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N^{ε}-Modified lysine containing inhibitors for SIRT1 and SIRT2

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ABSTRACT

Sirtuins catalyze the NAD$^+$ dependent deacetylation of N^{ε}-acetyl lysine residues to nicotinamide, O'-acetyl-ADP-ribose (OAADPR) and N^{ε}-deacetylated lysine. Here, an easy-to-synthesize Ac-Ala-Lys-Ala sequence has been used as a probe for the screening of novel N^{ε}-modified lysine containing inhibitors against SIRT1 and SIRT2. N^{ε}-Selenoacetyl and N^{ε}-isothiovaleryl were the most potent moieties found in this study, comparable to the widely studied N^{ε}-thioacetyl group. The N^{ε}-3,3-dimethylacryl and N^{ε}-isovaleryl moieties gave significant inhibition in comparison to the N^{ε}-acetyl group present in the substrates. In addition, the studied N^{ε}-alkanoyl, N^{ε}-α,β-unsaturated carbonyl and N^{ε}-aroyl moieties showed that the acetyl binding pocket can accept rather large groups, but is sensitive to even small changes in electronic and steric properties of the N^{ε}-modification. These results are applicable for further screening of N^{ε}-acetyl analogues.

1. Introduction

The mammalian sirtuin family of class III deacetylases includes seven enzymes, called SIRT1–SIRT7. Sirtuins are novel therapeutic targets to treat medically important age-associated diseases, such as cancer and metabolic, cardiovascular and neurodegenerative diseases.[1–4] Currently, there is intensive research to discover potent activators and inhibitors of SIRT1 and SIRT2. The most relevant therapeutic prospects for SIRT1 inhibitors are for the treatment of cancer, either induce cell death or prevent angiogenesis.[1,5–9] SIRT2 inhibitors have been demonstrated to rescue proteotoxicity in neurodegenerative diseases.[10,11] In addition to therapeutic studies, small molecular activators and inhibitors of sirtuins have been used as pharmacological tools in biological studies to reveal sirtuin functions in cells.[1–4] Thus, it is important to discover novel lead molecules for SIRT1 and SIRT2 inhibition.

Sirtuins catalyze deacetylation of N^{ε}-acetyl lysine residues of various substrate proteins. This NAD$^+$ dependent reaction has been thoroughly defined.[12] First binds an acetylated substrate,[13] which is followed by the binding of NAD$^+$ into a conformation,[14,15] where the glycosidic bond between the nicotinamide and ribosyl moieties of NAD$^+$ becomes strained. The correctly positioned acetyl oxygen acts as a nucleophile and forms an ADPR-peptidyl amidate (1′-*O*-alkylamidate),[16,17] consequently cleaving the glycosidic bond and releasing nicotinamide. At this stage, the pyridyl-nitrogen of the released nicotinamide can act as a competing nucleophile. This reversibility of the transglycosidation makes nicotinamide a physiological inhibitor for sirtuins.[18,19] In the following steps, the active site histidine acts as a base and activates the 2′-hydroxyl-ribose in the intermediate complex, which in turn attacks the 1′-*O*-alkylamidate carbon and forms a cyclic intermediate. This is subsequently cleaved by addition of water to form OAADPR and the deacetylated protein as the end products.

N^{ε}-Acetylated protein substrates provide a fresh starting point for the development of novel sirtuin inhibitors as several inhibitors binding putatively to NAD$^+$ binding site has already been published.[20–26] Fatkins et al. were the first to report N^{ε}-thioacetyl lysine containing peptides, derived from human p53 tumor suppressor protein, human α-tubulin and human acetyl-coenzyme A synthetase 2 proteins, as potent sirtuin inhibitors.[27,28] Later, their mechanism of inhibition has been fully elucidated.[29–31] So far, a limited number of different N^{ε}-modifications at the lysine side chain have been reported to inhibit sirtuins.[27,32–36]

We recently reported a series of N^{ε}-thioacetyl lysine containing tri-, tetra- and pentapeptides, based on alfa-tubulin and human tumor suppressor protein p53 sequences, and demonstrated the effect of peptide length and selection of the side chains for the SIRT1 and SIRT2 inhibitory activity.[33] It transpired that the +2 residue (the second residue towards the C-terminus calculating from

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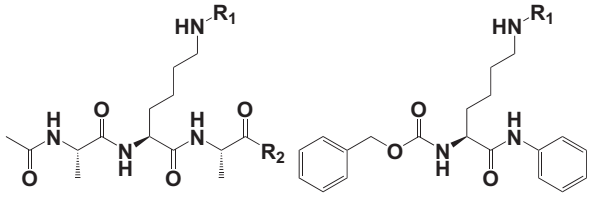


Figure 1. Ac-Ala-Lys(N^{ε}-R1)-Ala-R2 and Cbz-Lys(N^{ε}-R1)-NH-Ph were used as backbones for novel N^{ε}-modified SIRT1 and SIRT2 inhibitors.

the N^{ε}-thioacetyl lysine) was not crucial for the binding of the studied peptides to SIRT1 or SIRT2. However, the peptides lacking the −2 residue (the second residue towards the N-terminus calculating from the N^{ε}-thioacetyl lysine) could bind to SIRT1 but had significantly decreased affinity towards SIRT2. Furthermore it was shown that alanine replacements at the −1 and +1 positions of the pentapeptide sequences were allowed by SIRT1 and SIRT2.

Based on the above findings, we here report a simple and easy-to-synthesize Ac-Ala-Lys-Ala sequence which, with an appropriate N^{ε}-modification, has potential to interact with SIRT1 and SIRT2. In order to develop new SIRT1 and SIRT2 inhibitors, a series of different N^{ε}-modifications was studied on the novel backbone and Cbz-Lys-NH-Ph backbone, previously reported by Suzuki et al.[32,35] (Fig. 1). Altogether, we report 36 novel compounds (Table 1), some of them being very potent ones, and discuss their structure–activity relationships.

2. Chemistry

Compounds with Ac-Ala-Lys-Ala backbone were synthesized as shown in Scheme 1. If an N^{ε}-modification was introduced before solid phase peptide synthesis (SPPS), the carboxyl terminal of N^{α}-Fmoc-Lys-OH·HCl was first protected as a methyl ester, then the N^{ε}-site was modified, and in the end the carboxyl terminal was deprotected (a–e in Scheme 1). Alternatively, the N^{ε}-site of N^{α}-Fmoc-Lys-OH·HCl was directly modified (f in Scheme 1). Treatment of compounds **2** and **8** with Woollin's and Lawessoń reagents, respectively gave compounds **3** and **9** (c and d in Scheme 1). If an N^{ε}-modification was introduced after SPPS, the amino terminal of N^{ε}-Cbz-Lys-OH was protected with an Fmoc group prior to SPPS, the Cbz group was removed by hydrogenation on palladium after

Table 1
SIRT1 and SIRT2 activity assay results (95% confidence intervals for IC_{50} given in parentheses)

Compd	R1	R2	200 μM ± SD[a] (%)		IC_{50}[b] (μM)	
			SIRT1	SIRT2	SIRT1	SIRT2
Ac-Ala-Lys(N^{ε}-R1)-Ala-R2						
48	$CSCH_3$	OH	100 ± 1	96 ± 1	0.24 (0.20–0.28)	9.8 (8.7–11)
49	$CSeCH_3$	OH	100 ± 1	97 ± 2	0.37 (0.28–0.50)	7.2 (5.2–9.9)
50	$COCH_3$	OH	62 ± 1	42 ± 1	122 (105–142)	223 (152–328)
51	$COCH_3$	OCH_3	39 ± 3	57 ± 1	268 (160–448)	180 (122–266)
52	$COCH_3$	$NHCH_3$	66 ± 4	68 ± 1	88 (77–101)	60 (47–77)
53	$COC(CH_3)_3$	OCH_3	53 ± 2	52 ± 8	223 (151–330)	338 (270–422)
54	$COC(CH_3)_3$	$NHCH_3$	82 ± 1	77 ± 1	40 (36–45)	66 (59–74)
55	(*E*)-$COCH{=}CHCH_3$	OCH_3	20 ± 2	9 ± 1	nd[c]	nd
56	(*E*)-$COC(CH_3){=}CHCH_3$	OCH_3	39 ± 1	39 ± 3	nd	nd
57	$COCH{=}C(CH_3)_2$	OCH_3	78 ± 1	74 ± 1	46 (38–56)	69 (49–97)
58	$COCH{=}C(CH_3)_2$	$NHCH_3$	96 ± 1	95 ± 1	6.1 (5.0–7.4)	23 (18–30)
59	$COCH{=}C(CH_3)_2$	OH	95 ± 1	52 ± 3	11 (10–13)	77 (59–101)
60	(*Z*)-$COCH{=}C(CH_3CF_3)$	OH	33 ± 4	20 ± 3	nd	nd
61	$COCH_2CH(CH_3)_2$	OH	94 ± 1	76 ± 2	12 (10–14)	30 (23–40)
62	$CSCH_2CH(CH_3)_2$	OH	100 ± 3	99 ± 3	0.70 (0.53–0.91)	3.6 (2.5–5.2)
64	CO-(cyclohex-1-ene)	OCH_3	33 ± 3	54 ± 4	nd	129 (90–184)
65	COPh	OCH_3	12 ± 1	14 ± 2	nd	nd
66	CO(pyridin-3-yl)	OCH_3	9 ± 2	2 ± 3	nd	nd
67	CO(pyridin-2-yl)	OCH_3	4 ± 4	0 ± 4	nd	nd
68	$COCH_2$(pyridin-3-yl)	OCH_3	10 ± 4	0 ± 1	nd	nd
69	$COCH_2$(pyridin-2-yl)	OCH_3	26 ± 2	21 ± 2	nd	nd
70	CO(1*H*-pyrrol-2-yl)	$NHCH_3$	36 ± 2	26 ± 2	nd	nd
71	CO(1-CH_3-1*H*-pyrrol-2-yl)	$NHCH_3$	23 ± 2	41 ± 4	nd	nd
72	CO-(*p*-$N(CH_3)_2$)Ph)	$NHCH_3$	39 ± 2	34 ± 2	nd	nd
73	$COCH_2Cl$	OCH_3	19 ± 4	8 ± 2	nd	nd
74	$COCH_2Br$	OCH_3	48 ± 1	16 ± 3	1767 (921–3392)	nd
75	$COOCH_3$	OCH_3	8 ± 2	0 ± 1	nd	nd
76	COOBz	OCH_3	11 ± 2	3 ± 4	nd	nd
78	$CON(CH_3)_2$	OCH_3	24 ± 1	13 ± 2	nd	nd
79	SO_2CH_3	OCH_3	17 ± 2	8 ± 1	nd	nd
80	SO_2CF_3	OCH_3	15 ± 4	4 ± 3	nd	nd
81	SO_2(*p*-CH_3Ph)	OCH_3	1 ± 6	0 ± 3	nd	nd
82	SO_2(*p*-NO_2Ph)	OCH_3	0 ± 1	5 ± 3	nd	nd
Cbz-Lys(N^{ε}-R1)-NH-Ph						
84[35]	$CSCH_3$	—	95 ± 1	94 ± 1	9.9 (8.3–12)	15 (13–17)
89	$CSeCH_3$	—	98 ± 1	99 ± 1	1.8 (1.2–2.8)	2.7 (2.2–3.3)
86	$PS(CH_3)_2$	—	10 ± 4	27 ± 2	nd	nd
83	$COOC(CH_3)_3$	—	6 ± 1	13 ± 3	nd	nd
90[27,28,33]	H-His-Lys-Lys(thioAc)-Leu-Met-OH		100 ± 1	98 ± 1	0.33 (0.27–0.40)	6.4 (5.3–7.7)
91[33]	H-Lys-Lys(thioAc)-Leu-OH		100 ± 1	47 ± 1	0.57 (0.38–0.84)	151 (104–218)

