Chemical tools to monitor and control human proteasome activities

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CHAPTER 11

Enantioselective synthesis of adamantylalanine and carboranylalanine and their incorporation into the proteasome inhibitor bortezomib*

Introduction

The ubiquitin proteasome system (UPS) is responsible for the degradation of 80-90% of the proteins in eukaryotic cells. E1-E2-E3 enzymes can modify a protein destined for degradation with a poly-ubiquitin chain, which is recognized by the 19S cap of the 26S proteasome. The 19S cap removes the ubiquitin chain and unfolds the protein, which is then translocated in the 20S core particle (CP), where proteolysis takes place.[1] The hollow cylindrical shaped CP is composed out of four heptameric rings: two outer α-rings and two inner β-rings. The catalytic activities resides within the β-subunits: β1 (caspase-like, cleaves after acidic residues), β2 (trypsin-like, cleaves after basic residues) and β5 (chymotrypsin-like, cleaves after hydrophobic residues). Next to the constitutively expressed cCP (β1c, β2c and β5c) subunits, immune cells also express β-subunits with slightly altered substrate specificities (iCP: β1i, β2i and β5i).[2] Two proteasome inhibitors (PIs) are currently used in the clinic for the treatment of multiple myeloma (MM) and mantle cell lymphoma, namely bortezomib (Btz, Figure 1) and carfilzomib (Cfz), and several PIs are being evaluated in clinical trials.[3] PIs can provide useful information on the substrate specificity of each β subunit. The S1 and S3 pockets mainly determine the substrate specificities for each β-subunit. Large (aromatic) ring structures are well tolerated by the S1 pocket of β5i and β5i and the S3 pocket of β5c and β5i.[4, 5] However, introducing an adamantylalanine at P1 resulted in complete loss of activity.[5] The crystal structure of bortezomib in complex with yeast proteasome showed a spacious, solvent exposed S2 pocket, in which the P2 phenylalanine lacked interactions with the protein.[6]

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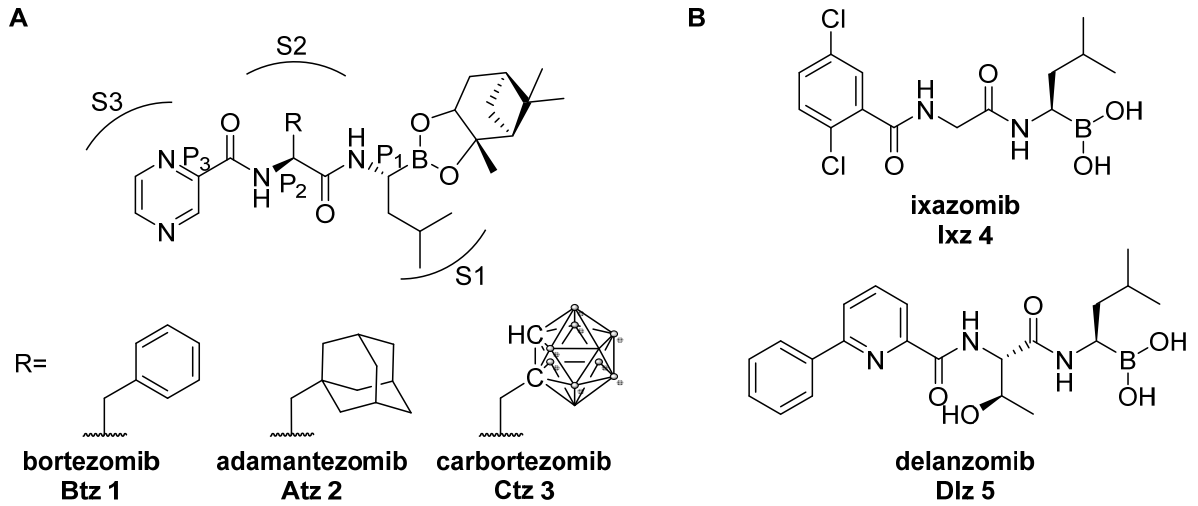


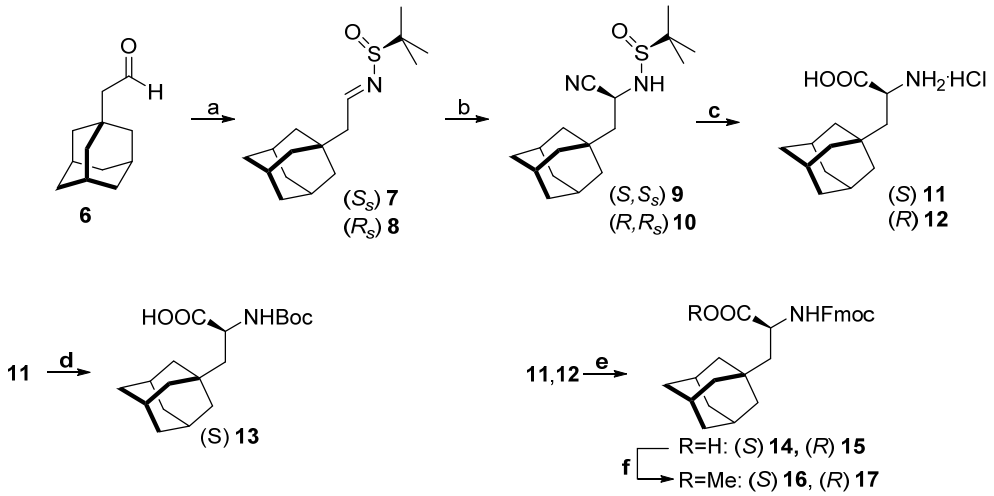
Figure 1. A) Structures of bortezomib **1** and the analogues adamantezomib **2** and carbortezomib **3** that are synthesized in this study. Enzyme pockets (S1, S2 and S3) and inhibitor residues (P1, P2 and P3) are indicated. B) Structures of boronic acid PIs currently in clinical trials: ixazomib **4** and delanzomib **5**.

Interestingly, ixazomib **4** (Ixz, P2: glycine) and to a lesser extent delanzomib **5** (Dlz, P2: threonine) (Fig. 1B) both show higher off-rates compared to Btz.[7] The high off-rate of Ixz is arguably responsible for its improved pharmacokinetic and pharmacodynamic properties.[8] This observation raised the question whether more sterically demanding moieties at P2 would affect binding properties of boronic acid PIs. To investigate this, Btz analogues were designed in which phenylalanine is replaced by adamantylalanine or *ortho*-carboranylalanine, leading to 'adamantezomib' (Atz) **2** and 'carbortezomib' (Ctz) **3** respectively. Carborane has roughly the same molecular volume as adamantane (148 Å vs. 136 Å), but is more hydrophobic.[9] Given their electronic structure, carboranes can be considered 'super-aromatic' and are therefore used as isosteres for phenyl groups. Due to their lipophilicity and stability, carboranes are often exploited to improve cell-permeability of lead compounds, and as a pharmacophore for hydrophobic interactions with receptors and proteins.[9] In addition, carboranes have been used in boron-neutron capture therapy (BNCT), a binary anti-cancer therapy based on the reaction between boron-10 and thermal neutrons, leading to high energy α-particles and lithium ions. When delivered in sufficient concentrations to the tumour, the energy released will ensure selective cell death in the tumour tissue.[10, 11] In this chapter, new synthetic routes towards adamantylalanine and carboranylalanine are described, which provided both amino acids in good yields and enantiomeric purity. These amino acids were incorporated in the Btz sequence and evaluated the resulting peptidic boronic esters as proteasome inhibitors.

Results and discussion

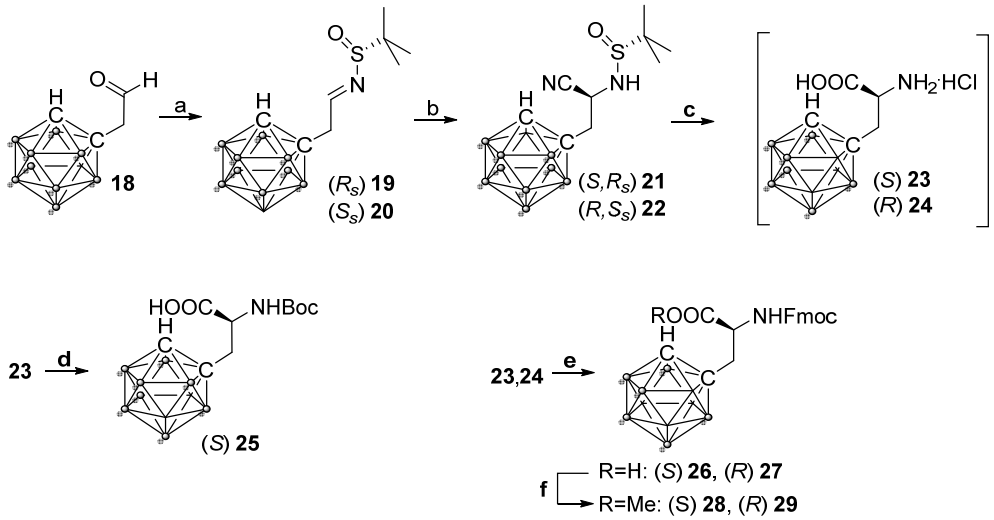
Synthesis

Two chiral auxiliaries have been reported in the asymmetric synthesis of adamantylalanine, namely (*S*)-α-methylbenzylamine[12] (poor enantiomeric excess) and sulfonamido-isobornane.[13] In addition, a moderate enantioselective conjugate addition of an adamantyl Grignard reagent to an optically active carbamatoacrylate has been reported[14] as well as the use of a chiral quaternary ammonium salt.[15] Also, various routes towards carboranylalanine have been reported, either starting from the highly toxic decaborane (the reaction of decaborane with propargyl glycine, recently optimized by Toppino *et al*.[16]) or starting from *ortho*-carborane, using either Evans' oxazolidinone[17] or Oppolzer's chiral camphorsultam[18] as the chiral auxiliaries. In this chapter, Ellman's N-*tert*-butylsulfinamide is used as the chiral auxiliary and the key step is the asymmetric Strecker reaction of a chiral imine.[19] The synthesis of adamantylalanine commenced with the condensation of aldehyde **6** with (*R)*- or (*S)*-N-*tert*-butyl-sulfinamide under Dean-Stark conditions providing imines **7** and **8** (Scheme 1).



