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Toward the Synthesis of Fluorinated Analogues of HCV NS3/4A Serine Protease Inhibitors Using Methyl α-Amino-β-fluoro-β-vinylcyclopropanecarboxylate as Key Intermediate

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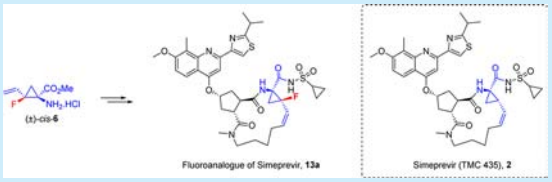
Supporting Information

ABSTRACT: Synthesis of fluorocyclopropyl building blocks, which constitute the core of various therapeutic agents against the hepatitis C virus, is described. The relevant methyl α-amino-β-fluoro-β-vinylcyclopropanecarboxylate has been used as a key intermediate for the total synthesis of a fluorinated analogue of Simeprevir (TMC 435), a HCV NS3/4A protease inhibitor.



The hepatitis C virus (HCV) is the leading cause of chronic liver diseases (liver fibrosis, cirrhosis, and hepatocellular carcinoma), afflicting over 3% of the world population.[1] Until recently, the most effective therapy has been based upon the use of PEGylated interferon-α in combination with the antiviral drug ribavirin.[2] Unfortunately, approximately 50% of genotype 1 infected patients respond with a sustained virological response, and the treatment is poorly tolerated.[3] Hence, the limited efficacy of the interferon-based therapy highlights the unmet medical need for more convenient therapeutics against this emerging infection.

In the past decades, the NS3/4A protease, which is an essential protein involved in the virus replication process,[4] became an unavoidable drug target for anti-HCV therapy through the proven antiviral effect in humans of the macrocyclic noncovalent inhibitor **1** (BILN 2061). Macrocycle **1**, discovered by Boehringer Ingelheim, displays a nanomolar activity against the NS3/4A protease (IC_{50} = 3 nM).[5] To date, this class of macrocylic peptidomimetics has been extensively explored by medicinal research programs.[6] Recent approvals of HCV protease inhibitors (Boceprevir, Telaprevir, and Simeprevir) and the NS5B RNA-dependent RNA polymerase inhibitor Sofosbuvir represent a major step forward. Among them, numerous new efficient therapeutic agents bear a cyclopropyl ring in their structural core (Figure 1).[7]

Indeed, these potent inhibitors are characterized by a 1-amino-1-carboxyl-2-vinylcyclopropane core, an essential subunit exhibiting an optimal fit in the hydrophobic S1 pocket of the NS3 protease.[8]

As part of our ongoing research program dedicated to the synthesis of functionalized monofluorocyclopropanes,[9] we decided to explore whether this valuable scaffold could be incorporated to modulate the pharmaceutical properties of such compounds. Indeed, the replacement of a hydrogen atom by a fluorine atom is a common and promising strategy in medicinal chemistry to alter the pharmacological properties of bioactive compounds such as solubility, lipophilicity, metabolic stability, and binding properties.[10] Moreover, the incorporation of a fluorine atom in a cyclopropane unit of biomolecules already proved its relevancy for the design of more powerful therapeutic agents.[9c,e]

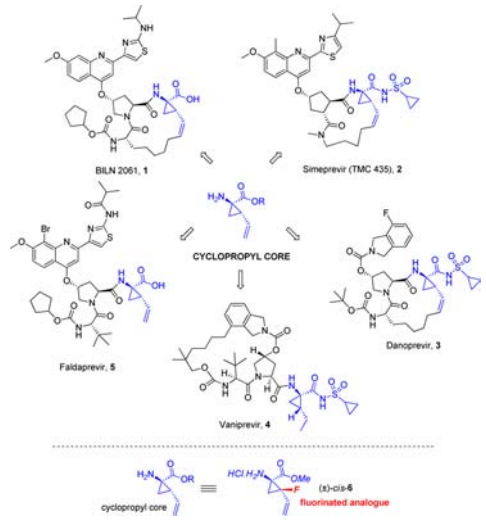


Figure 1. Inhibitors of the HCV NS3/4A serine protease.

