Selective Nucleophilic Chemistry in the Synthesis of 5-Carbamoyl-3-sulfanylmethylisoxazole-4-carboxylic Acids

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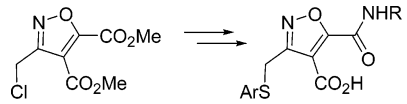
The solution-phase syntheses of 5-carbamoyl-3-sulfanylmethylisoxazole-4-carboxylic acids were accomplished from dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate by selective nucleophilic chemistry. For example, treatment of this trifunctionalized core with 3-bromobenzylamine and subsequent X-ray analysis identified the sole product as methyl 5-(3-bromobenzylcarbamoyl)-3-chloromethylisoxazole-4-carboxylate. Subjecting this amide/ester to thiophenol in the presence of 1 N NaOH completed the two-step transformation of this versatile starting material to the targeted 5-carbamoyl-3-sulfanylmethylisoxazole-4-carboxylic acid. Employing various amines and thiophenols, this chemistry was applied in the generation of a 90-compound library of druglike isoxazoles.

Introduction

Small-molecule combinatorial libraries are powerful tools for the interfacing of chemistry with biology because small molecules often have the ability to selectively manipulate the activity of biological systems.[1] Additionally, these libraries are not limited to one target, but can be screened multiple times against various biological targets with the possibility of producing multiple "hit" compounds. Although the rational design of small molecules has been successfully applied to the discovery of inhibitors for many biological enzymes, including kinases and proteases,[2] high-throughput screening offers a complementary approach for the identification of active, small molecules.[3]

Our interest in the design of small molecules as tools for the manipulation of biological systems based on combinatorial chemistry prompted us to study the chemistry of 3-chloromethylisoxazole-4,5-dicarboxylates (e.g., **2** in Figure 1). It has been shown that nitrogen-containing heteroaromatic scaffolds effectively serve as the basis for druglike small molecules.[1] Additionally, the biological activity of isoxazoles is well-documented in the literature, with these heterocycles having been shown to exhibit anticancer[4] as well as useful activities in conditions such as schizophrenia, hypertension, and Alzheimer's disease.[5] This diverse isoxazole-based biological activity[6] made trifunctional dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate an appealing starting material for diversity oriented synthesis.[7] Indeed, the three electrophilic positions available for diversification make it an ideal building block for both solution- and solid-phase library syntheses.[8] Collectively, these diversification points allow for the development of large numbers of compounds through reliable transformations—acylation and substitution reactions—making isoxazole **2** an ideal starting point for a small-molecule library. Herein, we report a route to **2**, as well, and its subsequent elaboration to a 90-compound combinatorial library of 5-alkylcarbamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acids (e.g., **5**).



2 **5**

Figure 1. 5-Alkylcarboamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acids from dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate.

Results and Discussion

The straightforward synthesis of dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate was accomplished as depicted in Scheme 1. Following literature procedures,[9] oxime **1** (Note: caution regarding the rash-causing nature of 1[9]) was prepared from commercially available chloroacetaldehyde and, without further purification, was employed in a 1,3-dipolar cycloaddition with dimethyl acetylenedicarboxylate to give starting isoxazole **2** in good yield (70%).

With this starting scaffold core in hand, we set out to evaluate the relative reactivity of its three electrophilic positions. As outlined in Scheme 2, reacting **2** with a primary amine could produce three monoaddition products. Paths A and B are the result of ester → amide transformations; path C is the consequence of S_N2 displacement ($-CH_2Cl \rightarrow -CH_2NHR$) followed by a potential intramolecular lactamization.

The reactivity of isoxazole **2** proved to be influenced greatly by the electron-withdrawing effects of the C4 and C5 esters. As evidenced by ^1H NMR of the crude reaction mixture, reacting **2** with 2 equiv of 3-bromobenzylamine gave a clean conversion (78% yield) through what was believed to be either a C4 or C5 ester → amide transformation. It proved difficult to determine which ester had reacted, which led us to pursue both crystallographic analysis and