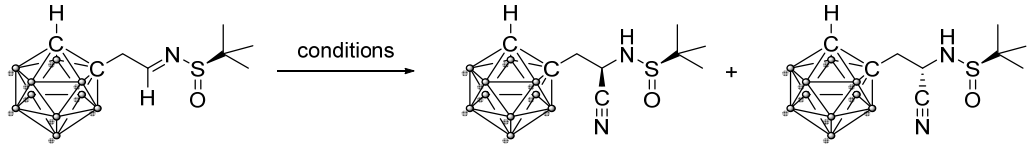
Scheme 1. Synthesis of (protected) adamantylalanine. Reagents and conditions: a. (*R)*- or (*S)*-N-*tert*-butyl-sulfinamide, toluene, 50°C, 100 mbar, **7** (S_s): 89%; **8** (R_s): 90%; b. Et_2AlCN, iPrOH, THF, **9** (*S,S*$_s$): 66%; **10** (*R,R*$_s$): 62%; *de*: 92%, >99% after crystallization; c. 6N HCl, reflux, **11** (*S*): 98%; **12** (*R*): 96%; d. Boc_2O, Na_2CO_3, H_2O, dioxane, 80%; e. FmocOSu, Na_2CO_3, H_2O, dioxane, **14** (*S*): 89%; **15** (*R*): 89%; f. $TMSCH_2N_2$, MeOH, **16** (*S*): 93%, 97.4 *ee*; **17** (*R*): 99%, 96.6 *ee.*

The asymmetric Strecker reaction using ethylisopropoxyaluminiumcyanide[20] (obtained by reacting Et_2AlCN with iPrOH) as the cyanide delivery agent yielded *syn* products **9** and **10** in 92% diastereomeric excess (*de*), which could be further increased by crystallization to >99% *de*. Subsequent hydrolysis under strong aqueous acidic conditions hydrolysed the nitrile and cleaved the chiral auxiliary, thereby providing amino acids **10** and **11** in high yield and purity.



Scheme 2. Synthesis of (protected) *ortho*-carboranylalanine. Reagents and conditions: a. *R*- or *S*-N-*tert*-butyl-sulfinamide, $CuSO_4$, DCM, **19** (R_s): 94%; **20** (S_s): 94%; b. CsF, TMSCN, DMF -50°C, **21** (S,R_s): 48%; **10** (R,S_s): 54%; *de*: 86%, >99% after crystallization; c. 6N HCl, reflux; d. Boc_2O, Et_3N, H_2O, THF, 85%; e. FmocOSu, Et_3N, H_2O, THF, (*S*): 94%; **15** (*R*): 81%; f. EDC·HCl, HOBT, MeOH, DCM, **28** (*S*): 79%, 85.5 *ee*; **29** (*R*): 78%, 90.5 *ee.*

Boc or Fmoc protection of adamantyalanine proceeded without any problem yielding compounds **13** (Boc), **14** and **15** (Fmoc). Fmoc protected compounds **14** and **15** were converted to the corresponding methyl esters **16** and **17** to allow chiral HPLC analysis, which revealed excellent enantiomeric excess (*ee*) for both enantiomers. Since condensation of carboranyl aldehyde **18**[21] with N-*tert* butylsulfinamide under Dean-Stark conditions resulted in significant enamine formation, the reaction was performed at room temperature in presence of an excess of $CuSO_4$, which dramatically increased the yield and prevented enamine formation (Scheme 2). Using the same conditions for the asymmetric Strecker reaction as used for the adamantylalanine synthesis resulted in a much lower diastereomeric ratio (*dr*) (Table 1, entry 1). Lewis acid catalyzed cyanation with $Sc(OTf)_3$ and TMSCN led to similar *syn* selectivity (Table 1, entry 2). The use of a catalytic amount of the Lewis base TBA-Ac at -78°C provided product **21b** in a good *dr*, (8:92, syn/anti, note the preference for *anti*-addition, Table 1, entry 3).[22] In order to further improve the *dr*, conditions reported by Li *et al.*, who used TMSCN and equimolar CsF at -50°C in *n*-hexane giving *syn*-addition, or in THF giving *anti* addition, were used.[23] As imine **19** is not soluble in *n*-hexane, the reaction was performed in THF, giving only poor selectivity with a slight preference for *anti*-addition product **21b** (Table 1, entry 4). Using a DMF/THF mixture at -60°C improved the *dr* (Table 1, entry 5). When only DMF was used at -50°C, the highest yield and *dr* (3:97, *syn/ anti;* 7:93 at larger scale) was obtained (Table 1, entries 6/7). Upon crystallization diastereomerically pure cyanosulfinamide **21b** was isolated in a reasonable yield. In the same manner sulfinimine **20** gave the mirror image **22**. The X-ray crystals structure confirmed the absolute stereochemistry of **21b** and **22** (Figure 2).[24]



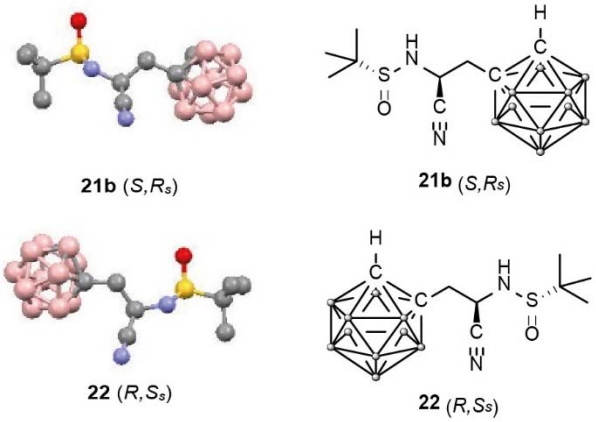
19 (R_S) **21a** (R,R_S) **21b** (S,R_S)

Entry	Reagents	Solvent	Temp (°C)	Yield (%)	*dr* (18a/18b)[a]
1	Et_2AlCN, iPrOH	THF	-78 to rt	78	71 : 29
2	TMSCN, $Sc(OTf)_3$	DCM	0 to rt	52	76 : 24
3	TMSCN, TBA-Ac[b]	DMF/THF (2:1)	-78	88	8 : 92
4	TMSCN, CsF[c]	THF	-50	n.d.	40 : 60
5	TMSCN, CsF[c]	DMF/THF (2:1)	-60	n.d.	7 : 93
6	TMSCN, CsF[c]	DMF	-50	92	3 : 97
7	TMSCN, CsF[d]	DMF	-50	98 (48)[e]	7 : 93 (1 < 99)
8	TMSCN, CsF[c]	Tol	-10	n.d.	67 : 33
9	TMSCN, CsF[c]	CH_2Cl_2	-10	n.d.	72 : 28

[a] The diastereomeric ratio was determined by ^{1}H-NMR, by comparing the integral of the CH_α. [b] 10 mol% TBA-Ac was added. [c] Conditions: 0.20 mmol **19**, 1.05 eq TMSCN, 1.05 eq CsF, 0.2M [d] Conditions: 3.5 mmol **19**, 2.2 eq TMSCN, 1.3 eq CsF, 0.2M [e] Between parentheses = yield of diastereomerically pure product after crystallization. n.d. = not determined.

Table 1. Optimization of the asymmetric Strecker reaction for the synthesis of carboranylalanine

Treatment of **21b** and **22** in refluxing aqueous 6N HCl yielded (*S*)- and (*R*)- carboranylalanine **23**/**24**, which could be converted into their Boc or Fmoc protected analogues without difficulties. Chiral HPLC analysis revealed excellent enantiomeric excess for both enantiomers. Both Boc-adamantylalanine and Boc-carboranylalanine were incorporated at the P2 position of Btz, using similar procedures as described before (see experimental).[25, 26] It was decided to leave the pinanediol protection in place, since it has been reported that boronic esters of this kind show similar activity as unprotected boronic acids.[25]



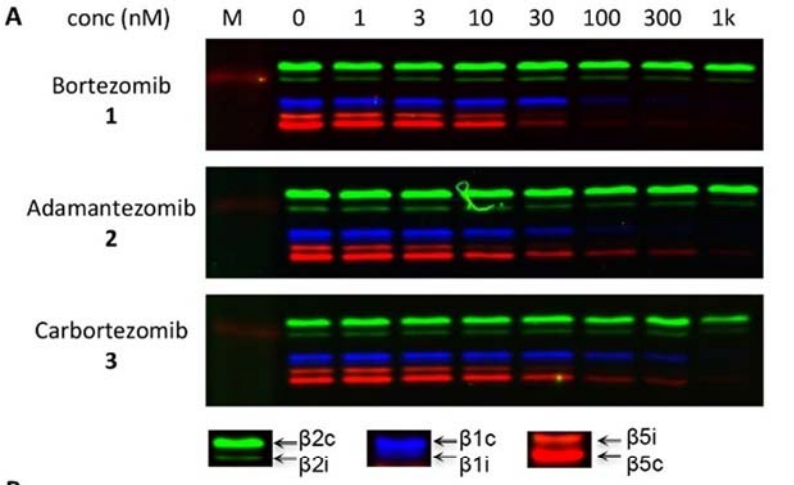
21b (S,R_S) **21b** (S,Rs)

22 (R,S_S) **22** (R,Ss)

Figure 2. X-ray crystal structures of (S,R_S)-cyanosulfinamide **21b** and (R,S_S)-cyanosulfinamide **22**.

Biological evaluation

The activity against all the cCP and iCP subunits was assessed by competitive activity-based protein profiling (ABPP) in lysate of Raji cells (human B-lymphoblastic cell line expressing all six active β subunits). In addition, the cell permeability and efficiency of proteasome inhibition was evaluated in living RPMI-8226 cells (MM cell line). Residual proteasome activity after incubation with inhibitors is labelled by the recently published activity-based probe (ABP) cocktail[7] (see chapter 2), which provides full resolution of all six active β subunits on SDS-PAGE (Figure 3). In Raji cell lysates, Atz closely resembles the activity of Btz, while Ctz shows slightly higher IC_{50} values for all β1 and β5 sites. All three inhibitors show a slight preference for β1i and β5i over their constitutive counterparts (Figure 3B). Evaluation in living RPMI-8226 cells showed similar potency of Atz and Ctz compared to Btz indicating good cell permeability (Figure 3). Interestingly, Atz and Ctz show a 4-5 fold preference for β5i over β5c, while Btz, Dlz and Cfz inhibit β5c and β5i with equal potency and Ixz shows only a <2 fold preference for β5i.



