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SOLUTION-PHASE COMBINATORIAL SYNTHESIS VIA THE OLEFIN METATHESIS REACTION

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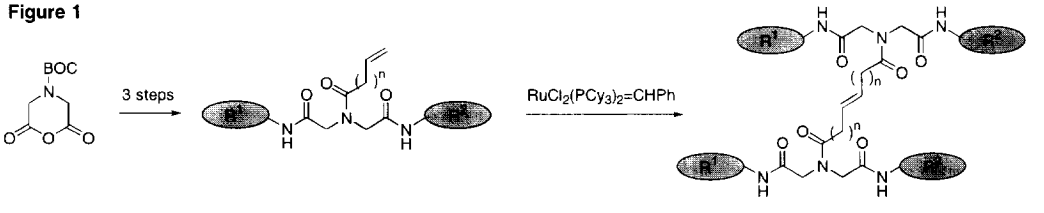
Abstract. The preparation of C_2-symmetric and unsymmetric chemical libraries by solution-phase techniques including the use of the olefin metathesis reaction to join and combinatorially randomize the length of a linking tether is detailed.

Ligand-induced receptor and protein dimerization or oligomerization have emerged as general mechanisms for signal transduction.[1] Members of several receptor families of significance for drug discovery have been established to utilize this mode of activation. These include protein tyrosine kinase receptors (homo- or heterodimerization),[2] cytokine receptors (homo- or heterodimerization),[3] serine/threonine kinase receptors (hetero-oligomerization)[4] and members of the TNF-receptor family (trimerization).[5] Within the cytokine receptor superfamily, the most extensively studied examples are the human growth hormone (hGHr),[6] prolactin (PRLr),[7] and erythropoietin (EPOr)[8] receptors, which form homodimers upon binding their endogenous ligands. Similarly, intracellular signal transduction often proceeds by ligand-induced protein–protein homo- or heterodimerization.[9]

Herein we report the development of an effective protocol for generating C_2-symmetrical or unsymmetrical chemical libraries suitable for probing receptor and protein homodimerization and heterodimerization events (Figure 1). To illustrate the approach, our initial efforts representing the assembly of a chemical library of 300 members (600 compounds including *cis/trans* isomers) by way of solution-phase combinatorial chemistry are detailed.[10-20]

Figure 1



The approach constitutes the dimerization and combinatorial randomization of the length of a linking tether joining two iminodiacetic acid diamides employing the olefin metathesis reaction.[21-23] The entire reaction sequence requires four steps and represents an extension of our solution-phase parallel synthesis of chemical libraries.[18] In addition to the advantages outlined in our original description of the approach, the solution-phase synthesis of the fragments permits their final direct linkage that would be precluded by more conventional solid-phase synthesis techniques.

