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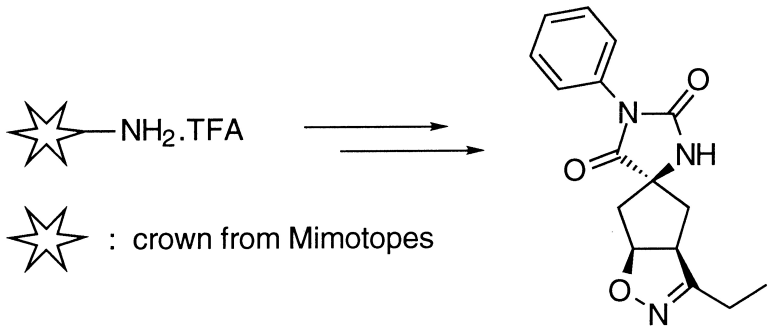

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Preparation of a 990-Member Chemical Compound Library of Hydantoin- and Isoxazoline-Containing Heterocycles Using Multipin Technology

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The development of a useful chemistry for the construction of polyfunctional heterocycles—first through solution and solid phase (resins) and then library production via SynPhase crowns—is reported. Bead-based synthetic work was done on Merrifield resin where treatment with benzylamine in the presence of DBU followed by reaction with 4-chloromethylbenzoyl chloride afforded amide-linked resin **9**. Finally, TFA·NH_2-polystyrene macro crowns were derivatized with 4-(hydroxymethyl)benzoic acid to afford pin **14** which was coupled with Boc-protected amino acid **2** in the presence of DIC to deliver pin **15**. Deprotection and reaction with phenyl isocyanate afforded urea functionalized pin **17** which underwent 1,3-dipolar cycloaddition reaction to give pin **19**. Finally, compound **20** was obtained with moderate diastereoselectivity (**20**:**21**::8:1) by the reaction of pin **19** with a catalytic amount of Et_3N.

Both hydantoin and isoxazoline heterocycles are structural elements which frequently impart biological activity. Indeed, a large number of hydantoins have been prepared for various biological applications,[1] both medicinal[2] and agrochemical.[3] The isoxazoline heterocycle has been used extensively to modulate various other biologically active motifs.[4]

The cornerstone of combinatorial chemistry is the design of analogue libraries using chemistry which allows for the incorporation of two or three elements of diversification. Solid-phase (SP) combinatorial chemistry has been successfully applied in the preparation of many chemical compound libraries, and over the past few years, a large variety of SP reactions have been developed.[5] Two of the important advantages of SP reactions are the use of excess of reagents to drive reactions to completion and the removal of resin-free side products through extensive washing. A less explored SP chemistry method employs Mimotopes Technologies SynPhase crowns.[6] Although chemical loading is generally lower than with beads, this system is more easily manipulated and washing is faster since the polymer chains are grafted to dimensionally stable plastic crowns. The crowns themselves are attached to plastic stems which can be conveniently handled in many formats (e.g., 8×12).

The development of synthetic strategies applicable to combinatorial chemistry using the Multipin Technology of Mimotopes Technologies is one of our interests. Herein, we report the successful transfer of a synthetic strategy for the construction of polyfunctional heterocycles from solution and solid phase (resins) to SynPhase crowns. With reliable solid-phase chemistry on pins, library preparation would set the stage for a thorough exploration of the biological properties of the resulting hydantoin/isoxazoline (isoxazolinoimidazolidinedione)-containing heterocycles.

In preliminary studies, we explored synthetic routes in solution and solid phase for the construction of novel isoxazolinoimidazolidinedione heterocycles about a central carbon core unit (Figure 1).[7] The generalized isoxazolinoimidazolidinedione scaffold **1** allows for the introduction of diversity on both the hydantoin and the isoxazoline moieties as well as the incorporation of various connecting groups (e.g., R_{II}—derived from unsaturated α-amino acids) between the two heterocycles.

