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Article

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TITLE PAGE

Discovery of *N*-substituted 3-amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acids as highly potent third generation inhibitors of human arginase I and II

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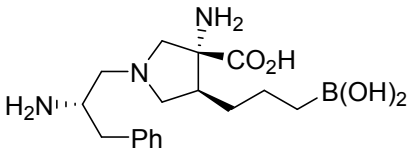
ABSTRACT

Recent efforts to identify new highly potent arginase inhibitors have resulted in the discovery of a novel family of (3*R*,4*S*)-3-amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acid analogs with up to a 1,000-fold increase in potency relative to the current standards, 2-amino-6-boronohexanoic acid (ABH) and *N*-hydroxy-nor-l-arginine (nor-NOHA). The lead candidate, with a *N*-2-amino-3-phenylpropyl substituent (NED-3238), example **43**, inhibits arginase I and II with $IC_{50's}$ of 1.3 nM and 8.1 nM respectively. Herein we report the design, synthesis and structure-activity relationships for this novel series of inhibitors, along with X-ray crystallographic data for selected examples bound to human arginase II.



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INTRODUCTION

Arginase is an ureohydrolase enzyme that converts L-arginine to urea and L-ornithine, thereby playing a key role in the urea cycle and in the regulation of nitric oxide (NO) homeostasis in mammals. There are two isoforms of the enzyme that differ in tissue specificity and subcellular distribution. Arginase I (Arg I) is a primarily expressed in the liver, while Arginase II (Arg II) is expressed in other organs and in the pituitary and thyroid glands. Although these isoforms are expressed from two different genes, Arg I and Arg II have similar enzymatic activities and they share an almost identical molecular structure in which a binuclear manganese cluster within the active-site plays a key role in chemical catalysis.[1]

It is becoming increasingly clear that the pathways for regulation of L-arginine metabolism are linked with a diversity of cardiovascular,[2] anti-inflammatory[3], autoimmune,[4] oncological,[5] and infectious diseases.[6] In many of these disorders, arginase is specifically dysregulated and its role in the urea cycle or NO homeostasis is disrupted. Specifically, disease pathologies frequently stem from a marked increase in arginase expression, leading to an increase in the production of polyamines, ornithine, and proline, along with a concomitant decrease in NO production.[7]

Efforts to understand the specific pharmacological role that arginase plays in diverse diseases have been limited by the availability of potent drug-like inhibitors. To date, much of the work in this area has relied on the modestly active first-generation arginase inhibitors such as (s)-2-amino-6-boronohexanoic acid (ABH), (s)-(2-boronoethyl)-L-cysteine (BEC) and *N*-hydroxy-nor-l-arginine (nor-NOHA) (Figure 1).

Figure 1.

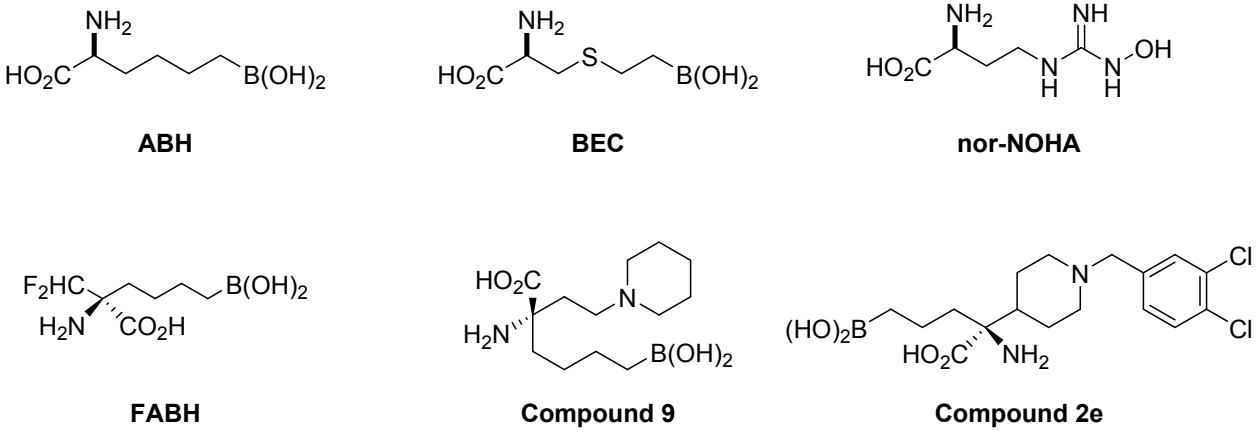


Figure 1. Early arginase inhibitors

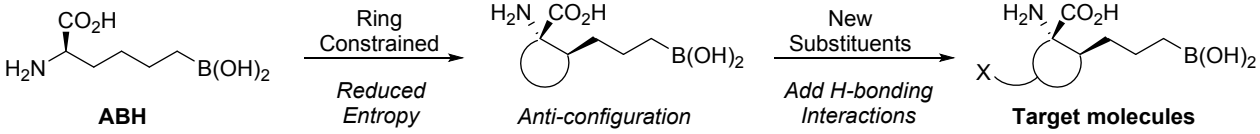
Although significant potency improvements were obtained with second-generation α,α-disubstituted amino acid inhibitors such as **FABH**,[8] **Compound 9**[9] and **Compound 2e**,[10] further optimization is needed. In our ongoing efforts to identify new, more potent and drug-like arginase inhibitors, we envisioned a novel ring-constrained series of boronic acid-based analogs where the critical elements of the pharmacophore would be fixed in a preferred binding orientation. Addition of an entropically-favorable ring constraint would potentially result in a new, significantly more potent class of arginase inhibitors. Herein we report our initial efforts to design, synthesize and evaluate a third-generation class of boronic acid-based arginase inhibitors and the discovery of **NED-3238**, which is the most potent arginase inhibitor reported to date.

INHIBITOR DESIGN AND SYNTHESIS

Evaluation of ABH[11] and our related second-generation series of analogs[9,10] co-crystalized with human arginase I (Arg I) and human arginase II (Arg II) led to the discovery that a constraint can be introduced by generating a five-membered ring that connects the first carbon of the ABH boronic acid side chain with the amino acid moiety. When the amine and the boronic acid side chain are positioned in an *anti*-orientation, conformational entropy is reduced without causing

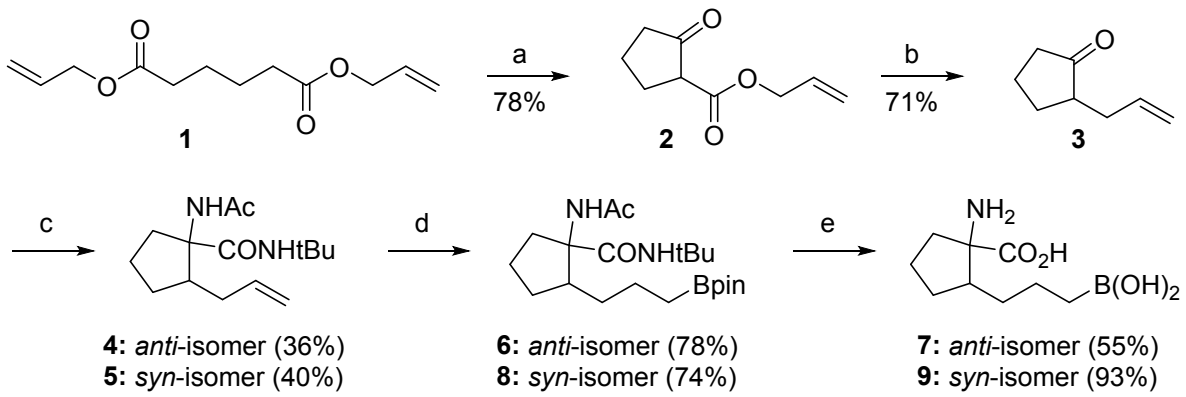
unfavorable van der Waals interactions with active site residues (Scheme 1). In addition, atoms within the ring can function as a scaffold for the introduction of additional substituents that can engage in hydrogen bonds with Asp 200 and Asp 202 (Arg II), forming active-site interactions analogous to those observed previously[9].

Scheme 1.



A cyclopentane derivative was selected as an ideal model compound to test this hypothesis. Since the three-carbon bridge would not be anticipated to have direct binding interactions with active site amino acids, any increase in potency could be attributed to the reduced entropy resulting from the ring constraint. This model compound was efficiently prepared using the chemistry illustrated in Scheme 2. Diekmann cyclization of diallyl adipate followed by palladium-mediated decarboxylative rearrangement gives the allyl ketone **3** in 56% yield (2 steps). The amino acid moiety is formed via the Ugi reaction with t-BuNC and NH_4OAc in trifluoroethanol, resulting in amino acid derivatives **4** and **5** as a mixture of *anti*- and *syn*-products that are easily separable by normal phase column chromatography. The desired *anti*-isomer, with the acetamide opposite the allyl sidechain, is isolated in 36% yield. Subsequent treatment with pinacolborane using $Ir_2Cl_2(COD)_2$ ethylenebis(diphenylphosphine) in dichloromethane gives exclusively the desired product with the anti-Markovnikov addition to the alkene. Deprotection with a mixture of 9 N hydrochloric acid and acetic acid at 130 ^oC gives the target compound (**7**) in approximately 9% overall yield from diallyl adipate. The *syn*-isomer (**9**) is prepared using the same method as described for intermediate **5**.

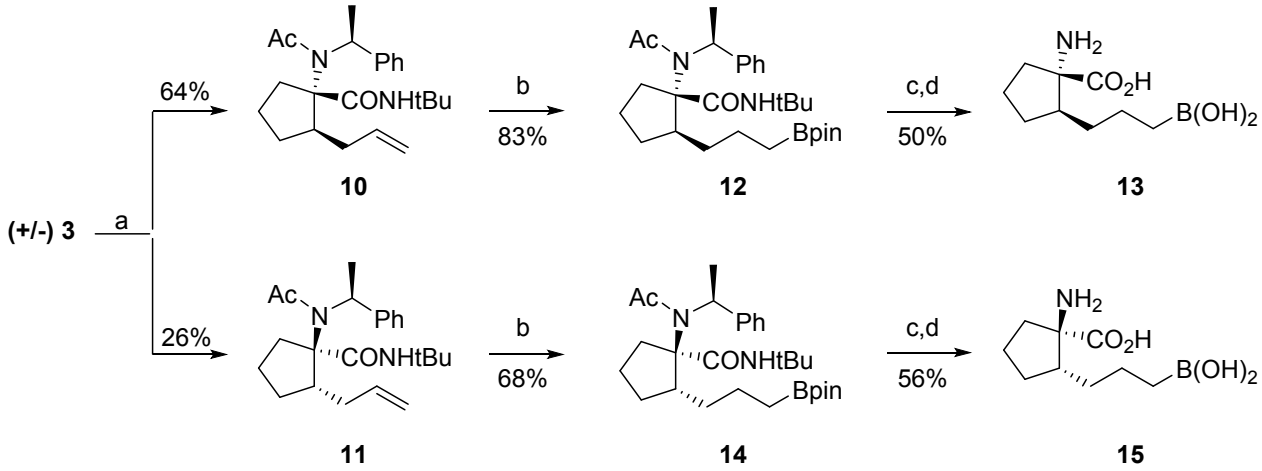
Scheme 2.



Reagents and conditions: (a) LiHMDS, THF, 0 ^{o}C; (b) $Pd(OAc)_2$, PPh_3 THF; (c) t-BuNC, CF_3CH_2OH, NH_4Ac, room temperature, 3 days; (d) pinacolborane, $Ir_2Cl_2(COD)_2$, dppe, dichloromethane; (e) 9 N HCl, AcOH, 130 ^{o}C, 12 h.

The single enantiomers were also prepared via the Ugi reaction. As illustrated in Scheme 3, use of (S)-α-methylbenzylamine with glacial acetic acid and *tert*-butylisonitrile gives diastereomers **10** and **11** which, after separation using normal phase column chromatography, are isolated in 64% and 26% yield respectively. It is interesting to note that use of the chiral amine in the Ugi reaction not only facilitates separation of the *R* and *S*-amino acids, but also eliminates formation of the undesired *syn*-diastereomers. With diastereomers **10** and **11** separated, hydroboration followed by cleavage of the chiral auxiliary using sodium in liquid ammonia and hydrolysis gives the individual enantiomers **13** and **15** in 27% and 10% yield respectively from ketone **3**.

Scheme 3.



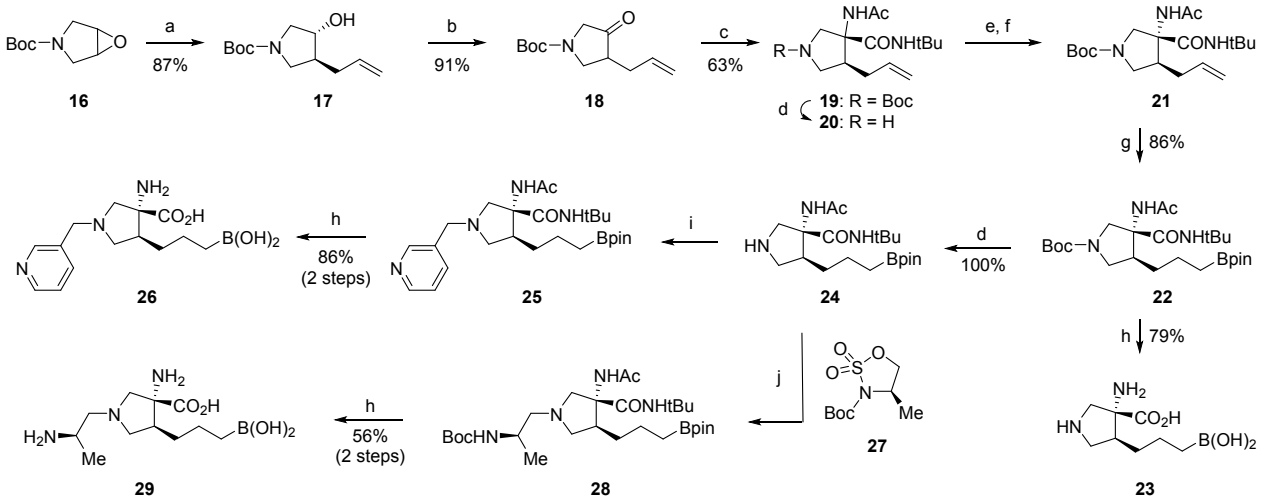
Reagents and conditions: (a) t-BuNC, (S)-a-methylbenzylamine, AcOH, CF_3CH_2OH, RT, 3 days (b) pinacolborane, $Ir_2Cl_2(COD)_2$, dppe, dichloromethane; (c) Na, ammonium; (d) 9 N HCl, AcOH, 130 ^oC, 12 h.

