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Synthesis of macrocyclic trypanosomal cysteine protease inhibitors

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Abstract

The importance of cysteine proteases in parasites, compounded with the lack of redundancy compared to their mammalian hosts makes proteases attractive targets for the development of new therapeutic agents. The binding mode of K11002 to cruzain, the major cysteine protease of *Trypanosoma cruzi* was used in the design of conformationally constrained inhibitors. Vinyl sulfone-containing macrocycles were synthesized via olefin ring-closing metathesis and evaluated against cruzain and the closely related cysteine protease, rhodesain.

Trypanosomes are parasitic protozoa responsible for several neglected diseases of global health importance including Chagas' disease and sleeping sickness. Chagas' disease, or American trypanosomiasis, is a chronic infection caused by the parasite, *Trypanosoma cruzi*, and is the leading cause of heart failure in many Latin American countries.[1] *T. cruzi* is transmitted to humans through the bite of the triatomine bug or by transfusion of infected blood. The overall prevalence of human infection is estimated at 16 to 18 million cases with 13,000 deaths reported each year.[2] *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are the pathogenic agents of human African trypanosomiasis, or sleeping sickness. These parasites live extracellularly in blood and tissue fluids of the mammalian host and are transmitted by the bite of tsetse flies. The disease is endemic in certain regions of sub-Saharan Africa, covering about 50 million people in 36 countries. It is estimated that 50,000 to 70,000 people are currently infected; if left untreated, the disease in humans is fatal.[3]

Current drug therapy for trypanosomal diseases is not always effective and is often hampered by severe side effects.[4] Thus, the identification of novel targets for trypanocidal agents is needed. One such target is the major cysteine protease of the parasitic organisms, which includes cruzain[5] in *T. cruzi* and rhodesain[6] in *T. brucei rhodesiense*. Both enzymes are clan CA proteases, share 70% similarity in primary structure, and are involved in critical roles in parasite survival, such as replication, penetration into host cells, nutrition at the expense of the host, and immunoevasion.[7] Selective inhibitors of cruzain have been demonstrated to cure *T. cruzi* infection both in cell culture screens and in mouse models of Chagas' disease. [8] In a

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recent report, a cruzain inhibitor was also found to be effective in treating Chagas' disease in beagle dogs.[9]

A large number of cysteine protease inhibitors have been reported to date, several classes of which are potent, irreversible inhibitors. [10, 11] Based on the pioneering studies by Hanzlik[12] and Palmer,[13] our group has developed peptidyl vinyl sulfone inhibitors of parasitic cysteine proteases. [14, 15] The vinyl sulfones serve as Michael acceptors for the nucleophilic active site cysteine, and the peptidic backbone contains several hydrogen bond acceptors that interact with complementary residues in the active site. Several cruzain-inhibitor complexes have been solved by X-ray crystallography, which displayed the active site Cys25 of cruzain covalently bound to the vinyl sulfone unit of the inhibitor.[16]

With the aim to improve upon the lead compounds from previous studies and to develop an inhibitor with a broad spectrum of activity against a variety of parasitic hosts, we were interested in the design of conformationally constrained vinyl sulfones. Limiting conformational flexibility of the inhibitor or ligand is a well-established strategy to improve binding energies by decreasing the entropic barrier to binding of a particular conformation. Hence, in principle, by tethering distal segments of the inhibitors to form a rigid structure with a conformation favorable to binding, selectivity and/or potency of the inhibitor can be improved. This approach has been implemented in the design of various biologically active molecules such as aspartyl protease inhibitors,[17] and Grb2 SH2 domain-binding ligands.[18]

The crystal structures of cruzain with bound vinyl sulfonyl inhibitors are instrumental in elucidating the key factors that contribute to inhibitor binding. Examination of the crystal structures of cruzain with vinyl sulfones such as K11002 (**1**) reveal several highly conserved binding interactions.[16] These include hydrogen bonding between the side chains of Gln19, His159, and Trp177 with the sulfonyl oxygen atoms, a hydrogen bond between the P_1 nitrogen with the Asp158 peptide carbonyl, a hydrogen bond between the P_2 carbonyl and the Gly66 amide, and a hydrogen bond between the P_2 amide nitrogen with the Gly66 carbonyl (Figure 1b). [16] The S_2 pocket is the primary recognition element for cruzain and all other enzymes in the papain class. The Phe side chain of K11002 and other inhibitors is deeply buried in the well-defined S_2 pocket. However, the S_1', S_1, and S_3 pockets are very shallow and poorly defined, therefore the P_1', P_1, and P_3 groups are highly solvent exposed. In addition, the urea carbonyl does not participate in any interactions with the enzyme and thus was assumed to be non-essential to inhibitor binding.

