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A Silanediol Inhibitor of the Metalloprotease Thermolysin: Synthesis and Comparison with a Phosphinic Acid Inhibitor[1]

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A silanediol inhibitor of the metalloprotease thermolysin was prepared for comparison to a known phosphinic acid inhibitor, providing the first comparison of these second-row element based transition-state analogues. Inhibition of thermolysin by the silanediol ($K_i = 41$ nM) was comparable to that of the phosphinic acid ($K_i = 10$ nM) even though the silanediol is uncharged and thereby lacks the intrinsic Coulombic attraction of the phosphinate anion to the active-site zinc cation. This silanediol protease inhibitor is the least sterically encumbered example prepared to date and, therefore, the most prone toward polymerization. Hydrolysis of a difluorosilane intermediate to the silanediol leads cleanly to a monomeric product.

Introduction

Metalloproteases are one of the four major classes of proteolytic enzymes,[2] distinguished by the presence of a zinc cation secured at the active site by three amino acids—usually two histidines and a glutamate—that coordinates and activates the scissile amide carbonyl.[3] Among this important class of proteolytic enzymes,[4] thermolysin has the distinction of being the first metalloprotease to have its detailed structure revealed by X-ray crystallography.[5] Thermolysin is considered to be one of *the* prototypical metalloproteases and to be structurally similar to important pharmaceutical targets.[6] Many inhibitors of this enzyme have been developed.[7,8]

Inclusion of this benchmark metalloprotease in our study of silanediol-based protease inhibitors presented the opportunity to compare the silanediol inhibitors to those utilizing phosphorus acids, two second-row elements. Several phosphorus-based enzyme inhibitors of thermolysin are known in which the scissile amide of the substrate has been replaced with a phosphinic acid (**1a**), a phosphinamide (**1b**), and a phosphinate ester (**1c**). All of these phosphorus-based groups are acidic, with pK_a values of 1.4−3.1,[9] and therefore carry a negative charge except under strongly acidic conditions. The charge of the anion provides a Coulombic attraction between the inhibitor and the active-site zinc cation. Dialkylsilanediols are less acidic, with a pK_a near 10−12,[10−12] and are neutral species at physiological pH, lacking a negative charge and its attraction to the active site zinc. This neutrality, however, would be expected to allow them to directly penetrate cell membranes,[13] a supposition born out in HIV protease inhibitor testing.[14] Comparison of the two inhibitors phosphinamide **1b** ($K_i = 9.1$ nM) and phosphinate **1c** ($K_i = 9000$ nM) has provided insight into the importance of the hydrogen bond from the nitrogen of the scissile amide group to the enzyme (as well as to solvent) for active-site binding.[9,15−19] Silanediol-based inhibitor analogues of **1b** and **1c** (substitution of PO_2H by SiO_2H_2) are unlikely to be useful, however, because of the hydrolytic instability of Si−N and Si−O bonds. Based on the strength of the Si−C bond, phosphinic acid

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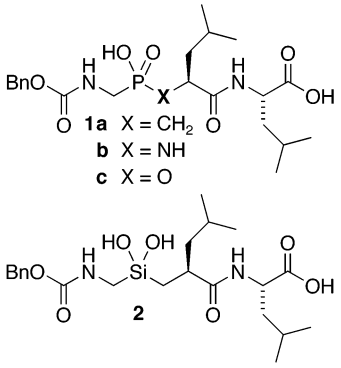
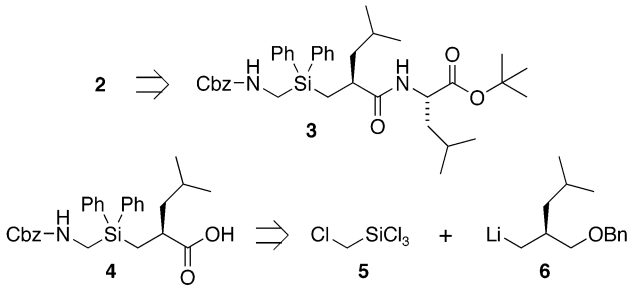


FIGURE 1. Phosphorus- and silicon-based thermolysin inhibitors.

SCHEME 1. Retrosynthetic Plan



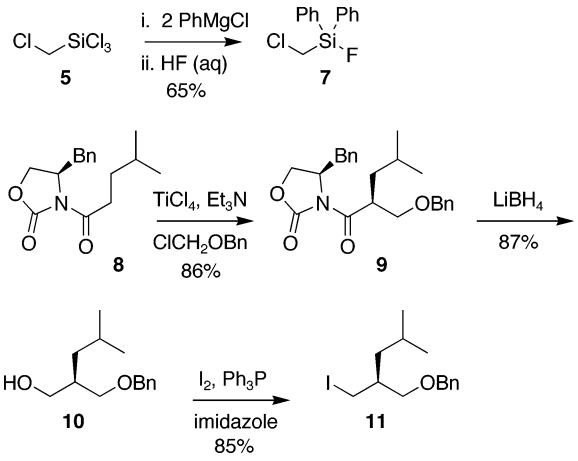
1a was an ideal target for comparison of phosphorus and silicon, in the form of structure **2** (Figure 1). We describe below the preparation of **2** and its evaluation as a thermolysin inhibitor.

Silanediols are well represented in the chemical literature[20,21] but have been best known for their propensity to undergo dehydration/condensation, forming siloxane polymers (silicones). They can, however, be stable. The stability of dialkylsilanediols is dependent in part on the steric effect of the alkyl groups. We have found that silanediol peptide mimics can be effective inhibitors of metallo- and aspartic proteases, such as angiotensin-converting enzyme (ACE)[22,23] and the HIV protease.[14] In this earlier work, the inhibitors were prepared as diphenylsilanes and hydrolyzed to silanediols with triflic acid. Following this successful approach, the synthetic plan for **2** anticipated diphenylsilane intermediate **3** as the immediate precursor, Scheme 1. Treatment of **3** with strong acid would hydrolyze the two phenyl−silicon bonds and the *tert*-butyl ester. The central silane amino acid **4** would be derived from commercially available chloromethyltrichlorosilane **5** and the enantiomerically pure lithium reagent **6**.

