Two Comparisons of the Performance of Positional Scanning and Deletion Synthesis for the Identification of Active Constituents in Mixture Combinatorial Libraries

Dale L. Boger,* Jae Kyoo Lee, Joel Goldberg, and Qing Jin

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

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Two libraries of 120 compounds each were prepared as individual compounds and as full mixtures. The corresponding scanning and deletion synthesis deconvolution libraries were prepared and tested (L-1210, IC_{50}) alongside the individual compounds and mixture libraries. This testing, where the properties of each compound in the mixtures were known, was used to compare the performance of scanning and deletion deconvolution libraries. Each has its own intrinsic strengths, with the former being capable of identifying multiple hits at the expense of accurately identifying the most potent library member, while the latter typically is more sensitive to identifying the most potent hit but at the expense of differentiating weaker activities. The protocols complement one another and together more thoroughly identify potent library members.

Introduction

Integrins are cell surface receptors that recognize extracellular matrix adhesive proteins such as fibrinogen, fibronectin, vitronectin, and VCAM-1 (vascular cell adhesion molecule-1).[1−3] These important biological targets are membrane-bound, heterodimeric glycoproteins, each consisting of an α subunit (approximately 1100 residues) and a β subunit (approximately 800 residues). The relative affinity and specificity for ligand binding are determined by the unique combinations of the different α and β subunits.[3] Of the members of this family of receptors, $\alpha_{IIb}\beta_3$ (GPIIb-IIIa), $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, and $\alpha_4\beta_1$ continue to be extensively studied. Some of the disease states that have a strong β integrin component in their etiologies are thrombosis (integrin $\alpha_{IIb}\beta_3$), unstable angina (integrin $\alpha_{IIb}\beta_3$), osteoporosis ($\alpha_v\beta_5$), and tumor metastasis ($\alpha_v\beta_3$ and $\alpha_v\beta_5$). These integrins bind the sequence Arg-Gly-Asp (RGD) as a common recognition motif within their putative ligands.[4−8]

Because of the crucial role that platelet $\alpha_{IIb}\beta_3$ plays in thrombosis, antagonists which compete for binding to $\alpha_{IIb}\beta_3$ are useful for the treatment of artial thrombotic conditions including unstable angina, myocardial infarction, and stroke. Nonpeptide RGD-based antagonists have been developed employing heterocyclic scaffolds or constrained linkers as templates for attachment of the crucial Arg and Asp side chain surrogates. Examples include the isoindolone-based agent **1**,[9] whose oral bioavailability and platelet aggregation inhibitory activity were enhanced by introduction of the sulfonamide functionality found in **2**.[10] Similarly, a benzodiazepine scaffold incorporating a piperidine, **3** (SB 214857),[11] exhibited $\alpha_{IIb}\beta_3$ selectivity, while that incorporating a properly spaced benzimidazole, **4** (SB 223245), exhibited $\alpha_v\beta_3$ selectivity (Figure 1).[12]

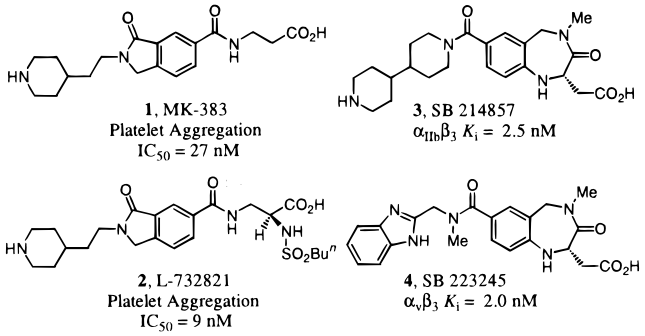

Figure 1.

The vitronectin receptor ($\alpha_v\beta_3$) is involved in many cell adhesion processes. Cheresh and co-workers have shown that in vivo inhibition of binding of these integrins to their native ligands interferes with angiogenesis and induces tumor regression.[13] In addition to its relevance to angiogenesis, $\alpha_v\beta_3$ has also been shown to play a role in mediating adhesion of osteoclasts to the bone matrix and in the migration of vascular smooth muscle cells. Therefore, antagonists of $\alpha_v\beta_3$ are envisioned as potential

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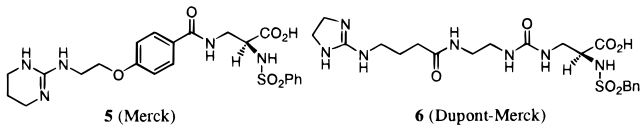

Figure 2.

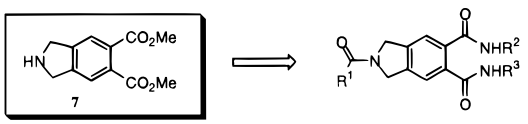

Figure 3.

therapeutic agents for the treatment of numerous diseases including diabetic retinopathy, cancer, osteoporosis, and restenosis. Recently a number of high-affinity ligands for $\alpha_v\beta_3$ possessing structures different from classical peptide frameworks (e.g., **5** and **6**, Figure 2) have been disclosed.[12,14,15]

In conjuction with an effort to evaluate a new class of RGD mimetics, we elected to use the opportunity to compare the performance of positional scanning and deletion synthesis to identify active constituents in mixture combinatorial libraries.[16] We prepared two small libraries of 120 compounds each as individual compounds along with the two full mixture libraries and the accompanying scanning[17] and deletion synthesis[18] deconvolution libraries. In this manner, the activity of each library member could be established and compared to the results derived from the scanning and deletion synthesis libraries. These libraries will be subjected to multiple screens including those designed to identify RGD antagonists, and herein we report the first of these results obtained in a cytotoxic assay. The results from the comparisons serve to highlight the complementary nature of the two deconvolution protocols.

Synthesis

Adopting a technically nondemanding multistep, solution-phase strategy for the preparation of chemical libraries which relies on the removal of excess reactants and reagents by liquid−liquid or liquid−solid extractions,[19−21] two small libraries based on the template **7** were prepared. The approach highlights the ease with which solution-phase mixture synthesis coupled with scanning and deletion synthesis deconvolution, which is conducted upfront for depository libraries subjected to multiple assays, can be used to identify new leads. Notably, the simple protocol of mixture synthesis typically cannot be conducted on the solid phase. Unlike solid-phase synthesis where the polymer-bound substrates must be the stoichiometry-limiting partner, either the substrates 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 to accommodate differential reaction rates, whereas the simpler procedure of mixture synthesis with limiting reagent stoichiometry may be used in solution to ensure all library members are generated. This only requires the ability to remove unreacted starting substrates. Although not possible with solid-phase synthesis, this was accomplished by aqueous acid/base extractions in each of the steps, which also served to remove reactants, reagents, and reagent byproducts, providing clean products.

