SUPPORTING INFORMATION:

Automated Design of Macrocycles for Therapeutic Applications: from Small Molecules to Peptides and Proteins

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Contents

Peptide example: hydrocarbon-stapled peptides targeting β-catenin (retrospective case study). One example of helix stabilization using ring-closing metathesis was done in Verdine's group by stapling a helical peptide targeting β-catenin by introduction of two α-methyl,α-alkenyl amino acids (4-pentenyl-alanine or 7-octenyl-alanine).[1] Here, two types of staplings of different linker lengths were attempted, *i,i+7* staples with D-,L- attachments and *i,i+4* staples with L-,L- attachments. In that study, three staple configurations were designed and tested (see Table S1). Of them, one stapling improved affinity to β-catenin. We applied our method to see whether this result could be reproduced in an automated, computational fashion. The peptidic precursor structure **7** was taken from pdb code 1qz7 (Figure S1). The linkers **1k** and **1l** (Table 1) were, according to the staples used by Grossman *et al.*, set as cyclization options, allowing the consideration of D- and L- attachment at each peptide position. The algorithm produced 64 cyclizations (Figure S1b) including the three (**7b-d**) which were synthesized and tested in the original paper. To accurately mimic the synthetic precursors, these three variants were post-processed by adding an α-methyl to the C_α atoms. Subsequently, these postprocessed molecules were scored by (i) conformational stability calculations of the bioactive substructure based on 100 ns

unrestrained MD simulations in explicit water in absence of the receptor and (ii) molecular mechanical minimization and MM/GBSA binding energy calculations with the Prime software (see minimized poses in Figure S1c). Table S1 shows the results of the scoring alongside experimental affinities, where measured. From the peptides synthesized in the paper, **7d** is correctly predicted as the most potent according to its predicted enthalpic score. For **7b** and **7c**, the enthalpic score is similar to the linear peptide, which is in line with the experimentally determined K_d values (see Table S1).

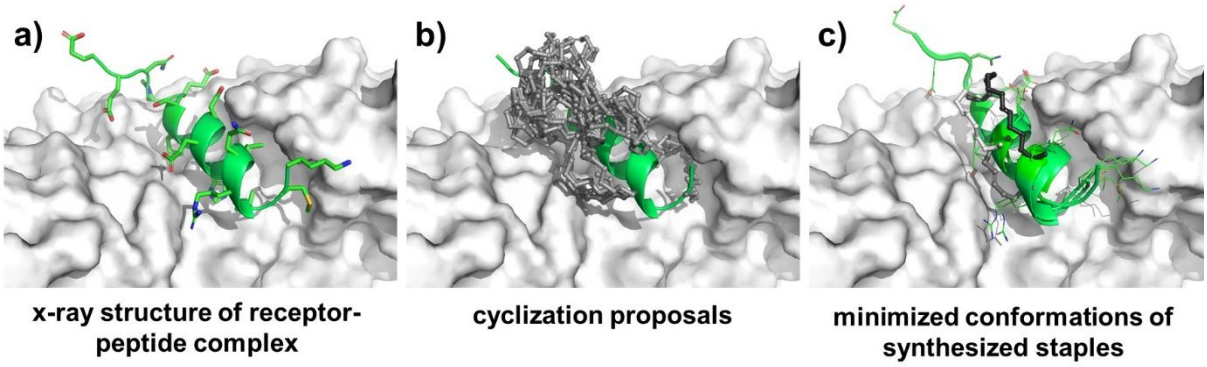


Figure S1. Stapling of a peptidic inhibitor of β-catenin. a) x-ray structure of the receptor-peptide complex (pdb code 1qz7). b) Cyclization proposals that were produced by the algorithm for two different linkers without constraints on stereochemistry. c) Predicted binding modes of **7b-7d** obtained from MM/GBSA energy minimization.

Table S1. Cyclized peptide inhibitors of β-catenin with predicted conformational and enthalpic scores and reported affinities.[1] The method predicts several staplings to be significantly more stable than those tested in the original manuscript.

a)

No	Sequence
7a	ENPESILDEHVQRVM-NH2

b)

No	Sequence modification vs 5a	computed properties		K_d (mM)[1]
		conformational score[a]	enthalpic score[b]	
7a		1.4	-50.8	~5
7b	(dOal[c]5,Pal12)-RCM	0.74	-45.7	~3
7c	(Pal[d]9,Pal13)-RCM	0.8	-52.3	~4
7d	(Pal5,Pal9)-RCM	0.71	-66.9	0.060 ± 0.002

[a]the conformational score was calculated as RMSD over the Cα residues of the bioactive region (residues 469-480). See Supplemental Information for details.

[b]the enthalpic score was obtained from Prime MM/GBSA.

[c]dOal = D-7-octenyl-alanine

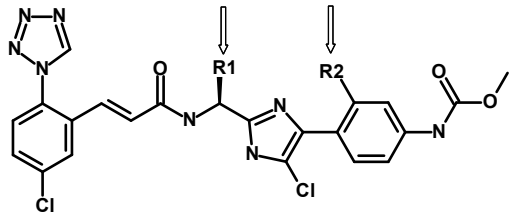
[d]Pal = 4-pentenyl-alanine

Small molecule example: Macrocyclic Factor XIa Inhibitors (retrospective case study).

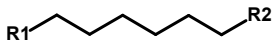
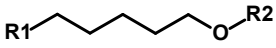
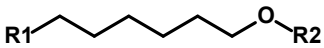
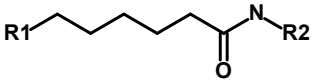
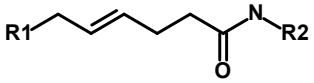
The discovery of novel macrocyclic factor XIa inhibitors based on an acyclic phenyl imidazole lead (Figure S2a) was described in 2017 by Corte *et al* (Bristol-Myers Squibb).[2] In this publication, several macrocyclic variants of the lead structure have been described that were obtained by structure-based drug design (see selected set of analogues in Table S2). We investigated whether these structures could be designed in an automated fashion with our approach. For this purpose, we generated a precursor structure for cyclization based on the published crystal structure of the acyclic phenyl imidazole lead (pdb code 4y8x, see Figure S2a and b) and provided the linkers shown in Table S2 as input for *in silico* cyclization. In all cases, linker orientations could be found that agree with the geometric constraints imposed by the attachment vectors of the precursor structure (see Figure S2c). For the estimation of conformational stability, a conformational sampling was performed using Prime-MCS (see as example the conformational ensemble of **8e** in Figure S2d). The predicted conformational scores, which were calculated by Prime-MCS, are provided in Table S2. Next, the cyclized ligands were docked with Glide to predict their protein-bound conformations and estimate their enthalpic scores (GlideScore). Notably, the most

potent macrocycle **8f** (Ki = 0.03 nM), was correctly predicted as (enthalpic) top-scorer. As shown in Figure S2e, the docking modes of all macrocycles are highly similar to the binding orientation of the acyclic lead structure. Figure S2f shows that the predicted binding mode of the macrocyclic compound **8e** is nearly identical to the x-ray structure of a very close analog (pdb code 5tku). In summary, this case study illustrates that our macrocyclization approach can (i) find small molecule macrocyclizations that maintain and stabilize the bioactive conformation of a noncyclic precursor ligand and (ii) identify the most potent analogues by conformational and enthalpic scoring.

