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Total Synthesis of K777: Successful Application of Transition-Metal-Catalyzed Alkyne Hydrothiolation toward the Modular Synthesis of a Potent Cysteine Protease Inhibitor

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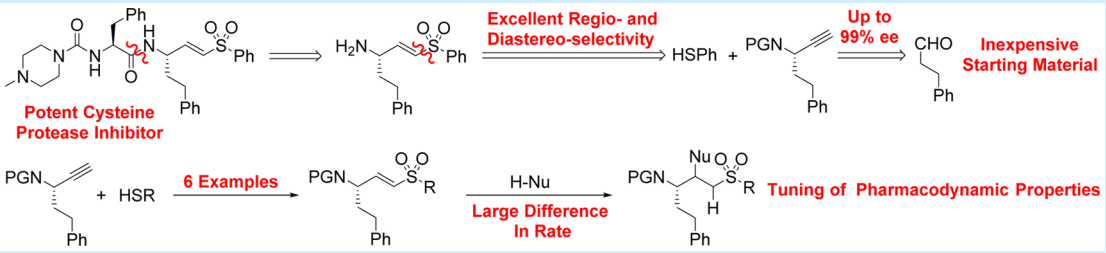
Supporting Information



ABSTRACT: We report the total synthesis of K777 and a series of analogues via alkyne hydrothiolation catalyzed by Wilkinson's complex ($ClRh(PPh_3)_3$). The alkyne hydrothiolation reactions proceeded with excellent regio- and diastereoselectivity to generate the desired *E*-linear vinyl sulfides in high yield. The use of Ellman's auxiliary generates the requisite propargyl amines in excellent enantiomeric excess (ee) and obviates the use of L-homophenylalanine, an expensive unnatural amino acid. The vinyl sulfone derivatives exhibit a large difference in rate toward Michael addition. Kinetic data are consistent with rate-limiting nucleophilic attack to generate the carbanion intermediate.

The design of peptidomimetic molecules for the inhibition of cysteine proteases is a popular strategy for the treatment of a variety of neglected diseases.[1−6] In particular, K777, a cysteine protease inhibitor, is gaining considerable attention for the treatment of American Trypanosomiasis (Chagas disease), a potentially fatal parasitic infection endemic in Latin American countries.[7,8] The vinyl sulfone of K777 irreversibly inhibits Cruzain, the major cysteine protease of Trypansoma cruzi, the causative organism of Chagas disease.[7−10] Inhibition occurs upon Michael addition of the active site cysteine residue to the electrophilic vinyl sulfone "warhead", resulting in a covalently bound enzyme complex.[11] K777 has shown comparable efficacy in preclinical studies to drugs currently in use without the multitude of significant side effects of those drugs.[9,12−16] As a consequence of the specificity of vinyl sulfone peptidomimetics, molecules with this functional group are becoming attractive drug candidates for the treatment of cancer and neglected global diseases, such as Malaria and African sleeping sickness.[2,4,5,17]

Given our interest in the development of catalytic methods for the formation of vinyl sulfides,[18−20] we elected to test such a strategy in the synthesis of K777. We anticipated that alkyne hydrothiolation with Wilkinson's catalyst would generate the *E*-linear vinyl sulfide, which upon oxidation would provide the requisite vinyl sulfone (**3**). Subsequent peptide coupling with the known fragment **2** would produce K777 (Scheme 1). Notably, this constitutes the first use of transition-metal-catalyzed alkyne hydrothiolation in total synthesis.[23]

This approach possesses several advantages over the reported preparation of K777,[11] most notably in eliminating L-homophenylalanine as a chiral building block. Although our approach does involve the use of a rhodium catalyst and the Ellman auxiliary (vide infra), both have been shown to be recyclable.[24,36,37] Additionally, analogues of K777 can be readily synthesized via alkyne hydrothiolation by simply varying the thiol employed. In comparison, Horner−Wadsworth−Emmons (HWE) olefination is used in the reported synthesis, which, in addition to low atom efficiency, requires the synthesis of each phosphonate reagent.[21,25]

