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Synthesis of novel potent hepatitis C virus NS3 protease inhibitors: Discovery of 4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid as a *N*-acyl-L-hydroxyproline bioisostere

Fredrik Thorstensson,[a] Fredrik Wångsell,[a] Ingemar Kvarnström,[a] Lotta Vrang,[b] Elizabeth Hamelink,[b] Katarina Jansson,[b] Anders Hallberg,[c] Åsa Rosenquist[a,b,*] and Bertil Samuelsson[b,d,*]

[a]*Department of Chemistry, Linköping University, S-581 83 Linköping, Sweden*
[b]*Medivir AB, Lunastigen 7, S-141 44 Huddinge, Sweden*
[c]*Department of Medicinal Chemistry, BMC, Uppsala University, Box 574, S-751 23 Uppsala, Sweden*
[d]*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*

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Abstract—Potent tetrapeptidic inhibitors of the HCV NS3 protease have been developed incorporating 4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid as a new *N*-acyl-L-hydroxyproline mimic. The hydroxycyclopentene template was synthesized in eight steps from commercially available (*syn*)-tetrahydrophthalic anhydride. Three different amino acids were explored in the P1-position and in the P2-position the hydroxyl group of the cyclopentene template was substituted with 7-methoxy-2-phenyl-quinolin-4-ol. The P3/P4-positions were then optimized from a set of six amino acid derivatives. All inhibitors were evaluated in an in vitro assay using the full-length NS3 protease. Several potent inhibitors were identified, the most promising exhibiting a K_i value of 1.1 nM.

1. Introduction

HCV-virus infection is a major health issue where infected are at risk of developing chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[1] Approximately 3% of the world population (~200 million people) are infected by the HCV virus, which spreads through blood contact and through exchange of bodily fluids.[2] After 20 years of chronic HCV infection ~20% of those infected develop cirrhosis which may (⩽5%) progress to liver cancer.[3] In the western world end-stage liver disease caused by chronic hepatitis C viral infection is one of the primary reasons for liver transplantation.[4] At present, there are no vaccines available and the currently most effective therapy consists of PEGylated α-interferons in combination with ribavirin, resulting in only ~50% sustained virological response in genotype 1 infected patients.[5] The limited efficacy of current therapies and the considerable side effects leading to discontinuation of therapy in some patient populations clearly highlight the need for new and more efficacious therapeutic options.

HCV is a single-stranded positive RNA-virus that encodes a polyprotein of approximately 3000 amino acids, which is proteolytically processed into at least four structural and six non-structural viral proteins by host enzymes and viral enzymes (NS2- and NS3-protease).[6] The NS3 protein is a bifunctional enzyme, which has protease activity at its N-terminal domain and RNA helicase/ATPase activity at its C-terminal domain. The NS3 protease is responsible for the autocatalytic cis cleavage of the NS3–NS4A junction and the trans cleavage at the NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B junctions.[7] The NS3 protease and NS5B RNA polymerase of HCV are currently the two most promising targets in the search for antiviral agents against HCV.[8]

Inhibitors of the NS3 protease generally fall into structural classes characterized as either non-covalent or

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* Corresponding authors. Tel.: +46 8 54683120; fax: +46 8 6083199 (Å.R.); tel.: +46 8 6083100; fax: +46 8 6083199 (B.S.); e-mail addresses: asa.rosenquist@medivir.se; bertil.samuelsson@medivir.se

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covalent inhibitors, the latter composed of peptides bearing a reactive center at the cleavage site trapping the catalytic Ser139 of the NS3 protease active site. Most advanced in this inhibitor class are **SCH503034**[9] and **VX-950**[10,11], both α-ketoamides (Fig. 1) which are both being evaluated in phase IIa clinical trials. A second structural class of NS3 protease inhibitors are the product based inhibitors exhibiting a P1[12] carboxylic acid,[13] for example, tetrapeptide **1**[13h] (Fig. 1). The most promising inhibitor from this class is the highly potent macrocyclic tripeptide inhibitor, **BILN 2061**, developed by researchers at Boehringer Ingelheim (Fig. 1). In a proof-of-concept study, a two-day, twice-daily treatment of **BILN 2061** (2× 200 mg) in patients infected with HCV genotype 1 decreased the viral load to nearly undetectable levels within 24–28 h after administration.[13h,14]

Several peptidomimetic inhibitors feature L-proline as a key building block, for example,[13] inhibitors of HIV-1,[15] angiotensin converting enzyme (ACE),[16] and thrombin.[17] In previous work from our laboratory, novel five-membered carbocyclic ring isosteres of proline have been disclosed furnishing thrombin inhibitors of low to modest activity (Fig. 2).[18] Recently, we reported the successful use of a novel trisubstituted cyclopentane moiety (**V**) in HCV NS3 protease inhibitors.[19] We now report on the synthesis of 4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid and its evaluation as a P2 bioisostere of *N*-acyl-L-hydroxy-proline in HCV NS3 protease inhibitors (Fig. 2). Several highly promising inhibitors have been developed from this work where one of the most potent inhibitors **39a** exhibits a K_i value of 1.3 nM (Fig. 10).

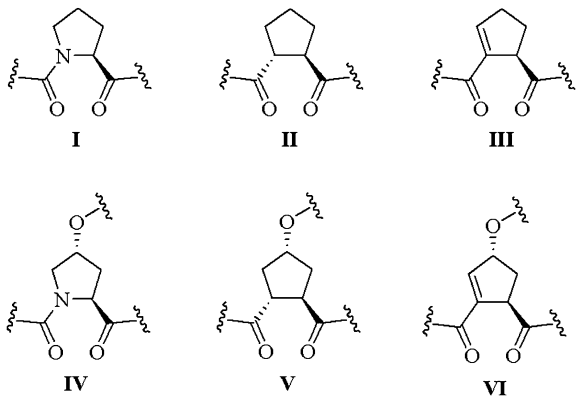


Figure 2. The previously reported **II** and **III** are examples of *N*-acyl-proline (**I**) mimetics used in inhibitors toward thrombin. Recently, we reported the successful use of **V** in HCV NS3 inhibitors. In the present work we use **VI** as a *N*-acyl-hydroxyproline isosteres (**IV**).

