A Library of 3-Aryl-4,5-dihydroisoxazole-5-carboxamides

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Parallel solution phase methods for the preparation of a 72-membered 3-aryl-4,5-dihydroisoxazole-5-carboxamide library is reported. The reaction order (nitrile oxide 1,3-dipolar cycloaddition followed by amide formation, or vice versa) was investigated both experimentally and computationally to determine which route would result in the highest yields, minimize purification efforts, and give higher 1,3-dipolar cycloaddition regioselectivity. Automated preparative HPLC was used to purify the final products to ≥90% purity on a 10+ mg scale.

Introduction

Many heterocyclic compounds are biologically active and have proven applications as pharmaceuticals.[1] Combinatorial chemistry tools have enabled the rapid synthesis of heterocyclic small molecule libraries, which can then be screened using high-throughput methods to generate lead compounds.[2] One such heterocycle of interest in library synthesis is the isoxazole ring and its variants, which are substructures in many pharmaceuticals currently on the market, specifically anti-inflammatory drugs and antibacterials.[3] These five-membered nitrogen/oxygen-containing heterocycles are formed in relatively high yields through a [3+2]-cycloaddition reaction.[4,5] Because of the biological relevance of these molecules, we set out to prepare a small molecule library containing an isoxazoline core (Figure 1). The work presented here, which is in collaboration with the National Institute for General Medical Sciences (NIGMS), targets the creation of pilot-scale discovery libraries. The targeted NIGMS collection will be used for high-throughput biological screening and represents a new approach to collaborative academic/nonprofit research.

Several approaches to this target scaffold were considered on the basis of both previous literature results as well as work conducted in our laboratory. As outlined in Figure 2, this effort eventually led us to use methyl acrylate as the dipolarophile starting reagent en route to a 72-member isoxazoline library. 1,3-Dipolar cycloaddition of nitrile oxides, in turn prepared in situ from oximes, introduced the first diversity point.[5] Subsequent ester saponification and coupling of the resulting 3-aryl-4,5-dihydroisoxazole-5-carboxylic acids with several different amines delivered a library of 3-aryl-4,5-dihydroisoxazole-5-carboxamides.

Results and Discussion

Several dipolarophile options (**A**–**D**; Figure 3) were considered vis-à-vis the targeted 3,5-disubstituted heterocycle. A review of pertinent literature indicated that cycloadditions of propiolate derivatives (option **A**) generally result in good yields of the isoxazole, but the regioselectivity of this dipolarophile is quite low.[6,7] As for propynamides (option **B**) there is limited literature, but reports indicate that product yields are generally not as high as with option **A** and, more importantly, the regioselectivity is generally poor, often delivering ~1.5:1 mixtures of 3,5- and 3,4-disubstituted heterocycles.[8] Surprisingly, in our hands, methyl propynamide gave 9:1 regioselectivity (3,5- vs. 3,4-disubstitution, respectively).

When considering options **C** and **D**, our investigation began with an analysis of the regioselectivity and isoxazoline yields from acrylamide, methacrylamide, but-2-enamide (e.g., crotyl amide), and acrylate dipolarophiles. According to the literature, an electron-deficient acrylate dipolarophile (option **D**) generally gives better regioselectivity than the corresponding acrylamide (option **C**) in 1,3-dipolar cycloaddition reactions.[9,10] To systematically test that premise, the various carboxamide alkenes required for this investigation were synthesized by coupling acryloyl, or methacryloyl, or crotyl chloride with the appropriate amine. These carboxamide alkene dipolarophiles were then employed in cycloaddition reactions with the nitrile oxide derived from 4-nitrobenzalde oxime. As delineated in Scheme 1, acrylamides (e.g., **4** → **7**) and methacrylamides (e.g., **5** → **8**) undergo 1,3-dipolar cycloaddition with high regioselectivity but only modest yields (~65%). Regioselectivity drops considerably with

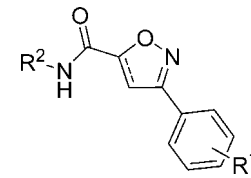

Figure 1. Isoxazoline and/or isoxazole library options.

