Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 3. Structure−Activity Studies of Ketomethylene-Containing Peptidomimetics

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The structure-based design, chemical synthesis, and biological evaluation of various ketomethylene-containing human rhinovirus (HRV) 3C protease (3CP) inhibitors are described. These compounds are comprised of a peptidomimetic binding determinant and an ethyl propenoate Michael acceptor moiety which forms an irreversible covalent adduct with the active site cysteine residue of the 3C enzyme. The ketomethylene-containing inhibitors typically display slightly reduced 3CP inhibition activity relative to the corresponding peptide-derived molecules, but they also exhibit significantly improved antiviral properties. Optimization of the ketomethylene-containing compounds is shown to provide several highly active 3C protease inhibitors which function as potent antirhinoviral agents (EC_{90} = <1 μM) against multiple virus serotypes in cell culture.

Introduction

The human rhinoviruses (HRVs) are members of the picornavirus family and are the single most significant cause of the common cold.[1,2] Recently, we described the discovery and development of novel HRV 3C protease (3CP) inhibitors which display in vitro antiviral activity against several rhinovirus serotypes.[3,4] These inhibitors are comprised of a substrate-derived tripeptide binding determinant which provides affinity for the target protease and a Michael acceptor moiety which irreversibly forms a covalent adduct with the active site cysteine residue of the 3C enzyme (e.g., compound **1**, Figure 1).[5−8] Although optimization of these tripeptides afforded relatively active antirhinoviral agents, we also sought to investigate related peptidomimetic 3CP inhibitors which might exhibit improved pharmacological properties (e.g., greater metabolic stability, improved oral bioavailability, etc.).[9] The results of our efforts to develop one series of peptidomimetic 3CP inhibitors are described below.

Inhibitor Design and Structure−Activity Studies

The replacement of a backbone amide moiety present in peptide-derived enzyme inhibitors with a ketomethylene isostere often affords peptidomimetic compounds with inhibition activities similar to the parent molecules (Chart 1).[10] We therefore wished to introduce such an isostere into a representative tripeptidyl 3CP inhibitor (compound **1**, Figure 1). Analysis of the HRV-2 3CP-**1** crystal structure[3] indicated that the backbone amide NH linking the P_2 Phe and P_3 Leu amino acid residues of **1** was highly solvent-exposed (Figure 2).[6] In addition, comparison with the uncomplexed 3CP crystal structure[11] suggested that the 3CP serine residue which formed a hydrogen bond with the P_2−P_3 amide NH of **1** was somewhat conformationally mobile (Ser-128, Figure 2). In contrast, the inhibitor P_1−P_2 and P_3−P_4 amide

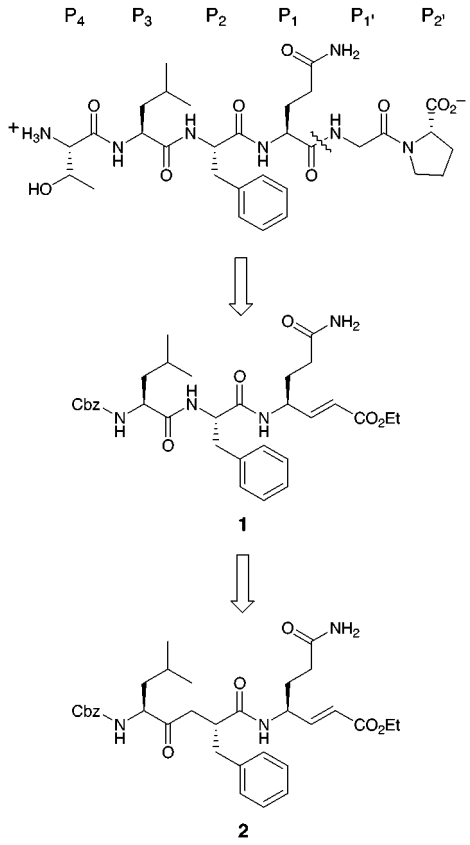

Figure 1. Design of peptidomimetic HRV 3CP inhibitors.

NH's were only slightly solvent-exposed and formed hydrogen bonds with relatively stationary 3CP residues. Replacement of the P_2−P_3 amide linkage with a ketomethylene dipeptide isostere was therefore anticipated to have less of an impact on inhibitor 3CP affinity than substitution of either of the two other amide bonds present in the tripeptidyl backbone of **1**.

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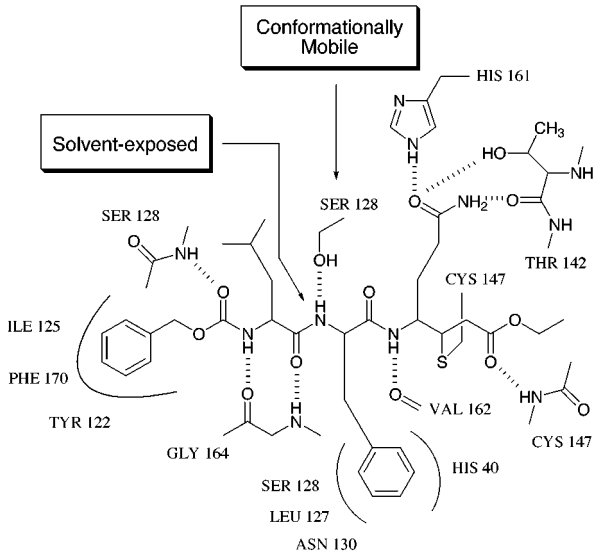
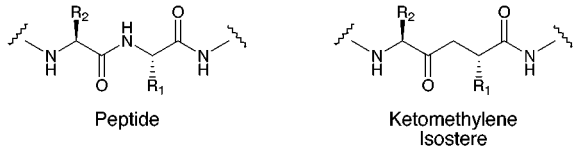


Figure 2. Schematic diagram of **1** bound in the HRV-2 3CP active site.[3] Hydrogen bonds are represented as dashed lines, and the residues which make up the enzyme binding subsites are depicted.

Chart 1



In the event, the ketomethylene-containing peptidomimetic compound **2** (Figure 1) displayed slightly reduced anti-3CP inhibitory activity but *improved* antiviral properties when compared with the tripeptide **1** (Table 1). The improved antiviral activity of **2** relative to **1** was consistent with increased cell membrane permeability due to the reduced potential of **2** to form hydrogen bonds with water.[12] As was observed for related tripeptidyl 3CP inhibitors, the ketomethylene-containing molecule **2** did not exhibit cytotoxicity in cell culture up to its solubility limit.[13] These encouraging findings prompted an extensive investigation of irreversible 3CP inhibitors which incorporate a P_2-P_3 ketomethylene dipeptide isostere to better define their potential for use as broad-spectrum antirhinoviral agents.

The development of such 3CP inhibitors commenced with the inclusion of several beneficial modifications discovered during previous studies of related tripeptide-derived molecules.[4] As before, the ethyl propenoate Michael acceptor was extensively employed for ketomethylene structure−activity studies due to its ease of preparation. Introduction of an N-terminal *S*-benzyl thiocarbamate moiety (**3**) into the ketomethylene inhibitor design increased anti-3CP activity approximately 5-fold relative to the corresponding carbamate-containing molecule (compare **2** and **3**, Table 1). Substitution of a P_3 valine amino acid residue in place of leucine further improved anti-3CP activity along with antiviral properties (compound **4**, Table 1). These two improvements paralleled those observed during the development of tripeptidyl 3CP inhibitors and suggested that other beneficial peptide modifications could also be directly incorporated into the ketomethylene series.

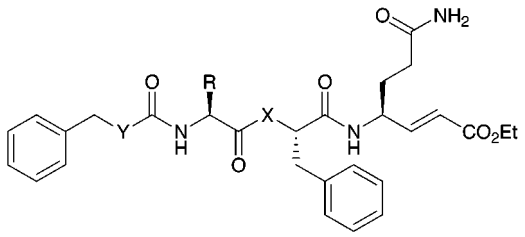
Accordingly, an N-terminal *S*-cyclopentyl thiocarbamate moiety, which afforded the most potent antiviral activity in the tripeptidyl 3CP inhibitor series,[4] was evaluated with several different P_2-P_3 ketomethylene dipeptide isosteres (Table 2). Inhibitors containing a Leu-Phe mimetic (**5**−**7**) were highly active antirhinoviral agents when tested against HRV-14 in cell culture, but they displayed significantly less potent antiviral properties against several other rhinovirus serotypes. In contrast, compounds incorporating a Val-Phe dipeptide isostere (**8**−**11**) exhibited sub-micromolar EC_{50} antirhinoviral activity against all serotypes examined.[14] Several inhibitors containing Phe-Phe peptidomimetics were also studied (**12** and **13**), but these compounds exhibited poorer antiviral activity than that displayed by the Val-Phe isosteres described above. In addition, a molecule containing a tBuGly-Phe dipeptide mimic (**14**) displayed potent antiviral activity against multiple rhinovirus serotypes. Similar to the 3CP inhibitors depicted in Table 1, the N-terminal *S*-cyclopentyl thiocarbamate-containing molecules did not exhibit cytotoxicity in cell culture up the limits of their solubility.[13]

