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Evaluation of an octahydroisochromene scaffold used as a novel SARS 3CL protease inhibitor



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ABSTRACT

An octahydroisochromene scaffold has been introduced into a known SARS 3CL protease inhibitor as a novel hydrophobic core to interact with the S2 pocket of the protease. An alkyl or aryl substituent was also introduced at the 1-position of the octahydroisochromene scaffold and expected to introduce additional interactions with the protease. Sharpless–Katsuki asymmetric epoxidation and Sharpless asymmetric dihydroxylation were employed to construct the octahydroisochromene scaffold. The introductions of the P1 site His-al and the substituent at 1-position was achieved using successive reductive amination reactions. Our initial evaluations of the diastereo-isomeric mixtures (**16a–d**) revealed that the octahydroisochromene moiety functions as a core hydrophobic scaffold for the S2 pocket of the protease and the substituent at the 1-position may form additional interactions with the protease. The inhibitory activities of the diastereoisomerically-pure inhibitors (**3a–d**) strongly suggest that a specific stereo-isomer of the octahydroisochromene scaffold, (1*S*, 3*S*) **3b**, directs the P1 site imidazole, the warhead aldehyde, and substituent at the 1-position of the fused ring to their appropriate pockets in the protease.

1. Introduction

In 2003, severe acute respiratory syndrome (SARS), a life-threatening pneumonia, spread across Southeast Asia and affected about 8500 patients with > 800 fatalities.^{1–3} The causative virus is a new betacoronavirus (βCoV) containing a 29.7-kb positive-strand RNA genome. Two large replicative polyproteins, pp1a (486 kDa) and pp1ab (790 kDa), are coded in the genome as precursor proteins to yield functional proteins.^{4–6} Among the functional proteins, a 3C-like cysteine protease (SARS 3CL^{Pro}) processes the polyprotein to produce itself as well as a variety of proteins necessary to re-construct the virus particles. Thus, SARS 3CL^{Pro} is thought to be an ideal target for anti-SARS agents. Numerous studies on SARS 3CL^{Pro} inhibitors have been reported,^{7,8} but no therapeutic agents are available at present.

In the course of our previous studies on SARS 3CL^{Pro} inhibitors,⁹ a substrate-based peptide aldehyde (**1**) was shown to be a potent inhibitor (IC₅₀ = 98 nM) for a mutant protease, R188I SARS 3CL^{Pro}.¹⁰ Analysis of the X-ray crystal structure of **1** complexed with R188I SARS 3CL^{Pro} revealed that the P2 site cyclohexyl ring of **1** interacts with the S2 pocket of the protease via a hydrophobic interaction. The distance between the 2-position carbon atom of the cyclohexyl-ring and α-nitrogen atom of cyclohexylalanine (Cha) was 3.48 Å (Fig. 1). This result

suggested that the cyclohexyl ring may be connected to the peptide chain via a methylene linker, yielding a new inhibitor scaffold containing a hydrophobic decahydroisoquinoline ring. The resulting decahydroisoquinoline inhibitor (**2**) showed inhibitory activity as expected, but with moderate potency (IC₅₀ = 63 μM).¹¹ Analysis of the X-ray crystal structure of inhibitor **2** complexed with R188I SARS 3CL^{Pro} revealed the lack of interactions with the P3 to P4 sites in substrate-based inhibitor **1**.

In this study, an octahydroisochromene scaffold was selected as a novel hydrophobic fused-ring in inhibitor **3** instead of decahydroisoquinoline to evaluate the effect of the configuration of the fused-ring structure and novel substituents on the ring system (Fig. 2).¹² The configuration of the octahydroisochromene ring in **3** can be easily controlled using a stereo-selective ring-closing reaction featuring the nucleophilic opening of an epoxide. The synthesis of the requisite oxygen-containing precursor used in the ring-closing reaction can be achieved using established stereo-selective reactions such as the Sharpless–Katsuki asymmetric epoxidation and Sharpless dihydroxylation reaction. In addition, the effect of the substituent at the 1-position in the fused-ring system of **3** may also be evaluated; the effects of different substituents in this position have not been assessed in our previous studies using the decahydroisoquinoline scaffold in **2**.¹² Thus,

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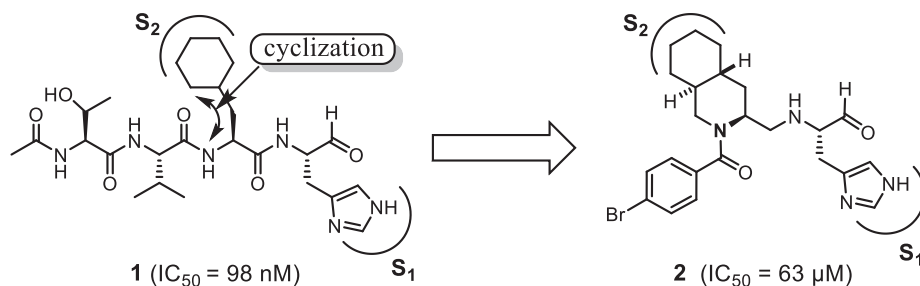


Fig. 1. The design of decahydroisoquinoline inhibitor 2.

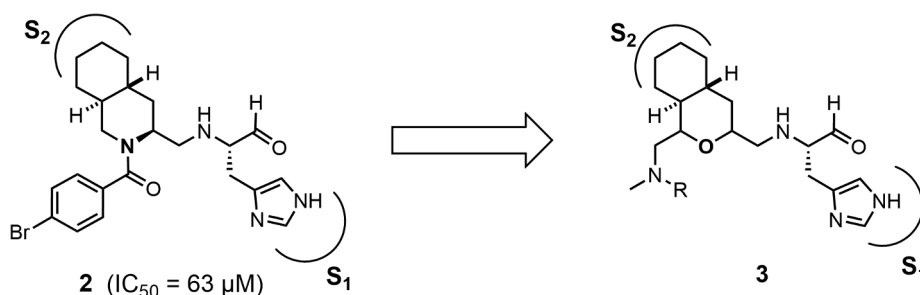


Fig. 2. The design of octahydroisochromene inhibitor 3 with four different substituents at the 1-position.

inhibitors containing an octahydroisochromene scaffold with four different substituents at the 1-position were designed and synthesized.

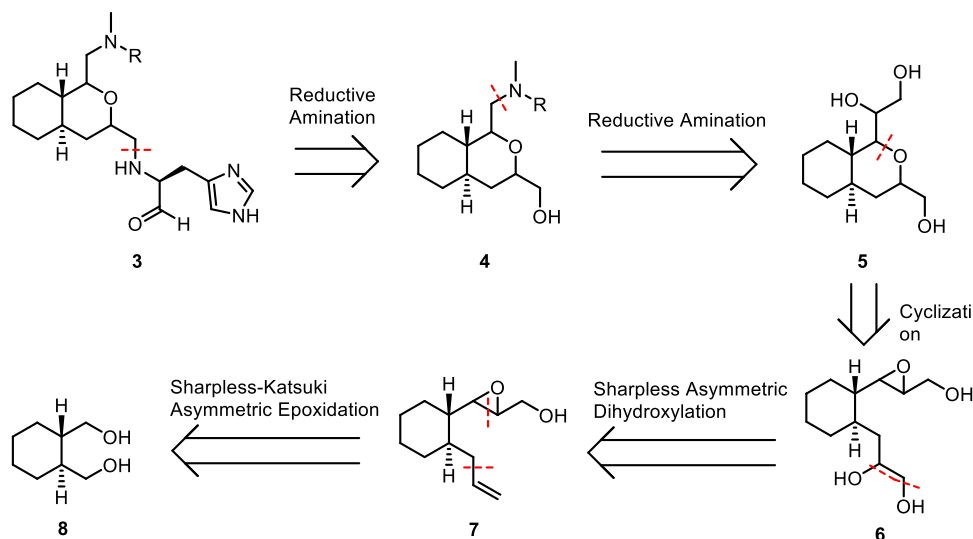
2. Results and discussion

2.1. Chemistry

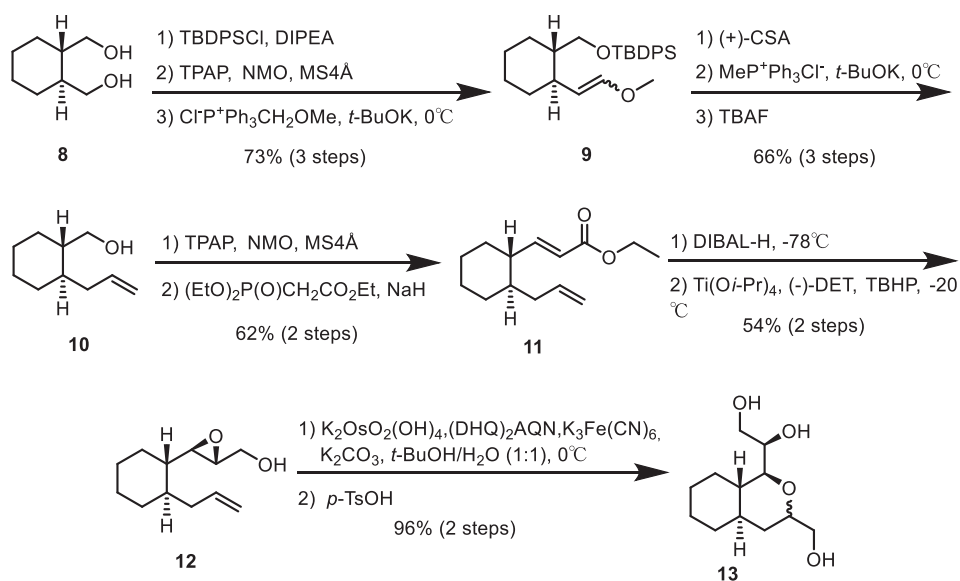
The retrosynthetic analysis of octahydroisochromene inhibitor 3 is shown in Scheme 1. (*S*)-2-Amino-3-imidazolyl propanal (His-al), the warhead part of 3, was to be introduced using a reductive amination reaction between His-al and octahydroisochromene derivative 4. The substituent at the 1-position of the octahydroisochromene ring in 4 was also introduced using a reductive amination reaction of the requisite amine and the aldehyde constructed via oxidative cleavage of the 1,2-diol in key intermediate 5. Construction of the octahydroisochromene ring in 5, a key step in the synthesis, may be carried out using an intramolecular nucleophilic addition reaction of the hydroxyl group to the epoxide in cyclohexyl derivative 6. The configuration of the

resulting ring structure in 5 can be controlled using a combination of the stereo-chemistry of the nucleophilic secondary hydroxyl group and electrophilic epoxide in 6, which make it possible to selectively form the four possible configurations of the ring system in 5. The configuration of the nucleophilic hydroxyl groups in 6 can be controlled using a Sharpless asymmetric dihydroxylation¹³ on the olefin in 7 and the configuration of the electrophilic epoxide in 7 can be selectively constructed using a Sharpless-Katsuki asymmetric epoxidation reaction¹⁴ on the allylic alcohol obtained upon the functional group conversion of commercially available diol 8.