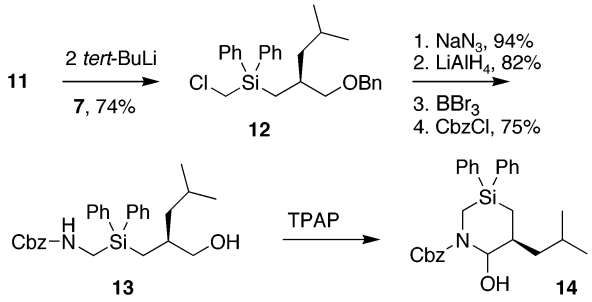
Results and Discussion

The two key components for assembly of silane **4** were prepared as described in Scheme 2. Treatment of chloromethyltrichlorosilane **5** with 2 equiv of phenylmagnesium chloride, followed by stirring the resulting mixture with aqueous 48% hydrofluoric acid, gave fluorosilane **7** in 65% distilled yield. Fluorosilanes have been convenient intermediates in our prior silanediol protease inhibitor syntheses[23,24] and continue to play an important role here (see below). Mono- and difluorosilanes are easily handled intermediates that are stable to moisture, yet are very reactive with organometallic reagents.

SCHEME 2. Synthesis of Key Intermediates



SCHEME 3. First Synthesis Approach



The iodide precursor of lithium reagent **6** was prepared using the Evans chiral auxiliary derivative of isohexanoic acid, **8**. Alkylation of the titanium enolate of **8** with benzyl chloromethyl ether gave **9** as a single diastereomer in 86% yield. Reduction of **9** with lithium borohydride[25] gave alcohol **10** (87%), which was converted to iodide **11** using iodine and triphenylphosphine (85%).[26,27]

Iodide **11** and fluorosilane **7** were coupled by initial conversion of **11** to lithium reagent **6** using 2 equiv of *tert*-butyllithium under the Bailey−Negishi conditions,[28,29] Scheme 3. Addition of silane **7** led to the isolation of **12** in 74% yield. Transformation of the hindered chloromethyl group to an amine was performed by displacement with azide and reduction of the product with lithum

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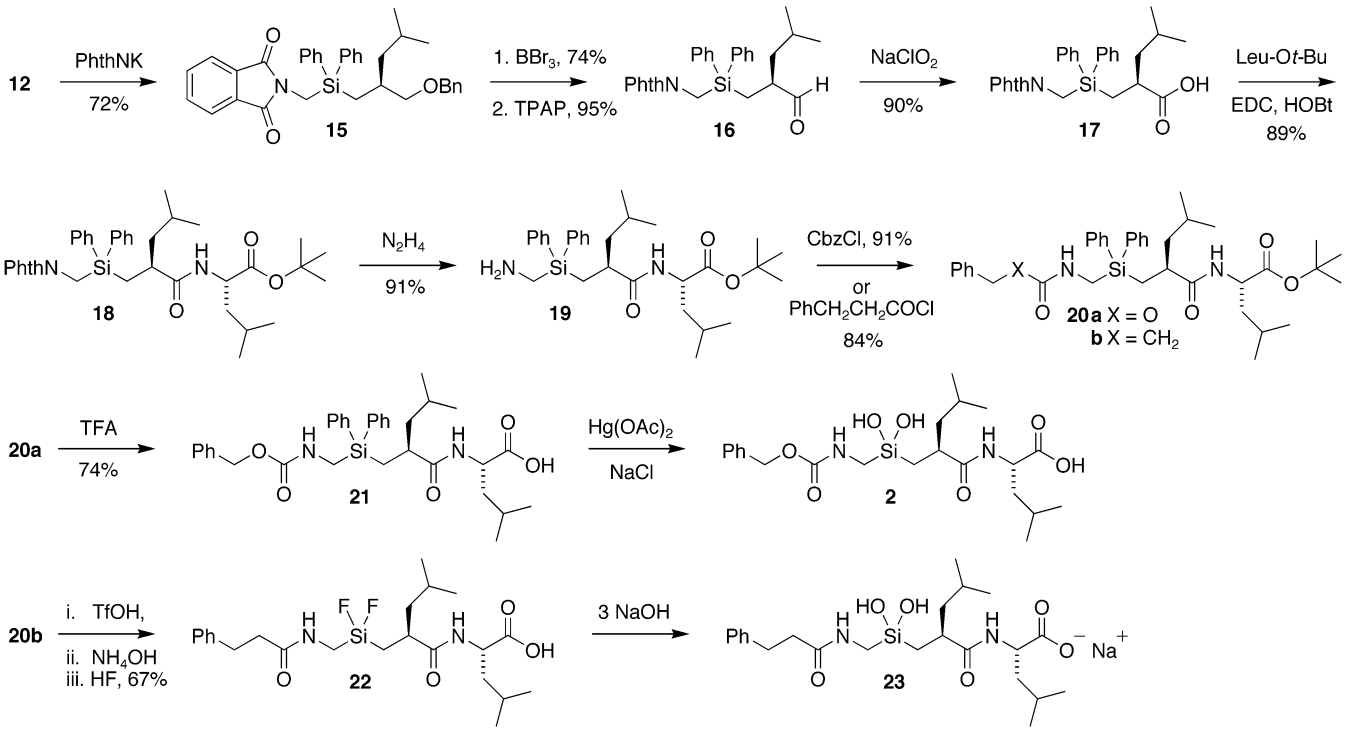
SCHEME 4. Synthesis of the Silanediol Inhibitor



aluminum hydride (77%, two steps). The resulting amino ether was cleaved to the amino alcohol with boron tribromide and then directly coupled with benzyl chloroformate yielding **13** (75%, two steps). Oxidation of the alcohol to an acid was initiated with TPAP oxidation to the aldehyde. Unfortunately, this aldehyde immediately cyclized to 4-hydroxy-1,3-azasilinane **14** (as a single diastereomer).[30] Attempts to avoid the formation of **14** or to use it to meet subsequent synthetic goals were frustrated, and so an alternative route was investigated.