Bead-based synthetic work was done on Merrifield resin where O-alkylation of the carboxylate salt leads to an ester linkage between the solid phase and the substrate. This linker was easily cleaved in the final hydantoin-forming intramolecular cyclization step. However, the functional groups available on SynPhase crowns are more limited than on resin; an aminomethyl linker was available but the desired hydroxymethyl linker was not. Therefore, we needed to search for a suitable spacer to bridge between the aminomethyl group and our requisite unsaturated α-amino acid. Obvious choices were 4-chloromethyl- and/or 4-hydroxymethyl benzoic acid which are both readily available. Prior to moving onto SynPhase crowns, we validated the overall reaction sequence both in solution with *N*-benzyl 4-chloromethylbenzamide (**1**) as a mimic linker (Scheme 1) and on SP where the Merrifield resin was treated with benzylamine to yield a modified aminomethyl resin. (Scheme 2). In both studies, we isolated the corresponding isoxazolinoimidazolidinedione in 30% and 25% overall yields (solution and Merrifield resin reactions, respectively).

In solution, benzylamine was coupled with 4-chloromethyl benzoyl chloride to give amide **1** which was in turn coupled

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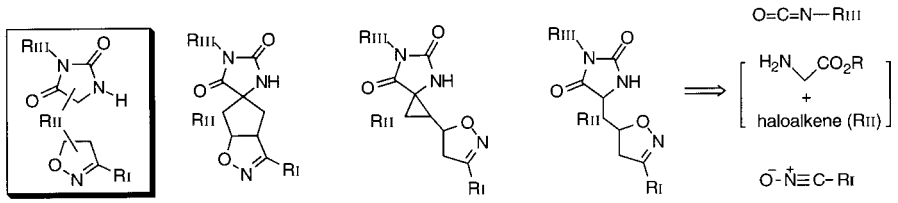
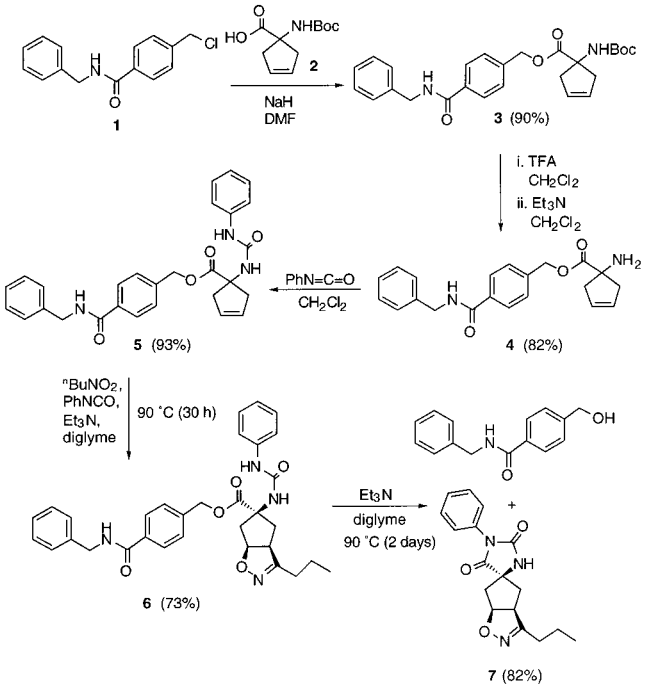


I

Figure 1. Novel isoxazoline-containing hydantoins.

Scheme 1. Solution-Phase Route to Isoxazolinoimidazolidinedione **7** Using *N*-Benzyl 4-Hydroxymethylbenzamide as a Leaving Group



with Boc-protected acid **2** to give ester **3**. Deprotection of the Boc group to afford amine **4** followed by treatment with phenyl isocyanate delivered urea derivative **5**. A hydrogen-bond-directed intermolecular 1,3-dipolar cycloaddition reaction[8] (i.e., urea NH···O—N≡CR)[7b] of the alkene in **5** with a Mukaiyama-generated nitrile oxide[9] was carried out to give intermediate **6**. This transformation (**5** → **6**) proceeds with excellent facial diastereoselectivity as evidenced by the base-mediated cyclo-elimination of only **7** (Scheme 1).

