Peptide-Based Inhibitors of the Hepatitis C Virus NS3 Protease: Structure−Activity Relationship at the *C*-Terminal Position

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The structure−activity relationship at the *C*-terminal position of peptide-based inhibitors of the hepatitis C virus NS3 protease is presented. The observation that the *N*-terminal cleavage product (DDIVPC-OH) of a substrate derived from the NS5A/5B cleavage site was a competitive inhibitor of the NS3 protease was previously described. The chemically unstable cysteine residue found at the P1 position of these peptide-based inhibitors could be replaced with a norvaline residue, at the expense of a substantial drop in the enzymatic activity. The fact that an aminocyclopropane carboxylic acid (ACCA) residue at the P1 position of a tetrapeptide such as **1** led to a significant gain in the inhibitory enzymatic activity, as compared to the corresponding norvaline derivative **2**, prompted a systematic study of substituent effects on the three-membered ring. We report herein that the incorporation of a vinyl group with the proper configuration onto this small cycle produced inhibitors of the protease with much improved in vitro potency. The vinyl-ACCA is the first reported carboxylic acid containing a P1 residue that produced NS3 protease inhibitors that are significantly more active than inhibitors containing a cysteine at the same position.

Introduction

It is estimated that up to 200 million people around the world are infected with the hepatitis C virus (HCV).[1,2] From this population, approximately 70% of individuals suffering from persistent infection will develop chronic hepatitis C. This progressive liver disease may lead to cirrhosis and hepatocellular carcinomas[3] and results in around 10 000 deaths annually in the United States alone. Standard therapies involve the use of pegylated interferon in combination with the broad spectrum antiviral agent ribavirin and are characterized by a limited efficacy (low sustained response rate) and severe side effects.[4]

The HCV was discovered in 1989 as the etiological agent for non-A and non-B hepatitis infections.[5] This enveloped RNA virus belongs to the Flaviviridae family. Its genome is composed of approximately 9600 base pairs and encodes a precursor polyprotein (ca. 3011 amino acids) that undergoes proteolytic maturation.[6,7] The NS3 is one of a number of mature proteins that result from the polypeptide processing. The *N*-terminal region of this NS3 protein exhibits proteolytic activity and can be considered as a chymotrypsin-like serine protease. The *C*-terminal portion of the protein contains an ATP-dependent RNA helicase. The NS3 protease is responsible for cleaving four sites on the polypeptide.[8−10] It possesses one structural zinc-binding domain and functions as a heterodimer complexed with the NS4A protein. This small polypeptide is an essential cofactor for efficient processing of the polyprotein by the NS3 protease.[10]

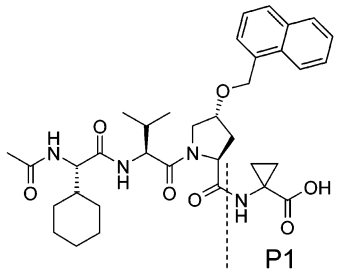

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Figure 1.

The NS3 protease is essential for viral replication, as shown by the lack of infectivity of an HCV RNA clone harboring a mutation in its catalytic domain (Ser-139-Ala), and therefore represents a prime target for drug discovery.[11]

We,[12] and others,[13] discovered that the *N*-terminal cleavage product (DDIVPC-OH) of a substrate derived from the NS5A/5B cleavage site is a competitive inhibitor of the NS3 protease. Early efforts using this hexapeptide as a starting point focused on finding a replacement for the unstable cysteine residue found at the P1[14] position (dimerization reaction). A structure−activity study performed on this cleavage product eventually led to the discovery of tetrapeptide **1** (Figure 1), a moderately potent ($IC_{50} = 14\ \mu M$), highly specific and competitive inhibitor,[15] possessing a chemically stable cysteine replacement at the *C*-terminal position. Related work carried out by Narjes et al.[16] led to the identification of 4,4-difluoro-2-aminobutyric acid (difluoro-Abu) as one possible P1 cysteine mimetic.

In this paper we present how the inhibitory activity of compound **1** was further improved through a structure−activity study carried out on the cyclopropane ring of the P1 position.

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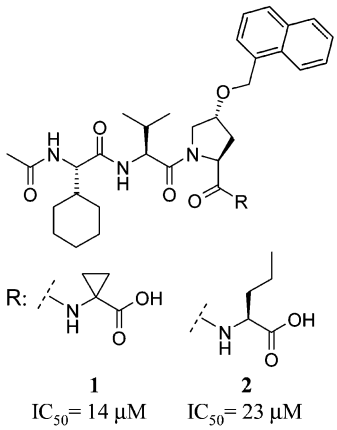


Figure 2.

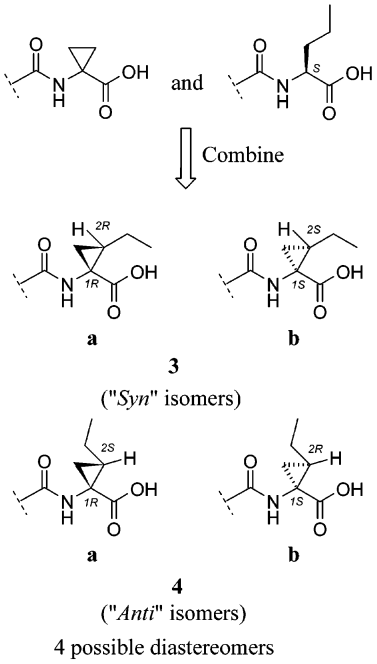


Figure 3.

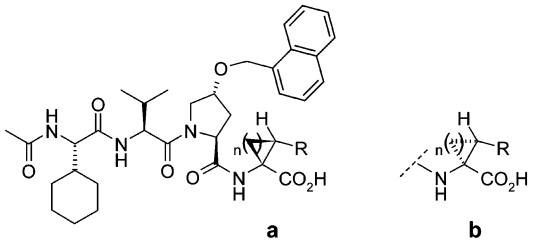
Results and Discussion

We have previously shown that norvaline[15a] and aminocyclopropane carboxylic acid[15b,c] (ACCA) (Figure 2) are two effective replacements for the cysteine residue at P1 of peptide-based inhibitors. It was anticipated that a gain in activity would be observed upon combining the beneficial effect of the cyclopropane ring with the propyl side chain of norvaline.

As depicted in Figure 3, the substituted cyclic amino acid resulting from this exercise can be viewed as a rigidified norvaline group (four possible diastereomers).

Isomers **3a**,**b**, which possess an ethyl substituent on a three-membered ring syn to the carboxylic acid group, were synthesized first. When these two "syn" methanonorvaline derivatives were introduced in a tetrapeptide series analogous to compound **1**, we discovered that one of them was well tolerated, giving a moderated increase in potency, while the other diastereomer was significantly less active (entry 3 in Table 1). The absolute configuration of these substituted ACCA derivatives was determined subsequently (see Chemistry section). As outlined in Figure 3, it was expected that the more active three-membered-ring isomer would correspond to

Table 1. ACCA Optimization



				IC50 (μm)[a]	
entry	compd	R	*n*	**a**	**b**
1	**2**	Et	0		23
2	**1**	H	1	14	
3	**14**	Et	1	4.8	32
4	**15**	Me	1	22	180
5	**16**	*n*-Pr	1	5.5	41
6	**17**	*i*-Pr	1	5.5	640
7	**18**	Bn	1	14	81
8	**19**	$PhCH_2CH_2$	1	22	90

[a] See main text for a comment on the establishment of the relative configuration at C1 and C2 on the cyclopropane ring.

3b (with the 1*S* configuration as with norvaline).[15d] However, a closer look at how the carboxylate group of both **3a** and **3b** would most likely bind in the active site of the enzyme suggested that this prediction was probably incorrect. The binding mode of the *C*-terminus of the helicase domain in the active site of the protease was revealed by the first crystal structure of the full-length NS3 protein with its *N*-terminus covalently linked to the NS4A peptide cofactor.[17] This X-ray crystal structure of a *C*-terminal carboxylate bound to the active site of the NS3/NS4A complex provided an atomic view of the interactions between the protease domain and the P-side product of the cis cleavage reaction (as highlighted in Figure 4A). In this structure, the P1 ϕ angle is −127.6°, properly positioning the carboxylate in the active site, where it is stabilized via several hydrogen bonds. These involve the side chains of catalytic residues His 57 and Ser 139, as well as the backbone amide protons of the oxyanion hole residues Gly 137 and Ser 139 (Figure 4A).

Application of the same carboxylate binding mode to both **3a** and **3b** (panels C and B, respectively) suggested that **3b** may not be the preferred rigidified norvaline mimic, as this analogue would thrust the ethyl side chain too deep into the small S1 binding pocket, resulting in unfavorable interactions with the Phe 154 side chain. On the other hand, the unnatural (1*R*,2*R*) configuration of **3a** allowed a better fit of the ethyl side chain into the pocket of interest (as illustrated in Figure 4C), resulting in a more potent analogue. This turned out to be the case when the **3a**- and **3b**-containing peptides were tested (Table 1, entry 3). The corresponding "anti" isomers (**4a**,**b**) were also prepared (see next section for a brief description of their synthesis) but did not offer any advantages over the "syn" ACCA derivatives and required two additional synthetic steps for their preparation (data not shown). The above results obtained for **3a** prompted us to carry out a more extensive structure−activity study centered around the cyclopropane residue that eventually culminated in the identification of more potent inhibitors of the NS3 protease.

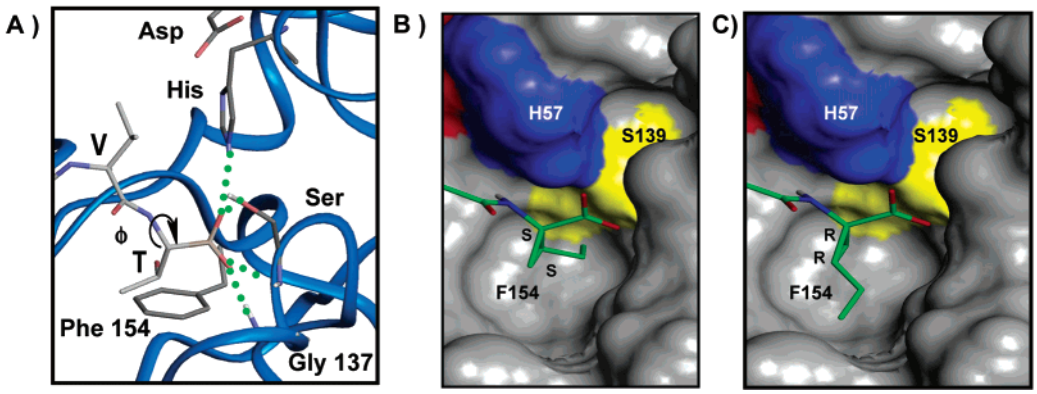
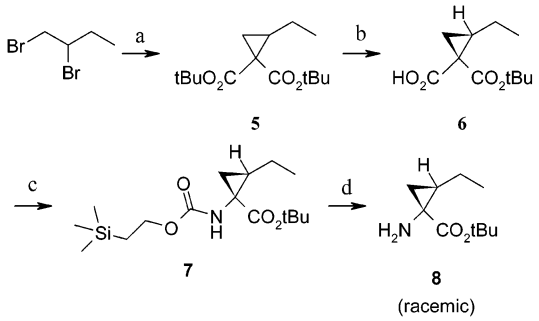


Figure 4. (A) Ribbon representation of the active site of the crystal structure of the engineered full-length NS3 protease−helicase protein (PDB entry 1CU1).[17] This structure provides an atomic view of the binding of the *C*-terminal carboxylic acid moiety of the helicase domain to the active site of the protease domain. (B) Binding model of the inhibitor P1 (1*S*)-amino-(2*S*)-ethylcyclopropanecarboxylic acid derivative (**3b**) to the active site of the NS3 protease domain. (C) Binding model of the inhibitor P1 (1*R*)-amino-(2*R*)-ethylcyclopropanecarboxylic acid derivative (**3a**) to the active site of the NS3 protease domain. In this model, the NS3 protein is represented as a Conolly surface, while the inhibitor P1 residue is represented in sticks and is colored by atom type (oxygen is red, nitrogen is blue, carbon is green, and hydrogen is light gray). For clarity, most of the hydrogen atoms were omitted.