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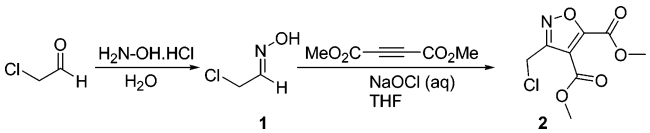
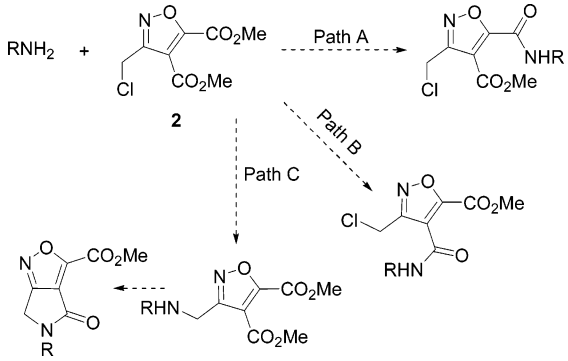
Scheme 1. Synthesis of Dimethyl 3-Chloromethylisoxazole-4,5-dicarboxylate



Scheme 2. Possible Monoaddition Outcomes in the Reaction of **2** with Primary Amines

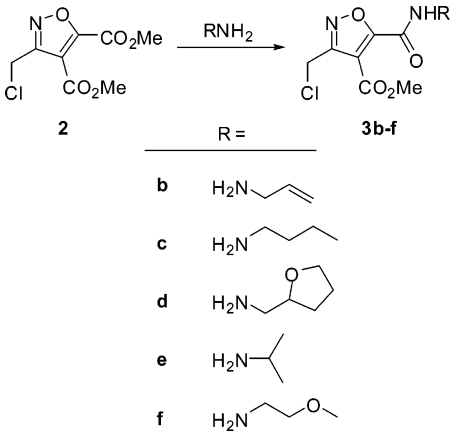


theoretical calculations as tools to evaluate the reactivity of the C4 and C5 carbomethoxy groups. Calculations on **2** using Gaussian 03[10] at the B3LYP/6-31G(d,p) level[11] and natural bond orbital (NBO) analysis[12] indicated that the charges on the carbons of the C4 and C5 carbomethoxy groups were nearly equivalent (≈ +0.8) and, not surprisingly, much more electron-deficient than the carbon of the C3 chloromethyl group (−0.46). Although these calculations suggested that the electron-deficient carbonyl centers were equally electrophilic, the NBO charges on C4 (−0.24) and C5 (+0.31) isoxazole carbons are quite disparate (see Figure 2). This along with steric considerations led us to speculate that the C5 carbomethoxy would perhaps be more reactive. The X-ray crystallographic structure depicted in Figure 2 unambiguously established that the C5 carbomethoxy was the sole source of reactivity, even with 2 equiv of amine (**2** → **3a**).

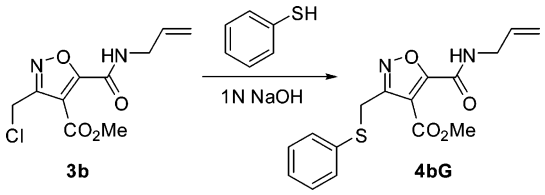
To ensure that this ester → amide selectivity was not due to the particular reactivity of 3-bromobenzylamine, we reacted **2** with five additional amines. As shown in Scheme 3, the C5 amide was formed exclusively in all cases, as verified by the selective disappearance of the C5 methoxy protons in the ^{1}H NMR spectra, with yields ranging from 72 to 92%.

With selective aminolysis of the C5 carbomethoxy group of **2** established, efforts turned to the introduction of a second

Scheme 3. Amines Used for Selective C5 Amide Bond Formation



Scheme 4. Chloride Displacement with Thiophenol



diversity element through the S_N2 displacement of chloride from the C3 chloromethyl group by thiophenols. As outlined in Scheme 4, we reasoned that reacting, for example, **3b** with 1 equiv of thiophenol in the presence of 1 N NaOH would deliver sufamylisoxazole **4bG**.

Surprisingly, the yield of isolated ester **4bG** was dramatically lower than anticipated, which prompted us to investigate the side reactions of this chloride displacement reaction. The electron deficiency at the C4 position provided a clue and led us to consider two potential side reactions: ester → thioester or ester → acid. Of these, hydrolysis of the ester seemed more likely. To test this supposition, the one-pot conversion of **3b** → **5bG** (see Figure 3) was attempted in the presence of 1 equiv of thiophenol and 3 equiv of NaOH. Indeed, carboxylic acid **5bG** was obtained in 90% yield.