Synthesis of key intermediate 5 was achieved starting from a commercially available compound (8) according to the route shown in Scheme 2. Monoprotection of diol 8 using *tert*-butyl diphenylsilyl chloride (TBDPSCl) followed by oxidation of the unprotected alcohol in the resulting product via a Ley-Griffith oxidation reaction using tetrapropyl ammonium perruthenate (TPAP) gave the corresponding aldehyde. The product was used with any purification, and reacted with (methoxymethyl)triphenylphosphonium chloride to give 9. The



Scheme 1. Retrosynthetic analysis of octahydroisochromene inhibitor 3.



Scheme 2. Synthetic route used to prepare compound 13.

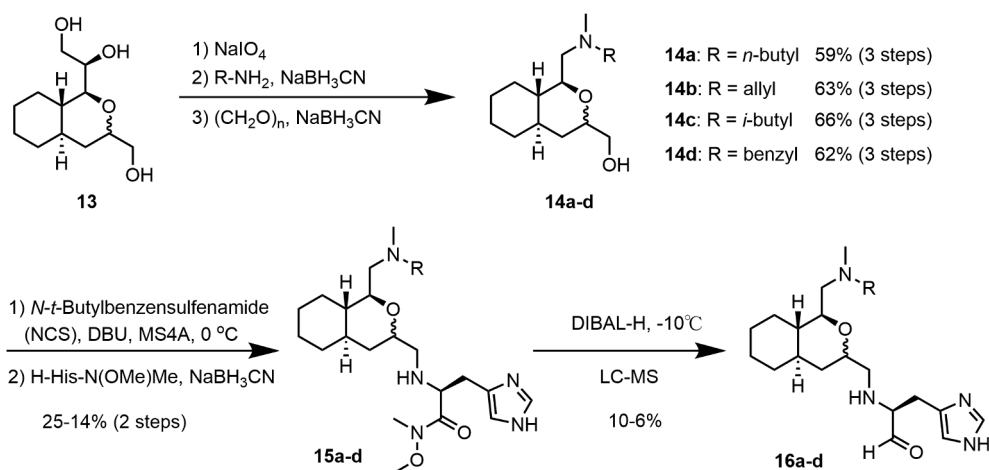
methoxyvinyl group in **9** was then converted to the corresponding aldehyde upon treatment with 10-camphorsulfonic acid. The isolated aldehyde was then reacted with methyl triphenylphosphonium chloride to yield a terminal olefin. The TBDPS group in the product was then removed upon treatment with tetra-*n*-butylammonium fluoride (TBAF) to give **10**. The primary alcohol in **10** was oxidized via a Ley–Griffith oxidation reaction and the resulting aldehyde homologated using a Horner–Wadsworth–Emmons (HWE) reaction upon reaction with ethyl 2-(diethoxyphosphoryl)acetate to give **11**. The ethyl ester in **11** was reduced with diisobutyl aluminum hydride (DIBAL-H) and the resulting allylic alcohol stereo-specifically oxidized via a Sharpless–Katsuki asymmetric epoxidation reaction using *tert*-butyl hydroperoxide (TBHP) in the presence of titanium tetraisopropoxide (TTIP) and (*S*, *S*)-diethyltartrate ((-)-DET) to yield **12** as a single diastereomer. The terminal olefin in **12** was then oxidized via a Sharpless asymmetric dihydroxylation reaction using potassium osmate (VI) dehydrate ($K_2OsO_2(OH)_4$) and potassium ferricyanide ($K_3Fe(CN)_6$) in the presence of hydroquinone (anthraquinone-1,4-diyl) diether ((DHQ)₂AQN). The formation of the expected diol was confirmed by ¹H NMR spectroscopy with the simultaneous production of a small amount of the cyclized product (**13**) formed via the nucleophilic addition of the resulting alcohol. Thus, the mixture was treated with *p*-toluenesulfonic acid without any further purification to convert the remaining dihydroxylated product into the desired cyclized product (**13**). However, whether product **13** was formed as a single diastereoisomer or an inseparable mixture of diastereoisomers could not be determined using ¹H NMR spectroscopy. Thus, the product was converted into the final products containing different substituents at the 1-position of the octahydroisochromene scaffold to evaluate whether the fused ring structure would function as a novel inhibitor scaffold.

Conversion of key intermediate **13** into the final product was carried out according to the route shown in Scheme 3. The terminal 1,2-diol in **13** was oxidatively cleaved using sodium periodate and the resulting aldehyde subjected to a reductive amination reaction with four different amines in the presence of $NaBH_3CN$. Each aminated product was then methylated with paraformaldehyde to give **14a–d**. The primary alcohol in each product was oxidized using *N-tert*-butylbenzylsulfonamide/1-chloropyrrolidine-2,5-dione (NCS) and the resulting aldehydes were reductively condensed with H-His-N(OMe)Me to give their corresponding Weinreb amides (**15a–d**).¹⁵ Each product was finally reduced to their corresponding aldehyde upon treatment with DIBAL-H and purified by LC/MS to give the desired aldehyde product (**16a–d**),

which exhibited a broad single peak in LC.

To access whether the octahydroisochromene derivatives containing substituents at the 1-position of the ring system function as a novel inhibitor scaffold, the inhibitory activity of compounds **16a–d** toward SARS 3CL^{pro} was preliminarily evaluated by the IC₅₀ values obtained using the procedure described below. As summarized in Table 1, compound **16a** bearing an *n*-butyl group showed moderate inhibitory activity, whereas **16c** bearing an isobutyl group showed no inhibitory activity. Compounds **16b** and **16d** bearing allyl and benzyl substituents, respectively showed weak activity. Although the absolute configurations of **16a** to **16d** were not identified, these results strongly suggested that the octahydroisochromene scaffold can function as a novel hydrophobic scaffold in the SARS 3CL^{pro} inhibitor and may also interact with the protease at the 1-position of the fused-ring system.

Confirmation of the configuration of compounds **16a–d** was then conducted based on the above findings. Since it was unclear whether key intermediate **13** used for the above syntheses was formed as a single diastereomer or an inseparable mixture of diastereoisomers using ¹H NMR spectroscopy, the ¹³C NMR spectrum of **13** was examined and two signals were detected for the methine carbon atom neighboring an oxygen atom in the octahydroisochromene scaffold (Fig. S-1). This result strongly suggests that triol **13** obtained using the Sharpless asymmetric dihydroxylation reaction using (DHQ)₂AQN as a ligand was formed as a 1.5:1 diastereoisomeric mixture (Scheme 2 and entry 1 in Table 2). Thus, the reaction conditions used in the asymmetric dihydroxylation were evaluated focusing on the chiral ligand, a key factor in the stereo-selectivity of the reaction.¹⁶ Precursor **12** was treated with $K_2OsO_2(OH)_4$ and $K_3Fe(CN)_6$ with or without the chiral ligand as stated above (Scheme 2) and the products immediately converted into triol **13** upon treatment with *p*-toluenesulfonic acid. The diastereoisomeric ratio of the products was estimated using the methine carbon atom signal in the ¹³C NMR spectrum. Table 2 shows that in the absence of the chiral ligand, an equal amount of both the possible diastereoisomers was obtained (entry 4). A slight preference for one diastereomer was observed when hydroquinone (anthraquinone-1,4-diyl) diether ((DHQ)₂AQN) was used as the chiral ligand, as in Scheme 2 (Table 2, entry 1). In contrast, a much better preference for one diastereoisomer was observed when 1,4-bis(dihydroquinone)phthalazine ((DHQ)₂PHAL) or hydroquinidine-2,5-diphenyl-4,6-pyrimidinediyl diether ((DHQ)₂Pyr) was used as the chiral ligand. (DHQ)₂Pyr (entry 3) showed a slightly better diastereoisomeric ratio (dr = 7:1) than (DHQ)₂PHAL (entry 2, dr = 6:1), which made it technically feasible to isolate the major



Scheme 3. Synthetic route used to prepare inhibitors 16a-d.

product. As expected, the diastereoisomeric ratio was inverted when hydroquinidine-2,5-diphenyl-4,6-pyrimidinediyl diether ((DHQD)₂Pyr) was used as the chiral ligand (entry 5, dr = 1:15).

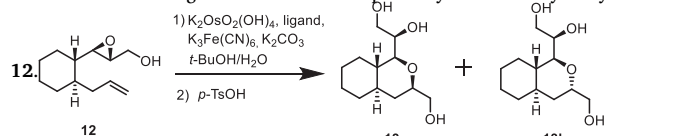
To determine the configuration of each diastereomer (13a and 13b), the major product obtained after the asymmetric dihydroxylation reaction using (DHQ)₂Pyr as the chiral ligand (Table 2, entry 3) was isolated and purified using preparative thin layer chromatography (PTLC). In the ¹H NMR spectrum of the major product, a nuclear Overhauser effect (NOE) was observed between the protons at the 1- and 3-positions of the octahydroisochromene ring and between the protons at the 3-position and bridgehead α-position, which indicates that these protons adopt a *cis*-α-configuration (Fig. S-1a). The coupling constant between the proton at the 1-position and the neighboring proton of the secondary alcohol was observed to be 9.7 Hz, which also indicates the *cis*-configuration of the protons. Taken together, it was estimated that the configuration of the major product was (1-*S*, 3-*R*) 13a, as shown in Table 1.¹⁷ Although the confirmed structure of 13a was the opposite to that expected for the asymmetric dihydroxylation reaction, it has been occasionally reported depending on the substrate used.^{18,19} In this specific substrate, steric hindrance due to the epoxy alcohol interferes with the ordinary interactions with the chiral ligand to direct attack of potassium osmate to the terminal olefin from the less sterically hindered face (Fig. S-1c).

Based on these results, the stereo-selective synthesis of all the possible diastereoisomers derived from the oxygen atom in the octahydroisochromene ring were then carried out to evaluate the steric effect of the fused ring on their inhibitory activity. In these syntheses, the separation and purification of the major product was achieved in the final step of the synthesis using LC/MS instead of purifying intermediate 13 (Scheme 3). In addition, an *n*-butyl group was employed as the suitable substituent at the 1-position in accordance with our preliminary evaluation of the inhibitory activity using the diastereoisomeric mixtures of compounds 16a-d (Table 1).