We anticipated that an appropriately protected propargyl amine (**4**) could serve as a chiral building block en route to K777 (Scheme 1). The asymmetric synthesis of **4** could be accomplished via a diastereoselective ethynylation of a sulfinimide derived from Ellman's auxiliary.[26,27] To this end, condensation of hydrocinnamaldehyde (**5**) with (*S*)-*N*-*tert*-butanesulfinamide (**6**) gave the corresponding sulfinimide (**7**) in 92% yield (Scheme 2). Addition of (trimethylsilylethynyl)-magnesium chloride at low temperature provided **8** as a single diastereomer in 73% yield by NMR.[38] Desilylation with *tert*-butylammonium fluoride (TBAF), hydrolysis of the sulfinamide, and diprotection of the free amine as a *tert*-butylcarbonate (Boc) provided the target propargyl amine (**4**) in 79% yield over three steps.

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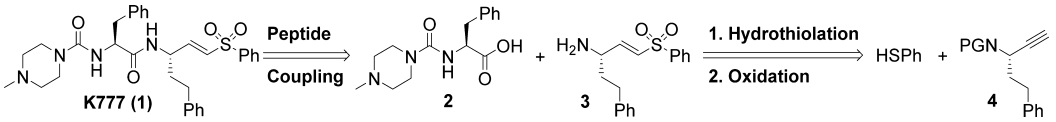
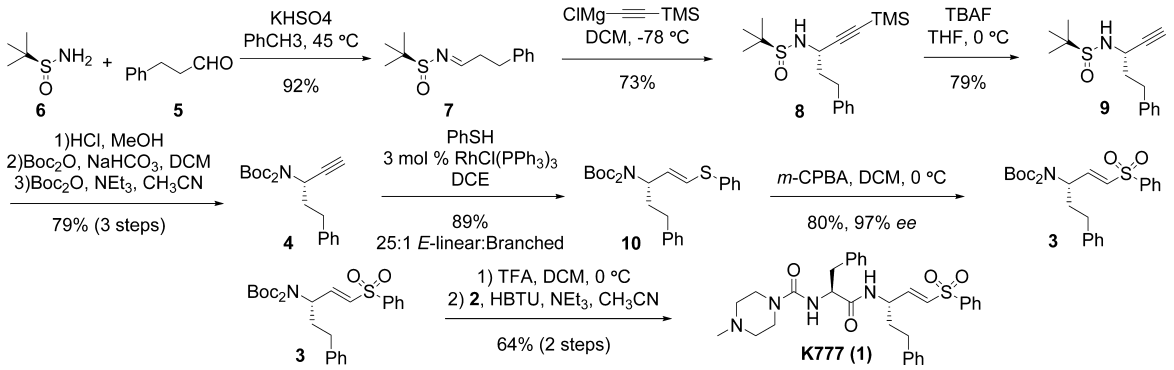
Scheme 1. Alkyne Hydrothiolation Approach to K777



Scheme 2. Synthesis of K777



With alkyne **4** in hand, we were poised to investigate alkyne hydrothiolation with Wilkinson's catalyst. Initial studies revealed that double protection of the amine was required for the highly selective formation of the *E*-linear vinyl sulfide; a monoprotected amine resulted in poor selectivity for the *E*-linear product.[28]

Gratifyingly, Boc-protected propargyl amine **4** underwent alkyne hydrothiolation with excellent regio- and stereoselectivity, in the presence of 3 mol % $ClRh(PPh_3)_3$ in DCE at room temperature, to produce the *E*-linear vinyl sulfide (**10**) in 89% yield (25:1 *E*-linear/branched). Subsequent oxidation with *m*-CPBA provided vinyl sulfone **3** in 80% yield and 97% ee. Lastly, deprotection of vinyl sulfone (**3**) with TFA, followed by amide coupling with the known peptide **2**,[22] afforded K777 (**1**) in 64% yield. The overall yield of this 10-step synthesis is 21% and is competitive with Palmer's reported yield of 34%,[21] considering the extra steps involved to generate the homophenylalanine stereocenter. Given the success of this strategy, we elected to study a series of other arene thiols as a means to generate analogues of K777.

