S U P P O R T I N G I N F O R M A T I O N

Peptide-Boronic Acid Inhibitors of Flaviviral Proteases: Medicinal Chemistry and Structural Biology

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1. General information and materials

Reagents and amino acids were purchased from Sigma Aldrich (Germany) and Carbolution Chemicals (Germany). No further purification steps were performed unless otherwise indicated. All solvents were used as obtained from the commercial sources. If anhydrous solvents are stated in synthetic procedures, these solvents were either purchased as anhydrous or dried using molecular sieve. Air and water-sensitive reagents and reactions were generally handled under nitrogen atmosphere. The reaction progress was monitored by TLC on Merck silica gel plates 60 F254. Detection was executed with a UV chamber (Vilber Lourmat) at 254 nm or with potassium permanganate staining. Normal phase chromatographic purification was performed on a Biotage Isolera One purification system using silica gel (0.060-0.200 mm), KP-Sil cartridges and UV monitoring at two different wavelengths (e.g. 254, 280 nm). RP-phase purifications were executed using an ÄKTA purifier (GE Healthcare) system with Reprospher C18-DE, Dr. Maisch (5 µm) 30 x 16 mm, and 120 x 16 mm pre- and main columns and UV detection. Nuclear magnetic resonance spectra were recorded on Varian Mercury Plus (300 MHz) and Varian NMR System 500 (500 MHz) instruments at 300 K. Chemical shifts (δ) are given in parts per million (ppm), coupling constants (J) given in Hertz (Hz) and multiplicity is reported using standard abbreviations. $CDCl_3$, acetone-d_6, DMSO-d_6, CD_3OD and D_2O were used as solvents and internal standards. Mass spectrometry was performed on a Bruker micrOTOF-Q II (ESI) instrument. High resolution mass data were generated using the same instrument with sodium formate or low concentration tuning mix (ESI-L, Agilent Technologies) as calibrating reagents. Analytical HPLC was performed on a Jasco HPLC system with UV detector and ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany, 5 µm, 50 x 2 mm column.

2. Biochemistry and cell culture

Expression and Purification of Dengue Protease (DENVpro) and West Nile Virus Protease (WNVpro). The DENVpro of serotype 2 and WNVpro NS2B-NS3 constructs were used as described before. In both constructs the core sequence of NS2B is covalently ligated to the protease NS3 domain by a glycine-serine linker (GGGGSGGGG).[1-2] Transformation of the pET28a plasmids (Novagen, Germany), expression of the His-tagged proteins in *Escherichia coli* BL 21 λ (DE3) cells, and purification by nickel affinity chromatography were performed according to an established protocol.[3-4]

Expression and Purification of Zika virus protease (ZIKVpro). The linked (GGGGSGGGG) ZIKVpro NS2B-NS3 construct, consisting of 47 NS2B and 170 NS3 residues was designed and transformed as described elsewhere.[5] Protein expression and purification were also exactly executed as recently described.[5] In short, the N-terminally His-tagged protease was overexpressed in *Escherichia coli* BL21-Gold (DE3) and purified by nickel affinity chromatography. The His-tag was cleaved using a thrombin cleavage site and the protein was purified again using nickel affinity chromatography and subsequent anion exchange and gel filtration.

DENVpro and WNVpro Enzymatic Assays. The assays were performed as previously described.[3-4, 6-10] In short, continuous assays were performed on a BMG Labtech Fluostar OPTIMA microtiter fluorescence plate reader using black 96 well V-bottom plates (Greiner Bio-One) with an excitation wavelength of 320 nm and a monitored emission wavelength of 405 nm. The inhibitors (various concentrations from 10 mM stock solutions in DMSO) were preincubated with the DENVpro (100 nM) or WNVpro (150 nM) in assay buffer (50 mM Tris-HCl pH 9, ethylene glycol (10% v/v), 0.0016% Brij® 58) for 15 min. Afterwards, the enzymatic reaction

was initiated by addition of FRET substrate (final concentration 50 μM; Abz-Nle-Lys-Arg-Arg-Ser-3-(NO_2)Tyr for DENVpro and Abz-Gly-Leu-Lys-Arg-Gly-Gly-3-(NO_2)Tyr for WNVpro). The substrates were synthesized by standard solid phase peptide synthesis using the Fmoc approach.[8] Final assay volume for every well was 100 μl. The enzymatic activity was determined as fluorescence increase (RFU/s) and monitored for 15 min. All determinations were calculated in relation to a positive control (without inhibitor) and performed in triplicate. IC_{50}-values were calculated from at least seven different inhibitor concentrations at appropriate enzyme concentration (at least the double of the lowest inhibitor concentration; assay walls were excluded by analyzing even lower enzyme and inhibitor concentrations) using Prism 5.0 (Graphpad Software, Inc.). K_i values were determined from four IC_{50} determinations at different substrate concentrations (50, 100, 150, 200 μM). IC_{50} values were plotted against the substrate concentrations, followed by linear regression fit which revealed the K_i value as y-intercept (Cheng-Prusoff equation).[11-12]

ZIKVpro Enzymatic Assays. The assay was exactly performed as recently described elsewhere.[5] In short, the assay was performed using a buffer of 10 mM Tris-HCl pH 8.5, 20% glycerol, 1 mM CHAPS, 1 mM TCEP and a fluorescent substrate (Bz-Nle-Lys-Lys-Arg-AMC; Biosyntan). The fluorescence signal from released AMC was monitored at 460 nm with excitation at 360 nm, using an Flx800 fluorophotometer (BioTek). Initial velocities were determined from the linear section of the curves. For the determination of IC_{50} values, 5 nM protease was incubated for 10 min with various inhibitor concentrations between 6 nM and 50 μM at 37°C. The reaction was initiated by adding the substrate at a concentration of 10 μM to each well at a final volume of 50 μL. IC_{50} values were calculated using GraphPad Prism 6.0 (GraphPad). For the determination of K_i, the assay was performed with different final

concentrations of compound **cn-716** (0.01, 0.05, 0.25, 1.00, 3.00 μM) and substrate (5, 10, 20, 40, 80, 160, 320 μM). At each compound concentration, 5 nM protease was incubated with the compound for 10 min at 37 °C. Subsequently, the reaction was initiated by addition of the corresponding concentration series of substrate described above to a final volume of 50 μL. The K_i was calculated by using the GraphPad Prism 6.0 software (GraphPad) in the competitive inhibition mode.

Thrombin and Trypsin Enzymatic Assays. The inhibition of catalytic activity of thrombin and trypsin was performed as previously described.[3, 6-7, 9, 13] In short, black 96 well V-bottom plates (Greiner Bio-One, Germany), a BMG Labtech Fluostar OPTIMA microtiter fluorescence plate reader, operating at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, and a buffer consisting of 50 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.05% Tween 20 were used. The inhibitors were preincubated with thrombin (10 nM) or trypsin (1 nM) at various concentrations for 15 min. The cleavage reaction was initiated by addition of the Boc-Val-Pro-Arg-AMC substrate (Bachem, Germany) at a final concentration of 50 μM. The activity of thrombin was determined as fluorescence increase (RFU/s) and monitored for 10 min. Determinations were calculated in relation to a positive control (without inhibitor) and performed in triplicate. IC_{50}-values were calculated from seven different inhibitor concentrations using Prism 5.0 (Graphpad Software, Inc.).

Table S1. Results of inhibitor activity against thrombin and trypsin. Standard deviations are not shown unless they exceed 10%.

Compound		Structure	Thrombin (μM)[a]		Trypsin (μM)[b]	
X = $B(OH)_2$	X = NH_2		X = $B(OH)_2$	X = NH_2	X = $B(OH)_2$	X = NH_2
1	**18**	Bz-**Orn**-X	13.4%	5.7%	IC_{50} = 0.74 ± 0.04	n.i.
2	**19**	Bz-**Arg**-X	IC_{50} = 0.23 ± 0.01	7.2%	IC_{50} = 0.33 ± 0.01	n.i.
3	**20**	Bz-**(4-NH_2)Phe**-Arg-X	IC_{50} = 1.02 ± 0.08	0.3%	IC_{50} = 0.044 ± 0.001	1.7%
4	**21**	Bz-**[4-(CH_2NH_2)]**Phe-Arg-X	IC_{50} = 0.52 ± 0.02	2.6%	IC_{50} = 0.051 ± 0.002	n.i.
5	**22**	Bz-**(3-guanidinyl)Phg**-Arg-X	IC_{50} = 0.27 ± 0.01	8.0%	IC_{50} = 0.013 ± 0.001	n.i.
6	**23**	Bz-(3-guanidinyl)**Phe**-Arg-X	IC_{50} = 0.23 ± 0.01	15.9%	IC_{50} = 0.015 ± 0.001	n.i.
7	**24**	Bz-**(4-guanidinyl)**Phe-Arg-X	IC_{50} = 0.25 ± 0.01	8.5%	IC_{50} = 0.042 ± 0.001	33.5%
8	**25**	**4-*t*Bu**Bz-(4-guanidinyl)Phe-X	IC_{50} = 0.25 ± 0.01	38.2%	IC_{50} = 0.22 ± 0.02	30.8%

All measurements were performed in triplicate; n.i.= no inhibition

[a] Thrombin (10 nM) with Boc-Val-Pro-Arg-AMC substrate (K_m = 16 μM). Percentage inhibition values are reported from 25 μM test compound assayed with 50 μM substrate. IC_{50} values base on a substrate concentration of 50 μM.

[b] Trypsin (1 nM) with Boc-Val-Pro-Arg-AMC substrate (K_m = 11 μM). Percentage inhibition values are reported from 50 μM test compound assayed with 50 μM substrate. IC_{50} values base on a substrate concentration of 50 μM.

Table S2. Results of inhibitor activity of peptidyl-carboxylic acids against DENV protease, WNV protease, thrombin and trypsin. Standard deviations are not shown unless they exceed 10%.

Compound	Structure	DENV protease[a]	WNV protease[b]	Thrombin[c]	Trypsin[d]
26	Bz-**Arg**-OH	n.i.	8.1%	3.8%	39.7%
27	Bz-**(4-NH_2)Phe**-Arg-OH	2.0%	12.0%	5.1%	14.4%
28	Bz-**[4-(CH_2NH_2)]**Phe-Arg-OH	1.1%	6.4%	1.3%	34.5%
29	Bz-**(4-guanidinyl)**Phe-Arg-OH	5.6%	4.1%	n.i.	19.3%

All measurements were performed in triplicate; n.i.= no inhibition

[a] Dengue serotype 2 NS2B-NS3 protease (100 nM) with Abz-Nle-Lys-Arg-Arg-Ser-3-(NO_2)Tyr substrate (K_m = 105 µM). Percentage inhibition values are reported from 50 µM test compound assayed with 50 µM substrate.

[b] WNV NS2B-NS3 protease (150 nM) with substrate Abz-Gly-Leu-Lys-Arg-Gly-Gly-3-(NO_2)Tyr (K_m = 212 µM). Percentage inhibition values are reported from 50 µM test compound assayed with 50 µM substrate.

[c] Thrombin (10 nM) with Boc-Val-Pro-Arg-AMC substrate (K_m = 16 µM). Percentage inhibition values are reported from 25 µM test compound assayed with 50 µM substrate. IC_{50} values base on a substrate concentration of 50 µM.

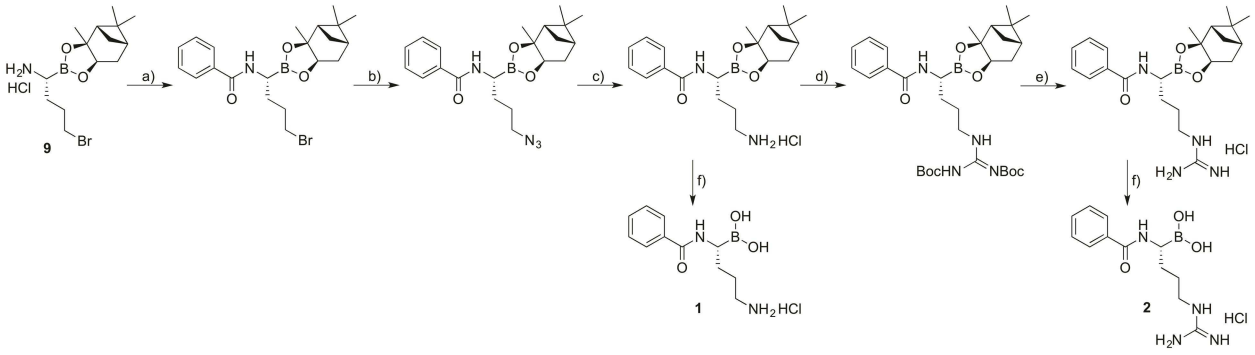
[d] Trypsin (1 nM) with Boc-Val-Pro-Arg-AMC substrate (K_m = 11 µM). Percentage inhibition values are reported from 50 µM test compound assayed with 50 µM substrate. IC_{50} values base on a substrate concentration of 50 µM.

Cell Viability Assay. 10^4 Huh-7 cells per well were seeded into 96-well plates in 50μl DMEM supplemented with 10 % FBS and incubated overnight at 37°C. Following this, the cells were infected with WT DENV serotype 2 with an MOI of 1 or with WT WNV with an MOI of 0.1 in presence of the respective concentration of the tested compound. Each concentration was assayed in triplicates. After incubation for 48 h at 37 °C, the medium was harvested; the triplicates were pooled and stored at -80 °C. 50 μl of fresh DMEM was added to the cells and

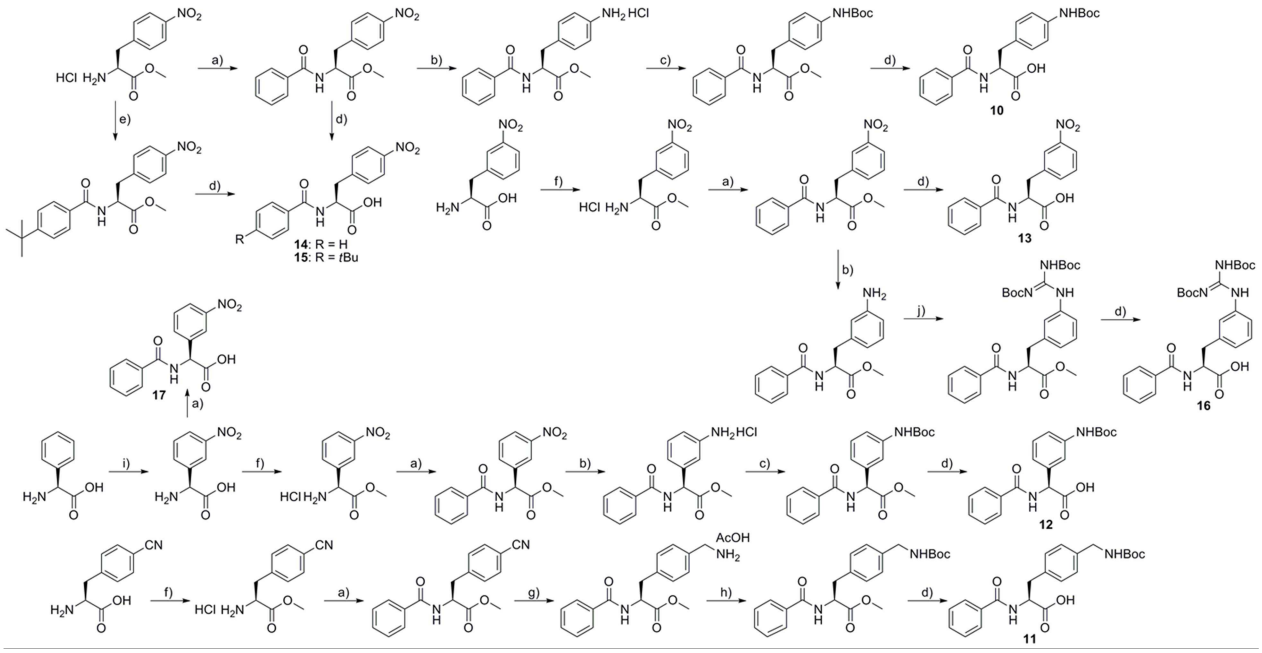
cell viability was determined using Cell-Titer Glo® Luminescent Viability Assay. A gradient of non-toxic concentrations was used for determination of the virus yield reduction by plaque assay using Vero E6 cells.

Virus Yield Reduction Assay. Vero E6 cells were seeded in 24-well plates with a density of 2.5 x 10^5 cells per well in DMEM supplemented with 10 % FBS. After overnight incubation at 37 °C, the cells were infected with the harvested virus supernatant. The virus containing medium was diluted with DMEM ranging from 10^{-1} to 10^{-6} for DENV and from 10^{-2} to 10^{-7} for WNV before infection. After incubation of the cells with 100 μl of the virus dilution at 37 °C with agitation for 1 h, the medium was removed and 1 ml of plaque medium was added. After further incubation for 7 days for DENV or 3 days for WNV at 37 °C the cells were fixed with 5 % (v/v) formaldehyde for 2 h, stained with crystal violet and plaques were counted. EC50 values were calculated with OriginPro 8.5 using a nonlinear dose reponse curve fit.

