Multistep Convergent Solution-Phase Combinatorial Synthesis and Deletion Synthesis Deconvolution

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Abstract: A solution-phase convergent versus linear, divergent solid-phase synthesis of chemical libraries is illustrated enlisting the 2-fold dimerization of iminodiacetic acid diamides ultimately incorporating eight variable groups. The first dimerization is conducted with ω-alkene carboxamide derivatives of iminodiacetic acid which sets up the second dimerization conducted with the olefin metathesis reaction. This latter reaction randomizes the linking tether length adding a ninth degree of diversification suitable for the discovery of receptor dimerization antagonists and their linkage into potential receptor dimerization agonists. Unlike the divergent synthesis of libraries which is amendable to solid-phase synthesis techniques, such convergent syntheses are especially suited for solution-phase synthesis and are precluded by conventional solid-phase techniques since the combining components typically would be on mutually exclusive phases. Two mixture libraries of 476 775 and 114 783 975 compounds were prepared in five steps from four ω-alkene carboxamides and 10 or 20 amines, respectively. Deconvolution of the library mixtures by positional scanning or a complementary technique we introduce as deletion synthesis can be conducted up front for depository libraries subjected to multiple assays. For convergent dimerizations such as that illustrated herein, only deletion deconvolution can provide information on all components of the mixture including the unsymmetrical combinations.

Combinatorial chemistry has undergone rapid development providing a new paradigm for drug discovery since its introduction with peptide, oligonucleotide, and antibody libraries.[1,2] As a consequence of the extension from linear peptide and oligonucleotide synthesis, most chemical approaches have relied on linear solid-phase synthesis.[3] A less commonly employed complement is the development of strategies for solution-phase combinatorial synthesis.[4] Our interest in studying receptor activation via dimerization[5] and the potential of enlisting a single approach for the discovery of antagonists and their conversion to agonists[6] was an important deliberation underlying our pursuit of multistep solution-phase combinatorial chemistry.[7−14] Inherent in all solid-phase syntheses including that of combinatorial

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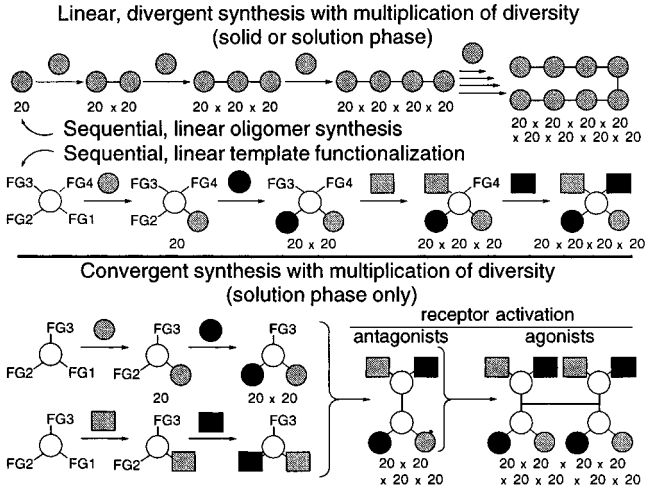


