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GLUTAMINE-DERIVED ALDEHYDES FOR THE INHIBITION OF HUMAN RHINOVIRUS 3C PROTEASE

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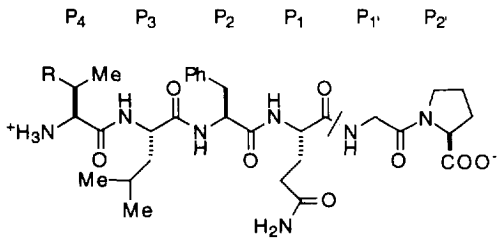
Abstract. Peptide aldehydes have been synthesized and evaluated as inhibitors of human rhinovirus 3C protease. Those inhibitors containing a C-terminal glutamine aldehyde were prepared using newly developed methodology involving reduction of the corresponding glutarimide, which can be easily prepared from *N*-protected glutamine. Low molecular weight (<500) compounds with low to submicromolar inhibitory activity in both isolated enzyme and in vitro translation assays have been identified.

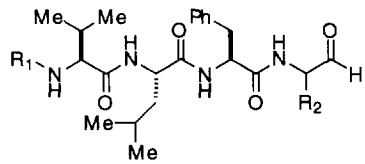
The human rhinoviruses are members of the picornavirus family, and are the primary cause of the common cold.[1] These positive-strand RNA viruses encode a single polyprotein that is proteolytically processed at Gln-Gly by human rhinovirus 3C protease ($3C^{pro}$), a virally encoded enzyme essential for virus replication.[2,3] $3C^{pro}$ is a thiol protease which possesses a Cys-His-Glu catalytic triad analogous to that found in serine proteases.[4] The lack of homology between $3C^{pro}$ and common mammalian enzymes has made it an attractive target for inhibition and antiviral therapy, although few inhibitors are known.[5-8]

Truncated peptides have been prepared previously by Colonno and coworkers to determine the structural requirements for cleavage by $3C^{pro}$, and the hexapeptide Thr-Leu-Phe-Gln-/-Gly-Pro appears to be a minimal effective substrate (Figure 1). The residues Gly and Pro at $P_{1'}$ and $P_{2'}$ respectively are essential for cleavage by the enzyme, while substitution of Val for Thr at P_4 affords a superior substrate.[9] In our laboratories, we desired to prepare peptidomimetic inhibitors of $3C^{pro}$ for use in assay validation and protein NMR studies. Since peptide aldehydes had previously been reported to be effective inhibitors of the mammalian thiol protease papain,[10,11] we elected to construct related aldehydes incorporating amino acids known to be tolerated by $3C^{pro}$.[8,9]

Figure 1



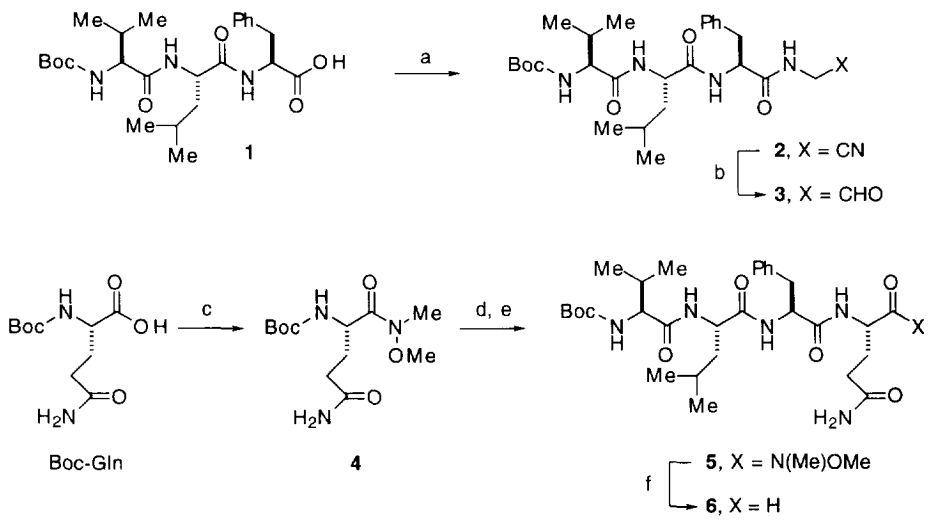
Colonno minimal HRV $3C^{pro}$ substrate
R = Me or OH



initial peptide aldehyde synthesis targets
R_1 = Boc or H
R_2 = H or $CH_2CH_2CONH_2$