To avoid the unwanted cyclization leading to **14**, the nitrogen was introduced as a phthalimide, Scheme 4. Treatment of chloromethyl **12** with potassium phthalimide gave **15** in 72% yield. Excess boron tribromide then removed the benzyl ether (74%). Oxidation of the alcohol to aldehyde **16** proceeded without incident (95%), and Pinnick oxidation[31] to the acid **17** occurred in high yield (90%). Coupling of acid **17** with the *tert*-butyl ester of l-leucine using EDC/HOBt led to **18** (89%). Intermediate **18** was formed as a single diastereomer, confirming that the stereochemical integrity of the chiral center in **11** had not been compromised. Removal of the phthalimide group with hydrazine gave the corresponding amine **19** in high yield. Treatment of this amine with benzyl chloroformate gave silane **20a** (91%).

Carbamate-protected amines have been dealkylated using triflic acid with preservation of the carbamate group.[32] This precedent, however, did not translate to a stable Cbz group during triflic acid mediated hydrolysis of **20a**, and use of our standard hydrolysis conditions led to product mixtures missing the benzyl ester group (NMR). Despite a series of experiments to remove the phenyl groups under strongly acidic conditions, we were unable to produce the desired silanediol **2**. Nevertheless, the phenyl groups could be hydrolyzed using mercuric ion as the electrophile. Because these conditions were mild enough to preserve the *tert*-butyl ester, this group was removed first using standard trifluoroacetic acid conditions, to give acid **21** (74%). Exposure of diphenylsilane **21** to an excess of mercuric acetate in methylene chloride at 0 °C led rapidly to cleavage of both silicon-phenyl bonds. While this provided the desired **2** in acceptable overall yield, separation of this product from all of the mercury salts and organomercury products proved to be difficult. The amalgamated problems of Cbz group preservation with the undesirable use of mercury reagents led us to modify our synthetic target. Some motivation for avoiding the use of mercury reagents in this last stage of the synthesis was to ensure confidence in the subsequent thermolysin inhibition data.[33] We therefore changed our synthetic target to **23**, substituting the isosteric 3-phenyl propionamide group for the benzyl carbamate group.

Inspection of the crystal structures of phosphinic acid **1a** and related inhibitors bound to thermolysin indicated that the alcohol oxygen of the Cbz group was not involved in hydrogen bonding to the enzyme. Anticipating that replacing the benzyloxy group of **2** with a phenethyl group would lend stability under the acidic conditions of silanediol deprotection, but would have little consequence to the enzyme inhibition, we coupled amine **19** with 3-phenyl propionyl chloride to give **20b** (84%).

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Consistent with these expectations, treatment of **20b** with triflic acid resulted in the rapid loss of the *tert*-butyl ester and the two phenyl groups from silicon, without alteration of the rest of the molecule. This hydrolysis reaction is believed to involve the participation of the amide groups flanking the silane, and neutralization of the triflic acid with ammonium hydroxide was used to ensure that any cyclized intermediates were hydrolyzed.[23] The resulting silanediol **23** was clearly not homogeneous, however, as determined by inspection of the proton NMR spectrum. This was not entirely unexpected, as silanediol **23** had the least steric hindrance of any protease inhibitor we had prepared and therefore the most potentially prone to siloxane formation.

Steric hindrance is a key element of silanediol stability.[21,34,35] Diethylsilanediol polymerizes at one-tenth of the rate of dimethylsilanediol, and di-*tert*-butylsilanediol cannot be polymerized. Therefore, the rather limited steric hindrance of the aminomethyl component of **23** was of some concern. Moreover, silanediol polymerization is promoted by both acid and base catalysts[36,37]—both employed in the hydrolysis of **20b**. The two silanols of **23** are also diastereotopic, and therefore, any siloxane formation between two molecules of **23** will transform both silicon atoms to stereogenic centers, leading to diastereomeric mixtures.

The silanediol product resulting from treatment of **20b** with triflic acid followed by ammonium hydroxide was clearly a mixture. In an attempt to modify the acid-catalyzed hydrolysis yet retain its simplicity and generality, we followed the ammonium hydroxide treatment with addition of aqueous hydrofluoric acid. Strong acid and hydrofluoric acid are both commonly used for peptide deprotection. Aqueous HF will react with a broad set of silicon−heteroatom bonds, replacing the heteroatoms with fluoride. This mild procedure was reported by Eaborn in 1952 as a general method to prepare fluorosilanes from chlorosilanes, silyl ethers, and silanols.[38] The resulting fluorosilanes are stable to moisture (under neutral and acidic conditions) yet are very reactive toward nucleophiles. For the deprotection of **20b**, the addition of hydrofluoric acid led to the formation of a single isomeric and crystalline **22** that precipitated from the reaction mixture and was purified by filtration (67%). This acetone-soluble product had two resonances in the ^{19}F NMR spectrum, consistent with the presence of two nonequivalent fluorine atoms.

Suspension of **22** in water and addition of sodium hydroxide led to a rapid conversion of the fluorine signals in the ^{19}F NMR to a single absorption at −120 ppm, consistent with the generation of sodium fluoride. The resulting solution of silanediol **23** was monomeric and stable in aqueous solution. The ^{13}C NMR spectrum clearly showed all the 21 carbons of the structure,

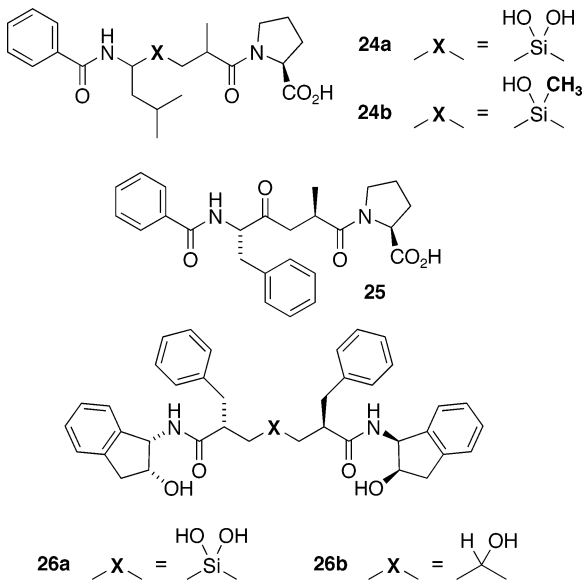

FIGURE 2. Inhibitors of ACE and the HIV protease.

including the carbonyls and the four diastereotopic methyl groups (see the Supporting Information).