Scheme 1[a]



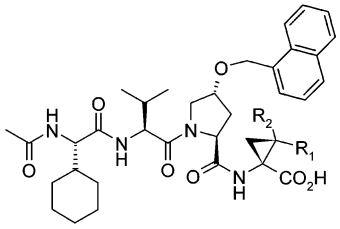
[a] Reagents and conditions: (a) di-*tert*-butyl malonate, 50% aqueous NaOH, $BnEt_3N^+Cl^-$; (b) ^{t}BuOK, H_2O, Et_2O, 0 °C to room temperature; (c) Et_3N, DPPA, benzene; 2-(trimethylsilyl)ethanol, reflux; (d) 1.0 M TBAF, THF, room temperature reflux.

Chemistry. (i) Synthesis of ACCA Derivatives. The ethyl-substituted cyclopropane derivatives presented above were prepared according to Scheme 1. This approach is not limited to the formation of the ethyl-substituted three-membered ring and is actually quite general for the preparation of several other derivatives (see Table 1), simply by changing the structure of the bis-electrophile. As reported,[18] cyclic sulfate derivatives have been found to be a useful alternative to the dibromide in this cyclization reaction (see Experimental Section).

Thus, the acid ester **6** was obtained from di-*tert*-butyl malonate after dialkylation[19] followed by a selective saponification reaction.[20] The racemic "syn" aminoester **8** was prepared via a Curtius rearrangement followed by a fluoride-induced deprotection performed on the intermediate carbamate **7**.[21] In the case of the "anti" isomer, the intermediate acid **6** was first converted to the corresponding allyl ester and then treated with anhydrous HCl in order to deprotect the *tert*-butyl ester. The revealed carboxylic acid was finally transformed into an amine functionality using the sequence of reactions exposed above for compound **7**.

Conversely, simple 2,2-dialkylated derivatives could be generated using the procedure of Verhé.[22] This alternative methodology allowed us to synthesize the 2,2-diethyl ACCA derivative **20** (see entry 3 in Table 2).

Table 2. Further Optimization of ACCA Derivatives



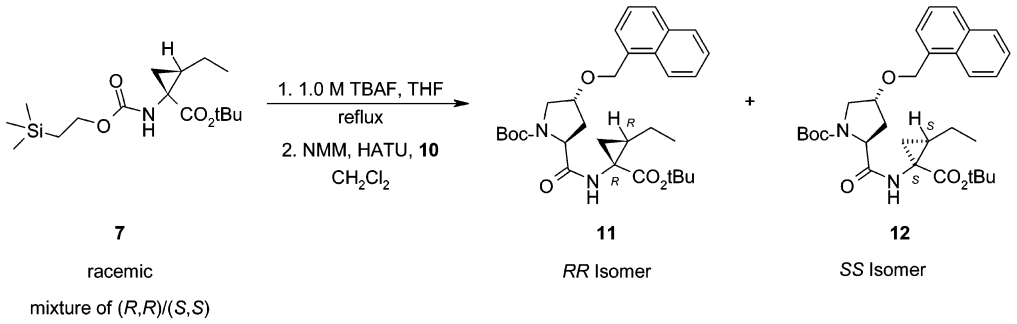
entry	compd	R_1	R_2	IC_{50} (μm)
1	**1**	H	H	14
2	**14**	Et	H	4.8
3	**20**[a]	Et	Et	310
4	**21**	vinyl	H	0.63

[a] Only the result for the more potent diastereomer is shown. The relative configuration at C1 and C2 on the cyclopropane ring is tentatively assigned.

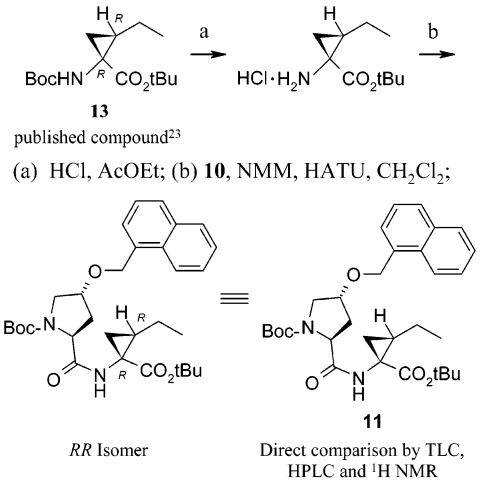
All P1's were incorporated into the tripeptide Ac-Chg-Val-Pro-(4*R*)-(1-naphthylmethoxy)-OH **9** (left-hand side of compound **1**) using standard solution-phase peptide-coupling reaction conditions.

(ii) Absolute Configuration Determination of ACCA Derivatives. The determination of the absolute configuration of the most active ethyl ACCA derivative was done as shown in Schemes 2 and 3. Carbamate **7** (racemic) was deprotected under standard conditions, and the resulting amine was coupled to (4*R*)-1-(*tert*-butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline **10** (homochiral). The mixture of dipeptide diastereomers was purified by flash chromatography to afford a pure sample of both isomers **11** and **12**. Independently, dipeptide **11** was prepared using a homochiral cyclopropane derivative of known absolute configuration[23] (Scheme 3). Thus, compound **13** (homochiral, 1*R*,2*R* isomer) was treated with a solution of hydrogen chloride in ethyl acetate in order to selectively remove the acid-sensitive carbamate in the presence of the *tert*-butyl ester group.[24] The resulting hydrochloride salt was coupled to **10**. HPLC, TLC, and ^{1}H NMR comparison of this authentic sample with the dipeptide **11** allowed us

Scheme 2



Scheme 3



to establish that the absolute configuration of the most active P1 fragment is (1*R*,2*R*) (Figure 3, structure **3a**).

Structure−Activity Study on ACCA Derivatives. As mentioned previously, the encouraging biological results obtained with an inhibitor having fragment **3a** in the P1 position prompted us to expand further on this observation. We were particularly interested by substituent effects on the cyclopropane ring. Thus, various ACCA derivatives were synthesized, and the results obtained are collected in Table 1.

Two different diastereomers (**a** and **b**) were made and tested for each R group. Notice that the relative configuration at C1 and C2 on the cyclopropane ring of these isomers has been proven only for compounds **14a**,**b** (Table 1)**,** as described above. In all the other cases, the configuration is tentatively assigned on the basis of the observation that the fast-eluting material on TLC at the tetrapeptide ester stage will always provide the most potent inhibitor after the ester saponification step. For the ethyl case, this would correspond to the isomer **a** (entry 3). An ethyl group (**14a**) afforded a modest 3-fold improvement of potency over the simple cyclopropane analogue **1**. A small decrease or increase in the length of the R group, from an ethyl to a methyl or a propyl substituent (entries 4 and 5), did not result in an improvement of potency. The effect of branching was subsequently evaluated. When an isopropyl group was introduced, an equally potent compound was produced (entry 6, compound **17a**). The incorporation of a larger substituent such as a phenyl group onto the cyclopropane ring, with two different tethers incorporated between the rings, led to a decrease in potency (entries 7 and 8). From the above observations, a two-carbon atom chain appears to be slightly preferred for activity (entry 3, R = Et). At this point, a more focused SAR study aiming at the optimization of the best R group identified above was performed.

The model presented earlier in Figure 4 (panel C) suggests that introduction of another substituent adjacent to the ethyl group already present on the cyclopropane ring may be tolerated and possibly better fill the S1 pocket. To evaluate this possibility, disubstitution was examined (Table 2). Unfortunately, the introduction of a *gem*-diethyl group led to a less potent inhibitor (entry 3). Another simple, two-carbon atom group was considered: a small vinyl substituent. When this particular group was introduced, we observed an appreciable gain in potency (entry 4). Thus, the formal replacement of one hydrogen atom of the three-membered ring by a small planar group (vinyl) produced an inhibitor with a 22-fold improved inhibitory activity (entries 1 and 4). The difference in potency observed between the ethyl and the vinyl substituents (entries 2 and 4) may be due, at least in part, to a beneficial electronic interaction between the π electrons of the vinyl group and the phenyl group of Phe 154, which is in close proximity (see Figure 4).

Further evaluation of this newly discovered P1 fragment was then carried out by introducing it in three different series of inhibitors that we reported previously[25,26] (see Table 3). These related series differ only by the nature of the substituent located at the 4 position of the P2 proline residue.

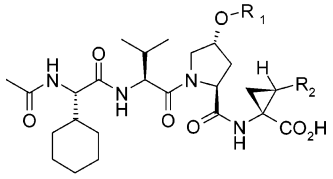
As can be seen from Table 3, the introduction of vinyl-ACCA residue in three different series provides a 29- to 34-fold improvement in potency over the simple cyclopropane derivative.

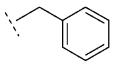
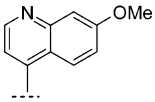
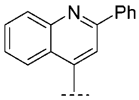
The importance of the vinyl-ACCA[27] residue in these series of inhibitors can be further demonstrated if one compares with the activity found for the corresponding cysteine derivative (which is the amino acid found in the natural substrate of the NS3 protease) and the two other residues that were the starting points for this study (Table 4). IC_{50} results indicate that not only is the vinyl-ACCA derivative (entry 4) more potent than the corresponding norvaline and ACCA residues (entries 2 and 3, respectively), but it is also around 14 times more active than cysteine itself (entry 1)! Recent direct comparison with the P1 cysteine mimetic developed by the IRBM/Merck group (difluoro-Abu[16]) also demonstrated the superiority of the vinyl-ACCA residue.[28]

Conclusion

The approach of combining a cyclopropane ring and the side chain of norvaline to produce novel P1 residues

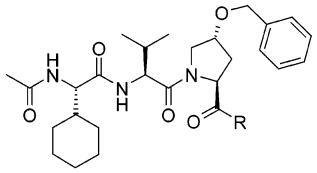
Table 3. Effects of the Introduction of Vinyl-ACCA in Two Other Inhibitor Series

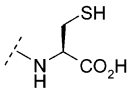
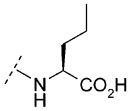
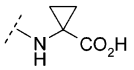
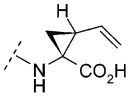


Entry	Compd	R_1	R_2	IC_{50} (μM)	
1	**22**		H	330	**34X**
2	**23**[a]		Vinyl	9.7	
3	**24**		H	0.85	**29X**
4	**25**		Vinyl	0.029	
5	**26**		H	0.55	**29X**
6	**27**		Vinyl	0.019	

[a] Prepared by sequential couplings using racemic vinyl-ACCA and diastereomer separation at the dipeptide stage by flash chromotography.

