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Discovery of potent, selective 4-fluoroproline-based thrombin inhibitors with improved metabolic stability

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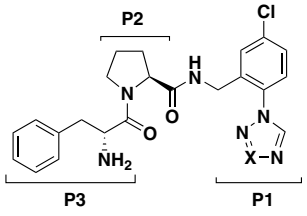
Abstract—Previous reports from our laboratories described potent tripeptide thrombin inhibitors which incorporate heterocycle-substituted chlorophenyl groups in the P1 position. Using these as lead compounds for further optimization, we identified sites of metabolism and designed analogs with 4-fluoroproline in P2 and cyclopropane-containing side chains in P3 as an approach to reducing metabolism and improving their oral pharmacokinetic performance. The large (300-fold) difference in potency between analogs containing (4*R*)- and (4*S*)-4-fluoroproline was rationalized by analyzing inhibitor–enzyme interactions in crystal structures of related compounds and by molecular modeling which indicated that the more potent (4*R*)-4-fluoroproline isomer stabilizes a proline ring conformation that is preferred for binding to the enzyme. An optimal compound from this work, **41**, exhibits high potency in a coagulation assay in human plasma (2×APTT = 190 nM), excellent selectivity versus the digestive enzyme trypsin (K_i = 3300 nM), and excellent oral bioavailability in dogs with moderate clearance (F = 100%, CL = 12 mL/min/kg).

1. Introduction

The serine protease thrombin is a critical trypsin-like enzyme in the blood coagulation cascade. Thrombin mediates the conversion of fibrinogen to fibrin and is also the most potent stimulator of platelet aggregation known. A direct thrombin inhibitor would therefore be expected to have therapeutic applications in the treatment of disease states involving undesirable clot formation, including the treatment of deep vein thrombosis and pulmonary embolism. We and others have previously described the development of potent thrombin inhibitors based on a D-Phe-Pro-Arg tripeptide motif.[1] Recent reports from these laboratories described the discovery of novel heterocycle-substituted chlorophenyl P1 groups and their incorporation into this classical tripeptide scaffold, giving rise to potent inhibitors such as **1** and **2**.[2]



1 (X = CH): K_i (thrombin) = 1.8 nM
2 (X = N): K_i (thrombin) = 0.33 nM

When dosed orally in dogs at 1 mg/kg, **1** and **2** exhibited modest plasma levels (AUC = 0.6 and 1 μM h, respectively) with short half-lives (1.7 and 2.5 h, respectively). Incubation of these compounds with microsomes obtained from rat, dog, monkey, and human liver tissues indicated that

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no metabolism had occurred on the triazole moiety in P1; however, extensive metabolic oxidation of the central P2 proline and the P3 side chain had occurred in all species. As an approach to improving the pharmacokinetic properties of **1** and **2**, we therefore focused our efforts on designing analogs which had the potential for greater stability toward oxidative metabolism in the P2–P3 portion of the molecule. Fluorination has been shown to be an effective strategy for reducing oxidative metabolism of susceptible functional groups,[3] and therefore we sought to improve the metabolic stability in P2 by incorporating 4-fluoroproline, the preparation of which has been described in the literature.[4] Additionally, we sought to incorporate novel amino acids in P3 which were designed to improve the metabolic stability of the side chain. This manuscript describes the synthesis, conformational properties, and biological profiles of analogs of **1** and **2** containing such modifications.

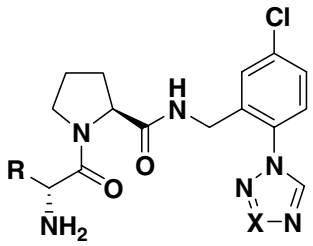
2. Chemistry

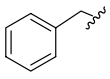
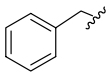
Synthesis of the compounds listed in Tables 1 and 2 began with assembly of the P1–P2 cores via standard amino acid coupling of the P1 benzylamines[2] to the appropriately functionalized Boc-protected prolines. Deprotection of the intermediate prolinamides followed by a second amino acid coupling to the protected P3 amino acids and removal of the protecting group afforded the desired targets. The 4-fluoroproline P2 amino acids were prepared according to procedures previously described in the literature.[4] The P2 amino acid Boc-L-proline and the P3 amino acid Boc-*tert*-butyl-D-alanine are commercially available, and the remaining amino acids incorporated into our inhibitor scaffold were synthesized from readily available starting materials as described below.

The syntheses of the novel Boc-3-(1-methylcyclopropyl)-D-alanine **6**, (2*R*)-2-azido-3-(1-chlorocyclopropyl)propanoic acid (**13**, precursor to the chlorocyclopropyl alanine P3 side chain), and Boc-cyclopropylvaline **20** have not been previously reported in the literature. We devised a three-step route to **6** starting with alkylation of the Williams chiral auxiliary **3**[5] with methallyl bromide. Cyclopropanation of the resulting olefin **4** and reductive removal of the auxiliary afforded acid **6** (Scheme 1).

Asymmetric synthesis of (2*R*)-2-azido-3-(1-chlorocyclopropyl)propanoic acid **13** was accomplished in several

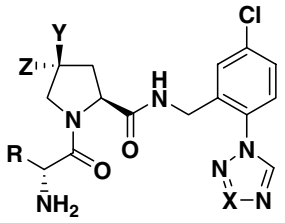
Table 1. P3 amino acid analogs



Compound	R	X	Thrombin K_i (nM)	2×APTT (nM)	Trypsin K_i (nM)	Trypsin/2× APTT ratio	Dog Oral AUC (μM h)*	Dog Oral $t_{1/2}$ (h)
1		CH	1.8	430	4100	9.5	0.57[a]	1.7
2		N	0.31	230	590	2.6	0.99[b]	2.5
37		CH	0.61	210	9500	45	2.1[c]	2.1
38		N	0.09	140	1400	10	1.8[d]	2.6
39		CH	0.60	170	6200	36	2.8[e]	3.4
40		N	0.08	100	850	8.5	1.1	3.6

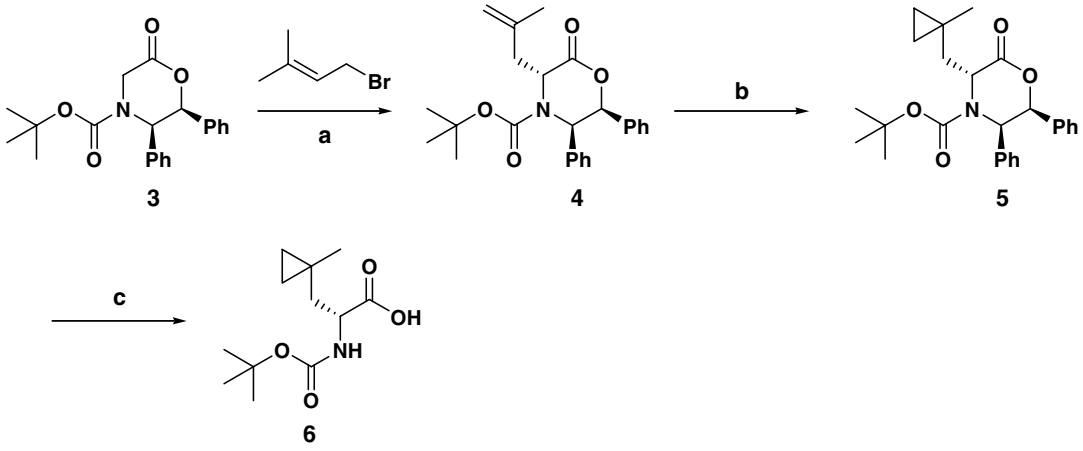
* Values based on 1 mpk dose; see notes below.
[a] Data normalized to 1 mpk dose. Raw data: dose = 0.65 mpk, AUC = 0.37 μM h.
[b] Data normalized to 1 mpk dose. Raw data: dose = 0.75 mpk, AUC = 0.74 μM h.
[c] Data normalized to 1 mpk dose. Raw data: dose = 0.85 mpk, AUC = 1.78 μM h.
[d] Data normalized to 1 mpk dose. Raw data: dose = 0.85 mpk, AUC = 1.51 μM h.
[e] Data normalized to 1 mpk dose. Raw data: dose = 0.90 mpk, AUC = 2.55 μM h.

Table 2. P2 fluoroproline analogs



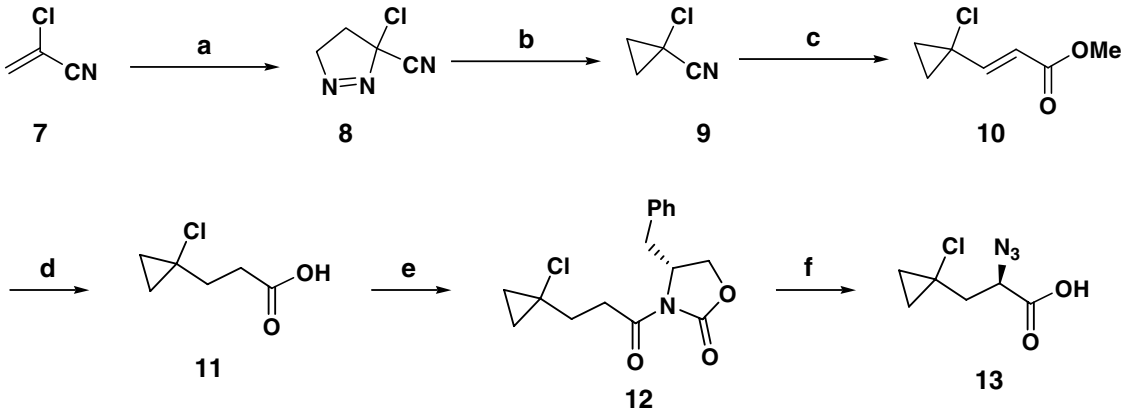
Compound	R	X	Y	Z	Thrombin K_i (nM)	2×APTT (nM)	Trypsin K_i (nM)	Trypsin/2× APTT ratio	Dog Oral AUC (μM h)*	Dog Oral $t_{1/2}$ (h)
39		CH	H	H	0.60	170	6200	36	2.8[a]	3.4
40		N	H	H	0.08	100	850	8.5	1.1	3.6
41		CH	H	F	0.37	190	3300	17	5.0	2.8
42		CH	F	H	110	—	26,000	—	—	—
43		CH	F	F	3.6	—	4600	—	—	—
44		N	F	F	0.55	190	520	2.7	2.8[b]	4.0
45a[13]		CH	H	F	1.3	540	13,000	24	4.5	2.0
46a[13]		CH	H	F	0.32	260	3600	14	1.5	1.4

* Values based on 1 mpk dose; see notes below.
[a] Data normalized to 1 mpk dose. Raw data: dose = 0.90 mpk, AUC = 2.55 μM h.
[b] Data normalized to 1 mpk dose. Raw data: dose = 0.85 mpk, AUC = 2.39 μM h.



Scheme 1. Reagents and conditions: (a) NaHMDS, THF, −70 °C; (b) CH_2N_2, $Pd(OAc)_2$, THF/Et_2O, 0 °C; (c) Li/NH_3(l), THF.