Inhibition of thermolysin by **23** was determined using the commercially available enzyme and a commercially available profluorescent substrate described by Feder et al.[39] Dixon plot of the data gave a K_i = 41 nM, a value intriguingly similar to the K_i reported for the phosphinate analogue **1a** (10 nM). This suggests that the silanediol binds to the enzyme in a manner similar to the phosphorus inhibitors **1**. Little is known about the propensity of silanols for interaction with zinc ions (for a review of silanol−metal interactions, see ref 21; see also ref 23). The crystal structure of **1b** bound to thermolysin shows a single oxygen of the phosphinamide group within contact distance of the active site zinc.[3] A very similar conformation of the silanediol **23** complex with thermolysin has now been determined and will be reported elsewhere.

Silanediol **23** is the third example of an effective protease inhibitor built around a central silanediol transition state analogue and the second example of metalloprotease inhibition by such a structure. Silanediol **24a**, Figure 2, was prepared as an analogue of ketone **25**. Silanediol **24a**, a mixture of diastereomers, was found to inhibit angiotensin-converting enzyme (ACE, a metalloprotease) with an IC_{50} value of 14 nM, comparing favorably with the 1 nM IC_{50} of **25**.[23] Replacement of one of the hydroxyls of the silanediol group of **24a** with a methyl group, to give methylsilanol **24b**, eliminated the inhibition of ACE (IC_{50} > 3000 nM), consistent with the silanediol providing a critical interaction with the enzyme.

The C_2 symmetric silanediol **26a** was prepared as an inhibitor of the C_2 symmetric HIV protease and as an analogue of carbinol inhibitor **26b**. The HIV protease is an aspartic protease, with two aspartic acid residues at the active site that catalyze the scissile amide bond hydrolysis through charge and hydrogen bonding inter-

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actions. Silanediol **26a** and carbinol **26b** were found to inhibit the enzyme with IC_{50} values of 2.7 and 0.38 nM, respectively.[14] Silanediol **26a** was also found to be effective for protection of cells from HIV infection by the virus, demonstrating that this neutral structure could effectively cross cell membranes. The first silanediol inhibitors of a serine protease have recently been reported.[40]

Conclusions

The finding that silanediol **23** inhibits thermolysin (K_i = 41 nM) at concentrations similar to those for phosphinic acid **1a** (K_i = 10 nM) suggests that the uncharged silanediol group can provide an interaction with the positively charged active site zinc ion of thermolysin that is comparable to that of the phosphinic acid anion. This may seem surprising when considering the charge carried by the phosphinic acid anion and its expected attraction to the active site zinc. Binding of the phosphinic acid anion to the enzyme, however, would be expected to require substantially more solvent reorganization than would the neutral silanediol. Efforts to more completely define the utility of silanediols as central components of protease inhibitors, and related studies are continuing.

Experimental Section

(Chloromethyl)fluorodiphenylsilane (7).[41] To a 0 °C solution of chloromethyltrichlorosilane **5 (**4.60 mL, 35.1 mmol) in ether (40 mL) was added phenylmagnesium chloride (36.0 mL of a 2 M solution in THF, 72.0 mmol) over 1 h. The resulting solution was then refluxed for 24 h. After being cooled to rt, the solvent was removed using a rotary evaporator and the residue was taken up in a mixture of ethanol (50 mL) and 48% aqueous HF (4.20 mL). After the mixture was stirred for 48 h, water (10 mL) and CH_2Cl_2 (30 mL) were added. The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organics were washed successively with water (50 mL) and saturated NaCl (50 mL) and then dried over Na_2SO_4. Concentration and distillation (145−146 °C, 3 mm Hg) gave **7** as a clear oil (5.74 g, 65%): IR (neat) 3091, 1591, 1429, 1126, 884, 737, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93−7.60 (m, 10H), 3.49 (d, J = 3.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.5, 131.3, 130.2 (d, J_{C-F} = 64 Hz), 128.2, 26.2 (d, J_{C-F} = 76 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −170.2; MS *m/e* (rel intensity) 273 (MNa, 12),201 (35), 179 (21), 165 (18), 155 (21).

(*R*)-4-Benzyl-3-[(*S*)-2-benzyloxymethyl-4-methylvaleroyl]oxazolidin-2-one (9).[41] To a 0 °C solution of (*R*)-4-benzyl-3-(4-methylvaleroyl)oxazolidin-2-one **8** (10.70 g, 38.86 mmol) in methylene chloride (100 mL) under a nitrogen atmosphere was added a 1.0 M $TiCl_4$ solution (40.0 mL of a 1.0 M solution in methylene chloride, 40.0 mmol) followed by diisopropylethylamine (7.0 mL, 40 mmol). The solution was stirred for 1 h. Benzyloxymethyl chloride (13.3 g, 84.8 mmol) was added, and the mixture was stirred for 7 h at 0 °C. Aqueous saturated ammonium chloride (75 mL) was added, and after being stirred for 15 min the aqueous phase was extracted with methylene chloride (3 × 40 mL). The combined organics were washed with 50 mL of saturated NaCl solution and dried over Na_2SO_4. Concentration and flash chromatography (gradient of 1:20 to 1:10 ethyl acetate/hexanes) afforded **9** (13.20 g, 86%) as a colorless solid: mp 54 °C; $[\alpha]^{20}_D$ −38.5 (*c* 0.26, CH_2Cl_2); R_f 0.33 (1:5 ethyl acetate/hexanes); IR (KBr) 3029, 1762, 1693, 1394, 1362, 1214, 1109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33−7.17(m, 10H), 4.71 (m, 1H), 4.54 (d, J = 3.9 Hz, 2H), 4.39 (m, 1H), 4.14 (m, 2H), 3.70 (m, 2H), 3.24 (dd, J = 3.3, 13.5 Hz, 1H), 2.64 (dd, J = 9.3, 13.5 Hz, 1H), 1.64 (m, 2H), 1.34 (m, 1H), 0.92 (d, J = 3.9 Hz, 3H), 0.90 (d, J = 2.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 153.1, 138.1, 135.4, 129.4, 128.8, 128.3, 127.6, 127.5, 127.2, 73.0, 71.9, 65.7, 55.3, 41.4, 37.9, 37.6, 26.1, 22.7; exact mass (EI) MH^+ calcd for $C_{24}H_{30}NO_4$ 396.2175, found 396.2162.

