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Parallel Synthesis of An Oligomeric Imidazole-4,5-dicarboxamide Library

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Abstract

A library of oligomeric compounds was synthesized based on the imidazole-4,5-dicarboxylic acid scaffold along with amino acid esters and chiral diamines derived from amino acids. The final compounds incorporate non-polar amino acids (Leu, Phe, Trp), polar amino acids (Ser, Asp, Arg), and neutral amino acids (Gly, Ala), and were designed to be useful in screening for inhibitors of protein-protein interactions. Many of the protected and deprotected oligomers show evidence of conformational isomers persistent at room temperature in aqueous solution. A total of 317 final oligomers, out of 441 targeted compounds, were obtained in high analytical purity and of sufficient quantity in order to submit them for high-throughput screening as part of the NIH Roadmap.

Introduction

The design and preparation of compound libraries for application in high-throughput screening is a well-known approach to hit identification in drug discovery research.¹,² The library design criteria are generally target dependent, with the choice of scaffold or building blocks determined by considering known bioactive compounds or hypothesis or both.³⁻⁵

Protein-protein interactions (PPI) are significant events in signaling within and between cells, and as such are potential targets for intervention by small molecules in order to modulate or treat diseases related to these signals.⁶⁻¹⁰ The design of inhibitors for PPIs has, in the past, been considered intractable due to the size and flatness of the associating surfaces. Yet, most buried interfaces rely on “hot spots” that require relatively few residues in order to obtain the majority of the thermodynamic driving force for the PPI.¹¹⁻¹² Thus, targeting a PPI “hot spot” is a practical approach to reduce the overall size and molecular weight of a potential PPI inhibitor.

Good progress toward the design and discovery of inhibitors of PPIs has been reported in recent years by generating a suitable mimic of one of the two protein surfaces.¹³⁻¹⁸ In general these inhibitors mimic secondary structures, such as β-turns,³⁻¹⁰ β-strands,¹⁹ or an α-helix,⁹,¹⁰,²⁰ in order to accurately display important residues or “hot spots” of the PPI in a proper orientation.
This approach can yield compounds with drug-like or nearly drug-like features;\textsuperscript{21,22} therefore, the use of inhibitors of PPIs as therapeutic agents is anticipated.\textsuperscript{23}

We have previously employed the imidazole-4,5-dicarboxylic acid scaffold in the design of CD81 proteomimetics that were based on the comparable distance between carbon atoms of the imidazole-4,5-dicarboxamide (I45DC) substituents with the spacing of side chains in a critical \( \alpha \)-helix within the PPI.\textsuperscript{24,25} These first-generation oligomeric I45DCs were symmetric and utilized two L-amino acids as well as a \( N,N' \)-dialkylalkanamine in their design and synthesis.\textsuperscript{26} Selected oligomers were effective as inhibitors of the CD81-hepatitis C glycoprotein E2 interaction, thereby supporting our design hypothesis.\textsuperscript{24}

The second-generation oligomeric I45DCs in this project likewise anticipate the distance between substituents on each I45DC to match the distance between side chains found along the length of an \( \alpha \)-helix or adjacent along one side of a \( \beta \)-strand.\textsuperscript{25} The total of four amino acid side chains per oligomer in this design is expected to be a significant improvement and doubles the number of pharmacophoric side chains as compared with the first-generation oligomers. Although only a few amino acids are often involved in a PPI hot spot,\textsuperscript{11,12} we nonetheless reason the oligomers in this project have greatly improved odds of yielding inhibitors of PPIs when employed in high-throughput screening.