3. Chemistry



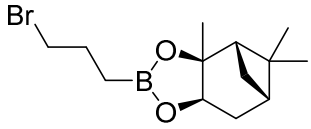
Scheme S1. Synthesis of derivatives **1** and **2**. a) BzCl, DIPEA, DCM, 0 °C; b) NaN_3, DMF, 100 °C; c) H_2, Pd/C, MeOH, HCl (aq.); d) bis-Boc-pyrazole-1-carboxamidine, DMAP, MeOH; e) TFA, DCM, HCl (g), dioxane; f) $PhB(OH)_2$, water, diethyl ether, HCl (aq.).



Scheme S2. Synthesis of precursor amino acids. a) BzCl, DIPEA, DCM, 0 °C; b) H_2, Pd/C, MeOH, HCl (aq.); c) Boc_2O, DIPEA, DCM; d) LiOH, THF, H_2O, 0 °C; e) 4-*tert*-butylbenzoic acid, HATU, HOAt, DIPEA, DCM; f) $SOCl_2$, MeOH; g) H_2, Pd/C, AcOH; h) Boc_2O, $NaHCO_3$, MeOH; i) H_2SO_4, HNO_3; j) bis-Boc-pyrazole-1-carboxamidine, DMAP, DIPEA, MeOH.

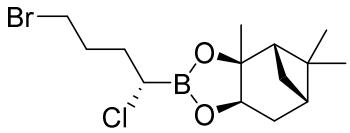
3.1 Synthesis of precursor 9

Synthesis of cn-581



A mixture of cathecholborane (35 mmol, 3.8 ml) and allyl bromide (36 mmol, 3.2 ml) was stirred at 100 °C for 3 h, cooled to room temperature and added dropwise to a solution of (1S,2S,3R,5S)-(+)-pinanediol (4.95 g, 29 mmol) in dry THF (35 ml) at 0 °C. The mixture was allowed to warm up to room temperature and stirred for 2 h. Afterwards, the solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-581** as a colorless liquid (5.60 g, 64%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.85 (s, 3H), 0.98 (t, *J* = 7.4 Hz, 2H), 1.10 (d, *J* = 10.9 Hz, 1H), 1.30 (s, 3H), 1.39 (s, 3H), 1.80-2.07 (m, 5H), 2.23 (m, 1H), 2.33 (m, 1H), 3.45 (t, *J* = 6.9 Hz, 2H), 4.27 (dd, *J* = 8.7, 2.0 Hz, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 24.0, 26.5, 27.1, 27.6, 28.6, 35.5, 36.3, 38.1, 39.5, 51.3, 77.7, 85.6 ppm; MS (ESI): *m/z*: 323.1 $[M+Na]^+$.

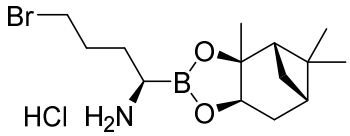
Synthesis of cn-582



To a solution of **cn-581** (5.5 g, 18.25 mmol) in cyclohexane (40 ml) and dry THF (20 ml) at -20 °C was added dry dichloromethane (1.5 ml, 23.5 mmol) followed by dropwise addition of LDA solution (22.5 ml, 22.5 mmol, 1 M in THF/hexane) over 45 min. Afterwards, a cold (-20 °C) solution of $ZnCl_2$ (30 ml, 30 mmol, 1 M in diethyl ether) was added and the resulting mixture was allowed to warm up to room temperature overnight. After evaporation of the solvents and purification by flash chromatography (cyclohexane/ethyl acetate), **cn-582** was obtained as a colorless liquid (5.27 g, 83%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.85 (s, 3H), 1.17 (d, *J* = 11.2

Hz, 1H), 1.31 (s, 3H), 1.43 (s, 3H), 1.87-2.18 (m, 7H), 2.23-2.41 (m, 2H), 3.42-3.52 (m, 3H), 4.38 (dd, *J* = 8.7, 1.9 Hz, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 23.9, 26.4, 27.0, 28.4, 30.4, 32.5, 33.0, 35.2, 38.2, 39.3, 51.1, 78.6, 86.9 ppm; MS (ESI): *m/z*: 371.1 $[M+Na]^+$.

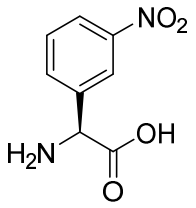
Synthesis of 9 (cn-584)



To a solution of potassium bis(trimethylsilyl)amide (1.75 g, 8.77 mmol) in dry toluene (20 ml) was added a solution of **cn-582** (2.70 g, 7.71 mmol) in dry THF (20 ml) dropwise at -40 °C. Afterwards, the mixture was allowed to warm up to room temperature overnight. The solvent was evaporated and hexane (75 ml) was added before filtration using celite. The filtrate was collected and the solvent was evaporated. The residue was dissolved in hexane (75 ml) and hydrogen chloride (4 ml, 16 mmol, 4 M in dioxane) was added at -40 °C. The resulting mixture was stored at -30 °C overnight, before the solvent was evaporated again. The addition of hexane (75 ml) resulted in a slow formation of a precipitate, which was collected by filtration to obtain **9** (cn-584) as a colorless solid (1.69 g, 60%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.83 (s, 3H), 1.16 (d, *J* = 11.1 Hz, 1H), 1.29 (s, 3H), 1.43 (s, 3H), 1.89-2.37 (m, 9H), 3.00 (m, 1H), 3.45 (m, 2H), 4.42 (d, *J* = 7.6 Hz, 1H), 8.32 (br s, 3H) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 23.9, 26.6, 27.0, 28.3, 28.5, 29.6, 33.0, 35.0, 38.1, 39.4, 51.1, 78.9, 87.8 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{25}BBrNO_2$: 330.1237, found: 330.1240.

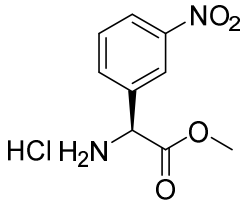
3.2 Synthesis of amino acids

Synthesis of cn-634



To a solution of L-phenylglycine (25 g, 165 mmol) in concentrated sulfuric acid (80 ml) was added a mixture of nitric acid (65%, 30 ml) and concentrated sulfuric acid (25 ml) dropwise at 0 °C. The resulting mixture was kept at 0 °C overnight, before poured on ice. Aqueous ammonia solution (25%) was added until the pH reached a value of 5. The resulting precipitate was collected and washed with water and diethyl ether. After recrystallization from ethanol (500 ml) crude **cn-634** was obtained as colorless solid (15.1 g, 47%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.25 (d, *J* = 6.2 Hz, 1H), 7.40-7.88 (m, 2H), 8.08-8.40 (m, 2H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_8H_9N_2O_4$: 197.0557, found: 197.0559.

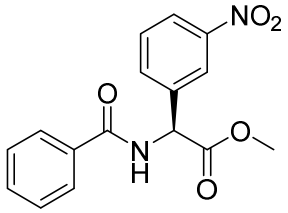
Synthesis of cn-636



Thionyl chloride (6 ml, 83 mmol) was added dropwise to methanol (60 ml) at 0 °C. The mixture was allowed to warm up to room temperature before **cn-634** (11.7 mg, 59 mmol) was added. The solution was refluxed for 1 h and the solvent was totally evaporated. The resulting residue was recrystallized from methanol/diethyl ether to obtain **cn-636** as colorless solid (8.2 g, 56%). ^{1}H-NMR (300 MHz, CD_3OD): δ = 3.84 (s, 3H), 5.48 (s, 1H), 7.78 (t, *J* = 8.2 Hz, 1H), 7.92 (m, 1H), 8.37 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.44 (t, *J* = 2.2 Hz, 1H) ppm; ^{13}C-NMR (75 MHz, CD_3OD):

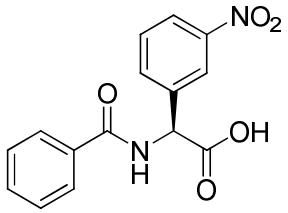
δ = 54.5, 56.8, 124.4, 126.1, 132.2, 135.3, 135.7, 150.2, 169.4 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_9H_{11}N_2O_4$: 211.0713, found: 211.0718.

Synthesis of cn-637



A solution of **cn-636** (1.85 g, 7.5 mmol), benzoyl chloride (1.08 ml, 9.3 mmol) and DIPEA (3.0 ml, 17.2 mmol) in dichloromethane (50 ml) was stirred for 2 h at 0 °C and allowed to warm up to room temperature overnight. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-637** as colorless solid (1.96 g, 83%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.82 (s, 3H), 5.38 (d, *J* = 6.3 Hz, 1H), 7.44 (br d, *J* = 6.2 Hz, 1H), 7.45-7.60 (m, 4H), 7.85 (m, 3H), 8.21 (ddd, *J* = 8.2, 2.3, 1.1 Hz, 1H), 8.32 (t, *J* = 2.2 Hz, 1H) ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{16}H_{14}N_2NaO_5$: 337.0795, found: 337.0802.

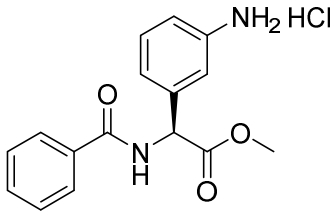
Synthesis of 17 (cn-705)



A solution of **cn-634** (200 mg, 1.02 mmol) in 20 mL dry DCM was chilled on ice-bath (0 °C, N_2) and DIPEA (900 µl, 5.10 mmol) and benzoyl chloride (150 µl, 1.27 mmol) were added. The resulting product was dried under reduced pressure and extracted with dichloromethane and 0.1 N HCl (aq). The organic layer was washed with brine and dried over magnesium sulfate, dried under reduced pressure and then purified by flash chromatography to obtain **cn-705** as yellow

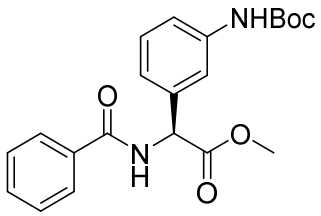
oil. The oil was crystallized from methylene chloride/hexane to afford **cn-705** as yellow solid (150 mg, 49 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.85 (s, 1H), 7.42-7.69 (m, 4H), 7.77 (d, *J* = 8.80 Hz, 1H), 7.83-8.06 (m, 3H), 8.17-8.31 (m, 2H), 8.42 (s, 1H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{14}N_2NaO_5$: 301.0819, found: 301.0819.

Synthesis of cn-640



A mixture of **cn-637** (1.7 g, 5.4 mmol) and palladium on carbon (10%, 400 mg, 0.38 mmol) in methanol (50 ml) and aqueous hydrochloric acid (1N, 5 ml) was stirred at room temperature under hydrogen atmosphere (1 bar) overnight. The mixture was filtered using celite, the solvent was evaporated and crude **cn-640** was obtained as pale red solid (1.71 g, 85%). ^{1}H-NMR (300 MHz, CD_3OD): δ = 3.78 (s, 3H), 5.84 (s, 1H), 7.39 (dt, *J* = 7.3, 2.0 Hz, 1H), 7.44-7.52 (m, 2H), 7.54-7.62 (m, 4H), 7.87 (m, 2H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{17}N_2O_3$: 285.1234, found: 285.1237.

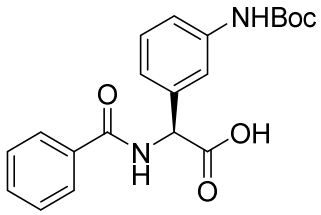
Synthesis of cn-642



A solution of **cn-640** (482 mg, 1.5 mmol), di-*tert*-butyl dicarbonate (655 mg, 3.0 mmol) and DIPEA (0.6 ml, 3.5 mmol) in dichloromethane (20 ml) was stirred for 2 days at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-642** as colorless solid (435 mg, 75%). ^{1}H-NMR (300

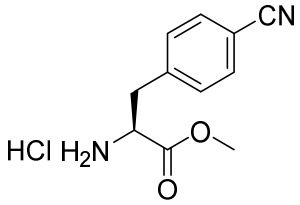
MHz, $CDCl_3$): δ = 1.51 (s, 9H), 3.78 (s, 3H), 5.74 (d, *J* = 7.1 Hz, 1H), 6.54 (br s, 1H), 7.11 (m, 1H), 7.16 (br d, *J* = 7.1 Hz, 1H), 7.31 (m, 1H), 7.37-7.55 (m, 5H), 7.83 (m, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.3, 53.0, 56.7, 80.7, 117.4, 118.7, 121.7, 127.2, 128.6, 129.7, 131.9, 133.6, 137.4, 139.0, 152.5, 166.5, 171.3 ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{21}H_{24}N_2NaO_5$: 407.1577, found: 407.1585.

Synthesis of 12 (cn-648)



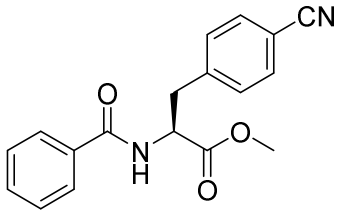
To a solution of **cn-642** (240 mg, 0.63 mmol) in THF (20 ml) at 0 °C was added a cold solution of lithium hydroxide (80 mg, 3.3 mmol) in water (20 ml) and the mixture was stirred at 0 °C for 15 min. After addition of aqueous monosodium phosphate solution (1N, 30 ml), aqueous hydrochloric acid (1N) was added drop wise until the pH reached a value of 4. After standard extraction with ethyl acetate and brine, drying over magnesium sulfate and solvent evaporation, crude **12** (cn-648) was obtained as a colorless solid (165 mg, 70%). ^{1}H-NMR (300 MHz, CD_3OD): δ = 1.50 (s, 9H), 5.59 (s, 1H), 7.16 (m, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.37 (m, 1H), 7.45 (m, 2H), 7.53 (m, 2H), 7.86 (m, 2H) ppm; ^{13}C-NMR (75 MHz, CD_3OD): δ = 28.8, 59.3, 81.1, 119.4, 119.8, 123.4, 128.7, 129.7, 130.2, 133.0, 135.4, 139.4, 141.1, 155.4, 169.9, 174.5 ppm; HRMS (ESI): *m/z* $[M–H]^-$ calcd for $C_{20}H_{21}N_2O_5$: 369.1456, found: 369.1457.

Synthesis of lw-111



L-4-Cyanophenylalanine (1.3 g, 6.83 mmol) was dissolved in methanol (20 ml) and cooled on ice. Thionyl chloride (2.48 ml, 34.17 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight. The mixture was then concentrated under reduced pressure to obtain crude **lw-111** as colorless solid (1.62 g, quant.). ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.75 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 4.40 (t, 1H), 3.81 (s, 3H), 3.34-3.41 (m, 1H), 3.19-3.28 (m, 1H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{13}N_2O_2$: 205.0972, found: 205.0969.

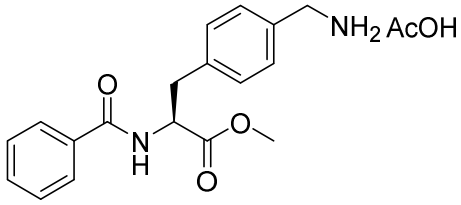
Synthesis of lw-112



A solution of **lw-111** (1.61 mg, 6.67 mmol) in dichloromethane (50 ml) was chilled on ice-bath and DIPEA (5.81 ml, 33.34 mmol) and benzoyl chloride (0.96 ml, 8.34 mmol) was added. The reaction mixture was warmed to room temperature and stirred overnight. The solvent was evaporated and the residue was dissolved in water and acidified to a pH value of 3. The aqueous phase was extracted with ethyl acetate and the combined organic layers were washed with brine. The organic solvent was dried over magnesium sulfate and removed under reduced pressure. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **lw-112** as pale yellow oil (1.83 g, 89%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 1.0 Hz, 2H),

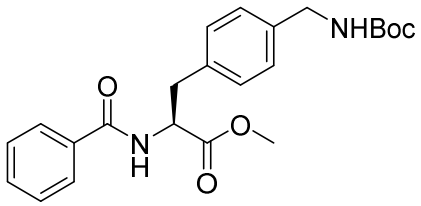
7.52-7.65 (m, 3H), 7.42-7.51 (m, 2H), 7.29 (d, *J* = 2.9 Hz, 2H), 6.69 (d, *J* = 7.1 Hz, 1H), 5.08-5.18 (m, 1H), 3.80 (s, 3 H), 3.37-3.48 (m, 1H), 3.22-3.34 (m, 1H); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{18}H_{16}N_2NaO_3$: 331.1053, found: 331.1051.

