Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 2. Peptide Structure−Activity Studies

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The structure-based design, chemical synthesis, and biological evaluation of various peptide-derived human rhinovirus (HRV) 3C protease (3CP) inhibitors are described. These compounds are comprised of an ethyl propenoate Michael acceptor moiety and a tripeptidyl binding determinant. The systematic modification of each amino acid residue present in the binding determinant as well as the N-terminal functionality is described. Such modifications are shown to provide irreversible HRV-14 3CP inhibitors with anti-3CP activities (k_{obs}/[I]) ranging from 60 to 280 000 M^{-1} s^{-1} and antiviral EC_{50}'s which approach 0.15 μM. An optimized inhibitor which incorporates several improvements identified by the structure−activity studies is also described. This molecule displays very rapid irreversible inhibition of HRV-14 3CP (k_{obs}/[I] = 800 000 M^{-1} s^{-1}) and potent antiviral activity against HRV-14 in cell culture (EC_{50} = 0.056 μM). A 1.9 Å crystal structure of an *S*-alkylthiocarbamate-containing inhibitor complexed with HRV-2 3CP is also detailed.

Introduction

The preceding paper describes the discovery of irreversible inhibitors of human rhinovirus (HRV) 3C proteases (3CPs) which display in vitro antiviral activity against several rhinovirus serotypes.[1] These inhibitors are comprised of a substrate-derived tripeptide binding determinant which provides affinity for the target proteases and a Michael acceptor moiety which forms a covalent adduct with the active site cysteine residue of the 3C enzymes (Figure 1).[2,3] Efforts to optimize the Michael acceptor portion of these molecules are detailed in the preceding paper.[1] In an attempt to further improve and define this class of 3CP inhibitors, modification of the peptidyl binding determinant was also undertaken. The results of this study are described below.

Structure−Activity Studies

Exploration of peptidyl structure−activity relationships began by truncation of the previously studied[1] tripeptide inhibitor **3** (Table 1). A molecule derived from a single amino acid (**1**) displayed poor 3CP inhibition properties and no measurable antiviral activity (EC_{50}).[4] Similarly, a dipeptide inhibitor (**2**) exhibited significantly reduced anti-3CP and antiviral properties relative to the tripeptide compound **3**. These data indicated that peptidyl inhibitors composed of at least three amino acids were required for effective recognition of the target protease, and subsequent structure−activity studies were therefore conducted with tripeptide-derived molecules. A systematic approach was utilized in which one amino acid component of **3** was modified while the other two remained unchanged. In addition, alteration of the

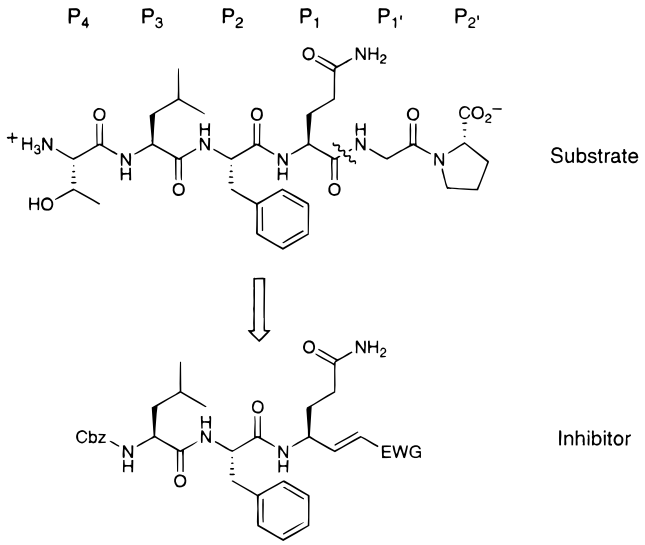

Figure 1. Design of irreversible HRV 3CP inhibitors. EWG = electron-withdrawing group.

N-terminal Cbz moiety present in **3** was examined upon completion of the amino acid variations. Due to its ease of preparation, the ethyl propenoate Michael acceptor was employed extensively for such structure−activity studies, although it was anticipated that this moiety might undergo in vivo metabolism.

Tripeptidyl structure−activity studies commenced with modification of the P_1 glutamine residue present in the irreversible 3CP inhibitor **3** (Table 2).[3] Since crystallographic analysis of the HRV-2 3CP−**3** complex[1] indicated that the glutamine amide formed several hydrogen bonds with the protein, it was expected that

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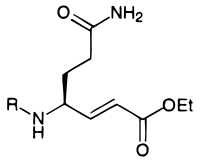
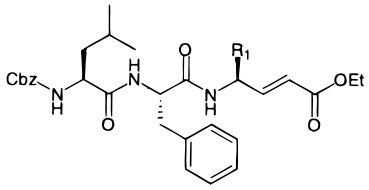
Table 1. Truncated 3C Protease Inhibitors



compd no.	R	prep[a]	formula[b]	$k_{obs}/[I]$ (M^{-1} s^{-1})[c,d]	EC_{50} (μM)[c,d]	CC_{50} (μM)[d]
1	Cbz	A	$C_{17}H_{22}N_2O_5$	4.5	>100	>100
2	Cbz-L-Phe	A	$C_{26}H_{31}N_3O_6$	400	5.6	>100
3	Cbz-L-Leu-L-Phe	A	$C_{32}H_{42}N_4O_7$	25 000	0.54	>320

[a] Method of preparation: see Scheme 1. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Serotype 14. [d] See ref 1 for assay method and error.

Table 2. Substitutions of the P_1 Side Chain (Tr = CPh_3)



compd no.	R_1	prep[a]	formula[b]	$k_{obs}/[I]$ (M^{-1} s^{-1})[c,d]	EC_{50} (μM)[c,d]	CC_{50} (μM)[d]
3	$CH_2CH_2C(O)NH_2$	A	$C_{32}H_{42}N_4O_7$	25 000	0.54	>320
4	$CH_2CH_2C(O)NHTr$	A	$C_{51}H_{56}N_4O_7$	0	100	>320
5	$CH_2CH_2C(O)NH(CH_3)$	A	$C_{33}H_{44}N_4O_7$	750	5.6	>100
6	$CH_2CH_2C(O)N(CH_3)_2$	A	$C_{34}H_{46}N_4O_7$	60	4.0	>100
7	$CH_2CH_2CO_2H$	A	$C_{32}H_{41}N_3O_8$	500	14	>100
8	$CH_2CH_2C(O)CH_3$	A	$C_{33}H_{43}N_3O_7$	1 400	1.6	>100
9	$CH_2CH_2CH(OH)CH_3$[e]	A	$C_{33}H_{45}N_3O_7$	0	>100	>100
10	$CH_2CH_2S(O)CH_3$[e]	B	$C_{32}H_{43}N_3O_7S$	2 200	1.6	>100
11	$CH_2CH_2SO_2CH_3$	B	$C_{32}H_{43}N_3O_8S$	60	>100	>100
12	$CH_2NHC(O)CH_3$	B	$C_{32}H_{42}N_4O_7$	800	2.2	>320
13	$CH_2NHC(O)NH_2$	A	$C_{31}H_{41}N_5O_7$	3 500	32	>100
14	$CH_2OC(O)NH_2$	A	$C_{31}H_{40}N_4O_8$	5 600	1.6	>320
15	CH_2OH	A	$C_{30}H_{39}N_3O_7$	0	>190	190

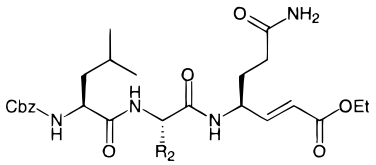
[a] Method of preparation: see Scheme 1. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Serotype 14. [d] See ref 1 for assay method and error. [e] 1:1 Mixture of diastereomers.

alteration of this moiety would result in reduced 3CP inhibition. In the event, alkyl substitution of the primary amide nitrogen led to significant or complete loss of anti-3CP properties (**4**−**6**). Similar results were obtained by replacement of the glutamine amide with a variety of isosteres (**7**−**12**). The low activity displayed by compounds **4**, **6**, **11**, and **12** was somewhat surprising since related peptide aldehydes are reported to exhibit either good 3CP inhibition levels or measurable anti-rhinoviral properties.[5,6] Inclusion of various heteroatoms in the glutamine side chain also reduced activity toward 3CP (**13** and **14**), but this reduction was not as severe as that resulting from the amide modifications described above. Replacement of the glutamine residue with serine afforded a compound (**15**) which did not inhibit 3CP and further confirmed that the presence of a P_1 glutamine was essential for potent 3CP inhibition by peptidic Michael acceptors.

Variation of the P_2 phenylalanine moiety present in the tripeptidyl inhibitor **3** was also examined (Table 3).[3] Removal or truncation of the amino acid side chain resulted in significant loss of 3CP inhibitory activity (**16**−**22**) while saturation of the aryl group afforded a compound with relatively good anti-3CP properties (**23**). Crystallographic analysis of the HRV-2 3CP−**3** complex[1] indicated that additional functionality could be incorporated at the 4-position of the aryl ring without adversely affecting 3CP inhibition. Accordingly, many compounds containing 4-substituted phenylalanine residues were prepared and evaluated as 3CP inhibitors (**24**−**35**). Several of these molecules exhibited increased inhibitory properties when tested against HRV-14 3CP. However, in some cases this increase in activity was accompanied by a decrease against proteases derived from other HRV serotypes (compare **28** and **33** with **3**). The varied activity displayed by these inhibitors against proteases derived from different HRV serotypes is believed to be due to amino acid variations in this portion of the enzyme.[7,8] In addition, as illustrated by compounds **27**, **30**, **34**, and **35**, not all 4-substituted Phe-containing inhibitors showed increased activity toward HRV-14 3CP. Substitution of other aryl moieties in place of the phenyl ring present in **3** typically reduced 3CP inhibition properties somewhat (**36** and **37**), although a thiophene-containing molecule (**38**) displayed anti-3CP properties nearly identical to that of the parent compound. Two other amino acids which occur at the P_2 position in 3CP substrates (Glu and Thr) were also included in the inhibitor design.[2] However, the resulting compounds (**39** and **40**) displayed significantly reduced anti-3CP activity relative to the Phe-containing inhibitor **3**.

Modification of the P_3 amino acid residue present in tripeptidyl inhibitors such as **3** was undertaken as well (Table 4).[3] Analysis of the HRV-2 3CP-**3** crystal structure[1] indicated that the leucine side chain of **3** did not appreciably contact the protein and was highly solvent-exposed. A wide variety of functionality was therefore

Table 3. Substitutions of the P_2 Side Chain



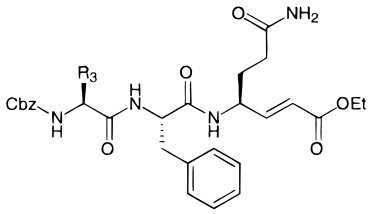
compd no.	R_2	prep[a]	formula[b]	serotype[c]	k_{obs}/[I] (M^{-1} s^{-1})[d]	EC_{50} (μM)[d]	CC_{50} (μM)[d]
3	CH_2Ph	A	$C_{32}H_{42}N_4O_7$		25 000	0.54	>320
				16	6 500	2.3	
				2	2 000	1.6	
16	H	A	$C_{25}H_{36}N_4O_7$		slow	141	>320
17	CH_3	A	$C_{26}H_{38}N_4O_7$		1 300	20	>320
18	CH_2CH_3	C	$C_{27}H_{40}N_4O_7$		4 500	6.0	>320
19	$CH_2CH_2CH_3$	C	$C_{28}H_{42}N_4O_7$		2 300	5.0	>320
				16	1 200	ND	
				2	600	ND	
20	$CH_2CH(CH_3)_2$	A	$C_{29}H_{44}N_4O_7$		2 300	5.4	>320
21	CH_2SCH_3	C	$C_{27}H_{40}N_4O_7S$		2 900	8.9	>320
22	$CH_2SCH_2CH_3$	C	$C_{28}H_{42}N_4O_7S$		3 000	10	>320
23	CH_2Cyclohexyl	C	$C_{32}H_{48}N_4O_7$		16 300	1.9	>320
				16	3 500	ND	
				2	700	ND	
24	CH_2Ph(4-F)	D	$C_{32}H_{41}FN_4O_7 \cdot 1.25H_2O$		46 000	1.8	>320
				16	9 200	ND	
				2	2 400	5.6	
25	$CH_2Ph(4\text{-}CH_3)$	D	$C_{33}H_{44}N_4O_7$		59 400	0.18	>320
				16	5 300	ND	
				2	1 400	ND	
26	CH_2Ph(4-OH)	D	$C_{32}H_{42}N_4O_8$		11 300	5.3	>320
27	CH_2Ph(4-OAc)	D	$C_{34}H_{44}N_4O_9$		1 200	11	>320
28	$CH_2Ph(4\text{-}OCH_3)$	C	$C_{33}H_{44}N_4O_8 \cdot 0.25H_2O$		29 000	1.7	>320
				16	3 400	ND	
				2	1 100	14	
29	$CH_2Ph(4\text{-}OCH_2CH_3)$	C	$C_{34}H_{46}N_4O_8$		4 200	>320	>320
30	$CH_2Ph(4\text{-}OPO_3H_2)$	D	$C_{32}H_{43}N_4O_{11}P$		1 200	14	>320
31	$CH_2Ph(4\text{-}CH_2OH)$	C	$C_{33}H_{44}N_4O_8 \cdot 0.83H_2O$		82 300	0.55	>320
32	$CH_2Ph(4\text{-}CH_2OCH_3)$	C	$C_{34}H_{46}N_4O_8$		7 100	39	>320
33	$CH_2Ph(4\text{-}CH_2CH_2OH)$	C	$C_{34}H_{46}N_4O_8 \cdot 1.0H_2O$		43 100	3.5	>320
				16	1 300	ND	
				2	700	ND	
34	CH_2Ph(4-CN)	D	$C_{33}H_{41}N_5O_7$		10 800	5.6	>320
35	$CH_2Ph[4\text{-}C(O)NH_2]$	D	$C_{33}H_{43}N_5O_8$		9 600	>177	177
36	CH_2(2-Imidazole)[e]	A	$C_{31}H_{41}F_3N_6O_9$		6 800	27	>320
37	CH_2(2-*N*-Methylimidazole)[e]	A	$C_{32}H_{43}F_3N_6O_9$		8 900	10	>320
38	CH_2(2-Thienyl)	D	$C_{30}H_{40}N_4O_7S$		20 000	0.56	>100
				16	4 500	ND	
				2	1 800	ND	
39	$CH_2CH_2CO_2H$	A	$C_{28}H_{40}N_4O_9$		200	>320	>320
40	$CH(R\text{-}OH)CH_3$	C	$C_{27}H_{40}N_4O_8 \cdot 0.25H_2O$		1 800	56	>320

[a] Method of preparation: see Scheme 1. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Serotype 14 unless otherwise noted. [d] See ref 1 for assay method and error. [e] TFA salt. ND = not determined.

expected to be tolerated at this position within the inhibitor design. As was observed for the P_2 phenylalanine residue, removal or truncation of the P_3 amino acid side chain reduced 3CP inhibitory properties (**41** and **42**). However, incorporation of several other aliphatic (**43**−**50**) and/or aromatic (**51**−**55**) moieties at the P_3 position often improved anti-3CP and antiviral activity relative to the leucine-containing inhibitor described above (compare to compound **3**). Amino acids containing hydroxylated side chains (**56**−**59**) also afforded active 3CP inhibitors when included at the P_3 position, although such compounds exhibited antiviral activities somewhat weaker than similar aliphatic molecules (compare **43** with **57**). Predictably, molecules containing ionizable P_3 residues (**60**−**64**) displayed significantly reduced antiviral properties compared to related nonionizable inhibitors, presumably due to poorer membrane permeability.

