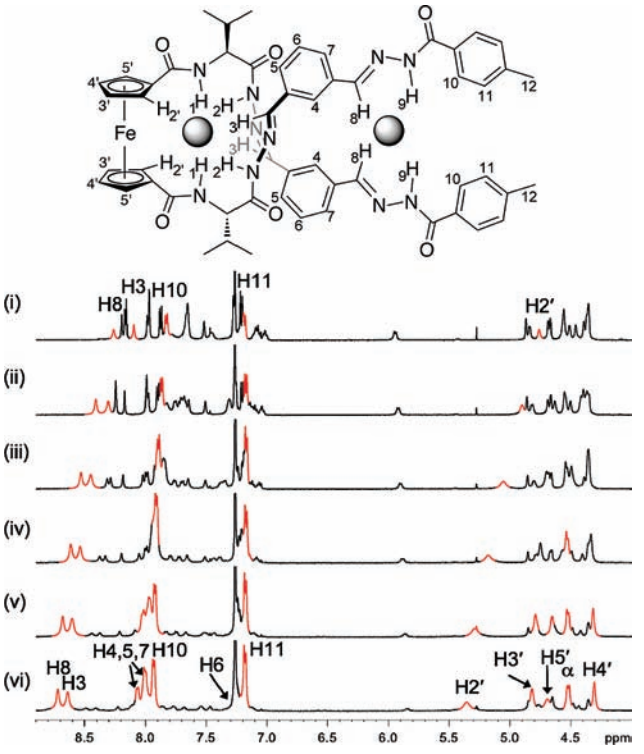

Figure 4. Partial ^{1}H NMR spectra (298 K) of **HIVIH** (4 mM, 96:4 $CDCl_3/CD_3OD$) in the presence of (i) 0, (ii) 2, (iii) 4, (iv) 6, (v) 8, and (vi) 10 mM $Bu_4NH_2PO_4$. Resonances corresponding to $\mathbf{HIVIH_A}$ are colored in red, and assigned protons are labeled accordingly.

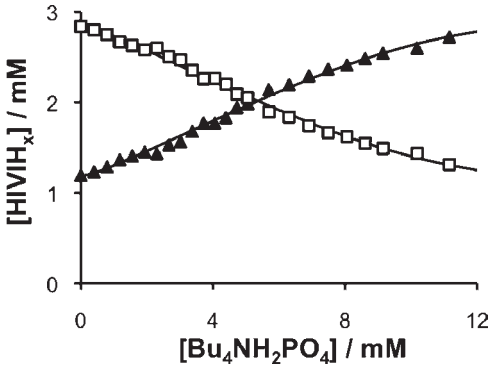

Figure 5. Experimentally determined concentrations of $\mathbf{HIVIH_A}$ (▲) and $\mathbf{HIVIH_B}$ (□) in the presence of increasing amounts of $Bu_4NH_2PO_4$ along with fitted curves for a 2:1 binding model generated using DCL*fit*.[14]

with $K_1K_2(\mathbf{HIVIH_A})$ = 800 000 M^{-2}, $K_1K_2(\mathbf{HIVIH_B})$ = 100 000 M^{-2}, and $K_1 \ll K_2$.

During the course of the NMR titration, significant downfield shifts in the spectrum of $\mathbf{HIVIH_A}$ were observed for imine protons H3 (0.55 ppm) and H8 (0.45 ppm), Cp proton H2″ (0.65 ppm), and hydrazone NH protons H2 (1.6 ppm) and H9 (0.65 ppm).[16] These protons evidently act as hydrogen-bond donors to bind the two $H_2PO_4^-$ ions, possibly supplemented by additional interactions between the imine nitrogens and the anions' hydrogen-bond donors.

Of the many theoretically possible isomers of **HIVIH** resulting from cis or trans amides and *E* or *Z* imines,[3c,17] a single isomer was selected as the best host for **HIVIH**. A nuclear Overhauser effect spectroscopy (NOESY) experiment was performed on a solution of **HIVIH** (4 mM) and $Bu_4NH_2PO_4$ (24 mM) to help elucidate the specific conformation of the hydrazone moieties in the receptor when bound to $H_2PO_4^-$. The spectrum showed NOE cross-peaks from H2 to H3 and from H8 to H9, indicating that the imines were both in an *E* conformation; additional NOE cross-peaks from H9 to H10 were seen, suggesting that the outer hydrazone amides were in a trans conformation.