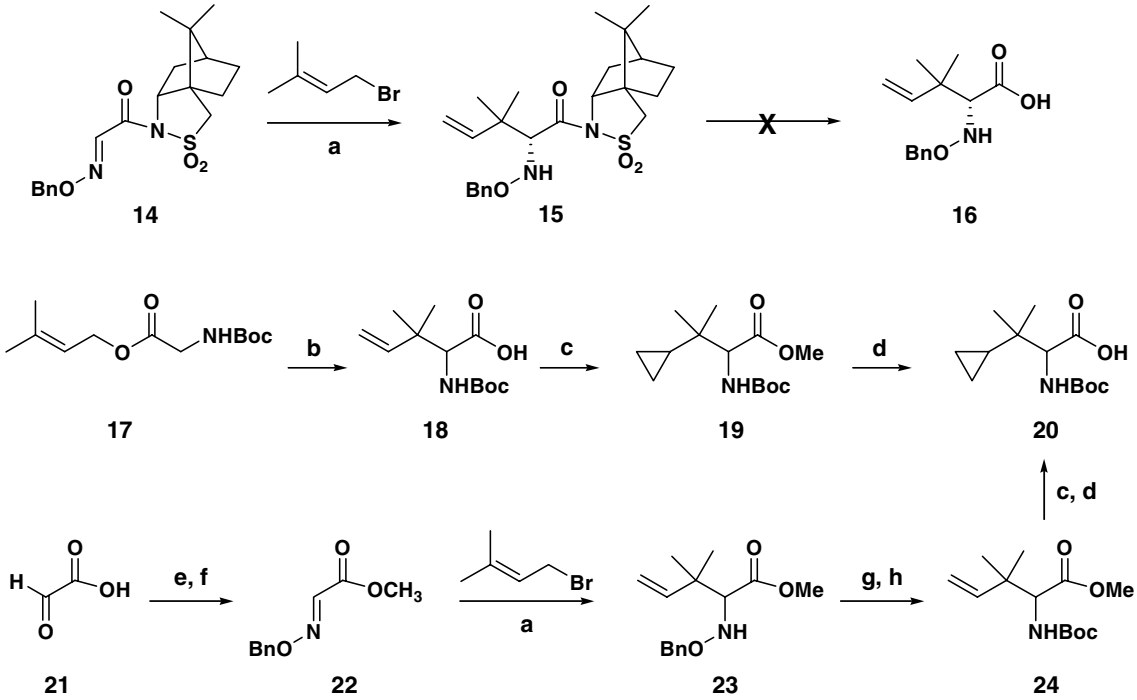
steps starting from commercially available 2-chloroacrylonitrile **7** (Scheme 2). Treatment of **7** with an ethereal solution of diazomethane at 0 °C afforded the intermediate dihydropyrazole adduct **8** which was thermally decomposed to yield 1-chlorocyclopropanecarbonitrile **9**. DIBAL reduction of nitrile **9** followed by Wittig olefination of the resulting aldehyde with (carbomethoxymethylene)-triphenylphosphorane afforded the α,



Scheme 2. Reagents and conditions: (a) MNNG, 40% aq KOH, Et_2O, 0 °C; (b) benzene, reflux; (c) i—DIBAL, toluene −78–0 °C; ii—$Ph_3P{=}CHCO_2Me$, toluene, rt; (d) i—H_2 (balloon), 10% Pd–C, MeOH, rt; ii—LiOH·H_2O, 1:1:1 THF/MeOH/H_2O, rt; (e) i—$(CH_3)_3CC(O)Cl$, Et_3N, THF, −20 °C; ii—(4*R*)-4-benzyl-1,3-oxazolidin-2-one, LiCl, rt; (f) i—KHMDS, toluene, −78 °C; ii—trisyl azide, warm to 30 °C; iii—LiOH·H_2O, 2:2:1 THF/MeOH/H_2O, 0 °C to rt.

β-unsaturated ester **10**. Catalytic hydrogenation of the double bond of **10** followed by ester hydrolysis with lithium hydroxide in an aqueous THF–methanol mixture afforded 3-(1-chlorocyclopropyl)propanoic acid **11**. Acid **11** was coupled to the chiral auxiliary (4*R*)-4-benzyl-1,3-oxazolidin-2-one to produce **12** which underwent stereoselective azidation with potassium bis(trimethylsilyl)amide and triisopropylbenzene sulfonyl azide.[6] Removal of the chiral auxiliary under basic conditions afforded the azido acid **13**, which was subsequently coupled to the P1–P2 core prior to reduction of the azide to the desired P3 amine.

We initially designed an asymmetric synthesis of Boc-cyclopropylvaline **20** via the known chiral sultam oxime precursor **15** (Scheme 3).[7] Removal of the chiral auxiliary of **15** and cyclopropanation of the resulting intermediate **16** would then have afforded the desired amino acid core. However, attempts to cleave the chiral auxiliary of **15** under basic,[8] Lewis acidic[9] or phase transfer conditions[10] were unsuccessful in our hands. We consistently observed either no reaction or the formation of products which appeared to arise from the reaction of nucleophiles (e.g., hydroxide or peroxide) at the sulfur atom of **15** resulting in ring opening of the sultam,



Scheme 3. Reagents and conditions: (a) Zn, satd aq NH_4Cl, THF, rt; (b) LDA, TMSCl, THF, −78 °C then 60 °C; (c) CH_2N_2, Et_2O, $Pd(OAc)_2$, 0 °C to rt; (d) LiOH·H_2O, THF–H_2O, 40 °C; (e) $BnONH_2$·HCl, NaOAc, 1:1 MeOH–H_2O, rt; (f) DCC, MeOH, DMAP, CH_2Cl_2, 0 °C to rt; (g) $Mo(CO)_6$, CH_3CN, H_2O, reflux; (h) Boc_2O, CH_3CN, 0 °C to rt.

rather than from the desired reaction at the carbonyl center of **15** to liberate the free acid **16** and the sultam auxiliary. We attributed these results to steric shielding imposed on the carbonyl carbon of **15** by both the adjacent neopentyl-like α-carbon center and the bulky sultam.

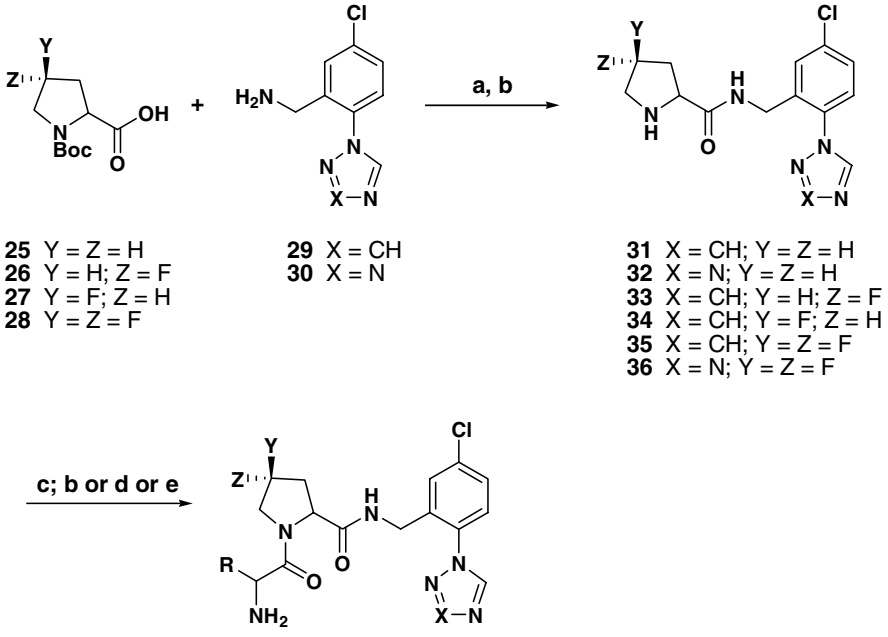
We thus resorted to an achiral synthesis of **20** via an ester enolate Claisen rearrangement[11] of **17** (prepared by DCC-mediated coupling of Boc-glycine and 3-methylbut-2-en-1-ol) to give 2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylpent-4-enoic acid **18** (Scheme 3). Treatment of **18** with excess diazomethane in the presence of $Pd(OAc)_2$ afforded methyl ester **19**. Hydrolysis of ester **19** to give the desired racemic Boc-cyclopropylvaline **20** required heating, which was consistent with our earlier hypothesis of reduced reactivity at the carbonyl carbon of this scaffold due to steric shielding by the adjacent neopentyl-like α-carbon.

Alternatively, acid **20** could be prepared via an achiral variant of the oxime chemistry described above[7] in much higher overall yield than the Claisen route. Thus, glyoxylic acid **21** was condensed with *O*-benzylhydroxylamine and the resulting benzyloxyiminoacetic acid was esterified by treatment with DCC and methanol to give methyl ester **22**. Imino ester **22** was then treated with methallyl bromide in the presence of zinc and saturated aqueous ammonium chloride solution in THF to obtain key olefin intermediate **23**, which underwent molybdenum hexacarbonyl-mediated removal of the benzyloxy group[12] followed by Boc protection of the intermediate amino ester to give **24**. Cyclopropanation of **24** followed by basic hydrolysis afforded the desired protected cyclopropylvaline **20**.

Standard EDC coupling of Boc-L-proline **25** or the Boc-protected 4-fluoroprolines **26**,[4] **27**,[4] or **28**[4] to the benzylamines **29** or **30** (prepared as described in Young et al.[2]) followed by HCl-mediated cleavage of the Boc group afforded the P1–P2 cores **31**–**36** as hydrochloride salts (Scheme 4). EDC-mediated coupling of the protected P3 amino acids Boc-*tert*-butyl-D-alanine, **6** or **20** to intermediates **31**–**36** followed by TFA or HCl-mediated removal of the Boc group afforded the target compounds **37**–**44** and **46**. The diastereomers of **46** were resolved by chiral chromatography to afford compound **46a** (the more potent diastereomer) and its isomer **46b**.[13] Compound **45** was prepared via EDC coupling of the azido acid **13** to the P1–P2 intermediate **33** followed by triphenylphosphine-mediated reduction of the azide to the amine. Some epimerization occurred during the reduction and the diastereomeric amines were separated by chiral chromatography to afford **45a** (the more potent diastereomer) and its isomer **45b**.[13]

3. Biological methods

Inhibition constants (K_i values) of test compounds were determined in an in vitro assay using human thrombin and trypsin as previously described.[14,15] The 2×APTT value is defined as the concentration of test compound required to double the activated partial thromboplastin time of human plasma, and was determined using previously described methods.[15] Pharmacokinetic



25 Y = Z = H
26 Y = H; Z = F
27 Y = F; Z = H
28 Y = Z = F

29 X = CH
30 X = N

31 X = CH; Y = Z = H
32 X = N; Y = Z = H
33 X = CH; Y = H; Z = F
34 X = CH; Y = F; Z = H
35 X = CH; Y = Z = F
36 X = N; Y = Z = F

Scheme 4. Reagents and conditions: (a) **25** or **26** or **27** or **28**, EDC, HOAT, DMF, rt; (b) 4 M HCl–dioxane, EtOAc, 0 °C; (c) **6** or **13** or **20** or Boc-*tert*-butyl-D-alanine, EDC, HOAT, DIEA, DMF, rt; (d) TFA, CH_2Cl_2, rt; (e) (Compound **45** only) i—Ph_3P, THF, 0 °C; ii—H_2O, 40 °C, 2 h then rt, 66 h.

studies were carried out in conscious male beagle dogs ($n = 2$) weighing 10–12 kg. Test compounds were dosed orally as amorphous trifluoroacetate or hydrochloride salts or free bases, either as a solution or as a suspension in 0.5% methocel, 1% methocel or 5% dextrose-PEG200; data were subsequently normalized to a 1 mg/kg dose. Blood samples were collected via the jugular vein at regular intervals over an 8 h time period, with a final sample taken 24 h post-dosing. Plasma samples were assayed by solid-phase extraction followed by quantitative LCMS/MS analysis against a standard curve. Metabolite identification studies were carried out by incubation of rat, dog, monkey or human liver microsomes (4 mg/mL) with 10 μM solutions of test compound, 100 mM potassium phosphate buffer, 6 mM magnesium chloride, and 1 mM EDTA in the presence of either 1 mM NADPH or 1 mM NADPH + 1 mM UDPGA at 37 °C for 2 h with monitoring via LCMS/MS analysis. Standard error in the measurement of thrombin and trypsin K_i values, 2×APTT values, and oral AUC values is ±20%.

