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# Capped Dipeptide α-Ketoacid Inhibitors of the HCV NS3 Protease

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**Abstract**—The N-terminal aminoacid of α-ketotripeptide inhibitors of the hepatitis C virus NS3 protease can be replaced with an α-hydroxy acid, leading to capped dipeptide inhibitors such as **20** with an $IC_{50}$ value of 3.0 μM. The importance of the lipophilic side chain interactions at S3 of the protease and the requirement of the capping residue with R configuration have been explained by molecular modeling studies.


With 200–300 million people[1] in the world believed to be affected, and no safe and generally effective therapy or vaccine presently available, hepatitis C is a critically important target for the biomedical community. In the US alone, some 10,000 persons die each year from the dire complications of infection, a number that is expected to triple by 2010. The hepatitis C virus (HCV) was discovered more than a decade ago[2] and remains a major hurdle for drug discovery. Of several putative viral enzyme targets, the NS3 serine protease has been intensively studied because of its central role in gene-product maturation and viral reproduction. A recent important study has shown that NS3 protease is necessary for HCV replication in chimpanzee.[3]

However, clinically relevant compounds—low molecular weight, orally bioavailable, cell-penetrant entities—have yet to be identified. The reason can be found in the unique structural characteristics of the target itself, the NS3/NS4 protease/co-factor complex: the featureless and highly polar substrate binding region underlies the need for unusually large, polyanionic decapeptide substrate molecules which make NS3 particularly challenging for small molecule drug design.

Contrariwise, the medical and scientific pay-off for success is clear.

We have attempted to utilize the original polycarboxy hexapeptide inhibitors[4] as a starting point for developing low molecular weight drug-like leads.

Recently we reported the evolution of potent tripeptides α-ketoacid inhibitors of HCV NS3 serine protease.[5] The strategy was predicated upon two primary discoveries in a series of hexapeptide inhibitors: (i) that 4,4-difluoro-2-aminobutyric acid (difluoroAbu) is an excellent and chemically inert P1 replacement for cysteine in NS3 protease inhibitors;[6] (ii) that the α-ketoacid is a particularly effective serine trap for NS3.[7] The choice of the difluoroAbu/α-ketoacid fragment as an anchor enabled the development of the tripeptide series (e.g., **1**, $IC_{50}$ 0.38 μM) from our initial hexapeptide lead[5] (Fig. 1).

A thorough appreciation of the molecular interactions underlying the effectiveness of the difluoroAbu/α-ketoacid anchor has emerged from crystal structure analysis of two protease/cofactor/inhibitor ternary complexes.[8]

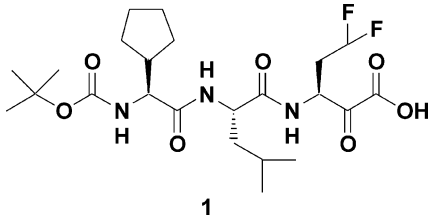


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**Figure 1.** Tripeptide lead ($IC_{50}$ 0.38 μM).

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As a next step towards more drug-like (i.e., less peptidic) compounds, we examined the possibility to replace the N-terminal aminoacid with a different residue. In exploratory work (Table 1) based essentially on privileged structures[9] we found activity only with compounds **8** ($IC_{50}=18$ μM) and **9** ($IC_{50}=7$ μM) in which the hydrophobic interaction with S3 of the protease is provided by a mandelic ether or thioether moiety.

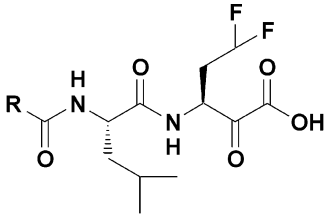
Molecular modeling based on analogy to other serine proteases predicted a canonical double hydrogen bonding interaction between Ala-157 at S3 of the protease and the P3 aminoacid of the inhibitor (Fig. 2). In addition, hydrophobic interactions between the S3 surface (Cys-159, Val-132) and the inhibitor P3 side chain were considered to be crucial for effective binding.