When appropriately functionalized, **7** contains a rigid bicyclic core which enables it to function as a Arg-Gly-Asp (RGD) mimic. Its symmetrical structure contains three positions which can be functionalized with a variety of nucleophiles and acylating agents, enabling the synthesis of libraries with three points of diversity (Figure 3).

Removal of the protecting group on the nitrogen of the starting template allows for the first functionalization by *N*-acylation and provides monoamides **8**, which can be purified by simple acid/base liquid−liquid extraction, removing unreacted starting material, reagents, and reaction byproducts. For the second functionalization of the diester, hydrolysis affords the corresponding diacid, which is activated as the cyclic anhydride. Suitable amines (R^2NH_2) can be added to open the anhydride to provide the diamide with release of a third functionalization site ($-CO_2H$). The released carboxylic acid functionality may be used for the purification of the expected products, allowing removal of starting material, reagents, and reaction byproducts by simple liquid−liquid extraction. Functionalization of the released acid, utilizing a protocol similar to that of the second functionalization, allows for additional diversity to be introduced onto the rigid template. In each step of the sequence the reactants, unreacted starting materials, and reagents and their byproducts can be removed by simple extractions, providing the intermediates and the final compounds in high purities (Scheme 1).

The template synthesis required *N*-Boc protection of propargylamine and subsequent alkylation, effected by treatment with NaH (1.1 equiv, DMF, 25 °C, 30 min) followed by propargyl bromide (1.2 equiv, 0−25 °C, 5 h), to generate **14** (85%, two steps), Scheme 2. Treatment of *N*-Boc-4-aza-1,6-heptadiyne (**13**) and dimethyl acetylenedicarboxylate (4 equiv) with $(PPh_3)_3RhCl$ (0.02 equiv, EtOH, reflux, 18 h) followed by deprotection of the Boc group afforded the desired isoindoline template **7** in 26% overall yield.[22]

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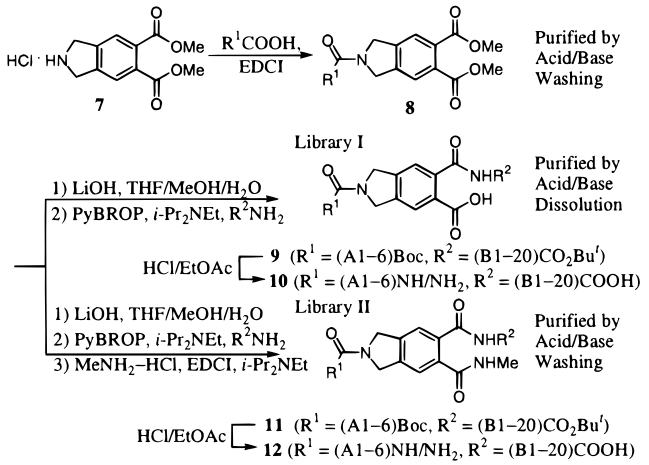
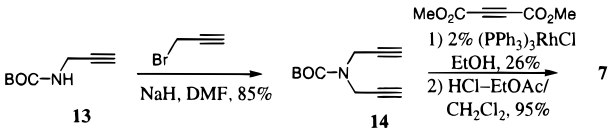
Scheme 1



Scheme 2



The approach outlined in Scheme 1 was shown to dependably deliver pure individual compounds in large quantities (25−60 mg), and two small libraries of 120 individual members were assembled. Each library member contains a free carboxylic acid that mimics the aspartic acid (D) in the RGD tripeptide and an amine at the opposite end of the structure to mimic the arginine (R), Figure 4. The varying length, rigidity, and structural properties introduced by the template and the linkages as well as variations in the basicity of the amino group provided a rich array of potential RGD mimetics. Simultaneous deprotection of the amine and carboxylic acid protecting groups provided 120 individual compounds (library I), 6 scanning sublibraries for R^1, 20 scanning sublibraries for R^2, 6 deletion sublibraries for R^1, 20 deletion sublibraries for R^2, and 1 full mixture of 120 compounds. Library II was prepared by the same method but with the additional coupling of methylamine to the remaining carboxylic acid of library I, affording another set of 120 individual compounds (library II), 6 scanning sublibraries for R^1, 20 scanning sublibraries for R^2, 6 deletion sublibraries for R^1, 20 deletion sublibraries for R^2, and 1 full mixture of 120 compounds.

To mimic the arginine, six different amino acids (**A1**−**A6**) (R^1CO_2H) were attached to **7** with the water-soluble coupling reagent EDCI (1−1.1 equiv, DMF, 15 h). Successive washing of the crude products diluted in EtOAc with aqueous acid (10% aqueous HCl) and saturated aqueous $NaHCO_3$ served to remove unreacted amine, R^1CO_2H, and EDCI and its byproducts and provided the pure momoamides **8** (>95% pure by ^1H NMR). Each of the six monoamides was converted to its dicarboxylic acid by treatment with LiOH in THF/MeOH/H_2O (3/1/1), and then partitioned into 20 portions. Each portion was treated with 1 of 20 different amines (**B1**−**B20**) (1.0 equiv) and PyBROP (1.05 equiv, 2−2.2 equiv of *i*-Pr_2NEt, DMF, 25 °C, 16 h, 25−100%) to afford the 120 individual diamides **9** (library I), which were purified by sequential 10% aqueous HCl and saturated aqueous NaCl extractions from EtOAc to remove unreacted amine, unreacted starting material, and PyBROP and its byproducts (Table 1). The triamides **11** were prepared with sequential addition of $MeNH_2$·HCl (2 equiv), EDCI (2.2 equiv, 2−2.2 equiv of *i*-Pr_2NEt, DMF, 25 °C, 4 h, 25−95%) to **9**, affording another set of 120 individual compounds **11** denoted as library II (30−95%, Table 2). The lower yielding reactions were compromised by the water solubility of the product. The resulting diamides **9** and the triamides **11** were treated with 3.9 M HCl in EtOAc to deprotect simultaneously the amine and carboxylic acid protecting groups with the formation of a white precipitate, which was collected by filtration or evaporation of solvent. Irrespective of the reaction efficiency or product recovery and without deliberate reaction optimization, the purities of the resulting individual compounds were uniformly satisfactory (≥80−95%) and the identities of the products were confirmed by matrix characterization (HRMS, ^1H NMR, and IR).

