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# Design and synthesis of novel fluoro amino acids: synthons for potent macrocyclic HCV NS3 protease inhibitors

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ABSTRACT

The Hepatitis C Virus (HCV) is a major health hazard and its infection is a leading cause of chronic liver disease world wide. In our efforts toward the discovery of a back up to our first clinical candidate, Boceprevir (SCH 503034), we approached the depeptidization of the molecule through macrocyclization. Herein we report the design and synthesis of fluoro amino acids with desired stereochemistry required for the synthesis of macrocyclic inhibitors with fluorine at various positions of the aliphatic chain. Biological activities of representative examples are also reported.



Around 3% of the world population is affected by Hepatitis C Virus and its infection is a leading cause of chronic liver desease.[1a–d] The current standard of care treatment is a combination of subcutaneous pegylated interferon-α with oral nucleoside drug ribavirin.[2] Due to the urgent need for a more tolerable and efficacious regimen, the last decade has seen an emergence of therapies targeting the key enzymes involved in the replication and maturation of the virus.[3] Extensive efforts have been focused on NS3-4A protease research[4] recently and a number of novel inhibitors including SCH 503034 (Fig. 1) Boceprevir[5] are reported.

Modeling studies and the X-ray crystal structure on enzyme surface for Boceprevir, the clinical candidate, revealed the proximity of P2 and P4 residues as well as the P1 and P3 residues. We envisioned the macrocyclization of these two units[6] as an effort to depeptidize the HCV protease inhibitor. Macrocyclization has been an approach widely used for depeptidization and there are reports which show that this type of modification could improve the physicochemical properties with improved Pharmacokinetics (PK).[7] Encouraged by the results from the fluorinated congener of Boceprevir[8] we decided to block the metabolic site of the aliphatic chain of the P1 or P3 with fluorine. Previous results from our group[6] showed that a 16-membered macrocycle would be optimal for P1–P3 ring. Herein we report the design and synthesis of the desired fluoro amino acids as synthons for macrocyclic inhibitors.

Retrosynthetic analysis of the 16-membered macrocycle **2** is depicted in Figure 2. Tripeptide **3** was obtained by the sequential coupling of the proline derivative **5** with the fluoro amino acid **4**, hydrolysis of the corresponding methyl ester followed by the coupling of the allyl glycine **6**. Ring-closing metathesis (RCM)[9] was used as the key step for the construction of macrocycle **2**.

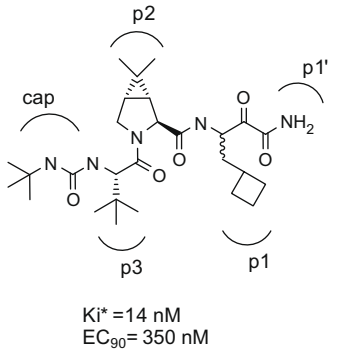


**Figure 1.** Boceprevir (SCH 503034).

The synthesis of the fluoro amino acid **4** started with the 6-oxo piperidine carboxylic acid derivative **7** (Scheme 1). Grignard addition of the butenyl magnesium bromide **8** to the piperidinone **7** resulted in the keto amino acid **9** with the desired stereochemistry. The Grignard reagent was synthesized in situ with the alkenyl bromide and magnesium turnings in THF. Keto amino acid **9** was converted to the corresponding difluoro derivative **10** with DAST. We were interested in investigating the effect of fluorine at both P1 and P3 units of the macrocycle. Difluoroamino acid **10** was used as the synthon for both 10,10-difluoromacrocycle and 12,12-difluoromacrocycle at P3 and P1, respectively (color coded blue in Fig. 3). Boc deprotection of the amino acid **10** afforded the amine salt **11** that will be coupled with the proline derivative **5** to get the fluoro side chain at the P3 position. Ester hydrolysis of the

