A Combinatorial Library Approach to Artificial Receptor Design

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Received July 5, 1995

Recently there has been great success in the generation of large libraries of synthetic ligands[1] via *linear* sequences of chemical transformations (e.g., peptides,[2] oligonucleotides,[3] benzodiazepines,[4] etc.). In the main these have been small molecules used for binding into the active sites of biological receptors. The strategy is less applicable to synthetic molecules with the size, rigidity, and shape to allow selective recognition of small substrates.[5] Such artificial receptor libraries could have important applications in the identification of novel sensors and catalysts. In nature, antibodies achieve binding site diversity via a *convergent* combination of light and heavy chains in the FAB fragment (shown schematically in Figure 1). Each of these chains has a constant domain and a variable domain linked through a hinge region. In essence, the two strongly associating constant domains act as an invariant anchor to enforce proximity of the two variable domains, each directing three hypervariable loops to form the antigen binding site.[6] This separation of function, into template (constant) and binding (variable) domains, allows for large changes in the antigen recognition site (e.g., length, sequence, and conformation of the hypervariable loops) without significant disruption in the overall structure of the protein.

Our interest lay in applying this *convergent* combinatorial approach to the formation of synthetic receptor libraries. Our strategy was to employ as the constant domain a strong metal-binding ligand (such as terpyridine) linked to a variable substrate-binding domain (such as polar, hydrophobic, or charged groups).[7] Addition of metal ion to the subunits should have no effect on the substrate recognition site but instead should cause the recruitment of two (or more) metal-chelating (constant) regions, leading to the formation of a well-defined substrate-binding region. For example, stepwise reaction of two different terpyridine subunits with $RuCl_3$ will give an octahedral Ru−bis-terpy complex[8] in which two recognition sites are held in a fixed relationship (as in Figure 2). In this two-chelate system n different variable domains can be easily combined to form $n(n + 1)/2$ different receptors.[9]

To test this idea we have prepared a family of terpyridine derivatives linked through the 5-position to thiourea, hydroxyl, diphenylmethane, and crown ether variable domains (**2**, **3**, **4**, and **5**, respectively). The key 5-methyl-2,2′:6′,2″-terpyridine (**1**)

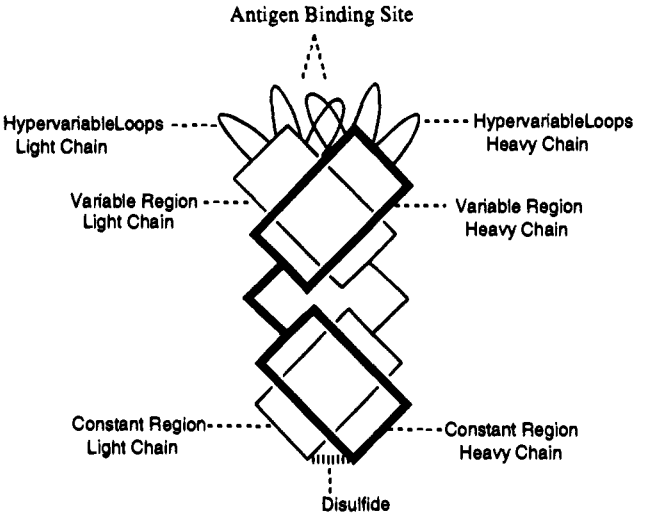


Figure 1. Generalized structure for the Fab fragment of immunoglobulins.

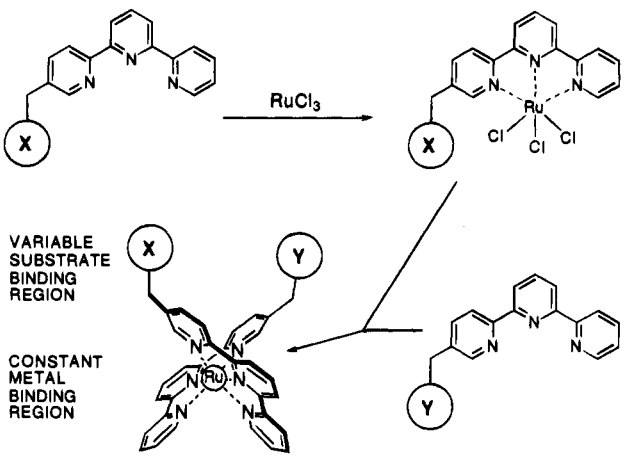
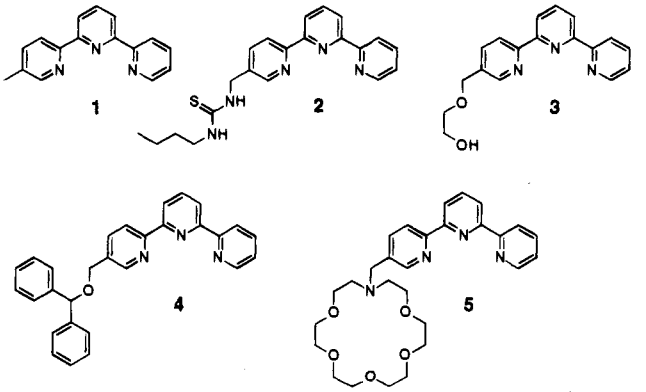