(*R*)-2-Benzyloxymethyl-4-methyl-pentan-1-ol 10. To a 0 °C solution of **9** (13.0 g, 34.6 mmol) in diethyl ether (110 mL) under a nitrogen atmosphere was added $LiBH_4$ (20.0 mL of a 2 M solution in THF, 40.0 mmol), and the resulting mixture was stirred for 2.5 h. A 1 N NaOH solution was then added slowly until both layers were clear (60 mL). The aqueous phase was extracted with ether (3 × 50 mL), and the combined organics were washed with saturated NaCl (50 mL) and dried over Na_2SO_4. The solution was then concentrated in vacuo, diluted with hexanes, and filtered. The filtrate was concentrated and purified by flash chromatography (1:20−1:10 ethyl acetate/hexanes) to afford **10** as a clear oil (5.42 g, 87%): $[\alpha]^{20}_D$ +15.4 (*c* 1.17, CH_2Cl_2); R_f 0.24 (1:5 ethyl acetate/hexanes); IR (neat) 3414.8, 2954.8, 1454.2, 1098.4, 697.2 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.4−7.2 (m, 5H), 4.51 (d, J = 2.7 Hz, 2H), 3.69 (dd, J = 3.6, 10.8 Hz, 1H), 3.59 (m, 2H), 3.44 (dd, J = 7.8, 9 Hz, 1H), 2.94 (s, 1H), 1.94 (m, 1H), 1.60 (septet, J = 6.3 Hz, 1H), 1.12 (m, 2H), 0.89 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 128.3, 127.5, 127.4, 73.9, 73.2, 65.9, 38.2, 37.1, 25.2, 22.6; exact mass (EI) MH^+ calcd for $C_{14}H_{23}O_2$ 223.2698, found 223.2696.

(*S*)-2-Benzyloxymethyl-1-iodo-4-methylpentane (11). To a solution of alcohol **10** (4.80 g, 21.6 mmol) in a mixture of diethyl ether (40 mL) and CH_3CN (20 mL) were added triphenylphosphine (6.30 g, 24.0 mmol) and imidazole (1.63 g, 23.9 mmol), and the solution was cooled to 0 °C. A solution of iodine (6.04 g, 23.8 mmol) in ether (20 mL) was added slowly and then stirred for 3 h at 0 °C. After dilution with ether (50 mL), the solution was successively washed with saturated $Na_2S_2O_3$ (30 mL), saturated $CuSO_4$ (30 mL), and saturated NaCl (50 mL) and then dried over anhydrous Na_2SO_4. Concentration and flash chromatography (2:98−1:10 ethyl acetate/hexanes) gave **11** as a clear oil (6.08 g, 85%): $[\alpha]^{20}_D$ −43.8 (*c* 0.32, CH_2Cl_2); R_f 0.63 (1:20 ethyl acetate/hexanes); IR (neat) 2954.8, 1452.3, 1102.3, 697.2 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.4−7.2 (m, 5H), 4.55 (s, 2H), 3.6−3.3 (m, 4H), 1.58 (m, 2H), 1.20 (m, 2H), 0.89 (t, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.2, 127.5, 127.1, 73.2, 40.5, 37.0, 24.8, 22.9, 22.3, 13.8; exact mass (EI) MNH_4^+ calcd for $C_{14}H_{25}NOI$ 350.0981, found 350.0991.

(*S*)-(2-Benzyloxymethyl-4-methylpentyl)chloromethyldiphenylsilane (12). To a −78 °C solution of **11** (4.04 g, 12.2 mmol) in ether (120 mL) was added *tert*-butyllithium (18.0 mL of a 1.5 M solution in pentane, 27.0 mmol). The solution was stirred for 1 h at 78 °C and a further 1 h at rt. The solution was cooled to 0 °C, and **7** (2.90 g, 11.6 mmol) was added. After 1 h, the solution was warmed to rt and stirred for 40 h. Saturated NH_4Cl (45 mL) was added, the aqueous phase was extracted with ether (3 × 30 mL), and the combined organics were washed successively with water (10 mL) and saturated NaCl (10 mL) and then dried over Na_2SO_4. Concentration and flash chromatography (1:200−1:100 ethyl acetate/hexane) afforded **12** as a colorless oil (3.93 g, 74%): $[\alpha]^{20}_D$ −16.2 (*c* 0.52, $CHCl_3$); R_f 0.19 (1:39 ethyl acetate/hexane); IR (neat), 3069.5, 2953.8, 1427.2, 1111.9, 734.8, 697.2 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.6−7.2 (m, 15H), 4.28 (d, J = 3.9 Hz, 2H), 3.32 (s, 2H), 3.26 (m, 2H), 2.0 (m, 1H), 1.6−1.8 (m, 5H), 0.78 (d, J = 2.7 Hz, 3H), 0.76 (d, J = 2.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.7, 135.1, 135.0, 134.1, 129.7, 128.2, 127.9, 127.8, 127.5, 127.3, 75.2, 72.7, 44.5, 32.3, 28.7, 25.2, 22.7, 22.6, 14.6; exact mass (FAB) M − H^+ calcd for $C_{27}H_{32}ClOSi$ 435.1911, found 435.1913.