Having completed the structure−activity studies described above, a comparison was made between one of the more potent ketomethylene-containing compounds (**9**) and an analogous tripeptidyl inhibitor (**15**). Examination of EC_{90} antiviral activity against several HRV serotypes clearly indicated that the peptidomimetic **9** was the superior antiviral agent and was at least 10-fold more potent than **15** for each serotype studied (Table 3).[14] Similar improvements in antiviral activity were noted when comparing other ketomethylene-containing 3CP inhibitors with the corresponding peptidyl compounds (data not shown). Replacement of the N-terminal thiocarbamate moiety present in **9** with an amide derived from 5-methylisoxazole-3-carboxylic acid[15] afforded a molecule which retained sub-micromolar EC_{90} antiviral activity when tested against several HRV serotypes (**16**, Table 3).[14] The above data indicate that the antirhinoviral activity displayed by tripeptidyl Michael acceptors may be substantially increased by reducing the number of amide NH's present in the molecules and suggest that further improvements in such activity might be realized by additional amide modifications.[16]

Synthesis

The ketomethylene-containing 3CP inhibitors utilized in this study were prepared by three different synthetic methods (A−C). The particular method employed to synthesize a given compound is indicated in Tables 1−3. An example of synthetic method A, which incorporates elements from previously described preparations of hydroxyethylene dipeptide isosteres,[17] is provided by the synthesis of compound **9** (Schemes 1 and 2). Isobutyraldehyde was condensed with vinylmagnesium bromide to provide allylic alcohol **17** in good yield.[18] This material was not purified but was instead subjected to a homologation protocol which involved transesterification with diethyl malonate, Claisen rearrangement, and subsequent basic hydrolysis. The resulting carboxylic acid (**18**) was transformed into acid chloride **19**,[19] and this product was coupled with (1*R*,2*R*)-(−)-pseudoephedrine to provide the corresponding *N*-acylated compound **20**

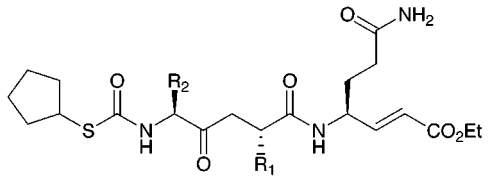
Table 1



compd no.	R	X	Y	prep.[a]	formula[b]	k_{obs}/[I] (M^{-1} s^{-1})[c,d]	EC_{50} (μM)[c,d]	CC_{50} (μM)[d]
1	$CH_2CH(CH_3)_2$	NH	O	ref 3	$C_{32}H_{42}N_4O_7$	25 000	0.54	>320
2	$CH_2CH(CH_3)_2$	CH_2	O	A	$C_{33}H_{43}N_3O_7$	17 400	0.36	>320
3	$CH_2CH(CH_3)_2$	CH_2	S	A	$C_{33}H_{43}N_3O_6S$	87 600	0.68	>100
4	$CH(CH_3)_2$	CH_2	S	A	$C_{32}H_{41}N_3O_6S$	500 000	0.19	>100

[a] Method of preparation: see Experimental Section. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Serotype-14. [d] See ref 3 for assay method and error.

Table 2



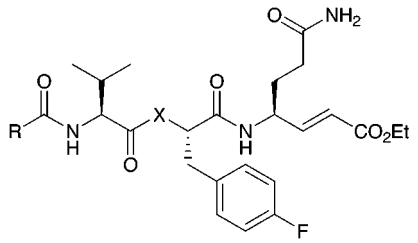
compd no.	R_1	R_2	prep.[a]	formula[b]	serotype	k_{obs}/[I] (M^{-1} s^{-1})[c]	EC_{50} (μM)[c]	CC_{50} (μM)[c]
5	CH_2Ph	$CH_2CH(CH_3)_2$	A	$C_{31}H_{45}N_3O_6S$	14	55 700	0.19	>100
6	$CH_2Ph(4\text{-}F)$	$CH_2CH(CH_3)_2$	B	$C_{31}H_{44}FN_3O_6S$	14	67 200	0.28	>100
					1A	ND	0.60	
					2	ND	0.71	
					10	ND	1.6	
7	$CH_2Ph(4\text{-}CH_3)$	$CH_2CH(CH_3)_2$	B	$C_{32}H_{47}N_3O_6S$	14	243 000	0.16	>100
					1A	ND	0.56	
					2	ND	1.0	
					10	ND	1.4	
8	CH_2Ph	$CH(CH_3)_2$	A	$C_{30}H_{43}N_3O_6S$	14	255 000	0.020	>100
					2	ND	0.10	
					10	ND	0.035	
9	$CH_2Ph(4\text{-}F)$	$CH(CH_3)_2$	A	$C_{30}H_{42}FN_3O_6S{\cdot}0.25H_2O$	14	293 000	0.020	>100
					1A	ND	0.17	
					2	ND	0.13	
					10	ND	0.16	
10	$CH_2Ph(4\text{-}CH_3)$	$CH(CH_3)_2$	A	$C_{31}H_{45}N_3O_6S$	14	850 000	0.0060	>100
					1A	ND	0.16	
					2	ND	0.13	
					10	ND	0.060	
11	$CH_2Ph(4\text{-}CF_3)$	$CH(CH_3)_2$	A	$C_{31}H_{42}F_3N_3O_6S$	14	150 000	0.050	>100
					1A	ND	0.18	
					2	ND	0.18	
					10	ND	0.16	
12	$CH_2Ph(4\text{-}F)$	CH_2Ph	B	$C_{34}H_{42}FN_3O_6S$	14	127 400	0.48	>100
13	$CH_2Ph(4\text{-}CH_3)$	CH_2Ph	B	$C_{35}H_{45}N_3O_6S$	14	404 000	0.14	>100
14	CH_2Ph	$C(CH_3)_3$	C	$C_{31}H_{45}N_3O_6S$	14	124 000	0.050	>100
					1A	ND	0.43	
					2	ND	0.36	
					10	ND	0.50	

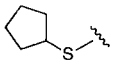
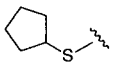
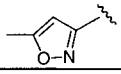
[a] Method of preparation: see Experimental Section. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] See ref 3 for assay method and error. ND = not determined.

in accordance with literature precedent.[20] Alkylation of the dianion derived from **20** with 4-fluorobenzyl bromide in the presence of excess lithium chloride then afforded the benzylated product **21** in good yield.[20,21] Exposure of **21** to a slight excess of *N*-bromosuccinimide under mildly acidic conditions at 0 °C followed by reflux for 45 min provided slightly impure bromo-γ-lactone **22** after careful flash column chromatography. This material was then converted to amino-lactone **24** by azide displacement and subsequent reduction in the presence of di-*tert*-butyl dicarbonate.

The desired ketomethylene dipeptide isostere was prepared from amino-lactone **24** by basic hydrolysis and TPAP-mediated oxidation[22] of the resulting hydroxy-acid (not shown, Scheme 2). The crude keto-acid thus obtained (**25**) was condensed with the known trityl-protected glutamine derivative **26**[3] to afford coupling product **27** in moderate yield. Removal of the N-terminal Boc protecting group from **27** under acidic conditions and derivatization of the resulting amine hydrochloride with cyclopentyl chlorothiolformate[23] then provided thiocarbamate **28** in good yield. The trityl protecting group present in **28** was removed by short exposure to trifluoroacetic acid (TFA) in the presence of triisopropylsilane to give inhibitor **9** in good yield.[24] Several different ketomethylene-containing molecules could be prepared utilizing method A by variation of the starting aldehyde (P_3), the benzylic halide employed to alkylate the γ,δ-unsaturated pseudoephedrine amide (P_2), and the derivatizing agent used for N-terminal modification

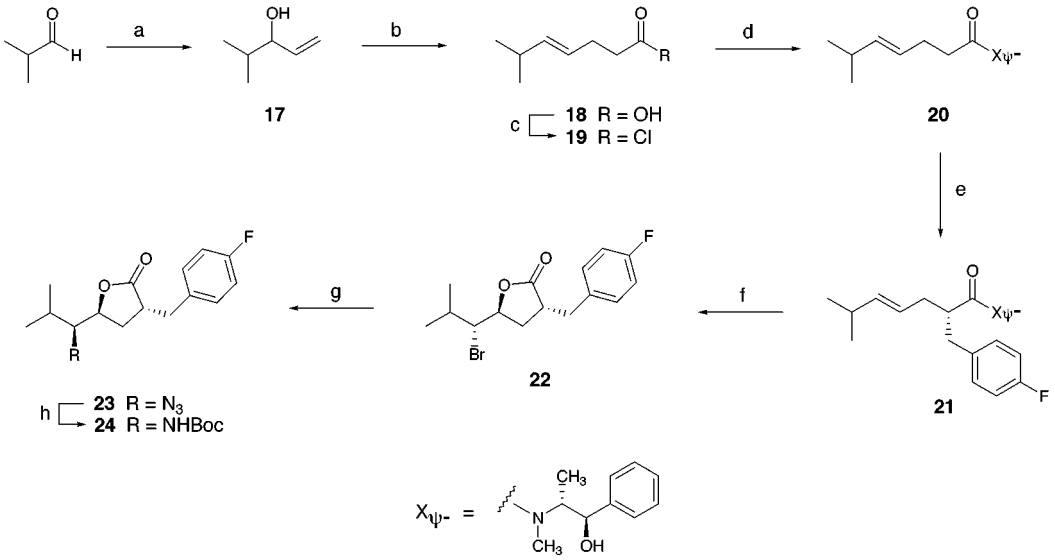
Table 3



Compd No.	R	X	Prep.[a]	EC_{90} vs Rhinovirus Serotype (μM)[b]			
				HRV-14	HRV-1A	HRV-2	HRV-10
9		CH_2	A	0.10	0.20	0.25	0.25
15		NH	ref 29	1.8	2.5	5.4	2.5
16		CH_2	A	0.32	0.63	0.28	0.79

[a] Method of preparation: see Experimental Section. [b] See ref 3 for assay method and error.