[a] Inhibition-% at 200 μM ± standard deviation with the Fluor de Lys based assay (n = 2–3).
[b] IC_{50} was determined with the Fluor de Lys based assay if inhibition-% at 200 μM was over 50% (repeated at least three times).
[c] Not determined.

SPPS, followed by N^{ε}-derivatization by coupling chemistry (g, i, j in Scheme 1). SPPS was performed on Wang-resin with Fmoc strategy (h in Scheme 1), using TBTU and DIPEA in the coupling phase and piperidine for the deprotection. Cleavage from the resin was achieved either with 1 M NaOH (aq) in dioxane or with a mixture of MeOH, DIPEA and DMF or with 8 M methylamine in EtOH giving a free carboxylic group or a methyl ester or a methyl amide at the C-terminus, respectively. Reference compounds **90** and **91** were synthesized as described previously.[33]

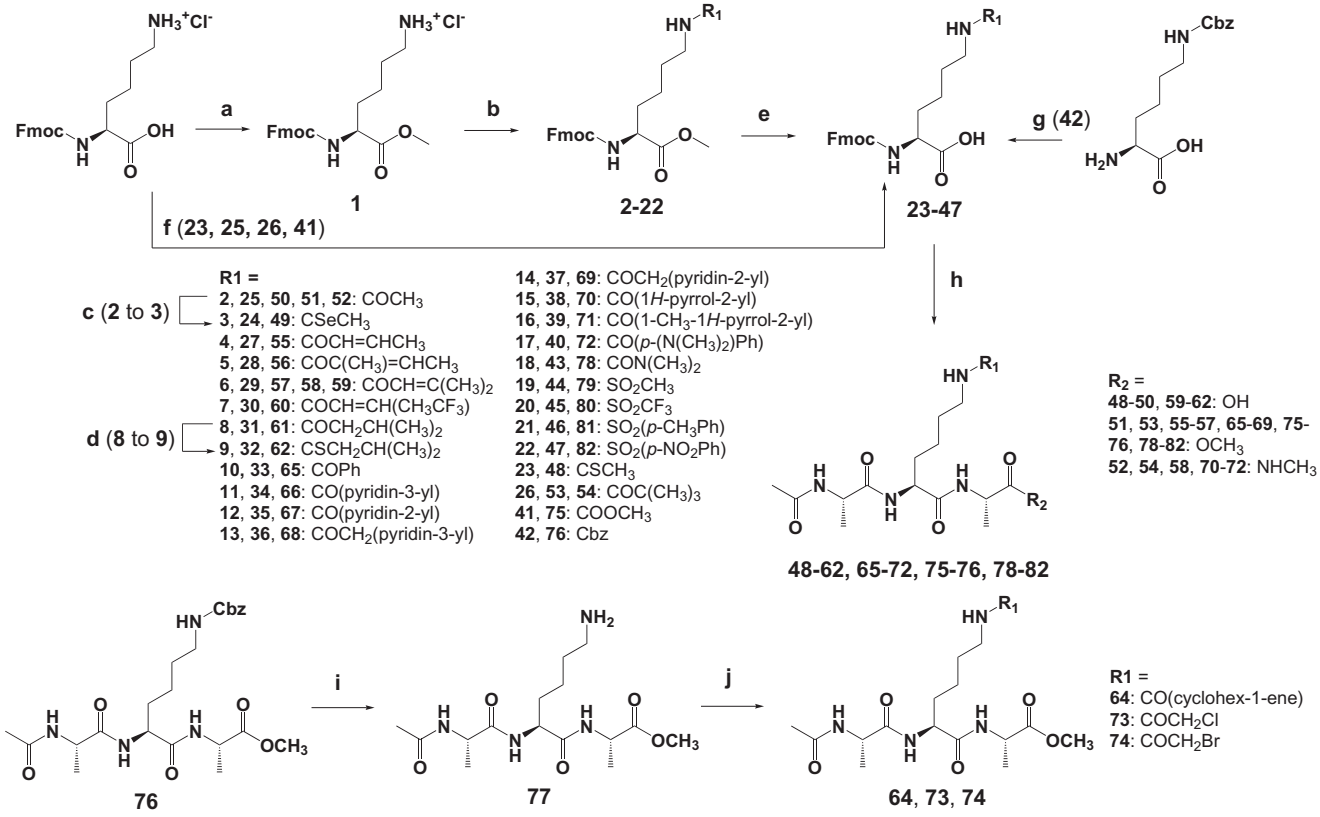
Compounds with Cbz-Lys-NH-Ph backbone were synthesized as shown in Scheme 2. N^{α}-Cbz-N^{ε}-Boc-Lys-OH was coupled with aniline using EDC·HCl and HOBt to give compound **83**. It was selectively Boc-deprotected in trifluoroacetic acid and the free N^{ε}-site was reacted with ethyl dithioacetate to gain compound **84**. To gain compound **86**, the Boc protection group of compound **83** was removed in 3.5 M HCl in EtOAc and the free N^{ε}-site was then coupled with dimethyl thiophosphinic chloride. Synthesis of compound **89** started by protection of N^{α}-Cbz-Lys-OH as a methyl ester followed by N^{ε}-acetylation using acetic anhydride to get **87**. Woollin's reagent was then used to selectively change acetamide to selenoacetamide (**88**), while the carbonyl oxygen in the methyl ester stays unchanged. The methyl ester was hydrolyzed in alkaline conditions and the carboxylic group was coupled with aniline to gain product **89**.

3. Results and discussion

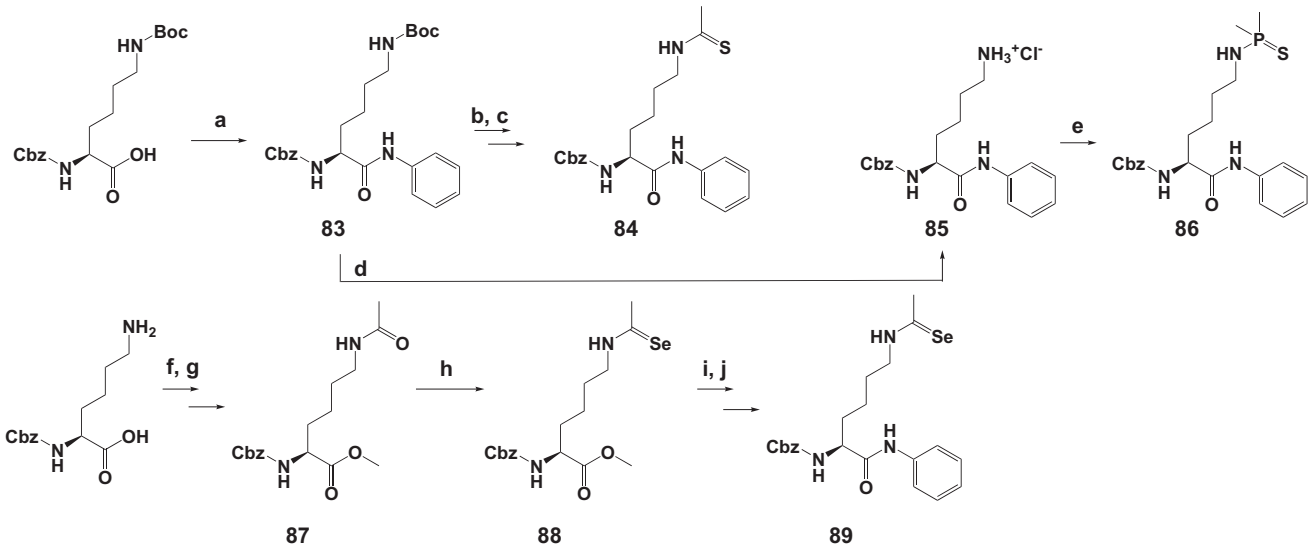
Our previous observations concerning the importance of −2 residue for SIRT2 binding and, that alanine replacements at the −1 and +1 positions are allowed by SIRT1 and SIRT2, encouraged us to synthesize tripeptide **48**, with the sequence Ac-Ala-Lys(thioAc)-Ala-OH. The resulting inhibitory activities were well in agreement with the previous observations and show that the −2 residue as such is not crucial for the recognition by SIRT2 because a plain acetyl group is sufficient to maintain affinity. Against SIRT1, compound **48** (IC_{50} = 0.24 μM) was equipotent with p53-based pentapeptide **90** (IC_{50} = 0.31 μM). Against SIRT2, compound **48** (IC_{50} = 9.8 μM) was only slightly less potent than pentapeptide **90** (IC_{50} = 6.3 μM) but 15–18 times more potent than the earlier reported tripeptides H-Asp-Lys(thioAc)-Thr-OH (IC_{50} = 175 μM) and H-Lys-Lys(thioAc)-Leu-OH (**91**) (IC_{50} = 151 μM).[33] For this reason, Ac-Ala-Lys-Ala was chosen as a backbone for the acetyl analogues presented in this study.