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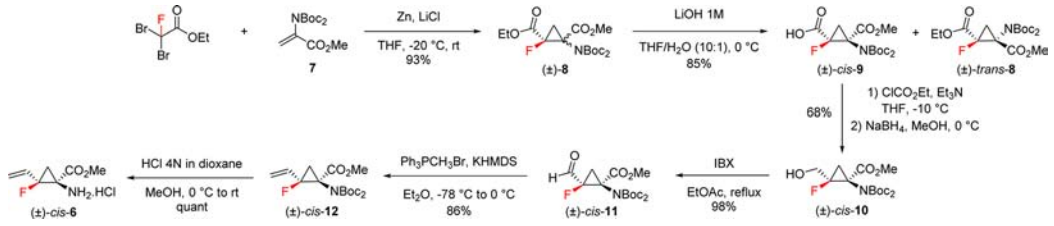
Scheme 1. Synthesis of the Fluorinated Vinylcyclopropane (±)-*cis*-6



Herein, we report a straightforward synthesis of the fluorinated analogue of the cyclopropyl core: methyl α-amino-β-fluoro-β-vinylcyclopropanecarboxylate building block (±)-*cis*-**6**. We report the total synthesis of a fluorinated analogue of **2** (Simeprevir, TMC 435), developed by Janssen Pharmaceutica, which was recently approved by the FDA (Figure 1),[11] from (±)-*cis*-**6**.

Fluorinated (±)-*cis*-**6** was prepared as outlined in Scheme 1. Recently, we developed a straightforward cyclopropanation process from commercially available ethyl dibromofluoroacetate and electron-deficient alkenes using Zn/LiCl combination.[9d] This methodology was successfully applied to the protected aminoacrylate **7** to generate the cyclopropane diester (±)-**8** as a mixture of diastereoisomers[12] (dr *cis*/*trans* = 59:41) in 93% yield on 0.1 mol scale. Subsequently, a diastereoselective and regioselective hydrolysis of the ethyl ester (±)-**8** at low temperature[13] provided after acid/base extraction the carboxylic acid (±)-*cis*-**9** along with the diester (±)-*trans*-**8** as highly enriched diastereoisomers. Primary alcohol (±)-*cis*-**10** was delivered from acid (±)-*cis*-**9** in 68% yield through mixed anhydride formation and subsequent reduction. Oxidation of (±)-*cis*-**10** with IBX gave quantitatively the corresponding aldehyde (±)-*cis*-**11**.[14] Then, (±)-*cis*-**11** was converted to the corresponding alkene (±)-*cis*-**12** in 86% yield via a Wittig reaction using triphenylmethylphosphonium bromide. Finally, removal of the *N*,*N*-(di-*tert*-butyloxycarbonyl) protecting group upon treatment with 4M HCl in dioxane led to the hydrochloride salt (±)-*cis*-**6** in a quantitative yield.

With these different fluorocyclopropyl building blocks in hand, we could have access to numerous fluorinated analogues of potent inhibitors of NS3/4A protease, as those described in Figure 1. Among them, in partnership with Janssen Research & Developement, we turned our attention toward the total synthesis of fluorinated analogue **13** of the Simeprevir **2** through a convergent strategy using four key building blocks, including the highly functionalized fluorinated cyclopropane (±)-*cis*-**6** (Figure 2). To our knowledge, a multistep synthesis based on a fluorinated vinylcyclopropane scaffold has never been reported in the literature. The retrosynthetic analysis of the corresponding fluorinated analogue **13** of Simeprevir **2** suggested that the cyclopropanesulfonamide **14** could be introduced by an amidation reaction. We envisioned that the introduction of the quinoline residue **15** onto the cyclopentyl alcohol **16** could be achieved by a Mitsunobu reaction. Finally, the 14-membered ring could be fashioned from the fluorinated cyclopropyl amino acid (±)-*cis*-**6** and the building block **16** by a ring-closing metathesis (RCM) and a peptide coupling (Figure 2).