Scheme 1[a]

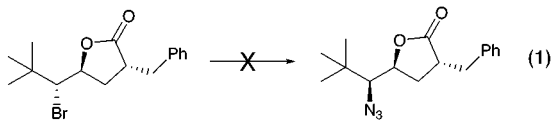


[a] Reagents and conditions: (a) ref 18; (b) 1.1 equiv of diethyl malonate, 0.1 equiv of $Ti(OEt)_4$, 80→125 °C, 3.5 h, then 190 °C, 9.5 h, then EtOH, 6.0 M KOH, reflux, 5 h, 98%; (c) 1.2 equiv of $SOCl_2$, $CHCl_3$, reflux, 30 min, 41%; (d) 0.87 equiv of (1*R*,2*R*)-(−)-pseudoephedrine, 1.13 equiv of Et_3N, THF, 0 °C, 15 min, 56%; (e) 3.1 equiv of LDA, 10.0 equiv of LiCl, THF, −78 °C, 1.8 h, then 3.0 equiv of (4-F)$PhCH_2Br$, 0 °C, 30 min, 94%; (f) 1.05 equiv of NBS, 5.0 equiv of HOAc, 4:1 THF:H_2O, 0 °C, 15 min, then reflux, 45 min, 80%; (g) 2.5 equiv of NaN_3, DMF, 50 °C, 67 h, 62%; (h) 1.4 equiv of $(Boc)_2O$, H_2/Pd/C, CH_3OH, 23 °C, 16 h, 62%.

(P_4). The ketomethylene-containing inhibitors prepared in this study were typically isolated as white solids by trituration and filtration as described previously for the synthesis of analogous tripeptides.[3,4] However, in several cases, flash column chromatography was utilized to further increase the purity of the desired compounds (see the Experimental Section).

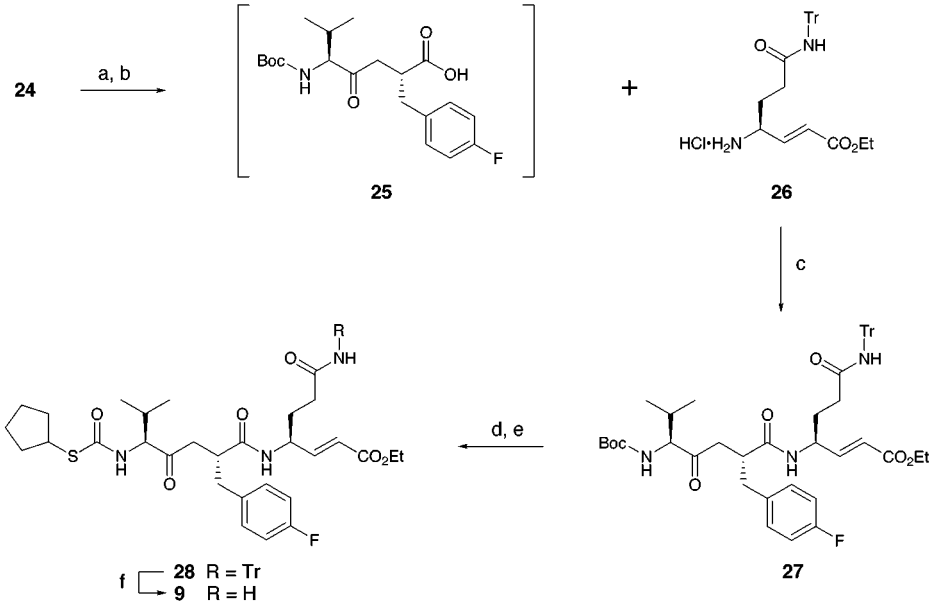
An example of preparation method B, which also incorporates elements from previously described syntheses of hydroxyethylene dipeptide isosteres,[25] is provided by the preparation of compound **12** (Scheme 3). Thus, the optically active epoxide **29**[26] was coupled with the lithium enolate of amide **30** to afford alcohol **31** in moderate yield. Subsequent transformation of **31** into amino-lactone **32** was then accomplished by exposure to mild acid. Compound **32** was converted into inhibitor **12** (via intermediates **33** and **34**) by a process analogous to the **24**→**9** conversion depicted in Scheme 2 (see the Experimental Section). As with synthetic method A, several different ketomethylene-containing inhibitors could be prepared via method B by altering the epoxide (P_3) or amide (P_2) components employed.

The final synthetic method used to prepare the peptidomimetic compounds described in this study is illustrated in Scheme 4 (method C).[27] This method was utilized to prepare the tBuGly-Phe-containing isostere **14** for which the azide displacement reaction analogous to the **22**→**23** conversion failed (equation 1, cf., Scheme



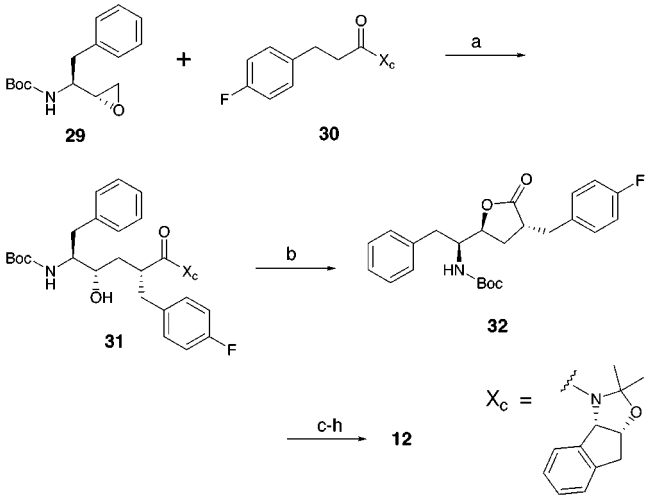
1). Cbz-protected L-*tert*-butylglycine was transformed into *β*-keto-ester **35** in moderate yield by activation with carbonyldiimidazole and subsequent displacement with the lithium enolate of *tert*-butyl acetate. Condensation

Scheme 2[a]



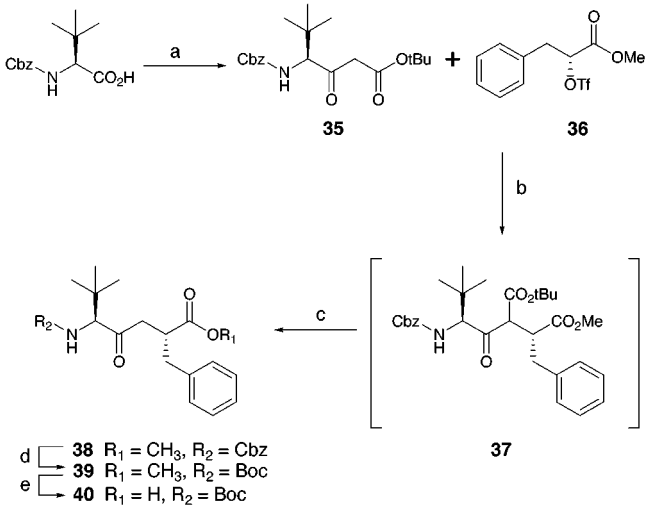
[a] Reagents and conditions (Tr = CPh_3): (a) 5.0 equiv of LiOH, DME, 23 °C, 30 min; (b) 2.0 equiv of NMO, 0.10 equiv of TPAP, 4 Å sieves, CH_2Cl_2, 23 °C, 1.3 h; (c) 1.2 equiv of **26**, 1.5 equiv of HOBt, 4.0 equiv of NMM, 1.5 equiv of EDC, CH_2Cl_2, 23 °C, 19 h, 53% from **24**; (d) HCl, 1,4-dioxane, 23 °C, 2 h; (e) 1.7 equiv of cyclopentyl chlorothiolformate, 3.0 equiv of $(i\text{-Pr})_2NEt$, CH_2Cl_2, 23 °C, 2 h, 59%; (f) 3.0 equiv of $(i\text{-Pr})_3SiH$, 1:2 TFA:CH_2Cl_2, 23 °C, 30 min, 82%.