Table 4. Vinyl-ACCA in Comparison with Various P1 Residues



Entry	Compd	R	IC_{50} (μM)
1	**28**		**140**
2	**29**		**530**
3	**22**		**330**
4	**23**		**9.7**

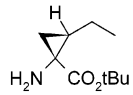
led, after optimization, to the discovery of a vinyl-ACCA, a derivative with improved potency compared to the starting point (between 22- and 34-fold). Its beneficial effect was demonstrated in four different series and allowed for the identification of a nanomolar tetrapeptide inhibitor (compound **27**, 19 nM). The vinyl-ACCA is the first reported carboxylic acid containing a P1 residue that produced inhibitors that are significantly more active than inhibitors containing a cysteine at the same position.

Experimental Section

General Methods. ^{1}H NMR spectra were obtained on a Bruker AMX400 spectrometer, and the chemical shifts are given in ppm, referenced to the internal deuterated solvent. Reagents and solvents, including anhydrous THF and CH_2Cl_2, were purchased from Aldrich Chemical Co. or VWR Scientific of Canada. The final compounds were purified by preparative C18 reversed-phase HPLC on a Whatman Partisil 10 ODS-3 column (2.2 × 50 cm, λ = 230 nm) using a linear gradient from 5% aqueous CH_3CN (containing 0.06% TFA) to 100% CH_3CN. HPLC homogeneities were determined under reversed-phase conditions using a C18 Vydac column (0.46 × 12.5 cm, 5 μM), a 35 min linear gradient at 1.5 mL/min flow from 5% CH_3CN-H_2O) (0.06% TFA) to 100% CH_3CN (0.06% TFA), and UV detection at 230 nm. Capillary electrophoresis was performed on a P/ACE MDQ capillary electrophoresis system with a PDA (purchased from Beckman Coulter, Fullerton, CA) and a fused-silica capillary, 50 cm length to detector, 60 cm total length, 50 μm internal diameter (purchased from Polymicro Technologies, Phoenix, AZ). The elution was monitored at 220 nm using a photodiode array detector and a data acquisition rate of 8 Hz. The capillary temperature and the sample temperature were 20 and 10 °C, respectively. The separation voltage was set at 30 kV. The separation electrolyte consisted of 50 mM Borax (pH 9.4). Mass spectral data were obtained in FAB mode on an MF 50 TATC instrument operating at 6 kV and 1 mA using thioglycerol or nitrobenzyl alcohol (NBA) as a matrix support. High-resolution mass spectrometry (HRMS) was preformed on a Micromass AutoSpecTOF apparatus using metastable atom bombardment ionization.

Flash chromatography was performed on Merck silica gel 60 (0.040−0.063 mm). Analytical thin-layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates.

Chemistry. Synthesis of *tert*-Butyl (1*R*,2*R*)/(1*S*,2*S*)-1-Amino-2-ethylcyclopropanecarboxylate (8).



(racemic)

(a) Preparation of Di-*tert*-butyl 2-Ethylcyclopropane-1,1-dicarboxylate (5). To a suspension of benzyltriethylammonium chloride (21.0 g, 92.2 mmol) in a 50% aqueous NaOH solution (92.4 g in 185 mL of H_2O) were successively added di-*tert*-butyl malonate (20.0 g, 92.5 mmol) and 1,2-dibromobutane (30.0 g, 138.9 mmol). The reaction mixture was vigorously stirred overnight at room temperature, and a mixture of ice and water was then added. The crude product was extracted with CH_2Cl_2 (3×) and sequentially washed with water (3×) and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was flash chromatographed (7 cm, 2−4% Et_2O in hexane) to afford the desired cyclopropane derivative **5** (19.1 g, 70.7 mmol, 76% yield): ^{1}H NMR ($CDCl_3$) δ 1.78−1.70 (m, 1H), 1.47 (s, 9H), 1.46 (s, 9H), 1.44−1.39 (m, 1H), 1.26−1.16 (m, 3H), 1.02 (t, 3H, J = 7.6 Hz); MS 293 (M + Na).

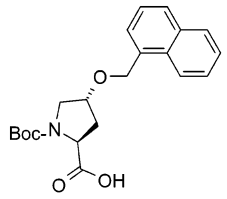
(b) Preparation of (1*S*,2*R*)/(1*R*,2*S*)-1-(*tert*-Butoxycarbonyl)-2-ethylcyclopropanecarboxylic Acid (6). To a suspension of potassium *tert*-butoxide (6.71 g, 59.8 mmol, 4.4 equiv) in dry ether (100 mL) at 0 °C was added H_2O (270 μL, 15.0 mmol, 1.1 equiv). After 5 min, diester **5** (3.68 g, 13.6 mmol) in ether (10 mL) was added to the suspension. The reaction mixture was stirred overnight at room temperature and then poured in a mixture of ice and water and washed with ether (3×). The aqueous layer was acidified with a 10% aqueous citric acid solution at 0 °C and extracted with AcOEt (3×). The combined organic layer was successively washed with water (2×) and brine. After the usual treatment (Na_2SO_4, filtration, and concentration), the desired acid **6** was isolated as a pale yellow oil (1.86 g, 8.68 mmol, 64% yield): ^{1}H NMR ($CDCl_3$) δ 2.09−2.01 (m, 1H), 1.98 (dd, J = 3.8, 9.2 Hz, 1H), 1.81−1.70 (m, 1H), 1.66 (dd, J = 3.0, 8.2 Hz, 1H), 1.63−1.56 (m, 1H), 1.51 (s, 9H), 1.0 (t, J = 7.3 Hz, 3H).

**(c) Preparation of *tert*-Butyl (1*R*,2*R*)/(1*S*,2*S*)-2-Ethyl-1-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)cyclo-

propanecarboxylate (7). To the acid **6** (2.02 g, 9.41 mmol) in dry benzene (32 mL) were successively added Et_3N (1.5 mL, 10.8 mmol, 1.14 equiv) and DPPA (2.2 mL, 10.2 mmol, 1.08 equiv). The reaction mixture was refluxed for 3.5 h, and then 2-(trimethylsilyl)ethanol (2.7 mL, 18.8 mmol, 2.0 equiv) was added. The reflux was maintained overnight, and then the reaction mixture was diluted with Et_2O and successively washed with a 10% aqueous citric acid solution, water, saturated aqueous $NaHCO_3$, water (2×), and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was purified by flash chromatography (5 cm, 10% AcOEt−hexane) to afford the desired carbamate **7** (2.6 g, 7.88 mmol, 84% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.1 (bs, 1H), 4.18−4.13 (m, 2H), 1.68−1.38 (m, 4H), 1.45 (s, 9H), 1.24−1.18 (m, 1H), 1.00−0.96 (m, 5H), 0.03 (s, 9H); MS (FAB) 330 (MH^+).

(d) Preparation of *tert*-Butyl (1*R*,2*R*)-1-Amino-2-ethylcyclopropanecarboxylate (8). To carbamate **7** (258 mg, 0.783 mmol) was added a 1.0 M TBAF solution in THF (940 μL, 0.94 mmol, 1.2 equiv). After 4.5 h, an additional amount of 1.0 M TBAF was added (626 μL, 0.63 mmol, 0.8 equiv). The reaction mixture was stirred overnight at room temperature, refluxed for 30 min, and then diluted with AcOEt. The solution was successively washed with water (2×) and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the desired amine **8** was isolated (84 mg, 0.453 mmol, 58% yield) as a pale yellow liquid: ^{1}H NMR ($CDCl_3$) δ 1.96 (bs, 2H), 1.60−1.40 (m, 2H), 1.47 (s, 9H), 1.31−1.20 (m, 1H), 1.14 (dd, J = 4.1, 7.3 Hz, 1H), 1.02 (dd, J = 4.1, 9.2 Hz, 1H), 0.94 (t, J = 7.3 Hz, 3H).

Synthesis of (4*R*)-1-(*tert*-Butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline (10).



Commercially available (4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxy-L-proline (5.0 g, 21.6 mmol) was dissolved in THF (100 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in oil, 1.85 g, 45.4 mmol) was added portionwise over 10 min, and the suspension was stirred at room temperature for 1 h. 1-(Bromomethyl)naphthalene[29] (8.0 g, 36.2 mmol) was then added, and the mixture was heated at reflux for 18 h. The mixture was poured into water (300 mL) and washed with hexane. The aqueous layer was acidified with 10% aqueous HCl and extracted twice with AcOEt. The organic layers were combined and washed with brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was purified by flash chromatography (49:49:2 hexane−AcOEt−AcOH) to give the desired compound **10** as a colorless oil (4.51 g, 56% yield): ^{1}H NMR (DMSO-d_6) (indicating the presence of two rotamers) δ 8.07−7.87 (m, 3H), 7.62−7.46 (m, 4H), 5.64−5.53 (m, 2H), 5.06 (bs, 1H), 4.31−4.20 (m, 2H), 3.41−3.31 (m, 1H), 2.16−2.05 (m, 1H), 1.36 and 1.21 (s, 9H).

Preparation of the Tripeptide Acid Fragment Ac-Chg-Val-Pro-(4*R*)-(1-naphthylmethoxy)-OH (9). (a) Preparation of (4*R*)-1-(*tert*-Butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline Allyl Ester. The acid **10** obtained above (4.45 g, 11.98 mmol) was dissolved in anhydrous CH_3CN (60 mL). DBU (2.2 mL, 14.4 mmol) and allyl bromide (1.1 mL, 13.2 mmol) were added successively, and the reaction mixture was stirred for 24 h at room temperature. The mixture was concentrated, and the resulting oil was diluted with AcOEt and water and successively washed with water (2×) and brine. The AcOEt layer was dried ($MgSO_4$), filtered, and evaporated to dryness. The yellow oil was purified by flash chromatography (eluent, hexane−AcOEt, 90:10 to 85:15) to provide (4*R*)-1-(*tert*-butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline allyl ester as a yellow oil (4.17 g, 85% yield): ^{1}H NMR ($CDCl_3$) (mixture of rotamers ca. 1:2) δ 8.04 (d, J = 8 Hz, 1H), 7.87 (d, J = 8 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.55−7.41 (m, 4H), 5.95−5.85 (m, 1H), 5.34−5.21 (m, 2H), 5.03−4.88 (m, 2H), 4.70−4.56 (m, 2H), 4.48 (dd, J = 7.3, 7.3 Hz) and 4.39 (dd, J = 7.6, 7.6 Hz) (1H total), 4.28−4.23 (m, 1H), 3.81−3.55 (m, 2H), 2.46−2.36 (m, 1H), 2.13−2.05 (m, 1H), 1.44 and 1.41 (s, 9H); MS (FAB) 412 MH^+.