B

IC_{50} (nM) Raji cell-lysate	β1c	β1i	β2c	β2i	β5c	β5i
Bortezomib **1**	33	14	1447	666	15	9.4
Adamantezomib **2**	15	6.2	1352	304	18	6.0
Carbortezomib **3**	93	36	1330	526	54	16
RPMI-8226 (intact cells)						
Bortezomib **1**	64	24	>1000	>1000	14	12
Adamantezomib **2**	20	6.4	>1000	>1000	51	9.1
Carbortezomib **3**	137	60	>1000	>1000	44	11
Ixazomib*	35	18	>1000	>1000	31	18
Delanzomib*	19	2.1	>1000	>1000	3.9	2.4
Carfilzomib*	130	49	34	15	1.6	3.2

Figure 3. Determination of inhibitory potency of Btz 1, adamantezomib 2, and carbortemib 3 by ABPP. A) Inhibitory profiles in intact RPMI-8226 cells after 1 h treatment. B) IC_{50} values as determined by ABPP in Raji cell lysates and RPMI-8226 intact cells. M=protein marker. *IC_{50} values of ixazomib, delanzomib and carfilzomib have been determined by ABPP before.[7]

In addition, Ctz shows lower- and Atz higher potency towards the β1 subunits compared to Btz, whereas all inhibitors show a preference for β1i over β1c. To gain more insight in the off-rate of Atz and Ctz, a wash-out experiment was performed in which Atz and Ctz were compared to Btz (low off-rate) and Ixz (high off-rate).[7, 8]

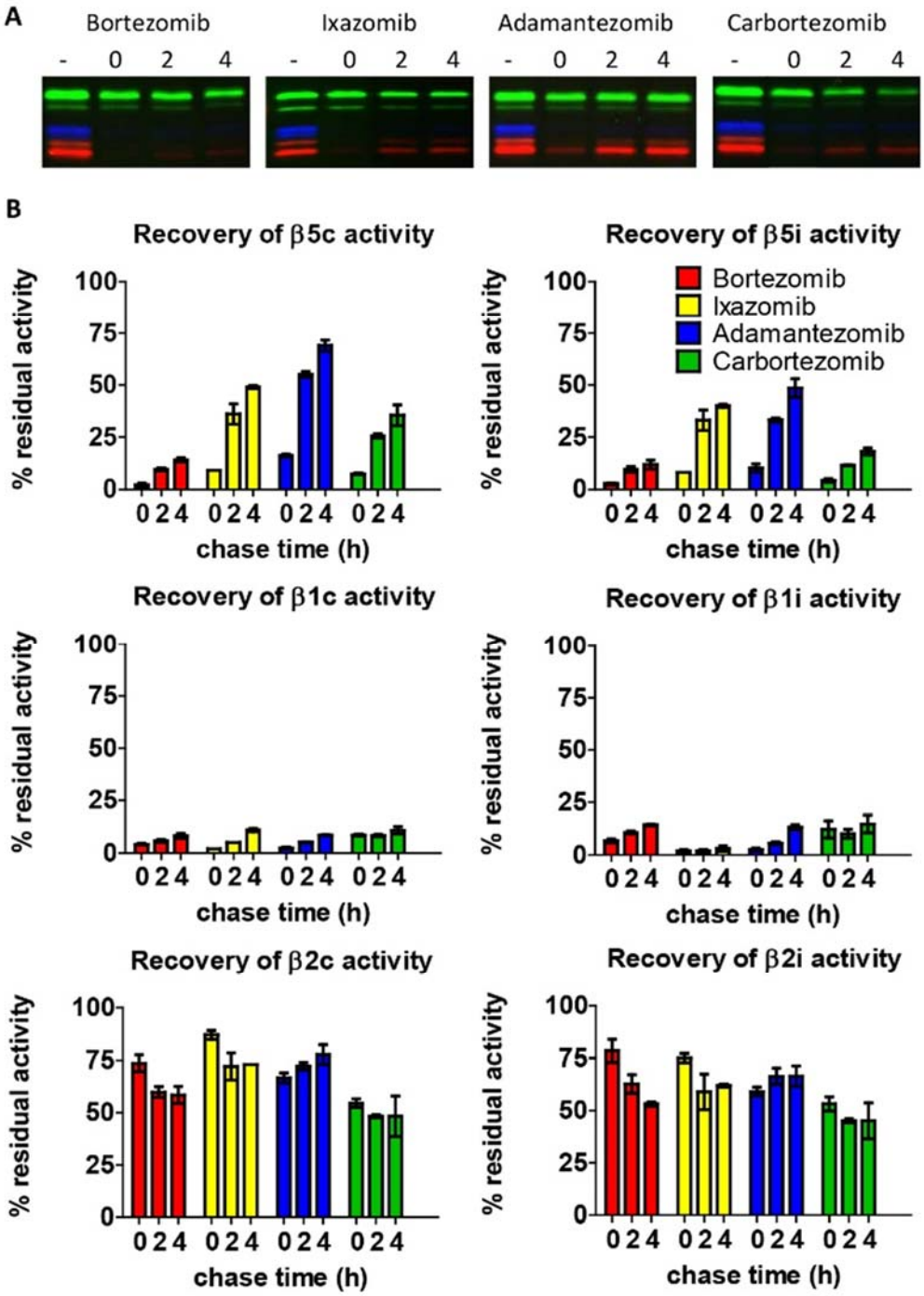


Figure 4. Inhibitor washout experiments. A) Inhibitory profilies of Btz 1, Ixz. Atz 2 and Ctz 3 in RPMI-8226 cells, which were treated with inhibitors for 1h, followed by washing of the cells and chase for 0, 2, or 4 h. B) Graphs show recovery for β5c and β5i. Error bars show ± SEM values. Quantifications of gel intensities have been corrected for gel loading (Coomassie). Wash-out data of Ixz used as determined before, see chapter 2.[7]

RPMI-8226 cells were incubated with the inhibitors in order to block the β1 and β5 sites.[27] Subsequently, the cells were washed and chased for multiple hours. The β5 sites show faster recovery when inhibited by Atz and Ctz, compared to Btz (Figure 4). Interestingly, Atz not only shows a much higher off-rate than Ctz for both β5 sites, it also shows faster recovery of the β5 activities than Ixz (69% vs. 49% for β5c after 4h). Remarkably, the off-rate of all compounds appears to be higher for β5c than β5i while the β1 subunits show comparable low recovery of activity for all inhibitors. The higher off-rate for β5c increases the β5i selectivity of Atz and Ctz. These data suggest that large P2 residues destabilize the inhibitor-proteasome-β5-subunit complexes.

Conclusion

This chapter describes new enantioselective synthetic routes for adamantylalanine and *ortho*-carboranylalanine. Incorporation of these amino acids at the P2 site of Btz led to potent proteasome inhibitors, with high off-rates and β5i selectivity. Atz and Ctz could thus be used to selectively block β5i with minimal co-inhibition of β5c. The incorporation of adamantylalanine or carboranylalanine at the P2 site of boronic acid PIs could be an important design parameter for the development of iCP selective PIs with similar pharmacodynamic and pharmacokinetic properties as Ixz. Finally, considering that carboranes are non-toxic and biologically stable, Ctz might also find its application in BNCT.

Experimental

Synthetic procedures

General procedures

Acetonitrile (ACN), dichloromethane (DCM), N,N-dimethylformamide (DMF), methanol (MeOH), diisopropylethylamine (DiPEA) and trifluoroacetic acid (TFA) were of peptide synthesis grade, purchased at Biosolve, and used as received. All general chemicals (Fluka, Acros, Merck, Aldrich, Sigma, Iris Biotech) were used as received. Traces of water were removed from reagents used in reactions that require anhydrous conditions by co-evaporation with toluene. Column chromatography was performed on Screening Devices b.v. Silica Gel, with a particle size of 40-63 µm and pore diameter of 60 Å. TLC analysis was conducted on Merck aluminium sheets (Silica gel 60 F254). Compounds were visualized by UV absorption (254 nm), by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid, a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water, or ninhydrin (0.75 g/L) and acetic acid (12.5 mL/L) in ethanol, where appropriate, followed by charring at ca. 150 °C. ^1H and ^{13}C-NMR spectra were recorded on a Bruker AV-400 (400 MHz) or AV-600 (600 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane, CD_3OD or $CDCl_3$ as internal standard. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile 50/50 (v/v) and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60,000 at m/z 400 (mass range m/z = 150-2,000) and dioctylpthalate (m/z = 391.28428) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Gemini C_{18} 50 × 4.60 mm column (detection at 200−600 nm) coupled to a Finnigan LCQ Advantage Max mass spectrometer with ESI. The applied buffers were H_2O, MeCN and 1.0% TFA in H_2O (0.1% TFA end concentration). Methods used are: 10→90% MeCN, 15.0 min (0→0.5 min: 10% MeCN; 0.5→10.5 min: gradient time; 10.5→12.5 min: 90% MeCN; 12.5→15.0 min: 90% → 10% MeCN), HPLC purification was performed on a Gilson HPLC system coupled to a C_4 Phenomenex Gemini 5µm 250×10 mm column and a GX281 fraction collector. Chiral HPLC analysis was performed using a Daicell Chiralcel OD column (250 x 5.4 mm) or a Chiralpak AD (250 x 5.4 mm), using hexane/isopropanol solvent mixtures, flowrate: 1 mL/min. All tested compounds are >95% pure on the basis of LC-MS and NMR. (1R)-4-(1-chloro-3-methyl(butyl)-2,9,9-trimethyl-3,5-dioxa-4-bora-tricyclo[6.1.1.02,6]decane[28], boronoleucine pinanediol ester[26] and bortezomib[28] were synthesized according to literature procedures.

Synthesis of (Fmoc/Boc-adamantylalanine(-OMe)

(S,E)-*N*-(2-(adamantan-1-yl)ethylidene)-*tert*-butyl-sulfinamide (7)

A solution of adamantylacetaldehyde (1.42 g, 8 mmol, 1 equiv.) and (*S*)-*tert*-butylsulfinamide (1.06 g, 8.8 mmol, 1.1 equiv.) in toluene (50 mL) was rotated overnight under continuous removal of water using a rotary evaporator (50°C, 100 mbar). After concentration, the crude product was purified by column chromatography (0→10% EtOAc:toluene) providing the title compound (2.02 g, 7.1 mmol, 90%). $[\alpha]_D^{20}$ = +253.8 (C=1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (t, *J* = 5.9 Hz, 1H), 2.34 – 2.14 (m, 2H), 1.93 (s, 3H), 1.72 – 1.46 (m, 12H), 1.16 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.37, 56.42, 50.17, 42.56, 42.18, 36.60, 33.50, 28.50, 28.42, 22.36. HRMS: calcd. for $C_{16}H_{28}NOS$ 282.18861 $[M+H]^+$; found 282.18854.

