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α-Ketoamides, α-Ketoesters and α-Diketones as HCV NS3 Protease Inhibitors

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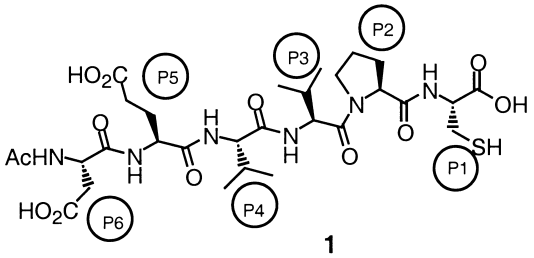
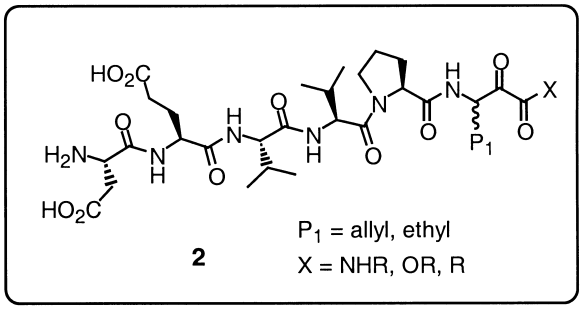
Abstract—Peptide-based α-ketoamides, α-ketoesters and α-diketones were designed, synthesized and evaluated against HCV NS3 protease. α-Ketoamides have the highest affinity among the three classes, with **8** being the most potent inhibitor with an IC_{50} of 340 nM.

The hepatitis C virus (HCV), first identified in 1989,[1] is the principal etiologic agent of both parenterally transmitted and sporadic non-A non-B hepatitis.[2] More than 170 million people worldwide are infected with HCV according to the World Health Organization. Current therapies for HCV infection include treatment with interferon-α and its combination with ribavirin.[3,4] These therapies have limited efficacy and are accompanied by frequent side effects.[3,4] Hence, a new treatment of the disease is of great interest. One of the most intensively studied and best understood targets for HCV antiviral therapy is the serine protease of the NS3 protein.[4,5] In this communication, we wish to report a class of peptide-based 1,2-dicarbonyl derivatives as HCV NS3 protease inhibitors.

Hexapeptide **1** was synthesized early on in our organization and found to inhibit HCV NS3 protease with an IC_{50} of 2.5 μM.[6] In an attempt to enhance the inhibitory potency, we chose to investigate the effect of serine traps. 1,2-Dicarbonyl derivatives, such as α-ketoamides, α-ketoesters, and α-diketones, are known serine traps and have been used as inhibitors of related serine proteases.[7] Therefore, we designed target **2** by incorporating α-ketoamides, α-ketoesters, and α-diketones into the peptide backbone of **1**. Based on the observation that HCV NS3 protease has a shallow and hydrophobic S1 pocket in the crystal structure[5] and on preliminary data showing the effectiveness of the allyl and ethyl P1 groups in a related series,[6] we replaced the P1 mercaptomethyl group in **1** with the less reactive allyl and ethyl groups in our new targets.[8,9]



1



2

Scheme 1 outlines the synthesis of α-ketoamide **8**. DL-Allylglycine **3** was converted to aldehyde **4** via a Wenreib amide intermediate. Cyanohydrin formation, hydrolysis and Boc protection provided α-hydroxycarboxylic acid **5**. Treatment of **5** with allylamine yielded the corresponding α-hydroxyamide **6**. After removal of Boc group, the resulting amine was reacted with pentapeptide **9**[10] to give α-hydroxyamide **7**. Finally,