2. Results and discussion

The ketone **5** was synthesized as previously reported from commercially available and inexpensive (*syn*)-tetrahydrophthalic anhydride **2** (Scheme 1).[20,21] The anhydride **2** was ring-opened to the corresponding diester and oxidatively cleaved to the dicarboxylic acid **3**, which was cyclized and decarboxylated to the *syn*-cyclopentanone **4**. The ketone **4** was α-brominated with cupric bromide and β-eliminated to the olefin **5** in 53% yield, using calcium carbonate.[22] The α,β-unsaturated ketone **5** was subsequently reduced to give the alcohol **6** in 92% yield

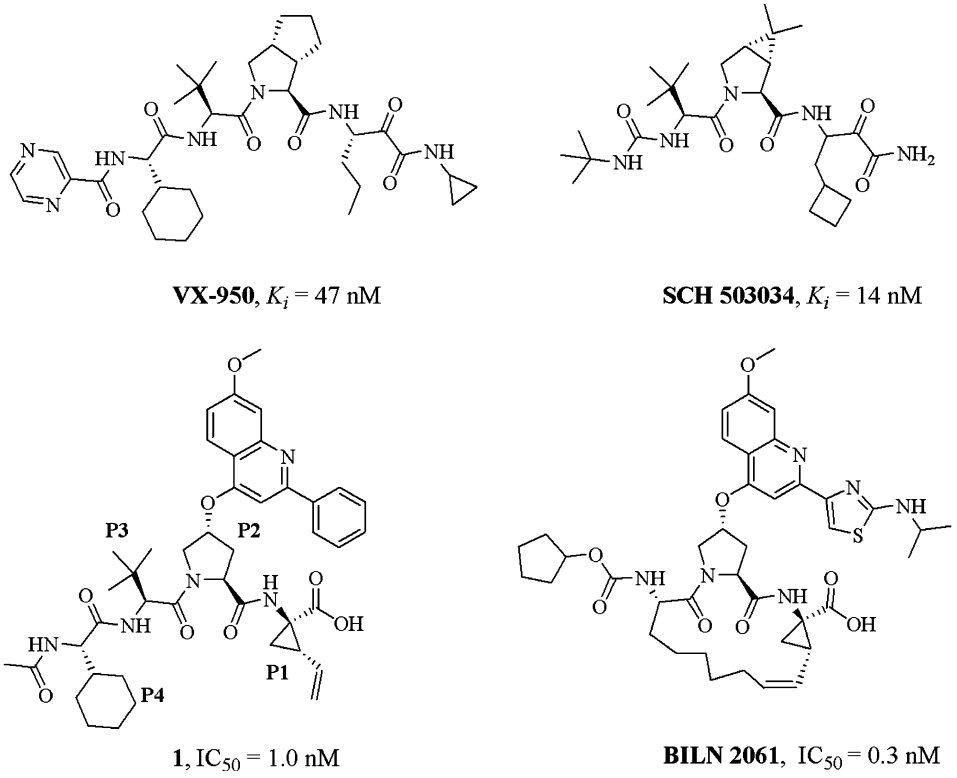
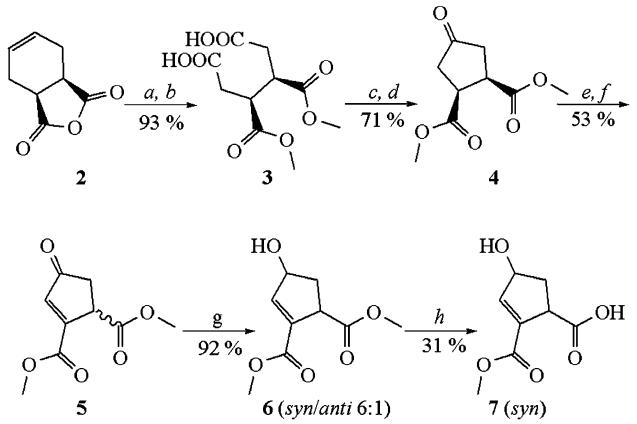


Figure 1. VX-950, **SCH 503034**, compound **1**, and **BILN 2061** are potent HCV inhibitors containing the P2-L-hydroxyproline moiety. Compound **38a** is the most potent inhibitor in our novel series and is containing the P2-hydroxycyclopentene moiety.



Scheme 1. Synthesis of the P2-template. Reagents and conditions: (a) *p*-toluene sulfonic acid monohydrate, methanol, reflux; (b) $KMnO_4$, water, 0 °C; (c) acetic anhydride, 130 °C; (d) NaOAc, 130 °C; (e) $CuBr_2$, THF, reflux; (f) $CaCO_3$, DMF, 100 °C; (g) $NaBH_4$, methanol, −30 °C; (h) LiOH, water/dioxane (1:1), 0 °C.

in a 6:1 diastereomeric ratio (*syn*/*anti*) using sodium borohydride at −30 °C.

The stereochemistry of the *syn*/*anti* diasteromers[23] of **6** was determined by NOESY NMR.[24] The NOESY-spectra of *syn*-**6** show that H_1 and H_4 interact with the same methylene hydrogen ($H_{5\beta}$), which is consistent with the *syn* configuration (Fig. 3). In the NOESY-spectra of *anti*-**6**, the H_4 interacts with $H_{5\alpha}$ and the H_1 with $H_{5\beta}$, which again is consistent with the *anti*-**6** isomer.

The dimethylester **6** (*syn*/*anti* 6:1) was regioselectively[25] mono-hydrolyzed using 1.0 equiv of LiOH in dioxane/water, yielding the diasteromeric acid **7** (*syn*/*anti* 6:1), which was recrystallized (hexane/diethyl ether) to the pure racemic acid **7** (*syn*) in 31% yield. The regiochemistry of the α,β-unsaturated acid **7** was determined with HMBC-spectra and the relevant correlations shown in Figure 4.[24]

The acid **7** (*syn*) was coupled with the three P1-building blocks (**A**–**C**) (Fig. 5), using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA) in DMF, to give the amides **8**–**10** in 52–69% yield (Scheme 2). The P1-building block **A** was commercially available, **B** was readily synthesized from commercially available amino acids, and **C** was synthesized from (1*R*,2*S*)-1-amino-2-vinyl-cyclopropanecarboxylic acid ethyl[26] using Fmoc-succinate and TBTA.[24,27,37] The vinylcyclopropyl amine

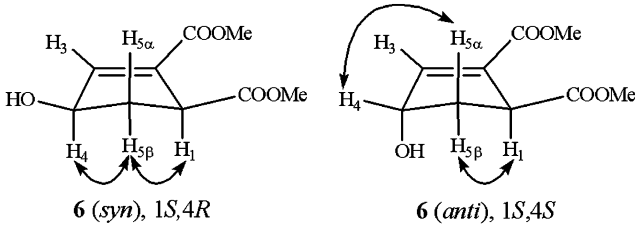


Figure 3. The relevant NOE-effects observed in **6** (*syn*) and **6** (*anti*).

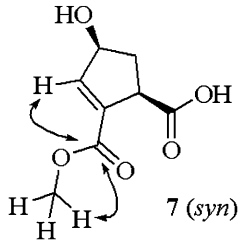


Figure 4. The relevant HMBC correlations observed for **7**.

