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# The Discovery of an Enantioselective Receptor for (−)-Adenosine from a Racemic Dynamic Combinatorial Library

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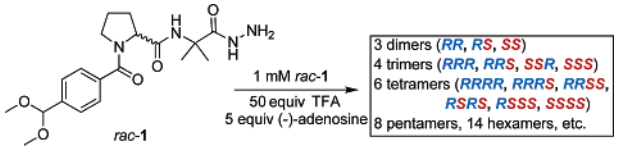
Dynamic combinatorial chemistry (DCC)[1] has developed into a powerful tool for the amplification and identification of new hosts for a broad range of guests, ultimately yielding such remarkably complex structures as the catenated dodecapeptide receptor for acetylcholine.[2] DCC experiments aimed at the discovery of enantioselective receptors have, however, not been reported, though diastereoselective receptor amplifications are known.[2,3]

We report herein two tools (laser polarimetry (LP) detection and pseudo-enantiomers) for the discovery and identification of a peptide-like enantioselective receptor for (−)-adenosine,[4,5] whose biological receptors represent important medicinal targets.[6] The receptor was elicited from a *racemic* dynamic combinatorial library (DCL), which has advantages of enhanced stereochemical diversity over enantiopure versions (Scheme 1) and ease of synthesis.

***Scheme 1.*** Templating a *rac*-**1** DCL with (−)-adenosine




Enantioselective receptors for (−)-adenosine could support the search for adenosine nucleotide receptor inhibitors.[6]

One difficulty with identifying enantioselective receptors from a DCL is that two conditions are required: (1) a binding propensity, and (2) a binding differential for the two enantiomers (diastereomeric host−guest complexes). While some of these data should be available by comparing the templating efficiency of an (*R*)- and an (*S*)-analyte for an enantiopure DCL, we sought a more direct single experiment protocol. We reasoned that templating a racemic DCL with an enantiopure guest would amplify the best host (condition 1), and if that receptor also bound the analyte enantioselectively, then the best matched enantiomer would be amplified over the mismatched antipode (condition 2).[7] The two methods described herein were designed to detect this enantio-amplification in the best host.

The classic method for detecting enantiomeric excess is via polarimetry, a powerful tool when matched with an HPLC separation (laser polarimeter (LP) detector).[8] Since achiral compounds and racemates are polarimetry silent, *only* receptors enriched in one enantiomer (i.e., from a diastereoselective host−guest complex) will give an LP signal and thus be detected. Creating a signal from an otherwise null background should, in principle, also enable the analysis of more complex libraries than previously possible.

For proof of principle studies, we chose the cyclic hydrazone family of DCC dipeptides; *rac*-**1**[2,9] is composed of a racemic proline unit and an achiral peptide. The addition of TFA deprotects the acetal and initiates reversible hydrazone exchange to form the cyclic oligomers (Scheme 1). Figure 1a shows the HPLC chromatogram (UV absorbance at 289 nm) for a nontemplated DCL (diastereomer resolution is not achieved).

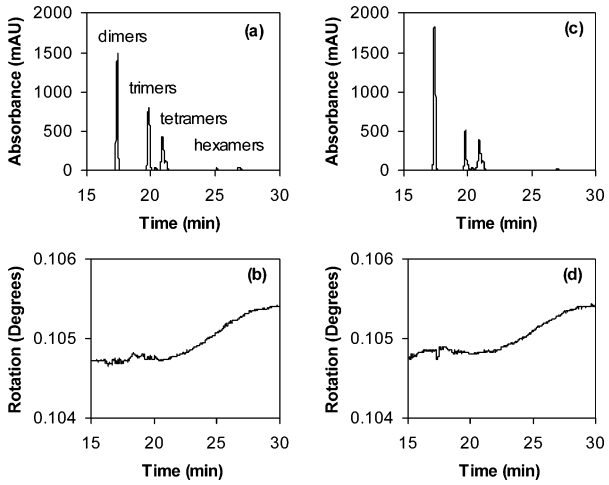



***Figure 1.*** (a) UV absorbance (289 nm) of untemplated DCL (1 mM in monomer). (b) LP of untemplated DCL. (c) UV absorbance (289 nm) of DCL + (−)-adenosine (1 mM *rac*-**1**, 5 mM (−)-adenosine). (d) LP of DCL + (−)-adenosine.