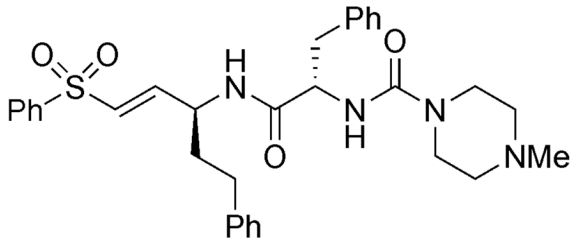
In designing a conformationally rigid inhibitor scaffold, we aimed to preserve the geometry of the peptide backbone and all of the critical hydrogen bonding interactions identified in Figure 1b. Since the P_1 side chain and the urea carbonyl are not involved in specific binding interactions to the enzyme, they were selected as the sites to form a conformationally restricted linkage. The distance between the urea carbonyl carbon and the γ-carbon of homoPhe in **1** when bound to cruzain is 4.51 Å, therefore we reasoned that a 10- or 11-membered macrocycle would provide the optimal ring size to preserve the bioactive peptide backbone conformation. In addition, introduction of a linking unit between these two units was expected not to interfere with enzyme binding, since the P_1 homoPhe residue is highly solvent exposed in the available crystal structures.[16] We anticipated that **2** would not be chemically stable, due to the acid labile aminal moiety. This was addressed by replacing the aminal unit with an α-amino amide as shown in **3**. Finally, we also elected to use leucine as the P_2 residue as shown in **4** and **5** to facilitate the extension of this strategy to the synthesis of inhibitors that might be effective against other parasitic CA proteases that are much more specific at P_2 than cruzain. We herein report the synthesis of two members of this class of compounds (**4** and **5**) and their inhibitory potency against cruzain and rhodesain.

As illustrated in Figure 2, we sought to assemble inhibitors **4** and **5** from scaffold **6**, which could be accessible from diene **7** via ring closing olefin metathesis. Diene **7**, in turn, could be prepared from either allylglycine or homoallylglycine which serves as the "P_1" fragment (**8** or **9**), leucine methyl ester (**10**), and α-hydroxyester **11**.

The synthesis of the "P_1" fragments of **8** and **9** is illustrated in Scheme 1. Weinreb amide **13** was prepared by functionalization of allylglycine (**12**) as described by Borzilleri et al.[19] Deprotection of the Boc group afforded amine **8** as a TFA salt. The synthesis of the Weinreb amide of homoallylglycine (**9**) began with asymmetric alkylation of pseudoephedrine glycinamide (**14**) [20] with 4-bromo-1-butene (**15**) to provide **16**. Removal of the chiral auxiliary followed by Boc protection of the amine and coupling of the resulting acid with *N*,*O*-dimethylhydroxylamine[21] gave Weinreb amide **18**. Finally, deprotection of the Boc group provided homoallylglycine **9**.

Allylboration of *t*-butyl glyoxylate **19**[22] with (*S*,*S*)-**20**[23] gave α-hydroxyester **11** with 74% ee [24] (Scheme 2). This material was carried onto the next two steps without further enantiomeric enrichment. The alcohol was converted to the corresponding triflate and this was used to alkylate leucine methyl ester (**10**) which provided **21** as a *ca*. 5:1 diastereomeric mixture which was separated by silica column chromatography. Amine **21** was protected with a Cbz group using *n*-butyllithium as the base, since attempted ring-closing metathesis of the amine corresponding to **24** was unsuccessful presumably due to catalyst poisoning.[25] The requirement for a strong base in this step is most likely due to the poor accessibility of the highly hindered amine proton. Selective hydrolysis of the methyl ester in compound **22**, followed by coupling with Weinreb amide **8** or **9** gave dienes **23** and **24**, respectively, which set the stage for macrocyclization via ring-closing olefin metathesis. [26] Macrocycle formation was achieved by treatment of either **23** or **24** with 20 mol % of second generation Grubbs' catalyst **25**[27] in 1,2-dichloroethane (0.8 mM substrate concentration). [28] Use of other solvents and higher reaction concentration led to substantial amounts of oligomers. The resulting alkene unit and Cbz group were removed by hydrogenation to yield **26** and **27**. Cleavage of the *t*-butyl ester by treatment with TFA, followed by coupling of the resulting carboxylic acid with benzyl amine gave **28** and **29**. Finally, the vinyl sulfone group was introduced by reduction of the Weinreb amide to the corresponding aldehyde, followed by Horner-Wadsworth-Emmons olefination with phosphonate **30**.[29] This yielded the targeted macrocyclic cysteine protease inhibitors **4** and **5** in 51 and 58% yield, respectively.