(b) Preparation of Boc-Val-Pro-(4*R*)-(1-Naphthylmethoxy) Allyl Ester. (4*R*)-1-(*tert*-Butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline allyl ester (2.89 g, 7.02 mmol) was treated with 4 N HCl/dioxane (30 mL). After 30 min at room temperature, the mixture was concentrated, and the crude hydrochloride salt was coupled to Boc-Val-OH (1.53 g, 7.73 mmol) with NMM (3.1 mL, 28.1 mmol) and TBTU (2.71 g, 8.43 mmol) in DCM (35 mL) for 3.5 h. The reaction mixture was diluted with EtOAc and successively washed with 10% aqueous citric acid (2×), saturated aqueous $NaHCO_3$ (2×), water (2×), and brine (1×). The AcOEt layer was dried ($MgSO_4$), filtered, and evaporated to dryness to provide the crude desired dipeptide as an ivory oil-foam (ca. 3.60 g, 100% yield): ^{1}H NMR ($CDCl_3$) δ 8.04 (bd, J = 8 Hz, 1H), 7.87 (bd, J = 7 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.56−7.40 (m, 4H), 5.93−5.85 (m, 1H), 5.34−5.28 (m, 1H), 5.24−5.19 (m, 2H), 5.04 (d, J = 12 Hz, 1H), 4.92 (d, J = 12 Hz, 1H), 4.67−4.60 (m, 3H), 4.31−4.26 (m, 2H), 4.11−4.09 (m, 1H), 3.72 (dd, J = 4, 11 Hz, 1H), 2.48−2.41 (m, 1H), 2.07−1.99 (m, 1H), 1.44−1.36 (m, 1H), 1.37 (s, 9H), 1.01 (d, J = 7 Hz, 3H), 0.93 (d, J = 7 Hz, 3H); MS (FAB) 509.3 (MH^-), 511.3 (MH^+), and 533.2 (M + Na).

(c) Preparation of Boc-Chg-Val-Pro-(4*R*)-(1-naphthylmethoxy) Allyl Ester. The crude dipeptide obtained above (ca. 7.02 mmol) was treated with 4 N HCl/dioxane (30 mL) as described above. The crude hydrochloride salt was coupled to Boc-Chg-OH·H_2O (2.13 g, 7.73 mmol) with NMM (3.1 mL, 28.09 mmol) and TBTU (2.71 g, 8.43 mmol) in CH_2Cl_2 (35 mL) as described for the synthesis of the above dipeptide fragment to provide the crude desired tripeptide as an ivory foam (ca. 4.6 g, 100% yield): ^{1}H NMR ($CDCl_3$) δ 8.06 (bd, J = 8 Hz, 1H), 7.87 (bd, J = 7.5 Hz, 1H), 7.82 (bd, J = 8 Hz, 1H), 7.57−7.40 (m, 4H), 6.46 (bd, J = 8.5 Hz, 1H), 5.94−5.84 (m, 1H), 5.31 (dd, J = 1, 17 Hz, 1H), 5.23 (dd, J = 1, 10.5 Hz, 1H), 5.03 (d, J = 12 Hz, 1H), 5.00−4.97 (m, 1H), 4.93 (d, J = 12 Hz, 1H), 4.63−4.59 (m, 4H), 4.29−4.27 (m, 1H), 4.10−4.07 (m, 1H), 3.92−3.86 (m, 1H), 3.72 (dd, J = 5, 11 Hz, 1H), 2.48−2.41 (m, 1H), 2.10−1.99 (m, 1H), 1.76−1.57 (m, 6H), 1.43 (s, 9H), 1.20−0.92 (m, 6H), 1.00 (d, J = 7 Hz, 3H), 0.93 (d, J = 7 Hz, 3H); MS (FAB) 648.5 (MH^-) and 672.4 (M + Na).

(d) Preparation of Ac-Chg-Val-Pro-(4*R*)-(1-naphthylmethoxy) Allyl Ester. The crude tripeptide obtained above (ca. 7.02 mmol) was treated with 4 N HCl/dioxane (30 mL) under standard conditions. The crude hydrochloride salt was further treated with acetic anhydride (1.33 mL, 14.1 mmol) and NMM (3.1 mL, 28.1 mmol) in CH_2Cl_2 (35 mL) as described above for the dipeptide fragment. The crude product was flash purified (eluent, hexane−AcOEt, 30:70) to provide the acetylated protected tripeptide as a white foam (3.39 g, 81% yield over three steps): ^{1}H NMR ($CDCl_3$) (mainly one rotamer) δ 8.06 (d, J = 8 Hz, 1H), 7.88 (bd, J = 8 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.58−7.41 (m, 4H), 6.37 (d, J = 9 Hz, 1H), 5.97 (d, J = 8.5 Hz, 1H), 5.94−5.84 (m, 1H), 5.31 (dd, J = 1, 17 Hz, 1H), 5.24 (dd, J = 1, 10.5 Hz, 1H), 5.05 (d, J = 12 Hz, 1H), 4.94 (d, J = 12 Hz, 1H), 4.66−4.57 (m, 4H), 4.31−4.22 (m, 2H), 4.11−4.05 (m, 1H), 3.73 (dd, J = 4.5, 11 Hz, 1H), 2.50−2.43 (m, 1H), 2.09−2.01 (m, 2H), 2.00 (s, 3H), 1.68−1.55 (m, 5H), 1.15−0.89 (m, 6H), 0.99 (d, J = 7 Hz, 3H), 0.91 (d, J = 7 Hz, 3H); MS (FAB) 590.3 (MH^-), 592.4 (MH^+), and 614.4 $(M + Na)^+$.

(e) Preparation of Ac-Chg-Val-Pro-(4*R*)-(1-naphthylmethoxy)-OH (9). To the acetylated tripeptide (3.39 g, 5.73 mmol) in a 1:1 mixture of anhydrous CH_3CN−DCM (30 mL) were successively added tetrakis(triphenylphosphine)palladium-(0) catalyst (172 mg, 0.15 mmol), triphenylphosphine (78 mg, 0.30 mmol), and pyrrolidine (516 μL, 6.19 mmol). The reaction mixture was stirred overnight at room temperature and then concentrated in vacuo. The residue was dissolved in a mixture of AcOEt and a 10% aqueous citric acid solution. The organic

layer was successively washed with 10% aqueous citric acid (2×), water (2×), and brine (2×). After the usual treatment ($MgSO_4$, filtration, and concentration), a crude light yellow foam product was obtained. This material was triturated in Et_2O−DCM (85:15) to provide, after filtration, the title tripeptide as an off-white solid (3.0 g, 95% yield): ^{1}H NMR ($CDCl_3$) δ 8.08 (d, J = 8 Hz, 1H), 8.04 (bd, J = 9 Hz, 1H), 7.88 (bd, J = 7.5 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.58−7.37 (m, 5H), 5.05 (d, J = 12 Hz, 1H), 4.94 (d, J = 12 Hz, 1H), 4.61 (t, J = 9.5, 19.5 Hz, 1H), 4.46−4.37 (m, 2H), 4.27 (bs, 1H), 4.17 (d, J = 11 Hz, 1H), 3.74 (dd, J = 4, 11 Hz, 1H), 2.49 (bdd, J = 7.5, 13 Hz, 1H), 2.17−2.09 (m, 1H), 2.04 (s, 3H), 2.03−1.94 (m, 1H), 1.79 (bd, J = 12.5 Hz, 1H), 1.62−1.43 (m, 5H), 1.08−0.85 (m, 5H), 1.00 (d, J = 7 Hz, 3H), 0.90 (d, J = 7 Hz, 3H); MS (FAB) 550.3 (MH^-).

Preparation of Compounds 14a and 14b. A partially resolved[27] mixture of (1*R*,2*R*) and (1*S*,2*S*) (2.9:1 ratio, respectively) forms of Boc-protected methyl 1-amino-2-ethylcyclopropanecarboxylate (44 mg, 0.18 mmol) was treated with a 4 N HCl/dioxane solution (2 mL). After 30 min at room temperature, the reaction mixture was concentrated. The residue was dissolved in CH_2Cl_2 (2 mL), and then NMM (80 μL, 0.73 mmol), the acid tripeptide component **9** (100 mg, 0.18 mmol), and HATU (83 mg, 0.22 mmol) were added. The reaction mixture was stirred overnight at room temperature, diluted with AcOEt, and successively washed with 10% aqueous citric acid (2×), saturated aqueous $NaHCO_3$ (2×), H_2O (2×), and brine. After the usual treatment, the residue was flash chromatographed (1 cm, 90% AcOEt−hexane) to afford a mixture of the two desired diastereomers (corresponding methyl ester of **14a** and **14b**) (5:1 ratio as estimated by HPLC at 220 nM) (58 mg, 0.086 mmol, 47% yield): ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.56−7.40 (m, 5H), 6.56 (bs, 1H), 5.97 (d, J = 8.6 Hz, 1H), 5.01 (d, J = 12.1 Hz, 1H), 4.96 (d, J = 11.8 Hz, 1H), 4.68−4.61 (m, 2H), 4.42 (dd, J = 4.1, 8.3 Hz, 1H), 4.28 (dd, J = 8.3, 15.3 Hz, 1H), 3.94 (dd, J = 3.2, 10.8 Hz, 1H), 3.67 (dd, J = 4.8, 10.5 Hz, 1H), 3.64 (s, 3H), 2.63−2.55 (m, 1H), 2.15−2.05 (m, 1H), 2.01 (s, 3H), 1.71−1.47 (m, 13H), 1.17−1.02 (m, 3H), 0.97−0.87 (m, 10H); MS 699 (M + Na).

The methyl esters (56 mg, 0.083 mmol) obtained above were dissolved in a mixture of absolute MeOH (1 mL) and THF (2 mL). The solution was cooled at 0 °C for the addition of hydrated lithium hydroxide (10 mg, 0.24 mmol) in water (1 mL). The reaction mixture was stirred for 30 min at 0 °C and then overnight at room temperature. Another quantity of hydrated lithium hydroxide (11 mg, 0.26 mmol) dissolved in water (0.5 mL) was added. An HPLC analysis of the mixture done 5 h later indicated that around 20% of starting ester was still present in the reaction mixture. To force the reaction to go to completion, a further 2.0 equiv of lithium hydroxide (7 mg, 0.17 mmol) in water (0.25 mL) was added. After 48 h at room temperature, the reaction mixture was diluted with water (5 mL) and extracted with ether (2×). The aqueous layer was acidified with citric acid and extracted with AcOEt (3×). The combined organic layer was successively washed with water (2×) and brine (1×). After the usual treatment, the crude mixture was purified by HPLC (Whatman10-ods-3, 2.2 × 50 cm, λ = 230 nm, linear gradient at 15 mL/min, 5% CH_3CN−H_2O for 10 min and then 5−58% CH_3CN−H_2O for 65 min and, finally, 58% CH_3CN−H_2O for 15 min) to afford compounds **14a** (29 mg, 0.044 mmol, 53% yield) and **14b** (3.5 mg, 0.0053 mmol, 6.4% yield). Homogeneity for **14a**: 93% by HPLC (contains 6% of **14b**) and 99.2% as evaluated by CE. Homogeneity for **14b**: 95% by HPLC (contains 1.6% of **14a**) and 95.6% by CE.

