Supporting Information

Novel Small Molecule Inhibitors of Activated Thrombin Activatable Fibrinolysis Inhibitor (TAFIa) from Natural Product Anabaenopeptin

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S1 Isolation and characterization of Anabaenopeptins

Cultivation of *P. rubescens*

Planktothrix rubescens strain Cya3 has been isolated from Lake Mondsee, while strain Cya14 has been isolated from Lake Irrsee (both located in Austria). The strains were classified as *P. rubescens* on the basis of morphology, PCR analysis and sequencing of various marker genes as previously described[1], and have been deposited under the accession numbers CBT 286 and CBT 287 in the culture collection of Cyano Biotech GmbH, Berlin, Germany. The strains were cultivated in BG11 medium[2] at 20 °C under continuous light (60-80 µmol m^{-2} s^{-1}) in 20 L scale photobioreactors and harvested semi-continuously over a period of several weeks. After harvest the biomass was freeze-dried.

Isolation of Anabaenopeptines

10g of the dried biomass from Cya3 cultivation was stirred for 60 min in 2,5l of a Methanol:Water (50:50) mixture. The solution was filtered, and the filtrate was put on an SPE column (MCI, CHP20P material, 50mm x 200 mm). A solid phase extraction using a NH4OAc (pH 4.6) : acetonitrile gradient (95:5 at 0 min to 0:100 at 20 min to 0:100 at 30 min) was performed applying a 90 ml/min flow rate and a fraction volume of 50 ml. Fractions 9-15 contained the compounds of interest and were therefore combined and freeze-dried, yielding 1.5 g of raw material. The raw material was dissolved in 15 ml of a Water:Methanol mixture (50:50) and further purified by reversed-phase chromatography. The separation was performed on a Waters® Sunfire RP-18 5µm (30 mm x 100 mm) using a 0,3% formic acid : acetonitrile gradient (95:5 at 0 min to 5:95 at 20 min to 5:95 at 30 min) applying a 50 ml/min

[1] a) Christiansen, G.; Kurmayer, R.; Liu, Q.; Börner, T. *Appl. Environ. Microbiol.* **2006**, 72, 117-123; b) Christiansen, G.; Molitor, C.; Philmus, B.; Kurmayer, R. *Mol. Biol. Evol.* **2008**, *25*, 1695–1704.

[2] Andersen, R. *Algal Culturing Techniques*.; Elsevier Academic Press, 2005; p. 596.

flow rate and a fraction collection triggered by the UV signal intensity at 220 nm. After freeze-drying of fractions, high purity material of Anabaenopeptin B (66 mg), Anabaenopeptin F (45 mg) and Oscillamide Y (24 mg) was obtained.

Anabaenopeptin C has been isolated from an extract obtained from Cya14 cultivation following a different protocol as outlined below:

Cell disruption and extraction: After completion of a 200 L-fermentation of CYA14, the culture broth was filtered and the remaining cells were freeze dried. The lyophilized material (~80 g) was transferred into a 2 L Schott bottle and suspended with 2 L MeOH/H2O (ratio 1:1). The suspension was dispersed for 5 min using an ultra turrax and then transferred into a french press. Cell disruption has been performed for 30 min at a pressure of 2250 bar. The extract (~2 L) was centrifuged for 30 min using a Heraeus cryofuge 8500 (4 °C, 5000 rpm). After 30 min the supernatant was decanted and the cell pellet was re-suspended in an ultrasonic bath with 2 L MeOH/H2O (ratio 1:1) for 30 min. Extract 2 was also centrifuged for 30 min at 5000 rpm. The supernatants were combined and filtered to give ~4 L crude exctract.

The crude extract (ca. 4 L) has been loaded onto a column (dimension: 160 x 200 mm) filled with ~3.0 L of CHP-20P (MCI® Gel, 75-150µ, Mitsubishi Chemical Corporation) material. Compounds were eluted at a flow rate of 240 ml/min using a gradient from 10 % to 100 % of 2-propanol in 0.65 M ammonium acetate buffer pH 7.0. Fractions have been collected every 4 min over a period of 40 min. The Anabaenopeptin containing fractions 6, 8 and 9 were freeze-dried and further purified.

Fraction 6 (~890 mg) was dissolved in methanol and split into two parts. Fraction 8 (~280 mg) and 9 (~130 mg) were combined and also dissolved in methanol. The three solutions were separately loaded onto a Waters Dynamax Pursuit C18 column (dimension: 41 mm x

100 mm, 10 µm) with a Waters XTerra® pre-column (dimension: 19 x 10 mm, 10 µm). Compounds were eluted with a gradient from 5 % to 95 % acetonitrile in water over a period of 43 min at a flow rate of 150 ml/min. The buffer (0.65 M ammonium acetate, pH 7.0) was pumped into the system with an additional pump at a flow rate of 2.0 ml/min. The eluents have been collected in 50 ml-fractions using UV-triggering. Anabaenopeptin-containing fractions have been pooled (run 1/2: fraction 6-8, run 3: fraction 3+4).

The pre-purified pool from step 3 was directly loaded onto a Waters Sunfire C18 column (dimension: 20 mm x 100 mm, 5 µm) with a Waters XTerra® pre-column (dimension: 19 x 10 mm, 10 µm). Compounds were eluted with a gradient from 5 % to 95 % acetonitrile in water over a period of 43 min at a flow rate of 45 ml/min. The buffer (0.65 M ammonium acetate, pH 7.0) was pumped into the system with an additional pump at a flow rate of 0.6 ml/min. The eluents have been collected in 1 ml-fractions using UV-triggering. Anabaenopeptin containing fractions have been pooled (fractions 8 and 9). After freeze-drying, 20 mg of Anabaenopeptin C have been obtained.

High resolution mass spectrometry and UV data:

Anabaenopeptin B: ESI+ m/z obs = 837.4625, m (neutral) = 836.4552, m (expected for $C_{41}H_{60}N_{10}O_9$) = 836.45448; UV: 208, 277 nm

Anabaenopeptin C: ESI+ m/z obs = 809.4580, m (neutral) = 808.45072, m (expected for $C_{41}H_{60}N_8O_9$ = 808.4483 UV: 206, 276 nm

Anabaenopeptin F: ESI+ m/z obs = 851,4803, m (neutral) = 850.4730, m (expected for $C_{42}H_{62}N_{10}O_9$) = 850.4701 UV: 208, 278 nm

Oscillamide Y: ESI+ m/z obs = 858.4386, m (neutral) = 857.43132, m(expected for $C_{45}H_{59}N_7O_{10}$) 857.4323 m UV: 222s, 227, 277 nm

^{1}H-1D- and 2D-NMR spectra were recorded on either a Bruker AVANCE 500 spectrometer operating at a proton frequency of 500.30 MHz and a ^{13}C-carbon frequency of 125.82 MHz or on a Bruker AVANCE 700 spectrometer operating at a proton frequency of 700.20 MHz and a ^{13}C-carbon frequency of 176.08 MHz. Both instruments were equipped with a 5 mm TXI cryo probe head. The 1D ^{13}C-spectra were recorded on a Bruker DRX 600 operating at a proton frequency of 600.20 MHz and a ^{13}C-carbon frequency of 150.94 MHz. This instrument was equipped with a room temperature ^{13}C-selective probe head. All experiments were carried out with samples of 3 - 5 mg compound dissolved in 600 µl d6-DMSO at 300 K. For structure elucidation and complete assignment of proton and carbon resonances 1D-^{1}H, 1D-^{13}C, DQF-COSY, ROESY (mixing time 150 ms, spinlock field 2 kHz), HSQC, and HMBC spectra were acquired. ^{1}H- and ^{13}C-chemical shifts were referenced to the solvent signals (^{1}H: 2.50 ppm, ^{13}C: 39.50 ppm).

Two-dimensional homonuclear experiments, DQF-COSY and ROESY, were performed with a spectral width of 10 ppm. Spectra were recorded with 512 increments in t_1 and 4096 complex data points in t_2. For each t_1 value 2 (DQF-COSY) or 8 (ROESY) transients were averaged, respectively.

For HSQC spectra 512 increments with 2048 complex data points in t_2 were collected using a sweep width of 10 ppm in the proton and 160 ppm in the carbon dimension. For each t_1 value 4 transients were averaged. The HMBC spectrum was acquired with a sweep width of 10 ppm in the proton and 200 ppm in the carbon dimension using a defocusing

delay of 62 ms (optimized for coupling constants of 8 Hz). A total of 16 transients were averaged for each of 512 increments in t_1, and 4096 complex points in t_2 were recorded.

Anabaenopeptin B **1a**

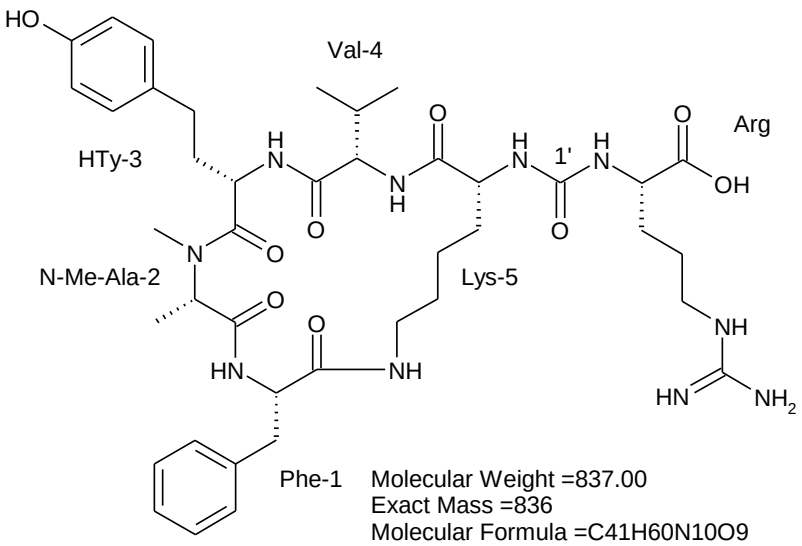


Fig. S1: Structure of *Anabaenopeptin B* **1a**

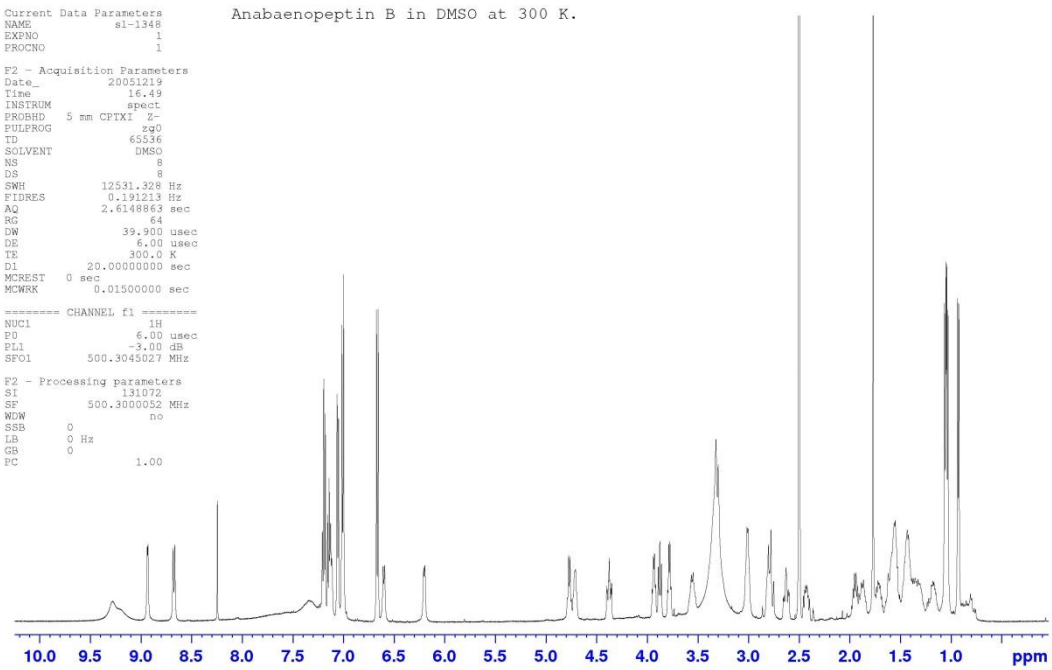


Fig. S2: ^{1}H-spectrum of *Anabaenopeptin B* **1a** in DMSO-d6 at 300 K.

