

## Libraries for Receptor-Assisted Combinatorial Synthesis (RACS). The Olefin Metathesis Reaction

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**Abstract:** A library of alkenes is generated using the olefin metathesis reaction, and converted to a set of diols suitable for a receptor assisted combinatorial synthesis (RACS) experiment with borate as a linker.

With the explosive growth in combinatorial research, new ways are being sought to expand the effective size of libraries that can be explored. Receptor-assisted combinatorial synthesis (RACS) is one of these.<sup>1,2,3</sup> The approach is based on the common observation that a composite ligand A--B generally binds to a receptor much more strongly than its fragments, A and B.<sup>4,5</sup> Further, should the ligand fragments A and B, bound together in an active site, undergo a reaction that joins them covalently to give A--B, an enzyme can actually synthesize its own inhibitor.<sup>6,7</sup>

This idea can be generalized (Figure 1). If ligand fragments A<sup>1-5</sup> and B<sup>1-5</sup> (where the superscripts indicate different components in the libraries A and B) undergo reversible reaction to form A--B composites, and if one of the composites (for example, A<sup>1</sup>--B<sup>2</sup>) has an affinity for a receptor, with a disassociation constant ( $K_{\text{diss}}$ ) of 1  $\mu\text{M}$ , allowing the mixture to equilibrate under conditions of dynamic equilibrium in the presence of receptor, it will remove its preferred composite (A<sup>1</sup>--B<sup>2</sup>) from the equilibrating mixture, if the concentration of the receptor is greater than  $K_{\text{diss}}$  for the complex between the receptor and A<sup>1</sup>--B<sup>2</sup>. This will pull the equilibrium in solution. Thermodynamic principles dictate that after the system achieves equilibrium, the receptor will be complexed with the tightest binding composite ligand (A<sup>1</sup>--B<sup>2</sup>) if the concentrations of A<sup>1</sup> and B<sup>2</sup> are greater than the concentration of the receptor.

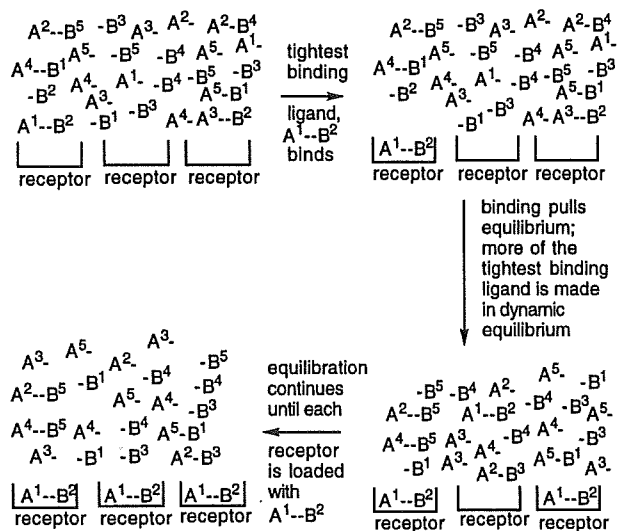


Figure 1. An idealized receptor assisted combinatorial synthesis (RACS) experiment, where a receptor selects its preferred composite ligand (A<sup>1</sup>--B<sup>2</sup>) from 5<sup>2</sup> = 25 combinations of two libraries of 5 ligand fragments associating and disassociating under conditions of dynamic equilibrium.

The effective library size in these experiments is the product of the degeneracy of the two libraries of ligand fragments. Thus, if the libraries of ligand fragments have 10<sup>5</sup> components, the number of composite A--B ligands is 10<sup>5</sup> squared, or 10<sup>10</sup> (the effective size of the library).

Many of the technologies are available to implement RACS experiments with specific biological receptors and ligands. We<sup>1</sup> and others<sup>8</sup> have reported Fourier transform mass spectroscopic methods that are suitable for analyzing the output of RACS experiments on large libraries. Affinity selection technology is in hand to separate receptor-ligand complex from library.<sup>9</sup> More recently, Huc and Lehn reported a preliminary RACS experiment on a library containing 12 composite ligands closely related to those known to inhibit carbonic anhydrase, built from two libraries of ligand fragments, one containing 3 components, the other containing 4 (3 x 4 = 12).<sup>2</sup> They observed a receptor-assisted increase (by perhaps a factor of 2) in the amount of one of the composite ligands.

The very small libraries used by Huc and Lehn account for the modest results that they obtained, and makes clear the importance of methods for generating libraries of adequate size to explore substantial areas of combinatorial space in a RACS experiment. We report here the first time methods that prepare soluble libraries of this type.