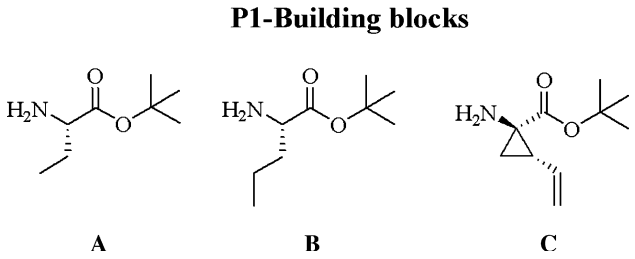
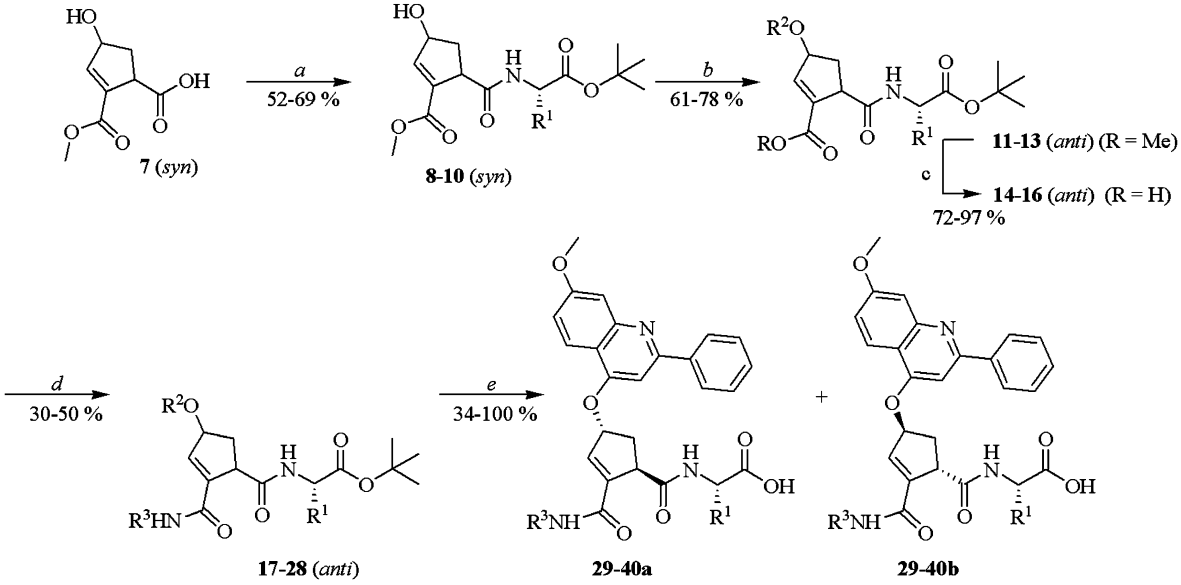


Figure 5. The P1-building blocks (**A**–**C**) ($H_2NCHR^1COO^tBu$).

C was coupled directly with the carboxylic acid **7** without prior purification due to rapid decomposition of the free amine at room temperature. The alcohols **8**–**10** were subsequently coupled with 7-methoxy-2-phenyl-quinolin-4-ol (**R²OH**) to give the ethers **11**–**13** in 61–78% yield using triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD) in THF.[26,28] The methyl esters **11**–**13** were hydrolyzed with lithium hydroxide in dioxane/water 1:1 at 0 °C to furnish the carboxylic acids **14**–**16** in 72–97% yield. The P3/P4 building blocks **D**–**I** were coupled with the acids **14**–**16** using HATU and DIPEA in DMF, which resulted in the *tert*-butyl esters **17**–**28** in 30–50% yield (Fig. 6). The P3/P4 building blocks **D**–**H** were synthesized from commercially available amino acids using standard protecting group chemistry.[24,29] The *tert*-butylesters **17**–**28** were hydrolyzed, using TFA and Et_3SiH in DCM, to yield the final products **29**–**40** in 34–100% yield.[30] Several of the diasteromeric compounds **17**–**40** could be separated[31] by chromatography and all isolated products were tested individually to determine HCV NS3/4A inhibitory properties (Table 1).

The final products **35a** and **35 b** are either of the 1*R*,4*R* or the 1*S*,4*S* stereochemistry in the cyclopentene ring (Fig. 7A) and the assignment of the diastereomers was based on ^1H-, ^{13}C-, COSY-, NOESY-, TOCSY-, HMBC-, HSQC-, and ROESY-spectra.[24] According to computer simulations the main significant NOE-differences between the diastereomers are the distance between $P2H_\alpha$ and P3NH (Fig. 7B).[24,32] In the ROESY-spectra[33] of **35a** and **35b** the two different diastereomers exhibit similar NOE cross-peak patterns and intensities except at the cross-peak $P2H_\alpha \leftrightarrow$ P3NH (Fig. 7C). The fact that **35a** has a weak NOE-interaction and **35b** has a medium one indicates that **35a** has the 1*R*,4*R*- and that **35b** has the 1*S*,4*S*-configuration, respectively. This annotation is also supported by the fact that diastereomer **35a** (K_i value of 30 nM), which closely resembles



Scheme 2. Synthesis of the final products. Reagents and conditions: (a) DIPEA, HATU, the P1-building blocks, (**A**–**C**, $H_2NCHR^1COO^tBu$), 0 °C, DMF; (b) the P2-substituent (7-methoxy-2-phenyl-quinolin-4-ol, **R^2OH**), PPh_3, DIAD, THF; (c) LiOH, dioxane/water (1:1); (d) DIPEA, HATU, the P3/P4-building blocks, (**D**–**I**, NH_2R^3), 0 °C, DMF; (e) triethyl silane, TFA, DCM.

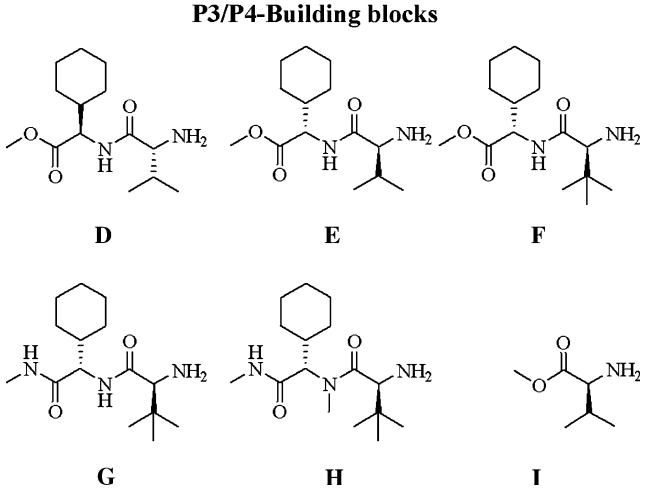


Figure 6. The P3/P4 building blocks (**D**–**I**) (R^3-NH_2).

the L-proline configuration, is a superior HCV NS3 protease inhibitor compared to **35b** (K_i > 2000 nM) (Table 1).