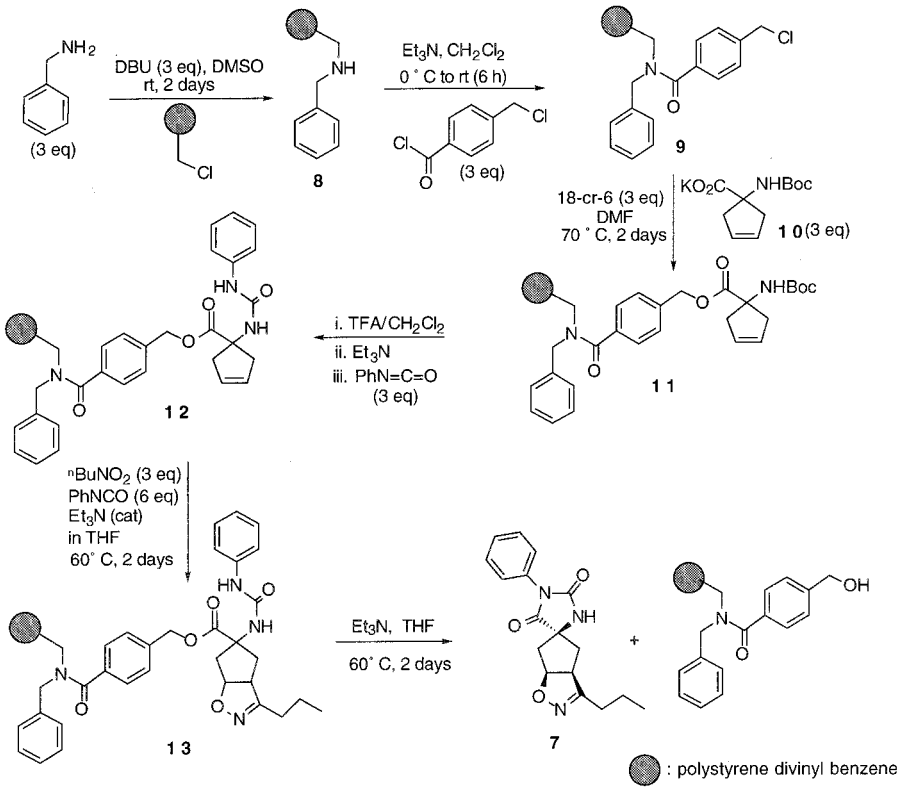
With this result in hand, we next investigated bead-based SP reactions using readily available Merrifield resin as a starting material. Treatment of Merrifield resin with benzylamine in the presence of DBU resulted in resin **8**. Subsequent reaction of **8** with 4-chloromethylbenzoyl chloride afforded amide-linked resin **9**. Potassium salt **10** was treated with resin **9** to give **11** which underwent Boc deprotection followed by reaction with phenylisocyanate to give resin **12**. 1,3-Dipolar cycloaddition reaction of resin **12** with a Mukaiyama-generated nitrile oxide was carried out to give resin **13**. Finally, isoxazolinoimidazolidinedione **7** was obtained (78% de) by the reaction of resin **13** with a catalytic amount of Et_3N (Scheme 2).

Having thus developed *N*-benzyl 4-hydroxymethylbenzamide as a suitable linker, we turned our attention to SynPhase crowns. The loading of a single crown is significantly lower than the loading of typical Merrifield resin. Therefore, we had to reevaluate some of our reaction conditions in order to avoid both large excesses of reagents and overly dilute reaction solutions. First, the starting pin, TFA·NH_2-polystyrene macro crown (12 μmol/crown, from Mimotopes),[10] was treated with Et_3N (5%) to deliver the free amine which was reacted with 4-(hydroxymethyl)benzoic acid, HOBt, and DIC to afford pin **14**. This pin was coupled with Boc-protected amino acid **2** in the presence of DIC to deliver pin **15** with an ester functional group. Deprotection and neutralization delivered free amine **16** which was reacted with phenyl isocyanate to afford the urea functionalized pin **17**. 1,3-Dipolar cycloaddition reaction of pin **17** with a Mukaiyama-generated nitrile oxide was carried out to give pin **19**. However, this [3+2] cycloaddition reaction required some modification since release of hydantoin **18** during **17** → **19** resulted at higher Et_3N concentrations. We were able to prevent this early and unwanted intramolecular cyclization by reducing the concentration of Et_3N (0.0075 M) and keeping the concentration of the nitroalkane and isocyanate equimolar at 0.5 M. Finally, compound **20** was obtained with moderate diastereoselectivity (**20**:**21**::8:1) by the reaction of pin **19** with catalytic amount of Et_3N (Scheme 3) (HPLC; Figure 2). The diastereoselectivity observed for compound **20** arises from the same interactions which explained the diastereoselective formation of **6** and **7**.