Synthesis of lw-114



lw-112 (1.81 g, 5.87 mmol) was dissolved in acetic acid (30 ml) and hydrogenated with palladium on carbon (10 %, 0.62 g, 0.59 mmol) at 40 °C for three hours. The mixture was filtered through celite and washed with methanol. The solvent was evaporated under reduced pressure. The resulting crude product was dissolved in methanol (3 ml) and cold diethyl ether was added until complete precipitation. After filtration **lw-114** was obtained as pale brown solid (789 mg, 36%). ^{1}H-NMR (300 MHz, acetone-d_6): δ = 7.78-7.87 (m, 2H), 7.66-7.74 (m, 1H), 7.38-7.60 (m, 4H), 7.24-7.30 (m, 2H), 4.83-4.96 (m, 1H), 4.38 (s, 2H), 3.70 (m, 3H), 3.21-3.28 (m, 1H), 3.06-3.18 (m, 1H), 1.95 (s, 5H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}N_2O_3$: 313.1547, found: 313.1547.

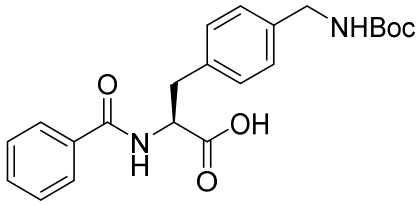
Synthesis of lw-117



lw-114 (0.78 g, 2.09 mmol) was dissolved in methanol (10 ml) and di-*tert*-butyl dicarbonate (0.9 ml, 4.2 mmol) and sodium hydrogen carbonate (0.74 g, 8.80 mmol) were added. The reaction mixture was stirred for three days at room temperature. The solvent was evaporated and the

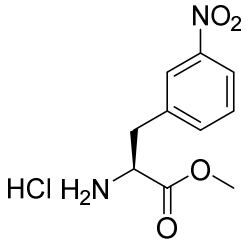
resulting crude product was dissolved in water and extracted with ethyl acetate. The combined organic layers were dried with magnesium sulfate and then evaporated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/ethyl acetate) to afford **lw-117** as colorless oil (514 mg, 60%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.74 (dd, *J* = 8.22, 1.0 Hz, 2H), 7.38-7.57 (m, 3H), 7.16-7.25 (m, 2H), 7.06-7.14 (m, 2H), 6.61 (d, *J* = 7.5 Hz, 1H), 5.09 (dt, *J* = 7.5, 5.5 Hz, 1H), 4.86 (br s, 1H), 4.28 (s, 2H), 3.77 (s, 3H), 3.15-3.35 (m, 2H), 1.46 (s, 8H) ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{28}N_2NaO_5$: 435.1890, found: 435.1895.

Synthesis of 11 (lw-118)



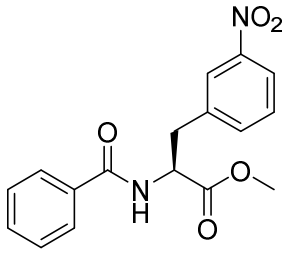
lw-117 (0.49 g, 1.19 mmol) was dissolved in THF (10 ml) and cooled on ice. Lithium hydroxide (0.14 g, 5.94 mmol) dissolved in water (10 ml) at 0 °C was added and the mixture was stirred on ice for 5 h. Afterwards, a solution of sodium hydrogen phosphate (1.2 g) in water (100 ml) was added. Aqueous hydrochloric acid (1N) was added dropwise until the pH reached a value of 3. The aqueous phase was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over magnesium sulfate and the solvent was evaporated under reduced pressure to afford a crude oil. The oil was crystallized from dichloromethane/hexane to afford **11** (lw-118) as colorless solid (442 mg, 93%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.72 (d, *J* = 7.1 Hz, 2H), 7.38-7.61 (m, 4H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.05-7.16 (m, *J* = 6.8 Hz, 2H), 6.69 (br s, 1H), 5.03-5.16 (m, 1H), 4.29 (s, 1H), 3.23-3.53 (m, 2H), 1.46 (s, 6H) ppm; HRMS (ESI): *m/z* $[M–H]^–$ calcd for $C_{22}H_{25}N_2O_5$: 397.1769, found: 397.1770.

Synthesis of cn-681



A cold solution of thionly chloride (3.65 ml, 50 mmol) in methanol (40 ml) was dropped into a solution of L-3-nitrophenylalanine (2.0 g, 9.5 mmol) in methanol (40 ml) at 0 °C and the mixture was stirred for 4 h at room temperature before the solvent was evaporated. The resulting residue was dried to obtain crude **cn-681** as pale brown solid (2.5 g, quant.). ^{1}H-NMR (300 MHz, acetone-d_6): δ = 3.69 (dd, *J* = 14.3, 3.7 Hz, 1H), 3.82 (s, 3H), 3.96 (dd, *J* = 14.2, 10.4 Hz, 1H), 5.35 (dd, *J* = 10.4, 4.3 Hz, 1H), 7.63 (t, *J* = 7.9 Hz, 1H), 8.13 (m, 2H), 8.44 (m, 1H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{10}H_{13}N_2O_4$: 225.0870, found: 225.0873.

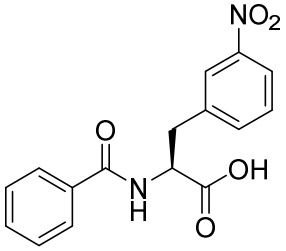
Synthesis of cn-686



A solution of **cn-681** (2.2 g, 8.5 mmol), benzoyl chloride (1.25 ml, 10.8 mmol) and DIPEA (3.0 ml, 17.2 mmol) in dichloromethane (80 ml) was stirred overnight at 0 °C. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-686** as colorless solid (1.1 g, 40%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.32 (dd, *J* = 13.8, 5.4 Hz, 1H), 3.47 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.82 (s, 3H), 5.14 (dd, *J* = 12.7, 5.6 Hz, 1H), 6.71 (d, *J* = 7.1 Hz, 1H), 7.42-7.56 (m, 5H), 7.75 (m, 2H), 8.04 (m, 1H), 8.13 (dt, *J* = 7.3, 2.1 Hz, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 37.6, 52.8, 53.4, 122.3, 124.2, 127.0, 128.7, 129.5,

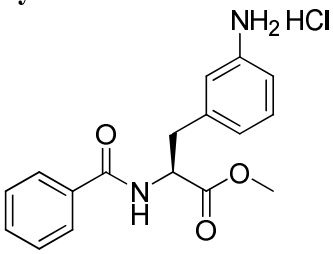
132.0, 133.5, 135.6, 138.1, 148.3, 167.0, 171.5 ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{17}H_{16}N_2NaO_5$: 351.0951, found: 351.0957.

Synthesis of 13 (cn-688)



To a solution of **cn-686** (329 mg, 1.0 mmol) in THF (25 ml) at 0 °C was added a cold solution of lithium hydroxide (120 mg, 5.0 mmol) in water (25 ml) and the mixture was stirred at 0 °C for 30 min. Aqueous hydrochloric acid (1N, 20 ml) and water (20 ml) were added and the resulting precipitate was collected, washed with water and dried to obtain **13** (cn-688) as colorless solid (260 mg, 83%). ^{1}H-NMR (300 MHz, CD_3OD): δ = 3.25 (dd, *J* = 13.9, 9.8 Hz, 1H), 3.50 (dd, *J* = 13.9, 5.0 Hz, 1H), 4.93 (dd, *J* = 9.8, 5.0 Hz, 1H), 7.42 (m, 2H), 7.53 (m, 2H), 7.71 (m, 3H), 8.10 (m, 1H), 8.21 (m, 1H) ppm; ^{13}C-NMR (75 MHz, CD_3OD): δ = 37.9, 55.3, 122.9, 125.4, 128.5, 129.7, 130.7, 133.0, 135.3, 136.9, 141.4, 149.8, 170.4, 174.3 ppm; HRMS (ESI): *m/z* $[M–H]^-$ calcd for $C_{16}H_{13}N_2O_5$: 313.0830, found: 313.0839.

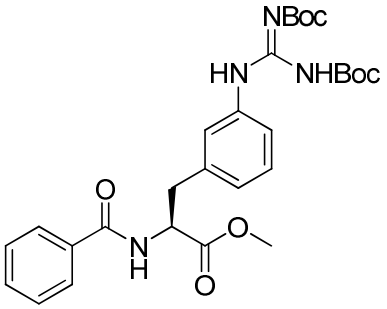
Synthesis of cn-689



Cn-686 (0.57 g, 1.74 mmol) was dissolved in 30 ml methanol (+ 1 mL 1 N HCl) and hydrogenated with palladium on carbon (10 %, 185 mg, 1.73 mmol) at room temperature overnight. The mixture was filtered through celite and washed with methanol. The solvent was evaporated under reduced pressure to obtain **cn-689** as yellow solid. No further purification was

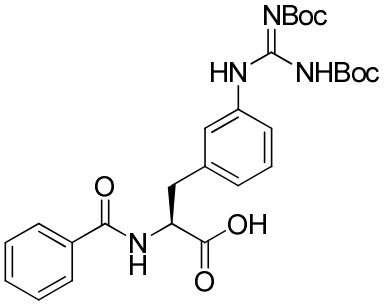
necessary (527 mg, 90 %). ^{1}H-NMR (300 MHz, CD_3OD): δ = 8.61 (d, *J* = 7.92 Hz, 1H), 7.74 (d, *J* = 7.04 Hz, 2H), 7.49 - 7.57 (m, 1H), 7.39-7.48 (m, 2H), 7.12-7.27 (m, 2H), 6.79-6.97 (m, 3H), 4.89-4.93 (m, 1H), 3.74 (s, 3H), 3.20-3.29 (m, 1H), 3.08 (dd, *J* = 9.46, 13.87 Hz, 1H) ppm; HRMS (ESI): (*m/z*) [M + H^+] calcd for $C_{17}H_{19}ClN_2O_3$: 299.1390, found: 299.1387.

Synthesis of cn-691



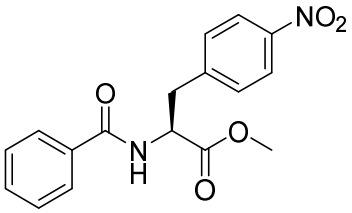
A mixture of **cn-689** (0.52 g, 1.55 mmol), bis-Boc-pyrazole-1-carboxamidine (0.72 g, 2.33 mmol), DMAP (35 mg, 0.31 mmol) and DIPEA (410 µL, 2.33 mmol) in methanol (30 mL) was stirred three days at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/ cyclohexane) to obtain **cn-691** as colorless oil (568 mg, 68 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 11.63 (br. s., 1H), 10.35 (s, 1H), 7.70-7.80 (m, 2H), 7.37-7.60 (m, 5H), 7.19-7.26 (m, 1H), 6.90 (d, *J* = 7.63 Hz, 1H), 6.65 (d, *J* = 7.48 Hz, 1H), 5.02-5.15 (m, 1H), 3.80 (s, 3H), 3.18-3.35 (m, 2H), 1.54 (s, 9H), 1.50 (s, 9H) ppm; HRMS (ESI) (*m/z*) [M + Na^+] calcd for $C_{28}H_{36}N_4O_7$: 563.2476, found: 563.2468.

Synthesis of 16 (cn-701)



Cn-691 (0.56 g, 1.04 mmol) was dissolved in 10 ml THF and cooled on ice bath (0 °C). Lithium hydroxid (124 mg, 5.18 mmol) was dissolved in 10 ml water (0 °C). The lithium hydroxid solution was added to the amino acid solution and stirred on ice bath for 30 min, and further stirring overnight at 4 °C. The aqueous phase was acidified to a pH value of 3 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with magnesium sulfate and the solvent was evaporated under reduced pressure to obtain **Cn-701** as white solid (473 mg, 86 %). ^{1}H-NMR (300 MHz, CD_3OD): δ =7.73 (d, *J* = 7.04 Hz, 2H), 7.36-7.56 (m, 5H), 7.28 (t, *J* = 7.85 Hz, 1H), 7.12 (d, *J* = 7.78 Hz, 1H), 4.88-4.93 (m, 1H) overlapped by water peak, 3.34-3.41 (m, 1H), 3.15 (dd, *J* = 9.39, 13.94 Hz, 1H), 2.01 (s, 1H), 1.99 (s, 1H), 1.50 (s, 18H) ppm; HRMS (ESI) (*m/z*) [M - H^+] calcd for $C_{27}H_{34}N_4O_7$: 525.2355, found: 525.2331.

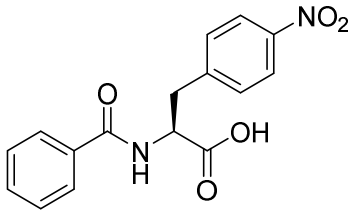
Synthesis of cn-620



To a solution of L-4-nitrophenylalanine methyl ester hydrochloride (1.3 g, 5.0 mmol) in dichloromethane (50 ml) were added DIPEA (2.1 ml, 12.5 mmol) and benzoyl chloride (0.72 ml, 6.25 mmol) successively at 0 °C. The solution was allowed to warm up to room temperature and stirred overnight. The solvent was evaporated and the residue was purified by flash

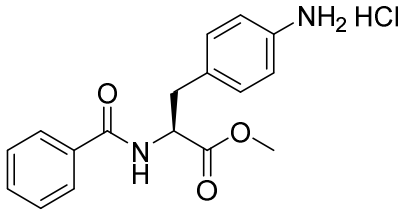
chromatography (cyclohexane/ethyl acetate) to obtain **cn-620** as colorless solid (1.56 g, 95%). ^{1}H-NMR (300 MHz, acetone-d_6): δ = 3.33 (dd, *J* = 13.9, 9.3 Hz, 1H), 3.47 (dd, *J* = 13.9, 5.4 Hz, 1H), 3.71 (s, 3H), 5.01 (m, 1H), 7.43 (m, 2H), 7.52 (m, 1H), 7.63 (m, 2H), 7.83 (m, 2H), 8.01 (br m, 1H), 8.17 (m, 2H) ppm; ^{13}C-NMR (75 MHz, acetone-d_6): δ = 37.8, 52.6, 54.6, 124.3, 128.2, 129.3, 131.5, 132.4, 135.1, 146.8, 148.0, 167.5, 172.5 ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{17}H_{16}N_2NaO_5$: 351.0951, found: 351.0953.

Synthesis of 14 (cn-672)



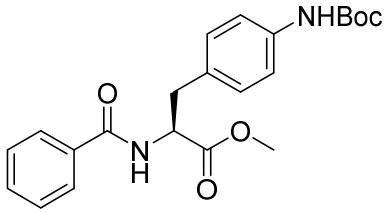
To a solution of **cn-620** (1.5 g, 4.6 mmol) in THF (40 ml) at 0 °C was added a cold solution of lithium hydroxide (360 mg, 15 mmol) in water (40 ml) and the mixture was stirred at 0 °C for 2 h. Aqueous hydrochloric acid (1N, 20 ml) and water (20 ml) were added. After extraction with ethyl acetate, drying over magnesium sulfate and solvent evaporation, crude **14** (cn-672) was obtained as a colorless solid (1.8 g, quant.). ^{1}H-NMR (300 MHz, CD_3OD): δ = 3.26 (dd, *J* = 13.9, 9.8 Hz, 1H), 3.49 (dd, *J* = 13.9, 5.0 Hz, 1H), 4.95 (dd, *J* = 9.8, 5.0 Hz, 1H), 7.42 (m, 2H), 7.51 (m, 3H), 7.72 (m, 2H), 8.15 (m, 2H) ppm; ^{13}C-NMR (75 MHz, CD_3OD): δ = 38.2, 55.2, 124.6, 128.5, 129.7, 131.6, 133.0, 135.3, 147.1, 148.5, 170.3, 174.3 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{16}H_{13}N_2O_5$: 313.0830, found: 313.0826.

Synthesis of cn-621



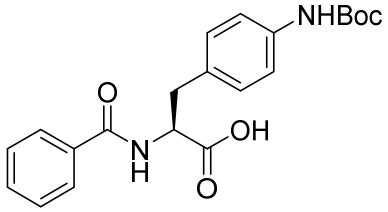
A mixture of **cn-620** (1.1 g, 3.35 mmol) and palladium on carbon (10%, 355 mg) in methanol (30 ml) and aqueous hydrochloric acid (1N, 3 ml) was stirred at room temperature under hydrogen atmosphere (1 bar) overnight. The mixture was filtered using celite and the solvent was evaporated to obtain crude **cn-621** as brown solid (960 mg, 86%). ^{1}H-NMR (300 MHz, acetone-d_6): δ = 3.23-3.48 (m, 2H), 3.70 (s, 3H), 4.96 (m, 1H), 7.40-7.58 (m, 7H), 7.83 (m, 2H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{19}N_2O_3$: 299.1390, found: 299.1389.