Scheme 3[a]



[a] Reagents and conditions: (a) 2.0 equiv of *n*-BuLi, THF, −78→0 °C, 1 h, 49%; (b) 1.0 equiv of TsOH·H_2O, 5:1 toluene:CH_2Cl_2, 23 °C, 13 h, 59%; (c) 5.0 equiv of LiOH, DME, 23 °C, 20 min; (d) 2.0 equiv of NMO, 0.10 equiv of TPAP, 4 Å sieves, 1:1 CH_3CN:CH_2Cl_2, 23 °C, 3 h; (e) 1.2 equiv of **26**, 1.3 equiv of HOBt, 4.0 equiv of NMM, 1.3 equiv of EDC, CH_2Cl_2, 23 °C, 22 h, 43% from **32**; (f) HCl, 1,4-dioxane, 23 °C, 1.5 h; (g) 1.5 equiv of cyclopentyl chlorothiolformate, 3.0 equiv of NMM, CH_2Cl_2, 0 °C, 30 min, 52%; (h) 2.7 equiv of $(i\text{-Pr})_3SiH$, 3:5 TFA:CH_2Cl_2, 23 °C, 30 min, 71%.

Scheme 4[a]



[a] Reagents and conditions (Tf = SO_2CF_3): (a) 1.1 equiv of CDI, THF, 23 °C, 1 h, then 2.1 equiv of LDA, 2.1 equiv of *t*-BuOAc, THF, −78 °C, 1 h, 44%; (b) 1.05 equiv of NaH, THF, 0 °C, 10 min, then 3.1 equiv of **36**, THF, 23 °C, 24 h; (c) 1:5 TFA:CH_2Cl_2, 23 °C, 24 h, 54% from **35**; (d) 1.3 equiv of $(Boc)_2O$, H_2/Pd/C, CH_3OH, 23 °C, 24 h, 70%; (e) 8.0 equiv of NaOH, CH_3OH, 0 °C, 2 h, 99%.

of the sodium salt of **35** with triflate **36** then provided the coupling product **37** which, without purification, was subsequently converted into Cbz-protected γ-keto-ester **38** in moderate overall yield. The N-terminal Cbz protecting group present in **38** was exchanged for a Boc moiety by hydrogenation in the presence of di-*tert*-butyl dicarbonate, and the resulting Boc-protected γ-keto-ester (**39**) was hydrolyzed under basic conditions to provide γ-keto-acid **40** in good yield.[28] Compound **40** was then converted to isostere **14** (via intermediates **41** and **42**) by a method analogous to the **25**→**9** conversion depicted in Scheme 2 above (see the Experimental Section).

Conclusions

The structure−activity studies described above confirmed the ability of Michael acceptor-containing molecules which incorporate ketomethylene dipeptide isosteres to function both as irreversible inhibitors of the human rhinovirus 3C protease and as potent antirhinoviral agents in cell culture. Such compounds typically display slightly reduced 3C protease inhibition activity relative to the corresponding peptide-derived molecules, but they also exhibit significantly improved antiviral

properties. Optimization of the ketomethylene-containing compounds afforded several highly active 3C protease inhibitors which display potent antiviral activity (EC_{90} = <1 μM) against multiple rhinovirus serotypes in cell culture. The pharmacological properties of the inhibitors described in this study will be detailed in subsequent publications.

Experimental Section

General descriptions of experimental procedures, reagent purifications, and instrumentation along with conditions for enzyme and antiviral assays are provided elsewhere.[3] ^{1}H NMR chemical shifts are reported in ppm (δ) downfield relative to internal tetramethylsilane, and coupling constants are given in hertz. A simplified naming system employing amino acid abbreviations is used to identify some intermediates and final products. When utilizing this naming system, italicized amino acid abbreviations represent modifications at the C-terminus of that residue where acrylic acid esters are reported as "E" (trans) propenoates. In addition, the terminology "$AA_1\Psi$-[$COCH_2$]-AA_2" indicates that, for any peptide sequence, two amino acids (AA_1 and AA_2) usually linked by an amide bond are replaced by a ketomethylene dipeptide isostere. The following abbreviations also apply: HOBt (1-hydroxybenzotriazole hydrate), EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), and CDI (1,1′-carbonyldiimidazole).

Representative Example of Preparation Method A. Synthesis of Ethyl-3-{cyclopentylSCO-L-ValΨ[$COCH_2$]-L-Phe(4-F)-L-*Gln*}-E-propenoate (9). ***trans*-6-Methylhept-4-enoic Acid (18).** Diethyl malonate (25.2 mL, 166 mmol, 1.1 equiv), **17**[18] (15.1 g, 151 mmol, 1 equiv), and titanium(IV) ethoxide (3.16 mL, 15.1 mmol, 0.1 equiv) were combined in a 100 mL pear-shaped flask equipped with a stir bar and a short path distillation head (with receiving flask). The reaction vessel was placed in an 80 °C oil bath, and the bath temperature was gradually raised to 125 °C over 2 h, keeping the distillation head temperature below 75 °C. After the mixture was stirred for 1.5 h at 125 °C, the bath temperature was gradually raised to 190 °C over 5 h, maintained at that temperature for 4.5 h, and then allowed to cool to room temperature overnight. EtOH (75 mL) and 6.0 M KOH (75 mL) were added to the reaction mixture which was warmed to reflux for 5 h and then allowed to cool. The resulting mixture was filtered through a medium fritted funnel, and the filtrate was concentrated, diluted with water (50 mL), and washed with Et_2O (50 mL). The aqueous phase was acidified to pH 1 with 12 M HCl and extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with brine (25 mL), dried over Na_2SO_4, and concentrated to give **18** (21 g, 98%) as a brown oil. This material was used without further purification: ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H, J = 6.5), 2.18−2.45 (m, 5H), 5.31−5.50 (m, 2H).

***trans*-6-Methylhept-4-enoic Acid Chloride (19).** Thionyl chloride (5.54 mL, 75.9 mmol, 1.2 equiv) and **18** (9.0 g, 63 mmol, 1 equiv) were dissolved in $CHCl_3$ (120 mL) and warmed to reflux for 30 min. Additional thionyl chloride (1.0 mL, 14 mmol, 0.22 equiv) was added, and the reflux was continued for 15 min more. The reaction mixture was concentrated, and the residue was distilled at vacuum line pressure (approximately 1 Torr), collecting the distillate between 32 and 36 °C to yield **19** (4.18 g, 41%) as a clear, colorless liquid: ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H, J = 6.8), 2.18−2.32 (m, 1H), 2.34−2.43 (m, 2H), 2.95 (t, 2H, J = 7.3), 5.26−5.38 (m, 1H), 5.49 (dd, 1H, J = 15.6, 6.5).

***trans*-(1′*R*,2′*R*)-6-Methylhept-4-enoic Acid (2′-Hydroxy-1′-methyl-2-phenylethyl)methyl Amide (20).** A solution of **19** (12.2 g, 76.2 mmol, 1.15 equiv) in THF (40 mL) was added dropwise over 10 min to a 0 °C solution of (1*R*,2*R*)-(−)-pseudoephedrine (10.95 g, 66.27 mmol, 1 equiv) and triethylamine (12.0 mL, 86.1 mmol, 1.3 equiv) in THF (200 mL). After being stirred for 15 min at 0 °C, the reaction mixture was poured into a separatory funnel and was washed with water (20 mL) and brine (2 × 40 mL). The organic layer was dried over Na_2SO_4 and was concentrated. The residue was purified by flash column chromatography (gradient elution, 40→60% EtOAc in hexanes) to give **20** (10.8 g, 56%) as a clear, colorless oil: R_f = 0.35 (50% EtOAc in hexanes); IR (cm^{-1}) 3382, 1622; ^{1}H NMR ($CDCl_3$, mixture of isomers) δ 0.96 (d, J = 6.8), 0.97 (d, J = 6.5), 1.11 (d, J = 6.9), 2.18−2.59 (m), 2.82 (s), 2.92 (s), 3.99−4.04 (m), 4.32−4.42 (m), 4.44−4.49 (m), 4.55−4.62 (m), 5.32−5.49 (m), 7.24−7.42 (m). Anal. ($C_{18}H_{27}NO_2$) C, H, N.

