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Combinatorial Synthesis of a Small-Molecule Library Based on the Vinyl Sulfone Scaffold

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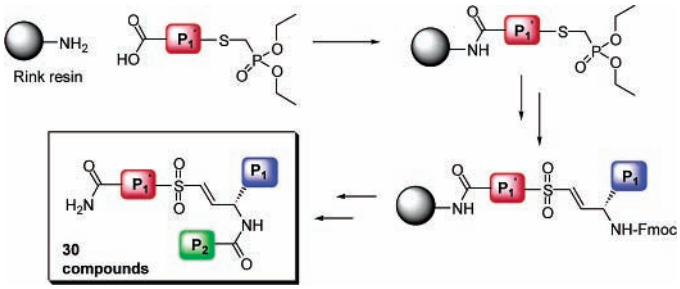
ABSTRACT



A 30-member library of small molecules based on the vinyl sulfone scaffold was prepared on rink amide resin, using solid phase-based reactions such as oxidation and Horner–Wadsworth–Emmons reaction. The library was designed such that three points of diversity were readily introduced in the library to accommodate the S_1', S_1, and S_2 binding pockets of different cysteine proteases, making the strategy suitable for high-throughput generation of potential cysteine protease inhibitors.

Cysteine proteases have drawn extensive attention from pharmaceutical industries in recent years since many enzymes belonging to this class are critically involved in major human diseases, including diseases related to viral infections (i.e., SARS), strokes, cancer, Alzheimer's disease, inflammation, arthritis, and many others,[1] making them excellent therapeutic targets. Over the last few decades, many research groups have developed chemical approaches capable of generating diverse small-molecule inhibitors that target different classes of cysteine proteases with various degrees of efficacy.[2] Among the different classes of inhibitors, molecules containing different types of Michael acceptors are extremely potent and highly specific toward cysteine proteases. They work by irreversibly inactivating the catalytic cysteine residue within the active site of the enzyme (Figure 1). Many of these inhibitors, including α,β-unsaturated ketones, acrylamides, vinyl sulfones, etc., have been successfully synthesized, and some have been tested in clinical experiments.[2−4]

With the emergence of microarray technologies and proteomics in the past few years,[5] peptidyl vinyl sulfones, in particular, have been shown to be extremely useful as

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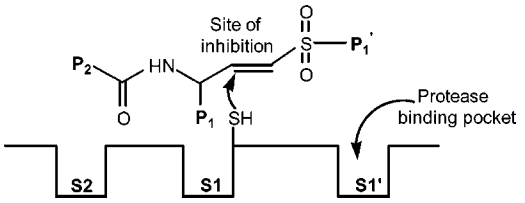
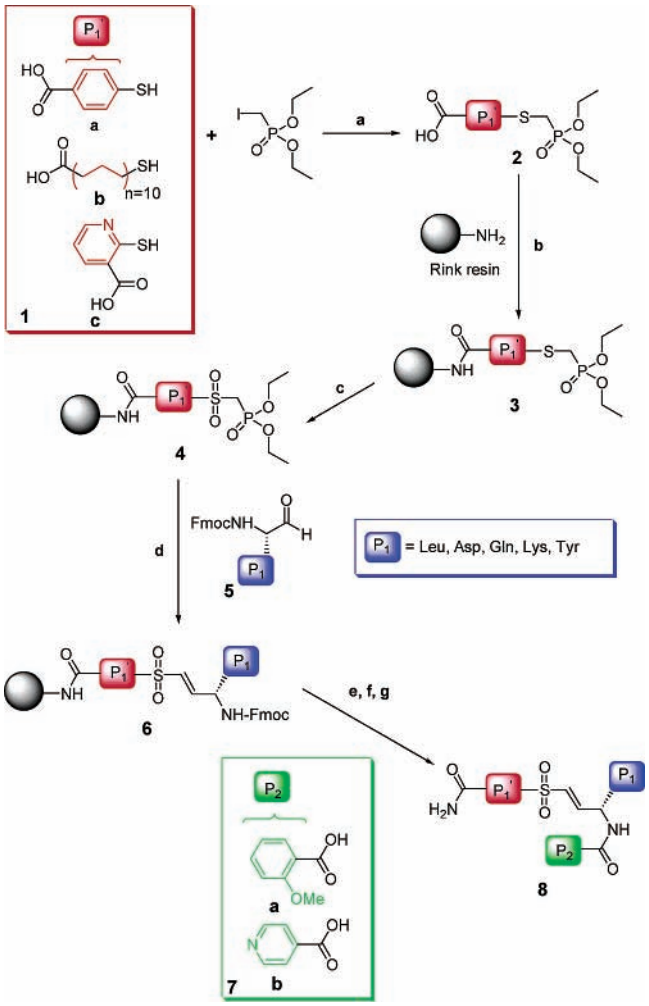