The number and type of amino acid building blocks that we employed for these oligomeric I45DCs was based on a compromise between those amino acids expected to be important in PPI hot spots and the number of compounds we could reasonably synthesize, purify, and characterize in parallel. Also guiding our selection was the knowledge that aromatic, polar, and ionoic amino acid side chains were of equal or greater significance to hydrophobic interactions in many PPIs, such as those in antibody-antigen or enzyme-protein inhibitor interactions.\textsuperscript{27,28} The oligomeric design uses combinations of two ethylenediamines along with two L-amino acids. Two of the three ethylenediamines used are chiral with Ala and Leu side chains, and were synthesized from their L-amino acids. The seven L-amino acids we chose included two aromatic residues (Tp and Phe), two hydrophobic residues (Leu and Ala), two charged residues (Asp and Arg) and one polar residue (Ser). The monomeric I45DCs were also differentially protected in order to allow selective deprotection of the amino and carboxylic acid termini before coupling in parallel to create a maximum of 441 unique oligomeric I45DCs. A total of 317 of these pure oligomeric I45DCs have been characterized and were obtained in sufficient quantity for submission to the Molecular Library Small Molecule Repository (MLSMR) for use in high-throughput screening as part of the NIH Roadmap.

**Results and Discussion**

Ethylenediamine was mono-protected to yield 12\{/\}, as shown in Scheme 1, by following a literature procedure.\textsuperscript{29} This compound was further protected with a known procedure\textsuperscript{30} to give 11\{/\} and then selectively deprotected to give 6\{/\} as the free amine, thereby providing the two differentially monoprotected ethylenediamines, 6\{/\} and 12\{/\} needed for oligomer synthesis. The other chiral diamines used in this project were synthesized from L-amino acids bearing either \( N \)-Boc or \( N \)-Cbz protecting groups as shown in Table 1. Boc-L-amino acids, 1\{2-3\}, were the starting materials for the chiral diamines 6\{2-3\} as shown in Scheme 2. The amino acids 1\{2-3\} were converted to their respective methyl esters, 2\{2-3\}, by slight modification of a known procedure,\textsuperscript{31} converted to amides 3\{2-3\} with an \( \text{NH}_2\text{OH} \) solution,\textsuperscript{32} and subsequently reduced to the chiral diamines 4\{2-3\} with \( \text{BH}_3\text{Si(CH}_3)_2 \) in THF.\textsuperscript{33} These intermediates were not purified, but first protected with Cbz-Cl to give 5\{2-3\} following purification.\textsuperscript{30} Deprotection of the Boc group in 5\{2-3\} with \( \text{CF}_3\text{CO}_2\text{H} \) in \( \text{CH}_2\text{Cl}_2 \) yielded 6\{2-3\}. Likewise, Cbz-L-amino acids, 7\{2-3\}, were starting materials for the chiral diamines 12\{2-3\} as shown in Scheme 3. The synthesis again proceeded through methyl esters 8\{2-3\},
amides 9{2-3}, reduced intermediates 10{2-3}, and the differentially-protected chiral diamines 11{2-3}. Hydrogenation then produced the final chiral diamines 12{2-3} needed for oligomer synthesis.

The oligomeric design uses the two chiral, mono-protected diamine chemsets 6 and 12 that were synthesized from amino acid amides, along with the two L-amino acid chemsets 14 and 18. The two halves of the oligomers come from chemsets 16 and 20 that are independently synthesized with orthogonal protecting groups in order to allow selective deprotection of these monomeric building blocks (Scheme 4).

Synthesis of the monomeric building blocks begins with the pyrazine diacid chloride, 13, which is prepared from imidazole-4,5-dicarboxylic acid as previously reported. The amino acid ester-substituted pyrazine chemsets 15 and 19 were likewise prepared by following methods previously reported, generally giving good to excellent yields of product (Table 3).

The Cbz and benzyl ester, respectively, for isolated chemset intermediates 16 and 20 were deprotected with 1 atm. H₂ and 5% Pd/C in MeOH. The reactions were stirred at room temperature from 4.5-29 h for chemset 16 and 11-30 h for chemset 20, at which point the reaction was complete as determined by TLC analysis. The reaction mixture was filtered through celite and the solvent removed under vacuum to afford chemsets 17 and 21, respectively, from chemsets 16 and 20, minus members 17{2,7}, 17{3,7}, and 21{3,5} for which starting materials were unavailable. The yields for chemset 17 ranged from 84-99% and averaged 94%, whereas the yields for chemset 21 ranged from 65-100% and averaged 89%. All of the products were suitable for use without further purification.