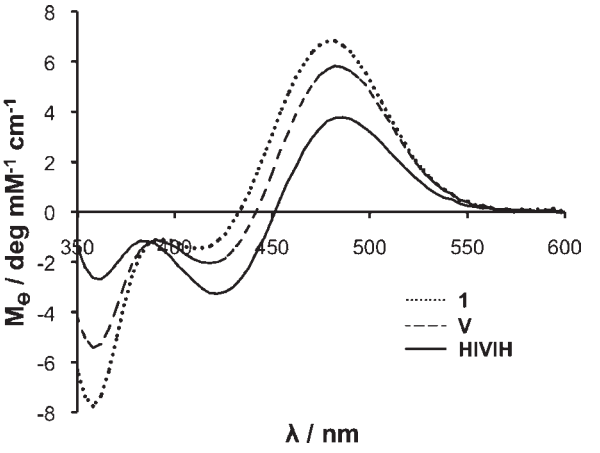

Figure 6. CD spectra of Fc−[CO−Val−OMe]$_2$ (**1**), **V**, and **HIVIH** (2 mM in 96:4 $CHCl_3$/MeOH).

The CD spectrum of the receptor **HIVIH** shows a positive peak at 485 nm suggestive of a *P*-helical Herrick conformation (Figure 6).[9,10] The spectrum mimics those of the building block **V** and the precursor Fc−[CO−Val−OMe]$_2$ (**1**) and was not significantly altered in the presence of 2 equiv of $H_2PO_4^-$. The suggested Herrick conformation, the structural information deduced from the NOESY spectrum, and the location of binding sites suggested by the NMR titration lead to the proposed helical binding of two $H_2PO_4^-$ anions to **HIVIH** depicted in Figure 1.

In the DCL templated with $H_2PO_4^-$, **HIVIH**, **HIVIVIH**, and **HIVIVIVIH** were amplified with amplification factors[18] of 1.6, 1.8 and 2.5, respectively. Since the amplification of oligomers containing many building blocks is entropically unfavorable, the amplification of **HIVIVIH** and **HIVIVIVIH** indicates either that they have an extremely high affinity for one or two $H_2PO_4^-$ ions (significantly stronger than the interaction between **HIVIH** and the two ions) or that they cooperatively bind more than two anions. **HIVIVIH** and **HIVIVIVIH** were synthesized in a kinetically controlled manner similar to that for **HIVIH**. Attempts to fully characterize the binding of $H_2PO_4^-$ to these longer receptors were hampered by the complexity of their NMR spectra resulting from the presence of multiple isomers. However, their behavior in the presence of $Bu_4NH_2PO_4$, when monitored using UV−vis titrations, Job plots, and CD spectroscopy, was similar to that of **HIVIH** (see the Supporting Information).

In conclusion, we have identified from a hydrazone-based DCL a linear receptor based on a valine-functionalized ferrocene that binds two molecules of $H_2PO_4^-$ cooperatively with K_1K_2 = 800 000 M^{-2}. This is the first example of a DCL in which templating has resulted in the amplification of such long and complex linear species in preference to macrocycles. This remarkable affinity for $H_2PO_4^-$ displayed by linear receptors illustrates how the use of dynamic combinatorial chemistry may provide access to unexpected receptors and recognition modes.

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A macrocycle may provide a predefined cavity for binding, but a lack of flexibility could inhibit its ability to adopt a suitable conformation to best bind a guest. In biological systems, evolution has allowed linear polymers such as proteins, DNA, RNA, and polysaccharides to generate recognition systems of extraordinary strength and subtlety. We propose that the efficiency of **HIVIH** as a receptor for $H_2PO_4^-$ results from a degree of preorganization provided by intramolecular hydrogen bonding combined with sufficient flexibility (due to its linearity and the rotational freedom of the ferrocene) to adapt its shape to optimize the intermolecular interactions between the host and the guests.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures; compound characterization; UV−vis spectra, titrations and Job plots; NMR titrations; VT and 2D NMR spectra; and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
jkms@cam.ac.uk

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