Synthesis of cn-623



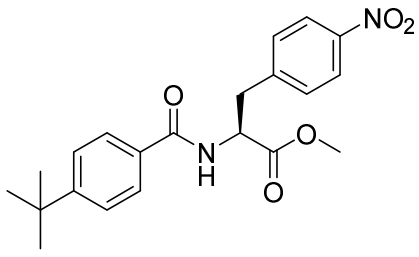
A solution of **cn-621** (335 mg, 1.0 mmol), di-*tert*-butyl dicarbonate (285 mg, 1.3 mmol) and DIPEA (0.45 ml, 2.5 mmol) in dichloromethane (10 ml) was stirred for 3 days at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-623** as pale yellow solid (300 mg, 75%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 9H), 3.18 (dd, *J* = 13.9, 5.3 Hz, 1H), 3.26 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.77 (s, 3H), 5.07 (m, 1H), 6.49 (br s, 1H), 6.68 (br d, *J* = 7.5 Hz, 1H), 7.05 (m, 2H), 7.30 (m, 2H), 7.43 (m, 2H), 7.52 (m, 1H), 7.74 (m, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.3, 37.2, 52.4, 53.5, 80.6, 118.6, 127.0, 128.6, 129.9, 130.3, 131.8, 133.9, 137.5, 152.7, 166.8, 172.0 ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{22}H_{26}N_2NaO_5$: 421.1734, found: 421.1751.

Synthesis of 10 (cn-627)



To a solution of **cn-623** (200 mg, 0.5 mmol) in THF (20 ml) at 0 °C was added a cold solution of lithium hydroxide (60 mg, 2.5 mmol) in water (20 ml) and the mixture was stirred at 0 °C for 1 h. After addition of aqueous monosodium phosphate solution (1N, 30 ml), aqueous hydrochloric acid (1N) was added drop wise until the pH reached a value of 4. After standard extractions with ethyl acetate and brine, drying over magnesium sulfate and solvent evaporation, crude **10** (cn-627) was obtained as a colorless solid (220 mg, quant.). ^{1}H-NMR (300 MHz, CD_3OD): δ = 1.49 (s, 9H), 3.07 (dd, *J* = 14.0, 9.4 Hz, 1H), 3.28 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.81 (dd, *J* = 9.4, 5.0 Hz, 1H), 7.18 (m, 2H), 7.30 (m, 2H), 7.43 (m, 2H), 7.52 (m, 1H), 7.72 (m, 2H), 8.80 (br s, 1H) ppm; ^{13}C-NMR (75 MHz, CD_3OD): δ = 28.8, 37.7, 55.9, 80.9, 120.1, 128.5, 129.7, 130.7, 132.9, 133.1, 135.5, 139.4, 155.5, 170.4, 175.1 ppm; HRMS (ESI): *m/z* $[M–H]^-$ calcd for $C_{21}H_{23}N_2O_5$: 383.1612, found: 383.1633.

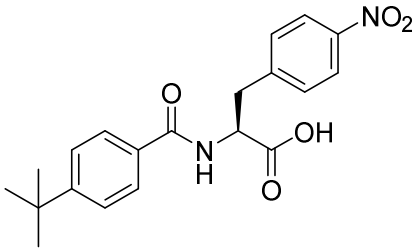
Synthesis of cn-717



4-*tert*-Butylbenzoic acid (450 mg, 2.5 mmol), HATU (950 mg, 2.5 mmol) and HOAt (340 mg, 2.5 mmol) were solved in dichloromethane (50 ml), before DIPEA (1.1 ml, 6.25 mmol) was added at 0 °C. L-4-Nitrophenylalanine methyl ester hydrochloride (650 mg, 2.5 mmol) was added and the solution was allowed to warm up to room temperature and stirred for 3 h. The

solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-717** as colorless solid (920 mg, 96%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.34 (s, 9H), 3.30 (dd, *J* = 13.8, 5.4 Hz, 1H), 3.46 (dd, *J* = 13.8, 5.9 Hz, 1H), 3.79 (s, 3H), 5.13 (m, 1H), 6.68 (br d, *J* = 7.1 Hz, 1H), 7.32 (m, 2H), 7.46 (m, 2H), 7.68 (m, 2H), 8.15 (m, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 31.1, 35.0, 37.8, 52.7, 53.2, 123.7, 125.7, 126.8, 130.3, 130.5, 143.9, 147.2, 155.7, 166.8, 171.5 ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{21}H_{24}N_2NaO_5$: 407.1577, found: 407.1564.

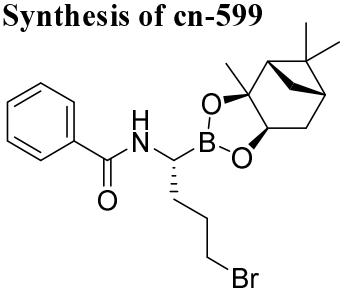
Synthesis of 15 (cn-718)



To a solution of **cn-717** (885 mg, 2.3 mmol) in THF (20 ml) at 0 °C was added a cold solution of lithium hydroxide (275 mg, 11.5 mmol) in water (20 ml) and the mixture was stirred at 0 °C for 2 h. Aqueous hydrochloric acid (1N, 5 ml) and water (10 ml) were added and the remaining THF was evaporated. The resulting precipitate was collected, washed with water and dried to obtain **15** (cn-718) as colorless solid (790 mg, 93%). ^{1}H-NMR (300 MHz, CD_3OD): δ = 1.33 (s, 9H), 3.25 (dd, *J* = 13.9, 9.8 Hz, 1H), 3.49 (dd, *J* = 13.9, 5.1 Hz, 1H), 4.94 (dd, *J* = 9.7, 5.0 Hz, 1H), 7.48 (m, 2H), 7.53 (m, 2H), 7.67 (m, 2H), 8.15 (m, 2H) ppm; ^{13}C-NMR (75 MHz, CD_3OD): δ = 31.7, 36.0, 38.2, 55.1, 124.6, 126.6, 128.4, 131.6, 132.3, 147.2, 148.5, 156.8, 170.2, 174.4 ppm; HRMS (ESI): *m/z* $[M–H]^–$ calcd for $C_{20}H_{21}N_2O_5$: 369.1456, found: 369.1464.

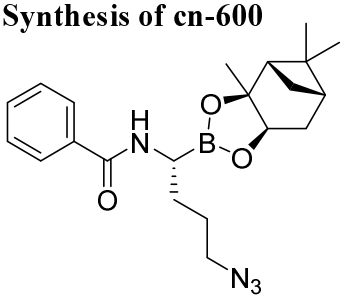
3.3 Synthesis of peptidyl-boronic acids, -amides and -carboxylic acids

Synthesis of cn-599



A solution of **9** (cn-584) (295 mg, 0.8 mmol), benzoyl chloride (0.15 ml, 1.3 mmol) and DIPEA (0.5 ml, 2.9 mmol) in dichloromethane (30 ml) was stirred overnight at 0 °C. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-599** as colorless oil (325 mg, 91%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.89 (s, 3H), 1.30 (s, 3H), 1.42, (d, *J* = 10.4 Hz, 1H), 1.46 (s, 3H), 1.75-2.08 (m, 7H), 2.20 (m, 1H), 2.37 (m, 1H), 3.12 (m, 1H), 3.45 (m, 2H), 4.34 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.02 (br s, 1H), 7.46 (m, 2H), 7.55 (m, 1H), 7.81 (m, 2H) ppm; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{21}H_{29}BBrNNaO_3$: 456.1320, found: 456.1321.

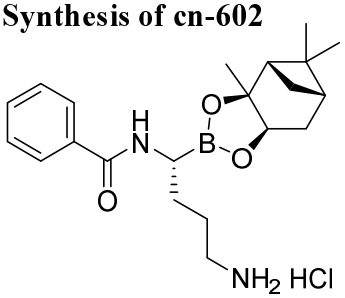
Synthesis of cn-600



A mixture of **cn-599** (315 mg, 0.73 mmol) and sodium azide (60 mg, 0.92 mmol) in DMF (40 ml) was heated to 100 °C for 1 h. Afterwards, the solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-600** as colorless solid (235 mg, 81%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89 (s, 3H), 1.30 (s, 3H), 1.41, (d, *J* = 10.6 Hz, 1H), 1.46 (s, 3H), 1.74-2.09 (m, 7H), 2.21 (m, 1H), 2.36 (m, 1H), 3.13 (m, 1H), 3.35 (m,

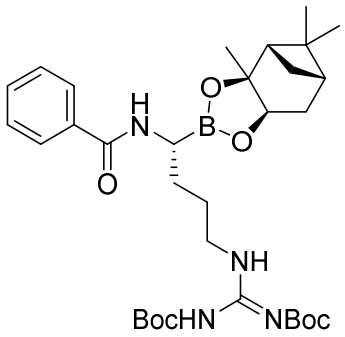
2H), 4.33 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.90 (br s, 1H), 7.46 (m, 2H), 7.56 (m, 1H), 7.82 (m, 2H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{30}BN_4O_3$: 397.2409, found: 397.2407.

Synthesis of cn-602



A mixture of **cn-600** (230 mg, 0.58 mmol) and palladium on carbon (10%, 230 mg) in methanol (40 ml) and aqueous hydrochloric acid (1N, 8 ml) was stirred at room temperature under hydrogen atmosphere (1 bar) overnight. The mixture was filtered using celite and the solvent was evaporated to obtain crude **cn-602** as pale yellow solid, which was directly used for the next synthetic steps (275 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{32}BN_2O_3$: 371.2504, found: 371.250

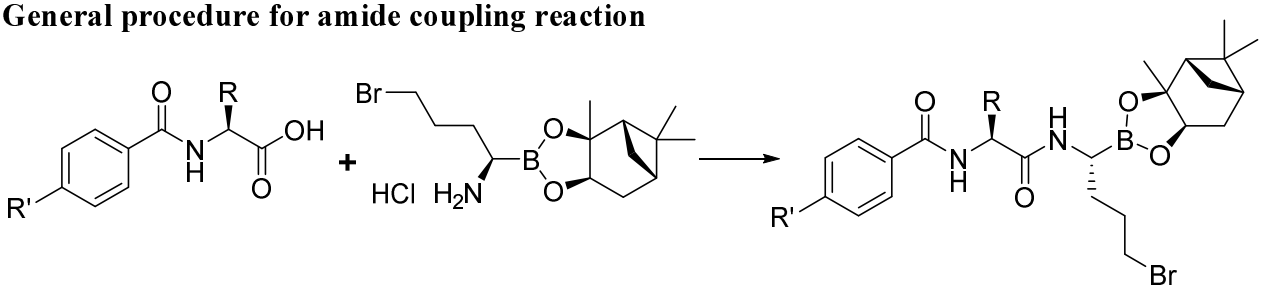
Synthesis of cn-607



A solution of **cn-602** (205 mg, 0.5 mmol), bis-Boc-pyrazole-1-carboxamidine (170 mg, 0.55 mmol) and DMAP (25 mg, 0.2 mmol) in methanol (15 ml) was stirred overnight at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain **cn-607** as colorless solid (175 mg, 57%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90 (s, 3H), 1.29 (s, 3H), 1.45, (d, *J* = 9.1 Hz, 1H), 1.47 (s, 3H), 1.52 (s,

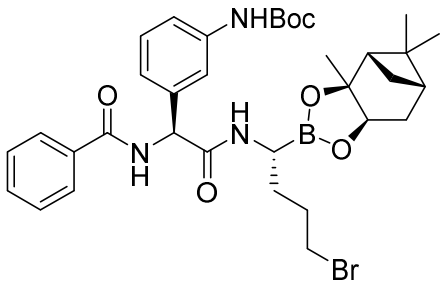
18H), 1.78-2.07 (m, 7H), 2.17 (m, 1H), 2.36 (m, 1H), 3.00 (m, 1H), 3.19 (m, 2H), 4.26 (m, 1H), 6.38 (br s, 1H), 7.44 (m, 2H), 7.56 (m, 1H), 8.00 (m, 2H) ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{32}H_{50}BN_4O_7$: 613.3773, found: 613.3756

General procedure for amide coupling reaction



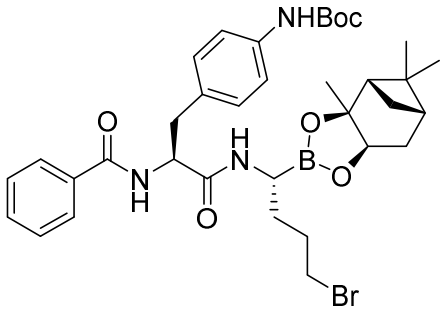
A solution of precursor acid (1 equiv.) and *N*-methylmorpholine (1.1 equiv.) was dissolved in THF and cooled to -15 °C, before IBCF (1.25-2.5 equiv.) was added. Afterwards a solution of **9** (cn-584) (0.9-1.4 equiv.) in dichloromethane was added dropwise, followed by a slightly delayed addition of DIPEA (0.9-1.2 equiv.) in dichloromethane at -15 °C over 30 min. Afterwards, the reaction mixture was allowed to warm up to room temperature and stirred overnight. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain a crude product, which was directly used for the next synthetic step.

cn-649



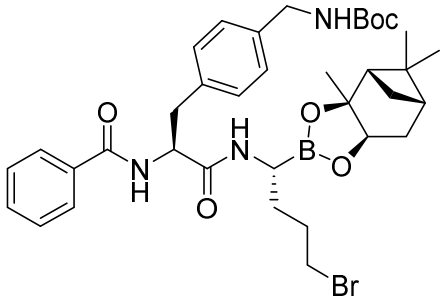
According to the general procedure, using **12** (cn-648) (160 mg, 0.43 mmol) and **9** (cn-584) (165 mg, 0.45 mmol) crude **cn-649** was obtained as pale yellow solid (150 mg, 49%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{34}H_{45}BBrN_3NaO_6$: 704.2483, found: 704.2502.

cn-628



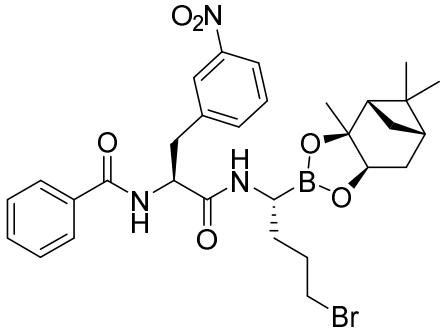
According to the general procedure, using **10** (cn-627) (192 mg, 0.5 mmol) and **9** (cn-584) (183 mg, 0.5 mmol) crude **cn-628** was obtained as colorless solid (195 mg, 56%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{35}H_{47}BBrN_3NaO_6$: 718.2640, found: 718.2627.

cn-673



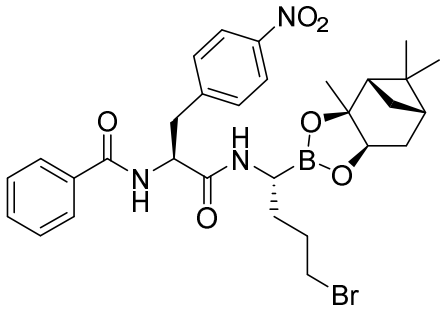
According to the general procedure, using **11** (lw-118) (200 mg, 0.5 mmol) and **9** (cn-584) (220 mg, 0.6 mmol) crude **cn-673** was obtained as colorless solid (210 mg, 59%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{36}H_{49}BBrN_3NaO_6$: 732.2796, found: 732.2789.

cn-692



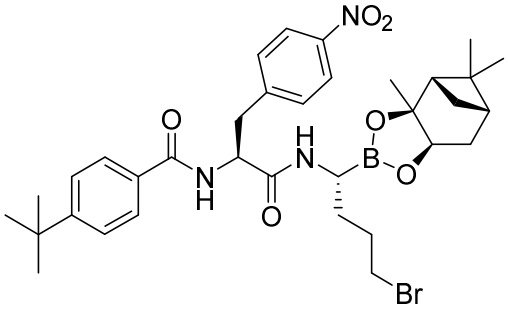
According to the general procedure, using **13** (cn-688) (236 mg, 0.75 mmol) and **9** (cn-584) (294 mg, 0.8 mmol) crude **cn-692** was obtained as colorless solid (215 mg, 46%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{30}H_{37}BBrN_3NaO_6$: 648.1856, found: 648.1853.

cn-678



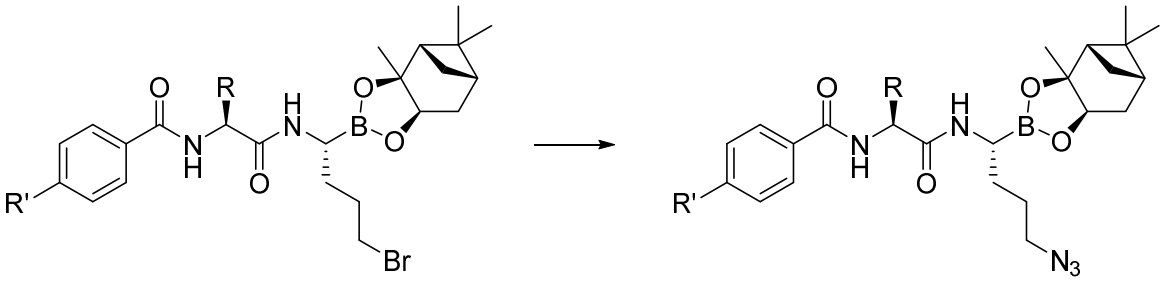
According to the general procedure, using **14** (cn-672) (350 mg, 1.1 mmol) and **9** (cn-584) (370 mg, 1.0 mmol) crude **cn-678** was obtained as pale yellow oil (270 mg, 43%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{30}H_{37}BBrN_3NaO_6$: 648.1856, found: 648.1852.

cn-719



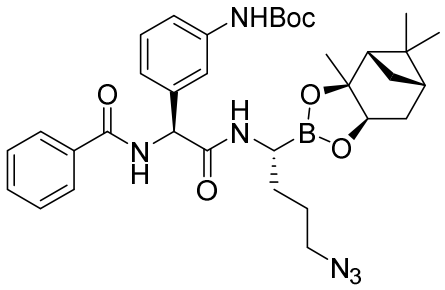
According to the general procedure, using **15** (cn-718) (185 mg, 0.5 mmol) and **9** (cn-584) (220 mg, 0.6 mmol) crude **cn-719** was obtained as a colorless solid (105 mg, 31%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{34}H_{45}BBrN_3NaO_6$: 704.2483, found: 704.2472.