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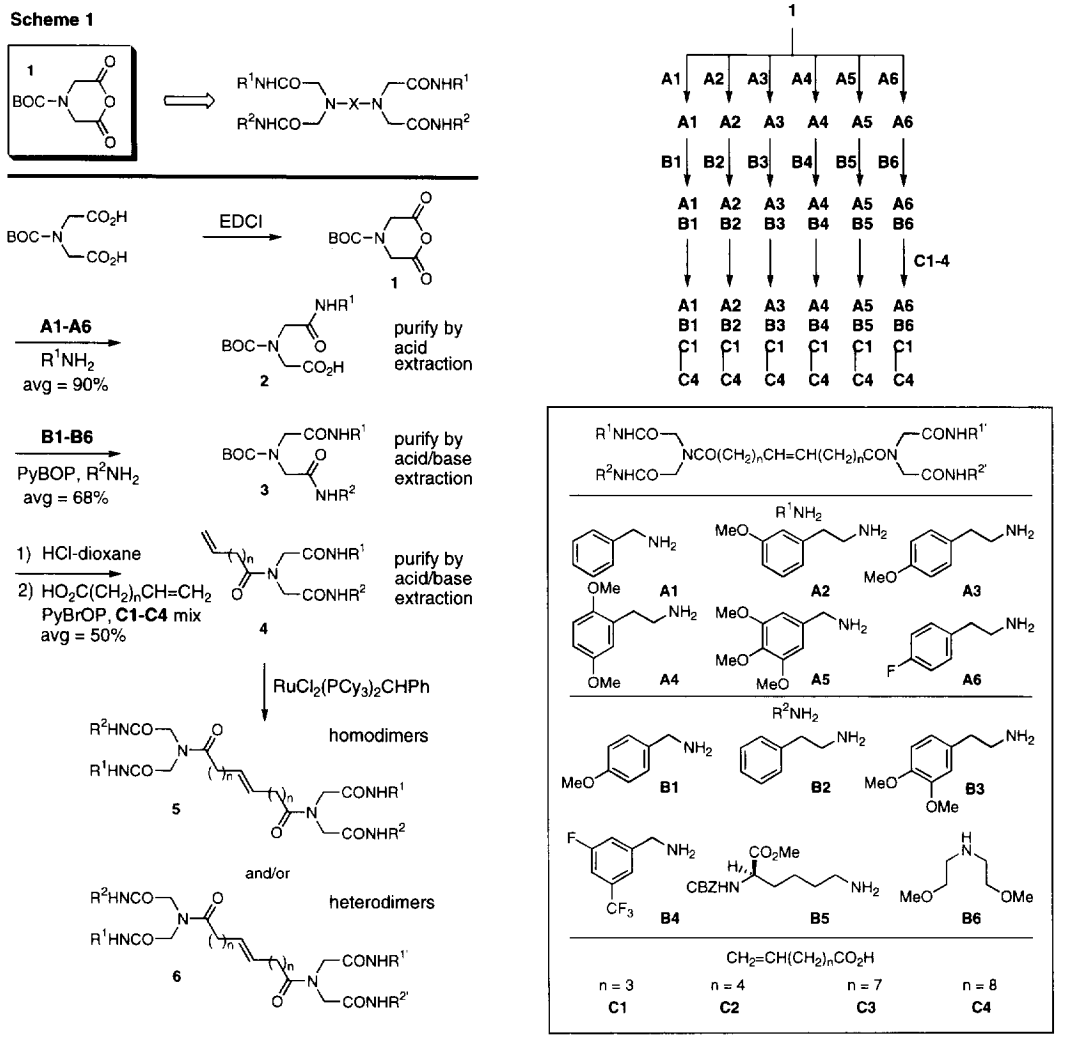
As such, the strategy is uniquely suited for taking advantage of such symmetrical diversity utilizing a limited number of synthetic steps. Moreover, this approach provides a unique opportunity to rapidly generate a statistically controlled diversity mixture. Although recent preliminary reports of the use of the olefin metathesis reaction for the cross-coupling of solution with resin bound olefins as well as for the release of resin bound olefins by ring closure metathesis have been disclosed,[23] its use to combinatorially randomize and statistically generate diversity has not been detailed. As described herein, the reaction seems particularly well suited for the solution-phase construction of homo- and heterodimerization libraries.

The precursors for our protypical library of 300 members (600 compounds including *cis/trans* isomers) were assembled from 6 iminodiacetic acid diamides each functionalized with 4 ω-alkene carboxylic acids, Scheme 1.

Scheme 1



The precursors were assembled in a matrix $6 \times 6 \times 4$ format with the 6 diagonal iminodiacetic acid diamides being prepared by parallel synthesis and with the last reaction conducted with a mixture of 4 ω-alkene carboxylic acids. As such, 24 precursors were assembled in three parallel steps as 6 mixtures each containing 4 compounds with variations in only the chain length of the terminal alkene representative of larger full matrix of 144 precursors to be prepared as 36 mixtures of 4 compounds each. For the development studies, the selection of the matrix diagonal **AXBX** combination represented in Table 1 for synthesis was simply a matter of convenience. Each of the three steps was conducted in solution employing acid/base extractions[18] to isolate and purify the intermediates and final precursors providing the desired pure products (≥95% pure) free of contaminants derived from unreacted starting materials, reagents, and reaction byproducts independent of the reaction yields (Table 1). In order to insure

Table 1. Yields (%) of the Library Precursors

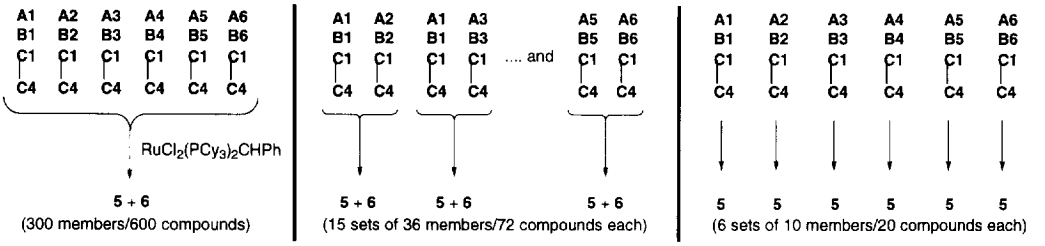
2	A1	A2	A3	A4	A5	A6
	93	91	89	88	76	100
3	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
B1	99					
B2		75				
B3			82			
B4				87		
B5					11	
B6						55
4	A1B1C1-4 61		A2B2C1-4 41		A3B3C1-4 53	
	A4B4C1-4 52		A5B5C1-4 25		A6B6C1-4 68	

