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Fused-ring structure of decahydroisoquinolin as a novel scaffold for SARS 3CL protease inhibitors

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ABSTRACT

The design and evaluation of a novel decahydroisoquinolin scaffold as an inhibitor for severe acute respiratory syndrome (SARS) chymotrypsin-like protease (3CL^{pro}) are described. Focusing on hydrophobic interactions at the S_2 site, the decahydroisoquinolin scaffold was designed by connecting the P_2 site cyclohexyl group of the substrate-based inhibitor to the main-chain at the α-nitrogen atom of the P_2 position via a methylene linker. Starting from a cyclohexene enantiomer obtained by salt resolution, *trans*-decahydroisoquinolin derivatives were synthesized. All decahydroisoquinolin inhibitors synthesized showed moderate but clear inhibitory activities for SARS 3CL^{pro}, which confirmed the fused ring structure of the decahydroisoquinolin functions as a novel scaffold for SARS 3CL^{pro} inhibitor. X-ray crystallographic analyses of the SARS 3CL^{pro} in a complex with the decahydroisoquinolin inhibitor revealed the expected interactions at the S_1 and S_2 sites, as well as additional interactions at the *N*-substituent of the inhibitor.

1. Introduction

Although the primary epidemic of SARS (Severe Acute Respiratory Syndrome)[1–3] affecting about 8500 patients and 800 dead was eventually brought under control, the recent identification of a SARS CoV (coronavirus)-like virus in Chinese bats[4,5] and of a novel coronavirus MERS-CoV (Middle East Respiratory Syndrome Corona Virus, previously known as human CoV-EMC) raise the possibility of a reemergence of SARS or related diseases.[6,7] Since no effective therapy exists for these viral infections, developing anti-SARS agents against future outbreaks remains a formidable challenge.

SARS is a positive-sense, single-stranded RNA virus featuring the largest known viral RNA which produces two large proteins with overlapping sequences, polyproteins 1a (~450 kDa) and 1ab (~750 kDa).[8–10] SARS 3CL (chymotrypsin like) protease (3CL^{pro}) is a key enzyme to cleave the polyproteins to yield functional polypeptides.[11,12] The 3CL^{pro} is a cysteine protease containing a Cys-His catalytic dyad and it exists as a homodimer; each monomer contains the catalytic dyad at each active site. Due to its functional importance in the viral life cycle, 3CL^{pro} is considered an attractive target for the structure-based design of drugs against SARS. Thus, numerous inhibitors of 3CL^{pro} have been reported including peptide-mimics[13–17] and small molecules derived from natural products,[18–20] anti-viral agents,[21,22] anti-malaria agents,[23] or high throughput screening.[24–27]

In the course of our own studies on the SARS 3CL^{pro} and its inhibitors,[28] we found that the addition of an extra sequence to the N- or C-terminus of the mature SARS 3CL^{pro} lowered the catalytic activity and that the mature SARS 3CL^{pro} is sensitive to degradation at the 188Arg/189Gln site, which causes a loss of catalytic activity. The stability of 3CL^{pro} is dramatically increased by mutating the Arg at the 188 position to Ile. The enzymatic efficiency of the R188I mutant was increased by a factor of more than 1×10^6. The potency of the mutant protease makes it possible to quantitatively evaluate substrate-based peptide-mimetic inhibitors easily by conventional HPLC using a substrate peptide containing no fluorescence derivatives. The evaluations revealed that a peptide aldehyde covering the P-site sequence of substrate, Ac-Ser-Ala-Val-Leu-NHCH($CH_2CH_2CON(CH_3)_2$)–CHO, inhibits the SARS 3CL^{pro} with an IC_{50} value of 37 μM. Systematic modification guided by the X-ray crystal structure of a series of peptide-mimics in a complex with R188I SARS 3CL^{pro} resulted in **1** with an IC_{50} value of 98 nM (Fig. 1).[13] All of the side-chain structures of **1** differed from the substrate sequence except at the P_3 site, where the side-chain was directed outward. Kinetic inhibition data for **1**

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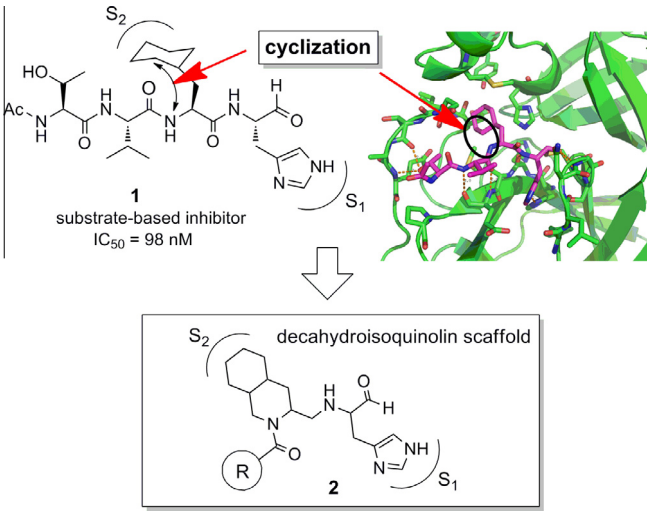


Figure 1. Design of a decahydroisoquinolin scaffold.

obtained from Lineweaver–Burk plots suggested that inhibitors containing an aldehyde at the C-terminus can be expected to function as competitive inhibitors.

In the present study, we designed a novel non-peptide inhibitor focusing on the interactions at the S_1 and S_2 sites of the 3CL^{pro} Confirmed to be critical to make the **1** potent competitive inhibitor. Among the key interactions clarified by X-ray crystallographic study, we focused on hydrophobic interactions at the cyclohexyl side-chain to design a novel inhibitor scaffold. Thus, the cyclohexyl ring is connected to the main-chain at an α-nitrogen atom of the P_2 position Cha (cyclohexylalanine) via a methylene linker to yield compound **2** (Fig. 1). The resulting decahydroisoquinolin scaffold of **2** is expected to keep the hydrophobic interactions at the cyclohexyl ring of the substrate-based inhibitor at the S_2 pocket. In addition, the resulting decahydroisoquinolin scaffold arranges the P_1 site imidazole and active site functional aldehyde at each required position, giving the fused-ring structure of decahydroisoquinolin as a scaffold for a novel inhibitor. The acyl substituent on the nitrogen in the decahydroisoquinolin scaffold may add an extra position for the interactions with the 3CL^{pro}.

2. Results and discussion

2.1. Chemistry

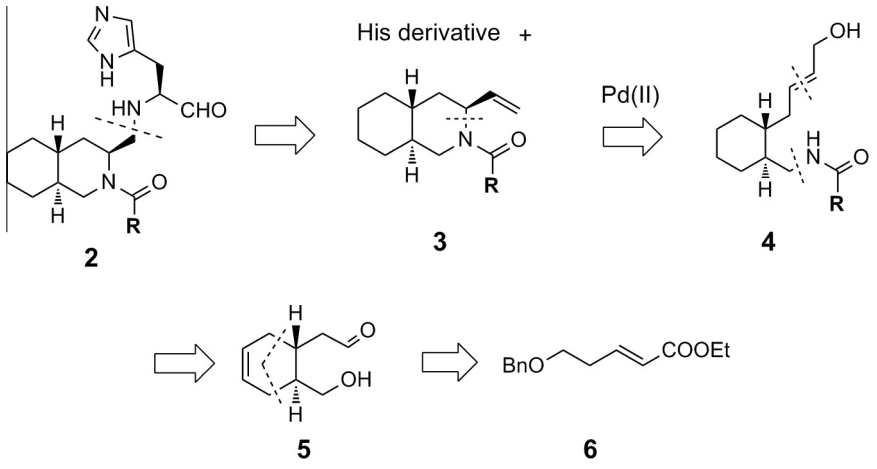
The retro synthetic route for the desired decahydroisoquinolin derivative **2** is shown in Scheme 1. The P_1 site His derivative could be introduced by a reductive amination reaction using an aldehyde derivative prepared by oxidative cleavage of the olefin bond of **3**. The *trans*-decahydroisoquinolin scaffold of **3** could be constructed via Pd-mediated stereoselective intra-molecular cyclization[29] by nucleophilic attack of a nitrogen atom to the Pd-activated olefin moiety of an allyl alcohol of **4**. The olefin structure of **4** could be constructed by a Horner–Emmons reaction utilizing an aldehyde of precursor **5**, and the amino group of **4** could be introduced by a Mitsunobu reaction to the alcohol of **5**. The six-membered ring structure of **5** could be constructed by a Diels–Alder reaction of known ester **6**[30] with butadiene.

Thus, the key intermediates **12** and **13**, a precursor of the Pd-mediated cyclization, were prepared according to the route shown in Scheme 2. The known ester **6** was first reacted with butadiene to construct the six-membered ring structure to yield **7** as an enantiomer mixture of 1,6-*trans*-substituted cyclohexene. The product was reduced with LAH and the resulting alcohol was then protected as *tert*-butyldiphenylsilyl ether to give **8**. The benzyl group was removed by catalytic hydrogenation, which reduced the cyclohexene to cyclohexane at the same time. The resulting hydroxyl group was then oxidized with PCC and the resulting aldehyde was then reacted with $(EtO)_2P(O)CH_2COOEt$ to yield **9**. The ethyl ester of **9** was reduced with DIBALH and the resulting alcohol was protected as acetyl ester to give **10**. After treatment with TBAF, the resulting alcohol was converted to the azide derivative **11** by a Mitsunobu reaction. Since the product **11** was rather unstable, **11** was immediately reduced to the corresponding amine. Without further purification, the amine derivative was coupled with *p*-phenylbenzoic acid using HBTU to yield **12** as an enantiomer mixture. Coupling with *p*-bromobenzoic acid was similarly conducted to yield a related derivative **13**.

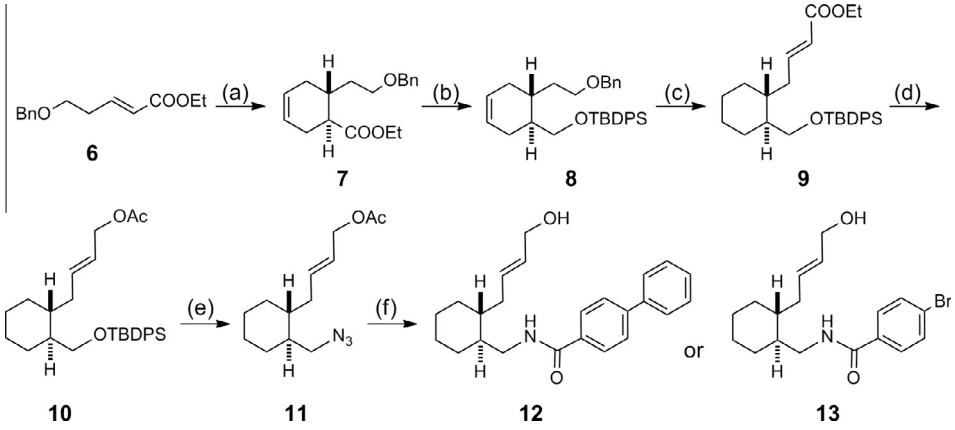
Construction of the decahydroisoquinolin scaffold was achieved as shown in Scheme 3. $(CH_3CN)_2PdCl_2$-mediated cyclization of **12/13** gave the desired *trans*-decahydroisoquinolin derivative **14/15** as a major product. The product was an enantiomer mixture which was thought to have the relative configuration of **14/15** due to the cyclization through a less hindered Pd-chelated intermediate. Thus, the vinyl substituent of the product **14/15** was thought to be axial, which was clearly confirmed by X-ray crystallographic studies of the inhibitor in a complex with the R188I mutant SARS 3CL^{pro} as discussed below. The olefin bond of **14/15** was oxidatively cleaved by the treatment with $K_2O_sO_2(OH)_4$ followed by $NaIO_4$ to yield aldehyde **16/17**. Reductive amination by H-His(Trt)-N(OCH_3)-CH_3 gave the coupling products **18** and **20** or **19** and **21** as a 1:1 diastereomer mixture which was separable on a reversed-phase column (YMC Pack ODS) by analytical HPLC (Fig. S1). The diastereomers could also be separated by conventional silica-gel column chromatography to yield diastereomers **18** and **20** or **19** and **21**, each having single peak on the above reversed-phase column. Each separated diastereomer was then treated with TFA to cleave the Trt group at the imidazole ring, and the product was reduced with DIBALH to yield the desired aldehyde **22/23** or **24/25**. Although the absolute configuration of each product was not determined at this stage, the purity of each product was confirmed by analytical HPLC. Since moderate but clear inhibitory activities were observed in a preliminary evaluation on the inhibitory potency of **22** and **24**, the identification of the stereo-structure was then conducted.

To separately prepare the above diastereomers and estimate the absolute configurations, cyclohexene carboxylic acid obtained by a Diels–Alder reaction was converted to a salt with (*R*)- or (*S*)-α-methylbenzylamine and resolved according to the literature procedure for (1*R*/6*S*,1*S*/6*R*)-6-(2-bromophenyl)cyclohex-3-ene-1-carboxylic acid **26**[31] (Scheme 4). Resolution of a carboxylic acid derived from compound **7** and compound **29** having the corresponding *p*-bromobenzyl group gave compounds showing the same polarimetric characters as the literature compounds.[31] (−) Carboxylic acid **27** or **30** was obtained by salt formation with (*R*)-α-methylbenzylamine and following salt-liberation with HCl, whereas the salt with (*S*)-α-methylbenzylamine gave (+) carboxylic acid **28** or **31**. Compared with the literature values, these results strongly suggest that **27** and **30** would have (1*R*,6*S*) and **28** and **31** would have (1*S*,6*R*) absolute configurations. Optical purity of each enantiomer was further confirmed using a chiral column (YMC CHIRAL Amylose-C) by HPLC (Fig. S2). Since the chemical yield from the *p*-bromobenzyl derivative **29** was superior to the benzyl derivative **7**, enantiomer **30** or **31** was used as the starting compound for the separate synthesis of decahydroisoquinolin diastereomers.