The activities of vinyl sulfones **4** and **5** as cysteine protease inhibitors were tested against cruzain and rhodesain. Recombinantly expressed cruzain or rhodesain was incubated with the inhibitor in sequential dilution followed by addition of Z-Phe-Arg-AMC as a fluorescent substrate. The increase in fluorescence produced by cleavage of the substrate allows determination of protease inhibition. IC_{50} values were determined in the liner portion of a plot of inhibition versus log of inhibitor concentration.



K11777

The results are summarized in Table 1. For comparison, K11777[8] was also assayed under similar conditions. The conformationally constrained compounds were substantially less active as inhibitors of cruzain and rhodesain compared to the acyclic vinyl sulfone. Owing to the very weak inhibitor potency, full kinetic analyses of these two inhibitors was not determined. Of the two macrocyclic inhibitors, **5** was the more effective, which suggests that the 11-membered macrocycle may have more flexibility to adopt a geometry that is more favorable for binding than the 10-membered ring scaffold of **4**. Our current hypothesis is that P_3 unit in **4** and **5** is sub-optimal compared to the P_3 units in K11002 and other dipeptidyl vinylsulfonyl inhibitors.[16] It is possible that the free amine in **4** or **5**, which will be protonated under conditions of the enzyme assay, may negatively impact the binding of these inhibitors to the enzyme targets. It is also possible that the urea units of acyclic cysteine protease inhibitors (c.f., K11002) are much more important to inhibitor binding than initially assumed. Studies addressing these issues via the synthesis and evaluation of a second-generation series of conformationally constrained vinylsulfonyl cysteine protease inhibitors will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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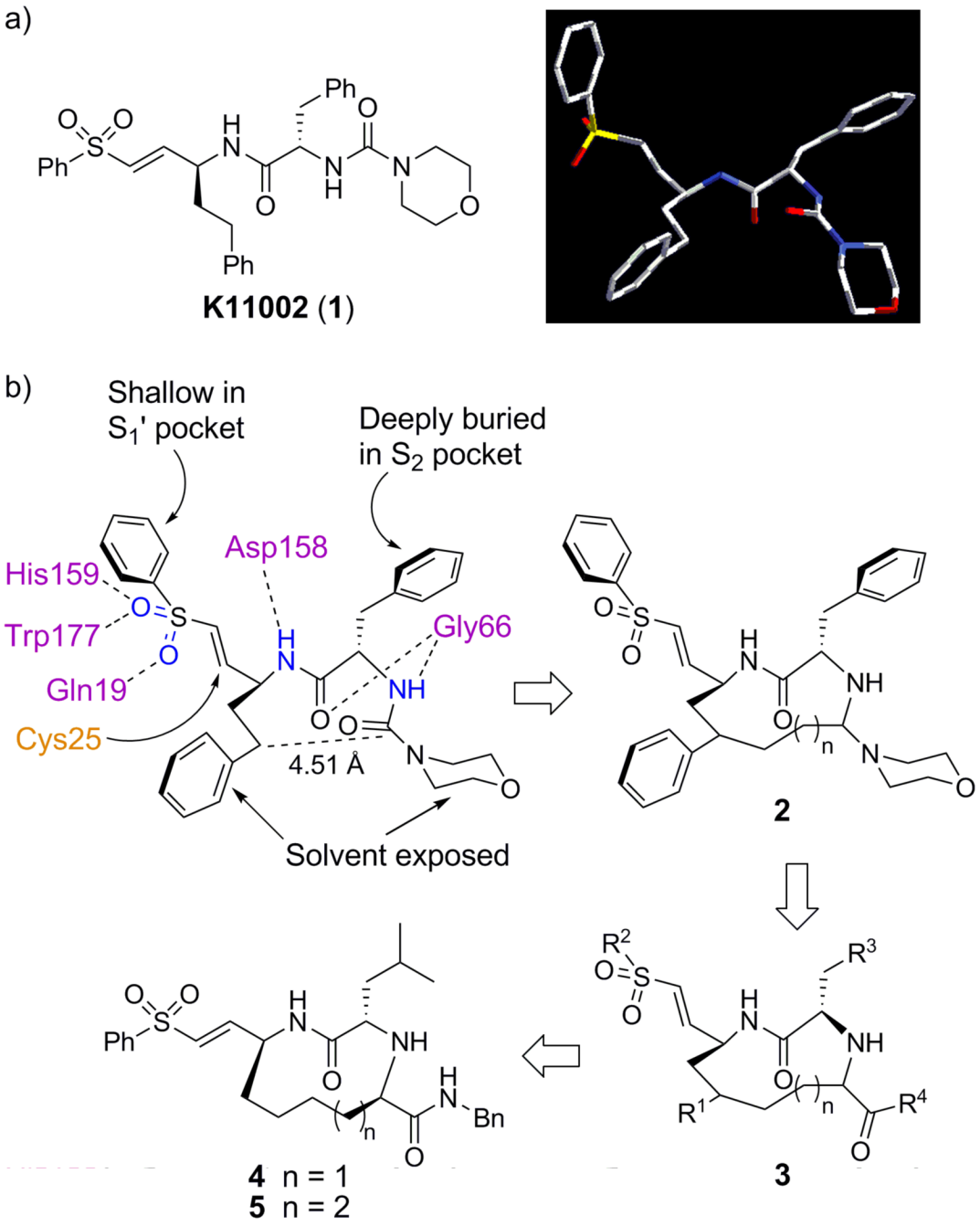