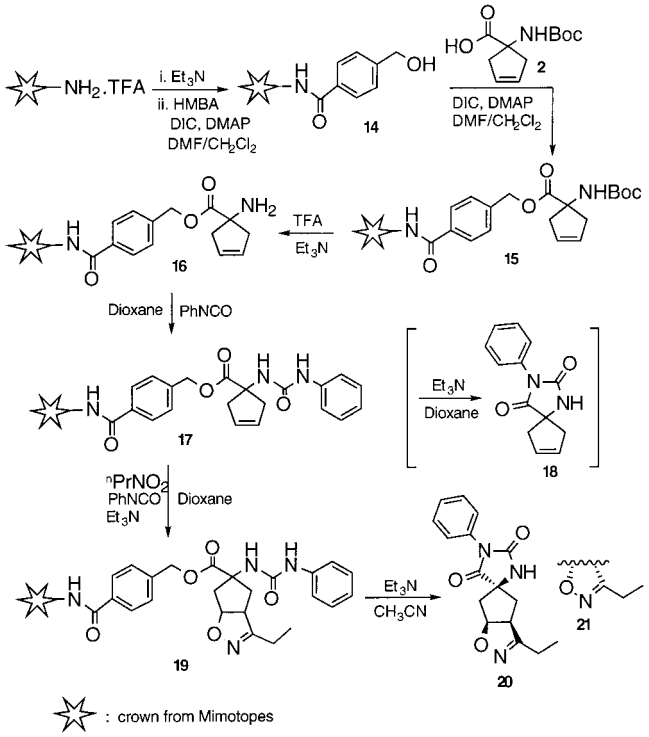
Reductive alkylation of the amino group of unsaturated amino ester **16** provides an additional point of diversity in the isoxazolinoimidazolidinedione target. Preliminary studies, in which the reductive alkylation was done prior to the urea formation and the [3+2] cycloaddition reaction, showed no product (**25**) due to early release of either the intermediate hydantoin (**24**) or the product (**25**) by cyclization at elevated temperature during the [3+2] cycloaddition reaction (Scheme 4).

Fortunately, switching the reaction sequence order to (i) isoxazoline formation on the Boc-protected amino ester, (ii) deprotection by TFA, (iii) reductive alkylation, (iv) urea formation, and (v) cleavage yielded the fully substituted isoxazolinoimidazolidinedione **25** more effectively. Subsequent reagent evaluations revealed that the full sequence including reductive alkylation is applicable only to a limited selection of isocyanates—generally those substituted by electron-donating groups. Therefore, we eliminated the reductive alkylation step from our library production, but we kept the reversed sequence of reactions (Scheme 5). Following an exhaustive reagent evaluation, we prepared a 990-compound library of isoxazolinoimidazolidinediones using building blocks consisting of 5 amino acids, 9 nitroalkanes, and 22 isocyanates (Figure 3).

Scheme 2. SP Route to Isoxazolinoimidazolidinedione **7** Using *N*-Benzyl 4-Hydroxymethylbenzamide as a Linker



Scheme 3. SynPhase Crown Route to Isoxazolinoimidazolidinedione **20** Using *N*-Benzyl 4-Hydroxymethylbenzamide as a Linker



Through our exploration of these routes to isoxazolinoimidazolidinedione heterocycles using Multipin Technology, a 5-fold diversity was achieved about the core building block R_{II}. Thus, the generalized isoxazolinoimidazolidinedione scaffold **I** allows for the introduction of diversity on both the hydantoin and the isoxazoline heterocycles as well as the incorporation of various connecting groups between these two heterocycles (Figure 4).