Table S2. Macrocyclic derivatives, their computed conformational and enthalpic scores and their experimentally measured Kd values.[2] The linkers shown in this table have been provided as input for the cyclization approach.



No	linker	computed properties	FXIa K_i

		conformational score[a]	enthalpic score[b]	[nM][2]
8a		2.7	-11.4	42
8b		2.3	-13.7	250
8c		2.4	-10.4	68
8d		2.9	-12.1	76
8e		2.3	-13.7	0.47
8f		2.6	-14.8	0.03

[a]the conformational score was calculated by Prime-MCS.

[b]the enthalpic score was obtained from GlideScore.

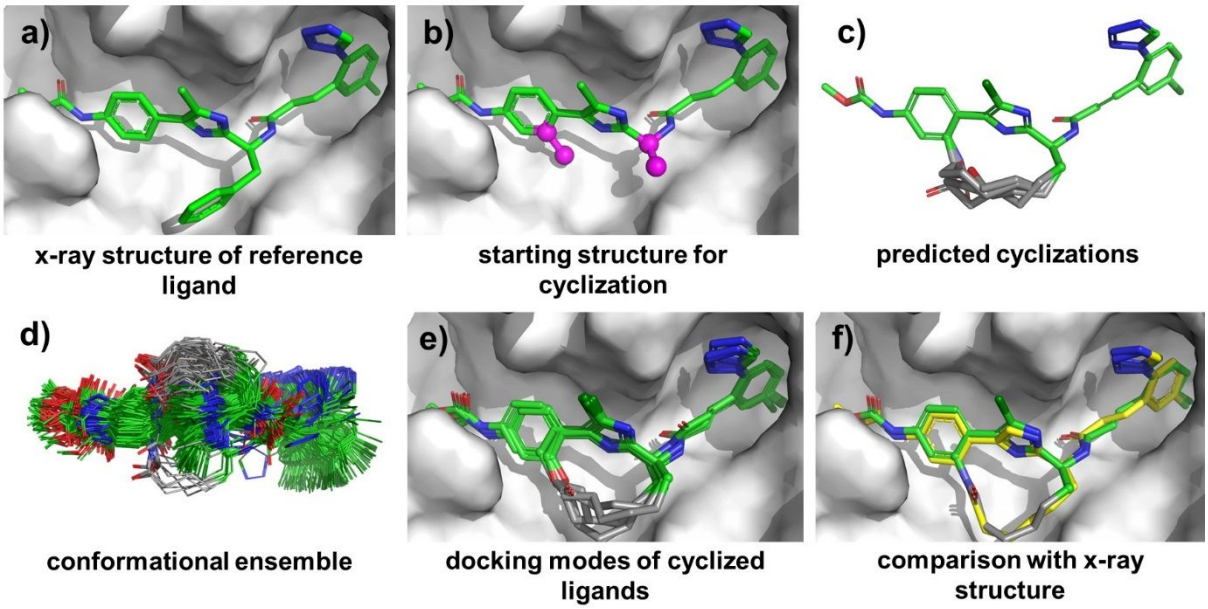


Figure S2. Macrocylization of a factor XIa inhibitor. a) x-ray structure of acyclic phenyl imidazole lead compound (pdb code 4y8x). b) The lead structure was modified to

obtain the starting structure for cyclization. The attachment vectors are indicated in magenta. c) Cyclized conformations of **8a-f** obtained after automated attachment of linkers (see Table S2) to the starting structure. d) Conformational ensemble obtained from Prime-MCS (shown for **8e**). e) Predicted binding modes of **8a-f** obtained from docking with Glide. f) Comparison of predicted binding mode of **8e** with the x-ray structure of a close analogue (pdb code 5tku).

Table S3. Analytical data of the synthesized peptides

No	found MIM (1)	calc. MIM	Retention time (min) (2)	Purity (%)
4a	4215.99	4218.00	13.07	95.18
4b	4210.022	4210.05	12.28	93.18
4c	4221.063	4221.08	13.56	95.58
4d	4178.046	4178.07	15.62	95.1
4e	4339.86	4339.01	13.35	95.60
4f	4337.987	4338.01	13.2	96.82
4g	4215.99	4218.00	13.01	92.03
4h	4178.046	4178.07	15.62	94.89
4i	4239.056	4239.08	13.45	92.48
4j	4253.071	4253.09	13.14	93.77
4k	4253.071	4253.09	13.48	92.84

1) MIM (monoisotopic mass) with mass analyzer: Agilent 6230 Accurate-Mass TOF, Agilent Jet Stream ESI
2) UPLC method) column: Acquity UPLC CSH C18 1.7µm, 2.1 * 150 mm, flow: 0.500 mL/min; solvent A: 100.0 % Water + 0.1%TFA, solvent B: 100.0 % Acetonitrile + 0.1%TFA; gradient: 80:20 (0 min) to 80:20 (3 min) to 25:75 (23 min) to 2:98 (23.5 min) to 2:98 (30.5 min) to 80:20 (31 min) to 80:20 (38 min)

Table S4: IUPAC names of the synthesized peptides.

No	IUPAC name
4a	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-3-carboxy-propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-guanidino-pentanoyl]amino]-5-guanidino-pentanoyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
4b	(4S)-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,5S,8S,11S,19S)-19-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-5-(4-aminobutyl)-8-(3-amino-3-oxo-propyl)-2-(hydroxymethyl)-3,6,9,13,20-pentaoxo-1,4,7,10,14-pentazacycloicosane-11-carbonyl]amino]-3-carboxy-propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-guanidino-pentanoyl]amino]-5-guanidino-pentanoyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-oxo-pentanoic acid
4c	(4S)-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-