Recently, Suzuki et al. presented a non-peptide sirtuin inhibitor Cbz-Lys(thioAc)-NH-Ph (**84**).[35] Although non-peptidic compounds possess beneficial properties, like better cell permeability, over peptidic ones, we wanted to compare these different types of structures in terms of affinity. Against SIRT1, the inhibitory activity of compound **84** was significantly lower in comparison to the peptidic structures **48** and **90**. However, against SIRT2 the inhibitory activity of compound **84** was only slightly decreased. Previously, we have shown that inhibition of SIRT2 requires longer sequences than inhibition of SIRT1.[33] Now obtained result indicates that it may be rather the size of the substrate than the peptidic nature, which is important.

N^{ε}-Thioacetyl lysine compounds have been reported to exploit the deacetylation mechanism and form a stalled intermediate, 1′-*S*-alkylamidate instead of 1′-*O*-alkylamidate in the normal deacet-



Scheme 1. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C to reflux, 45 min; (b) acid chloride or anhydride or TBTU activated carboxylic acid, pyridine or pyridine/DMF (1:1), 0 °C to rt, 2–16 h; (c) Woollin's reagent, toluene/benzene (1:1), 70 °C, overnight; (d) Lawessońs reagent toluene, 70 °C, overnight; (e) NaOH, 0.8 M $CaCl_2$ in *i*-PrOH/$H_2$0 (7:3), rt, 4–16 h; (f) **23**: ethyl dithioacetate, EtOH/10% (w/v) Na_2CO_3 (aq) (3:1), rt, overnight; **25** and **26**: appropriate anhydride, pyridine, 0 °C to rt, overnight; **41**: methyl chloroformate, $NaHCO_3$, dioxane/H_2O (1:1), 0 °C to rt, 3 h; (g) 9-fluorenylmethyl chloroformate, 0 °C to rt, 4 h; (h) solid phase peptide synthesis with Fmoc strategy: (coupling) appropriate carboxylic acid, TBTU, DIPEA, DFM, rt, 1 h; (Fmoc-deprotection) 20% piperidine/DMF; (cleavage) R_2 = OH: NaOH (1 M)/dioxane (2:6), rt, 1 h; R_2 = OCH_3: DIPEA/MeOH/DMF (1:5:5), heat at reflux, overnight; R_2 = $NHCH_3$: 8 M methylamine in ethanol, rt, 2 h; (i) 10% Pd on charcoal, MeOH, rt, 2 h; (j) **64** and **73**: TBTU activated carboxylic acid, DMF/pyridine (1:1), 0 °C to rt, 3 h; **74**: bromoacetyl bromide, DIPEA, 1 h at 0 °C, overnight at rt.



Scheme 2. Reagents and conditions (a) EDC·HCl, HOBt·H_2O, aniline, CH_3CN, rt, overnight; (b) TFA/EtOAC (1:1), rt, 2 h; (c) ethyl dithioacetate, Na_2CO_3, EtOH/H_2O (6:1), rt, overnight; (d) 3.5 M HCl in EtOAc, 0 °C to rt, overnight; (e) dimethylthiophosphinic chloride, DIPEA, pyridine, 0 °C to rt, overnight; (f) $SOCl_2$, MeOH, 0 °C to reflux, 45 min; (g) acetic anhydride, pyridine, 0 °C to rt, overnight; (h) Woollin's reagent, toluene, 70 °C overnight; (i) LiOH, MeOH/H_2O (3:1), 0 °C to rt, 2.5 h; (j) TBTU, aniline, pyridine/DMF (1:1), 0 °C to rt, 3 h.

ylation reaction.[12,27,29,30,34] Therefore, we synthesized N^{ε}-acetylated compounds **50–52** as reference compounds. The free carboxyl terminus of compound **50** was protected as a methyl ester (**51**), and methyl amide (**52**). All three compounds inhibited SIRT1 and SIRT2 in vitro with little or moderate differences in their inhibitory activities, compound **52** with the methyl amide being the most favorable modification (IC_{50} = 88 μM for SIRT1 and 60 μM for SIRT2). While N^{ε}-thioacetyl compound **48** slows the reaction kinetics, compounds **50–52** undergo a normal deacetylation reaction and can be considered as competitive substrates in the assay.

The excellent potency of N^{ε}-thioacetyl lysine possessing compounds, first reported by Fatkins et al.,[27] prompted us to synthesize the N^{ε}-selenoacetylated derivative **49**. The changes in the atomic radius and electronegativity are smaller between sulfur and selenium than between oxygen and sulfur. N^{ε}-Thioacetylation (**48**) and N^{ε}-selenoacetylation (**49**) gave almost equipotent inhibitory activities for SIRT1 and SIRT2. Encouraged by this result, we made N^{ε}-selenoacetylation on the Cbz-Lys-NH-Ph backbone also. Here, the change from N^{ε}-thioacetylation (**84**) to N^{ε}-selenoacetylation (**89**) increased the inhibitory activity 5.5-fold for both SIRT1 and SIRT2. Compound **89** is one of the most potent reported SIRT2 inhibitors (IC_{50} = 2.7 μM). It is likely, that N^{ε}-selenoacetyl lysine forms a stalled intermediate similar to N^{ε}-thioacetyl lysine.

In addition to the N^{ε}-thioacetylated and N^{ε}-selenoacetylated derivatives, several compounds were synthesized and tested to study different functionalities as a substitute for the N^{ε}-acetyl group and inhibit SIRT1 and SIRT2. In comparison to N^{ε}-acetylated reference compound **51**, carbamates **75** and **76**, dimethylurea **78**, and sulfonamides **79–82** were all significantly weaker inhibitors of SIRT1 and SIRT2 or did not inhibit them at all. Also N^{ε}-dimethylphosphorothioylated derivative **86** and N^{ε}-Boc protected compound **83** failed to show any significant inhibitory activity. These modifications are therefore considered to be mechanistically inactive.

The possible size restriction of the binding cavity was first studied with compounds **53** and **54**, which introduce a N^{ε}-pivaloyl group. Compound **53** showed equipotent SIRT1 inhibitory activity with the corresponding N^{ε}-acetylated compound **51**, but slightly decreased SIRT2 inhibition. However, compound **54** with methylamide at the C-terminus was two times more potent against SIRT1 (IC_{50} = 40 μM) and equipotent against SIRT2 (IC_{50} = 66 μM) in comparison to N^{ε}-acetylated compound **52**. These results suggest that the binding cavity accepts also larger moieties than the N^{ε}-acetyl group.

Compounds **55–57** and **64** were synthesized to study different N^{ε}-α,β-unsaturated carbonyl groups. (*E*)-N^{ε}-3-Methylacryl derivate **55** and (*E*)-N^{ε}-2,3-dimethylacryl derivate **56** inhibited only weakly if at all SIRT1 and SIRT2. The bulkier N^{ε}-cyclohex-1-enecarbonyl group (**64**) gave SIRT2 inhibition which was comparable to that caused by the N^{ε}-acetyl group (**51**). Surprisingly, the change to the N^{ε}-3,3-dimethylacryl group (**57**) increased inhibitory activity for both enzymes (IC_{50} = 46 μM for SIRT1 and 69 μM for SIRT2) in comparison to N^{ε}-acetylated compound **51**. There seems to be space for bulkier groups but the shape of a group has crucial effect on the inhibitory activity. To confirm this result, two more compounds with the N^{ε}-3,3-dimethylacryl group but with different modifications at the C-terminus were synthesized (**58** and **59**). They both were superior to their N^{ε}-acetylated reference compounds **52** and **50**, respectively. In agreement with the previous results, the most potent SIRT1 (IC_{50} = 6.1 μM) and SIRT2 (IC_{50} = 23 μM) inhibition was achieved with compound **58**, with the methyl amide at the C-terminus, while the free carboxylic group (**59**) was slightly preferred over the methyl ester (**57**) by SIRT1.

In the N^{ε}-3,3-dimethylacryl group, the carbonyl function is conjugated with a double bond which may increase its nucleophilicity. In order to study if this electron donating property is important for the inhibitory activity, we synthesized (*Z*)-N^{ε}-3-methyl-3-trifluoromethylacryl derivative **60** with electron withdrawing properties. It had significantly decreased activity compared to compound **59**. We also prepared compounds **73** and **74** with the electron withdrawing groups attached on the N^{ε}-acetyl group and they also showed decreased activity compared to the reference compound **51**. It seems that electron withdrawing groups near the nucleophilic oxygen abolish the inhibitory activity. Next we removed the acrylic double bond and synthesized N^{ε}-isovaleryl derivative **61**, with decreased nucleophilicity and increased flexibility. Compared to compound **59**, compound **61** was equipotent SIRT1 inhibitor (IC_{50} = 12 μM) and over two times more potent SIRT2 inhibitor (IC_{50} = 30 μM). The good inhibitory activity of compound **61** indi-

cates that, although electron distribution may play some role, the potency of compound **59** likely relies on complementary binding and lipophilic interactions. Next we wanted to see if the beneficial effects of a bulky lipophilic group and a thiocarbonyl group could be combined to the same molecule and synthesized N^{ε}-isothiovaleryl derivative **62**. It was clearly more potent SIRT1 (IC_{50} = 0.70 μM) and SIRT2 (IC_{50} = 3.6 μM) inhibitor than N^{ε}-isovaleryl derivative **61**. Compared to N^{ε}-thioacetyl derivative **48**, SIRT1 inhibition was slightly decreased and SIRT2 inhibition slightly increased.