In addition to alteration of the amino acid residues described above, modification of the N-terminal (P_4) functionality contained in the tripeptidyl inhibitors was also examined (Table 5).[3] Replacement of the Cbz group present in the parent compound **3** with other alkyl or benzylic carbamates either increased or reduced 3CP inhibitory properties depending on the structure of the appended moiety (**66**−**71**). Crystallographic analysis of the HRV-2 3CP−**3** complex[1] indicated that a significant gap existed between the inhibitor and the protein directly beneath the carbamate oxygen atom adjacent to the benzyl group. Such analysis suggested that incorporation of a larger moiety at this position would improve the 3CP affinity of the resulting inhibitor. Indeed, inclusion of a P_4 *S*-alkyl thiocarbamate in the inhibitor design (**72**−**75**) dramatically increased anti-3CP properties relative to similar carbamate-containing molecules (compare **3** with **75**). This improvement was

Table 4. Substitutions of the P_3 Side Chain

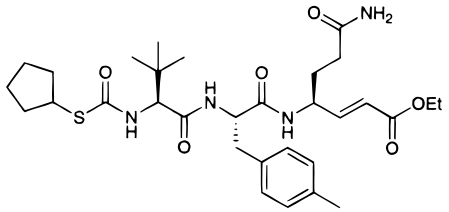


compd no.	R_3	prep[a]	formula[b]	k_{obs}/[I] (M^{-1} s^{-1})[c,d]	EC_{50} (μM)[c,d]	CC_{50} (μM)[d]
3	$CH_2CH(CH_3)_2$	A	$C_{32}H_{42}N_4O_7$	25 000	0.54	>320
41	H	A	$C_{28}H_{34}N_4O_7 \cdot 1.0H_2O$	3 800	5.6	>320
42	CH_3	A	$C_{29}H_{36}N_4O_7 \cdot 0.67H_2O$	11 300	2.0	>320
43	$CH(CH_3)_2$	C	$C_{31}H_{40}N_4O_7$	62 500	0.38	>320
44	$CH(S\text{-}CH_3)CH_2CH_3$	C	$C_{32}H_{42}N_4O_7$	31 700	0.79	>100
45	$C(CH_3)_3$	E	$C_{32}H_{42}N_4O_7 \cdot 0.75H_2O$	39 800	0.32	178
46	$CH_2CH_2SCH_3$	C	$C_{31}H_{40}N_4O_7S$	46 200	1.4	>100
47	CH_2SCH_3	E	$C_{30}H_{38}N_4O_7S \cdot 1.5H_2O$	35 200	0.18	>100
48	$CH(R\text{-}CH_3)SCH(CH_3)_2$	C	$C_{33}H_{44}N_4O_7S \cdot 0.50H_2O$	40 000	10	>100
49	Cyclohexyl	C	$C_{34}H_{44}N_4O_7$	36 200	1.0	>100
50	CH_2Cyclohexyl	D	$C_{35}H_{46}N_4O_7 \cdot 0.50H_2O$	39 000	1.2	>320
51	Ph[e]	D	$C_{34}H_{38}N_4O_7 \cdot 0.50H_2O$	20 700	ND	ND
52	CH_2Ph	B	$C_{35}H_{40}N_4O_7$	161 500	0.56	>320
53	CH_2SPh	D	$C_{35}H_{40}N_4O_7S$	141 200	0.12	>320
54	CH_2SCH_2Ph	D	$C_{36}H_{42}N_4O_7S$	97 000	0.20	>100
55	CH_2CH_2Ph	D	$C_{36}H_{42}N_4O_7$	57 000	0.25	>320
56	CH_2OH	C	$C_{29}H_{36}N_4O_8 \cdot 0.60H_2O$	39 900	1.8	>320
57	$CH(R\text{-}OH)CH_3$	C	$C_{30}H_{38}N_4O_8 \cdot 0.75H_2O$	38 900	1.8	>320
58	$C(CH_3)_2OH$	C	$C_{31}H_{40}N_4O_8 \cdot 0.25H_2O$	98 000	0.66	>100
59	$C(CH_3)_2CH_2OH$	A	$C_{32}H_{42}N_4O_8 \cdot 0.75H_2O$	40 000	1.3	>100
60	$(CH_2)_4NH_2$[f]	C	$C_{34}H_{44}F_3N_5O_9 \cdot 0.50H_2O$	18 600	205	>320
61	CH_2CH_2Morpholine[f]	C	$C_{36}H_{46}F_3N_5O_{10} \cdot 0.75H_2O$	35 000	5.1	>100
62	$CH_2(CH_2)_2$Morpholine[f]	C	$C_{37}H_{48}F_3N_5O_{10} \cdot 0.75H_2O$	43 300	7.1	>100
63	CH_2CO_2H	C	$C_{30}H_{36}N_4O_9$	35 000	2.4	>320
64	$CH_2CH_2CO_2H$	C	$C_{31}H_{38}N_4O_9 \cdot 0.25H_2O$	78 900	5.5	>320
65	$CH_2C(O)N(CH_3)_2$	C	$C_{32}H_{41}N_5O_8 \cdot 1.0H_2O$	22 600	5.9	>320

[a] Method of preparation: see Scheme 1. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Serotype 14. [d] See ref 1 for assay method and error. [e] 9:1 mixture of diastereomers. [f] TFA salt. ND = not determined.

the largest realized by a single modification during the structure−activity studies described herein and was subsequently shown crystallographically to result from significantly increased recognition of the target protease (see below). Inclusion of a variety of amides at the P_4 position (**76**−**84**) in the inhibitor design was tolerated but typically reduced anti-3CP and antiviral properties relative to the Cbz-containing compound **3**. One notable exception was compound **82** which displayed properties very similar to the parent molecule. Replacement of the P_4 carbamate group with a urea moiety significantly reduced both anti-3CP and antiviral activity (**85** and **86**), while incorporation of several substrate-inspired[2] acylated amino acids afforded tetrapeptides with good anti-3CP activity but poor antiviral properties (**87**−**89**).

Having completed the systematic structure−activity studies described above, an attempt was made to incorporate several modifications into a single compound. Thus, an inhibitor was conceived which contained functionalities shown above to increase both anti-3CP and antiviral activity relative to the parent compound **3**. The resulting molecule (**90**) displayed very rapid, irreversible inhibition of HRV-14 3CP and exhibited potent antiviral activity when tested against HRV-14 in cell culture. These results suggest that improvements realized by the structure−activity studies described above can be combined in an additive manner. The activity of **90** against HRV serotypes other than 14 as well as the examination of other functional group combinations will be described in future publications.



90

k_{obs}/[I] = 800,000 $M^{-1}s^{-1}$

EC_{50} = 0.056 μM

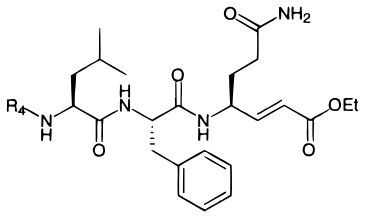
(Serotype-14)

X-ray Structure Analysis

Several crystal structures of 3CP−inhibitor complexes were obtained during the peptidyl structure−activity studies described above. These structures confirmed the binding geometries that the inhibitors adopted when complexed with 3CP and suggested locations for peptide side chain modifications. In particular, the previously detailed crystal structure of the HRV-2 3CP−**3** complex[1] inspired several improvements to the tripeptide inhibitor design. As illustrated above, the most significant of these was the incorporation of an N-terminal *S*-alkyl thiocarbamate moiety (compounds **72**−**75**). The crystallographic details of one such inhibitor complexed with 3CP are described below.

The 1.9 Å X-ray crystal structure of the covalent adduct formed between compound **75** and HRV-2 3CP[9] is shown in Figure 2. In general, the inhibitor bound

Table 5. Substitutions of the P_4 Moiety



compd no.	R_4	prep[a]	formula[b]	serotype[c]	k_{obs}/[I] (M^{-1} s^{-1})[d]	EC_{50} (μM)[d]	CC_{50} (μM)[d]
3	CO_2CH_2Ph	A	$C_{32}H_{42}N_4O_7$		25 000	0.54	>320
				16	6 500	2.3	
				2	3 400	1.6	
66	$CO_2CH_2Ph(2\text{-}CH_3)$	C	$C_{33}H_{44}N_4O_7 \cdot 0.50H_2O$		41 800	1.0	>100
67	$CO_2CH_2Ph(2\text{-}Cl)$	C	$C_{32}H_{41}ClN_4O_7 \cdot 0.50H_2O$		19 000	0.63	>100
68	$CO_2CH_2(4\text{-}Pyridine)$	F	$C_{31}H_{41}N_5O_7$		12 000	56	>320
69	CO_2CH_3	C	$C_{26}H_{38}N_4O_7 \cdot 0.25H_2O$		9 100	1.3	>320
70	CO_2-Cyclohexyl	C	$C_{31}H_{46}N_4O_7$		15 500	7.6	>100
71	$CO_2C(CH_3)_3$	A	$C_{29}H_{44}N_4O_7$		700	4.5	>100
72	$C(O)SCH_3$	C	$C_{26}H_{38}N_4O_6S$		69 800	1.1	>320
73	$C(O)SCH_2CH_3$	C	$C_{27}H_{40}N_4O_6S \cdot 0.25H_2O$		91 300	0.46	>320
				16	20 000	ND	
				2	8 900	1.0	
74	C(O)S-Cyclopentyl	C	$C_{30}H_{44}N_4O_6S \cdot 0.50H_2O$		114 000	0.18	>100
75	$C(O)SCH_2Ph$	D	$C_{32}H_{42}N_4O_6S$		280 000	0.27	>320
				16	75 000	ND	
				2	28 400	1.9	
76	C(O)-2-Naphthalene	F	$C_{35}H_{42}N_4O_6$		21 000	1.0	>320
77	C(O)Ph	F	$C_{31}H_{40}N_4O_6$		9 600	5.2	>320
78	C(O)Ph(4-OPh)	D	$C_{37}H_{44}N_4O_7 \cdot 1.0H_2O$		8 000	5.2	>320
79	$C(O)CH_3$	C	$C_{26}H_{38}N_4O_6 \cdot 0.25H_2O$		3 700	14	>320
80	$C(O)CH(CH_3)_2$	C	$C_{28}H_{42}N_4O_6 \cdot 0.25H_2O$		18 800	1.0	>320
81	$C(O)C(CH_3)_3$	E	$C_{29}H_{44}N_4O_6$		8 200	1.8	>100
82	C(O)-Cyclopentyl	C	$C_{30}H_{44}N_4O_6 \cdot 0.20H_2O$		23 700	0.60	>320
83	$C(O)CH_2OH$	C	$C_{26}H_{38}N_4O_7 \cdot 0.50H_2O$		2 100	30	>320
84	$C(O)CH_2CH_2OH$	C	$C_{27}H_{40}N_4O_7 \cdot 0.75H_2O$		4 500	19	>320
85	$C(O)NHCH_2Ph$	E	$C_{32}H_{43}N_5O_6 \cdot 0.25H_2O$		6 900	>100	>100
86	$C(O)N(CH_3)CH_2Ph$	E	$C_{33}H_{45}N_5O_6 \cdot 1.0H_2O$		3 200	5.6	>100
87	Ac-L-Val	E	$C_{31}H_{47}N_5O_7 \cdot 0.50H_2O$		26 700	63	>100
88	Ac-L-Ala	E	$C_{29}H_{43}N_5O_7$		43 400	20	>100
89	Ac-L-Thr	E	$C_{30}H_{45}N_5O_8 \cdot 0.50H_2O$		4 600	>100	>100

[a] Method of preparation: see Scheme 1. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Serotype 14 unless otherwise noted. [d] See ref 1 for assay method and error. ND = not determined.

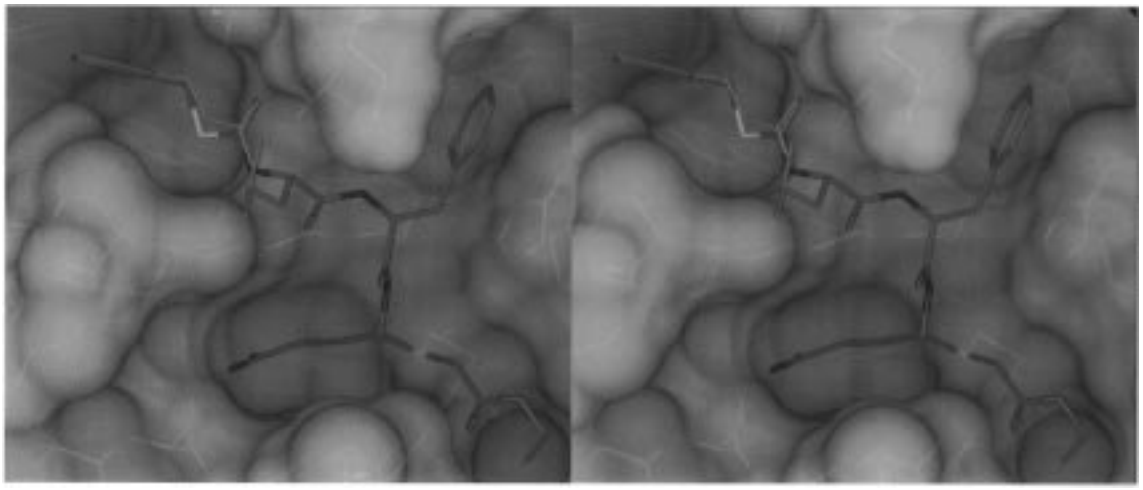
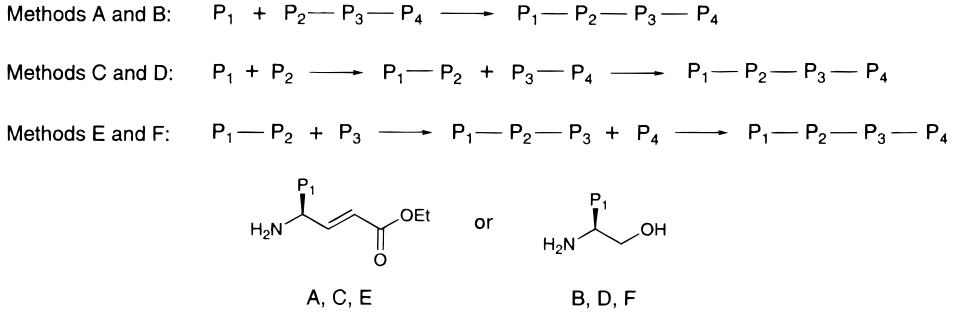


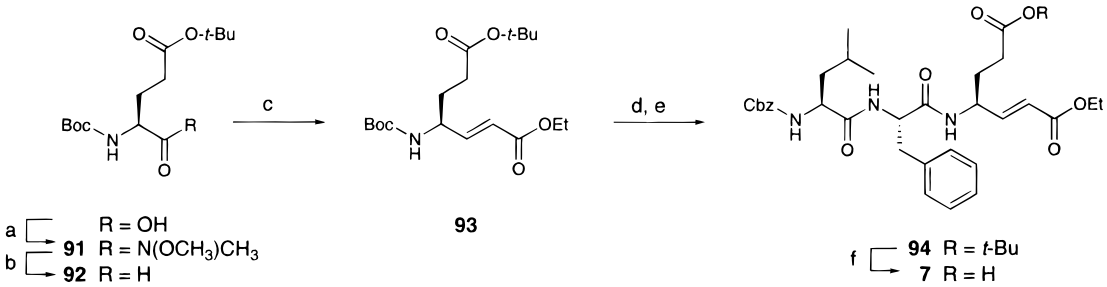
Figure 2. Stereoview of the crystal structure of **75** complexed with HRV-2 3CP. A portion of the water-accessible surface of the protein is shown in gray. A fragment of compound **3** from the HRV-2 3CP−**3** complex[1] is superimposed and is shown in orange.

to the enzyme in a manner similar to that observed previously for the related Cbz-containing molecule (**3**).[1] A covalent bond was observed between the 3CP active site cysteine residue (Cys-147) and the β-carbon of the Michael acceptor of **75**, and all hydrogen bonds observed in the 3CP−**3** complex were also present in the 3CP−**75** crystal structure. However, the sulfur atom present in **75** was buried more deeply into the 3CP S_4 binding pocket than the related oxygen atom contained in the Cbz moiety of **3** (Figure 2). The S atom placement also slightly altered the location of the appended benzyl group of **75** compared to that observed previously for

Scheme 1. Peptide Coupling Sequences Utilized to Prepare 3CP Inhibitors



Scheme 2[a]



[a] Reagents and conditions: (a) 1.0 equiv of isobutyl chloroformate, 1.0 equiv of $HCl \cdot HN(OCH_3)CH_3$, 2.0 equiv of NMM, CH_2Cl_2, 0 °C, 20 min → 23 °C, 30 min, 91%; (b) 2.25 equiv of DIBAL, THF, −78 °C, 1 h; (c) 1.0 equiv of $(EtO)_2POCH_2CO_2Et$, 1.0 equiv of $NaN(TMS)_2$, THF, −78 °C, 15 min, then crude **92**, −78 °C, 1 h → 0 °C, 10 min, 36% from **91**; (d) HCl in 1,4-dioxane, 23 °C, 3 h; (e) 1.0 equiv Cbz-L-Leu-L-Phe-OH, 1.2 equiv of HOBT, 4.0 equiv of NMM, 1.2 equiv of EDC, CH_2Cl_2, 23 °C, 14 h, 32%; (f) 1.5 equiv of $(i\text{-}Pr)_3SiH$, 1:2 TFA:CH_2Cl_2, 23 °C, 45 min, 72%.

compound **3**. In contrast to the 3CP−**3** complex, no gaps or spaces were observed between protein and ligand in the 3CP−**75** crystal structure. This optimized recognition of 3CP by the thiocarbamate portion of **75** is presumably responsible for the improved anti-3CP activity exhibited by the thiocarbamate-containing compounds described in this study.