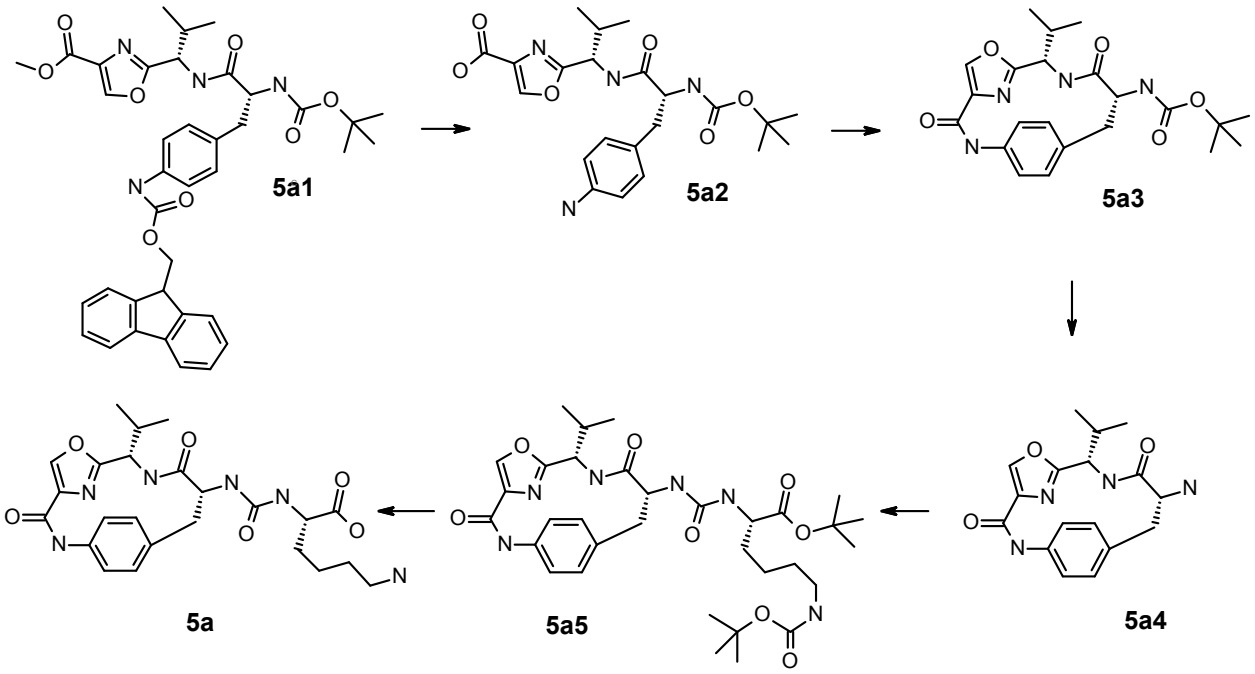
	amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,5S,8S,11R,15Z,20S)-20-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-5-(4-aminobutyl)-8-(3-amino-3-oxo-propyl)-2-(hydroxymethyl)-11,20-dimethyl-3,6,9,21-tetraoxo-1,4,7,10-tetrazacyclohenicos-15-ene-11-carbonyl]amino]-3-carboxy-propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-guanidino-pentanoyl]amino]-5-guanidino-pentanoyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-oxo-pentanoic acid
4d	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S,5S,8S,11S,15E,20R)-20-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-2-(carboxymethyl)-8-(3-guanidinopropyl)-5-(hydroxymethyl)-11,20-dimethyl-3,6,9,21-tetraoxo-1,4,7,10-tetrazacyclohenicos-15-ene-11-carbonyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
4e	(3S)-3-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-4-[[(1S)-2-[[(1S)-1-[[(1S)-1-[[(1S)-2-[[(1S)-4-amino-1-[[(5R,11S,14S,17S,29S,32S,35S,38S,41S,44S,47S,50R)-32-(4-aminobutyl)-5-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]-29-(2-amino-2-oxo-ethyl)-47-benzyl-41-(2-carboxyethyl)-11,14-bis(hydroxymethyl)-38-(1H-indol-3-ylmethyl)-35-isobutyl-44-[(1S)-1-methylpropyl]-7,10,13,16,22,25,28,31,34,37,40,43,46,49-tetradecaoxo-3,52-dithia-6,9,12,15,21,24,27,30,33,36,39,42,45,48-tetradecazatricyclo[52.3.1.017,21]octapentaconta-1(58),54,56-trien-50-yl]carbamoyl]-4-oxo-butyl]amino]-1-methyl-2-oxo-ethyl]carbamoyl]-4-guanidino-butyl]carbamoyl]-4-guanidino-butyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-4-oxo-butanoic acid;methane
4f	(3S)-3-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-

	3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-4-[[(1S)-2-[[(1S)-1-[[(1S)-1-[[(1S)-2-[[(1S)-4-amino-1-[[(7R,13S,16S,19S,31R,34S,37S,40S,43S,46S,49S,52R)-34-(4-aminobutyl)-7-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]-49-benzyl-43-(2-carboxyethyl)-13,16-bis(hydroxymethyl)-40-(1H-indol-3-ylmethyl)-37-isobutyl-46-[(1S)-1-methylpropyl]-9,12,15,18,24,27,30,33,36,39,42,45,48,51-tetradecaoxo-5,54,59-trithia-8,11,14,17,23,26,29,32,35,38,41,44,47,50-tetradecazatetracyclo[29.26.3.13,56.019,23]henhexaconta-1(57),2,56(61)-trien-52-yl]carbamoyl]-4-oxo-butyl]amino]-1-methyl-2-oxo-ethyl]carbamoyl]-4-guanidino-butyl]carbamoyl]-4-guanidino-butyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-4-oxo-butanoic acid
4g	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2R)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-3-carboxy-propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-guanidino-pentanoyl]amino]-5-guanidino-pentanoyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
4h	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S,5S,8S,11S,15E,20R)-20-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2R)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-2-(carboxymethyl)-8-(3-guanidinopropyl)-5-(hydroxymethyl)-11,20-dimethyl-3,6,9,21-tetraoxo-1,4,7,10-tetrazacyclohenicos-15-ene-11-carbonyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
4i	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S,5S,8S,11S,20S)-20-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-

	[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2R)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-3-carboxy-propanoyl]amino]-2,5-bis(3-guanidinopropyl)-8-methyl-3,6,9,14,21-pentaoxo-1,4,7,10,15-pentazacyclohenicosane-11-carbonyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(1S)-3-amino-1-[[2-[[2-[(2S)-2-[[(1S)-2-[[(1S)-2-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-3-oxo-propyl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
4j	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2R)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-3-carboxy-propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-guanidino-pentanoyl]amino]-5-guanidino-pentanoyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(9S,18S,21S,24S)-18-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-21-(hydroxymethyl)-2,5,8,12,20,23-hexaoxo-1,4,7,13,19,22-hexazabicyclo[22.3.0]heptacosan-9-yl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid
4k	(4S)-4-[[(2S,3S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-5-amino-2-[[(2S)-6-amino-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[(2R)-2-[[(2S)-2-amino-3-(1H-imidazol-4-yl)propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-phenyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-3-carboxy-propanoyl]amino]-4-methyl-pentanoyl]amino]-3-hydroxy-propanoyl]amino]hexanoyl]amino]-5-oxo-pentanoyl]amino]-4-methylsulfanyl-butanoyl]amino]-3-carboxy-propanoyl]amino]-3-hydroxy-propanoyl]amino]-5-guanidino-pentanoyl]amino]-5-guanidino-pentanoyl]amino]propanoyl]amino]-5-oxo-pentanoyl]amino]-3-carboxy-propanoyl]amino]-3-phenyl-propanoyl]amino]-3-methyl-pentanoyl]amino]-5-[[(1S)-2-[[(1S)-1-[[(1S)-5-amino-1-[[(9S,18S,21S,24S)-18-[[2-[[(1S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[[(1S)-2-amino-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-1-methyl-2-oxo-ethyl]amino]-2-oxo-ethyl]carbamoyl]-21-(hydroxymethyl)-2,5,8,15,20,23-hexaoxo-1,4,7,14,19,22-hexazabicyclo[22.3.0]heptacosan-9-yl]carbamoyl]pentyl]carbamoyl]-3-methyl-butyl]amino]-1-(1H-indol-3-ylmethyl)-2-oxo-ethyl]amino]-5-oxo-pentanoic acid

Synthesis of TAFIa inhibitors

Scheme 1. Synthesis of 5a.