Table 2. Yields (%) of the Sub-Library Reactions

	A1 B1 C1 C4	A2 B2 C1 C4	A3 B3 C1 C4	A4 B4 C1 C4	A5 B5 C1 C4	A6 B6 C1 C4
A1B1C1-C4	67	75	65	51	72	72
A2B2C1-C4	75	64	68	37	71	57
A3B3C1-C4	65	68	42	62	45	68
A4B4C1-C4	51	37	62	42	49	47
A5B5C1-C4	72	71	45	49	51	45
A6B6C1-C4	72	57	68	47	45	55
A1-6B1-6C1-4				62%		

that different coupling rates might not skew the equimolar mixture, the final coupling reaction with the **C1–C4** mixture (0.67 equiv) was conducted with excess secondary amine (1.0 equiv) and a prolonged reaction time (16 h) to guarantee complete consumption of the stoichiometry limiting carboxylic acid. The final library construction was accomplished in a single reaction providing a mixture of 300 members/600 compounds and by 15 pair-wise combinations (**A1B1C1–4 + A2B2C1–4**) providing 15 sub-libraries of 36 members/72 compounds containing two sets of homodimers as well as a defined set of heterodimers (Figure 2). Finally, the 6 sub-libraries of homodimers

Figure 2



containing 10 members/20 compounds were assembled in 6 reactions. There was no special significance to this choice of combinations except that each represents a significantly different mixture pool size which can be appropriately chosen to accomodate preferences in testing and deconvolution protocols. However, given the ease of conducting the reactions and the modest numbers of reactions that must be run to generate all three, this protocol does provide multisampling of the same compounds (each homodimer is generated in 5 of the 15 mixtures, indexed mixtures), and provides the opportunity for considerable deconvolution in a first pass assay. Final deconvolution of such mixtures by resynthesis of the individual components of the final precursors (last step) in the modest-sized 10- or 36-membered sub-libraries is straightforward. The dimerization and simultaneous combinatorial diversification of the linker greatly expands the diversity size of the initial compound collection in a minimum number of synthetic operations. For example, the full matrix represented in Table 1 (36 mixtures of 4 compounds each) derived from 16 diversity units (6 + 6 + 4) provides a total library size of 10,440 members (20,880 compounds including *cis/tran* isomers). Similarly, the expansion of such a library to 60 iminodiacetic acid diamides[18] followed by functionalization with a mixture of 4 ω-alkene carboxylic acids and olefin metathesis linking and randomization of the tethering chain would produce a library of 28,920 members (57,840 compounds including *cis/trans* isomers) from only 20 diversity units (6 + 10 + 4).

The assemblage of the full library of 600 compounds, the preparation of the complete set of 15 homo/heterodimer sub-libraries, and the 6 homodimer libraries described herein were conducted similarly (0.2–0.25 equiv $RuCl_2(PCy_3)_2$=CHPh, $CHCl_3$, reflux, 16 h) providing the mixture libraries in 75–23% (50% average, Table 2). The homodimer metathesis products **5** and **6** proved to be chromatographically similar to one another and substantially different from the precursors **4**, which in turn all behaved similarly. This additional and unanticipated bonus provided the opportunity to purify the final products, as mixtures, free from any potential starting materials. In our original design, the intention was to conduct the olefin metathesis reaction under conditions where all or essentially all **4** is consumed. While this proved to be the case, the simple chromatographic separation of the homodimer and heterodimer metathesis products from the precursors **4** permitted an additional level of purity control without compromising the mixture integrity. This final chromatographic purification/enrichment was used to remove the metathesis catalyst, its reaction byproducts, and the small amount of remaining reacting monomers and their exchange products with the catalyst. The latter minor byproducts containing a terminating styrene derived from reaction with the catalyst proved chromatographically similar to the starting monomers and were readily removed during this chromatographic enrichment. This was employed for the prototypical library generation detailed herein but in practice is probably unnecessary. Assay of the precursor mixtures **AXBXC1–4** along with the metathesis libraries would permit detection and identification of activity due to contaminant monomer precursors and that of any of the styrene-capped monomers which would be present only in minor amounts could be recognized and addressed upon resynthesis and deconvolution of the individual components of the small mixtures. Although

the similar properties of the individual components of the library mixtures precluded their separation/identification in the mixture by chromatographic means, ^{1}H NMR and especially MS unambiguously established the presence of each of the individual components. These details, studies of the individual metathesis reactions that first established the viabilitiy of the methodology, approximate *cis/trans* ratios, and similar reaction efficiencies for n > 2 as well as their extensions to larger libraries, and their evaluation will be disclosed in full accounts of this and related work.

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