To the best of our knowledge, few analogues have been reported in which the vinyl sulfone moiety was modified, despite this functionality being the reactive site in cysteine protease inhibition (Figure 1, P_1 and P_1' fragments).[22,29,30] The second-order inactivation constants of cruzain (k_i/K_I) have been reported for these analogues, including the sulfonate ester **12a**, and the benzyl and phenethyl sulfones **12b** and **12c**,

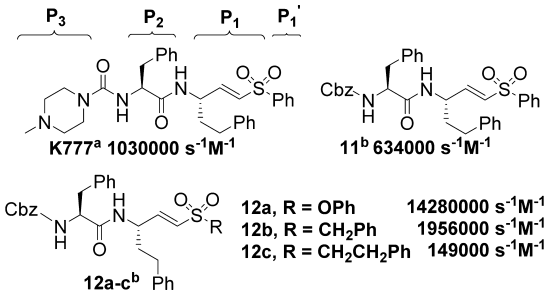


Figure 1. Second-order (k_i/K_I) inactivation rates of cruzain for K777 Analogues. [a] Reference 22. [b] Reference 29.

respectively. Sulfonamides were found to be poor inhibitors of cruzain. By comparing **11**, **12b**, and **12c**, the size of the P1′ derivative is seen to play a role in inhibition of cruzain, likely due to changes in binding affinity (K_I). However, comparing **12a** to **12b** reveals a large increase in reactivity for the more electrophilic sulfonate ester, likely due to an increase in k_i.[29]

We selected a series of *para*-substituted arene thiols, ranging from electron-rich to electron-poor, all providing good yields and excellent selectivity for the *E*-linear isomers **13**–**17** (Table 1). Notably, both *p*-chloro- and *p*-bromobenzene thiol were well-tolerated, without detectable cleavage of the C–Cl and C–Br bonds, respectively (entries 3 and 4). The vinyl sulfides **13**–**17** were then converted to the corresponding sulfones **18**–**22** upon oxidation with *m*CPBA. The vinyl sulfones **18**–**22** were subsequently deprotected with TFA and coupled with peptide **2**, providing analogues **23**–**27** in good yield.

Next we sought to understand the potential effect of varying the *para*-substituent (R) on cruzain inhibition. Following the work of Roush and co-workers,[31] we modeled the expected first-order rates of inhibition of cruzain (k_i) by measuring the solution phase rates of Michael addition for the series of substituted vinyl sulfones with a model thiol nucleophile (Table 2).

In polar, protic solvents such as methanol, addition of thiols to Michael acceptors involves two steps: nucleophilic attack to generate an enolate ion, followed by protonation.[33] Relative rates are highly dependent on the electrophilicity of the β-carbon and stabilization of the emerging enolate.[34,35] Consequently, Roush and co-workers found vinyl sulfonate ester **28** to be the most reactive Michael acceptor, reacting 25 times faster than vinyl sulfone **29** (Figure 2). This mimics the reactivity difference in cruzain inhibition between **12a** and **12b**.

Unsurprisingly, vinyl sulfones with electron-withdrawing substituents reacted much faster than those bearing electron-donating substituents. Compound **22** (R = CF_3, σ = 0.54)[32] reacted 16 times faster than compound **3** (R = H), and 49 times faster than **18** (R = OCH_3, σ = −0.27).[32] Comparison of half-lives is much more dramatic: whereas the half-life for compound **18** is 6 h at room temperature, the half-life for the CF_3 substituted vinyl sulfone is a mere 7 min.

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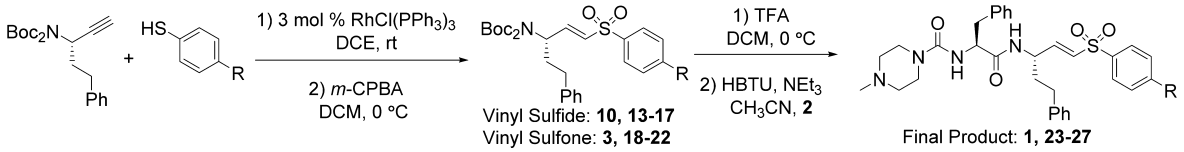
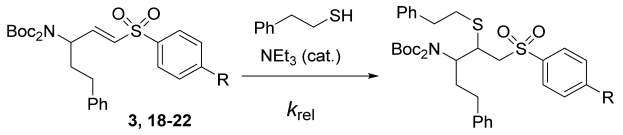
Table 1. Synthesis of *para*-Substituted Vinyl Sulfones



entry	R	vinyl sulfide	yield[a] (%)	*E*-linear: branched[b]	vinyl sulfone	yield[a] (%)	ee[c] (%)	final product	yield[a] (%)	overall yield[d] (%)
1	H	**10**	89	25:1	**3**	80	96	**1**	69	21
2	OCH_3	**13**	52	50:1	**18**	81	92	**23**	42	8
3	CH_3	**14**	81	50:1	**19**	79	98	**24**	78	21
4	Cl	**15**	88	50:1	**20**	68	99	**25**	37	9
5	Br	**16**	80	50:1	**21**	79	90	**26**	72	19
6	CF_3	**17**	84	50:1	**22**	61	98	**27**	41	9