The separated (1*S*,6*R*) enantiomer **31** was then used to synthesize the corresponding decahydroisoquinolin diastereomer **40** or **41** using basically the same route as above (Scheme 5i). (1*R*,6*S*) Enantiomer **30** was also employed for the syntheses of diastereo-



Scheme 1. Retro synthetic route for the decahydroisoquinolin derivative.



Scheme 2. Synthesis of intermediate **12** or **13**. Configurations in the racemic compounds **7**–**13** indicate the relative 1,6-*trans* configurations. Reagents: (a) 1,3-butadiene; (b) (1) LAH, (2) TBDPS-Cl/imidazole; (c) (1) H_2/Pd(OH)$_2$-C (2) PCC (3) $(EtO)_2P(O)CH_2COOEt$/NaH; (d) (1) DIBALH (2) Ac_2O/pyridine/DMAP; (e) (1) TBAF, (2) $(EtO)_2P(O)N_3$/DIAD/PPh_3; (f) (1) LAH, (2) 4-phenylbenzoic acid or 4-bromobenzoic acid/HBTU/DIPEA.

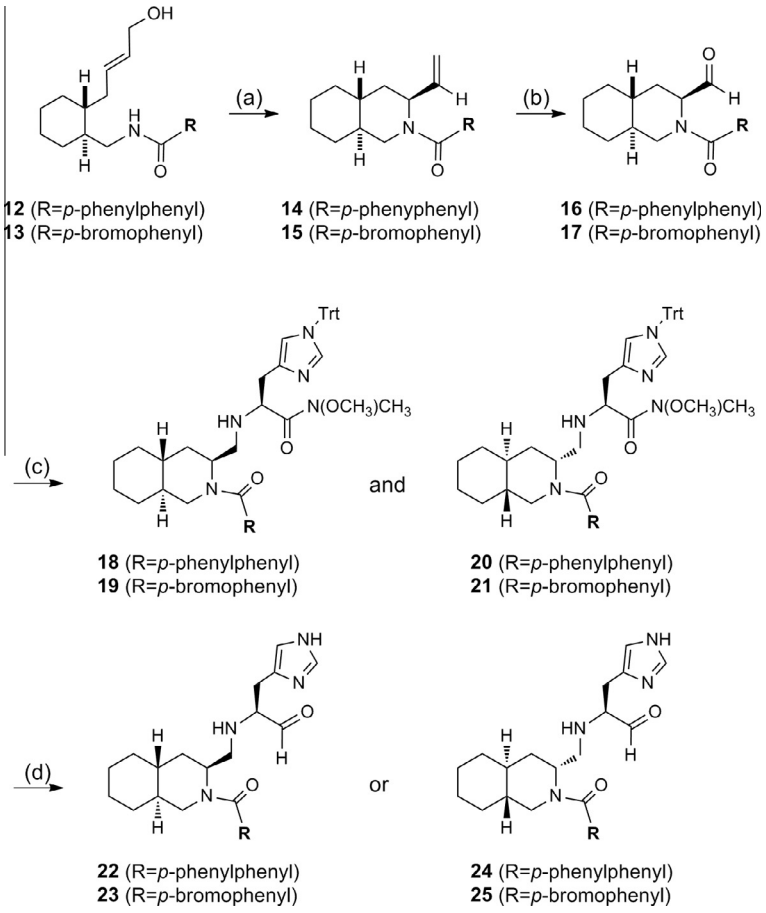
mer **44** or **45** (Scheme 5ii). The protected intermediate **38** (R = *p*-phenylphenyl) from **31** and the diastereomer **42** (R = *p*-phenylphenyl) from (1*R*,6*S*) enantiomer **30** were co-eluted with a previously synthesized diastereomixture of **18** and **20** on a reversed-phase column (YMC Pack ODS). Intermediate **38** had the same retention time as **18**, whereas intermediate **42** had the same retention time as **20** (Fig. S3). The comparison was also conducted on **39** and **43** having a *p*-bromophenyl *N*-substituent with the corresponding diastereomers **19** and **21**, and the same results as above were obtained (Fig. S4). These results clearly demonstrated that the two diastereomers **18** and **20** were derived from the *trans*-decahydroisoquinolin structure constructed from enantiomer **7**. Each protected diastereomer **38**/**39** and **42**/**43** thus synthesized was converted to the desired derivatives **40**/**41** and **44**/**45** without difficulty. Several analogs shown in Table 1 containing different *N*-acyl substituents of the decahydroisoquinolin scaffold were also prepared using the same synthetic route (Fig. S5).

2.2. Inhibitory activity

Digestion of the substrate peptide with R188I SARS 3CL^{pro} in the presence of decahydroisoquinolin derivatives of different concentrations was conducted according to the published procedure.[13] The inhibitory activities were evaluated based on IC_{50} values calculated from the decrease in the substrate digested by R188I SARS 3CL^{pro}; a typical sigmoidal curve used for estimation of the IC_{50} value is shown in Figure S6. As summarized in Table 1, synthesized decahydroisoquinolin derivatives all showed inhibitory activities for the mutant 3CL^{pro}. The results strongly suggest that the decahydroisoquinolin fused-ring can function as an inhibitor scaffold. Comparison of IC_{50} values of *trans*-decahydroisoquinolin diastereomers in *N*-4-phenylbenzoyl derivatives (**40** vs **44**) or *N*-4-bromobenzoyl derivative (**41** vs **45**) clearly showed that the (4a*R*,8a*S*) isomer is more potent than (4a*S*,8a*R*) isomer. The results suggest the importance of the interaction at the S_2 pocket of the mutant 3CL^{pro}. It was also demonstrated that a series of the *N*-benzoyl derivative was more potent than *N*-4-phenylbenzoyl derivatives. Substitution at the 4-position of the benzoyl substituent in **48** with halogen showed no significant effect on the inhibitory activity (**41** and **49**), whereas substitution at the 4-position of the phenyl group in the *N*-biphenylacyl derivative **40** gave a slightly more potent inhibitor than 2- or 3-substituted biphenyl derivatives (**46** and **47**). The results suggest that the substituent on the nitrogen atom of the decahydroisoquinolin scaffold may have some interactions with R188I SARS 3CL^{pro}.

2.3. Evaluation of the interactions

To clarify the interactions of a newly synthesized decahydroisoquinolin inhibitor with R188I SARS 3CL^{pro}, the structure of



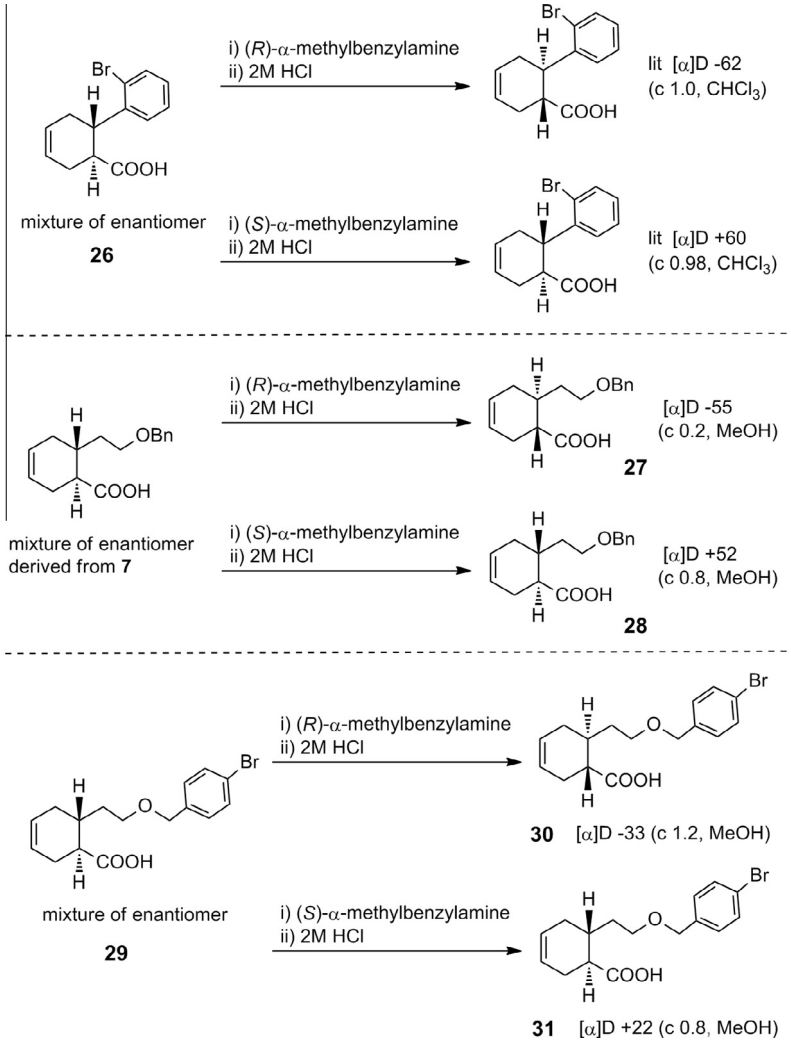
Scheme 3. Construction of the decahydroisoquinolin scaffold. Reagents: (a) $(CH_3CN)_2PdCl_2$; (b) $K_2OsO_2(OH)_4/NaIO_4$; (c) (1) H-His(Trt)-$N(OCH_3)CH_3/NaBH_3CN$; (d) (1) TFA, (2) DIBALH.

the protease in a complex with the inhibitor was revealed by X-ray crystallography. Subsequently, a co-crystal of the inhibitor with $3CL^{pro}$ was prepared and analyzed. Structures of the $3CL^{pro}$ in a complex with inhibitors **40**, **41**, and **44** were refined to resolutions of 1.60 Å, 2.42 Å, and 1.89 Å, respectively (PDB code 4TWY, 4TWW, and 4WY3). The data obtained are summarized in Table 2.

The overall structure of the $3CL^{pro}$ in complex with inhibitor **41** (IC_{50} = 63 μM) was first compared with the substrate-based inhibitor **1** (PDB code 3ATW) (Fig. 2). Basically, the decahydroisoquinolin inhibitor **41** was at the active site cleft of the $3CL^{pro}$ as observed in the highly potent inhibitor **1**. The aldehyde group and imidazole ring of His-al, as well as the decahydroisoquinolin structure of **41**, had an almost identical conformation with **1** and similarly interacted with $3CL^{pro}$. In contrast, the direction of the *p*-bromobenzoyl group was outward from $3CL^{pro}$ and opposite to the P_3 to P_4 sites of **1**. The *N*-*p*-bromobenzoyl group, however, was at the surface of $3CL^{pro}$, where additional hydrophobic interaction with Met of the $3CL^{pro}$ may be possible (Fig. S7).

The carbonyl carbon of the aldehyde group in **41** was detected at a distance of 2.43 Å from the active center thiol of Cys-145, and its electron density could be fitted to an sp^2 carbonyl carbon as in **1** (Fig. 3i). The results suggest that the decahydroisoquinolin inhibitor would function as a competitive inhibitor as do the peptide-aldehyde inhibitor **1**.[13] It was clearly confirmed that the decahydroisoquinolin scaffold of **41** took a *trans*-fused (4a*R*,8a*S*) configuration, as expected from the salt-resolution of enantiomixture **29**. It was also confirmed that the P_1 His-al substituent on the decahydroisoquinolin scaffold took an axial-configuration, as expected from the Pd(II)-mediated cyclization. The decahydroisoquinolin scaffold of **41** was inserted into a large S_2 pocket created by His-41, Met-49, Met-165, and Asp-187, as in the case of a parent peptide aldehyde inhibitor, and most of the S_2 pocket was occupied by the fused-ring structure of decahydroisoquinolin (Fig. 3i). The nitrogen atom of the P_1 site imidazole of **41** formed a hydrogen bond with the imidazole nitrogen of His-163, resulting in close fitting at the other side of the S_1 pocket formed from the Phe-140, Leu-141, and Glu-166 side chains of the protease (Fig. 3ii). These interactions, especially of the decahydroisoquinolin scaffold in the S_2 pocket, function to hold the P_1 site imidazole and terminal aldehyde tightly inside the active site cleft, which resulted in the compact fitting of the novel scaffold to the $3CL^{pro}$.

To evaluate the effects of absolute configuration of the decahydroisoquinolin scaffold, structures of the $3CL^{pro}$ in complex with (4a*R*,8a*S*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **40** and (4a*S*,8a*R*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **44** were compared (Fig. 4i). In both inhibitors, the P_1 site imidazole ring and the terminal aldehyde group had nearly the same interactions as in the (4a*R*,8a*S*)-*N*-bromobenzoyl decahydroisoquinolin inhibitor **41** described above. Due to the configuration change at the decahydroisoquinolin moiety, however, the (4a*S*,8a*R*) decahydroisoquinolin scaffold was clearly twisted compared to the (4a*R*,8a*S*) decahydroisoquinolin in the S_2 pocket (Fig. 4ii). This conformation change of the decahydroisoquinolin scaffold transferred to the direction of the *N*-substituent. Thus, the substituent of (4a*R*,8a*S*) decahydroisoquinolin **40** took nearly the same conformation as the *N*-*p*-bromobenzoyl inhibitor **41** located on the surface of the $3CL^{pro}$, whereas the substituent of (4a*S*,8a*R*) decahydroisoquinolin directed outside from the protease surface. These



Scheme 4. Resolution by salt formation.

conformational differences at the *N*-substituent, as well as the interactions at the S_2 pocket, explain the discrepancy in the inhibitory activity between (4a*R*,8a*S*) and (4a*S*,8a*R*) decahydroisoquinolin inhibitors (**41** vs **44**).