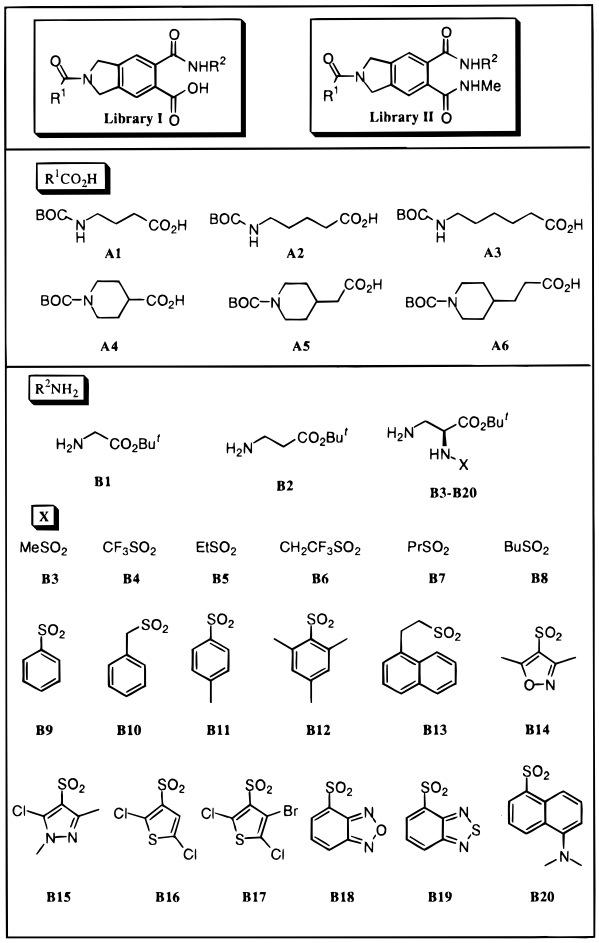


Figure 4.

For the formation of the triamides **11**, we compared the in situ formation without isolation of the diamide **9** with the stepwise process. Both afforded satisfactory results, and the in situ process was more convenient.

Following the preparation of the individual compounds (Tables 1 and 2), the full mixture libraries, 6 positional scanning sublibraries of **9** and **11** which contain only **A1**, **A2**, **A3**, **A4**, **A5**, or **A6** (**scanA**) and an equimolar mixture of the 20 amines R^2NH_2 (Table 3), and 20 positional scanning sublibraries, each of which contains one **B** subunit from **B1**−**B20** (**scanB**) and an equimolar mixture of the 6 acids R^1CO_2H (**A1**−**A6**) were produced (Table 3). Deletion sublibraries (**deleteA**) were also

Table 1. Yields (%) and Amounts (mg) for Compounds 8 and Diamides 9 (Library I)

	8 (A1) (88%)	**8 (A2)** (92%)	**8 (A3)** (87%)	**8 (A4)** (94%)	**8 (A5)** (99%)	**8 (A6)** (92%)
B1	28 mg, 79%	33 mg, 87%	28 mg, 71%	33 mg, 88%	28 mg, 71%	36 mg, 89%
B2	19 mg, 53%	30 mg, 81%	29 mg, 73%	35 mg, 90%	36 mg, 89%	35 mg, 84%
B3	24 mg, 52%	46 mg, 97%	37 mg, 83%	37 mg, 77%	46 mg, 92%	40 mg, 79%
B4	29 mg, 57%	38 mg, 72%	32 mg, 60%	31 mg, 57%	33 mg, 61%	37 mg, 66%
B5	34 mg, 72%	44 mg, 95%	35 mg, 70%	44 mg, 89%	43 mg, 84%	41 mg, 79%
B6	31 mg, 60%	22 mg, 40%	43 mg, 78%	44 mg, 81%	24 mg, 43%	42 mg, 72%
B7	43 mg, 88%	44 mg, 86%	43 mg, 85%	36 mg, 71%	34 mg, 65%	47 mg, 88%
B8	46 mg, 92%	40 mg, 79%	46 mg, 88%	32 mg, 62%	37 mg, 68%	45 mg, 82%
B9	46 mg, 90%	53 mg, 99%	52 mg, 95%	47 mg, 87%	54 mg, 97%	54 mg, 95%
B10	35 mg, 67%	41 mg, 75%	38 mg, 68%	46 mg, 83%	47 mg, 82%	49 mg, 83%
B11	19 mg, 36%	19 mg, 34%	26 mg, 47%	24 mg, 43%	35 mg, 62%	35 mg, 59%
B12	33 mg, 59%	39 mg, 68%	42 mg, 71%	43 mg, 74%	49 mg, 81%	51 mg, 83%
B13	52 mg, 88%	60 mg, 99%	42 mg, 68%	45 mg, 72%	58 mg, 91%	57 mg, 87%
B14	27 mg, 50%	24 mg, 47%	37 mg, 65%	34 mg, 61%	39 mg, 70%	46 mg, 78%
B15	47 mg, 82%	39 mg, 67%	31 mg, 52%	35 mg, 58%	31 mg, 51%	46 mg, 73%
B16	53 mg, 90%	48 mg, 79%	49 mg, 79%	45 mg, 72%	56 mg, 89%	58 mg, 90%
B17	51 mg, 76%	40 mg, 59%	55 mg, 79%	68 mg, 97%	58 mg, 82%	61 mg, 84%
B18	31 mg, 56%	30 mg, 53%	33 mg, 58%	36 mg, 62%,	42 mg, 71%	49 mg, 82%
B19	30 mg, 52%	35 mg, 59%	35 mg, 58%	37 mg, 61%	43 mg, 70%	49 mg, 78%
B20	13 mg, 22%	33 mg, 53%	25 mg, 39%	20 mg, 32%	18 mg, 28%	24 mg, 36%

Table 2. Yields (%) and Amounts (mg) for Triamides 11 (Library II)