Based on X-ray crystallography data and screening results from our previous series, we hypothesized that significant potency gains could be achieved by introducing a basic amine substituent to the ring opposite the amino acid moiety to form hydrogen / ionic bonding interactions with Asp 200 and Asp 202 (Arg II). To facilitate synthesis of these new targets, we selected a pyrrolidine-based ring system that would provide convenient access to a versatile late-stage intermediate that would facilitate lead optimization.

The pyrrolidine-based arginase inhibitors were synthesized using the chemistry outlined in Scheme 4. Commercially available *boc*-protected pyrrolidine epoxide **16** was treated with allyl magnesium bromide in diethylether to give alcohol **17**. Subsequent oxidation with sulfur trioxide pyridine complex gives the corresponding allyl ketone (**18)** in 79% yield (from epoxide). Treatment with our standard Ugi reaction conditions gives the protected amino acid as a 10:1 mixture of *anti* and *syn*-diastereomers that can be separated by normal phase column chromatography or

recrystallization. The desired *anti*-diastereomer is resolved into its individual enantiomers using a selective crystallization of the diastereomeric salts formed with dibenzoyl-L-tartrate [(2S,3S)-2,3-bis(benzoyloxy)-4-(isopropylamino)-4-oxobutanoic acid] in a methanol/isopropanol mixture. Using this procedure, the desired enantiomer **21** is obtained in 77% theoretical yield with an enantiomeric excess of 99.7% as determined by chiral HPLC after reintroduction of the boc-group. Subsequent hydroboration and hydrolysis gives unsubstituted pyrrolidine **23** in 68% yield (2 steps).

Scheme 4.



Reagents and conditions: (a) allyl magnesium bromide, diethyl ether, 0 ^{o}C; (b) sulfur trioxide-pyridine complex, DMSO, diisopropylethylamine, dichloromethane, 0 ^{o}C; (c) NH_4Ac, t-BuNC, CF_3CH_2OH, room temperature, 3 days; (d) TFA, dichloromethane; (e) (2S,3S)-2,3-bis(benzoyloxy)-4-(isopropylamino)-4-oxobutanoic acid, IPA/ MeOH; (f) BOC_2O, aq $NaHCO_3$, ethyl acetate; (g) pinacolborane, $Ir_2Cl_2(COD)_2$, dppe, dichloromethane; (h) 9 N HCl, AcOH, 130 ^{o}C, 12 h; (i) RCHO, $Na(OAc)_3BH$; (j) acetonitrile.

Selective removal of the boc-group from intermediate **22** using trifluoroacetic acid gives the corresponding amine (**24**) which is a highly versatile intermediate useful for a variety of alkylation, acylation and reductive amination reactions. As an example of the reductive amination reaction, treatment of nicotinaldehyde with sodium triacetoxyborohydride gives substituted amine **25**. Hydrolysis using our standard conditions gives 3-pyridyl analog **26** in 86% yield (2 steps) after purification by reverse phase HPLC. When the desired aldehyde, such as *N*-boc-(R)-2-aminopropanal, contains a chiral center adjacent to the aldehyde, the reductive amination reaction results in significant epimerization. To avoid this racemization, the methyl piperidine substituent can be introduced via alkylation chemistry using the corresponding cyclic sulfamate (**27**) which is prepared from *N*-boc-(R)-2-aminopropan-1-ol using the general method described by Alker.[12] Treatment of pyrrolidine intermediate **24** with sulfamate **27** in acetonitrile gives amine **28**, the alkylation product. Subsequent hydrolysis gives the target 2-aminopropane (**29**) as a single enantiomer in 10 steps with an approximate overall yield of 9%.

RESULTS AND DISCUSSION

All program compounds were tested for arginase activity using human recombinant Arg I and Arg II using a colorimetric assay that has been previously described.[13] Results from these experiments for selected examples are listed in Table 1. As hypothesized, introduction of the five-membered ring constraint resulted in improved potency relative to ABH, the parent compound. Racemic analog **8**, with the amine and butane boronic acid side chain in the *anti*-orientation has IC_{50}s of 380 nM and 1,280 nM for Arg I and Arg II respectively. More than 100-fold loss in Arg I potency is observed when these groups are in a *syn*-orientation as seen with Compound **9** (Arg I 48.0 μM; Arg II 79.2 μM). The single *anti*-enantiomer with the amino acid in the *S*-configuration

(**13**) is approximately 2-fold more active than the corresponding racemic compound. If a nitrogen atom is introduced in the center of the ring constraint (**23**) activity is increased about 2-fold relative to **13**, the corresponding all-carbon example (**23**, Arg I 110 nM; Arg II 440 nM). Figure 2 illustrates crystal structures of example **23** superimposed with ABH, each in their active conformations when bound to Arg II. The ring constraint allows for positioning of the amino acid and boronic acid moieties in nearly the same *anti*-orientation as observed with the unconstrained scaffold with less entropic cost. Also indicated in the figure is an additional contact between the pyrrolidine nitrogen and Asp 200, mediated by the water molecule W1 (H-bonds of 2.66 Å and 2.78 Å). This additional contact is likely responsible for the 2-fold increase in potency of **23** relative to the cyclopentane derivative (**13**).

Figure 2.

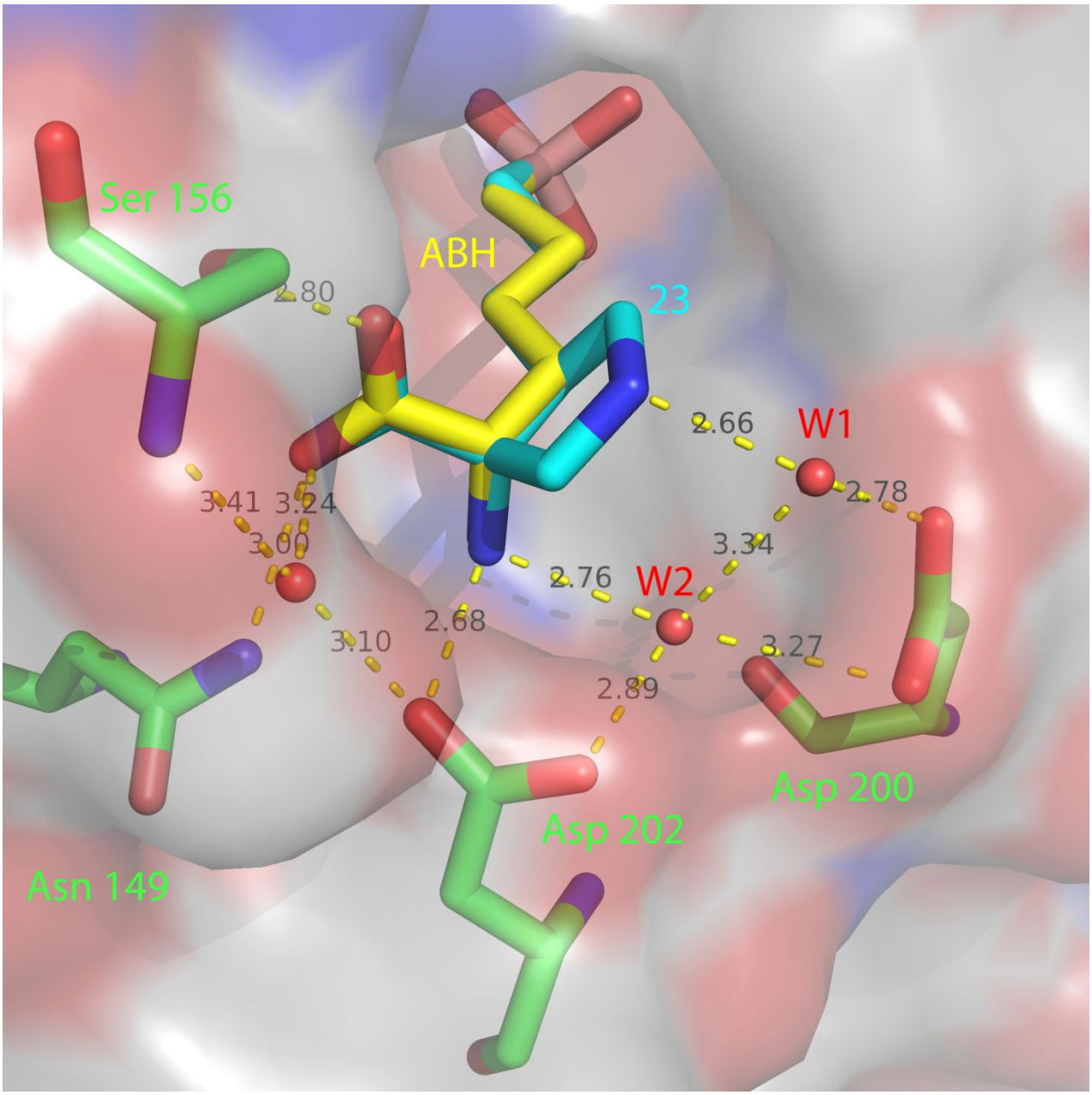


Figure 2. Superposition of ABH (yellow, IC_{50}: 2,551 nM) and constrained analog **23** (PDB ID 6Q37, cyan, IC_{50}: 440 nM) bound in the arginase II active site pocket.

Substitution of the pyrrolidine nitrogen with a simple isobutyl (**30**) or benzyl group (**31**) is tolerated but does not provide an improvement in activity. Similar results were observed for the pyridine derivatives **26**, **32, 33** though a trend is observed where potency increases for both Arg I

and II as the pyridine nitrogen moves from the *para*- to *meta*- and *ortho*-positions. Introduction of the 6-fluoro-substituent to the 2-pyridine results in about a 2-fold loss in activity, suggesting that potency is decreased with reduced nitrogen basicity. This decrease is most likely due to the electrostatic interaction with Asp 202 and/or Asp 200 strengthening with increasing nitrogen basicity. Addition of a 4-substituted pyrazole **(35** and **36)** or a 5-substituted thiazole (**38**) did not improve potency relative to the unsubstituted benzyl analog (**31**) indicating no significant hydrogen bonding interactions from the heteroatoms. Methylimidazole example **37** is about 3-fold more active than the benzyl analog suggesting a beneficial interaction, likely a weak electrostatic interaction with Asp 200. A dramatic increase in potency is observed with examples containing a basic primary or secondary amine positioned to make electrostatic contacts with Asp 202 and/or Asp 200. Examples **29**, **39**-**43** where a 2-aminoethyl moiety is substituted with a methyl (*R*-**29**, *S*-**39**), phenyl (*S*-**40**, *R*-**41**) or benzyl (*R*-**42**, *S*-**43**) moiety at the 2-position are all exceptionally potent, with the more active *R*-enantiomers having Arg I IC_{50} values of 7.7 nM, 2.5 nM and 1.3 nM, respectively. These are, by far, the most potent arginase inhibitors ever reported. Consistent with other examples in the series, the corresponding IC_{50} values for Arg II are 2 to 4-fold less active. Excellent potency is maintained when the basic amine is incorporated into a ring such as a morpholine, pyrrolidine or piperidine. With a morpholine, the *R*-enantiomer (**44**; Arg I IC_{50} 10 nM, Arg II IC_{50} 27 nM) is 2-fold more potent than the *S*-enantiomer (**45**; Arg I IC_{50} 19 nM, Arg II IC_{50} 65 nM). When the amine is used to form a pyrrolidine the *S*-enantiomer (**46**; Arg I IC_{50} 11 nM, Arg II IC_{50} 47 nM) and *R*-enantiomer (**47**; Arg I IC_{50} 10 nM, Arg II IC_{50} 37 nM) are nearly equipotent. In contrast, when the ring is expanded to form a piperidine, the *R*-enantiomer (**49**; Arg I IC_{50} 2.6 nM, Arg II IC_{50} 14 nM) is approximately 10-fold more potent than the corresponding *S*-enantiomer (**48**; Arg I IC_{50} 30 nM, Arg II IC_{50} 95 nM).

Analysis of example **49** co-crystallized with Arg II demonstrates a hydrogen bonding network between aspartic acid residues of the protein and the piperidine ring nitrogen (Figure 3). The piperidine nitrogen forms a direct hydrogen bond with Asp 200 (3.11 Å) and a second interaction with Asp 202 that is mediated by water molecule W2 (2.80 Å and 2.77 Å). It is interesting to note that the piperidine nitrogen in example **49** displaces water molecule W1 in example **23.** Which enables a direct contact with Asp 200, and not through a water molecule. This likely explains the large increase in potency (Arg II IC_{50} of 440 nM for **23** *vs* 14 nM for **49**). A 2D diagram of the contacts and the details of the inhibitor density in the initial difference maps are given in the supporting information.

Figure 3.