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2-([(2(*S*)-Benzyloxymethyl-4-methylpentyl)diphenylsilanyl]methyl)isoindole-1,3-dione (15). To a solution of **12** (2.67 g, 6.11 mmol) in anhydrous dimethylformamide (15 mL) at rt was added potassium phthalamide (2.11 g, 11.6 mmol), and the solution was then heated to 90−100 °C overnight. After the mixture was cooled to rt, ethyl acetate (15 mL), water (15 mL), and a pH 7 buffer solution (15 mL) were added. The aqueous portion was extracted with ethyl acetate (2 × 10 mL), and the combined organics were washed with water (10 mL) and saturated NaCl (10 mL) and then dried over Na_2SO_4. Concentration and flash chromatography (12:88 ethyl acetate/hexanes) gave **15** (2.45 g, 73%) as yellow oil: R_f 0.66 (1:5 ethyl acetate/hexanes); $[\alpha]^{20}_D$ −3.8 (*c* 1.68, $CHCl_3$); IR (neat) 3048.3, 2952.8, 1714.6, 1697.3, 1110.9, 1070.4 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.6−7.1 (m, 19 H), 4.15 (s, 2H), 3.70 (s,2H), 3.26 (dd, J = 4.8, 9.0 Hz, 1H), 3.15 (dd, J = 4.8, 9.0 Hz, 1H), 1.99 (sept, J = 6.6 Hz, 1H), 1.6−0.8 (m, 5H), 0.73 (t, J = 7.2 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 168.3, 138.7, 135.1, 135.0, 134.6, 134.5, 133.3, 132.2, 129.4, 128.2, 127.7, 127.6, 127.5, 127.3, 122.6, 75.1, 72.6, 44.6, 32.6, 26.8, 25.2, 22.8, 22.7, 16.4; exact mass (FAB) MNa^+ calcd for $C_{35}H_{37}NO_3NaSi$ 570.2440, found 570.2451.

2-([(2(*S*)-Hydroxymethyl-4-methylpentyl)diphenylsilanyl]methyl)isoindole-1,3-dione. To a solution of **15** (1.39 g, 2.54 mmol) in methylene chloride (20 mL) at −78 °C was added BBr_3 (7.60 mL of a 1 M solution in methylene chloride, 75.2 mmol). After 2 h, methanol (10 mL) was added slowly over 5 min, and then the solution was warmed to rt over 1 h. The solvent was removed by rotary evaporation, and the residue was taken up in methylene chloride (20 mL), washed with water (10 mL) and saturated NaCl (10 mL), and dried over Na_2SO_4. Concentration and flash chromatography using (3:7 ethyl acetate/hexanes) gave the title compound as a yellow oil (853.4 mg, 74%): R_f 0.59 (2:3 ethyl acetate/hexanes); $[\alpha]^{20}_D$ −36.1 (*c* 4.4, $CHCl_3$); IR (neat) 3549.8, 1760.0, 1705.0, 770.5, 737.7, 718.4 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.5−7.1 (m, 14H), 3.80 (AB quartet, J = 15.3, 15.3 Hz, 2H), 3.51 (dd, J = 3.9,10.8 Hz, 1H), 3.33 (dd, J = 6.3,10.8 Hz, 1H), 2.12 (br s, 1H), 1.79 (sept, J = 6.6 Hz,1H), 1.56−0.93 (m, 5H), 0.67 (d, J = 6.6 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 168.4, 135.0, 134.9, 134.2, 134.0, 133.5, 132.0, 129.6, 129.5, 127.9, 127.8, 122.7, 67.5, 44.0, 34.6, 26.8, 25.1, 22.8, 22.6, 15.9; exact mass (FAB) MNa^+ calcd for $C_{28}H_{31}NO_3SiNa$ 480.1971, found 480.1988.

2(*S*)-([(1,3-Dioxo-1,3-dihydroisoindol-2-ylmethyl)diphenylsilanyl]methyl)-4-methylpentanal (16). To a solution of 2-([(2(*S*)-hydroxymethyl-4-methylpentyl)diphenylsilanyl]methyl)isoindole-1,3-dione (1.784 g, 3.90 mmol) in dry methylene chloride (8 mL) were added *N*-methylmorpholine *N*-oxide (612 mg, 5.07 mmol), tetrapropylammonium perruthenate (59.6 mg, 0.17 mmol), and finely ground 4 Å molecular sieves (2.0 g). The solution was stirred for 2 h at rt and filtered through a pad of silica, eluting the silica with methylene chloride (150 mL). Concentration gave the crude aldehyde **16** (1.60 g, 95%): R_f 0.58 (1:4 ethyl acetate/hexanes); IR (neat) 1715, 1709.8, 1678.0, 720.4, 702.0 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.39 (d, J = 3.0 Hz, 1H), 7.7−7.2 (m, 14H), 3.7 (s, 2H), 2.5 (m, 1H), 1.7−1.1 (m, 5H), 0.69 (d, J = 6.3 Hz, 3H), 0.65 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.4, 168.1, 135.0, 134.9, 133.4, 133.1, 132.9, 131.8, 129.8, 129.7, 127.8, 127.7, 122.6, 45.6, 41.0, 26.4, 25.5, 22.6, 22.0, 12.7; exact mass (FAB) MNa^+ calcd for $C_{28}H_{29}NO_3SiNa$ 478.1814, found 478.1937.