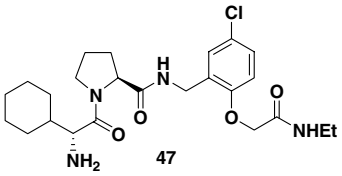
4. Results and discussion

As previously described,[2] thrombin inhibitors containing triazole- and tetrazole-substituted chlorophenyl P1 groups such as **1** and **2** exhibited high levels of selectivity (>1000-fold) for inhibiting thrombin versus the digestive serine protease, trypsin, in enzyme assays. In looking at the ratio of the K_i for inhibiting trypsin and the functional potency for inhibiting thrombin, that is, the 2×APTT value, however, the levels of selectivity are much lower. For example, the ratios of K_i values for inhibiting trypsin versus thrombin for **1** and **2** are 2300 and 1900, respectively; whereas the ratios using the 2×APTT values are 9.5 and 2.6 (see Table 1). Since the 2×APTT concentration approximates the plasma concentration required for therapeutic efficacy, we were interested in maintaining as large a window as possible between the 2×APTT potency and the K_i for inhibiting trypsin, and therefore express selectivity in this way.

Previous reports from these laboratories described the incorporation of P3 groups containing small, nonaromatic side chains as a means to obtain potent P2 proline-based thrombin inhibitors with excellent pharmacokinetic properties.[16] We adopted a similar strategy and screened several analogs of **1** and **2** containing commercially available nonaromatic amino acids in P3. From this screen we identified *tert*-butylalanine analogs **37** and **38**, which were 2-fold more potent than **1** and **2** in the 2×APTT assay and additionally exhibited improved selectivity versus trypsin. Both **37** and **38** showed a slight improvement in oral absorption compared to **1** and **2**, but no improvement in oral half-life was observed. We speculated that the methyl groups of the *tert*-butylalanine side chain in **37** and **38** could be potential sites for oxidative metabolism. It is known that cyclopropyl groups are more resistant to hydrogen abstraction (the initial step in oxidative metabolism) than alkyl groups,[17,18] and therefore we prepared analogs **39** and **40** which contain the novel amino acid methylcyclopropylalanine in P3. The potency and selectivity of methylcyclopropanes **39** and **40** were the same as the corresponding *tert*-butyl analogs **37** and **38**, and there was no significant change in the oral PK properties in dogs.

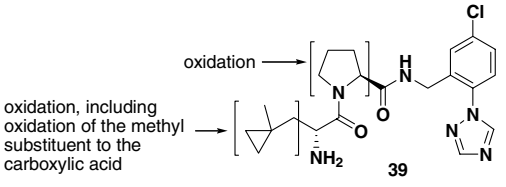
Exposure of **39** to microsomes from rat, dog, monkey, and human liver tissues indicated that oxidative metabolism was still occurring on the P3 side chain (including oxidation of the methyl group to a carboxylic acid) and on the P2 proline ring (Fig. 1). To follow up on these observations, we focused first on P2 metabolism and prepared analogs of **39** containing one or two fluorines at the proline ring 4-position (**41**–**43**, Table 2).

We were intrigued to find a nearly 300-fold difference in potency for inhibiting thrombin when comparing the two diastereomeric monofluoroproline derivatives **41** and **42**, with the (4*S*)-4-fluoroproline-derived analog **42** being almost 200-fold less potent than the des-fluoro analog **39**. A possible explanation for the loss in potency of **42** compared to **39** can be gleaned from the crystal structure of the related P2 proline-based inhibitor **47**[19] bound in the active site of thrombin (Fig. 2). In this structure, it is seen that the pro-(*S*) hydrogen at the proline ring 4-position of **47** is near to the aromatic side chains of residues Y60A and W60D, well within distance (3.4–3.7 Å) to be engaged in a CH-π-type interaction.[20] Substitution of fluorine for this hydrogen would then replace the weakly attractive CH-π interaction with a repulsive CF-π interaction.[21]



47

We were also interested in the effect of fluorination on proline ring conformation. The proline ring can adopt two main puckered conformations as depicted in Table 3, namely a 'syn' ring pucker, in which C4 points in the same direction as the substituent at C2, and an 'anti' ring pucker, in which C4 is oriented in the opposite direction to that of the C2 substituent. The anti pucker is the conformation observed in the majority of crystal structures of proline-based inhibitors bound in the thrombin active site (e.g., see the crystal structure in Fig. 2).[22] Energy calculations on model compounds **48**–**51** using MMFF and ab initio methods revealed



39

Figure 1. Observed metabolic profile upon incubation of compound **39** in rat, dog, monkey, and human liver microsomes.

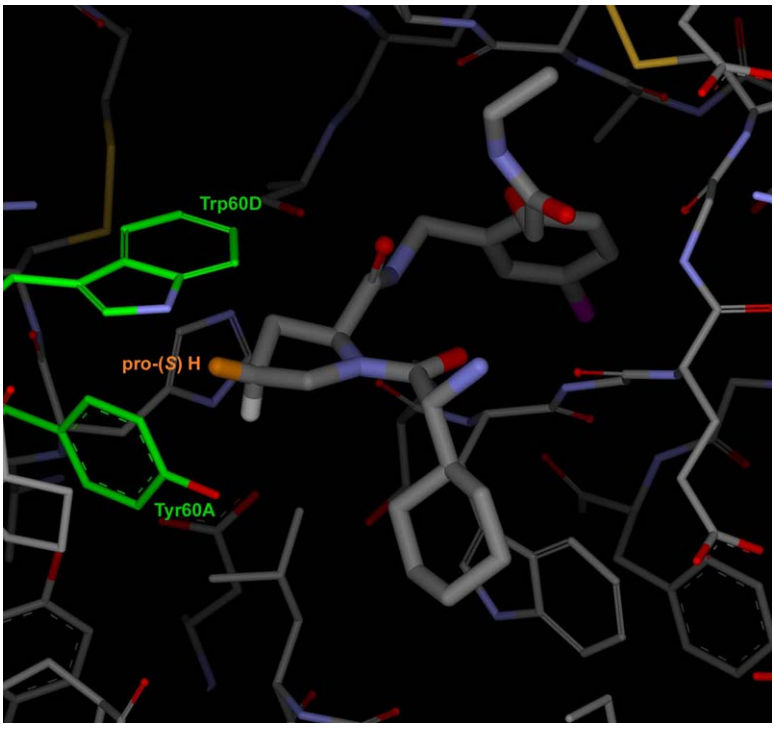
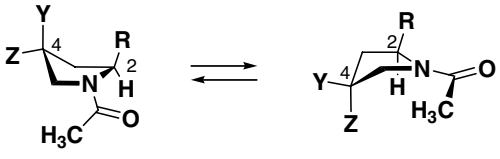
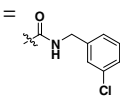


Figure 2. Crystal structure of compound **47** bound in the thrombin active site, from PDB structure code 1TA6.[19] The pro-(*S*) hydrogen is colored orange, and the aromatic rings of residues Y60A and W60D are shown in green. Also note the 'anti' pucker of the proline ring as described in the molecular modeling discussion above.

Table 3. Calculated energies of proline conformers of 4-fluoro and des-fluoro prolinamides



Model compound	$E_{anti} - E_{syn}$ (MMFF)[a] (kcal/mol)	$E_{anti} - E_{syn}$ (ab initio)[b] (kcal/mol)	Corresponding compound in Table 2	K_i (nM)
Y = Z = H (**48**)	+0.98	+0.29	**39**	0.60
Y = H, Z = F (**49**)	−1.2	−0.90	**41**	0.37
Y = F, Z = H (**50**)	+3.3	+1.8	**42**	110
Y = Z = F (**51**)	+1.1	+0.69	**43**	3.6

[a] R = 

for MMFF method.

[b] R = Me for ab initio method; data generated using density functional theory (DFT) calculations (B3LYP method).

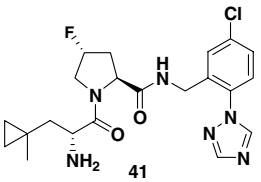
significant effects of fluorination on the relative energies of the syn and anti conformers (Table 3). Results from both computational methods indicate that relative to the unfluorinated system **48**, the equilibrium with the (4*R*) monofluoro diastereomer **49** is shifted toward the anti conformer and the equilibria with both the (4*S*) monofluoro diastereomer **50** and the difluoro compound **51** are shifted toward the syn conformer.[23] Thus, using the crystallographic evidence to infer that the anti conformer is the preferred conformer for binding to the enzyme, both sets of calculations on model compounds **48**–**51** correctly predict the rank order of potencies observed for compounds **39** and **41**–**43**.

Excellent potency in the 2×APTT assay was obtained with (4*R*)-4-fluoroprolyl triazole **41** and 4,4-difluoroprolyl tetrazole **44**, although trypsin selectivity for the latter is quite low. Compared to their non-fluorinated

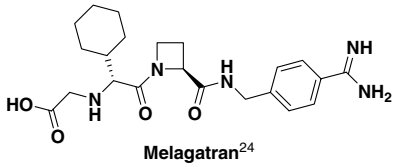
proline analogs **39** and **40**, however, no significant change in the oral PK properties in dogs was realized. Incubation of (4*R*)-4-fluoroprolyl triazole **41** with microsomes obtained from rat, dog, monkey, and human liver tissues revealed that the fluorine substituent on the proline ring had effectively suppressed metabolic transformations in P2—all metabolites arose exclusively from oxidative transformation of the P3 methylcyclopropyl alanine side chain. Based on this result and the P3 metabolites identified with **39**, we synthesized the analog of **41** in which the methyl group of the methylcyclopropylalanine P3 side chain is replaced with a chlorine atom. The resulting compound, **45a**, lost ~3-fold potency in the 2×APTT assay compared to **41**, and there was no improvement in the oral dog PK profile. Introduction of a gem-dimethyl substituent at the β-carbon of the P3 side chain to potentially block oxidation at that position gave rise to compound **46a**. Excellent 2×APTT potency resulted with this modification, and further, the compound showed good stability in the presence of hepatic microsomes across species. Performance in the oral dog PK assay, however, was unremarkable, with lower exposure and shorter half-life compared to **41**. Because at best only modest beneficial effects were observed in the PK assay with compounds containing several types of metabolism-reducing modifications, we concluded that compounds in this series are being cleared predominantly by non-oxidative and/or extrahepatic mechanisms.

5. Conclusion

In summary, structural modifications targeted at improving the pharmacokinetic properties of lead compounds **1** and **2** via reduction of metabolic oxidation in P2 and P3 led to the identification of several novel, potent inhibitors containing 4-fluoroproline in P2. The 300-fold difference in potency between the (4*R*)- and (4*S*)-4-fluoroproline diastereomers was rationalized by examining the crystal structure of a related compound to infer a destabilizing interaction between the fluorine of the less potent (4*S*)-4-fluoroproline diastereomer and the π-electrons of nearby aromatic amino acid side chains of the enzyme, and also by computational methods which indicated that the fluorine in the more potent (4*R*)-4-fluoroproline isomer stabilizes the proline ring conformation observed in enzyme–inhibitor crystal structures. Fluorination of the P2 proline was shown to effectively suppress oxidative metabolism of the proline ring in hepatic microsomes. Syntheses of several novel α-amino acids containing cyclopropyl side chains were developed with the goal of improving the metabolic stability of the P3 side chain. Compared to lead compound **1**, inhibitor **41** which contains (4*R*)-4-fluoroproline in P2 and methylcyclopropylalanine in P3 showed a 2-fold improvement in functional potency and trypsin selectivity, and a 10-fold improvement in oral plasma concentrations after a 1 mg/kg oral dose in dogs. Full PK characterization of **41** in dogs after a 1 mg/kg oral dose and a 1 mg/kg intravenous dose indicated that the compound was completely absorbed ($F = 100\%$) with a short half-life (intravenous $t_{1/2} = 2.0$ h), moderate clearance (12 mL/min/kg), and a low volume of distribution (1.7 L/kg). Compared to melagatran,[24] a thrombin inhibitor which has seen clinical use, **41** exhibited similar functional potency with greatly improved selectivity versus trypsin. Continued pharmacokinetic optimization of **41** holds the potential for delivering a therapeutically useful thrombin inhibitor suitable for once-daily oral dosing.