	A1	**A2**	**A3**	**A4**	**A5**	**A6**
B1	19 mg, 53%	17 mg, 46%	26 mg, 70%	18 mg, 49%	33 mg, 86%	37 mg, 92%
B2	26 mg, 71%	36 mg, 96%	27 mg, 68%	17 mg, 43%	31 mg, 78%	40 mg, 96%
B3	27 mg, 48%	33 mg, 70%	24 mg, 49%	31 mg, 64%	28 mg, 56%	22 mg, 44%
B4	35 mg, 69%	34 mg, 65%	36 mg, 67%	34 mg, 64%	36 mg, 65%	42 mg, 74%
B5	13 mg, 27%	27 mg, 56%	15 mg, 30%	19 mg, 38%	20 mg, 39%	13 mg, 25%
B6	32 mg, 61%	34 mg, 64%	34 mg, 61%	31 mg, 56%	40 mg, 71%	36 mg, 63%
B7	25 mg, 51%	28 mg, 57%	40 mg, 78%	42 mg, 82%	35 mg, 66%	39 mg, 73%
B8	32 mg, 64%	35 mg, 69%	38 mg, 72%	44 mg, 84%,	38 mg, 71%	46 mg, 83%
B9	50 mg, 97%	46 mg, 86%	35 mg, 64%	46 mg, 84%	51 mg, 91%	33 mg, 58%
B10	34 mg, 64%	37 mg, 68%	40 mg, 71%	39 mg, 70%	39 mg, 68%	44 mg, 74%
B11	27 mg, 49%	33 mg, 59%	36 mg, 63%	32 mg, 56%	40 mg, 68%	41 mg, 69%
B12	45 mg, 78%	39 mg, 67%	46 mg, 77%	48 mg, 78%	54 mg, 86%	51 mg, 80%
B13	48 mg, 78%	52 mg, 83%	47 mg, 73%	46 mg, 72%	56 mg, 86%	55 mg, 83%
B14	40 mg, 72%	30 mg, 53%	31 mg, 53%	33 mg, 56%	37 mg, 63%	25 mg, 42%
B15	18 mg, 31%	25 mg, 58%	24 mg, 39%	20 mg, 33%,	25 mg, 46%	27 mg, 42%
B16	47 mg, 77%	48 mg, 77%	51 mg, 81%	51 mg, 80%	52 mg, 81%	60 mg, 91%
B17	51 mg, 75%	39 mg, 56%	59 mg, 83%	59 mg, 83%	61 mg, 85%	52 mg, 70%
B18	25 mg, 43%	34 mg, 57%	34 mg, 57%	33 mg, 56%	37 mg, 60%	47 mg, 75%
B19	37 mg, 50%	37 mg, 49%	39 mg, 50%	35 mg, 45%	42 mg, 56%	49 mg, 61%
B20	14 mg, 22%	14 mg, 21%	25 mg, 38%	19 mg, 29%	17 mg, 26%	29 mg, 43%

Table 3. Scanning Sublibraries of 9 and 11, Synthesis Yields[a] (%) and Amounts (mg)

	scanA1[b]	**scanA2**	**scanA3**	**scanA4**	**scanA5**	**scanA6**	**Amix**
library I	32 mg, 60%	44 mg, 80%	39 mg, 67%	29 mg, 56%	37 mg, 64%	48 mg, 83%	35 mg, 63%
library II	30 mg, 55%	34 mg, 61%	41 mg, 72%	34 mg, 60%	33 mg, 56%	21 mg, 35%	38 mg, 66%
	scanB1[c]	**scanB2**	**scanB3**	**scanB4**	**scanB5**	**scanB6**	**scanB7**
library I	16 mg, 80%	19 mg, 91%	18 mg, 89%	20 mg, 72%	23 mg, 87%	23 mg, 80%	23 mg, 85%
library II	15 mg, 72%	17 mg, 79%	12 mg, 58%	41 mg, 73%	9 mg, 32%	20 mg, 76%	23 mg, 85%
	scanB8	**scanB9**	**scanB10**	**scanB11**	**scanB12**	**scanB13**	**scanB14**
library I	23 mg, 86%	25 mg, 91%	23 mg, 80%	19 mg, 63%	23 mg, 74%	24 mg, 79%	18 mg, 61%
library II	23 mg, 84%	23 mg, 81%	18 mg, 63%	19 mg, 62%	23 mg, 73%	27 mg, 82%	19 mg, 65%

	scanB15	**scanB16**	**scanB17**	**scanB18**	**scanB19**	**scanB20**
library I	21 mg, 69%	24 mg, 77%	30 mg, 84%	20 mg, 67%	22 mg, 71%	15 mg, 46%
library II	14 mg, 46%	23 mg, 72%	26 mg, 73%	18 mg, 58%	18 mg, 56%	13 mg, 38%

[a] Calculated on the basis of the average molecular weight for the mixture (reaction scale, 0.1 mmol). [b] Mixture synthesis but including only ***Xn*** for **scan*Xn***; e.g. **scanA1** includes only **A1** and **B1**−**B20** (reaction scale, 0.05 mmol). [c] Mixture synthesis but including only ***Xn*** for **scan*Xn***; e.g., **scanB1** includes only **B1** and **A1**−**A6** (reaction scale, 0.05 mmol).

prepared using the same protocol by the reaction of an equimolar mixture of 5 of 6 monoamides **8** and an equimolar mixture of the 20 amines R^2NH_2 (**B1**−**B20**) producing 6 sublibraries of 100 compounds, each lacking only one of the **A** subunits. The 20 **B** deletion sublibraries (**deleteB**) were prepared by the reaction of an equimolar mixture of the six monoamides **8** and 19 of the 20 amines R^2NH_2, producing 20 sublibraries of 114 compounds, each lacking only one of the **B** subunits (Table 4).