Figure 2. Metal-templated approach to formation of combinatorial receptor libraries.



was prepared from 2-acetyl-5-methylpyridine[10] and converted to its bromomethyl derivative (via NBS bromination) and thence

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(4) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10977.

(5) For a notable and elegant exception, see: Boyce, W.; Li, G.; Nestler, P.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7995.

(6) Brandon, C.; Tooze, J. *Introduction to Protein Structure*; Garlend: New York, 1991; p 187. Alzari, P. N.; Lascombe, M. B.; Pojak, R. J. *Annu. Rev. Immunol.* **1988**, *6*, 555.

(7) For an earlier example of this strategy using phenanthroline as the metal-binding ligand, see: Goodman, M. S.; Weiss, J.; Hamilton, A. D. *Tetrahedron Lett.* **1994**, 8943−8946.

(8) Evans, I. P.; Spencer, A.; Wilkinson, G. *J. Chem. Soc., Dalton Trans.* **1973**, *204*−209.

(9) The number of symmetrical receptors with two of the same ligands (X, X or Y, Y) is n. The number of unsymmetrical receptors formed from two different subunits (X, Y) is

$$n!/[(n-2)!2!] = n(n-1)/2$$

Thus, the total number of different receptors in the library from n different subunits is

$$n + n(n-1)/2 = n(n+1)/2$$

Since, in the octahedral geometry, each receptor exists as a pair of enantiomers, the total number of receptors from n subunits is $n(n + 1)$.

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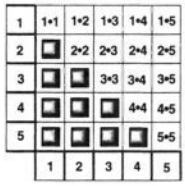

Figure 3. Combinatorial grid of the receptors formed from five subunits.[13]

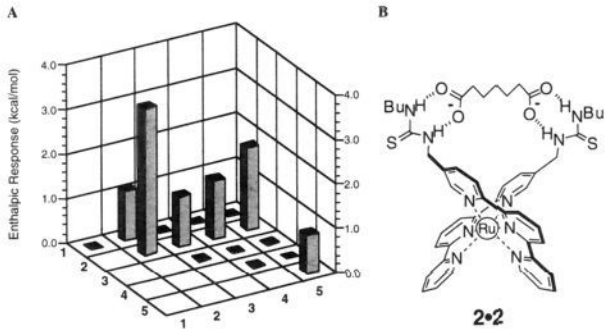

Figure 4. (A) Bis(tetrabutylammonium) pimelate enthalpic response to each receptor. (B) Proposed structure of complex with receptor **2·2**.

to **2** (via Gabriel amination and treatment with butyl isothiocyanate),[11] **3** (via reaction with ethylene glycol), **4** (via reaction with diphenylmethanol), and **5** (via reaction with aza-18-crown-6). The Ru(II) complexes can be prepared by a simple two-step procedure. For example, thiourea **2** was reacted with 1 equiv of $RuCl_3$ in refluxing ethanol to give **2**$RuCl_3$. This was divided into five portions, which were reacted with 1 equiv of **1**, **2**, **3**, **4**, and **5**, respectively, in ethylene glycol at 160 °C. The resulting bis-terpyridine complexes (**1·2**, **2·2**, **2·3**, **2·4**, and **2·5**) were then isolated as their PF_6^- salts.[12] Similar protocols with the other terpyridines led in a straightforward manner to a full combination of the five subunits and formation of a library of 15 different and isolated receptor samples, as shown in grid form in Figure 3.