The protected oligomer chemset 22, excluding those examples where monomeric intermediates 17{2,7}, 17{3,7}, and 21{3,5} were unavailable, were prepared in CH₂Cl₂ at room temperature over 20 h by coupling chemsets 17 and 21 with the water-soluble carbodiimide, EDC·HCl, and dimethylaminopyridine. The reaction mixtures were concentrated under vacuum and the product purified by column chromatography with a gradient ranging from EtOAc/hexanes to EtOAc/MeOH as the eluents, affording chemset 22.

Out of the 361 possible combinations for which monomeric chemset intermediates 17 and 21 were available, a total of 323 products were purified and yields determined, which were generally good (Table 4). The compounds of the protected oligomer chemset 22 were characterized by combined LC-MS and ¹H NMR spectroscopy (see supporting information). In a few cases a library member was identified by LC-MS, but was not completely characterized or carried forward due to insufficient amounts of material or contamination with impurities.
Quantitative deprotection of products of chemset 22 was done with 25% CF₃CO₂H in CH₂Cl₂ at room temperature over 2-18 h, using LC-MS analysis to determine when the reaction was complete.

Following removal of the solvents, the trifluoroacetate salt was exchanged for chloride by dissolving the deprotected oligomer chemset 23 in 0.90 mL of 10% aqueous MeOH with gentle heat as needed for solubilization and adding 100 μL of 1 M HCl. The solutions were immediately frozen in liquid N₂ before lyophilizing to dryness.

Interestingly, we observed two significant signals in the LC-MS analysis of 45 of the protected oligomer library compounds. In all cases both signals have an identical MS spectra that is consistent with the proposed structure. We hypothesize that this is evidence of two conformers that are stable under the conditions of the analysis. Indeed, conformational isomers have been reported for comparable oligomers containing two N-methylimidazole-4,5-dicarboxylic acid rings. A majority of the hypothesized conformational isomers that are observed have hydrophobic amino acid side chains located in both substituent amides of chemset, such as combinations of leucine or phenylalanine (see supporting information). However, we do not observe such conformational isomers in the protected oligomers containing tryptophan in chemset 21. It is possible that this is due to a preference for one conformer with the larger tryptophan substituent as compared with leucine or phenylalanine, or that these conformational isomers simply do not resolve in the LC-MS for those examples.

Our hypothesis of conformational isomers in protected oligomer chemset 22 is further supported by the fact that many compounds show broad and poorly defined ¹H NMR spectra, whereas others have comparably well defined signals. Figure 1 compares part of the LC-MS and ¹H NMR spectra for 22{2,1,2,2} with 22{2,1,3,2}. Oligomer 22{2,1,2,2} showed only a single peak in the LC-MS analysis while 22{2,1,3,2} shows two peaks hypothesized to be the conformational isomers. The ¹H NMR spectra in the aliphatic region for 22{2,1,2,2} is considerably more defined than the comparable region for 22{2,1,3,2}. The broadness of the ¹H NMR spectra for 22{2,1,3,2} is therefore hypothesized to result from the presence of conformational isomers having overlap in their ¹H NMR chemical shifts or the relative dynamics of the compound on the NMR time scale or both. It is noted, however, that a broad ¹H NMR spectrum did not necessarily mean that the oligomer showed two peaks in the LC-MS analysis, since there are examples of this behavior also. Among the potential explanations for this differing behavior include overlap in the retention time of persistent conformers in the LC conditions or that the change in solvent from aqueous CH₃CN (LC-MS) versus CDCl₃ (¹H NMR) alters the conformational behavior between the analyses.

One concern was that the two signals in the LC-MS analysis represent diasteromeric oligomers resulting from epimerization of a stereocenter under the coupling conditions. We did not expect the coupling conditions to cause in significant epimerization, and note that we observe two signals only in select cases for any given set of hydrophobic amino acids. This variability in behavior is strong evidence against epimerization as the explanation for our results.