The synthetic route used to prepare (1-*S*, 3-*R*) 3a is shown in

Table 2

Effect of the chiral ligand used in the Sharpless asymmetric dihydroxylation of



Entry	Ligand	Ratio of the mixture*	Yield of 13a + 13b
1	(DHQ) ₂ AQN	1.5 : 1	96
2	(DHQ) ₂ PHAL	6 : 1	85
3	(DHQ) ₂ Pyr	7 : 1	84
4	–	1 : 1	82
5	(DHQD) ₂ Pyr	1 : 15	85

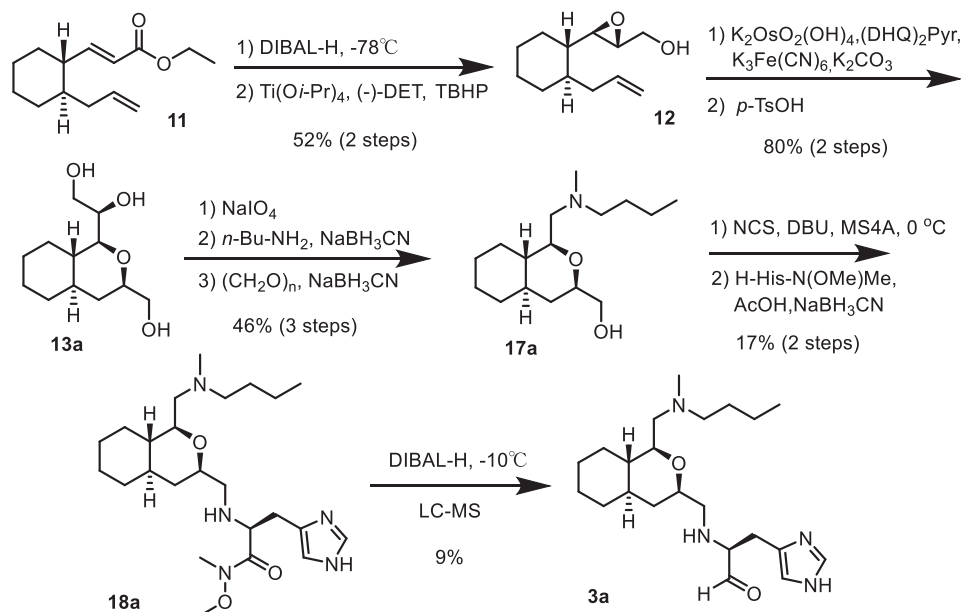
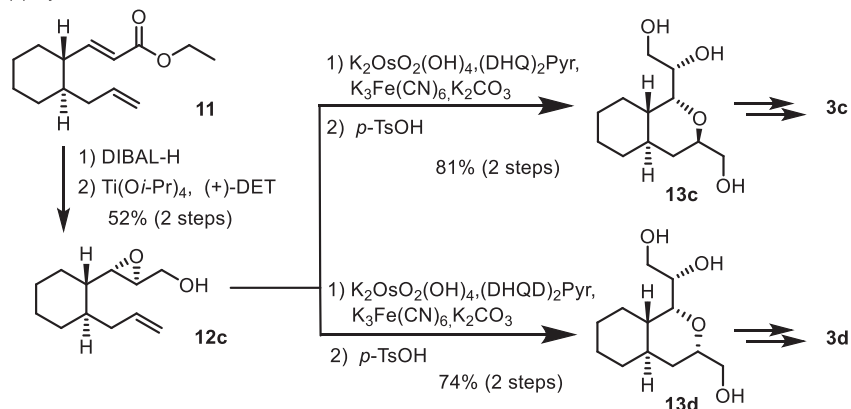
* The ratio of 13a and 13b was determined by ¹³C NMR analysis.

Scheme 4-(i). The allylic alcohol obtained from the reduction of ester 11 was oxidized via a Sharpless-Katsuki asymmetric epoxidation reaction using (–)-DET as the chiral ligand to give 12 as a single diastereoisomer. The terminal olefin in 12 was then oxidized via a Sharpless asymmetric dihydroxylation reaction using (DHQ)₂Pyr as the chiral ligand to give 13a as the major product (dr = 7:1). The mixture was then used for the following reactions without separating the major product. Oxidative cleavage of the 1,2-diol in 13a and the subsequent reductive amination reaction using *n*-butyl amine and paraformaldehyde were conducted as described for compound 14a (Scheme 3). The final coupling with H-His-N(OMe)Me followed by reduction with DIBAL-H was carried out as described for compound 16a (Scheme 3) to yield 3a as the major product. Since the diastereoisomeric ratio of the major product was relatively high (dr = 7:1), purification of the desired 3a could be achieved using LC/MS without difficulty. A diastereoisomer 3b with the configuration of (1-*S*, 3-*S*) was synthesized by employing a combination of (–)-DET and (DHQD)₂Pyr, which were used in the Sharpless-Katsuki asymmetric epoxidation reaction (yielding a single diastereoisomer)

Table 1

IC₅₀ values obtained for compounds 16a-d.

16a IC₅₀ ~100 μM
16b IC₅₀ ~420 μM
16c IC₅₀ >1600 μM
16d IC₅₀ ~400 μM

(i) Synthetic route to **3a**(ii) Synthetic route to **3c** and **3d****Scheme 4.** (i) Synthetic route used to prepare inhibitors (1-*S*, 3-*R*) **3a**. (ii) Synthetic route used to prepare inhibitors (1-*R*, 3-*R*) **3c** and (1-*R*, 3-*S*) **3d**.**Table 3**
IC₅₀ values obtained for inhibitors **3a–d**.

3a IC ₅₀ =260 μM	3b IC ₅₀ =95 μM	3c IC ₅₀ =780 μM	3d IC ₅₀ =440 μM

and the Sharpless asymmetric dihydroxylation reaction (*dr* = 1:15), respectively. The product was then purified using LC/MS with high homogeneity.

Further, diastereoisomers, **3c** and **3d**, were synthesized with the configurations of (1-*R*, 3-*R*) and (1-*R*, 3-*S*), respectively; this was achieved by employing synthetic routes as shown in [Scheme 4](#)-(ii). For the construction of the configuration of (1-*R*, 3-*R*) in **13c**, a combination of (+)-DET and (DHQ)₂Pyr was used, which were utilized in the Sharpless-Katsuki asymmetric epoxidation reaction (yielding a single diastereoisomer) and the Sharpless asymmetric dihydroxylation

reaction (*dr* = 8:1), respectively. Similarly, the construction for the configuration of (1-*R*, 3-*S*) in **13d** was performed using a combination of (+)-DET and (DHQD)₂Pyr, which were utilized in the Sharpless-Katsuki asymmetric epoxidation reaction (yielding a single diastereoisomer) and the Sharpless asymmetric dihydroxylation reaction (*dr* > 29:1), respectively. In both syntheses, the diastereo-excess (*dr*) was exceedingly high, which led to both the products, **13c** and **13d**, being obtained as single diastereoisomers. Likewise, each product was then converted to **3c** or **3d** as mentioned above and purified using LC/MS with high homogeneity.

2.2. Inhibitory activity

The inhibitory activities of **16a–d** and **3a–d** were evaluated based on the IC_{50} values obtained according to a literature procedure (Table 1 and 3).⁹ In brief, a peptide substrate, H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH₂, was incubated with R188I SARS 3CL^{PRO} at 37 °C for 2 h in the presence of each inhibitor and the resulting mixture was analyzed using analytical HPLC performed on a C18 reversed-phase column. The IC_{50} value of **16a** was estimated from the sigmoidal plot obtained from the decrease in the digested substrate (Fig. S-2). However, the as-obtained IC_{50} value (Table 1) was an approximate value and only indicated the inhibition tendency because the obtained plot was not well fitted to the expected sigmoidal curve due to the presence of only 40% of the desired diastereomer (derived from the diastereoisomeric mixture of **13**, dr = 1.5:1). Compound **16b** and **16d** showed ~50% inhibition at 400 μ M and **16d** showed weak inhibition even at 1600 μ M. In spite of these vague results, they strongly suggest that the octahydroisochromene ring functions as a novel hydrophobic scaffold in the SARS 3CL^{PRO} inhibitor. It was also expected that a medium size linear alkyl group at the 1-position, such as *n*-butyl in **16a**, can interact with SARS 3CL^{PRO} at a specific site, but bulkier substituents such as the branched group in **16c** or planar groups in **16b** and **16d** exhibit less interactions.

The IC_{50} values observed for the diastereoisomerically pure (**3a–d**) ranged from 95 to 780 μ M, a rather wide range, which clearly indicates the steric effect of the octahydroisochromene scaffold on the inhibitory activity (Table 3). Compound **3b** was most active (IC_{50} = 95 μ M) and had a (1*S*, 3*S*)-configuration in the ring system. Compound **3c** was the least active (IC_{50} = 780 μ M) with an enantiomeric (1*R*, 3*R*)-configuration when compared to **3b**. The results suggest that converting the stereocenter at the 1-position has a more significant effect on the inhibitory activity than changing the stereochemistry at the 3-position.

2.3. Interaction model for **3a–d** with SARS 3CL protease

The interactions between compounds **3a–d** and R188I SARS 3CL^{PRO} were proposed using a docking model constructed with Molecular Operating Environment (MOE) software. The fused-ring system containing the aldehyde warhead of the decahydroisoquinoline inhibitor complexed with the protease (PDB: 4TWW)¹¹ was used as a template for the docking model. The docking scores obtained for inhibitors **3a**, **3b**, and **3d** were similar (**3a**, -8.3615 kcal/mol; **3b**, -8.3982 kcal/mol; **3d**, -8.4354 kcal/mol), whereas the docking score for **3c** was rather low (-7.9265 kcal/mol). The docking mode for **3b** (IC_{50} = 95 μ M) showed nearly the same interactions with the protease as the parent decahydroisoquinoline inhibitor at its P1 site imidazole as well as those at the P2 site fused-ring system (Fig. 3a). The warhead aldehyde was also directed toward the active center of the protease, as-observed in the case of the decahydroisoquinolin inhibitor (Fig. 3a). In addition, the *n*-butyl substituent at the 1-position of the octahydroisochromene scaffold in **3b** was assumed to be situated on the surface of the protease near the S1' to S2' pockets (Fig. 3b). In contrast, the docking mode for

less potent inhibitor **3c** (IC_{50} = 780 μ M) showed different interactions at the P1 site imidazole and aldehyde warhead at the active center of the protease, although the interactions with the fused-ring system were expected to be similar to **3b** (Fig. 3c). The interactions with the *n*-butyl substituent at the 1-position of the octahydroisochromene scaffold in **3c** were different from those of inhibitor **3b**. The different modes of interaction observed for **3b** and **3c** were derived from the change in the configuration of the octahydroisochromene scaffold, which caused an 88% decrease in the inhibitory activity. To confirm the estimated interactions, X-ray crystallographic analysis of **3b** complexed with R188I SARS 3CL^{PRO} is now underway in our laboratory.