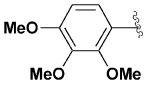
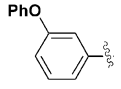
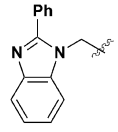
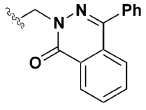
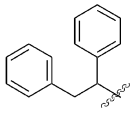
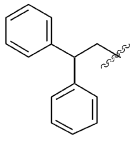
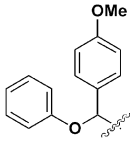
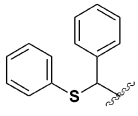
Accordingly, our first selection of a P3 aminoacid replacement, was a lipophilic α-hydroxy acid which we believed could simultaneously fulfill the hydrogen bonding and hydrophobicity requirements.

In analogy to α-aminoacids, we believed the conformation around the bond alpha to the carbonyl allows the hydrogen bonding requirement to be fulfilled and projects the P3 side-chain towards the S3 surface of the protease.

Based on this assumption, we assumed the *S*-isomer **10** of the alcohols to be the active isomer as depicted in Figure 3.

**Table 1.** Privileged structures derivatives as inhibitors of NS3/4A protease and $IC_{50}$



| Compd | R | $IC_{50}$, μM |
|---|---|---|
| **2** |  | na[a] |
| **3** |  | na[a] |
| **4** |  | na[a] |
| **5** |  | na[a] |
| **6** |  | > 30 |
| **7** |  | na[a] |
| **8** |  | 18[b] |
| **9** |  | 7[b] |

[a]na, Means $IC_{50} > 200$ μM.
[b]Only one of the two diastereoisomers is active.

## Synthesis

The synthesis of the capped dipeptide **19** from the previously reported intermediate **11**[5] is shown in Scheme 1. Condensation of intermediate **11** with (*R*)-hexahydromandelic acid **12** was followed by ozonolysis and quenching of the intermediate cyanoketone with MeOH to give the corresponding ketoester, which was then hydrolyzed to the desired ketoacid **19**.[10]

All crude acids were purified by HPLC; when it was necessary, the four diastereoisomers, resulting from the

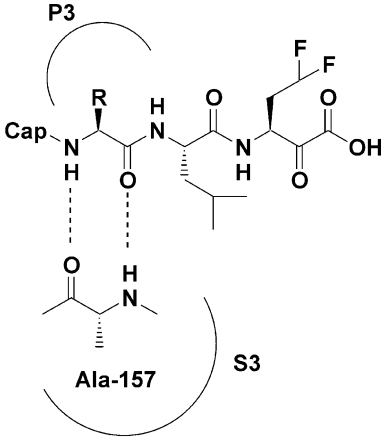
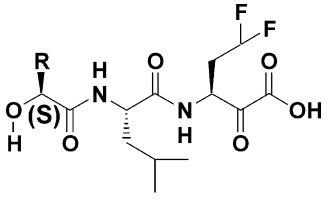



**Figure 2.** Canonical double hydrogen bonding between Ala 157 at S3 of the protease and P3 aminoacid of the inhibitor.



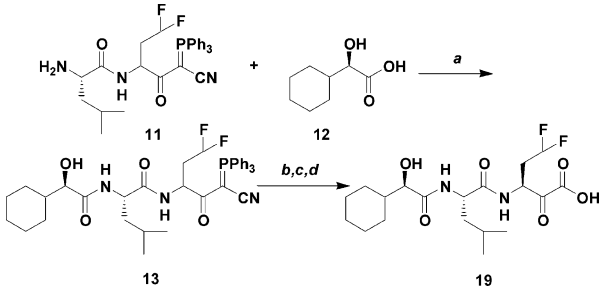
**10**

**Figure 3.** Predicted active conformation and active isomer.

use of racemic difluoroAbu and racemic mandelic acids, were separated by RP-HPLC as well.

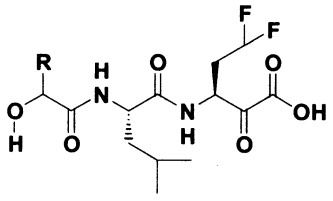
The more active diastereoisomer always eluted first and was later shown by use of chiral fragments, such as (*R*) or (*S*) mandelic acid and (*R*) hexahydromandelic acid, to have the *R* configuration at the capping residue.