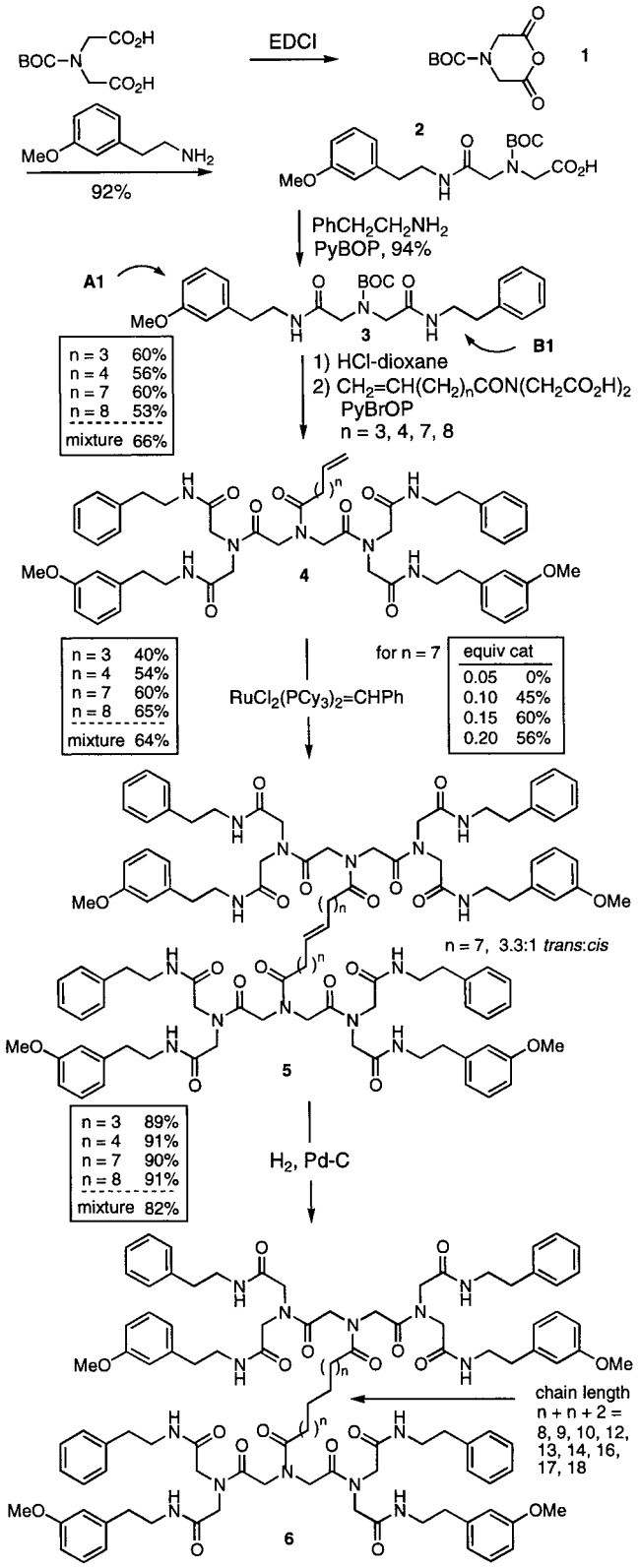
Figure 1.

libraries are their stepwise, linear preparation with the divergent introduction of diversity. This includes not only the linear synthesis of oligomer libraries composed of repeating monomers but also the sequential functionalizations enlisted for template libraries (Figure 1). Herein, we detail an illustrative example of the complementary power of the convergent versus divergent combination of a small number of monomers that is especially suited for the discovery of receptor antagonists and their linkage into potential receptor dimerization agonists.[6,15] Unlike a linear divergent synthesis which is amenable to both solution- or solid-phase synthesis, a convergent synthesis can only be conducted in solution and would be precluded by typical solid-phase techniques where the combining components would customarily be on mutually exclusive solid-phases.

The approach is illustrated employing the synthesis of iminodiacetic acid diamide libraries[7] with a 2-fold dimerization to convert the monomers first to dimers and then to tetramers incorporating eight variable groups. We wish to emphasize that this strategy is not limited to iminodiacetic acid diamide libraries which themselves resemble peptides and that nonamide-based templates may be incorporated into analogous sequential dimerizations. The first dimerization is conducted with ω-alkene carboxamide derivatives of iminodiacetic acid which sets up the second dimerization to provide tetramers conducted with the olefin metathesis reaction.[14−18] This latter reaction is conducted with a mixture of four ω-alkenes to join and randomize the linking tether length adding a ninth degree of diversification suited for simultaneously incorporating a linker of unknown optimal length. The mixture of four ω-alkenes (n = 3, 4, 7, 8) provides 16 heterodimer or 10 homodimer metathesis products, each produced as a trans/cis mixture (2−4:1).[14] Upon hydrogenation, this reduces to nine saturated chain lengths simplifying deconvolution.