Compound **14a**: ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H), 8.04 (d, J = 7.3 Hz, 1H), 7.94 (dd, J = 2.9, 7.3 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.84−7.80 (m, 2H), 7.57−7.51 (m, 3H), 7.47 (d, J = 8.0 Hz, 1H), 4.99 (d, J = 11.8 Hz, 1H), 4.90 (d, J = 11.8 Hz, 1H), 4.36 (dd, J = 8.3, 16.8 Hz, 1H), 4.34−4.31 (m, 1H), 4.20 (dd, J = 8.0, 16.2 Hz, 1H), 4.10 (d, J = 11.1 Hz, 1H), 3.75 (dd, J = 4.5, 11.4 Hz, 1H), 2.22−2.13 (m, 1H), 2.03−1.93 (m, 2H), 1.84 (s, 3H), 1.63−1.42 (m, 8H), 1.35−1.22 (m, 2H), 1.20 (dd, J = 4.5, 7.6 Hz, 1H), 1.03−0.88 (m, 11H), 0.85 (d, J = 6.7 Hz, 3H), 0.75 (dd, J = 6.7, 14.0 Hz, 1H); MS 685 (M + Na); HRMS calcd for $C_{37}H_{51}N_4O_7$ 663.375776, found 663.375321.

Compound **14b**: ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.83−7.80 (m, 2H), 7.58−7.44 (m, 4H), 4.99 (d, J = 12.1 Hz, 1H), 4.90 (d, J = 11.8 Hz, 1H), 4.37−4.32 (m, 2H), 4.26 (dd, J = 8.0, 15.9 Hz, 1H), 4.19 (dd, J = 7.3, 16.5 Hz, 1H), 4.11 (d, J = 10.5 Hz, 1H), 3.70 (dd, J = 4.4, 11.1 Hz, 1H), 2.25−2.17 (m, 1H), 2.04−1.90 (m, 2H), 1.84 (s, 3H), 1.62−1.32 (m, 8H), 1.29−1.22 (m, 4H), 1.03−0.88 (m, 9H), 0.85 (d, J = 6.7 Hz, 3H), 0.75 (dd, J = 6.4, 16.5 Hz, 1H); MS 685 (M + Na); HRMS calcd for $C_{37}H_{51}N_4O_7$ 663.375776, found 663.375977.

Chemical Resolution of the Dipeptides 11 and 12. The amine **8** was dissolved in anhydrous CH_2Cl_2 (120 mL). NMM (8.5 mL, 77.6 mmol), (4*R*)-1-(*tert*-butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline **10** (10.1 g, 27.2 mmol), and HATU (11.79 g, 31.03 mmol) were added successively. The reaction mixture was stirred at room temperature overnight and then worked up as described previously. The crude diastereomeric mixture was separated by flash chromatography (eluent, hexane−Et_2O, 25:75) to provide the dipeptide **11** (the less polar eluting spot) as a white foam (4.42 g, 64% of the theoretical yield) and **12** (the more polar eluting spot) as an ivory foam (4.0 g, 57% of theoretical yield). At this time, both isomers were separated but the absolute configuration was still not known.

Compound **11**: ^{1}H NMR ($CDCl_3$) (ca. 1:1 mixture of rotamers) δ 8.05 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.55−7.40 (m, 4 H), 7.37 and 6.45 (bs, 1H), 5.20−4.91 (m, 2H), 4.41−3.44 (m, 4H), 2.70−2.02 (m, 2H), 1.61−1.25 (m, 5H), 1.44 (s, 9H), 1.41 (s, 9H), 0.96 (t, J = 7.5 Hz, 3H); MS (FAB) 539 (MH^+).

Compound **12**: ^{1}H NMR ($CDCl_3$) (ca. 1:1 mixture of rotamers) δ 8.04 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 7.9 Hz, 1H), 7.54−7.32 (m, 4H), 7.32 and 6.37 (bs, 1H), 5.03−4.87 (m, 2H), 4.42−3.42 (m, 4H), 2.71−2.02 (m, 2H), 1.64−1.10 (m, 5H), 1.44 (s, 9H), 1.41 (s, 9H), 1.00 (t, J = 7.3 Hz, 3H); MS (FAB) 539 (MH^+).

Determination of the Absolute Configuration of Compounds 11 and 12 by Correlation with Known *tert*-Butyl (1*R*-Amino-2*R*)-ethylcyclopropanecarboxylate. *tert*-Butyl (1*R*,2*R*)-1-[(*tert*-butoxycarbonyl)amino]-2-ethylcyclopropanecarboxylate **13**[23] (13.2 mg, 0.046 mmol) was dissolved in 1 M HCl/EtOAc (240 μL) and stirred for approximately 48 h. The mixture was evaporated to dryness to provide its corresponding hydrochloride salt as a light yellow paste. This material was coupled to (4*R*)-1-(*tert*-butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline **10** (18 mg, 0.049 mmol) as described above, using NMM (20.3 μL, 0.185 mmol) and HATU (21.1 mg, 0.056 mmol) in CH_2Cl_2. The crude material was purified by flash chromatography (eluent, hexane−Et_2O, 50:50) to provide a pure dipeptide as an oil (7.7 mg, 31% yield). By TLC, HPLC, and ^{1}H NMR comparison, this dipeptide was found to be identical to the less polar compound **11** obtained above, thus identifying the absolute configuration of **11** as (1*R*,2*R*).

Preparation of Compounds 20a and 20b. (a) Preparation of 2,2-Diethyl-1-(methoxycarbonyl)cyclopropanecarboxylic Acid. To dimethyl 2,2-diethylcyclopropane-1,1-dicarboxylate[22] (1.24 g, 5.8 mmol) in methanol (6 mL) was added a 1 N aqueous NaOH solution (5.79 mL, 5.79 mmol, 0.97 equiv). The reaction mixture was stirred for 5 days at room temperature, and then the methanol was removed in vacuo. The aqueous layer was washed with ether (2×), acidified with a 10% aqueous citric acid solution, and extracted with ethyl acetate. After the usual treatment ($MgSO_4$, filtration, and concentration), the desired acid, 2,2-diethyl-1-(methoxycarbonyl)cyclopropanecarboxylic acid, was isolated as a colorless oil (1.08 g, 5.4 mmol, 93% yield): ^{1}H NMR ($CDCl_3$) δ 1.79 (d, J = 5.1 Hz, 1H), 1.69 (d, J = 5.1 Hz, 1H), 1.65−1.49 (m, 3H), 1.47−1.39 (m, 1H), 0.90−0.84 (m, 6H).

(b) Preparation of Methyl 1-Amino-2,2-diethylcyclopropanecarboxylate. To the acid obtained above (300 mg, 1.50 mmol) in dry THF (10 mL) were successively added triethylamine (229 μL, 1.65 mmol, 1.1 equiv) and DPPA (348 μL, 1.62 mmol, 1.08 equiv). The reaction mixture was refluxed

for 2 h, and then 2-(trimethylsilyl)ethanol (430 μL, 3.00 mmol, 2.0 equiv) was added. After 16 h under reflux, the reaction mixture was concentrated and then diluted with ethyl acetate. The organic layer was successively washed with 10% aqueous citric acid, (2×), saturated aqueous $NaHCO_3$ (2×), and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was flash chromatographed (3 cm, 10% AcOEt−hexane) to afford the desired carbamate (300 mg, 1.04 mmol, 70% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.1 (bs, 1H), 4.18−4.11 (m, 2H), 3.71 (s, 3H), 1.68−1.59 (m, 3H), 1.53−1.40 (m, 2H), 1.00−0.95 (m, 6H), 0.82 (t, J = 7.3 Hz, 3H), 0.03 (s, 9H).

To the carbamate obtained above (300 mg, 1.04 mmol) in dry THF (4 mL) was added a 1.0 M TBAF solution in THF (2.1 mL, 2.1 mmol, 2.0 equiv). The reaction mixture was refluxed for 30 min, and then AcOEt was added and the mixture was successively washed with sodium bicarbonate (2×) and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), methyl 1-amino-2,2-diethylcyclopropanecarboxylate was isolated (135 mg, 0.788 mmol, 75% yield) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.65 (s, 3H), 1.74−1.62 (m, 1H), 1.51−1.39 (m, 2H), 1.32 (d, J = 4.8 Hz, 1H), 0.94 (t, J = 7.3 Hz, 3H), 0.93−0.89 (m, 1H), 0.81 (t, J = 7.6 Hz, 3H), 0.67 (d, J = 4.4 Hz, 1H).

(c) Preparation of the Corresponding Methyl Ester of Compounds 20a and 20b. Coupling of the methyl 1-amino-2,2-diethylcyclopropanecarboxylate obtained above (45 mg, 0.26 mmol) to the tripeptide fragment **9** (145 mg, 0.26 mmol) was done as described for the preparation of compounds **14a** and **14b**. The crude material was flash chromatographed (3 cm, 100% AcOEt−hexane) to afford a mixture of the desired tetrapeptides (164 mg, 88% yield): ^{1}H NMR ($CDCl_3$) (mixture of two diastereomers) δ 8.05 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.70−7.64 (m, 1H), 7.57−7.40 (m, 4H), 6.51−6.43 (m, 1H), 5.95 (d, J = 8.6 Hz, 1H), 5.03−4.94 (m, 2H), 4.72−4.55 (m, 2H), 4.45−4.38 (m, 1H), 4.26 (dd, J = 7.0, 7.0 Hz, 1H), 3.96−3.90 (m, 1H), 3.69−3.58 (m, 1H), 3.65 and 3.61 (s, 3H), 2.62−2.47 (m, 1H), 2.07−1.96 (m, 2H), 2.04 and 2.01 (s, 3H), 1.71−1.57 (m, 10H), 1.50−1.30 (m, 1H), 1.19−1.14 (m, 2H), 1.01−0.79 (m, 16 H).

(d) Preparation of Compounds 20a and 20b. The mixture of esters (101 mg, 0.14 mmol) was saponified using the conditions described for the preparation of compounds **14a** and **14b**. The crude mixture was dissolved in acetic acid and purified by HPLC to afford the less polar isomer **20a** (96% homogeneous by HPLC and 99.2% by CE) and the more polar isomer **20b** (99% homogeneous by HPLC and 99.9% by CE). Note that the compound represented as **20** in Table 2, entry 2, corresponds to **20a** (**20b** was omitted for clarity).

Compound **20a**: ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 9.5 Hz, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.58−7.44 (m, 4H), 4.99 (d, J = 11.8 Hz, 1H), 4.89 (d, J = 11.8 Hz, 1H), 4.43 (dd, J = 8.0, 8.0 Hz, 1H), 4.37 (dd, J = 8.0, 8.0 Hz, 1H), 4.34−4.29 (m, 1H), 4.20 (dd, J = 7.6, 7.6 Hz, 1H), 4.11 (d, J = 11.4 Hz, 1H), 3.71 (dd, J = 4.5, 10.8 Hz, 1H), 2.15−2.06 (m, 1H), 2.01−1.92 (m, 2H), 1.84 (s, 3H), 1.68−1.42 (m, 9H), 1.34 (d, J = 5.1 Hz, 1H), 1.23−1.14 (m, 1H), 1.05−0.84 (m, 15H), 0.80 (t, J = 7.3 Hz, 3H), 0.71 (d, J = 5.1 Hz, 1H); MS 713 (M + Na); HRMS calcd for $C_{39}H_{55}N_4O_7$ 691.407076, found 691.406387.