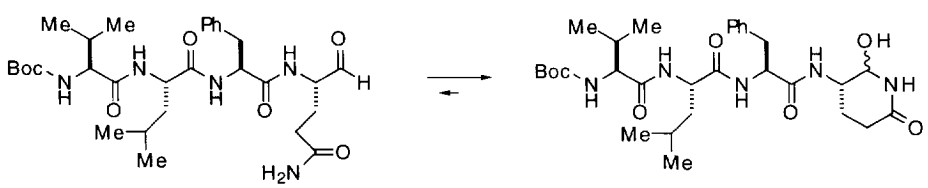
We began by coupling β-aminoacetonitrile with Boc-Val-Leu-Phe (**1**) to afford nitrile **2** (Scheme 1). Reduction of **2** with 5.5 equivalents of DiBAl-H provided the desired aldehyde for testing.[12] Aldehyde **3** was ascertained to be a moderate enzyme inhibitor, with an IC_{50} of 10 μM against human rhinovirus-14 3C protease (Table).[13] The preparation of the analogous inhibitor incorporating a P_1 glutamine side chain was undertaken next in an effort to more closely mimic the natural substrate. In our initial synthesis strategy, an *N*-methoxy-*N*-methyl amide was chosen as a latent aldehyde.[14] Coupling of *N*-methoxy-*N*-methyl amine with Boc-Gln yielded the corresponding amide **4**, albeit in only 1% yield (Scheme 1). Amine deprotection followed by carbodiimide-mediated coupling with Boc-Val-Leu-Phe provided amide **5**, which was then reduced with 6.5 equivalents of DiBAl-H to deliver the target aldehyde **6** (0.2% overall yield). As expected based on the substrate requirements of the enzyme, glutamine aldehyde **6** proved to be a relatively potent inhibitor of $3C^{pro}$ (IC_{50} = 0.6 μM), binding with at least 15-fold better affinity than glycine analog **3**.

Scheme 1



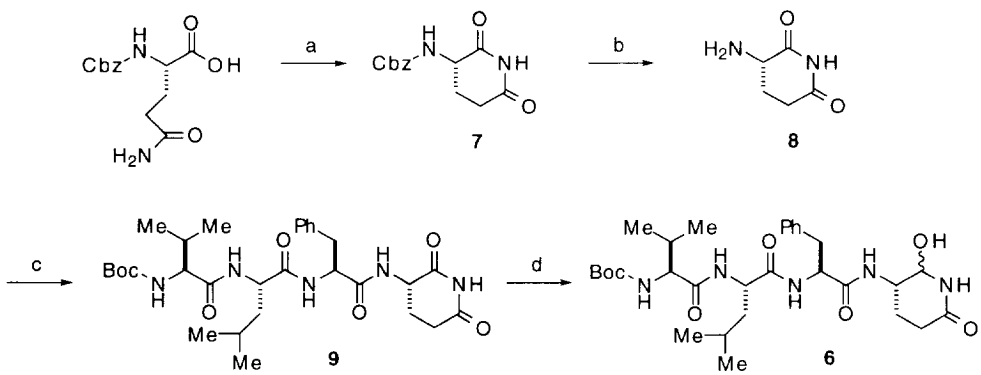
Reagents: (a) HCl-NH_2CH_2CN, DCC, HOBt, NMM, THF, 39%; (b) 5.5 equiv DiBAl-H, THF, -78 °C; MeOH, 21%; (c) HN(OMe)Me-HCl, NMM, DCC, HOBt, DMF, 1%; (d) 1.5 N HCl, dioxane, 91%; (e) **1**, DCC, HOBt, DMF, 40%; (f) 6.5 equiv DiBAl-H, THF, -78-0 °C, 32%.