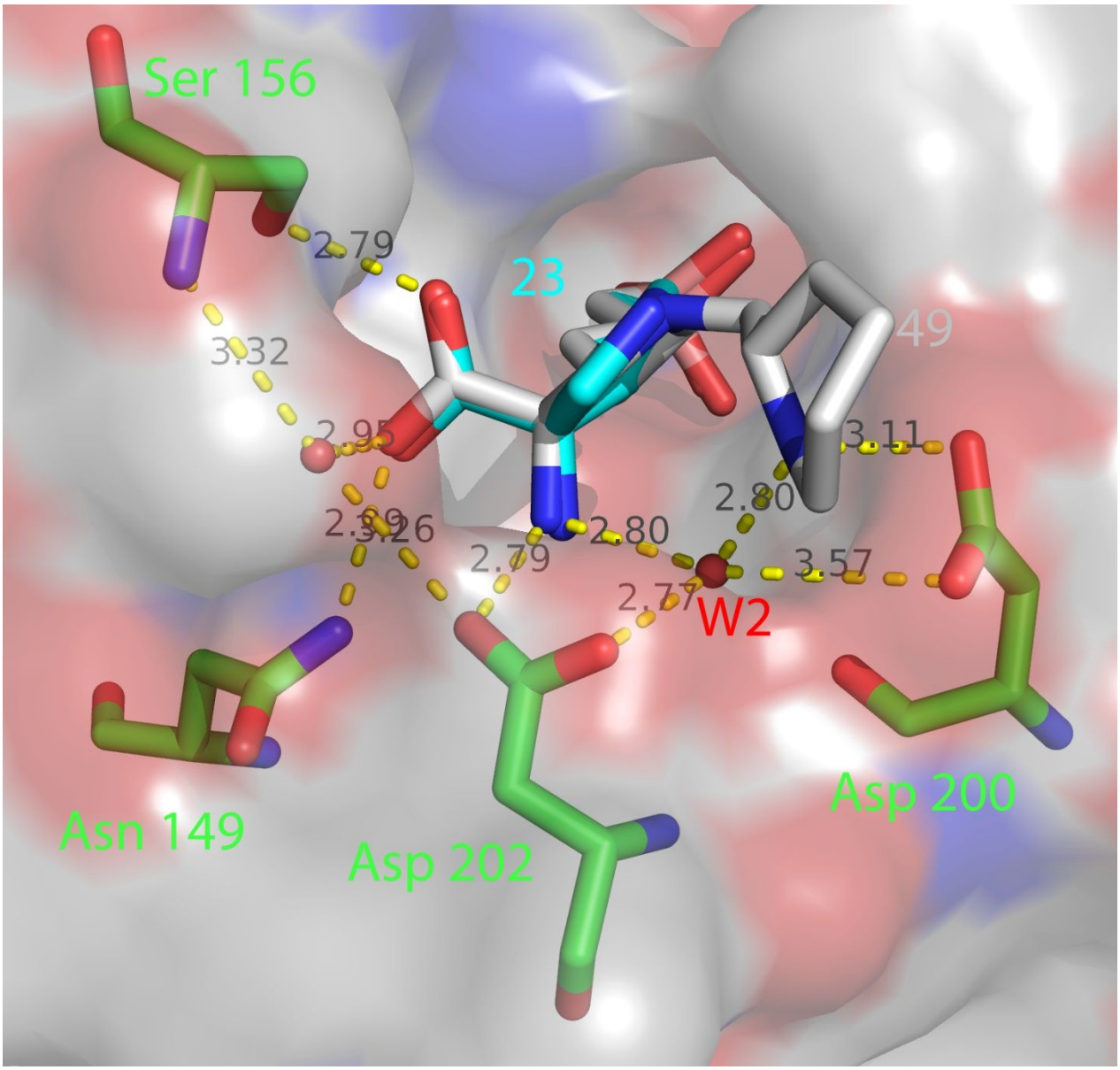
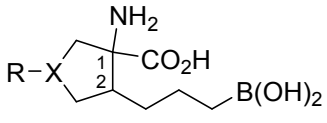
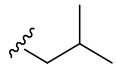
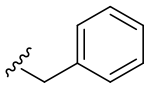
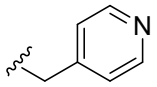
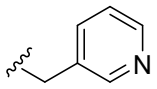
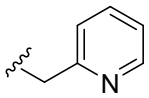
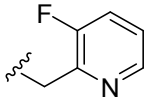
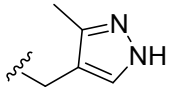
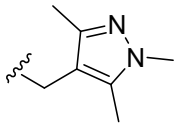
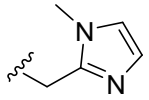
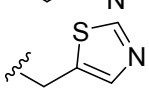
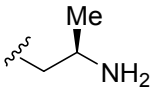
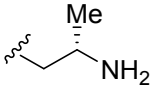
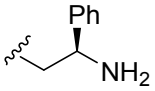
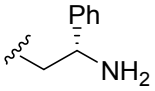
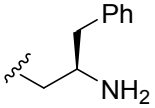
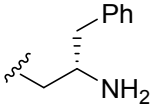
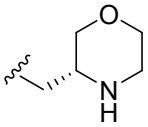
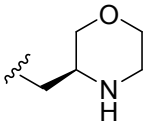
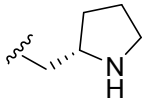
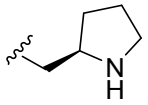
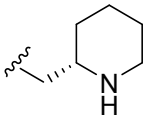
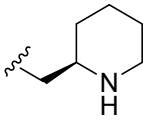


Figure 3. Crystal structures of example **49** (6Q39, grey, IC_{50}: 14 nM) bound in the Arg II active site pocket (green). Positions of ionic and hydrogen bonds (yellow) are indicated, along with bond distances (black). Example **23** is shown in cyan (6Q37, IC_{50}: 440 nM).

Table 1



Cmpd.	R	X	Isomer[a]	Inh. IC_{50}[b]	
				ARG I	ARG II
8	H	CH	(±)-Anti	380 nM	1,280 nM
9	H	CH	(±)-Syn	48.0 μM	79.2 μM
13	H	CH	1S,2S	210 nM	630 nM
15	H	CH	1R,2R	inactive	inactive
23	H	N	1S,2S	110 nM	440 nM
30		N	1S,2S	390 nM	1,610 nM
31		N	1S,2S	300 nM	1,540 nM
32		N	1S,2S	600 nM	1,860 nM
26		N	1S,2S	470 nM	1,370 nM
33		N	1S,2S	380 nM	1,270 nM
34		N	1S,2S	670 nM	2,160 nM
35		N	1S,2S	400 nM	2,180 nM
36		N	1S,2S	340 nM	2,230 nM
37		N	1S,2S	100 nM	330 nM
38		N	1S,2S	360 nM	850 nM

29		N	1S,2S	16 nM	37 nM
39		N	1S,2S	7.7 nM	16 nM
40		N	1S,2S	18 nM	62 nM
41		N	1S,2S	2.5 nM	9.5 nM
42		N	1S,2S	10 nM	27 nM
43 (NED-3238)		N	1S,2S	1.3 nM	8.1 nM
44		N	1S,2S	10 nM	27 nM
45		N	1S,2S	19 nM	65 nM
46		N	1S,2S	11 nM	47 nM
47		N	1S,2S	10 nM	37 nM
48		N	1S,2S	30 nM	95 nM
49		N	1S,2S	2.6 nM	14 nM
ABH				1,547 nM	2,551 nM
Nor-NOHA				1,360 nM	1,260 nM

[a] All compounds are prepared as single enantiomers with *S*-configuration (*S*), *R*-configuration (*R*) or as racemates (±). Stereochemistry of propylboronic acid side chain relative to carboxylic acid. [b]Arginase IC_{50} measurements using human recombinant protein (duplicate).

Figure 4.

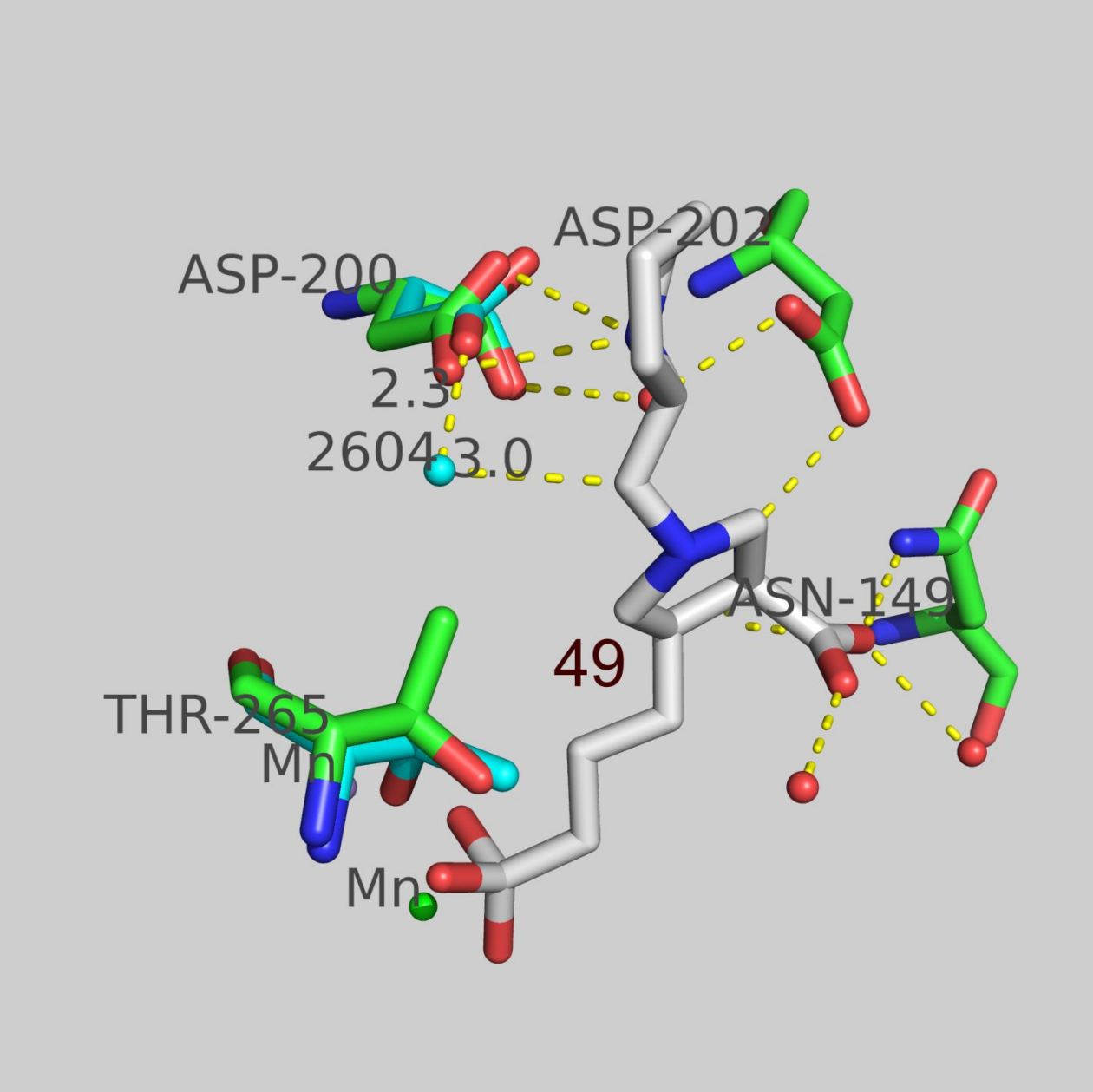


Figure 4. Superposition of the complex Arg II-Example 49 (6Q39, green protein carbons, grey inhibitor carbons) with the Arginase I unliganded structure (PDB entry 2ZAV, colored in cyan). Note the rotamer change of Thr 265, which opens the place for water 2604 in the Arg I structure. This water, which is not present in the Arg II complex, makes an H-bond with Asp 200 and is well positioned to make a contact with the inhibitor Example 49 (3.0 A distance).

As shown in Table 1, compounds in this series are generally 3~5 times more potent against Arg I than Arg II, while nor-NOHA is roughly equally potent with both enzymes. In an attempt to better understand this, the structure of the complex Arg II-Example **49** was superimposed with the nonliganded structure of Arg I (pdb entry 2ZAV, see figure 4). The superposition showed a change in the rotamer of Thr 265 in Arg II (Thr 246 in ARG I) which opens a space near the inhibitor occupied by water molecule 2604 in Arg I. This water molecule is well positioned to make a close contact with the inhibitor, which could explain the increase in potency. On the other hand, in the structure of Arg I with nor-NOHA (pdb entry 1HQH), Thr 246 makes an H-bond with nor-NOHA, fixing it in the same rotamer as that observed for Arg II, in agreement with the observed potency values.

CONCLUSIONS

In conclusion, a novel series of highly potent 3-amino-4-(3-boronopropyl) pyrrolidine-3-carboxylic acid-based inhibitors of arginase have been identified. Their increase in potency relative to the parent ABH template results from at least two structural changes in the scaffold: introduction of a ring constraint for reduction of conformational entropy, and incorporation of a basic amine side chain that forms additional ionic interactions with aspartic acids in the active site. For our lead compound, example **43** (**NED-3238**), the Arg I potency enhancements for these modifications are approximately 14-fold and 84-fold respectively, and when these values are combined, they represent a 1,190-fold enhancement relative to ABH. In addition to being exquisitely potent, compounds in this series also have physical-chemical properties consistent with drug-like structures.[14] It is hoped that this exciting new class of inhibitors will not only provide a path to

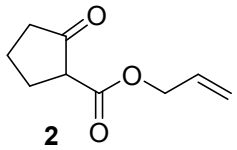
important new therapeutics, but will also facilitate our understanding of arginine metabolism and the role played by arginase in health and disease.

EXPERIMENTAL SECTION

Chemistry. General methods. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on Agilent DD2 400 MHz, 500 MHz or 600 MHz NMR spectrometers. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (internal standard) with coupling constants in hertz (Hz). Multiplicity is indicated by the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br). Mass spectra were recorded using an Advion Expression CMS mass spectrometer coupled with an Agilent 1260 analytical HPLC. Elemental analyses (C,H,N) were performed by Atlantic Microlab, Inc. (Norcross, Georgia) and are within ±4% of theory unless otherwise noted. Intermediate products from all reactions (non-polar compounds) were purified by either flash column chromatography or

medium pressure liquid chromatography using a Biotage Isolera One with SNAP cartridge unless otherwise indicated. All final products were purified on a Gilson 215 robotic liquid handler equipped with a Gilson ELSD detector and Phenomenex Synergi 4 μ Polar-RF preparative column (250 mm x 21.2 mm) or a Shimadzu Prominence LC-20AP preparative HPLC equipped with a Shimadzu ELSD-LT II using a Phenomenex Synergi 10 μ Polar-RF preparative column (250 mm x 50 mm). Analytical purity for all final compounds was determined to be > 99% by HPLC. This analysis was completed using an Agilent Eclipse XDB-Phenyl analytical HPLC column (3.5 μm; 4.6 x 150 mm) with a evaporative light scattering detector. Thin-layer chromatography using glass-backed silica plates containing a fluorescent indicator (0.25 mm, Whatman, Merck) was used to monitor reactions. Chromatograms were visualized using ultraviolet illumination, exposure to iodine vapors, or by dipping in an aqueous potassium permanganate solution. All starting materials were used without further purification. All reactions were carried out under an atmosphere of dried nitrogen or argon. Compound names are derived from the structures using ChemDraw Ultra 14.0.