41
K_i (thrombin) = 0.37 nM
K_i (trypsin) = 3300 nM
2xAPTT = 190 nM



Melagatran[24]
K_i (thrombin) = 1.1 nM
K_i (trypsin) = 7.5 nM
2xAPTT = 280 nM

6. Experimental

6.1. General

All starting materials were obtained from commercial sources and were used without further purification. 1-Methyl-3-nitro-1-nitrosoguanidine, used for the generation of diazomethane in situ, is abbreviated as MNNG throughout the text. 1-Hydroxy-7-azabenzotriazole is abbreviated as HOAT throughout the text. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride is abbreviated as EDC throughout the text. Anhydrous solvents were obtained from Aldrich in Sure-seal bottles. Commercial diethyl ether was used without further distillation or drying. MP-carbonate resin was purchased from Argonaut Technologies, which is now owned by Biotage AB. All reactions were carried out under nitrogen atmosphere unless otherwise noted. Normal-phase chromatography was carried out using silica gel 60F (230–400 mesh) or RediSep® silica cartridges (purchased from Teledyne Isco, Inc.). Reverse-phase preparative HPLC was carried out on a Gilson 215 HPLC unit using a YMC-Pack Pro C18 column (dimensions 150 × 20 mm, 5 μm particle size). NMR spectra were recorded in $CDCl_3$, d_6-DMSO or CD_3OD solution on Varian Unity Inova spectrometers as follows: ^1H NMR spectra were recorded at 300, 400 or 500 MHz as noted. Chemical shifts are expressed in ppm relative to tetramethylsilane and the 1H coupling constants (J) are quoted in Hertz (Hz). Low resolution mass measurement data (MS) were generally recorded on a Waters LC–MS using electrospray ionization (ESI) for the mass detector and the following HPLC conditions: Stationary phase: YMC Pro C18 5 μm 120 Å 3.0 × 50 mm column;

Mobile phase: A = H_2O containing 0.05% TFA by volume, B = CH_3CN containing 0.0425% TFA by volume; Gradient: 8:92 B/A to 100:0 B/A over 3.6 min; Flow Rate: 1.5–2.0 mL/min over 3.6 min; UV detection at 215 and/or 254 nm. High resolution accurate mass measurement data (HRMS) were obtained as follows: Electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectral data were acquired on a Bruker Daltonics 3T Fourier transform ion cyclotron resonance mass spectrometer (FT/ICR/MS). External/internal calibration was accomplished with polypropylene glycol.

6.2. *tert*-Butyl (3*R*,5*R*,6*S*)-3-(2-methylprop-2-enyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylate (4)

To a stirred solution of *tert*-butyl (5*R*,6*S*)-2-oxo-5,6-diphenylmorpholine-4-carboxylate **3** (1.5 g, 4.24 mmol) in dry THF (30 mL) was added dropwise a 1 M solution of NaHMDS in THF (4.67 mL, 4.67 mmol) at −78 °C under Ar. The mixture was stirred at this temperature for 30 min and was then treated with 1-bromo-2-methyl-3-propene (0.43 mL, 4.24 mmol) dropwise via syringe. The mixture was stirred at −78 °C for 30 min and the bath was then removed and the mixture stirred at room temperature overnight. The reaction was quenched by the addition of 20% aqueous NH_4Cl and the THF was removed at reduced pressure. The residue was partitioned between EtOAc and H_2O, and the aqueous layer extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated at reduced pressure to give a residue that was dissolved in $CHCl_3$ and chromatographed on a 35 g RediSep® column using a gradient elution of 0–50% EtOAc–hexane, 40 min at a flow rate of 40 mL/min. The pure fractions were combined and concentrated to give *tert*-butyl (3*R*,5*R*,6*S*)-3-(2-methylprop-2-enyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylate **4** as a colorless solid (785 mg, 46%). ^{1}H NMR (300 MHz, DMSO-d_6) mixture of rotamers: δ 7.35–7.00 (m, 8H), 6.52 (t, J = 6.3 Hz, 2H), 6.35–6.28 (m, 1H), 5.15–4.82 (m, 4H), 2.95–2.70 (m, 2H), 1.87 (s, 3H), 1.41 (s, 3H), 1.03 (s, 6H).

6.3. *tert*-Butyl-(3*R*,5*R*,6*S*)-3-[(1-methylcyclopropyl) methyl]-2-oxo-5,6-diphenylmorpholine-4-carboxylate (5)

To a stirred mixture of ether (8 mL) and 25% aqueous KOH (2.15 mL) at 0 °C was added MNNG (637 mg, 4.33 mmol) in small portions. After 5 min, the aqueous layer was removed via pipette, and the yellow organic layer was added to a solution of *tert*-butyl (3*R*,5*R*,6*S*)-3-(2-methylprop-2-enyl)-2-oxo-5,6-diphenylmorpholine-4-carboxylate **4** (785 mg, 1.93 mmol) in THF (10 mL) at 0 °C under Ar. To this stirred solution was added a very small portion of $Pd(OAc)_2$, resulting in mild gas evolution. After 10 min, a second portion of $Pd(OAc)_2$ was added. After 20 min, the reaction mixture was filtered through a pad of SiO_2 eluting with THF and the solvents removed at reduced pressure to give a white solid. NMR analysis indicated that this material contained approximately 50% of **4**. This material was treated with double the amount of diazomethane solution prepared as described above. The reaction mixture was allowed to stir overnight under Ar and was then quenched by the addition of a few drops of HOAc, then filtered as above to give 900 mg of an almost colorless solid that was chromatographed on a 35 g RediSep® column (loaded in $CHCl_3$, eluted with 0–50% EtOAc–hexane). The pure fractions were combined to give *tert*-butyl-(3*R*,5*R*,6*S*)-3-[(1-methylcyclopropyl)methyl]-2-oxo-5,6-diphenylmorpholine-4- carboxylate **5** as a colorless solid (701 mg, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) mixture of rotamers: δ 7.35–7.00 (m, 8H), 6.52 (t, J = 6.3 Hz, 2H), 6.35–6.28 (m, 1H), 5.18–4.85 (m, 2H), 2.5–1.95 (m, 2H), 1.40 (s, 4H), 1.22 (s, 3H), 1.01 (s, 9H).

6.4. *N*-(*tert*-Butoxycarbonyl)-3-(1-methylcyclopropyl)-D-alanine (6)

To a stirred solution containing ethanol (0.96 mL, 16.6 mmol), liquid ammonia (~40 mL), dry THF (20 mL), and *tert*-butyl (3*R*,5*R*,6*S*)-3-[(1-methylcyclopropyl) methyl]-2-oxo-5,6-diphenylmorpholine-4-carboxylate **5** (701 mg, 1.66 mmol) cooled to −78 °C was added lithium wire (230 mg, 33.3 mg-atom) in small pieces until a deep blue color persisted for 10 min. The reaction was quenched by careful addition of 20% aqueous NH_4Cl, the cold bath removed, and the ammonia allowed to evaporate overnight. The reaction mixture was diluted with water and extracted with three portions of ether. The aqueous phase was cooled in ice, acidified with 2 M HCl, and immediately extracted with three portions of EtOAc. The combined EtOAc layers were washed with brine, dried over Na_2SO_4, and concentrated at reduced pressure to give *N*-(*tert*-butoxycarbonyl)-3-(1-methylcyclopropyl)-D-alanine **6** as a colorless solid (347 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.99 (br d, J = 6.1 Hz, 1H), 4.45–4.35 (m, 1H), 1.84 (dd, J = 5.6, 14.2 Hz, 1H), 1.55 (dd, J = 8.5, 14.2 Hz, 1H), 1.46 (s, 9H), 1.11 (s, 3H), 0.40–0.25 (m, 4H).

6.5. 1-Chlorocyclopropanecarbonitrile (9)

MNNG (18.39 g, 125 mmol) was added in portions to a stirred two phase mixture of ether (180 mL) and 40% potassium hydroxide solution (55 mL) at 0 °C. After 15 min, the ether layer was decanted into a stirred solution of 2-chloroacrylonitrile (**7**) (7.98 mL, 100 mmol) in ether (300 mL) at 0 °C. After 15 min, acetic acid (1.50 mL) was added dropwise and the solution was dried over potassium hydroxide pellets, filtered, and evaporated in vacuo (200 mm Hg) to give 3-chloro-4,5-dihydro-3*H*-pyrazole-3-carbonitrile **8** as a clear oil. A solution of **8** in benzene (100 mL) was heated at reflux for 16 h and was then distilled, collecting the fraction boiling at 92 °C (200 mm Hg) to give 1-chlorocyclopropanecarbonitrile **9** (3.14 g, 31%) containing 22% by mass of 2-chlorobut-2-enenitrile (as a 20:1 mixture of isomers) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (m, 2H), 1.49 (m, 2H).

6.6. Methyl (2*E*)-3-(1-chlorocyclopropyl)prop-2-enoate (10)

DIBAL (33 mL of a 1 M solution in toluene) was added to a stirred solution of 1-chlorocyclopropanecarbonitrile

9 (3.02 g, 29.7 mmol, containing 22% by mass of 2-chlorobut-2-enenitrile) in toluene (30 mL) at −78 °C under nitrogen. The solution was warmed to 0 °C and after 30 min was quenched with 1 M hydrochloric acid (50 mL). Concentrated hydrochloric acid (6 mL) was added to give a cloudy mixture and sodium chloride was added to saturate the aqueous layer. The layers were separated and the organic layer was dried (Na_2SO_4) and filtered, washing the solids with a minimal volume of toluene. (Carbomethoxymethylene)triphenylphosphorane (9.94 g, 29.7 mmol) was added to the filtrate and the solution was stirred for 1 h. The volatiles were removed at reduced pressure (12 mm Hg, rt) and hexanes were added to the residue. The solids were removed by filtration and the filtrate was evaporated in vacuo. The residue was purified by flash column chromatography on silica (1:1 methylene chloride/hexanes) to give methyl (2*E*)-3-(1-chlorocyclopropyl)prop-2-enoate **10** (3.58 g, 75%) containing 11% by mass of methyl 4-chlorohexa-2,4-dienoate (as a mixture of isomers) as a crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.53 (d, J = 14.9 Hz, 1H), 6.17 (d, J = 14.9 Hz, 1H), 3.75 (s, 3H), 1.51 (m, 2H), 1.27 (m, 2H).

6.7. 3-(1-Chlorocyclopropyl)propanoic acid (11)

A suspension of 10% palladium on carbon (180 mg) in a solution of methyl (2*E*)-3-(1-chlorocyclopropyl)prop-2-enoate **10** (3.50 g, 21.8 mmol, containing 11% by mass of methyl 4-chlorohexa-2,4-dienoate) in methanol (60 mL) was stirred under an atmosphere of hydrogen (balloon). After 16 h, the mixture was filtered through Celite washing with ethyl acetate, and evaporated in vacuo. Lithium hydroxide (1.03 g, 24.5 mmol) was added to a solution of the residual oil in 1:1:1 THF/methanol/water (60 mL). After 30 min, the volatiles were evaporated in vacuo and the remaining solution was washed with ether. The aqueous solution was acidified with 1 M hydrochloric acid and was extracted with ether. The extract was dried (Na_2SO_4) and evaporated in vacuo to give 3-(1-chlorocyclopropyl)propanoic acid **11** (1.66 g, 50%) containing 20% by mass of hexanoic acid, as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.69 (t, J = 7.8 Hz, 2H), 1.99 (t, J = 7.8 Hz, 2H), 1.08 (m, 2H), 0.81 (m, 2H).