The approach was first established with the preparation of a series of individual precursors and their sequential symmetrical

Scheme 1



dimerizations as shown in Scheme 1. Thus, deprotection of **3** with 4 M HCl−dioxane (25 °C, 4 h) followed by the individual or mixture coupling with the four ω-alkene carboxamide derivatives of iminodiacetic acid provided **4** in good yields when conducted with PyBrOP (i-Pr$_2$NEt, DMF, 25 °C, 16 h). Conducting the reaction with excess amine (1.5 equiv, 3 molar equiv) and stoichiometry limiting dicarboxylic acid ensured dimerization and that the mixture synthesis proceeded to

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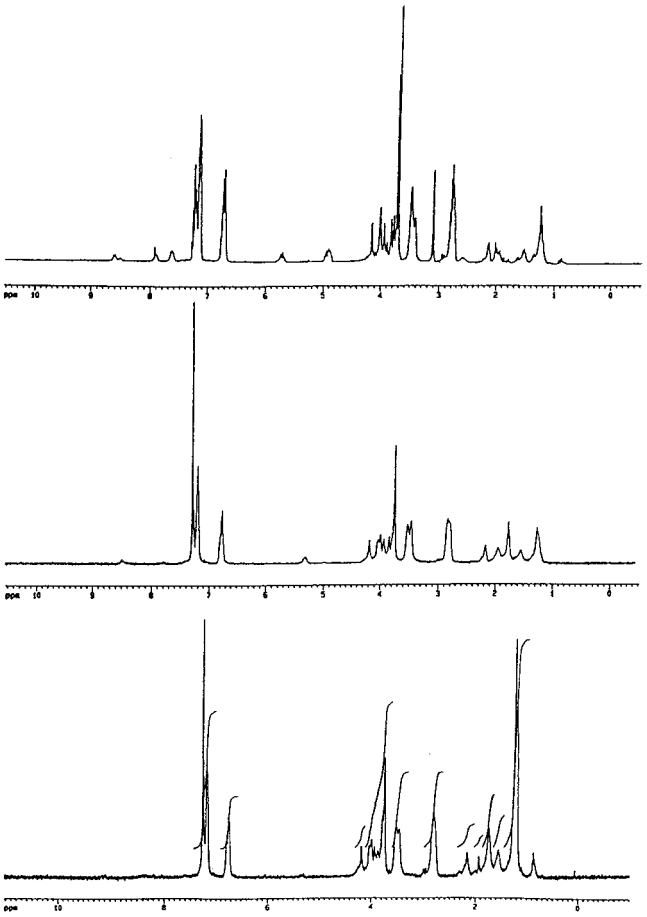


Figure 2. ^{1}H NMR of **4** mixture (four compounds): $n = 3$, 4, 7, and 8 (top), **5** mixture (10 compounds): $n = 3$, 4, 7, and 8 (middle), and **6** mixture (nine compounds): $n = 3$, 4, 7, and 8 (bottom).

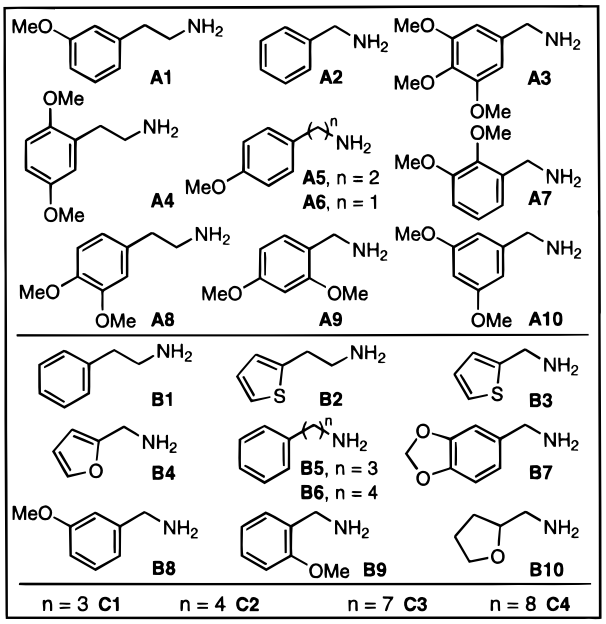


Figure 3.

completion regardless of relative coupling rates. Workup and purification by acid/base extraction provided the individual and mixture reaction products **4** ≥ 95% pure.