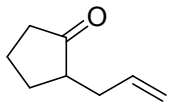
General Procedures for the Synthesis of the 1-Amino-2-(3-boronopropyl)cyclopentane-1-carboxylic acids and 3-Amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acids. The 1-amino-2-(3-boronopropyl)cyclopentane-1-carboxylic acids in Tables 1 were prepared using the methods illustrated in Schemes 1 and 2 which are detailed below. The 3-amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acids were prepared using the method described in Scheme 4. Details for compounds **23**, **26** and **43** are provided below. Analytical data for the remaining compounds are provided in the supplementary material.



2

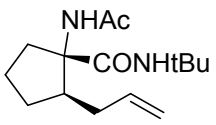
Preparation of anti-1-amino-2-(3-boronopropyl)cyclopentane-1-carboxylic acid (8). Step 1: synthesis of allyl 2-oxocyclopentanecarboxylate (2). A stirred solution of diallyl adipate (4.53 g, 20 mmol) in anhydrous tetrahydrofuran (100 mL) was cooled to 0 ^{o}C and treated with lithium bis(trimethylsilyl)amide (40 mL, 1.0 N in THF, 40 mmol). After the addition was complete, the solution was warmed to room temperature, stirred for an additional 2 h, re-cooled to 0 ^{o}C and quenched with acetic acid (2.53 mL, 44 mmol) in a dropwise manner. The turbid mixture was warmed to room temperature and filtered and concentrated. Purification by flash column chromatography (silica gel, dichloromethane) afforded allyl 2-oxocyclopentanecarboxylate, **2** (2.62 g, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.90 (ddt, J_1 = 16.5 Hz, J_2 = 10.9 Hz, J_3

= 5.7 Hz, 1 H), 5.33 (dd, J_1 = 17.2 Hz, J_2 = 1.9 Hz, 1 H), 5.23 (d, J = 10.4 Hz, 1 H), 4.63 (t, J = 6.1 Hz, 1 H), 3.17 (t, J = 9.0 Hz, 1 H), 2.34 – 2.27 (m, 4 H), 2.16 – 2.09 (m, 1 H), 1.91 – 1.82 (m, 1 H); ^{13}C NMR (600 MHz, D_2O): δ 212.26, 169.15, 131.82, 118.59, 65.97, 54.83, 38.17, 27.51, 21.07.; MS (CI): *m/z* for $C_9H_{12}O_3$: expected 168.1; found 169.1 $(M+H)^+$. Anal. Calcd for $C_9H_{12}O_3$: C 64.27; H, 7.19. Found C, 63.89; H, 7.05.

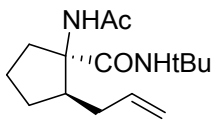


3

Step 2: synthesis of 2-allylcyclopentanone (3). While under a nitrogen atmosphere, a solution of palladium(II) acetate (51 mg, 0.23 mmol) and triphenylphosphine (0.24 g, 0.9 mmol) in anhydrous THF (20 mL) was heated to 65 °C and treated with a second solution of allyl 2-oxocyclopentanecarboxylate (**2**, 2.52 g, 15 mmol) in anhydrous THF (rapid gas evolution upon addition). After 45 minutes at 65 °C, the reaction mixture was cooled and concentrated. Purification by flash column chromatography (silica gel, dichloromethane) afforded 2-allylcyclopentanone (1.32 g, 71%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.76 (td, J_1 = 16.8 Hz, J_2 = 7.2 Hz, 1 H), 5.06 (d, J = 17.0 Hz, 1 H), 5.01 (d, J = 10.1 Hz, 1 H), 2.53-2.48 (m, 1 H), 2.31 (dd, J_1 = 18.9 Hz, J_2 = 8.6 Hz, 1 H), 2.22 – 1.97 (m, 5 H), 1.83-1.73 (m, 1 H), 1.62 – 1.52 (m, 1 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 220.72, 136.05, 116.52, 48.73, 38.31, 34.02, 29.10, 20.78.

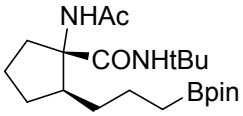


4



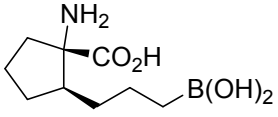
5

Step 3: synthesis of anti-1-acetamido-2-allyl-N-tert-butylcyclopentanecarboxamide (4) and syn-1-acetamido-2-allyl-N-tert-butylcyclopentanecarboxamide (5). A solution of 2-allylcyclopentanone, **3** (0.993 g, 8 mmol) and ammonium acetate (1.54 g, 20 mmol) in 2,2,2-trifluoroethanol (2.5 mL) was treated with *tert*-butyl isocyanide (1.81 mL, 16 mmol). After stirring at room temperature for 4 days, the solution was concentrated. Purification by flash column chromatography (silica gel, 60% ethyl acetate in heptane) afforded first, the *anti*-isomer, **4** (0.771g, 36%), then after increasing the polarity of the eluent (80% ethyl acetate in hexanes) the later migrating *syn*-isomer, **5** (0.851g, 40%), both obtained as white solids. *Anti*-isomer **(4)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.47 (brs, 1 H), 6.21 (brs, 1 H), 5.75-5.65 (m, 1 H), 4.99 (d, J = 18.4 Hz, 1 H), 4.97 (d, J = 11.0 Hz, 1 H), 2.46 (dtd, J_1 = 11.5 Hz, J_2 = 7.4 Hz, J_3 = 4.3 Hz, 1H), 2.40 (dt, J_1 = 14.1 Hz, J_2 = 8.8 Hz, 1 H), 2.25-2.18 (m, 1 H), 2.02-1.98 (m, 1 H), 1.97 (s, 3 H), 1.91-1.80 (m, 2 H), 1.79-1.72 (m, 2 H), 1.51-1.45 (m, 1 H), 1.32 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 171.47, 170.44, 137.15, 116.20, 70.85, 51.36, 46.66, 34.97, 34.38, 29.45, 28.81, 24.28, 21.56.; MS (CI): *m/z* for $C_{15}H_{26}N_2O_2$: expected 266.2; found 289.1 (M+Na), 166.1 (M-Boc+1), 265.1 (M-1); Anal. Calcd for $C_{15}H_{26}N_2O_2$: C 67.63; H, 9.84; N, 10.52. Found C, 67.57; H, 9.95; N, 10.26. *Syn*-isomer **(5)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.80 (brs, 1 H), 5.97 (brs, 1 H), 5.77 (ddt, J_1 = 17.1 Hz, J_2 = 10.0 Hz, J_3 = 6.9 Hz, 1 H), 5.04 (dd, J_1 = 17.1 Hz, J_2 = 1.6 Hz, 1 H), 4.99 (d, J = 10.1 Hz, 1 H), 2.48 (tdd, J_1 = 9.2 Hz, J_2 = 7.6 Hz, J_3 = 4.6 Hz, 1 H), 2.31-2.15 (m, 3 H), 1.99 (s, 3 H) , 1.91-1.81 (m, 2 H), 1.70-1.59 (m, 2 H), 1.51-1.42 (m, 1 H), 1.30 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 172.50, 170.79, 137.03, 116.29, 69.90, 51.03, 46.38, 34.50, 34.31, 29.10, 28.71, 24.18, 21.58.; MS (CI): *m/z* for $C_{15}H_{26}N_2O_2$: expected 266.2; found 289.1 (M+Na), 166.1 (M-Boc+1), 265.1 (M-1); Anal. Calcd for $C_{15}H_{26}N_2O_2$: C 67.63; H, 9.84; N, 10.52. Found C, 67.80; H, 9.99; N, 10.35.



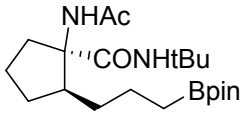
6

Step 4: synthesis of anti-1-acetamido-N-tert-butyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide (6). While under nitrogen, a stirred solution of *anti*-1-acetamido-2-allyl-*N*-tert-butylcyclopentanecarboxamide, **4** (0.599 g, 2.25 mmol) in anhydrous methylene chloride (9 mL) was treated with $Ir_2Cl_2(COD)_2$ (45 mg, 0.07 mmol) and DiPhos (54 mg, 0.136 mmol) and stirred at room temperature for 30 min. pinacolborane (0.65 mL, 4.48 mmol) was added dropwise and stirring was continued at room temperature for 20 h. The reaction mixture was poured into water (20 mL) and extracted with ethyl acetate (40 mL, then 2 x 15 mL), and the combined organic solution was washed with saturated aqueous sodium chloride (30 mL), dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (silica gel, 40-50% ethyl acetate in heptane) afforded *anti*-1-acetamido-*N*-*tert*-butyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide, **6** (0.695g, 78%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.22 (brs, 1 H), 6.13 (brs, 1 H), 2.44-2.30 (m, 2 H), 2.07-1.99 (m, 1 H), 1.98 (s, 3 H) , 1.97-1.91 (m, 1 H), 1.83-1.74 (m, 2 H), 1.51-1.36 (m, 3 H), 1.30 (s, 9 H), 1.22 (s, 12 H), 1.15-1.06 (m, 1 H), 0.82-0.67 (m, 2 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 171.78, 170.13, 83.06, 70.81, 51.34, 47.50, 34.47, 33.13, 29.96, 28.83, 24.94, 24.32, 22.99, 21.97, 11.53.; MS *m/z* for $C_{21}H_{39}BN_2O_4$: expected 394.3; found 417.2 (M+Na), 395.3 (M+1), 393.2 (M-1); Anal. Calcd for $C_{21}H_{39}BN_2O_4$: C 63.96; H, 9.97; N, 7.10. Found C, 64.06; H, 10.09; N, 7.22.



7

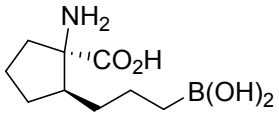
Step 5: synthesis of anti-1-amino-2-(3-boronopropyl)cyclopentanecarboxylic acid (7). A solution of *anti*-1-acetamido-*N*-*tert*-butyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide, **6** (400 mg, 1.01 mmol) in concentrated hydrochloric acid (1 mL), acetic acid (1 mL) and water (2 mL) in a pressure bottle was stirred for 30 min at 60°C, then capped and stirred for 18 h at 130 °C, cooled to room temperature, and uncapped. The solution was diluted with water (20 mL), extracted with toluene (20 mL) and concentrated. Purification by preparative HPLC afforded the desired product (55 mg, 55%) as an off-white foam. ^{1}H NMR (600 MHz, D_2O): δ 2.40 (dt, J_1 = 14.3 Hz, J_2 = 8.5 Hz, 1 H), 2.15-2.04 (m, 2 H), 1.97-1.85 (m, 2 H), 1.83-1.71 (m, 1 H), 1.54-1.41 (m, 3 H), 1.36-1.25 (m, 1 H), 1.24-1.13 (m, 1 H), 0.76 (ddd, J_1 = 15.1 Hz, J_2 = 8.8 Hz, J_3 = 6.2 Hz, 1 H), 0.70 (ddd, J_1 = 15.1 Hz, J_2 = 9.1 Hz, J_3 = 6.1 Hz, 1 H); ^{13}C NMR (600 MHz, D_2O): δ 173.78, 68.07, 49.21, 34.84, 31.66, 30.62, 22.64, 22.42, 14.02.; MS (CI): *m/z* for $C_9H_{18}BNO_4$: expected 215.1; found 198.1 (M-H_2O+1).



8

Preparation of syn-1-amino-2-(3-boronopropyl)cyclopentane-1-carboxylic acid (9). **Step 1: syn-1-acetamido-N-tert-butyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide (8).** While under nitrogen, a stirred solution of *syn*-1-acetamido-2-allyl-*N*-tert-butylcyclopentanecarboxamide, **5** (0.285 g, 1.07 mmol) in anhydrous methylene chloride (5.5 mL) was treated with $Ir_2Cl_2(COD)_2$ (22 mg, 0.032 mmol) and 1,2-bis(diphenylphosphino)ethane (26 mg, 0.06 mmol). After stirring at room temperature for 30 min, the solution was cooled to -10 °C (ice/ethanol bath) and treated with pinacolborane (0.23 mL, 1.61 mmol) dropwise. After the addition was complete, the bath was allowed to warm to room

temperature with stirring for 20 h. The reaction mixture was poured into water (20 mL) and extracted with ethyl acetate (40 mL, then 2 x 15 mL), and the combined organic solution was washed with saturated aqueous sodium chloride (30 mL), dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (silica gel, 40-50% ethyl acetate in heptane) afforded *syn*-1-acetamido-N-tert-butyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide, **7** (0.313g, 74%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.71 (bs, 1 H), 5.71 (bs, 1 H), 2.39-2.31 (m, 2 H), 2.13 (dt, J_1 = 13.6 Hz, J_2 = 7.4 Hz, 1 H), 2.01 (s, 3 H) , 1.95 (tt, J_1 = 13.6 Hz, J_2 = 7.4 Hz, 1 H), 1.68-1.60 (m, 2 H), 1.52-1.43 (m, 2 H), 1.42-1.32 (m, 2 H), 1.30 (s, 9 H) , 1.22 (s, 12 H), 1.13-1.02 (m, 1 H), 0.76 (m, 2 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 172.49, 170.75, 83.08, 70.02, 50.99, 47.56, 34.35, 32.50, 29.67, 28.74, 24.94, 24.34, 22.97, 21.90, 11.72.; MS (CI): *m/z* for $C_{21}H_{39}BN_2O_4$: expected 394.3; found 417.2 (M+Na), 395.3 (M+1), 393.2 (M-1).