### Results

Many linkages can be envisioned that permit ligand fragments to be joined under RACS conditions, including disulfide, imine, and thiohemiacetal. The libraries reported here use the 1,2-diol unit as the reactive functionality. 1,2-Diols can be joined under conditions of dynamic equilibrium as borate esters (Figure 2). The disassociation constants of borate-diol complexes are typically 10-100 mM<sup>2,10,11,12</sup>. The kinetics of ligand exchange are rapid, there is little cross reactivity with functional groups presented on a protein (unlike with disulfides, imines and thiohemiacetals), and the resulting borate ester "leads" can be converted to stable spiro ketals. Thus, these libraries are suitable for exploring RACS technology.

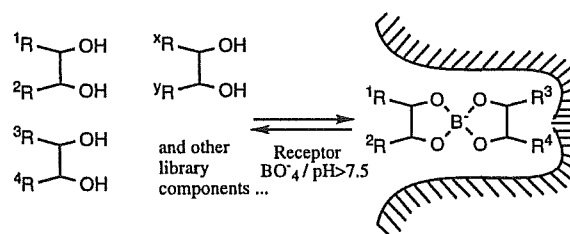


Figure 2. Diols can be assembled under conditions of dynamic equilibrium in a receptor-assisted combinatorial chemistry synthesis (RACS) experiment using borate as a linking group.

1,2-Disubstituted alkenes are convenient precursors for 1,2-diols. Further, alkenes with 1,2-substituents should be conveniently accessible using the olefin metathesis reaction (Figure 3) with terminal alkenes.<sup>13</sup>

This reaction generates volatile ethylene as a side product, and "cross-products" are commonly observed.<sup>14</sup> Further, Grubbs and his coworkers have recently developed a catalyst (1) for the metathesis reaction that tolerates a range of functional groups.<sup>15</sup>

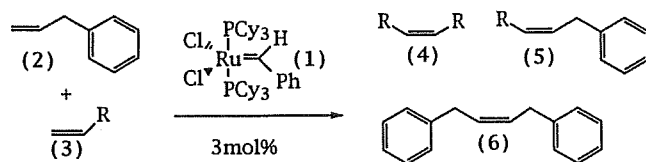


Figure 3. The olefin metathesis reaction used to generate libraries as precursors for a RACS synthesis.

To explore the scope and versatility of the metathesis reaction as a tool for generating combinatorial libraries, a series of reactions were first run where allylbenzene was reacted with a variety of functionalized and unfunctionalized alkenes (Figure 3). The results are collected in Table 1.

The results in Table 1 illustrate the scope of the metathesis reaction, which was successful with a range of aliphatic and aromatic (including halo and nitroaromatic) compounds. Alkenes carrying keto, ester, protected alcohol and ether functionalities reacted more slowly, although sufficient cross products 5 were formed to permit these alkenes to serve as the starting points for the preparation of a RACS library. Some of these alkenes gave a crossed-metathesis product but no detectable symmetric product (compounds 3ad, 3ae, 3al, 3ap). Mass balances suggested that some of the symmetrical products were formed, however, and the symmetrical products may have low volatilities and be poorly detected by GC-MS.

The metathesis reaction was not entirely tolerant of functional groups, however. Allylbromide (3aq), 4-penten-1-al (3ar), allylmethylsulfide (3as) and trityl protected allylamine (3at) gave detectable amounts of crossed metathesis products under these conditions, but in still lower yields. Entirely failing to give any detectable metathesis reactions were vinylanthracene, 2,6-dichlorostyrene, sclareol, nootkatone, allyldiphenylphosphine, allylamine, allylcyanide, pentenenitrile, allylimidazole, 4-vinylpyridine, N-vinylphthalimide, 2-(3-pentenyl)pyridine, allylrhodanine, and N-allyltrifluoroacetamide. This last result was somewhat surprising in light of work from the Grubbs group showing that amides were accepted by catalyst 1 in ring-closing metathesis reactions.<sup>16</sup>

Given this scope, a trial library sufficiently small to permit identification of all products was prepared from five alkenes that carried two alkane substituents, one aromatic substituent, and two esters (4-methyl-1-pentene (3aa), 1-heptene (3ab), allylbenzene (2), methyl-3-butenate (3ag) and ethyl-2-methyl-4-pentenoate (3ah)). All 15 of the expected products were detected by GC-MS (Figure 4), along with the corresponding reactants.