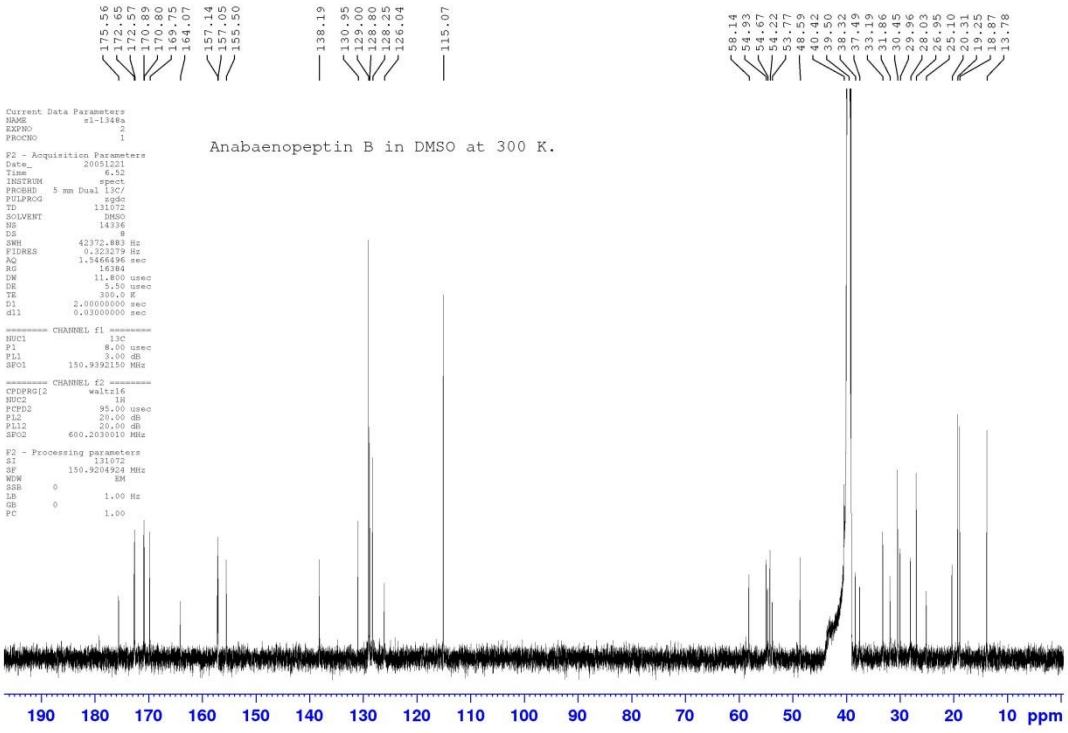


Fig. S3: ^{13}C-spectrum of *Anabaenopeptin B* **1a** in DMSO-d6 at 300 K.

Table S1: Chemical shifts of *Anabaenopeptin B* **1a** in DMSO-d6 at 300 K.

	^{1}H	^{13}C
Phe-1 NH	8.67	-
α	4.38	54.93
β	3.31/2.78	37.49
γ	-	138.19
δ	7.06	128.80
ε	7.19	128.25
ζ	7.14	126.04
C’	-	170.80
N-Me-Ala-2 NMe	1.77	26.95
α	4.77	54.22

	β	1.06	13.78
	C’	-	169.75
HTy-3	NH	8.93	-
	α	4.72	48.59
	β	1.88/1.71	33.19
	homo-β	2.63/2.43	30.45
	γ	-	130.95
	δ	7.01	129.00
	ε	6.67	115.07
	ζ	-	155.50
	ζ-OH	broad	-
	C’	-	170.89
Val-4	NH	7.01	-
	α	3.88	58.14
	β	1.95	29.96
	γ	1.04	18.87
	γ’	0.93	19.25
	C’	-	172.65
Lys-5	NH	6.60	-
	α	3.93	54.67
	β	1.60	31.86
	γ	1.31/1.17	20.31
	δ	1.44	28.03
	ε	3.56/2.81	38.32
	ζ-NH	7.13	-

	C’	-	172.57
Arg-6	NH	6.20	-
	α	3.78	53.77
	β	1.56	30.45
	γ	1.43/1.36	25.10
	δ	3.01	40.42
	ε	broad	-
	ζ	-	157.14
	ζ-NH_2	broad	-
	C’	-	175.56
	1’	-	157.05

Anabaenopeptin F **1b**

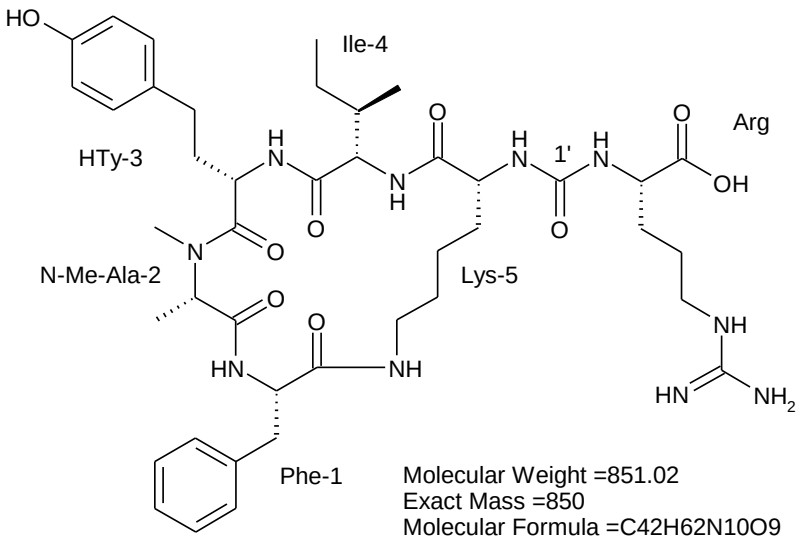


Fig. S4 Structure of *Anabaenopeptin F* **1b**

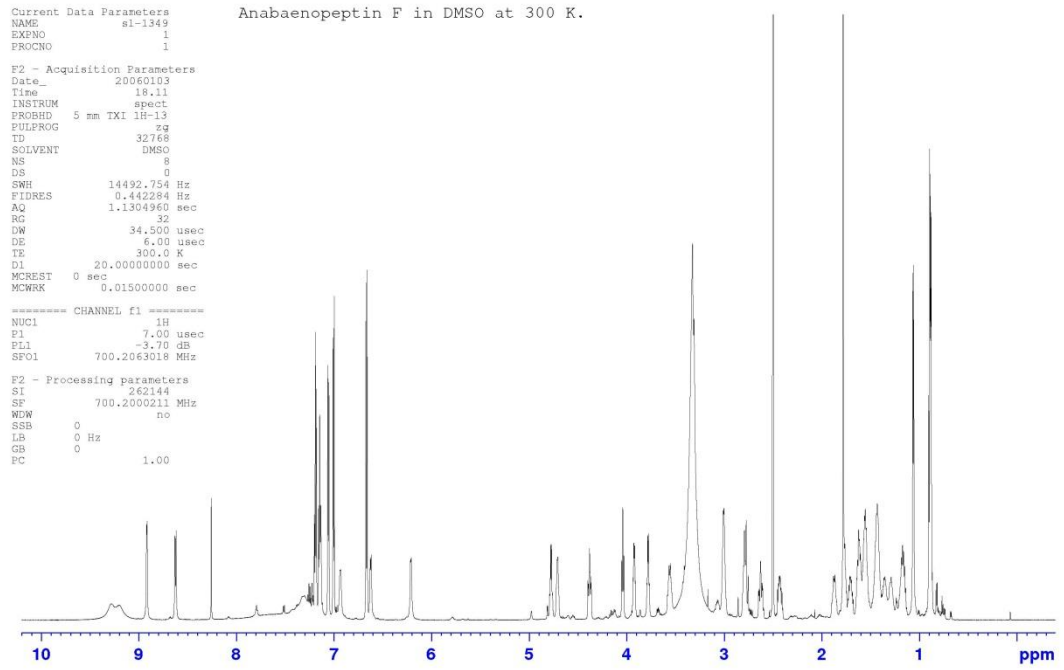


Fig. S5: ^{1}H-spectrum of *Anabaenopeptin F* **1b** in DMSO-d6 at 300 K.

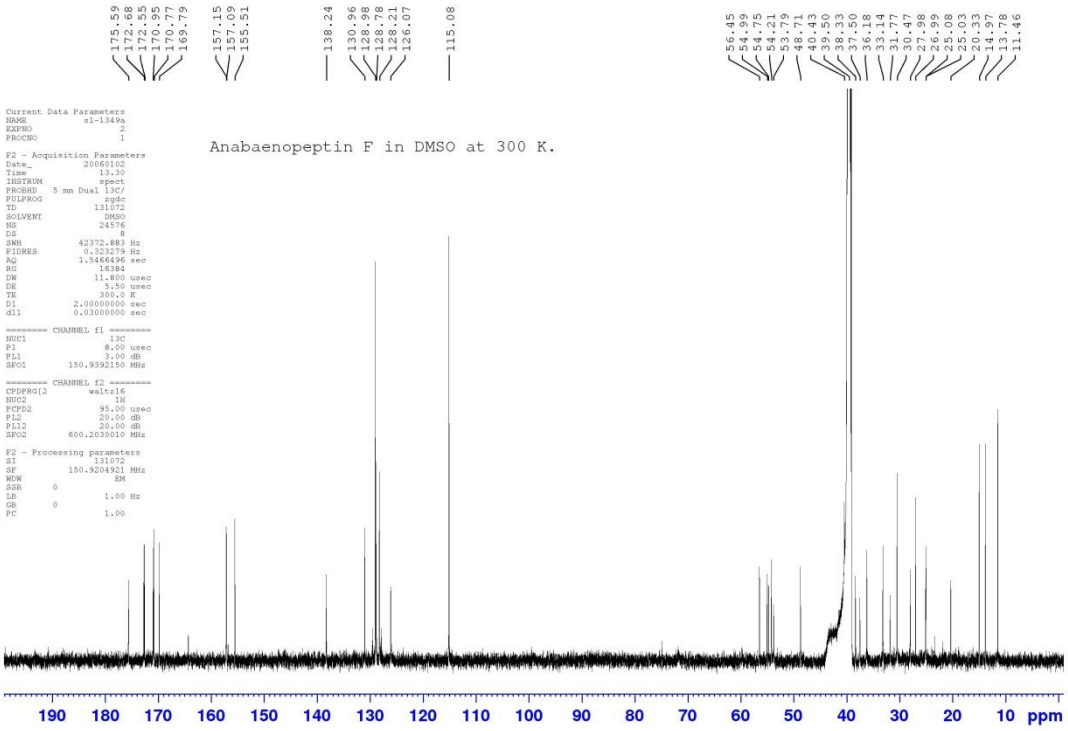


Fig. S6: ^{13}C-spectrum of *Anabaenopeptin F* **1b** in DMSO-d6 at 300 K.

Table S2: Chemical shifts of *Anabaenopeptin F* **<u>1b</u>** in DMSO at 300 K.