3. Conclusion

As an alternative non-peptide scaffold to decahydroisoquinoline^{11,12}, using an octahydroisochromene scaffold has been introduced into a fused-ring type SARS 3CL protease inhibitor as a novel hydrophobic core to interact with the S2 pocket of the protease. Alkyl and aryl substituents were also introduced to the 1-position of the octahydroisochromene scaffold to form additional interactions with the protease. Sharpless-Katsuki asymmetric epoxidation and Sharpless asymmetric dihydroxylation reaction were used to construct the four possible diastereoisomers of the octahydroisochromene scaffold by selecting the appropriate chiral ligand in each asymmetric reaction. The purity and stereochemistry of the synthesized key intermediate **13** were confirmed using ¹H NMR, NOE, and ¹³C NMR spectroscopy. Introductions of the P1 site His-al and substituent at the 1-position was achieved using successive reductive amination reactions.

Inhibitor **16a** was prepared as a diastereo-isomeric mixture derived from the Sharpless asymmetric dihydroxylation reaction and showed moderate inhibitory activity toward R188I SARS 3CL^{PRO}. In contrast, the diastereoisomeric mixtures of **16b**, **16c** and **16d**, which contain bulky or planar substituents at the 1-position, showed lower inhibitory activity. These results strongly suggested the functionality of the octahydroisochromene as a core hydrophobic scaffold for the S2 pocket of the protease and the additional interactions formed between the 1-position of the scaffold when the substituent is a linear alkyl group of moderate size. Based on these positive findings, the effects of the stereochemistry of the octahydroisochromene scaffold were examined and the results suggest that a specific configuration of the octahydroisochromene, (1*S*, 3*S*) **3b**, directed the P1 site imidazole and the warhead aldehyde to their counterpart pockets in the protease. In addition, the *n*-butyl substituent at 1-position of the fused-ring system in **3b** was also expected to form some interactions with the protease. In contrast, these functional groups in the enantiomeric octahydroisochromene inhibitor, (1*R*, 3*R*) **3c**, were probably misdirected causing an 88% decrease in the inhibitory activity. Thus, detailed analysis of these interactions based on X-ray crystal analysis of **3b** complexed with R188I SARS 3CL^{PRO} to optimize the substituent at the 1-position as well as an alternative warhead group are ongoing in our laboratory.

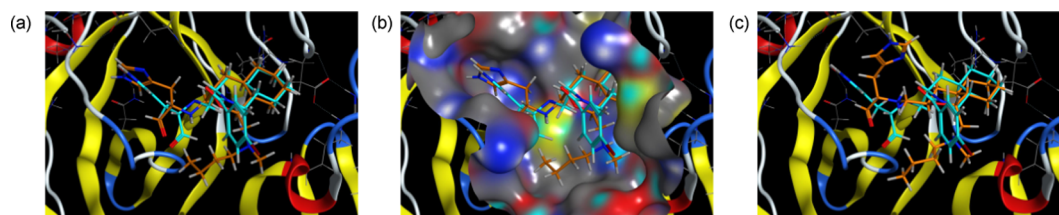


Fig. 3. Docking model for **3b** and **3c** with R188I SARS 3CL^{PRO}. (a) and (b) The interaction mode for **3b** with a GBVI/WSA dG score of -8.3982 kcal/mol. (c) The interaction mode for **3c** with a GBVI/WSA dG score of -7.9265 kcal/mol. Each docking model with R188I SARS 3CL^{PRO} was constructed using the X-ray crystal structure obtained for the SARS 3CL^{PRO} and decahydroisoquinoline inhibitor (PDB 4TWW) complex as a template. The possible binding mode was obtained using a docking simulation of each inhibitor and SARS 3CL^{PRO} using an automated template-guided docking protocol with the Amber10:EHT force field in the MOE 2019.0101 software package (Chemical Computing Group Inc., Montreal, Quebec, Canada).

4. Experimental

4.1. General

Silica gel 70PF 254 Plate-Wako was used for TLC and column chromatography was performed on Wakogel® 60 N (particle size, 63–212 μm) or Wakogel® C-300E (particle size, 45–75 μm). Low-resolution mass spectroscopy (LRMS) was recorded on Shimadzu LCMS-2010EV (ESI) and high-resolution mass spectroscopy (HRMS) was recorded on Shimadzu LCMS-IT-TOF (ESI) or JEOL GC mate II (EI) or JEOL SX-102A (FAB). ^1H NMR spectroscopy was recorded on a Bruker AV300 or Ascend500 spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to tetramethylsilane (0 ppm) or the residual solvent peak. ^{13}C NMR spectroscopy was recorded on the same spectrometer at 75 or 125 MHz using the residual solvent peak as an internal reference. Optical rotations were recorded using a Jasco P-2200 Polarimeter.

4.2. ((1*S*,2*S*)-2-((*tert*-Butyldiphenylsilyloxy)methyl)cyclohexyl)methanol

To a solution of diol **8** (14.4 g, 100 mmol) and DIPEA (87 mL, 500 mmol) in CH_2Cl_2 (200 mL) was added TBDPS-Cl (30 mL, 110 mmol) and the resulting mixture stirred at room temperature for 10 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The resulting residue was purified using silica gel column chromatography (hexane/EtOAc = 30:1) to give the protected alcohol (37.4 g, 98%) as a colorless oil. $[\alpha]_{\text{D}}^{20} + 6.1$ (c 0.48, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 7.68–7.65 (m, 4H), 7.47–7.36 (m, 6H), 3.65–3.54 (m, 4H), 3.18 (brs, 1H), 1.73–1.65 (m, 3H), 1.48–1.32 (m, 3H), 1.28–0.85 (m, 4H), 1.06 (s, 9H); ^{13}C NMR (75 MHz, CDCl_3) δ : 135.6, 133.1, 129.80, 127.78, 127.7, 69.0, 67.2, 44.7, 43.3, 29.9, 29.7, 26.8, 26.09, 26.05, 19.1; HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{34}\text{NaO}_2\text{Si}$ $[\text{M} + \text{Na}]^+$: 405.2626. Found: 405.2224.

4.3. (((1*S*,2*R*)-2-Allylcyclohexyl)methoxy)(*tert*-butyl)diphenylsilane

To a solution of the protected alcohol (37.4 g, 98.0 mmol) and TPAP (tetra-*n*-propyl ammonium perruthenate, 688 mg, 1.96 mmol) in CH_2Cl_2 (200 mL) was added NMO (*N*-methylmorpholine-*N*-oxide, 45.9 g, 392 mmol) at room temperature under an argon atmosphere. After stirring for 1 h, the reaction mixture was filtered through a pad of silica gel and the filtrate concentrated in vacuo. The resulting residue was used immediately in the next step without any purification. To a solution of *t*-BuOK (21.9 g, 196 mmol) in THF (200 mL) was added methoxymethyltriphenylphosphonium chloride (67.1 g, 196 mmol) at 0 °C under an argon atmosphere and the resulting mixture stirred for 1 h. The residue obtained from the TPAP oxidation reaction in THF (50 mL) was added dropwise at 0 °C and the resulting mixture stirred for 10 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 30:1) to give **9** (29.7 g, 74%, over 2 steps) as a colorless oil. **9**, which was used immediately in the next step. To a solution of (+)-camphorsulfonic acid (CSA; 25.4 g, 109 mmol) in CH_2Cl_2 (200 mL) was added to a solution of **9** (29.7 g, 73 mmol) in CH_2Cl_2 (200 mL) and the resulting mixture stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NaHCO_3 and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo to give the crude aldehyde, which was used immediately in the next step without any purification. To a solution of *t*-BuOK (16.3 g, 146 mmol) in THF (200 mL) was added methyltriphenylphosphonium bromide (52.1 g, 146 mmol) at 0 °C under an argon atmosphere and the resulting mixture stirred for 1 h. The crude

aldehyde in THF (40 mL) was added dropwise at 0 °C and the resulting mixture was stirred for 10 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 30:1) to give the olefin product (20.3 g, 72%, over 2 steps) as a colorless oil. $[\alpha]_{\text{D}}^{20} + 3.1$ (c 0.93, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 7.69–7.65 (m, 4H), 7.43–7.34 (m, 6H), 5.71 (dddd, $J = 16.8, 10.3, 8.0, 6.5$ Hz, 1H), 4.94–4.86 (m, 2H), 3.69–3.57 (m, 2H), 2.23–2.18 (m, 1H), 1.88–1.78 (m, 2H), 1.73–1.67 (m, 2H), 1.43–1.17 (m, 4H), 1.06 (s, 9H), 1.06–0.95 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 137.2, 135.6, 134.0, 129.49, 129.47, 127.56, 127.55, 115.6, 66.3, 43.8, 37.9, 31.3, 30.0, 26.9, 26.3, 26.1, 19.4; HRMS (FAB) calcd for $\text{C}_{26}\text{H}_{37}\text{OSi}$ $[\text{M} + \text{H}]^+$: 393.2614. Found: 393.2623.

4.4. ((1*S*,2*R*)-2-Allylcyclohexyl)methanol (**10**)

To a solution of the olefin (20.3 g, 52.0 mmol) in THF (150 mL) was added TBAF (1.0 M solution in THF; 78 mL, 78 mmol) at room temperature and the resulting mixture stirred for 10 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 20:1) to give **10** (7.30 g, 92%) as a colorless oil. $[\alpha]_{\text{D}}^{20} + 12.9$ (c 1.55, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 5.80 (dddd, $J = 16.8, 10.3, 7.9, 6.5$ Hz, 1H), 5.04–4.98 (m, 2H), 3.70 (dd, $J = 10.7, 2.9$ Hz, 1H), 3.56 (dd, $J = 10.8, 5.4$ Hz, 1H), 2.33–2.24 (m, 1H), 2.01–1.91 (m, 1H), 1.82–1.71 (m, 3H), 1.26–1.12 (m, 5H), 1.08–0.96 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 137.0, 115.8, 65.4, 43.7, 37.94, 37.92, 31.6, 29.5, 26.0, 25.8.

4.5. Ethyl (*E*)-3-((1*S*,2*R*)-2-allylcyclohexyl)acrylate (**11**)

To a solution of **10** (7.30 g, 47.4 mmol) and TPAP (330 mg, 0.940 mmol) in CH_2Cl_2 (160 mL) was added NMO (22.1 g, 189 mmol) at room temperature under an argon atmosphere. After stirring for 1 h, the reaction mixture was filtered through a pad of silica gel and the filtrate concentrated in vacuo. The resulting residue was used immediately in the next step without any purification. To a solution of NaH (60% dispersion in mineral oil; 2.40 g, 61.6 mmol) in THF (160 mL) was added triethyl phosphonoacetate (13 mL, 66 mmol) at –20 °C and the resulting mixture stirred for 20 min. The residue obtained from the TPAP oxidation of **10** in THF (25 mL) was added dropwise to the reaction mixture at –20 °C and stirred for 30 min. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 30:1) to give **11** (6.50 g, 62% over 2 steps) as a colorless oil. $[\alpha]_{\text{D}}^{20} + 10.3$ (c 1.68, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 6.81 (dd, $J = 15.8, 9.5$ Hz, 1H), 5.79 (dd, $J = 15.6, 0.6$ Hz, 1H), 5.72 (dddd, $J = 16.2, 11.0, 8.1, 6.2$ Hz, 1H), 5.00 (brs, 1H), 4.97–4.93 (m, 1H), 4.19 (q, $J = 7.1$ Hz, 1H), 2.22–2.17 (m, 1H), 1.91–1.65 (m, 7H), 1.34–1.21 (m, 4H), 1.29 (t, $J = 7.1$ Hz, 3H), 1.01–0.88 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 166.8, 153.5, 136.5, 120.9, 116.2, 60.1, 46.4, 41.0, 39.0, 32.6, 30.9, 25.9, 25.5, 14.3; HRMS (EI) calcd for $\text{C}_{14}\text{H}_{22}\text{O}_2$ $[\text{M}]^+$: 222.1620. Found: 222.1615.