6.8. (4*R*)-4-Benzyl-3-[3-(1-chlorocyclopropyl)propanoyl]-1,3-oxazolidin-2-one (12)

Triethylamine (3.89 mL, 27.9 mmol) was added to a stirred solution of 3-(1-chlorocyclopropyl)propanoic acid **11** (1.66 g, 11.2 mmol, 20% by mass of hexanoic acid) in THF (130 mL) and the solution was cooled to −20 °C (carbon tetrachloride/dry ice bath). Pivaloyl chloride (1.38 mL, 11.2 mmol) was added to give a precipitate. After 2 h, lithium chloride (0.525 g, 12.3 mmol) and (4*R*)-4-benzyl-1,3-oxazolidin-2-one (1.98 g, 11.2 mmol) were added and the mixture was warmed to room temperature. After 16 h, the mixture was filtered through a glass frit and the filtrate was evaporated in vacuo. The residual oil was partitioned between ethyl acetate and dilute hydrochloric acid. The organic layer was washed with water, sodium hydrogen carbonate solution, and brine, dried (Na_2SO_4), and evaporated to an oil. The crude product was purified by flash column chromatography on silica (ethyl acetate/hexanes gradient, 20–30% ethyl acetate) to give (4*R*)-4-benzyl-3-[3-(1-chlorocyclopropyl)propanoyl]-1,3-oxazolidin-2-one **12** (1.36 g, 40%) as a heavy oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–7.36 (m, 5H), 4.68 (m, 1H), 4.20 (m, 2H), 3.33–3.21 (m, 3H), 2.77 (dd, J = 9.7, 13.4 Hz, 1H), 2.07 (t, J = 7.7 Hz, 2H), 1.09 (m, 2H), 0.85 (m, 2H).

6.9. (2*R*)-2-Azido-3-(1-chlorocyclopropyl)propanoic acid (13)

A solution of (4*R*)-4-benzyl-3-[3-(1-chlorocyclopropyl)-propanoyl]-1,3-oxazolidin-2-one **12** (1.01 g, 3.29 mmol) in THF (10 mL) was added to a stirred solution of potassium bis(trimethylsilyl)amide (7.25 mL of a 0.5 M solution in toluene, 3.62 mmol) in THF (10 mL) at −78 °C under nitrogen. After 30 min, a solution of 2,4,6-triisopropylbenzenesulfonyl azide (1.27 g, 4.11 mmol) in THF (10 mL) was added and followed after 1 min by acetic acid (0.866 mL, 15.1 mmol). The resulting mixture was warmed to 30 °C and after 1 h was diluted with ethyl acetate and washed with brine which was basified with sodium hydrogen carbonate. The organic layer was dried (Na_2SO_4) and evaporated to an oil. Lithium hydroxide monohydrate (276 mg, 6.58 mmol) was added to a stirred solution of this crude material in 2:2:1 THF/methanol/water (25 mL) at 0 °C and the reaction was warmed to room temperature. After 16 h, excess saturated sodium hydrogen carbonate solution was added and the volatiles were evaporated in vacuo. Water was added to the mixture to dissolve the salts and the solution was washed with methylene chloride (4 times). The aqueous solution was acidified with concentrated hydrochloric acid and extracted with ether. The ether extract was dried (Na_2SO_4) and evaporated in vacuo to an oil which was purified by flash column chromatography on silica (1% acetic acid/hexanes/ethyl acetate gradient, 9–29% ethyl acetate) to give (2*R*)-2-azido-3-(1-chlorocyclopropyl)propanoic acid **13** (190 mg, 30%) containing 20% by mass of 3-(1-chlorocyclopropyl)propanoic acid, as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (br s, 1H), 4.43 (dd, J = 4.4, 9.5 Hz, 1H), 2.55 (dd, J = 4.2, 14.8 Hz, 1H), 1.76 (dd, J = 9.5, 14.8 Hz, 1H), 1.24–0.80 (m, 4H).

6.10. 3-Methylbut-2-enyl *N*-(*tert*-butoxycarbonyl) glycinate (17)

DCC (1 M in CH_2Cl_2, 26.2 mL, 26.2 mmol) was added to a stirred solution of Boc-glycine (4.24 g, 24.2 mmol), 3-methyl-2-buten-1-ol (2.15 g, 25.0 mmol), and DMAP (152 mg, 1.25 mmol) in anhydrous CH_2Cl_2 (155 mL) at 0 °C. The mixture was then warmed to room temperature while stirring overnight. The mixture was filtered twice to remove the precipitated dicyclohexylurea, washing the solids well with CH_2Cl_2. The filtrate was concentrated to a slightly colored oil. Silica gel chromatography (15% EtOAc–hexanes) afforded 3-methylbut-2-enyl *N*-(*tert*-butoxycarbonyl) glycinate **17** as a colorless oil (5.52 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (t, J = 7.2 Hz, 1H), 5.00 (br s, 1H), 4.65 (d, J = 7.2 Hz, 2H), 3.91 (d, J = 5.2 Hz, 2H), 1.76 (s, 3H), 1.72 (s, 3H), 1.45 (s, 9H).

6.11. 2-[(*tert*-Butoxycarbonyl)amino]-3,3-dimethyl pent-4-enoic acid (18)

LDA was prepared by the dropwise addition of BuLi (2.5 M in hexanes, 7.73 mL, 19.3 mmol) to a solution of anhydrous diisopropylamine (2.8 mL, 20.3 mmol) in anhydrous THF (65 mL) at 0 °C under nitrogen. The solution was stirred for 15 min at 0 °C and was then cooled to −78 °C. A solution of 3-methylbut-2-enyl *N*-(*tert*-butoxycarbonyl)glycinate **17** (2.24 g, 9.2 mmol) in anhydrous THF (5 mL) was added dropwise over 2 min. The mixture was stirred for 15 min at −78 °C and then treated with TMSCl (2.5 mL, 19.3 mmol). The mixture was then stirred at −78 °C for 5 min, then at room temperature for 30 min, then at 60 °C for 1 h. TLC after 1 h (50% EtOAc/hexanes, $KMnO_4$ stain) showed that most of the starting material had been consumed. After 1 h and 45 min, the reaction mixture was cooled to room temperature and quenched with MeOH (5 mL). The THF was removed in vacuo and the aqueous residue was extracted once with ether to remove any remaining starting material. The aqueous layer was cooled to 0 °C and adjusted to pH 3 by the dropwise addition of 1 N HCl. The mixture was saturated with solid NaCl and extracted immediately with EtOAc three times. The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo to give 2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylpent-4-enoic acid **18** as a beige, oily solid, which was further dried overnight under high vacuum (420 mg, 19%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.90–5.84 (m, 1H), 5.14–4.92 (m, 2H), 4.12 (br s, 1H), 1.42 (s, 9H), 1.10 (s, 6H).

6.12. Methyl *N*-(*tert*-butoxycarbonyl)-3-cyclopropyl valinate (19)

To a mixture of 5 N NaOH solution (24 mL) and Et_2O (30 mL) at 0 °C was added MNNG (2.29 g, 15.6 mmol), portionwise over 15 min. The mixture was stirred vigorously for 30 min and was then allowed to partition into two layers. The organic layer was transferred by pipet to a solution of 2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylpent-4-enoic acid **18** (316 mg, 1.30 mmol) in Et_2O (30 mL) at 0 °C. Immediately following transfer, a catalytic amount of $Pd(OAc)_2$ was added. The reaction mixture was stirred at 25 °C for 18 h. Acetic acid was added dropwise until the yellow reaction color diminished and bubbling ceased, and the solvent was then removed in vacuo. Purification by silica gel chromatography (hexanes to 20% EtOAc/hexanes) afforded methyl *N*-(*tert*-butoxycarbonyl)-3-cyclopropylvalinate **19** as a pale yellow oil (264 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.25–5.21 (br m, 1H), 4.21 (d, J = 9.6 Hz, 1H), 3.73 (s, 3H), 1.44 (s, 9H), 0.85 (s, 3H), 0.81–0.77 (m, 1H), 0.75 (s, 3H), 0.34–0.32 (m, 2H), 0.21–0.19 (m, 2H). MS (ESI): 272.2 $(M+H)^+$.

6.13. *N*-(*tert*-Butoxycarbonyl)-3-cyclopropyl-DL-valine (20)

To a stirred solution of methyl *N*-(*tert*-butoxycarbonyl)-3-cyclopropylvalinate **19** (248 mg, 0.91 mmol) in CH_3CN (15 mL) was added $LiOH{\cdot}H_2O$ (77 mg, 1.82 mmol, 2.0 equiv) in water (5 mL). The reaction mixture was stirred at 25 °C for 3 h then was warmed to 70 °C for 2 h. Acetonitrile was removed in vacuo, and the remaining aqueous solution was cooled to 0 °C and acidified to pH 3 by dropwise addition of 1 N HCl solution. Following saturation with NaCl, the solution was extracted into EtOAc three times. The organic layers were combined, dried over Na_2SO_4, and concentrated in vacuo to afford *N*-(*tert*-butoxycarbonyl)-3-cyclopropyl-DL-valine **20** as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.41 and 5.64 (rotamers, s, 1H), 5.23 (d, J = 8.8 Hz, 1H), 4.19 (d, J = 9.2 Hz, 1H), 1.45 (s, 9H), 0.91 (s, 3H), 0.88–0.84 (m, 1H), 0.82 (s, 3H), 0.36–0.34 (m, 2H), 0.26–0.23 (m, 2H). MS (ESI): 258.1 $(M+H)^+$.

6.14. Methyl (2*E*)-[(benzyloxy)imino]acetate (22)

Sodium acetate (32.8 g, 400 mmol) was added to a stirred solution of glyoxylic acid hydrate **21** (14.8 g, 200 mmol) and *O*-benzylhydroxylamine hydrochloride (31.9 g, 200 mmol) in 1:1 MeOH/water (300 mL). The mixture was stirred at room temperature overnight. The methanol was then removed in vacuo and the residue partitioned between EtOAc and water. The layers were separated and the aqueous layer was saturated with NaCl and exhaustively extracted with EtOAc. The combined organic extracts were dried (anhydrous $MgSO_4$), filtered, and concentrated in vacuo to afford (2*E*)-[(benzyloxy)imino]acetic acid as an oily colorless solid (32.2 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (s, 1H), 7.39–7.35 (m, 5H), 5.32 (s, 2H). DCC (6.33 g, 30.7 mmol), was added to a stirred mixture of the crude (2*E*)-[(benzyloxy)imino]acetic acid (5.00 g, 27.9 mmol), anhydrous MeOH (1.24 mL, 30.7 mmol) and DMAP (170 mg, 1.40 mmol) in anhydrous CH_2Cl_2 (180 mL) at 0 °C. The mixture was stirred overnight while warming to room temperature. The precipitated dicyclohexyl urea was removed by filtration and the filtrate was concentrated in vacuo. Purification of the residual oil by silica gel chromatography (20% EtOAc–hexanes) afforded methyl (2*E*)-[(benzyloxy)imino] acetate **22** as a clear, colorless oil (4.78 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (s, 1H), 7.38–7.33 (m, 5H), 5.30 (s, 2H), 3.85 (s, 3H).

6.15. Methyl 2-[(benzyloxy)amino]-3,3-dimethylpent-4-enoate (23)

Zinc dust (3.03 g, 46.6 mmol, 1.8 equiv) was added slowly in portions over 10 min to a stirred suspension of methyl (2*E*)-[(benzyloxy)imino]acetate **22** (4.98 g, 25.6 mmol, 1 equiv) and prenyl bromide (5.38 g, 4.16 mL, 36.1 mmol, 1.4 equiv) in THF (29 mL) and saturated aqueous ammonium chloride (131 mL) at room temperature. Upon complete addition of zinc, the mixture was stirred vigorously for 30 min and was then extracted twice with ether. The combined organic extracts were washed with water and brine, dried (anhydrous Na_2SO_4), filtered, and concentrated in vacuo. Further drying under high vacuum afforded methyl 2-[(benzyloxy)amino]-3,3-dimethylpent-4-enoate

23 as a clear, yellow oil (6.28 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.28 (m, 5H), 5.99 (d, J = 12.8 Hz, 1H), 5.77 (dd, J = 10.8, 17.2 Hz, 1H), 5.01–4.94 (m, 2H), 4.64 (s, 2H), 3.72 (s, 3H), 3.39 (d, J = 11.6 Hz, 1H), 1.03 (s, 3H), 1.01 (s, 3H).