	^{1}H	^{13}C
Phe-1 NH	8.62	-
α	4.38	54.99
β	3.31/2.77	37.50
γ	-	138.24
δ	7.06	128.78
ε	7.19	128.21
ζ	7.15	126.07
C’	-	170.77
N-Me-Ala-2 NMe	1.78	26.99
α	4.77	54.21
β	1.06	13.78
C’	-	169.79
HTy-3 NH	8.92	-
α	4.71	48.71
β	1.87/1.71	33.14
homo-β	2.63/2.43	30.47
γ	-	130.96
δ	7.00	128.98
ε	6.67	115.08
ζ	-	155.51
ζ-OH	broad	-
C’	-	170.95

Ile-4	NH	6.93	-
	α	4.04	56.45
	β	1.76	36.18
	β-Me	0.88	14.97
	γ	1.62/1.16	25.03
	δ	0.89	11.46
	C’	-	172.68
Lys-5	NH	6.62	-
	α	3.92	54.75
	β	1.61/1.56	31.77
	γ	1.29/1.18	20.33
	δ	1.44	27.98
	ε	3.56/2.79	38.33
	ζ-NH	7.14	-
	C’	-	172.55
Arg-6	NH	6.21	-
	α	3.78	53.79
	β	1.55	30.47
	γ	1.43/1.36	25.08
	δ	3.01	40.43
	ε	Broad	-
	ζ	-	157.15
	ζ-NH_2	broad	-
	C’	-	175.59
	1’	-	157.09

Anabaenopeptin C **1c**

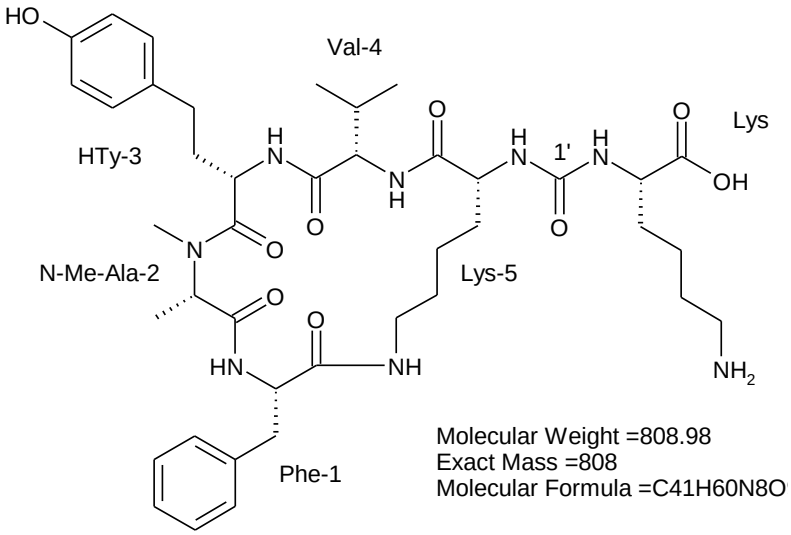


Fig. S7: Structure of *Anabaenopeptin C* **1c**

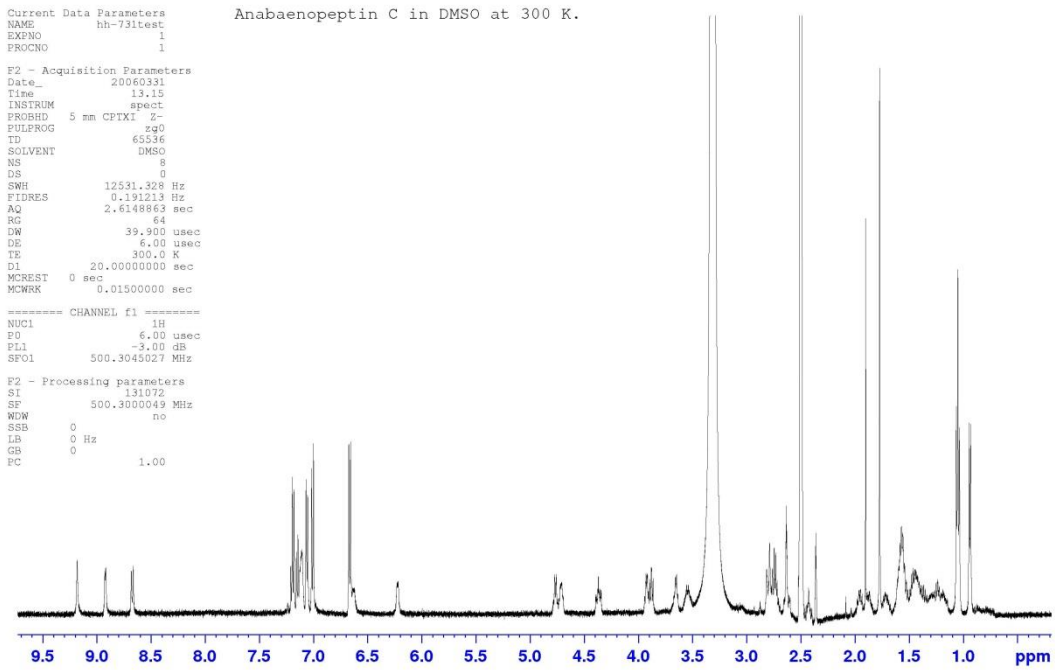


Fig. S8: ^{1}H-spectrum of *Anabaenopeptin C* **1c** in DMSO-d6 at 300 K.

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.94 (d, 3 H), 1.04 (d, 3 H), 1.06 (d, 3 H), 1.96 – 1.12 (m, 15 H), 1.77 (s, 3H), 2.74 (m, 1 H), 2.79 (m, 1 H), 3.54 (m, 1 H), 3.65 (m, 1 H), 3.88 (m, 1 H), 3.92 (m, 1 H), 4.37 (m, 1 H), 4.71 (m, 1 H), 4.77 (m, 1 H), 6.22 (d, 1 H), 6.63 (m, 1 H), 6.67 (d, 2 H), 7.01 (d, 2 H), 7.06 (d, 2 H), 7.11 (m, 2 H), 7.14 (m, 1H), 7.20 (t, 2 H), 8.67 (d, 1 H), 8.92 (d, 1 H), 9.18 (s, 1H)

Oscillamide Y **1d**

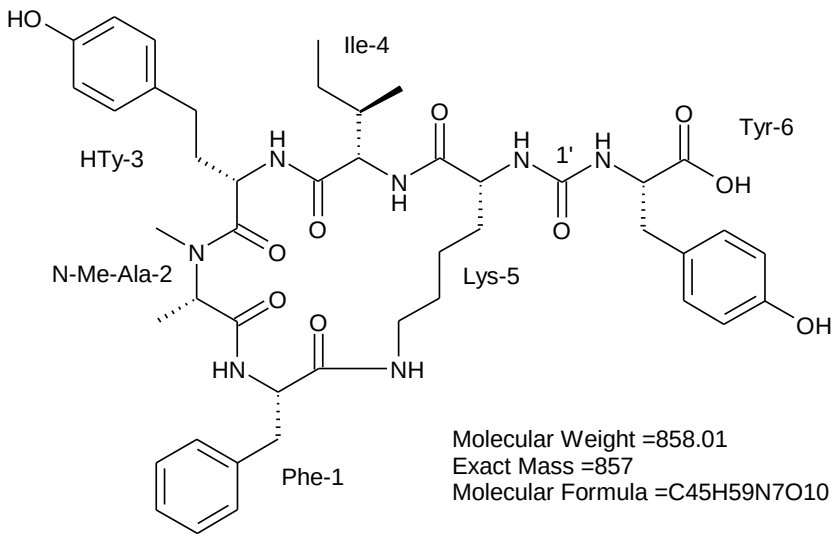


Fig. S9: Structure of *Oscillamide Y* **1d**

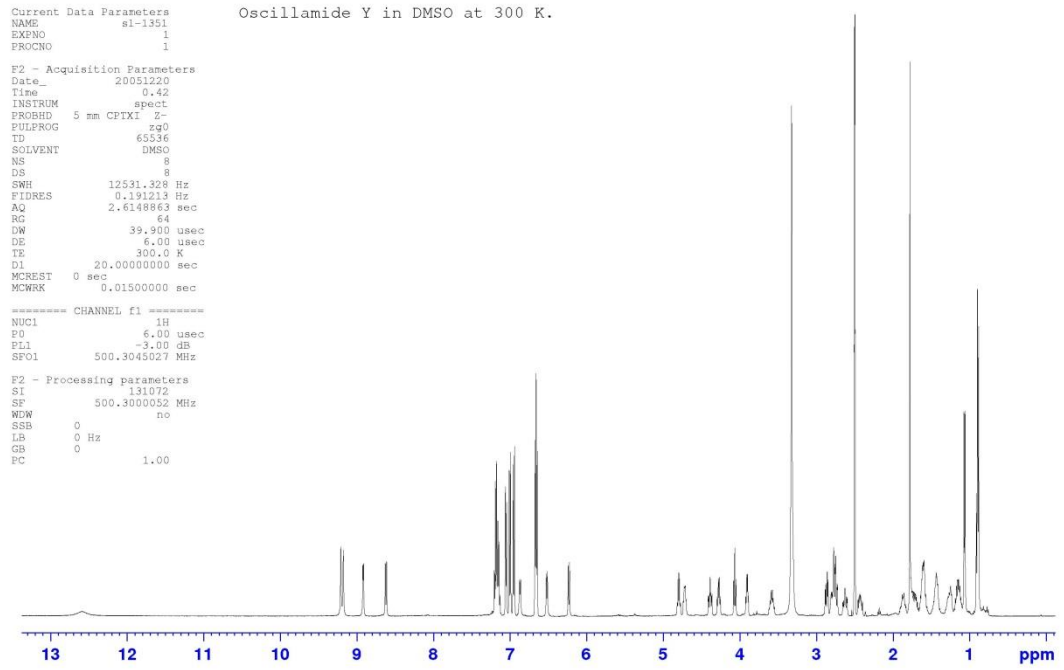


Fig. S10: ^{1}H-spectrum of *Oscillamide Y* **<u>1d</u>** in DMSO-d6 at 300 K.

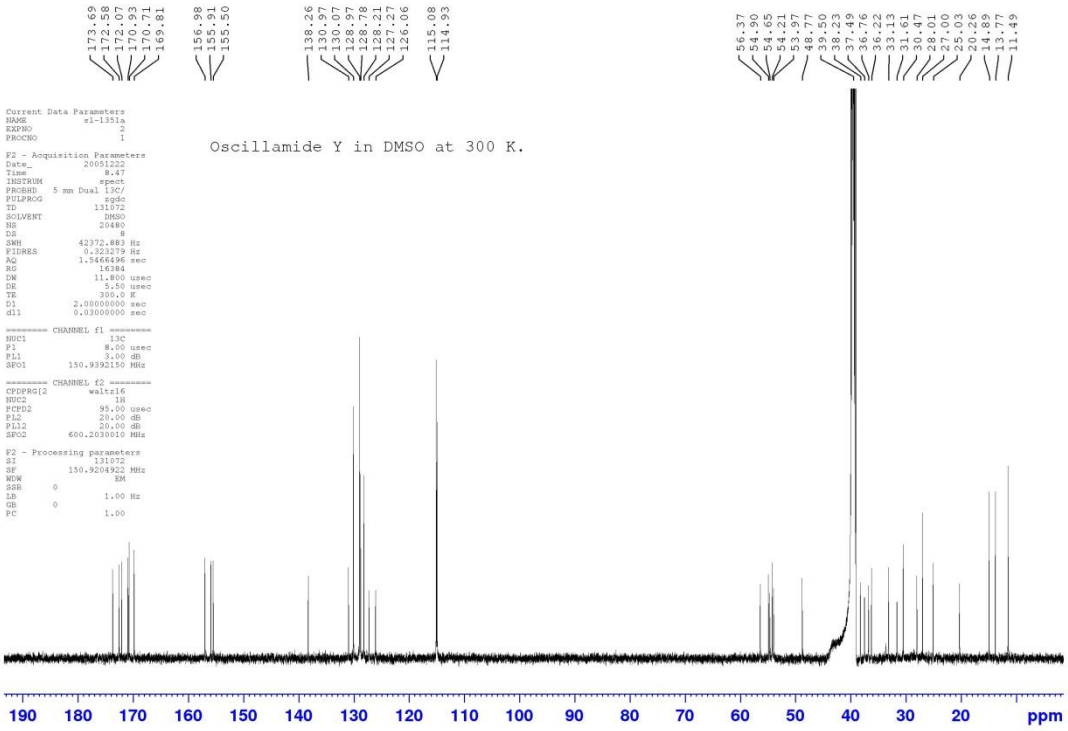


Fig. S11: ^{13}C-spectrum of *Oscillamide Y* **<u>1d</u>** in DMSO-d6 at 300 K.

Table S3: Chemical shifts of *Oscillamide Y* **1d** in DMSO-d6 at 300 K.

		^{1}H	^{13}C
Phe-1	NH	8.62	-
	α	4.38	54.90
	β	3.32/2.74	37.49
	γ	-	138.26
	δ	7.05	128.78
	ε	7.19	128.21
	ζ	7.15	126.06
	C’	-	170.71
N-Me-Ala-2	NMe	1.77	27.00
	α	4.79	54.21
	β	1.06	13.77
	C’	-	169.81
HTy-3	NH	8.92	-
	α	4.71	48.77
	β	1.87/1.70	33.13
	homo-β	2.62/2.43	30.48
	γ	-	130.97
	δ	7.00	128.97
	ε	6.66	115.08
	ζ	-	155.50
	ζ-OH	9.18	-
	C’	-	170.94

Ile-4	NH	6.87	-
	α	4.06	56.37
	β	1.76	36.22
	β-Me	0.88	14.89
	γ	1.61/1.15	25.03
	δ	0.89	11.49
	C’	-	172.58
Lys-5	NH	6.52	-
	α	3.90	54.65
	β	1.60	31.61
	γ	1.26/1.14	20.26
	δ	1.48	28.01
	ε	3.58/2.79	38.24
	ζ-NH	7.16	-
	C’	-	172.07
Tyr-6	NH	6.23	-
	α	4.27	53.97
	β	2.86/2.75	36.76
	γ	-	127.27
	δ	6.95	130.07
	ε	6.65	114.93
	ζ	-	155.91
	ζ-OH	9.21	-
	C’	-	173.69
	1’	-	156.98

S2 Data collection and refinement statistics TAFI inhibitor – CPB complexes

A mutated "tafinized" porcine carboxypeptidase B (T111-L416; SwissProt sequence) was used where 8 residues, closest to the active site that were different between CPB and TAFI, were mutated to their TAFI equivalents. These mutations were F175I, T302S, M309H, L311V, I355L, P357L, A359P and S362G. The recombinant protein was expressed in P. pastoris GS115.