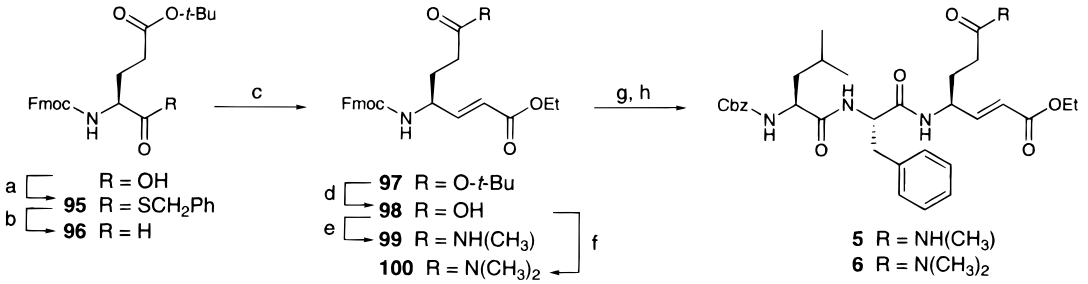
Synthesis

The tripeptide-derived Michael acceptors utilized in this study were prepared by a variety of related synthetic methods (A−F, Scheme 1). The particular method used to synthesize a given compound is indicated in Tables 1−5. Thus, an appropriately derivatized P_1 amino acid was either coupled with a dipeptide fragment (methods A and B) or iteratively modified with two individual amino acids (methods C−F). Whenever possible, commercially available dipeptides (e.g., Cbz-L-Leu-L-Phe-OH) and amino acids were employed, although some entities were prepared utilizing standard peptide synthesis techniques. The N-terminal P_4 moiety was often incorporated into a given P_3 amino acid (methods A−D) and was also introduced by modification of a tripeptidyl compound (methods E and F). As previously detailed, the ethyl propenoate Michael acceptor could be incorporated by derivitization of an appropriate P_1 amino acid residue (methods A, C, E) or by oxidation of a tripeptide alcohol with subsequent olefination of the resulting aldehyde (methods B, D, F).[1] In all cases, potentially reactive functionalities were masked with acid-labile protecting groups which were removed by treatment with trifluoroacetic acid (TFA) in the final synthetic step. Accordingly, the glutamine side chain present in many inhibitors was protected as an *N*-trityl amide during synthesis.[10] Primary amines, including those contained in α-amino acids, were masked as *tert*-butyl carbamates while carboxylic acids were protected as the corresponding *tert*-butyl esters. The latter derivatives usually survived acidic removal of the Boc moieties during peptide syntheses (see below). Hydroxyl groups were typically left unprotected during peptide coupling reactions.[11] Representative synthetic examples of methods A and D are described below and are detailed in the Experimental Section. The remaining methods involved chemistries identical to those described for A and D and altered only the sequence of peptide/amino acid coupling reactions (cf. Scheme 1). The preparations of several noncommercially available amino acids are also included.[12]

An example of synthetic method A is provided by the preparation of compound **7** (Scheme 2). Thus, *N*-α-Boc-L-glutamic acid γ-*tert*-butyl ester was transformed into aldehyde **92** by reduction of the corresponding Weinreb amide (**91**).[13,14] The crude aldehyde thus obtained was converted to the desired Michael acceptor by reaction with the sodium enolate of triethyl phosphonoacetate to afford ethyl ester **93** in moderate yield. This material was deprotected under acidic conditions (HCl), and the resulting amine salt was subjected to carbodiimide-mediated coupling with Cbz-L-Leu-L-Phe-OH to afford tripeptide **94** in modest yield.[15] The *tert*-butyl protecting group was removed by short exposure of **94** to TFA in the presence of triisopropylsilane to give compound **7** in good yield.[16] As described previously,[1] the tripeptide inhibitors prepared in this study were typically isolated as white solids by removal of the volatiles from the TFA reaction mixture, trituration of the resulting oil with Et_2O, and subsequent filtration.

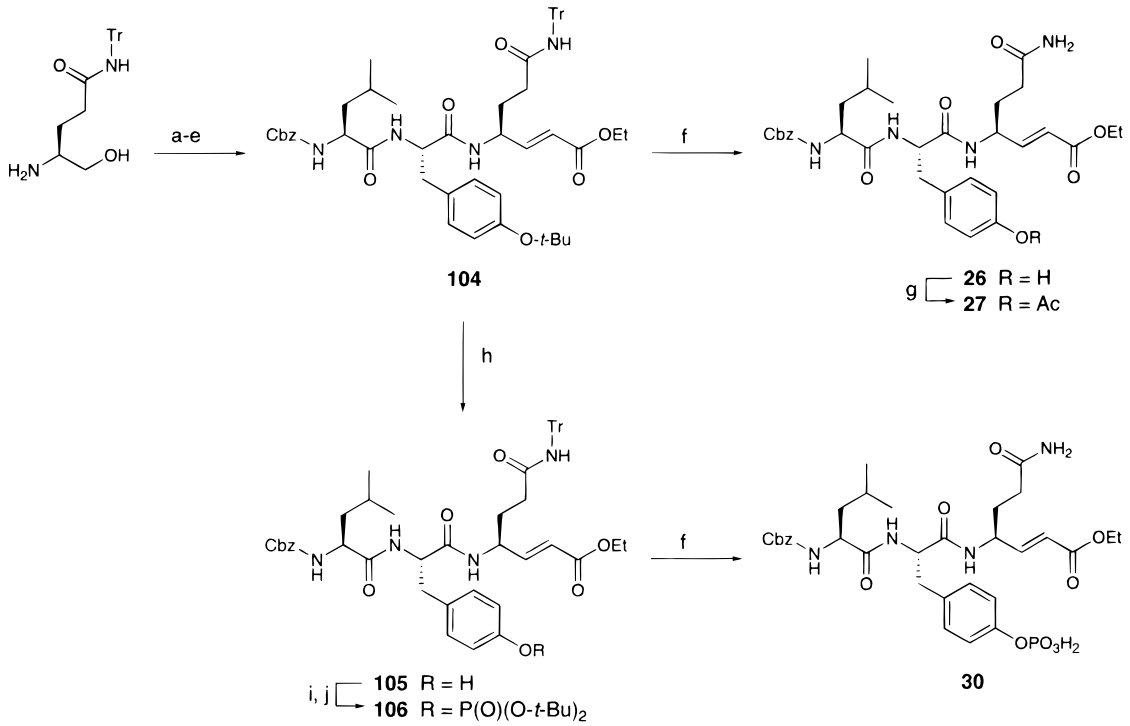
An alternate version of preparative method A which employed Fmoc-protected amino acid derivatives was

Scheme 3[a]



[a] Reagents and conditions: (a) 1.05 equiv of DCC, 2.0 equiv of BnSH, 0.10 equiv of DMAP, THF, 23 °C, 18 h, 60%; (b) 5.0 equiv of Et_3SiH, Pd/C, acetone, 23 °C, 15 min; (c) 1.5 equiv of $Ph_3P{=}CHCO_2Et$, THF, 23 °C, 24 h, 41% from **95**; (d) 1:4 TFA:CH_2Cl_2, 23 °C, 2 h, 79%; (e) 3.0 equiv of isobutyl chloroformate, 8.0 equiv of $(i\text{-}Pr)_2NEt$, 5.0 equiv of H_2NCH_3, CH_2Cl_2, 0 → 23 °C, 1 h, 67%; (f) 3.0 equiv of isobutyl chloroformate, 8.0 equiv of $(i\text{-}Pr)_2NEt$, 5.0 equiv of $HCl{\cdot}HN(CH_3)_2$, CH_2Cl_2, 0 → 23 °C, 40 min, 69%; (g) 9:1 piperidine:DMF, 23 °C, 30 min; (h) 2.0 equiv Cbz-L-Leu-L-Phe-OH, 3.0 of equiv HOBT, 5.0 equiv of NMM, 3.0 equiv of EDC, CH_2Cl_2, 23 °C, 24 h, 38%.

Scheme 4[a]



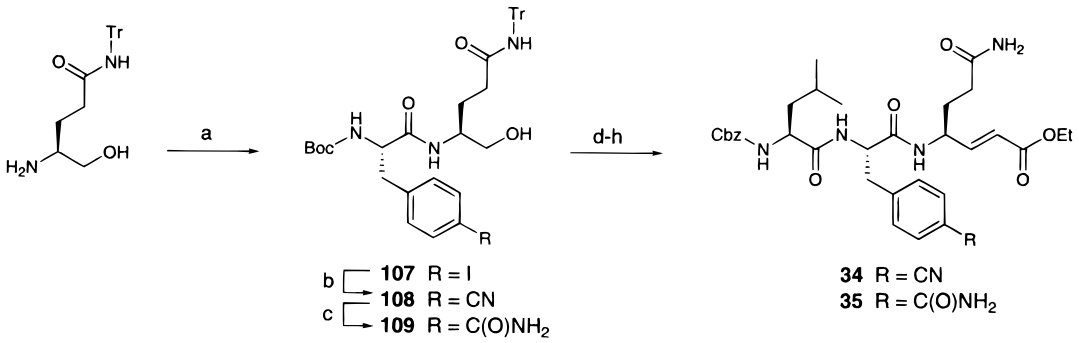
[a] Reagents and conditions (Tr = CPh_3): (a) 1.0 equiv of CDI, 1.0 equiv of Cbz-L-Tyr(OtBu)-OH, THF, 23 °C, 1 h, then 1.05 equiv of L-Tr-glutaminol,[6] overnight, 67%; (b) H_2/Pd/C, CH_3OH, 23 °C, 5 h; (c) 1.0 equiv of CDI, 1.0 equiv of Cbz-L-Leu-OH, THF, 23 °C, overnight, 58%; (d) 3.0 equiv of IBX, DMSO, 23 °C, 1.5 h, 93%; (e) 1.2 equiv of $Ph_3P{=}CHCO_2Et$, THF, 23 °C, overnight, 63%; (f) 1:10 TFA:CH_2Cl_2, 23 °C, 1−6 h, 24−95%; (g) 1.0 equiv of Ac_2O, 1.0 equiv of pyridine, 5:1 CH_2Cl_2:DMF, 23 °C, 2−3 h, 72%; (h) 3.0 equiv of $TiCl_4$, CH_2Cl_2, 0 °C, 1 h, 83%, (i) 2.0 equiv of tetrazole, 1.0 equiv of $(EtN)_2P[OC(CH_3)_3]_2$, THF, 23 °C, 2 h; (j) 1.2 equiv of *m*-CPBA, CH_2Cl_2, 0 °C, 30 min, 45%.

utilized for the synthesis of compounds **5** and **6** (Scheme 3). *N*-α-Fmoc-L-glutamic acid *γ*-*tert*-butyl ester was converted into aldehyde **96** by reduction of the corresponding *S*-benzyl thioester (**95**).[17] Crude **96** was condensed with (carbethoxymethylene)triphenylphosphorane to provide ethyl ester **97** in good yield after purification on silica gel. The *tert*-butyl protecting group was removed under acidic conditions, and the resulting carboxylic acid (**98**) was independently coupled with methylamine and dimethylamine hydrochloride to give amides **99** and **100**, respectively. Compound **99** was subsequently transformed into inhibitor **5** by Fmoc deprotection and coupling of the resulting amine with Cbz-L-Leu-L-Phe-OH. Similar manipulation of amide **100** afforded inhibitor **6**.

A modified version of preparative method D was utilized for the synthesis of inhibitors **26**, **27**, and **30** (Scheme 4). L-Tr-Glutaminol[6] was transformed into tripeptide **104** (via intermediates **101**−**103**) utilizing chemistries similar to those described previously for the preparation of related compounds (see the Experimental Section).[1,6] Brief exposure of **104** to TFA afforded compound **26** which was subsequently treated with acetic anhydride to provide inhibitor **27** in good yield. Alternatively, the *tert*-butyl protecting group present in **104** was selectively removed by treatment with $TiCl_4$ to give phenol **105** in excellent yield.[18] Coupling of **105** with di-*tert*-butyl diethylphosphoramidite and subsequent *m*-CPBA oxidation afforded phosphate ester **106**. Deprotection of **106** by treatment with TFA then provided inhibitor **30** in excellent yield.

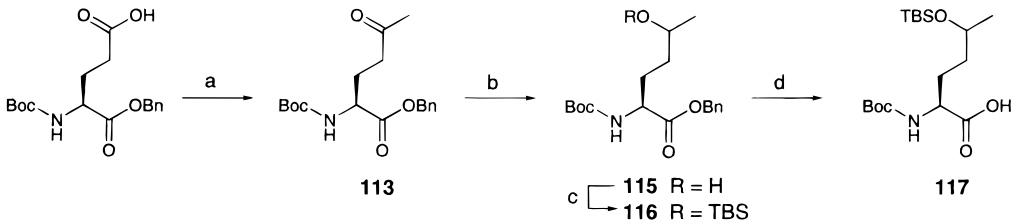
A second modification of preparative method D was employed in the synthesis of compounds **34** and **35** (Scheme 5). L-Tr-Glutaminol[6] was condensed with *N*-α-

Scheme 5[a]



[a] Reagents and conditions (Tr = CPh_3): (a) 1.0 equiv of CDI, 1.0 equiv of Boc-L-Phe(4-I)-OH, THF, 23 °C, 1 h, then 1.1 equiv of L-Tr-glutaminol,[6] overnight, 73%; (b) 2.0 equiv of KCN, 0.015 equiv of $Pd(PPh_3)_4$, THF, reflux, overnight, 85%; (c) H_2O_2, 3:2 3.0 M Na_2CO_3:EtOH, 23 °C, overnight, 91%; (d) HCl (gas), CH_2Cl_2, 23 °C, 5 min; (e) 1.0 equiv of CDI, 1.0 equiv of Cbz-L-Leu-OH, 1.05 equiv of Et_3N, THF, 23 °C, overnight, 47%; (f) 3.0 equiv of IBX, DMSO, 23 °C, 1.5 h, 86%; (g) 1.2 equiv of $Ph_3P{=}CHCO_2Et$, THF, 23 °C, overnight, 53%; (h) 1:10 TFA:CH_2Cl_2, 23 °C, 6 h, 71%.