***Table 1.*** Oligomer Quantities (from *rac*-**1**) (HPLC UV area %) in the Untemplated and (−)-Adenosine Templated Cases[a]

| | HPLC area % | | |
|---|---|---|---|
| library member | untemplated | templated | Δ |
| dimers | 44 | 56 | +12 |
| trimers | 29 | 21 | −8 |
| tetramers | 24 | 23 | −1 |
| hexamers | 3 | 0 | −3 |

[a] 1 mM *rac*-**1**, 5 mM (−)-adenosine, 50 mM TFA, 1% 2-methoxyethanol in $CH_2Cl_2$, 24 h equilibration.

When the DCL was templated with (−)-adenosine (5 equiv with respect to *rac*-**1**), the dimer was amplified at the expense of the higher order oligomers (Figure 1c). As shown in Table 1, the shift toward dimer and loss of the higher oligomers, while modest, is easily quantified by HPLC/UV. The increase from 44 to 56% dimer (Δ = +12%) and concomitant decrease in the higher oligomers suggests a dimer of modest binding ability. More interesting, however, was the appearance of a signal in the LP trace at a retention time corresponding to the dimer (Figure 1d). This signal clearly indicates that the dimer has had one of its homochiral diastereomers selectively enhanced over the other (the heterochiral dimer *RS* is achiral, Chart 1).

Additional evidence for an enantioselective amplification of **2** was obtained by examining the templating effect of (−)-adenosine

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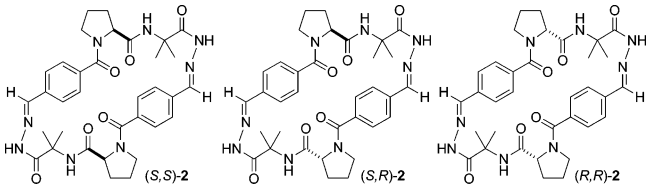
***Chart 1.*** Diastereomers of **2**




with an (*S*)-**1** and an (*R*)-**1** DCL. As shown in Figure 2a, the increase in dimer was significantly larger for the (*S*)-DCL than for the (*R*)-DCL, and this was especially true at low monomer concentrations (1 mM). We thus concluded that (*S*,*S*)-**2** was the enantioselective receptor for (−)-adenosine and that it was amplified selectively in the racemic DCL (Chart 1).[10]

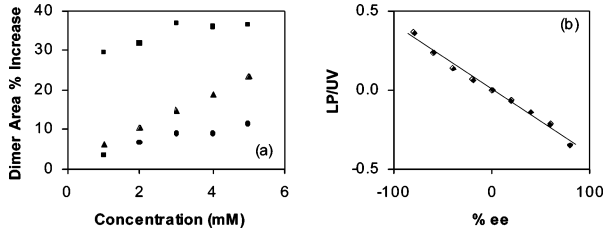
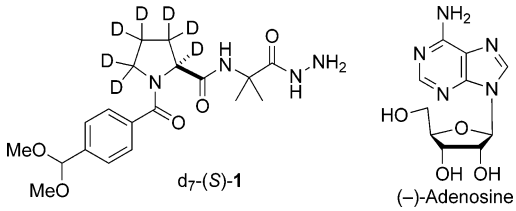



***Figure 2.*** (a) Amplification of **2** (% $\text{dimer}_{\text{templated}}$ − % $\text{dimer}_{\text{untemplated}}$) by (−)-adenosine in an (*S*)-**1** DCL (■), (*R*)-**1** DCL (▲), and *rac*-**1** DCL (●) versus initial monomer concentration. (b) Ratio of LP peak height to UV peak height for the dimer in an untemplated DCL versus % ee of **1**.