The purified protein was dissolved in 50 mM Tris-HCl, pH 7.5 and concentrated to 11 mg/mL. 1 µl of protein solution was equilibrated against 1 µL of reservoir solutions containing 16-20% PEG3350, 100 mM MES pH 5.5 and 50 mM ZnAcetate. Crystals were soaked with inhibitors by adding 1 µL of a 10 mM solution of inhibitor in DMSO to a CPB crystal in 9 µL reservoir solution. After overnight incubation, the crystal was transferred to a drop of 8 µL soakbuffer with 2 µL glycerol and the crystal was picked with a nylon loop and flash frozen in liquid nitrogen. Data were collected at the European Synchrotron Radiation Facility (ESRF).

The crystals diffracted to 1.94 and 2.18 Å resolution. The overall R_{meas} of 19.0 and 14.5% is higher as would be expected from the average I/σ. We do not have an explanation for this. The R_{meas} at low resolution is good (~5%) and also the quality of the electron density maps matches the specified resolution limits. It may be that our R_{meas} values are higher due to the high multiplicity of our high resolution data, as discussed in the paragraph "An additional problem with 'overall' reliability factor" in the original paper on R_{meas} by Diederichs and Karplus[3]. Based on the $CC_{1/2}$ > 0.5 criterion, the program aimless[4] suggest that our **3a** data should be cut around 2.08 to 2.23 Å, while the **3p** data should be cut around 1.75 to 1.78 Å, suggesting that the resolution cutoff for the **3p** data set has been too conservative and we may have discarded useful data[5].

[3] Diederichs, K., and Karplus, P. A. (1997) Improved R-factors for diffraction data analysis in macromolecular crystallography. *Nature Structural Biology* **4**, 269-275
[4] Evans, P. R., and Murshudov, G. N. (2013) How good are my data and what is the resolution? *Acta Crystallographica Section D* **69**, 1204-1214
[5] Diederichs, K., and Karplus, P. A. (2013) Better models by discarding data? *Acta Crystallographica Section D* **69**, 1215-1222

Data processing and scaling were carried out using the XDS package.[6] Model building and inhibitor fitting was done with Quanta and Coot[7] and refinement was done with Refmac[8] and Buster[9].

[6] Kabsch, W. (1988) Evaluation of single-crystal X-ray diffraction data from a position-sensitive detector. *Journal of Applied Crystallography* **21**, 916-924

[2] Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010) Features and development of Coot. *Acta Crystallographica Section D* **66**, 486-501

[3] Murshudov, G. N., Skubak, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F., and Vagin, A. A. (2011) REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallographica Section D* **67**, 355-367

[9] Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W.,Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2011). BUSTER version 2.11.5. Cambridge, United Kingdom: Global Phasing Ltd.

Inhibitor	**3a**	**3p**
Protein	tafCPB	tafCPB
Data collection		
Space group	$P4_12_12$	$P4_12_12$
Cell dimensions		
a, b, c (Å)	83.67 83.67 97.04	82.53 82.53 95.40
α, β, γ (°)	90.0 90.0 90.0	90.0 90.0 90.0
Resolution (Å)	83.74-2.18 (2.25-2.18)*	95.40-1.94 (2.05-1.94)*
$I / \sigma I$	7.9 (3.3)	22.6 (7.1)
Observed reflections	83545 (7224)	320858 (46139)
R_{meas} (%)[10]	19.0 (52.2)	14.9 (58.6)
$CC_{1/2}$[11]	0.981 (0.520)	0.997 (0.925)**
*CC**	0.995 (0.827)	0.999 (0.980)
Completeness (%)	99.3 (98.3)	100.0 (100.0)
Redundancy	4.5 (4.4)	12.8 (13.0)
Refinement		
Protein atoms	2446	2441
Inhibitor atoms	29	34
water molecules	329	463
zinc ions	3***	3***
Resolution (Å)	63.37-2.18 (2.31-2.18)*	62.41-1.94 (2.02-1.94)*
R_{work} (%)	21.9 (24.1)	16.6 (24.7)
R_{free} (%)	25.1 (30.6)	19.3 (28.4)
Average B-factors (Å^2)		
protein	17.7	11.3
inhibitor	28.6	9.5
water	24.7	32.0
zinc	25.4	14.2
rmsd bond lengths (Å)	0.008	0.007
rmsd bond angles (°)	0.92	0.91
rmsd ΔB (bonded atoms) (Å^2)[12]		
All protein atoms	4.87	4.59
Main chain-Main chain	2.62	2.56
Side chain-Side chain	6.89	6.41
Main chain-Side chain	3.21	2.68
rmsd ΔB (Non-bonded contacts) (Å^2)		
All protein atoms	8.06	8.11

* The highest resolution bin is given in brackets.

** Here the highest resolution bin is 1.99-1.94 Å

[10] As defined in formula 2 in reference 1.

[11] Calculated with the program Aimless[3].

[12] Calculated with Moleman2: Kleywegt, G. J. (1997) Validation of protein models from Cα coordinates alone. *Journal of Molecular Biology* **273**, 371-376

*** One catalytic zinc ion and two zinc ions present in crystal contacts due to the presence of 50 mM ZnAcetate in the crystallization cocktail.

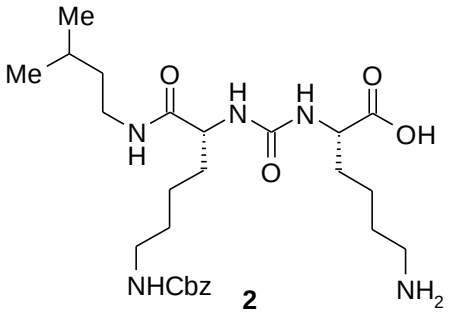
S3 Chemistry

All solvents used were commercially available and were used without further purification. Reactions were typically run using anhydrous solvents under an inert atmosphere of argon. Starting materials used were available from commercial sources. ^{1}H-NMR spectra were recorded in the indicated deuterated solvent at 400 or 500 MHz. Purity of all compounds tested in biological assays were determined to be of >95% purity by LCMS.

Library Synthesis of ureas.

All library compounds **3** in Table 2 were prepared following a 3-step procedure as described for (2S)-6-amino-2-[[(1R)-2-(cyclohexylamino)-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3c** below.

(2S)-6-amino-2-[[(1R)-5-(benzyloxycarbonylamino)-1-(isopentylcarbamoyl)pentyl]carbamoylamino]hexanoic acid **2**

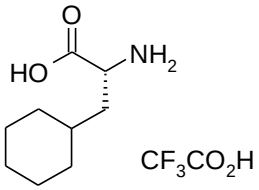


2

(2S)-6-amino-2-[[(1R)-5-(benzyloxycarbonylamino)-1-(isopentylcarbamoyl)pentyl]carbamoylamino]hexanoic acid **2** was prepared according to the general library procedure described below. 1H-NMR (DMSO-d_6, 500 MHz) δ 0.85 (dd, 6H, *J* = 1.3, 6.6 Hz), 1.13-1.70 (m, 16H), 2.75 (t, 2H, *J* = 7.5 Hz), 2.94 (q, 2H, *J* = 6.5 Hz), 2.98-3.14 (m, 2H), 4.06 (quintet, 2H, *J* = 6.9 Hz), 6.27 (d, 1H, *J* = 8.3 Hz), 6.42 (d, 1H, *J* = 8.3 Hz), 7.20 (t, 1H, *J*

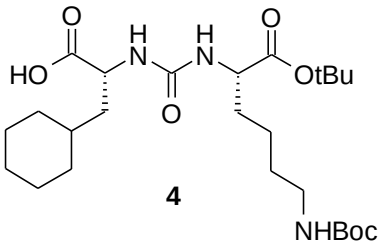
= 5.5 Hz), 7.28-7.39 (m, 5H), 7.78 (br, 3H), 7.86 (t, 1H, *J* = 5.6 Hz), 12.52 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 522.33, Found: 522.35.

*(*R)-2-Amino-3-cyclohexyl-propanoic acid trifluoroacetate



To a solution of commercially available (*R*)-2-tert-butoxycarbonylamino-3-cyclohexyl-propanoic acid (3.0g, 11.1 mmol) in 20 mL of dichloromethane was added 5 mL of trifluoroacetic acid and the mixture stirred at 20 °C for 14 h. The mixture was evaporated and 50 mL of H_2O was added to the remaining solid and the mixture was lyophilized to give 2.84g (90%) of *(R)*-2-Amino-3-cyclohexyl-propanoic acid trifluoroacetate as a colorless solid that was used in the next step without further purification.

(2R)-2-[[(1S)-1-tert-butoxycarbonyl-5-(tert-butoxycarbonylamino)pentyl]carbamoylamino]-3-cyclohexyl-propanoic acid **4**

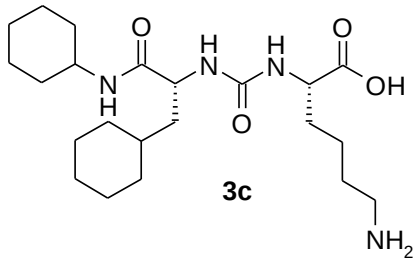


4

Commercially available (S)-2-Amino-6-tert-butoxycarbonylamino-hexanoic acid tert-butyl ester hydrochloride (1.95g, 5.75 mmol) in 30 ml of DMF was treated with NEt_3 (0.8 mL, 5.75 mmol) and 1,1´-carbonyl-diimidazole (0.93g, 5.75 mmol). The mixture was stirred at 20 °C for 30 min. *(R)*-2-amino-3-cyclohexyl-propanoic acid trifluoroacetate, 1.64g, 5.75 mmol) and

triethylamine (1.6 mL, 11.5 mmol) were added and the mixture heated to 80 °C to complete conversion of the intermediary imidazolide. Purification by flash chromatography on silicagel using dichloromethane/methanol as the eluent) afforded 2.1g (73%) of **4**. MS (ES+) Calcd.: $[M+H]^+$ 500.33, Found: 500.33.

(2S)-6-amino-2-[[(1R)-2-(cyclohexylamino)-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3c**.



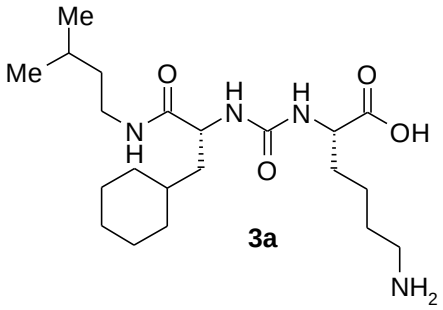
3c

A solution of ((2R)-2-[[(1S)-1-tert-butoxycarbonyl-5-(tert-butoxycarbonylamino)pentyl]carbamoylamino]-3-cyclohexyl-propanoic acid **4**, 80 mg, 0.16 mmol) and cyclohexylamine (23 mg, 0.16 mmol) in 3 mL of dichloromethane and 1 mL of DMF were treated with N-methyl-morpholine (53 µL, 0.48 mmol), 1-hydroxy-benzotriazole (28mg, 0.21 mmol), and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (37 mg, 0.19 mmol). The mixture was stirred at 20 °C for 14 h and extracted with dichloromethane/water. The organic phase was dried over $MgSO_4$ and evaporated. The crude product was dissolved in 4 mL of dichloromethane and 1.5 mL of trifluoroacetic acid was added, and the reaction stirred at 20 °C for 10 h. Preparative RP-HPLC using acetonitrile/water with 0.5% TFA as the eluent afforded 19 mg (22%) of **3c** as its trifluoroacetate salt. 1H-NMR (DMSO-d_6, 500 MHz) δ 0.77-0.90 (m, 2H), 1.04-1.40 (m, 14H), 1.45-1.74 (m, 14H), 2.75 (sextet, 2H, *J* = 6.0 Hz), 4.07 (dt, 1H, *J* = 5.9, 8.2 Hz), 4.14 (q, 1H, *J* =

6.5 Hz), 6.20 (d, 1H, *J* = 8.8 Hz), 6.35 (d, 1H, *J* = 8.4 Hz) 7.64 (br, 3H), 7.78 (d, 1H, *J* = 8.0 Hz), 12.56 (br, 1H); MS (ES-) Calcd.: $[M-H]^-$ 423.30, Found 423.44.

The following compounds were prepared by the same procedure.