*Methyl 2-((S)-1-((R)-3-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)phenyl)-2-((tert-butoxycarbonyl)amino)propanamido)-2-methylpropyl)oxazole-4-carboxylate (**5a1**).*

2.41 g (4.8 mmol) N-□-tert.-butyloxycarbonyl-D-4-(9-fluorenylmethoxycarbonyl)-aminophenylalanine and 1.5 g (4.8 mmol) methyl (S)-2-(1-amino-2-methylpropyl)oxazole-4-carboxylate TFA salt were dissolved in 25 mL DMF. 0.65 g (4.8 mmol) HOAT, 3.27 ml (4eq.) DIPEA and 1.83 g (4.8 mmol) HATU were added and the resulting mixture was stirred for 2 h at room temperature and then concentrated under vacuum. The resulting residue was dissolved in dichloromethane and washed with a saturated $NaHCO_3$ solution and afterwards with brine. The organic phase was concentrated under vacuum to give 4.2 g of crude methyl 2-((S)-1-((R)-3-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)phenyl)-2-((tert-butoxycarbonyl)amino)propanamido)-2-methylpropyl)oxazole-4-carboxylate, which was used without further purification in the next reaction.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 1.17 min (705.25 M+Na^+).

*2-((S)-1-((R)-3-(4-aminophenyl)-2-((tert-butoxycarbonyl)amino)propanamido)-2-methylpropyl)oxazole-4-carboxylic acid (**5a2**).*

3.27 g (4.79 mmol) Methyl 2-((S)-1-((R)-3-(4-((((9H-fluoren-9-yl)methoxy)-carbonyl)amino)phenyl)-2-((tert-butoxycarbonyl)amino)propanamido)-2-methylpropyl)oxazole-4-carboxylate were dissolved in 57 ml tetrahydrofuran and 19 ml methanol. 19 ml of a 1M LiOH solution in water were added and the resulting mixture was stirred for 1 h at room temperature. The reaction mixture was neutralized with a 2M HCl solution in water. The mixture was concentrated under vacuum and co-distilled with toluene to remove residual water. 2-((S)-1-((R)-3-(4-aminophenyl)-2-((tert-butoxycarbonyl)amino)propanamido)-2-methylpropyl)oxazole-4-carboxylic acid was obtained as crude product (quant. yield) and used as such in the cyclization reaction.

LCMS (FRA.M: 0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.46 min (468.30 M+Na^+).

*Tert-butyl ((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamate (**5a3**).*

2.14 g (4.79 mmol) of crude 2-((S)-1-((R)-3-(4-aminophenyl)-2-((tert-butoxycarbonyl)amino)propanamido)-2-methylpropyl)oxazole-4-carboxylic acid were dissolved in a mixture of 300 ml DMF and 1900 ml dichloromethane. 0.65 g (4.79 mmol) HOAt, 2.4 ml DIPEA and 1.82 g (4.79 mmol) HATU were added. The resulting mixture was stirred overnight at room temperature. After concentration under reduced pressure the resulting residue was taken up in dichloromethane and washed with a saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. Concentration under reduced pressure and

purification by flash-chromatography using a n-heptane / ethyl acetate gradient (10:1 – 1:1) gave 1.89 g (92% yield) tert-butyl ((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamate as colorless material.

LCMS (FRA.M: 0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.77 min (451.25 M+Na^+).

*(6R,9S,Z)-6-amino-9-isopropyl-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-2,7-dione (**5a4**).*

49.7 mg (0.12 mmol) crude tert-butyl ((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamate were added to a mixture of 2 ml TFA and 2 ml dichloromethane. After stirring for 1 h at room temperature the reaction mixture was concentrated under vacuum and co-distilled with toluene. The resulting residue was used as crude product in the next reaction.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.47 min (351.25 M+Na^+).

*tert-butyl N6-(tert-butoxycarbonyl)-N2-(((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamoyl)-L-lysinate (**5a5**).*

To a mixture of 52 mg (0.26 mmol) 4-nitrophenyl chloroformate in 2 ml dichloromethane was added slowly at 0°C a solution of 57 mg (0.12 mmol) (6R,9S,Z)-6-amino-9-isopropyl-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-2,7-dione TFA-salt and 40 µl DIPEA in 2 ml dichloromethane. The resulting mixture was allowed to come to room temperature and then stirred for 3 h. The reaction mixture was washed with a saturated $NaHCO_3$ solution, water and with brine. After drying over anhydrous $MgSO_4$ the organic phase was concentrated under reduced pressure to give crude 4-nitrophenyl ((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamate. Crude 4-

nitrophenyl ((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamate was taken up in 2 ml DMF. To that mixture a solution of 43.1 mg (130 µmol) of H-Lys(Boc)-OtBu * HCl and 40 µl DIPEA in 2 ml DMF were added and the resulting mixture was stirred for 1 h at room temperature. The mixture was concentrated under vacuum and used as crude product in the next reaction. Yield : 89 mg (quant. yield) crude product, colorless oil.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.92 min (679.35 $M+Na^+$).

*(((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamoyl)-L-lysine (**5a**).*

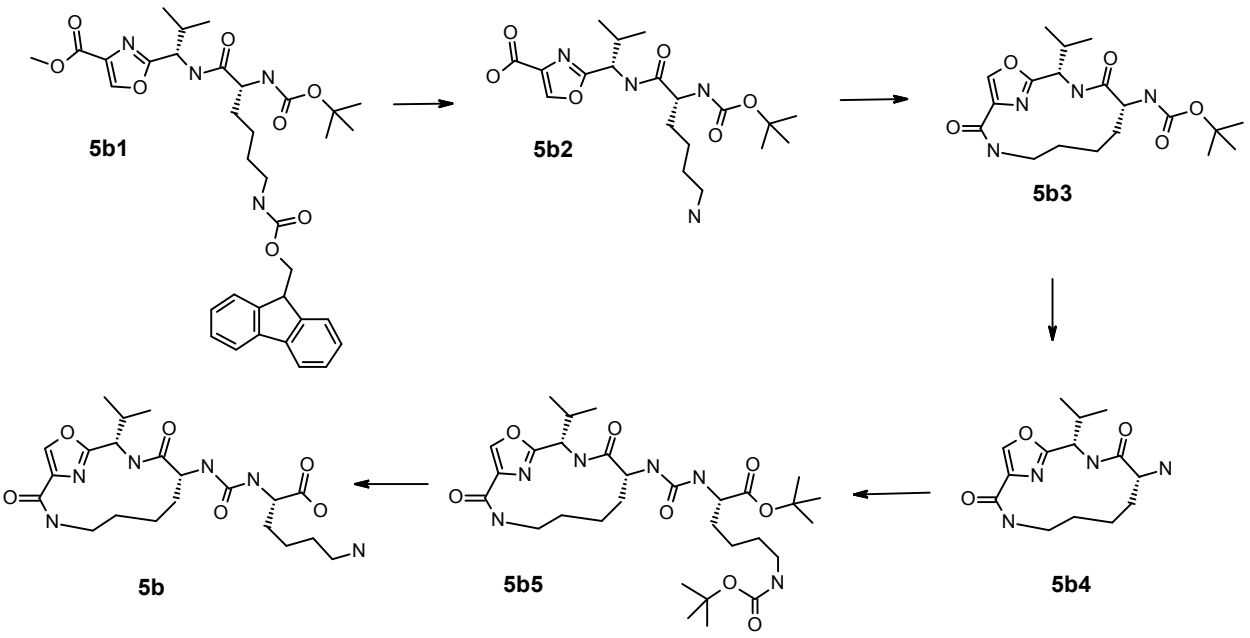
57 mg (90 µmol) tert-butyl N6-(tert-butoxycarbonyl)-N2-(((6R,9S,Z)-9-isopropyl-2,7-dioxo-3,8-diaza-1(4,2)-oxazola-4(1,4)-benzenacyclononaphane-6-yl)carbamoyl)-L-lysinate were added to a mixture of 1.9 ml TFA, 50 µl triisopropylsilane and 50 µl water and stirred for 1 h at room temperature. The mixture was concentrated under vacuum and co-distilled with toluene. Final purification by RP-HPLC gave 11 mg of the title compound in form of its TFA salt as white amorphous lyophilizate.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.48 min (501.25 $M+Na^+$).