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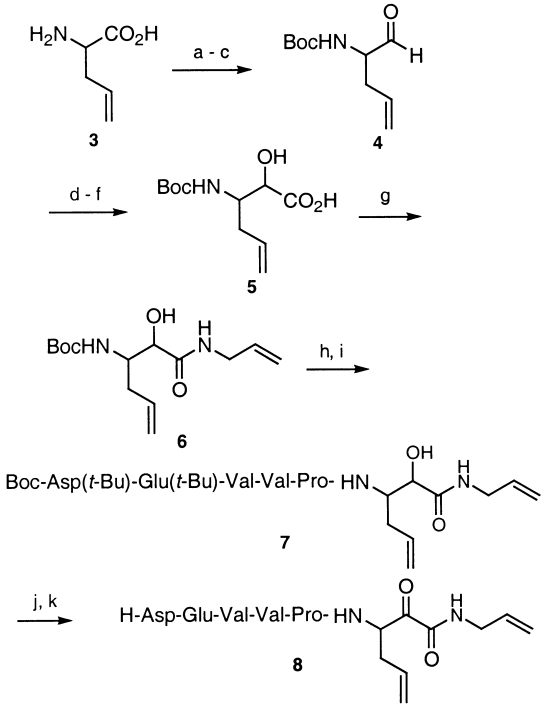
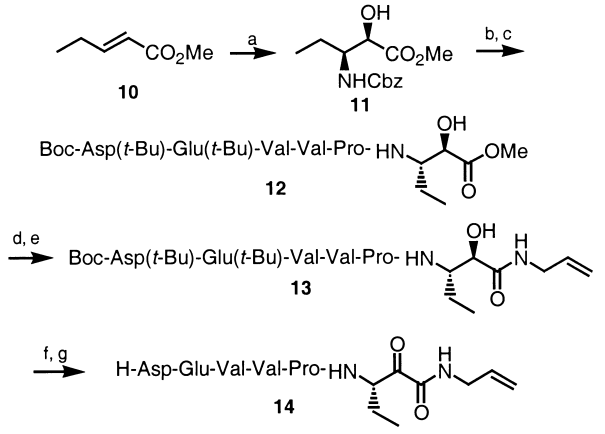
Dess–Martin oxidation[11] followed by acidic deprotection of the *t*-butyl groups completed the synthesis of α-ketoamide **8**. The α-ketoester **20** was synthesized following a similar sequence.

The synthesis of ketoamides with an ethyl P1 group is outlined in Scheme 2. Starting from methyl 2-pentenoate (**10**) Sharpless asymmetric aminohydroxylation catalyzed with $K_2OsO_2(OH)_4$ and $(DHQ)_2PHAL$ provided α-hydroxy-β-amino compound **11** in 78% yield and 83% ee.[12] The enantiomeric excess of **11** was improved to 95% by a single recrystalization from EtOAc/hexane. Reductive cleavage of the Cbz group followed by coupling with pentapeptide **9** provided the α-hydroxyester **12**. Ester **12** was then saponified with LiOH and coupled with allylamine to give the α-hydroxyamide **13**. Dess–Martin oxidation and deprotection gave the final product **14**.



Scheme 1. (a) $(Boc)_2O$, NaOH, THF/H_2O (95%); (b) MeONHMe HCl, BOP, TEA, CH_2Cl_2 (90%); (c) LAH, THF (91%); (d) $Me_2C(OH)CN$, TEA, CH_2Cl_2 (81%); (e) HCl, H_2O/dioxane; (f) $(Boc)_2O$, Na_2CO_3, H_2O (73% for two steps); (g) allylamine, BOP, DIEA, DMF (82%); (h) 4 N HCl in dioxane; (i) Boc-Asp(*t*-Bu)-Glu(*t*-Bu)-Val-Val-Pro-OH (**9**), BOP, DIEA, DMF (70% for two steps); (j) Dess–Martin oxidation (70%); (k) TFA, CH_2Cl_2 (95%).

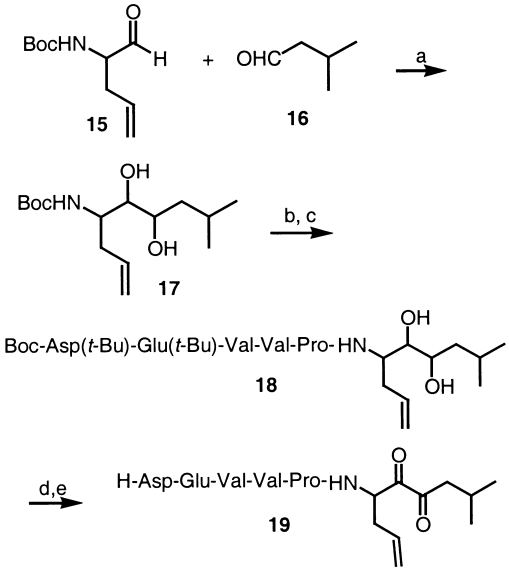


Scheme 2. (a) $(DHQ)_2PHAL$, $K_2[OsO_2(OH)_4]$, CbzNClNa, H_2O/*i*-PrOH (78%); (b) H_2, Pd/C, MeOH (95%); (c) **9**, BOP, DIEA, DMF (82%); (d) LiOH, THF/H_2O (95%); (e) allylamine, BOP, DIEA, DMF (83%); (f) Dess–Martin oxidation (71%); (g) TFA, CH_2Cl_2 (95%).