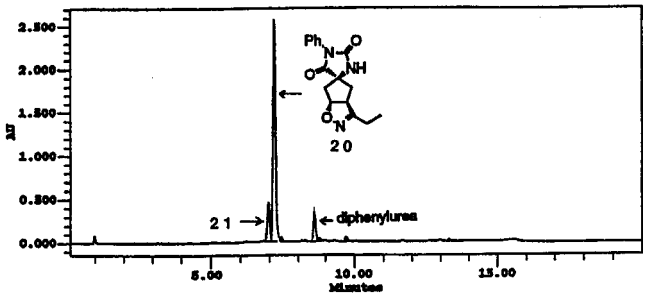
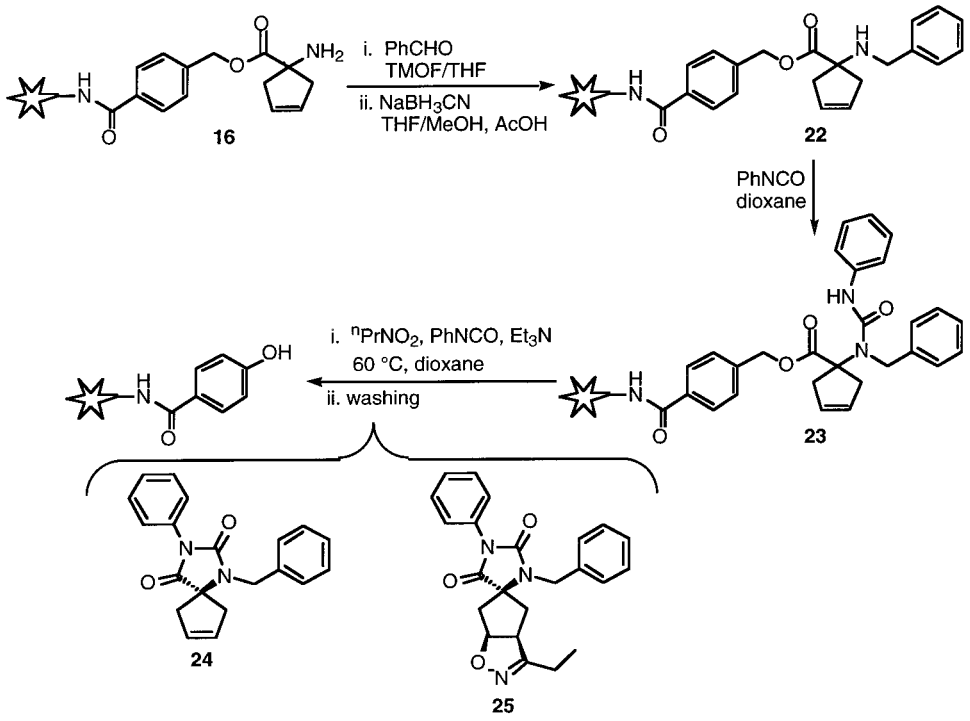


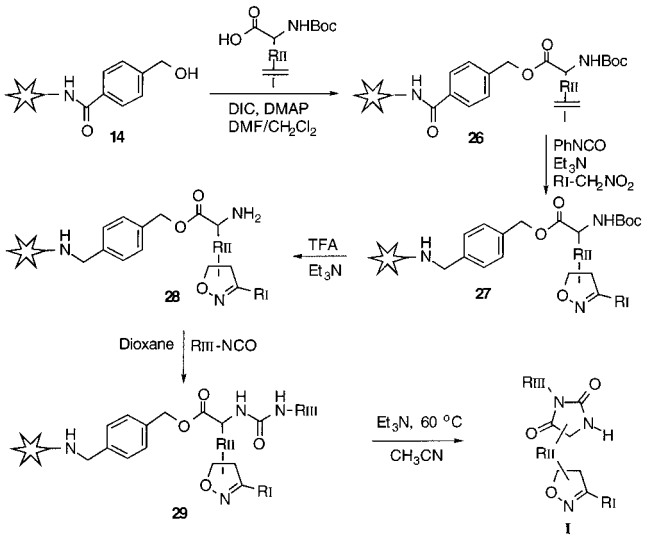
Figure 2. HPLC of the hydantoin **20** from pin **19**.

The desired isoxazolinoimidazolidinediones were obtained in 40−60% yield (2−3 mg, from 22 samples) from TFA·NH_2 functionalized pins (12 or 9.4 μmol/crown). While the anticipated diastereoselectivity (vide supra) was observed with some isoxazolinoimidazolidinediones **I** (i.e., library members obtained from R_{II}, amino acid **E**) due to optimal geometric positioning of the H-bond directing NH relative to the alkene, other precursors did not accommodate this interaction as effectively which led to much lower distereoselectivities. Library quality (i.e., presence and purity of target compounds) was checked through standardized MS (ES^-) and HPLC analyses of 10 random samples per 96-deep-well plate. An average of 80% of the samples showed an HPLC purity of greater than 70%, and the expected mass was detected in more than 90% of these samples.[11] All of these samples are being screened for biological activity by HTS (high throughput screening).