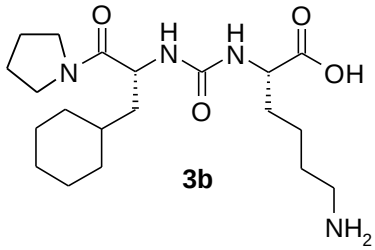
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-(isopentylamino)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3a**



3a

1H-NMR (DMSO-d_6, 400 MHz) δ 0.77-0.95 (m, 8H), 1.05-1.45 (m, 10H), 1.45-1.75 (m, 10H), 2.75 (sextett, 2H, *J* = 6.2 Hz), 2.95-3.05 (m, 1H), 3.12 (sextet, 1H, *J* = 6.9 Hz), 4.00-4.18 (m, 2H), 6.15-6.30 (m, 1H), 6.33-6.38 (m, 1H), 7.70-7.95 (m, 4H); MS (ES+) Calcd.: $[M+H]^+$ 413.31, Found 413.35.

(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-pyrrolidin-1-yl-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3b**

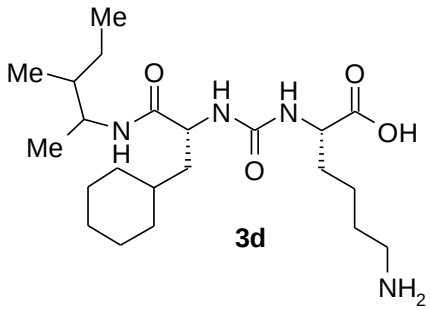


3b

1H-NMR (DMSO-d_6, 500 MHz) δ 0.80-1.00 (m, 2H) 1.00-1.20 (m, 3H), 1.20-1.40 (m, 5H), 1.45-1.70 (m, 8H), 1.70-1.95 (m, 5H), 2.77 (sextet, 2H, *J* = 6.0 Hz), 3.20-3.40 (m, 4H), 4.09 (dt,

1H, *J* = 8.1 Hz, 5.4 Hz), 4.45 (dt, 1H, *J* = 9.4 Hz, 4.1 Hz), 6.34 (q, 2H, *J* = 7.4 Hz), 7.55-7.75 (br, 3H), 12.6 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 397.28, Found: 397.33.

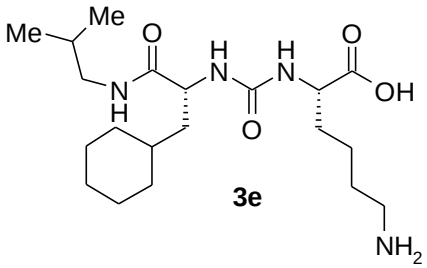
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-(1,2-dimethylbutylamino)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3d**



3d

1H-NMR (DMSO-d_6, 500 MHz) δ 0.76-0.87 (m, 9H), 0.90-1.42 (m, 15H), 1.47-1.76 (m, 10H), 2.75 (sextet, 2H, *J* = 6.6 Hz), 3.60-3.78 (m, 1H), 4.05-4.11 (m, 1H), 4.12-4.22 (m, 1H), 6.16-6.23 (m, 1H), 6.33-6.37 (m, 1H), 7.54-7.80 (m, 3H), 12.55 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 427.33, Found: 427.33.

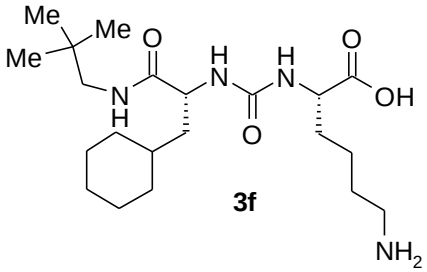
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-(isobutylamino)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3e**



3e

1H-NMR (DMSO-d_6, 500 MHz) δ 0.80-1.00 (m, 8H), 1.05-1.75 (m, 18H), 2.70-2.80 (m, 3H), 2.96 (quintet, 1H, *J* = 6.4 Hz), 4.09 (ddd, 1H, *J* = 13.3, 8.2, 3.1 Hz), 4.16 (ddd, 1H, *J* = 14.7 Hz, 8.2 Hz, 1.1 Hz), 6.22 (d, 1H, *J* = 8.5 Hz), 6.33 (d, 1H, *J* = 8.5 Hz), 7.55-7.70 (br, 3H), 7.92 (t, 1H, *J* = 5.8 Hz), 12.6 (br, 1H); MS (ES-) Calcd.: $[M-H]^-$ 397.28, Found: 397.24.

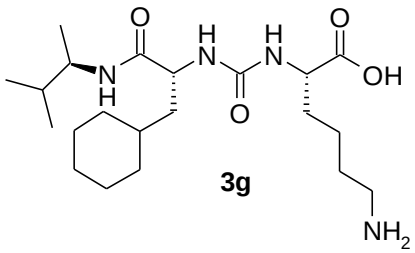
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-(2,2-dimethylpropylamino)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3f**



3f

1H-NMR (DMSO-d_6, 500 MHz) δ 0.79-0.90 (m, 2H), 0.82 (s, 9H), 1.04-1.76 (m, 17H), 2.69-2.79 (m, 3H), 3.02 (dd, 1H, *J* = 7.0, 13.5 Hz), 4.09 (dt, 1H, *J* = 5.3, 8.4 Hz), 4.22 (q, 1H, *J* = 5.3 Hz), 6.23 (d, 1H, *J* = 8.7 Hz), 6.34 (d, 1H, *J* = 8.3 Hz), 7.63 (br, 3H), 7.83 (t, 1H, (d, 1H, *J* = 6.4 Hz), 12.56 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 413.31, Found: 413.31.

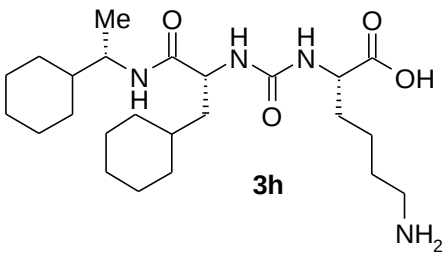
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-[[(1R)-1,2-dimethylpropyl]amino]-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3g**



3g

1H-NMR (DMSO-d_6, 500 MHz) δ 0.81 (dd, 6H, *J* = 1.8, 6.7 Hz), 0.80-0.90 (m, 2H), 0.97 (d, 3H, *J* = 6.8 Hz), 1.04-1.75 (m, 18H), 2.75 (m, 2H), 3.59 (d-quintet, 1H, *J* = 8.5, 6.8 Hz), 4.08 (dt, 1H, *J* = 5.0, 7.9 Hz), 4.18 (q, 1H, *J* = 7.7 Hz), 6.19 (d, 1H, *J* = 8.6 Hz), 6.36 (d, 1H, *J* = 8.4 Hz), 7.64 br, 3H), 7.75 (d, 1H, *J* = 8.5 Hz), 12.55 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 413.31, Found: 413.37.

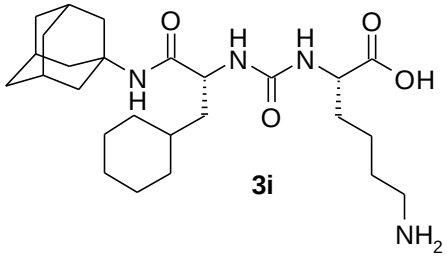
(2S)-6-amino-2-[[(1R)-2-[[(1S)-1-cyclohexylethyl]amino]-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3h**



3h

1H-NMR (DMSO-d_6, 500 MHz) δ 0.77-0.90 (m, 4H), 0.95 (d, 3H, *J* = 8.6 Hz), 1.04-1.75 (m, 26H), 2.75 (sextet, 2H, *J* = 5.7 Hz), 3.55 (q, 1H, *J* = 7.8 Hz), 4.09 (dt, 1H, *J* = 5.2, 8.2 Hz), 4.18 (q, 1H, *J* = 7.7 Hz), 6.20 (d, 1H, *J* = 8.7 Hz), 6.36 (d, 1H, *J* = 8.3 Hz), 7.62 (br, 4H), 12.56 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 453.34, Found: 453.40.

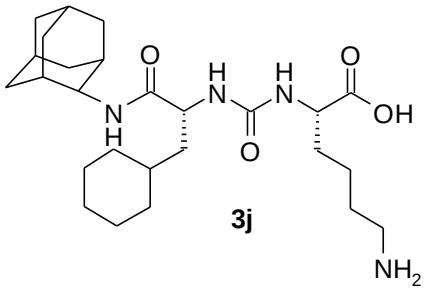
(2S)-2-[[(1R)-2-(1-adamantylamino)-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]-6-amino-hexanoic acid trifluoroacetate **3i**



3i

1H-NMR (DMSO-d_6, 500 MHz) δ 0.76-0.92 (m, 2H), 1.05-1.16 (m, 3H), 1.20-1.39 (m, 4H), 1.44-1.78 (m, 18H), 1.90 (s, 6H), 1.99 (s, 3H), 2.09 (s, 1H), 2.72-2.79 (m, 2H), 4.07 (dt, 1H, *J* = 5.0, 8.1 Hz), 4.12 (q, 1H, *J* = 7.6 Hz), 6.13 (d, 1H, *J* = 8.6 Hz), 6.37 (d, 1H, *J* = 8.6 Hz), 7.35 (s, 1H), 7.64 (br, 3H), 12.55 (br, 1H); MS (ES-) Calcd.: $[M-H]^-$ 475.32, Found: 475.25.

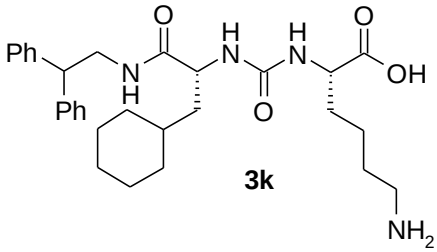
(2S)-2-[[(1R)-2-(2-adamantylamino)-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]-6-amino-hexanoic acid trifluoroacetate **3j**



3j

1H-NMR (DMSO-d_6, 500 MHz) δ 0.78-0.91 (m, 2H), 1.05-1.16 (m, 3H), 1.19-1.85 (m, 28H), 1.87-1.96 (m, 2H), 2.00 (d, 1H, *J* = 12.5 Hz), 2.72-2.78 (m, 2H), 3.80 (d, 1H, *J* = 6.6 Hz), 4.09 (dt, 1H, *J* = 5.0, 8.2 Hz), 4.30 (q, 1H, *J* = 6.6 Hz), 6.23 (d, 1H, *J* = 8.5 Hz), 6.35 (d, 1H, *J* = 8.4 Hz), 7.63 (m, 3H), 7.77 (d, 1H, *J* = 7.5 Hz), 12.55 (br, 1H); MS (ES-) Calcd.: $[M-H]^-$ 475.32, Found: 475.32.

(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-(2,2-diphenylethylamino)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3k**

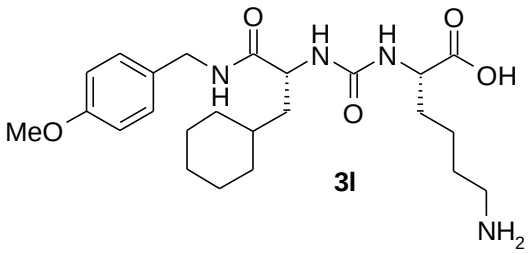


3k

1H-NMR (DMSO-d_6, 500 MHz) δ 0.63-0.75 (m, 2H), 0.94-1.17 (m, 6H), 1.25-1.33 (m, 2H), 1.45-1.71 (m, 10H), 2.70-2.78 (m, 2H), 3.49-3.60 (m, 1H), 3.81-3.88 (m, 1H), 4.01-4.09 (m, 1H), 4.17-4.26 (m, 1H), 6.16 (d, 1H, *J* = 8.6 Hz), 6.33 (d, 1H, *J* = 8.4 Hz), 7.14-7.39 (m, 10H),

7.63 br, 3H), 7.99 (t, 1H, *J* = 5.3 Hz), 12.59 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 523.33, Found: 523.34.

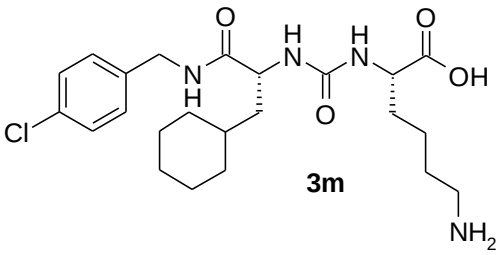
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-[(4-methoxyphenyl)methylamino]-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3l**



3l

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.76-0.90 (2H, m), 1.02-1.73 (m, 17H), 2.71-2.78 (m, 2H), 3.72 (s, 3H), 4.07-4.26 (m, 4H), 6.26 (d, 1H, *J* = 8.7 Hz), 6.34 (d, 1H, *J* = 8.4 Hz), 6.86 (d, 2H, *J* = 8.6 Hz), 7.15 (d, 2H, *J* = 8.6 Hz), 7.62 (br, 3H), 8.40 (t, 1H, *J* = 5.8 Hz), 12.60 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 463.29, Found: 463.28.