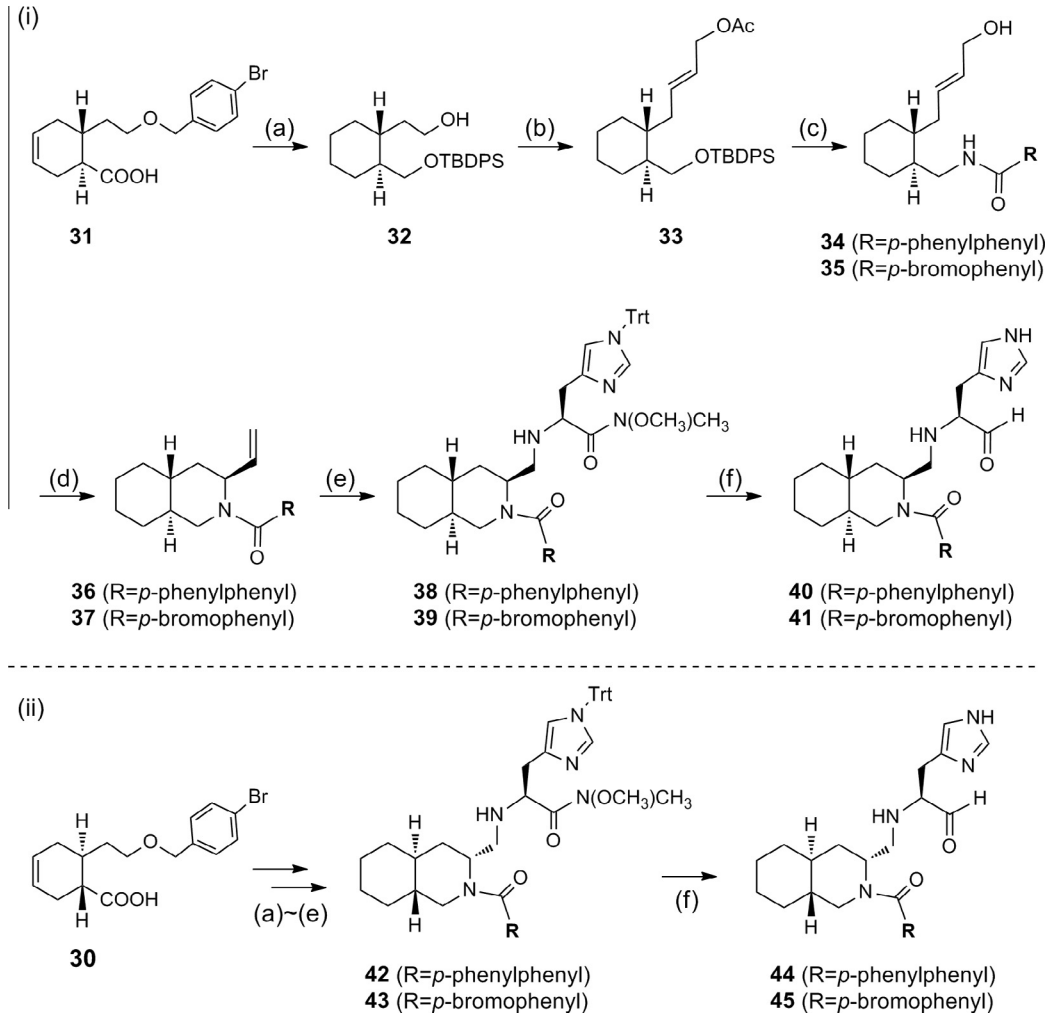
3. Conclusion

A novel non-peptide inhibitor based on the interactions at the S_1 and S_2 sites of SARS $3CL^{pro}$ was designed and synthesized. Focusing on cleavage site interaction at the S_1 site and hydrophobic interaction at the S_2 site, a decahydroisoquinolin scaffold was designed. Using a cyclohexene enantiomer obtained by salt resolution using chiral amine, the *trans*-decahydroisoquinolin derivative was synthesized as an enantiomer. Several analogs containing different *N*-substituents were also prepared similarly. All decahydroisoquinolin inhibitors showed moderate but clear inhibitory activities for SARS $3CL^{pro}$, which confirmed that the fused ring structure of the decahydroisoquinolin scaffold functions as an inhibitor for SARS $3CL^{pro}$. By X-ray crystallographic studies, it was confirmed that the decahydroisoquinolin inhibitors were at the active site cleft of $3CL^{pro}$, as observed in the highly potent peptide–aldehyde inhibitor. The decahydroisoquinolin scaffold was inserted into a large S_2 pocket and occupied most of the pocket. The P_1 site imidazole was inserted into the S_1 pocket as expected. These interactions were effective to hold the terminal aldehyde tightly inside the active site cleft, which resulted in the compact fitting of the novel scaffold to $3CL^{pro}$. The acyl substituent on the nitrogen in the decahydroisoquinolin scaffold was at the surface of the $3CL^{pro}$, where additional interactions with the $3CL^{pro}$ may be possible. Evaluations on the analogs focusing on the interactions at the *N*-substituent are now underway.

4. Experimental

4.1. General

All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. All commercial reagents were of the highest purity available. Analytical TLC was performed on silica gel (60 F-254, 0.25 mm Plates). Column chromatography was carried out on Wakogel C-200E (particle size, 75–150 μm) or Wakogel FC-40 (particle size, 20–40 μm). ^1H NMR spectra were recorded in $CDCl_3$ (unless otherwise stated) on agilent UNITY INOVA 400 NB, JEOL JNM-ECS 400, Bruker AM-300, or JEOL JNM-LA 500 spectrometers. Chemical shifts are expressed in ppm relative to tetramethylsilane (0 ppm) or $CHCl_3$ (7.28 ppm). The coupling constants are given in Hz. ^{13}C NMR spectra were recorded on the same spectrometers at 100 or 125 MHz, using the central resonance of $CDCl_3$ (δ_C 77.0 ppm) as the internal reference unless otherwise stated. High-resolution mass spectra



Scheme 5. Construction of the decahydroisoquinolin scaffold starting from the separated enantiomer. Reagents: (a) (1) IBCF/$NaBH_4$, (2) TBDPS-Cl/imidazole, (3) H_2/Pd-C/sat. $NaHCO_3$ aq.; (b) (1) PCC, (2) $(EtO)_2P(O)CH_2COOEt$/NaH, (3) DIBALH, (4) Ac_2O/pyridine/DMAP; (c) (1) TBAF, (2) $(EtO)_2P(O)N_3$/DIAD/PPh_3, (3) LAH, (4) 4-phenylbenzoic acid or 4-bromobenzoic acid/HBTU/DIPEA; (d) $(CH_3CN)_2PdCl_2$; (e) (1) $K_2OsO_2(OH)_4$/$NaIO_4$, (2) H-His(Trt)-N$(OCH_3)CH_3$/$NaBH_3CN$; (f) (1) TFA, (2) DIBALH.

(HRMS) were obtained on a JMS-HX-110A (FAB), and Shimadzu LCMS-IT-TOF (ESI). Low-resolution mass spectra (LRMS) were obtained on a Shimadzu LCMS-2010EV (ESI). Optical rotations were determined with a HORIBA SEPA-300 polarimeter. Preparative HPLC was performed using a COSMOSIL 5C18-ARII column (20×250 mm) with a linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 5.0 mL/min on a HITACHI LaChrom system (OD, 254 nm). For analytical HPLC, unless otherwise noted, a COSMOSIL 5C18-ARII column (4.6×150 mm) was employed with a linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 0.9 mL/min on a HITACHI LaChrom system (OD, 254 nm). The purity of the test compounds was determined by analytical HPLC. All test compounds showed $\geqslant$95% purity.

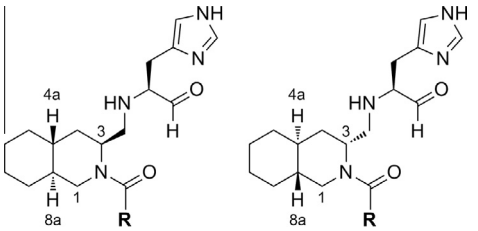
4.1.1. (1*S*/*R*,6*R*/*S*)-Ethyl 6-[2-(benzyloxy)ethyl]cyclohex-3-enecarboxylate 7

To a solution of 1,3-butadiene (20 wt% solution in hexane, 17 mL, 40 mmol) was added ester **6** (2.34 g, 10.0 mmol), heated at 250 °C for 60 h. After the reaction mixture was cooled to room temperature, water was added and the whole was extracted with AcOEt. The organic layer was washed with 1 M HCl and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give **7** (1.87 g, 65%) as a yellow pale oil. ^{1}H NMR (400 MHz): δ = 7.36–7.31 (m, 4H), 7.29–7.26 (m, 1H), 5.64 (m, 2H), 4.51 (d, J = 11.6 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.14 (q, J = 7.2 Hz, 2H), 3.54–3.50 (m, 2H), 2.41–2.35 (m, 1H), 2.31–2.20 (m, 3H), 2.09–2.04 (m, 1H), 1.84–1.73 (m, 2H), 1.54–1.45 (m, 1H), 1.25 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz): δ = 175.8, 138.5, 128.3, 127.6, 127.5, 125.7, 124.7, 72.8, 67.9, 60.2, 45.3, 33.7, 32.4, 29.9, 28.0, 14.2; HRMS (EI) calcd for $C_{18}H_{24}O_3$ $[M]^+$: 288.1725. Found: 288.1722.

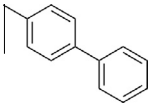
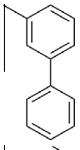
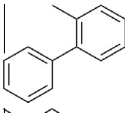
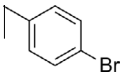
4.1.2. {(1*S*/*R*,6*R*/*S*)-6-[2-(Benzyloxy)ethyl]cyclohex-3-en-1-yl}methanol

To a suspension of $LiAlH_4$ (387 mg, 10.2 mmol) in ether (30 mL) was added **7** (1.47 g, 5.12 mmol) at 0 °C. After being stirred for 15 min at 0 °C, the reaction was quenched with H_2O. The mixture was warmed to room temperature and filtered through Celite and a silica gel layer, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to give a title alcohol (1.25 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.37–7.32 (m, 4H), 7.30–7.26 (m, 1H), 5.65–5.57 (m, 2H), 4.51 (s, 2H), 3.66 (dd, J = 10.8, 6.0 Hz, 1H), 3.62–3.48 (m, 3H), 2.14–2.09 (m, 2H), 2.01–1.75 (m, 5H), 1.66–1.59 (m, 1H), 1.55–1.48 (m, 1H); ^{13}C NMR (100 MHz): δ = 138.3, 128.4, 127.7, 127.6, 125.8, 125.5, 73.1, 68.5, 65.0, 39.7, 32.9, 31.0, 29.5, 26.7; HRMS (EI) calcd for $C_{16}H_{22}O_2$ $[M]^+$: 246.1620. Found: 246.1618.

Table 1
Inhibitory activities of the decahydroisoquinolin derivatives



decahydroisoquinolin inhibitor

R	IC_{50}	
	(3*S*,4a*R*,8a*S*)	(3*R*,4a*S*,8a*R*)
	**40** 108 μM	**44** 240 μM
	**46** 135 μM	
	**47** 135 μM	
	**48** 68 μM	
	**41** 63 μM	45 175 μM
	**49** 57 μM	

4.1.3. ({(1*S*/*R*,6*R*/*S*)-6-[2-(Benzyloxy)ethyl]cyclohex-3-en-1-yl}methoxy)(*tert*-butyl)diphenylsilane 8

TBDPS-Cl (3.6 mL, 13.1 mmol) was added to a solution of the above alcohol (2.92 g, 11.9 mmol) and imidazole (1.21 g, 17.8 mmol) in CH_2Cl_2 (30 mL) and the mixture was stirred for 16 h. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20:1) to give 8 (5.76 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.67–7.65 (m, 4H), 7.43–7.30 (m, 10H), 7.28 (m, 1H), 5.63–5.54 (m, 2H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.45 (d, *J* = 12.0 Hz, 1H), 3.68 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.62 (dd, *J* = 9.8, 7.0 Hz, 1H,), 3.54–3.45 (m, 2H), 2.17–2.06 (m, 2H), 2.02–1.95, (m, 1H), 1.87–1.80 (m, 2H), 1.73–1.67 (m, 2H), 1.51–1.42 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (100 MHz): δ = 138.6, 135.62, 135.61, 133.98, 133.95, 129.5, 128.3, 127.58, 127.56, 127.4, 125.8, 125.4, 72.9, 68.6, 65.9, 39.6, 32.9, 30.9, 29.1, 26.9, 26.7, 19.3; HRMS (FAB) calcd for $C_{32}H_{41}O_2Si$ $[M+H]^+$: 485.2876. Found: 485.2870.

4.1.4. 2-[(1*R*/*S*,6*S*/*R*)-6-{[(*tert*-Butyldiphenylsilyl)oxy]methyl}-cyclohexyl]ethanol

To a solution of **8** (3.40 g, 7.01 mmol) in CH_3OH/AcOEt/CH_2Cl_2 (10:10:1, 21 mL) $Pd(OH)_2$–C (610 mg) was added and stirred under a hydrogen gas atmosphere at room temperature for 12 h. The mixture was filtered through Celite and a silica gel layer, and the filtrate was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give a title alcohol (2.78 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.68–7.65 (m, 4H), 7.45–7.36 (m, 6H), 3.68–3.54 (m, 4H), 1.78–1.66 (m, 5H), 1.37–1.18 (m, 7H), 1.06 (s, 9H), 1.01–0.96 (m, 1H); ^{13}C NMR (100 MHz): δ = 135.69, 135.66, 133.92, 133.90, 129.55, 129.54, 127.60, 127.57, 66.6, 61.1, 44.5, 36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3; HRMS (FAB) calcd for $C_{25}H_{37}O_2Si$ $[M+H]^+$: 397.2563. Found: 397.2569.