4.6. (*E*)-3-((1*S*,2*R*)-2-Allylcyclohexyl)prop-2-en-1-ol

To a solution of **11** (6.50 g, 29.3 mmol) in CH_2Cl_2 (100 mL) was added DIBAL-H (1.0 M solution in hexane; 88 mL, 88 mmol) at –78 °C under an argon atmosphere and the resulting mixture stirred for 1 h. The reaction was quenched with CH_3OH , warmed to room temperature, filtered through a pad of silica gel, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/

EtOAc = 6:1) to give the allylic alcohol (4.10 g, 79%) as a colorless oil. $[\alpha]_D^{20} + 11$ (c 0.10, MeOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ : 5.81–5.47 (m, 3H), 4.99 (brs, 1H), 4.95–4.92 (m, 1H), 4.10 (d, $J = 5.4$ Hz, 1H), 2.30–2.22 (m, 1H), 1.82–1.58 (m, 7H), 1.25–1.12 (m, 4H), 0.98–0.84 (m, 1H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ : 137.9, 137.3, 128.7, 115.7, 63.9, 46.3, 41.4, 38.9, 33.7, 31.3, 26.2, 25.9.

4.7. ((2R,3R)-3-((1S,2R)-2-Allylcyclohexyl) oxiran-2-yl) methanol (**12**)

To a solution of diethyl *D*-tartrate (7.8 mL, 46 mmol) in CH_2Cl_2 (100 mL) was added titanium(IV) isopropoxide (13 mL, 46 mmol) at 0 °C under an argon atmosphere. After stirring for 20 min, the mixture was cooled to –20 °C and added to *t*-butyl hydroperoxide (6.0 M solution in toluene; 15 mL, 90 mmol). The allylic alcohol was then added dropwise to the reaction mixture at –20 °C and stirred for 6 h. The reaction was quenched with 10% potassium sodium (+)-tartrate tetrahydrate in water at 0 °C, extracted with CH_2Cl_2 and the organic layer concentrated in vacuo. 1 M NaOH in diethyl ether was added to the resulting residue at room temperature and stirred for 2 h. The resulting mixture was extracted with diethyl ether, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 6:1) to give **12** (3.10 g, 68%) as a colorless oil. $[\alpha]_D^{20} + 34$ (c 0.50, MeOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ : 5.83–5.69 (m, 1H), 5.03–4.98 (m, 2H), 3.95–3.92 (m, 1H), 3.70–3.63 (m, 1H), 2.99–2.98 (m, 1H), 2.72 (dd, $J = 8.7$, 2.1 Hz, 1H), 2.33–2.29 (m, 1H), 1.94–1.73 (m, 5H), 1.34–1.13 (m, 4H), 0.99–0.92 (m, 2H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ : 136.7, 116.2, 61.4, 59.7, 59.4, 44.6, 39.6, 38.6, 31.2, 29.8, 26.0, 25.4; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{20}\text{NaO}_2$ $[\text{M} + \text{Na}]^+$: 219.1361. Found: 219.1357.

Diastereomer of 12: yield 68%; $[\alpha]_D^{20} + 8.7$ (c 0.55, MeOH); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ : 5.89–5.75 (m, 1H), 5.06–4.98 (m, 2H), 3.95–3.90 (m, 1H), 3.64–3.59 (m, 1H), 2.88–2.87 (m, 1H), 2.80 (dd, $J = 8.0$, 2.3 Hz, 1H), 2.56–2.48 (m, 1H), 2.04–1.94 (m, 1H), 1.82–1.64 (m, 4H), 1.39–1.05 (m, 4H), 1.00–0.87 (m, 2H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ : 137.1, 116.0, 61.9, 60.0, 56.1, 44.7, 42.2, 38.5, 31.1, 29.1, 25.8, 25.6; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{20}\text{NaO}_2$ $[\text{M} + \text{Na}]^+$: 219.1361. Found: 219.1356.

4.8. (1R)-1-((1S,3RS,4aR,8aS)-3-(Hydroxymethyl)octahydro-1H-isochromen-1-yl) ethane-1,2-diol (**13**)

To a solution of $\text{K}_3\text{Fe}(\text{CN})_6$ (15.3 g, 46.5 mmol) in *t*-BuOH/ H_2O (1:1, 150 mL) at room temperature was added K_2CO_3 (6.42 g, 46.5 mmol), $(\text{DHQ})_2\text{AQN}$ (118 mg, 0.137 mmol), and $\text{K}_2\text{OsO}_2(\text{OH})_4$ (22.7 mg, 0.0894 mmol). After stirring for 20 min, the resulting mixture was cooled to 0 °C, **12** (3.01 g, 13.1 mmol) in *t*-BuOH/ H_2O (1:1, 20 mL) was added, and the resulting mixture stirred for 10 h. The reaction was quenched with aqueous Na_2SO_3 and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 20:1$) to give the triol as a colorless oil, which was used immediately in the next step without any further purification. To a solution of *p*-toluenesulfonic acid monohydrate (836 mg, 4.40 mmol) was added the triol in CH_2Cl_2 (50 mL) at room temperature and the resulting mixture stirred for 10 h. The reaction was quenched with saturated aqueous NaHCO_3 and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 20:1$) to give **13** (3.20 g, 96%) as a colorless oil. $^1\text{H NMR}$ (300 MHz, CDCl_3) δ : 4.13–4.07 (m, 1H), 3.87–3.75 (m, 2H), 3.67–3.53 (m, 2H), 3.50–3.29 (m, 2H), 1.79–1.37 (m, 6H), 1.25–1.18 (m, 3H), 1.10–0.88 (m, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , dr = 5:4) δ : 83.8, 78.8, 76.2, 74.2, 71.2, 70.9, 66.1, 62.4, 62.2, 60.4, 43.6, 43.2, 40.5, 36.2, 34.6, 33.5, 33.2, 27.3, 27.0, 25.9, 25.8, 25.7; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{22}\text{NaO}_4$ $[\text{M} + \text{Na}]^+$: 253.1416. Found: 253.1410.

4.9. ((1S,3RS,4aR,8aS)-1-((Butyl(methyl)amino)methyl)octahydro-1H-isochromen-3-yl) methanol (**14a**)

To a solution of **13** (800 mg, 3.48 mmol) was added NaIO_4 (1.00 g, 4.67 mmol) in THF (8.0 mL) and H_2O (8.0 mL) at room temperature. After the mixture was stirred for 10 min, the mixture was washed with brine, extracted with EtOAc, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The resulting residue was used immediately in the next step without any purification. The residue was added to *n*-butylamine (460 μL , 4.7 mmol) and sodium cyanotrihydroborate (640 mg, 10.2 mmol) in CH_2Cl_2 (15 mL) at room temperature. After stirring for 1 h, the reaction was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The resulting residue was used immediately in the next step without any purification. The amination product was added to paraformaldehyde (2.70 g) and sodium cyanotrihydroborate (640 mg, 10.2 mmol) in EtOH (14 mL) at room temperature and stirred for 12 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 15:1$) to give **14a** (468 mg, 59% over 3 steps) as a colorless oil. $^1\text{H NMR}$ (300 MHz, CDCl_3) δ : 4.04–4.02 (m, 1H), 3.56–3.51 (m, 1H), 3.49–3.36 (m, 1.6H), 3.28–3.21 (m, 0.4H), 2.58–2.57 (m, 1H), 2.52–2.42 (m, 1H), 2.35 (s, 1.2H), 2.33 (s, 1.8H), 2.35–2.15 (m, 3H), 1.84–1.49 (m, 6H), 1.43–1.17 (m, 4H), 1.08–0.82 (m, 9H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ : 79.3, 74.9, 74.5, 70.1, 65.6, 60.4, 59.2, 58.6, 58.0, 45.1, 44.8, 43.1, 40.1, 35.6, 34.0, 33.4, 32.9, 27.6, 27.3, 26.3, 25.8, 25.7, 25.6, 20.17, 20.12, 13.68, 13.65; HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{31}\text{NO}_2$ $[\text{M} + \text{H}]^+$: 270.2434. Found: 270.2432.