General procedure for nucleophilic substitution of bromide to azide



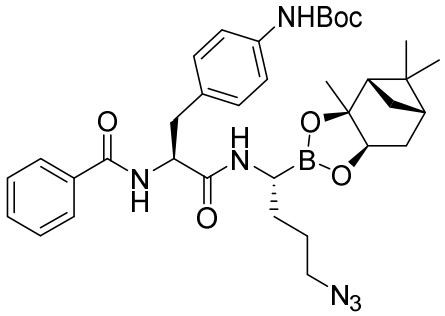
A mixture of precursor bromide (1 equiv.) and sodium azide (1.2-1.5 equiv.) in DMF was heated to 100 °C for 1 h. Afterwards, the solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain a crude product, which was directly used for the next synthetic step.

cn-650



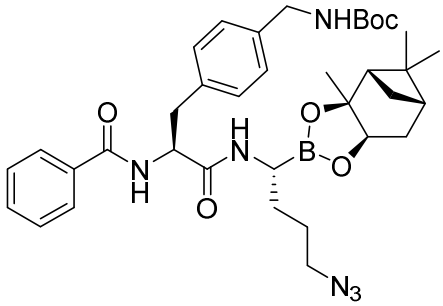
According to the general procedure, using **cn-649** (145 mg, 0.21 mmol) crude **cn-650** was obtained as colorless solid (120 mg, 88%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{34}H_{45}BN_6NaO_6$: 667.3392, found: 667.3375.

cn-629



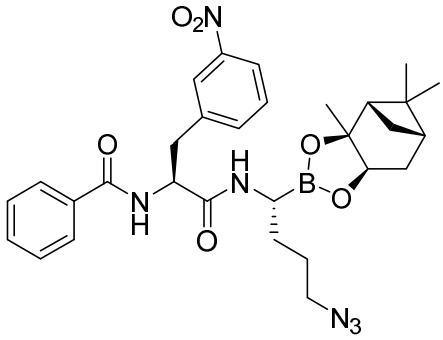
According to the general procedure, using **cn-628** (175 mg, 0.25 mmol) crude **cn-629** was obtained as colorless solid (130 mg, 79%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{35}H_{47}BN_6NaO_6$: 681.3548, found: 681.3550.

cn-674



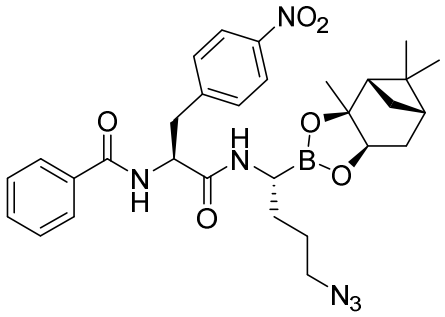
According to the general procedure, using **cn-673** (320 mg, 0.45 mmol) crude **cn-674** was obtained as colorless solid (240 mg, 79%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{36}H_{49}BN_6NaO_6$: 695.3705, found: 695.3712.

cn-693



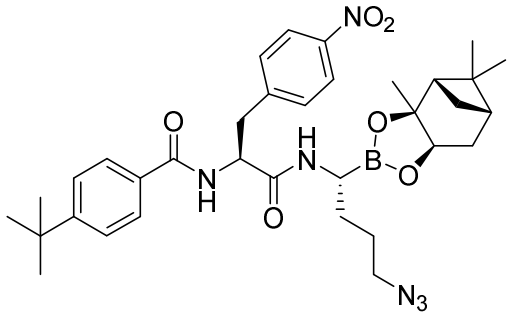
According to the general procedure, using **cn-692** (200 mg, 0.3 mmol) crude **cn-693** was obtained as colorless solid (120 mg, 69%). HRMS (ESI): *m/z* $[M–H]^-$ calcd for $C_{30}H_{36}BN_6O_6$: 587.2800, found: 587.2812.

cn-680



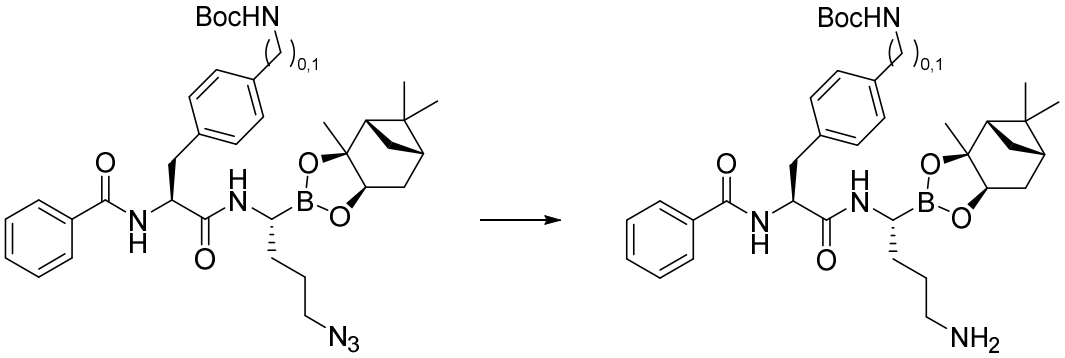
According to the general procedure, using **cn-678** (260 mg, 0.41 mmol) crude **cn-680** was obtained as colorless solid (160 mg, 66%). HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{30}H_{37}BN_6NaO_6$: 611.2765, found: 611.2744.

cn-725



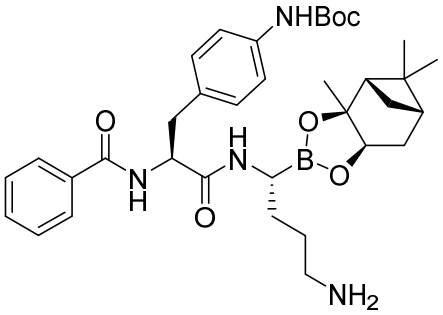
According to the general procedure, using **cn-719** (250 mg, 0.37 mmol) crude **cn-725** was obtained as pale yellow oil (175 mg, 74%). HRMS (ESI): *m/z* $[M–H]^–$ calcd for $C_{34}H_{44}BN_6O_6$: 643.3427, found: 643.3416.

General procedure for hydrogenation of azide substituents in Boc-protected compounds



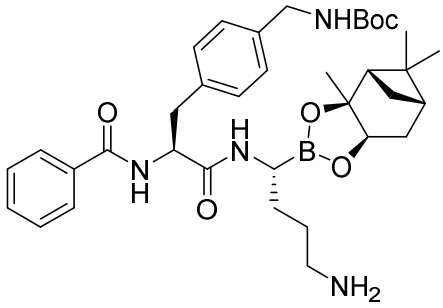
A mixture of precursor azide (1 equiv.) and palladium on carbon (10%, 0.1 equiv.) in methanol was stirred at room temperature under hydrogen atmosphere (1 bar) overnight. The mixture was filtered using celite, the solvent was evaporated and the crude product was directly used for the next synthetic step.

cn-630



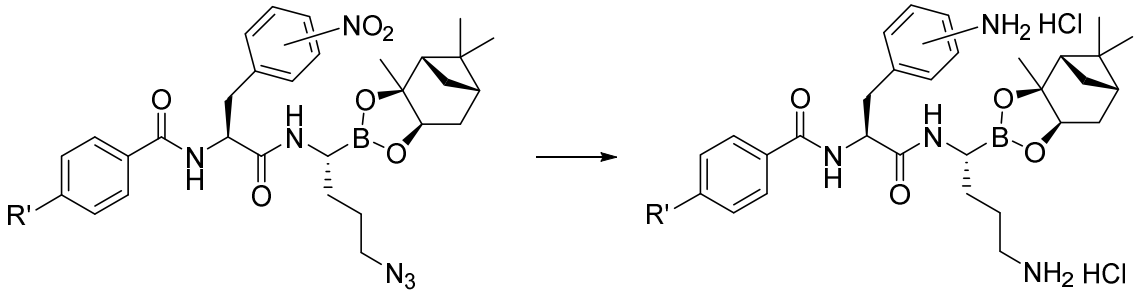
According to the general procedure, using **cn-629** (120 mg, 0.18 mmol) crude **cn-630** was obtained as colorless solid (110 mg, 97%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{35}H_{50}BN_4O_6$: 633.3824, found: 633.3845.

cn-675



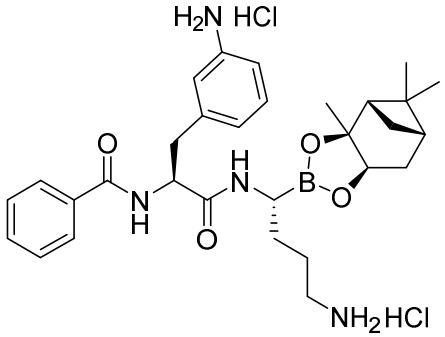
According to the general procedure, using **cn-674** (220 mg, 0.33 mmol) crude **cn-675** was obtained as colorless solid (200 mg, 94%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{36}H_{52}BN_4O_6$: 647.3981, found: 647.3957.

General procedure for parallel hydrogenation of nitro and azide substituents



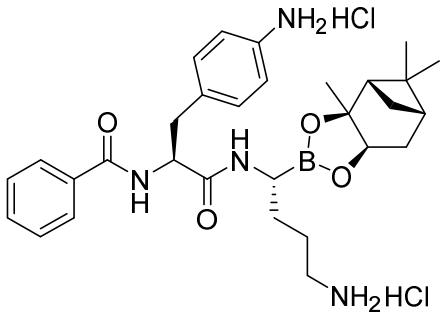
A mixture of precursor azide (1 equiv.) and palladium on carbon (10%, 0.2 equiv.) in methanol and aqueous hydrochloric acid (1N) was stirred at room temperature under hydrogen atmosphere (1 bar) overnight. The mixture was filtered using celite, the solvent was evaporated and the crude hydrochloride was directly used for the next synthetic step.

cn-694



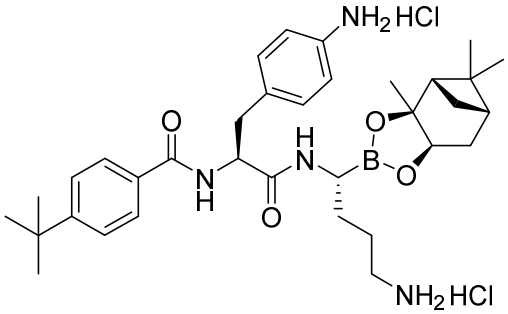
According to the general procedure, using **cn-693** (110 mg, 0.19 mmol) crude **cn-694** was obtained as pale yellow solid (110 mg, 96%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{42}BN_4O_4$: 533.3299, found: 533.3295.

cn-684



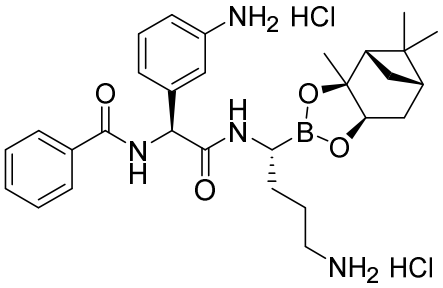
According to the general procedure, using **cn-680** (150 mg, 0.25 mmol) crude **cn-684** was obtained as pale yellow solid (135 mg, 89%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{30}H_{42}BN_4O_4$: 533.3299, found: 533.3302.

cn-726



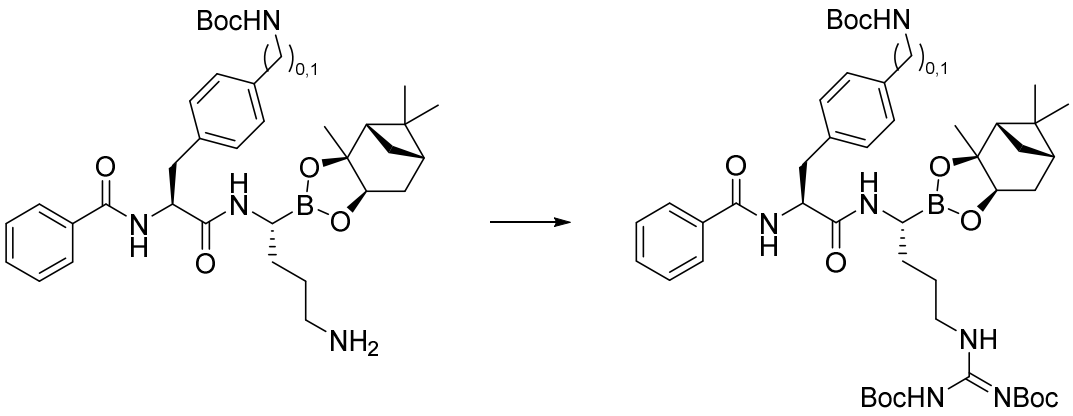
According to the general procedure, using **cn-725** (160 mg, 0.25 mmol) crude **cn-726** was obtained as pale yellow solid (155 mg, 94%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{50}BN_4O_4$: 589.3926, found: 589.3923.

Synthesis of cn-652



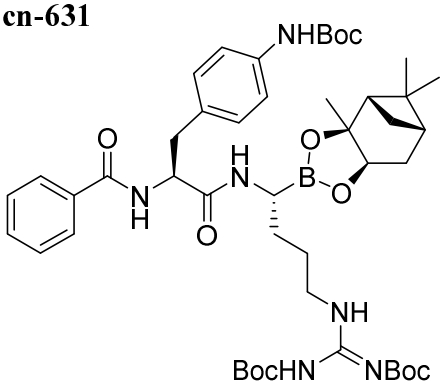
A mixture of **cn-650** (115 mg, 0.178 mmol) and palladium on carbon (10%, 30 mg) in methanol (20 ml) and aqueous hydrochloric acid (1N, 5 ml) was stirred at room temperature under hydrogen atmosphere (1 bar) overnight. The mixture was filtered using celite, the solvent was evaporated and the crude intermediate was solved in dichloromethane (10 ml) and hydrogen chloride in dioxane (4M, 2 ml, 8 mmol) was added. After 1 h, the solvent was evaporated to obtain crude **cn-652** as yellow solid (105 mg, 99%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{29}H_{40}BN_4O_4$: 519.3142, found: 519.3128.