6.16. Methyl 2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylpent-4-enoate (24)

Molybdenum hexacarbonyl (4.40 g, 16.7 mmol) was added to a stirred solution of methyl 2-[(benzyloxy)amino]-3,3-dimethylpent-4-enoate **23** (6.27 g, 23.8 mmol) in acetonitrile (355 mL) and water (23.5 mL). The mixture was heated to reflux for 2.5 h. The dark mixture was cooled to room temperature and then immersed in an ice bath. di-*tert*-butyl dicarbonate (1 M in THF, 28.6 mL, 28.6 mmol) was then added dropwise and the mixture was stirred overnight while warming to room temperature. The solvent was removed in vacuo and the resulting black tar was purified by silica gel chromatography (20% EtOAc–hexanes). The product fractions were combined and concentrated to afford methyl 2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylpent-4-enoate **24** as a pale yellow oil (3.49 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86–5.79 (m, 1H), 5.11–5.00 (m, 3H), 4.15 (d, J = 9.6 Hz, 1H), 3.71 (s, 3H), 1.43 (s, 9H), 1.09 and 1.08 (overlapping s, 6H).

6.17. Methyl *N*-(*tert*-butoxycarbonyl)-3-cyclopropylvalinate (19)

A biphasic mixture of 5 N aqueous sodium hydroxide (70 mL) and diethyl ether (90 mL) cooled to 0 °C was treated portionwise with MNNG (7.09 g, 48.2 mmol). The mixture was stirred for 30 min and was then allowed to partition into two layers. The ether layer was transferred via pipet to a stirred solution of methyl 2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylpent-4-enoate **24** (1.55 g, 6.02 mmol) in diethyl ether (90 mL) at 0 °C. After 5 min, the stirred mixture was treated with a catalytic amount of palladium (II) acetate (added spatula tip at a time until faint bubbling was observed) and the mixture stirred overnight at room temperature. The reaction was then quenched by dropwise addition of glacial acetic acid until bubbling ceased and the mixture was concentrated in vacuo. The residue was purified by silica gel chromatography (gradient elution 100% hexanes to 20% EtOAc–hexanes) to afford methyl *N*-(*tert*-butoxycarbonyl)-3-cyclopropylvalinate **19** as a yellow oil (879 mg, 54% yield). The ^{1}H NMR spectrum of this material is identical to that reported in **6.12** above.

6.18. *N*-(*tert*-Butoxycarbonyl)-3-cyclopropyl-DL-valine (20)

Methyl *N*-(*tert*-butoxycarbonyl)-3-cyclopropylvalinate **19** was hydrolyzed as described in **6.13** above to afford *N*-(*tert*-butoxycarbonyl)-3-cyclopropyl-DL-valine **20**. Spectral data are identical to those of the material prepared via the route described in Sections **6.10** through **6.13** above.

6.19. *N*-[5-Chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl] prolinamide (31)

A mixture of 1-(*tert*-butoxycarbonyl)proline **25** (1.03 g, 4.79 mmol, 1 equiv), 5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzylamine **29**[2] (998 mg, 4.79 mmol), EDC (1.38 g, 7.18 mmol, 1.5 equiv), and HOAT (326 mg, 2.39 mmol, 0.5 equiv) in DMF (5 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with water and extracted with EtOAc (×3). The combined organic extracts were dried (anhydrous Na_2SO_4) and concentrated in vacuo. The residue was purified by normal-phase medium pressure chromatography (gradient elution with 20–80% EtOAc–hexanes) to afford *N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-[(1-*tert*-butoxy)carbonyl] prolinamide (1.66 g, 85% yield). HCl gas was bubbled through a cold (0 °C) solution of this material (1.66 g, 4.07 mmol) in EtOAc (45 mL; a few drops of MeOH were added to aid dissolution) for 5 min. The mixture was then stirred at room temperature for 1 h. The solvent was removed in vacuo to afford the bis-hydrochloride salt of *N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]prolinamide **31** as an off-white, hygroscopic powder (1.66 g; 97% yield based on CHN analysis below). ^{1}H NMR (400 MHz, CD_3OD): δ 9.79 (s, 1H), 8.87 (m, 1H), 8.83 (s, 1H), 7.70 (d, J = 1.6 Hz, 1H), 7.61–7.60 (m, 2H), 4.44–4.41 (m, 2H), 4.30 (app t, J = 7.6 Hz, 1H), 3.39–3.31 (m, 2H), 2.46–2.40 (m, 1H), 2.18–1.92 (m, 3H). MS (ESI): 306.1 $(M+H)^+$. Elemental analysis: calcd for ($C_{14}H_{16}ClN_5O$ + 2.60 equiv HCl + 0.40 H_2O + 0.15 equiv Et_2O): C, 41.86%; H, 5.03%; N, 16.72%; found: C, 41.86%; H, 5.04%; N, 16.82%.

6.20. *N*-[5-Chloro-2-(1*H*-tetrazol-1-yl)benzyl] prolinamide (32)

A mixture of 1-(*tert*-butoxycarbonyl)proline **25** (513 mg, 2.4 mmol), 5-chloro-2-(1*H*-tetrazol-1-yl)benzylamine **30**[2] (500 mg, 2.4 mmol), EDC (686 mg, 3.6 mmol), and HOAT (162 mg, 1.2 mmol) in DMF (12 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with water and saturated aqueous potassium carbonate solution and was then extracted into EtOAc. The organic layer was washed with brine, and the combined aqueous washes were extracted once with CH_2Cl_2. The combined organic layers were dried (anhydrous Na_2SO_4) and concentrated. Silica gel chromatography (50–80% EtOAc–hexanes) afforded a yellow foam (935 mg, 96% yield). This material was then dissolved in EtOAc (46 mL) and gaseous HCl was bubbled through the solution for 10 min. The solvent was removed in vacuo and the resulting residue triturated with hexanes to afford the bis-hydrochloride salt of *N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]prolinamide **32** as a hygroscopic pale yellow solid (622 mg, 71% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 9.59 (s, 1H), 8.83 (br s, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.59 (dd, J = 2.0, 8.4 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 4.32 (m, 2H), 4.27–4.24 (m, 1H), 3.40–3.32 (m, 2H), 2.44–2.36 (m, 1H), 2.09–1.89 (m, 3H). MS (ESI): 307.1 $(M+H)^+$.

6.21. (4*R*)-*N*-[5-Chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide (33)

A mixture of (4*R*)-1-(*tert*-butoxycarbonyl)-4-fluoroproline **26**[4] (1.12 g, 4.79 mmol, 1 equiv), 5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzylamine **29**[2] (1.00, 4.79 mmol, 1 equiv), EDC (1.38 g, 7.19 mmol, 1.5 equiv), and HOAT (326 mg, 2.40 mmol, 0.5 equiv) in anhydrous DMF (14 mL) was stirred at room temperature for 2.5 h. Water and solid potassium carbonate were added and the mixture was extracted with EtOAc (×3). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give a yellow oil. Purification by silica gel chromatography (EtOAc eluent) afforded Boc-(4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide as a white foam (1.97 g, 97% yield). A stirred solution of this material (1.96 g, 4.62 mmol) in EtOAc (51 mL) was treated with HCl–dioxane solution (4 M, 21 mL, excess) at room temperature for 1 h, at which point LCMS indicated 97% conversion. Additional HCl–dioxane (4 M, 6 mL, 5 equiv) was added to drive the reaction to completion. After 30 min, the mixture was concentrated to dryness. Repeated trituration with ether afforded the bis-hydrochloride salt of (4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide **33** as a hygroscopic white solid (2.0 g, quantitative yield). ^{1}H NMR (400 MHz, CD_3OD): δ 9.37 (s, 1H), 8.84 (m, 1H), 8.57 (s, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.58–7.52 (m, 2H), 5.47 (app dt, J = 3.2, 51.6 Hz, 1H), 4.52–4.45 (m, 1H), 4.42–4.37 (m, 2H), 3.71–3.57 (m, 2H), 2.80–2.69 (m, 1H), 2.26–2.09 (m, 1H). HRMS (ESI): calcd for $(C_{14}H_{16}ClFN_5O)^+$, 324.1022; found: 324.1040.

6.22. (4*S*)-*N*-[5-Chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide (34)

A mixture of (4*S*)-1-(*tert*-butoxycarbonyl)-4-fluoro-L-proline **27**[4] (995 mg, 4.27 mmol), 5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzylamine **29**[2] (890 mg, 4.27 mmol), EDC (1.23 g, 6.40 mmol), and HOAT (290 mg, 2.13 mmol) in DMF (3 mL) was brought to pH 8 by dropwise addition of Hünig's base and stirred at room temperature for 18 h. The solvent was removed in vacuo. Purification by reverse-phase chromatography [95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 5:95 water (+0.1% TFA)/CH_3CN (+0.1% TFA) over 30 min] afforded a solid. The solid was dissolved in EtOAc, and the solution was washed with saturated aqueous K_2CO_3 solution (×3). The organic layer was then washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to afford Boc-(4*S*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide. This material was dissolved in CH_2Cl_2 (10 mL), and the solution was saturated with hydrogen chloride gas. After stirring for 2 h, the solvent was removed in vacuo to afford the bis-hydrochloride salt of (4*S*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide **34** as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 9.64 (s, 1H), 8.74 (s, 1H), 7.66–7.65 (m, 1H), 7.57–7.56 (m, 2H), 5.42 (br d, J = 51.2 Hz, 1H), 4.89–4.38 (m, 3H), 3.78–3.70 (m, 1H), 3.62–3.50 (m, 1H), 2.80–2.65 (m, 1H), 2.51–2.45 (m, 1H). HRMS (ESI): calcd for $(C_{14}H_{16}ClFN_5O)^+$, 324.1022; found: 324.1030.

6.23. *N*-[5-Chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4,4-difluoroprolinamide (35)

A mixture of 1-(*tert*-butoxycarbonyl)-4,4-difluoro proline **28**[4] (1.00 g, 3.98 mmol), 5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzylamine **29**[2] (831 mg, 3.98 mmol), EDC (1.14 g, 5.97 mmol), and HOAT (271 mg, 1.99 mmol) in DMF (3 mL) was brought to pH 8 by dropwise addition of Hünig's base and stirred at room temperature for 18 h. The solvent was removed in vacuo. Purification by reverse-phase chromatography [95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 5:95 water (+0.1% TFA)/CH_3CN (+0.1% TFA) over 30 min] afforded a solid. The solid was dissolved in EtOAc, and the solution was washed with saturated aqueous K_2CO_3 solution (×3). The organic layer was then washed with water and brine, dried (anhydrous Na_2SO_4), and concentrated in vacuo to afford Boc-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4,4-difluoroprolinamide. This material was dissolved in CH_2Cl_2 (10 mL), and the solution was saturated with hydrogen chloride gas. After stirring for 2 h, the solvent was removed in vacuo to afford the bis-hydrochloride salt of *N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4,4-difluoroprolinamide **35** as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 9.48 (s, 1H), 8.64 (s, 1H), 7.68–7.67 (m, 1H), 7.58–7.55 (m, 2H), 4.67–4.62 (m, 1H), 4.45–4.42 (m, 2H), 3.86–3.79 (m, 2H), 3.02–2.95 (m, 1H), 2.63–2.57 (m, 1H). HRMS (ESI): calcd for $(C_{14}H_{15}ClF_2N_5O)^+$, 342.0928; found: 342.0937.

6.24. *N*-[5-Chloro-2-(1*H*-tetrazol-1-yl)benzyl]-4,4-difluoroprolinamide (36)

A mixture of 1-(*tert*-butoxycarbonyl)-4,4-difluoroproline **28**[4] (200 mg, 0.80 mmol), 1-[5-chloro-2-(1*H*-tetrazol-1-yl)phenyl]methanamine **30**[2] (179 mg, 0.85 mmol), EDC (229 mg, 1.19 mmol), and HOAT (54 mg, 0.40 mmol) in DMF (4.4 mL) was stirred at room temperature for 2 h. The DMF was removed in vacuo and the residue was partitioned between EtOAc and saturated aqueous K_2CO_3. The layers were separated and the organic layer was washed with brine. The combined aqueous layers were extracted once with CH_2Cl_2 and the combined organic extract was then dried (anhydrous Na_2SO_4) and concentrated to an orange oil. Silica gel chromatography (60% EtOAc–hexanes) afforded 1-(*tert*-butoxycarbonyl)-*N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]-4,4-difluoroprolinamide as a white foam (309 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (s, 1H), 7.65 (br s, 1H), 7.48–7.26 (m, 3H), 4.45 (br m, 1H), 4.32–4.20 (m, 2H), 3.88–3.69 (m, 2H), 2.90 (br m, 1H), 2.55 (br m, 1H), 1.54 (br s, 9H). MS (ESI): 443.1 $(M+H)^+$.