Compounds **14b**, **14c**, and **14d** were prepared using the same synthetic procedure. **14b**: yield 63%, 3 steps; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 4:1 mixture of two diastereoisomers) δ : 6.12–5.98 (m, 1H), 5.54–5.46 (m, 2H), 4.18–4.07 (m, 2H), 3.96–3.90 (m, 1H), 3.74–3.38 (m, 3.2H), 3.33 (d, $J = 9.3$ Hz, 0.8H), 3.10 (dd, $J = 13.1$, 2.0 Hz, 0.8H), 3.03–3.02 (m, 0.2H), 2.94–2.92 (m, 0.2H), 2.84 (dd, $J = 12.9$, 9.9 Hz, 0.8H), 2.81 (s, 2.4H), 2.75 (s, 0.6H), 1.79–1.69 (m, 2H), 1.62–1.50 (m, 2H), 1.48–1.39 (m, 1H), 1.25–1.22 (m, 3H), 1.03–0.91 (m, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , 4:1 mixture of two diastereoisomers) δ : 128.1, 127.5, 124.9, 124.6, 78.9, 74.3, 74.2, 69.6, 65.4, 60.44, 60.37, 59.1, 57.9, 57.3, 44.9, 44.6, 42.4, 40.5, 39.8, 35.4, 33.9, 33.3, 32.81, 32.75, 27.3, 25.7, 25.6, 25.5; HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{28}\text{NO}_2$ $[\text{M} + \text{H}]^+$: 254.2122. Found: 254.2113. **14c**: yield 66%, 3 steps; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 3:2 mixture of two diastereoisomers) δ : 4.22–4.04 (m, 1H), 3.64–3.48 (m, 2H), 3.41 (td, $J = 9.4$, 3.1 Hz, 0.6H), 3.31 (dd, $J = 11.9$, 3.2 Hz, 0.4H), 3.13 (dd, $J = 13.4$, 2.0 Hz, 0.4H), 3.09–2.75 (m, 3.6H), 2.87 (s, 1.2H), 2.79 (s, 1.8H), 2.17–2.06 (m, 1H), 1.80–1.41 (m, 5H), 1.31–1.24 (m, 3H), 1.14–0.88 (m, 3H), 1.13 (d, $J = 6.6$ Hz, 1.2H), 1.08 (t, $J = 6.9$ Hz, 3H), 1.04 (d, $J = 6.6$ Hz, 1.8H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , 3:2 mixture of two diastereoisomers) δ : 79.0, 74.7, 74.5, 69.7, 66.5, 65.5, 64.4, 60.3, 59.7, 58.3, 45.0, 44.8, 44.1, 42.8, 40.0, 35.5, 34.0, 33.4, 32.92, 32.86, 27.4, 27.2, 25.8, 25.77, 25.69, 25.65, 25.5, 24.9, 24.5, 21.3, 21.0, 20.93, 20.90; HRMS (EI) calcd for $\text{C}_{16}\text{H}_{31}\text{NO}_2$ $[\text{M}]^+$: 269.2355. Found: 269.2355. **14d**: yield 62%, 3 steps; $^1\text{H NMR}$ (300 MHz, CDCl_3 , 1:1 mixture of two diastereoisomers) δ : 7.32–7.24 (m, 5H), 4.03–3.96 (m, 1H), 3.69–3.16 (m, 6H), 2.55–2.48 (m, 2H), 2.26 (s, 1.5H), 2.25 (s, 1.5H), 1.75–1.38 (m, 6H), 1.28–1.17 (m, 3H), 1.07–0.78 (m, 3H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , 1:1 mixture of two diastereoisomers) δ : 138.6, 138.2, 129.4, 129.2, 128.2, 127.1, 127.0, 79.3, 78.0, 72.90, 72.87, 66.2, 63.1, 62.5, 60.9, 59.8, 59.5, 45.0, 44.5, 43.5, 43.0, 40.6, 36.0, 34.6, 33.6, 33.4, 32.8, 28.0, 27.8, 26.2, 26.1, 26.0, 25.9; HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{30}\text{NO}_2$ $[\text{M} + \text{H}]^+$: 304.2277. Found: 304.2273.

4.10. (2S)-2-(((1S,3RS,4aR,8aS)-1-((Butyl(methyl)amino)methyl)octahydro-1H-isochromen-3-yl)methyl)amino)-3-(1H-imidazol-4-yl)-N-methoxy-N-methylpropanamide (15a)

To a solution of **14a** (30.0 mg, 0.12 mmol) in CH_2Cl_2 was added benzenesulfenamide (20 μL , 0.11 mmol), 1,8-diazabicyclo[5.4.0]-7-undecene (100 μL , 0.64 mmol), and *N*-chlorosuccinimide (44.0 mg, 0.66 mmol) at 0 °C in the presence of Molecular Sieves 4A and the resulting mixture was stirred for 2 h. The reaction was quenched with saturated aqueous NaHCO_3 and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The resulting residue was used immediately in the next step without any purification. To a solution of the product in CH_2Cl_2 (3 mL) was added *H*-His-*N*(OMe)Me (65.0 mg, 0.328 mmol), CH_3COOH (1 drop from syringe), and cyanotrihydroborate (20.7 mg, 0.329 mmol) at room temperature. After the mixture was stirred for 1 h, the reaction was quenched with saturated aqueous NaHCO_3 and extracted with CH_2Cl_2 . The organic layer was washed with saturated aqueous NH_4Cl and brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by LC-MS [linear gradient of B from 10 to 35% over 30 min (B: 0.05% CH_3COOH in CH_3CN ; A: 0.05% CH_3COOH in H_2O)] to give **15a** (18.0 mg, 18%, 2 steps) as a yellow oil. ^1H NMR (300 MHz, CDCl_3 , 3:2 mixture of two diastereomers) δ : 7.54 (s, 0.4H), 7.51 (s, 0.6H), 6.79 (s, 1H), 4.03–3.96 (m, 0.6H), 3.74–3.72 (m, 1H), 3.65 (s, 3H), 3.50–3.45 (m, 1.4H), 3.23 (s, 1.8H), 3.22 (1.2H), 3.23–3.22 (m, 1H), 2.98–2.86 (m, 2.6H), 2.75–2.57 (m, 3.4H), 2.52–2.42 (m, 4H), 2.37 (s, 1.8H), 2.34 (s, 1.2H), 1.78–1.46 (m, 6H), 1.39–1.26 (m, 5H), 1.08–0.85 (m, 3H), 0.91 (t, $J = 7.5$ Hz, 3H); HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{44}\text{N}_5\text{O}_3$ [$\text{M} + \text{H}$] $^+$: 450.3446. Found: 450.3439.

Compounds **15b**, **15c**, and **15d** were prepared using the same synthetic procedure. **15b**: yield 14%, 2 steps; ^1H NMR (300 MHz, CDCl_3 , 4:1 mixture of two diastereomers) δ : 7.55 (s, 0.2H), 7.52 (s, 0.8H), 6.81 (s, 1H), 5.87 (ddt, $J = 17.0, 10.3, 6.7$ Hz, 1H), 5.21–5.13 (m, 2H), 4.04–3.98 (m, 1H), 3.75–3.63 (m, 1H), 3.68 (s, 2.4H), 3.65 (s, 0.6H), 3.50–3.43 (m, 1.6H), 3.40–3.34 (m, 0.4H), 3.24 (s, 2.4H), 3.22 (s, 0.6H), 3.21–3.17 (m, 1H), 3.09 (dd, $J = 13.5, 6.6$ Hz, 1H), 2.97–2.85 (m, 2H), 2.79–2.63 (m, 2H), 2.58 (dd, $J = 13.5, 1.5$ Hz, 1H), 2.50–2.38 (m, 3H), 2.34 (s, 2.4H), 2.33 (s, 0.6H), 1.74–1.53 (m, 4H), 1.47 (dd, $J = 12.5, 1.5$ Hz, 1H), 1.39 (dd, $J = 3.9, 1.5$ Hz, 0.8H), 1.39 (dd, $J = 4.1, 1.4$ Hz, 0.2H), 1.25–1.16 (m, 3H), 1.10–0.82 (m, 2H); HRMS (EI) calcd for $\text{C}_{23}\text{H}_{39}\text{N}_5\text{O}_3$ [M] $^+$: 433.3053. Found: 433.3054. **15c**: yield 25%, 2 steps; ^1H NMR (500 MHz, CDCl_3 , 55:45 mixture of two diastereomers) δ : 7.56 (s, 0.45H), 7.55 (s, 0.55H), 6.81 (s, 1H), 3.99–3.97 (m, 0.55H), 3.81–3.75 (m, 1H), 3.64 (s, 3H), 3.49–3.42 (m, 1H), 3.27–3.20 (m, 0.45H), 3.224 (s, 1.6H), 3.216 (s, 1.4H), 2.95 (dd, $J = 15.0, 4.0$ Hz, 1H), 2.91–2.86 (m, 1H), 2.74–2.66 (m, 1.55H), 2.62–2.39 (m, 3.45H), 2.35 (s, 1.6H), 2.33 (s, 1.4H), 2.30–2.17 (m, 2H), 1.82–1.70 (m, 5.35H), 1.61–1.55 (m, 1.1H), 1.51–1.45 (m, 0.55H), 1.41–1.38 (m, 1H), 1.26–1.20 (m, 3H), 1.08–0.86 (m, 8H); HRMS (EI) calcd for $\text{C}_{24}\text{H}_{43}\text{N}_5\text{O}_3$ [M] $^+$: 449.3366. Found: 449.3364. **15d**: yield 17%, 2 steps; ^1H NMR (500 MHz, CDCl_3 , 2:1 mixture of two diastereomers) δ : 7.51–7.45 (m, 1H), 7.34–7.23 (m, 5H), 6.80 (s, 1H), 5.41–5.38 (m, 2H), 3.78–3.70 (m, 1H), 3.65–3.62 (m, 4H), 3.52–3.50 (m, 1H), 3.31–3.20 (m, 1H), 3.23 (s, 1H), 3.22 (s, 2H), 2.92–2.89 (m, 1H), 2.70–2.62 (m, 1H), 2.57–2.46 (m, 1H), 2.43–2.39 (m, 1H), 2.31 (s, 3H), 2.07–1.95 (m, 1H), 1.75–1.38 (m, 5H), 1.25–0.82 (m, 6H); HRMS (EI) calcd for $\text{C}_{27}\text{H}_{41}\text{N}_5\text{O}_3$ [M] $^+$: 483.3209. Found: 483.3214.

4.11. (2S)-2-(((1S,3RS,4aR,8aS)-1-((Butyl(methyl)amino)methyl)octahydro-1H-isochromen-3-yl)methyl)amino)-3-(1H-imidazol-4-yl)propanal (16a)

To a solution of **15a** (4.5 mg, 0.00932 mmol) in CH_2Cl_2 (1.5 mL) was added DIBAL-H (1.0 M solution in hexane, 200 μL , 0.20 mmol) at –10 °C under an argon atmosphere and the resulting mixture stirred for 30 min. The reaction was quenched with CH_3OH and concentrated in

vacuo. The residue was filtered and concentrated in vacuo. The resulting residue was purified by LC-MS [linear gradient of B from 5 to 35% over 30 min (B: 0.05% CH_3COOH in CH_3CN ; A: 0.05% CH_3COOH in H_2O)] to give **16a** (450 μg , 10%) as a yellow solid. ^1H NMR (500 MHz, CDCl_3 , 2:1 mixture of two diastereomers) δ : 8.85–8.84 (m, 0.6H), 8.75 (m, 0.4H), 8.25 (m, 0.6H), 8.08 (s, 0.4H), 7.27–7.24 (m, 1H), 4.71 (m, 1H), 4.34 (m, 0.4H), 3.93 (m, 0.6H), 3.81 (m, 1H), 3.69–3.62 (m, 3H), 3.45–3.44 (m, 1H), 3.35–3.29 (m, 1H), 3.21–3.12 (m, 3H), 2.98–2.90 (m, 1H), 2.94 (s, 2H), 2.93 (s, 1H), 1.84–1.65 (m, 6H), 1.46–1.29 (m, 6H), 1.67–0.89 (m, 7H); HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$: 391.3075. Found: 391.3028.