With the distinct reactivity of the various C3, C4, and C5 substituents defined, we turned our attention to the construction of a 90-compound library. Reacting amides **3b**−**f** with 18 thiophenol derivatives in the presence of 3 equiv of 1 N aq NaOH in methanol provided a straightforward route to the trifunctionalized target of generalized structure **5**. Workup

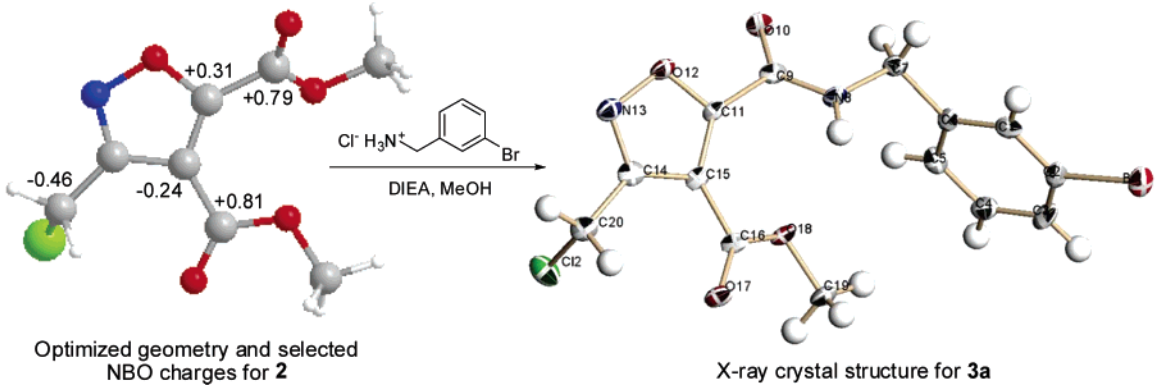


Figure 2. Optimized geometry and selected NBO charges for **2** as well as the X-ray crystal structure of methyl 5-(3-bromobenzylcarbamoyl)-3-chloromethylisoxazole-4-carboxylate (**3a**).

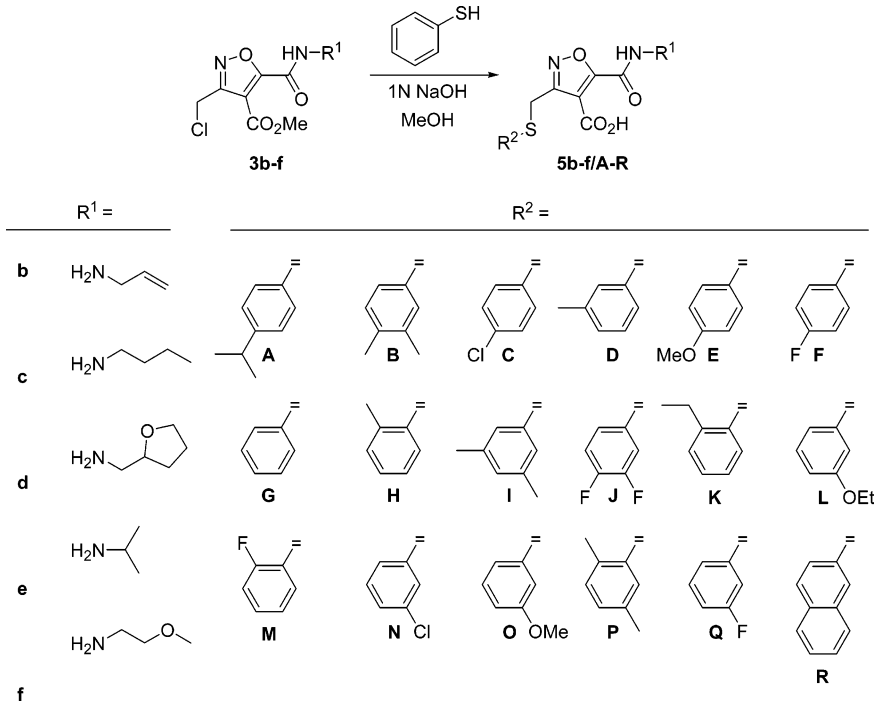


Figure 3. Ninety-member 5-alkylcarbamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acid library.

consisted of acidification with 1 N aq HCl, followed by solvent evaporation. The crude reaction mixture (80−90% yield and 88−94% crude purity prior to HPLC) was dissolved in CH_3CN/H_2O/DMF (3:1:4; 2 mL) and purified directly by HPLC to deliver the targeted 90-compound 5-alkylcarbamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acid library (Figure 3).

Conclusions

A two-pot conversion of dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate (**2**) to 5-alkylcarbamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acids (**5**) was accomplished by exploiting the distinct reactivities of the C3 chloromethyl and C4/C5 carbomethoxy substituents. Employing 5 primary amines and 18 thiophenols led to the preparation of a 90-member isoxazole-based library.