Scheme 4. Early Release of the Hydantoin Intermediate (**24**) or Final Product (**25**) by Cyclization at Elevated Temperature



Scheme 5. Optimized SynPhase Crown Route to Isoxazolinoimidazolidinedione Library **I**



Experimental Section

Reagents and General Methods. Boc-protected amino acids were synthesized from the protected glycine derivatives. Pins (9.4 μmol/crown) were supplied by Mimotopes. Except for phenylnitromethane,[12] eight nitro compounds and 22 isocyanates were purchased from Aldrich (Milwaukee, WI). Most chemical reactions were performed in polypropylene deep-well microtiter plates from Beckman (Fullerton, CA). The [3+2] cycloaddition and cleavage reactions were performed in a stainless steel container which can accommodate the pin holder block/microtiter plate assembly at elevated temperature. The pins, loaded in a holder block, were washed in a chemically resistant polypropylene bath and dried in a vacuum desiccator.

Incorporation of a Spacer to the TFA·NH_2-Crown. TFA·NH_2-crowns (9.4 μmmol/crown; 990) were treated with 1200 mL of 5% Et_3N in DMF and shaken for 1 h at room temperature. The pins were rinsed with DMF and DCM (1000 mL × 3 for each solvent) and dried in a vacuum desiccator. 4-(Hydroxymethyl)benzoic acid (HMBA) (21.28 g, 140 mmol) and HOBt (25.72 g, 168 mmol) were dissolved in 500 mL of cosolvent (DMF/DCM = 1/1), followed by the addition of DIC (17.6 g, 140 mmol). After addition of cosolvent (DMF/DCM = 1/1) to the above solution, the volume was adjusted to 700 mL (DMF/DCM = 1/1) and the neutralized pins were added to this solution. The reaction mixture was shaken overnight at room temperature at which time the pins were washed with DMF and DCM (1000 mL × 3 for each solvent) and dried. These pins were treated with 1000 mL of 10% ethanolamine in DMF for 1 h, washed with DMF and DCM (1000 mL × 3 for each solvent), and dried to give pin **14**.

Coupling Boc-Protected Amino Acids to the Pin 14 To Give the Pin 26. Each set of 198 pins (for the reaction with nine nitro compounds and 22 isocyanate derivatives) was coupled with each of the five Boc-protected amino acids. The typical procedure is as follows. A DMF/DCM (1/4, v/v; 100 mL) solution of one of the Boc-protected acids (20 mmol; for example, acid **E**) and DIC (2.52 g, 20 mmol) was added to a flask containing a set of 198 pins. A DMF/DCM (1/4, v/v; 100 mL) solution of DMAP (0.24 g, 2 mmol) was added, and the reaction mixture was shaken overnight at room temperature. The pins were washed with DMF and DCM (200 mL × 3 for each solvent) and dried to give pin **26**.

[3+2] Cycloaddition Reaction. To each row in a deep-well microtiter plate were added a 1.5 M solution (0.4 mL for each well) of each nitroalkane, a 1.5 M solution (0.4 mL for each well) of phenyl isocyanate, and a 0.0225 M solution (0.4 mL for each well) of Et_3N in dioxane. Pins (**26**) secured in a pin holder block were put into each deep-well microtiter