We studied also a series of bulky but flat five- and six-membered aromatic substituents at the N^{ε}-site (**65–72**). Compared to the aliphatic substituents, the aromatic structures differ in their electronic or steric properties in the the acetyl binding pocket. Compounds **65–67** and **70–72** have an aromatic ring directly attached to the carbonyl carbon and all of them failed to inhibit SIRT1 or SIRT2. Introduction of a methylene spacer between the carbonyl and aromatic groups (compounds **68** and **69**) or introduction of an electron donating substituent (**70–72**) didn't improve the inhibitory activity. Although nicotinamide is able to inhibit sirtuins by attacking the 1′-*O*-alkylimidate intermediate to regenerate NAD^+,[18,19,30] pyridyl derivatives **66–69** did not show any significant improvement in inhibitory activity compared to phenyl derivative **65**.

4. Conclusions

SIRT2 inhibitory activity of N^{ε}-thioacetylated tripeptides was increased one order of magnitude by simple acetylation of the *N*-terminus. This finding was combined with our previous observation that both SIRT1 and SIRT2 accept L-alanines at the positions surrounding N^{ε}-thioacetyllysine and, an Ac-Ala-Lys-Ala sequence was developed. The sequence is easily applicable for screening of different N^{ε}-modifications for SIRT1 and SIRT2 inhibition, it is easy to synthesize by solid phase peptide synthesis and, apart from the lysine, it does not have reactive side chains. It can be cleaved from resin as a free carboxylic acid, a methyl ester or a methyl amide and can be purified on silica.

Several different N^{ε}-modifications were studied with two different backbones. N^{ε}-Selenoacetylation gave equipotent or improved SIRT1 and SIRT2 inhibition compared to N^{ε}-thioacetylation. Although the applications of seleno-compounds are limited to research purposes, Cbz-Lys(selenoAc)-NH-Ph (**89**) was the most potent SIRT2 inhibitor (2.7 μM) found in this study and is among the most potent reported SIRT2 inhibitors. The studied bulky N^{ε}-alkanoyl and N^{ε}-aroyl groups showed that both SIRT1 and SIRT2 can accept rather large groups at the active site. However, they seem to be sensitive to even small changes in the electronic and steric properties of the N^{ε}-modification (see series **51–54**, **55–57**, **59–61** and **64–66**). N^{ε}-3,3-Dimethylacryl (**57–59**) and N^{ε}-isovaleryl (**61**) were the best aliphatic moieties found in this study. Further modification gave N^{ε}-isothiovaleryl derivative **62** which had slightly decreased SIRT1 but slightly increased SIRT2 inhibition when compared to N^{ε}-thioacetylated compound **48**.

5. Experimental section

5.1. General

All reagents and solvents were commercial high purity quality. Purification of the products was performed by CombiFlash® column chromatography on silica column or by crystallization. NMR spectra were recorded on a Bruker Avance 500 (Bruker Biospin, Switzerland) 500.1 MHz for ^1H and 125.8 MHz for ^{13}C. The chemical shifts are expressed in ppm relative to the shift of used solvent as an internal standard (^1H NMR: DMSO at 2.50 ppm, CH_3OH at 3.31 ppm and $CHCl_3$ at 7.26 ppm. ^{13}C NMR: $(CD_3)_2SO$ at 39.52 ppm, CD_3OD at 49.00 ppm and $CDCl_3$ at 77.16 ppm). Positive ion mass spectra (ESI-MS) were acquired with an LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electro spray ionization source. Combustion analysis for CHN was measured on Thermo Quest CE Instruments EA 1110 CHNS–O elemental analyzer. The purity of the compounds **60** and **72** was determined using Agilent 1100 HPLC (Agilent Technologies Inc., Waldbronn, Karlsruhe, Germany) with diode array detection, using reversed phase column (Zorbax Eclipse XDB C 18, 4.6 × 50 mm, 1.8 μm, Agilent Technologies, Palo Alto, CA, USA), 5% Solvent B (0.05% AcOH in CH_3CN) in solvent A (0.05% AcOH in H_2O) for 5 min, then linear gradient 5–80% B in A in 15 min with the flow rate 1 mL/min.

5.2. Manual solid phase peptide synthesis (SPPS)

Wang resin (polymer-bound *p*-alkoxy-benzyl alcohol) was used as solid support for the peptide synthesis of compounds **48–62**, **65–72**, **75**, **76** and **78–82**. Solid phase synthesis was performed in a 10 mL syringe equipped with a frit. Fmoc-Ala-Wang resin (loading 0.4–0.8 mmol/g) was swelled for 1 h in 3 mL of DMF. In the deprotection phase, the N^{α}-Fmoc protection group was removed with 5 mL of 20% (V/V) piperidine in DMF for 15 min and the resin was rinsed five times with DMF. In the coupling phase, the following N^{α}-Fmoc-amino acid or acetic acid (2–4 equiv) was preactivated (1–3 min) with the coupling reagent TBTU (2–4 equiv) and DIPEA (5–10 equiv) in 3–5 mL of DMF. This solution was added on the resin and the syringe was shaken at rt for 60 min. Then the resin was rinsed five times with DMF. The cycle of Fmoc-deprotection and coupling was repeated until the desired resin-bound peptide was completed. Before cleavage from the resin, the resin was washed once with AcOH, five times with DCM, and once with MeOH to remove the excess solvents and then dried under vacuum.

5.3. Cleavage of peptide-resin as carboxylic acid

The dried peptide-resin was pre-swelled in dioxane for 15 min. The excess of dioxane was removed and 8 mL of cool (0 °C) cleavage mixture of 1 M NaOH (aq)/dioxane (2 mL and 6 mL) was added on the peptide-resin. The reaction mixture was shaken 1 h at rt. The mixture was filtrated, filtrate was collected and the resin was washed with H_2O/dioxane (1:3), dioxane and H_2O. The filtrate was neutralized with 3 M HCl (aq) and the solvents were evaporated. Acetone was added on the residue and the mixture was filtrated to remove inorganic salts. The solvent of the filtrate was evaporated under reduced pressure to yield the crude product that was purified by column chromatography or by crystallization.

5.4. Cleavage of peptide-resin as methyl ester

The dry peptide-resin was placed in a flask. Cleavage mixture DIPEA/MeOH/DMF (1:5:5, 11–22 mL) was added and the reaction mixture was refluxed under argon atmosphere overnight. The mixture was filtrated, filtrate was collected and the resin was rinsed five times with MeOH/DMF (1:1). Solvent was evaporated from the combined filtrates under reduced pressure to yield the crude product that was purified by column chromatography or by crystallization.

5.5. Cleavage of peptide-resin as methyl amide

5 mL of 8 M methylamine in EtOH was added to the dry peptide-resin and the mixture was stirred for 2 h at rt. The resulting white precipitate was dissolved in MeOH and the resin beads were filtrated. The solvent of the filtrate was evaporated under reduced

pressure to yield the crude product that was purified by column chromatography or by crystallization.

5.6. Analytical data for compounds 48–76, 78–84, 86 and 89

5.6.1. (*S*)-2-((*S*)-2-((*S*)-2-Acetamidopropanamido)-6-ethanethioamidohexanamido)propanoic acid (48)

^{1}H NMR (CD_3OD): δ = 1.35 (d, ^3J = 7.2 Hz, 3H), 1.40 (d, ^3J = 7.3 Hz, 3H), 1.43–1.52 (m, 2H), 1.60–1.93 (m, 4H), 1.98 (s, 3H), 2.45 (s, 3H), 3.51–3.63 (m, 2H), 4.26–4.40 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.82, 17.86, 22.40, 24.10, 28.21, 32.81, 33.12, 46.97, 49.67, 50.62, 54.21, 173.26, 173.68, 175.22, 176.27 (br), 201.52. ESI-MS (*m/z*): 389.24 $[M+H]^+$. Anal. ($C_{16}H_{28}N_4O_5S$·1AcOH) C, H, N.

5.6.2. (*S*)-2-((*S*)-2-((*S*)-2-Acetamidopropanamido)-6-ethaneselenoamidohexanamido)propanoic acid (49)

^{1}H NMR (CD_3OD): δ = 1.34 (d, ^3J = 7.2 Hz, 3H), 1.40 (d, ^3J = 7.3 Hz, 3H), 1.44–1.54 (m, 2H), 1.64–1.94 (m, 4H), 1.98 (s, 3H), 2.54 (s, 3H), 3.58–3.69 (m, 2H), 4.26–4.43 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.62, 17.83, 22.39, 24.03, 27.99, 32.83, 37.18, 50.52, 50.62, 54.13, 173.25, 173.76, 175.20, 175.79, 204.95. ESI-MS (*m/z*): 437.19 $[M+H]^+$. Anal. ($C_{16}H_{28}N_4O_5Se$·0.2H_2O·0.8AcOH) C, H, N.

5.6.3. (*S*)-2-((*S*)-6-Acetamido-2-((*S*)-2-acetamidopropanamido) hexanamido)propanoic acid (50)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.17 (d, ^3J = 7.1 Hz, 3H), 1.26 (d, ^3J = 7.3 Hz, 3H), 1.19–1.41 (m, 4H), 1.42–1.72 (m, 2H), 1.78 (s, 3H), 1,83 (s, 3H), 2.90–3.02 (m, 2H), 4.09–4.29 (m, 3H), 7.68–7.78 (m, 1H), 7.78–7.86 (m, 1H), 7.96–8.05 (m, 1H), 8.05–8.11 (m, 1H), 12.49 (br, ^{1}H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.06, 18.04, 22.48, 22.56, 22.61, 28.81, 31.73, 38.41, 47.43, 48.23, 51.95, 168.93, 169.15, 171.27, 172.22, 173.95. ESI-MS (*m/z*): 373.1 $[M+H]^+$. Anal. ($C_{16}H_{28}N_4O_6$·0.7H_2O) C, H, N.