Figure 1. Binding of a vinyl sulfone to the active site of a cysteine protease.

activity-based probes for high-throughput profiling of cysteine proteases in both gel- and microarray-based experiments,[4,6] largely due to their negligible cross-reactivity toward other classes of enzymes.[2b] Under physiological conditions, the electrophilic, vinyl sulfone moiety of the inhibitor is not reactive toward most nucleophilic elements present in a biological species (i.e., amine and thiol groups in a protein).[3a] However, upon binding to the active site of an enzyme having a catalytic cysteine residue, i.e., a cysteine protease, the vinyl sulfone would react, at a highly specific rate, with the thiol in the cysteine residue, which results in the formation of a covalent enzyme−inhibitor adduct, leading to subsequent irreversible inactivation of the enzyme.[7]

A number of strategies have been developed for the chemical synthesis of both peptide- and nonpeptide-based vinyl sulfone libraries.[3,4] Traditionally, peptide vinyl sulfones were prepared by methods based on conventional solution-phase peptide synthesis.[3a] Recently, a number of solid-phase strategies have been developed.[3b−e,4] Overkleeft et al. first synthesized the N-terminal peptide fragment of the vinyl sulfone on a solid support, followed by nucleophilic cleavage/ligation using a desired vinyl sulfone-containing, C-terminal amino acid.[4b] Bogyo et al. reported solid-phase methods to generate positional-scanning combinatorial libraries of peptide vinyl sulfones, with variations at the P_2−P_4 positions of the library,[3b−e] by attaching a vinyl sulfone-containing aspartic acid at the P_1 position onto a rink amide resin via its side-chain carboxylic acid (Figure 1). However, this strategy is limited only to synthesis of peptide vinyl sulfones having carboxyl side chains at the P_1 positions (e.g., Asp and Glu). In our own work,[4a] we recently reported a solid-phase strategy for the synthesis of peptide vinyl sulfones having different P_1 residues. By anchoring the vinyl sulfone-derivatives onto 2-chlorotrityl resin via the phenolic alcohol moiety at the P_1' position, we were able to install diversities at any given P position of the inhibitor (i.e., P_1, P_2, etc.). None of these methods, however, allows the facile solid-phase synthesis of vinyl sulfone libraries having variations at the P_1' position, which is known to be critical for binding to the S_1' site of the enzyme active site. Herein, we report the first strategy for solid-phase synthesis of vinyl sulfone

Scheme 1. Solid-Phase Synthesis of Vinyl Sulfones[a]



[a] Reagents and conditions: (a) Cs_2CO_3 (2.1 equiv) or DBU (2.1 equiv), DMF, 6−12 h at room temperature, 60−85%. (b) Rink amide resin (0.7 mmol/g), DIC (3.3 equiv), HOBt (3.3 equiv), DIEA (6 equiv), DMF (10 mL/g resin), 6 h at room temperature, 0.65−0.7 mmol/g loading. (c) *m*-CPBA (3.5 equiv), DCM (10 mL/g resin), 1−1.5 h at room temperature, 95−100%. (d) LHMDS (5 equiv; 1.0 M in THF), 30 min with resin, washed with THF, then Fmoc-AA-CHO (4 equiv) in THF (20 mL/g resin), 2 h at room temperature, 0.2−0.4 mmol/g loading. (e) DBU/HOBt/DMF (2:1:97, 10 mL/g resin), 10 min at room temperature. (f) **7** (8 equiv), DIC (7.5 equiv), HOBt (7.5 equiv), DIEA (8.5 equiv), DMF, 6 h at room temperature. (g) TFA/DCM/H_2O (50:45:5, 15 mL/g resin), precipitate in cold ether.

molecules, which allows easy introduction of diversities at not only the P positions (e.g., P_1 and P_2) but also the P_1' position of the inhibitor. As proof-of-concept experiments, we have successfully applied this strategy to the synthesis of a 30-member small-molecule-based (i.e., MW < 500) vinyl sulfone library that installs three, five, and two different variables at P_1', P_1, and P_2 positions of the inhibitor, respectively (Scheme 1).

As shown in Scheme 1, steps in our synthesis include (a) solution-phase nucleophilic reaction between a commercially available thiol-containing acid, **1**, and diethyl iodomethyl phosphonate to generate the sulfide phosphonate **2**; (b)

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loading of **2** onto rink amide resin to give **3**; (c) solid-phase oxidation of **3** to give sulfone **4**; (d) solid-phase Horner−Emmons reaction with an amino acid-derived, Fmoc-protected aldehyde to give **6**; and (e) deprotection of Fmoc group under optimized conditions followed by (f) acylation and (g) TFA cleavage to release the desired vinyl sulfone **8** in solution. In contrast to what we previously reported,[4a] our current approach takes advantage of the successful implementation of both *m*-CPBA oxidation of sulfide **2** and its subsequent Horner−Emmons reaction on solid support with high yield and purity, thus making it possible to install three points of diversity (i.e., P_1', P_1, and P_2) within the vinyl sulfone scaffold.