***trans*-(1′*R*,2*S*,2′*R*)-6-Methyl-2-(4″-fluorobenzyl)hept-4-enoic Acid (2′-Hydroxy-1′-methyl-2-phenylethyl)methyl Amide (21).** *n*-Butyllithium (32.5 mL of a 1.6 M solution in hexanes, 52.0 mmol, 3.1 equiv) was added to a suspension of anhydrous lithium chloride (7.18 g, 169 mmol, 10 equiv) and diisopropylamine (7.80 mL, 55.7 mmol, 3.3 equiv) in THF (250 mL) at −78 °C. The reaction mixture was stirred for 30 min at −78 °C, then was maintained at 0 °C for 5 min, and was subsequently cooled again to −78 °C. A solution of **20** (4.91 g, 17.0 mmol, 1 equiv) in THF (50 mL) was added via cannula, and the resulting mixture was stirred at −78 °C for 1.75 h, maintained at 0 °C for 20 min, stirred at 23 °C for 5 min, and then was cooled again to 0 °C. A solution of 4-fluorobenzyl bromide (6.34 mL, 50.9 mmol, 3.0 equiv) in THF (15 mL) was added, and the reaction mixture was stirred at 0 °C for 30 min and then was partitioned between half-saturated NH_4Cl (230 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 150 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Purification of the residue by flash column chromatography (gradient elution, 20→40% EtOAc in hexanes) provided **21** (6.33 g, 94%) as a viscous oil: R_f = 0.38 (40% EtOAc in hexanes); IR (cm^{-1}) 3378, 1614; ^{1}H NMR ($CDCl_3$, mixture of isomers) δ 0.85−0.95 (m), 0.96 (d, J = 6.8), 2.10−2.32 (m), 2.34−2.46 (m), 2.58 (s), 2.67−2.79 (m), 2.82−2.94 (m), 3.00−3.18 (m), 3.94 (br), 4.37−4.52 (m), 5.24−5.42 (m), 5.44−5.56 (m), 6.89−7.01 (m), 7.08−7.14 (m), 7.19−7.38 (m). Anal. ($C_{25}H_{32}FNO_2$) C, H, N.

(1′*R*,3*R*,5*S*)-5-(1′-Bromo-2′-methylpropyl)-3-(4″-fluorobenzyl)dihydrofuran-2-one (22). *N*-Bromosuccinimide (2.93 g, 16.5 mmol, 1.05 equiv) was added in small portions over 10 min to a solution of **21** (6.24 g, 15.7 mmol, 1 equiv) and glacial acetic acid (4.49 mL, 78.4 mmol, 5.0 equiv) in a 4:1 mixture of THF and H_2O (165 mL) at 0 °C. The resulting yellow solution was stirred for 15 min at 0 °C, then was warmed to 23 °C, and subsequently refluxed for 45 min. After cooling to 23 °C, the reaction mixture was partitioned between half-saturated $NaHCO_3$ (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 200 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Flash chromatographic purification of the residue (gradient elution, 5→10% EtOAc in hexanes) gave **22** (4.14 g, 80%) as a pale yellow oil (containing approximately 5−10% unidentified impurities by ^{1}H NMR): R_f = 0.56 (25% EtOAc in hexanes); IR (cm^{-1}) 1772; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 3H, J = 6.5), 1.00 (d, 3H, J = 6.8), 2.05−2.35 (m, 3H), 2.83 (dd, 1H, J = 13.6, 8.4), 2.92−3.03 (m, 1H), 3.11 (dd, 1H, J = 13.6, 4.7), 3.90 (dd, 1H, J = 9.0, 3.7), 4.33−4.40 (m, 1H), 6.98−7.06 (m, 2H), 7.14−7.20 (m, 2H). Anal. ($C_{15}H_{18}BrFO_2$) C, H.

(1′*S*,3*R*,5*S*)-5-(1′-Azido-2′-methylpropyl)-3-(4″-fluorobenzyl)dihydrofuran-2-one (23). A suspension of sodium azide (1.90 g, 29.2 mmol, 2.5 equiv) and **22** (3.85 g, 11.7 mmol, 1 equiv) in DMF (40 mL) was heated at 50 °C for 67 h. The reaction mixture was cooled to 23 °C and was partitioned between half-saturated NaCl (200 mL) and a 1:1:1 mixture of EtOAc, hexanes, and acetone (2 × 200 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by flash column chromatography (gradient elution, 10→20% EtOAc in hexanes) to give **23** (2.10 g, 62%) as a white solid (containing approximately 5−10% unidentified impurities by ^{1}H NMR): mp 91−96 °C; R_f = 0.44 (25% EtOAc in hexanes); IR (cm^{-1}) 2097, 1772; ^{1}H NMR ($CDCl_3$) δ 0.99 (d, 3H, J = 6.5), 1.02 (d, 3H, J = 6.8), 1.95−2.20 (m, 3H), 2.78−2.88 (m, 1H), 2.94 (dd, 1H, J = 7.0, 4.2), 3.03−3.17 (m, 2H), 4.37−4.43 (m, 1H), 6.97−7.09 (m, 2H), 7.14−7.21 (m, 2H).

(1*S*,2′*S*,4′*S*)-{2-Methyl-1-[4′-(4″-fluorobenzyl)-5′-oxo-tetrahydrofuran-2′-yl]propyl}carbamic Acid *tert*-Butyl Ester (24). A suspension of **23** (2.02 g, 6.93 mmol, 1 equiv), di-*tert*-butyl dicarbonate (2.12 g, 9.71 mmol, 1.4 equiv), and Pd/C (10%, 0.20 g) in CH_3OH (100 mL) was stirred at 23 °C under a hydrogen atmosphere (balloon) for 16 h. The reaction mixture was vacuum-filtered through Whatman No. 3 paper and was concentrated. Purification of the residue by flash column chromatography (15% EtOAc in hexanes) provided **24** (1.58 g, 62%) as a white foam: $R_f = 0.80$ (5% MeOH in CH_2Cl_2); IR (cm^{-1}) 3331, 1766, 1702; ^1H NMR ($CDCl_3$) δ 0.93 (d, 3H, J = 6.8), 0.95 (d, 3H, J = 6.5), 1.41 (s, 9H), 1.71−1.83 (m, 1H), 1.95−2.06 (m, 1H), 2.16−2.27 (m, 1H), 2.80 (dd, 1H, J = 13.5, 8.6), 2.88−2.99 (m, 1H), 3.09 (dd, 1H, J = 13.5, 4.4), 3.32−3.40 (m, 1H), 4.42−4.48 (m, 2H), 6.95−7.03 (m, 2H), 7.11−7.18 (m, 2H). Anal. ($C_{20}H_{28}FNO_4$) C, H, N.

Ethyl-3-{Boc-L-ValΨ[$COCH_2$]-L-Phe(4-F)-L-(Tr-*Gln*)}-E-propenoate (27). Lithium hydroxide (9.62 mL of a 1.0 M aqueous solution, 9.62 mmol, 5.0 equiv) was added to a solution of **24** (0.703 g, 1.92 mmol, 1 equiv) in DME (25 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 min and then was partitioned between 10% $KHSO_4$ (50 mL) and CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was dissolved in CH_2Cl_2 (30 mL). Powdered 4 Å molecular sieves (0.70 g), 4-methylmorpholine *N*-oxide (0.451 g, 3.85 mmol, 2.0 equiv), and tetrapropylammonium perruthenate (0.068 g, 0.19 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 1.33 h at 23 °C and then was vacuum-filtered (twice) through Whatman No. 3 and No. 5 papers. The filtrate was concentrated under reduced pressure to provide a dark residue (intermediate **25**) which was dissolved in CH_2Cl_2 (30 mL). Crude ethyl-3-[H_2N-L-(Tr-*Gln*)]-E-propenoate·HCl[3] (**26**, 2.30 mmol, 1.2 equiv), 4-methylmorpholine (0.846 mL, 7.69 mmol, 4.0 equiv), HOBt (0.390 g, 2.89 mmol, 1.5 equiv), and EDC (0.553 g, 2.88 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 19 h at 23 °C and then was partitioned between brine (100 mL) and CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Purification of the residue by flash column chromatography (gradient elution, 30→40% EtOAc in hexanes) provided **27** (0.820 g, 53%) as a tan foam: $R_f = 0.50$ (50% EtOAc in hexanes); IR (cm^{-1}) 3307, 1708, 1666; ^1H NMR ($CDCl_3$) δ 0.67 (d, 3H, J = 6.8), 0.92 (d, 3H, J = 6.8), 1.28 (t, 3H, J = 7.2), 1.40 (s, 9H), 1.53−1.67 (m, 1H), 1.91−2.04 (m, 2H), 2.32−2.41 (m, 2H), 2.46−2.55 (m, 1H), 2.63 (dd, 1H, J = 12.1, 5.9), 2.69−2.80 (m, 1H), 2.83 (dd, 1H, J = 12.1, 8.2), 3.03 (dd, 1H, J = 17.7, 10.0), 4.05−4.11 (m, 1H), 4.17 (q, 2H, J = 7.2), 4.40−4.50 (m, 1H), 4.84 (d, 1H, J = 8.4), 5.38 (d, 1H, J = 15.7), 6.01 (d, 1H, J = 8.4), 6.60 (dd, 1H, J = 15.7, 5.0), 6.92−6.99 (m, 2H), 7.03−7.12 (m, 3H), 7.17−7.30 (m, 15H). Anal. ($C_{48}H_{56}FN_3O_7$) C, H, N.