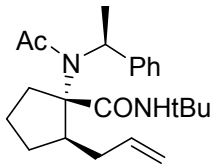


9

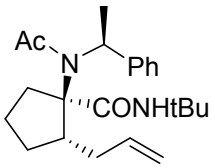
Step 2: syn-1-amino-2-(3-boronopropyl)cyclopentanecarboxylic acid (9) (syn-isomer, racemic)

A stirred mixture of *syn*-1-acetamido-*N*-tert-butyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide **8** (0.627 g, 1.59 mmol) in 6 N HCl (15 mL) was heated to 90 ^{o}C for 20 h, cooled to room temperature, and diluted with water (15 mL). The mixture was extracted with dichloromethane (2 x 15 mL) and concentrated in vacuo. The resulting residue was dissolved in methanol (5 mL) and diluted to a volume of 40 mL with ether and stirred for 1 hour, to remove solid tertbutylamine hydrochloride by filtration. The resulting filtrate, was concentrated, dissolved

in saturated aqueous ammonium hydroxide (15 mL), and re-concentrated (3 x). The resulting solid white residue was triturated using acetonitrile and dried to afford the target compound *syn*-1-amino-2-(3-boronopropyl) cyclopentanecarboxylic acid **9** (0.397g, 93%) as a white powder. ^{1}H NMR (600 MHz, D_2O): δ 2.40 (dt, J_1 = 14.4 Hz, J_2 = 8.5 Hz, 1 H), 2.08 (d, J_1 = 7.1 Hz, J_2 = 2.3 Hz, 2 H), 1.96-1.84 (m, 2 H), 1.83-1.70 (m, 1 H), 1.54-1.42 (m, 3 H), 1.36-1.25 (m, 1 H), 1.23-1.14 (m, 1 H), 0.77 (ddd, J_1 = 15.1 Hz, J_2 = 8.7 Hz, J_3 = 6.2 Hz, 1 H), 0.70 (ddd, J_1 = 15.3 Hz, J_2 = 9.2 Hz, J_3 = 6.2 Hz, 1 H); ^{13}C NMR (600 MHz, D_2O): δ 173.82, 68.08, 49.21, 34.85, 31.67, 30.63, 22.65, 22.43, 14.02; MS (+CI): m/z for $C_9H_{18}BNO_4$: expected 215.1; found 215.3 $(M+H)^+$. Anal. Calcd for $C_9H_{19}BClNO_4$: C 42.98; H, 7.61; N, 5.57. Found C, 42.69; H, 7.43; N, 5.60.



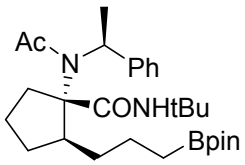
10



11

Preparation of (1S,2S)-1-amino-2-(3-boronopropyl)cyclopentane carboxylic acid (13). Step 1: synthesis of (1S,2R)-2-allyl-N-(tert-butyl)-1-(N-((S)-1-phenylethyl)acetamido) cyclopentanecarboxamide (10) and (1R,2S)-2-allyl-N-(tert-butyl)-1-(N-((S)-1-phenylethyl)acetamido) cyclopentanecarboxamide (11). A stirred solution of 2-(propene-3-yl)cyclopentanone, **3** (0.745 g, 6.0 mmol), (*S*)-α-methylbenzylamine (3.1 mL, 24 mmol) and glacial acetic acid (1.38 mL, 24 mmol) in methanol (5 mL) was treated with *tert*-butylisonitrile (2.04 mL, 18 mmol) and warmed to 60 °C for 2 days. After cooling to room temperature, the mixture was concentrated, diluted with dichloromethane (60 mL) and washed successively with water (50 mL) and saturated aqueous sodium chloride. The combined organic solution was dried over $MgSO_4$,

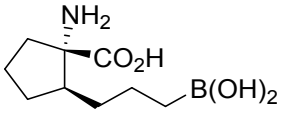
filtered and concentrated. Purification via column chromatography (silica gel, 5-30% ethyl acetate in heptane) afforded *anti*-diastereomer **10** (1.42 g, 3.83 mmol, 64%) and *syn*-diastereomer, **11** (0.57 g, 1.54 mmol, 26%), both as pale-yellow viscous oils. Compound **10**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.53 (d, J = 7.5 Hz, 2 H), 7.37 (t, J = 7.6 Hz, 2 H), 7.25 (m, 1 H), 6.33 (brs, 1 H), 5.74 (ddt, J_1 = 17.1 Hz, J_2 = 10.8 Hz, J_3 = 7.1 Hz, 1 H), 5.01-4.91 (m, 2 H), 4.91-4.74 (m, 1 H), 3.00-2.89 (m, 1 H), 2.55-2.42 (m, 2 H), 1.99-1.86 (m, 1 H), 1.75 (d, J = 7.0 Hz, 3 H), 1.85-1.56 (m, 7 H), 1.35 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 174.13, 171.89, 142.98, 137.10, 128.71, 127.03, 126.63, 116.12, 53.78, 51.36, 46.25, 36.08, 29.78, 29.65, 29.52, 28.74, 25.94, 21.94, 19.81.; MS (CI): *m/z* for $C_{23}H_{34}N_2O_2$: expected 370.3; found 393.8 (M+Na), 371.1 (M + 1). Compound **11**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.36 (t, J = 7.8 Hz, 2 H), 7.30 (d, J = 7.6 Hz, 2 H), 7.26 (m, 1 H), 7.41-7.20 (m, 5 H), 6.00 (brs, 1 H), 5.72 (brs, 1 H), 5.10-4.79 (m, 3 H), 3.15-2.96 (m, 1 H), 2.55-2.34 (m, 1 H), 2.34-2.18 (m, 1 H), 2.09-1.74 (m, 9 H), 1.71-1.53 (m, 3 H), 1.37 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 173.23, 172.11, 143.06, 136.91, 128.78, 126.91, 125.80, 116.12, 53.53, 51.46, 47.44, 37.15, 36.26, 30.27, 29.78, 28.72, 25.42, 22.45, 20.08.; MS (CI): *m/z* for $C_{23}H_{34}N_2O_2$: expected 370.3; found 393.2 (M+Na).



12

Step 2: synthesis of (1S,2S)-N-(tert-butyl)-1-(N-((S)-1-phenylethyl)acetamido)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentanecarboxamide (12). While under nitrogen, (1S,2R)-2-allyl-N-(tert-butyl)-1-(N-((S)-1-phenylethyl)acetamido) cyclopentanecarboxamide, **10** (1.00 g, 2.7 mmol) in anhydrous methylene chloride (14 mL) was

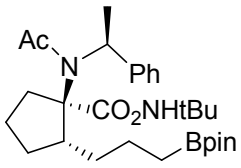
treated with chloro-1,5-cyclooctadiene iridium dimer (54 mg, 0.08 mmol, 3 mol%) and 1,2-bis(diphenylphosphino)ethane (64 mg, 0.16 mmol, 6 mol%). After stirring at room temperature for 30 min, the solution was cooled to -10 °C (ice/ethanol bath) and treated with pinacolborane (0.587 mL, 4.0 mmol) in a dropwise manner. After the addition was complete, the cooling bath was removed, and the mixture was allowed to warm to room temperature with continued stirring for 18 h. Water (4 mL) was added and stirring continued for 30 min, and the mixture extracted with ethyl acetate (30 mL, then 20 mL). The combined organic solution was washed successively with water and saturated aqueous sodium chloride (20 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (silica gel, 10-60% ethyl acetate in heptane) gave the subject compound (1.12 g, 83%) as a colorless viscous oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.58-7.49 (m, 2 H), 7.35 (t, *J* = 7.6 Hz, 2 H), 7.24 (t, *J* = 7.4 Hz, 1 H), 6.37 - 6.02 (brs, 1 H), 4.96 - 4.58 (m, 1 H), 3.04 - 2.95 (m, 1 H), 2.42 - 2.31 (m, 1 H), 2.11 - 1.97 (m, 1 H), 1.89 - 1.48 (m, 12 H), 1.37-1.26 (m, 11 H), 1.17 (d, *J* = 5.2 Hz, 12 H), 0.81 - 0.66 (m, 2 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 173.77, 172.17, 143.15, 128.85, 128.62, 126.97, 83.09, 53.38, 51.36, 46.79, 34.57, 29.80, 28.76, 28.65, 25.86, 24.98, 24.83, 23.48, 22.16, 19.30, 11.54. MS (CI): *m/z* for $C_{29}H_{47}BN_2O_4$: expected 498.4; found 521.3 (M+Na). Anal. Calcd for $C_{29}H_{47}BN_2O_4$: C 69.87; H, 9.50; N, 5.62. Found C, 69.50; H, 9.44; N, 5.63.



13

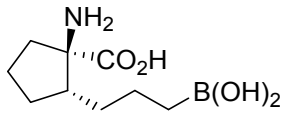
Step 3: synthesis of (1S,2S)-1-amino-2-(3-boronopropyl)cyclopentanecarboxylic acid (13). While under nitrogen, a cooled (-50 °C) solution of (1S,2S)-N-(tert-butyl)-1-(N-((S)-1-phenylethyl) acetamido)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentane

carboxamide (0.400 g, 0.802 mmol) in anhydrous tetrahydrofuran (5 mL) was treated with liquid ammonia (20 mL) and lithium wire (0.14 g, 20 mmol) in portions over several minutes. After 1.5 h at -40 to -50 °C, the deep blue reaction was quenched with solid ammonium chloride. Once the solution became colorless the cooling bath was removed allowing the reaction mixture to warm slowly to room temperature and the residual ammonia to evaporate over several hours. Water (3 mL) was added, and the mixture was washed with methylene chloride (3 x 30 mL). The remaining aqueous solution was concentrated. The resulting residue was dissolved in glacial acetic acid (1 mL), water (2 mL) and concentrated hydrochloric acid (1 mL), placed in a pressure bottle and stirred for 30 min at 60 °C, then capped, warmed to 130 °C with stirring for 18 h. After cooling to room temperature, the solution was diluted with water (20 mL), washed with toluene (20 mL) and concentrated. The crude product was purified by preparative HPLC to afford the subject compound (100 mg, 50%) as a white foam. ^{1}H NMR (600 MHz, D_2O): δ 2.40 (dt, J_1 = 14.4 Hz, J_2 = 8.5 Hz, 1 H), 2.08 (m, 2 H), 1.96-1.84 (m, 2 H), 1.83-1.70 (m, 1 H), 1.54-1.42 (m, 3 H), 1.36-1.25 (m, 1 H), 1.23-1.14 (m, 1 H), 0.77 (ddd, J_1 = 15.1 Hz, J_2 = 8.7 Hz, J_3 = 6.2 Hz, 1 H), 0.70 (ddd, J_1 = 15.3 Hz, J_2 = 9.2 Hz, J_3 = 6.2 Hz, 1 H); ^{13}C NMR (600 MHz, D_2O): δ 173.82, 68.08, 49.21, 34.85, 31.67, 30.63, 22.65, 22.43, 14.02.; MS (CI): *m/z* for $C_9H_{18}BNO_4$: expected 215.1; found 198.1 (M-H_2O+1). Anal. Calcd for $C_9H_{19}BClNO_4$: C 42.98; H, 7.61; N, 5.57. Found C, 42.69; H, 7.43; N, 5.60.



14

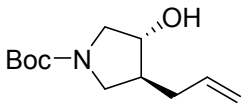
Preparation of (1R,2R)-1-amino-2-(3-boronopropyl)cyclopentane-1-carboxylic acid. Step 1: synthesis of N-((1R,2R)-1-(((tert-butylamino)oxy)carbonyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentyl)-N-((S)-1-phenylethyl)acetamide (14). While under nitrogen, (1R,2S)-2-allyl-N-(tert-butyl)-1-(N-((S)-1-phenylethyl)acetamido) cyclopentanecarboxamide(0.38 g, 1.03 mmol) in anhydrous methylene chloride (5.3 mL) was treated with chloro-1,5-cyclooctadiene iridium dimer (20 mg, 0.03 mmol, 3 mol%) and 1,2-bis(diphenylphosphino)ethane (24 mg, 0.06 mmol, 6 mol%). After stirring at room temperature for 30 min, the solution was cooled to -10 ^{o}C (ice/ethanol bath) and treated with pinacolborane (0.224 mL, 1.55 mmol) in a dropwise manner. After the addition was complete, the cooling bath was removed, and the mixture was allowed to warm to room temperature with continued stirring for 18 h. Water (3 mL) was added and stirring continued for 30 min, and the mixture extracted with ethyl acetate (20 mL, then 15 mL). The combined organic solution was washed successively with water (20 mL) and saturated aqueous sodium chloride (20 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (silica gel, 10-60% ethyl acetate in heptane) gave the subject compound (0.35 g, 68%) as a colorless viscous oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.44 - 7.13 (m, 5 H), 6.09 - 5.72 (brs, 1 H), 5.06 - 4.64 (m, 1 H), 3.17 - 2.92 (m, 1 H), 2.21 - 2.07 (m, 1 H), 2.05 - 1.75 (m, 9 H), 1.70 - 1.46 (m, 5 H), 1.42 - 1.30 (m, 10 H), 1.22 (d, J = 2.5 Hz, 12 H), 0.82 - 0.64 (m, 2 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 173.14, 172.65, 143.24, 128.70, 126.82, 125.85, 83.10, 53.51, 51.41, 48.13, 34.69, 30.41, 28.75, 25.48, 25.04, 24.99, 24.91, 24.83, 23.49, 22.92, 20.29, 11.65.; MS (CI): *m/z* for $C_{29}H_{47}BN_2O_5$: expected 514.3; found 537.4 (M+Na). Anal. Calcd for $C_{29}H_{47}BN_2O_4$: C 69.87; H, 9.50; N, 5.62. Found C, 70.39; H, 9.46; N, 5.99.