5.6.4. (*S*)-Methyl 2-((*S*)-6-acetamido-2-((*S*)-2-acetamidopropanamido)hexanamido)propanoate (51)

^{1}H NMR (CD_3OD): δ = 1.33 (d, ^3J = 7.2 Hz, 3H), 1.39 (d, ^3J = 7.3 Hz, 3H), 1.38–1.57 (m, 4H), 1.62–1.88 (m, 2H), 1.93 (s, 3H), 1.97 (s, 3H), 3.11–3.22 (m, 2H), 3.71 (s, 3H), 4.26–4.43 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.28, 17.84, 22.39, 22.58, 23.93, 29.88, 32.81, 40.21, 49.43, 50.58, 52.73, 54.16, 173.23, 173.23, 173.95, 174.52, 175.15. ESI-MS (*m/z*): 387.2 $[M+H]^+$. Anal. ($C_{17}H_{30}N_4O_6$) C, H, N.

5.6.5. (*S*)-6-Acetamido-2-((*S*)-2-acetamidopropanamido)-*N*-((*S*)-1-(methylamino)-1-oxopropan-2-yl)hexanamide (52)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.13–1.21 (m, 6H), 1.21–1.41 (m, 4H), 1.44–1.70 (m, 2H), 1.78 (s, 3H), 1.83 (s, 3H), 2.54–2.59 (m, 3H), 2.95–3.01 (m, 2H), 4.12–4.27 (m, 3H), 7.69–7.79 (m, 2H), 7.80–7.86 (m, 1H), 7.89–7.95 (m, 1H), 8.05–8.09 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.95, 18.30, 22.48, 22.61, 22.69, 25.55, 28.76, 31.32, 38.34, 48.12, 48.37, 52.49, 168.96, 169.28, 171.08, 172.40, 172.60. ESI-MS (*m/z*): 386.5 $[M+H]^+$. Anal. ($C_{17}H_{31}N_5O_5$·0.7H_2O·0.2hexane) C, H, N.

5.6.6. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-pivalamidohexanamido)propanoate (53)

^{1}H NMR (CD_3OD): δ = 1.17 (s, 9H), 1.34 (d, ^3J = 7.2 Hz, 3H), 1.39 (d, ^3J = 7.3 Hz, 3H), 1.35–1.57 (m, 4H), 1.62–1.89 (m, 2H), 1.97 (s, 3H), 3.12–3.22 (m, 2H), 3.71 (s, 3H), 4.27–4.43 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.30, 17.90, 22.40, 24.00, 27.89, 30.10, 32.85, 39.63, 40.37, 49.44, 50.57, 52.73, 54.25, 173.22, 173.99, 174.49, 175.14, 181.36. ESI-MS (*m/z*): 429.2 $[M+H]^+$, 451.3 $[M+Na]^+$. Anal. ($C_{20}H_{36}N_4O_6$·0.1H_2O) C, H, N.

5.6.7. (*S*)-2-((*S*)-2-Acetamidopropanamido)-*N*-((*S*)-1-(methylamino)-1-oxopropan-2-yl)-6-pivalamidohexanamide (54)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.07 (s, 9H), 1.13–1.30 (m, 8H), 1.31–1.42 (m, 2H), 1.44–1.71 (m, 2H),1.83 (s, 3H), 2.55–2.59 (m, 3H), 2.92–3.06 (m, 2H), 4.09–4.29 (m, 3H), 7.37–7.46 (m, 1H), 7.69–7.78 (m, 1H), 7.78–7.86 (m, 1H), 7.89–7.97 (m, 1H) 8.04–8.13 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 18.02, 18.34, 22.49, 22.69, 25.54, 27.47, 28.87, 31.37, 37.94, 38.61, 48.08, 48.33, 52.54, 169.21, 171.09, 172.34, 172.59, 177.17. ESI-MS (*m/z*): 428.3 $[M+H]^+$, 450.5 $[M+Na]^+$. Anal. ($C_{20}H_{37}N_5O_5$·0.2H_2O) C, H, N.

5.6.8. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-((*E*)-but-2-enamido)hexanamido)propanoate (55)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 7.1 Hz, 3H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.21–1.44 (m, 4H), 1.45–1.71 (m, 2H), 1.77 (d, ^3J = 6.7 Hz, 3H), 1.83 (s, 3H), 3.02–3.11 (m, 2H), 3.60 (s, 3H), 4.18–4.30 (m, 3H), 5.87 (d, ^3J = 15.4 Hz, 1H), 6.58 (dddd, ^3J = 15.4 Hz, 6.7 Hz, 1H), 7.76–7.89 (m, 2H), 7.99–8.08 (m, 1H), 8.20–8.30 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.79, 17.29, 18.04, 22.48, 22.57, 28.87, 31.72, 38.32, 47.52, 48.21, 51.82, 51.88, 126.02, 137.26, 164.74, 169.16, 171.45, 172.21, 172.92. ESI-MS (*m/z*): 413.2 $[M+H]^+$, 435.4 $[M+Na]^+$. Anal. ($C_{19}H_{32}N_4O_6$·0.1H_2O) C, H, N.

5.6.9. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-((*E*)-2-methylbut-2-enamido)hexanamido)propanoate (56)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 7.1 Hz, 3H), 1.27 (d, ^3J = 7.2 Hz, 3H), 1.21–1.45 (m, 4H), 1.45–1.71 (m, 2H), 1.67 (d, ^3J = 6.8 Hz, 3H), 1.72 (s, 3H), 1.82 (s, 3H), 3.02–3.11 (m, 2H), 3.60 (s, 3H), 4.18–4.30 (m, 3H), 6.28 (ddd, ^3J = 6.8 Hz, 1H), 7.64–7.73 (m, 1H), 7.78–7.87 (m, 1H), 7.98–8.08 (m, 1H), 8.20–8.30 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 12.34, 13.56, 16.78, 18.04, 22.47, 22.59, 28.91, 31.77, 38.78, 47.51, 48.18, 51.79, 51.94, 128.75, 132.04, 168.25, 169.10, 171.45, 172.19, 172.89. ESI-MS (*m/z*): 427.2 $[M+H]^+$, 449.4 $[M+Na]^+$. Anal. ($C_{20}H_{34}N_4O_6$·0.1H_2O) C, H, N.

5.6.10. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(3-methylbut-2-enamido)hexanamido)propanoate (57)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 6.9 Hz, 3H), 1.27 (d, ^3J = 7.2 Hz, 3H), 1.21–1.43 (m, 4H), 1.45–1.71 (m, 2H), 1.76 (s, 3H), 1.83 (s, 3H), 2.06 (s, 3H), 2.97–3.08 (m, 2H), 3.60 (s, 3H), 4.19–4.30 (m, 3H), 5.62 (s, 1H), 7.65–7.72 (m, 1H), 7.80–7.87 (m, 1H), 8.00–8.07 (m, 1H), 8.22–8.29 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.78, 18.03, 19.18, 22.47, 22.62, 26.72, 28.90, 31.76, 38.06, 47.51, 48.19, 51.81, 51.89, 119.22, 147.86, 165.85, 169.13, 171.45, 172.19, 172.91. ESI-MS (*m/z*): 427.2 $[M+H]^+$, 449.4 $[M+Na]^+$. Anal. ($C_{20}H_{34}N_4O_6$) C, H, N.

5.6.11. (*S*)-2-((*S*)-2-Acetamidopropanamido)-*N*-((*S*)-1-(methylamino)-1-oxopropan-2-yl)-6-(3-methylbut-2-enamido)hexanamide (58)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.13–1.21 (m, 6H), 1.21–1.31 (m, 2H), 1.31–1.42 (m, 2H), 1.44–1.71 (m, 2H), 1.76 (s, 3H), 1.83 (s, 3H), 2.06 (s, 3H), 2.54–2.62 (m, 3H), 2.96–3.08 (m, 2H), 4.11–4.29 (m, 3H), 5.62 (s, 1H), 7.65–7.72 (m, 1H), 7.72–7.78 (m, 1H), 7.79–7.87 (m, 1H), 7.88–7.96 (m, 1H) 8.04–8.12 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.97, 18.30, 19.19, 22.47, 22.77, 25.54, 26.73, 28.84, 31.38, 38.00, 48.09, 48.32, 52.46, 119.23, 147.87, 165.86, 169.23, 171.06, 172.38, 172.56. ESI-MS (*m/z*): 426.3 $[M+H]^+$, 448.4 $[M+Na]^+$. Anal. ($C_{20}H_{35}N_5O_5$·1H_2O·0.1hexane) C, H, N.

5.6.12. (*S*)-2-((*S*)-2-((*S*)-2-Acetamidopropanamido)-6-(3-methylbut-2-enamido)hexanamido)propanoic acid (59)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.17 (d, ^3J = 7.1 Hz, 3H), 1.26 (d, ^3J = 7.3 Hz, 3H), 1.20–1.42 (m, 4H), 1.44–1.71 (m, 2H), 1.76 (s,

3H), 1,83 (s, 3H), 2.06 (s, 3H), 2.96–3.07 (m, 2H), 4.11–4.31 (m, 3H), 5.62 (s, 1H), 7.64–7.71 (m, 1H), 7.80–7.87 (m, 1H), 8.01–8.06 (m, 1H), 8.06–8.11 (m, 1H), 12.51 (br, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.07, 18.05, 19.19, 22.47, 22.66, 26.74, 28.89, 31.76, 38.08, 47.45, 48.21, 51.96, 119.21, 147.88, 165.85, 169.13, 171.27, 172.22, 173.96. ESI-MS (*m/z*): 411.2 $[M+H]^+$. Anal. ($C_{19}H_{32}N_4O_6 \cdot 0.5H_2O \cdot 0.2$hexane) C, H, N.