LCMS (A: H2O+0.1%FA B:ACN+0.08%FA; 0.9ml/min; Grad: 95:5 (0min) to 5:95 (1.1min) 5:95 (1.7min)]: 0.64 min (501.36 [M+H]; 100% purity)

^{1}H NMR (DMSO-d_6, 500 MHz) δ [ppm]: 12.58 (s_b, 1H), 10.01 (s, 1H), 8.41 (s, 1H), 7.58-7.69 (m, 3H), 7.41 (d, 1H), 7.12 (d, 1 H), 6.80 (d, 1H), 6.62 (d, 1 H), 6.38-6.48 (dd, 2H), 6.25 (d, 1H), 4.50 (m, 1H), 4.28 (m, 1 H), 4.12 (m, 1H), 3.11 (m, 1H), 2.78 (m, 3H), 1.65-1.83 (m, 2 H), 1.48-1.59 (m, 3H), 1.28-1.40 (m, 2H), 0.69 (d, 3H), 0.61 (d, 3H); HRMS (ES^-) expected for C24H32N6O6 500.23833; found 500.23828.

Scheme 2. Synthesis of 5b.



*Methyl 2-((S)-1-((R)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)oxazole-4-carboxylate (**5b1**).*

440 mg (2.03 mmol) BOC-D-LYS(FMOC)-OH and 630 mg (2.03 mmol) methyl (S)-2-(1-amino-2-methylpropyl)oxazole-4-carboxylate TFA salt were dissolved in 15mL DMF. 277 mg (2.03 mmol) HOAT, 1.38 ml (4eq.) DIPEA and 770 mg (2.03 mmol) HATU were added and the resulting mixture was stirred for 6 h at room temperature and then concentrated under vacuum. The resulting residue was dissolved in dichloromethane and washed with a saturated $NaHCO_3$ solution and afterwards with brine. The organic phase was concentrated under vacuum and the resulting residue was purified by flash-chromatography using a 1:1 n-heptane / ethyl acetate mixture as solvent. Yield: 810 mg (62 %);

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 1.10 min (671.25 M+Na^+).

*2-((S)-1-((R)-6-amino-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)oxazole-4-carboxylic acid (**5b2**).*

580 mg (0.89 mmol) Methyl 2-((S)-1-((R)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)oxazole-4-carboxylate were dissolved in a mixture of 3 ml methanol and 9 ml tetrahydrofuran. After addition of 5 ml of a 1M LiOH solution in water the resulting mixture was stirred for 2 h at room temperature, then neutralized by the addition of a 1M HCl solution and then concentrated under reduced pressure. Without further purification 2-((S)-1-((R)-6-amino-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)oxazole-4-carboxylic acid was used in the subsequent reaction. Yield: 370 mg (quant.);

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 1.10 min (413.25 M+H^+).

*tert-butyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamate (**5b3**).*

370 mg (0.89 mmol) crude 2-((S)-1-((R)-6-amino-2-((tert-butoxycarbonyl)amino)-hexanamido)-2-methylpropyl)oxazole-4-carboxylic acid were dissolved in 350 mL DMF. 121 mg (0.89 mmol) HOAT, 340 mg (0.89 mmol) HATU and 0.46 ml (3eq.) DIPEA were added and the resulting mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane and washed with a sat. $NaHCO_3$ solution. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Final purification by preparative RP-HPLC gave tert-butyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazola-cycloundecaphane-5-yl)carbamate as colorless material. Yield: 166 mg (47% yield);

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.74 min (417.25 M+Na^+).

*(8R,11S,Z)-8-amino-11-isopropyl-3,10-diaza-1(2,4)-oxazolacycloundecaphane-2,9-dione (**5b4**).*

165 mg (0.42 mmol) Tert-butyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacyclo-undecaphane-5-yl)carbamate were dissolved in a mixture of 2.5 ml TFA and 2.5 ml dichloromethane and stirred for 1 h at room temperature. The mixture was concentrated under vacuum and co-distilled with toluene. 90 mg crude (8R,11S,Z)-8-amino-11-isopropyl-3,10-diaza-1(2,4)-oxazolacycloundecaphane-2,9-dione were obtained as TFA salt and used without further purification in the next reaction.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.40 min (295.25 $M+H^+$).

*Tert-butyl N6-(tert-butoxycarbonyl)-N2-(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamoyl)-L-lysinate (**5b5**).*

To a mixture of 51 mg (0.25 mmol) 4-nitrophenyl chloroformate in 5 ml dichloromethane was added slowly at 0°C a solution of 86 mg (0.21 mmol) (8R,11S,Z)-8-amino-11-isopropyl-3,10-diaza-1(2,4)-oxazolacycloundecaphane-2,9-dione TFA-salt and 80 µl DIPEA in 5 ml dichloromethane and 1 ml pyridine. The resulting mixture was allowed to come to room temperature and then stirred overnight. The reaction mixture was washed with a saturated $NaHCO_3$ solution, water and with brine. After drying over anhydrous $MgSO_4$ the organic phase was concentrated under reduced pressure to give crude 4-nitrophenyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamate. Crude 4-nitrophenyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamate was taken up in 3 ml DMF. To that mixture a solution of 78 mg (200 µmol) of H-Lys(Boc)-OtBu * HCl and 80 µl DIPEA in 2 ml DMF was added and the resulting mixture was stirred overnight at room temperature. The mixture was concentrated under vacuum and

the resulting residue was purified by flash chromatography using a n-heptane / ethyl acetate mixture (1:1) as eluent to give 130 mg Tert-butyl N6-(tert-butoxycarbonyl)-N2-(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamoyl)-L-lysinate in the form of a colorless oil.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.89 min (645.30 $M+Na^+$).

