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Hepatitis C virus NS3-4A serine protease inhibitors: SAR of new P1 derivatives of SCH 503034

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ABSTRACT

Substitutions on the P_1 cyclobutyl side chain of **SCH 503034** were studied by introduction of hydroxyl and fluoro substituents. Additionally, effects of fluoro substitution on other P1 moieties were evaluated.

Hepatitis C is the most prevalent liver disease. Viral hepatitis C (HCV), a small (+)-RNA virus, infects chronically an estimated 300 million people worldwide. Untreated HCV infections can progress to cirrhosis, hepatocellular carcinoma, and liver failure, a primary cause for liver transplantation. Subcutaneous and intramuscular injections of α-interferons (immune system booster) have been used since the late 1980s in the treatment of chronic hepatitis C. Currently, PEGylated-interferon in combination with antiviral drug REBETOL® (Ribavirin, USP) has brought the rate of long-term viral clearance (or sustained virological response) from <5% to approximately 50% in patients infected with the genotype 1. Although use of α-interferons is an integral component in the management of chronic hepatitis C infection, major adverse events can occur and subsequently result in dose reduction or discontinuation of treatment. Thus, several research groups have been working toward the development of a more effective, convenient, and tolerable treatment.[1]

Upon entering a liver cell, the hepatitis C virus dissociates liberating the viral RNA genome. The HCV genome serves as a template for cap-independent translation through an internal ribosome entry site (IRES) located in the 5′-untranslated region. The resulting polyprotein undergoes both co- and post-translational proteolytic maturation by host and virally encoded proteases. The NS3 serine protease, a pivotal enzyme required for maturation of hepatitis C virons, assists in processing of the HCV polyprotein by cleaving four downstream sites. Because of its central role in viral replication,[2] inhibition of HCV NS3 serine protease has been actively pursued as target for antiviral therapy.[3] Clinical candidates, **BILN 2061**, **VX-950**, and **SCH 503034** (Fig. 1), established proof of concept in human studies.[4]

During the SAR studies that led to the discovery of **SCH 503034**, cyclopropyl and cyclobutyl alanine were identified as the best P1 surrogates.[5] In an effort to further explore the P1 moiety, we were interested in introducing substituents on the cyclobutyl ring with the aim of blocking potential metabolic sites and thus improving the pharmacokinetic profile. Because of the interesting profile of a compound with fluoro-substituted P1 discovered earlier,[5] we planned to investigate fluoro-containing P1 entities that might enhance the overall profile of the resulting inhibitor. Herein, we report the results of our P1 exploration study.

Syntheses of the modified hydroxyl amide P1 fragments required for inhibitors **43**–**47**, **49**, and **51** (Table 2) were accomplished using Sharpless aminohydroxylation reaction.[6] Preparation

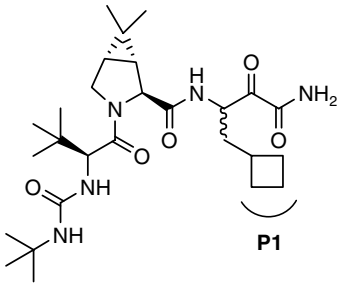

Figure 1. Structure of **SCH 503034.**

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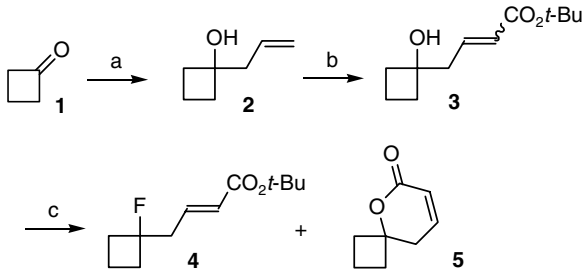
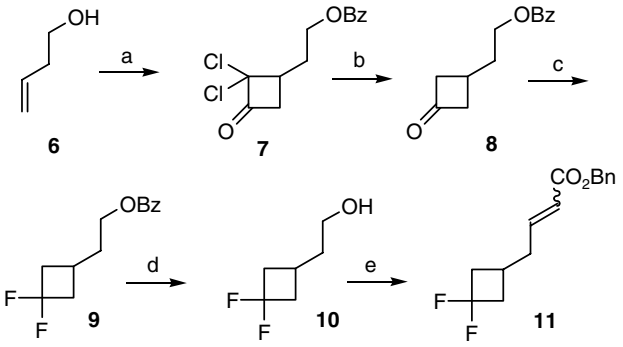
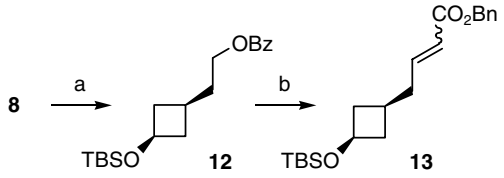