Experimental Section

Dimethyl 3-Chloromethylisoxazole-4,5-dicarboxylate (2). Hydroxyl amine (12.0 g, 174 mmol) was added to chloroacetaldehyde (20 mL, 155 mmol) and stirred at ambient temperature for 30 min.[5] The solution was extracted with ether (2 × 500 mL), dried over magnesium sulfate, filtered, and concentrated. To a THF (200 mL) solution of the resulting crude oxime cooled to 0 °C was added dimethyl acetylenedicarboxylate (12.65 mL, 103 mmol), followed by aq NaOCl (546 mL, 387 mmol; dropwise over 3 h). Upon completion of this addition, the solution was warmed overnight to room temperature. After 12 h, the mixture was concentrated under vacuum by rotoevaporation and extracted with dichloromethane (2 × 500 mL). The combined organic layer was dried over magnesium sulfate, filtered, and concentrated, and the crude product was purified by column chromatography (SiO_2; 90:10 hexane/ethyl acetate) to give **2** as a colorless oil (17.14 g, 70%): IR (neat) 1728, 1652, 1604; ^{1}H NMR δ 3.87 (s, 3H), 3.95 (s, 3H), 4.72 (s, 2H); ^{13}C NMR δ 161.7, 160.4, 160.1, 156.6, 113.8, 53.8, 53.1. HRMS (EI) for $C_8H_8ClNO_5$ calcd 233.0086, obsd 233.0087.

Methyl 5-Allylcarbamoyl-3-chloromethylisoxazole-4-carboxylate (3b). To a stirred solution of isoxazole **2** (2 g, 8.4 mmol) in MeOH (100 mL) was added dropwise a solution of allyl amine (760 μL, 16.8 mmol) in MeOH (20 mL). The mixture was stirred overnight at ambient temperature and concentrated under vacuum by rotoevaporation. The crude mixture was taken up in EtOAc (2 × 100 mL) and washed with water, 1 N HCl, and brine. The organic layer was dried over magnesium sulfate, filtered, concentrated, and purified by column chromatography (80:20, EtOAc/hexanes) to give **3b** as a white solid (1.95 g, 90%): mp 75.6−77.5 °C; IR (neat) 3290, 1717, 1657, 1605, 1557; ^{1}H NMR δ 4.02 (s, 3H), 4.10−4.13 (m, 2H), 5.22−5.24 (dd, 1H, J = 1.2, 9), 5.30−5.33 (dd, 1H, J = 1.8, 15.6 Hz), 5.89−5.96 (m, 1H), 9.48 (s, 1H); ^{13}C NMR δ 166.2, 163.1, 160.3, 154.5, 132.8, 117.6, 110.0, 53.8, 43.5, 35.5. HRMS (EI) for $C_{10}H_{11}ClN_2O_4$ calcd 258.0402, obsd 258.0406.

5-Allylcarbamoyl-3-(3,4-dimethylphenylsulfanylmethyl)isoxazole-4-carboxylic Acid (5aB). Compound **3a** (115 mg, 0.47mmol) was dissovled in methanol. To that solution was added 3,4-dimethylbenzenethiol (65 mg, 0.47mmol) and 1 N NaOH (0.47 mL, 0.47 mmol). The solution was stirred overnight at room temperature, concentrated, and purified by column chromatography (50:50, EtOAc/hexanes/0.05% HOAc) to give **5bB** as a white solid (146 mg, 90%): mp 115.9−117.3 °C. IR (neat) 1708, 1609, 1575, 1528, 1490; ^{1}H NMR δ 2..21 (s, 6H), 4.14−4.16 (t, 2H, J = 6 Hz), 4.41 (s, 2H), 5.31−5.36 (t, 2H, J = 10.8 Hz), 5.86−5.93 (m, 1H), 7.03−7.04 (d, 1H, J = 7.6 Hz), 7.11−7.13 (d, 1H, , J = 7.6 Hz), 7.26 (s, 1H); ^{13}C NMR δ 164.4, 159.3, 159.1, 157.9, 137.7, 136.5, 133.0, 131.3, 130.5, 129.2, 119.5, 115.4, 42.9,

29,7, 19.9, 19.7. HRMS (EI) for $C_{17}H_{18}N_2O_4S$ calcd 346.0982, obsd 346.0980.

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Supporting Information Available. Detailed experimental procedures for **3a**−**f**, **5**{*a*−*e*/*A*−*R*}, spectral data for **2**, **3a**−**e**, and 20 library members. HPLC traces for all library members and crystallographic data for **3a** are also available. This material is available free of charge via the Internet at http://pubs.acs.org.

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