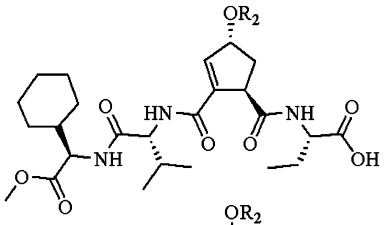
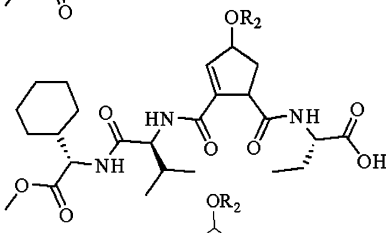
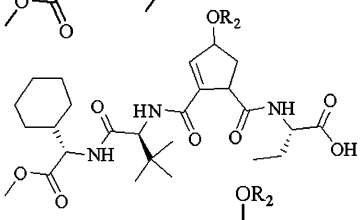
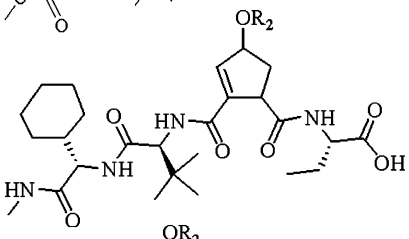
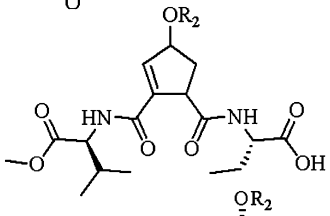
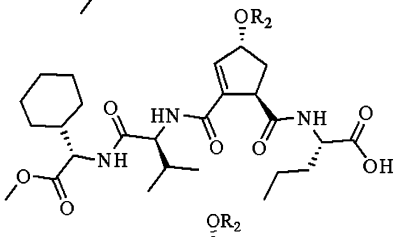
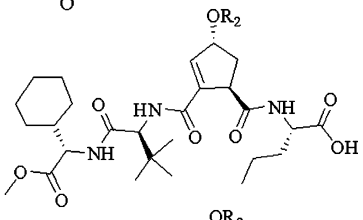
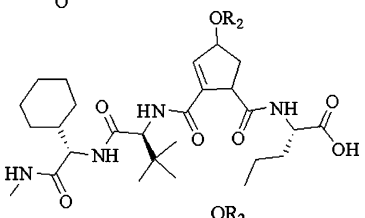
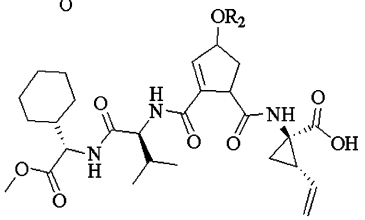
3. Biological data and structure–activity relationships

Inhibitors were evaluated in an in vitro assay comprising the full-length NS3 protein (Table 1). All of the HCV protease inhibitors synthesized contain the 7-methoxy-2-phenyl-quinolin-4-ol (**R^2OH**) group as P2-substituent,[13a,34] which is reported to stabilize the catalytic machinery in the correct geometry by shielding the catalytic triad of the protease from exposure to the solvent[35]and moreover which likely interacts with the helicase domain of the NS3 protein enhancing the overall activity.[36] The preferred stereochemistry of the P2 hydroxycyclopentene is analogous to the L-4-hydroxyproline residue exhibited in known HCV protease inhibitors.[8,13,26] Thus inhibitors **34a**, **35a**, **38a**, and **39a** show highly promising potencies, whereas inhibitors **34b**, **35b**, **38b**, and **39b** (Table 1) incorporating the enantiomeric hydroxycyclopentene are considerably less active. However, **39b** (K_i value of 23 nM) is still having an unexpectedly high potency for having the non-optimal P2 stereochemistry. Therefore, our inhibitor **39b** was aligned with compound **1**[13f] described by Boehringer Ingelheim using a molecular modeling alignment tool (GASP™) to elucidate whether it would be possible to find a conformation that would overlay well with **1** (Fig. 8). The overall alignment of **39b** and **1** is similar, except for the cyclopentene group in **39b** that adopts a different angle compared to the proline of **1**, forcing the P1–P2 amide into a different conformation and thereby losing the possibility to form a H-bond to the protein backbone of Arg 155. Apart from that, all side chains and H-bonding groups seem to have the same interaction possibilities as **1**. Similar SAR in P1 and P3 also indicates that the binding mode of the **b**-series is similar to the **a**-series.

At the P1-position the ethyl Gly amino acid **A** (Fig. 5) is roughly 4 times less potent than the corresponding propyl Gly amino acid **B**, cf. **32** and **36** (exhibiting K_i values of 280 and 67 nM, respectively). The most potent inhibitors were obtained using the (1*R*,2*S*)-1-amino-2-vinylcyclopropylcarboxylic acid **C** as P1-residue, cf. **35a** and **38a** (exhibiting K_i values of 30 and 1.1 nM, respectively).[37]

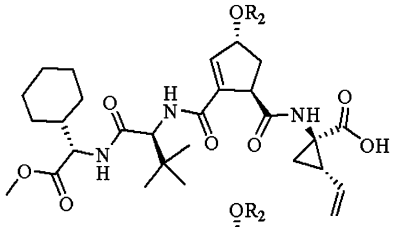
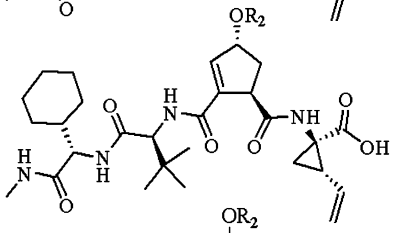
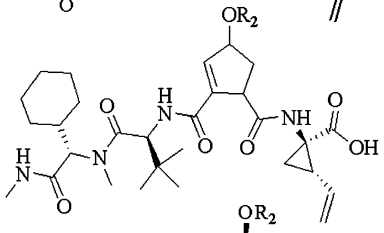
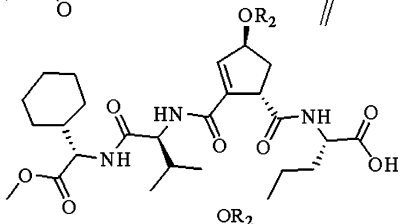
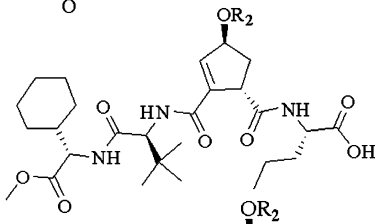
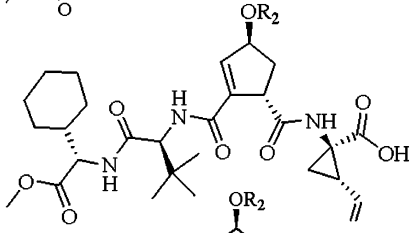
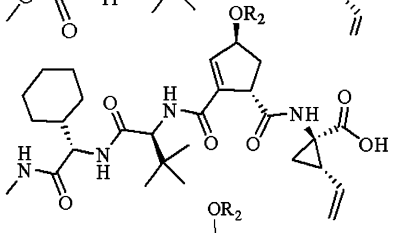
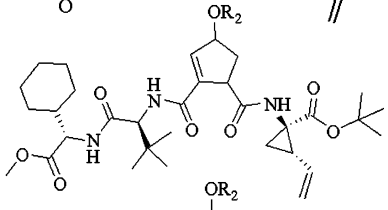
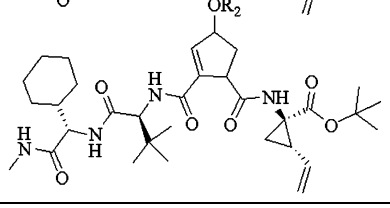
It has previously been reported that the P1-carboxylic acid forms a hydrogen bond with Ser139 which results in increased affinity to the HCV NS3 protease.[37] The present inhibitors fall into this general SAR, as can be seen by comparing, for example, the acids **38a** and **39a**

Table 1. Activity data

Compound	Structure	K_i (nM)
29a		>2000
30 (*anti*)		523
31 (*anti*)		503
32 (*anti*)		280
33 (*anti*)		>2000
34a		102
35a		30
36 (*anti*)		67
37 (*anti*)		24

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	K_i (nM)
38a		1.1
39a		1.3
40 (*anti*)		>5000
34b		2560
35b		>2000
38b		108
39b		23
26 (*anti*)		>2000
27 (*anti*)		>2000