Compound **20b**: ^{1}H NMR (DMSO-d_6) δ 8.33 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.82 (dd, J = 8.3, 16.9 Hz, 1H), 7.58−7.45 (m, 5H), 4.99 (d, J = 12.1 Hz, 1H), 4.91 (d, J = 12.1 Hz, 1H), 4.39 (dd, J = 7.6, 7.6 Hz, 1H), 4.36−4.32 (m, 2H), 4.19 (dd, J = 7.3, 7.3 Hz, 1H), 4.12 (d, J = 11.1 Hz, 1H), 3.71 (dd, J = 4.1, 11.1 Hz, 1H), 2.27−2.19 2.02−1.90 (m, 2H), 1.84 (s, 3H), 1.71 (dd, J = 7.0, 14.0 Hz, 1H), 1.64−1.39 (m, 10 H), 1.30 (dd, J = 7.0, 14 Hz, 1H), 1.04−0.70 (m, 18H); MS 713 (M + Na); HRMS calcd for $C_{39}H_{55}N_4O_7$ 691.407076, found 691.407126.

Preparation of Compounds 15a and 15b. These tetrapeptides were prepared using the procedure already described for the preparation of compounds **14a** and **14b**. 1,2-Dibromopropane was used instead of 1,2-dibromobutane for the cyclopropane ring elaboration. The diastereomers were separated at the ester stage.

Compound **15a** (91% homogeneous by HPLC; contains 5.7% of **15b**, 98.9% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.94 (dd, J = 2.9, 7.3 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.85−7.78 (m, 2H), 7.57−7.45 (m, 4H), 4.98 (d, J = 11.8 Hz, 1H), 4.90 (d, J = 12.1 Hz, 1H), 4.38−3.32 (m, 2H), 4.25 (dd, J = 8.0, 15.9 Hz, 1H), 4.19 (dd, J = 8.0, 16.2 Hz, 1H), 4.08 (d, J = 11.1 Hz, 1H), 3.76 (dd, J = 4.5, 11.1 Hz, 1H), 2.20−2.15 (m, 1H), 2.03−1.97 (m, 2H), 1.84 (s, 3H), 1.60−1.45 (m, 6H), 1.38−1.27 (m, 1H), 1.15 (d, J = 6.4 Hz, 3H), 1.05−0.90 (m, 6H), 0.89 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.76 (dd, J = 6.7, 13.0 Hz, 1H); MS 649 (MH^+), 671 (M + Na); HRMS calcd for $C_{36}H_{49}N_4O_7$ 649.360125, found 649.361314.

Compound **15b** (91% homogeneous by HPLC; contains 8.4% of **15a**, 99.0% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.35 (bs, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.94 (dd, J = 2.5, 7.3 Hz, 1H), 7.95−7.81 (m, 4H), 4.99 (d, J = 12.1 Hz, 1H), 4.89 (d, J = 11.8 Hz, 1H), 4.37−4.09 (m, 5H), 3.70 (dd, J = 4.1, 11.1 Hz, 1H), 2.23−2.15 (m, 1H), 2.05−1.92 (m, 2H), 1.84 (s, 3H), 1.65−1.17 (m, 6H), 1.13 (d, J = 6.0 Hz, 3H), 1.07−0.9 (m, 10H), 0.90 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.75 (dd, J = 6.7, 18.1 Hz, 1H); MS 649 (M + H), 671 (M + Na); HRMS calcd for $C_{36}H_{49}N_4O_7$ 649.360125, found 649.360923.

Preparation of Compounds 16a and 16b. (a) Preparation of 4-Propyl-1,3,2-dioxathiolane 2,2-Dioxide. To 1,2-pentanediol (5.0 g, 48.0 mmol) in carbon tetrachloride (48 mL) was added thionyl chloride (4.2 mL, 57.6 mmol). The reaction mixture was refluxed for 30 min and then cooled to 0 °C for the successive addition of CH_3CN (48 mL), $RuCl_3 \cdot H_2O$ (8 mg, 0.038 mmol), $NaIO_4$ (15.4 g, 72.0 mmol), and H_2O (72 mL). After being stirred at 0 °C for 15 min and for 1 h at room temperature, the reaction mixture was diluted with ether (380 mL) and successively washed with H_2O (20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), and brine (20 mL). The solution was dried over $MgSO_4$ and filtered through a short pad of silica gel. After concentration, a quantitative yield of the desired cyclic sulfate was obtained (8.0 g, 48.0 mmol).

(b) Preparation of Dimethyl 2-Propylcyclopropane-1,1-dicarboxylate. The crude intermediate prepared above (6.29 g, 37.8 mmol) was then added to a suspension of sodium hydride (3.2 g of a 60% dispersion in mineral oil, 80.0 mmol) and dimethyl malonate (5.0 g, 37.8 mmol) in dry THF (190 mL). The reaction mixture was refluxed for 36 h and then concentrated. To the residue was added water and AcOEt. The layers were separated, and the aqueous phase was further extracted with AcOEt (2×). The combined organic layer was successively washed with water (2×) and brine. After the usual treatment, the desired dimethyl 2-propylcyclopropane-1,1-dicarboxylate was isolated (7.61 g of crude material): ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 3.72 (s, 3H), 1.93−1.85 (m, 1H), 1.52−1.35 (m, 4H), 1.31−1.09 (m, 3H), 0.92 (t, J = 7.0 Hz, 3H).

(c) Preparation of (1*R*,2*R*)/(1*S*,2*S*)-1-(Methoxycarbonyl)-2-propylcyclopropanecarboxylic Acid. To the cyclopropane derivative obtained above (5.0 g, 24.97 mmol) in absolute MeOH (11 mL) was added a water solution (27 mL) of sodium carbonate (2.7 g, 25.47 mmol). The reaction mixture was stirred for 3 days at room temperature and then refluxed overnight. After removal of the MeOH in vacuo, the aqueous layer was extracted with ether (3×), acidified at 0 °C with a cold 10% aqueous HCl solution, and extracted with AcOEt (3×). The combined organic layer was washed with brine (2×). After the usual treatment, the desired monoacid was isolated as a yellow oil (3.37 g, 18.08 mmol, 72% yield): ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3H), 2.16−2.07 (m, 1H), 1.98 (dd, J = 4.1, 9.2 Hz, 1H), 1.73 (dd, J = 4.1, 8.6 Hz, 1H), 1.59−1.52 (m, 2H), 1.48−1.32 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H).

(d) Preparation of Compounds 16a and 16b. The above acid was successively treated with DPPA and TBAF and then coupled to the tripeptide fragment **9** as described above in the preparation of compounds **14a** and **14b**.

Compound 16a (95% homogeneous by HPLC; 98.0% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.38 (s, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.94 (s, J = 7.6 Hz, 1H), 7.90−7.79 (m, 3H), 7.59−7.42 (m, 4H), 4.98 (d, J = 12.1 Hz, 1H), 4.89 (d, J = 12.1 Hz, 1H), 4.39−4.15 (m, 4H), 4.10 (d, J = 10.8 Hz, 1H), 3.79−3.71 (m, 1H), 2.21−2.12 (m, 1H), 2.04−1.92 (m, 1H), 1.84 (s, 3H), 1.62−1.41 (m, 7H), 1.39−1.28 (m, 2H), 1.21−1.16 (m, 1H), 1.07−0.81 (m, 15 H); MS 677 (MH^+); HRMS calcd for $C_{38}H_{53}N_4O_7$ 677.391426, found 677.392437.

Compound 16b (87% homogeneous by HPLC; contains 12% of **16a**, 94.1% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.47 (s, 1H), 8.05 (d, J = 7.0 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.91−7.79 (m, 3H), 7.58−7.44 (m, 4H), 4.99 (d, J = 12.1 Hz, 1H), 4.90 (d, J = 12.1 Hz, 1H), 4.37−4.29 (m, 2H), 4.26 (dd, J = 7.6, 7.6 Hz, 1H), 4.19 (dd, J = 7.6, 7.6 Hz, 1H), 4.13 (d, J = 11.5 Hz, 1H), 3.69 (dd, J = 3.8, 10.8 Hz, 1H), 2.25−2.16 (m, 1H), 2.03−1.89 (m, 2H), 1.84 (s, 3H), 1.62−1.22 (m, 12 H), 1.05−0.79 (m, 15 H); MS 677 (MH^+); HRMS calcd for $C_{38}H_{53}N_4O_7$ 677.391426, found 677.392265.

Preparation of Compounds 17a and 17b. (a) Preparation of Boc-2,3-methano-leucine Allyl Ester. To a suspension of Boc-2,3-methano-leucine[18b] (53 mg, 0.22 mmol) in CH_3CN (2.0 mL) were successively added DBU (39 μL, 0.26 mmol) and allyl bromide (75 μL, 0.87 mmol). After 6 h at room temperature, another amount of DBU (10 μL, 0.067 mmol) and allyl bromide (38 μL,0.44 mmol) was added. The reaction mixture was stirred overnight at room temperature and then concentrated. The residue was dissolved in AcOEt and successively washed with 10% aqueous citric acid, 10% aqueous Na_2CO_3, water (2×), and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was flash chromatographed (1 cm, 5% AcOEt−hexane) to afford the desired allyl ester as a colorless oil (50 mg, 0.176 mmol, 81% yield): ^{1}H NMR ($CDCl_3$) δ 5.94−5.85 (m, 1H), 5.34 (dd, J = 1.6, 17.2 Hz, 1H), 5.22 (d, J = 10.5 Hz, 1H), 5.12 (bs, 1H), 4.67−4.56 (m, 2H), 1.64−1.59 (m, 1H), 1.44 (s, 9H), 1.28−1.17 (m, 2H), 1.02 (dd, J = 6.4, 14.0 Hz, 1H), 1.01 (d, J = 6.7 Hz, 3H), 0.91 (d. J = 6.7 Hz, 3H); MS 284 (MH^+), 306 (M + Na).

(b) Preparation of the Corresponding Allyl Ester of Compounds 17a and 17b. The ester obtained above (24.4 mg, 0.086 mmol) was treated with a 4 N HCl/dioxane solution (1 mL). After 30 min at room temperature, the reaction mixture was concentrated. The residue was dissolved in CH_2Cl_2 (1 mL) and successively treated with NMM (38 μL, 0.344 mmol), the tripeptide fragment **9** (50 mg, 0.90 mmol), and HATU (39 mg, 0.10 mmol) as for compounds **14a** and **14b**. After flash chromatography (1 cm, 80% AcOEt−hexane), the desired tetrapeptide was isolated (46.4 mg, 0.065 mmol, 75% yield): ^{1}H NMR ($CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.49−7.35 (m, 4H), 6.42 (bs, 1H), 6.10 (d, J = 8.6 Hz, 1H), 5.89 (d, J = 8.9 Hz, 1H), 5.81−5.71 (m 1H), 5.19 (dd, J = 1.3, 17.2 Hz, 1H), 5.10 (d, J = 1.3, 11.4 Hz, 1H), 4.95 (d, J = 12.4 Hz, 1H), 4.91 (d, J = 12.1 Hz, 1H), 4.64−4.42 (m, 3H), 4.39−4.31 (m, 1H), 4.27−4.16 (m, 1H), 4.14−4.02 (m, 2H), 3.92−3.83 (m, 1H), 3.63−3.57 (m, 1H), 2.61−2.51 (m, 2H), 2.02−1.95 (m, 1H), 1.95 (s, 3H), 1.63−1.48 (m, 6H), 1.21−0.72 (m, 20H); MS 739 (M + Na).