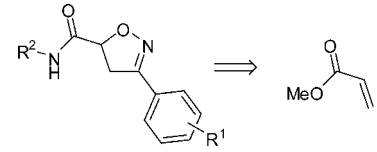

Figure 2. Isoxazoline-based library with two points of diversity.

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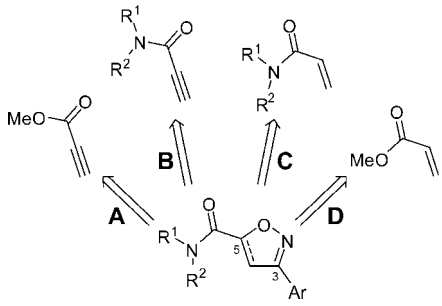
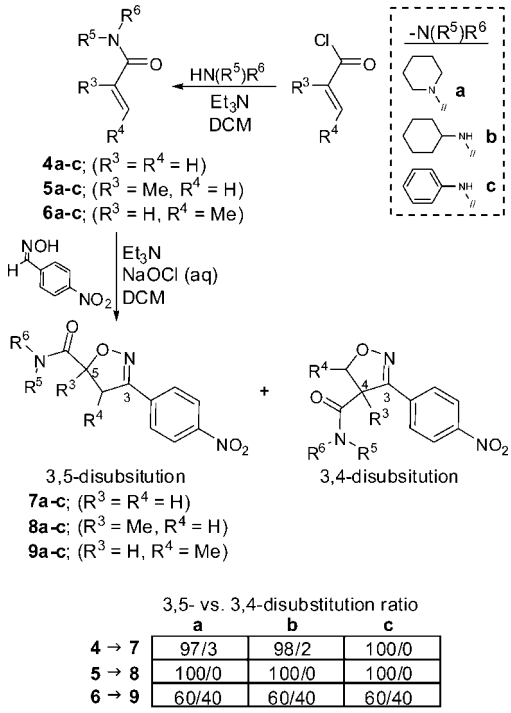


Figure 3. Possible routes to the target scaffold.

Scheme 1. 1,3-Dipolar Regioselectivity (3,5- vs. 3,4-disubstitution) of Amides **4–6**



3,5- vs. 3,4-disubstitution ratio

	a	b	c
4 → 7	97/3	98/2	100/0
5 → 8	100/0	100/0	100/0
6 → 9	60/40	60/40	60/40

crotyl amides, giving a nearly 1:1 mixture of regioisomeric products (e.g., **6** → **9**). This lack of regioselectivity is a consequence of opposing steric interactions between the 1,2-disubstituted dipolarophile and the nitrile oxide as well as changes in the HOMO/LUMO coefficients of crotyl versus acryl dipolarophiles. Literature reports for crotyl systems generally suggest variable yields and the regioselectivity is always lower, possibly with the 3,4-disubstituted regioisomer becoming the major product.[10–12] Conversely, the same cycloaddition reaction with methyl acrylate resulted in 3,5-disubstituted regioselectivity of ≥95% with yields generally of 90%.

To further direct our **A**–**D** option analysis, we decided to calculate the HOMO and LUMO energies of the dipolarophiles depicted in Figure 3 in the hope that this information would facilitate a systematic understanding of the 1,3-dipolar cycloaddition regioselectivity. To accomplish this, we calculated the HOMO and LUMO energies for both the dipole (fixed as 4-$NO_2C_6H_4C{\equiv}N^+{-}O^-$) as well as the various dipolarophiles depicted in Figure 3. Our calculated results (see Figure 4) predict that all four routes will be controlled by the dipole-LUMO–dipolarophile-HOMO interaction because these energy gaps are smaller than the competing dipole-HOMO–dipolarophile-LUMO energy gap[14a] (each dipole–dipolarophile interaction, HOMO–LUMO and LUMO–HOMO, affords a particular regioisomer). From our calculations, the dipole-LUMO–dipolarophile-HOMO energy gap is considerably smaller than the dipole-HOMO–dipolarophile-LUMO energy gaps for options **C** and **D** ($\Delta\Delta E$ > 0.07 eV). Option **A**'s dipole-LUMO–dipolarophile-HOMO versus dipole-HOMO–dipolarophile-LUMO difference in energy is <0.07 eV and the regioselectivity is quite low. Option **B** was calculated to have a dipole-LUMO–dipolarophile-HOMO versus dipole-HOMO–dipolarophile-LUMO difference in energy of >0.07 eV (in our hands, methyl propynamide gives 9:1 regioselectivity (3,5- vs. 3,4-disubstitution, respectively)). Option **B** is slightly different from options **A/C/D** in that the HOMO of methyl propynamide (not shown) has an orbital interaction between the alkyne and the electron-withdrawing carbonyl group.[14b]

