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Silanediol-Based Inhibitor of Thermolysin†

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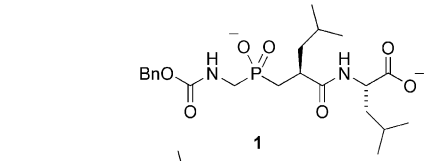
Abstract—The first silanediol inhibitor of thermolysin is reported, prepared by analogy with the Grobelny/Bartlett phosphinate inhibitor. A Cbz group on nitrogen proved to be unstable to the triflic acid mediated silanediol deprotection and was replaced with a dihydrocinnamoyl group. The silanediol was prepared in high purity by hydrolysis of a difluorosilane intermediate and proved to be an effective inhibitor, differing from the phosphinate by a factor of 4 ($K_i = 41$ nM).

Thermolysin is the archetype metalloprotease and has been one of the most intensively studied examples.[2] In the accepted mechanism for peptide hydrolysis, the active site zinc activates the scissile amide carbonyl, delivers water, and stabilizes the resulting tetrahedral intermediate.[3]

Several analogous metalloproteases are of significant pharmaceutical importance,[4] including angiotensin-converting enzyme (ACE),[5] and the matrix metalloproteases.[6] Additional metalloprotease targets of note include TNF-α converting enzyme,[7,8] anthrax lethal factor,[9] and the botulinum toxin.[10]

Silanediols are new transition state analogue inhibitors, effective with both aspartic[11] and metalloproteases.[12,13] Silanediol **3** (Fig. 1) has recently been described as an ACE inhibitor.[12,13] As a vehicle for comparison and understanding of protease inhibitors, thermolysin provides a useful benchmark. The Grobelny/Bartlett phosphinate **1**[14,15] is a low nanomolar inhibitor of thermolysin and an attractive starting point for design of a silanediol-based inhibitor. Silanediols are best known for their self-condensation, forming silicone polymers (siloxanes),[16] but silanol polymerization can be inhibited by steric effects. In comparison with **3** and the silanediol HIV protease inhibitors,[11] the steric environment around the silanediol in **2** is less hindering, providing a new level of silanediol exposure for evaluating the stability and utility of these new structures. Compound **1** would provide a nearly ideal comparison with silicon-based inhibitor **2** because the center of the tetrahedral transition state analogues of **1** and **2** are both second row elements. We describe here chemistry directed toward the synthesis of **2**.

A straightforward construction of **2** was expected to involve a protected version of **4** (Fig. 2), which could be generated from the commercially available chloromethyltrichlorosilane **5** and enantiomerically pure lithium reagent **6**. A diphenylsilane (**4**) was chosen as a silanediol



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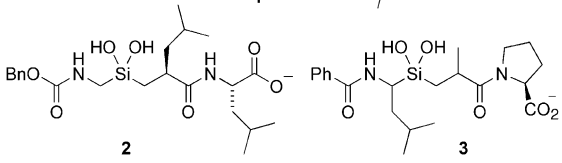
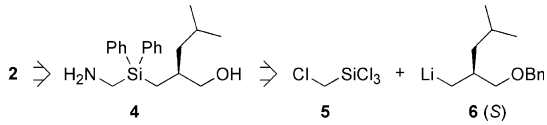


2 **3**

Figure 1. Phosphinate inhibitor of thermolysin **1**, silanediol analogue **2**, and ACE inhibitor **3**.



4 **5** **6** (*S*)

Figure 2. Synthesis of **2** via diphenylsilane **4**, derived from commercially available **5** and enantiomerically pure lithium reagent **6**.