(c) Preparation of Compounds 17a and 17b. The tetrapeptide obtained above (44.2 mg, 0.062 mmol) was dissolved in CH_3CN (1 mL) and successively treated with triphenylphosphine (0.84 mg, 0.0032 mmol), $Pd(PPh_3)_4$ (1.9 mg, 0.0016 mmol), and pyrrolidine (5.6 mL, 0.067 mmol). The reaction mixture was stirred overnight at room temperature. Since the reaction was not completed, the same amount of reagents was added again. The reaction mixture was stirred for another 4 h at room temperature, and then the CH_3CN was evaporated. The residue was dissolved in AcOEt and successively washed with 10% aqueous citric acid (2×), water (2×), and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was flash chromatographed (1 cm, AcOEt and then eluted with a mixture of $CHCl_3$, MeOH, andAcOH). The product obtained was dissolved in a mixture of CH_3CN and water and lyophilized to afford the desired acid **17a** as a white solid (24.4 mg, 0.036 mmol, 59% yield; 99% homogeneous by HPLC and 99.1% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.25 (bs, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.88−7.78 (m, 2H), 7.57−7.44 (m, 4H), 4.99 (d, J = 11.8 Hz, 1H), 4.89 (d, J = 12.1 Hz, 1H), 4.37 (dd, J = 8.3, 8.3 Hz, 1H), 4.32 (bs, 1H), 4.27 (dd, J = 8.0, 8.0 Hz, 1H), 4.19 (dd, J = 7.9, 7.9 Hz, 1H), 4.09 (d, J = 11.8 Hz, 1H), 3.74 (dd, J = 4.4, 11.1 Hz, 1H), 2.18−2.13 (m, 1H), 2.05−1.93 (m, 1H), 1.84 (s, 3H), 1.72−1.43 (m, 7H), 1.08−0.71 (m, 21H); MS 699 (M + Na); HRMS calcd for $C_{38}H_{53}N_4O_7$ 677.391426, found 677.390801.

The enantiomer of Boc-methano-leucine[18b] was converted to compound **17b** using the conditions described for the preparation of compound **17a**.

Compound **17b** (98% homogeneous by HPLC and 98.2% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.41 (bs, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.84−7.78 (m, 2H), 7.58−7.44 (m, 4H), 4.99 (d, J = 11.8 Hz, 1H), 4.91 (d, J = 12.1 Hz, 1H), 4.37−4.30 (m, 2H), 4.27 (dd, J = 7.6, 7.6 Hz, 1H), 4.19 (dd, J = 7.3, 7.3 Hz, 1H), 4.10 (d, J = 11.5 Hz, 1H), 3.74−3.67 (m, 1H), 2.25−2.16 (m, 1H), 2.05−1.89 (m, 2H), 1.84 (s, 3H), 1.62−1.40 (m, 7H), 1.30−1.22 (m, 1H), 1.16−0.86 (m, 12H), 0.96 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.77 (dd, J = 6.7, 15.9 Hz, 1H). MS 675 (M − H); HRMS calcd for $C_{38}H_{53}N_4O_7$ 677.391426, found 677.388923.

Preparation of Compounds 18a and 18b. Using 1,2-dibromopropylbenzene and the same conditions used for the generation of tetrapeptides **14a** and **14b**, the desired acids **18a** and **18b** were prepared.

Compound **18a** (92% homogeneous by HPLC; contains 5% of **18b**; 98.2% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.33 (bs, 1H), 8.04 (d, J = 8.9 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.89−7.78 (m, 1H), 7.56−7.44 (m, 4H), 7.30−7.13 (m, 5H), 4.98 (d, J = 11.8 Hz, 1H), 4.88 (d, J = 11.8 Hz, 1H), 4.36 (dd, J = 8.3, 8.3 Hz, 1H), 4.33−4.25 (m, 2H), 4.19 (dd, J = 7.6, 7.6 Hz, 1H), 4.08 (d, J = 11.1 Hz, 1H), 3.74 (dd, J = 4.5, 11.5 Hz, 1H), 2.94−2.80 (m, 2H), 2.20−2.11 (m, 1H), 2.04−1.93 (m, 2H), 1.84 (s, 3H), 1.63−1.42 (m, 7H), 1.38−1.30 (m, 1H), 1.12−0.81 (m, 6H), 0.89 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H), 0.75 (dd, J = 6.7, 18.8 Hz, 1H); MS 747 (M + Na); HRMS calcd for $C_{42}H_{53}N_4O_7$ 725.391426, found 725.393120.

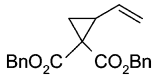
Compound **18b** (91% homogeneous by HPLC; contains 9% of **18a**; 97.5% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.48 (bs, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 7.3 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.84−7.77 (m, 2H), 7.58−7.44 (m, 4H), 7.32−7.15 (m, 5H), 4.98 (d, J = 12.1 Hz, 1H), 4.89 (d, J = 12.1 Hz, 1H), 4.38−4.25 (m, 2H), 4.18 (dd, J = 7.6, 7.6 Hz, 1H), 4.11 (d, J = 11.1 Hz, 1H), 3.74−3.67 (m, 1H), 2.91−2.75 (m, 2H), 2.25−2.16 (m, 1H), 2.04−1.90 (m, 2H), 1.83 (s, 3H), 1.69−1.40 (m, 7H), 1.29−1.22 (m, 1H), 1.09−0.92 (m, 6H), 0.89 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.73 (dd, J = 7.0, 15.3 Hz, 1H); MS 747 (M + Na); HRMS calcd for $C_{42}H_{53}N_4O_7$ 725.391426, found 725.393184.

Preparation of Compounds 19a and 19b. 4-Phenyl-1-butene was treated with bromine in CCl_4 to produce 1,2-dibromo-4-phenylbutane. This dibromide was used to synthesize the title compounds using the procedure already described for compounds **14a** and **14b**.

Compound **19a** (97% homogeneous by HPLC; 98.9% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H), 8.04 (d, J = 7.3 Hz, 1H), 7.94 (d, J = 7.3 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.85−7.80 (m, 2H), 7.58−7.45 (m, 4H), 7.28−7.23 (m, 2H), 7.19−7.13 (m, 3H), 4.98 (d, J = 12.1 Hz, 1H), 4.90 (d, J = 12.1 Hz, 1H), 4.39−4.30 (m, 2H), 4.27 (dd, J = 8.0, 8.0 Hz, 1H), 4.20 (dd, J = 8.3, 8.3 Hz, 1H), 4.10 (d, 11.4 Hz, 1H), 3.75 (dd, J = 4.1, 11.1 Hz, 1H), 2.69−2.59 (m, 2H), 2.22−2.14 (m, 1H), 2.05−1.94 (m, 2H), 1.84 (s, 3H), 1.82−1.73 (m, 2H), 1.63−1.33 (m, 6H), 1.26−1.20 (m, 1H), 1.07−0.91 (m, 6H), 0.89 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.76 (dd, J = 6.7, 14.0 Hz, 1H); MS 761 (M + Na); HRMS calcd for $C_{43}H_{55}N_4O_7$ 739.407076, found 739.405613.

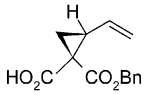
Compound **19b** (85% homogeneous by HPLC; contains 15% of **19a**; 98.1% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H), 8.05 (d, J = 7.3 Hz, 1H), 7.94 (d, J = 7.31 Hz, 1H), 7.88 (d, J = 7.9 Hz, 1H), 7.84−7.77 (m, 2H), 7.59−7.44 (m, 4H), 7.31−7.23 (m, 2H), 7.21−7.13 (m, 3H), 4.99 (d, J = 11.8 Hz, 1H), 4.90 (d, J = 11.8 Hz, 1H), 4.39−4.30 (m, 2H), 4.29 (dd, J = 8.0, 8.0 Hz, 1H), 4.19 (dd, J = 7.3, 7.3 Hz, 1H), 4.12 (d, J = 11.1 Hz, 1H), 3.74−3.67 (m, 1H), 2.71−2.53 (m, 2H), 2.26−2.15 (m, 1H), 2.04−1.92 (m, 2H), 1.84 (s, 3H), 1.79−1.70 (m, 2H), 1.65−1.35 (m, 6H), 1.32−1.20 (m, 1H), 1.07−0.92 (m, 6H), 0.89 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.4 Hz, 3H), 0.75 (dd, J = 6.0, 13.7 Hz, 1H); MS 761 (M + Na); HRMS calcd for $C_{43}H_{55}N_4O_7$ 739.407076, found 739.408618.

Preparation of Compound 21. (a) Preparation of Dibenzyl 2-Vinylcyclopropanedicarboxylate.



To a THF solution (850 mL) of potassium *tert*-butoxide (22 g, 196.04 mmol, 1.1 equiv) at −30 °C was slowly added dibenzyl malonate (50 g, 175.86 mmol). The internal temperature was maintained between −30 and − 10 °C throughout the addition. After 30 min, 1,4-dibromobutene (45.1 g, 210.84 mmol, 1.2 equiv) was added. The cooling bath was then removed. After 1.5 h, the reaction mixture was cooled to − 10° C for the second addition of potassium *tert*-butoxide (22.0 g, 196.0 mmol, 1.1 equiv). The cooling bath was again removed. One hour later, a mixture of ice and water was added to the reaction mixture. The two layers were separated, and the organic one was concentrated. The residue was combined with the initial aqueous layer, and the mixture was extracted with ether (3×). The combined organic layer was then washed with brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was flash chromatographed (12 cm, 3−6% AcOEt−hexane) to afford dibenzyl 2-vinylcyclopropanedicarboxylate as a colorless oil (30.87 g, 92 mmol, 52% yield): ^{1}H NMR ($CDCl_3$) δ 7.32−7.25 (m, 10H), 5.45−5.36 (m, 1H), 5.29−5.08 (m, 6H), 2.65−2.59 (m, 1H), 1.75 (dd, J = 4.8, 8.0 Hz, 1H), 1.60 (dd, J = 4.8, 9.2 Hz, 1H); MS 337 (MH^+).

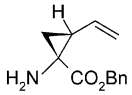
(b) Preparation of (1*R*,2*S*)/(1*S*,2*R*)-1-[(Benzyloxy)carbonyl]-2-vinylcyclopropanecarboxylic Acid.



(racemic)

To the diester obtained above (29.38 g, 87.3 mmol) in a mixture of THF (145 mL), *tert*-butyl alcohol (72 mL) and water (72 mL) at 0 °C was added lithium hydroxide monohydrate (4.03 g, 96.0 mmol, 1.1 equiv). The reaction mixture was stirred for 18.5 h at room temperature, and then an additional amount of hydroxide was added (916 mg, 0.25 equiv). After 3 days at room temperature, most of the THF and the *tert*-butyl alcohol were removed in vacuo. A 10% aqueous citric acid solution was added to the residue for acidification. The aqueous layer was extracted with ethyl acetate (3×). The combined organic extract was successively washed with water (2×) and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the desired monoacid was obtained as a colorless oil (16.35 g, 66.4 mmol, 76% yield): ^{1}H NMR ($CDCl_3$) δ 7.39−7.33 (m, 5H), 5.61−5.52 (m, 1H), 5.34 (d, J = 17.2 Hz, 1H), 5.22 (s, 2H), 5.14 (d, J = 10.4 Hz, 1H), 2.77−2.71 (m, 1H), 2.08 (dd, J = 4.8, 9.2 Hz, 1H), 1.98 (dd, J = 4.8, 8.4 Hz, 1H).