Compounds **16b**, **16c**, and **16d** were prepared using the same synthetic procedure. **16b**: yield 6%; ^1H NMR (300 MHz, CDCl_3) δ : 8.86 (m, 0.6H), 8.76 (m, 0.4H), 8.22–8.19 (m, 1H), 7.25–7.24 (m, 1H), 6.03–5.94 (m, 1H), 5.63–5.60 (m, 2H), 4.75–4.73 (m, 1H), 4.34–4.32 (m, 0.4H), 4.23 (m, 0.6H), 3.92–3.82 (m, 2H), 3.74–3.56 (m, 3H), 3.37 (m, 1H), 3.24–3.22 (m, 1H), 3.19–3.18 (m, 2H), 2.94 (s, 1.2H), 2.92 (s, 1.8H), 2.69–2.61 (m, 1H), 1.86–1.66 (m, 4H), 1.47–1.25 (m, 3H), 1.17–0.79 (m, 5H); HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{35}\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$: 375.2762. Found: 375.2755. **16c**: yield 9%; ^1H NMR (300 MHz, CDCl_3) δ : 8.62 (s, 1H), 8.07 (s, 1H), 7.41–7.38 (m, 1H), 4.77 (m, 1H), 4.37–4.32 (m, 2H), 3.96–3.81 (m, 2H), 3.75–3.63 (m, 3H), 3.26 (m, 3H), 2.98 (s, 3H), 2.98–2.92 (m, 1H), 2.24–2.15 (m, 2H), 1.85–1.64 (m, 6H), 1.42–1.29 (m, 4H), 1.12–1.03 (m, 7H); HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$: 391.3075. Found: 391.3068. **16d**: yield 10%; ^1H NMR (500 MHz, CDCl_3) δ : 8.33 (s, 1H), 8.07 (s, 1H), 7.54–7.52 (m, 5H), 7.28 (s, 1H), 4.78–4.74 (m, 1H), 4.51 (d, $J = 13.5$ Hz, 1H), 4.33–4.29 (m, 1H), 3.87 (m, 1H), 3.22–3.09 (m, 2H), 2.93 (s, 3H), 2.84–2.64 (m, 4H), 2.00–1.95 (m, 2H), 1.76–1.61 (m, 4H), 1.48–1.29 (m, 5H), 1.13–0.88 (m, 3H); HRMS (ESI) calcd for $\text{C}_{25}\text{H}_{37}\text{N}_4\text{O}_2$ [$\text{M} + \text{H}$] $^+$: 425.2918. Found: 425.2911.

4.12. (R)-1-((1S,3R,4aR,8aS)-3-(Hydroxymethyl)octahydro-1H-isochromen-1-yl)ethane-1,2-diol (13a)

To a solution of $\text{K}_3\text{Fe}(\text{CN})_6$ (15.3 g, 46.5 mmol) in *t*-BuOH/ H_2O (1:1, 150 mL) was added K_2CO_3 (6.42 g, 46.5 mmol), $(\text{DHQ})_2\text{Pyr}$ (121 mg, 0.137 mmol) and $\text{K}_2\text{OsO}_2(\text{OH})_4$ (22.7 mg, 0.0894 mmol) at room temperature. After stirring for 20 min, the mixture was cooled to 0 °C and **12** (3.06 g, 15.6 mmol) in *t*-BuOH/ H_2O (1:1, 20 mL) added, and the resulting mixture stirred for 10 h. The reaction was quenched with aqueous Na_2SO_3 and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 20:1$) to give the triol as a colorless oil, which was used immediately in the next step without any further purification. To a solution of *p*-toluenesulfonic acid monohydrate (836 mg, 4.40 mmol) was added the triol in CH_2Cl_2 (50 mL) at room temperature and the resulting mixture stirred for 10 h. The reaction was quenched with saturated aqueous NaHCO_3 and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH} = 20:1$) to give **13a** (2.87 g, 80%) as a colorless oil. $[\alpha]_D^{20} + 11.6$ (c 1.08, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 4.47 (brs, 1H), 4.22 (brs, 1H), 4.14–4.03 (m, 2H), 3.91–3.80 (m, 2H), 3.68–3.65 (m, 1H), 3.59 (dd, $J = 9.9, 3.0$ Hz, 1H), 3.51–3.36 (m, 1H), 3.32 (d, $J = 8.4$ Hz, 1H), 1.78–1.37 (m, 5H), 1.27–1.17 (m, 3H), 1.13–0.89 (m, 3H). ^{13}C NMR (75 MHz, CDCl_3 , major isomer dr = 6:1) δ : 76.3, 74.1, 70.1, 62.5, 60.4, 43.6, 36.2, 33.4, 33.1, 27.2, 25.8, 25.7; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{22}\text{NaO}_4$ [$\text{M} + \text{Na}$] $^+$: 253.1416. Found: 253.1414.

(1-*S*, 3-*R*)-**13b**, (1-*R*, 3-*R*)-**13c**, and (1-*R*, 3-*S*)-**13d** were prepared using the same synthetic procedure. **13b**: yield 85%, 2 steps; $[\alpha]_D^{20} + 12$ (c 0.48, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 4.53 (brs, 1H), 4.22 (brs, 1H), 3.86–3.81 (m, 2H), 3.68–3.60 (m, 1H), 3.57–3.48 (m, 3H), 3.45–3.40 (d, $J = 7.8$ Hz, 1H), 2.24 (brs, 1H), 1.79–1.62 (m,

4H), 1.44–1.39 (m, 1H), 1.29–1.21 (m, 3H), 1.07–0.92 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3) δ : 84.1, 78.9, 70.5, 66.1, 62.5, 43.2, 40.4, 34.6, 33.1, 27.0, 25.9, 25.8; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{22}\text{NaO}_4$ $[\text{M} + \text{Na}]^+$: 253.1416. Found: 253.1410. **13c**: yield 81%, 2 steps; $[\alpha]_{\text{D}}^{20}$ –16 (c 0.18, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 3.96 (dd, J = 8.3, 4.4 Hz, 1H), 3.86 (dd, J = 12.0, 6.3 Hz, 1H), 3.77–3.57 (m, 3H), 3.51 (dd, J = 11.7, 3.0 Hz, 1H), 3.51 (dd, J = 11.7, 7.8 Hz, 1H), 1.80–1.69 (m, 4H), 1.59–1.50 (m, 3H), 1.44–1.37 (m, 1H), 1.25–1.10 (m, 4H), 1.00–0.93 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ : 78.9, 72.8, 71.7, 66.0, 64.2, 40.1, 34.7, 32.3, 31.6, 27.6, 26.7, 26.0; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{22}\text{NaO}_4$ $[\text{M} + \text{Na}]^+$: 253.1416. Found: 253.1414. **13d**: yield 74%, 2 steps; $[\alpha]_{\text{D}}^{20}$ –12 (c 0.24, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 4.37 (brs, 1H), 4.04 (brs, 1H), 3.83 (dd, J = 11.3, 4.7 Hz, 1H), 3.75 (d, J = 10.5 Hz, 1H), 3.68 (dd, J = 9.0, 4.2 Hz, 1H), 3.61–3.50 (m, 2H), 3.46–3.38 (m, 2H), 1.81–1.65 (m, 3H), 1.54–1.41 (m, 3H), 1.36–1.17 (m, 3H), 1.00–0.94 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ : 76.9, 73.2, 69.4, 66.3, 64.0, 44.4, 34.8, 34.5, 34.2, 28.6, 27.0, 26.0; HRMS (ESI) calcd for $\text{C}_{12}\text{H}_{22}\text{NaO}_4$ $[\text{M} + \text{Na}]^+$: 253.1416. Found: 253.1410.

4.13. ((1*S*,3*R*,4*aR*,8*aS*)-1-((Butyl(methyl)amino)methyl)octahydro-1*H*-isochromen-3-yl) methanol (**17a**)

(1-*S*, 3-*R*)-**17a**, (1-*S*, 3-*S*)-**17b**, (1-*R*, 3-*R*)-**17c**, and (1-*R*, 3-*S*)-**17d** were prepared using the same synthetic procedure described for **14a**. **17a**: yield 46%, 3 steps; $[\alpha]_{\text{D}}^{20}$ + 35 (c 0.21, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 4.06 (d, J = 10.2 Hz, 1H), 4.01 (d, J = 10.2 Hz, 1H), 3.51–3.47 (m, 0.5H), 3.45–3.32 (m, 1.5H), 2.49–2.24 (m, 4H), 2.26 (s, 3H), 1.75–1.68 (m, 3H), 1.61–1.38 (m, 5H), 1.36–1.22 (m, 5H), 1.04–0.85 (m, 3H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 74.2, 70.6, 60.6, 59.5, 58.6, 44.7, 43.1, 35.7, 33.5, 32.9, 27.7, 27.0, 25.9, 25.6, 20.3, 13.8; HRMS (EI) calcd for $\text{C}_{16}\text{H}_{31}\text{NO}_2$ $[\text{M}]^+$: 269.2355. Found: 269.2352. **17b**: yield 43%, 3 steps; $[\alpha]_{\text{D}}^{20}$ + 13 (c 0.50, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 3.64–3.59 (m, 2H), 3.50–3.45 (m, 1H), 3.37 (td, J = 9.3, 4.0 Hz, 1H), 3.09–2.88 (m, 4H), 2.76 (s, 3H), 1.82–1.64 (m, 4H), 1.55–1.21 (m, 8H), 1.09–0.92 (m, 4H), 0.97 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 78.2, 77.8, 66.1, 59.6, 57.9, 45.1, 42.4, 40.4, 34.5, 33.3, 28.2, 27.7, 26.1, 25.9, 20.6, 14.0; HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{32}\text{NO}_2$ $[\text{M} + \text{H}]^+$: 270.2435. Found: 270.2430. **17c**: yield 44%, 3 steps; $[\alpha]_{\text{D}}^{20}$ –17 (c 0.18, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 4.07 (dd, J = 6.0, 5.1 Hz, 1H), 4.00 (dd, J = 10.5, 5.7 Hz, 1H), 3.66 (t, J = 12.0 Hz, 1H), 3.38 (dd, J = 12.3, 4.8 Hz, 1H), 2.96 (t, J = 12.8 Hz, 1H), 2.75–2.65 (m, 1H), 2.52–2.46 (m, 1H), 2.43 (s, 3H), 2.23 (dd, J = 12.6, 4.8 Hz, 1H), 1.78–1.64 (m, 4H), 1.63–1.22 (m, 10H), 1.00–0.88 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 72.6, 69.6, 65.2, 58.8, 57.0, 43.2, 40.7, 34.1, 32.7, 29.5, 27.8, 27.7, 26.6, 26.0, 20.5, 13.9; HRMS (EI) calcd for $\text{C}_{16}\text{H}_{31}\text{NO}_2$ $[\text{M}]^+$: 269.2355. Found: 269.2360. **17d**: yield 42%, 2 steps; $[\alpha]_{\text{D}}^{20}$ –14 (c 0.74, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 3.99–3.95 (m, 1H), 3.81–3.77 (m, 2H), 3.55 (dd, J = 6.6, 1.8 Hz, 1H), 3.48 (dd, J = 6.6, 4.2 Hz, 1H), 2.94 (dd, J = 8.1, 6.3 Hz, 1H), 2.76–2.68 (m, 1H), 2.55–2.44 (m, 2H), 2.35 (s, 3H), 2.24 (dd, J = 8.1, 1.8 Hz, 1H), 1.79–1.58 (m, 3H), 1.53–1.44 (m, 4H), 1.42–1.21 (m, 5H), 1.04–0.91 (m, 6H); ^{13}C NMR (75 MHz, CDCl_3 , 4:1 mixture of two conformers) δ : 78.7, 73.7, 70.3, 66.4, 65.9, 58.8, 57.9, 57.3, 53.0, 45.1, 44.3, 42.4, 40.3, 34.9, 34.32, 34.25, 34.0, 33.1, 28.7, 28.3, 27.5, 27.1, 26.6, 26.1, 25.9, 25.8, 20.6, 20.4, 14.0, 13.8; HRMS (EI) calcd for $\text{C}_{16}\text{H}_{31}\text{NO}_2$ $[\text{M}]^+$: 269.2355. Found: 269.2354.