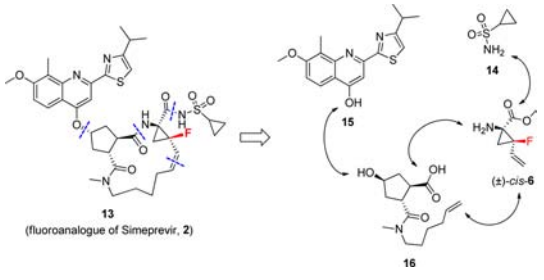


Figure 2. Retrosynthesis of the fluorinated analogue **13** of Simeprevir **2**.

Total synthesis of compound **13** is depicted in Scheme 2. We first achieved the peptide coupling reaction between (±)-*cis*-**6** and **16**[15,16] to obtain the crucial intermediate **17**. However, activation of the acid function of **16** with HBTU and *N*-methylmorpholine at 0 °C for 30 min, followed by the introduction of the amine (±)-*cis*-**6**, did not afford the expected product **17** due to two main side reactions: cyclopropane ring opening[17] from the conjugation of the free amine nitrogen lone pair in basic conditions with the double bond through the cyclopropane moiety and intramolecular cyclization in **16** between the alcohol and carboxylic acid functions in *cis* configuration, furnishing the corresponding lactone (see Supporting Information for more details). To prevent these side reactions, we introduced simultaneous fragments **16** and (±)-*cis*-**6**, along with HATU and *N*-methylmorpholine, at lower temperature (−15 °C), giving **17** as a mixture of two diastereoisomers (dr = 1:1) with a good 74% yield. Then, quinoline **15**[18] and intermediate **17** were connected via a Mitsunobu reaction using diisopropylazodicarboxylate and triphenylphosphine, giving compound **18** in 72% yield (dr = 1:1) (Scheme 2). To construct the macrocycle by RCM of the olefin, after optimization of reaction parameters, we found out that the macrocycle **19** could be obtained in 47% yield using Hoveyda−Grubbs II catalyst in 1,2-dichloroethane at 100 °C. Interestingly, each diastereoisomer gave rise to only one configuration of the macrocyclic double bond. These two diastereoisomeric products were efficiently separated by supercritical fluid chromatography to provide (7*R*,9*S*)-**19** and (7*S*,9*R*)-**19**.[19] The methyl ester function of intermediates **19** was subsequently hydrolyzed using lithium hydroxide at room temperature to provide the corresponding carboxylic acids (7*R*,9*S*)-**20** and (7*S*,9*R*)-**20** in quantitative and 82% yields, respectively.

To determine the configuration of the macrocyclic double bond, the two isomers of **20** were analyzed by NMR. The scalar coupling constant between the olefinic protons H11 and H12 (Figure 3) was measured on the ^{1}H NMR spectrum of each isomer. The observed values showed that the configuration of the newly formed double bond differs from one isomer to the other. Indeed, a large coupling constant of 15.9 Hz, characteristic of a $^3J_{trans}$, was assigned to (11,12*E*)-*cis*-**20**. A smaller one (12 Hz) was observed for (11,12*Z*)-*cis*-**20**. These conclusions were confirmed by ^{1}H−^{1}H NOESY. A significantly stronger NOE was observed between H11 and H12 in (11,12*Z*)-*cis*-**20** than in (11,12*E*)-*cis*-**20**, indicating that these two protons are closer in (11,12*Z*)-*cis*-**20** than in (11,12*E*)-*cis*-**20**. Finally, 1D ^{1}H−^{19}F HOESY