Figure 1.
(a) K11002 and its 3-D structure when bound to cruzain; (b) Design rationale for conformationally constrained inhibitors **4** and **5**.

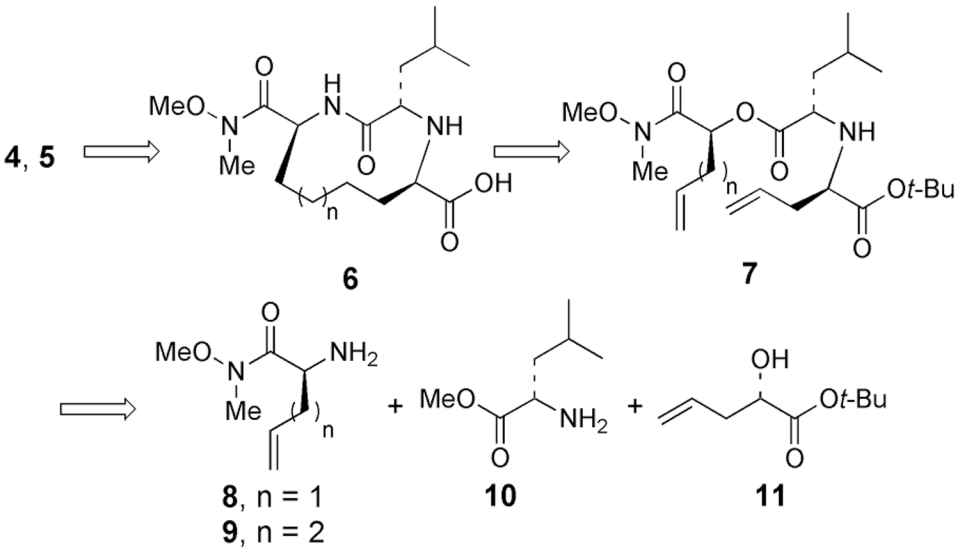
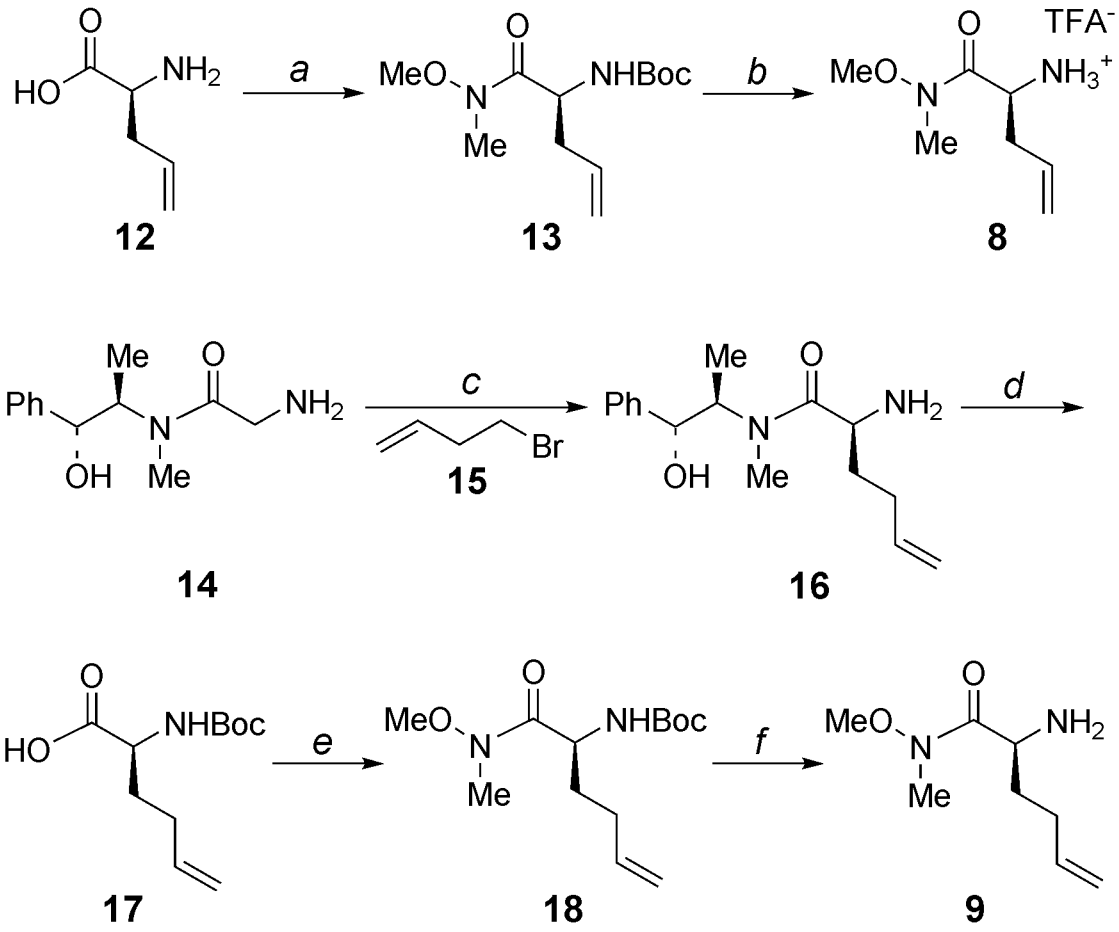
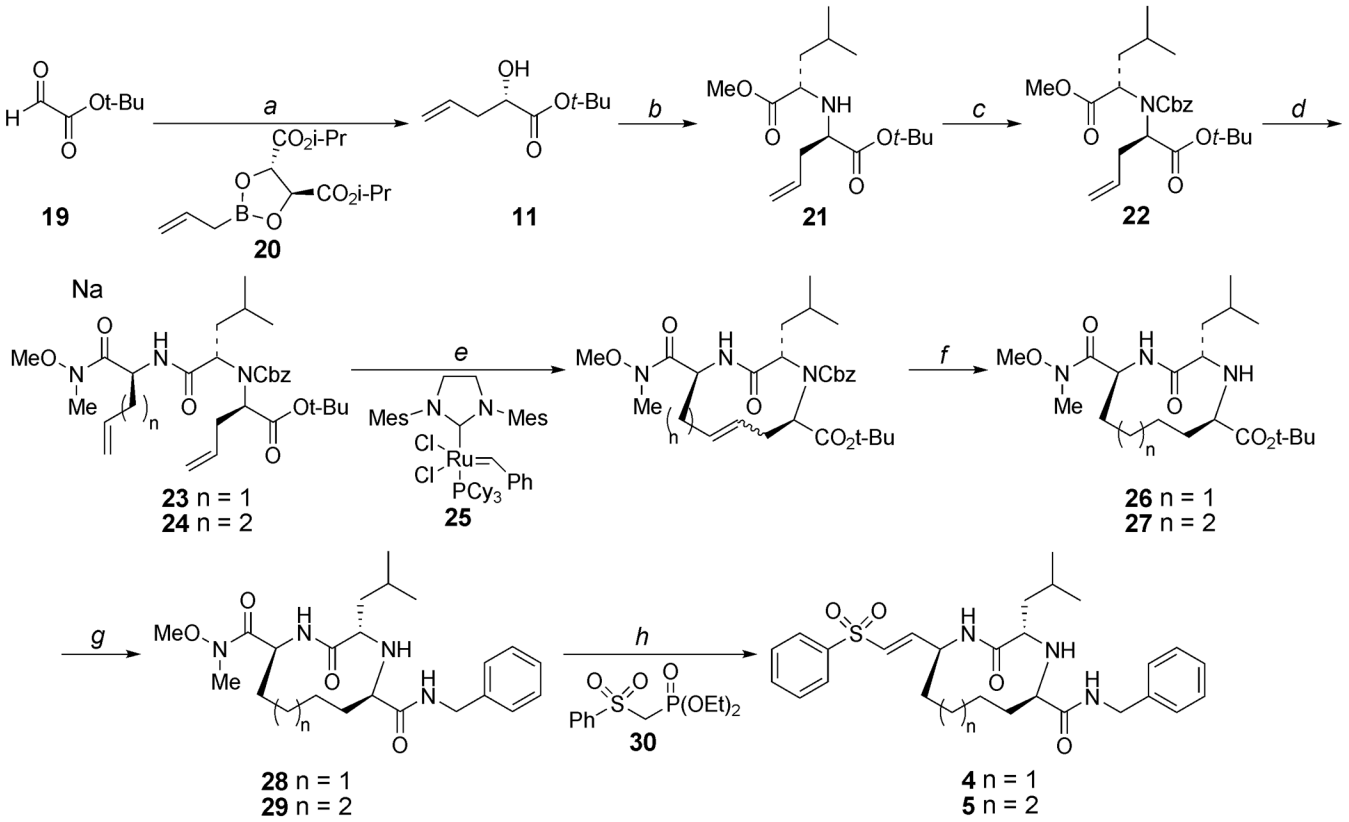