Analogous to prior studies,[14] olefin metathesis dimerization of the individual olefins **4** or the small mixture of **4** ($n = 3$, 4, 7, and 8) promoted by $RuCl_2(PCy_3)_2$=CHPh[16] (0.16 equiv, $CHCl_3$, reflux, 16 h) provided the individual products **5** or the small mixture of 10 products derived from the **4** mixture in good yields. This second dimerization produced a mixture of *trans* and *cis* olefins with the former predominating (3−4:1) and 0.15 equiv of catalyst was required for complete reaction. Because of distinct chromatographic mobilities of **4** and **5** regardless of the substitution, the individual compounds **5** as well as the mixture of **5** could be purified by chromatography (SiO_2). Final hydrogenation (0.1 wt equiv 10% Pd−C, CH_3OH, 1 atm H_2, 25 °C, 40 h) provided the individual compounds **6** and nine member mixture of **6**. In selected instances of the large library mixtures detailed below with functional groups sensitive to catalytic hydrogenation or in instances of apparent or contaminate poisoning of the catalyst, the reduction of the double bond could also be effected with diimide ($TsNHNH_2$, NaOAc, CH_3OH, 80 °C, 5 h). The integrity of the individual and mixture synthesis intermediates and products could be monitored by MS and their purity assessed by both HPLC and ^{1}H NMR (Figure 2). The latter proved especially valuable where the diagnostic olefinic resonances served as distinguishable signals by which

Scheme 2

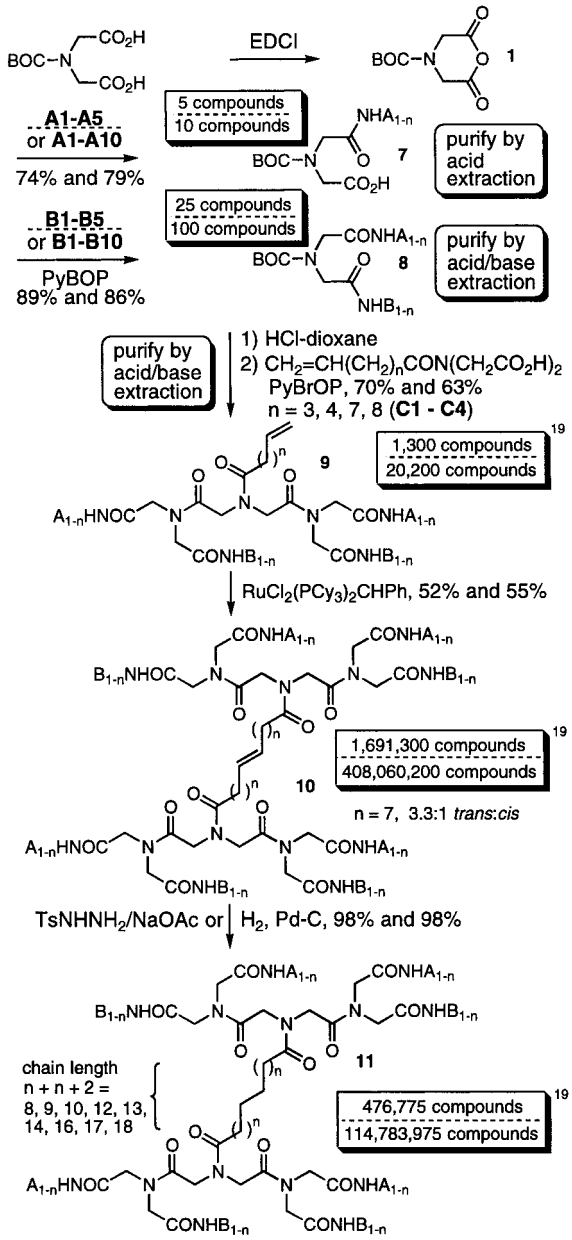


Table 1. Deconvolution Sublibraries, Synthesis % Yields[a] of Intermediates **7**−**10** and Final Sublibraries **11**