Figure 2



These encouraging data provided impetus for the exploration of a more efficient means for the preparation of **6** and related glutamine aldehyde-containing analogs. The ^{1}H NMR spectrum of **6** (CD_3OD) lacked an aldehyde peak, suggesting that it favored the cyclic tautomer illustrated in Figure 2. This observation provoked us to consider using a cyclic imide as an alternative glutamine aldehyde precursor.[15] There was ample precedent for the cyclization of glutamine derivatives to the corresponding imides.[16,17] It was also our belief that, due to both steric and electronic influences, reduction of the imide would take place preferentially at the desired carbonyl.[18] The preparation of the key imide intermediate **8** proceeded smoothly and in good yield (Scheme 2). Activation of Cbz-Gln as its *N*-hydroxysuccinimide ester followed by thermal cyclization afforded Cbz imide **7**.[17] Removal of the Cbz protecting group under transfer hydrogenation conditions proceeded uneventfully to deliver imide **8** in high enantiomeric purity.[19,20] Coupling of imide **8** with Boc-Val-Leu-Phe followed by regioselective reduction of **9** gave compound **6**, identical in all respects to material constructed by our earlier route.

Scheme 2



Reagents: (a) NHS, DCC, CH_2Cl_2, DMF; $CHCl_3$, 60 °C, 72%; (b) Pd black, 1,4-cyclohexadiene, EtOH, 99%; (c) **1**, DCC, HOBt, THF, 55%; (d) $NaBH_4$, EtOH, 75%.

With efficient synthesis methodology in hand, a variety of truncated analogs of **6** were prepared to determine the minimum requirements for activity (Table). Complete loss of activity was seen on excision of either the P_4 Val or P_4-P_3 Val-Leu residues (**10** and **11**). We speculated that **10** was a poor inhibitor due to unfavorable interactions between the enzyme and the *N*-terminal Boc group. In accord with prediction, the corresponding free amine **12** (IC_{50} = 1.0 μM) and acetamide derivative **13** (IC_{50} = 2.7 μM) proved to be moderate inhibitors of the enzyme. Compound **14** was prepared to determine the effect of removing the P_3 nitrogen, and the result was complete loss of activity. Replacement of the lipophilic leucine side chain at P_3 with glycine led to a 5-fold poorer inhibitor (**15** versus **13**).

Compounds with demonstrated $3C^{pro}$ activity were also examined in an in vitro translation assay for their ability to selectively inhibit the translation of rhinoviral RNA (Table and Figure 3).[21] For the five compounds examined, enzyme activity directly correlated with potency in the translation assay, with inhibitor **6** showing pronounced ability to inhibit rhinoviral RNA translation even at 5-10 μM concentrations. Inhibitor **6**

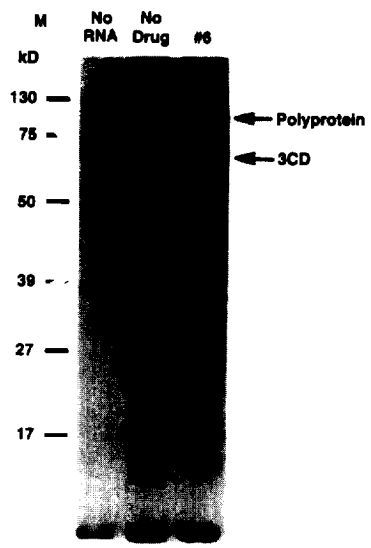
selectively inhibits the ability to produce mature virus-encoded proteins in this assay, and causes a build-up in the concentrations of precursor polyproteins as expected (Figure 3).

Table

Cpd. #	Compound	$3C^{pro}$ IC_{50} (μM)[a,b]	Translation Assay (μM)[b,c]
3	BocNH-Val-Leu-Phe-Gly-CHO	10±4 (n=3)	180
6	BocNH-Val-Leu-Phe-Gln-CHO	0.6±0.4 (n=5)	10 (n=2)
10	BocNH-Leu-Phe-Gln-CHO	>80	not tested
11	BocNH-Phe-Gln-CHO	>80	not tested
12	H_2N-Leu-Phe-Gln-CHO	1.0±0.4 (n=2)	toxic
13	AcNH-Leu-Phe-Gln-CHO	2.7	170
14	$(CH_3)_2CHCH_2CONH$-Phe-Gln-CHO	>80	>200
15	AcNH-Gly-Phe-Gln-CHO	16	not tested