Figure 2.
Disconnective analysis of inhibitors **4** and **5**.



Scheme 1.

(a) (i) Boc_2O, $NaHCO_3$, H_2O, THF, 0 to 23 °C, 12 h; (ii) $CH_3(CH_3O)NH{\bullet}HCl$, HOBT, NMM, EDC, CH_2Cl_2, 0 to 23 °C, 12 h, 84% (two steps); (b) TFA, CH_2Cl_2, quant.; (c) LDA, LiCl, THF, 0 °C, 29 h, 62% (de > 20:1); (d) (i) NaOH, H_2O, reflux, 3 h; (ii) Boc_2O, NaOH, H_2O, dioxane, 0 to 23 °C, 16 h, 82% (two steps); (e) $CH_3(CH_3O)NH{\bullet}HCl$, HOBT, NMM, EDC, $CHCl_3$, 0 to 23 °C, 14 h, 88%; (f) TFA, CH_2Cl_2, 0 23 °C, 1 h, quant.



Scheme 2.

(a) **16**, toluene, 4 Å mol. sieves, −78 °C, 3 h, 70% (74 % ee); (b) (i) Tf_2O, pyridine, CH_2Cl_2, 0 to 23 °C, 30 min; (ii) **10**, proton sponge, $CHCl_3$, −78 to 23 °C, 18 h, 70% (two steps); (c) CbzCl, *n*-BuLi, Et_2O, −78 to 23 °C, 72 h, 55%; (d) (i) NaOH, THF, MeOH, 0 °C; (ii) **8** or **9**, EDC, HOBT, NMM, CH_2Cl_2, 0 to 23 °C, 14 h (two steps), **23** (77%), **24** (68%); (e) **25**, 1,2-dichloroethane, 95 °C, 1 h; (f) H_2, 10% Pd/C, EtOAc, EtOH, 36 h, **26** (65% from **23**), **27** (67% from **24**); (g) (i) TFA, CH_2Cl_2, 6 h; (ii) benzylamine, EDC, HOBT, NMM, CH_2Cl_2, 0 to 23 °C, 14 h (two steps), **28** (89%), **29** (73%); (h) (i), $LiAlH_4$, THF, −10 °C 30 min; (ii) **30**, NaH, THF, 0 to 23 °C; 4–8 h (two steps), **4** (58%), **5** (51%).

Table 1

Inhibition of cruzain and rhodesain by vinyl sulfone derivatives.

Compounds	IC_{50} (μM)	
	Rhodesain	Cruzain[a]
4	10	>10
5	6	2
K11777	0.1	0.004