In addition to these regioselectivity issues, parallel library synthesis practicalities dictate that it is most effective to employ the more routine diversification reaction (here, amide formation) later in the reaction scheme and the less routine diversification reaction (here, the 1,3-dipolar cycloaddition) earlier. With this and these various regioselectivities in mind, we elected to employ methyl acrylate as the dipolarophile for our library.

The synthesis of the targeted 3-aryl-4,5-dihydroisoxazole-5-carboxamide library was accomplished as shown in Scheme 2. The oximes in chemset **10a–h**, prepared from the corresponding aldehydes,[13] were condensed with methyl acrylate using the Huisgen method.[5] Upon work-up, 3,5-dihydroisoxazole-5-carboxylates **11** were obtained in good yields (≥90%). These methyl esters were then saponified under standard conditions to give carboxylic acids **12**(90–95%).[15] EDC-mediated coupling of these acids with the various amines in reagent chemset **13A–I** introduced the second diversity point and gave the novel targeted library **14aA–hI** (≥65% overall yield). Attempts to effect a direct ester to amide conversion (**11** → **14**) proved to be quite variable.

By this three-step method, methyl acrylate plus eight different aryl oximes (**10a–h**, Figure 5) and nine different 1°-amines (**13A–I**, Figure 6) delivered a 72-member library of 3-aryl-4,5- dihydroisoxazole-5-carboxamides (**14**). Automated HPLC purification delivered each library member in regioisomerically and chemically pure form on a ≥10 mg scale. All 72 3-aryl-4,5-dihydroisoxazole-5-carboxamides were characterized by analytical LCMS to confirm structure and purity.

Finally, the observed regioselectivities of the 1,3-dipolar cycloaddition reactions reported here are the consequence of both steric and electronic interactions. Sterically, an antiarrangement of the acrylate carbomethoxy and nitrile oxide aryl moieties is preferred and this favors the 3,5-disubstituted isoxazoline (see Scheme 1). The role of electronic factors in determining regioselectivity is best understood in terms of frontier molecular orbital (FMO) theory. Both the HOMO and LUMO of monosubstituted alkenes with an electron-withdrawing group have larger molecular orbital coefficients at the unsubstituted carbon.[16] Regioselectivity in the ensuing cycload-