Given this success, a second, larger library was constructed from 17 alkenes containing a range of functionalities (allylbenzene (2), 4-methyl-1-pentene (3aa), 1-heptene (3ab), allylcyclopentene (3ac), vinylnaphthalene (3ad), allylphenylsulfone (3ae), allylbutyrate (3af), methyl-3-butenate (3ag), ethyl-2-methyl-4-pentenoate (3ah), 1,2-epoxy-5-hexene (3ai), allylanisole (3aj), safrole (3ak), allylcyclohexanone (3am), allylpentafluorobenzene (3an), 4-fluorostyrene (3ao), 3-nitrostyrene (3ap) and 4-penten-1-al (3ar). GC-

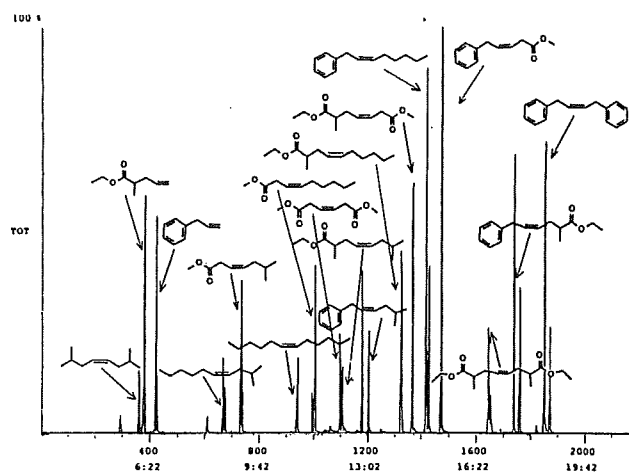


Figure 4. GC-MS spectra of a library with 15 products

MS analysis (Figure 5) showed the level of complexity expected for a library with a degeneracy of 153 compounds; of these ca. 10% could be explicitly identified.

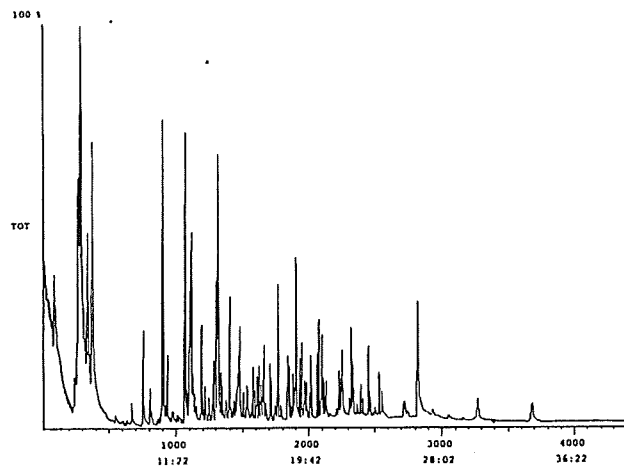


Figure 5. GC-MS spectra of a library with approx. 150 compounds

Lastly, a library of diols was prepared from the small alkene library using the Sharpless dihydroxylation reaction.<sup>17</sup> Because of poor separation of diols by GC, the success of the reaction was demonstrated by cleavage of the product diols with  $\text{IO}_4^-$  to give the derivative aldehydes,<sup>18</sup> which were identified by GC-MS. Each of the expected aldehydes were detected by GC-MS; volatile aldehydes were identified as their dinitrophenylhydrazone derivatives.<sup>19</sup> Last, a diol library was similarly constructed and analyzed, starting from the large library described above. Diversity on the order of that expected was also observed, although the assignment of specific structures of diols in libraries of this size is impossible. Degradation of the library with periodate yielded the expected aldehydes.

## Discussion

These results show that the metathesis reaction generates libraries of alkenes as precursors for diol libraries suitable for a RACS experiment. Epoxide libraries have also been prepared from these alkene libraries (data not shown). A wide variety of functionality is created by

metathesis. The primary disadvantage of the metathesis reaction is the intolerance of available catalysts to substrates containing nitrogen.

More generally, RACS offers an opportunity for application of libraries of all kinds generated in solution. To date, the majority of synthetic effort in combinatorial chemistry has focused on solid phase synthesis,<sup>20</sup> tagging methods,<sup>21,22</sup> and subtraction methods.<sup>23</sup> Much work remains to be done, however, to exploit the potential of RACS for exploiting libraries with the high effective sizes needed to permit combinatorial chemistry to become a "lead discovery" technology rather than a "lead enhancement" technology.