15

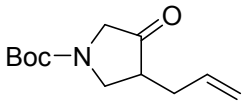
Step 2: synthesis of (1R,2R)-1-amino-2-(3-boronopropyl)cyclopentane-1-carboxylic acid (15). While under nitrogen, a cooled (-50 °C) solution of (N-((1R,2R)-1-(((tert-butylamino)oxy)carbonyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)cyclopentyl)-N-((S)-1-phenylethyl)acetamide (0.200 g, 0.402 mmol) in anhydrous tetrahydrofuran (5 mL) was treated with liquid ammonia (20 mL) and sodium (0.11 g, 43 mmol) in portions over several minutes. After 1.5 h at -40 to -50 °C, the deep blue reaction was quenched with solid ammonium chloride. Once the solution became colorless the cooling bath was removed allowing the reaction mixture to warm slowly to room temperature and the residual ammonia to evaporate over several hours. Water (3 mL) was added, and the mixture was washed with methylene chloride (3 x 30 mL). The remaining aqueous solution was concentrated. The resulting residue was dissolved in glacial acetic acid (1 mL), water (2 mL) and concentrated hydrochloric acid (1 mL), placed in a pressure bottle and stirred for 30 min at 60 °C, then capped, warmed to 130 °C with stirring for 18 h. After cooling to room temperature, the solution was diluted with water (20 mL), washed with toluene (20 mL) and concentrated. The crude product was purified by preparative HPLC to afford the subject compound (48 mg, 56%) as a white foam. ^{1}H NMR (600 MHz, D_2O): δ 2.40 (dt, J_1 = 14.4 Hz, J_2 = 8.5 Hz, 1 H), 2.08 (d, J_1 = 7.1 Hz, J_2 = 2.3 Hz, 2 H), 1.96-1.84 (m, 2 H), 1.83-1.70 (m, 1 H), 1.54-1.42 (m, 3 H), 1.36-1.25 (m, 1 H), 1.23-1.14 (m, 1 H), 0.77 (ddd, J_1 = 15.1 Hz, J_2 = 8.7 Hz, J_3 = 6.2 Hz, 1 H), 0.70 (ddd, J_1 = 15.3 Hz, J_2 = 9.2 Hz, J_3 = 6.2 Hz, 1 H); ^{13}C NMR (600 MHz, D_2O): δ 173.82, 68.08, 49.21, 34.85, 31.67, 30.63, 22.65, 22.43, 14.02.; MS (CI): *m/z*

for $C_9H_{18}BNO_4$: expected 215.1; found (M-H_2O+1): 198.1.; Anal. Calcd for $C_9H_{19}BClNO_4$: C 42.98; H, 7.61; N, 5.57. Found C, 42.69; H, 7.43; N, 5.60.



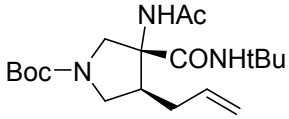
17

Preparation of (3R,4S)-3-amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acid (23). Step 1: synthesis of tert-butyl-trans-3-allyl-4-hydroxypyrrolidine-1-carboxylate (17). Allyl magnesium bromide (1,037 mL, 713 mmol, 0.69 M in diethyl ether) was cooled to 0 ^oC and carefully treated with *tert*-butyl 6-oxa-3-azabicyclo[3.1.0]hexane-3-carboxylate (60 g, 323.9 mmol) in anhydrous diethyl ether (324 mL, 1 M). After the addition was complete, the reaction mixture was stirred for 15 min, slowly quenched with saturated aqueous ammonium chloride (500 mL), extracted with diethyl ether (2 x 400 mL), dried over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (20-40% ethyl acetate in heptane) gave *tert*-butyl-*trans*-3-allyl-4-hydroxypyrrolidine-1-carboxylate **17** (64.33 g, 87% yield) as a pale-yellow oil. ^1H NMR (600 MHz, $CDCl_3$): δ 5.83-5.73 (m, 1 H), 5.06 (d, J = 17.6 Hz, 1 H), 5.04 (d, J = 10.8 Hz, 1 H), 4.04 (q, J = 4.9 Hz, 1 H), 3.58 (td, J_1 = 11.8 Hz, J_2 = 6.3 Hz, 2 H), 3.22 (dd, J_1 = 11.8 Hz, J_2 = 4.2 Hz, 1 H), 3.07 (dd, J_1 = 11.2 Hz, J_2 = 5.5 Hz, 1 H), 2.37 (brs, 1 H), 2.19 (dt, J_1 = 13.7 Hz, J_2 = 6.7 Hz, 1 H), 2.16-2.08 (m, 1 H), 2.02 (dt, J_1 = 14.4 Hz, J_2 = 7.4 Hz, 1 H), 1.44 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 154.86, 135.91, 116.94, 79.58, 74.55, 52.65, 48.96, 45.40, 35.82, 28.62.; MS (CI): *m/z* for $C_{12}H_{21}NO_3$: expected 227.2; found 250.1 (M+Na). Anal. Calcd for $C_{12}H_{21}N_1O_3$: C 63.41; H, 9.31; N, 6.16. Found C, 63.69; H, 9.59; N, 6.02.



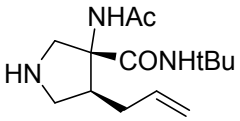
18

Step 2: synthesis of tert-butyl 3-allyl-4-oxopyrrolidine-1-carboxylate (18). While under nitrogen, an ice-cooled solution of *tert*-butyl 3-allyl-4-hydroxypyrrolidine-1-carboxylate **17** (64.33 g, 283 mmol) and diisopropylethylamine (142 mL, 858 mmol) in dichloromethane (282 mL, 1 M) was treated dropwise with a solution of sulfur trioxide pyridine complex (101.8 g, 640 mmol) in anhydrous DMSO (281.5 mL) at a rate to keep the reaction mixture below 10 °C. After the addition was complete, the mixture was stirred at 3 °C for 45 min, quenched with water (41 mL) and extracted with ether (500 mL, then 2 x 300mL). The combined organic solution was washed twice with water (100 mL), once with saturated aqueous sodium chloride (100 mL), dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (dichloromethane) gave *tert*-butyl 3-allyl-4-oxopyrrolidine-1-carboxylate **18** (58 g, 91% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.72 (td, J_1 = 17.0 Hz, J_2 = 7.0 Hz, 1 H), 5.09 (d, J = 17.1 Hz, 1 H), 5.07 (d, J = 10.2 Hz, 1 H), 4.10-3.94 (m, 1 H), 3.93-3.81 (m, 1 H), 3.66 (brd, J = 19.1 Hz, 1 H), 3.30 (dd, J_1 = 11.3 Hz, J_2 = 8.4 Hz, 1 H), 2.66 (brs, 1 H), 2.53 (dt, J_1 = 14.8 Hz, J_2 = 5.5 Hz, 1 H), 2.53 (dt, J_1 = 15.4 Hz, J_2 = 8.1 Hz, 1 H), 1.46 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 212.52 (211.99), 154.44, 134.16, 117.83, 80.49, 53.27 (52.83), 48.29 (47.70), 47.40 (46.91), 32.96, 28.50. Anal. Calcd for $C_{12}H_{19}N_1O_3$: C 63.98; H, 8.50; N, 6.22. Found C, 63.75; H, 8.67; N, 6.19.



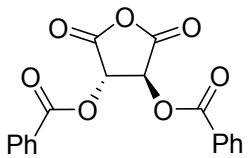
19

Step 3: synthesis of anti-tert-butyl 3-acetamido-4-allyl-3-(tert-butylcarbamoyl)pyrrolidine-1-carboxylate (19). A solution of tert-butyl 3-oxo-4-(prop-2-en-1-yl)pyrrolidine-1-carboxylate **18** (79.3 g, 0.352 mol) and ammonium acetate (135.7 g, 0.704 mol) in methanol (200 mL) was cooled to 0 ^{o}C and treated with *tert*-butylisocyanide (80.2 mL, 0.704 mol). After stirring at 0 ^{o}C for 2 h, the solution was allowed to warm to room temperature with continued stirred for 48 h. Once complete the solution was concentrated to remove most of the methanol, diluted with ethyl acetate (200 mL) and water (100 mL) and stirred for 1 h. The resulting solid was suction filtered and successively washed with water (1 x 100 mL) and cold ether (2 x 50 mL) to give the desired product as a 10 to 1 mixture of *anti* and *syn* isomers. The solid was suspended in a solution of ethyl acetate (400 mL), isopropyl ether (400 mL) and ethanol (2 mL) and warmed to 70 ^{o}C with stirring. After all the solid dissolved, stirring was discontinued, and the solution was allowed to slowly cool to room temperature overnight. The resulting precipitate was filtered, washed with ether (2 x 50 mL) and dried to give the desired isomer as a white powder **19** (82.1g, 63%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.49 (brs, 1 H), 6.37 (brs, 1 H), 5.70 (td, J_1 = 17.0 Hz, J_2 = 7.0 Hz, 1 H), 5.05 (d, J = 18.5 Hz, 1 H), 5.03 (d, J = 10.9 Hz, 1 H), 4.02 (d, J = 11.7 Hz, 1 H), 3.74 (d, J = 11.7 Hz, 1 H), 3.66 (t, J = 9.6 Hz, 1 H), 3.17 (brs, 1 H), 3.12-3.04 (m, 1 H), 2.24 (dt, J_1 = 13.3 Hz, J_2 = 5.2 Hz, 1 H), 2.01 (s, 3 H), 1.89-1.81 (m, 1 H), 1.46 (s, 9 H), 1.35 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 170.63, 169.26, 154.47, 135.39, 117.18, 80.32, 67.14, 51.71, 51.37, 48.93, 41.16, 33.06, 28.74, 28.53, 24.37.; MS (CI): *m/z* for $C_{19}H_{33}N_3O_4$: expected 367.3; found: 390.2 (M +Na), 268.2. (M-Boc+1). Anal. Calcd for $C_{19}H_{33}N_3O_4$: C 62.10; H, 9.05; N, 11.43. Found C, 62.39; H, 9.30; N, 11.51.



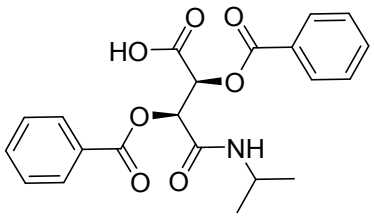
20

Step 4: synthesis of (3R,4S)-3-acetamido-4-allyl-N-(tert-butyl)pyrrolidine-3-carboxamide (20). A solution of boc-protected amine **19** (20.3 g, 55.24 mmol) in dichloromethane (400 mL) was cooled to 0 °C and treated with trifluoroacetic acid (80 mL, 19.8 mmol) dropwise via addition funnel. After the addition was complete, the solution was allowed to warm to room temperature with continued stirring until no starting material remained (approximately 2 h). The solution was concentrated, then charged with toluene and re-concentrated (3 x) to remove any excess trifluoroacetic acid. The resulting solid was dissolved in methanol (400 mL) and treated with DOWEX 550A-OH resin (approximately 120 g pre-washed with H_20 and methanol). After stirring for 2 hours the solution (pH 8.5) was filtered, concentrated, re-dissolved in dichloromethane and concentrated to the free base as a white foam. 14.35g (97%). ^{1}H NMR (600 MHz, CD_3OD): δ 5.81 - 5.09 (m, 1 H), 5.07 (d, J = 17.1 Hz, 1 H), 5.03 (d, J = 10.2 Hz, 1 H), 3.74 (dd, J_1 = 12.0 Hz, J_2 = 1.5 Hz, 1 H), 3.15 (dd, J_1 = 10.8 Hz, J_2 = 8.1 Hz, 1 H), 2.97 (d, J = 11.9 Hz, 1 H), 2.68 (dd, J_1 = 11.1 Hz, J_2 = 7.1 Hz, 1 H), 2.41-2.32 (m, 1 H), 2.31-2.22 (m, 1 H), 1.98 (s, 3H), 1.88 (td, J_1 = 12.7 Hz, J_2 = 8.3 Hz, 1 H), 1.33 (s, 9H); ^{13}C NMR (600 MHz, CD_3OD): δ 173.51, 172.00, 137.49, 117.03, 69.86, 56.14, 52.24, 50.97, 48.64, 35.15, 28.88, 22.80.; MS (CI): *m/z* for $C_{14}H_{25}N_3O_2$: expected 267.2; found: 268.2 (M +1).



(**1**)

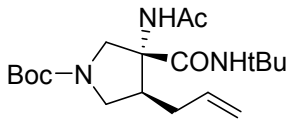
Step 5: synthesis of (3S,4S)-2,5-dioxotetrahydrofuran-3,4-diyl dibenzoate. A suspension of (+)-2,3-dibenzoyl-D-tartaric acid (300 g, 358.3 mmol) in acetic anhydride (600 mL) was warmed to 85 °C with stirring. After 2 h, the solution was cooled in an ice bath and the resulting suspension was filtered, washed with 1:1 hexanes / diethyl ether (500 mL) and dried in vacuo to afford (3S,4S)-2,5-dioxotetrahydrofuran-3,4-diyl dibenzoate (239 g, 84% yield) as a white crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08-8.06 (m, 4 H) 7.67-7.63 (m, 2 H) 7.51-7.47 (m, 4 H) 5.98 (s, 2 H).



(**2**)

Step 6: synthesis of (2S,3S)-2,3-bis(benzoyloxy)-4-(isopropylamino)-4-oxobutanoic acid. A solution of (3S,4S)-2,5-dioxotetrahydrofuran-3,4-diyl dibenzoate (88 g, 258.6 mmol) in ethyl acetate (132 mL) and THF (132 mL) was cooled to 0 °C and carefully treated with 2-aminopropane (26.6 mL, 310.3 mmol). Once the addition was complete, the ice bath was removed, and the solution stirred for an additional 2 h, then sequentially washed with 2 N HCl, and saturated aqueous sodium chloride, dried over sodium sulfate, filtered and concentrated. The crude product was recrystallized from methyl tert-butylether and hexanes to give (2S,3S)-2,3-bis(benzoyloxy)-4-(isopropylamino)-4-oxobutanoic acid (97.4 g, 94% yield) as a white powder. ^{1}H NMR (400MHz,

$CDCl_3$) δ 8.05-8.01 (m, 4 H) 7.67-7.51 (m, 2 H) 7.48-7.40 (m, 4 H) 6.02 (d, *J* = 3.4 Hz, 1 H) 5.98 (d, *J* = 3.4 Hz, 1 H) 4.12-4.04 (m, 1 H) 1.09 (d, *J* = 6.6 Hz, 3 H) 1.06 (d, *J* = 6.6 Hz, 3 H).