(R,E)-*N*-(2-(adamantan-1-yl)ethylidene)- *tert*-butyl-sulfinamide (8)

A solution of adamantylacetaldehyde (1.78 g, 10 mmol, 1 equiv.) and (*R*)-*tert*-butylsulfinamide (1.33 g, 11 mmol, 1.1 equiv.) in toluene (50 mL) was rotated overnight under continuous removal of water using a rotary evaporator (50°C, 100 mbar). After concentration, the crude product was purified by column chromatography (0→10% EtOAc:toluene) providing the title compound (2.50 g, 8.9 mmol, 89%). $[\alpha]_D^{20}$= -242.6 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (t, *J* = 5.9 Hz, 1H), 2.24 (m, 2H), 1.93 (s, 3H), 1.72 – 1.50 (m, 12H), 1.16 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.34, 56.41, 50.17, 42.57, 42.19, 36.64, 36.60, 33.50, 28.51, 28.42, 22.36. HRMS: calcd. for $C_{16}H_{28}NOS$ 282.18861 $[M+H]^+$; found 282.18849.

(S)-*N*-((S)-2-(adamantan-1-yl)-1-cyanoethyl)- *tert*-butyl-sulfinamide (9)

Et_2AlCN (1M in toluene, 28.3 mmol, 28.3 mL, 1.5 equiv.) was added to THF (55 mL), followed by the addition of iPrOH (57 mmol, 4.3 mL, 3 equiv.) resulting in some discolouration of the mixture, from red to slightly yellow. After stirring for 15 min, the Et_2AlCN/iPrOH solution was added in 25 min to a solution of sulfinamide **7** (5.32 g, 18.9 mmol, 1 equiv.) in THF (120 mL) at -78 °C. After stirring for 30 min at -78°C, the reaction mixture was let to warm up to RT. After stirring for 3 hour at RT, TLC showed full consumption of starting material. The reaction mixture was cooled to -78°C and quenched by the addition of 10% $NaHCO_3$ (40 mL). After warming up to RT, the mixture was diluted by $NaHCO_3$ and extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography (10→40% EtOAc:PE) provided the title compound (4.97 g, 16.1 mmol, 85%) as a 96:4 mixture of diastereomers. Recrystallization from DCM:n-hexane provided enantiomerially pure product (3.85 g, 12.5 mmol, 66%). $[\alpha]_D^{20}$= +25.8 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (td, *J* = 8.3, 5.2 Hz, 1H), 3.84 (d, *J* = 8.2 Hz, 1H), 2.01 – 1.93 (m, 3H), 1.84 (dd, *J* = 14.3, 8.3 Hz, 1H), 1.76 – 1.51 (m, 13H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.80, 57.03, 49.44, 42.33, 41.74, 36.74, 32.41, 28.45, 22.57. HRMS: calcd. for $C_{17}H_{29}N_2OS$ 309.19951 $[M+ H]^+$; found 309.19955

(R)-*N*-((R)-2-(adamantan-1-yl)-1-cyanoethyl)- *tert*-butyl-sulfinamide (10)

Et_2AlCN (1M in toluene, 11.85 mmol, 11.85 mL, 1.5 equiv.)was added to THF (24 mL), followed by the addition of iPrOH (23.7 mmol, 1.61 mL, 3 equiv.) resulting in some discolouration of the mixture, from red to slightly yellow. After stirring for 15 min, the Et_2AlCN/iPrOH solution was added in 25 min to a solution of sulfinamide **8** (2.29 g, 7.9 mmol, 1 equiv.) in THF (55 mL) at -78°C. After stirring for 30 min at -78 °C, the reaction mixture was let to warm up to RT. After stirring for 1 hour at RT, TLC showed full consumption of starting material. The reaction mixture was cooled to -78°C and quenched by the addition of 10% $NaHCO_3$ (20 mL). After warming up to RT, the mixture was diluted by $NaHCO_3$ and extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography (10→30% EtOAc:PE) provided the title compound (1.86 g, 6.0 mmol, 77%) as a 96:4 mixture of diastereomers. Recrystallization from DCM:n-hexane provided enantiomerially pure product (1.51 g, 4.9 mmol, 62%). $[\alpha]_D^{20}$= -25.8 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (td, *J* = 8.3, 5.2 Hz, 1H), 4.04 (d, *J* = 8.4 Hz, 1H), 1.95 (m, 3H), 1.82 (dd, *J* = 14.3, 8.2 Hz, 1H), 1.73 – 1.49 (m, 13H), 1.20 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.86, 56.97, 49.33, 42.25, 41.75, 36.69, 32.33, 28.39, 22.55. HRMS: calcd. for $C_{17}H_{29}N_2OS$ 309.19951 $[M+ H]^+$; found 309.19958

(S)-3-(adamantan-1-yl)-2-aminopropanoic acid hydrochloride (11)

Compound **9** (3.85 g, 12.5 mmol) was dissolved in 6N HCl (400 mL) and refluxed at 130°C overnight. The reaction mixture was cooled on ice, resulting in precipitation of the product. The precipitate was collected by filtration, washed with ice-cold water and dried under vacuum yielding the title product as a white solid (3.17 g, 12.2 mmol, 98%). $[\alpha]_D^{20}$= +16.8 (C=1, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 3.98 (t, *J* = 5.6 Hz, 1H), 2.00 (s, 3H), 1.89 – 1.38 (m, 14H). ^{13}C NMR (101 MHz, MeOD) δ 172.97, 49.85, 46.40, 42.93, 37.71, 33.31, 29.91. HRMS: calcd. for $C_{13}H_{22}NO_2$ $[M+ H]^+$ 224.16451; found 224.16451.

(R)-3-(adamantan-1-yl)-2-aminopropanoic acid (12)

Compound **10** (1.40 g, 4.5 mmol) was dissolved in 6N HCl (140 mL) and refluxed at 130°C overnight. The reaction mixture was cooled on ice, resulting in precipitation of the product. The precipitate was collected by filtration, washed with ice-cold water and dried under vacuum yielding the title product as a white solid (1.13 g, 4.4 mmol, 96%). $[\alpha]_D^{20}$= -16.6 (C=1, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 3.98 (t, *J* = 5.5 Hz, 1H), 2.00 (s, 3H), 1.89 – 1.37 (m, 14H). ^{13}C NMR (101 MHz, MeOD) δ 172.97, 49.87, 46.41, 42.94, 37.71, 33.32, 29.92. HRMS: calcd. for $C_{13}H_{22}NO_2$ [M+ H]$^+$ 224.16451; found 224.16454.

(S)-*N*-Boc-adamantylalanine-OMe (13)

To a solution of (*S*)-adamantylalanine **11** (1.0 g, 3.85 mmol, 1 equiv.) in H_2O (4.5 mL) at 0 °C was added Na_2CO_3 (466 mg, 8.01 mmol, 2.1 equiv.). After 5 min, Boc_2O (1.68 g, 7.7 mmol, 2.0 equiv.) in dioxane (13.2 mL) was added. After stirring overnight, LC-MS analysis showed incomplete conversion. Therefore, another 2 equiv.)alent Boc_2O (1.68 g, 7.7 mmol) in dioxane (9 mL) was added and after 2 h, LC-MS showed complete consumption of the starting material. The reaction mixture was diluted with H_2O and acidified to pH=4 using 0.5N HCl, followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4 and concentrated. Purification by column chromatography (0→5% MeOH/DCM) yielded the title compound (998 mg, 3.08 mmol, 80%). $[\alpha]_D^{20}$= -4.2 (C=0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.66 (s, 1H), 6.02 (s, 0.3H), 4.89 (d, *J* = 8.4 Hz, 0.7H), 4.38 (t, *J* = 7.7 Hz, 0.7H), 4.18 (s, 0.3H), 1.98 (s, 3H), 1.91 – 1.10 (m, 23H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.98, 155.28, 80.05, 49.75, 46.78, 42.24, 36.78, 32.59, 28.50, 28.31. HRMS: calcd. for $C_{18}H_{30}NO_4$ 324.21693 [M+H]$^+$; found 324.21695

(S)-*N*-Fmoc-adamantylalanine (14)

To a solution of (*S*)-adamantylalanine **11** (260 mg, 1 mmol, 1 equiv.) in H_2O (4.5 mL) and dioxane (3.3 mL) were added Fmoc-OSu (371 mg, 1.1 mmol, 1.1 equiv.) and Na_2CO_3 (222 mg, 2.1 mmol, 2.1 equiv.). After stirring overnight, the reaction mixture was diluted with H_2O and acidified to pH=2 using 1N HCl, followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4 and concentrated. Purification by column chromatography (0→2% MeOH/DCM) yielded the title compound (350 mg, 0.89 mmol, 89%). $[\alpha]_D^{20}$= -5.6 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (bs, 1H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 8.1 Hz, 2H), 7.39 (t, *J* = 7.0 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 2H), 5.19 (d, *J* = 8.8 Hz, 1H), 4.58 – 4.33 (m, 3H), 4.24 (t, *J* = 7.0 Hz, 1H), 1.98 (s, 3H), 1.80 – 1.22 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.87, 155.93, 143.99, 143.79, 141.40, 127.80, 127.15, 125.22, 125.14, 120.07, 67.19, 50.28, 47.27, 46.66, 42.43, 36.88, 32.78, 28.62. HRMS: calcd. for $C_{28}H_{32}NO_4$ [M+ H]$^+$ 446.23258; found 446.23254.