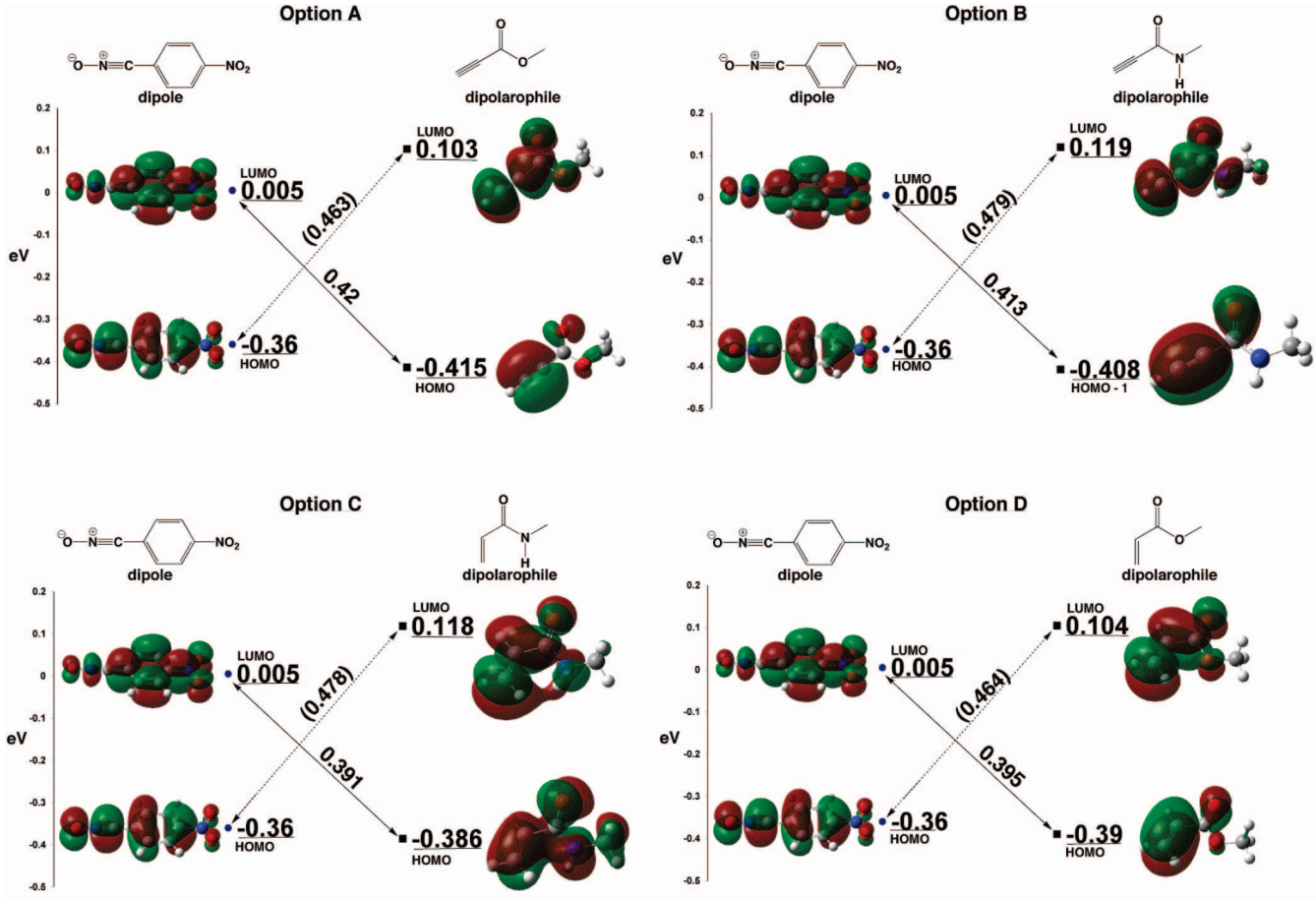
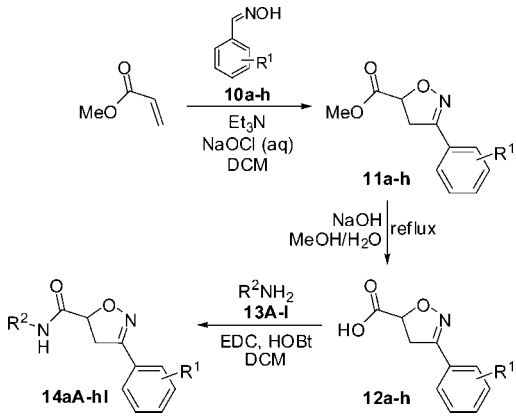


Figure 4. Calculations on HOMO–LUMO energy gaps for 1,3-dipolar reactions of possible synthetic route routes. Isovalue for molecular orbitals = 0.02.