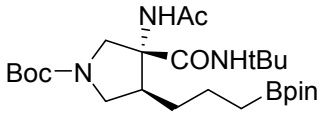


21

Step 7: synthesis of tert-butyl (3R,4S)-3-acetamido-4-allyl-3-(tert-butylcarbamoyl)pyrrolidine-1-carboxylate (21). A solution of racemic (*anti*)-3-acetamido-4-allyl-N-(tert-butyl)pyrrolidine-3-carboxamide (1.65 g, 6.17 mmol) in isopropanol (20 mL) was treated with a second solution of (2S,3S)-2,3-bis(benzoyloxy)-4-(isopropylamino)-4-oxobutanoic acid (2.46 g, 6.17 mmol) in warm 55% methanol/isopropanol (30 mL). After the solutions were combined and allowed to cool to ambient temperature, the desired salt slowly crystalized from the solution. After about 48 h the resulting crystalline material was filtered, washed with an ice-cooled solution of 33% methanol/isopropanol and dried to give the crude diastereomeric salt as a white crystalline solid. The crude salt was dissolved in a bi-phasic solution of ethyl acetate (25 mL) and saturated aqueous $NaHCO_3$ (25 mL), and treated with di-*tert*-butyl dicarbonate (1.01 g, 4.63 mmol). After stirring for 16-24 h, the organic layer was separated, filtered through a short pad of silica gel eluted with 30% ethyl acetate/hexane then 100% ethyl acetate and concentrated to give *tert*-butyl (3R,4S)-3-acetamido-4-allyl-3-(tert-butylcarbamoyl)pyrrolidine-1-carboxylate (**21**) as a white solid (0.87g, 77%-theoretical, 99.7% ee). Samples were analyzed by HPLC using a Gilson 215 Liquid Handler equipped with a PrepELS II Detector, Daicel Corporation Chiralpak IB 5µm (4.6

mm x 250 mm) column using 10% ethanol/ hexane, isocratic over 12 minutes with a flow rate of 1 mL/min.

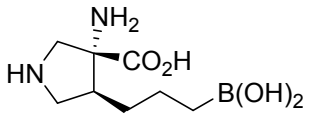
^{1}H NMR (600 MHz, $CDCl_3$): δ 6.69-6.31 (m, 2 H), 5.69 (td, J_1 = 16.9 Hz, J_2 = 7.0 Hz, 1 H), 5.04 (d, J = 17.1 Hz, 1 H), 5.02 (d, J = 10.1 Hz, 1 H), 3.96 (d, J = 11.7 Hz, 1 H), 3.76 (d, J= 11.8 Hz, 1 H), 3.63 (brs, 1 H), 3.33-2.96 (m, 2 H), 2.23 (dt, J_1 = 13.9 Hz, J_2 = 5.0 Hz, 1 H), 2.00 (s, 3 H), 1.84 (dt, J_1 = 14.1 Hz, J_2 = 9.1 Hz, 1 H), 1.44 (s, 9 H), 1.34 (s, 9 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 170.57, 169.30, 154.46, 135.38, 117.19, 80.36, 66.97, 51.75, 51.50, 48.94, 41.13, 33.07, 28.76, 28.54, 24.41. MS (CI): *m/z* for $C_{19}H_{33}N_3O_4$: expected 367.3; found: 390.2 (M +Na), 268.2 (M-Boc+1). Anal. Calcd for $C_{19}H_{33}N_3O_4$: C 62.10; H, 9.05; N, 11.43. Found C, 61.94; H, 9.01; N, 11.32.



22

Step 8: synthesis of (3R,4S)-3-acetamido-N-tert-butyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-3-carboxamide (22). While under nitrogen, a stirred solution of (3R,4S)-tert-butyl 3-acetamido-4-allyl-3-(tert-butylcarbamoyl)pyrrolidine-1-carboxylate (5.51 g, 15 mmol), chloro-1,5-cyclooctadiene iridium dimer (0.252 g, 0.375 mmol) and 1,2-bis(diphenylphosphino)ethane (0.299 g, 0.75 mmol) in anhydrous methylene chloride (80 mL) was cooled to -20 ^{o}C and treated with pinacolborane (3.30 mL, 22.5 mmol) dropwise. Once the addition was complete, the solution was placed in an ice bath and allowed to reach room temperature overnight (18 h). The mixture was quenched with water (75 mL), stirred 15 min, and extracted with ethyl acetate (400 mL, then 2 x 100 mL). The combined organic solution was washed with saturated aqueous sodium chloride (150 mL), dried over $MgSO_4$, filtered and concentrated.

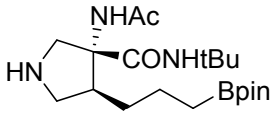
The resulting solid was recrystallized (2 crops) from acetonitrile to afford (3R,4S)-tert-butyl 3-acetamido-3-(tert-butylcarbamoyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-1-carboxylate (6.13g, 82%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): 6.50 (brs, 1 H), 6.24 (s, 1 H), 3.97 (brs, 1 H), 3.74 - 3.65 (m, 2 H), 3.17 - 2.88 (m, 2 H), 1.99 (s, 3 H), 1.44 (s, 9 H), 1.47 - 1.36 (m, 2 H), 1.36 - 1.29 (m, 1 H), 1.32 (s, 9 H), 1.21 (s, 12 H), 1.17 - 1.06 (m, 1 H), 0.77 (ddd, J_1 = 14.8 Hz, J_2 = 8.4 Hz, J_3 = 6.0 Hz, 1 H), 0.70 (ddd, J_1 = 15.6 Hz, J_2 = 9.1 Hz, J_3 = 6.4 Hz, 1 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 170.22, 169.58, 154.41, 83.12, 80.24, 51.66, 49.40, 41.82, 31.21, 28.76, 28.55, 24.94, 24.41, 22.73, 11.44. MS (CI): *m/z* for $C_{25}H_{46}BN_3O_6$: expected 495.4; found: 518.3 (M +Na), 396.3 (M-Boc+1); Anal. Calcd for $C_{25}H_{46}BN_3O_6$: C 60.60; H, 9.36; N, 8.48. Found C, 60.86; H, 9.58; N, 8.54.



23

Step 9: synthesis of (3R,4S)-3-amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acid (23). A solution of (3R,4S)-3-acetamido-N-tert-butyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-3-carboxamide (198 mg, 0.5 mmol) in glacial acetic acid (2 mL), water (2 mL) and concentrated hydrochloric acid (4 mL) in a pressure bottle was stirred for 2 h at 60 °C, then capped and heated to 130 °C for 18 h. After cooling to room temperature, the bottle was diluted with water (20 mL), washed with methylene chloride (20 mL) and concentrated. The resulting residue was re-dissolved in water (20 mL) and concentrated to remove excess hydrochloric acid, then dissolved in water (40 mL) and treated with DOWEX® 550A-OH resin (3 g) which had been rinsed with methanol. The mixture was stirred for 40 min, then filtered and the resin washed successively with water, methanol, methylene chloride, water, methanol, and methylene chloride.

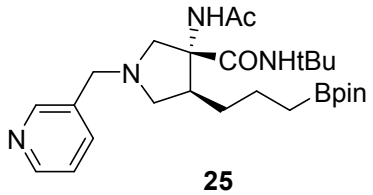
The resin was stirred with 1N HCl (15 mL) for 20 min and filtered. The filtrate was concentrated and purified by preparative HPLC (5-25% deionized water in acetonitrile with 0.1% trifluoroacetic acid). The product-containing fractions were concentrated, dissolved in 1 N HCl (10 mL) and concentrated (3x), then dissolved in water (10 mL), frozen, and lyophilized overnight to afford the subject compound (114 mg, 79%) as an off-white solid. ^{1}H NMR (600 MHz, D_2O): δ 4.02 (d, J = 13.0 Hz, 1 H), 3.85 (dd, J_1 = 11.8 Hz, J_2 = 8.3 Hz, 1 H), 3.60 (d, J =13.0 Hz, 1 H), 3.33 (t, J =11.8 Hz, 1 H), 2.75 - 2.66 (m, 1 H), 1.75 - 1.64 (m, 1 H), 1.49 - 1.34 (m, 1 H), 1.34 - 1.22 (m, 1 H), 0.78 (m, 2 H); ^{13}C NMR (600 MHz, D_2O): δ 170.36, 64.69, 50.52, 48.92, 44.94, 29.31, 22.01, 13.98. MS (CI): *m/z* for $C_8H_{17}BN_2O_4$: expected 216.1; found: 199.1 (m - H_2O + 1).



24

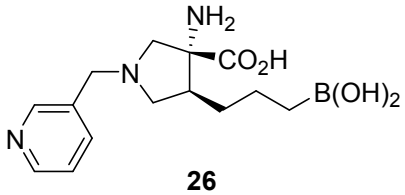
Synthesis of (3R,4S)-3-amino-4-(3-boronopropyl)-1-(pyridin-3-ylmethyl)pyrrolidine-3-carboxylic acid (26). Step 1: synthesis of (3R,4S)-3-acetamido-N-(tert-butyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-3-carboxamide (24). While under nitrogen, an ice-cooled solution of tert-butyl (3R,4S)-3-(tert-butylcarbamoyl)-3-acetamido-4-[3-(tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]pyrrolidine-1-carboxylate (10 g, 20.18 mmol) in anhydrous dichloromethane (200 mL) was treated with trifluoroacetic acid (50 mL, 673 mmol). After stirring for 3 h the solution was concentrated, diluted with toluene (10 mL) and re-concentrated (3x). The resulting oil was triturated with ether to give the subject compound as a trifluoroacetic acid salt (10.28 g, 20.18mmol, 100%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 6.85 (s, 1 H), 4.29 (d, J = 12.1 Hz, 1 H), 3.61 (dd, J_1 = 11.5 Hz, J_1 = 7.9 Hz, 1 H), 3.11 (d, J = 11.8 Hz, 1 H), 3.09 (d, J = 10.6 Hz, 2 H), 2.44 (tdd, J_1 = 10.9 Hz, J_2 = 7.9 Hz, J_3 = 3.0 Hz, 1

H), 2.03 (s, 3 H), 1.70 (tdd, J_1 = 10.3 Hz, J_2 = 7.9 Hz, J_3 = 3.1 Hz, 1 H), 1.51-1.38 (m, 1 H), 1.34 (s, 9 H), 1.24 (s, 12 H), 0.85 (dt, J_1 = 14.5 Hz, J_2 = 7.1 Hz, 1 H), 0.75 (dt, J_1 = 16.1 Hz, J_2 = 8.1 Hz, 1 H). ^{13}C NMR (600 MHz, CD_3OD): δ 174.23, 170.59, 163.04, 117.16, 84.43, 67.61, 54.74, 52.80, 47.23, 32.04, 28.84, 25.21, 25.13, 23.75, 22.49, 12.13.; MS (CI): *m/z* for $C_{20}H_{38}BN_3O_4$: expected 395.3; found: 396.3 (M +1). Anal. Calcd for $C_{22}H_{39}BF_3N_3O_6$: C 51.88; H, 7.72; N, 11.19. Found C, 51.65; H, 7.48; N, 8.28.



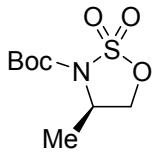
25

Step 2: synthesis of (3R,4S)-3-acetamido-N-(tert-butyl)-1-(pyridin-3-ylmethyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-3-carboxamide (25). A solution of (3R,4S)-N-tert-butyl-3-acetamido-4-[3-(tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]pyrrolidine-3-carboxamide trifluoroacetic acid salt (300 mg, 0.59 mmol) in 1,2-dichloroethane 6.5 mL was treated with triethylamine (0.245 mL), three drops of acetic acid and 3-pyridinecarboxaldehyde (110 ul, 1.17 mmol). After stirring for 30 min, sodium triacetoxyborohydride (0.375 g, 1.77 mmol) was added and stirring was continued for 18 hr. Once complete, the mixture was basified with saturated aqueous sodium carbonate (5 mL) and extracted with ethyl acetate (15 mL, 3x). The combined extracts were washed with water and saturated aqueous sodium chloride (25 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was used in the subsequent step without further purification.



26

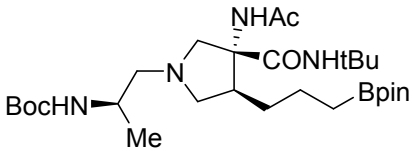
Step 3: synthesis of (3R,4S)-3-amino-4-(3-boronopropyl)-1-(pyridin-3-ylmethyl)pyrrolidine-3-carboxylic acid (26). A solution of the crude reductive amination product **25** in glacial acetic acid (2 mL), water (2 mL) and concentrated hydrochloric acid (4 mL) in a pressure bottle was stirred for 2 h at 60 °C, then capped and heated to 130 °C for 18 h. After cooling to room temperature, the bottle was uncapped, diluted with water (20 mL), washed with toluene (20 ml) and concentrated. The resulting residue was then purified by preparative HPLC. The product-containing fractions were concentrated, dissolved in 1 N HCl (10 mL) and re-concentrated (3x), then dissolved in water (10 mL), frozen, and lyophilized to afford the subject compound (211 mg, 86%) as an off-white solid. ^{1}H NMR (600 MHz, D_2O): δ 9.09 (d, J = 1.9 Hz, 1 H), 8.94 (d, J = 6.0 Hz, 1 H), 8.86 (dt, J_1 = 8.2 Hz, J_2 = 1.7 Hz, 1 H), 8.22 (dd, J_1 = 8.2 Hz, J_2 = 5.8 Hz, 1 H), 4.92 (d, J =13.7 Hz, 1 H), 4.83 (d, J = 13.8 Hz, 1 H), 4.13 (d, J = 13.1 Hz, 1 H), 3.97-3.88 (m, 2 H), 3.54 (t, J = 11.7 Hz, 2 H) , 2.75-2.60 (m, 1 H), 1.75-1.632 (m, 1 H), 1.44-1.23 (m, 3 H), 0.83-0.67 (m, 2 H); ^{13}C NMR (600 MHz, D_2O): δ 170.39, 148.98, 142.94, 142.84, 129.85, 128.14, 64.67, 59.02, 57.26, 55.03, 44.86, 29.22, 21.98, 13.94. MS (CI): *m/z* for $C_{14}H_{22}BN_3O_4$: expected 307.2; found: 332.2 (M+i-PrOH-2H_2O+1), 290.2 (M-H2O+1), 272.1 (M-2H_2O+1).