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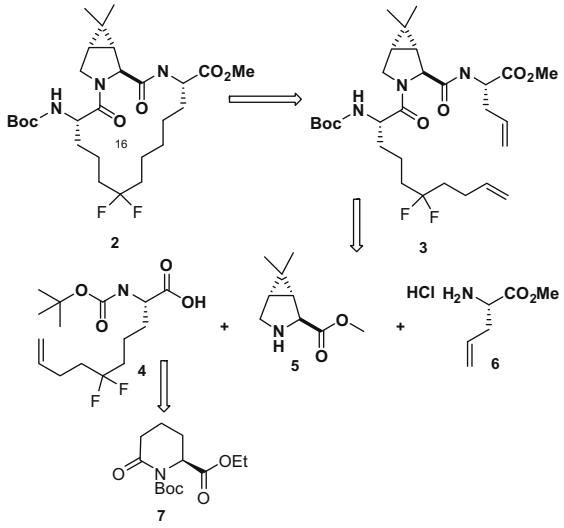
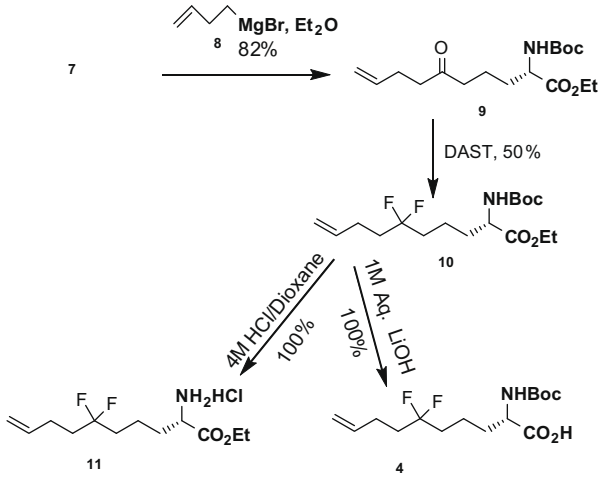


**Figure 2.** Retrosynthetic analysis of 10,10-difluoromacrocycle **2**.



**Scheme 1.** Synthesis of fluoro amino acid.

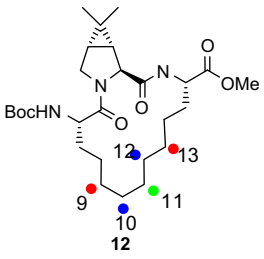
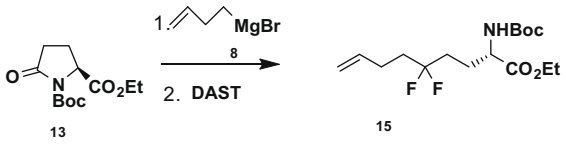


**Figure 3.** Macrocycle with color code for fluorine substitution.

amino acid **10** with LiOH resulted in the Boc-protected amino acid **4**. Incorporation of the fluorine at the desired position in the P1 unit was achieved via the coupling of the amino acid **4** with the dipeptide **25** under standard HATU conditions (Scheme 4).

In order to achieve the synthesis of macrocycles with fluorine at 9,9 and 13,13 positions (colored red in Fig. 3) we designed amino acid **15**[10] (scheme 2). Pyroglutamate derivative **13** was opened up with butenyl Grignard reagent[11] **8** as described before and the resulting keto amino acid **14** (Table 1) was fluorinated with DAST to afford **15**. Boc-protected amino acid of ester **15** or its free amino



**Scheme 2.** Synthesis of fluoro amino acid.

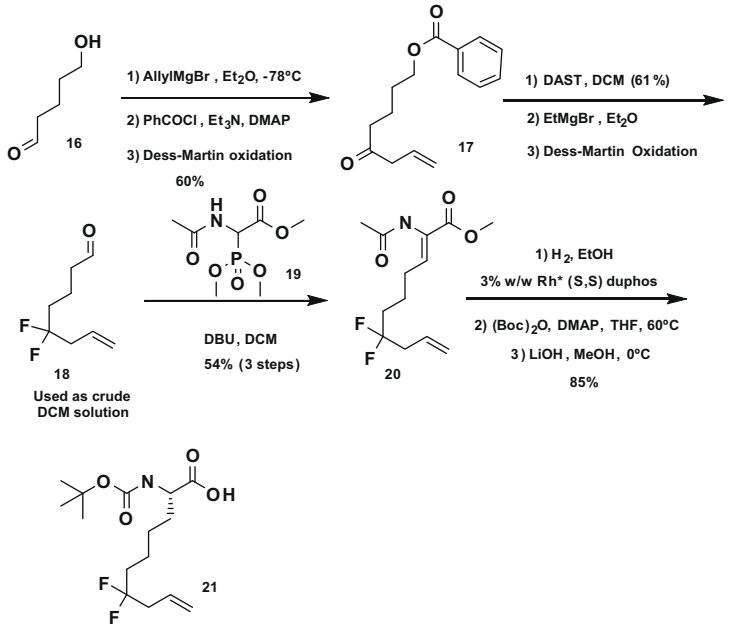
ester could also be used at both P1 and P3 areas to construct macrocycles with fluorine at 13,13 and 9,9 positions, respectively.