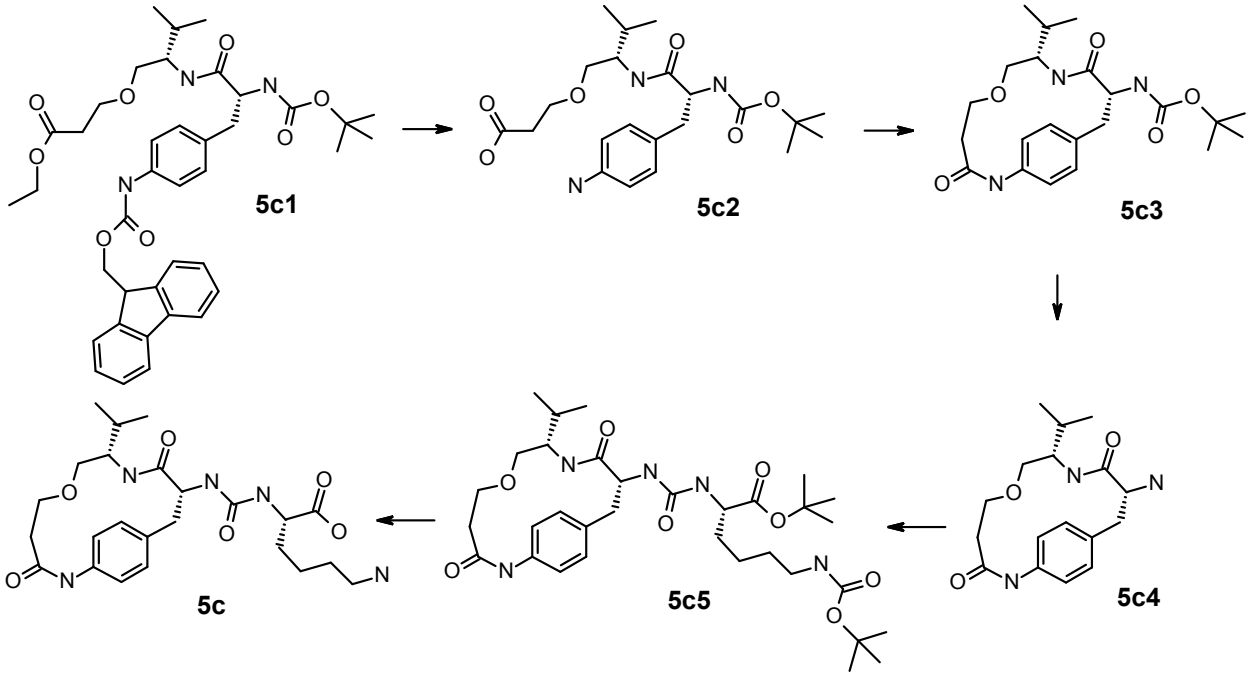
*(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamoyl)-L-lysine (**5b**).*

130 mg (0.21 mmol) Tert-butyl N6-(tert-butoxycarbonyl)-N2-(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-oxazolacycloundecaphane-5-yl)carbamoyl)-L-lysinate were added to a mixture of 2 ml TFA and 2 ml dichloromethane and stirred for 1 h at room temperature. The mixture was concentrated under vacuum and co-distilled with toluene. Final purification by preparative RP-HPLC gave 36 mg (30% yield) of the title compound in form of its TFA salt as white amorphous lyophilizate.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.46 min (467.25 $M+H^+$, 100% purity).

^1H NMR (DMSO-d_6, 500 MHz) δ [ppm]: 12.62 (s_b, 1H), 8.41 (s, 1H), 7.89 (m, 1H), 7.54-7.72 (m, 3H), 6.45 (d, 1H), 6.39 (d, 1 H), 4.28 (m, 1H), 4.05 (m, 1 H), 3.92 (m, 1H), 3.72 (m, 1H) 3.25-3.52 (m, 2H), 3.11 (m, 1H), 2.76 (m, 2H), 2.33 (m, 1H), 1.8 (m, 1H), 1.71 (m, 1H), 1.45-1.5 (m, 2H), 1.27-1.39 (m, 4H), 0.92 (dd, 6H); HRMS (ES^-) expected for C21H34N6O6 466.25398; found 466.25395.

Scheme 3. Synthesis of 5c.



*Ethyl (6R,9S)-6-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)benzyl)-9-isopropyl-2,2-dimethyl-4,7-dioxo-3,11-dioxa-5,8-diazatetradecan-14-oate (**5c1**).*

0.37 g (1.17 mmol) Ethyl (S)-3-(2-amino-3-methylbutoxy)propanoate and 0.59 g (1.17 mmol) N-□-tert.-butyloxycarbonyl-D-4-(9-fluorenylmethoxycarbonyl)-aminophenylalanine were dissolved in 10 ml DMF. 0.16 g (1.17 mmol) HOAt, 0.6 ml DIPEA and 0.44 g (1.17) mmol HATU were added and the resulting mixture was stirred at room temperature for 2 h. After concentration under reduced pressure the residue was taken up in dichloromethane and washed with a saturated $NaHCO_3$ solution and dried. The solvent was removed under vacuum and the resulting residue was purified by flash-chromatography using a heptane / ethyl acetate mixture (1:1) as eluent. 595 mg (74 % yield) of pure ethyl (6R,9S)-6-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)benzyl)-9-isopropyl-2,2-dimethyl-4,7-dioxo-3,11-dioxa-5,8-diazatetradecan-14-oate were obtained.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 1.23 min (710.30 M+Na^+).

*(6R,9S)-6-(4-Aminobenzyl)-9-isopropyl-2,2-dimethyl-4,7-dioxo-3,11-dioxa-5,8-diazatetradecan-14-oic acid (**5c2**).*

350 mg (0.52 mmol) ethyl (6R,9S)-6-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)benzyl)-9-isopropyl-2,2-dimethyl-4,7-dioxo-3,11-dioxa-5,8-diazatetradecan-14-oate were dissolved in 9 ml tetrahydrofuran and 3 ml methanol. 1.3 ml of a 1M LiOH solution in water were added and the resulting mixture was stirred for 1 h at room temperature. 1.5 ml of a 1M HCl solution in water were added. The mixture was concentrated under vacuum and co-distilled with toluene to remove residual water. (6R,9S)-6-(4-Aminobenzyl)-9-isopropyl-2,2-dimethyl-4,7-dioxo-3,11-dioxa-5,8-diazatetradecan-14-oic acid was obtained as crude product (quant. yield) and used as such in the cyclization reaction. LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN bis 1.4min;7%ACN 1.45min; 1.1ml/min): 0.67 min (460.25 M+Na^+).

*tert-butyl ((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamate (**5c3**).*

250 mg (0.51 mmol) of crude (6R,9S)-6-(4-aminobenzyl)-9-isopropyl-2,2-dimethyl-4,7-dioxo-3,11-dioxa-5,8-diazatetradecan-14-oic acid were dissolved in 225 ml of DMF. 70 mg (0.51 mmol) HOAt, 260 µl DIPEA and 195.4 mg (0.51 mmol) HATU were added. The resulting mixture was stirred for 1 h at room temperature. After concentration under reduced pressure the resulting residue was taken up in dichloromethane and washed with a saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. Concentration under reduced pressure gave 215 mg (quant. yield) of crude tert-butyl ((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamate.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.79 min (442.25 M+Na^+).