27

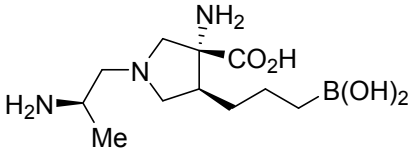
Synthesis of (3R,4S)-3-amino-1-((R)-2-aminopropyl)-4-(3-boronopropyl)pyrrolidine-3-carboxylic acid (29). Step 1: synthesis of tert-butyl (R)-4-methyl-1,2,3-oxathiazolidine-3-carboxylate 2,2-dioxide (27). While under nitrogen, a solution of (*R*)-*N*-Boc alaninol (1.2 g, 6.8 mmol), imidazole (1.88 g, 27.6 mmol), and triethylamine (2.06 mL, 14.7 mmol) in anhydrous dichloromethane (55 mL) was cooled to -50 ^{o}C and carefully treated thionyl chloride (0.56 mL, 7.7 mmol). After the addition was complete, the reaction mixture was warmed to 0-5 ^{o}C and stirred for an additional 4 h, quenched with ice-cooled water and extracted with dichloromethane (2x). The combined organic layer was washed successively with water and saturated aqueous sodium chloride, dried over sodium sulfate and concentrated to give the intermediate sulfuramidite as a pale-yellow oil (1.54 g) that was used immediately in the subsequent step.

The sulfuramidite (1.54 g, 6.8 mmol) in acetonitrile (54 mL) and water (10 mL) was cooled to 0 ^{o}C (ice bath) and sequentially treated with sodium (meta)periodate (1.7 g, 8.16 mmol) and ruthenium (III) chloride hydrate (14 mg, 0.062 mmol, 1 mol%). After 10 min the cooling bath was removed and stirring was continued for 20 min. Once complete, the mixture was diluted with saturated sodium bicarbonate (30 mL) and extracted with ethyl acetate (50 mL, 2x). The combined organic layer was dried over sodium sulfate, filtered and concentrated. Purification by flash column chromatography afforded the subject compound (1.43 g, 88%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.66 (dd, J_1 = 9.1 Hz, J_2 = 6.0 Hz, 1 H), 4.41 (pd, J_1 = 6.3 Hz, J_2 = 2.9 Hz, 1 H), 4.19 (dd, J_1 = 9.1 Hz, J_2 = 2.9 Hz, 1 H), 1.55 (s, 9 H), 1.50 (d, J = 6.4 Hz, 3 H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 148.59, 85.52, 71.44, 53.94, 28.09, 18.47. Anal. Calcd for $C_8H_{15}NO_5S$: C 40.50; H, 6.37; N, 5.90. Found C, 40.58; H, 6.45; N, 5.92.



28

Step 2: synthesis of tert-butyl ((R)-1-((3R,4S)-3-acetamido-3-(tert-butylcarbamoyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidin-1-yl)propan-2-yl)carbamate (28). A solution of (3R,4S)-N-tert-butyl-3-acetamido-4-[3-(tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]pyrrolidine-3-carboxamide (200 mg, 0.50 mmol) in CH_3CN (4 mL) was treated with (R)-tert-butyl 4-methyl-1,2,3-oxathiazolidine-3-carboxylate 2,2-dioxide (180 mg, 0.76 mmol) at room temperature. After stirring for 2 days, the solution was concentrated and used directly in the subsequent step without further purification.



29

Step 3: synthesis of (3R,4S)-3-amino-1-((R)-2-aminopropyl)-4-(3-boronopropyl)pyrrolidine-3-carboxylic acid (29). A solution of (3R,4S)-3-acetamido-1-((R)-2-aminopropyl)-N-(tert-butyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl)pyrrolidine-3-carboxamide in glacial acetic acid (2 mL), water (2 mL) and concentrated hydrochloric acid (4 mL) in a pressure bottle was stirred for 2 h at 60 °C, then capped and heated to 130 °C for 18 h. After cooling to room temperature, the bottle was diluted with water (20 mL), washed with toluene (20 mL) and concentrated. The resulting residue was purified by prep HPLC and lyophilized to give the subject compound (108 mg, 56%) as a white solid. ^{1}H NMR (600 MHz, D_2O): δ 4.17 - 4.02 (m, 3 H), 3.87 (d, J = 6.6 Hz, 1 H), 3.73 (dd, J_1 = 13.8 Hz, J_2 = 7.0 Hz, 1 H),

3.59 (dd, J_1 = 13.8 Hz, J_2 = 5.5 Hz, 1 H), 3.41 (t, J =11.8 Hz, 1 H), 2.85-2.77 (m, 1 H), 1.74 -1.66 (m, 1 H), 1.44 (d, J = 6.8 Hz, 3 H), 1.44 -1.28 (m, 3 H), 0.84 -0.70 (m, 2 H). ^{13}C NMR (600 MHz, D_2O): δ 170.13, 64.63, 59.96, 58.11, 58.09, 45.03, 43.89, 28.99, 21.96, 16.49, 13.92. MS (CI): *m/z* for $C_{11}H_{24}BN_3O_4$: expected 273.19; found 298.6 (M+i-PrOH-2H_2O+1), 281.6 (M+i-PrOH-2H_2O-NH_2+1), 256.5 (M-H_2O+1), 238.5 (M-2H_2O+1), 221.4 (M-2H_2O-NH2+1).

Pharmacology. Enzyme Preparation. Recombinant human Arg I cloned in pET-24a (Novagen) was overexpressed and purified using the procedure described previously. [15,16] Briefly, E. coli BL21(DE3) strain (Novagen) transformed with the pET16b plasmid, was grown in LB medium at 37 °C for 3h. Protein expression was induced by the addition of 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) (Euromedex). The pellet from a 6 L culture was disrupted by sonication in 10 mM Tris·HCl pH 8.0, 10 mM NaCl, 1 mM β-mercaptoethanol, 1mM $MnSO_4$ and centrifuged at 4 °C. Partial purification of arginase I was done by heating at 60 °C for 20 min, based on the heat stability of this protein.[17] After heating, the protein was clarified by centrifugation at 45,000 rpm for 30 min. The supernatant was further purified in a hitrap SP FF column of 5 mL (GE Healthcare) equilibrated with 10 mM Tris·HCl pH 8.0, 10 mM NaCl, 1 mM β-mercaptoethanol, 1 mM $MnSO_4$ and eluted with a KCl gradient.

A recombinant fully active truncated form of human Arg II was constructed to circumvent aggregation problems observed with the wild-type enzyme.[18,19] The truncation variant ΔM1-V23/ΔH331-I354 (114 kDa trimer) was constructed by site-directed mutagenesis using mature human arginase II cDNA. The following primers were used to generate the variant: sense mutagenic primer, TACTAACATATGGTCCACTCCGTTGCTGTGATAGGAGCC and antisense mutagenic primer, ACTGTACTCGAGTCAATGCCCTCCTTCTCTTGTCTGACCAAAGCTTGAAGC. The

truncated variant cloned in pET23b was overexpressed as described for Arg I. The pellet from a 6 L culture was disrupted by sonication in 10 mM Tris·HCl pH 8.0, 10 mM NaCl, 1mM β-mercaptoethanol, 1 mM $MnSO_4$ and centrifuged at 4 °C. As for Arginase I, partial purification was performed by heating at 60 °C for 20 min. After heating, the protein was clarified by centrifugation at 45,000 rpm for 30 min. Then ammonium sulfate was added to the supernatant to 60% saturation and after centrifugation the pellet was resuspended in 10 mM Tris·HCl pH 8.0, 10 mM NaCl, 1 mM β-mercaptoethanol, 1 mM $MnSO_4$. The resuspended protein was dialyzed in the same buffer and then applied to an anion exchange, capto Q column of 25 mL (GE Healthcare) and eluted with a KCl gradient.

Enzymatic Assays. Arginase activity was determined spectrophotometrically in a plate reader by measuring the evolution of urea (arginase catalyzes the conversion of $_L$-arginine to $_L$-ornithine and urea) using an approach adapted from published methods[13]. Enzymatic parameters for activity of Arg I and Arg II preparations were measured by plotting the initial velocity of reaction at increasing concentration of substrate L-arginine, resulting in parameters of K_m = 10.1 mM and k_{cat} = 664 sec^{-1} for Arg I and K_m = 23.3 mM and k_{cat} = 817 sec^{-1} for Arg II, which are consistent with reported values in the literature[20]. Inhibition of arginase activity by program compounds was followed spectrophotometrically in a plate reader at 520 nm. Specifically, the compound to be tested was dissolved in DMSO at an initial concentration 50-fold greater than its final concentration in the plate well. A 10 µl aliquot of this compound stock solution was added to 90 µl of the enzyme assay buffer (0.1 M sodium phosphate pH 7.4, 130 mM NaCl, 1 mg/ml ovalbumin (OVA)). Solutions of Arg I and Arg II were also prepared in the enzyme assay buffer (above), resulting in an arginase stock solution at a final concentration of 1000 ng/ml.

To each well of a 96-well microtiter plate, 30 µl of enzyme, 10 µl of the test compound solution and 10 µl of enzyme substrate solution ($_L$-arginine + manganese sulfate) were added. For wells that were used as positive controls, only the enzyme and its substrate were added, while wells used as negative controls contained only enzyme and manganese sulfate. After incubating the microtiter plate at 37°C for 60 minutes, 50 µl of concentrated acetic acid was added to each well to stop the reaction. The urea reaction product was measured using a colorimetric assay, in which 150 µl of colorimetric reagent was added to each well. This reagent, which is made immediately before use, is prepared by combining 1:1 volumes of Reagent A (4 mM o-phthaldialdehyde in 50 mM boric acid, 1 M sulfuric acid, 0.03% Brij-35 (w/v)) with Reagent B (4 mM N-(1-Naphthyl)ethylene-diamine dihydrochloride in 50 mM boric acid, 1 M sulfuric acid, 0.03% Brij-35 (w/v)). After adding the colorimetric reagent, the microtiter plate was allowed to stand for 10 minutes at room temperature to allow color development. Inhibition of arginase is computed by measuring the optical density (OD) of the reaction mixture at 530 nm and normalizing the OD value to the percent inhibition that is observed in the control. The normalized OD is then used to generate a dose-response curve by plotting the normalized OD values against log [concentration] and using regression analysis to compute the IC_{50} values.

In several cases, the measured IC_{50} values for program compounds were found to be very low (1-10 nM), and in this tight binding regime, it is necessary to ensure that equilibrium has been achieved, and the concentration of enzyme is low enough to accurately measure apparent affinity of the inhibitor. To that end, IC_{50} values of the high affinity compounds were measured at increasingly lower enzyme concentration (from 2 nM to 0.2 nM) and longer incubation times (up to 2 hr) to ensure that all IC_{50} values were accurate. Fortunately, for compounds with reported

IC_{50} values of 1.5n M-10 nM, acceptable signal to noise values were still achievable under these conditions, resulting in robust determinations for IC_{50} values in the low nanomolar regime.

X-Ray crystallography. Crystallization of human Arg II.

Crystals of the human Arg II-inhibitor complexes were obtained by co-crystallization of Arg II with 2 mM of example **23** or **49**. Crystals were obtained by the vapor diffusion method at 4 °C. Protein was concentrated at 5 mg/mL in 10 mM Tris-HCl pH 8.0, 10 mM NaCl, 1 mM β-mercaptoethanol, 0.2 mM $MnSO_4$. A screen allowed us to obtain crystals in the H3 condition from Silver Bullets screen (Hampton Research) using Tacsimate in the reservoir. Crystals appeared in a few days and grew up to dimensions of 0.3 mm x 0.3 mm x 0.5 mm. They were cryoprotected with a reservoir solution containing 30% ethylene glycol prior to flash cooling in liquid nitrogen.

Data Collection and Refinement.

For crystals of Arg II-example **23** and **49** complexes X-ray data were collected at 2.20 Å resolution and 2.21 Å respectively at a home source. The crystals belong to the space group $P4_22_12$. All data were processed with HKL2000.[21]

Structural Refinement.

The structures of the two complexes Arg II-example 23 and 49 were solved by molecular replacement with AMORE using the structure of a rat arginase protein complexed with a boronic acid inhibitor (Protein Data Bank ID: 1D3V) as a search model. Further crystallographic refinement involved several repeated cycles of conjugate gradient energy minimization and temperature factor refinement, and these were performed with the CCP4 suite[22]. Amino acid side-chains were fitted into 2Fo-Fc and Fo-Fc electron density maps. The final Fo-Fc maps indicated a clear electron density for each different inhibitor. Water molecules were fitted into difference maps and riding H-

atoms were introduced in the final cycles. The programs Phenix[23] and Coot[24] were used for refinement and fitting the models to the electron density. The atomic coordinates for structures of Arg II in complex with examples 23 and 49 have been deposited in the PDB (PDB IDs: 6Q37 and 6Q39, respectively). The figures were built with the PyMOL Molecular Graphics System (Schrödinger LLC). Data collection and refinement statistics for example **23** and **49** co-crystalized with Arg II are included as supplementary material in SI table 2.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxx.

Molecular formula strings

Characterization data for all target compounds (^{1}H NMR, ^{13}C NMR, LC/MS and analytical HPLC summary data) and sulfamate intermediates (^{1}H NMR, ^{13}C NMR and elemental analysis) not described above

HPLC optical purity determination chromatograms for compound **21**

Example arginase inhibition IC_{50} curves

X-ray crystallography data collection and refinement statistics

Example 2D diagrams of contacts

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Abbreviations Used

The following is a listing of non-standard abbreviations used in this manuscript. ARG, arginase; Bpin, pinacolborane; ABH, 2-amino-6-boronohexanoic acid; nor-NOHA, *N*-hydroxy-nor-l-arginine; DiPhos, 1,2-bis(diphenylphosphino)ethane; $Ir_2Cl_2(COD)_2$, bis(1,5-cyclooctadiene)diiridium(I) dichloride; dppe, 1,2-bis(diphenylphosphino)ethane; ELSD, evaporative light scattering detector

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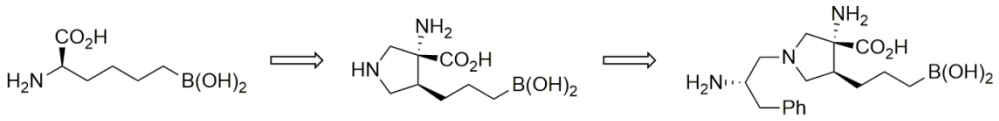

ABH

ArgI IC_{50}: 1,547 nM
ArgII IC_{50}: 2,551 nM

ArgI IC_{50}: 110 nM
ArgII IC_{50}: 440 nM

$ABPC_{APP}$

ArgI IC_{50}: 1.3 nM
ArgII IC_{50}: 8.1 nM

117x29mm (300 x 300 DPI)