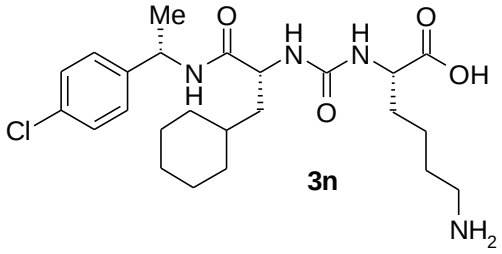
(2S)-6-amino-2-[[(1R)-2-[(4-chlorophenyl)methylamino]-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3m**



3m

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.78-0.89 (m, 2H), 1.04-1.72 (m, 17H), 2.35-2.38 (m, 2H), 4.12-4.30 (m, 4H), 6.31 (2d, 2H, *J* = 8.6 Hz), 7.25 (d, 2H, *J* = 8.6 Hz), 7.37 (d, 2H, *J* = 8.6 Hz), 7.6 (br, 2H), 8.50 (t, 1H, *J* = 6.0 Hz), 12.6 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 467.24, Found: 467.43.

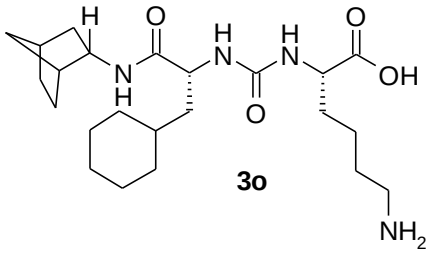
(2S)-6-amino-2-[[(1R)-2-[[(1S)-1-(4-chlorophenyl)ethyl]amino]-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3n**



3n

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.80-1.00 (m, 2H), 1.05-1.75 (m, 20H), 2.72-2.82 (m, 2H), 4.09 (ddd, 1H, *J* = 13.3 Hz, 8.0 Hz, 2.7 Hz), 4.87 (quintet, 1H, *J* = 7.4 Hz), 4.22 (ddd, 1H, *J* = 14.9 Hz, 8.4 Hz, 1.9 Hz), 6.22 (d, 1H, *J* = 8.7 Hz), 6.32 (d, 1H, *J* = 8.3 Hz), 7.33 (ddd, 4H, *J* = 22.1 Hz, 8.4 Hz, 2.1 Hz), 7.55-7.70 (br, 3H), 8.38 (d, 1H, *J* = 8.1 Hz), 12.6 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 481.26, Found: 481.23.

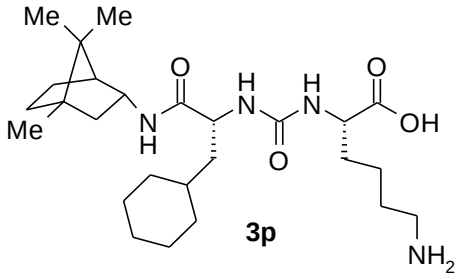
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-(norbornan-2-ylamino)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3o** (mixture of diastereoisomers)



3o

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.80-1.00 (m, 3H), 1.05-1.85 (m, 22H), 2.10-2.15 (m, 1H), 2.20-2.30 (m, 1H), 2.70-2.82 (m, 2H), 3.80-4.00 (m, 1H), 4.05-4.15 (m, 1H), 4.20-4.25 (m, 1H), 6.21 (dd, 1H, *J* = 8.6 Hz, 3.9 Hz), 6.35 (dd, 1H, *J* = 8.6 Hz, 3.1 Hz), 7.55-7.75 (br, 3H), 7.82 (d, 0.5H, *J* = 6.5 Hz), 7.94 (d, 0.5H, *J* = 7.2 Hz), 12.6 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 437.31, Found: 437.27.

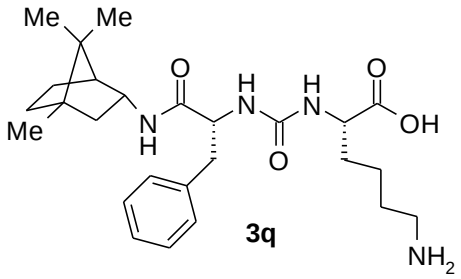
(2S)-6-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid hydrochloride **3p**



3p

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.79 (s, 3H), 0.88 (s, 3H), 0.80-1.78 (m, 25H), 2.07-2.13 (m, 1H), 2.70-2.79 (m, 2H), 3.99-4.03 (m, 1H), 4.07-4.12 (m, 1H), 4.19-4.26 (m, 1H), 6.24 (d, 1H, *J* = 8.5 Hz), 6.35 (d, 1H, *J* = 8.5 Hz) , 7.65 (d, 1H, *J* = 8.5 Hz), 7.88 (br s, 3H); MS (ES-) Calcd.: $[M-H]^-$ 477.34, $[M-H]^-$, Found: 477.36.

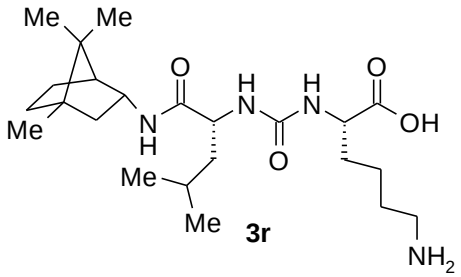
(2S)-6-amino-2-[[(1R)-1-benzyl-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3q**



3q

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H); 0.80 (s, 3H), 0.88 (s, 3H), 1.10-1.22 (m, 2H), 1.22-1.35 (m, 2H), 1.40-1.70 (m, 8H), 2.00-2.10 (m, 1H), 2.65-2.90 (m, 4H), 3.92-4.02 (m, 1H), 4.02-4.10 (m, 1H), 4.36-4.50 (m, 1H), 6.30-6.40 (m, 1H), 6.40-6.50 (m, 1H), 7.15-7.27 (m, 5H), 7.59 (d, 1H, *J* = 8.3 Hz), 7.9 (br, 3H); MS (ES+) Calcd.: $[M+H]^+$ 473.31, Found: 473.45.

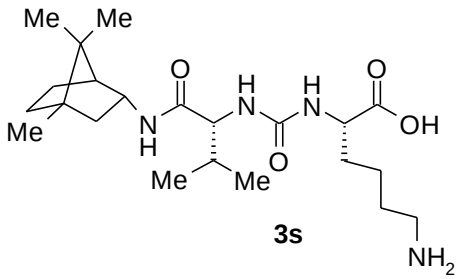
(2S)-6-amino-2-[[(1R)-3-methyl-1-[(4,7,7-trimethylnorbornan-2-yl)carbamoyl]butyl]carbamoylamino]hexanoic acid trifluoroacetate **3r**



3r

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.82 (s, 3H), 0.82-0.95 (m, 9H), 1.15-1.45 (m, 6H), 1.45-1.75 (m, 8H), 2.05-2.16 (m, 1H), 2.70-2.80 (m, 2H), 4.00-4.16 (m, 2H), 4.16-4.25 (m, 1H), 6.29 (d, 1H, *J* = 8.6 Hz), 6.39 (d, 1H, *J* = 8.3 Hz), 7.64 (d, 1H, *J* = 8.4 Hz), 7.70-7.85 (br s, 3H), 12.5 (br s, 1H); MS (ES+) Calcd.: $[M+H]^+$ 439.33, Found: 439.25.

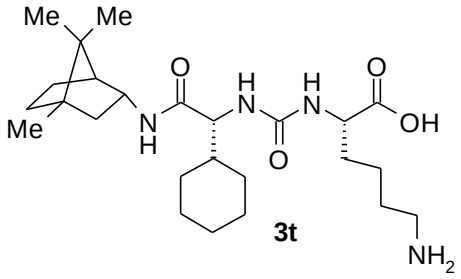
(2S)-6-amino-2-[[(1R)-2-methyl-1-[(4,7,7-trimethylnorbornan-2-yl)carbamoyl]propyl]carbamoylamino]hexanoic acid trifluoroacetate **3s**



3s

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.83 (s, 3H), 0.78-0.90 (m, 6H), 0.90 (s, 3H), 1.18-1.30 (m, 2H), 1.30-1.45 (m, 2H), 1.50-1.75 (m, 8H), 1.87 (sextett, 1H, *J* = 6.3 Hz), 2.11 (tt, 1H, *J* = 12.3 Hz, 3.5 Hz), 2.74 (sextett, 2H, *J* = 5.9 Hz), 4.00-4.15 (m, 3H), 6.28-6.40 (br d, 1H), 6.45-6.56 (br s, 1H), 7.80-7.90 (br s, 3H), 7.63 (d, 1H, *J* = 8.7 Hz); MS (ES+) Calcd.: $[M+H]^+$ 425.31, Found: 425.35.

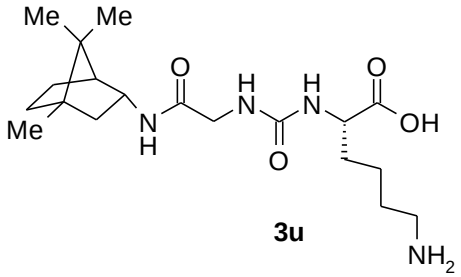
(2S)-6-amino-2-[[(1R)-1-cyclohexyl-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid hydrochloride **3t**



3t

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.69 (s, 3H), 0.82 (s, 3H), 0.83-1.38 (m, 10H), 0.88 (s, 3H), 1.45-1.73 (m, 13H), 2.11 (tt, 1H, *J* = 12.2 Hz, 4.3 Hz), 2.75 (sextett, 2H, *J* = 5.9 Hz), 3.98-4.11 (m, 3H), 6.25 (d, 1H, *J* = 9.1 Hz), 6.45 (d, 1H, *J* = 8.3 Hz), 7.61 (d, 1H, *J* = 8.5 Hz), 7.87 (br, 3H); MS (ES+) Calcd.: $[M+H]^+$ 465.34, Found: 465.44.

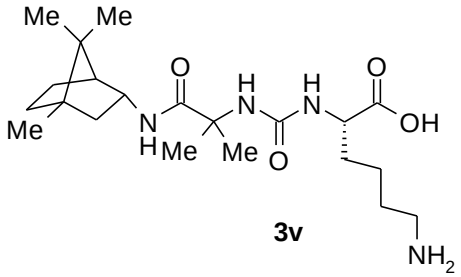
(2S)-6-amino-2-[[2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3u**



3u

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.83 (s, 3H), 0.90 (s, 3H), 1.18-1.30 (m, 2H), 1.30-1.45 (m, 2H), 1.45-1.75 (m, 8H), 2.05-2.20 (m, 1H), 2.70-2.90 (m, 2H), 3.68 (s, 2H), 4.00-4.15 (m, 2H), 6.00-6.45 (br s, 1H), 6.45-6.63 (br s, 1H), 7.63 (d, 1H, *J* = 8.7 Hz), 7.80-7.92 (br s, 3H); MS (ES+) Calcd.: $[M+H]^+$ 383.27, Found: 383.25.

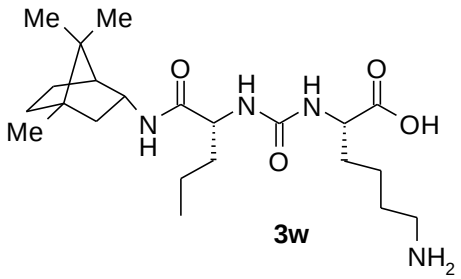
(2S)-6-amino-2-[[1,1-dimethyl-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3v**



3v

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.80 (s, 3H), 0.90 (s, 3H), 1.15-1.45 (m, 10H), 1.50-1.75 (m, 8H), 2.09 (tt, 1H, *J* = 11.9 Hz, 3.5 Hz), 2.70-2.80 (m, 2H), 4.00-4.10 (m, 2H), 6.35-6.50 (br s, 1H), 6.45-6.60 (br s, 1H), 7.65 (d, 1H, *J* = 8.7 Hz), 7.80-7.95 (br s, 3H), MS (ES+) Calcd.: $[M+H]^+$ 411.30, Found: 411.25.