5.6.13. (*S*)-2-((*S*)-2-((*S*)-2-Acetamidopropanamido)-6-((*Z*)-4,4,4-trifluoro-3-methylbut-2-enamido)hexanamido)propanoic acid (60)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 7.1 Hz, 3H), 1.25 (d, ^3J = 7.3 Hz, 3H), 1.21–1.34 (m, 2H), 1.35–1.45 (m, 2H), 1.45–1.72 (m, 2H), 1,82 (s, 3H), 2.16 (s, 3H), 3.04–3.14 (m, 2H), 4.09–4.29 (m, 3H), 6.47 (s, 1H), 7.84–7.91 (m, 1H), 8.01–8.11 (m, 2H), 8.35–8.43 (m, 1H), 12.41 (br, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 11.25, 17.12, 18.00, 22.43, 22.57, 28.44, 31.66, 38.44, 47.52, 48.24, 51.88, 123.77 (q, $^1J_{CF}$ 273.36 Hz), 134.37 (q, $^2J_{CF}$ 29.24 Hz), 125.44 (q, $^3J_{CF}$ 6.01 Hz), 163.34, 169.13, 171.18, 172.23, 173.92. ESI-MS (*m/z*): 465.18, 466.18, 467.18, 468.18 $[M-H]^-$. Anal. ($C_{19}H_{32}N_4O_6 \cdot 1.5$AcOH$\cdot 0.1$hexane) C, H, N. HPLC: t_R 9.31 min, area percent 97% at 260 nm.

5.6.14. (*S*)-2-((*S*)-2-((*S*)-2-Acetamidopropanamido)-6-(3-methylbutanamido)hexanamido)propanoic acid (61)

^{1}H NMR ($(CD_3)_2SO$): δ = 0.85 (d, ^3J = 6.1 Hz, 6H), 1.17 (d, ^3J = 7.1 Hz, 3H), 1.26 (d, ^3J = 7.3 Hz, 3H), 1.21–1.42 (m, 4H), 1.43–1.71 (m, 2H), 1.83 (s, 3H), 1.88–2.00 (m, 3H), 2.92–3.05 (m, 2H), 4.11–4.31 (m, 3H), 7.66–7.74 (m, 1H), 7.79–7.87 (m, 1H), 7.99–8.08 (m, 1H), 8.05–8.10 (m, 1H), 12.48 (br, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.07, 18.05, 22.29, 22.30, 22.47, 22.56, 25.45, 28.91, 31.69, 38.25, 44.75, 47.43, 48.20, 51.93, 169.08, 171.23, 171.24, 172.19, 173.93. ESI-MS (*m/z*): 413.4 $[M+H]^+$. Anal. ($C_{19}H_{34}N_4O_6 \cdot 0.5H_2O$) C, H, N.

5.6.15. (*S*)-2-((*S*)-2-((*S*)-2-Acetamidopropanamido)-6-(3-methylbutanethioamido)hexanamido)propanoic acid (62)

^{1}H NMR (CD_3OD): δ = 0.89–0.95 (m, 6H), 1.32–1.41 (m, 6H), 1.41–1.56 (m, 2H), 1.62–1.94 (m, 4H), 1.92 (s, 3H), 2.17–2.28 (m, 1H), 2.45 (d, ^3J = 7.4 Hz, 2H), 3.57–3.62 (m, 2H), 4.22–4.39 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.92, 18.32, 22.25, 22.28, 22.42, 24.26, 28.24, 30.28, 32.72, 46.63, 50.37, 50.65, 54.36, 56.02, 173.26, 173.44, 175.27, 177.37, 205.37. ESI-MS (*m/z*): 431.25 $[M+H]^+$, 453.30 $[M+Na]^+$. Anal. ($C_{19}H_{34}N_4O_5S \cdot 0.5H_2O \cdot 0.1$AcOH) C, H, N.

5.6.16. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(cyclohex-1-enecarboxamido)hexanamido)propanoate (64)

^{1}H NMR (CD_3OD): δ = 1.33 (d, ^3J = 7.2 Hz, 3H), 1.39 (d, ^3J = 7.4 Hz, 3H), 1.40–1.91 (m, 10H), 1.97 (s, 3H), 2.11–2.27 (m, 4H), 3.13–3.27 (m, 2H), 3.71 (s, 3H), 4.27–4.43 (m, 3H), 6.54–6.59 (m, 1H). ^{13}C NMR (CD_3OD): δ = 17.29, 17.87, 22.39, 22.72, 23.33, 24.03, 25.29, 26.35, 30.08, 32.82, 40.27, 49.45, 50.59, 52.72, 54.25, 134.38, 134.62, 171.69, 173.22, 173.99, 174.49, 175.14. ESI-MS (*m/z*): 453.21 $[M+H]^+$, 475.36 $[M+Na]^+$. Anal. ($C_{22}H_{36}N_4O_6$) C, H, N.

5.6.17. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-benzamidohexanamido)propanoate (65)

^{1}H NMR (CD_3OD): δ = 1.32 (d, ^3J = 7.2 Hz, 3H), 1.38 (d, ^3J = 7.3 Hz, 3H), 1.43–1.56 (m, 2H), 1.60–1.93 (m, 4H), 1.95 (s, 3H), 3.36–3.43 (m, 2H), 3.68 (s, 3H), 4.27–4.42 (m, 3H), 7.41–7.48 (m, 2H), 7.52 (t, ^3J = 7.4, 1H), 7.78–7.85 (m, 2H). ^{13}C NMR (CD_3OD): δ = 17.27, 17.83, 22.37, 24.07, 30.05, 32.88, 40.76, 49.45, 50.61, 52.71, 54.23, 128.29, 129.50, 132.53, 135.90, 170.30, 173.25, 173.98, 174.49, 175.16. ESI-MS (*m/z*): 449.2 $[M+H]^+$, 471.3 $[M+Na]^+$. Anal. ($C_{22}H_{32}N_4O_6 \cdot 0.1$hexane) C, H, N.

5.6.18. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(nicotinamido)hexanamido)propanoate (66)

^{1}H NMR (CD_3OD): δ = 1.32 (d, ^3J = 7.2 Hz, 3H), 1.38 (d, ^3J = 7.4 Hz, 3H), 1.44–1.56 (m, 2H), 1.60–1.93 (m, 4H), 1.95 (s, 3H), 3.36–3.48 (m, 2H), 3.68 (s, 3H), 4.25–4.42 (m, 3H), 7.49–7.57 (m, 1H), 8.20–8.28 (m, 1H), 8.63–8.71 (m, 1H), 8.94–9.01 (m, 1H). ^{13}C NMR (CD_3OD): δ = 17.26, 17.81, 22.37, 24.02, 29.91, 32.87, 40.84, 49.44, 50.62, 52.72, 54.17, 125.09, 132.21, 137.04, 149.15, 152.53, 167.79, 173.23, 173.95, 174.49, 175.16. ESI-MS (*m/z*): 450.2 $[M+H]^+$, 472.3 $[M+Na]^+$. Anal. ($C_{21}H_{31}N_5O_6 \cdot 0.1$hexane) C, H, N.

5.6.19. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(picolinamido)hexanamido)propanoate (67)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.15 (d, ^3J = 7.7 Hz, 3H), 1.26 (d, ^3J = 7.3 Hz, 3H), 1.22–1.37 (m, 2H), 1.47–1.73 (m, 4H), 1.82 (s, 3H), 3.24–3.34 (m, 2H), 3.59 (s, 3H), 4.20–4.29 (m, 3H), 7.56–7.61 (m, 1H), 7.79–7.84 (m, 1H), 7.95–8.05 (m, 3H), 8.21–8.26 (m, 1H), 8.60–8.65 (m, 1H), 8.72–8.78 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.76, 18.04, 22.46, 22.57, 28.95, 31.76, 38.69, 47.49, 48.15, 51.76, 51.92, 121.76, 126.34, 137.71, 148.29, 150.11, 163.64, 169.07, 171.40, 172.16, 172.86. ESI-MS (*m/z*): 450.2 $[M+H]^+$, 472.3 $[M+Na]^+$. Anal. ($C_{21}H_{31}N_5O_6 \cdot 0.2H_2O$) C, H, N.

5.6.20. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(2-(pyridin-3-yl)acetamido)hexanamido)propanoate (68)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.17 (d, ^3J = 7.0 Hz, 3H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.22–1.32 (m, 2H), 1.35–1.70 (m, 4H), 1.83 (s, 3H), 2.98–3.06 (m, 2H), 3.43 (s, 2H), 3.60 (s, 3H), 4.19–4.30 (m, 3H), 7.29–7.35 (m, 1H), 7.62–7.67 (m, 1H), 7.80–7.85 (m, 1H), 8.01–8.06 (m, 1H), 8.06–8.11 (m, 1H), 8.22–8.27 (m, 1H), 8.40–8.46 (m, 2H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.78, 18.02, 22.47, 22.49, 28.71, 31.66, 38.62, 39.23, 47.49, 48.20, 51.79, 51.81, 123.28, 132.14, 136.46, 147.54, 149.94, 169.13, 169.25, 171.43, 172.19, 172.89. ESI-MS (*m/z*): 464.2 $[M+H]^+$, 486.2 $[M+Na]^+$. Anal. ($C_{22}H_{33}N_5O_6 \cdot 0.6H_2O \cdot 0.1$hexane) C, H, N.

5.6.21. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(2-(pyridin-2-yl)acetamido)hexanamido)propanoate (69)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 7.0 Hz, 3H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.22–1.32 (m, 2H), 1.35–1.70 (m, 4H), 1.83 (s, 3H), 2.99–3.07 (m, 2H), 3.58 (s, 2H), 3.60 (s, 3H), 4.19–4.30 (m, 3H), 7.20–7.25 (m, 1H), 7.28–7.34 (m, 1H), 7.68–7.74 (m, 1H), 7.79–7.85 (m, 1H), 7.99–8.09 (m, 2H), 8.21–8.27 (m, 1H), 8.44–8.48 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.78, 18.03, 22.47, 22.52, 28.75, 31.68, 38.59, 44.91, 47.49, 48.19, 51.79, 51.84, 121.65, 123.67, 136.36, 148.81, 156.42, 168.87, 169.12, 171.43, 172.19, 172.89. ESI-MS (*m/z*): 464.3 $[M+H]^+$, 486.2 $[M+Na]^+$. Anal. ($C_{22}H_{33}N_5O_6 \cdot 0.2H_2O$) C, H, N.