Starting from three mercaptoacids (**1a**−**c**), the nucleophilic reaction proceeded smoothly with diethyl iodomethyl phosphonate in the presence of a suitable base (i.e., DBU or NaH for **1a** and **1c**, Cs_2CO_3 for **1b**, respectively) to give **2a**−**c** in good yields (60−85%). When aliphatic, 11-mercapto undecanoic acid **1b** was used with bases such as DBU, NaH, or NaOEt, the major product isolated upon workup was the disulfide, rather than the desired product **2b**. This problem was overcome by using Cs_2CO_3 as the base in the reaction, producing the desired product in high yield (85%). The sulfide phosphonates **2a**−**c** were conveniently loaded onto the rink amide resin using the standard DIC/HOBT/DIEA coupling procedures to afford the resin-bound sulfide phosphonates **3a**−**c** with high substitution levels (0.65−0.7 mmol/g). It should be noted that, while we prefer to perform step **a** in solution to generate **2a**−**c** from **1a**−**c**, which is commercially available and requires no further protection, one could alternatively (i) block the thiols in **1a**−**c** with a suitable protecting group, (ii) load them on the solid support, (iii) deprotect the thiol groups, and (iv) react with diethyl iodomethyl phosphonate on solid phase to give **3a**−**c** (data not shown). All subsequent steps (steps **c**−**g** in Scheme 1) were performed on the solid support. To optimize our synthetic conditions, a small portion of the resin following each step of synthesis was cleaved, and the product was analyzed directly by LC-MS to assess its % conversion (judged by disappearance of the starting material) as well as % purity (judged by the product HPLC profile; see Supporting Information for details). Oxidation of **3a**−**c** was accomplished by treatments of the resin with *m*-CPBA (5 equiv) for 1−1.5 h, to give **4a**−**c** in nearly quantitative yields (95−100%). Shorter oxidation times gave rise to the formation of the partially oxidized products, the sulfoxide phosphonates. Next, the resin-bound sulfone phosphonates, **4a**−**c**, were used as the precursors in the Horner−Emmons reaction. Horner−Emmons reaction is a valuable carbon−carbon bond-forming reaction that has been successfully applied in the solid-phase synthesis of olefins and some peptidyl Michael acceptors.[8] In our strategy, the reaction was used to generate the critical vinyl sulfone scaffold and at the same time introduce the P_1 diversity in the library. Most published Horner−Emmons protocols require the presence of an excess of a strong base in order for the reaction to go to completion. They are therefore not suitable for our synthesis because the presence of an excess of a strong base may (1) cause the Fmoc group in the aldehyde to be cleaved off prematurely, (2) lead to severe epimerization at the aldehyde chiral center, and (3) lead to potential epimerization in the vinyl sulfone product (i.e., **6** in Scheme 1).[9] Consequently, it is necessary to optimize the Horner−Emmons conditions in our strategy such that the conversion of **4** to **6** is highly quantitative, while maintaining premature Fmoc cleavage, as well as minimal epimerization in the reaction (Table 1).

Table 1. Horner−Emmons Reaction from **4** to **6** under Different Conditions

base	conditions[a]	equiv	% conversion[b] **4** → **6**	% purity[b]
LDA	50 min, −78 °C	6	30%	nd[c]
LDA	30 min, 0 °C	4	100%	64%
LHMDS	30 min, 25 °C	5	100%	>95%
DBU	90 min, 25 °C	8	<10%	nd[c]
KHMDS	60 min, 25 °C	5	65%	nd[c]
NaH	60 min, 25 °C	5	45%	38%

[a] LHMDS and LDA were 1 and 2 M solutions, respectively, in THF. Time indicated is the incubation time of resin **4** and the base, before addition of Fmoc-AA-CHO. [b] Determined by HPLC, including both epimers (see main text for discussion). All conditions shown in Table 1 were optimized to maintain epimerization to <10%. Other conditions caused substantial epimerization (data not shown). [c] nd = not determined.