Table 2
Data collection and refinement statistics for the R188I SARS 3CL protease in complexes with compounds **40**, **41**, and **44**

PDB ID	4TWY In complex with **40**	4TWW In complex with **41**	4WY3 In complex with **44**
Space group	*C*121	*P*1	*C*121
Unit cell parameters			
Length *a*	107.83	54.89	108.11
Length *b*	82.128	59.52	81.82
Length *c*	53.271	68.40	53.24
Angle α	90	93.11	90
Angle β	104.98	102.82	104.69
Angle γ	90	107.30	90
Resolution	1.60	2.42	1.89
Observations			
Unique observations	57,490	31,213	49,270
Redundancy	4.2	1.75	4.1
Completeness	88.6	94.3	93.2
Mean I/σ (I)	2.18 (at 1.60 Å)	9.96 (at 2.42 Å)	2.49 (at 1.89 Å)
R merge	0.08	0.05	0.07
Refinement			
Resolution range	25.3–1.60	66.1–2.42	30.6–1.89
R_{cryst}	0.29	0.23	0.27
R_{free}	0.32	0.26	0.30
RMSZ from ideal			
Bond length (Å)	0.93	0.73	0.86
Bond angle (°)	0.96	0.86	0.90

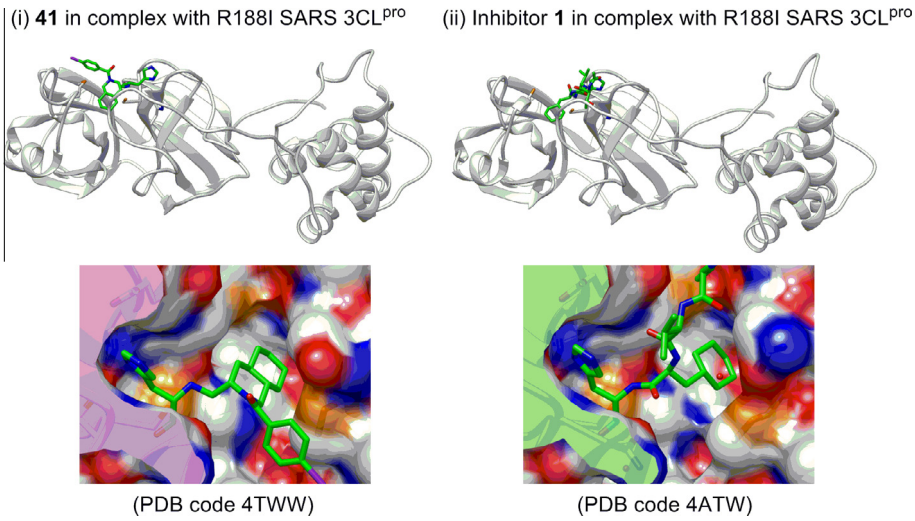


Figure 2. (i) X-ray structure of the inhibitor **41** in complex with R188I SARS 3CL^{pro} (PDB code 4TWW) and molecular graphics image around the P_1 and P_2 sites. (ii) X-ray structure of the inhibitor **1** in complex with R188I SARS 3CL^{pro} (Ref. 13; PDB code 4ATW) and molecular graphics image around the P_1 and P_2 sites.

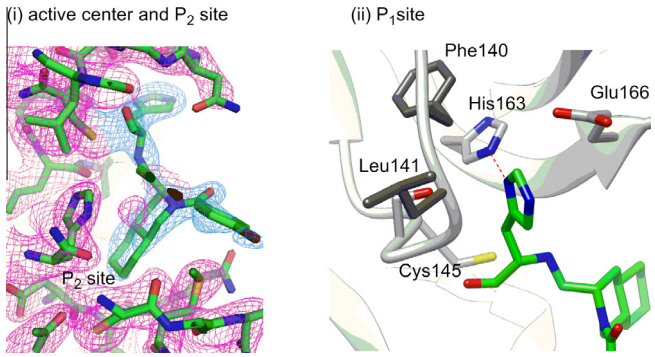


Figure 3. (i) Interactions of the inhibitor **41** with R188I SARS 3CL^{pro} at the active center and P_2 site. (ii) Interactions at the P_1site.

4.1.5. (*E*)-Ethyl 4-[(1*R*/*S*,2*S*/*R*)-2-{[(*tert*-butyldiphenylsilyl)oxy]methyl}cyclohex-1-yl]but-2-enoate 9

To a solution of PCC (3.45 g, 16.0 mmol) and Celite (3.5 g) in CH_2Cl_2 (20 mL), above alcohol (2.50 g, 6.30 mmol) was added at 0 °C. The temperature was gradually raised to room temperature. After being stirred for 6 h, the reaction mixture was filtered through a silica gel layer and the filtrate was concentrated. This compound was immediately used for the next step without purification. Triethylphosphonoacetate (1.5 mL, 7.7 mmol) was added to a suspension of NaH [60% in mineral oil (308 mg, 7.70 mmol)] in THF (10 mL) at −20 °C under an argon gas atmosphere and the mixture was stirred for 0.5 h. The oxidized product was added drop-wise to the reaction mixture and stirred for 1.5 h at −20 °C. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20:1) to give **9** (2.70 g, 92%, 2 steps) as a colorless oil. ^{1}H

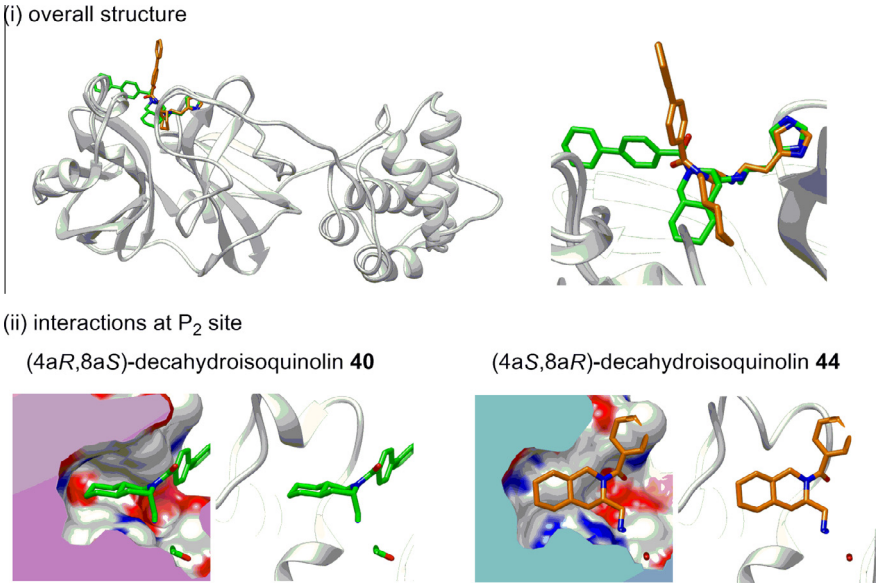


Figure 4. (i) X-ray structures of R188I SARS 3CL^{pro} in complex with (4a*R*/8a*S*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **40** (PDB code 4TWY) and (4a*S*/8a*R*)-*N*-4-phenylbenzoyl decahydroisoquinolin inhibitor **44** (PDB code 4WY3). (ii) Interactions of **40** and **44** at the P_2 site.

NMR (400 MHz): δ = 7.67–7.64 (m, 4H) 7.45–7.36 (m, 6H), 6.91 (ddd, J = 15.4, 8.8, 6.4 Hz, 1H), 5.72 (d, J = 15.6 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.63–3.57 (m, 2H), 2.38–2.32 (m, 1H), 1.97 (td, J = 14.8, 8.1 Hz, 1H), 1.79–1.76 (m, 1H), 1.71–1.69 (m, 4H), 1.54–1.49 (m, 1H), 1.32–1.18 (m, 4H), 1.29 (t, J = 7.2 Hz, 3H), 1.05 (s, 9H), 1.03–0.97 (m, 1H); ^{13}C NMR (100 MHz): δ = 166.6, 148.2, 135.62, 135.61, 133.82, 133.80, 129.59, 129.55, 127.62, 127.59, 122.4, 66.2, 60.1, 43.9, 37.8, 36.4, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3, 14.3; HRMS (FAB) calcd for $C_{29}H_{40}NaO_3Si$ [M+Na]$^+$: 487.2644. Found: 487.2651.

4.1.6. (*E*)-4-[(1*R*/*S*,2*S*/*R*)-2-{[(*tert*-Butyldiphenylsilyl)oxy]-methyl}cyclohexyl]but-2-en-1-ol

To a solution of **9** (1.92 g, 4.13 mmol) in CH_2Cl_2 (20 mL), DIBALH (1.0 mol/L solution in hexane, 12.4 mL, 12.4 mmol) was added at −78 °C. After being stirred for 15 min at the same temperature, the reaction was quenched with CH_3OH (5.0 mL). The mixture was warmed to room temperature, and filtered through Celite and a silica gel layer. The filtrate was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to give a title alcohol (1.74 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.68–7.65 (m, 4H), 7.44–7.36 (m, 6H), 5.64–5.48 (m, 2H), 4.04 (d, J = 6.0 Hz, 2H), 3.66 (dd, J = 10.0, 2.8 Hz, 1H), 3.58 (dd, J = 9.8, 5.4 Hz, 1H), 2.23–2.17 (m, 1H), 1.87–1.79 (m, 2H), 1.72–1.69 (m, 3H), 1.43–1.32 (m, 1H), 1.30–1.18 (m, 4H), 1.05 (s, 9H), 1.01–0.94 (m, 1H); ^{13}C NMR (100 MHz): δ = 135.64, 135.63, 134.0, 131.6, 130.2, 129.52, 129.50, 127.58, 127.55, 66.3, 63.8, 43.9, 38.1, 36.2, 31.7, 30.0, 26.9, 26.2, 26.1, 19.4; HRMS (FAB) calcd for $C_{27}h_{38}nao_2si$ [M+Na]$^+$: 445.2539. Found: 445.2541.

4.1.7. (*E*)-4-[(1*R*/*S*,2*S*/*R*)-2-{[(*tert*-Butyldiphenylsilyl)oxy]-methyl}cyclohexyl]but-2-en-1-yl acetate 10

To a solution of above alcohol (1.74 g, 4.11 mmol) in CH_2Cl_2 (20 mL), pyridine (0.50 mL, 6.2 mmol), acetic anhydride (0.59 mL, 6.19 mmol), and DMAP (50 mg, 0.41 mmol) were added at 0 °C. The mixture was stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give **10** (1.81 g, 95%) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.67–7.64 (m, 4H), 7.44–7.36 (m, 6H), 5.71–5.64 (m, 1H), 5.49–5.42 (m, 1H), 4.47 (d, J = 6.4 Hz, 2H), 3.65 (dd, J = 9.8, 3.0 Hz, 1H), 3.57 (dd, J = 10.0, 4.8 Hz, 1H), 2.23–2.18 (m, 1H), 2.05 (s, 3H), 1.87–1.79 (m, 2H), 1.71–1.68 (m, 3H), 1.43–1.35 (m, 1H), 1.30–1.18 (m, 4H), 1.05 (s, 9H), 1.00–0.94 (m, 1H); ^{13}C NMR (100 MHz): δ = 170.9, 135.6, 134.8, 133.94, 133.93, 129.5, 127.6, 125.0, 66.3, 65.3, 43.8, 38.0, 36.3, 31.7, 30.0, 26.9, 26.2, 26.1, 21.0, 19.3; HRMS (FAB) calcd for $C_{29}H_{40}NaO_3Si$ [M+Na]$^+$: 487.2644. Found: 487.2642.

4.1.8. (*E*)-4-[(1*R*/*S*,2*S*/*R*)-2-(Hydroxymethyl)cyclohexyl]but-2-en-1-yl acetate

To a solution of **10** (1.81 g, 3.89 mmol) in THF (20 mL), TBAF [1.0 M solution in THF (7.8 mL, 7.8 mmol)] was added at room temperature. After the mixture was stirred for 12 h, the reaction was quenched with saturated aqueous NH_4Cl and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 6:1) to give a title alcohol (1.03 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz): δ = 5.80–5.72 (m, 1H), 5.60–5.53 (m, 1H), 4.51 (d, J = 6.4 Hz, 2H), 3.69 (dd, J = 10.8, 3.2 Hz, 1H), 3.59 (dd, J = 10.8, 5.6 Hz, 1H), 2.33–2.27 (m, 1H), 2.06 (s, 3H), 2.02–1.90 (m, 1H), 1.81–1.79 (m, 1H), 1.74–1.67 (m, 3H), 1.37–1.11 (m, 5H), 1.05–0.95 (m, 1H); ^{13}C NMR (100 MHz): δ = 170.9, 134.5, 125.3, 65.7, 65.2, 43.8, 38.0, 36.4, 31.7, 29.5, 26.0, 25.8, 21.0; HRMS (FAB) calcd for $C_{13}H_{22}NaO_3$ [M+Na]$^+$: 249.1467. Found: 249.1460.