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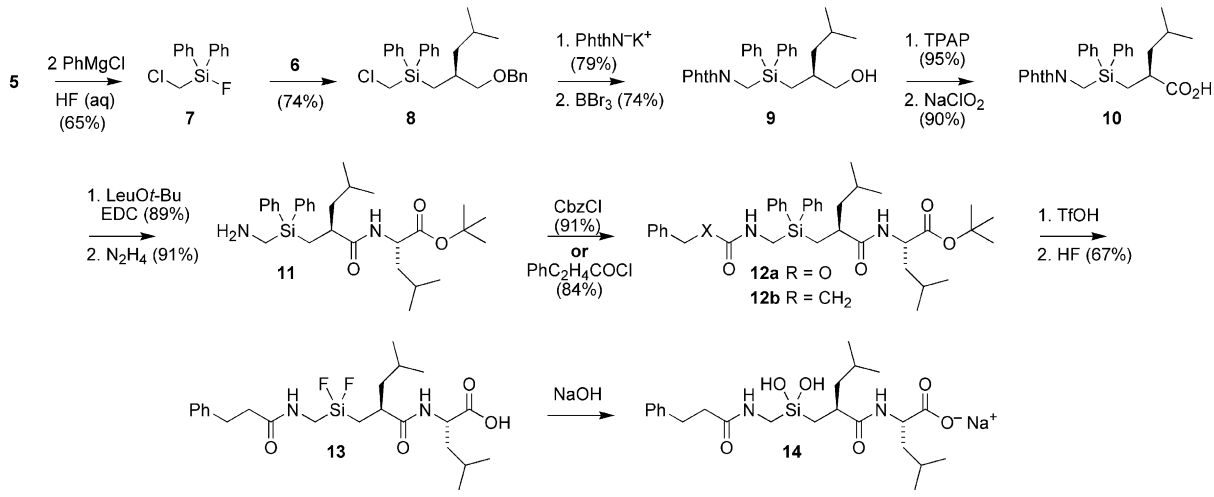


Scheme 1. Synthesis of silanediol **14**.

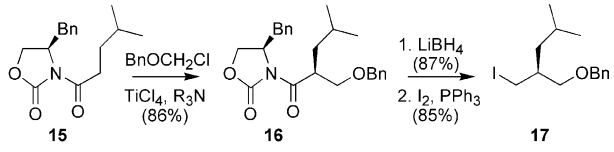
precursor based on the successful use of this strategy for preparation of **3**. The phenyl–silicon bond is stable to a wide range of chemical transformations, yet hydrolysis to a silanediol was readily achieved within the tripeptide analogue structure **3** under strongly acidic conditions.[13]

To prepare **2**, chloromethyltrichlorosilane (**5**) was coupled with two equivalents of phenylmagnesium chloride, followed by treatment of the resulting product with aqueous HF[17] (Scheme 1). The resulting fluorosilane (**7**) was isolated by distillation in 65% overall yield. Fluorosilanes were shown by Eaborn to be moisture stable reagents that undergo efficient coupling with organometallics.[17]

Lithium reagent **6** was prepared from the corresponding iodide **17** (Scheme 2) by metal-halogen exchange,[18,19] which in turn was prepared using Evans' asymmetric alkylation chemistry.[20]

Addition of **6** to fluorosilane **7** gave silane **8** in 74% yield (Scheme 1). Gabriel synthesis was used to convert the chloride of **8** to a protected amine. Treatment of **8** with potassium phthalimide in DMF, was followed by three equivalents of boron tribromide to cleave the benzyl ether, forming alcohol **9**. Notably, the potential reaction of the phenyl-silicon bond with this Lewis acid was not observed.[21,22] Oxidation of the alcohol to the acid **10** was performed in two stages, TPAP followed by sodium chlorite. Coupling of this acid with the *tert*-butyl ester of L-leucine gave the amide as a single diastereomer, confirming that epimerization of the stereogenic center of **10** had not occurred.