As with the protected oligomers, two significant LC-MS signals were observed in 54 of final compounds of chemset 23. Again, the general trend appears to be related to the presence of two hydrophobic amino acids in chemset 21, although there are exceptions in the deprotected oligomers just as there were for the protected oligomers. Importantly, some protected oligomers with two conformers did not show two conformers when deprotected (e.g., 23{2,2,3,2} and 23{2,2,3,3}), while others did not show two conformers until the oligomer was deprotected (e.g., 23{1,2,3,4} and 23{1,3,3,4}). This is yet further evidence against the formation of diasteromers in the coupling reactions, as diasteromeric compounds of chemset 22 would yield diastereomeric compounds of chemset 23.
As additional evidence in support of the conformational hypothesis, we performed variable-temperature LC-MS on four deprotected oligomers (23{1,2,3,3}, 23{1,3,3,4}, 23{2,5,3,3}, and 23{6,3,3}) that show evidence of two peaks in the LC-MS analysis (see supporting information). The analysis was run from 25 °C to 65 °C, and showed a steady decline in the relative amounts of the lesser conformer as compared to the major conformer. While we did not observe coalescence of the two conformers at 65 °C, it is possible that hydrophobic interactions could continually stabilize the conformation(s) in an aqueous environment. Nonetheless, the differing relative amounts of the two peaks support our hypothesis that these are conformational isomers rather than diastereomers.

It is commonplace to use VT-NMR spectroscopy in order to examine conformational isomerism in solution. We know from our previous experience with monomeric derivatives that self-association occurs at or above 1 mM in CDCl₃ and above 10 mM in DMSO-d₆.³⁹ Thus, VT-NMR was performed from 30 °C to 70 °C in DMSO-d₆ for 22{2,1,2,2} and 22{2,1,3,2} at 3 mM (see supporting information), resulting in similar ¹H NMR spectra observed for both compounds across the range of temperatures. We are unable to assign specific NHs to observed signals, but can nonetheless identify the two imidazole NHs as those signals around 13 ppm, the two amide NHs involved in intramolecular hydrogen bonding as the signals around 11 ppm, the three remaining amide NHs between 8-9 ppm, and the carbamate NH near 7 ppm, as shown in Figure 2 for 22{2,1,3,2}. From inspection it is then clear that there are conformational isomers present in the solution. For example, there are four signals for the two intramolecular hydrogen bonded hydrogens (b). An increase in temperature yields only modest changes on the chemical shifts of the NHs over this entire region. This has previously been reported for oligomeric I45DCs that form conformation isomers observable in DMSO-d₆ even at 100 °C.³⁷ Cooling the NMR sample of 22{2,1,3,2} to 30 °C from 70 °C yields the same spectrum as first recorded at 30 °C, indicating the stability of the compounds in a polar solvent at high temperatures.

We suggest that the conformational isomers may result from differing intramolecular hydrogen bonding interactions, particularly around the I45DC from chemset 21, and perhaps supported by the presence of hydrophobic interactions that protect the hydrogen bond from fast exchange during the LC-MS analysis and on the ¹H NMR time scale. We have previously observed two intramolecular hydrogen bonded conformations in dissymmetrically-disubstituted I45DCs and provided evidence that the favored hydrogen bond donor arose from increasing substitution adjacent the amide nitrogen.³⁸ Moreover, we have shown in model I45DCs that the intramolecular hydrogen bond is relatively strong and worth at least 14±1 kcal/mol, as well as observed in an aqueous environment.³⁹ The two conformations for 22{2,1,3,2} shown in Figure 2 are therefore real possibilities for explaining the observed behavior in both the LC-MS analysis and ¹H NMR spectra. It is reasonable that 22{2,1,2,2} would also adopt analogous intramolecular hydrogen bonded conformations, and the lack of a second hydrophobic side chain may increase the dynamics of the conformational exchange.