We accomplished this by adopting a two-step procedure, which involves treatments of the resin-bound sulfone phosphonates, **4a**−**c**, first with an excessively strong base (i.e., LHMDS) and then, upon removal of the base, with a Fmoc-protected amino acid aldehyde **5** (prepared on the basis of procedures previously described[4a]) under Horner−Emmons conditions. Typically, the reaction was performed under anhydrous conditions at room temperature. Resin **4** was treated with 1 M LHMDS solution (in THF) for 30 min and then washed with excess anhydrous THF under N_2. The Fmoc-AA-aldehyde solution (in THF) was subsequently added to the resin, and the reaction was further incubated for 1−2 h. To accurately determine the enantiomeric purity of the product generated from the Horner−Emmons reaction, upon completion of the reaction (step **d** in Scheme 1), the product, **6**, was first deprotected and then coupled to an optically pure amino acid. The resulting dipeptide was then cleaved off the resin, and analyzed directly by HPLC. The extent of epimerization in **6**, as well as its purity under different reaction conditions, was estimated from the resulting HPLC profile, by comparing the ratio of two separable peaks arising from the two diastereomeric dipeptides in the product mixture (as a result of epimerization in the Horner−Emmons

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(9) Epimerization of **6** could possibly occur in two ways. One, the chiral center in the starting material, amino aldehyde **5**, may be directly epimerized, leading to generation of both isomers in product **6**. Alternatively, deconjugation of the vinyl sulfone in product **6** to generate an allylic sulfone, followed by reconjugation, could also lead to direct epimerization of **6**. In our studies here, we cannot rule out either possibility.

reaction; see Supporting Information). As shown in Table 1, five different bases (e.g., DBU, LHMDS, KHMDS, LDA, NaH) were tried with different reaction times and temperatures. Only LHMDS under optimized reaction conditions (i.e., 5 equiv, 30 min, 25 °C) produced the best result, giving rise to quantitative conversion from **4** to **6** (100% conversion) and at the same time with nearly quantitative yield (>95% including both epimers). The degree of epimerization in **6** under these conditions was determined to be <10% (Supporting Information). Consequently, all of our subsequent work was carried out using the optimized conditions, unless otherwise specified. Different Fmoc-AA-aldehydes, **5a**−**e**, have been used together with different resins, **4a**−**c**, to generate the resin-bound vinyl sulfones, **6**, with high yields and purities and minimal epimerization.[10] We also observed only the trans isomers in all Horner−Emmons products **6**.

After the Horner−Emmons reaction, we continued to steps **e**−**g**, by deprotecting the Fmoc group in **6**, acylating the resulting free amine with different acids, **7a**,**b**, followed by TFA cleavage to generate the final products, **8**. It was found that, while the standard Fmoc cleavage conditions (20% piperidine in DMF for 30 min) worked well in step **e** for reactions involving resin **4b**, they failed with resins **4b**,**c**, generating mostly side products, which were subsequently determined to be adducts derived from the addition of piperidine to the double bond of the vinyl sulfone (Supporting Information). We reasoned that this may be due to the electron-withdrawing nature of the aromatic substituents at P_1' position in the two resins, which increase the reactivity in the vinyl sulfone moiety, making it more susceptible to attacks from nucleophiles such as piperidine. This is consistent with previous observations,[11] in which different derivatives of vinyl sulfones having varying P_1' substituents had different degrees of reactivity toward base-catalyzed additions of thiol compounds. We therefore modified the Fmoc cleavage conditions. By using a solution containing 2% DBU and 1% HOBt in DMF,[12] we were able to efficiently cleave Fmoc from different resins in 10 min while maintaining the integrity of the vinyl sulfone in the products. Upon Fmoc cleavage, the free amine was further acylated with either anisic acid (**7a**) or isonicotinic acid (**7b**) using standard DIC/HOBT/DIEA coupling methods. This installed the P_2 position in our vinyl sulfone library. The resulting final products were subsequently deprotected, cleaved off the resin using TFA/DCM/H_2O (5/4.5/0.5), and precipitated in cold ether. In all, a total of 30 different vinyl sulfones were prepared. The purity and identity of each product was assessed by direct analysis of the precipitate using LC-MS. All desired products were unambiguously confirmed and determined to have varying degrees of purity (40−95%; see Supporting Information). For products generated using resins **3a** and **3b**, the purity of the desired products ranges from 70 to 95%, which may be used directly for subsequent screenings without any further purification. However, for those vinyl sulfones generated from resin **3c**, the purity is typically between 40 and 70%. This low purity in products generated from resin **3c** may be due to the enhanced reactivity in the vinyl sulfone moiety in **3c**, as mentioned earlier, which could have caused side reactions in our synthesis.

In summary, we have developed the first strategy for solid-phase synthesis of a small-molecule library based on the vinyl sulfone scaffold, which allows facile installation of diversities not only at the P positions but, more importantly, at the P_1' position. This strategy utilizes multiple steps of solid-phase reactions, facilitating the easy preparation of potentially large arrays of small molecules that contain the important vinyl sulfone pharmacophore. Given the enormous interest in developing various protease inhibitors, this strategy may find broad applications in the studies of cysteine proteases.

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Supporting Information Available: Experimental details and characterizations of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Typical yields were ~90−95%, with high purity of the desired products (90%) and minimal epimerization (<10%).

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