Hydrazinolysis removed the phthalimide group to give amine **11** in high yield and without epimerization. This amine was coupled with benzyl chloroformate. Despite



Scheme 2. Preparation of enantiomerically pure iodide **17**.

what appeared to be adequate precedent for the stability of a benzyl carbamates to treatment with triflic acid,[23] a survey of conditions for triflic acid-mediated hydrolysis of the phenyl–silicon bonds in **12a** convinced us that it could not be accomplished without loss of this Cbz group. We therefore turned our attention to **12b**, substituting dihydrocinnamoyl for benzyloxycarbonyl.

Inspection of the crystal structure of inhibitor **1** bound to the thermolysin active site[24] shows that the Cbz benzyl group makes little contact with the enzyme, and therefore substitution of phenethyl **14** for benzyloxy **2** would be expected to have little, if any, effect on the binding.

Treatment of **12b** with an excess of triflic acid in methylene chloride at 0 °C, followed by addition of ammonium hydroxide, led to the removal of the *tert*-butyl ester and both phenyl groups on silicon. The addition of ammonium hydroxide serves to neutralize the excess triflic acid and to ensure that any bonds between the amides and the silicon that may have formed during the silicon-phenyl bond cleavage had been hydrolyzed.[11,12]

The silanediol product from this hydrolysis could be isolated directly, but was most cleanly generated by conversion of the product to a crystalline difluorosilane **13** that precipitated when the silanediol was stirred with aqueous HF. It is likely that the structure of **13** involves a hypervalent silicon.[25,26] When **13** was taken up in water and treated with three equivalents of sodium hydroxide,[17] the diastereotopic fluorines on silicon (^{19}F NMR in acetone-d_6, δ −119 and −124 ppm, $J = 20$ Hz,) rapidly hydrolyzed to give silanediol **14** that was spectroscopically pure.

Inhibition of thermolysin (Sigma) by **14** was evaluated using the substrate *N*-[3-(2-furyl)acryoyl]glycyl-L-leucinamide (FAGLA, Sigma) following the method of Feder.[27] Silanediol **14** showed competitive binding and a $K_i = 41$ nM. This value is only slightly higher than that reported for phosphinate **1**, with a $K_i = 11$ nM.[15]

The inhibition of this metalloprotease by a neutral species not known for its ability to coordinate metals (the silanediol) could be viewed as surprising. Eliminating

the negative charge associated with phosphinate **1**, a group that binds to the positively charged active site zinc, might be expected to result in a lower affinity for the enzyme. The effect of the acidity and related attributes of the phosphorus has received considerable attention, but all of the examples are negatively charged.[14,15,28] Notably, charged species carry solvation that must be displaced during a binding event.

The structure of **14** differs from that of **1** in two ways. The replacement of the Cbz group in **1** by the dihydrocinnamoyl group replaces an oxygen with a methylene group. This exchange was anticipated to have a minimal effect on the binding, based on inspection of the crystal structures of inhibitors bound to the thermolysin active site.[24] The substitution of silicon for phosphorus, an exchange of two second row elements, eliminates the charge on the transition state analogue. In addition, there are intrinsic differences in bond angles and bond lengths for the central silicon and phosphorous units.[29]

Conclusions

Silanediol **14** is an effective inhibitor of thermolysin, with a K_i similar to that of the Grobelny/Bartlett phosphinate **1**.

Silanediols, as potentially useful structural units, suffer from a reputation for irreversible condensation to form siloxane polymers. Some condensation of **14** may have been observed under the acidic and basic conditions used for hydrolysis of the phenyl–silicon bonds. Nevertheless, the transient conversion of crude silanediol to a difluorosilane and subsequent hydrolysis results in a very pure silanediol product. A similar hydrolysis of difluorosilanes to yield silanediols was reported by Eaborn in 1952[17] and a recent example was reported by Organ et al.[30] The resulting silanediol **14** can be precipitated as stable monomeric species, or left in aqueous solution for weeks (at least) without decomposition. Alternative chemistry to prepare **2**, the precise silanediol analogue of **1** carrying the Cbz group, is currently under study.

Acknowledgements

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