5.6.22. *N*-((*S*)-5-((*S*)-2-Acetamidopropanamido)-6-((*S*)-1-(methylamino)-1-oxopropan-2-ylamino)-6-oxohexyl)-1*H*-pyrrole-2-carboxamide (70)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.13–1.22 (m, 6H), 1.22–1.38 (m, 2H), 1.39–1.74 (m, 4H), 1.83 (s, 3H), 2.55–2.59 (m, 3H), 3.12–3.22 (m, 2H), 4.12–4.29 (m, 3H), 6.02–6.09 (m, 1H), 6.69–6.77 (m, 1H), 6.78–6.85 (m, 1H),7.68–7.76 (m, 1H), 7.79–7.86 (m, 1H), 7.89–7.98 (m, 2H), 8.04–8.12 (m, 1H) 11.32–11.42 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.98, 18.29, 22.47, 22.85, 25.53, 29.17, 31.39, 38.33, 48.09, 48.32, 52.56, 108.39, 109.59, 120.99, 126.44, 160.55, 169.26, 171.09, 172.36, 172.61. ESI-MS (*m/z*): 437.3 $[M+H]^+$, 459.4 $[M+Na]^+$. Anal. ($C_{20}H_{32}N_6O_5 \cdot 0.1H_2O$) C, H, N.

5.6.23. *N*-((*S*)-5-((*S*)-2-Acetamidopropanamido)-6-((*S*)-1-(methylamino)-1-oxopropan-2-ylamino)-6-oxohexyl)-1-methyl-1*H*-pyrrole-2-carboxamide (71)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.13–1.22 (m, 6H), 1.22–1.38 (m, 2H), 1.39–1.74 (m, 4H), 1.83 (s, 3H), 2.55–2.59 (m, 3H), 3.08–3.19 (m, 2H), 3.81 (s, 3H), 4.12–4.29 (m, 3H), 5.94–6.01 (m, 1H), 6.69–6.76 (m, 1H), 6.83–6.88 (m, 1H), 7.69–7.76 (m, 1H), 7.80–7.87 (m, 1H), 7.89–7.98 (m, 2H), 8.05–8.11 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.99, 18.30, 22.47, 22.87, 25.54, 29.12, 31.44, 36.10, 38.29, 48.09, 48.33, 52.56, 106.45, 111.90, 125.74, 127.37, 161.24, 169.23, 171.09, 172.35, 172.59. ESI-MS (*m/z*): 451.3 $[M+H]^+$, 473.4 $[M+Na]^+$. Anal. ($C_{21}H_{34}N_6O_5\cdot0.9H_2O\cdot0.1$hexane) C, H, N.

5.6.24. *N*-((*S*)-5-((*S*)-2-Acetamidopropanamido)-6-((*S*)-1-(methylamino)-1-oxopropan-2-ylamino)-6-oxohexyl)-4-(dimethylamino)benzamide (72)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.13–1.22 (m, 6H), 1.22–1.38 (m, 2H), 1.39–1.74 (m, 4H), 1.83 (s, 3H), 2.54–2.59 (m, 3H), 2.95 (s, 6H), 3.14–3.23 (m, 2H), 4.12–4.29 (m, 3H), 6.68 (d, ^3J = 8.9 Hz, 2H), 7.70 (d, ^3J = 8.9 Hz, 2H), 7.69–7.75 (m, 1H), 7.81–7.87 (m, 1H), 7.92–7.97 (m, 1H), 8.05–8.12 (m, 2H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.99, 18.30, 22.49, 22.89, 25.55, 29.10, 31.42, 38.91, 39.75, 48.11, 48.35, 52.59, 110.75, 121.43, 128.45, 151.97, 165.99, 169.26, 171.12, 172.36, 172.61. ESI-MS (*m/z*): 491.3 $[M+H]^+$, 513.4 $[M+Na]^+$. Anal. ($C_{24}H_{38}N_6O_5\cdot1.5H_2O$) C, H, N. HPLC: t_R 9.31 min, area percent 82% at 260 nm.

5.6.25. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(2-chloroacetamido)hexanamido)propanoate (73)

^{1}H NMR (CD_3OD): δ = 1.33 (d, ^3J = 7.2 Hz, 3H), 1.39 (d, ^3J = 7.3 Hz, 3H), 1.41–1.49 (m, 2H), 1.51–1.62 (m, 2H), 1.63–1.89 (m, 2H), 1.98 (s, 3H), 3.18–3.29 (m, 2H), 3.72 (s, 3H), 4.04 (s, 2H), 4.26–4.45 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.28, 17.84, 22.39, 23.83, 29.77, 32.76, 40.51, 43.23, 49.62, 50.59, 52.74, 54.14, 169.29, 173.26, 173.94, 174.52, 175.16. ESI-MS (*m/z*): 421.07$[M+H]^+$, 443.24 $[M+Na]^+$. Anal. ($C_{17}H_{29}ClN_4O_6$) C, H, N.

5.6.26. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(2-bromoacetamido)hexanamido)propanoate (74)

^{1}H NMR (CD_3OD): δ = 1.34 (d, ^3J = 7.2 Hz, 3H), 1.39 (d, ^3J = 7.3 Hz, 3H), 1.41–1.49 (m, 2H), 1.50–1.61 (m, 2H), 1.62–1.89 (m, 2H), 1.98 (s, 3H), 3.15–3.27 (m, 2H), 3.71 (s, 3H), 3.83 (s, 2H), 4.26–4.45 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.29, 17.85, 22.41, 23.82, 28.89, 29.66, 32.75, 40.65, 49.44, 50.61, 52.75, 54.13, 169.48, 173.25, 173.94, 174.54, 175.17. ESI-MS (*m/z*): 465.13 $[M+H]^+$, 487.30 $[M+Na]^+$. Anal. ($C_{17}H_{29}BrN_4O_6$) C, H, N.

5.6.27. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(methoxycarbonylamino)hexanamido)propanoate (75)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 7.1 Hz, 3H), 1.20–1.32 (m, 2H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.33–1.42 (m, 2H), 1.46–1.68 (m, 2H), 1.83 (s, 3H), 2.92–2.95 (m, 2H), 3.50 (s, 3H), 3.60 (s, 3H), 4.20–4.28 (m, 3H), 7.01–7.09 (m, 1H), 7.79–7.85 (m, 1H), 7.99–8.05 (m, 1H), 8.21–8.27 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.79, 18.04, 22.38, 22.47, 29.18, 31.74, 40.18, 47.53, 48.20, 51.11, 51.81, 51.92, 169.16, 171.44, 172.21, 172.21, 172.91. ESI-MS (*m/z*): 425.3 $[M+Na]^+$. Anal. ($C_{17}H_{30}N_4O_7\cdot0.1H_2O$) C, H, N.

5.6.28. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(benzyloxycarbonylamino)hexanamido)propanoate (76)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.16 (d, ^3J = 7.1 Hz, 3H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.21–1.33 (m, 2H), 1.34–1.70 (m, 4H), 1.82 (s, 3H), 2.91- 3.02 (m, 2H), 3.60 (s, 3H), 4.18–4.30 (m, 3H), 4.99 (s, 2H), 7.17–7.25 (m, 1H), 7.27–7.41 (m, 5H), 7.79–7.86 (m, 1H), 7.99–8.06 (m, 1H), 8.21–8.28 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.79, 18.04, 22.39, 22.46, 29.14, 31.73, 40.23, 47.51, 48.19, 51.79, 51.90, 65.10, 127.72, 127.72, 128.33, 137.26, 156.05, 169.12, 171.42, 172.19, 172.90. ESI-MS (*m/z*): 479.1 $[M+H]^+$, 501.3 $[M+Na]^+$. Anal. ($C_{23}H_{34}N_4O_7$) C, H, N.

5.6.29. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(3,3-dimethylureido)hexanamido)propanoate (78)

^{1}H NMR (CD_3OD): δ = 1.33 (d, ^3J = 7.2 Hz, 3H), 1.39 (d, ^3J = 7.3 Hz, 3H), 1.37–1.56 (m, 4H), 1.63–1.87 (m, 2H), 1.97 (s, 3H), 2.88 (s, 6H), 3.14–3.17 (m, 2H), 3.71 (s, 3H), 4.30–4.41 (m, 3H). ^{13}C NMR (CD_3OD): δ = 17.29, 17.90, 22.40, 23.94, 31.01, 32.89, 36.45, 36.45, 41.52, 49.51, 50.54, 52.71, 54.33, 161.20, 173.21, 174.04, 174.48, 175.13. ESI-MS (*m/z*): 416.1 $[M+H]^+$. Anal. ($C_{18}H_{33}N_5O_6\cdot1.0H_20\cdot1.0$MeOH) C, H, N.

5.6.30. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(methylsulfonamido)hexanamido)propanoate (79)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.17 (d, ^3J = 7.1 Hz, 3H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.21–1.35 (m, 2H), 1.38–1.71 (m, 4H), 1.83 (s, 3H), 2.87 (s, 3H), 2.85–2.93 (m, 2H), 3.61 (s, 3H), 4.18–4.29 (m, 3H), 6.84–6.93 (m, 1H), 7.78–7.87 (m, 1H), 7.98–8.06 (m, 1H), 8.19–8.28 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 16.79, 18.03, 22.32, 22.47, 29.16, 31.17, 39.21, 42.45, 47.53, 48.21, 51.83, 51.83 169.16, 171.42, 172.22, 172.91. ESI-MS (*m/z*): 423.1 $[M+H]^+$, 445.1 $[M+Na]^+$. Anal. ($C_{16}H_{30}N_4O_7S$) C, H, N.