*(8S,11R)-11-amino-8-isopropyl-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-3,10-dione (**5c4**).*

215 mg crude tert-butyl ((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamate were added to a mixture of 4,75 ml TFA, 130 µl water and 130 µl triisopropylsilane. After stirring for 1 h at room temperature the reaction mixture was concentrated under vacuum and co-distilled with toluene. The resulting residue was purified by preparative RP-HPLC. 96 mg (8S,11R)-11-amino-8-isopropyl-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-3,10-dione were obtained in form of its TFA salt as white amorphous lyophilizate.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.47 min (442.25 M+H).

*tert-butyl N6-(tert-butoxycarbonyl)-N2-(((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamoyl)-L-lysinate (**5c5**).*

To a mixture of 82 mg (0.41 mmol) 4-nitrophenyl chloroformate in 3 ml dichloromethane was added slowly at 0°C a solution of 80 mg (0.18 mmol) (8S,11R)-11-amino-8-isopropyl-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-3,10-dione TFA-salt and 70 µl DIPEA in 3 ml dichloromethane. The resulting mixture was allowed to come to room temperature and then stirred for 3 h. The reaction mixture was washed with a saturated $NaHCO_3$ solution, water and with brine. After drying over anhydrous $MgSO_4$ the organic phase was concentrated under reduced pressure to give crude 4-nitrophenyl ((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamate. Crude 4-nitrophenyl ((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamate was

taken up in 3 ml DMF. To that mixture a solution of 69 mg (0.20 mmol) of H-Lys(Boc)-OtBu * HCl and 70 µl DIPEA in 3 ml DMF were added and the resulting mixture was stirred overnight at room temperature. The mixture was concentrated under vacuum and the resulting residue was purified by flash chromatography using a n-heptane / ethyl acetate mixture (1:1) as eluent. 20 mg (17% yield) pure tert-butyl N6-(tert-butoxycarbonyl)-N2-(((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamoyl)-L-lysinate were obtained as colorless oil.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN bis 1.4min;7%ACN 1.45min; 1.1ml/min): 0.94 min (648.35 M+H).

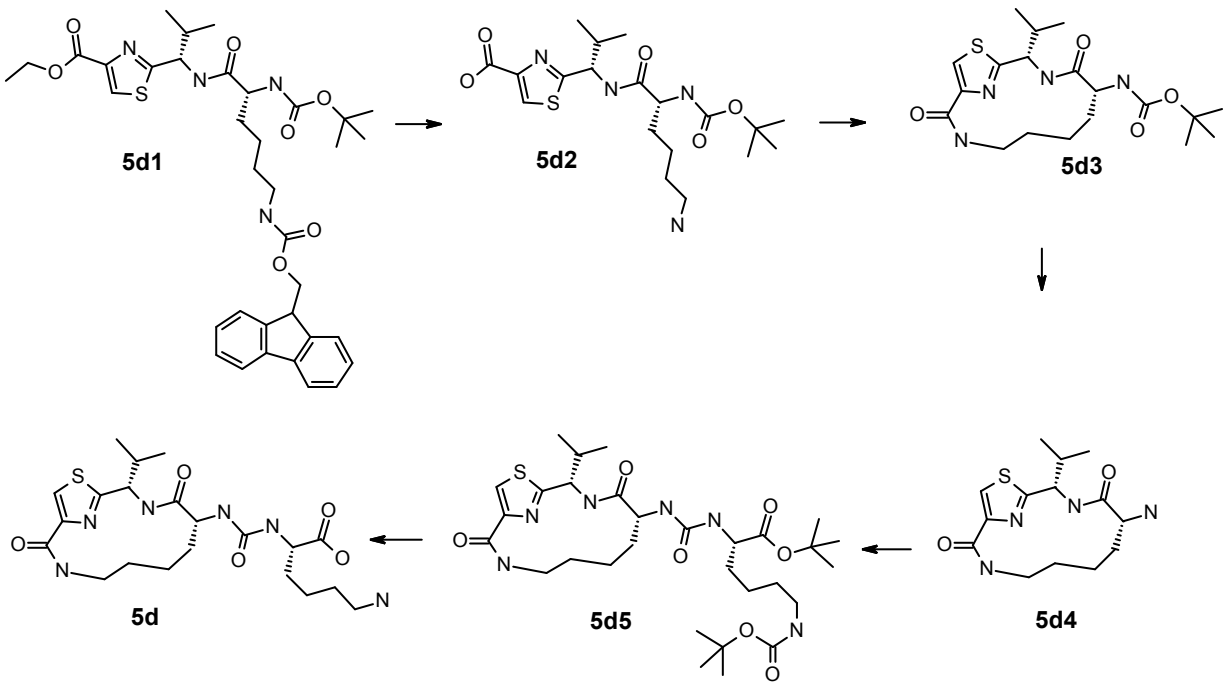
*(((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamoyl)-L-lysine (**5c**).*

20 mg (30 µmol) Tert-butyl N6-(tert-butoxycarbonyl)-N2-(((8S,11R)-8-isopropyl-3,10-dioxo-6-oxa-2,9-diaza-1(1,4)-benzenacyclododecaphane-11-yl)carbamoyl)-L-lysinate were added to a mixture of 0.95 ml TFA, 25 µl triisopropylsilane and 25 µl water and stirred for 2 h at room temperature. The mixture was concentrated under vacuum and co-distilled with toluene. Final purification by RP-HPLC gave 9 mg (74% yield) of the title compound in form of its TFA salt as white amorphous lyophilizate.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.33 min (492.25 M+H).

^{1}H NMR (DMSO-d_6, 500 MHz) δ [ppm]: 12.58 (s_b, 1H), 9.32 (s, 1H), 7.58-7.68 (m, 3H), 7.32 (d, 1H), 7.28 (m, 2H), 7.12 (d, 1 H), 6.62 (d, 1 H), 6.39 (d, 1H), 6.19 (d, 1H), 4.28 (m, 1 H), 4.10 (m, 1H), 3.21-3.48 (m, 5H) 3.14 (m, 2H), 2.89 (m, 2H), 2.75 (m, 2H), 2.41-2.49 (m, 1 H), 1.48-1.72 (m, 4H), 1.34 (m, 2H), 0.69 (d, 3H), 0.61 (d, 3H); HRMS (ES^-) expected for C24H37N5O6 491.27438; found 491.27434.

Scheme 4. Synthesis of 5d.