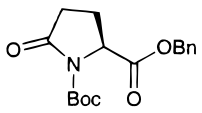
Scheme 6[a]



[a] Reagents and conditions [TBS = $Si(CH_3)_2tBu$]: (a) 1.0 equiv of isobutyl chloroformate, 1.0 equiv of NMM, CH_2Cl_2, 0 °C, 15 min, then 1.0 equiv of CH_3MgBr, THF, −78 °C, 45 min, 12%; (b) 1.25 equiv of $NaBH_4$, 5:1 THF:H_2O, 0 °C, 1.5 h, 74%; (c) 1.0 equiv of TBSOTf, 1.2 equiv of 2,6-lutidine, CH_2Cl_2, −78 °C, 1 h, 85%; (d) H_2/Pd/C, EtOAc, 23 °C, 3 h, 89%.

Boc-L-4-iodophenylalanine to give dipeptide **107** in good yield following silica gel chromatography. Palladium-mediated coupling of **107** with KCN[19] afforded nitrile **108** which was subsequently converted to amide **109** in good yield by partial hydrolysis with H_2O_2.[20] Nitrile **108** was transformed into inhibitor **34** (via intermediates **110**−**112**) utilizing chemistries related to those described above for the preparations of compounds **26** and **27** (see the Experimental Section). Similar elaboration of amide **109** afforded compound **35** in good overall yield (experimental not described).

The amino acid required for the preparation of compounds **8** and **9** was prepared by the method illustrated in Scheme 6. *N*-α-Boc-L-Glutamic acid α-benzyl ester was converted to methyl ketone **113** by sequential treatment with isobutyl chloroformate and methylmagnesium bromide. A significant amount of pyroglutamate **114** was also formed by this process, and



114

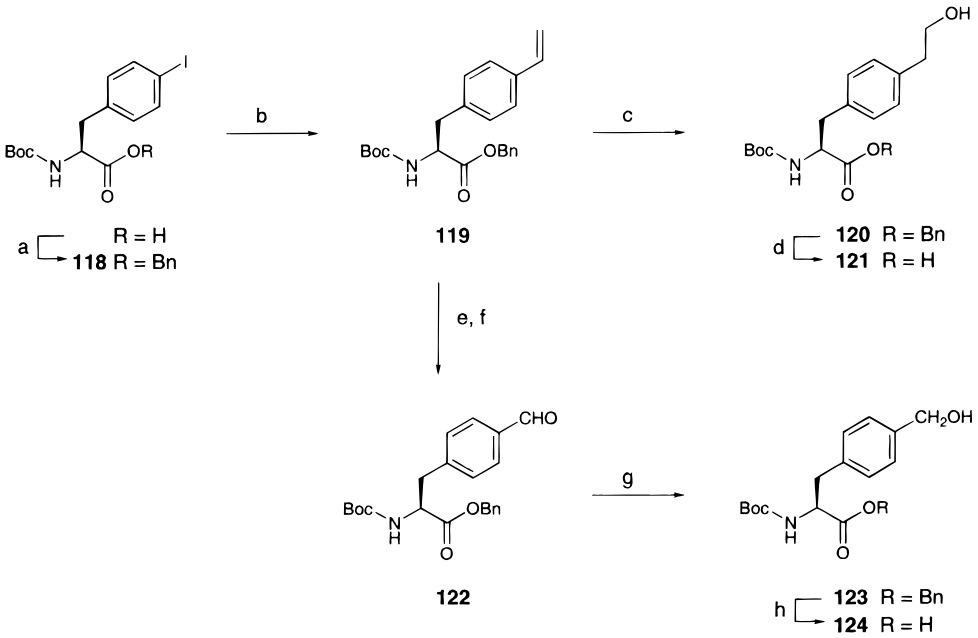
this entity may occur as an intermediate during the grignard-assisted production of **113**. Indeed, **114** could be converted to **113** in moderate yield (51%) by treatment with methylmagnesium bromide at low temperature.[21,22] Reduction of **113** with $NaBH_4$ followed by silylation of the resulting diastereomeric alcohols (**115**) afforded a 1:1 mixture of silyl ethers (**116**) after purification by flash column chromatography. Removal of the benzyl protecting group provided carboxylic acid **117** which was subsequently utilized in the syntheses of compounds **8** and **9**.[23]

The amino acids utilized in the synthesis of inhibitors **31**−**33** were prepared by the method depicted in Scheme 7. *N*-α-Boc-L-4-iodophenylalanine was converted to the corresponding benzyl ester **118** in good yield. Palladium-mediated coupling of **118** with tributyl(vinyl)-tin afforded intermediate **119** following purification on silica gel.[25] Hydroboration of **119** and subsequent debenzylation of the resulting alcohol (**120**) gave carboxylic acid **121** in moderate overall yield. Alternatively, osmium-catalyzed dihydroxylation of **119** followed by periodate-mediated cleavage of the resulting diol (not shown) provided aldehyde **122**.[26] This material was reduced with $NaBH_4$ and the alcohol thus obtained (**123**) was deprotected to give carboxylic acid **124** in good yield. Compounds **121** and **124** were subsequently utilized in the preparation of inhibitors **33** and **31**, respectively.[27]

Conclusions

The peptidyl structure−activity studies described above confirmed the ability of peptide-derived Michael acceptors to function as potent inhibitors of human rhinovirus 3C protease and further defined this class of antirhinoviral agents. The systematic variation of a lead tripeptidyl inhibitor (**3**) identified optimal amino acid residues and N-terminal moieties for obtaining high levels of anti-3CP and antiviral activity. The combination of several isolated improvements afforded a highly active 3CP inhibitor (**90**) which displayed potent antiviral activity (EC_{50} = 0.056 μM) against HRV-14 in cell culture.

Scheme 7[a]



[a] Reagents and conditions: (a) 1.2 equiv of DCC, 1.2 equiv of BnOH, 0.40 equiv of DMAP, CH_2Cl_2, 23 °C, 6 h, 87%; (b) 1.1 equiv of $Bu_3SnCH{=}CH_2$, 0.05 equiv of $Pd(PPh_3)_4$, 1,4-dioxane, 80 °C, 6 h, 83%; (c) 0.33 equiv of BH_3·THF, THF, 0 → 23 °C, 2 h, 49%; (d) 2.0 M NaOH, CH_3OH, 23 °C, 6 h, 89%; (e) 1.1 equiv of NMO, 0.03 equiv of OsO_4, 8:1 acetone:H_2O, 23 °C, 3 h, 61%; (f) 1.1 $NaIO_4$, 5:1 Et_2O:H_2O, 23 °C, 2 h; (g) 1.0 equiv of $NaBH_4$, EtOH, 0 °C, 20 min, 74%; (h) H_2/Pd/C, EtOAc, 23 °C, 6 h, 93%.

Experimental Section

General. All reactions were performed in septum-sealed flasks under a slight positive pressure of argon unless otherwise noted. All commercial reagents were used as received from their respective suppliers with the following exceptions. Tetrahydrofuran (THF) was distilled from sodium−benzophenone ketyl prior to use. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride prior to use. Flash column chromatography[28] was performed using silica gel 60 (Merck Art 9385). ^{1}H NMR spectra were recorded at 300 MHz utilizing either a Varian UNITY*plus* 300 or a General Electric QE-300 spectrometer equipped with Techmag operating software. Chemical shifts are reported in ppm (δ) downfield relative to internal tetramethylsilane, and coupling constants are given in hertz. Infrared absorption spectra were recorded using either a MIDAC Corp. or a Perkin-Elmer 1600 series FTIR. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Melting points were determined using a Mel-Temp II apparatus and are uncorrected. Experimental conditions for enzyme and antiviral assays are detailed elsewhere.[1]

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. The amino acids required for the preparation of inhibitors **10**−**12**,[29] **13**,[30] **14**-**15**,[32] **48**,[33] **53**,[34] **58**,[35] **61**−**62**,[36] **66**−**67**,[37] **69**,[38] **70**,[37] **72**−**75**,[39] **83**,[38] and **84**[40] were synthesized by standard techniques and/or literature methods. Similarly, the dipeptides required for the synthesis of compounds **36**−**37**,[41] **39**,[42] and **59**[43] were prepared by standard peptide chemistry methods and/or literature syntheses. Inhibitor **71** was prepared utilizing general method A in which the final detritylation step and penultimate peptide coupling step were transposed.[45]

Representative Example of Preparation Method A. Synthesis of Ethyl 3-(Cbz-L-Leu-L-Phe-L-*Glu*)-(*E*)-propenoate (7). [Boc-L-(OtBu)Glu]-N(OMe)Me (91). Isobutyl chloroformate (0.910 mL, 7.02 mmol, 1.0 equiv) was added to a solution of *N*-α-Boc-γ-trityl-L-glutamic acid (2.13 g, 7.02 mmol, 1 equiv) and 4-methylmorpholine (1.54 mL, 14.0 mmol, 2.0 equiv) in CH_2Cl_2 (80 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 15 min and then *N*,*O*-dimethylhydroxylamine hydrochloride (0.688 g, 7.02 mmol, 1.0 equiv) was added. The resulting solution was stirred at 0 °C for 20 min and at 23 °C for 30 min 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 **91** (2.20 g, 91%) as a clear oil: R_f = 0.43 (50% EtOAc in hexanes); IR (cm^{-1}) 3335, 2977, 1715, 1664; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.45 (s, 9H), 1.79−1.89 (m, 1H), 1.96−2.07 (m, 1H), 2.29−2.34 (m, 2H), 3.21 (s, 3H), 3.78 (s, 3H), 4.68 (s, br, 1H), 5.22 (d, 1H, J = 9.0). Anal. ($C_{16}H_{30}N_2O_6$) C, H, N.

[Boc-L-(OtBu)Glu]-H (92). Diisobutylaluminum hydride (9.53 mL of a 1.5 M solution in toluene, 14.3 mmol, 2.25 equiv) was added to a solution of **91** (2.20 g, 6.35 mmol, 1 equiv) in THF at −78 °C, and the reaction mixture was stirred at −78 °C for 1 h. Methanol (3 mL) and 1.0 M HCl (6 mL) were added sequentially, and the mixture was warmed to 23 °C. The resulting suspension was diluted with Et_2O (150 mL) and was washed with 1.0 M HCl (3 × 100 mL), half-saturated $NaHCO_3$ (100 mL), and water (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give crude **92** which was used immediately without further purification.

Ethyl 3-[Boc-L-(OtBu)*Glu*]-(*E*)-propenoate (93). Sodium bis(trimethylsilyl)amide (6.35 mL of a 1.0 M solution in THF, 6.35 mmol, 1.0 equiv) was added to a solution of triethyl phosphonoacetate (1.55 g, 6.35 mmol, 1.0 equiv) in THF (150 mL) at −78 °C, and the resulting solution was stirred for 15 min at that temperature. Crude **92** (6.35 mmol, 1 equiv) in THF (30 mL) was added via cannula, and the reaction mixture was stirred for 1 h at −78 °C, warmed to 0 °C for 10 min, and partitioned between 0.5 M HCl (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 (gradient elution, 10 → 20% EtOAc in hexanes) provided **93** (0.824 g, 36%) as a clear oil: R_f = 0.50 (30% EtOAc in hexanes); IR (cm^{-1}) 3357, 2979, 1723; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, J = 7.2), 1.44 (s, 9H), 1.45 (s, 9H), 1.65−1.96 (m, 2H), 2.29−2.34 (m, 2H), 4.19 (q, 2H, J = 7.2), 4.30 (s, br, 1H), 4.67 (s, br, 1H),

5.92 (dd, 1H, $J = 15.6, 1.6$), 6.82 (dd, 1H, $J = 15.6, 5.5$). Anal. ($C_{18}H_{31}NO_6$) C, H, N.

Ethyl 3-[Cbz-L-Leu-L-Phe-L-(OtBu)*Glu*]-(*E*)-propenoate (94). A solution of HCl in 1,4-dioxane (4.0 M, 9 mL) was added to a solution of **93** (0.766 g, 2.14 mmol, 1 equiv) in the same solvent (10 mL) at 23 °C. After 3 h, the volatiles were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL), and Cbz-L-Leu-L-Phe-OH (0.883 g, 2.14 mmol, 1.0 equiv), 1-hydroxybenzotriazole hydrate (HOBT, 0.347 g, 2.57 mmol, 1.2 equiv), 4-methylmorpholine (0.941 mL, 8.56 mmol, 4.0 equiv), and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.492 g, 2.57 mmol, 1.2 equiv) were added sequentially. The reaction mixture was stirred at 23 °C for 14 h and then was partitioned between water (150 mL) and EtOAc (2 × 150 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Flash chromatographic purification of the residue (3% CH_3OH in CH_2Cl_2) afforded **94** (0.439 g, 32%) as a white foam: $R_f = 0.51$ (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3289, 1722, 1646; ^1H NMR ($CDCl_3$) δ 0.88−0.92 (m, 6H), 1.29 (t, 3H, $J = 7.2$), 1.41 (s, 9H), 1.54−1.61 (m, 2H), 1.78−1.87 (m, 1H), 2.15−2.20 (m, 2H), 3.00−3.18 (m, 3H), 4.07−4.13 (m, 1H), 4.18 (q, 2H, $J = 7.2$), 4.55−4.67 (m, 2H), 4.94−5.16 (m, 3H), 5.75 (d, 1H, $J = 15.7$), 6.48 (d, 1H, $J = 7.5$), 6.58 (d, 1H, $J = 8.1$), 6.67 (dd, 1H, $J = 15.7, 5.6$), 7.16−7.39 (m, 11H). Anal. ($C_{36}H_{49}N_3O_8$) C, H, N.

Ethyl 3-(Cbz-L-Leu-L-Phe-L-*Glu*)-(*E*)-propenoate (7). Trifluoroacetic acid (6 mL) was added to a solution of **94** (0.420 g, 0.644 mmol, 1 equiv) and triisopropylsilane (0.20 mL, 0.976 mmol, 1.5 equiv) in CH_2Cl_2 (10 mL) at 23 °C. The reaction mixture was stirred for 45 min at 23 °C, CCl_4 (4 mL) was added, and the volatiles were removed under reduced pressure. The residue was triturated with Et_2O (10 mL), and the resulting white solid was collected by vacuum filtration, washed with Et_2O (3 × 10 mL), and air-dried to afford **7** (0.278 g, 72%): mp 180−183 °C; $R_f = 0.38$ (10% CH_3OH in CH_2Cl_2); IR (cm^{-1}) 3260, 1734, 1692, 1638; ^1H NMR (DMSO-d_6) δ 0.79 (d, 3H, $J = 10.3$), 0.81 (d, 3H, $J = 10.3$), 1.21 (t, 3H, $J = 7.2$), 1.28−1.39 (m, 2H), 1.41−1.59 (m, 2H), 1.61−1.83 (m, 1H), 2.13−2.24 (m, 2H), 2.84 (dd, 1H, $J = 13.6, 8.4$), 2.98 (dd, 1H, $J = 13.6, 6.1$), 3.95−3.99 (m, 1H), 4.10 (q, 2H, $J = 7.2$), 4.42−4.52 (m, 2H), 4.97 (d, 1H, $J = 12.6$), 5.04 (d, 1H, $J = 12.6$), 5.62 (d, 1H, $J = 15.6$), 6.68 (dd, 1H, $J = 15.6, 5.3$), 7.15−7.44 (m, 11H), 7.99 (d, 1H, $J = 8.1$), 8.06 (d, 1H, $J = 8.1$). Anal. ($C_{32}H_{41}N_3O_8$) C, H, N.