Ethyl-3-{cyclopentylSCO-L-ValΨ[$COCH_2$]-L-Phe(4-F)-L-(Tr-*Gln*)}-E-propenoate (28). A solution of HCl in 1,4-dioxane (4.0 M, 3 mL) was added to a solution of **27** (0.273 g, 0.339 mmol, 1 equiv) in the same solvent (3 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 2 h and then was concentrated. The residue was dissolved in CH_2Cl_2 (5 mL), and *N*,*N*-diisopropylethylamine (0.177 mL, 1.02 mmol, 3.0 equiv) and cyclopentyl chlorothiolformate[23] (0.095 mL, 0.576 mmol, 1.7 equiv) were added sequentially. The reaction mixture was stirred for 2 h at 23 °C and then was partitioned between brine (30 mL) and CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford **28** (0.166 g, 59%) as a white foam: $R_f = 0.24$ (40% EtOAc in hexanes); IR (cm^{-1}) 3307, 1713, 1654; ^1H NMR ($CDCl_3$) δ 0.69 (d, 3H, J = 6.8), 0.93 (d, 3H, J = 6.8), 1.29 (t, 3H, J = 7.2), 1.47−1.75 (m, 7H), 1.91−2.12 (m, 4H), 2.30−2.41 (m, 2H), 2.51 (dd, 1H, J = 17.2, 2.3), 2.63 (dd, 1H, J = 12.3, 5.9), 2.69−2.80 (m, 1H), 2.84 (dd, 1H, J = 12.3, 8.4), 3.01 (dd, 1H, J = 17.2, 10.0), 3.57−3.67 (m, 1H), 4.17 (q, 2H, J = 7.2), 4.33−4.50 (m, 2H), 5.39 (dd, 1H, J = 15.7, 1.7), 5.61 (d, 1H, J = 7.8), 6.11 (d, 1H, J = 8.1), 6.60 (dd, 1H, J = 15.7, 4.8), 6.92−7.00 (m, 2H), 7.03−7.12 (m, 3H), 7.18−7.32 (m, 15H). Anal. ($C_{49}H_{56}FN_3O_6S$·0.5H_2O) C, H, N.

Ethyl-3-{cyclopentylSCO-L-ValΨ[$COCH_2$]-L-Phe(4-F)-L-*Gln*}-E-propenoate (9). Triisopropylsilane (0.097 mL, 0.47 mmol, 3.0 equiv) and trifluoroacetic acid (3 mL) were added sequentially to a solution of **28** (0.132 g, 0.158 mmol, 1 equiv) in CH_2Cl_2 (6 mL), producing a bright yellow solution. This solution was stirred for 30 min at 23 °C, during which time it became colorless, then was concentrated. The residue was triturated with Et_2O (6 mL), and the resulting solid was collected by filtration, washed with Et_2O (2 × 5 mL), and then dried under vacuum to give **9** (0.077 g, 82%) as a white solid: mp = 215 °C (dec); $R_f = 0.45$ (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3413, 3296, 1715, 1649; ^1H NMR (DMSO-d_6) δ 0.75 (d, 3H, J = 6.5), 0.82 (d, 3H, J = 6.5), 1.21 (t, 3H, J = 6.9), 1.36−1.75 (m, 8H), 1.92−2.14 (m, 5H), 2.52−2.85 (m, 4H), 2.87−2.99 (m, 1H), 3.47−3.58 (m, 1H), 4.06−4.18 (m, 1H), 4.09 (q, 2H, J = 6.9), 4.25−4.36 (m, 1H), 5.41 (d, 1H, J = 15.6), 6.61 (dd, 1H, J = 15.6, 5.1), 6.74 (s, 1H), 6.98−7.23 (m, 5H), 8.01 (d, 1H, J = 8.4), 8.28 (d, 1H, J = 8.1). Anal. ($C_{30}H_{42}FN_3O_6S$·0.25H_2O) C, H, N.

Representative Example of Preparation Method B. Synthesis of Ethyl-3-{cyclopentylSCO-L-PheΨ[$COCH_2$]-L-Phe(4-F)-L-*Gln*}-E-propenoate (12). 3-(4′-Fluorophenyl)-propionic Acid *cis*-(1″*S*,2″*R*)-1″-Amino-2″-indanolacetonide Amide (30). Oxalyl chloride (6.14 mL, 70.4 mmol, 1.05 equiv) was added to a solution of 3-(4′-fluorophenyl)propionic acid (11.3 g, 67.2 mmol, 1 equiv) and DMF (0.03 mL, 0.39 mmol, 0.006 equiv) in benzene (150 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 1.5 h and then was concentrated. The resulting oil was dissolved in THF (30 mL) and was added to a 0 °C solution of *cis*-(1*S*,2*R*)-1-amino-2-indanol (10.0 g, 67.0 mmol, 1.0 equiv) and Et_3N (10.3 mL, 73.9 mmol, 1.1 equiv) in THF (250 mL). After being stirred for 20 min at 0 °C, the reaction mixture was partitioned between half-saturated NH_4Cl (150 mL) and EtOAc (2 × 150 mL). The organic layers were dried over Na_2SO_4 and were concentrated to afford a white solid. This material was dissolved in a mixture of CH_2Cl_2 (400 mL) and 2-methoxypropene (30 mL), and the resulting solution was treated with methanesulfonic acid (0.20 mL). After being stirred for 15 min at 23 °C, the reaction mixture was partitioned between half-saturated $NaHCO_3$ (150 mL) and CH_2Cl_2 (2 × 150 mL). The combined organic layers were dried over $MgSO_4$ and gravity-filtered. The filtrate was concentrated, and the residue was purified by flash column chromatography (gradient elution, 10→20% EtOAc in hexanes) to provide **30** (18.2 g, 83%) as a pale yellow oil: R_f = 0.52 (50% EtOAc in hexanes); IR (cm^{-1}) 2934, 1645; ^1H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.60 (s, 3H), 2.91−2.95 (m, 2H), 3.04−3.13 (m, 4H), 4.68−4.71 (m, 1H), 5.06 (d, 1H, J = 4.7), 6.94−7.00 (m, 2H), 7.02−7.30 (m, 6H). Anal. ($C_{21}H_{22}FNO_2$) C, H, N.

(1*S*,2′*S*,4′*R*)-{1-[4′-(4″-Fluorobenzyl)-5′-oxo-tetrahydrofuran-2′-yl]-2-phenylethyl}carbamic Acid *tert*-Butyl Ester (32). *n*-Butyllithium (13.5 mL of a 1.6 M solution in hexanes, 21.6 mmol, 2.0 equiv) was added to a solution of (1*R*,2*S*)-1-oxiranyl-2-phenylethyl)carbamic acid *tert*-butyl ester **29**[26] (2.85 g, 10.8 mmol, 1 equiv) and **30** (3.67 g, 10.8 mmol, 1.0 equiv) in THF (150 mL) at −78 °C. The reaction mixture was stirred for 5 min at −78 °C, maintained at 0 °C for 1 h and then was partitioned between 0.5 M HCl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 150 mL). The organic layers were dried over Na_2SO_4 and were concentrated. Flash chromatographic purification of the residue (gradient elution, 25→40% EtOAc in hexanes) gave the coupling product **31** (3.19 g, 49%) as a yellow oil contaminated with several minor impurities. This material was dissolved in a 5:1 mixture of toluene and CH_2Cl_2 (180 mL) and was treated with *p*-toluenesulfonic acid monohydrate (1.01 g, 5.31 mmol, 1.0 equiv) at 23 °C. After being stirred for 13 h at 23 °C, the reaction mixture was filtered through a medium fritted funnel, and the filtrate was partitioned between half-saturated $NaHCO_3$ (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 150 mL). The organic layers were dried over Na_2SO_4 and were concentrated. The residue was purified by flash column

chromatography (20% EtOAc in hexanes) to provide **32** (1.28 g, 59%) as a white foam: $R_f = 0.46$ (30% EtOAc in hexanes); IR (cm^{-1}) 3332, 2976, 1767, 1702; ^1H NMR ($CDCl_3$) δ 1.36 (s, 9H), 1.88−1.97 (m, 1H), 2.19−2.29 (m, 1H), 2.75−2.99 (m, 4H), 3.05 (dd, 1H, J = 13.5, 4.5), 3.93 (q, 2H, J = 8.5), 4.13−4.18 (m, 1H), 4.54 (d, 1H, J = 9.7), 6.91−6.98 (m, 2H), 7.08−7.32 (m, 7H). Anal. ($C_{24}H_{28}FNO_4$) C, H, N.