*Ethyl 2-((S)-1-((R)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)thiazole-4-carboxylate (**5d1**).*

2.37 g (5.05 mmol) Boc-D-Lys(Fmoc)-OH and 1.73 g (5.05 mmol) ethyl (S)-2-(1-amino-2-methylpropyl)thiazole-4-carboxylate TFA salt were dissolved in 15 mL DMF. 0.69 g (5.05 mmol) HOAT, 3.45 ml (4 eq.) DIPEA and 1.92 g (5.05 mmol) HATU were added and the resulting mixture was stirred for 3h at room temperature and then concentrated under vacuum. The resulting residue was dissolved in dichloromethane and washed with a saturated $NaHCO_3$ solution and afterwards with brine. The organic phase was concentrated under vacuum and the resulting residue was purified by flash-chromatography using a dichloromethane / methanol mixture as eluent to give 3.45 g (quant. yield) ethyl 2-((S)-1-((R)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)thiazole-4-carboxylate as colorless material.

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN bis 1.4min;7%ACN 1.45min; 1.1ml/min): 1.11 min (679.25 $M+H^+$).

*2-((S)-1-((R)-6-amino-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)thiazole-4-carboxylic acid (**5d2**).*

3.42 g (5.04 mmol) Ethyl 2-((S)-1-((R)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)thiazole-4-carboxylate were dissolved in a mixture of 15 ml methanol and 45 ml tetrahydrofuran. After addition of 15 ml of a 1M LiOH solution in water the resulting mixture was stirred for 1 h at room temperature, then neutralized by the addition of a 1M HCl solution and finally concentrated under reduced pressure. Without further purification crude 2-((S)-1-((R)-6-amino-2-((tert-butoxycarbonyl)amino)hexanamido)-2-methylpropyl)thiazole-4-carboxylic acid was used in the subsequent reaction. Yield: 2.3 g (quant.)

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.58 min (429.25 $M+H^+$).

*tert-butyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-thiazolacycloundecaphane-5-yl)carbamate (**5d3**).*

2.16 g (5.04 mmol) crude 2-((S)-1-((R)-6-amino-2-((tert-butoxycarbonyl)-amino)hexanamido)-2-methylpropyl)thiazole-4-carboxylic acid were dissolved in a mixture of 300 mL DMF and 1000 ml dichloromethane. 0.69 g (5.04 mmol) HOAT, 1.92 g (5.04 mmol) HATU and 2.6 ml DIPEA were added and the resulting mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane and washed with a sat. $NaHCO_3$ solution and with brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Final purification by flash chromatography using a n-heptane / ethyl acetate mixture

as gradient (from 10:1 to 1:1) gave tert-butyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-thiazola-cycloundecaphane-5-yl)carbamate as colorless material. Yield: 1.84 g (89%); LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.73 min (433.25 M+Na^+).

*(8R,11S,Z)-8-amino-11-isopropyl-3,10-diaza-1(2,4)-thiazolacycloundecaphane-2,9-dione (**5d4**).*

1.84 g (4.48 mmol) Tert-butyl ((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-thiazola-cycloundecaphane-5-yl)carbamate were dissolved in a mixture of 35 ml TFA, 1 ml triisopropylsilane and 1 ml water and stirred for 1 h at room temperature. The mixture was concentrated under vacuum and co-distilled with toluene. The resulting residue was purified by preparative RP-HPLC to give 0.6 g (29% yield) pure (8R,11S,Z)-8-amino-11-isopropyl-3,10-diaza-1(2,4)-thiazolacycloundecaphane-2,9-dione as trifluoroacetate.
LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.26 min (311.2 M+H^+).

*tert-butyl N6-(tert-butoxycarbonyl)-N2-(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-thiazolacycloundecaphane-5-yl)carbamoyl)-L-lysinate (**5d5**).*

110 mg (0.26 mmol) (8R,11S,Z)-8-amino-11-isopropyl-3,10-diaza-1(2,4)-thiazolacyclo-undecaphane-2,9-dione TFA-salt were dissolved in 3 ml DMF. 90 µl DIPEA were added and the resulting mixture was stirred for 15 min at room temperature. At 0 °C 42 mg (0.26 mmol) 1,1'-carbonyldiimidazole were added. After stirring for 30 minutes at 0°C a solution of 88 mg (0.26 mmol) H-Lys(Boc)-OtBu * HCl and 45 µl DIPEA in 2 ml DMF were added slowly and the resulting reaction mixture was stirred for 2 h at room temperature. Finally, the mixture was concentrated under reduced pressure and used without further purification in the next reaction. Yield: 240 mg (quant.);

LCMS (0 min 93%H_2O(0.05%TFA) 1.2min-95%ACN; 95%ACN to 1.4min;7%ACN 1.45min; 1.1ml/min): 0.37 min (483.25 M+H-Boc-tBu).

*(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-thiazolacycloundecaphane-5-yl)carbamoyl)-L-lysine (**5d**).*

165 mg (0.26 mmol) tert-butyl N6-(tert-butoxycarbonyl)-N2-(((2S,5R,Z)-2-isopropyl-4,11-dioxo-3,10-diaza-1(2,4)-thiazolacycloundecaphane-5-yl)carbamoyl)-L-lysinate were added to a mixture of 1.95 ml TFA, 50 µl tisisopropylsilane and 50 µl water and stirred for 1 h at room temperature. The mixture was concentrated under vacuum and co-distilled with toluene. Final purification by preparative RP-HPLC gave 74 mg (48% yield) of the title compound in form of its TFA salt as white amorphous lyophilizate.

LCMS (Eluent A: H2O+0.1%FA B:ACN+0.08%FA; 0.9ml/min; Gradient: 95:5 (0min) to 5:95 (1.1min) 5:95 (1.7min): 0.70 min (483.35 M+H, 95% purity)

^{1}H NMR (DMSO-d_6, 500 MHz) □ [ppm]: 12.68 (s_b, 1H), 8.15 (s, 1H), 7.73-7.80 (m, 1H), 7.54-7.69 (m, 3H), 7.40 (m, 1H), 6.51 (m, 2H), 4.83 (m, 1H), 4.08 (m, 1 H), 4.02 (m, 1H), 3.20 (m, 1H), 2.92 (m, 1H), 2.78 (m, 2H), 2.28 (m, 1H), 1.73 (m, 4H), 1.54 (m, 4H), 1.35 (m, 4H), 0.99 (d, 3H), 0.89 (d, 3 H); HRMS (ES^-) expected for C21H34N6O5S 482.23114; found 482.23112.

References

(1) Grossmann, T. N.; Yeh, J. T.-H.; Bowman, B. R.; Chu, Q.; Moellering, R. E.; Verdine, G. L. Inhibition of Oncogenic Wnt Signaling through Direct Targeting of β-Catenin. *Proc. Natl. Acad. Sci.* **2012**, *109* (44), 17942–17947. https://doi.org/10.1073/pnas.1208396109.

(2) Corte, J. R.; Fang, T.; Osuna, H.; Pinto, D. J. P.; Rossi, K. A.; Myers, J. E.; Sheriff, S.; Lou, Z.; Zheng, J. J.; Harper, T. W.; Bozarth, J. M.; Wu, Y.; Luettgen, J. M.; Seiffert, D. A.; Decicco, C. P.; Wexler, R. R.; Quan, M. L. Structure-Based Design of Macrocyclic Factor XIa Inhibitors: Discovery of the Macrocyclic Amide Linker. *J. Med. Chem.* **2017**, *60* (3), 1060–1075. https://doi.org/10.1021/acs.jmedchem.6b01460.