4.1.9. *N*-({(1*S*/*R*,2*R*/*S*)-2-[(*E*)-4-Hydroxybut-2-en-1-yl]cyclohexyl}methyl)-[1,1′-biphenyl]-4-carboxamide 12

DPPA (2.4 mL, 11 mmol) was added drop-wise to a solution of above alcohol (1.03 g, 4.56 mmol), triphenylphosphine (2.80 g, 10.8 mmol), and DEAD (40% solution in toluene, 4.2 mL, 10.8 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 16 h at the same temperature, and then the reaction mixture was concentrated. The residue was roughly purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give **11**. ^{1}H NMR (400 MHz): δ = 5.77–5.69 (m, 1H), 5.62–5.54 (m, 1H), 4.52 (d, J = 6.0 Hz, 2H), 3.40 (dd, J = 12.0, 3.2 Hz, 1H), 3.25 (dd, J = 12.2, 6.2 Hz, 1H), 2.29–2.24 (m, 1H), 2.06 (s, 3H), 2.00–1.91 (m, 1H), 1.80–1.65 (m, 4H), 1.34–1.29 (m, 2H), 1.27–1.11 (m, 3H), 1.05–0.95 (m, 1H).

The crude **11** was dissolved in ether (10 mL) and added to a suspension of $LiAlH_4$ (1.04 g, 27.4 mmol) in ether (10 mL) at 0 °C. The reaction was quenched with CH_3OH and concentrated. The mixture was stirred for 6 h under reflux. The reaction mixture cooled to room temperature and then quenched with CH_3OH and concentrated to give a corresponding amine derivative. ^{1}H NMR (400 MHz): δ = 5.63–5.48 (m, 2H), 3.94–3.92 (m, 2H), 2.81 (dd, J = 12.6, 3.0 Hz, 1H), 2.43 (dd, J = 12.8, 7.6 Hz, 1H), 2.20–2.15 (m, 1H), 1.97–1.86 (m, 1H), 1.79–1.75 (m, 1H), 1.69–1.60 (m, 3H), 1.24–1.09 (m, 5H), 1.05–0.96 (m, 1H).

The residue was used in the next step without purification. The crude product in CH_2Cl_2 (10 mL) was added to a solution of HBTU (4.32 g, 11.4 mmol), DIPEA (2.4 mL, 14 mmol), and 4-biphenyl carboxylic acid (903 mg, 4.56 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred for 3 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **12** (1.04 g, 63%, 3 steps) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.84–7.82 (m, 2H), 7.67–7.59 (m, 4H), 7.48–7.44 (m, 2H), 7.41–7.37 (m, 1H), 6.28 (br s, 1H), 5.79–5.67 (m, 2H), 4.10 (d, J = 4.4 Hz, 2H), 3.78 (ddd, J = 13.6, 6.0, 3.6 Hz, 1H), 3.20 (ddd, J = 13.7, 8.1, 5.9 Hz, 1H), 2.32–2.27 (m, 1H), 2.21–2.12 (m, 1H), 1.87–1.84 (m, 1H), 1.74–1.72 (m, 3H), 1.52–1.41 (m, 1H), 1.32–1.04 (m, 5H); ^{13}C NMR (100 MHz): δ = 167.2, 144.2, 140.0, 133.3, 131.2, 130.5, 128.9, 128.0, 127.3, 127.23, 127.17, 63.8, 43.3, 41.1, 39.6, 36.5, 31.9, 30.6, 26.0, 25.7; HRMS (EI) calcd for $C_{24}H_{29}NO_2$ [M]$^+$:363.2198. Found: 363.2207.

4.1.10. 4-Bromo-*N*-({(1*S*/*R*,2*R*/*S*)-2-[(*E*)-4-hydroxybut-2-en-1-yl]cyclohexyl}methyl)benzamide 13

A title compound was similarly prepared from **10** as above. Colorless oil; yield 50% (3 steps): ^{1}H NMR (400 MHz): δ = 7.64–7.61 (m, 2H), 7.58–7.56 (m, 2H), 6.16 (m, 1H), 5.78–5.66 (m, 2H), 4.10 (d, J = 4.8 Hz, 2H), 3.76 (ddd, J = 13.4, 5.8, 3.8 Hz, 1H), 3.16 (ddd, J = 13.7, 8.1, 5.9 Hz, 1H), 2.29–2.25 (m, 1H), 2.18–2.11 (m, 1H), 1.84–1.80 (m, 1H), 1.73–1.71 (m, 3H), 1.50–1.41 (m, 1H), 1.28–0.96 (m, 5H); ^{13}C NMR (100 MHz): δ = 166.5, 133.5, 131.8, 131.2, 130.4, 128.5, 126.0, 63.7, 43.3, 41.0, 39.6, 36.4, 31.9, 30.5, 26.0, 25.7; HRMS (EI) calcd for $C_{18}H_{24}BrNO_2$ [M]$^+$ 365.0990. Found 365.0996.

4.1.11. (1,1′-Biphenyl)-4-yl{(3*S*/*R*,4a*R*/*S*,8a*S*/*R*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl}methanone 14

To a solution of **12** (120 mg, 0.331 mmol) in dry CH_2Cl_2 (1 mL), $(CH_3CN)_2PdCl_2$ (15 mg, 0.056 mmol) was added at 0 °C under an argon gas atmosphere, and the mixture was stirred at the same

temperature for 4 h. The reaction mixture was filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 10:1) to give **14** (100 mg, 88%) as a colorless oil. ^{1}H NMR (400 MHz): δ = 7.64–7.58 (m, 4H), 7.49–7.43 (m, 4H), 7.38–7.35 (m, 1H), 5.87 (ddd, *J* = 17.5, 10.7, 3.7 Hz, 0.4H), 5.78 (ddd, *J* = 17.5, 10.7, 3.5 Hz, 0.6H), 5.55 (br s, 0.4H), 5.31–5.28 (m, 1H), 5.23–5.16 (m, 1H), 4.54 (br s, 0.6H), 4.49 (dd, *J* = 13.2, 4.0 Hz, 0.6H), 3.49 (dd, *J* = 13.0, 3.8 Hz, 0.4H), 2.86 (dd, *J* = 13.2, 11.6 Hz, 0.4H), 2.61 (dd, *J* = 12.8, 11.6 Hz, 0.6H), 1.84–1.52 (m, 5H), 1.47–1.18 (m, 5H), 1.15–1.13 (m, 0.4H), 1.03–0.98 (m, 1H), 0.90–0.84 (m, 0.6H); ^{13}C NMR (100 MHz): δ = 171.1, 170.4, 142.3, 142.2, 140.3, 137.1, 136.7, 135.4, 128.8, 127.69, 127.66, 127.4, 127.1, 126.8, 116.6, 116.1, 57.2, 50.8, 49.7, 43.5, 42.8, 41.9, 37.5, 36.8, 35.9, 32.9, 29.9, 29.7, 26.2, 26.1, 25.8, 25.7; HRMS (EI) calcd for $C_{24}H_{27}NO$ [M]$^+$: 345.2093. Found: 345.2090.

4.1.12. (4-Bromophenyl)((3*S*,4a*R*/*S*,8a*S*/*R*)-3-vinyloctahydroisoquinolin-2(1*H*)-yl)methanone 15

A title compound was similarly prepared as above. Colorless oil; yield 57%: ^{1}H NMR (400 MHz): δ = 7.56–7.50 (m, 2H), 7.29–7.27 (m, 2H), 5.84 (ddd, *J* = 17.4, 10.6, 3.8 Hz, 0.4H), 5.74 (ddd, *J* = 17.5, 10.7, 3.5 Hz, 0.6H), 5.49 (br s, 0.4H), 5.29–5.26 (m, 1H), 5.19–5.10 (m, 1H), 4.44 (dd, *J* = 13.4, 3.8 Hz, 0.6H), 4.39 (s, 0.6H), 3.33 (dd, *J* = 13.2, 3.6 Hz, 0.4H), 2.82 (dd, *J* = 13.0, 11.8 Hz, 0.4H), 2.57 (dd, *J* = 13.0, 11.4 Hz, 0.6H), 1.83–1.49 (m, 5H), 1.43–1.19 (m, 5H), 1.13–1.04 (m, 0.4H), 0.99–0.96 (m, 1H), 0.88–0.83 (m, 0.6H); ^{13}C NMR (100 MHz): δ = 170.2, 169.6, 136.9, 136.5, 135.4, 131.7, 131.6, 128.6, 128.0, 123.7, 123.6, 116.7, 116.2, 57.2, 50.8, 49.6, 43.5, 42.8, 41.8, 37.5, 36.7, 35.9, 32.8, 29.9, 29.6, 26.1, 26.0, 25.7, 25.6; HRMS (EI) Calcd for $C_{18}H_{22}BrNO$ [M]$^+$: 347.0885. Found: 347.0879.

4.1.13. (*S*)-2-({[(3*S*,4a*R*,8a*S*)-2-[(1,1′-Biphenyl)-4-carbonyl]-decahydroisoquinolin-3-yl]methyl}amino)-*N*-methoxy-*N*-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide 18

To a solution of $K_2OsO_2(OH)_4$ (3.1 mg, 0.0083 mmol) and *N*-methylmorpholine *N*-oxide (389 mg, 3.32 mmol), **14** (286 mg, 0.829 mmol) was added in THF/H_2O (3:1, 10 mL). After being stirred for 12 h, $NaIO_4$ (710 mg, 3.32 mmol) was added to the mixture. The resultant mixture was stirred for 30 min. The reaction was quenched with H_2O, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was roughly purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give **16**. ^{1}H NMR (400 MHz): δ = 9.69 (s, 0.75H), 9.65 (s, 0.25H), 7.69–7.54 (m, 5H), 7.49–7.37 (m, 4H), 5.50 (d, *J* = 6.4 Hz, 0.75H), 4.62–4.59 (m, 0.25H), 4.44 (d, *J* = 5.6 Hz, 0.25H), 3.69–3.65 (m, 0.75H), 2.81 (dd, *J* = 13.2, 11.6 Hz, 0.75H), 2.40 (t, *J* = 12.6 Hz, 0.25H), 2.33 (d, *J* = 13.6 Hz, 0.75H), 2.15 (dd, *J* = 13.6 Hz, 0.25H), 1.74–1.69 (m, 3H), 1.59–1.50 (m, 1H), 1.44–1.41 (m, 1H), 1.25–1.11 (m, 3H), 1.08–0.96 (m, 2H), 0.92–0.76 (m, 1H).

The product was used without further purification. To a solution of **16** and H-His(Trt)-N(OCH_3)CH_3 (410 mg, 0.930 mmol) in CH_2Cl_2 (1 mL), AcOH (0.05 mL, 0.8 mmol) was added. The mixture was stirred at room temperature for 2 h and then $NaBH_3CN$ (181 mg, 2.88 mmol) was added. The resultant mixture was stirred for 30 min. The reaction was quenched with 1 M HCl and the whole was extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography ($CHCl_3$/CH_3OH = 25:1) to give **18** and **20**.

*Compound **18**:* [80 mg, 13% (50% max.), 3 steps] as a colorless oil. $[\alpha]_D^{28}$ −20 (*c* 0.48, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.59–7.53 (m, 4H), 7.47–7.41 (m, 4H), 7.37–7.29 (m, 11H), 7.13–7.09 (m, 6H), 6.62 (m, 0.6H), 6.56 (m, 0.4H), 4.94 (br s, 0.6H), 4.41 (dd, *J* = 13.0, 3.0 Hz, 0.4H), 4.12–4.11 (m, 0.4H), 3.93 (m, 1H), 3.69 (s, 1.8H), 3.50 (s, 1.2H), 3.44–3.41 (m, 0.6H), 3.14 (s, 1.8H), 3.08 (s, 1.2H), 2.93–2.84 (m, 2.4H), 2.76–2.66 (m, 2H), 2.46 (t, *J* = 12.2 Hz, 0.6H), 1.80–1.70 (m, 3H), 1.61–1.54 (m, 1H), 1.43–1.17 (m, 6H), 1.08–0.85 (m, 2H); ^{13}C NMR (100 MHz): δ = 175.4, 175.2, 171.3, 170.5, 142.44, 142.38, 141.88, 141.86, 140.42, 140.35, 138.2, 138.1, 137.6, 137.2, 135.9, 135.7, 129.72, 129.66, 129.3, 128.8, 128.7, 127.91, 127.87, 127.54, 127.46, 127.4, 127.2, 127.1, 127.04, 126.99, 119.3, 115.6, 77.2, 75.03, 75.02, 61.6, 61.5, 57.8, 57.4, 55.5, 49.5, 48.3, 47.1, 46.6, 43.1, 42.6, 42.1, 36.4, 36.2, 34.4, 33.0, 32.9, 32.6, 32.2, 32.0, 29.9, 29.7, 26.14, 26.05, 25.8, 25.7; HRMS (EI) calcd for $C_{50}H_{53}N_5O_3$ [M]$^+$: 771.4148. Found: 771.4141.

*Compound **20**:* [75 mg, 12% (50% max.), 3 steps] as a colorless oil. $[\alpha]_D^{28}$ +32 (*c* 2.3, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.58–7.24 (m, 19H), 7.13–7.07 (m, 6H), 6.58 (m, 0.4H), 6.55 (m, 0.6H), 5.02–4.97 (m, 0.4H), 4.46 (dd, *J* = 13.2, 3.6 Hz, 0.6H), 4.13 (br s, 0.4H), 3.95 (m, 1H), 3.65 (s, 1.2H), 3.62–3.58 (m, 0.6H), 3.50 (s, 1.8H), 3.44 (dd, *J* = 13.4, 3.4 Hz, 0.4H), 3.14 (s, 1.2H), 3.11 (s, 1.8H), 3.01–2.94 (m, 1H), 2.89–2.81 (m, 2H), 2.65 (dd, *J* = 11.8, 6.6 Hz, 0.4H), 2.52 (dd, *J* = 12.0, 6.8 Hz, 0.6H), 2.50–2.44 (m, 0.6H), 2.26–2.24 (br s, 1H), 1.71–1.69 (m, 3H), 1.60–1.52 (m, 2H), 1.45–1.16 (m, 5H), 1.07–0.83 (m, 2H); ^{13}C NMR (100 MHz): δ = 175.6, 175.3, 171.1, 170.8, 142.44, 142.37, 141.9, 141.8, 140.41, 140.39, 138.12, 138.08, 137.5, 137.3, 135.7, 129.72, 129.66, 128.73, 128.68, 127.9, 127.5, 127.4, 127.1, 127.05, 127.03, 126.95, 119.5, 119.3, 77.2, 75.0, 61.6, 61.5, 57.7, 57.5, 55.4, 49.3, 48.4, 47.4, 47.2, 43.0, 42.8, 42.0, 36.7, 36.5, 34.6, 33.5, 33.00, 32.96, 32.3, 32.1, 29.9, 29.7, 29.6, 26.2, 26.0, 25.8, 25.7; HRMS (EI) calcd for $C_{50}H_{53}N_5O_3$ [M]$^+$: 771.4148. Found: 771.4154.