Scheme 2. Synthesis of the 3-Aryl-4,5-dihydroisoxazole-5-carboxyamide Library



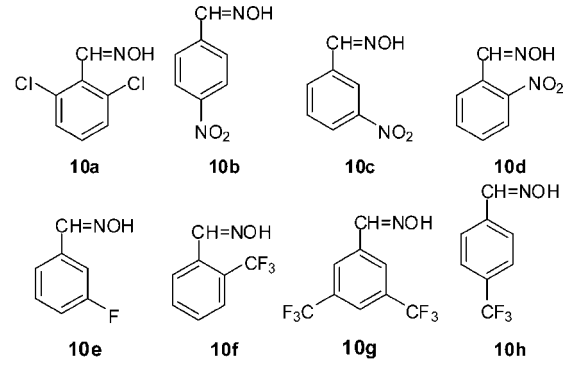


Figure 5. Aryl oxime chemset (**10a–h**).

dition reaction is then straightforward as long as one LUMO–HOMO interaction dominates (dipolarophile-HOMO–dipole-LUMO typically predominates with nitrile oxides).[17] However, regioselectivity can be diminished because of the relatively narrow HOMO–LUMO gap for nitrile oxides.[16] Houk and Sustmann have presented three types of HOMO–LUMO interactions which ultimately control the regioselectivity of the cycloaddition (Figure 7).[18,19] The two interactions represented here are for a dipolarophile with an electron-donating group (EDG) and a dipolarophile with an electron-withdrawing group (EWG). Acrylate, with its monosubstituted alkene and electron withdrawing carboalkoxy, gives high regioselectivity (>95:5 3,5-:3,4-disubstitution; the dipolarophile-HOMO–dipole-LUMO interaction leads to the 3,5-disubstituted isoxazoline).[16]

Conclusion

A three-step route consisting of methyl acrylate + nitrile oxide 1,3-dipolar cycloaddition, ester saponification, and carboxylic acid → carboxamide formation has been developed for the preparation of a library of unique 3-aryl-4,5-dihydroisoxazole-5-carboxamides. The protocol reported requires only purification of the final product.

Experimental Section

General Synthetic Procedures. All chemicals were purchased from commercial suppliers and used without

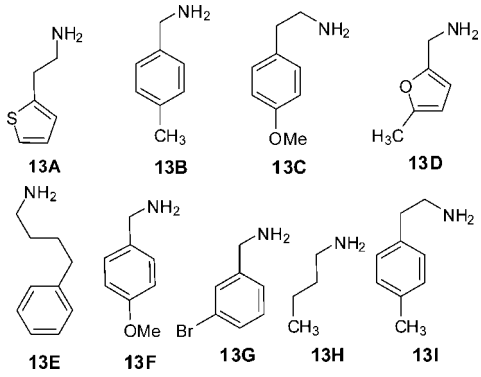


Figure 6. 1°-Amine chemset (**13A–I**).

further purification. Analytical TLC was carried out on precoated plates (silica gel 60, F254) and visualized with UV light. NMR spectra (^{1}H at 300, 400, and 600 MHz; ^{13}C at 75 and 100 MHz) were recorded in chloroform-d_1, methanol-d_4, and acetone-d_6 as solvents and chemical shifts are expressed in parts per million relative to TMS. The specifications of the LC/MS are as follows: electrospray (+) ionization, mass range 100–900 Da, 20 V cone voltage, and Xterra MS C_{18} column (2.1 mm × 50 mm × 3.5 μm). Concentration refers to rotary evaporation under reduced pressure. Regardless of crude reaction purity, and because of purity constraints required by the NIGMS, all 72 library members were purified by preparatory HPLC and characterized by LCMS to be greater than 90% purity.