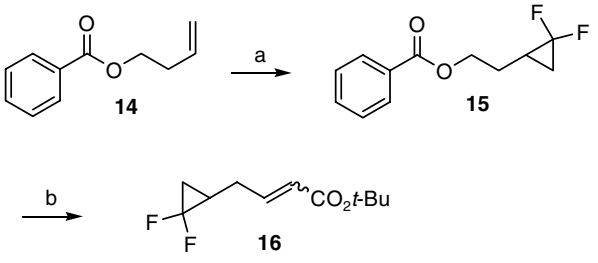
Scheme 1. Reagents and conditions: (a) AllylMgCl, THF, 0 °C; (b) O_3, DCM, Ph_3P=$CHCO_2t$-Bu, DCM (40% over three steps, *trans*/*cis*: 78/22); (c) DAST, DCM, 0 °C, 95% (based on trans isomer).



Scheme 2. Reagents and conditions: (a) i—Benzoyl chloride, Et_3N, CH_2Cl_2 (100%); ii—Trichloroacetyl chloride, Zn, Et_2O/DME (70%); (b) Zn, AcOH, 60 °C (90%); (c) DAST, CH_2Cl_2 (79%); (d) K_2CO_3, MeOH/THF/water; (e) Ph_3P=$CHCO_2Bn$, Dess–Martin's periodinane, CH_2Cl_2/DMSO (59% for three steps).

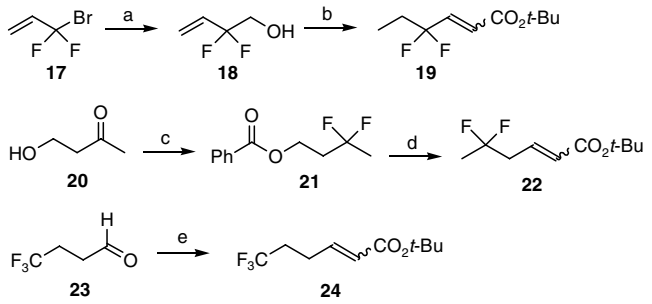


Scheme 3. Reagents and conditions: (a) i—L-Selectride, THF, −78 to 0 °C; ii—TBSCl, imidazole, DMAP (cat.), CH_2Cl_2 (88% for two steps); (b) Steps d and e as shown in Scheme 2.



Scheme 4. Reagents and conditions: (a) $FSO_2CF_2CO_2SiMe_3$, NaF, Toluene, 105 °C (73%); (b) i—EtMgBr, Et_2O, 0 °C; ii—Dess–Martin's periodinane, Ph_3P=$CHCO_2t$-Bu, DCM (92%)

of the α,β-unsaturated *tert*-butyl or benzyl ester precursors are described in Schemes 1–5. The 4-hydroxyl and 4-fluoro cyclobutyl moieties **3** and **4** were prepared as follows (Scheme 1). Allyl alcohol **2** was prepared by addition of allylmagnesium chloride to cyclobutanone **1**. Ozonolysis of the allyl moiety generated the corresponding aldehyde that was converted in situ to α,β-unsaturated



Scheme 5. Reagents and conditions: (a) In(0) (1.4 equiv), paraformaldehyde (10 equiv), LiI (10 mol %), DMF (100%), (b) i—H_2, 10% Pd/C, EtOAc (95%); ii—Dess–Martin's periodinane, DCM, 1 h, then Ph_3P=$CHCO_2t$-Bu, 16 h (76%); (c) i—PhCOCl, DCM, Et_3N, DMAP (cat.); (ii) DAST, DCM (50% two steps); (d) i—EtMgBr; ii—Dess–Martin's periodinane, Ph_3P=$CHCO_2t$-Bu, DCM (57%); (e) Ph_3P=$CHCO_2t$-Bu, DCM, (95%).

tert-butyl ester **3** via the Wittig reaction.[7] Fluorination of the 4-position was efficiently accomplished by subjecting alcohol **3** to DAST. Under these conditions, the hydroxyl of *cis*-olefin **3** cyclized readily to form the lactone **5**, whereas the trans isomer provided exclusively the desired fluorinated intermediate **4**.