4.1.14. (*S*)-2-({[(3*S*,4a*R*,8a*S*)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl]methyl}amino)-*N*-methoxy-*N*-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide 19

Compound **19** was similarly synthesized as **18**. Colorless oil; yield 11% (50% max., 3 steps): $[\alpha]_D^{28}$ −31 (*c* 0.83, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.47 (d, *J* = 8.4 Hz, 1.2H), 7.44 (d, *J* = 8.4 Hz, 0.8H), 7.34–7.31 (m, 10.8H), 7.22 (d, *J* = 8.4 Hz, 1.2H), 7.12–7.11 (m, 6H), 6.60 (br s, 0.6H), 6.55 (br s, 0.4H), 4.87 (m, 0.6H), 4.37 (dd, *J* = 13.2, 3.6 Hz, 0.4H), 4.10 (br s, 0.6H), 3.89 (br s, 0.4H), 3.78 (m, 0.6H), 3.64 (s, 1.8H), 3.51 (s, 1.2H), 3.24 (dd, *J* = 13.2, 3.6 Hz, 0.6H), 3.13 (s, 1.8H), 3.11 (s, 1.2H), 2.91–2.80 (m, 2.4H), 2.73–2.62 (m, 2H), 2.47–2.41 (m, 0.4H), 1.76–1.65 (m, 3.4H), 1.60–1.54 (m, 1.6H), 1.36–1.25 (m, 5H), 1.00–0.82 (m, 2H); ^{13}C NMR (100 MHz): δ = 175.5, 175.1, 170.4, 169.7, 142.5, 142.4, 138.3, 138.1, 137.7, 137.2, 135.9, 135.8, 131.52, 131.49, 129.75, 129.72, 128.7, 128.4, 127.94, 127.91, 123.24, 123.21, 119.26, 119.25, 77.2, 75.1, 61.6, 61.5, 57.8, 57.5, 55.6, 49.3, 48.4, 47.1, 46.6, 43.1, 42.6, 42.0, 36.4, 36.2, 34.5, 33.0, 32.9, 32.7, 32.3, 32.0, 29.9, 29.7, 26.1, 26.0, 25.8, 25.7; HRMS (EI) calcd for $C_{44}H_{48}BrN_5O_3$ [M]$^+$: 773.2941. Found: 773.2948.

4.1.15. (*S*)-2-({[(3*R*,4a*S*,8a*R*)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl]methyl}amino)-*N*-methoxy-*N*-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide 21

Compound **21** was similarly synthesized as **20**. Colorless oil; yield 11% (50% max., 3 steps): $[\alpha]_D^{28}$ +4.5 (*c* 0.42, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.47–7.43 (m, 2H), 7.37–7.29 (m, 12H), 7.12–7.10 (m, 6H), 6.55 (m, 1H), 4.98–4.95 (m, 0.4H), 4.40 (dd, *J* = 13.2, 3.6 Hz, 0.6H), 4.10 (br s, 0.4H), 3.90 (br s, 0.6H), 3.84–3.81 (m, 0.6H), 3.64–3.58 (m, 0.4H), 3.63 (s, 1.8H), 3.54 (s, 1.2H), 3.28 (dd, *J* = 13.2, 3.6 Hz, 0.4H), 3.13 (s, 1.8H), 3.11 (s, 1.2H), 2.98–2.91 (m, 1H), 2.86–2.74 (m, 2.6H), 2.60 (dd, *J* = 11.6, 6.0 Hz, 0.6H), 2.47–2.41 (m, 1.4H), 1.72–1.65 (m, 3H), 1.59–1.47 (m, 2H), 1.43–1.12 (m, 5H), 1.04–0.79 (m, 2H); ^{13}C NMR (100 MHz): δ = 175.6, 175.2, 170.3, 170.0, 142.42, 142.37, 138.2, 138.1, 137.35, 137.25, 135.70, 135.66, 131.47, 131.45, 129.73, 129.70, 128.9, 128.7, 127.93, 127.91, 123.32, 123.26, 119.5, 119.3, 77.2, 75.1, 75.0, 61.5, 57.5,

57.4, 55.5, 49.2, 48.4, 47.4, 47.2, 42.9, 42.8, 42.0, 36.6, 36.5, 34.7, 33.6, 33.0, 32.9, 32.3, 32.0, 29.8, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) calcd for $C_{44}H_{48}BrN_5O_3$ $[M]^+$: 773.2941. Found: 773.2944.

4.1.16. (*S*)-2-[({(3*S*,4a*R*,8a*S*)-2-[(1,1′-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl}methyl)amino]-3-(1*H*-imidazol-4-yl)-*N*-methoxy-*N*-methylpropanamide

TFA/CH_2Cl_2/TIS/H_2O (10:10:1.0:1.0, 5.5 mL) was added to **18** (40 mg, 0.052 mmol). The mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure. The residue was diluted with AcOEt and basified by saturated aqueous $NaHCO_3$. The whole was extracted with AcOEt and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/CH_3OH = 10:1) to give the de-tritylated product (25 mg, 90%) as a yellowish oil. $[\alpha]_D^{28}$ −33 (*c* 0.51, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.68–7.36 (m, 10H), 6.84 (s, 0.6H), 6.82 (s, 0.4H), 5.03–5.01 (m, 0.4H), 4.31–4.27 (m, 0.6H), 4.15 (br s, 0.6H), 3.86 (br s, 0.4H), 3.73 (s, 1.2H), 3.66 (s, 1.8H), 3.54–3.51 (m, 1H), 3.25 (s, 1.2H), 3.19 (s, 1.8H), 3.00–2.86 (m, 1H), 2.75–2.62 (m, 2H), 2.52–2.44 (m, 2H), 1.77–1.68 (m, 3.4H), 1.62–1.59 (m, 1.6H), 1.49–1.23 (m, 5H), 1.17–1.11 (m, 0.4H), 1.07–0.99 (m, 1H), 0.89–0.85 (m, 0.6H); ^{13}C NMR (100 MHz): δ = 174.4, 171.0, 143.1, 142.4, 140.2, 140.1, 135.8, 135.3, 135.2, 134.8, 128.84, 128.83, 128.2, 127.8, 127.7, 127.5, 127.20, 127.18, 127.14, 127.08, 77.2, 61.7, 59.8, 58.4, 55.7, 49.5, 49.4, 48.6, 48.1, 43.5, 42.6, 42.0, 36.7, 34.3, 34.1, 33.0, 32.9, 32.2, 30.0, 29.6, 26.2, 26.0, 25.8, 25.6; HRMS (EI) calcd for $C_{31}H_{39}N_5O_3$ $[M]^+$: 529.3053. Found: 529.3057.

Compounds **19**, **20**, and **21** were similarly treated with TFA/CH_2Cl_2/TIS/H_2O (10:10:1.0:1.0, 5.5 mL) as above to yield the corresponding de-tritylated products.

4.1.17. From 19: (*S*)-2-({[(3*S*,4a*R*,8a*S*)-2-(4-bromobenzoyl)-decahydroisoquinolin-3-yl]methyl}amino)-3-(1*H*-imidazol-4-yl)-*N*-methoxy-*N*-methylpropanamide

Yellowish oil; yield, 70%: $[\alpha]_D^{28}$ −33.9 (*c* 0.415, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.66 (s, 0.6H), 7.56–7.53 (m, 2H), 7.38 (s, 0.4H), 7.32 (d, *J* = 8.4 Hz, 1.2H), 7.19 (d, *J* = 8.4 Hz, 0.8H), 6.83 (s, 0.6H), 6.81 (s, 0.4H), 4.97–4.95 (m, 0.4H), 4.26–4.22 (m, 0.6H), 4.00–3.98 (m, 0.6H), 3.85–3.84 (m, 0.4H), 3.72 (s, 1.2H), 3.66 (s, 1.8H), 3.56–3.53 (m, 0.6H), 3.35 (dd, *J* = 13.4, 3.8 Hz, 0.4H), 3.24 (s, 1.2H), 3.20 (s, 1.8H), 2.99–2.83 (m, 2H), 2.71–2.60 (m, 2H), 2.54–2.41 (m, 2H), 1.77–1.58 (m, 4H), 1.51–1.33 (m, 1H), 1.30–1.17 (m, 5H), 1.05–0.80 (m, 2H); ^{13}C NMR (100 MHz): δ = 174.3, 173.1, 170.2, 135.8, 135.4, 135.3, 134.7, 131.9, 131.7, 129.3, 128.3, 124.2, 123.7, 77.2, 61.7, 59.7, 58.4, 55.8, 49.6, 49.4, 48.5, 48.0, 43.5, 42.6, 42.0, 36.6, 34.2, 34.0, 33.0, 32.8, 32.6, 29.9, 29.6, 26.1, 26.0, 25.8, 25.6; HRMS (EI) calcd for $C_{25}H_{34}BrN_5O_3$ $[M]^+$: 531.1845. Found: 531.1839.

4.1.18. From 20: (*S*)-2-[({(3*R*,4a*S*,8a*R*)-2-[(1,1′-biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl}methyl)amino]-*N*-methoxy-*N*-methyl-3-(1*H*-imidazol-4-yl)propanamide

Yellowish oil; yield, quantitative: $[\alpha]_D^{28}$ −41 (*c* 0.45, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.65–7.59 (m, 4H), 7.54 (s, 1H), 7.49–7.44 (m, 4H), 7.39–7.36 (m, 1H), 6.78 (m, 1H), 5.21–5.20 (m, 0.75H), 4.52–4.49 (m, 0.25H), 4.12 (m, 0.25H), 3.90–3.88 (m, 0.75H), 3.67 (s, 2.25H), 3.67–3.65 (m, 0.75H), 3.56 (s, 0.75H), 3.56–3.49 (m, 0.75H), 3.25 (s, 2.25H), 3.25–3.21 (m, 0.25H), 3.21 (s, 0.75H), 3.11–3.05 (m, 0.25H), 2.98–2.95 (m, 0.75H), 2.89–2.83 (m, 0.75H), 2.63–2.52 (m, 1.5H), 2.37 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.29 (m, 1H), 1.72 (br s, 2H), 1.62–1.41 (m, 4H), 1.30–1.22 (m, 4H), 1.19–1.06 (m, 0.75H), 1.00–0.85 (m, 1.25H); ^{13}C NMR (100 MHz): δ = 174.9, 171.6, 171.0, 142.4, 140.2, 135.6, 135.4, 135.2, 134.4, 128.9, 128.8, 127.7, 127.4, 127.23, 127.16, 127.1, 127.0, 77.2, 61.7, 58.5, 55.5, 49.4, 49.1, 47.5, 42.8, 42.3, 36.9, 36.8, 35.2, 34.3, 33.1, 32.9, 32.3, 29.9, 29.65, 29.56, 29.2, 26.2, 26.0, 25.8, 25.6; HRMS (EI) calcd for $C_{31}H_{39}N_5O_3$ $[M]^+$: 529.3053. Found: 529.3060.

4.1.19. From 21: (*S*)-2-({[(3*R*,4a*S*,8a*R*)-2-(4-bromobenzoyl) decahydroisoquinolin-3-yl]methyl}amino)-*N*-methoxy-*N*-methyl-3-(1*H*-imidazol-4-yl)propanamide

Yellowish oil; yield, 65%: $[\alpha]_D^{28}$ −27.7 (*c* 0.96, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.57–7.47 (m, 3H), 7.30–7.27 (m, 2H), 6.79 (s, 0.25H), 6.78 (s, 0.75H), 5.17–5.14 (m, 0.75H), 4.45 (dd, *J* = 13.4, 3.4 Hz, 0.25H), 3.94 (br s, 0.25H), 3.87–3.86 (m, 0.75H), 3.67 (s, 2.25H), 3.59 (s, 0.75H), 3.39 (dd, *J* = 13.6, 3.2 Hz, 0.75H), 3.25 (s, 2.25H), 3.21 (s, 0.75H), 3.19–3.16 (m, 0.75H), 3.07–3.01 (m, 0.25H), 2.98–2.89 (m, 1H), 2.82 (dd, *J* = 13.4, 11.8 Hz, 0.75H), 2.70–2.48 (m, 1.5H), 2.36 (dd, *J* = 12.2, 4.6 Hz, 0.75H), 2.30 (dd, *J* = 11.8, 5.8 Hz, 0.25H), 1.82–1.61 (m, 5H), 1.48–1.28 (m, 5H), 1.09–1.04 (m, 0.75H), 0.98–0.87 (m, 1.25H); ^{13}C NMR (100 MHz): δ = 174.9, 170.7, 170.2, 135.6, 135.31, 135.26, 134.5, 131.8, 131.6, 128.7, 128.4, 123.8, 123.5, 77.2, 61.7, 58.4, 58.0, 55.4, 49.5, 49.1, 47.5, 47.4, 42.8, 42.7, 42.2, 36.8, 36.7, 35.1, 34.2, 33.0, 32.9, 32.3, 29.8, 29.5, 29.2, 26.1, 26.0, 25.8, 25.6; HRMS (EI) Calcd. For $C_{25}H_{34}BrN_5O_3$ $[M]^+$: 531.1845. Found: 531.1839.