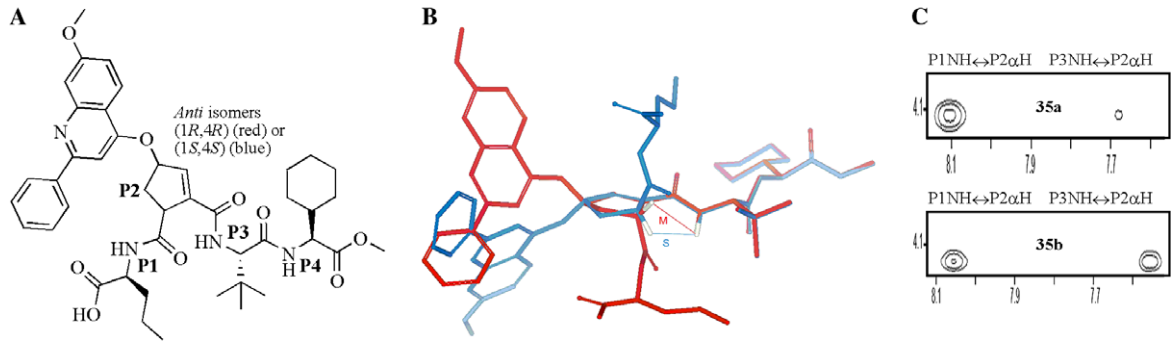


Figure 7. (A) Diastereomers **35**; (B) overlay of energy minimized **35a** (red) and **35b** (blue). Theoretical NOE interactions of P3NH ↔ P2αH is in **35a** medium (M) and in **35b** strong (S); (**C**) data extracted from ROESY-spectra[24] of **35a** and **35b** displaying the NOE-interactions between P3NH ↔ P2αH and P1NH ↔ P2αH.

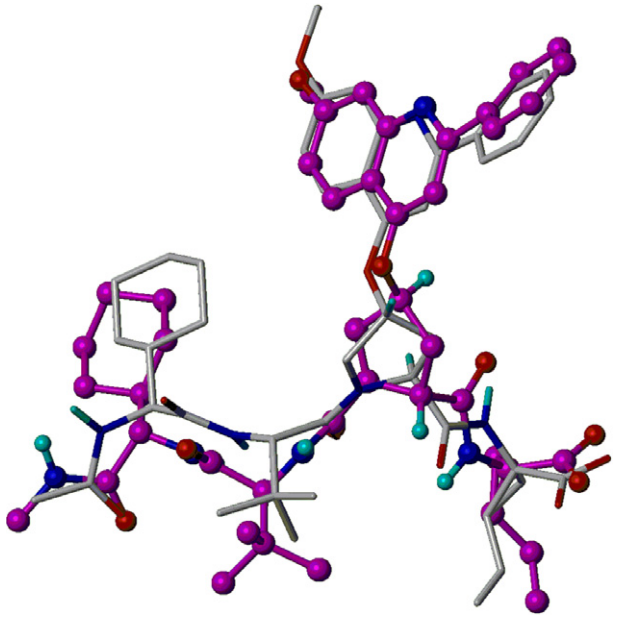


Figure 8. Compound **39b** (magenta) with the less active enantiomeric stereochemistry of the cylopentene has been superimpositioned with **1** (gray). The figure shows that it is possible to align **39b** with **1**, so that all side chains and all H-bonding groups except the P1–P2 amide overlay well with **1**, indicating a similar binding mode.

(K_i values of 1.1 and 1.3 nM, respectively) with the corresponding *tert*-butylesters **26** and **27**, both essentially devoid of inhibitory activity.

At the P3/P4 positions the *S* stereochemistry has been the preferred in all inhibitors reported in the literature.[13] In the present work the P2 cyclopentene scaffold introduces a reversal of direction in the peptide chain at the P2 position, resulting in inhibitors having two carboxy terminus. Moreover, a one carbon extension is introduced along the P3/P4 amino acid chain. Whilst this novel P2 hydroxycyclopentene template introduces substantial structural changes affecting the P3/P4 residues of the inhibitors it became clear from analyzing the 3D computer overlays of structures **1** and **39a** that the *S* stereochemistry would indeed be most favored at both the P3 and P4 positions (Fig. 9). The importance of the stereochemistry at the P3/P4 residues can be seen by comparing the *S,S*-inhibitor **30** (K_i value of 523 nM) with the corresponding *R,R*-inhibitor **29a** (K_i > 2000 nM).

The S3 pocket has a major role to rigidify and predefine the inhibitor in the extended conformation.[13f] It has also been shown that the P3 Val can be replaced by other hydrophobic P3 residues.[38] In the present series enhanced activity was observed when the P3 Val was replaced with *tert*-butyl glycine, cf. **34** and **35** (displaying K_i values of 102 and 30 nM, respectively). Attempts to shorten the inhibitors cf. the P1–P3 spanning derivative **33** (K_i > 2000 nM) were not successful.[39]

The P4 methyl ester derivative **38a** (K_i value of 1.1 nM) and the P4 methyl amide derivative **39a** (K_i value of 1.3 nM) are equipotent, which suggests that no hydro-

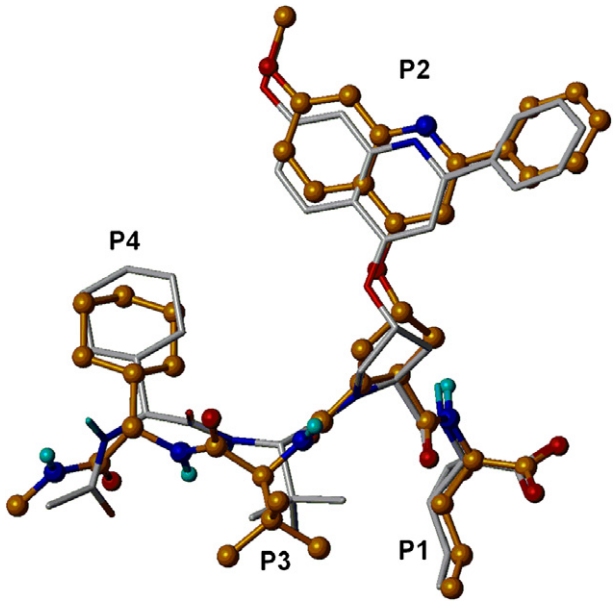
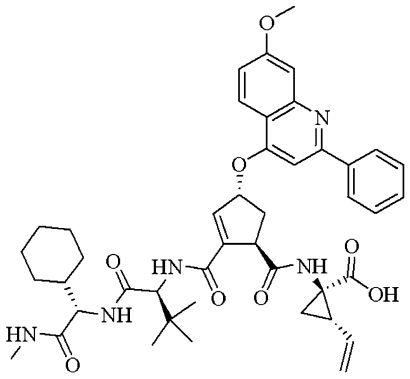
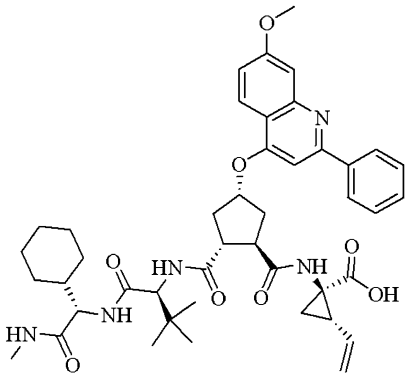


Figure 9. Compound **39a** (orange) with reversed direction of the peptide in P3 and P4, has been superimpositioned with **1** (gray), for comparison of the directionality of the side chains. The P3/P4 side chains of **39a** with *S* stereochemistry overlay well with the P3/P4 side chains of **1**.