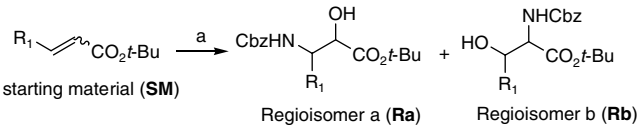
Synthesis of the Sharpless aminohydroxylation precursor **11** for 3,3-difluorocyclobutyl alanine derivative is shown in Scheme 2. Thus, commercially available butenol **6** was protected as the benzoate derivative and subjected to [2+2] cycloaddition with dichloroketene (prepared in situ from trichloroacetyl chloride and activated zinc) to provide the dichlorocyclobutanone derivative **7**.[8] Dechlorination with zinc and acetic acid afforded the cyclobutanone intermediate **8**. DAST treatment of **8** resulted in difluoro intermediate **9**. Removal of the benzoate functionality, followed by oxidation and Wittig reaction (in one pot) furnished the required α,β-unsaturated ester **11**.

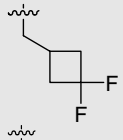
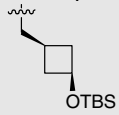
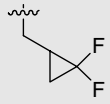
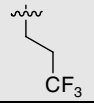
Preparation of the 3-hydroxycyclobutyl alanine precursor **13** is described in Scheme 3. Reduction of previously described cyclobutanone derivative **8** with L-Selectride essentially afforded the *cis*-alcohol[9] that was protected as the TBS-ether, **12**. Conversion of **12** to the α,β-unsaturated ester **13** was accomplished using procedures described above.

Preparation of the 2,2-difluorocyclopropyl alanine precursor was accomplished as shown in Scheme 4. Difluorocyclopropanation usually required the use of chlorodifluoroacetic acid sodium salt in diglyme at very high temperature.[10] Trimethylsilylfluorosulfonyl difluoroacetate[11] (TFDA), reported as a highly versatile source of difluorocarbene, was successfully used for the difluorocyclopropanation of benzoate **14**, and provided **15** in 73% yield. Hydrolysis of the benzoate ester with LiOH or KOH was unsuccessful, but was performed in a quantitative manner using ethylmagnesium bromide in Et_2O. The resulting alcohol was oxidized and converted in situ to *tert*-butyl ester **16** according to the aforementioned procedures.

Preparation of the Sharpless aminohydroxylation precursors **19**, **22**, and **24** for β-difluoro, γ-difluoro and trifluoro norvaline derivatives is shown in Scheme 5. Thus, 3-bromo-3,3-difluoropropene **17** was reacted with paraformaldehyde and stoichiometric amount of Indium(0) in DMF to generate alcohol **18** in very high yield.[12] The olefin of **18** was hydrogenated using Pd/C in EtOAc, and the alcohol was oxidized and converted in situ to *tert*-butyl ester **19** according to procedures described earlier. Benzoylation of 4-hydroxy-2-butanone followed by DAST treatment furnished the desired difluoro moiety **21** in 50% yield. After removal of the benzoate ester, the alcohol was processed as described above to provide **22**. Commercially available trifluoro-butyraldehyde **23** was used to generate *tert*-butyl ester **24**.

Table 1
Regioselectivity of Sharpless aminohydroxylation reaction



SM	R_1	R_a	R_b	Ratio R_a:R_b	Yield (%)
3		**3a**	**3b**	55:45	54
4		**4a**	**4b**	55:45	63
11[a]		**11a**	**11b**	100:0	28
13[a]		**13a**	**13b**	100:0	28
16		**16a**	**16b**	100:0	39
19		**19a**	**19b**	40:60	22
22		**22a**	**22b**	50:50	42
24		**24a**	**24b**	100:0	34

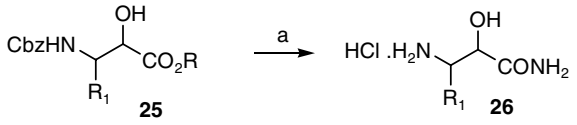
Reagents and conditions: (a) $CbzNH_2$ (3 equiv), $(DHQ)_2PHAL$ (0.05 equiv), $K_2OsO_2(OH)_4$ (0.04 equiv), *t*-BuOCl (3.05 equiv), NaOH (3.05 equiv), *n*PrOH/H_2O (22–63%).
[a] Benzyl ester was used instead of *t*-Bu ester.