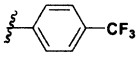
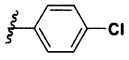
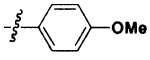
The L-configuration at P1 was in accord with the results from tripeptide work and recently published structures of enzyme-inhibitor complexes.[8]




**Scheme 1.** (a) WSCDI, HOBt, DCM (74%); (b) $O_3$, DCM, MeOH, −78 °C (64%); (c) MeOH, 1 N NaOH (69%); (d) RP-HPLC.

**Table 2.** SAR of capped dipeptide ketoacids



| Compd | R | P3 conf. | $IC_{50}$, μM |
|---|---|---|---|
| **14** |  | (R) | 41 |
| **15** |  | (R) | 28 |
| **16** |  | (R) | 13 |
| **17** |  | (R) | 13 |
| **18** |  | (R) | 18 |
| **19** |  | (R) | 7 |
| **20** |  | (R) | 3 |
| **21** |  | (S) | >100 |

## Results and Discussion

A selection of capped dipeptide analogues was prepared to investigate the hypothesis of replacing an amide or a carbamate by an alcohol, wherein the lipophilic substituents R and the stereochemistry of the alcohol were varied (Table 2).

Of these, certain aromatic and cyclohexyl analogues were active at the micromolar level.

Data for inhibition of the NS3/NS4A complex were obtained after 30 min incubation[11] with the inhibitor.

Comparing the two mandelic acid derivatives **20** and **21**, it was discovered that only one isomer was active. Contrary to our expectations, based on our simple hypothesis, the active isomer had the (*R*) configuration. Molecular modeling[12,13] suggests that the preferred conformation of the enzyme bound alcohols is not that depicted in Figure 3, but rather a rotamer which is favored by an intramolecular interaction between the amide N–H and the hydroxy O-atom as shown in Figure 4.

This pseudo ring conformation holds the alcohol fragment in a fixed conformation with the alcohol C-O bond antiperiplanar to the carbonyl group as opposed to the syn arrangement in the extended conformation of aminoacids.

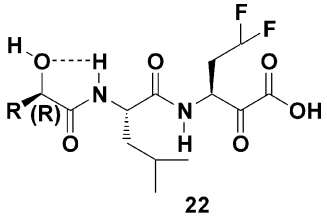



**Figure 4.** Preferred conformation of the enzyme-bound alcohol.

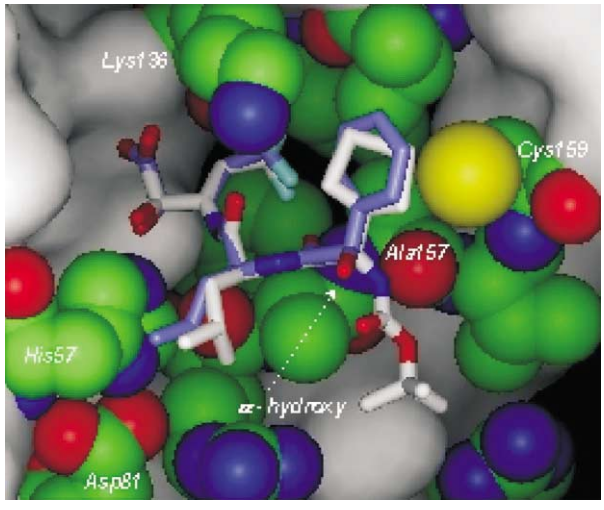



**Figure 5.** Superposition of tripeptide **1** (white) and the α-hydroxyacid **20** (blue) modeled into the active site of the NS3-protease according to the crystal structure.[8]

As a consequence the side chain in α-hydroxy acids with the *S*-configuration is not directed towards S3.

Modeling however shows for the corresponding compound with *R*-configuration (**20**) a good agreement in the side chain orientations of the α-hydroxy acid and aminoacid (Fig. 5).

In this conformation the α-hydroxy group is oriented away from the protein and can not form a hydrogen bond to Ala157 so that solvent water molecules can take the position occupied by the amino group in the aminoacids. The activity of isomer **20** underlines the importance of lipophilic interactions in S3.

In summary we have described the evolution from a N-protected tripeptide to a capped dipeptide showing that the entire P3 residue can be replaced by a small hydroxylated residue whose chirality is critical for binding recognition. Furthermore the lipophilic side chain interactions in P3 seem to be crucial for binding whereas significant activity can be achieved despite the absence of the P3 backbone hydrogen bond pair. We believe α-hydroxy carboxylic acids with a *R*-configuration to be of general interest as amino acid mimetics.

We think these results will provide an interesting stimulus towards more potent and less peptidic inhibitors of HCV NS3/NS4A Protease.

## Acknowledgements

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