The expected intramolecular hydrogen bonding conformations were a valuable design criteria as they fix the distance between amide substituents in order to approximate the separation of nearby side chains in α-helices and β-strands.²⁴,²⁵ The two different conformers would not affect that separation, but do alter the possible hydrogen bonding interactions with the imidazole ring, as hydrogen bond donor and acceptor groups on the ring have their relative positions switched. One conformation about a single I45DC of the oligomer, relative to the other I45DC conformer, may also be valuable as the oligomers optimize binding interactions at protein interfaces. In this way the conformational isomers could be of added value in the use of these oligomers in screening for inhibitors of protein-protein interactions.
Conclusion

A total of 317 final products in chemset 23 that were both analytically pure and of sufficient quantity were submitted as HCl salts to the Molecular Library Small Molecule Repository (MLSMR) for high-throughput screening by the as part of the NIH Roadmap. A total of 37 protected intermediates from chemset 22 where likewise submitted to the MLSMR. Chemical and biological data for the oligomers will be accessible free of charge at PubChem (http://pubchem.ncbi.nlm.nih.gov) as the compounds are incorporated into the database and subsequently screened by the Molecular Libraries Probes Production Centers Network (MLPCN). It is reasonably hypothesized that these oligomers will be valuable in the discovery of inhibitors against protein-protein interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Jennifer Parker for assistance with the submission of these compounds to the MLSMR and The National Institutes of General Medical Sciences for funding this research (P41 GM79589-02).

References

Scheme 1.
Scheme 2.
Scheme 3.
Scheme 4.
Figure 1.
Comparison of the LC-MS trace (boxed) and aliphatic region in the $^1$H NMR spectra of 22
d{2,1,2,2} (left) with 22{2,1,3,2} (right).
Figure 2.
A partial $^1$H NMR spectrum of 22{2,1,3,2} recorded at 3 mM in DMSO-$d_6$ at 30 °C. The imidazole NHs, amide NHs (intramolecular hydrogen bonded as well as free amide) and carbamate NH are labeled and indicate the presence of conformational isomers. Also shown in the 2-CH of the imidazole (e) at 7.8 ppm. Two intramolecularly hydrogen bonded conformations of 22{2,1,3,2} that differ about the I45DC acquired from chemset 21 are also shown (A and B). The arrows underneath each imidazole indicate the direction of the intramolecular hydrogen bond from donor to acceptor for that ring. The top conformer has the same direction for each hydrogen bond and the bottom conformer has opposing directions.
Table 1

\(N\)-Boc and Cbz protected amino acids, 1\{2-3\} and 7\{2-3\}, respectively, used in library synthesis.

\[
\begin{align*}
\text{Cmpd} & \quad \text{R}_1 & \quad \text{Cmpd} & \quad \text{R}_2 \\
1\{2\} & \quad \text{CH}_3 & \quad 7\{2\} & \quad \text{CH}_3 \\
1\{3\} & \quad \text{CH}_2\text{CH}(\text{CH}_3)_2 & \quad 7\{3\} & \quad \text{CH}_2\text{CH}(\text{CH}_3)_2
\end{align*}
\]

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**Table 2**

Protected amino acids, **14**{1-7} and **18**{1-7}, respectively, used in library synthesis.

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<th>Cmpd</th>
<th>R₃</th>
<th>Cmpd</th>
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<td>CH₃</td>
<td>18{1}</td>
</tr>
<tr>
<td>Cmpd</td>
<td>R₃</td>
<td>Cmpd</td>
</tr>
<tr>
<td>------</td>
<td>----</td>
<td>------</td>
</tr>
<tr>
<td>14{2}</td>
<td></td>
<td>18{2}</td>
</tr>
<tr>
<td>Cmpd</td>
<td>R₃</td>
<td>Cmpd</td>
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<tr>
<td>------</td>
<td>----</td>
<td>------</td>
</tr>
<tr>
<td>14[^1]</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>18[^1]</td>
</tr>
<tr>
<td>Cmpd</td>
<td>( R_3 )</td>
<td>Cmpd</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>14{4}</td>
<td></td>
<td>18{4}</td>
</tr>
<tr>
<td>14{5}</td>
<td>( \text{O(H) or O(CH}_3\text{)} )</td>
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</tr>
<tr>
<td>Cmpd</td>
<td>R₃</td>
<td>Cmpd</td>
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<tr>
<td>------</td>
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</tr>
<tr>
<td>14[6]</td>
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<tr>
<td>18[6]</td>
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Table 3

Pyrazines substituted with amino acid esters.