General Route to α,β-Unsaturated Amides. *N*-Phenylmethacrylamide (5c).[20] Methacryl chloride (10.7 mmol) was dissolved in DCM (100 mL) followed by addition of aniline (8.9 mmol) at 0 °C. Triethylamine (10.7 mmol) was then syringed into the reaction mixture and the solution was stirred overnight and allowed to warm to ambient temperature. The reaction was then concentrated and redissolved in EtOAc. The organic solution was then washed with 1 M HCl, water, saturated sodium bicarbonate, and brine. The organic layer was dried over magnesium sulfate, filtered, concentrated, and purified by column chromatography (30:70 EtOAc:hexanes) to give solid **5c** (65%). ^{1}H NMR δ 2.02 (s, 3H), 5.45 (s, 1H), 5.85 (s, 1H), 7.06–7.09 (m, 1H), 7.28–7.32 (m, 2H), 7.77–7.79 (m, 2H), 9.16 (s, NH). Amides **4a–c**, **5a–c**, and **6a–c** were prepared by appropriate modification of this general procedure.

General Procedure for 1,3-Dipolar Nitrile Oxide Cycloadditions. Methyl 3-(4-Nitrophenyl)-4,5-dihydroisoxazole-5-carboxylate (11b). Methyl acrylate (2.49 g, 28.9 mmol) was added to a DCM (300 mL) solution of 4-nitrobenzalde oxime (43.36 mmol) and the reaction was cooled to 0 °C. Bleach (6% aq NaOCl, 131 mL, 115.6 mmol) was added dropwise over 20 min and the resulting mixture was stirred overnight and allowed to warm to ambient temperature. Water (200 mL) was added and the product was extracted using DCM (2 × 300 mL). The combined organic layer was dried over magnesium sulfate, filtered, and concentrated to give solid **11b** (90%). Isoxazolines **11a–h** were prepared by this method and used without further purification.

For regioselectivity studies, isoxazolines **7a–c**, **8a–c**, and **9a–c** were prepared by this general procedure. Following concentration, these crude products were purified by HPLC purification (e.g., **7c**: 67% yield; ^{1}H NMR δ 3.74–3.88 (dd, 1H), 3.77–3.85 (dd, 1H), 5.33–5.36 (dd, 1H), 7.13–7.15 (dd, 1H), 7.32–7.34 (d, 2H), 7.56–7.57 (d, 2H), 7.84–7.85 (d, 2H), 8.26–8.27 (d, 2H), 8.46 (s, NH); ^{13}C NMR δ 171.4, 156.9, 149.1, 137.0, 134.8, 129.3, 127.9, 125.2, 124.2, 120.0, 79.3, 44.9, 24.3; LC-MS *m/z* 312.16 $[M + H]^+$ (Nova-Pak C_{18} Column, 99%, 200–400 nm).

General Procedure for Ester Saponification and Amide Formation. 3-(3-Fluorophenyl)-*N*-(4-methylbenzyl)-4,5-dihydroisoxazole-5-carboxamide(14eB). Isoxazoline **11e** (39.6 mmol) was added to a methanol (200 mL) + water (200 mL) solution followed by addition of NaOH pellets (198.2 mmol). The reaction mixture was refluxed overnight, cooled to ambient temperature, and concentrated. The resulting solution was acidified with aq HCl to pH <4 and then extracted using EtOAc (3 × 200 mL). The organic layers were dried over magnesium sulfate, filtered, and concentrated (95%). A 100 mg portion of solid crude **12e** (0.3876 mmol) was dissolved in DCM (5 mL) followed by addition of 4-methylbenzylamine (0.4263 mmol) and HOBt (50 mg, 0.0426 mmol). The solution was stirred at 0 °C for 30 min