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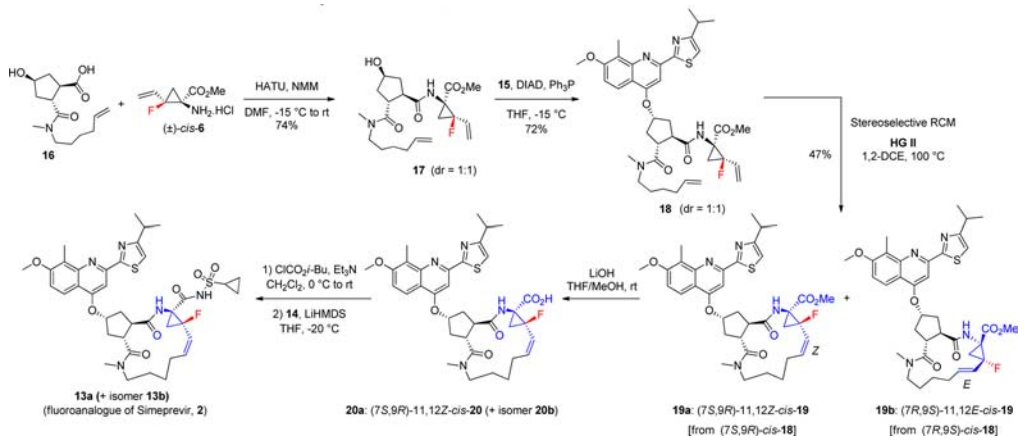
Scheme 2. Synthesis of the Fluoroanalogues 13 of Simeprevir



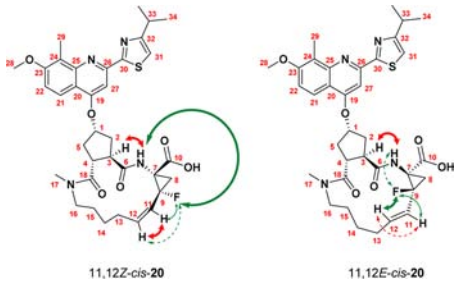


Figure 3. NOE/HOE observed for the both isomers of compound **20**. $^{1}H-^{1}H$ NOE and $^{1}H-^{19}F$ HOE are represented in red and green, respectively. Bold plain arrows, thin plain arrows, and dashed arrows are for intense, medium, and weak NOE/HOE, respectively.

(heteronuclear Overhauser effect spectroscopy) spectra were recorded for each isomer. In (11,12*Z*)-*cis*-**20**, strong F−H11 HOE and weak F−H12 HOE were observed, which are consistent with a double bond in *Z* configuration. On the contrary, in (11,12*E*)-*cis*-**20**, the strong HOE observed between F and H12 is in favor of the configuration shown in Figure 3.

Molecular modeling was necessary, in addition to NOESY and HOESY NMR analysis, to determine the absolute configuration of C7 and C9 of the fluorinated cyclopropyl. As the chosen chemical synthesis pathway was expected to lead only to compounds (11,12*E*)-*cis*-**20** and (11,12*Z*)-*cis*-**20**, these two stereoisomers were studied in silico using SYBYL 7.0 (Tripos. Inc., St. Louis, USA; see Supporting Information for more details). Our complementary studies of molecular modeling and NMR experiments allowed us to identify the two products synthesized as (7*S*,9*R*)-(11,12*Z*)-*cis*-**20** and (7*R*,9*S*)-(11,12*E*)-*cis*-**20**. The ^{19}F chemical shift difference observed in the two compounds (−163 ppm for (7*S*,9*R*)-(11,12*Z*)-*cis*-**20** and −187 ppm for (7*R*,9*S*)-(11,12*E*)-*cis*-**20**) confirms different chemical environments for the fluorine atom in each compound, probably linked to the position of the cyclopropyl moiety.

The RCM reaction turned out to be stereoselective, that is, (7*S*,9*R*)-*cis*-**18** giving only the *Z* geometry of a double bond (**19a**) with the (7*R*,9*S*)-*cis*-**18** enantiomer leading to the *E* stereoisomer **19b** (Scheme 2).