Alternate Example of Preparation Method A. Synthesis of Ethyl 3-[Cbz-L-Leu-L-Phe-L-(*N*-Me)*Gln*]-(*E*)-propenoate (5). Fmoc-L-(OtBu)Glu-SBn (95). 4-(Dimethylamino)pyridine (0.574 g, 4.70 mmol, 0.10 equiv) and 1,3-dicyclohexylcarbodiimide (10.2 g, 49.3 mmol, 1.05 equiv) were added sequentially to a solution of *N*-α-Fmoc-L-glutamic acid γ-*tert*-butyl ester (20.0 g, 47.0 mmol, 1 equiv) and benzyl mercaptan (11.0 mL, 94.0 mmol, 2.0 equiv) in THF (300 mL) at 23 °C. The cloudy reaction mixture was stirred at 23 °C for 18 and then was filtered through a medium fritted funnel. The pale yellow filtrate was concentrated and then was partitioned between EtOAc (250 mL) and 1.0 M HCl (150 mL). The organic layer was dried over Na_2SO_4 and concentrated, and the resulting oil was triturated with Et_2O (60 mL) and again filtered through a medium fritted funnel. The filtrate thus obtained was concentrated to provide a yellow oil which solidified on standing. The resulting solid was slurried with a 2:1 mixture of petroleum ether and Et_2O (150 mL), collected by vacuum filtration, washed with petroleum ether (4 × 100 mL), and air-dried to give **95** (15.0 g, 60%) as an off-white solid: mp 114−116 °C; $R_f = 0.80$ (50% EtOAc in hexanes); IR (cm^{-1}) 3333, 2982, 1725; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.91−1.98 (m, 1H), 2.16−2.37 (m, 3H), 4.07−4.53 (m, 6H), 5.63 (d, 1H, $J = 8.4$), 7.25−7.30 (m, 7H), 7.36−7.41 (m, 2H), 7.58−7.61 (m, 2H) 7.75 (d, 2H, $J = 7.5$). Anal. ($C_{31}H_{33}NO_5S$) C, H, N.

Fmoc-L-(OtBu)Glu-H (96). Triethylsilane (16.37 mL, 102.5 mmol, 5.0 equiv) was added slowly via addition funnel to a degassed suspension of **95** (10.9 g, 20.5 mmol, 1 equiv) and Pd/C (10%, 5.5 g) in acetone (500 mL) at 23 °C. The reaction mixture was stirred at that temperature for 15 min, then was filtered through Celite. The filtrate was concentrated to afford crude **96** (8.40 g) which was used without further purification.

Ethyl 3-[Fmoc-L-(OtBu)*Glu*]-(*E*)-propenoate (97). (Carbethoxymethylene)triphenylphosphorane (23.3 g, 68.7 mmol, 1.5 equiv) was added to a solution of **96** (18.3 g, 44.7 mmol, 1 equiv) in THF (200 mL) at 23 °C. The resulting mixture was stirred at 23 °C for 24 h and then was concentrated under reduced pressure. The residue was purified by flash column chromatography (15% EtOAc in hexanes) to afford **97** (13.9 g, 65%) as a white foam: $R_f = 0.68$ (50% EtOAc in hexanes); IR (cm^{-1}) 3339, 2979, 1721; ^1H NMR ($CDCl_3$) δ 1.26 (t, 3H, $J = 7.2$), 1.44 (s, 9H), 1.79−2.02 (m, 2H), 2.26−2.31 (m, 2H), 4.20 (q, 2H, $J = 7.2$), 4.35−4.48 (m, 4H), 5.07 (d, 1H, $J = 8.1$), 5.92 (d, 1H, $J = 15.5$), 6.82 (dd, 1H, $J = 15.5, 5.4$), 7.26−7.43 (m, 4H), 7.59 (d, 2H, $J = 7.2$), 7.77 (d, 2H, $J = 7.5$). Anal. ($C_{28}H_{33}NO_6$) C, H, N.

Ethyl 3-[Fmoc-L-*Glu*]-(*E*)-propenoate (98). Trifluoroacetic acid (50 mL) was added to a solution of **97** (13.9 g, 28.9 mmol) in CH_2Cl_2 (200 mL) at 23 °C. The reaction mixture was stirred for 2 h at 23 °C, and then the volatiles were removed under reduced pressure. The residue was triturated with a 1:1 mixture of Et_2O and hexanes (100 mL), and the resulting solid was collected by filtration. Recrystallization from Et_2O/hexanes afforded **98** (9.62 g, 79%) as a off-white solid: $R_f = 0.14$ (50% EtOAc in hexanes); IR (cm^{-1}) 3294, 2977, 1711; ^1H NMR (DMSO-d_6) δ 1.20 (t, 3H, $J = 7.2$), 1.64−1.82 (m, 2H), 2.22 (t, 2H, $J = 7.2$), 4.07−4.15 (m, 3H), 4.22 (t, 1H, $J = 7.2$), 4.31−4.33 (m, 2H), 5.82 (d, 1H, $J = 15.6$), 5.76 (dd, 1H, $J = 15.9, 6.0$), 7.29−7.43 (m, 4H), 7.58 (d, 1H, $J = 7.8$), 7.68−7.70 (m, 2H), 7.88 (d, 2H, $J = 7.2$). Anal. ($C_{24}H_{25}NO_6 \cdot 0.25H_2O$) C, H, N.

Ethyl 3-[Fmoc-L-(*N*-Me)*Gln*]-(*E*)-propenoate (99). *N*,*N*-Diisopropylethylamine (0.247 mL, 1.42 mmol, 3.0 equiv) and isobutyl chloroformate (0.184 mL, 1.42 mmol, 3.0 equiv) were added sequentially to a 0 °C slurry of **98** (0.200 g, 0.472 mmol, 1 equiv) in CH_2Cl_2 (5 mL). After the mixture was stirred for 40 min at 0 °C, additional *N*,*N*-diisopropylethylamine (0.411 mL, 2.36 mmol, 5.0 equiv) and a solution of methylamine in THF (2.0 M, 1.18 mL, 2.36 mmol, 5.0 equiv) were added sequentially. The reaction mixture was stirred for 40 min at 0 °C and for 20 min at 23 °C and then was partitioned between 1.0 M HCl (10 mL) and 10% CH_3OH in CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Flash chromatographic purification of the residue (2% CH_3OH in $CHCl_3$) gave **99** (0.137 g, 67%) as a white solid: mp 162 °C dec; $R_f = 0.46$ (10% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3306, 1716, 1692, 1643, 1539; ^1H NMR ($CDCl_3$) δ 1.29 (t, 3H, $J = 7.2$), 1.82−2.05 (m, 2H), 2.17−2.26 (m, 2H), 2.79 (d, 3H, $J = 4.7$), 4.14−4.23 (m, 3H), 4.26−4.48 (m, 3H), 5.57 (d, 1H, $J = 8.1$), 5.74 (s, 1H), 5.93 (d, 1H, $J = 15.6$), 6.83 (dd, 1H, $J = 15.6, 5.3$), 7.28−7.43 (m, 4H), 7.60 (d, 2H, $J = 7.2$), 7.76 (d, 2H, $J = 7.5$). Anal. ($C_{25}H_{28}N_2O_5 \cdot 0.50H_2O$) C, H, N.

Ethyl 3-[Fmoc-L-(*N*-Me$_2$)*Gln*]-(*E*)-propenoate (100). *N*,*N*-Diisopropylethylamine (0.197 mL, 1.13 mmol, 3.0 equiv) and isobutyl chloroformate (0.147 mL, 1.13 mmol, 3.0 equiv) were added sequentially to a 0 °C slurry of **98** (0.160 g, 0.378 mmol, 1 equiv) in CH_2Cl_2 (4 mL), and the resulting solution was stirred 40 min. A solution of *N*,*N*-diisopropylethylamine (0.329 mL, 1.89 mmol, 5.0 equiv) and dimethylamine hydrochloride (0.154 g, 1.89 mmol, 5.0 equiv) in CH_3CN (4 mL) was added dropwise, and the reaction mixture was allowed to warm to 23 °C, stirred for 40 min, and then partitioned between 1 M HCl (10 mL) and 10% CH_3OH in CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and were concentrated. Flash chromatographic purification of the residue (2% CH_3OH in $CHCl_3$) gave **100** (0.117 g, 69%) as a glass: $R_f = 0.62$ (10% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3284, 1713, 1631; ^1H NMR ($CDCl_3$) δ 1.29 (t, 3H, $J = 7.2$), 1.95−2.03 (m, 2H), 2.35−2.50 (m, 2H), 2.97 (s, 3H), 2.98 (s, 3H), 4.15−4.24 (m, 3H), 4.27−4.43 (m, 3H), 5.90−6.00 (m, 2H), 6.87 (dd, 1H, $J = 15.9, 5.3$), 7.27−7.43 (m,

4H), 7.56−7.63 (m, 2H), 7.76 (d, 2H, J = 7.5). Anal. ($C_{26}H_{30}N_2O_5$·0.25H_2O) C, H, N.

Ethyl 3-[Cbz-L-Leu-L-Phe-L-(*N*-Me)*Gln*]-(*E*)-propenoate (5). A solution of **99** (0.115 g, 0.263 mmol, 1 equiv) in 9:1 piperidine:DMF (2 mL) was stirred for 30 min at 23 °C and then was concentrated under reduced pressure. Toluene (25 mL) was added to the reside, the volatiles were subsequently removed under vacuum, and the resulting oil was dissolved in CH_2Cl_2 (8 mL). Cbz-L-Leu-L-Phe-OH (0.217 g, 0.526 mmol, 2.0 equiv), 4-methylmorpholine (0.145 mL, 1.32 mmol, 5.0 equiv), 1-hydroxybenzotriazole hydrate (0.107 g, 0.792 mmol, 3.0 equiv), and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.152 g, 0.793 mmol, 3.0 equiv) were then added sequentially. The reaction mixture was stirred for 24 h at 23 °C and then was loaded directly onto a flash column and eluted with 2% CH_3OH in $CHCl_3$ to give a white solid which smelled of 4-methylmorpholine. Evaporation from toluene (2 × 30 mL) afforded **5** (0.060 g, 38%) as a white solid: mp 224−225 °C; R_f = 0.46 (10% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3436, 3295, 1719, 16996, 1643, 1543; ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.5), 0.82 (d, 3H, J = 6.5), 1.21 (t, 3H, J = 7.2), 1.25−1.38 (m, 2H), 1.43−1.56 (m, 1H), 1.59−1.82 (m, 2H), 2.02−2.10 (m, 2H), 2.52 (d, 3H, J = 4.4), 2.84 (dd, 1H, J = 14.0, 9.3), 2.97 (dd, 1H, J = 14.0, 6.2), 3.93−4.03 (m, 1H), 4.10 (q, 2H, J = 7.2), 4.31−4.52 (m, 2H), 4.97 (d, 1H, J = 12.6), 5.04 (d, 1H, J = 12.6), 5.63 (d, 1H, J = 15.9), 6.68 (dd, 1H, J = 15.9, 5.6), 7.13−7.24 (m, 5H), 7.27−7.37 (m, 5H), 7.43 (d, 1H, J = 7.8), 7.59−7.66 (m, 1H), 8.00 (d, 1H, J = 8.1), 8.04 (d, 1H, J = 8.4). Anal. ($C_{33}H_{44}N_4O_7$) C, H, N.

Representative Example of Preparation Method D. Synthesis of Ethyl 3-(Cbz-L-Leu-L-Tyr-L-*Gln*)-(*E*)-propenoate (26), Ethyl 3-[Cbz-L-Leu-L-Tyr(OAc)-L-*Gln*]-(*E*)-propenoate (27), and Ethyl 3-[Cbz-L-Leu-L-Tyr(OPO$_3$H$_2$)-L-*Gln*]-(*E*)-propenoate (30). Cbz-L-Tyr(OtBu)-L-(Tr)glutaminol (101). Carbonyldiimidazole (0.74 g, 4.57 mmol, 1.0 equiv) was added to a solution of Cbz-L-Tyr(OtBu)-OH (1.69 g, 4.57 mmol, 1.0 equiv) in THF (45 mL) at 23 °C. After 1 h, L-Tr-glutaminol[6] (1.80 g, 4.79 mmol, 1.05 equiv) was added, and the reaction mixture was stirred overnight at 23 °C. The volatiles were removed under reduced pressure, and the residue was purified by flash column chromatography (gradient elution, 0 → 3% CH_3OH in $CHCl_3$) to give **101** (2.24 g, 67%) as a white glassy solid: R_f = 0.21 (3% CH_3OH in $CHCl_3$); IR (cm^{-1}) 1666, 1506, 1238; ^1H NMR (DMSO-d_6) δ 1.25 (s, 9H), 1.40−1.52 (m, 1H), 1.68−1.82 (m, 1H), 2.23−2.35 (m, 2H), 2.67−2.75 (m, 1H), 2.86−2.94 (m, 1H), 3.11−3.15 (m, 1H), 3.22−3.26 (m, 1H), 3.63−3.73 (m, 1H), 4.15−4.20 (m, 1H), 4.62−4.66 (m, 1H), 4.86 (d, 1H, J = 12.5), 4.91 (d, 1H, J = 12.5), 6.84 (d, 2H, J = 8.1), 7.15−7.31 (m, 22H), 7.42 (d, 1H, J = 8.5), 7.69 (d, 1H, J = 8.5), 8.48 (s, 1H). Anal. ($C_{45}H_{49}N_3O_6$) C, H, N.

Cbz-L-Leu-L-Tyr(OtBu)-L-(Tr)glutaminol (102). Carbonyldiimidazole (0.45 g, 2.76 mmol, 1.0 equiv) was added to a solution of Cbz-L-Leu-OH (0.73 g, 2.76 mmol, 1.0 equiv) in THF (28 mL) at 23 °C, and the reaction mixture was stirred for 1 h at that temperature. In a separate flask, a suspension of **101** (2.20 g, 3.03 mmol, 1.1 equiv) and Pd/C (10%, 0.22 g) in CH_3OH (20 mL) was stirred under a hydrogen atmosphere (balloon) until disappearance of the starting material was indicated by TLC analysis (5 h). The reaction mixture was filtered through Celite, and the filtrate was concentrated to afford a white glassy solid. This material was dissolved in THF (5 mL) and was added to the Cbz-L-Leu-OH solution prepared above. The reaction mixture was stirred overnight at 23 °C, and then the volatiles were removed under reduced pressure. Purification of the residue by flash column chromatography (gradient elution, 0 → 3% CH_3OH in $CHCl_3$) provided **102** (1.36 g, 58%) as a white glassy solid: R_f = 0.15 (3% CH_3OH in $CHCl_3$); IR (cm^{-1}) 1653, 1508, 1236; ^1H NMR (DMSO-d_6) δ 0.77−0.81 (m, 6H), 1.23 (s, 9H), 1.27−1.60 (m, 4H), 1.70−1.83 (m, 1H), 2.20−2.24 (m, 2H), 2.72−2.78 (m, 1H), 2.88−2.93 (m, 1H), 3.04−3.11 (m, 1H), 3.20−3.23 (m, 1H), 3.60−3.68 (m, 1H), 3.95−4.02 (m, 1H), 4.43−4.48 (m, 1H), 4.59−4.62 (m, 1H), 4.97 (d, 1H, J = 12.5), 5.02 (d, 1H, J = 12.5), 6.80 (d, 2H, J = 7.7), 7.08 (d, 2H, J = 7.7), 7.14−7.33 (m, 20H), 7.41 (d, 1H, J = 8.1), 7.63 (d, 1H, J = 7.7), 7.85 (d, 1H, J = 8.5), 8.51 (s, 1H). Anal. ($C_{51}H_{60}N_4O_7$) C, H, N.

Cbz-L-Leu-L-Tyr(OtBu)-L-(Tr)glutaminal (103). *o*-Iodoxybenzoic acid[24] (1.32 g, 4.73 mmol, 3.0 equiv) was added to a solution of **102** (1.32 g, 1.58 mmol, 1 equiv) in DMSO (16 mL) at 23 °C. After the mixture was stirred 1.5 h at 23 °C, the DMSO was removed under reduced pressure. The residue was twice diluted with CH_2Cl_2 (20 mL), and the volatiles were evaporated to remove any residual DMSO. The resulting residue was then triturated with EtOAc (80 mL), and the white solid thus obtained was filtered off. The filtrate was washed with a 1:1 mixture of 5% $Na_2S_2O_3$ and 5% $NaHCO_3$ solution (80 mL), water (80 mL), and brine (80 mL), dried over Na_2SO_4, and concentrated to give **103** (1.23 g, 93%) as a white glassy solid. This material was used immediately without further purification: ^1H NMR (DMSO-d_6) δ 0.78−0.81 (m, 6H), 1.23 (s, 9H), 1.25−1.35 (m, 1H), 1.45−1.65 (m, 2H), 1.82−1.90 (m, 1H), 2.25−2.30 (m, 2H), 2.77−2.82 (m, 1H), 2.93−2.98 (m, 1H), 3.95−4.05 (m, 2H), 4.53−4.56 (m, 1H), 4.94−5.03 (m, 2H), 6.82 (d, 2H, J = 8.5), 7.10−7.33 (m, 22H), 7.39 (d, 1H, J = 8.5), 7.97 (d, 1H, J = 7.7), 8.38 (d, 1H, J = 6.3), 8.59 (s, 1H), 9.20 (s, 1H).