Ethyl-3-{Boc-L-PheΨ[COCH$_2$]-L-Phe(4-F)-L-(Tr-*Gln*)}-E-propenoate (33). Lithium hydroxide (7.6 mL of a 1 M aqueous solution, 7.6 mmol, 5.0 equiv) was added to a solution of **32** (0.630 g, 1.52 mmol, 1 equiv) in DME (8 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 20 min and then was partitioned between 0.5 M HCl (100 mL) and EtOAc (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was dissolved in a 1:1 mixture of CH_2Cl_2 and CH_3CN (100 mL). 4-Methylmorpholine *N*-oxide (0.357 g, 3.05 mmol, 2.0 equiv), powdered 4 Å molecular sieves (0.70 g), and tetrapropylammonium perruthenate (0.054 g, 0.153 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 3 h at 23 °C and then was filtered through Celite. The filtrate was concentrated under reduced pressure to provide a brown oil which was dissolved in CH_2Cl_2 (40 mL). Crude ethyl-3-[H_2N-L-(Tr-*Gln*)]-E-propenoate·HCl[3] (**26**, 1.27 mmol, 1.2 equiv), HOBt (0.268 g, 1.98 mmol, 1.3 equiv), 4-methylmorpholine (0.670 mL, 6.09 mmol, 4.0 equiv), and EDC (0.380 g, 1.98 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred for 22 h at 23 °C and then was partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 150 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Purification of the residue by flash column chromatography (40% EtOAc in hexanes) provided **33** (0.558 g, 43%) as a white solid: mp = 89−100 °C; $R_f = 0.44$ (50% EtOAc in hexanes); IR (cm^{-1}) 3316, 2972, 1708, 1665; ^1H NMR ($CDCl_3$) δ 1.29 (t, 3H, J = 7.2), 1.35 (s, 9H), 1.95−2.05 (m, 1H), 2.34−2.39 (m, 2H), 2.46 (d, 1H, J = 16.8), 2.57−2.99 (m, 7H), 4.17 (q, 2H, J = 7.2), 4.27−4.33 (m, 1H), 4.48 (s, br, 1H), 4.58 (d, 1H, J = 6.9), 5.42 (d, 1H, J = 15.3), 6.08 (d, 1H, J = 8.4), 6.62 (dd, 1H, J = 15.3, 4.8), 6.93−7.19 (m, 6H), 7.21−7.29 (m, 19H); Anal. ($C_{52}H_{56}FN_3O_7$) C, H, N.

Ethyl-3-{cyclopentylSCO-L-PheΨ[COCH$_2$]-L-Phe(4-F)-L-(Tr-*Gln*)}-E-propenoate (34). A solution of HCl in 1,4-dioxane (4.0 M, 8 mL) was added to a solution of **33** (0.302 g, 0.354 mmol, 1 equiv) in the same solvent (10 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 1.5 h and then was concentrated. The resulting oil was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C, and 4-methylmorpholine (0.117 mL, 1.06 mmol, 3.0 equiv) and cyclopentyl chlorothiolformate[23] (0.087 mL, 0.528 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 30 min at 0 °C and then was partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was chromatographed on silica gel (gradient elution, 30→40% EtOAc in hexanes) to afford **34** (0.163 g, 52%) as a white solid: mp = 75−85 °C; $R_f = 0.48$ (50% EtOAc in hexanes); IR (cm^{-1}) 3314, 1710, 1655; ^1H NMR ($CDCl_3$) δ 1.29 (t, 3H, J = 7.2), 1.48−1.67 (m, 6H), 1.97−2.03 (m, 2H), 2.29−2.42 (m, 2H), 2.54−2.97 (m, 8H), 3.54−3.63 (m, 1H), 4.18 (q, 2H, J = 7.2), 4.51−4.58 (m, 2H), 5.44 (dd, 1H, J = 15.6, 1.7), 5.59 (d, 1H, J = 6.9), 5.90 (d, 1H, J = 7.2), 6.16 (d, 1H, J = 8.4), 6.64 (dd, 1H, J = 15.6, 5.0), 6.91−7.08 (m, 5H), 7.14−7.29 (m, 20H). Anal. ($C_{53}H_{56}FN_3O_6S$) C, H, N.

Ethyl-3-{cyclopentylSCO-L-PheΨ[COCH$_2$]-L-Phe(4-F)-L-*Gln*}-E-propenoate (12). Triisopropylsilane (0.10 mL, 0.488 mmol, 2.7 equiv) and trifluoroacetic acid (6 mL) were added sequentially to a solution of **34** (0.160 g, 0.181 mmol, 1 equiv) in CH_2Cl_2 (10 mL), producing a bright yellow solution. The reaction mixture was stirred for 30 min at 23 °C and then carbon tetrachloride (6 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH_3OH in CH_2Cl_2) to afford **12** (0.082 g, 71%) as a white solid: mp = 210−212 °C; $R_f = 0.10$ (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3284, 1717, 1637; ^1H NMR (DMSO-d_6) δ 1.21 (t, 3H, J = 6.8), 1.33−1.72 (m, 8H), 1.90−2.07 (m, 4H), 2.49−3.07 (m, 7H), 3.43−3.47 (m, 1H), 4.09 (q, 2H, J = 6.8), 4.33−4.35 (m, 2H), 5.37−5.46 (m, 1H), 6.59−6.67 (m, 1H), 6.77 (s, br, 1H), 7.00−7.28 (m, 9H), 8.04 (d, 1H, J = 7.8), 8.46 (d, 1H, J = 7.5), 8.53 (d, 1H, J = 7.5). Anal. ($C_{32}H_{42}FN_3O_6S$) C, H, N.

Representative Example of Preparation Method C. Synthesis of Ethyl-3-{cyclopentylSCO-L-tBuGlyΨ[COCH$_2$]-L-Phe(4-F)-L-*Gln*}-E-propenoate (14). Cbz-L-tBuGly-OH. Benzyl chloroformate (6.42 mL, 43.68 mmol, 1.12 equiv) and freshly prepared aqueous NaOH solution (1.0 M, 39.0 mL, 39.0 mmol, 1 equiv) were added simultaneously to a solution of H_2N-L-tBuGly-OH (5.12 g, 39.0 mmol, 1 equiv) in 1,4-dioxane (80 mL) at 0 °C. The reaction mixture was warmed to 23 °C and was stirred at that temperature for 18 h. The resulting solution was concentrated under reduced pressure to ∼40 mL volume and then was partitioned between 0.5 M HCl (100 mL) and CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated to give Cbz-L-tBuGly-OH (10.34 g, 100%) as a colorless oil. This material was used without further purification: IR (cm^{-1}) 3336, 1521, 1232; ^1H NMR ($CDCl_3$) δ 1.02 (s, 9H), 1.26 (s, 1H), 4.21 (d, 1H, J = 9.9), 5.13 (s, 2H), 5.35 (d, 1H, J = 9.0), 7.33−7.34 (m, 5H).

(*S*)-4-Benzyloxycarbonylamino-5,5-dimethyl-3-oxo-hexanoic Acid *tert*-Butyl Ester (35). To a solution of Cbz-L-tBuGly-OH (20.53 g, 77.4 mmol, 1 equiv) in THF (150 mL) was added CDI (13.81 g, 85.14 mmol, 1.1 equiv) at 23 °C. The resulting mixture was stirred at 23 °C for 1 h. In a separate flask, *n*-butyllithium (101.6 mL of a 1.6 M solution in hexanes, 162.5 mmol, 2.1 equiv) was added to a solution of diisopropylamine (22.78 mL, 162.5 mmol, 2.1 equiv) in THF (100 mL) at −78 °C. The reaction mixture was stirred for 15 min at −78 °C, warmed to 0 °C for 5 min, and then cooled back to −78 °C. A solution of *tert*-butyl acetate (21.9 mL, 162.5 mmol, 2.1 equiv) in THF (10 mL) was added via cannula, and the resulting mixture was stirred at −78 °C for 10 min. The Cbz-L-tBuGly-OH/CDI solution prepared above was then added dropwise to the lithium enolate at −78 °C. The resulting mixture was stirred at −78 °C for 1 h, quenched with 1 M HCl (100 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography on silica gel (10% EtOAc in hexanes) to afford **35** (12.1 g, 44%, contaminated with ∼10% minor impurities) as a pale yellow oil: IR (cm^{-1}) 1717, 1508, 1265, 739; ^1H NMR ($CDCl_3$) δ 1.09 (s, 9H), 1.44 (s, 9H), 3.50 (s, 2H), 4.29 (d, 1H, J = 9.3), 5.03 (s, 2H), 5.35−5.42 (m, 1H), 7.34 (s, 5H).

(*R*)-3-Phenyl-2-trifluoromethanesulfonyloxypropionic Acid Methyl Ester (36). 2,6-Lutidine (1.72 mL, 14.8 mmol, 3.3 equiv) and trifluoromethanesulfonic anhydride (2.50 mL, 14.8 mmol, 3.3 equiv) were added sequentially to a solution of (*R*)-2-hydroxy-3-phenylpropionic acid methyl ester (2.53 g, 14.06 mmol, 3.1 equiv) in CH_2Cl_2 (30 mL) at 0 °C. The resulting pink mixture was stirred at 0 °C for 30 min and then was partitioned between 0.5 M HCl (100 mL) and a 1:1 mixture of EtOAc in hexanes (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated to afford **36**. All of this material was utilized in the subsequent transformation without further purification.