[a]Isolated yield following column chromatography. [b]Determined by ^{1}H NMR spectroscopy. [c]Determined by chiral HPLC separation. Details are provided in the Supporting Information. [d]Linear yield from hydrocinnamaldehyde. Yield of **2** is 90%.

Table 2. Rates of Michael Addition of 2′-(Phenethyl)thiol with *para*-Substituted Vinyl Sulfones[a]



entry	R	σ[b]	k_{rel}	$t_{1/2}$ (min)
1	OCH_3 (**18**)	−0.27	0.34	360
2	CH_3 (**19**)	−0.17	0.52	231
3	H (**3**)	0	1	121
4	Cl (**20**)	0.23	3.49	35
5	Br (**21**)	0.23	1.35	90
6	CF_3 (**22**)	0.54	16.4	7

[a]Pseudo-first-order reaction rates were determined by ^{1}H NMR spectroscopy. Reactions performed in triplicate. [b]Reference 32.

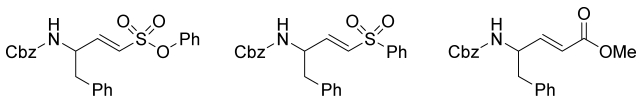


Figure 2. Selection of Michael acceptors assayed by Roush and co-workers (ref 31).

Measuring the rates of reaction for a series of *para*-substituted compounds allowed us to generate a Hammett plot for the reaction of 2′-(phenethyl)thiol with vinyl sulfones (Figure 3). The high positive ρ value of 2.0 is consistent with rate-limiting nucleophilic attack of the thiol.

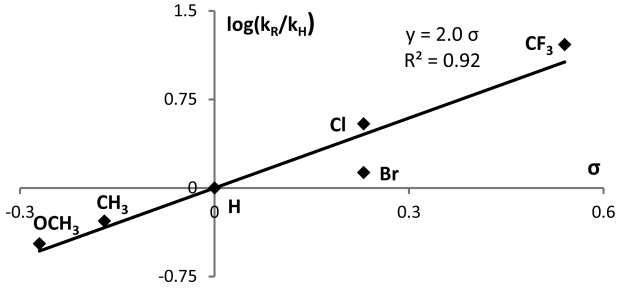


Figure 3. Hammett plot for the reaction of 2′-(phenethyl)thiol with *para*-substituted vinyl sulfones.

The successful application of alkyne hydrothiolation in the synthesis of K777 and analogues **23**−**27** illustrates the potential of this method for the formation of biologically active cysteine protease inhibitors. The use of Ellman sulfinamides in the diastereoselective synthesis of propargyl amines helps avoid the use of the expensive L-homophenylalanine starting material that had been used in the other reported synthesis. Kinetic measurements demonstrate that variation in the P_1' moiety of K777 can have a significant impact in the reactivity of the vinyl sulfone as a Michael acceptor. Our methodology provides a facile method for varying the substitution of the vinyl sulfone using commercially available and inexpensive thiols. This approach offers a direct avenue to tailor the pharmacodynamic properties of K777 derivatives. The Hammett plot for the reaction between a series of *para*-substituted vinyl sulfones and 2-phenethanethiol revealed a large positive ρ value of 2.0. This is consistent with rate-limiting generation of the α-carbanion succeeding nucleophilic attack of the thiol.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03535.

Experimental details, characterizations, ^{1}H, ^{13}C, and ^{19}F NMR spectra for new compounds, and HPLC spectra for ee % determinations (PDF)

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Notes

The authors declare no competing financial interest.

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(38) Substitution of **9** with Mosher's acid chloride revealed a 97% diastereomeric excess (see Supporting Information).

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