A solution of 1-(*tert*-butoxycarbonyl)-*N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]-4,4-difluoroprolinamide (460 mg, 1.04 mmol) in EtOAc (5.8 mL) at room temperature was treated with a 4 M solution of HCl in dioxane (1 mL, excess). After 1.5 h, additional 4 M HCl–dioxane

(1 mL) was added and the mixture stirred at room temperature overnight. The solvent was removed and the residue dried in vacuo to afford the bis-hydrochloride salt of *N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]-4,4-difluoroprolinamide **36** as a yellow gum (425 mg, 98%). ^{1}H NMR (400 MHz, CD_3OD): δ 9.56 (s, 1H), 8.85 (s, 1H), 7.70 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 2.3, 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 4.57 (app t, J = 8.7 Hz, 1H), 4.34 (br s, 2H), 3.88–3.74 (m, 2H), 3.14–2.88 (m, 1H), 2.65–2.51 (m, 1H). MS (ESI): 343.0 $(M+H)^+$.

6.25. General EDC coupling and deprotection procedure for the preparation of compounds 37–44 and 46

The following is a representative procedure for the coupling of compounds **31**–**36** with the Boc-protected P3 amino acids and subsequent deprotection to afford compounds **37**–**44** and **46** unless otherwise noted: A 0.2 M solution of the Boc-protected P3 amino acid (1 equiv) in DMF was treated with one of prolinamides **31**–**36** (1 equiv, compounds **31**–**36** used as their bis-hydrochloride salts unless otherwise noted), EDC (1.5 equiv), and HOAT (0.5 equiv). The pH of the mixture was adjusted to 6–8 by addition of Hünig's base and the reaction mixture was then stirred at room temperature for 18 h. The solvent was removed in vacuo and the resulting residue was dissolved in 2:1 CH_2Cl_2/TFA (0.08 M based on starting acid) and stirred for 1 h. The solvent was again removed in vacuo and purification by reverse-phase chromatography [gradient elution on a YMC Pro C18 150 × 20 mm, 5 μm column with 95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 50:50 water (+0.1% TFA)/CH_3CN (+0.1% TFA) unless otherwise noted] followed by concentration of product fractions and trituration of the resulting residues with ether afforded the title compounds as the trifluoroacetate salts. Alternatively, the crude coupling product could be purified by reverse-phase chromatography then deprotected by stirring with HCl–dioxane solution (4 M, 10 equiv) at room temperature for 1 h. The reaction progress was monitored by LCMS and additional HCl–dioxane was added as needed to drive the reaction to completion. Upon complete conversion the solvent was removed in vacuo and the residue stripped twice from methanol then triturated with ether to afford the hydrochloride salts of the title compounds.

6.26. 4-Methyl-D-leucyl-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]prolinamide (37)

Compound **37** was prepared from Boc-*tert*-butyl-D-alanine (32 mg, 0.13 mmol, 1 equiv) and *N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]prolinamide **31** (50 mg, 0.13 mmol, 1 equiv) according to general procedure **6.25** and was isolated as the trifluoroacetate salt (55 mg, 77% yield). LCMS t_R = 1.12 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.35 (br s, 2H), 8.23 (s, 1H), 8.09 (t, J = 5.6 Hz, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.35 (dd, J = 2.0, 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 4.41–4.38 (m, 1H), 4.29 (dd, J = 5.6, 15.6 Hz, 1H), 4.20–4.12 (m, 2H), 3.87–3.83 (m, 1H), 3.50–3.45 (m, 1H), 2.12–2.02 (m, 3H), 1.85–1.72 (m, 3H), 0.96 (s, 9H). HRMS (APCI): calcd for $(C_{21}H_{29}ClN_6O_2)^+$, 433.2113; found: 433.2104.

6.27. 4-Methyl-D-leucyl-*N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]prolinamide (38)

Compound **38** was prepared from Boc-*tert*-butyl-D-alanine (32 mg, 0.13 mmol, 1 equiv) and *N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]prolinamide **32** (50 mg, 0.13 mmol, 1 equiv) according to general procedure **6.25** and was isolated as the trifluoroacetate salt (56 mg, 77% yield). LCMS t_R = 1.15 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.18 (s, 1H), 8.45 (br s, 2H), 8.00 (t, J = 5.2 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.41 (dd, J = 2.0, 8.4 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 4.41–4.34 (m, 2H), 4.25 (dd, J = 4.8, 14.8 Hz, 1H), 4.00–3.94 (m, 1H), 3.89–3.85 (m, 1H), 3.53–3.46 (m, 1H), 2.25–2.22 (m, 1H), 2.09–1.85 (m, 5H), 1.00 (s, 9H). HRMS (APCI): calcd for $(C_{20}H_{28}N_7O_2Cl)^+$, 434.2066; found: 434.2065. Elemental analysis: calculated for ($C_{20}H_{28}N_7O_2Cl$ + 1.15 equiv TFA + 0.70 equiv H_2O + 0.10 equiv Et_2O): C, 46.60%; H, 5.44%; N, 16.76%; found: C, 46.59%; H, 5.35%; N, 16.76%.

6.28. 3-(1-Methylcyclopropyl)-D-alanyl-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]prolinamide (39)

Compound **39** was prepared from Boc-3-(1-methylcyclopropyl)-D-alanine **6** (75 mg, 0.31 mmol) and *N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl] prolinamide **31** (94 mg of the free base, 0.31 mmol) according to general procedure **6.25** with the following changes: the intermediate coupling product was purified by normal-phase silica gel chromatography on an ISCO Combiflash system (10 g RediSep® column eluting with 93:7 $CHCl_3$/saturated NH_3–MeOH). This purified product (124 mg, 0.23 mmol, 74% yield from the coupling step) was then deprotected and purified as described in procedure **6.25** and was isolated as the trifluoroacetate salt (white solid, 55 mg, 43% yield). LCMS t_R = 0.95 min. ^{1}H NMR (400 MHz, CD_3OD): δ 8.80, (s, 1H), 8.22 (s, 1H), 7.67 (d, J = 2.4 Hz, 1H), 7.50 (dd, J = 2.4, 8.4 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 4.39 (d, J = 15.6 Hz, 1H), 4.36–4.30 (m, 2H), 4.22 (d, J = 16.0 Hz, 1H), 3.83–3.78 (m, 1H), 3.68–3.62 (m, 1H), 2.28–2.19 (m, 1H), 2.14–1.88 (m, 4H), 1.48 (dd, J = 8.2, 14.6 Hz, 1H), 1.16 (s, 3H), 0.49–0.28 (m, 4H). HRMS (ESI): calcd for $(C_{21}H_{28}ClN_6O_2)^+$, 431.1957; found: 431.2004. Elemental analysis: calcd for ($C_{21}H_7ClN_6O_2$ + 1.70 equiv TFA): C, 46.91%; H, 4.63%; N, 13.45%; found: C, 46.74%; H, 4.96%; N, 13.68%.

6.29. 3-(1-Methylcyclopropyl)-D-alanyl-*N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]prolinamide (40)

Compound **40** was prepared from Boc-3-(1-methylcyclopropyl)-D-alanine **6** (75 mg, 0.31 mmol) and *N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]-L-prolinamide **32** (117 mg, 0.31 mmol) according to general procedure **6.25** (coupling step allowed to proceed for two days at room temperature) and was isolated as the bis hydrochloride salt (83 mg, 53%). LCMS t_R = 1.10 min. ^{1}H NMR (500 MHz, CD_3OD): δ 9.54 (s, 1H), 7.73 (d,

J = 2.5 Hz, 1H), 7.57 (dd, J = 2.0, 8.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 4.33–4.30 (m, 3H), 4.20 (d, J = 15.5 Hz, 1H), 3.83–3.78 (m, 1H), 3.66–3.61 (m, 1H), 2.25–2.18 (m, 1H), 2.11–1.99 (m, 3H), 1.93–1.87 (m, 1H), 1.49 (dd, J = 8.2, 14.8 Hz, 1H), 1.16 (s, 3H), 0.49–0.42 (m, 2H), 0.39–0.35 (m, 1H), 0.32–0.29 (m, 1H). HRMS (APCI): calcd for $(C_{20}H_{27}ClN_7O_2)^+$, 432.1909; found: 432.1905.

6.30. 3-(1-Methylcyclopropyl)-D-alanyl-(4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoro prolinamide (41)

Compound **41** was prepared from Boc-3-(1-methylcyclopropyl)-D-alanine **6** (33 mg, 0.14 mmol) and (4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide **33** (60 mg, 0.14 mmol) according to general procedure **6.25** and was isolated as the trifluoroacetate salt (42 mg, 55% yield). LCMS t_R = 1.12 min. ^{1}H NMR (400 MHz, CD_3OD): δ 8.79 (s, 1H), 8.21 (s, 1H), 7.68 (d, J = 2.4 Hz, 1H), 7.49 (dd, J = 2.4, 8.4 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 5.38 (br d, J = 52.4 Hz, 1H), 4.47–4.38 (m, 2H), 4.26–4.20 (m, 2H), 4.03–3.83 (m, 2H), 2.59–2.51 (m, 1H), 2.18–1.97 (m, 2H), 1.52 (dd, J = 8.0, 14.8, 1H), 1.14 (s, 3H), 0.49–0.29 (m, 4H). HRMS (ESI): calcd for $(C_{21}H_{27}ClFN_6O_2)^+$, 449.1868; found: 449.1855.

6.31. 3-(1-Methylcyclopropyl)-D-alanyl-(4S)-*N*-[5-chloro-2-(1*H*-1,2, 4-triazol-1-yl)benzyl]-4-fluoro-L-prolinamide (42)

Compound **42** was prepared from Boc-3-(1-methylcyclopropyl)-D-alanine **6** (30 mg, 0.12 mmol) and (4*S*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide **34** (49 mg, 0.12 mmol) according to general procedure **6.25** and was isolated as the bis-trifluoroacetate salt (white powder, 28 mg, 34% yield). LCMS t_R = 1.13 min. ^{1}H NMR (400 MHz, CD_3OD): δ 8.77(s, 1H), 8.21 (s, 1H), 7.61 (d, J = 2.0 Hz, 1H), 7.47 (dd, J = 2.0, 8.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 5.37 (br d, J = 53.4 Hz, 1H), 4.59 (d, J = 10.0 Hz, 1H), 4.38–4.10 (m, 4H), 3.99–3.87 (m, 1H), 2.60–2.33 (m, 2H), 2.11–2.06 (m, 1H), 1.50–1.41 (m, 1H), 1.16 (s, 3H), 0.50–0.27 (m, 4H). HRMS (APCI): calcd for $(C_{21}H_{27}ClFN_6O_2)^+$, 449.1868; found: 449.1857.

6.32. 3-(1-Methylcyclopropyl)-D-alanyl-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4,4-difluoro prolinamide (43)

Compound **43** was prepared from Boc-3-(1-methylcyclopropyl)-D-alanine **6** (30 mg, 0.12 mmol) and *N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4,4-difluoroprolinamide **35** (51 mg, 0.12 mmol) according to general procedure **6.25** and was isolated as the bis-trifluoroacetate salt (white powder, 31 mg, 37% yield). LCMS t_R = 1.26 min. ^{1}H NMR (400 MHz, CD_3OD): δ 8.77 (s, 1H), 8.21 (s, 1H), 7.64 (s, 1H), 7.50–7.43 (m, 2H), 4.85–4.55 (m, 1H), 4.43–4.37 (m, 1H), 4.28–4.15 (m, 3H), 4.08–4.03 (m, 1H), 2.83–2.77 (m, 1H), 2.45–2.40 (m, 1H), 2.06–2.01 (m, 1H), 1.53–1.47 (m, 1H), 1.14 (s, 3H), 0.48–0.29 (m, 4H). HRMS (ESI): calcd for $(C_{21}H_{26}ClF_2N_6O_2)^+$, 467.1769; found: 467.1760.