5.6.31. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(trifluoromethylsulfonamido)hexanamido)propanoate (80)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.17 (d, ^3J = 7.1 Hz, 3H), 1.27 (d, ^3J = 7.3 Hz, 3H), 1.23–1.37 (m, 2H), 1.42–1.72 (m, 4H), 1.82 (s, 3H), 3.06–3.15 (m, 2H), 3.61 (s, 3H), 4.19–4.29 (m, 3H), 7.80–7.88 (m, 1H), 7.99–8.07 (m, 1H), 8.21–8.29 (m, 1H), 9.27–9.38 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 17.28, 17.75, 22.35, 23.44, 30.91, 32.66, 44.75, 49.45, 50.67, 52.76, 54.02, 121.50 (q, $^1J_{CF}$ = 321.05 Hz), 173.34, 173.85, 174.53, 175.19. ESI-MS (*m/z*): 477.06 $[M+H]^+$, 499.22 $[M+Na]^+$. Anal. ($C_{16}H_{27}F_3N_4O_7S\cdot0.1$hexane) C, H, N.

5.6.32. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(4-methylphenylsulfonamido)hexanamido)propanoate (81)

^{1}H NMR (CD_3OD): δ = 1.32 (d, ^3J = 7.2 Hz, 3H), 1.38 (d, ^3J = 7.3 Hz, 3H), 1.35–1.43 (m, 2H), 1.43–1.52 (m, 2H), 1.55–1.82 (m, 2H), 1.97 (s, 3H), 2.43 (s, 3H), 2.79–2.87 (m, 2H), 3.71 (s, 3H), 4.26–4.42 (m, 3H), 7.37 (d, ^3J = 7.9 Hz, 2H), 7.72 (d, ^3J = 8.2 Hz, 2H). ^{13}C NMR (CD_3OD): δ = 17.28, 17.78, 21.42, 22.39, 23.39, 30.17, 32.65, 43.79, 49.43, 50.60, 52.54, 54.11, 128.05, 128.05, 130.71, 130.71, 138.95, 144.56, 173.29, 173.90, 174.53, 175.13. ESI-MS (*m/z*): 499.08 $[M+H]^+$, 521.27 $[M+Na]^+$. Anal. ($C_{22}H_{34}N_4O_7S\cdot0.1$hexane) C, H, N.

5.6.33. (*S*)-Methyl 2-((*S*)-2-((*S*)-2-acetamidopropanamido)-6-(4-nitrophenylsulfonamido)hexanamido)propanoate (82)

^{1}H NMR (CD_3OD): δ = 1.33 (d, ^3J = 7.2 Hz, 3H), 1.38 (d, ^3J = 7.3 Hz, 3H), 1.36–1.44 (m, 2H), 1.44–1.55 (m, 2H), 1.55–1.82 (m, 2H), 1.97 (s, 3H), 2.88–2.98 (m, 2H), 3.71 (s, 3H), 4.24–4.43 (m, 3H), 8.08 (d, ^3J = 8.8 Hz, 2H), 8.41 (d, ^3J = 8.7 Hz, 2H). ^{13}C NMR (CD_3OD): δ = 17.28, 17.77, 22.38, 23.57, 30.21, 32.63, 43.86, 49.51, 50.63, 52.78, 54.02, 125.43, 125.43, 129.39, 129.39, 147.95, 151.42, 173.32, 173.85, 174.54, 175.15. ESI-MS (*m/z*): 530.04 $[M+H]^+$, 552.22 $[M+Na]^+$. Anal. ($C_{21}H_{31}N_5O_9S\cdot0.5H_2O$) C, H, N.

5.6.34. (*S*)-Benzyl 6-(*tert*-butoxycarbonylamino)-1-oxo-1-(phenylamino)hexan-2-ylcarbamate (83)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.19–1.42 (m, 4H), 1.35 (s, 9H), 1.62 (m, 2H), 2.89 (m, 2H), 4.11 (m, 1H), 5.03 (s, 2H), 6.78 (br, 1H), 7.04 (t, ^3J = 7.3 Hz, 1H), 7.17–7.37 (m, 7H), 7.55 (d, ^3J = 7.7 Hz, 1H), 7.60 (d, ^3J = 8.1 Hz, 2H), 10.01 (s, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 22.9, 28.3,

29.2, 31.5, 39.6, 55.4, 65.4, 77.3, 119.2, 123.2, 127.7, 127.8, 128.3, 128.7, 137.0, 139.0, 155.6, 156.1, 171.1. ESI-MS (*m/z*): 456.01 $[M+H]^+$, 478.30 $[M+Na]^+$. Anal. ($C_{25}H_{33}N_3O_5$) C, H, N.

5.6.35. (*S*)-Benzyl 6-ethanethioamido-1-oxo-1-(phenylamino)hexan-2-ylcarbamate (84)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.29–1.47 (m, 2H), 1.50–1.72 (m, 4H), 2.36 (s, 3H), 3.45 (m, 2H), 4.14 (m, 1H), 5.03 (s, 2H), 7.05 (m, 1H), 7.17–7.37 (m, 7H), 7.57–7.61 (m, 3H), 9.96 (br, 1H), 10.02 (s, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 22.74, 27.10, 31.87, 34.28, 45.73, 55.28, 67.58, 120.24, 120.24, 124.91, 128.19, 128.19 128.53, 128.78, 128.78, 129.19, 129.19, 136.03, 137.44, 156.88, 170.02, 201.22. ESI-MS (*m/z*): 414.24 $[M+H]^+$, 436.26 $[M+Na]^+$. Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N.

5.6.36. (*S*)-Benzyl 6-(dimethylphosphorothioylamino)-1-oxo-1-(phenylamino)hexan-2-ylcarbamate (86)

^{1}H NMR ($(CD_3)_2SO$): δ = 1.26–1.49 (m, 4H), 1.57–1.72 (m, 2H), 1.62 (s, 3H), 1.65 (s, 3H), 2.78 (m, 2H), 4.10–4.19 (m, 2H), 5.03 (s, 2H), 7.04 (t, ^3J = 7.3 Hz, 1H), 7.18–7.37 (m, 7H), 7.57 (d, ^3J = 8.1 Hz, 1H), 7.60 (d, ^3J = 7.7 Hz, 2H), 10.02 (s, 1H). ^{13}C NMR ($(CD_3)_2SO$): δ = 22.7 (d, $^1J_{CP}$ = 2.9 Hz), 23.0, 23.4 (d, $^1J_{CP}$ = 3.1 Hz), 30.9, 31.0, 31.6, 55.5, 65.4, 119.2, 123.3, 127.7, 127.8, 128.3, 128.7, 137.0, 139.0, 156.1, 171.1. ESI-MS (*m/z*): 448.25 $[M+H]^+$. Anal. ($C_{22}H_{30}N_3O_3PS$) C, H, N.

5.6.37. (*S*)-Benzyl 6-ethaneselenoamido-1-oxo-1-(phenylamino)hexan-2-ylcarbamate (89)

^{1}H NMR ($CDCl_3$): δ = 1.38–1.55 (m, 2H), 1.65–1.98 (m, 4H), 2.54 (s, 3H), 3.55–3.76 (m, 2H), 4.32–4.46 (m, 1H), 5.02–5.17 (m, 2H), 5.77–5.89 (m, 1H), 7.09 (t, ^3J = 7.3 Hz, 1H), 7.22–7.38 (m, 7H), 7.46 (d, ^3J = 8.1 Hz, 1H), 8.60–8.69 (m, 1H). ^{13}C NMR ($CDCl_3$): δ = 22.74, 26.76, 32.16, 49.33, 55.25, 67.47, 120.32, 120.48, 124.96, 128.01, 128.46, 128.72, 129.12, 135.96, 137.38, 156.92, 170.45, 205.04. ESI-MS (*m/z*): 462.20 $[M+H]^+$, 484.21 $[M+Na]^+$. Anal. ($C_{25}H_{27}N_3O_3Se$) C, H, N.

5.7. In vitro assay for SIRT1 and SIRT2 activities

The Fluor de Lys fluorescence assays were based on the method described in the BioMol product sheet using BioMol KI177 substrate for SIRT1 and KI179 substrate for SIRT2. Determined K_m for SIRT1 substrate was 58 μM and for SIRT2 substrate 198 μM.[33]

Briefly, assays were carried out using Fluor de Lys acetylated 40 μM SIRT1- or 138 μM SIRT2-peptide substrate (concentrations were 70% of K_m values), 500 μM NAD^+ (N6522, Sigma), recombinant GST-SIRT1/2-enzyme and SIRT assay buffer (HDAC assay buffer, KI143, supplemented with 1 mg/ml BSA, A3803, Sigma). GST-SIRT1-enzyme and GST-SIRT2-enzyme were produced as described recently.[23,25] The buffer, SIRT1/2- peptide substrate, NAD^+ and DMSO/compounds in DMSO (2.5 μl in 50 μl total volume of reaction mixture; DMSO from Sigma, D2650) for testing were preincubated for 5 min at rt. The reaction was started by adding the SIRT1- or SIRT2-enzyme. The reaction mixture was incubated for 1 h at 37 °C. After that Fluor de Lys developer (KI176) plus 2 mM nicotinamide in 50 μL were added and incubation was continued for 45 min at 37 °C. Fluorescence readings were obtained using the VictorTM 1420 Multilabel Counter (Wallac, Finland) with excitation wavelength 355 nm and emission 460 nm.

The IC_{50} values were based on 9-point dose-response determination (2000 μM, 1000 μM, 100 μM, 10 μM, 1 μM, 0.1 μM, 0.01 μM, 0.001 μM and 0.0001 μM) where more necessary dose points were added between the critical concentrations depending on the compound. Each experiment was repeated at least three times and calculated using Graph Pad Prism Software version 4.03 (© 19922005 GraphPad Software, Inc.). The SIRT1 and SIRT2 assays differ in their active enzyme concentrations and consequently, SIRT1 and SIRT2 IC_{50} values cannot be directly compared.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.035.

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