39a, K_i = 1.3 nM



41, K_i = 16 nM

Figure 10. NS3/4A inhibition values for the P2-cyclopentene inhibitor **39a** and the corresponding P2-cyclopentane inhibitor **41**.

gen donating bonds are required at this position. In contrast *N*-methylation of the P4-NH derivative **39a** to give P4-NMe derivative **40** (K_i > 5000 nM) results in loss of inhibitory activity, suggesting that the P4-NH is involved in a hydrogen bond donation network with the NS3-enzyme.

As can be seen from Figure 10, it is evident that the most potent inhibitor in the previously synthesized P2-cyclopentane series[19] is considerably less active than the corresponding inhibitor of the P2-cyclopentene series.[40]

4. Conclusion

Eighteen HCV NS3 protease inhibitors have been synthesized all encompassing the novel 4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid, a *N*-acyl-L-hydroxyproline mimic, in the P2-position. These inhibitors display two carboxy terminus where the P2 template induces a reversal of direction of the P3/P4 amino acid chain. In spite of these different features as compared with inhibitors previously reported in the literature very potent inhibitors have been identified. The most potent inhibitor in this series displays an impressive K_i value of 1.1 nM.

5. Experimental

5.1. HCV NS3 protease enzyme assay[41]

The inhibition assay with HCV protease was performed using recombinant full-length NS3 enzyme (Poliakov et al.) and NS4A (KKGSVVIVGRIVLSGK, Gunnar Lindeberg, Department of Medicinal Chemistry, Uppsala University, Sweden) in final concentrations of 3.5 nM and 14 μM, respectively.[42] The test compounds were dissolved and diluted in DMSO and were added to the assay buffer containing 50 mM Hepes, pH 7.5, 40% glycerol, 0.1% Chaps, and 10 mM DTT. The maximum final DMSO concentration in the assay was 1%. After a pre-incubation for 30 min at room temperature, the enzyme reaction was started by adding the FRET substrate Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-ψ-[COO]Ala-Ser-Lys(DABCYL)-NH2 (RET S1, AnaSpec, San Jose, CA, USA) to a final concentration of 2 μM. The enzyme activity was continuously measured over time (20 min) in a fluorescence reader (Fluorocan Ascent, ThermoLab systems, Stockholm, Sweden) with 355 nm as excitation and 500 nm as emission wavelengths, respectively. IC_{50} values were calculated by non-linear fitting into the equation $(1 - v_i/v_o) = (I)/((I) + IC_{50})$ and the K_i value was calculated from the IC_{50} value using the equation. $K_i = IC_{50}/(1 + S/K_m)$ assuming a competitive enzyme inhibition.

5.2. General methods

Standard ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 300 instrument using $CDCl_3$ or methanol-d_4 with TMS as an internal standard. NOESY spectra that were used to determine the structure of *syn*-**6** and *anti*-**6** were recorded on Varian 300 instrument using $CDCl_3$ with TMS as an internal standard. ^{1}H-, ^{13}C-, HMBC-, HSQC-, TOCSY-, NOESY-, ROESY-, and COSY-NMR that were used to determine the structure of **35a** and **35b**, and determining the regiochemistry of **7** were recorded on a Varian 500 instrument using methanol-d_4 or DMSO-d_6. In recorded NMR spectra with diasteromeric mixtures the presumed diasteromeric peaks were put into brackets with the general formula [nn.n & nn.n] for ^{13}C NMR and [n.nn & n.nn, (x, yH)] for ^{1}H NMR. Preparative HPLC was performed on a Gynkotek (pump: P580, detector: UVD 170S, software: Chromeleon) using a Kromasil 100-10-C18 (250× 20 mm) column. Mixtures of deionized water and methanol were used as mobile phase. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and charring with ethanol/sulfuric acid/acetic acid/*p*-anisaldehyde (90:3:1:2) for visualization. MALDI-TOF analysis was performed using a Voyager-DE STR Biochemistry Workstation, in positive mode. Using the matrix recipe: 10 mg α-cyano-4-hydroxy-*trans*-cinnamic acid dissolved in 0.5 mL of 0.1% TFA solution and 0.5 mL acetonitrile. LC/MS was performed on a Gilson system (Column: Phenomenex C18 250 × 15 mm and Phenomenex C18 150 × 4.6 mm for preparative and analytical runs, respectively; Pump: Gilson gradient pump 322; UV/Vis-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Gilson

Fraction Collector FC204, software: Gilson UniPoint ver. 4.0 and Xcalibur ver. 1.3) using methanol with 0.1% formic acid and deionized water with 0.1% formic acid as mobile phase. Concentrations were performed under diminished pressure (1–2 kPa) at a bath temperature of 40 °C. All low-temperature reactions were accomplished by submerging the reaction vessels into an ethanol bath that had been cooled by additions of liquid nitrogen. Organic phases were dried over magnesium sulfate monohydrate. All degassing of solvents were achieved using ultrasonification (Ultrasonik 104×) for at least 2 h. Optical rotation was performed on a Perkin-Elmer 141. Drying of solvents and reagents: THF was refluxed over sodium/benzophenone and distilled onto 4 Å MS; filtration was achieved using filter paper Munktell, OOH quality.

5.3. Synthesis of the P2-template

5.3.1. ((1*S*,4*R*) and (1*R*,4*S*))-4-Hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid dimethyl ester (*syn*-6). To a cold solution (−30 °C) of **5** (3.18 g, 16.1 mmol) dissolved in MeOH (23 mL), $NaBH_4$ (0.66 g, 17.5 mmol) was added. After seven minutes the excess $NaBH_4$ was destroyed by adding brine (80 mL). The mixture was concentrated and extracted with ethyl acetate (3× 80 mL). The combined organic phases were dried, filtered, and concentrated which gave the diasteromeric mixture (*syn*/*anti* 6:1) of **6** (3.0 g, 92%) as a yellow oil. The major compound *syn*-**6** could further be purified by flash chromatography (toluene/ethyl acetate 3:1) but the *anti*-**6** was always polluted with the *syn*-**6**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69–1.77 (m, 1H), 2.43–2.56 (m, 1H), 3.55 (s, 3H), 3.57 (s, 3H), 3.68–3.70 (m, 1H), 4.66–4.4.69 (m, 1H) 6.70–7.72 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 38.3, 48.0, 52.2, 52.9, 75.7, 137.0, 146.3, 164.6, 175.9.