General procedure for synthesis of *N,N'*-di(Boc)-protected guanidines



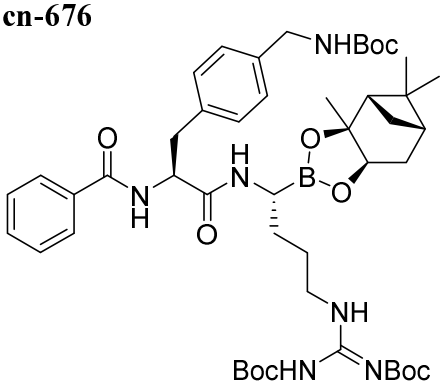
A solution of amine precursor (1 equiv.), bis-Boc-pyrazole-1-carboxamidine (1.25 equiv.) and DMAP (0.25 equiv.) in methanol was stirred 1-3 days at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain a crude product, which was directly used for the final deprotection steps.

cn-631



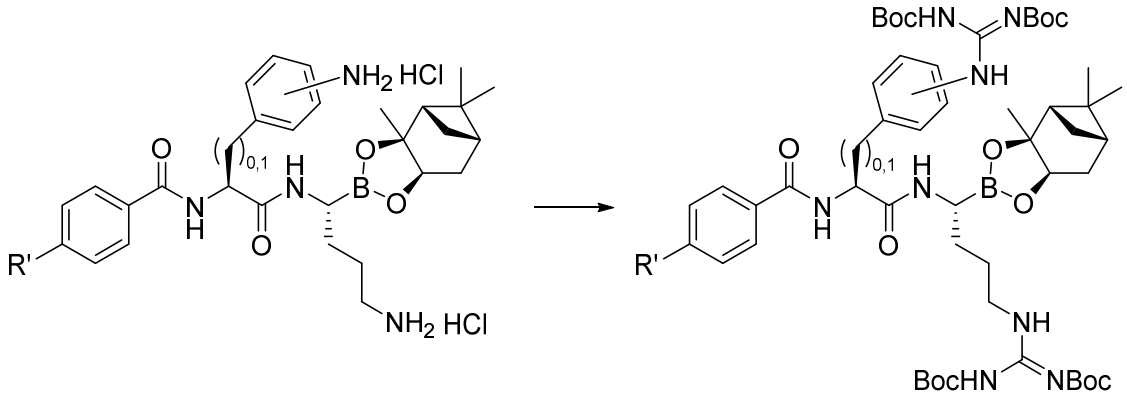
According to the general procedure, using **cn-630** (100 mg, 0.15 mmol) crude **cn-631** was obtained as colorless solid (80 mg, 59%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{46}H_{68}BN_6O_{10}$: 875.5092, found: 875.5053.

cn-676



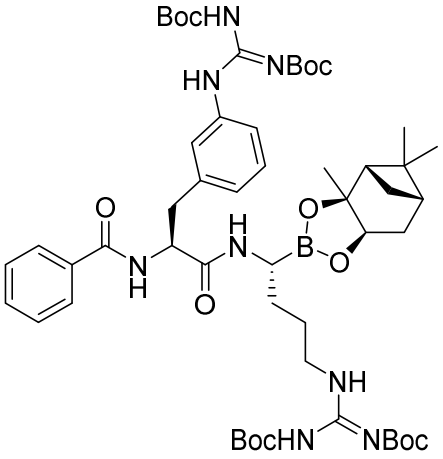
According to the general procedure, using **cn-675** (195 mg, 0.3 mmol) crude **cn-676** was obtained as colorless oil (175 mg, 66%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{47}H_{70}BN_6O_{10}$: 889.5249, found: 889.5228.

General procedure for synthesis of double *N*,*N*'-di(Boc)-protected guanidines



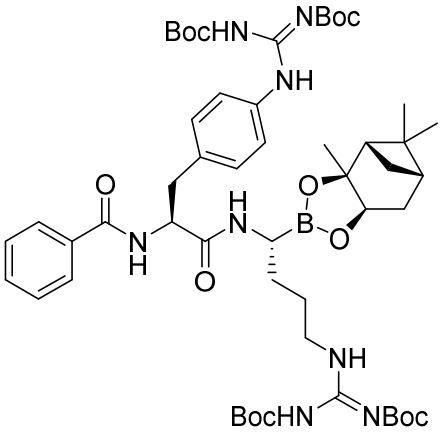
A solution of diamine precursor (1 equiv.), bis-Boc-pyrazole-1-carboxamidine (2.5 equiv.), DMAP (0.5 equiv.) and DIPEA (2.1 equiv.) in methanol was stirred 2-5 days at room temperature. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/ethyl acetate) to obtain a crude product, which was directly used for the final deprotection steps.

cn-695



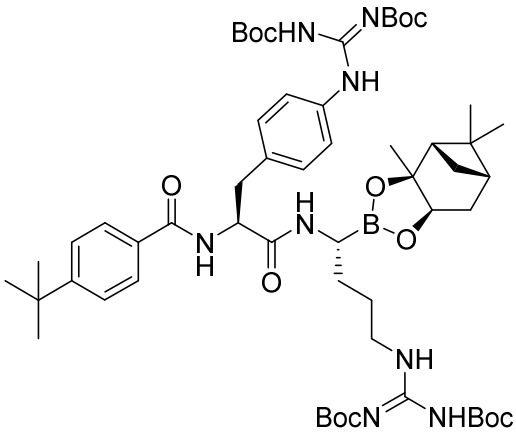
According to the general procedure, using **cn-694** (105 mg, 0.17 mmol) crude **cn-695** was obtained as colorless solid (75 mg, 44%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{52}H_{78}BN_8O_{12}$: 1017.5836, found: 1017.5787.

cn-685



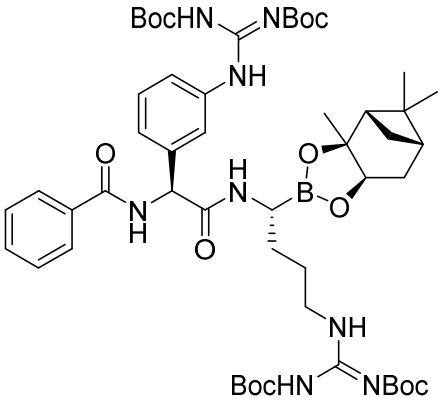
According to the general procedure, using **cn-684** (125 mg, 0.2 mmol) crude **cn-685** was obtained as colorless solid (90 mg, 44%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{52}H_{78}BN_8O_{12}$: 1017.5836, found: 1017.5796.

cn-727



According to the general procedure, using **cn-726** (150 mg, 0.225 mmol) crude **cn-727** was obtained as colorless solid (125 mg, 51%). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{56}H_{86}BN_8O_{12}$: 1073.6462, found: 1073.6452.

cn-653

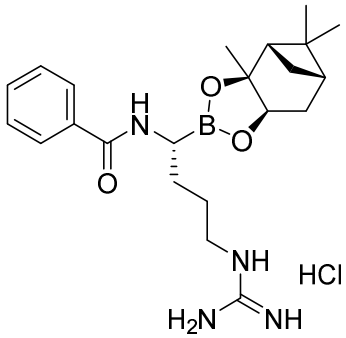


According to the general procedure, using **cn-652** (100 mg, 0.17 mmol) crude **cn-653** was obtained as colorless oil (35 mg, 21%). HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{51}H_{74}BN_8O_{12}$: 1001.5533, found: 1001.5535.

General procedure for multiple Boc-deprotection

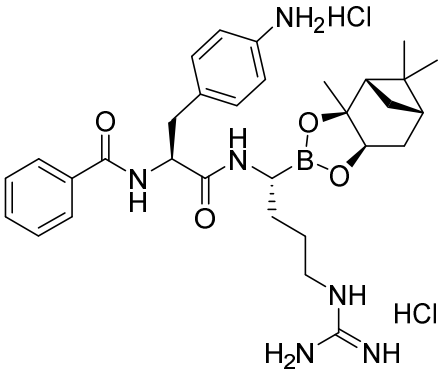
A solution of Boc-protected precursor was dissolved in dichloromethane (5-15 ml) and TFA (1 ml) was added. The mixture was stirred at room temperature 4-15 h and the solvent was evaporated. The residue was triturated with hydrogen chloride (1 ml, 4 M in dioxane) and the solvent was evaporated again to obtain a crude product, which was directly used for the final pinanediol deprotection.

cn-608



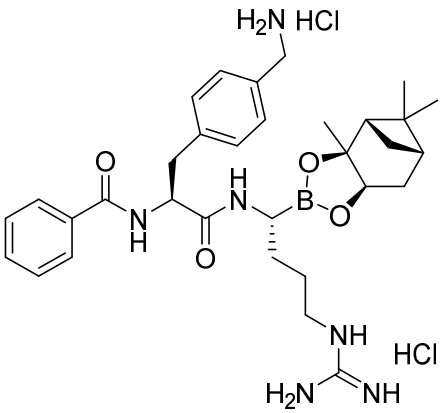
According to the general procedure, using **cn-607** (60 mg, 0.1 mmol) crude **cn-608** was obtained as brown solid (45 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}BN_4O_3$: 413.2722, found: 413.2795.

cn-632



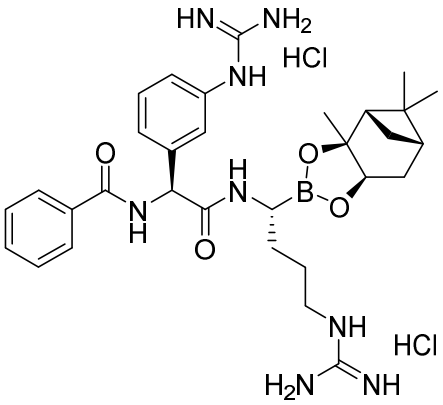
According to the general procedure, using **cn-631** (65 mg, 0.075 mmol) crude **cn-632** was obtained as brown solid (65 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{31}H_{44}BN_6O_4$: 575.3517, found: 575.3514.

cn-713



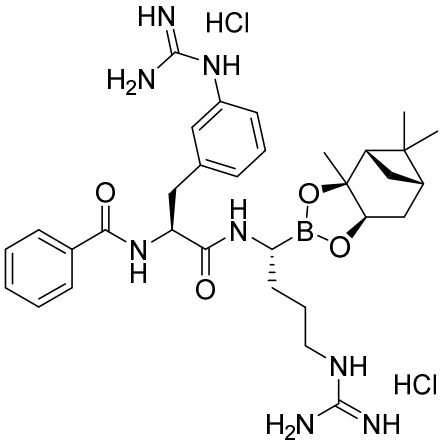
According to the general procedure, using **cn-676** (160 mg, 0.18 mmol) crude **cn-713** was obtained as brown solid (120 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{32}H_{46}BN_6O_4$: 589.3674, found: 589.3672.

cn-655



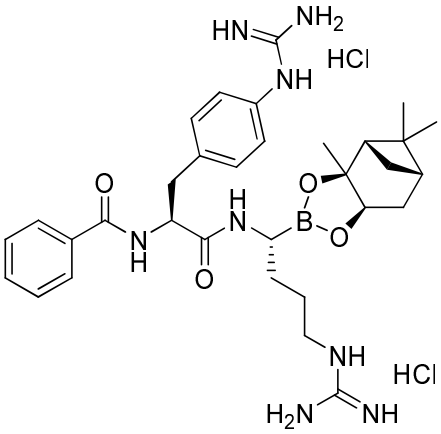
According to the general procedure, using **cn-653** (33 mg, 0.033 mmol) crude **cn-655** was obtained as brown solid (27 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{31}H_{44}BN_8O_4$: 603.3573, found: 603.3532.

cn-712



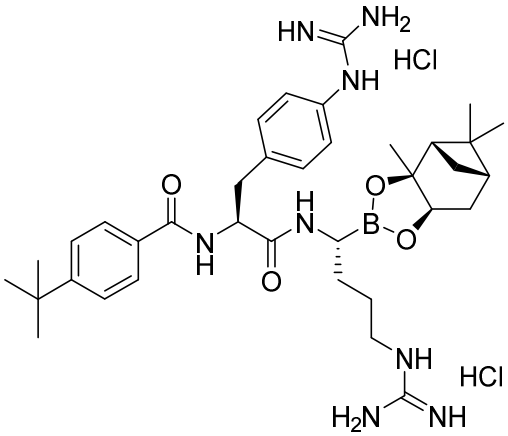
According to the general procedure, using **cn-695** (70 mg, 0.069 mmol) crude **cn-712** was obtained as brown solid (65 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{32}H_{46}BN_8O_4$: 617.3735, found: 617.3736.

cn-711



According to the general procedure, using **cn-685** (85 mg, 0.0835 mmol) crude **cn-711** was obtained as brown solid (80 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{32}H_{46}BN_8O_4$: 617.3735, found: 617.3728.

cn-728



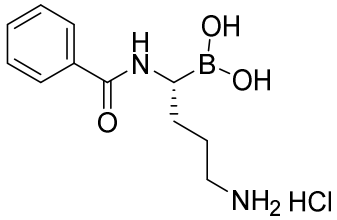
According to the general procedure, using **cn-727** (115 mg, 0.11 mmol) crude **cn-728** was obtained as brown oil (110 mg, quant.). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{36}H_{54}BN_8O_4$: 673.4362, found: 673.4388.

General procedure for final pinanediol ester cleavage

A two-phase mixture of pinanediol ester (1 equiv.) and phenylboronic acid (4-5 equiv.) in water (containing some drops of 1N hydrochloric acid) and diethyl ether was stirred at room

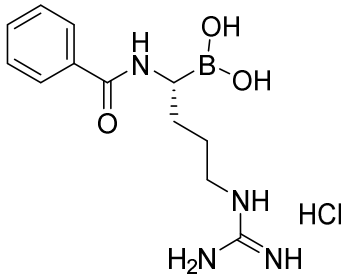
temperature overnight. Afterwards, the phases were separated and the aqueous phase was extracted with diethyl ether for three times. The aqueous phase was concentrated and directly used for purification by preparative HPLC on an ÄKTA Purifier (GE Healthcare, Germany), with an RP-18 pre and main column (Rephospher, Dr. Maisch GmbH, Germany, C18-DE, 5 µm, 30 x 16 mm and 120 x 16 mm). The conditions were: eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in methanol, flow rate: 8 ml/min, gradient: 10% B (2.5 min), 100% B (23.5 min), 100% B (26 min) 10% B (26.1 min), 10% B (30 min). Detection was performed at 214, 254, and 280 nm. The excess of methanol was evaporated and hydrochloric acid (1 ml, 1N) was added before freeze-drying.

1 (cn-604)



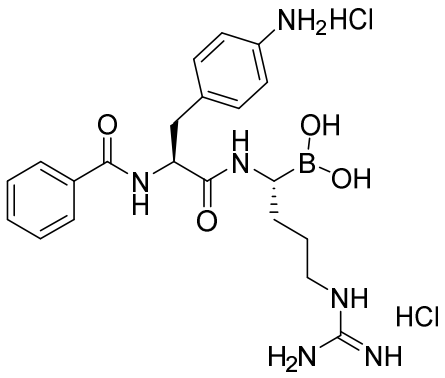
According to the general procedure, using crude **cn-602** (11.7 mg, 0.032 mmol) **1** (cn-604) was obtained as colorless solid (3.5 mg, 40%). ^{1}H-NMR (500 MHz, D_2O): δ = 1.62 (m, 2H), 1.80 (m, 2H), 2.70 (t, *J* = 7.6 Hz, 1H), 3.06 (t, *J* = 7.4 Hz, 2H), 7.59 (td, *J* = 7.5, 1.8 Hz, 2H), 7.73 (tt, *J* = 7.6, 1.2 Hz, 1H), 7.93 (dd, *J* = 8.4, 1.2 Hz, 2H) ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{11}H_{16}BN_2O_2$: 219.1299, found: 219.1337.

2 (cn-609)



According to the general procedure, using crude **cn-608** (0.1 mmol) **2** (cn-609) was obtained as colorless solid (19 mg, 60%). ^{1}H-NMR (500 MHz, D_2O): δ = 1.61 (m, 2H), 1.71 (m, 2H), 2.70 (t, *J* = 7.5 Hz, 1H), 3.24 (t, *J* = 6.9 Hz, 2H), 7.59 (td, *J* = 7.5, 1.8 Hz, 2H), 7.73 (tt, *J* = 7.5, 1.3 Hz, 1H), 7.92 (dd, *J* = 8.5, 1.2 Hz, 2H) ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{12}H_{18}BN_4O_2$: 261.1517, found: 261.1514.

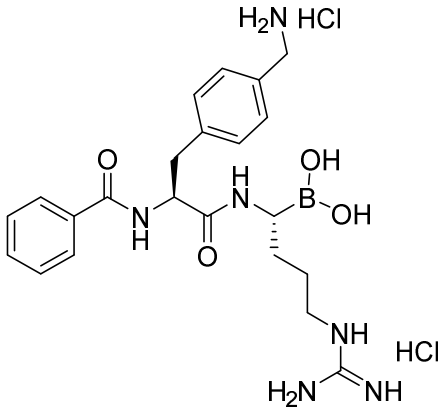
3 (cn-633)



According to the general procedure, using crude **cn-632** (0.075 mmol) **3** (cn-633) was obtained as colorless solid (15 mg, 39%). ^{1}H-NMR (500 MHz, D_2O): δ = 1.41-1.58 (m, 4H), 2.67 (m, 1H), 3.14 (t, *J* = 6.7 Hz, 2H), 3.18 (dd, *J* = 13.7, 9.7 Hz, 1H), 3.36 (dd, *J* = 13.9, 6.3 Hz, 1H), 4.97 (dd, *J* = 9.3, 6.2 Hz, 1H), 7.32 (m, 2H), 7.46 (m, 4H), 7.61 (m, 3H) ppm; ^{13}C-NMR (125 MHz, D_2O): δ = 25.8, 27.0, 36.0, 40.9, 52.8, 123.0, 127.1, 128.7, 128.9, 130.8, 132.5, 132.6,

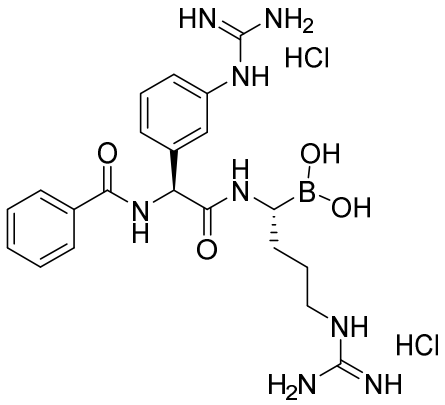
137.4, 170.9, 174.6 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{30}BN_6O_4$: 441.2420, found: 441.2400.