Ethyl 3-[Cbz-L-Leu-L-Tyr(OtBu)-L-(Tr-*Gln*)]-(*E*)-propenoate (104). (Carbethoxymethylene)triphenylphosphorane (0.61 g, 1.76 mmol, 1.2 equiv) was added to a solution of **103** (1.23 g, 1.47 mmol, 1 equiv) in THF (30 mL) at 23 °C, and the reaction mixture was stirred overnight at that temperature. The volatiles were then removed in vacuo, and the residue was purified by flash column chromatography (gradient elution, 0 → 2% CH_3OH in $CHCl_3$) to provide **104** (0.84 g, 63%) as a white glassy solid: R_f = 0.20 (2% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3293, 1653, 1508; ^1H NMR (DMSO-d_6) δ 0.77−0.81 (m, 6H), 1.21 (t, 3H, J = 7.0), 1.23 (s, 9H), 1.25−1.37 (m, 2H), 1.49−1.56 (m, 1H), 1.63−1.65 (m, 2H), 2.23−2.28 (m, 2H), 2.76−2.81 (m, 1H), 2.91−2.98 (m, 1H), 3.92−3.98 (m, 1H), 4.10 (q, 2H, J = 7.0), 4.35−4.40 (m, 1H), 4.42−4.47 (m, 1H), 4.95 (d, 1H, J = 12.5), 5.02 (d, 1H, J = 12.5), 5.72 (d, 1H, J = 15.4), 6.70 (dd, 1H, J = 15.4, 5.5), 6.80 (d, 2H, J = 8.1), 7.08 (d, 2H, J = 8.5), 7.14−7.32 (m, 20H), 7.41 (d, 1H, J = 8.1), 7.93 (d, 1H, J = 8.1), 8.06 (d, 1H, J = 8.1), 8.58 (s, 1H). Anal. ($C_{55}H_{64}N_4O_8$) C, H, N.

Ethyl 3-(Cbz-L-Leu-L-Tyr-L-*Gln*)-(*E*)-propenoate (26). Trifluoroacetic acid (1 mL) was added to a solution of **104** (0.43 g, 0.47 mmol) in CH_2Cl_2 (10 mL) at 23 °C, and the reaction mixture was stirred at that temperature for 6 h. The volatiles were then removed under reduced pressure, and the residue was purified by flash column chromatography (gradient elution, 0 → 4% CH_3OH in $CHCl_3$) to give **26** (0.068 g, 24%) as a white solid: mp 190−195 °C dec; IR (cm^{-1}) 1643, 1539; ^1H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.6), 0.83 (d, 3H, J = 6.6), 1.21 (t, 3H, J = 7.0), 1.23−1.27 (m, 2H), 1.45−1.55 (m, 1H), 1.64−1.80 (m, 2H), 2.04−2.09 (m, 2H), 2.71−2.78 (m, 1H), 2.83−2.89 (m, 1H), 3.92−3.97 (m, 1H), 4.11 (q, 2H, J = 7.0), 4.33−4.39 (m, 2H), 4.97 (d, 1H, J = 12.5), 5.05 (d, 1H, J = 12.5), 5.71 (d, 1H, J = 15.8), 6.60 (d, 2H, J = 7.7), 6.72 (dd, 1H, J = 15.8, 5.5), 6.75 (s, br, 1H), 6.96 (d, 2H, J = 8.1), 7.20 (s, br, 1H), 7.29−7.34 (m, 5H), 7.45 (d, 1H, J = 7.7), 7.91 (d, 1H, J = 8.1), 8.04 (d, 1H, J = 7.7), 9.04 (s, br, 1H). Anal. ($C_{32}H_{42}N_4O_8$) C, H, N.

Ethyl 3-[Cbz-L-Leu-L-Tyr(OAc)-L-*Gln*]-(*E*)-propenoate (27). Acetic anhydride (0.013 mL, 0.12 mmol, 1.0 equiv) was added to a solution of **26** (0.075 g, 0.12 mmol, 1 equiv) and pyridine (0.01 mL, 0.12 mmol, 1.0 equiv) in a 5:1 mixture of CH_2Cl_2 and DMF (3.0 mL) at 23 °C. The reaction mixture was stirred at 23 °C, and additional small amounts of pyridine and acetic anhydride were added until TLC analysis indicated that all starting material was consumed. The volatiles were then removed in vacuo, and the residue was purified by flash column chromatography (gradient elution, 0 → 3% CH_3OH in $CHCl_3$) to give **27** (0.058 g, 72%) as a white solid: mp 233−234 °C; R_f = 0.29 (7% CH_3OH in $CHCl_3$); IR (cm^{-1}) 1651, 1539, 1227; ^1H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.6), 0.83 (d, 3H, J = 6.6), 1.21 (t, 3H, J = 7.0), 1.28−1.36 (m, 2H), 1.43−1.55 (m, 1H), 1.66−1.74 (m, 2H), 2.04−2.07 (m, 2H), 2.23 (s, 3H),

2.79−2.82 (m, 1H), 2.88−2.97 (m, 1H), 3.93−3.97 (m, 1H), 4.11 (q, 2H, J = 7.0), 4.36−4.41 (m, 1H), 4.45−4.51 (m, 1H), 4.98 (d, 1H, J = 12.5), 5.04 (d, 1H, J = 12.5), 5.69 (d, 1H, J = 15.4), 6.71 (dd, 1H, J = 15.4, 5.5), 6.77 (s, 1H), 6.96 (d, 2H, J = 8.1), 7.20 (s, 1H), 7.21 (d, 2H, J = 7.4), 7.31−7.35 (m, 5H), 7.44 (d, 1H, J = 7.7), 8.02−8.05 (m, 2H). Anal. ($C_{34}H_{44}N_4O_9$) C, H, N.

Ethyl 3-[Cbz-L-Leu-L-Tyr-L-(Tr-*Gln*)]-(*E*)-propenoate (105). Titanium(IV) chloride (0.19 mL, 1.71 mmol, 3.0 equiv) was added dropwise to a solution of **104** (0.52 g, 0.57 mmol, 1 equiv) in CH_2Cl_2 (6 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The organic layer was washed with brine (25 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated, and the residue was purified by flash column chromatography (gradient elution, 0 → 2% CH_3OH in $CHCl_3$) to give **105** (0.40 g, 83%) as a white glassy solid: R_f = 0.18 (7% CH_3OH in $CHCl_3$); IR (cm^{-1}) 3325, 1707, 1655, 1516; ^1H NMR (DMSO-d_6) δ 0.77−0.81 (m, 6H), 1.20 (t, 3H, J = 7.0), 1.31−1.36 (m, 2H), 1.44−1.53 (m, 1H), 1.60−1.70 (m, 2H), 2.20−2.26 (m, 2H), 2.69−2.74 (m, 1H), 2.82−2.87 (m, 1H), 3.94−3.97 (m, 1H), 4.10 (q, 2H, J = 7.0), 4.34−4.41 (m, 2H), 4.94 (d, 1H, J = 12.5), 5.03 (d, 1H, J = 12.5), 5.69 (d, 1H, J = 15.8), 6.70 (dd, 1H, J = 15.8, 5.5), 6.95 (d, 2H, J = 7.5), 7.14−7.35 (m, 20H), 7.42 (d, 1H, J = 7.7), 7.85 (d, 1H, J = 7.7), 8.04 (d, 1H, J = 8.1), 8.58 (s, 1H), 9.13 (s, 1H). Anal. ($C_{51}H_{56}N_4O_8 \cdot 0.50H_2O$) C, H, N.

Ethyl 3-{Cbz-L-Leu-L-Tyr[OP(O)(OtBu)$_2$]-L-(Tr-*Gln*)}-(*E*)-propenoate (106). Tetrazole (0.07 g, 0.95 mmol, 2.0 equiv) and di-*tert*-butyl diethylphosphoramidite (0.14 mL, 0.47 mmol, 1.0 equiv) were added sequentially to a solution of **105** (0.40 g, 0.47 mmol, 1 equiv) in THF (5 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 2 h and then was cooled to 0 °C. A solution of *m*-CPBA (57−86%, 0.095 g, 0.55 mmol, 1.2 equiv) in CH_2Cl_2 (2 mL) was added via cannula, and the reaction mixture was stirred for 30 min at 0 °C and then was concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (25 mL) and 10% $Na_2S_2O_5$ (25 mL), and the organic layer was washed with H_2O (25 mL) and brine (25 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (gradient elution, 0 → 2% CH_3OH in $CHCl_3$) to give **106** (0.22 g, 45%) as a white glassy solid: R_f = 0.15 (5% CH_3OH in $CHCl_3$); IR (cm^{-1}) 1717, 1669, 1508, 1267, 1007; ^1H NMR (DMSO-d_6) δ 0.76−0.81 (m, 6H), 1.20 (t, 3H, J = 7.0), 1.25−1.50 (m, 3H), 1.41 (s, 18H), 1.70−1.80 (m, 2H), 2.29−2.39 (m, 2H), 2.81−2.88 (m, 1H), 2.97−3.04 (m, 1H), 3.97−4.03 (m, 1H), 4.10 (q, 2H, J = 7.0), 4.41−4.44 (m, 1H), 4.52−4.60 (m, 1H), 4.94 (d, 1H, J = 12.5), 5.03 (d, 1H, J = 12.5), 5.72 (d, 1H, J = 15.8), 6.72 (dd, 1H, J = 15.8, 5.5), 7.00 (d, 1H, J = 8.1), 7.14−7.32 (m, 22H), 7.41 (d, 1H, J = 7.7), 7.95 (d, 1H, J = 7.7), 8.09 (d, 1H, J = 7.7), 8.58 (s, 1H).

Ethyl 3-[Cbz-L-Leu-L-Tyr(OPO$_3$H$_2$)-L-*Gln*]-(*E*)-propenoate (30). Trifluoroacetic acid (0.4 mL) was added to a solution of **106** (0.19 g, 0.18 mmol) in CH_2Cl_2 (4 mL) at 23 °C, and the reaction mixture was stirred at that temperature for 1 h. The volatiles were then removed under reduced pressure, and the residue was triturated with a 2:1 mixture of Et_2O and EtOAc (15 mL). The resulting white precipitate was filtered, washed with Et_2O (2 × 10 mL), and then air-dried to give **30** (0.12 g, 95%) as a white solid: mp 190−195 °C dec; IR (cm^{-1}) 1699, 1655, 1539; ^1H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.6), 0.83 (d, 3H, J = 6.6), 1.21 (t, 3H, J = 7.0), 1.29−1.36 (m, 2H), 1.48−1.58 (m, 1H), 1.67−1.82 (m, 2H), 2.04−2.08 (m, 2H), 2.80−2.85 (m, 1H), 2.92−3.00 (m, 1H), 3.95−4.03 (m, 1H), 4.11 (q, 2H, J = 7.0), 4.37−4.50 (m, 2H), 4.97 (d, 1H, J = 12.5), 5.05 (d, 1H, J = 12.5), 5.73 (d, 1H, J = 15.8), 6.74 (dd, 1H, J = 15.8, 5.5), 6.77 (s, br, 1H), 7.02 (d, 2H, J = 8.5), 7.15 (d, 2H, J = 8.1), 7.21 (s, br, 1H), 7.28−7.34 (m, 5H), 7.45 (d, 1H, J = 7.7), 7.99 (d, 1H, J = 8.1), 8.07 (d, 1H, J = 7.7). Anal. ($C_{32}H_{43}N_4O_{11}P$) C, H, N.

Alternate Example of Preparation Method D. Synthesis of Ethyl 3-[Cbz-L-Leu-L-Phe(4-CN)-L-*Gln*]-(*E*)-propenoate (34). Boc-L-Phe(4-I)-L-(Tr)glutaminol (107). Carbonyldiimidazole (1.02 g, 6.27 mmol, 1.0 equiv) was added to a solution of Boc-L-Phe(4-I)-OH (2.45 g, 6.27 mmol, 1.0 equiv) in THF (60 mL) at 23 °C. After 1 h, L-Tr-glutaminol[6] (2.58 g, 6.90 mmol, 1.1 equiv) was added, and the reaction mixture was stirred overnight at 23 °C. The volatiles were removed under reduced pressure, and the residue was purified by flash column chromatography (gradient elution, 0 → 3% CH_3OH in $CHCl_3$) to give **107** (3.42 g, 73%) as a white glassy solid: R_f = 0.08 (3% CH_3OH in $CHCl_3$); IR (cm^{-1}) 1665, 1491; ^1H NMR (DMSO-d_6) δ 1.28 (s, 9H), 1.42−1.49 (m, 1H), 1.64−1.78 (m, 1H), 2.23−2.28 (m, 2H), 2.64−2.72 (m, 1H), 2.85−2.91 (m, 1H), 3.14−3.22 (m, 1H), 3.25−3.31 (m, 1H), 3.63−3.66 (m, 1H), 4.05−4.09 (m, 1H), 4.66−4.69 (m, 1H), 6.85 (d, 1H, J = 8.5), 7.06 (d, 2H, J = 8.1), 7.15−7.28 (m, 15H), 7.59−7.64 (m, 3H), 8.49 (s, 1H). Anal. ($C_{38}H_{42}IN_3O_5$) C, H, N.

Boc-L-Phe(4-CN)-L-(Tr)glutaminol (108). Potassium cyanide (0.13 g, 2.00 mmol, 2.0 equiv) and $Pd(PPh_3)_4$ (0.017 g, 0.015 mmol, 0.015 equiv) were added sequentially to a solution of **107** (0.75 g, 1.00 mmol, 1 equiv) in THF (10 mL) at 23 °C. The reaction mixture was then refluxed overnight, and the solvent was subsequently removed in vacuo. The residue was purified by flash column chromatography (gradient elution, 0 → 2% CH_3OH in $CHCl_3$) to give **108** (0.55 g, 85%) as a white glassy solid: R_f = 0.13 (5% CH_3OH in $CHCl_3$); IR (cm^{-1}) 2228, 1663, 1491; ^1H NMR (DMSO-d_6) δ 1.26 (s, 9H), 1.40−1.53 (m, 1H), 1.696−1.69 (m, 1H), 2.23−2.30 (m, 2H), 2.77−2.85 (m, 1H), 2.98−3.05 (m, 1H), 3.17−3.24 (m, 1H), 3.26−3.33 (m, 1H), 3.64−3.70 (m, 1H), 4.14−4.20 (m, 1H), 4.68 (t, 1H, J = 5.5), 6.93 (d, 1H, J = 8.5), 7.15−7.28 (m, 15H), 7.44 (d, 1H, J = 8.5), 7.66 (d, 1H, J = 8.5), 7.73 (d, 2H, J = 8.1), 8.48 (s, 1H). Anal. ($C_{39}H_{42}N_4O_5$) C, H, N.

Boc-L-Phe(4-carboxamide)-L-(Tr)glutaminol (109). Hydrogen peroxide (30%, 0.45 mL) was added to a solution of **108** (0.49 g, 0.76 mmol) in a 1.5:1 mixture of 3.0 M Na_2CO_3 and EtOH (2.5 mL) at 23 °C. The cloudy reaction mixture was stirred for 24 h at 23 °C, and then additional H_2O_2 (30%, 0.50 mL) was added. After again stirring overnight at 23 °C, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc (30 mL) and H_2O (30 mL), and the organic layer was washed with brine (15 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated to afford **109** (0.46 g, 91%) as a white solid which was used without further purification: IR (cm^{-1}) 1663, 1493; ^1H NMR (DMSO-d_6) δ 1.27 (s, 9H), 1.42−1.53 (m, 1H), 1.68−1.76 (m, 1H), 2.23−2.29 (m, 2H), 2.73−2.82 (m, 1H), 2.95−3.04 (m, 1H), 3.14−3.22 (m, 1H), 3.25−3.33 (m, 1H), 3.64−3.73 (m, 1H), 4.12−4.18 (m, 1H), 4.66−4.69 (m, 1H), 6.88 (d, 1H, J = 8.5), 7.15−7.28 (m, 16H), 7.30 (d, 2H, J = 8.5), 7.65 (d, 1H, J = 8.5), 7.77 (d, 2H, J = 8.1), 7.88 (s, br, 1H), 8.48 (s, 1H). Anal. ($C_{39}H_{44}N_4O_6$) C, H, N.