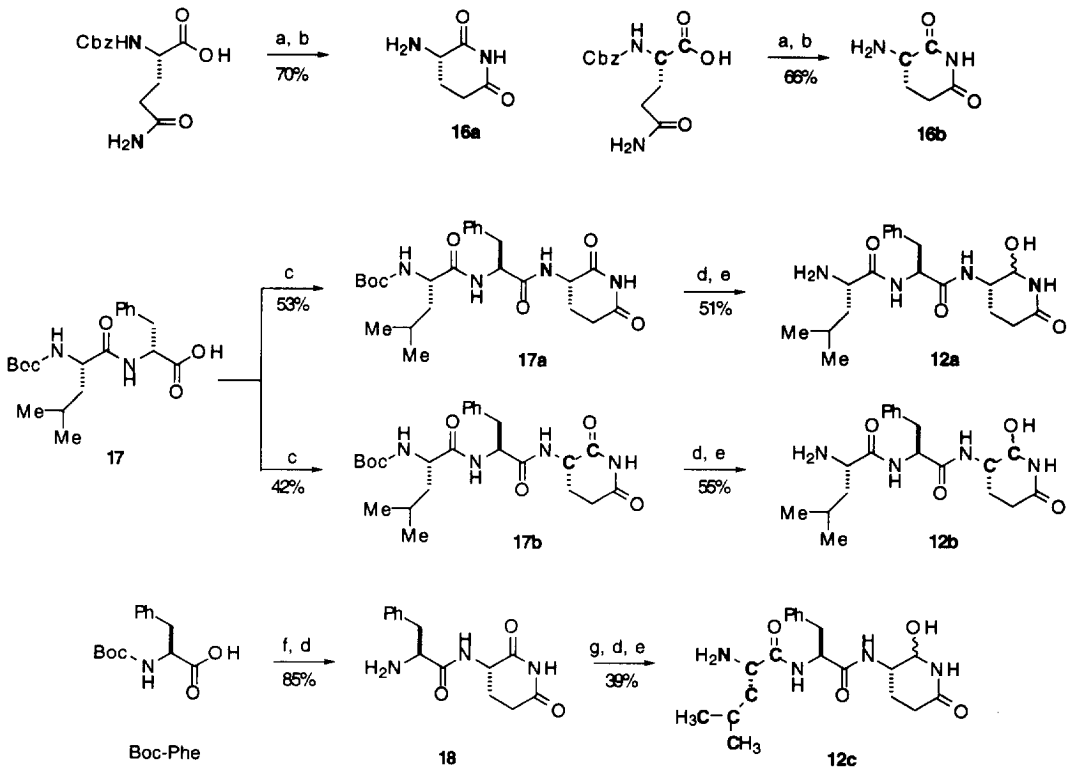
[a]Ref. 13. [b]Unless otherwise indicated, n=1. [c]Ref 21.

Figure 3



In an effort to gain a better appreciation for enzyme-inhibitor interactions, isotopically labelled derivatives of the relatively water soluble inhibitor **12** were prepared to facilitate NMR studies with $3C^{pro}$ (Scheme 3).[12] Inhibitors **12a** and **12b**, bearing isotopic labels on the glutamine aldehyde residue, were prepared in a manner directly analogous to Scheme 2. By contrast, a modified route was devised for the construction of inhibitor **12c** to enable late introduction of isotopically labelled leucine at P_3. A detailed account of protein NMR studies with **12a-c** will be the topic of a separate paper.[22]

Scheme 3



Bolded atoms correspond to the following isotopic labels: **C** = ^{13}C, **N** = ^{15}N

Reagents: (a) NHS, DCC, CH_2Cl_2, DMF; $CHCl_3$, 60 °C; (b) Pd black, 1,4-cyclohexadiene, EtOH; (c) **16a** or **16b**, DCC, HOBt, THF; (d) TFA, CH_2Cl_2; (e) $NaBH_4$, EtOH; (f) DCC, HOBt, THF, **8**; (g) Boc-Leu (^{15}N, $^{13}C_6$), DCC, HOBt, THF.

In summary, several small peptide aldehydes have been synthesized and studied as inhibitors of human rhinovirus 3C protease. Those inhibitors containing a C-terminal glutamine aldehyde were prepared by newly developed methodology involving reduction of the corresponding glutarimide, which can be easily prepared from *N*-protected glutamine. Low molecular weight (<500) compounds with low to submicromolar enzyme inhibitory activity have been identified, and several of these inhibitors also curb the processing of viral polyproteins in an in vitro translation assay at micromolar concentrations. Further studies on these novel inhibitors are currently in progress and will be reported in due course.

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