A unique synthesis (Scheme 3) was designed for the symmetrical fluoro analog (11,11-difluoro derivative, green colored in Fig. 3). 5-Hydroxy pentanal **16** was treated with allyl magnesium bromide to the corresponding allyl alcohol. Protection of the primary alcohol with the benzoyl group followed by oxidation of the secondary alcohol with Dess–Martin Periodinane resulted in the allyl ketone **17**. Difluorination of **17** was achieved with DAST in 61% isolated yield. Basic hydrolysis attempts of the benzyl ester under aqueous conditions were unsuccessful. Thus, the difluorocompound was further treated with ethyl magnesium bromide and the alcohol was oxidized with Dess–Martin Periodinane to get the aldehyde **18**. Aldehyde **18** was treated with the phosphonate **19** to get the unsaturated amino acid **20**. Enantioselective reduction of enamide **20** was accomplished with 8% w/w of chiral rhodium catalyst and the resulting acetamide was exchanged with Boc-protecting group. The Boc methyl ester was then hydrolyzed to the chiral amino acid **21** with LiOH in 85% combined yield in three steps.

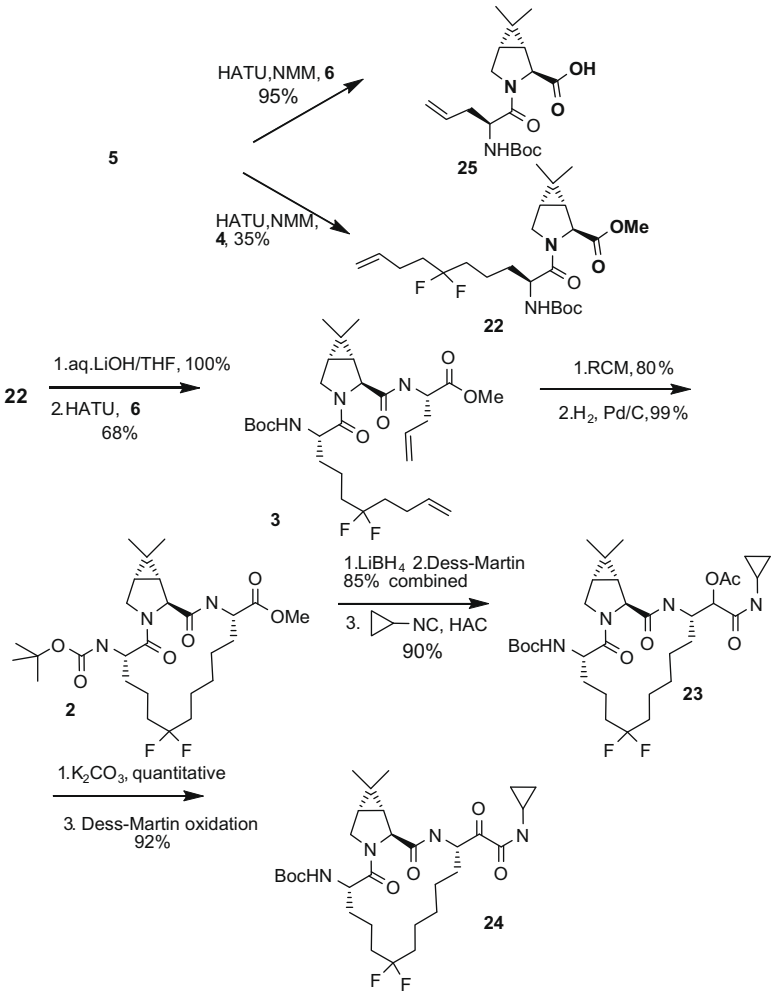
Incorporation of fluorine in the macrocycle was outlined with a representative example shown in Scheme 4. Dipeptide **22** was synthesized via a coupling of the difluoroamino acid **4** and the P2 proline methyl ester **5**. The synthesis of the P2 proline derivative **5** was reported in earlier publications.[12] The dipeptide **22** was then hydrolyzed under basic conditions to the acid which was then coupled to the allyl glycine **6** to obtain the tripeptide **3**. Although the initial attempts for the Ring-closing Metathesis with Grubbs first generation catalyst were not successful, we were able to convert the tripeptide **3** to the corresponding 16-membered macrocycle in good yield using Grubbs second generation catalyst. The resulting isomeric mixture of olefins was hydrogenated using Pd/C at 1 atm. pressure with an excellent yield to afford the methyl ester **2**. Reduction of the methyl ester **2** to the corresponding alcohol with lithium borohydride followed by oxidation with Dess–Martin periodinane to the aldehyde and the Passerini reaction with cyclopropyl isocyanide resulted in the acetyl amide **23**.[6c] Deprotection of the acetyl group followed by the oxidation of the hydroxyl group resulted in the ketoamide **24**.