Biological Assay: Cytotoxic Activity. The 240 individual compounds **9** and **11** constituting the protected precursors to libraries I and II as well as the corresponding mixture libraries and the scanning and deletion synthesis deconvolution libraries were assayed for in

Table 4. Deletion Sublibraries of Libraries I and II, Synthesis Yields[a] (%) and Amounts (mg)

	deleteA1[b]	**deleteA2**	**deleteA3**	**deleteA4**	**deleteA5**	**delete A6**	**Amix**
library I	20 mg, 70%	19 mg, 67%	18 mg, 65%	17 mg, 60%	20 mg, 70%	22 mg, 78%	35 mg, 63%
library II	19 mg, 66%	21 mg, 75%	15 mg, 68%	19 mg, 65%	16 mg, 60%	21 mg, 75%	38 mg, 66%
	deleteB1	**deleteB2**	**deleteB3**	**deleteB4**	**deleteB5**	**deleteB6**	**deleteB7**
library I	22 mg, 77%	17 mg, 60%	22 mg, 78%	21 mg, 75%	26 mg, 90%	24 mg, 86%	22 mg, 79%
library II	21 mg, 74%	26 mg, 93%	18 mg, 65%	18 mg, 63%	20 mg, 70%	19 mg, 67%	23 mg, 80%
	deleteB8	**deleteB9**	**deleteB10**	**deleteB11**	**deleteB12**	**deleteB13**	**deleteB14**
library I	22 mg, 78%	20 mg, 70%	24 mg, 86%	18 mg, 65%	20 mg, 72%	24 mg, 86%	23 mg, 83%
library II	17 mg, 60%	23 mg, 79%	23 mg, 73%	23 mg, 73%	18 mg, 64%	21 mg, 73%	19 mg, 66%

	deleteB15	**deleteB16**	**deleteB17**	**deleteB18**	**deleteB19**	**deleteB20**
library I	21 mg, 76%	19 mg, 69%	18 mg, 65%	15 mg, 55%	17 mg, 63%	14 mg, 49%
library II	11 mg, 39%	23 mg, 80%	19 mg, 68%	17 mg, 60%	18 mg, 13%	15 mg, 54%

[a] Calculated on the basis of the average molecular weight for the mixture (reaction scale, 0.1 mmol). [b] Mixture synthesis but deleting only ***Xn*** for **delete*Xn***; e.g., **deleteA1** includes **A2**−**A6** and **B1**−**B20** (reaction scale, 0.06 mmol).

Table 5. Cytotoxic Activity (L-1210 IC_{50}) of Mixture, Scanning, and Deletion Deconvolution Sublibraries for 9

L-1210[a] IC_{50} (μM) for full mixture **9** = 34

scanning deconvolution[b]						deletion deconvolution[c]					
library	IC_{50}	library	IC_{50}	library	IC_{50}	library	IC_{50}	library	IC_{50}	library	IC_{50}
scanA1	46	scanB1	>100	scanB11	>100	deleteA1	40	deleteB1	32	deleteB11	34
scanA2	47	scanB2	>100	scanB12	61	deleteA2	42	deleteB2	38	deleteB12	32
scanA3	40	scanB3	>100	scanB13	**26**	deleteA3	37	deleteB3	30	deleteB13	**44**
scanA4	41	scanB4	>100	scanB14	>100	deleteA4	**61**	deleteB4	25	deleteB14	34
scanA5	**33**	scanB5	>100	scanB15	>100	deleteA5	40	deleteB5	34	deleteB15	30
scanA6	35	scanB6	>100	scanB16	33	deleteA6	29	deleteB6	32	deleteB16	37
		scanB7	**30**	scanB17	**22**			deleteB7	**61**	deleteB17	35
		scanB8	>100	scanB18	>100			deleteB8	32	deleteB18	30
		scanB9	>100	scanB19	>100			deleteB9	36	deleteB19	34
		scanB10	>100	scanB20	>100			deleteB10	21	deleteB20	32

[a] L-1210 (mouse leukemia) cytotoxic activity. [b] Structure **9**, **scanA1** mixture includes **A1** and **B1**−**B20**. [c] Structure **9**, **deleteA1** mixture includes **A2**−**A6** and **B1**−**B20**.

vitro cytotoxic activity in a L-1210 assay,[18,23] Tables 5−8. Active compounds may be recognized from a gain in activity within the scanning deconvolution sublibraries or a loss in activity in the deletion synthesis deconvolution sublibraries relative to the full mixtures. Several elements of the comparisons are worth noting. First, the IC_{50} values established for the mixtures corresponded beautifully to the average values calculated from the components of the mixtures where this was possible. Thus, in this particular assay, the components of the mixtures are not acting independently. Rather, and perhaps because of their structural similarity and likelihood of acting at a common target with near equivalent potencies, they act cooperatively. With library I, scanning deconvolution revealed the second and fifth most potent agents, which were essentially indistinguishable from the third and fourth most potent agents. It did not, however, adequately detect the most potent agent **9**, **A4B7**. Nonetheless, potent leads relative to the remainder of the library were detected. In the case of library I, deletion synthesis deconvolution uniquely identified **A4B7** as the most potent agent and further identified **A4B13**, the fourth most potent agent. Thus, deletion synthesis deconvolution proved more sensitive to identifying the most potent library member. More significantly, the two protocols together revealed the identity of four of the top five most potent members of the library, and both protocols identified **B7** and **B13** as key residues. This level of lead identification from the mixture libraries would have required the synthesis of three compounds based on the scanning deconvolution results (**A5B7**, **A513**, and **A5B17**, two of which would have been active)

(23) The assay was run 2−3 times each in duplicate, and the results are reported as an average IC_{50}, standard deviation ±10% with each assay. Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918.

Table 6. Cytotoxic Activity (IC_{50}, μM) for Individual Compounds 9

	A1	A2	A3	A4	A5	A6	Avg vs mixture
B1	>100	>100	>100	>100	>100	>100	
B2	>100	>100	>100	>100	88	75	
B3	>100	>100	>100	>100	>100	>100	
B4	>100	57	>100	>100	>100	>100	
B5	>100	>100	>100	>100	>100	>100	
B6	>100	>100	>100	>100	>100	>100	
B7	>100	>100	>100	5	>100	>100	
B8	>100	>100	>100	>100	68	65	
B9	>100	>100	>100	>100	>100	>100	
B10	>100	62	63	>100	>100	45	
B11	>100	58	67	>100	90	35	
B12	37	66	81	72	47	37	57 vs 61
B13	31	32	65	26	19	36	35 vs 26
B14	>100	>100	>100	>100	69	>100	
B15	>100	>100	>100	70	>100	37	
B16	34	62	67	56	54	32	51 vs 33
B17	31	33	30	25	28	31	30 vs 22
B18	>100	77	>100	>100	>100	>100	
B19	>100	>100	>100	28	>100	>100	
B20	86	>100	81	77	>100	34	

and two compounds from the deletion synthesis deconvolution (**A4B7** and **A4B13**, both of which would have been active).