2(*S*)-([(1,3-Dioxo-1,3-dihydroisoindol-2-ylmethyl)diphenylsilanyl]methyl)-4-methylpentanoic Acid (17). To a solution of **16** (609 mg, 1.34 mmol) in *tert*-butyl alcohol (16 mL) and water (4.0 mL) were added 2-methyl-2-butene (5 mL, 9.438 mmol) and sodium dihydrogenphosphate (50 mg, 0.41 mmol). After the mixture was stirred for 20 min at rt, $NaClO_2$ (401 mg, 4.4 mmol) was added and stirring continued for 2 h. Saturated NH_4Cl (12 mL) and methylene chloride (10 mL) were added, and the aqueous phase was extracted with methylene chloride (3 × 10 mL). The combined organics were washed with saturated NaCl (15 mL), dried over Na_2SO_4, concentrated, and purified by flash chromatography (1:19 methanol/methylene chloride) to give **17** as a foam (570 mg, 90%): mp 49−50 °C; $[\alpha]^{20}_D$ −2.46 (*c* 3.66 CH_2Cl_2); R_f 0.50 ($MeOH/CH_2Cl_2$ 1:15); IR (neat) 3457.2, 3070.5, 1776.3, 1760.9, 789.8, 678.0 cm^{-1}; ^1H NMR (300 MHz, CDCl3) δ 7.6−7.2 (m, 14H), 3.71 (s, 2H), 2.54 (m, 1H), 1.7−1.1 (m, 5H), 0.72 (d, J = 6.6 Hz, 3H), 0.67 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 182.8, 168.1, 135.1, 135.0, 133.4, 132.9, 132.0, 129.7, 127.7, 122.6, 44.2, 38.8, 26.3, 25.9, 22.8, 21.6, 16.4; exact mass (FAB) MNa^+ calcd for $C_{28}H_{29}NO_4SiNa$ 494.1764, found 494.1768.

2(*S*)−(2(*S*)−([(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-diphenyl-silanyl]-methyl)-4-methyl-pentanoylamino)-4-methyl-pentanoic acid *tert*-butyl ester (18). To a 0 °C solution of acid **17** (1.40 g, 3.0 mmol) in dry methylene chloride (30 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (633 mg, 3.30 mmol) and HOBt (447 mg, 3.31 mmol) and the mixture allowed to stir for 1 h. Diisopropylethylamine (0.65 mL, 3.71 mmol) and L-leucine *tert*-butyl ester hydrochloride (0.675 g, 3.0 mmol) were added, and the solution was stirred and warmed to rt over 24 h. The solution was diluted with methylene chloride (10 mL), washed with saturated NH_4Cl (2 × 5 mL), saturated $NaHCO_3$ (5 mL), water (5 mL), and saturated NaCl (5 mL), and then dried over Na_2SO_4. Concentration and flash chromatography (1:4 ethyl acetate/hexanes) gave **18** (1.70 g, 89%) as an oil: $[\alpha]^{20}_D$ −41.6 (*c* 1.85 CH_2Cl_2); R_f 0.21 (EtOAc/hexanes 1:5); IR (neat) 3385.8, 2955.7, 1730.1, 1708.8, 1675.1, 1385.8, 1154.3, 718.4 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.6−7.2 (m, 14H), 5.84 (d, J = 8.0 Hz, 1H), 4.29 (q, J = 6.8 Hz, 1H), 3.75 (d, J = 6.8 Hz, 2H), 2.42 (m, 1H), 1.6−1.0 (m, 8H), 1.40 (s, 9H), 0.86 (d, J = 6.4 Hz, 3H): 0.84 (d, J = 6.4 Hz, 3H): 0.70 (d, J = 6.4 Hz, 3H), 0.68 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8, 172.1, 168.2, 135.0, 133.8, 133.4, 133.3, 132.0, 129.8, 127.9, 127.8, 122.7, 81.4, 51.2, 44.4, 41.7, 40.3. 27.9, 26.4, 25.8, 24.8, 22.9, 22.5, 22.3, 21.9, 16.5; exact mass (EI), MH^+ calcd for $C_{38}H_{49}N_2O_5Si$ 641.3411, found 641.3480.

2(*S*)-(2(*S*)-[(Aminomethyldiphenylsilanyl)methyl]-4-methylpentanoylamino)-4-methylpentanoic Acid *tert*-Butyl Ester (19). To a solution of **18** (1.15 g, 1.80 mmol) in ethanol (18 mL) was added hydrazine (0.34 mL, 10.6 mmol). The solution was heated to reflux for 2 h and then cooled to rt. The solution was filtered through a pad of Celite, washing with ether. Concentration and flash chromatography (1:1 ethyl acetate/hexanes) gave **19** as an oil (845 mg, 91%): $[\alpha]^{20}_D$ −32.1 (*c* 4.76 CH_2Cl_2); R_f 0.30 (100% ethyl acetate); IR (neat) 3297.1, 3068.6, 2931.6, 2956.7, 1731.0, 1659.6, 1367.4, 1153.4, 729.0 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.4−7.2 (m, 10H), 6.43 (d, J = 7.5 Hz, 1H), 4.21 (q, J = 6.9 Hz, 1H), 2.65 (s, 2H), 2.28 (m, 1H), 1.6−1.0 (m, 10H), 1.30 (s, 9H), 0.78 (d, J = 4.2 Hz, 3H): 0.76 (d, J = 4.5 Hz, 3H), 0.63 (t, J = 4.5 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 176.1, 172.2, 134.9, 134.8, 134.4, 134.3, 129.7, 128.1, 128.0, 81.3, 51.3, 45.3, 41.6, 39.8, 28.1, 28.0, 25.8, 24.9, 22.9, 22.5, 22.2, 15.6; exact mass (FAB) MH^+ calcd for $C_{30}H_{47}N_2O_3Si$ 511.3379, found 511.3379.