	Scanning Deconvolution[b]						Deletion Deconvolution[c]					
7	scanA1	92%					**7**	B1-B5	84%			
	scanA2	91%										
	scanA3	81%										
	scanA4	96%										
	scanA5	94%										
8	scan A1	75%	scanB1	90%			**8**	deleteA1	76%	deleteB1	81%	
	scanA2	62%	scanB2	73%				deleteA2	71%	deleteB2	84%	
	scanA3	73%	scanB3	67%				deleteA3	84%	deleteB3	84%	
	scanA4	83%	scanB4	81%				deleteA4	71%	deleteB4	90%	
	scanA5	77%	scanB5	90%				deleteA5	76%	deleteB5	95%	
9	scanA1	70%	scanB1	67%	scanC1	85%	**9**	deleteA1	85%	deleteB1	74%	deleteC1	76%
	scanA2	69%	scanB2	82%	scanC2	82%		deleteA2	83%	deleteB2	62%	deleteC2	82%
	scanA3	68%	scanB3	79%	scanC3	83%		deleteA3	90%	deleteB3	68%	deleteC3	75%
	scanA4	76%	scanB4	79%	scanC4	75%		deleteA4	82%	deleteB4	55%	deleteC4	82%
	scanA5	84%	scanB5	80%				deleteA5	80%	deleteB5	69%		
10	scanA1	51%	scanB1	51%	scanC1	49%	**10**	deleteA1	43%	deleteB1	51%	deleteC1	51%
	scanA2	58%	scanB2	63%	scanC2	37%		deleteA2	48%	deleteB2	63%	deleteC2	48%
	scanA3	51%	scanB3	51%	scanC3	44%		deleteA3	55%	deleteB3	51%	deleteC3	42%
	scanA4	48%	scanB4	52%	scanC4	54%		deleteA4	48%	deleteB4	52%	deleteC4	51%
	scanA5	53%	scanB5	59%				deleteA5	45%	deleteB5	59%		
11	scanA1	91%	scanB1	91%	scanC1	89%	**11**	deleteA1	93%	deleteB1	91%	deleteC1	91%
	scanA2	88%	scanB2	93%	scanC2	97%		deleteA2	88%	deleteB2	93%	deleteC2	95%
	scanA3	91%	scanB3	91%	scanC3	94%		deleteA3	95%	deleteB3	91%	deleteC3	95%
	scanA4	88%	scanB4	92%	scanC4	94%		deleteA4	88%	deleteB4	92%	deleteC4	90%
	scanA5	93%	scanB5	89%				deleteA5	95%	deleteB5	89%		

[a] Calculated based on an average molecular weight for the mixtures. **7**−**9** were isolated with an acid/base extraction purification that typically provides products >95% pure. **10** was isolated by chromatography (see text) and diagnostic olefin ^{1}H NMR resonances used to establish purity and completion of reaction (see Figure 2). **11** was typically pure after filtration removal of catalyst and required no further purification. [b] Mixture synthesis (Scheme 2, Figure 3) but including only Xn for scanXn, *e.g.*, scanA1 includes only A1 and B1−B5, C1−C4. [c] Mixture synthesis (Scheme 2, Figure 3) but deleting only Xn for deleteXn, *e.g.*, deleteA1 includes A2−A5, B1−B5, C1−C4.

to monitor the appearance or disappearance of **4**−**6** even in the large mixture libraries detailed below.

With the conditions in hand, two libraries of 10^6 or 10^8 compounds were assembled in an analogous 4−5 steps enlisting only 10 or 20 amines (**A1**−**A5**/**A1**−**A10** and **B1**−**B5**/**B1**−**B10**) and the four ω-alkene carboxamide derivatives of *N*-BOC-iminodiacetic acid (**C1**−**C4**), Figure 3 and Scheme 2.[19] This can be conducted by mixture synthesis, mix and split synthesis,[20] or by partial parallel/mixture synthesis[14] with smaller pool sizes. However, given the ease with which positional scanning[22] or deletion synthesis deconvolution can identify an active lead, the simpler process of mixture synthesis was used. Unlike solid-phase synthesis where the polymer-bound substrate typically must be the stoichiometry limiting reaction partner, either the substrate or the reacting attachment groups may be limiting in solution-phase chemistry. This dictates the use of mix and split synthesis for the solid-phase in order to accommodate differential reaction rates,[21] whereas the simpler protocol of mixture synthesis with limiting reagent stoichiometry (e.g., **B1**−**B10**) may be used in solution to ensure all library members are generated. The implementation of the latter only requires the ability to remove unreacted starting substrate. Although not possible with solid-phase synthesis, this was accomplished by aqueous acid/base extractions in the first three steps of Scheme 2 which also served to remove reactants, reagents, and reagent byproducts providing clean products.