With library II, both scanning and deletion synthesis deconvolution would have identified the most potent library member **11**, **A5B17**. In this instance, scanning deconvolution uniquely identified this agent as the most potent, while the deletion deconvolution identification was not unique. The deconvolution of the leads from the library II scanning (one compound, **A5B17**) and deletion synthesis (three compounds, **A5B6**, **A5B14**, and the same **A5B17**) libraries would have required the individual synthesis of only three compounds.

Although a more complete assessment of the more weakly active leads could be derived from an examination of the weaker scanning or more active deletion synthesis libraries, they would not have improved the performance at identifying the most active compounds in the mixtures. More importantly, the same level of confidence in the coverage provided by either deconvolution protocol alone was not observed and would require a more extensive deconvolution to ensure adequate identification. For example, the preparation of 9−12 compounds based on the scanning deconvolution results from library I would have been required before **A4B7** would have been identified. The screening of **9**−**12** in other assays is in progress, and the results of the comparative performance of the scanning and deletion synthesis deconvolution will be forthcoming.

Experimental Section

***N*-(*tert*-Butyloxy)carbonyl Dipropargylamine (14).** A solution of *N*-(*tert*-butyloxy)carbonyl propargylamine (**13**; 33.36 g, 215 mmol) in 50 mL of DMF was treated portionwise (4×) with 60% NaH (10.4 g) at 0 °C. After being stirred for 30 min at 25 °C, 39 mL of an 80% solution of propargyl bromide in toluene was added. The reaction mixture was stirred for an additional 5 h at 25 °C, and then quenched with the addition of ice−water. The mixture was extracted with Et_2O (3 × 200 mL), and the combined extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was fractionally distilled under high vacuum (57−60 °C/0.05 Torr or 76 °C/0.1 Torr) to give 34.5 g of **14** as a colorless liquid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.16 (s, 4H), 2.21 (t, J = 2.0 Hz, 2H), 1.47 (s, 9H); IR (film) ν_{max} 2976, 2962, 1725 cm^{-1}, FABHRMS (NBA−NaI) *m*/*z* 216.1006 (M + Na^+, $C_{11}H_{15}NO_2$ requires 216.1000).

Dimethyl Isoindoline-5,6-dicarboxylate (7). A solution of **14** (10.4 g, 53.9 mmol) and dimethyl acetylenedicarboxylate (30.7 g, 216 mmol) in 110 mL of absolute EtOH was degassed by bubbling N_2 through the solution for 10 min. To this was added 1.0 g (0.02 equiv) of Wilkinson's catalyst [$(Ph_3P)_3RhCl$] at 25 °C. After being warmed at reflux for 18 h, the reaction mixture was cooled to 25 °C and then concentrated in vacuo. The resulting brown residue was diluted in 200 mL of Et_2O, and the precipitate was removed by filtration over Celite. The filtrate was concentrated and the crude product purified by column chromatography (SiO_2, 20% EtOAc/hexane) to give 4.60 g (26%) of the dimethyl *N*-(*tert*-butyloxycarbonyl) isoindoline-5,6-dicarboxylate as a white solid: mp 121−123 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 and 7.56 (two s, 2H), 4.70 and 4.67 (two s, 4H), 3.88 (s, 6H), 1.50 (s, 9H); IR (film) ν_{max} 2976, 2962, 1731, 1698 cm^{-1}; FABHRMS (NBA−NaI) *m*/*z* 358.1277 (M + Na^+, $C_{17}H_{21}NO_6$ requires 358.1267). A solution of the *N*-Boc-isoindoline (4.6 g, 13.7 mmol) in 50 mL of CH_2Cl_2 was treated with 20 mL of 3.9 M HCl solution in EtOAc at 25 °C, resulting in a pale gray precipitate. After being stirred for 5 h at 25 °C, the resulting precipitate was collected by filtration, rinsed with CH_2Cl_2, and then dried under vacuum to give 3.5 g (95%) of the HCl salt of **7** as a gray powder: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (s, 2H), 4.61 (s, 4H), 3.79 (s, 6H); IR (film) ν_{max} 3310, 2985, 2967, 1698 cm^{-1}; FABHRMS (NBA−NaI) *m*/*z* 236.0923 (M + Na^+, $C_{12}H_{13}NO_4$ requires 236.0923).

General Procedure for the First Functionalization, Preparation of 8 (A1). A solution of **7** (0.81 g, 3.0 mmol), R^1CO_2H [**A1**, 4-((*tert*-butyloxycarbonyl)amino)butanoic acid (0.60 g, 3 mmol)], and *i*-Pr_2NEt (1.23 mL, 2 equiv) in anhydrous DMF (30 mL) was treated with EDCI (0.58 g, 1 equiv). After being stirred for 16 h at 25 °C, the reaction mixture was poured into a separatory funnel containing 50 mL of 10% aqueous HCl. Extraction with EtOAc (2 × 50 mL) followed by washing of the combined organic extracts with 10% aqueous HCl, saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 1.10 g (87%) of the monoamide **8** (**A1**)[24] as a foamy solid.

General Procedure for Preparation of the Diamides 9 and 10 (Library I) (A1B1). A solution of **8** (**A1**; 1.10 g, 2.6 mmol) in 16 mL of 25% MeOH−THF was treated with a solution of LiOH·H_2O (0.43 g, 4 equiv) in 4 mL of H_2O at 25 °C. After being stirred for 2−3 h at 25 °C, the reaction mixture was diluted with H_2O (20 mL) and poured into a separatory funnel. The mixture was washed with EtOAc to remove the unreacted starting material, and then the aqueous phase was

(24) Characterization data are provided in the Supporting Information.