5.3.2. (1*R*,4*R*) and (1*S*,4*R*)-Dimethyl-4-hydroxycyclopent-2-ene-1,2-dicarboxylate (*anti*-6). The alcohol *syn*-**6** (0.23 g, 1.15 mmol), benzoic acid (0.16 mL, 1.4 mmol), triphenylphosphine (0.37 g, 1.4 mmol) and DIAD (0.32 mL, 1.6 mmol) were dissolved in dry THF (5.7 mL) and stirred at room temperature. After two hours the solvent was removed by evaporation to give a yellow oil. The residue was purified by flash chromatography (toluene/ethyl acetate 18:1) to give a colorless oil. The oil was diluted in MeOH (6 mL) and cooled in an ice bath. To the solution sodium methoxide (0.57 mmol, 1 M) was slowly added while stirring. After one hour the reaction was terminated and the solvent was removed by evaporation. The residue was purified by flash chromatography (toluene/ethyl acetate 1:1) to give the alcohol *anti*-**6** as a yellow oil. ^{1}H NMR (300 MHz $CDCl_3$): δ 2.04–2.13 (m, 1H) 2.37–2.46 (m, 1H), 3.65 (s, 3H), 3.71 (s, 3H) 3.87–3.92 (m. 1H), 5.05–5.15 (m, 1H), 6.79–6.81 (m, 1H); ^{13}C NMR (75.5 MHz $CDCl_3$): δ 38.9, 48.6, 52.2, 52.4, 75.7, 136.4, 146.6, 164.7, 174.5.

5.3.3. (1*S*,4*R*) or (1*R*,4*S*)-4-Hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid 2-methyl ester (7). To an ice-cold solution of a diasteromeric mixture of **6** (2.5 g, 12.3 mmol) dissolved in dioxane and water (1:1, 50 mL), LiOH (0.3 g, 12.3 mmol) was added. After two and a half hours the mixture was co-evaporated with toluene and methanol. Purification by flash chromatography (toluene/ethyl acetate 3:1 + 1% AcOH) gave a *syn*/*anti* (6:1) mixture (1.6 g, 68%), which was recrystallized twice from hexane/diethyl ether yielding pure *syn*-**7** (0.7 g, 31%) as white crystals (mp 94–98 °C). ^{1}H NMR (300 MHz, CD_3OD): δ 1.78–1.89 (m, 1H), 2.70–2.84 (m, 1H), 3.56–3.71 (m, 1H), 3.76 (s, 3H), 4.81–4.90 (m, 1H), 6.76–6.81 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 38.0, 48.0, 52.4, 75.7, 137.0, 146.2, 165.0 178.4.

5.4. Method used for synthesis of the amides 8–10

5.4.1. ((3*S*,5*R*) and (3*R*,5*S*))-5-((*S*)-1-*tert*-Butoxycarbonyl-butylcarbamoyl)-3-hydroxy-cyclopent-1-enecarboxylic acid methyl (9). To an ice cooled solution of **7** (0.20 g, 1.1 mmol) and **B** (2-amino-pentanoic acid *tert*-butyl ester) (0.24 g, 1.4 mmol) in DMF (7 mL), DIPEA (0.18 g, 1.4 mmol) and HATU (0.53 g, 1.4 mmol) were added. After two hours the solution was concentrated and purified by flash chromatography (toluene/ethyl acetate 3:1). This gave **9** as a yellow oil (0.22 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84–0.96 (m, 3H), 1.14–1.39 (m, 2H), [(1.44 and 1.49) (s, 9H)], 1.50–1.60 (m, 1H), 1.61–1.85 (m, 1H), 1.97–2.10 (m, 1H), 2.11–2.28 (m, 1H), 3.57–3.68 (m, 1H), [(3.73 and 3.76) (s, 3H)], 4.30–4.50 (m, 1H), 4.63–4.73 (m, 1H), 6.80–6.95 (m, 1H), 6.95–7.00 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ [13.9 and 14.0], [18.5 and 18.7] [28.1 and 28.2], [34.7 and 34.8], 37.6, [50.3 and 50.5], [52.0 and 52.2], [53.4 and 53.6], [75.0 and 75.1], [82.2 and 82.4], [137.1 and 137.2], [146.1 and 146.3], 165.1, [171.2 and 171.5], [173.3 and 173.5].

5.5. Synthesis of the ethers 11–13

5.5.1. ((3*R*,5*R*) and (3*S*,5*S*))-5-((*S*)-1-*tert*-Butoxycarbonyl-butylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid methyl ester (12). To an ice cooled solution of **9** (0.23 g, 0.67 mmol) in dry THF (5 mL), 7-methoxy-2-phenyl-quinolin-4-ol (0.22 g, 0.88 mmol) and triphenylphosphine (0.23 g, 0.88 mmol) were added. Then DIAD (0.18 g, 0.88 mmol) was dissolved in THF (2 mL) and added dropwise to the solution. After one hour the mixture was concentrated and purified by flash chromatography (toluene/ethyl acetate 3:1). This gave **12** as a white powder (0.30 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88–1.00 (m, 3H), 1.18–1.43 (m, 2H), [(1.45 and 1.50) (s, 9H)], 1.53–1.65 (m, 1H), 1.66–1.85 (m, 1H), 2.29–2.43 (m, 1H), 3.10–3.25 (m, 1H), [(3.79 and 3.83) (s, 3H)], 3.97 (s, 3H), 4.05–4.20 (m, 1H), 4.38–4.50 (m, 1H), 6.03–6.13 (m, 1H), 6.65–6.90 (m, 1H), 7.04–7.18 (m, 3H), 7.40–7.56 (m, 4H), 8.00–8.12 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, [18.6 and 18.8], [28.2 and 28.3], [34.9 and 35.0], 36.2, [50.2 and 50.5], 52.4, 53.2, 55.7, 82.0, 82.3, 98.4, 107.8, 118.4, 123.1, 127.8, 129.0, 129.1, 129.2, 129.5, 132.2, 138.7, 140.5, 142.4, 152.0, 159.2, [161.0 and 161.4], [164.0 and 164.2], [171.2 and 171.3].

5.6. Method used for synthesis of the carboxylic acids 14–16

5.6.1. ((3*R*,5*R*) and (3*S*,5*S*))-5-((*S*)-1-*tert*-Butoxycarbonyl-butylcarbamoyl)-3-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-1-enecarboxylic acid (15). The methyl ester **12** (0.35 g, 0.61 mmol) was dissolved in dioxane/water (1:1, 7 mL) and LiOH (0.031 g, 1.3 mmol) was added. The reaction mixture was stirred overnight and then diluted with toluene/methanol (1:1, 10 mL) and concentrated. This gave the lithium salt of **15** (0.32 g, 90%) as a brown powder. ^{1}H NMR (300 MHz, CD_3OD): δ 0.90–1.00 (m, 3H), 1.18–1.30 (m, 1H), 1.38–1.42 (m, 1H), [(1.44 and 1.53) (s, 9H)], 1.60–1.82 (m, 2H), 2.30–2.40 (m, 1H), 3.00–3.17 (m, 1H), 3.98 (s, 3H), 4.01–4.12 (m, 1H), 4.20–4.30 (m, 1H), 5.95–6.05 (m, 1H), 6.78–6.82 (m, 1H), 7.10–7.30 (m, 3H), 7.45–7.60 (m, 3H), 8.00–8.15 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 12.9, (18.7 and 18.80, 23.1, 27.1, 35.7, (50.1 and 50.3), (53.5 and 53.7), 54.8, 81.4, (82.9 and 83.0), 98.9, 106.3, 117.9, 123.1, 125.2, 127.8, 128.0, 128.6, 128.7, 129.3, 135.3, 135.6, 140.3, 151.0, 160.3, 161.9, 170.7, 174.1.