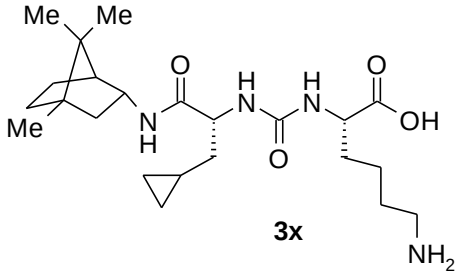
(2S)-6-amino-2-[[(1R)-1-[(4,7,7-trimethylnorbornan-2-yl)carbamoyl]butyl]carbamoylamino]hexanoic acid hydrochloride **3w**



3w

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.69 (s, 3H), 0.83 (s, 3H), 0.85 (t, 4H, *J* = 7.2 Hz), 0.89 (s, 3H), 1.14-1.72 (m, 15H), 2.11 (tt, 1H, *J* = 11.9 Hz, 3.6 Hz), 2.75 (sextett, 2H, *J* = 5.9 Hz), 3.98-4.11 (m, 2H), 4.16 (q, 1H, *J* = 6.3 Hz), 6.30 (d, 1H, *J* = 8.1 Hz), 6.45 (d, 1H, *J* = 8.0 Hz), 7.65 (d, 1H, *J* = 8.5 Hz), 7.80 (br, 3H), 12.55 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 425.31, Found: 425.36.

(2S)-6-amino-2-[[(1R)-1-(cyclopropylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3x**

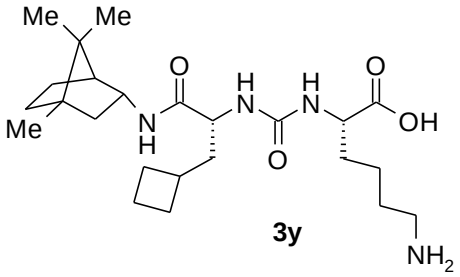


3x

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.03 (d, 2H, *J* = 6.4 Hz), 0.36 (t, 2H, *J* = 7.2 Hz) 0.62-0.67 (m, 1H), 0.70 (s, 3H), 0.83 (s, 3H), 0.84-0.89 (m, 1H), 0.90 (s, 3H), 1.19-1.29 (m, 4H), 1.34 (sextet, 2H, *J* = 6.8 Hz), 1.45-1.73 (m, 6H), 2.11 (tt, 1H, *J* = 12.8 Hz, 3.2 Hz), 2.75 (m, 2H), 4.01-4.07 (m, 1H), 4.09 (q, 1H, *J* = 6.4 Hz), 4.25 (q, 1H, *J* = 6.8 Hz), 6.31 (d, 1H, *J* = 8.0 Hz), 6.46 (d, 1H, *J* = 8.0

Hz), 7.58 (d, 1H, *J* = 8.4 Hz), 7.60 (br, 3H), 12.50 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 437.31, Found: 437.25.

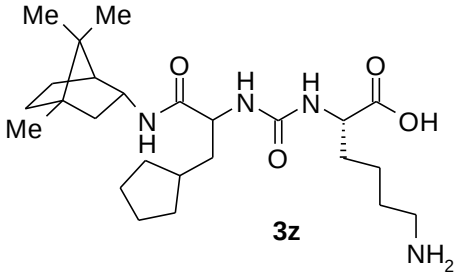
(2S)-6-amino-2-[[(1R)-1-(cyclobutylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3y**



3y

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.82 (s, 3H), 0.85 (d, 1H, *J* = 4.9 Hz), 0.88 (s, 3H), 1.14-1.38 (m, 4H), 1.45-1.80 (m, 13H), 1.90-2.01 (m, 2H), 2.10 (t, 1H, *J* = 11,8 Hz), 2.26 (quintet, 1H, *J* = 7.4 Hz), 2.75 (sextet, 2H, *J* = 5.7 Hz), 3.96-4.14 (m, 3H), 6.23 (br, 1H), 6.43 (br, 1H), 7.66 (d, 1H, *J* = 8.5 Hz), 7.78 (br, 3H); MS (ES+) Calcd.: $[M+H]^+$ 451.33, Found: 451.25.

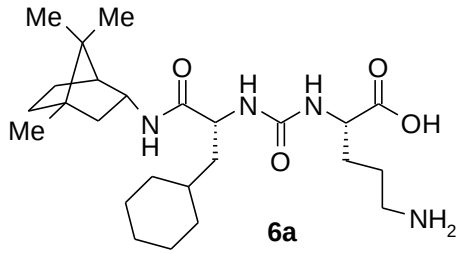
(2S)-6-amino-2-[[1-(cyclopentylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanoic acid hydrochloride **3z** (mixture of diastereomers)



3z

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.69 (s, 3H), 0.82 (s, 3H), 0.83-0.87 (m, 1H), 0.88 (s, 3H), 0.99-1.78 (m, 22H), 2.10 (t, 1H, *J* = 12.0 Hz), 2.74 (sextet, 2H, *J* = 5.7 Hz), 4.02-4.22 (m, 3H), 6.29 (br, 1H), 6.49 (br, 1H), 7.66 (d. 1H, *J* = 8.5 Hz), 7.82 (br, 3H); MS (ES+) Calcd.: $[M+H]^+$ 465.34, Found: 465.30.

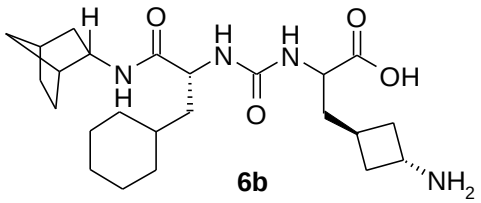
(2S)-5-amino-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]pentanoic acid hydrochloride **6a**



6a

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.82 (s, 3H), 0.83-0.87 (m, 3H), 0.88 (s, 3H), 1.07-1.43 (m, 8H), 1.50-1.78 (m, 13H), 2.10 (t, 1H, *J* = 11.6 Hz), 2.78 (br, 2H), 3.96-4.05 (m, 1H), 4.09-4.15 (m, 1H), 4.21 (q, 1H, *J* = 6.5 Hz), 6.33 (d, 1H, *J* = 8.5 Hz), 6.42 (d, 1H, *J* = 8.4 Hz), 7.64 (d, 1H, *J* = 8.5 Hz), 7.80 (br, 3H); MS (ES+) Calcd.: $[M+H]^+$ 465.34, Found: 465.35.

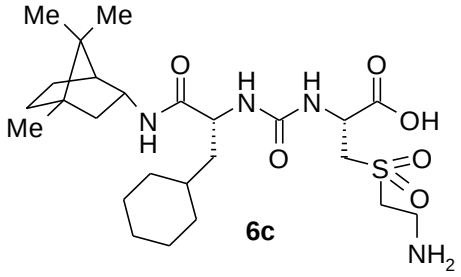
3-(3-aminocyclobutyl)-2-[[(1R)-1-(cyclohexylmethyl)-2-(norbornan-2-ylamino)-2-oxo-ethyl]carbamoylamino]propanoic acid trifluoroacetate **6b** (mixture of diastereomers)



6b

MS (ES-) Calcd.: $[M-H]^-$ 447.30, Found: 447.40.

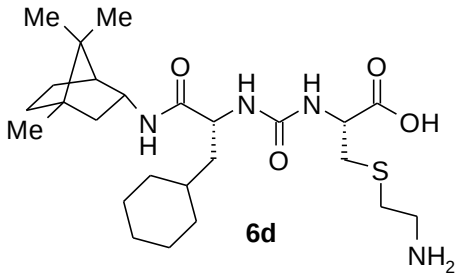
(2R)-3-(2-aminoethylsulfonyl)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]propanoic acid trifluoroacetate **6c**



6c

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.69 (s, 3H), 0.80-0.94 (m, 3H), 0.83 (s, 3H), 0.89 (s, 3H), 1.06-1.44 (m, 8H), 1.55-1.76 (m, 8H), 2.06-2.14 (m, 1H), 3.10-3.27 (m, 2H), 3.47-3.55 (m, 2H), 3.58-3.72 (m, 1H), 3.97-4.05 (m, 1H), 4.12-4.23 (m, 1H), 4.53-4.66 (m, 1H), 6.58-6.71 (m, 2H), 7.77, 7.64 (2d, 1H, *J* = 8.5 Hz), 8.12 (br, 3H), 13.09 (br, 1H); MS (ES+) Calcd.: [M+H] 529.31, Found: 529.17.

(2R)-3-(2-aminoethylsulfanyl)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]propanoic acid trifluoroacetate **6d**

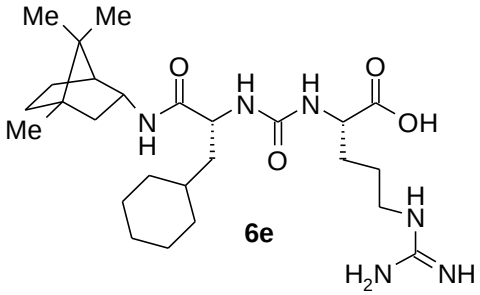


6d

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.68 (s, 3H), 0.89-0.81 (m, 3H), 0.82 (s, 3H), 0.88 (s, 3H), 1.08-1.42 (m, 8H), 1.56-1.76 (m, 8H), 2.06-2.14 (m, 1H), 2.62-2.84 (m, 3H), 2.89-3.01 (m, 3H), 3.97-4.04 (m, 1H), 4.23 (q, 1H, *J* = 6.3 Hz), 4.35 (q, 1H, *J* = 6.0 Hz), 6.44 (d, 1H, *J* = 8.7 Hz), 6.52

(d, 1H, *J* = 8.5 Hz), 7.66 (d, 1H, *J* = 8.5 Hz), 7.86 (br, 3H), 12.86 (br, 1H); MS (ES+) Calcd.: [M+H] 497.32, Found: 497.23.

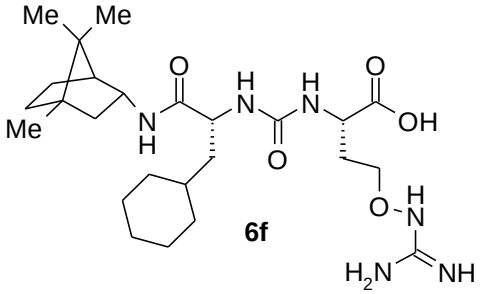
(2S)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]-5-guanidino-pentanoic acid trifluoroacetate **6e**



6e

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.68 (s, 3H), 0.80 (s, 3H), 0.88 (s, 3H), 0.80-0.95 (m, 2H), 1.00-1.80 (m, 21H), 2.05-2.15 (m, 1H), 3.05-3.15 (m, 2H), 3.97-4.05 (m, 1H), 4.10-4.20 (m, 1H), 4.25-4.35 (m, 1H), 6.20 (d, 0.5H, *J* = 8.6 Hz), 6.30 (d, 0.5H, *J* = 8.7 Hz), 6.35 (d, 0.5H, *J* = 8.6 Hz), 6.40 (d, 0.5H, *J* = 8.3 Hz), 6.50-7.45 (br, 4H), 7.45-7.53 (m, 1H), 7.65 (t, 1H, *J* = 8.6 Hz), 12.6 (br s, 1H); MS (ES+) Calcd.: $[M+H]^{+}$ 507.37, Found: 507.40.

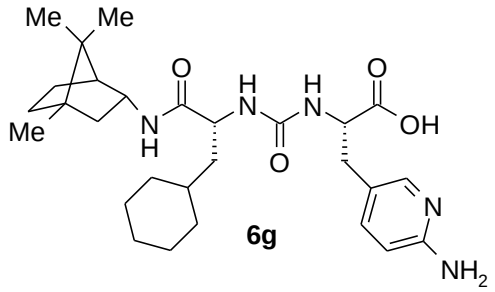
(2S)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]-4-guanidinooxy-butanoic acid **6f**



6f

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.68 (s, 3H), 0.82 (s, 3H), 0.88 (s, 3H), 0.81-0.89 (m, 4H), 1.00-1.42 (m, 9H), 1.51-1.82 (m, 10H), 2.04-2.15 (m, 3H), 6.27 (d, 0.5H, *J* = 8.3 Hz), 6.37 (d, 0.5H, *J* = 8.6 Hz), 6.43 (d, 0.5H, *J* = 8.6 Hz), 6.50 (d, 0.5H, *J* = 8.8 Hz), 7.56-7.72 (m, 4H), 10.96 (d, 1H, *J* = 11.1 Hz), 12.77 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 509.35, Found: 509.44.