To estimate the enantioenrichment of (*S*,*S*)-**2** over (*R*,*R*)-**2** in the racemic DCL, new DCLs (nontemplated) were synthesized using variable quantities of (*S*)-**1** and (*R*)-**1**. Plotting the ratio of the dimer's LP and UV signals versus the enantiomeric excess of **1** generated a crude calibration curve for the dimer's enantiomeric excess (Figure 2b). This calibration curve yielded an ee of ∼21% for the dimer in the templated racemic DCL (1 mM, Figure 1c and 1d).[11] This value compares favorably with some of the best peptide-based receptors for chiral ammonium ions.[4a,12]

More convenient for this and future studies would be an in situ method for directly measuring the amount of (*S*,*S*)-**2**, (*R*,*R*)-**2**, and (*S*,*R*)-**2** under the templating conditions. To this end, $d_7$-(*S*)-**1** was prepared (from $d_7$-proline) and mixed with (*R*)-**1** to generate a pseudo-racemic monomer. The resulting unique mass signature of the diastereomeric library components was expected to enable MS analysis for diastereo- and enantio-composition assessment ((*R*,*R*)-**2** = $M^+$, (*S*,*R*)-**2** = $M^+$+7, and (*S*,*S*)-**2** = $M^+$+14).




LC-MS analysis of the untemplated library indicated a roughly statistical mixture of dimers: $d_{14}$-(*S*,*S*)-**2**:(*R*,*R*)-**2**:$d_7$-(*R*,*S*)-**2** was 19:15:66%.[13] Under templating conditions, however, the amount of heterodimer drops and the ratio of $d_{14}$-(*S*,*S*)-**2** to (*R*,*R*)-**2** increases to 1.7:1 (34:20:45%), an enantio-ratio that is similar to that obtained from the calibration curve in Figure 2 (22% ee = 1.5:1). The pseudo-enantiomer methodology thus enables the direct measurement of the amplified receptor's enantiomeric and diastereomeric ratios.[14]

These results demonstrate that it is indeed possible to elicit an enantioselective receptor from a racemic DCL. The demonstrated applicability of laser polarimetric detection and pseudo-racemic monomers to first identify and then pinpoint the stereochemical identity of interesting receptors from complex libraries suggests an effective strategy for continued efforts to discover enantioselective receptors. Enantioselectivity in the recognition of a nucleotide bodes well for the feasibility of future endeavors aimed at discovering selective receptors for important biological ligands, especially considering the ease with which the racemic library members may be altered and screened.

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**Supporting Information Available:** Experimental details for monomer synthesis and DCL conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Concentrating a single enantiomer into the amplified receptor causes a concomitant buildup of its mirror image into the other members of the DCL. This effect reduces the LP signal from these enantioenriched components and dilutes the entropic cost of deracemization.

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(10) (*S*,*S*)-**2** gives a levorotatory (negative phase) peak in the LP (Figure 1d).

(11) This analysis accounts for statistical quantities of the achiral heterodimer, but may not account for templating induced changes in its concentration.

(12) Cyclic peptides selectively bind chiral quaternary ammonium ions. See: (a) Heinrichs, G.; Vial, L.; Lacour, J.; Kubik, S. *Chem. Commun.* **2003**, 1252−1253. For achiral binding, see: (b) Madison, V.; Deber, C. M.; Blout, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 4788−4798 and references therein.

(13) The ratio of (*R*,*R*)-**2** to (*S*,*S*)-**2** was not exactly 1:1 in the pseudo-racemate.

(14) Direct characterization of the differential binding properties of **2** and (−)-adenosine was hindered by poor solubility in nonacidic templating solutions (where library exchange does not occur), perhaps reasonably suggesting that the actual analyte is protonated. See: Kampf, G.; Kapinos, L. E.; Griesser, R.; Lippert, B.; Sigel, H. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1320−1327.

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