Cbz-L-tBuGlyΨ[COCH$_2$]-L-Phe-OMe (38). A solution of **35** (1.64 g, 4.51 mmol, 1 equiv) in THF (100 mL) was added dropwise to a stirred suspension of NaH (0.19 g of a 60% dispersion in mineral oil, 4.74 mmol, 1.05 equiv) in THF (100 mL) at 0 °C. After the mixture was stirred for 10 min, a solution of **36** (prepared above) in CH_2Cl_2 (10 mL) was added dropwise. The resulting mixture was stirred at 23 °C for 24 h and then was partitioned between 1.0 M HCl (50 mL) and EtOAc (3 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated to provide the crude coupling product (**37**) as a pale yellow oil. Without further purification, the above oil was dissolved in CH_2Cl_2 (10 mL), treated with trifluoroacetic acid (2 mL), and

then maintained at 23 °C for 24 h. After dilution with CH_2Cl_2 (50 mL), the resulting solution was washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (10% EtOAc in hexanes) to afford **38** (1.01 g, 54%) as a pale yellow oil: R_f = 0.41 (25% EtOAc in hexanes); IR (cm^{-1}) 1711, 1514, 1233; ^1H NMR ($CDCl_3$) δ 0.97 (s, 9H), 2.58−2.76 (m, 2H), 2.96−3.17 (m, 3H), 3.62 (s, 3H), 4.17 (d, 1H, J = 8.1), 5.06−5.10 (s, 2H), 5.32 (d, 1H, J = 8.6), 7.12−7.36 (m, 10H). Anal. ($C_{25}H_{31}NO_5 \cdot 0.25H_2O$) C, H, N.

Boc-L-tBuGlyΨ[COCH$_2$]-L-Phe-OMe (39). A sample of 10% Pd on C (0.110 g) was added to a solution of **38** (0.513 g, 1.33 mmol, 1 equiv) and di-*tert*-butyl dicarbonate (0.378 g, 1.73 mmol, 1.3 equiv) in CH_3OH (10 mL) at 23 °C. The reaction mixture was stirred at 23 °C under an H_2 atmosphere (balloon) overnight and then was filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford **39** (0.366 g, 70%) as white solid: mp = 98−99 °C; R_f = 0.54 (25% EtOAc in hexanes); IR (cm^{-1}) 1707, 1497, 1367; ^1H NMR ($CDCl_3$) δ 0.97 (s, 9H), 1.40 (s, 9H), 2.60−2.78 (m, 2H), 2.95−3.19 (m, 3H), 3.63 (s, 3H), 4.07−4.11 (m, 2H), 5.07 (d, 1H, J = 9.3), 7.13−7.32 (m 5H). Anal. ($C_{25}H_{31}NO_5$) C, H, N.

Boc-L-tBuGlyΨ[COCH$_2$]-L-Phe-OH (40). A sample of 2.0 M NaOH (3.35 mL, 6.7 mmol, 8.0 equiv) was added to a solution of **39** (0.328 g, 0.84 mmol, 1 equiv) in CH_3OH (6 mL) at 0 °C over 10 min. The reaction mixture was stirred at 0 °C for 2 h and then was partitioned between 10% $KHSO_4$ (80 mL) and CH_2Cl_2 (2 × 100 mL). The organic layers were dried over Na_2SO_4 and were concentrated to give **40** (0.315 g, 99%) as a white solid which was used without further purification: IR (cm^{-1}) 2960, 1710, 1498; ^1H NMR ($CDCl_3$) δ 0.94 (s, 9H), 1.39 (s, 9H), 2.60−2.80 (m, 2H), 2.95−3.16 (m, 3H), 4.08 (d, 2H, J = 9.3), 5.09 (d, 1H, J = 9.6), 7.12−7.31 (m, 5H).

Ethyl-3-{Boc-L-tBuGlyΨ[COCH$_2$]-L-Phe-L-(Tr-*Gln*)}-E-propenoate (41). HOBt (0.170 g, 1.26 mmol, 1.5 equiv), 4-methylmorpholine (0.77 mL, 2.52 mmol, 3 equiv), and EDC (0.247 g, 1.26 mmol, 1.5 equiv) were added sequentially to a solution of ethyl-3-[H_2N-L-(Tr-*Gln*)]-E-propenoate·HCl[3] (**26**, 1.01 mmol, 1.2 equiv) and **40** (0.315 g, 0.84 mmol, 1 equiv) in CH_2Cl_2 (10 mL) at 23 °C. The reaction mixture was stirred at 23 °C overnight and then was partitioned between water (100 mL) and CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by flash column chromatography (35% EtOAc in hexane) to afford **41** (0.474 g, 70%) as a white foam: R_f = 0.58 (50% EtOAc in hexanes); IR (cm^{-1}) 1702, 1669, 1494, 1169; ^1H NMR ($CDCl_3$) δ 0.85 (s, 9H), 1.30 (t, 3H, J = 7.2), 1.41 (s, 9H), 1.56−1.65 (m, 1H), 1.95−2.02 (m, 1H), 2.22−2.42 (m, 2H), 2.62−2.88 (m, 4H), 3.09−3.18 (m, 1H), 4.00 (d, 1H, J = 8.7), 4.17 (t, 2H, J = 7.2), 4.46−4.51 (m, 1H), 4.93 (d, 1H, J = 8.7), 5.37 (d, 1H, J = 15.9), 5.69 (d, 1H, J = 9.3), 6.54 (dd, 1H, J = 15.9, 4.8), 7.19−7.31 (m, 21H). Anal. ($C_{49}H_{59}N_3O_7$) C, H, N.

Ethyl-3-{cyclopentylSCO-L-tBuGlyΨ[COCH$_2$]-L-Phe-L-(Tr-*Gln*)}-E-propenoate (42). A solution of HCl in 1,4-dioxane (4.0 M, 4 mL) was added dropwise to a solution of **41** (0.441 g, 0.55 mmol, 1 equiv) in the same solvent at 23 °C. The reaction mixture was stirred for 2 h at 23 °C and then was concentrated to provide the amine salt as a white foam. This material was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C, and 4-methylmorpholine (0.181 mL, 1.65 mmol, 3 equiv) and cyclopentyl chlorothiolformate[23] (0.135 mL, 0.82 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred at 0 °C for 30 min and then was partitioned between H_2O (50 mL) and a 1:1 mixture of EtOAc and hexanes (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. The residue was purified by flash column chromatography (35% EtOAc in hexane) to provide **42** (0.347 g, 76%) as a white foam: R_f = 0.48 (50% EtOAc in hexanes); IR (cm^{-1}) 1718, 1656, 1493, 1186; ^1H NMR ($CDCl_3$) δ 0.86 (s, 9H), 1.27 (t, 3H, J = 7.2), 1.56−1.78 (m, 6H), 1.95−2.16 (m, 4H), 2.22−2.39 (m, 2H), 2.60−2.90 (m, 4H), 3.05−3.14 (m, 1H), 3.59−3.64 (m, 1H), 4.16 (q, 2H, J = 7.2), 4.31 (d, 1H, J = 8.4), 4.48 (m, 1H), 5.41 (dd, 1H, J = 15.9, 1.8), 5.67 (d, 1H, J = 8.7), 5.82 (d, 1H, J = 9.0), 6.56 (dd, 1H, J = 15.6, 5.1), 7.19−7.31 (m, 21H). Anal. ($C_{50}H_{59}N_3O_6$) C, H, N.

Ethyl-3-{cyclopentylSCO-L-tBuGly-Ψ[COCH$_2$]-L-Phe-L-*Gln*}-E-propenoate (14). Triisopropylsilane (0.077 mL, 0.38 mmol, 1.0 equiv) and trifluoroacetic acid (4 mL) were added sequentially to a solution of **42** (0.318 g, 0.38 mmol, 1 equiv) in CH_2Cl_2 (4 mL) at 23 °C, producing a bright yellow solution. After the mixture was stirred for 30 min, no yellow color remained. The volatiles were removed under reduced pressure, and the resulting white solid was triturated with Et_2O (10 mL) and filtered to give **14** (0.204 g, 91%): mp 65−68 °C; R_f = 0.32 (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 1715, 1652, 1520, 1193; ^1H NMR ($CDCl_3$) δ 0.96 (s, 9H), 1.31 (t, 3H, J = 7.2), 1.45−1.72 (m, 6H), 1.96−2.13 (m, 4H), 2.23 (t, 2H, J = 7.5), 2.68−2.79 (m, 2H), 2.84−2.95 (m, 2H), 3.11−3.21 (m, 1H), 3.59−3.69 (m, 1H), 4.18 (q, 2H, J = 7.2), 4.36 (d, 1H, J = 8.1), 4.52−4.59 (m, 1H), 5.37 (s, br, 1H), 5.42 (dd, 1H, J = 15.9, 1.5), 5.80 (d, 1H, J = 9.0), 5.90 (d, 1H, J = 8.4), 6.46 (s, br, 1H), 6.61 (dd, 1H, J = 15.9, 5.1), 7.18−7.30 (m, 5H). Anal. ($C_{31}H_{45}N_3O_6S$) C, H, N.

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(14) The antirhinoviral properties of certain 3CP inhibitors were determined under similar conditions against several additional HRV serotypes in cell culture. In general, the activity observed for a given compound vs serotypes 1A, 2, and 10 was representative of its broad-spectrum antiviral properties. Serotype 14 nearly always proved to be the most susceptible to the antiviral agents under study. Full details of these experiments will be presented elsewhere (Patick, A. K. Manuscript in preparation).

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(29) Compound **15** was synthesized by a method analogous to that reported previously for the preparation of tripeptide-derived, irreversible 3CP inhibitors. See refs 3 and 4 above.

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