Sharpless aminohydroxylation reaction rapidly provided the desired hydroxyl ester moieties. Interestingly, we noticed that the regioselectivity outcome of the reaction depended on the nature of the substrate. As highlighted in Table 1, regioselectivity was poor when the hydroxyl or fluorine substitution was closer to the olefin moiety. The undesired regioisomer (**R_b**, Table 1) was predominantly formed when ester **19**, bearing a difluoro substituion α to the olefin, was used as starting material. When the fluorine substitution was moved one carbon away to the β position, regioselectivity was improved with both regioisomers isolated in approximately 1/1 ratio (**4** and **22**, Table 1). Similar outcome was observed for ester **3** having hydroxyl moiety β to the olefin. Ultimately, we found out that the reaction was totally regioselective favoring the desired regioisomer (**R_a**, Table 1) when the α,β-unsaturated esters had the substitution (fluorine or ether) moved to the γ or δ position (**11**, **13**, **16**, and **24**, Table 1).

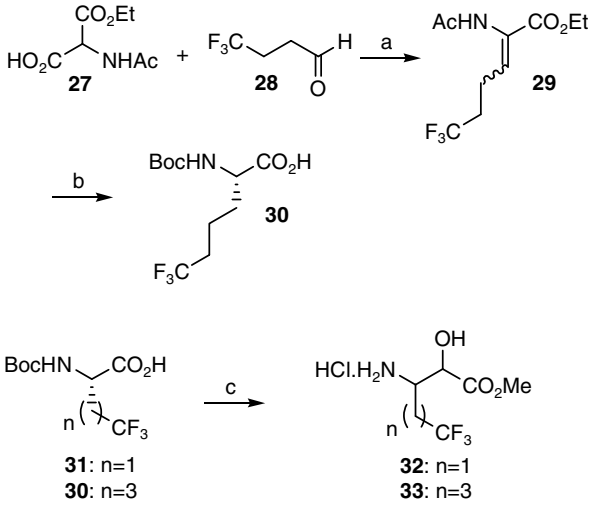
Deprotection of esters of type **25** generated hydroxyacids,[13] which were subjected to standard coupling condition with ammonium chloride. Removal of the amino protecting group delivered the P1 primary hydroxyamides **26** for further processing (Scheme 6).

Syntheses of other modified P1 derivatives (trifluoro Abu and trifluoro norleucine) are described in Scheme 7. Thus, Knoevenagel reaction was used to prepare dehydroaminoacid **29** in 56% yield from ethyl-2-acetamidomalonate **27** and trifluoro butyraldehyde**28**. Asymmetric hydrogenation of **29** using Rh[(*S*,*S*) ethyl duphos][14] followed by amino protection and one-pot hydrolysis provided **30** in excellent yield. *t*-Boc-L-trifluoro norleucine **30** and commercially available *t*-Boc-L-trifluoro Abu **31** were converted to the corresponding Weinreb amide, and subjected to $LiAlH_4$ reduction to give the corresponding aldehydes in high yield. Conversion to cyanohydrins by treatment with acetone cyanohydrin and potassium cyanide followed by acidic hydrolysis using methanolic HCl provided the desired hydroxyl esters **32** and **33** in good overall yield.

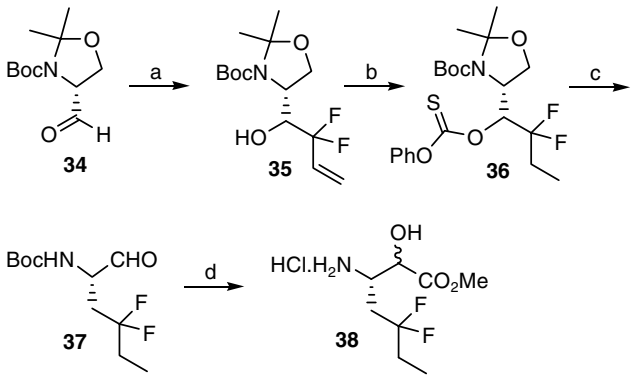
Synthesis of γ-difluoro norleucine P1 fragment is described in Scheme 8. Thus, (*R*)-Garner aldehyde **34** was reacted with 3-bromo-3,3-difluoropropene and stoichiometric amount of indium(0) to generate alcohol **35** in very high yield. The olefin of **35** was hydrogenated using PtO_2 in EtOAc, and the resulting alcohol was reductively deoxygenated via phenylthionocarbonate **36**, by treatment with TTMSSiH and AIBN in refluxing toluene. Chemoselective removal of the acetonide moiety followed by Dess–Martin oxidation delivered aldehyde **37** in 57% yield. As described earlier in Scheme 7, cyanohydrin chemistry was also used to prepare the desired hydroxy methylester **38**.