The synthesis of the α-diketone **19** is described in Scheme 3. Key transformations include intermolecular pinacol coupling under McMurry conditions[13] to introduce diol **17** and Dess–Martin oxidation to give the diketone moiety in **19**.

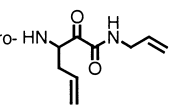
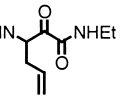
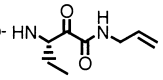
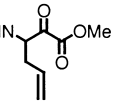
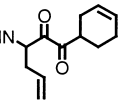
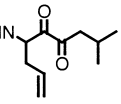
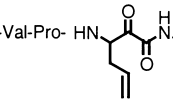
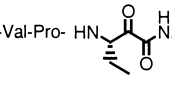
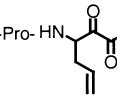
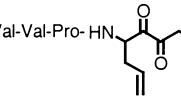
The HCV protease (NS3) inhibitory data is summarized in Table 1.[14] The α-ketoamides prepared in this study are active inhibitors, with the best compound (**8**) having an IC_{50} of 0.34 μM. Ketoamides of allylamine and ethylamine gave comparable potency (entries 1 and 2). As predicted from modeling, the analogue with an ethyl group in P1 is also active. The IC_{50}'s for ethyl and allyl P1 analogues are comparable (entries 1 and 3). However, since **8** is a 1:1 mixture of P1 C_α isomers and only the L isomer is expected to be potent, the allyl P1 may be slightly better than the ethyl group. Replacing the α-ketoamide with an α-ketoester or α-diketone reduced the potency by an order of magnitude (entries 4–6). Blocking of the N terminus and the side chains of Asp and Glu with *t*-butyl groups also slightly reduced the potency in the α-ketoamide series (entries 7 and 8). Interestingly, this variation resulted in total loss of the inhibitory activity in α-ketoester and α-diketone series (entries 9 and 10).

In summary, a series of α-ketoamides, α-ketoesters and α-diketones were designed, synthesized, and found to be HCV NS3 protease inhibitors. Among them, α-ketoamides provided the most potent HCV NS3 protease inhibitors.



Scheme 3. (a) $VCl_3(THF)_3$, Zn, CH_2Cl_2 (59%); (b) 4 N HCl in dioxane; (c) **9**, BOP, DIEA, DMF (67% for two steps); (d) Dess–Martin oxidation (47%); (e) TFA, CH_2Cl_2 (90%).

Table 1.

Entry	Structure	IC_{50} (μM)
1	H-Asp-Glu-Val-Val-Pro- HN  **8**	0.34
2	H-Asp-Glu-Val-Val-Pro- HN  **21**	0.35
3	H-Asp-Glu-Val-Val-Pro- HN  **14**	0.42
4	H-Asp-Glu-Val-Val-Pro- HN  **20**	4.34
5	H-Asp-Glu-Val-Val-Pro- HN  **22**	4.23
6	H-Asp-Glu-Val-Val-Pro- HN  **19**	4.80
7	Boc-Asp(*t*-Bu)-Glu(*t*-Bu)-Val-Val-Pro- HN  **23**	0.57
8	Boc-Asp(*t*-Bu)-Glu(*t*-Bu)-Val-Val-Pro- HN  **24**	0.84
9	Boc-Asp(*t*-Bu)-Glu(*t*-Bu)-Val-Val-Pro- HN  **25**	>30
10	Boc-Asp(*t*-Bu)-Glu(*t*-Bu)-Val-Val-Pro- HN  **26**	>30

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14. The inhibition of HCV protease activity was determined using a modification of the reported method (Taliani et al. *Anal. Biochem.* **1996**, *240*, 60). The assay measures the increase in fluoresence on cleavage by the catalytic domain of type 1A HCV protease of a self quenching depsi-peptide substrate. Compounds were serially diluted in buffer, then preincubated with 4 nM enzyme for 30 min at room temperature in white 96 well plates. After addition of substrate, fluoresence was measured at 5 min intervals for 40 min. (Ex = 360 nm/Em = 530 nm).