(c) Preparation of Benzyl (1*S*,2*S*)/(1*R*,2*R*)-1-Amino-2-vinylcyclopropanecarboxylate.

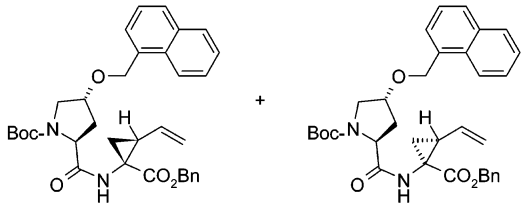


(racemic)

The acid obtained above (16.35 g, 66.4 mmol) was converted to the corresponding trimethylsilyl carbamate using the conditions already described for the preparation of compounds **20a** and **20b**. The crude material was flash chromatographed (8.5 cm, 10−15% AcOEt−hexane) to afford the desired carbamate (11.0 g, 47% yield) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.33 (bs, 5H), 5.79−5.70 (m, 1H), 5.28 (d, J = 17.2 Hz, 1H), 5.26 (bs, 1H), 5.18 (d, J = 12.4 Hz, 1H), 5.16 (d, J = 12.4 Hz, 1H), 5.10 (d, J = 10.8 Hz, 1H), 4.15−4.11 (m, 2H), 2.21−2.15 (m, 1H), 1.86−1.83 (m, 1H), 1.58−1.52 (m, 1H), 0.99−0.88 (m, 2H), 0.018 (s, 9H); MS 384 (M + Na).

To the carbamate (10.4 g, 28.8 mmol) was added a 1.0 M solution of tetrabutylammonium fluoride in THF (65 mL, 65.0 mmol). The reaction mixture was stirred for 20 h at room temperature, and then the THF was removed in vacuo. The residue was diluted with AcOEt and successively washed with water (2×) and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the crude amine was isolated (6.82 g).

(d) Preparation of Dipeptide Fragments.



To **(**4*R*)-1-(*tert*-butoxycarbonyl)-4-(1-naphthylmethoxy)-L-proline **10** (11.8 g, 31.8 mmol) in acetonitrile (100 mL) was added TBTU (10.7 g, 33.3 mmol). To the crude amine obtained above (ca. 28.8 mmol) in acetonitrile (80 mL) was added NMM (9.5 mL, 86.4 mmol). This solution was added to the acid solution, and the reaction mixture was stirred overnight at room temperature. The acetonitrile was then removed in vacuo, and the residue was dissolved in AcOEt and successively washed with 10% aqueous citric acid (2×), saturated aqueous $NaHCO_3$ (2×), and brine. After the usual treatment ($MgSO_4$, filtration, and concentration), the residue was flash chromatographed (9 cm, 25−35% AcOEt−hexane) to afford the less polar diastereomer (4.33 g, 7.6 mmol), the more polar diastereomer (4.05 g, 7.1 mmol), and a mixture of both compounds (0.93 g, 1.6 mmol).

(e) Preparation of Compound 21. Sequential coupling of the less polar diastereomer with Boc-Val-OH, Boc-Chg-OH, and acetic anhydride, followed by a saponification reaction of the benzyl ester under the conditions already described above, afforded the desired acid **21**.

Compound **21** (>99% homogeneous by HPLC; 95.8% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.52 (s, 1H), 8.06−7.77 (m, 5H), 7.58−7.43 (m, 4H), 5.74−5.64 (m, 1H), 5.26−4.88 (m, 4 H), 4.39−3.83 (m, 5H), 3.76 (dd, J = 4.2, 10.8 Hz, 1H), 2.21−2.16 (m, 1H), 2.06−1.93 (m, 3H), 1.84 (s, 3H), 1.69−1.42 (m, 7H), 1.22 (dd, J = 4.8, 9.2 Hz, 1H), 1.12−0.92 (m, 4H), 0.9 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.75 (dd, J = 6.7, 13.4 Hz, 1H); MS 661 (MH^+), 683 (M + Na); HRMS calcd for $C_{37}H_{49}N_4O_7$ 661.360125, found 661.360768.

Preparation of Compound 22. This inhibitor was prepared using commercially available building blocks and standard coupling conditions (TBTU).

Compound **22** (>99% homogeneous by HPLC; 99.4% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.73 (s, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.38−7.25 (m, 5H), 4.53 (d, J = 11.9 Hz, 1H), 4.45 (d, J = 11.7 Hz, 1H), 4.33−4.15 (m, 4H), 4.04 (d, J = 11.1 Hz, 1H), 3.68 (dd, J = 4.2, 11.2 Hz, 1H), 2.23−2.15 (m, 1H), 2.02−1.89 (m, 2 H), 1.84 (s, 3H), 1.69−1.42 (m, 6H), 1.39−1.31 (m, 1H), 1.28−1.20 (m, 1H), 1.13−0.82 (m, 6H), 0.88 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.78 (dd, J = 7.4, 7.4 Hz, 1H); MS 585 (MH^+); HRMS calcd for $C_{31}H_{45}N_4O_7$ 585.328825, found 585.331500.

Preparation of Compound 23. This inhibitor (98% homogeneous by HPLC; 95.7% homogeneous by CE) was pre-

pared using racemic vinyl-ACCA (see the preparation of compound **21** above). After coupling to the Boc-Hyp(O-Bn)-OH, the less polar diastereomer was isolated and converted to the final compound using sequential coupling and standard conditions (TBTU).

Compound **23** (98% homogeneous by HPLC; 95.7% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 8.52 (s, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.82 (d, J = 8.9 Hz, 1H), 7.38−7.24 (m, 5H), 5.74−5.63 (m, 1H), 5.19 (dd, J = 1.6, 17.5 Hz, 1H), 5.06 (d, J = 11.1 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 4.45 (d, J = 11.8 Hz, 1H), 4.35−4.15 (m, 4H), 4.00 (d, J = 11.1 Hz, 1H), 3.72 (dd, J = 4.1, 10.8 Hz, 1H), 2.21−2.11 (m, 1H), 2.05−1.91 (m, 3H), 1.84 (s, 3H), 1.71−1.43 (m, 6H), 1.22 (dd, J = 5.1, 9.5 Hz, 1H), 1.12−0.82 (m, 5H), 0.89 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.79 (dd, J = 7.0, 1.3 Hz, 1H); MS 611 (MH^+), 633 ($M + Na^+$); HRMS calcd for $C_{33}H_{46}N_4O_7$ 611.344475, found 611.342000.

Preparation of Compound 24. This compound has been described previously.[25]

Preparation of Compound 25. This compound was prepared using conditions already described above.

Compound **25** (99% homogeneous by HPLC; 97.6% homogeneous by CE): ^{1}H NMR (DMSO-d_6) δ 9.06 (d, J = 6.5 Hz, 1H), 8.59 (s, 1H), 8.26 (d, J = 10.0 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.54−7.40 (m, 3H), 5.77−5.61 (m, 2H), 5.21 (d, J = 17.4 Hz, 1H), 5.07 (d, J = 11.4 Hz, 1H), 4.50 (d, J = 11.9 Hz, 1H), 4.38 (dd, J = 8.4, 8.4 Hz, 1H), 4.19 (dd, J = 8.0, 8.0 Hz, 1H), 4.13 (dd, J = 7.8, 7.8 Hz, 1H), 4.06 (dd, J = 2.9, 11.9 Hz, 1H), 3.99 (s, 3H), 2.05−1.94 (m, 2H), 1.83 (s, 3H), 1.71−1.36 (m, 7H), 1.24 (dd, J = 4.9, 9.2 Hz, 1H), 1.15−0.84 (m, 6H), 0.93 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.4 Hz, 3H), 0.78 (dd, J = 6.0, 14.0 Hz, 1H); MS 611 (MH^+), 633 ($M + Na^+$); HRMS calcd for $C_{33}H_{46}N_4O_7$ 611.344475, found 611.342000.

Preparation of Compounds 26 and 27. These compounds have been described previously.[26]

Computational Chemistry (Figure 4). The binding models for the inhibitor P1 residues (Figure 4) were generated using the INSIGHT II software package (Accelerys Inc., San Diego, CA). Both (*R*,*R*) and (*S*,*S*) isomers of the P1 ACCA were built and docked into the substrate-binding region of the X-ray crystal structure of the apo NS3 protease.[30] Specifically, the P1 residues were appropriately oriented in order to mimic the conformation of the *C*-terminal threonine amino acid observed in the crystal structure of the full-length NS3 protease−helicase protein. In this conformation, the P1 ϕ angle is 127.6°, and the carboxylate is properly oriented to make hydrogen bonds with the side chains of the active-site residues His 57 and Ser 139 as well as with the backbone amide protons of the oxyanion hole residues Gly 137 and Ser 139.

Biological Assay. Reported IC_{50} values are averages of duplicate experiments on two separate samples (four measurements) and were determined using the radiometric assay described below.

NS3−NS4A Protein Radiometric Assay. The NS3−NS4A protein was produced using a recombinant baculovirus system and purified as previously described.[31] The enzymatic assay was performed in 50 mM Tris-HCl, pH 8.0, 0.25 M sodium citrate, 0.01% *n*-dodecyl-β-D-maltoside, 1 mM TCEP. A 25 μM concentration of the substrate DDIVPC-SMSYTW-OH, a 1 nM concentration of biotin-DDIVPC-SMSY[^{125}I]TW-OH, and various concentrations of inhibitor were incubated with 6 nM NS3−NS4A protein for 2 h at 23 °C. The reaction was terminated by the sequential addition of 0.5 N NaOH and 1 M MES, pH 5.8. The separation of the biotinylated substrate from products was performed by adding avidin-coated agarose beads (Pierce) to the assay mixture and incubating for 1 h at 23 °C. Following filtration on a Millipore MADP N65 plate, the amount of SMS[^{125}I-Y]TW-OH product found in the filtrate was quantitated using a TopCount detector (Perkin-Elmer) and allowed for the calculation of the percentage of inhibition. A nonlinear curve fit using the Hill model was then applied to the % inhibition−concentration data, and 50% effective concentration (IC_{50}) was calculated through the use of SAS (Statistical Software System, SAS Institute Inc., Cary, NC).

Acknowledgment. We thank Mr. Ewald Welchner for all the enzymatic assays. We also thank Mr. Michael Little and Mr. Sylvain Bordeleau for analytical support. Professor Charette (Université de Montréal), who kindly provided us with a sample of compound **13** in a homochiral form, is gratefully acknowledged. We are grateful to Dr. Nathalie Goudreau for her input in the computational chemistry section of this document and to Ms. Elise Ghiro for the preparation of compound **21**. Finally, we also thank Drs. Pierre Beaulieu and Peter White for proofreading the manuscript.

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(27) The enzymatic resolution of vinyl-ACCA has been performed successfully. Details regarding the resolution of ACCA derivatives will be reported elsewhere. The absolute configuration at C1 and C2 on the cyclopropane ring of vinyl-ACCA was established by hydrogenation of the unsaturation and comparison with the corresponding ethyl-ACCA derivative for which the structure had been proven (see Scheme 3).

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