Scheme 6. Reagents and conditions: (a) i—R = *t*-Bu: 50% TFA/DCM; R = Bn: K_2CO_3, MeOH, H_2O; ii—NH_4Cl, HATU, DMF, DIPEA; iii—H_2, 10% Pd/C, EtOH; iv—2.0 N HCl in Et_2O.



Scheme 7. Reagents and conditions: (a) i—Pyridine (10 equiv), rt, 5 min; ii—Ac_2O (3.2 equiv), 10 °C–rt, 2 h (58%, over two steps); (b) i—H_2, EtOH, Rh[(*S*,*S*) ethyl duphos] (3% w/w) (85%); ii—$(Boc)_2O$ (2 equiv), DMAP (0.2 equiv), THF, 60 °C; iii—LiOH (2 equiv), THF, 60 °C (95% over two steps); (c) i—HCl·HN(OMe)Me, EDCI, NMM, CH_2Cl_2, −10 °C, ii—LAH, THF, −30 °C (100%); iii—acetone cyanohydrin (1.5 equiv), KCN (3 mol %), nBu_4NI (3 mol %), H_2O–Heptane (1:3); iv—HCl/MeOH, 60 °C, 18 h (*n* = 1: 53%, *n*=3: 44%, over four steps).



Scheme 8. Reagents and conditions: (a) 2,2-difluorobromopropene (1.4 equiv), I-n(0), DMF, LiI (10 mol %) (98%); (b) i—H_2, EtOAC, PtO_2 (20 mol %) (64%); ii—*n*-BuLi (1.1 equiv), THF, −78 °C, phenyl-chlorothionocarbonate (1.5 equiv) (76%); (c) i—TTMSSiH (1.1 equiv), AIBN (20 mol %), PhMe, reflux; (ii) 0.5 M TFA/DCM; iii—Dess–Martin's periodinane, DCM (57%, three steps); (d) i—acetone cyanohydrin (1.5 equiv), KCN (3 mol %), nBu_4NI (3 mol %), H_2O–Heptane (1:3); ii—HCl/MeOH, 60 °C, 18 h (51%, two steps).

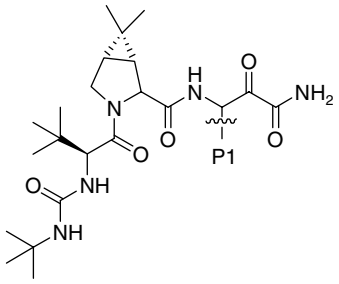
Intermediates **32**, **33**, and **38** were converted to the corresponding hydroxyamides following the procedure described in Scheme 6. Compounds summarized in Table 2 were assembled according to the general Scheme 9. Thus, acid **39**[5] was coupled with the appropriate P1 hydroxyamides **26** following standard HATU coupling methodology. Oxidation of the primary hydroxyamide moiety was done using Moffatt conditions to yield the desired inhibitors of type **40**.[15] Oxidation of **41** bearing the 3,3-difluoro norvaline hydroxyamide moiety resulted in the exclusive formation of **42**.

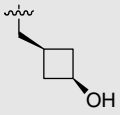
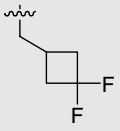
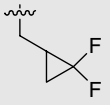
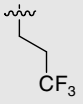
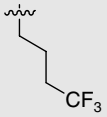
Recently, we reported our efforts in the HCV NS3 serine protease inhibitor area culminating in the discovery of **SCH 503034** that was advanced for human clinical studies.[5] During the course of our investigations, we identified cyclopropyl and cyclobutylalanine as the best P1 moieties, with the latter being incorporated in **SCH 503034**. In an effort to improve the overall profile of the inhibitor, we decided to further explore the P1 moiety, via introduction of hydroxyl or fluoro substitution.[16]

Incorporation of hydroxyl substituent at the 1-position of cyclobutyl ring resulted in inhibitor **43**, that was essentially inactive when tested in the HCV NS3 serine protease continuous assay.[17] Moving the hydroxyl group to 3-position afforded inhibitor **44** with improved potency (K_i^* = 160 nm) compared to **43**. However, inhibitor **44** was still less potent than **SCH 503034**, thus proving that polar hydroxyl group was not tolerated in the enzyme S1-binding pocket. Then, we turned our attention toward fluorine substitution, since our early studies[5] with fluoro-containing P1 were encouraging. Introduction of fluorine at the 1-position of cyclobutyl ring provided compound **45**, with measurable potency (K_i^* = 210 nM) and rat oral AUC (0.31 μM h). Interestingly, the 3,3-difluorocyclobutyl alanine P1-containing inhibitor **46** exhibited improved potency (K_i^* = 98 nM) and rat PK (po, 3 mpk, AUC = 1.1 μM h). While the PK profile was encouraging for inhibitor **46**, it exhibited weak cell-based replicon[18] potency (EC_{90} = 1200 nM).