<table>
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<tr>
<th>compound</th>
<th>amino acid ester</th>
<th>yield (%)</th>
<th>compound</th>
<th>amino acid ester</th>
<th>yield (%)</th>
</tr>
</thead>
</table>

ND: Yield was not determined and the member was used without purification
Yields of protected oligomer chemset 22.

Table 4

| 17 | 21 | (1,1) | (2,1) | (3,1) | (1,2) | (2,2) | (3,2) | (1,3) | (2,3) | (3,3) | (1,4) | (2,4) | (3,4) | (1,5) | (2,5) | (3,5) | (1,6) | (2,6) | (3,6) | (1,7) | (2,7) | (3,7) |
|----|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 51 | 53 | 71    | 58    | 48    | 58    | 63    | 61    | 76    | 61    | 52    | 38    | 83    | 70    | 65    | 47    | 67    | 84    |
| 50 | 56 | 51    | 44    | 50    | 51    | 47    | 69    | 53    | 47    | 48    | 41    | 51    | 46    | 39    | 43    | 66    | 45    |
| 36 | 24 | 41    | 44    | 25    | 42    | 51    | 29    | 33    | 37    | 36    | 53    | 51    | 35    | 40    | 76    |
| 84 | 56 | 53    | 42    | 62    | 69    | 60    | 66    | 73    | 58    | 53    | 44    | 60    | 58    | 50    | 56    | 68    | 68    |
| 70 | 46 | 57    | 64    | 49    | 44    | 68    | 78    | 58    | 51    | 34    | 36    | 39    | 63    | 37    | 46    | 56    | 56    |
| 58 | 49 | 60    | 59    | 67    | 66    | 68    | 65    | 51    | 63    | 60    | 47    | 54    | 63    | 73    | 69    | 79    |
| 53 | 51 | 67    | 71    | 96    | 47    | 70    | 68    | 74    | 67    | 60    | 36    | 53    | 52    | 65    | 64    | 66    | 81    |
| 60 | 53 | 43    | 59    | 46    | 55    | 66    | 71    | 49    | 49    | 39    | 49    | 38    | 61    | 42    | 41    | 68    | 62    |
| 64 | 47 | 62    | 58    | 61    | 52    | 63    | 62    | 43    | 45    | 52    | 44    | 55    | 65    | 48    | 56    | 76    |
| 63 | 40 | 53    | 57    | 51    | 63    | 64    | 60    | 51    | 76    | 52    | 46    | 51    | 71    | 56    | 45    | 50    | 74    |
| 87 | 59 | 75    | 73    | 85    | 83    | 83    | 78    | 74    | 77    | 61    | 63    | 68    | 63    | 64    | 90    | 30    |
| 86 | 79 | 60    | 77    | 65    | 57    | 90    | 60    | 57    | 78    | 64    | 51    | 77    | 62    | 63    | 77    |
| 82 | 66 | 78    | 74    | 60    | 63    | 72    | 82    | 68    | 73    | 49    | 38    | 61    | 70    | 47    | 56    | 81    | 70    |
| 62 | 66 | 69    | 67    | 75    | 80    | 74    | 75    | 82    | 66    | 63    | 60    | 44    | 57    | 73    | 50    | 56    | 66    |
| 63 | 50 | 42    | 66    | 51    | 40    | 47    | 47    | 35    | 55    | 42    | 35    | 35    | 58    | 41    | 84    | 54    |
| 62 | 33 | 69    | 57    | 70    | 67    | 68    | 74    | 70    | 56    | 61    | 37    | 48    | 68    | 67    | 62    | 67    | 70    |
| 38 | 49 | 52    | 58    | 52    | 44    | 49    | 66    | 58    | 27    | 47    | 43    | 41    | 37    | 46    | 58    | 52    | 56    |
| 49 | 48 | 39    | 48    | 54    | 34    | 53    | 42    | 31    | 42    | 53    | 29    | 61    | 58    | 36    |
| 69 | 49 | 60    | 37    | 65    | 32    | 52    | 49    | 50    |       |       |       |       |       |       |       |       |       |       |       |       |
Areas shaded in gray indicate regions of chemset space for which intermediates were not obtained.