4.14. (S)-2-(((1*S*,3*R*,4*aR*,8*aS*)-1-((Butyl(methyl)amino)methyl)octahydro-1*H*-isochromen-3-yl)methyl)amino)-3-(1*H*-imidazol-4-yl)-*N*-methoxy-*N*-methylpropanamide (**18a**)

(1-*S*, 3-*R*)-**18a**, (1-*S*, 3-*S*)-**18b**, (1-*R*, 3-*R*)-**18c**, and (1-*R*, 3-*S*)-**18d** were prepared using the same synthetic procedure described for **15a**. **18a**: yield 17%, 2 steps; $[\alpha]_{\text{D}}^{20}$ + 17 (c 0.55, MeOH); ^1H NMR (300 MHz, CDCl_3) δ : 7.54 (s, 1H), 6.79 (s, 1H), 3.99–3.96 (m, 1H),

3.84–3.81 (m, 1H), 3.66–3.64 (m, 1H), 3.61 (s, 3H), 3.56–3.48 (m, 1H), 3.13 (s, 3H), 2.98–2.90 (m, 2H), 2.77–2.61 (m, 4H), 2.56–2.32 (m, 3H), 2.49 (s, 3H), 1.74–1.66 (m, 5H), 1.58–1.44 (m, 3H), 1.39–1.17 (m, 5H), 0.96–0.90 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H); HRMS (EI) calcd for $\text{C}_{24}\text{H}_{43}\text{N}_5\text{O}_3$ $[\text{M}]^+$: 449.3366. Found: 449.3361. **18b**: yield 13%, 2 steps; $[\alpha]_{\text{D}}^{20}$ + 36 (c 0.050, MeOH); ^1H NMR (500 MHz, CDCl_3) δ : 7.54 (s, 1H), 6.79 (s, 1H), 3.74–3.73 (m, 1H), 3.64 (s, 3H), 3.48 (td, J = 8.8, 2.0 Hz, 1H), 3.36–3.34 (m, 1H), 3.27–3.20 (m, 1H), 3.22 (s, 3H), 2.92 (dd, J = 15.0, 3.5 Hz, 1H), 2.75–2.67 (m, 3H), 2.59 (d, J = 13.5 Hz, 2H), 2.52–2.44 (m, 4H), 2.36–2.31 (m, 2H), 2.35 (s, 3H), 1.77–1.66 (m, 4H), 1.59 (d, J = 13.5 Hz, 1H), 1.54–1.44 (m, 2H), 1.39–1.16 (m, 4H), 1.33 (q, J = 7.5 Hz, 2H), 1.07–0.86 (m, 2H), 0.92 (t, J = 7.5 Hz, 3H). **18c**: yield 19%, 2 steps; $[\alpha]_{\text{D}}^{20}$ –7.2 (c 0.42, MeOH); ^1H NMR (500 MHz, CDCl_3 , 4:1 mixture of two conformers) δ : 7.55 (s, 0.8H), 7.52 (s, 0.2H), 6.80 (s, 1H), 3.97–3.93 (m, 1H), 3.85 (dd, J = 8.5, 4.0 Hz, 0.8H), 3.80–3.77 (m, 1.2H), 3.66 (s, 2.4H), 3.64 (s, 0.6H), 3.21 (s, 3H), 2.96 (dd, J = 15.0, 3.5 Hz, 0.8H), 2.91 (dd, J = 15.0, 4.0 Hz, 0.2H), 2.78–2.64 (m, 2.4H), 2.58–2.40 (m, 5.2H), 2.37 (s, 2.4H), 2.32 (s, 0.6H), 2.29–2.28 (m, 0.2H), 2.24 (dd, J = 13.3, 3.3 Hz, 0.2H), 1.78–1.57 (m, 6H), 1.52–1.16 (m, 8H), 1.06–0.89 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); HRMS (EI) calcd for $\text{C}_{24}\text{H}_{43}\text{N}_5\text{O}_3$ $[\text{M}]^+$: 449.3366. Found: 449.3365. **18d**: yield 22%, 2 steps; $[\alpha]_{\text{D}}^{20}$ –3.1 (c 0.72, MeOH); ^1H NMR (500 MHz, CDCl_3 , 4:1 mixture of two conformers) δ : 7.58 (s, 0.2H), 7.53 (s, 0.8H), 6.80 (s, 1H), 4.02–4.00 (m, 0.8H), 3.83–3.76 (m, 1.8H), 3.72–3.67 (m, 0.2H), 3.64 (s, 3H), 3.61–3.56 (m, 0.2H), 3.22 (s, 2.4H), 3.20 (s, 0.6H), 3.00 (dd, J = 13.3, 10.3 Hz, 0.8H), 2.43 (dd, J = 15.0, 4.0 Hz, 1.2H), 2.73–2.64 (m, 2.8H), 2.58–2.46 (m, 2.4H), 2.43 (dd, J = 11.8, 2.8 Hz, 0.8H), 2.37 (s, 3H), 2.30 (dd, J = 13.3, 2.8 Hz, 1H), 1.77–1.65 (m, 4H), 1.53–1.48 (m, 4H), 1.42–1.28 (m, 3H), 1.24–1.20 (m, 2H), 1.05–0.89 (m, 3H), 0.91 (t, J = 7.3 Hz, 3H); HRMS (EI) calcd for $\text{C}_{24}\text{H}_{43}\text{N}_5\text{O}_3$ $[\text{M}]^+$: 449.3366. Found: 449.3369.

4.15. (S)-2-(((1*S*,3*R*,4*aR*,8*aS*)-1-((Butyl(methyl)amino)methyl)octahydro-1*H*-isochromen-3-yl)methyl)amino)-3-(1*H*-imidazol-4-yl)propanal (**3a**)

(1-*S*, 3-*R*)-**3a**, (1-*S*, 3-*S*)-**3b**, (1-*R*, 3-*R*)-**3c**, and (1-*R*, 3-*S*)-**3d** were prepared using the same procedure described for **16a**. **3a**: yield 9%; ^1H NMR (300 MHz, CDCl_3) δ : 7.90 (s, 1H), 7.72–7.69 (m, 1H), 6.96 (m, 1H), 4.68 (m, 1H), 4.30 (m, 2H), 4.15–4.12 (m, 2H), 3.78–3.65 (m, 3H), 3.45–3.43 (m, 1H), 3.34–3.34 (m, 2H), 3.00–2.79 (m, 4H), 1.85–1.53 (m, 6H), 1.48–1.22 (m, 7H), 1.20–0.90 (m, 6H); HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_4\text{O}_2$ $[\text{M} + \text{H}]^+$: 391.3075. Found: 391.3068. **3b**: yield 7%; ^1H NMR (500 MHz, CDCl_3) δ : 8.79–8.74 (m, 1H), 8.10 (s, 1H), 7.19–7.18 (m, 1H), 4.71 (m, 1H), 3.90 (d, J = 10.3 Hz, 1H), 3.83–3.79 (m, 1H), 3.63–3.61 (m, 3H), 3.44 (m, 1H), 3.28–3.24 (m, 1H), 3.16–3.10 (m, 3H), 2.99–2.91 (m, 1H), 2.97 (s, 3H), 1.83–1.59 (m, 6H), 1.42–1.28 (m, 6H), 1.15–0.88 (m, 7H); HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_4\text{O}_2$ $[\text{M} + \text{H}]^+$: 391.3075. Found: 391.3077. **3c**: yield 6%; ^1H NMR (300 MHz, CDCl_3) δ : 8.35 (m, 1H), 8.07 (s, 1H), 7.28 (s, 1H), 4.65 (m, 1H), 4.34 (m, 2H), 4.26–4.20 (m, 2H), 3.25–3.19 (m, 3H), 3.15–3.10 (m, 3H), 2.94–2.93 (m, 4H), 1.91–1.62 (m, 7H), 1.62–1.37 (m, 7H), 1.24–1.01 (m, 5H); HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_4\text{O}_2$ $[\text{M} + \text{H}]^+$: 391.3075. Found: 391.3068. **3d**: yield 7%; ^1H NMR (500 MHz, CDCl_3) δ : 8.20 (s, 1H), 7.98 (m, 1H), 7.21 (s, 1H), 4.63 (m, 1H), 4.34 (m, 1H), 4.21 (m, 1H), 4.04–4.02 (m, 1H), 3.65 (m, 1H), 3.53 (m, 1H), 3.40–3.36 (m, 2H), 3.22–3.20 (m, 2H), 3.11–2.87 (m, 5H), 1.87–1.57 (m, 7H), 1.46–1.23 (m, 6H), 1.08–0.90 (m, 3H), 1.03 (t, J = 7.3 Hz, 3H); HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_4\text{O}_2$ $[\text{M} + \text{H}]^+$: 391.3074. Found: 391.3068.

4.16. Estimation of the IC_{50} values

The peptide substrate (H-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-NH₂)⁹ 111 μM in a solution of 20 mM Tris-HCl buffer pH 7.5 containing 7 mM DTT (25 μL) was incubated with R1881 SARS 3CL^{pro} (56 nM)⁹ at 37 °C for 2 h in the presence of various concentrations of

the inhibitors. The mixture was eluted on an analytical HPLC column [Cosmosil 5C18 (4.6 × 150 mm)] using CH₃CN in aqueous 0.1% TFA (10–20% over 30 min) as the eluent and the cleavage rates were calculated from the reduction in the substrate peak area. Each IC₅₀ value was obtained from the sigmoidal dose–response curve (Fig. S-2 for a typical sigmoidal curve). Each experiment was repeated in triplicate and the results reported as the average value.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Determination of the configuration of triol **13** using the nOe spectra, typical sigmoidal curves used to obtain IC₅₀ values, and NMR data of the as-synthesized compounds.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2019.115273>.

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- The major product of entry 5 in Table 1 gave a clear nOe signal between the protons at the 1-position and bridgehead α-position. In addition, no nOe signals were observed between the protons at the 1- and 3-positions as well as between the 3-position and bridgehead α-position (Figure S-1b). However, although unclear, an nOe signal between the protons at the 1-position and methylene group at the 3-position of the fused ring was observed. These results strongly suggest the major product has a (1R, 3S) configuration.
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