4 (cn-716)



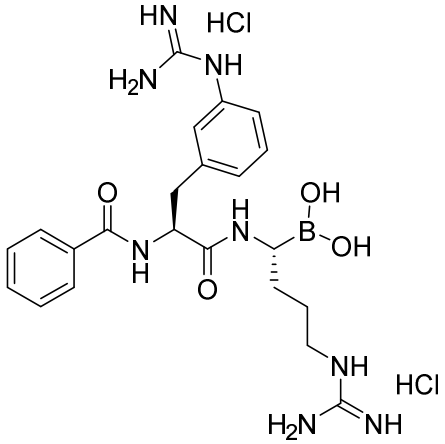
According to the general procedure, using crude **cn-713** (0.18 mmol) **4** (cn-716) was obtained as colorless solid (15 mg, 16%). ^{1}H-NMR (500 MHz, D_2O): δ = 1.38-1.54 (m, 4H), 2.65 (m, 1H), 3.13 (t, *J* = 6.8 Hz, 2H), 3.17 (dd, *J* = 13.9, 9.5 Hz, 1H), 3.33 (dd, *J* = 13.8, 6.4 Hz, 1H), 4.12 (s, 2H), 4.96 (dd, *J* = 9.5, 6.5 Hz, 1H), 7.38 (m, 4H), 7.27 (m, 2H), 7.61 (m, 3H) ppm; ^{13}C-NMR (125 MHz, D_2O): δ = 25.8, 27.1, 36.3, 40.9, 42.7, 52.8, 127.1, 128.7, 129.1, 129.9, 131.4, 132.5, 132.6, 137.2, 170.9, 174.8 ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{22}H_{30}BN_6O_3$: 437.2471, found: 437.2463.

5 (cn-657)



According to the general procedure, using crude **cn-655** (0.033 mmol) **5** (cn-657) was obtained as colorless solid (4 mg, 23%). ^{1}H-NMR (300 MHz, D_2O): δ = 1.47-1.61 (m, 4H), 2.82 (m, 1H), 3.13 (m, 2H), 5.75 (s, 1H), 7.37 (m, 1H), 7.43 (m, 1H), 7.51 (m, 3H), 7.62 (m, 2H), 7.78 (m, 2H) ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{21}H_{28}BN_8O_3$: 451.2376, found: 451.2362.

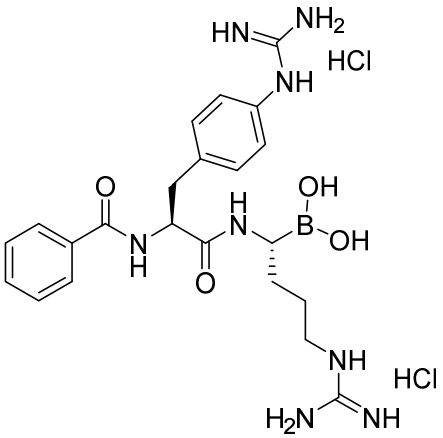
6 (cn-715)



According to the general procedure, using crude **cn-712** (0.069 mmol) **6** (cn-715) was obtained as colorless solid (14 mg, 37%). ^{1}H-NMR (500 MHz, D_2O): δ = 1.41-1.56 (m, 4H), 2.65 (m, 1H), 3.13 (m, 2H), 3.17 (dd, *J* = 13.8, 9.3 Hz, 1H), 3.33 (dd, *J* = 13.8, 6.4 Hz, 1H), 4.96 (dd, *J* = 9.4, 6.3 Hz, 1H), 7.21 (m, 2H), 7.32 (m, 1H), 7.41 (m, 1H), 7.48 (m, 2H), 7.62 (m, 3H) ppm; ^{13}C-NMR (125 MHz, D_2O): δ = 25.9, 27.1, 36.3, 40.9, 52.8, 124.4, 126.4, 126.5, 127.2, 128.7, 130.3,

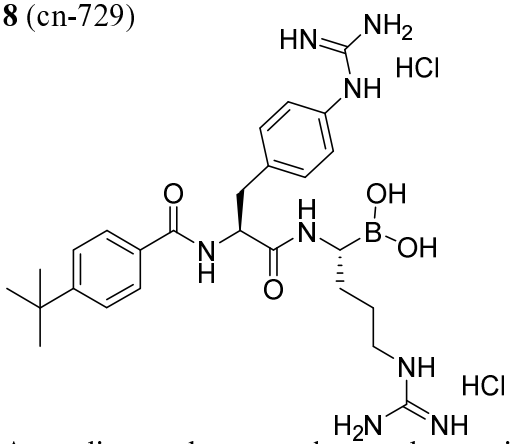
132.5, 132.6, 134.3, 138.2, 170.8, 174.6 ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{22}H_{30}BN_8O_3$: 465.2532, found: 465.2518.

7 (cn-714)



According to the general procedure, using crude **cn-711** (0.0835 mmol) **7** (cn-714) was obtained as colorless solid (15 mg, 33%). ^{1}H-NMR (300 MHz, D_2O): δ = 1.37-1.57 (m, 4H), 2.65 (m, 1H), 3.13 (m, 2H), 3.17 (dd, *J* = 13.8, 9.3 Hz, 1H), 3.32 (dd, *J* = 13.9, 6.7 Hz, 1H), 4.94 (dd, *J* = 9.3, 6.8 Hz, 1H), 7.25 (m, 2H), 7.41 (m, 2H), 7.50 (m, 2H), 7.63 (m, 3H) ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{22}H_{30}BN_8O_3$: 465.2532, found: 465.2527.

8 (cn-729)



According to the general procedure, using crude **cn-728** (0.11 mmol) **8** (cn-729) was obtained as colorless solid (24 mg, 36%). ^{1}H-NMR (500 MHz, D_2O): δ = 1.29 (s, 9H), 1.38-1.58 (m, 4H), 2.64 (m, 1H), 3.13 (t, *J* = 6.7 Hz, 2H), 3.17 (dd, *J* = 13.8, 9.3 Hz, 1H), 3.32 (dd, *J* = 13.8, 6.6 Hz,

1H), 4.94 (dd, *J* = 9.1, 6.7 Hz, 1H), 7.25 (m, 2H), 7.40 (m, 2H), 7.56 (m, 2H), 7.63 (m, 2H) ppm; ^{13}C-NMR (125 MHz, D_2O): δ = 25.8, 27.1, 30.2, 34.4, 36.2, 40.9, 52.9, 125.8, 125.9, 127.2, 129.6, 130.7, 133.1, 136.0, 156.8, 170.5, 174.8 ppm; HRMS (ESI): *m/z* $[M–H_2O+H]^+$ calcd for $C_{26}H_{38}BN_8O_3$: 521.3159, found: 521.3151.

General procedure for synthesis of peptidyl-amides 18-25

All peptidyl-amides were synthesized analogously to the Fmoc protocol using Rink amide resin, using either commercially available or pre-synthesized amino acids. Rink amid resin was swelled in DCM overnight before Fmoc was removed by 25 % piperidine solution for 10 min. The procedure was repeated for another 5 min. After Fmoc removal the resin was washed wit DMF, DCM and DMF. Subsequently, amino acid (3.0 equiv) and HATU (3.0 equiv) were dissolved in 400-500 µL DMF and DIPEA (5.0 equiv) was added. The reaction mixture was added to the resin and stirred for at least one hour. After coupling the resin was again washed with DMF, DCM and DMF. The procedure was repeated for all amino acids. Benzoic acid and *tert*-butyl benzoic acid as N-terminal caps were coupled to the peptide substituted resin by adding a mixture of cap (3.0 equiv), HATU (3.0 equiv) and DIPEA (5.0 equiv). The already benzoyl protected amino acids (**10**, **11**, **16**) (2.0 equiv) were coupled with HATU (2.0 equiv) and diisopropylethylamine (2.2 equiv) to the sequence. The reaction mixture was stirred for three hours. The resin was washed with DMF, DCM and DEE. The resin was dried overnight and the peptide was cleaved by the standard procedure (92.5 % TFA, 5.0 % TIPS and 2.5 % water) for 2.5 hours. After precipitation in cold diethyl ether peptides were purified by preparative RP-HPLC on an ÄKTApurifier, GE Healthcare (Germany) with a RP-18 column (Rephospher, Dr.

Maisch GmbH, Germany, C18-DE, 5 µm, 30 x 16 mm and 120 x 16 mm). All peptides were freeze-dried and characterized by HR-ESI and ^{1}HNMR.

Synthesis of 22 (lw-211)

22 was synthesized as described previously.[13] In short, to a presynthesized sequence of compound **17** coupled to Pbf-protected arginine on Rink amide resin (scale: 63 µM) was added tin(II)chloride dihydrate (815 mg) and diisopropylethylamine (200 µL) in 2 mL of DMF and shaken overnight. The resin was washed and a mixture of bis-boc-pyrazole-1-carboxamidine (150 mg) and DMAP (20 mg) in 2 mL of DMF was added and shaken overnight. The resin was washed, dried overnight and the peptide was cleaved using the standard procedure.

Table S3. Analytical data of peptidyl-amides **18-25**

Compound	**Molecular Formula**	**$[M+H]^+$ m/z calcd**	**$[M+H]^+$ m/z found**
18 (lw-214)	$C_{12}H_{17}N_3O_2$	236.1394	236.1389
19 (lw-208)	$C_{13}H_{19}N_5O_2$	278.1612	278.1619
20 (lw-218)	$C_{22}H_{29}N_7O_3$	440.2405	440.2396
21 (lw-220)	$C_{23}H_{31}N_7O_3$	454.2561	454.2559
22 (lw-211)	$C_{22}H_{29}N_9O_3$	468.2466	468.2440
23 (lw-221)	$C_{23}H_{31}N_9O_3$	482.2623	482.2613
24 (lw-206)	$C_{23}H_{31}N_9O_3$	482.2623	482.2597
25 (lw-219)	$C_{27}H_{39}N_9O_3$	538.3249	538.3239

18: ^{1}H-NMR (500 MHz, D_2O): δ = 7.79 (d, *J* = 7.46 Hz, 2H), 7.59-7.66 (m, 1H), 7.49-7.56 (m, 2H), 4.52 (dd, *J* = 5.53, 8.83 Hz, 1H), 3.05 (t, *J* = 7.49 Hz, 2H), 1.75-2.06 (m, 4H) ppm.

19: ^{1}H-NMR (500 MHz, D_2O): δ = 7.79 (d, *J* = 7.95 Hz, 2H), 7.59-7.66 (m, 1H), 7.49-7.56 (m, 2H), 4.49 (dd, *J* = 5.96, 7.98 Hz, 1H), 3.24 (t, *J* = 6.88 Hz, 2H), 1.66-2.02 (m, 5H) ppm.

20: ^{1}H-NMR (500 MHz, D_2O): δ = 7.65 (d, *J* = 7.46 Hz, 2H), 7.57-7.62 (m, 1H), 7.46-7.52 (m, 2H), 7.43 (d, *J* = 7.21 Hz, 2H), 7.33 (d, *J* = 6.79 Hz, 2H), 4.78-4.82 (m, 1H) overlapped by solvent peak, 4.32 (dd, *J* = 5.01, 8.86 Hz, 1H), 3.18-3.30 (m, 2H), 3.15 (t, *J* = 7.43 Hz, 2H), 1.77-1.87 (m, 1H), 1.53-1.74 (m, 3H) ppm.

21: ^{1}H-NMR (500 MHz, D_2O): δ = 7.67 (d, *J* = 9.29 Hz, 2H), 7.58-7.63 (m, 1H), 7.46-7.52 (m, 2H), 7.36-7.41 (m, 3H), 4.20 (dd, *J* = 4.71, 9.60 Hz, 1H), 4.13 (s, 2H), 3.19-3.27 (m, 2H), 3.08 (t, *J* = 7.67 Hz, 2H), 1.75-1.86 (m, 2H), 1.55-1.66 (m, 2H), 1.35 (td, *J* = 7.90, 15.38 Hz, 2H) ppm.

22: ^{1}H-NMR (500 MHz, D_2O): δ = 7.78 (d, *J* = 7.21 Hz, 3H), 7.57-7.67 (m, 2H), 7.46-7.55 (m, 3H), 4.49 (dd, *J* = 5.44, 8.99 Hz, 1H), 3.23 (t, *J* = 6.85 Hz, 3H), 1.62-2.04 (m, 6H) ppm.

23: ^{1}H-NMR (500 MHz, D_2O): δ = 7.64-7.72 (m, 2H), 7.58-7.64 (m, *J* = 17.70 Hz, 1H), 7.47-7.53 (m, 2H), 7.39-7.47 (m, 1H), 7.27-7.33 (m, 1H), 7.18-7.23 (m, 2H), 4.32 (dd, *J* = 5.29, 9.14 Hz, 1H), 3.13-3.27 (m, 3H), 3.03-3.12 (m, 1H), 1.52-1.89 (m, 4H), 1.19-1.36 (m, 2H) ppm.

24: ^{1}H-NMR (500 MHz, D_2O): δ = d 7.69 (d, *J* = 7.15 Hz, 2H), 7.58-7.63 (m, 1H), 7.47-7.53 (m, 2H), 7.37 (d, *J* = 8.44 Hz, 2H), 7.25 (d, *J* = 8.44 Hz, 2H), 4.32 (dd, *J* = 5.26, 9.11 Hz, 1H), 3.20 (d, *J* = 8.01 Hz, 2H), 3.12 - 3.17 (m, 2H), 1.52-1.85 (m, 6H) ppm.

25: ^{1}H-NMR (500 MHz, D_2O): δ = 7.66 (d, *J* = 7.70 Hz, 2H), 7.58 (d, *J* = 8.31 Hz, 2H), 7.36 (d, *J* = 7.58 Hz, 2H), 7.24 (d, *J* = 7.64 Hz, 2H), 4.28-4.34 (m, 1H), 3.20 (d, *J* = 8.07 Hz, 2H), 3.12-3.17 (m, 2H), 1.51-1.87 (m, 6H), 1.30 (s, 9H) ppm.

General procedure for synthesis of peptidyl-carboxylic acids 26-29

All peptidyl-carboxylic acids were synthesized analogously to the protocol described for peptidyl-amides by using presubstituted Fmoc-Arg(Pbf)-Wang resin. In short, Wang resin was

swelled in DCM overnight before Fmoc was removed by 25 % piperidine solution for 10 min and again 5 min. After Fmoc removal and washing steps (DMF, DCM, DMF) the amino acids (3.0 equiv) and HATU (3.0 equiv) were dissolved in 400 µL DMF and DIPEA (5.0 equiv) was added. The reaction mixture was added to the resin and stirred for at least one hour. After coupling and washing the procedure was repeated for benzoic acid. After the last coupling the resin was washed with DMF, DCM and DEE. The resin was dried overnight and the peptide was cleaved by the standard procedure (92.5 % TFA, 5.0 % TIPS and 2.5 % water) for 2.5 hours. After precipitation in cold diethyl ether peptides were purified by preparative RP-HPLC on an ÄKTApurifier, GE Healthcare (Germany) with a RP-18 column (Rephospher, Dr. Maisch GmbH, Germany, C18-DE, 5 µm, 30 x 16 mm and 120 x 16 mm). All peptides were freeze-dried and characterized by HR-ESI.