5.7. Method used for synthesis of the *tert*-butylesters 17–28

5.7.1. (*S*)-2-{[(1*R*,4*R*)-2-{(*R*)-1-[((*S*)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-pentanoic acid *tert*-butyl ester (23a) and (*S*)-2-{[(1*S*,4*S*)-2-{(*R*)-1-[((*S*)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-pentanoic acid *tert*-butyl ester (23b). To an ice-cold DMF (1 mL) solution of **15** (60 mg, 0.10 mmol) the amine **F** (42 mg, 0.15 mmol), DIPEA (19 mg, 0.15 mmol) and HATU (62 mg, 0.16 mmol) were added. After two and a half hours the mixture was concentrated and purified by flash chromatography (toluene/ethyl acetate 3:1) (25 mg, 50%). The diastereomers **23** were separated using HPLC (90% MeOH + 0.2% TEA) to **23a** (6 mg, 6%) and **23 b** (9 mg, 10%). Compound **23a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82–0.90 (m, 3H), 1.01 (s, 9H), 1.05–1.40 (m, 7H), 1.46 (s, 9H), 1.50–1.80 (m, 8H), 2.20–2.35 (m, 1H), 3.07–3.25 (m, 1H), 3.73 (s, 3H), 3.97 (s, 3H), 4.11 (d, J = 7.96 Hz, 1H), 4.38–4.52 (m, 3H), 6.03–6.12 (m, 1H), 6.24 (d, J = 8.79 Hz, 1H), 6.63 (s, 1H), 6.82 (d, J = 9.06 Hz, 1H), 7.07–7.27 (m, 2H), 7.36 (d, J = 7.96 Hz, 1H), 7.41–7.55 (m, 4H), 8.01–8.10 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 14.0, 18.8, 26.1, 26.8, 28.2, 28.6, 29.6, 34.9, 35.6, 36.2, 40.9, 50.7, 52.4, 53.3, 55.7, 57.3, 60.8, 82.0, 82.7, 98.4, 105.2, 107.7, 115.2, 118.4, 123.2, 127.9, 129.0, 129.4, 131.1, 135.1, 138.4, 142.4, 153.3, 159.6, 161.6, 164.2, 170.1, 171.3, 172.2. Compound **23b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90–0.98 (m, 3H), 1.04 (s, 9H), 1.08–1.40 (m, 7H), 1.44 (s, 9H), 1.55–1.90 (m, 8H), 2.20–2.38 (m, 1H), 3.10–3.22 (m, 1H), 3.73 (s, 3H), 3.97 (s, 3H), 4.02–4.15 (m, 1H), 4.35–4.48 (m, 3H), 6.00–6.08 (m, 1H), 6.72 (s, 1H), 6.90 (d, J = 9.06 Hz, 1H), 7.09–7.20 (m, 3H), 7.44–7.55 (m, 5H), 8.03–8.11 (m, 3H).

5.8. Method used for synthesis of the final compounds 29–40

5.8.1. (*S*)-2-{[(1*R*,4*R*)-2-{(*R*)-1-[((*S*)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-pentanoic acid (35a) and (*S*)-2-{[(1*S*,4*S*)-2-{(*R*)-1-[((*S*)-Cyclohexyl-methoxycarbonyl-methyl)-carbamoyl]-2,2-dimethyl-propylcarbamoyl}-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-cyclopent-2-enecarbonyl]-amino}-pentanoic acid (35b). A mixture of the *tert*-butyl ester **23a** and **23b** (28 mg, 0.034 mmol), triethyl silane (8.7 mg, 0.075 mmol) was dissolved in DCM (1 mL) and cooled to 0 °C using an ice-bath. TFA (1 mL) was added and two hours later the mixture was concentrated. The diastereomers were separated by the use of preparatory HPLC (65% MeOH + 0.2% TEA) to the chiral compounds **35a** (15 mg, 55%) and **35b** (12 mg, 45%). After lyophilization **35a** and **35b** were collected as white powders. Compound **35a:** $[\alpha]_D^{22}$ +155.8; ^{1}H NMR (300 MHz, CD_3OD): δ 0.90–0.97 (m, 3H), 1.03 (s, 9H), 1.05–1.50 (m, 7H), 1.50–1.80 (m, 8H), 2.43–2.55 (m, 1H), 2.77–2.90 (m, 1H), 3.68 (s, 3H), 3.96 (s, 3H), 4.20–4.30 (m, 2H), 4.31–4.40 (m, 1H), 4.45–4.50 (m, 1H), 6.03–6.11 (m, 1H), 6.98 (s, 1H), 7.12–7.19 (m, 1H), 7.36 (s, 1H), 7.41 (d, J = 2.2 Hz, 1H), 7.50–7.60 (m, 3H), 8.03–8.10 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 13.1, 19.1, 26.1, 28.7, 28.9, 29.5, 34.3, 34.8, 35.9, 40.1, 50.8, 51.2, 54.8, 55.0, 57.9, 60.7, 83.5, 99.1, 106.0, 115.2, 118.2, 123.3, 127.8, 128.0, 128.7, 128.8, 129.7, 135.2, 139.8, 143.7, 150.6, 160.1, 162.2, 165.2, 171.7, 172.2, 173.4. HRMS m/z 771.3988 $[(M+H)^+$ calcd for $C_{43}H_{55}N_4O_9^+$ 771.3969]. Compound **35b**: $[\alpha]_D^{22}$ −72.3; ^{1}H NMR (300 MHz, CD_3OD): δ 0.90–0.97 (m, 3H), 1.02 (s, 9H), 1.07–1.35 (m, 7H), 1.53–1.90 (m, 8H), 2.46–2.61 (m, 1H), 2.76–2.88 (m, 1H), 3.69 (s, 3H), 3.96 (s, 3H), 4.15–4.35 (m, 2H), 4.37–4.41 (m, 1H), 4.42–4.47 (m, 1H), 6.02–6.12 (m, 1H), 7.02 (s, 1H), 7.16 (dd, J = 2.47, 9.34 Hz, 1H), 7.32 (s, 1H), 7.40 (d, J = 2.47 Hz, 1H), 7.48–7.58 (m, 3H), 8.03–8.12 (m, 3H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 13.0, 18.8, 25.9, 26.0, 28.8, 29.4, 34.2, 34.8, 36.3, 39.9, 48.8, 50.5, 51.1, 54.8, 57.9, 60.5, 82.8, 99.0, 106.0, 115.1, 118.2, 123.1, 127.8, 127.9, 128.7, 129.0, 129.5, 136.7, 139.8, 142.8, 150.6, 160.1, 162.0, 162.2, 164.7, 172.1, 173.5. HRMS m/z 771.4006 $[(M+H)^+$ calcd for $C_{43}H_{55}N_4O_9^+$ 771.3969].

5.9. Modeling

The alignments of **39a** and **39b** with compound **1** were obtained from energy minimizations and GASP™ (Genetic Algorithm Similarity Program) included in Sybyl 7.1 (Tripos Inc. 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA). The compounds (**1**, **39a**, and **39b**) were built in an extended conformation and minimized. The alignment of **39a** and **39b** with **1**, respectively, was run in 10 cycles for each compound. The final results from the alignments are shown in Figures 8 and 9.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.10.044.

References and notes

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