(R)-*N*-Fmoc-adamantylalanine (15)

To a solution of *R*-adamantylalanine **12** (260 mg, 1 mmol, 1 equiv.) H_2O (4.5 mL) and dioxane (3.3 mL) were added Fmoc-OSu (371 mg, 1.1 mmol, 1.1 equiv.) and Na_2CO_3 (222 mg, 2.1 mmol, 2.1 equiv.). After stirring overnight, the reaction mixture was diluted with H_2O and acidified to pH=2 using 1N HCl, followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4 and concentrated. Purification by column chromatography (0→2% MeOH/DCM) yielded the title compound (350 mg, 0.89 mmol, 89%). $[\alpha]_D^{20}$= +5.6 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (bs, 1H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.66 – 7.58 (m, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.31 (dd, *J* = 14.8, 7.4 Hz, 2H), 5.28 – 5.01 (m, 1H), 4.58 – 4.39 (m, 3H), 4.27 (t, *J* = 6.8 Hz, 1H), 2.01 (s, 3H), 1.82 – 1.28 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.11, 156.11, 144.10, 143.99, 141.57, 127.85, 127.21, 125.17 125.13, 120.10, 67.43, 50.54, 47.53, 46.87, 42.63, 37.05, 32.86, 28.84. HRMS: calcd. for $C_{28}H_{32}NO_4$ [M+ H]$^+$ 446.23258; found 446.23257.

(S)-*N*-Fmoc-adamantylalanine-OMe (16)

To a solution of Fmoc protected adamantyl-alanine **14** (50 mg, 0.11 mmol, 1 equiv.) in MeOH (1 mL) was added slowly added $TMSCH_2N_2$ (2M in hexanes, added until solution stayed clear, 7 equiv.) in total). After concentration

of the reaction mixture, the crude product was purified by column chromatography (0→10% EtOAc/PE) providing the title product (48 mg, 0.10 mmol, 93%). *ee*: 97.4% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralcell OD). $[\alpha]_D^{20}$= -5.8 (C=0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.35 – 7.28 (m, 2H), 5.11 (d, *J* = 8.9 Hz, 1H), 4.53 – 4.33 (m, 3H), 4.24 (t, *J* = 7.1 Hz, 1H), 3.73 (s, 3H), 1.97 (s, 3H), 1.77 – 1.29 (m, 14H). HRMS: calcd. for $C_{29}H_{34}NO_4$ 460.24824 $[M+H]^+$; found 460.24820.

(R)-*N*-Fmoc-adamantylalanine-OMe (17)

To a solution of Fmoc protected adamantylalanine **15** (50 mg, 0.11 mmol, 1 equiv.) in MeOH (1 mL) was added slowly added $TMSCH_2N_2$ (2M in hexanes, added until solution stayed clear, 9 equiv. in total). After concentration of the reaction mixture, the crude product was purified by column chromatography (0→10% EtOAc/PE) providing the title product (50 mg, 0.11 mmol, 99%). *ee*: 96.6% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralcell OD). $[\alpha]_D^{20}$ = +5.6 (C=0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.5, 1.0 Hz, 2H), 5.10 (d, *J* = 8.9 Hz, 1H), 4.55 – 4.32 (m, 3H), 4.24 (t, *J* = 7.1 Hz, 1H), 3.73 (s, 3H), 1.97 (s, 3H), 1.76 – 1.14 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.21, 155.60, 143.97, 143.78, 141.32, 127.69, 127.05, 125.12, 125.06, 119.98, 67.00, 52.36, 50.29, 47.22, 46.91, 42.39, 36.82, 32.64, 28.56. HRMS: calcd. for $C_{29}H_{34}NO_4$ 460.24824 $[M+H]^+$; found 460.24823).

Synthesis of (Fmoc/Boc-carboranylalanine(-OMe)

(*R*,E)- *N* -[2-(1',2'-Dicarba-*closo*-dodecaboranyl)ethylidene]-*tert*-butyl-sulfinamide (19)

To a solution of aldehyde **18** (0.96 g, 5.18 mmol, 1 equiv.) in dry DCM (25 mL) was added (*R*)-*tert*-butylsulfinamide (0.69 g, 5.7 mmol, 1.1 equiv.) and anhydrous $CuSO_4$ (2.65 g, 16.6 mmol, 3.2 equiv.) at rt under an argon atmosphere. TLC showed complete conversion of starting material after stirring overnight. The suspension was vacuum filtrated over a Whatman glass microfiber filter, washed with DCM and concentrated under reduced pressure. Flash column chromatography (5% → 30% EtOAc in pentane) afforded the sulfinimine **19** as a white powder (1.41 g, 4.88 mmol, 94%). $[\alpha]_D^{20}$ = -231.4 (*c* = 1.0, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (t, *J* = 5.3 Hz, 1H), 3.84 (s, 1H), 3.54 – 3.29 (m, 2H,), 3.16 – 1.48 (m, 10H), 1.21 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.29, 69.85, 60.55, 57.73, 42.69, 22.55. ^{11}B NMR (128 MHz, $CDCl_3$) δ -1.82, -4.92, -8.87, -11.31, -12.69. HRMS (m/z): calcd. for $C_8H_{24}B_{10}NOS$ 290.25777 $[M+H]^+$, found 290.25791.

(*S,* E)-*N*-[2-(1',2'-Dicarba-*closo*-dodecaboranyl)ethylidene]-*tert*-butyl-sulfinamide (20)

To a solution of aldehyde **18** (2.65 g, 14.2 mmol, 1 equiv.) in dry DCM (70 mL) was added (*S*)-*tert*-butylsulfinamide (1.89 g, 15.6 mmol, 1.1 equiv.) and anhydrous $CuSO_4$ (7.25 g, 45.4 mmol, 3.2 equiv.) at rt under an argon atmosphere. TLC showed complete conversion of starting material after stirring overnight. The suspension was vacuum filtrated over a Whatman glass microfiber filter, washed with DCM and concentrated under reduced pressure. Flash column chromatography (10% → 50% EtOAc in pentane) afforded the sulfinimine **20** as a white powder (3.86 g, 13.3 mmol, 94%). $[\alpha]_D^{20}$ = +239.0° (*c* = 1.0, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (t, *J* = 5.3 Hz, 1H), 3.82 (s, 1H), 3.51 – 3.28 (m, 2H), 3.21 – 1.50 (m, 10H), 1.21 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.29, 69.84, 60.52, 57.76, 42.72, 22.58. ^{11}B NMR (128 MHz, $CDCl_3$) δ -1.87, -4.94, -8.91, -11.36, -12.78. HRMS (m/z): calcd. for $C_8H_{24}B_{10}NOS$ $[M+H]^+$ 290.25777, found 290.25797.

(S_s)-(+)-*N*-[(*R*)-1-Cyano-2-(1',2'-dicarba-*closo*-dodecaboranyl)ethyl]-*tert*-butylsulfinamide (21)

Sulfinamide **19** (1.74 g, 6.00 mmol, 1 equiv.) was dissolved in dry DMF (30 mL) and cooled to -50 °C under an argon atmosphere. CsF (hygroscopic!) (1.00 g, 6.60 mmol, 1.1 equiv.) was added, followed by the dropwise addition of TMSCN (0.83 mL, 6.60 mmol, 1.1 equiv.). The mixture turned bright yellow and stirring was kept at -50 °C. After 24 h additional CsF (0.27 g, 1.80 mmol, 0.3 equiv.) was added as well as TMSCN (0.23 mL, 1.80 mmol, 0.3 equiv.). TMSCN (0.3 equiv.) was added two times more after 43 h and 67 h until TLC showed complete

conversion of the starting material after 71 h. The reaction was quenched with a sat. aq. NH_4Cl solution (50 mL) and water (100 mL) was added. The aqueous layer was extracted with EtOAc (3 x 150 mL) and the combined organic layers were washed with brine (1 x 400 mL), dried over $MgSO_4$, filtrated and concentrated by rotary evaporation. The product was co-evaporated three times with toluene to remove leftover DMF and purified by flash column chromatography (30% →60% EtOAc in pentane) to yield cyanosulfinamide **21** (1.77 g, 5.58 mmol, 93%) as a pale yellow powder in a diastereomeric ratio of 93:7 (anti/syn, determined by ^{1}H NMR). Recrystallization from EtOH/pentane at -20 °C afforded cyanosulfinamide 21 as white crystals (1.03 g, 3.24 mmol, 54%) as a single diastereomer (de ≥ 99%). $[\alpha]_D^{20}$ = +62.1° (*c* = 2.2, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 4.71 (s, 1H), 4.51 (dd, *J* = 9.1, 5.0 Hz, 1H), 3.00 (dd, *J* = 15.4, 9.2 Hz, 1H), 2.90 (dd, *J* = 15.4, 5.0 Hz, 1H), 3.21 – 1.43 (m, 10H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 119.19, 72.20 $_b$), 63.72, 58.24, 48.08, 42.22, 22.68. ^{11}B NMR (128 MHz, MeOD) δ -2.38, -5.01, -9.35, -11.70, -12.75. HRMS (m/z): calcd. for $C_9H_{24}B_{10}N_2OS$ $[M+H]^+$ 317.26863, found 317.26900.

(R_s)-(+)-*N*-[(*S*)-1-Cyano-2-(1',2'-dicarba-*closo*-dodecaboranyl)ethyl]-*tert*-butylsulfinamide (22)

Sulfinamide **20** (1.01 g, 3.50 mmol, 1 equiv.) was dissolved in dry DMF (18 mL) and cooled to -50 °C under an argon atmosphere. CsF (hygroscopic!) (0.69 g, 4.60 mmol, 1.3 equiv.) was added, followed by the dropwise addition of TMSCN (0.57 mL, 4.60 mmol, 1.3 equiv.). The mixture turned bright yellow and stirring was kept at -50 °C. After 24 h additional TMSCN (0.13 mL, 1.10 mmol, 0.3 equiv.) was added and stirring was maintained at -50 °C for two days. TMSCN (0.3 equiv.) was added two times more after 92 h and 97 h until TLC showed complete conversion of the starting material after 99 h. The reaction was quenched with a sat. aq. NH_4Cl solution (30 mL) and water (60 mL) was added. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (1 x 300 mL), dried over $MgSO_4$, filtrated and concentrated by rotary evaporation, which gave cyanosulfinamide 22 (1.09 g, 3.43 mmol, 98%) as an orange/yellow solid which was pure according to ^{1}H-NMR-analysis in a diastereomeric ratio of 93:7 (anti/syn, determined by ^{1}H NMR). Recrystallization from EtOH/pentane at -20 °C afforded cyanosulfinamide **22** as yellow crystals (0.54 g, 1.69 mmol, 48%) as a single diastereomer (de ≥ 99%). $[\alpha]_D^{20}$ = -59.4° (*c* = 2.2, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 4.70 (s, 1H), 4.50 (dd, *J* = 9.1, 5.0 Hz, 1H), 3.00 (dd, *J* = 15.4, 9.2 Hz, 1H), 2.89 (dd, *J* = 15.4, 5.0 Hz, 1H), 3.20 – 1.47 (m, 10H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 119.17, 72.15, 63.68, 58.21, 48.03, 42.18, 22.68. ^{11}B NMR (128 MHz, MeOD) δ -2.36, -4.97, -9.33, -11.71, -12.71. HRMS (m/z): calcd. for $C_9H_{25}B_{10}N_2OS$ $[M+ H]^+$ 317.26863, found 317.26905.