4.1.20. (*S*)-2-[({(3*S*,4a*R*,8a*S*)-2-[(1,1′-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl}methyl)amino]-3-(1*H*-imidazol-4-yl)-propanal 22

To a solution of above de-tritylated product of **18** (33 mg, 0.61 mmol) in CH_2Cl_2 (1 mL), DIBALH (1.0 mol/L solution in hexane, 1.2 mL, 1.2 mmol) was added drop-wise at −78 °C. The reaction mixture was stirred for 5 min. The reaction was quenched with CH_3OH and concentrated. The residue was dissolved in CH_3OH and filtered through a silica gel layer. The filtrate was concentrated. The residue was purified by HPLC to give **22** (10.5 mg, 28%) as a colorless oil. $[\alpha]_D^{28}$ −3.2 (*c* 0.48, CH_3OH); ^1H NMR (500 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.80 (br s, 1H), 7.75–7.73 (m, 2H), 7.67–7.65 (m, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.50 (br s, 1H), 7.48–7.45 (m, 2.5H), 7.40–7.36 (m, 1.5H), 5.13 (m, 1H), 4.82 (dd, *J* = 8.4, 3.2 Hz, 1H), 3.88–3.79 (m, 2H), 3.63–3.59 (m, 1H), 3.43–3.40 (m, 1H), 3.34 (s, 1H), 2.97 (t, *J* = 12.6 Hz, 1H), 1.77–1.68 (m, 5H), 1.45–1.34 (m, 5H), 1.06–0.98 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD, referenced to CD_3OD): δ = 175.1, 175.0, 163.0, 162.7, 144.70, 144.66, 141.1, 141.04, 135.5, 134.86, 134.80, 129.87, 129.86, 129.00, 128.97, 128.92, 128.0, 127.9, 118.6, 95.0, 94.9, 61.3, 61.0, 50.7, 50.5, 49.6, 47.2, 47.1, 43.2, 43.1, 37.59, 37.56, 35.2, 33.5, 30.2, 26.9, 26.5, 23.1, 22.9; HRMS (ESI) calcd for $C_{29}H_{35}N_4O_2$ $[M+H]^+$: 471.2760. Found: 471.2760.

4.1.21. (*S*)-2-((((3*S*,4a*R*,8a*S*)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl)methyl)amino)-3-(1*H*-imidazol-4-yl)propanal 23

A title compound **23** was synthesized from the de-tritylated product of **19** as above. Colorless oil; yield, 36%: $[\alpha]_D^{28}$ −1.1 (*c* 0.40, CH_3OH); ^1H NMR (400 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.72 (br s, 1H), 7.66–7.64 (m, 2H), 7.46 (br s, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 5.11–5.03 (m, 1H), 4.78 (dd, *J* = 11.0, 3.0 Hz, 1H), 3.85–3.75 (m, 2H), 3.47–3.39 (m, 2H), 3.26–3.24 (m, 1H), 2.96–2.84 (m, 1H), 1.78–1.54 (m, 5H), 1.43–1.22 (m, 5H), 1.07–0.93 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD, referenced to CD_3OD): δ = δ = 174.1, 173.5, 163.1, 162.6, 135.7, 135.45, 135.39, 133.0, 130.39, 130.35, 130.2, 125.84, 125.78, 118.8, 118.7, 95.1, 94.9, 61.3, 61.0, 50.64, 50.58, 43.3, 43.2, 37.71, 37.70, 37.68, 35.25, 35.22, 33.7, 30.4, 30.3, 27.0, 26.6, 23.2, 23.0; HRMS (ESI) calcd for $C_{23}H_{30}BrN_4O_2$ $[M+H]^+$: 473.1552. Found: 473.1543.

4.1.22. (*S*)-2-[({(3*R*,4a*S*,8a*R*)-2-[(1,1′-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl}methyl)amino]-3-(1*H*-imidazol-4-yl)propanal 24

A title compound **24** was synthesized from the de-tritylated product of **20** as above. Colorless oil; yield, 30%: $[\alpha]_D^{29}$ −2.3 (*c* 0.61, CH_3OH); ^1H NMR (500 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.76 (s, 1H), 7.74 (d, *J* = 6.4 Hz, 2H), 7.66 (d, *J* = 6.0 Hz, 2H), 7.56 (d, *J* = 6.4 Hz, 2H), 7.48–7.45 (m, 3.5H), 7.40–7.37 (m, 1.5H), 5.09 (br s, 1H), 3.86–3.75 (m, 2H), 3.64–3.59 (m, 1H), 3.55–3.48 (m, 1H), 3.35–3.32 (m, 1H), 3.28–3.26 (m, 1H), 2.93–2.91 (m, 1H), 1.79–1.66 (m, 5H), 1.47–1.28 (m. 5H), 1.07–0.97 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD, referenced to CD_3OD): δ = 175.5, 175.4, 163.1, 162.8, 145.0, 141.2, 135.7, 135.6, 134.9, 130.1, 129.2, 129.1, 128.2, 128.1, 119.0, 95.4, 95.1, 62.1, 61.6, 50.8, 43.2, 43.1, 37.7, 35.5, 35.4, 33.8, 33.7, 30.4, 27.0, 26.6, 24.5, 24.1; LRMS (ESI) calcd for $C_{29}H_{35}N_4O_2$ $[M+H]^+$: 471.28. Found: 471.30.

4.1.23. (*S*)-2-({[(3*R*,4a*S*,8a*R*)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl]methyl}amino)-3-(1*H*-imidazol-4-yl)propanal 25

A title compound **25** was synthesized from the de-tritylated product of **21** as above. Colorless oil; yield, 28%: $[\alpha]_D^{29}$ −7.8 (*c* 0.36, CH_3OH); ^1H NMR (500 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.72 (br s, 1H), 7.66–7.62 (m, 2H), 7.45 (s, 1H), 7.43–7.36 (m, 2H), 5.06 (m, 1H), 3.83–3.75 (m, 2H), 3.49–3.46 (m, 2H), 3.34–3.33 (m, 1H), 3.28–3.23 (m, 1H), 2.92–2.85 (m, 1H), 1.76–1.58 (m, 5H), 1.44–1.26 (m, 5H), 1.06–0.93 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD, referenced to CD_3OD): δ = 174.43, 174.35, 163.1, 162.8, 135.7, 135.6, 135.3, 133.0, 130.3, 125.9, 118.8, 95.4, 95.1, 62.1, 61.6, 50.7, 43.1, 43.0, 37.7, 37.6, 35.4, 35.3, 33.7, 30.3, 27.0, 26.6, 24.6, 24.1; LRMS (ESI) calcd for $C_{23}H_{30}BrN_4O_2$ $[M+H]^+$: 473.16. Found: 473.25.

4.1.24. (1*S*,6*R*)-6-{2-[(4-Bromobenzyl)oxy]ethyl}cyclohex-3-enecarboxylic acid 31

To a solution of 1,3-butadiene (20 wt% solution in toluene, 108 mL, 255 mmol) was added (*E*)-ethyl 5-[(4-bromobenzyl)oxy]pent-2-enoate[32] (20.0 g, 63.9 mmol), and the mixture was heated at 225 °C for 60 h. After the reaction mixture was cooled to room temperature, water was added and the whole was extracted with AcOEt. The organic layer was washed with 1 M HCl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 35:1) to give an ethyl ester of **29**, (1*S*/*R*, 6*R*/*S*)-ethyl 6-{2-[(4-bromobenzyl)oxy]ethyl)}cyclohex-3-enecarboxylate, (11.7 g, 50%) as a yellow pale oil. ^1H NMR (400 MHz): δ = 7.47–7.45 (m, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 5.65 (m, 2H), 4.43 (dd, *J* = 18.8, 12.0 Hz, 2H) 4.14 (q, *J* = 7.2 Hz, 2H), 3.52–3.49 (m, 2H), 2.41–2.20 (m, 4H), 2.08–2.03 (m, 1H), 1.81–1.72 (m, 2H), 1.53–1.46 (m, 1H), 1.26 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz): δ = 175.8, 137.5, 131.4, 129.2, 125.7, 124.8, 121.3, 72.1, 68.1, 60.3, 45.3, 33.7, 32.4, 29.9, 28.1, 14.3; HRMS (EI) Calcd for $C_{18}H_{23}BrO_3$ $[M]^+$: 366.0831. Found: 366.0826.

The above ester (31.8 g, 86.6 mmol) was dissolved in 2 M NaOH/THF (1:1, 100 mL). After being stirred for 15 h under reflux, the reaction mixture was cooled to room temperature. The mixture was acidified with 2 M HCl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue purified by silica gel column chromatography (hexane/AcOEt = 3:1). The product was dissolved in AcOEt (300 mL) and then (*S*)-(−)-phenylethylamine (11 mL, 87 mmol) was added. After 12 h, the solid was collected by suction filtration. The free acid was liberated from the salt by treatment with 2 M HCl and extraction with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give **31** [7.63 g, 26% (50% max.)] as a colorless oil. $[\alpha]_D^{28}$ +22 (*c* 0.78, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.47–7.45 (m, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.69–5.66 (m, 2H), 4.44 (dd, *J* = 17.0, 12.2 Hz, 2H) 3.56–3.49 (m, 2H), 2.47–2.20 (m, 4H), 2.12–2.07 (m, 1H), 1.91–1.75 (m, 2H), 1.60–1.51 (m, 1H); ^{13}C NMR (100 MHz): δ = 181.1, 137.3, 131.5, 129.3, 125.7, 124.5, 121.4, 72.2, 68.0, 44.9, 33.6, 32.1, 29.5, 27.7; HRMS (EI) calcd for $C_{16}H_{19}BrO_3$ $[M]^+$: 338.0518. Found: 338.0520.

4.1.25. [(1*S*,6*R*)-6-{2-[(4-Bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methanol

To a solution of **31** (7.70 g, 22.7 mmol) in THF (80 mL), Et_3N (6.4 mL, 46 mmol) and IBCF (4.5 mL, 34 mmol) were added at −20 °C. After being stirred for 15 min at the same temperature, $NaBH_4$ (3.47 g, 91.2 mmol) and H_2O (10 drops from a pipette) was added. The mixture was warmed up to room temperature and then the reaction was quenched with saturated aqueous NH_4Cl. The whole was extracted with AcOEt and the organic layer was washed with brine and dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1) to give a title alcohol (5.68 g, 77%) as a colorless oil. $[\alpha]_D^{29}$ +22 (*c* 0.65, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.48–7.46 (m, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.65–5.58 (m, 2H), 4.45 (s, 2H), 3.68 (dd, *J* = 10.8, 6.4 Hz, 1H), 3.62 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.58–3.47 (m, 2H), 2.15–2.09 (m, 2H), 2.00–1.76 (m, 4H), 1.66–1.49 (m, 3H); ^{13}C NMR (100 MHz): δ = 137.4, 131.5, 129.3, 125.8, 125.5, 121.4, 72.3, 68.7, 65.0, 39.7, 32.9, 31.1, 29.5, 26.6; HRMS (EI) calcd for $C_{16}H_{21}BrO_2$ $[M]^+$: 324.0725. Found: 324.0732.

4.1.26. {[(1*S*,6*R*)-6-{2-[(4-Bromobenzyl)oxy]ethyl}cyclohex-3-en-1-yl]methoxy}(*tert*-butyl)diphenylsilane

TBDPS-Cl (5.0 mL, 19 mmol) was added to a solution of above alcohol (5.66 g, 17.4 mmol) and imidazole (1.43 g, 21.0 mmol) in CH_2Cl_2 (50 mL), and the mixture was stirred for 8 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 30:1) to give a di-protected alcohol compound (9.81 g, quant.) as a colorless oil. $[\alpha]_D^{28}$ +18.6 (*c* 1.74, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.67–7.64 (m, 4H), 7.44–7.34 (m, 8H), 7.17 (d, *J* = 8.4 Hz, 2H), 5.63–5.54 (m, 2H), 4.41 (dd, *J* = 15.2, 12.0 Hz, 2H), 3.68 (dd, *J* = 9.8, 5.4 Hz, 1H), 3.62 (dd, *J* = 10.0, 6.8 Hz, 1H), 3.50–3.46 (m, 2H), 2.16–1.96 (m, 3H), 1.87–1.81 (m, 2H), 1.71–1.68 (m, 2H), 1.48–1.44 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (100 MHz): δ = 137.7, 135.61, 135.60, 133.94, 133.92, 131.4, 129.5, 129.1, 127.6, 125.8, 125.3, 121.2, 72.1, 68.7, 65.9, 39.6, 32.9, 30.8, 29.0, 26.9, 26.7, 19.3; HRMS (FAB) Calcd. For $C_{32}H_{40}BrO_5$ $[M+H]^+$: 563.1981. Found: 563.1988.

4.1.27. 2-[(1*R*,2*S*)-2-{[(*tert*-Butyldiphenylsilyl)oxy]methyl}cyclohexyl]ethanol 32

To a solution of above di-protected alcohol (9.81 g, 17.4 mmol) in CH_3OH/EtOAc/saturated aqueous $NaHCO_3$ (5:5:1, 110 mL), Pd-C (3.8 g) was added, and the mixture was stirred under a hydrogen gas atmosphere at room temperature for 6 h. The mixture was filtered through Celite and a silica gel layer, and the filtrate was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/AcOEt = 6:1) to give **32** (6.90 g, quant.) as a colorless oil. $[\alpha]_D^{28}$ +12 (*c* 0.65, $CHCl_3$); ^1H NMR (400 MHz): δ = 7.68–7.65 (m, 4H), 7.43–7.36 (m, 6H), 3.67–3.56 (m, 4H), 1.78–1.70 (m, 5H), 1.37–1.21 (m, 6H), 1.06 (s, 9H), 1.02–0.96 (m, 1H); ^{13}C NMR (100 MHz): δ = 135.69, 135.66, 133.91, 133.89, 129.55, 129.54, 127.60, 127.57, 66.5, 61.0, 44.5,

36.5, 35.5, 31.9, 30.0, 26.9, 26.1, 26.0, 19.3; HRMS (FAB) calcd for $C_{25}H_{37}O_2Si$ $[M+H]^+$: 397.2563. Found: 397.2558.