Since fluorine substitution seemed to enhance the binding potency when compared to polar hydroxyl group, difluorocyclopropyl alanine P1 containing inhibitor **47** was prepared next. The enzyme potency improved further (K_i^* = 50 nM), while the replicon potency was still not optimal (EC_{90} = 1000 nM). Introduction of fluoro substituent on the straight chain P1 moieties was then studied. While trifluoro Abu at P1 (inhibitor **48**) exhibited reasonable binding potency (K_i^* = 120 nM), trifluoro nor-Leu P1 inhibitor, **50**, was far less active (K_i^* = 1300 nM), clearly indicating the limits of S1 pocket.

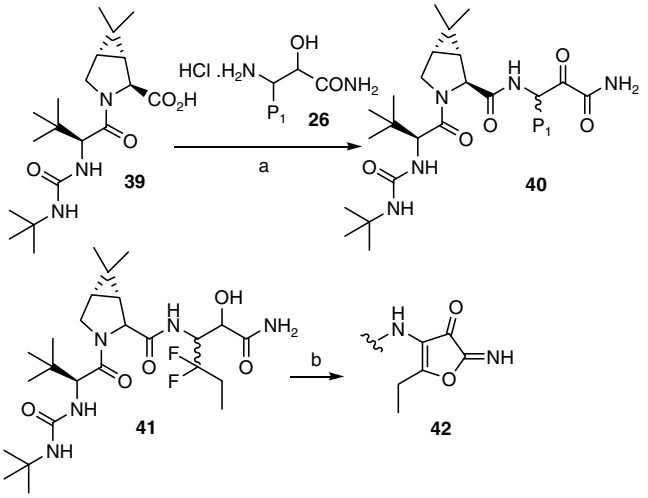
Table 2
Profile of synthesized inhibitors



Compound	P1	K_i^* (nM)	EC_{90} (nM)	Rat AUC[b]
SCH 503034		14	350	1.52[c]
43		163,000	nt[a]	nt
44		160	nt	nt
45		210	nt	0.31[d]
46		98	1200	1.1[d]
47		50	1000	nt
48		120	nt	nt
49		65	600	0.02[d]
50		1300	nt	nt
51		230	nt	nt
52		360	nt	nt

[a] Compounds with K_i^* > 100 nM were not tested (nt) for replicon activity.
[b] AUC (μM h).
[c] Rat po was dosed at 10 mpk.
[d] Rat po was dosed at 3 mpk.

Interestingly, trifluoro nor-Val at P1 (inhibitor **49**) retained most of the enzyme potency (K_i^* = 65 nM), including replicon EC_{90} of



Scheme 9. Reagents and conditions: (a) i—HATU, DMF, DIPEA, −20 °C, 24 h; (ii) Cl_2CHCO_2H, EDCI·HCl, DMSO; (b) Cl_2CHCO_2H, EDCI·HCl, DMSO.

600 nM. Further modifications at the P1 position, via introduction of difluoro susbstituent at the γ-position of nor-Val or nor-Leu (**51** and **52**) afforded less potent inhibitors.

In summary, we have prepared several HCV NS3 protease inhibitors containing hydroxyl or fluoro P1 moieties. Sharpless asymmetric aminohydroxylation reaction was used as a key step to efficiently generate the desired hydroxy ester intermediates. Interestingly, we noticed that regioselectivity of the reaction was greatly influenced by the position of the fluoro or hydroxyl/ether substituent on the olefin substrate. Incorporation of fluoro or hydroxyl functionality on the cyclobutyl or cyclopropyl ring resulted in diminished inhibitor potency. However, difluoro cyclobutyl P1-containing inhibitor **46** afforded the best rat PK profile, for the series reported here. Fluorination of straight chain alkyl moieties yielded potent inhibitors but lacked desirable rat PK properties. Thus, the SAR studies described above clearly demonstrate the stringent requirements at the P1 position for both, enzyme inhibition and replicon potency.

Acknowledgments

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References and notes

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