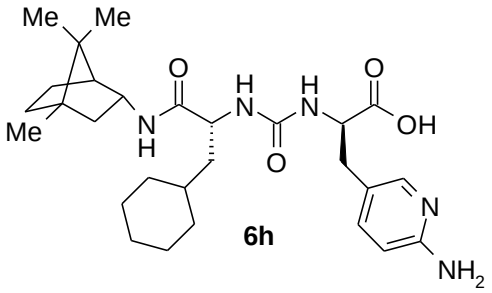
(2S)-3-(6-amino-3-pyridyl)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]propanoic acid trifluoroacetate **6g**



6g

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.68 (s, 3H), 0.82 (s, 3H), 0.88 (s, 3H), 0.80-1.00 (m, 2H), 1.00-1.40 (m, 9H), 1.50-1.75 (m, 8H), 2.05-2.15 (m, 1H), 2.71 (dd, 1H, *J* = 14.1 Hz, 9.0 Hz), 2.94 (dd, 1H, *J* = 13.8 Hz, 5.1 Hz), 3.95-4.05 (m, 1H), 4.17 (dd, 1H, *J* = 14.6 Hz, 8.1 Hz), 4.34 (dt, 1H, *J* = 8.5 Hz, 5.1 Hz), 6.24 (d, 1H, *J* = 9.0 Hz), 6.43 (d, 1H, *J* = 9.0 Hz), 6.91 (d, 1H, *J* = 9.0 Hz), 7.68 (d, 1H, *J* = 8.5 Hz), 7.74 (s, 1H), 7.78 (dd, 1H, *J* = 8.7 Hz, 2.1 Hz), 7.85-8.00 (br s, 2H), 13.6 (br s, 1H); MS (ES+) Calcd.: [M+H] 514.34, Found: 514.50.

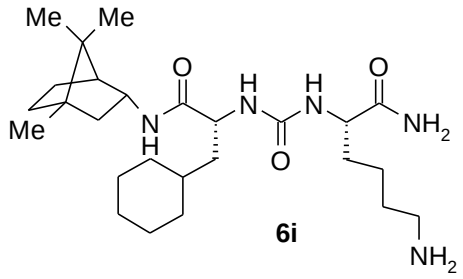
(2R)-3-(6-amino-3-pyridyl)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]propanoic acid trifluoroacetate **6h**



6h

^{1}H-NMR (DMSO-d_6, 500MHz) δ 0.68 (s, 3H), 0.82 (s, 3H), 0.88 (s, 3H), 0.80-1.00 (m, 2H), 1.00-1.40 (m, 9H), 1.50-1.75 (m, 8H), 2.05-2.15 (m, 1H), 2.75 (dd, 1H, 14.2 Hz, 7.7 Hz), 2.94 (dd, 1H, *J* = 14.2 Hz, 5.0 Hz), 3.95-4.05 (m, 1H), 4.15 (dd, 1H, *J* = 14.2 Hz, 8.2 Hz), 4.30 (dt, 1H, *J* = 7.5 Hz, 5.5 Hz), 6.25 (d, 1H, *J* = 8.7 Hz), 6.35 (d, 1H, *J* = 7.9 Hz), 6.91 (d, 1H, *J* = 8.9 Hz), 7.65 (d, 1H, *J* = 8.4 Hz), 7.73 (s, 1H), 7.75 (dd, 1H, *J* = 9.4 Hz, 1.7 Hz), 7.85-8.00 (br s, 2H), 13.6 (br s, 1H); MS (ES+) Calcd.: [M+H] 514.34, Found: 514.55.

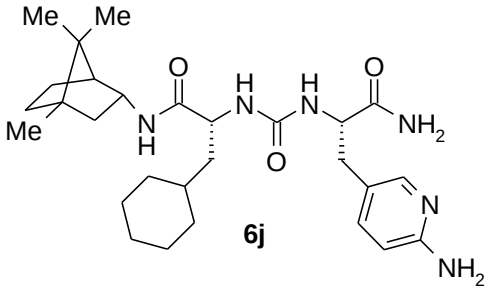
(2S)-6-amino-2-[[1-(cyclopentylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]hexanamide trifluoroacetate **6i**



6i

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (s, 3H), 0.81 (s, 3H), 0.88 (s, 3H), 0.80-0.90 (m, 2H), 1.05-1.80 (m, 23H), 2.07-2.13 (m, 1H), 2.70-2.79 (m, 2H), 3.95-4.03 (m, 1H), 4.07-4.12 (m, 1H), 4.15-4.25 (m, 1H), 6.25 (d, 1H, *J* = 8.4 Hz), 6.28 (d, 1H, *J* = 8.5 Hz), 7.00 (s, 1H), 7.35 (s, 1H), 7.62 (d, 1H, *J* = 8.7 Hz), 7.60-7.75 (br s, 3H); MS (ES+) Calcd.: $[M+H]^+$ 478.38, Found: 478.38.

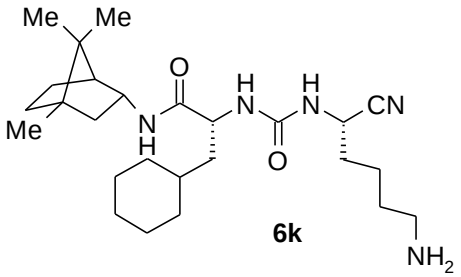
(2S)-3-(6-amino-3-pyridyl)-2-[[(1R)-1-(cyclohexylmethyl)-2-oxo-2-[(4,7,7-trimethylnorbornan-2-yl)amino]ethyl]carbamoylamino]propanamide hydrochloride **6j**



6j

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.65 (d, 3H, *J* = 11.7 Hz), 0.82 (s, 3H), 0.87 (s, 3H), 0.81-0.86 (m, 3H), 1.00-1.38 (m, 9H), 1.55-1.73 (m, 8H), 2.07 (t, 1H, *J* = 11.6 Hz), 2.58-2.67 (m, 1H), 2.79-2.87 (m, 1H), 3.96-4.03 (m, 1H), 4.08-4.16 (m, 1H), 4.26-4.36 (m, 1H), 6.26-6.38 (m, 2H), 6.90 (t, 1H, *J* = 9.1 Hz), 7.11 (s, 1H), 7.48 (s, 1H), 7.63 (dd, 1H, *J* = 8.5 Hz), 7.68 (s, 1H), 7.71-7.76 (m, 1H), 7.94 (br, 2H), 13.74 (br, 1H); MS (ES+) Calcd.: $[M+H]^+$ 513.36, Found: 513.41.

(2R)-2-[[(1S)-5-amino-1-cyano-pentyl]carbamoylamino]-3-cyclohexyl-N-(4,7,7-trimethylnorbornan-2-yl)propanamide trifluoroacetate **6k**



6k

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 0.68 (d, 3H, *J* = 6.6 Hz), 0.82 (s, 3H), 0.88 (s, 3H), 0.81-0.87 (m, 3H), 1.06-1.39 (m, 9H), 1.55-1.78 (m, 8H), 2.10 (t, 1H, *J* = 11.5 Hz), 2.82 (t, 1H, *J* = 6.9 Hz), 3.96-4.04 (m, 1H), 4.25 (quintet, 1H, *J* = 7.3 Hz), 4.66 (sextet, 1H, *J* = 6.9 Hz), 5.84 (br, 2H), 6.38 (d, 1H, *J* = 8.3 Hz), 6.61 (d, 1H, *J* = 7.6 Hz), 7.29 (d, 1H, *J* = 8.7 Hz), 7.74 (t, 1H, *J* = 9.5 Hz), 7.80 (s, 1H); MS (ES+) Calcd.: $[M+H]^+$ 495.34, Found: 495.34.

S4 In Vitro Methods for the Determination of IC50s of TAFIa

The prepared substances were tested for TAFIa inhibition using the Actichrome plasma TAFI activity kit from American Diagnostica (Pr. No. 874). This entailed adding 29 µL of assay buffer (20 mM Hepes, 150 mM NaCl, pH 7.4) and 10 µL of TAFIa (American Diagnostica Pr. No. 874TAFIA; 2.5 µg/mL) to 1 µL of 5 mM DMSO solution of the substance and incubating in a 96 half-well microtiter plate at room temperature for 15 minutes. The enzymic reaction was started by adding 10 µL of TAFIa developer (prediluted 1:2 with water). The time course of the reaction was followed at 420 nm in a microtiter plate reader (SpectraMax plus 384; Molecular Devices) for 15 minutes. The IC50 was calculated from the averaged values (duplicate determination) of serial dilutions of the substance with the aid of the Grafit 4 software (Erithacus Software, UK).

S5 Metabolic stability on liver microsomes

Incubation conditions with hepatic microsomal fractions and further experimental conditions used throughout were as follows: microsomal proteins concentration = 1 mg/mL, bovine serum albumin (BSA) concentration = 1 mg/mL; substrate concentration = 5 µM; incubation duration = 20 min; cytochrome P-450 monooxygenases (CYPs) and flavin-containing monooxygenases (FMOs) cofactor = 1 mM NADPH. Enzyme activity was stopped with 1 volume of acetonitrile (ACN). Hepatic microsomal fractions: from Swiss CD1 male mouse (m7), Sprague−Dawley male rat (m21), humans (pool of H-19, six donors). Inhibitor: quinidine at a final concentration of 8 µM (20-fold its Ki for CYP2D6) was used for the specific and potent inhibition of enzyme reactions catalyzed by CYP2D6. Ketoconazole at a final concentration of 1.5 µM (100-fold its Ki for CYP3A4) was used for the specific and potentinhibition of enzyme reactions catalyzed by CYP3A4. For each test compound and for each microsomal preparation, three incubations were prepared: absolute reference in buffer (without enzyme material, i.e., microsomes); incubation without NADPH cofactor (with microsomal fractions); incubation with NADPH (with microsomal fractions). For most compounds, biotransformation, as observed in hepatic microsomal fractions in the presence of the NADPH cofactor, consists of oxidative reactions catalyzed by either CYP or FMO. In these conditions, the percentage of total metabolism, which corresponds to oxidative metabolism, was determined as follows: [% total metabolism] ≈ [% oxidative metabolism] = [1(UCpeak area − NADPH UC peak area + NADPH)] × 100, where NADPH corresponds to the enzyme cofactor for oxidation reactions catalyzed by either CYP or FMO, and UC represents the unchanged compound.

S6 IC50 determination for CYP P450 enzyme inhibition

The in vitro procedure for IC50 determination of a test compound as direct, reversible inhibitor against CYP3A4, CYP2D6 and CYP2C9 in human liver microsomes (HLM) was as follows: inhibition of the turn-over of probe substrates of CYP3A4 (Midazolam 3µM, 10 minutes and Testosterone 50µM, 30 minutes), CYP2D6 (Dextromethorphan 5µM, 30 minutes) and CYP2C9 (Diclofenac 5 µM, 10 minutes) to their specific metabolites i.e. 1´-Hydroxymidazolam, 6ß-Hydroxytestosterone, Dextrorphan and 4´-Hydroxydiclofenac by NCEs was evaluated. For scientific reasons, CYP3A4 inhibition was studied with two different probe substrates.

The specific metabolites were quantified by LC-MS/MS analysis.

CYP Inhibition conditions: 50 mM Phosphate buffer (no BSA in incubation medium), 0.5 mM EDTA, 6 mM MgCl2, 1mM NADPH, 0.1 or 0.2 mg microsomal protein/mL, maximum 0.5% DMSO with test compound(s) concentration range including 30, 10, 3, 1, 0.3 µM). Incubations were carried out at 37°C. Incubations were terminated at the appropriate time with acetonitrile containing an appropriate internal standard.

S7 Permeability testing using CACO-2 TC7 cells

Cellular permeability was tested using CACO-2 TC7 cells at passages 20 to 70, 21 to 28 days post seeding on filters (HTS plate membrane PET 1 µm, 3 wells). Transport medium forapical compartment : Hank's balanced salt solution; HEPES 10 mM; 0.5 % BSA; adjusted pH 6.5; for basal compartment : Hank's balanced salt solution; HEPES 10 mM; 5 % BSA; adjusted pH 7.4. Test compound concentration was 20 µM; incubation duration = 120 minutes under agitation at 37°C without CO_2.

Sampling was done at Time 0 (Apical compartment) and Time 120 (Apical and Basal compartments), Calibration curve used 3 concentration levels (at least) and transport in the "Apical-to-Basal" direction was evaluated. Following protein precipitation with acetonitrile and their removal by centrifugation, supernatant fluids were analysed by UPLC/ESI-MS-MS or equivalent assays.

Permeability values were calculated as follows:

$$\text{Permeability coefficient (in nm/sec)} = \frac{\text{Amount Basal at time}\,120}{\text{Time} * \text{Filter Area} * \text{Apical concentration at time}\,0}$$

S8 Molecular Modelling Methods

Virtual library enumeration processes were carried out using Pipeline Pilot (Pipeline Pilot, Scitegic Inc., San Diego, CA.). Docking was carried out with GOLD. Docking poses were scored and ranked using DrugScore.