4.1.28. (*S*)-2-[({(3*S*,4a*R*,8a*S*)-2-[(1,1′-Biphenyl)-4-carbonyl]decahydroisoquinolin-3-yl}methyl)amino]-3-(1*H*-imidazol-4-yl)propanal 40

Title compound was prepared from **32** according to the same procedure[33] employed for the synthesis of **22** starting from enantiomer mixture **7**. Colorless solid; yield, 30%: $[\alpha]_D^{28}$ −4.3 (*c* 0.83, CH_3OH); ^1H NMR (500 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.81 (br s, 1H), 7.75–7.73 (m, 2H), 7.67–7.65 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.51 (br s, 1H), 7.48–7.45 (m, 2.5H), 7.40–7.36 (m, 1.5H), 5.14–5.13 (m, 1H), 4.81 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.89–3.80 (m, 2H), 3.63–3.59 (m, 1H), 3.44–3.39 (m, 1H), 3.34 (s, 1H), 2.97 (t, *J* =12.6 Hz, 1H), 1.82–1.62 (m, 5H), 1.45–1.28 (m, 5H), 1.09–0.89 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD, referenced to CD_3OD): δ = 175.24, 175.17, 163.2, 162.8, 144.9, 144.8, 141.21, 141.20, 135.6, 135.03, 134.97, 130.1, 129.22, 129.18, 129.12, 128.2, 128.1, 118.98, 118.95, 95.0, 94.9, 61.3, 60.9, 50.73, 50.69, 49.8, 47.1, 47.0, 43.33, 43.30, 37.8, 37.7, 35.4, 33.7, 30.4, 27.0, 26.6, 23.1, 22.9; HRMS (ESI) Calcd. For $C_{29}H_{35}N_4O_2$ $[M+H]^+$: 471.2760. Found: 471.2765.

Compounds **41**, **44**, and **45**–**49** listed in Table 1 were similarly prepared as above.

4.1.29. Compound 41

4.1.29.1. (S)-2-({[(3S,4aR,8aS)-2-(4-Bromobenzoyl)decahydroisoquinolin-3-yl]methyl}amino)-3-(1H-imidazol-4-yl)-propanal 41. Colorless solid; yield, 23%: $[\alpha]_D^{28}$ −0.64 (*c* 0.88, CH_3OH); ^1H NMR (400 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.80 (br s, 1H), 7.65–7.63 (m, 2H), 7.49 (br s, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 5.11 (m, 1H), 4.81–4.78 (m, 1H), 3.86–3.78 (m, 2H), 3.47–3.34 (m, 2H), 2.97–2.91 (m, 1H), 1.75–1.60 (m, 5H), 1.42–1.24 (m, 5H), 1.04–0.96 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD, referenced to CD_3OD): δ = 174.2, 174.1, 163.2, 162.8, 135.6, 135.44, 135.39, 132.9, 130.40, 130.36, 130.0, 125.8, 125.7, 119.0, 118.9, 95.0, 94.9, 61.2, 60.8, 50.64, 50.60, 43.24, 43.22, 37.69, 37.66, 35.25, 35.23, 33.7, 30.4, 30.3, 27.0, 26.6, 23.1, 22.9; HRMS (ESI) calcd for $C_{23}H_{30}BrN_4O_2$ $[M+H]^+$: 473.1552. Found: 473.1546.

4.1.30. Compound 44

Colorless solid; yield, 31%: $[\alpha]_D^{28}$ −1.62 (*c* 1.23, CH_3OH); ^1H NMR (500 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.75 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.47–7.45 (m, 3.5H), 7.40–7.37 (m, 1.5H), 5.09 (br s, 1H), 3.80 (m, 2H), 3.66–3.63 (m, 1H), 3.51 (m, 1H), 3.26 (m, 1H), 2.93–2.91 (m, 1H), 1.77–1.68 (m, 5H), 1.45–1.35 (m, 5H), 1.07–0.97 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD, referenced to CD_3OD): δ = 175.5, 175.4, 163.2, 162.8, 144.9, 141.2, 135.6, 135.5, 134.9, 130.1, 129.2, 129.1, 128.2, 128.1, 119.1, 95.2, 95.0, 62.0, 61.4, 50.8, 43.12, 43.10, 37.73, 37.69, 35.5, 35.4, 33.7, 30.4, 27.0, 26.6, 24.4, 23.9; HRMS (ESI) calcd for $C_{29}H_{35}N_4O_2$ $[M+H]^+$: 471.2760. Found: 471.2756.

4.1.31. Compound 45

Colorless solid; yield, 30%: $[\alpha]_D^{28}$ −6.1 (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.82 (br s, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.49 (s, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 5.06 (m, 1H), 4.83 (m, 1H), 3.86–3.76 (m, 2H), 3.51–3.43 (m, 2H), 3.27–3.25 (m, 1H), 2.93–2.90 (m, 1H), 1.76–1.66 (m, 5H), 1.44–1.30 (m, 5H), 1.04–0.96 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD, referenced to CD_3OD): δ = 174.43, 174.35, 163.1, 162.8, 135.6, 135.5, 135.3, 133.0, 130.4, 125.9, 119.1, 95.3, 95.0, 61.8, 61.3, 50.7, 43.03, 43.01, 37.7, 37.6, 35.4, 35.3, 33.7, 30.33, 30.31, 27.0, 26.6, 24.4, 23.9; HRMS (ESI) calcd for $C_{23}H_{30}BrN_4O_2$ $[M+H]^+$: 473.1552. Found: 4731537. Found: 4731537.

4.1.32. Compound 46

Colorless solid; yield, 31%: $[\alpha]_D^{29}$ −6.7 (*c* 0.10, CH_3OH); ^1H NMR (400 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.66 (br s, 1H), 7.79–7.73 (m, 2H), 7.64–7.55 (m, 4H), 7.48–7.45 (m, 3.5H), 7.41–7.37 (m, 1.5H), 5.19–5.18 (m, 1H), 4.77 (dd, *J* = 12.6, 3.2 Hz, 1H), 3.87–3.79 (m, 2H), 3.63–3.58 (m, 1H), 3.46–3.40 (m, 1H), 3.36–3.34 (m, 0.5H), 3.25–3.23 (m, 1.5H), 2.99–2.93 (m, 1H), 1.79–1.62 (m, 5H), 1.42–1.21 (m, 5H), 1.10–0.89 (m, 2H); LRMS (ESI) calcd for $C_{29}H_{35}N_4O_2$ $[M+H]^+$: 471.28. Found: 471.35.

4.1.33. Compound 47

Colorless solid; yield, 27% (obtained as the mixture of a diastereomer derived from Pd-mediated cyclization): ^1H NMR (400 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.84 (br s, 1H), 7.59–7.38 (m, 11H), 5.03 (m, 1H), 4.81 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.90 (m, 1H), 3.69–3.59 (m, 1H), 3.41 (m, 1H), 3.34 (s, 1H), 3.27–3.24 (m, 1H), 2.93–2.87 (m, 1H), 2.61–2.54 (m, 1H), 1.59–1.56 (m, 2H), 1.50 (d, *J* = 10.4 Hz, 1H), 1.42 (d, *J* = 12.4 Hz, 1H), 1.18–1.08 (m, 2H), 0.99–0.88 (m, 3H), 0.70–0.61 (m, 1H), 0.46–0.44 (m, 1H); LRMS (ESI) calcd for $C_{29}H_{35}N_4O_2$ $[M+H]^+$: 471.28. Found: 471.35.

4.1.34. Compound 48

Colorless solid; yield, 25%: $[\alpha]_D^{29}$ −3.7 (*c* 0.15, CH_3OH); ^1H NMR (400 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.61 (br s, 1H), 7.52–7.46 (m, 6H), 7.45–7.41 (m, 1H), 5.13–5.11 (m, 2H), 4.77 (dd, *J* = 11.8, 3.4 Hz, 1H), 3.79–3.66 (m, 1H), 3.56–3.50 (m, 1H), 3.42–3.32 (m, 1H), 3.26–3.20 (m, 1H), 2.97–2.91 (m, 1H), 1.78–1.59 (m, 5H), 1.40–1.20 (m, 5H), 1.08–0.86 (m, 2H); LRMS (ESI) calcd for $C_{23}H_{31}N_4O_2$ $[M+H]^+$: 395.24. Found: 395.30.

4.1.35. Compound 49

Colorless solid; yield, 18%: $[\alpha]_D^{29}$ −3.6 (*c* 0.18, CH_3OH); ^1H NMR (400 MHz, CD_3OD, referenced to residual CH_3OH): δ = 8.68 (br s, 1H), 7.56–7.53 (m, 2H), 7.44 (br s, 1H), 7.25–7.19 (m, 3H), 5.10 (m, 1H), 4.77 (dd, *J* = 11.4, 3.2 Hz, 1H), 3.84–3.75 (m, 2H), 3.52–3.47 (m, 1H), 3.40–3.34 (m, 1H), 3.25–3.23 (m, 1H), 2.96–2.89 (m, 1H), 1.78–1.65 (m, 5H), 1.43–1.23 (m, 5H), 1.05–0.93 (m, 2H); LRMS (ESI) calcd for $C_{23}H_{30}FN_4O_2$ $[M+H]^+$: 413.24. Found: 413.35.

4.2. Estimation of IC_{50} values

Peptide substrate [H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH_2][28] (111 μM) in a reaction solution (25 μL of 20 mM Tris–HCl buffer pH 7.5 containing 7 mM DTT) was incubated with the R188I SARS $3CL^{pro}$[28] (56 nM) at 37 °C for 60 min in the presence of various inhibitor concentrations at 37 °C for 60 min. The cleavage reaction was monitored by analytical HPLC [Cosmosil 5C18 column (4.6 × 150 mm), a linear gradient of CH_3CN (10–20%) in an aq0.1% TFA over 30 min], and the cleavage rates were calculated from the reduction in the substrate peak area. Each IC_{50} value was obtained from the sigmoidal dose-response curve (see Fig. S1 for a typical sigmoidal curve). Each experiment was repeated 3 times and the results were averaged.

4.3. X-ray crystallography

The purified SARS $3CL^{pro}$ in 20 mM Bis–Tris pH 5.5, 10 mM NaCl, and 1 mM DTT was concentrated to 8 mg/mL.[13] Crystals of SARS $3CL^{pro}$ were grown at 4 °C using a sitting-drop vapor diffusion method by mixing it with an equal volume of reservoir solution containing 100 mM MES pH 6.2, 5–10% PEG20000, and 5 mM DTT. Cubic-shaped crystals with dimensions of 0.3 mm × 0.3 mm × 0.3 mm grew within 3 days. The crystals were then soaked for 24 h with reservoir-based solution of 100 mM MES

pH 6.2, 5–8% PEG20000, and 5 mM DTT containing 3 mM of **40** or **44**. Crystals were then transferred into a cryobuffer of 100 mM MES pH 6.2, 10% PEG20000, 5 mM DTT, 15% ethylene glycol containing 3 mM of **40** or **44**, and flash-frozen in a nitrogen stream at 100 K. X-ray diffraction data of SARS $3CL^{pro}$ in complexes with inhibitor **40** or **44** were collected at the SPring-8, beamline BL44XU with a Rayonix MX300HE CCD detector at a wavelength of 0.900 Å.

Crystals of SARS $3CL^{pro}$ in a complex with **41** were obtained by co-crystallization using sitting-drop vapor diffusion at 4 °C and mixing an equal volume of protein-inhibitor complex (final inhibitor concentration of 3 mM) and a reservoir solution containing 100 mM MES pH 6.0, 5–6% PEG20000, and 5 mM DTT. Cubic-shaped crystals with dimensions of 0.2 mm × 0.2 mm × 0.2 mm were obtained within 3 days. Crystals were transferred into cryobuffer with 100 mM MES pH 6.0, 6% PEG20000, 5 mM DTT, 15% ethylene glycol, and 3 mM of **41** and then flash-frozen in a nitrogen stream at 100 K. X-ray diffraction data were collected on a Rigaku RAXIS VII imaging-plate detector at a wavelength of 1.5418 Å equipped with an in-house rotating anode FR-E/Super Bright X-ray generator and Confocal VariMax (VariMax HF) optics system.

The structures of SARS $3CL^{pro}$ in a complex with inhibitors were determined by molecular replacement using the Molrep[34] program with a R188I SARS $3CL^{pro}$ structure (PDB code 3AW1[13]) as the search model. Rigid body refinement and subsequent restrained refinement protocols were performed with the program Refmac 5[35] of the CCP package.[36] The Coot program[37] was used for manual model rebuilding. Water molecules were added using Coot only after the refinement of protein structures had converged. Ligands generated on JLigand[38] software were directly built into the corresponding difference in electron density, and the model was then subjected to an additional round of refinement. The figures for structural representation were generated on Pymol[39] or chimera[40] software.

5. PDB ID codes

4TWY, 4TWW, and 4WY3.

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Supplementary data

Supplementary data (the HPLC data for the evaluation of purities using a reversed-phase or chiral column, typical sigmoidal curves used to obtain IC_{50} values, and NMR data of synthesized compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.12.028.

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