A number of approaches can be envisioned for screening the receptor library for strong binding to a set of target substrates.[14] One direct method involves using microcalorimetry to measure the enthalpic response on addition of a polar substrate to solutions of the receptor library.[15] For example, the receptor library was screened for binding to bis(tetrabutylammonium) pimelate in DMSO, and the results are represented graphically in Figure 4. Receptor **2·2** gave the largest response, which, by ^{1}H NMR titration, corresponded to a $K_a > 10^4$ M^{-1} in d_6-DMSO ($K_a = 6.0 \times 10^3$ M^{-1} in 5% D_2O/d_6-DMSO).[11] The role of the metal in this strong binding is twofold. First, it imparts a

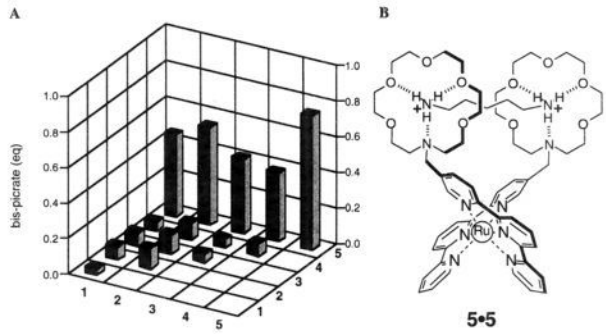

Figure 5. (A) Pentane-1,5-diylbis(ammonium) picrate extraction into 5% CH_3CN/CH_2Cl_2 solutions (0.23 mM) of each receptor. (B) Proposed structure of complex with receptor **5·5**.

favorable chelate effect by recruiting two thiourea hydrogen-bonding sites. The corresponding thiourea−methyl receptor **1·2** binds tetrabutylammonium phenylacetate in d_6-DMSO with $K_a = 2.1 \times 10^3$ M^{-1}.[16] Second, the positively charged metal provides an additional electrostatic stabilization of the complex with negatively charged pimelate or phenylacetate substrates. In the absence of an adjacent metal center, dialkylthioureas bind to tetrabutylammonium carboxylates in d_6-DMSO with $K_a = 3.0 \times 10^2$ M^{-1}.[17]

A second screening strategy involves measuring the ability of solutions of the receptors to effect a solid−liquid extraction of a target substrate. For example, binding to pentane-1,5-diylbis(ammonium) ions can be screened by addition of the solid bis-picrate salt to 5% CH_3CN/CH_2Cl_2 solutions of the receptor library. Colorimetric analysis (represented graphically in Figure 5) shows that receptor **5·5** extracts nearly 1 equiv of the diammonium salt into 5% CH_3CN/CH_2Cl_2 while receptors **1·5** and **2·5** extract lesser amounts. Detailed analysis of the binding to **5·5** gives a 1:1 stoichiometry (from Job's analysis), a binding constant in CD_3CN of 3.2×10^4 M^{-1}, and chemical shift changes that are consistent with the structure shown in Figure 5B. Dimerization of binding sites gives less advantage in this case (for propylammonium picrate binding to mono crown **1·5**, $K_a = 1.2 \times 10^4$ M^{-1}) due presumably to a repulsive effect of the positively charged metal complex on the bis-cationic substrate.

In summary, we have developed a novel metal-template approach for the formation of libraries of artificial receptors. Simple screening methods allow the rapid identification of those receptors that bind strongly to diverse substrates. We are currently extending this library approach to more complex variable binding regions (such as peptides) and to metal complexes that will recruit three or more binding regions to the recognition site.

Acknowledgment. We thank the National Institutes of Health (GM35208) for support of this work.

Supporting Information Available: Details of the screening for tetrabutylammonium phenylacetate binding to the library and titration data, curve-fitting analysis, and details of NMR changes for all reported K_a values (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(12) If necessary, purification can be effected by silica column chromatography.

(13) The squares in Figure 3 represent degeneracy since, for example, **1·2** and **2·1** are identical about a C_2 axis. Each receptor exists as two enantiomers, meaning that the number of distinct receptors resulting from the combination of five subunits is 30.

(14) Colorimetric methods have been effective with other metal-templated receptors,[7] and later modifications of the Ru complexes should also be amenable to this as well as fluorescence detection.

(15) One equivalent of dicarboxylate solution (40 μL, 8.43 mM) was added to a solution of the receptor (1.34 mL, 0.25 mM), and the enthalpic response was measured using an ITC microcalorimeter (MicroCal Inc.). Values were corrected to the response from dilution of the carboxylate solution into pure DMSO. Exothermic responses are shown as positive values, while endothermic responses are shown as baseline.

(16) For a calorimetrically-derived screen of tetrabutylammonium phenylacetate binding to the receptor library, see supporting information.

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