6.33. 3-(1-Methylcyclopropyl)-D-alanyl-*N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]-4,4-difluoroprolinamide (44)

Compound **44** was prepared from Boc-3-(1-methylcyclopropyl)-D-alanine **6** (61 mg, 0.15 mmol) and *N*-[5-chloro-2-(1*H*-tetrazol-1-yl)benzyl]-4,4-difluoroprolinamide **36** (91 mg, 0.24 mmol) according to general procedure **6.25** and was isolated as the bis-trifluoroacetate salt, a fluffy white solid after lyophilization (71 mg, 70%). LCMS t_R = 1.25 min. ^{1}H NMR (400 MHz, CD_3OD): δ 9.52 (s, 1H), 8.76–8.75 (m, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.57 (dd, J = 2.0, 8.4 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 4.54 (dd, J = 6.4, 9.2 Hz, 1H), 4.39–4.33 (m, 1H), 4.28–4.03 (br m, 4H), 2.86–2.74 (m, 1H), 2.48–2.36 (m, 1H), 2.04 (dd, J = 6.0, 14.4 Hz, 1H), 1.50 (dd, J = 8.2, 14.4 Hz, 1H), 1.16 (s, 3H), 0.51–0.29 (br m, 4H). HRMS (APCI): calcd for $(C_{20}H_{25}ClF_2N_7O_2)^+$, 468.1721; found: 468.1723.

6.34. 3-(1-Chlorocyclopropyl)alanyl-(4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoro prolinamides (45a and 45b)

A mixture of (2*R*)-2-azido-3-(1-chlorocyclopropyl)propanoic acid **13** (105 mg, 0.56 mmol), (4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide **33** (200 mg, 0.56 mmol, 1.0 equiv), EDC (160 mg, 0.83 mmol, 1.5 equiv), and HOAT (38 mg, 0.28 mmol, 0.5 equiv) in DMF (1 mL) was brought to pH 8 by dropwise addition of Hünig's base and stirred at room temperature for 18 h. The solvent was removed in vacuo. Purification by reverse-phase chromatography [95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 5:95 water (+0.1% TFA)/CH_3CN (+0.1% TFA)] afforded an oil. The product was dissolved in EtOAc, and the solution was washed with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted twice into EtOAc. The organic layers were combined, dried over Na_2SO_4, and concentrated in vacuo to afford (4*R*)-1-[(2*R*)-2-azido-3-(1-chlorocyclopropyl)propanoyl]-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoro-L-prolinamide as the free base (156 mg, 57%). LCMS t_R = 1.91 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.32 (s, 1H), 7.60 (d, J = 2 Hz, 1H), 7.43 (dd, J = 2.0, 8.4 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 5.34 (br d, J = 52.4 Hz, 1H), 4.64 (app t, J = 8.0 Hz, 1H), 4.32 (d, J = 6.0 Hz, 2H), 4.25 (dd, J = 6.0, 7.6 Hz, 1H), 4.16–4.08 (m, 1H), 3.83–3.71 (m, 1H), 2.66–2.06 (m, 3H), 2.05–1.98 (m, 1H), 1.20–0.77 (m, 4H). MS (ESI): 495.3 $(M+H)^+$. A solution of this material (156 mg, 0.32 mmol) in THF (2 mL) at 0 °C was treated with triphenylphosphine (87 mg, 0.33 mmol, 1.05 equiv). After stirring for 1 h at 0 °C, water (114 μL, 6.30 mmol, 20 equiv) was added. The reaction mixture was warmed to 40 °C for 2 h then allowed to stir at 25 °C for 66 h. The solvent was removed in vacuo, and the residue was purified by reverse-phase chromatography [95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 50:50 water (+0.1% TFA)/CH_3CN (+0.1%

TFA)] to afford 130 mg (59%) of a diastereomeric mixture of bis-trifluoroacetate salts. LCMS t_R = 1.08 min. The components were separated by chiral HPLC (Chiralcel OD 5 × 50 cm column, eluting at 75 mL/min with 1:1 A/B, where A = 0.1% diethylamine/hexanes and B = 2-propanol) to afford compound **45a** (earlier eluting diastereomer; free base t_R = 5.21 min on Chiralcel OD 250 × 4.6 mm column, eluting at 1.0 mL/min with 1:1 A/B as defined above) as a white solid (58 mg, 39%). The later eluting diastereomer (free base t_R = 7.38 min on Chiralcel OD 250 × 4.6 mm column, eluting at 1.0 mL/min with 1:1 A/B as above; 14 mg, 9.5%) contained residual diethylamine and was further purified by reverse-phase chromatography [95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 50:50 water (+0.1% TFA)/CH_3CN (+0.1% TFA)] to give the bis-trifluoroacetate salt as an oil, **45b** (1.4 mg, 0.6%). Diastereomer **45a**: ^{1}H NMR (400 MHz, CD_3OD): δ 8.80 (s, 1H), 8.21 (s, 1H), 7.72 (d, J = 1.6 Hz, 1H), 7.48 (dd, J = 2.0, 8.4 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 5.37 (br d, J = 52.4 Hz, 1H), 4.50–4.45 (m, 1H), 4.38–4.27 (m, 3H), 4.03–3.85 (m, 2H), 2.61–2.51 (m, 1H), 2.20–2.03 (m, 2H), 1.94–1.88 (m, 1H), 1.09–0.88 (m, 3H), 0.78–0.73 (m, 1H). HRMS (ESI): calcd for $(C_{20}H_{24}Cl_2FN_6O_2)^+$, 469.1317; found: 469.1288. Diastereomer **45b**: ^{1}H NMR (300 MHz, CD_3OD): δ 8.82 (s, 1H), 8.23 (s, 1H), 7.69 (d, J = 2.4 Hz, 1H), 7.51 (dd, J = 2.1, 8.4 Hz, 1H), 7.46 (d, J = 8.7 Hz, 1H), 5.41 (br d, J = 52.2 Hz, 1H), 4.61–4.56 (m, 2H), 4.44–4.37 (m, 1H), 4.25–4.10 (m, 2H), 3.99–3.82 (m, 1H), 2.64–2.41 (m, 2H), 2.25–1.99 (m, 2H), 1.21–1.11 (m, 2H), 1.01–0.96 (m, 2H). MS (ESI): 469.2 $(M+H)^+$.

6.35. 3-Cyclopropylvalyl-(4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamides (46a and 46b)

N-(*tert*-butoxycarbonyl)-3-cyclopropylvaline **20** (519 mg, 2.02 mmol 1.6 equiv) and (4*R*)-*N*-[5-chloro-2-(1*H*-1,2,4-triazol-1-yl)benzyl]-4-fluoroprolinamide **33** (500 mg, 1.26 mmol, 1 equiv) were coupled according to general procedure **6.25**. The crude mixture of diastereomers was purified by reverse-phase chromatography [gradient elution on a YMC Pro C18 150 × 20 mm, 5 μm column with 95:5 water (+0.1% TFA)/CH_3CN (+0.1% TFA) to 5:95 water (+0.1% TFA)/CH_3CN (+0.1% TFA)] to afford an oily white foam which was taken up in MeOH (5 mL) and shaken with MP-carbonate resin (2.73 mmol/g, 1.35 g, 4 equiv) for 1 h. The resin was filtered off, washing well with MeOH and CH_2Cl_2, and the filtrate concentrated in vacuo to give a foamy solid (634 mg, 89% yield). A portion of this purified mixture of diastereomers (61 mg, 0.11 mmol) was dissolved in dioxane (2 mL) and the solution cooled to 0 °C. Excess HCl–dioxane (2 mL of a 4 M solution) was added and the mixture stirred at room temperature. After 4 h, additional HCl–dioxane (0.2 mL) was added and the mixture stirred for 1 h. The solvent was removed in vacuo to afford a white solid (65 mg). The diastereomers were separated by chiral chromatography (Chiralpak AD 5 × 50 cm 20 μm column, isocratic elution at 50 mL/min with 40:10:50 hexanes/MeOH/*i*PrOH containing 1 mL/L of diethylamine). The fractions of each diastereomer were concentrated and dried under high vacuum overnight to remove residual diethylamine. The hydrochloride salts were prepared by dissolution of each diastereomer in MeOH (1 mL) followed by addition of an HCl–ether solution (2 M, 2 mL) with stirring. The solvents were removed in vacuo to give the title compound **46a** (free base t_R = 4.7 min on an analytical Chiralpak AD 4.6 × 250 mm 10 μm column, isocratic elution at 1 mL/min with 40:10:50 hexanes/MeOH/*i*PrOH containing 1 mL/L of diethylamine) as a white powder (42% yield) and its diastereomer **46b** (free base t_R = 8.2 min on an analytical Chiralpak AD 4.6 × 250 mm 10 μm column, isocratic elution at 1 mL/min with 40:10:50 hexanes/MeOH/*i*PrOH containing 1 mL/L of diethylamine) as a white powder (47% yield; total yield of both diastereomers from deprotection step = 89%). Diastereomer **46a**: ^{1}H NMR (400 MHz, CD_3OD): δ 8.91 (s, 1H), 8.29 (s, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.53 (dd, J = 2.4, 8.8 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 5.36 (br d, J = 51.6 Hz, 1H), 4.50 (app t, J = 8.8 Hz, 1H), 4.42 (d, J = 15.2 Hz, 1H), 4.28–4.22 (m, 2H), 4.10 (s, 1H), 3.88–3.76 (m, 1H), 2.52–2.20 (m, 1H), 2.18–1.97 (m, 1H), 0.99 (s, 3H), 0.91 (s, 3H), 0.90–0.86 (m, 1H), 0.46–0.41 (m, 2H), 0.34–0.31 (m, 2H). HRMS (ESI): calcd for $(C_{22}H_{29}ClFN_6O_2)^+$, 463.2019; found: 463.2023. Elemental analysis: calcd for ($C_{22}H_{28}ClFN_6$ O_2 + 2.10 equiv HCl + 0.15 equiv ether): C, 49.30%; H, 5.78%; N, 15.26%; found: C, 49.33%; H, 6.01%; N, 15.33%. Diastereomer **46b**: ^{1}H NMR (400 MHz, CD_3OD): δ 9.17 (s, 1H), 8.44 (s, 1H), 7.74 (s, 1H), 7.51–7.46 (m, 2H), 5.36 (br d, J = 52.0 Hz, 1H), 4.63 (dd, J = 7.2, 9.6 Hz, 1H), 4.42 (d, J = 16.0 Hz, 1H), 4.24–4.15 (m, 3H), 3.88–3.75 (m, 1H), 2.60–2.49 (m, 1H), 2.21–2.01 (m, 1H), 0.99 (s, 3H), 0.97–0.91 (m, 1H), 0.84 (s, 3H), 0.45–0.41 (m, 2H), 0.37–0.33 (m, 2H). HRMS (ESI): calcd for $(C_{22}H_{29}ClFN_6O_2)^+$, 463.2019; found: 463.2025. Elemental analysis: calcd for ($C_{22}H_{28}ClFN_6O_2$ + 2.50 equiv HCl + 0.80 equiv H_2O + 0.30 equiv ether): C, 47.17%; H, 5.99%; N, 14.23%; found: C, 47.15%; H, 5.99%; N, 14.26%.

6.36. Molecular modeling

Molecular mechanics optimizations were performed with MacroModel[25] using the MMFFs force field and a distance-dependent dielectric of 4r. Ab initio calculations were performed with Gaussian 03.[26] Starting geometries from MMFFs calculations were optimized at the B3LYP/6-31G** level. Frequency calculations were performed at the same level to determine zero-point energy corrections; all frequencies were positive.

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