Table S4. Analytical data of peptidyl-carboxylic acids **LW-262 - LW-265**

Compound	**Molecular Formula**	**$[M+H]^+$ m/z calcd**	**$[M+H]^+$ m/z found**
26 (lw-262)	$C_{13}H_{18}N_4O_3$	279.1452	279.1454
27 (lw-263)	$C_{22}H_{28}N_6O_4$	441.2245	441.2239
28 (lw-264)	$C_{23}H_{30}N_6O_4$	455.2401	455.2395
29 (lw-265)	$C_{23}H_{30}N_8O_4$	483.2463	483.2457

3.4 Purity of final compounds used for biochemical and biological studies

The purity of all compounds was tested at a concentration of 50 µM in water (diluted from 10 mM compound stocks in DMSO). The measurements were determined by analytical HPLC on a Jasco HPLC system with UV detector and RP-18 column (ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany, 5 µm, 50 x 2 mm). The conditions were: eluent A: water (0.1% TFA), eluent

B: acetonitrile (0.1% TFA), flow rate: 1 ml/min, gradient: 1% B (0.2 min), 100% B (3.5 min), 100% B (4.5 min), 1% B (4.6 min), 1 %B (5 min).

Table S5. HPLC-purity data for compounds **1-8**, **18-25** and **LW-262 - LW-265**

Compound	Retention time (HPLC)	HPLC purity
1	1.37 min	90%[a]
2	1.62 min	≥ 95%[a]
3	1.62 min	≥ 95%[a]
4	1.73 min	≥ 95%
5	1.76 min	≥ 95%
6	1.79 min	≥ 95%
7	1.76 min	≥ 95%
8	2.21 min	≥ 95%[a]
18	1.07 min	≥ 95 %
19	1.54 min	≥ 95 %
20	1.57 min	≥ 95 %
21	1.62 min	≥ 95 %
22	1.59 min	55 %[b]
23	1.71 min	≥ 95 %
24	1.70 min	≥ 95 %
25	2.14 min	≥ 95 %
26	1.55 min	92%
27	1.55 min	94%
28	1.58 min	≥ 95 %
29	1.60 min	≥ 95 %

[a] Chromatographically non-separable peak shoulders were detected, which may derive from various conformations, cyclic condensations or protonation states of the actual compound.

[b] A chromatographically non-separable peak shoulder was detected, which was identified as precursor without enzymatic activity.

4. Co-crystallization and X-ray structure analysis

Recombinant production of the WNV-NS2B-NS3 protease

Cloning and expression of a DNA construct encoding the WNV NS2B-NS3 protease (NS2B-NS3^{pro}) with a covalent Gly_4-Ser-Gly_4 linker between NS2B and NS3 was performed as described previously, as was the purification of the protease.[14] The construct comprised 47 residues (49-96) of NS2B and 170 residues (1-170) of NS3; the C-terminal lysine of NS2B was replaced by alanine, in order to avoid autoproteolytic cleavage in the NS2B-NS3^{pro} junction region.

Crystallization and diffraction data collection

The purified protein in 25 mM Tris pH 8.5, 5% glycerol, was concentrated to 49.8 mg/ml by using an Amicon YM10 membrane (EMD Millipore), and mixed with compound **4** at 20 mM final concentration. Following overnight-incubation at 4°C, crystallization screens were run using a Phoenix™ robot (Art Robbins), with application of the sitting-drop vapor-diffusion method at 18°C. The drops contained 0.25 µl of protein and 0.25 µl of reservoir solution, to be equilibrated against 75 µl reservoir solution. Five commercially available screening kits were used: MD1-01 & 02 (Molecular Dimensions), SaltRx™, PEG/Ion™ 1 & 2, Index™, and PEG Rx™ 1 & 2 (Hampton Research). Within two days, tiny crystallites were detected in the presence of 0.2 M magnesium formate dihydrate, 20% polyethylene glycol (PEG) 3350, pH 7.0. Subsequent optimization involved equilibration of 1 µl protein-inhibitor solution mixed with 1 µl mother liquor containing 0.2 M magnesium formate and 20% PEG 3350, pH 7.0, against 500 µl reservoir solution at 18°C. Optimized crystals were protected by a cryobuffer consisting of 80% reservoir, 20% glycerol, and 20 mM of compound **4**, and flash-cooled in liquid nitrogen prior to data collection. Diffraction data were collected at 100 K at synchrotron beamline P11 of PETRA

III (DESY, Hamburg), using a Pilatus 6M fast detector at λ = 0.9919 Å. The data set was processed using the program XDSAPP[15] and scaled using SCALA from the CCP4 suite of programs.[16-17] The space group was determined as $P2_12_12_1$, with unit-cell parameters *a* = 36.21 Å, *b* = 96.56 Å, *c* = 186.92 Å. Diffraction data statistics are listed in Supplementary Table S6.

Structure elucidation and refinement

The structure was elucidated by the molecular replacement method using MOLREP from the CCP4 suite.[17-18] The structure of the complex between the WNV NS2B-$NS3^{pro}$ and the inhibitor 3,4-dichlorophenylacetyl-Lys-Lys-GCMA (PDB code: 2YOL)[14] was selected as search model. The rotation/translation search located 3 complex molecules in the asymmetric unit. A molecular model for compound **4** was generated by using JLIGAND[17, 19] and built into clear F_o-F_c difference density in the substrate-binding site of the protease. Most atoms of the inhibitor were well-defined by difference density at 3σ above the mean of this map. The structure was refined using REFMAC5 version 5.8.0131.[17, 20-21] Refinement statistics are summarized in Table S6.

Table S6. Diffraction data collection and refinement statistics

	WNV-NS2B-NS3 with cn716
Data collection statistics	
X-ray source	DESY P11
Wavelength [Å]	0.9919
Space group	$P2_12_12_1$ (No.19)
Unit cell dimensions [Å]	a = 36.42, b = 96.96, c = 187.86
Number of complex molecules per asymmetric unit	3
Resolution range [Å] [a]	46.94 - 1.50 (1.58 - 1.50)
Number of observations	350,519 (51,736)
Number of unique reflections	105,999 (15,393)
Completeness [%]	98.6 (99.3)
Mean I/σ(I)	15.6 (2.5)
Multiplicity	3.3 (3.4)
R_{merge} [%]	0.045 (0.557)
R_{pim} [b] [%]	0.028 (0.344)
$CC_{1/2}$	0.999 (0.749)
Refinement statistics	
R_{cryst} [c] /R_{free} [d] [%]	15.92 / 18.58
r.m.s. deviation in bond lengths (Å)	0.0252
r.m.s. deviation in bond angles (°)	2.34
Average B-factor for all atoms (Å^2)	21.043
Number of protein atoms	4711
Number of inhibitor atoms	111
Number of water atoms	637
Ramachandran plot	
Residues in preferred regions (%)	96.42
Residues in additionally allowed regions (%)	3.41
Residues in outlier regions (%)	0.17

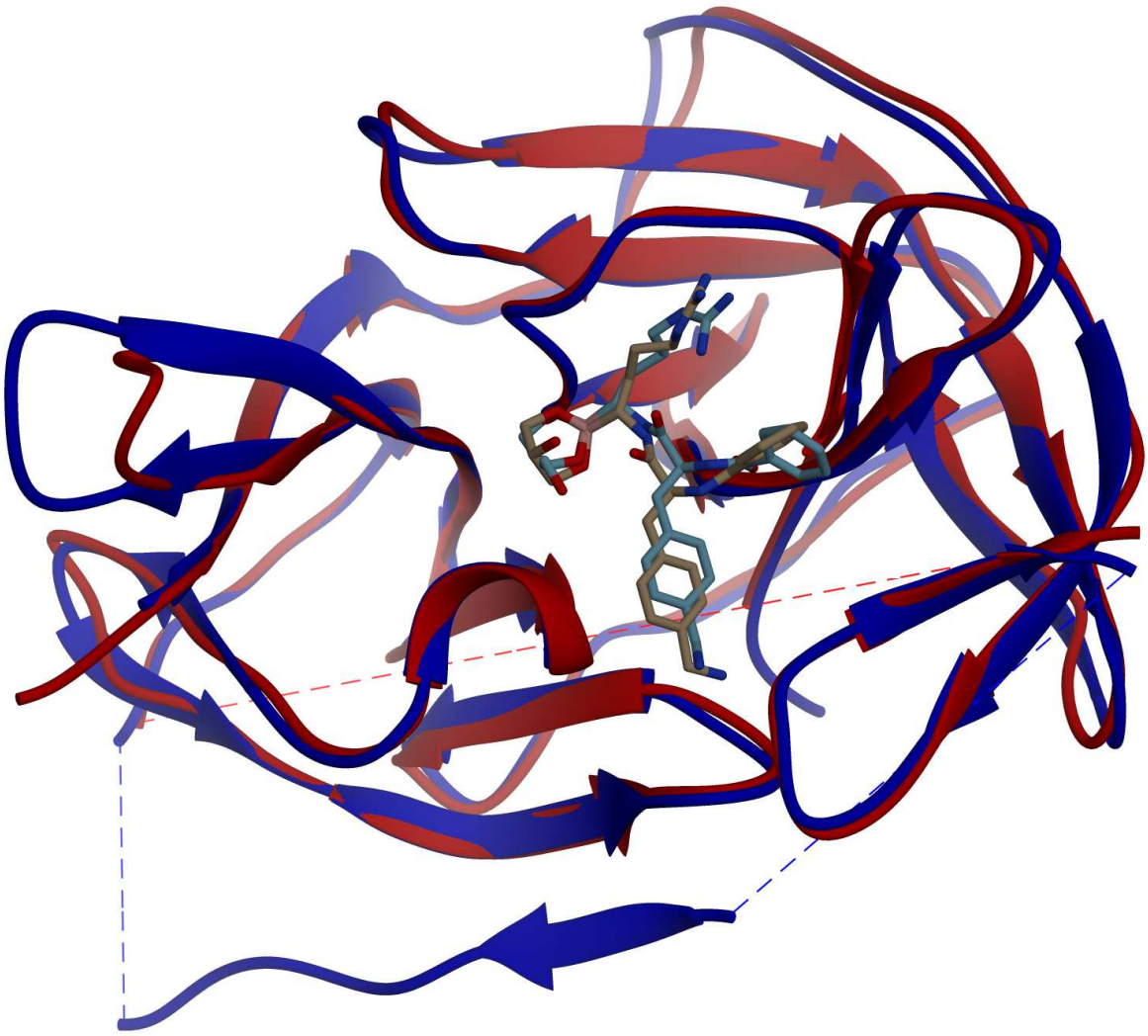
a The highest resolution shell is shown in parentheses.

b (ref. 9); $R_{pim} = \frac{\sum_{hkl} \sqrt{1/n-1} \sum_{i=1}^{n} \left| I_i(hkl) - \bar{I}(hkl) \right|}{\sum_{hkl} \sum_{i=1}^{n} I_i(hkl)}$

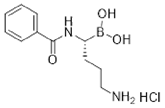
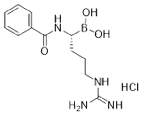
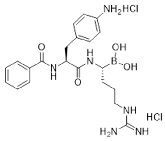
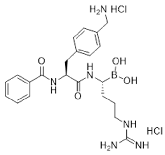
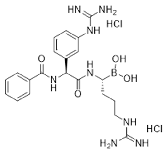
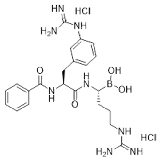
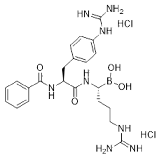
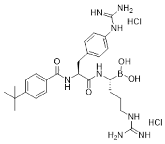
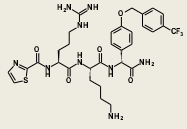
c $R_{cryst} = \frac{\sum_{hkl} \left| F_o(hkl) - F_c(hkl) \right|}{\sum_{hkl} \left| F_o(hkl) \right|}$

d R_{free} was calculated for a test set of reflections (5%) omitted from the refinement.

Figure S1. Overlay of the West Nile Virus and Zika protease structures in complex with compound **4**. The figure was created by applying the MatchMaker function of the Chimera software to the A chains in the 5LC0 and 5IDK structures. 5LC0 (ZIKV) is represented as red ribbons and with the ligand carbons in grey, 5IDK (WNV) as blue ribbons and with the ligand carbons in light blue.



5. SAR Table with full structures and reference compounds from the literature. (Table S7)

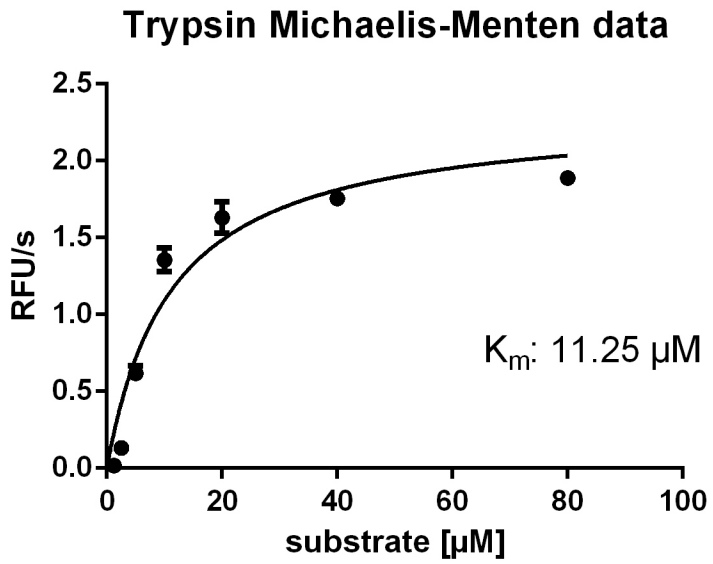
Cpd. ID	Source	Structure	Inhibition of DENV protease[a]	Inhibition of WNV protease[b]	Inhibition of ZIKV protease[c]	Cellular activity of the boronic acids (X=B(OH)$_2$)		
		K_i and IC50 values in µM; % inhibition values at [inhibitor] = 50 µM				Cyto-toxicity[d]	Antiviral effect EC_{50} (µM)[e]	
						CC_{50} (µM)	DENV	WNV
1	This work		8.3%	30%	IC_{50} > 50	> 100	7.5%	33%
2	This work		51.9%	18%	IC_{50} > 50	> 100	12%	19%
3	This work		IC_{50} = 7.4 K_i = 4.2	IC_{50} = 7.4 K_i = 6.0	IC_{50} > 40	10.1	44% (3.1 µM)	EC_{50} = 1.5
4	This work		IC_{50} = 0.066[f] K_i = 0.051[f]	IC_{50} = 0.11[g] K_i = 0.082[g]	IC_{50} = 0.25 K_i = 0.04	> 100	EC_{50} = 30	EC_{50} = 38
5	This work		IC_{50} = 0.26 K_i = 0.22	IC_{50} = 0.14 K_i = 0.12	IC_{50} = 1.2	> 100	24%	47%
6	This work		IC_{50} = 0.11 K_i = 0.091	IC_{50} = 0.31 K_i = 0.30	IC_{50} = 1.9	≥ 100	EC_{50} = 48	38%
7	This work		IC_{50} = 0.036[f] K_i = 0.027[f]	IC_{50} = 0.071[h] K_i = 0.065[h]	IC_{50} = 0.83	> 100	EC_{50} = 18	EC_{50} = 25
8	This work		IC_{50} = 0.11[f] K_i = 0.078[f]	IC_{50} = 0.21 K_i = 0.16	IC_{50} = 2.1	> 100	EC_{50} = 19	30%
83	Behnam et al., J. Med. Chem. 2015[22]		IC_{50} = 0.018 K_i = 0.012	IC_{50} = 0.050 K_i = 0.039	n.d.	> 100	EC_{50} = 20	EC_{50} = 23
21	Yin et al., BMCL 2005[23]	Bz-Nle-Lys-Arg-Arg-B(OH)2	K_i = 0.043	n.d.	n.d.	n.d.	n.d.	n.d.

Footnotes for compounds 1–8, see references for information on other compounds. All measurements were performed in triplicate. Standard deviations are always below 10%. Percentage inhibition values determined at 50 µM inhibitor concentration. K_i values were calculated from measurements at substrate concentrations of 50, 100, 150, and 200 µM. [a] Dengue serotype 2 NS2B-NS3 protease (100 nM unless stated otherwise). Substrate: 50 µM Abz-Nle-Lys-Arg-Arg-Ser-3-(NO_2)Tyr (K_m = 105 µM).

[b] WNV NS2B-NS3 protease (150 nM unless stated otherwise). Substrate: 50 µM Abz-Gly-Leu-Lys-Arg-Gly-Gly-3-(NO_2)Tyr (K_m = 212 µM). [c] ZIKV NS2B-NS3 protease (5 nM). Substrate: 10 µM Bz-Nle-Lys-Lys-Arg-AMC (K_m = 18 µM). [d] Cytotoxicity was assayed up to a maximum concentration of 100 µM. [e] Plaque assay results for DENV and WNV. Percentage inhibition values at 50 µM or at the highest non-toxic inhibitor concentration. [f] 50 nM DENV protease. [g] 100 nM WNV protease. [h] 75 nM WNV protease.

6. **Determination of the trypsin/Boc-Val-Pro-Arg-AMC Km value.**

(Figure S2)



7. References

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