The deconvolution of the 10^6 library, which may be tested under conditions providing realistic concentrations of the individual components, by positional scanning[22] requires five sublibraries of **11** each of which contains only **A1**, **A2**, **A3**, **A4**, or **A5** (scanA), five sublibraries of **11** each of which contains only **B1**, **B2**, **B3**, **B4**, or **B5** (scanB), and four sublibraries of **11** each of which contains only **C1**, **C2**, **C3**, or **C4** (scanC). For example scanA1 refers to a mixture synthesis enlisting only **A1** and the full **B1**−**B5** and **C1**−**C4** mixtures. They were prepared concurrent with the full library mixture requiring 14 additional mixture syntheses and, in principle, provides the lead identities in a single round of testing (Table 1).[23] As a complement, we also introduce a protocol we refer to as deletion synthesis deconvolution. It was conducted simultaneously by constructing 14 sublibrary mixtures (Table 1), each lacking only one different member of the variable units and the libraries are screened for a loss versus gain in activity. For example, deleteA1 refers to the full mixture synthesis lacking only the inclusion of **A1**. Typically, the deletion synthesis deconvolution mixtures lack what the scanning mixtures contain and their combination reconstitute the full mixture.[23]

Although it is not possible to unambiguously establish the accuracy of the deletion synthesis deconvolution strategy on a large mixture library since the activity of each member cannot be determined, we conducted initial assessments with small libraries where the activity of each member could be established. One representative example is detailed in Table 2. The small library of 16 compounds was prepared for illustration purposes as individual compounds following protocols we have described in detail.[7] The full mixture and the eight scanning and eight deletion deconvolution sublibrary mixtures were synthesized following protocols detailed herein in Schemes 1 and 2. Their

(19) The combination of two nonidentical libraries of x and y members provides $x \times y$ members. When a library of x members is symmetrically dimerized, the combination provides $x(x+1)/2$ members. With **A1**−**A10**, **B1**−**B10**, **C1**−**C4**: for **9**, $n = (100 \times 101/2) \times 4$. For **10**, $n = (100 \times 101/2) \times 20 + \{(100 \times 101/2)[(100 \times 101/2) - 1]/2\} \times 32$. For **11**, $n = (100 \times 101/2) \times 9 + \{(100 \times 101/2)[(100 \times 101/2) - 1]/2\} \times 9$.

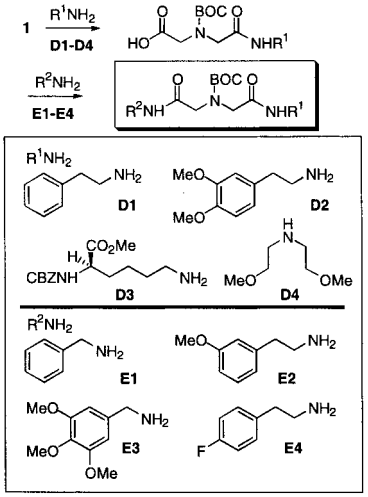
(20) Furka, A.; Sebestyen, F.; Asgedorn, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487. Sebestyen, F.; Dibo, G.; Kovacs, A.; Furka, A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 413.

(21) The solid-phase synthesis exception enlists an excess of the reacting monomers in adjusted concentrations to accommodate the different reaction rates and requires that this information be available at the onset of the mixture synthesis: see ref 22.

(22) Dooley, C. T.; Houghten, R. A. *Life Sci.* **1993**, *52*, 1509.

(23) This is true in the generalized case when a single residue within the library is incorporated once at a unique location. With dimerization libraries such as those disclosed herein, scanning deconvolution would fail to detect unique activities for unsymmetrical incorporations, e.g., structures containing both **A1** and **A5**. Here, deletion deconvolution sublibraries are required and would be expected to detect a loss of activity with the sublibraries lacking **A1** and **A5**. In the example provided in Table 3 for **9**, the number of compounds insensitive to scanning deconvolution is 800 out of 1300, while in principle all are sensitive to deletion deconvolution. Expanding this to the 20 200 compounds in **9** prepared from **A1**−**A10**, **B1**−**B10**, and **C1**−**C4**, 16 200 out of 20 200 compounds are insensitive to scanning deconvolution.