(*S*)-(-)-*N*-Boc-*o*-carboranylalanine (25)

Cyanosulfinamide **21** (520 mg, 1.64 mmol, 1 equiv.) was dissolved in 6N HCl (aq., 10 mL) at rt and refluxed overnight. The mixture was co-evaporated three times with toluene to remove all solvent to afford unprotected amino acid as the HCl salt **23**. Subsequently, the amino acid was redissolved in THF/water (8 mL, 1:1) at rt under an argon atmosphere and Boc_2O (537 mg, 2.46 mmol, 1.5 equiv.) and Et_3N (0.69 mL, 4.92 mmol, 3 equiv.) were added and the mixture was stirred overnight. The solvent was removed under reduced pressure and co-evaporated with toluene (3x). Flash column chromatography (100% DCM → 20% MeOH in DCM) gave the Boc protected amino acid **25** as an off-white powder (462 mg, 1.39, 85%). $[\alpha]_D^{20}$ = -19.7° (*c* = 2.3, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 4.52 (s, 1H), 4.15 (d, *J* = 8.8 Hz, 1H), 2.92 (d, *J* = 15.2 Hz, 1H), 2.64 (dd, *J* = 15.0, 10.6 Hz, 1H), 3.14 – 1.56 (m, 10H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 157.67, 80.90, 74.51, 63.18, 54.60, 39.55, 28.71. ^{11}B NMR (128 MHz, MeOD) δ -2.64, -5.55, -9.57, -11.40, -13.00. HRMS (m/z): calcd. for $C_{10}H_{26}B_{10}NO_4$ + CH_3CN $[M+CH_3CN+H]^+$ 373.31270, found 373.31299.

(*S*)-(-)-*N*-Fmoc-*o*-carboranylalanine (26)

Boc-carboranylalanine **25** (166 mg, 0.50 mmol, 1 equiv.) was dissolved in 4M HCl in dioxane (2.5 mL, 10 mmol, 20 equiv.) at rt and stirred for 1.5 h. The mixture was co-evaporated three times with toluene to remove all solvents to afford the unprotected amino acid as the HCl salt. Subsequently, the amino acid (80 mg, 0.30 mmol,

1 equiv.) was redissolved in THF/water (3 mL, 1:1) at 0°C under an argon atmosphere and FmocOSu (121 mg, 0.36 mmol, 1.2 equiv.) and Et_3N (125 µL, 0.90 mmol, 3 equiv.) were added. After 1h at 0°C the mixture was stirred overnight at rt. The reaction was acidified with 0.1M HCl (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (5% -> 20% MeOH in DCM) gave the Fmoc protected amino acid **26** as a clear oil (128 mg, 0.28 mmol, 94%). $[\alpha]_D^{20}$ = -9.6° (*c* = 2.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.74 (d, J = 7.5 Hz, 2H), 7.61 – 7.43 (m, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.28 (t, J = 7.3 Hz, 2H), 5.59 – 5.31 (m, 1H), 4.53 – 4.43 (m, 1H), 4.43 – 4.33 (m, 1H), 4.33 – 4.21 (m, 1H), 4.17 (t, J = 5.6 Hz, 1H), 3.68 (s, 1H), 2.99 (s, 12H), 2.89 (d, J = 13.6 Hz, 1H), 2.66 – 2.53 (m, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 174.17, 156.21, 143.47, 143.32, 141.39, 128.05, 127.29, 125.00, 120.23, 77.48, 77.16, 76.84, 71.69, 67.59, 61.26, 53.67, 47.04, 38.58. ^{11}B NMR (128 MHz, CDCl3) δ -2.01, -5.15, -9.15, -11.57. HRMS (m/z): calcd. for $C_{20}H_{28}B_{10}NO_4$ $[M+H]^+$ 454.30261, found 454.30215.

(*R*)-(+)-*N*-Fmoc-*o*-carboranylalanine (27)

Cyanosulfinamide **22** (250 mg, 0.80 mmol, 1 equiv.) was dissolved in 6M HCl (aq., 8 mL) at rt and refluxed overnight. The mixture was co-evaporated three times with toluene to remove all solvents to afford the unprotected amino acid as the HCl salt. Subsequently, the amino acid (106 mg, 0.40 mmol, 1 equiv.) was redissolved in THF/water (4 mL, 1:1) at 0°C under an argon atmosphere and FmocOSu (160 mg, 0.47 mmol, 1.2 equiv.) and Et_3N (167 µL, 1.2 mmol, 3 equiv.) were added. After 1h at 0°C the mixture was stirred overnight at rt. The reaction was acidified with 0.1M HCl (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (5% -> 20% MeOH in DCM) gave the Fmoc protected amino acid **27** as a clear oil (147 mg, 0.32 mmol, 81%). $[\alpha]_D^{20}$ = +9.5° (*c* = 2.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.73 (d, J = 7.5 Hz, 2H), 7.51 (d, J = 6.8 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.27 (t, J = 7.4 Hz, 2H), 5.63 – 5.46 (m, 1H), 4.52 – 4.40 (m, 1H), 4.40 – 4.28 (m, 1H), 4.28 – 4.19 (m, 1H), 4.19 – 4.09 (m, 1H), 3.67 (s, 1H), 3.10 – 1.42 (m, 10H), 2.86 (d, J = 14.5 Hz, 1H), 2.55 (dd, J = 15.3, 8.9 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 173.75, 156.34, 143.49, 143.31, 141.39, 128.07, 127.30, 125.00, 120.24, 77.48, 77.16, 76.84, 71.80, 67.60, 61.28, 53.66, 47.02, 38.54. ^{11}B NMR (128 MHz, CDCl3) δ -2.02, -5.06, -9.13, -11.49. ESI-HRMS (m/z): calcd. for $C_{20}H_{28}B_{10}NO_4$ $[M+H]^+$ 454.30261, found 454.30206.

(*S*)-(-)-*N*-Fmoc-*o*-carboranylalanine methyl ester (28)

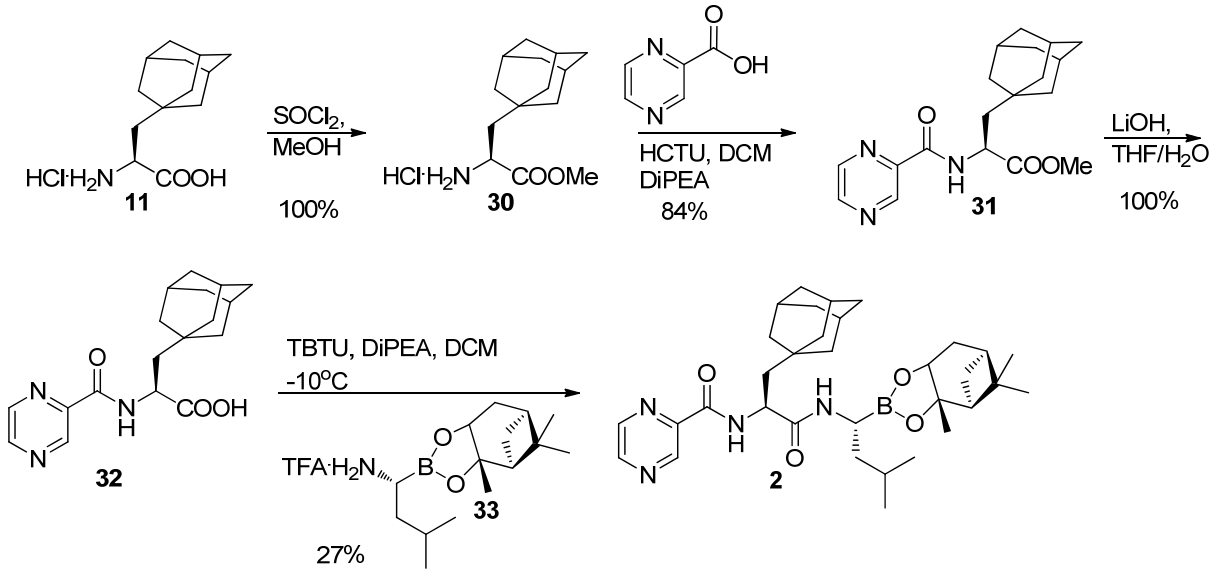
Fmoc-carboranylalanine **26** (114 mg, 0.25 mmol, 1 equiv.) was dissolved in DCM (2 mL) at 0°C under an argon atmosphere. HOBt (46 mg, 0.33 mmol, 1.3 equiv.), EDC·HCl (62 mg, 0.33 mmol, 1.3 equiv.) and MeOH (0.5 mL) were added and the reaction was stirred at rt overnight. The mixture was diluted with EtOAc (25 mL) and washed with 0.1M HCl (2 x 25 mL), sat. aq. $NaHCO_3$ (2 x 25 mL) and brine (1 x 25 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (10% -> 40% EtOAc in pentane) gave the fully protected amino acid **28** as a clear oil (92 mg, 0.20 mmol, 79%). $[\alpha]_D^{20}$ = -10.0° (*c* = 1.6, $CHCl_3$). *ee*: 90.6% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralpak AD). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 6.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.37 (d, J = 8.2 Hz, 1H), 4.57 – 4.46 (m, 1H), 4.46 – 4.37 (m, 1H), 4.31 (s, 1H), 4.20 (t, J = 6.3 Hz, 1H), 3.77 (s, 1H), 3.73 (s, 3H), 3.03 – 1.46 (m, 10H), 2.91 (d, J = 14.6 Hz, 1H), 2.61 (dd, J = 14.9, 7.9 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 170.54, 155.79, 143.61, 143.49, 141.44, 127.97, 127.25, 125.00, 120.19, 77.48, 77.16, 76.84, 71.72, 67.31, 60.96, 53.56, 53.31, 47.15, 39.07. ^{11}B NMR (128 MHz, CDCl3) δ -2.09, -5.01, -9.14, -11.41, -12.71. HRMS (m/z): calcd. for $C_{21}H_{30}B_{10}NO_4$ $[M+H]^+$ 468.31830, found 468.31741.