To end our synthesis sequence, we activated carboxylic acid moiety **20** with 1,1′-carbodiimidazole in refluxing THF for 90 min,[19] followed by introduction of the cyclopropylsulfonamide **14** in the presence of DBU at 50 °C, but this led to substrate decomposition. Treatment of **20a** or **20b** with HATU and DIEA in DMF at 0 °C for 30 min and subsequent introduction of **14** in the presence of DMAP and DBU was also unsuccessful even if this procedure proved to be efficient for the peptide coupling reaction of a vinylcyclopropyl amino acid.[20] Finally, activation of **20a** and **20b** via a mixed anhydride strategy at 0 °C for 18 h, followed by reaction with the previously deprotonated cyclopropylsulfonamide **14** by a LiHMDS solution,[21] afforded the final targets (7*S*,9*R*)-(11,12*Z*)-*cis*-**13** (**13a**) and (7*R*,9*S*)-(11,12*E*)-*cis*-**13** (**13b**) in 13[22] and 83% yield, respectively (Scheme 2).

Both **13a**, which is the exact analogue of Simeprevir, and **13b** were then submitted to biological assay to compare their potency as anti-HCV agent with Simeprevir **2** (Table 1).[11b]

Table 1. Antiviral Activity

compound	Huh7-RepEC_{50}[a]
2	8.1 nM
13a	15 μM
13b	>25 μM

[a]50% effective concentration (EC_{50}) was determined in the Huh-7 replicon cell line containing the subgenomic bicistronic HCV genotype 1b replicon clone ET and luciferase readout.

Antiviral activity of fluoroanalogues was low compared to that of Simeprevir. Analysis of the crystal structure of the TMC435 bound to its NS3/4A protease target[8] shows a particular orientation of the macrocycle to promote the establishment of a hydrogen bond between the inhibitor and the protease. A particular orientation of the cyclopropane also allows the positioning of the acylsulfonamide group in the area S1′. Our 3D models obtained by molecular modeling for compounds **20a** and **20b** show a slight rotation of P1−P2 *backbone* peptide, which could harm the electrostatic interactions (with Lys136:NZ) and intermolecular hydrogen bond (with Arg155:O).[23] Moreover, the orientation of the fluorocyclopropane in our two model compounds seems unfavorable for positioning the acylsulfonamide group in the region of the catalytic serine residue (Ser139) and also causes steric hindrance with the lysine residue (Lys136) and the possible loss of hydrogen bonding with Gly137:NH.[23] These structural changes were only caused by the fluorine atom in **13a**, probably due to orbital interactions between fluorine and oxygen of the macrocycle. In the case of **13b**, the changes were caused by the fluorine atom and the double bond configuration. These structural modifications, in both cases, should hamper the

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interactions between inhibitor and target, explaining the loss of activity of the two fluoroanalogue compounds.[24]

In conclusion, we developed an efficient synthesis of versatile fluorinated cyclopropane amino acid building blocks that can be used in the synthesis of promising new HCV NS3/4A protease inhibitors, therapeutic agents against the hepatitis C virus. We showed that these fluorinated scaffolds could be used efficiently in the total synthesis of the Simeprevir analogue. The antiviral activity of the fluoroanalogues was quite low, probably because the fluorine atom induced some modification in the structure of the macrocycle.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedure, characterization data, computational data, and copies of ^{1}H, ^{13}C, and ^{19}F NMR spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01216.

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Notes

The authors declare no competing financial interest.

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(22) Reaction not optimized, carried out only one time.

(23) See Supporting Information for more details.

(24) As requested by one of the reviewers, log *D* and log *P* of Simeprevir **2** and **13a** were calculated (Stardrp software, Optibrium). The following values were obtained: log *D* = 3.40 (**2**); 3.54 (**13a**); log *P* = 4.45 (**2**); 4.34 (**13a**).

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