Table 2. IC_{50} (L-1210, μM)[a]



mixture	IC_{50} = 40 μg/mL		
deletion deconvolution[b]			
deleteD1	28		
deleteD2	32		
deleteD3	> 75 (20% inhibition)		
deleteD4	50		
deleteE1	34		
deleteE2	> 75 (32% inhibition)		
deleteE3	51		
deleteE4	50		
Identifies D3E2 as the most potent constituent of the mixture.			
confirmation			
D1E1	100	D2E1	>100
D1E2	32	D2E2	>100
D1E3	>100	D2E3	>100
D1E4	32	D2E4	>100
D3E1	31	D4E1	>100
D3E2	0.1	D4E2	>100
D3E3	32	D4E3	>100
D3E4	30	D4E4	>100
scanning deconvolution[c]			
scanD1	28	scanE1	41
scanD2	> 100	scanE2	20
scanD3	25	scanE3	36
scanD4	> 100	scanE4	20
Suggests D3E2, D3E4, D3E1, D3E3 may be active and should be examined and also suggests D1E2, D1E4, D1E1 and D1E3 are active and need be examined.			

[a] Mouse leukemia cytotoxic assay (ref 25). [b] DeleteD1 indicates D2-4E1-4 mixture. [c] ScanD1 indicates D1E1-4 mixture.

side-by-side testing in a simple cytotoxic assay (L-1210) established that scanning deconvolution accurately identified the most potent library member through selective loss of activity from the deleteD3 and deleteE2 sublibraries. The testing of the scanning deconvolution sublibraries identified a number of candidate structures responsible for the activity, albeit not with an unambiguous identification of the most potent, and identified a number of weakly active compounds as well as the most potent compound in the small library. These and related comparisons suggest that the two methods are complementary and that deletion synthesis is more sensitive to establishing the most active lead in a library that contains few hits albeit at the expense of identifying weaker leads, whereas positional scanning is more effective at identifying the weaker leads especially in libraries with multiple hits but at the expense of accurately identifying

Table 3. Cytotoxic Activity of Mixture (1300 Compounds) and Deconvolution Sublibraries for **9**[a]

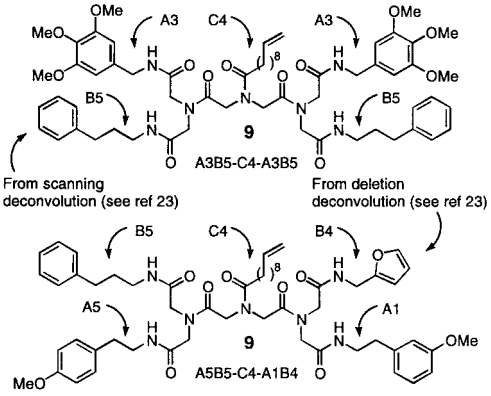
L-1210[a] IC_{50} (μg/mL) for full mixture **9** = 36 μg/mL					
scanning deconvolution[b]					
library	IC_{50}	library	IC_{50}	library	IC_{50} (μg/mL)
scanA1	32	scanB1	32	scanC1	178
scanA2	32	scanB2	55	scanC2	25
scanA3	3.5	scanB3	245	scanC3	32
scanA4	18	scanB4	251	scanC4	20
scanA5	32	scanB5	10		
deletion deconvolution[c]					
library	IC_{50}	library	IC_{50}	library	IC_{50} (μg/mL)
deleteA1	38	deleteB1	28	deleteC1	32
deleteA2	27	deleteB2	32	deleteC2	32
deleteA3	32	deleteB3	32	deleteC3	32
deleteA4	30	deleteB4	45	deleteC4	40
deleteA5	40	deleteB5	240		

[a] L-1210 (mouse leukemia) cytotoxic activity (ref 25). [b] Structure **9**, scanA1 mixture includes only A1, B1−B5, C1−C4. [c] Structure **9**, deleteA1 mixture includes A2−A5, B1−B5, C1−C4.