(*R*)-(+)-*N*-Fmoc-*o*-carboranylalanine methyl ester (29)

Fmoc-carboranylalanine **27** (102 mg, 0.22 mmol, 1 equiv.) was dissolved in DCM (2 mL) at 0°C under an argon atmosphere. HOBt (40 mg, 0.29 mmol, 1.3 equiv.), EDC·HCl (56 mg, 0.29 mmol, 1.3 equiv.) and MeOH (0.5 mL) were added and the reaction was stirred at rt overnight. The mixture was diluted with EtOAc (25 mL) and washed with 0.1M HCl (2 x 25 mL), sat. aq. $NaHCO_3$ (2 x 25 mL) and brine (1 x 25 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (10% -> 40% EtOAc in pentane) gave the fully protected amino acid **29** as a clear

oil (80 mg, 0.17 mmol, 78%). $[\alpha]_D^{20}$ = +10.6° (*c* = 1.6, $CHCl_3$). *ee*: 86.6% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralpak AD). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 6.5 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.38 (d, J = 8.3 Hz, 1H), 4.56 – 4.46 (m, 1H), 4.45 – 4.37 (m, 1H), 4.36 – 4.26 (m, 1H), 4.20 (t, J = 6.4 Hz, 1H), 3.77 (s, 1H), 3.74 (s, 3H), 3.13 – 1.36 (m, 10H), 2.91 (d, J = 13.2 Hz, 1H), 2.61 (dd, J = 15.4, 8.0 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 170.58, 155.78, 143.60, 143.48, 141.43, 127.97, 127.25, 125.00, 120.19, 77.48, 77.16, 76.84, 71.70, 67.31, 60.96, 53.54, 53.32, 47.13, 39.06. ^{11}B NMR (128 MHz, CDCl3) δ -2.08, -4.98, -9.15, -11.40, -12.64. HRMS (m/z): calcd. for $C_{21}H_{30}B_{10}NO_4$ $[M+H]^+$ 468.31830, found 468.31784.

Synthesis of adamantezomib



Scheme 3. Syntesis of adamantezomib **2** starting from enantiopure S-adamantylalanin*e*

(S)-methyl 3-(adamantan-1-yl)-2-aminopropanoate hydrochloride (30)

To a solution of (*S*)-adamantylalanine **11** (519 mg, 2 mmol, 1 equiv.) in MeOH (10 mL) was added $SOCl_2$ (435 µL, 6 mmol, 3 equiv.). After refluxing for 3 hours, the solvent was removed by evaporation providing the product in a quantitative yield. ^{1}H NMR (400 MHz, MeOD) δ 4.07 (t, *J* = 5.0 Hz, 1H), 3.84 (s, 3H), 1.99 (s, 3H), 1.89 – 1.65 (m, 7H), 1.63 – 1.49 (m, 7H). ^{13}C NMR (101 MHz, MeOD) δ 171.89, 53.79, 49.95, 46.16, 42.78, 37.64, 33.16, 29.80.

(S)-methyl 3-(adamantan-1-yl)-2-(pyrazine-2-carboxamido)propanoate (31)

To a solution of pyrazinecarboxylic acid (74 mg, 0.6 mmol, 1.2 equiv.) and HCTU (248 mg, 0.6 mmol, 1.2 equiv.) in DCM was added DiPEA (304 µL, 1.75 mmol, 3.5 equiv.). After stirring for 5 min, methylester **30** (137 mg, 0.5 mmol, 1 equiv.) was added and the resulting mixture was stirred for 3 hours. The reaction mixture was evaporated and dissolved in EtOAc and washed with 0.1N HCl (2x) and sat. $NaHCO_3$ (2x), dried over Na_2SO_4 and concentrated. Column chromatography (30→50% EtOAc:PE) provided the title compound (144 mg, 0.42 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (d, *J* = 1.1 Hz, 1H), 8.73 (d, *J* = 2.4 Hz, 1H), 8.59 – 8.47 (m, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 4.82 (td, *J* = 8.8, 3.3 Hz, 1H), 3.71 (s, 3H), 1.91 (s, 3H), 1.74 (dd, *J* = 14.6, 3.3 Hz, 1H), 1.58 (dt, *J* = 31.6, 11.9 Hz, 13H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.48, 162.48, 147.53, 144.54, 144.05, 142.79, 52.56, 48.50, 46.83, 42.31, 36.78, 32.73, 28.51. HRMS (m/z): calcd. for $C_{18}H_{26}N_3O_3$ $[M+H]^+$ 330.18122, found 330.18118

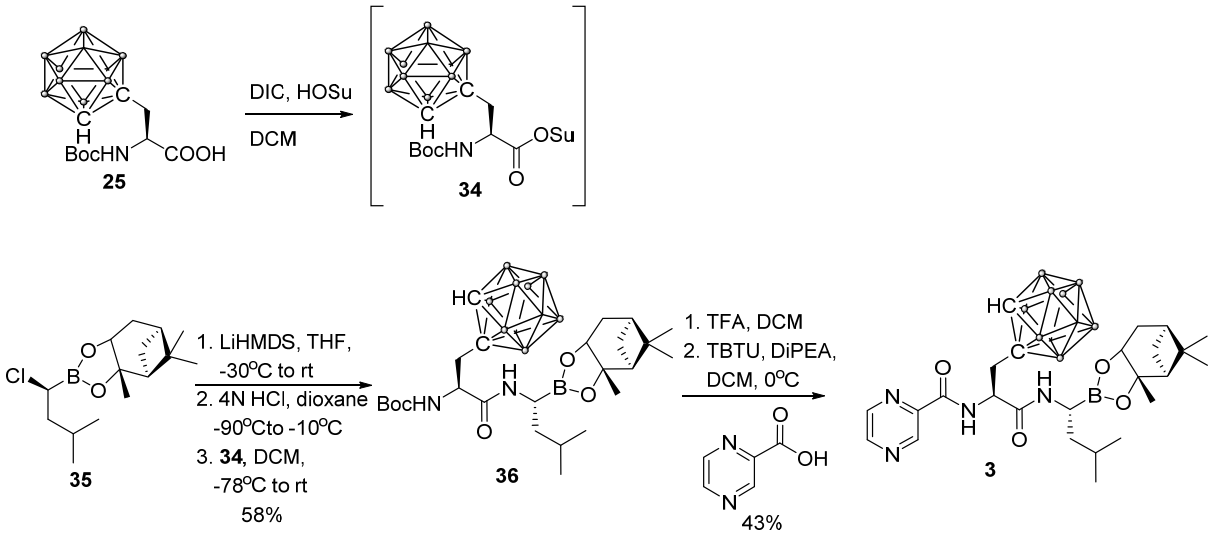
(S)-3-(adamantan-1-yl)-2-(pyrazine-2-carboxamido)propanoic acid (32)

To a solution of methyl ester **31** (144 mg, 0.42 mmol) in THF (5 mL) was added LiOH (11 mg, 0.46 mmol, 1.1 equiv.) in H_2O (1 mL). After 1.5 hours, 4 mg LiOH was added since TLC-analysis showed remaining starting material. After 15 min, TLC-analysis showed complete conversion of starting material and the reaction mixture was diluted by the addition of EtOAc. 1N HCl was added and the mixture was extracted with EtOAc (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated, providing the title compound in a quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.47 (bs, 1H), 9.36 (s, 1H), 8.76 (s, 1H), 8.56 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 4.83 (s, 1H), 1.91 (s, 3H), 1.83 (d, *J* = 14.0 Hz, 1H), 1.69 – 1.45 (m, 13H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.70, 162.44, 146.97, 144.08, 143.96, 142.95, 48.36, 46.42, 42.11, 36.59, 32.61, 29.57, 28.33.

(1*S*, 2*S*, 3*R*, 5*S*) - Pinanediol - *N* - pyrazinoyl - L - adamantylalanine - L - boronoleucine 'Adamantezomib' (2)

To a solution of TBTU (35.3 mg, 0.11 mmol, 1.1 equiv.), boronoleucine 33 (33.7 mg, 0.1 mmol, 1 equiv.) and dipeptide **32** in DCM at -10°C was added DiPEA (52.3 µL, 0.3 mmol, 3 equiv.). After stirring for 2 hours at -10°C, TLC analysis (5% MeOH:DCM) indicated complete conversion of starting material. The reaction mixture was concentrated and the residue was dissolved in EtOAc, washed with 0.1N HCl (2x), sat. $NaHCO_3$ (2x) and brine, dried over Na_2SO_4, filtered and concentrated. Column chromography (0→2% MeOH:DCM) followed by HPLC purification (C4, 50-90% MeCN, 0.1 % TFA, 10 min gradient) provided the title compound (15.67 mg, 0.027 mmol, 27%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.40 (d, *J* = 1.3 Hz, 1H), 8.79 (d, *J* = 2.4 Hz, 1H), 8.56 (dd, *J* = 2.3, 1.6 Hz, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 6.44 (d, *J* = 4.7 Hz, 1H), 4.71 (td, *J* = 8.0, 4.9 Hz, 1H), 4.31 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.21 (dt, *J* = 9.1, 5.9 Hz, 1H), 2.33 (ddt, *J* = 13.9, 8.8, 2.3 Hz, 1H), 2.22 – 2.15 (m, 1H), 2.02 – 1.94 (m, 5H), 1.92 (tt, *J* = 5.8, 3.1 Hz, 1H), 1.85 (dt, *J* = 14.5, 2.6 Hz, 1H), 1.73 – 1.60 (m, 7H), 1.59 – 1.56 (m, 6H), 1.56 – 1.43 (m, 3H), 1.40 (s, 3H), 1.30 (s, 3H), 1.27 (d, *J* = 10.8 Hz, 1H), 0.89 (t, *J* = 6.4 Hz, 6H), 0.86 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.68, 162.78, 147.62, 144.54, 144.15, 142.89, 85.74, 77.84, 51.58, 48.62, 45.93, 42.55, 40.17, 39.78, 38.32, 36.96, 35.75, 32.41, 28.74, 28.67, 27.29, 26.51, 25.75, 24.21, 23.14, 22.26. LC-MS (linear gradient 50 → 90% MeCN, 0.1% TFA, 15 min): R_t (min): 9.30 (ESI-MS (m/z): 577.20. HRMS (m/z): calcd. for $C_{33}H_{50}BN_4O_4$ $[M+H]^+$ 577.39196, found 577.39203