Cbz-L-Leu-L-Phe(4-CN)-L-(Tr)glutaminol (110). Anhydrous HCl gas was bubbled through a solution of **108** (0.84 g, 1.30 mmol) in CH_2Cl_2 (3 mL) at 23 °C for 5 min. The resulting precipitate was filtered and washed with Et_2O (2 × 10 mL) to give a white crystalline solid. In a separate flask, carbonyldiimidazole (0.14 g, 0.84 mmol, 1.0 equiv) was added to a solution of Cbz-L-Leu-OH (0.22 g, 0.84 mmol, 1.0 equiv) in THF (9 mL) at 23 °C, and the reaction mixture was stirred for 1 h at that temperature. A portion of the amine hydrochloride salt prepared above (0.52 g, 0.89 mmol, 1.05 equiv) and Et_3N (0.13 mL, 0.89 mmol, 1.05 equiv) were added sequentially, and the reaction mixture was stirred at 23 °C overnight. The volatiles were removed under reduced pressure, and the residue was purified by flash column chromatography (gradient elution, 0 → 2% CH_3OH in $CHCl_3$) to give **110** (0.31 g, 47%) as a white glassy solid: R_f = 0.18 (5% CH_3OH in $CHCl_3$); IR (cm^{-1}) 2228, 1647, 1520, 1238; ^1H NMR (DMSO-d_6) δ 0.76 (d, 3H, J = 6.6), 0.80 (d, 3H, J = 6.6), 1.22−1.30 (m, 2H), 1.41−1.46 (m, 2H), 1.67−1.81 (m, 1H), 2.20−2.34 (m, 2H), 2.83−2.91 (m, 1H), 3.04−3.09 (m, 1H), 3.17−3.24 (m, 1H), 3.28−3.33 (m, 1H), 3.61−3.70 (m, 1H), 3.89−3.95 (m, 1H), 4.51−4.59 (m, 1H), 4.65−4.72 (m, 1H), 4.97 (d, 1H, J = 12.5), 5.02 (d, 1H, J = 12.5), 7.14−7.33 (m, 16H), 7.39 (d, 2H, J = 8.1),

7.66 (d, 2H, J = 8.1), 7.72 (d, 1H, J = 8.8), 7.94 (d, 1H, J = 8.5), 8.53 (s, 1H).

Cbz-L-Leu-L-Phe(4-CN)-L-(Tr)glutaminal (111). *o*-Iodoxybenzoic acid[24] (0.31 g, 1.10 mmol, 3.0 equiv) was added to a solution of **110** (0.29 g, 0.37 mmol, 1 equiv) in DMSO (4 mL) at 23 °C. After 1.5 h of stirring at 23 °C, the reaction mixture was concentrated under reduced pressure. The residue was twice diluted with CH_2Cl_2 (10 mL), and the volatiles were evaporated to remove any residual DMSO. The residue was then triturated with EtOAc (50 mL), and the resulting white solid was filtered off. The filtrate was washed with a 1:1 mixture of 5% $Na_2S_2O_3$ and 5% $NaHCO_3$ solution (50 mL), water (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated to afford **111** (0.25 g, 86%) as a white glassy solid. This material was used immediately without further purification: ^{1}H NMR (DMSO-d_6) δ 0.77 (d, 3H, J = 6.6), 0.80 (d, 3H, J = 6.6), 1.21−1.34 (m, 2H), 1.41−1.50 (m, 1H), 1.58−1.68 (m, 1H), 1.87−1.93 (m, 1H), 2.28−2.38 (m, 2H), 2.88−2.96 (m, 1H), 3.10−3.16 (m, 1H), 3.91−4.00 (m, 2H), 4.61−4.67 (m, 1H), 4.94−5.03 (m, 2H), 7.14−7.38 (m, 21H), 7.42 (d, 2H, J = 8.1), 7.68 (d, 2H, J = 8.1), 8.05 (d, 1H, J = 8.5), 8.42 (d, 1H, J = 7.0), 8.63 (s, 1H), 9.28 (s, 1H).

Ethyl 3-[Cbz-L-Leu-L-Phe(4-CN)-L-(Tr-*Gln*)]-(*E*)-propenoate (112). (Carbethoxymethylene)triphenylphosphorane (0.13 g, 0.37 mmol, 1.2 equiv) was added to a solution of **111** (0.25 g, 0.31 mmol, 1 equiv) in THF (6 mL) at 23 °C, and the reaction mixture was stirred at that temperature overnight. The volatiles were then removed under reduced pressure, and the residue was purified by flash column chromatography (gradient elution, 0 → 0.75% CH_3OH in $CHCl_3$) to give **112** (0.14 g, 53%) as a white glassy solid: R_f = 0.15 (3% CH_3OH in $CHCl_3$); IR (cm^{-1}) 2228, 1649, 1516; ^{1}H NMR (DMSO-d_6) δ 0.77 (d, 3H, J = 6.6), 0.79 (d, 3H, J = 6.6), 1.21 (t, 3H, J = 7.0), 1.24−1.32 (m, 2H), 1.42−1.30 (m, 1H), 1.54−1.75 (m, 2H), 2.20−2.35 (m, 2H), 2.88−2.92 (m, 1H), 2.95−3.08 (m, 1H), 3.92−3.98 (m, 1H), 4.12 (q, 2H, J = 7.0), 4.32−4.40 (m, 1H), 4.55−4.59 (m, 1H), 4.96 (d, 1H, J = 12.5), 5.02 (d, 1H, J = 12.5), 5.54 (d, 1H, J = 15.8), 6.68 (dd, 1H, J = 15.8, 5.5), 7.13−7.34 (m, 21H), 7.51 (d, 2H, J = 8.1), 7.66 (d, 2H, J = 8.1), 8.07−8.08 (m, 2H), 8.58 (s, 1H).

Ethyl 3-[Cbz-L-Leu-L-Phe(4-CN)-L-*Gln*]-(*E*)-propenoate (34). Trifluoroacetic acid (0.4 mL) was added to a solution of **112** (0.12 g, 0.14 mmol) in CH_2Cl_2 (4 mL) at 23 °C, and the reaction mixture was stirred at room temperature for 6 h and then was concentrated under reduced pressure. The residue was triturated with a 2:1 mixture of Et_2O and EtOAc (15 mL), and the white solid thus obtained was collected by vacuum filtration, washed with Et_2O (2 × 20 mL), and air dried to give **34** (0.063 g, 71%) as a white solid: mp 225−227 °C dec; IR (cm^{-1}) 2230, 1653, 1535; ^{1}H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.6), 0.82 (d, 3H, J = 6.6), 1.24 (t, 3H, J = 7.0), 1.41−1.53 (m, 1H), 1.61−1.80 (m, 4H), 2.02−2.07 (m, 2H), 2.88−2.95 (m, 1H), 3.02−3.07 (m, 1H), 3.95−3.99 (m, 1H), 4.13 (q, 2H, J = 7.0), 4.39−4.42 (m, 1H), 4.53−4.60 (m, 1H), 4.98 (d, 1H, J = 12.5), 5.04 (d, 1H, J = 12.5), 5.55 (d, 1H, J = 15.8), 6.71 (dd, 1H, J = 15.8, 5.5), 6.76 (s, br, 1H), 7.19 (s, br, 1H), 7.24−7.43 (m, 8H), 7.67 (d, 2H, J = 8.1), 8.08 (d, 1H, J = 8.5), 8.12 (d, 1H, J = 8.1). Anal. ($C_{33}H_{41}N_5O_7$) C, H, N.

Preparation of Amino Acid 117. Methyl Ketone 113. Isobutyl chloroformate (3.94 mL, 30.4 mmol, 1.0 equiv) was added to a solution of *N*-α-Boc-L-glutamic acid α-benzyl ester (10.3 g, 30.4 mmol, 1 equiv) and 4-methylmorpholine (3.34 mL, 30.4 mmol, 1.0 equiv) in CH_2Cl_2 (150 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 15 min and then was partitioned between water (150 mL) and CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated to provide a colorless oil.

This material was dissolved in THF (200 mL), and the resulting solution was cooled to −78 °C. Methylmagnesium bromide (21.7 mL of a 1.4 M solution in toluene/THF, 30.4 mmol, 1.0 equiv) was added, and the reaction mixture was stirred at −78 °C for 45 min and then was partitioned between 0.5 M HCl (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 (gradient elution, 30 → 50% EtOAc in hexanes) provided **113** (1.24 g, 12%) and **114**[46] (3.35 g, 35%) both as clear oils: **113**: R_f = 0.65 (50% EtOAc in hexanes); IR (cm^{-1}) 3364, 1740, 1713; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.84−1.96 (m, 2H), 2.09 (s, 3H), 2.38−2.59 (m, 2H), 4.30−4.32 (m, 1H), 5.11−5.15 (m, 2H), 5.20 (d, 1H, J = 12.1), 7.27−7.36 (m, 5H). Anal. ($C_{18}H_{25}NO_5$) C, H, N.

Alcohol 115. Sodium borohydride (0.175 g, 4.63 mmol, 1.25 equiv) was added to a solution of **113** (1.24 g, 3.70 mmol, 1 equiv) in a 5:1 mixture of THF and H_2O (80 mL) at 0 °C. After 1.5 h, the reaction mixture was partitioned between 0.5 M HCl (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. The residue was purified by flash column chromatography (gradient elution, 40 → 50% EtOAc in hexanes) to provide **115** (0.93 g, 74%) as a colorless oil: R_f = 0.43 (50% EtOAc in hexanes); IR (cm^{-1}) 3371, 2972, 1710; ^{1}H NMR ($CDCl_3$, 1:1 mixture of diastereomers) δ 1.13−1.16 (m), 1.32−1.55 (m), 1.43 (s), 1.64−1.99 (m), 3.73−3.81 (m), 4.38 (s, br), 5.13 (d, J = 12.1), 5.22 (d, J = 12.1), 7.32−7.40 (m). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

Silyl Ether 116. *tert*-Butyldimethylsilyl trifluoromethanesulfonate (0.633 mL, 2.76 mmol, 1.0 equiv) was added to a solution of alcohol **115** (0.93 g, 2.76 mmol, 1 equiv) and 2,6-lutidine (0.385 mL, 3.31 mmol, 1.2 equiv) in CH_2Cl_2 (150 mL) at −78 °C. The reaction mixture was stirred at −78 °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 combined organic layers were dried over Na_2SO_4 and were concentrated. Flash chromatographic purification of the residue (20% EtOAc in hexanes) afforded **116** (1.07 g, 85%) as a colorless oil: R_f = 0.68 (30% EtOAc in hexanes); IR (cm^{-1}) 3367, 2958, 1717; ^{1}H NMR ($CDCl_3$, 1:1 mixture of diastereomers) δ −0.11 to −0.08 (m), 0.76 (s), 0.96−0.99 (m), 1.20−1.38 (m), 1.33 (s), 1.48−1.93 (m), 3.61−3.71 (m), 4.23 (s, br), 5.02 (d, J = 12.1), 5.11 (d, J = 12.1), 7.16−7.25 (m). Anal. ($C_{24}H_{41}NO_5Si$) C, H, N.

Amino Acid 117. A suspension of **116** (0.240 g, 0.531 mmol) and Pd/C (10%, 0.040 g) in EtOAc (30 mL) was stirred at 23 °C under a hydrogen atmosphere (balloon) for 3 h. The mixture was filtered through Celite, and the filtrate was concentrated to afford **117** (0.172 g, 89% crude yield) as a colorless oil: R_f = 0.10 (30% EtOAc in hexanes); IR (cm^{-1}) 3321 (br), 2958, 1714; ^{1}H NMR ($CDCl_3$, 1:1 mixture of diastereomers) δ 0.05−0.06 (m), 0.89 (s), 1.13−1.15 (m), 1.45 (s), 1.48−1.57 (m), 1.66−1.98 (m), 3.82−3.87 (m), 4.28 (s, br), 5.06−5.18 (m). Anal. ($C_{17}H_{35}NO_5Si$) C, H, N.

Preparation of Amino Acids 121 and 124. Boc-L-Phe-(4-I)-OBn (118). 1,3-Dicyclohexylcarbodiimide (1.20 g, 5.92 mmol, 1.2 equiv) and 4-(dimethylamino)pyridine (0.239 g, 1.96 mmol, 0.40 equiv) were added sequentially to a solution Boc-L-Phe(4-I)-OH (1.93 g, 4.9 mmol, 1 equiv) and benzyl alcohol (0.61 mL, 5.92 mmol, 1.2 equiv) in CH_2Cl_2 (100 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 6 h and then was filtered. The filtrate was partitioned between H_2O (100 mL) and CH_2Cl_2 (2 × 100 mL), and the combined organic layers were dried over Na_2SO_4 and were concentrated. The residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford **118** (2.05 g, 87%) as a white solid: mp 111−112 °C; R_f = 0.72 (50% EtOAc in hexanes); IR (cm^{-1}) 2975, 1712, 1487, 1124; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 2.95−3.06 (m, 2H), 4.57−4.63 (m, 1H), 4.98 (d, 1H, J = 7.5), 5.06−5.20 (m, 2H), 6.75 (d, 2H, J = 7.8), 7.26−7.29 (m, 2H), 7.35−7.40 (m, 3H), 7.52 (d, 2H, J = 8.1). Anal. ($C_{21}H_{24}INO_4$) C, H, N.

Boc-L-Phe(4-CH=CH$_2$)-OBn (119). Tributyl(vinyl)tin (1.60 mL, 5.32 mmol, 1.1 equiv), several crystals of 2,6-di-*tert*-butyl-4-methylphenol, and $Pd(PPh_3)_4$ (0.280 g, 0.242 mmol, 0.05 equiv) were added sequentially to a solution of **118** (2.33 g, 4.84 mmol, 1 equiv) in 1,4-dioxane (40 mL) at 23 °C. The reaction mixture was stirred at 80 °C for 6 h, cooled to 23 °C, and partitioned between H_2O (100 mL) and CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4

and were concentrated. The residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford **119** (1.53 g, 83%) as a dark brown oil: $R_f = 0.72$ (50% EtOAc in hexane); IR (cm^{-1}) 2969, 1711, 1495, 1165; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 3.06−3.08 (m, 2H), 4.60−4.64 (m, 1H), 4.96−4.99 (d, 1H, $J = 7.8$), 5.07−5.19 (m, 2H), 5.22 (d, 1H, $J = 5.7$), 5.68−5.74 (d, 1H, $J = 17.4$), 6.67 (dd, 1H, $J = 17.4, 10.8$), 6.99 (d, 2H, $J = 7.8$), 7.26−7.29 (m, 4H), 7.32−7.37 (m, 3H).