Table 4. Activity for Compounds Identified by Deconvolution[a]

from scanning deconvolution		from deletion deconvolution	
compound	IC_{50} (μg/mL)	compound	IC_{50} (μg/mL)
A3B5-C4-A3B5	0.6	A5B5-C4-A5B5	3.2
A3B1-C4-A3B1	22	A1B5-C4-A1B5	6.2
A3B2-C4-A3B2	40	A5B4-C4-A5B4	11
A4B5-C4-A4B5	3.2	A1B4-C4-A1B4	10
A4B1-C4-A4B1	7.6	A5B5-C4-A5B4	5.5
A4B2-C4-A4B2	18	A1B5-C4-A1B4	11
		A5B5-C4-A1B5	4.9
		A5B4-C4-A1B4	4.4
		A5B5-C4-A1B4	0.8
		A1B5-C4-A5B4	12

[a] L-1210 (mouse leukemia) cytotoxic activity (ref 25).



the most potent agent.[23,24] The implementation of either, or better both, in conjunction with the solution-phase preparation of library mixtures provides a powerful approach to lead discovery that permits the rapid preparation and screening of large numbers of compounds.

In an additional illustration of the complementary nature of the scanning and deletion deconvolution strategies, the in vitro cytotoxic activity (L-1210)[25] of the modest sized mixture **9** (Scheme 2, 1300 compounds) was examined alongside the 14 scanning and 14 deletion deconvolution libraries for **9** (Table 1). This represents a special case of a mixture library assembled

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(25) Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918. A procedure is provided in the Supporting Information.

in a convergent manner that incorporates both symmetrical and unsymmetrical dimers. Scanning deconvolution as implemented is incapable of revealing activities from unsymmetrical dimerizations (e.g., activity requiring incorporation of both **A1** and **A2**)[23] but would be especially sensitive to activity arising from symmetrical dimerizations (e.g., activity due to incorporation of **A1** twice). Complementing this, the deletion synthesis deconvolution libraries are sensitive to the activities arising from both unsymmetrical and symmetrical dimerizations making it a requisite deconvolution protocol for such libraries. The results of the testing and deconvolution are summarized in Table 3. The scanning deconvolution results indicate potent activities originating from **A3** and **A4**, **B5** and **B1**, **C4** and **C2**, the most potent scan sublibraries. Deletion deconvolution reveals activity originating from **A1** and **A5**, **B4** and **B5**, and **C4**, the least potent delete sublibraries. Of these, **B5** and **C4** were featured prominently in both deconvolution protocols. Synthesis of the individual compounds implicated by the testing of the deconvolution libraries was carried out, and the results of their examination are summarized in Table 4. This included both the symmetrical and unsymmetrical combinations, and their evaluation provided an especially interesting set of results. The most potent agent examined ($IC_{50} = 0.6\ \mu g/mL$) was found to be the symmetrical combination of **A3**, **B5**, and **C4**. Its structure follows that deduced directly from the scanning deconvolution and two of the incorporated units were also implicated in the deletion deconvolution (**B5** and **C4**). The next most potent agent ($IC_{50} = 0.8\ \mu g/mL$) was the unsymmetrical compound incorporating **A1**, **A5**, **B4**, **B5**, and **C4** and was revealed only through the deletion deconvolution. Its presence and activity could not have been deduced from the scanning deconvolution sublibraries.

Conclusions

The combined use of solution-phase mixture synthesis and the two deconvolution protocols is simple and technically nondemanding even for large compound libraries, applicable to convergent as well as linear syntheses, less demanding than solid-phase mix and split syntheses or tagging,[2] and unlike iterative,[26] SURF[27] or recursive deconvolution,[28] can be conducted up front for depository libraries subjected to multiple screening assays. The two identification protocols of scanning and deletion deconvolution are complementary to one another and their combined use provides a powerful method for lead identification within large mixture libraries. In selected instances, including libraries generated by sequential dimerizations, only deletion deconvolution can survey all library members and would be a requisite deconvolution protocol.

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Supporting Information Available: Experimental procedures and characterization for the preparation of **6** and their precursors, the characterization of **D3E2** (Table 2), the characterization of **A3B5-C4-A3B5** and **A5B5-C4-A1B4** (Table 4), and details of the cytotoxic assays (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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