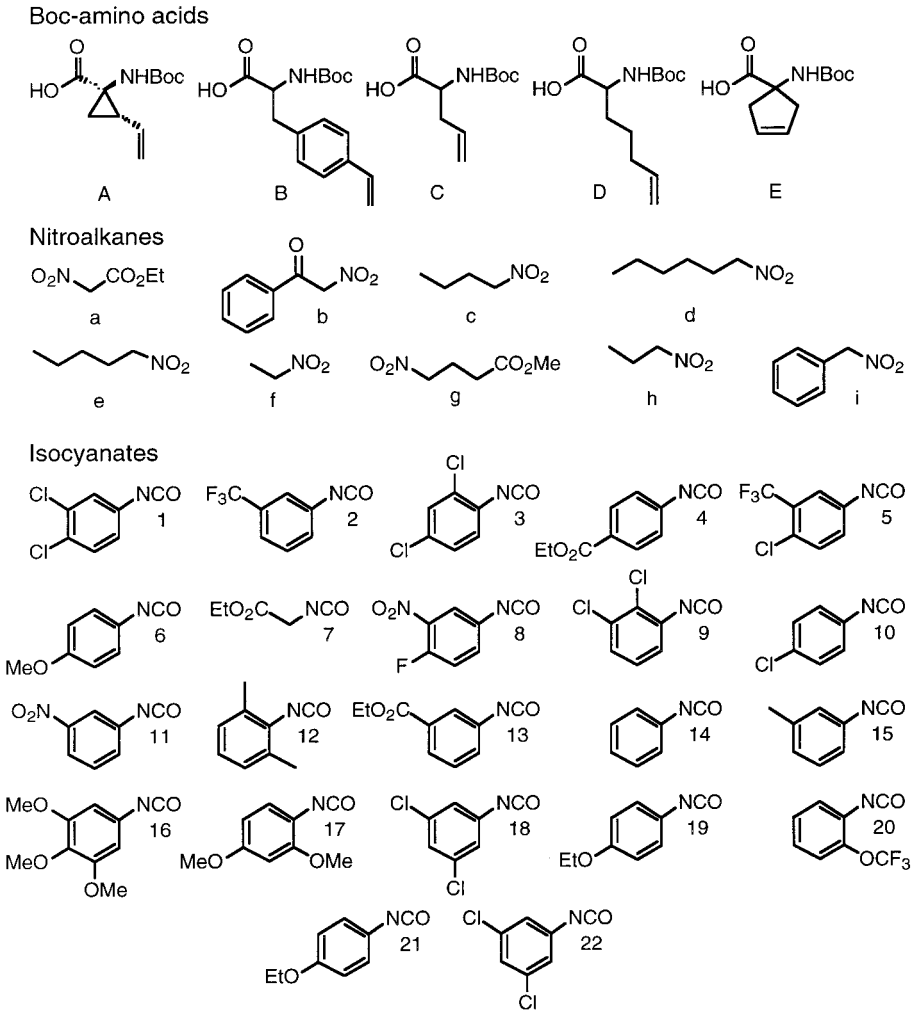


Figure 3. Building blocks used for isoxazolinoimidazolidinedione library production.

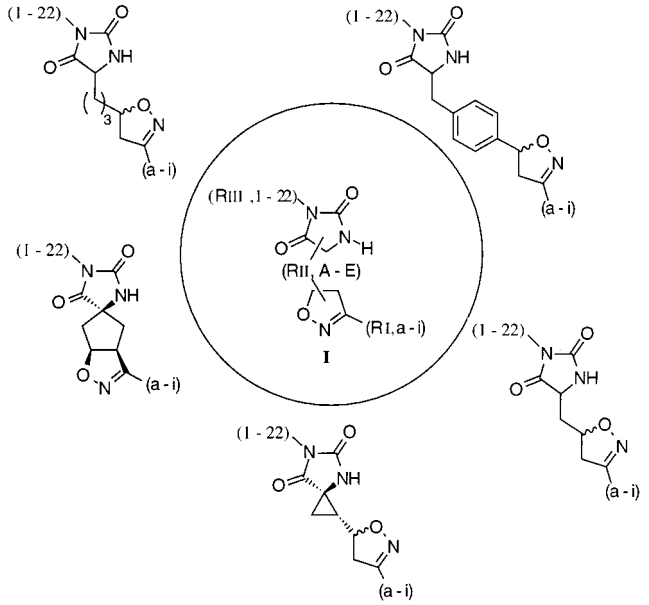


Figure 4. Diversity in isoxazolinoimidazolidinedione **I** from R_I (from nitroalkanes **a**−**i**), R_{II} (from amino acids **A**−**E**), and R_{III} (from isocyanates **1**−**22**).

plate containing the above solution, and the assembled set (pin holder block plus deep-well plate) was placed into a stainless steel container with the lid tightly sealed where the reaction mixture was incubated overnight at 60 °C. The pins, secured in a pin holder block, were washed with dioxane and DMF in a chemically resistant polypropylene bath and dried. The [3+2] cycloaddition reaction was repeated twice more (as above but for 6 h). Finally, pins in a pin holder block were washed with dioxane, DMSO (60 °C), DMF, THF, and DCM (250 mL for each solvent) in a chemically resistant polypropylene bath and dried to give pin **27**.