2(*S*)-[2(*S*)-((Diphenyl-[(3-phenylpropionylamino)methyl]silanyl)methyl)-4-methylpentanoylamino]-4-methylpentanoic Acid *tert*-Butyl Ester (20b). To a 0 °C solution of **19** (707 mg, 1.52 mmol) in ether (21 mL) with saturated $NaHCO_3$ (6.0 mL) was added 3-phenylpropionyl chloride (0.25 mL, 1.65 mmol). After 1.5 h, saturated NH_4Cl was added, and the aqueous phase was extracted with ether (3 × 10 mL). The combined organics were washed with saturated NaCl (15 mL) and dried over Na_2SO_4. Concentration and flash chromatography (2:98−1:4 ethyl acetate/hexanes) gave **20b** as a colorless oil (0.821 g, 84%): $[\alpha]^{20}_D$ −33.0 (*c* 4.5, CH_2Cl_2); R_f 0.46 (1:2 ethyl acetate/hexanes); IR (neat) 3282.6, 3027.1, 2955.7, 1735.8, 1644.2, 1538.1, 1367.5, 1153.4, 699.2 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.5−7.2 (m, 15H), 6.74 (br s, 1H), 6.05 (d, J = 8.1 Hz, 1H), 4.33 (q, J = 6.3 Hz, 1H), 3.58 (dd, J = 6.3, 15.6 Hz, 1H), 3.23 (dd, J = 4.5, 15.6 Hz, 1H), 2.98 (t, J = 10.5 Hz, 2H), 2.56−2.51 (m, 3H), 1.7−1.3 (m, 8H), 1.50 (s, 9H), 0.95

(t, 6H), 0.90 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 176.5,172.4, 171.9, 141.1, 134.8, 134.7, 134.2, 133.6, 129.9, 129.8, 128.4, 128.3, 128.2, 128.1, 126.0, 81.7, 51.5, 45.8, 41.6, 40.4, 38.4, 31.9, 28.0, 26.1, 25.6, 24.9, 22.6, 22.4, 15.0; ^{29}Si NMR (99 MHz, $CDCl_3$) δ −10.2; exact mass (FAB) MH^+ calcd for $C_{39}H_{55}N_2O_4Si$ 643.3931, found 643.3944.

2(*S*)-[2(*S*)-((Difluoro[(3-phenylpropionylamino)methyl]silanyl)methyl)-4-methylpentanoylamino]-4-methylpentanoic Acid (22). To a 0 °C solution of **20b** (109 mg, 0.17 mmol) in methylene chloride (6.0 mL) was added trifluoromethanesulfonic acid (0.25 mL, 2.8 mmol). The mixture was stirred for 35 min at 0 °C, and then ammonium hydroxide (0.30 mL of a 14.8 N solution) was added. The solution was stirred for an additional 35 min at 0 °C, and hydrofluoric acid (0.20 mL of a 48% solution) was then added. The solution was stirred for 10 min. The colorless precipitate was collected to give **22** (53.7 mg, 67%): mp 106−108 °C; $[\alpha]^{20}_D$ −16.2 (*c* 0.21, acetone); IR (neat) 3378, 3310, 1720, 1623, 1600, 1541, 1208, 871 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 8.62 (bs, 1H), 7.29−7.25 (m, 5H), 5.61 (s, 1H), 4.50 (q, J = 3.3 Hz, 1H), 2.98 (t, J = 7.5 Hz, 2H), 2.68 (m, 3H), 2.34 (d, J = 3.0 Hz, 2H), 1.81 (m, 1H), 1.67−1.60 (m, 4H), 1.26 (m, 1H), 1.06 (m, 2H), 0.93−0.90 (m, 9H), 0.84 (d, J = 6.3 Hz, 3H); ^{13}C NMR (63 MHz, acetone-d_6) δ 178.2,177.1, 174.1, 141.1,129.2, 129.1, 127.0, 51.4, 45.6, 41.5, 41.4, 40.8, 35.1, 31.8, 26.5, 25.4, 23.4, 22.8, 21.9; ^{19}F NMR (235 MHz, Acetone-d_6) δ −119 (d, J = 20 Hz), −124 (d, J = 20 Hz); exact mass (FAB) MNa^+ calcd for $C_{23}H_{36}N_2O_4F_2NaSi$ 493.2310, found 493.2303.

2-(*S*)-[2-(*S*)-((Dihydroxy[(3-phenylpropionylamino)-methyl]silanyl)methyl)-4-methylpentanoylamino]-4-methylpentanoate Sodium Salt (23). To a solution of **22** (30 mg, 63.7 μmol) in D_2O (0.5 mL) at rt was added NaOH (10 mg, 0.25 mmol) in D_2O (0.1 mL). The reaction was monitored by ^{19}F NMR. After 20 min, the reaction was complete as judged by the disappearance of the two fluorine signals of **22** and the appearance of a single fluoride signal: ^{1}H NMR (400 MHz, D_2O) δ 7.26−7.11 (m, 5H), 4.06 (dd, J = 6.4, 9.2 Hz, 1H), 2.79 (t, J = 7.2 Hz, 2H), 2.48−2.35 (m, 5H), 1.50−1.29 (m, 5H), 1.17−1.10 (m, 1H), 0.91−0.82 (m, 9H), 0.69 (d, J = 6.4 Hz, 3H), 0.61 (t, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, 1% CD_3CN in D_2O) δ 181.0, 180.6, 176.3, 141.6, 129.6, 129.3, 127.3, 54.5, 44.7, 41.8, 40.5, 38.4, 32.4, 30.0, 26.6, 25.4, 23.6, 23.3, 22.1, 21.7, 19.9;

Enzyme Assay. Thermolysin was obtained from a commercial supplier and used without purification. Stock solutions of Tris (50 mM), NaBr (2.5 M), and $CaCl_2$ (10 mM), pH 7.0 were prepared and stored at 4 °C. A stock solution of FAGLA (*N*-[3-(2-furyl)acryloyl]glycyl-L-leucinamide) was prepared in DMF, diluting with buffer to a final concentration of 0.1 M Tris, 0.1 M NaBr, and 2.5 mM $CaCl_2$, pH 7.0 (final concentration of DMF, 2.5%).

The enzyme and inhibitor **22** were incubated for 15 min at 25 °C in a temperature-regulated cuvette, and then the FAGLA solution was added to give a substrate concentration of either 0.8 or 1.5 mM. Initial velocities were determined for ≤10% reaction and were performed in triplicate for each inhibitor concentration, interspersing controls without inhibitor after every two runs with inhibitor.

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Supporting Information Available: Proton and carbon NMR spectra for all new compounds, the Dixon plot, and experimental details for the preparation of **13**, **14**, **20a**, **21**, and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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