Improvement in the yield of the fluorination was achieved with Deoxofluor as the fluorinating agent. One representative example is outlined in Table 1. Change of fluorinating agent from DAST to deoxofluor for ketone **14** improved the yield almost twofold. The yield for the coupling of amino acid of ester **14** with proline **5** was low (30%). Twofold betterment in the yield in two steps was achieved by reversing the sequence of coupling and fluorination. Thus, coupling of the amino acid of keto ester **14** with the proline **5** (98% isolated yield) followed by reaction of the resulting dipeptide **26** with deoxofluor afforded the corresponding difluoro dipeptide in 70% yield.

Biological activities of selected fluoromacrocycles are outlined in Table 2. All inhibitors were tested in the HCV continuous enzymatic assay[13] using the NS4A-tethered single chain NS3 serine protease.[14] The $K_i^*$ values reflected the equilibrium constant determined by the reversible covalent bond formed between the ketone and serine and other interactions between the inhibitors and the enzyme.[15] The concentration required for the inhibition of 90% of virus replication, $EC_{90}$, was obtained as a measure of replicon




**Scheme 3.** Synthesis of fluoromacrocyclic precursor for the symmetrical (11,11-difluoro) analog.




**Scheme 4.** Synthesis of fluoromacrocycles.

**Table 1**
Fluorination of amino acids

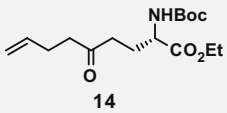
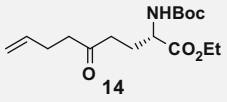
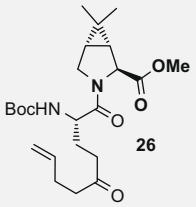
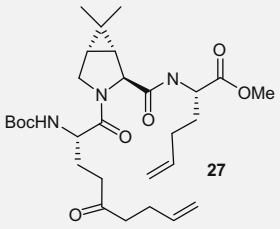
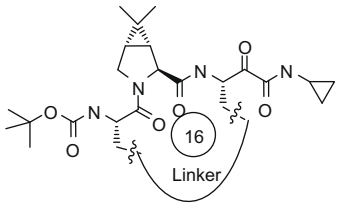
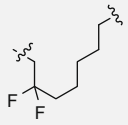
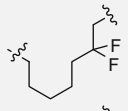
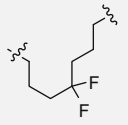
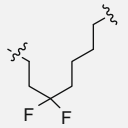
| Reagent | Ketoamino acid | Yield (%) |
|---|---|---|
| DAST |  **14** | 40 |
| Deoxo-fluor |  **14** | 75 |
| Deoxo-fluor |  **26** | 70 |
| Deoxo-fluor |  **27** | 50 |

**Table 2**
Biological activity of fluorinated macrocycles



| Compound | Linker | $K_i^*$ (μM) | $EC_{90}$ (μM) |
|---|---|---|---|
| **28** |  | 0.096 | 0.30 |
| **29** |  | 0.42 | 0.80 |
| **30** |  | 0.6 | NA |
| **24** |  | 0.4 | 0.900 |

cellular potency.[16] Inhibitors were tested for the activity against one of the most structurally closely related serine protease, human neutrophil elastase (HNE) to determine the selectivity between HCV and HNE.

In conclusion, we have designed and accomplished the syntheses of new fluoroamino acids in such a way as to have the difluoro-substitution at various desired positions of the macrocycles, at both the P3 and the P1 positions. Macrocyclization of the fluoro olefins were achieved via Grubbs Ring-closing metathesis using second generation catalyst. These macrocycles are biologically active against the HCV NS3 serine protease.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.010.

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