Boc Deprotection. The Boc protecting group was removed by treatment of the pins (**27**) with 250 mL of TFA/DCM (1:1) for 1 h at room temperature. Subsequent treatment with 250 mL of 5% Et_3N in DMF for 30 min at room temperature in a chemically resistant polypropylene bath followed by washing with DMF and DCM (250 mL × 3 for each solvent) gave pin **28**.

Urea Formation. To the each column in a deep-well microtiter plate was added a 0.5 M solution (1.2 mL for each well) of each isocyanate derivative in dioxane. Pins (**28**), in a pin holder block, were immersed into the deep-well plate, and the block/plate assembly was placed into a desiccator to minimize the decomposition of each isocyanate (moisture-mediated urea formation). After 12 h at room temperature, the pins were washed with dioxane, DMSO (60 °C), DMF, THF, and DCM (250 mL × 3 for each solvent) in a polypropylene bath and dried to give pin **29**.

Cleavage from the Crown Support. The final hydantoin library was released from the support by placing the pins (**29**), in a pin holder block, into deep-well plates containing a 0.05 M solution (1.2 mL for each well) of Et_3N in acetonitrile. The block/plate assembly was placed into a stainless steel container with the lid tightly sealed, and the reaction mixture was incubated overnight at 60 °C. After removing the pins, the solvent in each well was removed by centrifugal vacuum (Speed Vac) to afford the final hydantoin library in each well.

Analytical Evaluation of the Library. Evaluation of the isoxazolinoimidazolidinedione library was accomplished by LC-MS as well as Open-Access MS. Specifications are as follows. LC-MS (Micromass Quattro II with a Waters 2690 and a Waters PDA 966): *Ionization mode* electronspray (+/−); *Mass range* 100−1500 Da, 30 V cone voltage; *Column* YMC ODS-AQ 125 mm × 2 mm i.d. 120A S-5 μm; *Gradient* A (water with 0.01% TFA), B (acetonitrile with 0.01% TFA) (0 min 0% B, 15 min 100% B, 20 min 100% B, 21 min 0% B, 27 min 0% B). LC: *Column* Rainin, Microsorb-MV 86-200-F3, C18 (3ym), no. F6 10497; *Gradient* A (water with 0.1% TFA), B (water/acetonitrile 1:9 with 0.1% TFA), (0 min 0% B, 1 min 0% B, 12 min 100% B, 14 min 100% B, 15 min 0% B, 20 min 0% B). FIMS (Micromass Platform II with a HP 1100 binary pump and a HP 1100 VWD and a Pal CTC): *Ionization mode* electronspray (+/−); *Mass range* 100−1500 Da, 30 V cone voltage; 50 μL loop injection with water/acetonitrile (20/80).

Spectroscopic Data. FTIR, ^{1}H NMR, ^{13}C NMR, mps, and EAs for the representative compounds[13] (prepared from solution or resins, for example, **A-c-14** (**A** from 1-((*tert*-butoxycarbonyl)amino)-2-vinylcyclopropane-1-carboxylic acid, **c** from nitrobutane, and **14** from phenyl isocyanate in Figure 3), **A-i-10**, **A-c-10**, **A-h-10**, **C-i-10**, **C-i-14**, **C-f-14**, **E-c-10**, **E-c-14**, **E-h-14**) from the library are available in the previously published papers.[7]

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Supporting Information Available. ^{1}H NMR/^{13}C NMR and IR spectra for compounds **A-c-14**, **A-i-10**, **A-c-10**, **A-h-10**, **C-i-10**, **C-i-14**, **C-f-14**, **E-c-10**, **E-c-14**, and **E-h-14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) (a) Chiron Technololgies Pty. Ltd., 11 Duerdin Street, Clayton Victoria 3168, Australia. (b) For further details on SynPhase crowns and the Multipin Technology, see: www.chirontechnologies.com.au.

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(11) (a) Our results (80% of samples with 70% purity) are below the *Journal of Combinatorial Chemistry*'s standard of 80% of samples with 80% purity. (b) Some of the samples, especially the library derived from the component **a** in building block R_I, did not show an exact mass in the spectroscopic conditions due to fragmentaton.

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(13) Our results (spectral data for 10 library members) are below the *Journal of Combinatorial Chemistry*'s standards of ^{1}H NMR, ^{13}C NMR, and IR spectra for 20 library members.

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