Table 7. Cytotoxic Activity of Mixture, Scanning, and Deletion Deconvolution Sublibraries for 11

L-1210[a] IC_{50} (μM) for full mixture **11** = 32

scanning deconvolution[b]						deletion deconvolution[c]					
library	IC_{50}	library	IC_{50}	library	IC_{50}	library	IC_{50}	library	IC_{50}	library	IC_{50}
scanA1	85	scanB1	90	scanB11	82	deleteA1	28	deleteB1	32	deleteB11	33
scanA2	40	scanB2	54	scanB12	68	deleteA2	32	deleteB2	32	deleteB12	36
scanA3	36	scanB3	>100	scanB13	32	deleteA3	34	deleteB3	35	deleteB13	37
scanA4	40	scanB4	69	scanB14	87	deleteA4	35	deleteB4	32	deleteB14	42
scanA5	28	scanB5	85	scanB15	>100	deleteA5	52	deleteB5	33	deleteB15	33
scanA6	37	scanB6	>100	scanB16	36	deleteA6	41	deleteB6	39	deleteB16	34
		scanB7	>100	scanB17	19			deleteB7	34	deleteB17	40
		scanB8	>100	scanB18	>100			deleteB8	30	deleteB18	34
		scanB9	77	scanB19	>100			deleteB9	33	deleteB19	36
		scanB10	73	scanB20	50			deleteB10	20	deleteB20	34

[a] L-1210 (mouse leukemia) cytotoxic activity. [b] Structure **11**, scanA1 mixture includes A1, B1–B20.
[c] Structure **11**, deleteA1 mixture includes A2–A6, B1–B20.

acidified with 10% aqueous HCl. Extraction into 70 mL of $CHCl_3$/*i*-PrOH (1/1) followed by washing of the organic phase with saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 0.92 g (90%) of the diacid as a pale yellow powder: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.71 (s, 1H), 7.70 (s, 1H), 4.94 (s, 2H), 4.79 (s, 2H), 3.12 (d, J = 6.8 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 1.75 (quintet, J = 7.0 Hz, 2H), 1.41 (s, 9H); IR (film) ν_{max} 3336, 2952, 2867, 1724, 1646 cm^{-1}; FABHRMS (NBA−CsI) m/z 553.0929 (M + Cs$^+$, $C_{19}H_{24}N_2O_7$ requires 553.0951). A solution of the diacid (39 mg, 0.1 mmol) and *i*-Pr_2NEt (65 μL, 4 equiv) in anhydrous DMF (2 mL) was treated with PyBROP (50 mg, 1.1 equiv) at 25 °C. After being stirred for 1 h at 25 °C, Gly-O*t*-Bu (**B1**; 19 mg, 1.1 equiv) was added, and the mixture was stirred for an additional 15 h at 25 °C. The reaction mixture was poured into a separatory funnel containing 20 mL of 10% aqueous HCl. Extraction into EtOAc (2 × 20 mL) followed by washing of the combined organic portions with 10% aqueous HCl (4 × 20 mL) and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 46 mg (92%) of **9** (**A1B1**) as a foamy solid: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.91 (d, J = 9.2 Hz, 1H), 7.50 (d, J = 9.2 Hz, 1H), 4.93 (d, J = 4.3 Hz, 2H), 4.79 (d, J = 4.8 Hz, 2H), 3.97 (s, 2H), 3.12 (t, J = 6.8 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 1.82 (quintet, J = 7.0 Hz, 2H), 1.48 (s, 9H), 1.40 (s, 9H); IR (film) ν_{max} 3325, 2978, 2933, 1711, 1654 cm^{-1}; FABHRMS (NBA−CsI) m/z 638.1499 (M + Cs$^+$, $C_{25}H_{39}N_3O_8$ requires 638.1478). A solution of **9** (**A1B1**; 46 mg, 0.92 mmol) in 0.5 mL of EtOAc was treated with 1.5 mL of a 3.9 M HCl solution in EtOAc. After being stirred for 16 h at 25 °C, 2 mL of Et_2O was added to the resulting white suspension to precipitate the product. The mixture was allowed to stand for 30 min before the solvent was removed by decantation. Evaporation of the residual solvent under a N_2 stream followed by drying under vacuum provided **10**[24] (**A1B1**; 32 mg, 100%) as a fine powder.

General Procedure for Preparation of the Triamides 11 and 12 (Library II) (A1B1). A solution of the diacid prepared from **8** (**A1**; 39 mg, 0.1 mmol), and *i*-Pr_2NEt (65 μL, 4 equiv) in anhydrous DMF (2 mL) was treated with PyBROP (50 mg, 1.1 equiv). After being stirred for 1 h at 25 °C, Gly-O*t*-Bu (**B1**; 19 mg, 1.1 equiv) was added, and the mixture was stirred for an additional 15 h at 25 °C. To this mixture were added *i*-Pr_2NEt (65 μL, 4 equiv), methylamine hydrochloride (13 mg, 2 equiv), and EDCI (38 mg, 2 equiv) at 25 °C. The reaction mixture was stirred for an additional 5 h and then poured into a separatory funnel containing 10 mL of 10% aqueous HCl. Extraction into EtOAc (2 × 20 mL) followed by washing of the combined organic portions with 10% aqueous HCl (4 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 45 mg (86%) of **11 (A1B1)** as a foam: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.87−7.49 (m, 2H), 4.95 (d, J = 4.3 Hz, 2H), 4.79 (d, J = 4.8 Hz, 2H), 3.97 (s, 2H), 3.14 (t, J = 6.8 Hz, 2H), 2.86 (s, 3H), 2.49 (t, J = 7.2 Hz, 2H), 1.84 (quintet, J = 7.0 Hz, 2H), 1.48 (s, 9H), 1.40 (s, 9H); IR (film) ν_{max} 3325, 2978, 2933, 1711, 1654 cm^{-1}; FABHRMS (NBA−CsI) m/z 651.1445 (M + Cs$^+$, $C_{26}H_{42}N_4O_7$ requires 638.1468). A solution of **11** (**A1B1**; 40 mg, 0.08 mmol) in 0.3 mL of EtOAc was treated with 1 mL of a 3.9 M HCl solution in EtOAc at 25 °C, resulting in a white precipitate. After being stirred for 16 h at 25 °C, the solvent and excess HCl were removed by evaporation under a N_2 stream to give **12**[24] (**A1B1**; 28 mg, 100%) as a powder.

General Procedure for the A-Scan (Library I). A solution of the diacid derived from **8** (**A1**; 39 mg, 0.1 mmol) in DMF was treated with PyBROP (57 mg, 1.1 equiv) and *i*-Pr_2NEt (65 μL, 4 equiv) at 25 °C. After being stirred for 30 min, the reaction mixture was treated with 1 mL of a 0.1 M solution of the 20 amines **B1**−**B20** in DMF. After being stirred for 16 h at 25 °C, the reaction mixture was poured into a separatory funnel containing 20 mL of 10% aqueous HCl. Extraction into EtOAc (2 × 20 mL) followed by washing of the combined organic portions with 10% aqueous HCl (4 × 20 mL) and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 43 mg of **9**, **scanA1** mixture as a foamy solid. A solution of this bisprotected **scanA1** mixture (43 mg, 0.62 mmol, average MW 700) in 0.5 mL of EtOAc was treated with 1.5 mL of a 3.9 M HCl solution in EtOAc. After being stirred for 16 h at 25 °C, 2 mL of Et_2O was added into the resulting white suspension to precipitate the product. The mixture was allowed to stand for 30 min before the solvent was removed by decantation. Evaporation of the residual solvent under a N_2 stream followed by drying under vacuum provided the **10**, **scanA1** library (32 mg, 100%) as a fine powder.