### Experimental part

**Metathesis.** Reagents were degassed by freezing and thawing under vacuum. In a typical metathesis reaction, a solution of alkene (1.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was degassed and added to **1** (25 mg, catalyst mw 822.97, ca. 2 mol%) by cannula transfer. The mixture was stirred at 45–50 °C under Ar for 6 h. The mixture was filtered (silica gel, 2 g) to remove catalyst. An aliquot (0.2 mL) was then removed, dissolved in  $\text{CH}_2\text{Cl}_2$  (1 mL) and resolved by GC-MS (Finnigan GCQ,  $\text{EI}^+$ -mode, 10°C/min, 50–250°C). For product analysis, biphenyl was used as an internal standard. This in turn permitted the use of the external standards for **2**, **3**, and **6** to determine the amount of each of these in the product mixture. As crude metathesis product mixtures were examined, and no significant amounts of side products were observed, **5** could be calculated as the difference between the amount of **2** added initially and the amount of **2** plus **6** (taken twice) in the product mixture. Amounts for **4** were obtained by difference given amounts of starting material, **3**, and **5**. Except where noted by \* in Table 1, peaks for **4** were seen by GC-MS, and displayed response factors between 0.5 and 2. To prepare libraries, a solution of alkenes (0.38 mmol each, 17 alkenes incorporated, for a total of 6.46 mM alkene) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was degassed and then added to **1** (100 mg, 2 mol %). After 6 h at 45–50°C, the mixture was analyzed by GC-MS as above.

**Sharpless dihydroxylation.** The reagent<sup>17</sup> was prepared by dissolving  $\text{K}_3\text{Fe}(\text{CN})_6$  (1.25 g),  $\text{K}_2\text{CO}_3$  (0.5 g),  $(\text{DHQD})_2\text{PHAL}$  (6 mg),  $(\text{DHQ})_2\text{PHAL}$  (6 mg) and  $\text{K}_2\text{OsO}_2(\text{OH})_4$  (2 mg) in  $\text{H}_2\text{O}$  (6 mL) and  $t\text{BuOH}$  (6 mL) at RT. The crude alkene library (1 mL, ca. 1.25 mmol alkene) and methanesulfonamide (119 mg) were added. The mixture was stirred (12 h, RT) and treated with sodium sulfite (1.6 g). The diols were extracted ( $\text{EtOAc}$ , 3x30 mL) and dried ( $\text{Na}_2\text{SO}_4$ ), the solvent evaporated, the mixture analyzed by GC-MS as above.

### References and Notes

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Table 1. Reaction of allylbenzene with olefins under metathesis conditions (products and reactants given in mole % after 6 h; see experimental). Values with \* were for compounds not directly observed; mol % was obtained by difference (see text).

Alkene 2, 3	Compound no.	mole % of 2	mole % of 3	mole % of 6	mole % of 5	mole % of 4
	<b>2</b>	4.4		95.6		
	<b>3aa</b>	1.0	35.7	19.3	42.2	1.9
	<b>3ab</b>	Trace	Trace	2.7	94.7	2.7
	<b>3ac</b>	2.6	Trace	12.7	56.1	28.5
	<b>3ad</b>	2.5	8.8	30.7	30.6	27.5*
	<b>3ae</b>	5.4	10.7	18.7	49.3	16.0*
	<b>3af</b>	12.3	5.0	6.3	66.3	10.0
	<b>3ag</b>	17.7	3.6	1.8	68.0	8.9
	<b>3ah</b>	2.7	4.2	28.3	37.3	27.6
	<b>3ai</b>	25.3	20.6	15.8	20.1	18.2
	<b>3aj</b>	1.2	2.4	18.7	59.5	18.2
	<b>3ak</b>	1.9	2.0	24.0	48.1	24.0
	<b>3al</b>	2.9	3.7	17.6	59.3	17.0*
	<b>3am</b>	27.6	22.6	7.9	31.6	10.3
	<b>3an</b>	Trace	Trace	8.3	83.5	8.3
	<b>3ao</b>	6.1	40.9	24.0	22.4	6.6
	<b>3ap</b>	3.1	53.3	26.1	16.6	1.0*
	<b>3aq</b>	40.1	51.1	5.5	2.7	<0.5*
	<b>3ar</b>	46.0	49.5	1.9	2.1	<0.5*
	<b>3as</b>	46.0	48.9	2.0	2.6	<0.5*
	<b>3at</b>	47.0	49.0	1.5	1.9	<0.5*

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- (18) Aliquots (0.2 mL) of diol library in THF (0.8 mL) were treated with aq. NaIO<sub>4</sub> (100 mg, 0.8 mL, 10 min, RT) and analyzed by GC-MS.
- (19) Aldehyde solution (0.5 mL, filtered) was added to a solution (1 mL) of dinitrophenylhydrazine (100 mg in 4 mL MeOH, 0.2 mL H<sub>2</sub>SO<sub>4</sub>). After 1 h at RT, the mixture was analyzed by GC-MS.
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