Boc-L-Phe(4-CH_2CH_2OH)-OBn (120). A solution of BH_3·THF (1.0 M in THF, 0.40 mL, 0.40 mmol, 0.33 equiv) was added dropwise to a solution of **119** (0.467 g, 1.21 mmol, 1 equiv) in THF (2 mL) at 0 °C. The reaction mixture was stirred at 23 °C for 2 h and then H_2O (3 mL) and $NaBO_3$·$4H_2O$ (0.186 g, 1.21 mmol, 1.0 equiv) were added. The resulting mixture was stirred at 23 °C for 1 h and then was partitioned between H_2O (10 mL) and Et_2O (4 × 10 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (50% EtOAc in hexanes) to afford **120** (0.238 g, 49%) as a yellow oil: $R_f = 0.30$ (50% EtOAc in hexanes); IR (cm^{-1}) 3376, 1709, 1165; ^1H NMR ($CDCl_3$) δ 1.37 (s, 9H), 2.82 (t, 2H, $J = 6.6$), 3.05 (m, 2H), 3.83 (q, 2H, $J = 6.3$), 4.58−4.63 (m, 1H), 4.97 (d, 1H, $J = 8.7$), 5.08−5.20 (m, 2H), 6.98 (d, 2H, $J = 7.8$), 7.09 (d, 2H, $J = 7.5$), 7.30−7.38 (m, 5H). Anal. ($C_{23}H_{29}NO_5$·$0.75H_2O$) C, H, N.

Boc-L-Phe(4-CH_2CH_2OH)-OH (121). Sodium hydroxide (2.0 M in H_2O, 1.76 mL, 3.52 mmol, 8.0 equiv) was added to a solution of **120** (0.176 g, 0.44 mmol, 1 equiv) in CH_3OH (5 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 6 h and then was partitioned between 10% $KHSO_4$ (50 mL) and CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated to give crude **121** (0.121 g, 89%). This material was used without further purification: ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.81 (t, 2H, $J = 6.6$), 3.05−3.17 (m, 2H), 3.80−3.87 (m, 2H), 4.57−4.60 (m, 1H), 4.96−4.99 (d, 1H, $J = 8.7$), 7.05−7.16 (m, 2H), 7.26−7.37 (m, 2H).

Boc-L-Phe(4-CHO)-OBn (122). 4-Methylmorpholine *N*-oxide (0.22 g, 1.88 mmol, 1.1 equiv) and OsO_4 (0.02 M solution in H_2O, 2.57 mL, 0.051 mmol, 0.03 equiv) were added sequentially to a solution of **119** (0.654 g, 1.71 mmol, 1 equiv) in an 8:1 mixture of acetone and H_2O (9 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 3 h, and then $Na_2S_2O_5$ (1 g) and H_2O (50 mL) were added carefully. The resulting mixture was stirred at 23 °C for 20 min and then was partitioned between EtOAc (2 × 100 mL) and H_2O (50 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. The residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford the corresponding diol (0.44 g, 61%) as an off-white foam: $R_f = 0.12$ (50% EtOAc in hexanes); IR (cm^{-1}) 3376, 1706, 1500, 1165; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H), 2.21 (s, br, 1H), 2.61 (s, br, 1H), 3.00−3.13 (m, 2H), 3.56−3.64 (m, 2H), 3.68−3.75 (m, 1H), 4.58−4.64 (m, 1H), 4.74−4.77 (m, 1H), 5.00 (d, 2H, $J = 7.8$), 5.08−5.19 (m, 2H), 7.02 (d, 2H, $J = 7.5$), 7.09−7.26 (m, 3H), 7.29−7.39 (m, 4H).

This material (0.44 g, 1.05 mmol, 1 equiv) was dissolved in Et_2O (10 mL) at 23 °C, and a solution of $NaIO_4$ (0.247 g, 1.15 mmol, 1.1 equiv) in H_2O (2 mL) was added. The reaction mixture was stirred at 23 °C for 2 h and then was partitioned between H_2O (50 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated to give crude **122** as a colorless oil. This material was used without further purification: IR (cm^{-1}) 1699, 1507, 1167; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 3.08−3.28 (m, 2H), 4.63−4.68 (m, 1H), 5.02−5.04 (d, 1H, $J = 7.8$), 5.07−5.21 (m, 2H), 7.18 (d, 2H, $J = 8.1$), 7.30−7.41 (m, 5H), 7.72 (d, 2H, $J = 8.1$), 9.96 (s, 1H).

Boc-L-Phe(4-CH_2OH)-OBn (123). Sodium borohydride (0.040 g, 1.05 mmol, 1.0 equiv) was added to a solution of **122** (0.40 g, 1.05 mmol, 1 equiv) in EtOH (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and then was partitioned between H_2O (50 mL) and a 1:1 mixture of EtOAc and hexanes (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and were concentrated. Purification of the residue by flash column chromatography (25% EtOAc in hexanes) provided **123** (0.298 g, 74%) as a white foam: $R_f = 0.88$ (50% EtOAc in hexanes); IR (cm^{-1}) 3378, 1699, 1506, 1184; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.68 (m, 1H), 3.07−3.09 (m, 2H), 4.58−4.60 (m, 1H), 4.64 (d, 2H, $J = 4.5$), 4.97 (d, 1H, $J = 8.4$), 5.08−5.20 (m, 2H), 7.02 (d, 2H, $J = 7.8$), 7.22 (d, 2H, $J = 7.8$), 7.28−7.37 (m, 5H). Anal. ($C_{22}H_{27}NO_5$) C, H, N.

Boc-L-Phe(4-CH_2OH)-OH (124). A suspension of **123** (0.28 g, 0.73 mmol) and Pd/C (0.060 g) in EtOAc (10 mL) was stirred at 23 °C under an H_2 atmosphere (balloon) for 6 h. The reaction mixture was filtered thought Celite, and the filtrate was concentrated to afford crude **124** (0.20 g, 93%) as a colorless oil. This material was used without further purification: ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H), 3.13 (m, 2H), 4.59 (m, 1H), 4.66 (s, 2H), 4.97−5.00 (d, 1H $J = 8.1$), 7.19 (d, 2H, $J = 8.7$), 7.28 (d, 2H, $J = 8.7$).

Protein−Ligand Crystal Structure Determination. Serotype 2 human rhinovirus 3C protease was incubated with a 3-fold molar excess of compound **75**, and the complex was concentrated to a final protein concentration of 10 mg/mL. Equal volumes of the protein/ligand stock solution and a 2.0 M solution of ammonium sulfate buffered with 100 mM ADA pH 6.5 were mixed and allowed to incubate at 21 °C for 1 h. This solution was then passed through a Centrex 0.45 μM filter and used immediately for crystallization experiments. Crystallization was carried out at 13 °C using a hanging drop vapor diffusion method in which 3 μL drops of the protein/inhibitor complex were mixed with an equal volume of reservoir solution on plastic coverslips and sealed over individual reservoir wells containing a solution of 0.5 M ammonium sulfate, 1.5 M Na/K phosphate, and 100 mM ADA pH 6.6 buffer. Following a 4 h equilibration at 13 °C, a microcrystal of HRV-2 3CP−**3**[1] was added to each drop using a Hampton microneedle to induce growth of HRV-2 3CP−**75** crystals. Crystals of the complex typically reached dimensions of 0.5 × 0.35 × 0.15 mm in 7−10 days.

A single crystal measuring 0.3 × 0.2 × 0.1 mm (space group $P2_12_12$; $a = 61.56$, $b = 77.68$, $c = 33.95$ Å) was prepared for low-temperature data collection by serial transfer to artificial mother liquor solutions of increasing glycerol concentration. When fully equilibrated against an artificial mother liquor containing 25% glycerol, the crystal was flash frozen. X-ray diffraction data were collected at −170 °C using a MAR imaging plate and processed with DENZO.[47] The resulting data were 95% complete to a resolution of 1.9 Å with R(sym) = 3.9%.

Protein atomic coordinates from the HRV-2 3CP-**3** structure determination[1] were used to initiate rigid body refinement in X-PLOR[48] followed by simulated annealing and conjugate gradient minimization protocols. Placement of the inhibitor **75**, addition of ordered solvent, and further rounds of refinement proceeded as described for the HRV-2 3CP−**3** complex.[1] The final R factor was 21.2% (11 618 reflections with $F > 2\sigma(F)$). The root-mean-square deviations from ideal bond lengths and angles were 0.017 Å and 2.9°, respectively. The final model consisted of all atoms for residues 1−180 (excluding side chains for residues 12, 55, and 65) plus 111 water molecules.

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(28) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(29) The tripeptide aldehydes required for the synthesis of compounds **10** and **12** were prepared as described in ref 6. The tripeptide aldehyde required for the synthesis of compound **11** was prepared from Cbz-L-Leu-L-Phe-L-(SO)methioninol[6] by the following sequence: (i) *m*-CPBA; (ii) IBX.[24]

(30) Compound **13** was prepared from Cbx-L-Leu-L-Phe-L-(*N*-Boc-aminoalaninal) (ref 6) by the following sequence: (i) $Ph_3P{=}CHCO_2Et$, (ii) TFA, (iii) $[(4\text{-}NO_2)PhO]_2CO$, NH_3.[31]

(31) Izdebski, J.; Pawlak, D. A New Convenient Method for the Synthesis of Symmetrical and Unsymmetrical *N*,*N*′-Disubstituted Ureas. *Synthesis* **1989**, 423−425.

(32) The olefin intermediate required for the preparation of compound **15** was prepared from *tert*-butyl (*S*)-(-)-4-formyl-2,2-dimethyl-3-oxazolidine-carboxylate by reaction with (carbethoxymethylene)triphenylphosphorane. Inhibitor **14** was prepared from compound **15** by the following sequence: (i) chloroacetyl isocyanate; (ii) $NaHCO_3$, H_2O, THF, EtOH. See: Richter, H. G. F.; Angehrn, P.; Hubschwerlen, C.; Kania, M.; Page, M. G. P.; Specklin, J.-L.; Winkler, F. K. Design, Synthesis, and Evaluation of 2β-Alkenyl Penam Sulfone Acids as Inhibitors of β-Lactamases. *J. Med. Chem.* **1996**, *39*, 3712−3722.

(33) The benzyl ester of the desired *N*-Cbz-protected amino acid was obtained by a combination of the following: (a) Nakajima, K.; Oda, H.; Okawa, K. Studies on 2-Aziridinecarboxylic Acid. IX. Convenient Synthesis of Optically Active *S*-Alkylcysteine, *threo*-*S*-Alkyl-β-methylcysteine, and Lanthionine Derivatives *via* the Ring-opening Reaction of Aziridine by Several Thiols. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 520−522. (b) Willems, J. G. H.; Hersmis, M. C.; de Gelder, R.; Smits, J. M. M.; Hammink, J. B.; Dommerholt, F. J.; Thijs, L.; Zwanenburg, B. Synthesis and Crystal Structure of Enantiopure *N*-Tritylaziridin-2-yl-methanols from L-Serine and L-Threonine. *J. Chem. Soc., Perkin Trans. 1* **1997**, 963−967. The free carboxylic acid was prepared by the following sequence: (i) $CH_2{=}CHCH_2OH$, cat. $Ti(O\text{-}i\text{-}Pr)_4$, 100 °C; (ii) Morpholine, $Pd(PPh_3)_4$.

(34) Marzoni, G.; Kaldor, S. W.; Trippe, A. J.; Shamblin, B. M.; Fritz, J. E. A Convenient, Large Scale Synthesis of N-CBZ-(*S*-Phenyl)-L-Cysteine. *Synth. Commun.* **1995**, *25*, 2475−2482.

(35) The free amino acid was obtained as described in: Shao, H.; Goodman, M. An Enantiomeric Synthesis of *allo*-Threonines and *β*-Hydroxyvalines. *J. Org. Chem.* **1996**, *61*, 2582−2583. The *N*-Cbz-protected amino acid was prepared as follows: CbzCl, $NaHCO_3$, *i*-PrOH.

(36) The *tert*-butyl esters of the amino acids required for the preparation of **61** and **62** were synthesized by the reductive coupling of morpholine with the aldehydes derived from *N*-α-Cbz-L-aspartic acid α-*tert*-butyl ester and *N*-α-Cbz-L-glutamic acid α-*tert*-butyl ester, respectively. See: (a) Valerio, R. M.; Alewood, P. F.; Johns, R. B. Synthesis of Optically Active 2-(*tert*-Butyloxycarbonylamino)-4-dialkoxyphosphorylbutanoate Protected Isosteres of *O*-Phosphonoserine for Peptide Synthesis. *Synthesis* **1988**, 786−789. (b) Faust, J.; Schreiber, K. Der Normalisierungsfaktor für die Tomatenmutante *chloronerva*; Synthese des (*S*)-Piperidin-2-carbonsäureanalogons von Nicotianamin. *Z. Chem.* **1989**, *29*, 20−21. The free carboxylic acids were prepared by treatment of the *tert*-butyl esters with TFA.

(37) The amino acids required for the synthesis of compounds **66**, **67**, and **70** were prepared by addition of 2-methylbenzyl alcohol, 2-chlorobenzyl alcohol, or cyclohexanol, respectively, to (*S*)-(−)-2-isocyanato-4-methylvaleric acid methyl ester (benzene, 50 °C) followed by basic hydrolysis (LiOH, CH_3OH, H_2O).

(38) The amino acids required for the preparation of compounds **69** and **83** were synthesized by reaction of methyl chloroformate (Et_3N) or glycolic acid (EDC, HOBt, 4-methylmorpholine), respectively, with H_2N-L-Leu-OtBu·HCl and subsequent acidic deprotection (TFA).

(39) The amino acids required for the synthesis of compounds **72**−**75** were prepared by reaction of the appropriate chlorothiolformate with H_2N-L-Leu-OtBu·HCl (Et_3N) and subsequent acidic deprotection (TFA). Benzyl chlorothiolformate and cyclopentyl chlorothiolformate were prepared in a manner similar to that described in the following: Eden, J. M.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hunter, J. C.; Martin, K.; Pritchard, M. C.; Rahman, S. S.; Richardson, R. S.; Roberts, E. Rationally Designed 'Dipeptoid' Analogues of Cholecystokinin (CCK): *N*-Terminal Structure-Affinity Relationships of α-Methyl-Tryptophan Derivatives. *Eur. J. Med. Chem.* **1993**, *28*, 37−45.

(40) The amino acid utilized in the preparation of compound **84** was synthesized by coupling of H_2N-L-Leu-OtBu·HCl with *β*-propiolactone ($AlMe_3$) and subsequent acidic deprotection (TFA).

(41) Cbz-L-Leu-OH was coupled with H_2N-L-His-OMe·2HCl or H_2N-L-His(1-Me)-OMe·HCl (EDC, HOBt) and the resulting dipeptides were hydrolyzed under basic conditions (NaOH, CH_3OH, H_2O).

(42) *N*-α-Cbz-L-glutamic acid *γ*-*tert*-butyl ester was esterified with 2-(trimethylsilyl)ethanol (EDC). The Cbz group present in the resulting di-ester was removed (H_2, Pd/C), and the free amine was coupled with Cbz-L-Leu-OH (EDC, HOBt, 4-methylmorpholine). Removal of the 2-(trimethylsilyl)ethyl moiety (Bu_4NF) provided the desired dipeptide.

(43) (*S*)-3-Azido-4,4-dimethyldihydrofuran-2-one[44] was coupled with H_2N-L-Phe-OtBu·HCl ($AlMe_3$), and the product thus obtained was converted to the desired dipeptide by the following sequence: (i) H_2, Pd/C; (ii) CbzCl, 4-methylmorpholine; (iii) TFA.

(44) Freskos, J. N. Use of *R*-Pantolactone in the Synthesis of L-*tert*-Leucine Derivatives. *Synth. Commun.* **1994**, *24*, 557−563.

(45) The dipeptide required for the preparation of **71** was synthesized by coupling Boc-L-Leu-OSu with H_2N-L-Phe-OBn·HCl [$(i\text{-Pr})_2$NEt] and subsequent debenzylation (H_2, Pd/C).

(46) Carpino, L. A.; Mansour, E.-L. M. E. Protected *β*- and *γ*-Aspartic and Glutamic Acid Fluorides. *J. Org. Chem.* **1992**, *57*, 6371−6373.

(47) Otwinowski, Z. DENZO in Data Collection and Processing. In *Proceedings of the CCP4 Study Weekend*; Sawyer, L., Isaacs, N., Bailey, S., Eds.; SERC Daresbury Laboratory: Warrington, UK, 1993; pp 55−62.

(48) X-PLOR, version 3.1. Brünger A. T. *X-PLOR v3.1 Manual*; Yale University Press: New Haven, CT, 1992.

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