Synthesis of carbortezomib



Scheme 4. Synthesis of carbortezomib **3** starting from enantiopure L-N-Boc-carboranylalanine

(1*S*,2*S*,3*R*,5*S*)-Pinanediol -*N*- Boc-L-carboranylalanine-L-borono-leucine (36)

(*S*)-*N*-Boc carboranylalanine **25** (83 mg, 0.25 mmol, 1.25 equiv.) in dry CH_2Cl_2 (0.2M) under an argon atmosphere at rt, was treated with *N*-hydroxysuccinimide (52 mg, 0.45 mmol, 2.25 equiv.) and *N*,*N*'-diisopropylcarbodiimide (57 mg, 0.45 mmol, 2.25 equiv.). The mixture was stirred until TLC showed complete conversion of the starting material, after 6 h, yielding the crude OSu ester. Separately, chloroboronate 35 (57 mg, 0.2 mmol, 1 equiv.) was dissolved in dry THF (0.2M) at -30 °C under an argon atmosphere and treated with LiHMDS (0.26 mL, 1M in THF, 1.3 equiv.). The mixture was slowly warmed to rt and re-cooled to -90 °C when TLC indicated complete conversion of the starting material typically after 5 h. HCl (0.23 mL, 4N in 1,4-dioxane, 4.5 equiv.) was added and the reaction was allowed to warm to -10°C. The mixture was cooled again to -80 °C and DiPEA (12 equiv.) was added, followed by the crude OSu ester solution. The reaction was stirred overnight and allowed to warm up to rt. The mixture was filtrated over a Whatmann glass microfiber filter and concentrated by rotary evaporation. Column chromatography (10% → 30% EtOAc in pentane) afforded dipeptide **36** as a colourless oil (67 mg, 0.12 mmol, 58%). ^{1}H NMR (400 MHz, MeOD) δ 4.55 (s, 1H, $C_{Carb}H$), 4.34 (dd, J = 9.6, 3.5 Hz, 1H), 4.21 (dd, J = 8.5, 1.7 Hz, 1H), 3.03 – 1.58 (m, 10H), 2.87 – 2.76 (m, 2H), 2.62 (dd, J = 15.6, 9.7 Hz, 1H), 2.40 – 2.30 (m, 1H), 2.19 – 2.11 (m, 1H), 1.96 (t, J = 5.5 Hz, 1H), 1.91 – 1.85 (m, 1H), 1.79 (dt, J = 14.3, 2.6 Hz, 1H), 1.75 – 1.67 (m, 1H), 1.46 (s, 9H), 1.42 – 1.32 (m, 6H), 1.29 (s, 3H), 0.92 (dd, J = 6.5, 3.6 Hz, 6H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 175.22, 156.99, 85.16, 81.27, 77.82, 73.91, 63.32, 53.23, 52.88, 41.16), 41.12, 40.97 (HSQC confirmed), 39.56, 39.21, 37.22, 29.49, 28.68, 27.68, 27.33, 26.61, 24.54, 23.43, 22.48. ^{11}B NMR (128 MHz, MeOD) δ 21.56, -2.51, -5.57, -9.57, -11.81, -13.11. HRMS (m/z): calcd. for $C_{25}H_{51}B_{11}N_2O_5$ $[M+H]^+$ 579.49725, found 579.49817.

(1*S*, 2*S*, 3*R*, 5*S*) - Pinanediol - *N* - pyrazinoyl - L - carboranylalanine - L - boronoleucine 'carbortezomib' (3)

Dipeptide **52** (23 mg, 40 µmol, 1 equiv.) was dissolved in dry DCM/TFA (1:1, 1.5 mL) at rt under an argon atmosphere. After 40 min the solvents were removed by co-evaporation with toluene (3x) and the deprotected dipeptide re-dissolved in dry DCM (1.5 mL) and cooled to 0 °C. 2-Pyrazinecarboxylic acid (8 mg, 60 µmol, 1.5 equiv.), TBTU (20 mg, 60 µmol, 1.5 equiv.) and DiPEA (20 µL, 120 µmol, 3 equiv.) were added and the mixture was stirred for 1 h at 0 °C. The DCM was removed by rotary evaporation and redissolved in EtOAc (20 mL). The organic layer was washed with 0.1M aq. HCl (2 x 20 mL), 2% aq. $NaCO_3$ (2 x 20 mL) and brine (1 x 20 mL). The EtOAc layer was then dried over $MgSO_4$, filtrated and concentrated by rotary evaporation. Flash column chromatography (20 → 50% EtOAc in pentane) followed by HPLC purification (C_{18}, 80→86% MeCN, 0.1 % TFA, 12 min gradient) and lyophilisation, afforded the title compound **3** as a white powder (10.08 mg, 17.24 µmol, 43%). ^{1}H NMR at 303 K (600 MHz, MeOD) δ 9.26 (d, *J* = 1.3 Hz, 1H), 8.82 (d, *J* = 2.4 Hz, 1H), 8.76 – 8.68 (m, 1H), 4.86 (dd, *J* = 8.8, 4.3 Hz, 1H), 4.59 (s, 1H), 4.25 (dd, *J* = 8.7, 1.9 Hz, 1H), 3.04 (dd, *J* = 15.8, 4.3 Hz, 1H), 2.97 – 2.88 (m, 2H), 2.78 – 1.57 (m, 10H), 2.38 – 2.32 (m, 1H), 2.19 – 2.13 (m, 1H), 1.95 (t, *J* = 5.5 Hz, 1H), 1.90 – 1.85 (m, 1H), 1.79 (dt, *J* = 14.3, 2.5 Hz, 1H), 1.69 (dtt, *J* = 20.5, 13.3, 6.6 Hz, 1H), 1.47 – 1.36 (m, 2H), 1.35 (s, 3H), 1.35 – 1.32 (m, 1H), 1.29 (s, 3H, $C_qCH_3CH_3$), 0.91 – 0.84 (m, 9H). ^{13}C NMR (151 MHz, MeOD) δ 173.52, 165.38, 148.92, 145.71, 144.99, 144.86, 85.72, 78.23, 73.97, 63.65, 53.15, 52.35, 41.13, 40.94, 40.28, 39.30, 39.24, 37.03, 29.35, 27.63, 27.30, 26.60, 24.47, 23.28, 22.52. ^{11}B NMR (128 MHz, MeOD) δ 22.40, -2.51, -5.47, -9.54, -11.59, -12.94. LC-MS (linear gradient 50 → 90% MeCN, 0.1% TFA, 15 min): R_t (min): 9.81 (ESI-MS (m/z): 585.20. HRMS : calcd. for $C_{25}H_{46}B_{11}N_4O_4$ $[M+H]^+$ 585.46237, found 585.46251.

Biochemical methods

General methods

Lysates of cells were prepared by treating cell pellets with 4 volumes of lysis buffer containing 50 mM Tris pH 7.5, 2 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 2 mM ATP, and 0.05% digitonin for 15-60 min. Protein concentration was determined using Qubit® protein assay kit (Thermofisher). All cell lysate labelling experiments were performed in assay buffer containing 50 mM Tris pH 7.5, 2 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 2 mM ATP. Cell lysate labelling and competition experiments were performed at 37°C. The 10x concentrated ABP cocktail is composed of: 1 µM Cy5-NC-001, 0.3 µM BODIPY(FL)-LU-112, 1 µM BODIPY(TMR)-NC-005-VS, mixed in DMSO. Prior to fractionation on 12.5% SDS-PAGE (TRIS/glycine), samples were boiled for 3 min in a reducing gel loading buffer. The 7.5x10 cm (L x W) gels were run for 15 min at 80V followed by 120 min at 130V. In-gel fluorescence in the wet gel slabs was directly detected on a ChemiDoc™ MP System using Cy2 setting to detect BODIPY(FL)-LU-112, Cy3 settings to detect BODIPY(TMR)-NC-005-VS and Cy5 settings to detect Cy5-NC-001.

Competition experiments in cell lysate

Cell lysates (diluted to 10-15 µg total protein in 9 µL buffer) were exposed to the inhibitors (10x stock in DMSO) at indicated concentrations for 1 h at 37 °C, followed by addition of probe cocktail (1.1 µL) and SDS-PAGE as described in general methods. Intensities of bands were measured by fluorescent densitometry and divided by the intensity of bands in mock-treated extracts. Average values of three independent experiments were plotted against inhibitor concentrations. IC_{50} (inhibitor concentrations giving 50% inhibition) values were calculated using GraphPad Prism software.

Competition experiments in living RPMI-8226 cells

RPMI-8226 were cultured in RPMI-1640 media supplemented with 10% fetal calf serum, GlutaMAX™, penicillin, streptomycin in a 5% CO_2 humidified incubator. 5-8 × 10^5 cells/mL were exposed to inhibitors for 1 h at 37 °C. Cells were harvested and washed twice with PBS. Cell pellets were treated with lysis buffer on ice for 15 min, followed by centrifugation at 14000 rpm for 10 min. Proteasome inhibition in the obtained cell lysates was determined using the method described above (60 min incubation with ABP cocktail). Intensities of bands were measured by fluorescent densitometry and divided by the intensity of bands in mock-treated extracts. Average values of three independent experiments were plotted against inhibitor concentrations. IC_{50} (inhibitor concentrations giving 50% inhibition) values were calculated using GraphPad Prism software.

Inhibitor washout experiments

5x 10^5 RPMI-8226 cells were treated with 1 µM of inhibitor (1% DMSO end concentration) at 37°C. After 1 h, the cells were washed with medium (2x) and incubated at 37°C for 0, 2 or 4 hours. The cells were harvested and washed with PBS, lysed in standard lysis buffer for 15 min, followed by centrifugation at 14000 rpm for 5 min. Proteasome inhibition in the obtained cell lysates was determined using the method described above (30 min incubation with ABP cocktail). Intensities of bands were measured by fluorescent densitometry and divided by the intensity of bands in mock-treated extracts and corrected for gel loading using Coomassie staining. Average values of three independent experiments are reported.

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