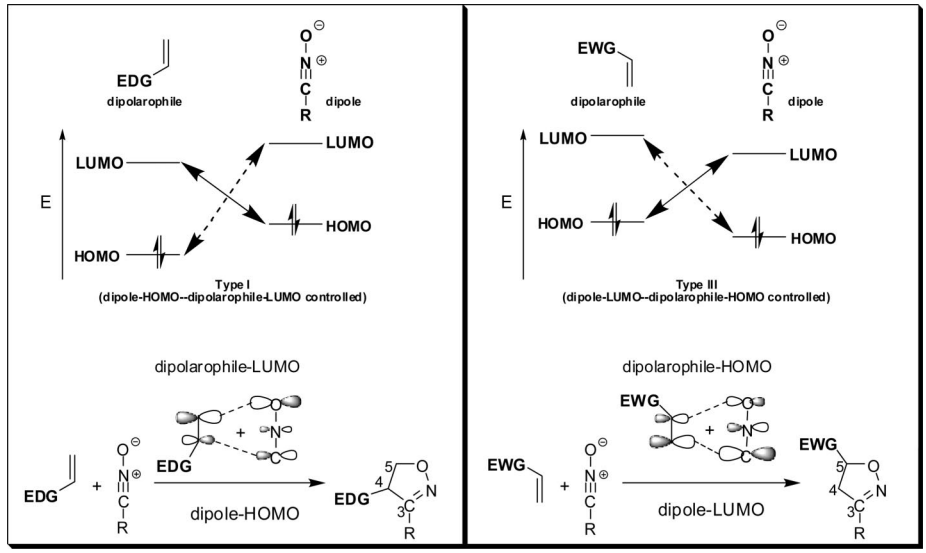


Figure 7. Two of three types of HOMO–LUMO interactions that control regioselectivity.

followed by addition of EDC (81 mg, 0.042 mmol). The reaction was allowed to warm to ambient temperature overnight followed by concentration and resolvation in EtOAc (5 mL). This organic layer was then washed with 1 M HCl, water, saturated sodium bicarbonate, and brine; it was then dried over sodium sulfate, filtered, and concentrated. Purification by preparative HPLC delivered **14eB** as a solid (65%): ^{1}H NMR δ 2.32 (s, 1H), 3.66–3.70 (d, 1H), 3.68–3.71 (d, 1H), 4.27–4.55 (dd, 1H), 4.32–4.50 (dd, 1H), 5.16–5.22 (dd, 1H), 7.05 (s, 1H), 7.10–7.18 (m, 4H), 7.38–7.41 (m, 3H); ^{13}C NMR δ 170.6, 164.6, 156.7, 137.7, 134.4, 130.8–130.7 (d), 129.7, 128.0, 123.1, 118.2, 114.2, 79.4, 43.3, 39.7, 21.3; LC-MS *m/z* 313.10 $[M + H]^+$ (Nova-Pak C_{18} Column, 99%, 200–400 nm). Isoxazolines **14aA–hI** were prepared by this method and, after purification by HPLC, gave these 3-aryl-4,5-dihydroisoxazole-5-carboxamides as both solids and viscous oils in 50–80% yield.

Computational Methods

Geometry optimizations were carried out at the MP2/6–31G(d)[21] level of theory and characterized by frequency analysis. All calculations were performed with Gaussian03.[22]

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Note Added after ASAP Publication. There was an error in Figure 6 in the version published ASAP on October 3, 2007; the corrected version was published ASAP on November 12, 2007.

Supporting Information Available. Spectra data (^{1}H NMR and LCMS) for 20 members of the library as well as for isoxazolines **7a–c**, **8a–c**, and **9a–c** are provided (PDF). This information is available free of charge via the Internet at http://pubs.acs.org.

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(14) (a) The dipole–LUMO–dipolarophile–HOMO energy gaps (given over the solid arrows in Figure 4) are option **A** (0.420 eV, methyl propiolate); option **B** (0.413 eV, *N*-methylpropiolamide); option **C** (0.373 eV, *N*-methylacrylamide); and option **D** (0.395 eV, methyl acrylate). The dipole–HOMO–dipolarophile–LUMO energy gaps, given parenthetically over the dashed arrows in Figure 4, are all larger than the dipole–LUMO–dipolarophile–HOMO energy gaps. That said, there are subtle differences between the calculated HOMO–LUMO energy gaps that are dominating the dipole–dipolarophile interactions. (b) For all the other HOMOs observed for the dipolarophiles, the alkene or alkyne is not conjugated into the carbonyl. For comparative consistency, a molecular orbital where the alkyne is not conjugated into the carbonyl was located at HOMO-1. Regardless of whether the system utilizes the HOMO or HOMO-1, the dipole–LUMO–dipolarophile–HOMO interaction still has a smaller energy gap than the dipole–HOMO–dipolarophile–LUMO interaction.
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