Table 8. Cytotoxic Activity (IC_{50}, μM) for Individual Compounds 11

	A1	A2	A3	A4	A5	A6	Avg vs mixture
B1	>100	56	76	46	43	>100	
B2	25	45	32	79	67	67	53 vs 54
B3	>100	>100	>100	>100	45	49	
B4	70	45	89	75	85	67	72 vs 69
B5	>100	>100	>100	>100	98	27	
B6	27	34	32	52	44	39	38 vs > 100
B7	>100	57	85	50	>100	69	
B8	>100	>100	>100	>100	95	53	
B9	>100	74	74	85	>100	48	
B10	>100	49	63	>100	53	77	
B11	>100	39	40	>100	88	65	
B12	48	39	47	>100	39	50	
B13	32	45	33	32	32	33	34 vs 32
B14	>100	37	91	20	71	72	
B15	81	64	>100	>100	36	58	
B16	32	32	30	51	46	33	37 vs 36
B17	33	31	23	23	5	21	23 vs 19
B18	>100	93	76	>100	>100	93	
B19	>100	>100	67	>100	>100	>100	
B20	67	41	70	72	71	34	59 vs 50

General Procedure for the B-Scan (Library I). A solution which contains 0.01 mmol of each of the six diacids (total 0.06 mmol) derived from **8** (**A1**−**A6**) in DMF (1.2 mL) was treated with *i*-Pr_2NEt (32 μL, 4 equiv) and PyBROP (30 mg, 1.1 equiv) at 25 °C. After 30 min, it was treated with amine **B1** (10 mg, 1.1 equiv) and then stirred for 16 h at 25 °C. The reaction mixture was poured into a separatory funnel containing 20 mL of 10% aqueous HCl. Extraction into EtOAc (2 × 20 mL) followed by washing of the combined organic portions with 10% aqueous HCl (4 × 20 mL) and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 22 mg of the **9**, **scanB1** mixture of library I as a foamy solid. A solution of this bisprotected **scanB1** mixture (22 mg, average MW 560) in 0.5 mL of EtOAc was treated with 1 mL of a 3.9 M HCl solution in EtOAc. After being stirred for 16 h at 25 °C, 2 mL of Et_2O was added into the resulting white suspension to precipitate the product. The mixture was allowed to stand for 30 min followed by the removal of the solvent by decantation. Evaporation of the residual solvent under a N_2 stream followed by drying under vacuum provided the desired product (16 mg) as a fine powder.

General Procedure for the Deletion Deconvolution Library, DeleteA1 (Library I). A stock solution (0.05 M) of **B1**−**B20** was prepared in DMF, and a 0.1 M solution in DMF of each diacid derived from monoamide **8** was prepared. A 1 dram vial was charged with 0.1 mL of each of the five diacid− stock solutions (**A2**−**A6**), *i*-Pr_2NEt (30 μL, 4 equiv), and PyBROP (28 mg, 1.1 equiv). After 30 min, it was treated with 1 mL (0.05 M) of the **B1**−**B20** mixture solution. After being stirred for 16 h at 25 °C, the reaction mixture was poured into a separatory funnel containing 20 mL of 10% aqueous HCl. Extraction into EtOAc (2 × 20 mL) followed by washing of the combined organic portions with 10% aqueous HCl (4 × 20 mL) and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 25 mg (70%, average MW 716) of the bisprotected **deleteA1** mixture of library I as a foamy solid. This mixture was treated with 1.5 mL of a 3.9 M HCl solution in EtOAc. After being stirred for 16 h at 25 °C, 2 mL of Et_2O was added into the resulting white suspension to precipitate the product. The mixture was allowed to stand for 30 min before the solvent was removed by decantation. Evaporation of the residual solvent by a N_2 stream followed by drying under vacuum provided the desired product (20 mg) as a fine powder.

General Procedure for the Deletion Deconvolution Library, DeleteB1 (Library I). A stock solution (0.05 M) of each amine from **B1** to **B20** was prepared by dilution in DMF, and a 0.1 M solution in DMF of each diacid derived from monoamide **8** was also prepared. A 1 dram vial was charged with 80 μL of the 0.1 M solution of each of the six diacids **A1**−**A6**, *i*-Pr_2NEt (30 μL, 4 equiv), and PyBROP (28 mg, 1.1 equiv). After 30 min, 50 μL (2.5 μmol) of each of the 19 amines **B2**−**B20** was added to the mixture. After being stirred for 16 h at 25 °C, the reaction mixture was poured into a separatory funnel containing 20 mL of 10% aqueous HCl. Extraction into EtOAc (2 × 20 mL) followed by washing of the combined organic portions with 10% aqueous HCl (4 × 20 mL) and saturated aqueous NaCl, drying over Na_2SO_4, and evaporation provided 25 mg (70%, average MW 716) of the **9**, **deleteA1** library I as a foam. This product was treated with 1.5 mL of 3.9 M HCl solution in EtOAc. After being stirred for 16 h at 25 °C, 2 mL of Et_2O was added into the resulting white suspension to precipitate the product. The mixture was allowed to stand for 30 min before the solvent was removed by decantation. Evaporation of the residual solvent by a N_2 stream followed by drying under vacuum provided **10**, **deleteA1** (20 mg) as a fine powder.

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Supporting Information Available: ^1H NMR spectra of **7** and characterization data and ^1H NMR spectra for **8** (**A1**−**A6**) and **9**−**12** [**A1B1**, **A2B2**, **A3B3**, **A4B4**, **A5B5**, **A6B6**, **A1B7**, **A2B8**, **A3B9**, **A4B10**, **A5B11**, **A6B12**, **A1B13**, **A2B14**, **A3B15**, **A4B16**, **A5B17** (**9**, **A3B17**), **A6B18** (**9**, **A2B18**), **A1B19** (**9**, **A5B19**), **A2B20**]. This material is available free of charge via the Internet at http://pubs.acs.org.

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